Supporting Information

Twisted intramolecular charge transfer plus aggregation-enhanced emission active based quinoxaline luminogen: photophysical properties and a light-up fluorescent probe for glutathione

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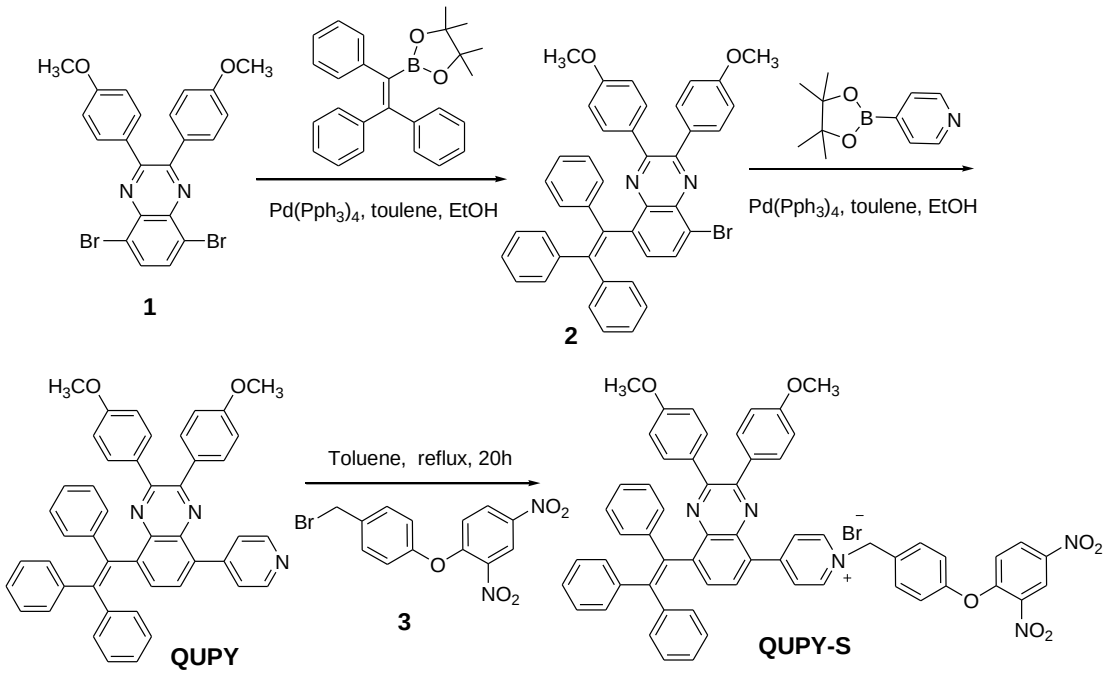
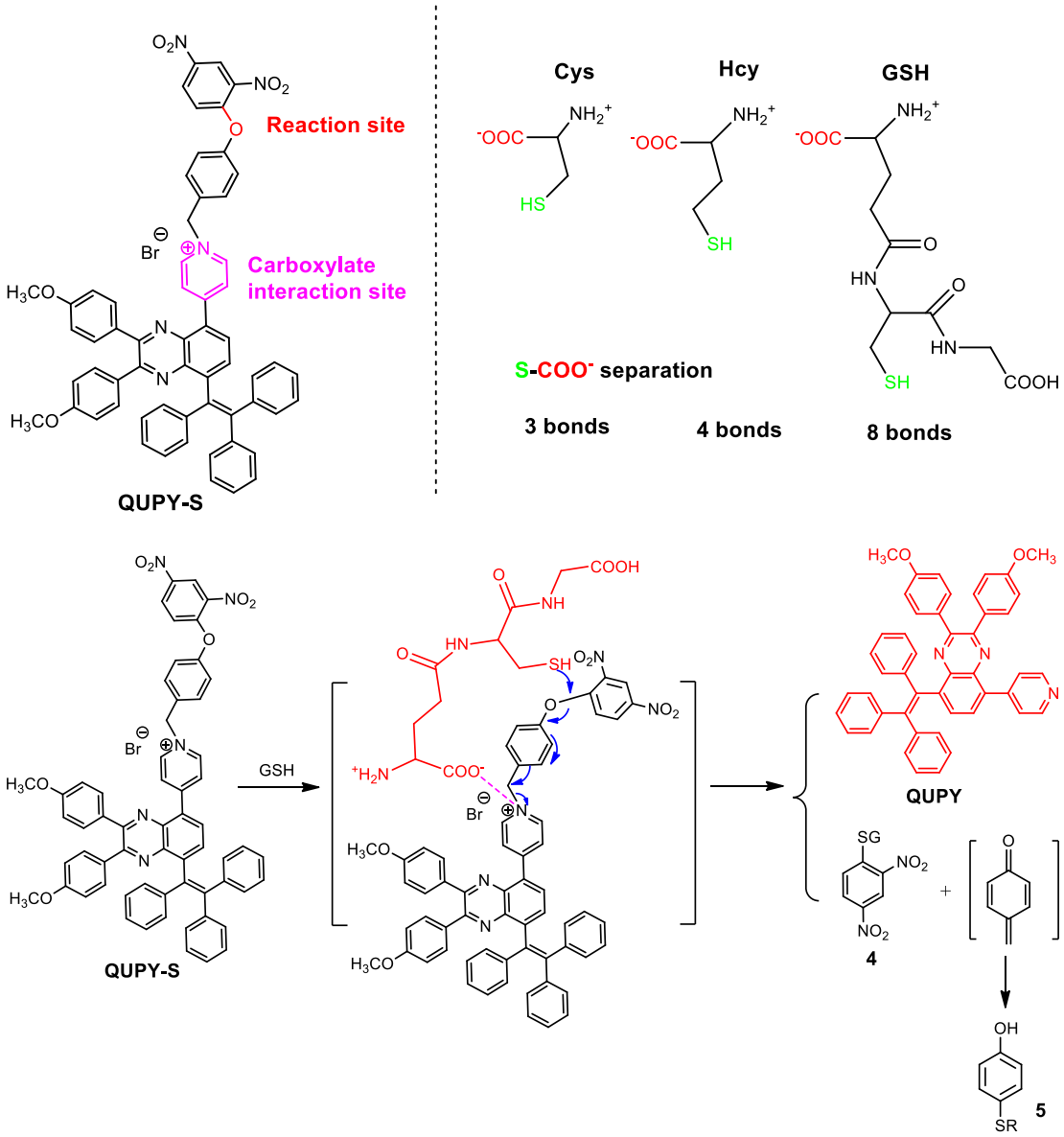


Scheme S1 Synthetic routs of **QUPY-S**.



Scheme S2 QUPY-S plausible mechanism for selective reaction of to GSH.

1. Synthesis

1.1 Synthesis of 5-bromo-2,3-bis(4-methoxyphenyl)-8-(1,2,2-triphenylvinyl)quinoxaline (2)

Under a nitrogen atmosphere, a solution of **1** (100 mg, 0.20 mmol), 4,4,5,5-tetramethyl-2-(1,2,2-triphenylvinyl)-1,3,2-dioxaborolane (171.9 mg, 0.45 mmol), $Pd(PPh_3)_4$ (23.2 mg, 0.02 mmol) in 25 mL toluene and 5 mL ethanol, then Na_2CO_3 (218 mg, 2 mmol) in 5 mL H_2O were mixed together. The mixture was stirred and heated at 95 °C for 12h. After cooling to room temperature, the solution was poured into water and extracted with dichloromethane. The organic layer was then dried over Na_2SO_4, filtered and solvent was removed under vacuum. The solid was purified by chromatography on silica gel (petroleum ether / dichloromethane, 40:1 as eluent) to give **2** as yellow solid in 54% yield (66.7 mg). m. p. 127-129 ºC. ^1H NMR ($CDCl_3$, 400 MHz, δ, ppm): 7.75 (d, 1H), 7.54 (d, 2H), 7.31 (d, 3H), 7.15 (s, 5H), 7.05 (s, 5H), 6.93 (m, 5H), 6.83 (d, 2H), 6.74 (d, 2H). HRMS (ESI, *m/z*), $[M + H]^+$ *calcd*. for $C_{42}H_{31}BrN_2O_2$, 675.6117, found, 676.1674 $[M+H]^+$

