Unlike RNA-TBA (rTBA), iso-rTBA, the 2'-5'-linked RNA-thrombin-binding aptamer, is a functional equivalent of TBA

Supporting Information

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Experimental details

Oligonucleotide synthesis

3'-5'-linked TBAand 2′–5′-linked iso-rTBAwere synthesized in-house on a Bioautomation Mermade-4 DNA synthesizer employing β-cyanoethyl phosphoramidite chemistry. The 2′-deoxy-3′-phosphoramidites were obtained from Innovassynth Technologies (I) Ltd. and 3′-TBDMS-protected2′-phosphoramidites, from Glen Research. Universal columns procured from Bioautomation and an extended coupling time of 10 s were used for 2′–5′-linked oligomer synthesis. Oligonucleotides were cleaved from the solid support by treating with aqueous ammonia at 60° C for 6 h and then concentrated.#Post-synthesis, deprotection of the TBDMS group was achieved by treatment with triethylamine trihydrofluoride for 2 h, followed by purification. The random RNA oligomer, 5'-r(CUGAAAUCGGUU)-3', was purchased from Gene Link™.

HPLC analysis

Oligonucleotides were purified by RP-HPLC on a C18 column using a Waters system (Waters Delta 600e quaternary solvent delivery system, 2998 photo-diode array detector and Empower2 chromatography software). An increasing gradient of acetonitrile in 0.1 M triethyl ammonium acetate (pH 7.0) was used. The molecular weights of all oligonucleotides were verified by MALDI-TOFanalysis.

CD studies

CD spectra were recorded on a Jasco J-815 CD spectrometer equipped with a Jasco PTC-424S/15 peltier system. 5 mm path-length quartz cuvettes were used for a sample volume of 2 ml and strand concentration of 5 μM in potassium phosphate buffer (10 mM, pH 7.2) containing 100 mM KCl. Oligomers in buffer were annealed by heating at 95 °C for 5 min, then slowly cooled to room temperature, followed by refrigeration for 3 to 4 h before use. Spectral scans over a range of 320 nm to 200 nm were collected as accumulations of 3 scans at a scanning rate of 100 nm min^{-1}. CD melting was performed by monitoring CD intensity at 295 nm against temperature over the range 5–90 °C at a heating rate of 3 °C per min.

For thombin-binding studies, repetitive amounts (0.22 μM per addition) of thrombin were added to an aqueous solution of iso-rTBA.

UV studies

UV-absorbance scans of the TBA and iso-rTBA oligomers were recorded using 10 mm pathlength quartz cells on an Analytik Jena SPECORD® 200 plus spectrometer equipped with a peltier-controlled temperature controller and at a scanning speed of 5 nm sec^{-1}. The TBA and iso-rTBA oligomers (5 μM strand concentration) were annealed in potassium phosphate buffer (10 mM, pH 7.2), containing 100 mM KCl. The oligomer concentration was calculated on the basis of absorbance from molar extinction coefficients of the corresponding nucleobases of DNA. Thermal difference spectra (TDS) were obtained by subtracting the UV-absorbance spectral scan of the sample at temperatures below (i.e., 10 °C) from that above (i.e., 90 °C) the melting temperature (T_m).The TDS factor is the absolute value of the ratio of $\Delta A_{240\ nm}/\Delta A_{295\ nm}$, where ΔA is the difference, at a given λ, between the absorbance above (i.e., 90 °C) and at a given temperature, T, below the melting temperature (where T = 5 °C, 10 °C,…) etc., upto the melting temperature, T_m.

For duplex studies, 2.5 μM each strand was taken in PBS (10 mM phosphate buffer containing 2.7 mM KCl and 137 mM NaCl, pH 7.4).

NMR experiments

^{1}H NMR spectra were acquired on a Bruker AvanceIIIHD#700 NMR spectrometer operating at 700.13 MHz for ^{1}H using a 5 mm TXI probe.#The spectral window was set to 20#ppm with the carrier positioned on the water signal.#Supression of water signal was achieved by using#a#standard Bruker pulse sequence incorporating#excitation sculpting with selective RF pulses and gradients. Spectra were recorded with a relaxation delay of 4s and 4096 scans.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 iso-rTBA was taken at 200 μM concentration in 90:10 v/v H_2O:D_2O with or without 100 mM KCl in 3 mm NMR tubes for recording spectra.

