Electronic Supplementary Material (ESI) for Analytical Methods.

Electronic Supplementary Information

A Thiol-Anchored Solvatochromic and Fluorogenic Molecular Rotor for Covalent Protein Labeling in SDS-PAGE and Mitochondria Specific Fluorescence Imaging

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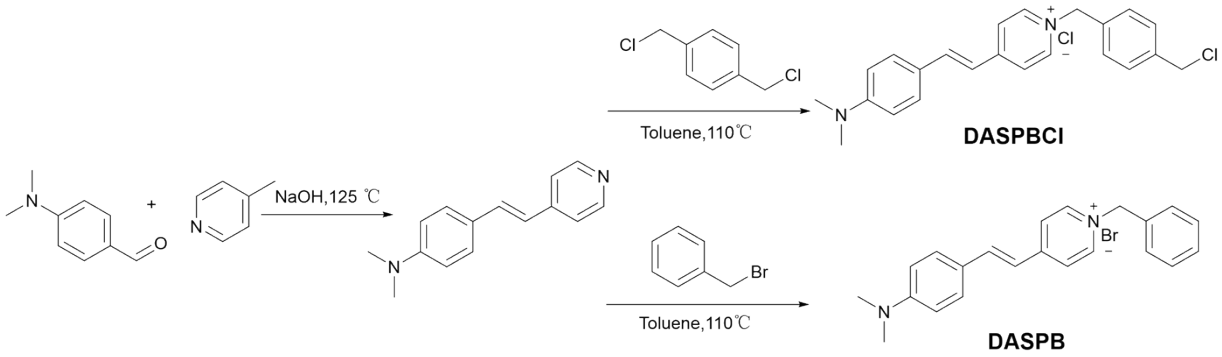

Scheme S1. Synthetic of the probes **DASPBCl** and **DASPB**.

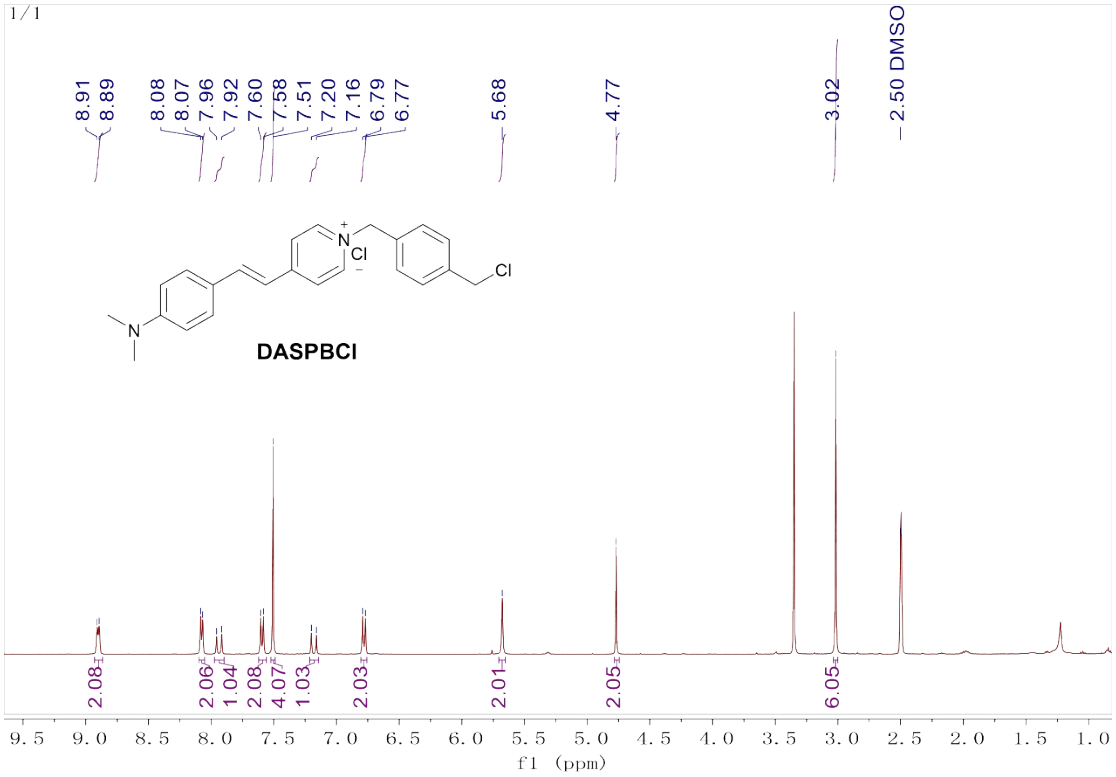

Fig S1. ^{1}H NMR spectrum of **DASPBCl**.

