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TPE based aggregation induced emission fluorescent sensors for viscosity of liquid and mechanical properties of hydrogel

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ABSTRACT

Two amphiphilic TPE *E*/*Z* isomers with aggregation induced emission (AIE) property have been synthesized and characterized. The logarithmic fluorescent intensity of the two molecules was in positive relationship with logarithmic viscosity of liquid. To note, the *Z*-TPE isomer exhibited more sensitivity in the viscosity of liquid sensing in comparison with the corresponding *E*-TPE counterpart (around 1.80 folds). Furthermore, two molecules could be used as fluorescent sensors for mechanical properties (viscosity and storage modulus) of hydrogel as well. In addition, two sensors displayed low cytotoxicity in normal tissue cell line (L929) within the concentration range of 2–10 μmol/L. These results potentially promised their applications as fluorescent sensors for mechanical properties in the fields of biological and biomedical.

Tetraphenyl ethylene (TPE) and the derivates were distinguished with their aggregation induced emission (AIE) property, which allowed fluorescent emission in aggregating state [1-2]. The reason was documented to be restricted intramolecular rotation (RIR) effect [3]. In solution, non-emissive decay pathway of TPE in excited state privileged and almost no fluorescence would be observed [4]. Upon aggregation or in solid state, rotation of phenyl rings was blocked and TPE would regain its fluorescent emission [5]. Due to this unique fluorescent property, various TPE derivates have been widely applied in the fields of fluorescent imaging, fluorescent sensing, disease diagnosis and organic optoelectronics [6-17]. Currently, TPE has been recognized as versatile building block of functional materials with fluorescent property [18].

The mechanical property of cellular microenvironment and extracellular matrix (ECM) has been reported to influence a plethora of biological behaviors [19-23]. For instance, the nano-viscosity of fluid could influence enzyme catalyzed DNA cleavage rate [24]. ECM stiffness was reported to affect intracellular rheology of cancer cells and was related to cancer metastasis [25-28]. Currently, biocompatible 3D hydrogels were regarded as one of promising systems for mimicking *in vivo* ECM of cancer cell [29-31]. Therefore, a probe for viscosity of liquid and mechanical parameters of hydrogel is of great significance in biological and biomedical fields. In this aspect, fluorescent molecular rotors have been applied as efficient liquid viscosity sensor [32-33]. Yet, these probes generally require complicated synthetical procedures and expensive fluorescence life imaging microscope (FLIM) [34]. Liquid viscosity could affect intramolecular rotation of phenyl rings on TPE core. Therefore, fluorescence intensity of TPE was in positive relationship with the viscosity of surrounding liquid [35]. A handful TPE based liquid viscosity sensors with cellular organelle targeting capabilities have been synthesized and succeeded in revealing the relationship of viscosity and cellular function [11-36]. These TPE based probes advanced in simple synthetical procedure and avoidance of expensive instruments. On top of the fluid viscosity sensing, TPE derivates were applied in the description of hydrogel degradation [37]. In principle, the viscosity of fluid confined to micro network inside hydrogel could also be sensed by TPE derivates. To some extent, this micro fluid viscosity could reflect the macro mechanical properties of hydrogel [38]. On base of that, TPE could potentially be applied in mechanical property sensing of hydrogel.

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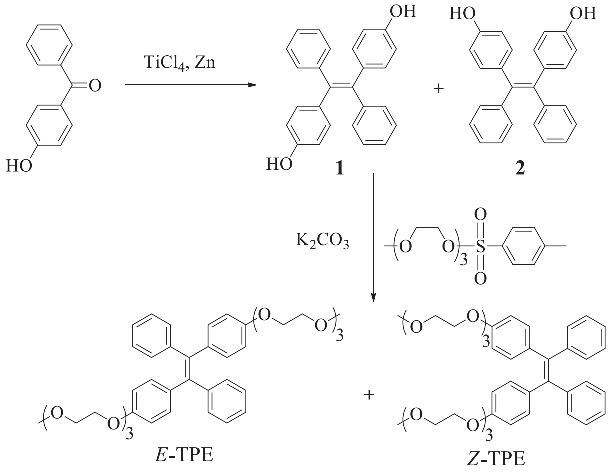

Fig. 1. Synthetical route for *E*-TPE and *Z*-TPE.

