ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2014, **50**, 1725

Received 12th November 2013,
Accepted 5th December 2013

DOI: 10.1039/c3cc48625g

www.rsc.org/chemcomm

Molecular luminogens based on restriction of intramolecular motions through host–guest inclusion for cell imaging†

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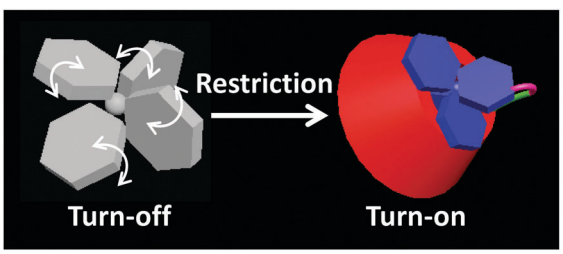
We developed a new strategy to restrict the motions of AIE molecules through host–guest inclusion, affording a catalogue of new molecular luminogens.

Luminogenic molecules with aggregation-induced emission (AIE) characteristics show strong light emission in the solid state and have attracted increasing interest recently due to their potential applications in biosensing, electroluminescent devices, cell imaging and so on.[1] Since our group reported the first AIE molecule in 2001,[2] a large number and a wide variety of AIE molecules have been synthesized based on the mechanism of restriction of intramolecular motions (RIM). Jiang and coworkers restricted the rotation of tetraphenylethene (TPE) by its confinement in the intermolecular network, affording a catalogue of emissive conjugated microporous polymers.[3] Our group enhanced the emission efficiency of TPE in the solution state by locking its phenyl rings *via* covalent bonding.[4] Dinca and coworkers immobilized functionalized TPE within rigid metal–organic framework through coordination polymerization, which turned its fluorescence on.[5] While chemical approaches have proved to be efficient in restricting the intramolecular motions, they involve variations in energy levels and molecular structures, both of which play important roles in the light emission process. This makes the effect of RIM process ambiguous and plausible. Thus, development of physical processes with minimal chemical reactions to restrict the intramolecular motions is highly desirable.

Host–guest inclusion has attracted considerable attention in recent years for its wide applications in nano-machines, smart materials and so on.[6] The guest molecules are accommodated inside the cavity of the host driven by physical interactions such as hydrophobic interaction, which offers a new opportunity for the preparation of new AIE luminogens. However, the utilization of host–guest inclusion to restrict the motions of AIE molecules is unprecedented to the best of our knowledge.

Herein, we report a new strategy for constructing single molecular luminogens through host–guest inclusion between cyclodextrins (CDs) and TPE. As a proof-of-concept demonstration, TPE functionalized with α-, β- or γ-CD is selected as a model compound. We demonstrated that the phenyl rings of TPE resided in the cavity of CD, which restricted their motions and hence turned the fluorescence of non-emissive TPE on (Scheme 1). Since only physical interaction is involved, the effect of motion restriction on the light emission process can be identified. Moreover, given their high emission efficiency and good biocompatibility inherited from TPE and CD, the fluorescent inclusion complexes are promising for biological applications.

The CD-decorated tetraphenylethenes (TPE–CDs) were synthesized by esterification reaction of CD with mono-carboxylic acid-substituted TPE (TPE–CO_2H Scheme S1, ESI†). Detailed procedures for the synthesis of TPE–CO_2H and TPE–CDs were described in the ESI.† All the intermediates and desirable products were characterized using NMR and mass spectrometry and satisfactory results were obtained



Scheme 1 Schematic illustration of tetraphenylethene trapped inside the cavity of cyclodextrin.

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† Electronic supplementary information (ESI) available: Materials and instruments, synthesis and characterization of the TPE-CDs, and NMR, mass, UV and CD spectra are supplied as supporting information. See DOI: 10.1039/c3cc48625g

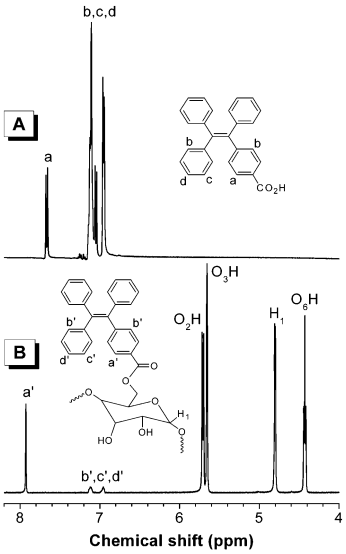
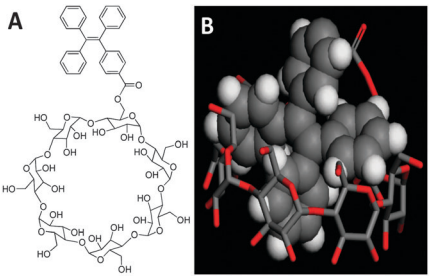


