Supplementary Information

Intrastrand backbone-nucleobase interactions stabilize unwound right-handed helical structures of heteroduplexes of L-αTNA/RNA and SNA/RNA

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Supplementary Figures 1 – 8 ••••••••• S2-S7
Supplementary Tables 1 – 5 ••••••••• S8-S13
Supplementary Figure 1. Melting curves and CD spectra of L-αTNA/RNA, and SNA/RNA, and RNA/RNA duplexes.

Absorbance versus temperature curves for L8a/R8Br (a) and S8a/R8Br (b). Solution conditions were 10 mM HEPES (pH 7.4), 100 mM CaCl2, and 2.5 μM oligonucleotide. (c) CD spectra of L8a/R8Br, S8a/R8Br, and R8a/R8b recorded at 5 ºC. Solution conditions for CD measurements were 10 mM HEPES (pH 7.4), 100 mM CaCl2, 4.0 μM oligonucleotide.

Supplementary Figure 2. Electron densities of 3’ terminal C8 of RNA strands were not observed in L-αTNA/RNA

In the stick representations, carbon atoms are coloured magenta and cyan. N, O, and P atoms are coloured as in Fig. 1. Contours represent the 2Fo-Fc electron density map at the 1.0 σ level.
Supplementary Figure 3. SNA-RNA complexes observed in the asymmetric units in the crystal structure of S8a/R8Br.

In the stick representations, carbon atoms in RNAs (R8Br-3 and R8Br-4), RNAs (R8Br-5 and R8Br-6), SNAs (S8a-1 and S8a-2), and SNAs (S8a-3 and S8a-4) are coloured in magenta, cyan, green, and yellow, respectively. N, O, and P atoms are coloured as in Fig. 1. The base-pairing patterns in the asymmetric units are shown below the structures. Watson-Crick and Hoogsteen base pairs are indicated as black circles and dashed lines, respectively.
Supplementary Figure 4. Electron densities of 3’ terminal regions of the RNA strands in SNA/RNA.

In the stick renderings, atoms are coloured magenta, green, cyan, and yellow for carbon. N, O, and P atoms are coloured as in Fig. 1. Contours represent the $2F_o-F_c$ electron density map at the 1.0 σ level.

Supplementary Figure 5. Close up views of superposition of duplex structures of L-αTNA/RNA (cyan) and PNA/RNA (purple).

In the stick renderings, atoms are coloured cyan and purple for carbon N, O, and P atoms are coloured as in Fig. 1.
Supplementary Figure 6. nanoESI-MS analysis of mixture of R8a and R7b.

Mass spectrum of solution of R8a and R7b collected under non-denaturing conditions in negative ionization mode. Calculated masses (singly charged states) are as follows: R8a (red), 2650.7; R7b (purple), 2195.3; and R8a/R7b duplex (green), 4741.7.
Supplementary Figure 7. Melting curves and CD spectra of homo duplexes of L-aTNA, SNA, and RNA.

Absorbance versus temperature curves for L-T8a/L-T7b (a), S8a/S7b (b), and R8a/R7b (c). Solution conditions were 10 mM HEPES (pH 7.4), 100 mM CaCl₂, and 2.5 μM oligonucleotide. (d) CD spectra of solution of L-T8a/L-T7b and S8a/S7b recorded at 5 °C. Solution conditions were 10 mM HEPES (pH 7.4), 100 mM CaCl₂, and 4.0 μM oligonucleotide.
Supplementary Figure 8. Steric crash between methyl group of D-aTNA and oxygens of neighbouring phosphate group in right-handed helix structure model.