Herein, we wish to report the syntheses of TPE based sensors for fluid viscosity and mechanical parameters of hydrogel. The resulting *E*/*Z*-TPE isomers succeeded in fluid viscosity and hydrogel mechanical parameters sensing. Noticeability, *Z*-TPE showed more sensitivity in viscosity of fluid sensing in comparison with the corresponding *E*-TPE (1.8 times). To best of our knowledge, this is the first report of TPE based sensor for both fluid viscosity and mechanical parameters of hydrogel.

As shown in Fig. 1, we employed a straight-forward synthetical route for compounds *E*/*Z*-TPE. Mixed precursors **1** and **2** were afforded in the yield of 80% through typical titanium tetrachloride catalyzed Mcmurry reaction by using 4-hydroxybenzophenone as starting material. The mixture was subsequently reacted with tosyl activated oligoethylene glycol to afford desired product *E* and *Z*-TPE. After carefully purification using silica chromatography, the yield for *E* and *Z*-TPE amounted to 51% and 52%, respectively. The detailed synthetical procedures and corresponding ^{1}H NMR, ^{13}C NMR and high-resolution mass spectroscopies were shown in supporting information (Figs. S5-S12 in Supporting information). The structures of *E* and *Z*-TPE were confirmed by ^{1}H NMR. As shown in Fig. 2, doublets of protons of substituted phenyl ring (H_a, H_b, H_a' and H_b') indicated pure *E* and *Z* isomers. In case of a mixture, the protons would be triplets. H_a and H_b in *E*-TPE slightly upfield shifted in comparison with that of H_a' and H_b' in *Z*-TPE ($\Delta\delta = -0.03$ ppm). On the contrary, H_c, H_d and H_e in *E*-TPE were observed to slightly downfield shift in comparison with that of H_c', H_d' and H_e' in *Z*-TPE. The resonance signal pattern of individual *E*/*Z*-TPE isomer matched previously TPE isomers [39].

The photophysical properties were investigated. Both compounds displayed UV–vis absorption with maxima absorbance centered at 260 and 338 nm in ddH$_2$O (Fig. S1 in Supporting information). These absorbances should be assigned to π-π^* electronic transition of TPE [40]. The amphiphilic nature of the two molecules facilitate self-assembling in water (10 µmol/L), thus a typical TPE fluorescent emission (centered at 491 nm, Fig. S1) has been observed.

TPE derivates were reported to be sensitive towards microenvironment, *e.g.*, fluid viscosity [10]. Yet, the impact of configuration on sensitivity remained to be investigated. The current *E*- and *Z*-TPE isomers (10 µmol/L) displayed different fluorescent emission intensity in relatively low viscous liquid (pure ethylene glycol, 31 cp). In details, the fluorescent quantum yield of *E*-TPE was around two times than that of *Z*-TPE (data not shown). Tang reported similar oligoethylene glycol bearing TPE with *Z*-configuration has slightly lower fluorescent quantum yield in comparison with the *E*-configuration isomer due to different assembling behavior [41]. In our case, *E*-TPE (10 µmol/L) showed an amorphous morphology (Fig. 3a) and *Z*-TPE (10 µmol/L) was in a micelle-like morphology (Fig. 3b) as characterized by TEM. The different assembly behavior together with fluorescent quantum yields in water was in good agreement with previous result, indicating the assembling behavior could be the reason for varied fluorescent emission properties of the isomers [41].

We tuned viscosity of liquid by changing glycerol fraction in ethylene glycol. Both isomers displayed viscosity dependent fluorescent increase. As shown in Fig. 4a, *E*-TPE showed an approxi-

