Electronic Supplementary Material (ESI) for Analyst

A ratiometric fluorescent probe for hydrophobic proteins in aqueous solution based on aggregation-induced emission

Lu Peng, Ruirui Wei, Kai Li, Zhaojuan Zhou, Panshu Song and Aijun Tong*

Department of Chemistry, Tsinghua University, Beijing 100084, PR China. Fax: +86-10-62782485; Tel: +86-10-62787682; E-mail: tongaj@mail.tsinghua.edu.cn.

Table of content Page

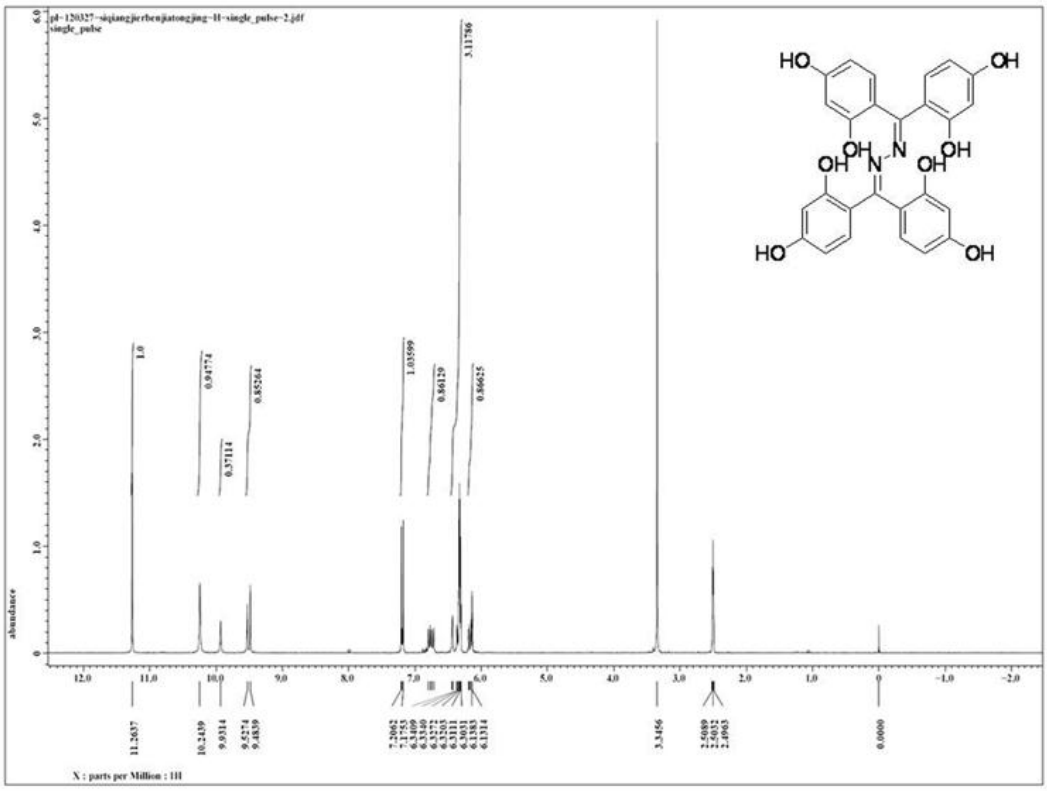


Figure. S1. ^{1}H NMR spectrum of **1.**

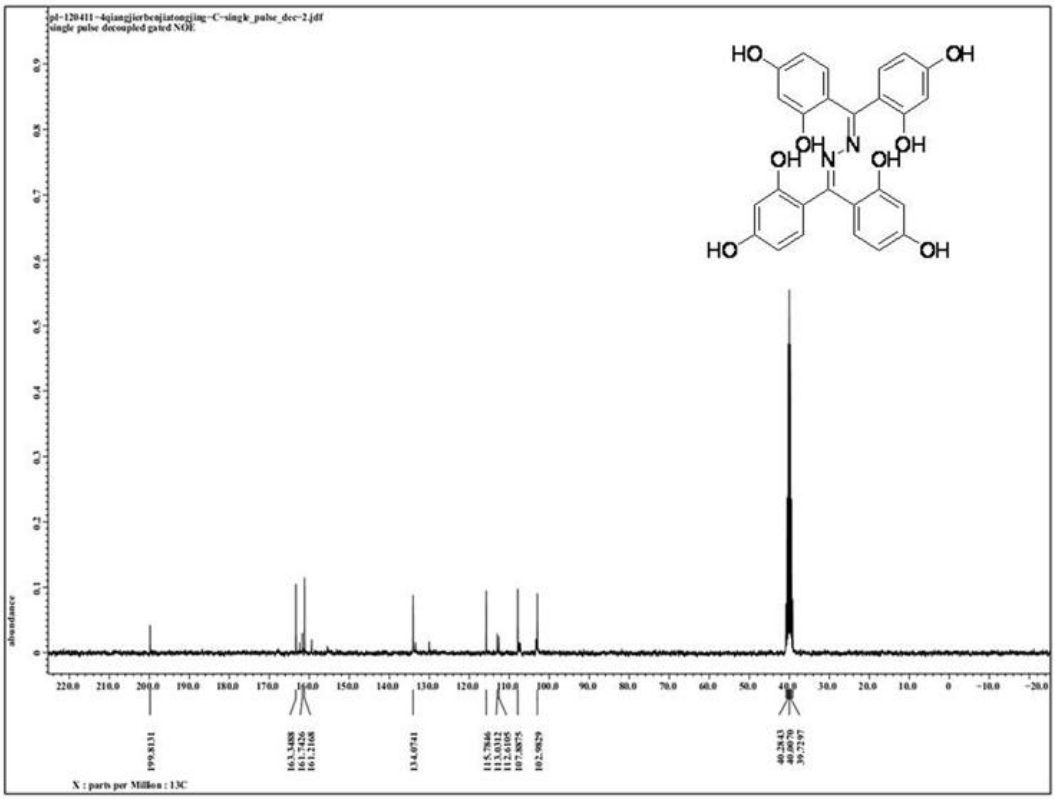
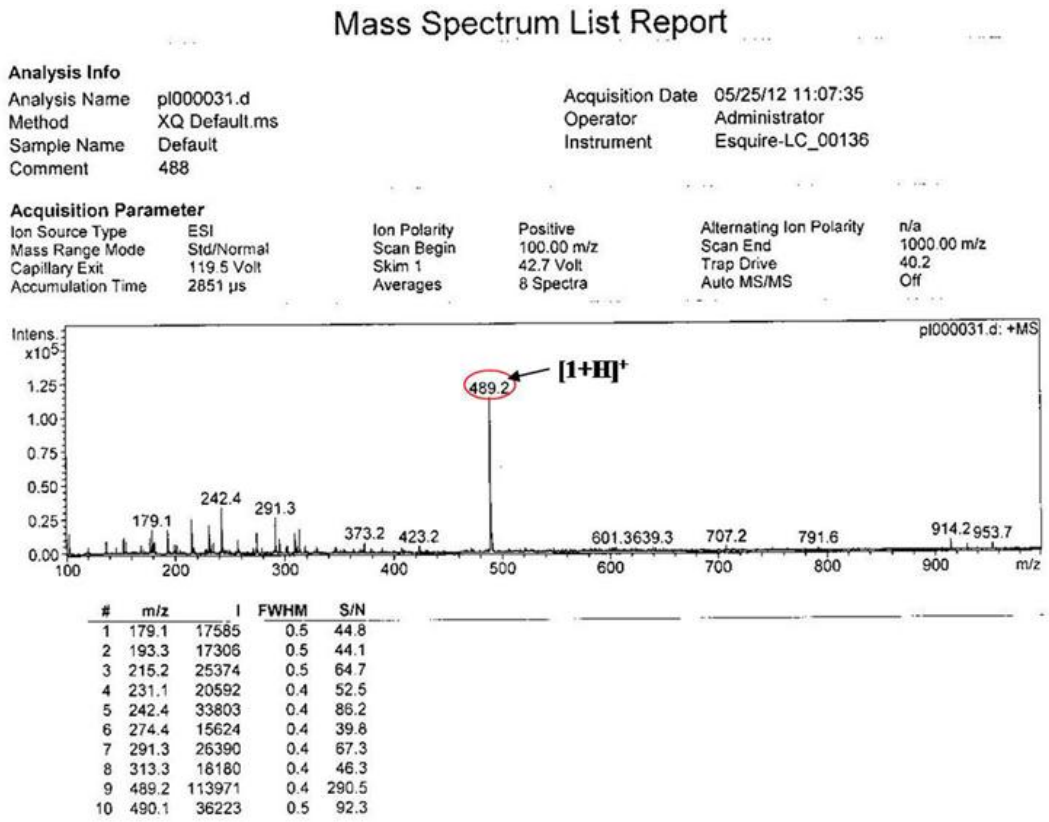


