Solution Structure and Thermodynamics of 2′,5′ RNA Intercalation

Eric D. Horowitz[1], Seth Lilavivat[1], Benjamin W. Holladay[1], Markus W. Germann[2], and Nicholas V. Hud[1]

[1]Parker H. Petit Institute of Bioengineering and Bioscience, Georgia Institute of Technology, School of Chemistry and Biochemistry, Atlanta, GA, USA 30332-0400
[2]Georgia State University, Departments of Chemistry and Biology, Atlanta, GA, USA 30302-4098

Supporting Information

2′,5′ RNA ^{1}H Assignments

	H6/H8	H5	H1′	H2′	H3′	H4′	H5′/5″	imino
G1	7.89	*na*	5.89	4.98	4.42	4.15	3.70/2.75	11.87
C2	7.44	5.67	5.72	4.15	4.16	3.49	3.91/3.85	*na*
C3	7.48	5.48	6.02	4.70	4.47	4.25	4.05	*na*
G4	7.45	*na*	5.68	4.64	4.72	4.19	--	11.30
C5	7.09	4.70	5.88	4.48	4.45	4.30	4.11	*na*
G6	7.39	*na*	5.71	4.70	4.18	4.16	--	11.22
G7	7.11	*na*	5.73	4.48	4.16	4.27	4.14	12.72
C8	7.20	5.20	5.56	3.90	3.85	3.99	4.08/3.87	*na*

2′,5′ RNA ^{31}P Assignments

G1pC2	C2pC3	C3pG4	G4pC5	C5pG6	G6pG7	G7pC8
-0.81 (-0.86)	-0.25 (-0.10)	0.87 (-0.18)	-1.23 (-0.61)	0.68 (-0.16)	-0.51 (-0.75)	-0.57 (0.15)

Proflavine ^{1}H Assignments

H1/H8	H2/H7	H4/H5	H9
6.08	6.62	5.97	7.62

All assignments are for the proflavine-RNA complex at 282 K. Values given in parentheses for ^{31}P assignments are for the unintercalated duplex.

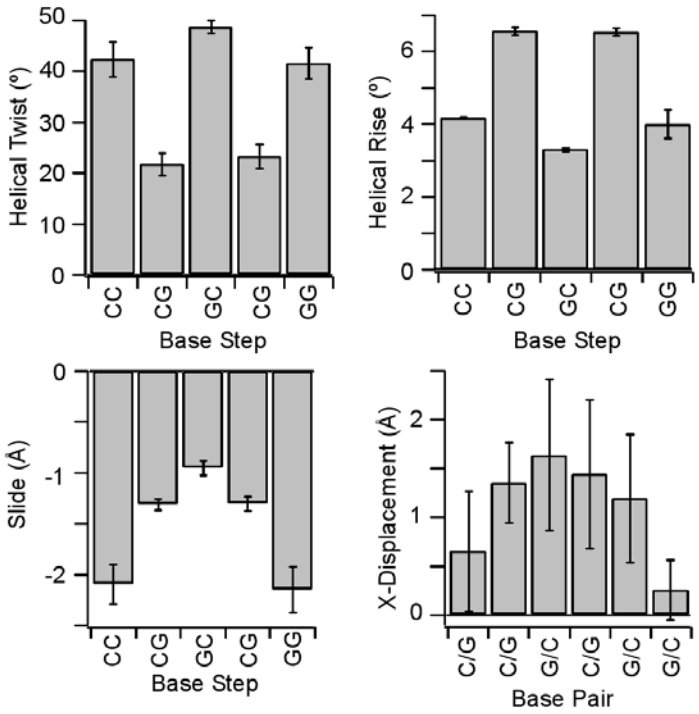

Figure S1. Bar graphs of average helical parameters for the 10 lowest energy structures of the 2′,5′ RNA duplex $(GCCGCGGC)_2$ with proflavine bound at the two CpG steps. Only the central five out of the seven base steps are shown. Error bars indicate standard deviations.