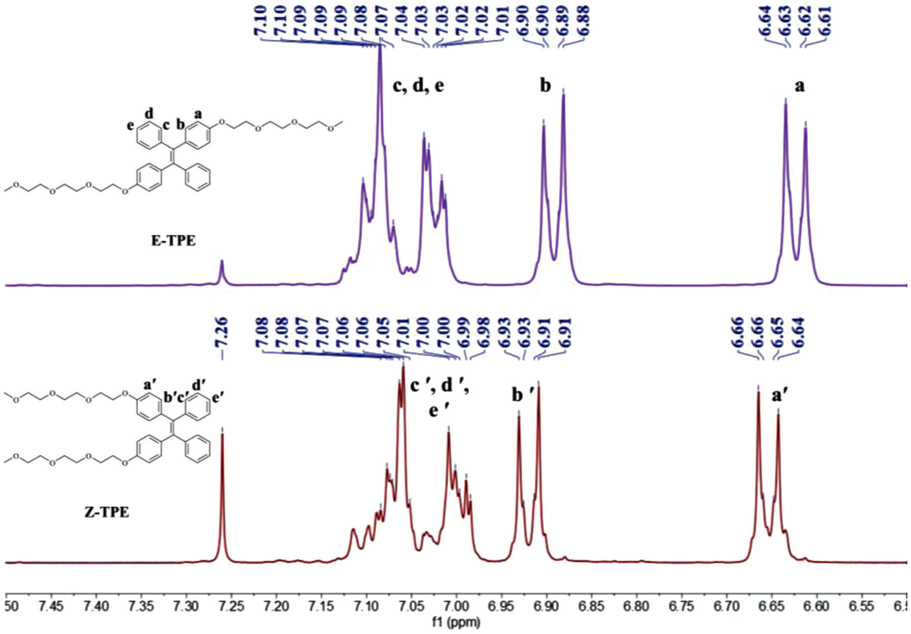

Fig. 2. Partial ^{1}H NMR spectra of *E*-TPE and *Z*-TPE (CDCl$_3$, 300 MHz).

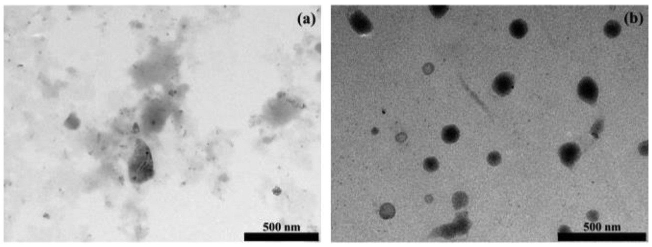

Fig. 3. TEM images of *E*-TPE (a) and *Z*-TPE (b). Scale bar = 500 nm. Acc. voltage was 100 kV. Magnificence for (a) and (b) were 25.0 K and 30.0 K.

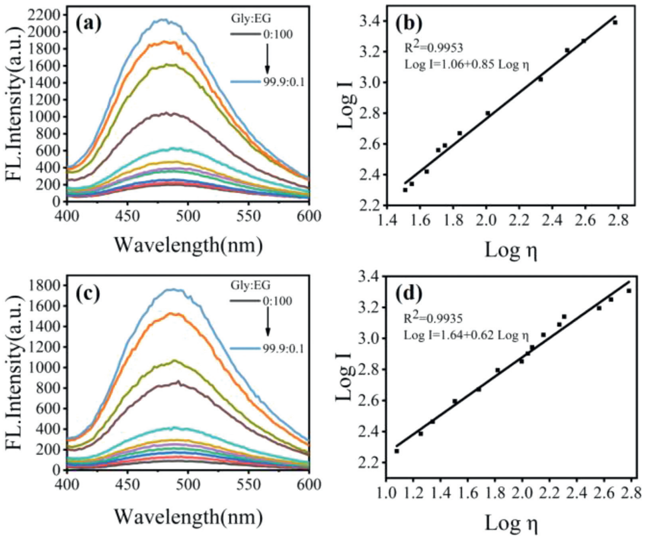

Fig. 4. Fluorescence emission spectra of *E*-TPE (a) and *Z*-TPE (c) in mixture of ethylene glycol and glycerol with different fraction of glycerol (0-99.90%). Plots of logarithmic fluorescent intensity (Log I) of *E*-TPE (b) and *Z*-TPE (d) *vs.* logarithmic mixture viscosity (Log η). [*E*/*Z*-TPE] = 10 µmol/L, excitation wavelength was 340 nm.

mately 11 fold increase of fluorescence intensity in 99.90% glycerol solution (609 cp) in comparison to the condition of pure ethylene glycol (37 cp). The logarithmic fluorescent emission intensity of *E*-TPE displayed excellent linear relationship (R^2 = 0.9953) with logarithmic viscosity of the testing solvent (Fig. 4b). The enhanced emission intensity should be due to fluid viscosity induced restriction of phenyl ring on TPE that block the non-radioactive decay pathway [39]. In addition, the fluorescence maxima of *E*-TPE displayed a slightly blue shift in high viscous medium (around 12 nm in 99.90% glycerol). Similar phenomena were observed in other TPE based viscosity sensor, and was supposed to be the influence of vibrational energy level in viscous solution [34]. *Z*-TPE was found to be more sensitive towards microenvironment viscosity. As shown in Fig. 4c, increasing glycerol fraction to 99.90% resulted a 19.82-folds enhancement of the fluorescent intensity (around 1.8 times sensitive towards viscosity in comparison to *E*-TPE). The logarithmic fluorescent emission intensity of *Z*-TPE also displayed excellent linear relationship (R^2 = 0.9935) with logarithmic viscosity of the testing solvent (Fig. 4d). The fluorescence from the agarose has been ruled out according to the spectra of hydrogel with different agarose concentration (Fig. S2 in Supporting information).

