SUPPORTING INFORMATION

Huang, Y., Chen, C., and I. M. Russu

Dynamics and Stability of Individual Base Pairs In Two Homologous RNA-DNA Hybrids

1. Conformation of the two RNA-DNA hybrids at increasing concentrations of ammonia

Determination of base-pair opening parameters relies upon measurements of the exchange rates of imino protons as a function of the concentration of ammonia. We have found that increasing the total concentration of ammonia up to 600 mM does not affect the chemical shifts of non-exchangeable and exchangeable protons in the two hybrids investigated. This finding is illustrated in Figure S1 for the imino protons of hybrid II. These results demonstrate that, over the range of ammonia concentrations investigated, the conformation of the two RNA-DNA hybrids does not change.

2. Assignment of the imino proton resonances in the two RNA-DNA hybrids investigated

The imino proton resonances of the two hybrids were assigned using ^{1}H-^{1}H NOESY and ^{15}N-edited HSQC. The ^{15}N-edited HSQC experiments were carried out on hybrid samples in which the RNA strand contained ^{15}N-labeled guanines. These experiments allow direct

observation of the guanine imino protons from the RNA strand. The imino proton resonances from thymines and uracils were distinguished in the ^{1}H-^{1}H NOESY spectra from their connectivities to adenine H2 resonances.

RNA-DNA Hybrid I

The ^{15}N-edited imino proton resonances of the guanines in the RNA strand (panel B in Figure S2) occur at the following spectral positions: 12.25 ppm, 12.53 ppm, 12.82 ppm, 13.02 ppm and 13.08 ppm. The RNA strand contains six guanines. The resonance of the last guanine (G_{14}) is not observed due to fraying at the end of the duplex.

The imino protons from thymines and uracils show the expected NOESY connectivities to adenine-H2 protons (Figure S3). Their resonances are at 13.49 ppm, 13.58 ppm and 13.94 ppm. The cross-peak between the resonances at 13.94 and 13.58 ppm (left panel, Figure S3) assigns these two resonances to T_{11} and T_{12}, which are next to each other in the base sequence. The resonance at 13.49 ppm is assigned to U_6 by default.

The remaining resonances in the spectrum (panel A in Figure S2) are from the guanines in the DNA strand. Their chemical shifts are: 12.73 ppm, 12.88 ppm and 13.20 ppm. Integration of the resonance at 13.20 ppm indicated that it originates from two imino protons. The resonance of the first guanine (G_1) is not observed due to fraying at the end of the duplex.

In the ^{1}H-^{1}H NOESY spectrum, the only cross-peak between imino protons of RNA guanines occurs at 12.82 and 13.08 ppm. This assigns the resonances at 12.82 and 13.08 ppm to the imino protons in G_9 and G_{10}, which are the only guanines located next to each other in the RNA strand. The resonance at 12.82 ppm has a cross-peak to the thymine imino proton resonance at 13.94 ppm. This assigns the resonance at 12.82 ppm to G_{10} imino proton, and the

resonance at 13.94 ppm to T_{11} imino proton. The other resonance, at 13.08 ppm, is assigned to G_9 imino proton. The cross-peak between the T_{11} resonance at 13.94 ppm and the resonance at 13.58 ppm assigns the latter resonance to T_{12} imino proton. The cross-peak between the imino proton resonance of T_{12} and the resonance at 12.25 ppm assigns the resonance at 12.25 ppm to G_{13} imino proton.

For the imino proton resonances of the guanines in the DNA strand, the resonance at 12.88 ppm has two cross-peaks: one with another DNA guanine imino proton resonance at 13.20 ppm and the other with U_6 imino proton (13.49 ppm). This assigns the resonance at 12.88 ppm to G_7 imino proton, and one of the resonances at 13.20 ppm to G_8 imino proton.

The NOESY spectrum also shows a cross-peak between a guanine imino proton from the DNA strand (12.73 ppm) and a guanine imino proton from the RNA strand (12.53 ppm). The resonance at 12.53 ppm has a connectivity to A_6-H2 proton; hence, it is assigned to G_5 imino proton. Then, the other resonance (at 12.73 ppm) is from G_4 imino proton.

