

Supplementary Material

Mechanism of RNA Double Helix-Propagation at Atomic Resolution

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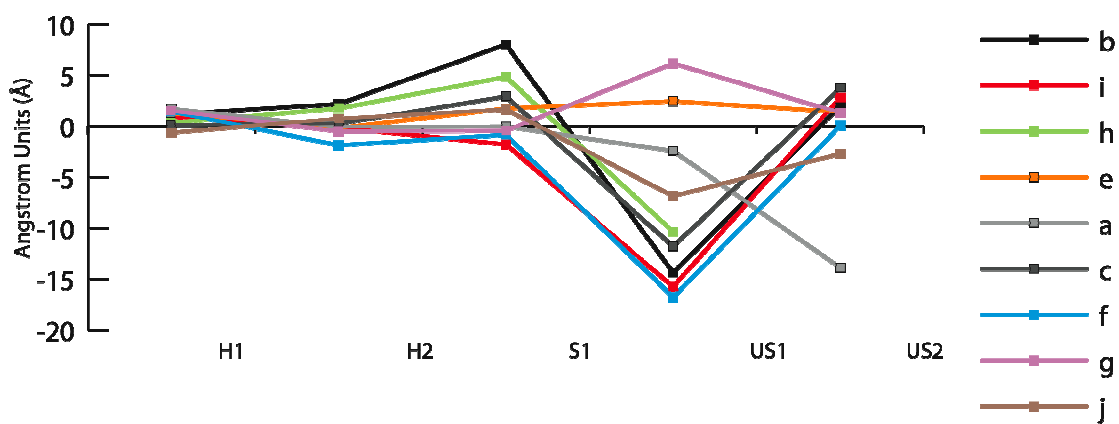
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Analysis of Local Helical parameters of data-mined helix junctions:

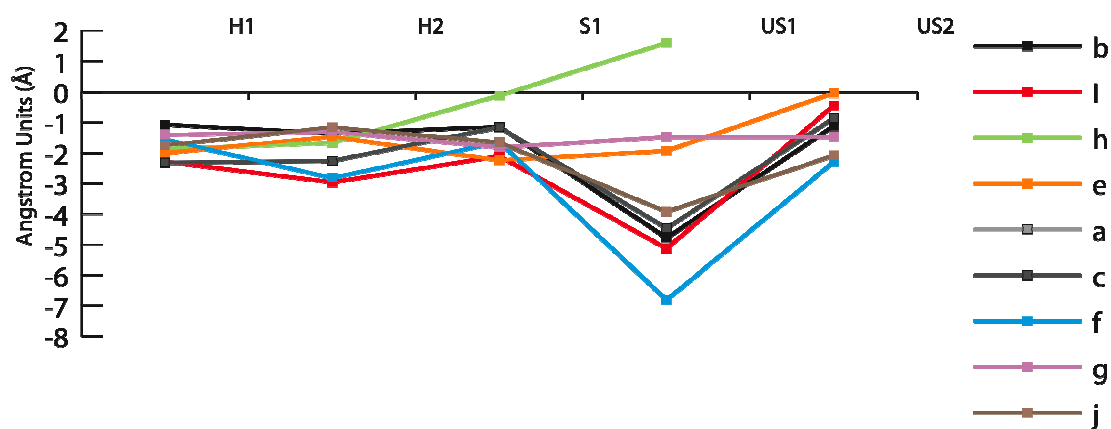
Graphical representation of 3DNA outputs

