Electron-nuclear interactions as probes of domain motion in proteins

Boaz Shapira and James H. Prestegard^{a)}*Complex Carbohydrate Research Center, University of Georgia, Athens, Georgia 30602, USA*

(Received 27 September 2009; accepted 2 February 2010; published online 17 March 2010)

Long range interactions between nuclear spins and paramagnetic ions can serve as a sensitive monitor of internal motion of various parts of proteins, including functional loops and separate domains. In the case of interdomain motion, the interactions between the ion and NMR-observable nuclei are modulated in direction and magnitude mainly by a combination of overall and interdomain motions. The effects on observable parameters such as paramagnetic relaxation enhancement (PRE) and pseudocontact shift (PCS) can, in principle, be used to characterize motion. These parameters are frequently used for the purpose of structural refinements. However, their use to probe actual domain motions is less common and is lacking a proper theoretical treatment from a motional perspective. In this work, a suitable spin Hamiltonian is incorporated in a two body diffusion model to produce the time correlation function for the nuclear spin-paramagnetic ion interactions. Simulated observables for nuclei in different positions with respect to the paramagnetic ion are produced. Based on these simulations, it demonstrated that both the PRE and the PCS can be very sensitive probes of domain motion. Results for different nuclei within the protein sense different aspects of the motions. Some are more sensitive to the amplitude of the internal motion, others are more sensitive to overall diffusion rates, allowing separation of these contributions. Experimentally, the interaction strength can also be tuned by substitution of different paramagnetic ions or by varying magnetic field strength (in the case of lanthanides) to allow the use of more detailed diffusion models without reducing the reliability of data fitting. © 2010 American Institute of Physics. [doi:10.1063/1.3328644]

I. INTRODUCTION

Many proteins above 20 kDa in molecular weight can be viewed as being assembled from two or more independently folded domains. In fact, a recent survey of the Protein Data Bank suggests that 36% of all depositions are multiple domain proteins with domains having an average molecular weight of 17.7 kDa.¹ These domains are often sufficiently independent that they execute motions relative to one another. These motions can be important in adapting to interactions with other proteins, in binding a variety of ligands, and in regulating various signaling events.^{2,3} Such motion can range in time scales from nanoseconds to milliseconds. NMR is one of the only available methods that can provide dynamical information on the atomic level over this range of time scales. It also can simultaneously provide information on the amplitude of motion and the relative position of domains. Slow time scales can be monitored experimentally by relaxation dispersion techniques.⁴ The nanosecond time scale, on which we will focus here, can be probed by nuclear spin relaxation through its dependence on the correlation function for the magnetic interactions. In the protein NMR community, the most commonly analyzed interactions are short range dipolar interactions ($^{15}\text{N}-^1\text{H}$, $^{13}\text{C}-^1\text{H}$, etc.). When domain motion exists, these interactions are typically modulated by local motions (N-H bond bending, peptide plane oscillations, methyl rotation, etc), by domain motion, and by overall diffusion of the protein. To interpret the con-

tributions of these short range interactions to spin relaxation, the three contributions must be separated in the analysis. This is a challenging task because the nuclei see the net effect of the three types of motion, not individual contributions to the effect. Here we focus on longer range interactions, paramagnetic relaxation enhancement (PRE) and pseudocontact shift (PCS), which are less sensitive to local motions and more sensitive to domain motions. We show that a variety of time scales and levels of coupling between domains can be probed by adopting the Slowly Relaxing Local Structure (SRLS) model of Polimeno and Freed^{5,6} to the analysis of PRE.

There have been previous attempts to deal with deconvolution of multiple motions effecting local interactions. In the work by Ryabov and Fushman,⁷ the three components of motion, internal motion, domain motion, and overall tumbling, are considered. However, in the model presented, it is assumed that these are statistically independent. As all motions modulate the same interaction, this assumption can be used only when time separation exists between the three modes. Another attempt in the literature fits relaxation data with an extended model free formalism that allows both fast and slow local motions,⁸ and it is suggested that slow time scales of internal motion may originate from domain motion.^{9,10} Physical interpretation, however, becomes problematic when the slow time scale and overall motion are similar. Recently Wong *et al.*¹¹ have addressed the effect of slow internal motion on the overall motion by introducing a model with a jump between two states having different dif-

^{a)}Electronic mail: jpresteg@ccrc.uga.edu.

fusion tensors, but as pointed out by the authors, application may be limited for realistic situations with more than two discrete states by the increase in numbers of parameters with the number of states. A final approach is that of Meirovitch, which implements the SRLS model in a study of nuclear spin relaxation.^{12–14} The SRLS model does not require assumptions about statistical independence or time separation and allows a representation of multiple states by mode coupling between two diffusing bodies; one body, which contains an internal probe, moves with respect to a second body, which represents the entire protein. In some cases, as Meirovitch showed, the motion of the probe can be equated to the motion of a domain on which the probe is located.^{15,16} However, it is not clear how well domain motion can be characterized when local backbone dynamics are also present.

It can be argued that many of the challenges in extracting information about domain motion using the approaches discussed above originates in a focus on short-range diamagnetic interactions (dimensions on the order of a bond length). PREs and PCSs differ primarily in that they are longer range, producing measurable effects over distances of several nanometers. At distances of several nanometers, the subnanometer fluctuations from local motions will produce much smaller effects on most observables, making effects from domain motions more observable. Recent years have seen an increased use of paramagnetic effects in refining structures of macromolecules. A rigid body model is often assumed, in which the length of a dipolar interaction vector between the ion and an NMR-observable nucleus is extracted from the PRE and/or the PCS. Applications have included effects from paramagnetic ions (natively present or substituted) in metal binding sites of proteins,^{17–19} as well as nitroxide spin labels covalently attached to native or mutationally introduced cysteines,^{20–22} or bound as a part of a nitroxide-tagged ligand.^{19,23}

Even in the case of paramagnetic interactions, local and domain motions can have an adverse effect on extraction of geometric parameters for structure refinement. For rigid single body proteins, the interacting vector is modulated in orientation simply by the overall motion. However, when the paramagnetic center is considered to exist in a separate domain, the vector can be modulated in direction and magnitude by both overall and interdomain motions. Corrections must then be made to distances extracted for structural refinement. Some work on dynamic effects has been motivated by the need to make these corrections.^{21,24–26}

Less work has been done for the purpose of characterizing motions. However, relaxation dispersion experiments have been used to extract microsecond to millisecond time scales of some motions that modulate PCSs,^{27,28} and very recently Xue *et al.*²⁹ have demonstrated the use of PRE in a disordered protein to study its diffusion behavior. One complexity that arises if PREs are to be used to extract domain motion is that these motions can be on time scales that are not very different from that of overall molecular tumbling and treatments that assume large separations of time scales cannot be used. Using an appropriate diffusion model and a coupling potential to connect domains, both rate constants and amplitudes of domain motion can be extracted. PREs

and PCSs for different nuclei in the protein can also be monitored in concert to generate sufficient data to define more complex models of motion.

This work will develop a theory, based on diffusion models for domain motion and mode couplings between domains, to provide a more general approach to the analysis of domain motions, and simulations using the theory will show the sensitivity of paramagnetic effects to these motions. A simple two body diffusion model is utilized based on the SRLS model of Polimeno and Freed.^{5,6} With this model, there is no need to assume time separation between internal and overall motion, and the model accounts for coupling between the two bodies in terms of a mean field potential. The model is incorporated into a time dependent spin Hamiltonian and an appropriate correlation function is developed to describe nuclear spin relaxation. Based on simulations, suggestions are made for placement of paramagnetic sites and nuclear observables that can facilitate extraction of information from experimental observables. Different types of paramagnetic interactions and their application are then discussed.

II. THEORY

A. NMR observables

We focus here on just two of the easily measured observables in paramagnetic systems, PREs in the Curie relaxation limit, usually measured from paramagnetic contributions to the transverse relaxation rates (R_2 s) of proton containing sites in proteins, and PCSs, usually measured from chemical shift offsets of ^{15}N – ^1H peaks in heteronuclear single quantum coherence spectra.³⁰ These provide the convenience of being able to measure both using a single type of ion, usually a lanthanide with a short electron spin lifetime.

