Electronic Supplementary Information (ESI)

Highly Sensitive Nucleic Acid Stain Based on Amino-Modified Tetraphenylethene: The Influence of Configuration

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Experimental Details

Instrumentation. ^{1}H NMR and ^{13}C NMR spectra were measured on a MECUYRVX300 or 600 spectrometer. Elemental analyses of carbon, hydrogen, and nitrogen were performed on a Vario EL III microanalyzer. 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.

Fluorescence Measurements. Subsequent fluorescence titration experiments were carried out at room temperature by addition of DNA into the deionized water or buffer solutions with 10 μM dyes. The mixtures were vortex mixed and stood for 10 minutes prior to the measurements.All the titration experiments werecarried out three times for calculating error bars.

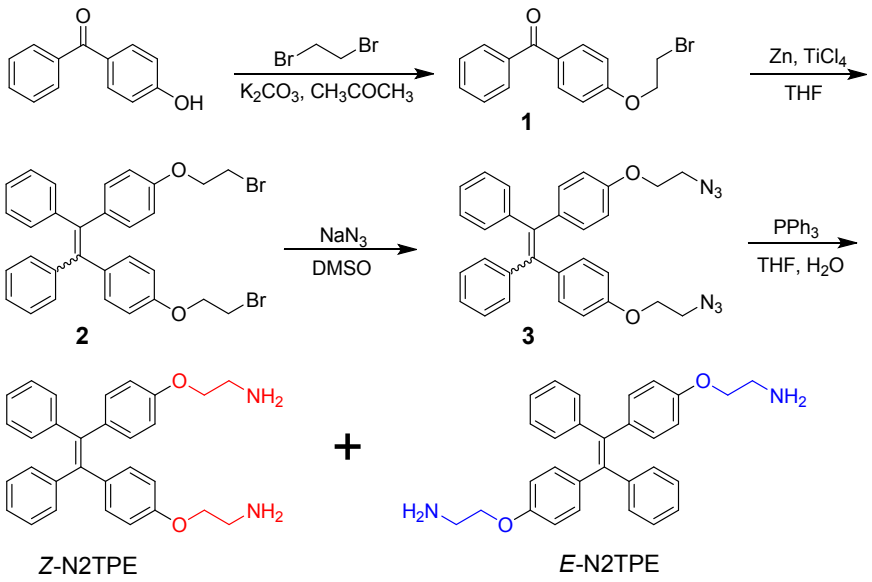
Electrophoresis and gel conditions. DNA was electrophoresed in 1.0mm-thick 18% polyacrylamide gelsin 1*TBE buffer (89 mM Tris, 89 mM boricacid, 2 mM EDTA, pH 8.3) at158 V/cm for 1.2 h. To determine sensitivity limits for oligodeoxynucleotides, dilutions containing 40ng to 10ng random-sequence oligonucleotide size markers (a mixture of equal mass oligonucleotides with 10, 20, and 30 based in length) in formamide loading buffer were electrophoresed in lane 1 to 3. To determine sensitivity limits for dsDNA in polyacrylamidegels, dilutions containing 133.6 ng to 16.7 ng per lane Ultra Low Range DNA ladder were used in lane 4 to 8, which means 12 ng to 1ng per band at 300 bp and 42 ng to 3.5 ng per band at 50 bp.

The gels were incubated in 10 μM dye-containing solutions for 30 min, then photographed using a 300-nm UV transillumination. EB staining was peformed using a 10 uM solution of dye in deionized water, while the gel wasstained for 30 min in a light-proof container. No special destaining was performed for any of thesedyes, but briefly washed two times with water. For sensitivity comparisons, the photographic conditions were optimized for gels stained by dyes.

Materials. Oligonucleotides(X10, X20, X30) were purchased from Sangon Biotech (Shanghai) Co., Ltd. ctDNA was purchased fromSigma. GeneRuler Ultra Low Range DNA Ladder was purchased from Thermo Scientific. All the other reagents were commercially available and used without further purification.

