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Structure-Dependent *cis*/*trans* Isomerization of Tetraphenylethene Derivatives: Consequences for Aggregation-Induced Emission

Chong-Jing Zhang, Guangxue Feng, Shidang Xu, Zhenshu Zhu, Xianmao Lu, Jien Wu, and Bin Liu**

Abstract: *The isomerization and optical properties of the cis and trans isomers of tetraphenylethene (TPE) derivatives with aggregation-induced emission (AIEgens) have been sparsely explored. We have now observed the tautomerization-induced isomerization of a hydroxy-substituted derivative, TPETH-OH, under acidic but not under basic conditions. Replacing the proton of the hydroxy group in TPETH-OH with an alkyl group leads to the formation of TPETH-MAL, for which the pure cis and trans isomers were obtained and characterized by HPLC analysis and NMR spectroscopy. Importantly, cis-TPETH-MAL emits yellow fluorescence in DMSO at −20 °C whereas trans-TPETH-MAL shows red fluorescence under the same conditions. Moreover, the geometry of cis- and trans-TPETH-MAL remains unchanged when they undergo thiol–ene reactions to form cis- and trans-TPETH-cRGD, respectively. Collectively, our findings improve our fundamental understanding of the cis/trans isomerization and photophysical properties of TPE derivatives, which will guide further AIEgen design for various applications.*

Stereochemistry plays an important role in modern organic chemistry with significant influence in the material and life sciences. The stereochemistry of carbon–carbon double bonds holds great implications for drug discovery and dye development. Geometric c*is* and *trans* isomers have been reported to differ in their pharmacodynamic and pharmacokinetic properties.[1] For example, *trans*-tamoxifen is a potent estrogen receptor antagonist, which is used for the treatment of breast cancer, whereas *cis*-tamoxifen is a full estrogen agonist.[2] Carbon–carbon double bonds are also built-in elements of fluorescent dyes whose *cis* and *trans* isomers display different optical properties.[3] The elucidation of the characteristic properties of *cis* and *trans* geometric isomers and their isomerization is thus not only useful for fundamental studies but also offers a unique opportunity to explore their practical applications.

Tetraphenylethene (TPE) based derivatives generally show aggregation-induced emission (AIE); they are non-emissive in the molecularly dissolved state but become highly emissive in the aggregated state.[4] These unique AIE properties are the opposite to the aggregation-caused quenching (ACQ) observed for conventional fluorophores.[5] AIEgens thus provide a novel and complementary approach for the design of new-generation fluorescent probes.[6] TPE, as an iconic AIEgen, has attracted tremendous research interest. Thus far, multiple functional groups have been attached to the phenyl rings of TPE to fine-tune its optical properties for sensing, imaging, and therapeutic applications.[7] Previous studies have shown that the majority of the TPE derivatives do not undergo photoinduced *cis*/*trans* isomerization under the conditions normally applied for photoluminescence spectroscopy.[8] The few separated isomers display different mechanochromic properties,[8a] and the corresponding probes show distinct biological responses to target enzymes.[9] These findings highlight the importance of obtaining and investigating the *cis*/*trans* isomers of TPE derivatives. However, to obtain TPE derivatives as pure isomers is not trivial. For example, palladium-catalyzed coupling reactions can be employed to directly synthesize pure isomers,[10] but generally mainly the *cis* product is formed while the isomeric purity of the obtained product is not very high.[8b] Another strategy is to attach bulky groups to the TPE core to improve isomer separation on a silica gel column.[8a] After successful separation, confirmation of the *cis*/*trans* geometry is another challenging task. Current methods rely on growing single crystals for X-ray diffraction to confirm the isomer geometry.[8] This approach is tedious and only applicable to those isomers that can easily grow into single crystals. Owing to these difficulties, mixtures of the *cis* and *trans* isomers were reported and used in most studies,[11] which has compromising effects on the functions and properties of these molecules in various applications. With these considerations in mind, we herein designed and synthesized various analogues of a tetraphenylethenethiophene derivative (TPETH), namely TPETH-OH, TPETH-MAL, and TPETH-cRGD (Scheme 1). Our results indicate that 1) TPETH-OH undergoes tautomerization-induced *cis*/*trans* isomerization under acidic but not under basic conditions; 2) c*is*- and *trans*-TPETH-MAL can be easily resolved by HPLC and their structures were confirmed by COSY and NOESY spectroscopy; 3) the fluorescence of *cis*-TPE is blue-shifted compared to that of the *trans* isomer in DMSO at −20 °C, a property that