Since the restriction of intramolecular rotation (RIR) effect contributes to fluorescence emission of TPE molecules, we suspected *E*- and *Z*-TPE may have different rotational energy barriers. According to DFT calculations (Supporting information), only slight difference in rotational barrier is observed between the two isomers; the Gibbs free energy barriers at room temperature and ambient pressure are from 8.4 kcal/mol to 9.7 kcal/mol for both isomers (Fig. S3 in Supporting information), in good agreement with those for similar compounds [37]. This suggests that there should be other reasons for the different sensitivity. Alternatively, we suspect hydrogen bonds between solvent and the oligoethylene glycol side-chains on TPE could serve as an external factor that influencing the rotation of the phenyl rings. In the situation of *Z*-TPE, which the oligoethylene glycol side chains stay in close distance, a successive hydrogen bonds bridge mediated by solvent molecules may form between the two side chains. This successive hydrogen bonds bridge might produce extra rotational energy barrier of the phenyl rings. Whereas the oligoethylene glycol chains stay relative far apart in *E*-TPE, the corresponding successive hydrogen bonds bridge would be less stable or even hard to form. Therefore, *E*-TPE was supposed to be less sensitive towards hydrogen bond formation solvent molecules in contrast to *Z*-TPE.

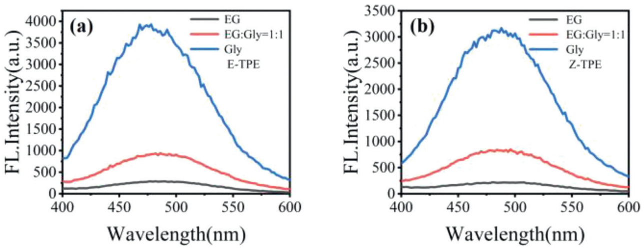

Fig. 5. Fluorescence emission spectra of *E*-TPE (a) and *Z*-TPE (b) in mixture of ethylene glycol and glycerol with different fraction of glycerol (0-99.9%) in the presence of 1 mol/L acetic acid. [*E*/*Z*-TPE] = 10 µmol/L, excitation wavelength was 340 nm.

In order to verify this hypothesis, we measured the fluorescent spectra of the two TPE isomers in solvents composed of different ratio of ethylene glycol and glycerol in the presence of 1 mol/L acetic acid to eliminate potential hydrogen bonds interactions. Both TPE isomers displayed fluorescence enhancement with increasing of glycerol in acidic solution. In 99.90% percentage of glycerol, *E* (Fig. 5a) and *Z*-TPE (Fig. 5b) showed 13.74- and 14.20-folds increase of fluorescence intensity enhancement, respectively. The similar fluorescence enhancement ratio in the presence of acid suggests hydrogen bond indeed played partial role in the RIR effect dominated fluorescence enhancement.

On top of liquid viscosity sensing, we further attempted to measure the mechanical properties of hydrogels using synthesized viscosity sensors. The hydrogels were fabricated straightforwardly by cooling the ddH_2O solution of low-melting point agarose with varied concentrations (0.20%, 0.30%, 0.40%, 0.50% and 0.60%) below 37 °C. The mechanical properties of hydrogels were first characterized. The mechanical properties were first measured using a rheometer. The viscosity and storage modulus (G') of the corresponding hydrogels were in positive relationship with the concentration of agarose. The viscosity of the hydrogel reduced with increase of shear rate, showing a pseudo-plastic behavior.

We next added *E* and *Z*-TPE (10 µmol/L) during the fabrication of these hydrogels, respectively. The hydrogels showed fluorescence emission centered around 490 nm, indicating existence of AIE effect in the hydrogels. As shown in Figs. 6a and d, the G' and viscosity of hydrogels was correlated with the fluorescent emission intensity centered around 490 nm. The logarithmic fluorescence intensity of *E*-TPE containing hydrogel was displayed good linear relationship with logarithmic value of both G' and viscosity (R^2 = 0.9487 and 0.9622, respectively, Figs. 6b and c). Similarly, logarithmic fluorescence intensity of *Z*-TPE containing hydrogel also showed good linear relationship with logarithmic value of G' and viscosity (R^2 = 0.9912 and 0.9891, respectively, Figs. 6e and f). The viscosity and G' of the corresponding hydrogels were in positive relationship with the concentration of agarose. The fluorescence emission intensity of both TPE isomers increased with the concentration of agarose indicated the fluorescence intensity

