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Heteronuclear Transverse Relaxation in AMX, AX_2, and AX_3 Spin Systems

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The development of multidimensional double- and triple-resonance NMR spectroscopy has enabled the determination of solution structures of proteins with molecular weights in the 20–25 kDa range (*1, 2*). Because these new techniques give rise to the assignment of a large number of ^{15}N and ^{13}C chemical shifts they also open up the possibility of studying protein dynamics at each position along the protein backbone or side chain through the measurement of the relaxation properties of ^{15}N and/or ^{13}C nuclei. Recently a number of one- (*3, 4*) and two-dimensional (*5–7*) NMR experiments have been developed that enable the indirect measurement of relaxation properties of insensitive nuclei such as ^{15}N and ^{13}C with high sensitivity. A number of studies of backbone and side-chain dynamics in proteins based on ^{15}N and ^{13}C relaxation measurements have begun to emerge (*7–11*).

Despite the potential that NMR holds for the extraction of information relating to protein dynamics the interpretation of the relaxation data is by no means trivial. The extraction of accurate relaxation rates of ^{13}C nuclei in methylene or methyl groups, for example, is complicated by cross-correlation effects between ^1H–^{13}C dipolar interactions (*12, 13*) as well as interference between ^1H–^{13}C dipolar interactions and chemical-shift anisotropy (*14*). While it is possible to remove the effects of correlation between dipolar interactions and chemical-shift anisotropy (*15–17*), dipolar cross-correlation effects can not be eliminated and can result in substantial errors in measured relaxation times if not taken into account properly (*18*). Equations describing the effects of dipolar cross correlation on ^{13}C T_1 and ^1H–^{13}C NOE measurements have been derived by Werbelow and Grant (*12*). In addition, Vold and Vold have derived similar expressions for ^{13}C transverse relaxation rates for a number of different spin systems attached to molecules tumbling in the extreme narrowing limit (*13*). Given the increased interest in measuring relaxation rates of side-chain carbons and in particular methyl groups in proteins, it is useful to derive expressions describing the transverse relaxation of ^{13}C nuclei due to dipolar interactions in AMX, AX_2, and AX_3 spin systems for arbitrary values of tumbling time, τ_c.

The relaxation of the transverse elements of the density matrix, ρ, for a spin system of arbitrary complexity can be calculated using Redfield theory (*19*) according to

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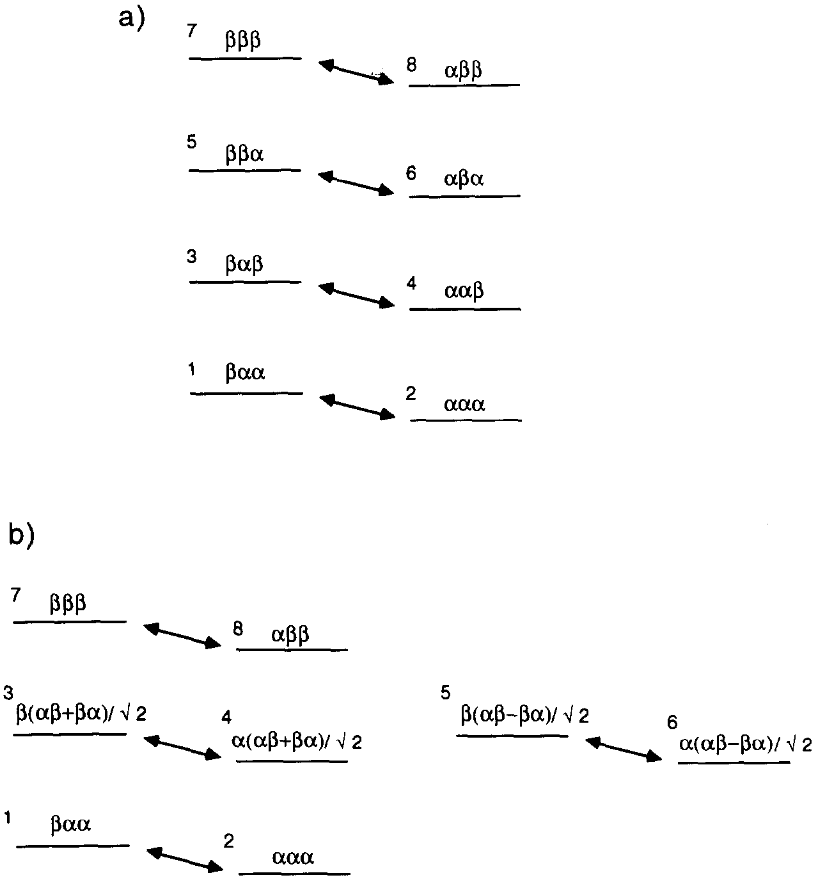

