Supporting Information

Nucleic Acid-Induced Aggregation and Pyrene Excimer Formation

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1. General experimental details

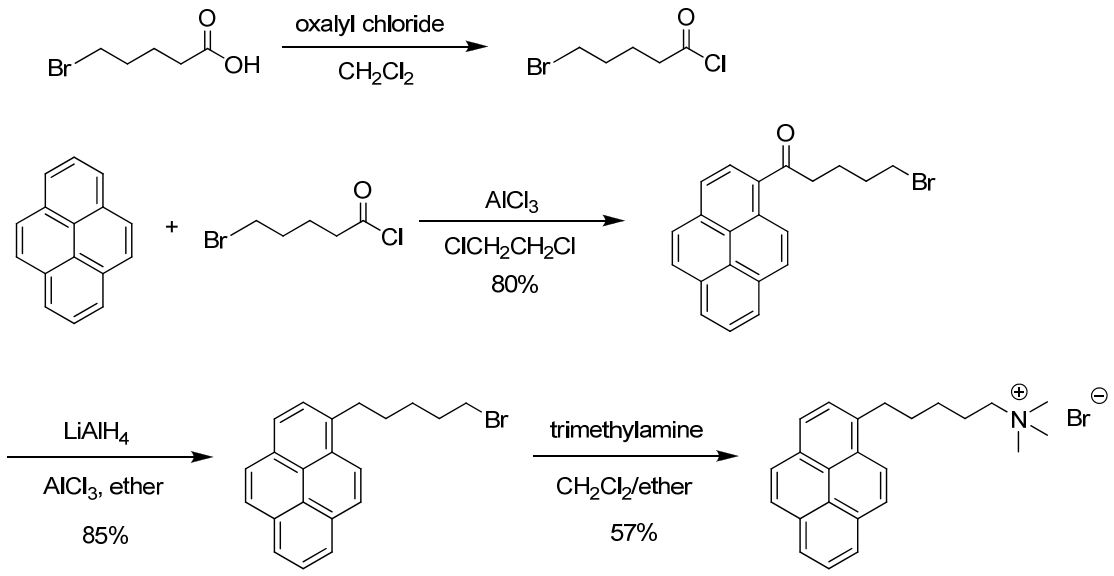
Apparatus. ^{1}H NMR spectra were obtained with a Bruker DPX-300 (300 MHz) or Bruker AVANCE 400 (400 MHz) Fourier transform NMR spectrometer at ambient temperature with chemical shifts reported in parts per million (ppm) relative to tetramethylsilane. Splitting patterns are reported as s (singlet), d (doublet), t (triplet), and multiplet (m). Coupling constants (J) are given in Hz. Mass spectra were obtained using a Finnigan MATLCQ electrospray ionization-ion trap mass spectrometer. UV-Vis absorption spectra were obtained using a Cary 500 spectrophotometer (Varian) equipped with a xenon flash lamp. Emission spectra were obtained using an LS-55 (Perkin Elmer) spectrofluorometer with excitation and emission bandwidths of 5 nm. The emission spectra were collected at an excitation wavelength of 352 nm. The I_E/I_M value was defined as the intensity ratio of pyrene probe excimer emission (at 485 nm) to monomer emission (at 377 nm). The spectra shown in the figures were all normalized at 377 nm. Microquartz cuvettes with a 10-mm path length and 2-mm window width were used for UV-vis and emission measurements. All spectra were obtained at an ambient temperature of about 22 ºC.

