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Amino-Modified Tetraphenylethene Derivatives as Nucleic Acid Stain: Relationship between the Structure and Sensitivity

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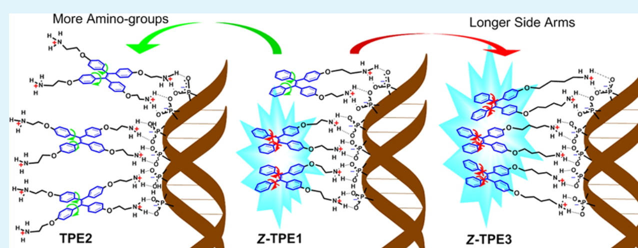
Supporting Information

ABSTRACT: A series of new amino-functionalized tetraphenylethene (TPE) derivatives were designed and synthesized to study the effect of molecular structures on the detection of nucleic acid. Contrastive studies revealed that the number of binding groups, the length of hydrophobic linking arm and the configuration of TPE molecule all play important roles on the sensitivity of the probes in nucleic acid detection. *Z*-TPE3 with two binding amino groups, long linking arms, and cis configuration was found to be the most sensitive dye in both solution and gel matrix. *Z*-TPE3 is able to stain dsDNA with the lowest amount of 1 ng and exclusively stain 40 ng of short oligonucleotide with only 10 nt. This work is of important significance for the further design of TPE probes as biosensors with higher sensitivity.



KEYWORDS: *nucleic acid stain, gel electrophoresis, fluorescence, tetraphenylethene, Z/E configuration*

INTRODUCTION

Oligonucleotides have been widely used in biochemical sciences and molecular medicine as research tools and a class of therapeutic agents against diseases, such as antisense oligodeoxynucleotides.[1] In all applications, oligonucleotides need to be highly purified and quantitatively characterized by polyacrylamide gel electrophoresis (PAGE), which is one of the valuable techniques for the separation of nucleic acids in life sciences. Therefore, it is of important significance to develop rapid, highly sensitive, and cost-effective methods for the detection and visualization of oligonucleotide in gel matrix. On the other hand, fluorescence probes are powerful analytical tools for nucleic acid detection,[2] which offer superb sensitivity, relatively rapid and easy operation approach.[3] Most classic fluorescent dyes, such as ethidium bromide (EB),[4] TOTO,[5] YOYO,[6] and SYBR stains,[7] have been developed for specific targeting of double-strand DNA by intercalation or groove binding, thus many of them do not show significant fluorescence response for single-strand DNA without secondary structures.[8] Although it has been commercialized as nucleic acid stain in PAGE,[9,10] EB is reported to be a toxic and mutagenic agent because of its intercalating property.[11−13] The alternatives of EB, such as SYBR-based dyes, which have been found to be highly sensitive but less carcinogenic,[14] are too expensive for high-throughput detection. In recent years, tetraphenylethene (TPE) derivatives have been widely explored as bioprobes for detecting biopolymers.[15−18] Many fluorescent dyes aggregate when dispersed in aqueous media or bound to biological polymers in large quantities, accompanying self-quenching that results in drastic reductions of their fluorescence signals.[19,20] In contrast, the tetraphenylethene (TPE) fluorogen is nonemissive when molecularly dispersed in solution, but becomes highly emissive when aggregated because of the restriction of intramolecular rotation (RIR) that prohibits energy dissipation *via* nonradiative channels.[21−23] This aggregation-induced emission (AIE) characteristic makes the TPE-based dyes "abnormal" from the conventional fluorescent probes and having more advantages as biosensors.[24,25] Tang et al. felicitously designed a series of TPE derivatives with tetraalkylammonium cation as "light up" probes for DNA detection by use of electrostatic interaction. These TPE derivatives were further used as a new kind of DNA stain in gel electrophoresis with the lowest detection limit of 0.25 μg.[26−30]

Recently, we reported two TPE derivatives (TPE1, Scheme 1) by introducing amino group to strengthen the interaction between TPE dye and nucleic acid through hydrogen bond, and thus developed simple, universal and highly sensitive methods for the detection of dsDNA and oligonucleotide in both solution and gel matrix.[31] In this article, to study the relationship between the structures of amino-modified TPE derivatives and their binding ability with nucleic acids and consequent sensitivity for the detection, we designed and synthesized a series of new amino-modified TPE derivatives. First, the four amino groups were attached to TPE molecules, but the resulted compound, namely TPE2 (Scheme 1), was not as sensitive as TPE1 with two amino groups as we previously reported.[31] In view of this result, we then designed and

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Scheme 1. Structures of Amino-Modified Tetraphenylethene