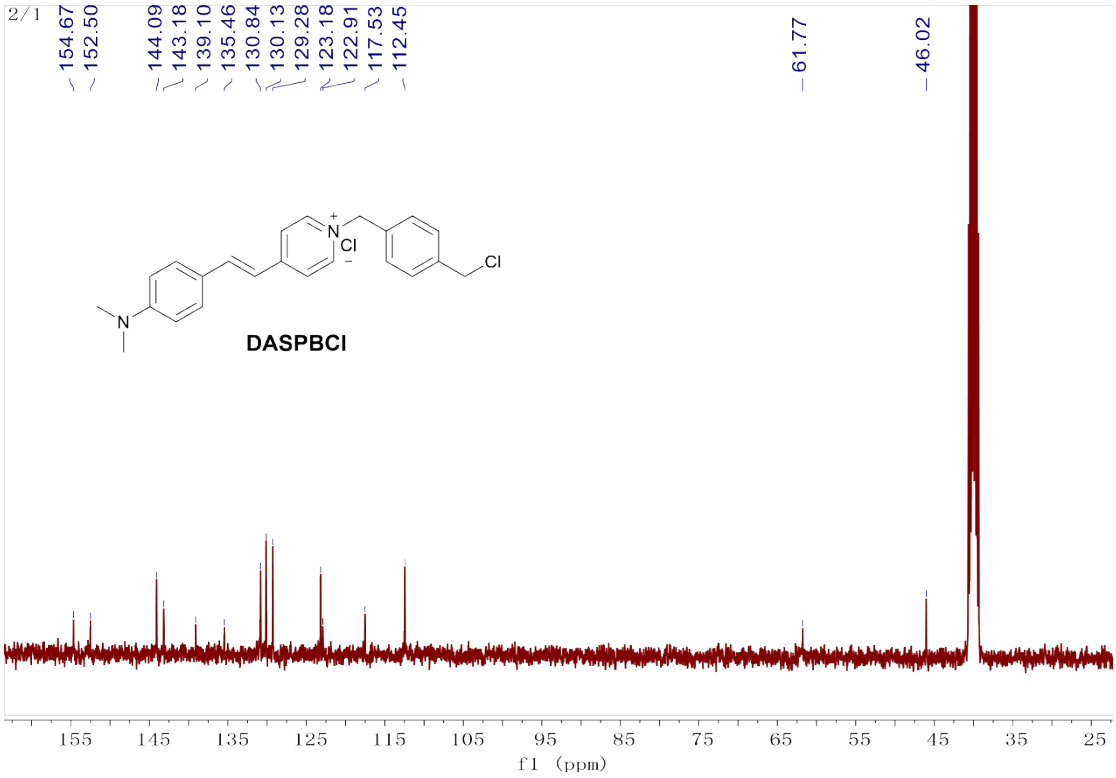

Fig S2. ^{13}C NMR spectrum of **DASPBCl**.

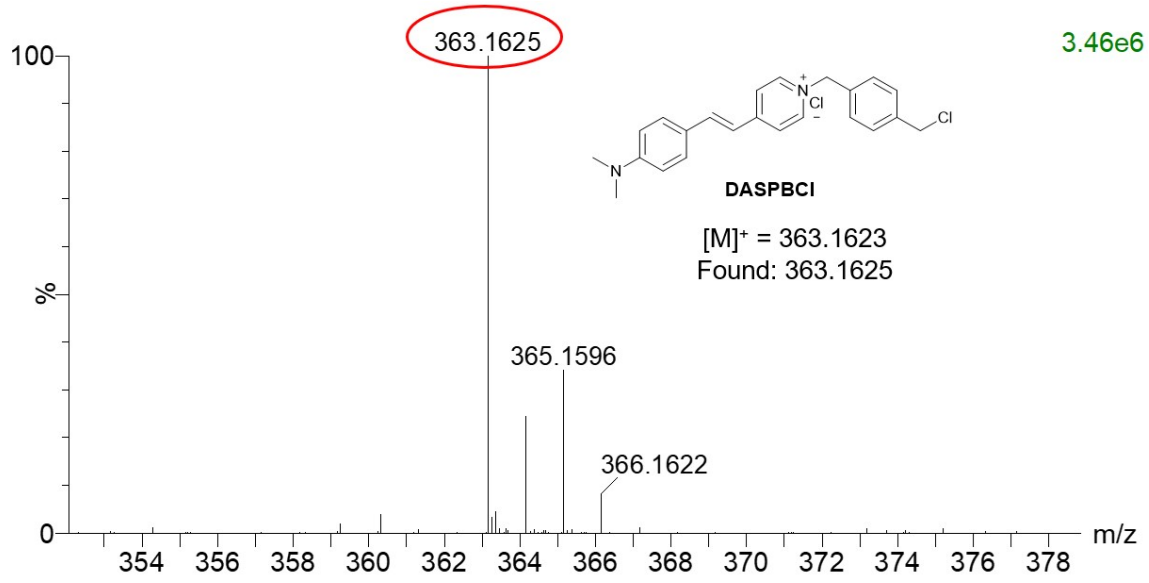

Fig S3. HRMS of **DASPBCl**.

