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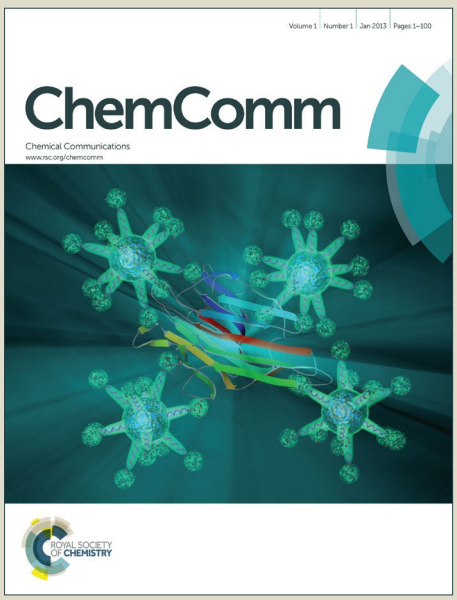

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COMMUNICATION

Hydrophobicity-Induced Prestaining for Protein Detection in Polyacrylamide Gel Electrophoresis

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An AIE fluorescent surfactant is first used to prestain protein by ultrastrong hydrophobic interaction between fluorescent surfactants and proteins, distinguishing from the most widely used poststaining strategies by employing AIE molecules with weak hydrophobic characteristics. A mixture of proteins with variable molecular weights has been detected.

Fluorescent staining has been identified as one of the most reliable and popular methods for biological detections of a trace amount of proteins after polyacrylamide gel electrophoresis (PAGE).[1] Although poststaining fluorescence methods have an excellent limit of detection in most cases, they require a time-consuming gel staining and destaining procedure in order to detect the desired fluorescent bands of proteins in the gel.[2] Alternatively, prestaining fluorescence methods for protein analysis in gel are achieved by covalent reactions between the reactive groups of fluorescent dyes and specific amino acids of proteins prior to electrophoresis.[3] Although covalently prestaining fluorescence methods have a much shorter staining process, and a better signal-to-noise ratio, most of them are cumbersome and change the structure of proteins, resulting in the migration rate change of proteins in gel during electrophoresis.[4] In addition, both prestaining and poststaining fluorescence methods are embarrassed by the aggregation-caused-quenching effect of high concentrations of fluorescent dyes.[2,3]

The use of luminescent materials with aggregation-induced emission (AIE) property has provided the opportunity to overcome the aggregation-caused-quenching.[5] A number of efforts have been put into the development of AIE materials for protein detection in PAGE.[6,7] Although these systems provided satisfactory detection sensitivity for protein, almost all of these configurations were based on the time-consuming poststaining methods through weak hydrophobic interaction[6] or a prestaining method by cumbersome covalent reactions between protein and AIE materials.[7] Reasoning that a simple and rapid prestaining method might be explored through a strong hydrophobic interaction between protein and AIE materials.

To achieve this reasoning, herein, we introduce a novel strategy to prestain proteins for PAGE using a tetraphenylethene-sodium dodecyl sulfonate (TPE-SDS) AIE dye with a long hydrophobic chain, which was previously designed and synthesized by our group (Fig. S1).[8] The luminescent TPE-SDS molecules could easily aggregate with proteins through hydrophobic interaction between TPE-SDS molecules and proteins in PAGE. The fluorescence intensity of the TPE-SDS-protein composite linearly responded to the amount of proteins in solution. The dramatic fluorescence turn-on enabled direct fluorescence detection for protein in PAGE without the need of poststaining and washing steps. This finding thus provides an attractive and simple prestaining method for detection of protein through simple noncovalent interactions.

