A Structural Mode-Coupling Approach to $^{15}$N NMR Relaxation in Proteins

Vitali Tugarinov, Zhichun Liang, Yury E. Shapiro, Jack H. Freed, and Eva Meirovitch

Contribution from the Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel, and Baker Laboratory of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14853-1301

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Abstract: The two-body Slowly Relaxing Local Structure (SRLS) model was applied to $^{15}$N NMR spin relaxation in proteins and compared with the commonly used original and extended model-free (MF) approaches. In MF, the dynamic modes are assumed to be decoupled, local ordering at the N–H sites is represented by generalized order parameters, and internal motions are described by effective correlation times. SRLS accounts for dynamical coupling between the global diffusion of the protein and the internal motion of the N–H bond vector. The local ordering associated with the coupling potential and the internal N–H diffusion are tensors with orientations that may be tilted relative to the global diffusion and magnetic frames. SRLS generates spectral density functions that differ from the MF formulas. The MF spectral densities can be regarded as limiting cases of the SRLS spectral density. SRLS-based model-fitting and model-selection schemes similar to the currently used MF-based ones were devised, and a correspondence between analogous SRLS and model-free parameters was established. It was found that experimental NMR data are sensitive to the presence of mixed modes. Our results showed that MF can significantly overestimate order parameters and underestimate local motion correlation times in proteins. The extent of these digressions in the derived microdynamic parameters is estimated in the various parameter ranges, and correlated with the time scale separation between local and global motions. The SRLS-based analysis was tested extensively on $^{15}$N relaxation data from several isotropically tumbling proteins. The results of SRLS-based fitting are illustrated with RNase H from E. coli, a protein extensively studied previously with MF.

Introduction

The ability to interpret nuclear spin relaxation properties in terms of microdynamic parameters turned NMR into a powerful method for elucidating protein dynamics.\(^1\) The amide $^{15}$N spin in proteins is a particularly useful probe, relaxed predominantly by dipolar coupling to the amide proton and $^{15}$N chemical shift anisotropy (CSA).\(^3\) The experimental NMR observables ($^{15}$N T1, T2, and $^{15}$N–$^1$H NOE acquired at one or more magnetic fields) are controlled by the global and local dynamic processes experienced by the N–H bond vector. The model-free (MF) approach in its original\(^4,\)\(^5\) and extended\(^6\) forms is currently the most popular means of analyzing experimental NMR data in terms of microdynamic parameters associated with the N–H bond vector motions. One of the fundamental assumptions underlying the MF formulation is that the global diffusion of the protein and the internal motion of the N–H bond vector are not correlated (coupled). This “decoupling” approximation allows one to separate the two types of motions and represents the autocorrelation function, C(t), of the overall dynamic process as a product of the global, $C_o(t)$, and the internal, $C_i(t)$, correlation functions:\(^4,\)\(^5\)

$$\text{C}(t) = \text{C}_o(t) \text{C}_i(t) \tag{1}$$

The resulting spectral density function, $J(\omega)$, is given by a weighted sum of Lorentzians. This assumption is preserved in the extended MF treatment\(^6\) although the resulting time scale separation between the global diffusion and the slow local (nanosecond) motion is typically only about 1 order of magnitude.

Recently, a semiempirical mode-coupling diffusion approach was developed\(^7,\)\(^8\) and applied to the derivation of local dynamics in proteins.\(^9\) However, experimental $^{15}$N T1 patterns could not be reproduced for magnetic fields exceeding 8.4 T, where the contribution of local motions becomes significant. This approach is based on molecular dynamics simulations and cannot reliably account for slower motions in proteins.\(^3\) The common MF approach also has been extended by applying the Gaussian Axial Fluctuations (GAF) model,\(^10\)\(^–\)\(^12\) which interprets the generalized...
order parameter in terms of fast fluctuations about three orthogonal axes, but otherwise preserves the MF spectral density.