FIG. 1. Energy-level diagram of an isolated AMX spin system (A = ^{13}C, M,X = ^{1}H) (a) and an isolated AX$_2$ spin system (b) with the wavefunctions written in a direct product basis set (AMX) and an irreducible basis representation (AX$_2$). The eigenstates are numbered from 1 to 8. The first spin state in wavefunction $|k\rangle$ corresponds to the ^{13}C spin state and the remaining spin states are associated with the proton spins.

$$\frac{d\nu}{dt} = -\mathbf{R}\nu, \tag{1}$$

where ν is a vector consisting of density elements, ρ_{ij}, corresponding to transverse magnetization and R_{kl} is a relaxation matrix element. Expressions for R_{kl} are given elsewhere (*12, 19–21*). Typically, ^{13}C transverse relaxation rates are measured using a Carr–Purcell–Meiboom–Gill (CPMG) pulse scheme (*22, 23*) with the spacing between successive 180° ^{13}C pulses, δ, satisfying the condition $\delta \ll 1/(2J_{AX})$, where J_{AX} is the one-bond heteronuclear coupling constant. Vold and Vold have shown that only in this limit is the measured transverse relaxation time independent of δ (*24*). Moreover, a choice of $\delta \ll 1/(2J_{AX})$ ensures that antiphase heteronuclear magnetization does not evolve during δ and does not contribute to the relaxation. This is important, especially for macromolecular applications, where it has recently been shown (*25–27*) that the transverse relaxation rate of antiphase heteronuclear magnetization

TABLE 1

Transverse Relaxation of Spin A for a Weakly Coupled AMX Spin System, where A = ^{13}C, M,X = ^{1}H

$\nu_1 = \rho_{1,2}$	$\omega_1 = \omega_C - \pi J_{AM} - \pi J_{AX}$
$\nu_2 = \rho_{3,4}$	$\omega_2 = \omega_C - \pi J_{AM} + \pi J_{AX}$
$\nu_3 = \rho_{5,6}$	$\omega_3 = \omega_C + \pi J_{AM} - \pi J_{AX}$
$\nu_4 = \rho_{7,8}$	$\omega_4 = \omega_C + \pi J_{AM} + \pi J_{AX}$

$\Gamma_1 = 1/2 J_{MX}(\omega_M) + 1/2 J_{MX}(\omega_X) + J_{AX}(\omega_A + \omega_X) + 1/2 J_{AX}(\omega_A) + 1/2 J_{AX}(\omega_X) + 1/6 J_{AX}(\omega_A - \omega_X) + 2/3 J_{AX}(0) + J_{AM}(\omega_A + \omega_M) + 1/2 J_{AM}(\omega_A) + 1/2 J_{AM}(\omega_M) + 1/6 J_{AM}(\omega_A - \omega_M) + 2/3 J_{AM}(0)$

$\Gamma_2 = K_{XAM}(\omega_A) + 4/3 K_{XAM}(0)$

$R_{11} = \Gamma_1 + \Gamma_2 + 2 J_{MX}(\omega_M + \omega_X)$

$R_{12} = 1/2\,[J_{AX}(\omega_X) - J_{MX}(\omega_X)]$

$R_{13} = 1/2\,[J_{AM}(\omega_M) - J_{MX}(\omega_M)]$

$R_{14} = -2 J_{MX}(\omega_M + \omega_X)$

$R_{22} = \Gamma_1 - \Gamma_2 + 1/3 J_{MX}(\omega_M - \omega_X)$

$R_{23} = -1/3 J_{MX}(\omega_M - \omega_X)$

$R_{24} = R_{13}$

$R_{33} = R_{22}$

$R_{34} = R_{12}$

$R_{44} = R_{11}$

$R_{ij} = R_{ji}$

Note. ν_i and R_{ij} are the elements of $\boldsymbol{\nu}$ and $\mathbf{R}$, respectively (see Eq. [1]), ω_i is the angular frequency of the multiplet component denoted by ν_i, ω_C is the ^{13}C angular frequency, and J_{ij} is the scalar coupling constant between spins i and j. The density element ρ_{ij} connects the eigenstates $|i\rangle$ and $|j\rangle$ indicated in Fig. 1.

