Electronic Supplementary Information

Unimolecular antiparallel G-quadruplex folding topology of 2'-5'- isoTBA sequences remains unaltered by loop composition

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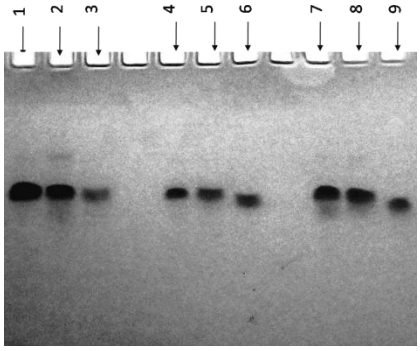
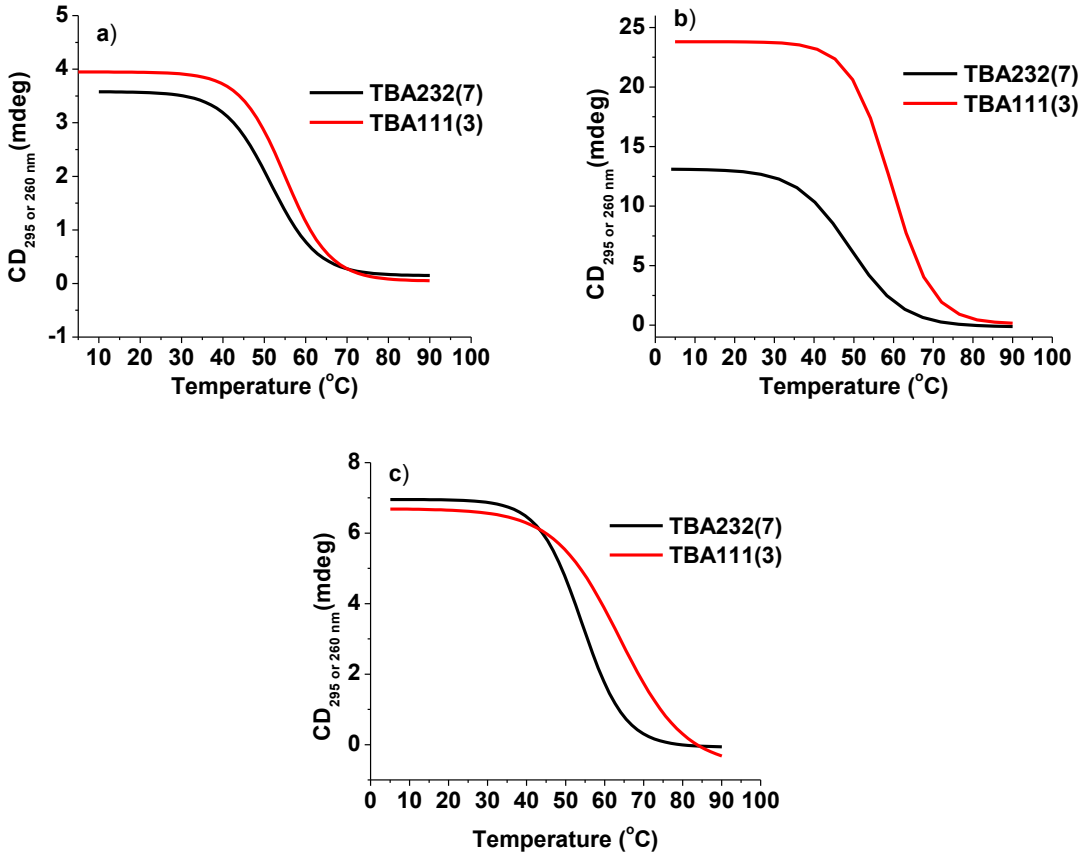
Contents:

1. Denaturing polyacrylamide gel mobility assay:



SI Figure S1: Denaturing polyacrylamide gel mobility assay of TBA and isoTBA sequences in DI-water without any added cations. Sample loading- 3µL+3µL formamide; Gel containing 7 M urea. Lane1- Scrambled TBA sequence, Lane2- TBA232(7), Lane3-isoTBA232(7), Lane4-TBA222(6), Lane5- TBA131(5), Lane6- TBA111(3), Lane7-isoTBA222(6), Lane8-isoTBA131(5), Lane9- isoTBA111(3). The bands were visualized by UV-shadowing.

