

Mode-Coupling SRLS versus Mode-Decoupled Model-Free N–H Bond Dynamics: Mode-Mixing and Renormalization

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Received: April 28, 2003; In Final Form: June 20, 2003

The common approach to N–H motion in proteins is model-free (MF), where the global ($\mathbf{R}^C$) and local ($\mathbf{R}^L$) motions are assumed decoupled. We have recently applied to N–H bond dynamics the slowly relaxing local structure (SRLS) model, which accounts rigorously for mode-coupling. The original and extended MF formulas are perturbational expansions of SRLS with respect to the local ordering, $(S_0^2)^2$, when $\mathbf{R}^L \gg \mathbf{R}^C$. Their functional form, number of terms equal to the number of dynamic modes, is implied by mode-decoupling, and the free diffusion eigenvalue, $1/\tau = 6\mathbf{R}^L$, by the absence of strong-potential-induced renormalization. However, for N–H motion, $(S_0^2)^2$ is high and in the extended MF regime $\mathbf{R}_\perp^L \approx \mathbf{R}^C$. Although the functional form of the original MF formula is largely valid for $\mathbf{R}^C/\mathbf{R}^L \leq 0.01$ and $(S_0^2)^2 \geq 0.8$, τ_e MF represents the significantly reduced potential-dependent renormalized value of τ . Hence, the application of this formula to calculate NMR variables is appropriate in this parameter range, but associating τ_e with the local motion correlation time is inappropriate. Means to derive τ from τ_e are provided. For a cosine squared potential, the cone-model-based MF formula that relates τ_e to τ can also be used. Deriving τ from τ_e is important for proper characterization of the site-specific local motion and in the context of τ -dependent MF functionalities. Mode-coupling dominates the extended MF regime where SRLS must be invariably used. Eigenmode and spectral density analysis is provided in this study for the two parameter ranges associated with N–H bond motion.

Introduction

NMR spin relaxation in proteins^{1–3} is commonly analyzed with the model-free (MF) approach.^{4–6} The spin-bearing entities are attached physically to the protein, engaging in both local and global motion. MF assumes that these dynamic modes are decoupled and treats simplified geometric situations. In a recent study,⁷ we applied the two-body slowly relaxing local structure (SRLS) approach developed by Freed and co-workers^{8,9} to ¹⁵N–¹H relaxation. SRLS accounts rigorously for coupling between the local and global motions and treats the global diffusion ($\mathbf{R}^C$), the local diffusion ($\mathbf{R}^L$), the local ordering ($\mathbf{S}$) related to the coupling potential, and the magnetic interactions as asymmetric tensors of arbitrary orientation, providing thereby important information related to protein structure.^{10–12} SRLS can be considered the generalization of MF. Previous studies^{7,13,14} indicated that the experimental data are sensitive to the enhancements inherent in SRLS.

SRLS yields MF in a perturbational approach with respect to the local ordering in the $\mathbf{R}^L \gg \mathbf{R}^C$ limit.^{15,16} Dynamical decoupling requires both separating the time scales and rendering the potential very weak. The original MF formula is obtained for parallel axial ordering (S_0^2) and isotropic $\mathbf{R}^L$.¹⁵ The extended MF formula is obtained for rhombic ordering (S_0^2 and S_2^2) and axial $\mathbf{R}^L$.¹⁶ These perturbational expansions consist of two (in the original MF regime) and three (in the extended MF regime)

terms featuring free diffusion eigenvalues. The functional forms of the perturbational spectral densities are implied by the absence of mode-coupling. The free-diffusion eigenvalues for local motion are implied by the absence of the renormalization phenomenon, which becomes important in the limit of $\mathbf{R}^L \gg \mathbf{R}^C$ when the local potential is high.^{17,18} Renormalization reduces τ significantly in accordance with the magnitude (and shape) of the potential. For N–H bond motion, the local potential is typically high and the rates $\mathbf{R}_\perp^L$ and $\mathbf{R}^C$ are comparable in the extended MF regime. Hence, the conditions underlying the validity of the perturbational spectral densities are not fulfilled. Renormalization dominates the original MF regime, and mode-coupling dominates the extended MF regime. Pertinent implications are elucidated in this study based on comparative examination of eigenvalues, associated weighting factors, and spectral densities.

The functional form of the original MF formula is found to be a good approximation of the SRLS spectral density for $\mathbf{R}^C/\mathbf{R}^L \leq 0.01$ and $(S_0^2)^2 \geq 0.8$, as mode-coupling effects are relatively small. On the other hand, the effective correlation time τ_e MF represents the renormalized value of τ . Means to derive τ from τ_e are provided. The retrieval of the free-diffusion correlation time, τ , is important for meaningful characterization of the dynamic properties of the various N–H sites and in the context of local-motion-dependent functionalities. Currently, this is not done. Renormalization reduces τ severalfold,¹⁸ with significant implications to the results of data fitting.^{7,13,14} The use of T_1/T_2 as a criterion for identifying rigid spins,^{19,20} and the determination of the range of validity of the reduced spectral density approach,^{21,22} may be also affected by using τ_e instead of τ .

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The functional form of the extended MF formula⁶ is shown to be a very poor approximation to the SRLS spectral density. In the extended MF regime, SRLS must be invariably used.

In summary, the MF formulas are presented in this study as perturbational SRLS expansions with their validity range exceeded when applied to N–H bond motion. The consequences of renormalization dominating the original MF regime, and mode-coupling the extended MF regime, are investigated with a detailed analysis of eigenmodes and spectral densities.

Theoretical Background

General Form of SRLS. Please see the subsection “ η_{xy} in the SRLS Approach” in the Theoretical Background section of ref 23.

MF Formulas as Perturbational SRLS Expansions. For $\mathbf{R}^L \gg \mathbf{R}^C$ and simplified geometric cases, the SRLS spectral density yields the MF formulas in a perturbational approach with respect to the local ordering.^{15,16} For isotropic $\mathbf{R}^C$, collinear CSA and DD frames and axial ordering it was shown¹⁵ that

$$C_K(t) = C_K^1(t) + C_K^2(t) + C_K^3(t) \quad (1)$$

with $C_K(t)$ denoting the components of the time correlation function. Because $\mathbf{R}^L$ is also approximated as isotropic, only the $K = 0$ component contributes, with $\tau = \tau_0 = \tau_{\perp} = \tau_{\parallel}$. C^1 is associated with the freely diffusing local mode, C^2 is the perturbational correction associated with the global mode, and C^3 is the perturbational correction to C^1 , reflecting the statistical dependence between the global and local motions.¹⁵ Fourier transformation leads to

$$J(\omega) = j_{K=0}(\omega) = \tau_0/(1 + \omega^2\tau_0^2) + (S_0^2)^2\tau_m/(1 + \omega^2\tau_m^2) - (S_0^2)^2\tau_0/(1 + \omega^2\tau_0^2) \quad (2)$$