Here, we report on the analysis of NMR $^{15}$N relaxation data using the Slowly Relaxing Local Structure (SRLS) model. SRLS was implemented originally\(^\text{(13,14)}\) as an approximate theory appropriate for small local ordering and applied to ESR spin probes and NMR in liquid crystals.\(^\text{(14)}\) In recent years SRLS was developed into a comprehensive rigorous stochastic-two-body mode-coupling theory\(^\text{(15,16)}\) and applied to ESR studies of biomolecular dynamics.\(^\text{(17–19)}\) In the latter application the two coupled modes represent the global motion of the macromolecule and the internal motion of the spin-bearing moiety. In the context of amide $^{15}$N spin relaxation in proteins, SRLS is a powerful theoretical tool that rigorously accounts for the dynamical coupling between the global diffusion of the protein and the local diffusion of the N–H bond vector. In SRLS, the global diffusion, the local diffusion, the local ordering, and the magnetic interactions are tensors that may be tilted relative to one another. The time-independent geometric relations contain important information related to protein structure. The SRLS theory can be viewed as a generalization of MF. For low ordering SRLS was shown theoretically to converge to MF in the motional narrowing limit.\(^\text{(15)}\) In the present study the computational SRLS methodology\(^\text{(15,16)}\) was adapted to the calculation of spectral densities for NMR spin relaxation in the case of isotropic global tumbling. The SRLS model was parametrized in a way very similar to the conventionally employed MF parametrization, and used to fit NMR experimental relaxation data. Our results show that model-free can significantly overestimate order parameters and underestimate local motion correlation times. The extent of these digressions in the derived microdynamic parameters was estimated in the various parameter ranges, and correlated with the time scale separation between the local and global motions.

**Theory**

The fundamentals of SRLS theory, discussed recently in the context of biomolecular dynamics, are directly applicable to N–H bond vector motions in proteins. The coordinate frames required to describe the SRLS model are depicted in Figure 1a. The laboratory frame (L) is a space-fixed frame with its $z$-axis along the applied magnetic field. CSA and D are the $^{15}$N chemical shift anisotropy and the N–H dipolar magnetic tensor frames, respectively. The dipolar tensor frame has its $z$-axis aligned along the N–H bond (Figure 1b). The Euler angles $\Omega_{\text{LD}}$ and $\Omega_{\text{LSA}}$ are the usual stochastic variables of magnetic resonance spin relaxation, modulated by local motion of the N–H bond vector and the global molecular tumbling. The internal diffusion frame (M) relates to the local N–H bond vector motions. The M frame can be tilted relative to the N–H bond vector (or D frame) by a set of time-independent Euler angles $\Omega_{\text{MD}}(\Omega_{\text{SD}}, \beta_{\text{MD}}, \gamma_{\text{MD}})$. If we assume the local motion to be axially symmetric only two angles, $\beta_{\text{MD}}$ and $\gamma_{\text{MD}}$, remain. The global diffusion frame (C) is a molecule-fixed frame determined mainly by the protein shape. The N–H bond vector diffuses in a highly anisotropic environment (due to geometrical and structural/motional restrictions) and experiences a mean orienting potential with symmetry axes that may be different from the C frame. We therefore define an internal ordering (director) frame (C') that is fixed relative to the C frame. For isotropic global tumbling the distinction between C and C' frames vanishes and they become the same (cage frame in Figure 1a, denoted as C below). To summarize, the local diffusion of the N–H bond vector and the local ordering induced by the globally tumbling surroundings (cage) are represented by tensors that
may be tilted relative to the N–H bond vector (D frame) and the CSA tensor.

The N–H bond vector motions and the cage are dynamically coupled by a potential $U(\Omega_{CM})$ that depends on their relative orientation through time-dependent Euler angles $\Omega_{CM}(t)$. The coupling potential tends to align the N–H bond vector toward the $z$-axis of the ordering frame. In the simplest case of axially symmetric local ordering it is given by:

$$ U(\Omega_{CM})/k_B T = -c_{20}D_{20}^2(\Omega_{CM}) = -u(\Omega_{CM}) $$

where $k_B$ is the Boltzmann constant, $T$ the temperature in K, $c_{20}$ the potential strength in units of $k_B T$, and $D_{20}^2$ the Wigner rotation matrix element. A conventional order parameter can be related to $c_{20}$ as:

$$ S = \langle D_{20}^2(\Omega_{CM}(t)) \rangle $$

where

$$ \langle D_{20}^2(\Omega_{CM}(t)) \rangle = \int D_{20}^2(\Omega) \exp[c_{20}D_{20}^2(\Omega)] \, d\Omega / \int \exp[c_{20}D_{20}^2(\Omega)] \, d\Omega. $$

A plot of the squared order parameter, $S^2$, versus $c_{20}$ is shown in Figure 2. It should be noted that the very definition of $S$ requires axial (or lower) symmetry of the internal diffusion tensor.

