

Supplementary information for

Near-infrared fluorescent detection of glutathione *via* reaction-promoted assembly of squaraine-analyte adducts

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Materials. Natural amino acids, Hcy and CTAB were purchased from Xiaan Wolsen Bio. Reagents Co. (Xiaan, China) and were used as received. Cationic squaraine dye, **SQ1**, was synthesized and purified as reported previously.^{S1} Glutathione dimethyl was prepared according to the reported literature.^{S2}

Measurements

Absorption and emission spectra were collected by using a Shimadzu 1750 UV-visible spectrometer and a RF-5301 fluorescence spectrometer (Japan), respectively.

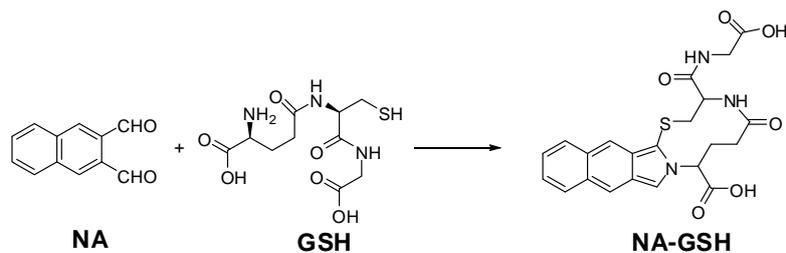
Sample Preparation and Titration. Stock solutions of OPA and GSH were mixed at 25⁰C with the molar ratio of OPA to GSH, 3.3:1 in 20 mM borate buffer (pH= 9.0) for 30 min. Stock solution of **SQ1** (5.0×10⁻⁴ M) was prepared in ethanol and diluted in buffer solution to 5.0×10⁻⁶ M for titration experiments. OPA-GSH solutions were added to **SQ1** buffer solution, UV and fluorescence spectra were monitored after 30 min.

Preparation of human blood samples. The procedure for preparation of human blood samples is followed the reported literature.^{S3} Human blood samples were collected from healthy volunteers treated in the local Medical Hospital. All samples were obtained by venipuncture and collected in heparinized vacutainer tubes. Then, a 200 μL aliquot of the blood was deproteinized by mixing immediately with 400 μL of cold 10% Cl₃CCOOH. After vortex mixing, the mixture was centrifuged at 8000 rpm for 10 min. A total of 400 μL of the supernatant was collected. The obtained supernatant was ready for assays.

S1. Y. Xu, Z. Li, A. Malkovskiy, S. Sun and Y. Pang, *J. Phys. Chem. B*, 2010, 114, 8574.

S2. J. R. Falek, Bhavani Sangras and Jorge H. Capdevila, *Bioorg. Med. Chem.*, 2007, 15, 1062.

S3. D. Tian, Z. Qian, Y. Xia and C. Zhu, *Langmuir*, 2012, 28, 3945.



Scheme S1. Illustration of reaction of GSH with 2,3-naphthalenedialdehyde (NA).

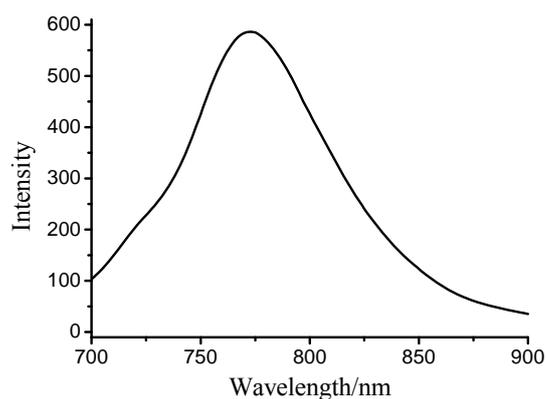


Fig. S1 Excitation spectrum of derivative mixed with NA and GSH in 20 mM borate buffer (pH=9.0). The molar ratio of NA to GSH was fixed as 3.3 : 1.

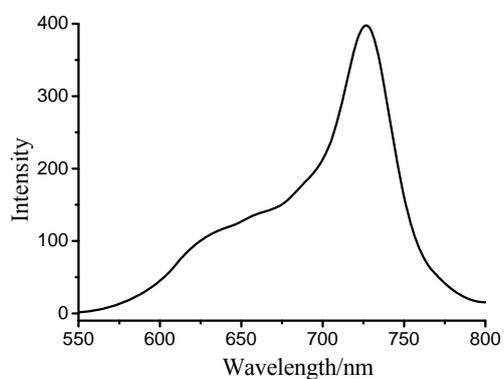


Fig. S2 Excitation spectrum of derivative mixed with OPA and GSH in 20 mM borate buffer (pH=9.0). The molar ratio of OPA to GSH was fixed as 3.3 : 1.

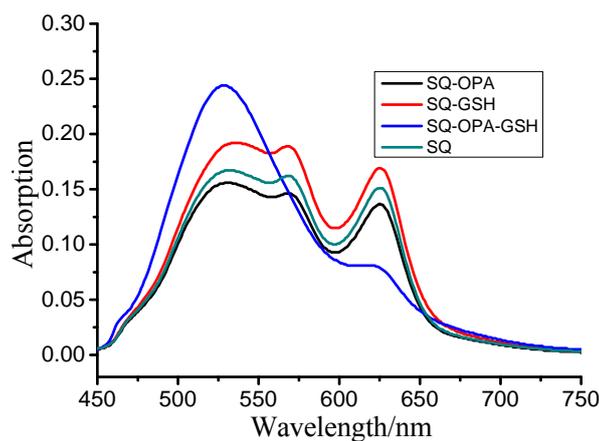


Fig. S3 Absorption spectra of **SQ1** (5.0×10^{-6} M) in the absence and the presence of OPA, GSH and the mixture of OPA and GSH (OPA-GSH) in 20 mM borate buffer (pH = 9.0) at 25°C ([OPA] = 1.95×10^{-3} M, [GSH] = 6.5×10^{-4} M).

