Supporting Information

Thrombin binding aptamer G-quadruplex stabilized by pyrene-modified nucleotides

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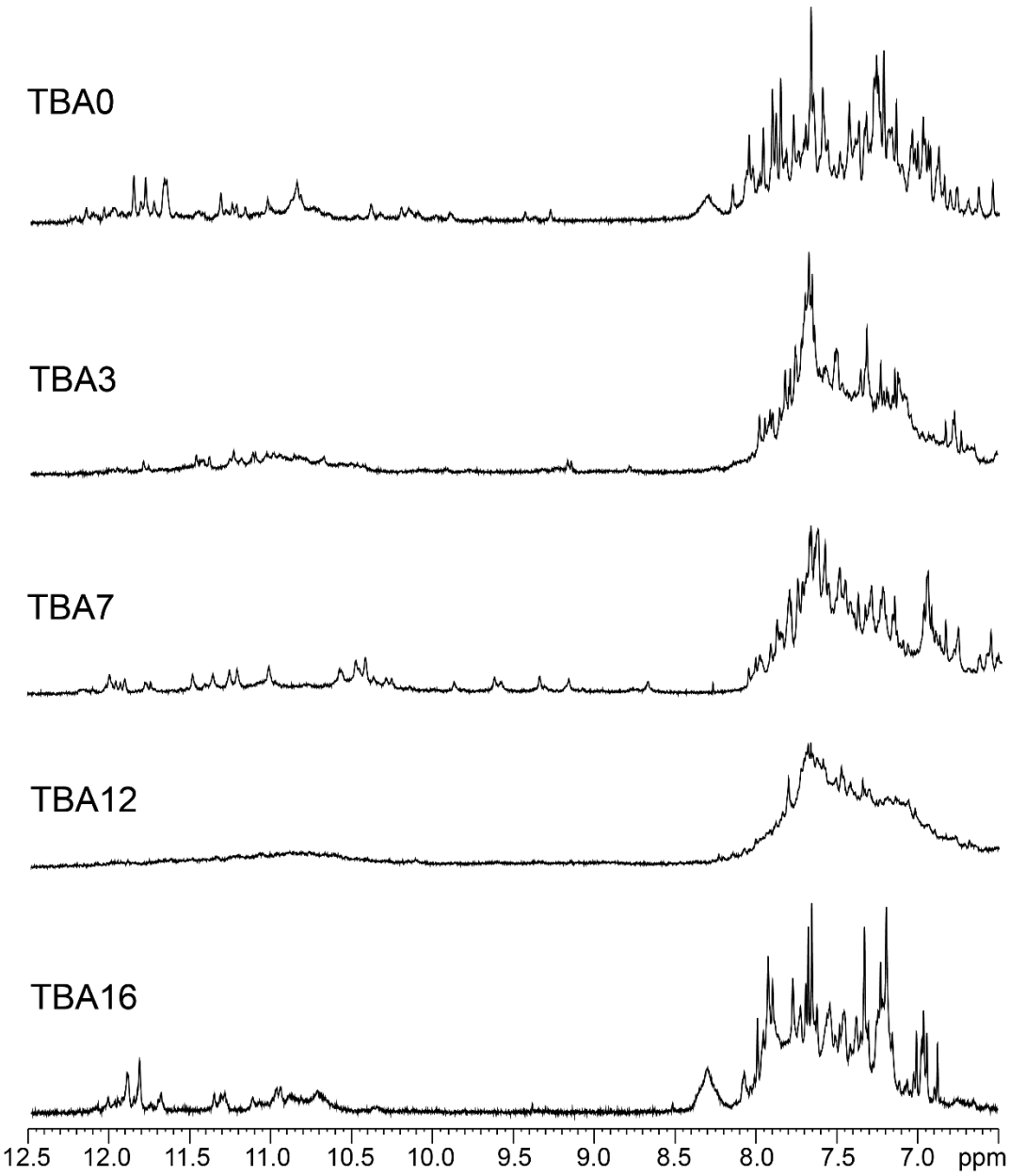

Figure S1. Imino and aromatic regions of ^{1}H NMR spectra of TBA0, TBA3, TBA7, TBA12 and TBA16 in a 50 mM KCl solution at 25 °C.