This expression for $J(\omega)$ is formally analogous to the original MF formula,^{4,5} with the effective MF correlation time for internal motion, τ_e , corresponding to τ_0 and the MF squared generalized order parameter, S^2 , to $(S_0^2)^2$. Note, however, that τ_0 is a free diffusion correlation time, whereas τ_e is an effective correlation time. This will be discussed in detail in the following. An earlier approach to this was discussed by Polnaszek and Freed.¹⁸

Lin and Freed¹⁶ extended this treatment to asymmetric ordering, wherein both principal values of the irreducible tensor $\mathbf{S}$, S_0^2 and S_2^2 , enter the calculation. $\mathbf{R}^L$ was considered to be axially symmetric, with all three components $K = 0, 1$, and 2 of eq 1 contributing. Fourier transformation of eq 1 yields

$$j_K(\omega) = \tau_K/(1 + \omega^2\tau_K^2) + (S_K^2)^2[\tau_m/(1 + \omega^2\tau_m^2) - \tau_K/(1 + \omega^2\tau_K^2)] \quad (3)$$

The spectral density $J^x(\omega)$ (cf. eq 11 of ref 23) (it is assumed here that the ¹⁵N CSA tensor is axially symmetric and collinear with the dipolar ¹⁵N–¹H tensor, hence $x = cc = dd = cd$) is given by

$$J^x(\omega) = [d_{00}^2(\beta_{MD})]^2 j_{K=0}(\omega) + 2[d_{01}^2(\beta_{MD})]^2 j_{K=1}(\omega) + 2[d_{02}^2(\beta_{MD})]^2 j_{K=2}(\omega) = (1.5 \cos \beta_{MD} - 0.5)^2 j_{K=0}(\omega) + 3 \sin^2 \beta_{MD} \cos^2 \beta_{MD} j_{K=1}(\omega) + 0.75 \sin^4 \beta_{MD} j_{K=2}(\omega) \quad (4)$$

Let us assume that $\beta_{MD} = 90^\circ$. In this case

$$J^x(\omega) = 0.25 j_{K=0}(\omega) + 0.75 j_{K=2}(\omega) \quad (5)$$

TABLE 1: Renormalized Eigenvalues $[(S_0^2)^2$ and $\mathbf{R}^C/\mathbf{R}^L$, Input Values]

$(S_0^2)^2$	$\mathbf{R}^C/\mathbf{R}^L$	$1/\tau_{e(\text{cone})}^a$	$1/\tau_{\text{ren}}^b$	$1/\tau_{\text{ren_SRLS}}^c$
0.96			112.6	153.0
0.90	0.01	51.2	59.4	58.2
0.85	0.01	32.3	39.6	37.2
0.80	0.01	24.4	29.4	27.6

^a Effective MF rate for the diffusion-in-a-cone model calculated with eq A4 of ref 4, in units of the diffusion rate D . ^b Renormalized value of τ calculated with eq 7, in units of $\mathbf{R}^L$. ^c Renormalized eigenvalue of the dominant local motion term in the SRLS spectral density, in units of $\mathbf{R}^L$.

and by substituting $j_{K=0}(\omega)$ and $j_{K=2}(\omega)$ from eq 3 into eq 5, one obtains

$$J^x(\omega) = [0.25(S_0^2)^2 + 0.75(S_2^2)^2]\tau_m/(1 + \omega^2\tau_m^2) + 0.25[1 - (S_0^2)^2]\tau_0/(1 + \omega^2\tau_0^2) + 0.75[1 - (S_2^2)^2]\tau_2/(1 + \omega^2\tau_2^2) \quad (6)$$

This is formally analogous to the extended MF formula⁶ with τ_s corresponding to τ_0 and τ_f to τ_2 . The squared generalized order parameters S_s^2 and S_f^2 are given by linear combinations of $(S_0^2)^2$ and $(S_2^2)^2$.

Renormalization of τ by Strong Local Potentials. Renormalization was studied previously by Freed et al.^{17,18} in the context of a spin-bearing particle reorienting in a strong static potential exerted by a locally ordered medium. This model is analogous to the limiting case of SRLS, and the original MF approach, where $\mathbf{R}^L \gg \mathbf{R}^C$ implies a global diffusion axis, C , that reorients only over a much longer time scale. It was shown^{17,18} that for a highly ordered prolate top the relaxation of a nonequilibrium orientation happens by the molecular z axis (Z_M in SRLS) being rapidly restored to the director z axis (C in SRLS). The respective correlation time, denoted τ_{ren} in the following, is given by¹⁸

$$\tau_{\text{ren}} = 1/(3C_0^2\mathbf{R}^L) \quad (7)$$

This result for frozen global motion can be employed in the present case by using a Born–Oppenheimer type approximation where one first solves for the fast local motion for frozen global motion.¹⁸ The SRLS analysis accounts automatically and rigorously for this renormalization. In the $\mathbf{R}^L \gg \mathbf{R}^C$ limit, the SRLS spectral density comprises a main local motion term with weighting factor close to $[1 - (S_0^2)^2]$ (see below). We found that its eigenvalue, denoted $1/\tau_{\text{ren_SRLS}}$, is in agreement with $1/\tau_{\text{ren}}$ given by eq 7 as shown in Table 1. The error implied by using eq 7 does not exceed 6.5% for $(S_0^2)^2 \geq 0.8$ and $\mathbf{R}^C/\mathbf{R}^L \leq 0.01$.

The original MF formula is given by⁴

$$J(\omega) = j_{K=0}(\omega) = S^2\tau_m/(1 + \omega^2\tau_m^2) + [1 - S^2]\tau_e'/(1 + \omega^2\tau_e'^2) \quad (8)$$

where $1/\tau_e' = 1/\tau_e + 1/\tau_m \approx 1/\tau_e$. The effective correlation time τ_e is defined as the area of the local motion correlation function divided by $[1 - S^2]$, assuming τ_e is in the extreme motional narrowing limit.⁴ Hence, by definition, τ_e MF represents $\tau_{\text{ren_SRLS}}$. It was pointed out previously⁴ that τ_e can be related to the diffusion rate for local motion and the generalized order parameter, S^2 , within the framework of a particular model. The relation for the diffusion-in-a-cone model is given by eq A4 of ref 4. Table 1 shows $1/\tau_{e(\text{cone})}$ in units of the diffusion-in-a-cone rate D , along with $1/\tau_{\text{ren}}$ and $1/\tau_{\text{ren_SRLS}}$ in units of $\mathbf{R}^L$. It

