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The Influence of Methyl Rotor Dynamics on Hydrogen Relaxation Networks: Derivation of Spectral Densities in Model-Free Form

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Abstract: Nuclear magnetic resonance spectral densities corresponding to three-site jump and continuous diffusion models for methyl rotation have been derived for methyl-non-methyl and methyl-methyl dipolar interactions. These spectral densities have been cast in model-free form such that they may be used to make direct contact with experimentally accessible measurements. It is shown that the calculation of an order parameter $\tilde{S}^2$ and an average $\langle r^{-6}\rangle$, required for the spectral density functions, can be efficiently performed for any arbitrary geometry and could easily be included in a comprehensive relaxation matrix-based refinement of molecular models. Comparisons are presented which detail the range of variation in these parameters as a function of geometry for the two models considered. Simulations of the nuclear Overhauser effect for a protein using a full relaxation matrix treatment emphasize the need for the rigorous treatment of methyl rotor dynamics, as opposed to the commonly used rigid rotor or pseudoatom approximations.

Introduction

A comprehensive view of the fast internal dynamics of proteins is becoming increasingly accessible by a variety of nuclear magnetic resonance (NMR) techniques.[1-7] Much work has been focused on the analysis of heteronuclear relaxation experiments, notably the interpretation of T_1 and T_2 relaxation times and the steady-state NOE.[8-11] Accurate interpretation of these measurements to yield useful dynamic information relies on either the specification of spectral density functions derived from detailed motional models[12-21] or, more generally, the use of a model-independent form of the spectral density which depends upon a small number of parameters.[16,17]

While investigations of heteronuclear relaxation are becoming increasingly common, few researchers have as yet proposed similar detailed analyses of homonuclear hydrogen-hydrogen relaxation. Though significant progress has been made in the development of algorithms required to employ a comprehensive rate matrix treatment to the analysis of NOESY spectra,[22-27] little progress has been made in the explicit and exact treatment of the role of intrinsic internal dynamics on the definition of the elements of the rate matrix. For the most part, current applications of rate-matrix-based refinement of model structures based upon structural constraints derived from NMR parameters have assumed an isotropically tumbling rigid rotor model.[28-33] With a few notable exceptions,[34,35] most attempts at including dynamic effects in this context have been empirical in nature.

Perhaps the most accessible point at which one can begin to address the issue of internal dynamics is a consideration of the effects of internal motions of methyl hydrogens. Though significant progress has been in this area,[34] an important consideration has not been met. That is, the treatment of methyl rotor dynamics ought to provide a direct path to experimentally accessible observables. In this paper, we derive equations for the spectral densities corresponding to dipolar interactions involving methyl hydrogen spin groups. These spectral densities have been cast in a form that allows calculation of generalized order parameters directly comparable to those that could, given sufficient data, be experimentally determined using the model-free theory of Lipari and Szabo.[16,17] We present such equations both for the case of single methyl hydrogens interacting with any other spin which is not also a methyl hydrogen (H^{Me}-X case) and also for the previously undocumented case of two interacting methyl groups (H^{Me}-H'^{Me}). Equations for both situations are given for a multisite jump model and for diffusive behavior. Simulations are presented which detail the effect of the full range of geometric variables on the order parameter and provide a means of both gauging the effects of such internal motions and interpreting actual experi-

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mental results. Previous work[12,19,21,34] has dealt only with models presented in form which are not intuitively related to the measurement of order parameters and, in addition, has not investigated the full range of possible geometries.

Theory

For the case of diffusive internal motions which are fast on the time scale of overall molecular reorientation, the autocorrelation function $C(t)$ describing the motion of a vector in the magnetic field can be factored into a product of two functions, one describing overall reorientation and one describing the internal motion:[16]

$$C(t) = C_0(t)\cdot\tilde{C}_I(t) \tag{1}$$

The function $\tilde{C}_I(t)$ describes internal motion in the frame of reference rigidly attached to the molecule. The formal definition of the autocorrelation function is given by[36]

$$\tilde{C}_I(t) = \left\langle \frac{P_2(\hat{\mu}(0)\cdot\hat{\mu}(t))}{r^3(0)r^3(t)} \right\rangle \tag{2}$$

where r is the length of the internuclear vector $\mathbf{r}$, and $\mu = \mathbf{r}/r$ is a unit vector coincident with $\mathbf{r}$. The simplest form of such a function has been given by Lipari and Szabo,[16,17] and its justification is not elaborated here. This form is given as

$$\tilde{C}_I(t) = \tilde{S}^2 + (\langle r^{-6}\rangle - \tilde{S}^2)e^{-t/\tau_e} \tag{3}$$

where $\langle r^{-6}\rangle = \tilde{C}_I(0)$, the value of the autocorrelation function at time $t = 0$, $\tilde{S}^2$ is the order parameter, and τ_e is an effective correlation time for the internal motion. The order parameter $\tilde{S}^2$ is defined as the limiting value of $\tilde{C}_I(t)$ at long times

$$\tilde{C}_I(\infty) = \tilde{S}^2 = \sum_{m=-2}^{2} \left| \left\langle \frac{C_{2m}(\Omega)}{r^3} \right\rangle \right|^2 \tag{4}$$

and is a measure of the amplitude of variations in both the length and angular orientation of the internuclear vector $\mathbf{r}$. The complete autocorrelation function and the related spectral density for the case of overall isotropic molecular tumbling with correlation time τ_m are given by

$$C(t) = \frac{1}{5}[\tilde{S}^2 e^{-t/\tau_m} + (\langle r^{-6}\rangle - \tilde{S}^2)e^{-t/\tau}]$$

$$J(\omega) = 2\int_0^\infty C(t)\cos(\omega t)\,\mathrm{d}t = \frac{2}{5}\left[\frac{\tilde{S}^2\tau_m}{1+\omega^2\tau_m^2} + \frac{(\langle r^{-6}\rangle - \tilde{S}^2)\tau}{1+\omega^2\tau^2}\right] \tag{5}$$

where

$$\frac{1}{\tau} = \frac{1}{\tau_m} + \frac{1}{\tau_e}$$

On the basis of this equation, it can be seen that $\langle r^{-6}\rangle$ represents a static average, applicable when internal motions are slow compared to the overall molecular tumbling. In contrast, $\tilde{S}^2$ contains the information about internal motions that are faster than molecular tumbling and is both an orientational and a radial average.