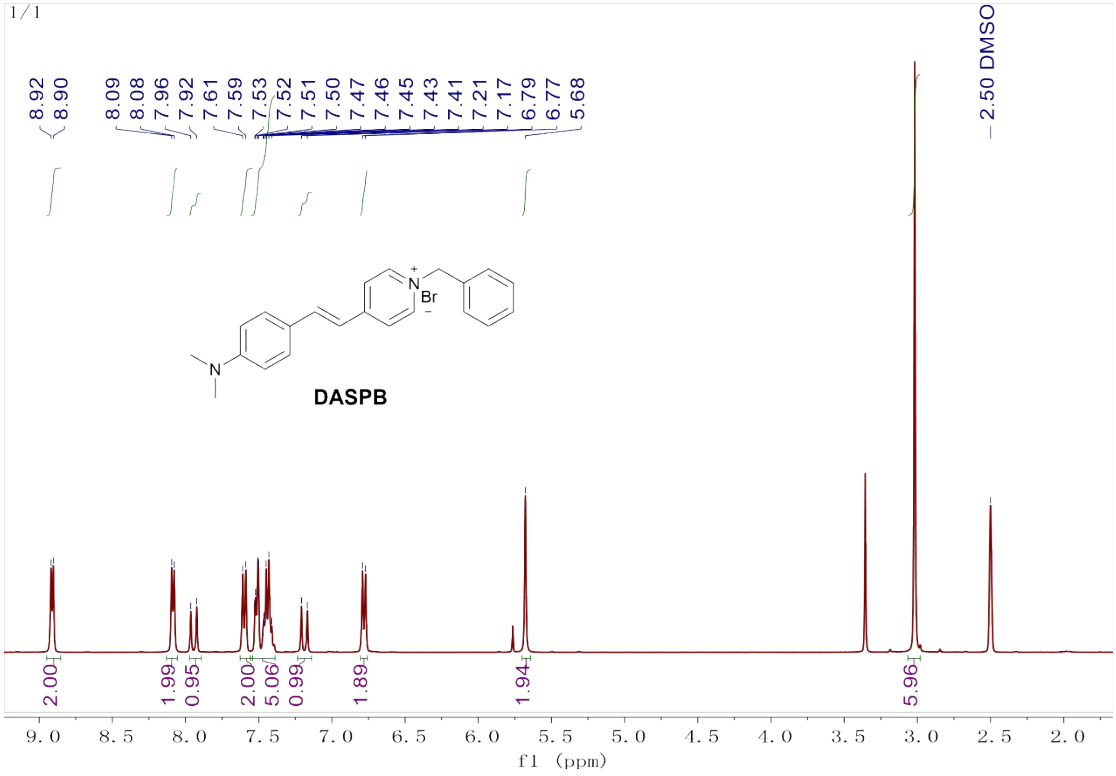

Fig S4. [1]H NMR spectrum of **DASPB**.

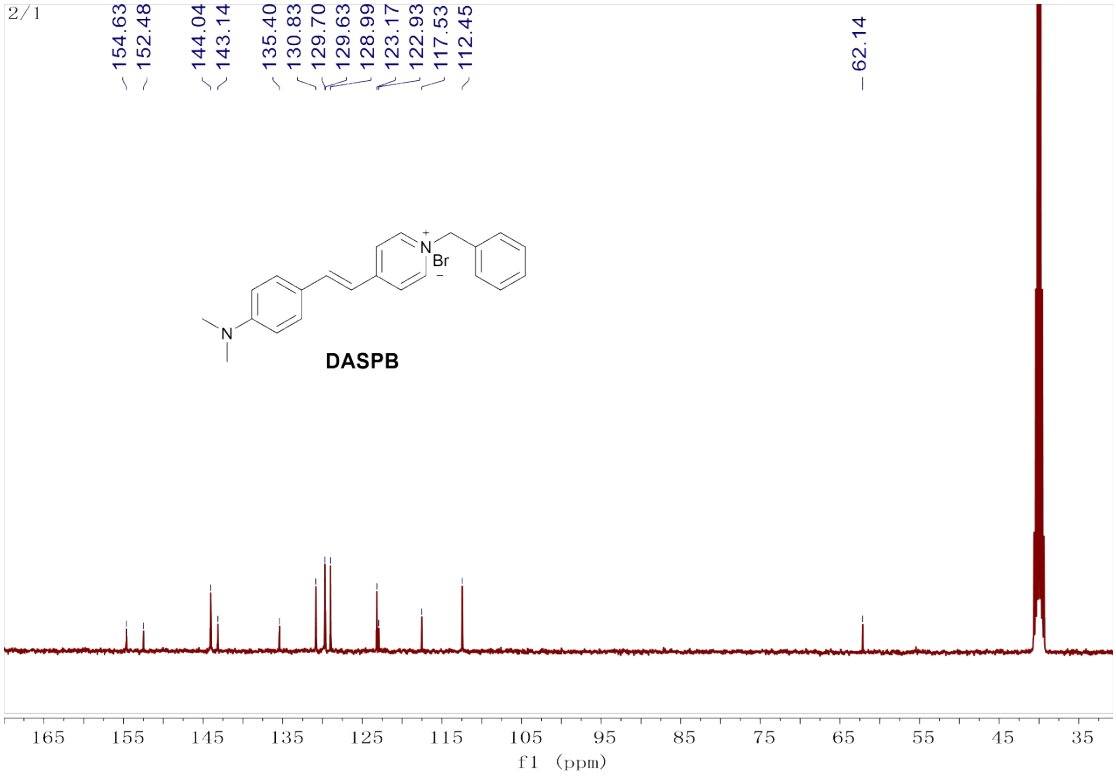

Fig S5. [13]C NMR spectrum of **DASPB**.

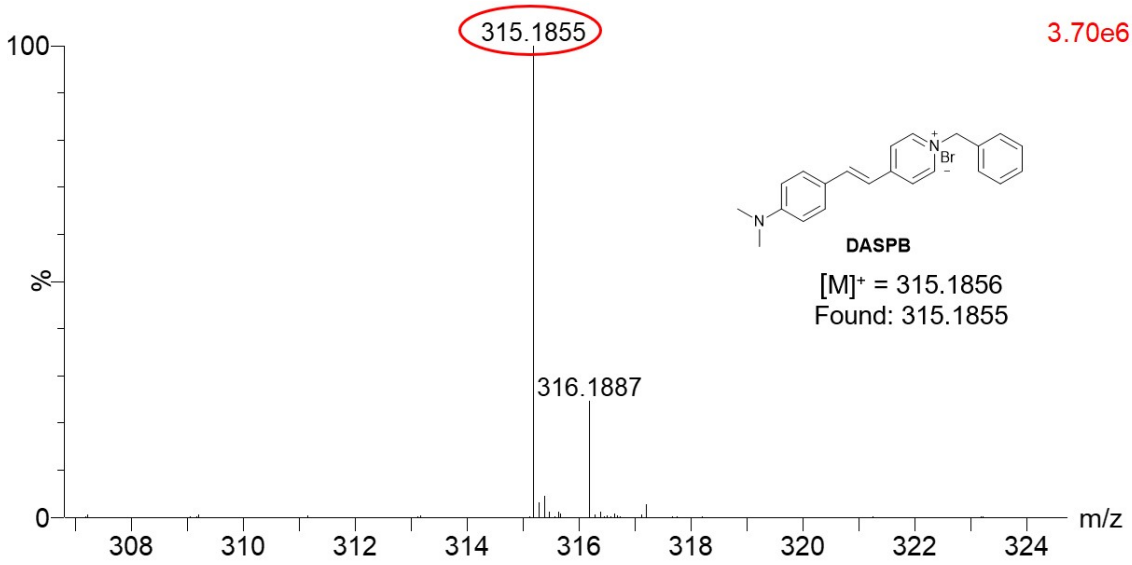

Fig S6. HRMS of **DASPB**.

