A Protein-supported Fluorescent Reagent for the Highly-sensitive Detection of Mercury Ions in Aqueous Solution and Live Cells

(Supporting Information)

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Experimental

1) Synthesis and Characterization of sodium dansyl-L-aspartic acid (DLD, 1)

In ice bath, the solution of dansyl chloride (0.20 g; 0.74 mmol) in anhydrous acetone (10.0 ml) was added slowly to that of L-aspartic acid methyl ester (0.11g; 0.68 mmol) and 0.2 ml tri-ethylamine in anhydrous acetone (20.0 ml) and the mixture was stirred for 0.5h. Then the reaction was continued for 12 h at the room temperature, and the process was monitored by TLC (30:1, chloroform to methanol; R_f = 0.5). After the solvent was removed under reduced pressure, the solid was dissolved in chloroform firstly and then was washed with the saturated Na_2CO_3 to alkalescence (pH~7. 5), with hydrochloric acid to weak acidity (pH~6.2), and finally with water to neutral. The organic solution was filtered after drying over anhydrous $MgSO_4$. After evaporation of solvent, purification of **DLD**-ester was performed by using column chromatography on silica gel (elution with chloroform) which resulted in a 48.0 % yield. Mild hydrolysis of the **DLD**-ester was performed in 1 M NaOH solution (room temperature, 12 h), followed by the column chromatography on silica gel (elution with 5:1, chloroform to methanol; R_f = 0.5) and resulted in a 40.0 % yield of dansyl-L-aspartic acid. Sodium dansyl-L-aspartic acid (**DLD, 1**) was available by adjusting pH value of dansyl-L-aspartic acid solution to basic. ^{1}H NMR (500 MHz, D_2O): δ = 8.435-7.365 (m, 6 H, dansyl (**H**)), δ = 3.865 (m, 1 H, -C**H),** δ = 2.840 (s, 6 H, -N(CH_3)), δ =2.351-2.229 (m, 2H, -CH_2COOH). Mass spectral data (MALDI-TOF-MS): for $C_{16}H_{16}N_2SO_6^{2-}$ calcd, 364.0; found 365.1.

2) Detection of Hg^{2+} in live cells

2.1 B16-F10 Cells Culture. B16-F10 cells were cultured according to the reported protocol (S. S. Mitchell, B. Nicholson, S. Teisan, K. S. Lam and B. C. M. Potts *J. Nat.*

Prod. 2004, **67**, 1400-1402.): cells were cultured in plastic petri dishes containing DMEM medium supplemented with 10% (v/v) fetal bovine serum (FBS), 1% (v/v) penicillin/streptomycin, 2.0 mM L-glutamine and 10.0 mM HEPES. All cells were maintained in their respective media at 37 °C, 5% CO_2, and 95% humidified air. Cells were plated 24 h before study into 6 well plates for two groups and at the time of study, the medium was changed with a fresh 2% serum one.

2.2 Fluorescence Imaging Experiments. 30.0 μM **1** (from a 500 μM stock solution) was added to each well except control of cultured B16-F10 cells both in group I and II. The cells were incubated for 30 min at 37 °C, at which point the medium was removed and the cells were washed twice by PBS (pH 7.4). After that, fluorescence microphotographs were taken for group I.

After cells were treated with 30.0 μM **1** for 30 min and then washed twice with PBS, fresh medium with 100.0 μM $HgCl_2$ was added to group II. After further 30 min exposure, cells in group II were washed several times with PBS until neutral conditions (pH is about 7.0) and fluorescence microphotographs were recorded for them.

The experiments were performed with an Olympus Bx51 microscope and an excitation wavelength of 350 nm was selected. All photographs were taken using a 20× objective lens.

