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A highly sensitive nucleic acid stain based on amino-modified tetraphenylethene: the influence of configuration†

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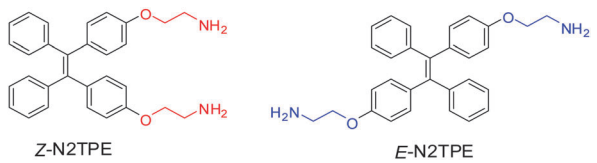
We designed and synthesized a new amino-functionalized tetraphenylethene (TPE) derivative as a highly sensitive dye for the detection of dsDNA and oligonucleotide in both solution and a gel matrix. We further revealed that the *cis* configuration dye showed a much higher sensitivity than its *trans* isomer for the first time.

Gel electrophoresis is one of the most valuable techniques for the separation of nucleic acids in life sciences.[1] The visualization of nucleic acid bands in a gel matrix relies on the generation of a contrast image through a readout species, which includes organic fluorescent dyes, silver stain, zinc-imidazole *etc.*[2] In particular, fluorescent probes are usually preferred in view of sensitivity and convenience. Commercial dyes widely used as markers in gel electrophoresis are based on fluorescent enhancement triggered by intercalating into or groove binding to DNA, such as Ethidium Bromide (EB), Gel Star, and SYBR stains.[2b,g] Among these fluorescent dyes, EB is a commonly used nucleic acid stain because of its high sensitivity and low cost.[3] But EB is reported to be a toxic and mutagenic agent because of its intercalating property upon binding to nucleic acids.[4] To be specific, EB has been shown to inhibit replication in several organisms by interfering with DNA synthesis[5] and causes frame shift mutations in bacteria.[4d,6] Besides, due to its binding mode of intercalating into DNA, EB is less sensitive in detecting single-stranded DNA, especially oligonucleotides without secondary structures.[7] Some alternatives of EB, such as SYBR-based dyes, have been found to be highly sensitivite but less carcinogenic.[8] However, the availability of these products at high price is disadvantageous to high-throughput detection. Thus, it is a pressing concern to develop less-toxic, cost-effective and highly sensitive dyes for the quantitation and detection of nucleic acids in a gel matrix.

In recent years, tetraphenylethene (TPE) derivatives have been widely studied in the field of cell labeling,[9] DNA and protein detection.[10] Unlike the conventional self-quenched fluorescent probes, TPE-based dyes are non-emissive as unassociated monomers, but become strongly fluorescent upon aggregation at high concentrations.[11] This novel aggregation-induced emission (AIE) effect is proposed to be caused by restriction of intramolecular rotation (RIR) that prohibits energy dissipation *via* non-radiative channels, leading to high quantum yields in aggregated states.[12] Tang *et al.* felicitously designed a series of TPE derivatives with tetraalkylammonium cations as "light up" probes for DNA detection.[10d] These probes bind to DNA through electrostatic interaction, quite unlike the classical intercalators or groove binders, and thus they were further used as a new kind of DNA stain for gel electrophoresis with the lowest detection limit of 0.25 μg.[7a] In addition, it is also reported that some TPE-based probes are cytocompatible and pose no threat of toxicity to living cells.[10d,e]

In this communication, aiming to develop simple, universal and highly sensitive dyes for the detection of nucleic acids in a gel matrix, we designed and synthesized a new amino-functionalized TPE derivative (Scheme 1). We expected that the introduction of an amino group would strengthen the interaction between the dye and nucleic acid by the formation of a hydrogen bond, and thus the new dye could detect not only dsDNA but also oligonucleotides with high sensitivity in both solution and a gel matrix. We further revealed that the *cis* configuration dye showed a much higher sensitivity than its



Scheme 1 The structures of *Z*-N2TPE and *E*-N2TPE.

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trans isomer for the first time. The *cis* configuration dye can stain 10 ng of short oligonucleotide with only 20 nt, and 1 ng of dsDNA with 300 bp in polyacrylamide gel electrophoresis (PAGE). The detection limits are significantly lower than those of the commonly used nucleic acid stain of EB.