[*] Dr. C. Zhang, Dr. G. Feng, S. Xu, Dr. Z. Zhu, Prof. Dr. X. Lu, Prof. Dr. B. Liu
Department of Chemical and Biomolecular Engineering
National University of Singapore
4 Engineering Drive 4, Singapore, 117585 (Singapore)
E-mail: cheliub@nus.edu.sg
Dr. J. Wu
Department of Chemistry, National University of Singapore
3 Science Drive 3, Singapore, 117543 (Singapore)
E-mail: chmwujie@nus.edu.sg
Prof. Dr. B. Liu
Institute of Materials Research and Engineering
3 Research Link, Singapore 117602 (Singapore)
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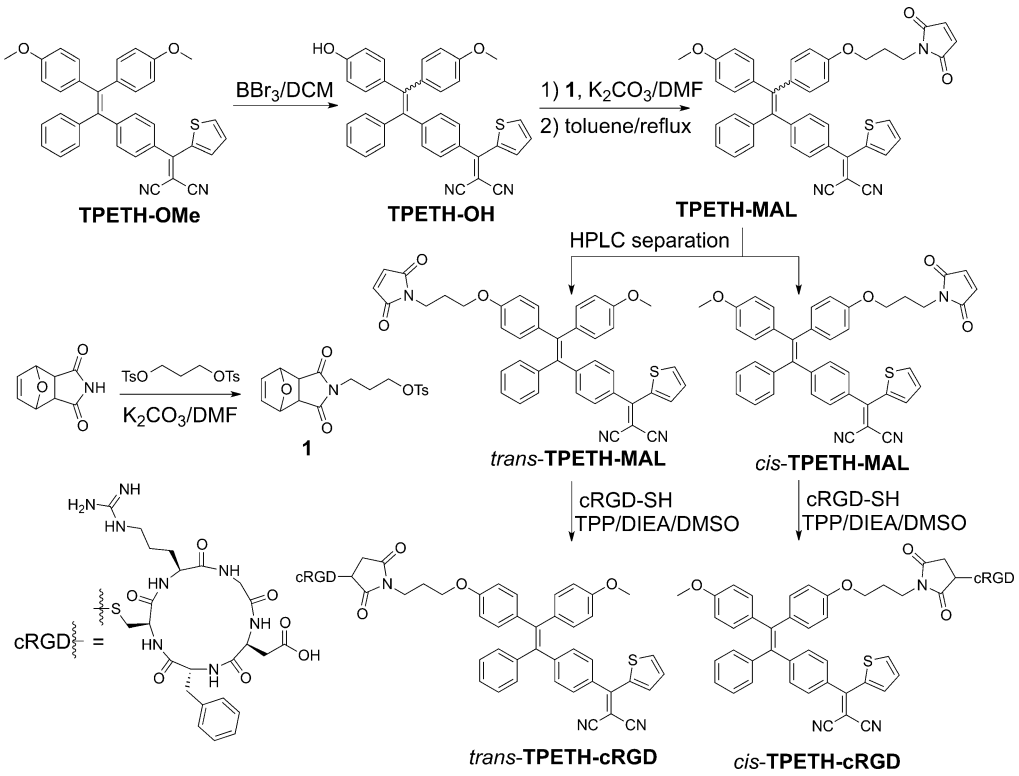

Scheme 1. Synthesis of TPETH-OH, TPETH-MAL, and TPETH-cRGD.

could potentially be employed to distinguish between the *cis* and *trans* isomer with the naked eye; and 4) the geometry of the TPE core remains unchanged during a reaction of the maleimide (MAL) groups with thiols to form the biological probe TPETH-cRGD.

