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Hydrophobicity-Induced Prestaining for Protein Detection in Polyacrylamide Gel Electrophoresis

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Experimental section

Chemicals and Instruments

Bovine serum albumin (BSA, 66 kDa) was purchased from Aladdin. α-lactalbumin (α-La, 14.4 kDa) and lactate dehydrogenase (LDH, 140 kDa) were obtained from Sigma Aldrich. acrylamide, methylene bis-acrylamide, ammonium persulfate, N,N,N',N'-Tetramethylethylenediamine (TEMED), tris (hydroxymethyl) aminomethane, aminoacetic acid (glycine), coomassie brilliant blue R-250 (CBB) were obtained from (BioRoYee) BioRoYee Biochemical. Co. Ltd. Guanidine hydrochloride (GndHCl) was obtained from Macklin (Shanghai Macklin Biochemical. Co. Ltd). Glycerol, methanol and acetic acid were obtained from Beijing Chemical Works. 8-Anilino-1-naphthalenesulfonic acid (1,8-ANS) was obtained from TCI (Shanghai) Development Co. Ltd. All the chemicals were analytical grade. TPE-SDS was synthesized by the procedures according to our previous work.[1] BSPOTPE was synthesized according to the procedures in the literature.[2] Fluorescence spectra were measured with a F-7000 fluorescence spectrophotometer from HATICHI. CD spectra data were recorded on a JASCO J-810 spectropolarimeter (JASCO Japan). Milli-Q Academic A10 (Millipore, Bedford,

MA) was used to purified water. DYCZ-24DN vertical electrophoresis tanks and DYY-7C electrophoresis steady voltage instruments were used as electrophoresis system (Liuyi Instrument Factory, Beijing, China). After PAGE, gels were employed a Bio-Rad ChemiDoc MP gel-imaging system for imaging.

Samples preparation and experimental methods

Fluorescence measurements

The fluorescence spectra were employed a 325 nm wavelength for the excitation of sample solutions in the range of 390−600 nm. Samples used for exploring the linear correlation between TPE-SDS and BSA were containing 20 μM TPE-SDS and a series of concentrations of BSA (0.1−500 μg/mL) in PBS buffer. Solutions containing 20 μM TPE-SDS and different kinds of proteins were also prepared to explore the variable response of TPE-SDS to the same concentration of BSA, LDH and α-La.

Circular dichroism measurements

For the CD spectra measurements, 100 μg/mL BSA was incubated with different concentration of TPE-SDS (0−100 μM) for 30 min before measurements. A 5 mm quartz cuvette and a step resolution of 0.5 nm were used to record the CD signals from 190 to 250 nm in order to investigate secondary structure changes of BSA in the presence of TPE-SDS. Proteins secondary structure was then calculated by CDPro software programs, and the obtained statistical results were shown in Table S1.

Zeta Potential measurements

To investigate the charge variation of TPE-SDS at different conditions, 100 μg/mL of BSA samples containing different concentrations of TPE-SDS were prepared and their Zeta Potentials

were measured by a Malvern Zetasizer.

Protein electrophoresis

BSA prestaining loading buffers were prepared containing 50 μM TPE-SDS, different concentrations of BSA and a certain concentration of glycerol, respectively. Another loading buffer containing 100 μg/mL BSA and 50 μM BSPOTPE was also prepared. Different concentrations of proteins were mixed together to get the loading buffers for PAGE experiments. In this experiment, proteins were separated using 4.5 % stacking and 10 % resolving acrylamide gel. Each loading well was added in 20 μL loading buffer. The electrophoresis voltage was 120 V in stacking gel and turned down to 100 V in resolving gel. After electrophoresis, gels were directly imaged by the gel-imaging system. Additionally, CBB solution was used as a poststaining reagent containing 0.1 % w/v CBB R250, 10 % methanol and 7 % acetic acid. A 1 h staining process and another 24 h destaining process were operated in an aqueous solution containing 10 % methanol and 10 % acetic acid on a rotary shaker.

Proteins surface hydrophobicity measurements

In this experiment, 8.0 mmol 1,8-ANS solution was prepared in 0.01 M PBS buffer. 20 μL 1,8-ANS stock solution was added to 2.0 mL different concentrations of protein solutions. A 344 nm wavelength was employed for the excitation of 1,8-ANS-protein conjugates. The surface hydrophobicity (S_0) was then calculated from the initial slope of enhanced fluorescence intensity vs. protein concentration plot. Duplicate analyses were applied to ensure the veracity of results.