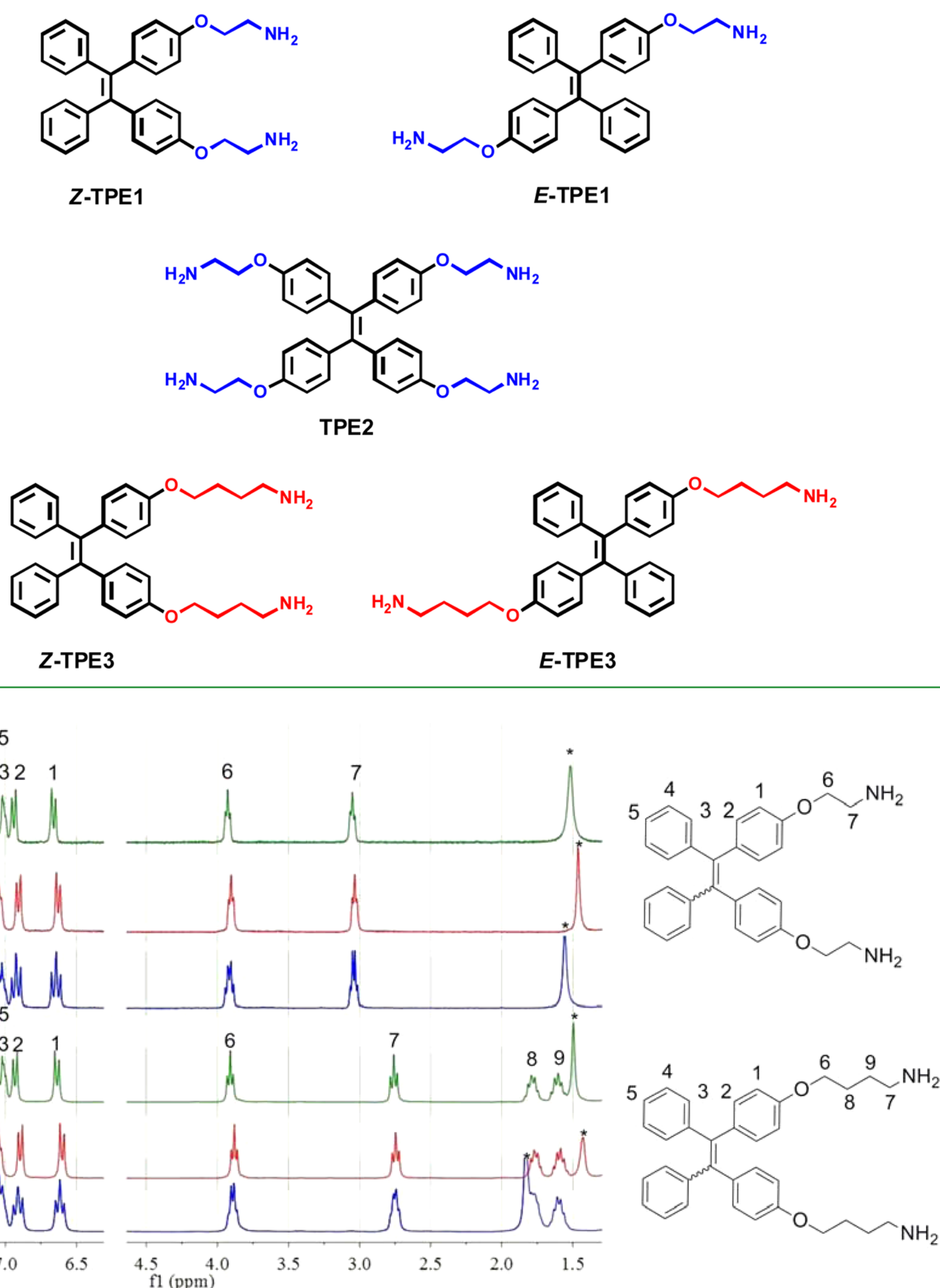

Figure 1. ^{1}H NMR spectra of cis, trans, and mixture of TPE1 and TPE3 in $CDCl_3$. The solvent peaks are marked with asterisks.

synthesized a new TPE derivative, namely TPE3 (Scheme 1), with the longer linking arms than TPE1. We also successfully obtained pure cis and trans configuration of TPE3 (*Z*-TPE3 and *E*-TPE3), and investigated the configurational effect on the sensitivity for the detection of nucleic acids.

RESULTS AND DISCUSSION

Synthesis and Characterization. Similar to the synthesis of TPE1 we previously reported,[31] the amino groups were introduced into the TPE frame through the nucleophilic substitution of bromine by azid group, then followed the reduction of azid groups (Scheme S1 in the Supporting Information). The pure *Z*-TPE3 and *E*-TPE3 were separated by careful chromatography. Since the configurations of *Z*-TPE1 and *E*-TPE1 were identified in our previous work, the configurations of *Z*-TPE3 and *E*-TPE3 were distinguished by comparing their ^{1}H NMR spectra with those of *Z*-TPE1 and *E*-TPE1. As Figure 1 presented, their ^{1}H NMR spectra in the aromatic region were hardly influenced by the side arms. Although the cis and trans isomers showed similar spectra, there were some slight differences in identifying the configurations. Both *Z*-TPE1 and *Z*-TPE3 showed doublets at ca. 6.6 (H1) and 6.9 (H2) ppm, which were downfield-shifted from those of *E*-TPE1 and *E*-TPE3. All the new TPE

