

Supporting Information:

Measurement of Methyl Axis Orientations in Invisible, Excited States of Proteins by Relaxation Dispersion NMR Spectroscopy

Andrew J. Baldwin, D. Flemming Hansen, Pramodh Vallurupalli and Lewis E. Kay

Complete reference (11) -Shen, Y.;Lange, O.;Delaglio, F.;Rossi, P.;Aramini, J.M.;Liu, G.;Eletsky, A.;Wu, Y.;Singarapu, K.K.;Lemak, A.;Ignatchenko, A.; Arrowsmith, C.H.; Szyperski, T.; Montelione, G.T.; Baker, D.; Bax, A. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 4685-4690.

Effects of Spin-Flips on Chemical Exchange: The ^{13}C spin of a methyl group is scalar coupled to its three directly attached protons with scalar coupling constant J_{CH} , giving rise to four distinct carbon transitions in NMR spectra, reflecting the different spin-states of the proton spins. The four lines (multiplet components) correspond to the density elements $C_+|\alpha\alpha\alpha\rangle\langle\alpha\alpha\alpha|$, $C_+[|\alpha\alpha\beta\rangle\langle\alpha\alpha\beta|+|\alpha\beta\alpha\rangle\langle\alpha\beta\alpha|+|\beta\alpha\alpha\rangle\langle\beta\alpha\alpha|]$, $C_+[|\beta\beta\alpha\rangle\langle\beta\beta\alpha|+|\beta\alpha\beta\rangle\langle\beta\alpha\beta|+|\alpha\beta\beta\rangle\langle\alpha\beta\beta|]$, $C_+|\beta\beta\beta\rangle\langle\beta\beta\beta|$ of Table 1 (see text) and are denoted by $C_+^{\alpha\alpha\alpha}, C_+^{\alpha\alpha\beta}, C_+^{\alpha\beta\beta}, C_+^{\beta\beta\beta}$ in what follows. These lines are centered at the frequencies

$$\{\omega - 3\pi(J_{\text{CH}} + D_{\text{CH}}), \omega - \pi(J_{\text{CH}} + D_{\text{CH}}), \omega + \pi(J_{\text{CH}} + D_{\text{CH}}), \omega + 3\pi(J_{\text{CH}} + D_{\text{CH}})\}$$
 with

intrinsic transverse relaxation rates of $\{R_{2,\text{int}}^{\alpha\alpha\alpha}, R_{2,\text{int}}^{\alpha\alpha\beta}, R_{2,\text{int}}^{\alpha\beta\beta}, R_{2,\text{int}}^{\beta\beta\beta}\}$. The time-dependence of each of the methyl-carbon magnetization components in the free precession limit,

assuming a two-site exchange model $A \xrightleftharpoons[k_{\text{BA}}]{k_{\text{AB}}} B$ can be written as

$$\frac{d}{dt} \vec{V} = - \begin{pmatrix} \rho^{A,\alpha\alpha\alpha} & -k_{BA} & -0.5R_{sel}^A & 0 & 0 & 0 & 0 & 0 \\ -k_{AB} & \rho^{B,\alpha\alpha\alpha} & 0 & -0.5R_{sel}^B & 0 & 0 & 0 & 0 \\ -1.5R_{sel}^A & 0 & \rho^{A,\alpha\alpha\beta} & -k_{BA} & -R_{sel}^A & 0 & 0 & 0 \\ 0 & -1.5R_{sel}^B & -k_{AB} & \rho^{B,\alpha\alpha\beta} & 0 & -R_{sel}^B & 0 & 0 \\ 0 & 0 & -R_{sel}^A & 0 & \rho^{A,\alpha\beta\beta} & -k_{BA} & -1.5R_{sel}^A & 0 \\ 0 & 0 & 0 & -R_{sel}^B & -k_{AB} & \rho^{B,\alpha\beta\beta} & 0 & -1.5R_{sel}^B \\ 0 & 0 & 0 & 0 & -0.5R_{sel}^A & 0 & \rho^{A,\beta\beta\beta} & -k_{BA} \\ 0 & 0 & 0 & 0 & 0 & -0.5R_{sel}^B & -k_{AB} & \rho^{B,\beta\beta\beta} \end{pmatrix} \vec{V} \quad [S1]$$

