Supplementary Material to Accompany

Molecular Dynamics Correctly Models the Unusual Major Conformation of the GAGU RNA Internal Loop and with NMR Reveals an Unusual Minor Conformation

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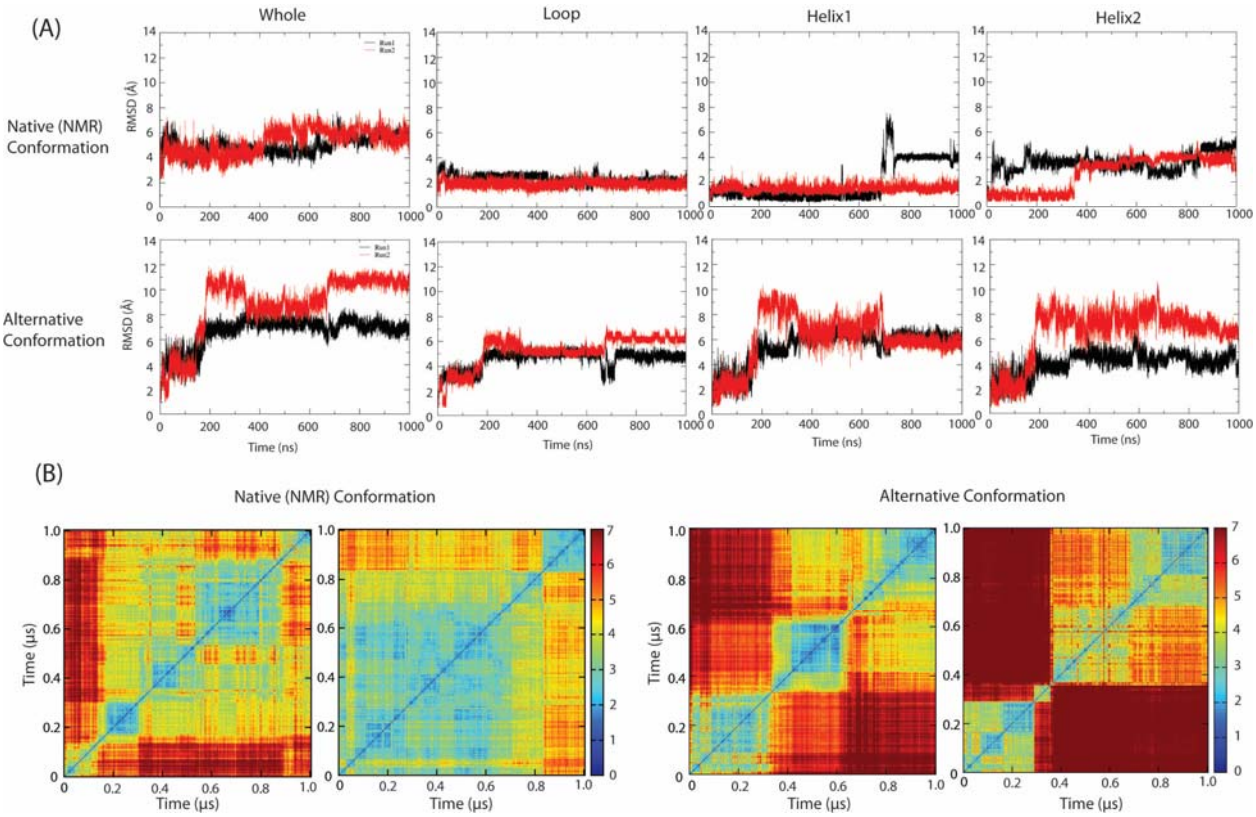


Figure S1. (A) Plots of RMSD as a function of simulation time for whole duplex, loop and helical regions for GAGU sequence in its NMR and alternative conformation simulated with ff99 force field. Columns refer to RMSD of whole duplex, loop region and the two helical regions respectively. Data in rows corresponds to the simulations starting from native (NMR) conformations or alternative (maximum base pair) conformation. Data from different trajectories are given in black and red color. **(B)** 2D RMSD plots of 2 simulations of GAGU duplex with ff99 force field in its native (NMR) conformation on the left and its alternative (maximally paired) conformation on the right.