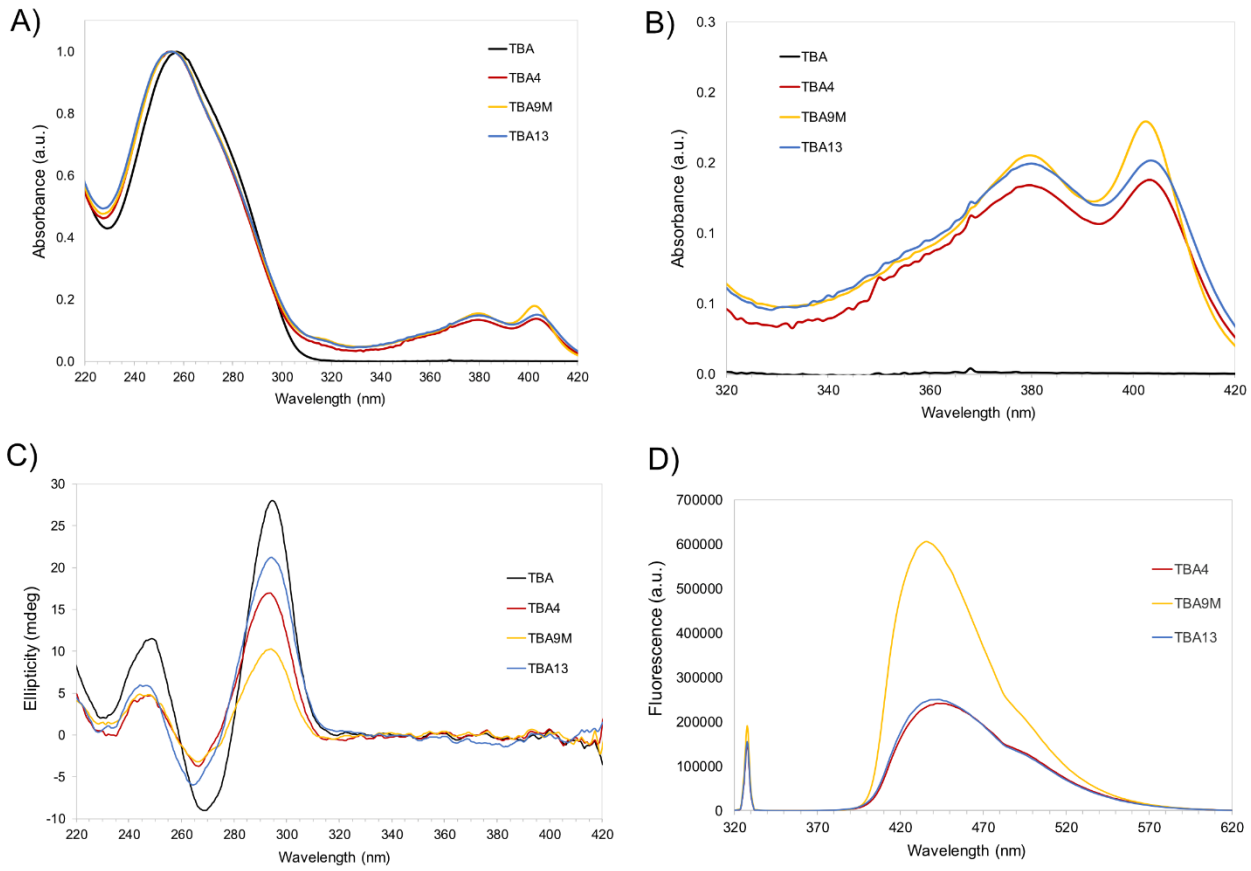

Figure S2. A,B) Normalized absorbance spectra of TBA, TBA4, TBA9^{M} and TBA13 samples. C) CD-spectra of 50 µM TBA, TBA9^{M} and TBA13. D) Fluorescence emission spectra of monomeric 150 µM TBA4, TBA9^{M} and TBA13 G-quadruplexes in 50 mM KCl solution at 25 °C. Excitation wavelenght of 330 nm was used.