Figure 1S: 3'(1) junctions

a. Shift

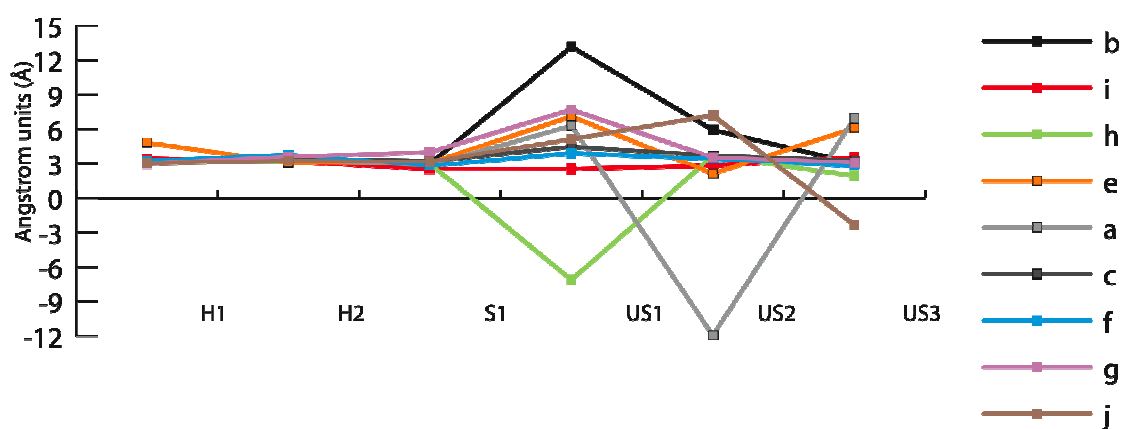


b. Slide



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c. Rise



d. Area of overlap

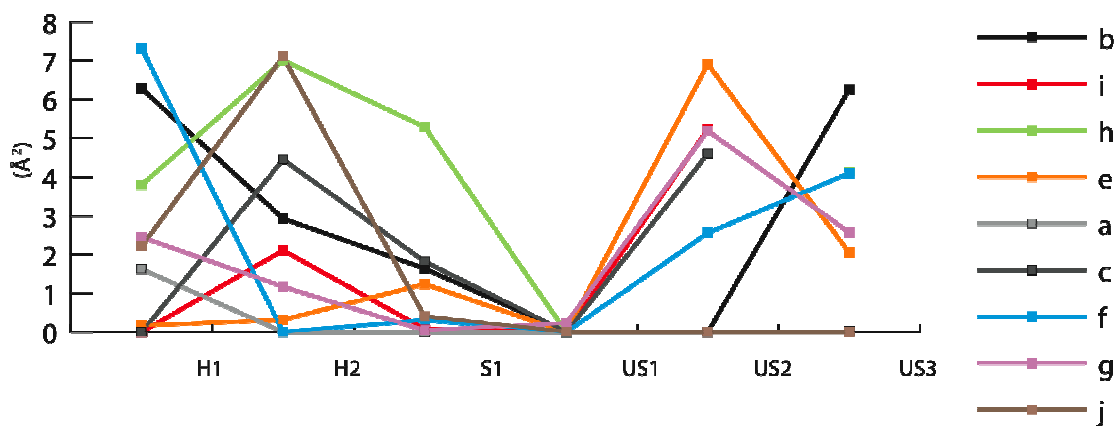


Figure 1S. Local helix parameters at 3'(1) junctions (Table 1). Helix parameters for 3' strands at junctions are used in the analysis. The junctions were analyzed using 3DNA. The 3' strand was treated as a single strand for this analysis. H1 and H2: base-paired residues within the terminal helix region; S1: first stacked, unpaired 3' strand base; US1:

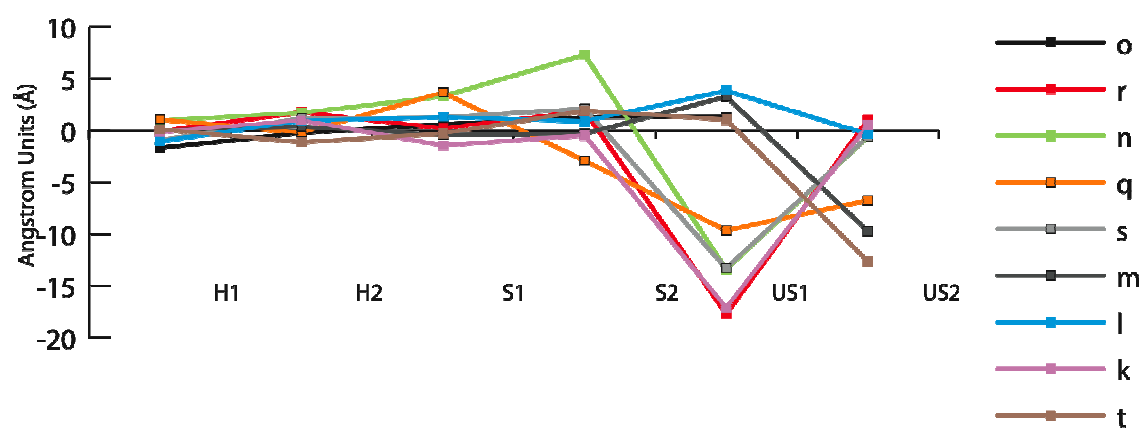
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first unpaired, unstacked 3' strand base, US2: second unpaired, unstacked 3' strand base, US3: third unpaired, unstacked 3' strand base. Unstacked bases (US) are defined as those bases that are not stacked at that particular helix junction and are therefore assigned to the single stranded segment of the junction. However, within the single stranded region bases may be stacked.