The synthetic route of N2TPE is shown in Scheme S1 (ESI†). Intermediate **1** was synthesized by substitution of 4-hydroxybenzophenone with 1,2-dibromoethane. Subsequently, intermediate **2** was obtained through titanium-catalyzed McMurry coupling reaction. The azide compound **3** obtained by the reaction of **2** with NaN_3 was reduced to give the final product, named N2TPE, which is obviously a mixture of the *cis* and *trans* isomers. The pure *Z*-N2TPE and *E*-N2TPE were separated by careful chromatography with yields of 41% and 44%, respectively. The pure isomers showed doublets at *ca.* 6.6 (H1) and 6.9 (H2) ppm, as well as triplets at *ca.* 3.9 (H6) and 3.0 (H7) ppm assigned to the ethylene group, while their mixture showed mutiplets due to the overlap of these peaks (Fig. S1, ESI†). The configuration of the *cis* isomer (*Z*-N2TPE) was further determined by single-crystal X-ray analysis of its dihydrochloride (Fig. S1, ESI†).

N2TPE was initially exploited to detect DNA. Fig. 1A presents the fluorescence response of N2TPE to X30 (synthetic oligonucleotide with 30 nt). Addition of X30 in the concentration range from 0 to 0.5 μM led to a progressive increase in the emission intensity of N2TPE at 480 nm. The FL intensity is 61 fold its original value when the DNA concentration is 0.5 μM (Fig. 1B). The non-emissive N2TPE became highly luminescent upon binding to DNA. We speculated that the intramolecular motions of the N2TPE were restricted through both hydrogen-bond and electrostatic interaction between the N2TPE and DNA, consequently the fluorescence became ''turn on''.

Similar to other TPE derivatives used in DNA detection,[10] N2TPE is a mixture of *cis* and *trans* configurational isomers. The stereochemistry was usually ignored in the study of TPE-based compounds due to the difficulty of separation after preparation by the McMurry reaction. Recently, Tang *et al.* reported pure *cis*/*trans* TPE derivatives, but their difference as bio/chemosensor is still to be explored.[13] By the aid of the pure *Z*-N2TPE and *E*-N2TPE isomers, we explored the configurational effects on DNA detection. The fluorescence spectra were recorded in deionized water (Fig. 2A and B). *Z*-N2TPE is almost not emissive, while *E*-N2TPE is weak emissive. Upon addition of

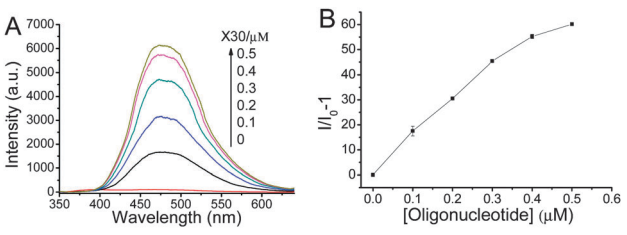

Fig. 1 (A) Fluorescence titration of X30 to N2TPE in deionized water. (B) Plot of $I/I_0 - 1$ at 480 nm *versus* X30 concentration. I_0 = emission intensity in the absence of oligonucleotide. X30 is a synthetic oligonucleotide with 30 nt. [N2TPE] = 10 μM; λ_{ex} = 330 nm, λ_{em} = 480 nm, error bars are ±SD.

