

Characterizing μ s-ms Exchange in Labeled and Unlabeled Nucleic Acids by Carbon $R_{1\rho}$ NMR Spectroscopy

Alexandar L. Hansen, Evgenia N. Nikolova, Anette Casiano-Negrone, Hashim M. Al-Hashimi*
Department of Chemistry and Biophysics, University of Michigan, 930 N. University, Ann Arbor, MI, 48109

Supplementary Methods

Sample Preparation and Assignment

The uniformly $^{13}\text{C}/^{15}\text{N}$ labeled A-site rRNA sample was prepared by *in vitro* transcription using synthetic double stranded DNA templates containing a T7 promoter and the A-site rRNA sequence of interest (Integrated DNA Technologies, Inc.), T7 RNA polymerase (Takara Mirus Bio, Inc.), and $^{13}\text{C}/^{15}\text{N}$ labeled nucleotide triphosphates (Cambridge Isotopes, Inc.). The RNA was purified by 20% (w/v) denaturing polyacrylamide gel electrophoresis containing 8M urea and 1x TBE followed by electroelution in 20 mM Tris pH 8 buffer and ethanol precipitation. The RNA pellet was dissolved and exchanged into NMR buffer (15 mM sodium phosphate, 0.1 mM EDTA, and 25 mM NaCl at pH ~6.4) using a Centricon Ultracel YM-3 concentrator (Millipore Corp.). The final NMR sample had an RNA concentration of ~1mM, NMR buffer and 10% D_2O . The A-site rRNA NMR spectra were assigned using conventional NMR methods such as 3D exchangeable ^1H - ^{15}N NOESY-HSQC, 3D non-exchangeable ^1H - ^{13}C NOESY-HSQC, 2D HCN ^1H , HCCH-COSY (correlates H2/H8 resonances)^{2,3}, 2D IP-COSY (correlated H5/H6 resonances)^{4,5}. The assignments were further verified with previous assignments.⁶

Unmodified DNA oligonucleotides were purchased from IDT, Inc. (Coralville, IA) and purified by standard desalting. The gel filtration grade 1,N6-ethenoadenine modified oligonucleotide (5' GATCCTeACCTTCG 3') was purchased from Midland Certified Reagent Company, Inc. (Midland, TX). The sequences for the control (A-DNA) and damaged (eA-DNA) duplex were identical (5' CGAAGGTAGGATC[G]/ [C]GATCCTXCCTTCG 3') except for the damaged residue (X = eA or A) and a terminal base pair substitution (in brackets) introduced in A-DNA to prevent spectral overlap of the target Adenine. The DNA oligos were resuspended in 10mM Na-MES (pH 6.1), 100 mM NaCl, 0.1 mM DTT, 0.1 mM EDTA buffer at ~ 200 μM concentration. Duplexes were annealed by mixing an equal molar ratio of the complementary DNA strands, heating for 2 min at 95°C and gradual cooling (~ 30 min) at room temperature. DNA preparations were further washed 3X in resuspension buffer by micro-centrifugation using an Amicon Ultra-4 centrifugal filter (3 kDa cutoff), concentrated to ~ 250 μl (~5 mM) for NMR studies and supplied with 10% D_2O . Exchangeable and non-exchangeable protons of the unlabeled DNA duplexes were assigned using conventional NMR methods (^1H , ^1H -NOESY)

at 25°C in 10% D₂O.⁷ The assignments for the adenine adduct were consistent with previous assignments.⁸ All 2D ¹³C, ¹H HSQC spectra of aromatic and sugar resonances were acquired at natural abundance.