1.2 Synthesis of 2,3-bis(4-methoxyphenyl)-5-(pyridin-4-yl)-8-(1,2,2-triphenylvinyl)quinoxaline (QUPY)

Under a nitrogen atmosphere, a solution of **2** (123.4 mg, 0.20 mmol), pyridin-4-ylboronic acid (36.9 mg, 0.3 mmol), $Pd(Pph_3)_4$ (23.1 mg, 0.02 mmol) in 25 mL toluene and 5 mL ethanol, then Na_2CO_3 (218 mg, 2 mmol) in 5 mL H_2O were mixed together. The mixture was stirred and heated at 95 °C for 18h. After cooling to room temperature,

the solution was poured into water and extracted with dichloromethane. The organic layer was then dried over Na_2SO_4, filtered and solvent was removed under vacuum. The solid was purified by chromatography on silica gel (petroleum ether / dichloromethane, 10:1 as eluent) to give **3** as yellow solid in 45% yield (55.6 mg). m. p. 157-159 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.72 (d, 2H), 7.75 (d, 2H), 7.59 (d, 2H), 7.42 (d, 2H),7.35 (d, 2H), 7.20-7.12 (m, 10H), 7.05 (m, 2H), 6.95 (m, 3H), 6.81(m, 4H), 3.85 (s, 3H), 3.82 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 160.30, 160.25, 150.87, 149.24, 146.18, 143.91, 143.73, 143.67, 143.51, 143.11, 139.85, 138.36, 138.24, 137.88, 137.88, 135.84, 131.63, 131.40, 131.31, 130.95, 130.95, 130.95, 130.95, 130.58, 130.58, 130.58, 130.58, 129.13, 129.13, 129.13, 129.13, 127.72, 127.44, 127.44, 127.44, 127.44, 126.71, 126.58, 126.58, 126.17, 125.57, 125.57, 125.57, 113.64, 113.36, 55.01, 55.01. HRMS calcd. for $C_{47}H_{35}N_3O_2$: 673.7997, found 674.2802 $[M+H]^+$

1.3 Synthesis of 4-(2,3-bis(4-methoxyphenyl)-8-(1,2,2-triphenylvinyl)quinoxalin-5-yl)-1-(4-(2,4-dinitrophenoxy)benzyl)pyridin-1-ium bromide (QUPY-S)

Compound **2** (0.2 mmol, 70.5 mg) and compound **QUPY** (0.20 mmol, 134.7 mg) were dissolved in 10 mL toluene, and then the mixture was refluxed at 110 ˚C for 24 hr. The organic layer was then dried over Na_2SO_4, filtered and solvent was removed under vacuum. The solid was purified by chromatography on silica gel (petroleum ether / dichloromethane, 10:1 as eluent) to afford pure **QUPY-S** as orange solid in 60% yield (113 mg). m. p. 201-204 °C. ^{1}H NMR(400 MHz, $CDCl_3$): δ (ppm) 9.55 (d, 2H), 8.80 (s, 1H), 8.46 (d, 2H), 8.31 (dd, 1H), 8.02 (s, 1H), 7.91 (d, 2H),7.70 (d, 1H), 7.61 (d, 1H),

7.38 (d, 2H), 7.29 (d, 2H), 7.18-7.15 (m, 12H), 6.97-6.86 (m, 5H), 6.85 (d, 2H), 6.77 (d, 2H), 6.40 (s, 2H), 3.85 (s, 3H), 3.82 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 60.75, 160.63, 155.52, 155.05, 152.31, 152.17, 148.14, 148.14, 148.14, 144.73, 143.60, 143.28, 143.11, 142.55, 142.06, 139.95, 139.89, 139.89, 137.54, 137.54, 137.48, 132.36, 132.16, 131.24, 131.24, 131.24, 131.24, 130.78, 130.67, 130.67, 130.62, 130.51, 130.48, 129.10, 128.94, 128.94, 127.80, 127.64, 127.64, 127.64, 127.64, 127.60, 127.60, 127.05, 127.05, 127.05, 126.99, 126.52, 122.13, 121.29, 121.21, 119.66, 119.59, 114.14, 114.09, 113.54, 113.50, 62.34, 55.42, 55.31. HRMS calcd. for $C_{60}H_{44}N_5O_7^+$: 947.0207, found 948.3264 [**M**+H] $^+$

2. Detection of GSH in blood serum.

The deproteinized serum sample for the detection of GSH was prepared as followings. 1.5 mL of serum was added into ultrafiltration tube and centrifuged at 7,000 g for 25 min at 4 °C. After centrifugation, the filtrate was collected, which was further diluted with PBS buffer (10 mM, pH 7.4) to form 10% (v/v) serum solution. Then, 10% serum solution was added into **QUPY-S** (10 μM) in DMSO/PBS buffer (v/v, 1/1), which was incubated for 20 min at 37 °C before recording the fluorescence spectrum. Alternatively, different known concentrations of GSH (2, 4, 6, 8 μM) were added into the 10% (v/v) serum solution, and the respective fluorescence spectra were measured after incubation with **QUPY-S** in the same manner.

3 Cellular imaging

3.1 Cell culture

HeLa cells were cultured in high-glucose Dulbecco's Modified Eagle's Medium (H-

DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin streptomycin at 37 ^{0}C in a humidified environment containing 5% CO_2. Before the experiment, the cells were precultured until confluence was reached.

3.2 Cell imaging

HeLa cells were seeded in the 12-well plate and cultured in H-DMEM with 10% FBS at 37 ^{0}C in a humidified environment containing 5% CO_2. After 80% confluence, the medium was removed and the adherent cells were rinsed twice with 1 × PBS. **QUPY-S** in DMEM medium with FBS at 1 μM was then added to the culture plate. After incubation for 2 hours, the cells were washed three times with 1 × PBS buffer. The nuclei were stained by 4',6-diamidino-2-phenylindole for 10 min. The cell monolayer was then washed twice with 1 × PBS buffer and imaged by laser confocal fluorescence microscopy.

Sub-cellular localization was used a fully-motorized inverted microscope system (Olympus IX83-DSU, Japan) with a Photometrics EMCCD (Evolve 512 Delta).