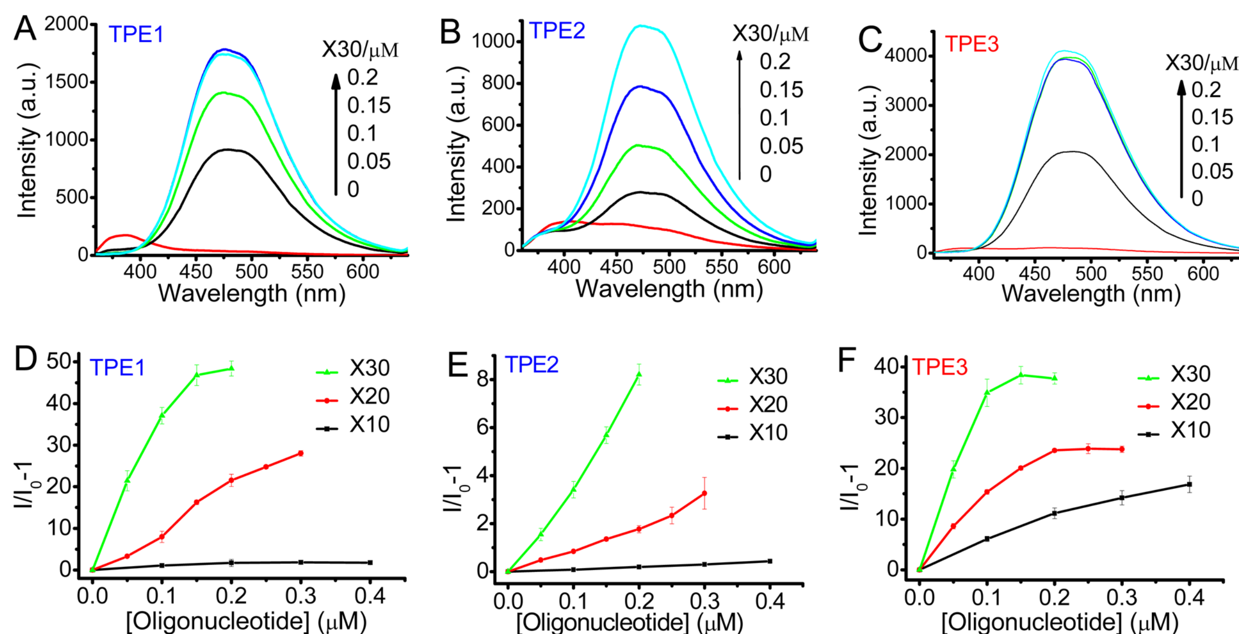

Figure 2. Fluorescence titration of X30 to (A) TPE1, (B) TPE2, and (C) TPE3 in deionized water. Plot of $I/I_0 - 1$ of (D)TPE1, (E) TPE2, and (F) TPE3 at 480 nm versus the oligonucleotides concentration. I_0 = emission intensity in the absence of oligonucleotides. [TPE1] = [TPE2] = [TPE3] = 5 μM; λ_{ex} = 330 nm, λ_{em} = 480 nm. Error bars are ± SD. X10, X20, X30 are synthetic oligonucleotides with length of 10, 20, and 30 nt, respectively.

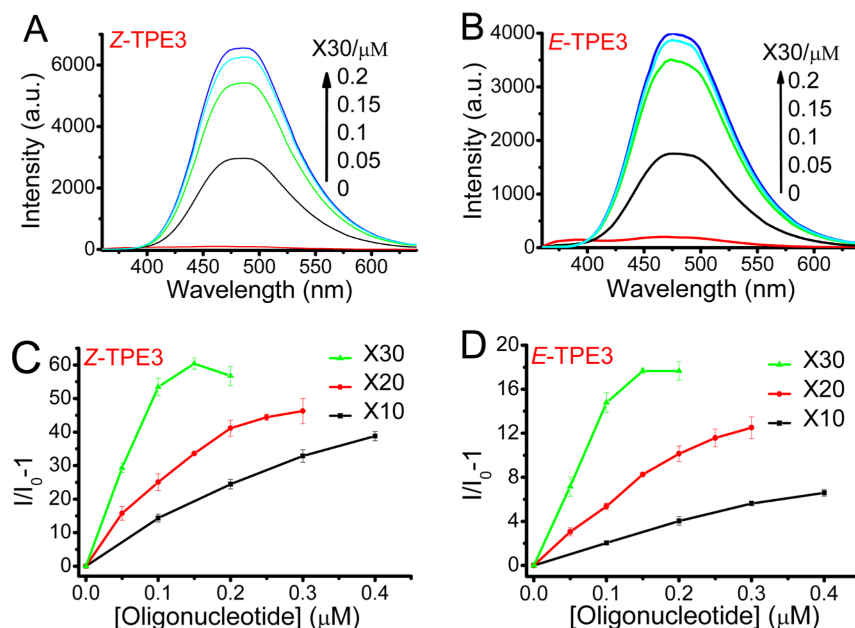

Figure 3. Fluorescence titration of X30 to (A) *Z*-TPE3 and (B) *E*-TPE3 in deionized water. Plot of $I/I_0 - 1$ at 480 nm versus the oligonucleotides (X10, X20, and X30) concentration of (C) *Z*-TPE3 and (D) *E*-TPE3. I_0 = emission intensity in the absence of oligonucleotides. [*Z*-TPE3] = [*E*-TPE3] = 5 μM; λ_{ex} = 330 nm, λ_{em} = 480 nm. Error bars are ± SD. X10, X20, X30 are synthetic oligonucleotides with length of 10, 20, and 30 nt, respectively.

derivatives were adequately verified by ^{1}H and ^{13}C NMR spectroscopy, and mass spectrometry (see Experimental Section).

Fluorescence Response in Aqueous Solution. Before exploiting the potential of the new derivatives, namely TPE2 and TPE3, as DNA satin in PAGE, we first investigated their fluorescence response to nucleic acid in aqueous solution. For comparison, the previously reported compound of TPE1 was also tested under the identical conditions with only 5 μM in this work.[31] For TPE1 and TPE3, the mixtures of 1:1 ratio of cis and trans configuration isomers were used. The fluorescence titrations with X30 (oligonucleotide with length of 30 nt) in deionized water are shown in Figure 2.

All the probes showed significant fluorescence enhancement upon the addition of X30. When the X30 concentration reached 0.2 μM, the fluorescent enhancement of TPE1, TPE2 and TPE3 were 48, 9, and 38-fold the original value (Figure 2). We speculate that the intramolecular motions of the TPE molecules were restricted when interacted with the DNA strand in water through both hydrogen-bond and electrostatic

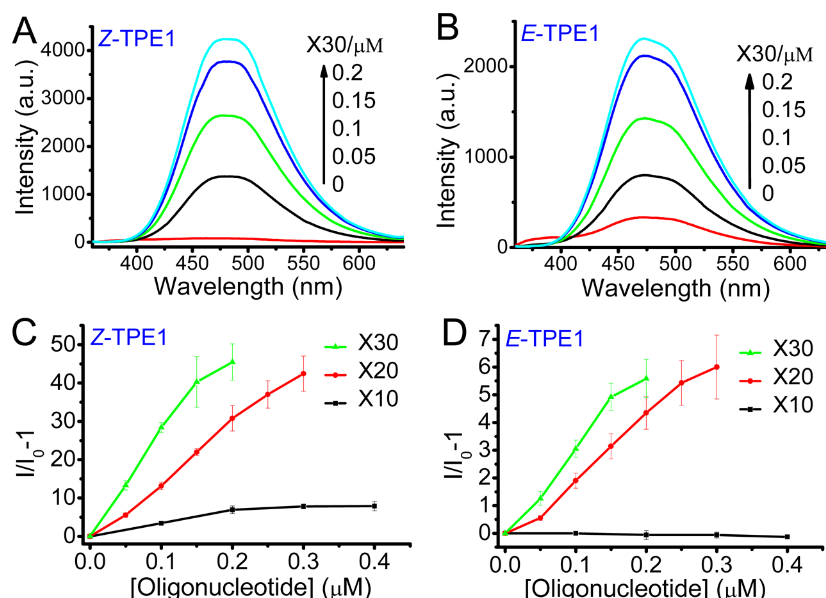

Figure 4. Fluorescence titration of X30 to (A) *Z*-TPE1 and (B) *E*-TPE1 in deionized water. Plot of $I/I_0 - 1$ at 480 nm versus the oligonucleotides (X10, X20, and X30) concentration of (C) *Z*-TPE1 and (D) *E*-TPE1. I_0 = emission intensity in the absence of oligonucleotides. [*Z*-TPE1] = [*E*-TPE1] = 5 μM; λ_{ex} = 330 nm, λ_{em} = 480 nm. Error bars are ± SD. X10, X20, X30 are synthetic oligonucleotides with length of 10, 20, and 30 nt, respectively.