For the specific case of the interaction of a single spin (X) with a methyl hydrogen (H) undergoing rotations about the methyl symmetry axis, we may express the order parameter $\tilde{S}^2$ as a function of two variable geometric factors and a sum over N sites for a discrete jump model or as an integration over the entire range of values when simple rotational behavior is considered, i.e., when $N \rightarrow \infty$. We begin by specifying the coordinates of the methyl hydrogen H in a reference frame convenient for the calculation of $\tilde{S}^2$. We consider the spin X to be at the origin of our molecular frame and the center of mass of the three methyl hydrogens, designated M, to be along the positive z-axis. We assume a standard tetrahedral geometry for the methyl carbon–hydrogen bond, which also fixes the distance d_{HM} as a constant which is identical for all methyls. In general, this is not a necessary assumption and the distance could be specified for individual methyls. As pointed out by Tropp,[34] the relative geometry of the methyl hydrogen with respect to the spin X will depend on the distance d_{MX} and the angle α, defined as the angle given by C–M–X where C indicates the position of the methyl carbon. This angle can range between 0° and 180°. For a discrete jump site model, the rotational conformer of the methyl hydrogen must also be defined. This is denoted by the angle ϕ^{Me}, the azimuthal angle for the vector HM about its methyl symmetry axis. We conveniently define ϕ^{Me} by reference to the angle between the vectors given by the cross products (C–M) × (H–M) and (X–M) × (C–M). In addition, the methyl carbon C is considered to lie solely in the x–z plane ($y^c = 0$) and, more specifically, to be in the $+x$ quadrant ($x^c \geq 0$). In this scheme, $\phi^{Me} = \phi^{mol}$, the azimuthal angle in our molecular frame, for $\alpha = 0°$. For $\alpha = 90°$ and $\phi^{Me} = 0°$, the methyl hydrogen lies along the z-axis at a distance $d_{HX} = d_{HM} + d_{MX}$. These definitions are summarized graphically in Figure 1.

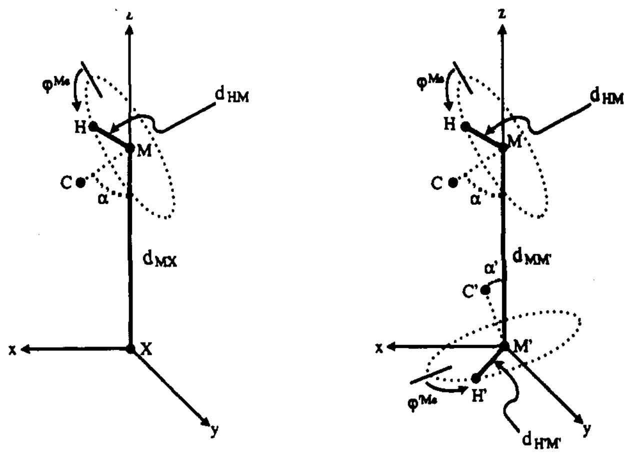

Figure 1. Definition of the geometries used in the derivation of autocorrelation functions for methyl–X (left) and methyl–methyl (right) dipolar interactions. Hydrogens are drawn coming out of the page ($+y$ quadrant). In the double methyl case, χ is defined by the C–M–M′–C′ dihedral angle.

Table I. Spherical Harmonics Defining the Three-Site Jump Model for Methyl Rotation

m	$\frac{C_{2m}(\theta_i,\varphi_i,r_i)}{r_i^3}$	$\frac{C_{2m}(x_i,y_i,z_i)}{r_i^3}$
0	$\frac{1}{2}\frac{(3\cos^2\theta_i - 1)}{r_i^3}$	$\frac{1}{2}\left(\frac{3z_i^2}{r_i^5} - \frac{1}{r_i^3}\right)$
±1	$\mp\left(\frac{3}{2}\right)^{1/2}\frac{\sin\theta_i\cos\theta_i}{r_i^3}e^{\pm i\phi_i}$	$\mp\left(\frac{3}{2}\right)^{1/2}\left(\frac{x_iz_i \pm iy_iz_i}{r_i^5}\right)$
±2	$\pm\left(\frac{3}{8}\right)^{1/2}\frac{\sin^2\theta_i}{r_i^3}e^{\pm 2i\varphi_i}$	$\pm\left(\frac{3}{8}\right)^{1/2}\left(\frac{(x_i^2 - y_i^2) \pm i2x_iy_i}{r_i^5}\right)$

Using the above definitions, the coordinates of a methyl hydrogen as a function of d_{HM}, α, d_{MX}, and ϕ^{Me} are given by

$$x^H = d_{HM}\cos\alpha\cos\phi^{Me} \qquad y^H = d_{HM}\sin\phi^{Me}$$
$$z^H = d_{MX} + d_{HM}\sin\alpha\cos\phi^{Me} \tag{6}$$

Subsequent algebraic manipulation allows the distance r to be expressed as

$$r = d_{HX} = (d_{MX}^2 + d_{HM}^2 + 2d_{MX}d_{HM}\sin\alpha\cos\phi^{Me})^{1/2} \tag{7}$$

Using these equations, the generalized order parameter for a three-site jump model can be calculated as the average over three orientations of ϕ^{Me}. For each spherical harmonic C_{2m}, the angle brackets represent the average over $i = 1..3$, corresponding to $\phi^{Me}_i = \phi^{Me} + \Delta\phi^{Me}_i$, where $\Delta\phi^{Me}_i = 0, \pm 120°$. Table I contains the

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various C_{2m} expressed both in polar form and by way of Cartesian coordinates. Given the above coordinate frame definitions, the Cartesian forms are particularly simple when calculating $\tilde{S}^2$ for any model involving discrete jump sites. For this model, the averages are given by

$$\langle\langle(\ldots)\rangle\rangle = \frac{1}{N}\sum_{i=1}^{N}(\ldots)_i \tag{8}$$

with $N = 3$. One might also wish to examine the case in which all values of ϕ^{Me} are equally accessible with no preferred rotational conformer. In this case, the averages become integrals of the form

$$\langle\langle(\ldots)\rangle\rangle = \frac{1}{2\pi}\int_0^{2\pi}(\ldots)\,\mathrm{d}\phi^{\mathrm{Me}} \tag{9}$$

Each function C_{2m} is expanded in terms of the parameters d_{HM}, α, d_{MX}, and ϕ^{Me}. For the cases $m \neq 0$, the imaginary portions of C_{2m} are odd functions, dependent on terms in $\sin\phi^{\mathrm{Me}}$, and integrate to 0. The remaining functions are given below and can be expressed in terms of complete elliptical integrals of the first and second kind.

$$\left\langle\frac{C_{20}}{r^3}\right\rangle = \frac{3}{2}\left(d_{\mathrm{MX}}^2\left\langle\frac{1}{r^5}\right\rangle + 2d_{\mathrm{MX}}d_{\mathrm{HM}}\sin\alpha\left\langle\frac{\cos\phi^{\mathrm{Me}}}{r^5}\right\rangle + d_{\mathrm{HM}}^2\sin^2\alpha\left\langle\frac{\cos^2\phi^{\mathrm{Me}}}{r^5}\right\rangle\right) - \frac{1}{2}\left\langle\frac{1}{r^3}\right\rangle$$

$$\left\langle\frac{C_{2\pm1}}{r^3}\right\rangle = \mp\left(\frac{3}{2}\right)^{1/2}\left(d_{\mathrm{MX}}d_{\mathrm{HM}}\cos\alpha\left\langle\frac{\cos\phi^{\mathrm{Me}}}{r^5}\right\rangle + d_{\mathrm{HM}}^2\sin\alpha\cos\alpha\left\langle\frac{\cos^2\phi^{\mathrm{Me}}}{r^5}\right\rangle\right)$$

$$\left\langle\frac{C_{2\pm2}}{r^3}\right\rangle = \pm\left(\frac{3}{8}\right)^{1/2}\left(d_{\mathrm{HM}}^2(1+\cos^2\alpha)\left\langle\frac{\cos^2\phi^{\mathrm{Me}}}{r^5}\right\rangle - d_{\mathrm{HM}}^2\left\langle\frac{1}{r^5}\right\rangle\right) \tag{10}$$

These equations make use of the identities derived in the Appendix.