Anticlotting measurements

Clotting time experiments were performed at 37 °C on a Start-Max (Stago) coagulation analyzer. Each experiment was repeated at least thrice; the standard deviation was ±1 s. Each commercial reagent was re-constituted according to the manufacturer's protocol. Bovine

thrombin (Tulip Diagnostics, 0.1 NIH unit) was incubated with TBA or iso-rTBA as applicable at 0.25 μM oligomer concentration for 1.5 min before addition to fibrinogen from human plasma (Aldrich, 3.5 μM). The clotting time (s) was measured as the time taken from the addition of thrombin till the polymerization of fibrin.

Stability of oligonucleotides to cleavage by RNase A

The reactions were performed in 1.5 ml plastic tubes with screw cap immersed in a water bath at 37 °C. Incubation was carried out in 50 mM MOPS buffer at pH 7.0. The samples were prepared by adding the oligonucleotides (iso-rTBA, rTBA, random RNA or TBA at 7.5 μM concentration) to a solution of the RNase enzyme (0.05 U) in MOPS buffer. Aliquots were collected at specific times after initiation of reaction, the enzyme was inactivated by heating at 90 °C for 3 min, and samples were analyzed by HPLC to determine the quantity of oligonucleotide remaining intact at each time point.

SVPD stability study

TBA and iso-rTBA (7.5 μM) stability to hydrolysis by snake venom phosphodiesterase (SVPD) was studied at 37 °C in 100 mM Tris-HCl buffer (pH 8.5) containing 15 mM $MgCl_2$, 100 mM NaCl, and SVPD (0.015U). Aliquots were removed at successive time intervals, heated at 90 °C for 3 min to inactivate the nuclease, and analyzed by RP-HPLC to measure the percentage of oligonucleotides remaining intact.

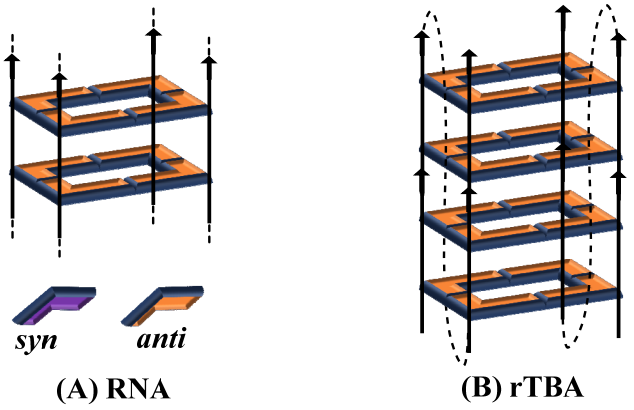


Figure S1. Schematic representation of parallel G-quadruplexes generally observed with RNA (A) and a possible bimolecular quadruplex for rTBA (B) *Syn* and *anti* conformations of guanine are represented in purple and orange colour respectively.