Fig. 1 ^{1}H NMR spectra of (A) TPE–CO_2H and (B) TPE–β-CD in DMSO-d_6.

corresponding to their molecular structures (Fig. S1–S11; ESI†). The ^{1}H NMR spectrum of TPE–β-CD as well as that of TPE–CO_2H is shown in Fig. 1 as an example. A distinct signal attributed to the resonances of the phenyl protons adjacent to the ester group (a′ in Fig. 1) was observed at δ 7.9. The absorptions of other protons (b′, c′ and d′) at δ 7.1 and 6.9 were broad and weak, which was related to the slow relaxation due to the restriction of their intramolecular motions.[7] Nuclear overhauser enhancement (NOE) cross-peaks between the TPE protons and the interior protons of CD cavity were observed in the 2D ROESY NMR spectrum of TPE–β-CD, indicating that they were closely coupled.[8] This also suggested that most of the phenyl rings of TPE were immobilized in the CD cavity (Fig. S12, ESI†). On the other hand, no NOE signals were detected between the resonance peak at δ 7.9 and those inside the β-CD cavity because they were far away from each other. Similar results were found in TPE–α-CD and TPE–γ-CD (Fig. S13 and S14, ESI†). From these results, the geometric structure of TPE–β-CD and the optimized chemical structure of TPE are schematically illustrated in Scheme 2 and Scheme S2 (ESI†). While most of phenyl rings are restricted by cyclodextrin, some of them are still free for motion.

The ^{1}H NMR signals of TPE–β-CD were concentration-dependent. The peaks at δ 7.1 and 6.9 disappeared in highly concentrated solution (Fig. S15, ESI†), which illustrated the formation of an intermolecular complex. It has been well known that adamantane has a strong and specific interaction with cyclodextrin.[9] Adamantane enters preferentially into the cavity of cyclodextrin to form the inclusion complex as compared with phenyl derivatives. After mixing with adamantane, the absorption peaks at δ 7.1 and 6.9 were clearly intensified (Fig. S16, ESI†) because part of the phenyl rings were driven out from the cavity of β-CD owing to the strong and specific interaction of adamantane with CD. This further confirmed the formation of the intermolecular complex in high concentrated solution.

The effect of cavity size of the CD on the RIM process was then investigated. Similarly to TPE–β-CD, the dilute solution of TPE–α-CD exhibited two broad peaks at δ 7.1 and 6.9, which disappeared in high concentrated solution (Fig. S17, ESI†).



Scheme 2 (A) Chemical structure and (B) schematic illustration of the geometric structure of TPE–β-CD.

In contrast, these peaks were still observed in the concentrated solution of TPE–γ-CD, demonstrating the less restriction of γ-CD on the mobility of TPE (Fig. S18, ESI†). Taking the signal at δ 7.9 as a reference, TPE–α-CD and TPE–γ-CD exhibited the weakest and strongest absorption peaks at δ 7.1 and 6.9, respectively. The smaller cavity of α-CD restricted the motions of phenyl rings of TPE to a greater extent than γ-CD with a larger cavity size (Table S1, ESI†), thus resulting in weak aromatic signals.

The UV spectra of all the TPE–CDs showed a broad absorption band at 314 nm, which was blue-shifted from that of the TPE derivative (TPE–C2) carrying no CD substituent (Fig. S19, ESI†). This indicated that the TPE unit of TPE–CDs adopted a more twisted conformation in order to fit into the cavity of CD driven by hydrophobic interaction between the two components. Such interaction was verified using circular dichroism (CD) spectroscopy. A positive signal appeared in the absorption region of TPE in the CD spectra of all the TPE–CDs, suggesting that the CD unit induced the TPE moiety to pack in a helical fashion (Fig. S20, ESI†).[10]

The fluorescence (FL) behaviors of the TPE–CDs are shown in Fig. 2. The dilute dimethylsulfoxide (DMSO) solution of TPE emitted weakly at 470 nm due to the intramolecular motions of the phenyl rings, which had efficiently consumed the energy of the excited states through nonradiative relaxation channels. In a sharp contrast, TPE–β-CD showed a stronger FL at 410 nm, which was 60 nm blue-shifted from that of unsubstituted TPE (Fig. S21, ESI†). The enhanced fluorescence revealed that the motions of the phenyl rings of TPE–β-CD were somewhat restricted and stemmed from the formation of the inclusion complex. The TPE unit included in the CD cavity possessed a more

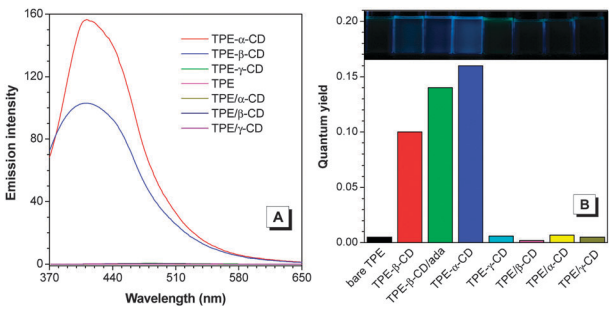


Fig. 2 (A) Fluorescence spectra and (B) quantum yield of TPE–CDs, bare TPE and TPE–CD mixtures (1 : 1 molar ratio) in DMSO solutions. Inset in (B): fluorescent photos of TPE–CDs, bare TPE and TPE–CD mixtures (1 : 1 molar ratio) in DMSO solutions taken under UV irradiation. Concentration: 5 mM.

