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A tetraphenylethene-based red luminophor for an efficient non-doped electroluminescence device and cellular imaging†

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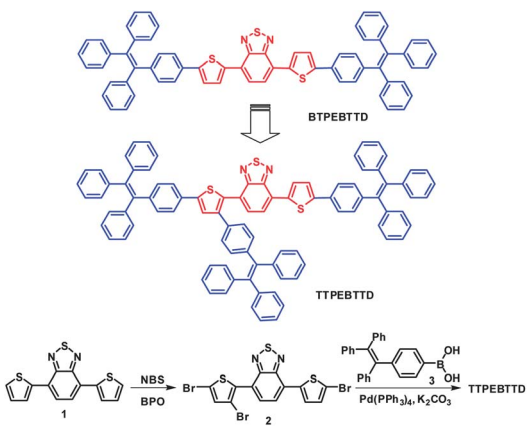
An efficient red luminophor (TTPEBTTD) consisting of a 4,7-di(thiophen-2-yl)benzo-2,1,3-thiadiazole core and tetraphenylethene peripheries is developed. The non-doped electroluminescence device based on TTPEBTTD radiates red light with high efficiency up to 3.7%. The nanoparticles of TTPEBTTD are promising fluorescent visualizers for cellular imaging with low cytotoxicity.

Red organic luminescent materials are keenly pursued for their diversiform potential applications in optoelectronics and biotechnology. To achieve red emission, the dye molecules are generally constructed from fused planar rings with extended conjugation[1] or possess strong dipoles stemmed from π-conjugated electron-donating and accepting groups. Examples of such luminogens include pyran-containing dyes (*e.g.* DCM),[2] Nile red,[3] benzo-2,1,3-thiadiazole-based chromophores[4] and BODIPYs.[5] Although these red dyes are quite emissive in the solution state, they are highly susceptible to concentration quenching due to the strong dipole–dipole interactions and/or intermolecular π–π interactions. As a result, their nanoparticles or solid films can only offer weak emissions.[6] Adopting branched and non-planar molecular architectures is a widely used approach in molecular design to disrupt chromophore aggregation and alleviate π–π interactions.[7–9] However, there are only limited reports available in the literature that present the preparation of efficient red luminescent materials with high brightness and good color fidelity in the solid state.

In our previous studies on efficient luminescent materials, we found that a series of propeller-like molecules were non-fluorescent when molecularly dissolved in solutions but were induced to emit intensely by aggregate formation.[10,11] Such a novel phenomenon was coined as aggregation-induced emission (AIE) and was rationalized to be caused by the restriction of intramolecular rotation (IMR) in the aggregate state, which blocks the nonradiative relaxation channel and populates excitons that relax radiatively. Such finding has paved a new path for researchers to design and synthesize efficient luminophors. Among various AIE-active luminogens, tetraphenylethene (TPE) is an archetypal molecule with a simple chemical structure but possesses a marvellous AIE characteristic.[11] TPE and its derivatives have found multifarious applications as emitters for organic light-emitting diodes (OLEDs), fluorescent chemosensors,[12] bioprobes,[13] *etc.* In addition, TPE is a versatile building block for the construction of efficient solid-state emitters. For example, covalent melding of TPE with conventional chromophores with aggregation-caused quenching effect at the molecular level can generate AIE luminogens with high solid-state photoluminescence (PL) efficiencies and outstanding electroluminescence (EL) performances.[11,14] Recently, we prepared a red luminophor BTPEBTTD (Scheme 1) by end-capping 4,7-di(thiophen-2-yl)benzo-2,1,3-thiadiazole (BTTD) with TPE units. The non-doped OLED based on BTPEBTTD exhibited red EL emission at 668 nm. The luminance and efficiency attained by the



Scheme 1 Chemical structures of BTPEBTTD and TTPEBTTD, and the synthetic route to TTPEBTTD.

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† Electronic supplementary information (ESI) available: Synthetic procedures, EL device fabrication, synthesis of TTPEBTTD-DSPE NPs, cell culture and imaging, cytotoxicity of TTPEBTTD-DSPE NPs, Fig. S1–S5, and other materials. See DOI: 10.1039/c2jm31482g

device, however, were not satisfactory,[14e] probably due to the strong intermolecular interaction between the linear and planar BTTD segments, which promotes the formation of detrimental species such as excimers and exciplexes. To enlarge the family of red luminophors, we elaborate a new luminophor, named TTPEBTTD, by the attachment of an additional TPE unit as a branch to the BTPEBTTD core (Scheme 1). Since the PL and EL spectra of the TPE film overlap well with the absorption spectrum of BTPEBTTD (Fig. S1 in the ESI†), it is envisioned that efficient energy transfer from the TPE side group to the BTPEBTTD segment occurs in the solid state.[15] At the same time, the branched conformation of TTPEBTTD can hamper close packing between molecules, which minimizes the energy loss through nonradiative pathways. These effects work collectively to endow the resulting luminophor with novel light-emitting property. Herein, we show how this luminogen is prepared and present its thermal and photophysical properties as well as its applications as host emitter in the OLED and fluorescent visualizer for intracellular imaging.