Figure. S2. ^{13}C NMR spectrum of **1.**



#	m/z	I	FWHM	S/N
1	179.1	17585	0.5	44.8
2	193.3	17306	0.5	44.1
3	215.2	25374	0.5	64.7
4	231.1	20592	0.4	52.5
5	242.4	33803	0.4	86.2
6	274.4	15624	0.4	39.8
7	291.3	26390	0.4	67.3
8	313.3	18180	0.4	46.3
9	489.2	113971	0.4	290.5
10	490.1	36223	0.5	92.3

Figure. S3. ESI mass spectrum of **1.**

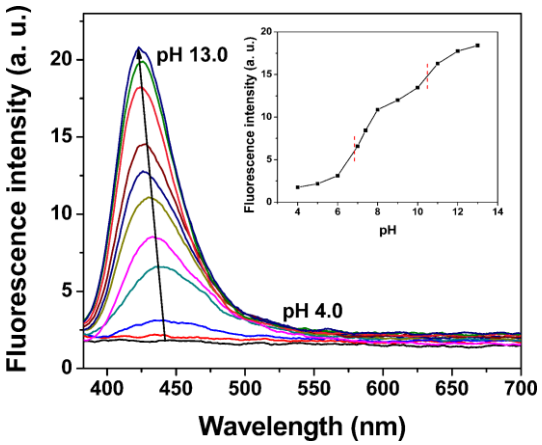


Figure. S4. Fluorescence spectra of **2** (10 μM) in 10 mM PBS at pH range from 4.0-13.0. Inset: effect of pH on the fluorescence intensity at 436 nm. Excitation is performed at 363nm.