As electron spin relaxation times become very short, fluctuations due to electron spin relaxation become ineffective in nuclear spin relaxation. However, because of the Boltzmann distribution over electron spin states, the electron magnetic moment does not average to zero, and modulation of nuclear spin interactions with this residual moment can still contribute to nuclear spin relaxation. This residual relaxation contribution, Curie relaxation, is particularly important at high fields because of the approximately linear increase of the average paramagnetic moment with magnetic field. The moment can be expressed in terms of the magnetic susceptibility tensor, χ , and the magnetic field, B_0 as $B_0 \cdot \chi$.^{31,32} The corresponding R_2 relaxation contribution is usually given as follows:

$$R_2^{\text{Curie}} = \frac{1}{5} \left(\frac{1}{4\pi} \right)^2 \gamma_I^2 B_0^2 \chi_{\text{iso}}^2 \left(\frac{1}{r^6} \right) \times [4J(0) + 3J(\omega_I)]. \quad (1)$$

In this expression, χ is well approximated by its isotropic part, χ_{iso} (see Appendix A), γ_I is the magnetogyric ratio for spin $\frac{1}{2}$ nucleus, I , at a distance r from the paramagnetic center. The $J(\omega)$ (spectral density functions sampled at frequency ω) are derived from a motional correlation function $[C(t)]$ by Fourier transformation and normalization as given in Eq. (2). The factor, A , contains the average of $1/r^6$ and the interaction specific constants of Eq. (1),

$$J(\omega) = \frac{2}{A} \int_0^\infty C(t) \cos(\omega t) dt = \sum_i \frac{w_i \tau_i'}{1 + (\omega \tau_i')^2}. \quad (2)$$

When stochastic processes are involved, the correlation function can be written as a series of decaying exponentials in which the amplitudes and time scales of motion are embodied in the weighting factors (w_i) and effective correlation times (τ_i'), which in the Curie limit, characterize contributions of overall molecular tumbling, domain motions, and various internal molecular motions. However, the correlation function for modulation of a single interaction ultimately comes from an appropriately normalized integral over the product of samplings of the space part of the fluctuating dipolar interaction (F) separated in time by t as shown in Eq. (3).

$$C(t) = \int_0^\infty F(t') F(t' + t) dt' = A \sum_i w_i \exp[-t/\tau_i]. \quad (3)$$

It is the time course of the fluctuations in F which must be embodied in any model for domain motion.

Like the PRE, the PCS depends on the space part of the dipolar interaction (F). However, here, it is a simple average over the interaction with the anisotropic part of the susceptibility tensor that enters, $\tilde{\chi}$, rather than the isotropic part, χ_{iso} .^{30,31} The distance dependence is also different in the PCS as it enters only once as $1/r^3$. As will be shown in Appendix A, a convenient expression for the PCS can be cast as an ensemble average over the scalar product between the dipolar tensor representation of F , $F^{(2)}$, and the second rank susceptibility tensor $\tilde{\chi}^{(2)}$ as follows:

$$\delta_{\text{PCS}} = \frac{\sqrt{5}}{12\pi} \int_X \sum_{k=-2}^2 C(2, k, 2, -k|00) F_k^2 \tilde{\chi}_{-k}^2 P_{\text{eq}}(X) dX. \quad (4)$$

Here $C(2, k, 2, -k|00)$ are Clebsch–Gordan coefficients and $P_{\text{eq}}(X)$ refers to the equilibrium probability distribution function of the stochastic variable X . In high resolution NMR, the overall tumbling of the molecule is assumed to sample orientations isotropically and $P_{\text{eq}}(X)$ would represent only a distribution over internal motion variables.

B. Domain motion

As mentioned above, both the PREs and PCSs reflect motional properties of a system under study through the spatial part of the dipolar interaction tensor. The F 's are conveniently expressed in terms of angular dependent spherical harmonics written in a molecular fixed frame with Euler angles $\Omega_{BA} = \alpha_{BA}, \beta_{BA}, \gamma_{BA}$ and the dipolar vector, $\vec{r}_{BA}$. The letters A and B denote the positions of the ion and the nuclei, respectively. Wigner matrix elements, $D_{m',m}^\ell(\Omega)$, are used to transform F from the molecular frame to the laboratory frame with the Euler angles $\Omega = \alpha, \beta, \gamma$.

$$F_m^2(\Omega_{BA}) = \sum_{m'} \frac{Y_{m'}^2(\Omega_{BA})}{r_{BA}^3} D_{m',m}^2(\Omega). \quad (5)$$

For a rigid molecule, the angles, Ω_{BA} , and the distance, r_{BA} , for a particular interaction vector are fixed and all time de-

FIG. 1. Two body model. Point A represents the paramagnetic ion. Point B is the position of the observable nucleus and point C is the origin of the ordering frame around which body 2 rotates. The dashed arrow represents the z direction of the ordering frame which is defined in the frame of body 1 and connects to body 2 through point C. The dipolar interaction vector $\vec{r}_{BA}$ varies due to the combined motions of the two vectors $\vec{r}_{AC}$, $\vec{r}_{BC}$ as described in the text.

pendence is in the Wigner elements, $D_{m',m}^\ell(\Omega)$. For a molecule with domain motion and interaction vectors that span domains, both Ω_{BA} and r_{BA} still contain time dependence that can contribute to spin relaxation. These time dependences are expected to be correlated and treatment can be very complex. Below we introduce a two domain model that is fairly general but eliminates the complexity of explicitly considering time variations in both distance and angle.

The model is depicted in Fig. 1. It is a two body model with a paramagnetic center located in body 2 and a nuclear observation site located in body 1. Assuming the motion of one body relative to the other can be represented as a rotation about point C, the distance dependence associated with domain motion can be reduced to a function of two stochastic angular variables, Ω_1 and Ω_2 . Ω_1 represents the Euler angles relating the body 1 frame to the laboratory frame. Ω_2 represents the Euler angles relating the body 2 frame to the body 1 frame. The electron to nuclear vector, $\vec{r}_{BA}$ is written in terms of two fixed length vectors, $\vec{r}_{AC}$ and $\vec{r}_{BC}$ as $\vec{r}_{BA} = \vec{r}_{BC} - \vec{r}_{AC}$. The vector $\vec{r}_{BA}$ varies due to the combined motions of the two vectors $\vec{r}_{AC}$, $\vec{r}_{BC}$. The vector, $\vec{r}_{BC}$, located rigidly on body 1, varies in direction through Ω_1 . The vector, $\vec{r}_{AC}$, fixed on body 2, varies with Ω_2 . The range of motion can be limited by introducing a restoring potential that is defined relative to an interdomain ordering frame, and the time scale of motion can be adjusted by choosing domains of different sizes or simply assigning them different diffusional properties.

With the relation between the three vectors $\vec{r}_{BA}$, $\vec{r}_{BC}$, $\vec{r}_{AC}$ as described above, we can make use of the spherical Neumann function³³ to express the dipolar tensor elements in Eq. (5), as follows:

$$\begin{aligned}
F_m^2(\Omega_{BA}) &= \frac{Y_m^2(\Omega_{BA})}{(r_{BA})^3} \\
&= \sqrt{\frac{4\pi}{24}} \sum_{\ell=0}^{\ell_{\max}} (-1)^\ell \sqrt{\frac{(2\ell+4)!}{(2\ell+1)!}} \frac{|r_{AC}|^\ell}{|r_{BC}|^{\ell+3}} \{Y^{(\ell)}(\Omega_{AC}) \\
&\quad \otimes Y^{(\ell+2)}(\Omega_{BC})\}_m^2.
\end{aligned} \quad (6)$$

Here, Ω_{AC} are the angles of the vector $\vec{r}_{AC}$ on body 2 with respect to the ordering frame. Ω_{BC} are the angles defining the direction of vector $\vec{r}_{BC}$ fixed on body 1 with respect to the ordering frame. To guarantee convergence of Eq. (6) with a finite number for $\ell_{\max}$, it is required that $|\vec{r}_{AC}| < |\vec{r}_{BC}|$. In the case with $|\vec{r}_{AC}| > |\vec{r}_{BC}|$, the letters *A* and *B* need to be interconverted. This expression can be substituted in Eq. (5) to obtain an expression that contains time dependence in the angles Ω for the movement of body 1 (Ω_1 in Fig. 1) and the angles Ω_{AC} for the movement of body 2 with respect to body 1 (Ω_2 in Fig. 1).

