

## Supplementary Material

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**Table-S1:** Occurrence of different types of tandem non-canonical motifs within the crystal structure database. Motifs containing protonated basepairs or modified bases are not reported.

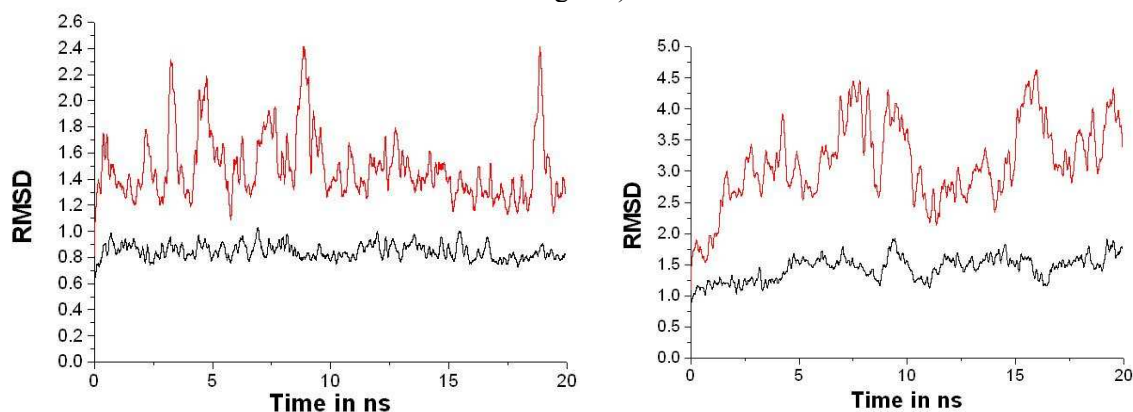
| Motif                                        | RNA Type   | Organism                                                                                                                  |
|----------------------------------------------|------------|---------------------------------------------------------------------------------------------------------------------------|
| G:A S:HT<br>A:G H:ST                         | 23S rRNA   | <i>Haloarcula marismortui</i><br><i>Thermus thermophilus</i><br><i>Escherichia coli</i><br><i>Deinococcus radiodurans</i> |
|                                              | 16S rRNA   | <i>Escherichia coli</i><br><i>Thermus thermophilus</i>                                                                    |
|                                              | Riboswitch | Synthetic                                                                                                                 |
| A:A s:hT<br>A:U H:WT<br>A:G H:ST             | 16S rRNA   | <i>Escherichia coli</i><br><i>Thermus thermophilus</i>                                                                    |
| G:A S:HT<br>A:G H:ST<br>A:G H:ST             | 23S rRNA   | <i>Haloarcula marismortui</i><br><i>Escherichia coli</i><br><i>Thermus thermophilus</i>                                   |
| U:G S:WC<br>U:U W:WC                         | 23S rRNA   | <i>Haloarcula marismortui</i>                                                                                             |
| G:A S:HT<br>A:U H:WT<br>A:G H:ST             | 23S rRNA   | <i>Thermus thermophilus</i>                                                                                               |
| A:G W:WC<br>A:G W:WC                         | 23S rRNA   | <i>Thermus thermophilus</i><br><i>Escherichia coli</i>                                                                    |
| G:A S:HT<br>G:A S:HT<br>A:G H:ST<br>U:U W:WC | 16S rRNA   | <i>Thermus thermophilus</i><br><i>Escherichia coli</i>                                                                    |
| G:A S:HT<br>G:A S:HT<br>A:G H:ST             | 23S rRNA   | <i>Thermus thermophilus</i>                                                                                               |
| U:U W:WC                                     | 23S rRNA   | <i>Haloarcula marismortui</i>                                                                                             |
| U:U W:WC                                     | IRES RNA   | <i>Cricket paralysis virus</i>                                                                                            |
| G:A S:HT<br>A:G H:ST<br>G:G H:zT             | 23S rRNA   | <i>Escherichia coli</i>                                                                                                   |
| G:G z:HT<br>U:A W:HT<br>A:G H:ST             | 16S rRNA   | <i>Thermus thermophilus</i> (in very few structures)                                                                      |
| U:C W:WC<br>U:U W:WC                         | 23S rRNA   | <i>Deinococcus radiodurans</i> (in very few structures)                                                                   |