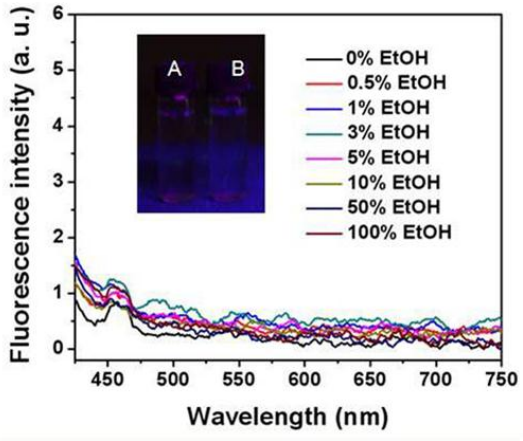
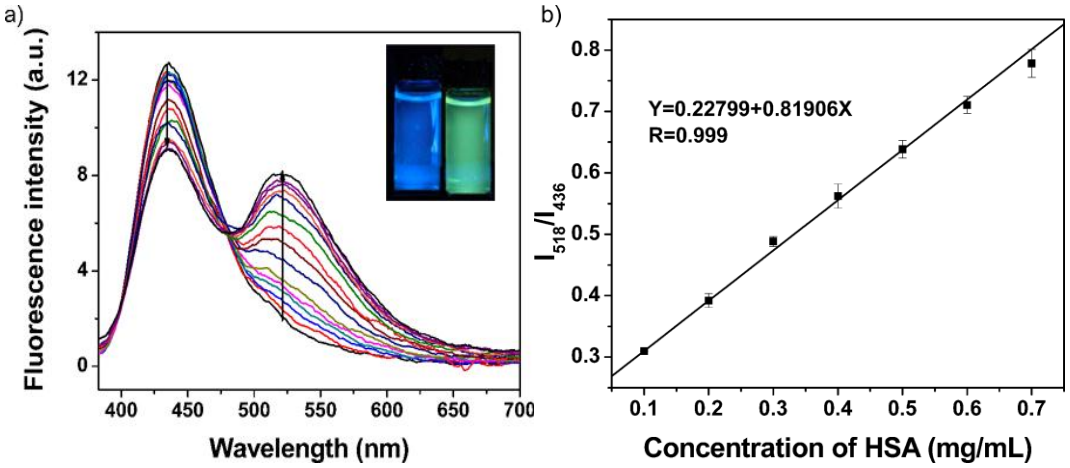


Figure. S5. Fluorescence spectra of **2** (10 μM) in different volume fractions of Ethanol-hexane solution. Excitation is performed at 405 nm. Insets A and B: photographs of **2** (10 μM) in hexane and ethanol under a UV lamp (365 nm).



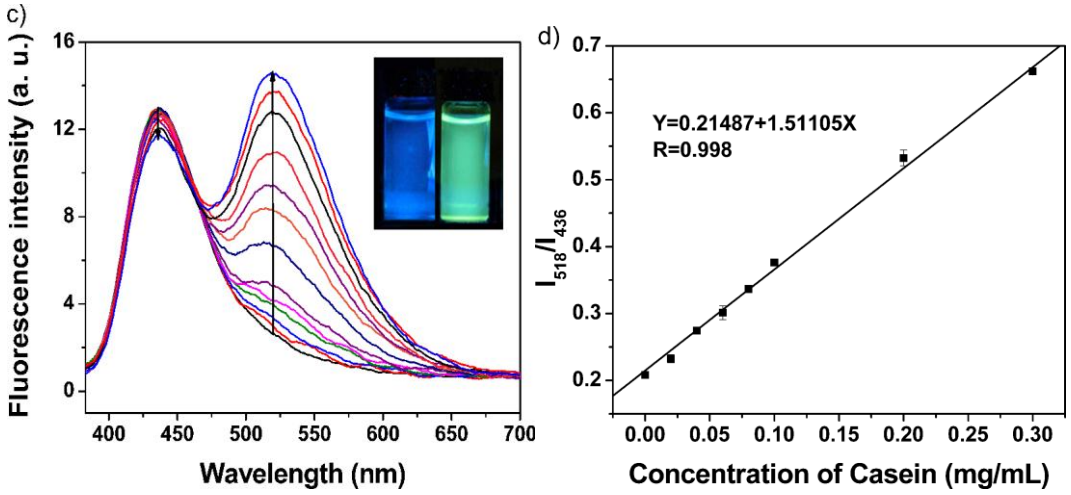


Figure. S6. Fluorescence spectra of **1** (10 μM) upon addition of various concentrations of (a) HSA (0-1.0 mg/mL) and (c) casein (0-1.0 mg/mL) in 10 mM PBS buffer at pH 7.4. Inset: photographs of **1** (30 μM) before and after addition of HSA or casein (1.0 mg/mL) under a UV lamp (365 nm). Ratiometric calibration curve of I_{518}/I_{436} as a function of (b) HSA and (d) casein concentrations. Excitation wavelength was set at 363 nm.