C. Two body diffusion model

The time dependence of the system can be described using the SRLS model.^{5,6} This is a form of the Smoluchowski equation in which two rigid bodies rotate with two self-diffusion tensors while a mean field potential couples their motion. It imposes no further restrictions on the time scales of motion. To simplify the system, one of the bodies will be represented as a sphere that rotates around its center of mass (body 2). The latter assumption makes the shape of the macromolecule independent of interdomain motion and justifies neglecting variations in frictional forces.

The diffusion equation is given by

$$\frac{\partial}{\partial t} P(X_0|X, t) = -\hat{\Gamma} P(X_0|X, t), \quad (7)$$

where $P(X_0|X, t)$ is the conditional probability distribution function with the following initial conditions:

$$P(X_0|X, 0) = \delta(X - X_0),$$

$$P(X_0|X, \infty) = P_{\text{eq}}(X),$$

where X represents the stochastic variables Ω_1, Ω_2 . $\hat{\Gamma}$ is the diffusion operator given by the SRLS model [Eq. (3) in Ref. 13]. The overall motion is assumed isotropic so P_{eq} is a function only of the orientation of body 2 relative to body 1,

$$P_{\text{eq}} = \exp[-U(\Omega_2)/k_B T] / \int d\Omega_2 \exp[-U(\Omega_2)/k_B T]. \quad (8)$$

For mathematical convenience, the restraining potential, $U(\Omega_2)$, will be expanded in terms of first rank Wigner functions,

$$\begin{aligned}
U(\Omega_2) &= c_0^1 D_{0,0}^1(\Omega_2) + c_1^1 [D_{1,-1}^1(\Omega_2)/D_{-1,1}^1(\Omega_2) \\
&\quad + D_{-1,1}^1(\Omega_2)/D_{1,-1}^1(\Omega_2)].
\end{aligned} \quad (9)$$

While one could extend this description, this particular expression restricts the motion of body 2 to wobble about a director within the body 1 frame. It will be convenient to

present the effect of this potential in terms of order parameters defined as follows:

$$S_0^1 = \int D_{0,0}^1(\Omega_2) P_{\text{eq}}(\Omega_2) d\Omega_2, \quad (10a)$$

$$\begin{aligned}
S_1^1 &= \int [D_{1,-1}^1(\Omega_2)/D_{-1,1}^1(\Omega_2) \\
&\quad + D_{-1,1}^1(\Omega_2)/D_{1,-1}^1(\Omega_2)] P_{\text{eq}}(\Omega_2) d\Omega_2.
\end{aligned} \quad (10b)$$

For spin relaxation processes, we consider the autocorrelation function connecting the time dependencies described above to the spectral densities in our spin relaxation formulas. This is written as follows:³⁴

$$C(t) = \int dX \delta f(X)^* \exp(-\hat{\Gamma} t) P_{\text{eq}}(X) \delta f(X), \quad (11)$$

Here, $\delta f = f - \langle f \rangle$, where f is the spatial function and the average component $\langle f \rangle$ vanishes as the overall distribution is isotropic. δf can be defined as

$$\delta f = \sum_{m'=-2}^2 D_{m',m}^2(\Omega_1) F_{m'}^2(\Omega_{BA}), \quad (12)$$

where $F_{m'}^2(\Omega_{BA})$ can be evaluated using Eq. (6) with Ω_1 and Ω_2 describing the stochastic variation of Ω_{BC} and Ω_{AC} [Eqs. (B4) and (B5)]. The diffusion operator $\hat{\Gamma}$ in Eq. (11) can be symmetrized as³⁵ $\tilde{\Gamma} = P^{-1/2} \hat{\Gamma} P^{1/2}$ allowing formal solutions and expression of the correlation function in the Dirac notation,³⁶

$$C_{f,f}(t) = \langle \delta f P_{\text{eq}}^{1/2} | e^{-\tilde{\Gamma} t} | \delta f P_{\text{eq}}^{1/2} \rangle / \langle |\delta f|^2 P_{\text{eq}} \rangle. \quad (13)$$

To provide a practical means of simulating the correlation function, it is expanded onto a complete basis set of orthonormal functions,

$$C_{f,f}(t) = \sum_{\lambda, \lambda'} \langle \delta f P_{\text{eq}}^{1/2} | \lambda \rangle \langle \lambda | e^{-\tilde{\Gamma} t} | \lambda' \rangle \langle \lambda' | \delta f P_{\text{eq}}^{1/2} \rangle / \langle |\delta f|^2 P_{\text{eq}} \rangle. \quad (14)$$

The choice for a basis set is given by the direct product of normalized Wigner matrices in Ω_1 and Ω_2 ,^{5,6}

$$|\lambda\rangle = |j_1 m_1 k_1\rangle \otimes |j_2 m_2 k_2\rangle, \quad (15)$$

where

$$|j_1 m_1 k_1\rangle = \sqrt{\frac{2j_1+1}{8\pi^2}} D_{m_1, k_1}^{j_1}(\Omega_1)$$

and

$$|j_2 m_2 k_2\rangle = \sqrt{\frac{2j_2+1}{8\pi^2}} D_{m_2, k_2}^{j_2}(\Omega_2). \quad (16)$$

Following Freed and co-workers,^{37,38} we will simulate the correlation function numerically by employing the Lanczos algorithm. This algorithm has been shown to be a powerful tool for the efficient diagonalization of large sparse symmetric matrices, as exists in our case.

In order to use the Lanczos algorithm, one needs to calculate the matrix elements of $\tilde{\Gamma}$ and a starting vector $\mathbf{v}_1 = \langle \delta f P_{\text{eq}}^{1/2} | \lambda \rangle / \sqrt{\langle \delta f^2 P_{\text{eq}} \rangle}$, which will be used as a first step in a recursion procedure. The matrix elements are given by $\tilde{\Gamma}_{\lambda', \lambda} = \langle \lambda' | \tilde{\Gamma} | \lambda \rangle$ with $\tilde{\Gamma}$ defined in Eq. (3) of Ref. 13, and $|\lambda\rangle$ being the basis set elements from Eqs. (15) and (16). The actual calculations of $\tilde{\Gamma}_{\lambda', \lambda}$ are demonstrated in several works.^{5,6} In Appendix B, we give the solution for the starting vector $\mathbf{v}_1$, which is unique to our model.

Evaluating Eq. (13), we end up with a finite sum of decaying exponents with eigenvalues, $1/\tau_i$, and normalized weighting factors, w_i , related to the fraction of interaction space sampled,

$$C_{f,f}(t) = A \sum_i w_i e^{-t/\tau_i}, \quad (17)$$

where A is the mean square fluctuation given by the value of the correlation function at time zero,

$$A = \xi \langle \delta f^2 P_{\text{eq}}(\Omega_2) \rangle. \quad (18)$$

In the calculations below, we explicitly consider amplitude factors, A , and spectral density functions, $J(\omega)$. Substituting Eq. (12) together with Eq. (6) into Eq. (18) leads to

$$A = 8\pi^2 \xi \int \frac{1}{r_{BA}^6(\Omega_2)} P_{\text{eq}}(\Omega_2) d\Omega_2 = 8\pi^2 \xi \left(\frac{1}{\bar{r}_{BA}^6} \right). \quad (19)$$

Here, ξ is a constant specific to the relaxation mechanism and the bar denotes averaging of $1/r^6$ as before. The spectral density function, which is the Fourier transform of the normalized correlation function, is then

$$J(\omega) = \sum_i \frac{w_i \tau_i}{1 + (\omega \tau_i)^2}. \quad (20)$$

Using the same diffusion model, the PCS calculations can be accomplished by substituting the expression for $P_{\text{eq}}(\Omega_2)$ [Eq. (8)], $F_k^2(\Omega_{AB})$ [Eq. (6)], and $\chi_{-k}^2(\Omega_2)$ values into Eq. (4) (see Appendix A).