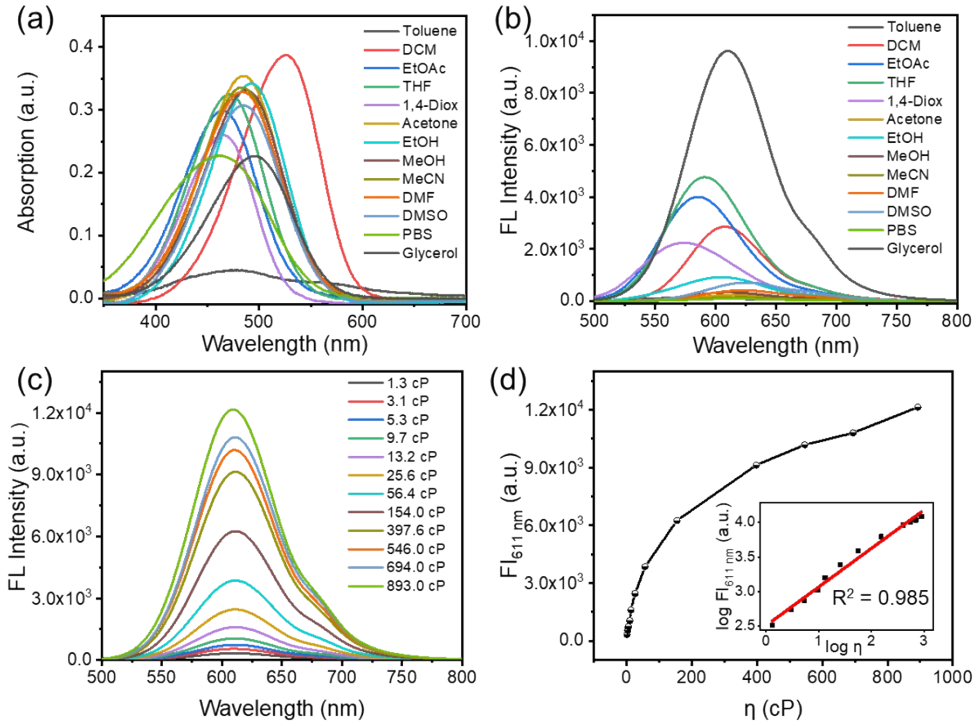

Fig S7. (a) Absorption spectra of **DASPB** (10 μM) in different solvents. (b) Fluorescence spectra of **DASPB** (10 μM) in different solvents. (c) Fluorescence spectra of **DASPB** (10 μM) with the variation of solution viscosity (methanol-glycerol system), λex = 480 nm. (d) The relationship between fluorescence intensity (FI) at 611 nm and viscosity (η) of methanol-glycerol solution. Inset: The linear relationship between log $FI_{611\ nm}$ and log η. (log FI = 0.557log η + 2.509, R^2 = 0.985).

Table S1 The spectral properties of probe **DASPBCl** in different solvents

Solvent	λ_{abs}/nm	λ_{em}/nm	Stokes shift/nm	ε/×10^4 M^{-1}cm^{-1}	Φ/%
Toluene	480	570	90	0.54	5.6
1,4-Diox	467	579	112	1.73	9.8
DCM	522	606	84	2.91	2.4
EtOAc	465	585	120	2.06	7.3
THF	472	590	118	2.31	10.2
Acetone	486	618	132	2.76	0.7
EtOH	494	604	110	2.82	2.4
MeOH	490	611	121	2.75	0.9
MeCN	485	620	135	2.66	0.4
DMF	484	624	140	2.54	5.5
DMSO	486	626	140	2.29	3.0
PBS	465	612	147	1.49	1.0
Gycerol	497	611	114	1.86	33.4

Table S2 The spectral properties of probe **DASPB** in different solvents

Solvent	λ_{abs}/nm	λ_{em}/nm	Stokes shift /nm	ε/×10^{4}M^{-1}cm^{-1}	Φ/%
Toluene	480	572	92	0.45	4.3
1,4-Diox	465	574	109	2.60	13.5
DCM	526	608	82	3.87	3.0
EtOAc	465	586	121	2.98	11.8
THF	471	591	120	3.26	18.9
Acetone	485	618	133	3.54	0.9
EtOH	492	605	113	3.42	2.3
MeOH	488	611	123	3.31	1.0
MeCN	483	620	137	3.36	0.5
DMF	484	623	139	3.29	2.6
DMSO	485	625	140	3.08	2.7
PBS	462	615	153	2.27	0.6
Gycerol	496	611	115	2.26	35.4

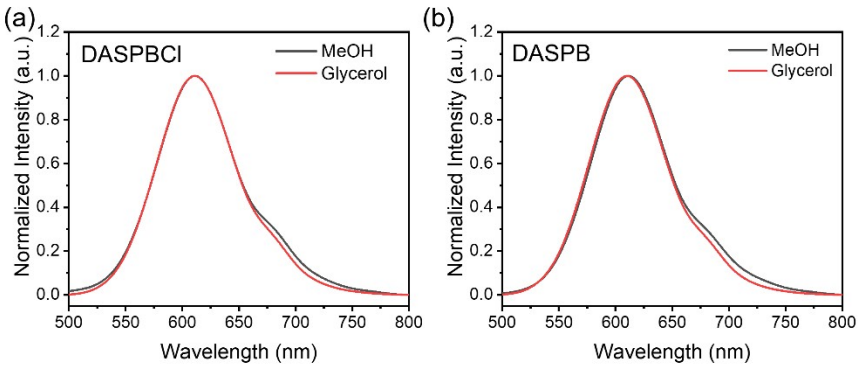

Fig. S8. The normalized fluorescence spectra of probe **DASPBCl** (a) and **DASPB** (b) in methanol and glycerol.