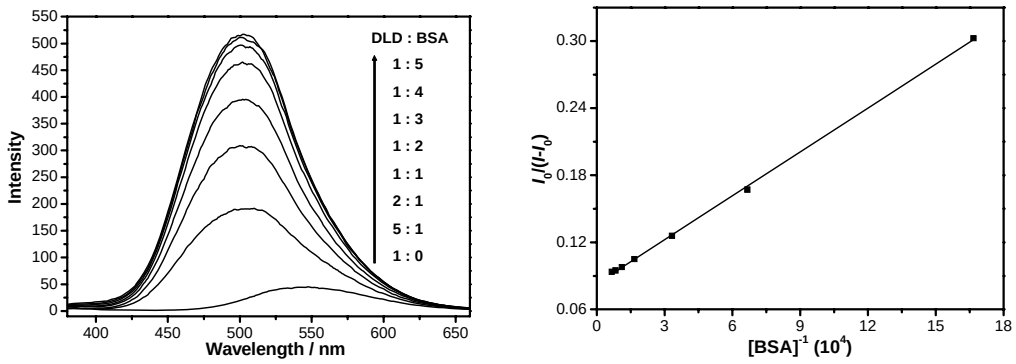


Fig. S1 Fluorescence emission spectra of **1** (30.0 μM) in the presence of BSA with various equiv. in 50.0 mM NaAc solution (pH 6.7).

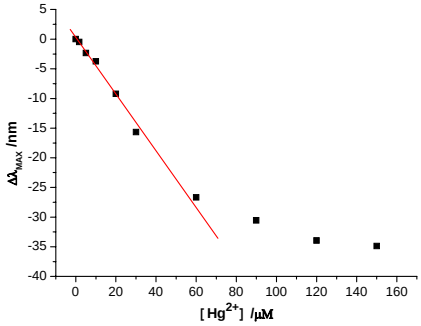


Fig. S2 The band shifts of the maximal emission peak ($\Delta\lambda_{MAX}$) of **1**/BSA (30.0 μM : 15.0 μM) in changing with various equiv. of Hg^{2+} in 50.0 mM NaAc solution (pH 6.7). Linear relationship can be observed between $\Delta\lambda_{MAX}$ and Hg^{2+} concentration within a range of 0.0~60.0 μM, estimating a detection limit of **1**/BSA to Hg^{2+} at 0.5 μM.

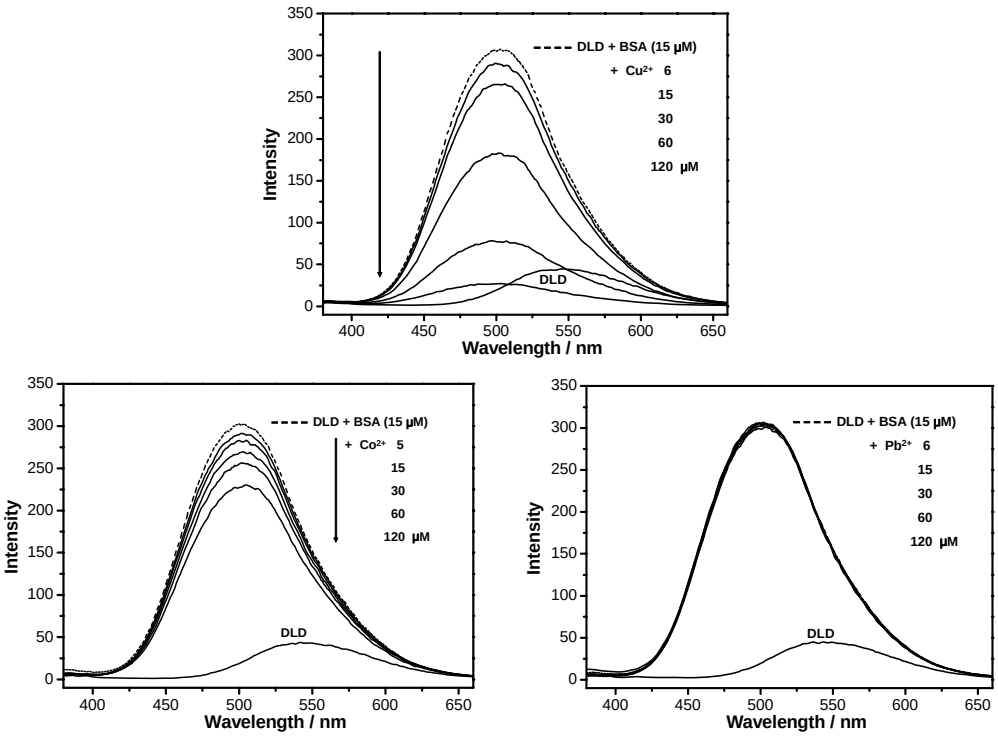


Fig. S3 Fluorescence emission spectra of **1**/BSA (2 : 1) with the addition of Cu^{2+}, Co^{2+} and Pb^{2+} in 50.0 mM NaAc solution (pH 6.7). The results of Na^{+}, Mg^{2+}, K^{+}, Ca^{2+}, Cr^{3+}, Mn^{2+}, Fe^{2+}, Ni^{2+}, Zn^{2+}, Ag^{+}, Cd^{2+} are similar with that of Pb^{2+}.

