

Supporting Information Table 1. Scalar couplings measured with the H1C1C2 experiment for helix-35.<sup>a</sup>

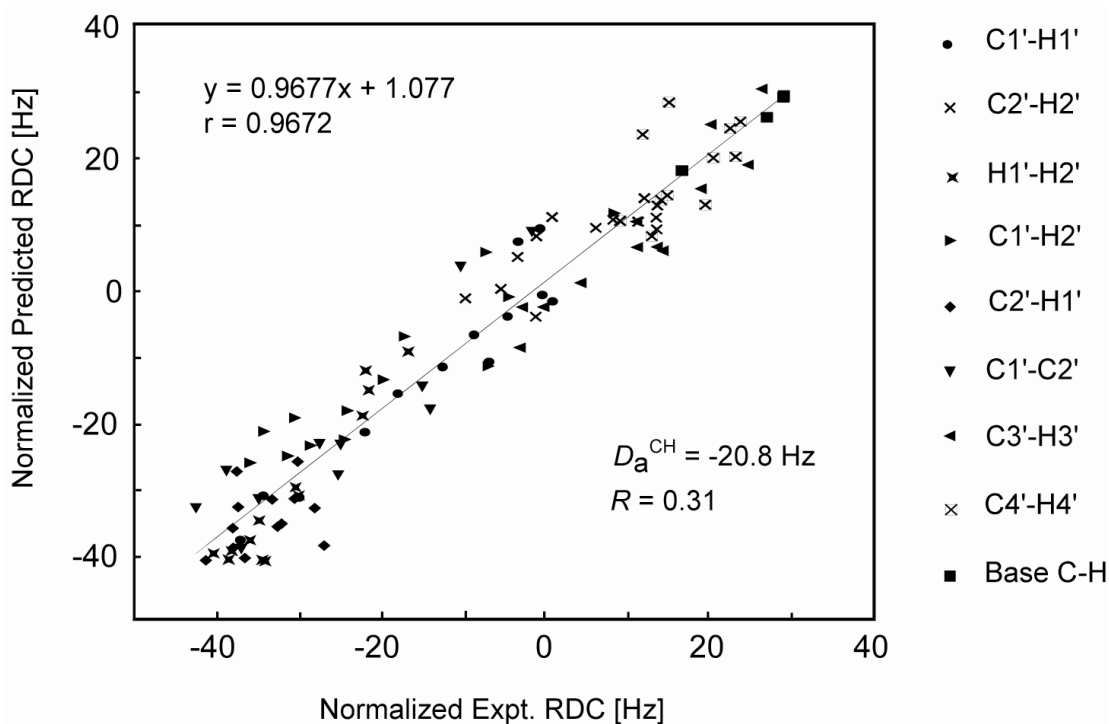
	JC1'-H1'	JC2'-H2'	JC1'-H2'	JC2'-H1'	JH1'-H2' <sup>b</sup>
<b>G738</b>	176.68 ± 0.10	158.22 ± 0.09	2.42 ± 0.10	-1.50 ± 0.09	0.79 ± 0.16
<b>G739</b>	176.33 ± 0.07	157.90 ± 0.07	2.86 ± 0.07	-1.85 ± 0.07	0.67 ± 0.12
<b>C740</b>	180.45 ± 0.07	159.72 ± 0.06	1.70 ± 0.07	-2.14 ± 0.06	1.07 ± 0.12
<b>U741</b>	179.91 ± 0.08	158.90 ± 0.07	2.11 ± 0.08	-2.43 ± 0.07	0.78 ± 0.13
<b>A742</b>	175.54 ± 0.08	157.02 ± 0.08	2.33 ± 0.08	-1.98 ± 0.08	1.02 ± 0.14
<b>A743</b>	175.86 ± 0.07	157.54 ± 0.06	2.76 ± 0.07	-1.78 ± 0.06	0.94 ± 0.12
<b>U744</b>	178.71 ± 0.12	158.81 ± 0.11	1.60 ± 0.12	-2.63 ± 0.11	0.93 ± 0.21
<b>G745</b>	173.44 ± 0.20	157.27 ± 0.24	1.13 ± 0.20	-2.28 ± 0.24	1.73 ± 0.39
<b>U747</b>	173.97 ± 0.21	157.78 ± 0.28	0.14 ± 0.21	-2.46 ± 0.28	3.32 ± 0.40
<b>G748</b>	168.51 ± 0.28	151.37 ± 0.34	-1.29 ± 0.28	-3.28 ± 0.34	4.24 ± 0.50
<b>A749</b>	168.65 ± 0.16	158.10 ± 0.20	-0.93 ± 0.16	-2.45 ± 0.20	3.84 ± 0.31
<b>A750</b>	171.60 ± 0.80	157.25 ± 2.50	-2.28 ± 1.00	-1.48 ± 0.93	5.36 ± 2.10
<b>A751</b>	170.32 ± 0.42	153.30 ± 0.52	0.50 ± 1.00	-3.44 ± 0.44	3.75 ± 0.90
<b>A752</b>	171.10 ± 1.04	153.02 ± 1.36	1.00 ± 2.00	-1.96 ± 0.57	2.37 ± 2.00
<b>A753</b>	175.94 ± 0.70	155.85 ± 0.91	2.13 ± 0.50	-3.40 ± 0.30	1.88 ± 0.64
<b>U754</b>	179.98 ± 0.08	158.96 ± 0.08	2.24 ± 0.08	-2.81 ± 0.08	0.89 ± 0.14
<b>U755</b>	178.89 ± 0.08	158.54 ± 0.08	2.03 ± 0.08	-2.76 ± 0.08	1.22 ± 0.13
<b>A756</b>	175.14 ± 0.12	157.18 ± 0.13	2.14 ± 0.12	-1.74 ± 0.13	1.23 ± 0.21
<b>G757</b>	175.57 ± 0.07	157.76 ± 0.06	2.85 ± 0.07	-2.32 ± 0.06	0.84 ± 0.11
<b>C758</b>	179.61 ± 0.07	158.97 ± 0.06	1.96 ± 0.07	-2.31 ± 0.06	0.84 ± 0.11
<b>C759</b>	180.00 ± 0.06	158.55 ± 0.06	1.92 ± 0.06	-2.67 ± 0.06	0.82 ± 0.10
<b>C760</b>	174.88 ± 0.04	155.89 ± 0.03	0.54 ± 0.04	-2.60 ± 0.03	1.78 ± 0.05

