The physical basis of model-free analysis of NMR relaxation data from proteins and complex fluids

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NMR relaxation experiments have provided a wealth of information about molecular motions in macromolecules and ordered fluids. Even though a rigorous theory of spin relaxation is available, the complexity of the investigated systems often makes the interpretation of limited datasets challenging and ambiguous. To allow physically meaningful information to be extracted from the data without commitment to detailed dynamical models, several versions of a model-free (MF) approach to data analysis have been developed. During the past 2 decades, the MF approach has been used in the vast majority of all NMR relaxation studies of internal motions in proteins and other macromolecules, and it has also played an important role in studies of colloidal systems. Although the MF approach has been almost universally adopted, substantial disagreement remains about its physical foundations and range of validity. It is our aim here to clarify these issues. To this end, we first present rigorous derivations of the three well-known MF formulas for the time correlation function relevant for isotropic solutions. These derivations are more general than the original ones, thereby substantially extending the range of validity of the MF approach. We point out several common misconceptions and explain the physical significance of the approximations involved. In particular, we discuss symmetry requirements and the dynamical decoupling approximation that plays a key role in the MF approach. We also derive a new MF formula, applicable to anisotropic fluids and solids, including microcrystalline protein samples. The so-called slowly relaxing local structure (SRLS) model has been advanced as an alternative to the MF approach that does not require dynamical decoupling of internal and global motions. To resolve the existing controversy about the relative merits of the SRLS model and the MF approach, we formulate and solve a planar version of the SRLS model. The analytical solution of this model reveals the unphysical consequences of the symmetrical two-body Smoluchowski equation as applied to protein dynamics, thus refuting the widely held belief that the SRLS model is more accurate than the MF approach. The different results obtained by analyzing data with these two approaches therefore do not indicate the importance of dynamical coupling between internal and global motions. Finally, we explore the two principal mechanisms of dynamical coupling in proteins: torque-mediated and friction-mediated coupling. We argue by way of specific analytically solvable models that torque-mediated coupling (which the SRLS model attempts to capture) is unimportant because the relatively slow internal motions that might couple to the global motion tend to be intermittent (jump-like) in character, whereas friction-mediated coupling (which neither the SRLS model nor the MF approach incorporates) may be important for proteins with unstructured parts or flexibly connected domains. © 2009 American Institute of Physics. [doi:10.1063/1.3269991]

I. INTRODUCTION

Over the past several decades, NMR relaxation has played a major role in unraveling the molecular-level dynamical properties of a wide range of biological and colloidal systems. In the motional narrowing regime, where the conventional perturbation theory of spin relaxation applies,1 the accessible information about molecular dynamics is contained in the spectral density function, \( J(\omega) \), the one-sided Fourier transform of the time correlation function (TCF), \( C(t) \). The multiscale structure and dynamics of complex systems make the interpretation of \( J(\omega) \) challenging unless it has been characterized exhaustively. In favorable cases, the frequency dependence (or dispersion) of \( J(\omega) \) can be mapped over five orders of magnitude by varying the NMR frequency.2 The extensive datasets provided by such magnetic relaxation dispersion experiments make it possible to discriminate among specific dynamical models of high complexity. On the other hand, in \(^2\text{H}, \ ^{13}\text{C}, \) or \(^{15}\text{N} \) relaxation studies of conformational dynamics in proteins and other biomolecules, information about \( J(\omega) \) is usually limited to a few frequencies. To physically interpret such data, the spectral density function, or the associated TCF, must be expressed in terms of a small number of parameters. There is
then a considerable risk of overinterpretation since the limited data may be consistent with several physically distinct models.

Such concerns led to the development of interpretational frameworks based on approximations of a general nature, allowing the TCF to be parametrized in a physically meaningful way without commitment to a specific dynamical model. This approach originated in the 1970s, when orientational order parameters were used to link first-order line splittings to second-order relaxation rates in ordered fluids such as liquid crystals and bilayer membranes. A formal derivation of a general TCF formula for isotropic fluids, known as the two-step model, was presented in 1981 (Ref. 7) and had then already been used in NMR relaxation studies of micelles and proteins.

In the following year, Lipari and Szabo, apparently overlooking the substantial body of preceding work in this area, presented a general TCF formula that came to be known as the model-free (MF) approach. While the Lipari–Szabo formula has been used extensively in the protein field, the nearly equivalent two-step formula has usually been preferred in applications to micelles, microemulsions, and other colloidal systems. Our focus here is on applications to protein solutions, but the new results derived and the conclusions reached are valid for a wide range of complex molecular systems. We refer to the closely related Lipari–Szabo and two-step formulas, along with their underlying approximations, as MF-A and MF-B, respectively. Both MF-A and MF-B address the case with one internal and one global motion. A third version of the MF approach, which we call MF-C, has been proposed for handling the case of internal motions on two distinct time scales, in addition to the global motion.

Although the MF approach has been almost universally adopted, substantial disagreement remains about its physical foundations and range of validity. This unfortunate situation has arisen, at least partly, because all three MF formulas were originally derived under unduly restrictive conditions. Furthermore, in the presentation of two of the MF formulas, formal rigor was traded for accessibility and brevity. As a result, the wide range of validity of the three MF formulas has not been fully appreciated. Furthermore, the differences and similarities between the MF-A and MF-B formulas have not been fully clarified.

In a series of papers, Meirovitch, Freed, and co-workers vigorously criticized the MF approach, arguing that it is physically unreasonable and that it may produce a qualitatively incorrect picture of protein dynamics. According to these authors, the principal deficiency of the MF approach is its neglect of dynamical coupling between internal (conformational fluctuations) and global (usually protein tumbling) motions. To rectify this perceived deficiency, Freed and co-workers developed the so-called slowly relaxing local structure (SRLS) model, which is based on a two-body Smoluchowski equation (SE). First applied (in a simpler form) to probe motion in liquid-crystalline solvents, the SRLS model has more recently been used to describe protein dynamics. More sophisticated versions of the SRLS model have also been used by Polimeni, Moro, Freed, and co-workers to analyze the rotational dynamics of a probe molecule interacting with a “cage” of solvent molecules.

The SRLS model differs fundamentally in spirit from the MF approach. While the SRLS model is restricted to small-step diffusive internal motion in a potential of mean torque (POMT) of specified form, the essence of the MF approach is its independence of any detailed specification of the dynamical mechanism. It is clear, therefore, that the SRLS model cannot be regarded as a generalization of the MF approach. Conversely, the MF formulas cannot be regarded as limiting cases of the SRLS model. On the other hand, it is not at all clear that, as invariably assumed, the SRLS model provides a more accurate description of protein dynamics than does the MF approach even for the particular choice of POMT and reorientation mechanism on which the SRLS model is based. If this is not the case, there is little incentive to sacrifice the analytical simplicity of the MF approach for the considerable computational burden of the SRLS model.

Our objective here is to clarify the physical basis of the MF approach and to unambiguously define its range of validity. Our strategy for achieving this goal is twofold. First, in Sec. II, we present rigorous formal derivations of the three MF formulas under significantly more general conditions than were imposed in the original publications. Specifically, for MF-A, we remove the limitation to uniaxial spin-lattice interaction tensor, isotropic internal mobility tensor, and coincident principal frames for these tensors. For MF-B, we remove the restriction to weak anisotropy of the internal motion. These generalizations do not alter the simple form of the MF formulas, but they enable a more general interpretation of the parameters. Explicit expressions are presented here that allow order parameters and internal correlation functions to be calculated for specific dynamical models. In Sec. II, we also discuss cross-TCFs and we derive a novel MF formula applicable to microcrystalline proteins and other solids.

The second part of our scrutiny of the MF approach is a detailed analysis of dynamical coupling, which is neglected in the MF approach but included (in some form) in the SRLS model. This analysis is divided into three sections. In Sec. III, we formulate and compare the two decoupling approximations invoked in the MF approach: the superposition approximation (MF-A and MF-C) and the adiabatic approximation (MF-B and MF-C). The issue of dynamical coupling is conceptually subtle and the SRLS model’s lack of analytical transparency hampers a direct comparison with the MF approach. Therefore, in Sec. IV, we present a simplified version of the SRLS model, which can be solved analytically. This planar SRLS model retains the dynamical complexity of the full SRLS model, but the geometry is simplified. In the adiabatic (time-scale separated) limit, the planar SRLS model reduces to the planar version of the popular diffusion-in-a-cone model. In other limits, our analysis shows that the SRLS model makes counterintuitive predictions that we attribute to the inappropriate theoretical foundations of the model rather than to dynamical coupling between internal and global motions. This conclusion refutes the widely held belief that the SRLS model is more accurate than the MF
approach. Finally, in Sec. V, we explore the two principal mechanisms of dynamical coupling in proteins: torque-mediated and friction-mediated coupling. We argue by way of specific analytically solvable models that torque-mediated coupling (which SRLS attempts to capture) is unimportant because the relatively slow internal motions that might couple to the global motion tend to be intermittent (jumplike) in character, whereas friction-mediated coupling (which neither MF nor SRLS incorporates) may be important for proteins with unstructured parts or flexibly connected domains.

II. MODEL-FREE APPROACH

In the motional narrowing regime, all autocorrelated nuclear spin relaxation observables measured in isotropic solution report on the time autocorrelation function of a nuclear spin-lattice interaction tensor $V$,

$$C(t) = \langle V(0); V(t) \rangle,$$ (2.1)

where the angular brackets denote an ensemble average. The rank-2 traceless symmetric tensor $V$ typically describes the magnetic dipole-dipole, magnetic shielding anisotropy, or electric quadrupole interaction.\(^1\) The scalar contraction (indicated by a colon) can be evaluated in any fixed coordinate system provided that the components of $V$ are expressed in the same frame at times 0 and $t$. The natural choice is the laboratory-fixed coordinate frame (L), with the $z_L$ axis along the external magnetic field. Cross-correlated relaxation observables report on cross-TCFs that, under certain conditions, can be obtained by a trivial extension of the treatment of auto-TCFs (Sec. II D).

If the scalar contraction is expanded in laboratory-frame spherical tensor components $V_m^{(L)}$, Eq. (2.1) yields

$$C(t) = \sum_{m=-2}^{2} \langle V_m^{(L)*}(0)V_m^{(L)}(t) \rangle = 5 \langle V_0^{(L)*}(0)V_0^{(L)}(t) \rangle,$$ (2.2)

where the asterisk denotes conjugation. The equality of the five TCFs in the sum follows from the isotropy of the solution,\(^39\) which also implies that $\langle V \rangle = 0$. Because $V(t)$ must be independent of $V(0)$ in the limit $t \rightarrow \infty$, it follows that the TCF decays to 0, that is, $C(\infty) = 0$. In Sec. II E, we consider anisotropic systems (solids and liquid crystals), where the TCFs in the sum of Eq. (2.2) depend on the index $m$.

Ubiquitous subpicosecond vibrational (or librational) motions average the spin-lattice interaction tensor $V$, but these motions are usually too fast to contribute directly to spin relaxation. Henceforth, we regard $V$ as the vibrationally averaged spin-lattice interaction tensor and we define the interaction frame (F) as the principal frame wherein $V$ is diagonal. By expanding the scalar contraction in the F-frame components $V_m^{(F)}$, we can express the initial value of the TCF as

$$C(0) = \frac{5}{3} \sum_{m=-2}^{2} |V_m^{(F)}|^2 = (V_0^{(F)})^2 \left( 1 + \frac{\eta^2}{3} \right) = V_{\text{eff}}^2,$$ (2.3)

where $\eta$ is the asymmetry parameter of the $V$ tensor, conventionally defined as\(^1\)

<table>
<thead>
<tr>
<th>Table I. Definition of coordinate systems.</th>
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<tbody>
<tr>
<td>Symbol</td>
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<tr>
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<tr>
<td>F (D, CSA, Q)</td>
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<tr>
<td>I (M)</td>
</tr>
<tr>
<td>D (C’)</td>
</tr>
<tr>
<td>G (C)</td>
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<tr>
<td>L (L)</td>
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$^a$Symbols used by Freed et al. (Ref. 18) are given within parentheses.

$$\eta = \sqrt{6} \frac{V_{zz}^{(F)}}{V_{xx}^{(F)} - V_{yy}^{(F)}}.$$ (2.4)

In the following, we shall work with a dimensionless $V$ tensor normalized such that

$$V : V = \text{Tr} \{VV\} = 1.$$ (2.5)

In expressions relating relaxation observables to TCFs (or spectral density functions), the latter should thus be multiplied by $V_{\text{eff}}^2$. With this normalization, the TCF decays to 0 from an initial value of 1,

$$C(0) = 1, \quad C(\infty) = 0.$$ (2.6)

A TCF with these properties, known as reduced TCF, is denoted by $C(t)$ throughout this paper. We shall also encounter TCFs, denoted by $G(t)$, that do not have (both of) these properties. Such TCFs can always be reduced by the transformation,

$$C(t) = \frac{G(t) - G(\infty)}{G(0) - G(\infty)}.$$ (2.7)

In the simplest and most general versions of the MF approach, MF-A and MF-B, the TCF is assumed to be governed by two kinds of motion, involving internal and global degrees of freedom. To obtain a simple parametrization of the TCF, two approximations must be invoked. The first is a decoupling approximation asserting that the internal and global motions are (effectively) independent. The physical basis of this approximation is elucidated in Secs. III–V. The second approximation is a symmetry requirement. Depending on how these two approximations are formulated, one obtains MF formulas that differ in physical content and in range of validity. It is straightforward to extend the MF approach to three or more motions. This can be done in several ways, depending on which approximations are invoked. Here, we consider the widely used three-motion MF-C formula.

In order to apply the approximations, the time dependence in the laboratory-frame component $V_0^{(L)}(t)$ in Eq. (2.2) must be formally linked to the relevant internal and global degrees of freedom. This is done by transforming the tensor component from the laboratory frame (L) to the interaction frame (F) via three additional coordinate frames denoted as I, D, and G (Table I). The I and G frames are the principal frames for the internal and global mobility tensors, respectively. The symmetry of each mobility tensor can be isotropic, uniaxial, or biaxial, corresponding to one, two, or three distinct principal components. The director frame D is the external reference frame for the local POMT that acts on the
“body” that executes the internal motion. If a second internal motion is present, as in MF-C, two additional frames, \(I'\) and \(D'\), are needed (Sec. II C).

