Communications

Modulating aggregation-induced emission via a non-conjugated linkage of fluorophores to tetraphenylethenes†

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A fluorophore consisting of two fluorescent moieties could display unusual optical behaviors that are unattainable in a single-fluorophore compound. Herein we reported two “dual-fluorophore” dyes: DNS-linked tetraphenylethenes demonstrate bright aggregation-induced emission, while NBD-linked tetraphenylethenes exhibit aggregation-caused quenching. Our results have important implications for engineering emission behaviors of molecular aggregates for practical applications.

The exploitation of highly emissive fluorescent dyes has attracted considerable research interests, owing to their broad applications in photoelectronic materials,1 biological sensors,2 chemosensors,3 luminescent materials,4 and so on. However, most of the organic fluorescent dyes are weakly emissive or non-emissive in the solid state due to the notorious aggregation caused quenching (ACQ) effect.5 In contrast, Tang’s group discovered an aggregation-induced emission (AIE) phenomenon which is exactly opposite to ACQ.6 Since then, various AIE compounds, many of which are conjugated tetraphenylethylene (TPE) derivatives, have been reported and used in numerous applications.7,8 Recently, TPE backbones have been expanded via non-conjugated incorporation of other fluorescent dyes, such as rhodamine9 and borondipyrromethene (BODIPY)10 to further adjust their optical behaviors for different applications. For example, Jia and Ma et al. linked a rhodamine compound to TPE via an amino-containing alkyl chain. The resulting compound showed a mechanochromic fluorescence with a sequential tricolor change upon grinding.9d Liu et al. synthesized a rhodamine-modified TPE derivative containing a tumor-targeting RGD peptide; this compound could be used to monitor the generation of singlet oxygen during the photodynamic ablation of tumor cells.9f

In this communication, we demonstrate that the AIE behaviors of TPE could be effectively adjusted via the non-conjugated linkage of one additional fluorophore. We have attached two commercially available dyes, a dansyl dye (DNS) and 4-methylamino-7-nitrobenzo-2,1,3-oxadiazole (NBD), to TPE (Scheme 1). Our results show that the fluorescence intensity of TPE-DNS experienced a slight decrease, followed by a significantly AIE induced enhancement, as the ratio of water content increases in the acetonitrile–water binary mixture. In contrast, TPE-NBD displayed a monotonous drop in the emission intensity and an ACQ characteristic as more water was added. We have analysed the underlying mechanism for these distinct behaviors via both experimental measurements and quantum chemical calculations based on (time-dependent) density functional theory [(TD)-DFT]. In addition, we have demonstrated that both

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Scheme 1  The structures of compounds TPE-MA, TPE-DNS, TPE-NBD, TPE, Bu-DNS and Bu-NBD.

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TPE-DNS and TPE-NBD showed good biocompatibility for live cell imaging.

We synthesized TPE-DNS and TPE-NBD via a one-step reaction of \(\{4(1,2,2\text{-triphenylvinyl})\text{phenyl}\}_\text{methanamine (TPE-MA) with dansyl chloride (DNS-Cl) or 4-chloride-7-nitrobenzo-2,1,3-oxadiazole (NBD-Cl) in the presence of Et}_3\text{N, respectively (yields: 30–53%). Theamine group in TPE-MA allows easy linkage of other fluorophores to TPE (Scheme 1).}\)

After that, we studied AIE behaviors of TPE-DNS and TPE-NBD in the binary mixture of CH\(_3\text{CN}\) and water. The UV-Vis absorption spectra of TPE-DNS exhibit the main absorption band peaked at \(\approx 305\) nm (Fig. S1A, ESI†). This absorption band resulted from the overlapping absorptions from both TPE and DNS moieties, as reflected by the UV-Vis absorption spectra of two reference compounds—TPE and Bu-DNS (Scheme 1 and Fig. S2A, S3A, ESI†). Indeed, time-dependent density functional theory (TD-DFT) calculations predict two intense and close absorption transitions for TPE-DNS in acetonitrile at \(\approx 344\) and \(\approx 380\) nm, respectively (Fig. S5 and Table S1, ESI†). The first transition (dominated by HOMO → LUMO+1) was mainly assigned to the TPE moiety whereas the electron densities involved in the second transition (dominated by HOMO−1 → LUMO) were largely delocalized in the DNS unit. In contrast, TPE-NBD displayed two distinct absorption bands, peaked at \(\approx 459\) nm and \(\approx 346\) nm, respectively (Fig. S1B, ESI†). These two peaks are attributed to the absorption of NBD and TPE fragments, respectively, according to our studies on reference compounds TPE and Bu-NBD as well as TD-DFT calculations (Scheme 1, Fig. S2A, S6A and Table S1, ESI†). Nevertheless, no obvious shifts in the UV-Vis absorption spectra of TPE-DNS and TPE-NBD were observed when we varied the volume fractions of water (\(f_\text{w}\)) in the CH\(_3\text{CN}\)–water mixture.