TABLE 2: Eigenvalues, $1/\tau^C$ and $1/\tau^L$, and Weighting Factors, W^C and W^L , of the Global Motion Term and the Dominant Local Motion Term in the Spectral Density for SRLS, Its Perturbation Limit (Italics), and MF. The Italicized Data under “Mode-Coupling Region with $R_{\parallel}^L \gg R_{\perp}^L$ ” Pertain to the Reduced Extended MF Spectral Density. MM, Mixed SRLS Modes. $(S_0^2)^2$ and R^C/R^L , Input Values^a

	$(S_0^2)^2$	R^C/R^L	$1/\tau^C$	W^C	$1/\tau^L$	W^L	MM	K
perturbation limit								
	0.05	0.0015	0.009	0.05	6.00	0.95		0
	0.05	0.0015	0.009	0.05	5.95	0.413	0.005	0
					7.08	0.296		0
					5.58	0.236		0
$R^L \gg R^C$ and high $(S_0^2)^2$ limit								
<i>pert</i>	0.70	0.0045	0.027	0.70	6.00	0.30		0
mf			0.027	0.70	16.0 ^a	0.30		0
srls			0.027	0.69	17.0	0.25	0.06	0
<i>pert</i>	0.80	0.0045	0.027	0.80	6.00	0.20		0
mf			0.027	0.80	24.4 ^a	0.20		0
srls			0.027	0.79	27.3	0.18	0.03	0
<i>pert</i>	0.80	0.01	0.06	0.80	6.00	0.20		0
mf			0.06	0.80	24.4 ^a	0.20		0
srls			0.059	0.79	27.5	0.18	0.03	0
<i>pert</i>	0.85	0.0045	0.027	0.85	6.00	0.15		0
mf			0.027	0.85	32.3 ^a	0.15		0
srls			0.027	0.83	37.4	0.14	0.03	0
<i>pert</i>	0.85	0.01	0.06	0.85	6.00	0.15		0
mf			0.06	0.85	32.3 ^a	0.15		0
srls			0.059	0.83	37.7	0.14	0.03	0
mode-coupling region with $R_{\parallel}^L \gg R_{\perp}^L$		$R^C/R_{\perp}^L$						
	0.51 ^b	0.57	3.42	0.51	6.0	0.49		0
	0.51 ^c	0.57	2.30	0.75	14.1	0.19	0.06	0
	0.51 ^d	0.57	3.00	0.73	15.1	0.25	0.02	1
	0.51 ^e	0.57	5.00	0.90	17.1	0.09	0.01	2

^a $1/\tau^L$ MF values were calculated using eq A4 of ref 4 (see also $\tau_{\text{e(cone)}}$ in Table 1). ^b Model-free analogue of the SRLS $K = 0$ component; corresponding coefficient in the expression for $J(\omega)$ in eq 9 is $Sf^2 = 0.68$. $1/\tau^L$ represents $1/\tau_s$. ^c Corresponding coefficient in the expression for $J^{\text{dd}}(\omega)$ of eq 11 of ref 23 is $A(\text{dd}) = 0.68$ for $\beta_{\text{MD}} = 20^\circ$. ^d Corresponding coefficient in the expression for $J^{\text{dd}}(\omega)$ of eq 11 of ref 23 is $B(\text{dd}) = 0.31$ for $\beta_{\text{MD}} = 20^\circ$. ^e Corresponding coefficient in the expression for $J^{\text{dd}}(\omega)$ of eq 11 of ref 23 is $C(\text{dd}) = 0.01$ for $\beta_{\text{MD}} = 20^\circ$.

can be seen that corresponding $1/\tau_e$ and $1/\tau_{\text{ren}} \approx 1/\tau_{\text{ren,SRLS}}$ values are similar in magnitude, in agreement with the definition of τ_e . It appears that diffusion-in-a-cone is a good approximation for Brownian motion in a cosine squared potential for $R^L \gg R^C$ and high $(S_0^2)^2$. Thus, τ can be derived from τ_e after fitting using eq 7 above or eq A4 of ref 4, to overcome the fact that the MF calculation does not account for renormalization.

Results and Discussion

1. Perturbation Limit. In the context of a comprehensive comparison among various spectral densities, we compare the perturbational expansion given by eq 2 with the SRLS spectral density calculated using eq 11 of ref 23 with $K = 0$, for parameters typical of the perturbation limit. Calculations were carried out for $c_0^2 = 0.04$ $k_B T[(S_0^2)^2 = 0.05]$ and $R^C/R^L = 0.0015$. The first term of the perturbational expansion, with a small eigenvalue $1/\tau^C = 1/\tau_m = 6R^C$ and weighting factor $W^C = (S_0^2)^2$, is associated with the global motion and the second term, with a large eigenvalue $1/\tau^L = 1/\tau$ and weighting factor $W^L = 1 - (S_0^2)^2$, with the local motion. R^L was set equal to 1 to obtain $\omega \equiv \omega/R^L$ and $j(\omega) \equiv j(\omega)R^L$. The respective parameters are given italicized in Table 2 under “perturbation limit”. The SRLS eigenvalues and weighting factors and the combined mixed-mode contribution denoted MM are shown in Table 2 in the same section in regular printing type. It can be seen that the $1/\tau^C$ and W^C values generated by the two calculations are identical. Three local motion SRLS eigenvalues with magnitude close to the free diffusion eigenvalue $1/\tau = 6$ (in units of R^L), and weighting factors adding up to 0.945, were obtained. These eigenvalues were found to be given predominantly by the eigenvalue $1/\tau$. The MM contribution, on the order

of 0.5%, is negligible. Clearly, the SRLS spectral density calculated with the general SRLS theory is practically identical to the perturbational expansion. Although this result was expected in general, the quantitative aspects of this comparison are of interest.

