A mitochondria-targeting fluorescent probe for the selective detection of glutathione in living cells

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Materials and instruments

All the solvents and reagents are of commercial quality and without further purification. ^{1}H and ^{13}C NMR spectra were recorded on an Advance Bruker 400M spectrometer and referenced to solvent signals. Mass spectra were obtained on a Bruker Apex IV Fourier Transform Mass Spectrometer. Fluorescence spectra were determined on a Hitachi 4500 spectrophotometer. Absorption spectra were determined on a Shimadzu UV-1601PC UV-Visible spectrophotometer. Confocal fluorescence imaging was performed with Nikon A1R MP multiphoton microscopy.

Cell culture fluorescence imaging

HeLa cells were cultured in culture media (DMEM/F12 supplemented with 10% FBS, 50 unit/mL penicillin, and 50 μg/mL of streptomycin) at 37 °C under a humidified atmosphere containing 5% CO_2 for 24 h. The cells were seeded in a 6-well plate at a density of 104 cells per well in culture media. Confocal fluorescence imaging was performed with Nikon A1R MP multiphoton microscopy with a 60 × oil-immersion objective lens. Green fluorescence was excited at 488 nm with a Si laser and emission was collected by a 500-550 nm band pass filter. Red fluorescence was excited at 561 nm and emission was collected by a 570-620 nm band pass filter.

Colocalization fluorescence imaging.

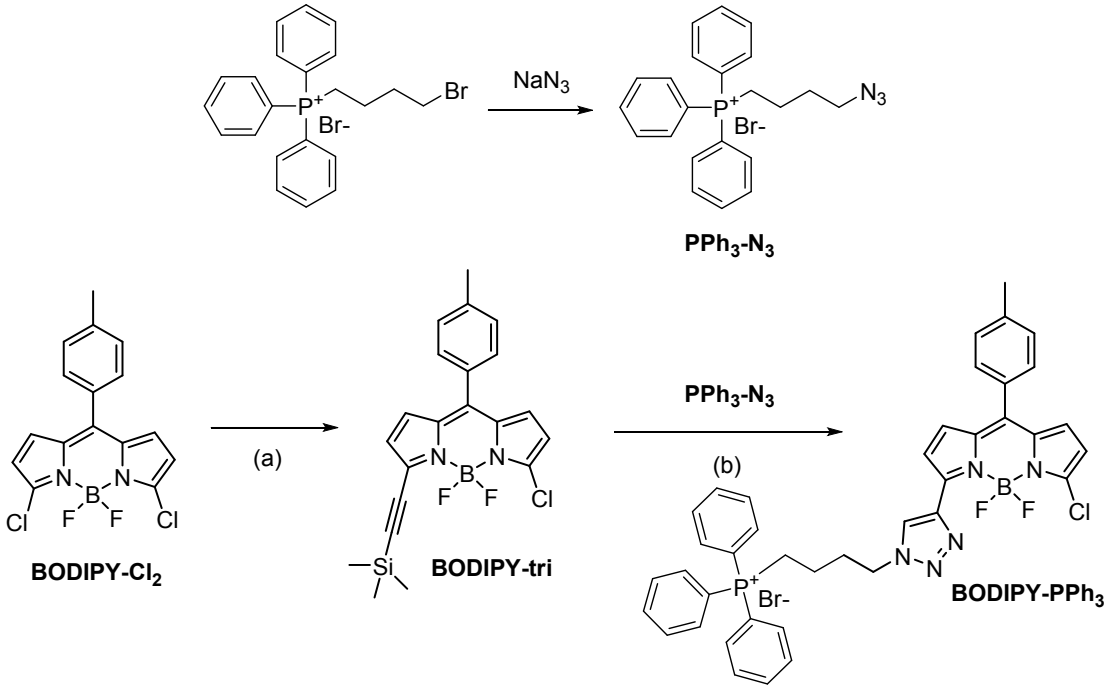
HeLa cells were incubated with GSH (1 mM) for 20 min, washed with PBS for three times, and then costained with **BODIPY-PPh$_3$** (2 μM) and Rhodamine 123 (2 μM) for 20 min under 5 % CO_2 at 37 °C.