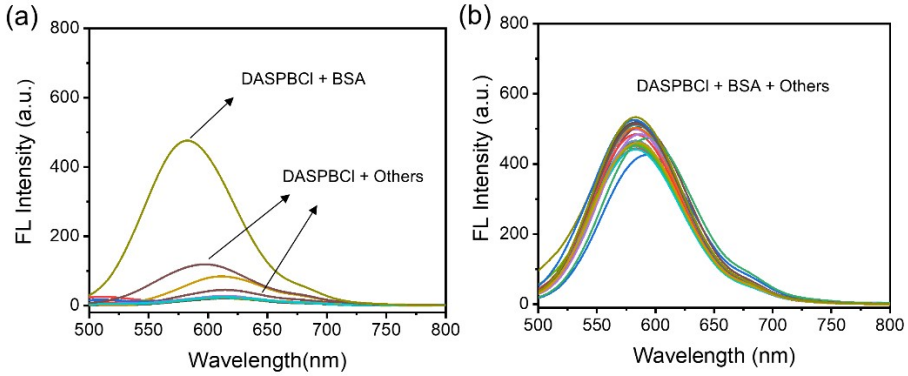

Fig S9. The selective test and the anti-interference detection of **DASPBCl** with BSA. (a) The selective test of **DASPBCl** probe to inorganic ions, amino acids and proteins for BSA. (b) The anti-interference detection of BSA by **DASPBCl** probe in the presence of various active substances. The concentration of all additives was 100 μM, except BSA (10 μM, 0.66 mg/mL) and myoglobin (44 μM, 0.66 mg/mL). λex = 480 nm.

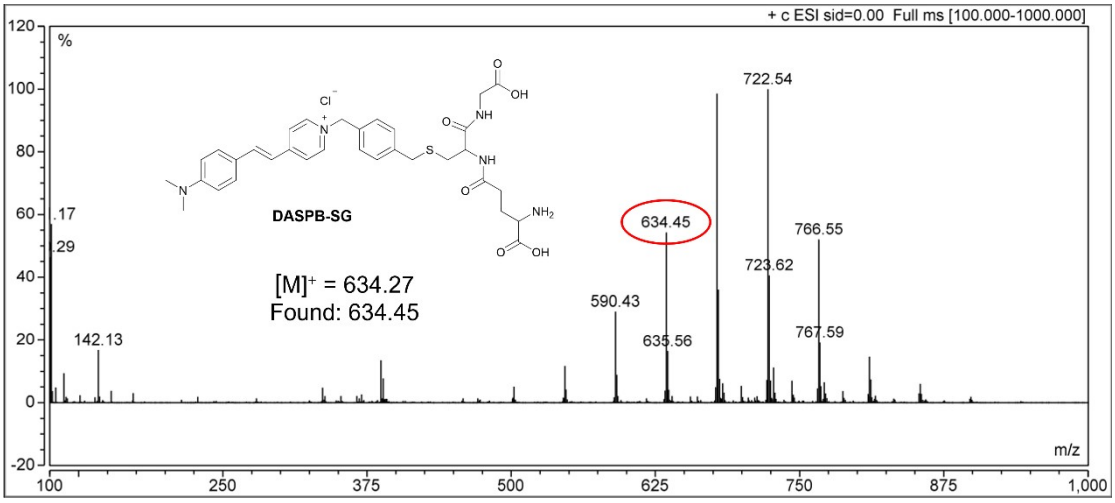

Fig S10. ESI-MS of the reaction solution of **DASPBCl** and GSH.

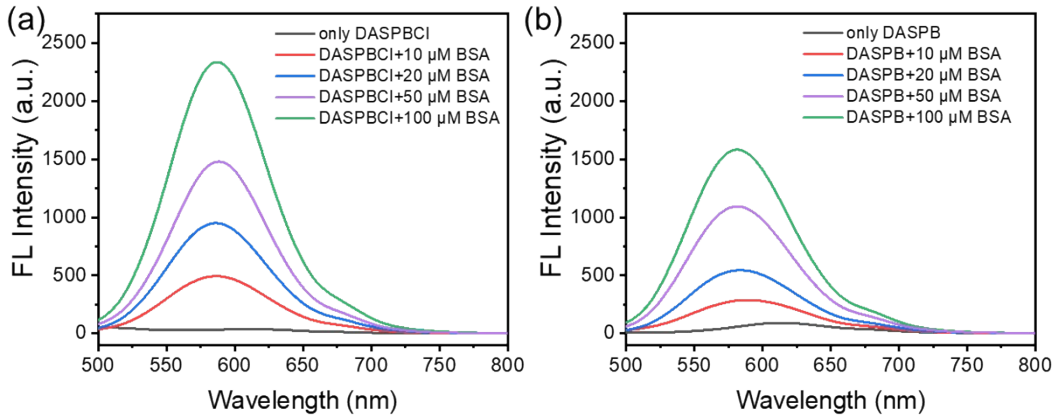

Fig. S11. The fluorescence spectra of (a) **DASPBCl** (10 μM) and (b) **DASPB** (10 μM) upon the addition of different concentrations of BSA.