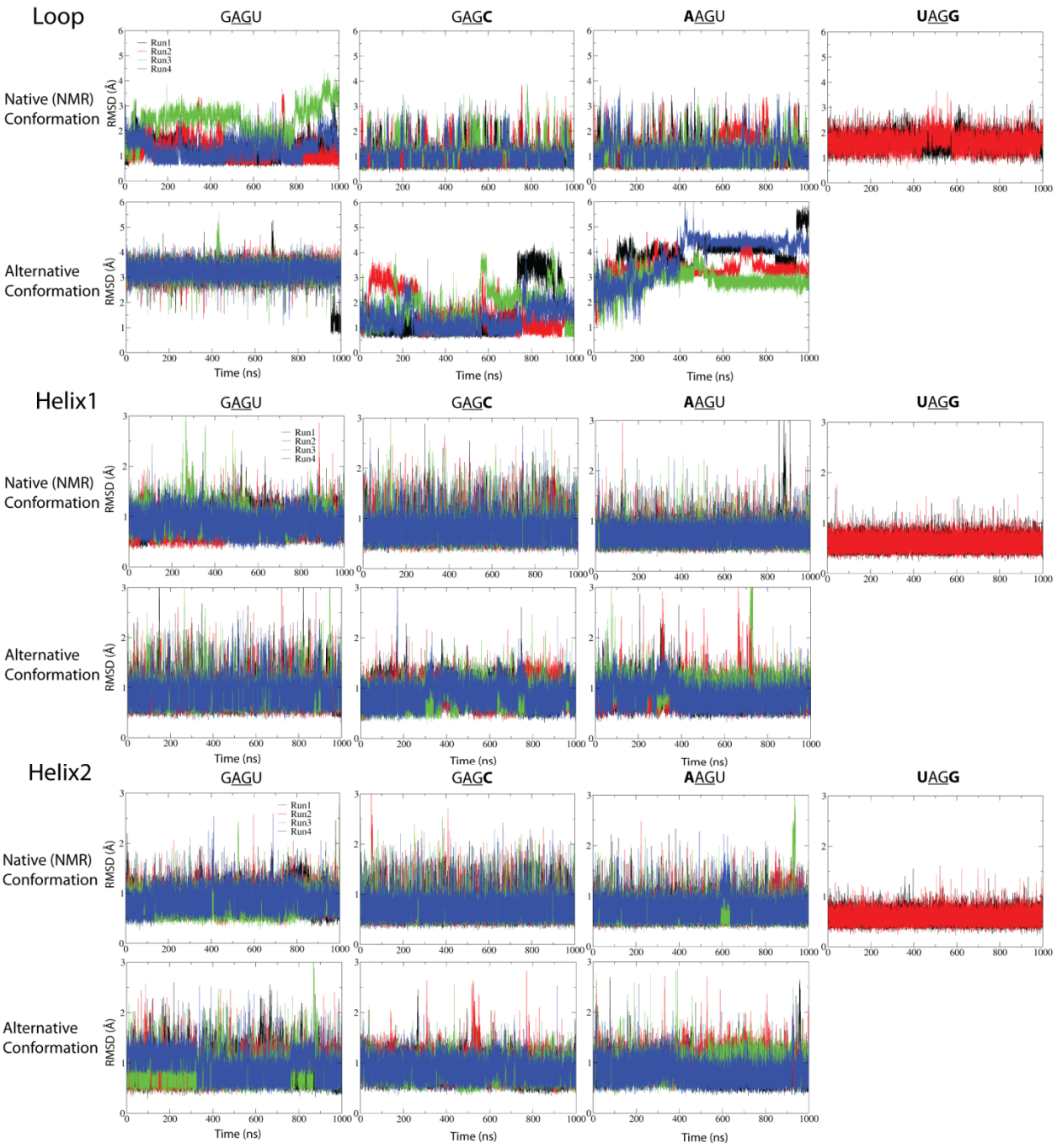
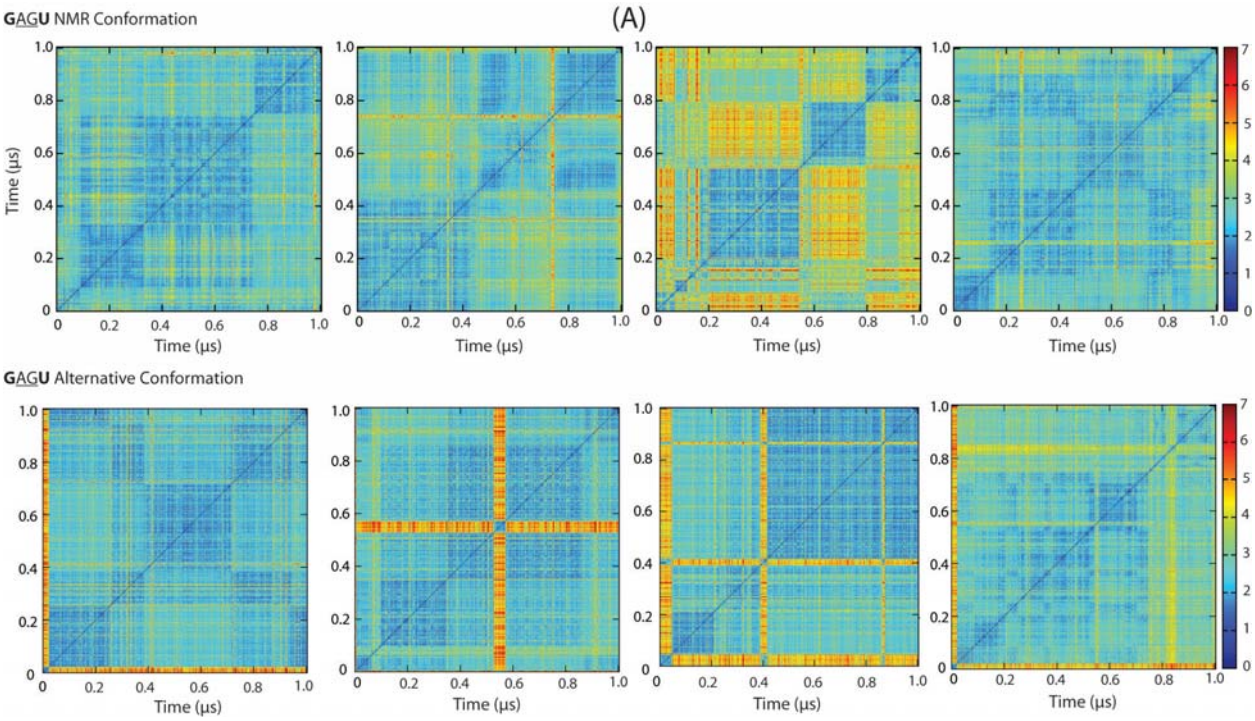