Selective ¹³C R_{1ρ} Pulse Sequence

The selective R_{1ρ} pulse sequence is shown in Figure 1 of main paper. Solid, narrow bars represent hard 90° pulses, while the open, narrow ¹³C pulses apply a tip angle $\theta = \text{arccot}(|\Omega|/\omega_{13C})$, where Ω is the ¹³C resonance offset from the spinlock and ω_{13C} is the spinlock field strength. Open rectangles represent periods of continuous-wave irradiation for water presaturation (ca. 10 Hz), cross polarization (ω_{CP} , ca. 100 Hz), decoupling CH DD/CSA cross-correlated relaxation and ¹J_{CH} evolution (ω_{1H} , ca. 8-10 kHz), and the R_{1ρ} spinlock (ω_{13C} , 100 – 3500 Hz). Spinlock powers were calibrated as described previously.^{9,10} Additional purge elements at the end of the ω_{1H} spinlock are included to aid with water suppression. Decoupling during acquisition is accomplished with a 3.5 kHz GARP sequence. For uniformly ¹³C/¹⁵N samples 2.4 kHz and 1.0 kHz GARP decoupling is used on ¹³C and ¹⁵N channels, respectively. Application of prolonged high power spinlocks is known to cause sample heating effects. These effects can be on the order of several degrees, depending on the length and power of the spinlocks applied as well as different buffer conditions. In order to maintain a constant level of rf power applied to the sample, a heat compensation block is used after acquisition for a time $T_{\text{comp}} = T_{\text{max}} - T$, where T_{max} is the maximum relaxation delay used in the series. Variations in the ¹³C spinlock power were not accounted for the dispersion study given the constant high power proton spinlock used in all studies. Heat compensation is applied far off resonance to prevent perturbations of the proton magnetization. The nearly constant rf irradiation of the proton channel affords excellent water suppression even for ribose and C5 resonances near the water signal. Between points a and d, the ¹H carrier is placed on-resonance with the signal of interest while between b and c, the carbon spinlock is placed at a desired offset Ω from the ¹³C frequency of the resonance of interest. The optional ζ delay (dotted line) can be used to suppress ¹³C signals with similar ¹H frequencies, where $\zeta = \pi/(2\delta)$ and $\delta/(2\pi)$ is the carbon offset (in Hz) of the undesirable signal. In uniformly labeled samples, the presence of large homonuclear scalar couplings severely compromises the efficiency of the ζ delay and therefore should not be used on pyrimidine C5 or C6 or ribose resonances and only isolated spins ($|\delta| > \omega_{cp}$) should be studied. Simulations show that the effect is negligible for purine C2 and C8 resonances with $|\delta|/2\pi$ greater than 25 Hz. Delay τ_{eq} allows equilibration of the exchanging spins, optimally $\sim 3/k_{ex}$, and is set to 5 ms. Gradients 1,2, and 3 are applied for 1 ms with SMSQ1.100 profiles and amplitudes of 4.3, 8.9, 8.3, 9.7, and

6.1 G/cm, respectively. The phase cycle is $\phi_1 = \{8(y) 8(-y)\}$, $\phi_2 = \{-x x\}$, $\phi_3 = \{4(x) 4(-x)\}$, $\phi_4 = \{2(x) 2(-x)\}$, $\phi_{\text{rec}} = \{x -x -x x -x x x -x -x x x -x x -x x\}$. For $\Omega > 0$ (resonance downfield of spinlock), $\phi_3 = -y$, $\phi_4 = y$. For $\Omega < 0$, $\phi_3 = y$, $\phi_4 = -y$. Data for eA20 C5 and C2 were acquired using 1536 and 2048 transients, respectively, by setting the selective heteronuclear Hartman-Hahn transfers on-resonance with the desired peak yielding signal:noise ratios $> 40:1$ at $T = 0$.

Calculating Hartman-Hahn contributions to relaxation

Heteronuclear cross polarization can be achieved with high efficiency as long as $\omega_{\text{CP}}/(2\pi)$ is larger than $|J_{\text{CH}}| \sqrt{3}/4$. For nucleic acids, a value for $\omega_{\text{CP}}/(2\pi)$ of ~ 70 - 100 Hz can therefore be used to minimize transfers to nearby carbon resonances (within about $1.5 \times \omega_{\text{CP}}$).^{11,12} When using uniformly labeled samples, care must be taken to avoid Hartman-Hahn matching conditions owing to sizable scalar couplings (8-12 Hz) to remote carbons in the aromatic bases and large one-bond homonuclear couplings in both sugar and bases. The maximum efficiency of Hartman-Hahn transfer between spins I and S is given by¹³

$$A_{\text{HAHA}} = \left(1 + \left(\frac{\omega_{\text{eff},I} - \omega_{\text{eff},S}}{J_{\text{IS}}(1 + \cos(\theta_I - \theta_S))/2} \right)^2 \right)^{-1} \quad (\text{S1})$$