$[2A_{(x,y)}X_z]$ can be significantly larger than the rate of decay of in-phase transverse magnetization $[A_{(x,y)}]$.

In the limit that $\delta \ll 1/(2J_{AX})$, during the CPMG interval the individual multiplet components of spin A (^{13}C) become locked along the applied RF field and hence all components evolve with the same frequency. This allows cross relaxation to occur efficiently among the individual lines of the multiplet with the rates given by the off-diagonal elements of **R**. This effect is analogous to the exchange of magnetization that occurs between density elements corresponding to Z magnetization in NOESY experiments (*28, 29*).

In contrast, for the case where $\delta \gg 1/(2J_{AX})$ many of the density elements corresponding to individual multiplet components evolve at different frequencies (frequencies separated by some multiple of J_{AX}) and cross relaxation among these elements becomes inefficient. In general, in this limit efficient cross relaxation will occur only among degenerate density elements. Expressed in an equivalent way, if two basis vectors of the spin-density matrix oscillate at different frequencies, then magnetization transfer between the vectors is blocked. The vectors are nonsecular with respect to each other (*20, 21*).

Figure 1a shows an energy-level diagram and associated wavefunctions for an AMX spin system (A = ^{13}C, M,X = ^{1}H) with the transitions which give rise to A single-

TABLE 2

Transverse Relaxation of Spin A in an AX_2 Spin System

$\nu_1 = \rho_{1,2}$ $\qquad$ $\omega_1 = \omega_C - 2\pi J_{AX}$

$\nu_2 = \rho_{3,4}$ $\qquad$ $\omega_2 = \omega_C$

$\nu_3 = \rho_{7,8}$ $\qquad$ $\omega_3 = \omega_C + 2\pi J_{AX}$

$\nu_4 = \rho_{5,6}$ $\qquad$ $\omega_4 = \omega_C$

$R_{11} = 2J_{XX}(2\omega_X) + J_{XX}(\omega_X) + 2J_{AX}(\omega_A + \omega_X) + J_{AX}(\omega_A) + J_{AX}(\omega_X) + 1/3 J_{AX}(\omega_A - \omega_X) + 4/3 J_{AX}(0) + K_{XAX}(\omega_A) + 4/3\, K_{XAX}(0)$

$R_{12} = -J_{XX}(\omega_X) + 1/2\, [J_{AX}(\omega_X) + K_{XAX}(\omega_X)]$

$R_{13} = -2J_{XX}(2\omega_X)$

$R_{14} = 1/2\, [J_{AX}(\omega_X) - K_{XAX}(\omega_X)]$

$R_{22} = 2J_{XX}(\omega_X) + 2J_{AX}(\omega_A + \omega_X) + J_{AX}(\omega_A) + J_{AX}(\omega_X) + 1/3\, J_{AX}(\omega_A - \omega_X) + 2/3 J_{AX}(0) + 2\, K_{XAX}(\omega_A + \omega_X) - K_{XAX}(\omega_A) + K_{XAX}(\omega_X) + 1/3\, K_{XAX}(\omega_A - \omega_X) - 2/3\, K_{XAX}(0)$

$R_{23} = R_{12}$

$R_{24} = 2/3\, [J_{AX}(0) - K_{XAX}(0)]$

$R_{33} = R_{11}$

$R_{34} = R_{14}$

$R_{44} = 2J_{AX}(\omega_A + \omega_X) + J_{AX}(\omega_A) + J_{AX}(\omega_X) + 1/3 J_{AX}(\omega_A - \omega_X) + 2/3\, J_{AX}(0) - 2\, K_{XAX}(\omega_A + \omega_X) - K_{XAX}(\omega_A) - K_{XAX}(\omega_X) - 1/3\, K_{XAX}(\omega_A - \omega_X) - 2/3\, K_{XAX}(0)$

$R_{ij} = R_{ji}$

Note. ν_i, R_{ij}, ω_i, and J_{ij} are defined as in the note to Table 1.

