Beyond the Decoupling Approximation in the Model Free Approach for the Interpretation of NMR Relaxation of Macromolecules in Solution

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Abstract: The model free (MF) approach allows straightforward extraction of generalized order parameters and correlation times for internal and overall bond vector reorientational fluctuations from NMR spin relaxation measurements for macromolecules in solution. The drawback of this approach is the use of a decoupling approximation that neglects correlations between internal and overall molecular motions. These correlations are significant when fluctuation amplitudes are less than the size of the "cage" that restricts the bond vector. In this regime, motion of the bond vector is only indirectly affected by molecule fluctuations through the orienting potential for the local director axis. By separating the regimes of large and small amplitude molecular fluctuations, an expression for the spectral density function is derived that does not invoke the decoupling approximation, but that relies on the same macroscopic level of description as the original MF approach. The parameters of the spectral density function provide insight into the effects on the MF parameters that result from violation of the decoupling approximation.

The Lipari−Szabo (LS) model free (MF) approach[1,2] provides a simple description of the correlation function $C(t)$ for stochastic fluctuations that modulate nuclear magnetic spin interactions for molecules in solution. The spectral density function $J(\omega)$ given by the cosine Fourier transformation of $C(t)$ provides a link between molecular motions and NMR spin relaxation arising from, for example, dipole−dipole, chemical shift anisotropy, and quadrupole interactions.[3] For dipole−dipole relaxation of two nuclear spins, $C(t)$ describes fluctuations in the orientation of the internuclear vector in the laboratory reference frame. The LS MF approach has been utilized extensively to extract from NMR experiments such characteristics as generalized order parameters and correlation times for internal and overall molecular motions for directly bonded dipole-coupled spin pairs.[4−9]

As originally formulated, the LS MF approach assumes that, when the internal bond motion and overall molecular motion are independent, the correlation function $C(t)$ can be factorized as

$$C(t) = C_o(t)C_i(t) \qquad (1)$$

where $C_o(t)$ is the autocorrelation function for overall molecular motion characterized by the correlation time τ_m, and $C_i(t)$ is the autocorrelation function for internal bond motion at a given molecule orientation characterized by the correlation time τ_e. Equation 1 assumes that internal bond motion in the molecular reference frame is independent of overall rotational tumbling in the laboratory reference frame. The validity of this assumption has already attracted attention in the past.[10,11]

Despite its simplicity, eq 1 reproduces correctly the model-independent behavior of the correlation function in two limits: (a) $\tau_e/\tau_m \gg 1$, when the bond vector could be considered as being fixed in the molecular reference frame, and (b) $\tau_e/\tau_m \ll 1$, which is an adiabatic limit, characterized by the average value of the bond vector orientation in the molecular reference frame.

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In the former limit, $C_i(t) = 1$, and in the latter limit, $C_i(t) = S^2$, where S is the generalized order parameter introduced by LS. Equation 1 also is valid for arbitrary ratios τ_e/τ_m if the local geometry of the bond environment is preserved at any moment of time, that is, if no retardation occurs between the local structure and the overall molecule motion.

Random distortion of the bond local geometry caused by overall molecular motion provides another mechanism for bond vector dynamics, in addition to the conventional LS model. This mechanism is subject to the following conditions: (a) the bond vector undergoes restricted rotational diffusion or jumpwise motion[1,9,11] in the orienting potential of a local director axis, (b) the orienting potential fluctuates due to overall molecular tumbling, and (c) the mean angular displacement of the molecule for the "dephasing" time τ_e is less than the size of the "cage" that restricts the local bond motion. Under these circumstances, the angle between the bond vector and the director axis will be modulated by molecular reorientation, and the criteria necessary for application of the decoupling approximation are violated.

The paper derives correlation and spectral density functions for bond vector reorientations using a macroscopic level of description similar to MF, and with the same set of the observable parameters, but without invoking the decoupling approximation. The correlation and spectral density functions have the same functional form as is given by the LS approach; however, the interpretations of the generalized order parameters and correlation times are different when neither of the conditions $\tau_e/\tau_m \gg 1$ or $\tau_e/\tau_m \ll 1$ are satisfied. In agreement with microscopic models, such as the slowly relaxing local structure (SRLS) formalism,[11] the present treatment indicates that MF overestimates generalized order parameters when internal motions are slow enough to violate the assumption of time scale separation.