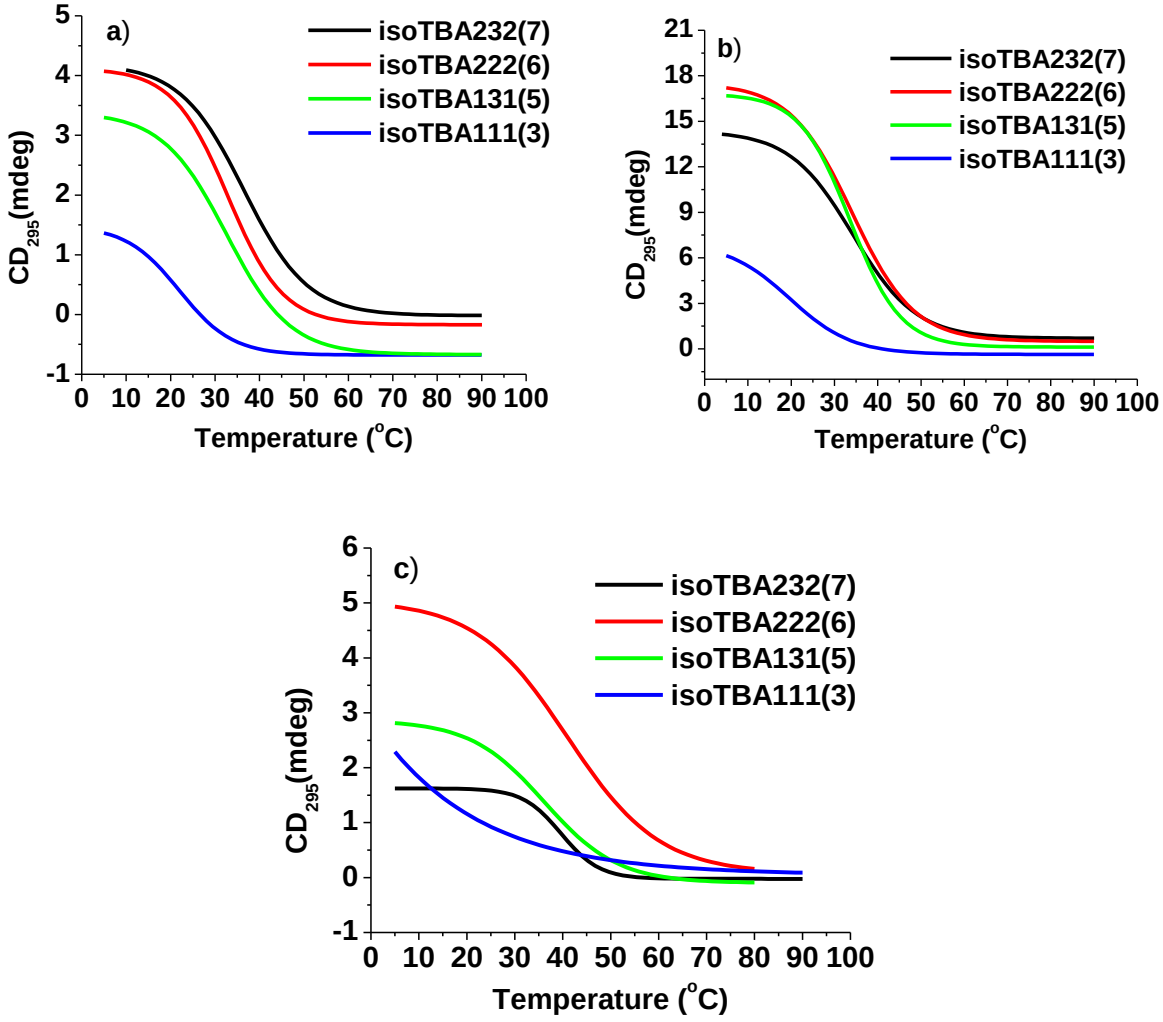
2. CD-T_m of TBA232(7) and TBA111(3)



SI Figure S2: CD amplitude at 295 nm for **TBA232(7)** and at 260 nm for **TBA111(3)** plotted versus temperature during heating for **TBA232(7)** and **TBA111(3). a)** 5 µM strand concentration, in potassium phosphate buffer (0.01 M, pH 7.2) containing 100 mM KCl **b)** 20 µM strand

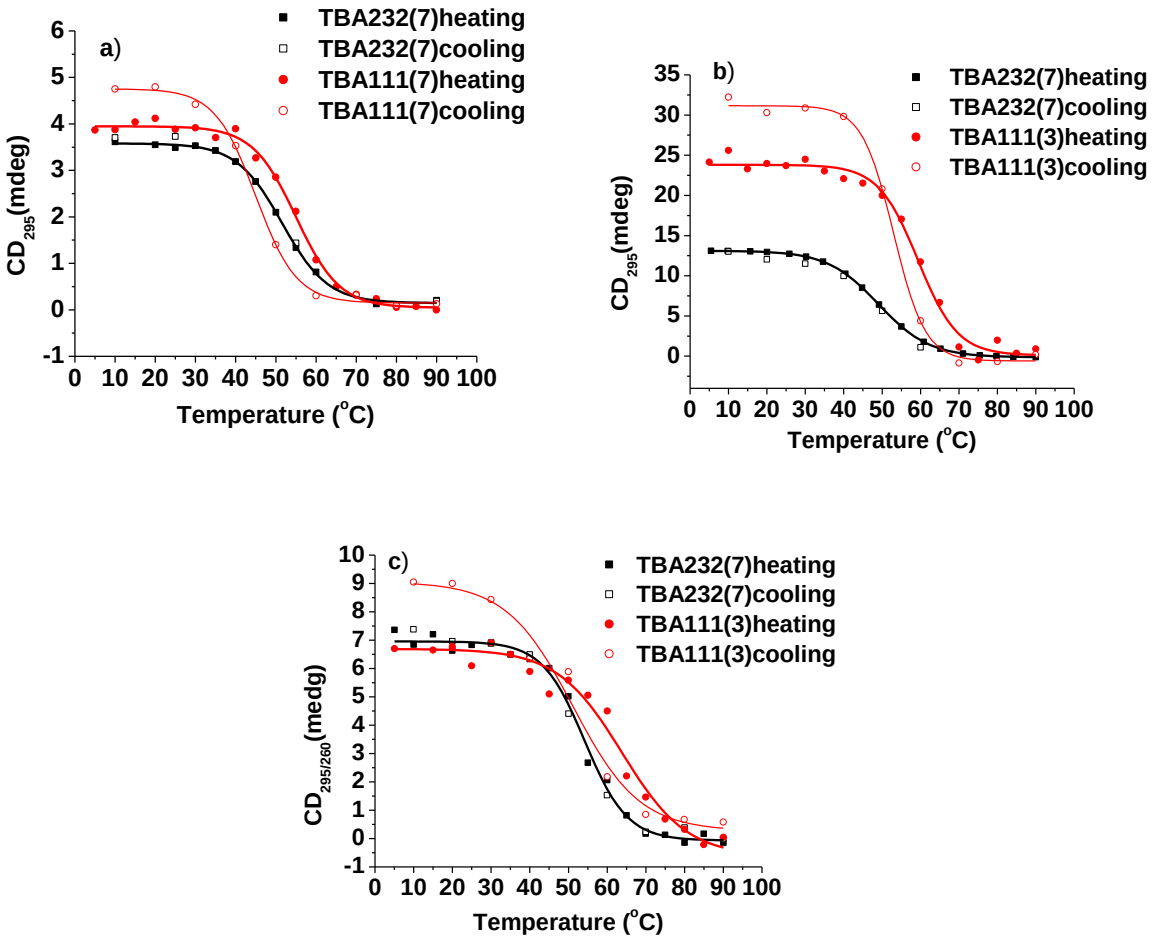
concentration, in potassium phosphate buffer (0.01 M, pH 7.2) containing 100 mM KCl **c)** 5 µM strand concentration, in potassium phosphate buffer (0.01 M, pH 7.2) containing 500 mM KCl.