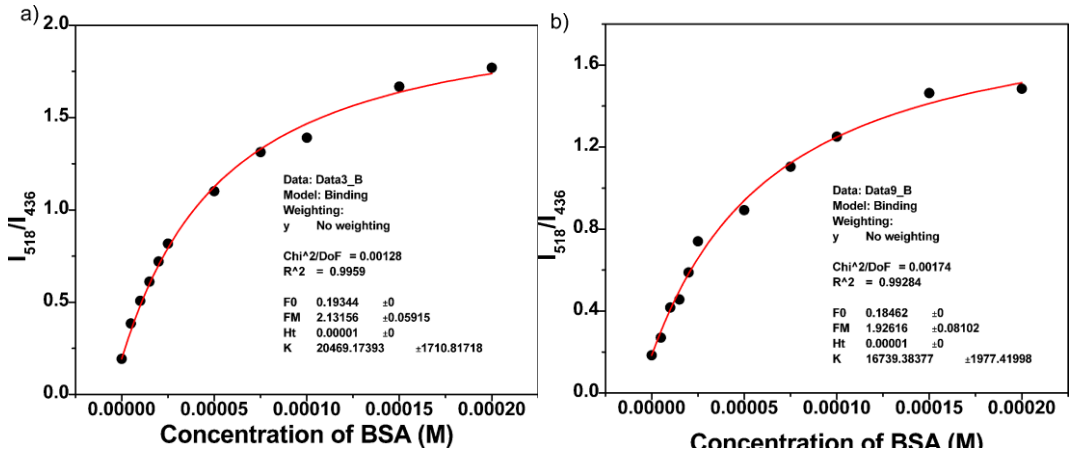


Figure. S7. The binding curve of **1** (10×10^{-6} M) and BSA (0-200 μM) at different temperatures of (a) 20℃ and (b) 40℃ in 10 mM PBS at pH 7.4. Excitation is performed at 363 nm.

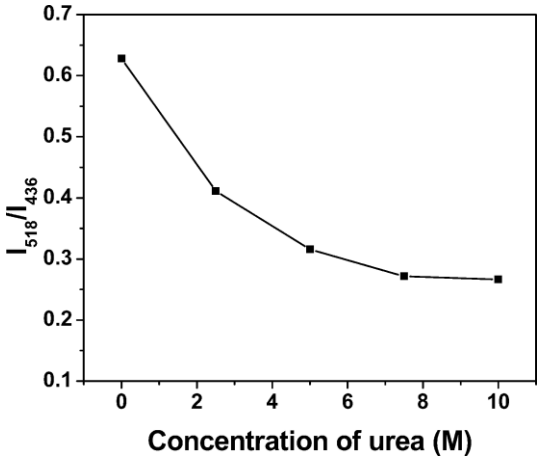


Figure. S8. The effects of different concentrations of urea (0-10 M) on the ratio ($I_{518}/_{436}$) of **1** (10 μM) and BSA (1.0 mg/mL) in 10 mM PBS at pH 7.4. Excitation is performed at 363 nm.

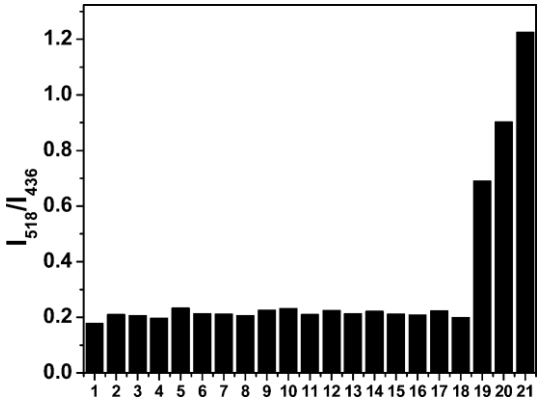


Figure. S9. Fluorescence intensity ratio (I_{518}/I_{436}) of **1** (10 μM) upon addition of 50 μM different metal ions in 10 mM PBS buffer at pH 7.4. 1: blank, 2: Ag^{+}, 3: Al^{3+}, 4: Ba^{2+}, 5: Ca^{2+}, 6: Cd^{2+}, 7: Co^{2+}, 8: Cu^{2+}, 9: Fe^{3+}, 10: Fe^{2+}, 11: Hg^{2+}, 12: K^{+}, 13: Li^{+}, 14: Mg^{2+}, 15: Mn^{2+}, 16: Ni^{2+}, 17: Pb^{2+}, 18: Zn^{2+}, 19: BSA (1.0 mg/mL), 20: HSA (1.0 mg/mL), 21: casein (1.0 mg/mL).

