On the Use of the Stochastic Liouville Equation in Nuclear Magnetic Resonance: Application to $R_{1\rho}$ Relaxation in the Presence of Exchange

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ABSTRACT: The nuclear magnetic resonance (NMR) spin relaxation rate constant in the rotating frame of reference $R_{1\rho}$ provides information on microsecond to millisecond (μs-ms) timescale kinetic processes that stochastically modulate the resonance frequencies of nuclear spins in molecules. Expressions for $R_{1\rho}$ are presented for two-site chemical exchange using both the conventional perturbation approach for evolution of the density operator in the interaction frame of reference and the stochastic Liouville equation (SLE) for evolution of the average density operator in the rotating frame of reference. The former approach is limited to the fast-exchange regime, in which the chemical exchange kinetics are faster than the frequency difference between spins in the two sites. The latter approach provides approximate expressions for $R_{1\rho}$ that are accurate when chemical exchange is not fast. Expressions for $R_{1\rho}$ that are accurate over the widest range of experimental conditions facilitate the interpretation of chemical exchange phenomena.
Concepts Magn Reson Part A 19A: 134–148, 2003

KEY WORDS: NMR relaxation; Redfield theory; master equation; Stochastic Liouville equation; Bloch-McConnell equations; rotating frame relaxation; chemical exchange; exchange regime

INTRODUCTION

Nuclear magnetic resonance (NMR) is a particularly useful technique for studying the dynamics of molecules over a wide range of timescales (*1–4*). Numerous relaxation measurements are sensitive to motions on the ps-ns timescale and thus provide valuable information on the microdynamics of biological macromolecules such as proteins (*5*). Moreover, dedi-

Received 29 April 2003; revised 28 July 2003; accepted 28 July 2003

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Concepts in Magnetic Resonance Part A, Vol. 19A(2) 134–148 (2003)
Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/cmr.a.10091

cated experiments, including $R_{1\rho}$ and R_2 relaxation measurements, are sensitive to molecular motions or kinetics occurring on a much slower timescale, in the microsecond to millisecond (μs-ms) range (*5*). Interpretation of data for $R_{1\rho}$, R_2, and related experiments that probe μs-ms timescales require a model for both relaxation and exchange processes against which the acquired data can be fitted. Two approaches have been used to tackle this problem. The most widespread approach, and the first method proposed historically, postulates the independance of both phenomena. Consequently, a model for chemical exchange is superimposed to the dynamic equations accounting for evolution of the spin system (including relaxation). This approach led to the celebrated Bloch-McConnell equations (*6*). These equations can not treat spin systems for which the chemical exchange process alters scalar coupling constants; instead, a density operator treatment is required in such instances. Nonetheless, the basic idea used in the density operator approach is conceptually similar to the Bloch-McConnell equations because a master equation of the Redfield type is combined with a given model for exchange (*7*). A second approach, initiated by Wennerström (*8*), combines relaxation and exchange processes in the relaxation superoperator as given by Abragam (*9*). Therefore, both processes are treated at the same level, but the range of validity of this approach is restricted to the so-called fast-exchange regime.

This study reviews some general theoretical aspects pertaining to the description of chemical exchange and to effect chemical exchange on relaxation measurements. Application of the stochastic Liouville equation (SLE) to $R_{1\rho}$ relaxation experiments will be emphasized. The relaxation rate constant $R_{1\rho}$ characterizes the decay of magnetization spin locked along the direction of the effective magnetic field in the rotating frame of reference for a spin system undergoing radio-frequency (RF) irradiation. Recently, $R_{1\rho}$ experiments have been developed to elucidate intramolecular conformational changes, ligand binding, and folding of proteins and other biological macromolecules (*5*). In these experiments, the dependence of $R_{1\rho}$ on the amplitude and frequency of the RF field is used to determine the rate constants, site populations, and Larmor frequencies for nuclear spins affected by the kinetic process (*5*). Analytical expressions for $R_{1\rho}$ that reproduce experimental results over the largest possible range of parameters are desirable both for analysis of data and for physical insight (*10, 11*). The SLE is a convenient way to describe the dynamics of a system driven by a stochastic process, such as chemical exchange, without restriction to particular timescales. A derivation of the SLE will be given and specialized to the case, important in practice, of a Markov sudden jump process, which is commonly used in the description of chemical exchange. Then, reviewing recent results (*11, 12*), a Laplace transform approach will be used to obtain an approximate solution for the evolution of the average density operator $\langle\rho\rangle(t)$. The special case of two-site exchange will be emphasized and new analytical approximate expressions that have been obtained recently will be outlined (*11, 12*). For the sake of clarity, some aspects of the Wennerström theory (*8*) will be recalled and the analytical formula for $R_{1\rho}$ will be obtained following this approach (*13*) and will be given for comparison.

A STOCHASTIC JUMP MODEL FOR CHEMICAL EXCHANGE

The description of the evolution of a spin system subject to chemical exchange can be approached from two different standpoints, which are very different in essence. One is a macroscopic approach, introduced by Gutowsky (*14*) and generalized by McConnell (*6*). The other is based on the theory as developed by Kubo (*15*) to describe the evolution of a magnetic moment in which its resonance frequency jumps between N different values, $\omega_1, \ldots, \omega_N$. This latter approach is somewhat generalized here in the case of a spin system that can be described by a Hamiltonian $\mathcal{H}(t)$ stochastically modulated by a jump process.

Description of a Markov Jump Process

The stochastic process used to describe chemical exchange commonly is assumed to be a Markov jump process. Thus, the stochastic variable ζ (which may encompass the Larmor resonance frequency ω_0 as well as the relaxation rate constants R_1 and R_2, for instance) takes N values $\zeta = \zeta_1, \ldots, \zeta_N$ and is characterized by a transition probability between states that does not depend on previous history. For a particular realization of the stochastic process, the variable ζ takes n different values ζ_k at times t_k: $(\zeta_n, t_n; \zeta_{n-1}, t_{n-1}; \cdots \zeta_1, t_1)$. The Markov property, i.e., the absence of memory of the process, is expressed by

$$P(\zeta_n, t_n | \zeta_{n-1}, t_{n-1}; \cdots \zeta_1, t_1) = P(\zeta_n, t_n | \zeta_{n-1}, t_{n-1}) \qquad [1]$$

where $P(\zeta_n, t_n | \zeta_{n'}, t_{n'})$ is the probability for the process being in state ζ_n at time t_n given that it was in state $\zeta_{n'}$ at time $t_{n'}$ and is denoted $P_{nn'}$. Such a process obeys the so-called Chapman-Kolmogorov equation (*16*):

$$P_{kj}(t) = \sum_{m} P_{km}(h) P_{mj}(t - h) \qquad [2]$$

where $P_{kj}(t)$ is the transition probability from state j to state k. The following assumptions are made concerning these transition probabilities (*17*):

$$P_{kj}(t) = \gamma_{kj} t + o(t) \qquad [3]$$

$$P_{jj}(t) = 1 - \gamma_j t + o(t) \qquad [4]$$

$$\gamma_j = \sum_{k} \gamma_{kj} \qquad [5]$$

and $\gamma_{jj} = 0$ by definition. Equation [2] can be written as

$$P_{kj}(t) = \sum_{m, m \neq k} (\gamma_{km} h + o(h)) P_{mj}(t - h) + (1 - \gamma_k h + o(h)) P_{kj}(t - h) \qquad [6]$$

where $o(h))/h \to 0$ for $h \to 0$. The Kolmogorov differential equation is obtained from Eq. [6] by grouping the terms in $P_{kj}(t)$, dividing by h, and taking the limit $h \to 0$:

$$\frac{d}{dt} P_{kj}(t) = \sum_{m} \gamma_{km} P_{mj}(t) - \gamma_k P_{kj}(t) \qquad [7]$$

This can be expressed as a master equation for the vector of transition probabilities:

$$\frac{d}{dt} P = \mathbf{\Gamma} P, \qquad [8]$$

where $\Gamma_{kj} = \gamma_{kj}$, $\Gamma_{jj} = -\gamma_j$, and $P = [P_{1l}(t), P_{2l}(t), \ldots, P_{Nl}(t)]^t$. From the foregoing definitions, the probability that no jump takes place during time t while the system is in site i is

$$e^{-\gamma_i t} \qquad [9]$$

while the probability that a jump occurs from site i to site j between t and $t + dt$ is given by

$$\gamma_{ji} e^{-\gamma_i t} dt \qquad [10]$$

Derivation of the SLE

The evolution of the spin density operator is given by the Liouville equation (*18*):

$$\frac{d}{dt} \boldsymbol{\rho}(t) = -i\mathscr{L}(t)\boldsymbol{\rho}(t) \qquad [11]$$

where $\mathscr{L}(t)$ is the Liouvillian operator, which is stochastically modulated by the jump process. The average evolution operator for the density matrix $\boldsymbol{\rho}(t)$ is calculated by

$$\mathbf{U}(t) = \left\langle \exp\left(-i \int_{0}^{t} \mathscr{L}(t') dt' \right) \right\rangle \qquad [12]$$

Suppose that starting initially in site i_0 at time t_0 the stochastic jumps occur in a particular sequence at times $t_1, t_2, \ldots, t_{k-1}, t_k$, where they take the values $(i_1, i_2, \ldots, i_{k-1}, i)$, respectively, with $i_j = 1, \ldots, N$. Using the same foregoing notation, the evolution of the spins thus is given by

$$\begin{aligned} &\mathbf{U}(t_0; t_1; t_2, \ldots, t_k; t)\boldsymbol{\rho}(t_0) \\ &\quad = e^{-\gamma_i(t-t_k)} \exp(-i\mathscr{L}_i(t - t_k)) \\ &\quad \gamma_{i,i_{k-1}} e^{-\gamma_{i_{k-1}}(t_k - t_{k-1})} \exp(-i\mathscr{L}_{i_{k-1}}(t_k - t_{k-1})) \cdots \\ &\quad \gamma_{i_2 i_1} e^{-\gamma_{i_1}(t_2 - t_1)} \exp(-i\mathscr{L}_{i_1}(t_2 - t_1)) \\ &\quad \gamma_{i_1 i_0} e^{-\gamma_{i_0}(t_1 - t_0)} \exp(-i\mathscr{L}_{i_0}(t_1 - t_0)) \boldsymbol{\rho}(t_0) dt_k \cdots dt_1 \end{aligned} \qquad [13]$$

