

Dipolar relaxation and nuclear Overhauser effects in nonrigid molecules: The effect of fluctuating internuclear distances

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A simple treatment of the dipolar contribution to nuclear magnetic relaxation is developed for molecules in which conformational fluctuations modulate the relevant internuclear distances. Expressions are given for AX system spectral densities using a general model in which conformational fluctuations occur as random jumps among discrete conformations, while the molecule as a whole undergoes rotational diffusion as either a spherical or a symmetric top. Approximations valid for proton spin systems are given for cases in which the jumping rates are either fast or slow compared to rotational diffusion; the results are independent of the jumping rates. Similar results are obtained for cross-correlation spectral densities. For complex spin systems, e.g., $A_n1M_n2X_n3\dots$, the cross-relaxation constants σ_{ij} , which couple pairs of magnetizations, depend only upon autocorrelation and spectral densities and are thus easily obtained from the AX system results. Measurement of σ_{ij} by time resolved Overhauser effect experiments is discussed with special attention to the problems raised by dipolar cross-correlation and spin diffusion. Numerical calculations of σ_{ij} are given for several geometries describing dipolar relaxation of a rotating methyl proton and a fixed nonmethyl proton in a slowly tumbling macromolecule.

INTRODUCTION

Expressions for dipolar correlation functions in molecules with conformational freedom have been given for a variety of spin systems and motional models,¹⁻⁸ but most calculations to date are subject to the restriction that the relevant internuclear distances remain fixed. The problem of dipolar relaxation in nonrigid molecules with fluctuating internuclear distances has been studied by Woessner⁷ and by Rowan *et al.*⁸ Their results are fundamental but of limited applicability, due to the choice of specialized motional models and the assumption of extreme narrowing conditions. We have extended those results; our treatment is straightforward and general, and leads to simple analytical expressions for both auto- and cross-correlation spectral densities in several motional limits of interest.

We emphasize the calculation of spectral densities for the AX spin system, where dipolar cross correlation does not enter the problem; some results on cross-correlation spectral densities are relegated to an Appendix. This emphasis precludes a completely general treatment of longitudinal relaxation in higher order spin systems where correlated motions involving three and four nuclei may be important.⁹⁻¹² Nevertheless, even in complex systems, such as AMX , AX_2 , or AX_3 , the cross-relaxation terms which couple the z component expectation values of the various spin angular momenta can be calculated from AX system spectral densities. Furthermore, as discussed below, these cross-relaxation terms may often be measured directly by following the early time evolution of one magnetization following rapid, selective saturation of another. Thus, the present treatment should apply to cross-relaxation phenomena in a variety of spin systems, and is specifically aimed at interpreting proton-proton Overhauser effects involving methyl groups in biological macromolecules.

Since we are concerned with molecules in solution, we must account for overall molecular tumbling as well

as for internal motions. We shall assume that the two are uncorrelated. The tumbling will be described as rotational diffusion, as either a spherical or symmetric top¹³⁻¹⁵; the internal motion will be described as jumps between discrete conformations. Regarding each conformation as a distinct chemical species, we assume that the interconversions among species are governed by a system of first order rate equations. Jump models of this type have been widely used to describe internal molecular motions¹⁻⁸ and prove to be well suited for the inclusion of time-varying internuclear distances in the calculation of dipolar correlation functions.

We shall sketch the treatment of a general N site jumping model which leads to useful results in certain motional limits. We shall then consider a specific three site model in detail and give some numerical calculations of cross-relaxation rates. Finally, we discuss the measurement of cross-relaxation rates by nuclear Overhauser effect experiments.

CALCULATION OF CORRELATION FUNCTIONS AND SPECTRAL DENSITIES

We begin by formulating the relaxation problem with-out reference to details of internal motions. Readers primarily interested in applications may skim most of this section.

The dipolar Hamiltonian is

$$\hat{H} = \left(\frac{6\pi}{5}\right)^{1/2} \frac{\gamma_a \gamma_x \hbar^2}{r_{ax}^3(t)} \sum_{m=-2}^2 (-1)^m \hat{T}_{2m}^{(ax)} Y_{2-m}(\Phi_{ax}^{lab}(t)), \quad (1)$$

where the Y_{2m} are spherical harmonics normalized according to Rose,¹⁶ the \hat{T}_{2m} are the second rank irreducible tensor operators defined by Werbelow and Grant,⁹ $r_{ax}(t)$ is the time dependent internuclear distance, and $\Phi_{ax}^{lab}(t)$ specifies the polar angles of the internuclear vector in a laboratory frame of coordinates whose z axis coincides with the applied dc magnetic field. Other

symbols have their usual meanings. We shall drop the subscripts on γ and Φ since no ambiguity can result in the AX system. For convenience, expressions for the Y_{2m} and T_{2m} are collected in Appendix A.

The longitudinal relaxation equations for an AX system are^{17,18}

$$\frac{dI_z^{(a)}}{dt} = -\rho_a(I_z^{(a)} - I_0^{(a)}) - \sigma_{ax}(I_z^{(x)} - I_0^{(x)}), \quad (2.1)$$

$$\frac{dI_z^{(x)}}{dt} = -\rho_x(I_z^{(x)} - I_0^{(x)}) - \sigma_{ax}(I_z^{(a)} - I_0^{(a)}), \quad (2.2)$$

where I_z 's are the z component expectation values of spin angular momentum (loosely referred to herein as "magnetizations"), the zero subscripts indicate the equilibrium values, ρ_a and ρ_x are spin-lattice relaxation rates, and σ_{ax} is the cross-relaxation rate. In terms of spectral densities, the various relaxation rates are⁹

$$\rho_a = \frac{6\pi}{5} (\gamma_a \gamma_x \hbar)^2 \left[\frac{1}{3} J^{00}(\omega_a - \omega_x) + J^{11}(\omega_a) + 2J^{22}(\omega_a + \omega_x) \right], \quad (3.1)$$

$$\rho_x = \frac{6\pi}{5} (\gamma_a \gamma_x \hbar)^2 \left[\frac{1}{3} J^{00}(\omega_a - \omega_x) + J^{11}(\omega_x) + 2J^{22}(\omega_a + \omega_x) \right], \quad (3.2)$$

$$\sigma_{ax} = \frac{6\pi}{5} (\gamma_a \gamma_x \hbar)^2 \left[2J^{22}(\omega_a + \omega_x) - \frac{1}{3} J^{00}(\omega_a - \omega_x) \right], \quad (3.3)$$

where ω_a and ω_x are the A and X spin Larmor frequencies, respectively. The spectral densities are Fourier cosine transforms of correlation functions

$$J^{mm'}(\omega) = \int_0^\infty C^{mm'}(t) \cos \omega t dt. \quad (4)$$

The correlation functions are given by

$$C^{mm'}(t) = \left\langle \frac{Y_{2m}(\Phi^{1ab}(0)) Y_{2m'}(\Phi^{1ab}(t))}{r^3(0) r^3(t)} \right\rangle, \quad (5)$$

where the angular brackets signify an ensemble average over all configurations of the system.