A typical AIE-active molecule, sodium 1,2-bis[4-(3-sulfonatopropoxyl)phenyl]-1,2-diphenylethene (BSPOTPE, Fig. S1), has been reported to bind proteins after PAGE through hydrophobic interaction between BSPOTPE and protein.[6b] The superior performances of BSPOTPE motivated us to employ this AIE molecule as a prestaining reagent for proteins. Although BSA could remarkably enhance the fluorescence intensity of BSPOTPE (Fig. S2), the desired fluorescent band of BSA in the gel after prestaining could hardly be observed under 365 nm UV light after PAGE; while a clear emissive band of BSA demonstrated the strong hydrophobic interaction between TPE-SDS and BSA (Fig. 1A). On the other hand, a clear band could be observed after a poststaining process of coomassie brilliant blue (CBB) solution for BSA (Fig 1B). These results showed that the hydrophobic interactions between BSPOTPE and BSA were so weak that BSPOTPE cannot bind to BSA tightly. As a result,

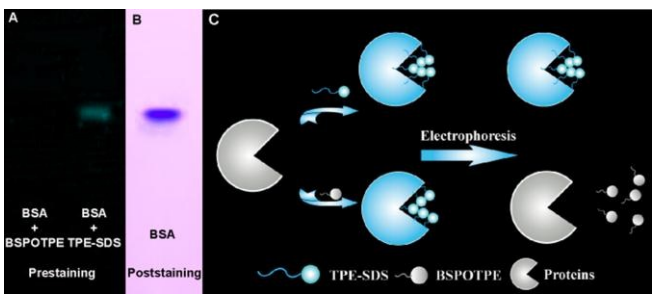

Fig. 1 (A) Gel comparison of 2.0 μg BSA by 50 μM BSPOTPE or TPE-SDS prestaining under a 365nm UV light; (B) gel imaging of 2.0 μg BSA by CBB poststaining under daylight; (C) different hydrophobic interaction between BSA and TPE-SDS /BSPOTPE by PAGE process.

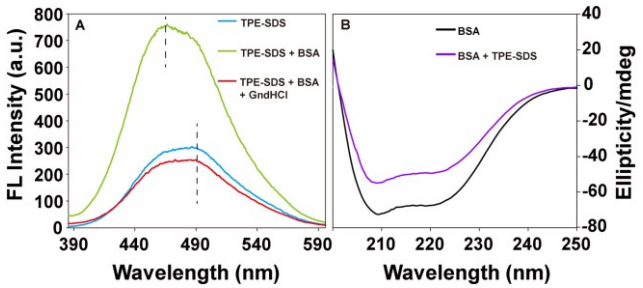

Fig. 2 (A) Fluorescence spectra of 20 μM TPE-SDS in the presence or absence of 100 μg/mL BSA and 100 μg/mL BSA-bound 20 μM TPE-SDS in the presence of 6.0 M GndHCl; (B) CD spectra of 100 μg/mL BSA in the presence or absence of 20 μM TPE-SDS.

BSPOTPE can be separated from BSA during the electrophoresis process. Meanwhile, the strong hydrophobic interactions between TPE-SDS and BSA made it possible for TPE-SDS to become an ideal prestaining reagent for proteins.

Fig. 2A showed that 20 μM TPE-SDS exhibited a weak fluorescence intensity because the critical micelle concentration (CMC) of TPE-SDS was 30 μM.[8] However, when TPE-SDS was mixed with BSA, there was an obvious increase in the fluorescence intensity of TPE-SDS, along with an increase of its fluorescence quantum yield from 6.15% to 18.6%. The enhancement of fluorescence emissions of TPE-SDS may be attributed to the formation of the TPE-SDS aggregates inside the hydrophobic pocket of BSA.[9] On the other hand, a blue shift of the emission peak of TPE-SDS from 490 nm to 472 nm could be observed in the presence of BSA (Fig. 2A). The blue–shifted emission may be attributed to the fact that the distorted conformation of TPE-SDS was formed in the hydrophobic cavities of BSA, leading to a decrease in the conjugation extent.[10] In addition, the fluorescence intensity of TPE-SDS was returned to its original state after the addition of 6.0 M GndHCl (Fig. 2A). This phenomenon could be explained that the GndHCl may break down the spatial structure of BSA folds by intruding into the hydrophobic cavities of BSA. During the unfolding process of BSA, TPE-SDS was gradually released from hydrophobic cavities of BSA into the aqueous solution.[6b]