The partial average of the density operator $\tilde{\boldsymbol{\rho}}_i(t)$ such that the ζ is in state i at time t and averaged over all realizations and initial conditions i_0 is given by

$$\begin{aligned} \tilde{\boldsymbol{\rho}}_i(t) = \sum_{i_0} \Bigg[& \delta_{i i_0} e^{-\gamma_{i_0}(t - t_0)} \mathbf{U}(t; t_0)) \boldsymbol{\rho}(t_0) + \sum_{k=1}^{\infty} \sum_{i_1, i_2, \ldots, i_{k-1}=1}^{N} \\ & \times \int_{t_0}^{t} dt_k \int_{t_0}^{t_k} dt_{k-1} \cdots \int_{t_0}^{t_2} dt_1 e^{-\gamma_i(t - t_k)} \mathbf{U}_{i_k}(t; t_k) \\ & \times \gamma_{i,i_{k-1}} e^{-\gamma_{i_{k-1}}(t_k - t_{k-1})} \mathbf{U}_{i_{k-1}}(t_k; t_{k-1}) \cdots \\ & \gamma_{i_2 i_1} e^{-\gamma_{i_1}(t_2 - t_1)} \mathbf{U}_{i_1}(t_2; t_1) \gamma_{i_1 i_0} e^{-\gamma_{i_0}(t_1 - t_0)} \mathbf{U}_{i_0}(t_1; t_0) \boldsymbol{\rho}(0) p_{i_0} \Bigg] \end{aligned}$$

$$[14]$$

in which the notation $U_{i_k}(t_{k+1};\ t_k) = \exp(-i\mathcal{L}_{i_k}(t_{k+1} - t_k))$ has been introduced and p_k is the population of site k. This very complicated expression gives the formal solution of the SLE.

The differential form of Eq. [14] is more amenable in the present context. The differential equation is derived by considering the evolution of one of the U_k's in the short time interval $(t - h,\ t)$. The evolution under $\mathcal{L}_k$ is given by

$$\mathbf{U}_k(t) = \exp(-i\mathcal{L}_k h) \sum_j P_{kj}(h)\mathbf{U}_j(t - h) \quad [15]$$

For small h this can be written

$$\mathbf{U}_k(t) = (1 - i\mathcal{L}_k h)\left\{ \sum_{j,j\neq k} P_{kj}(h)\mathbf{U}_j(t - h) + P_{kk}(h)\mathbf{U}_k(t - h)\right\} \quad [16]$$

Using Eqs. [3] and [4]:

$$\mathbf{U}_k(t) = (1 - i\mathcal{L}_k h)\left\{ \sum_j (\gamma_{kj}h + o(h))\mathbf{U}_j(t - h) + (1 - \gamma_k h + o(h))\mathbf{U}_k(t - h)\right\} \quad [17]$$

which can be expressed as

$$\mathbf{U}_k(t) - \mathbf{U}_k(t - h) = (-i\mathcal{L}_k - \gamma_k)\mathbf{U}_k(t - h)h + \sum_j \mathbf{U}_j(t - h)\gamma_{kj}h + o(h) \quad [18]$$

Finally, dividing by h and taking the limit $h \rightarrow 0$, the differential equation obeyed by the matrices $\mathbf{U}_k$ is obtained:

$$\frac{d}{dt}\mathbf{U}_k(t) = (-i\mathcal{L}_k - \gamma_k)\mathbf{U}_k(t) + \sum_j \gamma_{kj}\mathbf{U}_j(t) \quad [19]$$

Equation [19] actually is a system of N matrix differential equations. Thus, solving Eq. [19] amounts to solving a matrix differential system of size $N \times M$, where M is the size of the Liouvillian $\mathcal{L}$. Introducing the direct product of the spin space and the site space (probability space), Eq. [19] can be recast in the form:

$$\frac{d}{dt}\mathbf{U}(t) = (\mathbf{\Lambda} + \mathbf{\Xi})\mathbf{U}(t) \quad [20]$$

The operators $\mathbf{\Lambda}$ and $\mathbf{\Xi}$ of Eq. [20] are defined as

$$\mathbf{\Lambda} = \begin{pmatrix} \mathbf{L}(\zeta_1) & 0 & \cdots & 0 \\ 0 & \mathbf{L}(\zeta_2) & \cdots & 0 \\ 0 & 0 & \ddots & 0 \\ 0 & 0 & 0 & \mathbf{L}(\zeta_N) \end{pmatrix} = \sum_{i=1,N} \mathbf{P}_i \otimes \mathbf{L}(\zeta_i) \quad [21]$$

$$\mathbf{\Xi} = \mathbf{\Gamma} \otimes \mathbf{1}_{\text{spin}} \quad [22]$$

The notation $\mathbf{L}(\zeta_k) = -i\mathcal{L}_k$ was introduced and $\mathbf{P}_i$ is the projector onto the state corresponding to the value $\zeta = \zeta_i$:

$$[\mathbf{P}_i]_{mn} = \delta_{im}\delta_{in} \quad [23]$$

$\mathbf{1}_{\text{spin}}$ is the identity matrix of dimension $M \times M$ acting on the Hilbert space of the spins.

To continue, the average density matrix $\langle\boldsymbol{\rho}\rangle(t)$ is introduced as

$$\langle\boldsymbol{\rho}\rangle(t) = \sum_{k=1}^{N} \tilde{\boldsymbol{\rho}}_k(t) \quad [24]$$

where $\tilde{\boldsymbol{\rho}}(t) = (\tilde{\boldsymbol{\rho}}_1(t),\ \tilde{\boldsymbol{\rho}}_2(t),\ \ldots,\ \tilde{\boldsymbol{\rho}}_N(t))^t$. Following the foregoing notation, each $\tilde{\boldsymbol{\rho}}_k(t)$ represents a density matrix with value $\zeta = \zeta_k$ of the stochastic variable at time t. Therefore, $\tilde{\boldsymbol{\rho}}(t)$ also obeys the same evolution equation as the corresponding evolution operator $\mathbf{U}$ in Eq. [20]:

$$\frac{d}{dt}\tilde{\boldsymbol{\rho}}(t) = (\mathbf{\Lambda} + \mathbf{\Xi})\tilde{\boldsymbol{\rho}}(t) \quad [25]$$

This equation is very general and applies to a broad range of problems. In fact, any stochastic process consisting of instantaneous Markovian jumps during which the state of the spins remains unchanged can be treated in this manner. The Liouvillian also can be redefined as

$$\mathcal{L} \rightarrow \mathcal{L} + i\hat{\mathbf{\Gamma}} \quad [26]$$

so as to include the relaxation superoperator $\hat{\mathbf{\Gamma}}$ (*18*). In the simple case where the spin dynamics can be described by the phenomenological Bloch equations for the macroscopic magnetization, Eq. [25] reduces to the Bloch-McConnell equations (*6*). Finally, the stochastic variable ζ, generally is associated with the resonance frequency, but it also can be defined as a set of totally correlated random variables. For instance, if each site is associated with different resonance frequency and relaxation rate constants, then ζ should be defined as the set of parameters $\{\omega_0, R_1, R_2, \cdots\}$.

A BRIEF SURVEY OF A MICROSCOPIC APPROACH TO EXCHANGE

In the preceding section, the problem of chemical exchange in NMR was approached by considering the evolution of the density operator subject to random modulation of the resonance frequency. Relaxation and chemical exchange were introduced independantly in a phenomenological way by superimposing a master equation describing the evolution of the spin system with the exchange jump process to yield the SLE. An alternative approach was introduced by Wennerström (*8*), based on the perturbative analysis of spin relaxation as presented by Abragam (*9*), and applied by others to the study of $R_{1\rho}$ (*13, 19*). In this method, the evolution of a spin system that undergoes both random coupling with the lattice and chemical exchange is treated by considering the elementary coupling and exchange processes on an equal footing.

The use of this approach for $R_{1\rho}$ relaxation will be outlined in order to provide results for comparison with solutions of the SLE to be presented. First, relaxation will be considered in the absence of chemical exchange, but in the presence of an applied RF field (*20, 21, 22*). Relaxation will be calculated for spin operators in a tilted rotating frame in which its z-axis is aligned along the direction of the effective field in the rotating frame. Subsequently, this result will be extended to include chemical exchange.

