Supplementary Information

Optimized Selection of Slow-Relaxing ^{13}C Transitions in Methyl Groups of Proteins: Application to Relaxation Dispersion

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Density matrix analysis of the pulse scheme in Figure 3A: derivation of optimal angles α and β.

The density matrix describing the state of the magnetization in a $^{13}CH_3$ spin-system can be represented as a tensor product, $C \otimes \rho$, where $C \in \{C_x, C_y, C_z, E\}$, C_l is a ^{13}C spin operator, E is the 2x2 identity matrix, and ρ describes the state of ^1H magnetization. The latter is constructed from a basis set of 8 ^1H eigenstates $|n\rangle$ formed by linear combinations of $|i,j,k\rangle$ ($i,j,k \in \{\alpha,\beta\}$) (see Figure 1; main text). Further, the density matrix ρ and ^1H RF pulse operators can be separated into two parts (each of dimension 4x4) corresponding to the $I = 3/2$ ($\rho^{3/2}$) and $I = 1/2$ ($\rho^{1/2}$) manifolds, as they evolve independently of each other under the effect of RF field. In the following, we concentrate on the transformations of the matrices ρ keeping in mind that the state of the full (16x16) density matrix can be obtained by the tensor product above.

Following isolation of the inner, slow-relaxing ^1H transitions at the beginning of the pulse scheme in Figure 3A, the density matrices ρ are given by,

$$\rho_1^{3/2} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix} \tag{S1}$$

for the $I = 3/2$ manifold, and

$$\rho_1^{1/2} = \frac{1}{2}\begin{bmatrix} 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 \\ 0 & 0 & 1 & 0 \end{bmatrix} \tag{S2}$$

for the two $I = 1/2$ manifolds. The evolution of $\rho_1^{3/2}$ and $\rho_1^{1/2}$ under the effect of an RF pulse with flip-angle α, is given by,

$$\rho(\alpha) = e^{-i\alpha I_y}\rho e^{i\alpha I_y} \tag{S3}$$

where the operators of a ^1H pulse applied with phase y (I_y) have the form,

$$I_y^{3/2} = i\begin{bmatrix} 0 & -\sqrt{3}/2 & 0 & 0 \\ \sqrt{3}/2 & 0 & -1 & 0 \\ 0 & 1 & 0 & -\sqrt{3}/2 \\ 0 & 0 & \sqrt{3}/2 & 0 \end{bmatrix} \tag{S4}$$

for the $I = 3/2$ manifold, and

$$I_y^{1/2} = i\begin{bmatrix} 0 & -1/2 & 0 & 0 \\ 1/2 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1/2 \\ 0 & 0 & 1/2 & 0 \end{bmatrix} \tag{S5}$$

for the $I = 1/2$ manifolds, and operate on the column-vectors of the eigenfunctions $[|1\rangle, |2\rangle, |3\rangle, |4\rangle]^T$ and $[|5\rangle, |6\rangle, |7\rangle, |8\rangle]^T$, respectively, where the ^{1}H eigenfunctions are defined in the energy level diagram of Figure 1, and the superscript 'T' denotes transposition.

The form of the density matrix ρ of each manifold after the ^{1}H pulse with flip-angle α can be calculated via the expansion of the expression in Eq. (S3) in powers of I_y as described in the Supplementary Information of Tugarinov et al. (2020). After the ^1H_y pulse with flip-angle α and the pulsed-field gradient g4 in the pulse scheme of Figure 3A, the density matrices are given by,

$$\rho_2^{3/2}(\alpha) = \frac{1}{4}\begin{bmatrix} -3\sin^3\alpha & 0 & 0 & 0 \\ 0 & 9\sin^3\alpha - 8\sin\alpha & 0 & 0 \\ 0 & 0 & 8\sin\alpha - 9\sin^3\alpha & 0 \\ 0 & 0 & 0 & 3\sin^3\alpha \end{bmatrix} \tag{S6}$$

for the $I = 3/2$ manifold, and

$$\rho_2^{1/2}(\alpha) = \frac{1}{2}\begin{bmatrix} -\sin\alpha & 0 & 0 & 0 \\ 0 & \sin\alpha & 0 & 0 \\ 0 & 0 & -\sin\alpha & 0 \\ 0 & 0 & 0 & \sin\alpha \end{bmatrix} \tag{S7}$$

for the $I = 1/2$ manifolds. Note that only the ^{1}H polarization terms (diagonal elements) 'survive' after application of the gradient g4. Eq. (2) of the main text is constructed by summation of the elements of

$\rho_2^{3/2}(\alpha)$ and $\rho_2^{1/2}(\alpha)$ that give rise to (subsequently selected) slow-relaxing ^{13}C coherences when the magnetization is transferred to ^{13}C nuclei, namely, the elements [2,2] of the matrix in Eq. (S6) and [1,1] and [3,3] of the matrix in Eq. (S7), and the result multiplied by a factor of '-2' to account for the fact that the full density matrix after the application of the first ^{13}C 90° pulse with phase *x*, is described by the products $-C_y \otimes \rho_2^{3/2}$ and $-C_y \otimes \rho_2^{1/2}$ for the two manifolds, respectively. The optimal value of the angle α, $\alpha_{opt} = \sin^{-1}(2/3)$, is determined by maximizing the elements of the density matrices corresponding to the slow-relaxing ^{13}C transitions in Eq. (2) as described in the main text.