The time-dependent part of the spin Hamiltonian for this two-body system is given by:

$$ \hat{H} = \sum_{\mu=CSA,D} \sum_{l=0,2} \sum_{m=-l}^{l} \sum_{m'=-l}^{l} \sum_{m''=-l}^{l} \hat{X}_{\mu,L}^{(lm)} D_{mm'}^{(lm)}(\Omega_{LM}) \hat{D}_{mm''}^{(lm)}(\Omega_{MCSA}) F_{\mu,CSA}^{lm} $$

where $\hat{X}_{\mu,L}^{(lm)}$ stands for the $m$th component of the $l$th ($l = 0, 2$) rank irreducible spherical tensor or tensor operator $X$ (where $X$ is either a spin operator $\hat{A}$ or a magnetic tensor $F$) defined in the N frame, with $\mu$ specifying the kind of interaction ($^{15}$N CSA or $^{15}$N–$^1$H dipolar). $D_{mm'}^{(lm)}(\Omega_{LM})$ are Wigner rotation matrix elements which relate the N frame to the N$'$ frame. The detailed form of $\hat{X}_{\mu,L}^{(lm)}$ and $F_{\mu,CSA}^{lm}$ can be found elsewhere.

The dynamic effects of the global and the local diffusion are incorporated into the spectral density through the diffusion operator:

$$ \hat{\Gamma} = \hat{\Gamma}_{global}(\Omega_{LC}) + \hat{\Gamma}_{local}(\Omega_{LM}) + F_{\mu,CSA}^{lm}(\Omega_{CM}) $$

where $\hat{\Gamma}$ is the infinitesimal rotation operator associated with this motion (and the super 2 implies the square), and $C = 1/(6\tau_m)$ is the diffusion constant for the global motion. The internal motion is given by an axially symmetric diffusion tensor:

$$ \hat{\Gamma}_{local}(\Omega_{LM}) = R_z \hat{J}_2^z + (R_{\parallel} - R_z) \hat{J}_2^z $$

where $\hat{J}_z$ is the infinitesimal rotation operator for the local motion with the $z$ component, $\hat{J}_2^z$, and $R_{\parallel}$ and $R_z$ are the principal values of the axial local diffusion tensor. The local motion within the macromolecule (cage) is restricted by the internal orienting potential $U(\Omega_{CM})$ (eq 2), which couples the global and local motions. The last two terms in eq 5 reflect the contributions to $\hat{\Gamma}$ due to $U(\Omega_{CM})$. $\hat{\Gamma}_{global}$ and $\hat{\Gamma}_{local}$ are functions of the Euler angles $\Omega_{CM}$ that transform the M frame into the C frame, which can be further expressed as $(-\Omega_{LC} + \Omega_{LM})$ (Figure 1a). The operator expressions for $\hat{\Gamma}_{global}$ and $\hat{\Gamma}_{local}$ are given by:

$$ F_{\mu,CSA}^{lm}(\Omega_{CM}) = (1/2)[R_{\parallel}^{\mu} \hat{J}_2^{\mu} u] + (R_{\parallel}^{\mu} - R_z^{\mu}) \hat{J}_2^{\mu} u - 1/4[R_z^{\mu} \hat{J}_2^{\mu} u] \hat{J}_2^{\mu} u + R_z^{\mu} \hat{J}_2^{\mu} u ] $$

and

$$ F_{\mu,CSA}^{lm}(\Omega_{CM}) = (1/4)R_z^{\mu} \hat{J}_2^{\mu} u - (\hat{J}_z^{\mu} u \hat{J}_z^{\mu} u) $$

This constitutes an effective two-body model for which a Smoluchowski equation representing the rotational diffusion of two interacting rotors is solved. The solution features three eigenvalues (correlation times) for the local motion when $S^2 = 0$:

$$ (\tau_K)^{-1} = 6R_{\parallel}^2 + K^2(R_{\parallel}^2 - R_z^2) $$

Each $K$ value leads to its own spectral density component. Even for $S^2 > 0$ the jk=±1(ω) and jk=±2(ω) components are mainly dominated by local motions, whereas the jk=0(ω) component represents mixed modes between the global and the local ($R_z^2$) motions. The “measurable” spectral density is then constructed out of the three jk(ω) components by incorporation of the orientation-dependent functions that multiply the spin operators in the spin Hamiltonian.