The master equation describing the relaxation of the density operator in the interaction frame $\boldsymbol{\rho}^{\ddagger}(t)$ is (*9, 23*)

$$\frac{d}{dt}\boldsymbol{\rho}^{\ddagger}(t) = -\frac{1}{2}\int_{-\infty}^{+\infty} \overline{[\mathcal{H}_1^{\ddagger}(t), [\mathcal{H}_1^{\ddagger}(t-\tau), \boldsymbol{\rho}^{\ddagger}(t) - \boldsymbol{\rho}_0^{\ddagger}]]}\,d\tau \qquad [27]$$

in which $\mathcal{H}_1^{\ddagger}(t)$ is the interaction frame representation of the time-dependent stochastic part of the Hamiltonian and satisfies $\overline{\mathcal{H}_1(t)} = 0$. The laboratory frame Hamiltonian is

$$\mathcal{H}(t) = \mathcal{H}_0 + \mathcal{H}_{\mathrm{RF}}(t) + \mathcal{H}_1(t) = \mathcal{H}_0 + \mathcal{H}_{\mathrm{RF}}(t) + \sum_{q=-2}^{2}\sum_{p} F_2^q(t)\mathbf{A}_{2p}^q \qquad [28]$$

in which $\mathcal{H}_{\mathrm{RF}}(t)$ is the RF Hamiltonian, and $F_2^q(t)$ and $\mathbf{A}_{2p}^q$ are the spatial and spin tensor components of the stochastic interactions that couple the spin system to the lattice (*9, 23*). The spin tensor operators are chosen to satisfy the condition $\omega_{\mathrm{RF}} \sum_{i=1}^{n} [I_{zi}, \mathbf{A}_{2p}^q] = \omega_p^q \mathbf{A}_{2p}^q$, so that

$$\exp\left\{i\omega_{\mathrm{RF}}t\sum_i I_{zi}\right\}\mathbf{A}_{2p}^q \exp\{-i\omega_{\mathrm{RF}}t\sum_i I_{zi}\} = \mathbf{A}_{2p}^q \exp\{i\omega_p^q t\} \qquad [29]$$

In these equations, ω_{RF} is the angular frequency of the applied RF field, and the sum extends over n nuclear spins. Consequently, homonuclear spins are treated as like spins (*23*). In the rotating frame,

$$\begin{aligned}\tilde{\mathcal{H}}(t) &= \sum_i \Omega_i I_{zi} + \omega_1 \sum_i I_{xi} + \tilde{\mathcal{H}}_1(t)\\ &= \sum_i \Omega_i I_{zi} + \omega_1 \sum_i I_{xi} + \sum_{p,q} F_2^q(t)\mathbf{A}_{2p}^q \exp\{i\omega_p^q t\}\end{aligned} \qquad [30]$$

in which the applied B_1 field is assumed to have x phase and amplitude (defined by the Rabi frequency) $\omega_1 = -\gamma B_1$, $\Omega_i = \omega_{0i} - \omega_{\mathrm{RF}}$ is the resonance offset, and ω_{0i} is the Larmor frequency of the ith spin. Next, $\mathbf{U}$ is defined as the unitary transformation from the rotating frame to the frame in which its z-axis is aligned along the effective field direction. In this instance, the Hamiltonian $\mathcal{H}'(t)$ expressed in the tilted frame is related to $\tilde{\mathcal{H}}(t)$ by $\mathcal{H}'(t) = \mathbf{U}\tilde{\mathcal{H}}(t)\mathbf{U}^{-1}$, where

$$\mathbf{U} = \exp\left\{i\sum_i \theta_i I_{yi}\right\} \qquad [31]$$

and the tilt angle in the rotating frame for the ith nuclear spin is $\tan\theta_i = \omega_1/\Omega_i$. In the tilted rotating frame,

$$\mathcal{H}'(t) = \sum_i \omega_{ei} I'_{zi} + \sum_{p,q} F_2^p(t)\mathbf{U}\mathbf{A}_{2p}^q\mathbf{U}^{-1}\exp\{i\omega_p^q t\} \qquad [32]$$

in which $\omega_{ei} = (\Omega_i^2 + \omega_1^2)^{1/2}$.

The fluctuating part of the Hamiltonian in the interaction representation $\mathcal{H}_1^{\ddagger}(t)$ is given by

$$\mathcal{H}_1^{\ddagger}(t) = \exp\left\{i \sum_i \omega_{ei} I_{zi} t\right\} \mathcal{H}_1'(t) \exp\left\{-i \sum_i \omega_{ei} I_{zi} t\right\}$$

$$= \sum_{p,q} e^{i\omega_p^q t} F_2^q(t) \exp\left\{i \sum_i \omega_{ei} I_{zi} t\right\}$$

$$\times \ \mathbf{U}\mathbf{A}_{2p}^q \mathbf{U}^{-1} \exp\left\{-i \sum_i \omega_{ei} I_{zi} t\right\} \quad [33]$$

The transformation $\mathbf{U}\mathbf{A}_{2p}^q\mathbf{U}^{-1}$ denotes the operator $\mathbf{A}_{2p}^q$ expressed in the rotated system of operators $\{\mathbf{B}_k'\}$:

$$\mathbf{U}\mathbf{A}_{2p}^q\mathbf{U}^{-1} = \sum_k b_{pqk} \mathbf{B}_k' \quad [34]$$

and $b_{pqk} = \langle \mathbf{B}_k'^{\dagger} \mathbf{U}\mathbf{A}_{2p}^q \mathbf{U}^{-1} \rangle$. Consequently,

$$\mathcal{H}_1^{\ddagger}(t)$$

$$= \sum_{p,q,k} b_{pqk} F_2^q(t) \exp\left\{i \sum_i \omega_{ei} I_{zi} t\right\} \mathbf{B}_k' \exp\left\{-i \sum_i \omega_{ei} I_{zi} t\right\} \quad [35]$$

In addition, the operators $\mathbf{B}_k'$ are chosen to satisfy the condition: $\Sigma_i\ \omega_{ei}[I_{zi}', \mathbf{B}_k'] = \omega_k \mathbf{B}_k'$, so that, finally,

$$\mathcal{H}_1^{\ddagger}(t) = \sum_{p,q,k} F_2^q(t) b_{pqk} \mathbf{B}_k' \exp\{i(\omega_p^q + \omega_k)t\} \quad [36]$$

Equation [36] is substituted into Eq. [27] to give

$$\frac{d}{dt}\boldsymbol{\rho}^{\ddagger}(t) = -\frac{1}{2} \sum_{p,q,k} \sum_{p',q',k'} \cdot\ e^{i(\omega_p^q + \omega_k + \omega_{p'}^{q'} + \omega_{k'})t} b_{pqk} b_{p'q'k'}$$

$$[\mathbf{B}_k', [\mathbf{B}_{k'}', \boldsymbol{\rho}^{\ddagger}(t) - \boldsymbol{\rho}_0^{\ddagger}]]$$

$$\times \int_{-\infty}^{+\infty} \overline{F_2^q(t) F_2^{q'}(t-\tau)} e^{-i(\omega_{p'}^{q'} + \omega_{k'})\tau} d\tau \quad [37]$$

This equation is transformed back to the tilted rotating frame to yield

$$\frac{d}{dt}\boldsymbol{\rho}'(t) = -i \sum_i \omega_{ei} [I_{zi}', \boldsymbol{\rho}'(t)]$$

$$-\frac{1}{2} \sum_{p,q,k} \sum_{p',q',k'} e^{i(\omega_p^q + \omega_{p'}^{q'})t} b_{pqk} b_{p'q'k'} [\mathbf{B}_k', [\mathbf{B}_{k'}', \boldsymbol{\rho}'(t) - \boldsymbol{\rho}_0']]$$

$$\times \int_{-\infty}^{+\infty} \overline{F_2^q(t) F_2^{q'}(t-\tau)} e^{-i(\omega_{p'}^{q'} + \omega_{k'})\tau} d\tau \quad [38]$$

The orthogonality of the spherical harmonic functions and the spherical symmetry of an isotropic liquid require that $q' = -q$. The secular approximation, i.e., neglecting fast oscillating terms that average to zero, requires $p' = p$. Thus, one gets

$$\frac{d}{dt}\boldsymbol{\rho}'(t) = -i \sum_i \omega_{ei} [I_{zi}', \boldsymbol{\rho}'(t)]$$

$$-\frac{1}{2} \sum_{p,q,k,k'} \int_{-\infty}^{+\infty} \overline{F_2^q(t) F_2^{-q}(t-\tau)} e^{-i(\omega_p^q + \omega_{k'})\tau} d\tau$$

$$\times\ b_{pqk} b_{p(-q)k'} [\mathbf{B}_k', [\mathbf{B}_{k'}', \boldsymbol{\rho}'(t) - \boldsymbol{\rho}_0']]$$

$$= -i \sum_i \omega_{ei} [I_{zi}', \boldsymbol{\rho}'(t)] - \frac{1}{2} \sum_{p,q,k,k'} j^q(\omega_p^q + \omega_{k'})$$

$$\times\ b_{pqk} b_{p(-q)k'} [\mathbf{B}_k', [\mathbf{B}_{k'}', \boldsymbol{\rho}'(t) - \boldsymbol{\rho}_0']] \quad [39]$$

in which

$$j^q(\omega) = \mathrm{Re} \int_{-\infty}^{+\infty} \overline{F_2^q(t) F_2^{-q}(t-\tau)} e^{-i\omega\tau} d\tau \quad [40]$$

is the spectral density function $\boldsymbol{\rho}_0' = \mathbf{U}\boldsymbol{\rho}_0\mathbf{U}^{-1}$ and dynamic frequency shifts have been neglected.