3. CD-T_m of isoTBA232(7), isoTBA222(6) , isoTBA131(5) and isoTBA111(3)



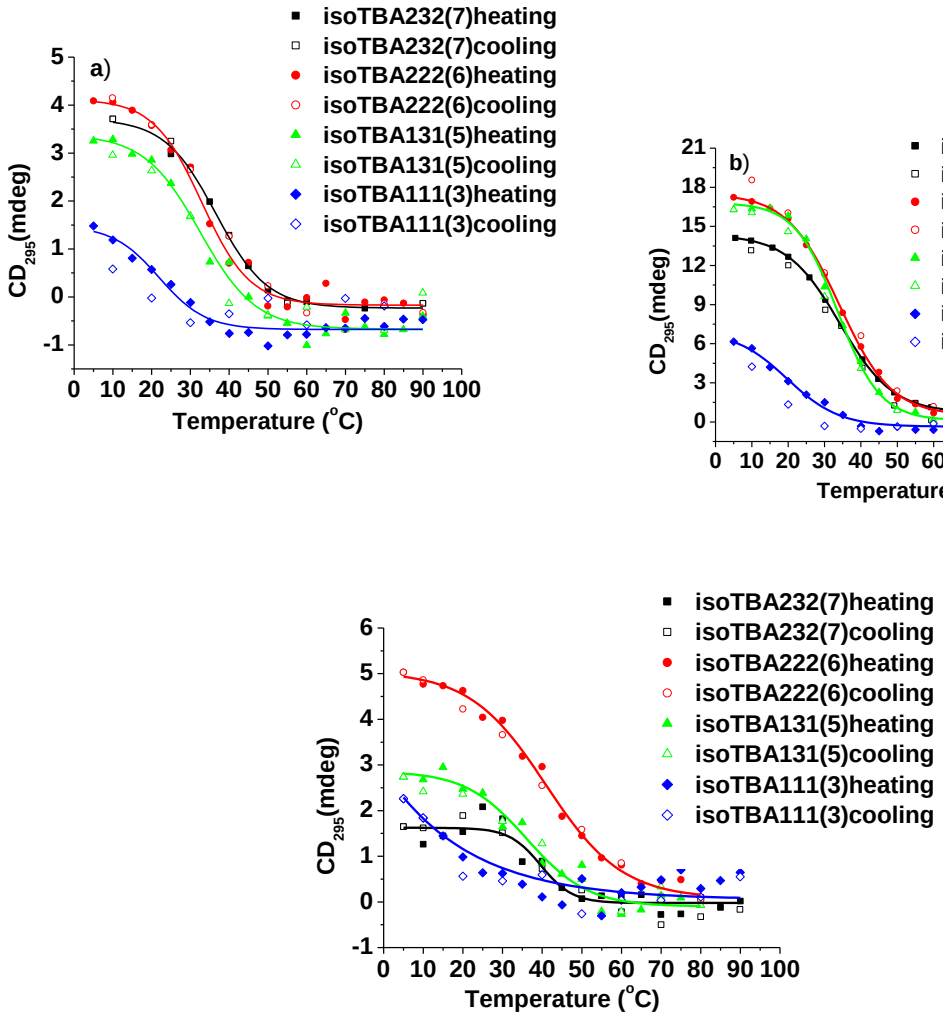
SI Figure S3: CD amplitude at 295 nm versus temperature during heating for **isoTBA232(7)**, **isoTBA222(6)**, **isoTBA131(5)** and **isoTBA111(3)** in presence of **a)** 5 µM strand concentration, in potassium phosphate buffer (0.01 M, pH 7.2) containing 100 mM KCl **b)** 20 µM strand concentration, in potassium phosphate buffer (0.01 M, pH 7.2) containing 100 mM KCl **c)** 5 µM strand concentration, in potassium phosphate buffer (0.01 M, pH 7.2) containing 500 mM KCl

4. CD hysteresis of TBA232(7) and TBA111(3)



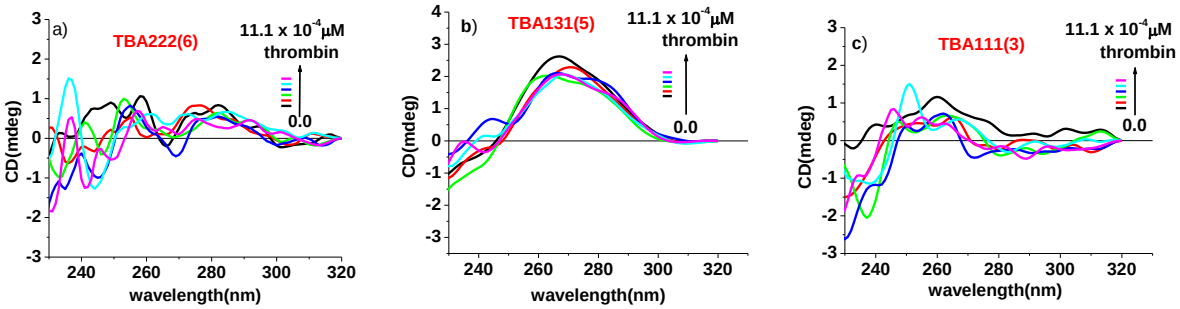
SI Figure S4: CD hysteresis –CD amplitude at 295 nm for **TBA232(7)** and at 260 nm for **TBA111(3)** plotted versus temperature during heating and cooling in presence of **a)** 5 µM strand concentration, in potassium phosphate buffer (0.01 M, pH 7.2) containing 100 mM KCl **b)** 20 µM strand concentration, in potassium phosphate buffer (0.01 M, pH 7.2) containing 100 mM KCl **c)** 5 µM strand concentration, in potassium phosphate buffer (0.01 M, pH 7.2) containing 500 mM KCl.

