Analyst

Electronic Supplementary Information

Rosamine with Pyronine-Pyridinium Skeleton: Unique Mitochondrial Targetable Structure for Fluorescent Probes

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1. Figures

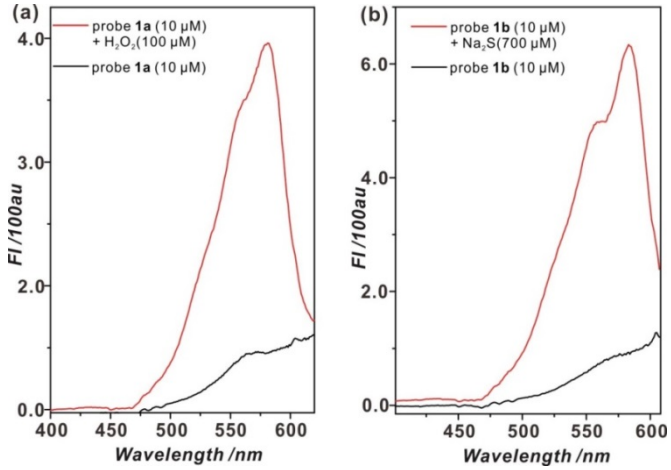

Fig. S1 Excitation spectra of probes **1a–b** (10 μM, λ_{em} = 604 nm) towards the detected substances in PBS buffer (20 mM, pH=7.4) containing 10% DMSO. (a) Excitation spectra of probe **1a** in the absence and presence of H_2O_2; (b) excitation spectra of probe **1b** in the absence and presence of Na_2S.

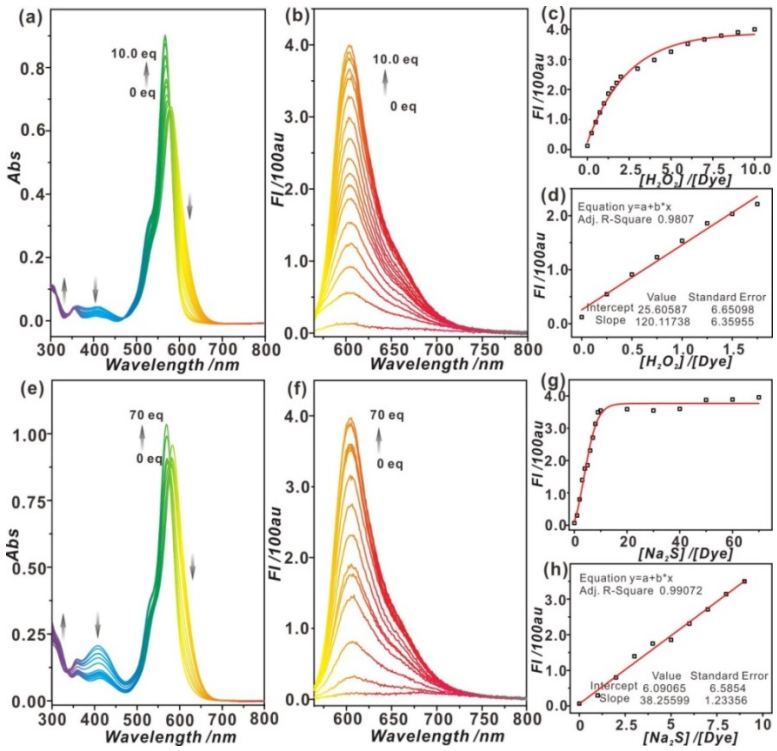

Fig. S2 Absorption and emission titration of probes **1a–b** (10 μM) toward the detected substances in PBS buffer (20 mM, pH=7.4) containing 10% DMSO. (a) Absorption spectra of probe **1a** in the presence of different concentrations of H_2O_2 (0–10.0 eq.); (b) emission spectra of probe **1a**; (c) fluorescence-intensity changes at 604 nm of probe **1a**; (d) plot of the fluorescence intensity of **1a** upon addition of H_2O_2 (0–2.0 eq.); (e) absorption spectra of probe **1b** in the presence of different concentrations of Na_2S (0–70.0 eq.); (f) fluorescence spectra of probe **1b**; (g) fluorescence-intensity changes at 604 nm of probe **1b**; (h) plot of the fluorescence intensity of **1b** upon addition of Na_2S (0–9.0 eq.).