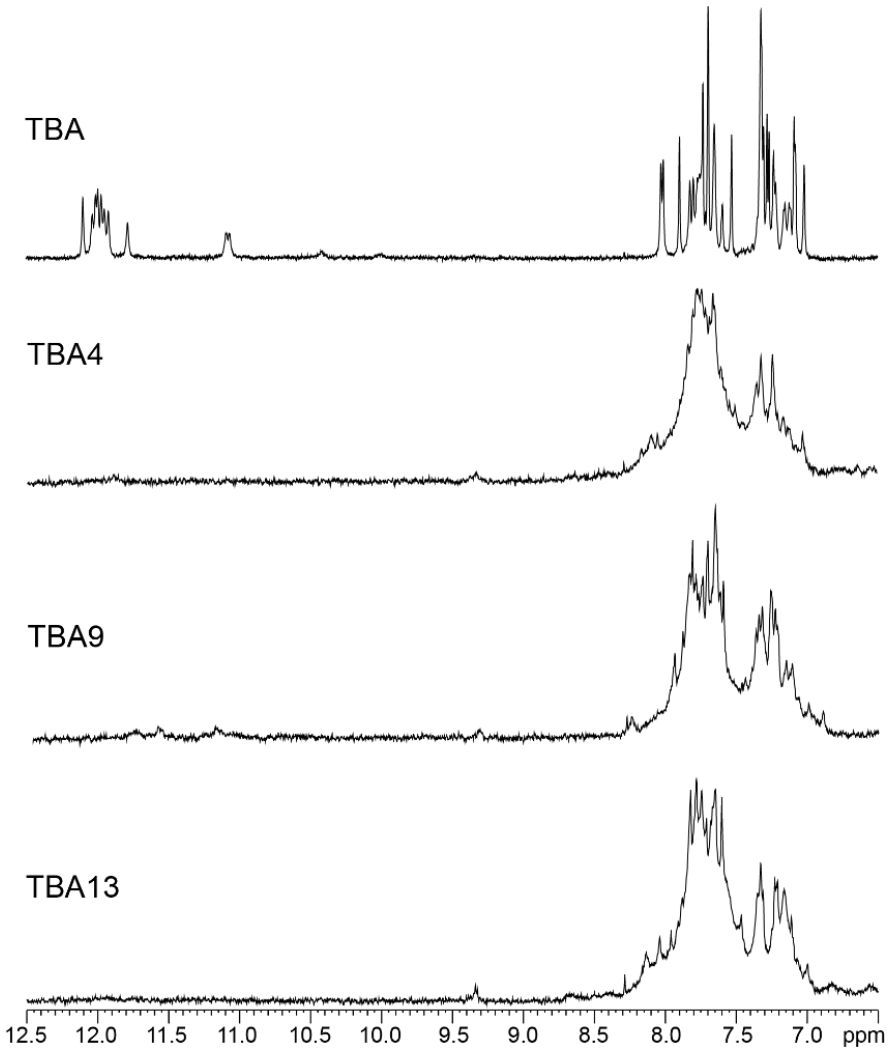

Figure S3. Imino and aromatic regions of ^{1}H NMR spectra of TBA, TBA4, TBA9 and TBA13 in a 50 mM NaCl solution at 25 °C.