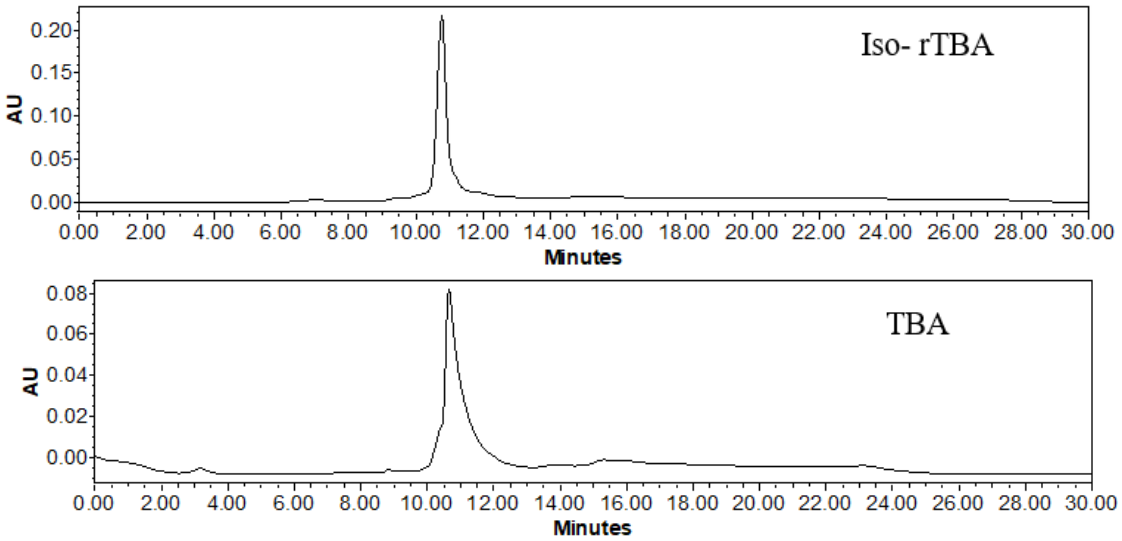
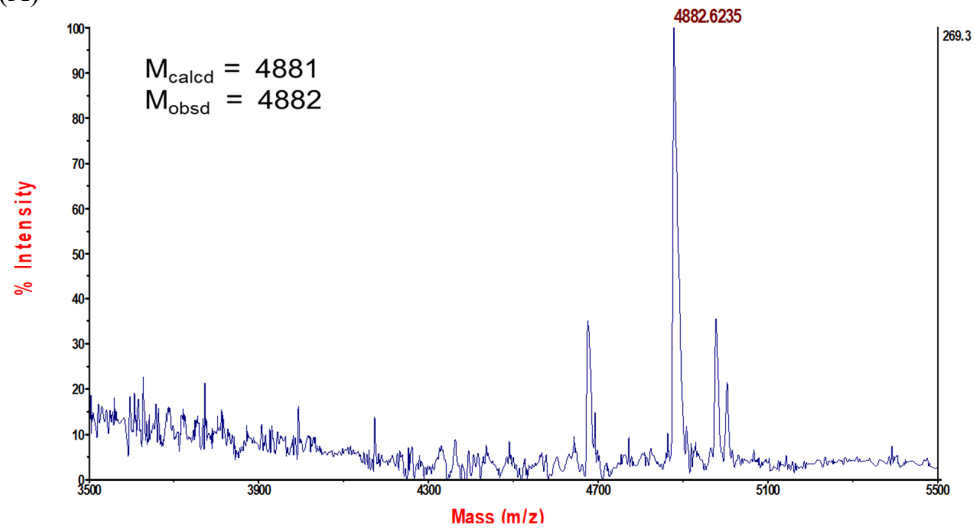


Figure S2. HPLC chromatograms of iso-rTBA and TBA.

(A)



(B)

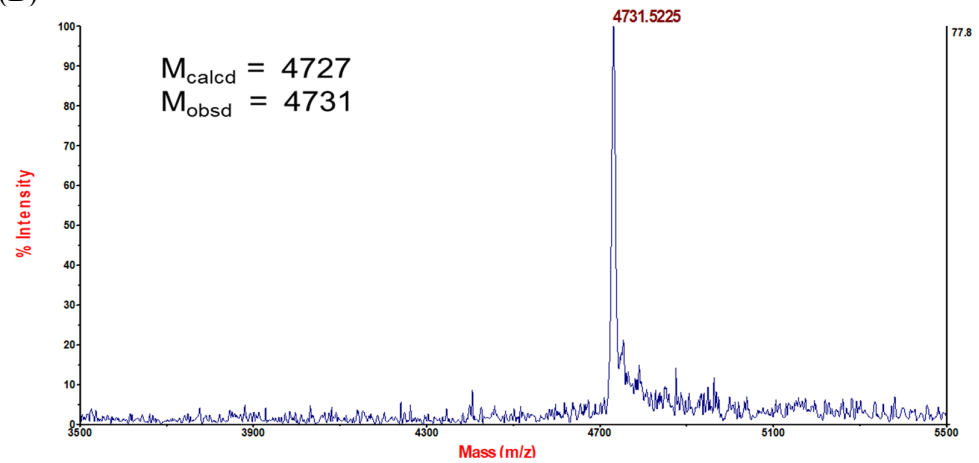


Figure S3. MALDI-TOF spectra of (A) iso-rTBA and (B) TBA.