<sup>a</sup> All values in Hertz.<sup>b</sup> Reported values have been divided by 0.8 to correct for change in H2' spin state during the final Rance-Kay transfer.

Supporting Information Table 2. Dipolar couplings measured with the H1C1C2 experiment for helix-35.<sup>a</sup>

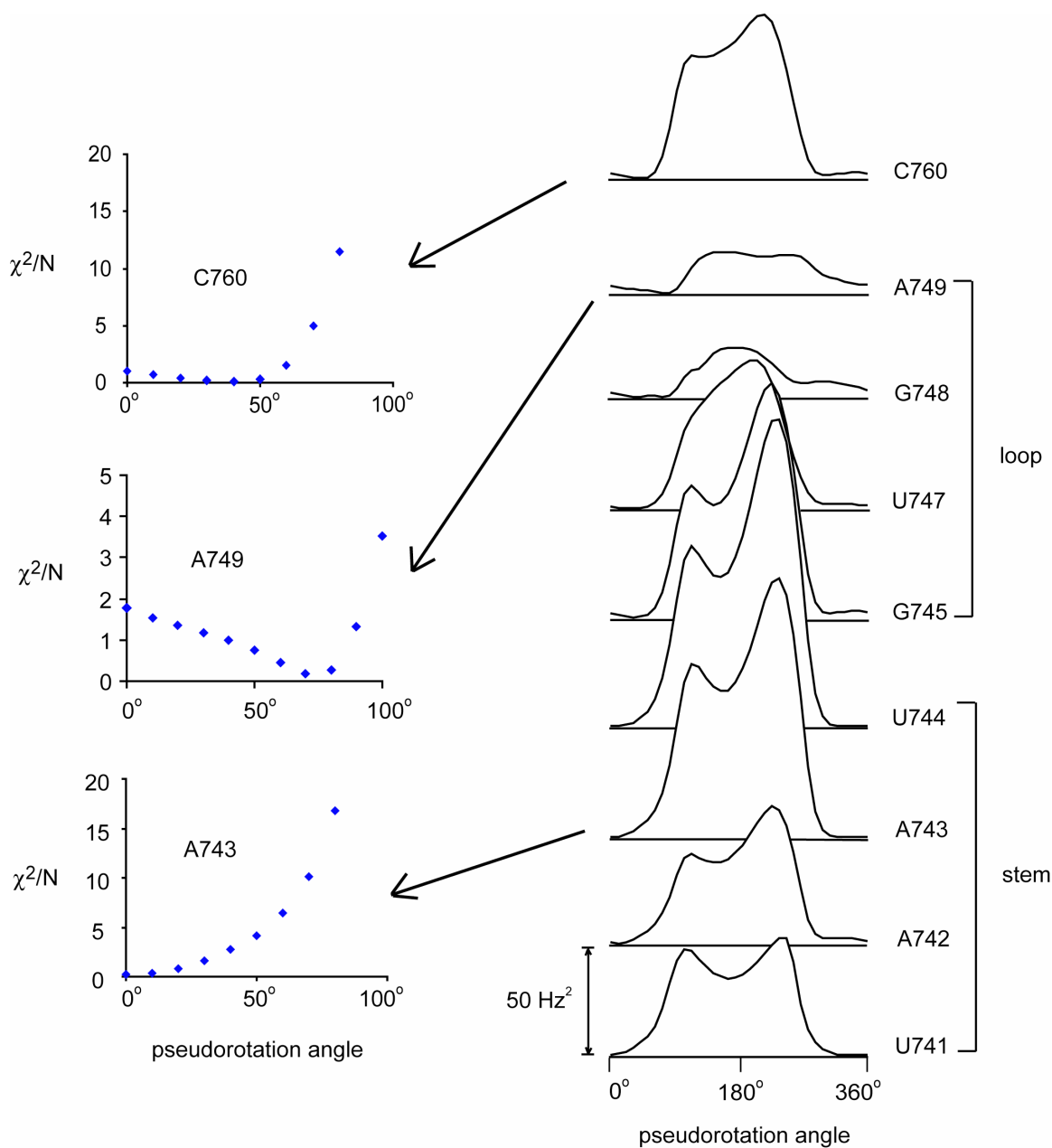
	DC1'-H1'	DC2'-H2'	DC1'-H2'	DC2'-H1'	DH1'-H2' <sup>b</sup>
<b>G738</b>	-29.8 ± 0.2	12.1 ± 0.1	1.1 ± 0.2	-4.9 ± 0.1	-4.5 ± 0.2
<b>G739</b>	-37.0 ± 0.2	11.3 ± 0.1	-0.9 ± 0.2	-3.9 ± 0.1	-5.8 ± 0.1
<b>C740</b>	-34.2 ± 0.1	8.3 ± 0.1	-2.2 ± 0.1	-4.0 ± 0.1	-8.2 ± 0.1
<b>U741</b>	-21.8 ± 0.1	0.8 ± 0.1	-2.6 ± 0.1	-3.7 ± 0.1	-9.4 ± 0.1
<b>A742</b>	-12.4 ± 0.2	-1.1 ± 0.1	-3.1 ± 0.2	-4.2 ± 0.1	-9.7 ± 0.2
<b>A743</b>	-4.5 ± 0.1	-5.4 ± 0.1	-4.0 ± 0.1	-5.0 ± 0.1	-10.9 ± 0.1
<b>U744</b>	1.0 ± 0.2	-1.2 ± 0.2	-4.1 ± 0.2	-4.8 ± 0.2	-9.3 ± 0.2
<b>G745</b>	-2.2 ± 0.4	8.7 ± 0.3	-3.5 ± 0.4	-4.9 ± 0.3	-6.7 ± 0.5