A general solution of Eq. [39] requires consideration of particular relaxation mechanisms. However, if $\omega_{ei}\tau_c \ll 1$, in which τ_c is the correlation time for stochastic fluctuations of the Hamiltonian $\mathcal{H}_1(t)$, then,

$$j^q(\omega_p^q + \omega_{k'}) \approx j^q(\omega_{0p}^q) \quad [41]$$

where ω_{0p}^q is defined by $[\mathcal{H}_0, \mathbf{A}_{2p}^q] = \omega_{0p}^q \mathbf{A}_{2p}^q$ and $j^q(\omega_{0p}^q)$ is the spectral density function in the absence of the RF field, such as would be calculated for conventional laboratory frame relaxation (*23*). For solution NMR spectroscopy, this assumption usually is well justified. For example, if $\tau_c = 20$ ns and $\omega_{ei} = 2\pi \times 10^5\ \mathrm{s}^{-1}$, then $\omega_{ei}\tau_c = 0.06$. Using this assumption,

$$\begin{aligned}\frac{d}{dt}\boldsymbol{\rho}'(t) &= -i\sum_i \omega_{ei}[I'_{zi}, \boldsymbol{\rho}'(t)] \\ &\quad - \frac{1}{2}\sum_{pq} j^q(\omega_{0p}^q)\sum_{kk'} b_{pqk}b_{p(-q)k'}[\mathbf{B}'_k, [\mathbf{B}'_{k'}, \boldsymbol{\rho}'(t) - \boldsymbol{\rho}'_0]] \\ &= -i\sum_i \omega_{ei}[I'_{zi}, \boldsymbol{\rho}'(t)] - \frac{1}{2}\sum_{pq} j^q(\omega_{0p}^q) \\ &\quad \times [\mathbf{U}\mathbf{A}_{2p}^q\mathbf{U}^{-1}, [\mathbf{U}\mathbf{A}_{2p}^{-q}\mathbf{U}^{-1}, \boldsymbol{\rho}'(t) - \boldsymbol{\rho}'_0]] \\ &= -i\sum_i \omega_{ei}[I'_{zi}, \boldsymbol{\rho}'(t)] \\ &\quad - \frac{1}{2}\sum_{pq} j^q(\omega_{0p}^q)\mathbf{U}[\mathbf{A}_{2p}^q, [\mathbf{A}_{2p}^{-q}, \mathbf{U}^{-1}\boldsymbol{\rho}'(t)\mathbf{U} - \boldsymbol{\rho}_0]]\mathbf{U}^{-1}\end{aligned} \quad [42]$$

The conventional laboratory frame relaxation superoperator is (*9, 23*):

$$\hat{\boldsymbol{\Gamma}} = -\frac{1}{2}\sum_{pq} j^q(\omega_{0p}^q)[\mathbf{A}_{2p}^q, [\mathbf{A}_{2p}^{-q}, \cdot\,]] \quad [43]$$

Substituting this expression into Eq. [42] yields the desired result:

$$\begin{aligned}\frac{d}{dt}\boldsymbol{\rho}'(t) &= -i\sum_i \omega_{ei}[I'_{zi}, \boldsymbol{\rho}'(t)] \\ &\quad + \mathbf{U}\hat{\boldsymbol{\Gamma}}\{\mathbf{U}^{-1}\boldsymbol{\rho}'(t)\mathbf{U} - \boldsymbol{\rho}_0\}\mathbf{U}^{-1}\end{aligned} \quad [44]$$

The physical meaning of Eq. [44] can be illustrated more clearly by expanding the density operator in an orthogonal set of basis operators. Thus, the cross-relaxation rate constant between two basis operators $\mathbf{B}'_m$ and $\mathbf{B}'_n$ in the tilted frame is given by (*23*)

$$\begin{aligned}\Gamma_{mn} &= \langle \mathbf{B}'^{\dagger}_m\mathbf{U}\hat{\boldsymbol{\Gamma}}\{\mathbf{U}^{-1}\mathbf{B}'_n\mathbf{U}\}\mathbf{U}^{-1}\rangle / \langle \mathbf{B}'^{\dagger}_m\mathbf{B}'_m\rangle \\ &= \langle \mathbf{U}^{-1}\mathbf{B}'^{\dagger}_m\mathbf{U}\hat{\boldsymbol{\Gamma}}\{\mathbf{U}^{-1}\mathbf{B}'_n\mathbf{U}\}\rangle / \langle \mathbf{B}'^{\dagger}_m\mathbf{B}'_m\rangle\end{aligned} \quad [45]$$

in which the last line is obtained using the cyclic property of the trace. Thus, for any relaxation mechanism that satisfies Eq. [41], cross-relaxation rate constants between operators in the tilted, spin-locked, reference frame are obtained by projecting the operators back into the untilted rotating frame and then calculating relaxation rate constants as usual, with the condition that all homonuclear spins are treated as like spins. For example, in the absence of exchange, the relaxation rate constant for I'_{zi} magnetization is obtained immediately as

$$R_{1\rho} = R_{1i}\cos^2\theta_i + R_{2i}\sin^2\theta_i \quad [46]$$

where R_{1i} and R_{2i} are the laboratory frame relaxation rate constants.

The foregoing formalism now must be generalized to include chemical exchange. The derivation follows the same general approach as previously shown. For simplicity, only a single spin that exchanges between N sites is considered. The laboratory frame spin Hamiltonian is written

$$\mathcal{H}(t) = \sum_{j=1}^{N} p_j\omega_{0j}I_z + \mathcal{H}_{\mathrm{RF}}(t) + \mathcal{H}_1(t) \quad [47]$$

where p_j is the equilibrium population of site j and

$$\mathcal{H}_1(t) = \sum_j [f_j(t) - p_j]\omega_{0j}I_z + \sum_{j,p,q} f_j(t)F_{2j}^q(t)\mathbf{A}_{2p}^q \quad [48]$$

In Eq. [48], the first summation on the right side is associated with the fluctuating Zeeman Hamiltonian caused by the exchange process and the second summation is the stochastic Hamiltonian associated with the lattice coupling. The $f_j(t)$ is a stochastic function that accounts for jumps between N sites, such that $f_j(t) = 1$ when the nucleus is in site j at time t and $f_j(t) = 0$ otherwise. By definition, $\overline{f_j(t)} = p_j$. As usual, jumps are considered to occur infinitely fast so that no evolution of the spin system takes place during a jump. In the rotating frame, the fluctuating part of the Hamiltonian is given by

$$\tilde{\mathcal{H}}_1(t) = \sum_j [f_j(t) - p_j]\omega_{0j}I_z + \sum_{j,p,q} f_j(t)F_{2j}^q(t)\mathbf{A}_{2p}^q e^{i\omega_p^q t} \quad [49]$$

Now, introducing the average Larmor frequency, resonance offset, tilt angle, and effective field, respectively, as

$$\begin{cases}\bar{\omega}_0 = \sum_j p_j\omega_{0j} \\ \bar{\Omega} = \sum_j p_j\Omega_{0j} = \bar{\omega}_0 - \omega_{\mathrm{RF}} \\ \tan\theta = \omega_1/\bar{\Omega} \\ \omega_{\mathrm{eff}} = (\bar{\Omega}^2 + \omega_1^2)^{1/2}\end{cases} \quad [50]$$

$\mathbf{U} = \exp\{i\theta I_y\}$ transforms the rotating frame into the frame in which its z-axis is aligned along the effective field direction for the average resonance offset. The fluctuating Hamiltonian is then given by

$$\mathcal{H}'_1(t) = \sum_j [f_j(t) - p_j]\omega_{0j}(I'_z\cos\theta + I'_x\sin\theta) + \sum_{j,p,q} f_j(t)F^q_{2j}(t)\mathbf{U}\mathbf{A}^q_{2p}\mathbf{U}^{-1}e^{i\omega^q_p t} \quad [51]$$

Finally, in the interaction frame, the stochastic part of the Hamiltonian is given by

$$\mathcal{H}^{\ddagger}_1(t) = \sum_j [f_j(t) - p_j]\omega_{0j} \sum_{k=-1}^{1} \mathbf{B}'_k\exp\{ik\omega_{\text{eff}}t\} + \sum_{j,p,q} f_j(t)F^q_{2j}(t)\mathbf{U}\mathbf{A}^q_{2p}\mathbf{U}^{-1}e^{i\omega^q_p t} \quad [52]$$

in which

$$\mathbf{B}'_{-1} = (1/\sqrt{2})I'^{-}\sin\theta$$
$$\mathbf{B}'_0 = I'_z\cos\theta$$
$$\mathbf{B}'_1 = (1/\sqrt{2})I'^{+}\sin\theta \quad [53]$$

As before, the interaction frame Hamiltonian is substituted into Eq. [27], the equation is transformed back to the tilted rotating frame, and the conditions $q' = -q$ and $p' = p$ are invoked. The resulting expression is

$$\begin{aligned}\frac{d}{dt}\boldsymbol{\rho}'(t) = {}& -i\omega_{\text{eff}}[I'_z, \boldsymbol{\rho}'(t)] - \frac{1}{2}\sum_k j_{\text{ex}}(k\omega_{\text{eff}}) \times [\mathbf{B}'_k, [\mathbf{B}'_{-k}, \boldsymbol{\rho}'(t) - \boldsymbol{\rho}'_0]] \\ & - \frac{1}{2}\sum_{j,j',p,q}\int_{-\infty}^{+\infty} \overline{f_j(t)f_{j'}(t-\tau)F^q_{2j}(t)F^{-q}_{2j'}(t-\tau)} \times \exp\{-i\omega^q_p\tau\}d\tau \\ & \times \mathbf{U}[\mathbf{A}^q_{2p}, [\mathbf{A}^{-q}_{2p}, \mathbf{U}^{-1}\boldsymbol{\rho}'(t)\mathbf{U} - \boldsymbol{\rho}_0]]\mathbf{U}^{-1} \quad [54]\end{aligned}$$

in which

$$j_{\text{ex}}(\omega) = \text{Re}\sum_{j,j'}\int_{-\infty}^{+\infty} \omega_{0j}\omega_{0j'}\overline{[f_j(t) - p_j][f_{j'}(t-\tau) - p_{j'}]} \times \exp\{-i\omega\tau\}d\tau = -2\sum_{j=2,N} |\langle u_1|\omega|u_j\rangle|^2\lambda_j/(\lambda_j^2 + \omega_e^2) \quad [55]$$