TPETH-OH and TPETH-MAL were synthesized as shown in Scheme 1. Briefly, TPETH-OMe[12] was treated with tribromoborane to remove one methyl group and yield TPETH-OH, which further underwent alkylation and deprotection to generate TPETH-MAL. TPETH-OH was formed as a mixture of the *cis* and *trans* isomers, which could not be separated by column chromatography on silica gel. However, TPETH-OH can be easily separated by HPLC using a reverse-phase column with acetonitrile/water containing 0.1 % TFA as the eluent. TPETH-OH can easily undergo *cis*/*trans* isomerization, and it takes less than twelve hours for the separated pure isomers to reach equilibrium (1:1 ratio; Figure 1 B; see also the Supporting Information, Figure S4). We propose that the mutual conversion is due to keto–enol tautomerization for TPETH-OH. As shown in Figure S5, when the phenol tautomerizes to the keto form, the central double bond becomes a single bond (shown in red). This single bond could rotate freely to yield a transient isomer 2, which will further tautomerize from the keto form to the phenol form to complete the isomerization process. When three equivalents of diisopropylethylamine (DIEA) had been added to the HPLC eluent, no isomerization was observed within 24 hours (Figure 1 C), indicating that the proton is essential for this isomerization.

TPETH-OH displays absorption and emission maxima at 450 nm in DMSO and 638 nm in DMSO/water (1:100, v/v), respectively (Figures S6 A, B). The photoluminescence (PL) spectra of TPETH-OH were recorded in DMSO/water mixtures with different water fractions (f_w). With a gradual increase in f_w, the emission of TPETH-OH becomes more intense (Figure S6 B), a characteristic AIE phenomenon. The PL spectra of TPETH-OH under acidic and basic conditions are similar (Figure S6 C), indicating that the *cis*/*trans* isomerization does not affect its fluorescence in DMSO/water (1:100, v/v).

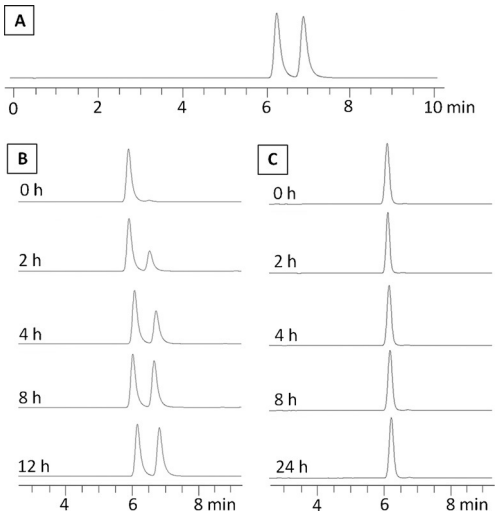

Figure 1. A) HPLC spectrum of TPETH-OH. B) Time-dependent HPLC traces of purified isomer 1 (eluted first). C) Time-dependent HPLC traces of purified isomer 1 in the presence of DIEA (3 equiv).

When the proton of the hydroxy group in TPETH-OH was replaced by an alkyl group, TPETH-MAL was obtained, the isomers of which were also successfully separated using HPLC (Figure S7). The first eluted isomer has a retention time of 7.5 min whereas the second one has a retention time of 8.0 min. The NMR spectra indicate that these two isomers of TPETH-MAL were obtained in high purity (Figures S8 and S9).