<b>U747</b>	-4.9 ± 0.4	13.1 ± 0.4	0.2 ± 0.4	-3.7 ± 0.4	-3.2 ± 0.4
<b>G748</b>	4.6 ± 0.6	-3.0 ± 0.5	0.6 ± 0.6	-2.0 ± 0.5	-2.9 ± 0.6
<b>A749</b>	7.9 ± 0.3	-6.6 ± 0.3	-1.3 ± 0.3	-1.5 ± 0.3	-2.0 ± 0.3
<b>A750</b>	3.8 ± 3.0	-4.7 ± 3.5	-1.1 ± 1.5	-1.9 ± 1.0	-3.5 ± 2.5
<b>A751</b>	-0.5 ± 0.6	4.8 ± 1.5	-2.1 ± 1.5	-1.0 ± 0.7	-3.9 ± 1.0
<b>A752</b>	-15.1 ± 1.7	7.8 ± 1.6	2.7 ± 3.3	-3.9 ± 1.7	-0.7 ± 2.2
<b>A753</b>	-23.5 ± 1.3	5.0 ± 2.0	-2.4 ± 1.5	-3.4 ± 1.1	-8.6 ± 2.0
<b>U754</b>	-17.8 ± 0.2	-9.7 ± 0.1	-4.5 ± 0.2	-4.2 ± 0.1	-10.3 ± 0.2
<b>U755</b>	-6.7 ± 0.1	-3.4 ± 0.1	-4.7 ± 0.2	-3.5 ± 0.1	-10.4 ± 0.2
<b>A756</b>	-0.3 ± 0.2	6.2 ± 0.2	-3.7 ± 0.3	-5.4 ± 0.2	-9.2 ± 0.2
<b>G757</b>	-0.5 ± 0.1	13.6 ± 0.1	-3.1 ± 0.2	-5.0 ± 0.1	-8.1 ± 0.1
<b>C758</b>	-3.2 ± 0.1	13.5 ± 0.1	-0.9 ± 0.2	-4.9 ± 0.1	-6.0 ± 0.1
<b>C759</b>	-8.5 ± 0.1	13.6 ± 0.1	-0.6 ± 0.1	-4.4 ± 0.1	-5.9 ± 0.1
<b>C760</b>	-9.0 ± 0.1	20.6 ± 0.1	-1.5 ± 0.1	-4.4 ± 0.1	-4.1 ± 0.1