where $\boldsymbol{\omega}$ has elements $\omega_{0i}\delta_{ij}$, λ_j and $|u_j\rangle$ are the jth eigenvalue and eigenvector, respectively, of the symmetrized matrix $\mathbf{S}^{-1}\Gamma\mathbf{S}$, and $S_{ij} = p_i^{1/2}\delta_{ij}$. Using the property that the lattice functions in site i average to zero, the cross-terms between the chemical shift modulation and the usual relaxation terms are discarded in Eq. [54]. If the timescale for chemical exchange is much longer than the correlation time τ_c, $\overline{f_j(t)f_{j'}(t-\tau)F^q_{2j}(t)F^{-q}_{2j'}(t-\tau)}$ in the last term of Eq. [54] can be replaced by $p_j\overline{F^q_{2j}(t)F^{-q}_{2j'}(t-\tau)}\delta_{jj'}$ (*8*); this approximation is well justified for μs-ms timescale chemical exchange in biological macromolecules. Using this assumption,

$$\frac{d}{dt}\boldsymbol{\rho}'(t) = -i\omega_{\text{eff}}[I'_{zi}, \boldsymbol{\rho}'(t)] - \frac{1}{2}\sum_k j_{\text{ex}}(k\omega_{\text{eff}})[\mathbf{B}'_k, [\mathbf{B}'_{-k}, \boldsymbol{\rho}'(t) - \boldsymbol{\rho}'_0]] + \sum_j p_j\mathbf{U}\hat{\boldsymbol{\Gamma}}_j\{\mathbf{U}^{-1}\boldsymbol{\rho}'(t)\mathbf{U} - \boldsymbol{\rho}_0\}\mathbf{U}^{-1} \quad [56]$$

in which $\hat{\boldsymbol{\Gamma}}_j$ is the relaxation superoperator (Eq. [43]) for the spin in the jth site. Following the derivation of Eq. [45], the cross-relaxation rate constant for two operators $\mathbf{B}'_m$ and $\mathbf{B}'_n$ in the tilted frame is given by

$$\Gamma_{mn} = \frac{1}{2}\delta_{mn}\sum_k j_{\text{ex}}(k\omega_e)\langle\mathbf{B}'^{\dagger}_m[\mathbf{B}'_k, [\mathbf{B}'_{-k}, \mathbf{B}'_m]]\rangle/\langle\mathbf{B}'^{\dagger}_m\mathbf{B}'_m\rangle + \sum_j p_j\langle\mathbf{U}^{-1}\mathbf{B}'^{\dagger}_m\mathbf{U}\hat{\boldsymbol{\Gamma}}_j\{\mathbf{U}^{-1}\mathbf{B}'_n\mathbf{U}\}\rangle/\langle\mathbf{B}'^{\dagger}_m\mathbf{B}'_m\rangle \quad [57]$$

The first term in Eq. [57] is the contribution to relaxation of the operator $\mathbf{B}'_m$ because of exchange broadening. As can be seen, chemical exchange does not lead to cross-relaxation between different spin operators. The second term in Eq. [57] is simply the site population weighted average rate constant for cross-relaxation between the operators $\mathbf{B}'_m$ and $\mathbf{B}'_n$ in the absence of exchange in the tilted frame defined by the average resonance offset.

Using Eq. [57], rotating frame relaxation rate constants for I'_z and I'_x operators then are given by, respectively,

$$\begin{aligned}R_{1\rho} &= \frac{1}{2}\sin^2\theta j_{\text{ex}}(\omega_{\text{eff}}) + \cos^2\theta\sum_j p_jR_{1j} + \sin^2\theta\sum_j p_jR_{2j} \\ &= \frac{1}{2}\sin^2\theta j_{\text{ex}}(\omega_{\text{eff}}) + \bar{R}_1\cos^2\theta + \bar{R}_2\sin^2\theta \quad [58]\end{aligned}$$

$$R_{2\rho} = \frac{1}{2}\cos^2\theta j_{ex}(0) + \frac{1}{4}\sin^2\theta j_{ex}(\omega_{eff}) + \sin^2\theta \sum_j p_j R_{1j} + \cos^2\theta \sum_j p_j R_{2j}$$
$$= \frac{1}{2}\cos^2\theta j_{ex}(0) + \frac{1}{4}\sin^2\theta j_{ex}(\omega_{eff}) + \bar{R}_1\sin^2\theta + \bar{R}_2\cos^2\theta \quad [59]$$

in which $\bar{R}_1$ and $\bar{R}_2$ are the population-averaged relaxation rate constants in the absence of chemical exchange. Only the population-averaged quantities θ, $\bar{R}_1$, and $\bar{R}_2$ appear in Eq. [58]; none of the individual tilt angles or relaxation rate constants appear explicitly. This is a consequence of the assumption that chemical exchange is sufficiently fast to allow the use of the perturbation approach of Eq. [27].

Equations [57] and [58] are general; however, Eq. [55] can be solved explicitly only for certain kinetic schemes describing the exchange process. The simplest case is the two-site model in which the spin exchanges between sites *A* and *B* (*8*):

$$A \underset{k_b}{\overset{k_a}{\rightleftharpoons}} B \quad [60]$$

Although simple, this particular kinetic scheme is of rather general relevance as far as chemical exchange is concerned because a number of more complicated processes can be treated by defining pseudo–first-order rate constants (*5*). The relative site populations p_a and p_b satisfy the detailed balance relationship: $p_b k_b = p_a k_a$ and $k_{ex} = k_a + k_b$. For convenience in the remainder of this work, the following quantities are defined here:

$$\Omega_a = \omega_{0a} - \omega_{RF}$$
$$\Omega_b = \omega_{0b} - \omega_{RF}$$
$$\delta = \Omega_b - \Omega_a$$
$$\sigma = p_b\Omega_a + p_a\Omega_b$$
$$\omega_{aeff}^2 = \Omega_a^2 + \omega_1^2$$
$$\omega_{beff}^2 = \Omega_b^2 + \omega_1^2 \quad [61]$$

where ω_{0a} and ω_{0b} are the Larmor frequencies, R_{1a} and R_{1b} are the longitudinal relaxation rate constants, and R_{2a} and R_{2b} are the transverse relaxation rate constants of spins in sites *A* and *B*, respectively. Other quantities are defined in Eq. [50]. The geometric relationship between these quantities is illustrated in Fig. 1. For the two-site model, the result is

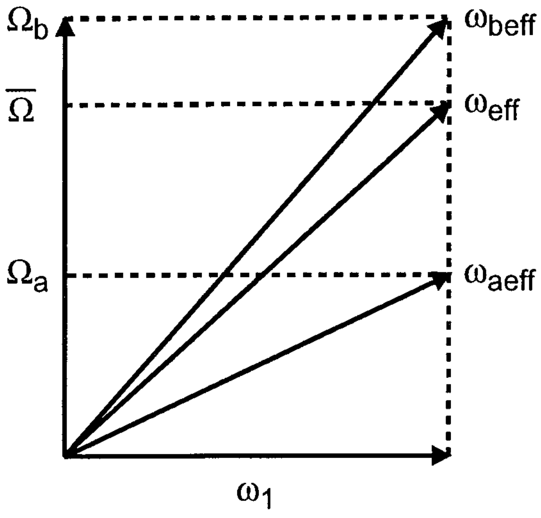

Figure 1 Resonance offsets and effective fields in the rotating frame of reference. Variables are defined in the text.

$$j_{ex}(\omega) = 2p_a p_b \delta^2 k_{ex}/(k_{ex}^2 + \omega^2) \quad [62]$$

which yields (*13*)

$$R_{1\rho} = \bar{R}_1\cos^2\theta + \bar{R}_2\sin^2\theta + \sin^2\theta p_a p_b \delta^2 k_{ex}/(k_{ex}^2 + \omega_{eff}^2) \quad [63]$$

The range of validity of Wennerström's theory is of immediate importance. The timescales involved in the definition of fast exchange include the correlation time τ_c, the characteristic exchange time ($\tau_{ex} = 1/k_{ex}$), and the relaxation time constants, given by the reciprocals of the relaxation rate constants. In the relaxation master equation in Eq. [27] (and see Eq. [40]), the limits of integration are $-\infty$ to ∞ inasmuch as the integrand is zero for times longer than τ_c (*9*). Equation [55] shows that this condition also must hold for τ_{ex}. The perturbative method also imposes the requirement that $t \ll 1/|\hat{\Gamma}|$; therefore, τ_{ex} obeys the inequality: $\tau_{ex} \ll 1/|\Gamma|$. Wennerström originally developed this microscopic approach to laboratory frame R_2 relaxation, for which he found $R_2 = \bar{R}_2 + p_a p_b \delta^2 k_{ex}$. Hence, a perturbative approach, requiring the condition $k_{ex} \gg R_2$ gives $k_{ex}^2 \gg p_a p_b \delta^2$, as obtained from the Bloch-McConnell equation for fast exchange.