The AIE behavior of TPE-DNS was clearly demonstrated in its fluorescence spectra. The emission peaks of TPE-DNS exhibited a complicated solvatochromic shift (Fig. 1a). In pure CH\(_3\text{CN}\), TPE-DNS displayed a yellow-green fluorescence at \(\approx 509\) nm and the absolute fluorescence quantum yield was 32.4%. With increasing \(f_\text{w}\), the emission intensity of TPE-DNS gradually decreased in conjunction with a red-shift in the peak emission wavelengths and the absolute fluorescence quantum yield showed a slight reduction (from 32.4% to 28.6%) (Fig. 1b and c). The red shift is mainly due to the intramolecular charge transfer (ICT) effect of the DNS moiety in response to the increasing solvent polarity of the CH\(_3\text{CN}\)–water mixture, as \(f_\text{w}\) increases from 0 to 60% (Fig. S3B and S4A, ESI†). When \(f_\text{w}\) increased to >60%, the emission intensity of TPE-DNS increased dramatically. Simultaneously, the emission peak experienced a blue-shift from 537 to 492 nm with enhanced fluorescence quantum yields of up to 94.1% (Fig. 1a and b); the emission color changed from yellow to blue-green (Fig. 1c). We attributed the intensification and blue shift of TPE-DNS emissions to considerable molecular aggregation and AIE, resulting from the TPE moiety (Fig. S2B, ESI†). The formation of molecular aggregates was further proved by dynamic light scattering (DLS) and transmission electron microscopy (TEM) (Fig. 1d and Fig. S7, ESI†). For example, the effective diameter of TPE-DNS aggregates amounts to \(\approx 156\) nm in the acetonitrile–water mixture (Fig. 1d). In comparison to the aggregation turn-on threshold of TPE-DNS (\(f_\text{w} = 60\%)\), the aggregation of TPE (the reference compound) becomes apparent only when \(f_\text{w}\) increases to 80% (Fig. S2, ESI†). These results suggested that the introduction of a DNS fluorophore reduces the solubility of TPE-DNS and lowers the AIE turn-off threshold to a small \(f_\text{w}\) value.

In contrast, TPE-NBD displayed a different aggregation behaviour. TPE-NBD showed a green emission peaked at 520 nm in pure CH\(_3\text{CN}\) and the absolute fluorescence quantum yield was 1.6% (due to the emission of the NBD moiety, excited at 450 nm). Along with increasing \(f_\text{w}\), the emission intensity of TPE-NBD gradually decreased and the fluorescence quantum yield dropped to 0.9%, in conjunction with a red-shift from 520 nm to 554 nm (Fig. 2). This change in fluorescence colors is partly attributed to the ICT effect of the NBD moiety (Fig. S4B and S6, ESI†). Similarly, the drop of fluorescence intensities in more aqueous solvents (with increasing \(f_\text{w}\)) is partially related to the intensive hydrogen bond interactions around the monomers of the NBD moiety.\(^{13}\) Interestingly, by varying \(f_\text{w}\) from 0 to 99%, we did not observe a noticeable AIE emission of the TPE moiety throughout the course (even when excited at 340 nm; Fig. S8, ESI†).

Although there is a lack of noticeable AIE emissions from the TPE moiety, both DLS experiments and TEM images clearly proved the formation of molecular aggregates in the TPE-NBD solution when the water fraction reached 90% (Fig. 2d and Fig. S7B, ESI†). The absence of TPE emissions in these aggregates is likely caused by efficient Förster resonance energy transfer from the TPE to the NBD moieties (Fig. S2B and S6A, ESI†). Moreover, although considerable energy is transferred to
the NBD moiety, the fluorescence of NBD seems to be significantly quenched in TPE-NBD aggregates.