The rotational transformations between these frames are conveniently expressed with the aid of the rank-2 Wigner rotation matrix,

\[
V^{(L)}_{0}(t) = \sum_{n} \sum_{p} \sum_{q} \sum_{r} D^{n}_{q}(\Omega_{LG})D^{p}_{n}(\Omega_{GD})D^{q}_{r}(\Omega_{DF}(t))V^{(F)}_{r},
\]

(2.8)

where the argument of the Wigner functions is the set of Euler angles, \(\Omega_{AB}\), that carry frame A into frame B.\(^{40}\) Here and in the following, all sums run from \(-2\) to \(+2\). By regarding the vibrationally averaged principal-frame components \(V^{(F)}_{r}\) in Eq. (2.8) as time-independent constants, we exclude from consideration “intramolecular” relaxation mechanisms, such as magnetic dipole-dipole relaxation induced by translational diffusion.\(^1\) Furthermore, the Euler angles \(\Omega_{DF}\) are regarded as constant parameters that define the relative orientation of the principal frames for the internal mobility tensor and the vibrationally averaged spin-lattice interaction tensor. It is convenient to define the time-independent geometrical coefficients,

\[
\sigma_{q} = \sum_{r} D^{2}_{qr}(\Omega_{DF})V^{(F)}_{r}.
\]

(2.9)

It follows Eqs. (2.4) and (2.5) that these coefficients may be written as

\[
\sigma_{q} = \left(1 + \frac{\eta^{2}}{3}\right)^{-1/2}\left\{D^{2}_{q0}(\Omega_{DF}) + \frac{\eta}{6}[D^{2}_{q2}(\Omega_{DF}) + D^{2}_{q-2}(\Omega_{DF})]\right\},
\]

(2.10)

and that they are normalized as

\[
\sum_{q} |\sigma_{q}|^{2} = 1.
\]

(2.11)

The internal and global motions are described by the time-dependent Euler angles \(\Omega_{DF}(t)\) and \(\Omega_{LG}(t)\), respectively. The Euler angles \(\Omega_{GD}\) in Eq. (2.8) are regarded as constant parameters that define the relative orientation of the global mobility frame and the local director frame. With the time dependence made explicit, Eq. (2.8) can now be written as

\[
V^{(L)}_{0}(t) = \sum_{n} \sum_{p} \sum_{q} D^{n}_{0n}(\Omega_{LG}(t))D^{p}_{np}(\Omega_{GD})D^{q}_{q}(\Omega_{DF}(t))\sigma_{q}.
\]

(2.12)

The TCF is obtained by substituting this expression into Eq. (2.2),

\[
C(t) = 5\sum_{n} \sum_{n'} \sum_{p} \sum_{p'} \sum_{q} \sum_{q'} D^{n}_{0n}(\Omega_{LG}(t))D^{n'}_{np}(\Omega_{GD})\sigma_{q}\sigma_{q'}
\]

\[
\times \langle D^{2}_{0n}(\Omega_{LG}(t))D^{2}_{0n'}(\Omega_{LG})D^{p}_{pq}(\Omega_{DF})D^{p'}_{pq'}(\Omega_{DF})\rangle,
\]

(2.13)

where the superscript 0 on the Euler angles signifies the initial time. In the following subsections, we show how this expression is simplified by invoking dynamical decoupling approximations and by imposing static or dynamic symmetry constraints. Static symmetries constrain the \(L\)- and \(D\)-frame projection indices \(m\) and \(p\), respectively. For example, the isotropic symmetry of the solution allowed us to set \(m=0\) in Eq. (2.2). Symmetries of the global and internal mobility tensors constrain the \(G\)- and \(I\)-frame projection indices \(n\) and \(q\), respectively.

### A. Model-free version A

In MF-A, decoupling of internal and global motions is imposed by a superposition approximation, formulated as a condition of statistical independence of these motions (Sec. III A). The ensemble average in Eq. (2.13) can then be replaced by a product of ensemble averages over the internal and global degrees of freedom, respectively,

\[
\langle D^{2}_{0n}(\Omega_{LG})D^{2}_{0n'}(\Omega_{LG})\rangle = \delta_{nn'}\exp(-t/\tau_{global})
\]

(2.14)

The second approximation in MF-A is a requirement of isotropic global motion, meaning that

\[
5\langle D^{2}_{0n}(\Omega_{LG})D^{2}_{0n'}(\Omega_{LG})\rangle = 5\delta_{nn'}\exp(-t/\tau_{global}) = \delta_{nn'}C_{global}(t).
\]

(2.15)

This form is valid for rotational diffusion of a compact spherical protein, in which case \(\tau_{global}=1/(6D_{G})\) is the rank-2 rotational correlation time of a spherical-top rotor with rotational diffusion coefficient \(D_{G}.\(^{41}\) However, Eq. (2.15) also describes exchange-mediated orientational randomization for a ligand or solvent molecule bound to a rotationally immobilized protein,\(^{42-44}\) in which case \(\tau_{global}\) equals the mean residence time \(\tau_{res}\) of the bound species. If both of these processes occur simultaneously, and if they are statistically independent, the global correlation time is given by

\[
\frac{1}{\tau_{global}} = 6D_{G} + \frac{1}{\tau_{res}}.
\]

(2.16)

Because the global TCF in Eq. (2.15) is independent of the \(G\)-frame projection index \(n\), Eqs. (2.13)–(2.15) yield a factorized TCF,

\[
C(t) = C_{global}(t)C_{internal}(t),
\]

(2.17)

with the internal TCF given by

\[
C_{internal}(t) = \sum_{q} \sum_{q'} \alpha_{q}\alpha_{q'}\sum_{p} \langle D^{2}_{pq}(\Omega_{DF})D^{2}_{pq}(\Omega_{DF})\rangle.
\]

(2.18)

Lipari and Szabo\(^{10}\) did not elaborate on the meaning of “statistical independence,” but simply stated (correctly) that it leads to the factorization of the TCF as in Eq. (2.17). In Secs. III–V, we discuss the physical significance of this approximation in detail.

It follows from the unitarity of the Wigner rotation matrix\(^{40}\) and the normalization of the geometric coefficients \(\alpha_{q}\) in Eq. (2.11) that \(C_{internal}(0)=1\). However, the internal TCF does not decay to 0. The asymptotic TCF \(C_{internal}(\infty)\) is
obtained from Eq. (2.18) by noting that the orientation \( \Omega_{\text{DI}} \) at infinite time must be independent of the initial orientation \( \Omega_{\text{IF}}^{0} \). The ensemble average of the product of Wigner functions can then be replaced by the product of ensemble averaged Wigner functions, yielding

\[
G_{\text{internal}}(\infty) = S^{2},
\]  
(2.19)

with the generalized rank-2 orientational order parameter \( S \) given by

\[
S^{2} = \sum_{p} \left| \sum_{q} \sigma_{q} (D^{2}_{pq}(\Omega_{\text{DI}})) \right|^{2}.
\]  
(2.20)

Combination of Eqs. (2.7), (2.15), (2.17), and (2.19) now yields the MF-A formula,

\[
C(t) = \exp(-t/\tau_{\text{global}}) [S^{2} + (1 - S^{2})C_{\text{internal}}(t)].
\]  
(2.21)

The reduced internal TCF \( C_{\text{internal}}(t) \) is defined explicitly as

\[
C_{\text{internal}}(t) = \frac{G_{\text{internal}}(0) - S^{2}}{1 - S^{2}},
\]  
(2.22)

with \( G_{\text{internal}}(0) \) given by Eq. (2.18). If the internal motion is fast compared to the Larmor frequency (extreme motional narrowing) and compared to the global motion, then the spin relaxation rates depend on the internal motion only via the time integral of \( C_{\text{internal}}(t) \). This integral defines an effective correlation time,

\[
\tau_{\text{internal}} = \int_{0}^{\infty} dt \ C_{\text{internal}}(t).
\]  
(2.23)

However, if the internal motion is slower, then Eq. (2.21) must be supplemented with an assumption about the (exponential or otherwise) decay of \( C_{\text{internal}}(t) \).

Equations (2.18) and (2.20)–(2.22) represent the MF-A in its most general form. We now show how these general results reduce to more familiar expressions when additional symmetry is present. The Lipari–Szabo treatment\(^{10} \) was restricted to a uniaxial interaction tensor, \( \eta = 0 \), in which case Eq. (2.10) reduces to \( \sigma_{q} = D^{2}_{pq}(\Omega_{\text{IF}}^{0}) \). It was also assumed that the I and F frames coincide \( (\Omega_{\text{IF}}^{0} = 0) \), whereby \( \sigma_{q} = \delta_{q0} \) so Eq. (2.20) reduces to

\[
S^{2} = \sum_{p} \left| (D^{2}_{pq}(\Omega_{\text{DI}})) \right|^{2}.
\]  
(2.24)

Equation (2.20) is the generalization of this familiar result to a biaxial spin-lattice tensor with arbitrary orientation relative to the internal mobility tensor. If, in addition to \( \eta = 0 \) and \( \Omega_{\text{IF}}^{0} = 0 \), the POMT is uniaxial in the I frame (meaning that \( z_{\text{D}} \) is an \( n \)-fold symmetry axis with \( n \geq 3 \)), then \( (D^{2}_{pq}(\Omega_{\text{DI}})) \) vanishes unless \( p = 0 \) so Eq. (2.24) reduces further to

\[
S = \langle P_{2}(\cos \beta_{\text{DI}}) \rangle.
\]  
(2.25)

With Eq. (2.20) it is straightforward to compare relaxation data pertaining to different (autocorrelated) spin-lattice interaction tensors in the same molecular fragment, such as the magnetic dipole-dipole and shielding anisotropy of a \( ^{13}\text{C} \) or \( ^{15}\text{N} \) spin or the electric field gradient tensors of the \( ^{2}\text{H} \) and \( ^{17}\text{O} \) spins in an internal water molecule.\(^{45} \) If no symmetry is present, the generalized order parameter \( S \) depends on 25 independent partial order parameters \( \langle D^{2}_{pq}(\Omega_{\text{DI}}) \rangle \). If the local POMT is uniaxial in the I frame, only the five order parameters \( \langle D^{2}_{pq}(\Omega_{\text{DI}}) \rangle \) are nonzero. They are linearly related to the direction cosines that constitute Saupe’s traceless symmetric Cartesian ordering tensor. On the other hand, if the local POMT is uniaxial in the D frame, then only the five terms with \( p = 0 \) survive.

The general result for the internal TCF, Eqs. (2.18) and (2.22), simplifies considerably in special cases. If the internal mobility tensor is uniaxial, then only terms with \( q = q’ \) survive in Eq. (2.18). If the internal mobility tensor is isotropic, as assumed by Lipari and Szabo,\(^{10} \) then

\[
\langle D^{2}_{pq}(\Omega_{\text{IF}}^{0})D^{2*}_{pq}(\Omega_{\text{DI}}) \rangle = \delta_{q0}\delta_{q’0}\langle D^{2}_{00}(\Omega_{\text{DI}})D^{2*}_{00}(\Omega_{\text{DI}}) \rangle,
\]  
(2.26)

in analogy with Eq. (2.15). Inserting this into Eq. (2.18) and making use of the unitarity of the Wigner rotation matrix (which, in this case, reduces to the spherical harmonic addition theorem) and the normalization (2.11), we obtain with Eq. (2.22),

\[
C_{\text{internal}}(t) = \frac{\langle P_{2}(\mathbf{e}_{1}(0) \cdot \mathbf{e}_{1}(t)) \rangle - S^{2}}{1 - S^{2}},
\]  
(2.27)

where \( \mathbf{e}_{1} \) is the unit vector along the \( z_{1} \) axis and \( S \) is given by Eq. (2.24). More generally, group-theoretical methods can be used to implement any internal symmetry constraints on the order parameter and the TCF.\(^{46} \)

**B. Model-free version B**

Many proteins deviate substantially from spherical shape, so the requirement of isotropic global motion limits the applicability of the MF-A formula (2.21). However, MF-B does not suffer from this limitation. MF-B is also based on two approximations, albeit not the same ones as in MF-A. To set the stage for these approximations, we split \( V_{\text{L}L}^{\text{L}}(t) \) in two parts:

\[
V_{\text{L}L}^{\text{L}}(t) = V_{\text{L}L}^{\text{L}}(t) + V_{\text{D}D}^{\text{L}}(t),
\]  
(2.28)

with

\[
V_{\text{L}L}^{\text{L}}(t) = (V_{\text{D}D}^{\text{L}}(t))_{\text{DF}} = \bar{V}_{\text{L}L}^{\text{L}}(t),
\]  
(2.29a)

\[
V_{\text{D}D}^{\text{L}}(t) = V_{\text{L}L}^{\text{L}}(t) - \bar{V}_{\text{L}L}^{\text{L}}(t).
\]  
(2.29b)

The overbar indicates that the tensor component has been averaged over the internal degrees of freedom \( \Omega_{\text{DF}} \). Upon substituting Eq. (2.28) into Eq. (2.2), we obtain two auto-TCFs and two cross-TCFs,

\[
C(t) = G_{ss}(t) + G_{ff}(t) + G_{sf}(t) + G_{fs}(t).
\]  
(2.30)

In MF-B, decoupling of internal and global motions is imposed by an *adiabatic approximation* that requires the internal motion to be much faster than the global motion (Sec. III B). If this is the case, then \( V_{\text{L}L}^{\text{L}}(t) \) and \( V_{\text{D}D}^{\text{L}}(t) \) fluctuate on disjoint time scales so the cross-TCFs in Eq. (2.30) vanish, leaving

\[
C(t) = G_{ss}(t) + G_{ff}(t),
\]  
(2.31)

where
\[ G_s(t) = 5 \langle V_0^{(L)}(0) \rangle V_0^{(L)}(t) \], \quad (2.32a) 
\[ G_q(t) = 5 \langle [V_0^{(L)}(0) - V_0^{(L)}(0)] [V_0^{(L)}(t) - V_0^{(L)}(t)] \rangle. \quad (2.32b) \]

Equation (2.31) may be contrasted with Eq. (2.17), where we have a product rather than a sum of partial TCFs. However, when the internal motion is much faster than the global motion, the MF-A formula (2.21) is also a sum of terms associated exclusively with global and internal dynamics.

Combination of Eqs. (2.12) and (2.29a) yields for the slowly fluctuating component
\[ \langle V_0^{(L)}(t) \rangle = \sum_n \sum_p \sum_q D_{np}^{2\ast} (\Omega_{GL}(t)) D_{pq}^{2\ast} (\Omega_{GD}) D_{qr}^{2\ast} (\Omega_{DP}) \sigma_q^s. \quad (2.33) \]

The second approximation in MF-B is the requirement that the local POMT is uniaxial in the D frame, meaning that
\[ \langle D_{pq}^{2\ast} (\Omega_{DP}) \rangle = \delta_{pq} D_{0q}^{2\ast} (\Omega_{DD}). \quad (2.34) \]

Using Eqs. (2.32a), (2.33), and (2.34), we can express the “slow” TCF as
\[ G_s(t) = S^2 C_{\text{global}}(t), \quad (2.35) \]
with the reduced global TCF \( C_{\text{global}}(t) \) defined as in Eq. (2.7) and the generalized order parameter \( S \) given by
\[ S^2 \equiv \sum_q \sigma_q^s \langle D_{0q}^{2\ast} (\Omega_{DD}) \rangle^2. \quad (2.36) \]

This is seen to be a special case of Eq. (2.20), where now only the \( p=0 \) term survives the averaging in the uniaxial local environment.

The explicit form of the reduced global TCF in Eq. (2.35) is
\[ C_{\text{global}}(t) = 5 \sum_n \sum_{n'} D_{nn'}^{2\ast} (\Omega_{GD}) \times \langle D_{00}^{2\ast} (\Omega_{LG}) D_{0q}^{2\ast} (\Omega_{LG}) \rangle. \quad (2.37) \]

From the spatial isotropy of the solution and the orthogonality of the Wigner functions, \( \langle \rangle \), it follows that
\[ \langle D_{00}^{2\ast} (\Omega_{LG}) \rangle = 0, \quad (2.38a) \]
\[ \langle D_{00}^{2\ast} (\Omega_{LG}) D_{00}^{2\ast} (\Omega_{LG}) \rangle = \delta_{\text{int}} \frac{1}{2}, \quad (2.38b) \]
which, together with the unitarity of the Wigner rotation matrix, show that \( C_{\text{global}}(t) \) decays from 1 to 0, as expected for a reduced TCF. However, no assumption has been made about the form of this decay. For example, the decay is triexponential for symmetric-top rotational diffusion, \( \frac{1}{4} \)
\[ C_{\text{global}}(t) = (1 - 3 \xi/2) e^{-\lambda_g t} + 3 \xi (1 - \xi) e^{-\lambda_f t} + \frac{3}{4} \xi^2 e^{-\lambda_s t}, \quad (2.39) \]
with \( \xi = \sin^2 \beta_{GD} \) and \( \lambda_g = 6D_{00}^{2\ast} + n^2 (D_{00}^{2\ast} - D_{00}^{2\ast}) \).

