Supporting Information

A highly sensitive “switch-on” fluorescent probe for protein quantification and visualization based on aggregation-induced emission

Fangfang Wang, a Jiying Wen, a Lingyun Huang, b Jinjiu Huang a and Jin Ouyang * a

College of Chemistry, Beijing Normal University, Beijing 100875, China
Materials and Methods

Reagents

All of reagents that were used were analytical grade. The reagents 4-Methoxystryrene, 9,10-Dibromoanthracene, Palladium (II) acetate, 1,3-Propanesultone, K$_3$PO$_4$ were purchased from Alfa Aesar and used without further purification. The dry N,N-Dimethylacetamide (DMAc) was bought from Sigma-aldrich. The Boron tribromide (99.99%), Sodium ethylate, Sodium acetate, Acetic acid, Coomassie Brilliant Blue R250, Methanol and Ethanol were obtained from Sinopharm Chemical Reagent Co. (China). The Acrylamide, Ammonium persulfate, N,N’-methylenebisacrylamide (Bis), N,N,N’,N’-tetramethylethlenediamine (TEMED), Tris(hydroxymethyl)aminomethane (Tris), Aminoacetic acid (glycine), DNA marker and heparin sodium were from Sino-American Biotechnology Co. (Beijing, China). MilliQ water (Millipore, Bedford, MA) was used to prepare the solution. The human serum samples from healthy people were obtained from the affiliated Hospital of Beijing Normal University.

Instruments

The $^1$HNMR spectra were recorded on a Bruker Avance III 400 MHZ spectrometer. The excitation and emission spectra were measured with a SHIMADZU RF-5301PC spectrophotometer. The electrophoresis system consisted of DYCZ-24D and DYCZ-21 vertical electrophoresis tanks and DYY-6C and DYY-6B electrophoresis steady voltage instruments (Liuyi Instrument Factory, Beijing, China). The detection and imaging of the gels after staining were recorded using a VILBER FUSION-SL5-3500. Single MS experiments were carried out with a TOF delayed extraction MALDI mass spectrometer (Bruker, Autoflex).

Synthesis of 9,10-Bis(4-methoxystryryl)anthracene (1)
All the reaction vessels were oven dried and cooled under N₂ atmosphere. 9,10-Dibromoanthracene (1.02 g, 3 mmol), 4-Methoxystyrene (0.96 g, 7.2 mmol), K₃PO₄ (1.92 g, 9 mmol) and Pd(OAc)₂ (60 mg) were dissolved in 30 mL of dry DMAc. The mixture solution was stirred at 110 °C for 24 h in an oil bath. After cooling to room temperature, the reaction mixture was poured into 50 mL water and then extracted with 50 mL CH₂Cl₂ for six times. Subsequently, the organic extracts were washed with saturation salt, dried with MgSO₄ and concentrated with rotary evaporators. Finally, the crude product was chromatographed over silica gel. The column was eluted with a mixture (4:1) of petroleum ether and dichloromethane to yield 1.26 g (95%) of a yellow powder. \(^{1}\)HNMR (CDCl₃, 400 MHz): δ = 8.39-8.42 (m, 4H), 7.79 (d, 2H), 7.63 (d, 4H), 7.45-7.47 (m, 4H), 7.00 (d, 4H), 6.88 (d, 2H), 3.89 (s, 6H).

**Synthesis of 9,10-Bis(4-hydroxystyryl)anthracene (2)**

0.67 g of 1 (1.5 mmol) and 20 mL of dry dichloromethane (DCM) were added into a 100 mL flask. The mixture was placed into liquid nitrogen-ethanol at -78 °C. A solution of 1.51 g of boron tribromide (6 mmol) in 10 mL dry CH₂Cl₂ was added carefully to the mixture while stirring. Then the reaction mixture was stirred overnight and allowed to warm to room temperature slowly. After that, 15 mL water was added carefully to the reaction mixture to make the product and unreacted boron tribromide hydrolyze. The organic phase was separated, dried with MgSO₄ and concentrated with to dryness under vacuum. Finally, the crude product was chromatographed over silica gel. The column was eluted with a mixture (3:1) of petroleum ether and acetone to yield 0.52 g (84%) of a yellow powder. \(^{1}\)HNMR (DMSO-\(d₆\), 400 MHz): δ = 9.65 (s, 2H), 8.35-8.37 (m, 4H), 7.86 (d, 2H), 7.62 (d, 2H), 7.51-7.53 (m, 4H), 6.84 (d, 4H), 6.79 (d, 2H).
Synthesis of 9,10-bis[4-(3-sulfonatopropoxyl)-styryl]anthracene sodium salt (3)