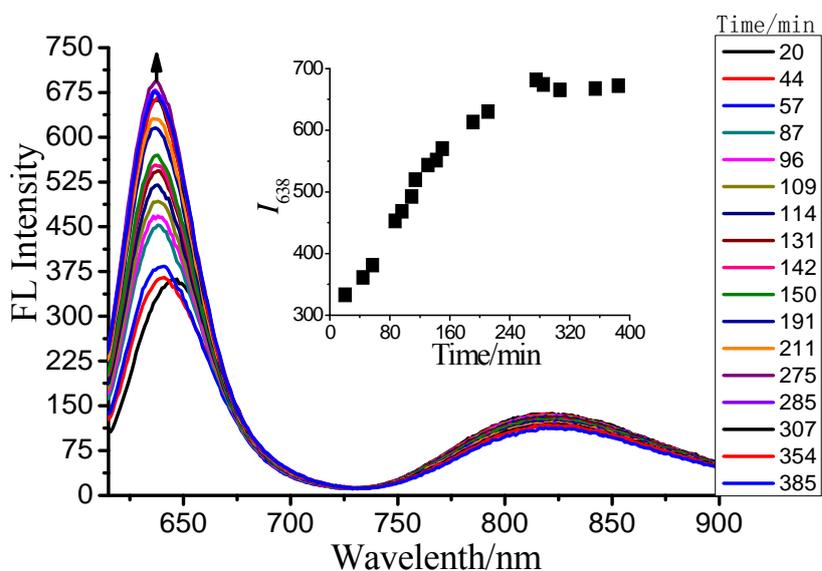


Fig. S4 Variation in the emission spectra of **SQ1** (5.0×10^{-6} M) in the presence of OPA in 20 mM borate buffer (pH = 9.0) with increasing reaction time as indicated ([OPA] = 1.95×10^{-3} M, [GSH] = 6.5×10^{-4} M. Excitation wavelength at 600 nm). Inset: the relationship between fluorescence intensity at 638 nm and reaction time.

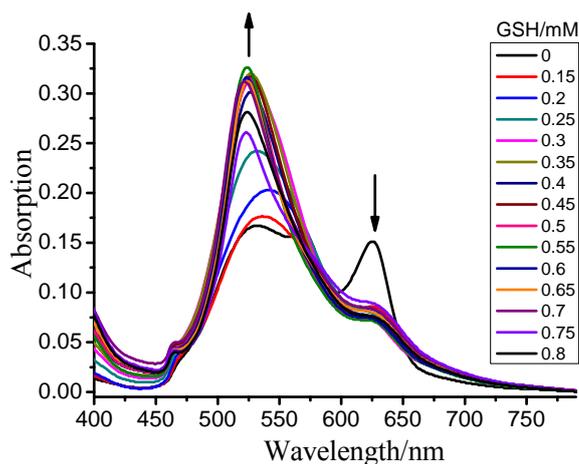


Fig. S5 Variation in the absorption spectra of **SQ1** (5.0×10^{-6} M) in 20 mM borate buffer (pH = 9.0) with increasing concentrations of GSH as indicated (the molar ratio of OPA to GSH was fixed to 3.3 : 1).

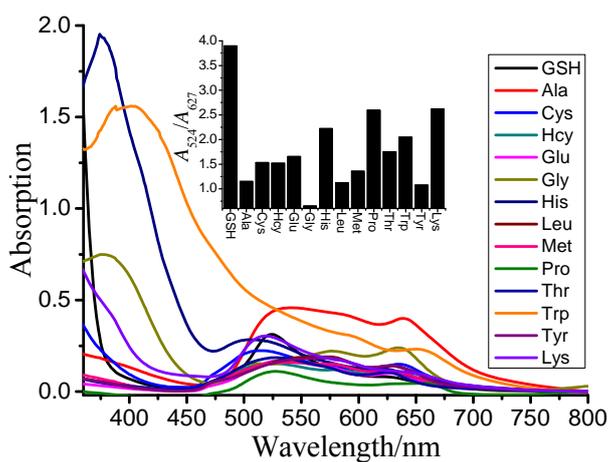


Fig. S6 Absorption spectra of **SQ1** (5.0×10^{-6} M) in the presence of OPA and 11 natural amino acids and Hcy in 20 mM borate buffer (pH = 9.0) ([OPA] = 1.95×10^{-3} M, [amino acids] = [Hcy] = [GSH] = 6.5×10^{-4} M). Inset: the ratio absorption A_{524}/A_{627} in the presence of OPA and 11 natural amino acids and Hcy.