The correlation function may be written in terms of spherical harmonics in a molecular frame of coordinates by use of the Wigner matrices. Assuming the internal motion to be uncorrelated with the overall tumbling, we have³

$$C^{mm'}(t) = \sum_{n,n'} \langle D_{mn}^{2*}(\Omega(0)) D_{m'n'}^{2*}(\Omega(t)) \rangle \times \left\langle \frac{Y_{2n}(\Phi^{mol}(0)) Y_{2n'}(\Phi^{mol}(t))}{r^3(0) r^3(t)} \right\rangle. \quad (6)$$

Here the D_{mn}^2 are the Wigner rotation matrix elements defined by Rose,¹⁶ $\Omega(t)$ specifies the time-dependent Euler angles which carry the coordinate vectors of the laboratory frame into those of the molecule frame, and $\Phi^{mol}(t)$ gives the polar angles of the internuclear vector in the molecule frame. The angular brackets around the D_{mn}^2 indicate the ensemble average over molecular tumbling; these have been given by several authors for the various symmetries of the rotational diffusion tensor.¹³⁻¹⁵ The brackets around the Y_{2n} indicate the average over internal motions, calculation of which requires that we introduce a motional model.

We consider a molecule which may exchange among several discrete conformations. Each conformation is characterized by the length and orientation of the AX internuclear vector, with the orientation given relative to some fixed frame in the molecule [cf. Eq. (6)].

The interchange of conformations is governed by a system of first order rate equations which are conveniently written in terms of the fractions of molecules populating the various conformations:

$$\frac{dP_i}{dt} = \sum_{j=1}^N A_{ij} P_j, \quad (7)$$

where P_j is the fraction of molecules in the j th conformation, N is the number of conformations, $-A_{ii}$ is the rate constant for a jump from the i th conformation, and A_{ij} ($i \neq j$) is the rate constant for a jump from the j th to the i th conformation. For an ensemble of molecules in conformational equilibrium, the P_i are constants which we denote $\langle P_i \rangle$. Since $\sum_{i=1}^N \langle P_i \rangle = 1$, it is easy to show from kinetic arguments that

$$\sum_i A_{ij} = 0. \quad (8)$$

Due to the dynamic nature of equilibrium, $P_i(t)$ may be regarded as the time dependent probability of finding a hypothetical labeled molecule in the i th conformation at time t . Then the conditional probability $P(i|j, t)$ to find the molecule in the j th conformation at a time t , given that it occupied the i th conformation at time zero, is given by the solution of Eq. (7), i. e., $P_j(t)$, subject to the initial conditions

$$P_j(0) = \delta_{ij}, \quad j = 1, 2, \dots, N. \quad (9)$$

It can be shown that in most cases of interest, the conditional probabilities will be of the form¹⁹

$$P(i|j, t) = \langle P_j \rangle + \sum_{k=1}^{N-1} c_{ij}^{(k)} \exp(-\lambda_k t), \quad (10)$$

where λ_k and $c_{ij}^{(k)}$ are related to the eigenvalues and eigenvectors of the matrix of rate constants. The $\langle P_j \rangle$ are easily obtained from the rate constants by detailed balancing arguments, and satisfy the equation $\sum_{j=1}^N \langle P_j \rangle A_{ij} = 0$. Also, since $P(i|j, 0) = \delta_{ij}$, we have

$$\sum_{k=1}^{N-1} c_{ij}^{(k)} = \delta_{ij} - \langle P_j \rangle. \quad (11)$$

Since the *a priori* probability to find a molecule in the j th conformation is just $\langle P_j \rangle$, the average over internal motion in Eq. (6) is given by²⁰

$$\left\langle \frac{Y_{2n}(\Phi^{mol}(0)) Y_{2n'}(\Phi^{mol}(t))}{r^3(0) r^3(t)} \right\rangle = \sum_{i,j=1}^N \langle P_i \rangle P(i|j, t) \frac{Y_{2n}(\Phi_i^{mol}) Y_{2n'}(\Phi_j^{mol})}{r_i^3 r_j^3}, \quad (12)$$

where the subscripts on Φ and r indicate their values in the various conformations. Then, allowing for spherical or axial symmetry of the rotational diffusion tensor, and employing the definitions

$$\tau^{-1} = 6D, \quad (13.1)$$

$$\tau_k^{-1} = 6D + \lambda_k, \quad (13.2)$$

$$\tau_{n0}^{-1} = 6D_1 + n^2(D_{11} - D_1), \quad (13.3)$$

$$\tau_{nk}^{-1} = \tau_{n0}^{-1} + \lambda_k, \quad (13.4)$$

where D is the isotropic rotational diffusion constant and D_{\parallel} and D_{\perp} are, respectively, the diffusion constants for rotation parallel and perpendicular to the molecular symmetry axis, a straightforward calculation from Eq. (4), (6), (10), and (12) gives the spectral densities

$$J^{mm'}(\omega) = \frac{\delta_{m-m'}}{5} (-1)^m \sum_{n=-2}^2 \left[\frac{\tau}{1 + \omega^2 \tau^2} \left| \sum_{i=1}^N \langle P_i \rangle \frac{Y_{2n}(\Phi_i^{\text{mol}})}{r_i^3} \right|^2 + \sum_{i,j=1}^N \sum_{k=1}^{N-1} \frac{\tau_k}{1 + \omega^2 \tau_k^2} \langle P_i \rangle c_{ij}^{(k)} \frac{Y_{2n}(\Phi_i^{\text{mol}}) Y_{2n}^*(\Phi_j^{\text{mol}})}{r_i^3 r_j^3} \right] \quad (\text{spherical top}), \quad (14.1)$$

$$J^{mm'}(\omega) = \frac{\delta_{m-m'}}{5} (-1)^m \sum_{n=-2}^2 \left[\frac{\tau_{n0}}{1 + \omega^2 \tau_{n0}^2} \left| \sum_{i=1}^N \langle P_i \rangle \frac{Y_{2n}(\Phi_i^{\text{mol}})}{r_i^3} \right|^2 + \sum_{i,j=1}^N \sum_{k=1}^{N-1} \frac{\tau_{nk}}{1 + \omega^2 \tau_{nk}^2} \langle P_i \rangle c_{ij}^{(k)} \frac{Y_{2n}(\Phi_i^{\text{mol}}) Y_{2n}^*(\Phi_j^{\text{mol}})}{r_i^3 r_j^3} \right] \quad (\text{symmetric top}) \quad (14.2)$$

where the vertical brackets indicate the modulus of the complex quantity within. For the spherical top, Φ^{mol} may be taken with respect to any convenient molecular coordinate system; for the symmetric top, the principle axis system of the rotational diffusion tensor must be employed.

The expressions in Eqs. (14) are of limited use as written since the constants λ_k and $c_{ij}^{(k)}$ cannot be specified in general. Later we shall discuss motional limits in which these constants drop out; meanwhile we turn to a more explicit model for the internal motions.