From the remaining resonances, one originates from the RNA strand (13.02 ppm) and the other from the DNA strand (13.20 ppm). They are assigned by default to G_3 and G_2 imino proton, respectively.

RNA-DNA Hybrid II

The imino proton resonances from thymines and uracils are distinguished from their NOESY connectivities to adenine-H2 protons (Figure S4). Two of these resonances are at 14.06 and 14.22 ppm. Their NOESY connectivity assigns them to U_{11} and U_{12}, which are next to each other in the RNA base sequence. The remaining resonance (13.41 ppm) is assigned to T_6 imino proton.

The ^{15}N-edited imino proton resonances of the guanines in the RNA strand (panel B in Figure S5) occur at 12.81 ppm, 12.88 ppm, 13.07 ppm and 13.19 ppm. One cross-peak is observed between the resonance at 12.81 ppm and that at 13.07 ppm. The cross-peak must originate from G_7 and G_8, the only guanines in the RNA strand that are located next to each other. The resonance at 12.81 ppm has a cross-peak to the T_6 imino proton resonance (13.41 ppm). This assigns the resonance at 12.81 ppm to G_7 imino proton. Then, the other resonance (13.07 ppm) is from G_8 imino proton.

The two other RNA guanine resonances, at 12.88 and 13.19 ppm, must originate from the remaining guanines in the RNA strand, i.e., G_2 and G_4. In the unedited spectrum (panel A in Figure S5), each of these two resonances overlaps with one DNA guanine resonance. In the NOESY spectrum, a cross-peak is observed between the resonance at 12.88 ppm and that at 13.19 ppm. This cross-peak cannot correspond to the two RNA guanines (G_2 and G_4) because these are not adjacent in the base sequence. Instead, the cross-peak must originate from G_9 and G_{10}, which are the only adjacent guanines in the DNA strand. The guanine resonance at 12.88 ppm has a NOESY connectivity to the uracil imino proton resonance at 14.22 ppm. This assigns the resonance at 12.88 ppm to G_{10}, the resonance at 14.22 ppm to U_{11} and the resonance at 13.19 ppm to G_9.

The resonance of G_5 imino proton (12.32 ppm) is assigned based on its connectivity to A_6-H2 proton (Figure S4). This resonance also has a cross-peak to one of the resonances at 12.88 ppm. This assigns the latter resonance to G_4 imino proton. Then, the remaining RNA guanine resonance at 13.19 ppm is assigned by default to G_2 imino proton. The cross-peak between the resonance at 13.19 ppm and that at 12.74 ppm assigns the resonance at 12.74 ppm to G_3 imino

proton. The remaining DNA guanine resonance at 12.69 ppm is assigned by default to G_{13} imino proton.

Figure Legends

Figure S1

The NMR resonances of imino protons for a sample of hybrid II in which the RNA strand was specifically labeled with ^{15}N at guanines, at different ammonia concentrations. The upper panel shows ^{15}N-edited spectra and the lower panel shows ^{14}N-edited spectra. The total concentration of ammonia for each spectrum is indicated on the left.

Figure S2

The NMR resonances of imino protons in hybrid I. (A) All imino proton resonances. (B) ^{15}N-edited imino proton resonances for a sample of hybrid I in which the RNA strand contained ^{15}N-labeled guanines. Experimental conditions: 100 mM NaCl, 0.5 mM EDTA and 1 mM triethanolamine at pH 8.3 and at 10 °C.

Figure S3

Selected regions of the ^{1}H-^{1}H NOESY spectrum of hybrid I. The connectivities between imino protons are indicated by full lines. The connectivities between imino protons and adenine-H2 protons are indicated by interrupted lines. Experimental conditions: 100 mM NaCl, 0.5 mM EDTA and 1 mM triethanolamine at pH 8.3 and at 10 °C.

Figure S4

Selected regions of the ^{1}H-^{1}H NOESY spectrum of hybrid II. The connectivities between imino protons are indicated by full lines. The connectivities between imino protons and adenine-

H2 protons are indicated by interrupted lines. Experimental conditions: 100 mM NaCl, 0.5 mM EDTA and 1 mM triethanolamine at pH 8.3 and at 10 °C.