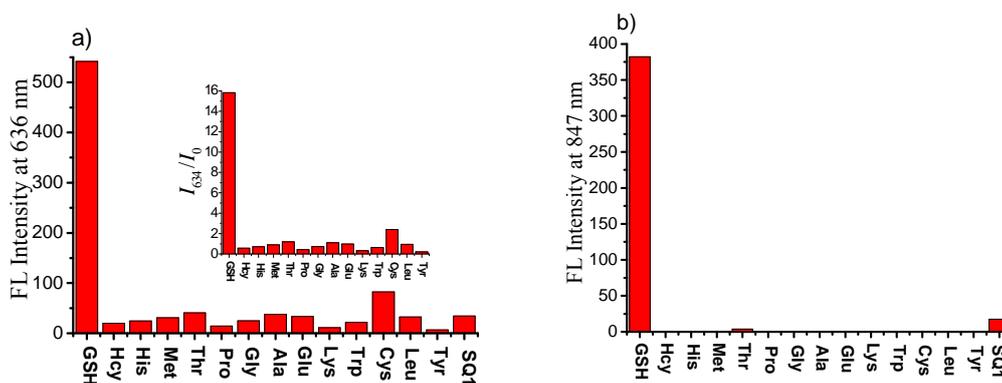


Fig. S9 UV-vis spectra of **SQ1** (5.0×10^{-6} M) in 20 mM borate buffer (pH = 9.0) containing OPA upon addition of glutathione dimethyl (the molar ratio of OPA to glutathione dimethyl was fixed as 3.3:1).

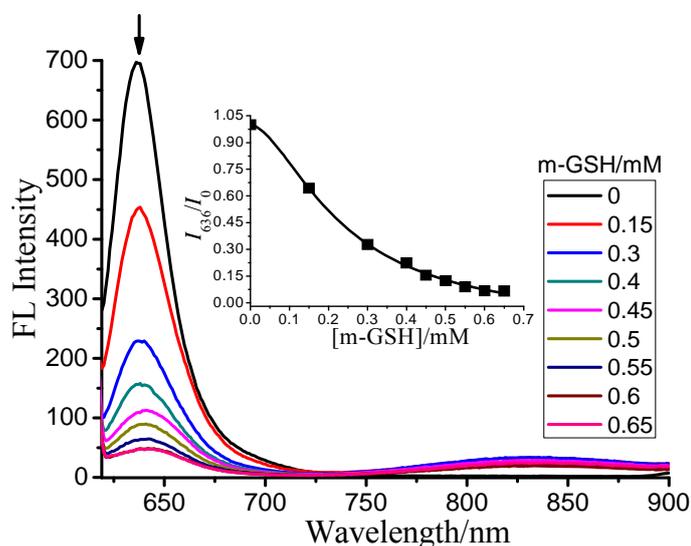


Fig. S10 UV-vis spectra of **SQ1** (5.0×10^{-6} M) in 20 mM borate buffer (pH = 9.0) containing OPA upon addition of glutathione dimethyl (the molar ratio of OPA to glutathione dimethyl was fixed as 3.3:1). Inset: the relative fluorescence change at 636 nm upon addition of glutathione dimethyl.

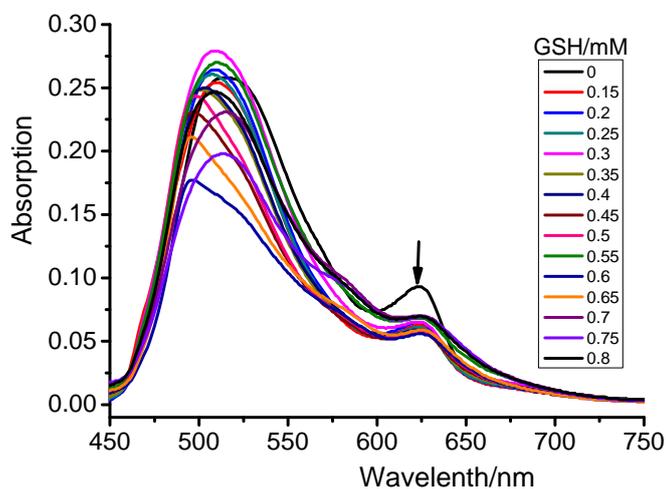


Fig. S11 Variation in the absorption spectra of **SQ1** (5.0×10^{-6} M) in 10 mM HEPES buffer (pH=7.4) with increasing concentrations of GSH as indicated. The molar ratio of OPA to GSH was fixed as 3.3 : 1.

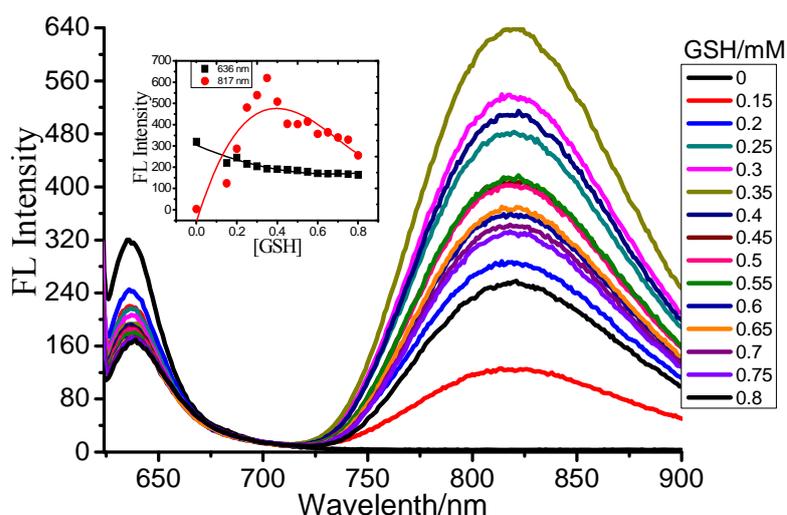


Fig. S12 Variation in the emission spectra of **SQ1** (5.0×10^{-6} M) in 10 mM HEPES buffer (pH=7.4) with increasing concentrations of GSH as indicated (The molar ratio of OPA to GSH was fixed as 3.3:1. Excitation wavelength at 610 nm). Inset: The fluorescence intensity change at 636 and 817 nm with increasing concentrations of GSH, where the red and black lines stand for curvilinear fit of fluorescent change at 817 and 636 nm respectively with increasing of GSH concentration.