III. CALCULATIONS

A. Specific models for observables

The theory presented above will now be used to evaluate the effects of interdomain motion on PRE and PCS observables. To simplify interpretation, two specific models have been constructed and calculations have been done for four different nuclear positions in each as depicted in Fig. 2 below. As in the general theory developed above, dipolar vector $\vec{r}_{BA}$ is given by its composing vectors $\vec{r}_{BC}$ and $\vec{r}_{AC}$. The effect of interdomain motion on $\vec{r}_{BA}$, depends on the relative orientation of $\vec{r}_{BA}$ with respect to the ordering frame and the ratio of the two vectors, $\vec{r}_{BC}$ and $\vec{r}_{AC}$. The ratio between the two vectors $|r_{<}|/|r_{>}|$ is smaller than one when $r_{>}$ and $r_{<}$ are the larger and smaller vectors among $\vec{r}_{BC}$, $\vec{r}_{AC}$. Nuclei in positions 1 and 2 in Fig. 2 are located on body 1 with the same vector ratio $|r_{<}|/|r_{>}|=0.7$. Their dipolar vectors point in different directions with respect to the ordering frame. The nucleus in position 3, also located on body 1, has a vector

FIG. 2. Two body model with 4 observed nuclei denoted with numbers 1–4. Nuclei 1–3 are located on body 1. Nucleus 4 is located on body 2. The ion is also located on body 2. The coupling between the two bodies is directed along the longitudinal axis of body 1. Body 2 rotates by β_2 around any axis perpendicular to the director. In (a), the ratio between the diffusion rate of the two bodies $D_2^{\parallel}/D_1^{\parallel}=1.3$. In (b), the ratio is $D_2^{\parallel}/D_1^{\parallel}=10.4$.

ratio of $|r_{<}|/|r_{>}|=0.29$. Point 4 has the same vector ratio as point 1 on body 1, $|r_{<}|/|r_{>}|=0.7$, but it is located on body 2. Figures 2(a) and 2(b) have the same allocation of points with respect to the ion. Since the absolute dipolar vector distances enter only through an overall scaling constant, distances are considered to be normalized. The four observation points are expected to display different sensitivities to different types of motion and illustrate a basis for a detailed characterization of domain motion.

Domain motion is embodied in the movement of body 2 relative to body 1. Body 2 diffuses about point C under the influence of an orienting potential that is defined relative to the ordering frame anchored in body 1. The motional properties have been simplified by assuming body 2 to be spherical and body 1 to be axially symmetric with the center of body 2 on the axis. An axially symmetric diffusion tensor has been assigned to body 1. The difference between the models in Figs. 2(a) and 2(b) is that the ratio of the body 2 diffusion constant and the body 1 diffusion constants, $D_2/D_1^{\parallel}$, varies from 1.3 in Fig. 2(a) to 10.4 in Fig. 2(b). The positions of the four points between the two models [Figs. 2(a) and 2(b)] have been adjusted to keep the ratios of $\vec{r}_{AC}$ to $\vec{r}_{BC}$ for each point between Figs. 2(a) and 2(b), fixed. The molecular equivalent of fixing the overall diffusion constants would be adjusting the shape of body 1 to compensate for the size of body 2.

A specific type of ordering for body 2 relative to body 1 has been selected by making c_1^1 very large in the potential of Eq. (9). This allows sampling of β_2 values, without rotation about the z axis of body 2. It is equivalent to requiring $\alpha_2 = -\gamma_2$. Under these conditions, S_1^1 is effectively set to one. In the simulations below, S_0^1 will be varied from 0.65 to 1 by varying c_0^1 .

B. Mean square interaction variations

The relaxation rates R_1 and R_2 (PREs) depend on the two following parts: the mean square interaction amplitude, A , (through the average of $1/r^6$) and the spectral density functions (through the various weighting factors and correlation

FIG. 3. The effect of domain motion coupling on the amplitude of the relaxation interaction. Nuclei in positions 1–3 are represented with triangles, circles, and x's respectively. Symbols that are connected with lines represent A/A_{stat} . Symbols with no connecting lines represent $\sqrt[6]{A/A_{\text{stat}}}$.

times). We first examine how the amplitude of interdomain motion is reflected at different observation points as a function of the coupling potential. By examining the ratio of amplitudes calculated for cases with domain motion (A) and without domain motion (A_{stat}), any scaling factors for distances cancel and one calculation serves for evaluation of both models. In Fig. 3 we show the ratio A/A_{stat} for points 1–3 (triangle, circle, x). Ratios for point 4 are not shown; point 4 is in body 2 and A/A_{stat} is fixed at a value of one. The symbols connected by lines are the raw ratio. The symbols not connected by lines are the sixth root of the ratio. Point 1 proves to be the most sensitive to the amplitude of the motion. For low ordering, the amplitude of the interaction is almost 20 times larger than A_{stat} . Point 2 has the same ratio between the vectors $|r_{<}|/|r_{>}|$ as for point 1 but the excursions of $\hat{r}_{AC}$ do not allow as close an approach of ion and nucleus. For point 3, the amplitude of the interaction is much less sensitive to the ordering. This results from the fact that as the ratio of the vectors decreases the fluctuation in the distance is less affected by interdomain motion.

The PRE is frequently used to extract paramagnetic center to nuclear distances. For paramagnetic complexes with short electronic relaxation times, but ones still long enough to dominate Curie contributions, (see Sec. II A), a single term in the spectral density function pertains ($w_1=1$) and the relaxation rates R_1 , R_2 could, in principle serve as a direct measure of the amplitude A of the dipolar interaction. In the case where there is no motional modulation of the interaction vector, the expression $r_{\text{stat}}=r_{BA} \sqrt[6]{A/A_{\text{stat}}}$ results. The symbols not connected by lines in Fig. 3 ($\sqrt[6]{A/A_{\text{stat}}}$), show that there can be substantial errors if this interpretation is used in the presence of motion. For points with relatively large vector ratios $|r_{<}|/|r_{>}|$ (points 1 and 2), errors can be quite large ($\sim 50\%$ for an S_0^1 order parameter of 0.7, Saupe order parameter of about 0.4). It is also important to note that the level of error varies depending on placement of the point, so that picking a point for internal distance calibration is a less than perfect approach.

C. Spectral density, $J(0)$, variations

In the next calculation, the behavior of the spectral density function $J(0)$ as a function of both the coupling and the

FIG. 4. $J(0)$ analysis as a function of shape and ordering. Nuclei in positions 1–4 are represented with triangles, circles, x's, and squares respectively. Points that are connected with lines represent the model of Fig. 2(a) with $D_2/D_1=1.3$. Points with no connecting lines represent the model of Fig. 2(b) with $D_2/D_1=10.4$.

diffusion rates is examined. Most observations of paramagnetic relaxation are made using transverse relaxation experiments (R_2). In high magnetic fields, for ^1H as well as for ^{13}C , R_2 s are dominated by $J(0)$ contributions. Again the effects of domain motion are most easily visualized by considering the ratio of relaxation rate contributions in the presence and absence of motion, $J(0)/J(0)_{\text{stat}}$. Figure 4 presents the ratio $J(0)/J(0)_{\text{stat}}$ for nuclei in positions 1–4. Each term in the $J(0)$ expression makes a contribution proportional to its correlation time, τ_i , and its weighting factor, w_i . It can be seen that for all four points $J(0)$ increases as the ordering increases. This is expected. With no coupling, the two subunits rotate with their individual self-diffusion rates. As coupling increases (increased S_0^1), the diffusion rate of each subunit become restricted by motions of the other. For complete order, $[J(0)/J(0)_{\text{stat}}=1]$, the two bodies rotate as one with an overall diffusion rate that is slower than those of its components.

To understand the different behavior of $J(0)$ for nuclei in different positions, one must consider the origin of the τ_i s and w_i s in the various components of $J(0)$. In general, the decay rates, $1/\tau_i$, which are the eigenvalues of the diffusion matrix $\tilde{\Gamma}$, are common to all the observed nuclei, regardless of their position within the protein. The difference between the four points arises from the weighting factors, w_i . The w_i s are produced by projecting the starting vector, $\mathbf{v}_1$, on the eigenfunctions of $\tilde{\Gamma}$. The dipolar interaction at each point generates a different starting vector and the projection selects the eigenmodes to which a particular vector is sensitive.

