Electronic Supplementary Information

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

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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
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):

![Graphs showing CD signal changes](image)

**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:

![Graphs showing CD signal changes](image)

**SI Figure S7:** Changes in CD signal upon addition of thrombin to TBA or isoTBA sequences annealed in potassium phosphate buffer (0.01 M, 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)

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<th>Sequence code</th>
<th>$T_m$ at 5 µM strand concentration in buffer containing 100 mM KCl and thrombin</th>
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<tr>
<td></td>
<td>heat</td>
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<tr>
<td>TBA232 (7)</td>
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<tr>
<td>TBA111(3)</td>
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<tr>
<td>isoTBA232(7)</td>
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<tr>
<td>isoTBA222(6)</td>
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<td>isoTBA131(5)</td>
<td>34</td>
</tr>
<tr>
<td>isoTBA111(3)</td>
<td>&lt;10</td>
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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

**TBA232(7)**
- $M_{\text{calcd}} = 4726.1$
- $M_{\text{obsd}} = 4727.04$

**TBA222(6)**
- $M_{\text{calcd}} = 4396.9$
- $M_{\text{obsd}} = 4396.95$

**TBA131(5)**
- $M_{\text{calcd}} = 4117.7$
- $M_{\text{obsd}} = 4116.6$
TBA111(3)
$M_{\text{calc}} = 3484.3$
$M_{\text{obsd}} = 3481.5$

isoTBA232(7)
$M_{\text{calc}} = 4726.1$
$M_{\text{obsd}} = 4731.04$

isoTBA222(6)
$M_{\text{calc}} = 4396.9$
$M_{\text{obsd}} = 4392.8$
**isoTBA131(5)**

M\text{calc}d - 4117.7  
M\text{obsd} - 4121.9

**isoTBA111(3)**

M\text{calc}d - 3484.3  
M\text{obsd} - 3483.5

**scrambled TBA**

5'-GGTGTTGGTTGTTGGT-3'  
M\text{calc}d - 4726.1  
M\text{obsd} - 4730.9