5. CD hysteresis of isoTBA(7), isoTBA222(6) , isoTBA131(5) and isoTBA111(3)



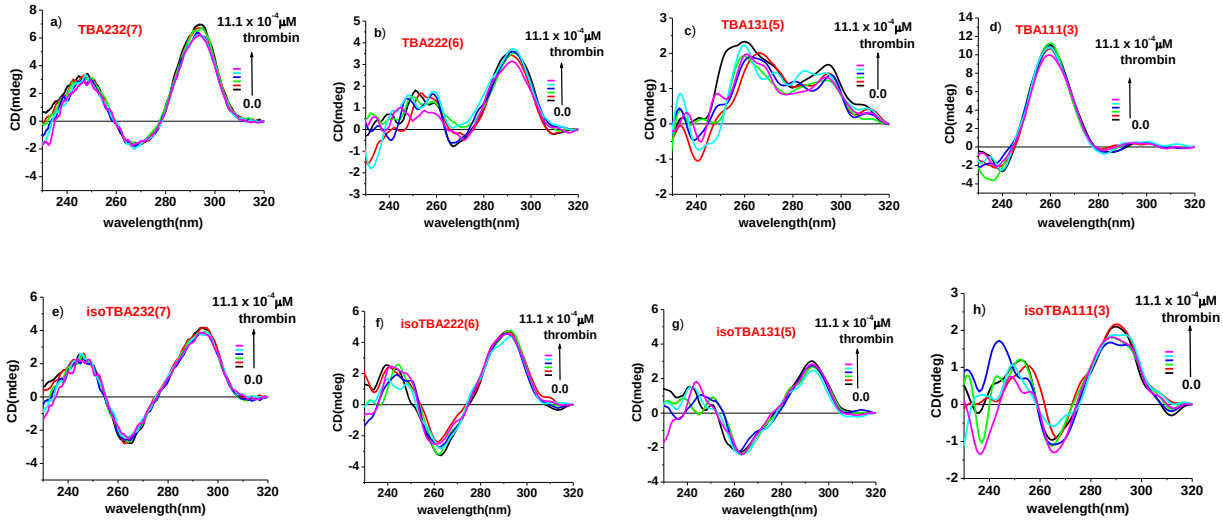
SI Figure S5: CD hysteresis –CD amplitude at 295 nm versus temperature during heating and cooling experiments for of **isoTBA232(7)**, **isoTBA222(6)** , **isoTBA131(5)** and **isoTBA111(3)** in potassium phosphate buffer (0.01M, pH 7.2) in presence of **a)** 5 µM strand concentration, 100 mM KCl **b)** 20 µM strand concentration, 100 mM KCl **c)** 5 µM strand concentration, 500 mM KCl

6. Binding to thrombin in DI-water a)TBA222(6), b)TBA131(5), c) TBA111(3):



SI Figure S6: Changes in CD signal upon addition of thrombin to TBA sequences annealed in DI-water **a) TBA222(6), b) TBA131(5), c) TBA111(3)**

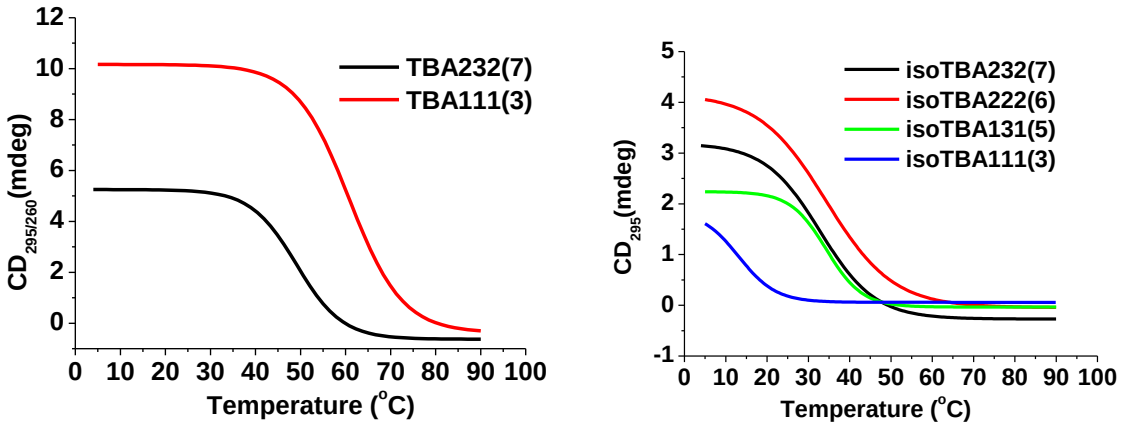
7. Binding of TBA and isoTBA sequences to thrombin in potassium phosphate buffer (0.01 M, pH 7.2) containing 100 mM KCl:



SI Figure S7: Changes in CD signal upon addition of thrombin to TBA or isoTBA sequences annealed in potassium phosphate buffer (0.01M, pH 7.2) containing 100 mM KCl **a) TBA232(7), b) TBA222(6), c) TBA131(5), d) TBA111(3) e) isoTBA232(7), f) isoTBA222(6), g) isoTBA131(5), h) isoTBA111(3).**