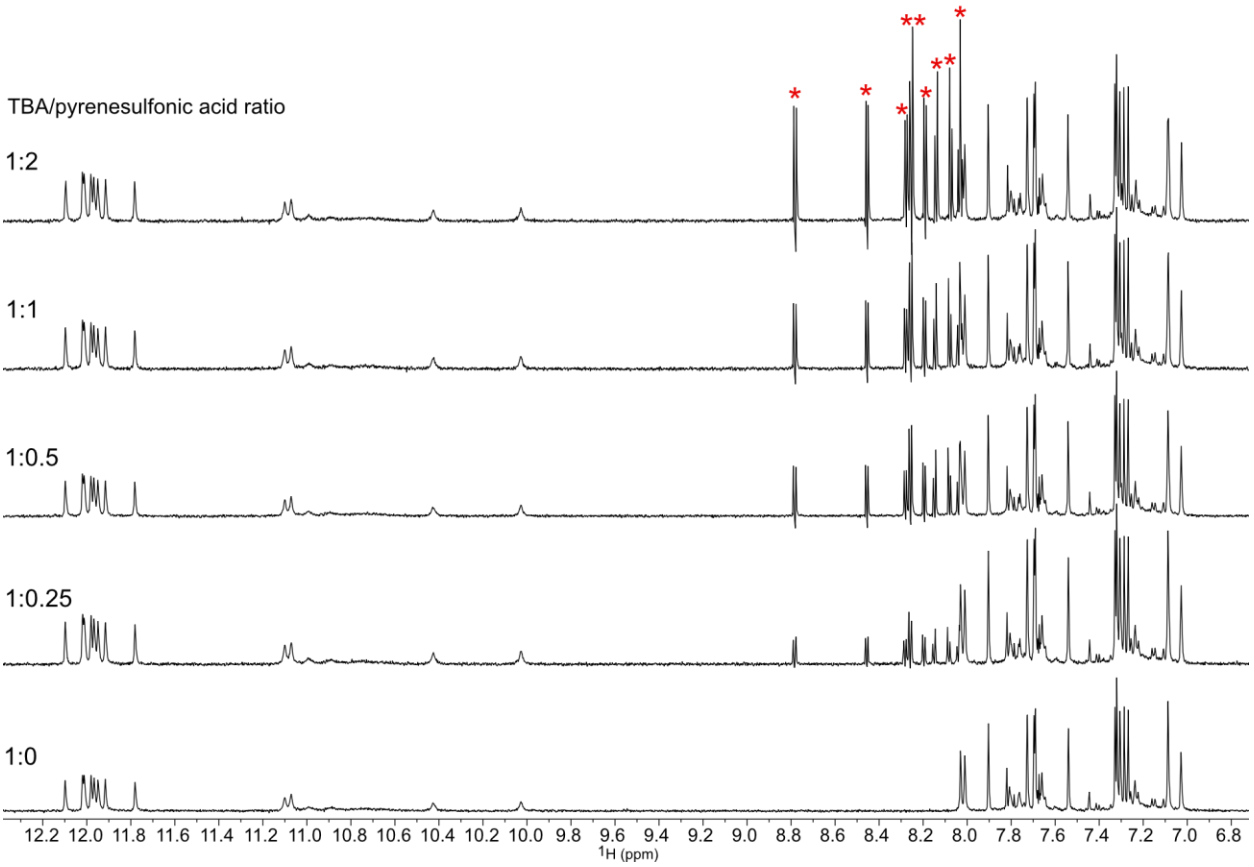

Figure S4. Imino and aromatic regions of ^{1}H NMR spectra of 100 µM TBA sample in a 50 mM KCl solution at 25 °C with increasing additions of 1-pyrenesulfonic acid sodium salt. Proton signals of pyrene group are marked with red asterisks.

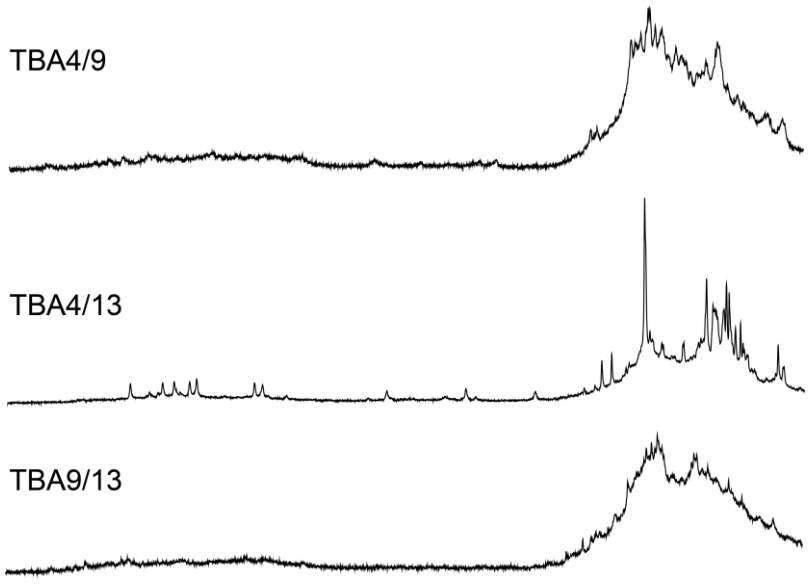

Figure S5. Imino and aromatic regions of ^{1}H NMR spectra of TBA4/9, TBA4/13 and TBA9/13 in a 50 mM KCl solution at 25 °C.