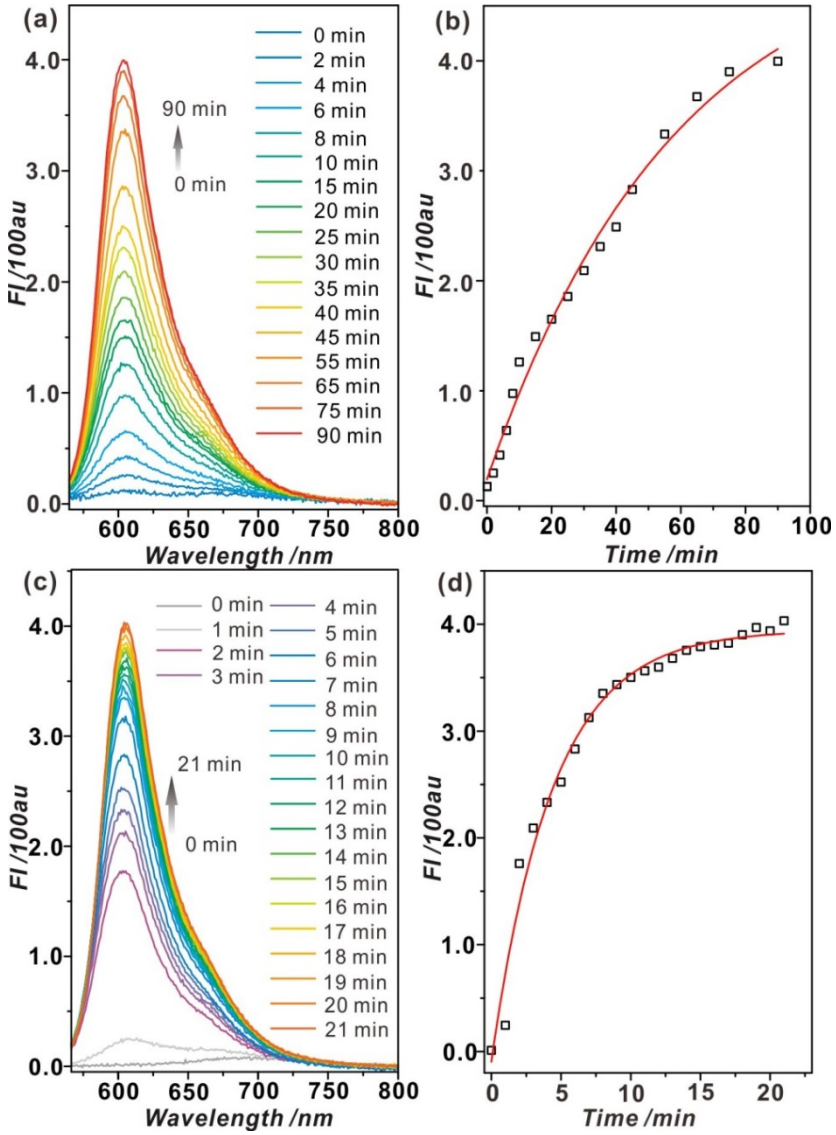

Fig. S3 Time-dependent fluorescence-intensity changes of probes **1a–b** (10 μM) towards the detected substances. (a, c) Time-dependent emission spectra; (b, d) plots of fluorescent intensities at 604 nm towards time. (a, b) Probe **1a** in the presence of H_2O_2 (100 μM); (c, d) probe **1b** in the presence of Na_2S (700 μM).

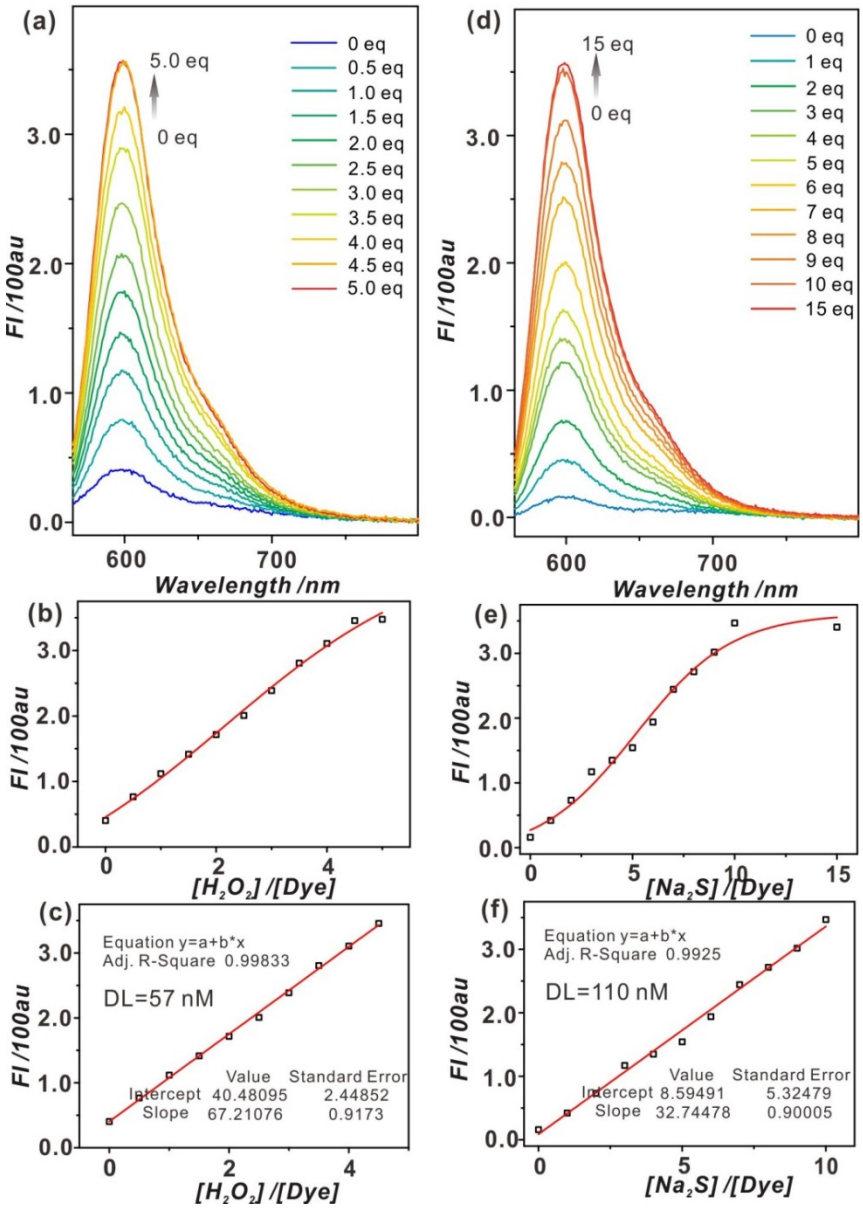

Fig. S4 Determinations of detection limits. (a) Fluorescence spectra of probe **1a** (1 μM) in PBS buffer solution in presence of different concentrations of H_2O_2 (0–5.0 eq.); (b) fluorescence intensity changes at 604 nm of probe **1a** with the amount of H_2O_2 (0–5.0 eq.); (c) plot of the fluorescence intensity of **1a** upon addition of H_2O_2 (0–4.5 eq.); (d) fluorescence spectra of probe **1b** (1 μM) in presence of different concentrations of Na_2S (0–15.0 eq.); (e) fluorescence intensity changes of probe **1b** with the amount of Na_2S (0–15.0 eq.); (f) plot of the fluorescence intensity of **1b** upon addition of Na_2S (0–10.0 eq.).