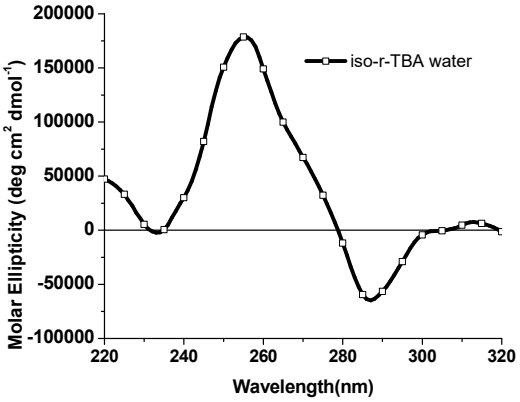


Figure S4. CD spectra of iso-rTBA in water.

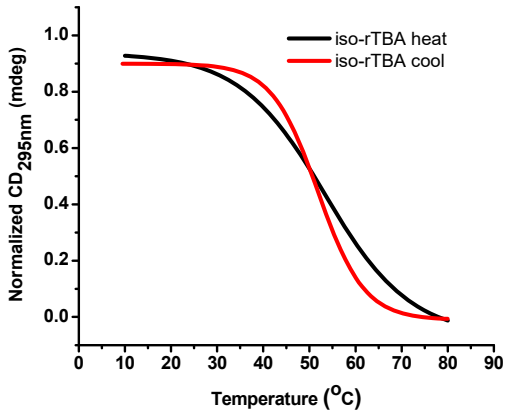


Figure S5. CD- heating and cooling plots for iso-rTBA. Oligomers were taken at a concentration of 5 μM in 10 mM potassium phosphate buffer (pH 7.2) containing 100 mM KCl. Each experiment was performed at least thrice.

Table S1. UV-T_m data for iso-rTBA in comparison to TBA.

Strand conc (μM)	Iso-rTBA (°C)				TBA (°C)			
	T_m (heat)	T_m (cool)	ΔT_m (20μM-5μM)	ΔT_m (heat-cool)	T_m (heat)	T_m (cool)	ΔT_m (20μM-5μM)	ΔT_m (heat-cool)
5	49	51	-1	-2	51	50	-2	+1
20	48	50		-2	49	-		-

Experiments were performed in 10 mM potassium phosphate buffer, pH 7.2, containing 100 mM KCl. Each experiment was performed at least thrice and the values are accurate to ± 1 °C.

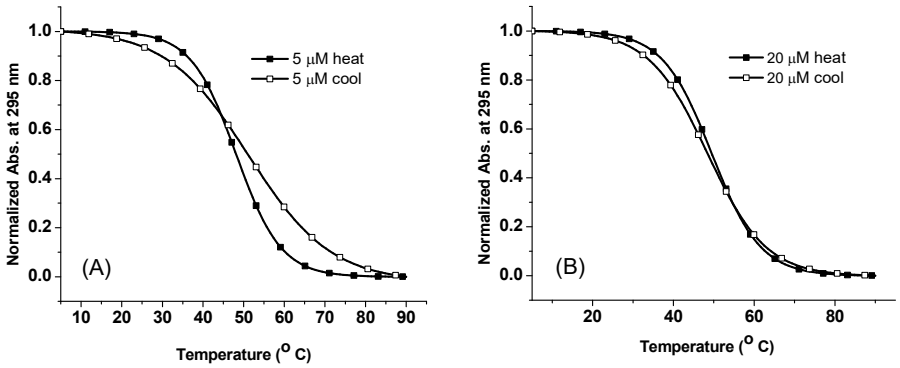


Figure S6. Normalized UV plots for heating and cooling cycles of iso-rTBA taken at (A) 5 μM and (B) 20 μM concentration, monitored at 295 nm. Buffer: 10 mM potassium phosphate buffer, pH 7.2, containing 100 mM KCl.

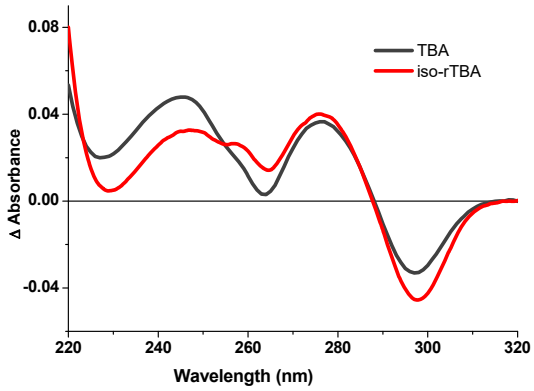


Figure S7. UV-TDS for TBA and iso-rTBA taken at 5 μM concentration in 10 mM potassium phosphate buffer, pH 7.2, containing 100 mM KCl.