where $\omega_{\text{eff},X} = (\omega_1^2 + \Omega_X^2)^{1/2}$ is the effective spinlock strength at spin X, J_{IS} is the scalar coupling constant, and $\theta_X = \text{atan}(\omega_1/\Omega_X)$ is the tip angle of the magnetization of spin X with respect to the static magnetic field. In the present study, the chemical shifts of quaternary carbons in purine bases were determined using a TROSY relayed HCCH-COSY experiment.² Data with computed $A_{\text{HAHA}} > 1\%$ for the C2-C4 or C2-C6 couplings (using a J_{CC} of -1 Hz) and $> 0.1\%$ for C2-C5 couplings ($J_{\text{CC}} = 11$ Hz) were removed from the analysis (Supplementary Table 1)^{5,7}. Mono-exponential decays were observed for all offset/power combinations for the C2 spins of A08, A10, A92, and A93 and C1' of A93 in the bacterial ribosomal A-site (for examples, see Supplementary Figure 2).

Analyzing chemical exchange data

Chemical exchange in the ribosomal A-site was determined to be near intermediate exchange ($k_{\text{ex}} \approx |\Delta\omega|$) on the NMR timescale. Under these conditions, the simple expression for fast chemical exchange, $R_{1\rho} = R_1 \cos^2\theta + R_{2,0} \sin^2\theta + \sin^2\theta \Phi_{\text{ex}} k_{\text{ex}}/(\omega_{\text{eff}}^2 + k_{\text{ex}}^2)$, where $\Phi_{\text{ex}} = p_a p_b \Delta\omega^2$ is used as a single fitting parameter and $\omega_{\text{eff}} = (\omega_{13\text{C}}^2 + \Omega^2)^{1/2}$, is not adequate to

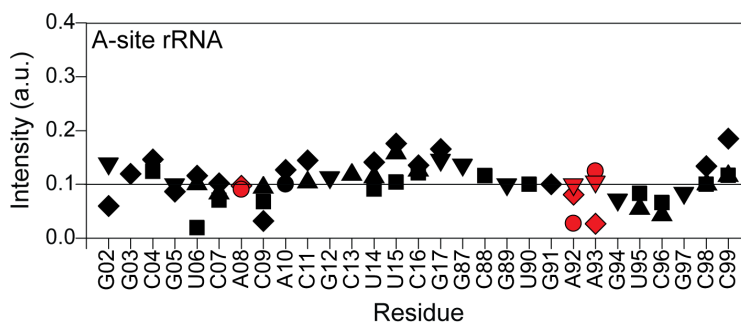
accurately determine chemical exchange parameters.¹⁴ Here, chemical exchange parameters were determined using the expression for asymmetric two-site chemical exchange,

$$R_{1\rho} = R_1 \cos^2 \theta + R_{2,0} \sin^2 \theta + \sin^2 \theta \frac{p_a p_b \Delta \omega^2 k_{ex}}{(\Omega + \Delta \omega)^2 + \omega_1^2 + k_{ex}^2} \quad (S2)$$

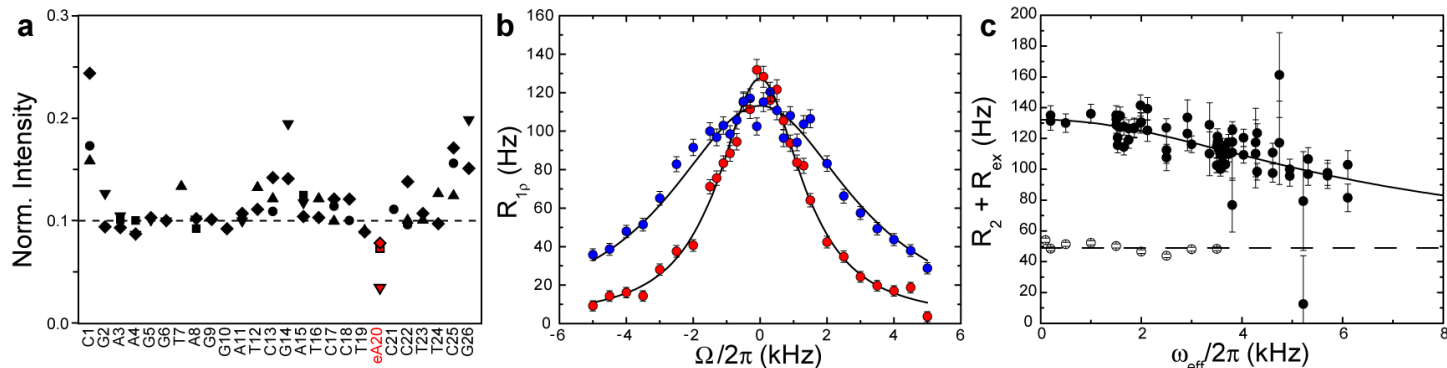
where $\Omega \approx \Omega_A$ is the resonance offset from the spinlock carrier, $\tan(\theta) = \omega_1 / \Omega_{avg}$, $\Delta \omega = \Omega_B - \Omega_A$, $\Omega_{avg} = p_a \Omega_A + p_b \Omega_B$.