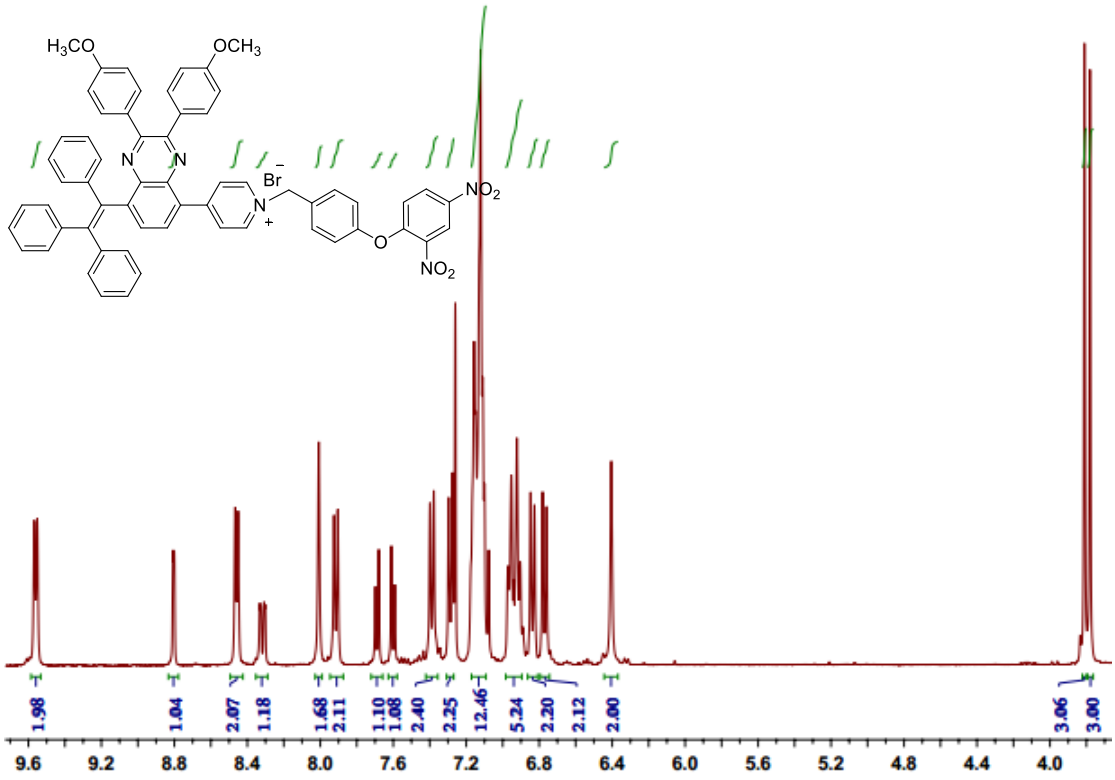


Figure S1 ^{1}H NMR spectrum of **QUPY-S** ($CDCl_3$, 400 MHz).

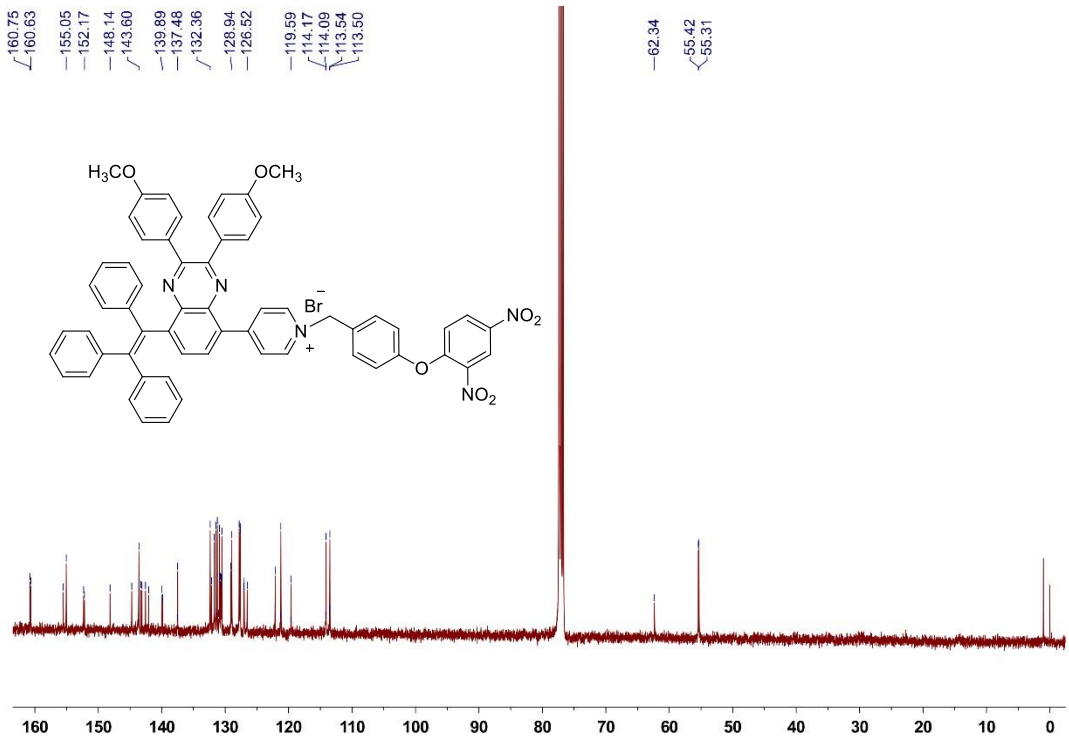


Figure S2 ^{13}C NMR spectrum of **QUPY-S** ($CDCl_3$, 100 MHz).