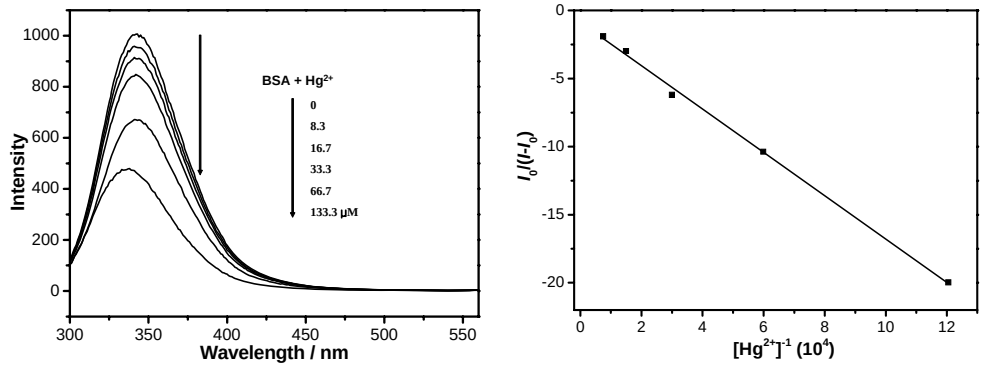


Fig. S4 (left) Fluorescence spectra of BSA (6.6 μM) in the presence of various concentration of Hg^{2+} in 50.0 mM NaAc solution (pH 6.7), λ_{ex} = 280 nm; (right) Estimation of binding constant for BSA with Hg^{2+}, the plot was calculated based on the Stern-volmer equation and a 1 : 1 binding model: $I_0/(I\text{-}I_0)$ *vs* $[Hg^{2+}]^{-1}$.

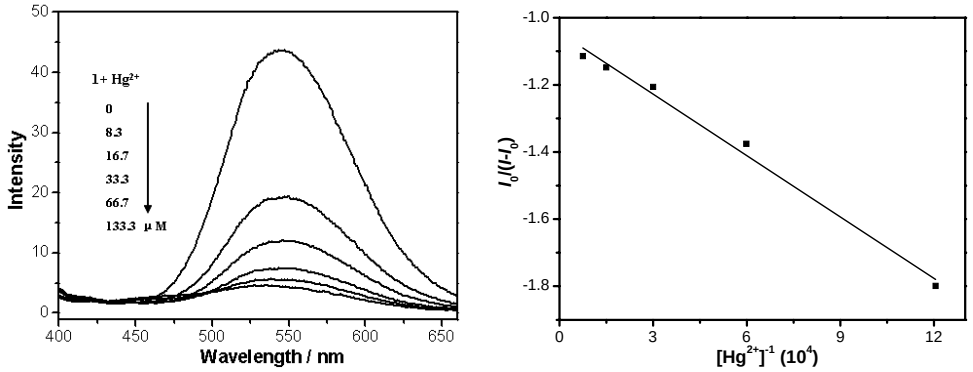


Fig. S5 (left) Fluorescence spectra of **1** (30.0 μM) in the presence of various concentration of Hg^{2+} in 50.0 mM NaAc solution (pH 6.7), λ_{ex} = 340 nm; (right) Estimation of binding constant for **1** with Hg^{2+}, the plot was calculated based on the Stern-volmer equation and a 1 : 1 binding model: $I_0/(I\text{-}I_0)$ *vs* $[Hg^{2+}]^{-1}$.