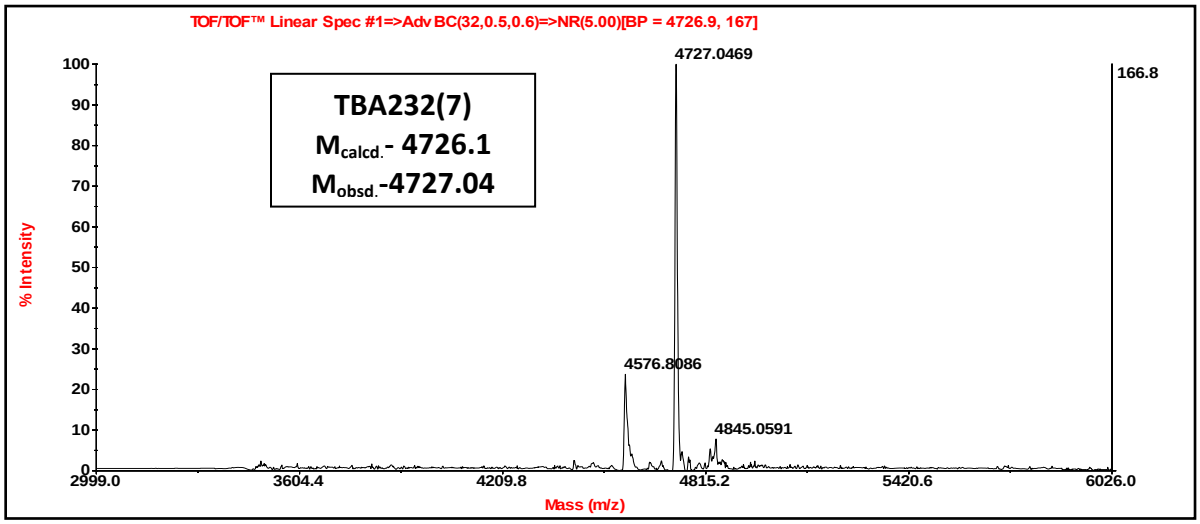
8. CD-T_m for 5 µM strand concentration in potassium phosphate buffer (0.01 M, pH 7.2) containing 100 mM KCl and thrombin for TBA232(7), TBA111(3) and isoTBA232(7), isoTBA222(6) isoTBA131(5), isoTBA111(3)

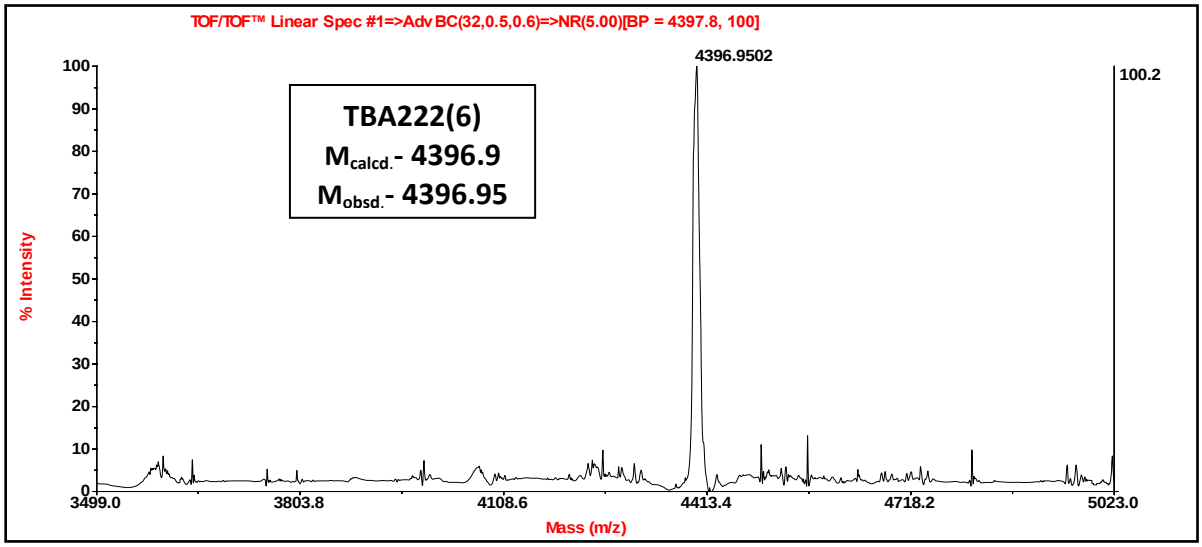
Sequence code	T_m at 5 µM strand concentration in buffer containing100 mM KCl and thrombin	
	heat	cool
TBA232 (7)	**53**	**53**
TBA111(3)	**60**	**48**
isoTBA232(7)	**38**	**38**
isoTBA222(6)	**34**	**34**
isoTBA131(5)	**34**	**31**
isoTBA111(3)	**<10**	**<10**