**Table-S2: RMSD-Values**

| Helix | Total RMSD | RMSD of Central Region |
|-------|------------|------------------------|
| 1N32  | 1.49(0.28) | 0.85(0.09)             |
| 2AW4  | 3.14(0.68) | 1.45(0.22)             |
| RNA11 | 2.77(0.59) | 1.33(0.31)             |

**Figure – S1: RMSD Plots for 1N32 (left) and 2AW4 (right)**

(Red lines show the deviation for the complete helices whereas black lines are for the central regions)

**Table-S3: Intra-basepair parameters for the helix RNA11**

(Standard deviation values are given within parentheses; average values have been calculated leaving the first 2ns runtime to exclude the probable equilibration period. It was seen that equilibration were attained at the early stages of simulation.)

| Basepair     | Buckle      | Opening     | Propeller   | Stagger   | Shear     | Stretch   |
|--------------|-------------|-------------|-------------|-----------|-----------|-----------|
| <b>RNA11</b> |             |             |             |           |           |           |
| 1G:22C W:WC  | -45.1(94.7) | 2.7(34.7)   | 11.5(46.6)  | 3.6(6.2)  | -3.5(3.6) | 0.1(4.2)  |
| 2A:21U W:WC  | 19.9(48.7)  | -16.5(36.0) | -25.6(28.5) | -1.7(3.1) | 1.2(3.0)  | 1.6(2.2)  |
| 3A:20U W:WC  | -1.9(13.9)  | 16.1(21.9)  | -12.2(11.0) | 0.2(0.9)  | -3.9(3.2) | 2.2(1.2)  |
| 4C:19G W:WC  | -1.3(13.0)  | 49.0(25.7)  | -8.5(9.7)   | 0.1(0.8)  | 1.3(1.0)  | 4.4(1.2)  |
| 5G:18C W:WC  | -16.1(10.8) | 1.8(4.4)    | -12.8(7.5)  | -0.4(0.4) | -0.3(0.4) | 2.9(0.1)  |
| 6C:17G W:WC  | -2.5(10.1)  | 1.4(5.0)    | -12.6(7.3)  | -0.2(0.4) | 0.4(0.4)  | 2.9(0.1)  |
| 7U:16A W:WC  | 1.1(9.1)    | 2.0(5.9)    | -13.1(8.2)  | -0.2(0.4) | 0.0(0.3)  | 2.9(0.1)  |
| 8G:15C W:WC  | -4.5(9.4)   | 0.6(4.1)    | -10.7(7.5)  | -0.2(0.4) | -0.2(0.4) | 2.9(0.1)  |
| 9G:14C W:WC  | 2.9(13.0)   | -0.0(4.1)   | -6.7(8.3)   | 0.0(0.4)  | -0.2(0.4) | 2.9(0.1)  |
| 10A:13U W:WC | -24.0(25.0) | -76.4(20.6) | -26.3(20.3) | -1.2(1.4) | 5.2(1.5)  | -0.7(1.2) |
| 11G:12C W:WC | -7.5(15.8)  | -32.4(96.2) | -0.4(12.7)  | 2.7(1.8)  | -0.1(1.4) | -0.6(2.4) |

**Table-S4: Inter-basepair step parameters for the helix RNA11**

(Standard deviation values are given within parentheses; average values have been calculated leaving the first 2ns runtime to exclude the probable equilibration period. It was seen that equilibration were attained at the early stages of simulation.)