Following selection of the slow-relaxing ^{13}C transitions by the element enclosed in the solid box in the pulse scheme of Figure 3A (equivalent to zeroing the elements [1,1] and [4,4] of the matrix in Eq. (S6)), the density matrices ρ for the optimal value of angle $\alpha = \alpha_{opt}$, are given by,

$$\rho_3^{3/2} = \frac{2}{3}\begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix} \tag{S8}$$

for the $I = 3/2$ manifold, and

$$\rho_3^{1/2} = \frac{1}{3}\begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & -1 \end{bmatrix} \tag{S9}$$

for the $I = 1/2$ manifolds, where the signs of the matrices in Eqs. (S6) and (S7) are inverted for consistency with the main text. Application of the ^1H_y pulse with flip-angle β and the cycling of the phase of this pulse with concomitant reversal of the receiver phase followed by selection of the slow-relaxing ^{1}H components in the rest of the pulse scheme (the element enclosed in the second dashed box in Figure 3A), provides the following forms of the density matrices,

$$\rho_4^{3/2}(\beta) = \frac{1}{6}\begin{bmatrix} 0 & 0 & 0 & (1/4)\{3\sin 3\beta - 9\sin\beta\} \\ 0 & 0 & 8\sin\beta - 9\sin^3\beta & 0 \\ 0 & 8\sin\beta - 9\sin^3\beta & 0 & 0 \\ (1/4)\{3\sin 3\beta - 9\sin\beta\} & 0 & 0 & 0 \end{bmatrix} \tag{S10}$$

for the $I = 3/2$ manifold, and

$$\rho_4^{1/2}(\beta) = \frac{1}{3}\begin{bmatrix} 0 & \sin\beta & 0 & 0 \\ \sin\beta & 0 & 0 & 0 \\ 0 & 0 & 0 & \sin\beta \\ 0 & 0 & \sin\beta & 0 \end{bmatrix} \tag{S11}$$

for the $I = 1/2$ manifolds. Note that in actuality, a ^{1}H pulse with flip-angle β and *phase x* is applied in the pulse scheme of Figure 3A. This provides results equivalent to our treatment as far as the detected ^{1}H magnetization at the end of the experiment is concerned, as the evolution of the ^{1}H magnetization with respect to the $^1J_{CH}$ coupling is not considered explicitly here.

The anti-diagonal form of the matrix in Eq. (S10) is ensured by: (1) the cycling of the phase of the ^{1}H pulse with flip-angle β with concomitant inversion of the receiver phase that leads to the elimination of all ^{1}H coherences of even order (0 and 2); and (2) elimination of the fast-relaxing ^{1}H coherences (elements {[1,2]; [2,1]} and {[3,4]; [4,3]} of the matrix in Eq. (S10)) by the element enclosed in the second dashed box in the pulse scheme of Figure 3A. The triple-quantum ^{1}H magnetization remains intact (elements [1,4] and [4,1] of the matrix in Eq. (S10)), but is not observable at the end of the experiment.

Eq. (3) of the main text can be obtained by taking the trace of the product of the observation operator (I_-; represented by a 8x8 matrix for each manifold) and the matrices $E \otimes \rho_4^{3/2}$ and $E \otimes \rho_4^{1/2}$ (see Eqs. (S10) and (S11)). The optimal angle β, $\beta_{opt} = \sin^{-1}(\sqrt{10/27})$, is obtained by maximizing the expression in Eq. (3) as described in the main text.

Materials and Methods

NMR Samples. The samples of {U-[^{15}N,^{2}H]; Ileδ1-[$^{13}CH_3$]; Leu,Val-[$^{13}CH_3$,$^{12}CD_3$]}-labeled ubiquitin and ΔST-DNAJB6b were prepared as described previously by Ceccon et al. (2016) and Karamanos et al. (2019), respectively. Sample conditions were as follows: for ubiquitin, 1.3 mM-ubiquitin, 20 mM sodium phosphate, pH 6.5 (uncorrected), and 50 mM NaCl; for ΔST-DNAJB6b, 200 μM ΔST-DNAJB6b, 20 mM sodium phosphate, pH 7.0 (uncorrected), and 50 mM NaCl. Both samples were dissolved in 99.9 % D_2O.

NMR Spectroscopy. All spectra were recorded on a 600 MHz, AVANCE HD Bruker spectrometer equipped with a triple-axis (x, y, z) gradient cryogenic probe and were processed and analyzed using the NMRPipe/NMRDraw suite of programs (Delaglio et al. 1995) and associated software. NMR experiments recorded with the pulse schemes of Figures 2A and 3A on ubiquitin (5 °C and 25 °C) and ΔST-DNAJB6b (25 °C) samples were typically obtained with 8 and 16 scans/FID, respectively, (128; 512) complex points in (t_1; t_2), and an inter-scan recovery delay of 1 s, resulting in net acquisition times of ~40 and ~80 min, respectively, per experiment.

Methyl ^{13}C single-quantum CPMG relaxation dispersion experiments on the samples of ubiquitin (5 °C) and ΔST-DNAJB6b (15 °C) were recorded with the pulse scheme of Figure 4A (with optimized selection of the slow-relaxing ^{13}C transitions) and the scheme of Lundström et al. (2007) using CPMG frequencies (ν_{CPMG}) ranging from 0 (reference experiment without relaxation delay) to 1000 Hz. A constant-time relaxation period T of 50 ms and 30 ms were used in the experiment of Lundström et al. (2007) for ubiquitin and ΔST-DNAJB6b samples, respectively, while the delay T was extended to 80 ms and 50 ms in the experiment of Figure 4A for ubiquitin and ΔST-DNAJB6b, respectively. The CPMG experiments recorded with the pulse scheme of Figure 4A and that of Lundström et al. (2007) were collected with 32 and 16 scans/FID, (128; 512) complex points in (t_1; t_2), and an inter-scan recovery delay of 1.5 s, resulting in acquisition times of ~3.8 hrs and ~1.9 hrs per 2D spectrum, respectively.

Supplementary References

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