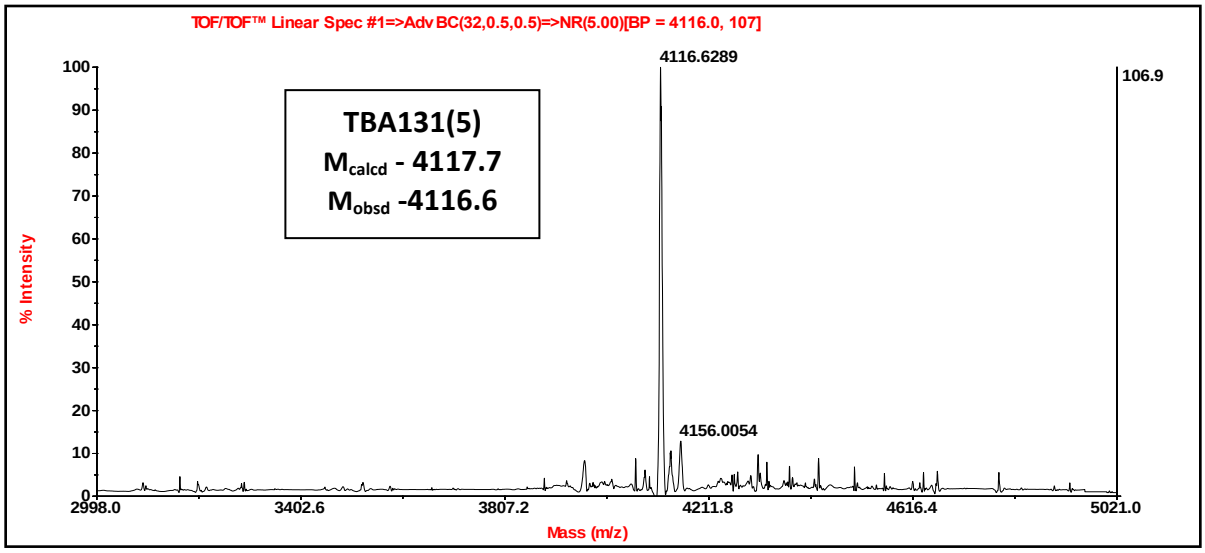


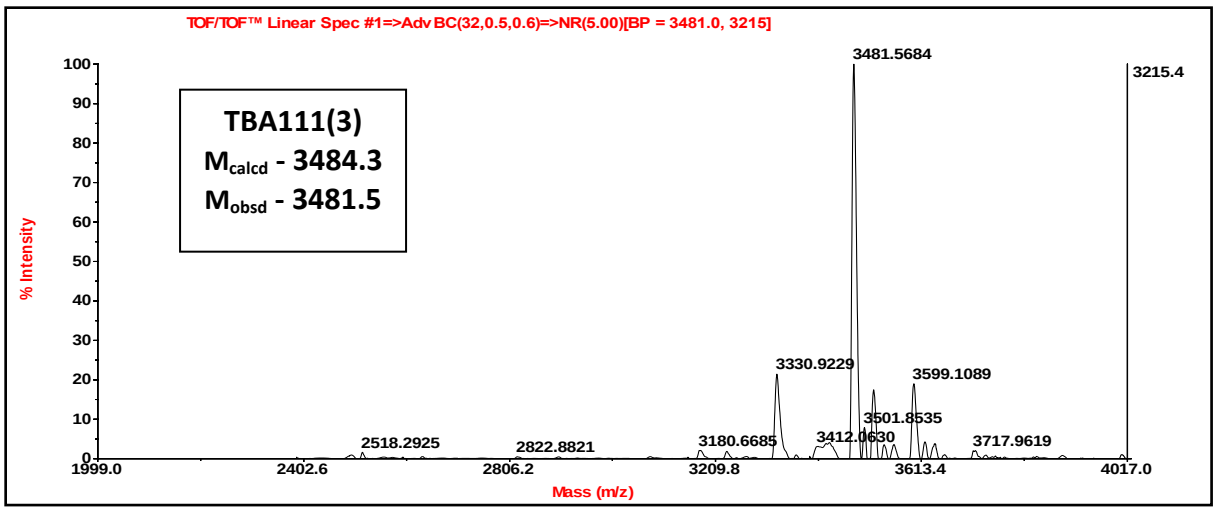
SI Figure S8: CD amplitude at 260 nm for TBA111(3) and at 295 nm for all other sequences versus temperature during heating of 5 µM sequences annealed in potassium phosphate buffer (0.01M, pH 7.2) containing 100 mM KCl after addition of thrombin **a) TBA232(7), b) TBA222(6), c) TBA131(5), d) TBA111(3) e) isoTBA232(7), f) isoTBA222(6), g) isoTBA131(5), h) isoTBA111(3).**

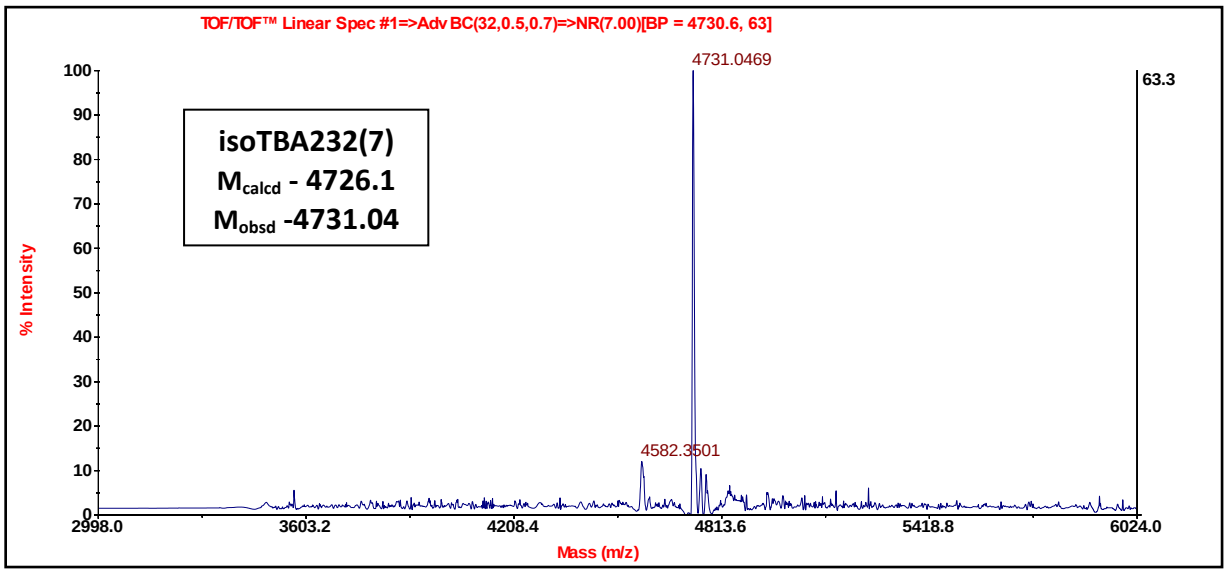
9. MALDI-TOF spectra of TBA and isoTBA sequences