Once the pure isomers of TPETH-MAL had been obtained, they were analyzed by NMR spectroscopy to confirm their geometry. Both COSY and NOESY NMR spectra were recorded for the two isomers. We here use the isomer with a retention time of 8.0 min as an example to illustrate the geometry analysis. In the ^{1}H NMR spectrum, the resonance at $\delta = 3.75$ ppm corresponds to the H13 protons, and the resonance at $\delta = 3.92$ ppm is due to the H11 protons (Figures S10–S12). Following this assignment and the observation of clear correlations in the NOESY spectrum (Figure S13), the protons with $\delta = 6.62$ and 6.66 ppm are H1 and H4 in ring B and H5 and H8 in ring A, respectively. The resonance at $\delta = 6.96$ ppm was assigned to H6 and H7 in ring A because of their strong correlation with H5 and H8 (Figure 2). Among the four aromatic rings in TPETH-MAL, only ring C has the ABX coupling pattern, suggesting that the two protons (H9, H10) with $\delta = 7.07$ ppm are from ring C. As can be seen in Figure 2, the cross-peaks in the green circles indicate clear correlations between H6/H7 and H9/H10 in the NOESY spectrum, which indicates that ring A and ring C are close in space, that is, on the same side of the central double bond. Therefore, the isomer with a retention time of 8.0 min is the *cis* isomer (*cis*-TPETH-MAL), whereas the other one is the *trans* isomer (*trans*-TPETH-MAL; for its NMR spectra, see Figures S14–S18). The approach of using NMR spectroscopy to confirm the *cis* and *trans* geometry could potentially be applied to other AIEgens and other molecules, especially if their crystallization is difficult.

After the structural confirmation, we first measured the spectroscopic properties of the two isomers at 24 °C. *cis*-TPETH-MAL and *trans*-TPETH-MAL have identical UV absorption spectra with an absorption maximum at 440 nm (Figure 3 A). Both are non-emissive in DMSO as they are in a molecularly dissolved state. They become very emissive with an emission maximum at 635 nm in aqueous media (DMSO/water, 1:100, v/v; Figure 3 B). Laser light scattering studies indicate that *cis*-TPETH-MAL and *trans*-TPETH-MAL form aggregates in aqueous media with average diameters of 66 nm and 77 nm, respectively (Figures 3 C, D). Collectively, these results indicate that both *cis*-TPETH-MAL and *trans*-TPETH-MAL are AIE active.

When *cis*-TPETH-MAL and *trans*-TPETH-MAL were dissolved in DMSO (2 mM) and stored for 12 min at −20 °C, the solutions became solid with red fluorescence upon excitation with a UV lamp (Figure 4 A). As DMSO has a low melting point of 19 °C, the solidification of the solution at low temperature restricted the free intramolecular motion of the *cis*- and *trans*-TPETH-MAL molecules, turning on the fluorescence. As the storage time was increased, the solidified DMSO solution of *trans*-TPETH-MAL emitted stronger red fluorescence, whereas the solidified DMSO solution of *cis*-TPETH-MAL exhibited a change in the fluorescence color from red to yellow (Figure 4 A). This color change implies that the conformation of *cis*-TPETH-MAL changed with storage time. Next, we tested whether the same phenomena were observed in other solvents. As shown in Figure 4 B,

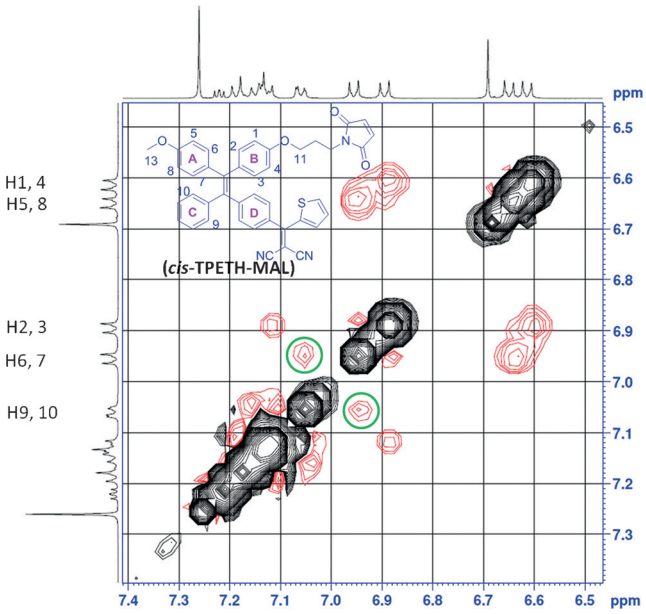

Figure 2. NOESY spectrum of *cis*-TPETH-MAL.