We consider a variant of the "three site hindered rotation model" which has been used to describe the rotation of methyl groups.^{1,3} The X nucleus executes rotational jumps of magnitude $2\pi/3$ rad about an axis fixed in the molecule; the A nucleus is fixed at some point off the rotation axis; a single rate constant λ describes the jumping between sites. The conditional probability is given by^{1,3}

$$P(i|j, t) = (1/3) [1 + \exp(-\lambda t) (3\delta_{ij} - 1)], \quad (15.1)$$

$$\lambda_1 = 3\lambda. \quad (15.2)$$

Then, with the following definitions:

$$f(n) = \left| \sum_{i=1}^3 \frac{Y_{2n}(\Phi_i^{\text{mol}})}{3r_i^3} \right|^2, \quad (16.1)$$

$$g(n) = (1/3) \sum_{i=1}^3 \left| \frac{Y_{2n}(\Phi_i^{\text{mol}})}{r_i^3} \right|^2 - f(n), \quad (16.2)$$

the spectral densities are

$$J^{mm'}(\omega) = \frac{\delta_{m-m'}}{5} (-1)^m \left[\sum_{n=-2}^2 \frac{\tau f(n)}{1 + \omega^2 \tau^2} + \sum_{n=-2}^2 \frac{\tau_1 g(n)}{1 + \omega^2 \tau_1^2} \right] \quad (\text{spherical top}), \quad (17.1)$$

$$J^{mm'}(\omega) = \frac{\delta_{m-m'}}{5} (-1)^m \left[\sum_{n=-2}^2 \frac{\tau_{n0} f(n)}{1 + \omega^2 \tau_{n0}^2} + \sum_{n=-2}^2 \frac{\tau_{n1} g(n)}{1 + \omega^2 \tau_{n1}^2} \right] \quad (\text{symmetric top}). \quad (17.2)$$

THE BEHAVIOR OF THE SPECTRAL DENSITIES IN VARIOUS SITUATIONS

The behavior of the spectral densities will depend upon the relative magnitudes of the diffusion constants, the jumping rates, and the Larmor frequency. The following limiting cases are of interest for cross relaxation in proton spin systems. Some similar results for cross-correlation spectral densities are given in Appendix B.

Case 1: Isotropic rotation diffusion: $\omega_0/6D \gg 1$, $\lambda_k \gg 6D$

This approximates the situation of a slowly tumbling globular macromolecule with a rapid internal motion. Under these conditions the cross-relaxation rate constant $\sigma_{\alpha\alpha}$ will be dominated by the zero-quantum spectral density, i. e.,

$$\sigma_{\alpha\alpha} \approx -\frac{2\pi}{5} (\gamma_H \hbar)^2 J^{00}(\omega_a - \omega_x). \quad (18)$$

Then, since $\tau \gg \tau_k$ and $(\omega_a - \omega_x)\tau \ll 1$, we find from Eqs. (14) and (17)

$$J^{00}(\omega_a - \omega_x) \approx J^{00}(0) = \frac{\tau}{5} \sum_{n=-2}^2 \left| \sum_{i=1}^N \frac{\langle P_i \rangle Y_{2n}(\Phi_i^{\text{mol}})}{r_i^3} \right|^2 \quad (N \text{ site case}), \quad (19.1)$$

$$J^{00}(0) = \frac{\tau}{5} \sum_{n=-2}^2 f(n) \quad (\text{three site case}). \quad (19.2)$$

These expressions deserve some comment. We emphasize their simplicity, and the fact that they are amenable to hand computation. The important quantity appearing in each is the squared modulus of the weighted average over conformations of Y_{2n}/r^3 ; the appearance of r^3 rather than r^6 in the denominator shows that relatively large internuclear distances can contribute significantly to cross relaxation. If, for some i , $r_i^3 \ll r_j^3$ for all $j \neq i$, application of the spherical harmonic addition theorem to Eq. (19.1) yields

$$J^{00}(0) = \frac{\tau}{4\pi} \frac{\langle P_i \rangle^2}{r_i^6}. \quad (20)$$

In the limit where $r_i \rightarrow r_j$, $\Phi_i^{\text{mol}} \rightarrow \Phi_j^{\text{mol}}$ for all i and j , Eqs. (19.1) and (19.2) reduce exactly to the spectral densities for two nuclei rigidly situated a distance r apart. Also, in this limit $g(n) = 0$ identically.

Case 2: Isotropic rotational diffusion: $\omega_0/6D \ll 1$, $6D \gg \lambda_k$

These conditions apply to a small molecule with a hindered internal motion which is slow compared to molecular tumbling. Since $\tau \approx \tau_k$, all spectral densities may be written from Eqs. (14.1) and (17.1) by application of the spherical harmonic addition theorem:

$$J^{mm'} = \frac{\delta_{m-m'}}{4\pi} (-1)^m \tau \sum_{i=1}^N \frac{\langle P_i \rangle}{r_i^3} \quad (N \text{ site case}), \quad (21.1)$$

$$J^{mm'} = \frac{\delta_{m-m'}}{4\pi} (-1)^m \tau \sum_{i=1}^3 \frac{1}{3r_i^3} \quad (\text{three site case}). \quad (21.2)$$

In contrast to case 1 above, the weighted average here involves r^6 rather than r^3 . Physically, this corresponds to rapid averaging over the internal motions in case 1

versus rapid averaging over molecular tumbling in case 2.

Case 3: Symmetric top rotational diffusion:

$$\omega_0/6D_{\perp} \gg 1, \lambda_k; D_{\parallel} \gg D_{\perp}$$

This case applies to a rigid, approximately cylindrical macromolecule with internal motion and tumbling about the cylinder axis both rapid compared to tumbling end over end. This might describe double helical DNA pieces of moderate length. With $\tau_{00} \gg \tau_{10}, \tau_{20}$, and τ_{nk} , the zero-quantum spectral densities will again dominate cross relaxation; they assume the particularly simple forms

$$J^{00}(0) = \frac{\tau_{00}}{5} \left| \sum_{i=1}^N \langle P_i \rangle \frac{Y_{20}(\Phi_i^{\text{mol}})}{r_i^3} \right|^2 \quad (N \text{ site case}), \quad (22.1)$$

$$J^{00}(0) = \frac{\tau_{00}}{5} f(0) \quad (\text{three site case}). \quad (22.2)$$

A less extreme approximate would be $\lambda_k \gg D_{\parallel} \approx D_{\perp}$, which leads to slightly more complicated expressions. The availability of independent experimental and theoretical values for D_{\perp} as a function of cylinder length^{21,22} should aid in the application of Eq. (22).