The differential sensitivity for various points is illustrated by comparing results for the four points, as shown in Fig. 4. First let us compare between points 3 and 4. For Point 3, most of the dipolar vector resides on body 1. This means that for any solution of the $\tilde{\Gamma}$ matrix, whether with high or low ordering, this observable will pick up eigenmodes that associate mostly with body 1. As body 2 is smaller than body 1 (Fig. 2), a limited sensitivity to changes in diffusion rates of body 2 would result. This is demonstrated in Fig. 4, where x's connected by lines are generated using the model of Fig.

2(a) where diffusion of body 2 is relatively slow ($D_2/D_1^{\parallel}=1.3$) and x's not connected by lines are generated using the model of Fig. 2(b) where the diffusion of body 2 is relatively fast ($D_2/D_1^{\parallel}=10.4$). The value of $J(0)$ is smaller than $J(0)_{\text{stat}}$ by less than 20% for point 3 over most of the ordering range for both models. The model of Fig. 2(b) actually has less variation, even with the faster diffusion rate of body 2, because the rapid motions contribute less to the overall $J(0)$.

Position 4 (squares) shows a behavior that is quite different. The starting vector for this position will reflect only decay rates associated with body 2. As the coupling increases, the effective diffusion rate for this body becomes more equivalent to that of the larger body 1 and $J(0)$ increases. The effect is more dramatic when the diffusion rate for body 2 is high relative to the diffusion rate of body 1. Hence, the squares that are not connected by lines vary more sharply with order. The reduction in $J(0)$ at an S_0^1 order parameter of 0.7 may at first seem extreme, but one must remember that the order parameter being used is not the usual Saupe order parameter. An S_0^1 of 0.7, if the model uniformly sampled space in a cone would correspond to a cone angle of 57° . The corresponding Saupe order parameter for this angle would be 0.42.

The correlation function behaves differently for nuclei in positions 1 and 2. For both points, the dipolar vectors sample motion from both domains to a significant extent. Hence, dipolar interactions are modulated directly by motion of the two subunits. The eigenmodes being sampled by $\mathbf{v}_1$ are mixed modes of the two bodies regardless their relative diffusion rates or the coupling strength. At low ordering, the decay rates include effects of uncorrelated self-diffusion of the two domains decreasing the overall effective correlation time and lowering $J(0)$. Moreover, there are substantial fluctuations in the distance between the ion and the nuclei for these points. As the ordering decreases, the rates of these fluctuations will become larger than the rate of isotropic sampling, and the weighting factors for these modes will have to include a distance dependent factor that scales down the extreme short distance weighting of the $1/r^6$ in the amplitude term, A . The sigmoidal shape of the order dependence can be traced to a “phase change” from a two body uncorrelated behavior ($S_0^1=0$) to one body correlated behavior ($S_0^1=1$).

D. R_2 relaxation rates.

In comparing the R_2 relaxation rates that originate from the product of amplitude and spectral density factors, it is observed that, although the amplitude A and the $J(0)$ components can individually deviate dramatically from the static case, their combination in R_2 displays less deviation (Fig. 5). Nevertheless, for points 1–3 the deviation in the R_2 values from the static case is substantial, deviating by more than a factor of 2 in the weak coupling limit for both points 1 and 2. This is largely due to the effect of $1/r^6$ averaging in the amplitude factor, but as discussed above, the effects are reduced by additional distance dependence in the $J(0)$ weighting factors. Point 4 is an exception in that it is entirely de-

FIG. 5. R_2/R_2^{static} analysis as a function of shape and ordering. Nuclei in positions 1–4 are represented with triangles, circles, x's, and squares respectively. Symbols that are connected with lines represent a molecular shape with $D_2/D_1^{\parallel}=1.3$. Symbols with no connecting line represent a molecular shape with $D_2/D_1^{\parallel}=10.4$.

pendent

on $J(0)$ since the distance to the paramagnetic center is fixed in body 2.

Note that the errors can be significant if one attempts to extract distances from R_2 measurements. Using the distance from the ion position to point 4 as an internal reference, ignoring motion, and attempting to get a distance for point one from the ratio of R_2 s at a S_0^1 value of 0.67 and a $D_2/D_1^{\parallel}$ ratio of 1.3, one would get a distance of 0.76 times that of the minimum energy distance. If one attempted to correct this distance using $1/r^6$ averaging over allowed molecular structures (here approximated by A factors from Fig. 3), one would over estimate the distance by 23%.

E. PCSs

PCSs are apparent in many samples that also display Curie contributions to spin relaxation, particularly those with fast relaxing lanthanide ions. They also can reflect domain motions in similar ways and can be analyzed in terms of the formalism presented above. However, they are purely geometry dependent as opposed to being both geometry and time scale dependent, simplifying their interpretation. Their magnitude depends on the distance of a nuclear observation point from a paramagnetic center, much as the A parameter in the PRE effect, but the dependence differs in two important ways. (i) The dependence on $\vec{r}_{BA}$ comes in as the third power rather than the sixth power. This means the PCS is less sensitive to fluctuations in distance with domain motion. (ii) The value δ^{PCS} , includes averaging over both the $1/r^3$ distance dependent factor and a factor that depends on the angles of the ion to nucleus vector relative to the axes of the susceptibility tensor of the ion. Those two components contribute in opposite directions and lead to a further reduction in sensitivity to domain motion. In other words, the ratio $\delta^{\text{PCS}}/\delta_{\text{stat}}^{\text{PCS}}$ will be affected even less by the amplitude of the motion than the average over only the distance part $\langle 1/r^3 \rangle / \langle 1/r \rangle^3$. We demonstrate this point in Fig. 6 by plotting both $\delta^{\text{PCS}}/\delta_{\text{stat}}^{\text{PCS}}$ (lines) and $\langle 1/r^3 \rangle / \langle 1/r \rangle^3$ (dotted lines) for the three nuclei in position 1–3. When distance and angular parts

FIG. 6. PCS as a function of the ordering: $\delta_{PCS}^{PCS}/\delta_{PCS}^{static}$ is plotted with lines connecting symbols, $\langle 1/r^3 \rangle / \langle 1/r \rangle^3$ is plotted without lines connecting points. Nuclei in position 1–3 are represented with triangles, circles, and x's, respectively.

are combined the $\delta_{PCS}^{PCS}/\delta_{stat}^{PCS}$ ratio deviates from 1 by less than 50% for all three points. If a distance is extracted using a static model to interpret PCSs maximum errors in extracting distance is just 13% for the cases simulated here.

IV. DISCUSSION AND SUMMARY

A. General observations

The theory presented above provides a very general approach to simulation of a number of types of nuclear spin interactions. For spin relaxation, the approach is free of assumptions about separation of time scales for overall and internal motions. This is particularly important for domain motions in proteins as these are likely to occur on time scales similar to overall motion. In the simulations, we have focused on a simple two domain model to minimize the time spent in calculations and simplify interpretation of results. We have also focused on simulation of paramagnetic effects (PREs and PCSs) as these are interactions that span distances appropriate for the investigation of domain motion. The results show a dramatic, but expected, dependence of observables on domain motion. More importantly the results show different dependencies for PREs and PCSs, different dependencies for PREs in the fast, slow, and Curie limits for electron spin relaxation times, and different dependencies for different nuclear observation points. Understanding the origin of these different dependencies helps in anticipating errors that can be introduced when attempting to interpret PREs or PCSs under rigid body or simple $1/r^6$ averaging assumptions. More importantly, understanding the origin of differences may ultimately allow one to deduce more accurate models for domain motion from these observables.

Let us first summarize implications for errors introduced if geometric parameters are extracted from various types of measurement assuming a rigid molecular model. Interpreting distances extracted from PRE measurements using an ion having electron spin relaxation times faster than overall tumbling and most relevant internal motions, but still slow enough for effective nuclear spin relaxation (Mn^{2+} , Gd^{3+}), will underestimate minimum energy distances when motion is present. However, for these cases a well defined $1/r^6$ av-

erage will pertain. If a distribution of molecular structures is available (from molecular dynamics simulations, for example), measurements can be compared to this simple $1/r^6$ average. This simple treatment cannot, however, be assumed to apply when electron moment lifetimes become longer (nitroxides spin labels or metal ions giving strong Curie contributions). Here, the simulations show that attempts to compensate for errors using $1/r^6$ averaging, over compensate and lead to over estimation of minimum energy distances. PCSs, arising in the case of paramagnetic metal ion centers with anisotropic susceptibilities and very rapidly relaxing electron spin states, offer a more promising route to extraction of geometric information in that the nature of motional averaging does not depend on motional or electron spin relaxation time scales. The distance dependence is also softer, $1/r^3$, and when averaging over motion occurs, angular and distance terms tend to compensate, reducing errors from interpretation based on a rigid model. Comparison to averages calculated using distributions of structures from molecular simulations is also straightforward, but somewhat more laborious because of the presence of both distance and angular dependence.