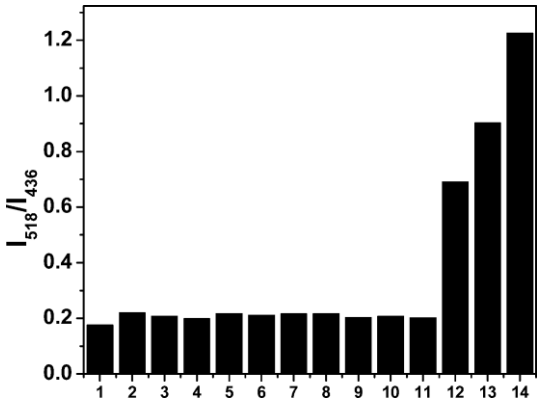


Figure. S10. Fluorescence intensity ratio (I_{518}/I_{436}) of **1** (10 μM) upon addition of 50 μM different anions in 10 mM PBS buffer at pH 7.4. 1: blank, 2: AcO^{-}; 3: Br^{-}, 4: Cl^{-}, 5: ClO_3^{-}, 6: ClO_4^{-}, 7: CNS^{-}, 8: CO_3^{2-}, 9: F^{-}, 10: H_2O_2, 11: I^{-}, 12: BSA (1.0 mg/mL), 13:

HSA (1.0 mg/mL), 14: casein (1.0 mg/mL).

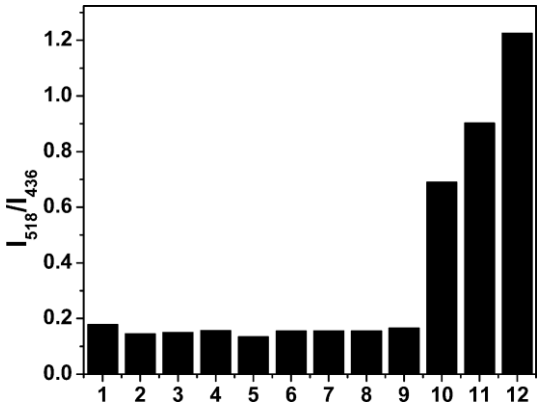
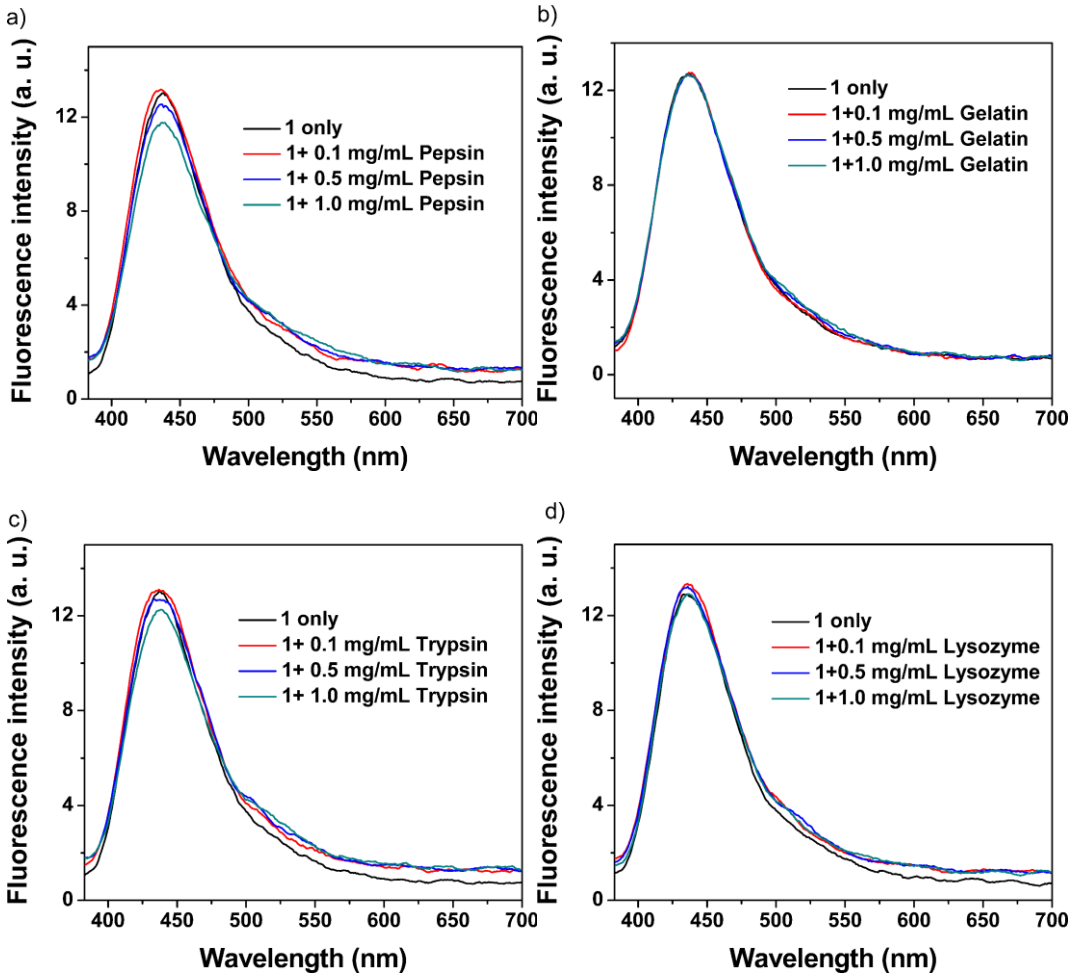


