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The Effects of Cross Correlation and Cross Relaxation on the Measurement of Deuterium T_1 and $T_{1\rho}$ Relaxation Times in $^{13}CH_2D$ Spin Systems

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In principle, NMR spectroscopy can provide a wealth of information about molecular dynamics extending over a wide range of motional time scales. Studies to date have focused to a large extent on backbone dynamics through measurement of ^{15}N relaxation properties in molecules uniformly ^{15}N labeled (*1–4*). An attractive feature of backbone ^{15}N relaxation studies is that the relaxation of an ^{15}N–NH spin pair can simply be described by the ^{15}N–^{1}H dipolar interaction and to a smaller extent by ^{15}N chemical-shift anisotropy (*5*). While cross-correlation effects do exist between these relaxation mechanisms, methods have been developed for their efficient removal (*6–8*). Extension of the methods developed for probing backbone dynamics to the study of side-chain motions has proven to be difficult. One approach involves either uniform (*9, 10*) or fractional (*11*) labeling of the molecule with ^{13}C; in the case of side chains, however, the majority of spin systems are of the AX_2, AMX (methylene), or AX_3 (methyl) variety, and cross-correlation effects between the various dipolar spin pairs can significantly complicate the interpretation of the relaxation data (*12–14*). Unfortunately, such effects are difficult or impossible to remove, and the detailed experiments necessary to exploit the information content of such cross-correlation terms are often not possible for complex molecules such as proteins.

With these problems in mind, we have recently developed a novel ^{2}H-based relaxation approach for studying dynamics of methyl-containing side chains. The method makes use of a uniformly ^{13}C-, fractionally ^{2}H-labeled sample and selects specifically for $^{13}CH_2D$ methyl groups. Because of the poor resolution and sensitivity of direct-observe ^{2}H spectroscopy, the experiments measure the relaxation properties of the deuteron indirectly by recording a series of constant-time two-dimensional ^{1}H–^{13}C correlation spectra. Excellent resolution is therefore afforded, and high-quality spectra are obtained in only 2–3 hours of measuring time for sample concentrations on the order of 1 m*M*. The experimental and theoretical details of the approach have been published (*15*), and an application to the study of the side-chain dynamics of an SH2 domain from phospholipase C-γ1 in the presence and absence of a 12-residue phosphopeptide has been reported (*16*). The goal of the present Communication is to examine in detail the effects of cross correlation and cross relaxation on the fidelity of the ^{2}H relaxation methods presented previously. We show that accurate ^{2}H T_1 and $T_{1\rho}$ values can be easily obtained.

For completeness, a brief description of the experiments that have been developed is provided here. The magnetization-transfer pathway in either the T_1 or $T_{1\rho}$ experiment can be described as

$$^{1}\mathrm{H} \xrightarrow{J_{\mathrm{CH}}} {}^{13}\mathrm{C}\,(t_1) \xrightarrow{J_{\mathrm{CD}}} {}^{2}\mathrm{D}\,(T) \xrightarrow{J_{\mathrm{CD}}} {}^{13}\mathrm{C} \xrightarrow{J_{\mathrm{CH}}} {}^{1}\mathrm{H}\,(t_2), \quad [1]$$

where the active couplings involved in each transfer process are indicated above the arrows and t_1, t_2 denote acquisition times. During the delay T, the magnetization of interest is of the form $I_zC_zD_z$ or $I_zC_zD_y$, depending on whether a T_1 ($I_zC_zD_z$) or $T_{1\rho}$ ($I_zC_zD_y$) experiment is recorded. The decay of these triple-spin terms proceeds at this point in the sequence. At the completion of the delay T, the magnetization is returned to protons for detection by reversing the first half of the transfer pathway. Fourier transformation of the resultant data set generates a (^{13}C, ^{1}H) correlation map. A set of two-dimensional spectra is obtained, with each member of the set recorded with a different value of T. The intensities of (^{13}C, ^{1}H) correlations as a function of T provide a direct measure of the relaxation rates of the triple-spin terms, $I_zC_zD_z$ or $I_zC_zD_y$. In order to obtain the relaxation of pure deuterium spin order, D_z or D_y, it is necessary to record an additional experiment which measures the decay of longitudinal order I_zC_z and subtract the decay rate of the double-

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TABLE 1[a]