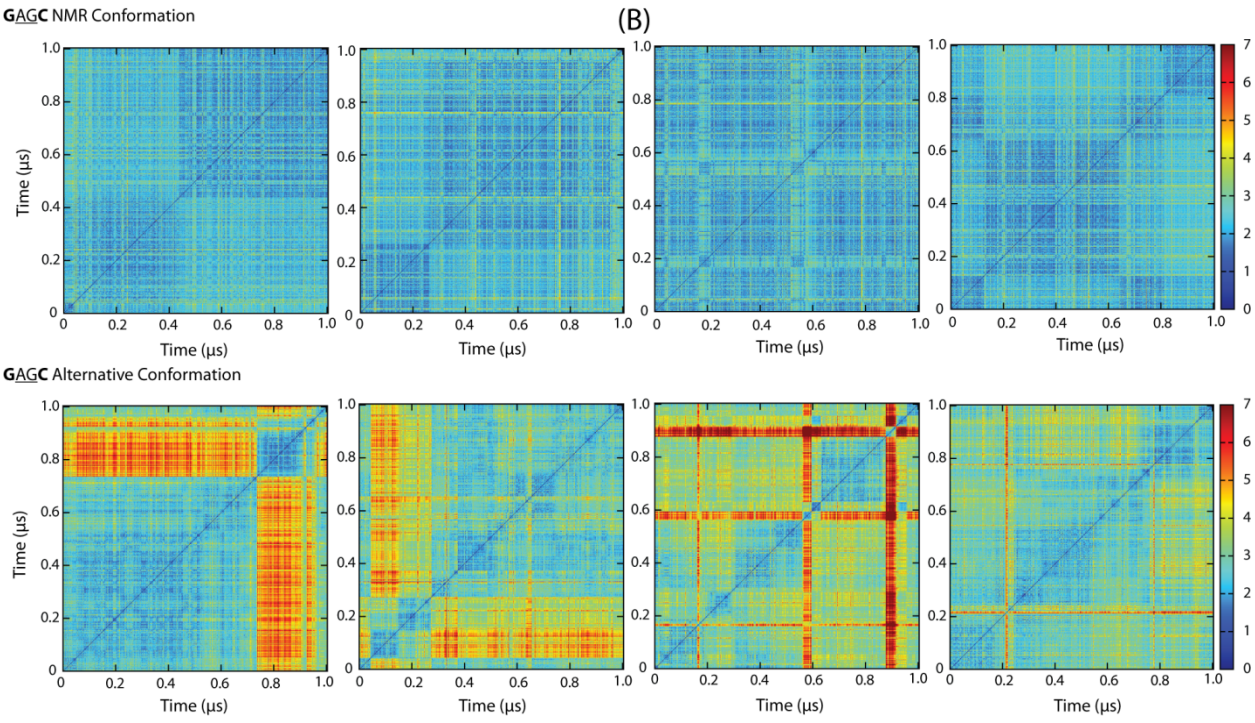