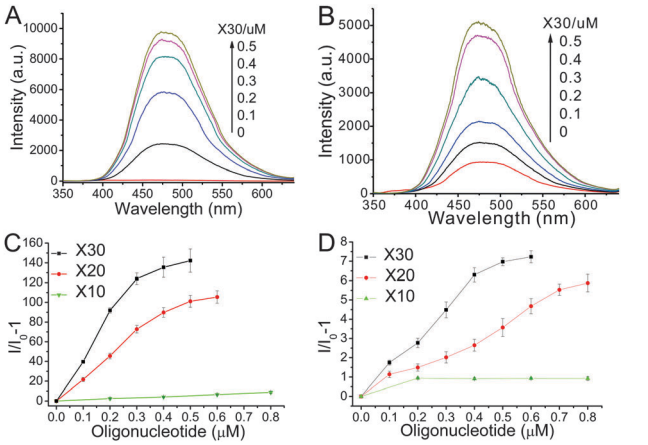

Fig. 2 Fluorescence titration of X30 to *Z*-N2TPE (A) and *E*-N2TPE (B) in deionized water. Plot of $I/I_0 - 1$ at 480 nm *versus* the oligonucleotide (X10, X20, and X30) concentration of *Z*-N2TPE (C) and *E*-N2TPE (D). I_0 = emission intensity in the absence of oligonucleotides. [*Z*-N2TPE] = 10 μM. [*E*-N2TPE] = 10 μM; λ_{ex} = 330 nm, λ_{em} = 480 nm, error bars are ±SD. X10, X20, X30 are synthetic oligonucleotides with a length of 10 nt, 20 nt and 30 nt, respectively.

oligonucleotide at the same concentration, the fluorescent intensity of *Z*-N2TPE was about two times higher than that of *E*-N2TPE. When the X30 concentration reached 0.5 mM, the fluorescent enhancement of *Z*-N2TPE was 143 fold its original value, in contrast the fluorescent enhancement of *E*-N2TPE was only 8 fold. This should be attributed to neighbouring group participation. The *cis* configuration with two close interaction centers could bind DNA with higher affinity than its *trans* isomer. To study the effect of DNA chain length, oligonucleotides with different lengths were tested. As shown in Fig. 2C and D, with the reduction of the length of the oligonucleotides from X30 to X10, the fluorescent enhancement was substantiallly decreased. When the oligonucleotide length is as short as X10 with only 10 nt, it affords less interaction sites with *Z*-N2TPE or *E*-N2TPE, and thus the aggregation may not be enough to turn on the fluorescence. We also consider ctDNA (calf thymus DNA, a natural dsDNA) as a model analyte for dsDNA detection (Fig. S2–S3, ESI†).

The ''turn on'' fluorescent response and high sensitivity of the newly designed TPE derivatives in detecting DNA in aqueous solution inspired us to further explore their application as a DNA stain in gel electrophoresis. We still used the pure *Z*-N2TPE and *E*-N2TPE as the stain to investigate the configurational effect (Fig. 3A and B). For comparison, we also tested the widely used stain of EB (Fig. 3C) and the mixture of *Z*/*E* isomers under the identical conditions (Fig. 3D). After running polyacrylamide gel electrophoresis (PAGE) in a tris-boric acid-EDTA (TBE) solution, the gel was stained by 10 mM solution.

We firstly tested the ssDNA as shown in lane 1–3 of Fig. 3A–D. After staining by *Z*-N2TPE, the bands of X20 and X30 with 10 ng loading can be seen, while the band of X10 is not detectable. With the increasing amount of DNA from 10 ng to 40 ng, the bands of X20 and X30 become distinct to identify, while 40 ng of X10 is still undetectable (Fig. 3A, lane 1 to 3).