Table S1. Synthetic ssDNA used in this study

ID	sequence
X30	5'-GGTGCTAACT GGTGCTAACT GGTGCTAACT -3'
X20	5'-GGTGCTAACT GGTGCTAACT -3'
X10	5'-GGTGCTAACT-3'



Scheme S1 The synthetic route of N2TPE

(4-(2-bromoethoxy)phenyl)(phenyl)methanone (1). To a mixture of potassium carbonate (4.35 g, 30 mmol) and (4-hydroxyphenyl)(phenyl)methanone (1.98 g, 10 mmol) in acetone (50 mL), 1,2-dibromoethane (9.96 g, 10 mmol) was added at room temperature. The mixture was heated to reflux and stirred for 12 h. After filtration, the filtrate was concentrated, then purified by silica gel chromatography using chloroform as elute. The product was obtained as white power in 74% yield (2.22 g). ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.84 (d, J = 8.7Hz, 2H), 7.76 (d, J = 7.5Hz, 2H), 7.57 (m, 1H), 7.48 (t, J = 6.9Hz, 2H), 6.98 (d, J = 6.9Hz, 2H), 4.38 (t, J = 7.5Hz, 2H), 3.68 (t, J = 7.5Hz, 2H).

1,2-bis(4-(2-bromoethoxy)phenyl)-1,2-diphenylethene (2). To a suspension of **1** (1.83 g, 6 mmol) and Zn dust (0.78 g, 12 mmol)in 50 mL of THF bath in ice water, $TiCl_4$ (0.7 mL, 6 mmol) was added slowly under 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 83% yield (1.44 g). ^{1}H NMR (300MHz, $CDCl_3$), δ(ppm): 7.10-7.01 (m, 10H), 6.96-6.90 (m, 4H), 6.68-6.61 (m, 4H), 4.23-4.13 (m, 4H), 3.62-3.52 (m, 4H).

1,2-bis(4-(2-azidoethoxy)phenyl)-1,2-diphenylethene (3). A mixture of **2** (0.82 g, 1.42 mmol) and NaN_3 (0.24 g, 3.69 mmol) in DMSO (15 mL) was stirred at 80℃for 3 h. After cooling to the room temperature, the mixture was pouring into water. The precipitation was purified by silica gel chromatography using chloroform/petroleum ether (1:4, v/v) as eluent. The product was obtained as yellow oil in 98% yield (0.70 g). ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.15-7.00 (m, 10H), 7.00-6.90 (m, 4H), 6.69-6.62 (m, 4H), 4.11-4.04 (m, 4H), 3.59-3.52 (m, 4H).

***Z*-N2TPE and *E*-N2TPE.** A mixture of **3** (0.5 g, 1 mmol) and PPh_3 (1.07 g, 4 mmol) in THF (120 ml) and H_2O (20 mL) was stirred at 60℃ 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*-N2TPE and *E*-N2TPE were obtained as white powers in 41% yield (0.18 g) and 44% yield (0.19 g), respectively. A single crystal of *cis*isomer in the form of dihydrochloride was obtained by diffusion of ether into a methanol solution in presence of 2 equiv of hydrochloric acid solution.

Z-N2TPE. ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.07 (m, 6H), 7.02 (m, 4H),6.95 (d, J = 8.1Hz, 4H), 6.67 (d, J = 8.1Hz, 4H), 3.92 (t, J = 5,1Hz, 4H), 3.05 (t, J = 5,1Hz, 4H).^{13}C NMR (150 MHz, $CDCl_3$), δ(ppm): 157.31, 144.21, 139.70, 136.63, 132.60, 131.46, 127.63, 126.27, 113.68, 69.90, 41.61. Anal. Calcd for $C_{30}H_{30}N_2O_2$: C, 79.97; H, 6.71; N, 6.22; Found: C, 79.78; H, 6.75; N, 6.31. ESI-MS: *m/z*$[M+H]^+$ 451

*E-N2TP*E. ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm):7.10 (m, 6H), 7.05-7.02 (m, 4H), 6.92 (d, J = 8.7Hz, 4H), 6.64 (d, J = 8.7 Hz, 4H), 3.90 (t, J = 4.8Hz, 4H), 3.03 (t, J = 4.8Hz, 4H).^{13}C NMR (150 MHz, $CDCl_3$), δ(ppm): 157.30, 144.28, 139.66, 136.54, 132.62, 131.43, 127.75, 126.27, 113.58, 69.92, 41.61. Anal. Calcd for $C_{30}H_{30}N_2O_2$: C, 79.97; H, 6.71; N, 6.22; Found: C, 79.82; H, 6.50; N, 6.06. ESI-MS: *m/z*$[M+H]^+$ 451