$$\vec{V} = (\mathbf{C}_+^{A,\alpha\alpha\alpha}, \mathbf{C}_+^{B,\alpha\alpha\alpha}, \mathbf{C}_+^{A,\alpha\alpha\beta}, \mathbf{C}_+^{B,\alpha\alpha\beta}, \mathbf{C}_+^{A,\alpha\beta\beta}, \mathbf{C}_+^{B,\alpha\beta\beta}, \mathbf{C}_+^{A,\beta\beta\beta}, \mathbf{C}_+^{B,\beta\beta\beta})^\dagger$$

where,

$$\begin{aligned} \rho^{A,\alpha\alpha\alpha} &= R_{2,int}^{A,\alpha\alpha\alpha} - i(\omega_A - 3\pi(J_{CH} + D_{CH}^A)) + k_{AB} + 1.5R_{sel}^A \\ \rho^{B,\alpha\alpha\alpha} &= R_{2,int}^{B,\alpha\alpha\alpha} - i(\omega_B - 3\pi(J_{CH} + D_{CH}^B)) + k_{BA} + 1.5R_{sel}^B \\ \rho^{A,\alpha\alpha\beta} &= R_{2,int}^{A,\alpha\alpha\beta} - i(\omega_A - \pi(J_{CH} + D_{CH}^A)) + k_{AB} + 1.5R_{sel}^A \\ \rho^{B,\alpha\alpha\beta} &= R_{2,int}^{B,\alpha\alpha\beta} - i(\omega_B - \pi(J_{CH} + D_{CH}^B)) + k_{BA} + 1.5R_{sel}^B \\ \rho^{A,\alpha\beta\beta} &= R_{2,int}^{A,\alpha\beta\beta} - i(\omega_A + \pi(J_{CH} + D_{CH}^A)) + k_{AB} + 1.5R_{sel}^A \\ \rho^{B,\alpha\beta\beta} &= R_{2,int}^{B,\alpha\beta\beta} - i(\omega_B + \pi(J_{CH} + D_{CH}^B)) + k_{BA} + 1.5R_{sel}^B \\ \rho^{A,\beta\beta\beta} &= R_{2,int}^{A,\beta\beta\beta} - i(\omega_A + 3\pi(J_{CH} + D_{CH}^A)) + k_{AB} + 1.5R_{sel}^A \\ \rho^{B,\beta\beta\beta} &= R_{2,int}^{B,\beta\beta\beta} - i(\omega_B + 3\pi(J_{CH} + D_{CH}^B)) + k_{BA} + 1.5R_{sel}^B \end{aligned} \quad [S2]$$

In eqs [S1,S2] the superscript ‘†’ indicates transpose and $R_{2,int}^{j,k}$, $j \in (A,B)$, $k \in \{\alpha\alpha\alpha, \alpha\alpha\beta, \alpha\beta\beta, \beta\beta\beta\}$ are the intrinsic transverse relaxation rates of each of the multiplet components of state j and $R_{sel}^A (R_{sel}^B)$ is the selective ^1H longitudinal relaxation rate for state A(B) that is given by the difference in relaxation rates between $4C_Z H_Z H_Z$ and $2C_Z H_Z$, $R_{sel} = R_1(4C_Z I_Z I_Z) - R_1(2C_Z I_Z)$. In the analysis of all the dispersion data we have assumed that the intrinsic relaxation rates are the same in ground and excited states ($R_{2,int}^{j,A} = R_{2,int}^{j,B}$, $R_{sel}^A = R_{sel}^B$).