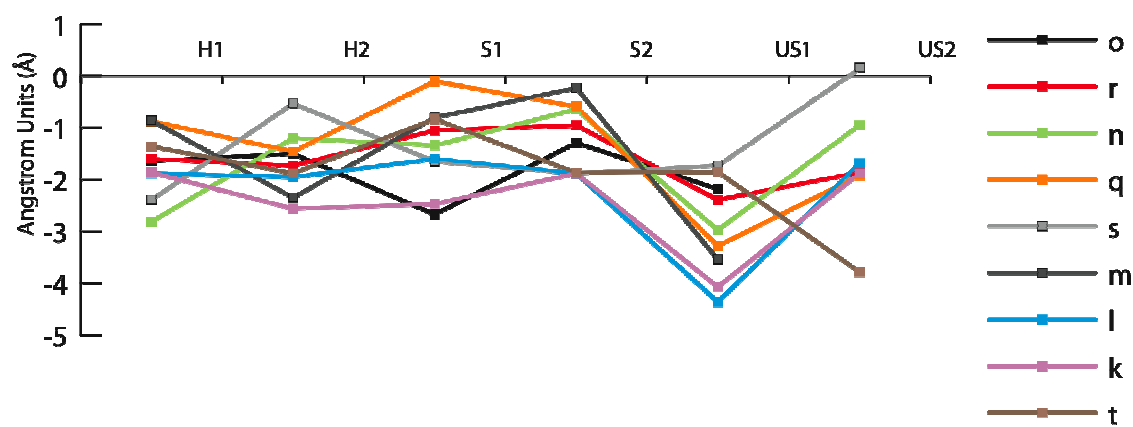
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Figure 2S: 3'(2) junctions