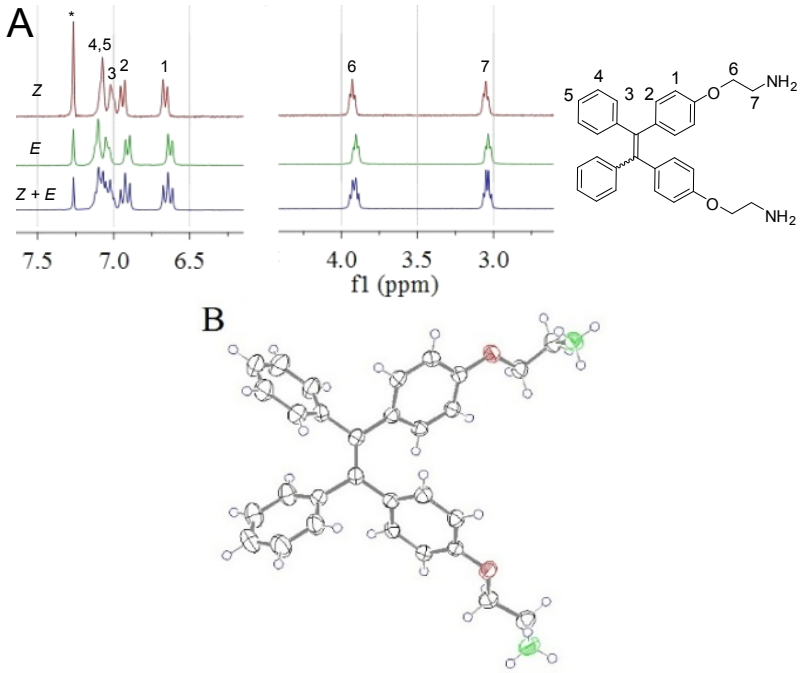


Fig. S1 (A) ^{1}H-NMR spectra of *cis*, *trans* and mixture of amino-functionalized TPE in $CDCl_3$. The solvent peaks are marked with asterisks. (B) Oak ridge thermal ellipsoidal plot (ORTEP) diagram of *Z*-N2TPE·H^+. Counterions have been omitted for clarity.

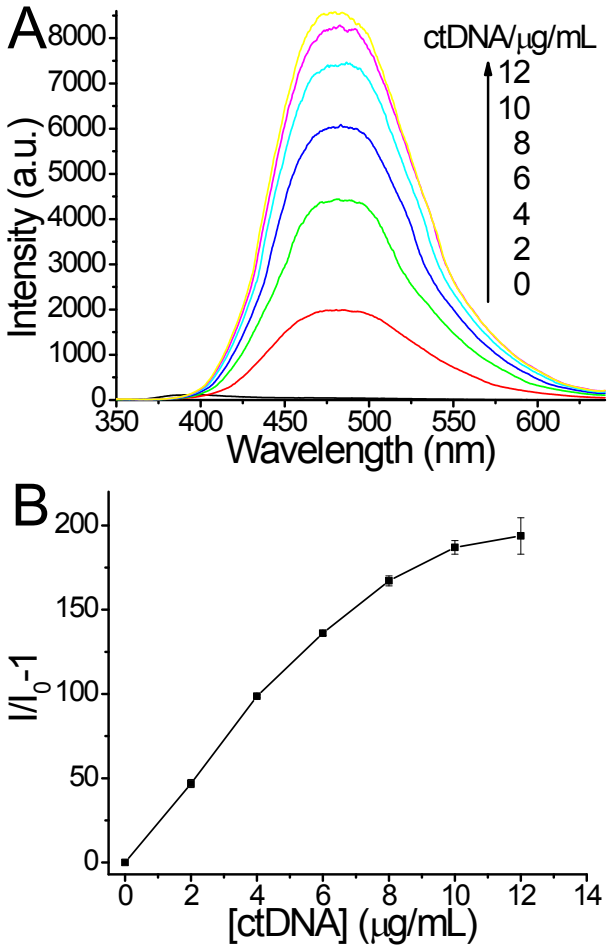


Fig. S2 (A) Fluorescence titration of ctDNA to 10 μM *Z*-N2TPEin deionized water. (B) Plot of I/I_0 - 1 at 480 nm versus ctDNA concentration. I_0 = emission intensity in the absence of oligonucleotide. ctDNA is Calf Thymus DNA, a natural dsDNA. [*Z*-N2TPE] = 10 μM; λ_{ex} = 330 nm, λ_{em} = 480 nm, Error bars are±SD.