Table S1 – Summary of residue specific chemical shift and RDC changes

		$\Delta\omega_C$ (ppm)		Direct RDC (Hz)		e) ΔD (CPMG) (Hz)	f) $D_E^*=(D_G-\Delta D)/k$ (Hz)	J (Hz)	
		a) Direct	b) CPMG	c) Holo (40mg/ml)	d) Apo (50mg/ml)			g) Apo	h) Holo
25°C	Leu18, δ 1	-0.262	-0.271 ± 0.003	8.9 ± 0.2	0.7 ± 0.3	-9.5 ± 0.3	8.23 ± 0.36	124.98 ± 0.26	124.75 ± 0.08
	Leu18, δ 2	0.316	0.359 ± 0.003	8.5 ± 0.4	-0.1 ± 0.2	-9.4 ± 0.4	7.54 ± 0.39	125.33 ± 0.20	124.95 ± 0.26
	Val21, γ 1	0.002	0.017 ± 0.005	-0.3 ± 0.4	-5.0 ± 0.2	-8.9 ± 0.6	3.16 ± 0.53	125.64 ± 0.13	125.62 ± 0.31
	Val21, γ 2	0.031	0.043 ± 0.005	9.5 ± 0.2	3.4 ± 0.3	-10.0 ± 0.6	10.80 ± 0.56	125.89 ± 0.18	125.82 ± 0.08
	Ile26, δ 1	0.075	0.101 ± 0.004	-6.2 ± 0.2	-5.5 ± 0.2	2.5 ± 0.5	-6.43 ± 0.44	125.43 ± 0.19	125.35 ± 0.15
	Leu27, δ 1	-0.025	-0.034 ± 0.009	-4.3 ± 0.3	-6.5 ± 0.2	-2.7 ± 1.5	-3.04 ± 1.18	125.54 ± 0.14	125.55 ± 0.11
	Ile29, δ 1	0.002	0.005 ± 0.002	-14.2 ± 0.6	0.5 ± 0.2	22.3 ± 0.3	-17.62 ± 0.30	124.81 ± 0.13	124.88 ± 0.13
	Val32, γ 1			-10.5 ± 0.3					125.89 ± 0.13
	Val32, γ 2			-8.7 ± 0.3					126.09 ± 0.19
	Leu38, δ 1	-0.318	-0.335 ± 0.002	-15.2 ± 0.2	-1.5 ± 0.3	17.7 ± 0.4	-15.50 ± 0.41	125.08 ± 0.30	125.15 ± 0.15
	Leu38, δ 2	0.494	0.506 ± 0.004	6.0 ± 0.2	-0.9 ± 0.2	-8.1 ± 0.5	5.77 ± 0.45	124.66 ± 0.05	124.48 ± 0.13
	Leu41, δ 1	-0.035	-0.052 ± 0.006	3.6 ± 0.2	1.4 ± 0.6	-3.2 ± 0.9	3.74 ± 0.86	124.96 ± 0.16	125.02 ± 0.04
	Leu41, δ 2	-0.008	-0.016 ± 0.006	5.5 ± 0.2	1.9 ± 0.2	-7.4 ± 0.8	7.51 ± 0.64	124.76 ± 0.14	124.81 ± 0.04
	Leu49, δ 1	-2.360	-2.331 ± 0.013	5.5 ± 0.3	-0.2 ± 0.2	-7.4 ± 1.5	5.80 ± 1.22	125.16 ± 0.13	124.75 ± 0.17
	Leu49, δ 2	-0.263	-0.282 ± 0.002	12.5 ± 0.2	1.3 ± 0.5	-14.5 ± 0.4	12.69 ± 0.53	124.48 ± 0.34	124.61 ± 0.04
	Val55, γ 1	0.364	0.412 ± 0.003	3.0 ± 0.3	6.3 ± 0.3	4.9 ± 0.4	1.12 ± 0.41	125.89 ± 0.18	125.89 ± 0.07
	Val55, γ 2	0.242	0.265 ± 0.003	20.6 ± 0.3	5.8 ± 0.3	-19.4 ± 0.5	20.30 ± 0.49	126.16 ± 0.19	126.22 ± 0.15
Leu57, δ 1	0.046	0.062 ± 0.005	14.0 ± 0.3	9.5 ± 0.2	-12.2 ± 0.6	17.44 ± 0.53	125.13 ± 0.12	125.08 ± 0.29	
Leu57, δ 2	0.005	0.005 ± 0.006	-0.1 ± 0.3	2.3 ± 0.2	7.5 ± 0.7	-4.23 ± 0.63	124.71 ± 0.14	124.61 ± 0.18	
5°C	Leu18, δ 1	-0.266	-0.325 ± 0.005	9.3 ± 0.2	0.3 ± 0.3	-12.0 ± 0.8	10.46 ± 0.76		
	Leu18, δ 2	0.316	0.358 ± 0.007	9.2 ± 0.3	0.3 ± 0.2	-7.1 ± 1.5	6.26 ± 1.28		
	Val21, γ 1	0.016	0.013 ± 0.010	-0.2 ± 0.2	-6.2 ± 0.2	-10.8 ± 1.2	3.92 ± 1.07		
