Quantitation, Visualization, and Monitoring of Conformational Transitions of Human Serum Albumin by a Tetraphenylethene Derivative with Aggregation-Induced Emission Characteristics

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**References**

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**Preparation of Artificial Urine.** An aqueous solution referred to as “artificial urine” was prepared according to the recipe provided by Brooks and Keevil. The solution contains 1.1 mM of lactic acid, 2.0 mM of citric acid, 25 mM of sodium bicarbonate, 170 mM of urea, 2.5 mM of calcium chloride, 90 mM of sodium chloride, 2.0 mM of magnesium sulfate, 10 mM of sodium sulfate, 7.0 mM of potassium dihydrogen phosphate, 7.0 mM of dipotassium hydrogen phosphate, and 25 mM of ammonium chloride, all mixed in Millipore water. The pH value of the solution was adjusted to 6.0 through the addition of aliquots of 1.0 M hydrochloric acid.

**MTT Cytotoxicity Assay.** HeLa cells were plated into a 96-well plate at a concentration of $5 \times 10^3$ cells/0.1 mL/well and treated at 37 °C for 48 h with different concentrations (5, 10, 20, 40, and 80 μM)
of BSPOTPE solutions in the aqueous buffer. After the treatment, 20 μL of PBS containing 5 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well and the plate was incubated at 37 °C for 2–4 h. To solubilize the intracellular formazan produced by active mitochondria in the living cells, 100 μL of detergent solution (10% w/v sodium dodecyl sulfate in 10 mM HCl) per well was added and the plate was incubated overnight in the dark at room temperature. The absorbance at 595 nm was measured on a spectrophotometric plate reader and used for calculation of relative cell viability, where cells treated with PBS only represents 100% viability.

![Graph](image)

**Figure S1.** Viability of HeLa cells incubated in the presence of different concentrations of BSPOTPE for 48 h.

**FRET Measurement.** It is well known that FRET is a useful tool to evaluate the distance between a pair of donor and acceptor. We thus employed FRET as a spectroscopic ruler to measure the distance between Trp-214 in domain II of HSA and BSPOTPE luminogen. The distance between BSPOTPE and Trp-214 can be calculated from the following eq:

\[
E = \frac{1}{1 + \left( \frac{r}{R_0} \right)^6} = 1 - \frac{F}{F_0}
\]  

(S1)
where $E$ is the efficiency of energy transfer, $r$ is the average distance between the donor and acceptor, and $R_0$ is the Förster distance at which the energy transfer efficiency is 50%:

$$R_0^6 = \frac{9Q_0(\ln 10)\kappa^2 J}{128\pi^2 n^4 N_A}$$  \hfill (S2)

In eq S2, $Q_0$ is the fluorescence quantum yield of the donor in the absence of the acceptor, $n$ is the refractive index of the medium, $N_A$ is the Avogadro’s number, $\kappa^2$ is the dipole orientation factor and is equal to 2/3 for random orientation in fluid solution, and $J$ is the spectral overlap integral calculated as follows:

$$J = \frac{\int f_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda}{\int f_D(\lambda)d\lambda}$$  \hfill (S3)

where $f_D(\lambda)$ is the normalized donor emission spectrum and $\varepsilon_A$ is the molar extinction coefficient of the acceptor.

**Figure S2.** Overlap between absorption (Ab) spectrum of BSPOTPE and emission (Em) spectrum of HSA.
According to eqs S1–S3, when \( n = 1.36 \) and \( Q_0 = 0.15 \), we get \( J = 4.41 \times 10^{-15} \text{ M}^{-1} \text{ cm}^3 \) and \( R_0 = 20.57 \text{ Å} \). Without GndHCl, \( E = 0.47 \) and \( r = 2.10 \text{ nm} \). The average distance between HSA and Trp-214 in HSA is within the range from 2 to 8 nm and \( 0.5R_0 < r < 1.5R_0 \), indicative of a high probability of energy transfer between the donor and the acceptor. The distance becomes longer in the presence of GndHCl. At 1.5 M of GndHCl, the average distance is equal to 2.65 nm. The distance, however, is shortened to 2.20 nm at 2.0 M of GndHCl, which is the concentration where a stable molten-globule intermediate is observed during the unfolding process.

**Figure S3.** FL spectra of BSPOTPE in the presence of different concentrations of GndHCl. \([\text{BSPOTPE}] = 5 \mu\text{M}; [\text{HSA}] = 2 \mu\text{M}; \lambda_{\text{ex}} = 295 \text{ nm}\).

**DSC Thermogram.** Thermal transitions of HSA were investigated by DSC using a TA instruments DSC Q1000 at a heating rate of 1 °C/min from −10 °C to 150 °C. All the scans were analyzed using the software of Universal Analysis 2000 to derive the transition temperatures.
**Figure S4.** DSC thermograms of HSA in the presence of 0, 0.8, 1.5, 2.0, and 6.0 M of GndHCl recorded under nitrogen at a scanning rate of 1 °C/min.

**pH-Dependent FL Analysis.** FL spectra of BSPOTPE were recorded in the aqueous buffers over a wide pH range in the presence and absence of HSA.

**Figure S5.** FL intensities of BSPOTPE at 470 nm in the presence and absence of HSA at different pH values. [BSPOTPE] = 5 μM; [HSA] = 0.4 μM; λ<sub>ex</sub> = 350 nm.
Computational Modeling. Ligand molecules were treated as flexible to enjoy full torsional freedom during modeling. First, the crystal structure of HSA complexed with 4Z,15E-bilirubin-IX-alpha was retrieved from the Protein Data Bank (PDB 2vue)⁴ as training data (www.pdb.org). 4Z,15E-Bilirubin-IX-alpha and HSA were separated. The structure of HSA was evaluated and optimized by MolProbity.⁵ The buried water molecules were predicted and assigned by Dowser.⁶ For both the ligand and HSA, hydrogen atoms were added, Gasteiger charges were assigned, but non-binding apolar hydrogen atoms were omitted for clarity. 4Z,15E-Bilirubin-IX-alpha was docked back to HSA by AutoDock 4.2⁷ with the maximum searching space. Different parameter sets were examined until AutoDock successfully predicted the binding site of 4Z,15E-bilirubin-IX-alpha on HSA with a rmsd of less than 3 Å between the conformation from the computational modeling and that from the crystal structure. Then the high resolution crystal structure of HSA (PDB 1e78)⁸ was used to study the binding of BSPOTPE with HSA in a similar process. The docking between HSA and BSPOTPE was performed for 50 runs with the optimized parameters. The resulted 50 docked conformations were summarized and analyzed using AutoDock Tools.⁹ The binding details were illustrated with Pymol (http://www.pymol.org).

![Figure S6](image-url)  
**Figure S6.** Summary of 50 docked conformations clustered with a rmsd tolerance of 2.0 Å. For each cluster, the horizontal and vertical axes represent the lowest binding energy and the number of docked conformations in that cluster, respectively. The three clusters with larger numbers are marked as clusters 1–3.
References