Assuming that the $^{15}$N CSA tensor is axially symmetric and collinear with the dipolar N–H tensor (θ = 0 in Figure 1b) the spectral density for $^{15}$N CSA and $^{15}$N–{`H} dipolar relaxation in the coordinate frame of the local motion is given by:

\[ J(\omega) = A_{J=k=0}(\omega) + B_{J=k=1}(\omega) + C_{J=k=2}(\omega) \]  

(11)

where \( A = (1.5 \cos^2 \beta_{MD} - 0.5)^2 \), \( B = 3 \sin^2 \beta_{MD} \cos^2 \beta_{MD} \), \( C = 0.75 \sin^4 \beta_{MD} \), and \( \beta_{MD} \) is the "diffusion tilt" angle between the molecular diffusion axis \( \mathbf{Z}_A \) and the N–H bond. In the present study the SRLS parameters featured by \( J(\omega) \) include three diffusion rate constants, \( R^2, R^2_L, R^2_R \), one potential parameter, \( c_{2D} \), and the polar angle \( \beta_{MD} \) (diffusion tilt) between the M and D frames (Figure 1). Special cases include the following: (1) isotropic fast local diffusion (local correlation time \( \tau_L = (6R^2)^{-1} \approx 6(6R^2)^{-1} \), implying \( \beta_{MD} = 0 \); then, \( J(\omega) = J_{k=0}(\omega) \), and the NMR relaxation data can be fit with one \( c_{2D} \) (if \( \tau_L \) is negligibly small) or two \( c_{2D} \) and \( \tau_L \) free parameters, in complete analogy with the original model-free formulation; 4, 5 (2) very anisotropic slow local motion (\( \tau_L \approx \tau_M \approx \tau_L \) and \( \tau_L \to 0 \), denoted VALM below; then the last two terms in eq 11 are negligibly small compared to \( A_{J=0}(\omega) \), provided \( \beta_{MD} \approx 54.7^\circ \), and NMR data can be reproduced with three free parameters \( c_{2D}, \tau_L, \beta_{MD} \). The coefficient \( A \) in eq 11 is formally analogous to the squared order parameter \( S_2^2 \), while \( S_{SRLS} \) (where \( S_{SRLS} \) is calculated using eq 3) is formally analogous to \( S_2^2 \), in the extended MF formula recast for \( \tau_L \to 0 \):  

\[ J(\omega) = S_2^2(1 + \omega^2 \tau_{MD}^2) + (1 - S_2^2)2/(1 + \omega^2 \tau_{MD}^2) \]  

(12)

where \( S_2 \) and \( \tau_{MD} \) are order parameters associated with the slow and fast local motions, respectively, \( \tau_{MD} \) is the correlation time of the global motion, and \( \tau_{MD} \) is the effective correlation time of the slow local motion. Within the scope of VALM the fast local motion represents diffusion about an axis close to the N–H bond (\( \mathbf{Z}_A \) in Figure 1b), and the slow local motion represents diffusion of the axis itself (about \( \mathbf{Y}_A \)). A formal correspondence between the extended MF order parameter \( S = S_2S_1 \) and the SRLS order parameter, \( S_{SRLS} \), can be established with the relation:  

\[ S = S_{SRLS}(1.5 \cos^2 \beta_{MD} - 0.5) \]  

(13)

If NMR data at more than one magnetic field are available, VALM can be extended to any degree of anisotropy in \( R^2 \) by including an additional free parameter \( \tau_L \). Then all the \( J_k(\omega) \) components contribute to \( J(\omega) \) in eq 11. A formal analogy is thus established between the extended MF formulation and VALM. 22 The global correlation time \( \tau_{MD} \) is not considered a free parameter in the present context, where we focus on the microdynamic parameters; it should be determined independently.

In principle, the CSA and dipolar magnetic tensors are not collinear (Figure 1b). Then, in the local diffusion coordinate frame the dipolar and CSA spectral densities differ. The CSA spectral density can then be corrected for noncollinearity 22 with \( \Delta J(\omega, \theta, \beta_{MD}, \gamma_{MD}) \) calculated by applying two consecutive rotations: from the M frame to the D frame and from the D frame to the CSA frame. In its most general form this correction term can be expressed as:  

\[ \Delta J(\omega) = 3/4 \{[J_{k=0}(\omega) - J_{k=1}(\omega)]F_A - [J_{k=1}(\omega) - J_{k=2}(\omega)]F_B \} \]  

(14)

The term \((1 - S_2^2)\omega/(1 + \omega^2 \tau_{MD})^2\), which should be added to eq 12 if no assumptions about \( \tau_L \) are made, is formally analogous to the second term on the right-hand side of eq 11, as \( S_2 = (1.5 \cos^2 \beta_{MD} - 0.5) \) and \( (1 - S_2^2) \approx 3 \sin^2 \beta_{MD} \cos^2 \beta_{MD} \) for small \( \beta_{MD} \). The third term of eq 11 can be neglected, as \( \sin^4 \beta_{MD} \) is very small in this case.


\[ F_A \] and \( F_B \) are complex trigonometric functions of the angles \( \theta, \beta_{MD}, \) and \( \gamma_{MD} \).