2. $R^L \gg R^C$ and High $(S_0^2)^2$ Limit. Eigenmodes. Calculations were carried out for $(S_0^2)^2 = 0.7-0.85$ and $R^C/R^L = 0.0045$. For $(S_0^2)^2 \geq 0.8$, the value of $R^C/R^L = 0.01$ was also used. The results appear in Table 2 under “ $R^L \gg R^C$ and high $(S_0^2)^2$ limit”, using the parameter notations outlined above. In this case, $1/\tau^L$ denotes the main local motion eigenvalue. The perturbational expansion data (labeled “pert”) appear italicized. The MF and SRLS data are given in regular printing type, and are labeled “mf” and “srls”, respectively. The pert data were obtained using eq 2, the srls data were obtained using eq 11 of ref 23 with $K = 0$, and the mf data were obtained using eq 8 with $S^2 = (S_0^2)^2$. The same input of $(S_0^2)^2$ and R^C/R^L , as outlined in Table 2, was used for all three calculation types. We first compare the pert and srls data. W^C and W^L show small discrepancies and the MM contribution is on the order of 3% for $(S_0^2)^2 \geq 0.8$, pointing out relatively small mode-coupling effects on the eigenvectors of the SRLS solution, which determine the weights in the spectral density. The pert global motion eigenvalue, $1/\tau^C$, is preserved in SRLS within a good approximation. On the other hand, the pert and srls local motion eigenvalues, $1/\tau^L$, differ significantly. In the perturbation limit $1/\tau^L = 6$ (in units of R^L) and in SRLS $1/\tau^L$ is given by the renormalized eigenvalue, $1/\tau_{\text{ren,SRLS}}$, which is in good agreement with $1/\tau_{\text{ren}}$ given by eq 7 (Table 1). $1/\tau_{\text{ren,SRLS}}$ exceeds the free diffusion eigenvalue of 6 approximately 3–6 times for $0.7 \leq (S_0^2)^2 \leq 0.85$. MF features $1/\tau^C$, W^C , and W^L as in pert, and $1/\tau^L$ as in srls. Hence, mode-

TABLE 3: Coefficients $A(x)$, $B(x)$, and $C(x)$ for an Axial ^{15}N CSA Tensor Obtained with $\theta = -16^\circ$ (Ref 24) and Euler Angles $\Theta = (0^\circ, \beta_{\text{MD}}, \gamma_{\text{MD}})$ for the Transformation from the DD to the M Frame^a

spectral density function	$\beta_{\text{MD}} = 0^\circ, \gamma_{\text{MD}} = 0^\circ$			$\beta_{\text{MD}} = 20^\circ, \gamma_{\text{MD}} = -90^\circ$		
	$A(x)$	$B(x)$	$C(x)$	$A(x)$	$B(x)$	$C(x)$
$J^{\text{dd}}(\omega)$	1.00	0.00	0.00	0.68	0.31	0.01
$J^{\text{cc}}(\omega)$	0.79	0.21	0.00	0.52	0.45	0.03
$J^{\text{cd}}(\omega)$	0.89	0.00	0.00	0.60	0.29	0.00

^a x denotes dd, cc, or cd, in accordance with $J^x(\omega)$.

TABLE 4: Coefficients $A(x)$, $B(x)$, and $C(x)$ for a Rhombic ^{15}N CSA Tensor Obtained Using the Principal Axes Orientation of the ^{15}N CSA and ^{15}N - ^1H Dipolar Tensors as Given in Ref 25 and Euler Angles $\Theta = (0^\circ, \beta_{\text{MD}}, \gamma_{\text{MD}})$ for the Transformation from the DD to the M Frame^a

spectral density function	$\beta_{\text{MD}} = 0^\circ, \gamma_{\text{MD}} = 0^\circ$			$\beta_{\text{MD}} = 20^\circ, \gamma_{\text{MD}} = -90^\circ$		
	$A(x)$	$B(x)$	$C(x)$	$A(x)$	$B(x)$	$C(x)$
$J^{\text{dd}}(\omega)$	1.00	0.00	0.00	0.68	0.31	0.00
$J^{\text{cc}}(\omega)$ axial	0.71	0.00	0.00	0.47	0.35	0.00
$J^{\text{cc}}(\omega)$ rhombic	0.00	0.00	0.00	0.00	0.00	0.00
$J^{\text{cd}}(\omega)$ axial	0.84	0.00	0.00	0.56	0.28	0.00
$J^{\text{cd}}(\omega)$ rhombic	0.00	0.00	-0.05	0.00	0.10	0.00

^a x denotes dd, cc, or cd, in accordance with $J^x(\omega)$.

coupling effects exist in the original MF regime but are quite small for $(S_0^2)^2 \geq 0.8$ and $\mathbf{R}^C/\mathbf{R}^L \leq 0.01$. On the other hand, renormalization is a large effect in this parameter range. It should be accounted for in MF by using eq 7 to derive the physical correlation time, τ , from the effective renormalized correlation time, τ_e , obtained by fitting. For a cosine squared potential, eq A4 of ref 4 can also be used (Table 1) to this end. In SRLS, renormalization is accounted for automatically and the fitting returns τ .

Spectral Densities—General. To gain further insight into N–H bond dynamics, we examine below typical examples of the spectral densities $j_K(\omega)$ and $J^x(\omega)$ that underlie the expressions for the NMR variables. The functions $j_K(\omega)$ are the building blocks of a particular dynamic model.^{7–9} The functions $J^x(\omega)$ are the building block for a particular geometric implementation of the dynamic model considered.^{7–9} The index x differentiates among $J^{\text{cc}}(\omega)$, $J^{\text{dd}}(\omega)$, and $J^{\text{cd}}(\omega)$, where c denotes the ^{15}N CSA interaction and d denotes the ^{15}N - ^1H dipolar interaction. The functions $J^x(\omega)$ depend on the angles θ , β_{MD} , and γ_{MD} which determine the coefficients $A(x)$, $B(x)$, and $C(x)$ in eq 11 of ref 23. The Wigner rotation from the CSA frame to the DD frame was performed according to eq 10 of ref 23 with $\Theta = (0, -16, 0)$.²⁴ The Wigner rotation from the DD frame to the M frame was performed with $\Theta = (0, \beta_{\text{MD}}, \gamma_{\text{MD}})$. For the ps regime, the assumption that $\mathbf{R}^L$ is isotropic implies $\beta_{\text{MD}} = 0^\circ$ and independence of the calculation on γ_{MD} .⁷ For the ns regime (to be discussed below), we found in previous work¹³ that $\beta_{\text{MD}} \approx 20^\circ$ and $\gamma_{\text{MD}} \approx -90^\circ$ are typical values for N–H bond motion in proteins. Table 3 shows the $A(x)$, $B(x)$, and $C(x)$ values for $\beta_{\text{MD}} = 0^\circ$ and $\gamma_{\text{MD}} = 0$, as well as $\beta_{\text{MD}} = 20^\circ$ and $\gamma_{\text{MD}} = -90^\circ$, for an axial ^{15}N CSA tensor. Table 4 shows the $A(x)$, $B(x)$, and $C(x)$ values for $\beta_{\text{MD}} = 0^\circ$ and $\gamma_{\text{MD}} = 0$, as well as $\beta_{\text{MD}} = 20^\circ$ and $\gamma_{\text{MD}} = -90^\circ$, for a rhombic ^{15}N CSA tensor, with the CSA $\rightarrow$ DD frame transformation given by the Euler angles $\Omega = (90^\circ, -16^\circ, 0^\circ)$.²⁵ As already mentioned, a rhombic ^{15}N CSA tensor also features rhombic components of $J^{\text{cc}}(\omega)$ and $J^{\text{cd}}(\omega)$ (eq 10 of ref 23). It can be seen that noncollinearity between the CSA and dipolar frames entails a 21% [11%] reduction in $A(\text{cc})$ [$A(\text{cd})$] versus $A(\text{dd})$ (Table 3 for $\beta_{\text{MD}} = 0^\circ$ and $\gamma_{\text{MD}} = 0^\circ$). Taking into account the rhombicity of the ^{15}N

