Supplementary Information

Functional *iso*DNA aptamers: Modified thrombin binding aptamers with 2‘-5‘-linked sugar phosphate backbone (*iso*TBA)

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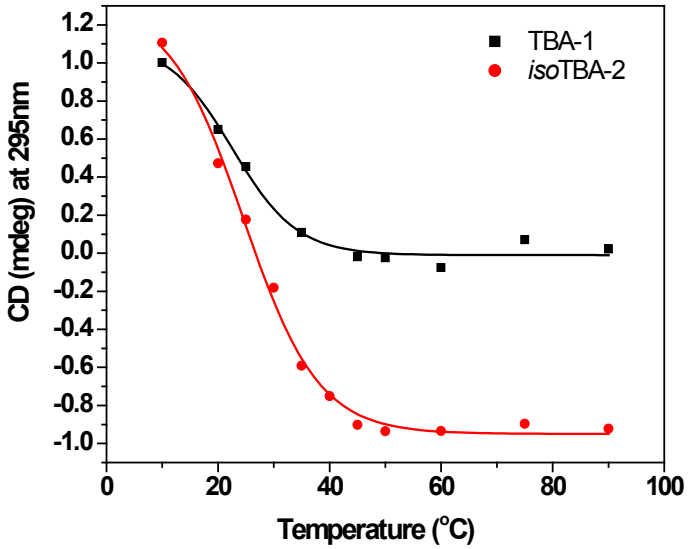
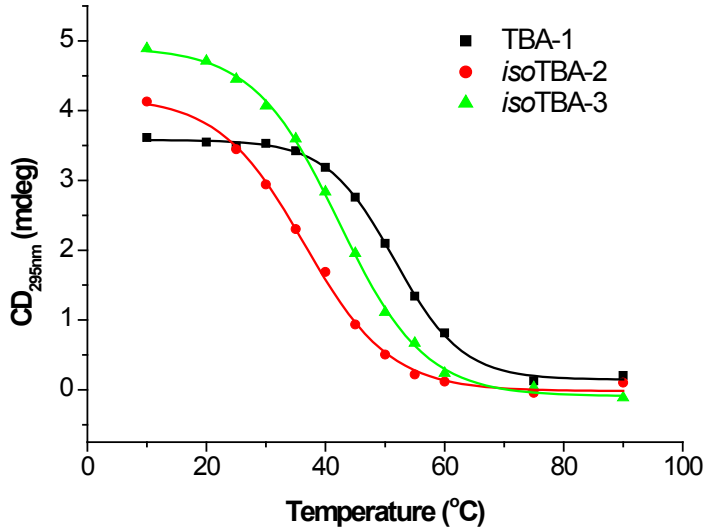
Contents