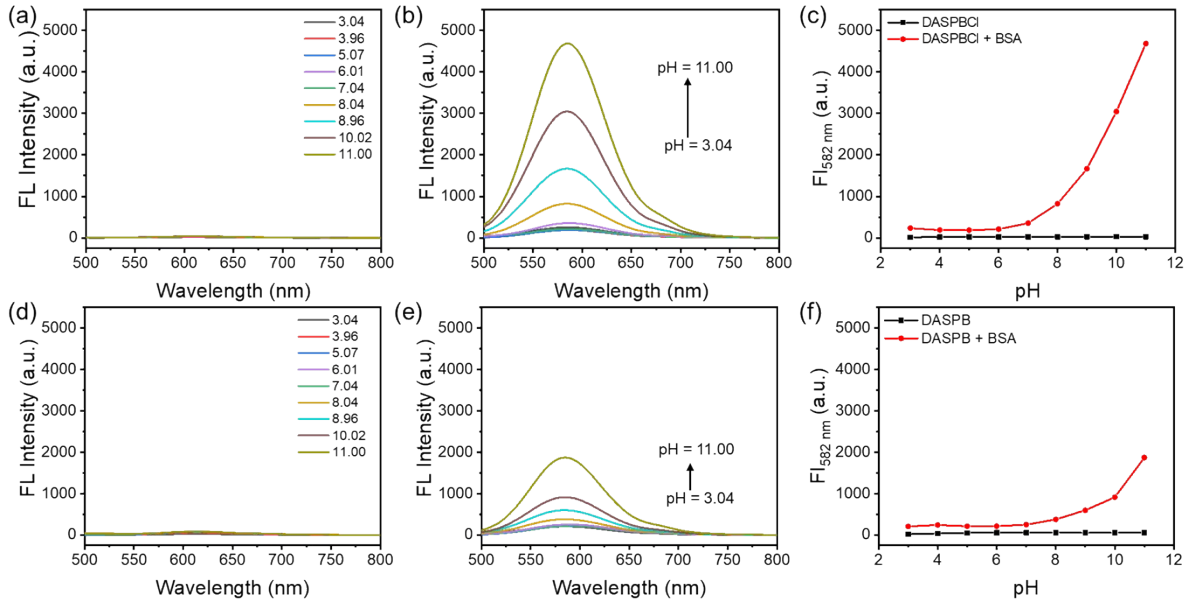

Fig S12. (a) The fluorescence intensity of **DASPBCl** in PBS buffer in different pH from 3 to 11. (b) The fluorescence intensity of **DASPBCl** with BSA in PBS buffer with different pH. (c) The fluorescence intensity of **DASPBCl** at 582 nm. (d) The fluorescence intensity of **DASPB** in PBS buffer in different pH from 3 to 11. (e) The fluorescence intensity of **DASPB** with BSA in PBS buffer with different pH. (f) The fluorescence intensity of **DASPB** at 582 nm. The probe concentration is 10 μM, and the BSA concentration is 10 μM. λex = 480 nm.

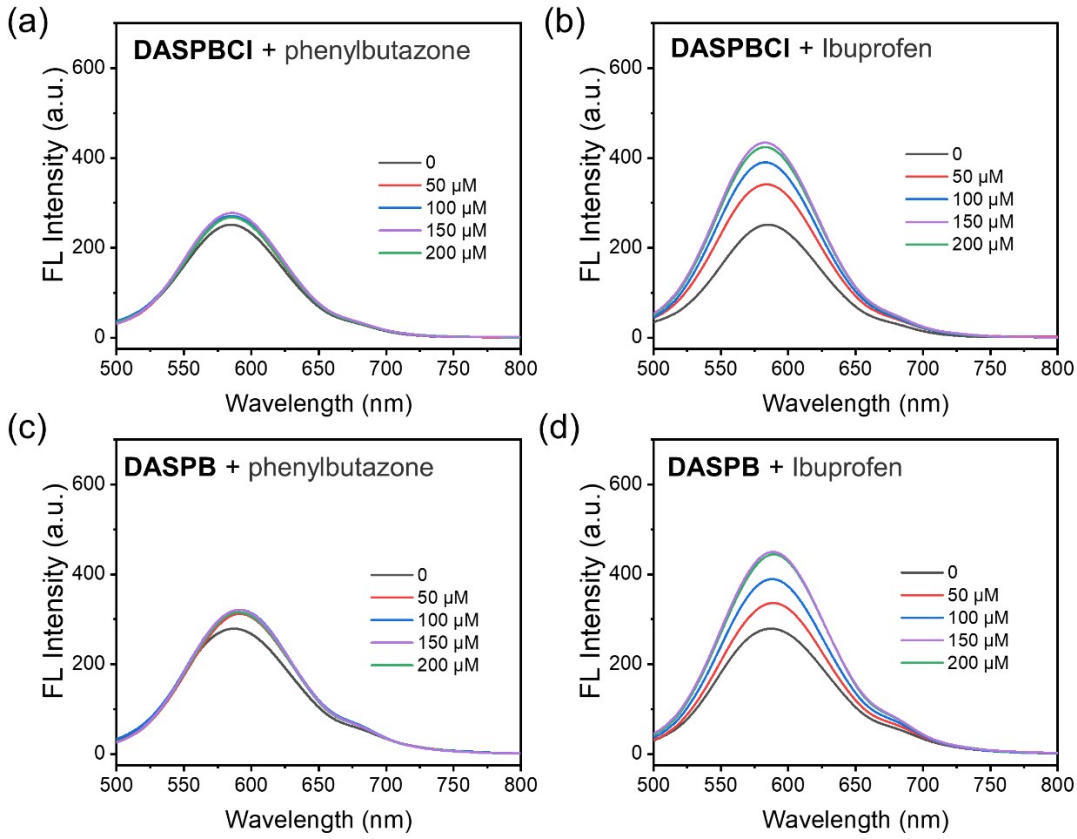

Fig. S13. The fluorescence spectra of **DASPBCl**-BSA (10 μM) complex with the addition of 0-200 μM phenylbutazone (a) and ibuprofen (b); The fluorescence spectra of **DASPB**-BSA (10 μM) complex with the addition of 0-200 μM phenylbutazone (c) and ibuprofen (d), respectively.