The analysis was implemented using Origin v7.0383 (OriginLab Corporation). The best fit parameters, as determined from F-statistics at the 99% confidence level (Supplementary Table 2), yield R_2 of 33.71 ± 0.11 and 22.68 ± 0.42 Hz, $\Delta \omega$ of -0.96 ± 0.02 and -4.33 ± 0.11 ppm for A08 and A93, respectively, a p_b of 4.60 ± 0.12 %, and k_{ex} of 3133 ± 77 s⁻¹. The best fit R_2 for A10 is 34.70 ± 0.07 Hz.

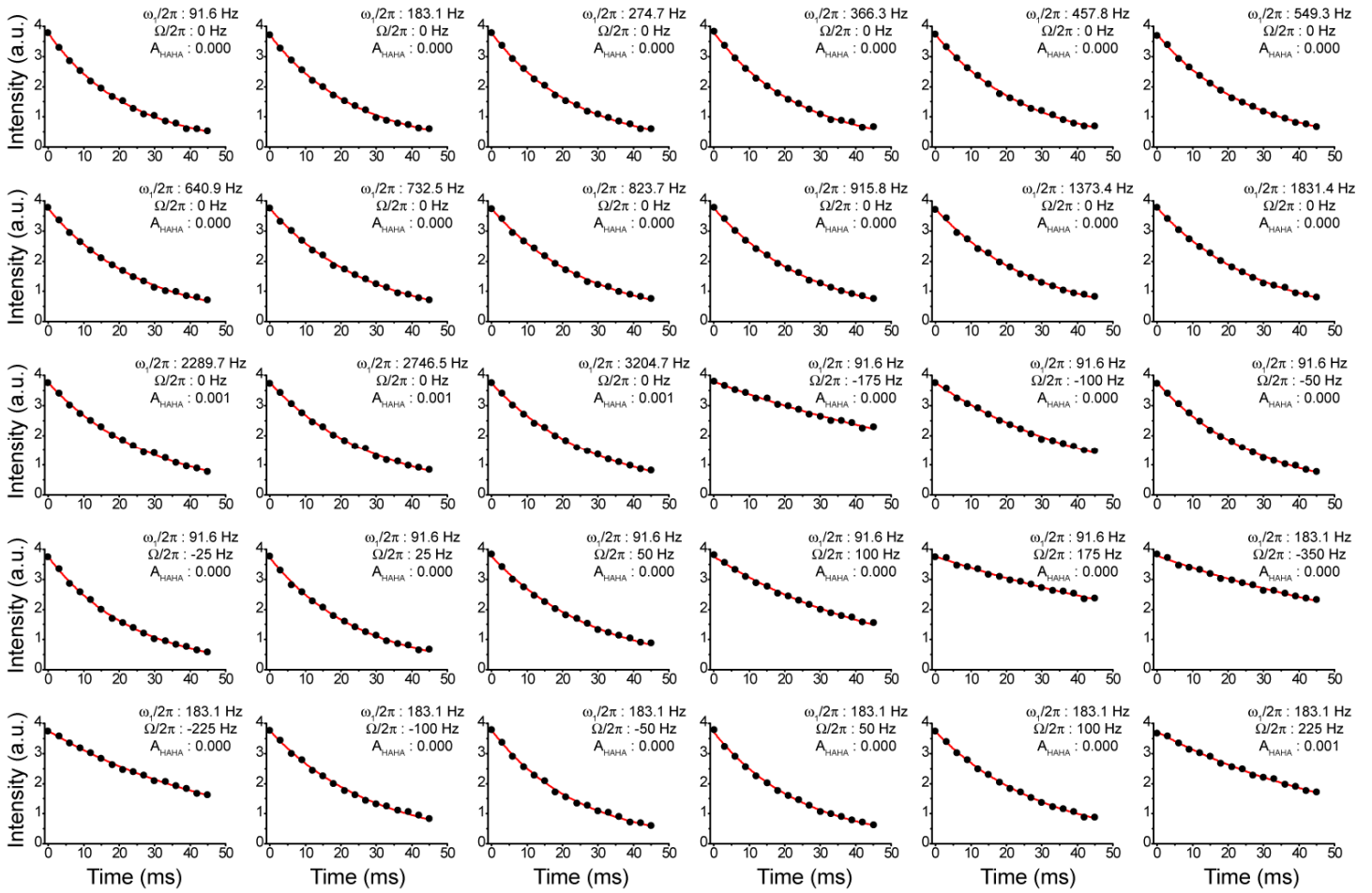
Chemical exchange in the unlabeled damaged DNA was determined to be fast ($k_{ex} \gg |\Delta \omega|$) on the NMR timescale. Under these conditions, the chemical shift difference between the exchanging states becomes inseparable from the populations and therefore the simple expression for fast exchange was used. The best fit parameters for the C2 resonance of the damaged base are $k_{ex} = 3.9 \pm 1.2 \times 10^4$ sec⁻¹, $R_1 = 2.87 \pm 0.93$ Hz, $R_{2,0} = 53 \pm 32$ Hz, and $\Phi_{ex} = 3.11 \pm 2.16 \times 10^6$ sec⁻².