CD-T_m of TBA-1 and *iso*TBA-2 with Na^+

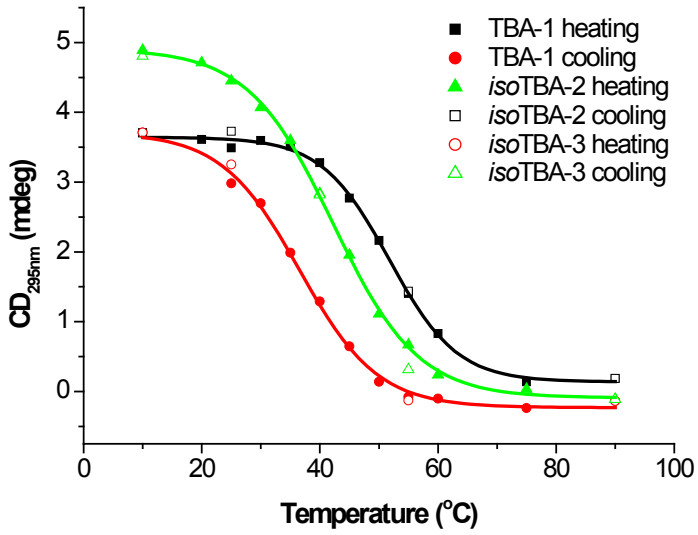


CD-T_m of TBA-1, *iso*TBA-2 and *iso*TBA-3 with K^+



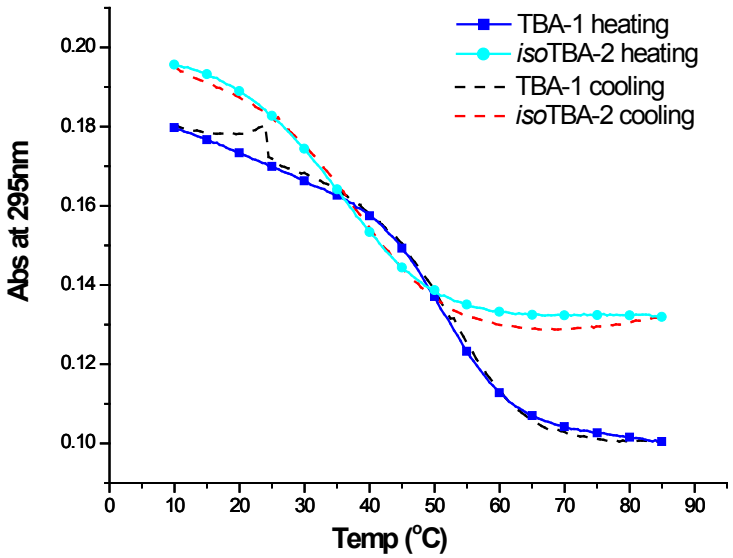
SI- Figure S 1 CD amplitude at 295 nm versus temperature during heating to get T_m of **TBA-1**, ***iso*TBA-2** and ***iso*TBA-3** in presence of Na^+ and K^+. (strand concentration of 5 µM in 10mM Na-phosphate buffer (pH 7.5) containing 100mM NaCl for Na^+ and 10mM K-phosphate buffer (pH 7.5) containing 100mM KCl for K^+).

CD hysteresis

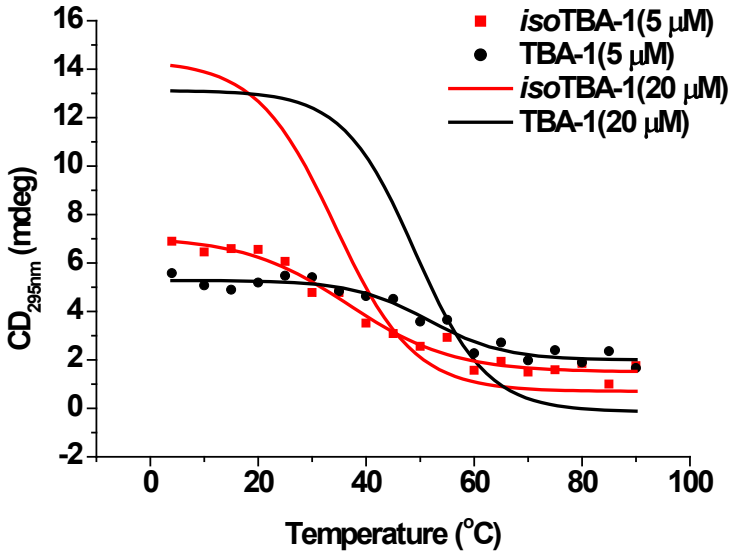


SI- Figure S2. CD hysteresis –CD amplitude at 295 nm versus temperature during heating and cooling experiments for **TBA-1, *iso*TBA-2 , *iso*TBA-3** with a strand concentration of 5 µM in 10mM K-phosphate buffer (pH 7.5) containing 100mM KCl.