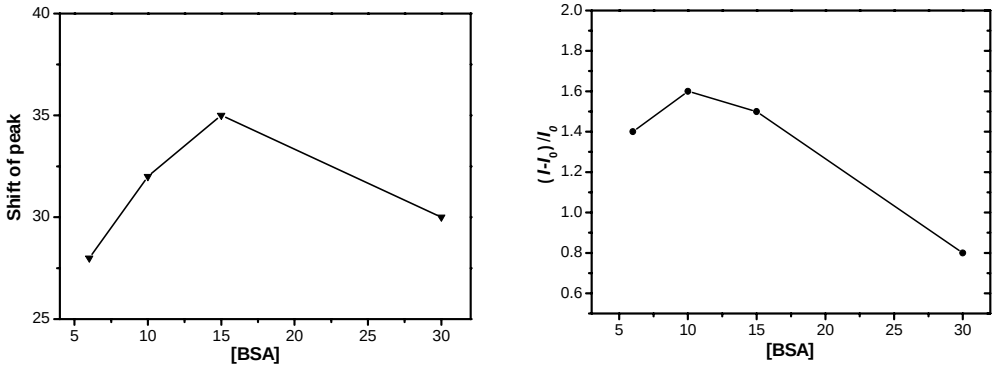


Fig. S6 [BSA]-dependence of the fluorescent response of the complex of **1**/BSA to Hg^{2+} (50.0 μM) on the band-shift ($\Delta\lambda_{MAX}$, left) and relative intensity changes ($(I\text{-}I_0)/I_0$, right) in 50.0 mM NaAc

solution (pH 6.7), respectively.

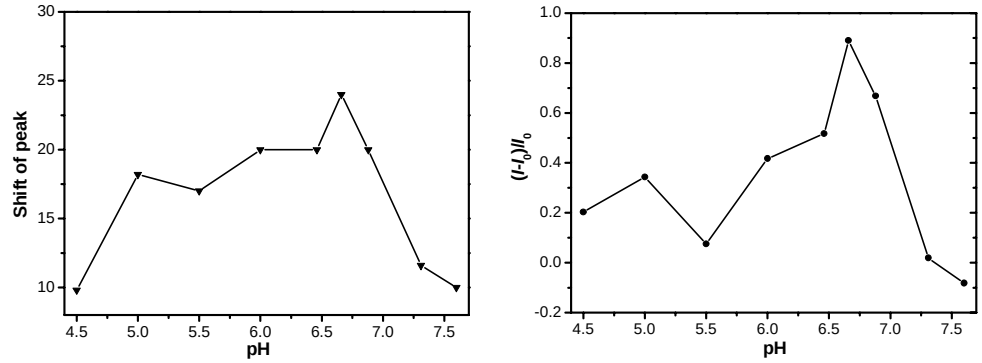


Fig. S7 pH-dependence of the fluorescence response of the **1**/BSA (30.0 μM / 15.0 μM) to Hg^{2+} (60.0 μM) on the band-shift (left) and relative intensity changes (right) in 50.0 mM NaAc solution.

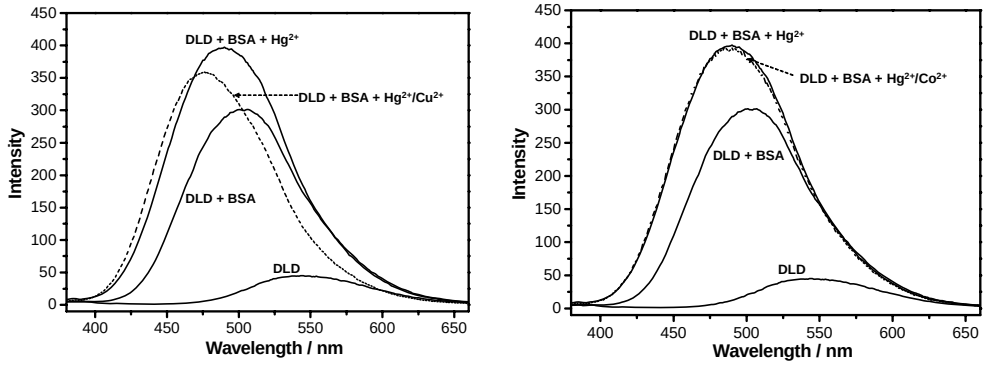


Fig. S8 Fluorescence emission spectra of **1** (30.0 μM), **1**/BSA (2 : 1) and **1**/BSA/M^{n+} (2 : 1 : 2). (M^{n+} represent Hg^{2+}, Hg^{2+}/Cu^{2+} and Hg^{2+}/Co^{2+}, respectively, in 50.0 mM NaAc solution (pH 6.7). The results demonstrate that the co-existence of Cu^{2+} reduces the intensity response but accentuates blue-shift of fluorescent peak, while no obvious interference of other metal ions as Na^{+}, Ca^{2+}, Fe^{2+}, Zn^{2+}, Co^{2+}, Cr^{3+} *etc*. can be observed.