Ratiometric fluorescence imaging of GSH

HeLa cells were treated and incubated with with GSH (1 mM) for 20 min. After washing with PBS, the cells were further incubated with **BODIPY-PPh$_3$** (2 μM) in culture media for 15 min. For the control experiment, the cells were treated with 1

mM NEM for 30 min. After washing with PBS, the cells were further incubated with **BODIPY-PPh$_3$** (2 μM) for 15 min.

Synthesis



Scheme S1. Synthesis of **BODIPY-PPh$_3$**. (a) trimethylsilylacetylene, Pd(PPh$_3$)$_2$Cl$_2$, CuI, toluene, Et$_3$N; (b) TBAF, TERN, CuI, CH$_2$Cl$_2$.

The (4-bromobutyl)triphenylphosphonium bromide (0.478g, 1 mmol) in 10 mL DMF was stirred and NaN_3 (0.195 g, 3 mmol) was added. The mixture was stirred at 85℃ overnight. After cooling to rt, CH_2Cl_2 was added and the mixture was washed with water to remove DMF. The organic phase was dried over $MgSO_4$ and the solvent was evaporated to obtain **PPh$_3$-N$_3$** (0.398 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.89-7.69 (m, 15H), 3.98 (m, 2H), 3.44 (t, 2H, J = 4.4 Hz), 2.03 (m, 2H), 1.73 (m, 2H).

BODIPY-Cl$_2$ (350 mg, 1 mmol) was dissolved in toluene (50 mL), then copper(I) iodide (10 mg, 0.05 mmol), bis(triphenylphosphine)palladium(II) chloride (35 mg, 0.05 mmol) were added under nitrogen. 1 mL of triethylamine was added to this mixture through a syringe, followed by trimethylsilylacetylene (142 μL, 1 mmol).

After stirring at 65 °C for 2 h, the solvent was evaporated under reduced pressure. The residue was purified by chromatography on silica gel (dichloromethane / petroleum ether = 1/4 as eluent) to give **BODIPY-tri** (185 mg, 45%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.39 (d, 2H, *J* = 8.0 Hz), 7.32 (d, 2H, *J* = 8.0 Hz), 6.87 (d, 1H, *J* = 4.4 Hz), 6.81 (d, 1H, *J* = 4.4 Hz), 6.63 (d, 1H, *J* = 4.4 Hz), 6.42 (d, 1H, *J* = 4.4 Hz), 2.46 (s, 3H), 0.32 (s, 9H).

BODIPY-tri (46 mg, 0.11 mmol), **PPh_3-N_3** (50 mg, 0.11 mmol), copper iodide (13 mg, 0.07 mmol) and tris[2-(dimethylamino)ethyl]amine (TREN, 10 uL，0.07mmol) were added to 15 mL dry dichloromethane and stirred for 30 min under nitrogen atmosphere. After adding tetrabutylammonium fluoride (TBAF, 40 mg, 0.15 mmol), the mixture continued to stir and rt for 3 hours. The reaction mixture was washed three times with water and extracted with dichloromethane. The organic layer was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The residue was purified by chromatography on silica gel (dichloromethane: methanol 10:1 as eluant). The product was further purified by recrystallization from dichloromethane/n-hexane to give **BODIPY-PPh_3** as a red solid (11.5 mg, 12.3%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.55 (s, 1H), 7.82-7.65 (m, 15H), 7.42 (d, 2H, *J* = 8.0 Hz), 7.34 (m, 3H), 7.00 (d, 1H, *J* = 4.0 Hz), 6.80 (d, 1H, *J* = 4.4 Hz), 6.39 (d, 1H, *J* = 4.0 Hz), 4.62 (m, 2H), 3.98 (m, 2H), 2.47 (m, 5H), 1.64 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 149.72, 143.74, 141.39, 141.28, 139.11, 136.72, 135.05, 135.02, 133.73, 133.63, 132.49, 130.56, 130.53, 130.44, 130.32, 129.65, 126.14, 120.39, 118.39, 117.67, 117.54, 49.39, 22.05, 21.54, 21.47, 19.29. ESI-HRMS: calculated for $C_{40}H_{35}BClF_2N_5P^+$ 700.23742, found 700.23750.