The above equations demonstrate how to calculate $\tilde{S}^2 = \tilde{C}_{\mathrm{I}}(\infty)$. In order to completely specify the autocorrelation function, one must also derive expressions for $\tilde{C}_{\mathrm{I}}(0) = \langle r^{-6}\rangle$. For the 3-site jump model, this is easily calculated as the sum

$$\langle r^{-6}\rangle = \frac{1}{3}\sum_{i=1}^{3}(d_{\mathrm{MX}}^2 + d_{\mathrm{HM}}^2 + 2d_{\mathrm{MX}}d_{\mathrm{HM}}\sin\alpha\cos\phi_i^{\mathrm{Me}})^{-3} \tag{11}$$

In the case of full integration over all ϕ^{Me}, the above is replaced by

$$\begin{aligned}\langle r^{-6}\rangle &= ((d_{\mathrm{MX}}^2 + d_{\mathrm{HM}}^2)^2 - (2d_{\mathrm{MX}}d_{\mathrm{HM}}\sin\alpha)^2)^{-3/2} \\ &\quad\times P_2\left(\frac{d_{\mathrm{MX}}^2 + d_{\mathrm{HM}}^2}{((d_{\mathrm{MX}}^2 + d_{\mathrm{HM}}^2)^2 - (2d_{\mathrm{MX}}d_{\mathrm{HM}}\sin\alpha)^2)^{1/2}}\right) \\ &= \frac{2(d_{\mathrm{MX}}^2 + d_{\mathrm{HM}}^2)^2 + (2d_{\mathrm{MX}}d_{\mathrm{HM}}\sin\alpha)^2}{2((d_{\mathrm{MX}}^2 + d_{\mathrm{HM}}^2)^2 - (2d_{\mathrm{MX}}d_{\mathrm{HM}}\sin\alpha)^2)^{5/2}}\end{aligned} \tag{12}$$

In deriving the fully integrated forms for $\tilde{S}^2$ and $\langle r^{-6}\rangle$ above, we have taken advantage of the fact that the internuclear distance has the general form $r = (a + b\cos\phi^{\mathrm{Me}})^{1/2}$ where a and b are constants. This dictates the expression of $\tilde{S}^2$ in terms of complete elliptical integrals and results in the above simple expression for $\langle r^{-6}\rangle$ which has the form $(2a^2 + b^2)/2(a^2 - b^2)^{5/2}$.

The equations for $\tilde{S}^2$ and $\langle r^{-6}\rangle$ for two hydrogens, each of which is a methyl hydrogen on separate methyl groups, can be examined in detail in a similar fashion. We equate the position of a hydrogen (H) from one methyl with the expressions above, where the origin is now identified with the center of mass of the second methyl group, designated M′. The same definitions apply to the geometry of the first methyl hydrogen as were given above, with the substitution of M′ for X. To define the geometry of the second methyl hydrogen, we must specify not only the angle α' given by C′–M′–M but also a dihedral angle (χ) describing the relative orientations of the two methyl symmetry axes. The dihedral is defined by C–M–M′–C′. The rotational conformer of the second methyl hydrogen is specified by ϕ'^{Me} and is found in reference to the angle between the cross products (C′–M′) × (H′–M′) and (M–M′) × (C′–M′) with $\phi'^{\mathrm{Me}} = \phi'^{\mathrm{mol}}$ when $\alpha' = 0$. Again, ideal methyl geometry is assumed such that the distance $d_{\mathrm{H'M'}}$ is constant and equal to d_{HM}, and we do not further distinguish the two quantities below, although this is merely a convenience and is not required for describing the system. Hence, the coordinates for the second methyl hydrogen are given by (see Figure 1)

$$\begin{aligned}x^{\mathrm{H'}} &= d_{\mathrm{HM}}(\cos\alpha'\cos\chi\cos\phi'^{\mathrm{Me}} + \sin\chi\sin\phi'^{\mathrm{Me}}) \\ y^{\mathrm{H'}} &= d_{\mathrm{HM}}(\cos\chi\sin\phi'^{\mathrm{Me}} - \cos\alpha'\sin\chi\cos\phi'^{\mathrm{Me}}) \\ z^{\mathrm{H'}} &= -d_{\mathrm{HM}}\sin\alpha'\cos\phi'^{\mathrm{Me}}\end{aligned} \tag{13}$$

The distance $r = d_{\mathrm{HH'}}$ is found simply by the usual vector equation for the length of the vector H–H′. When this equation is explicitly expanded, a rather complex expression is found which has the following general form $r = (a + b\cos\phi^{\mathrm{Me}} + c\cos\phi'^{\mathrm{Me}} + d\cos\phi^{\mathrm{Me}}\cos\phi'^{\mathrm{Me}} + e\sin\phi^{\mathrm{Me}}\sin\phi'^{\mathrm{Me}} + f\cos\phi^{\mathrm{Me}}\sin\phi'^{\mathrm{Me}} + g\sin\phi^{\mathrm{Me}}\cos\phi'^{\mathrm{Me}})^{1/2}$ where a–g are constants. To calculate the order parameter and the average $\langle r^{-6}\rangle$ for a 3-site jump model, the identical equations for the spherical harmonics (Table I) as for the single methyl case are used, where now the coordinates in the functions are for the difference vector given by the coordinates $[(x^{\mathrm{H}}-x^{\mathrm{H'}}),(y^{\mathrm{H}}-y^{\mathrm{H'}}),(z^{\mathrm{H}}-z^{\mathrm{H'}})]$. Note that the averages involve sums of the form

$$\langle\langle(\ldots)\rangle\rangle = \frac{1}{NN'}\sum_{i=1}^{N}\sum_{j=1}^{N'}(\ldots)_{i,j} \tag{14}$$

with $N,N' = 3$. The complicated form of r prevents a derivation of useful integrals for the case of diffusive behavior among $N,N' \to \infty$ equivalent sites, since this would involve double integration over ϕ^{Me} and ϕ'^{Me}. Therefore, to calculate the order parameter and average $\langle r^{-6}\rangle$, a sum over discrete sites is always employed. However, computer simulations indicate that an approximated full integration using large values for N and N' converges rapidly for reasonable step sizes. Using $N,N' = 180$, the sum can be performed rapidly and the values determined converge to at least 10 significant digits.

Results

The parameters $\tilde{S}^2$ and $\langle r^{-6}\rangle$ enter into the definition of the spectral densities, which in turn determine the NMR dipolar relaxation rates.[16,17] Accordingly, we have examined these parameters for a range of geometries.

Methyl-X Cross-Relaxation. A common simplification for simulating the relaxation behavior of methyl groups is the use of either the completely rigid model with fixed distances for all three hydrogens or the pseudoatom treatment where the center of mass of the three methyl hydrogens is used as a rigid distance. Neither appropriately accounts for the motional averaging of the $\mathrm{H^{Me}}$–X vector, and the latter approximation may inappropriately neglect the intramethyl $\mathrm{H^{Me}}$–$\mathrm{H^{Me}}$ contribution to the overall relaxation.