NUMERICAL RESULTS

We have calculated σ_{α} for three representative geometries which illustrate the cross relaxation between methyl and nonmethyl protons in macromolecules. Similar calculations for small molecules are discussed in Ref. 8. We emphasize that σ_{α} is the cross-relaxation rate between a pair of protons, in this case a nonmethyl proton and a single proton on a methyl group (e.g., CHD₂, neglecting the deuterons). The equations describing cross relaxation between a nonmethyl proton and three methyl protons are easily deduced as a special case of Eq. (24).

Results are given for the three site jump model of Eqs. (18) and (19.2). We assume rapid jumping of the methyl proton ($\lambda^{-1} = 10^{-10}$ sec) and slow isotropic tumbling of the molecular frame [$(6D)^{-1} = 3 \times 10^{-8}$ sec]. In this motional limit, σ_{α} depends both upon internuclear distances and geometrical factors. A convenient specification of the relevant geometrical factors is given by the angle α , defined as follows: We first define the vector \mathbf{r} from the nonmethyl proton to the geometrical center of the triangle defined by the three positions of the methyl proton, and the vector \mathbf{s} from the methyl carbon to the center of the methyl triangle. Then $\cos \alpha = \mathbf{r} \cdot \mathbf{s} / (r \cdot s)$.

In the first geometry $\alpha = 90^\circ$, i.e., the nonmethyl proton lies in the plane of the methyl protons. We assume that the methyl and nonmethyl protons are eclipsed in one of the methyl orientations; results for the staggered conformation lie within 20% of those for the eclipsed. Values of σ_{α} are plotted as a function of $|\mathbf{r}|$ in Fig. 1 (filled circles). For comparison we include the results based on two naive "approximations" to the correct spectral density (19.2). The first approximation (open triangles, Fig. 1) consists of substituting Eq. (21.2) for Eq. (19.2); the second (open circles, Fig. 1) consists of taking

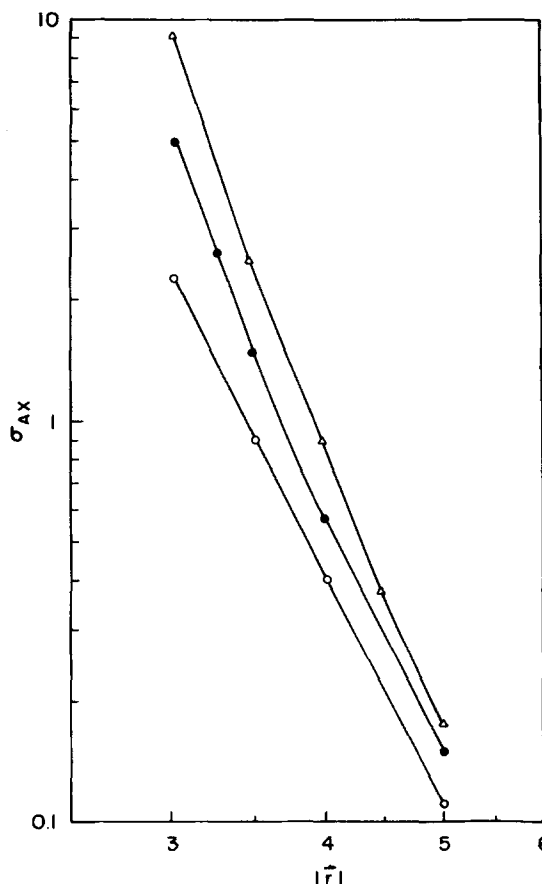


FIG. 1. The cross relaxation rate σ , in sec^{-1} , between a rotating methyl proton and a fixed nonmethyl proton for the equatorial geometry described in the text ($\alpha = 90^\circ$). The distance $|\mathbf{r}|$ between the nonmethyl proton and geometrical center of the triangle defined by the three positions accessible to the methyl proton is given in Å. Filled circles give correct results calculated from Eq. (19.2); open triangles and open circles are approximations calculated from Eqs. (21.2) and (23), respectively. Refer to the text for full details.

$$J^{00}(0) = \frac{\tau}{4\pi} \frac{1}{|\mathbf{r}|^6}, \quad (23)$$

where \mathbf{r} is defined above. The arithmetic mean of the two incorrect results gives a fair estimate of the correct value for σ_{α} .

The second geometry is that of the pyrimidine base thymine. In this case, $\alpha = 42^\circ$, $|\mathbf{r}| = 3$ Å. The calculation is given for cross relaxation between a methyl proton and the C-6 proton. Bond lengths and angles are taken from Ref. 23. The eclipsed conformation yields $\sigma_{\alpha} = 3.1 \text{ sec}^{-1}$; the staggered conformation gives 2.8 sec^{-1} .

In the third geometry $\alpha = 180^\circ$, i.e., the nonmethyl proton lies on the methyl rotation axis. The values of σ_{α} as a function of $|\mathbf{r}|$ are plotted in Fig. 2 (closed circles). The methyl-nonmethyl interproton distance is constant in this case which we include because the comparison to the other geometries is instructive. In addition to the correct calculation based on Eq. (19.2) (filled circles, Fig. 2), we also include the approximation of Eq. (23) (open triangles, Fig. 2).

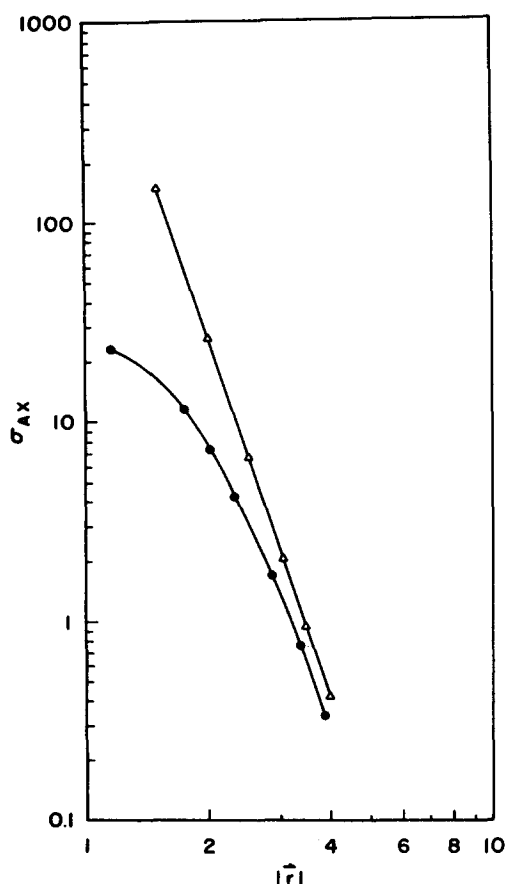


FIG. 2. The cross relaxation rate σ , in sec^{-1} , between a rotating methyl and fixed nonmethyl proton for the axial geometry described in the text ($\alpha = 180^\circ$). The distance $|r|$ is defined in Fig. 1 and in the text. Filled circles give correct results calculated from Eqs. (19.2); open triangles give approximate results calculated from Eq. (23). Additional details are given in the text.