Figure. S11. Fluorescence intensity ratio (I_{518}/I_{436}) of **1** (10 μM) upon addition of 50 μM different amino acids in 10 mM PBS buffer at pH 7.4. 1: blank, 2: Asp; 3: Cys, 4: Glu, 5: His, 6: Lys, 7: Phe, 8: Pro, 9: Thr, 10: BSA (1.0 mg/mL), 11: HSA (1.0 mg/mL), 12: casein (1.0 mg/mL).



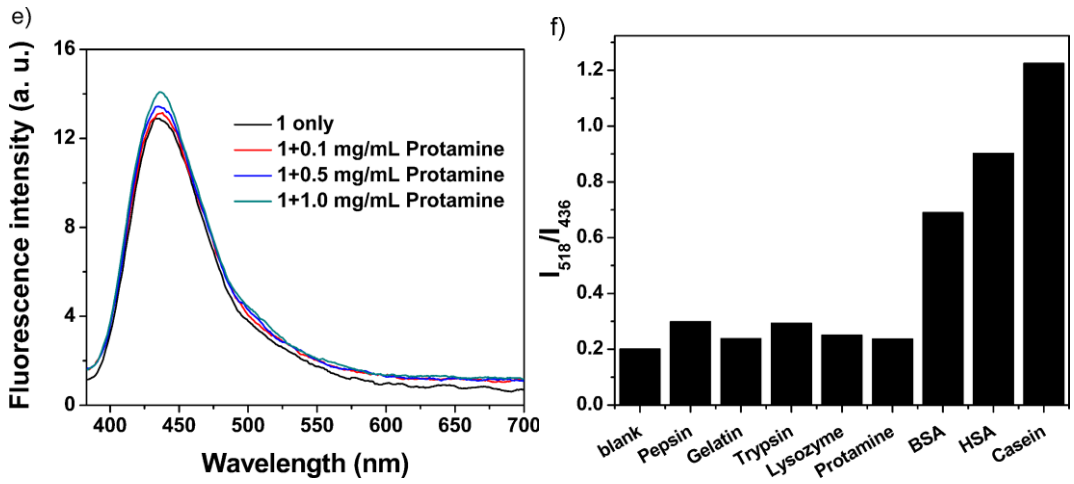


Figure. S12. Fluorescence spectra of **1** (10 μM) upon addition of 1.0 mg/mL proteins (a) pepsin, (b) gelatin, (c) trypsin, (d) lysozyme and (e) protamine in 10 mM PBS buffer at pH 7.4. (f): the ratio (I_{518}/I_{436}) of **1** (10 μM) upon addition of 1.0 mg/mL proteins.