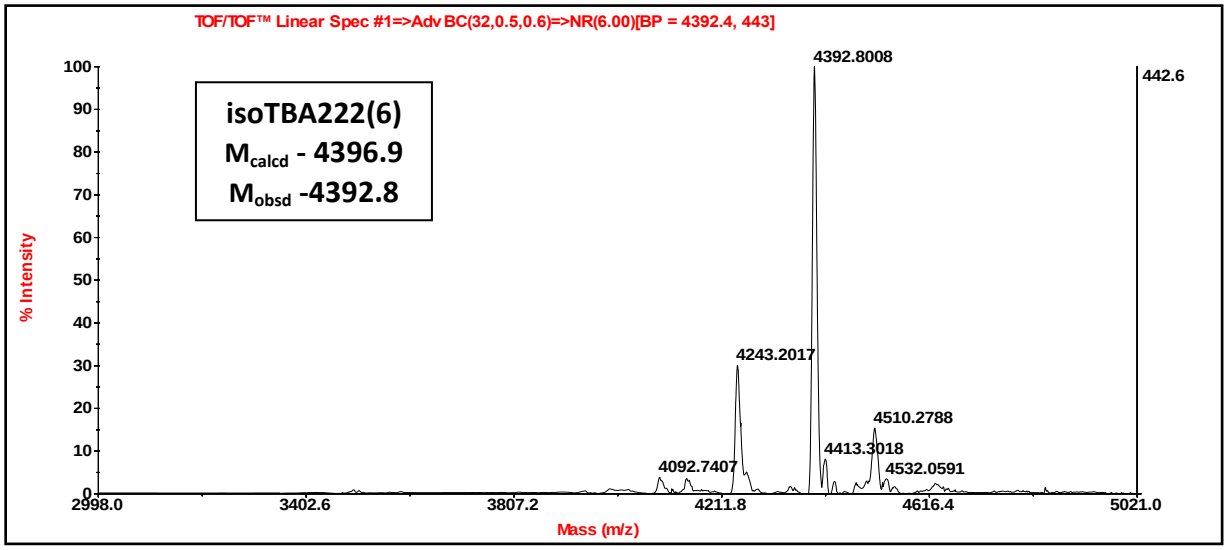


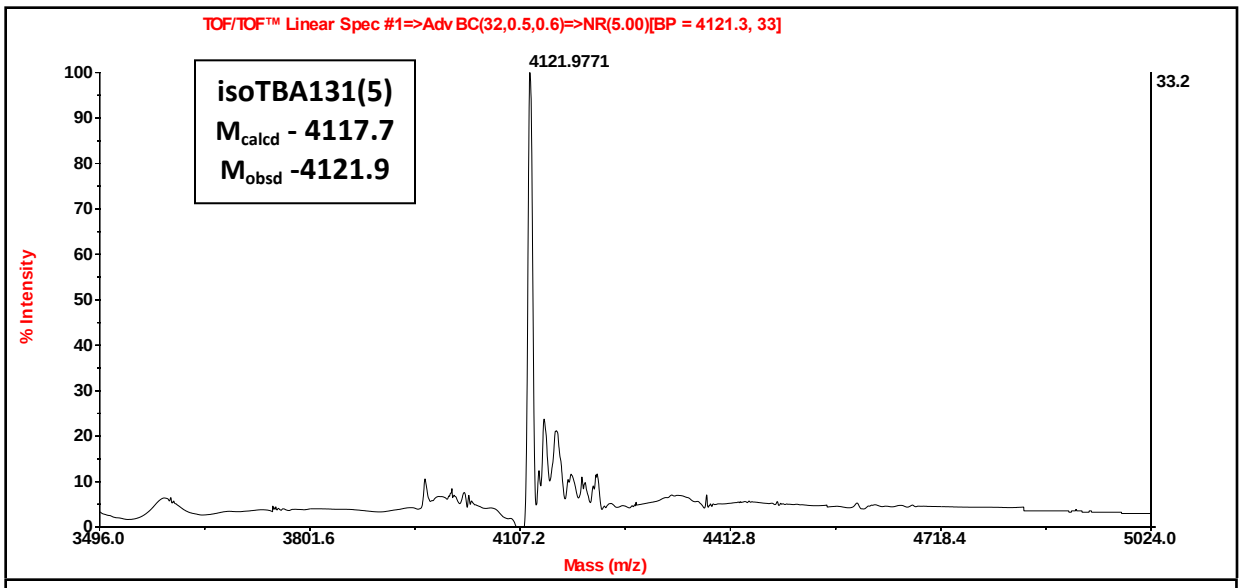


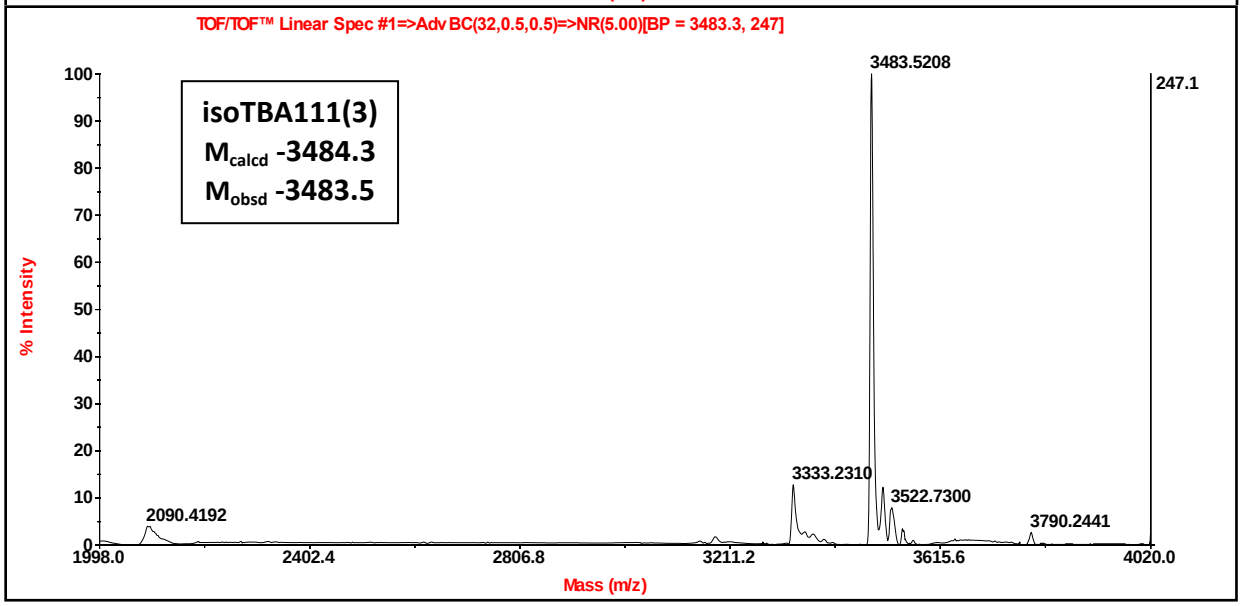


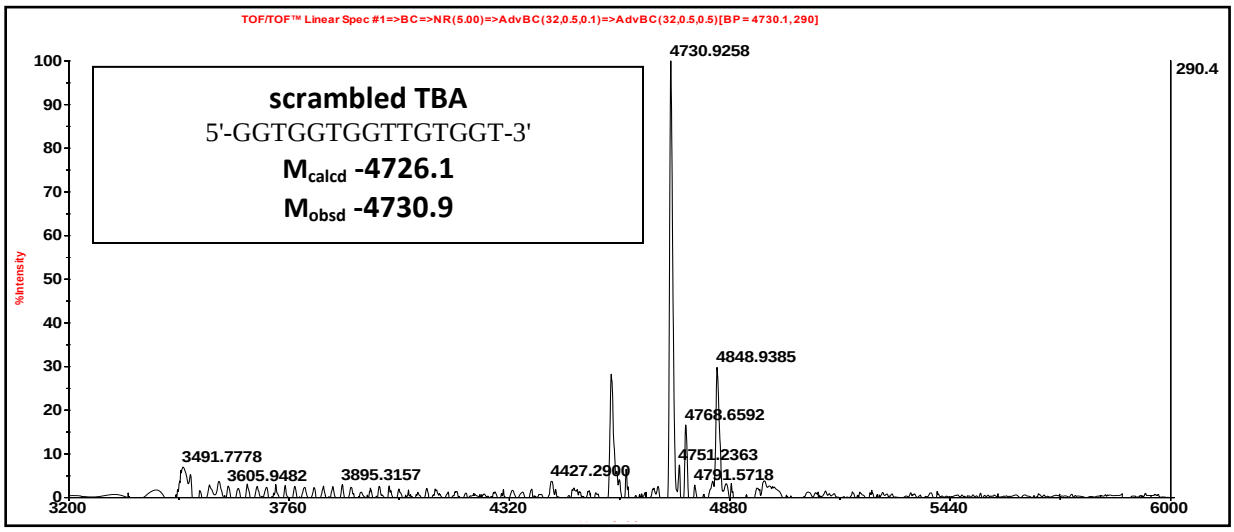

TOF/TOF™ Linear Spec #1=>Adv BC(32,0.5,0.6)=>NR(5.00)[BP = 3481.0, 3215]
TBA111(3)
Mcalcd - 3484.3
Mobsd - 3481.5
% Intensity
Mass (m/z)
3481.5684
3215.4
3330.9229
3599.1089
3501.8535
3412.0630
3180.6685
3717.9619
2518.2925
2822.8821
1999.0
2402.6
2806.2
3209.8
3613.4
4017.0


TOF/TOF™ Linear Spec #1=>Adv BC(32,0.5,0.7)=>NR(7.00)[BP = 4730.6, 63]
isoTBA232(7)
Mcalcd - 4726.1
Mobsd -4731.04
% Intensity
Mass (m/z)
4731.0469
63.3
4582.3501
2998.0
3603.2
4208.4
4813.6
5418.8
6024.0


TOF/TOF™ Linear Spec #1=>Adv BC(32,0.5,0.6)=>NR(6.00)[BP = 4392.4, 443]
isoTBA222(6)
Mcalcd - 4396.9
Mobsd -4392.8
% Intensity
Mass (m/z)
4392.8008
442.6
4243.2017