Theory

A general expression for an autocorrelation function $C(t)$ that describes bond orientational fluctuations for molecules in isotropic solution is written in the form[1]

$$C(t) = \frac{1}{5}\langle P_2(\mu(t) \cdot \mu(0)) \rangle \qquad (2)$$

where $\mu(t)$ is the unit radius vector along the direction of the bond, $\langle ... \rangle$ indicates the ensemble average over the distribution of bond orientations, and $P_2(x) = (3x^2 - 1)/2$. Using the addition theorem for the spherical harmonics, eq 2 can be expressed as

$$C(t) = \frac{4\pi}{25} \sum_{m=-2}^{2} \langle Y_2^m[\Omega(t)]Y_2^{m*}[\Omega(0)]\rangle \qquad (3)$$

where $\Omega(t)$ and $\Omega(0)$ are the solid angles with respect to the laboratory reference frame, and $Y_2^m[\Omega(t)]$ are the spherical harmonic functions.

The calculation of the time evolution of the correlation functions $\langle Y_2^m[\Omega(t)]Y_2^{m*}[\Omega(0)]\rangle$ can be reduced to the calculation of the time evolution of the average values $Y_2^m[\Omega(t)]$. Indeed, according to the definition of the correlation function

$$\langle Y_2^m[\Omega(t)]Y_2^{m*}[\Omega(0)]\rangle =$$
$$\int d\Omega \int d\Omega_0 G(\Omega_0,0)G(\Omega,t|\Omega_0,0)Y_2^m(\Omega)Y_2^{m*}(\Omega_0) \quad (4)$$

in which $G(\Omega,t|\Omega_0,0)$ is the conditional probability density that the solid angle is equal to Ω at time t given that $\Omega = \Omega_0$ at $t = 0$, and $G(\Omega_0,0)$ is the stationary probability density that $\Omega = \Omega_0$ at $t = 0$. Thus, the correlation function in eq 4 can be expressed as

$$\langle Y_2^m[\Omega(t)]Y_2^{m*}[\Omega(0)]\rangle = \langle\langle Y_2^m[\Omega(t)]\rangle_{\Omega(0)}Y_2^{m*}[\Omega(0)]\rangle \quad (5)$$

where $\langle Y_2^m[\Omega(t)]\rangle_{\Omega(0)}$ is the average value of $Y_2^m[\Omega(t)]$ under the initial conditions $\langle Y_2^m[\Omega(t=0)]\rangle = Y_2^m[\Omega(0)]$.

As an initial step, the correlation function $C(t)$ is calculated by neglecting overall molecular motion and assuming a simple relaxation law for the components $\langle Y_2^m[\Omega(t)]\rangle$:

$$\frac{d}{dt}\langle Y_2^m[\Omega(t)]\rangle = -\frac{1}{\tau_e}(\langle Y_2^m[\Omega(t)]\rangle - \langle Y_2^m\rangle^0) \qquad (6)$$

with a single correlation time τ_e. $\langle Y_2^m\rangle^0 = \langle Y_2^m\rangle^0_{\Omega_{LM}}$ is the thermal equilibrium average value of $Y_2^m[\Omega(t)]$ at fixed molecule orientation, where Ω_{LM} represents the Euler angles between the local molecule axis and the laboratory reference frame. Combining eqs 3, 5, and 6 using the initial conditions specified above yields a differential equation for $C(t)$:

$$\frac{dC(t)}{dt} = -\frac{1}{\tau_e}\left[C(t) - \frac{1}{5}S^2\right] \qquad (7)$$