Numerical simulations showed that the sensitivity of \( ^{15N} T_1, T_2, \) and \( ^{15N} - [\{^1H] \) NOE to \( \gamma_{MD} \) increases with magnetic field strength because of the augmented CSA contribution. In general, the sensitivity of \( T_1 \) and \( T_2 \) to \( \gamma_{MD} \) variations is limited, while NOEs are approximately twice less sensitive to \( \gamma_{MD} \) than \( T_1 \) and \( T_2 \). To avoid an excess of free variables we fixed \( \gamma_{MD} \) at 90°. Then the perpendicular local motion represents excursions of the \( \mathbf{Z}_A \) axis out of the peptide plane approximately about the \( \mathbf{CM}(i-1) - \mathbf{CM}(i) \) axis (Figure 1b).

After the spectral density function \( J(\omega) \) has been constructed out of its fundamental \( j_x(\omega) \) components by using eq 11, the measurable \( ^{15N} \) relaxation quantities \( ^{15N} T_1, ^{15N} T_2, \) and \( ^{15N} - [\{^1H \) NOE values are calculated as a function of \( J(0), J(\omega), J(\omega), J(\omega), \) \( J(\omega) \), using standard expressions for NMR spin relaxation. 3, 24

**Methods and Calculations**

The complete SRLS computational strategy, including the optimal choice of the basis set, was described previously. 15, 16 The calculation of SRLS spectral densities is computationally intensive for \( c_{2D} \) values exceeding \( \sim 10 \) \( S^2 \) exceeding \( \sim 0.81 \) and/or very fast internal motions. Therefore, we used precalculated two-dimensional grids of \( J(0), J(\omega), J(\omega), J(\omega), \) \( J(\omega) \) to fit experimental \( ^{15N} T_1, T_2, \) and \( ^{15N} - [\{^1H \) NOE data. The \( j_k \) grids of spectral density values at the five frequencies were constructed under the assumption of isotropic global motion for sets of \( c_{2D} \) and \( \tau_L \) or \( \tau_L \) values. An axial \( ^{15N} \) chemical shielding tensor with \( \tau_L = 170 \text{ ppm} \) and \( \theta = \pm 16^\circ \), 23 were used in these calculations. The \( c_{2D} \) grid dimension spanned the values between 0 (\( S^2 = 0 \)) and 40 (\( S^2 = 0.95 \)), and the \( \tau_L \) dimension spanned the values between 0.0005\( r_a \) and 1.4\( r_a \). A two-dimensional polynomial interpolation on the pre-constructed grid using Neville’s algorithm 23 was employed for spectral density evaluation in the course of model fitting. The spectral density values at a fixed frequency are smooth functions of both \( c_{2D} \) and \( \tau_L \) or \( \tau_L \) and can be reliably interpolated. The interpolation errors in both the \( c_{2D} \) and \( \tau_L \) grid dimensions were estimated to be at least 1 order of magnitude smaller than the errors in microdynamic parameters assessed with currently available experimental NMR techniques.

The fitting of experimental NMR data was based on target function minimization. For measurements carried out at one magnetic field the target function for spin \( i \) was defined as the sum of the squared differences between experimental and calculated \( T_i, \) \( T_i, \) and NOE values divided by the squared random errors:  

\[ x_i^2 = \left[ (T_i^{\text{obs}} - T_i^{\text{calc}})|\sigma_{T_i}|^2 + (|T_i^{\text{obs}} - T_i^{\text{calc}})|\sigma_{T_i}|^2 + (|\text{NOE}_{\text{obs}} - \text{NOE}_{\text{calc}})|\sigma_{\text{NOE}}|^2 \right] \]  

(15)