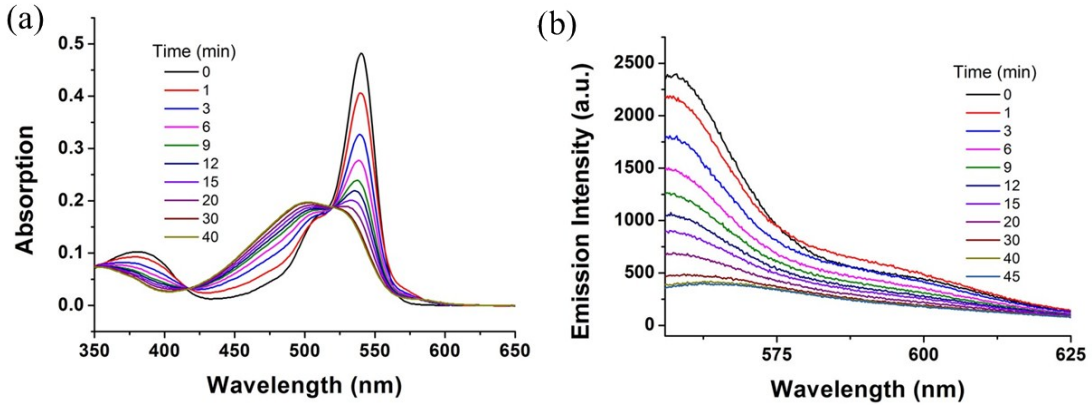


Fig. S1. Time-dependent (a) absorption and (b) emission spectra of **BODIPY-PPh$_3$** (10 μM) in the presence of 1 mM Cys in acetonitrile/ HEPES buffer (5:95, v/v, 20 mM, pH 7.4) at 37 °C. λ_{ex} = 550 nm.

