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A Turn-on and Colorimetric Metal-free Long Lifetime Fluorescent Probe and Its Application for Time-Resolved Luminescent Detection and Bioimaging of Cysteine

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Reagents and materials

Common reagents used in the experiments were all of analytical grade. Acryloylchloride was purchased from Tianjing Damao Chemical Reagent Factory. Bovine serum albumin and other proteins were purchased from Sangon Biotech (Shanghai) Co. Ltd. All the reactions were carried out under nitrogen atmosphere with dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Silica gel (100-200 mesh) was used for flash column chromatography.

General information

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a VARIAN INOVA-400 spectrometer with chemical shifts reported as ppm (in DMSO or methanol, TMS as internal standard). Mass spectrometric data were obtained on a Q-TOF Micro mass spectrometry. The following abbreviations are used to indicate the multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Absorption spectra were measured on a Perkine Elmer Lambda 35 UV-Vis spectrophotometer. Fluorescence measurements were performed on a VARIAN CARY Eclipse Fluorescence Spectrophotometer. Cell imaging measurements were obtained with

spectral confocal microscopes (Olympus, FV1000). The time-resolved luminescence imaging measurements were carried out on a laboratory-useluminescence microscope. The microscope, equipped with a 30 W xenon flash-lamp (Pulse 300, Photonic Research Systems Ltd.), UV-2A filters (Nikon, excitation filter, 510-560 nm; dichroic mirror, 400 nm; emission filter, > 420 nm) and a time-resolved digital black-and- white CCD camera system (Photonic Research Systems Ltd.) was used for the time-resolved luminescence imaging measurement with the conditions of delaytime, 15 μs; gatetime, 100 ms; lamp-pulse width, 6 ms; and exposure time, 300 s. The time-resolved luminescence images are shown in pseudo-color treated by Simple PCI software.

Cell culture and staining

MCF-7 cell lines were cultured in DEME (Invitrogen) supplemented with 10% FCS (Invitrogen). Then the pretreated MCF-7 cells were stained with **DCF-MPYM-thiol** (20 μM) for 120 min at ambient temperature and then imaged with confocal fluorescence microscopy or time-resolved fluorescence microscopy. And a total of 40 μL BSA (10 mM) was added into the system and incubated at 37 ^{o}C in 5% CO_2 for 2 h. Cells were rinsed by clean PBS three times before imaging. For the dye's test, the fluorescent imaging pictures were obtained with an almostly equal parameter for control.

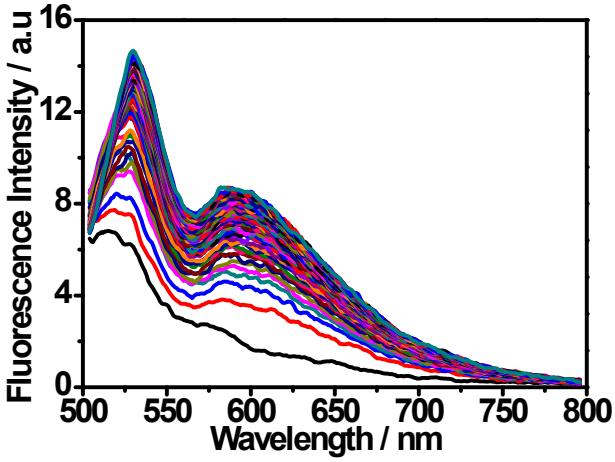


Figure S1. Fluorescence changes of **DCF-MPYM-thiol** (5.0 μM in PBS buffer) after incubation with 100 μL (10 mM) of cysteine. Each spectrum was recorded after 1 min, excitation at 485 nm.

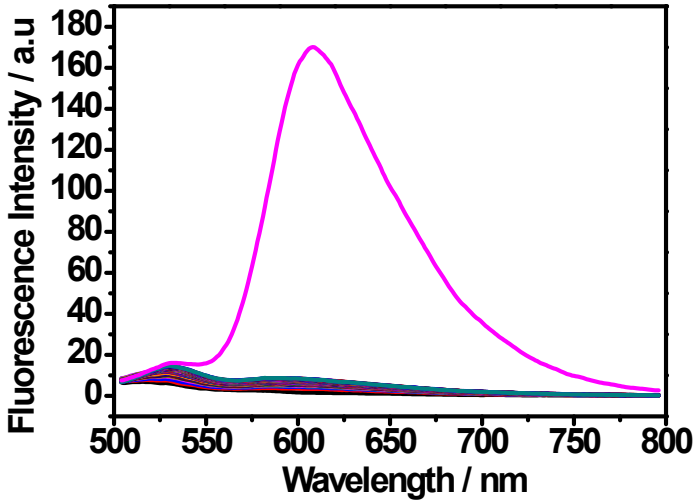


Figure S2. Fluorescence changes of **DCF-MPYM-thiol** (5.0 μM in PBS buffer) after incubation with 100 μL (10 mM) of cysteine. Each spectrum was recorded after 1 min, excitation at 485 nm. And then add 100 μL (25 mg/mL) BSA into the detection system.