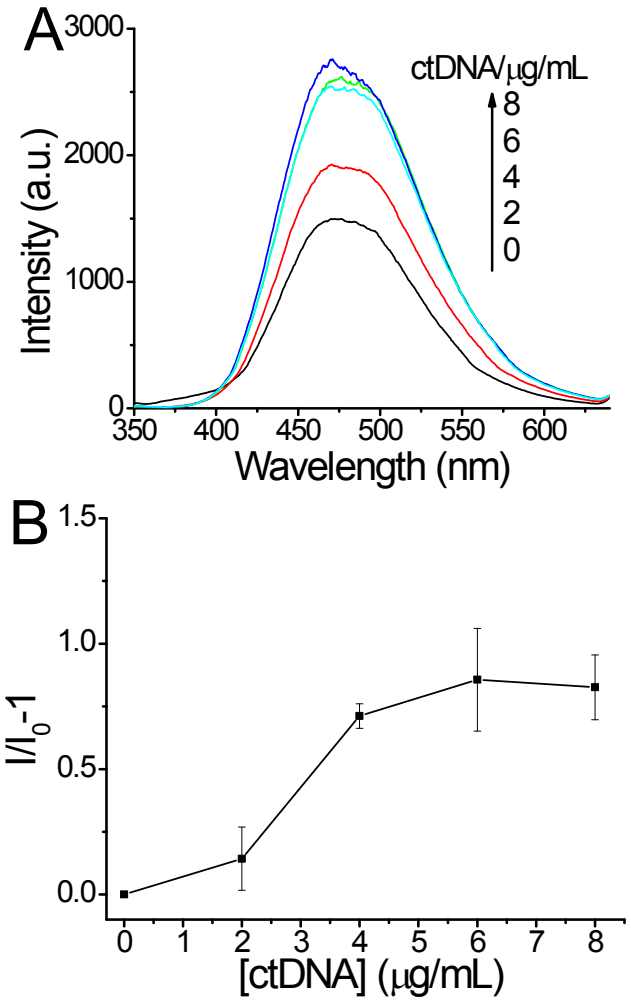
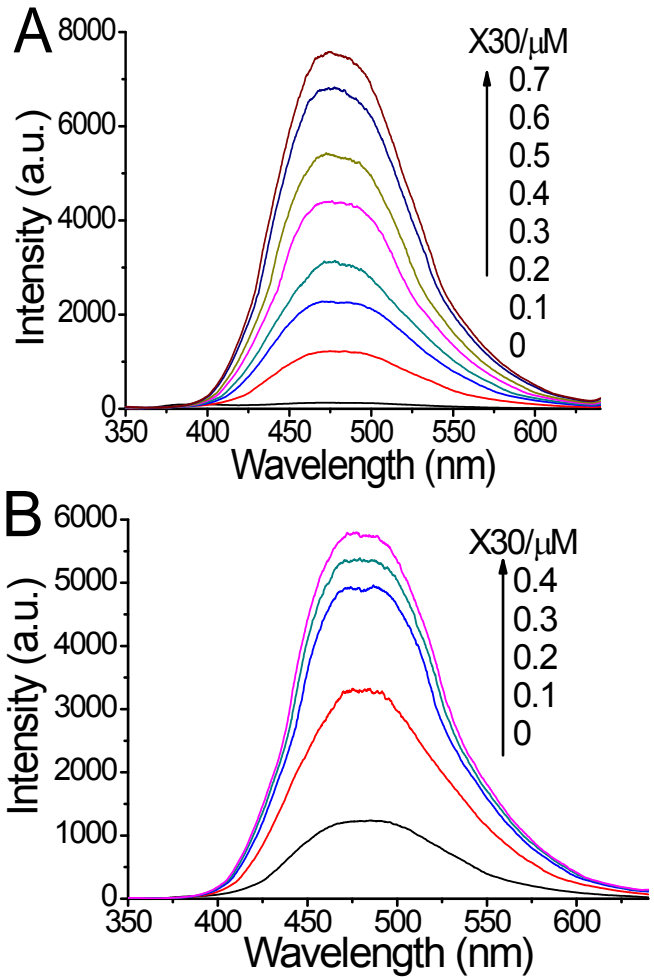


Fig. S3 (A) Fluorescence titration of ctDNA to 10 μM *E*-N2TPEin deionized water. (B) Plot of I/I_0 - 1 at 480 nm versus ctDNA concentration. I_0 = emission intensity in the absence of oligonucleotide. ctDNA is Calf Thymus DNA, a natural dsDNA. [*E*-N2TPE] = 10 μM; λ_{ex} = 330 nm, λ_{em} = 480 nm, Error bars are±SD.