Materials. Chemicals were purchased from Aldrich, Alfa Aesar, or Sigma and used as received. Organic solvents were dried and distilled before use. Water was doubly distilled and passed through a Millipore deionization system. PAGE purified nucleic acid and nuclease S1 were purchased from Shanghai Sangon Biological Engineering Technology & Service Co. Ltd. Poly(dA)$_{25}$, poly(dT)$_{25}$, poly(dC)$_{25}$, and poly(dG)$_{25}$ were 25-base-long

oligonucleotides of simple repeats of dA, dT, dC, and dG, respectively. Oligo$(dN)_{25}$ has the following sequence: 5'-AGC TCG TTT AGT GAA CCG TCA GAT C-3', and oligo$(dM)_{25}$ has the following sequence: 5'-GAT CTG ACG GTT CAC TAA ACG AGC T-3'. These two sequences are complementary to each other. Duplex DNA was obtained by a simple annealing procedure using equimolar amounts of the complementary single stranded nucleic acid strands [oligo$(dN)_{25}$ + oligo$(dM)_{25}$]. The formation of duplex DNA was confirmed by nuclease digestion studies. Single-stranded nucleic acid could be degraded by nuclease S1, whereas duplex DNA could not. Unless otherwise specified, a buffer solution containing 1 mM MOPS and 1 mM NaCl at pH 7.5 was used throughout the current investigation.

2. Synthesis of the positively charged pyrene probe (compound 1)

The synthetic route of compound **1** is shown in scheme 1.[s1, s2] Its successful synthesis was confirmed by ^{1}H NMR, ^{13}C NMR, and mass spectrometry. ^{1}H NMR (DMSO-d_6 – D_2O, 400 MHz): δ (ppm) 1.41-1.51 (m, 2H), 1.74-1.93 (m, 4H), 3.04 (s, 9H), 3.23-3.29 (m, 2H), 3.40 (t, 2H, J = 7.6 Hz), 8.01 (d, 1H, J = 7.9 Hz), 8.08-8.20 (m, 3H), 8.24-8.33 (m, 4 H), 8.40 (d, 1H, J = 9.3 Hz). ^{13}C NMR (DMSO-d_6 – D_2O, 100 MHz): δ (ppm) 22.9, 26.4, 31.7, 33.2, 53.3, 66.6, 124.4, 125.0, 125.1, 125.7, 125.9, 127.2, 127.4, 128.2, 128.4, 128.5, 128.9, 130.1, 131.3, 131.7, 137.7. ESI-MS: *m/z* calculated for $C_{24}H_{28}N$: 330.2, found: *m/z* 330.2.



Scheme 1

3. Determination of the binding constant via UV-vis titration

5 μL of compound **1** (400 μM) and various amounts of DNA were mixed in a MOPS buffer solution, and pure water was added to make a final total volume of 200 μL. The corresponding UV-vis spectra were recorded. The binding constant was calculated according to the following equation: $[DNA]/(\varepsilon_A - \varepsilon_F) = [DNA]/(\varepsilon_B - \varepsilon_F) + 1/K_b(\varepsilon_B - \varepsilon_F)$, where ε_A, ε_B and ε_F were $A_{obs}/[Py]$, the extinction coefficient for compound **1** in the fully bound form, and the extinction coefficient for free compound **1**, respectively.[s3] A plot was made of $[DNA]/(\varepsilon_A - \varepsilon_F)$ versus [DNA]. The binding constant (K_b) could then be obtained by the ratio of the slope to the intercept.

4. Determination of nucleic acid and compound 1 binding ratio (Job's plot)

Thirteen sample solutions containing various amounts of DNA and compound **1** in MOPS buffer solution were prepared. The total concentration of DNA base +

compound **1** was kept constant at 180 μM, and the total sample volume was 0.2 mL. The concentration of compound **1** varied from 180 to 0 μM, and the concentration of the DNA base was from 0 to 180 μM. UV-vis spectra of the sample mixtures were recorded. A plot of ΔA against X was made, where ΔA equaled the absolute value of $A_{obs} - \varepsilon_{base}[DNA] - \varepsilon_{py}[py]$. A_{obs} was the absorbance of the corresponding sample solution, ε_{base} and ε_{py} were the extinction coefficients for free DNA and compound **1** at 342 nm, and X was the molar fraction of DNA base.