In buffer solution (pH=7.4), two carboxyl groups on isoindole-GSH derivatives were partly protonated, the driving force of self-assembly through electrostatic interactions between **SQ1**-OPA-GSH is weak, so isoindole-GSH is unable effectively to induce deaggregates of squaraines, correspondingly no fluorescent change at 630 nm can be detected. The fluorescent increasing at 820 nm is attributed to FRET from peripheral dye molecules of squaraine aggregation (donors) to isoindole-GSH derivatives (acceptors). The amount of donors is fixed, so excitation at 600 nm the acceptors fluorescence at 820 nm first went up and then saturated with increasing of GSH concentration.

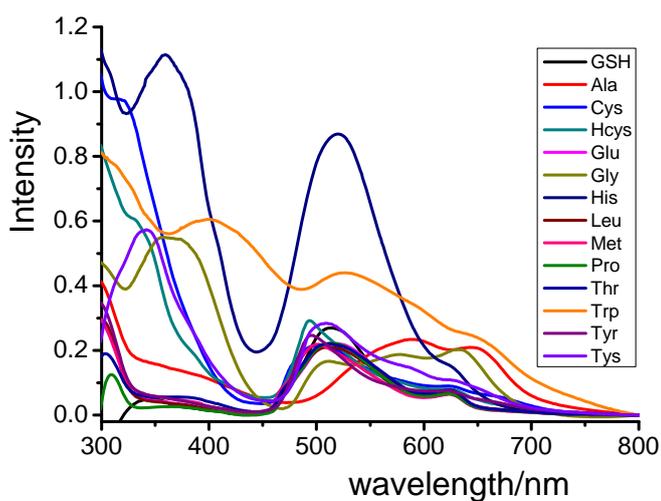


Fig. S13 Absorption spectra of **SQ1** (5.0×10^{-6} M) in the presence of OPA and 11 natural amino acids and Hcy in 10 mM HEPES buffer (pH=7.4) ($[OPA] = 1.95 \times 10^{-3}$ M, $[amino\ acids] = [Hcy] = [GSH] = 6.5 \times 10^{-4}$ M).

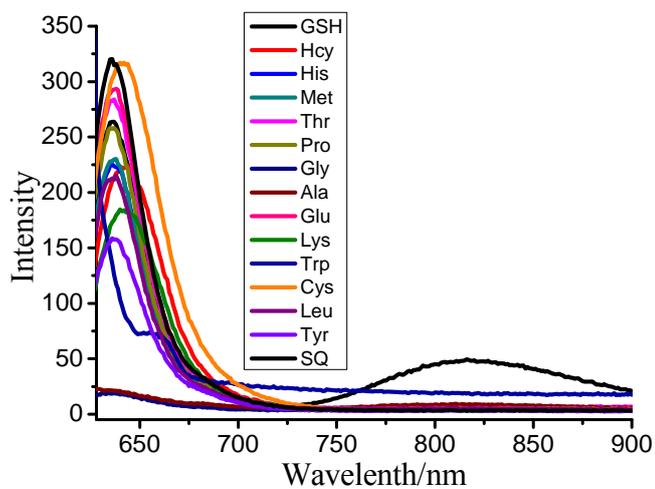


Fig. S14 Fluorescence spectra of **SQ1** (5.0×10^{-6} M) in the presence of OPA and 11 natural amino acids, Hcy in 10 mM HEPES buffer (pH=7.4) ($[OPA] = 1.95 \times 10^{-3}$ M, $[amino\ acids] = [Hcy] = [GSH] = 6.5 \times 10^{-4}$ M. Excitation wavelength was at 610 nm).

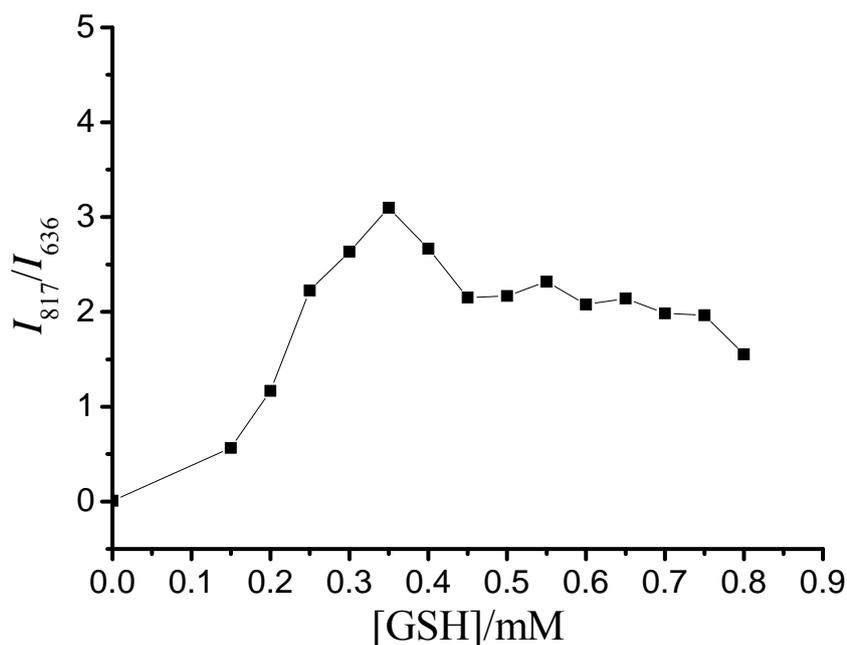


Fig. S15 The relative fluorescence intensity (I_{817}/I_{636}) change of **SQ1** (5.0×10^{-6} M) in 10 mM HEPES buffer (pH=7.4) with increasing concentrations of GSH as indicated (The molar ratio of OPA to GSH was fixed as 3.3:1. Excitation wavelength was at 610 nm).