TABLE 5: Coefficients $A(x)$, $B(x)$, and $C(x)$ for a Rhombic ^{13}CO CSA Tensor Obtained Using the Principal Axes Orientation of the ^{13}CO CSA and ^{13}CO - $^{13}\text{C}^\alpha$ Dipolar Tensor as Given in Ref 26 [where the Euler Angles for the Transformation from the CSA to the DD Frame are $\Theta = (90^\circ, 156^\circ, 0^\circ)$]^a

spectral density function	$\beta_{\text{MD}} = 0^\circ, \gamma_{\text{MD}} = 0^\circ$			$\beta_{\text{MD}} = 20^\circ, \gamma_{\text{MD}} = -90^\circ$		
	$A(x)$	$B(x)$	$C(x)$	$A(x)$	$B(x)$	$C(x)$
$J^{\text{dd}}(\omega)$	1.00	0.00	0.00	0.68	0.31	0.01
$J^{\text{cc}}(\omega)$ axial	0.25	0.00	0.75	0.11	0.31	0.46
$J^{\text{cc}}(\omega)$ rhombic	0.38	0.50	0.00	0.23	0.49	0.00
$J^{\text{cd}}(\omega)$ axial	-0.50	0.00	0.00	-0.27	0.00	-0.01
$J^{\text{cd}}(\omega)$ rhombic	0.00	0.00	0.00	0.00	-0.21	0.00

^a x denotes dd, cc, or cd, in accordance with $J^x(\omega)$.

CSA tensor entails an 8% [5%] reduction in $A(\text{cc})$ (axial) [$A(\text{cd})$ (axial)] versus their axial ^{15}N CSA tensor counterparts (Tables 3 and 4 for $\beta_{\text{MD}} = 0^\circ$ and $\gamma_{\text{MD}} = 0^\circ$). This should be taken into consideration when precise structural information is derived using the MF formulas, where $J^{\text{cc}}(\omega) = J^{\text{dd}}(\omega)$.

For comparison, we also present the coefficients associated with the ^{13}CO - $^{13}\text{C}^\alpha$ system in Table 5. The rhombicity of the ^{13}CO CSA tensor (absolute values $\sigma_{11} = -70.5$, $\sigma_{22} = -4.8$ and $\sigma_{33} = 65.8$, $\sigma_{\text{iso}} = 175.9$ ppm) and the relative orientation with respect to the dipolar ^{13}CO - $^{13}\text{C}^\alpha$ tensor given by $\Theta = (90, 156, 0)$ ^{25–28} are significantly larger than the analogous parameters of the ^{15}N - ^1H spin system. This entails larger diversity among the coefficients $A(x)$, $B(x)$, and $C(x)$, associated in this case with three axial and two rhombic components (eq 10 of ref 23). The Euler angles $\Theta = (0^\circ, 20^\circ, -90^\circ)$ were used for the transformation from the DD to the M frame in the ns regime.

Spectral Densities—Table 2 Data. In Figure 1, parts a–c, we show the SRLS $j_K(\omega)$ functions calculated with $\mathbf{R}^C/\mathbf{R}^L = 0.0045$, $(S_0^2)^2 = 0.8$ [$C_0^2 = 9.87$ k_BT], $\tau_m = 15.1$ ns, and $\theta = -16^\circ$. The original MF spectral density obtained with the same parameters is indistinguishable from SRLS $j_{K=0}(\omega)$ on the scale of Figure 1a. For $\mathbf{R}^L \gg \mathbf{R}^C$, the tensor $\mathbf{R}^L$ may be approximated as isotropic. In this case, $\beta_{\text{MD}} = 0$ and the contributions of the SRLS components $j_{K=1}(\omega)$ and $j_{K=2}(\omega)$ will be zero, as $B(x) = C(x) = 0$ in eq 11 of ref 23. This is certainly a good approximation in the low-frequency region dominated by the global motion. However, in the high-frequency region, the $K = 1$ and 2 components, absent in MF, may contribute for β_{MD} angles that are not very small. Because in the $\mathbf{R}^L \gg \mathbf{R}^C$ regime data fitting^{7,13,14} leads to models where $\beta_{\text{MD}} = 0^\circ$, it can be concluded that the experimental data are not sensitive enough to pick up the contribution of the $j_{K=1}(\omega)$ and $j_{K=2}(\omega)$ components in this case. The curves labeled 1, 2, and 3 in Figure 1d represent $J^{\text{dd}}(\omega)$, $J^{\text{cc}}(\omega)$, and $J^{\text{cd}}(\omega)$, respectively (eq 11 of ref 23 and Table 3 for $\beta_{\text{MD}} = 0^\circ$ and $\gamma_{\text{MD}} = 0^\circ$). The difference between $J^{\text{dd}}(\omega)$ and $J^{\text{cc}}(\omega)$, implied by the noncollinearity between the CSA and dipolar tensors, is significant. The inset of Figure 1d shows $J^x(\omega)$ for the rhombic ^{15}N CSA case. The axial components associated with the rhombic tensor are very similar to the corresponding functions associated with the axial CSA tensor, whereas the rhombic components are close to zero. Although the effect of CSA rhombicity appears to be small, it was found to be significant in some cases.²⁹ The effect of ^{13}CO CSA rhombicity on the relaxation rates is expected to be large for the ^{13}CO - $^{13}\text{C}^\alpha$ system, as illustrated in Figure 1e where we show the corresponding $J^x(\omega)$ functions. The curves labeled 1, 2, 3, 4, and 5 represent the functions $J^{\text{dd}}(\omega)$, the axial component of $J^{\text{cc}}(\omega)$, the rhombic component of $J^{\text{cc}}(\omega)$, the axial component of $J^{\text{cd}}(\omega)$, and the rhombic component of $J^{\text{cd}}(\omega)$, respectively.