<sup>a</sup> All values in Hertz.<sup>b</sup> Reported values have been divided by 0.8 to correct for change in H2' spin state during the final Rance-Kay transfer.

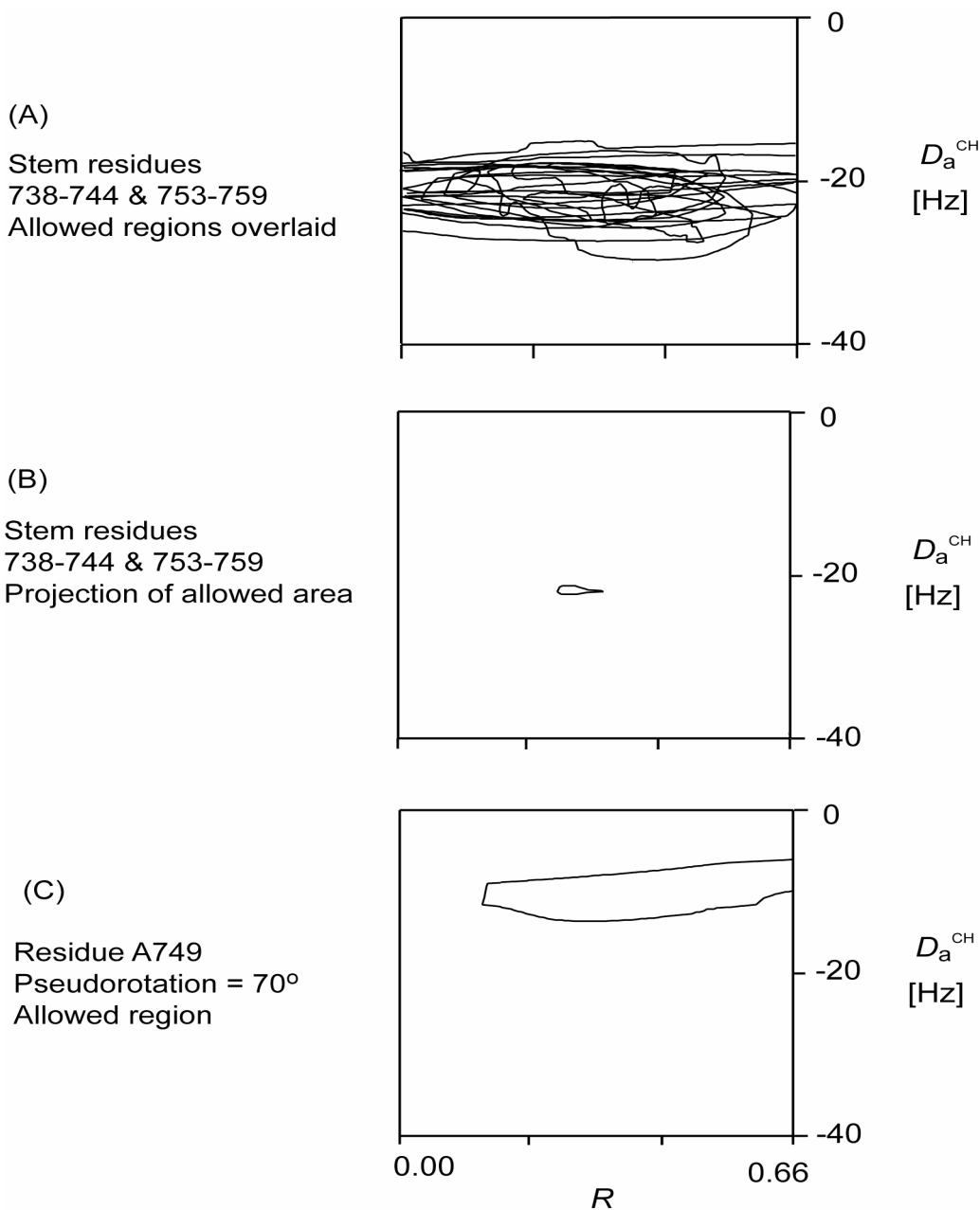


**Supporting information Figure 1:** Correlation plot of normalized predicted RDCs measured for the 12 nucleotides comprising the stem region of helix-35<sup>ψ746</sup> RNA (G738-A743 and C759-U754) vs experimental couplings obtained from an SVD fit of the data to the 1QCU structure. Also included in the fit are four one-bond carbon-proton base RDCs (C8-H8 for A756, G757; C6-H6 for C740, U741) for nucleotides that matched the base type of the 36-fold averaged X-ray structure oligomer, modeled as a repeat of 18 GC basepairs (Klosterman, P. S., Shah, S. A., Steitz, T. A., *Biochemistry* 38, 14784,1999).

*Note: A labelling error in the scale of the above plot has been corrected in this version of the Supporting Information.*



**Supporting Information Figure 2.** Residual of the SVD fit ( $\chi^2/N$ ) between normalized experimental  $D(C1'H1')$ ,  $D(C2'H2')$ ,  $D(C3'H3')$ ,  $D(C4'H4')$ ,  $D(C1'H2')$ ,  $D(C2'H1')$ ,  $D(H1'H2')$  and  $D(C1'C2')$  dipolar couplings and values best fitted for a ribose with a pucker amplitude of 