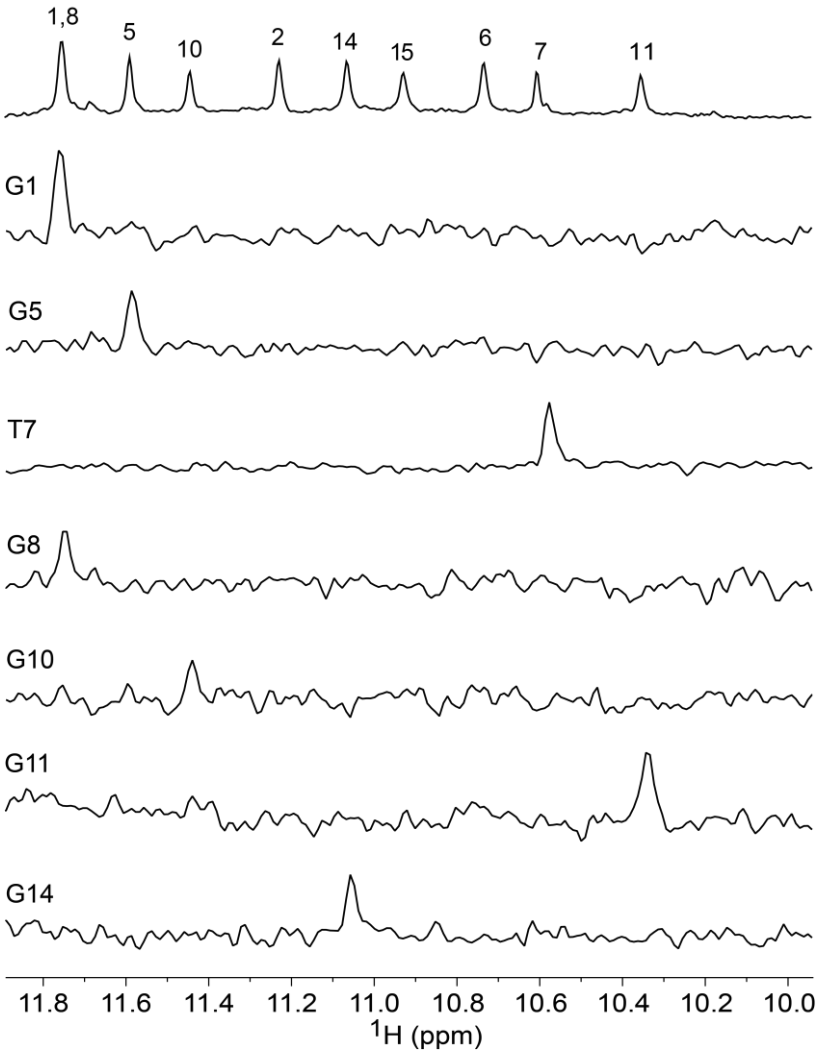

Figure S6. Imino regions of 1D ^{15}N-edited HSQC NMR spectra of site-specifically labeled TBA9^{D} in a 100 mM KCl solution at 25 °C.