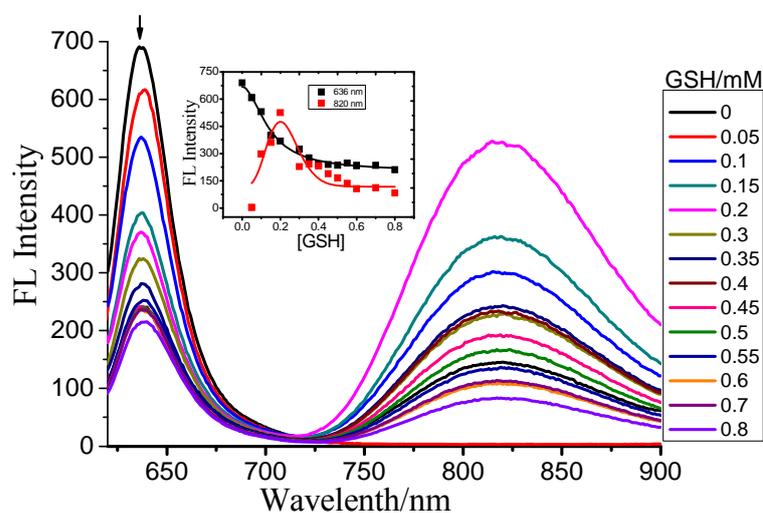


Fig. S16 Variation in the emission spectra of **SQ1** (5.0×10^{-6} M) in 10 mM HEPES buffer (pH=6.0) with increasing concentrations of GSH as indicated (the molar ratio of OPA to GSH was fixed as 3.3:1. Excitation wavelength was at 600 nm). Inset: fluorescence intensity change at 636 and 820 nm with increasing concentrations of GSH.

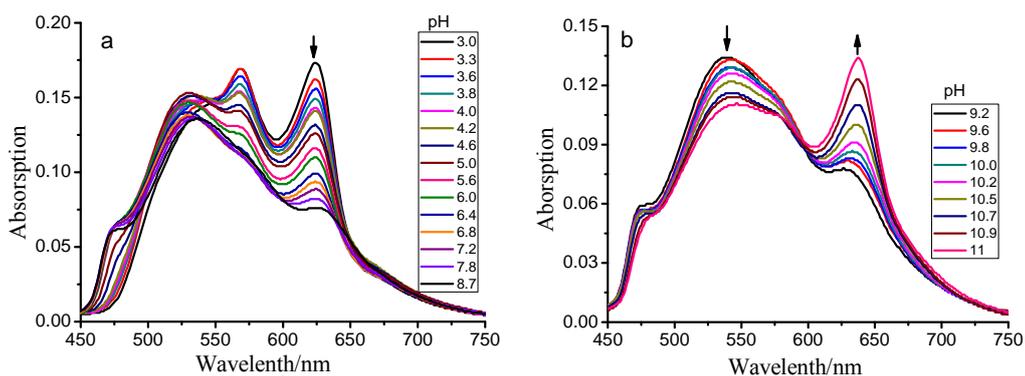


Fig. S17 Variation in the absorption spectra of **SQ1** (5.0×10^{-6} M) in 20 mM borate buffer (pH = 9.0) with pH change of solution as indicated. The molar ratio of OPA to GSH was fixed as 3.3:1.

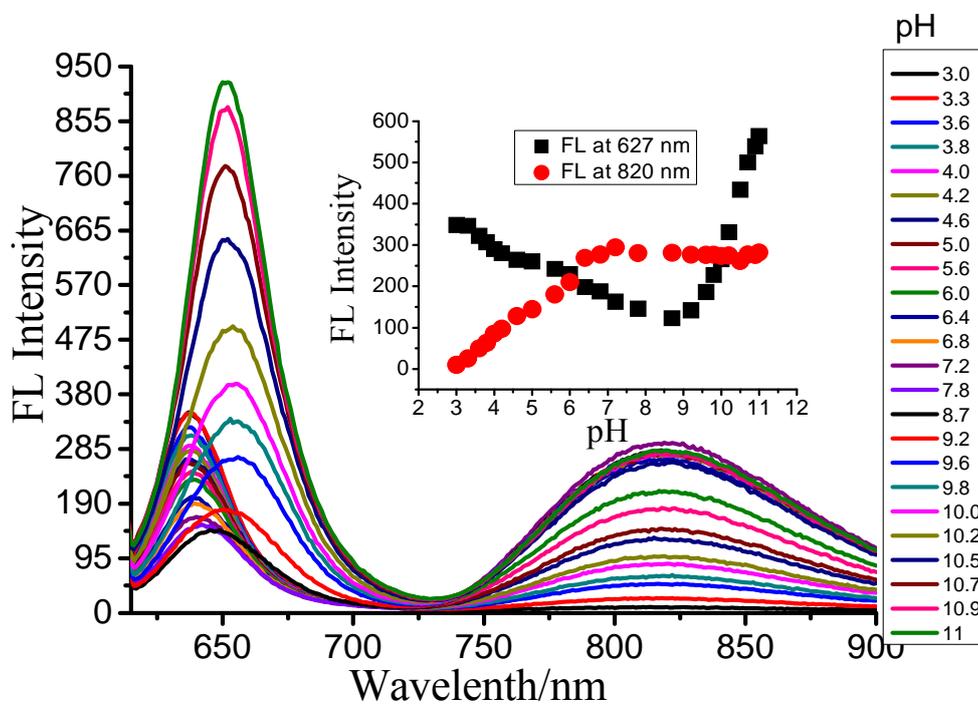


Fig. S18 Variation in the emission spectra of **SQ1** (5.0×10^{-6} M) in solution with increasing pH of solution as indicated (the molar ratio of OPA to GSH was fixed as 3.3:1. Excitation wavelength was at 600 nm). Inset: the fluorescence intensity change of **SQ1** (5.0×10^{-6} M) at 637 and 820 nm in solution with increasing pH of solution.