$$\begin{aligned}
\rho_{1,1} &= \mathrm{AR(Q)} + \mathrm{AR(CD)} + K^{\mathrm{d}}(\mathrm{CD})[3J(\omega_{\mathrm{D}}) + 12J(\omega_{\mathrm{C}})] + 2\mathrm{AR(HD)} \\
&\quad + K^{\mathrm{d}}(\mathrm{HD})[6J(\omega_{\mathrm{D}}) + 6J(\omega_{\mathrm{D}})^{\mathrm{HD-HD}} + 12J(\omega_{\mathrm{H}})] + \mathrm{AR(HC)} \\
&\quad + K^{\mathrm{d}}(\mathrm{HC})\,[3J(\omega_{\mathrm{H}}) + 6J(\omega_{\mathrm{C}}) + 6J(\omega_{\mathrm{C}})^{\mathrm{HC-HC}}] \\
&\quad + \mathrm{AR(HH)} + R(\mathrm{H}, \mathrm{H}_k) \\
\rho_{1,2} &= \mathrm{CR(Q, CD)} + 0.5\mathrm{CR(CD)} + 2\mathrm{CR(HC, HD)} \\
\rho_{1,3} &= 4\mathrm{CR(Q, HD)} - 2\mathrm{CR(HD)}/3 + 8\mathrm{CR(HH, HD)} + 8\mathrm{CR(HC, CD)} \\
\rho_{1,4} &= \mathrm{CR(HC)} + 6\mathrm{CR(HH, HC)} + 6\mathrm{CR(HD, CD)} \\
\rho_{1,5} &= -0.5\mathrm{CR(HC)} - 3\mathrm{CR(HH, HC)} \\
\rho_{1,6} &= 8\mathrm{CR(HD)}/3 + 16\mathrm{CR(HC, CD)} + 16\mathrm{CR(HH, HD)} \\
\rho_{1,7} &= 4\mathrm{CR(HC, HD)} \\
\rho_{1,8} &= 4\mathrm{CR(HD)}/3 - 4\mathrm{CR(HH, HD)} \\
\rho_{1,9} &= -4\mathrm{CR(HD)}/3 - 8\mathrm{CR(HH, HD)} \\
\rho_{1,10} &= \Sigma K^{\mathrm{d}}(\mathrm{HH_k})[-2J(0) + 12J(2\omega_{\mathrm{H}})] \\
\rho_{2,1} &= 12\mathrm{CR(Q, CD)} + 6\mathrm{CR(CD)} + 24\mathrm{CR(HC, HD)} \\
\rho_{2,2} &= \mathrm{AR1(Q)} + 3\mathrm{AR(CD)} + K^{\mathrm{d}}(\mathrm{CD})[9J(\omega_{\mathrm{D}})] + 10\mathrm{AR(HD)}/3 \\
&\quad + K^{\mathrm{d}}(\mathrm{HD})[18J(\omega_{\mathrm{D}}) + 18J(\omega_{\mathrm{D}})^{\mathrm{HD-HD}} + 4J(\omega_{\mathrm{H}})] \\
&\quad + \mathrm{AR(HC)} + K^{\mathrm{d}}(\mathrm{HC})[3J(\omega_{\mathrm{H}})] + \mathrm{AR(HH)} + R(\mathrm{H}, \mathrm{H}_k) \\
\rho_{2,3} &= 72\mathrm{CR(HD, CD)} + 24\mathrm{CR(HH, HC)} \\
\rho_{2,4} &= 12\mathrm{CR(Q, HD)} + 6\mathrm{CR(HD)} + 24\mathrm{CR(HH, HD)} \\
\rho_{2,5} &= -4\mathrm{CR(HD)} - 12\mathrm{CR(HH, HD)} \\
\rho_{2,6} &= 0 \\
\rho_{2,7} &= -4\mathrm{AR(HD)}/3 + 8K^{\mathrm{d}}(\mathrm{HD})J(\omega_{\mathrm{H}}) \\
\rho_{2,8} &= 2\mathrm{CR(HC)} - 12\mathrm{CR(HH, HC)} \\
\rho_{2,9} &= 0 \\
\rho_{2,10} &= 0 \\
\rho_{3,1} &= 12\mathrm{CR(Q, HD)} + 2\mathrm{CR(HD)} + 12\mathrm{CR(HH, HD)} + 24\mathrm{CR(HC, CD)} \\
\rho_{3,2} &= 0.5\mathrm{CR(HC)} + 3\mathrm{CR(HH, HC)} + 18\mathrm{CR(HD, CD)} \\
\rho_{3,3} &= \mathrm{AR1(Q)} + \mathrm{AR(CD)}/3 + K^{\mathrm{d}}(\mathrm{CD})[9J(\omega_{\mathrm{D}}) + 4J(\omega_{\mathrm{C}})] + 2\mathrm{AR(HD)}/3 \\
&\quad + K^{\mathrm{d}}(\mathrm{HD})[18J(\omega_{\mathrm{D}}) + 18J(\omega_{\mathrm{D}})^{\mathrm{HD-HD}} + 8J(\omega_{\mathrm{H}})] \\
&\quad + K^{\mathrm{d}}(\mathrm{HC})[6J(\omega_{\mathrm{H}}) + 6J(\omega_{\mathrm{C}}) + 6J(\omega_{\mathrm{C}})^{\mathrm{HC-HC}}] \\
&\quad + \mathrm{AR1(HH)} + 2\mathrm{R(H, H_k)} \\
\rho_{3,4} &= 3\mathrm{CR(Q, CD)} - 0.5\mathrm{CR(CD)} + 12\mathrm{CR\ (HC, HD)} \\
\rho_{3,5} &= -6\mathrm{CR(HC, HD)} \\
\rho_{3,6} &= -4\mathrm{AR(CD)}/3 + 8K^{\mathrm{d}}(\mathrm{CD})J(\omega_{\mathrm{C}}) - 8\mathrm{AR(HD)}/3 + 16K^{\mathrm{d}}(\mathrm{HD})J(\omega_{\mathrm{H}}) \\
\rho_{3,7} &= 0 \\
\rho_{3,8} &= \mathrm{AR(HC)} + 8\mathrm{AR(HD)}/3 - 4K^{\mathrm{d}}(\mathrm{HD})J(\omega_{\mathrm{H}}) \\
&\quad - 3K^{\mathrm{d}}(\mathrm{HC})J(\omega_{\mathrm{H}}) - 3K^{\mathrm{d}}(\mathrm{HH})J(\omega_{\mathrm{H}}) \\
\rho_{3,9} &= 4\mathrm{AR(HD)}/3 - 8K^{\mathrm{d}}(\mathrm{HD})J(\omega_{\mathrm{H}}) \\
\rho_{3,10} &= 0 \\
\rho_{4,1} &= 2\mathrm{CR(HC)} + 24\mathrm{CR(HD, CD)} + 12\mathrm{CR(HH, HC)} \\
\rho_{4,2} &= 4\mathrm{CR(Q, HD)} + 2\mathrm{CR(HD)}/3 + 4\mathrm{CR(HH, HD)} \\
\rho_{4,3} &= 4\mathrm{CR(Q, CD)} - 2\mathrm{CR(CD)}/3 + 16\mathrm{CR(HC, HD)} \\
\rho_{4,4} &= \mathrm{AR(Q)} + \mathrm{AR(CD)} + K^{\mathrm{d}}(\mathrm{CD})[3J(\omega_{\mathrm{D}})] + 2\mathrm{AR(HD)} \\
&\quad + K^{\mathrm{D}}(\mathrm{HD})[6J(\omega_{\mathrm{D}}) + 6J(\omega_{\mathrm{D}})^{\mathrm{HD-HD}} + 24J(\omega_{\mathrm{H}})] + 2\mathrm{AR(HC)} \\
&\quad + K^{\mathrm{d}}(\mathrm{HC})[6J(\omega_{\mathrm{H}}] + \mathrm{AR1(HH)} + 2\mathrm{R}(\mathrm{H}, \mathrm{H}_k) \\
\rho_{4,5} &= -\mathrm{AR(HC)} - 12K^{\mathrm{d}}(\mathrm{HD})J(\omega_{\mathrm{H}}) - 3K^{\mathrm{d}}(\mathrm{HC})J(\omega_{\mathrm{H}}) - 3K^{\mathrm{d}}(\mathrm{HH})J(\omega_{\mathrm{H}}) \\
\rho_{4,6} &= 8\mathrm{CR(CD)}/3 + 32\mathrm{CR(HC, HD)} \\
\rho_{4,7} &= 4\mathrm{CR(HD)}/3 + 8\mathrm{CR(HH, HD)} \\
\rho_{4,8} &= -8\mathrm{CR(HC, HD)} \\
\rho_{4,9} &= -16\mathrm{CR(HC, HD)} \\
\rho_{4,10} &= 0 \\
\rho_{5,1} &= 24\mathrm{CR(HD, CD)} \\
\rho_{5,2} &= 4\mathrm{CR(Q, HD)} - 2\mathrm{CR(HD)}/3 \\
\rho_{5,3} &= 0 \\
\rho_{5,4} &= 12K^{\mathrm{d}}(\mathrm{HD})J(\omega_{\mathrm{D}})^{\mathrm{HD-HD}}
\end{aligned}$$