Computational modeling

TPE-SDS was treated as a full torsional freedom probe during modeling. The crystal structures of BSA (3v03), LDH (3H3F) and α-La (3B0O) were obtained from Protein Data Bank as modeling data (www.rcsb.org). The structures of proteins were evaluated and optimized by MolProbity.[3] Water molecules and organics were removed by Pymol (www.pymol.org). Only

polar hydrogen atoms were added to the ligand and proteins for the clarification of calculate results. After the examination of different parameter set for the modeling, we chose the optimized parameters to dock TPE-SDS to the proteins for 50 runs using the Lamarckian genetic algorithm by Autodock 4.2.[4] The obtained 50 docked conformations were analyzed by Autodock Tools.[5] Further analysis was carried out for the hydrophobic interaction between TPE-SDS and proteins by Chimera.[6]

Supporting Figures

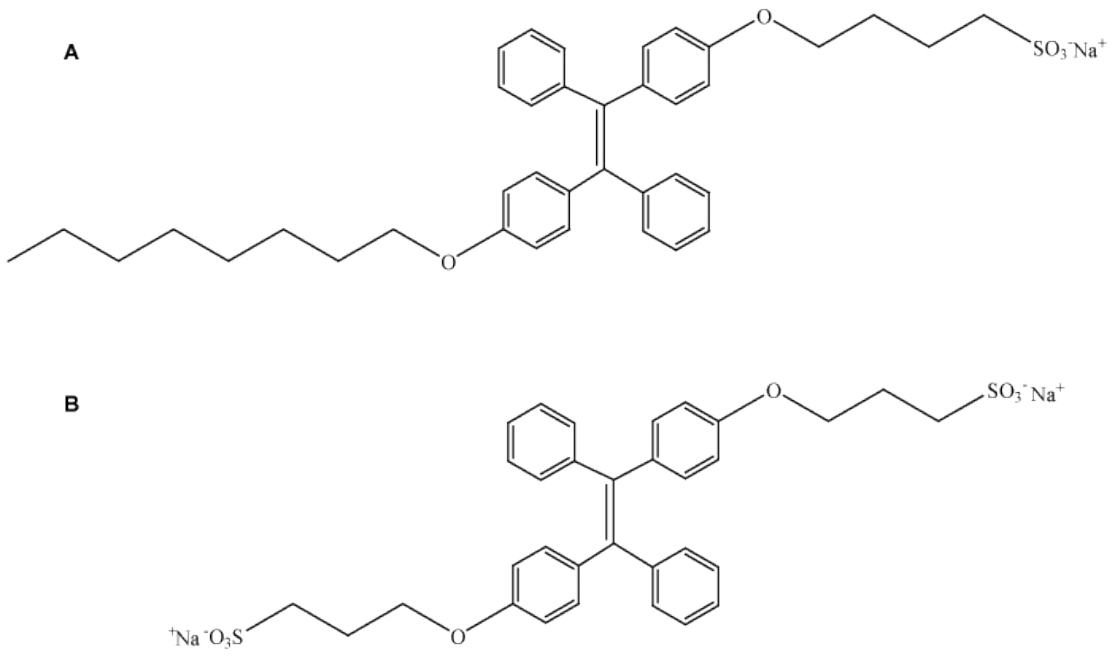

Fig. S1 Chemical structure of TPE-SDS (A) and BSPOTPE (B).

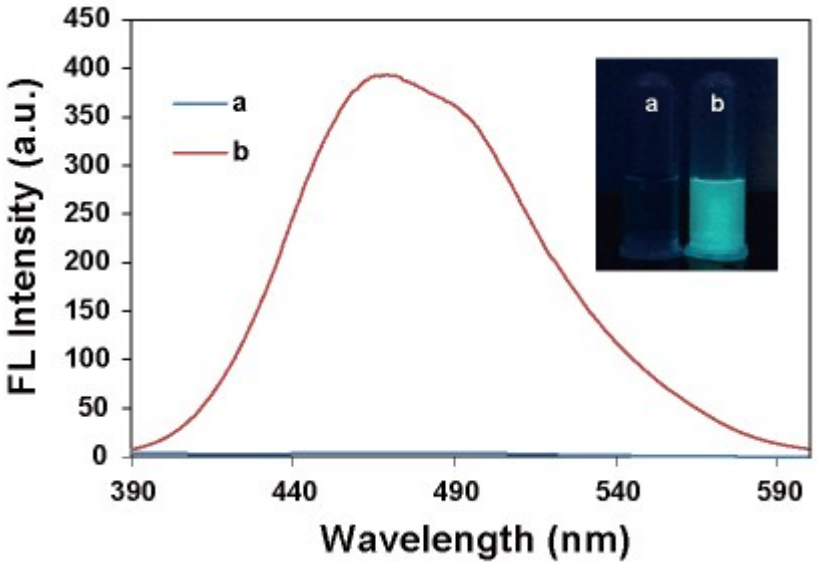

Fig. S2 Fluorescence spectra of 20 μM BSPOTPE in the absence (a) or in the presence (b) of 100 μg/mL BSA. Inset: photo of 20 μM BSPOTPE solution in the presence or absence of 100 μg/mL BSA.