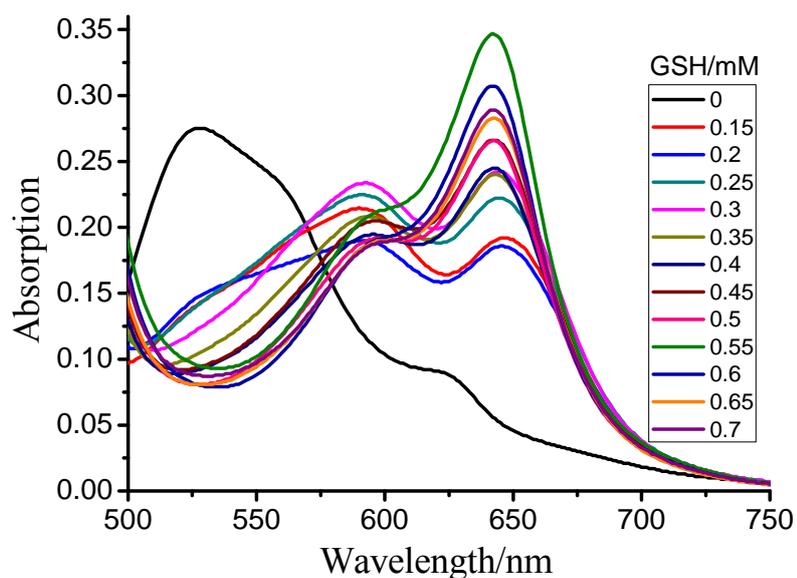


Fig. S19 Variation in the absorption spectra of **SQ1** (5.0×10^{-6} M) in 20 mM borate buffer (pH=9.0) in the presence of NA with increasing concentrations of GSH as indicated. The molar ratio of NA to GSH was fixed as 3.3 : 1.

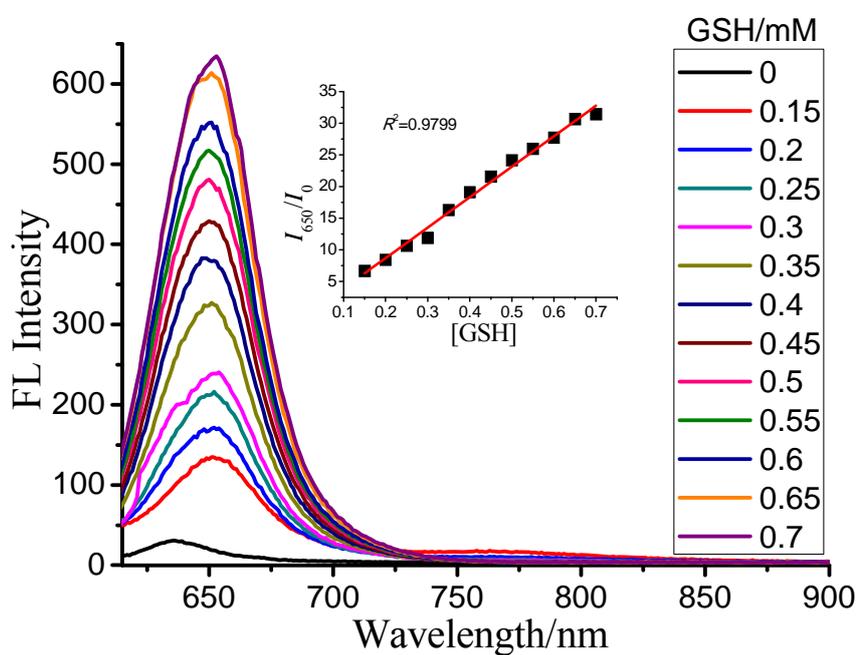


Fig. S20 Variation in the emission spectra of **SQ1** (5.0×10^{-6} M) in 20 mM borate buffer (pH = 9.0) with increasing concentrations of GSH as indicated (the molar ratio of NA to GSH was fixed as 3.3:1. Excitation wavelength at 600 nm).

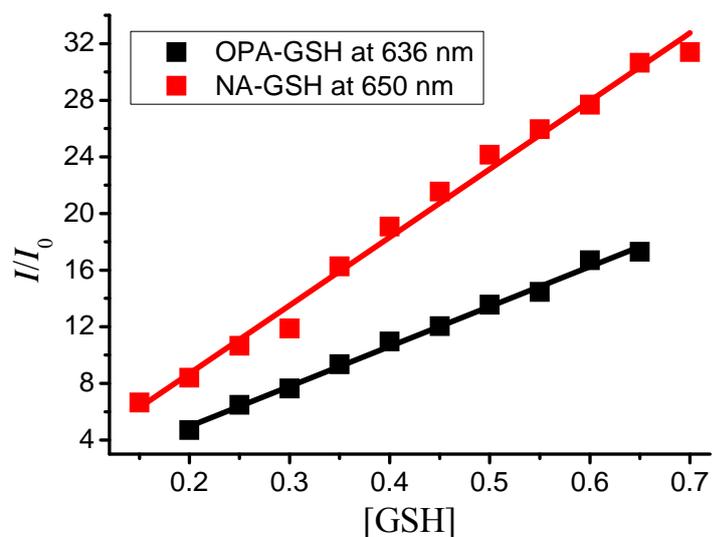


Fig. S21 The relative fluorescence change of **SQ1** (5.0×10^{-6} M) in 20 mM borate buffer (pH = 9.0) in the presence of OPA or NA with increasing concentrations of GSH as indicated (the molar ratio of aldehyde to GSH was fixed as 3.3:1. Excitation wavelength at 600 nm).

