Supporting Information

Accurate measurement of ^{15}N-^{13}C residual dipolar couplings in nucleic acids

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Table S1. One-bond ^{15}N-^{13}C isotropic J couplings in helix-35ψ.

Nucleotide	$^1J_{N1/9-C1'}$ (Hz)	$^1J_{N1/9-C6/8}$ (Hz)	$^1J_{N1/9-C2/4}$ (Hz)
G37	-10.33 ± 0.19	-12.43 ± 0.23†	-18.35 ± 0.38
G38	-9.36 ± 0.04	-11.76 ± 0.05	-18.64 ± 0.07
G39	-9.10 ± 0.03	-11.93 ± 0.03	-18.43 ± 0.05
C40	-9.72 ± 0.01	-13.37 ± 0.02†	-12.79 ± 0.03
U41	-10.00 ± 0.02	-13.22 ± 0.02	-17.91 ± 0.03
A42	-9.22 ± 0.02	-12.15 ± 0.03†	-18.24 ± 0.05
A43	-9.08 ± 0.02	-11.75 ± 0.04	-18.18 ± 0.05
U44	-10.24 ± 0.04	-13.07 ± 0.03	-17.82 ± 0.07
G45	-9.85 ± 0.13	-11.48 ± 0.13	-18.56 ± 0.21
U47	-11.55 ± 0.06	-13.22 ± 0.03	-18.65 ± 0.04
G48	-10.40 ± 0.23	-11.61 ± 0.03	-18.93 ± 0.05
A49	-10.70 ± 0.06	-12.04 ± 0.02	-18.60 ± 0.04
A50	-10.71 ± 0.19	-11.84 ± 0.03	-18.53 ± 0.05
A51	-10.32 ± 0.17	-12.47 ± 0.20†	-18.40 ± 0.35
A53	-9.19 ± 0.05*	-11.99 ± 0.06	-18.28 ± 0.10
U54	-9.81 ± 0.02	-13.11 ± 0.03	-17.70 ± 0.05
U55	-10.04 ± 0.02	-13.10 ± 0.03	-17.70 ± 0.04
A56	-9.43 ± 0.04	-12.09 ± 0.04†	-18.34 ± 0.07
G57	-9.19 ± 0.02	-11.83 ± 0.03	-18.49 ± 0.05
C58	-9.74 ± 0.01	-13.32 ± 0.02†	-12.76 ± 0.03
C59	-9.87 ± 0.01	-13.57 ± 0.02	-12.95 ± 0.04
C60	-10.87 ± 0.01	-13.62 ± 0.02	-13.31 ± 0.03
Adenine (7)	*-9.81 ± 0.74*	*-12.05 ± 0.23*	*-18.37 ± 0.15*
Guanine (6)	*-9.71 ± 0.57*	*-11.84 ± 0.33*	*-18.57 ± 0.21*
Cytidine (4)	*-10.05 ± 0.55*	*-13.47 ± 0.15*	*-12.95 ± 0.25*
Uridine (5)	*-10.33 ± 0.70*	*-13.14 ± 0.07*	*-17.95 ± 0.40*

All $^1J_{N1/9-C1'}$ and $^1J_{N1/9-C6/8}$ values are obtained from 3D MQ-HCN-QJ ($^1J_{N1/9-C1'}$) and 3D TROSY-HCN-QJ ($^1J_{N1/9-C6/8}$) experiments, respectively, which are compensated for natural ^{13}C abundance effects (see text), unless specified otherwise (see below). The $^1J_{N1/9-C2/4}$ values are obtained from 3D MQ-HCN-QJ ($^1J_{N1/9-C2/4}$) and 3D TROSY-HCN-QJ ($^1J_{N1/9-C2/4}$) experiments and the values reported in the table are *not* corrected for natural ^{13}C abundance effects. Based on the $^1J_{N1/9-C1'}$ and $^1J_{N1/9-C6/8}$ results (see below), the true $^1J_{N1/9-C2/4}$ couplings are expected to differ from the values in given in the table by approximately -1 Hz, i.e., we expect the average $^1J_{N9-C4}$ for adenine bases to be ca. -19.4 Hz. For $^1J_{N1/9-C2/4}$, average values of 3D MQ-HCN-QJ ($^1J_{N1/9-C2/4}$) and 3D TROSY-HCN-QJ ($^1J_{N1/9-C2/4}$) experiments were used where available (pairwise rmsd for a set of 11 couplings measured using both experiments was 0.08 Hz), except for the loop nucleotides U47-A50 where values obtained from the 3D TROSY-HCN-QJ ($^1J_{N1/9-C2/4}$) experiment are given, due to low S/N of these correlations in 3D MQ-HCN-QJ spectra (see text). Uncertainties were calculated based on the S/N ratios in reference spectra as described in Table 1. Also given are the average J couplings according to nucleotide type, with the number of nucleotides used to calculate the average J value given in parentheses.

* $^1J_{N1/9-C1'}$ is obtained by correcting the value obtained from 3D TROSY-HCN-QJ ($^1J_{N1/9-C1'}$) experiment by -0.89 Hz. The average difference due to natural ^{13}C abundance effects between the apparent J value obtained using 3D MQ-HCN-QJ ($^1J_{N1/9-C1'}$) and TROSY-HCN-QJ ($^1J_{N1/9-C1'}$) was J(MQ) - J(TROSY) = -0.89±0.15 Hz for 15 nucleotides, where couplings could be obtained using both methods.