interaction between the amino groups and DNA phosphodiester skeleton, consequently the fluorescence became "turn on".

The fluorescence titrations with different length of oligonucleotides were also performed. Oligonucleotides (X10, X20, and X30) with repeated sequences of X10 from 10 nt to 30 nt were used for excluding the effect of sequence diversity. At the same concentration of total phosphodiester units, these oligonucleotides have the same amount of binding sites to interact with the probes. However, as the length of the oligonucleotide increased from 10 nt to 30 nt, the fluorescence enhancement of all the TPE probes was significantly increased (see Figure S1 in the Supporting Information). This indicated that the bound TPE molecules could interact each other by hydrophobic aggregation that lead to further restriction of the TPE units.

Through comparing the fluorescence titrations to different TPE probes, it turned out that TPE2 with four amino groups showed much lower fluorescent enhancement than other probes. First, it is probably that although TPE2 has more recognition sites for binding DNA compared to TPE1 and TPE3, not all of the sites of TPE2 can bind DNA, and the rotation of the unbound linking arms dissipated energy *via* nonradiative channels. Second, the unbound protonated amino groups may repel each other through electrostatic interaction, which is not conducive to the hydrophobic aggregation of TPE units. Third, TPE2 has relatively higher background fluorescence in water presumably because its molecules may easily form aggregates by intermolecular hydrogen-bond interaction.

When detecting X10 with only 10 nt, TPE1 and TPE2 showed no fluorescent enhancement, whereas TPE3 still exhibited fluorescence "turn on" response. We speculate that long hydrophobic arm of TPE3 make it easier to aggregate when bound to the DNA strand. In addition, the hydrophobic interactions between the long arms and DNA strands also play an important role in the interaction between the TPE derivatives and the DNA strands. These factors lead to more efficient emission of TPE3 than that of TPE1.

The *Z*/*E* configuration effect on the DNA detection was investigated by using pure isomers of the probes. Panels A and B in Figure 3 presented the fluorescence response of *Z*-TPE3 and *E*-TPE3 to X30, respectively. The *Z*-TPE3 is almost nonemissive, whereas *E*-TPE3 is weakly emissive in the absence of DNA, which is similar to the situation of *Z*-TPE1 and *E*-TPE1 (Figure 4A, B). Upon addition of oligonucleotide under the identical concentration, the fluorescent intensity of *Z*-TPE3 was much higher than that of *E*-TPE3. When the X30 concentration reached 0.2 μM, the fluorescence enhancement of *Z*-TPE3 was 58-fold original value, but that of *E*-TPE3 was only 18-fold original value. Thus, we prove again that the cis configuration isomer has superior sensitivity over trans configuration isomer, which is attributed to neighboring group participation. Moreover, *Z*-TPE3 and *E*-TPE3 with longer linking arms displayed higher sensitivity than *Z*-TPE1 and *E*-TPE1 respectively (Figure 3 vs Figure 4), which was in line with the experiments of the mixtures. From the plot of $I/I_0 - 1$ at 480 nm versus the oligonucleotides, *Z*-TPE3 and *E*-TPE3 can detect 0.2 μM X10 in solution, whereas *Z*-TPE1 and *E*-TPE1 showed no response. Besides, we also used ctDNA (calf thymus DNA, a natural dsDNA) as a model analyte for dsDNA detection (see Figures S11−S13 in the Supporting Information). All the amino-modified TPE molecules we used for dsDNA detection had a higher value of $(I/I_0 - 1)$ than TTAPE-Me (a TPE derivative with four tetraalikylammonium cations) with titration of ctDNA by the same concentration as reported.[32]

DNA Stain in Gel Electrophoresis. The highly sensitive fluorescent response of the TPE derivatives in detecting DNA in aqueous solution inspired us to further explore their application in DNA stain in gel electrophoresis. Ten micromolar aqueous solution of TPE1 (1:1 ratio of cis and trans configuration isomers) was used to stain DNA with similar sensitivity compared with EB in our previous work.[31] Herein,

Figure 5. Fluorescence staining of nucleic acids in polyacrylamide gels by (A) TPE2, (B) TPE1, and (C) TPE3. Oligonucleotide size marker (X10, X20, and X30) with equal nanogram amounts of each oligounucleotide were loaded in lanes 1 to 3. Lane 1, 10 ng; lane 2, 20 ng; lane 3, 40 ng per band; ultra low range dsDNA ladder (10, 15, 20, 25, 35, 50, 75, 100, 150, 200, and 300 bases) were loaded in lanes 4 to 8. Lane 4, 1 ng; lane 5, 2 ng; lane 6, 4 ng; lane 7, 6 ng; lane 8, 12 ng per band at 300 bp. Concentration of dyes: 5 μM. Staining time: 30 min.