$$\begin{aligned}
\rho_{5,5} &= \mathrm{AR(Q)} + \mathrm{AR(CD)} + 2\mathrm{AR(HD)} + K^{\mathrm{d}}(\mathrm{CD})[3J(\omega_{\mathrm{D}})] \\
&\quad + 6K^{\mathrm{d}}(\mathrm{HD})J(\omega_{\mathrm{D}}) - 6K^{\mathrm{d}}(\mathrm{HD})J(\omega_{\mathrm{D}})^{\mathrm{HD-HD}} \\
\rho_{5,6} &= 0 \\
\rho_{5,7} &= 8\mathrm{CR(HD)}/3 \\
\rho_{5,8} &= -2\mathrm{CR(CD)}/3 + 4\mathrm{CR(Q, CD)} \\
\rho_{5,9} &= 8\mathrm{CR(CD)}/3 \\
\rho_{5,10} &= 0 \\
\rho_{6,1} &= 2\mathrm{CR(HD)} + 24\mathrm{CR(HC, CD)} + 12\mathrm{CR(HH, HD)} \\
\rho_{6,2} &= 0.5\mathrm{CR(HC)} \\
\rho_{6,3} &= -2\mathrm{AR(CD)}/3 - 4\mathrm{AR(HD)}/3 + 4K^{\mathrm{d}}(\mathrm{CD})J(\omega_{\mathrm{C}}) + 8K^{\mathrm{d}}(\mathrm{HD})J(\omega_{\mathrm{H}}) \\
\rho_{6,4} &= \mathrm{CR(CD)} + 12\mathrm{CR(HC, HD)} \\
\rho_{6,5} &= -6\mathrm{CR(HC, HD)} \\
\rho_{6,6} &= 8\mathrm{AR(CD)}/3 + 16\mathrm{AR(HD)}/3 + K^{\mathrm{d}}(\mathrm{CD})[8J(\omega_{\mathrm{C}})] \\
&\quad + K^{\mathrm{d}}(\mathrm{HD})[16J(\omega_{\mathrm{H}})] \\
&\quad + K^{\mathrm{d}}(\mathrm{HC})[6J(\omega_{\mathrm{H}}) + 6J(\omega_{\mathrm{C}}) + 6J(\omega_{\mathrm{C}})^{\mathrm{HC-HC}}] + 2R(\mathrm{H}, \mathrm{H}_k) \\
\rho_{6,7} &= 3\mathrm{CR(HH, HC)} \\
\rho_{6,8} &= 2\mathrm{AR(HD)}/3 + \mathrm{AR(HC)} - 4K^{\mathrm{d}}(\mathrm{HD})J(\omega_{\mathrm{H}}) - 3K^{\mathrm{d}}(\mathrm{HC})J(\omega_{\mathrm{H}}) \\
\rho_{6,9} &= -8\mathrm{AR(HD)}/3 - 8K^{\mathrm{d}}(\mathrm{HD})J(\omega_{\mathrm{H}}) - 3K^{\mathrm{d}}(\mathrm{HH})J(\omega_{\mathrm{H}}) \\
\rho_{6,10} &= 0 \\
\rho_{7,1} &= 24\mathrm{CR(HC, HD)} \\
\rho_{7,2} &= -2\mathrm{AR(HD)}/3 + 4K^{\mathrm{d}}(\mathrm{HD})J(\omega_{\mathrm{H}}) \\
\rho_{7,3} &= 0 \\
\rho_{7,4} &= 24\mathrm{CR(HH, HD)} \\
\rho_{7,5} &= 2\mathrm{CR(HD)} - 12\mathrm{CR(HH, HD)} \\
\rho_{7,6} &= 24\mathrm{CR(HH, HC)} \\
\rho_{7,7} &= \mathrm{AR(HH)} + 8\mathrm{AR(HD)}/3 + \mathrm{AR(HC)} + K^{\mathrm{d}}(\mathrm{HC})[3J(\omega_{\mathrm{H}})] \\
&\quad + K^{\mathrm{d}}(\mathrm{HD})[8J(\omega_{\mathrm{H}})] + R(\mathrm{H}, \mathrm{H}_k) \\
\rho_{7,8} &= 0 \\
\rho_{7,9} &= 2\mathrm{CR(HC)} - 12\mathrm{CR(HH, HC)} \\
\rho_{7,10} &= 0 \\
\rho_{8,1} &= 6\mathrm{CR(HD)} + 24\mathrm{CR(HC, CD)} + 12\mathrm{CR(Q, HD)} \\
\rho_{8,2} &= \mathrm{CR(HC)} + 18\mathrm{CR(HD, CD)} \\
\rho_{8,3} &= 12K^{\mathrm{d}}(\mathrm{HC})J(\omega_{\mathrm{C}})^{\mathrm{HC-HC}} + 36K^{\mathrm{d}}(\mathrm{HD})J(\omega_{\mathrm{D}})^{\mathrm{HD-HD}} \\
\rho_{8,4} &= 0 \\
\rho_{8,5} &= -0.5\mathrm{CR(CD)} + 3\mathrm{CR(Q, CD)} \\
\rho_{8,6} &= 0 \\
\rho_{8,7} &= 0 \\
\rho_{8,8} &= \mathrm{AR1(Q)} + \mathrm{AR(CD)}/3 + 6\mathrm{AR(HD)} + 2\mathrm{AR(HC)} \\
&\quad + K^{\mathrm{d}}(\mathrm{CD})[4J(\omega_{\mathrm{C}}) + 9J(\omega_{\mathrm{D}})] + 18K^{\mathrm{d}}(\mathrm{HD})J(\omega_{\mathrm{D}}) \\
&\quad - 18K^{\mathrm{d}}(\mathrm{HD})J(\omega_{\mathrm{D}})^{\mathrm{HD-HD}} + 6K^{\mathrm{d}}(\mathrm{HC})J(\omega_{\mathrm{C}}) \\
&\quad - 6K^{\mathrm{d}}(\mathrm{HC})J(\omega_{\mathrm{C}})^{\mathrm{HC-HC}} \\
\rho_{8,9} &= -4\mathrm{AR(CD)}/3 + 8K^{\mathrm{d}}(\mathrm{CD})J(\omega_{\mathrm{C}}) \\
\rho_{8,10} &= 0 \\
\rho_{9,1} &= 24\mathrm{CR(HC, CD)} \\
\rho_{9,2} &= \rho_{9,3} = \rho_{9,4} = 0 \\
\rho_{9,5} &= \mathrm{CR(CD)} \\
\rho_{9,6} &= 12K^{\mathrm{d}}(\mathrm{HC})J(\omega_{\mathrm{C}})^{\mathrm{HC-HC}} \\
\rho_{9,7} &= \mathrm{CR(HC)} \\
\rho_{9,8} &= -2\mathrm{AR(CD)}/3 + 4K^{\mathrm{d}}(\mathrm{CD})J(\omega_{\mathrm{C}}) \\
\rho_{9,9} &= 8\mathrm{AR(CD)}/3 + 2\mathrm{AR(HC)} + K^{\mathrm{d}}(\mathrm{CD})[8J(\omega_{\mathrm{C}})] \\
&\quad + 6K^{\mathrm{d}}(\mathrm{HC})J(\omega_{\mathrm{C}}) - 6K^{\mathrm{d}}(\mathrm{HC})J(\omega_{\mathrm{C}})^{\mathrm{HC-HC}} \\
\rho_{9,10} &= 0 \\
\rho_{10,1} &= \Sigma K^{\mathrm{d}}(\mathrm{HH}_k)[-J(0) + 6J(2\omega_{\mathrm{H}})] \\
\rho_{10,2} &= \rho_{10,3} = \rho_{10,4} = \rho_{10,5} = \rho_{10,6} = \rho_{10,7} = \rho_{10,8} = \rho_{10,9} = 0 \\
\rho_{10,10} &= \rho_{1,1} + R(\mathrm{H}, H_k)
\end{aligned}$$