D-aTNA structure model was produced by attachment of methyl group at C1’ position of SNA crystal structure. Methyl group is located in the minor groove. D-aTNA backbone structure is shown as stick and sphere representation. Protons are omitted. C is coloured in yellow. N, O, and P atoms are coloured as in Fig. 1.
| L-\(\alpha\)TNA/RNA\[^b\] | X-displacement [Å] | Inclination [deg] | Helical rise [Å] | Helical twist [deg]\[^c\] | Tilt [deg] \[^c\] | Slide [Å] | Roll [deg] \[^c\] |
|---------------------|------------------|------------------|------------------|-------------------|----------------|----------|----------------|
| GC/gc               | -4.8             | 7.6              | 3.0              | 31.7              | 10.7           | -2.2     | 4.1            |
| CU/ag               | -7.3             | 15.7             | 2.5              | 23.7              | -0.2           | -2.2     | 6.4            |
| UG/ca               | -6.4             | 5.2              | 3.0              | 21.8              | 0.3            | -2.2     | 2.0            |
| GC/gc               | -8.2             | 0.2              | 3.1              | 18.7              | 2.3            | -2.7     | 0.1            |
| C\textsubscript{\text{Br}}U/ag | -5.8             | -5.4             | 3.8              | 20.9              | -3.3           | -2.5     | -1.9           |
| \textsubscript{\text{Br}}UG/ca | -3.5             | -7.3             | 3.3              | 26.1              | 1.8            | -2.0     | -3.3           |
| ca/\textsubscript{\text{Br}}UG | -3.5             | -4.1             | 3.0              | 24.9              | -3.1           | -1.7     | -1.8           |
| ag/C\textsubscript{\text{Br}}U | -5.6             | -2.2             | 3.6              | 22.3              | 1.4            | -2.3     | -1.9           |
| gc/GC               | -10.8            | 9.6              | 2.5              | 16.3              | -0.3           | -2.6     | 2.7            |
| ca/UG               | -5.8             | -0.6             | 3.1              | 23.4              | -4.3           | -2.4     | -0.3           |
| ag/CU               | -9.1             | -7.2             | 3.7              | 16.0              | 0.3            | -2.9     | -2.0           |
| gc/GC               | -6.3             | 7.3              | 2.9              | 27.5              | -2.5           | -2.6     | 3.5            |

[^a]: The duplex regions are highlighted in light blue background. All data were calculated using 3DNA-Web.  
[^b]: Capitals indicate RNA and small letters indicate L-\(\alpha\)TNA.  
[^c]: Local base-pair step parameters.
**Supplementary Table 2. Helical parameters of SNA/RNA duplex.**