Figure S2. Plots of RMSD as a function of simulation time for loop and helical regions for all simulated sequences in both of their conformations. Columns refer to different sequences and rows to conformations as labeled in the graphs. Different trajectories are given in black, red, blue and green color.


GAGU NMR Conformation
(A)
GAGU Alternative Conformation
Time (μs)
Time (μs)


GAGC NMR Conformation
(B)
GAGC Alternative Conformation
Time (μs)
Time (μs)

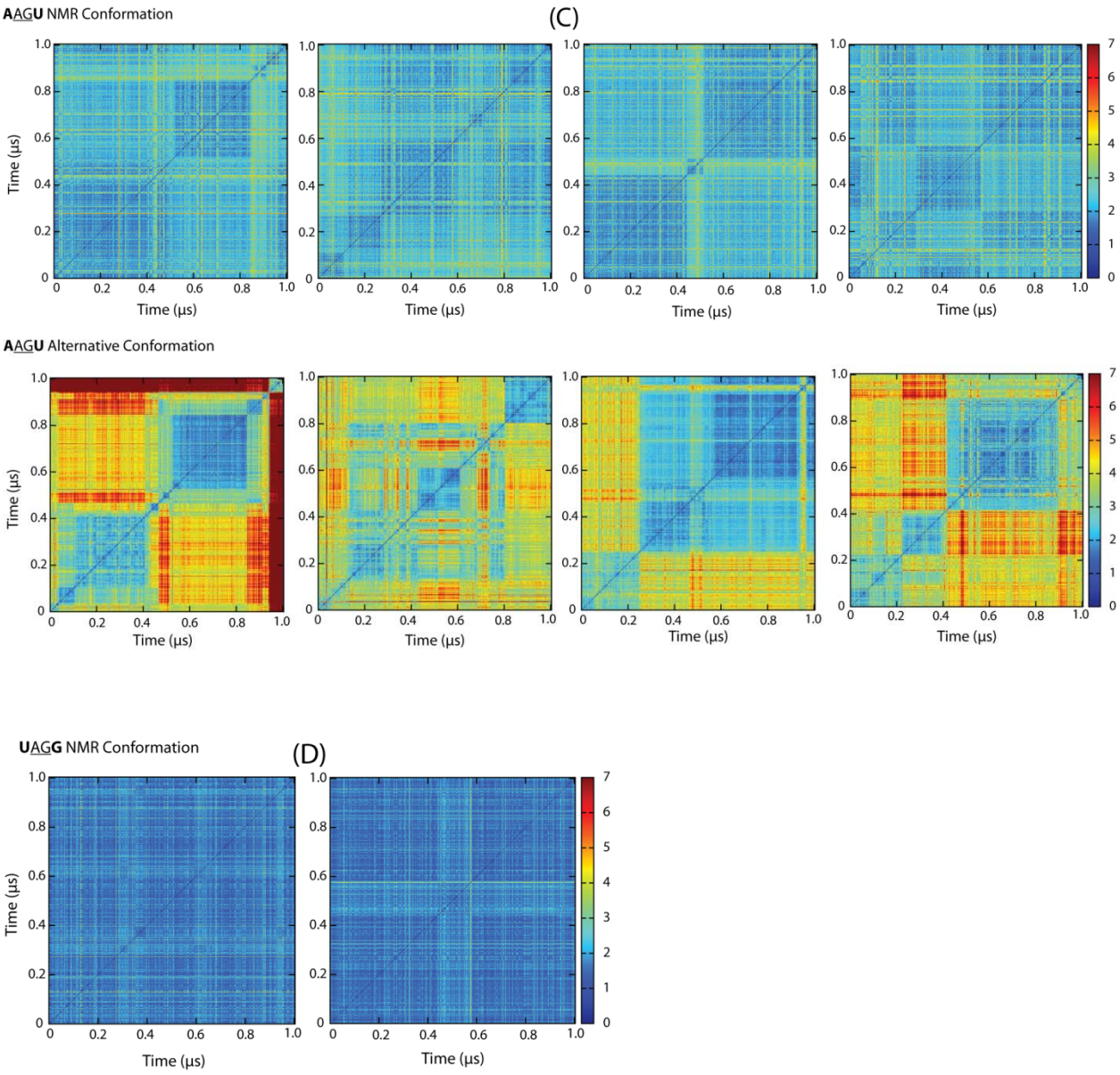


Figure S3. 2D RMSD plots for all studied sequences, their conformations and the 4 simulations. For a given sequence data from different simulations is given in columns and for different conformations in rows as labeled in plots. **(A)** GAGU sequence, including