The MF-B formula now follows Eqs. (2.31) and (2.35),
\[ C(t) = S^2 C_{\text{global}}(t) + (1 - S^2) C_{\text{internal}}(t), \quad (2.40) \]
with the reduced internal TCF defined as \( C_{\text{internal}}(t) = G_q(t)/ (1 - S^2) \). It follows from Eqs. (2.32b), (2.33), (2.34), (2.36), and (2.37) and the unitarity of the Wigner rotation matrix that \( C_{\text{internal}}(t) \) is given by Eqs. (2.18) and (2.22), just as for MF-A. The original derivation of the MF-B formula (2.40) differs in two respects from the present one. First, the generalized order parameter was defined as \( A = (1 + \tau^2/3)^{1/2} \). Second, an approximation of weak anisotropy for the internal motion was invoked, which is actually not needed. The MF-B formula (2.40) is thus more general than suggested by its original derivation.

**C. Model-free version C**

When slow internal motions occur, they are usually superimposed on faster internal motions. To handle such situations, Claro and co-workers\(^1\) proposed what has come to be known as the extended MF approach. This is actually a hybrid of MF-A and MF-B and we refer to it as MF-C. The tensor component \( V_0^{(L)}(t) \) is now modulated by three kinds of motion (global, slow internal, and fast internal), so a general treatment requires two new coordinate frames in addition to the five frames in Table I. We associate the D and I frames with the slow internal motion and we introduce the analogous frames \( \text{D'} \) and \( \text{I'} \) to describe the fast internal motion.

As in MF-A, the global motion is assumed to be isotropic and statistically independent of the internal motions. The total TCF therefore factorizes as in Eq. (2.17) with \( C_{\text{global}}(t) \) given by Eq. (2.15) and \( C_{\text{internal}}(t) \), which now represents internal motions on two time scales, given by
\[ G_{\text{internal}}(t) = \sum_p \langle V_p^{(D)}(0) \rangle V_p^{(D)}(t), \quad (2.41) \]
with
\[ V_p^{(D)}(t) = \sum_p \sum_q D_{pq}^{2\ast} (\Omega_{DD}(t)) D_{qr}^{2\ast} (\Omega_{DI}) D_{rs}^{2\ast} (\Omega_{DI'}(t)) \sigma_r^s. \quad (2.42) \]

The Euler angles \( \Omega_{DD}(t) \) and \( \Omega_{DI'}(t) \) are modulated by the slow and fast internal motions, respectively, while the fixed Euler angles \( \Omega_{DI} \) define the relative orientation of the mobility frame for the slow internal motion and the director frame for the fast internal motion.

Next, the tensor components \( V_p^{(D)}(t) \) are split into two parts as in Eq. (2.28),
\[ V_p^{(D)}(t) = V_{p,s}^{(D)}(t) + V_{p,d}^{(D)}(t), \quad (2.43) \]
with
\[ V_{p,s}^{(D)}(t) = \langle V_p^{(D)}(t) \rangle_{\Omega_{DI}} = \overline{V_p^{(D)}}(t), \quad (2.44a) \]
\[ V_{p,d}^{(D)}(t) = V_p^{(D)}(t) - \overline{V_p^{(D)}}(t), \quad (2.44b) \]

The overbar now indicates that the tensor component has been averaged over the fast internal degrees of freedom \( \Omega_{DI'} \). Upon substituting Eq. (2.43) into Eq. (2.41), we obtain two auto-TCFs and two cross-TCFs. We now assume that the fast internal motion is much faster than the slow internal motion (adiabatic approximation) so that the cross-TCFs vanish and...
\[ G_{\text{internal}}(t) = G_{\text{internal}}^{\text{ss}}(t) + G_{\text{internal}}^{\text{ff}}(t), \]  

where

\[ G_{\text{internal}}^\text{ss}(t) = \sum_p \langle \overline{V}_p^\text{D}(0) \overline{V}_p^\text{D}(t) \rangle, \]  

\[ G_{\text{internal}}^\text{ff}(t) = \sum_p \langle [V_p^\text{D}(0) - \overline{V}_p^\text{D}(0)][V_p^\text{D}(t) - \overline{V}_p^\text{D}(t)] \rangle. \]

As the fourth and last approximation in MF-C, we require the POMT for the fast internal motion to be uniaxial in the D' frame so that

\[ \langle D_{\alpha}^\text{D'}(\Omega_{D'1'}) \rangle = \delta_{\alpha0} D_{\alpha}^\text{D}(\Omega_{D'1'}). \]  

Using Eqs. (2.42), (2.44a), and (2.47), we can express the slow internal TCF as

\[ G_{\text{internal}}^\text{ss}(t) = S^2_{\text{fast}} [S^2_{\text{slow}} + (1 - S^2_{\text{slow}})C_{\text{internal}}], \]  

with the generalized order parameters \( S_{\text{fast}} \) and \( S_{\text{slow}} \) given by

\[ S_{\text{fast}}^2 = \sum_q \sigma_q(D_{q0}^\text{D}(\Omega_{D'1'}))^2, \]

\[ S_{\text{slow}}^2 = \sum_p \sum_q D_{q0}^\text{D}(\Omega_{D'})D_{pq}^\text{D}(\Omega_{D})^2. \]

The reduced slow internal TCF, defined as in Eq. (2.7), is given by

\[ C_{\text{internal}}^\text{slow}(t) = C_{\text{internal}}(t) - S^2_{\text{slow}} \frac{1 - S^2_{\text{slow}}}{1 - S^2_{\text{fast}}}, \]  

with

\[ G_{\text{internal}}^\text{slow}(t) = \sum_p \sum_q D_{q0}^\text{D}(\Omega_{D'})D_{pq}^\text{D}(\Omega_{D})^2 \]

\[ \times \sum_p D_{q0}^\text{D}(\Omega_{D})D_{pq}^\text{D}(\Omega_{D})^2. \]

Combination of Eqs. (2.15), (2.17), (2.45), (2.46b), and (2.48) now yields the MF-C formula:

\[ C(t) = \exp(-\tau_{\text{global}})[S^2_{\text{fast}} C_{\text{slow}} + (1 - S^2_{\text{slow}})C_{\text{internal}}(t)] \]

\[ + (1 - S^2_{\text{fast}})C_{\text{internal}}^\text{fast}(t). \]  

The reduced fast internal TCF \( C_{\text{internal}}^\text{fast}(t) \), defined as in Eq. (2.7), is given by Eqs. (2.18) and (2.22) with \( S \) replaced by \( S_{\text{fast}} \) and \( \Omega_{D} \) replaced by \( \Omega_{D'1'} \). As seen from its derivation, the MF-C formula (2.53) requires all four types of approximation used in the MF-A and MF-B treatments. As expected, Eq. (2.53) reduces to the MF-A result (2.21) when \( S_{\text{fast}} = 1 \) (no fast internal motion) or when \( S_{\text{slow}} = 1 \) (no slow internal motion). Effective correlation times \( \tau_{\text{global}}^\text{slow} \) and \( \tau_{\text{global}}^\text{fast} \) can be defined as the time integral of \( C_{\text{slow}} \) and \( C_{\text{fast}} \), as in Eq. (2.23).

If the fast internal mobility tensor is isotropic, the fast internal reduced TCF becomes

\[ C_{\text{fast}}(t) = \frac{P_2(e_{1}\cdot e_{1}(t)) - S^2_{\text{fast}}}{1 - S^2_{\text{fast}}}. \]  

This result follows Eqs. (2.26), (2.51), and (2.52) and the unitarity of the Wigner rotation matrix.

D. Cross correlations

Cross-correlated relaxation observables report on the cross-TCF

\[ C_{AB}(t) = \langle V_A^\text{D}(0) V_B^\text{D}(t) \rangle, \]  

where \( V_A \) and \( V_B \) are two different spin-lattice interaction tensors. Provided that these tensors have a fixed (time-independent) geometrical relationship, it is straightforward to generalize the three MF formulas. The main difference in the derivations is that the initial value of the partial internal TCF is no longer 1. Each of the interaction tensors is taken to be anisotropic so that

\[ C_{AB}(t) = \exp(-\tau_{\text{global}})[S^2_{AB} + (1 - S^2_{AB})C_{AB}(t)]. \]  

with \( S^2_{AB} \) and \( C_{AB} \) given by the obvious generalizations of Eq. (2.20).
and of Eqs. (2.18) and (2.22),

$$C_{\text{internal}}(t) = \kappa_{AB}^2 - S_{AB}^2, \quad (2.62)$$

with

$$G_{\text{internal}}(t) = \sum_{q, q'} \sigma_{q}^A \sigma_{q'}^B \sum_{p} (D_{pq}^2(\Omega_{D1})) (D_{pq}^{2*}(\Omega_{D1})). \quad (2.63)$$

The formula (2.60) has been given previously for the special case of uniaxial interaction tensors ($\eta_A = \eta_B = 0$). In general, there is no simple relation between the cross-TCF $C_{AB}(t)$ and the auto-TCF $C_{AA}(t)$. However, if the internal mobility tensor is isotropic, we obtain from Eqs. (2.26), (2.57), (2.60), (2.62), and (2.63),

$$C_{AB}(t) = \kappa_{AB} C_{AA}(t). \quad (2.64)$$

This result was previously obtained for the special case of uniaxial interaction tensors, where Eq. (2.59) applies, and under the additional (but unnecessary) restriction to small-amplitude internal motion.\(^{49}\)

### E. Anisotropic systems

Although the MF approach was originally conceived in connection with relaxation studies of anisotropic systems,\(^{3-6}\) specific dynamical models have usually been used to interpret relaxation data from solids\(^ {50}\) and liquid crystals.\(^ {51,52}\) In many cases, only local motions are important so there is no need for dynamical decoupling. However, in situations where both global and internal/local motions occur, suitably modified MF formulas are applicable. We refer to such “solid-adapted” versions of the MF formulas derived in Sec. II A and II B as MF-A/S and MF-B/S. Formulas of MF-B/S type (based on the adiabatic approximation) were derived in the original MF-B paper.\(^ {7}\) For applications of MF-B/S to liquid crystals, group-theoretical methods can be used to fully exploit the rotational symmetries of mesophases and supramolecular structures.\(^ {46}\)

Solid-state magic-angle-spinning (MAS) NMR relaxation studies of microcrystalline protein samples have the potential to reveal internal motions that cannot be probed by dipole-dipole, shielding anisotropy, or electric quadrupole induced relaxation in a solution of freely tumbling proteins.\(^ {53,54}\) So far, the interpretation of such data has been based on specific models\(^ {53}\) or on the initial-slope approximation.\(^ {50,54}\) Here, we present a rigorous derivation of a MF-A/S formula that is applicable to such data.

For an anisotropic system, the autocorrelated relaxation observables depend on three independent laboratory-frame TCFs ($m=0,1,2$),

$$\tilde{G}_m^{(L)}(t) = \tilde{G}_{-m}^{(L)}(t) = (\tilde{V}_m^{(L)}(0) \tilde{V}_m^{(L)}(t)), \quad (2.65)$$

involving the fluctuating part of the tensor component

$$\tilde{V}_m^{(L)}(t) = V_m^{(L)}(t) - \langle V_m^{(L)} \rangle. \quad (2.66)$$

Introducing a crystal-fixed frame C and a MAS frame M with $z_M$ as spinning axis, one obtains\(^ {50}\)

$$\tilde{G}_m^{(C)}(t) = \sum_k \left[ d_m^2(\beta_{LM}) \right] \sum_{k'} \sum_k d_m^2(\beta_{MC}) d_{k'}^2(\beta_{MC}) \times \exp[i(k' - k) \gamma_{MC}] G_{kk'}^{(C)}(t). \quad (2.67)$$

For a single-crystal sample (without MAS), the M frame is superfluous and we can set $\beta_{LM} = 0$. With $d_m^2(0) = \delta_m$, Eq. (2.67) then shows how the relaxation observables depend on the orientation ($\beta_{LC}$, $\gamma_{LC}$) of the crystal with respect to the external magnetic field.\(^ {46}\) For a MAS experiment with $\beta_{LM} = 54.74^\circ$ on a polycrystalline sample, each spectral component has contributions from microcrystals with different orientations ($\beta_{MC}$, $\gamma_{MC}$) relative to the spinning axis, and the decay of the spectral density is obtained as a powder average of the relaxation decays associated with each microcrystal orientation.\(^ {30,53,54}\)

The desired dynamical information is contained in the crystal-frame TCFs $G_{kk'}^{(C)}(t)$. There are 25 of them, but microscopic time-reversal invariance ensures that at most 15 are independent.\(^ {46}\) This number is further reduced if rotational symmetry is present. Here, we shall assume that the global POMT is uniaxial in the C frame. Then, all off-diagonal ($k \neq k'$) TCFs vanish and Eq. (2.67) reduces to

$$\tilde{G}_m^{(L)}(t) = \sum_k \left[ d_m^2(\beta_{LM}) \right] \sum_{k'} \sum_k d_m^2(\beta_{MC}) d_{k'}^2(\beta_{MC}) G_{kk'}^{(C)}(t). \quad (2.68)$$

with only three distinct crystal-frame TCFs ($k=0,1,2$),

$$\tilde{G}_k^{(C)}(t) = \tilde{G}_{-k}^{(C)}(t) = \tilde{G}_k^{(C)}(t) - \tilde{G}_k^{(C)}(\infty), \quad (2.69)$$

$$\tilde{G}_k^{(C)}(t) = \langle V_k^{(C)}(0) V_k^{(C)}(t) \rangle. \quad (2.70)$$

As in MF-A, we consider the case of global and internal motions that modulate the Euler angles $\Omega_{CG}(t)$ and $\Omega_{DG}(t)$, respectively. Transforming the crystal-frame tensor component $V_k^{(C)}$ as in Eq. (2.8), we can express the crystal-frame TCF $G_k^{(C)}(t)$ as in Eq. (2.13), but without the factor of 5 and with $D_{m0}^0(\Omega^0_{LG}) D_{m0}^{2*}(\Omega^0_{LG})$ replaced by $D_{m0}^2(\Omega^0_{LG}) D_{m0}^{2*}(\Omega_{CC})$. We now introduce the two MF-A approximations. The superposition approximation allows us to factorize the ensemble average as in Eq. (2.14) and the requirement of an isotropic
global mobility tensor implies that

\[
\langle D_{t_0}^2(\Omega_{CG})D_{t_0}^{2*}(\Omega_{CG}) \rangle = \delta_{t_0}\langle D_{t_0}^2(\Omega_{CG})D_{t_0}^{2*}(\Omega_{CG}) \rangle = \delta_{t_0}G_{\text{global},k}(t),
\]

(2.71)

In contrast to Eq. (2.15) for the isotropic case, the global TCF defined here depends on the index \( k \) and it is not in reduced form. The limits of this TCF are

\[
G_{\text{global},k}(0) = A_k,
\]

(2.72a)

\[
G_{\text{global},k}(\infty) = \delta_{t_0}\delta_{0}S^2_{\text{global}},
\]