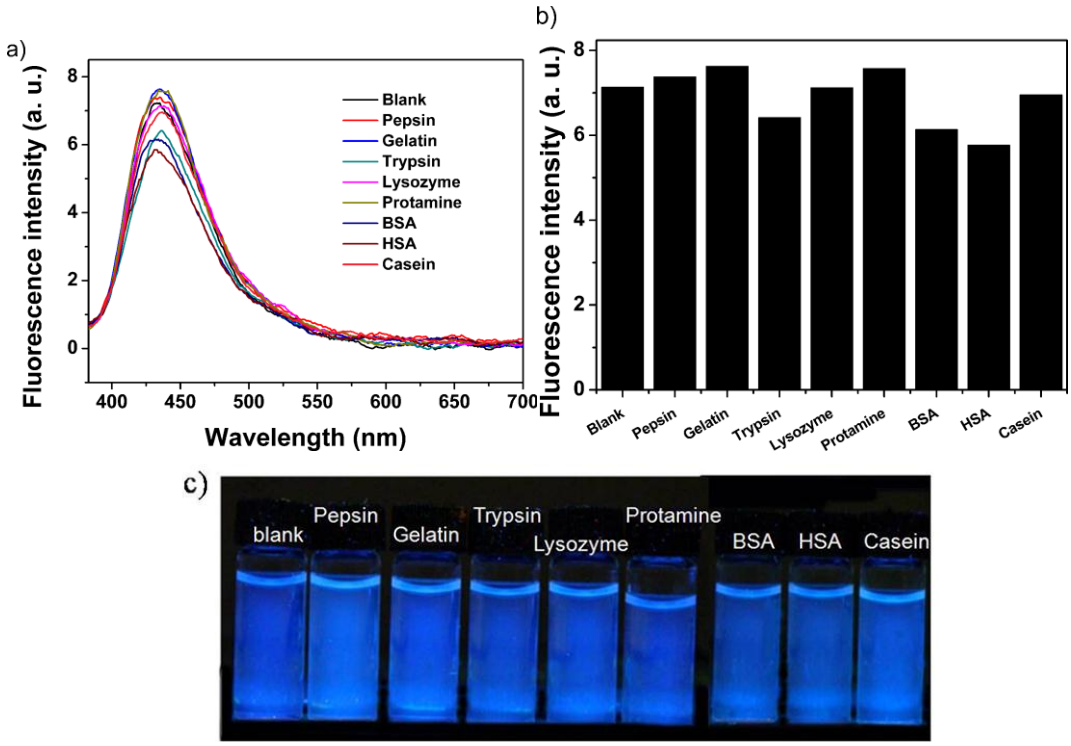


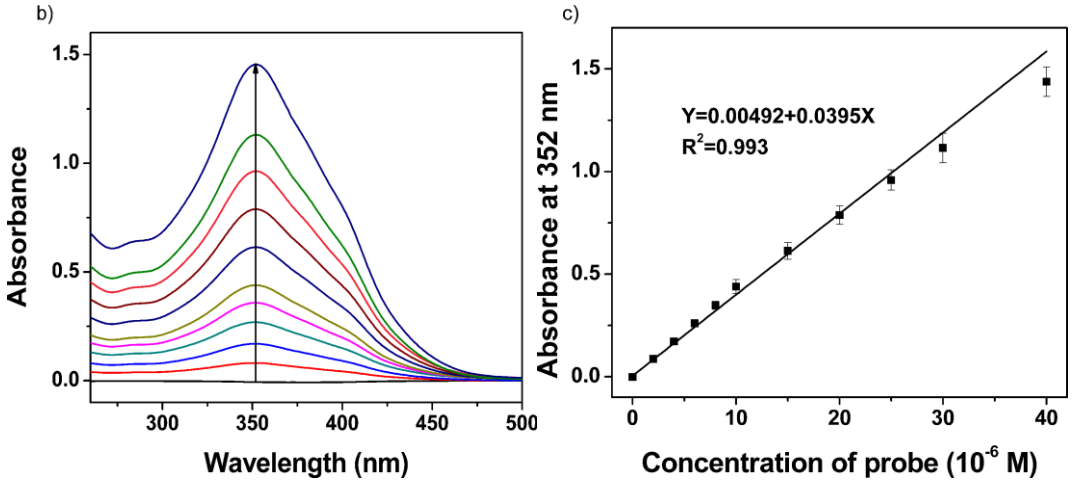
Figure. S13. (a) Fluorescence spectra and (b) fluorescence intensity of **2** (10 μM) at 436 nm upon addition of various kinds of proteins (1.0 mg/mL) in 10 mM PBS buffer at 7.4. Excitation wavelength was set at 363 nm. (c) Photographs of **2** (10 μM) upon addition of various kinds of proteins (1.0 mg/mL) in 10 mM PBS buffer at pH 7.4 under a UV lamp (365 nm).

Calculation of the amount of the probe bound to BSA

First, 400 μL solutions (10 mM PBS, pH 7.4) of 10 μM BSA (4.0 nmol) and different amount of probe (20.0 nmol and 40.0 nmol) were prepared. Then, the solution was filtered by using Amicon ultra 10K centrifugal filter device to separate the probe-BSA complex and the rest probe unbound to BSA. After centrifugation (10000 r/min, 10 min) for 3 times, the filtrate was obtained and diluted to 1.0 mL. As shown in Figure S14a, the filtrate emitted blue fluorescence which indicated the excessive probe unbound to BSA was separated into the filtrate. The absorption spectra were recorded to quantify the probe unbound to BSA according to the calibration curve of probe **1** (Figure S14c). The amount of the probe bound to BSA was calculated through the following equation,

$$n_{bound}=n_{feeding}-n_{unbound}= n_{feeding}-1.0\ mL\times(A_{352}-0.00492)/0.0395$$

where $n_{feeding}$ is the total amount of probe **1** before separation; A_{352} is the absorbance of the filtrate (diluted to 1.0 mL) at 352 nm; 0.00492 and 0.0395 are the intercept and slop of the calibration curve (Figure S14b), respectively. .