the data for 2 simulations of NMR conformation using force field ff99, **(B)** GAGC sequence, **(C)** AAGU sequence and **(D)** UAGG sequence

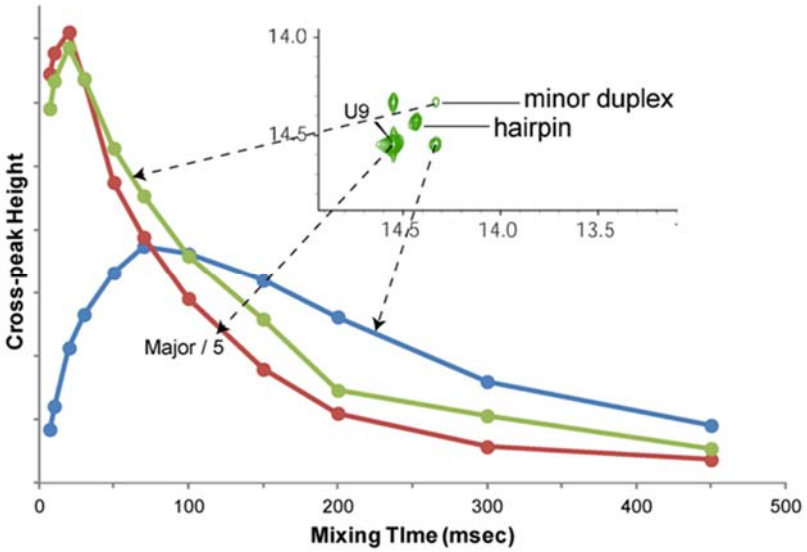


Figure S4. Intensity of 2D NOESY (inset) major-conformation diagonal (red), minor-conformation diagonal (green), and conformational-exchange cross-peak (blue) of U9-H3 (imino proton) as a function of mixing time in the self-complementary duplex 5'GACGAGUGUCA/3'ACUGUGAGCAG. The intensity for the major-conformation diagonal peak has been divided by five in the graph (red line). Temperature is 1°C. Cross-peaks between signals from GAGU major and minor conformations will occur in 2D NOESY spectra when major-to-minor transitions occur during the NOESY "mixing" time.