Equation 7 has been obtained using the relations

$$(4\pi/5) \sum_{m=-2}^{2} |Y_2^m[\Omega(t)]|^2 = 1$$

$$(4\pi/5) \sum_{m=-2}^{2} |\langle Y_2^m\rangle^0|^2 = S^2 \qquad (8)$$

where S^2 is the generalized order parameter and is independent of the choice of the molecular orientation. Integration of eq 8 gives

$$C(t) = \frac{1}{5}(e^{-t/\tau_e} + S^2(1 - e^{-t/\tau_e})) \qquad (9)$$

Equation 9 is a limiting case of the total correlation function including overall molecular motion in the limit $\tau_m \rightarrow \infty$. It coincides with the LS expression in the same limit. Because eq 9 is the solution of eq 7, the present approach and the LS MF formalism are, in the limit $\tau_m \rightarrow \infty$, based on the same model of bond vector fluctuations, which is distinct from the diffusion model for bond vector motion employed in SRLS formalism.[11]

To take into account the overall molecular motion, the components $\langle Y_2^m\rangle^0$ in eq 6 are replaced by the time-dependent "quasiequilibrium" values $\langle Y_2^m(t)\rangle^{eq}$, whose time dependence arises from the overall molecular reorientation. Thus, eq 6 is recast as

$$\frac{d}{dt}\langle Y_2^m[\Omega(t)]\rangle = -\frac{1}{\tau_e}(\langle Y_2^m[\Omega(t)]\rangle - \langle Y_2^m(t)\rangle^{eq}) \qquad (10)$$

The value of $\langle Y_2^m(t)\rangle^{eq}$ has a clear physical meaning according to its definition:

$$\langle Y_2^m(t)\rangle^{\text{eq}} = \int d\Omega_{\text{LM}} P(\Omega_{\text{LM}}, t) \langle Y_2^m[\Omega(t)]\rangle_{\Omega_{\text{LM}}}^0 \qquad (11)$$

where $P(\Omega, t)$ is the time-dependent probability density for a given molecular orientation in the laboratory reference frame and is a solution of a rotational diffusion equation; that is, eq 10 neglects the effect of the bond vector on the overall motion. $\langle Y_2^m(t)\rangle^{\text{eq}} \rightarrow 0$ at $t \rightarrow \infty$ because all orientations of the molecule in isotropic solution have equal probability at equilibrium. The time dependence of $\langle Y_2^m(t)\rangle^{\text{eq}}$ for isotropic molecular rotational diffusion is described phenomenologically by

$$\langle Y_2^m(t)\rangle^{\text{eq}} = \langle Y_2^m\rangle^0 e^{-t/\tau_m} \qquad (12)$$

The approach based on equations similar to eq 10 has proven to be successful in describing kinetic phenomena in various systems with orientational ordering (see, e.g., refs 12,13). Combining eqs 3, 5, and 10 using the initial conditions specified above results in a differential equation for $C(t)$:

$$\frac{dC(t)}{dt} = -\frac{1}{\tau_e}\left[C(t) - \frac{1}{5}S^2 e^{-t/\tau_m}\right] \qquad (13)$$

whose solution is

$$C(t) = \frac{1}{5}\left(e^{-t/\tau_e} + S^2\frac{\tau_m}{\tau_m - \tau_e}(e^{-t/\tau_m} - e^{-t/\tau_e})\right)$$
$$= \frac{1}{5}e^{-t/\tau_m}(e^{-t/\tilde{\tau}_e} + \tilde{S}^2(1 - e^{-t/\tilde{\tau}_e})) \qquad (14)$$

where

$$\tilde{S}^2 = \frac{\tau_m}{\tau_m - \tau_e}S^2 \qquad (15)$$

$$\tilde{\tau}_e = \frac{\tau_m}{\tau_m - \tau_e}\tau_e \qquad (16)$$

In contrast to the correlation function given by eq 14, the LS MF correlation function is

$$C^{\text{LS}}(t) = \frac{1}{5}e^{-t/\tau_m}(e^{-t/\tau_e} + S^2(1 - e^{-t/\tau_e})) \qquad (17)$$

and is a solution of the equation

$$\frac{dC^{\text{LS}}(t)}{dt} = -\frac{1}{\tau_m}C^{\text{LS}}(t) - \frac{1}{\tau_e}\left[C^{\text{LS}}(t) - \frac{1}{5}S^2 e^{-t/\tau_m}\right] \qquad (18)$$

The additional term $-(1/\tau_m)C^{\text{LS}}(t)$ in eq 18 describes synchronous variations in the average orientation of the bond vector and the molecular orientation induced through the geometrical constraints imposed by a rigid bond environment. As a consequence, dynamics of the bond vector in the molecular reference frame do not depend on overall tumbling, contrary to the dynamics described by eq 14.