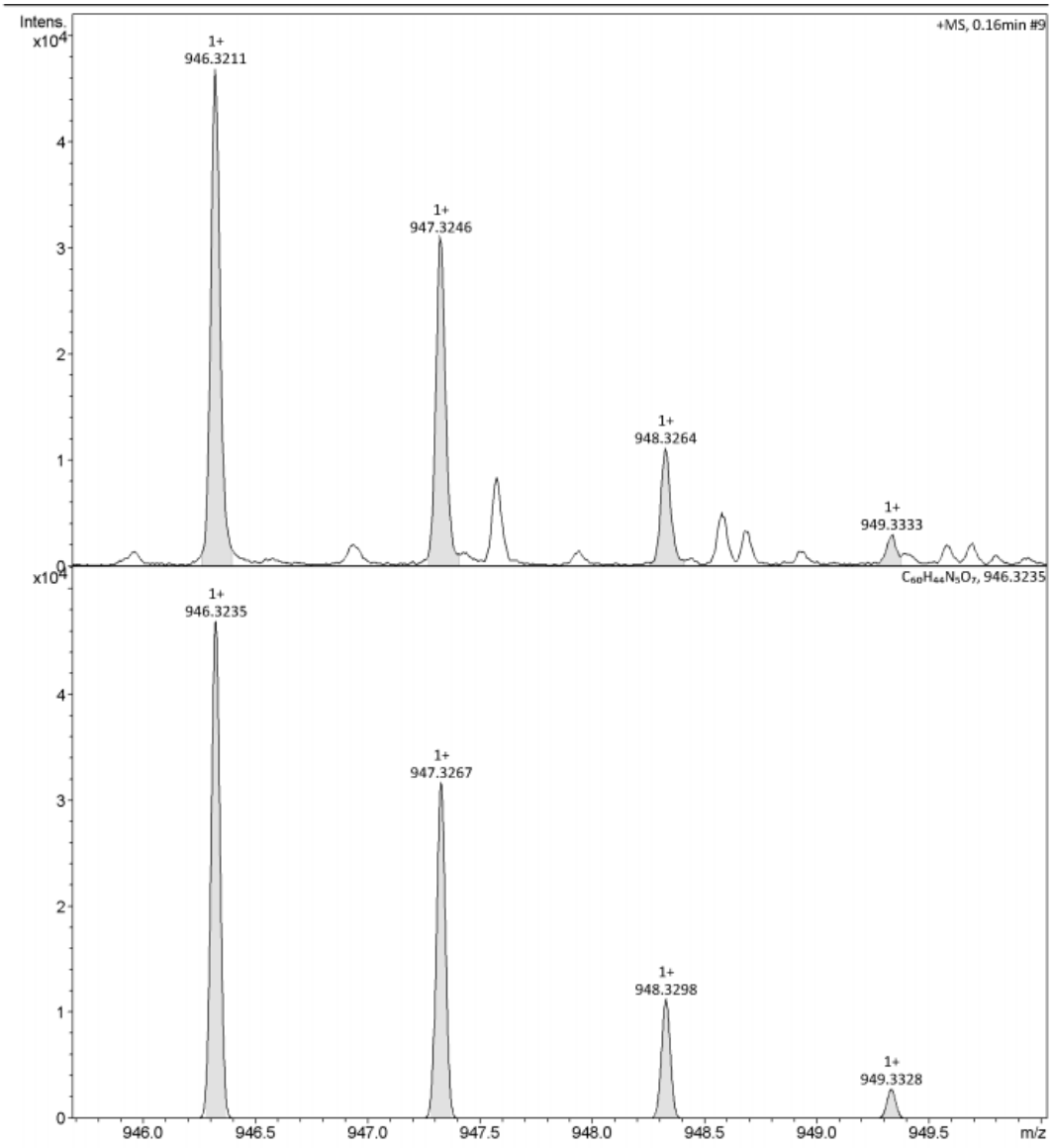


Figure S3 HRMS-ESI spectrum of **QUPY-S**

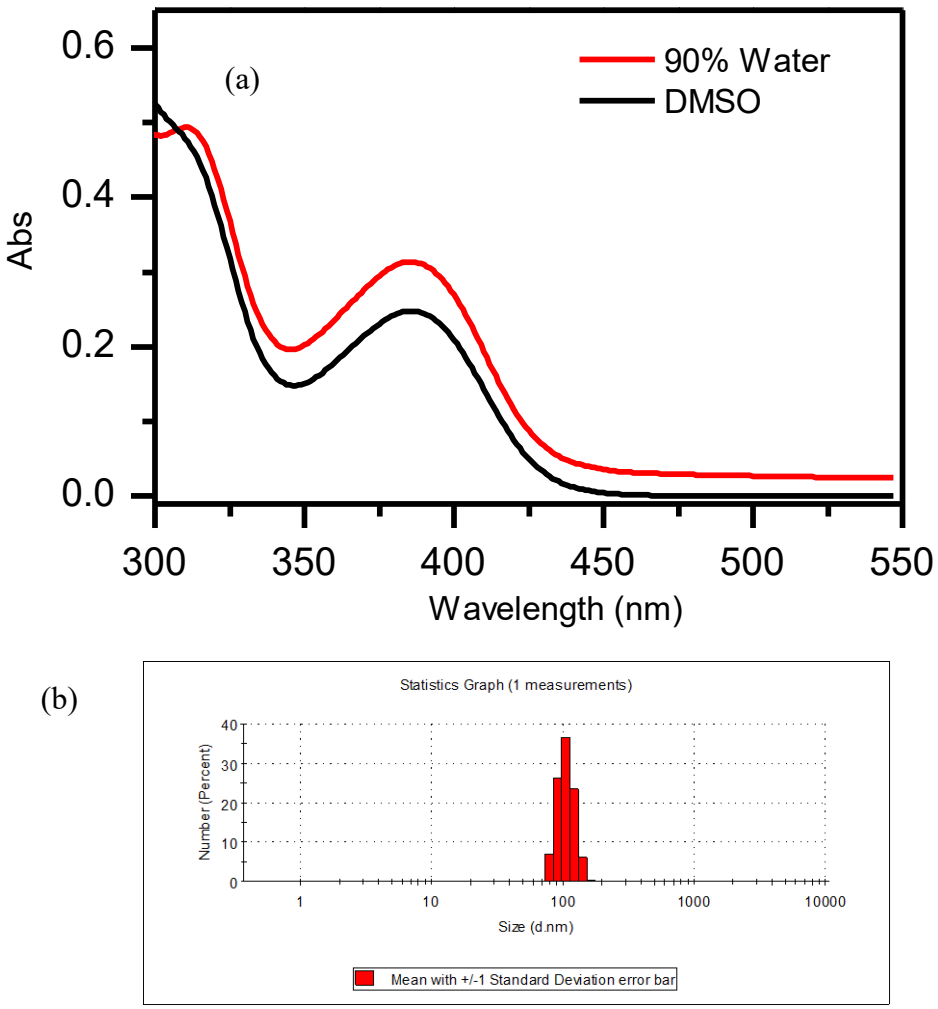
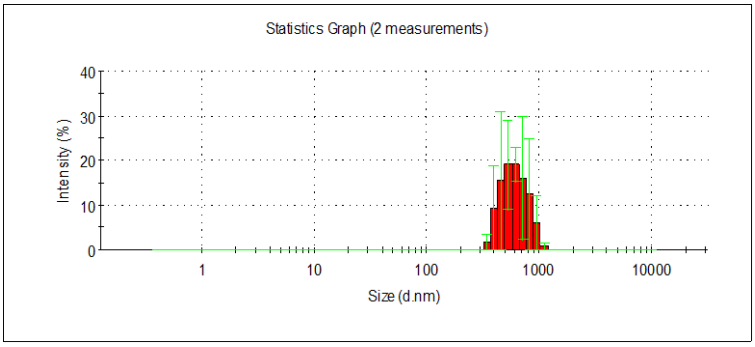


Figure S4 (a) UV-vis absorption spectra of **QUPY** (10 μM) in DMSO and DMSO/water (1/9, v/v). (b) DLS spectrum of **QUPY** (10 μM) in DMSO/water (1/9, v/v).

(a)



(b)

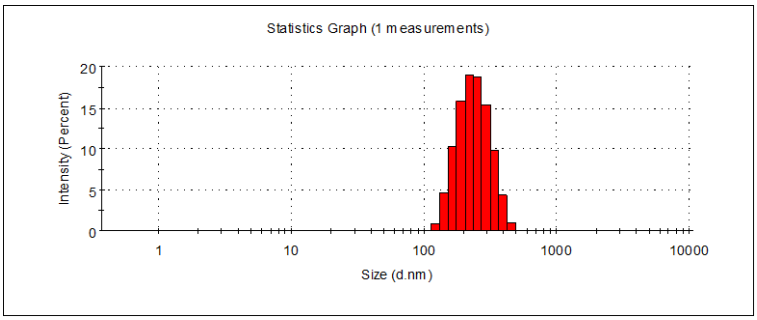


Figure S5 (a) DLS spectrum of **QUPY-S** (10 μM) in DMSO/water (3/7, v/v). (b) DLS spectrum of **QUPY-S** (10 μM) in DMSO/water (1/9, v/v).