5. DNA cleavage with nuclease S1

Nuclease plays important roles in many biological processes, such as DNA replication, transcription, and translation. Therefore, the detection of nuclease activity has many potential applications. At present, several traditional methods, such as electrophoresis, high performance liquid chromatography (HPLC), and the enzyme-linked immunosorbent assay (ELISA) are used for the detection of nuclease activity, but in general they are not very satisfactory because they are time-consuming, laborious, and expensive.[s4-s8]

Nuclease S1 was subsequently selected in our study. It is widely used in molecular biology research because of its single-stranded nucleic acid specificity. It has a molecular weight of about 34 kDa and exists as a monomer. Its optimal working pH range is 4.0 – 4.6 and requires the presence of Zn^{2+} for activation. It is a single-strand-specific nuclease that exhibits both endo- and exolytic hydrolytic activity for the phosphodiester bonds of

DNA and RNA, yielding > 90% 5'-phosphomononucleotide end products. It has been used for the digestion of hairpin loops in DNA or RNA duplexes, the removal of non-annealed polynucleotide tails, and the conversion of superhelical DNA into the linear form.

Assay procedure: a total volume of 25 μL of the reaction mixture including single-stranded nucleic acid, nuclease S1 (1.13 U), and buffer was mixed and incubated at ambient temperature (22 ºC) for 5, 10, 15, 20, 25, and 30 min (final buffer concentration: 2 mM CH_3COONa, 5 mM NaCl, 0.1 mM $ZnCl_2$, pH 4.5). Then 162 μL of compound **1** (400 μM), 36 μL of buffer (10 mM MOPS, 10 mM NaCl, pH 7.5), and 137 μL of water were added, and the emission spectra were immediately measured. The percentage decrease of the excimer emission indicated the extent of nucleic acid cleavage by nuclease S1.

6. Detection of DNA single point mutation

Mixtures of equal amounts of single-stranded oligonucleotides (ssDNA-0 + ssDNA-1, and ssDNA-0 + ssDNA-2) were diluted with buffer (MOPS-NaCl, pH 7.5) to the desired concentration (total sample volume: 60 μL), incubated at 95 ºC in a hot water bath for 5 min, and annealed at 37.5 ºC for 15 min. The samples were then moved quickly into a refrigerator (-20 ºC) and stored for 5 min before the digestion experiments. This rapid cooling process ensured that the mismatched ssDNA had no chance to form duplex structures and had no influence on normal duplex DNA. The samples were then digested

by S1 nuclease (1.13 U) at 20 ºC for 20 minutes. After S1 nuclease digestion, compound **1** (180 μM) was added, and the emission spectra were measured.

Sequences of ssDNA:

ssDNA-0 5'-CGGTTCGCTA -3' (10-mer)

ssDNA-1 5'-TAGCGAACCG-3' (normal)

ssDNA-2 5'-TAGCTAACCG-3' (mutant)

References:

(s1) Atik, S. S.; Kwan, C. L.; Singer, L. A. *J. Am. Chem. Soc.* **1979**, *101*, 5696-5702.

(s2) Boal, A. K.; Rotello, V. M. *J. Am. Chem. Soc.* **2002**, *124*, 5019-5024.

(s3) Rescifina, A.; Chiacchio, U.; Piperno, A.; Sortino, S. *New J. Chem.* **2006**, *30*, 554-561.

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(s5) Halford, S. E.; Goodall, A. J. *Biochemistry* **1988**, *27*, 1771-1777.

(s6) McLaughlin, L. W.; Benseler, F.; Graeser, E.; Piel, N.; Scholtissek, S. *Biochemistry* **1987**, *26*, 7238-7245.

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(s8) Jeltsch, A.; Fritz, A.; Alves, J.; Wolfes, H.; Pingoud, A. *Anal. Biochem.* **1993**, *213*, 234-240.

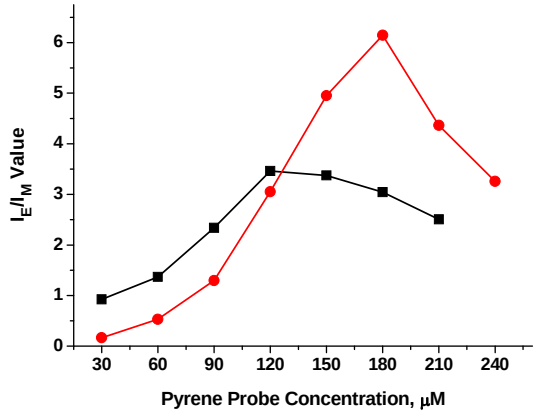


Figure S1. Change in the I_E/I_M value when 7.2 μM of poly(dT)$_{25}$ (■) and poly(dA)$_{25}$ (●) were mixed with different concentrations of compound **1**.