Supplementary Figure 1. Normalized intensity measurements from HSQC experiments of the C1'H1' (diamonds), C2H2 (circles), C5H5 (squares), C6H6 (▲), and C8H8 (▼) spins. Internal loop adenines are highlighted in red.



Supplementary Figure 2. Evidence for chemical exchange in an unlabelled eA damaged DNA. (a) Normalized intensity measurements from HSQC experiments of the C1'H1' (diamonds), C2H2 (squares), C5H5 (circles), C6H6 (▲), and C8H8 (▼) spins. The square and triangle for residue eA20 represent C5H5 and C2H2 of the damaged base, respectively. (b,c) Relaxation dispersion profiles for C2 of eA20. Data was collected in a constant-time fashion with $R_{1p} = \ln(I_0/I_1)/T$, where I_0 and I_1 are the intensities of the signal at T is 0 and 8 ms, respectively. The experiments had a total acquisition time of 62 hours (1 hour per power/offset combination). Solid lines represent the best fit to the fast exchange model. (b) Offset and power dependence of R_{1p} . Shown are spinlock powers of 1.5 and 3.5 kHz in red and blue, respectively. (c) The complete effective-field dependence of $R_2 + R_{ex}$. Errors are calculated as $\sigma_{R_{1p}}/\sin^2\theta$, where $\sigma_{R_{1p}}$ is the R_{1p} measurement error. A sequence where eA20 is replaced with an adenine was used as a control (open symbols, C8 spin of A20). Dashed line represents the average R_2 of 49.0 Hz.



Supplementary Figure 3. Examples of mono-exponential decays for the C2 of A08 at a variety of offsets and spinlock powers, indicated in the frames along with the maximum calculated A_{HAHA} value.

Offset (Hz)	Power (Hz)	A08			A10		
		A _{HAHA} - C4	A _{HAHA} - C5	A _{HAHA} - C6	A _{HAHA} - C4	A _{HAHA} - C5	A _{HAHA} - C6
0	91.58	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0	183.14	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0	274.65	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0	366.25	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0	457.91	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0	549.26	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0	640.89	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0	732.45	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000
0	823.72	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000
0	915.76	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000
0	1373.35	0.0000	0.0000	0.0002	0.0001	0.0000	0.0000
0	1831.39	0.0000	0.0000	0.0003	0.0001	0.0000	0.0001
0	2289.71	0.0001	0.0000	0.0005	0.0002	0.0000	0.0001
0	2746.50	0.0001	0.0000	0.0008	0.0003	0.0000	0.0001
0	3204.65	0.0001	0.0000	0.0011	0.0004	0.0000	0.0002
-175	91.58	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-100	91.58	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-50	91.58	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-25	91.58	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
25	91.58	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
50	91.58	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
100	91.58	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
175	91.58	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-350	183.14	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-225	183.14	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-100	183.14	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-50	183.14	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
50	183.14	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
100	183.14	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
225	183.14	0.0000	0.0000	0.0078	0.0000	0.0000	0.0000
350	183.14	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002
-850	457.91	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-625	457.91	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-475	457.91	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-325	457.91	0.0001	0.0000	0.0000	0.0002	0.0000	0.0000
325	457.91	0.0000	0.0000	0.0001	0.0000	0.0000	0.0002
475	457.91	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
625	457.91	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
850	457.91	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-1700	915.76	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-1100	915.76	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-650	915.76	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-450	915.76	0.0003	0.0000	0.0000	0.0001	0.0000	0.0000
450	915.76	0.0000	0.0000	0.0001	0.0000	0.0000	0.0002
650	915.76	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1100	915.76	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1700	915.76	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-2500	2289.71	0.0000	0.0280	0.0000	0.0000	0.0030	0.0000
2500	2289.71	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-1800	2746.50	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000
1800	2746.50	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
-2000	3204.65	0.0000	0.0001	0.0000	0.0000	0.0002	0.0000
2000	3204.65	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
2800	3204.65	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
3500	3204.65	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
4800	3204.65	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6000	3204.65	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Supplementary Table 1. Hartman-Hahn efficiencies calculated for the C2 spins in A08 and A10 using equation S1.

