Supplementary information for

Conjugatd Polyelectrolyte as a Colorimetric and Fluorescent Probe for the Detection of Glutathione

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Materials. All chemicals were purchased from Aldrich and Beijing Chem. Reagents Co. (Beijing, China) and were used as received. Water-soluble polythiophene derivative, PMTPA, was synthesized and purified as reported previously.[S1]

Sample Preparation. Typically, the in-situ premodification reaction of GSH with OPA was performed as the following procedures: stock solutions of OPA and GSH were mixed at 25 ^{o}C to give a mixture with the molar ratio of OPA to GSH, 1.5:1, in 20 mM borate buffer (pH = 9.0). For experiments operated at physiological pH, stock solutions of OPA and GSH were mixed at 25 ^{o}C to give a mixture with the molar ratio of OPA to GSH, 10:1, in 10 mM HEPES buffer (pH = 7.4).

Control experiments for addressing the selectivity of PMTPA toward GSH were carried out at the identical conditions. 20 Natural amino acids, Hcy and BSA were premodified by reacting with OPA for 30 min, respectively, and then the probe PMTPA was added into the OPA/analyte mixture to give a solution containing 0.15 mM PMTPA, 0.5 mM analyte and 0.75 mM OPA. The sample was measured by UV-visible spectrometer immediately.

Titration Experiments. To exclude the influence of dilution on the equilibrium of the premodified reaction between OPA and GSH, dilution of the reaction mixture of OPA and GSH was monitored by absorption spectroscopy. It was found that there is a linear relationship between the absorbance of isoindole-GSH and GSH concentration (Fig. S0), indicating that diluting has scarcely influence on the reaction equilibrium in the concentration examined here.

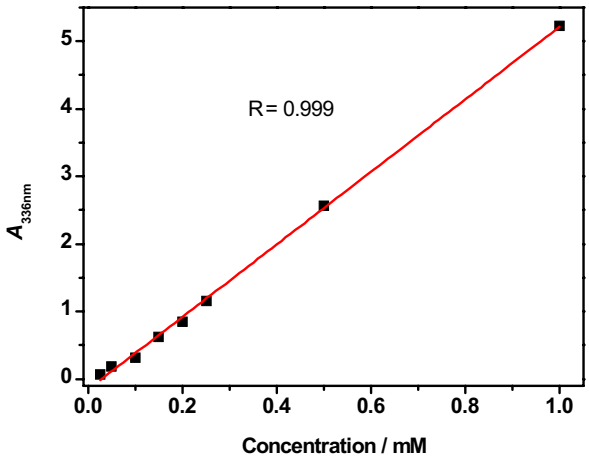


Fig. S0 Plot of the absorbance of a mixture of OPA/GSH at 336 nm against the concentration of GSH in 20 mM borate buffer at 25 ^{o}C. [OPA]/[GSH] = 1.5:1.

Measurements

Absorption and emission spectra were collected by using a Hitachi 3010 UV-visible spectrometer and a LS55 fluorescence spectrometer (PerkinElmer), respectively. Circular dichroism spectra were acquired on a Jasco J-815 spectropolarimeter.

Reference

S1 C. Li, M. Numata, A.-H. Bae, K. Sakurai and S. Shinkai, *J. Am. Chem. Soc.*, 2005, **127**, 4548.