† $^1J_{N1/9-C6/8}$ is obtained by correcting the value obtained from 3D MQ-HCN-QJ ($^1J_{N1/9-C6/8}$) experiment by -1.44 Hz. The average difference due to natural ^{13}C abundance effects between the apparent J value obtained using 3D MQ-HCN-QJ ($^1J_{N1/9-C6/8}$) and TROSY-HCN-QJ ($^1J_{N1/9-C6/8}$) was J(TROSY) - J(MQ) = -1.44±0.20 Hz for 15 nucleotides, where couplings could be obtained using both methods.

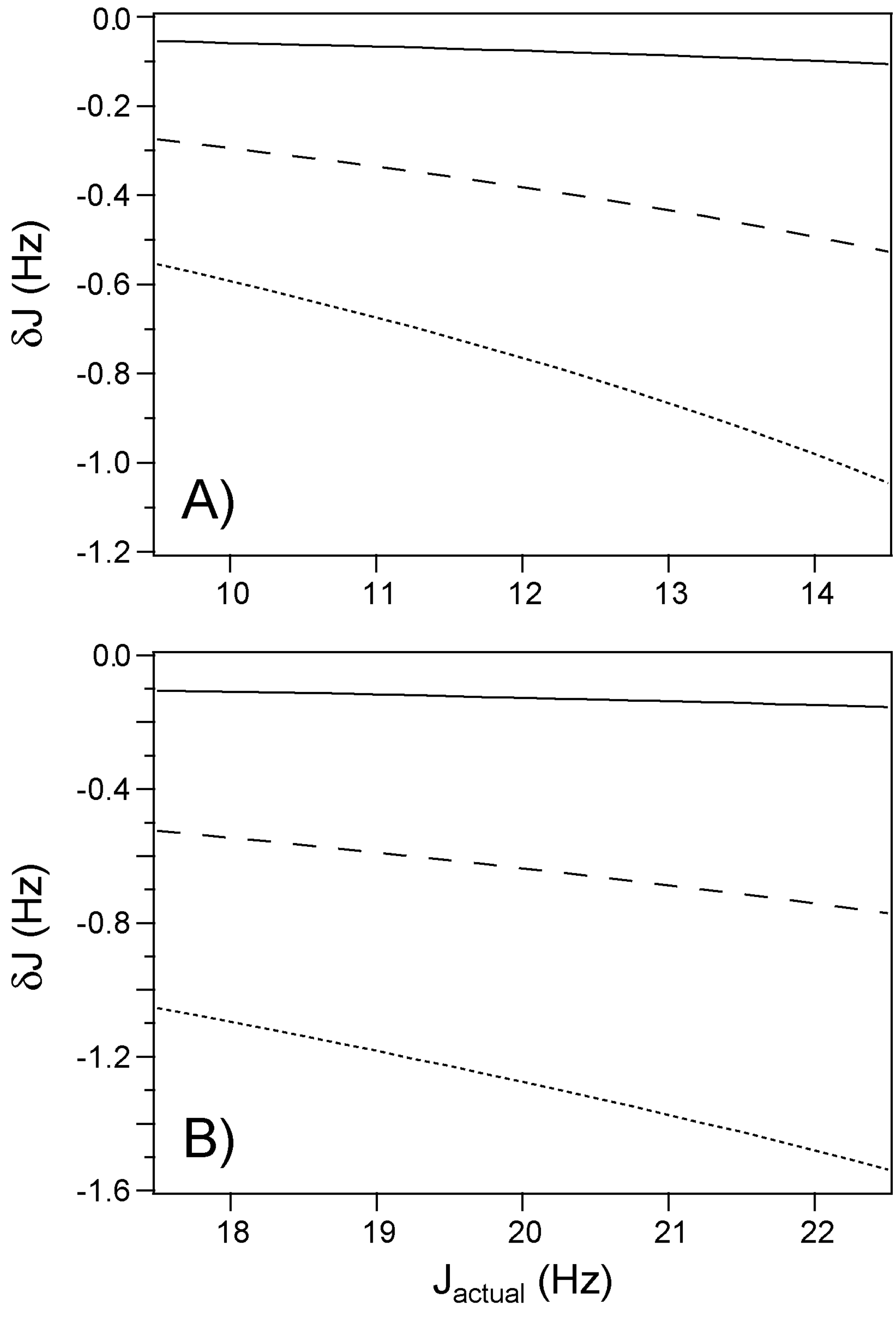

Figure S1. Simulations of the effect of incomplete ^{13}C enrichment on measurement of $^{1}J_{NC}$ using MQ-HCN-QJ and TROSY-HCN-QJ methods (see text). Plots of difference, δJ, between the apparent J coupling extracted using Equation 1 of the main text and the actual J coupling are shown for ^{13}C labeling efficiencies of 99% (solid line), 95% (dashed line) and 90% (dotted line) for target ^{15}N-^{13}C J-couplings of 12 Hz (A) and 20 Hz (B). Note that, even though the underestimate of $^{1}J_{NC}$ is relatively large, the error in the measured $^{1}D_{NC}$ results from the difference in $^{1}J_{NC}$, where these errors largely cancel.