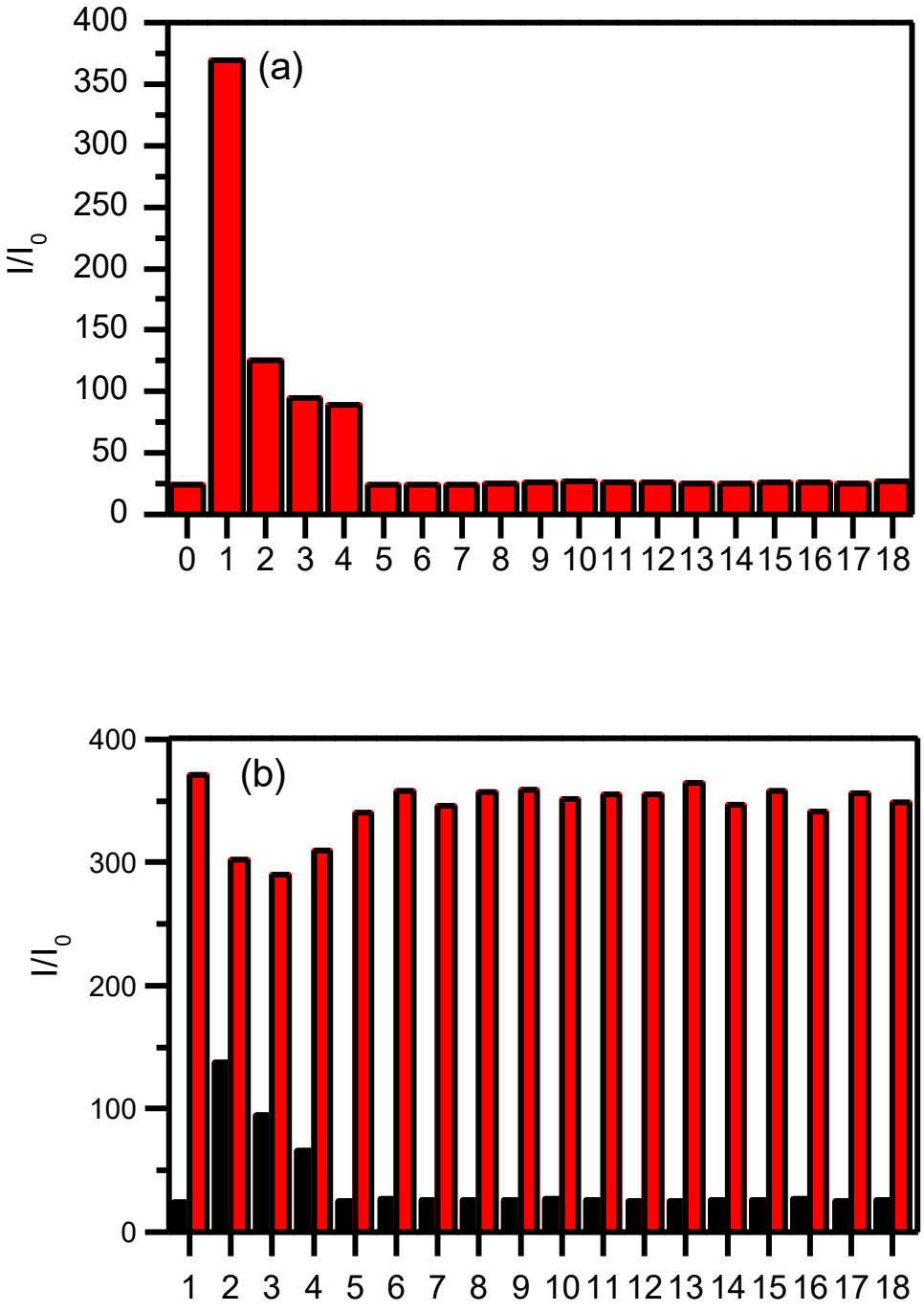


Figure S6 (a) The selectivity of fluorescence intensity of **QUPY-S** (10 μM) after treatment with 10 equiv of various analytes in DMSO/PBS buffer (1:1, v/v) at 37 °C and incubated for 90 min. (b) The fluorescence intensity of **QUPY-S** (10 μM) after treatment with 20 equiv of various analytes in DMSO/PBS buffer (1:1, v/v,

10 mM, pH 7.4) at 37 °C prior to (black bars) and after (red bars) addition of 100 μM GSH to the individual probe/analyte solution and incubated for 90 min respectively. (0) blank; (1) GSH; (2) NaSH; (3) Cys; (4) Hcy; (5) Asp; (6) Leu; (7) Ile; (8) Gly; (9) Phe; (10) Ala; (11) Thr; (12) Ser; (13) Pro; (14) Try; (15) Lys; (16) Arg; (17) Val; (18) Tyr.

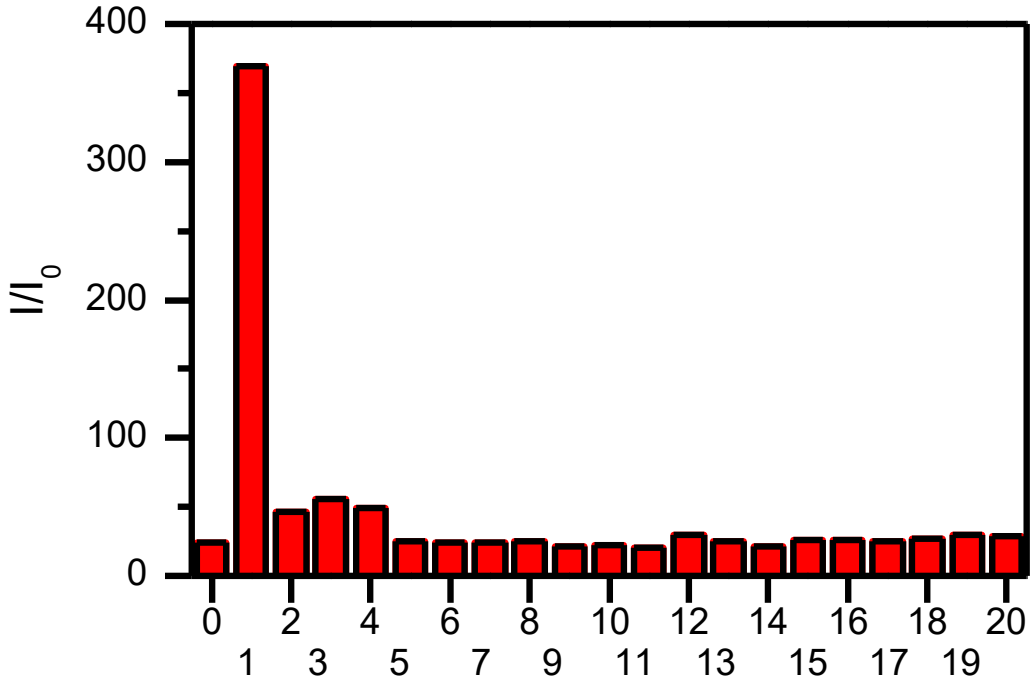


Figure S7 The selectivity of fluorescence intensity of **QUPY-S** (10 μM) after treatment with 10 equiv of various ions in DMSO/PBS buffer (1:1, v/v) at 37 °C and incubated for 90 min. (0) blank; (1) GSH; (2) HSO_4^-; (3) HSO_3^-; (4) $S_2O_5^{2-}$; (5)SO_4^{2-}; (6) SO_3^{2-}; (7) I^-; (8) Br^-; (9) Cl^-; (10) F^-; (11) NO_2^-; (12) PO_4^{3-}; (13) CH_3COO^-; (14)SCN^-; (15) CN^-; (16) Fe^{3+}; (17) Zn^{2+}; (18) Mg^{2+}; (19) Cu^{2+}; (20). Mn^{2+}

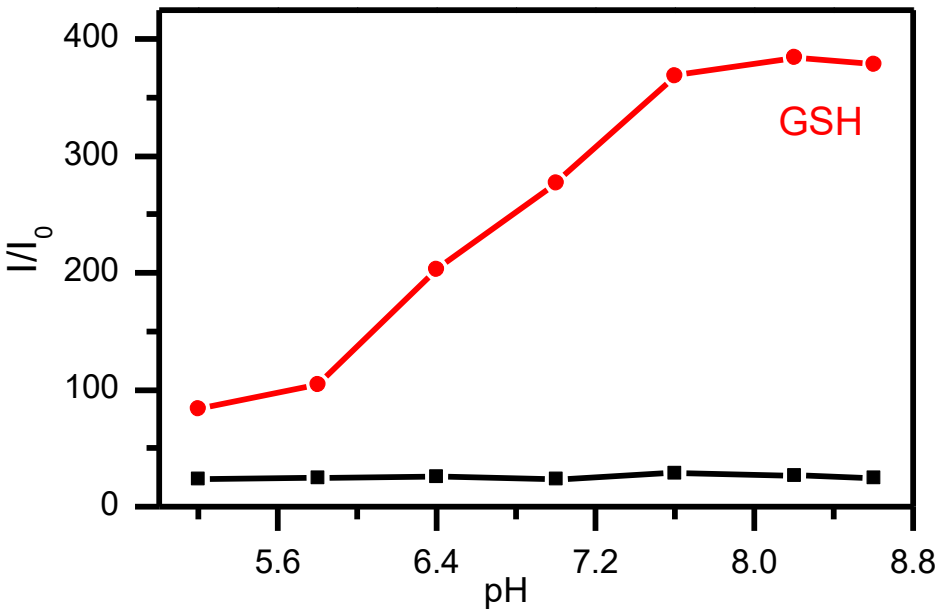


Figure S8 The effect of pH (5.2, 5.8, 6.4, 7.0, 7.6, 8.2, 8.6) on the fluorescence intensity of **QUPY-S** (10 μM) in DMSO/PBS buffer (1:1, v/v) upon addition of 100 μM GSH at 37 °C and incubated for 90 min.