quantum magnetization indicated by arrows. The elements of ν and **R** are defined in Table 1, where $J_{MX}(\omega)$, $J_{AM}(\omega)$, and $J_{AX}(\omega)$ correspond to auto spectral density terms due to homonuclear MX dipolar interactions and heteronuclear AM and AX dipolar interactions, respectively. $K_{MAX}(\omega)$ is a spectral density term due to cross-correlation between the AM and AX vectors. For the case of spins A, M, and X attached to a molecule undergoing isotropic motion, $J_{ij}(\omega)$ and $K_{iji'}(\omega)$ are given by

$$\begin{aligned} J_{ij}(\omega) &= 3[\gamma_i^2\gamma_j^2 h^2/(40\pi^2 r_{ij}^6)]\tau_c/[1 + (\omega\tau_c)^2] \\ K_{iji'}(\omega) &= 3[\gamma_i^2\gamma_j^2 h^2/(40\pi^2 r_{ij}^3 r_{i'j}^3)]P_2(\cos\theta_{iji'})\tau_c/[1 + (\omega\tau_c)^2], \end{aligned} \qquad [2]$$

where γ_i is the gyromagnetic ratio of spin i, h is Planck's constant, r_{ij} is the length of the vector connecting spins i and j, P_2 is a second-order Legendre polynomial, $\theta_{iji'}$ is the angle between vectors ij and $i'j$, and τ_c is the correlation time for the isotropic tumbling. More complicated expressions for the spectral densities exist for molecules tumbling anisotropically (*12, 30, 31*).

In the limit that $\delta \ll 1/(2J_{AX})$, $1/(2J_{AM})$, cross relaxation occurs among the four multiplet components associated with transverse A magnetization with rates given by the off-diagonal elements of **R** indicated in Table 1. In contrast, for the case where $\delta \gg 1/(2J_{AX})$, $1/(2J_{AM})$ and when $J_{AM} \neq J_{AX}$, the individual lines of the multiplet evolve separately and all of the off-diagonal elements of **R** are zero in this limit. For the case where $J_{AM} = J_{AX}$, carbon multiplet components corresponding to the transitions $\beta\beta\alpha \leftrightarrow \alpha\beta\alpha$ and $\beta\alpha\beta \leftrightarrow \alpha\alpha\beta$ are degenerate and hence evolve at the same frequency irrespective of the relative values of δ and $1/J_{AM} = 1/J_{AX}$. Therefore, cross