The use of $\langle r^{-6}\rangle$ alone, in place of a fixed distance, accounts for averaging only on time scales slower than molecular tumbling. Such averaging weights the shortest distances more, which may lead to a smaller effective $\mathrm{H^{Me}}$–X distance compared to the distance to the methyl center of mass d_{MX}. This is most apparent when $\alpha = 90°$; however, for the axially symmetric case with $\alpha = 0°$, the distance $r = d_{\mathrm{HX}}$ is equal for all sites and is longer than the distance d_{MX}. The value of $\langle r^{-6}\rangle$ was calculated for a range of values of α and d_{MX} for the 3-site jump model, and the effective distance $\langle r^{-6}\rangle^{-1/6}$ versus ϕ^{Me} for the range $0° \leq \phi^{\mathrm{Me}} \leq 120°$ is shown in Figure 2.

The appropriate model which accounts for fast internal motions is given by eq 3. To examine the effect on the order parameter

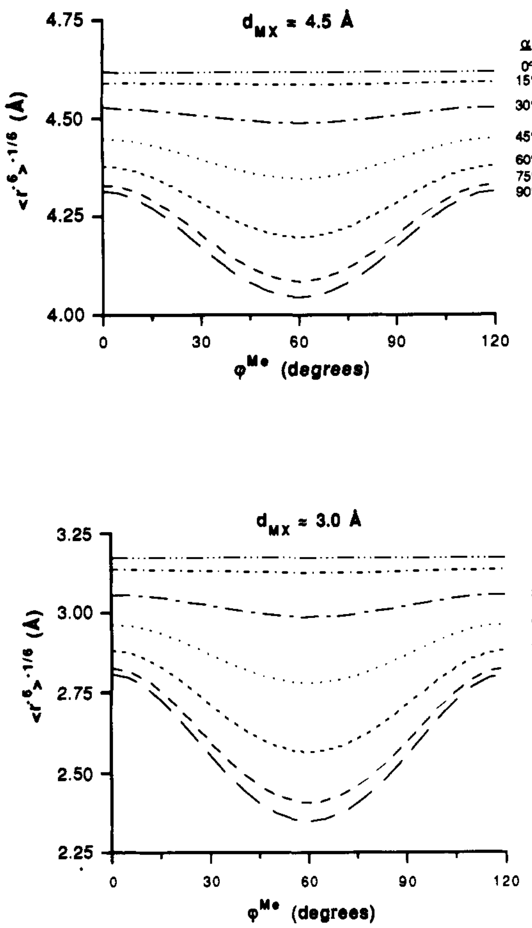

Figure 2. Simulated values of the effective distance given by $\langle r^{-6}\rangle^{-1/6}$ versus methyl rotamer ϕ^{Me} for the case of a single methyl undergoing motions described by a 3-site jump model. The values were calculated for the range $0° \leq \alpha \leq 90°$ and pseudoatom distances d_{MX} of 4.5 Å (top panel) and 3.0 Å (bottom panel). An ideal tetrahedral methyl geometry was assumed with a C–H bond length of 1.09 Å.

$\tilde{S}^2$ for the fast motions compared to simple $\langle r^{-6}\rangle$ averaging, we first rewrite the internal autocorrelation function as

$$\tilde{C}_I(t) = \langle r^{-6}\rangle(\tilde{S}'^2 + (1 - \tilde{S}'^2)e^{-t/\tau_e}) \quad \text{where } \tilde{S}'^2 \equiv \tilde{S}^2/\langle r^{-6}\rangle \tag{15}$$

This equation is superficially analogous to the form of the autocorrelation function when only angular variations are considered and all internuclear distances are fixed. We note that in such a case $\tilde{S}'^2 = S^2$ and can range from 0 to 1.[16,17] In the other extreme where only radial variations contribute to the internal motion, $\tilde{S}^2 = \langle r^{-3}\rangle^2$ and will always be $\leq\langle r^{-6}\rangle$. Thus we expect that $\tilde{S}'^2$ will always be ≤ 1. The values of $\tilde{S}^2$ were calculated for a range of values of α and d_{MX} for the 3-site jump model, and the ratio $\tilde{S}'^2 = \tilde{S}^2/\langle r^{-6}\rangle$ was plotted versus ϕ^{Me} (Figure 3). As the order parameter is the dominant factor for extremely fast internal motions in the limit where τ_e approaches 0, the effective distance $(\tilde{S}^2)^{-1/6}$ was also plotted versus ϕ^{Me} (Figure 4). Similar calculations were performed for the case of diffusive behavior, where knowledge of the value of ϕ^{Me} is irrelevant. The variations with respect to α and d_{MX} are qualitatively similar to those for the 3-site jump model. We have therefore tabulated $(\tilde{S}^2)^{-1/6}$ for α = 0,90° at a number of values of d_{MX} (Table II). In all of the above cases, the results are symmetric about $\alpha = 90°$.

Intramethyl Cross-Relaxation. The case of two methyl hydrogens belonging to the same methyl group is a special case of axial symmetry, where we have a rigid H–H distance fixed by the tetrahedral geometry but the rotation about the methyl symmetry axis yields the following effective order parameter of $(P_2(\cos \pi/2))^2/r_{HH}{}^6 = {}^1/_4 r_{HH}{}^{-6}$ in the case of fast internal motions.[12] The complete $\tilde{C}_I(t)$ would be written as $\tilde{C}_I(t) = r_{HH}{}^{-6}({}^1/_4 - {}^3/_4 e^{-t/\tau_e})$.

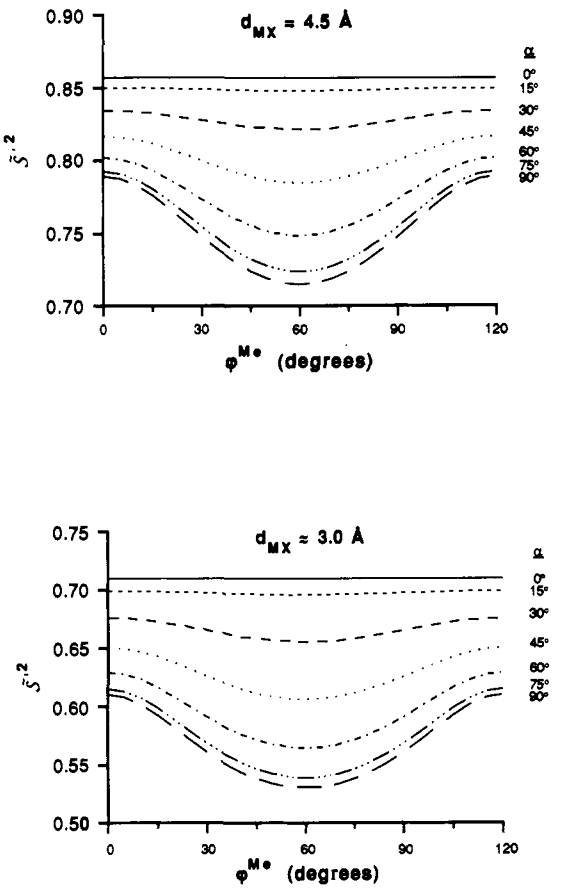

Figure 3. Ratio $\tilde{S}'^2 = \tilde{S}^2/\langle r^{-6}\rangle$ versus methyl rotamer ϕ^{Me} for the case of a single methyl undergoing motions described by a 3-site jump model. The values were calculated for the range $0° \leq \alpha \leq 90°$ and pseudoatom distances d_{MX} of 4.5 Å (top panel) and 3.0 Å (bottom panel). An ideal tetrahedral methyl geometry was assumed with a C–H bond length of 1.09 Å.

