

## COMMUNICATIONS

 $R_{1\rho}$  Relaxation outside of the Fast-Exchange Limit

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Chemical-exchange effects in NMR spectroscopy enable kinetic processes to be characterized at equilibrium in solution. Beginning with the Bloch–McConnell equations, new expressions are derived for the spin relaxation rate constant in the rotating frame,  $R_1$ , for chemical exchange between two sites that have distinct magnetic environments and Larmor frequencies. The results are accurate provided that the spin relaxation decay is dominated by a single exponential damping constant and are applicable to a wider range of conditions than existing theoretical descriptions. The new expressions for  $R_1$  will be particularly useful in analyzing experimental data when exchange is not fast and site populations are unequal. © 2002 Elsevier Science

**Key Words:** chemical exchange; asymmetric populations; rotating-frame relaxation.

## 1. INTRODUCTION

Chemical reaction kinetics can be quantified by nuclear magnetic resonance (NMR) spectroscopy using nuclear spin relaxation in the presence of a radiofrequency (rf) field (for reviews see Refs. (1, 2)). The characteristic relaxation rate constant is called  $R_{1\rho}$ , and the experiment itself is referred to as a  $R_{1\rho}$  or  $T_{1\rho}$  ( $T_{1\rho} = 1/R_{1\rho}$ ) rotating-frame relaxation measurement. In recent years,  $R_{1\rho}$  experiments have been applied to elucidate intramolecular conformational changes, ligand binding, and folding of proteins and other biological macromolecules (2). In experimental studies, the dependence of  $R_{1\rho}$  on experimental conditions, such as the amplitude and the 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. The most commonly used theoretical expression for  $R_{1\rho}$  requires that exchange kinetics be fast on the chemical shift time scale, i.e., the chemical-exchange rate constant is much greater than the difference between the Larmor frequencies of the exchanging nuclear spins (3). An expression also has been reported that is applicable to all kinetic regimes provided that one of the sites is much more populated than others, the frequency of the applied rf field coincides with the population

average Larmor frequency, and the longitudinal relaxation rate  $R_1$  equals the transverse relaxation rate  $R_2$  (4). In the present work, new expressions for  $R_{1\rho}$  are derived that generalize these existing results.

## 2. BLOCH–MCCONNELL EQUATION

We consider a chemical reaction or conformational transition that exchanges a nuclear spin between two sites *A* and *B* with distinct magnetic environments,



in which  $k_a$  is the rate constant for the forward reaction and  $k_b$  is the rate constant for the reverse reaction. Although the reaction is depicted in Eq. [1] as first-order, higher-order ligand-binding or oligomerization reactions can be treated by defining pseudo-first-order rate constants (2). The kinetic process is studied by NMR spectroscopy while the system remains in chemical equilibrium.

The resonance offsets in the rotating frame are defined as (6)

$$\delta_a = \Omega_a - \omega_{rf}, \quad [2]$$

$$\delta_b = \Omega_b - \omega_{rf}, \quad [3]$$

in which  $\Omega_a$  and  $\Omega_b$  are the Larmor frequencies of sites *A* and *B*, respectively, and  $\omega_{rf}$  is the frequency of the applied rf field. The exchange rate  $k = k_a + k_b$  is referred to as slow, intermediate, or fast on the chemical shift time scale, if it is much smaller than, comparable to, or much greater than the difference between the two Larmor frequencies. The Larmor frequencies are proportional to the value of the static magnetic field of the NMR spectrometer; therefore, the time scale of the exchange process can depend on the NMR spectrometer utilized.