USING THE LAPLACE TRANSFORM TO SOLVE THE SLE

The goal of this section is to illustrate a frequency domain approach for solving the SLE (Eq. [25]). The Laplace transform of $\tilde{\boldsymbol{\rho}}(t)$ is defined by

$$\tilde{\boldsymbol{\rho}}[s] = \int_0^\infty \tilde{\boldsymbol{\rho}}(t) e^{-st} dt \qquad [64]$$

The main useful properties of the Laplace transformation in the present context are the transformation of differential and convolution equations into algebraic equations. Thus, the Laplace transform of Eq. [25] gives

$$\tilde{\boldsymbol{\rho}}[s] = \mathbf{K}\tilde{\boldsymbol{\rho}}(t_0) \qquad [65]$$

where $\tilde{\boldsymbol{\rho}}(t_0)$ is the initial value of the marginal average in the time domain and the resolvent $\mathbf{K}$ is given by

$$\mathbf{K} = (s - \boldsymbol{\Lambda} - \boldsymbol{\Xi})^{-1} \qquad [66]$$

The average $\langle\boldsymbol{\rho}\rangle(s)$ can be calculated as

$$\begin{aligned}\langle\boldsymbol{\rho}\rangle(s) &= \langle\mathbf{K}\rangle\boldsymbol{\rho}(0) \\ &= [\langle\psi_0| \otimes \mathbf{1}_{\text{spin}}]\boldsymbol{\rho}(0)\mathbf{K}[|\phi_0\rangle \otimes \mathbf{1}_{\text{spin}}] \qquad [67]\end{aligned}$$

in which $|\phi_0\rangle = (p_1, p_2, \ldots, p_N)^t$ and $\langle\psi_0| = (1, \ldots, 1)$. Given the structure of the SLE, the evolution of $\tilde{\boldsymbol{\rho}}_i(t)$ is described by a set of $N \times M$ coupled differential equations, where N is the number of sites and M is the dimension of the $\tilde{\boldsymbol{\rho}}(t)$ vector (in the superoperator representation). As can be seen from Eqs. [65] and [66], the problem amounts to finding the poles of $\mathbf{K}$ or, equivalently, the eigenvalues of $\boldsymbol{\Lambda} - \boldsymbol{\Xi}$. An equivalent formulation of the problem can be made in terms of matrices of size $M \times M$ only, which reduces the dimensionality of the problem and leads to an interesting way of deriving approximate solutions (*12*). The presentation of the method is beyond the scope of this study, and the reader is referred to Ref. *12* for a complete derivation. Although the solutions of Eqs. [65] and [66] are obtained easily by standard numerical methods, formal analytical solutions can be obtained only in very simple cases. The focus of the remainder of this study is on the solution of the SLE for the simple case of two-site chemical exchange and $R_{1\rho}$ relaxation. The stochastic variable ζ takes two values $\zeta_{a,b}$ and the exchange matrix $\boldsymbol{\Gamma}$ (Eq. [8]) is given by

$$\boldsymbol{\Gamma} = \begin{pmatrix} -k_a & k_b \\ k_a & -k_b \end{pmatrix} \qquad [68]$$

with the equilibrium population distribution

$$|\phi_0\rangle = \begin{pmatrix} p_a \\ p_b \end{pmatrix} = \begin{pmatrix} k_b/k \\ k_a/k \end{pmatrix} \qquad [69]$$

The average resolvent can be written in the form (*12*)

$$\langle\mathbf{K}\rangle = (s - \mathbf{D}_0(s))^{-1} \qquad [70]$$

in which

$$\mathbf{D}_0(s) = \bar{\mathbf{L}} + p_a p_b \boldsymbol{\Delta}[s + k_{\text{ex}} - \mathbf{C}]^{-1}\boldsymbol{\Delta} \qquad [71]$$

where

$$\boldsymbol{\Delta} = \mathbf{L}_b - \mathbf{L}_a \qquad [72]$$

$$\mathbf{L} = p_a\mathbf{L}_a + p_b\mathbf{L}_b \qquad [73]$$

$$\mathbf{C} = p_b\mathbf{L}_a + p_a\mathbf{L}_b \qquad [74]$$

and $\mathbf{L}_{a,b} = \mathbf{L}(\zeta_{a,b})$. The average resolvent in the Laplace domain is obtained by computations involving only $M \times M$ matrices that are one half the size of the matrices involved in the product space. The time domain solution is obtained by applying an inverse Laplace transform to Eqs. [67], [70], and [71] to obtain the following integro-differential equation for the average density operator

$$\frac{d}{dt}\langle\boldsymbol{\rho}\rangle(t) = \bar{\mathbf{L}}\langle\boldsymbol{\rho}\rangle(t) + p_a p_b \boldsymbol{\Delta} \int_0^t e^{-(k_{\text{ex}} - \mathbf{C})(t-t')}\boldsymbol{\Delta}\langle\boldsymbol{\rho}\rangle(t')dt' \qquad [75]$$

In many practical experimental situations, the long-term evolution of $\langle\boldsymbol{\rho}\rangle(t)$ is of primary interest, and the relevant limit is for $s \rightarrow 0$ in the Laplace domain. Thus, for small s

$$\begin{aligned}\mathbf{D}_0^{(1)}(s) &\approx \bar{\mathbf{L}} + p_a p_b \boldsymbol{\Delta}\{\mathbf{1}_{\text{spin}} - s(k_{\text{ex}} - \mathbf{C})^{-1}\} \\ &\quad \times (k_{\text{ex}} - \mathbf{C})^{-1}\boldsymbol{\Delta} \approx \bar{\mathbf{L}} + p_a p_b \boldsymbol{\Delta}(k_{\text{ex}} - \mathbf{C})^{-1}\boldsymbol{\Delta} \\ &\quad - s p_a p_b \boldsymbol{\Delta}(k_{\text{ex}} - \mathbf{C})^{-2}\boldsymbol{\Delta} \qquad [76]\end{aligned}$$

A formal inverse Laplace transform of Eq. [76] gives a simple differential equation for the average of the density operator

$$\frac{d}{dt}\langle \boldsymbol{\rho} \rangle(t) = \mathbf{L}_0^{(1)}\langle \boldsymbol{\rho} \rangle(t) \qquad [77]$$

with

$$\mathbf{L}_0^{(1)} = [\mathbf{1} + p_a p_b \boldsymbol{\Delta}(k_{\text{ex}} - \mathbf{C})^{-2}\boldsymbol{\Delta})]^{-1} \\ [\mathbf{L} + p_a p_b \boldsymbol{\Delta}(k_{\text{ex}} - \mathbf{C})^{-1}\boldsymbol{\Delta}] \qquad [78]$$

Equation [77] represents the long time approximation of the exact evolution of Eq. [75].

A REMARK ON THE DEFINITION OF THE EXCHANGE REGIME

Equation [78] provides a definition of the fast-exchange regime. If

$$\mathbf{1}_{\text{spin}} + p_a p_b \boldsymbol{\Delta}(k_{\text{ex}} - \mathbf{C})^{-2}\boldsymbol{\Delta} \approx \mathbf{1}_{\text{spin}} \qquad [79]$$

then Eq. [78] is equivalent to $\mathbf{D}_0(0)$ and

$$\frac{d}{dt}\langle \boldsymbol{\rho} \rangle(t) = \mathbf{D}_0(0)\langle \boldsymbol{\rho} \rangle(t) \qquad [80]$$

The use of these equations will be illustrated for transverse relaxation in the laboratory frame. Using the raising and lowering basis operators, the matrix $\mathbf{L}_{a,b}$ for spins in sites a and b are given by

$$\mathbf{L}_{a,b} = \begin{pmatrix} -R_{2a,b} + i\Omega_{a,b} & 0 \\ 0 & -R_{2a,b} - i\Omega_{a,b} \end{pmatrix} \qquad [81]$$

The matrices

$$\bar{\mathbf{L}} = \begin{pmatrix} -\bar{R}_2 + i\bar{\Omega} & 0 \\ 0 & -\bar{R}_2 - i\bar{\Omega} \end{pmatrix} \qquad [82]$$

$$\boldsymbol{\Delta} = \begin{pmatrix} R_{2a} - R_{2b} + i\delta & 0 \\ 0 & R_{2a} - R_{2b} - i\delta \end{pmatrix} \qquad [83]$$

$$\mathbf{C} = \begin{pmatrix} -p_b R_{2a} - p_a R_{2b} + i\sigma & 0 \\ 0 & -p_b R_{2a} - p_a R_{2b} - i\sigma \end{pmatrix} \qquad [84]$$

are all diagonal. In the absence of an applied RF field, the resonance offset does not affect relaxation and σ can be set to zero. Furthermore, in the current example, $|\delta| \gg |R_{2a} - R_{2b}|$ and $k \gg R_{2a,b}$ are assumed. Using these conditions,

$$p_a p_b \boldsymbol{\Delta}(k_{\text{ex}} - \mathbf{C})^{-2}\boldsymbol{\Delta} = -p_a p_b \delta^2 k_{\text{ex}}^{-2} \mathbf{1}_{\text{spin}} \qquad [85]$$

and Eq. [79] implies

$$p_a\, p_b \delta^{-2} \ll k_{\text{ex}}^2 \qquad [86]$$

This is the usual fast-exchange condition for transverse relaxation. If Eq. [86] is satisfied, then

$$\mathbf{D}_0(0) = \bar{\mathbf{L}} - p_a\, p_b \delta^2 / k_{\text{ex}} \mathbf{1}_{\text{spin}} \qquad [87]$$

gives the expected result for fast-exchange contribution to relaxation of transverse magnetization (*23*):

$$R_2 = p_a\, R_{2a} + p_b\, R_{2b} + p_a\, p_b\, \delta^2 / k_{\text{ex}} \qquad [88]$$

The definition of the fast exchange regime is based on the "cross"-average of the Hamiltonian governing the evolution of the spin system in both states (see **C**, Eq. [72]). In the case of laboratory frame relaxation, the usual criterion for fast exchange is obtained; however, different definitions of the exchange regime may arise depending on particular circumstances. This point will be shown in the example developed in the following section.

APPLICATION TO EXCHANGE $R_{1\rho}$: EXTENSION OF THE FAST-EXCHANGE LIMIT

In a recent study (*12*), the calculation of the relaxation rate constant in the rotating frame $R_{1\rho}$ for isolated spins was performed using the techniques introduced in the previous section for two-site exchange. The exact eigenvalues of $\mathbf{L}_0^{(1)}$ given by Eq. [78] can be obtained as analytic solutions to a third-order characteristic polynomial; however, the resulting expressions are complicated and approximations are necessary to obtain simple analytic formula. Furthermore, as noted by Trott and Palmer (*11*), the RF field inhomogeneity over the volume of the NMR sample, translated into the eigenvalue inhomogeneity, results in rapid averaging of the oscillatory (i.e., corresponding to nonreal eigenvalues) components to zero. Thus, the problem of finding the dominant relaxation rate reduces to finding the largest (least negative) real eigenvalue λ of Eq. [78].