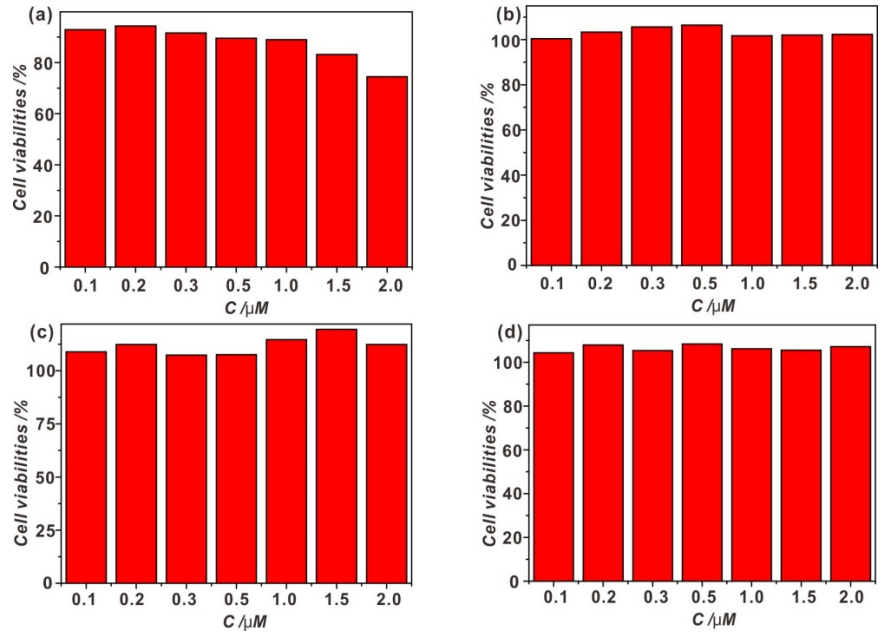

Fig. S5 Percentages of HeLa (a, c) and Ges-1 (b, d) cell viabilities remaining after cell treatment with probes **1a-b** (untreated cells were considered to have 100% survival). (a, b) Cells treated with probe **1a**; (c, d) cells treated with probe **1b**. Cell viabilities were assayed by the CCK-8 method.

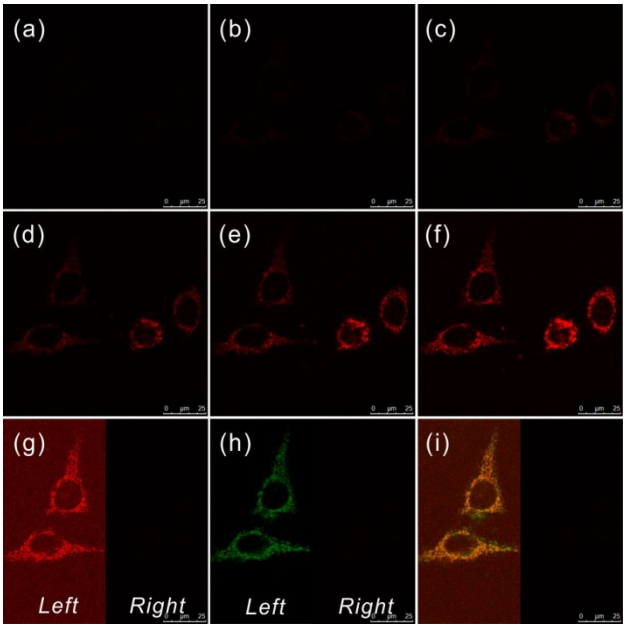

Fig. S6 Fluorescence confocal images of HeLa cancer cells with probe **1a** (200 nM) and standard MitoTracker FM (50 nM). (a) Confocal images (red channel) of cells with probe **1a** before addition of H_2O_2; (b–f) confocal images of cells with probe **1a** after addition of H_2O_2 (2 μM) for 15 min, 30 min, 45 min, 60 min, 90 min; (g) left, enhanced background light of (a); right, the right half of (a); (h) left, confocal images (green channel) of cells with MitoTracker Green FM; right, the right half of (a); (i) merged images of (g) and (h).

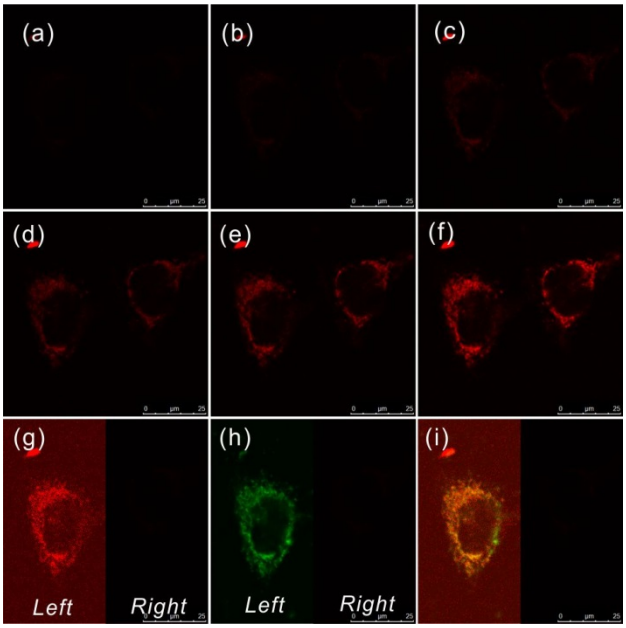

Fig. S7 Fluorescence confocal images of Ges-1 normal cells with probe **1a** (200 nM) and standard MitoTracker FM (50 nM). (a) Confocal images (red channel) of cells with probe **1a** before addition of H_2O_2; (b–f) confocal images of cells with probe **1a** after addition of H_2O_2 (2 μM) for 15 min, 30 min, 45 min, 60 min, 90 min; (g) left, enhanced background light of (a); right, the right half of (a); (h) left, confocal images (green channel) of cells with MitoTracker Green FM; right, the right half of (a); (i) merged images of (g) and (h).