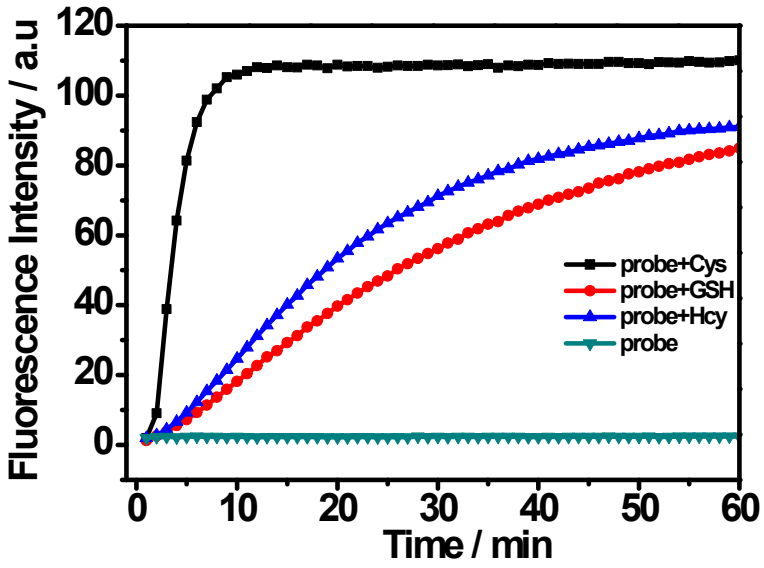


Figure S3. Time course of the fluorescence response (λ_{em} = 620 nm) of probe **DCF-MPYM-thiol** (3.0 μM in CH_3CN/PBS buffer = 1/1), regarding the presence of 62.6 equiv of Cys, Hcy and GSH, excitation wavelength is 485 nm.

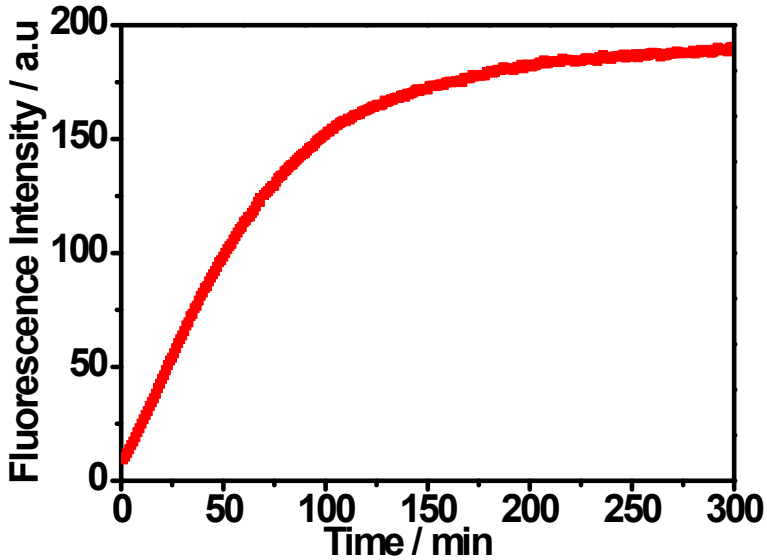


Figure S4. Time course of the fluorescence response (λ_{em} = 625 nm) of probe **DCF-MPYM-thiol** (3.0 μM in CH_3CN/PBS buffer = 1/1), regarding the presence of 62.6 equiv of Hcy with an excitation at 485 nm.