The SRLS-based dynamic models employed in the fitting procedure are summarized in Table 1. In model 1 the local motion is so fast (\( \tau_L \to 0 \)) that its effect on the spectral density is negligible. This assumption is equivalent in practice to fixing \( \tau_L \) at the lowest value for which the SRLS spectral densities could be calculated. In model 2 it is assumed that the internal motion can be approximated as isotropic (\( \tau_L = \tau_0 \)). This model is analogous to the original MF formulation. Models 3 and 4 are derived from models 1 and 2, respectively, by addition of the free parameter \( R_a \) to the transverse relaxation rate expressions, to account for possible exchange processes on the microsecond to millisecond time scale. For models 1–4, \( \beta_{MD} = 0 \), hence \( J(\omega) = J_{k=0}(\omega) \) in eq 11 and the correction \( \Delta J(\omega) \) in eq 14 depends solely on

**Table 1.** SRLS Models Used to Fit Experimental $^{15}$N NMR Relaxation Data Acquired at One Magnetic Field

<table>
<thead>
<tr>
<th>model no.</th>
<th>parameters</th>
<th>SRLS model description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$c_{0}(S_{0})$</td>
<td>very fast internal motion ($\tau \rightarrow 0$)</td>
</tr>
<tr>
<td>2</td>
<td>$c_{20}(S_{2})$, $\tau(\tau_{l})$</td>
<td>isotropic internal motion</td>
</tr>
<tr>
<td>3</td>
<td>$c_{20}(S_{2})$, $R_{ex}$</td>
<td>model 1 with exchange term</td>
</tr>
<tr>
<td>4</td>
<td>$c_{20}(S_{2})$, $R(\tau_{l})$, $R_{ex}$</td>
<td>model 2 with exchange term</td>
</tr>
<tr>
<td>5</td>
<td>$c_{20}(S_{2})$, $\beta_{0}/S_{2}$, $\tau_{l}(\tau_{l})$</td>
<td>very anisotropic slow local motion</td>
</tr>
</tbody>
</table>

* Analogous MF parameters are shown in parentheses. For model 1 the $\tau \rightarrow 0$ assumption is practically equivalent to fixing $\tau$ at the lowest value for which the SRLS spectral densities can be calculated. For model 5 it is assumed that $\tau_{l} \rightarrow 0$. This assumption is equivalent to neglecting the $j_{K}=1(\omega)$ and $j_{K}=2(\omega)$ spectral density components.

The low-frequency regions of SRLS $j_{K}=0(\omega)$, $j_{K}=1(\omega)$, and $j_{K}=2(\omega)$ (solid lines) and MF $J(\omega)$ (dashed lines) functions calculated for $\tau_{m} = 4.0$ ns for several values of the internal motion correlation time ($\tau_{l}$). The internal motion was assumed to be isotropic.

**Figure 3.** The low-frequency regions of SRLS $j_{K}=0(\omega)$, $j_{K}=1(\omega)$, and $j_{K}=2(\omega)$ (solid lines) and MF $J(\omega)$ (dashed lines) functions calculated for $\tau_{m} = 4.0$ ns for several values of the internal motion correlation time ($\tau_{l}$). The internal motion was assumed to be isotropic.

**Table 2.** Parameters for the SRLS Spectral Density Components

<table>
<thead>
<tr>
<th>parameter</th>
<th>value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c_{0}$</td>
<td>0.1</td>
</tr>
<tr>
<td>$c_{20}$</td>
<td>0.05</td>
</tr>
<tr>
<td>$\tau_{l}$</td>
<td>0.1 ns</td>
</tr>
<tr>
<td>$\tau_{m}$</td>
<td>1 ns</td>
</tr>
</tbody>
</table>

The described model fitting and model-selection schemes were implemented in a fitting program used for SRLS data analysis. Error estimation of the fitted parameters was carried out using 100 Monte Carlo simulations.

The 2D grids were generated on a Microway workstation equipped with a 500 MHz Digital Alpha 21264 processor and a 500 MB memory. The CPU time required was 10 days for the $j_{K}=0(\omega)$ grid, and 20 days for each of the $j_{K}=1(\omega)$ and $j_{K}=2(\omega)$ grids, with several $c_{20}$ values exceeding 20. Once the grids were generated, they could be rapidly and repeatedly utilized in fitting experimental data.