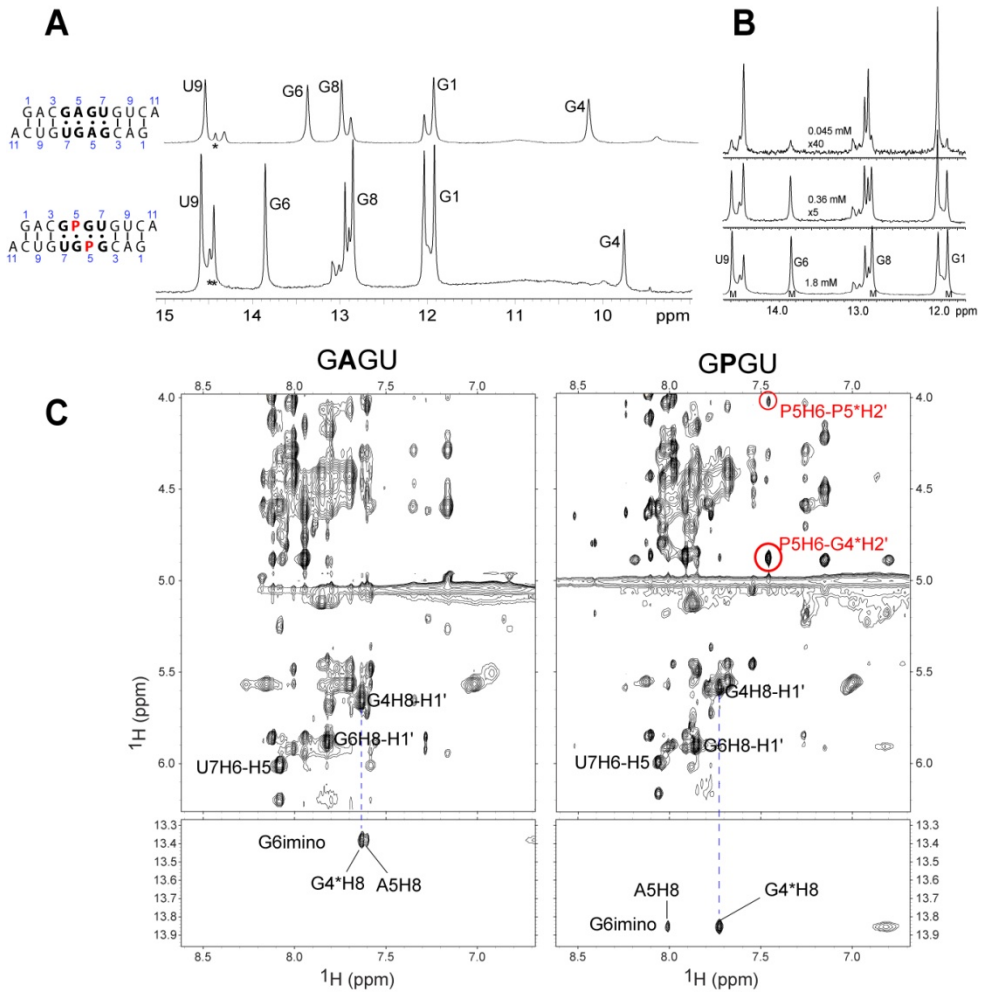


Figure S5. NMR spectra comparing unmodified, self-complementary GAGU, r(GACG**A**GUGUCA)$_2$, with the same sequence with purine (P) substituted for A5, r(GACG**P**GUGUCA)$_2$. Purine differs from adenine by substitution of the adenine amino group with a proton (P5H6). (A) 1D spectra of the imino region of GAGU (top; 2.0 m<u>M</u>) and GPGU (bottom; 1.8 m<u>M</u>). Peak labels are adjacent to the major duplex conformation. Peaks associated with U9 and labeled with asterisks (*) are from hairpin conformations. Evidently, the ratio of duplex:hairpin is lower for GPGU than for GAGU. (B) 1D spectra of GPGU as a function of concentration (up to 40-fold dilution) demonstrating that peaks that are not part of the major duplex are due to hairpin conformation(s) as evidenced by constant or increasing intensity with decreasing strand concentration. (C) 2D NOESY NMR spectra of (left) GAGU, and (right) GPGU. Cross-peaks with black labels indicate the presence of the major (U7 bulged) conformation in both samples (large H8-H1′ peaks due to syn G4 and G6; upfield shifted U7H5 and U7H6 due to bulge; G6H1-G4*H8 cross-peak due to WC/Hoogsteen G6/G4 pair). Cross-peaks with red labels are cross-strand interactions between P5H6 and G4*H2′ and between P5H6 and P5*H2′ that are expected if P5 in GPGU is positioned similarly to A5 in GAGU (Figure 5). Temperature is 0°C for both spectra. For GAGU, strand concentration is 2 mM, mixing time is 150 msec. For GPGU, strand concentration is 1.8 mM, mixing time is 100 msec.