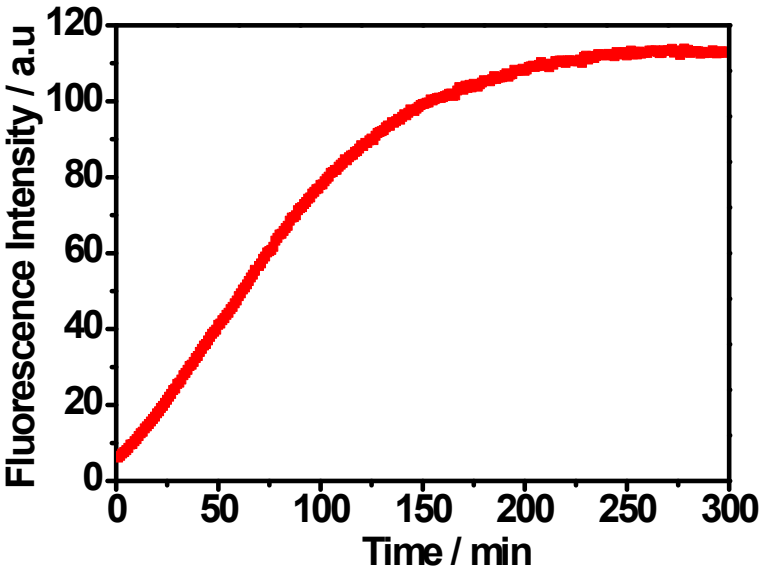


Figure S5. Time course of the fluorescence response (λ_{em} = 625 nm) of probe **DCF-MPYM-thiol** (3.0 μM in CH_3CN/PBS buffer = 1/1), regarding the presence of 62.6 equiv of GSH with an excitation at 485 nm.

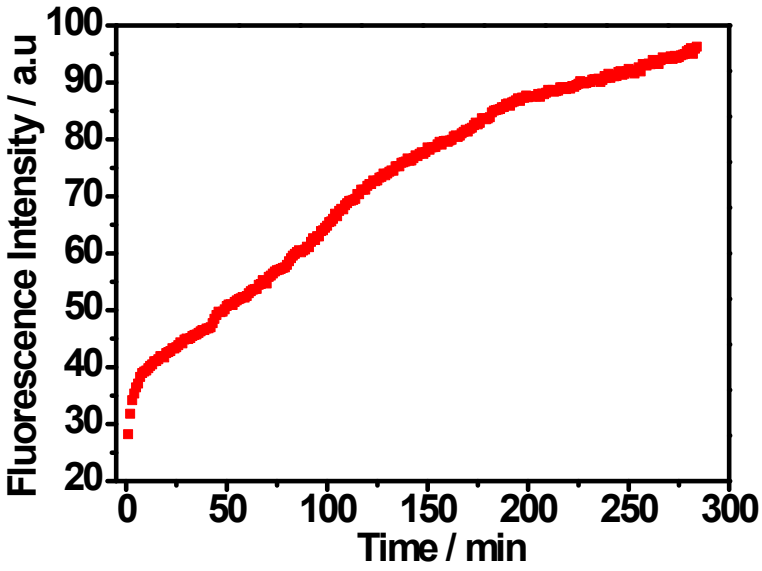


Figure S6. Time course of the fluorescence response (λ_{em} = 625 nm) of probe **DCF-MPYM-thiol** (3.0 μM in newborn-calf serum sample), with an excitation at 485 nm.

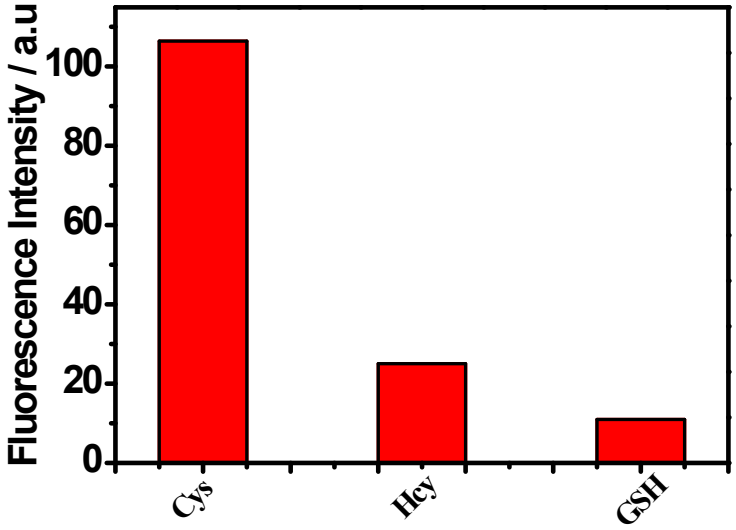


Figure S7. Fluorescence intensity at 620 nm of **DCF-MPYM-thiol** (3.0 μM in CH_3CN/PBS buffer = 1/1) upon addition of the same concentration of Cys (133.0 μM), Hcy (133.0 μM), GSH (133.0 μM) after 10 min, excitation wavelength is 485 nm.