| SNA/RNA | X-displacement [Å] | Inclination [deg] | Helical rise [Å] | Helical twist [deg] | Tilt [deg] | Slide [Å] | Roll [deg] |
|---------|---------------------|-------------------|------------------|---------------------|-----------|----------|-----------|
| SR-1    |                     |                   |                  |                     |           |          |           |
| GC/gc   | -2.9                | -6.1              | 3.4              | 30.6                | -2.1      | -1.9     | -3.2      |
| CU/ag   | -5.6                | -2.8              | 3.1              | 20.2                | -5.4      | -2.1     | -1.0      |
| UG/ca   | -10.8               | 29.9              | 2.1              | 18.2                | -0.5      | -1.9     | 9.0       |
| GC/gc   | -6.8                | 1.5               | 3.2              | 22.3                | -0.9      | -2.6     | 0.6       |
| C,brU/ag| -5.8                | 1.1               | 3.5              | 22.0                | 1.0       | -2.2     | 0.4       |
| brUG/ca | -3.5                | -3.8              | 3.0              | 24.3                | -3.3      | -1.7     | -1.6      |
| ca/brUG | -3.3                | -2.2              | 3.0              | 28.8                | 2.9       | -1.8     | -1.1      |
| ag/C,brU| -6.2                | -7.3              | 3.8              | 17.9                | -1.0      | -2.4     | -2.3      |
| gc/GC   | -6.5                | 3.6               | 3.1              | 23.2                | 3.7       | -2.4     | 1.4       |
| ca/CG   | -8.9                | -17.5             | 3.6              | 11.6                | 3.5       | -2.9     | -3.4      |
| ag/CC   | -5.2                | 17.1              | 2.8              | 32.0                | 1.0       | -1.9     | 9.3       |
| gc/GC   | -2.5                | -8.1              | 3.4              | 33.7                | 1.7       | -1.9     | -4.7      |
| SR-2    |                     |                   |                  |                     |           |          |           |
| GC/gc   | -5.2                | 2.0               | 3.4              | 30.5                | 4.8       | -2.6     | 1.0       |
| CU/ag   | -7.3                | 6.6               | 2.8              | 21.4                | -1.5      | -2.3     | 2.5       |
| UG/ca   | -4.6                | -5.5              | 3.6              | 20.9                | 3.7       | -2.0     | -2.0      |
| GC/gc   | -7.5                | 9.8               | 3.1              | 22.0                | 3.3       | -2.4     | 3.7       |
| C,brU/ag| -7.8                | 5.1               | 3.8              | 20.0                | -3.1      | -2.4     | 1.8       |
| brUG/ca | -3.8                | -5.9              | 3.0              | 25.5                | 2.8       | -2.0     | -2.6      |
| ca/brUG | -3.6                | -5.4              | 3.3              | 25.7                | -4.3      | -1.9     | -2.4      |
| ag/C,brU| -4.7                | -6.5              | 3.5              | 23.3                | 2.2       | -2.3     | -2.6      |
| gc/GC   | -8.4                | 5.1               | 3.1              | 20.2                | -2.5      | -2.7     | 1.8       |
| ca/UG   | -5.8                | 2.2               | 2.1              | 20.9                | -4.1      | -2.0     | 0.8       |
| ag/CU   | -7.8                | 18.8              | 3.1              | 22.5                | 1.9       | -2.1     | 7.2       |
| gc/GC   | -2.0                | -2.5              | 3.4              | 44.1                | -2.4      | -1.6     | -1.9      |

[a] The duplex regions are highlighted in light blue background. All data were calculated using 3DNA-Web. [b] Capital letters indicate RNA and small letters indicate SNA. [c] Local base pair step parameters.
Supplementary Table 3. Torsion angles (deg.) in L-αTNA/RNA hetero duplexes.