The time evolution of the magnetization components for the spins in state *A* ( $M_{ax}$ ,  $M_{ay}$ ,  $M_{az}$ ) and in state *B* ( $M_{bx}$ ,  $M_{by}$ ,  $M_{bz}$ ) is described by the Bloch–McConnell equation (5, 6)

$$\frac{d}{dt} \begin{pmatrix} M_{ax} \\ M_{bx} \\ M_{ay} \\ M_{by} \\ M_{az} \\ M_{bz} \end{pmatrix} = \begin{pmatrix} -k_a - R_2 & k_b & -\delta_a & 0 & 0 & 0 \\ k_a & -k_b - R_2 & 0 & -\delta_b & 0 & 0 \\ \delta_a & 0 & -k_a - R_2 & k_b & -\omega_1 & 0 \\ 0 & \delta_b & k_a & -k_b - R_2 & 0 & -\omega_1 \\ 0 & 0 & \omega_1 & 0 & -k_a - R_1 & k_b \\ 0 & 0 & 0 & \omega_1 & k_a & -k_b - R_1 \end{pmatrix} \begin{pmatrix} M_{ax} \\ M_{bx} \\ M_{ay} \\ M_{by} \\ M_{az} \\ M_{bz} \end{pmatrix} + R_1 \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ M_{a0} \\ M_{b0} \end{pmatrix}. \quad [4]$$

$R_1$  and  $R_2$  are the intrinsic longitudinal and transverse relaxation rates, respectively, resulting from processes other than chemical exchange. For the situations considered herein, numerical simulations indicate that the effect of the differences between the intrinsic relaxation rates for species in sites  $A$  and  $B$  on  $R_{1\rho}$  is negligible as long as  $R_1$  and  $R_2$  in Eq. [4] are understood as population average values.  $M_{a0}$  and  $M_{b0}$  are the thermal equilibrium magnetizations in sites  $A$  and  $B$ , respectively, and are proportional to the site populations  $p_a = k_b/k$  and  $p_b = k_a/k$ . The amplitude of the rf field is denoted by  $\omega_1$  and is defined by its Rabi frequency (6). Equation [4] is a first-order linear differential equation with constant coefficients. Its solution has the form

$$\mathbf{M}(t) = \sum_{n=1}^6 e^{\lambda_n t} \mathbf{I}_n + \mathbf{S}, \quad [5]$$

where  $\lambda_n$  is the  $n$ th eigenvalue of the matrix in Eq. [4],  $\mathbf{I}_n$  is proportional to the corresponding eigenvector, and  $\mathbf{S}$  is the stationary solution.

For realistic experimental conditions, numerical simulations establish that four of the six eigenvalues are complex with a relatively large imaginary part, while the remaining two eigenvalues are real and negative. In practice,  $\omega_1$  varies at different points within the macroscopic NMR sample due to instrumental imperfections. The  $\omega_1$  inhomogeneity introduces variability in the values of the eigenvalues and in the orientation of the eigenbasis. While the latter effect is insignificant, eigenvalue inhomogeneity results in rapid averaging of the oscillatory (i.e., corresponding to nonreal eigenvalues) components to zero. In many cases of interest, the two real eigenvalues are very different in magnitude, and, on experimentally accessible time scales, the larger (least negative) real eigenvalue dominates the evolution of the magnetization components, making the relaxation decay essentially monoexponential. Thus, the problem of finding the relaxation rate  $R_{1\rho}$  reduces to finding the largest real eigenvalue  $\lambda$  of the matrix in Eq. [4]:

$$R_{1\rho} = -\lambda. \quad [6]$$

### 3. APPROXIMATE SOLUTIONS

The eigenvalue problem is equivalent to finding the roots of the determinant:

$$\begin{vmatrix} -k_a - \xi & k_b & -\delta_a & 0 & 0 & 0 \\ k_a & -k_b - \xi & 0 & -\delta_b & 0 & 0 \\ \delta_a & 0 & -k_a - \xi & k_b & -\omega_1 & 0 \\ 0 & \delta_b & k_a & -k_b - \xi & 0 & -\omega_1 \\ 0 & 0 & \omega_1 & 0 & -k_a + r - \xi & k_b \\ 0 & 0 & 0 & \omega_1 & k_a & -k_b + r - \xi \end{vmatrix} = 0, \quad [7]$$

where, for convenience, two new variables have been introduced:

$$\xi = \lambda + R_2, \quad [8]$$

$$r = R_2 - R_1. \quad [9]$$

The 106 terms that result from direct expansion of Eq. [7] can be grouped by powers of  $\xi$  and  $r$  into 18 nonzero terms:

$$\sum_{\substack{0 \leq i \leq 6 \\ 0 \leq j \leq 2}} a_{ij} \xi^i r^j = 0. \quad [10]$$