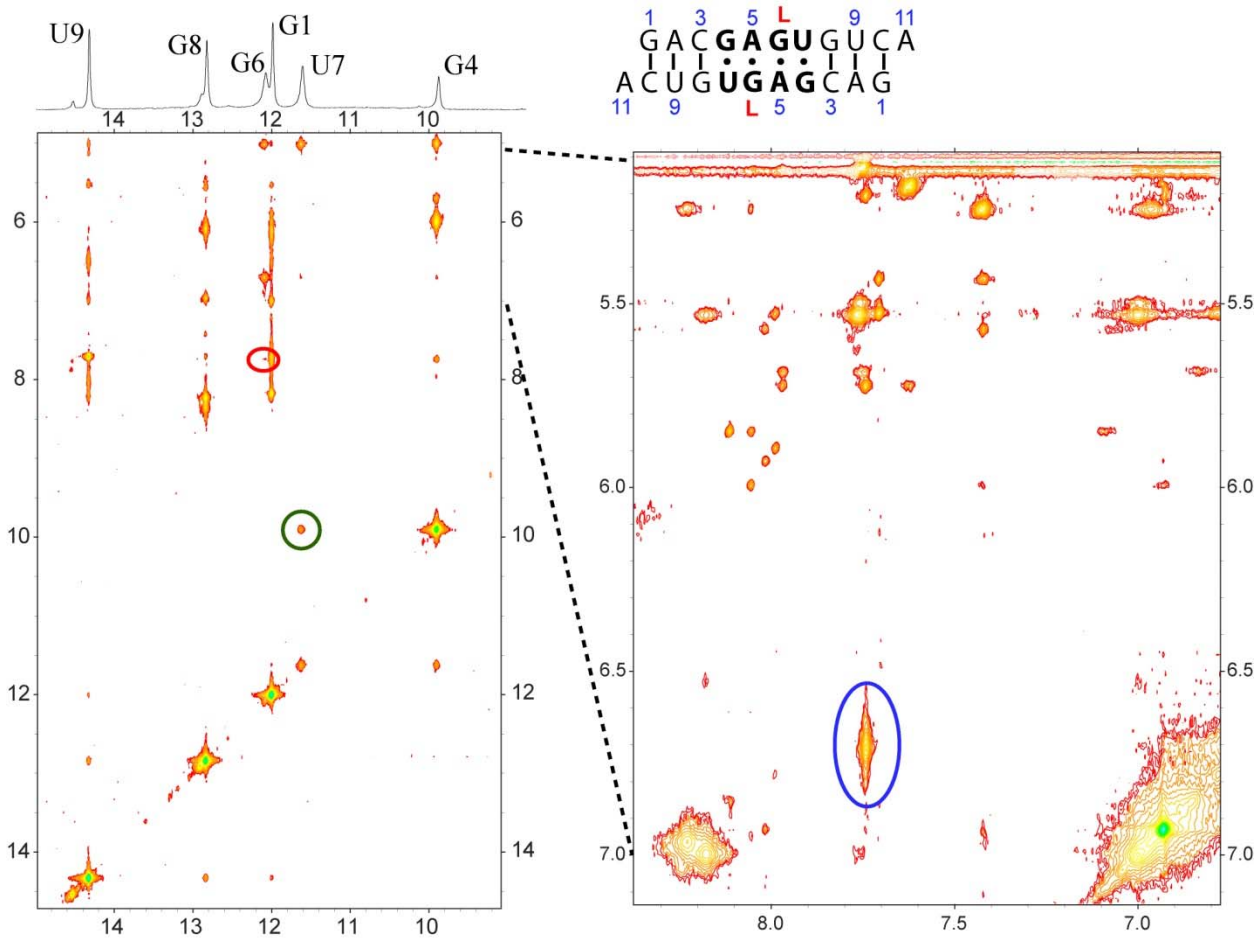
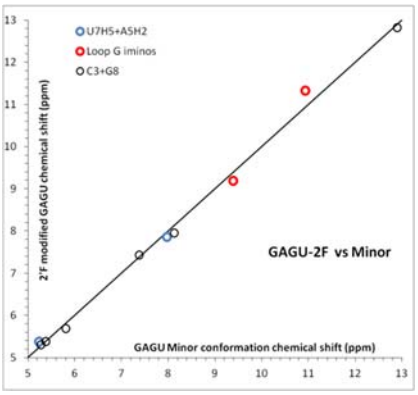


Figure S6. 2D NOESY of 6L: residue G6/6* is replaced with guanosine LNA. Temperature is 1°C; mixing time is 75 msec. Red, blue and green circles correspond to NOE cross-peaks between protons indicated by arrows in Figure 6C and 6D.