|       | α     | β    | γ     | δ     | ε     | ζ     | χ     |
|-------|-------|------|-------|-------|-------|-------|-------|
| **R8Br-1**<sup>a</sup> |       |      |       |       |       |       |       |
| G1    | ---   | ---  | 15.0  | 92.2  | -153.9 | -62.2 | -174.9 |
| C2    | -122.4 | -168.1 | 91.1  | 72.4  | -141.1 | -81.8 | -168.8 |
| U3    | -45.9 | 167.8 | 48.3  | 76.4  | -161.4 | -71.1 | -162.9 |
| G4    | -73.4 | 178.8 | 59.7  | 75.7  | -160.8 | -62.6 | -165.4 |
| C5    | -103.4 | 165.2 | 103.1 | 69.8  | -148.4 | -85.1 | -177.3 |
| BrU6  | -49.2 | 159.0 | 58.1  | 79.1  | -167.3 | -77.7 | -172.8 |
| G7    | -77.4 | -164.1 | 58.4  | 90.3  | ---   | ---   | -149.7 |
| **R8Br-2**<sup>a</sup> |       |      |       |       |       |       |       |
| G1    | ---   | ---  | -81.0 | 93.4  | -142.9 | -67.1 | -178.9 |
| C2    | -79.5 | -177.2 | 60.6  | 74.6  | -156.4 | -76.5 | -174.2 |
| U3    | -66.7 | 172.8 | 75.4  | 78.2  | -157.5 | -78.6 | -174.2 |
| G4    | -73.2 | 179.5 | 61.3  | 72.1  | -151.8 | -75.5 | -167.1 |
| C5    | -70.5 | 161.7 | 79.9  | 75.5  | -155.9 | -79.8 | -173.9 |
| BrU6  | -69.3 | 173.2 | 65.4  | 81.1  | -147.7 | -89.5 | -166.5 |
| G7    | -66.7 | 169.7 | 56.0  | 77.3  | ---   | ---   | -154.8 |
| **LT8a**<sup>b</sup> |       |      |       |       |       |       |       |
| G1    | ---   | ---  | 117.6 | -62.5 | -105.1 | 50.8  |       |
| C2    | 90.5  | 177.3 | -62.7 | -68.2 | -149.7 | 66.8  |       |
| A3    | 85.9  | 179.7 | -57.2 | -71.6 | -135.2 | 68.3  |       |
| G4    | 86.0  | 172.6 | -54.8 | -59.1 | -135.6 | 48.9  |       |
| C5    | 92.5  | 170.0 | -53.3 | -63.3 | -134.0 | 46.8  |       |
| A6    | 89.7  | 173.6 | -53.7 | -68.7 | -142.1 | 68.8  |       |
| G7    | 78.0  | 174.8 | -48.8 | -76.5 | -142.5 | 81.5  |       |
| C8    | 86.7  | 170.3 | -68.5 | 137.3 | ---   | ---   |       |
| **LT8a-2**<sup>b</sup> |       |      |       |       |       |       |       |
| G1    | ---   | ---  | 106.8 | -57.2 | -167.6 | 53.9  |       |
| C2    | 88.0  | 172.7 | -64.5 | -62.6 | -150.8 | 63.5  |       |
| A3    | 76.7  | -179.0 | -57.8 | -63.9 | -128.6 | 45.4  |       |
| G4    | 92.8  | 171.9 | -56.9 | -61.2 | -126.8 | 53.5  |       |
| C5    | 103.9 | 158.0 | -56.8 | -59.9 | -140.1 | 48.9  |       |
| A6    | 79.9  | 179.0 | -51.7 | -60.3 | -132.1 | 54.9  |       |
| G7    | 88.9  | 165.2 | -52.3 | -62.3 | -140.9 | 62.3  |       |
| C8    | 93.1  | 170.3 | -71.8 | 128.0 | ---   | ---   |       |

<sup>a</sup>All values were calculated using 3DNA-Web. <sup>b</sup>All values were measured using pymol.
Supplementary Table 4. Torsion angles (deg.) of SNA/RNA hetero duplexes.

|        | α    | β    | γ    | δ   | ε    | ζ    | χ    |
|--------|------|------|------|-----|------|------|------|
| R8Br-3 | G1   | ---  | ---  | 71.6| 81.1 | -140.2| -78.6| 176.0|
|        | C2   | -83.6| 173.5| 70.4| 80.8 | -144.6| -80.2| -168.4|
|        | U3   | -60.2| 166.0| 58.2| 70.8 | -127.7| -87.9| -156.5|
|        | G4   | -85.5| 141.7| 92.7| 70.3 | -150.6| -79.5| -176.2|
|        | C5   | -56.0| 165.2| 64.1| 75.0 | -156.3| -74.7| -171.1|
|        | BrU6 | -74.5| 169.9| 73.9| 83.0 | -158.5| -87.4| -164.7|
|        | G7   | -63.9| 174.3| 72.4| 141.7| -94.4 | -69.0| -136.1|
| R8Br-4 | G1   | ---  | ---  | -79.7| 109.8| -133.9| 8.7  | 178.5|
|        | C2   | -135.1| 73.5| 168.7| 96.2 | -105.6| -91.2| 175.3|
|        | U3   | -59.3| 165.7| 31.2| 75.6 | -150.5| -41.4| -160.2|
|        | G4   | -128.3| 110.7| 157.7| 82.1 | -139.4| -79.3| -177.5|
|        | C5   | -54.5| 172.5| 51.5| 74.8 | -177.5| -84.9| -165.3|
|        | BrU6 | 162.1| -151.4| 175.0| 93.0 | -121.9| -86.6| 176.2|
|        | G7   | -74.5| 167.0| 56.8| 78.0 | -154.2| -123.2| -162.0|
| R8Br-5 | G1   | ---  | ---  | 8.9 | 83.4 | 148.3 | 39.2 | -176.7|
|        | C2   | 147.1| 132.0| 151.7| 87.4 | -143.1| -75.6| -173.4|
|        | U3   | -67.7| 179.2| 56.4| 80.1 | -160.1| -78.9| -167.2|
|        | G4   | -72.9| 173.9| 62.8| 76.9 | -157.3| -70.7| -163.8|
|        | C5   | -83.0| 169.6| 82.6| 74.4 | -152.7| -84.1| -171.5|
|        | BrU6 | -64.8| 166.0| 67.5| 79.0 | -153.1| -83.5| -172.4|
|        | G7   | -60.7| 177.3| 50.0| 79.7 | -152.5| -117.1| -159.2|
| R8Br-6 | G1   | ---  | ---  | -93.1| 99.3 | -133.8| -74  | -179.5|
|        | C2   | -58.5| 171.5| 45.8| 79.1 | -138.8| -85.1| -167.2|
|        | U3   | -50.3| 168.5| 45.3| 74.1 | -155.8| -74.6| -165.1|
|        | G4   | -61.7| 167.9| 62.4| 74.4 | -166.7| -70.4| -162.9|
|        | C5   | 144.7| -171.1| -170| 83.2 | -151.6| -70.5| -176.9|
|        | BrU6 | -78.2| 174.7| 69.5| 82.7 | -155.6| -85.6| -170.9|
|        | G7   | -67.0| 177.9| 54.2| 81.2 | ---  | ---  | -153.8|