In practice,  $R_1$  and  $R_2$  are of the order of  $10 \text{ s}^{-1}$ , and the relaxation rate during the  $R_{1\rho}$  experiment has to be slower than  $10^3 \text{ s}^{-1}$  to be observed experimentally. Therefore,  $r$  and  $\xi$  are small parameters and, recalling that the relaxation decay is monoexponential, Eq. [10] can be linearized by keeping only those terms with  $i + j \leq 1$ . The solution of the linearized equation for  $\xi$  yields:

$$\xi = \frac{\sin^2 \theta p_a p_b \delta^2 (r - k) + \cos^2 \theta (\omega_1^2 + k^2 + \delta_a^2 \delta_b^2 / \delta_a^2) r}{\omega_{a\text{eff}}^2 \omega_{b\text{eff}}^2 / \omega_{\text{eff}}^2 + k^2}, \quad [11]$$

in which

$$\bar{\Omega} = p_a \Omega_a + p_b \Omega_b, \quad [12]$$

$$\Delta \Omega = \bar{\Omega} - \omega_{rf}, \quad [13]$$

$$\delta = \delta_b - \delta_a = \Omega_b - \Omega_a, \quad [14]$$

$$\omega_{a\text{eff}}^2 = \delta_a^2 + \omega_1^2, \quad [15]$$

$$\omega_{b\text{eff}}^2 = \delta_b^2 + \omega_1^2, \quad [16]$$

$$\omega_{\text{eff}}^2 = \Delta\Omega^2 + \omega_1^2, \quad [17]$$

$$\theta = \arctan(\omega_1/\Delta\Omega). \quad [18]$$

Returning to the original variables in Eqs. [8] and [9] and neglecting  $r$  compared to  $k$ , the linearized expression for the relaxation rate constant is given by

$$R_{1\rho} = R_1 \cos^2 \theta + R_2 \sin^2 \theta + \frac{\sin^2 \theta p_a p_b \delta^2 k}{\omega_{\text{aeff}}^2 \omega_{\text{beff}}^2 / \omega_{\text{eff}}^2 + k^2}. \quad [19]$$

In the fast-exchange limit,  $\omega_{\text{aeff}}^2 \omega_{\text{beff}}^2 / \omega_{\text{eff}}^2 + k^2 \approx \omega_{\text{eff}}^2 + k^2$ . Therefore, [19] agrees with the previously derived expression for the relaxation rate constant in the fast-exchange limit (3):

$$R_{1\rho} = R_1 \cos^2 \theta + R_2 \sin^2 \theta + \frac{\sin^2 \theta p_a p_b \delta^2 k}{\omega_{\text{eff}}^2 + k^2}. \quad [20]$$

Equation [19] generalizes Eq. [20] and is one of the main results of the present work. In the fast-exchange limit,  $R_{1\rho}$  does not depend on  $\delta$ ,  $p_a$ , and  $p_b$  separately, but only on their combination  $p_a p_b \delta^2$ . Therefore  $\delta$ ,  $p_a$ , and  $p_b$  cannot be determined independently of each other. In contrast, outside of the fast-exchange limit, independent determination of  $\delta$ ,  $p_a$ , and  $p_b$  is possible due to the dependence of the denominator in Eq. [19] on these parameters through  $\omega_{\text{aeff}}$  and  $\omega_{\text{beff}}$ .