(2.72b)

where we have introduced the mean-square fluctuation amplitudes.

\[
A_k = \langle [D_{t_0}^2(\beta_{CG})]^2 \rangle = \begin{cases} 
\frac{1}{2} + \frac{3}{4}\delta_{t_0}S^2_{\text{global}} + \frac{1}{32}Q_{\text{global}}, & k = 0 \\
\frac{1}{2} + \frac{3}{4}\delta_{t_0}S^2_{\text{global}} - \frac{1}{32}Q_{\text{global}}, & k = 1 \\
\frac{1}{2} - \frac{3}{4}\delta_{t_0}S^2_{\text{global}} + \frac{3}{32}Q_{\text{global}}, & k = 2 
\end{cases}
\]

(2.73)

and the rank-2 and rank-4 global order parameters

\[
S_{\text{global}} = \langle P_2(\cos \beta_{CG}) \rangle, \tag{2.74a}
\]

\[
Q_{\text{global}} = \langle P_4(\cos \beta_{CG}) \rangle. \tag{2.74b}
\]

The model-independent expressions in Eq. (2.73) were obtained with the aid of the Clebsch–Gordan series. The order parameters are readily evaluated for specific models. For example, if the global motion is modeled as diffusion in a cone,

\[
S_{\text{global}} = \frac{1}{2}\cos \alpha(1 + \cos \alpha), \tag{2.75a}
\]

\[
Q_{\text{global}} = \frac{1}{8}\cos \alpha(1 + \cos \alpha)(7 \cos^2 \alpha - 3). \tag{2.75b}
\]

Combining Eq. (2.71) with the counterpart of Eq. (2.13) and making use of the unitarity of the Wigner rotation matrix, we find that the crystal-frame TCF factorizes as

\[
\tilde{G}_{k}^{(C)}(t) = G_{\text{global},k}(t)G_{\text{internal},t}, \tag{2.76}
\]

with the internal TCF \( G_{\text{internal},t} \) given by Eq. (2.18). Finally, we obtain the MF-A/S formula by combining Eqs. (2.69), (2.76), (2.72), and (2.22),

\[
\tilde{G}_{k}^{(C)}(t) = \delta_{t_0}\delta_{0}S^2_{\text{global}}(1 - S^2)C_{\text{internal},t} + (A_k - \delta_{t_0}\delta_{0}S^2_{\text{global}})C_{\text{global},k}(t)[S^2 + (1 - S^2)C_{\text{internal},t}], \tag{2.77}
\]

with the (internal) order parameter \( S \) given by Eq. (2.20), the reduced internal TCF \( C_{\text{internal},t} \) by Eqs. (2.18) and (2.22), and the reduced global TCF given by

\[
C_{\text{global},k}(t) = \langle D_{t_0}^2(\Omega_{CG})D_{t_0}^{2*}(\Omega_{CG}) \rangle = \frac{A_k - \delta_{t_0}\delta_{0}S^2_{\text{global}}}{A_k - \delta_{t_0}\delta_{0}S^2_{\text{global}}}.
\]

In the limit of vanishing amplitude for the global motion, \( \beta_{CG} \to 0 \), we have \( A_k = \delta_{0} \) and \( S_{\text{global}} = 1 \), so Eq. (2.77) reduces to

\[
\tilde{G}_{k}^{(C)}(t) = (A_k - \delta_{0}S^2_{\text{global}})S^2_{\text{global}}C_{\text{global},k}(t), \tag{2.79}
\]

if the internal motion is so fast that it does not contribute directly to relaxation, then Eq. (2.77) yields

\[
\tilde{G}_{k}^{(C)}(t) = (A_k - \delta_{t_0}\delta_{0}S^2_{\text{global}})S^2_{\text{global}}C_{\text{global},k}(t). \tag{2.80}
\]

In the absence of a global POMT, when \( f_{eq}(\Omega_{CG}) = 1/(8\pi^2) \), we have \( S_{\text{global}} = 0 \) and \( A_k = 1/5 \). Furthermore, the global TCF in Eq. (2.78) becomes independent of \( k \). It then follows Eqs. (2.68) and (2.77) that

\[
G_{m}(t) = 3\exp(-t/t_{\text{global}})[S^2 + (1 - S^2)C_{\text{internal},t}]. \tag{2.81}
\]

Because this result is independent of \( m \), Eq. (2.2) shows that the MF-A/S formula reduces to the isotropic MF-A formula (2.21), as expected in this limit.

\section{III. DECOUPLING APPROXIMATIONS}

\subsection{A. Superposition approximation}

Theories of condensed-phase dynamics usually employ a stochastic description of the relevant degrees of freedom. The dynamical variables \( \Omega_{CG}(t) \) and \( \Omega_{eq}(t) \) in the MF approach (MF-A or MF-B) are thus regarded as stochastic processes governed by a set of time-dependent probability distributions. The partial TCFs appearing in Eq. (2.13) can then be expressed as

\[
\langle D_{t_0}^2(\Omega_{CG})D_{t_0}^{2*}(\Omega_{CG}) \rangle = \int d\Omega_{LG} \int d\Omega_{DG} P_{\text{eq}}(\Omega_{LG},\Omega_{DG})\langle D_{t_0}^2(\Omega_{LG})D_{t_0}^{2*}(\Omega_{DG}) \rangle
\]

(3.1)

When multiplied by \( d\Omega_{LG} d\Omega_{DG} \), the joint propagator \( P(\Omega_{LG},\Omega_{DG},t|\Omega_{LG}',\Omega_{DG}') \) gives the probability of having, at time \( t \), Euler angles to within \( d\Omega_{LG} \) of \( \Omega_{LG} \) and to within \( d\Omega_{DG} \) of \( \Omega_{DG} \), given that they were \( \Omega_{LG}' \) and \( \Omega_{DG}' \) initially. The propagator has the limiting values

\[
P(\Omega_{LG},\Omega_{DG},0|\Omega_{LG}',\Omega_{DG}') = \delta(\Omega_{LG} - \Omega_{LG}')\delta(\Omega_{DG} - \Omega_{DG}'), \tag{3.2a}
\]

\[
\lim_{t \to \infty} P(\Omega_{LG},\Omega_{DG},t|\Omega_{LG}',\Omega_{DG}') = P_{eq}(\Omega_{LG},\Omega_{DG}). \tag{3.2b}
\]
The joint equilibrium distribution $P_{eq}(\Omega_{LG}, \Omega_{Di})$ is the normalized Boltzmann distribution
\[
P_{eq}(\Omega_{LG}, \Omega_{Di}) = \frac{\exp[-U(\Omega_{LG}, \Omega_{Di})/(k_B T)]}{\int d\Omega_{LG} d\Omega_{Di} \exp[-U(\Omega_{LG}, \Omega_{Di})/(k_B T)]},
\]
(3.3)
where $U(\Omega_{LG}, \Omega_{Di})$ is the POMT that governs the orientation of the I frame relative to the L frame. It is usually a good approximation to assume that this potential is separable,
\[
U(\Omega_{LG}, \Omega_{Di}) = U(\Omega_{LG}) + U(\Omega_{Di}).
\]
(3.4)
It then follows from Eq. (3.3) that the joint equilibrium distribution factorizes,
\[
P_{eq}(\Omega_{LG}, \Omega_{Di}) = P_{eq}(\Omega_{LG}) P_{eq}(\Omega_{Di}),
\]
(3.5)
where the marginal equilibrium distributions on the right are defined as
\[
P_{eq}(\Omega_{LG}) = \int d\Omega_{Di} P_{eq}(\Omega_{LG}, \Omega_{Di}),
\]
and similarly for $P_{eq}(\Omega_{Di})$. When Eq. (3.5) holds, the two sets of stochastic variables $\Omega_{LG}$ and $\Omega_{Di}$ are said to be statistically independent.\textsuperscript{55} For an isotropic solution sample, where $U(\Omega_{LG})$ is a constant, Eq. (3.5) becomes
\[
P_{eq}(\Omega_{LG}, \Omega_{Di}) = \frac{1}{8 \pi} P_{eq}(\Omega_{Di}).
\]
(3.7)
The analogous definition of statistical independence for the stochastic processes $\Omega_{LG}(t)$ and $\Omega_{Di}(t)$ is that the joint propagator factorizes,
\[
P(\Omega_{LG}, \Omega_{Di}, t\mid \Omega_{LG}^0, \Omega_{Di}^0) = P(\Omega_{LG}, t\mid \Omega_{LG}^0) P(\Omega_{Di}, t\mid \Omega_{Di}^0),
\]
(3.8)
where the marginal propagators are defined as
\[
P(\Omega_{LG}, t\mid \Omega_{LG}^0) = \int d\Omega_{Di} P_{eq}(\Omega_{Di}^0)
\]
\[
\times \int d\Omega_{Di} P(\Omega_{LG}, \Omega_{Di}, t\mid \Omega_{LG}^0, \Omega_{Di}^0),
\]
(3.9)
and similarly for $P(\Omega_{Di}, t\mid \Omega_{Di}^0)$. Because the propagators evolve toward the corresponding equilibrium distributions [see Eq. (3.2b)], it is clear that Eq. (3.8) implies Eq. (3.5). The condition (3.8) may be regarded as a superposition approximation, stating that the total motion of I relative to L is a pure superposition of the motion of I relative to D and the motion of G relative to L. (Note that the D frame is fixed relative to the G frame.) This is not true, in general. However, when the two motions are statistically independent, then the TCF in Eq. (3.1) factorizes into a product of global and internal TCFs, as in Eq. (2.14). This is readily seen by combining Eqs. (3.1), (3.5), and (3.8).

To formulate the physical requirements for statistical independence, we assume that the stationary multivariate process $\{\Omega_{LG}(t), \Omega_{Di}(t)\}$ is Markovian. The joint propagator then obeys the master equation\textsuperscript{55}
\[
\frac{\partial}{\partial t} P(\Omega_{LG}, \Omega_{Di}, t\mid \Omega_{LG}^0, \Omega_{Di}^0) = \mathcal{L}(\Omega_{LG}, \Omega_{Di}) P(\Omega_{LG}, \Omega_{Di}, t\mid \Omega_{LG}^0, \Omega_{Di}^0),
\]
(3.10)
where the evolution operator $\mathcal{L}(\Omega_{LG}, \Omega_{Di})$ acts on $\Omega_{LG}$ and $\Omega_{Di}$ and also may depend parametrically on these variables. Associated with the master equation is the initial condition in Eq. (3.2a) and a set of boundary conditions that we express in terms of an operator $B(\Omega_{LG}, \Omega_{Di}),$
\[
B(\Omega_{LG}, \Omega_{Di}) P(\Omega_{LG}, \Omega_{Di}, t\mid \Omega_{LG}^0, \Omega_{Di}^0) = 0.
\]
(3.11)
Statistical independence requires that the evolution operator and the boundary conditions are separable,
\[
\mathcal{L}(\Omega_{LG}, \Omega_{Di}) = \mathcal{L}(\Omega_{LG}) + \mathcal{L}(\Omega_{Di}),
\]
(3.12a)
\[
B(\Omega_{LG}, \Omega_{Di}) = B(\Omega_{LG}) + B(\Omega_{Di}).
\]
(3.12b)
When these conditions are satisfied, the solution to Eq. (3.10) can be written on the factorized form (3.8). This is readily seen by inserting Eqs. (3.8) and (3.12a) into Eq. (3.10) to obtain
\[
\frac{1}{P(\Omega_{LG}, t\mid \Omega_{LG}^0)} \left[ \left( \frac{\partial}{\partial t} - \mathcal{L}(\Omega_{LG}) \right) P(\Omega_{LG}, t\mid \Omega_{LG}^0) \right] = - \frac{1}{P(\Omega_{Di}, t\mid \Omega_{Di}^0)} \left[ \left( \frac{\partial}{\partial t} - \mathcal{L}(\Omega_{Di}) \right) P(\Omega_{Di}, t\mid \Omega_{Di}^0) \right],
\]
and by noting that this equality holds generally only if both the bracketed expressions are identically zero.

**B. Adiabatic approximation**

The adiabatic approximation, like the Born–Oppenheimer approximation in molecular quantum mechanics, is based on an assumption of time-scale separation of different degrees of freedom. The stochastic processes $\Omega_{LG}(t)$ and $\Omega_{Di}(t)$ are said to be time-scale separated if there exists a time interval $t^*$ during which $\Omega_{LG}(t)$ remains constant, while $\Omega_{Di}(t)$ has become independent of its initial value. Then,\textsuperscript{29,35}
\[
P(\Omega_{LG}, \Omega_{Di}, t\mid \Omega_{LG}^0, \Omega_{Di}^0) = \left\{ \begin{array}{ll}
\delta(\Omega_{LG} - \Omega_{LG}^0) P(\Omega_{Di}, t\mid \Omega_{Di}^0; \Omega_{LG}), & t < t^* \\
P(\Omega_{LG}, t\mid \Omega_{LG}^0) P_{eq}(\Omega_{Di}; \Omega_{LG}), & t > t^*.
\end{array} \right.
\]
(3.13)
On the short time scale ($t < t^*$), there is no slow motion, and on the long time scale ($t > t^*$), the fast variables $\Omega_{Di}$ are completely slaved to the slow variables $\Omega_{LG}$. In the absence of external fields or, more generally, if the POMT is separable as in Eq. (3.4), the fast propagator and the fast (conditional) equilibrium distribution become independent of the slow variables. The adiabatic approximation can then be expressed in the stronger form\textsuperscript{56}
\[
P(\Omega_{LG}, \Omega_{Di}, t\mid \Omega_{LG}^0, \Omega_{Di}^0) = \left\{ \begin{array}{ll}
\delta(\Omega_{LG} - \Omega_{LG}^0) P(\Omega_{Di}, t\mid \Omega_{Di}^0), & t < t^* \\
P(\Omega_{LG}, t\mid \Omega_{LG}^0) P_{eq}(\Omega_{Di}), & t > t^*.
\end{array} \right.
\]
(3.14)
Because the propagator in Eq. (3.14) factorizes at all times,
the stochastic processes $\Omega_{LG}(t)$ and $\Omega_{D}(t)$ are, in fact, statistically independent. Time-scale separation in the sense of Eq. (3.14) thus implies statistical independence, but the reverse is not true. In other words, time-scale separation is a stronger condition than statistical independence. Thus, $\Omega_{LG}(t)$ and $\Omega_{D}(t)$ can be statistically independent even though they fluctuate on the same time scale (see Sec. V A).