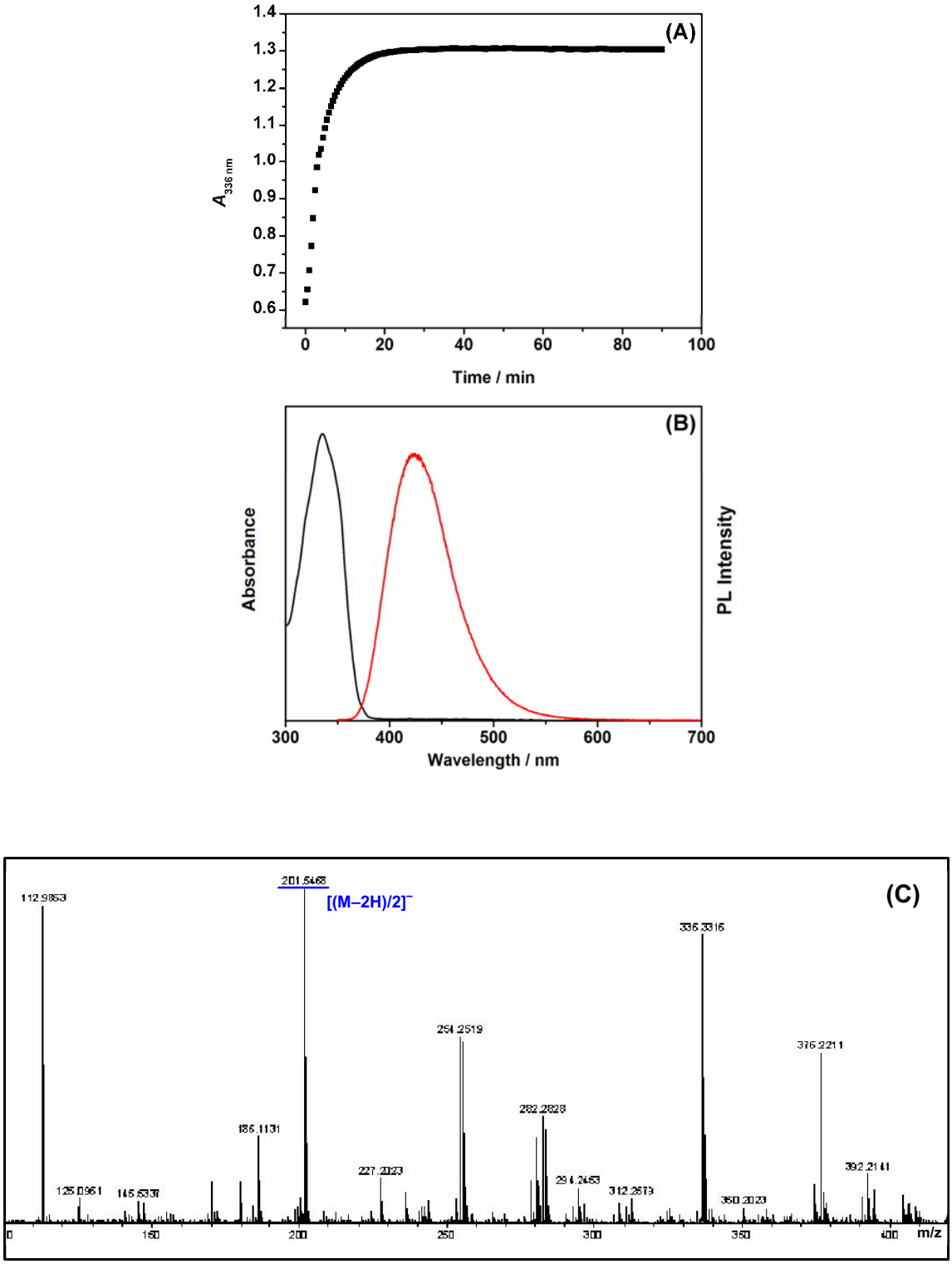


Fig. S1 (A) Time course of the absorbance of a mixture of OPA/GSH at 336 nm in 20 mM borate buffer (pH = 9.0); (B) Absorbance and emission spectra (λ_{ex} = 340 nm) and (C) ESI-Mass spectrum of a mixture of OPA/GSH in 20 mM borate buffer (pH = 9.0). [OPA] = 7.5×10^{-4} M; [GSH] = 5.0×10^{-4} M.