The linearized expression given by Eq. [19], the fast-limit expression given by Eq. [20], and the exact numerical solution to (Eq. [7]) are compared in Fig. 1. For the indicated conditions, the linearized result agrees very well with the exact result for all values of  $k$ ; in contrast, the fast-limit expression agrees well with the exact result when  $k/\delta > 4$ , but fails for exchange processes that are slower. Numerical simulations for a wider range of conditions indicate that the linearized approximation is accurate except for cases in which site populations are nearly equal

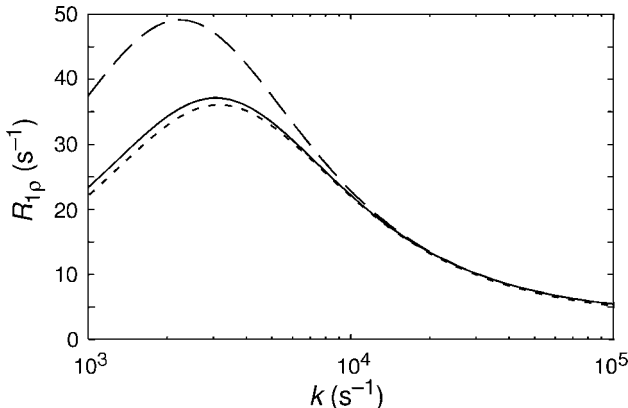


FIG. 1. Exchange rate dependence of  $R_{1\rho}$ . Results are calculated for (—) exact numerical solution, (· · ·) Eq. [19], and (- · -) Eq. [20]. Calculations used  $\omega_1 = 1000 \text{ s}^{-1}$ ,  $\Delta\Omega = 2000 \text{ s}^{-1}$ ,  $p_b/p_a = 0.3$ ,  $\delta = 2400 \text{ s}^{-1}$ ,  $R_1 = 1.5 \text{ s}^{-1}$ , and  $R_2 = 11 \text{ s}^{-1}$ .

and exchange is not in the fast limit. Under these conditions, the assumption that the relaxation decay is dominated by a single damping constant is violated.

In many systems of practical interest, the free energy difference between sites is greater than  $k_B T$ ; consequently, the site populations are unequal because even small differences in energy translate into large population differences through the Boltzman equation (2, 7). Equation [19] can be simplified further if one of the sites is much more populated than the other. In the asymmetric-populations limit,  $p_a \gg p_b$ ; consequently,  $\delta_a \approx \Delta\Omega$  and  $\omega_{\text{eff}} \approx \omega_{\text{aeff}}$ . Using these relations to simplify Eq. [19], the relaxation rate constant in the asymmetric-populations limit becomes

$$R_{1\rho} = R_1 \cos^2 \theta + R_2 \sin^2 \theta + \frac{\sin^2 \theta p_a p_b \delta^2 k}{\omega_{\text{beff}}^2 + k^2}. \quad [21]$$

Equation [21] generalizes the expression previously reported for the special conditions  $R_1 = R_2$  and  $\omega_{rf} = \bar{\Omega}$  (4). The new result allows both  $\omega_1$  and  $\omega_{rf}$  to be varied experimentally.

The effective relaxation rate is defined as the relaxation rate constant in the limit  $\omega_1 \rightarrow \infty$  or  $\Delta\Omega \rightarrow \infty$  and, from Eq. [19], is given by

$$R_{\text{eff}} = R_1 \cos^2 \theta + R_2 \sin^2 \theta. \quad [22]$$

The exchange relaxation rate constant is defined as (8)

$$R_{\text{ex}} \equiv \frac{R_{1\rho} - R_{\text{eff}}}{\sin^2 \theta}. \quad [23]$$

Values of  $R_1$  and  $R_2$  can be determined independently from  $R_{1\rho}$  (2), which allows  $R_{\text{ex}}$  to be defined experimentally. From Eqs. [19], [22] and [23], the linearized expression for  $R_{\text{ex}}$  is given by

$$R_{\text{ex}} = \frac{p_a p_b \delta^2 k}{\omega_{\text{aeff}}^2 \omega_{\text{beff}}^2 / \omega_{\text{eff}}^2 + k^2}. \quad [24]$$

Similarly, from Eqs. [3], [16], [19], [22], and [23], the asymmetric-populations limit expression for  $R_{\text{ex}}$  is

$$R_{\text{ex}} = \frac{p_a p_b \delta^2 k}{(\Omega_b - \omega_{rf})^2 + \omega_1^2 + k^2}. \quad [25]$$