In a Cartesian operator basis in the rotating frame, the matrix $\mathbf{L}_{a,b}$ for spins in sites a and b are given by

$$\mathbf{L}_{a,b} = \begin{pmatrix} -R_{2a,b} & -\Omega_{a,b} & 0 \\ \Omega_{a,b} & -R_{2a,b} & -\omega_1 \\ 0 & \omega_1 & -R_{1a,b} \end{pmatrix} \qquad [89]$$

For the cases of interest here, numerical simulations indicate that effects of differences between the intrinsic relaxation rate constants in the two sites are negligible for $k \gg |R_{2b} - R_{2a}|$ and $k \gg |R_{1b} - R_{1a}|$; under these conditions, the relaxation rate constants for individual sites can be replaced by the population averaged values. Therefore, $R_{1a} = R_{1b} = \bar{R}_1$ and $R_{2a} = R_{2b} = \bar{R}_2$, are assumed to be identical in sites a and b and the stochastic variable is taken to be $\zeta_{a,b} = \{\delta_{a,b}\}$. Further simplification is obtained by neglecting $\bar{R}_1$ and $\bar{R}_2$ in $\mathbf{C}$, because $k_{ex} \gg \bar{R}_1$, $\bar{R}_2$. With these approximations

$$\boldsymbol{\Delta} = \begin{pmatrix} 0 & \delta & 0 \\ -\delta & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix} \qquad [90]$$

$$\bar{\mathbf{L}} = \begin{pmatrix} -\bar{R}_2 & -\bar{\Omega} & 0 \\ \bar{\Omega} & -\bar{R}_2 & -\omega_1 \\ 0 & \omega_1 & -\bar{R}_1 \end{pmatrix} \qquad [91]$$

$$\mathbf{C} = \begin{pmatrix} 0 & -\sigma & 0 \\ \sigma & 0 & -\omega_1 \\ 0 & \omega_1 & 0 \end{pmatrix} \qquad [92]$$

As discussed previously, for transverse relaxation, Eq. [79] defines the range of validity of the present approach. A reasonable (sufficient, but not necessary) constraint for Eq. [79] to be valid is that the eigenvalues of $\mathbf{1} + p_a p_b \boldsymbol{\Delta}(k_{ex} - \mathbf{C})^{-2}\boldsymbol{\Delta}$ are close to unity, which constrains their sum and product and implies

$$|\mu(a + c)| < 1$$

$$|\mu^2(b^2 + ac)| < 1 \qquad [93]$$

in which the following definitions have been introduced:

$$a = \sigma^2(k_{ex}^2 - \omega_1^2) - (k_{ex}^2 + \omega_1^2)^2$$

$$b = 2\sigma k^3$$

$$c = k_{ex}^2(\sigma^2 - k_{ex}^2 + \omega_1^2)$$

$$T = \sigma^2 + k_{ex}^2 + \omega_1^2$$

$$d = p_a p_b \delta^2$$

$$\mu = \frac{d}{k_{ex}^2 T^2} \qquad [94]$$

When the conditions of Eqs. [79] and [93] are not satisfied, Eq. [77] no longer describes the evolution of the average density operator; however, the value of $R_{1\rho}$ obtained from the largest eigenvalue still may be accurate. Notably, analytical solutions for $R_{1\rho}$ that have been reported previously in the (*10, 13*) correspond to particular cases where one of the parameters (k_{ex}, σ, ω_1, p_a, or δ) alone causes μc to tend to zero.

If $k_{ex} \gg R_{1\rho}$ and $\mu c \ll 1$, the characteristic polynomial for Eq. [78] can be linearized with respect to the eigenvalues λ, $\bar{R}_2$, and $\bar{R}_1$ to yield (*12*)

$$R_{1\rho} = \bar{R}_1\cos^2\theta + \frac{1}{\gamma}\bar{R}_2\sin^2\theta + \frac{1}{\gamma}\,\frac{\sin^2\theta p_a p_b \delta^2 k_{ex}}{\omega_{aeff}^2\omega_{beff}^2/\omega_{eff}^2 + k_{ex}^2 - 2\sin^2\theta p_a p_b \delta^2 + (1-\gamma)\omega_1^2} \qquad [95]$$

where the definitions of the effective fields and tilt angle, given in Eq. [61] are modified to

$$\omega_{eff}^2 = \Delta\Omega^2 + \gamma\omega_1^2$$

$$\omega_{aeff}^2 = \delta_a^2 + \gamma\omega_1^2$$

$$\omega_{beff}^2 = \delta_b^2 + \gamma\omega_1^2$$

$$\theta = \arctan(\sqrt{\gamma}\omega_1/\Delta\Omega) \qquad [96]$$

and $\gamma = 1 + \mu c$. When $\gamma \rightarrow 1$, $R_{1\rho}$ has the value corresponding to the linearized eigenvalue of $\mathbf{D}_0(0)$, i.e.:

$$R_{1\rho} = \bar{R}_1\cos^2\theta + \bar{R}_2\sin^2\theta + \frac{\sin^2\theta p_a p_b \delta^2 k_{ex}}{\omega_{aeff}^2\omega_{beff}^2/\omega_{eff}^2 + k_{ex}^2 - 2\sin^2\theta p_a p_b \delta^2} \qquad [97]$$

with the effective fields and tilt angle defined by Eq. [61] (*12*). The result derived by Trott and Palmer (*11*) by linearizing the eigenvalue problem for the Bloch-McConnell equations directly is given by

$$R_{1\rho} = \bar{R}_1\cos^2\theta + \bar{R}_2\sin^2\theta + \sin^2\theta\,\frac{p_a p_b \delta^2 k_{ex}}{\omega_{aeff}^2\omega_{beff}^2/\omega_{eff}^2 + k_{ex}^2} \qquad [98]$$

in which the effective field and tilt angles are defined by Eq. [61]. Each of these expressions reduces to the fast-exchange result given by Eq. [63] when $k_{ex} \gg \delta$.

The new expressions of Eqs. [95] and [97] differ from Eq. [98] in several respects. First, both new expressions contain an additional term $-2\sin^2\theta p_a p_b \delta^2$ in the denominator. This term vanishes

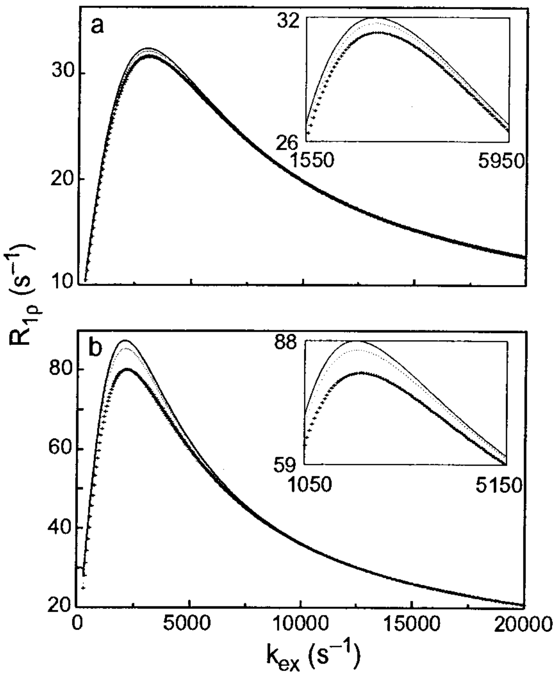

Figure 2 Values of $R_{1\rho}$ obtained using Eq. [98] *(11)* (+++), Eq. [95] (···), and the numerical solution to the 6 × 6 Bloch-McConnell equations (—) for (a) $p_b/p_a = 0.15$ and (b) $p_b/p_a = 0.5$. Calculations used $\delta = 2{,}200$ s^{-1}, $\bar{\Omega} = 1{,}500$ s^{-1}, and $\omega_1 = 1{,}000$ s^{-1}.

for asymmetric populations $p_a \gg p_b$ but provides increased accuracy when the site populations are not highly skewed. Second, the parameter γ appears in the expression for $R_{1\rho}$ in Eq. [95]. Its presence involves a function of k_{ex}, ω_1, and σ in the definition of the local and effective fields ω_{aeff}, ω_{beff}, and ω_{eff}, respectively. Figure 2 compares $R_{1\rho}$ values calculated using Eqs. [95], [97], and [98] as a function of the exchange rate k_{ex} for different populations of the major site. Equations [95] and [97] provide increased accuracy when the site populations are not highly skewed as a result of the term $-2\sin^2\theta p_a p_b \delta^2$ appearing in the denominator of these equations but not in Eq. [98]. This term vanishes for asymmetric populations $p_a \gg p_b$. Equation [95] provides the highest accuracy because of the additional parameter γ that enters the definition of the local and effective fields ω_{aeff}, ω_{beff}, and ω_{eff}, respectively.

The dependence of $R_{1\rho}$ on the carrier frequency ω_{RF} provides a signature for the fast-exchange limit. If exchange is fast, Eq. [63] shows that identical results are obtained for $\bar{\Omega}$ and $-\bar{\Omega}$, obtained by positioning the carrier symmetrically above and below the average Larmor frequency $\bar{\omega}_0$. In contrast, if exchange is not fast and δ is positive (negative), larger (smaller) values of $R_{1\rho}$ are observed when $\omega_{RF} > \bar{\omega}_0$ than when $\omega_{RF} < \bar{\omega}_0$.