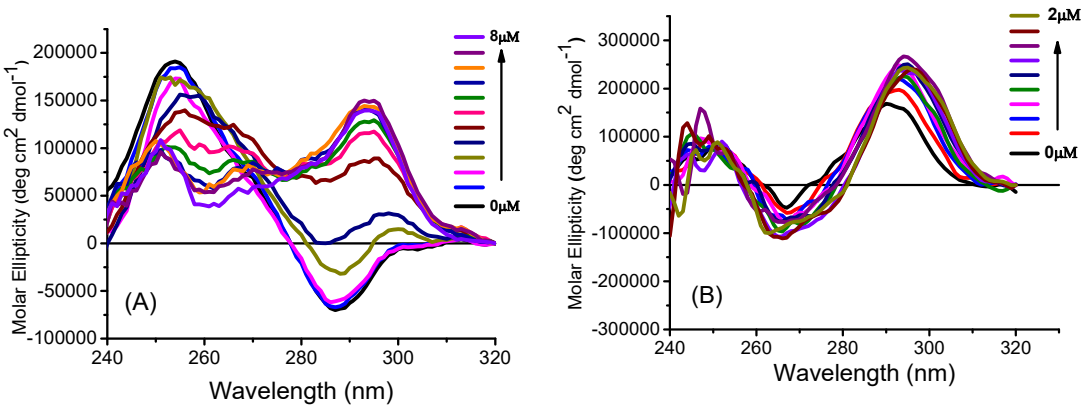


Figure S8. Changes in the CD signal of (A) iso-rTBA and (B) TBA (5 μM each) upon addition of thrombin.

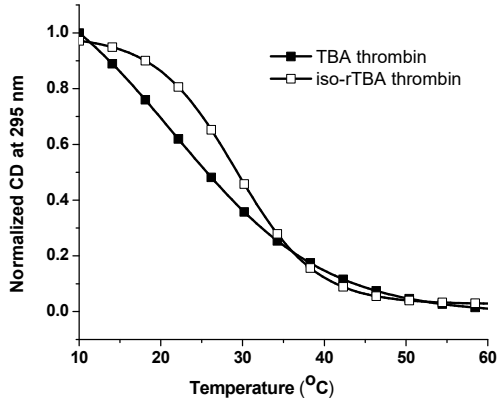


Figure S9. CD melting plots of iso-rTBA in comparison to TBA in complex with thrombin.

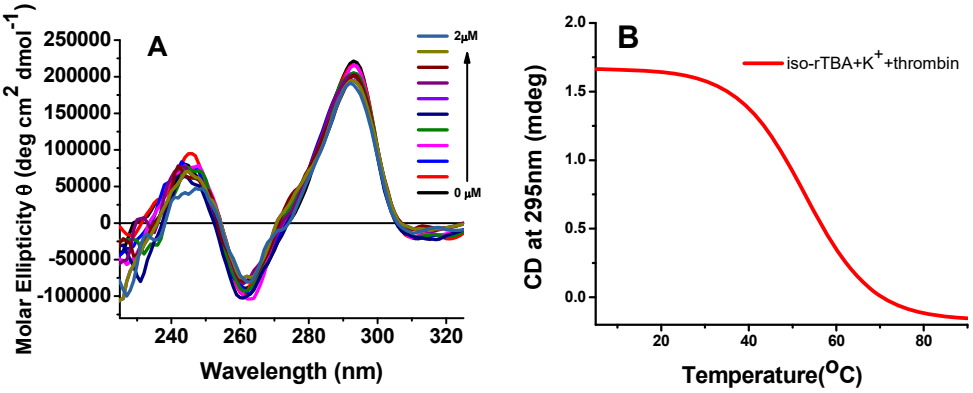


Figure S10. (A) CD spectra of iso-rTBA upon addition of thrombin and (B) CD melting plot of iso-rTBA after thrombin addition. Experiments were performed in water containing 100 mM KCl.