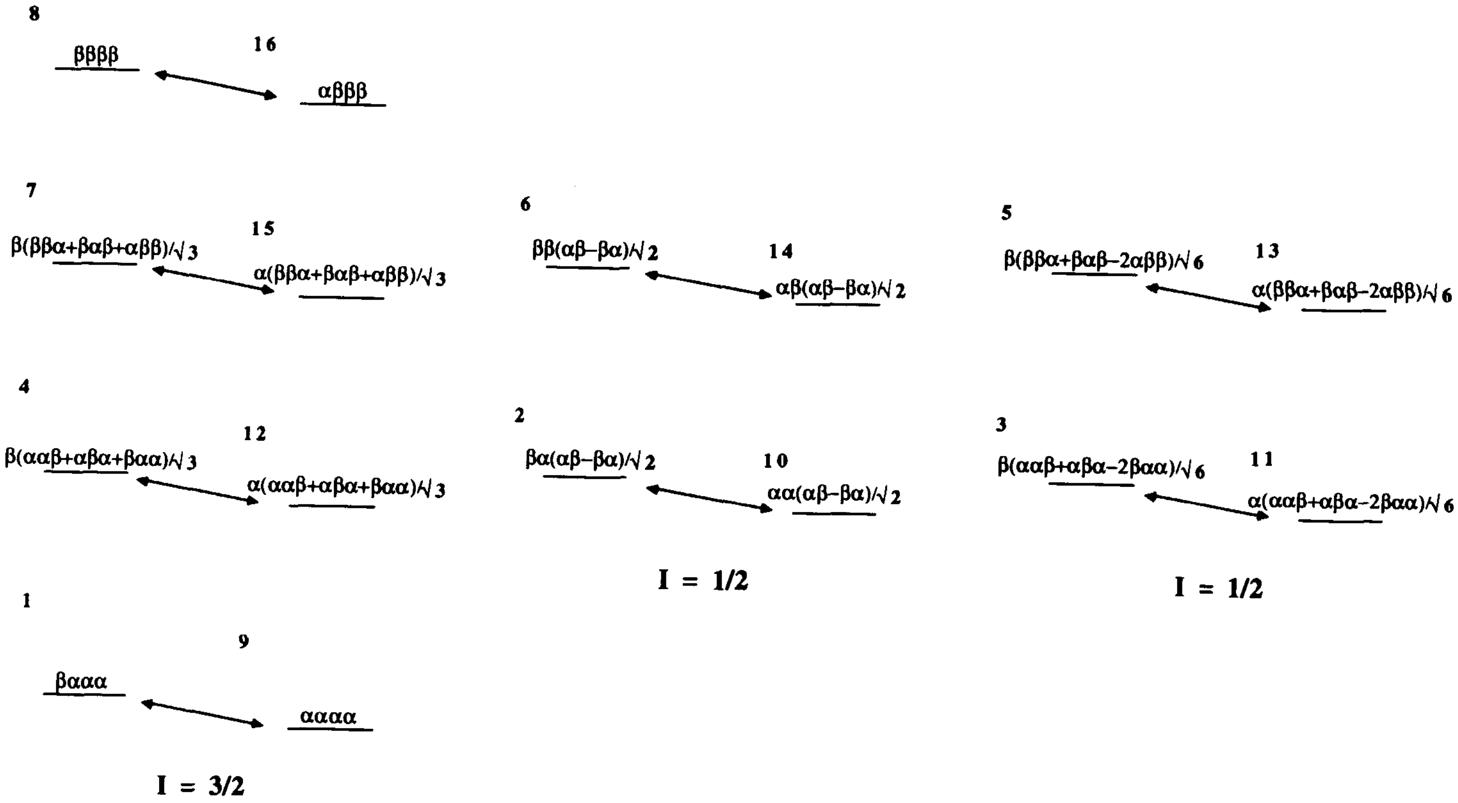

FIG. 2. Energy-level diagram of an isolated AX_3 spin system with the wavefunctions written in an irreducible basis representation. Each of the eigenstates is numbered from 1 to 16. The wavefunctions are grouped into three manifolds and the total spin angular momentum, I, associated with each manifold is indicated. The first spin state in wavefunction $|k\rangle$ corresponds to the ^{13}C spin state and the remaining spin states are associated with the proton spins.

TABLE 3

Transverse Relaxation of Spin A in an AX$_3$ Spin System

$\nu_1 = \rho_{1,9}$	$\omega_1 = \omega_C - 3\pi J_{AX}$
$\nu_2 = \rho_{4,12}$	$\omega_2 = \omega_C - \pi J_{AX}$
$\nu_3 = \rho_{7,15}$	$\omega_3 = \omega_C + \pi J_{AX}$
$\nu_4 = \rho_{8,16}$	$\omega_4 = \omega_C + 3\pi J_{AX}$
$\nu_5 = (1/\sqrt{2})(\rho_{2,10} + \rho_{3,11})$	$\omega_5 = \omega_C - \pi J_{AX}$
$\nu_6 = 1/\sqrt{2})(\rho_{6,14} + \rho_{5,13})$	$\omega_6 = \omega_C + \pi J_{AX}$

$R_{11} = 3J_{AX}(\omega_A + \omega_X) + 3/2J_{AX}(\omega_X) + 3/2J_{AX}(\omega_A) + 1/2J_{AX}(\omega_A - \omega_X) + 2J_{AX}(0) + 6J_{XX}(2\omega_X) + 3J_{XX}(\omega_X) + 3K_{XAX}(\omega_A) + 4K_{XAX}(0) + 3K_{XXX}(\omega_X)$

$R_{12} = 1/2J_{AX}(\omega_X) + K_{XAX}(\omega_X) - 2J_{XX}(\omega_X) - 4K_{XXX}(\omega_X)$

$R_{13} = -2J_{XX}(2\omega_X) - 4K_{XXX}(2\omega_X)$

$R_{14} = 0$

$R_{15} = (2)^{-1/2}[J_{AX}(\omega_X) - J_{XX}(\omega_X) - K_{XAX}(\omega_X) + K_{XXX}(\omega_X)]$

$R_{16} = 2(2)^{1/2}[-J_{XX}(2\omega_X) + K_{XXX}(2\omega_X)]$

$R_{22} = 3J_{AX}(\omega_A + \omega_X) + 3/2J_{AX}(\omega_X) + 3/2J_{AX}(\omega_A) + 1/2J_{AX}(\omega_A - \omega_X) + 10/9J_{AX}(0) + 2J_{XX}(2\omega_X) + 5J_{XX}(\omega_X) + 2J_{XX}(0) + 4K_{XAX}(\omega_A + \omega_X) + 2K_{XAX}(\omega_X) - K_{XAX}(\omega_A) + 2/3K_{XAX}(\omega_A - \omega_X) - 4/9K_{XAX}(0) + 4K_{XXX}(2\omega_X) + K_{XXX}(\omega_X) - 2K_{XXX}(0)$