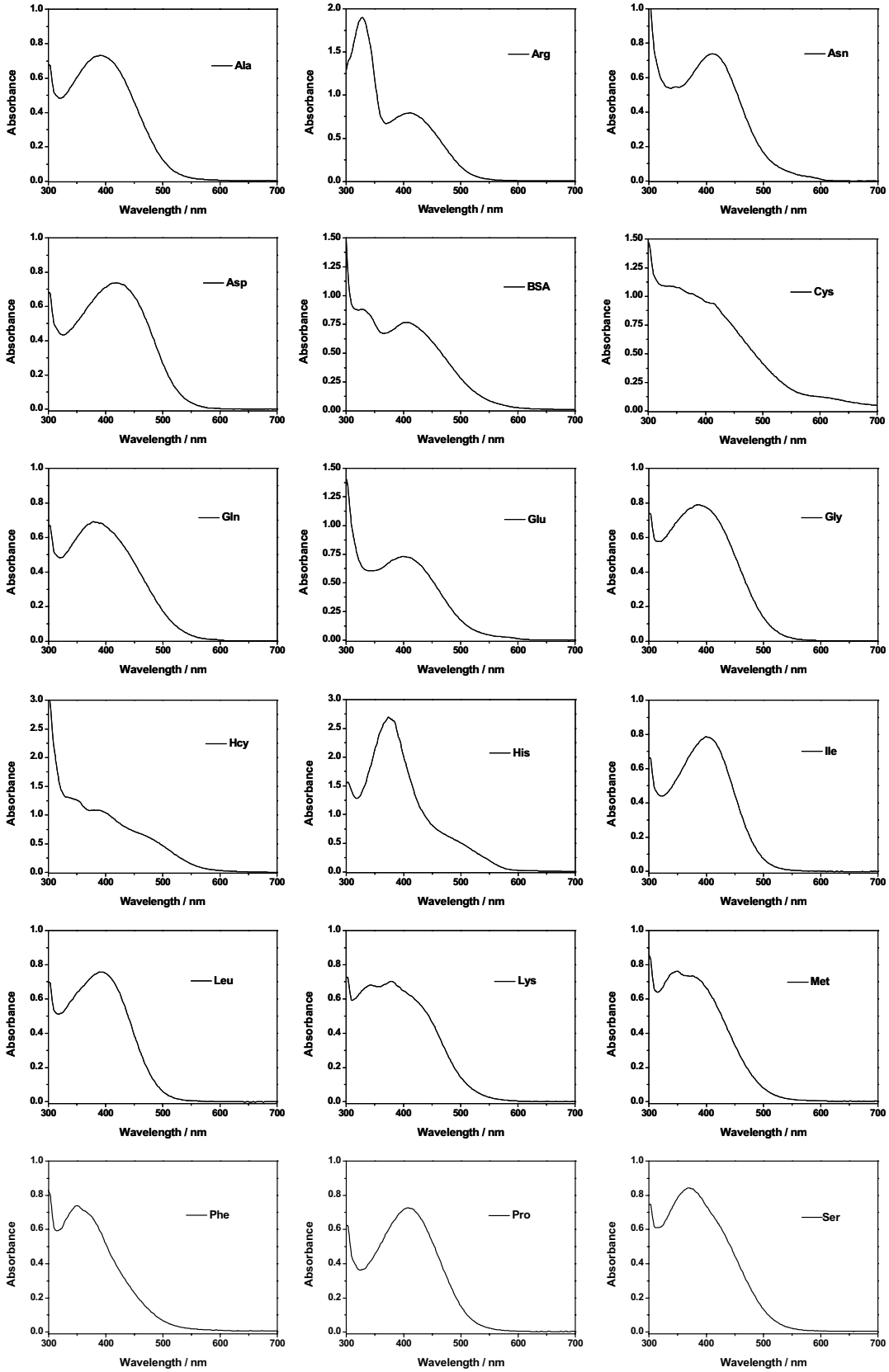
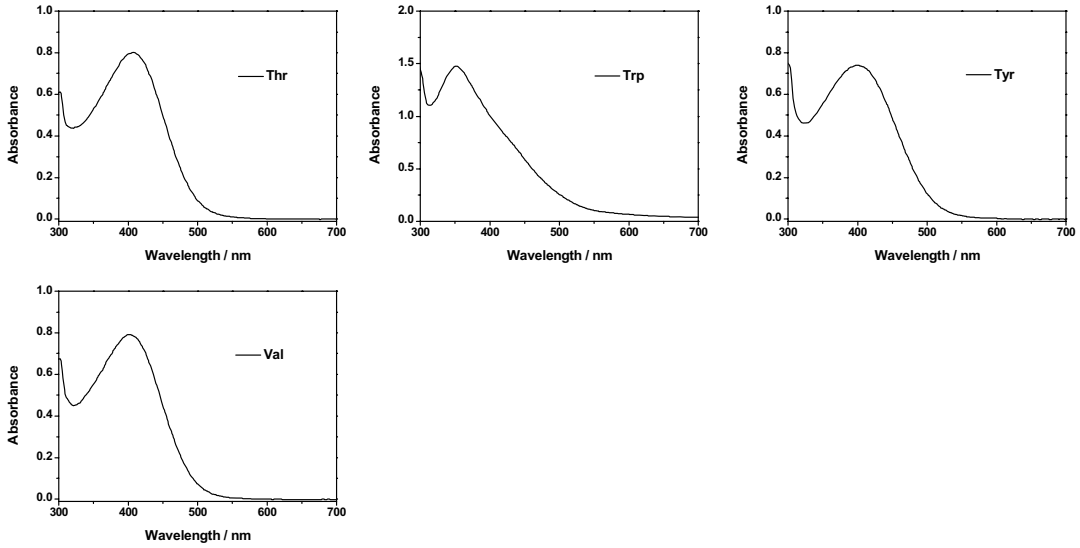