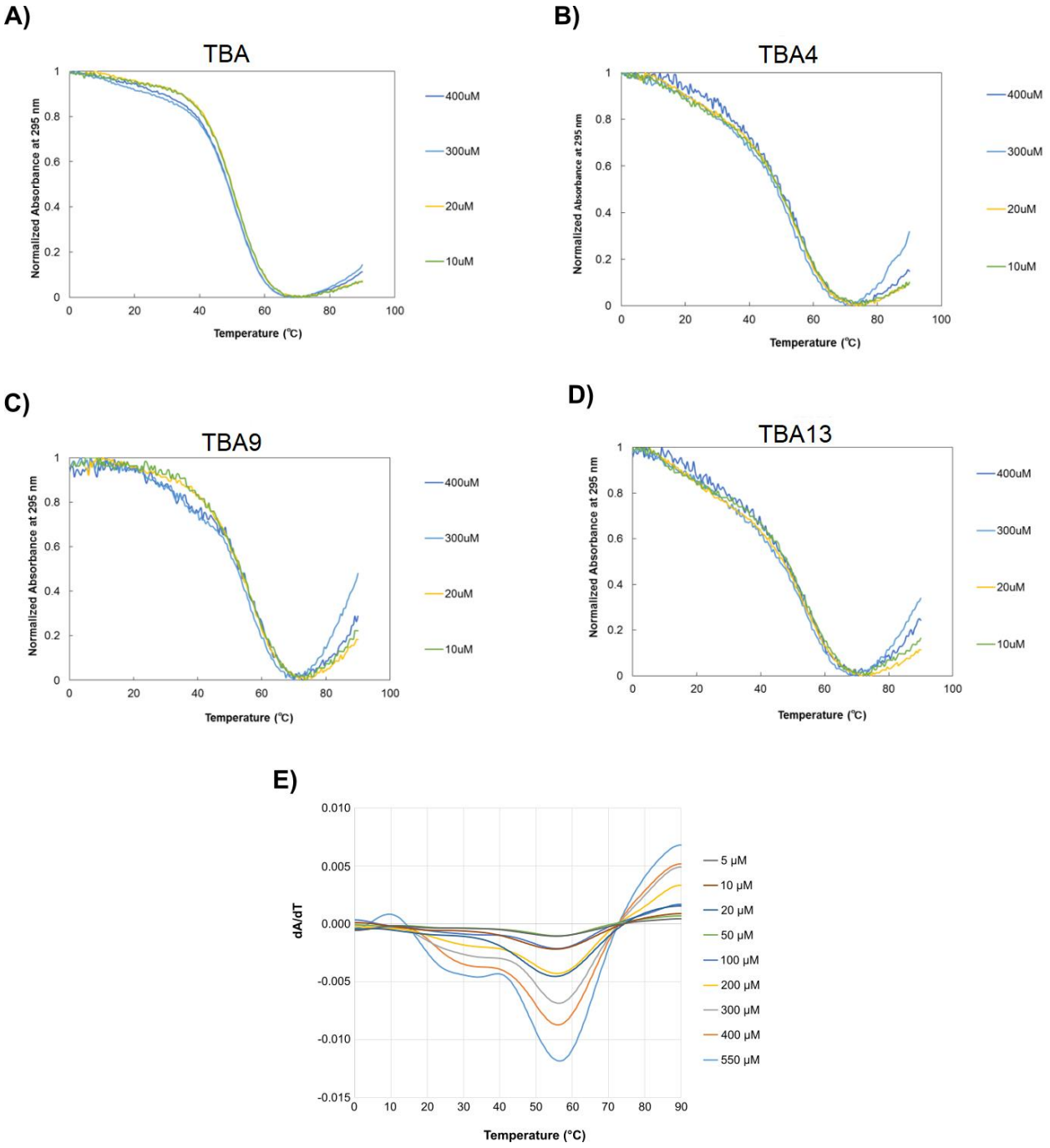

Figure S7. (A-D) Normalized UV melting curves for TBA, TBA4, TBA9 and TBA13 at 10, 20, 300 and 400 μM oligonucleotide concentrations in a buffer containing 50 mM KCl. (E) First derivative of absorbance at 295 nm vs temperature for TBA9 at different oligonucleotide concentrations.