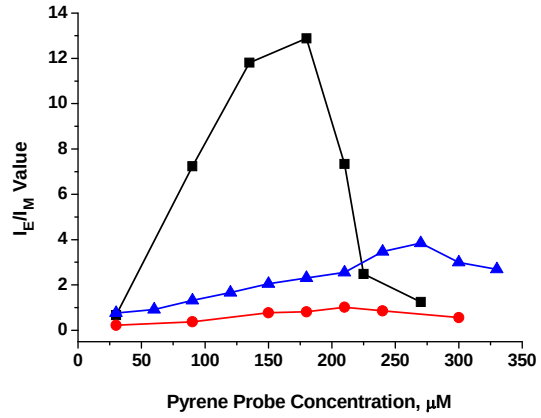


Figure S2. Change in the I_E/I_M value when 7.2 μM of poly(dG)$_{25}$ (■) and poly(dC)$_{25}$ [at pH 8.5 (▲) or pH 5.0 (●)] were mixed with different concentrations of compound **1**. The medium used for poly(dG)$_{25}$ was 1 mM MOPS and 1 mM NaCl, pH 7.5. Two buffers were used for poly(dC)$_{25}$. They were 1 mM HOAc-NaOAc and 1 mM NaCl, pH 5.0, or 1 mM TAPS and 1 mM NaCl, pH 8.5.

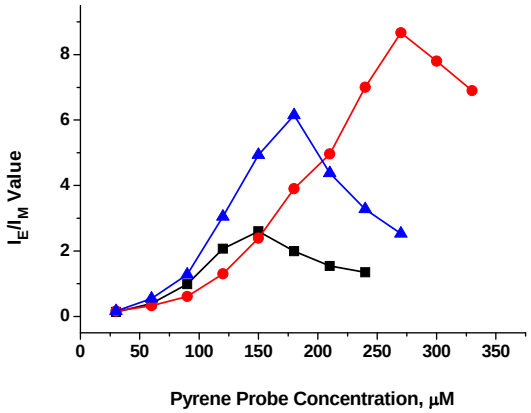


Figure S3. Change in the I_E/I_M value when 3.6 (■), 7.2(▲), or 10.8 (●) μM of poly(dA)$_{25}$ were mixed with different concentrations of compound **1**.

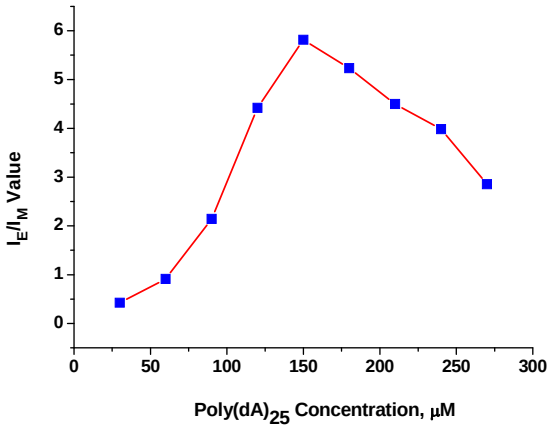


Figure S4. Change in the I_E/I_M value when 150 μM of compound **1** was mixed with different concentrations of poly(dA)$_{25}$.