B. Characterizing domain motion

The most promising implication of the results presented is that comparison of measurements of different types, made at different nuclear sites, may provide a reliable indicator for the presence of domain motion and ultimately a better way to characterize domain motion. It is certainly possible to measure PREs and PCSs at a large number of sites in a molecule (for example, amide protons attached to ^{15}N in a uniformly ^{15}N labeled protein), and structures for individual domains might be known and individually well modeled as rigid entities. The mere failure to extract distances from multiple PREs or PCSs that are consistent with a single rigid model for domain placement, will signal the presence of motion.

At a more quantitative level, a search procedure can be employed to find a set of parameters for the stochastic model that optimize a fit to experimental data. Even with the simple, highly symmetric, SRLS model of Fig. 2, the number of parameters is moderately large (seven); three for the diffusion constants, $D_{\perp}$, $D_{\parallel}$, D^2 , two for the angles defining the director of the restoring potential in the body 1 frame (assumed to be along the principal diffusion axis and pointing through the pivot point, C, to the paramagnetic ion), one to position the pivot point along this axis, and one constant, c_0^1 , to define the degree of motional coupling ($c_0^1 \rightarrow \infty$ mimics wobbling in a cone). The dozens of observables available from just paramagnetic relaxation effects of a single ion may be adequate to independently define a model of this motional complexity. The unique projections of starting vectors ($\mathbf{v}_i^1 s$), from the paramagnetic ion to various nuclear sites in a fully labeled ^{15}N protein with a paramagnetic center, should provide independent views of the dynamics. By altering the position of the paramagnetic ion in the protein, as is often done through cysteine mutagenesis in nitroxide spin label studies,³⁹ one can effectively multiply the number of observables. Given the computational capacity of modern computer

clusters, a brute force grid search for optimum parameters is likely to be applicable for this minimal parameter model. Target functions can be defined as sums of squared differences between experimental and calculated observables, with the terms weighted by factors that reflect relative errors, and the set of parameters yielding the minimum value selected. Ranges for some of the parameters can be constrained based on structural information, or fixed with additional experimental data on non-paramagnetic analogs, to facilitate computation. For more complex models with larger numbers of parameters, more sophisticated optimization procedures will have to be considered.⁴⁰

C. Extending experimental measurements

It is important to emphasize the potential complementarity of various types of data when extending treatment to more complex cases. As illustrated, PCS measurements complement R_2 measurements of Curie contributions when rapidly relaxing paramagnetic ions are used (certain lanthanides, for example) in that they provide a measure of distance and angle averaging independent of time scale. It is also possible to complement the PCS distance measurements by measuring R_2 contributions from ions such as Gd^{3+} which provide a measure of a pure $1/r^6$ average with a negligible sensitivity to the time scales of domain motions. Residual dipolar coupling (RDC) measurements also provide a complement in that they can provide a measure of pure orientational averaging by interdomain motions.⁴¹ In all cases, measurements must, of course, be free of artifacts. R_2 s, in particular can be contaminated by chemical exchange. The existence of PCSs can exaggerate the problem by providing additional chemical shift contributions that can be modulated by domain motion and local changes in susceptibility tensors.^{27,28} Many of these contributions can be detected and eliminated using relaxation dispersion experiments.²⁷ Explicit measurement of exchange effects can also add another dimension to motional investigation. Relaxation dispersion experiments, in particular, sense longer time scale motions that do not contribute to the R_2 expressions presented here.

It is also useful to sample correlation functions at multiple frequency points. The potential utility of R_1 observation has not been discussed. Paramagnetic contributions to R_1 are smaller and were omitted from our simulations on this basis. However, they do contain frequency dependence that can complement the vector projection approach to separating contributions of various spectral density components. The possibility of measurement at multiple fields then adds to this advantage. The reason for the small size of R_1 is that expressions for spin-lattice relaxation rates, and PRE contributions to those rates, do not contain a $J(0)$ term. This is easily seen with an example where Curie contributions dominate. For a proton observed at 800 MHz in a rigid molecule with a rotational correlation time of 10^{-8} s, R_2^{Curie} is approximately 40 times R_1^{Curie} . In practice, this means that the PRE contribution to R_1 will be of the order of 2 s^{-1} , a small number. However, for resonances that can still be observed with reasonable resolution and sensitivity (25 Hz line broadening due to a PRE), 2 s^{-1} can be a significant contribution to the total R_1 , and it

can be accurately measured. There are also other paramagnetic R_1 contributions that become more significant at lower field (contributions from nitroxide spin labels, for example) that could provide spectral density sampling at other frequencies.⁴² While not presented in detail, the methodology presented above is fully capable of simulating R_1 effects under these alternate conditions.

D. Limitations to the current treatment

There are several limitations in the theoretical treatment and simulation models that we have presented. In many cases, these limitations can be addressed by extension of the general principles applied. With respect to theory we have considered only a single type of interaction; a dipole-dipole interaction between a nucleus of spin $\frac{1}{2}$ and a dipole associated with a paramagnetic center. Most sites in experimental systems have additional interactions; dipolar interactions with other nearby nuclear spins (DD), chemical shift anisotropy (CSA) interactions from the anisotropic electron distribution around the nucleus being observed, etc. As all of these interactions are represented by second rank tensors, they are easily treated individually using irreducible tensor formalism similar to those presented here. However, if they act simultaneously with PREs, cross correlation between PREs and the additional interactions (DD, CSA) also need to be considered. While this introduces complexity, it also provides additional observables. Such observables have already been used effectively in applications with rigid models or models with more limited motional descriptions.⁴³ Incorporation in the more general motional treatment provided here would clearly be advantageous.

With respect to the motional model, the one presented is very general in terms of the range of motional time scales that it can accommodate, but it is very limited in geometry and the types of motion allowed. First, it treats just two domains, and it neglects the possibility of internal motion of large amplitude. Fast internal motions can be accommodated using time separation treatments.⁴⁴ These will simply add an additional order parameter and rapid motion correlation time. It is also possible to accommodate this by imbedding a third body that contains our observation site and using the same SRLS formalism used by Freed and co-workers.¹²⁻¹⁴

In terms of the types of motion treated in our simulations, even the two body model presented here incorporates simplifying assumptions; body 1 is treated as axially symmetric and body 2 is treated as a sphere. It is possible to treat body shape more generally by using full diffusion tensors. The diffusion of macromolecule with two subunits joined by hinges or swivels has been treated in several places.⁴⁵⁻⁴⁸ Moro⁴⁷ showed how one can define a macromolecule diffusion tensor as a function of interdomain motion and combine it with the Smoluchowski equation. This route can be incorporated in the formalism presented here. The primary limitation to experimental application is the additional parameters introduced.

The type of motion is also restricted to rotation of one body relative to another about a point, restrained by a potential. A potential expanded in first order Wigner elements was

used, but this can of course be generalized by including other terms. Pure rotational descriptions of domain motion are convenient in terms of expressing relaxation equations and using them to produce simulations. However, they exclude motions that might result in less restricted distance variations such as in domains connected by a more flexible linker. This limitation can be addressed to a certain extent by adding a third domain, with rotation point D, as an intermediary between body 1 and body 2. Three fixed vectors ($\vec{r}_{AD}, \vec{r}_{DC}, \vec{r}_{CB}$) with appropriate correlated angular displacements can then mimic a pure distance variation between points A and B. Other more direct approaches have also been taken to the introduction of additional translational diffusion modes.⁴⁹ Recent work by Xue *et al.*²⁹ demonstrates implementation of a restricted translational diffusion model to extract dynamics from PRE effects in unfolded proteins.