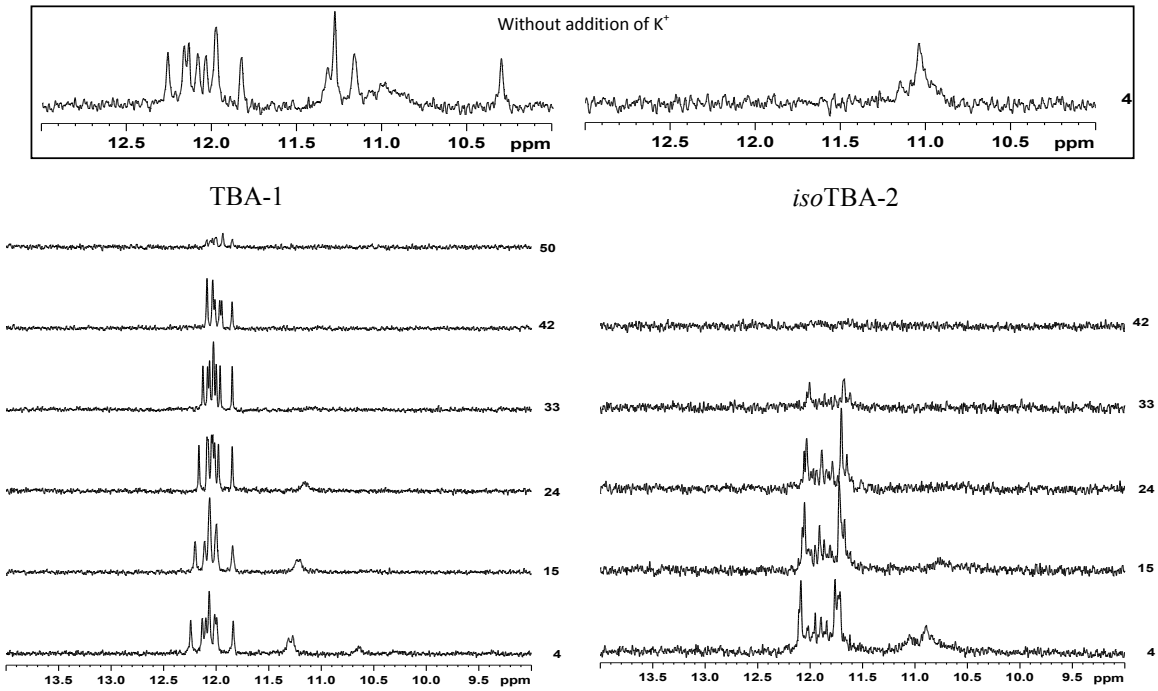
UV-hysteresis:



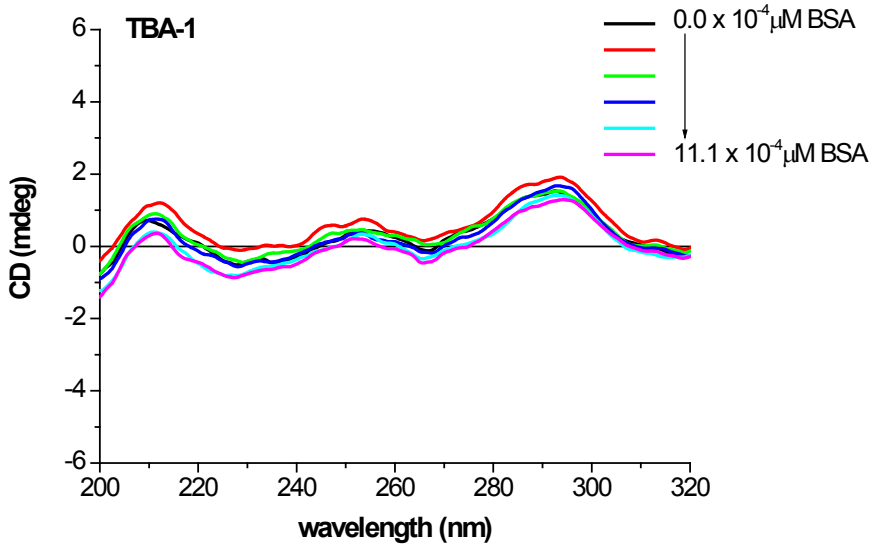
SI- Figure S3. UV hysteresis–UV absorbance at 295 nm versus temperature during heating and cooling experiments for **TBA1** and ***iso*TBA-2** with strand concentration of 5 µM in 10mM K-phosphate buffer (pH 7.5) containing 100mM KCl for each respectively.