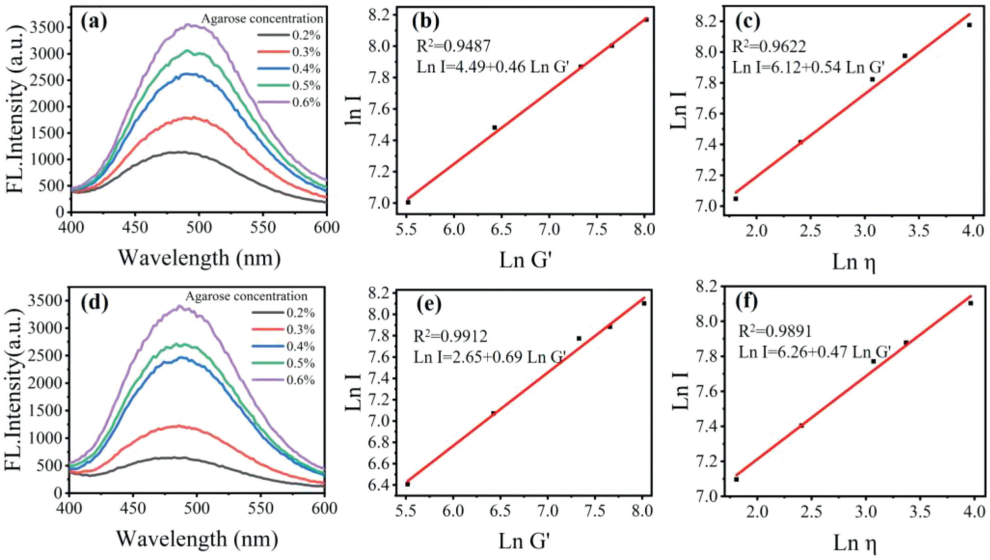

Fig. 6. Fluorescence emission spectra of *E*-TPE (a) and *Z*-TPE (d) in hydrogels composed of different concentration of agarose (0.20%–0.60%). [*E*/*Z*-TPE] = 10 µmol/L, excitation wavelength was 340 nm. Plots of natural logarithmic fluorescent intensity of *E*-TPE (b) and *Z*-TPE (e) *vs.* natural logarithmic storage modulus (G′) of hydrogel. Plots of natural logarithmic fluorescent intensity of *E*-TPE (c) and *Z*-TPE (f) *vs.* natural logarithmic viscosity of hydrogel. [*E*/*Z*-TPE] = 10 µmol/L, excitation wavelength was 340 nm.

could reflect its mechanical properties. Given the fact that agarose constitutes the network structure inside hydrogels, increased concentration of agarose contributes to the enhanced viscosity and G' of hydrogels. In the presence of more agarose, the grid size would become smaller. The liquid inside each grid might become less mobile and more viscous due to more hydrogen bond interactions. This might explain the enhancement of fluorescent intensity in the presence of more agarose loading. Finally, the two sensors displayed low cellular toxicity in mouse fibroblast cell line (L929) within the concentration range of 2–10 µmol/L. This promised their feasibility of future biomedical applications (Fig. S4 in Supporting information).

In conclusion, two oligoethylene glycol bearing TPE isomers (*E*-TPE and *Z*-TPE) have been synthesized and characterized. The fluorescent intensity of both compounds was in positive relationship with viscosity of liquid within the range of 31–690 cp (R^2 = 0.9953 and 0.9935 for *E* and *Z*-TPE, respectively). In details, *Z*-TPE was more sensitive in viscosity sensing in comparison with *E*-TPE (around 1.80 times). Computational simulation suggests the rotational energy barriers for the two compounds are practically identical. Addition of high concentration of acid in viscous medium afford similar fluorescent enhancing ratio of two compounds, indicating the different hydrogen bond interacting strength might be the reason for different viscosity sensing performance. Furthermore, mechanical parameters (viscosity and storage modulus G′) of hydrogel were also in positive relationship with the fluorescent intensity of both TPE isomers. The reason was believed to be restricted intramolecular rotation effect as well. Finally, both compounds displayed neglectable cellular toxicity. These results allowed them to be potentially used as promising sensors of liquid viscosity and hydrogel mechanical parameters in biological and biomedical fields.

Declaration of competing interest

The authors declare no confliction of interest.

Acknowledgments

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2021.06.092.

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