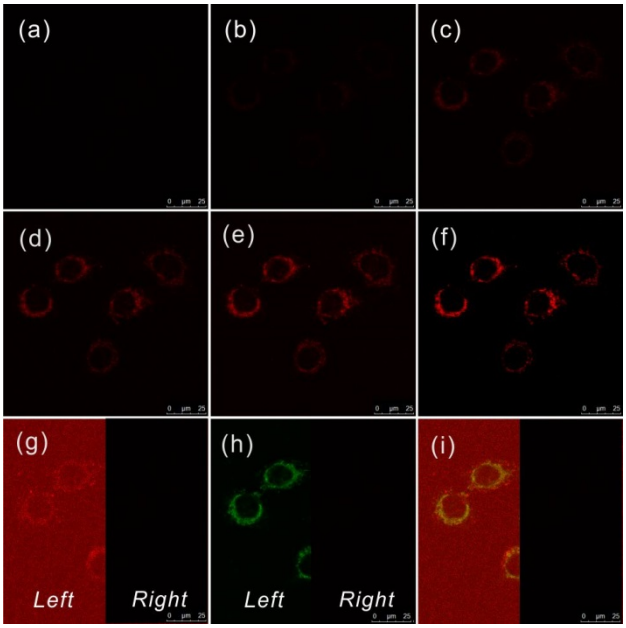

Fig. S8 Fluorescence confocal images of HeLa cancer cells with probe **1b** (200 nM) and standard MitoTracker FM (50 nM). (a) Confocal images (red channel) of cells with probe **1b** before addition of Na_2S; (b–f) confocal images of cells with probe **1b** after addition of Na_2S (20 μM) for 5 min, 10 min, 15 min, 20 min, 25 min; (g) left, enhanced background light of (a); right, the right half of (a); (h) left, confocal images (green channel) of cells with MitoTracker Green FM; right, the right half of (a); (i) merged images of (g) and (h).

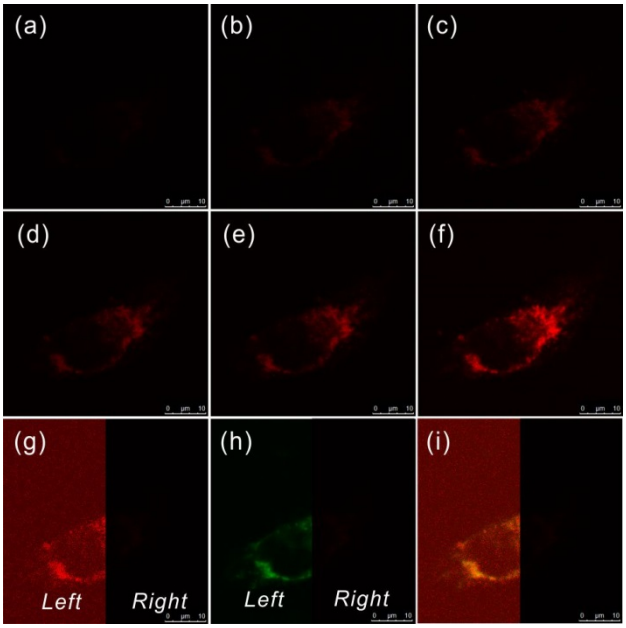

Fig. S9 Fluorescence confocal images of Ges-1 normal cells with probe **1b** (200 nM) and standard MitoTracker FM (50 nM). (a) Confocal images (red channel) of cells with probe **1b** before addition of Na_2S; (b–f) confocal images of cells with probe **1b** after addition of Na_2S (20 μM) for 5 min, 10 min, 15 min, 20 min, 25 min; (g) left, enhanced background light of (a); right, the right half of (a); (h) left, confocal images (green channel) of cells with MitoTracker Green FM; right, the right half of (a); (i) merged images of (g) and (h).