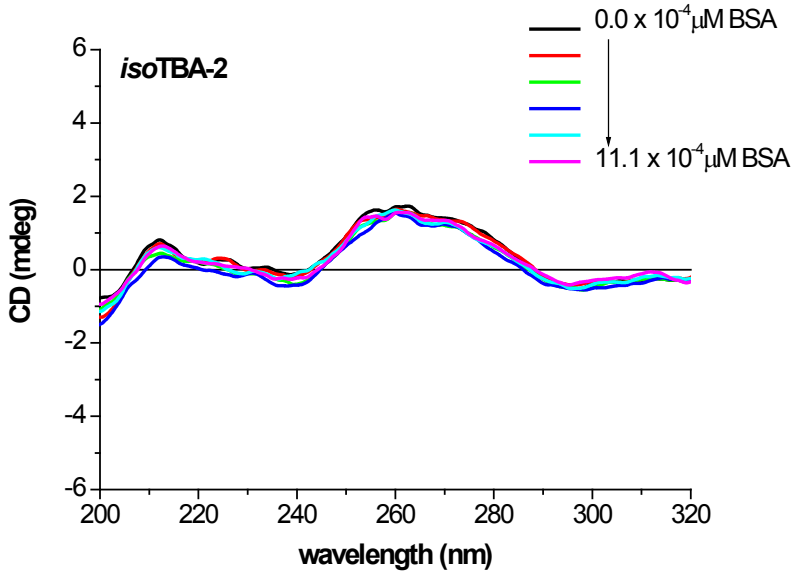


SI- Figure S4. CD melting plots of **TBA-1** and ***iso*TBA-2** at 5 μM (T_m = 52 °C and 37 °C respectively) and 20 μM concentration (T_m = 49 °C and 34 °C respectively) in buffer containing 100mM KCl.



SI- Figure S5. Quadruplex imino proton spectral region for **TBA-1** and ***iso*TBA-2** in the presence of K^+ from 4°C to 50°C. Inset shows the imino proton spectral region in the absence of K^+ at 4°C.