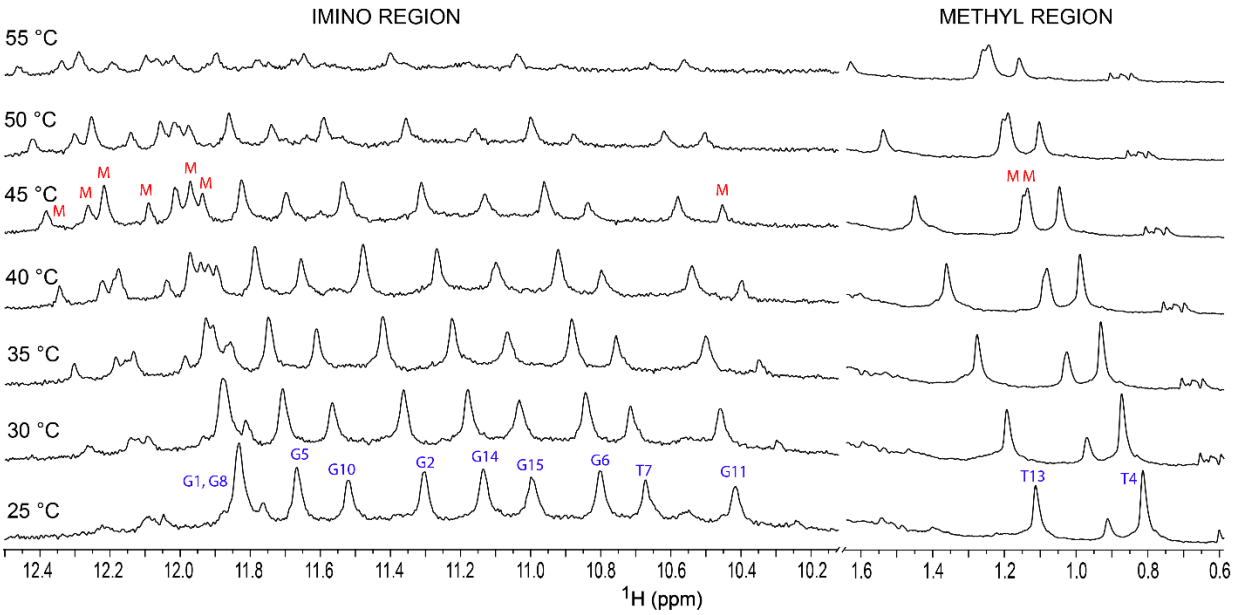

Figure S8. Imino and methyl regions of ^{1}H NMR spectra of TBA9 in a 100 mM KCl solution at temperatures ranging from 25 to 55 °C.

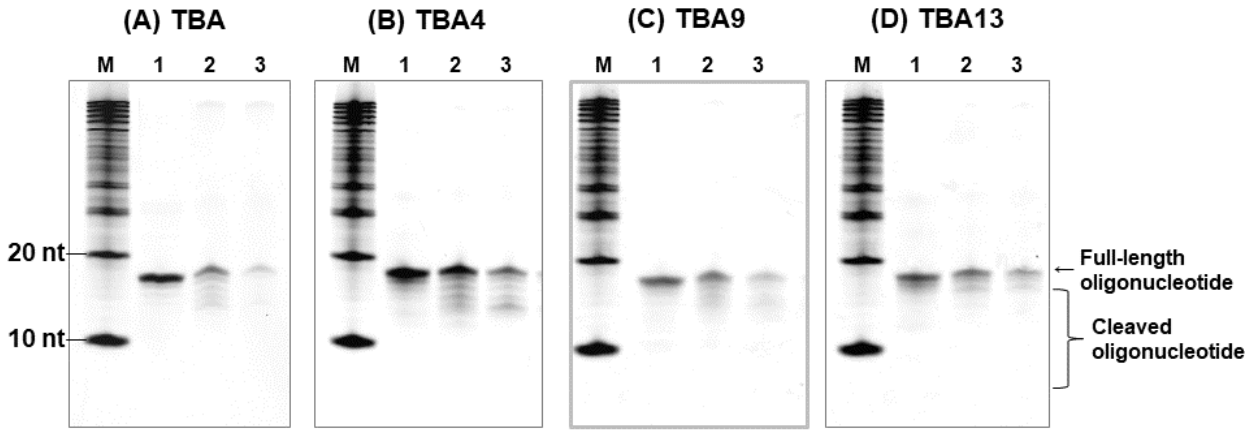

Figure S9. Denaturing gel electrophoresis of (a) TBA, (b) TBA4, (c) TBA9, and (d) TBA13 after the addition of human serum at 37 °C. Samples incubated for 0, 1, and 3 hours were loaded on lanes 1 to 3, respectively. Lane M indicated a DNA size maker.