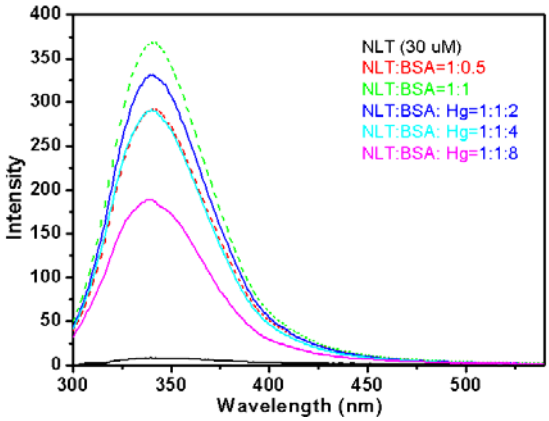


Fig. S9 Fluorescence emission spectra of naphthalene-L-tryptophan (NLT) (30.0 μM), NLT/BSA and NLT/BSA/Hg^{2+} in different concentrational ratio, respectively, in 50.0 mM NaAc solution (pH 6.7).

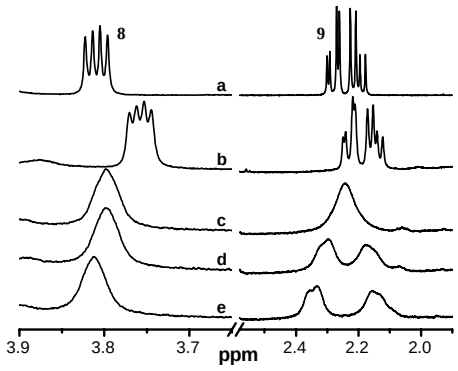
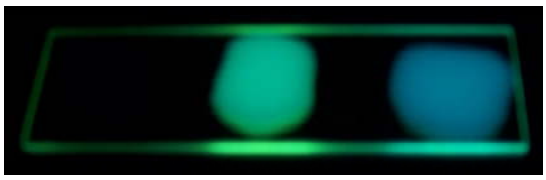


Fig. S10 Region of the ^1H NMR spectra of (a) **1** (10.0 mM), (b) **1**/BSA (1 : 0.01), (c) **1**/BSA/Hg^{2+} (1 : 0.01 : 0.5), (d) **1**/BSA/Hg^{2+} (1 : 0.01 : 1) and (e) **1**/BSA/Hg^{2+} (1 : 0.01 : 1.5) in D_2O.



(a) **(b)** **(c)**

Fig. S11 Fluorescence photograph of (a) **1**, (b) **1** and fetal bovine serum (FBS) in a molar ratio of 2 : 1 and (c) **1**/FBS/Hg^{2+} in a molar ration of 2 : 1 : 6 under illumination with 365 nm light.

Table S1. Proton chemical shifts (δ, in ppm) of **1** and its association with Hg^{2+}; atomic numbering is shown in Fig. 1 in text**.**

	H(3)	H(1)	H(7)	H(2)	H(6)	H(5)	H(4)	H(8)	H(9)
1	8.376 d	8.212 d	8.186 d	7.589 t	7.572 t	7.323 d	2.791 s	3.809 f	2.281d, 2.200 d
1/BSA (100:1)	8.345 d	8.183 d	8.157 d	7.556 t	7.530 t	7.289 d	2.738 s	3.758 f	2.232 d, 2.146 d
1/BSA/Hg^{2+} (100:1:50)	8.364 d	8.198 d	8.271 d	7.586 t	7.586 t	7.362 d	2.811 s	3.789 s	2.241 s
1/BSA/Hg^{2+} (100:1:100)	8.350 d	8.183 d	8.308 d	7.586 d	7.586 d	7.383 d	2.831 s	3.798 s	2.241 s, 2.177 s
1/BSA/Hg^{2+} (100:1:150)	8.347	8.196 d	8.347	7.061 d	7.061 d	7.410 d	2.858 s	3.812 s	2.345 d, 2.143 d