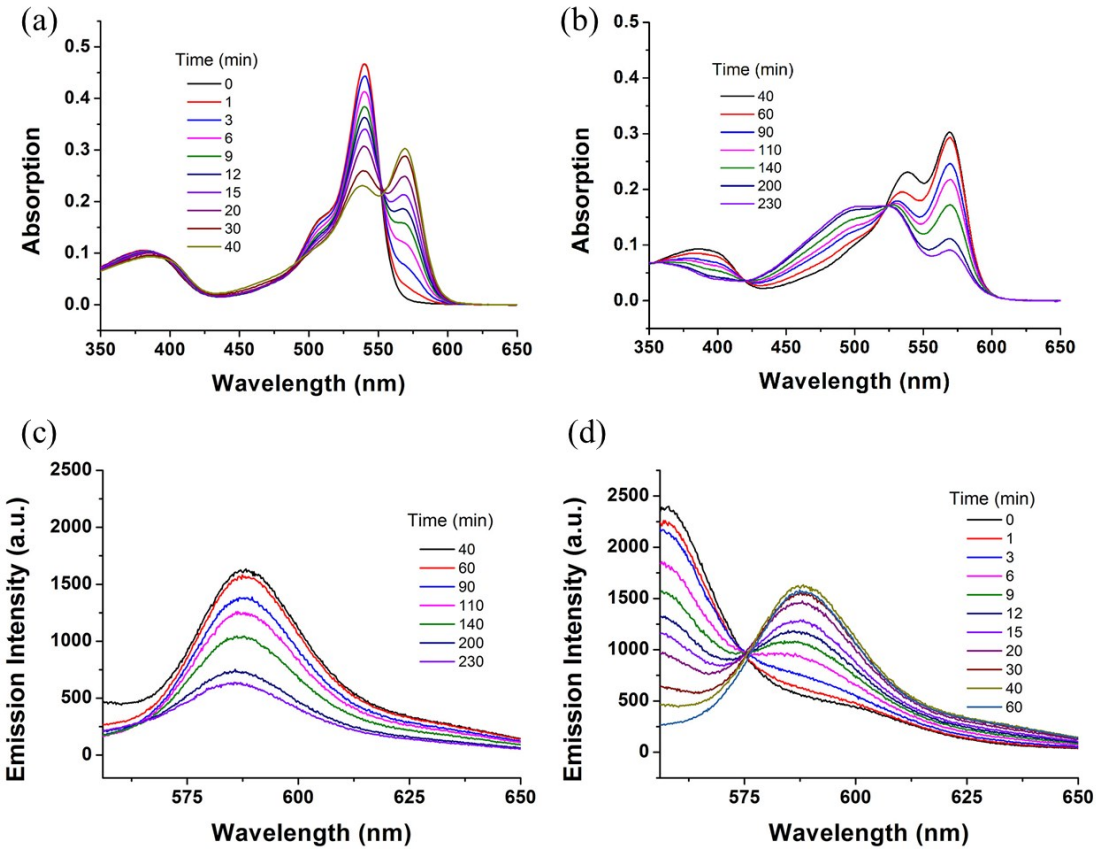


Fig. S2. Time-dependent (a,b) absorption and (c,d) emission spectra of **BODIPY-PPh$_3$** (10 μM) in the presence of 1 mM Hcy in acetonitrile/ HEPES buffer (5:95, v/v, 20 mM, pH 7.4) at 37 °C. λ_{ex} = 550 nm.