Figure S5

The NMR resonances of imino protons in hybrid II. (A) All imino proton resonances. (B) ^{15}N-edited imino proton resonances for a sample of hybrid II in which the RNA strand contained ^{15}N-labeled guanines. Experimental conditions: 100 mM NaCl, 0.5 mM EDTA and 1 mM triethanolamine at pH 8.3 and at 10 °C.

Figure S1

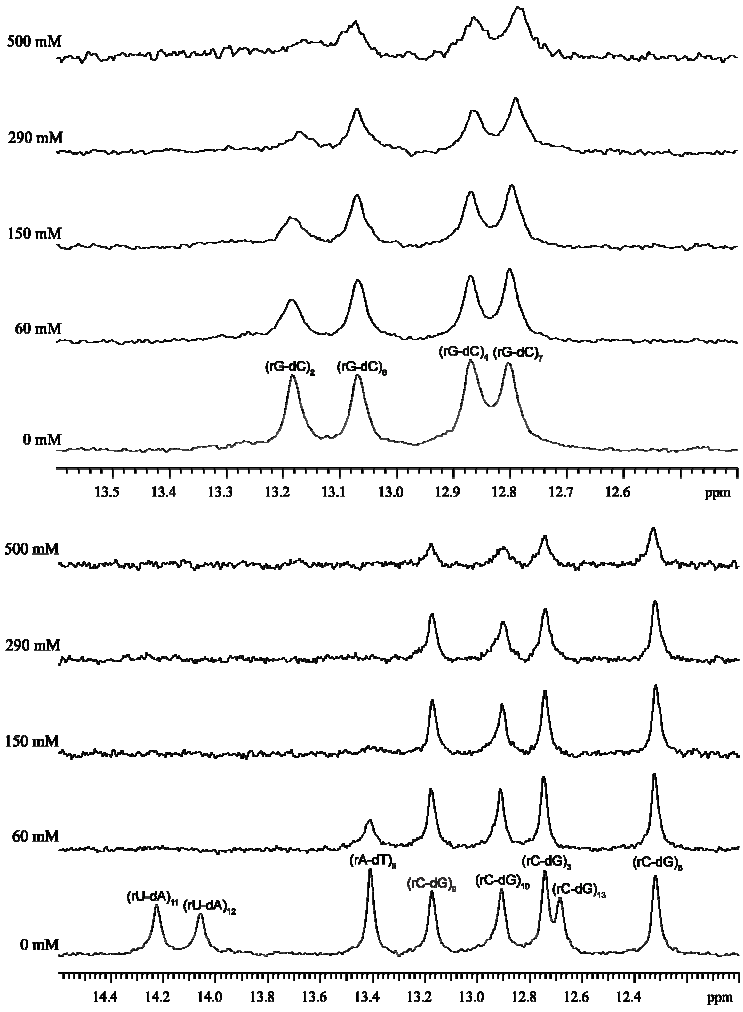

500 mM
290 mM
150 mM
60 mM
0 mM
(rG-dC)2
(rG-dC)6
(rG-dC)4
(rG-dC)7
13.5 13.4 13.3 13.2 13.1 13.0 12.9 12.8 12.7 12.6 ppm
500 mM
290 mM
150 mM
60 mM
0 mM
(rU-dA)11
(rU-dA)12
(rA-dT)8
(rC-dG)5
(rC-dG)10
(rC-dG)3
(rC-dG)13
(rC-dG)9
14.4 14.2 14.0 13.8 13.6 13.4 13.2 13.0 12.8 12.6 12.4 ppm