Finally, from Eqs. [13], [17], [20], [22], and [23] the fast-limit expression for  $R_{\text{ex}}$  is:

$$R_{\text{ex}} = \frac{p_a p_b \delta^2 k}{(\bar{\Omega} - \omega_{rf})^2 + \omega_1^2 + k^2}. \quad [26]$$

The asymmetric-populations limit expression given by Eq. [25], the fast-limit expression given by Eq. [26], and the exact numerical solution to Eqs. [6], [7], and [23] are compared

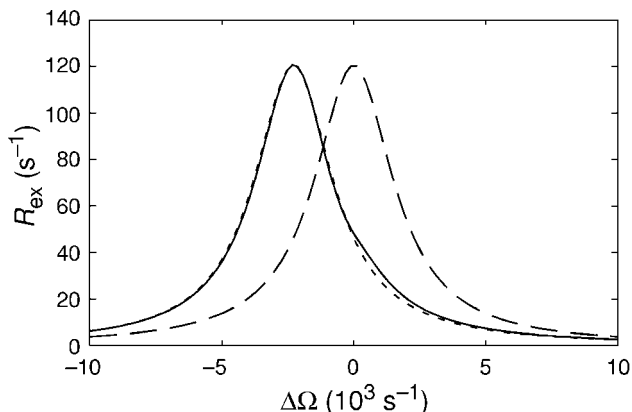


FIG. 2. Offset dependence of  $R_{ex}$ . Results are calculated for (—)  $R_{ex}$  obtained from Eq. [23] using the exact numerical solution, (· · ·) Eq. [25], and (---) Eq. [26]. Calculations used  $\omega_1 = 1000 \text{ s}^{-1}$ ,  $k = 1500 \text{ s}^{-1}$ ,  $p_b/p_a = 0.05$ ,  $\delta = 2400 \text{ s}^{-1}$ ,  $R_1 = 1.5 \text{ s}^{-1}$ , and  $R_2 = 11 \text{ s}^{-1}$ .

in Fig. 2. For the indicated conditions, in which  $k/\delta = 0.6$ , results calculated using Eq. [25] agree very well with the exact numerical results; in contrast, the fast-limit expression yields dramatically different predictions.

As indicated by Eq. [25] and Fig. 2, the maximum value of  $R_{ex}$  occurs when the rf frequency is resonant with the Larmor frequency of the minor site  $B$ . Thus, in the asymmetric-populations limit, varying the resonance offset at a single static magnetic field strength provides an experimental approach to determining  $\Omega_b$  even if  $p_b$  is too low to permit direct observation of the corresponding spectral line (7).

#### 4. CONCLUSION

Chemical-exchange effects in NMR spectroscopy provide powerful approaches for characterizing kinetic processes, including intramolecular conformational changes, ligand binding, and folding of proteins and other biological macromolecules (2).

TABLE 1

Assumptions Inherent to Existing and New Theoretical Expressions

Assumptions	Davis <i>et al.</i> (3)	Meiboom (4)	Linearized solution	Asymmetric- populations limit
$\omega_{rf} = \Omega$	No	Yes	No	No
$R_1 = R_2$	No	Yes	No	No
$k_a + k_b \gg \delta$	Yes	No	No	No
$k_a \ll k_b$	No	Yes	No	Yes

Herein, new expressions have been presented that generalize existing theoretical descriptions for the spin relaxation rate constant in the rotating frame,  $R_{1\rho}$ , for two-site exchange phenomena. The resulting expressions given in Eq. [19], and Eq. [21] are accurate provided that the relaxation decay is dominated by a single exponential damping constant. Table 1 summarizes the range of applicability of the existing and new theoretical results.

The new expressions for  $R_{1\rho}$  are expected to be very valuable for analyzing experimental data when exchange is not fast and site populations are unequal. In particular, in the asymmetric-populations limit, the  $R_{1\rho}$  experiment allows complete characterization of exchange kinetics using data recorded at a single static magnetic field strength. In contrast, to characterize a system outside of the fast-exchange limit, the Carr–Purcell–Meiboom–Gill experiment must be performed at multiple static magnetic field strengths, which requires inconvenient use of different NMR spectrometers subject to systematic variability (9).

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