E. Prospects for experimental applications

The development of a general theory and suggestions for application protocols are only valuable if there are systems of interest with important questions that can be addressed. We mention just a few to illustrate potential applications in the biomolecular area. A classic case in which domain motions are important is that of calmodulin. Calmodulin is a Ca^{2+} sensor protein that in response to variations in Ca^{2+} concentration regulates a variety of intracellular processes.⁵⁰ The molecule is basically a two domain protein with these domains connected by a linker that allows reorientation for cooperative binding of peptide motifs in target proteins. Each domain contains two Ca^{2+} binding EF-hand structures and the variable occupancy of these structures influences peptide binding. Recently, mutational studies have uncovered a species that preferentially binds a Ln^{3+} ion in one site, providing a two domain system with a single paramagnetic site in one of the domains. Bertini *et al.*⁵¹ have collected PCS and RDC data on this system and used these data to establish the presence of motional averaging among a distribution of conformers. Using two different lanthanide ions more than 100 ^{15}N – ^1H PCSs were measured for each of the two domains demonstrating the ability to collect a wealth of paramagnetic data on this system. Adding PREs to the existing data would clearly allow application of the methods described here and description of the motion in terms of a set of domain centered diffusional constants, relative domain orientation, and a restoring potential.

Another example stems from work on the carbohydrate binding protein, Galectin-3. Galectin-3 is involved in a variety of process that regulate the movement and distribution of glycosylated proteins at the cell surface.⁵² This protein does not have a native metal binding site, but an expression construct has been modified to produce a protein with a short peptide extension that binds lanthanide ions.¹⁹ Lanthanide tags are intended to provide a general approach to structural analysis of proteins. In the Galectin-3 case, the tag allowed measurement of more than 30 RDCs and more than 30 PCSs for ^{15}N – ^1H sites in the protein and a limited set of ^{13}C – ^1H data on a bound ligand. Arguments were made supporting the validity of a rigid structure and the position of the bound

ligand was determined using the data. However, lanthanide tags are in general not rigid¹⁸ and characterization of motion of the tag domain relative to the protein domain can easily become a prerequisite for structural applications. The Galectin-3 system suggests the possibility of collecting adequate data to carry out this characterization using the protocols described here.

In summary, an approach to analysis of domain motions in proteins based on various SRLS formalisms offers much promise. There are challenges to extending the formalism to more realistic models and challenges in using the theory to extract model parameters from experimental data. However, it is easy to see routes to meeting some of these challenges and we expect them to be met in the future.

ACKNOWLEDGMENTS

B.S. would like to thank the following people for useful discussions during the course of this work: Dr. Diego Frezzato, Professor Giorgio Moro and Professor Antonino Polimeno from Padova University, Dr. Golan Bel from Los Alamos National Laboratory, Dr. Lex Vega from the University of Delaware, and Professor Zeev Luz from the Weizmann Inst. This work was financially supported by Award No. P41RR005351 from the National Center For Research Resources, by Award No. R01-GM033225 from the National Institute of General Medical Sciences, and by a research grant from the Herbert J. Seligmann Charitable Trust.

APPENDIX A: DERIVATION OF CURIE RELAXATION AND PCS EXPRESSIONS

In Appendix A, we derive the expressions for the Curie relaxation mechanism which leads to Eq. (1) and to the expression for the PCS in Eq. (4). In Cartesian coordinates the Curie Hamiltonian is written as³²

$$\mathbf{H}_{\text{Curie}} = \mathbf{I} \cdot \mathbf{T} \cdot \chi \cdot \mathbf{B}, \quad (\text{A1})$$

where χ is the second rank and symmetric susceptibility tensor which is proportional to the anisotropic g tensor, $\mathbf{B}$ is the external magnetic field vector, and $\mathbf{T}$ is the dipolar second rank tensor.

As Vega and Fiat³² showed for a rigid molecule, Eq. (A1) can be written in the irreducible representation as

$$\mathbf{H}_{\text{Curie}} = \sum_{\ell} \sum_m \sum_{m'} (-1)^m Q_{-m}^{\ell} K_m^{\ell} D_{m',m}^{\ell}(\Omega_1), \quad (\text{A2})$$

where $Q^{(\ell)}$ represents the spin part of the interaction in the laboratory frame with

$$Q_0^0 = -(1/\sqrt{3})\mathbf{I}_z, \quad (\text{A3a})$$

$$Q_0^1 = 0, \quad Q_{\pm}^1 = -(1/\sqrt{2})\mathbf{I}_{\pm}, \quad (\text{A3b})$$

$$Q_0^2 = (2/\sqrt{6})\mathbf{I}_z, \quad Q_{\pm 1}^2 = \mp (1/2)\mathbf{I}_{\pm} Q_{\pm 2}^2 = 0. \quad (\text{A3c})$$

$K^{(\ell)}$ is the space part given by separating the 3×3 matrix $\mathbf{T} \cdot \chi$ into its trace ($\ell=0$), antisymmetric ($\ell=1$), and symmetric ($\ell=2$) irreducible components. The Wigner matrix ele-

ments $D_{m',m}^\ell(\Omega_1)$, transform $K_{m'}^\ell$ from the body 1 frame to the Laboratory frame.

Within the scope of the two body model (Fig. 1 in the text), where the dipolar vector, $\vec{r}_{BA}$, spans across the two domains, T and χ depend in different ways on the interdomain variable Ω_2 . In our specific examples, the ion is located on body 2 and so χ is fixed on body 2. Its dependency on the variable Ω_2 would be realized only through the transformation to the body 1 frame. In the irreducible representations, it is written as follows:

$$\chi = \chi_{\text{iso}} + \sum_{m'} D_{m',m}^\ell(\Omega_2) \tilde{\chi}_{m'}^2, \quad (\text{A4})$$

where $\tilde{\chi}^{(2)}$ is the anisotropic susceptibility second rank tensor, χ_{iso} is the isotropic susceptibility component, and the $D_{m',m}^\ell(\Omega_2)$ are the Wigner matrix elements transforming $\tilde{\chi}^2$ from the body 2 frame to the body 1 frame.

The dependency of the dipolar tensor T on the variable Ω_2 is more complicated as already illustrated in the text (Sec. II B): T is expressed in the spherical harmonic representation as $F^{(2)}(\Omega_{BA})$ in Eq. (6) and in Eqs. (B4) and (B5).

The tensor products between $\tilde{\chi}^{(2)}$, χ_{iso} and $F^{(2)}$ to give $K^{(\ell)}$, are given using the following angular momentum coupling formulas:³³

$$K_0^0 = -\sqrt{5/3} \{F^{(2)}(\Omega_{BA}) \otimes \tilde{\chi}^{(2)}\}_{00}^0, \quad (\text{A5a})$$

$$K_m^1 = \sqrt{5/4} \{F^{(2)}(\Omega_{BA}) \otimes \tilde{\chi}^{(2)}\}_m^1, \quad (\text{A5b})$$

$$K_m^2 = -\sqrt{7/12} \{F^{(2)}(\Omega_{BA}) \otimes \tilde{\chi}^{(2)}\}_m^2 + \{F^{(2)}(\Omega_{BA}) \otimes \chi_{\text{iso}}\}_m^2. \quad (\text{A5c})$$

When averaging over the full ensemble, as we do for PCSs, only K_0^0 gives a nonvanishing average. Hence, the scalar product between $F^{(2)}$ and $\tilde{\chi}^{(2)}$ in Eq. (A5a) correspond to the PCS observable. We can write Eq. (A5a) explicitly as

$$K^{(0)} = -\sqrt{5/3} \sum_{k=-2}^2 C(2,k,2,-k|00) F_k^2 \tilde{\chi}_{-k}^2, \quad (\text{A6})$$

where $C(2,k,2,-k|00)$ are Clebsch-Gordan coefficients.

Averaging Eq. (A6) over the full ensemble leads to Eq. (4) in the text.

For spin relaxation, the four elements in Eqs. (A5a)–(A5c) should, in principle, be considered. However, it turns out that, for most lanthanides, the values in $\tilde{\chi}^{(2)}$ ($\Delta\chi_{\text{ax}}, \Delta\chi_{\text{th}}$), are about 1% of the isotropic part χ_{iso} , and the coefficient for the autocorrelation function would have square dependencies on those values. Hence, autocorrelation functions generated using Eqs. (A5a) and (A5b) can be neglected as their contributions to relaxation are about $\sim 0.01\%$ with respect to the autocorrelation function of Eq. (A5c). As for the latter, neglecting the anisotropic susceptibility contribution (the first term) can result in an error of $\sim 1\%$ which means that it is safe to reduce Eq. (A5c) to the form

$$K_m^2 \equiv \chi_{\text{iso}} F_m^2. \quad (\text{A7})$$

This in effect represents a through space dipolar interaction with an induced moment of constant value and leads to the relaxation rates as written in Eq. (1).