| Base-doublet     | Tilt        | Roll       | Twist      | Shift     | Slide     | Rise     |
|------------------|-------------|------------|------------|-----------|-----------|----------|
| RNA11            |             |            |            |           |           |          |
| 1G:22C::2A:21U   | -13.2(32.5) | 24.0(41.2) | 14.3(51.6) | -0.6(2.2) | -0.9(2.4) | 1.0(5.2) |
| 2A:21U::3A:20U   | -4.5(8.3)   | 7.3(19.2)  | 42.9(16.8) | 0.3(1.3)  | -2.0(1.1) | 3.4(1.0) |
| 3A:20U::4C:19G   | -0.5(2.7)   | 13.4(7.4)  | 12.6(9.8)  | 0.4(0.9)  | -1.1(0.7) | 3.5(0.3) |
| 4C:19G::5G:18C   | 0.3(3.8)    | 12.9(6.4)  | 30.2(4.6)  | -1.0(0.5) | -2.8(0.6) | 3.5(0.3) |
| 5G:18C::6C:17G   | 0.1(2.4)    | 7.0(4.9)   | 28.0(3.1)  | -0.0(0.4) | -1.1(0.5) | 3.3(0.2) |
| 6C:17G::7U:16A   | 2.4(2.8)    | 10.6(5.4)  | 30.2(3.2)  | 0.3(0.4)  | -1.6(0.4) | 3.3(0.2) |
| 7U:16A::8G:15C   | -0.1(2.6)   | 14.4(6.0)  | 30.1(3.1)  | 0.0(0.4)  | -1.4(0.4) | 3.5(0.2) |
| 8G:15C::9G:14C   | -1.7(3.3)   | 9.3(6.0)   | 28.7(4.8)  | -0.2(0.7) | -1.9(0.3) | 3.3(0.2) |
| 9G:14C::10A:13U  | -10.1(30.0) | 11.8(21.3) | 2.9(34.6)  | -1.1(2.2) | -1.3(1.7) | 3.7(1.3) |
| 10A:13U::11G:12C | 14.4(47.2)  | -1.3(39.0) | 31.7(36.2) | 1.3(1.2)  | 1.4(2.3)  | 3.3(1.1) |

**Table –S5: Comparison of Intra-basepair Parameters of the non-canonical basepairs as Calculated by X3DNA and NUPARM**

|                     | <u>Buckle</u> | <u>Open</u> | <u>Propeller</u> | <u>Stagger</u> | <u>Shear</u> | <u>Stretch</u> |
|---------------------|---------------|-------------|------------------|----------------|--------------|----------------|
| <b>1N32 Crystal</b> |               |             |                  |                |              |                |
| <b>X3DNA</b>        |               |             |                  |                |              |                |
| 780A:802A           | 1.29          | -18.23      | -19.15           | 0.25           | 6.42         | -3.84          |
| 781A:801U           | 3.70          | -92.85      | -7.36            | -0.57          | -4.51        | -2.65          |
| 782A:800G           | 12.00         | -1.46       | -14.10           | 0.14           | -6.54        | -3.79          |
| <b>NUPARM</b>       |               |             |                  |                |              |                |
| 780A:802A           | -19.10        | 0.99        | 3.30             | -0.15          | 2.75         | 2.83           |
| 781A:801U           | -3.99         | 3.50        | -6.96            | -0.25          | -0.41        | 2.87           |
| 782A:800G           | -15.49        | 14.77       | -10.51           | 0.11           | 2.77         | 3.10           |
| <b>2AW4 Crystal</b> |               |             |                  |                |              |                |
| <b>X3DNA</b>        |               |             |                  |                |              |                |
| 537G:556A           | -1.93         | -2.01       | -1.65            | -0.03          | 6.67         | -4.12          |
| 538A:555G           | -2.48         | -7.91       | -0.73            | 0.06           | -6.84        | 3.82           |
| <b>NUPARM</b>       |               |             |                  |                |              |                |
| 537G:556A           | -1.83         | -13.90      | -1.59            | 0.01           | 2.50         | 3.19           |
| 538A:555G           | -0.36         | 7.11        | 2.63             | 0.03           | 2.88         | 3.21           |