2. Appendix

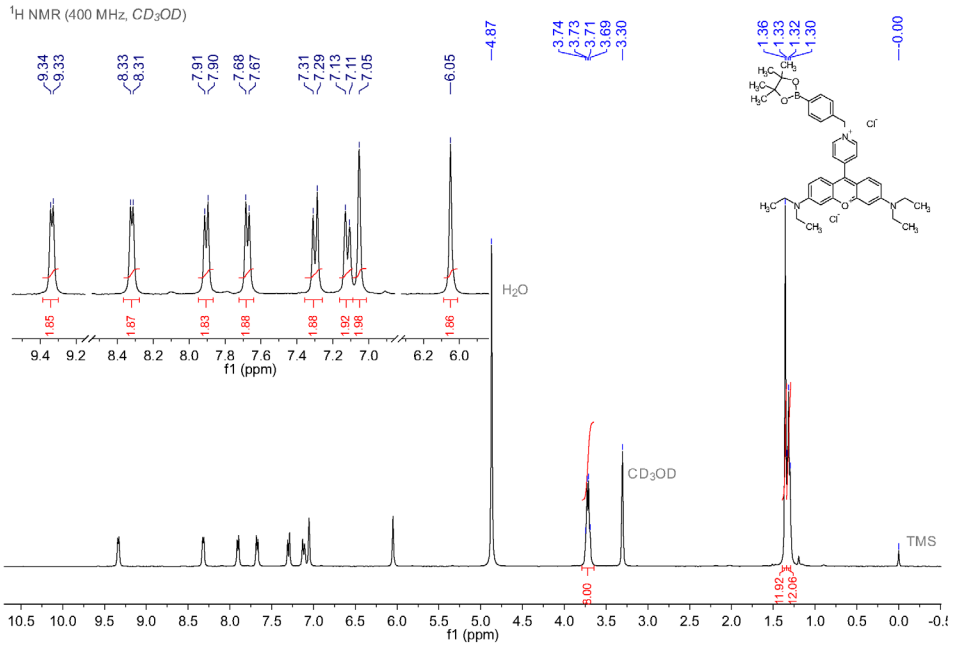

Fig. S10 ^{1}H NMR of probe **1a**

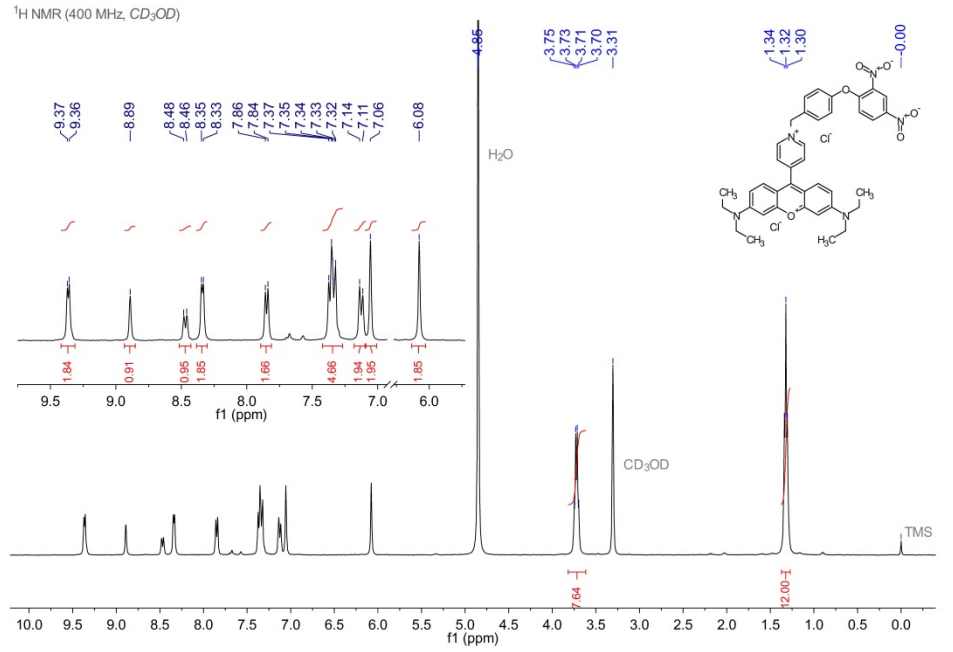

Fig. S11 ^{1}H NMR of probe **1b**.

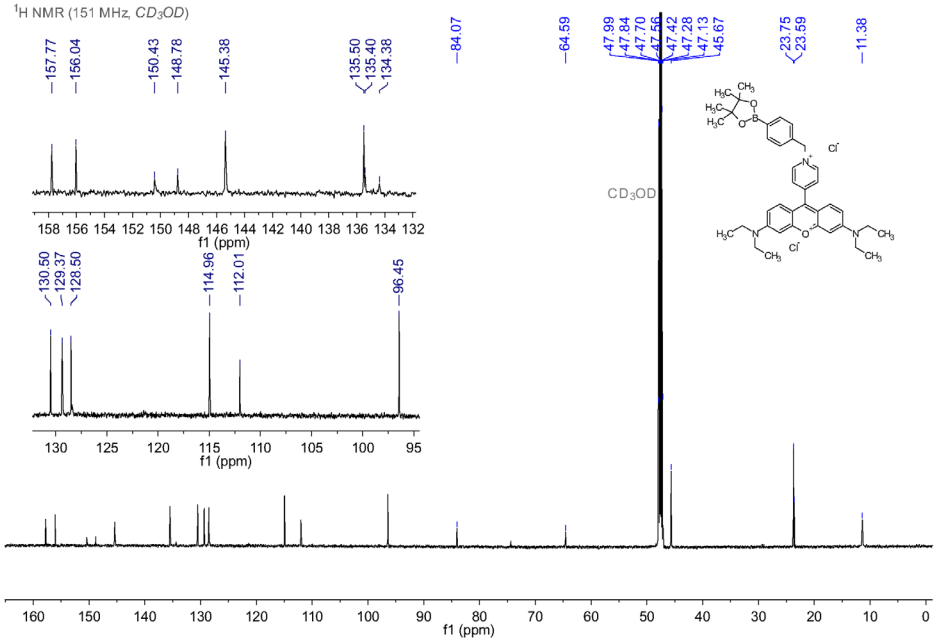

Fig. S12 ^{13}C NMR of probe **1a**.

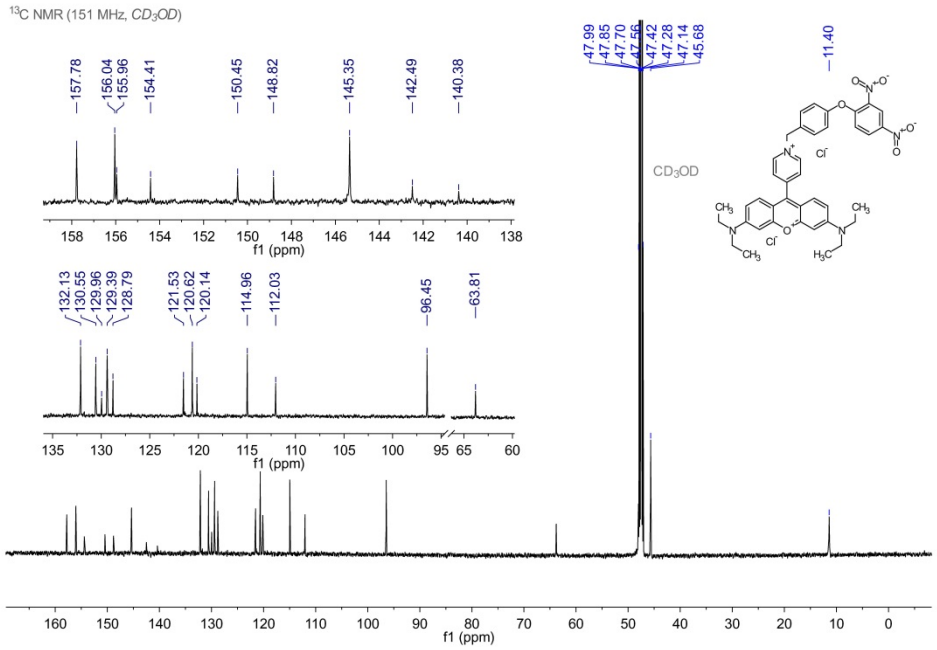

Fig. S13 ^{13}C NMR of probe **1b**.