Figure S2

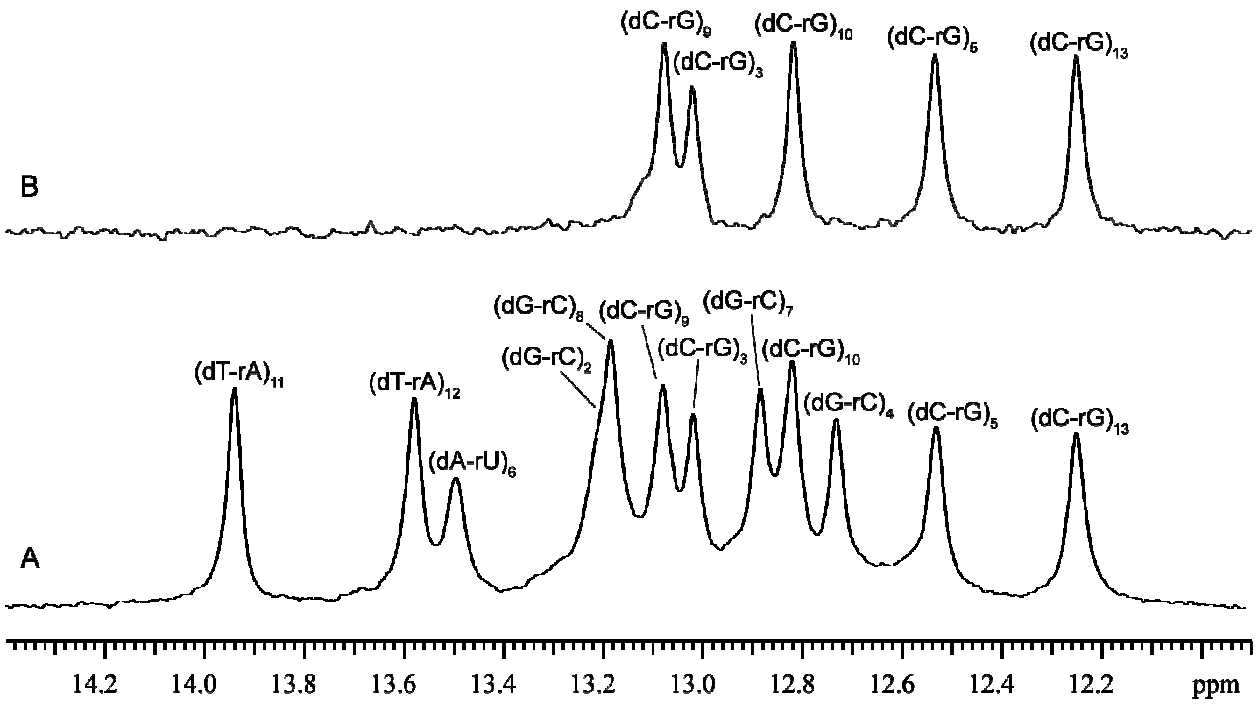

(dC-rG)9
(dC-rG)10
(dC-rG)6
(dC-rG)13
(dC-rG)3
B
(dG-rC)8
(dC-rG)9
(dG-rC)7
(dG-rC)2
(dC-rG)3
(dC-rG)10
(dT-rA)11
(dT-rA)12
(dG-rC)4
(dC-rG)5
(dC-rG)13
(dA-rU)6
A
14.2
14.0
13.8
13.6
13.4
13.2
13.0
12.8
12.6
12.4
12.2
ppm