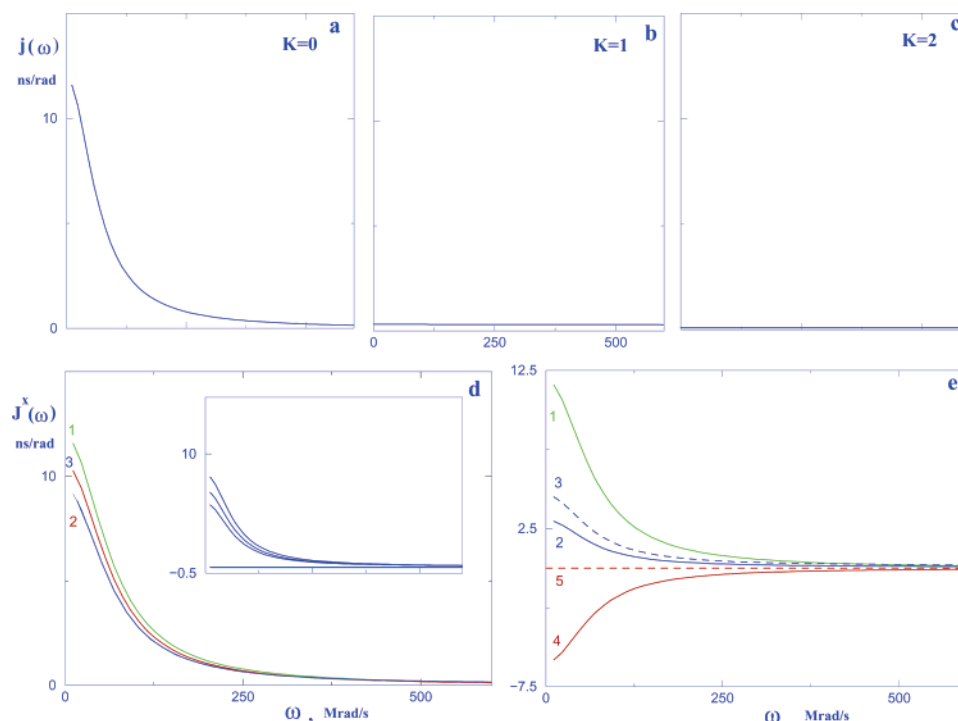


Figure 1. SRLS spectral densities as a function of ω obtained with $\mathbf{R}^C/\mathbf{R}^L = 0.0045$, $\tau_m = 15.1$ ns, $c_0^2 = 9.87$ [$(S_0^2)^2 = 0.8$]. (a–c) $j_K(\omega)$ with $K = 0, 1$, and 2 (blue). The function in green shown in Figure 1a is the original MF spectral density obtained with $(S_0^2)^2 = 0.8$, $\tau_m = 15.1$ ns, and $\mathbf{R}^C/\mathbf{R}^L = 0.0045$. (d) $J^{dd}(\omega)$, 1 (green); $J^{cc}(\omega)$, 2 (blue); and $J^{cd}(\omega)$, 3 (red) obtained from the functions $j_K(\omega)$ in Figure 1a–c and the data given in Table 3 for $\beta_{MD} = 0^\circ$ and $\gamma_{MD} = 0^\circ$, using eq 11 of ref 23. Inset of Figure 1d: $J^{dd}(\omega)$, $J^{cd}(\omega)$ (axial), $J^{cc}(\omega)$ (axial), $J^{cc}(\omega)$ (rhombic), and $J^{cd}(\omega)$ (rhombic), from the highest to the lowest curve, obtained from the $j_K(\omega)$ functions in Figure 1a–c, using the coefficients given in Table 4 for $\beta_{MD} = 0^\circ$ and $\gamma_{MD} = 0^\circ$. (e) $J^{dd}(\omega)$, 1 (green); $J^c(\omega)$ (axial), 2 (solid blue); $J^c(\omega)$ (rhombic), 3 (dashed blue); $J^{cd}(\omega)$ (axial), 4 (solid red); and $J^{cd}(\omega)$ (rhombic), 5 (dashed red) for the $^{13}\text{CO}-^{13}\text{C}\alpha$ spin system obtained from the functions $j_K(\omega)$ in Figure 1a–c and the data given in Table 5 for $\beta_{MD} = 0^\circ$ and $\gamma_{MD} = 0^\circ$, using eq 11 of ref 23. For Figure 1a–d, ^{15}N CSA of $\sigma_{||} - \sigma_{\perp} = -170$ ppm, $r_{\text{NH}} = 1.02$ Å, and $\theta = 16^\circ$ were used. For Figure 1e, ^{13}CO CSA data given in ref 26, and $r_{\text{CO}-\text{C}\alpha} = 1.52$ Å, were used.

The diversity featured is indicative of the potential inherent in SRLS for the analysis of ^{13}C -related cross-correlated relaxation.

3. Mode-Coupled Region. When the time scale separation between the global and local motions is not so large, as in the extended MF regime, mode-coupling is pervasive, and the complete SRLS spectral density must be used. A typical example for axial potentials, featuring the input parameters $c_0^2 = 4 k_B T$, $\mathbf{R}^C/\mathbf{R}^L = 0.57$, and $\mathbf{R}_{||}^L \gg \mathbf{R}_{\perp}^L$, similar to results obtained previously with data fitting,^{13,14} is shown under “mode-coupling region with $\mathbf{R}_{||}^L \gg \mathbf{R}_{\perp}^L$ ” in Table 2. Only the dominant global and local motion eigenvalues and weighting factors are shown for $K = 0, 1$, and 2 . Because MF does not feature $K = 1$ and 2 contributions, it is necessary to simplify eq 11 of ref 23 to enable meaningful comparison between SRLS and MF. Assuming that $B(x)j_{K=1}(\omega)$ and $C(x)j_{K=2}(\omega)$ contribute negligibly when $\mathbf{R}_{||}^L \rightarrow \infty$, which applies to N–H bond motion, the SRLS spectral density, $J^{dd}(\omega)$, was shown previously⁷ to be formally analogous to the reduced extended MF spectral density. The latter is given by

$$J(\omega) = j_{K=0}(\omega) = Sf^2 \{ S_s^2 \tau_m / (1 + \omega^2 \tau_m^2) + [1 - S_s^2] \tau_s' / (1 + \omega^2 \tau_s'^2) \} \quad (9)$$

where $1/\tau_s' = 1/\tau_s + 1/\tau_m$, with τ_s denoting the slow local motion correlation time. The SRLS and MF spectral densities described correspond to model 5 in the model selection hierarchy.^{30,7} The generalized MF order parameter Sf^2 is formally analogous to the SRLS coefficient $A(\text{dd}) = (1.5 \cos^2 \beta_{MD} - 0.5)^2$ in eq 11 of ref 23, and the MF function multiplying Sf^2 in eq 9 is formally analogous to $j_{K=0}(\omega)$ in eq 11 of ref 23. It should be pointed

out that the various analogies drawn here are merely formal, with the meaning of the constituents compared being very different.