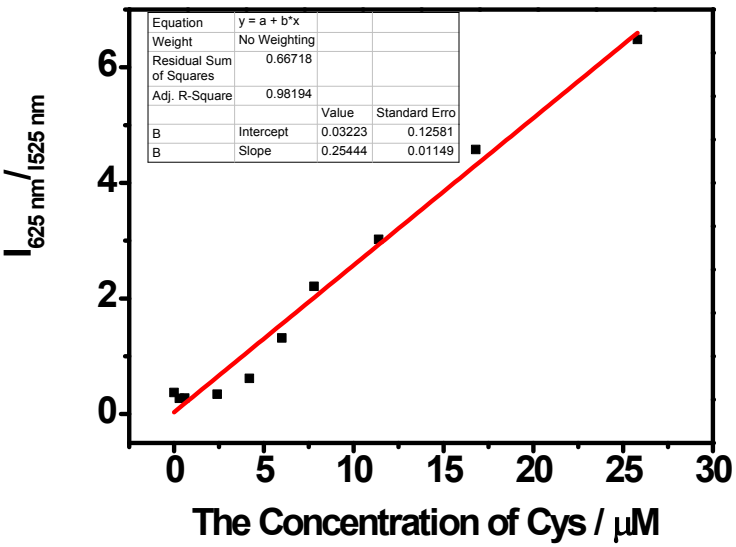


Figure S8. Fluorescence intensity ratio at 625 nm and 525 nm of **DCF-MPYM-thiol** (3.0 μM in CH_3CN/PBS buffer = 1/1) upon addition of Cys (0–25.8 μM), excitation wavelength is 485 nm.

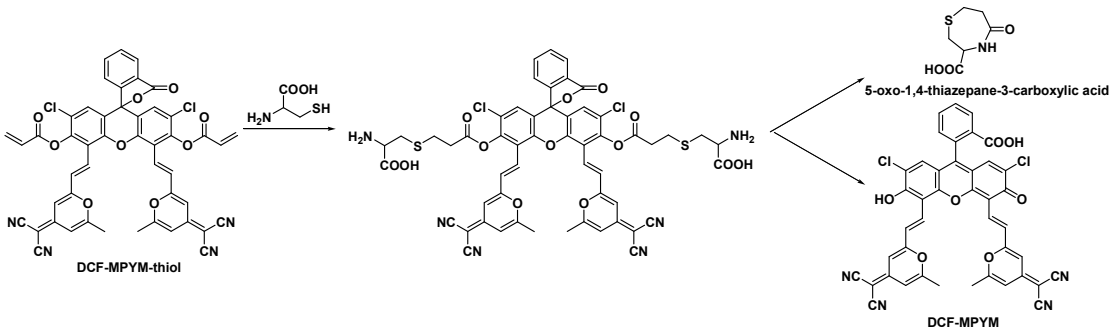


Figure S9. A plausible mechanism of reaction between **DCF-MPYM-thiol** and Cys.