TTPEBTTD was prepared according to the synthetic route shown in Scheme 1. The detailed procedures and characterization data are given in the ESI†. Intermediates **1** and **3** were prepared according to our previously published methods.[14e] Bromination of **1** with *N*-bromosuccinimide (NBS) in the presence of benzoyl peroxide (BPO) in refluxed carbon tetrachloride gave an unprecedented intermediate **2**, which afforded the target product TTPEBTTD in a moderate yield *via* Suzuki coupling reaction with **3**. TTPEBTTD is soluble in common organic solvents, such as THF, toluene and chloroform. Thermogravimetric analysis and differential scanning calorimetry measurement reveal that TTPEBTTD is thermally and morphologically very stable, losing merely 5% of its weight at 465 °C (T_d) and showing a high glass-transition temperature (T_g) of 174 °C (Fig. S2†).

TTPEBTTD absorbs at 490 nm in THF solution, which is 20 nm blue-shifted from that of BTPEBTTD (Fig. 1). This is indicative of a lower molecular conjugation. When its dilute THF solution (10 μM) is photoexcited, it emits intense red light peaked at 620 nm. The fluorescence quantum yield (Φ_F) estimated using rhodamine B (Φ_F = 50% in ethanol) as standard is reasonably high and equal to 18%, implying that the IMR process of the TPE units has not quenched completely the light emission of the luminogen.[14e] The TTPEBTTD film is also emissive and emits at 646 nm, nicely meeting the requirement for the construction of red OLEDs. The PL spectrum of the TTPEBTTD film is red-shifted from that of the solution by 26 nm. Compared with BTPEBTTD (38 nm),[14e] such an extent of emission shift is small, presumably due to its branched conformation, which better hampers the interactions between the TTPEBTTD molecules. The PL spectra in both solution and solid states are independent of the excitation wavelength. TTPEBTTD can be excited by lights in the absorption region of TPE (~330 nm) owing to the energy transfer from the TPE units to the BTPEBTTD core.

To better understand the photophysical property of TTPEBTTD, density functional theory (DFT) calculation was carried out using a suite of Gaussian 03 program. The nonlocal density functional of B3LYP with 6-31G(d) basis sets was used for the calculation. The optimized molecular structure and molecular amplitude plots for the HOMO and LUMO of TTPEBTTD are shown in Fig. 2. Due to the steric congestion caused by the additional TPE unit, the backbone of TTPEBTTD is highly twisted. This shortens its effective conjugation length, endowing it with a wider band-gap (2.37 eV) than that of BTPEBTTD (2.28 eV).[14e] The theoretical study thus nicely explains the hypsochromic shifts in the absorption and emission of TTPEBTTD from those of BTPEBTTD. The HOMO of TTPEBTTD is dominated by the orbitals from the backbone composed of the BTTD core and TPE terminals. The TPE branch, however, contributes less to the energy level, suggesting that it is only weakly conjugated with the main segment. The LUMO, on the other hand, is dominated by the orbitals from the BTTD core and almost no contribution from the TPE units is observed. The HOMO and LUMO energy levels of TTPEBTTD determined by cyclic voltammetry are all lower than the calculated values and are equal to −5.57 eV and −3.68 eV, respectively (Fig. S3†).

To prevent concentration quenching of most red dyes, doping is a widely used approach in OLED fabrication. However, such method is rather complicated. Generally, efficient doping is achieved at a low concentration, where the dopant molecules are well segregated to promote good energy transfer. Inefficient energy transfer from host to dopant will result in a mixture of two emission profiles and hence an unsaturated color light. Because a precise and consistent control of the dopant concentration is required, this makes the doping system a challenging task for high speed and mass production.[6] In this regard, efficient red emitters are crucial and highly desirable for the fabrication of non-doped OLEDs. To investigate the EL property of TTPEBTTD, a non-doped OLED with a configuration of ITO/NPB (60 nm)/TTPEBTTD (20 nm)/TPBi (40 nm)/LiF (1 nm)/Al (100 nm) was fabricated by vapor deposition processes in which TTPEBTTD served as emitting layer, *N*,*N*-bis(1-naphthyl)-*N*,*N*-diphenylbenzidine (NPB), and

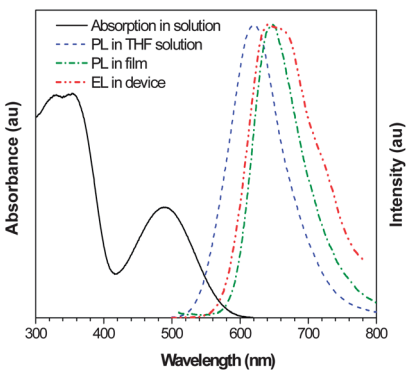

Fig. 1 Absorption, PL and EL spectra of TTPEBTTD.