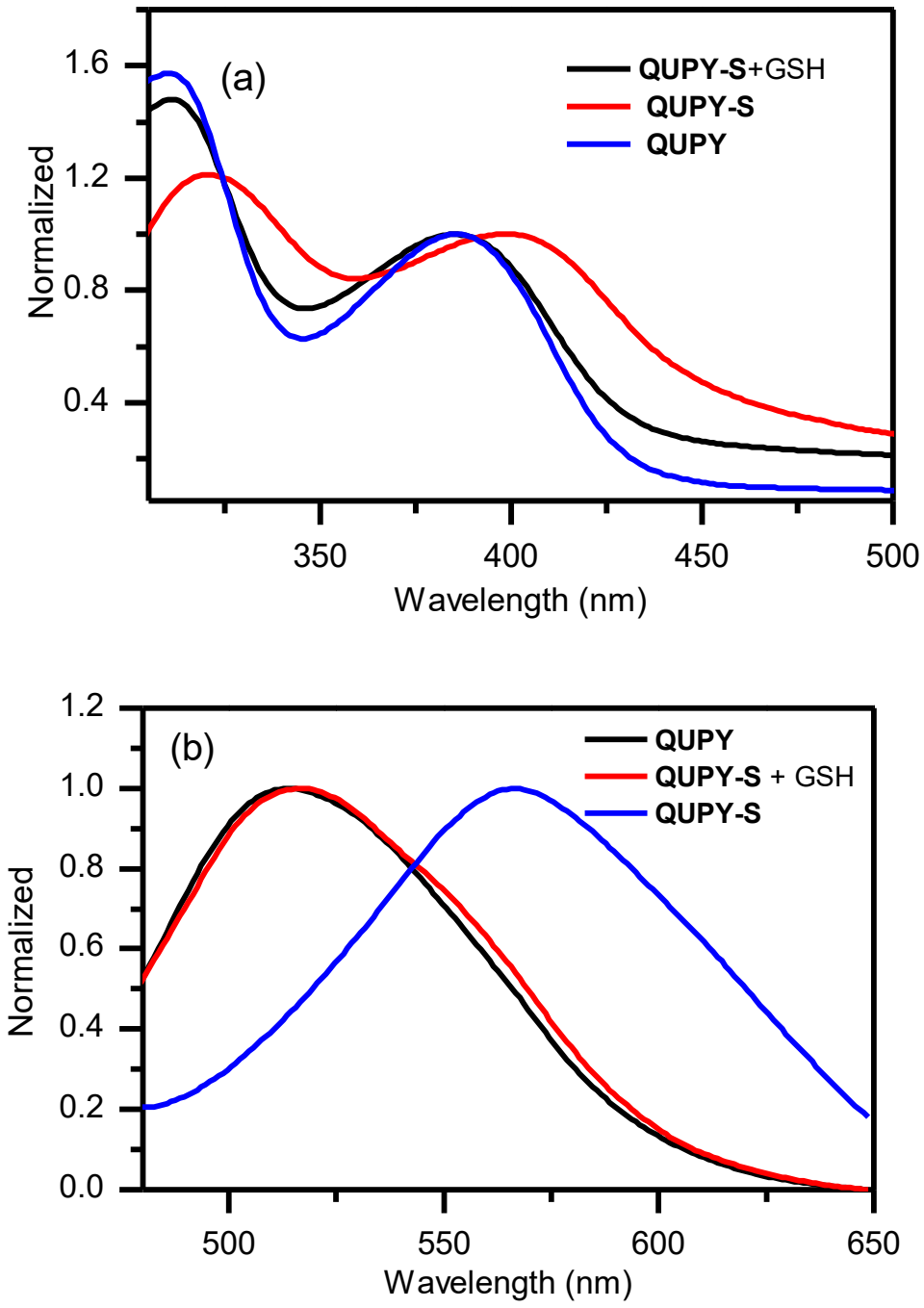


Figure S9 (a) Normalized UV-vis absorption and (b) emission spectra of **QUPY-S** (10 μM) prior to (black), after addition (red) of GSH (100 μM) and **QUPY** in DMSO/PBS (v/v = 1:1) at 37 °C for 90 min.

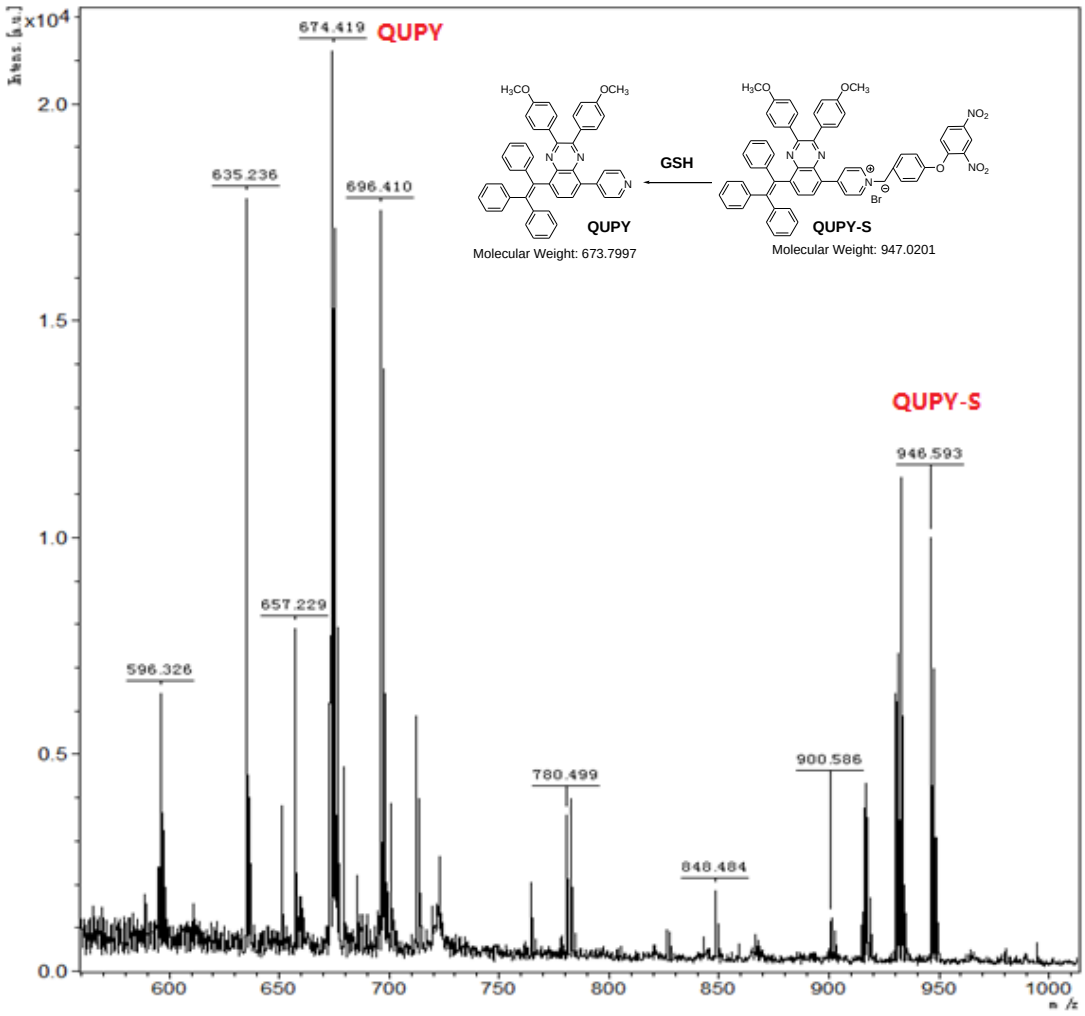


Figure S10 The ESI-MS spectrum in the range of 550 to 1000 of **QUPY-S** (10 μM) in presence of 100 μM GSH.