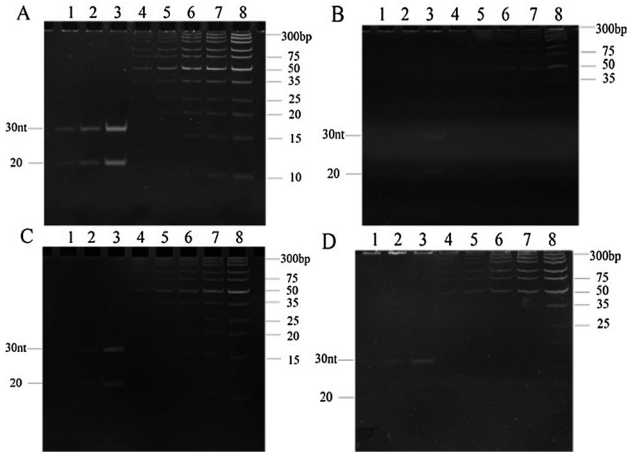

Fig. 3 Fluorescence staining of nucleic acids in polyacrylamide gels by *Z*-N2TPE (A), *E*-N2TPE (B), EB (C), and N2TPE (D). Oligonucleotide size markers (X10, X20, and X30) with equal nano-gram amounts of each oligounucleotide were loaded in lane 1 to 3. Lane 1: 10 ng, lane 2: 20 ng, lane 3: 40 ng per band; ultra low range dsDNA ladder (10, 15, 20, 25, 35, 50, 75, 100, 150, 200, and 300 bases) were loaded in lane 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: 10 μM. Staining time: 30 min.

In contrast, the bands of X20 and X30 stained by *E*-N2TPE or the reference EB are not clear to identify even with a loading amount of up to 40 ng (Fig. 3B and C, lane 3). The superior sensitivity of *Z*-N2TPE over *E*-N2TPE for the DNA stain in the gel accords with the results of DNA detection in an aqueous solution (Fig. 2C and D).

We then tested dsDNA as shown in lane 4–8 of Fig. 3A–D. Similar to the case of the ssDNA length, with the increasing dsDNA fragment size from 10 to 300 bp, the stained bands become gradually clear. After staining by *Z*-N2TPE, the band of 50 bp dsDNA can be seen with only 3.5 ng, and becomes distinct with increasing amount of DNA (Fig. 3A, lane 4 to 8). By contrast, the band of 50 bp dsDNA stained by *E*-N2TPE is still not clear even with a DNA amount of up to 42 ng (Fig. 3B, lane 8). The detection limits with *Z*-N2TPE and EB as the stain are collected in Table 1, in which the results of EB accord with those reported in the literature.[7] In the range of 10–50 bp dsDNA, the detection limit of *Z*-N2TPE is *ca.* one-third the magnitude of EB. The lowest detectable limits are 1 ng and 4 ng in the range from 75 to 300 bp for *Z*-N2TPE and EB, respectively. It is obvious that *Z*-N2TPE is superior to the commonly-used commercial DNA stain of EB. We finally used the mixture of *Z*-N2TPE and *E*-N2TPE (1 : 1 ratio by weight) as the stain, and observed an average result between *Z*-N2TPE and *E*-N2TPE (Fig. 3D). This result means that the mixture of *Z*/*E* isomers could also be used as the DNA stain. Though the sensitivity is not as high as the case of *Z*-N2TPE, there is no need of separation between the *Z*/*E* isomers which would be advantageous to its real application in terms of the stain cost.

Table 1 Detection limits of *Z*-N2TPE and EB as stains for oligonucleotides and dsDNA[a]

	Z-N2TPE (ng)	EB (ng)
Oligonucleotides (nt)		
30	10	40
20	10	40
Ultra low range dsDNA (bp)		
75–300	1	4
50	<3.5	7.5
35	2.5	7.5
25	5	15
20	5.3	15.9
25	9.5	>19
10	12	>24

[a] The detection limit 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 threefold smaller than the numbers listed here.

In conclusion, we developed a new amino-functionalized TPE derivative as a simple, universal and highly sensitive dye for the detection of nucleic acids in a gel matrix. By the aid of the pure *cis* and *trans* configuration TPE isomers, we demonstrated the significant differences of *Z*/*E* isomers in DNA detection and as nucleic acid stains for the first time. The *cis* configuration dye showed a much higher sensitivity than its *trans* isomer. This reveals that many other stereo isomers may differ in affinity to bind many analytes. The ultra-low detection limits and universality superior to EB make it promising in real application.

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