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[a] The summation term in some of the elements of ρ includes all neighboring proton spins, H_k, that contribute to the relaxation of the methyl proton spins, denoted by H in the table. Equations [5] and [6] are used to evaluate the spectral-density terms listed in the table. For example, $\rho_{1,1}$ is made up of terms of the form AR(Q) and AR(CD), among others. The ''Q'' and ''CD'' in AR(Q) and AR(CD) indicate that these terms originate from the deuterium quadrupolar and the carbon–deuterium dipolar interactions, respectively. Such terms are defined at the bottom of the table. Note that a term of the form $K^{\mathrm{d}}(\mathrm{HD})[6J(\omega_{\mathrm{D}}) + 6J(\omega_{\mathrm{D}})^{\mathrm{HD-HD}} + 12J(\omega_{\mathrm{H}})]$ is also present in $\rho_{1,1}$. All the elements in this term originate from the proton–deuterium dipolar interaction [''HD'' in $K^{\mathrm{d}}(\mathrm{HD})$]. The first element, $6J(\omega_{\mathrm{D}})$, is an auto-correlation spectral density, while the second element $6J(\omega_{\mathrm{D}})^{\mathrm{HD-HD}}$ is a cross term between $^1\mathrm{H}_1$–$^2\mathrm{D}$ and $^1\mathrm{H}_2$–$^2\mathrm{D}$ dipolar interactions, where H_1 and H_2 are $\mathrm{CH_2D}$ methyl protons. (Auto-correlation spectral densities are denoted by $J(\omega)$, while cross-correlation spectral densities are denoted by $J(\omega)^{ij-kl}$). Finally, terms of the form CR(Q, CD) or CR(HC, HD), for example (see $\rho_{1,2}$), originate from cross correlation between deuterium quadrupolar and carbon–deuterium dipolar interactions [CR(Q, CD)] or interference between proton–carbon and proton–deuterium dipolar interactions [CR(HC, HD)].

TABLE 1—*Continued*

$R(\mathrm{H}, \mathrm{H}_k) = \Sigma K^{\mathrm{d}}(\mathrm{HH}_k)[J(0) + 3J(\omega_{\mathrm{H}}) + 6J(2\omega_H)]$

$K^{\mathrm{d}}(\mathrm{CD}) = \gamma_{\mathrm{C}}^2\gamma_{\mathrm{D}}^2\hbar^2/(4r_{\mathrm{CD}}^6)$

$K^{\mathrm{d}}(\mathrm{HD}) = \gamma_{\mathrm{H}}^2\gamma_{\mathrm{D}}^2\hbar^2/(4r_{\mathrm{HD}}^6)$

$K^{\mathrm{d}}(\mathrm{HC}) = \gamma_{\mathrm{H}}^2\gamma_{\mathrm{C}}^2\hbar^2/(4r_{\mathrm{HC}}^6)$

$K^{\mathrm{d}}(\mathrm{HH_k}) = \gamma_{\mathrm{H}}^4\hbar^2/(4r_{\mathrm{HH}k}^6)$

$K^{\mathrm{d}}(\mathrm{HH}) = \gamma_{\mathrm{H}}^4\hbar^2/(4r_{\mathrm{HH}}^6)$

$\mathrm{CR(Q, CD)} = \frac{3}{8}(e^2qQ\gamma_{\mathrm{C}}\gamma_{\mathrm{D}}/r_{\mathrm{CD}}^3)J(\omega_{\mathrm{Q}})^{\mathrm{Q-CD}}$