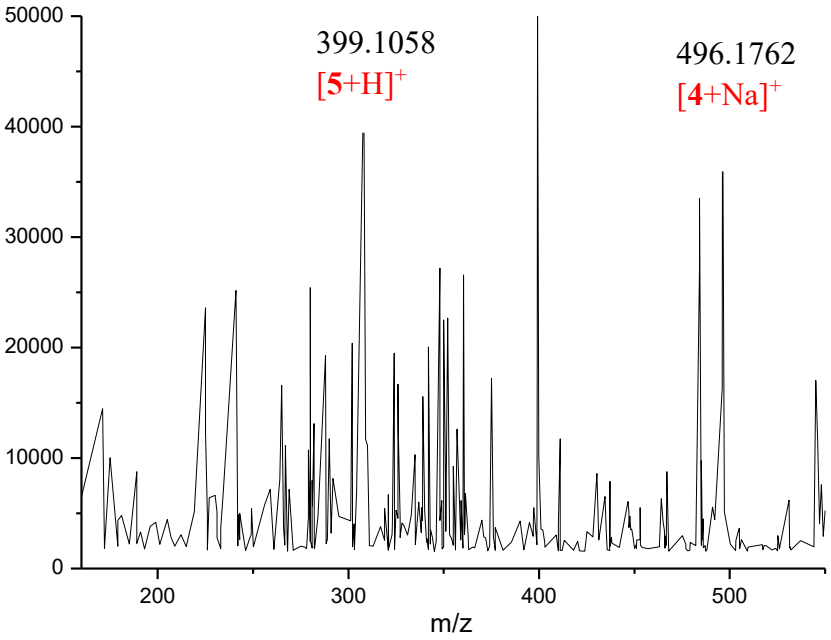


Figure S11 The ESI-MS spectrum in the range of 200 to 550 of **QUPY-S** (10 μM) in presence of 100 μM GSH.

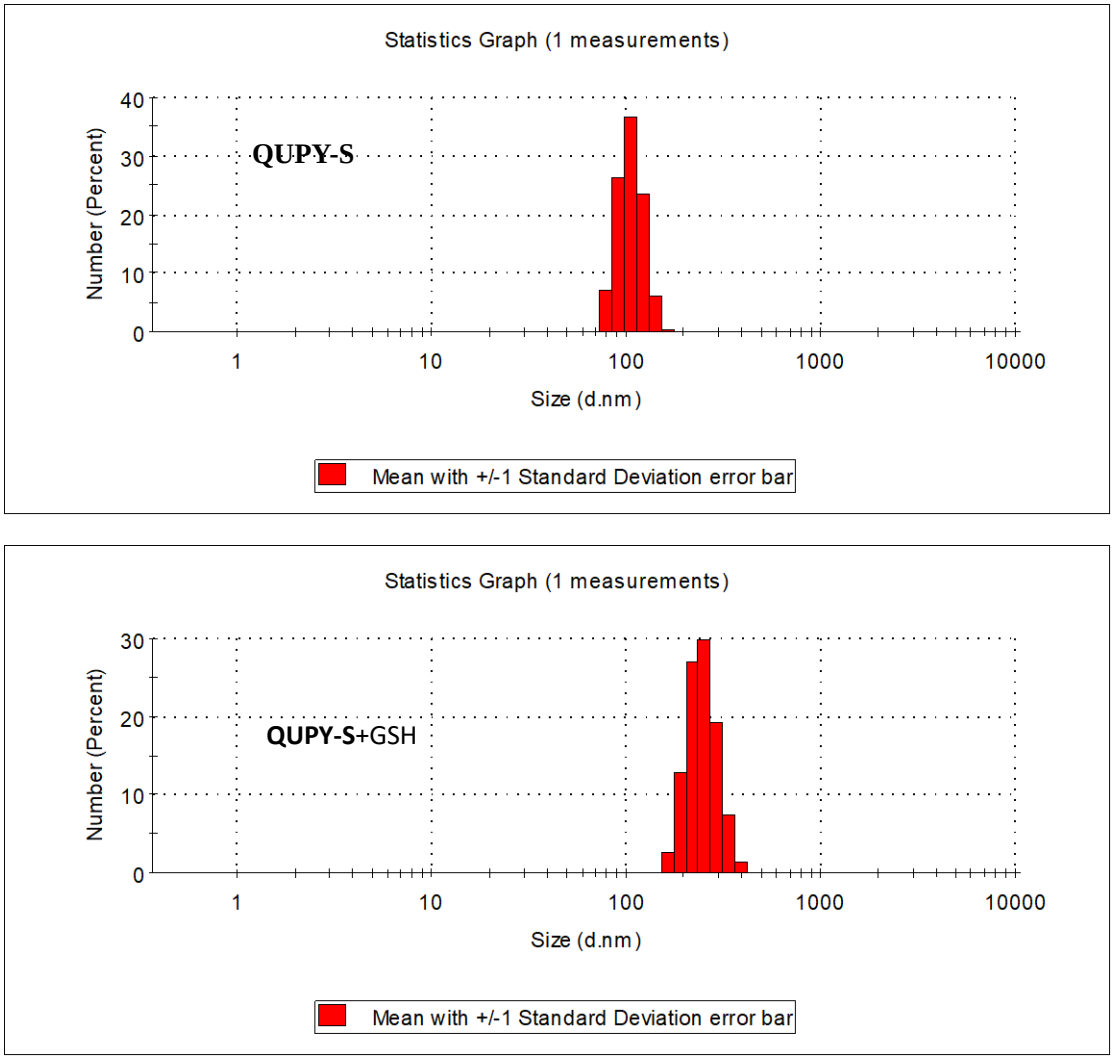


Figure S12 DLS test of **QUPY-S** (10 μM) prior to and after the addition of 100 μM GSH in DMSO/PBS buffer (v/v, 1/1) at 37°C.

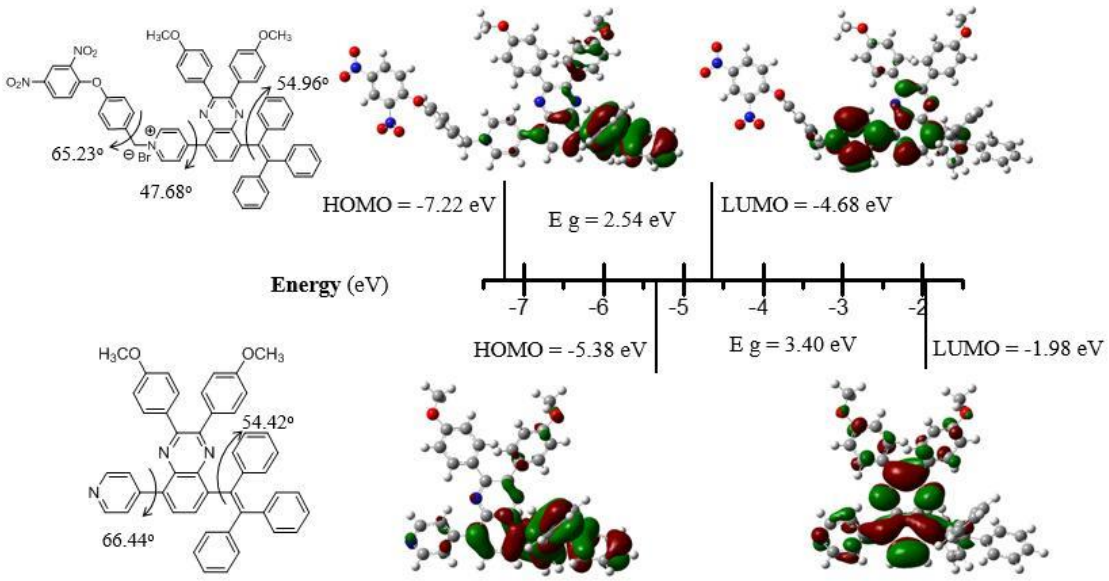


Figure S13 Optimized molecular conformation and molecular orbital amplitude plots of HOMO and LUMO energy levels of **QUPY-S** and **QUPY**.