The corresponding SRLS and MF dominant global and local motion eigenvalues and weighting factors appear in Table 2 under “mode-coupling region with $\mathbf{R}_{||}^L \gg \mathbf{R}_{\perp}^L$ ”. The SRLS parameters, which are associated with $j_{K=0}(\omega)$ of eq 11 of ref 23, indicate very clearly that modemixing takes place. For example, for $K = 0$ $1/\tau^C = 2.3$ as compared to the free diffusion global motion eigenvalue of $1/\tau_m = 3.42 = 6 \times 0.57$, and $1/\tau^L = 14.1$ as compared to the value of $1/\tau_s' = 6$ one would obtain in the absence of modemixing. It can be seen clearly that MF can only try to reproduce SRLS *technically* with force-fitted parameters. This was borne out previously by data fitting^{7,13,14} and is also illustrated in Figure 2a. It is concluded that the reduced extended MF formula, and, on the basis of similar considerations, the extended MF formula, constitute poor approximations of the corresponding mode-coupled SRLS spectral densities. If used in data fitting schemes, these MF formulas will generate highly inaccurate force-fitted microdynamic parameters, which as such provide very limited physical insight.

The following comment is in order. It can be seen in Figure 2 that for $\beta_{MD} = 20^\circ$ the term $B(\text{dd})j_{K=1}(\omega)$ contributes significantly to $J^{dd}(\omega)$, as $j_{K=1}(\omega)$ is on the same order of magnitude as $j_{K=0}(\omega)$ and $B(\text{dd}) = 0.31$ (Table 2). In a previous study,¹⁴ this contribution was estimated to exceed 20%. As pointed out in that study, the spectral density corresponding to model 5, where $B(x)j_{K=1}(\omega)$ is ignored, is a poor approximation to the exact spectral density when $\beta_{MD} \approx 20^\circ$. In SRLS,

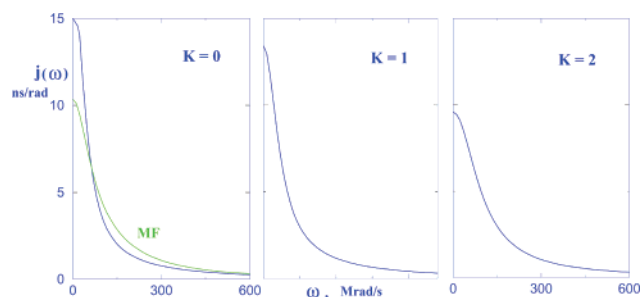


Figure 2. Spectral densities $j_K(\omega)$ as a function of ω obtained with $\mathbf{R}^C/\mathbf{R}^L = 0.57$, $\mathbf{R}_\perp^L/\mathbf{R}_\parallel^L \rightarrow 0$, $\tau_m = 15.1$ ns, and $c_0^2 = 4 k_B T [(S_0^2)^2 = 0.51]$ for $K = 0, 1$, and 2 (blue). The function in green in Figure 2a is the reduced extended MF spectral density obtained with the same parameters outlined above and $S^2 = 0.68$, which corresponds to $(1.5 \cos^2 \beta_{MD} - 0.5)^2$ with $\beta_{MD} = 20^\circ$.

significant improvement can be achieved by using model 6, where the spectral density does feature the $B(x)j_{K=1}(\omega)$ term. In MF, the efficacy of using model 6 instead of model 5 is reduced, as mixed modes and the $K = 1$ contribution are absent. The term added to the reduced extended MF formula to obtain the extended MF formula is merely a fast local motion mode mimic of $C(\text{dd})j_{K=2}(\omega)$ (eq 6). It is of interest to note that in cases where $\beta_{MD} \approx 20^\circ$ SRLS fitting selected predominantly models 6 and 8 (where R_{ex} is added as free parameter to model 6) rather than models 5 and 7 (where R_{ex} is added as free parameter to model 5) when models 5–8 were offered as options.^{13,14} This indicates that SRLS leads to physically meaningful, rather than technical, minima.

4. SRLS versus Previous Theories. We conclude this section with a comment on the SRLS concept versus the previous theories for treating N–H bond motion.³¹ All of the previous theories assume that $C(t) = C^C(t) C^L(t)$, where $C(t)$, $C^C(t)$, and $C^L(t)$ denote the time correlation functions for overall, global, and local motion, respectively. They differ by the form proposed for $C^L(t)$, and the means for deriving this function, but rely on the following common assumptions. (1) The local diffusion frame L reorients with respect to the global diffusion frame C that is taken to be fixed, rendering the L-frame motion a one-body problem. (2) S^2 represents the total mean-squared amplitude of fast oscillations representing all internal motions, with the spatial restrictions intrinsically axially symmetric. This conceptualization of S^2 is only valid when the internal motions are in the extreme motional narrowing limit and much faster than the global motion. (3) The local geometry is oversimplified because the fast local fluctuations are considered to be centered at the N–H bond, which is taken to be collinear with the symmetry axis of the collinear axial ^{15}N CSA and ^{15}N – ^1H dipolar magnetic tensors. In contrast to the picture outlined above, SRLS is a stochastic model solving a coupled two-body problem of general geometry. (1) The N–H bond vector is considered reorienting in a local environment which is itself relaxing usually on a longer time scale. The longer time relaxation of the local environment is provided by the global tumbling of the protein. The faster internal N–H dynamics is readjusting itself to the changing orientation of the protein, which it must follow. This provides the coupling between the global and local motions, materialized by a coupling potential exerted by the local environment at the N–H bond. The general SRLS approach allows one to consider comparable rates of dynamics. The solution of the coupled diffusion equation uses basis sets which include products of functions that span the orientations of the N–H bond vector and functions that span the orientations of the macromolecule, where both are relative to the same fixed

laboratory frame. Thus, SRLS is a generalization of the standard methods of solving the diffusion equation for a static ordering potential^{17,18} to the case where the ordering potential is itself reorienting.^{8,9} (2) The order parameters are ensemble averages defined in terms of the orienting potentials. These equilibrium quantities are independent of the diffusion tensors. (3) The local geometry is general in nature, with asymmetric local diffusion, local ordering, and magnetic tensors. The asymmetry of the local diffusion tensor and/or the local ordering tensor distinguishes the components $j_{K=1}(\omega)$ and $j_{K=2}(\omega)$, which are ignored in all of the previous theories. However, the distinction of the $K = 1$ and 2 components is important when the magnetic tensors are not axially symmetric or are axially symmetric but with their principal axes tilted relative to the local diffusion and ordering frame. In a previous study,¹⁴ the contribution of the $K = 1$ term in eq 11 of ref 23 to $J(\omega)$ was estimated to be at least 20% because of “diffusion tilt”. For other geometries, the $K = 2$ term may also contribute significantly, and for smaller time scale separations between $\mathbf{R}^L$ and $\mathbf{R}^C$, the $K = 1$ and 2 contributions will increase further. Although the previous theories, featuring the $K = 0$ contribution only, were developed for fast local motions, in many cases, slow local motions were also treated.

