Electronic Supplementary Material (ESI) for RSC Advances

Supplementary Information

Specific Nucleic Acid Detection Based on Fluorescent Light-up Probe from Fluorogens with Aggregation-Induced Emission Characteristics

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Materials and Experimental Methods

The chemicals were purchased from Sigma Aldrich, and used directly without further purification. The oligonucleotides were ordered from Biosearch Technologies, Inc. The ^{1}H and ^{13}C NMR spectra were measured on a Bruker ARX 400 NMR spectrometer using chloroform-d as solvent and tetramethylsilane (TMS) as internal reference. UV-vis spectra were recorded on a Shimadzu UV-1700 spectrometer. Photoluminescence (PL) spectra were measured on a Perkin Elmer LS-55 equipped with a xenon lamp excitation source and a Hamamatsu (Japan) 928 PMT, using 90 degree angle detection for solution samples. Concentrations of oligonucleotides were determined using NanodropTM spectrophotometer at absorbance of 260 nm. All UV-vis and PL spectra were collected at 24 ± 1 ^{o}C. The average particle size and size distribution of TPE-N_3 was determined by laser light scattering (LLS) with particle size analyzer (90 Plus, Brookhaven Instruments Co. USA) at a fixed angle of 90^{o} at room temperature. HPLC purification was conducted using reverse-phase high-pressure liquid chromatography (HPLC, Shimadzu) on a 250×1 mm Kromasil C-18 analytical column connected to a variable wavelength monitor. The following gradient system was used at two detection wavelengths of 260 nm and 350 nm at a flow rate of 0.8 mL per minute: phase A is ammonium acetate (50 mmol/L) buffer; phase B is acetonitrile. Flow rate was set at 0.8 mL/min; 0-25 minutes 0-40% phase B in A, 25-30 minutes 40% B in A, 30-35 minutes 40%-0% B in A. MALDI-TOF was conducted using Bruker Autoflux using 3-hydroxypicolinic acid as the matrix.

Synthesis of 1-((4-azidomethyl) phenyl)-1,2,2-triphenylethene, TPE-N_3. Into a 250 mL two necked round bottom flask, 1-((4-bromomethyl)phenyl)-1,2,2-triphenylethene (1.70 g, 4 mmol) and sodium azide (0.39 g, 6 mmol) were dissolved in dimethylsulfoxide under nitrogen. The mixture was stirred at room temperature overnight. A large amount (100 mL) of water was then

added and the solution was extracted three times with diethyl ether. The organic layers were combined, dried over magnesium sulfate and concentrated. The crude product was purified by silica-gel chromatography using hexane and chloroform mixture (3:1, v/v) as eluent to give TPE-N_3 as a white solid (1.5 g, 97% yield). ^{1}H NMR ($CDCl_3$, 400 MHz), δ (TMS, ppm): 7.13-7.06 (m, 9H), 7.06-6.98 (m, 10H), 4.24(s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ (TMS, ppm): 53.91, 125.90, 126.02, 126.99, 127.04, 127.09, 130.67, 131.11, 131.22, 132.61, 139.62, 140.82, 142.83, 142.90, and 143.27. HR-MS (MALDI-TOF): m/z 387.1342 [$(M)^+$, calculated 387.1735].

Synthesis of TPE-DNA_p conjugates via click reaction. Click conjugation of custom oligonucleotides with TPE-N_3 was carried out by preparing an initial aqueous solution via mixing of alkyne-labeled custom oligonucleotide sequence (5'-alkyne-AGC ACC CAC ATA GTC AAG AT -3', thereafter named DNA_p sequence, 100 nmol)) and TPE-N_3 (150 nmol) in a mixture (1 mL) of deionized water and dimethylsulfoxide (1:1, v/v). Freshly prepared aqueous solution of sodium ascorbate (200 nmol, 15 μL in deionized water) was added to the mixture above, followed by copper (II) sulphate (50 nmol, 30 μL in deionized water). The mixture was stirred overnight at room temperature before reverse HPLC purification. Quantitative analysis reveals a coupling yield of 80%. The peak with a retention time of 5 minutes was collected and the solution was lyophilized to yield colorless solids, which were kept at –20 ^{o}C. Deionized water was added to dissolve the TPE-DNA_p conjugates to yield a stock solution with known concentrations.