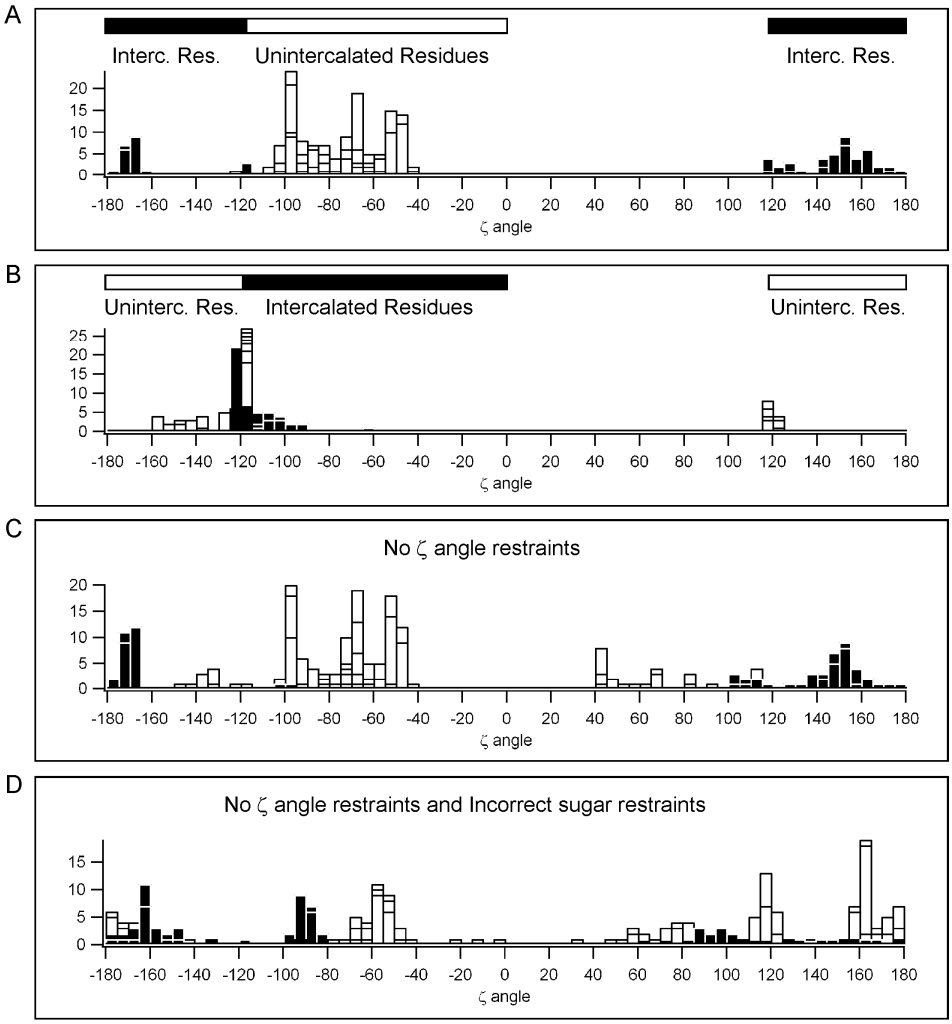

Figure S2. Zeta angles compiled from the 30 lowest energy structures after the second annealing refinement. Intercalated residue restraints correspond to ζ angles on the C3pG4 and C5pG6 phosphates. Unintercalated residue restraints correspond to all other ζ angles. A) ζ angles defined by what has been previously observed for 3′,5′-linked nucleic acids. B) ζ angles opposite of what was previously observed for 3′,5′-linked nucleic acids. C) No ζ angle restraints. D) No ζ angle restraints and the opposite sugar pucker restraints with respect to what is observed by NMR spectroscopy.