Table II. Effective Distances $(\tilde{S}^2)^{-1/6}$ Obtained from Dipolar Interactions between a Single Hydrogen and a Methyl Group Undergoing Diffusive Rotation

	$(\tilde{S}^2)^{-1/6}$ (Å)	
d_{MX} (Å)	$\alpha = 0°$	$\alpha = 90°$
2.5	2.93	2.27
3.0	3.38	2.81
3.5	3.81	3.37
4.0	4.30	3.86
4.5	4.74	4.38
5.0	5.26	4.89
6.0	6.22	5.91

For rotational rates slower than overall tumbling, the complete autocorrelation function reduces to that of the rigid case with no internal motions.

Intermethyl Cross-Relaxation. Similar calculations can be performed as above to compare the behavior of H^{Me}–H'^{Me} relaxation when accounting for both static $\langle r^{-6}\rangle$ averaging and $\tilde{S}^2$ scaling due to fast internal motions. However, it is more difficult to view the results since they are a function of up to five variables in the case of a jump model. The quantities $\langle r^{-6}\rangle$ and $\tilde{S}^2$ have been calculated for an ideal geometry of geminal methyl groups such as for the two methyls of a valine or leucine side chain. For this case, $d_{MM'}$ = 3.093 Å, $\alpha = \alpha' = 35.25°$, and $\chi = 0°$. Note that ϕ^{Me} and ϕ'^{Me} equal to zero corresponds to staggered conformations with respect to the carbon backbone. Figure 5 presents a contour plot for a double 3-site jump model, showing $\tilde{S}^2/\langle r^{-6}\rangle$ versus ϕ^{Me} and ϕ'^{Me}. The effective distance $(\tilde{S}^2)^{-1/6}$ for this case ranges from 3.16 to 3.42 Å.

Simulations were performed to examine the diffusive model for the full range of α, α', and χ. Results are qualitatively similar

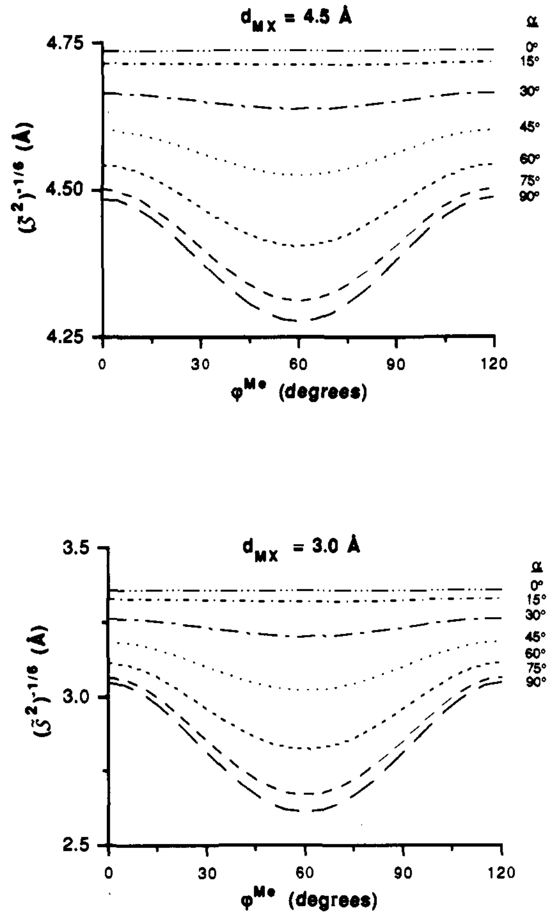

Figure 4. Simulated values of the effective distance given by $(\bar{S}^2)^{-1/6}$ versus methyl rotamer ϕ^{Me} for the case of a single methyl undergoing motions described by a 3-site jump model. The values were calculated for the range $0° \leq \alpha \leq 90°$ and pseudoatom distances d_{MX} of 4.5 Å (top panel) and 3.0 Å (bottom panel). An ideal tetrahedral methyl geometry was assumed with a C–H bond length of 1.09 Å.

to the single methyl cases; the minimum order parameter occurs when $\alpha,\alpha' = 0,180°$, and the maximum occurs for $\alpha = \alpha' = 90°$ and $\chi = 0,180°$. The range for the effective distance $(\bar{S}^2)^{-1/6}$ for the case of $d_{MM'}$ = 4.0 Å is 3.69–4.48 Å.

NOE Time Courses. The effects of the various treatments of methyl rotor dynamics on the time dependence of NOE cross peaks were investigated using a working model for the solution structure of human ubiquitin as an example system. NOESY simulations were performed using a rate-matrix-based calculation assuming rigid rotor isotropic tumbling for all dipolar relaxation rates except for those involving methyl hydrogens.[37] The importance of explicit treatment of methyl rotor dynamics becomes quite important in cases where the primary distance defining the methyl–X interaction is to be directly determined from the time course of the NOE. For example, short-range $(H_\alpha,H_N)_{i,i+1}$ and long-range $(H_\alpha,H_\beta)_{i,i+3}$ NOEs are extremely important constraints for the definition of α-helical protein secondary structure.[38,39] As shown in Figure 6, the rigid rotor treatment, and often the simple pseudoatom treatment, leads to an underestimated distance relative to the exact treatments. Interestingly, in most cases examined the three-site jump and continuous diffusion models for methyl rotation give rise to similar NOE time courses. In the case of fixed (and assumed) geometry (such as the CH–CH_3 fragment of alanine and the $CH(CH_3)_2$ fragment of leucine and valine), the effect of inappropriate treatment of methyl group relaxation on structure determination will be second order in the sense that errors in describing the evolution of populations will be manifested only in the spin diffusion regime. This is fortunate, as the variance between the rigid rotor and simple pseudoatom treatments and the three-site jump and continuous diffusion treatments is quite large (Figure 7).

Discussion

A primary motivation of the work presented here is to allow for the efficient but rigorous treatment of methyl rotations during simulations of NOESY spectra. Such simulations are required for the quantitative iterative refinement of molecular models on the basis of relaxation matrix methods. Here it is shown how to calculate the autocorrelation functions and spectral densities for both the 3-site jump and continuous diffusion motional models for dipolar interactions involving a single methyl or two methyls in a form which is directly comparable to the model-free form of Lipari and Szabo.[16,17] The equations for the parameters $\bar{S}^2$ and $\langle r^{-6}\rangle$ have been expressed in terms of the minimal geometric parameters describing any orientation of a methyl with respect to its interacting partner, independent of the particular choice of coordinate system. Note, however, that these parameters are simple to derive from a set of coordinates using standard vector operations. This allows one to simulate the expected relaxation parameters analytically as a function of geometry as well as to include such models in a full-scale spectral simulation.[37] The computational cost of implementing these models is insignificant when compared to the total computational effort required.