A
Intensity (a.u.)
8000
6000
4000
2000
0
350
400
450
500
550
600
Wavelength (nm)
X30/μM
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
B
Intensity (a.u.)
6000
5000
4000
3000
2000
1000
0
350
400
450
500
550
600
Wavelength (nm)
X30/μM
0.4
0.3
0.2
0.1
0

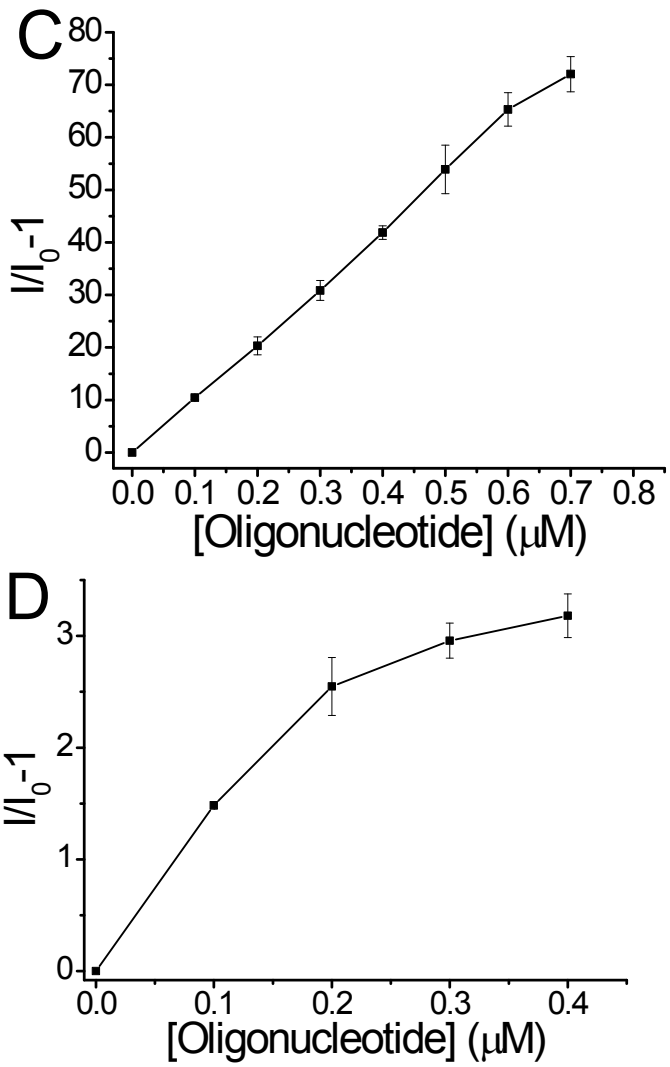
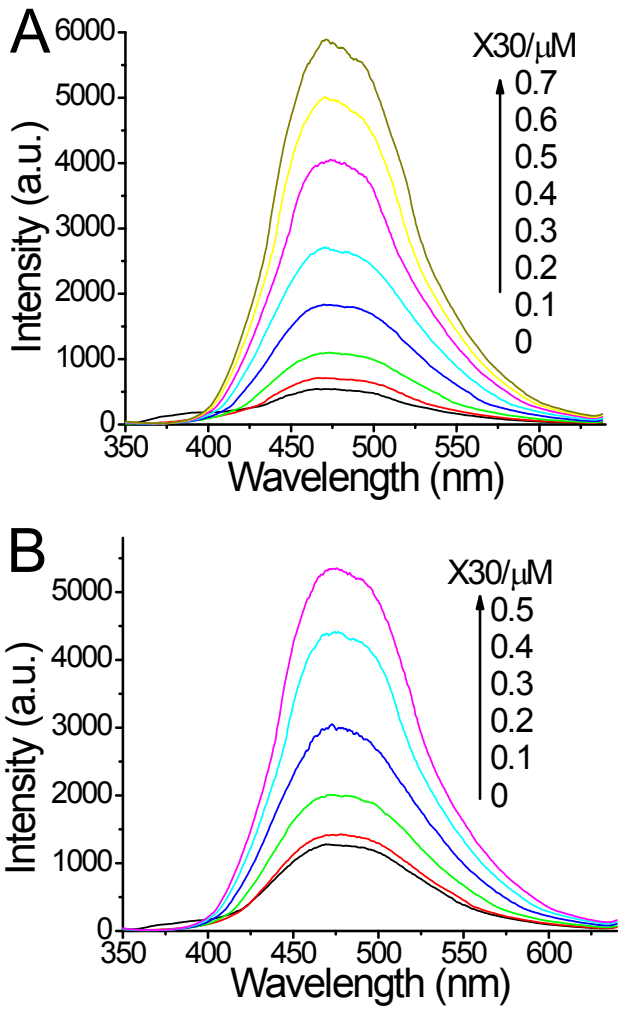