RELATIONSHIP BETWEEN THEORETICAL APPROACHES

Now, the relationship between the results of the perturbation and SLE approaches will be considered. The perturbation analysis was performed in the tilted rotating frame, whereas the SLE was solved using the untilted rotating frame. The eigenvalues of Eq. [78] do not depend on the basis chosen; however the Cartesian operator representation (giving rise to the definitions of Eqs. [89]–[92]) can be transformed using $\mathbf{U} = \exp\{i\theta I_y\}$ as before to give an expression for $\mathbf{U}\mathbf{L}_0^{(1)}\mathbf{U}^{-1}$ in the tilted rotating frame. The secular approximation then consists of neglecting the off-diagonal elements of the matrix representation of $\mathbf{U}\mathbf{L}_0^{(1)}\mathbf{U}^{-1}$, in which case $R_{1\rho}$ is given by the (3, 3) matrix element

$$R_{1\rho} = \bar{R}_1\cos^2\theta + \bar{R}_2\sin^2\theta + \frac{\sin^2\theta p_a p_b \delta^2 k_{ex}}{\sigma^2 + \omega_1^2 + k_{ex}^2} \qquad [99]$$

This equation differs from Eqs. [95], [97], and [98]; consequently, the latter three equations contain contributions from nonsecular interactions in the tilted frame.

To illustrate this point, the effective RF fields in the tilted interaction frame (rotating about the I_z' axis with frequency ω_{eff}) are shown in Fig. 3. The effective fields $\omega_{aeff}^{\ddagger}$ and $\omega_{beff}^{\ddagger}$ are not colinear with I_z'. Thus,

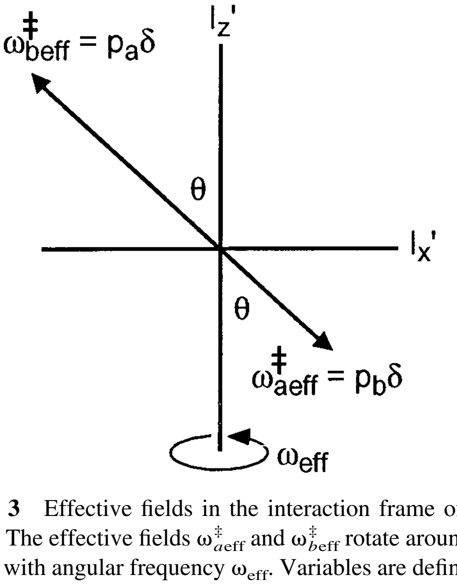

Figure 3 Effective fields in the interaction frame of reference. The effective fields $\omega_{aeff}^{\ddagger}$ and $\omega_{beff}^{\ddagger}$ rotate around the z'-axis with angular frequency ω_{eff}. Variables are defined in the text.

magnetization components that are spin locked along the effective field directions for sites A and B contain projections onto both the I'_x and I'_z operators that give rise to nonsecular contributions to $R_{1\rho}$ in Eqs. [95], [97], and [98]. Conversely, Eq. [99] is accurate under conditions where the effective fields are close to colinear with I'_z or vanish in the interaction frame. Three limiting cases can be established. First, if $\omega_{\text{eff}} \gg \delta$, then precession in the interaction frame averages the transverse components of the effective fields to zero and the effective fields for sites A and B become colinear with I'_z. As a result, the secular approximation is valid and Eqs. [95], [97], [98], and [99] become equivalent to Eq. [63]. Consequently, for sufficiently large ω_{eff}, the fast-exchange limit formula is applicable even to chemical exchange processes outside of the fast-exchange regime. Second, when exchange is fast, $k_{\text{ex}} \gg R_{1\rho}$, which implies $k_{\text{ex}}^2 \gg p_a p_b \delta^2$, the effective fields shown in Fig. 3 are replaced by the population averaged values $|\omega_{a\text{eff}}^{\ddagger}| = |\omega_{b\text{eff}}^{\ddagger}| = p_a p_b \delta$. Therefore, the net population averaged effective field in the interaction frame vanishes. In this fast-exchange limit, Eqs. [95], [97], [98], and [99] once again become equivalent to Eq. [63]. Third, if the populations are highly skewed, $p_a \gg p_b$, then the effective field $\omega_{a\text{eff}}^{\ddagger}$ approaches zero and the secular approximation also is valid for spins in the A sites. In this limit, $\Omega_a \approx \bar{\Omega}$ and Eqs. [95], [97], [98], and [99] become

$$R_{1\rho} = \bar{R}_1\cos^2\theta + \bar{R}_2\sin^2\theta + \frac{\sin^2\theta p_a p_b \delta^2 k_{\text{ex}}}{\omega_{b\text{eff}}^2 + k_{\text{ex}}^2} \qquad [100]$$

which was obtained previously by Trott and Palmer (*11*). This equation also reduces to Eq. [63] when $k_{\text{ex}} \gg \delta$ but also is accurate for a range of parameters outside of the fast-exchange regime (*11*).

EXPERIMENTAL RESULTS

Numerous investigations of $R_{1\rho}$ relaxation in proteins have been reported (reviewed in Ref. *5*). With one exception (*24*), these studies were completed before Eqs. [95], [97], and [98] had been reported in the literature; consequently, the fast-limit expression, (Eq. [63]) was used to fit experimental data. Recently, a ^{15}N off-resonance $R_{1\rho}$ spin relaxation study of a Leu99 to Ala point mutant of T4 lysozyme has been presented that used Eq. [98] to fit the relaxation data (*24*). For this system, $p_b = 0.034$ and Eqs. [95], [97], [98], and [100] give nearly identical predictions. Figure 4 shows the results of fitting Eqs. [63] and [98] to the data for the backbone amide ^{15}N of Gly 110. As shown, in Fig. 4(a), the maximum value of $R_{1\rho}$ is obtained for $\bar{\Omega} \neq 0$; as discussed previously, this is a hallmark of exchange outside of the fast-exchange limit. Figure 4(b) shows the dependence of $R_{1\rho}$ on $\sin^2\theta$. For fast exchange, identical values of $R_{1\rho}$ are predicted for RF frequencies symmetrically placed relative to $\bar{\omega}_0$. In contrast, the results for Gly 110 exhibit larger values of $R_{1\rho}$ for identical values of $\sin^2\theta$ when $\omega_{\text{RF}} > \bar{\omega}_0$ than when $\omega_{\text{RF}} < \bar{\omega}_0$. This result also is characteristic of chemical exchange outside of the fast-exchange limit. The good agreement between the experimental values of $R_{1\rho}$ and the fitted values from Eq. [98], as contrasted with Eq. [63], provides the first experimental confirmation of the new expressions for $R_{1\rho}$.

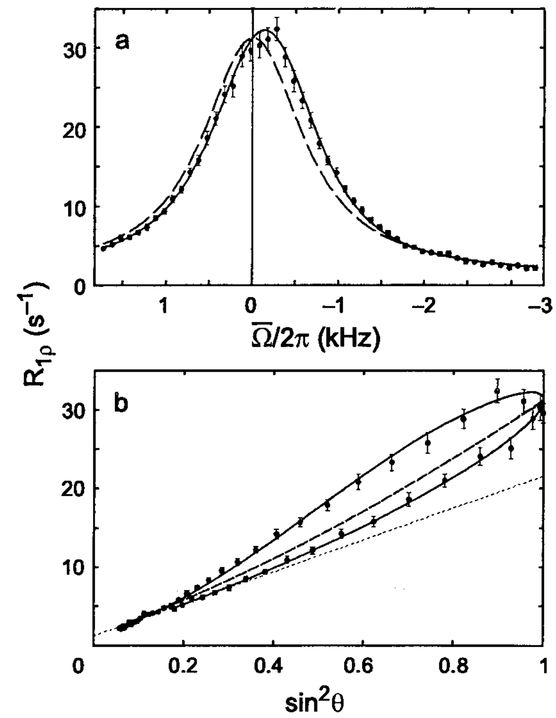

Figure 4 The $R_{1\rho}$ for the backbone ^{15}N of Gly 110 of the Leu99 to Ala mutant of T4 lysozyme as functions of (a) $\bar{\Omega}$ and (b) $\sin^2\theta$. Solid lines are the fits to Eq. [98] and dashed lines are fits to Eq. [63]. The thin dashed line in panel (b) shows the profile expected in the absence of chemical exchange. Data were acquired with $\omega_1 = 5060$ s^{-1} and fitting was performed assuming $k_{\text{ex}} = 1450$ s^{-1} and $p_b = 0.034$, as obtained independently from Carr-Purcell-Meiboom-Gill (CPMG) dispersion measurements (*25*). The optimized value of $\delta = 3020 \pm 50$ s^{-1}. Reproduced from Korzhnev et al., J Biomol NMR (2003) 26:39–48, with permission from Kluwer Academic Publishers.

CONCLUSION

A general treatment of chemical exchange effects in NMR spectroscopy based on the long-time behavior of the average density operator within formalism of the SLE (*12*) has been described. Approximate formulas for the $R_{1\rho}$ relaxation rate constant in the rotating frame of reference derived using this approach have been compared with the fast-exchange limit results obtained using the conventional perturbation approach (*13*). Experimental results for the Leu99 to Ala point mutant of T4 lysozyme have been presented that validate the new expressions for $R_{1\rho}$ (*24*). The new results are more accurate when chemical exchange does not approach the fast-exchange limit and are essential for interpretation of chemical exchange processes in proteins and other biological macromolecules.

ACKNOWLEDGMENTS

We thank L.E. Kay (University of Toronto) for providing a preprint of Ref. *25*. Daniel Abergel acknowledges support from a NATO fellowship. This work was supported by National Institutes of Health (NIH) grant GM59273 awarded to Arthur G. Palmer.

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