In the above discussion, the LS mechanism of bond fluctuations was compared to the proposed mechanism, which takes

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into account distortions of the local bond environment accompanied by the effect of the director orienting potential. To take into account the coexistence of both mechanisms, which is most likely to occur in actual molecules, the ad hoc assumption can be made, by comparing eqs 13 and 18, that the correlation function in the intermediate regime is governed by the equation:

$$\frac{dC(t)}{dt} = -\frac{1}{\tau_m'}C(t) - \frac{1}{\tau_e}\left[C(t) - \frac{1}{5}S^2 e^{-t/\tau_m}\right] \qquad (19)$$

with $\tau_m' \geq \tau_m$. Equation 19 reduces to eq 13 for $\tau_m' \rightarrow \infty$ and to eq 18 for $\tau_m' = \tau_m$. The meaning of eq 19 in the intermediate regime follows from its solution

$$C(t) = \frac{1}{5}e^{-t/\tau_m'}\left(e^{-t/\tau_e} + \frac{\tau_m''}{\tau_m'' - \tau_e}S^2(e^{-t/\tau_m''} - e^{-t/\tau_e})\right)$$
$$= \frac{1}{5}e^{-t/\tau_m}(e^{-t/\tilde{\tau}_e} + \tilde{S}^2(1 - e^{-t/\tilde{\tau}_e})) \qquad (20)$$

where

$$\frac{1}{\tau_m} = \frac{1}{\tau_m'} + \frac{1}{\tau_m''} \qquad (21)$$

$$\tilde{S}^2 = \frac{\tau_m''}{\tau_m'' - \tau_e}S^2 \qquad (22)$$

$$\tilde{\tau}_e = \frac{\tau_m''}{\tau_m'' - \tau_e}\tau_e \qquad (23)$$

Although eqs 14, 17, and 20 have the form of a product between the overall rotational correlation function, $C_o(t) = \exp(-t/\tau_m)/5$, and a second correlation function, given in large brackets, eqs 14 and 20, unlike eq 17, do not incorporate the decoupling approximation of eq 1, because the parameter $\tilde{\tau}_e$ depends on both τ_e and τ_m.

As shown by eqs 20 and 21, eq 19 assumes that the correlation frequency $1/\tau_m$ due to overall molecular rotational diffusion can be decomposed into two independent contributions $1/\tau_m'$ and $1/\tau_m''$. The latter frequency is associated with small amplitude molecular fluctuations such that the local axis displacement for time $t \approx \tau_e < \tau_m''$ is less than the size of the cage restricting the bond vector motion. The rigid bond limit $\tau_e \rightarrow \infty$ thus corresponds to $\tau_m'' \rightarrow \infty$ and therefore to $\tau_m' \rightarrow \tau_m$. In this context, τ_e is the analogue of a dephasing time that prevents the accommodation of large displacements. In the regime of small fluctuations, the bond vector dynamics are affected by the orienting potential for the director axis. In contrast, the frequency $1/\tau_m'$ is associated with large amplitude molecule fluctuations for which the bond vector orientation is strongly affected by displacements of the cage environment. As shown by the second line of eq 20, the correlation function has the same functional form as $C^{\text{LS}}(t)$ in eq 17, but the effective order parameters and correlation times are scaled according to eqs 22 and 23. Examples of the correlation functions calculated using eqs 14, 17, and 20 are shown in Figure 1.