MEASUREMENT OF CROSS RELAXATION RATES IN PROTON SYSTEMS BY NUCLEAR OVERHAUSER EFFECT (NOE) EXPERIMENTS

Measurement of σ_{α} is straightforward in a two-spin system, where the conventional steady state NOE experiment¹⁸ presents no difficulties. However, the measurement of the analogous cross-relaxation rates σ_{ij} in multispin systems may be complicated by multiple higher order NOE's (e. g., "spin diffusion" in macromolecules)^{24,25} and dipolar cross-correlation effects.⁹⁻¹² Our object here is to describe a time resolved NOE experiment which often permits direct measurement of the various σ_{ij} in highly complex proton systems. Similar experiments have been proposed,^{26,27} but to our knowledge, certain of their advantages and limitations have not been fully discussed.

We consider a multiproton system with weak scalar coupling, such as $A_{n_1}M_{n_2}P_{n_3}X_{n_4}\dots$. The naive extension of Eqs. (2) to this case is a system of equations of the type¹⁸

$$n_i^{-1} \frac{d\tilde{I}_i^{(i)}}{dt} = -\rho_i(\tilde{I}_i^{(i)} - \tilde{I}_0^{(i)}) - \sum_{j(i \neq j)} \sigma_{ij}(\tilde{I}_i^{(j)} - \tilde{I}_0^{(j)}), \quad (24)$$

where the tilde indicates magnetizations summed over

equivalent spins, n_i is the number of spins of type i , σ_{ij} is defined as in Eq. (3.3), and the form of ρ_i does not concern us. The experiment is based on a two pulse sequence: saturate and observe. A saturation pulse of duration τ is applied to selectively perturb a particular magnetization, say the k th. Saturation (with minimal Torrey oscillations)²⁸ will occur in a time comparable to T_2 of the irradiated spins, and will cause no perturbation of the nonirradiated magnetizations, provided that

$$\gamma H_1 \approx T_{2k}^{-1} = T_{2j}^{-1} \ll |\sigma_{jk}|, \quad (25.1)$$

$$\rho_k \ll \gamma H_1 \ll \Delta\omega_{jk}, \quad (25.2)$$

for all $j \neq k$, where $(\pi T_{2k})^{-1}$ is the k th spin linewidth, $\Delta\omega_{jk}$ is the chemical shift in Hz between the j th and k th spins, H_1 is the radio frequency amplitude, and other symbols have been defined earlier. If τ is appreciably longer than T_{2k} , the nonirradiated magnetizations will gradually depart from their equilibrium values, due to the coupling terms σ_{ij} . The values of these magnetizations at the end of the saturation pulse are then sampled with a suitable observation pulse. This two pulse cycle is performed for several τ values, starting with $\tau = T_{2k}$ and progressively increasing; this procedure yields the various $I_x^{(j)}$ ($j \neq k$), as functions of τ . For suitable H_1 , the time dependence of the irradiated magnetization during the saturation interval is exponential:

$$\tilde{I}_x^{(k)}(\tau) = \tilde{I}_0^{(k)} \exp(-c\tau), \quad (26)$$

where c is of the order of T_{2k}^{-1} . Then, for τ small enough so that

$$|\tilde{I}_x^{(j)} - \tilde{I}_0^{(j)}| \ll \tilde{I}_0^{(j)} \quad (27)$$

for all $j \neq k$, it follows from Eqs. (24) and (26) that the time evolution of the nonirradiated magnetization during the saturation pulse is given approximately by

$$n_j^{-1} \frac{d\tilde{I}_x^{(j)}}{dt} = \sigma_{jk}[1 - \exp(-c\tau)]\tilde{I}_0^{(k)}. \quad (28)$$

Since c can be measured, Eq. (28) can always be solved, and a fit of the experimental values of $\tilde{I}_x^{(j)}(\tau)$ to the solution yields σ_{jk} directly. For sufficiently large c , $\tilde{I}_x^{(j)}(\tau)/n_j$ will be linear in time, with slope $= \sigma_{jk}\tilde{I}_0^{(k)}$. Note the simplicity of Eq. (28) compared to the cumbersome expressions involved in extracting the σ_{jk} from conventional steady state NOE data.²⁹

Because of the brief saturation pulse, extensive cross-relaxation among the nonirradiated spins (second and higher order NOE) will not occur; thus, specific first order NOE's may be observed even in macromolecules. This has been amply verified in experimental studies of proteins and nucleic acids using τ values in the range 10 to 200 msec.^{25,30,31} Most of these studies have used difference spectroscopy to observe NOE's from resolved to unresolved resonances.

The conditions (25) will often be satisfied for protons in macromolecules, but will fail for small molecules in the extreme narrowing limit. For extreme narrowing, a standard 180- τ -90 sequence is preferable to the saturate-observe sequence, and the appropriate modifications of Eq. (28) must be made.

So far we have ignored dipolar cross correlation ef-

fects; we shall now show that Eq. (28) is often correct even when these effects are properly accounted for. First we summarize the cross-correlation problem, and then turn to the justification of Eq. (28). Certain details are relegated to Appendix C; for comprehensive discussion we refer the reader to the recent review of Werbelow and Grant.⁹

The general form of the dipolar correlation function in a multispin system is⁹

$$C_{ijkl}^{mm'}(t) = \left\langle \frac{Y_{2m}(\Phi_{ij}^{\text{lab}}(0)) Y_{2m'}(\Phi_{kl}^{\text{lab}}(t))}{r_{ij}^3(0) r_{kl}^3(t)} \right\rangle, \quad (29)$$

where the index pairs ij and kl refer to pairs of nuclei, and $i < j$, $k < l$. When $i = k$ and $j = l$ we have the autocorrelation function which is essentially identical to $C^{mm'}(t)$ of Eq. (5). When $i \neq j$ and/or $k \neq l$ we obtain the so-called cross-correlation functions. Cross-correlation spectral densities are defined by analogy to Eq. (4).

It is well known and can be shown quite generally (cf. Appendix C) that cross correlation does not enter the calculation of the σ_{ij} and ρ_i . Rather, the effect of cross correlation is to introduce additional variables into the relaxation equations. These variables are coupled to the magnetizations by rate constants which are linear functions of the cross-correlation spectral densities only (cf. Appendix C) so that neglect of cross correlation gives Eq. (24). The correct relaxation equations a spin system such as $A_{n_1}M_{n_2}P_{n_3}X_{n_4} \dots$ will be of the form

$$\begin{aligned} n_i^{-1} \frac{d\bar{I}_z^{(i)}}{dt} = & -\rho_i(\bar{I}_z^{(i)} - \bar{I}_0^{(i)}) \\ & - \sum_{j(\neq i)} \sigma_{ij}(\bar{I}_z^{(j)} - \bar{I}_0^{(j)}) - \sum_k \zeta_{ik}(R^{(k)} - R_0^{(k)}), \end{aligned} \quad (30.1)$$

$$\begin{aligned} \frac{dR_i}{dt} = & -\eta_i(R^{(i)} - R_0^{(i)}) - \sum_j \xi_{ij}(\bar{I}_z^{(j)} - \bar{I}_0^{(j)}) \\ & - \sum_{m(\neq i)} \xi_{im}(R^{(m)} - R_0^{(m)}), \end{aligned} \quad (30.2)$$

where the R 's are additional variables with the zero subscript indicating their equilibrium values, and the ζ , η , and ξ are relaxation rates involving the cross-correlation spectral densities. Other symbols have been defined earlier. Some of the properties of the R 's are discussed in Appendix C; for now it suffices to note that⁹⁻¹¹ (a) the R 's are expectation values of suitably constructed multispin operators, (b) the R 's and I 's are mutually orthogonal to each other, somewhat in the sense of normal modes in vibration problems, and (c) the number of additional variables depends on the spin system; the choice of these variables is not unique.