IV. TWO-BODY SMOLUCHOWSKI APPROACH

A. Reduction to two dimensions

In this section we consider a simple but nontrivial model where the internal and global motions take place in a two-dimensional (2D) space. Coordinate frames and sets of Euler angles $\Omega$ in three dimensions correspond to vectors and angles $\varphi$ in two dimensions. The orientations of the internal mobility vector I (taken to coincide with the spin-lattice interaction vector $\mathcal{F}$) and of the global mobility vector $G$ (taken to coincide with the local director $D$), both defined with respect to the laboratory-fixed vector $L$, are denoted by $\varphi_{LG}$ and $\varphi_{LG}$. The 2D orientational TCF corresponding to the TCF in Eq. (2.2) is

$$C(t) = \langle \exp(ik[\varphi_{LG}(t) - \varphi_{LG}(0)]) \rangle$$

$$= \int_0^{2\pi} d\varphi_{LG} P_{eq}(\varphi_{LG}) \exp(-ik\varphi_{LG})$$

$$\times \int_0^{2\pi} d\varphi_{LG} P(\varphi_{LG}, \varphi_{LG}, t|\varphi_{LG}, 0) \exp(ik\varphi_{LG}) \exp(ik\varphi_{LG}).$$

(4.1)

The relative orientation of the I and G vectors is constrained by the local POMT, which is taken to depend only on the internal coordinate $\varphi_{LG}$. Furthermore, the system is assumed to be isotropic. Then, in analogy with Eq. (3.7),

$$P_{eq}(\varphi_{LG}, 0) = \frac{1}{2\pi} P_{eq}(\varphi_{LG}).$$

(4.2)

It will prove convenient to introduce the modified marginal propagator

$$Q(\varphi_{LG}, t|\varphi_{LG}) = \frac{1}{2\pi} \int_0^{2\pi} d\varphi_{LG} \exp(-ik\varphi_{LG})$$

$$\times \int_0^{2\pi} d\varphi_{LG} \exp(ik\varphi_{LG}) P(\varphi_{LG}, \varphi_{LG}, t|\varphi_{LG}, 0).$$

(4.3)

in terms of which Eq. (4.3) can be expressed as

$$C(t) = \int_0^{2\pi} d\varphi_{LG} \exp(-ik\varphi_{LG}) P_{eq}(\varphi_{LG})$$

$$\times \int_0^{2\pi} d\varphi_{LG} \exp(ik\varphi_{LG}) Q(\varphi_{LG}, t|\varphi_{LG}).$$

(4.4)

B. Planar SRLS model

In this subsection, we formulate and solve a 2D version of the SRLS model, which assumes that the I and G vectors (or the “bodies” to which they are attached) undergo independent rotational diffusion, with diffusion coefficients $D_I$ and $D_G$, but are coupled via their mutual interaction.\cite{18, 20, 32}

We shall analyze this planar SRLS model for the case of a

FIG. 1. Definition of the geometrical parameters in the planar SRLS model. The G vector bisects the $2\alpha$ angular range of the “wedge” and is fixed to the “protein” (shaded).
for hard repulsive interaction that confines the I vector to a restricted angular range of width $2\alpha$ centered on the G vector (Fig. 1). Thus,

$$P_{\text{eq}}(\varphi_{\text{GL}}^0) = \frac{1}{2\alpha}. \quad (4.7)$$

The SRLS model assumes that the coupled evolution of the I and G vectors is governed by a two-body Smoluchowski equation (SE). For the excluded-volume interaction considered here, there is no mean torque. The two-body SE therefore reduces to a two-body (free) diffusion equation and the POMT enters the problem solely via the boundary conditions. Apart from the effect of their mutual interaction, the I and G vectors rotate independently relative to the laboratory-fixed L vector. The propagator $P(\varphi_{\text{LG}}, \varphi_{\text{LL}}, t | \varphi_{\text{LG}}^0, \varphi_{\text{LL}}^0)$ thus obeys the bivariate diffusion equation

$$\frac{\partial}{\partial t} P(\varphi_{\text{LG}}, \varphi_{\text{LL}}, t | \varphi_{\text{LG}}^0, \varphi_{\text{LL}}^0) = D_G \frac{\partial^2}{\partial \varphi_{\text{LG}}^2} + D_L \frac{\partial^2}{\partial \varphi_{\text{LL}}^2} P(\varphi_{\text{LG}}, \varphi_{\text{LL}}, t | \varphi_{\text{LG}}^0, \varphi_{\text{LL}}^0) \quad (4.8)$$

on the domain $\mathcal{D}$ defined by the shaded region in Fig. 2(a). The initial condition is

$$P(\varphi_{\text{LG}}, \varphi_{\text{LL}}, 0 | \varphi_{\text{LG}}^0, \varphi_{\text{LL}}^0) = \delta(\varphi_{\text{LG}} - \varphi_{\text{LG}}^0) \delta(\varphi_{\text{LL}} - \varphi_{\text{LL}}^0). \quad (4.9)$$

The boundary conditions follow from the $2\pi$ periodicity of the variables $\varphi_{\text{LG}}$ and $\varphi_{\text{LL}}$ from the normalization of the joint propagator

$$\int_0^{2\pi} d\varphi_{\text{LG}} \int_0^{2\pi} d\varphi_{\text{LL}} P(\varphi_{\text{LG}}, \varphi_{\text{LL}}, 0 | \varphi_{\text{LG}}^0, \varphi_{\text{LL}}^0) = 1, \quad (4.10)$$

and from the confinement condition

$$-\alpha < \varphi_{\text{LL}} - \varphi_{\text{LG}} < \alpha, \quad (4.11)$$

imposed by the mutual interaction of the I and G vectors. As a result of these three conditions, the propagator and its derivatives obey periodic boundary conditions along the dashed lines in Fig. 2(a), while reflecting boundary conditions apply along the solid lines in Fig. 2(a). A reflecting boundary means that the normal component of the probability flux $J$ vanishes. For example, along the line $\varphi_{\text{LL}} = \varphi_{\text{LG}} + \alpha$,

$$u \cdot J = -u \cdot \nabla P = D_G \frac{\partial P}{\partial \varphi_{\text{LG}}} \bigg|_{\varphi_{\text{LG}}=\varphi_{\text{LG}}+\alpha} - D_L \frac{\partial P}{\partial \varphi_{\text{LL}}} \bigg|_{\varphi_{\text{LL}}=\varphi_{\text{LG}}+\alpha} = 0,$$

(4.12)

where $u = (-1,1)/\sqrt{2}$ is the outward-pointing unit normal for the line considered.

The joint propagator $P(\varphi_{\text{LG}}, \varphi_{\text{LL}}, t | \varphi_{\text{LG}}^0, \varphi_{\text{LL}}^0)$ evolves toward the joint equilibrium distribution

$$P_{\text{eq}}(\varphi_{\text{LG}}, \varphi_{\text{LL}}) = P_{\text{eq}}(\varphi_{\text{LG}}) P_{\text{eq}}(\varphi_{\text{LL}})$$

$$= \begin{cases} 1, & \{\varphi_{\text{LG}}, \varphi_{\text{LL}}\} \in \mathcal{D} \\ 0, & \{\varphi_{\text{LG}}, \varphi_{\text{LL}}\} \notin \mathcal{D}. \end{cases} \quad (4.13)$$

Because $P_{\text{eq}}(\varphi_{\text{LG}}, \varphi_{\text{LL}})$ cannot be written as a product of the marginal equilibrium distributions $P_{\text{eq}}(\varphi_{\text{LG}})$ and $P_{\text{eq}}(\varphi_{\text{LL}})$, it is clear that the stochastic variables $\varphi_{\text{LG}}$ and $\varphi_{\text{LL}}$ are statistically dependent. Therefore, the stochastic processes $\varphi_{\text{LG}}(t)$ and $\varphi_{\text{LL}}(t)$ cannot be statistically independent under any conditions. With regard to the condition (3.12), we note that the diffusion operator in Eq. (4.8) is separable, but the boundary condition in Eq. (4.12) is not.

While $\{\varphi_{\text{LG}}, \varphi_{\text{LL}}\}$ are the natural variables for formulating the two-body Smoluchowski (or diffusion) equation (4.8), they are not the most convenient variables for analyzing the coupled dynamics of the I and G vectors in terms of internal and global motions. We therefore transform the independent variables from $\{\varphi_{\text{LG}}, \varphi_{\text{LL}}\}$ to $\{\varphi_{\text{LG}}, \varphi_{\text{GL}}\}$, with the internal coordinate $\varphi_{\text{GL}}$ defined by Eq. (4.2) (see also Fig. 1). The Jacobian of this transformation is unity, so the transformed diffusion equation is obtained simply by applying the chain rule to Eq. (4.8) with the result

$$\frac{1}{(D_L + D_G)} \frac{\partial}{\partial t} P(\varphi_{\text{LG}}, \varphi_{\text{GL}}, t | \varphi_{\text{LG}}^0, \varphi_{\text{GL}}^0) = \left[ \gamma \frac{\partial^2}{\partial \varphi_{\text{LG}}^2} - 2 \gamma \frac{\partial}{\partial \varphi_{\text{LG}}} \frac{\partial}{\partial \varphi_{\text{GL}}} + \frac{\partial^2}{\partial \varphi_{\text{GL}}^2} \right] P(\varphi_{\text{LG}}, \varphi_{\text{GL}}, t | \varphi_{\text{LG}}^0, \varphi_{\text{GL}}^0), \quad (4.14)$$
where we have introduced the dimensionless dynamical parameter
\[ \gamma = \frac{D_G}{D_1 + D_G}. \]  

Equation (4.14) is obeyed in the domain defined by the shaded region in Fig. 2(b). Periodic boundary conditions apply along the dashed lines \( \varphi_{GL} = 0 \) and \( \varphi_{GL} = 2\pi \), while reflecting boundary conditions apply along the solid lines \( \varphi_{GL} = -\alpha \) and \( \varphi_{GI} = \alpha \). The latter conditions are obtained by transforming Eq. (4.12), yielding
\[ \frac{\partial P}{\partial \varphi_{GI}} \bigg|_{\varphi_{GI} = \pm \alpha} = \gamma \frac{\partial P}{\partial \varphi_{LG}} \bigg|_{\varphi_{LG} = \pm \alpha}. \]  

Finally, we have the initial condition
\[ P(\varphi_{LG}, \varphi_{GI}, 0) = \delta(\varphi_{LG} - \varphi_{LG}^0) \delta(\varphi_{GI} - \varphi_{GI}^0). \]  

The cross term in the evolution operator of Eq. (4.14) implies that the stochastic processes \( \varphi_{LG}(t) \) and \( \varphi_{GI}(t) \) are not statistically independent, in general. However, the joint propagator \( P(\varphi_{LG}, \varphi_{GI}, t | \varphi_{LG}^0, \varphi_{GI}^0) \) evolves toward a joint equilibrium distribution that factorizes
\[ P_{eq}(\varphi_{LG}, \varphi_{GI}) = P_{eq}(\varphi_{LG}) P_{eq}(\varphi_{GI}) = \frac{1}{4\pi \alpha}. \]  

The new independent variables are thus statistically independent. Therefore, the stochastic processes \( \varphi_{LG}(t) \) and \( \varphi_{GI}(t) \) may be statistically independent under certain conditions.

The diffusion equation (4.14) can also be obtained from the two-body SE used by Freed and co-workers by taking the limit of vanishing POMT in the 2D version of Eq. (3) in Ref. 20. For the continuous POMT employed by these authors, the diffusion equation contains additional terms, which, however, do not violate the superposition approximation. The breakdown of the superposition approximation is caused by the cross term, which is present in Eq. (4.14) as well as in Eq. (3) of Ref. 20. The effect of the additional terms that appear for a continuous POMT is to reduce the deviation from the superposition approximation. Our model, with a square-well POMT, thus emphasizes this deviation, which should vanish in the strong-coupling limit.

The boundary value problem defined by Eqs. (4.14)–(4.17) does not have an analytical solution. However, as shown in Appendix A of Ref. 57, the modified marginal propagator \( Q(\varphi_{GI}, t | \varphi_{GI}^0) \) can be obtained analytically. By way of Eq. (4.6), we can then also obtain the TCF analytically. The result can be recast in the functional form of the MF-A formula (2.21),
\[ C(t) = \exp(-t/\tau_{global}) [S^2 + (1 - S^2) C_{internal}(t)], \]  

but the parameters must now be regarded as apparent quantities. The apparent global correlation time is
\[ \tau_{global} = \frac{1}{k^2 (D_G + D_1)} = \frac{1}{k^2 D_G (1 - \gamma)}. \]  

The apparent order parameter is
\[ S = \text{sinc}(\alpha), \]  

with \( \text{sinc}(x) = \sin(x)/x \) the un-normalized cardinal sinc function and
\[ \alpha = (1 - \gamma)k \alpha. \]  

The apparent reduced internal TCF is given by
\[ C_{internal}(t) = \sum_{n=1}^{\infty} A_n \exp \left[ - \left( \frac{n\pi}{2\alpha} \right)^2 (D_1 + D_G)t \right], \]  

with relative mode amplitudes
\[ A_n = \left( \frac{\alpha^2}{1 - \sin^2 \sigma} \right) \left[ \frac{1 - (-1)^n \cos(2\sigma)}{(n\pi)^2 - \sigma^2} \right]. \]  

The sum in Eq. (4.23) converges rapidly and under most conditions it can be truncated after the first term. For example, \( A_1 = 0.90 \) and \( A_2 = 0.09 \) for \( \sigma = 1.22 \), which corresponds to \( 2\alpha = 70^\circ \) for \( \gamma = 0 \) and \( k = 2 \). For stronger confinement (smaller \( \alpha \)) or slower internal motion (larger \( \gamma \)), the dominance of the first term is even stronger. The decay of the apparent internal TCF \( C_{internal}(t) \) is thus very nearly exponential under most conditions (Fig. 3). The effective internal correlation time is obtained by integrating Eq. (4.23), as in Eq. (2.23), and then summing the resulting trigonometric series,
\[ \tau_{internal} = \frac{2\alpha^2}{(D_1 + D_G)} \left[ \frac{1}{\alpha^2} - \frac{\sin^2 \sigma}{3(1 - \sin^2 \sigma)} \right]. \]  

C. Adiabatic limit

The adiabatic limit of the SRLS model corresponds to \( D_1 \gg D_G \) and, hence, \( \gamma \ll 1 \). In this limit, the adiabatic approximation invoked in MF-B is valid. Since, in addition, the \( G \) vector (which coincides with the director \( D \) in the planar SRLS model) is a symmetry axis for the internal motion, the TCF in Eq. (4.19) can be identified with the MF-B formula (2.40),
where the adiabatic limit is indicated by a $0$ subscript or superscript. In the adiabatic limit, the global TCF $C^0_{\text{global}}(t)$ depends only on $D_G$ and the internal TCF $C^0_{\text{internal}}(t)$ depends only on the internal parameters $D_I$ and $\alpha$. In addition, the order parameter $S_0$ is a true equilibrium property, depending on the interaction parameter $\alpha$, but not on the dynamical parameters $D_I$ and $D_G$. These expectations are confirmed by taking the $\gamma \to 0$ limit of Eqs. (4.20)—(4.24). The TCF in Eq. (4.19) then reduces to Eq. (4.26) with

$$S_0 = \text{sinc}(k\alpha),$$

$$C^0_{\text{global}}(t) = \exp\left(-\frac{t}{\tau^0_{\text{global}}(t)}\right) = \exp(-k^2D_G t),$$

$$C^0_{\text{internal}}(t) = \sum_{n=1}^{\infty} A^0_n \exp\left[-\left(\frac{n\pi}{2\alpha}\right)^2 D_I t\right],$$

and Eq. (4.25) reduces to

$$\tau^0_{\text{internal}} = \frac{2}{k^2D_I} \left[1 - \frac{\sin^2\kappa}{3(1-\sin^2\kappa\alpha)}\right].$$

Because the global motion in the planar SRLS model is isotropic, the TCF in Eq. (4.26) also coincides with the MF-A formula (2.21). In other words, the planar SRLS model has sufficient symmetry to make the MF-A and MF-B formulas equivalent in the adiabatic limit.