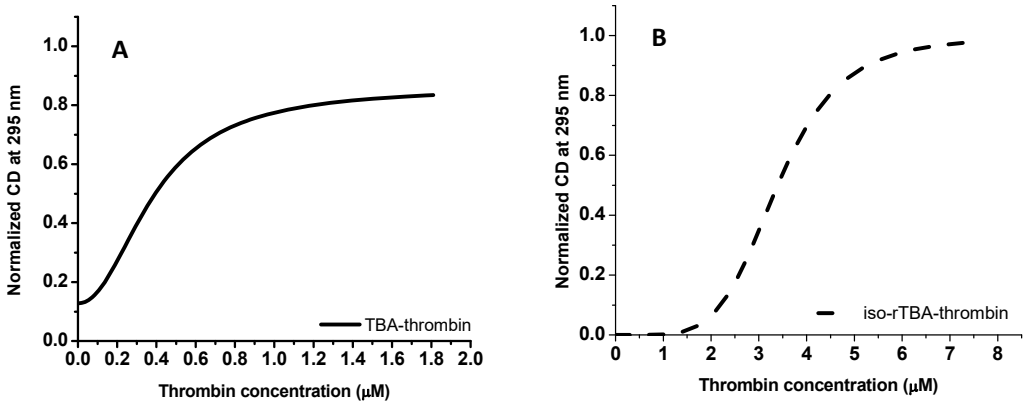


Figure S11. CD saturation binding curves for TBA and iso-rTBA with thrombin. The plots were fitted with the Hill equation for non-linear variation using Microcal Origin software.

K_d values were obtained from the Hill plots of CD Vs. thrombin concentration using Microcal Origin software and are equivalent to $1/K_a$.

Table S2. Dissociation constants obtained from the CD saturation binding curves.

Oligomer	K_d
TBA	3.8×10^{-7}M
iso-rTBA	3.4×10^{-6} M

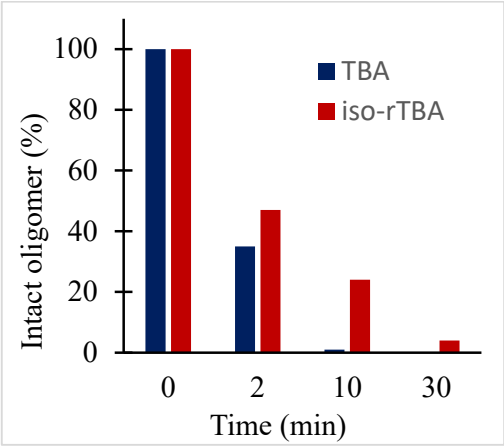


Figure S12. Stability of oligomers (7.5 µM) at 37 °C to hydrolysis by SVPD (0.015 U); 100 mM Tris-HCl buffer, pH 8.5 containing 15 mM $MgCl_2$, 100 mM NaCl. TBA: indigo; iso-rTBA: red.

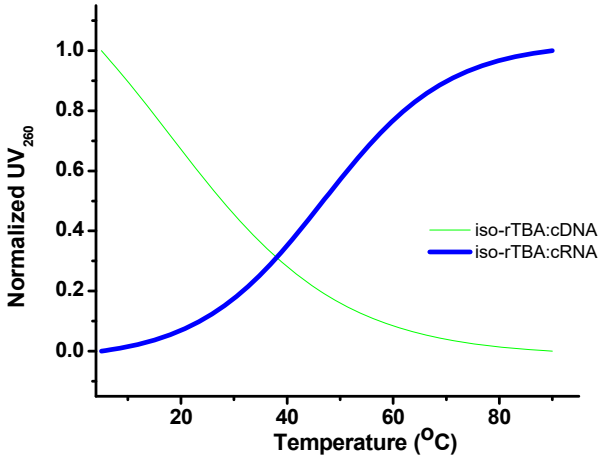


Figure S13. UV-T_m profiles of iso-rTBA:cDNA and iso-rTBA:cRNA duplexes in phosphate buffered saline (PBS, 10 mM phosphate buffer containing 2.7 mM KCl and 137 mM NaCl, pH 7.4). Strand concentration 2.5 µM each strand.

Table S3. UV-melting data of iso-rTBA duplexes with complementary DNA/RNA.

Duplex	UV-T_m (°C)
Iso-rTBA:cDNA	Not detected (non-binding)
Iso-rTBA:cRNA	47

Each experiment was repeated thrice and the value indicated is an average of the measurements, and is accurate to ±1 °C. cDNA: 5'- CCAACCACACCAACC-3'; cRNA: 5'- r (CCAACCACACCAACC)-3'.