Fig. S2 Absorption spectra of PMTPA (1.5×10^{-4} M) in the presence of OPA and 20 natural amino acids, Hcy and BSA in 20 mM borate buffer (pH = 9.0). [OPA] = 7.5×10^{-4} M; [amino acids] = [Hcy] = [BSA] 5.0×10^{-4} M. (to be continued)



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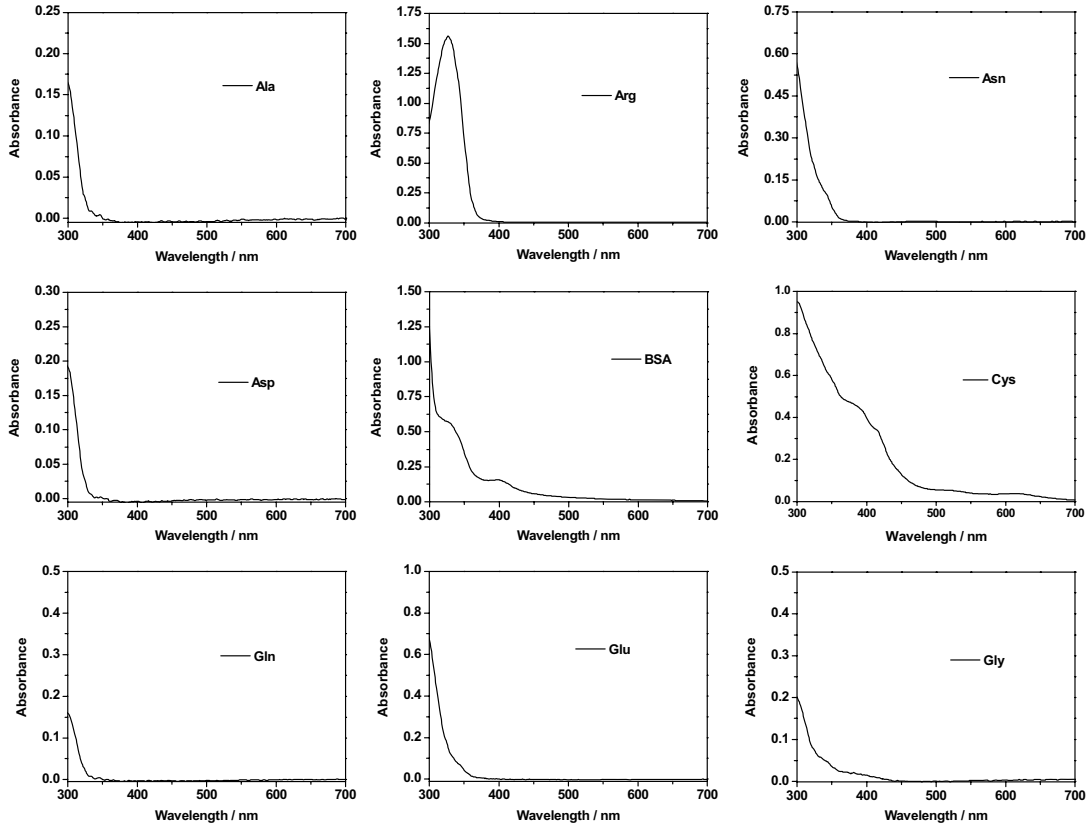