Fourier transformation leads to the following forms of the spectral density functions:
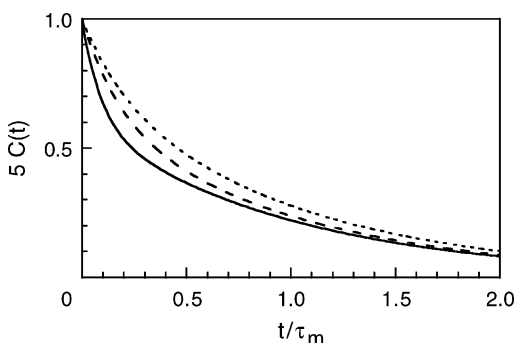
Figure 1. Plots are shown for (- - -) the correlation function of eq 14, (—) the LS correlation function of eq 17, and (— — —) the correlation function of eq 20. Curves were calculated for $\tau_e = 0.2\ \tau_m$, $\tau_m' = 1.5\ \tau_m$, and $S^2 = 0.6$.

$$J^{LS}(\omega) = \frac{2}{5}\left(\frac{(1-S^2)\tau}{1+\omega^2\tau^2} + \frac{S^2\tau_m}{1+\omega^2\tau_m^2}\right) \qquad (24)$$

with $1/\tau = 1/\tau_e + 1/\tau_m$ for the LS correlation function of eq 17, and

$$J(\omega) = \frac{2}{5}\left(\frac{(1-\tilde{S}^2)\tilde{\tau}}{1+\omega^2\tilde{\tau}^2} + \frac{\tilde{S}^2\tau_m}{1+\omega^2\tau_m^2}\right) \qquad (25)$$

with $1/\tilde{\tau} = 1/\tilde{\tau}_e + 1/\tau_m$ for eq 20. These two spectral density functions have the same functional form and would yield equivalent fits to experimental data; however, as indicated by eqs 22 and 23, the interpretations of the generalized order parameters and correlation times are different.
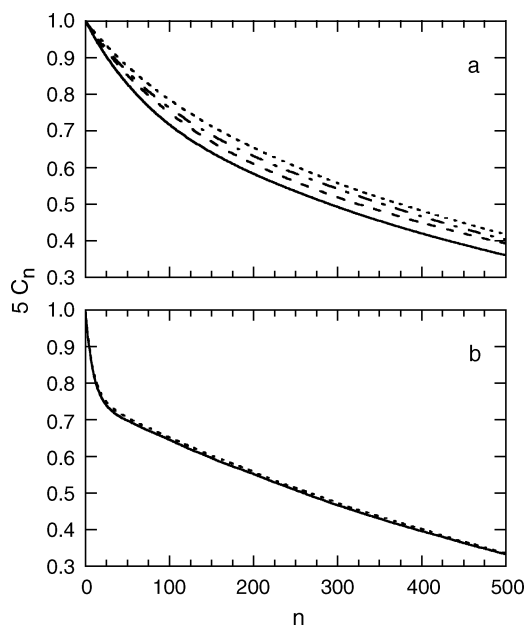
Simulations

The predictions of the theoretical model were supported qualitatively by Monte Carlo simulations. Overall tumbling of the director axis was treated as a random walk on the surface of a sphere with an angular step size $\Delta\theta_D = 0.01$ and a random step direction drawn from a uniform distribution on $[0, 2\pi]$. Internal motion of the bond vector was modeled using an axially symmetric potential $U(\theta, \phi)/kT = U_0 [1 - P_2(\cos\theta)]$ in which $U_0 = 8kT$, and k is the Boltzmann constant. Random steps of the bond vector had an angular step size $\Delta\theta_M = 0.01$ and a random step direction drawn from a uniform distribution on $[0, 2\pi]$. The Metropolis algorithm was used to accept or reject internal steps of the bond vector at a given orientation of the director axis. The relative scaling of the effective decay constant for overall and internal motions was adjusted by varying the number of internal steps, p, taken for each overall step. Coordinates of the bond vector were recorded after every $m = 10$ overall steps. A total of $N = 5 \times 10^5$ coordinates μ_i were used to calculate the correlation function according to the expression:

$$C_n = \frac{1}{5(N-n)}\sum_{i=1}^{N-n} P_2(\mu_i \cdot \mu_{i+n}) \qquad (26)$$

for $0 \le n \le 500$.