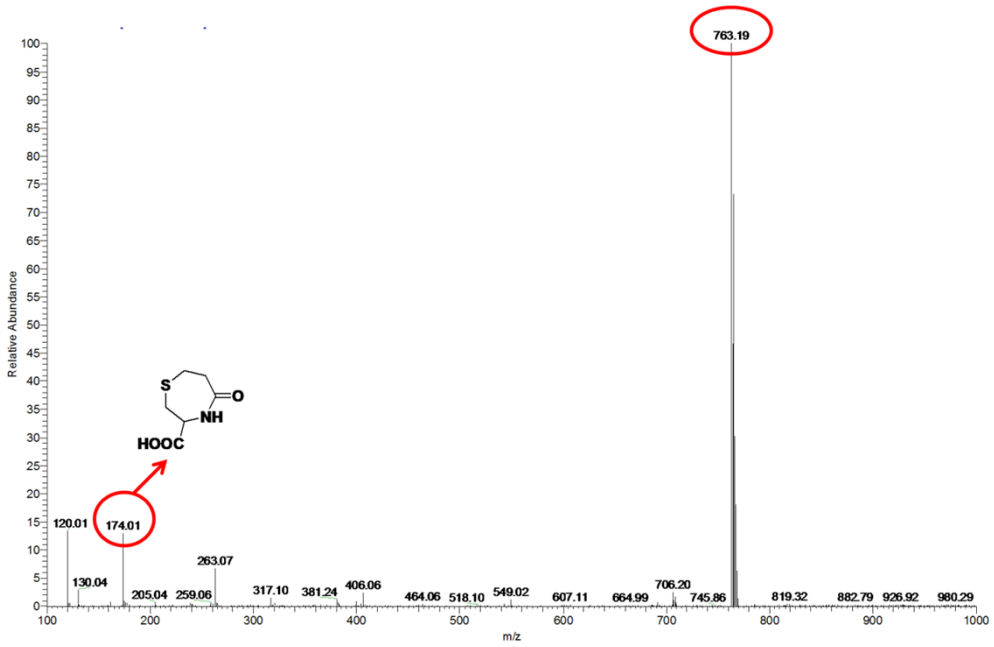


Figure S10. The API-ES mass of product obtained by mixing sensor **DCF-MPYM-thiol** and Cys.

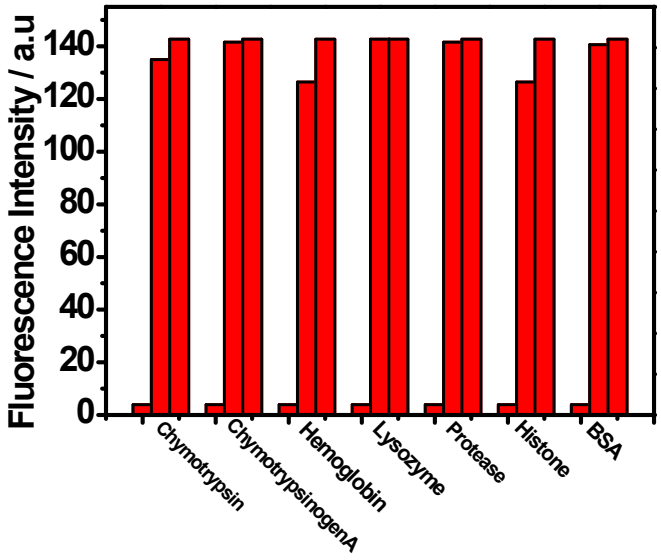


Figure S11. Fluorescence response at 625 nm of free **DCF-MPYM-thiol** (5.0 μM in CH_3CN/PBS buffer = 1/1), probe + proteins (55.0 μM) + Cys, and probe + Cys. The selectivity of **DCF-MPYM-thiol** to different kinds of proteins, the excitation wavelength is 485 nm. The columns from the left to the right stand for free **DCF-MPYM-thiol**, probe + protein + Cys, and probe + Cys, respectively.

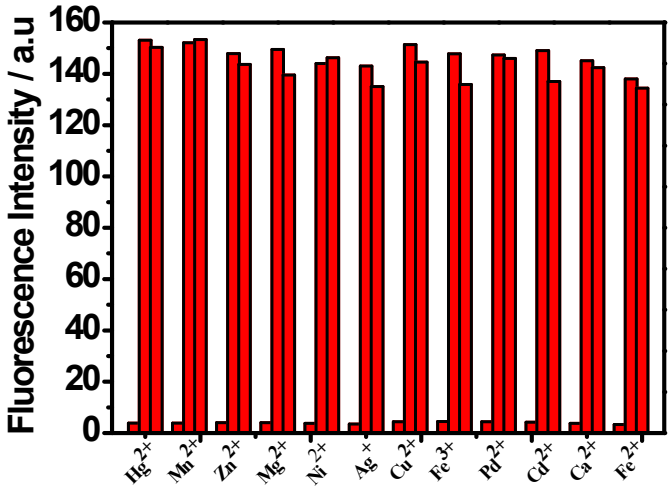


Figure S12. Fluorescence response at 625 nm of free **DCF-MPYM-thiol** (5.0 μM in CH_3CN/PBS buffer = 1/1), probe + Cys, and probe + cations (500.0 μM) + Cys. Each column was recorded after 10 min. λ_{ex} = 485 nm, λ_{em} = 625 nm. The columns from the left to the right stand for free **DCF-MPYM-thiol**, probe + Cys, and probe + cation + Cys, respectively.