The adiabatic limit of the planar SRLS model is the 2D analog of the widely used 3D diffusion-in-a-cone model. The 3D model does not admit an analytical solution, but the order parameter, $S_0 = \cos(1+\cos\alpha)\alpha/2$, and the effective correlation time $\tau^0_{\text{internal}}$ (Ref. 38) can be expressed in closed form. The order parameter for the cone model is numerically close to the order parameter in Eq. (4.27) for $k=2$ (Fig. 4). The 2D order parameter with $k=2$ vanishes when the I vector orientation is constrained to a half-circle ($\alpha=90^\circ$). Similarly, for the 3D cone model, the rank-2 order parameter $S = \langle P_2(\cos\beta) \rangle$ vanishes when uniformly averaged over a hemisphere ($\alpha=90^\circ$). Furthermore, the effective internal correlation time $\tau^0_{\text{internal}}$ has almost the same dependence on $\alpha$ for the two models, the difference being essentially a constant factor that accounts for the faster sampling of angular space in 2D than in 3D (Fig. 4). This close correspondence ensures that the conclusions drawn here on the basis of a 2D model (with rank $k=2$) are quantitatively relevant to real 3D systems.

**D. Physical interpretation**

Outside the adiabatic limit, that is, when $\gamma$ is not $\ll 1$, the SRLS model cannot be fully described by the MF approach. The TCF can still be expressed on the MF-A form, as in Eq. (4.19), but the parameters do not have the same physical meaning as in the MF approach and they cannot be interpreted in terms of the explicit expressions given in Sec. II.

Since it depends on the dynamical parameter $\gamma$, the apparent order parameter $S$ in Eq. (4.21) is no longer a true equilibrium property determined solely by the internal POMT (here parametrized via the confinement angle $\alpha$). Therefore, $1-S^2$ cannot be interpreted as a measure of orientational disorder of the I vector, related to its configurational entropy. When the protein tumbles faster or the internal motion slows down, $\gamma$ increases and thus $1-S^2$ decreases (Fig. 5). This is a large effect. For example, for $\alpha=30^\circ$ and $D_G=D_I$ (corresponding to $\gamma=0.5$), $1-S^2$ is reduced from the adiabatic ($D_G=D_I$) value by a factor of 3.6 (from 0.316 to 0.088).

In the adiabatic limit, the effective internal correlation time in Eq. (4.31) does not depend on $D_G$. In contrast, the apparent effective internal correlation time in Eq. (4.25) decreases with increasing $\gamma$ (Fig. 6). Because the apparent internal TCF is strongly dominated by the first exponential term in Eq. (4.23), the decrease in $\tau^0_{\text{internal}}$ in Fig. 6 can be attributed to the appearance of the relative diffusion coefficient $D_I+D_G$ in the exponent of Eq. (4.23) rather than to the $D_G$ dependence of the mode amplitude $A_n$. To an excellent approximation, we can therefore obtain $\tau^0_{\text{internal}}$ from the adiabatic-limit expression (4.31) simply by replacing $D_I$ with $D_I+D_G$ (Fig. 6).
Figure 7 shows the total TCF $C(t)$ and the internal TCF $C_{\text{internal}}(t)$ for the SRLS model, as well as the corresponding quantities $C_{\text{global}}(t)$ and $C_{\text{internal}}^0(t)$ in the adiabatic limit. Because the apparent internal motion in the SRLS model involves the relative diffusion coefficient $D_1+D_G$, $C_{\text{internal}}(t)$ decays faster than $C_{\text{internal}}^0(t)$ and the difference can, to an excellent approximation, be accounted for simply by replacing $D_1$ in Eq. (4.29) with $D_1+D_G$ (see insets in Fig. 7). Because of the faster (apparent) internal motion and because of the larger (apparent) order parameter, the total TCF for the SRLS model is relatively less affected by the internal motion. In other words, $C(t)$ in Eq. (4.19) is more strongly dominated by the first term $S^2 \exp(-t/\tau_{\text{global}})$ than it is in the adiabatic (MF) limit.

The apparent global correlation time $\tau_{\text{global}}$ in Eq. (4.20) depends not only on the protein mobility ($D_G$) but also on the internal mobility ($D_1$). It is evident from Eqs. (4.20) and (4.25) that $\tau_{\text{internal}}$ is dominated by the fastest process (the largest of $D_1$ and $D_G$), whereas $\tau_{\text{global}}$ is dominated by the slowest process (the smallest of $D_1$ and $D_G$). For this reason, $\tau_{\text{internal}}$ can never exceed $\tau_{\text{global}}$ in the SRLS model (Fig. 8). In the adiabatic regime ($D_1 \gg D_G$), $\tau_{\text{internal}}$ is determined by $D_1$ and $\tau_{\text{global}}$ is determined by $D_G$, as in the MF approach. In the opposite regime of slow internal motion ($D_1 \ll D_G$), $\tau_{\text{internal}}$ is determined by $D_G$ and $\tau_{\text{global}}$ is determined by $D_1$. In this regime, $S=1$ so the total TCF is $C(t)=\exp(-k^2D_1)$. In the limit $D_1=0$ (no internal motion), the SRLS model thus yields $C(t)=1$. In the strong-coupling limit, corresponding to $\alpha=0$, Eqs. (4.19)–(4.24) yield nonzero $\gamma$,

$$
C(t) = C_{\text{global}}(t) = \exp\left[-k^2\left(\frac{1}{D_G} + \frac{1}{D_1}\right)^{-1} t\right],
$$

that is, the TCF still depends on the rate ($D_1$) of internal motion even though the amplitude of the internal motion is zero.

Thus, while the SRLS model coincides with the MF approach in the adiabatic regime ($D_1 \gg D_G$), it makes predictions in other limits that contradict physical intuition. For example, when the internal motion is much slower than the global motion ($D_1 \ll D_G$), it should have no effect on spin relaxation and the TCF should be the same as for a rigid protein, $C(t) = \exp(-k^2D_G)$. However, the SRLS model predicts that $C(t) = \exp(-k^2D_1t)$ in this limit. This striking discrepancy cannot be attributed to a dynamical coupling between the internal and global motions because such a coupling can only exist when the two motions occur on the same time scale (Sec. III).

The origin of the counterintuitive predictions of the SRLS model can be traced back to its foundation, the two-body Šmolučowski (or diffusion) equation (4.8). This equa-
tion (and its associated boundary conditions) is completely symmetrical in I and G. In particular, because it describes overdamped motion, Eq. (4.8) ignores the fact that the protein has a much larger moment of inertia than the small molecular fragment that executes the internal motion. The two-body SE is an appropriate model for two similarly sized molecules coupled by a POMT that tends to align a vector (I) in one molecule with a vector (G) in the other molecule. In the strong-coupling limit, the two molecules should rotate as a single rigid body with a friction coefficient that is the sum of the friction coefficients of the individual molecules. Because the diffusion coefficient is inversely related to the friction coefficient, this intuition is seen to be in accord with Eq. (4.32). We thus conclude that the counterintuitive predictions of the SRLS model result not from dynamical coupling of internal and global motions but from the failure of the two-body SE to describe the inherent asymmetry of the dynamical problem (local conformational fluctuations in a tumbling protein) to which the SRLS model has been applied by Meirovitch, Freed, Polimeni, and co-workers.6,20,22-27

V. DYNAMICAL COUPLING IN PROTEINS

In NMR relaxation studies of conformational motion in proteins, dynamical coupling can enter in two ways. First, because of the covalent connectivity of the backbone and the dense packing of side chains, the motions of proximal bond vectors or molecular fragments are always coupled to some extent. Examples include the rotation of the three C—H bonds in a methyl group,58 crankshaft-like coupled torsional transitions in a polymer chain,59 and wobbling of a planar peptide unit carrying an 15N—H bond and a 13C' shielding anisotropy tensor.60 This type of dynamical coupling, involving spin-lattice interaction tensors at two different nuclear sites, gives rise to experimentally accessible cross-TCFs.61 In the limit of total correlation, where the relative orientation of the two interaction tensors is fixed, it is straightforward to generalize the MF treatment to cross-TCFs (Sec. II D).

Dynamical coupling between different sites may also affect the internal-motion part of the auto-TCFs. Since the MF approach does not rely on specific dynamical models, it can accommodate this type of dynamical coupling via Eq. (2.18) or Eq. (2.52). Of course, the MF parameters from multiple sites are not independent if the internal motions at these sites are coupled. To simplify the simultaneous analysis of multisite autocorrelated relaxation data, several schemes have thus been proposed based on molecular dynamics trajectories,62 analytical models,63 or a combination thereof.64 Although these schemes incorporate dynamical coupling, it is not possible to prove the existence of dynamical coupling between internal motions at different sites by analyzing autocorrelated relaxation data.

The second type of dynamical coupling, and the one that we are concerned with here, is the coupling between two motions that modulate the same spin-lattice interaction tensor at the same nuclear site. Specifically, we focus on the coupling between internal and global motions that both modulate the orientation of a given interaction tensor, specified by the Euler angles $\Omega_{1,2}(t)$. If these motions are dynamically coupled, the MF approach is not rigorously valid. As noted in Sec. III, the internal and global motions can be dynamically coupled only if they occur on the same time scale. Therefore, only relatively slow internal motions, on time scales of $10^{-10}$ s or longer, are relevant. On these time scales, atomic motions are overdamped and inertia plays no role. Provided that the solvent can be approximately treated as a continuous viscous fluid, the dynamics of a protein comprising N atoms can then be described by an N-particle SE, featuring friction (or diffusion) coefficients for each atom and a force field describing the mutual interactions of the N atoms. A familiar example of this level of description is the Rouse-Zimm model of polymer dynamics.65 A reduction or contraction of the N-particle SE to only two degrees of freedom would produce a non-Markovian evolution equation with time-dependent force and friction coefficients.66 The Markovian two-body SE on which the SRLS model is based ignores all such memory effects. As applied to protein dynamics, the two-body SE must therefore be regarded as a phenomenological model, the validity of which must be judged according to its ability to describe real systems over the relevant range of parameter values. There is thus no guarantee that the SRLS model improves upon the much simpler MF approach under the conditions where it has been applied. In fact, the analysis of the planar SRLS model in Sec. IV indicates that this is not the case.

To develop a realistic model that incorporates dynamical coupling, it is helpful to have a clear physical picture of the underlying mechanism. Since the motions are overdamped (diffusive), dynamical coupling must be either torque mediated or friction mediated. In Sec. V A, we argue that torque-mediated dynamical coupling, which the SRLS model attempts to describe, is generally not important in folded proteins. In Sec. V B, we consider friction-mediated dynamical coupling, which is not included in the SRLS model.

A. Persistent and intermittent motions

As noted above, dynamical coupling can only arise if the internal motion is relatively slow. Diffusive conformational motions in proteins can be slow either because they experience a large friction (and thus a small rotational diffusion coefficient $D_\tau$) or because one or more potential barriers have to be surmounted. In applications of the SRLS model to proteins, only the former possibility has been considered.

In the absence of barriers, $D_\tau$ must be smaller than $D_c$, in order for the correlation times $\tau_{\text{internal}}$ and $\tau_{\text{global}}$ to be comparable (since the internal motion is restricted), which is necessary for dynamical coupling to develop. Generally, the friction coefficient increases with the size of the rotating body and with the correlation time of the fluctuating torque exerted on it. While the torque on the “I-body” might decay more slowly than the solvent-generated torque acting on the entire protein, it is not clear that this could offset the effect of the size difference.

On the other hand, it is clear that most slow conformational motions within densely packed native proteins are activated processes, involving torsional or other barriers in the POMT. Broadly speaking, we can classify diffusive internal
motions as persistent or intermittent. Persistent or continuous motions, such as libration in a potential well, tend to be much faster than protein tumbling and therefore cannot be dynamically coupled. The reaction coordinate of an intermittent or infrequent motion features at least one barrier that is large compared to k_BT. Such motions are not intrinsically slow (D_1 is not small) but involve a long time scale because a successful barrier crossing is typically preceded by many unsuccessful attempts. If the barrier is sufficiently large, an intermittent rotational motion can be accurately modeled in terms of instantaneous jumps between discrete orientations. In NMR jargon, this type of motion is usually referred to as “conformational exchange.”

Consider an intermittent internal motion where the internal orientational variable Ω_GI only can assume the discrete values Ω^ν GI, ν = 1, 2, ..., N. The internal orientation can then be represented by the state index ν and the joint propagator can be written as P(Ω_GI, ν, t | Ω^0 GI, ν^0). The evolution of this propagator is described by a set of N coupled master equations (ν = 1, 2, ..., N),

$$\frac{\partial}{\partial t} P(\Omega_{GI}, \nu, t | \Omega^0_{GI}, \nu^0) = \sum_{\mu=1}^{N} \mathcal{L}(\Omega_{GI}, \nu, \mu) P(\Omega_{GI}, \mu, t | \Omega^0_{GI}, \nu^0),$$

(5.1)

with the evolution operators

$$\mathcal{L}(\Omega_{GI}, \nu, \mu) = \mathcal{L}(\Omega_{GI}) + W(\nu | \mu) - \delta_{\nu \mu} \sum_{\nu' = 1}^{N} W(\mu' | \nu).$$

(5.2)

Here, W(ν | μ) is the transition rate from state μ to state ν. From the point of view of dynamical coupling, the critical feature in this model is the physically reasonable assumption that the transition rates are independent of the global motion. In other words, the probability per unit time for a jump from one state to another is the same whether the protein is fixed or if it is tumbling. The evolution operator (5.2) is then separable as in Eq. (3.12a), and the superposition approximation, Eq. (3.8), is valid. If the internal motion is intermittent, it is thus statistically independent from the global motion even if the internal and global motions take place on the same time scale. Under such conditions, the MF-A approach is valid but not the MF-B approach.

As an illustration of the foregoing general remarks, we show in Appendix B of Ref. 57 that the planar model in Fig. 1 with intermittent jumps between the two internal states φ = ± α yields a TCF on the MF-A form,

$$C_0(t) = \exp(-t/\tau^0_{\text{global}})[S_0^2 + (1 - S_0^2)\exp(-t/\tau^0_{\text{internal}})].$$

(5.3)

As before, the global correlation time is τ^0_{global} = 1/(k^2D_G). The internal motion is characterized by the conformational exchange time τ^0_{internal} related to the mean residence times τ_- and τ_+ in the two states as

$$\frac{1}{\tau^0_{\text{internal}}} = \frac{1}{\tau_-} + \frac{1}{\tau_+},$$

(5.4)

and the order parameter S_0 is related to the jump angle 2α and the equilibrium populations P_- and P_+ in the two states as

$$S_0 = (1 - 4P_- P_+ \sin^2 k\alpha)^{1/2}.$$  

(5.5)

B. Hydrodynamic coupling

The most important mechanism for coupling the internal and global motions in a protein is a conformational change that alters the shape of the protein-solvent interface. We refer to this type of dynamical coupling as hydrodynamic coupling, since it is mediated by the global friction generated by the aqueous solvent, which is usually modeled at a hydrodynamic (continuum-solvent) level. Hydrodynamic coupling is particularly important for intrinsically unstructured or denatured proteins, but it may also be significant for proteins with extended flexible loops or flexibly connected rigid domains. In such cases, the analytical simplicity of the MF approach may have to be sacrificed for computationally intensive simulation-based interpretation schemes.