Figure S3

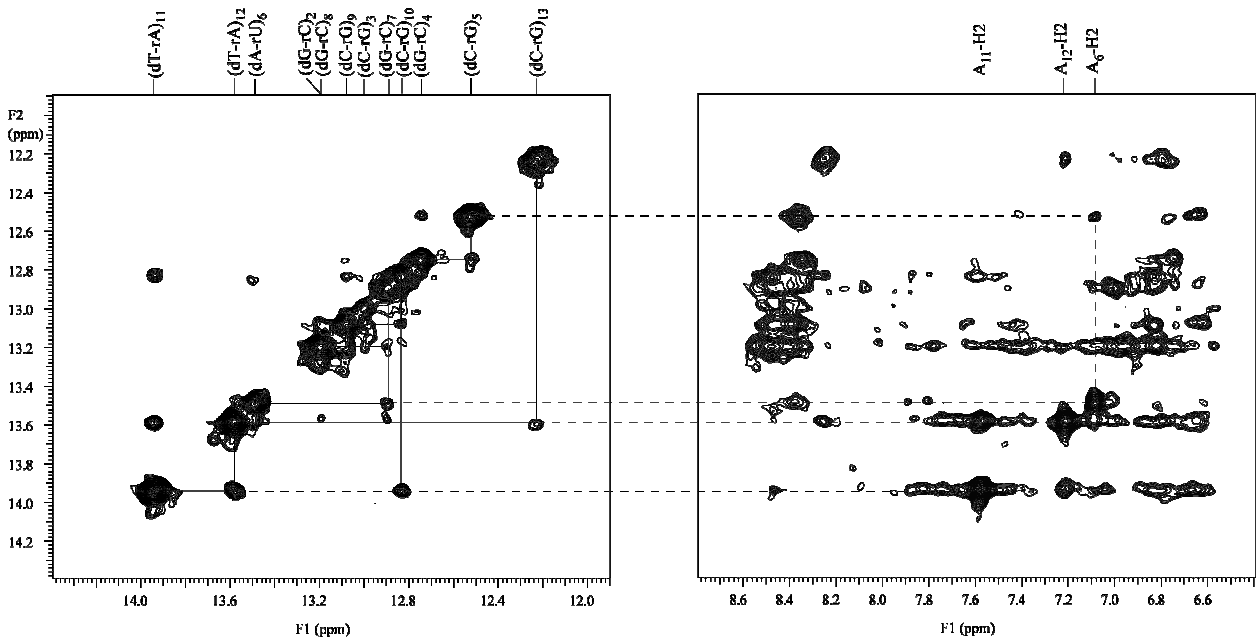

(dT-rA)11
(dT-rA)12
(dA-rU)6
(dG-rC)2
(dG-rC)8
(dC-rG)9
(dC-rG)3
(dG-rC)7
(dC-rG)10
(dG-rC)4
(dC-rG)5
(dC-rG)13
A11-H2
A12-H2
A6-H2
F2 (ppm)
F1 (ppm)
F1 (ppm)

