Fluorescent "light-up" bioprobes based on tetraphenylethylene derivatives with aggregation-induced emission characteristics†

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Aggregation in poor solvents and complexation with calf thymus DNA and bovine serum albumin turn "on" the fluorescence of tetraphenylethylene derivatives, due to the restriction of intra-molecular rotations of the dyes in the aggregates and complexes.

The study of detection of biomacromolecules by fluorescence (FL) probes is emerging as an active area of research due to its potential applications in biological science and engineering.[1] The FL-based technique offers high sensitivity, low background noises, and wide dynamic ranges.[2] Upon complexation with proteins and DNAs, FL of the bioprobes can be enhanced/quenched and/or red/blue-shifted, thus enabling visual observation of the biomacromolecular species. The most useful bioprobes are those "turn-on" sensors, whose FL is activated by analytes.[3] Several probes for DNA detection based on FL enhancement have been developed, such as phenanthridine and acridine derivatives.[4] FL enhancements induced by proteins have been reported for fluorescamine, *o*-phthaldialdehyde, NanoOrange, SYPRO, and Nile Red.[5] FL intensities of cyanine and acridizinium dyes have been found to increase dramatically upon complexation with DNA and proteins.[6] Water-soluble "light-up" probes for the detection of biomacromolecules such as DNAs and proteins are, however, still rare.

Many FL dyes aggregate when dispersed in aqueous media or bound to biological polymers in large quantities. Self-quenching often accompanies the aggregation of the dyes, resulting in drastic reductions in their FL signals. This aggregation-caused quenching (ACQ) has been a thorny problem in the development of efficient bioprobes and biosensors.[7] Recently, we and others have observed a phenomenon exactly opposite to the ACQ, that is, aggregation-induced emission (AIE): some nonemissive dyes can be induced to emit efficiently by the aggregate formation.[8–10] AIE molecules with high quantum yields (Φ_F up to 0.85) and various emission colors (blue, green, yellow and red) have been reported.[8,9] While the AIE dyes have been used for the construction of efficient optical and photonic devices,[11] the possibility of employing them as bioprobes for detecting biopolymers have been virtually unexplored.[12] In this work, we synthesized a group of AIE-active tetraphenylethylene (TPE) derivatives 1–4 (Chart 1) and examined the utility of water-soluble cationic salts 3 and 4 as bioprobes. In the aqueous buffer solutions, these non-emissive fluorophores become highly emissive upon binding to protein and DNA molecules through noncovalent, such as hydrophobic and electrostatic, interactions.

The TPE derivatives were prepared by the synthetic route shown in Scheme S1 (ESI†). Reactions of 1,2-bis(4-hydroxyphenyl)-1,2-diphenylethene with α,ω-dibromoalkanes in the presence of sodium hydride yielded TPEs 1 and 2, whose quaternizations by NEt₃ gave salts 3 and 4, respectively. Molecular structures of the TPEs were characterized by spectroscopic techniques, from which satisfactory analysis data were obtained (ESI†). Dyes 1 and 2 are soluble in common organic solvents such as acetonitrile (AN), chloroform and THF but insoluble in water. Salts 3 and 4, on the other hand, are soluble in water as well as DMF and DMSO.

Dilute solutions of 1 and 2 in AN are practically nonluminescent. Addition of nonsolvent water into the AN solutions can turn on the emissions of the dyes. From the molecular solution in AN to the aggregate suspension in an AN–water mixture (1 : 99 by volume), the FL intensity of 1 at 476 nm is increased by ~240 fold (Fig. 1A). Its absorption maximum shifts from 310 nm in the solution to 330 nm in the suspension. The excitation maximum of 1 locates at 330 nm (Fig. S1, ESI†), coinciding well with its absorption maximum. The formation of nanoscopic aggregates of 1 is suggested by the level-off tail in the visible region of its absorption spectrum due to the Mie effect of the nanoparticles.[13] Evidently, the emission of 1 is induced by the aggregate formation, or in other words, 1 is AIE-active.

The change of Φ_F value of 1 with water fraction in the AN–water mixture further reveals its AIE characteristics (Fig. 1B). In the mixtures with water fractions below ~40%, 1 exhibits negligibly small Φ_F values (~0.5%, ESI†) because the dye molecules are genuinely dissolved in the mixtures. The Φ_F value of 1 starts to increase when the water fraction is increased to
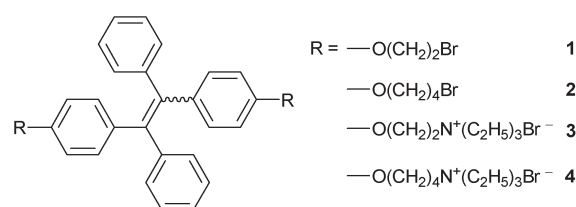
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† Electronic supplementary information (ESI) available: preparation and characterization details for dyes 1–4; absorption, emission and excitation spectra of their aggregate suspensions and biopolymer complexes; effects of solution concentration and solvent viscosity on their photophysical properties; and temperature dependence of ¹H NMR spectra of 1 and associated ln δ_{fwhm} *vs* 1/T plot. See DOI: 10.1039/b608425g