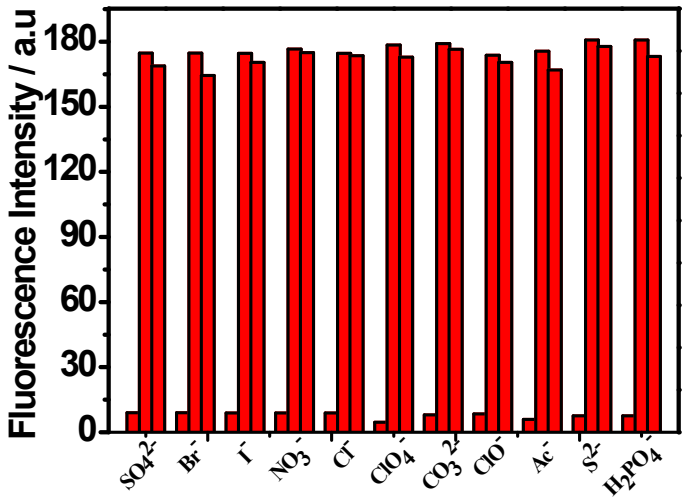


Figure S13. Fluorescence response at 625 nm of free **DCF-MPYM-thiol** (5.0 μM in CH_3CN/PBS buffer = 1/1), probe + Cys, and probe + cations (500.0 μM) + Cys. Each column was recorded after 10 min. λ_{ex} = 485 nm, λ_{em} = 625 nm. The columns from the left to the right stand for free probe, probe + Cy and probe + anion + Cys, respectively.