after running polyacrylamide gel electrophoresis (PAGE) in tris-boric acid-EDTA (TBE) solution, the gel was stained by only 5 μM aqueous solution of all the dyes for 30 min. TPE2, the mixtures of *Z*/*E* isomers of TPE1 and TPE 3 (1:1 ratio by weight) were compared first. As Figure 5 shows, the DNA bands of the gel stained by TPE2 under UV illumination is hardly to identify, whereas the bands stained by TPE3 can be clearly seen. We tested the ssDNA as shown in lanes 1−3 and dsDNA as shown 4−8, and the detection limits with TPE2, TPE1, and TPE3 as stains are collected in Table 1. After stained by TPE2, the bands of X30 with only 10 ng loading can be seen, and become distinct to identify with the increasing amount of DNA from 10 ng to 40 ng. In contrast, the bands of X30 stained by TPE1 or TPE2 are not clear to indentify even with a loading amount of up to 40 ng. It is noticeable that the bands of X10 with only 10 nt can be detected by TPE3, while even 10 μM aqueous solution of TPE1 failed to stain the bands of X10 in gel matrix in our previous work,[31] which accords with the results of oligouncleotide detection in aqueous solution (Figure 2E, F, black line). As a supplement to ssDNA, ultra low range dsDNA ladder with fragment size from 10 to 300 bp were used as model of dsDNA to be tested. As summarized in Table 1, the detection limits of TPE3 is ca. one-fourth magnitude of TPE1.

Table 1. Detection limits of TPE1, TPE2, and TPE3 as Stains for Oligonucleotides and dsDNA[a]

	stains (ng/band)		
	TPE1	TPE2	TPE3
oligonucleotides (nt)			
30	40	>40	10
20	>40	na	40
10	na	na	40
ultra low range dsDNA (bp)			
75−300	4	na	1
50	14	>42	<3.5
35	15	na	5
25	na	na	7.5
20	na	na	8
15	na	na	19
10	na	na	24

[a]The detection limits per band is defined as that amount of nucleic acid which forms an easily detectable clear band. The absolute limit of detection is approximately two- or 3-fold smaller than the numbers listed here. na: not available.

By using pure isomers of the probes, the *Z*/*E* configuration effect on the DNA stain was also investigated. As Figure 6 and Table 2 presented, *Z*-TPE3 and *E*-TPE3 with longer linking arms showed higher sensitivity than *Z*-TPE1 and *E*-TPE1 as stain in gel matrix. In addition, the cis configuration isomers performed better than the trans configuration isomers, which accords with the detection results in water as discussed above. Among these TPE molecules, *Z*-TPE3 exhibited the highest sensitivity as a stain, even higher than EB with twice concentration (10 μM) as we reported before.[31] Besides, although the *trans* configuration isomers slightly lowered the sensitivity of TPE3 as a mixture, the detection limits of TPE3 are still low enough as stain compared with EB, which is less sensitive in detecting single-stranded DNA, especially oligonucleotides without second structure.

■ CONCLUSION

In summary, a series of new amino-functionalized TPE derivatives were designed and synthesized to reveal the relationship between molecular structures and sensitivity for nucleic acid detection. The sensitivities of TPE1 and TPE3 with only two amino-groups were much higher than TPE2 with four amino-groups. The cis isomers of both TPE1 and TPE3 showed higher sensitivities than their trans isomers. *Z*-TPE3 was found to be the most sensitive dye both in both solution and gel matrix. *Z*-TPE3 can exclusively stain 40 ng of short oligonulceotide with only 10 nt, which cannot be implemented by *Z*-TPE1 or EB.[31] The relationship between the structures of TPE molecules and sensitivity for DNA detection makes this work significant for the further design of TPE-based biosensors with higher sensitivity.

■ EXPERIMENTAL SECTION

Materials. Oligonucleotides (X10, X20, X30) were purchased from Sangon Biotech (Shanghai) Co., Ltd. The sequence of DNA is shown in Table S1 (see the Supporting Information). ctDNA was purchased from Sigma. Gene Ruler Ultra Low Range DNA Ladder was purchased from Thermo Scientific. All the other reagents were commercially available and used without further purification.

Instrumentation. ^{1}H NMR and ^{13}C NMR spectra were measured on a MECUYRVX300 in $CDCl_3$ and CD_3OD. Mass spectra were measured on a Micromass-ZQ mass spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectrophotometer. Water was purified using a Millipore filtration system. The polyacrylamide gel electrophoresis products were scanned by BioDoc-it imaging system with LMS-26E Transilluminator.

Fluorescence Measurements. TPE dyes were dissolved in DMSO to get a 5 mM solution. When carrying out the fluorescence

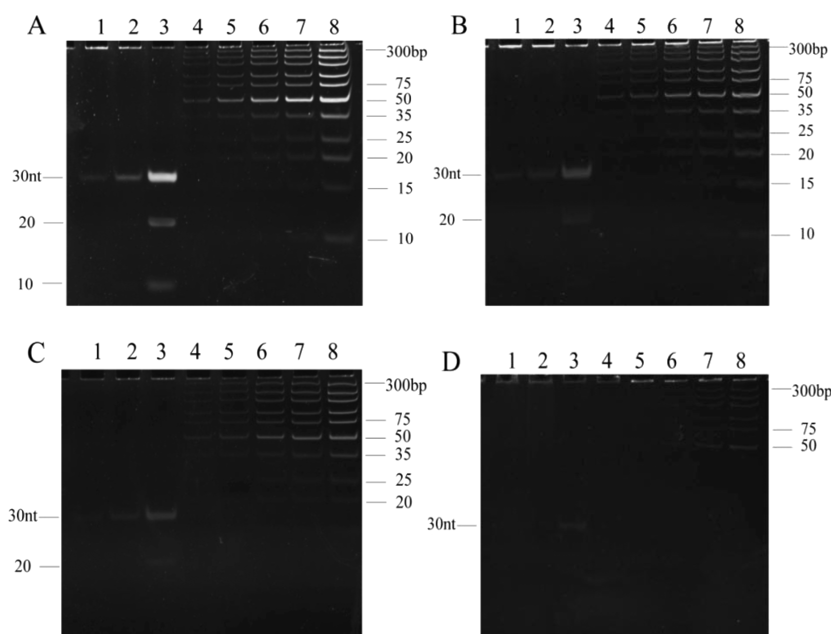

Figure 6. Fluorescence staining of nucleic acids in polyacrylamide gels by (A) *Z*-TPE3, (B) *E*-TPE3, (C) *Z*-TPE1, and (D) *E*-TPE1. Oligonucleotide size marker (X10, X20, and X30) with equal nanogram amounts of each oligounucleotide were loaded in lanes 1 to 3. Lane 1, 10 ng; lane 2, 20 ng; lane 3, 40 ng per band; ultra low range dsDNA ladder (10, 15, 20, 25, 35, 50, 75, 100, 150, 200, and 300 bases) were loaded in lanes 4 to 8. Lane 4, 1 ng; lane 5, 2 ng; lane 6, 4 ng; lane 7, 6 ng; lane 8, 12 ng per band at 300 bp. Concentration of dyes: 5 μM. Staining time: 30 min.