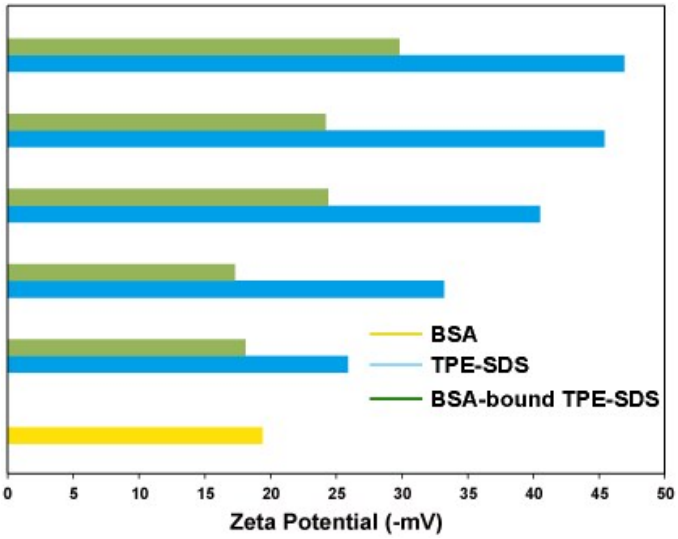

Fig. S3 Zeta potential measurements of 100 μg/mL BSA, different concentrations of TPE-SDS (1 μM−20 μM), and a mixture of 100 μg/mL BSA and different concentrations of TPE-SDS.

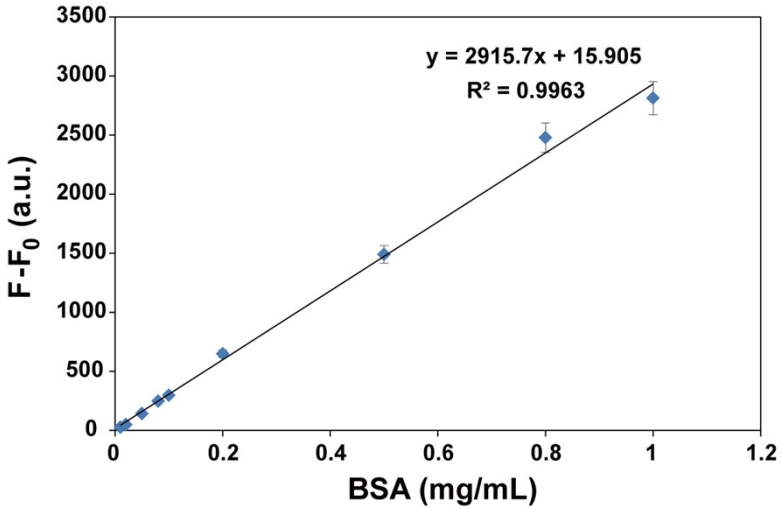

Fig. S4 Linear response of 1,8-ANS to BSA at the BSA concentration range of 0.01-1 mg/mL. F is the fluorescence intensity of the mixture solution of 20 μL 8.0 mmol 1,8-ANS with 2.0 mL different concentrations of BSA. F_0 is the fluorescence intensity of the solution of 20 μL 8.0 mmol 1,8-ANS with 2.0 mL PBS buffer. S_0 is the slope of linearity curve = 2915.7.