Acquisition Parameter					
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	120 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	300.0 Vpp	Set Divert Valve	Waste

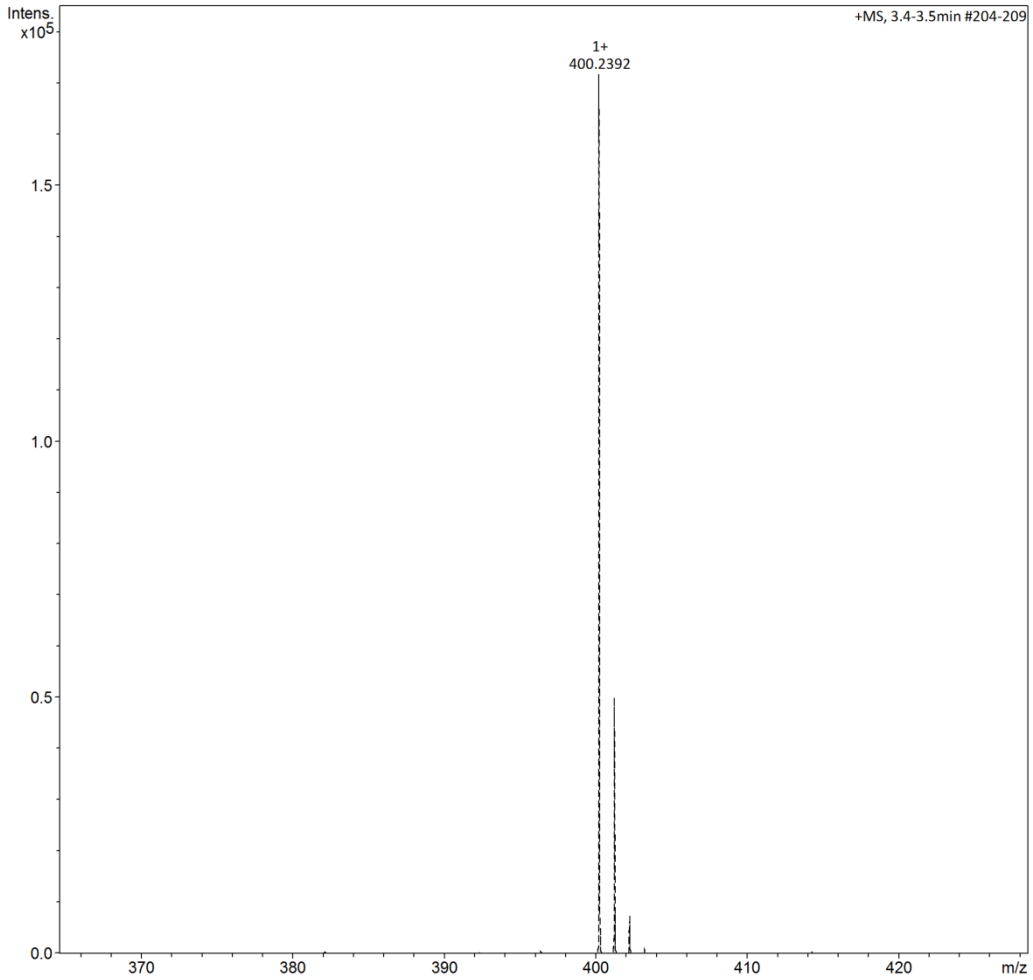

Fig. S14 HRMS(ESI$^+$) of compound **3**

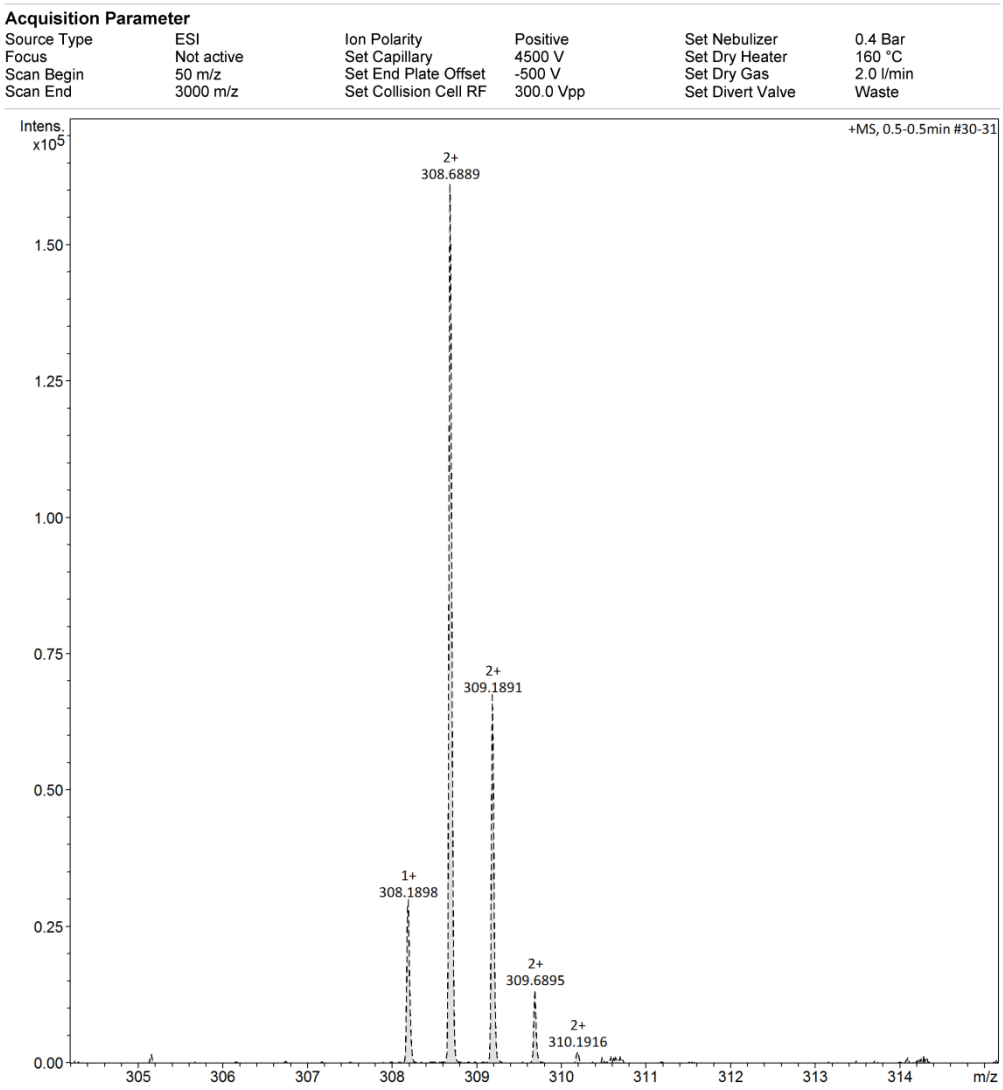