Hydrodynamic coupling is only significant if all of the following three conditions are satisfied: (i) the global motion is rotational diffusion of the entire protein (rather than exchange of a bound ligand or solvent molecule), (ii) the internal motion substantially alters the shape of the protein, and (iii) the internal and global motions occur on the same time scale. The evolution operator in Eq. (3.10) is then of the form

$$\mathcal{L}(\Omega_{GI}, \Omega_G) = -\mathbf{L}_G : \mathbf{D}_G(\Omega_G) : \mathbf{L}_G + \mathcal{L}(\Omega_G),$$

(5.6)

where \mathbf{L}_G is the angular momentum operator acting on the Euler angles Ω_GI. Because the global rotational diffusion tensor \mathbf{D}_G(\Omega_G) depends on the internal configuration Ω_GI, the evolution operator is not separable, as required for statistical independence [see Eq. (3.12a)].

While shape-preserving interdomain motions have been treated within the MF context, the effect of hydrodynamic coupling on the TCF has been considered only recently. To illustrate the consequences of hydrodynamic coupling in a simple and transparent way, we analyze an extended version of the model in Sec. V A, where now the global diffusion coefficient depends on the internal state: D_G(\varphi^c GI) = D^c G. This is essentially a 2D version of the “diagonal model” considered in Ref. 68. For simplicity, we assume that the two states are equally populated: P_- = P_+ = 1/2. We show in Appendix C of Ref. 57 that the TCF for this model can be recast in the MF-A form,

$$C(t) = \exp(-t/\tau_{\text{global}})[S^2 + (1 - S^2)\exp(-t/\tau_{\text{internal}})],$$

(5.7)

but with apparent parameters. The apparent correlation times are

$$\tau_{\text{global}} = \left[ k^2 D_G - \frac{\left(1 + \delta^2 - 1\right)}{2 u^0_{\text{internal}}} \right]^{-1},$$

(5.8)

$$\tau_{\text{internal}} = \frac{u^0_{\text{internal}}}{1 + \delta^2},$$

(5.9)

with u^0_{\text{internal}} given by Eq. (5.4) and
In the fast-exchange limit, where \( |\delta| \ll 1 \), the three apparent parameters in Eq. (5.7) reduce to the corresponding parameters in Eq. (5.3) with \( P_{\text{iso}} = P_{\text{uni}} \), that is, \( \tau_{\text{global}} = \tau^0_{\text{global}} \), \( \tau_{\text{internal}} = \tau^0_{\text{internal}} \), and \( S = S_0 = \cos \kappa \alpha \). The dynamical coupling thus vanishes if \( |\delta| \ll 1 \), which is a weaker condition than the adiabatic condition \( \tau_{\text{internal}} \ll \tau_{\text{global}} \) if \( D^+_G \) and \( D^-_G \) differ relatively little.

In the slow-exchange limit, where \( |\delta| \gg 1 \), Eqs. (5.8)–(5.13) yield \( 1/\tau_{\text{global}} = k^2 D^+_G \) and \( 1/\tau_{\text{global}} + 1/\tau_{\text{internal}} = k^2 D^-_G \) and \( S = 1/\sqrt{2} \). The TCF in Eq. (5.7) is then a population-weighted superposition,

\[
C(t) = \frac{1}{\sqrt{2}} \exp(-k^2 D^+_G t) + \frac{1}{\sqrt{2}} \exp(-k^2 D^-_G t).
\]

The internal motion thus has no effect on the TCF, as expected since the condition \( |\delta| \gg 1 \) implies that \( \tau_{\text{internal}} \gg \tau_{\text{global}} \). This is in contrast to the SRLS model, where the global motion has no effect on the TCF in this limit (Sec. IV D).

VI. CONCLUDING REMARKS

A. Validity of the MF approach

In Fig. 9 we summarize the decoupling approximations and symmetries required to obtain each of the four MF formulas considered in Sec. II. The first three of these formulas (MF-A, MF-B, and MF-C) are more general than the original versions, while the last one (MF-A/S) is new. The generalized MF formulas have the same functional form as the original, widely used formulas, but their range of validity is extended by the more general form of order parameters and reduced TCFs. For example, our generalized treatment shows that the MF-A formula (2.21) is valid for any type of internal motion, as long as it is superimposed on, and hence dynamically decoupled from, the global motion. In particular, it is not necessary to assume, as did Lipari and Szabo,\(^{10}\) that the internal mobility tensor is uniaxial, that the spin-lattice interaction tensor is uniaxial, and that the principal frames (I and F) of these tensors coincide.

1. Time-scale separation

It is often asserted that MF-A requires the internal motion to be much faster than the global motion. However, as explained in Sec. III, time-scale separation is sufficient, but not necessary, for statistical independence (superposition). Moreover, slow conformational dynamics in folded proteins are likely to be intermittent, with infrequent barrier crossings. The time scale of intermittent motions is related to the mean lifetimes of the conformational states, which may be long. However, the actual motion, within each potential well and during the infrequent barrier crossings, is fast. For such motions, the superposition approximation is expected to be accurate on all time scales (Sec. V A). Unless the internal motion alters the shape of the protein substantially (Sec. V B), MF-A should therefore be accurate in the vast majority of cases. This expectation is supported by several analyses based on long molecular dynamics simulations of proteins.\(^{69–71}\)

2. Order parameters

Unlike the rank-2 spin-lattice interaction and mobility tensors, which can only have isotropic, uniaxial or biaxial symmetry, the POMT \( U (\Omega_D) \) is related to two frames and its symmetry group is the direct product of the symmetry groups associated with the two frames.\(^{46}\) The direct-product group determines the symmetry selection rules on the partial order parameters \( \langle D^2_{pq} (\Omega_D) \rangle \). Thus, for example, the POMT may be uniaxial in the D frame or in the I frame or in both. A D-frame-uniaxial POMT, such as a prolate-shaped body confined within a circular cone, yields the selection rule \( p = 0 \).
An I-frame-uniaxial POMT, such as a cylindrical body confined within an elliptical cone, yields the selection rule \( q = 0 \). In either of these cases, the five independent partial order parameters are linearly related to the five independent components of a Cartesian traceless, symmetric ordering tensor of rank 2. However, in the general case, there are 25 distinct partial order parameters.

The generalized order parameter \( S \) in Eq. (2.20) retains all 25 partial order parameters \( \langle D_{pq}^2(\Omega_{DF}) \rangle \) since no assumptions about the symmetry of the POMT were made in the derivation of the MF-A formula. It represents the complete model-independent information about the POMT available from autocorrelated relaxation observables. [The reduced internal TCF \( C_{\text{internal}}(t) \) also contains information about the POMT, but this information cannot be separated from the kinetics of the internal motion in a model-independent way.] The order parameter \( S \) is usually interpreted as a measure of the spatial restriction imposed by the POMT or the angular amplitude of the internal motion. Such naive geometrical interpretations can be misleading, since the single number \( S \) only represents a particular “projection” of the orientational distribution function \( P_{\text{eq}}(\Omega_{DF}) \). In Eq. (2.20), the partial order parameters \( \langle D_{pq}^2(\Omega_{DF}) \rangle \) are associated with the internal dynamical variables \( \Omega_{DF} \), but \( S \) also depends on the fixed Euler angles \( \Omega_{IF} \) via the geometrical coefficients \( \sigma_q \). More information about the POMT can thus be obtained by comparing, with the aid of Eq. (2.20), order parameters from two or more noncoincident spin-lattice tensors attached to the same rigidly moving fragment.45,72 By using Eqs. (2.3)–(2.5) and (2.9), we can recast Eq. (2.20) on the form

\[
S^2 = \left( 1 + \frac{2}{3} \right)^{-1} \times \sum_p \left| \langle D_{pq}^2(\Omega_{DF}) \rangle + \frac{\eta}{6} (\langle D_{pq}^2(\Omega_{DF}) \rangle + \langle D_{pq}^2(\Omega_{DF}) \rangle) \right|^2.
\]

(6.1)

Unless the F and I frames happen to coincide \( (\Omega_{IF}=0) \), the Euler angles \( \Omega_{DF} \) are not the natural dynamical variables and the quantities \( \langle D_{pq}^2(\Omega_{DF}) \rangle \) in Eq. (6.1) are not the natural partial order parameters. The I frame is usually defined in such a way that the POMT \( U(\Omega_{DF}) \) is minimized when \( \Omega_{DF} = 0 \). Then, for example, the partial order parameter \( \langle D_{00}^2(\Omega_{DF}) \rangle \) does not necessarily assume a maximum value of 1 for complete ordering of the fragment to which the F frame is attached. In applications to dipolar relaxation data, Eq. (6.1) with \( \eta=0 \) is generally used. Estimates of configurational entropy for the degrees of freedom associated with the observable internal motion are commonly derived from the order parameter with the aid of a one-parameter representation of the POMT that assumes that the minimum-energy configuration corresponds to the bond vector (for example, the peptide N–H bond) being aligned with the local director \( z_D \).73–77 Of course, one can always define the D frame so that this condition is satisfied, but the POMT then cannot be modeled in terms of a single parameter unless it happens to be uniaxial in both the D and F frames.

3. Exponential decay of internal TCF

None of the MF formulas in Sec. II make any assumptions about the mathematical form of the reduced internal TCF(s). In contrast, both MF-A and MF-C were originally presented with the additional approximation of exponentially decaying internal TCF(s).10,17 As noted by Lipari and Szabo, this approximation is not needed when the internal motion is in the extreme motional narrowing regime.10 They also made the important observation that when the exponential approximation is numerically accurate, which is often the case, then the decay time is \textit{exactly} the effective correlation time defined in Eq. (2.23). Nevertheless, for conceptual and practical reasons, we prefer to retain the generality of the basic MF formulas by not invoking further approximations until they are needed. The most important advantage of the MF approach is that it separates the available information about the internal motion into an equilibrium quantity, \( S \), and a kinetic quantity, \( C_{\text{internal}}(t) \). The order parameter \( S \) is not a true equilibrium property since it is “filtered” by the global motion (internal motions \textit{much} slower than the global motion are not manifested in \( S \)), but it is independent of the mechanistic details of the internal motion, such as the symmetry of the internal mobility tensor. This advantage is most decisive when the internal motion is much faster than the global motion, as is usually the case. Then, because the internal and global relaxation dispersions do not overlap, \( S \) can be determined without the need for realistic modeling of \( C_{\text{internal}}(t) \).

4. Symmetry of internal motion

In the original derivation of MF-A,10 it was assumed that the internal mobility tensor is isotropic. As shown by the derivation in Sec. II A, this assumption is unnecessary. The MF-A formula (2.21) is valid for any kind of internal motion, with \( C_{\text{internal}}(t) \) given explicitly by Eqs. (2.18) and (2.22). Of course, a subsequent single-exponential approximation is likely to be less numerically accurate if the internal mobility tensor has lower symmetry (for example, if two different internal diffusion coefficients are involved). However, this complication does not affect the validity of the basic MF-A formula (2.21) or the accuracy of the order parameter derived with the aid of this formula. This general conclusion is consistent with the results of stochastic simulations for a particular dynamical model.21

5. Anisotropic global motion

Lipari and Szabo argued that the MF-A formula, although no longer rigorous, remains an accurate approximation also for anisotropic global motion.10 For a uniaxial (symmetric-top) rotational diffusion tensor, the factor \( \text{exp}(-t/\tau_{\text{global}}) \) in Eq. (2.21) can then simply be replaced by the reduced global TCF in Eq. (2.39). However, Lipari and Szabo tested this approximation for the diffusion-in-a-cone model with internal diffusion much faster than global diffusion. Both approximations in MF-B are then valid so, in effect, they tested the MF-B formula (2.40), which is exact under these conditions. The good numerical agreement found can thus not be taken as proof for the validity of MF-A with
anisotropic global motion under other conditions, notably when the internal motions are not time-scale separated.

**B. Validity of the SRLS model**

Freed and co-workers\(^{18,20,22–27}\) describe the SRLS model as a more accurate generalization of the MF approach. Specifically, they argue that the SRLS model is more accurate because it incorporates a number of features that they believe are neglected in the MF approach. These features are (in their own words) as follows:\(^{20}\)

1. a proper treatment of general features of local geometry,
2. rhombic local ordering,
3. axial local motion, and
4. rigorous account of mode coupling.

We now examine each of these claims.

**1. Local geometry**

Freed et al. asserted that the MF approach is limited to the case where the principal frames of the spin-lattice interaction and internal mobility tensors coincide, that is, when \(\Omega_{IL}=0\). This simplifying assumption was made by Lipari and Szabo,\(^10\) but it is not required to obtain the MF-A formula (2.21). As seen from Eq. (2.9), the geometrical coefficient \(\sigma_g\), which appears in the definition (2.20) of the generalized order parameter and in the definition (2.18) of the internal TCF, allows for arbitrary relative orientation of the I and F frames.

**2. Local ordering**

In general, the POMT can be expanded in the complete basis of Wigner functions,

\[
U(\Omega_{DF}) = -\sum_{L=0}^{\infty} \sum_{p=q=L}^{L} C_{pq} D_{pq}^L(\Omega_{DF}).
\]

Freed and co-workers\(^{18,20,22–27}\) approximated this infinite series by one or two terms. Their most general potential model is

\[
U(\Omega_{DF}) = -C_{20} D_{20}^2(\Omega_{DF}) - C_{22} [D_{02}^2(\Omega_{DF}) + D_{0-2}^2(\Omega_{DF})].
\]

For this particular model, which presupposes cylindrical symmetry in the D frame and \(D_{2h}\) symmetry in the I frame, only two of the 25 partial order parameters survive,

\[
S_{20} = \langle D_{20}^2(\Omega_{DF}) \rangle = \frac{1}{2} (3\cos^2\varphi_{DF} - 1),
\]

\[
S_{22} = \langle D_{02}^2(\Omega_{DF}) \rangle + \langle D_{0-2}^2(\Omega_{DF}) \rangle = \frac{\sqrt{6}}{2} (\sin^2\varphi_{DF} \cos 2\gamma_{DF}).
\]

These are referred to as “axial” and “rhombic” order parameters.\(^{18,20,22–27}\)

Freed et al.\(^{18,20,24–27}\) insisted that MF-A is valid only for an “axially symmetric” POMT, by which they mean that \(S_{20}\) but not \(S_{22}\) is included in MF-A. However, as explained in Sec. VI A 2, MF-A is completely general as regards the symmetry of the POMT. Even MF-B is based on a more general POMT than the SRLS model. As seen from Eq. (2.36), the order parameter in MF-B features 5, in general distinct, partial order parameters \(\langle D_{00}^0(\Omega_{DF}) \rangle\). Therefore, \(S\) cannot be expressed in terms of \(S_{20}\) and \(S_{22}\) alone. Apart from the imposed symmetries, the validity of the POMT model in Eq. (6.3) is limited by the omission of the leading terms (of rank \(L=1\)) in the expansion (6.2). For example, the interaction of the fragment dipole with the local electric field gives rise to a rank-1 potential.

**3. Internal mobility tensor**

As explained in Sec. VI A 4, the original restriction to an isotropic internal mobility tensor\(^10\) is not required to obtain the MF-A formula (2.21). In fact, the internal TCF in Eq. (2.18) allows for a biaxial internal mobility tensor and is thus more general than the SRLS model, which assumes a uniaxial tensor.

**4. Dynamical coupling**

The principal justification for replacing the popular MF approach with the computationally intensive and less transparent SRLS model is the asserted ability of the latter to account rigorously for dynamical coupling between internal and global motions.\(^{18,20,24–27}\) Freed et al. assumed that the SRLS model is more accurate than the MF approach and, therefore, when they analyzed the same data with either SRLS or MF and obtained different results, they attributed the difference to the shortcomings of MF. However, the present analysis indicates that these differences have two principal causes.