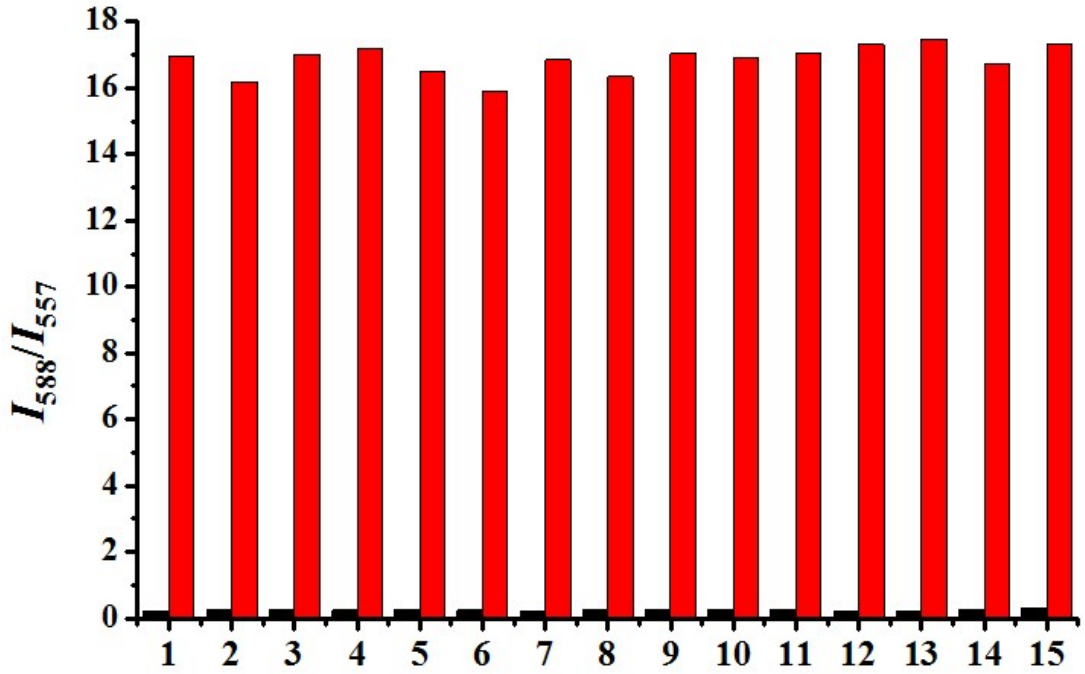


Fig. S3. Ratios of fluorescence intensity (I_{588}/I_{557}) of **BODIPY-PPh$_3$** (10 μM) in the presence various analytes. Black bars represent the addition of a single analyte (1 mM): (1) none, (2) Al^{3+}, (3) Zn^{2+}, (4) Na^{+}, (5) Mg^{2+}, (6) Fe^{3+}, (7) Ca^{2+}, (8) Val, (9) Ser, (10) Thr, (11) Lys, (12) Glu, (13) His, (14) Arg, (15) Ala. Red bars represent the subsequent addition of GSH (1 mM) to the mixture. All experiments were performed in acetonitrile/HEPES buffer (5:95 v/v, 20mM, pH 7.4) at 37 °C, and data was obtained 1h after addition of the analytes and GSH. λ_{ex} = 550 nm