Fig. S15 HRMS(ESI$^+$) of probe **1a**

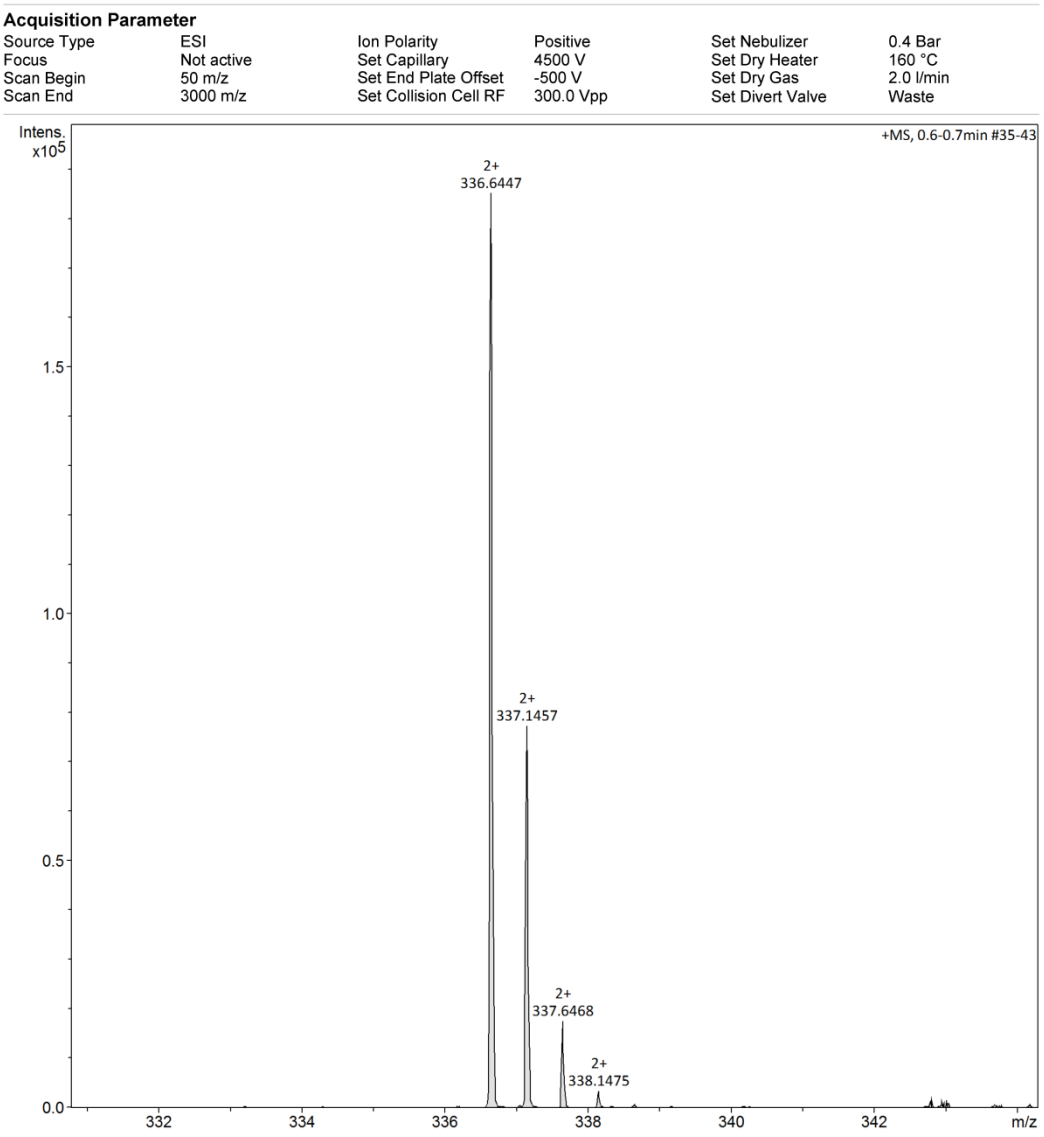

Fig. S16 HRMS(ESI$^+$) of probe **1b**

Acquisition Parameter

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Scan End	3000 m/z	Set Collision Cell RF	300.0 Vpp	Set Divert Valve	Waste