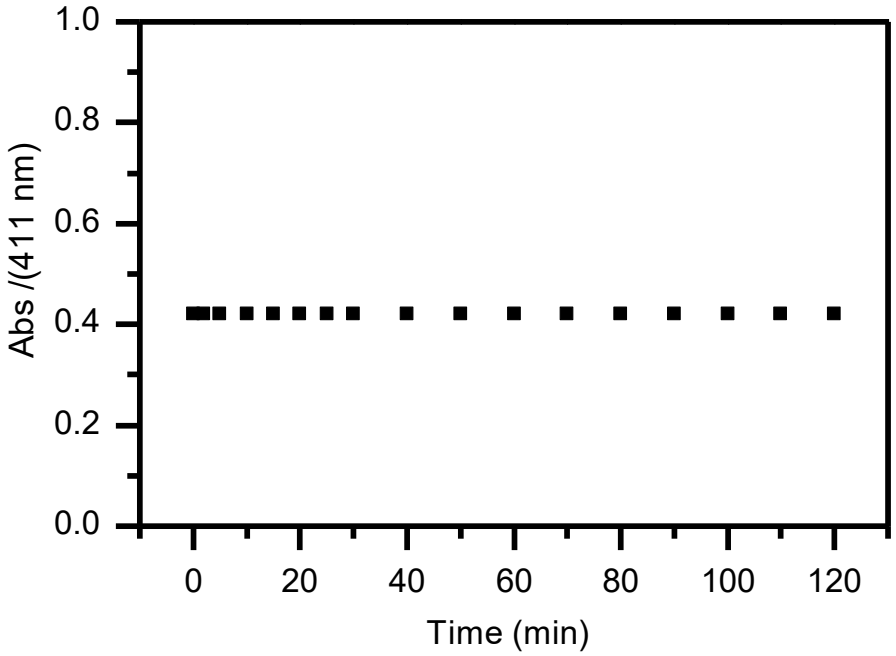


Figure S14 The photostability of **QUPY-S** in DMSO/PBS buffer (v/v, 1/1) under continuous 365 nm irradiation.

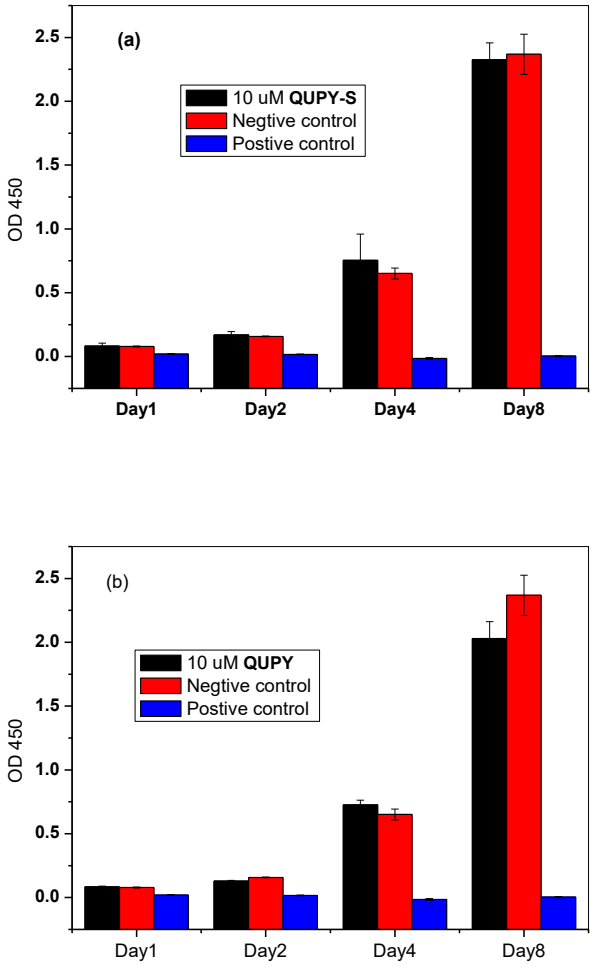


Figure S15 Cell viability of HeLa cells incubated with (a) **QUPY-S** and (b) **QUPY**.

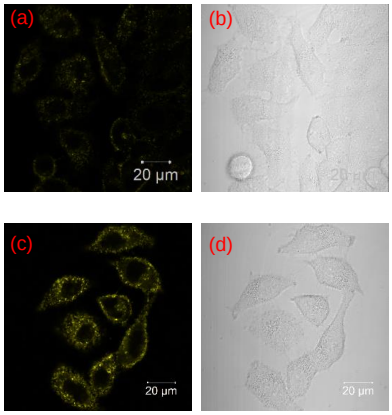


Figure S16 Lambda mode of confocal fluorescence images of HeLa cells. (a) Cells incubated with **QUPY-S** (10 μM) for 30 min; (c) Cells incubated with **QUPY-S** (20 μM) for 30 min. (b and d, phase contrast images; a and b, fluorescence images).

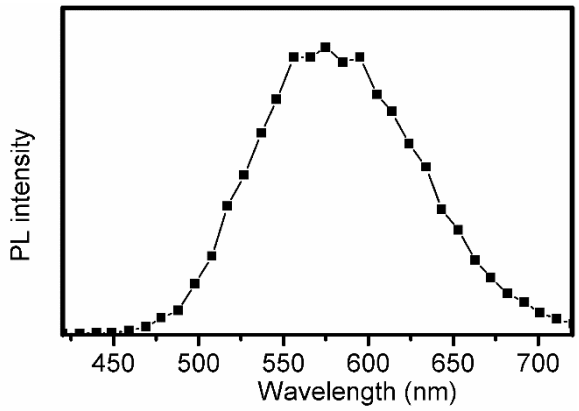


Figure S17 Fluorescence spectrum of HeLa cells stained with **QUPY-S** (10 μM) for 30 min.

Table S1. The comparison of **QUPY-S** with GSH probes available in the literatures.

No.	References	Detection system	Fluorescence pattern	LOD	Selectivity
1	Present work	PBS/DMSO (1:1, v/v)	Turn-on	434 nM	High
2	ACS Appl. Mater. Interfaces 2018, 10, 12141−12149	PBS buffer	Turn-on	36.9 nM	Low
3	J. Am. Chem. Soc. 2011, 133, 11132–11135	MOPS buffer	Ratiometric	--	Low
4	Chem. Eur. J. 2015, 21, 4747–4754	DMF/PBS (4:6, v/v)	Turn-on	0.03 μM	Low
5	Sensors and Actuators B 159 (2011) 142–147	Ethanol/PBS (3:7, v/v)	Ratiometric	0.178 mM	Low
6	ACS Appl. Mater. Interfaces 2015, 7, 12809−12813	PBS buffer with 2% DMF	Turn-on	--	High
7	Analyst, 2013, 138, 7169–7174	CH_3CN/H_2O (3:7, v/v)	Turn-on	411 nM	High
8	RSC Adv., 2014, 4, 52583–52589	PBS solution	Turn-on	87 nM.	Low
9	Chem. Sci., 2014, 5, 2177–2183	DMF/PBS (3:7, v/v)	Ratiometric	--	Low
10	J. Am. Chem. Soc. 2012, 134, 18928−18931	Acetonitrile/HEPES (5:95 v/v)	Turn-on	86 nM.	High
11	J. Am. Chem. Soc. 2014, 136, 5351−5358	HEPES containing 10% DMSO.	Turn-on	--	High
12	Biosensors and Bioelectronics 2016, 85, 164–170.	PBS/DMSO (1:1, v/v)	Turn-on	109 nM	High
13	Biosensors and Bioelectronics 71 (2015) 68–74	PBS buffer	Turn-on	122 nM	High
14	Biosensors and Bioelectronics 81 (2016) 341–348	PBS containing 5% DMSO	Turn-on	--	Low
15	Chem. Commun. 2014, 50, 15439–15442	PBS buffer (pH = 7.4, 1% CH_3CN)	Turn-on	--	Low

Table S2. Determination of GSH in blood serum samples with **QUPY-S** (n=3)

Sample	GSH added (μM)	GSH found (μM)	Recovery (%)	RSD (n=3, %)
BSA	-	1.67	-	1.27
BSA + GSH	2	3.48	94.8	2.13
BSA + GSH	4	5.71	100.7	2.87
BSA + GSH	6	7.58	98.8	1.63
BSA + GSH	8	8.89	91.9	2.46