**Results and Discussion**

The low-frequency regions of all three $K$ components of $J(\omega)$, calculated for isotropic global motion with $\tau_{m} = 4$ ns, are shown in Figure 3 as a function of the time scale separation between the local and global motions. The $j_{K}=1(\omega)$ and $j_{K}=2(\omega)$ components (Figure 3a–h,i–j) are dominated by the local motion and are almost independent of $c_{20}$. They contribute significantly to the “measurable” $J(\omega)$ only for slow local motions. The $j_{K}=0(\omega)$ component is similar to the MF spectral density (Figure 3a–d). In general, model-free underestimates spectral density values at low frequencies and slightly overestimates them at higher ones. These differences become more significant for higher values of $c_{20}$ (not shown) and slower local motions, i.e., with decreasing time scale separation between the local motion and the overall tumbling (cf. Figure 3a–d). It is important to note that for low ordering MF was shown to represent a limiting case of the SRLS theory. It was also shown that a small SRLS coupling potential has the same effect as anisotropic local diffusion, which manifests as an increase in $j_{K}=0(\omega)$ as compared to isotropic local diffusion. It can be shown that SRLS...
available NMR techniques the typical experimental error in the spectral density, i.e. a Lorentzian function of the local motion to a much larger extent than the contributions due to mixed modes. The relative difference between the global and local motions, the higher is the extent of the two modes (Figure 5c). The smaller the time scale separation which in this case determines the time scale separation between the local and the global motions also controls the relative differences in the slow motion correlation times (Figure 5b). For models with slow (very anisotropic) internal motions the relative overestimation by MF was found to be weakly field dependent in the range of 11.7–18.7 T and almost independent of the volume fraction of the slow motion correlation times (Figure 5b). For models with slow (very anisotropic) internal motions the relative differences in the obtained order parameters are approximately twice higher (cf. Figure 5c,d). For a fixed local motion correlation time these differences are strongly dependent on \( \tau_m \), which in this case determines the time scale separation between the two modes (Figure 5c). The smaller the time scale separation between the global and local motions, the higher is the extent to which \( S^2 \) is overestimated by MF. The time scale separation between the local and the global motions also controls the relative differences in the slow motion correlation times (Figure 5d). These results indicate that in the parameter range relevant for folded proteins the order parameters are significantly overestimated, whereas local motion correlation times are considerably underestimated by MF.

Theoretical \( ^{15}N \) T1, T2, \( ^{15}N-\{^{1}H\} \) NOE, and T1/T2 curves, calculated as a function of \( \tau_1 \) with both SRLS and MF for \( S^2 = 0.786 \), \( \tau_m = 12 \) ns, and 14.1 T magnetic field. Numerical simulations were carried out using SRLS-derived synthetic \( ^{15}N \) T1, T2, and NOE data sets with subsequent parametric fitting using MF. Figure 5a illustrates relative errors in the derived order parameters (\( S^2_{\text{MF}} - S^2_{\text{SRLS}} \)) for spins with negligible local motions. In this regime the extent of \( S^2 \) overestimation by MF was found to be weakly field dependent in the range of 11.7–18.7 T and almost independent of the global correlation time. The \( \tau_m \) values calculated from T1/T2 ratios using MF were essentially the same as those obtained using SRLS. The correlation times for fast internal motions were underestimated by MF more than 2-fold (Figure 5b). For models with slow (very anisotropic) internal motions the relative differences in the obtained order parameters are approximately twice higher (cf. Figure 5c,d). For a fixed local motion correlation time these differences are strongly dependent on \( \tau_m \), which in this case determines the time scale separation between the two modes (Figure 5c). The smaller the time scale separation between the global and local motions, the higher is the extent to which \( S^2 \) is overestimated by MF. The time scale separation between the local and the global motions also controls the relative differences in the slow motion correlation times (Figure 5d). These results indicate that in the parameter range relevant for folded proteins the order parameters are significantly overestimated, whereas local motion correlation times are considerably underestimated by MF.

Figure 4. Theoretical curves of (a) \( ^{15}N \) T1 (ms), (b) \( ^{15}N-\{^{1}H\} \) NOE, (c) \( ^{15}N \) T2 (ms), and (d) the T1/T2 ratio as a function of the local motion correlation time \( \tau_1 \) (ps) for SRLS (solid lines) and MF (dashed lines) calculated for \( S^2 = 0.786 \), \( \tau_m = 12.0 \) ns, and 14.1 T magnetic field.