Four different protocols were used to simulate the effects of the decoupling approximation. In protocol 1, in addition to the internal jumps driven by the Metropolis algorithm, the bond vector was rotated in a manner identical to that of the director

Figure 2. Correlation functions simulated with $S^2 = 0.75$ and (a) $\tau_e/\tau_m = 0.12$ or (b) $\tau_e/\tau_m = 0.012$, as determined from independent simulations of correlation functions for overall and internal motions. Simulation protocols are described in the text. (—) Data simulated for protocol 1, yielding fitted apparent values of (a) $S^2 = 0.76$, $\tau_e/\tau_m = 0.12$ and (b) $S^2 = 0.75$, $\tau_e/\tau_m = 0.014$. (- - -) Data simulated for protocol 2, yielding fitted apparent values of (a) $S^2 = 0.87$, $\tau_e/\tau_m = 0.12$ and (b) $S^2 = 0.76$, $\tau_e/\tau_m = 0.014$. (— — —) Data simulated for protocol 3, yielding fitted apparent values of (a) $S^2 = 0.80$, $\tau_e/\tau_m = 0.13$; for clarity, data are not shown for (b). (— — —) Data simulated for protocol 4, yielding fitted apparent values of (a) $S^2 = 0.83$, $\tau_e/\tau_m = 0.12$; for clarity, data are not shown for (b).

axis at each overall step; thus, the relative orientation of the bond vector and the director axis was unchanged during an overall step. In protocol 2, the orientation of the bond vector was not changed during an overall step. In protocol 3, the bond vector was rotated along with the director axis for one-half of the overall steps and left unchanged for the other half; the decision whether to rotate the bond vector at a given overall step was made randomly. In protocol 4, the bond vector was rotated in the same direction as the overall step, but the step size was reduced by a factor drawn from the uniform distribution on $[0, 1]$. Correlation functions were simulated for overall rotational diffusion alone by using the coordinates of the director axis. Correlation functions were simulated for internal motions in the absence of overall diffusion by setting the overall step size to zero.

Figure 2 shows simulated correlation functions for the above four protocols. Independent simulations of overall rotational diffusion gave $\tau_m = 660$; in this approach, $\tau_m = 2/(3m\Delta\theta_D^2)$. Independent simulations of internal bond vector motion in the absence of overall tumbling gave $S^2 = 0.75$ and either $\tau_e/\tau_m = 0.12$ or $\tau_e/\tau_m = 0.014$ for 2 or 20 internal steps per overall step, respectively; in this approach, $\tau_e/\tau_m = (1 - S^2)(\Delta\theta_D^2/p\Delta\theta_M^2)$. In agreement with the similar functional forms of eqs 17 and 20, all simulated correlation functions for protocols 1–4 were accurately fit by the LS MF correlation function of eq 17; fitted parameters are given in the figure caption. Protocol 1 embodies the assumptions of the LS MF approach. Accordingly, fitted parameters were identical to those obtained from independent simulations of overall and internal motion. In agreement with eq 1, the simulated correlation function was indistinguishable

from the correlation function obtained as the product of the correlation functions for separate independent simulations of overall and internal motions. Protocol 2 embodies the largest retardation or "cage" effects because the instantaneous orientation of the bond vector is not affected by an overall jump of the director axis. In agreement with the theoretical results given by eqs 14 and 15, the apparent value of S^2 (corresponding to $\tilde{S}^2$ in eq 22) is overestimated by ~10% when $\tau_e/\tau_m = 0.12$, but the apparent value of S^2 approaches the expected value when $\tau_e/\tau_m = 0.014$. Models 3 and 4 embody partial retardation effects. Accordingly, the degree by which the apparent value of S^2 is overestimated is reduced as compared to protocol 2, as expected from eqs 20 and 22. For the parameter ranges examined, the simulated correlation functions are very weakly dependent on τ_e, and nearly identical fits are obtained if τ_e is fixed at the value expected for independent internal motion or at the value given by eq 23.