|        | α    | β    | γ    | δ   | ε    | ζ    |      |
|--------|------|------|------|-----|------|------|------|
| S8a-1  | G1   | ---  | ---  | -55.2| -58.7| -161.3| 67.2 |
|        | C2   | 88.2 | -168.2| -68.2| -50.3| -147.6| 64.3 |
|        | A3   | 75.4 | -168.2| -65.4| -53.9| -158.7| 60.9 |
|        | G4   | 70.6 | -164.8| -50.2| -70.3| -137.7| 76.7 |
|        | C5   | 74.1 | -177.0| -54.0| -55.1| -157.0| 45.8 |
|        | A6   | 132.7| 173.1| -64.5| -58.3| -143.9| 65.4 |
|        | G7   | 86.4 | -173.0| -56.4| -52.1| -156.7| 68.1 |
|       | C8    | -163.3 | -73.8 | 35.8 | --- | --- |
|-------|-------|--------|-------|------|-----|-----|
| S8a-2 |       |        |       |      |     |     |
| G1    | 83.9  | 174.9  | -65.8 | -53.3| -178.7| 59.2 |
| C2    | 70.7  | -175.7 | -57.5 | -61.9| -151.1| 61.7 |
| A3    | 68.5  | -176.6 | -52.8 | -65.7| -157.2| 62.5 |
| G4    | 79.8  | -173.8 | -57.9 | -56.3| -163.9| 74.7 |
| C5    | 54.9  | -161.2 | -51.5 | -58.2| -140.1| 79.2 |
| A6    | 80.9  | 178.1  | -60.1 | -64.5| -160.2| 73.8 |
| G7    | 90.4  | -82.4  | 158.8 | -160.1| 73.8 |
| C8    |       |        |       |      |     |     |
| S8a-3 |       |        |       |      |     |     |
| G1    | 70.6  | -160.6 | -61.8 | -70.8| -169.9| 73.3 |
| C2    | 68.6  | -162.9 | -58.1 | -61.5| -139.1| 73.1 |
| A3    | 68.4  | -175.3 | -55.8 | -62.5| -161.1| 68.0 |
| G4    | 72.7  | -173.3 | -58.3 | -56.6| -144.8| 63.2 |
| C5    | 89.4  | 176.4  | -65.4 | -65.5| -157.1| 78.3 |
| A6    | 67.2  | -167.0 | -49.2 | -68.1| -129.8| 69.8 |
| G7    | -154.2| -177.9 | -179.7| -67.6| 73.8 |
| C8    |       |        |       |      |     |     |
| S8a-4 |       |        |       |      |     |     |
| G1    | 89.6  | -168.5 | -58.5 | -54.9| -155.2| 70.8 |
| C2    | 67.2  | -162.6 | -59.5 | -55.8| -155.6| 60.7 |
| A3    | 81.6  | -170.8 | -64.1 | -57.8| -140.4| 73.8 |
| G4    | 77.9  | 173.3  | -58.2 | -63.2| -155.4| 81.7 |
| C5    | 58.7  | -164.7 | -51.5 | -61.4| -138.1| 92.2 |
| A6    | 90.0  | -175.1 | -60.8 | -54.9| -161.4| 57.8 |
| G7    | 89.7  | 178.8  | -52.7 | -73.9| 57.8 |
| C8    |       |        |       |      |     |     |