twisted conformation, thus resulting in a blue-shift in the fluorescence. Compared to TPE–α-CD, TPE–β-CD showed a weaker FL at 410 nm but emitted stronger than TPE–γ-CD. The physical mixtures of TPE and CDs (TPE–CDs) radiated weakly at 470 nm in DMSO solutions under the same experimental conditions. The quantum yields of the TPE–CDs and their physical mixtures were determined using quinine sulfate as a reference (Fig. 2B). Bare TPE showed a low quantum yield (0.5%) in DMSO. On the other hand, TPE–β-CD exhibited a 20-fold higher value. The value was further enhanced by mixing with adamantane, presumably due to the further activation of the RIM process by the bulky size of adamantane. Among the TPE–CDs, TPE–α-CD exhibited the highest FL quantum yield because the smaller cavity of α-CD restricted the motions of phenyl rings more efficiently than the larger-sized γ-CD cavity. Since all the TPE–CD mixtures exhibited low quantum yields, it demonstrated that covalent bonding of TPE with cyclodextrin is crucial for the formation of the inclusion complex. The melding of TPE and CD into one molecule had brought the two units close together, making the inclusion of the phenyl rings to the cavity of CD more easy.

To further verify the mechanism of the emission enhancement, time-resolved fluorescence measurements were carried out. The emission of bare TPE decayed exponentially with a lifetime of 1.41 ns (Fig. S22 and Table S2, ESI†). In contrast, the excited state of TPE–β-CD relaxed mainly through a slow pathway with a lifetime of 8.23 ns. More interestingly, TPE–β-CD showed a much lower non-radiative decay rate constant (10.2×10^7 s^{-1}) and by a far higher radiative decay rate constant (1.94×10^7 s^{-1}) as compared to bare TPE (70.6×10^7 s^{-1} and 0.355×10^7 s^{-1}, respectively, for bare TPE). In bare TPE solution, the energy of the excited states was annihilated efficiently by active intramolecular motions, which led to high non-radiative decay rate and low radiative decay rate as well as a significantly shortened lifetime. On the other hand, the motions of the TPE unit were restricted in TPE–β-CD, which blocked the non-radiative relaxation pathway and populated excitons that underwent radiative decay. Consequently, this gave rise to a much lower non-radiative decay rate and higher radiative decay rate as well as a longer emission lifetime. The time-resolved FL spectrum of TPE–α-CD resembled that of TPE–β-CD, while the lifetime of TPE–γ-CD was short and comparable to bare TPE.

Taking advantage of their high quantum yield and good biocompatibility inherited from TPE and CD,[1,11] we explored the application of the TPE–CDs in biological science. As depicted in Fig. 3, the cytoplasm of HeLa cells incubated with TPE–β-CD emitted upon UV irradiation, revealing that TPE–β-CD was able to penetrate the cell membrane and worked as a fluorescent visualizer for intracellular imaging.

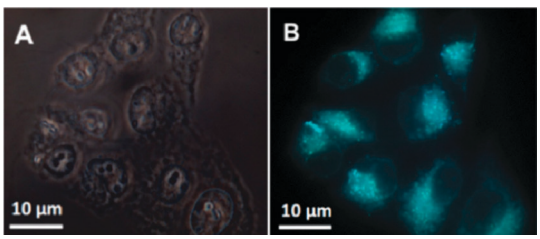


Fig. 3 (A) Bright-field and (B) fluorescent images of HeLa cells incubated with TPE–β-CD.

In summary, we have developed a new strategy to restrict the motions of AIE molecules through host–guest inclusion, leading to the formation of a catalogue of new molecular luminogens. The phenyl rings of TPE were included in the cavity of cyclodextrins, resulting in enhanced fluorescence due to the restriction of their motions. The quantum yield of the TPE–CDs increased upon decreasing the cavity size of the cyclodextrin. The TPE–CDs were biocompatible and could be utilized to image the cytoplasm of living cells.

This work was partially supported by the National Basic Research Program of China (973 Program; 2013CB834701), the Research Grants Council of Hong Kong (HKUST2/CRF/10 and N_HKUST620/11) and the University Grants Committee of Hong Kong (AoE/P-03/08). B.Z.T. is thankful for the support from Guangdong Innovative Research Team Program of China (201101C0105067115). G.D.L is thankful for the support from the Hong Kong Scholar Program (XJ2011047) and NSFC (21374136).

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