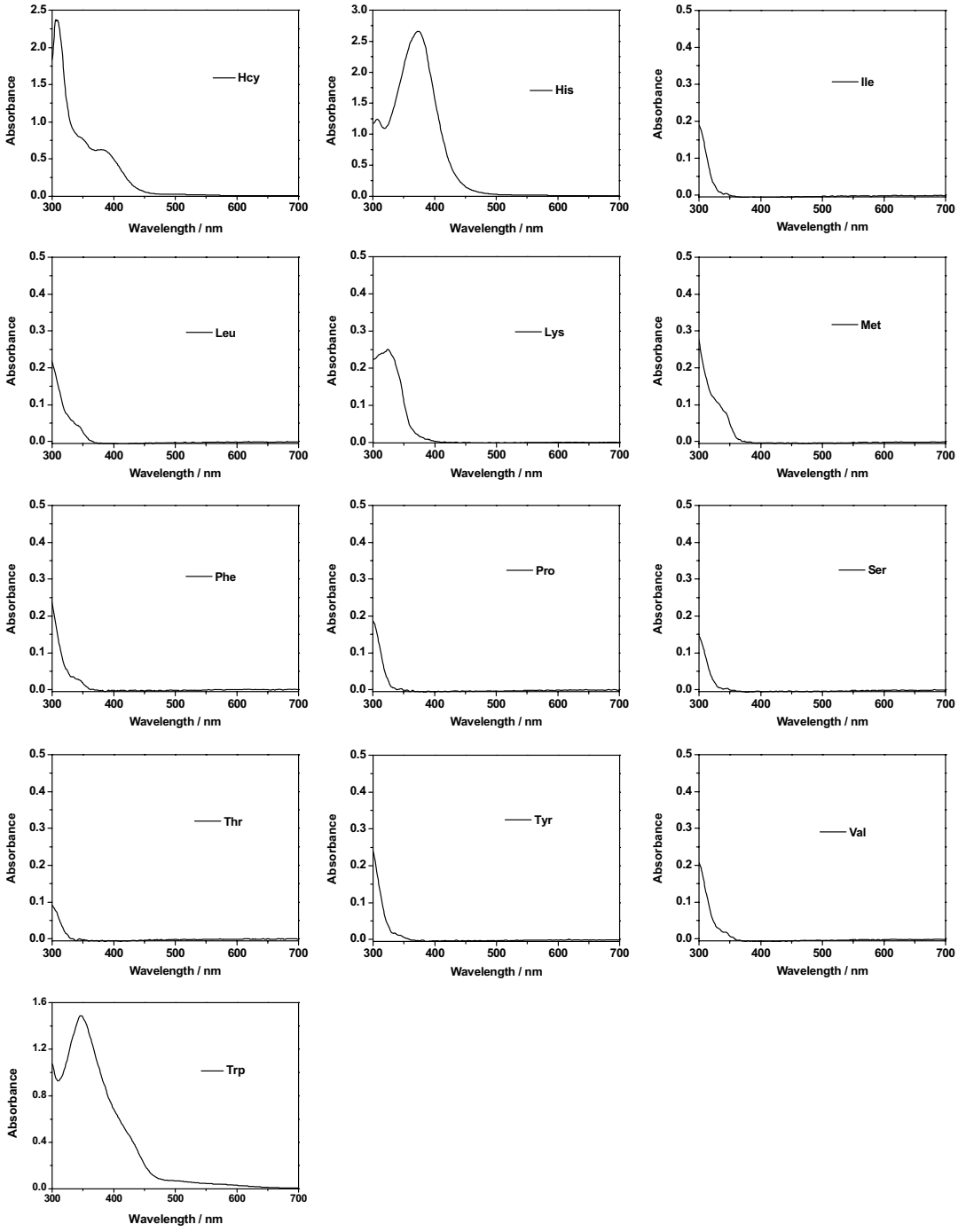


Fig. S3 Absorption spectra of a mixture of OPA with 20 natural amino acids, Hcy and BSA, respectively, in 20 mM borate buffer (pH = 9.0). [OPA] = 7.5×10^{-4} M; [amino acids] = [Hcy] = [BSA] = 5.0×10^{-4} M. (to be continued)