$\mathrm{CR(Q, HD)} = \frac{3}{8}(e^2qQ\gamma_{\mathrm{H}}\gamma_{\mathrm{D}}/r_{\mathrm{HD}}^3)J(\omega_{\mathrm{Q}})^{\mathrm{Q-HD}}$

$\mathrm{CR(HH, HC)} = \gamma_{\mathrm{H}}^3\gamma_{\mathrm{C}}\hbar^2/(4r_{\mathrm{HH}}^3r_{\mathrm{HC}}^3)J(\omega_{\mathrm{H}})^{\mathrm{HH-HC}}$

$\mathrm{CR(HH, HD)} = \gamma_{\mathrm{H}}^3\gamma_{\mathrm{D}}\hbar^2/(4r_{\mathrm{HH}}^3r_{\mathrm{HD}}^3)J(\omega_{\mathrm{H}})^{\mathrm{HH-HD}}$

$\mathrm{CR(HC, HD)} = \gamma_{\mathrm{H}}^2\gamma_{\mathrm{C}}\delta_{\mathrm{D}}\hbar^2/(4r_{\mathrm{HC}}^3r_{\mathrm{HD}}^3)J(\omega_{\mathrm{H}})^{\mathrm{HC-HD}}$

$\mathrm{CR(HC, CD)} = \gamma_{\mathrm{H}}\gamma_{\mathrm{C}}^2\gamma_{\mathrm{D}}\hbar^2/(4r_{\mathrm{HC}}^3r_{\mathrm{CD}}^3)J(\omega_{\mathrm{C}})^{\mathrm{HC-CD}}$

$\mathrm{CR(HD, CD)} = \gamma_{\mathrm{H}}\gamma_{\mathrm{C}}\gamma_{\mathrm{D}}^2\hbar^2/(4r_{\mathrm{HD}}^3r_{\mathrm{CD}}^3)J(\omega_{\mathrm{D}})^{\mathrm{HD-CD}}$

$\mathrm{CR(HC)} = K^{\mathrm{d}}(\mathrm{HC})[-J(\omega_{\mathrm{H}} - \omega_{\mathrm{C}}) + 6J(\omega_{\mathrm{H}} + \omega_{\mathrm{C}})]$

$\mathrm{CR(HD)} = K^{\mathrm{d}}(\mathrm{HD})[-J(\omega_{\mathrm{H}} - \omega_{\mathrm{D}}) + 6J(\omega_{\mathrm{H}} + \omega_{\mathrm{D}})]$

$\mathrm{CR(CD)} = K^{\mathrm{d}}(\mathrm{CD})[-J(\omega_{\mathrm{C}} - \omega_{\mathrm{D}}) + 6J(\omega_{\mathrm{C}} + \omega_{\mathrm{D}})]$

$\mathrm{AR(HC)} = K^{\mathrm{d}}(\mathrm{HC})[J(\omega_{\mathrm{H}} - \omega_{\mathrm{C}}) + 6J(\omega_{\mathrm{H}} + \omega_{\mathrm{C}})]$

$\mathrm{AR(HD)} = K^{\mathrm{d}}(\mathrm{HD})[J(\omega_{\mathrm{H}} - \omega_{\mathrm{D}}) + 6J(\omega_{\mathrm{H}} + \omega_{\mathrm{D}})]$

$\mathrm{AR(CD)} = K^{\mathrm{d}}(\mathrm{CD})[J(\omega_{\mathrm{C}} - \omega_{\mathrm{D}}) + 6J(\omega_{\mathrm{C}} + \omega_{\mathrm{D}})]$

$\mathrm{AR(Q)} = \frac{3}{16}\,(e^2qQ/\hbar)^2[J(\omega_{\mathrm{Q}}) + 4J(2\omega_{\mathrm{Q}})]$

$\mathrm{AR1(Q)} = \frac{3}{16}\,(e^2qQ/\hbar)^2[3J(\omega_{\mathrm{Q}})]$

$\mathrm{AR(HH)} = \gamma_{\mathrm{H}}^4\hbar^2/(4r_{\mathrm{HH}}^6)[3J(\omega_{\mathrm{H}}) + 12J(2\omega_{\mathrm{H}})]$

$\mathrm{AR1(HH)} = \gamma_{\mathrm{H}}^4\hbar^2/(4r_{\mathrm{HH}}^6)[6J(\omega_{\mathrm{H}})]$

order magnetization from the decay rate of the triple-order terms,

$$1/T_1(\mathrm{D}) = 1/T_1(I_zC_zD_z) - 1/T_1(I_zC_z)$$
$$1/T_{1\rho}(\mathrm{D}) = 1/T_{1\rho}(I_zC_zD_y) - 1/T_1(I_zC_z). \qquad [2]$$

At first glance, it might appear more practical to refocus the magnetization so that at the start of the T delay, D_z or D_y is present. In practice, however, the very rapid transverse relaxation of the deuterium spins would significantly deteriorate the sensitivity of such experiments.

A justification of Eq. [2] has been provided previously (*15*), where it was also shown in a semi-quantitative manner that the effects of cross correlation between the many different relaxation mechanisms that contribute to the relaxation of the triple-spin terms, $I_zC_zD_z$ and $I_zC_zD_y$, are insignificant. In the present Communication, the effects of cross correlation and cross relaxation are considered in detail, and it is shown that Eq. [2] is indeed accurate for proteins with molecular weights that are currently amenable for study by NMR (see below).

In what follows, we first derive the equations describing the relaxation of $I_zC_zD_z$, where only the spins within the methyl group and adjacent proton spins are considered. The Hamiltonian which gives rise to the relaxation can be expressed as

$$H = H^{\mathrm{q}}(\mathrm{D}) + H^{\mathrm{dd}}(\mathrm{ID}) + H^{\mathrm{dd}}(\mathrm{CD}) + H^{\mathrm{dd}}(\mathrm{IC}) + H^{\mathrm{dd}}(\mathrm{I_1I_2}) + \sum_k H^{\mathrm{dd}}(\Pi_k), \qquad [3]$$

where $H^{\mathrm{q}}(\mathrm{D})$ is the quadrupolar Hamiltonian for spin D and $H^{\mathrm{dd}}(\mathrm{AB})$ is the dipolar Hamiltonian describing the interactions between spins A and B. In Eq. [3], D, $\mathrm{I_1}$, $\mathrm{I_2}$, and C are the $^{13}\mathrm{CH_2D}$ methyl deuterium, proton 1, proton 2, and carbon spins, respectively, $\mathrm{I} = \mathrm{I_1} + \mathrm{I_2}$, and I_k is a neighboring proton spin that is dipolar coupled to methyl spins $\mathrm{I_1}$ and $\mathrm{I_2}$.