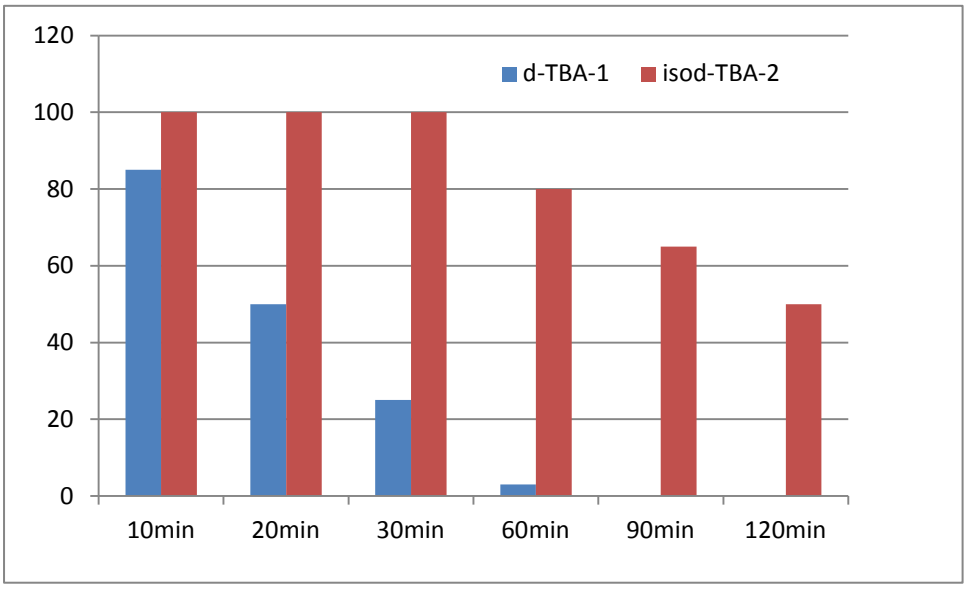




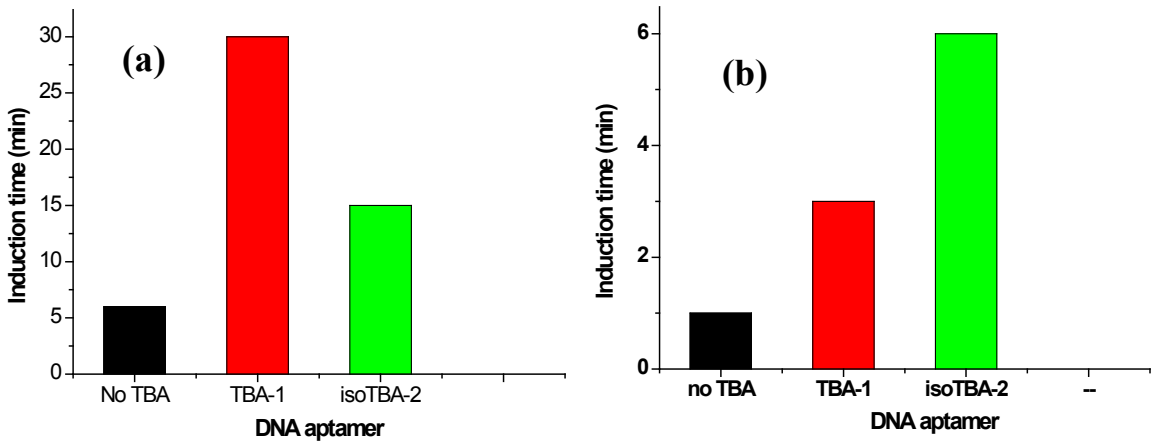
SI- Figure S6. CD of **TBA-1** and ***iso*TBA-2** with incremental additions of Bovine Serum Albumin (BSA) at 4°C.

Nuclease resistance study

Oligonucleotides:	TBA-1 and *iso*TBA-2
Concentration of ONs	7.5 µM
SVPD concentration	5mg/ml
Volume used	2µlit SVPD/200µlit ONs



SI- Figure S7. Stability of the aptamers **TBA-1** and ***iso*TBA-2** towards snake venom phosphodiesterase (SVPD) enzyme, plotted as % intact oligomer Vs time.



SI- Figure S8. Clotting assay induction time for **TBA-1** and ***iso*TBA-2** (a) in the absence and (b) in the presence of SVPD. **TBA-1** and ***iso*TBA-2** used at 0.037 µM strand concentration.

Experimental procedures

Oligonucleotides both 3'-5'and 2'-5'were synthesized in-house on a Bioautomation Mermade-4 DNA synthesizer employing β-cyanoethyl phosphoramidite chemistry. The 2'-deoxy-3'-phosphoramidites, Uridine 3'-OTBDMS CED phosphoramidite (used for modified *iso*TBA-3 sequence) were obtained from ChemGenes and 3'deoxy-2'-phosphoramidites were obtained from Glen Research. Universal columns procured from Bioautomation were used for 2'-5' oligomer synthesis. Oligonucleotides after post-synthetic treatment were desalted by passing through Pharmacia NAP-5 columns then purified by RP-HPLC on a C18 column using a Waters system (Waters Delta 600e quaternary solvent delivery system and 2998 photodiode array detector and Empower2 chromatography software). An increasing gradient of acetonitrile in 0.1M triethylammonium acetate (pH 7.0) was used.

Mass spectra of the oligomers were obtained by MALDI-ToF mass spectrometry. The MALDI-ToF spectra were recorded on Voyager-De-STR (Applied Biosystems). The matrix used for analysis was THAP (2', 4', 6'-trihydroxyacetophenone).