0.54 g of 2 (1.3 mmol) and 20 mL of anhydrous ethanol was added into 100 mL flask under nitrogen. Then a solution of NaOEt (0.20 g, 3.0 mmol) in anhydrous ethanol (20 mL) was added by dropwise to the above mixture and stirred until the solution color turned to orange-red. After that, 1,3-propanesultone (0.37 g, 3 mmol) in ethanol (20 mL) was added to the reaction mixture. The mixture was vigorously stirred overnight and a yellow product was precipitated out from the solution. Finally, the product was filtered and then washed with ethanol and acetone two times to give 0.78 g (85.4%) of a yellow powder. \(^1\)HNMR (DMSO-\(d_6\), 400 MHz): \(\delta = 8.37 (m, 4H), 7.96 (d, 2H), 7.74 (d, 4H), 7.53-7.56 (m, 4H), 7.01 (d, 4H), 6.86 (d, 2H), 4.11-4.14 (m, 4H), 2.50-2.58 (m, 4H), 2.01-2.06 (m, 4H).

Protein Electrophoresis

Nondenaturing 1D and 2D polyacrylamide gel electrophoresis was carried out as previously described. For 1D-PAGE, 40 \(\mu\)L of serum was mixed with 360 \(\mu\)L of loading buffer, which consisted of bromophenol blue (80 \(\mu\)L, 0.02%, w/v), glycerol (80 \(\mu\)L, 20%, v/v), and H\(_2\)O (200 \(\mu\)L); the loading volume for each well was 15 \(\mu\)L. The voltage as the samples migrated through the stacking gels was 120 V. When the samples entered the resolving gels, the voltage was turned down to 90 V and kept constant for 3 h. For 2D-PAGE, 15 \(\mu\)L of human serum was diluted with the loading buffer (glycerol, bromophenol blue and deionized water) to 90 \(\mu\)L. Then, the sample was applied to a gel strip (7 cm, pH = 4-6). The serum proteins were isoelectrically focused at 200 V for 30 min and then at 400 V for 16 h. The anode solution was H\(_3\)PO\(_4\) (0.03 M); the cathode solution was NaOH (0.1 M). Then, the focused strip was placed onto a slice of polyacrylamide gel and overlaid with 1% low melt agarose at 60 °C; finally, the strips were subject to electrophoresis at 90 V for 3 h.

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Protein detection after PAGE

After 1D-PAGE and 2D-PAGE, the gels were stained with 9,10-bis[4-(3-sulfonatopropoxy)-styryl]anthracene sodium salt (BSPSA) at room temperature. The staining solution was prepared as follows: the dye (BSPSA) was dissolved in H₂O at a concentration of 1 mg/100 mL, and then 4 mL of HAc was added to the BSPSA solution. After PAGE, the native gel (6 cm × 8 cm × 1.5 cm) was immersed in the staining solution (40 mL) and gently shaken for 10 min on an orbital shaker. After staining, the gel was recorded by a gel-imaging system.

Results and Discussion

To investigate the photophysical properties of the protein reporter BSPSA in vitro, we recorded its fluorescence spectra in the buffer solution (pH = 7.4) at 25 °C. The BSPSA in buffer solution is feebly luminescent as shown in Fig. S1A. However, a significant enhancement of the emission intensity comes about when human serum albumin (HSA) is gradually introduced, and the fluorescence enhancement can be distinguished by naked eye when illuminating the buffer solution with a UV lamp (365 nm) as shown in the inset of Fig. S1A. For instance, when the concentration of HSA reached 0.32 μM, the fluorescence intensity of BSPSA at 535 nm was enhanced by 202 times. As shown in Fig. S1B, the enhancement rate of FL intensity is high at lower HSA concentrations ([HSA] < 0.16) and remains the same when the HSA concentration is higher than 1.28 μM. In addition, a blue shift of the emission peak is associated with the enhancement of FL, it might be because the structure of BSPSA is symmetric and its molecules may pack well during the gradual addition of HSA, which might make the molecular structure more rigid.¹ Furthermore, a good linear relationship was observed between the FL enhancement \( (I/I_0-1) \) at 535 nm and the concentration of HSA (in the range of 0-80 μM) as displayed in the inset of Fig. S1B. It is obvious
that BSPSA can be used as a “switch-on” or “light-up” FL bioprobe for protein detection and quantification.