Chart 1 Structures of tetraphenylethene derivatives 1–4.
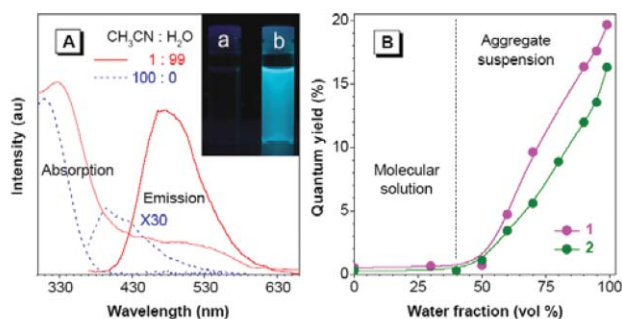
Fig. 1 (A) Absorption and emission spectra of solutions of **1** (10 μM) in AN and AN–water mixture (1 : 99 v/v). Inset: Photographs of solutions of **1** in (a) AN and (b) the AN–water mixture taken under illumination of a UV lamp. (B) Dependence of fluorescence quantum yields of solutions of **1** and **2** on the solvent composition of AN–water mixture. λ_{ex} = 350 nm.

~50%, at which the solvating power of the mixture is worsened to such an extent that the dye molecules begin to aggregate. The Φ_F value reaches ~20% at a water content of 99%, which is ~40-fold higher than that of its AN solution. The absolute Φ_F values of the aggregates should be much higher than the relative Φ_F values given in Fig. 1B, because the determination of the latter did not take into consideration the strong absorption caused by the Mie effect of the aggregates.[14]

Dye **2** shows similar AIE behaviours (Table S1 and Fig. S3, ESI†). Salts **3** and **4** are soluble in water. Addition of methanol, AN, THF and dioxane to their water solutions cannot cause the salts to aggregate, possibly due to their amphiphilic nature. Their emissions in the mixtures remain as faint as those in the water solutions. However, increasing the concentrations of the salts can increase their Φ_F values, implying that the salts are also AIE-active (Fig. S3, ESI†).

Complexation of the water-soluble AIE dyes with calf thymus (ct) DNA and bovine serum albumin (BSA) were investigated by spectrometric titrations in aqueous phosphate buffer (pH = 7.0) at 25 °C. Stock solutions of **3** and **4** (0.25 mM) were prepared (ESI†). The mixture of 100 μL stock solution of **3** with 9.9 mL buffer emits faintly at 395 nm with a side band at 462 nm (Fig. S4, ESI†). Its absorption maximum locates at 311 nm, with a molar absorptivity of 12400 M^{-1} cm^{-1}. Upon addition of the DNA, FL intensity of **3** is increased by 5.4 fold. Meanwhile its emission maximum shifts to ~462 nm, giving a Stokes shift as large as 134 nm. In the DNA concentration range of 0–100 μg mL^{-1}, the plot of the FL intensity (*I*) at 462 nm as a function of DNA concentration (*c*) is a linear line with a correlation coefficient of 0.996. Addition of BSA to buffer solution of **3** induces a similar effect. The linear range of the $I/I_o - 1$ *vs.* *c* plot in this case is 0–50 μg mL^{-1}. The excitation maximums of the solutions of **3** containing BSA and ct DNA both locate at 328 nm (Fig. S5, ESI†).

The effects of the biopolymers on the FL properties of **4** are much more pronounced. As can be seen from Fig. 2, I/I_o values as high as 16.3 and 23.8 are achieved when 300 μg mL^{-1} ct DNA and 500 μg mL^{-1} BSA are added into the solutions of **4**, respectively. Clearly **4** is a more sensitive bioprobe. The excitation maximum is at 328 nm and the Stokes shift is ~135 nm. The linear ranges of **4** are narrower: 0–20 μg mL^{-1} for DNA and 0–40 μg mL^{-1} for BSA. It now becomes obvious that the AIE salts **3** and **4** can be
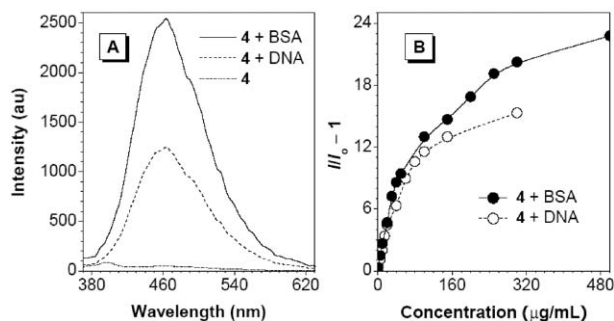

Fig. 2 (A) Emission spectra of **4** (2.5 μM) in an aqueous phosphate buffer (pH = 7) and in the buffers containing 300 μg mL^{-1} ct DNA and 500 μg mL^{-1} BSA. (B) Plots of fluorescence intensities of buffer solutions of **4** at 463 nm *vs.* concentrations of ct DNA and BSA.

used as light-up bioprobes for DNA and protein detection. The probing sensitivity and linear range can be tuned by modifying their structures.