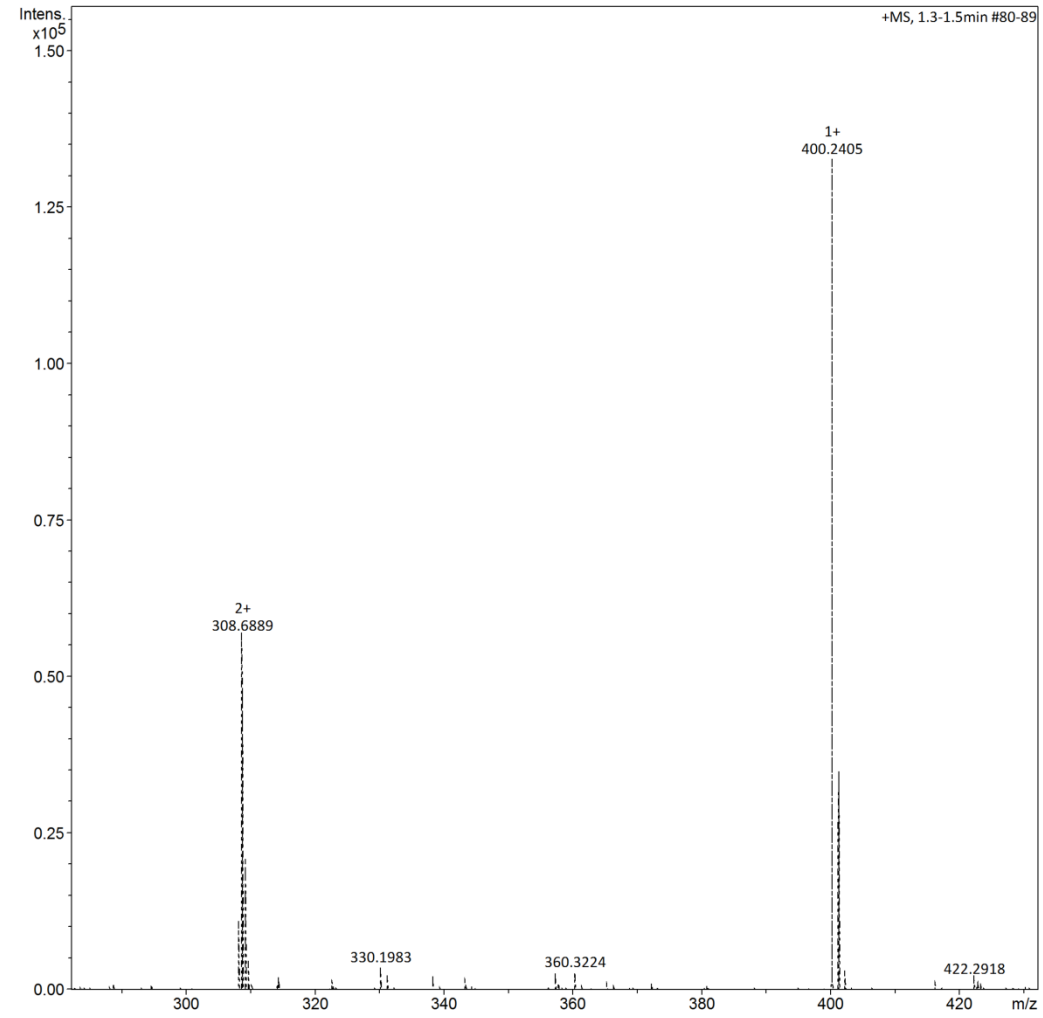

Fig. S17 Mass spectra of **1a**+H_2O_2

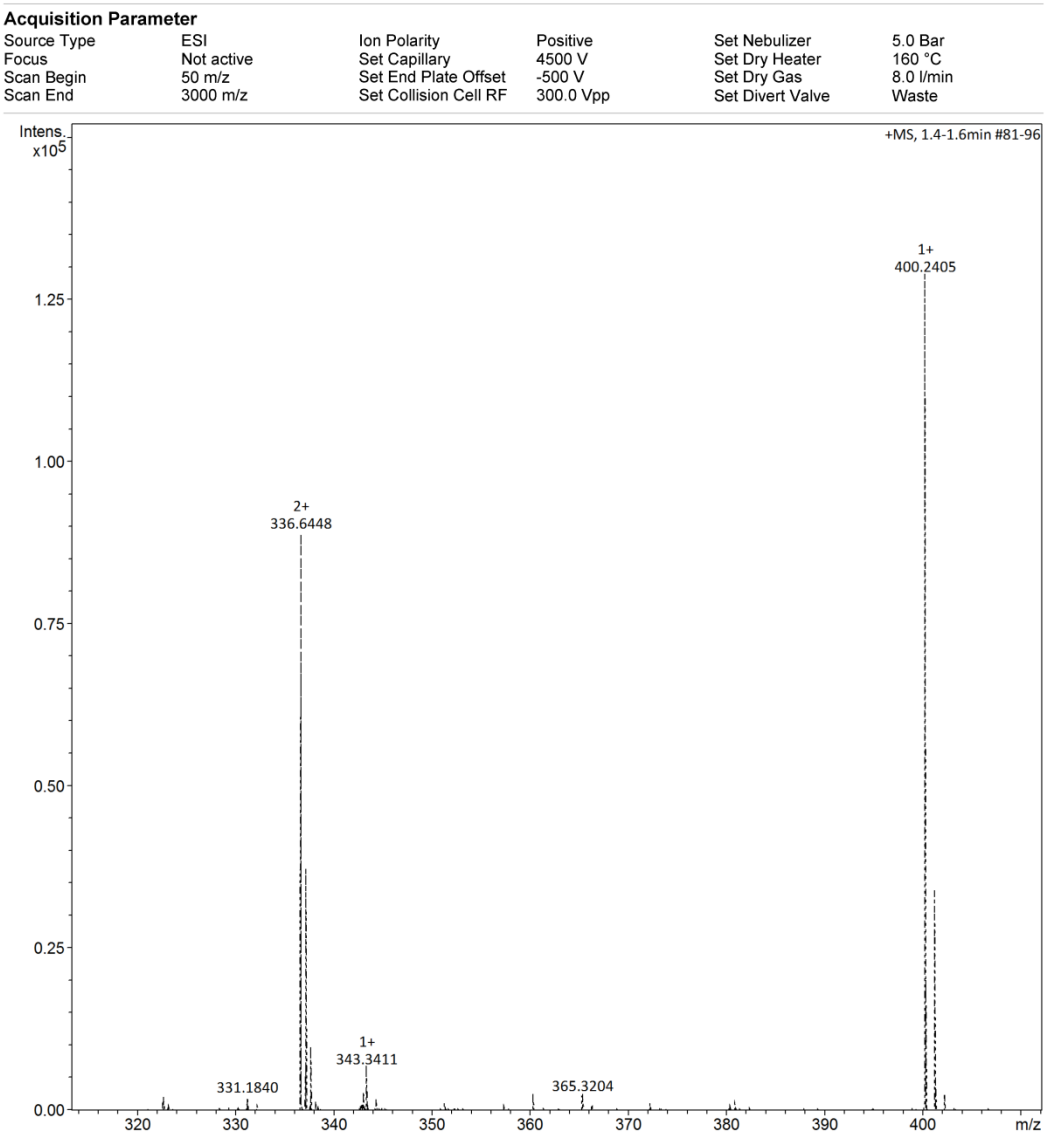

Fig. S18 Mass spectra of **1b**+Na_2S.