An extremely lengthy calculation which includes the effects of cross correlation between all of the relaxation interactions given in Eq. [3] as well as the effects of cross relaxation between all spins within the methyl group and between methyl protons and neighboring proton spins can be performed, following the commutator method of Abragam (*17*). A set of coupled equations describing the relaxation of $I_zC_zD_z$ results and is given by

$$d\mathbf{M}/dt = -\boldsymbol{\rho}\cdot\mathbf{M}, \qquad [4]$$

where $\mathbf{M}$ is a magnetization vector which can be written as follows $\mathbf{M} = [\langle I_zC_zD_z\rangle, \langle I_z(3D_z^2 - D^2)\rangle, \langle I_z^2C_z(3D_z^2 - D^2)\rangle, \langle I_z^2D_z\rangle, \langle D_z\rangle, \langle I_z^2C_z\rangle, \langle I_z\rangle, \langle C_z(3D_z^2 - D^2)\rangle, \langle C_z\rangle, \langle I_{kz}C_zD_z\rangle]$, $D^2 = D(D + 1) = 2$, $\boldsymbol{\rho}$ is a relaxation matrix with diagonal terms, ρ_{ii}, describing the auto relaxation rates of the elements of $\mathbf{M}$ and cross terms, ρ_{ij}, describing the cross relaxation between elements i and j. In $\mathbf{M}$, the value I_z is given by $I_z = I_{1z} + I_{2z}$. The elements of ρ are listed in Table 1. Note that, in the limit of an isolated $^{13}\mathrm{CH_2D}$ spin system, considered initially, the terms proportional to $R(\mathrm{H}, \mathrm{H}_k)$ in ρ are set to zero, $\rho_{i,10} = \rho_{10,i} = 0$, $\{i = 1\text{–}10\}$, and the last term in $\mathbf{M}$ is ignored. The influence of neighboring spins, I_k, will be considered shortly.

The spectral-density function used to evaluate the elements of $\boldsymbol{\rho}$ can be expressed as (*13*)

$$J(\omega)^{ij-kl} = \tfrac{2}{5}\{S^2(ij, kl)\tau_{\mathrm{m}}/[1 + (\omega\tau_{\mathrm{m}})^2] + [P_2(\mathbf{u}_{ij}\cdot\mathbf{u}_{kl}) - S_{\mathrm{f}}^2(ij, kl)]\tau_1/[1 + (\omega\tau_1)^2] + S_{\mathrm{f}}^2(1 - S_{\mathrm{s}}^2)\tau_2/[1 + (\omega\tau_2)^2]\}, \qquad [5]$$

where τ_{m} is the overall rotation time, $1/\tau_1 = 1/\tau_{\mathrm{m}} + 1/\tau_{\mathrm{f}}$, $1/\tau_2 = 1/\tau_{\mathrm{m}} + 1/\tau_{\mathrm{s}}$, τ_{f} is the correlation time for the rotation about the methyl symmetry axis, τ_{s} is the correlation time describing motion of the symmetry axis, $S_{\mathrm{f}}^2(ij, kl) = P_2(\cos\,\theta_{ij})P_2(\cos\,\theta_{kl})$, S_{s}^2 is the order parameter of the methyl symmetry axis, $S^2(ij, kl) = S_{\mathrm{s}}^2\cdot S_{\mathrm{f}}^2(ij, kl)$, and $P_2(\mathbf{u}_{ij}\cdot\mathbf{u}_{kl})$ is given by (*13*)

$$P_2(\mathbf{u}_{ij}\cdot\mathbf{u}_{kl}) = P_2(\cos\,\theta_{ij})P_2(\cos\,\theta_{kl}) + \tfrac{3}{4}\sin\,2\theta_{ij}\sin\,2\theta_{kl}\cos(\phi_{ij} - \phi_{kl}) + \tfrac{3}{4}\sin^2\theta_{ij}\sin^2\theta_{kl}\cos\,2(\phi_{ij} - \phi_{kl}). \qquad [6]$$

In Eq. [6], $\mathbf{u}_{\mathrm{AB}}$ is a unit vector describing the orientation of the interaction vector AB in a frame that is fixed in the macromolecule and (θ_{AB}, ϕ_{AB}) is the polar angle of $\mathbf{u}_{AB}$. When $ij = kl$, $J(\omega)^{ij-kl}$ is an auto-correlation spectral-density term and is simply designated as $J(\omega)$. If $ij \neq kl$, $J(\omega)^{ij-kl}$ is a cross-correlation term arising from interactions between ij and kl. Note that such terms can originate from dipolar interactions between dipoles ij and kl where $ij \neq kl$ or may be due to interactions involving different relaxation mechanisms. For example, in Table 1, $J(\omega)^{\mathrm{HD-HD}}$ is a cross-correlation spectral density arising from dipole cross terms involving $^1H_1-^2D$ and $^1H_2-^2D$ (H_1 and H_2 are $^{13}CH_2D$ protons). In contrast, $J(\omega)^{\mathrm{Q-CD}}$ is the cross-correlation spectral-density function arising from interference between quadrupolar and $^{13}C-^2D$ dipolar interactions. In the limit that cross-correlation terms vanish and an isolated $^{13}CH_2D$ spin system is considered, the value of $\rho_{1,1}$ in Table 1 is identical to the value for $1/T_1(I_zC_zD_z)$, $I_z = I_z^i + I_z^i$, given by Eq. [3.1] in Ref. (*15*).