First, by not recognizing the true generality of the MF approach, Freed et al. interpreted the MF parameters too narrowly. For example, they identified the generalized order parameter \(S\) of MF-A with their axial order parameter \(S_{20}\) rather than with the more general equation (2.20). Second, our analysis of the planar SRLS model shows that this model produces unphysical results that are not attributable to dynamical coupling (Sec. IV). This applies also to the version of the SRLS model used by Freed et al. For example, for \(D_{02}/D_{00}=0.57\) the planar SRLS model (with a hard repulsive POMT) yields \(\tau_{global}/\rho_{global}=1.57\), not far from the value 1.49 obtained with Freed’s version of the SRLS model (with a \(\cos^2\varphi_{DF}\) POMT).\(^{25}\) This substantial slowing down of protein tumbling by the internal motion is an artifact of the SRLS model, but Freed et al. regarded it as a real effect and they argued that the MF approach is inaccurate because it does not predict such behavior.\(^{24}\) We conclude that the SRLS model lacks rigor and is less accurate than the MF approach (which neglects dynamical coupling) under the conditions where dynamical coupling is expected to be most important (when \(D_{02}=D_{00}\)). In particular, protein tumbling times extracted from SRLS analysis are likely to be inaccurate.\(^{78}\)

Freed and co-workers\(^{20}\) asserted that the internal and global motions in the SRLS model are dynamically decoupled only if these motions occur on different time scales (adiabatic limit) and if the local ordering is either very weak or very strong (so that the order parameter is either \(\ll 1\) or...
close to 1). However, our analysis of the planar SRLS model (Sec. V) shows that all effects of dynamical coupling vanish in the adiabatic limit without any condition on the order parameter (or confinement angle \( \alpha \)). This behavior is consistent with the general fact that time-scale separation implies statistical independence (Sec. III).

The conclusions of the present study regarding the effect of dynamical coupling of internal and global motions on the analysis of NMR relaxation data may be summarized in the following points.

1. If the internal motion is much faster than the global motion, there can be no dynamical coupling and the MF approach is rigorously valid.

2. If the internal and global motions occur on the same time scale, they may be dynamically coupled. However, in folded proteins, internal motions on this time scale tend to be intermittent, and the superposition approximation invoked in MF-A is then valid as long as the internal motion does not alter the shape of the protein substantially (hydrodynamic coupling).

3. In their present forms, neither the SRLS model nor the MF approach treats hydrodynamic coupling.

4. In the SRLS model, the internal motion can be slow only if the diffusion coefficient \( D_I \) is small, but in folded proteins slow internal motions usually involve barrier crossings. The SRLS model is therefore not applicable to typical slow internal motions in folded proteins.

5. Even under the rare conditions where nonhydrodynamic coupling may be significant, the usefulness of the SRLS model is compromised by its unphysical foundation: a two-body SE that ignores the inherent asymmetry between internal and global degrees of freedom.

6. The SRLS model is rigorous only in the adiabatic limit (\( D_I \gg D_G \)), where it offers no advantage over the more general MF approach.

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(Appendix A), for the planar 2-state jump model (Appendix B), and for the planar 2-state jump model with hydrodynamic coupling (Appendix C).

The physical basis of model-free analysis of NMR relaxation data from proteins and complex fluids

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APPENDIX A – TCF FOR SRLS MODEL

Here we derive an analytical expression for the TCF $C(t)$ for the planar SRLS model by first solving the boundary value problem for the modified marginal propagator $Q(\varphi_{GI}, t \mid \varphi_{GI}^0)$ defined in Eq. (4.5). To simplify the notation, we use a reduced time variable $\tau \equiv (D_I + D_G) t$ and we denote $\varphi_{GI}$ by $\varphi$.

Multiplying Eq. (4.14) by $\exp(-ik\varphi_{LG}^0) \exp(ik\varphi_{LG})$ and integrating $\varphi_{LG}$ and $\varphi_{LG}$ from 0 to $2\pi$ using the periodic boundary conditions, we find that the modified marginal propagator satisfies the parabolic partial differential equation

$$\frac{\partial}{\partial \tau} Q(\varphi, \tau \mid \varphi^0) = \mathcal{L} Q(\varphi, \tau \mid \varphi^0), \quad -\alpha < \varphi < \alpha, \quad t > 0. \quad (A1)$$

The differential operator is

$$\mathcal{L} = \frac{\partial^2}{\partial \varphi^2} + i 2 \gamma k \frac{\partial}{\partial \varphi} - \gamma k^2, \quad (A2)$$

with $\gamma$ defined in Eq. (4.15). Similarly, from Eqs. (4.16) and (4.17) we obtain the boundary conditions

$$\frac{\partial}{\partial \varphi} Q(\varphi, \tau \mid \varphi^0) \bigg|_{\varphi = \pm \alpha} + i \gamma k Q(\pm \alpha, \tau \mid \varphi^0) = 0, \quad (A3)$$

and the initial condition

$$Q(\varphi, 0 \mid \varphi^0) = \delta(\varphi - \varphi^0). \quad (A4)$$

The separation ansatz

$$Q(\varphi, \tau) = U_n(\varphi) \exp(-i \gamma k \varphi) \exp(-\lambda_n t), \quad (A5)$$
yields the eigenvalue equation
\[
\left[ \frac{d^2}{d\varphi^2} - \gamma (1 - \gamma) k^2 \right] U_n(\varphi) = -\lambda_n U_n(\varphi) , \quad -\alpha < \varphi < \alpha , \quad (A6)
\]
with Neumann boundary conditions
\[
\left. \frac{dU_n}{d\varphi} \right|_{\varphi = \pm \alpha} = 0 . \quad (A7)
\]
Since the differential operator within brackets in Eq. (A6), as well as the boundary conditions (A7), are self-adjoint (Hermitian), the eigenvalues \( \lambda_n \) are real and the eigenfunctions \( U_n(\varphi) \) constitute a complete orthogonal set. With suitable normalization, we thus have
\[
\int_{-\alpha}^{\alpha} d\varphi U_n^*(\varphi) U_p(\varphi) = \delta_{np} , \quad (A8)
\]
It then follows from Eq. (A5) that \( Q(\varphi, \tau \mid \varphi^0) \) can be expanded as
\[
Q(\varphi, \tau \mid \varphi^0) = \exp[-i \gamma k (\varphi - \varphi^0)] \sum_{n=0}^{\infty} U_n(\varphi) U_n^*(\varphi^0) \exp(-\lambda_n \tau) . \quad (A9)
\]
By combining Eqs. (4.6), (4.7) and (A9), we can express the TCF as an eigenmode expansion:
\[
C(\tau) = \sum_{n=0}^{\infty} C_n \exp(-\lambda_n \tau) , \quad (A10)
\]
with real-valued and non-negative mode amplitudes
\[
C_n = \frac{1}{2\alpha} \left| \int_{-\alpha}^{\alpha} d\varphi \exp[i (1 - \gamma) k \varphi] U_n(\varphi) \right|^2 . \quad (A11)
\]
Noting that $C(0) = 1$, Eq. (A10) immediately yields the sum rule

$$\sum_{n=0}^{\infty} C_n = 1. \quad (A12)$$

It now only remains to solve the eigenvalue equation (A6). For $\gamma = 0$, Eqs. (A6) and (A7) constitute a standard eigenvalue problem with eigenvalues $\lambda_n = (n\pi/2\alpha)^2$ and orthonormal eigenfunctions

$$U_n(\phi) = \begin{cases} 
\frac{1}{\sqrt{2\alpha}}, & n = 0, \\
\frac{1}{\sqrt{\alpha}} \cos \left[\frac{n\pi}{2\alpha} (\phi - \alpha)\right], & n = 1, 2, 3, \ldots.
\end{cases} \quad (A13)$$

Since the $\gamma (1 - \gamma) k^2$ term can be incorporated in the eigenvalue on the right-hand side of Eq. (A6), it is clear that the eigenfunctions $U_n(\phi)$ are independent of $\gamma$, and are thus given by Eq. (A13), and that the eigenvalues are

$$\lambda_n = \left(\frac{n\pi}{2\alpha}\right)^2 + \gamma (1 - \gamma) k^2, \quad n = 0, 1, 2, \ldots. \quad (A14)$$

Finally, we substitute the eigenfunctions from Eq. (A13) into Eq. (A11) and integrate to obtain

$$C_0 = \left(\frac{\sin \sigma}{\sigma}\right)^2, \quad (A15)$$

and

$$C_n = \frac{\sigma^2 [1 - (-1)^n \cos(2\sigma)]}{[(n\pi/2)^2 - \sigma^2]^2}, \quad n = 1, 2, 3, \ldots, \quad (A16)$$

where we have defined

$$\sigma \equiv (1 - \gamma) k \alpha. \quad (A17)$$
In Sec. IV, we have redefined the mode amplitudes as $A_n = C_n/(1 - C_0)$. 
Here we derive the TCF for the planar model with intermittent jumps between the two internal states $\nu_+$ and $\nu_-$ with orientations $\varphi_{\text{GI}}(\nu_{\pm}) = \pm \alpha$ and mean residence times $\tau_{\pm}$. The joint propagator obeys the master equation (5.1):

\[
\frac{\partial}{\partial t} P(\varphi_{\text{LG}}, \nu_+, t | \varphi_{\text{LG}}^0, \nu^0) = \left[ D_G \frac{\partial^2}{\partial \varphi^2} - \frac{1}{\tau_+} \right] P(\varphi_{\text{LG}}, \nu_+, t | \varphi_{\text{LG}}^0, \nu^0) + \frac{1}{\tau_-} P(\varphi_{\text{LG}}, \nu_-, t | \varphi_{\text{LG}}^0, \nu^0),
\]

and a similar equation with the state indices $+$ and $-$ interchanged. The boundary conditions are periodic in $\varphi_{\text{LG}}$ and the initial condition is

\[
P(\varphi_{\text{LG}}, \nu, 0 | \varphi_{\text{LG}}^0, \nu^0) = \delta(\varphi_{\text{LG}} - \varphi_{\text{LG}}^0) \delta_{\nu \nu^0}.
\]

Defining the modified marginal propagator $Q(\nu, t | \nu^0)$ as in Eq. (4.5), we obtain from Eq. (B1)

\[
\frac{d}{dt} Q(\nu_+, t | \nu^0) = - \left[ k^2 D_G + \frac{1}{\tau_+} \right] Q(\nu_+, t | \nu^0) + \frac{1}{\tau_-} Q(\nu_-, t | \nu^0),
\]

and a similar equation with $+$ and $-$ interchanged. The initial condition is

\[
Q(\nu, 0 | \nu^0) = \delta_{\nu \nu^0}.
\]

We define an auxiliary quantity $\tilde{Q}(\nu, t | \nu^0)$ through

\[
Q(\nu, t | \nu^0) \equiv \tilde{Q}(\nu, t | \nu^0) \exp(-k^2 D_G t),
\]
and write the resulting evolution equations in matrix form as

$$\frac{d}{dt} \tilde{Q} = L \tilde{Q}, \quad (B6)$$

where $\tilde{Q}$ is a column vector with elements $\tilde{Q}(\nu_\pm, t | \nu^0)$ and $L$ is the rate matrix

$$L = \frac{1}{\tau^0_{\text{internal}}} \begin{pmatrix} -P_- & P_+ \\ P_- & -P_+ \end{pmatrix}, \quad (B7)$$

where $\tau^0_{\text{internal}}$ is the conformational exchange time defined in terms of the mean residence times $\tau_-$ and $\tau_+$ as in Eq. (5.4). To obtain this form, we have made use of the detailed balance condition

$$\frac{P_-}{\tau_-} = \frac{P_+}{\tau_+}, \quad (B8)$$

where $P_-$ and $P_+$ are the equilibrium populations in the two states, normalized as

$$P_- + P_+ = 1. \quad (B9)$$

Equation (B6) can be solved, for example, by symmetrizing the matrix $L$ and then finding its eigenvalues and eigenvectors. The result is

$$\tilde{Q}(\nu_+, t | \nu^0) = P_+ + P_- \exp(-t/\tau^0_{\text{internal}}), \quad (B10a)$$

$$\tilde{Q}(\nu_-, t | \nu^0) = P_- \left[1 - \exp(-t/\tau^0_{\text{internal}})\right], \quad (B10b)$$

and two similar equations with + and − interchanged.
The TCF is obtained from the discrete internal-state version of Eq. (4.6):

\[ C_0(t) = \sum_{\nu_0} \exp[-ik\varphi_{G1}(\nu_0)] P_{eq}(\nu_0) \sum_{\nu} \exp[i k \varphi_{G1}(\nu)] Q(\nu, t | \nu_0). \]  

(B11)

Combining Eqs. (B5), (B10) and (B11), and making use of the normalization (B9), we obtain the desired result

\[ C_0(t) = \exp(-k^2D_G t) \left\{ 1 - 4P_- P_+ \sin^2 k\alpha [1 - \exp(-t/\tau_{\text{internal}})] \right\}, \]  

(B12)

which can be rearranged on the MF-A form as in Eq. (5.3).
Here we derive the TCF for the two-state jump model in Appendix B with uniform internal distribution \( P_+ = P_- = 1/2 \), but with a global diffusion coefficient that depends on the internal state: \( D_G(\nu) = D^\pm_G \) for \( \varphi = \pm \alpha \). The evolution of the modified marginal propagator is governed by Eq. (B3) with \( D_G \) replaced by \( D^\pm_G \). In place of Eq. (B5), we define
\[
Q(\nu, t | \nu^0) \equiv \tilde{Q}(\nu, t | \nu^0) \exp(-k^2 D_G t),
\] (C1) with the mean global diffusion coefficient
\[
\bar{D}_G \equiv \frac{1}{2} (D^+_G + D^-_G).\] (C2)
The quantity \( \tilde{Q}(\nu, t | \nu^0) \) obeys Eq. (B6) with the symmetric rate matrix
\[
L = \lambda \begin{pmatrix}
-\delta - 1 & 1 \\
1 & \delta - 1
\end{pmatrix},\] (C3)
with
\[
\lambda \equiv \frac{1}{2 \tau^0_{\text{internal}}},\] (C4)
\[
\delta \equiv k^2(D^+_G - D^-_G) \tau^0_{\text{internal}}.\] (C5)
and \( \tau^0_{\text{internal}} \) defined in Eq. (5.4).
The eigenvalues and eigenvectors of \( L \) are readily obtained, leading to

\[
\tilde{Q}(\nu_+, t | \nu_+) = \frac{1}{1 + \Delta^2} \exp(-\lambda t) \left[ \exp(\kappa \lambda t) + \Delta^2 \exp(-\kappa \lambda t) \right],
\]

(C6a)

\[
\tilde{Q}(\nu_-, t | \nu_-) = \frac{1}{1 + \Delta^2} \exp(-\lambda t) \left[ \Delta^2 \exp(\kappa \lambda t) + \exp(-\kappa \lambda t) \right],
\]

(C6b)

\[
\tilde{Q}(\nu_+, t | \nu_-) = \tilde{Q}(\nu_-, t | \nu_+) = \frac{\Delta}{1 + \Delta^2} \exp(-\lambda t) \left[ \exp(\kappa \lambda t) - \exp(-\kappa \lambda t) \right],
\]

(C6c)

with

\[
\kappa \equiv \sqrt{1 + \delta^2},
\]

(C7)

\[
\Delta \equiv \delta + \kappa.
\]

(C8)

The TCF in Eqs. (5.7) – (5.13) is obtained by combining Eqs. (C1), (C6) and (B11).