Bold data were excluded from analysis. Spinlock offsets of $(\Omega_{C1'} + \Omega_{C2'})/2 \pm \omega_1$ ($\Omega_{C1'} = 87.8$ ppm, $\Omega_{C2'} = 74.1$ ppm) were avoided to prevent Hartman-Hahn matching during the experiments on C1' of A93.

Residue	N	F-statistic	p-value	R ₂ (Hz)	$\Delta\omega/2\pi$ (Hz)	p _b (%)	k _{ex} (s ⁻¹)
<i>Individual</i>							
A08 C2	56	1092.79	0	37.85 ± 0.06	--	--	--
				33.87 ± 0.12	199.7 ± 15.9	2.28 ± 0.34	2830.9 ± 106.0
A10 C2	56	0.83039	0.483333	34.70 ± 0.07	--	--	--
				34.64 ± 0.08	111.4 ± 259.8	0.79 ± 165.3	48.4 ± 10269.5
A93 C1'	64	2078.012	0	56.34 ± 0.22	--	--	--
				22.29 ± 0.46	641.1 ± 16.7	4.63 ± 0.13	3339.7 ± 110.5
<i>Shared p_b, k_{ex}</i>							
A08 C2	120	3.8989	2.30714E-2	33.71 ± 0.11	144.3 ± 2.8	4.60 ± 0.12	3133.3 ± 76.7
A93 C1'				22.68 ± 0.42	653.9 ± 16.1		

Supplementary Table 2. Chemical exchange parameters and statistical analysis indicating the presence of two separate motions. In bold are the final choice of parameters.

Supplementary References

1. Sklenar, V., Peterson, R.D., Rejante, M.R. & Feigon, J. *J. Biomol. NMR* **3**, 721-727 (1993).
2. Simon, B., Zanier, K. & Sattler, M. *J. Biomol. NMR* **20**, 173-176 (2001).
3. Furtig, B., Richter, C., Wohnert, J. & Schwalbe, H. *ChemBioChem* **4**, 936-962 (2003).
4. Xia, Y.L. et al. *Magn. Reson. Chem.* **43**, 372-379 (2005).
5. Fiala, R., Munzarova, M.L. & Sklenar, V. *J. Biomol. NMR* **29**, 477-490 (2004).
6. Fourmy, D., Yoshizawa, S. & Puglisi, J.D. *J. Mol. Biol.* **277**, 333-345 (1998).
7. Wijmenga, S.S. & van Buuren, B.N.M. *Prog. Nucl. Magn. Reson. Spectrosc.* **32**, 287-387 (1998).
8. Kouchakdjian, M. et al. *Biochemistry* **30**, 1820-1828 (1991).
9. Guennegues, M., Berthault, P. & Desvaux, H. *J. Magn. Reson.* **136**, 118-126 (1999).
10. Hansen, A.L. & Al-Hashimi, H.M. *J. Am. Chem. Soc.* **129**, 16072-16082 (2007).
11. Pelupessy, P., Chiarparin, E. & Bodenhausen, G. *J. Magn. Reson.* **138**, 178-181 (1999).
12. Pelupessy, P. & Chiarparin, E. *Concepts Magn. Reson.* **12**, 103-124 (2000).
13. Bax, A. & Davis, D.G. *J. Magn. Reson.* **63**, 207-213 (1985).
14. Palmer, A.G. & Massi, F. *Chem. Rev.* **106**, 1700-1719 (2006).