35°, as a function of the pseudorotation phase angle, for representative stem and loop residues of Helix-35 $\psi$ <sup>746</sup>. Expanded regions are shown on the left for A743, A749, and C760.



**Supporting Information Figure 3.** Contour plots of systematic ( $D_a^{\text{CH}}, R$ ) grid search results, where experimental dipolar couplings are best fitted to (A,B) a C3'-endo ribose (in all cases allowing the orientation of the alignment tensor to vary during the fit, but keeping  $D_a^{\text{CH}}$  and  $R$  fixed), and (C) to a ribose with a pseudorotation phase of  $70^\circ$  and a pucker amplitude of  $35^\circ$ . (A) Superposition of the contours that mark ( $D_a^{\text{CH}}, R$ ) regions, where a fit within experimental error can be obtained for all 12 stem riboses. (B) Projection of the 12 superimposed grid search results of panel (A), displaying the common ( $D_a^{\text{CH}}, R$ ) region where all 12 riboses can simultaneously satisfy the dipolar couplings to within experimental error. (C) Results for loop nucleotide A749, indicating a much reduced  $D_a^{\text{CH}}$  and a relatively poorly defined P. All contours are drawn at  $\chi^2/N = 4 \text{ Hz}^2$ , i.e., assuming a 2 Hz random error in the normalized couplings.