	Val21, γ 2	0.029	0.031 ± 0.011	9.3 ± 0.2	2.9 ± 0.3	-9.8 ± 1.4	10.72 ± 1.19		
	Ile26, δ 1	0.075	0.128 ± 0.005	-6.4 ± 0.3	-6.5 ± 0.2	1.7 ± 0.8	-7.00 ± 0.71		
	Ile27, δ 1	-0.014	-0.027 ± 0.021	-4.6 ± 0.3	-7.9 ± 0.2	-2.9 ± 3.5	-4.25 ± 3.01		
	Ile29, δ 1	0.026	0.005 ± 0.005	-14.1 ± 1.0	-0.4 ± 0.2	24.8 ± 0.6	-21.33 ± 0.56		
	Val32, γ 1	-1.449	-1.468 ± 0.019	-11.1 ± 0.4		13.9 ± 3.1	-11.79 ± 2.59		
	Val32, γ 2	1.864	1.823 ± 0.022	-9.0 ± 0.2		12.6 ± 3.8	-10.67 ± 3.22		
	Leu38, δ 1	-0.343	-0.364 ± 0.006	-15.5 ± 0.2	-1.1 ± 0.3	18.0 ± 0.9	-16.18 ± 0.85		
	Leu38, δ 2	0.576	0.562 ± 0.008	6.0 ± 0.2	-0.5 ± 0.2	-7.1 ± 1.0	5.57 ± 0.90		
	Leu41, δ 1	-0.033	-0.051 ± 0.012	3.5 ± 0.4	0.7 ± 0.6	-5.5 ± 1.7	5.18 ± 1.55		
	Leu41, δ 2	0.001	0.004 ± 0.009	6.5 ± 0.4	1.7 ± 0.2	-10.4 ± 1.1	10.27 ± 0.96		
	Leu49, δ 1	-2.418	-2.469 ± 0.028	4.7 ± 0.2	-0.1 ± 0.2	-0.5 ± 3.9	0.32 ± 3.31		
	Leu49, δ 2	-0.358	-0.360 ± 0.005	13.4 ± 0.2	2.7 ± 0.5	-12.9 ± 0.9	13.25 ± 0.87		
	Val55, γ 1	0.368	0.469 ± 0.012	2.8 ± 0.4	8.2 ± 0.3	2.4 ± 2.0	4.95 ± 1.75		
	Val55, γ 2	0.257	0.273 ± 0.006	22.1 ± 0.5	6.5 ± 0.3	-18.3 ± 1.3	21.01 ± 1.17		
Leu57, δ 1	0.052	0.075 ± 0.009	14.7 ± 0.1	10.2 ± 0.2	-14.4 ± 1.6	20.87 ± 1.39			
Leu57, δ 2	0.044	0.081 ± 0.007	0.3 ± 0.3	2.5 ± 0.2	1.9 ± 1.2	0.55 ± 1.01			

a) Carbon chemical shift difference $\omega_G-\omega_H$ measured directly from HSQC experiments. b) Values of $\Delta\omega_C=\omega_G-\omega_E$ obtained through fitting CPMG data encompassing all four lines and the CW experiment at both 500 and 800 MHz. All signs are those obtained from the direct measurement. Signs experimentally from HSQC/HMQC spectra are indicated with black boxes. c/d) Directly measured RDC values of the holo state (c) and the apo state (d). e) Values of $\Delta D=D_G-D_E$ obtained through fitting CPMG data encompassing all four lines and the CW experiment at both 500 and 800 MHz. f) The excited state RDC values adjusted to take into account differences in the amount of alignment media in samples used for direct measurements of the holo state (D_H) and CPMG measurements (D_E); $D_E^*=D_E/k=(D_G-\Delta D)/k$, ($k=1.24$ at 25°C and 1.17 at 5°C). D_E^* is directly comparable to D_H . Values of k are determined from the slopes of the best fit lines to the correlations indicated in Figures 6d,h. (c). g/h) J coupling values of the apo (g) and holo (h) states.