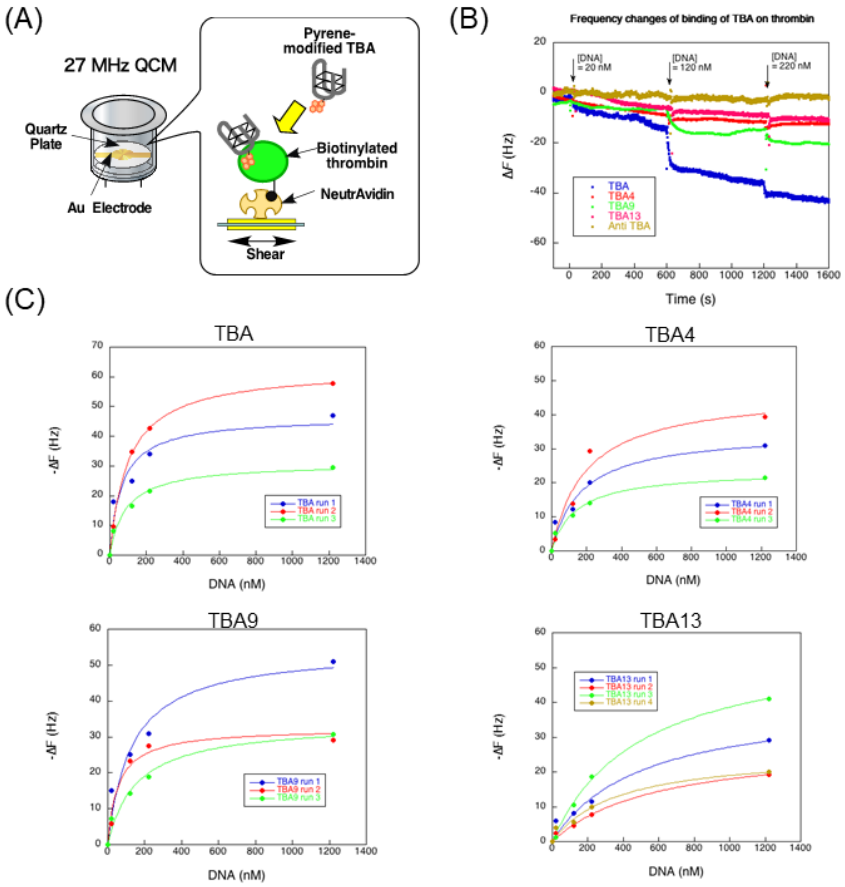

Figure S10. Binding assay of pyrene-modified TBA to human thrombin immobilized on quartz crystal microbalance (QCM). (A) Schematic illustration of assay for the binding of pyrene-modified TBA to thrombin by using QCM. (B) Frequency changes of QCM by the addition of native TBA (blue), TBA4 (red), TBA9^{M} (green), and TBA13 (pink). As a control experiment, antisense of the TBA sequence (Anti TBA; yellow) was injected. Black arrows indicate the timings of injection of the DNAs with the indicated amount. (C) Binding isotherms of pyrene-modified TBA to thrombin immobilized on QCM. All the data were corrected from three individual experiments. All the assays were carried out in potassium buffer pH 6.9 at 25 °C. The plots were fitted by Langmuir adsorption model as follows: $-\Delta F = [\mathrm{DNA}]*(-\Delta F_{max})/([\mathrm{DNA}] + K_D)$.

Table S1. Degradation results of TBA oligonucleotides under human serum conditions.

Oligonucleotide	Incubation time (hours)	Extend of degradation (%)
TBA	0	100
	1	41.6
	3	10.0
TBA4	0	100
	1	72.5
	3	39.8
TBA9	0	100
	1	67.5
	3	28.4
TBA13	0	100
	1	70.2
	3	39.2

Table S2. Binding parameters of pyrene-modified TBA analouges to thrombin immobilized on QCM.

Oligonucleotide	K_D (nM)
TBA	85.1 ± 17.7
TBA4	166 ± 29.3
TBA9	93.6 ± 51.0
TBA13	523 ± 91.6
Anti TBA*	No binding

*Non-binding control DNA sequence is complementary to TBA.