Compared to the most commonly used method for dealing with dipolar interactions involving methyl groups, that of using a pseudomethyl atom at the center of mass of the three methyl hydrogens, the exact models for 3-site jump and diffusive behavior both predict relaxation behavior which depends markedly on the specific geometry of the spin system. This is evident in the comparison of relaxation parameters as a function of ϕ^{Me} in the case of the jump models and as a function of the angle α and the distance to the methyl center of mass in all models. The appropriate model involves averages of quantities dependent on both $1/r^6$ ($\langle r^{-6}\rangle$) and $1/r^3$ ($\bar{S}^2$), with the latter term dominating in the fast motional regime. Other averages, such as $1/\langle r^3\rangle^2$ used in a recent treatment,[32] are not appropriate as they do not correspond to physically possible systems, improperly weight short distances, and ignore the effect of angular reorientation. We note that for the single methyl interaction, the case of axial symmetry ($\alpha = 0,180°$) always leads to an order parameter $\bar{S}^2 = \langle P_2(x)\rangle^2/r^6$, where r is constant and $x = d_{MX}/r$, and thus results in a different average compared to the simple $\langle r^{-6}\rangle$ average, contrary to previous claims.[40] In the more general case, the order parameter will be less than the value of $\langle r^{-3}\rangle^2$ since angular reorientation occurs concurrently with distance fluctuations. Thus the order parameter will always result in an effectively smaller $1/r^6$ average compared to either $\langle r^{-3}\rangle^2$ or $\langle r^{-6}\rangle$ (Figure 3). The order parameter will be the main factor perturbing the cross-relaxation rate in the limit of vanishingly small values of τ_e. In such cases, the usual rigid rotor cross-relaxation rate is simply scaled by the value of $\bar{S}^2 \cdot r_0$,[6] where r_0 is the assumed rigid distance. As is seen in the simulations of the protein ubiquitin, the inclusion of methyl rotor dynamics does, in most cases, substantially decrease the initial rate of cross-peak buildup, proportional at $t_{mix} = 0$ to the cross-relaxation rate. Thus the use of buildup rates for distance estimations in biomolecular structure determinations, without consideration of methyl rotor dynamics, will result in overestimation of the distances in such cases, as the rate will appear too low. For some geometries and dipolar environments, the pseudomethyl treatment fortuitously approximates the exact treatments. It is also evident that for $d_{MX} \gg d_{HM}$, the effect of the methyl hydrogen rotation has a diminishing effect on the relaxation of spin X. The same can be said for the interaction of two methyls at long distances $d_{MM'}$. Comparison of both $\bar{S}^2$ and $\langle r^{-6}\rangle$ to the distance d_{MX} indicates

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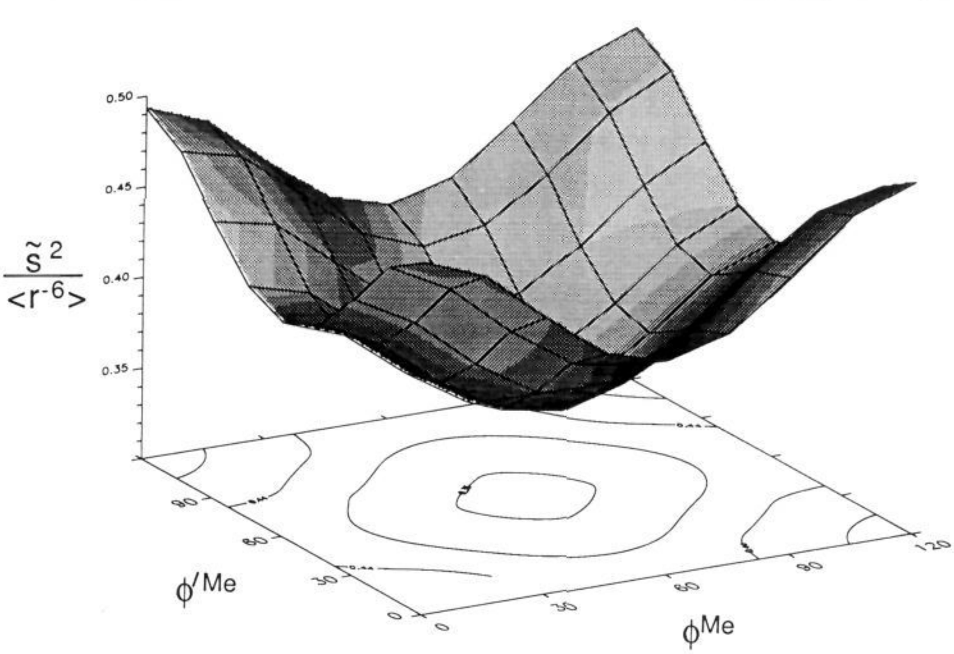

Figure 5. Ratio $\tilde{S}'^2 = \tilde{S}^2/\langle r^{-6}\rangle$ versus methyl rotamers ϕ^{Me} and ϕ'^{Me} for the case of geminal methyl groups where both methyls are undergoing motions described by a 3-site jump model. The values were calculated assuming ideal tetrahedral methyl geometries with C–H bond lengths of 1.09 Å, both angles α and $\alpha' = 35.25°$, the dihedral angle $\chi = 0°$, and the distance d_{MM} between pseudomethyls of 3.093 Å.