Circular dichroism (CD) spectra were employed to research the change of the secondary structure of BSA in the presence of different concentrations of TPE-SDS.[11] As shown in Fig. 2B, the two minima characteristic peaks intensity of BSA at 208 and 222 nm in CD spectra exhibited a slight change in the presence of 20 μM TPE-SDS. On the other hand, the calculation results from the CDPro software programs showed that TPE-SDS could barely change the secondary structure of BSA in a series of concentrations (Table S1).[12] In addition, ζ potential measurements were used to investigate the charge variation of TPE-SDS at different conditions.[13] Fig. S3 showed that the ζ potentials of 100 μg/mL BSA and the pure 1.0 μM TPE-SDS were −19.4 mV and −25.9 mV, respectively. However, the ζ potential of the mixture solution of 100 μg/mL BSA and 1.0 μM TPE-SDS was −18.1 mV. Moreover, the ζ potential of the mixture solution was always lower than that of the pure TPE-SDS solution when the concentration of TPE-SDS gradually increased. These results further confirmed that the TPE-SDS can only intrude into the BSA cavities by strong hydrophobic interactions, realizing electrophoresis separation of proteins as a novel prestaining reagent.

The changes of fluorescence intensity of TPE-SDS were used to explore the concentration correlativity between TPE-SDS and BSA. Fig. 3 showed that there was an obvious increase of the fluorescence intensity of TPE-SDS with increasing the concentration of BSA up to 100 μg/mL. The detection limit of BSA was as low as 0.1 μg/mL. The linear curve of fluorescence enhancement (I/I_0−1) could be obtained in the BSA concentration range of 0.1−1.5 μg/mL with a high linear correlation coefficient (R^2 = 0.9965), where I_0 is the fluorescence signal in the absence of BSA and I is the fluorescence signal in the presence of BSA. These results indicated that TPE-SDS could be employed as an efficient fluorescence probe for detection of BSA.

Next, we tried to investigate the possibility of TPE-SDS as a new prestaining reagent for proteins. The results showed that TPE-SDS could hardly run within the stacking gel during the electrophoresis process due to the strong hydrophobic interactions between TPE-SDS and polyacrylamide (lane 7 in Fig. 4A). When BSA was prestained by TPE-SDS, TPE-SDS could be packaged in the hydrophobic cavities of BSA. Therefore, the TPE-SDS-BSA composite could easily run during the electrophoresis process. The detection limit of BSA could be 0.05 μg under 365 nm UV light after PAGE. In addition, the fluorescence bands of BSA could be easily observed under the

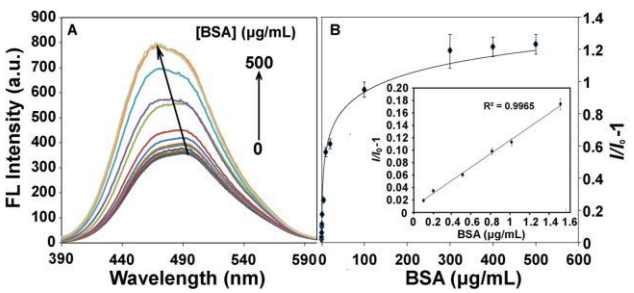

Fig. 3 (A) Fluorescence spectra of 20 μM TPE-SDS in the presence of different concentration of BSA (0−500 μg/mL); (B) the concentration correlativity between 20 μM TPE-SDS and BSA. I_0 is the fluorescence intensity of 20 μM TPE-SDS, I is the fluorescence intensity of the mixture solution of 20 μM TPE-SDS with different concentrations of BSA. Inset: linear response of 20 μM TPE-SDS to BSA at the concentration range of 0.1−1.5 μg/mL.