Discussion and Conclusion

If the decoupling approximation is violated because distortion of the bond vector local geometry results from overall molecular tumbling, then the theoretical analysis of bond vector orientational fluctuations presented herein demonstrates that values of the generalized order parameter obtained using the LS MF formalism will be overestimated unless $\tau_e/\tau_m \ll 1$. This theoretical result is confirmed qualitatively by simulations of correlation functions for simple motional models exhibiting "cage" effects. The microscopic SRLS treatment also demonstrates that violation of the decoupling approximation results in overestimation of the generalized order parameter.[11] Furthermore, in both approaches, the degree of overestimation of the generalized order parameter depends on the time scale separation between internal and overall motion. Concurrence between these two methods suggests that these conclusions are robust. Therefore, the present treatment allows simple estimation of the size of the errors in the generalized order parameter that result from violation of the decoupling approximation.

The spectral density functions presented herein are based on a macroscopic model distinct from the diffusion model employed by SRLS formalism. Consequently, while the two approaches yield similar qualitative conclusions about the effects of violation of the decoupling approximation on the generalized order parameter, identical quantitative predictions are not to be expected. In addition, the macroscopic approach also suggests that violation of the decoupling approximation leads to overestimation of the internal correlation time τ_e, in contrast to the underestimation predicted by the SRLS formalism.[11]

As shown by eq 22 and in Figure 3, the correction factor for S^2, as compared to the LS MF estimate, is more significant for smaller molecules because the ratio τ_e/τ_m is larger. Overestimation of S^2 may be important in the studies of the temperature dependence of S,[14,15] because the ratio τ_e/τ_m is itself temperature dependent (it decreases as the temperature decreases). Consequently, violation of the decoupling approximation potentially introduces an artificial temperature dependence to S.

The MF formalism has been applied extensively to characterize conformational fluctuations in proteins.[6–8] The internal motions of interest for a bond vector in a globular protein result,
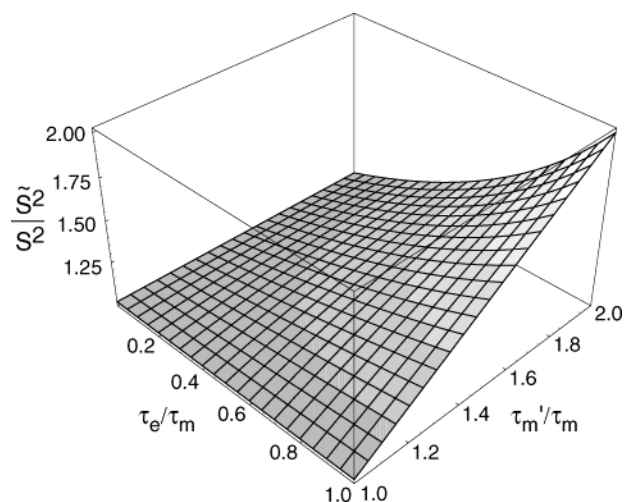
Figure 3. Plot of $\tilde{S}^2/S^2$ calculated using eq 22 for $0 \leq \tau_e \leq \tau_m$ and $\tau_m \leq \tau_m{}' \leq 2\,\tau_m$.

to a first approximation, from the interplay between relatively soft potentials, such as dihedral angle fluctuations, and relatively stiff potentials, such as repulsive van der Waals interactions. The effects of very high-frequency covalent bond stretches can be incorporated into an effective coupling constant for the stochastic process.[16] In the hydrophobic core of a protein, atoms are nearly closely packed, and the van der Waals interactions would be expected to result in very limited "cage" effects and relatively small deviations from the LS MF results. In contrast, surface-exposed loops and amino acid side chains are not necessarily closely packed with other protein atoms, and "cage" effects leading to violations of the decoupling approximation would be expected to be more significant. In agreement with this simple view, the largest differences between the LS MF and SRLS analyses of experimental NMR data for the backbone $^{15}N-^{1}H$ bond vectors in the protein ribonuclease H are observed for amino acid residues in loops with values of $\tau_e > 1$ ns, as compared to $\tau_m = 9.28$ ns.[11]

In conclusion, on the basis of the principles of nonequilibrium statistical mechanics, a simple phenomenological treatment of internal and overall orientational fluctuations for macromolecules in solution has been proposed that avoids invoking the decoupling approximation. The resulting spectral density function has the same form and contains the same set of macroscopic parameters as is present in the original LS approach; however, additional contributions to the generalized order parameters and internal correlation times arise from the effect of asynchronous fluctuations of the bond vector and the local director axis.

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