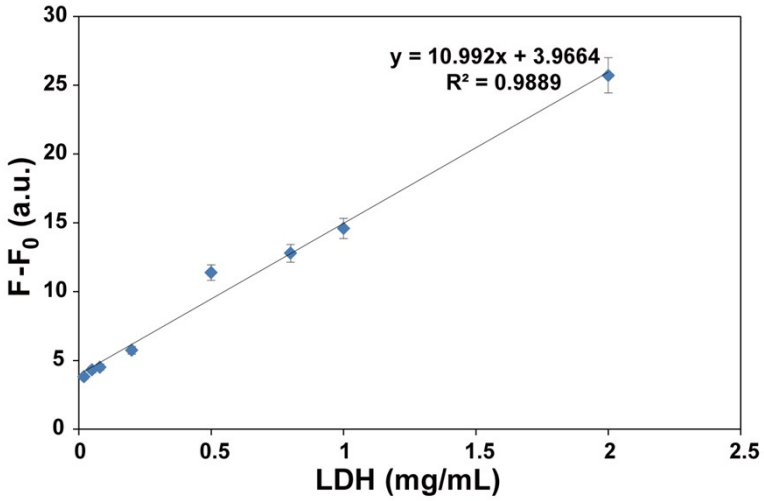

Fig. S5 Linear response of 1,8-ANS to LDH at the LDH concentration range of 0.01-2.5 mg/mL. F is the fluorescence intensity of the mixture solution of 20 μL 8.0 mmol 1,8-ANS with 2.0 mL different concentrations of LDH. F_0 is the fluorescence intensity of the solution of 20 μL 8.0 mmol 1,8-ANS with 2.0 mL PBS buffer. S_0 is the slope of linearity curve = 10.992.

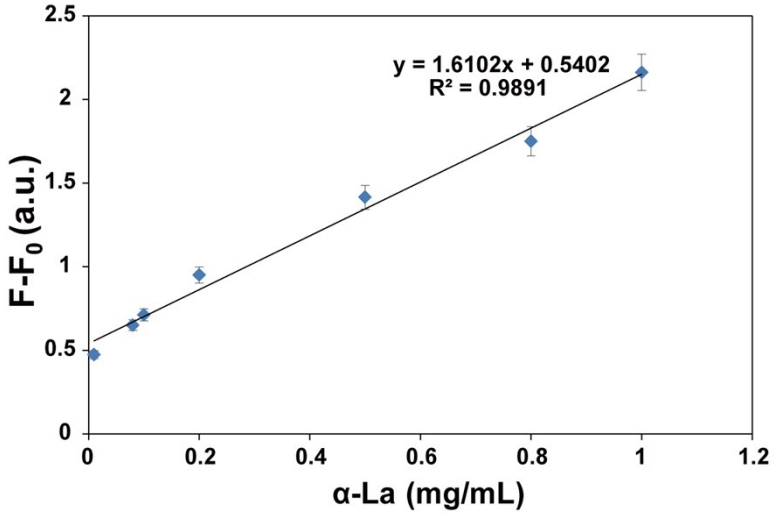

Fig. S6 Linear response of 1,8-ANS to α-La at the α-La concentration range of 0.01-1 mg/mL. F is the fluorescence intensity of the mixture solution of 20 μL 8.0 mmol 1,8-ANS with 2.0 mL different concentrations of α-La. F_0 is the fluorescence intensity of the solution of 20 μL 8.0 mmol 1,8-ANS with 2.0 mL PBS buffer. S_0 is the slope of linearity curve = 1.6102.