Table 2. Detection Limits of *Z*-TPE1, *E*-TPE1, *Z*-TPE2 and *E*-TPE2 as stains for Oligonucleotides and dsDNA.[a]

	stain (ng/band)			
	Z-TPE1	*E*-TPE1	*Z*-TPE3	*E*-TPE3
oligonucleotides (nt)				
30	40	>40	10	20
20	na	na	40	40
10	na	na	40	na
ultra low rang dsDNA (bp)				
75–300	4	12	1	2
50	10.5	>42	<3.5	3.5
35	15	na	5	7.5
25	>15	na	7.5	7.5
20	na	na	8	16
15	na	na	19	19
10	na	na	<24	>24

[a]The detection limits per band is defined as that amount of nucleic acid which forms an easily detectable clear band. The absolute limit of detection is approximately two- or 3-fold smaller than the numbers listed here. na: not available.

titration experiment, 1 μL of 5 mM DMSO solution was added to the fluoremeter quartz cuvette with 1 mL of deionized water or buffer solution, then followed the addition of DNA. The mixtures were vortex mixed and stood for 8 min prior to the measurements. All the titration experiments were carried out three times for calculating error bars.

Electrophoresis and Gel Conditions. DNA was electrophoresed in 1.0 mm thick 18% polyacrylamide gelsin 1*TBE buffer (89 mM Tris, 89 mM boricacid, 2 mM EDTA, pH 8.3) at 158 V/cm for 1.2 h. To determine sensitivity limits for oligodeoxynucleotides, dilutions containing 40 ng to 10 ng random-sequence oligonucleotide size markers (a mixture of equal mass oligonucleotides 10, 20, and 30 bases in length) in formamide loading buffer were electrophoresed in lanes 1 to 3. To determine sensitivity limits for dsDNA in polyacrylamidegels, we used dilutions containing 133.6 to 16.7 ng per lane ultra low range DNA ladder in lanes 4 to 8, which means 12 ng to 1 ng per band at 300 bp and 42 ng to 3.5 ng per band at 50 bp.

TPE stains were dissolved in DMSO to get a 10 mM solution, then it was diluted by deionized water according to 1:2000 ratio to afford a 5 μM solution at last. The gels were incubated in 5 μM dye-containing solutions for 30 min, then photographed using a 300 nm UV transillumination. No special destaining was performed for any of thesedyes, but briefly washed two times with water. For sensitivity comparisons, the photographic conditions were the same.

Synthesis of TPE Molecules. Compound **4** were synthesized according to the reported literatures.[17] All the other reagents were commercially available and used without further purification.

TPE2. A mixture of **4** (2.47 g, 3 mmol) and NaN_3 (975 mg, 15 mmol) in DMF (15 mL) was stirred at 80 °C for 3 h. After cooling to the room temperature, the mixture was pouring into water. The precipitation **5** was obtained without further purification for next step. A mixture of **5** (2.02 g, 3 mmol) and PPh_3 (3.93 g, 15 mmol) in THF (49 mL) and H_2O (7 mL) was stirred at 60 °C overnight. After concentration, the residue was purified by silica gel chromatography using chloroform/methanol/ammonium hydroxide (60:10:1, v/v/v) as eluent. The product was obtained as white power in 90% yield (1.54 g). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.92 (d, J = 8.4 Hz, 8H), 6.64 (d, J = 8.7 Hz, 8H), 3.03 (t, J = 5.1 Hz, 8H), 3. 90 (t, J = 5.1 Hz, 8H). ^{13}C NMR (75 MHz, CD_3OD) δ (ppm): 158.68, 139.99, 133.64, 133.64, 114.75, 70.11, 41.82. ESI-MS: m/z $[M + H]^+$ 568.71(M^+)

*1,2-Bis(4-(4-bromobutoxy)phenyl)-1,2-diphenylethene (**2**).* To a suspension of **1** (1.98 g, 6 mmol) and Zn dust (0.78 g, 12 mmol) in 50 mL of THF bath in ice water was added $TiCl_4$ (0.7 mL, 6 mmol) slowly under an Ar atmosphere. The mixture was heated to reflux and stirred for 12 h. After filtration, the filtrate was concentrated, then purified by silica gel chromatography using petroleum ether/chloroform (4:1, v/v) as eluent. The product was obtained as white power in 79% yield (1.52 g). ^{1}H NMR (300 MHz, $CDCl_3$), δ(ppm): 7.11–7.01 (m, 10H), 6.94–6.88 (m, 4H), 6.64–6.58 (m, 4H), 3.94–3.84 (m, 4H), 3.49–3.43 (m, 4H), 2.06–1.98 (m, 4H), 1.92–1.85 (m, 4H).

Z-TPE3 and E-TPE3. A mixture of **2** (0.9 g, 1.42 mmol) and NaN_3 (0.24 g, 3.69 mmol) in DMF (50 mL) was stirred at 80 °C for 3 h. After cooling to the room temperature, the mixture was pouring into water. The precipitation **3** was obtained without further purification. A

mixture of **3** (0.56 g, 1 mmol) and PPh_3 (1.07 g, 4 mmol) in THF (120 mL) and H_2O (20 mL) was stirred at 60 °C overnight. After concentration, the residue was purified by silica gel chromatography using chloroform/methanol/ammonium hydroxide (100:10:1, v/v/v) as eluent. The *Z*-TPE3 and *E*-TPE3 were obtained as white powers in 43% yield (0.22 g) and 45% yield (0.23 g), respectively.