From the form of Eqs. (30) it can be seen that the extra variables are coupled to the magnetizations, to each other, and to the lattice; this may greatly complicate the relaxation pathways of the system. Due to such complications and the difficulty of calculating the ζ , η , and ξ , it has been common practice to ignore cross correlation and work with Eq. (24).¹⁸ In general, this practice is not justified, although in many cases the errors incurred are certainly small.⁵

We now consider the time resolved experiment in light of Eq. (30). Due to the orthogonality of the R 's and I 's, it is possible to selectively saturate a given magnetization without perturbing either the other magnetizations or the extra variables, if in addition to Eq. (25) we have the conditions

$$|\zeta_{jk}| \leq |\sigma_{jk}| \quad (31)$$

for all $j \neq k$, where the k th magnetization is irradiated. Then for τ small enough so that

$$|R^{(j)} - R_0^{(j)}| \ll |R^{(j)}|, \quad (32)$$

it follows from Eqs. (25) and (30) that Eq. (28) is approximately correct.

The validity of Eq. (28) rests on Eqs. (25), (27), (31), and (32). Verification of the degree to which conditions (25) are satisfied is straightforward. The linewidth, chemical shift differences, and H_1 are all measurable, and the selectivity of saturation may be determined by experimental trial. Furthermore, the σ_{ij} and ρ_i may often be estimated *a priori* to fair accuracy, if they have not been measured. Verification of Eq. (27) involves the measurement of magnetizations and therefore presents no problems.

On the other hand, verification of Eqs. (31) and (32) is problematical since (a) the relevant R 's are often unobservable⁹ and, (b) *a priori* estimation of the ζ may be difficult since their analytical forms may not be known, and are likely to be cumbersome in any case. No general prescriptions can be given; we shall briefly discuss the situation for macromolecules where the condition $\omega_0/6D \gg 1$ holds.

We assume the overall spin systems may be effectively decomposed into a loosely coupled network of "islands,"³² where each island constitutes a relatively simple system, e. g., AX_3 . Werbelow and Grant have tabulated the ζ_{ik} and $R^{(k)}$ (in notation different from ours) for many of the common simple spin systems. Examination of these tabulations shows that many of the ζ vanish identically because of selection rules. Furthermore, because of the Lorentzian form of the spectral densities, the condition that $\omega_0/6D \gg 1$ insures that only those spectral densities whose arguments are zero or $\Delta\omega_{ij}$ will contribute significantly to the remaining ζ . Then, since auto- and cross-correlation spectral densities are of similar magnitudes,⁹ it is not difficult to obtain rough estimates of the relevant ζ 's. It appears to be a safe generalization that oftentimes the ζ_{ik} and σ_{ik} are approximately equal, so that Eqs. (31) and therefore (32) will be satisfied, given Eqs. (24) and (26). For example, we consider the AX_3 system with saturation of the X_3 magnetization. Then, with the simplifications noted above, only two R 's will couple significantly to the X_3 magnetization; for convenience we denote the coupling coefficients ζ_{1x} and ζ_{2x} (${}^a\Gamma_{23}^{AX_3}$ and ${}^a\Gamma_{24}^{AX_3}$, respectively, in the notation of Ref. 9). Assuming the equality of auto- and cross-correlation spectral densities, a simple calculation gives

$$\frac{3}{2} |\zeta_{1x}| \approx |\sigma_{ax}| \approx \frac{3}{2} |\zeta_{2x}|. \quad (33)$$

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APPENDIX A

Expressions (A1) give the second rank tensor operators defined in Ref. 9 for a pair of spins i and j and expressions (A2) give the second rank spherical harmonics, normalized according to Rose¹⁶.

$$\hat{T}_{20}^{(ij)} = \frac{-1}{\sqrt{6}} [4\hat{I}_z^{(i)}\hat{I}_z^{(j)} - (\hat{I}_+^{(i)}\hat{I}_-^{(j)} + \hat{I}_-^{(i)}\hat{I}_+^{(j)})], \quad (\text{A1})$$

$$\hat{T}_{2\pm 1}^{(ij)} = \pm (\hat{I}_z^{(i)}\hat{I}_\pm^{(j)} + \hat{I}_\pm^{(i)}\hat{I}_z^{(j)}), \quad (\text{A2})$$

$$\hat{T}_{2\pm 2}^{(ij)} = -\hat{I}_\pm^{(i)}\hat{I}_\pm^{(j)}, \quad (\text{A3})$$

$$Y_{20} = \sqrt{\frac{5}{4\pi}} [(3/2)\cos^2\theta - 1/2], \quad (\text{A2.1})$$

$$Y_{21} = -\sqrt{\frac{15}{8\pi}} \sin\theta \cos\theta e^{i\phi}, \quad (\text{A2.2})$$

$$Y_{22} = (1/4)\sqrt{\frac{15}{2\pi}} \sin^2\theta e^{2i\phi} \quad (\text{A2.3})$$

$$Y_{l-m} = (-1)^m Y_{lm}^*. \quad (\text{A2.4})$$

APPENDIX B

The dipolar cross-correlation function has been defined in Eq. (29) of the text. The calculation of autocorrelation spectral densities given in Eqs. (5) through (14) can easily be extended to the cross-correlation case, using Eq. (29) rather than Eq. (5) as the starting point. The calculation is facilitated by some changes in notation which avoid overly cumbersome subscripting. For the length of the vector connecting the i th and j th nuclei of interest, denoted by r_{ij} in Eq. (29), we substitute the symbol r ; for r_{ki} we substitute r' ; likewise, we substitute Φ and Φ' for Φ_{ij} and Φ_{ki} . The value of r in the i th conformation [cf. Eqs. (5)–(12)] is denoted r_i ; the value of Φ' in the j th conformation is Φ'_j , and so on.