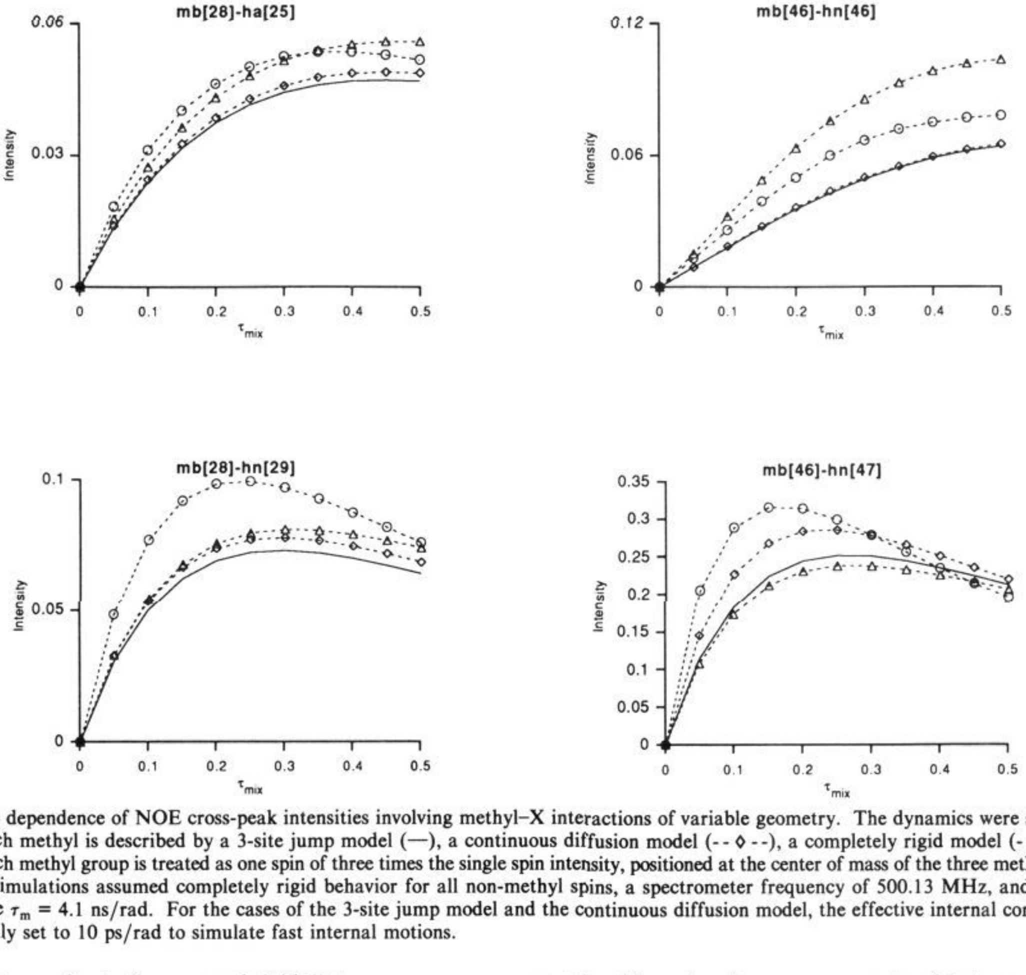

Figure 6. Time dependence of NOE cross-peak intensities involving methyl–X interactions of variable geometry. The dynamics were simulated for cases where each methyl is described by a 3-site jump model (—), a continuous diffusion model (- - ◇ - -), a completely rigid model (- - ○ - -), and a model where each methyl group is treated as one spin of three times the single spin intensity, positioned at the center of mass of the three methyl hydrogens (- - △ - -). All simulations assumed completely rigid behavior for all non-methyl spins, a spectrometer frequency of 500.13 MHz, and a molecular correlation time $\tau_m = 4.1$ ns/rad. For the cases of the 3-site jump model and the continuous diffusion model, the effective internal correlation time τ_e was arbitrarily set to 10 ps/rad to simulate fast internal motions.

that a simple correction to the assumed rigid distance can vary over an interval ±0.5 Å at short distances and progressively less at larger distances, as shown for $\tilde{S}^2$ in Figure 8.

The model-free parameters of Lipari and Szabo[16,17] have been used successfully to measure the unique dynamic information contained in relaxation measurements of heteronuclei.[8-11] A similar approach is, in principle, equally applicable to the studies of ^1H–^1H relaxation. The specific models here allow a means of reconciling theoretical predictions with experimental data. Given accurate measurements of model-free parameters from the re-

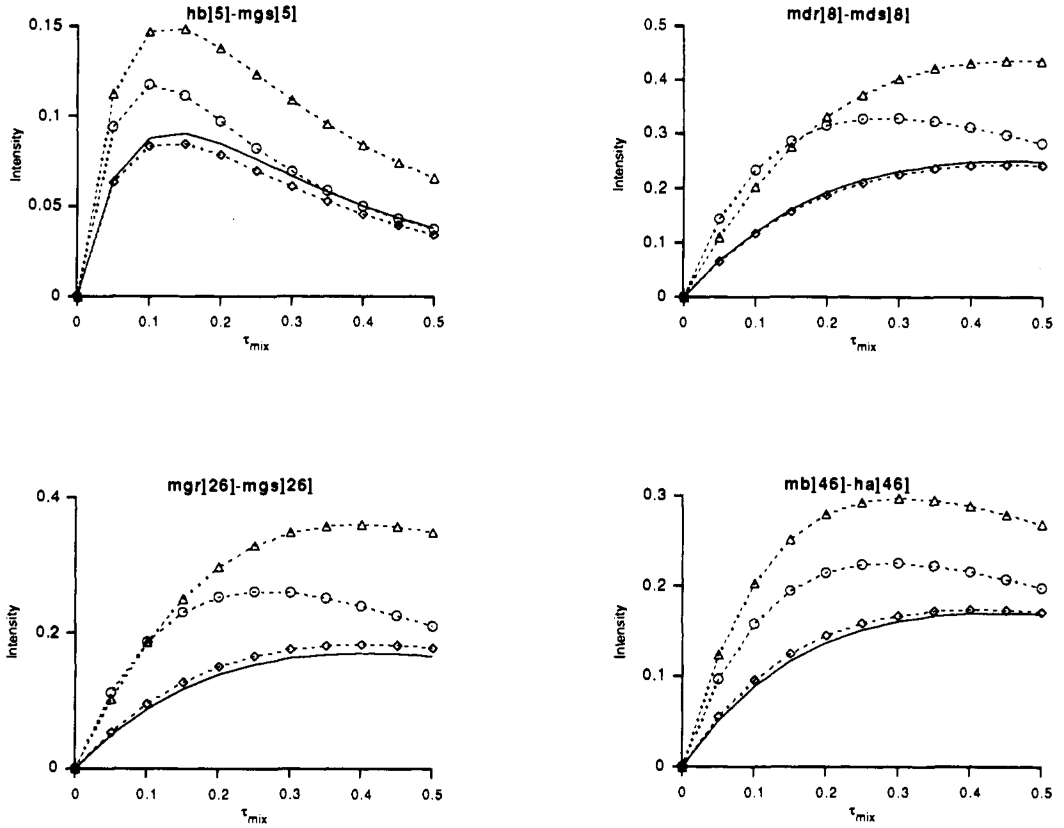

Figure 7. Time dependence of NOE cross-peak intensities involving intraresidue methyl–X and methyl–methyl interactions of fixed geometry. The dynamics were simulated for cases where each methyl is described by a 3-site jump model (—), a continuous diffusion model (- - ◇ - -), a completely rigid model (- - ○ - -), and a model where each methyl group is treated as one spin of three times the single spin intensity, positioned at the center of mass of the three methyl hydrogens (- - △ - -). All simulations assumed completely rigid behavior for all non-methyl spins, a spectrometer frequency of 500.13 MHz, and a molecular correlation time τ_m = 4.1 ns/rad. For the cases of the 3-site jump model and the continuous diffusion model, the effective internal correlation time τ_e was arbitrarily set to 10 ps/rad to simulate fast internal motions.

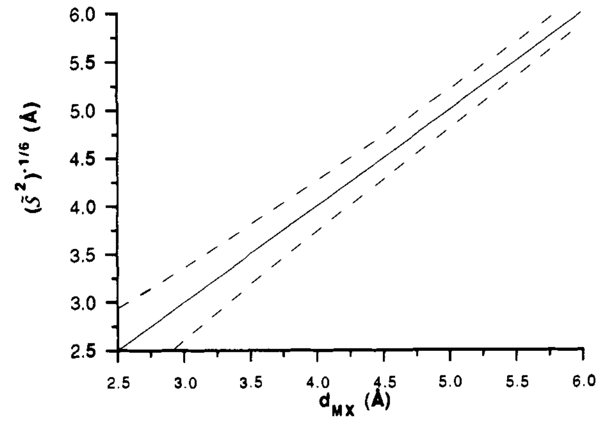

Figure 8. Limits of the effective distance given by $(\bar{S}^2)^{-1/6}$ versus distance to the pseudomethyl center d_{MX} for the case of a single methyl undergoing motions described by either a 3-site jump model or continuous diffusion. The dashed lines encompass the extremes calculated for the full range of values of α and ϕ^{Me}. An ideal tetrahedral methyl geometry was assumed with a C–H bond length of 1.09 Å.

laxation behavior observed in biomolecules, distinguishing between alternative models for the motions of methyl hydrogens is possible. We stress that distinguishing between the two models here, diffusive and jump, cannot be done on the basis of experimental data from one interaction but requires multiple relaxation rates involving a single methyl with various surrounding partners. The case of geminal methyl–methyl interactions might be simply addressed by comparison of results for several different pairs of geminal methyls. Within the limits of ideal bond lengths and geometries, the diffusive model should give similar results in all cases.