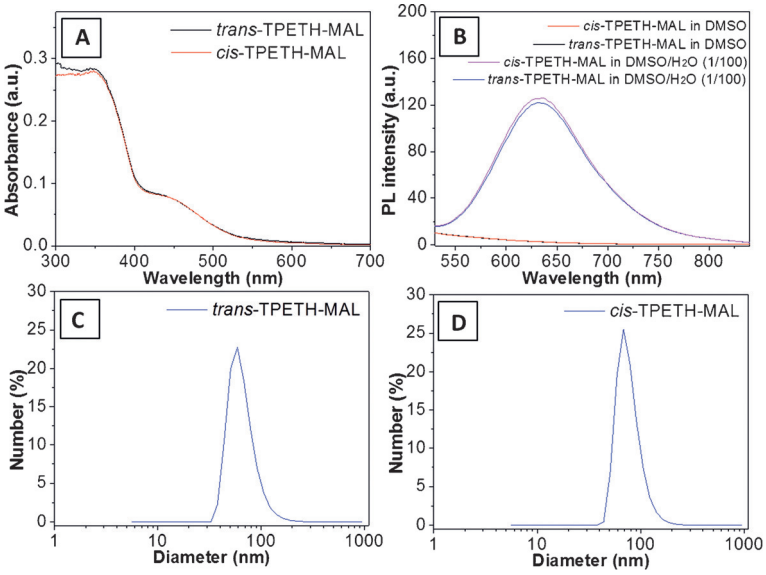

Figure 3. A) UV/Vis absorption spectra of 10 μM *cis*- and *trans*-TPETH-MAL in DMSO. B) PL spectra of *cis*- and *trans*-TPETH-MAL in DMSO and DMSO/water (1:100, v/v), $\lambda_{ex} = 440$ nm. C, D) Hydrodynamic diameters measured by laser light scattering (LLS) for 10 μM solutions of *trans*-TPETH-MAL (C) and *cis*-TPETH-MAL (D) in a mixture of DMSO/water (1:100, v/v). All measurements were conducted at 24 °C.

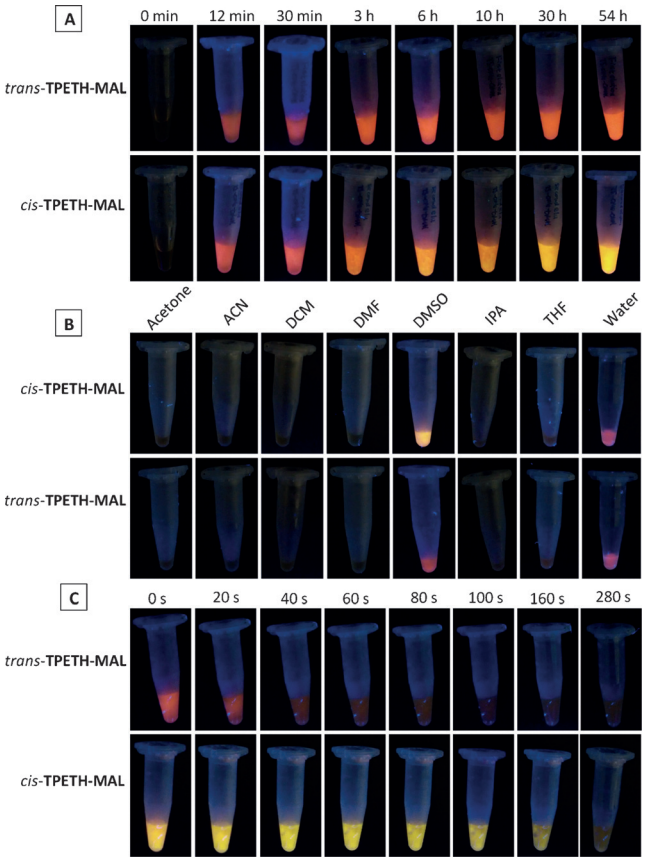

Figure 4. A) Photographs showing the fluorescence of *trans*-TPETH-MAL (2 mM, top row) and *cis*-TPETH-MAL (2 mM, bottom row) in DMSO at −20 °C. B) Photographs showing the fluorescence of *cis*-TPETH-MAL (1 mM, top row) and *trans*-TPETH-MAL (1 mM, bottom row) in different solvents kept at −20 °C for 3 days. C) Photographs showing the fluorescence of *trans*-TPETH-MAL (2 mM, top row) and *cis*-TPETH-MAL (2 mM, bottom row) in DMSO, allowed to warm from −20 °C to room temperature for various periods of time. All photographs were taken with 365 nm excitation.