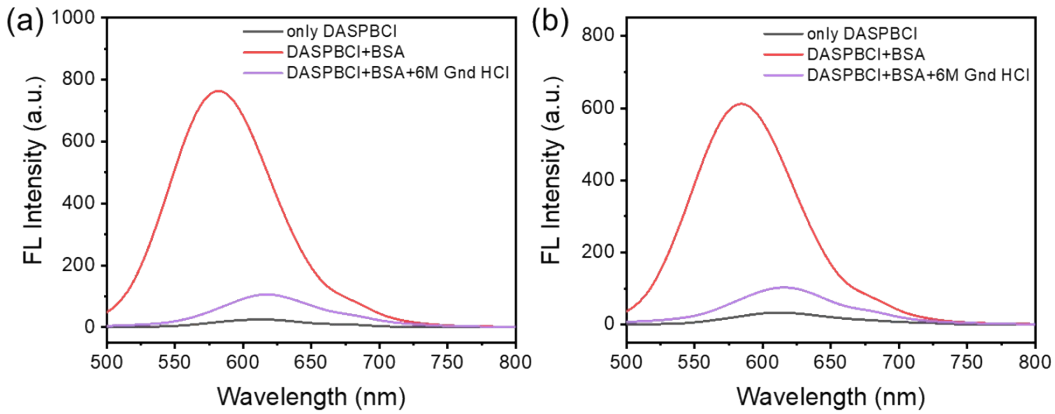

Fig S14. (a) Fluorescence spectra of **DASPBCl** with BSA (10 μM) and BSA pretreated with 6 M GndHCl in PBS for 30 min. (b) Fluorescence spectra of **DASPBCl** with BSA (10 μM) and followed by the addition of 6 M GndHCl for 30 min. λex = 480 nm.

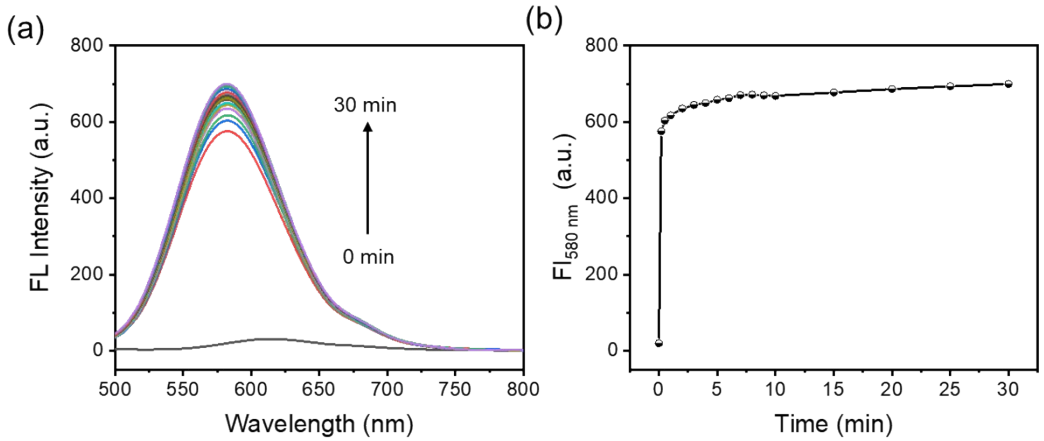

Fig S15. (a) Time-dependent fluorescence spectra of **DASPBCl** (10 μM) in PBS with BSA (10 μM). λex = 480 nm. (b) The fluorescence intensity (580 nm) of **DASPBCl** (10 μM) at different time.

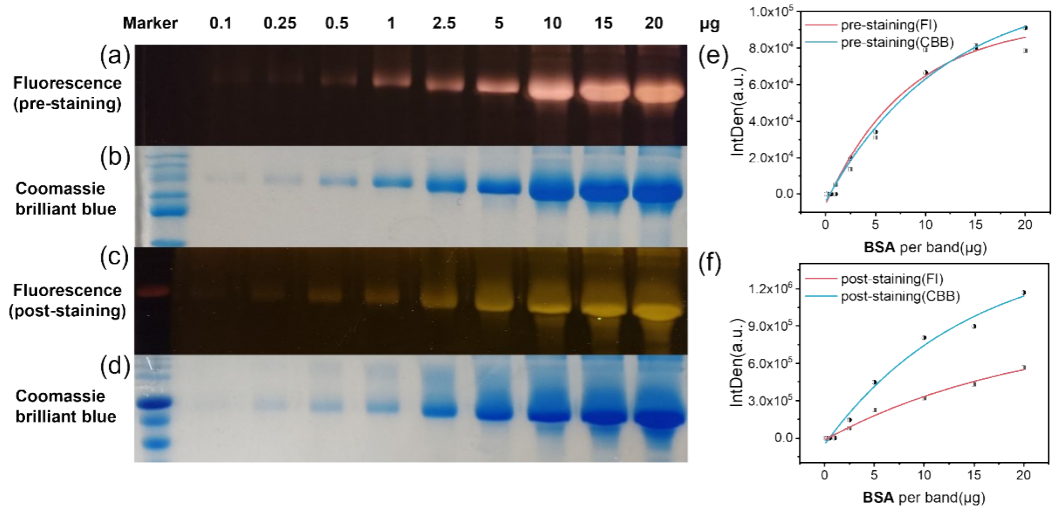

Fig S16. (a) SDS-PAGE fluorescence image of **DASPBCl** pre-stained BSA with various loaded amounts (0-20 μg) of protein and (b) image of the same gel stained with CBB. (c) Plots of fluorescence and CBB versus amount of BSA in the SDS-PAGE assay. (d) SDS-PAGE fluorescence image of **DASPBCl** poststained BSA with various loaded amounts and (e) image of the same gel stained with CBB. (f) Plots of fluorescence and CBB versus amount of BSA in the SDS-PAGE assay.