An efficient energy transfer from TPE to NBD moieties in TPE-NBD was further proved by physically mixing TPE and Bu-NBD in the acetonitrile–water solution (Fig. S9, ESI†). The average distance between TPE and NBD moieties becomes larger in the physical mixture than that in TPE-NBD, thereby suppressing the potential energy transfer. Indeed, we observed a weak emission peak due to the TPE aggregates at ~450 nm in the physical mixture, when fNBD increased to >60%. The appearance of this peak fully matches incomplete energy transfer from TPE to Bu-NBD in the physical mixture.

The starkly different emission characteristics of TPE-NBD and TPE-DNS aggregates also motivated us to explore their intermolecular interactions in the solid state. We obtained the crystal structures of TPE-DNS and TPE-NBD via X-ray diffraction experiments (Fig. 3a, b and Tables S2, S3, ESI†). In packed TPE-NBD molecules, strong hydrogen bond interactions connected the neighbouring NBD moieties in a head-to-tail (and J-aggregate like) manner (O·H–N distance: 2.888 Å; Fig. 3c). The strong dipole–dipole interactions between these polar NBD moieties lead to significantly red-shifted emissions peaked at 592 nm in the solid state (Fig. S10, ESI†), in comparison to ~520 nm in acetonitrile. Besides, we noticed weak π–π stacking interactions between the two layers of NBD moieties (Fig. S11, ESI†). Consequently, intermolecular hydrogen bond and π–π stacking interactions greatly reduce the emission intensities, making TPE-NBD an ACQ compound.14

Likewise, hydrogen bond interactions are also present between the DNS moieties in the TPE-DNS crystal (O·H–N distance: 2.840 Å; Fig. 3d), but no π–π stacking interactions are observed. These hydrogen bond interactions could diminish the fluorescence of DNS. Nevertheless, there is a lack of efficient FRET from TPE to DNS, due to the mismatch between the TPE emission and DNS absorption spectra. Consequently, bright emissions from the aggregated TPE moieties endow TPE-DNS with an AIE characteristic. A similar AIE phenomenon is also observed in the physical mixture of TPE and Bu-DNS, due to the AIE of TPE molecules (Fig. S9, ESI†).

On account of the fluorescence properties of TPE-DNS and TPE-NBD, we applied them in bioimaging of live cells. Firstly, the cytotoxicity of TPE-DNS and TPE-NBD at different concentrations was evaluated by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in HeLa cells. Both TPE-DNS and TPE-NBD exhibited low cellular toxicity and good biocompatibility. After 24 hours of incubation, the cell viability of HeLa cells remains at >88% at dye concentrations of up to 10 μM and at >83% at dye concentrations of up to 200 μM (Fig. S12, ESI†).

Next, we incubated HeLa cells in TPE-DNS and TPE-NBD solutions (5 μM) at 37 °C for different durations (0, 15, 30, 45, and 60 min), and then washed these cells with PBS. The fluorescence images were collected using a confocal microscope (LCFM). In cells stained with TPE-DNS and TPE-NBD, weak emissions were detected after 15 min of incubation, mainly in the cytoplasm of HeLa cells (Fig. 4 and Fig. S13, ESI†). Moreover, the fluorescence intensity gradually increased at a longer incubation time. Overall, these bioimaging experiments indicated that TPE-NBD and TPE-DNS had good cell permeability, and could be deployed in fluorescence bioimaging.

In conclusion, based on TPE-MA and its facile reaction, we demonstrate that the AIE effect is adjustable via a non-conjugated linkage of tetraphenylethane (TPE) to another fluoroscent moiety (i.e., DNS or NBD). We find that TPE-DNS demonstrates a higher tendency to molecular aggregation than unsubstituted TPE and displays a bright AIE. In contrast, TPE-NBD exhibited aggregation caused quenching (ACQ), due to an efficient energy transfer from TPE to NBD moieties and strong intermolecular interactions between NBD moieties. Finally, both TPE-DNS and TPE-NBD involve simple synthesis
and exhibit excellent biocompatibility and cell permeability in bioimaging of live cells. We expect that our strategy provides new insights into modulation of AIE behaviors of TPE-based materials for fluorescence applications.

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Notes and references