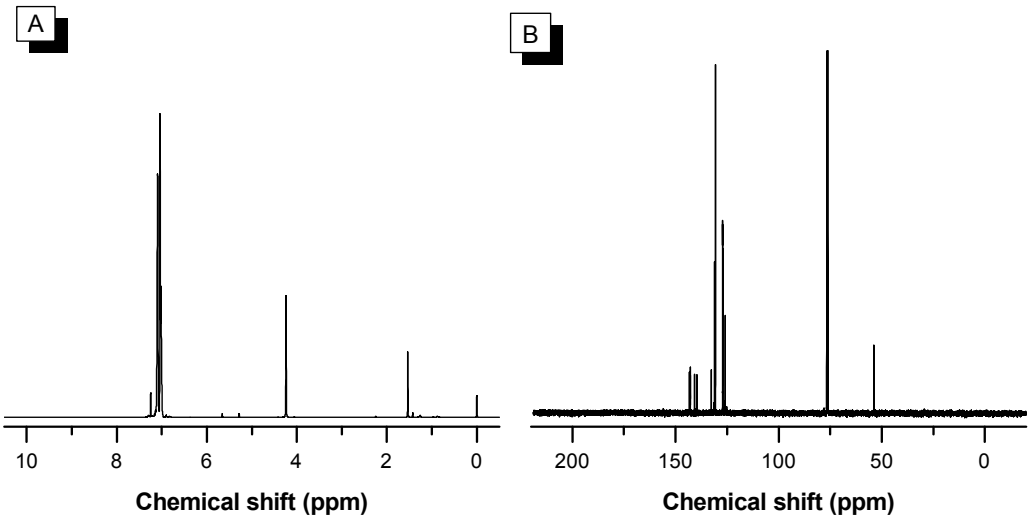

Figure S1 (A) ^{1}H and (B) ^{13}C NMR spectra of compound TPE-N_3 in $CDCl_3$.

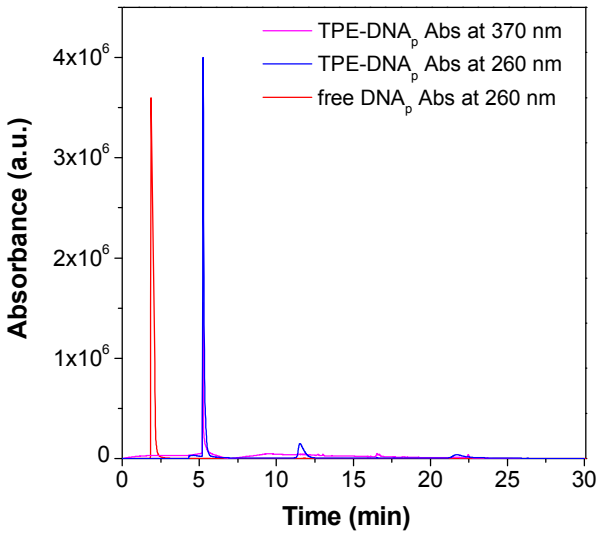

Figure S2 Reverse phase-HPLC spectra of free DNA_p oligonucleotides (first peak in red) and TPE-DNA_p conjugates (second peak in both blue and pink).

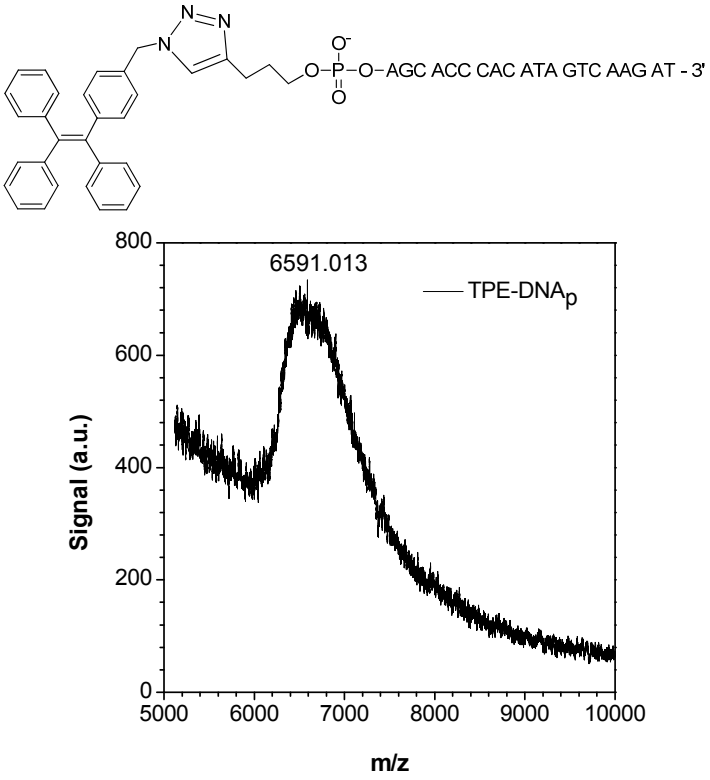

Figure S3 Chemical structure and MALDI-TOF spectrum of TPE-DNAp, showing a molecular weight at 6591.013 .

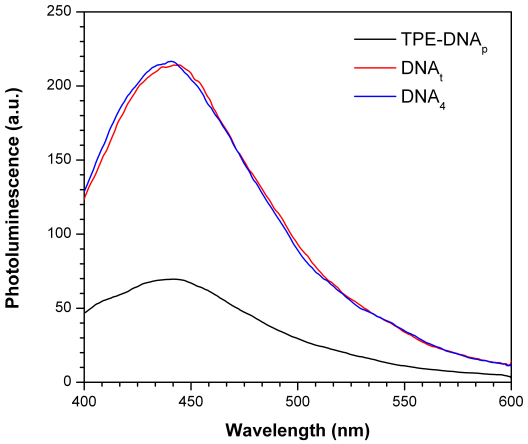

Figure S4 Fluorescence spectra of TPE-DNAp (1 μM) upon hybridization with its complementary strands (1 μM each). Sequence of DNA_4 is 5'-ATC TTG ACT ATG TGG GTG CTA GTG TAC CAG-3' (30 bp), with the first 20 bp being the same as DNA_t (20 bp).