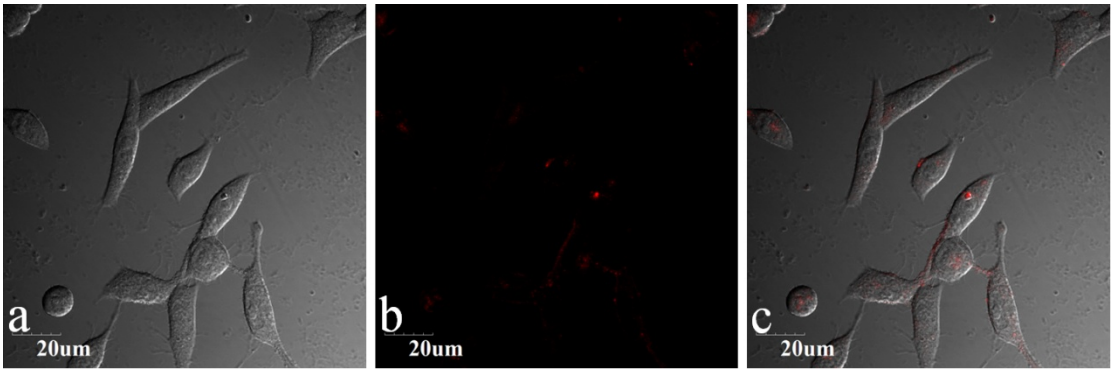
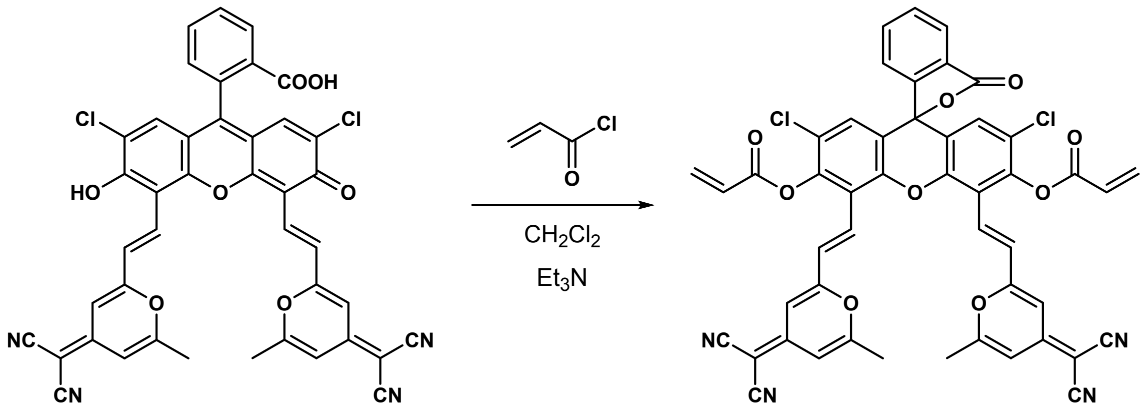
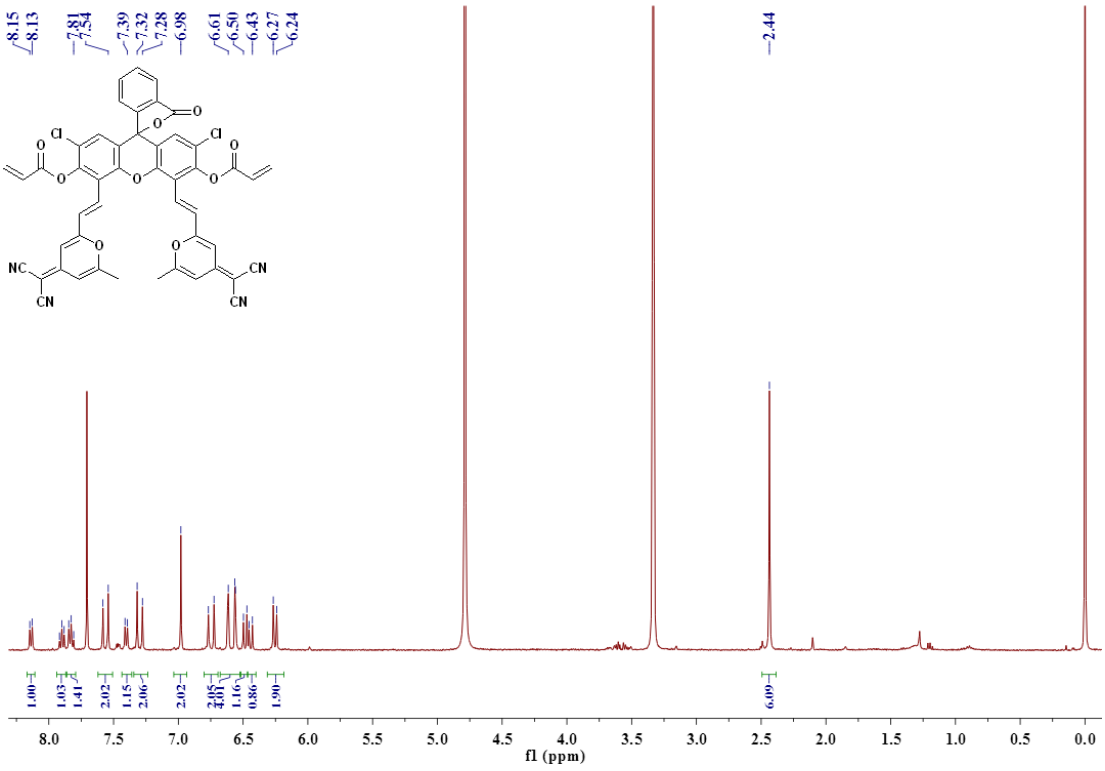


Figure S14. Confocal fluorescence images of MCF-7 cells pre-incubated with 500 μM NEM for 50 min and then treated with 15 μM of **DCF-MPYM-thiol** for 120 min and BSA (40 μL, 10 mM) at 37℃. After 120 min incubation: (a) bright field image of cells for **DCF-MPYM-thiol**; (b) fluorescent image excited at 488 nm for **DCF-MPYM-thiol** red emission (600±20 nm); (c) the overlay of (a), (b).