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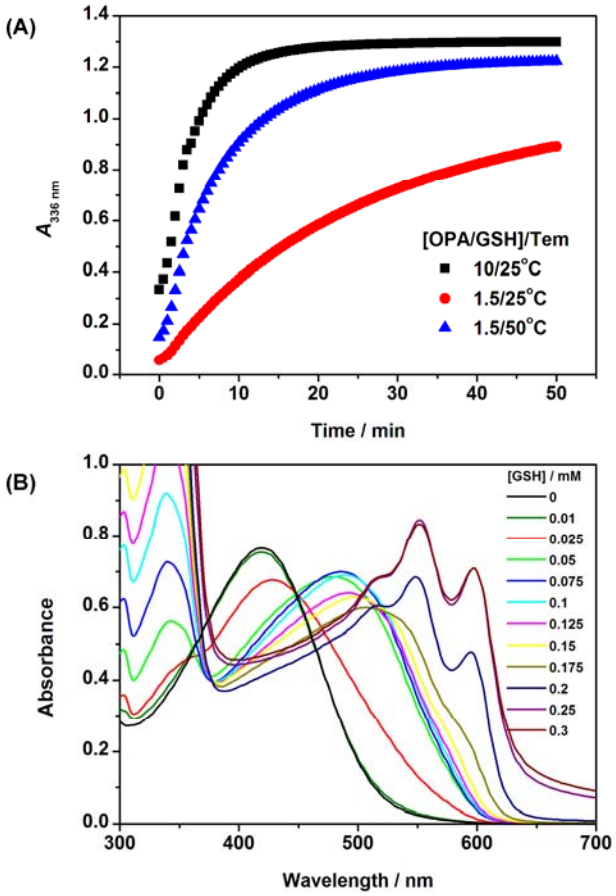

Fig. S4 (A) Time course of the absorbance of a mixture of OPA/GSH at 336 nm in 10 mM HEPES buffer (pH = 7.4). [GSH] = 5.0×10^{-4} M. Black: [OPA]/[GSH] = 10:1, 25 °C; Blue: [OPA]/[GSH] = 1.5:1, 50 °C; Red: [OPA]/[GSH] = 1.5:1, 25 °C. (B) Variation in the absorption spectra of PMTPA (1.5×10^{-4}M) in 10 mM HEPES buffer (pH = 7.4) with increasing concentrations of GSH as indicated. [OPA]/[GSH] = 10:1.