Table S1. Chemical shifts at 1 °C of unmodified GAGU resonances that can be assigned in both major and minor conformations, and the same resonances in modified sequences 2′F, 6L, and 6I (see Figure 8 for description). Minor conformation assignments are based on NMR exchange cross-peaks to major conformation peaks previously assigned (Kennedy, 2012). Assignments in bold could not be resolved from the major conformation. [a]From the non-self-complementary sequence. [b]Inosine imino. Graphs to the right of the table show correlation of GAGU minor conformation shifts (horizontal axis) with sugar-modified sequences 6L and 2′F (vertical axes). Apparently, except for G6 iminos, chemical shifts do not substantially distinguish the two conformations. Loop residue H1′ and H8/6 shifts are not plotted because these shifts are chemically influenced by the sugar modification, particular so in 2′F.

Resonance		Major	Minor	2'F	6L	6I
G1	H1	11.94	12.05	12.03	12.00	11.98
	H8	8.11	8.13	8.14	8.11	8.09
	H1'	5.85	**5.85**	5.87	5.85	5.83
A2	H2	7.58	7.77	7.74	7.71	7.68
	H8	7.95	8.08	8.09	8.06	8.03
	H1'	5.95	6.01	6.02	5.99	5.98
C3	H5	4.98	5.27	5.31	5.24	5.22
	H6	7.16	7.38	7.44	7.43	7.35
	H1'	5.06	5.38	5.38	5.43	5.37
G4	H1	10.17	9.39	9.19	9.91	9.64
	H8	7.62	7.35	7.50	7.42	7.41
	H1'	5.66	5.61	5.98	5.57	5.57
A5	H2	7.68	7.97	7.86	7.74	7.79
	H8	7.60	7.73	7.55	8.02	8.04
	H1'	5.73	5.67	6.06	5.93	5.89
G6	H1	13.38	10.93[a]	11.33	12.09	14.9[b]
	H8	7.82	7.56	7.25	6.93	7.54
	H1'	5.89	5.46	5.83	5.21	5.75
	H2'	5.68	4.90	4.89	4.53	
U7	H3				11.63	11.63
	H5	6.01	5.24	5.38	5.18	5.13
	H6	8.09	7.57	7.54	7.63	7.36
	H1'	6.21	5.58	5.74	5.72	5.48
G8	H1	12.99	12.89	12.83	12.84	12.92
	H8	7.80	8.12	7.96	7.97	7.98
	H1'	5.06	5.81	5.69	5.69	5.67
U9	H3	14.55	14.33	14.20	14.33	14.27
	H5	5.12	**5.12**	5.02	5.13	5.08
	H6	7.86	**7.86**	7.77	7.75	7.72
	H1'	5.46	5.59	5.63	5.50	5.47
C10	H5	5.56	**5.56**	5.54	5.53	5.51
	H6	7.70	7.77	7.80	7.76	7.73
	H1'	5.49	5.57	5.56	5.53	5.51
A11	H2	7.28	**7.28**	7.24	7.22	7.23
	H8	8.01	8.04	8.02	7.99	7.98
	H1'	5.92	**5.92**	5.92	5.90	5.88



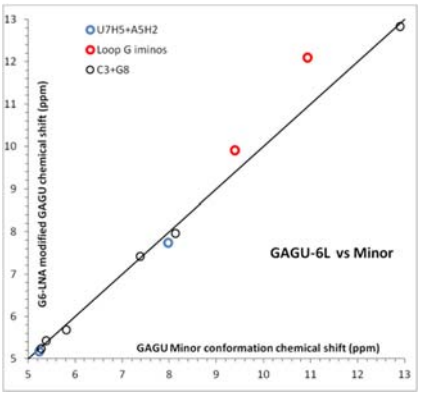


Table S2. Carbon-13 chemical shifts at 1 °C of unmodified GAGU in the major conformation.

	G1	A2	C3	G4	A5	G6	U7	G8	U9	C10	A11
C1′	93.25	93.10		89.96	89.69	92.14	90.23		93.67	93.82	91.87
C8/C6	138.71	139.44	140.02	143.23	140.09	143.35	144.49	138.07	141.97	140.74	139.97