The effects of cross correlation between the various relaxation mechanisms indicated in Eq. [3] have been evaluated by numerical integration of Eq. [4] for a wide range of motional parameters τ_m, τ_s, τ_f, S_s^2. In all of the calculations considered in this Communication, integration of Eq. [4] extends to $1.5/\rho_{1,1}$, and the decay curve generated is fitted to a single exponential. In the simulations, we have assumed ideal tetrahedral geometry for the methyl group and used a value of 165 kHz for the quadrupolar coupling constant, e^2qQ/h (*18*). Considering $0 \leqslant S_s^2 \leqslant 1$, $0 \leqslant \tau_f \leqslant 150$ ps, $0 \leqslant \tau_s \leqslant 1$ ns, and $2 \leqslant \tau_m \leqslant 25$ ns, the value of $\rho_{1,1}$ differs from the decay of $I_zC_zD_z$ (isolated $^{13}CH_2D$ spin system) by no more than 3%. In addition, the right- and left-hand sides of Eq. [2] differ by less than 3%. (Note that a coupled set of equations describing the relaxation of I_zC_z has also been derived). The effects of cross correlation are largest, in general, when the relaxation rate of $I_zC_zD_z$ is smallest which occurs for $\tau_s = \tau_f = 0$. For nonzero values of τ_s and τ_f, errors introduced by Eq. [2] are even smaller. For example, for $\tau_m = 20$ ns, $\tau_s = 0.5$ ns, $\tau_f = 35$ ps, and $S_s^2 = 0.5$, the difference between the right- and left-hand sides of Eq. [2] is less than 0.5%. These values of τ_s, τ_f, and S_s^2 are typical for methyl-containing side chains (*16, 19*). Figure 1a shows simulated decay curves for the triple-spin term, $I_zC_zD_z$, in the case of an isolated spin system and in the presence (○) and absence (—) of cross correlation obtained with the following parameters: $\tau_m = 20$ ns, $\tau_s = 0.5$ ns, $\tau_f = 35$ ps, and $S_s^2 = 0.5$. Figure 1b illustrates the evolution of the remaining eight elements of $\mathbf{M}$. In this case, $\rho_{1,1}:\rho_{1,2}:\rho_{1,3}:\rho_{1,4}:\rho_{1,5}:\rho_{1,6}:\rho_{1,7}:\rho_{1,8}:\rho_{1,9} = 100:0.97:3.33:2.52:-1.22:0.32:0.11:0.04:-0.06$ and $\rho_{1,1}:\rho_{2,1}:\rho_{3,1}:\rho_{4,1}:\rho_{5,1}:\rho_{6,1}:\rho_{7,1}:\rho_{8,1}:\rho_{9,1} = 100:11.63:10.10:5.20:0.28:0.39:0.63:10.21:0.31$. The simulated $1/T_1(I_zC_zD_z)$ rate is 0.46% smaller than $\rho_{1,1}$. Clearly, the effects of cross correlation on measured values of $T_1(I_zC_zD_z)$ are negligible.

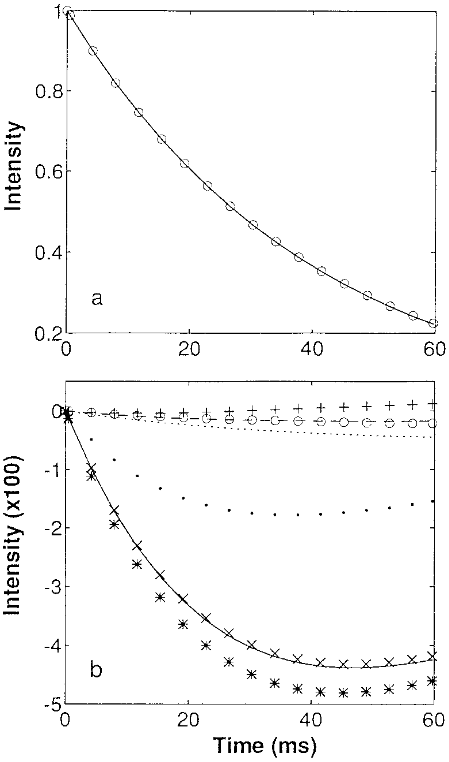

FIG. 1. (a) Simulated decay curves for $I_zC_zD_z$ for an isolated $^{13}CH_2D$ methyl group in the presence (○) and absence (—) of cross correlation. (b) Evolution of the remaining eight elements of M, arising due to cross correlation between the relaxation mechanisms listed in Eq. [3]. Evolution of $\langle I_z(3D_z^2 - D^2)\rangle$, $\langle I_z^2C_z(3D_z^2 - D^2)\rangle$, $\langle I_z^2D_z\rangle$, $\langle D_z\rangle$, $\langle I_z^2C_z\rangle$, $\langle I_z\rangle$, $\langle C_z(3D_z^2 - D^2)\rangle$, $\langle C_z\rangle$ are indicated by (*), (×), (· · ·), (+), (---), (···), (—), (○), respectively. The motional parameters $\tau_m = 20$ ns, $\tau_s = 0.5$ ns, $\tau_f = 35$ ps, and $S_s^2 = 0.5$ were employed in the simulation.

Up to this point, the discussion has focused on an isolated $^{13}CH_2D$ methyl group. In complex macromolecules, methyl groups are in proximity to a large number of protons which can contribute to the relaxation of the methyl protons. In what follows, the effects of neighboring protons (i.e., protons not in the methyl group of interest) are included by placing a single proton, H_k, at a distance of 2 Å from the methyl carbon along the three-fold averaging axis. It is straightforward to calculate that for the case of a 50% randomly deuterated SH2 domain phosphopeptide complex (*20*), the effective distance between a methyl carbon and the neighboring protons in the sample is approximately 2 Å (*15*). In the calculations of $^1H-^1H$ cross relaxation, we have neglected cross-correlation effects between the methyl spins and the proton H_k. The auto-correlation contributions arising from the additional spin, H_k, have been calculated and are included in Table 1. Note that, unlike the case for an isolated CH_2D methyl, the final term in $\mathbf{M}$, $\langle I_{kz}C_zD_z\rangle$, must now be included in the calculations since its evolution is coupled to the relaxation of $I_zC_zD_z$. Contributions from relaxation between the methyl proton spins and proton H_k are included with the term $R(H, H_k)$ in the elements of ρ_{ij} ($1 \leqslant i, j \leqslant 10$). In all calculations, the auto-relaxation rate of $\langle I_{kz}C_zD_z\rangle$, $\rho_{10,10}$, is assumed to differ from the auto-relaxation rate of $\langle I_zC_zD_z\rangle$, $\rho_{1,1}$, only in that an additional contribution from the methyl proton–H_k dipolar interaction is included in the relaxation of $\langle I_{kz}C_zD_z\rangle$. This takes into account the fact that spin H_k can be relaxed by two CH_2D methyl protons, while only a single external proton, H_k, relaxes the methyl protons.