Then, the cross-correlation analog of Eq. (6) is

$$C_{ijkl}^{mm'}(t) = \sum_{n,n'} \langle D_{mn}^{2*}(\Omega(0)) D_{m'n}^{2*}(\Omega(t)) \rangle \times \left\langle \frac{Y_{2n}(\Phi^{\text{mol}}(0)) Y_{2n'}(\Phi'^{\text{mol}}(t))}{r^3(0)r'^3(t)} \right\rangle, \quad (\text{B1})$$

which leads to the analog of Eq. (12):

$$\left\langle \frac{Y_{2n}(\Phi^{\text{mol}}(0)) Y_{2n'}(\Phi'^{\text{mol}}(t))}{r^3(0)r'^3(t)} \right\rangle = \sum_{i,j=1}^N \langle P_j \rangle P(i|j,t) \times \frac{Y_{2n}(\Phi^{\text{mol}}(0)) Y_{2n'}(\Phi'^{\text{mol}}(t))}{r_i^3 r_j'^3}. \quad (\text{B2})$$

The calculation of spectral densities from this point proceeds exactly as in the text, leading to the following general expression for the symmetric top spectral densities:

$$J^{mm'}(\omega) = \frac{\delta_{m-m'}}{5} (-1)^m \sum_{n=2}^2 \sum_{i,j=1}^N \frac{Y_{2n}(\Phi_i^{\text{mol}}) Y_{2n}^*(\Phi_j'^{\text{mol}})}{r_i^3 r_j'^3} \times \left\langle \frac{\langle P_i \rangle \langle P_j \rangle \tau_{n0}}{1 + \omega^2 \tau_{n0}^2} + \sum_{k=1}^{N-1} \frac{\langle P_i \rangle C_{ij}^{(k)} \tau_{nk}}{1 + \omega^2 \tau_{nk}^2} \right\rangle. \quad (\text{B3})$$

The corresponding expression for the spherical top is easily obtained by comparison of Eq. (B3) with Eqs. (14.1) and (14.2).

The cross-correlation spectral densities are similar in form to the autocorrelation spectral densities, and show similar behavior in the various motional limits discussed in the text. Corresponding to Eq. (19.1) we obtain

$$J^{00}(0) = \frac{\tau}{5} \sum_{n=2}^2 \sum_{i,j=1}^N \langle P_i \rangle \langle P_j \rangle \frac{Y_{2n}(\Phi_i^{\text{mol}}) Y_{2n}^*(\Phi_j'^{\text{mol}})}{r_i^3 r_j'^3}. \quad (\text{B4})$$

Corresponding to Eq. (21.1) we have

$$J^{mm'} = \delta_{m-m'} \frac{\tau}{5} (-1)^m \sum_{n=2}^2 \sum_{i=1}^N \langle P_i \rangle \frac{Y_{2n}(\Phi_i^{\text{mol}}) Y_{2n}^*(\Phi_i'^{\text{mol}})}{r_i^3 r_i'^3}. \quad (\text{B5})$$

Corresponding to Eq. (22.1) we have

$$J^{00}(0) = \frac{\tau_{00}}{5} \sum_{i,j=1}^N \langle P_i \rangle \langle P_j \rangle \frac{Y_{20}(\Phi_i^{\text{mol}}) Y_{20}(\Phi_j'^{\text{mol}})}{r_i^3 r_j'^3}. \quad (\text{B6})$$

APPENDIX C

For many types of spin systems it is well known that the dipolar relaxation rates σ_{ij} and ρ_i which directly couple the magnetizations to each other and to the lattice contain no contribution from dipolar cross correlation. It is also known that the *autocorrelation* functions do not contribute to the direct coupling between the magnetizations and the additional variables. However, to our knowledge, no simple explicit proof of these facts has been published, although arguments which suffice to establish such a proof have been given in different contexts.^{11,33,34} We shall present a somewhat informal proof, for a system of spins 1/2 with weak scalar coupling (e.g., $A_{n1}M_{n2}P_{n3}X_{n4} \dots$) undergoing intramolecular dipolar relaxation in an isotropic solvent.

Our starting point is the relaxation equation for the spin density operator $\hat{\sigma}$. In the Schrödinger picture,²⁰

$$\frac{d\hat{\sigma}}{dt} = -\frac{1}{\hbar^2} \int_0^\infty \left[\hat{H}(t), \left[\exp\left(\frac{-i}{\hbar} \hat{H}_0 \tau\right) \hat{H}(t-\tau) \times \exp\left(\frac{i}{\hbar} \hat{H}_0 \tau\right), \hat{\sigma} - \hat{\sigma}_0 \right] \right] d\tau, \quad (\text{C1})$$

where $H(t)$ is the relaxation Hamiltonian, H_0 is the nuclear Zeeman Hamiltonian, $\hat{\sigma}_0$ is the equilibrium spin density operator, and an ensemble average over non-spin variables is understood. Note that all operators are denoted by capped letters.

Fano³⁵ has shown that the density operator may be expanded as a linear combination of spin operators:

$$\sigma = \sum_a C_a(t) \hat{O}_a. \quad (\text{C2})$$

The operators O_a may be chosen to be mutually orthogonal in the sense that

$$\text{Tr}(\hat{O}_a \hat{O}_b) = k \delta_{ab}, \quad (\text{C3})$$

where k is a constant. Then

$$C_a(t) = \text{Tr}(\hat{O}_a \hat{\sigma}) / \text{Tr}(\hat{O}_a \hat{O}_a), \quad (\text{C4})$$

so that the $C_a(t)$ are essentially the normalized expecta-

tion values of the operators \hat{O}_a . For the equilibrium spin density operator, the \hat{O}_a will comprise the various z component spin angular momentum operators (summed over equivalent spins), and the $C_a(t)$ will be magnetizations. In nonequilibrium situations, the collection of \hat{O}_a must include more complicated operators in addition to the $\sum \hat{I}_z$. The expectation values of the more complicated O_a are the additional variables referred to in the text. Many of these expectation values correspond to nonobservable quantities such as the individual doublet and quartet contributions to methyl proton magnetizations.

Substituting Eq. (C2) into Eq. (C1) and using the identity

$$\text{Tr}A[B, [C, D]] = \text{Tr}[A, B][C, D],$$

we obtain

$$\frac{dC_a}{dt} = [\text{Tr}(\hat{O}_a^\dagger \hat{O}_a)]^{-1} \sum L_{ab} C_b, \quad (\text{C5.1})$$

$$L_{ab} = -\frac{1}{\hbar^2} \text{Tr} \int_0^\infty [\hat{O}_a^\dagger, \hat{H}(t)] \times \left[\exp\left(-\frac{i}{\hbar} \hat{H}_0 \tau\right) \hat{H}(t - \tau) \exp\left(\frac{i}{\hbar} \hat{H}_0 \tau\right), \hat{O}_b \right] d\tau. \quad (\text{C5.2})$$

The Eqs. (C5.1) are equivalent to Eqs. (30) in the text. It will soon become apparent that L_{ab} is a function of the various dipolar spectral densities. Our proof consists in showing that L_{ab} contains *only autocorrelation* spectral density when C_a and C_b are magnetizations and that L_{ab} contains *only cross-correlation* spectral densities when C_a is a magnetization but C_b is an "additional variable" (or *vice versa*). The proof is trivial once certain features of the \hat{O} 's are understood.