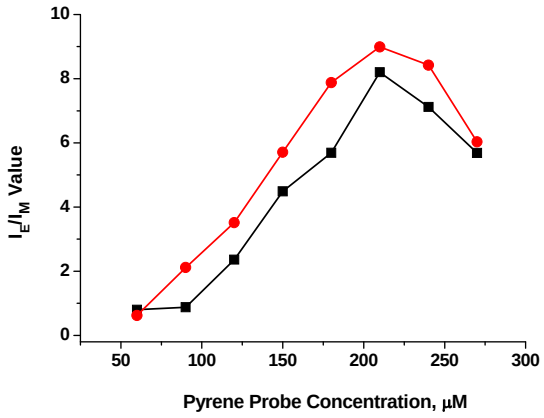


Figure S5. Change in the I_E/I_M value when 180 μM of compound **1** was mixed with 7.2 μM of poly(dM)$_{25}$ (■), or 3.6 μM of duplex DNA [poly(dN)$_{25}$ + poly(dM)$_{25}$] (●).

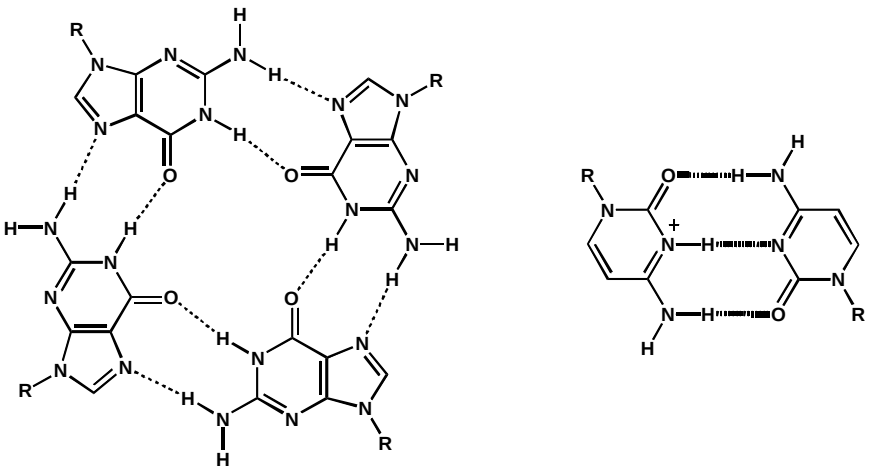


Figure S6. Left: Hydrogen bonding among four guanine bases in a G-quadruplex structure. Right: Hydrogen bonding in the cytosine-protonated cytosine (C^+ – C) base pair.

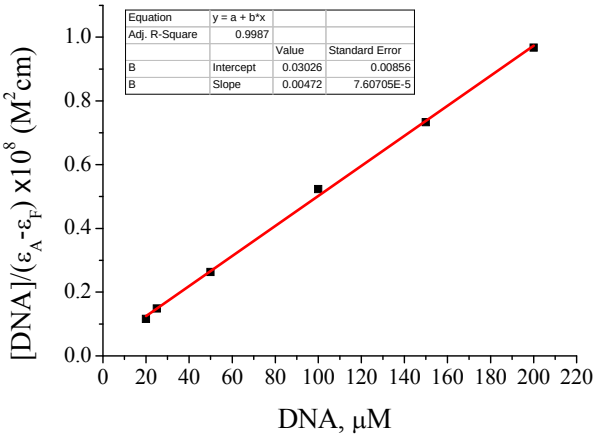


Figure S7. Binding constant curve fit. Compound **1** binding to poly(dN)$_{25}$.

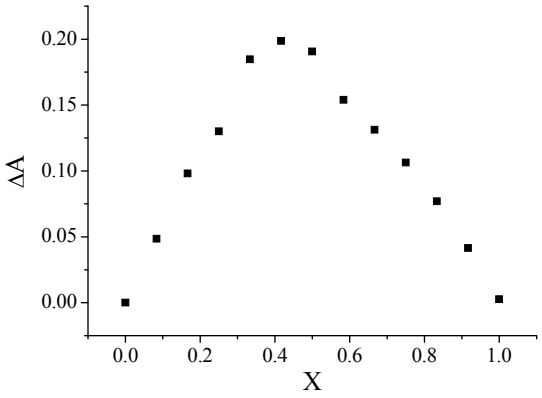


Figure S8. Job's plot, compound **1** binding to poly(dN)$_{25}$.