Numerical simulations clearly establish that there can be a very significant contribution to the decay of $I_zC_zD_z$ from proton–proton spin flips. For example, for values of τ_m, τ_s, τ_f, and S_s^2 of (10 ns, 0.5 ns, 35 ps, 0.5), close to 20% of the decay rate is due to dipolar contributions from the neighboring proton, H_k. The effects become even larger as a function of increasing τ_m. Nevertheless, for the range of motional parameters considered, $0 \leqslant S_s^2 \leqslant 1$, $0 \leqslant \tau_f \leqslant 150$ ps, $0 \leqslant \tau_s \leqslant 1$ ns, and $2 \leqslant \tau_m \leqslant 25$ ns, the left- and right-hand sides of Eq. [2] differ by less than 2.5% (i.e., $[1/T_1(I_zC_zD_z) - 1/T_1(I_zC_z)]/1/T_1(D)] < 0.025$), indicating that subtraction of the I_zC_z decay rate from the measured decay of $I_zC_zD_z$ effectively removes the contributions from proton spin flips. For example, for the specific case of $\tau_m = 15$ ns, $\tau_s = 0$ ns, $\tau_f = 0$ ps, $S_s^2 = 1$, the value of $T_1(I_zC_zD_z)$ calculated by integrating Eq. [4] to $1.5/\rho_{1,1}$ is a factor of 1.7 times shorter than $T_1(D)$. Nevertheless, the values of $[1/T_1(I_zC_zD_z) - 1/T_1(I_zC_z)]$ and $1/T_1(D)$ differ by only 0.2%. For the case of $\tau_m = 15$ ns, $\tau_s = 0.5$ ns, $\tau_f = 35$ ps, $S_s^2 = 0.5$, $T_1(I_zC_zD_z)$ is 1.2 times smaller than $T_1(D)$ while the value of $[1/T_1(I_zC_zD_z) - 1/T_1(I_zC_z)]$ differs from $1/T_1(D)$ by only 0.2%, as well. It is important to indicate that because the decay of I_zC_z can be very nonexponential for large values of τ_m and very small values of τ_s and τ_f (i.e., $\tau_s = \tau_f = 0$), only the initial decay of I_zC_z should be measured. For this reason, the decay rate of I_zC_z is obtained with the same delay values T (see Eq. [1]) that are used to measure the decay of $I_zC_zD_z$ (*15, 16*).

$T_{1\rho}$ measurements are performed by applying a weak B_1 (^2H) RF field in the transverse plane to spin-lock deuterium transverse magnetization (*15*). It is straightforward to show that cross-correlation effects between the deuterium quadrupolar interaction and both the deuterium–carbon and the deuterium–proton dipolar interactions result in the coupling of $I_zC_zD_{z'}$ to the cross-relaxation terms,

$$\begin{aligned} &\tfrac{3}{8}\sin^2\theta\cos\theta(e^2qQ\gamma_D\gamma_C/r^3_{CD})J(\omega_e)^{Q-CD}\langle I_z(3D^2_{z'}-D^2)\rangle \\ &\quad + \tfrac{3}{2}\sin^2\theta\cos\theta(e^2qQ\gamma_D\gamma_H/r^3_{HD})J(\omega_e)^{Q-HD} \\ &\quad \times \langle I_z^2C_z(3D^2_{z'}-D^2)\rangle, \qquad [7] \end{aligned}$$

where z' points along the direction of the effective field formed by the vector sum of the residual Zeeman field and the B_1 field, $\tan\theta = \omega_1/\Delta\omega$, $\omega_e = \sqrt{(\omega_1^2 + \Delta\omega^2)}$, ω_1 is the spin-lock field strength, $\Delta\omega$ is the resonance offset, γ_i is the gyromagnetic ratio of spin i, and r_{ij} is the distance between spins i and j. When $\omega_1 \gg \Delta\omega$, $\cos\theta \sim 0$, $z' \rightarrow y$ (for a B_1 field along y) and the above cross-correlation terms vanish. In addition, so long as the relation $\Delta\omega \ll \omega_1 \ll 1/\tau_m$ is satisfied, $T_{1\rho}(I_zC_zD_{z'}) = T_2(I_zC_zD_y)$ (*15*). Note that other cross-correlation effects are still present during the spin-lock period (for example, interference between the two $^1H-^{13}C$ dipolar interactions). However, these effects do not contribute $J(\omega_e) \sim J(0)$ terms to the relaxation expressions, while the decay of $\langle I_zC_zD_y\rangle$ is dominated by the $J(0)$ term arising from the deuterium quadrupolar interaction, and, hence, cross-correlation effects are completely negligible in this case as well.

Calculations have been performed to analyze the effects of cross correlation and cross relaxation on measured decay values of $I_zC_zD_y$ in the case of an additional proton spin, H_k. The results establish that $[1/T_{1\rho}(I_zC_zD_y) - 1/T_1(I_zC_z)]/1/T_2(D)] < 0.025$ for the full range of motional parameters considered above, indicating that Eq. [2] is valid for $T_{1\rho}$ measurements as well.

The results from the present set of calculations indicate that, despite the fact that a complex set of equations governs the decay of triple-spin magnetization ($I_zC_zD_z$ or $I_zC_zD_y$), it is surprisingly simple to extract accurate deuterium relaxation rates, $1/T_1(D)$ and $1/T_{1\rho}(D)$. The accuracy of the method is no doubt the result of the fact that by far the dominant contribution to the decay of the triple-spin terms arises from the deuterium quadrupolar interaction, and that the contribution to the relaxation from adjacent proton spins can be accounted for by the measurement of the decay of I_zC_z two-spin longitudinal order. The ^2H relaxation experiments provide, therefore, a simple approach for obtaining information about side-chain dynamics in proteins and are a useful complement to existing methods for measuring backbone motions.

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