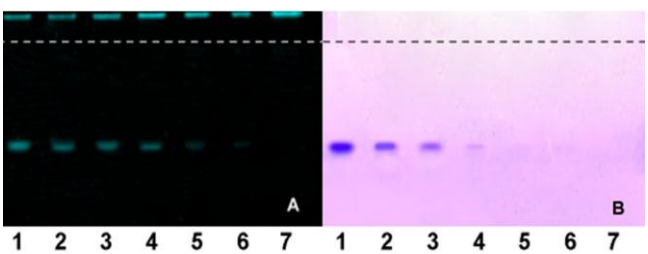

Fig. 4 (A) PAGE analysis of different amounts of BSA by 50 μM TPE-SDS prestaining technique; (B) PAGE analysis of different amounts of BSA by CBB poststaining method. Lanes correspond to the BSA bands containing amounts of (1) 2.0 μg, (2) 1.0 μg, (3) 0.5 μg, (4) 0.2 μg, (5) 0.1 μg, (6) 0.05 μg, (7) 0 μg, respectively.

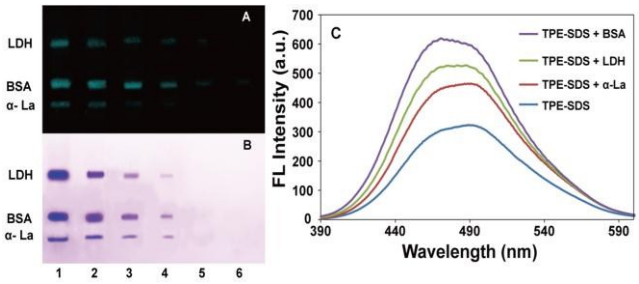

Fig. 5 Comparison of sensitivity of proteins by PAGE method (A) the proposed method; (B) CBB method. A series of concentrations of proteins were loaded in the wells (1) 2.0 μg, (2) 1.0 μg, (3) 0.5 μg, (4) 0.2 μg, (5) 0.1 μg, (6) 0.05 μg, respectively. (C) Fluorescence spectra of 20 μM TPE-SDS in the presence and absence of 100 μg/mL different kinds of proteins.

excitation of a 365 nm UV flashlight in gel during the electrophoresis process. Interestingly, the extra TPE-SDS would be retained with the loading wells after the saturation adsorption by BSA. Therefore, an additional washing step could be left out after prestaining. In contrast, a controlled experiment was performed on the other gel under the same electrophoresis conditions, except that the gel was poststained by CBB solution (Fig. 4B). Interestingly, both of the methods had a similar protein migration rate. However, in comparison with the conventional CBB method, the proposed fluorescence method exhibited a lower detection limit (0.05 μg *vs* 0.2 μg) without a background fluorescence interference, making it suitable for PAGE analysis.

The separation of three kinds of proteins with different molecule weights was further carried out using the proposed prestaining method, including α-lactalbumin (α-La), 14.4 kDa, BSA, 66 kDa, and lactic dehydrogenase (LDH), 140 kDa. Fig. 5A indicated that the separation of three fluorescence protein bands could be directly observed under 365 nm excitation. The detection limit of each protein in gel was 0.1 μg for LDH, 0.05 μg for BSA, and 0.2 μg for α-La, respectively. The surface hydrophobicity (S_0) of three kinds of proteins (α-La, LDH and BSA) was determined by a traditional method, in which 8-anilino-1-naphthalenesulfonic acid (1,8-ANS) was used as the fluorescence probe.[14,15] The results showed that the S_0 value increased from α-La, LDH and BSA (Fig. S4–S6), indicating that the interactions of TPE-SDS with BSA/LDH were tight by strong hydrophobic effect, while this interactions between TPE-SDS and α-La were weak as a result of a relatively weak hydrophobicity of α-La.[16] In addition, as shown in Fig. 5C, the proposed fluorescence probe exhibited the variable response to the same concentration of BSA, LDH and α-La (each 100 μg/mL). More interestingly, the emission peak of LDH-bound TPE-SDS showed a blue shift, similar as BSA-bound TPE-SDS; however, the emission peak of α-La-bound TPE-SDS kept constant. These phenomena might be attributed to the differences of the microenvironment between α-La and BSA/LDH.[17] Finally, a controlled gel experiment was carried out by poststaining with CBB, which had a higher detection limit with background signal (Fig. 5B), clearly demonstrating the feasibility of the developed fluorescence prestaining method for detection of proteins.