Fig. S4 Fluorescence titration of X30 to *Z*-N2TPE in 10 mM HEPES pH = 5.0 (A) and pH = 7.0 (B). Plot of I/I_0 - 1 at 480 nm versus X30 concentration in 10 mM HEPES pH = 5.0 (C) and pH = 7.0 (D). I_0 = emission intensity in the absence ofoligonucleotide.X30 is a syntheticoligonucleotide with 30nt. [*Z*-N2TPE] = 10 μM; λ_{ex} = 330 nm, λ_{em} = 480 nm, Error bars are±SD.


A
Intensity (a.u.)
Wavelength (nm)
X30/μM
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
B
Intensity (a.u.)
Wavelength (nm)
X30/μM
0.5
0.4
0.3
0.2
0.1
0

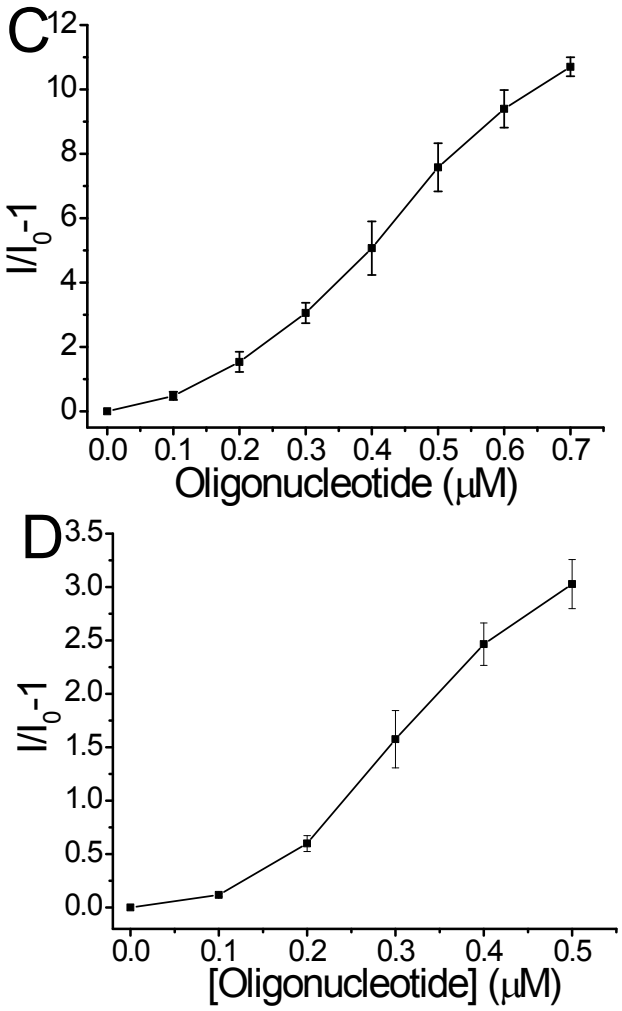


Fig. S5 Fluorescence titration of X30 to *E*-N2TPE in 10 mM HEPES pH = 5.0 (A) and pH = 7.0 (B). Plot of I/I_0 - 1 at 480 nm versus X30 concentration in 10 mM HEPES pH = 5.0 (C) and pH = 7.0 (D). I_0 = emission intensity in the absence ofoligonucleotide.X30 is a syntheticoligonucleotide with 30nt. [*E*-N2TPE] = 10μM; λ_{ex} = 330 nm, λ_{em} = 480 nm, Error bars are±SD.