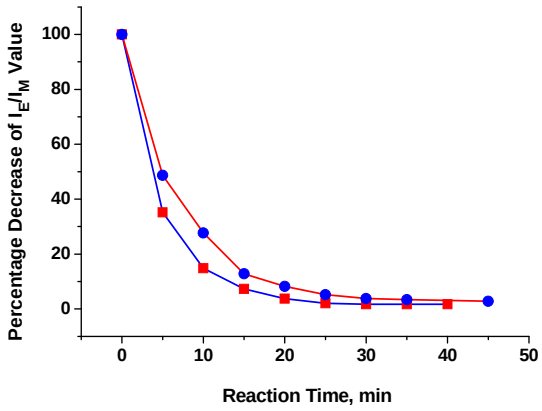


Figure S9. Percentage decrease of the I_E/I_M value when nuclease S1 (1.13 U) was incubated with a mixture of compound **1** and poly(dA)25 (■) [or poly(dN)25 (●)] for different periods of time.

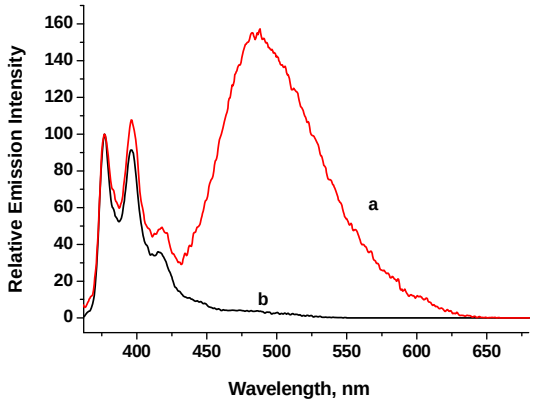
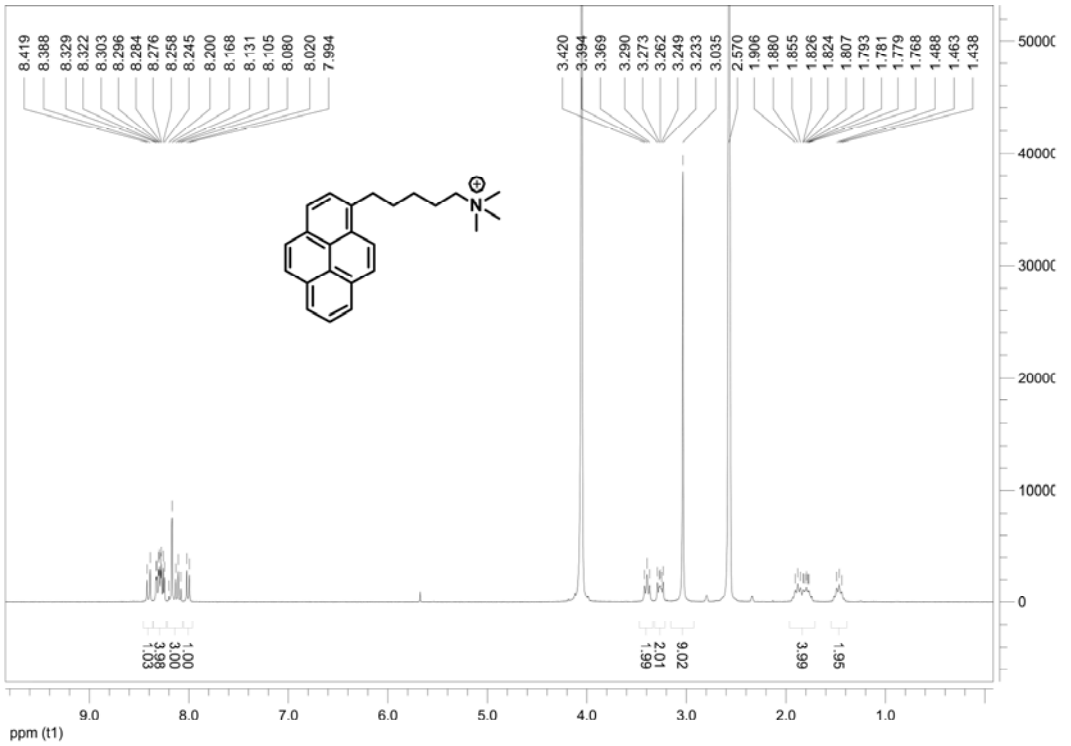
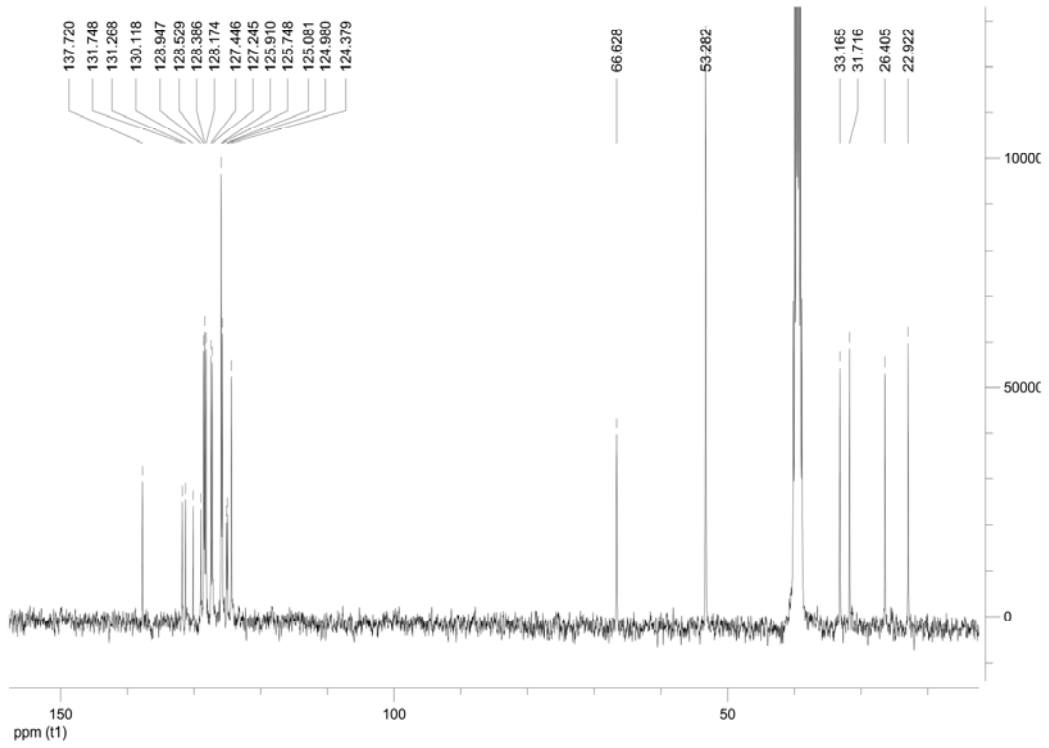