Previous work has treated the dynamics of methyls by considering limited motional regimes or specific geometries. In particular, rotational correlation functions for cases of a rigid dipolar vector undergoing axially symmetric diffusion or jump behavior have been considered by Woessner.[20] A case of methyl–methyl relaxation with the simplified geometry where $\alpha = \alpha'$ = 0 for both staggered and eclipsed hydrogen conformations has also been considered as a jump model.[21] The derivation presented here is general for any conformation and considers the case of diffusive behavior. The work of Tropp[34] details the single methyl case for jump models and can be shown to give results identical to those of the equations presented here for isotropic overall motion. Comparing the equations for the methyl–X case to those of Tropp, we note the equivalence of the sum

$$\sum_{m=-2}^{2} f(m) = \sum_{m=-2}^{2}\left|\frac{1}{3}\sum_{i=1}^{3}\frac{C_{2m}(\Omega_i^{\mathrm{mol}})}{r_i^3}\right|^2 \tag{16}$$

to our formulation of $\bar{S}^2$. The function

$$\sum_{m=-2}^{2} g(m) = \sum_{m=-2}^{2}\left(\frac{1}{3}\sum_{i=1}^{3}\left|\frac{C_{2m}(\Omega_i^{\mathrm{mol}})}{r_i^3}\right|^2 - f(m)\right) \tag{17}$$

can be separated and the sums rearranged such that

$$\begin{aligned}\sum_{m=-2}^{2} g(m) &= \frac{1}{3}\sum_{i=1}^{3}\left(\sum_{m=-2}^{2}\left|\frac{C_{2m}(\Omega_i^{\mathrm{mol}})}{r_i^3}\right|^2\right) - \sum_{m=-2}^{2} f(m)\\ &= \frac{1}{3}\sum_{i=1}^{3}\frac{1}{r_i^6} - \sum_{m=-2}^{2} f(m)\\ &= \langle r^{-6}\rangle - \bar{S}^2\end{aligned} \tag{18}$$

These expressions make it clear that the overall autocorrelation function for this model can be rigorously factored into a product of two autocorrelation functions, one describing overall tumbling and one describing the internal motions (eq 1). Also, it is easy to demonstrate, by comparison to the treatment here, that τ_e is the rate for methyl jumps (λ_1^{-1} of Tropp[34]). When internal motions are determined by more than one distinct correlation time such as for diffusive behavior[20] or the case of two independent methyls,[21] then the time constants cannot be uniquely determined by the value of τ_e.[16,17] Further work is in progress to detail the exact relationship of these rates to the definition of τ_e.

We have thus far considered only the case of overall isotropic reorientation. If one assumes overall anisotropic motion with two correlation times τ_1 and τ_2,[16,17] it can be seen in the limit where $\tau_e \ll \tau_{1,2}$ (or $\lambda_1^{-1} \ll \tau_{r0}$ in Tropp[34]) that the internal correlation function can still be rigorously factored out of the total autocorrelation function. For biomolecules of interest, molecular correlation times are on the order of nanoseconds, and this limiting behavior should be seen for methyl rotations. The treatment of fast internal methyl dynamics presented here should therefore be valid even for the case of anisotropic overall motion (see refs 16 and 17).

It is useful to consider modifications involved when methyl rotations are superimposed on additional motions. For relaxation of ^{13}C or ^{15}N nuclei, observed order parameters may be a product of order parameters about successive independent rotational axes.[19] This is not directly applicable to the more general case involving methyl rotations considered here, since the relative orientation defined by the vectors d_{MX} or $d_{MM'}$ and the various angles α, α', or χ may not remain constant irrespective of whether covalent bond lengths and angles are assumed rigid. Additional internal motions could result in a time-dependent change in these parameters or even the distance d_{HM}. In general, this is not trivial to model, and the observed order parameters may be larger or smaller than predicted here. For cases where the geometric parameters are maintained in a time-independent, rigid orientation (assuming constant bond lengths and bond angles), a factorization of the internal motions into two or more order parameters may be possible. This is the case for single methyls whose bonded carbon is separated from a relaxing spin by one or two bonds (such as the β carbon and α hydrogen of alanine).

Although we have ignored the effects of cross correlation, the equations used here can be easily adapted to the calculation of cross-correlation functions.[18,34] Such calculations result in relaxation rates which enter only as additional terms in the rate matrix describing dipolar relaxation and would thus modify the behavior of cross-peak buildup in NOESY spectra at longer mixing times. The order parameters and autocorrelation functions derived here are unaffected.

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Appendix

Equations 10 depend on the following identities for the integrals:

$$\begin{aligned}
\left\langle \frac{1}{r^5} \right\rangle &= \frac{4a}{3(a^2 - b^2)}\left\langle \frac{1}{r^3} \right\rangle - \frac{1}{3(a^2 - b^2)}\left\langle \frac{1}{r} \right\rangle \\
\left\langle \frac{\cos \varphi^{Me}}{r^5} \right\rangle &= \frac{a}{3b(a^2 - b^2)}\left\langle \frac{1}{r} \right\rangle - \frac{a^2 + 3b^2}{3b(a^2 - b^2)}\left\langle \frac{1}{r^3} \right\rangle \qquad \text{(A1)} \\
\left\langle \frac{\cos^2 \varphi^{Me}}{r^5} \right\rangle &= \frac{2a^2 - 3b^2}{3b^2(a^2 - b^2)}\left\langle \frac{1}{r} \right\rangle - \frac{2a(a^2 - 3b^2)}{3b^2(a^2 - b^2)}\left\langle \frac{1}{r^3} \right\rangle
\end{aligned}$$

The averages for $1/r$ and $1/r^3$ can be succinctly written in terms of the complete elliptical integrals of the first and second kind:

$$\begin{gathered}
\left\langle \frac{1}{r^3} \right\rangle = \frac{2(\mathrm{a} + \mathrm{b})^{1/2}}{\pi(a^2 - b^2)}E(k) \qquad \left\langle \frac{1}{r} \right\rangle = \frac{2}{\pi(\mathrm{a} + \mathrm{b})^{1/2}}K(k) \\
a = d_{MX}{}^2 + d_{HM}{}^2 \qquad b = 2d_{MX}d_{HM} \sin \alpha \qquad \text{(A2)} \\
k = \left(\frac{2b}{a + b}\right)^{1/2}
\end{gathered}$$

The transformation of eqs 10 to the form for elliptical integrals makes use of the following change of variables and the fact that the functions involved are even:

$$\begin{gathered}
\varphi^{Me} \rightarrow 2\varphi' \\
(a + b \cos \varphi^{Me})^{n/2} \rightarrow (a + b)^{n/2}(1 - k^2 \sin^2 \varphi')^{n/2} \qquad \text{(A3)} \\
\frac{1}{\pi}\int_0^{\pi} d\varphi^{Me} \rightarrow \frac{2}{\pi}\int_0^{\pi/2} \mathrm{d}\varphi'
\end{gathered}$$

All of the above integrals can be expressed in related forms for which standard solutions exist.