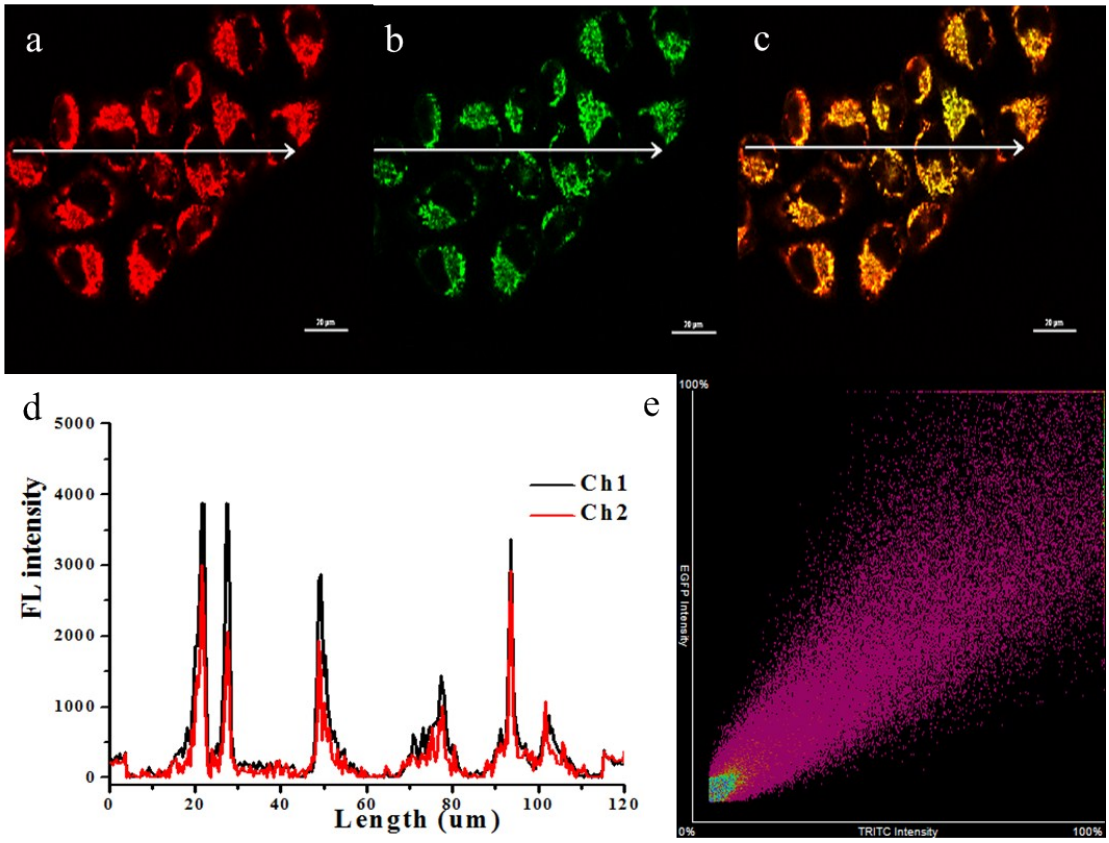
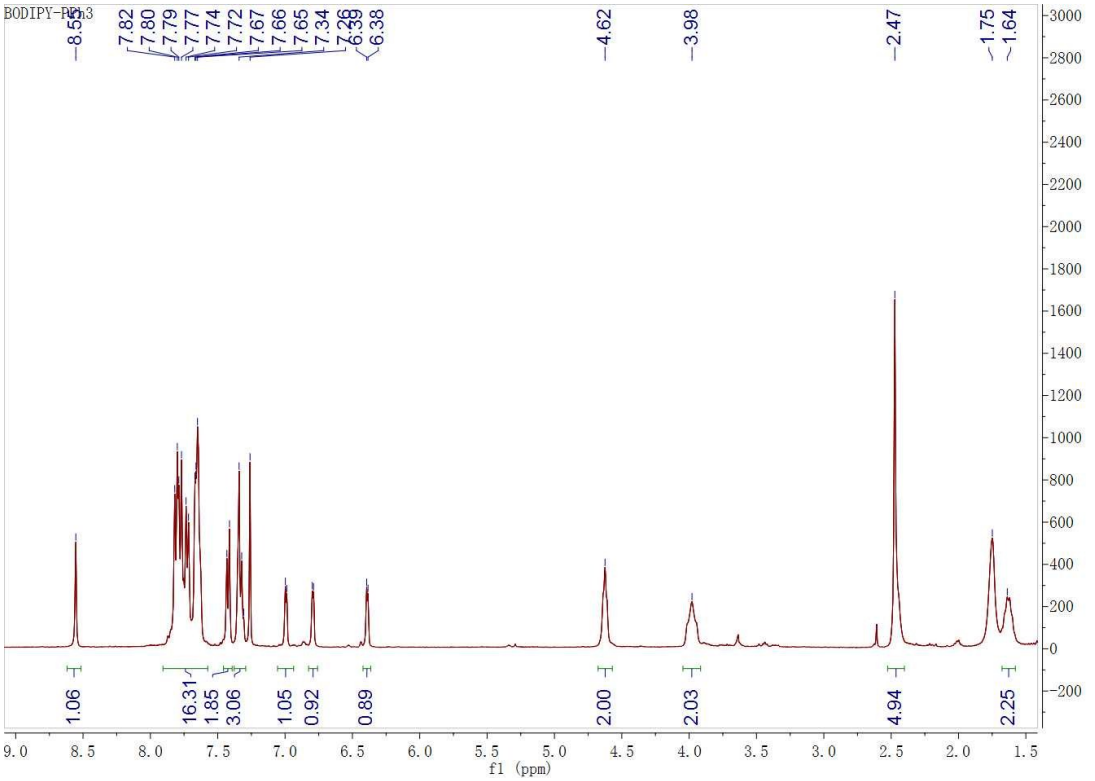
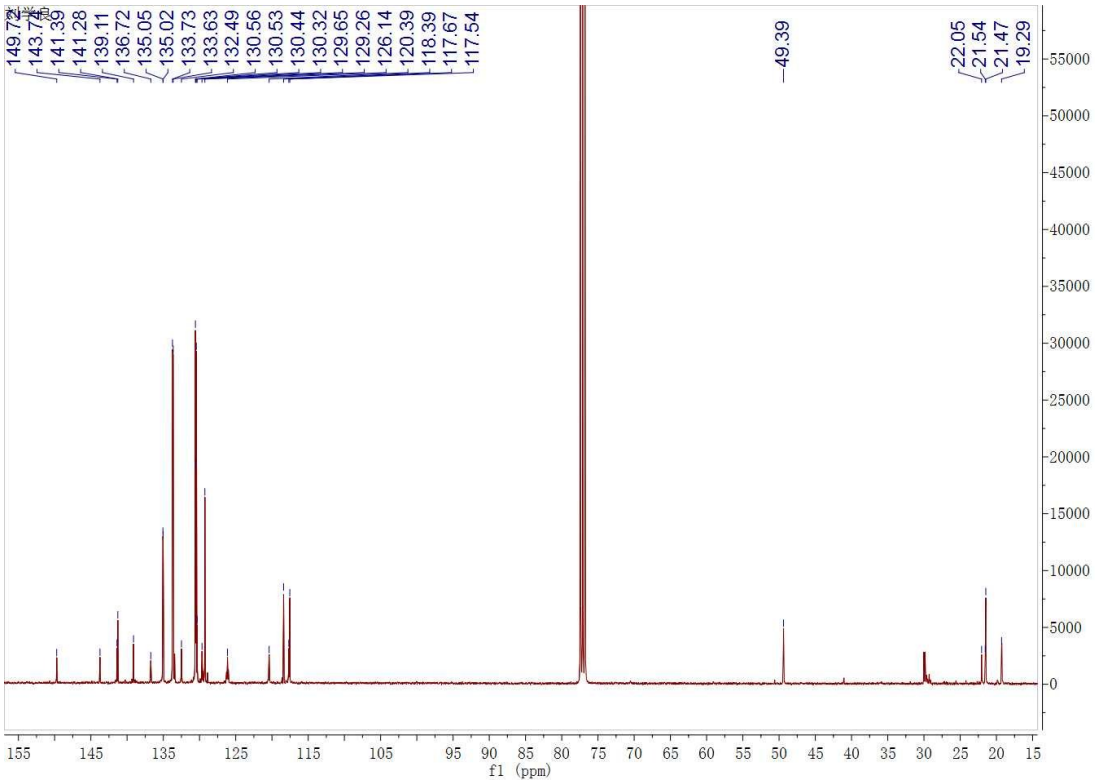
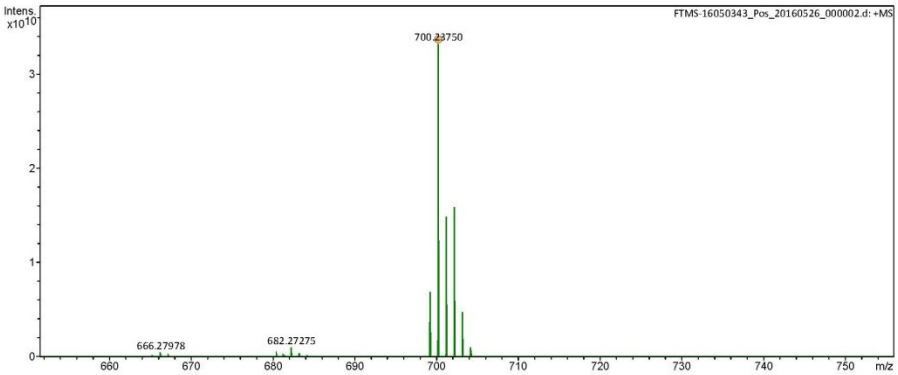


Fig. S4. Multiple cells colocalization of Rh123 and **BODIPY-PPh$_3$** in mitochondria of HeLa cells. HeLa cells were pretreated with GSH (1mM) for 20 min, and then co-stained with (a) Rh123 (2 μM, Channel 1 (Ch1), λ_{ex} 488 nm; λ_{em} 500−550 nm) and **BODIPY-PPh$_3$** (2 μM, Channel 2 (Ch2), λ_{ex} 561 nm; λ_{em} 570−620 nm). (c) Merged image of (a) and (b). (d) Intensity profile of region of interest 1 (ROI 1) cross the HeLa cell costained with Rh 123 and **BODIPY-PPh$_3$**. (e) Intensity scatter plot of Ch1 and Ch2.

^{1}H NMR, ^{13}C NMR and HRMS spectra of BODIPY-PPh$_3$







Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
700.237505	1	C40H35BClF2N5P	100.00	700.237424	0.9	1.3	35.1	25.5	even	ok