CD spectra were recorded on Jasco J-815 CD Spectrometer equipped with a Jasco PTC-424S/15 peltier system. 2 mm path-length quartz cuvettes were used for a sample volume 500 µL and strand concentration of 5 µM in 10 mM Na/K-phosphate buffer (pH 7.5) containing 100mM NaCl/KCl respectively. Oligomers prepared in buffer were annealed by heating at 95 °C for 5 minutes then slowly cooling to room temperature followed by refrigeration for 5 to 6 hours before use. Spectral scans were collected over a wavelength range 200- 320 nm at a scanning rate of 100 nm min^{-1}. Three scans were averaged for each sample. CD thermal denaturation studies of the TBA sequences with and without thrombin and BSA were performed.

UV-Thermal denaturation studies of the TBA oligomers was performed using a 10 mm quartz cell in a Cary 300 Bio UV-Visible Varian Spectrophotometer . The TBA oligomers (5µM concentration) were annealed in a 10 mM potassium phosphate buffer, pH 7.5, 100mM KCl. The concentration was calculated on the basis of absorbance from molar extinction coefficients of the corresponding nucleobases of DNA/*iso*DNA. Absorbance versus temperature profiles were obtained by monitoring the absorbance at 295 nm from 10–85°C at a ramp rate of 0.5°C per minute. Both melting and annealing profiles were obtained to check reversibility of the process. A stream of dry nitrogen was gently applied through the sample compartment to prevent condensation of water on the cuvette at low temperatures.

All the NMR measurements were performed on a Bruker AV 500 NMR spectrometer operating at 500.13 MHz for 1H using a 5mm BBFO probe. Samples (150 uL of ~100µM solution) were prepared in a standard 3mm NMR tube in 10% D2O and 90% H2O. Water suppression was achieved by using a standard Bruker watergate W5 pulse sequence with gradients. 2000 transients were collected with an acquisition time of 3.54 sec and a pulse delay of 1 sec. The raw data were processed with a Gaussian

function for improvement of signal to noise ratio. Temperature during the measurements was controlled by means of a Bruker BVT 3000 unit.

HPLC purified and lyophilised TBA-1 and *iso*TBA-2 were dissolved in 150 µl of 10mM K-phosphate buffer pH7.5 containing 100mM KCl and lyophilized, then diluted in 90% v/v H_2O /10% v/v D_2O to 100 µM. 3mm NMR tubes were used for scanning the spectra. One-dimensional spectra were acquired at 4, 15, 24, 33,42, 50 ^{0}C. One-dimensional spectra of TBA-1 and *iso*TBA-2 only in 90% v/v H_2O /10% v/v D_2O (100 µM) at 4^0 C were scanned to show that *iso*TBA-2 did not form quadruplex in the absence of KCl while TBA-1 does form a quadruplex in H_2O alone.

Anti-thrombin activity measurements were done by using Varian Cary 300 Bio UV-Visible Spectrophotometer to measure % transmittance change over time. 0.1NIH unit of thrombin (50 NIH/ml, bovine thrombin, Fibroscreen reagent, Tulip Diagnostics (P) LTD.) was added to the TBA-1, *iso*TBA-2 aptamers dissolved in water to a concentration of 3.7 x 10^{-8}M and incubated for 15 minutes at 25^0 C. This was then added to a 1.0 ml fibrinogen solution (Sigma product No F 3879) 3.0 x 10^{-6} M, in saline and the transmittance was measured at 2 minute intervals for 90 minutes.

Enzymatic hydrolysis of the aptamers TBA-1, *iso*TBA-2 (7.5µM) was carried out at 37^0C in 100µl buffer (100mM Tris-HCl (pH 8.5), 15mM $MgCl_2$, 100mM NaCl) and SVPD (2µG, 1.2 x 10^{-4} U) Aliquots were taken at several time ponts. Each aliquot was heated at 90^0C for 2 min to inactivate the nuclease enzyme. The intact oligomer at each time point was monitored by RP-HPLC. Percentage of intact oligomer was plotted against time to show the degradation of oligomers with respect to time.