**Figure 5.** Relative errors of SRLS and MF parameters associated with numerical simulations using the synthetic SRLS data fit with MF, shown as percent deviations: (a) $S^2_{\text{MF}} - S^2_{\text{SRLS}} / S^2_{\text{SRLS}}$ versus $S^2_{\text{SRLS}}$ for magnetic fields of 11.7 (dotted line), 14.1 (solid line), and 18.7 T (dashed line) for $t_\text{f} = 0$ and $t_\text{m} = 12$ ns. (b) $|t_{\text{MF}} - t_{\text{SRLS}}| / t_{\text{SRLS}}$ versus $t_{\text{SRLS}}$ for magnetic fields denoted as in panel a, for $S^2_{\text{SRLS}} = 0.786$ and $t_\text{m} = 12$ ns. (c) $|S^2_{\text{MF}} - S^2_{\text{SRLS}}| / S^2_{\text{SRLS}}$ versus $S^2_{\text{SRLS}}$ for $t_\text{f} = 4.8$ ns and $S^2_\text{f} = 0.734$. Data are given for $t_\text{m} = 8$ (squares), 12, and 16 ns (circles). For $t_\text{m} = 12$ ns the field dependence is also shown, as denoted in panel a. (d) $|t_{\text{MF}} - t_{\text{SRLS}}| / t_{\text{SRLS}}$ versus $t_{\text{SRLS}}$ for $S^2_\text{SRLS} = 0.366$ and $S^2_\text{f} = 0.734$. Data are given for $t_\text{m} = 12$ ns the field dependence is also shown, as denoted in panel a.

The SRLS-based analysis was tested extensively on $^{15}$N relaxation data from several isotropically tumbling proteins. RNase H from *E. coli,* extensively studied previously with MF,26,29 is chosen here as an illustrative example of the results of the SRLS-based fitting. The anisotropy of the global tumbling of RNase H was shown29 to be low ($\gamma_{\text{MD}} < 0.73$). Data are given for $t_\text{m} = 8$ (squares), 12, and 16 ns (circles). For $t_\text{m} = 12$ ns the field dependence is also shown, as denoted in panel a.

If NMR relaxation data acquired at more than one magnetic field are available, both angles $\hat{p}_{\text{MD}}$ and $\gamma_{\text{MD}}$ can be determined in principle. These angles fix the orientation of the local diffusion and local ordering axes, while their magnitude is determined by the local surroundings of the N–H bond vector. Thus, local structure affects local geometry via dynamical coupling. Domain motions of the kind encountered in many enzymes30–32 exemplify more indirect elements of dynamic structure, where mode-coupling is expected to have important implications. In such cases SRLS is expected to help correlate structural dynamics with function.

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The $j_{k-1}(\alpha)$ and $j_{k-2}(\alpha)$ spectral density contributions are associated with axial local diffusion and ordering tensors and nonzero “diffusion tilt” angles ($\beta_{MD}$). Models with $\beta_{MD} \neq 0$ (VALM) are therefore indicative of the fact that MF effective correlation times and generalized order parameters are no longer adequate descriptors of internal motions and local ordering. Rather, tensorial properties must be assigned to these variables. We found that experimental $^{15}$N NMR relaxation data featuring slow motions cannot be reproduced with eq 11 using the assumption of isotropic local diffusion. This is actually the consequence of the relatively high axial local ordering and small time scale separation between $t_m$ and $t_r$.

It is of interest to pinpoint the basic tenets of the mode-coupling diffusion theory\textsuperscript{7–9} and the GAF model\textsuperscript{10–12} in the SRLS context. The mode-coupling diffusion theory as applied to $^{15}$N relaxation in proteins could not account for data acquired above 8.4 T, apparently because it focuses on the weight of the global diffusion mode, but precludes the manifestation of mixed modes. Nevertheless, Lee and Wand\textsuperscript{38} assigned problems with the interpretation of a single-body SRLS model provide ample opportunities for a physically meaningful interpretation of NMR relaxation data in proteins. It can be applied to other NMR heteronuclei\textsuperscript{37,39}.

In summary, the theoretical treatment of $^{15}$N protein relaxation data is extended in this study to account for dynamical coupling between global and local motions. The tensorial properties of the two-body SRLS model provide ample opportunities for a physically meaningful interpretation of NMR relaxation data in proteins. It can be applied to other NMR heteronuclei\textsuperscript{37,39}.
and anisotropic global tumbling. It is expected that exploring the option of complete anisotropic ordering for high order parameters and fast local motions will also become feasible.

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Supporting Information Available: Table 1S giving the results of SRLS fitting of E. coli RNase H 15N relaxation data including estimated errors of the best-fit parameters (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.