For our purposes these features can be best illustrated by examining some representative examples. For convenience we choose some of the \hat{O} 's for the AX_2 system, as tabulated by Wang and Grant¹¹:

$$\hat{O}_1 = \hat{I}_z^{(a)}, \quad (\text{C6.1})$$

$$\hat{O}_2 = \hat{I}_z^{(x1)} + \hat{I}_z^{(x2)}, \quad (\text{C6.2})$$

$$\hat{O}_3 = \hat{I}_z^{(x1)} \hat{I}_z^{(x2)} + (1/2)(\hat{I}_+^{(x1)} \hat{I}_-^{(x2)} + \hat{I}_+^{(x2)} \hat{I}_-^{(x1)}), \quad (\text{C6.3})$$

$$\hat{O}_4 = \hat{O}_1 \hat{O}_3. \quad (\text{C6.4})$$

We observe the following: (1) Each of the \hat{O}_a consists of sums of products of the individual spin operators $\hat{I}_z^{(i)}$, $\hat{I}_+^{(j)}$, and $\hat{I}_-^{(k)}$. (2) Each product contains one and only one operator acting on a particular spin. (For systems containing spins with $I > \frac{1}{2}$, each product may contain repeated operators acting on a given spin.) (3) In each product, the total number of raising operators equals the total number of lowering operators, i. e., only "zero projection" operator products appear. (4) In a given sum of products, the total number of single spin operators in each product is the same for all products. This provides a hierarchy of complexity for the \hat{O}_a and we shall refer to "type 1," "type 2," and "type N " operators and variables, where, for example, \hat{O}_1 and \hat{O}_2 are of type 1 and \hat{O}_4 is of type 3. Note that item (3) above implies that the *only* type 1 variables are magnetizations.

Introducing the dipolar Hamiltonian

$$\hat{H}(t) = \left(\frac{6\pi}{5}\right)^{1/2} \hbar^2 \sum_{i < j} \sum_{m=-2}^2 (-1)^m \gamma_i \gamma_j \frac{\hat{T}_{2m}^{(ij)} Y_{2-m}(\Phi_{ij}^{ab}(t))}{\gamma_{ij}^3(t)} \quad (\text{C7})$$

into (C5.2), a straightforward calculation gives

$$L_{ab} = -\frac{1}{\hbar^2} \text{Tr} \sum_{i < j} \sum_{\mu, \nu=-2}^2 \int_0^\infty C_{ij\mu\nu}^{\mu\nu}(t) (-1)^{\mu+\nu} [\hat{O}_a^\dagger, \hat{T}_{2\mu}^{(ij)}] \times \left[\exp\left(-\frac{i}{\hbar} \hat{H}_0 \tau\right) \hat{T}_{2\nu}^{(kl)} \exp\left(\frac{i}{\hbar} \hat{H}_0 \tau\right), \hat{O}_b \right] d\tau, \quad (\text{C8})$$

where³⁶

$$C_{ij\mu\nu}^{\mu\nu}(t) = \delta_{\mu\nu} C_{ij\mu\nu}^{00}(t).$$

We then consider the form of L_{ab} when C_a and C_b are magnetizations, i. e.,

$$\hat{O}_a = \sum_i \hat{I}_z^{(i)}, \quad (\text{C10.1})$$

$$\hat{O}_b = \sum_j \hat{I}_z^{(j)}, \quad (\text{C10.2})$$

where the sums are over groups of equivalent spins.

Substituting Eq. (A1) into Eq. (C8) and noting that

$$\exp\left(-\frac{i}{\hbar} \hat{H}_0 t\right) \hat{I}_z^{(j)} \exp\left(\frac{i}{\hbar} \hat{H}_0 t\right) = \hat{I}_z^{(j)} \exp[\pm i\omega_0(j)t], \quad (\text{C11.1})$$

$$\exp\left(-\frac{i}{\hbar} \hat{H}_0 t\right) \hat{I}_z^{(j)} \exp\left(\frac{i}{\hbar} \hat{H}_0 t\right) = \hat{I}_z^{(j)}, \quad (\text{C11.2})$$

it is clear that in evaluating Eq. (C.8) we need only consider terms of the type

$$\text{Tr}[\hat{I}_z^{(m)}, \hat{I}_z^{(i)} \hat{I}_z^{(j)}][\hat{I}_z^{(k)} \hat{I}_z^{(l)}, \hat{I}_z^{(n)}], \quad (\text{C12.1})$$

$$\text{Tr}[\hat{I}_z^{(m)}, \hat{I}_z^{(i)} \hat{I}_z^{(j)}][\hat{I}_z^{(k)} \hat{I}_z^{(l)}, \hat{I}_z^{(n)}], \quad (\text{C12.2})$$

$$\text{Tr}[\hat{I}_z^{(m)}, \hat{I}_z^{(i)} \hat{I}_z^{(j)}][\hat{I}_z^{(k)} \hat{I}_z^{(l)}, \hat{I}_z^{(n)}], \dots \quad (\text{C12.3})$$

When only two distinct spin indices appear in Eq. (C.12), e. g., $m = i = k \neq j = l = n$, then the coefficients of the expressions (C.12) in (C.8) will be autocorrelation functions. If more than two distinct indices appear, the coefficients will be cross-correlation functions. Since $\text{Tr} \hat{I}_z = \text{Tr} \hat{I}_z = 0$, it is clear by inspection that the expressions (C.12) will vanish identically when indices referring to five or six distinct spins appear. It is a straightforward exercise to show that this is also true for three or four distinct indices. The expressions in Eq. (C.12) will be nonzero only when not more than two distinct spin indices appear. If $C_a \neq C_b$, the above reasoning shows that the cross-correlation contribution to all σ_{ij} vanishes identically. If $C_a = C_b$, the same reasoning shows that the cross-correlation contribution to all the ρ_i also vanishes.

The proof that the autocorrelation contributions to the coupling between magnetizations and additional variables proceeds along similar lines. For example, for type 3 variables (see above) we have, instead of Eq. (C.12) expressions of the type

$$\text{Tr}[\hat{I}_z^{(i)}, \hat{I}_z^{(i)} \hat{I}_z^{(j)}][\hat{I}_z^{(i)} \hat{I}_z^{(j)}, \hat{I}_z^{(i)} \hat{I}_z^{(j)} \hat{I}_z^{(k)}], \quad (\text{C13.1})$$

$$\text{Tr}[\hat{I}_z^{(i)}, \hat{I}_z^{(i)} \hat{I}_z^{(j)}][\hat{I}_z^{(i)} \hat{I}_z^{(j)}, \hat{I}_z^{(i)} \hat{I}_z^{(j)} \hat{I}_z^{(k)}], \dots \quad (\text{C13.2})$$

which are shown to vanish by inspection. Identical arguments hold for variables of type > 3 . For type 2 vari-

ables, symmetry arguments show that all coupling terms (auto- and cross-correlation) to the magnetizations will vanish identically.^{9,11}

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