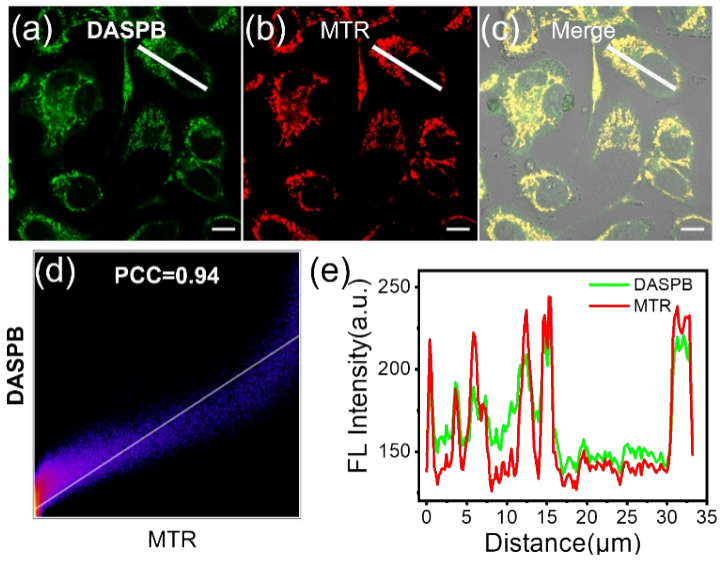

Fig S17. Fluorescence images of HeLa cells co-incubated with (a) **DASPB** (10 μM, 1 h) and (b) MTR (0.2 μM, 1 h). (c) Merged images. (d) Pearson's colocalization coefficient of **DASPB** with MTR (PCC =0.94). (e) Fluorescence intensity distribution of a linear ROI across the cells. **DASPB**: λex = 488 nm, λem = 500−590 nm; MTR: λex = 561 nm, λem = 591−700 nm. Scale bar: 10 μm.

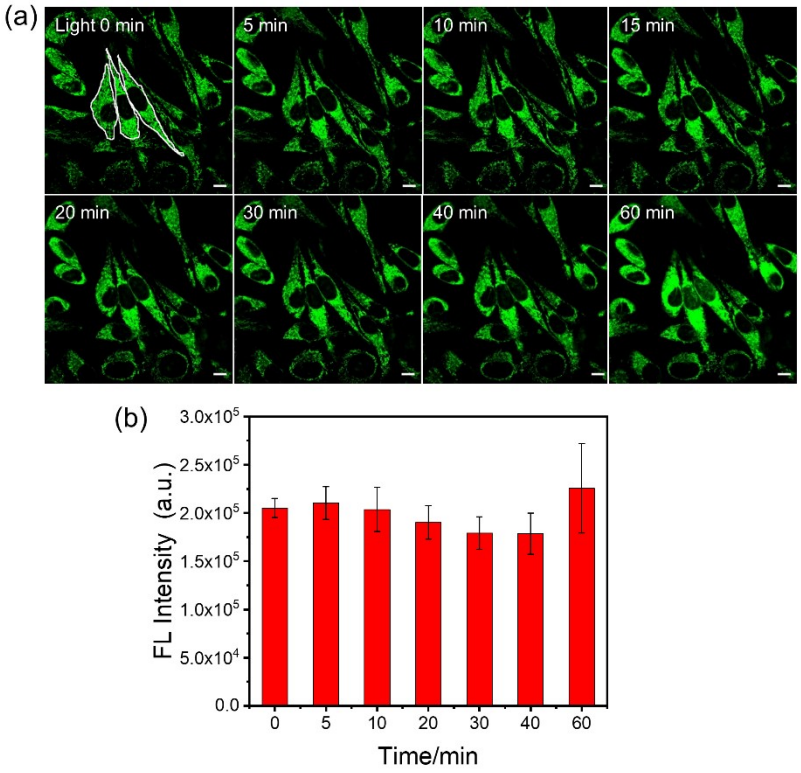

Fig. S18. (a) Fluorescence image of HeLa cells incubated with **DASPBCl** for 30 min under 488 laser irradiations for 60 min. (b) The calculated fluorescence intensity in (a). λex = 488 nm, λem = 500-700 nm. Scale bar: 10 μm.

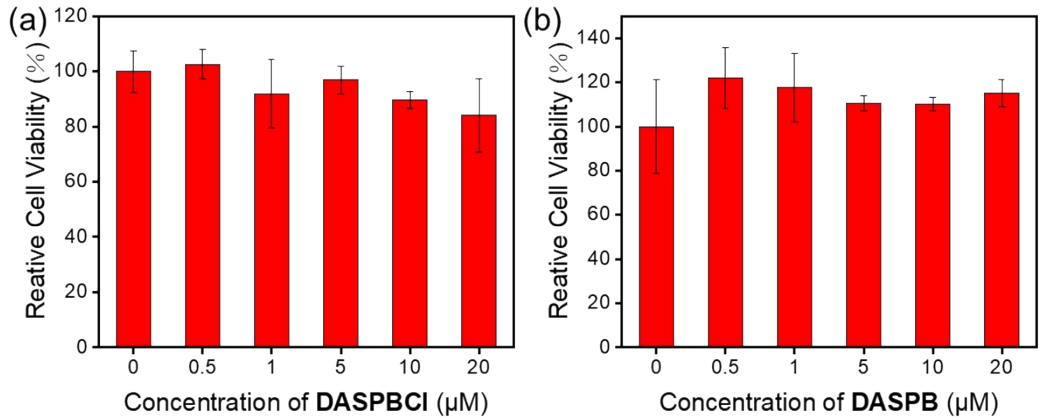

Fig S19. General cytotoxicity of probe **DASPBCl** (a) and **DASPB** (b) in living HeLa cells calculated by MTT assay. HeLa cells were incubated with probes (0-20 μM) for 24 h. Data are showed as the mean ± SD.