In order to investigate the protein-to-protein viability of BSPSA, the fluorescence spectral changes of BSPSA was monitored in the presence of a variety of proteins (BSA, HSA, IgG, transferrin, pepsin, papain, etc) with isoelectric points ranging from 1.0 to 9.6. The results are shown in the Fig. S2. From the figure, we could found that BSPSA has response to all 12 proteins tested, and the responses to HSA, IgG and BSA are more sensitive than those of the other proteins. The protein-to-protein variations of BSPSA were comparable to the most frequently used and commercially available protein dyes CBB-R250. Based on these results, BSPSA is a good FL probe for detecting the complete serum proteins.

For quantitative analysis, the relative intensities of protein bands in Fig. 2 were analyzed using the densitometric analysis software of VILBER FUSION SL7-3500. Taking ferritin for instance, the linear dynamic ranges for CBB-R250 and SYPRO-Ruby were 46.95-187.50 ng, while BSPSA-based imaging demonstrated the linearity over 11.70-187.50 ng (R^2=0.9987). The corresponding quantitative data are shown in Fig. S3 in the ESI†. In addition, the detection results would not be affected by other biomolecules (such as DNA and heparin sodium) or the properties of buffer solutions within the optimal staining time (Fig. S4 and S5, ESI†).
**Fig. S1** (A) The effect of increasing concentration of HSA on the fluorescence spectra of BSPSA. Inset: the corresponding photographs taken under excitation of a UV lamp. (B) Changes in the FL intensity at 535 nm with different concentration of HSA; $I_0 =$ FL intensity in the absence of HSA. Inset: linear region of the binding isotherm of BSPSA to HSA. [BSPSA] = 1.4 μM; $\lambda_{ex} = 404$ nm.

**Fig. S2** Dependence of the FL intensity of BSPSA on different proteins in PBS buffer. [protein] = 100 μg/mL; [BSPSA] = 1.4 μM.
**Fig. S3** The relationship between the relative fluorescent intensity of the scanned bands (ferritin) in the gel and the concentration of ferritin. (A) BSPSA staining; (B) CBB-R250 staining; (C) SYPRO-Ruby staining.

**Fig. S4** BSPSA-based fluorescent imaging after staining with BSPSA for 20 min (A) and 2 h (B). Samples loaded in lane 1-3 were a mixture of HMW protein marker, 100 bp DNA marker I and...
heparin sodium; Samples loaded in lane 4-5, lane 6-8 and lane 9-10 were 100 bp DNA Ladder I, HMW protein marker and heparin sodium, respectively.

Fig. S5 The images achieved in the BSPSA solutions with different pH values [(A) pH = 1.8; (B) pH = 3.6; (C) pH = 4.5; (D) pH = 6.2; (E) pH = 7.4 and (F) pH = 8.3] and the optimal staining time for different pH values (G). The samples loaded in the lanes are HMW protein marker.
**Fig. S6** The detection of human serum after 1D-PAGE. A) BSPSA-based fluorescent imaging. B) CBB-R250 staining. C) SYPRO-Ruby staining. D) Silver staining.

**Fig. S7** Sensitivity comparison (A) BSPSA-based fluorescent imaging, (B) SYPRO-Ruby staining, (C) Silver staining, (D) CBB-R250 staining [dilution ratio of the serum samples: 1/2 (1), 1/5 (2), 1/10 (3), 1/20 (4), 1/40 (5), 1/80 (6), 1/100 (7), 1/200 (8), 1/300 (9) and 1/400 (10)].
**Fig. S8** The relationship between the relative fluorescent intensity of the scanned bands (transferrin) in the gel and the concentration of transferrin. (A) BSPSA staining; (B) SYPRO-Ruby staining; (C) silver staining; (D) CBB-R250 staining.
References