solutions of *cis*- and *trans*-TPETH-MAL in acetone, acetonitrile (ACN), dichloromethane (DCM), dimethylformamide (DMF), isopropyl alcohol (IPA), and tetrahydrofuran (THF) emit weak fluorescence as the solutions did not solidify at −20 °C. However, aqueous dispersions of both isomers emitted red fluorescence. As *cis*- and *trans*-TPETH-MAL form aggregates in water, this observation implies that the subtle conformation alteration at low temperature does not occur in the aggregated state. Moreover, when the solidified DMSO solutions at −20 °C were transferred to the bench at room temperature, *trans*-TPETH-MAL became almost non-emissive within 40 s but *cis*-TPETH-MAL remained emissive for up to 5 min while the fluorescence intensity decreased with time (Figure 4C).

These different optical properties of *cis*- and *trans*-TPETH-MAL could be ascribed to their structural differences. In *cis*-TPETH-MAL, the two bulky side chains are located on the same side of the double bond, which leads to more steric hindrance than in *trans*-TPETH-MAL (Figure S19). This difference in the steric hindrance between the two isomers directly affects the intramolecular motion of the molecules, which is more restricted for *cis*-TPETH-MAL. When kept at −20 °C for a few hours, *cis*-TPETH-MAL will gradually adopt a more stable state to yield blue-shifted fluorescence. Accordingly, intramolecular motion is much easier in *trans*-TPETH-MAL than in *cis*-TPETH-MAL, which is in agreement with the observation that the fluorescence of the former disappears faster when its solidified DMSO solution is slowly warmed to room temperature. The different optical properties of *cis*-TPETH-MAL and *trans*-TPETH-MAL could also be used to distinguish them with the naked eye.

As thiol–ene reactions between maleimides and thiols are widely used for bioprobe synthesis,[13] we were interested in whether the purified isomers would be able to maintain their geometry during such reactions. cRGD-SH was selected to react with both *cis*-TPETH-MAL and *trans*-TPETH-MAL (Scheme 1). This reaction proceeded fast in the presence of triphenylphosphine and DIEA. The final conjugation products (*cis*-TPETH-cRGD and *trans*-TPETH-cRGD) were obtained in good yields, and their structures were confirmed by ^{1}H NMR spectroscopy and high-resolution mass spectrometry (Figures S20–S23). Comparison of the ^{1}H NMR spectra of the conjugation products and *cis*-TPETH-MAL and *trans*-TPETH-MAL revealed that the resonance of the proton highlighted in blue is shifted downfield in *trans*-TPETH-MAL compared to that of *cis*-TPETH-MAL (Figures 5A,B). As shown in Figures 5C,D, the same pattern was also observed for the *cis*/*trans* isomers of TPETH-cRGD, which indicates that the geometry of the AIEgens is not changed by the reaction.

Finally, we employed *cis*-TPETH-cRGD for cell imaging. *cis*-TPETH-cRGD has almost the same absorption spectrum as TPETH-MAL when dissolved in DMSO (Figure S24). Because of the high hydrophilicity of the cRGD group, *cis*-TPETH-cRGD has a lower fluorescence than *cis*-TPETH-MAL in aqueous media (Figure S24). As the cRGD group is able to target cells with integrin overexpression (e.g., MDA-