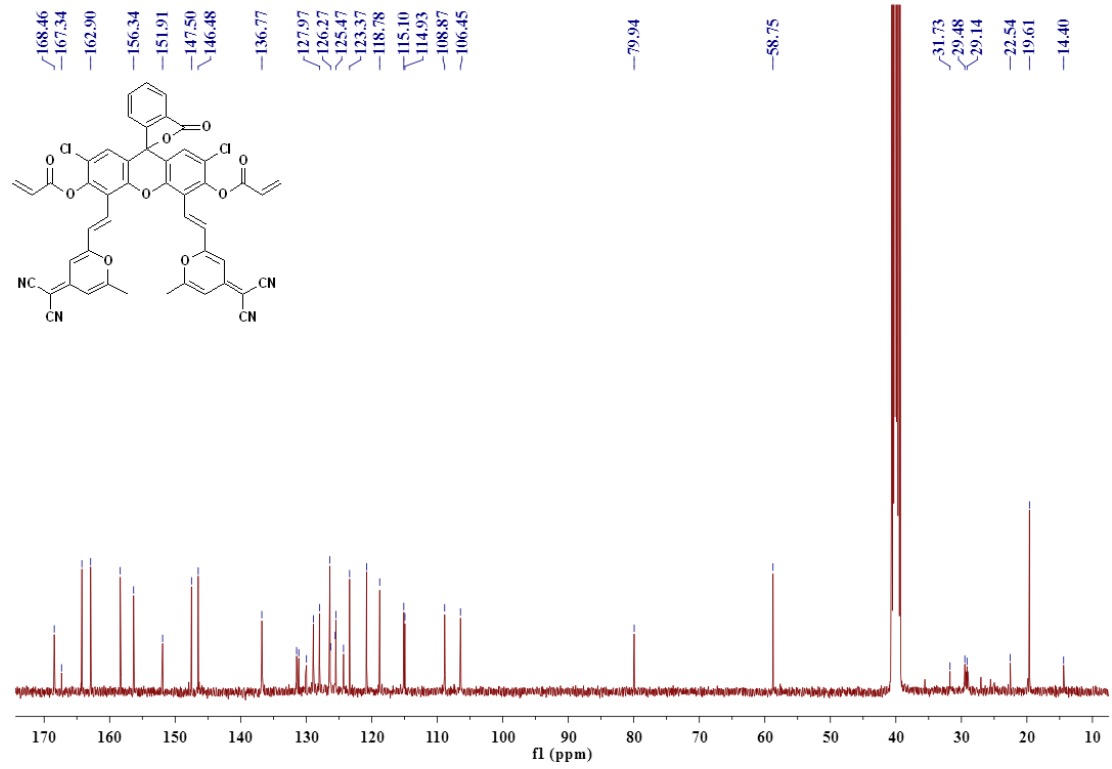


2',7'-dichloro-4',5'-bis((E)-2-(4-(dicyanomethylene)-6-methyl-4H-pyran-2-yl)vinyl)-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-3',6'-diyl diacrylate Compound DCF-MPYM-thiol：DCF-MPYM (12.6 mg, 0.0165 mmol) was dissolved in 20 mL anhydrous CH_2Cl_2, acryloylchloride (10 eq, 15 μL), Et_3N (10 eq, 30 μL) were added dropwise at 0 ^oC. After stirring at this temperature 90 min, the mixture was stirred at room temperature and stirred overnight. After evaporation of under reduced pressure, product **DCF-MPYM-thiol** (5.0 mg, 0.01035 mmol, yield 24%) was afforded from chromatography of the crude product on silica gel using CH_2Cl_2/CH_3OH (250/2) as eluent. R_f = 0.30, (5% CH_3OH in dichloromethane). ^1H NMR (400 MHz, CD_3OD) δ 8.14 (d, *J* = 7.7 Hz, 1H), 7.90 (t, *J* = 7.2 Hz, 1H), 7.83 (t, *J* = 7.6 Hz, 1H), 7.56 (d, *J* = 16.4 Hz, 2H), 7.40 (d, *J* = 7.6 Hz, 1H), 7.30 (d, *J* = 16.4 Hz, 2H), 6.98 (s, 2H), 6.75 (d, *J* = 17.3 Hz, 2H), 6.68 – 6.53 (m, 4H), 6.48 (d, *J* = 10.5 Hz, 1H), 6.44 (d, *J* = 10.6 Hz, 1H), 6.25 (d, *J* = 10.7 Hz, 2H), 2.44 (s, 6H). ^{13}C NMR (100 MHz, CD_3OD) δ 168.46, 167.34, 164.24, 162.90, 158.38, 156.34, 151.91, 147.50, 146.48, 136.77, 131.47, 131.13, 129.96, 128.90, 127.97, 126.39, 126.27, 125.57, 125.47, 124.30, 123.37, 120.78, 118.78, 115.10, 114.93, 108.87, 106.45, 79.94, 58.75, 31.73, 29.48, 29.14, 22.54, 19.61, 14.40. MS (ESI+Tof) m/z Found 872.0964 $[M+Na]^+$, calculated 872.11 for $C_{48}H_{26}Cl_2N_4O_9$.

^{1}H-NMR spectrum of compound **DCF-MPYM-thiol**



^{13}C spectrum of compound **DCF-MPYM-thiol**



HRMS spectrum of compound **DCF-MPYM-thiol**