Figure S4

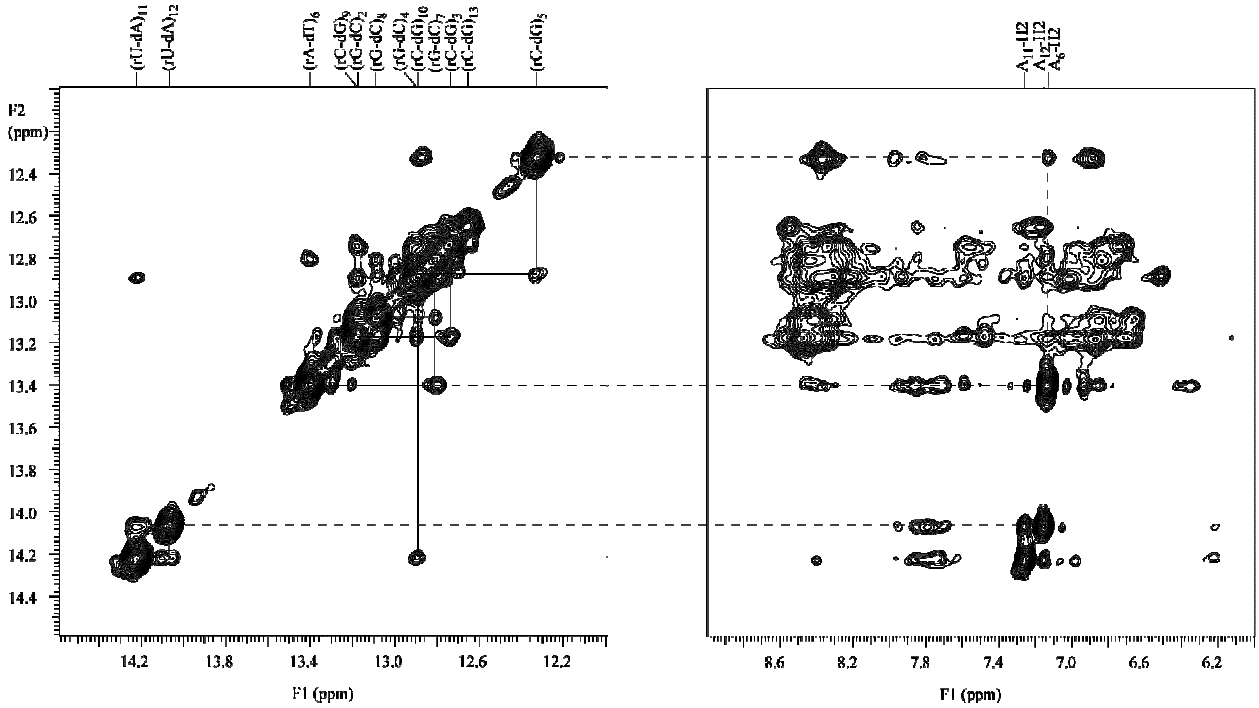
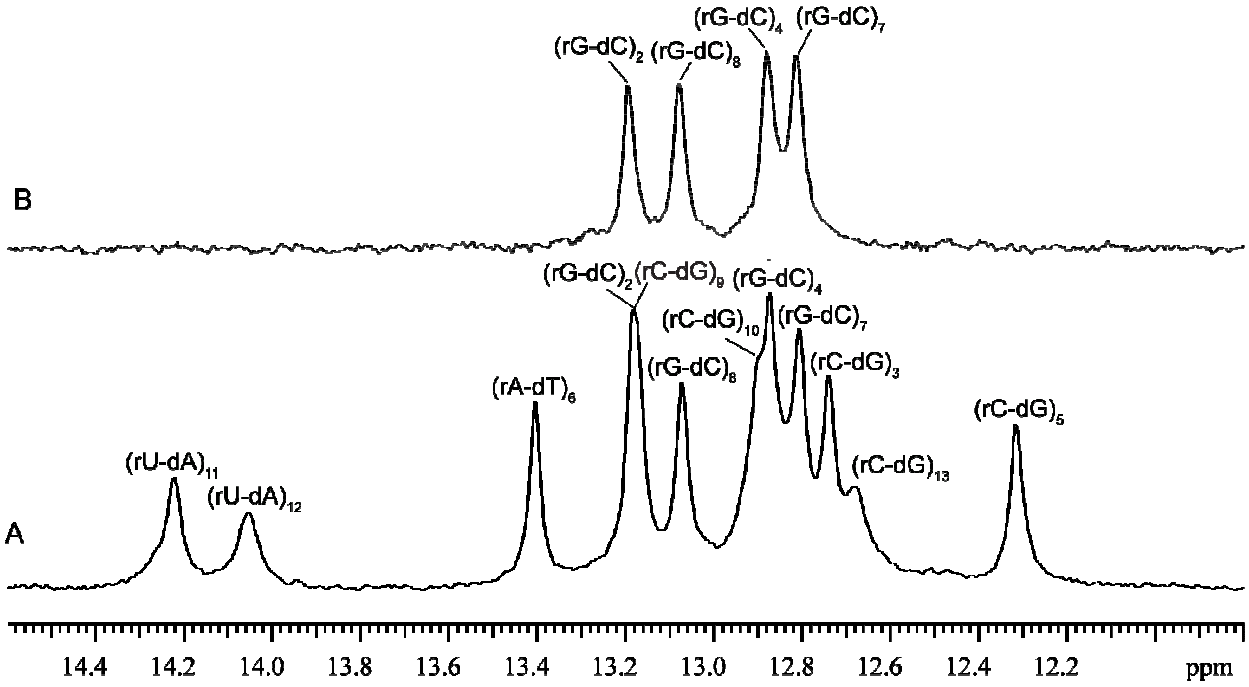


Figure S5


(rG-dC)4
(rG-dC)7
(rG-dC)2
(rG-dC)8
B
(rG-dC)2
(rC-dG)9
(rG-dC)4
(rC-dG)10
(rG-dC)7
(rG-dC)8
(rC-dG)3
(rA-dT)6
(rC-dG)5
(rU-dA)11
(rC-dG)13
(rU-dA)12
A
14.4
14.2
14.0
13.8
13.6
13.4
13.2
13.0
12.8
12.6
12.4
12.2
ppm