a. Shift

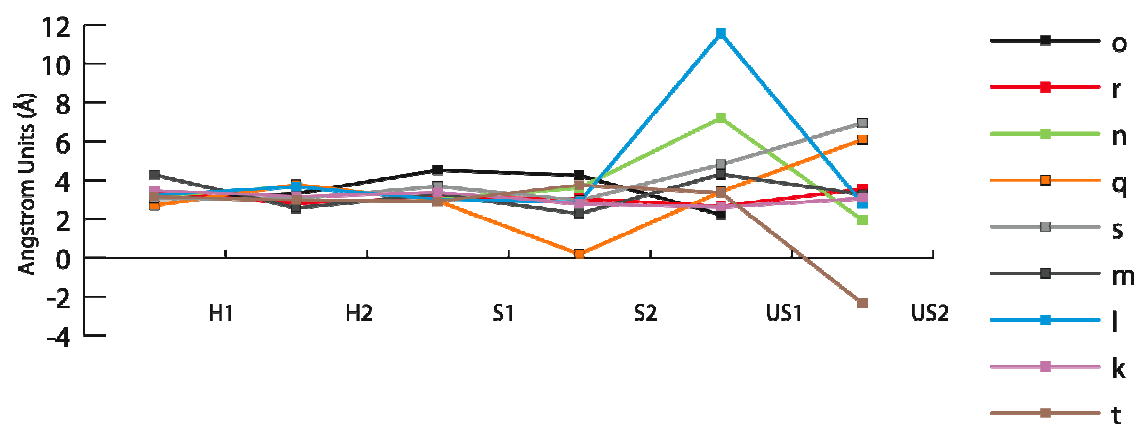


b. Slide



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c. Rise



d. Area of overlap

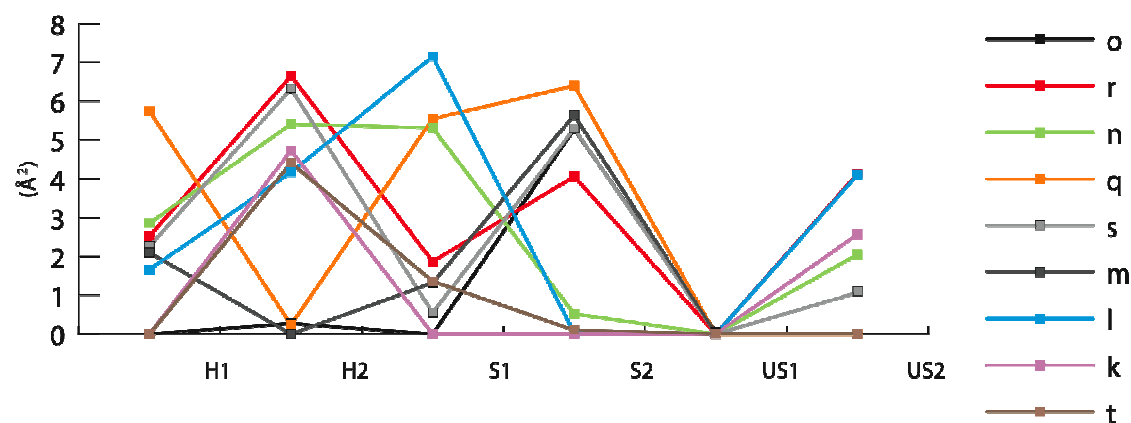


Figure S2. Local helix parameters at 3'(2) junctions (Table 1). H1 and H2: base-paired residues within the terminal helix region; S1: first stacked, unpaired 3' strand base; S2: second stacked, unpaired 3' strand base; US1: first unpaired, unstacked 3' strand base, US2: second unpaired, unstacked 3' strand base. Unstacked bases (US) are defined as

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those bases that are not stacked at that particular helix junction and are therefore assigned to the single stranded segment of the junction. However, within the single stranded region bases may be stacked.

Details of Stacked Junctions

3'(1) Junctions

Nine 3'(1) junctions are observed (Figure 7). All observed 3'(1) junctions close with 5'G-C3' base pairs (9 of 9 junctions, Figure 9A, Table 1). In seven of the 3'(1) junctions a 3' ss purine stacks on the closing base pair. In the other two, a U stacks on the closing base pair. There is no obvious sequence consensus of the next two 3' ss residues.

A purine at the first ss position of the 3' strand prefers interstrand or both-strand stacking to intrastrand stacking. Seven 3'(1) junctions show purine stacking upon the opposing G of the closing base pair. Only when there is a U at the first 3' ss position is intrastrand stacking observed, resulting in a pyrimidine-pyrimidine base stack.

3'(2) Junctions

This group, with nine members (Figure 8), is observed with the same frequency as 3'(1) junctions. 3'(2) Junctions most commonly close with 5'G-C3' base pairs (6 of 9 junctions, Figure 9b, Table 1). Two 3'(2) junctions close with 5'C-G3' base-pairs and

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one with an 5'A-U3' base-pair. The first 3' ss residue is commonly U (5 of 9) or A (4 of 9). There is no obvious sequence preference at the second 3' ss position.

The 3' ss base stacks on the purine of the closing base pair in 6 of 9 junctions. In four of six 3'(2) junctions that close with 5'G-C3' base-pairs, the 3' ss base stacks on the 5' purine base in interstrand and in both-strand stacking. In both junctions with 5'C-G3' closing base-pair, the 3' ss base stacks on the 3' purine base in intrastrand stacking. Intrastrand stacking is the predominant mode of stacking of 3'(2) junctions and is seen in 5 of 9 junctions.

Junction o is partially stacked, and is a possible intermediate in the stacking/unstacking reaction. This junction is intermediate between a 3'(2) and a 3'(3). The minimum distance between base atoms of G181 and U180 is 3.3 Å [N7 (G181) to O3(U180)]. No other atoms are within vdw contact. The bases are not on parallel planes, with a tip angle of -43°. *Junction m* maybe a partially stacked and is intermediate between 3'(1) and 3'(2). The roll between base U437 and G438 is 41°, slightly over the cut-off. However, four pairs of atoms in these two bases are within in vdw contact [3.2 Å, O4(U437) - C6(G438), 2.9 Å C5(U437) - N7(G438), 3.0 Å C6(U437) - N7(G438) and 3.4 Å, C4(U437) - C5(G438)]. The rise is 4 Å.