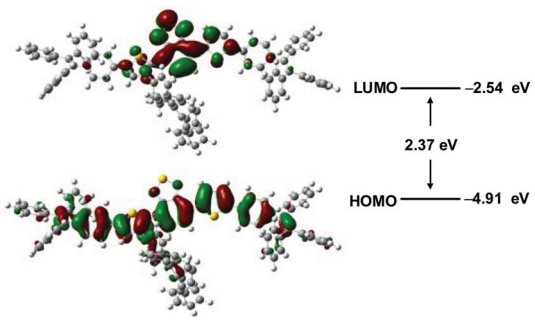

Fig. 2 Optimized molecular structure and molecular orbital amplitude plots of HOMO and LUMO energy levels of TTPEBTTD calculated using the B3LYP/6-31G(d) basis set.

2,2′,2″-(1,3,5-benzinetriyl)tris(1-phenyl-1-*H*-benzimidazole) (TPBi) functioned as hole- and electron-transporting layer, respectively. Due to the matching of the energy levels between NPB, TTPEBTTD, and TPBi layers, which facilitates the charge injection to the TTPEBTTD layer, the device is turned on at a relatively low voltage of 4.2 V. The device radiates intense red light at 650 nm (Fig. 3) with Commission International d'Eclairage (CIE) chromaticity coordinates of (0.67, 0.32). The maximum luminance (L_{max}), maximum current efficiency ($\eta_{C,max}$) and maximum external quantum efficiency ($\eta_{ext,max}$) attained by the device are 3750 cd m^{-2}, 2.4 cd A^{-1}, and 3.7%, respectively, which are much better than those of EL devices of BTPEBTTD (1640 cd m^{-2}, 0.4 cd A^{-1} and 1.0%)[14e] and other red dyes reported previously.[6] The efficient energy transfer from the TPE branch to the BTPEBTTD backbone and the obstruction of strong intermolecular interactions between the BTTD cores may be the possible reasons for the good EL property of TTPEBTTD. Although the device configuration is yet to be optimized, the good EL data clearly demonstrate the great potential of TTPEBTTD as red emitter for OLED application.

Red fluorescent dyes are in high demand for applications in biological systems because they can be excited by long-wavelength excitation sources that cause low harm to cells. However, most organic luminogens become weakly fluorescent when they are dispersed in aqueous media or accumulated in cells due to the emission quenching by molecular aggregation. Our recent research demonstrates that red luminogens with TPE units can serve as biocompatible stains for *in vitro* and *in vivo* cell imaging, thanks to their efficient emission in the aggregate state.[13b] To further explore the practical application of TTPEBTTD, we employed it as fluorescent visualizer for cellular imaging. The TTPEBTTD-loaded DSPE nanoparticles (TTPEBTTD-DSPE NPs) were synthesized by a modified nanoprecipitation method using 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (DSPE-PEG_{2000}) as the encapsulation material. Encapsulating TTPEBTTD NPs in the DSPE-PEG_{2000} matrix renders the resulting NPs with excellent biocompatibility.[16] The TTPEBTTD-DSPE NPs are formed spontaneously upon sonication and the obtained NPs can be stored for several months without obvious aggregation. The morphology of the TTPEBTTD-DSPE NPs was studied by high-resolution transmission electron microscopy. As shown in Fig. S4A†, the spherical NPs can be clearly visualized as black dots due to the high electron density of TTPEBTTD molecules. The average hydrodynamic diameter of the TTPEBTTD-DSPE NPs measured by laser scattering is 63 nm with a narrow size distribution (Fig. S4B†). The TTPEBTTD-DSPE NPs absorb at 500 nm and emit at 670 nm (Fig. S5†). The Φ_F measured using rhodamine 6G in ethanol as standard is 14%, which is close to the value (12%) of bare TTPEBTTD nanoparticles but is still much higher than those of Nile red (0%) and DCM (5%) in the solid state.