[a] All values were calculated using 3DNA-Web. [b] All values were measured using pymol.
Supplementary Table 5. C–O distances (Å) between backbone and neighboring nucleobase in L-aTNA and SNA of L-aTNA/RNA and SNA/RNA[^a]

| L-aTNA in L-aTNA/RNA | SNA in SNA/RNA |
|-----------------------|----------------|
| O5'-C8/C6 | O5'-C6' | O2-C6' | O5'-C8/C6 | O5'-C6' | O2-C6' |
| **tT8a-1** | | |
| G1-C2 | 3.4 | 3.5 | - | G1-C2 | 3.4 | 3.5 | - |
| C2-A3 | 3.3 | 3.5 | 3.3 | C2-A3 | 3.1 | 3.3 | 3.2 |
| A3-G4 | 3.5 | 3.3 | - | A3-G4 | 3.2 | 3.5 | - |
| G4-C5 | 3.6 | 3.5 | - | G4-C5 | 3.7 | 3.6 | - |
| C5-A6 | 3.2 | 3.5 | 3.3 | C5-A6 | 3.3 | 3.5 | 3.1 |
| A6-G7 | 3.4 | 3.6 | - | A6-G7 | 3.3 | 3.4 | - |
| G7-C8 | 3.0 | 3.7 | - | G7-C8 | 3.2 | 3.6 | - |
| **tT8a-2** | | |
| G1-C2 | 3.6 | 3.5 | - | G1-C2 | 3.5 | 3.6 | - |
| C2-A3 | 3.2 | 3.4 | 3.1 | C2-A3 | 3.2 | 3.4 | 3.3 |
| A3-G4 | 3.5 | 3.4 | - | A3-G4 | 3.5 | 3.6 | - |
| G4-C5 | 4.0 | 3.4 | - | G4-C5 | 3.3 | 3.4 | - |
| C5-A6 | 3.2 | 3.2 | 3.4 | C5-A6 | 3.3 | 3.6 | 3.3 |
| A6-G7 | 3.8 | 3.4 | - | A6-G7 | 3.2 | 3.6 | - |
| G7-C8 | 3.2 | 4.0 | - | G7-C8 | 3.0 | 4.8 | - |
| **S8a-3** | | |
| G1-C2 | 3.6 | 3.5 | - |
| C2-A3 | 3.2 | 3.5 | 3.2 |
| A3-G4 | 3.3 | 3.5 | - |
| G4-C5 | 3.5 | 3.4 | - |
| C5-A6 | 3.2 | 3.5 | 3.3 |
| A6-G7 | 3.4 | 3.2 | - |
| G7-C8 | 3.2 | 5.0 | - |
| **S8a-4** | | |
| G1-C2 | 4.0 | 3.9 | - |
| C2-A3 | 3.1 | 3.3 | 3.2 |
| A3-G4 | 3.6 | 3.2 | - |
| G4-C5 | 3.4 | 3.5 | - |
| C5-A6 | 3.1 | 3.4 | 3.4 |
| A6-G7 | 3.4 | 3.4 | - |
| G7-C8 | 3.5 | 3.6 | - |

[^a] All values were measured using pymol.