Supporting Information

AIE and ESIPT Based Kinetic Resolved Fluorescent Probe for Biothiols

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Figure S1. The pH titration of probe2 in water (contains 1% DMSO).



Figure S2.Time-dependence of fluorescence intensity of probe **AIE-S** (10 μ M) upon addition of different biothiols (Cys, GSH and Hcy) in DMSO–PBS buffer (10 μ M, pH 7.4, 1:99, v/v) at 25 °C. There are four groups. The first is 20 min, the second is 40 min, the third is 120 min and the the forth group is 240 min. The black, green and red represent Cys, GSH and Hcy, respectively.



Figure S3.Time-dependent of fluorescence kinetics spectra of probe **AIE-S** (10 μ M) upon addition of different concentration GSH (20 μ M and 100 μ M) in DMSO–PBS buffer (10 μ M, pH 7.4, 1:99, v/v) at 25 °C. All the reactions are monitored every 0.02s at 505 nm. $\lambda_{ex} = 340$ nm.



Figure S4. Selectivity of **AIE-S** towards Cys. Fluorescence response of **AIE-S** (10 μ M) at 505 nm in the presence of different cation (0.5 mM) in a PBS/DMSO mixture (99:1, v/v) with excitation wavelength: 340 nm. Each spectrum was recorded after 20 min of reaction in a mixture of PBS and DMSO (99 : 1, v/v) at r.t. Cations: Hg²⁺; Co²⁺; Cr³⁺; Cd²⁺; Fe³⁺; Ni²⁺;Zn²⁺; Cu²⁺; Al³⁺; Mg²⁺; Ca²⁺ and Ag⁺.



Figure S5. Selectivity of **AIE-S** towards Cys. Fluorescence response of **AIE-S** (10 μ M) at 505 nm in the presence of different anions (0.5 mM) in a PBS/DMSO mixture (99:1, v/v) with excitation wavelength: 340 nm. Each spectrum was recorded after 20 min of reaction in a mixture of PBS and DMSO (99 : 1, v/v) at r.t. Anions: F⁻, Cl⁻, Br⁻, I⁻, NO₃⁻, NO²⁻, SCN, CO₃²⁻, SO₄²⁻, SO₃²⁻, HS⁻ and S²⁻.



Figure S6. Cell cytotoxicity test by MTT assays. Blue and orange mean probe **AIE-S** and salicylaldazine dye **2**, respectively. The concentration is 1μ M, 10μ M, 50μ M and 100μ M, respectively.



Figure S7.¹H NMR spectrum of salicylaldazine dye 2in CDCl₃ solution.



Figure S8.¹³C NMR spectrum of salicylaldazine dye2 in CDCl₃ solution



Figure S9.¹H NMR spectrum of AIE-S in CDCl₃ solution.



Figure S10.¹³C NMR spectrum of AIE-S in CDCl₃ solution.



Figure S11. ESI-MS spectrum of AIE-S.