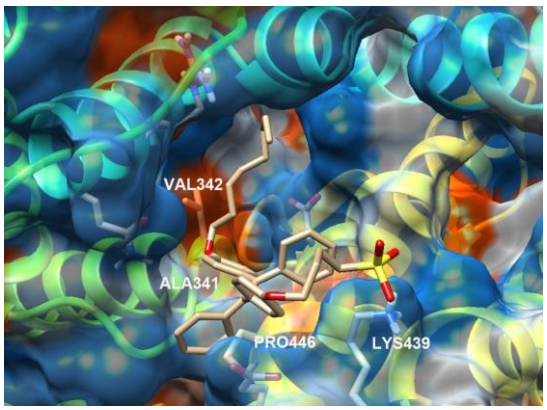

Fig. 6 Details of the binding conformation of TPE-SDS-BSA conjugate with the lowest binding free energies from 50 docked conformations clustered with a rmsd tolerance of 2.0 Å. TPE-SDS and amino acid residues were shown as a stick representation using the color of white for carbon, blue for nitrogen, red for oxygen and yellow for sulfur. For the surface of the protein, hydrophobic domain was red-colored, the hydrophilic domain was blue-colored, respectively.

The computation modeling was further used for understanding the probable binding mode between TPE-SDS and proteins,[18] all the procedures were shown in Supporting Information. For the TPE-SDS-BSA conjugate, we chose one of the 50 docked conformations with the lowest binding energy (−3.71 kcal/mol) to analyze the binding sites of TPE-SDS in BSA (Fig. 6). The small gibbs free energies indicate a highly spontaneous and energetically favorable system for the conjugate. In this binding conformation, TPE-SDS was found to bind in the hydrophobic cavity of BSA. VAL342, ALA341 and PRO446 were contacted with the hydrophobic part of the luminogen by hydrophobic interaction. On the other side, it was found that no amino acid residues accommodate the aromatic core of TPE-SDS *via* a cation-π interaction. All the mentioned interactions rigidify the distorted conformation of TPE-SDS and make the luminogen more emissive in the binding state. Similarly, the details of LDH and α-La binding with TPE-SDS were shown in Fig. S7 and Fig. S8. The results showed that the lowest binding energy for TPE-SDS-LDH and TPE-SDS-α-La was −7.18 kcal/mol and −2.42 kcal/mol, respectively. LEU71, LEU69 and LEU39 in LDH and ILE72 in α-La were found to contact with the hydrophobic part of the luminogen by hydrophobic interaction. In addition, no cation-π interaction was found between protein and TPE-SDS.

In summary, a synthetical AIE molecule, TPE-SDS, could be tightly bound into the protein cavities by strong hydrophobic interaction, distinguishing from weak hydrophobic characteristics of the widely used AIE molecules (*e.g.*, BSPOTPE). In comparison with the covalently prestaining techniques, TPE-SDS-based fluorescent method exhibited some advantages, such as simplicity, rapid staining procedure, stability and convenience. Therefore, TPE-SDS could be employed as a novel protein prestaining reagent in the PAGE analysis. The applicability and reliability of the proposed methodology have been demonstrated by detecting different proteins. This work opens up a new strategy to prestain proteins with superior performances. Further studies of the TPE-SDS-based fluorescence probe in biological applications are currently in progress.

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Notes and references

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