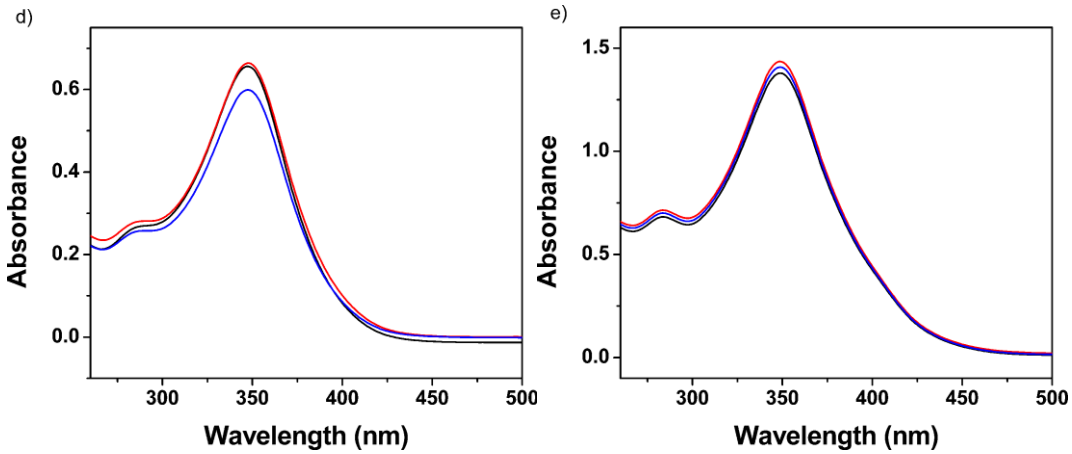


Figure. S14. (a) Photographs of probe **1** (50 μM, 20.0 nmol) and BSA (10 μM, 4.0 nmol) in 10 mM PBS buffer at 7.4 under a UV lamp (365 nm). (b) Absorption spectra of various concentration of **1** (0-40.0 μM) in 10 mM PBS buffer at 7.4 and (c) the corresponding calibration curve of absorbance at 352 nm. Absorption spectra of (d) the filtrate of 4.0 nmol BSA and 20.0 nmol probe **1**, (e) the filtrate of 4.0 nmol BSA and 40.0 nmol probe **1**. (both d and e were repeated three times)

The amount of the probe bound to BSA and stoichiometry were listed in the following table, confirming the 1:1 binding mode between probe **1** and BSA.

Table S1 The amount of probe **1** bound to BSA and stoichiometry

BSA feeding amount (nmol)	**1** feeding amount (nmol)	**1** binding amount (nmol)	Stoichiometry (**1**:BSA)	R.S.D. (%)
4.0	20.0	4.27	1.06:1	3.5
	40.0	4.86	1.21: 1	2.7

Fluorescence imaging of BSA bands in PAGE gels with probe 1

First, 400 μL solutions (10 mM PBS, pH 7.4) of 10 μM probe **1** and different concentration of BSA (1.0, 5.0, 10.0 and 20.0 mg/mL) were prepared. Then, the solution was filtered by using Amicon ultra 10K centrifugal filter device to separate the probe-BSA complex and the rest probe unbound to BSA. After centrifugation (10000 r/min, 10 min) for 3 times, the upper liquid was collected as protein samples for carrying out native-PAGE electrophoresis. The loading volume for each well was 9.0 μL. The voltage as the samples migrated through the stacking gels was 150 V. When the samples entered the resolving gels, the voltage was turned to 300 V and kept constant for 3 h.

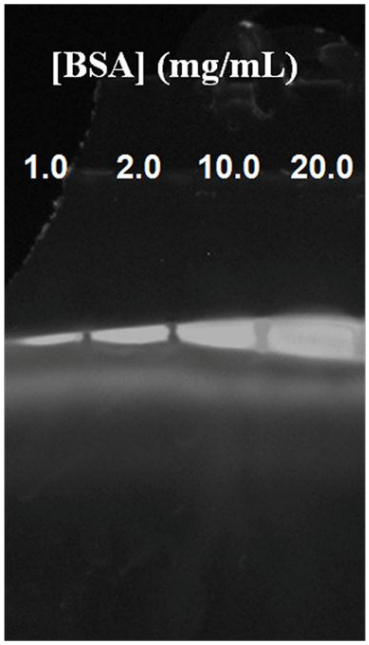


Figure. S15. Fluorescence imaging of BSA bands in PAGE gels with probe **1** (10 μM). The concentrations of BSA were 1.0, 5.0, 10.0 and 20.0 mg/mL (from left to right).

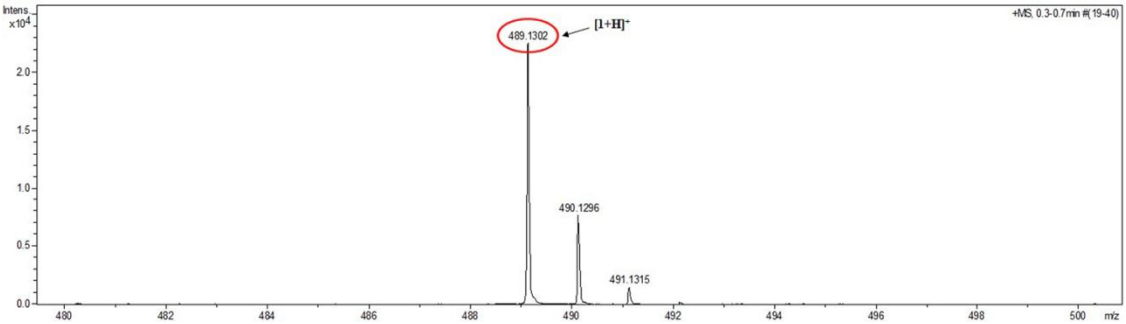


Figure. S16. High resolution mass spectrum of **1.**