Z-TPE3. ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.08−7.07 (m, 6H), 7.03−7.00 (m, 4H), 6.95 (d, J = 8.4 Hz, 4H), 6.66 (d, J = 8.4 Hz, 4H), 3.91 (t, J = 6.3 Hz, 4H), 2.76 (t, J = 6.6 Hz, 4H), 1.83−1.74 (m, 4H), 1.65−1.55 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 157.59, 144.46, 139.85, 136.58, 132.74, 131.65, 127.77, 126.38, 113.83, 67.73, 42.10, 30.43, 26.94. ESI-MS: m/z $[M + H]^+$ 507

E-TPE3. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.10−7.08 (m, 6H), 7.05−7.02 (m, 4H), 6.90 (d, J = 8.4 Hz, 4H), 6.61 (d, J = 8.7 Hz, 4H), 3.87 (t, J = 6.3 Hz, 4H), 2.74 (t, J = 6.9 Hz, 4H), 1.81−1.72 (m, 4H), 1.63−1.53 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 157.59, 144.55, 139.84, 136.53, 132.77, 131.64, 127.90, 126.40, 113.74, 67.68, 42.07, 30.32, 26.90. ESI-MS: m/z $[M + H]^+$ 507

■ ASSOCIATED CONTENT

Supporting Information

Synthetic routes of the new amino-modified tetraphenylethene derivatives and additional spectra on fluorescence studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Driver, S. E.; Robinson, G. S.; Flanagan, J.; Shen, W.; Smith, L. E. H.; Thomas, D. W.; Roberts, P. C. Oligonucleotide-Based Inhibition of Embryonic Gene Expression. *Nat. Biotechnol.* **1999**, *17*, 1184−1187.

(2) Arya, D. P. New Approaches Toward Recognition of Nucleic Acid Triple Helices. *Acc. Chem. Res.* **2011**, *44*, 134−146.

(3) Thomas, S. W.; Joly, G. D.; Swager, T. M. Chemical Sensors Based on Amplifying Fluorescent Conjugated Polymers. *Chem. Rev.* **2007**, *107*, 1339−1386.

(4) Rentzeperis, D.; Medero, M.; Marky, L. A. Thermodynamic Investigation of the Association of Ethidium, Propidium and Bis-ethidium to DNA Hairpins. *Bioorg. Med. Chem.* **1995**, *3*, 751−759.

(5) Rye, H. S.; Glazer, A. N. Interaction of Dimeric Intercalating Dyes with Single-Stranded DNA. *Nucleic Acids Res.* **1995**, *23*, 1215−1222.

(6) Glazer, A. N. R.; Hays, S. Stable Dye-DNA Intercalation Complexes as Reagents for High-Sensitivity Fluorescence Detection. *Nature* **1992**, *359*, 859−861.

(7) Tuma, R. S.; Beaudet, M. P.; Jin, X.; Jones, L. J.; Cheung, C.-Y.; Yue, S.; Singer, V. L. Characterization of SYBR Gold Nucleic Acid Gel Stain: a Dye Optimized for Use with 300-nm Ultraviolet Trans-illuminators. *Anal. Biochem.* **1999**, *268*, 278−288.

(8) Rye, H. S.; Yue, S.; Wemmer, D. E.; Quesada, M. A.; Haugland, R. P.; Mathies, R. A.; Glazer, A. N. Stable Fluorescent Complexes of Double-stranded DNA with Bis-intercalating Asymmetric Cyanine Dyes: Properties and Applications. *Nucleic Acids Res.* **1992**, *20*, 2803−2812.

(9) Glazer, A. N.; Peck, K.; Mathies, R. A. A Stable Double-Stranded DNA-ethidium Homodimer Complex: Application to Picogram Fluorescence Detection of DNA in Agarose Gels. *Proc. Natl. Acad. Sci. U. S. A.* **1990**, *87*, 3851−3855.

(10) Dragan, A. I.; Bishop, E. S.; Strouse, R. J.; Casas-Finet, J. R.; Schenerman, M. A.; Geddes, C. D. Metal-Enhanced Ethidium Bromide Emission: Application to dsDNA Detection. *Chem. Phys. Lett.* **2009**, *480*, 296−299.

(11) Singer, V. L.; Lawlor, T. E.; Yue, S. Comparison of SYBR® Green I Nucleic Acid Gel Stain Mutagenicity and Ethidium Bromide Mutagenicity in the Salmonella/Mammalian Microsome Reverse Mutation Assay (Ames Test). *Mutat. Mutat. Res., Genet. Toxicol. Environ. Mutagen.* **1999**, *439*, 37−47.

(12) Ohta, T.; Tokishita, S.-i.; Yamagata, H. Ethidium Bromide and SYBR Green I Enhance the Genotoxicity of UV-Irradiation and Chemical Mutagens in E. coli. *Mutat. Mutat. Res., Genet. Toxicol. Environ. Mutagen.* **2001**, *492*, 91−97.

(13) Ouchi, R. Y.; Manzato, A. J.; Ceron, C. R.; Bonilla-Rodriguez, G. O. Evaluation of the Effects of a Single Exposure to Ethidium Bromide in Drosophila Melanogaster (Diptera-Drosophilidae). *Bull. Environ. Contam. Toxicol.* **2007**, *78*, 489−493.

(14) Kirsanov, K. I.; Lesovaya, E. A.; Yakubovskaya, M. G.; Belitsky, G. A. SYBR Gold and SYBR Green II Are not Mutagenic in the Ames Test. *Mutat. Res., Genet. Toxicol. Environ. Mutagen.* **2010**, *699*, 1−4.

(15) Wang, Z.; Chen, S.; Lam, J. W. Y.; Qin, W.; Kwok, R. T. K.; Xie, N.; Hu, Q.; Tang, B. Z. Long-Term Fluorescent Cellular Tracing by the Aggregates of AIE Bioconjugates. *J. Am. Chem. Soc.* **2013**, *135*, 8238−8245.