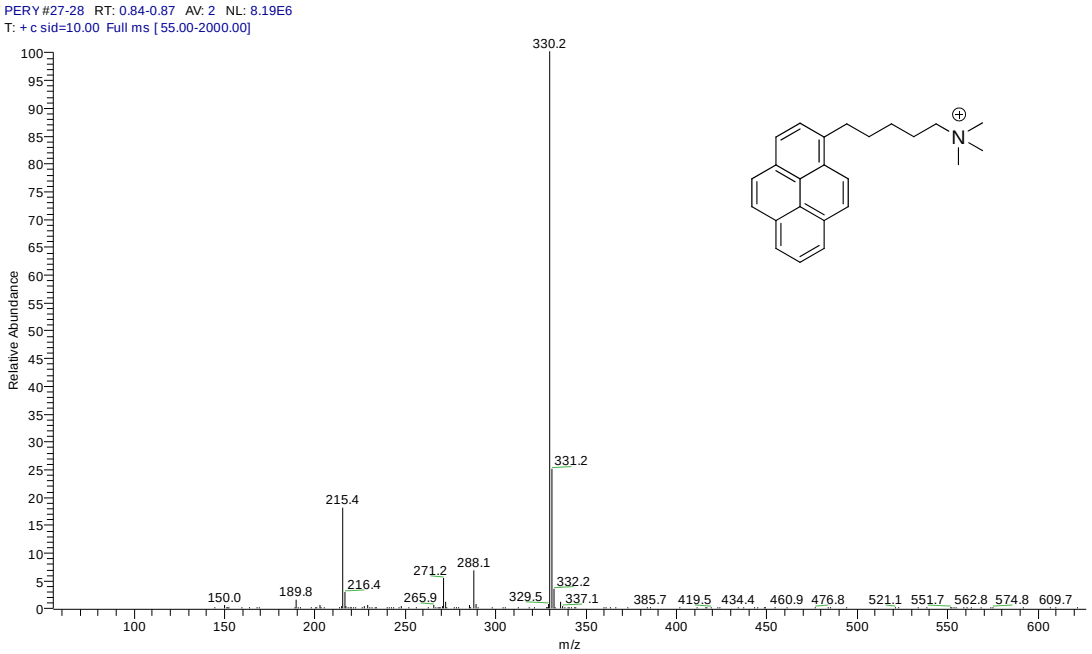
Figure S10. Detection of an SNP in 10-mer duplex DNA. Emission spectra of fully matched duplex DNA (line a) and duplex DNA with a single base mismatch (G to T) (line b) after nuclease S1 digestion. Conditions: 180 μM of compound **1**; 9 μM of duplex DNA; 1.13 U nuclease S1.