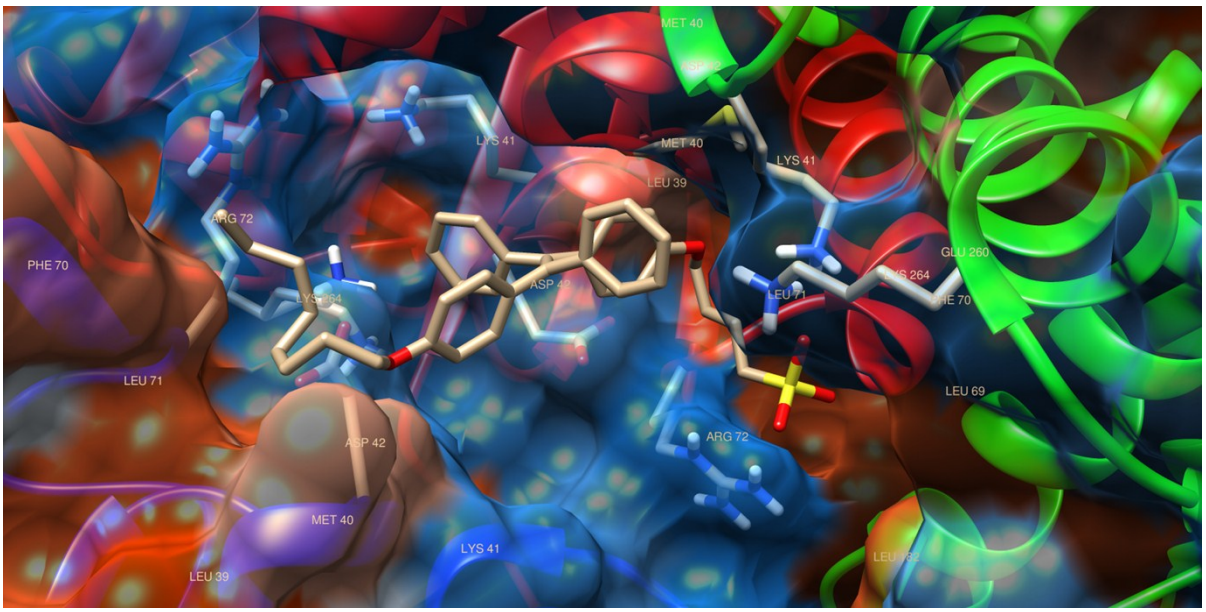

Fig. S7 Details of the binding conformation of TPE-SDS-LDH conjugate with the lowest binding free energies from 50 docked conformations clustered with a rmsd tolerance of 2.0 Å. TPE-SDS and amino acid residues were shown as a stick representation using the color of white for carbon, blue for nitrogen, 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.

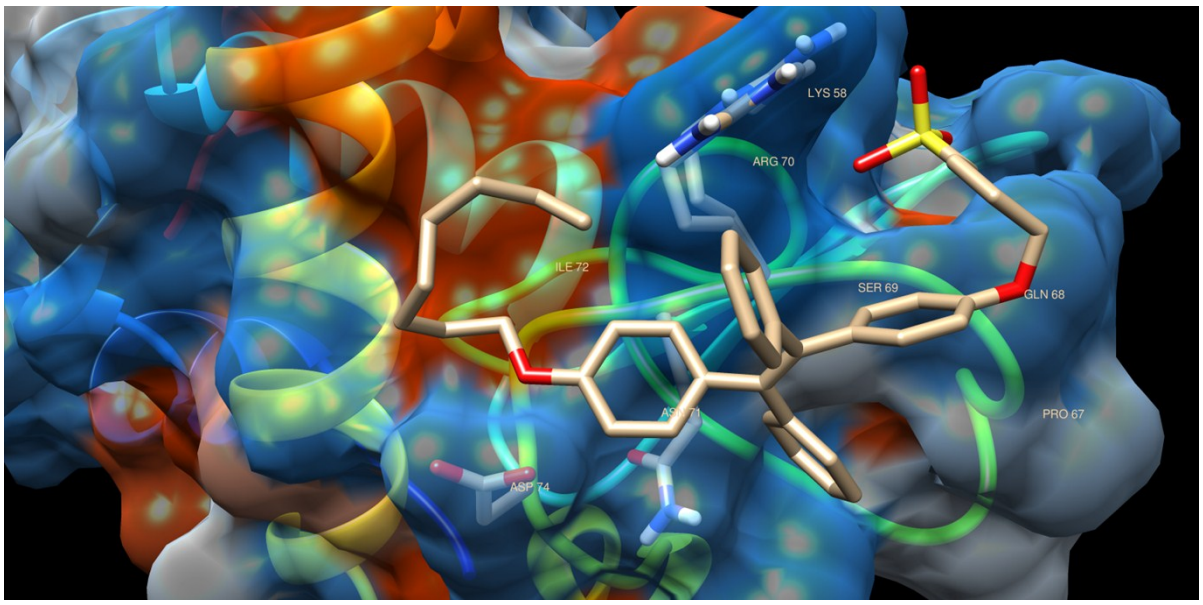

Fig. S8 Details of the binding conformation of TPE-SDS-α-La conjugate with the lowest binding free energies from 50 docked conformations clustered with a rmsd tolerance of 2.0 Å. TPE-SDS and amino acid residues were shown as a stick representation using the color of white for carbon, blue for nitrogen, 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.

Table S1. Comparison of Secondary structure of 100 μg/mL BSA bound with 0−100 μM TPE-SDS calculated by CDPro software programs.

PROT	H(r)	H(d)	S(r)	S(d)	Trn	Unrd	Sum
BSA	**0.586**	**0.225**	**0.001**	**0.001**	**0.056**	**0.152**	**1.020**
BSA+TPE-SDS (5)	**0.583**	**0.226**	**0.000**	**0.003**	**0.068**	**0.163**	**1.044**
BSA+TPE-SDS (10)	**0.589**	**0.228**	**-0.001**	**0.000**	**0.056**	**0.152**	**1.023**
BSA+TPE-SDS (20)	**0.594**	**0.225**	**-0.002**	**0.000**	**0.061**	**0.161**	**1.039**
BSA+TPE-SDS (30)	**0.576**	**0.225**	**0.001**	**0.004**	**0.069**	**0.165**	**1.041**
BSA+TPE-SDS (50)	**0.572**	**0.226**	**0.001**	**0.006**	**0.074**	**0.167**	**1.041**
BSA+TPE-SDS (100)	**0.578**	**0.226**	**0.001**	**0.004**	**0.068**	**0.165**	**1.042**

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