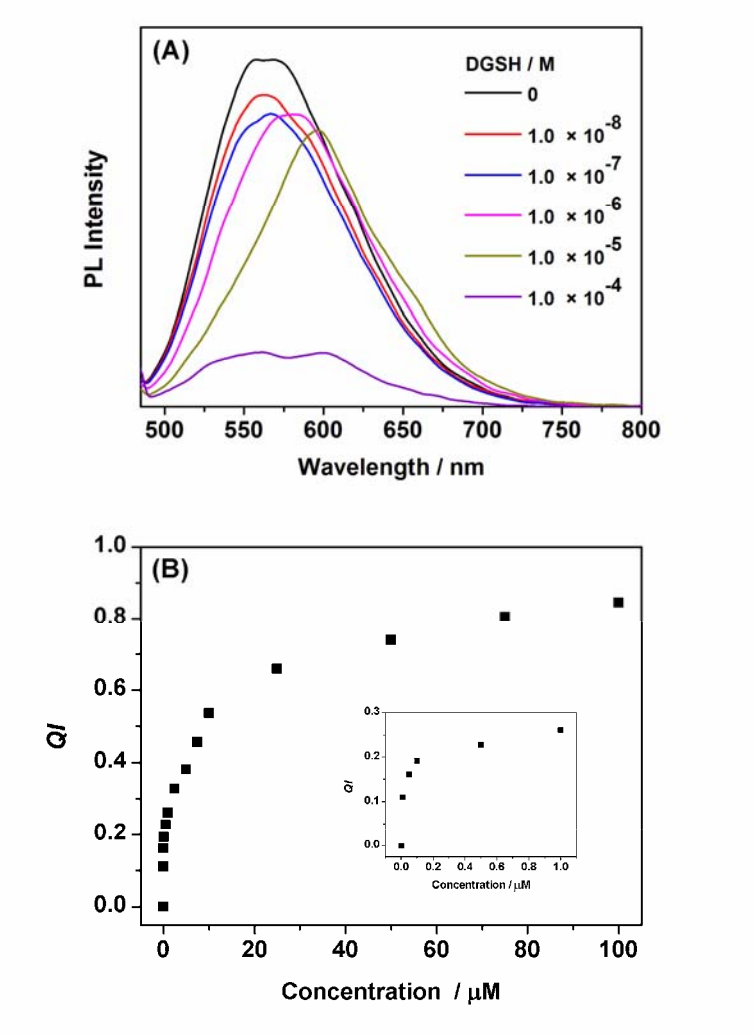

Fig. S5 (A) Variation in the emission spectra of PMTPA (1.5×10^{-5} M) in 10 mM HEPES buffer (pH = 7.4) with increasing concentrations of GSH as indicated. The molar ratio of OPA to GSH was fixed as 1.5:1. Excitated wavelength λ_{ex} = 475 nm. (B) Fluorescence quenching of PMTPA (1.5×10^{-5} M) by isoindole-GSH at various concentrations. The fluorescence quenching $QI = [(I_0–I)/I_0] \times 100\%$; I_0 is the fluorescence intensity at 557 nm of a solution of PMTPA (1.5×10^{-5} M); I is the fluorescence intensity at 557 nm of a solution of PMTPA (1.5×10^{-5} M) in the presence of different amounts of GSH. Inset: plot of QI vs GSH concentration at lower concentration.

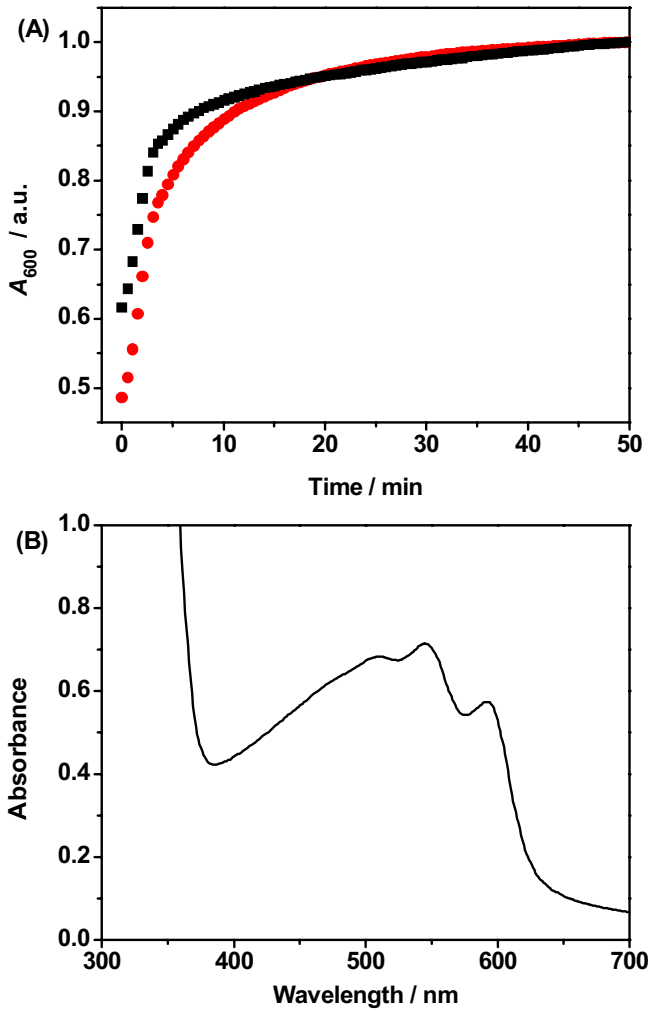

Fig. S6 (A) Time course of the absorbance at 600 nm of a solution of PMTPA (1.5×10^{-4} M) in 10 mM phosphate buffer (pH = 7.4, black square) and in phosphate buffered saline (pH 7.4 with 137 mM NaCl, 2 mM KCl and 10 mM phosphate buffer, red circle) upon addition of 2.0 mM OPA and 0.2 mM GSH at 25 °C. The absorbance at the terminal points was normalized for comparison. (B) Absorption spectrum of PMTPA (1.5×10^{-4} M) in phosphate buffered saline (pH 7.4 with 137 mM NaCl, 2 mM KCl and 10 mM phosphate buffer) upon addition of 2.0 mM OPA and 0.2 mM GSH at 25 °C after attaining thermodynamic equilibrium (1h).