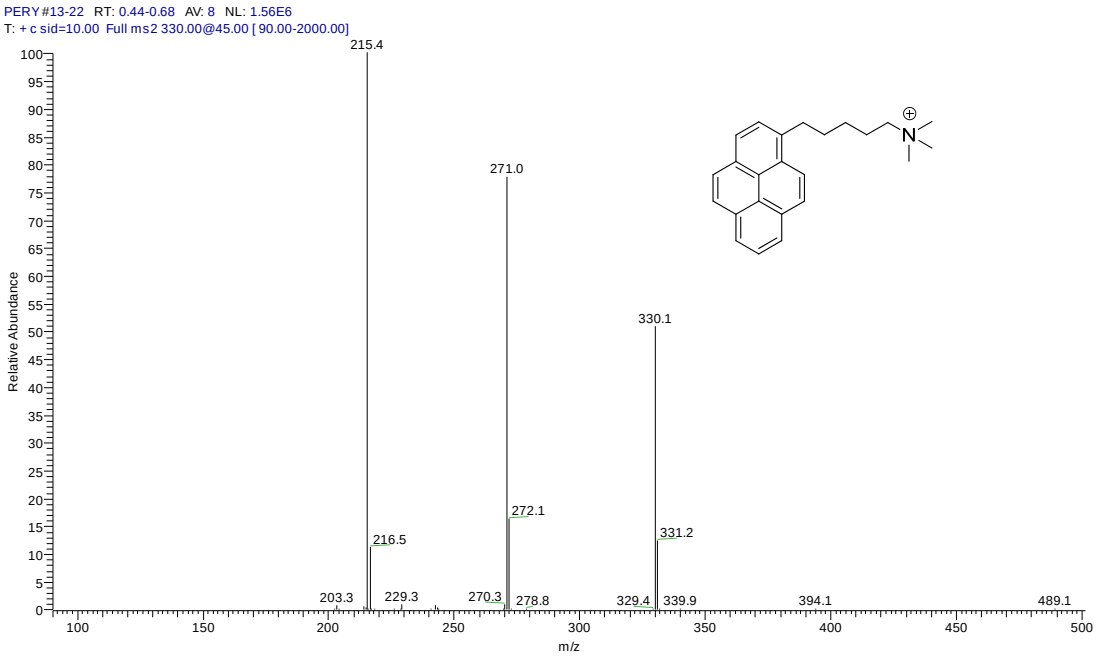
^{1}H NMR spectrum of compound **1**



^{13}C NMR spectrum of compound **1**



ESI-MS spectrum of compound **1**



ESI-MS spectrum of compound **1**