(16) Wang, F.; Wen, J.; Huang, L.; Huang, J.; Ouyang, J. A Highly Sensitive "Switch-on" Fluorescent Probe for Protein Quantification and Visualization Based on Aggregation-Induced Emission. *Chem. Commun.* **2012**, *48*, 7395−7397.

(17) Zhu, Z.; Xu, L.; Li, H.; Zhou, X.; Qin, J.; Yang, C. A Tetraphenylethene-Based Zinc Complex as a Sensitive DNA Probe by Coordination Interaction. *Chem. Commun.* **2014**, *50*, 7060−7062.

(18) Hu, R.; Gomez-Duran, C. F. A.; Lam, J. W. Y.; Belmonte-Vazquez, J. L.; Deng, C.; Chen, S.; Ye, R.; Pena-Cabrera, E.; Zhong, Y.; Wong, K. S.; Tang, B. Z. Synthesis, Solvatochromism, Aggregation-Induced Emission and Cell Imaging of Tetraphenylethene-Containing BODIPY Derivatives with Large Stokes Shifts. *Chem. Commun.* **2012**, *48*, 10099−10101.

(19) Mergny, J.-L.; Maurizot, J.-C. Fluorescence Resonance Energy Transfer as a Probe for G-Quartet Formation by a Telomeric Repeat. *ChemBioChem.* **2001**, *2*, 124−132.

(20) Tong, H.; Hong, Y.; Dong, Y.; Hau; Lam, J. W. Y.; Li, Z.; Guo, Z.; Guo, Z.; Tang, B. Z. Fluorescent "Light-up" Bioprobes Based on Tetraphenylethylene Derivatives with Aggregation-Induced Emission Characteristics. *Chem. Commun.* **2006**, 3705−3707.

(21) Luo, J.; Xie, Z.; Lam, J. W. Y.; Cheng, L.; Chen, H.; Qiu, C.; Kwok, H. S.; Zhan, X.; Liu, Y.; Zhu, D.; Tang, B. Z. Aggregation-Induced Emission of 1-Methyl-1,2,3,4,5-Pentaphenylsilole. *Chem. Commun.* **2001**, 1740−1741.

(22) Ding, D.; Li, K.; Liu, B.; Tang, B. Z. Bioprobes Based on AIE Fluorogens. *Acc. Chem. Res.* **2013**, *46*, 2441−2453.

(23) Hong, Y.; Lam, J. W. Y.; Tang, B. Z. Aggregation-Induced Emission. *Chem. Soc. Rev.* **2011**, *40*, 5361−5388.

(24) Hong, Y.; Meng, L.; Chen, S.; Leung, C. W. T.; Da, L.-T.; Faisal, M.; Silva, D.-A.; Liu, J.; Lam, J. W. Y.; Huang, X.; Tang, B. Z. Monitoring and Inhibition of Insulin Fibrillation by a Small Organic Fluorogen with Aggregation-Induced Emission Characteristics. *J. Am. Chem. Soc.* **2011**, *134*, 1680−1689.

(25) Shi, H.; Kwok, R. T. K.; Liu, J.; Xing, B.; Tang, B. Z.; Liu, B. Real-Time Monitoring of Cell Apoptosis and Drug Screening Using Fluorescent Light-Up Probe with Aggregation-Induced Emission Characteristics. *J. Am. Chem. Soc.* **2012**, *134*, 17972−17981.

(26) Hong, Y.; Häußler, M.; Lam, J. W. Y.; Li, Z.; Sin, K. K.; Dong, Y.; Tong, H.; Liu, J.; Qin, A.; Renneberg, R.; Tang, B. Z. Label-Free

Fluorescent Probing of G-Quadruplex Formation and Real-Time Monitoring of DNA Folding by a Quaternized Tetraphenylethene Salt with Aggregation-Induced Emission Characteristics. *Chem.—Eur. J.* **2008**, *14*, 6428−6437.

(27) Hong, Y.; Xiong, H.; Lam, J. W. Y.; Häußler, M.; Liu, J.; Yu, Y.; Zhong, Y.; Sung, H. H. Y.; Williams, I. D.; Wong, K. S.; Tang, B. Z. Fluorescent Bioprobes: Structural Matching in the Docking Processes of Aggregation-Induced Emission Fluorogens on DNA Surfaces. *Chem.—Eur. J.* **2010**, *16*, 1232−1245.

(28) Wang, J.; Mei, J.; Hu, R.; Sun, J. Z.; Qin, A.; Tang, B. Z. Click Synthesis, Aggregation-Induced Emission, E/Z Isomerization, Self-Organization, and Multiple Chromisms of Pure Stereoisomers of a Tetraphenylethene-Cored Luminogen. *J. Am. Chem. Soc.* **2012**, *134*, 9956−9966.

(29) Li, S.; Langenegger, S. M.; Haner, R. Control of Aggregation-Induced Emission by DNA Hybridization. *Chem. Commun.* **2013**, *49*, 5835−5837.

(30) Jin, J. K.; Chen, X.; Liu, Y.; Qin, A.; Sun, J.; Tang, B. Z. Detection of ctDNA with Water Soluble Tetraphenylene-Based Fluorescence Probe. *Gaofenzi Xuebao* **2011**, *9*, 1079−1085.

(31) Xu, L.; Zhu, Z.; Zhou, X.; Qin, J.; Yang, C. A Highly Sensitive Nucleic Acid Stain Based on Amino-Modified Tetraphenylethene: the Influence of Configuration. *Chem. Commun.* **2014**, *50*, 6494−6497.

(32) Hong, Y.; Chen, S.; Leung, C. W. T.; Lam, J. W. Y.; Tang, B. Z. Water-Soluble Tetraphenylethene Derivatives as Fluorescent "Light-Up" Probes for Nucleic Acid Detection and Their Applications in Cell Imaging. *Chem.—Eur. J.* **2013**, *8*, 1806−1812.