3'(3+) Junctions

This group contains stacked ss regions varying in length from three to eleven residues. Eight 3'(3+) junctions are identified among the 31 ss-ds junctions. These

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junctions show greater variation in closing base pair than the 3'(2') junctions. Four of the 3'(3+) junctions close with 5'G-C3' base pairs, three close with 5'C-G3' and one closes with 5'A-U3' (Table 1). The first 3' ss base is most commonly C (4 of 8 junctions), while A occurs twice and U and G once each (Figure 9C). The stack of *junction x* may be greater than 4 bases; however, residues are missing in the PDB coordinate file.

The stacking mode of 3'(3+) ss-ds junctions is either intrastrand or interstrand. Both-strand stacking is not observed. *Junctions u, x* and *y* exhibit intrastrand stacking and close with 5'C-G3' base-pairs. The 3' ss base stacks exclusively on the 3' G of the closing base pair. *Junctions w, v, d, z* and *ba* exhibit interstrand stacking. These junctions close with a 5'G-C3' base-pair. *Junction z* closes with an 5'A-U3' base-pair. The first 3' ss base is stacked on the A.

Some base-base steps in 3'(3+) junctions are intermediate between stacked and unstacked. These partially stacked/unstacked states are not at the junction but are within more distal regions of the stack.

3'(2.5) junctions. In *junctions y* and *z* the first two 3' ss bases are stacked, followed by intermediate stacking/unstacking between the second and third bases. In *junction y* one pair of atoms of the second and third bases of the 3' strand are in vdw contact [3.7 Å, N3(C930) - C5(C931)]. In *junction z*, the rise between the second and third 3' ss bases is within the stacking criterion of 3.4 Å; however there is no axial overlap. A slide of 2.2 Å unstacks the bases so that no atoms are within vdw contact. The

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3' stack continues, with other partially stacked steps, for another 8 residues. The poorest base stacking is at pyrimidine-pyrimidine steps and pyrimidine-purine steps.

3'(1.5) or 3'(10) junction. In *junction u*, the 3' strand stack extends for 10 residues, with partial stacking at some steps. Only two pairs of atoms of bases C1301 and G1302 are within vdw contact [3.2 Å, N3(C1301)-N7(G1302) and 3.4 Å, C2(C1301)-C8(G1302)]. Between bases C(1303) and U(1304), two pairs of atoms in vdw contact [3.5 Å, N3(C1303) - C5(U1304) and 3.9 Å C2(C1303) - C6(C1304)]. Similarly, two-pairs of atoms are in contact between C(1305) and U(1306) [3.6 Å, N3(C1305) - C5(U1306) and 3.9 Å C2(C1305) - C6(C1306)]. Residues extending from 1306 to 1310 are stacked with an average overlap area of 2.8 Å². Hence, *junction u* can be classified as either a 3'(1.5) junction, assuming to the shortest ss 3' stack, or as a 3'(10) junction, assuming the longest ss 3' stack.

5' Stacked Junctions

Stacking of the 5' single strand on the closing base-pair is infrequent, and is observed in only three of 31 junctions.

Two 5' stacked junctions close with 5'C-G3' base-pairs and one closes with 5'G-C3'. In one junction, a C on the 5' strand stacks on the closing base-pair. In the other two junctions a purine stacks upon the closing base pair. Observed 5' stacked junctions exhibit intrastrand stacking exclusively. In *junction be* the closing base-pair is 5'G-C3', followed by five consecutive stacked bases of the 5' strand [5'(5) junction]. The first four

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bases of the stack are purines. In *junction bd*, the closing base pair is 5'C-G3'. This junction displays intermediate stacking/unstacking between the second and third ss residues [5'(2.5) junction]. In *junction bc*, the extent of overlap between the first ss base of the 5' strand and the 5'G-C3' closing base-pair is small. Only two atom pairs are in vdw contact [5'(0.5) junction]. The remainder of the stack is well formed.

References

Lu, X. J.; Olson, W. K. Nucleic Acids Res. **2003**, 31, 5108.