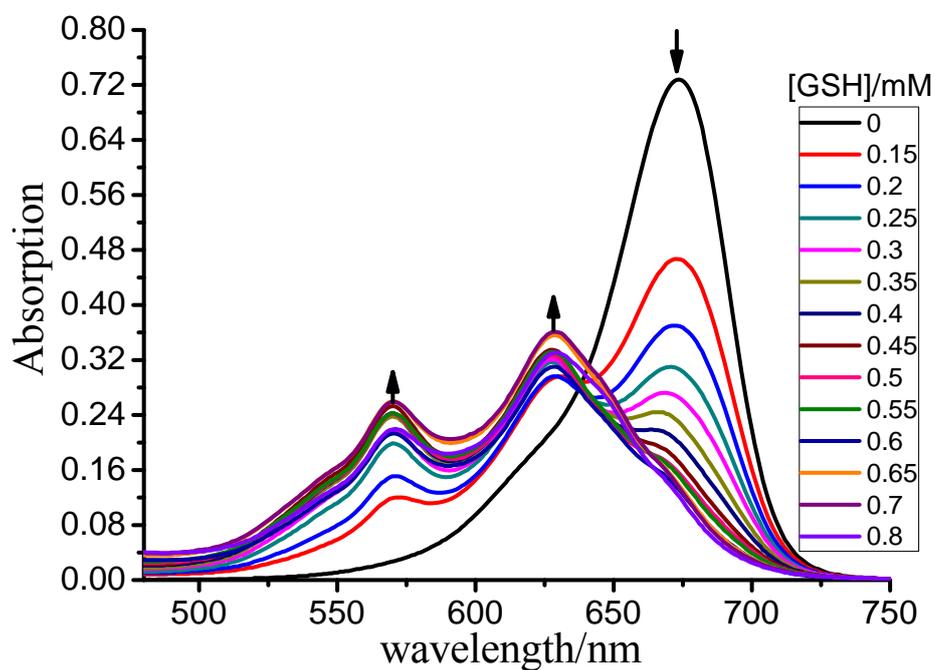
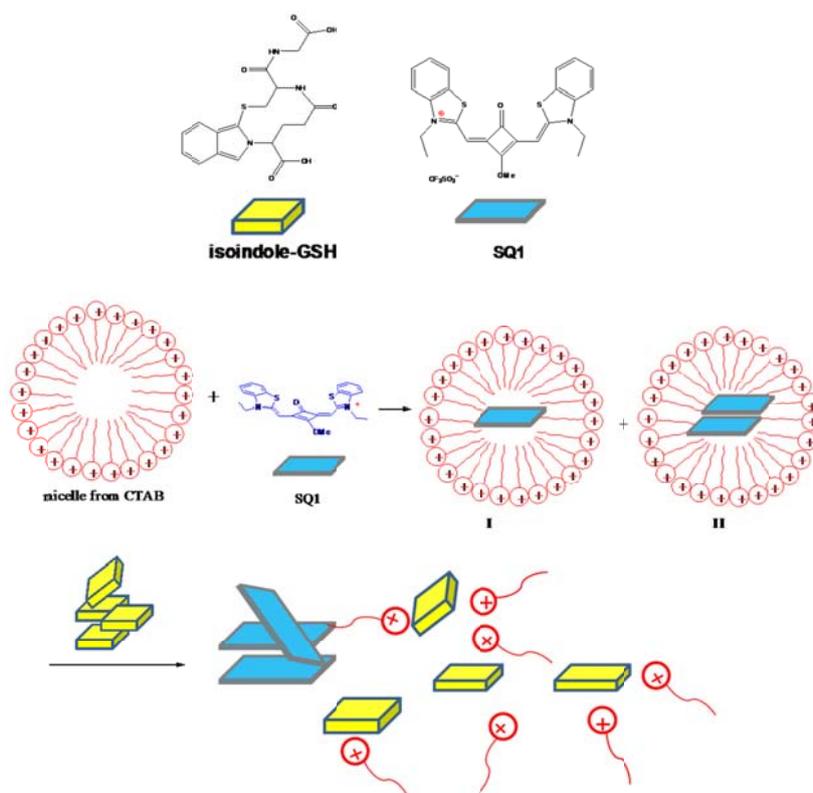


Fig. S22 Variation in the absorption spectra of **SQ1** (5.0×10^{-6} M) in 10 mM HEPES buffer (pH = 7.4) in the presence of 0.05 wt% CTAB with increasing concentrations of GSH as indicated (the molar ratio of OPA to GSH was fixed to 3.3 : 1).



Scheme S2 Plausible interaction between isoindole-GSH, **SQ1** and CTAB in buffer solution in the presence of CTAB.