4510.2788
4413.3018
4092.7407
4532.0591
2998.0
3402.6
3807.2
4211.8
4616.4
5021.0


TOF/TOF™ Linear Spec #1=>Adv BC(32,0.5,0.6)=>NR(5.00)[BP = 4121.3, 33]
isoTBA131(5)
Mcalcd - 4117.7
Mobsd -4121.9
4121.9771
33.2
% Intensity
Mass (m/z)
3496.0
3801.6
4107.2
4412.8
4718.4
5024.0


TOF/TOF™ Linear Spec #1=>Adv BC(32,0.5,0.5)=>NR(5.00)[BP = 3483.3, 247]
isoTBA111(3)
Mcalcd -3484.3
Mobsd -3483.5
3483.5208
247.1
3333.2310
3522.7300
3790.2441
2090.4192
% Intensity
Mass (m/z)
1998.0
2402.4
2806.8
3211.2
3615.6
4020.0


TOF/TOF™ Linear Spec #1=>BC=>NR(5.00)=>AdvBC(32,0.5,0.1)=>AdvBC(32,0.5,0.5)[BP = 4730.1, 290]
scrambled TBA
5'-GGTGGTGGTTGTGGT-3'
Mcalcd -4726.1
Mobsd -4730.9
4730.9258
290.4
4848.9385
4768.6592
4751.2363
4791.5718
3491.7778
3605.9482
3895.3157
4427.2900
%Intensity
3200
3760
4320
4880
5440
6000