Regarding the origin of the emission induced by the addition of the biomacromolecules, the correlation with the AIE nature of the dyes must be considered. In both cases, similar shifts in the FL maximums (from 390–399 nm to 463–478 nm) are observed. The excitation spectra of the biopolymer-induced emissions are also similar to those of the AIEs for the TPE derivatives. These facts lead to a natural conclusion that the strong blue emissions are from the same excited species.

We have proposed that the restriction of intramolecular rotations in the aggregates of AIE dyes may have blocked their nonradiative channels, thus making them highly emissive.[15] If the AIE process of the TPE dyes follows the same mechanism, they should become emissive in the solutions with high viscosities at low temperatures, because under these conditions their intramolecular rotations would be hampered. The FL behaviours of **4** were thus investigated in a highly viscous glycerol–water (99 : 1 by volume) mixture at different temperatures. At 25 °C, the glycerol–water solution of **4** emits a strong blue light of 467 nm with a Stokes shift of 147 nm (Fig. 3), demonstrating that the high viscosity indeed helps. As the solution temperature is decreased from 25 to −5 °C, the FL intensity of **4** is increased just as expected. Its excitation maximum locates at 328 nm (Fig. S6, ESI†), close to those of its nanoscopic aggregates and its complexes with the biopolymers.
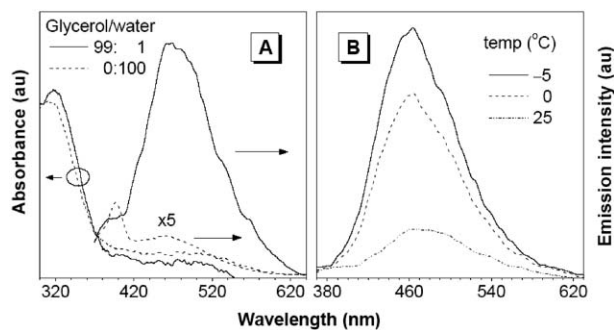
Fig. 3 (A) Absorption and emission spectra of **4** (2.5 μM) in water and a glycerol–water mixture at 25 °C. (B) Emission spectra of **4** (2.5 μM) in glycerol–water (99 : 1 by volume) mixture at different temperatures.

It is well known that fast conformational exchanges caused by fast intramolecular rotations give sharp NMR resonance peaks, which can be broadened by cooling because the rotations and hence the exchanges become slower at lower temperatures.[16] Dynamic NMR experiments of a dichloromethane solution of **1** reveals that its resonance peaks are broadened with a decrease in temperature (Fig. S7, ESI†). The plot of $\ln \delta_{fwhm}$ *vs.* $1/T$ gives a linear line (Fig. S8, ESI†), suggesting a single mechanism for the peak broadening. All these results confirm that the restriction of intramolecular rotations plays a crucial role in the AIE process.

We now may conjure up the picture of how the emissions of the TPE salts are turned on by the addition of the biomacromolecules. In the buffer solutions containing the DNA and BSA, the cationic amphiphilic dyes bind to the biomacromolecules *via* noncovalent interactions, such as electrostatic attraction (especially for the negative-charged DNA) and hydrophobic effect (particularly for the protein with hydrophobic pockets in its native folding structure). When docked on the surfaces of the biopolymers and in the cavities of their folding structures, the dye molecules aggregate with the aid of strong electronic and hydrophobic interactions between their aryl rings. This suppresses intramolecular rotations of the dye molecules, which in turn impedes their radiationless transitions and activates their FL processes. Thanks to the AIE nature, the emissions of the TPE–biopolymer complexes are greatly intensified with increasing concentration, as can be seen from the example given in Fig. S9 (ESI†) for the **4**–BSA complex. This is truly remarkable, because conventional FL probes suffer from the ACQ problem at high dye concentrations.[7]

In summary, in this work, we have successfully developed AIE-active, water-soluble, cationic dyes for protein and DNA detection in the aqueous media for the first time. The non-emissive dye solutions become emissive upon addition of the DNA and BSA. These AIE dyes exhibit large molar absorptivities, high quantum yields and wide Stokes shifts and are thus ideal "turn-on" FL bioprobes. The restriction of their intramolecular rotations is believed to play a critical role in their AIE processes. We envision that any molecule whose electronic conjugation is affected by the twisting of multiple pendants around its core due to involved steric effects can be AIE active. This work opens a wide avenue for utilizing AIE luminophors as FL probes in the area of biological research. Further studies of other AIE dyes with green, yellow and red emissions for detecting biological species are currently under way in our laboratories.

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