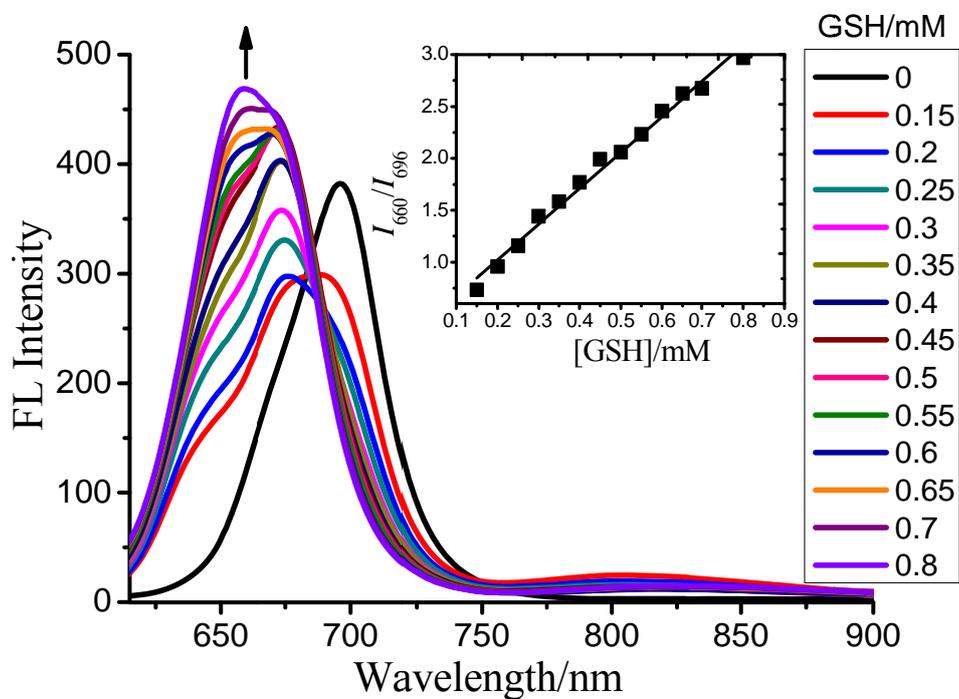


Fig. S23 Variation in the emission spectra of **SQ1** (5.0×10^{-6} M) in 10 mM HEPES buffer (pH=7.4) in the presence of 0.05 wt% CTAB with increasing concentrations of GSH as indicated (The molar ratio of OPA to GSH was fixed as 3.3:1. Excitation wavelength at 610 nm). Inset: The ratio

fluorescence intensity change at 660 and 696nm (I_{660}/I_{696}) with increasing concentrations of GSH.

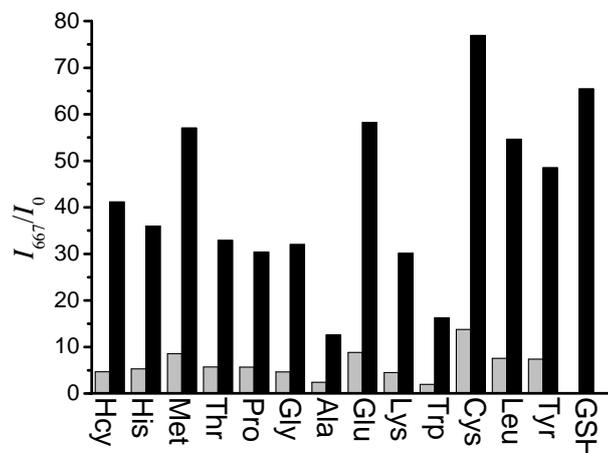


Fig. S24 Emission intensity change (I_{667}/I_0) of **SQ1** (5.0×10^{-6} M) at 640 nm in 10 mM HEPES buffer (pH = 7.4) containing OPA and 0.05 wt% CTAB in the presence of different amino acids and Hcy with the excitation at 600 nm (gray bar) after 0.5 h. Black bars represent the intensity with subsequent addition of GSH ($[OPA] = 1.95 \times 10^{-3}$ M, $[amino\ acids] = [Hcy] = [GSH] = 6.5 \times 10^{-4}$ M). I_0 indicates the fluorescence intensity of free amino acids or Hcy while I_{640} indicated the fluorescence intensity upon addition of amino acids.

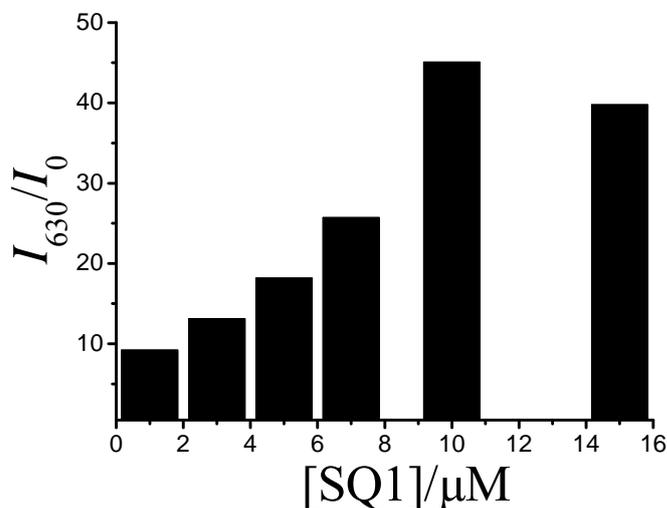


Fig. S25 The relative fluorescence intensity change at 630 nm of different concentration of **SQ1** in 20 mM borate buffer (pH = 9.0) at 25°C before and after addition of GSH. ($[SQ1]:[OPA]:[GSH] = 1:390:130$. Excitation wavelength at 600 nm).