Conclusions

The original and extended MF formulas are perturbational expansions of SRLS. However, in their application to N–H bond dynamics, the perturbational conditions are violated. As a consequence, the original MF regime is dominated by renormalization, and the extended MF regime is dominated by mode-coupling. The functional form of the original MF formula is appropriate within a good approximation for $(S_0^2)^2 \geq 0.8$ and $\mathbf{R}^C/\mathbf{R}^L \leq 0.01$, as mode-coupling effects are small in this parameter range. On the other hand, τ_e MF was found to represent the significantly reduced renormalized value of τ . Therefore, when the magnitude of the local motion correlation time is involved, τ should be recovered from τ_e . Means to accomplish this are provided. For a cosine squared potential, eq A4 of ref 4 can also be used. The functional form of the extended MF formula, dominated by mode-coupling, is inappropriate and perforce the parameters it features are highly inaccurate. In this case, the SRLS spectral density must be invariably used.

Acknowledgment. This work was supported by the Israel Science Foundation grant number 520/99-16.1 to E.M., the Damadian Center for Magnetic Resonance research at Bar-Ilan University, and a Grant from NIH/NCRR to J.H.F. E.M. gratefully acknowledges the hospitality of CCBB, University of Pittsburgh Medical School, Pittsburgh, PA, where she spent her sabbatical year 2002/2003, in the course of which this work was finalized.

References and Notes

- (1) Kay, L. E. *Nat. Struct. Biol.* **1998**, *5*, 513–517.
- (2) Ishima, R.; Torchia, D. A. *Nat. Struct. Biol.* **2000**, *7*, 740–743.
- (3) Peng, J. W.; Wagner, G. In *Methods in Enzymology*; James, T. L., Oppenheimer, N. J., Eds.; Academic Press: New York, 1994; Vol. 239, pp 563–595.
- (4) Lipari, G.; Szabo, A. *J. Am. Chem. Soc.* **1982**, *104*, 4546–4559.
- (5) Lipari, G.; Szabo, A. *J. Am. Chem. Soc.* **1982**, *104*, 4559–4570.
- (6) Clore, G. M.; Szabo, A.; Bax, A.; Kay, L. E.; Driscoll, P. C.; Gronenborn, A. M. *J. Am. Chem. Soc.* **1990**, *112*, 4989–4991.
- (7) Tugarinov, V.; Liang, Z.; Shapiro, Y. E.; Freed, J. H.; Meirovitch, E. *J. Am. Chem. Soc.* **2001**, *123*, 3055–3063.
- (8) Polimeno, A.; Freed, J. H. *Adv. Chem. Phys.* **1993**, *83*, 89–210.
- (9) Polimeno, A.; Freed, J. H. *J. Phys. Chem.* **1995**, *99*, 10995–11006.
- (10) Liang, Z.; Freed, J. H. *J. Phys. Chem. B* **1999**, *103*, 6384–6396.

- (11) Barnes, J. P.; Liang, Z.; Mchaourab, H. S.; Freed, J. H.; Hubbell, W. L. *Biophys. J.* **1999**, 76, 3298–3306.
- (12) Liang, Z.; Freed, J. H.; Keyes, R.; Bobst, A. M. *J. Phys. Chem. B* **2000**, 104, 5372–5381.
- (13) Tugarinov, V.; Shapiro, Y. E.; Liang, Z.; Freed, J. H.; Meirovitch, E. *J. Mol. Biol.* **2002**, 315, 171–186.
- (14) Shapiro, Yu. E.; Kahana, E.; Tugarinov, V.; Liang, Z.; Freed, J. H.; Meirovitch, E. *Biochemistry* **2002**, 41, 6271–6281.
- (15) Freed, J. H. *J. Chem. Phys.* **1977**, 66, 4183–4199.
- (16) Lin, W. J.; Freed, J. H. *J. Phys. Chem.* **1979**, 83, 379–401.
- (17) Polnaszek, C. F.; Bruno, G. V.; Freed, J. H. *J. Chem. Phys.* **1973**, 58, 3185–3199.
- (18) Polnaszek, C. F.; Freed, J. H. *J. Phys. Chem.* **1975**, 79, 2283–2306.
- (19) Kay, L. E.; Torchia, D. A.; Bax, A. *Biochemistry* **1989**, 28, 8972–8979.
- (20) Pawley, N. H.; Wang, C.; Koide, S.; Nicholson, L. K. *J. Biomol. NMR* **2001**, 20, 149–165.
- (21) Farrow, N. A.; Zhang, O.; Szabo, A.; Torchia, D. A.; Kay, L. E. *J. Biomol. NMR* **1995**, 6, 153–162.
- (22) Ishima, R.; Nagayama, K. *J. Magn. Res. Ser. B* **1995**, 108, 73–76.
- (23) Meirovitch, E.; Shapiro, Y. E.; Tugarinov, V.; Liang, Z.; Freed, J. H. *J. Phys. Chem. B* **2003**, 107, 9883.
- (24) Fushman, D.; Tjandra, N.; Cowburn, D. *J. Am. Chem. Soc.* **1998**, 120, 10947–10952.
- (25) Cornilescu, G.; Bax, A. *J. Am. Chem. Soc.* **2000**, 122, 10143–10154.
- (26) Fischer, M. W. F.; Zeng, L.; Pang, Y.; Hu, W.; Majumdar, A.; Zuiderweg, E. R. P. *J. Am. Chem. Soc.* **1997**, 119, 12629–12642.
- (27) Pellechia, M.; Pang, Y.; Wang, L.; Kurochkin, A. V.; Kumar, A.; Zuiderweg, E. R. P. *J. Am. Chem. Soc.* **1999**, 121, 9165–9170.
- (28) Pang, Y.; Zuiderweg, E. R. P. *J. Am. Chem. Soc.* **2000**, 122, 4841–4842.
- (29) Boyd, J.; Redfield, C. *J. Am. Chem. Soc.* **1999**, 121, 7441–7442.
- (30) Mandel, A. M.; Akke, M.; Palmer, A. G., III. *Biochemistry* **1996**, 35, 16009–16023.
- (31) Palmer, A. G., III. *Annu. Rev. Biophys. Biomol. Struct.* **2001**, 30, 129–155.