$R_{23} = 2/3J_{AX}(\omega_X) + 4/3K_{XAX}(\omega_X)$

$R_{24} = R_{13}$

$R_{25} = \{4(2)^{1/2}/9\}[J_{AX}(0) - K_{XAX}(0)] + (2)^{1/2}[K_{XXX}(0) - J_{XX}(0)]$

$R_{26} = \{(2)^{1/2}/6\}[J_{AX}(\omega_X) - K_{XAX}(\omega_X)] + 3(2)^{-1/2}[K_{XXX}(\omega_X) - J_{XX}(\omega_X)]$

$R_{33} = R_{22}$

$R_{34} = R_{12}$

$R_{35} = R_{26}$

$R_{36} = R_{25}$

$R_{44} = R_{11}$

$R_{45} = R_{16}$

$R_{46} = R_{15}$

$R_{55} = 3J_{AX}(\omega_A + \omega_X) + 3/2J_{AX}(\omega_X) + 3/2J_{AX}(\omega_A) + 1/2J_{AX}(\omega_A - \omega_X) + 14/9J_{AX}(0) + 2J_{XX}(2\omega_X) + 2J_{XX}(\omega_X) + J_{XX}(0) - 2K_{XAX}(\omega_A + \omega_X) - K_{XAX}(\omega_X) - K_{XAX}(\omega_A) - 1/3K_{XAX}(\omega_A - \omega_X) - 8/9K_{XAX}(0) - 2K_{XXX}(2\omega_X) - 2K_{XXX}(\omega_X) - K_{XXX}(0)$

$R_{56} = 5/6J_{AX}(\omega_X) - 1/3K_{XAX}(\omega_X)$

$R_{66} = R_{55}$

$R_{ij} = R_{ji}$

Note. ν_i, R_{ij}, ω_{ij}, and J_{ij} are defined as in the note to Table 1. The spectral densities $K_{XXX}(\omega)$ are cross-correlation spectral densities arising from ^{1}H–^{1}H dipolar interactions involving proton pairs ij and ik ($j \neq k$). The other spectral densities are defined in the text.

relaxation will occur between the density elements $\rho_{3,4}$ and $\rho_{5,6}$ corresponding to these transitions (see Fig. 1) for all values of δ with a rate given by R_{23} in Table 1.

Figure 1b shows an energy-level diagram and associated wavefunctions for an AX$_2$ spin system written in an irreducible basis representation with the A transitions marked with arrows. The elements of ν and **R** for this spin system are indicated in Table 2. As with the AMX spin system, in the limit that $\delta \ll 1/(2J_{AX})$, cross relaxation between individual density elements occurs with rates, R_{ij} ($i \neq j$), given in Table 2. For the case where $\delta \gg 1/(2J_{AX})$, all of the off-diagonal elements are zero with the exception of $R_{2,4}$, which connects the degenerate transitions $\rho_{3,4}$ and $\rho_{5,6}$. This is analogous to the case where $J_{AM} = J_{AX}$ in the AMX spin system. Prestegard and Grant have derived identical equations for the A spin transverse relaxation rate in an AX_2 spin system in the limit that $\delta \gg 1/(2J_{AX})$ (*32*).

The energy-level diagram and associated wavefunctions for an AX_3 spin system are given in Fig. 2 with single-quantum A transitions indicated with arrows. The elements of ν and **R** for the AX_3 case are defined in Table 3. In the limit where $\delta \ll 1/(2J_{AX})$ all multiplet components are locked along the RF axis and cross relaxation between individual components occurs with the rates given in Table 3. In the limit where $\delta \gg 1/(2J_{AX})$ only those density elements ν_2, ν_5 and ν_3, ν_6 are degenerate and hence the only nonzero off-diagonal elements of **R** are $R_{2,5}$ and $R_{3,6}$ (i.e., the elements of **R** that connect ν_2 with ν_5 and ν_3 with ν_6).

In summary, in this Note we have presented the equations describing the transverse relaxation of isolated AMX, AX_2, and AX_3 spin systems for the cases where the spacing between successive refocusing pulses in the CPMG sequence is either much smaller or much larger than $1/(2J_{AX})$ or $1/(2J_{AM})$. It is clear that, as suggested by Werbelow and Grant for longitudinal relaxation (*12*), the effects of cross correlation can be significant and cannot be neglected in the interpretation of transverse relaxation rates used to study molecular dynamics.

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