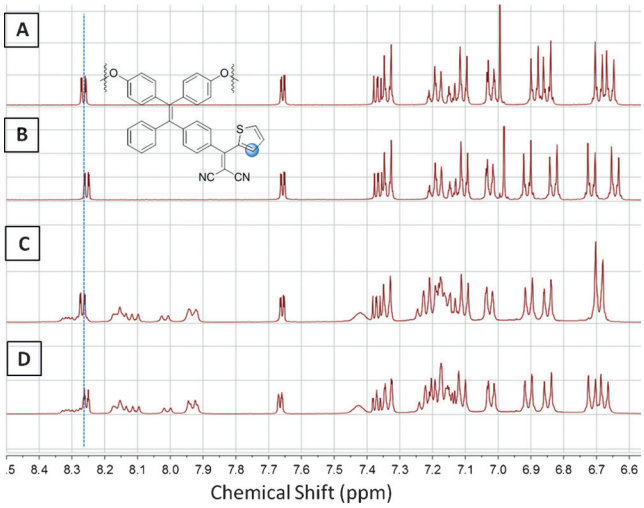

Figure 5. ^{1}H NMR spectra of *trans*-TPETH-MAL (A), *cis*-TPETH-MAL (B), *trans*-TPETH-cRGD (C), and *cis*-TPETH-cRGD (D) in [D_6]DMSO. The peaks indicated by the blue line belong to the proton in the thiophene ring highlighted in blue.

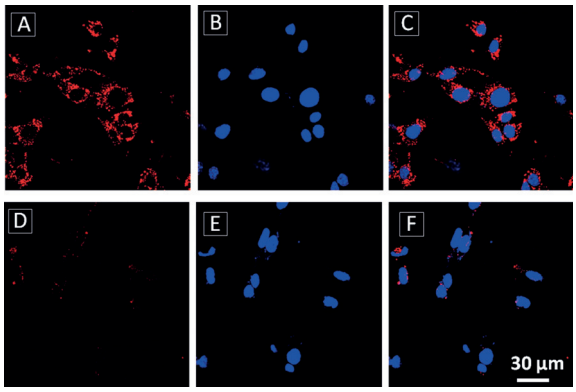

Figure 6. Fluorescence images of MDA-MB-231 (A–C) and NIH-3T3 (D–F) cells treated with *cis*-TPETH-cRGD (10 mM) for 2 hours. A, D) Images obtained with *cis*-TPETH-cRGD; $\lambda_{ex} = 405$ nm, $\lambda_{em} > 560$ nm long pass filter. B, E) Images obtained with the nucleus tracker DAPI; $\lambda_{ex} = 405$ nm, $\lambda_{em} = 430$–470 nm. C, F) Overlays of the images shown in A, B and D, F. Scale bar: 30 mm.

MB-231),[14] the uptake of *cis*-TPETH-cRGD into MDA-MB-231 cells is much higher than that into normal NIH-3T3 cells (Figure 6), and the fluorescence intensity of the probe is eight times higher in MB-MDA-231 than in NIH-3T3 (Figure S25A), indicating that the probe could be used to selectively image integrin-overexpressing cancer cells. Moreover, no obvious cytotoxicity was observed for probe concentrations of 1 to 20 μM (Figure S25B).

In conclusion, we have studied the *cis/trans* isomerization and optical properties of the *cis* and *trans* isomers of various TPE-based AIEgens. With a free hydroxy group, TPETH-OH undergoes *cis*/*trans* isomerization under acidic conditions but not under basic conditions, which was ascribed to keto–enol tautomerization. By replacing the proton of the hydroxy group in TPETH-OH with an alkyl group, the pure *cis* and *trans* isomers of TPETH-MAL were obtained and characterized by HPLC and NMR spectroscopy. *cis*-TPETH-MAL and *trans*-TPETH-MAL have roughly the same spectroscopic properties at room temperature. However, *cis*-TPETH-MAL emits yellow fluorescence and *trans*-TPETH-MAL emits red fluorescence when they are stored in DMSO at −20 °C for a few hours. The differences in the photophysical properties can be ascribed to the higher steric hindrance in *cis*-TPETH-MAL than in *trans*-TPETH-MAL. Finally, the geometry of TPETH-MAL is maintained in a thiol–ene reaction, and the generated probe, *cis*-TPETH-cRGD, can selectively stain integrin-overexpressing cancer cells. This study improves our fundamental understanding of the *cis*/*trans* isomerization and photophysical properties of TPE derivatives, which offers new opportunities to explore the unique properties of AIEgens.

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