The application of TTPEBTTD-DSPE NPs for *in vitro* cellular imaging was studied by confocal laser scanning microscopy (CLSM). After incubation with TTPEBTTD-DSPE suspension (2 μM dye) for 2 h at 37 °C in culture medium, MCF-7 breast cancer cells were fixed and the cell nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI). The cells were subsequently imaged by CLSM with 488 nm laser excitation and the fluorescence signals were collected above 650 nm for the NPs and 560 nm for DAPI. As shown in Fig. 4A, intense red fluorescence from the cellular cytoplasm is observed. The homogeneous distribution of TTPEBTTD-DSPE NPs in the cytoplasm with strong fluorescence suggests that the encapsulation of TTPEBTTD NPs by DSPE has resulted in an efficient intracellular uptake of the formulated NPs. Moreover, all the NPs are nontoxic as the stained cells are in good healthy conditions. To prove this, the cytotoxicity of the NPs was evaluated through the investigation of the metabolic viability of MCF-7 breast cancer cells in the presence of various concentrations of TTPEBTTD-DSPE NPs (Fig. 5). The cell viability remains ~100% within 72 h under the experimental conditions, indicating the low cytotoxicity of the TTPEBTTD-DSPE NPs

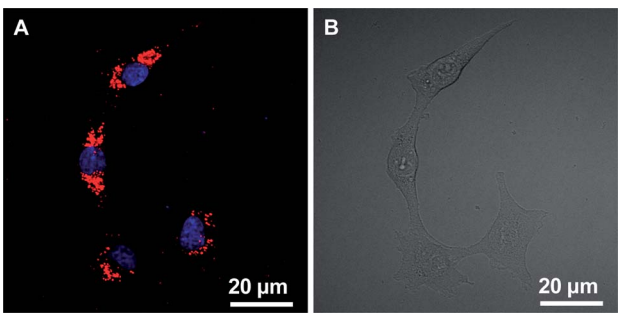

Fig. 4 Confocal images of MCF-7 breast cancer cells after incubation with TTPEBTTD-DSPE NPs for 2 h at 37 °C ([TTPEBTTD] = 2 μM). The fluorescence was recorded under 488 nm excitation wavelength with a 650 nm longpass filter. The blue signal indicates cell nuclei stained by DAPI.

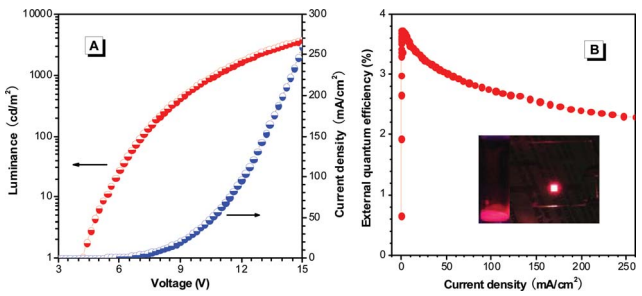

Fig. 3 (A) Changes in luminance and current density with voltage and (B) external quantum efficiency *versus* current density curve of the multilayer EL device of TTPEBTTD. Inset in panel (B): photographs of TTPEBTTD powders taken under UV illumination and TTPEBTTD-based OLED.

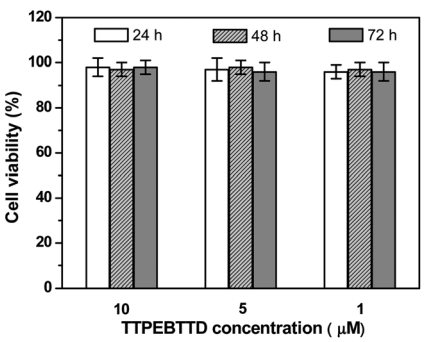

Fig. 5 Metabolic viability of MCF-7 breast cancer cells after incubation with TTPEBTTD-loaded DSPE suspension at different TTPEBTTD concentrations for 24, 48 and 72 h, respectively.

and demonstrating that the NPs are good red fluorescent visualizers for intracellular imaging with high emission contrast.

In summary, in this work, an efficient red luminescent material was synthesized by decoration of traditional chromophores with AIE-active TPE units. The attachment of an additional TPE unit to BTPEBTTD generates TTPEBTTD, which absorbs and emits at shorter wavelengths. The steric effect of the TPE side group has made the luminogen more twisted and hence lowered its molecular conjugation. TTPEBTTD is thermally and morphologically stable with high T_d and T_g of 465 °C and 174 °C, respectively. It performs outstandingly as emitter in the non-doped OLED, showing much higher EL efficiencies than its parent BTPEBTTD. The TTPEBTTD-based OLED radiates red light at 650 nm (CIE = 0.67, 0.32) with a L_{max} of 3750 cd m^{-2} and a high $\eta_{ext,max}$ of 3.7%. In addition, TTPEBTTD shows potential application as remarkable red fluorescent visualizer for intracellular imaging. The TTPEBTTD-DSPE NPs can be internalized in cells without causing adverse effects. All these results demonstrate that the new luminogen possesses multifunctional properties for an array of high-technological applications in optoelectronics and bioscience.

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