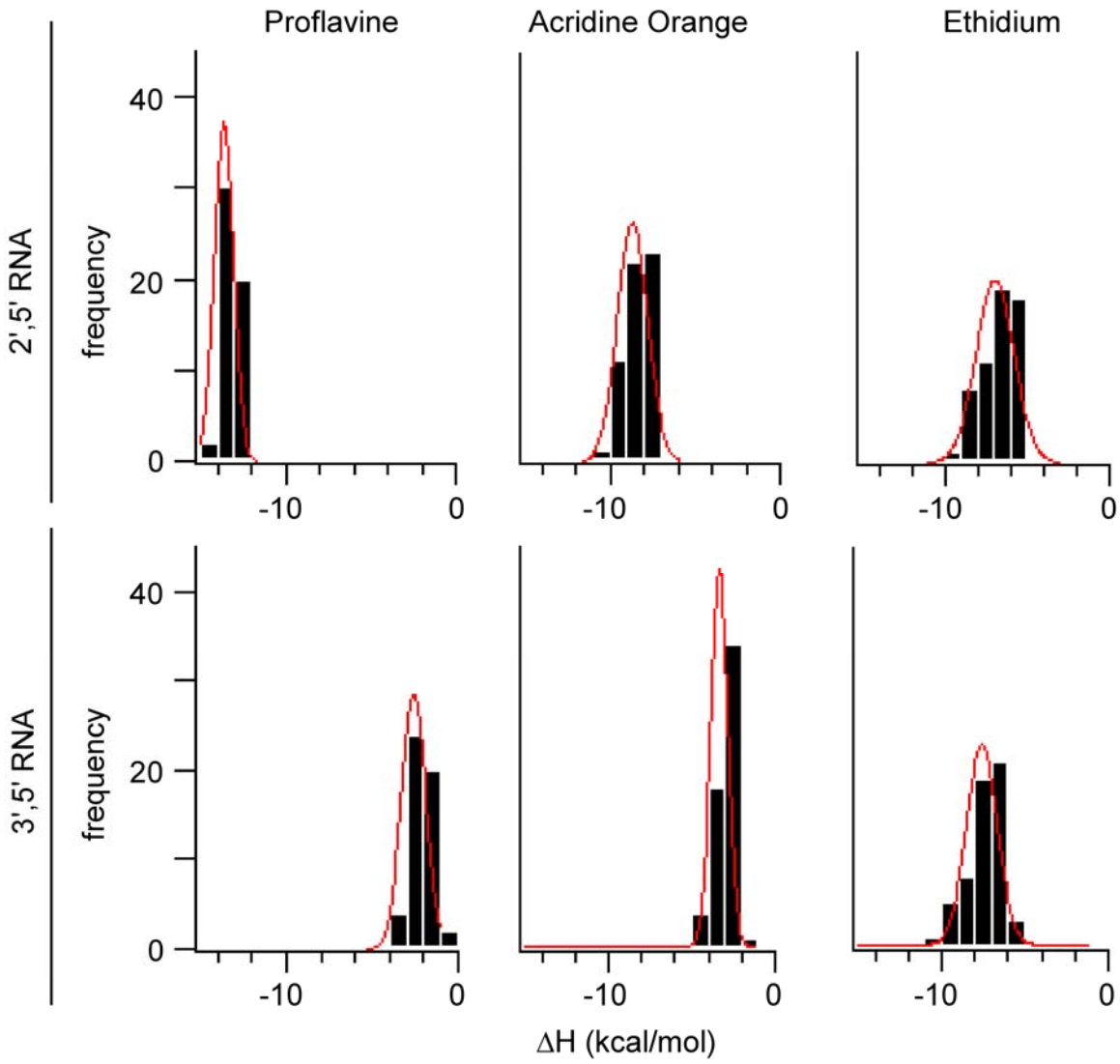

Figure S3. Histograms showing the enthalpy distributions obtained from model-free ITC.

	$\Delta G_{2',5'}$	$\Delta G_{3',5'}$	$\Delta H_{2',5'}$	$\Delta H_{3',5'}$	$-T\Delta S_{2',5'}$	$-T\Delta S_{3',5'}$
Proflavine	-7.8	-5.9	-13.6	-2.6	+5.8	-3.3
Acridine orange	-6.4	-5.3	-8.7	-3.4	+2.3	-1.9
Ethidium	-6.0	-6.4	-6.9	-7.6	+0.9	+1.2

Figure S4. Enthalpy (ΔH) and entropy (-TΔS) values for the intercalation of 2′,5′ RNA and 3′,5′ RNA with proflavine, acridine orange, and ethidium at 25 °C. Data shown are in kcal/mol.

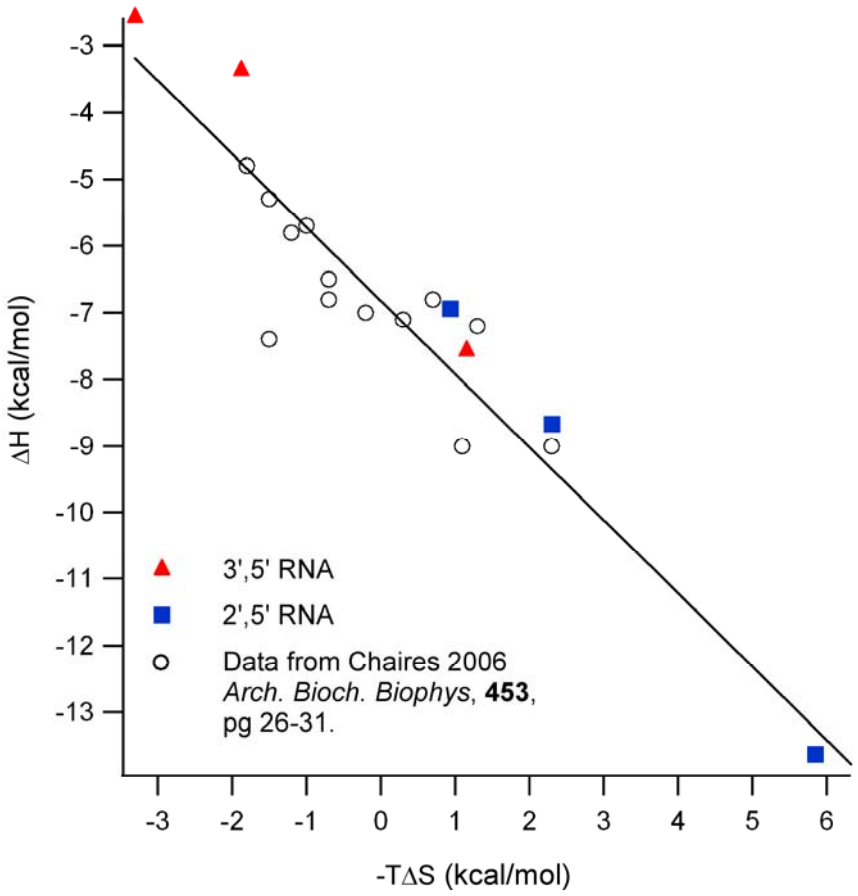

Figure S5. The linear relationship between enthalpy and entropy for intercalators binding to nucleic acids. The compilation of data reproduced from the work of Chaires[1] shows the linear relationship for intercalators binding to DNA. The same trend is observed for 3′,5′ RNA as well as 2′,5′ RNA.

(1) Chaires, J. B., A thermodynamic signature for drug-DNA binding mode. *Arch. Biochem. Biophys.* **2006,** 453, 26-31.