APPENDIX B: CALCULATING THE STARTING VECTORS

The starting vectors are essentially a projection of the interaction, δf [Eq. (14)], on the basis set functions used to describe the motional modes.

$$\mathbf{v}_1 = \langle \lambda | \delta f P_{\text{eq}}^{1/2} \rangle / \sqrt{\langle |\delta f|^2 P_{\text{eq}} \rangle}. \quad (\text{B1})$$

Substituting Eqs. (14)–(16) into the nominator of Eq. (B1):

$$\begin{aligned} \langle \lambda | \delta f P_{\text{eq}}^{1/2} \rangle &= \langle j_1, m_1, k_1 | \otimes \langle j_2, m_2, k_2 | \delta f P_{\text{eq}}^{1/2} \rangle \\ &= \sqrt{\frac{j_2(j_2+1)}{8\pi^2}} \int_{\Omega_1} D_{m_1, k_1}^{j_1*}(\Omega_1) \\ &\quad \times \sum_{m'=-2}^2 D_{m', m}^2(\Omega_1) d\Omega_1 \int_{\Omega_2} \sqrt{\frac{j_2(j_2+1)}{8\pi^2}} \\ &\quad \times D_{m_2, k_2}^{j_2*}(\Omega_2) F_{m'}^2(\Omega_{BA}) P_{\text{eq}}^{1/2}(\Omega_2) d\Omega_2. \end{aligned} \quad (\text{B2})$$

Using Wigner matrix properties,³³ the integration over Ω_1 can be eliminated and we left with

$$\begin{aligned} \langle j_1, m_1, k_1 | \otimes \langle j_2, m_2, k_2 | \delta f P_{\text{eq}}^{1/2} \rangle &= \delta_{j_1, 2} \delta_{m_1, m'} \delta_{k_1, m} \int_{\Omega_2} \sqrt{\frac{j_2(j_2+1)}{8\pi^2}} \\ &\quad \times D_{m_2, k_2}^{j_2*}(\Omega_2) F_{m'}^2(\Omega_{BA}) P_{\text{eq}}^{1/2}(\Omega_2) d\Omega_2. \end{aligned} \quad (\text{B3})$$

Expanding the expression given in Eq. (6) in the text, $F_{m'}^2(\Omega_{BA})$ can be put into the explicit form

$$\begin{aligned} F_{m'}^2(\Omega_{BA}) &= \frac{Y_m^2(\Omega_{BA})}{(r_{BA})^3} \\ &= \sqrt{\frac{4\pi}{24}} \sum_{\ell=0}^{\ell_{\text{max}}} (-1)^\ell \sqrt{\frac{(2\ell+4)!}{(2\ell+1)!}} \frac{|r_{AC}|^\ell}{|r_{BC}|^{\ell+3}} \\ &\quad \times \sum_{n=-\ell}^{\ell} C(\ell, -n; \ell+2, m'+k|2, m') \\ &\quad \times Y_{-n}^\ell(\Omega_{AC}) Y_{m'+k}^{\ell+2}(\Omega_{BC})_{m'}. \end{aligned} \quad (\text{B4})$$

In the presented model, the vector $\vec{r}_{AC}$ is on body 2. Therefore, it can be described with fixed Euler angles Ω_{AC}^2 relative to the principal axis system of body 2. The corresponding spherical harmonics, $Y^{(\ell)}(\Omega_{AC}^2)$, will be transformed to the body 1 frame with the stochastic variable Ω_2 ,

$$Y_{-n}^\ell(\Omega_{AC}) = \sum_{n'=-\ell}^{\ell} D_{n', -n}^\ell(\Omega_2) Y_{n'}^\ell(\Omega_{AC}^2). \quad (\text{B5})$$

Substituting Eq. (B5) into Eq. (B4) and putting the results into Eq. (B3) we get the following expression:

$$\begin{aligned}
& \langle j_1, m_1, k_1 | \otimes \langle j_2, m_2, k_2 | \delta f P_{\text{eq}}^{1/2} \rangle \\
&= \delta_{j_1,2} \delta_{m_1,m'} \delta_{k_1,m} \sqrt{\frac{4\pi}{(4)!}} \sum_{\ell=0}^{\ell_{\max}} \sum_{n=-\ell}^{\ell} (-1)^{\ell} \\
&\quad \times \sqrt{\frac{(2\ell+4)!}{(2\ell+1)!}} C(\ell, -n; \ell+2, m' + n | 2, m') \\
&\quad \times \frac{r_{BC}^{\ell}}{r_{AB}^{\ell+3}} \sum_{n'=-\ell}^{\ell} Y_{n'}^{\ell}(\Omega_{AC}^2) Y_{k+m'}^{\ell+2}(\Omega_{BC}) \sqrt{\frac{(2j_2+1)}{8\pi^2}} \\
&\quad \times \int_{\Omega_2} D_{m_2,k_2}^{j_2*}(\Omega_2) D_{n',-n}^{\ell}(\Omega_2) P_{\text{eq}}(\Omega_2) d\Omega_2. \quad (\text{B6})
\end{aligned}$$

Next we expand $P_{\text{eq}}^{1/2}(\Omega_2)$ in terms of Wigner functions using the potential as defined in Eq. (9). In our simulation, since we define the z axis of body 2 to be along the vector $\vec{r}_{AC}$ (Fig. 2), the results of the correlation function are independent of any value of c_1^1 in the calculation of $\mathbf{v}_1$, and we can set $c_1^1=0$ (it is still important to include the c_1^1 in the calculation of $\tilde{\Gamma}$). Hence we can write

$$P_{\text{eq}}^{1/2}(\Omega_2) = \sum_{J=0}^{J_{\max}} \varepsilon_0^J D_0^J(\Omega_2). \quad (\text{B7})$$

In practice $J_{\max}$ ranges between 0 and 30 for $0 \leq S_0^1 \leq 0.95$. Substituting Eq. (B7) into Eq. (B6) gives

$$\begin{aligned}
& \langle j_1, m_1, k_1 | \otimes \langle j_2, m_2, k_2 | \delta f P_{\text{eq}}^{1/2} \rangle \\
&= \delta_{j_1,2} \delta_{m_1,m'} \delta_{k_1,m} \sqrt{\frac{4\pi}{(4)!}} \sum_{\ell=0}^{\ell_{\max}} \sum_{n=-\ell}^{\ell} (-1)^{\ell} \\
&\quad \times \sqrt{\frac{(2\ell+4)!}{(2\ell+1)!}} C(\ell, -n; \ell+2, m' + n | 2, m') \\
&\quad \times \frac{r_{BC}^{\ell}}{r_{AB}^{\ell+3}} \sum_{n'=-\ell}^{\ell} Y_{n'}^{\ell}(\Omega_{AC}^2) Y_{k+m'}^{\ell+2}(\Omega_{BC}) \sqrt{\frac{(2j_2+1)}{8\pi^2}} \\
&\quad \times \sum_J \varepsilon_0^J \int_{\Omega_2} D_{m_2,k_2}^{j_2*}(\Omega_2) D_{n',-n}^{\ell}(\Omega_2) D_0^J(\Omega_2) d\Omega_2. \quad (\text{B8})
\end{aligned}$$

Integration over three Wigner functions can be written as³³

$$\begin{aligned}
& \int_{\Omega_2} D_{m_2,k_2}^{j_2*}(\Omega_2) D_{n',-n}^{\ell}(\Omega_2) D_0^J(\Omega_2) d\Omega_2 \\
&= \frac{8\pi^2}{2j_2+1} \delta_{k_2,-n} \delta_{m_2,n'} C(J, 0, \ell, n' | j_2, m_2) \\
&\quad \times C(J, 0, \ell, -n | j_2, k_2). \quad (\text{B9})
\end{aligned}$$

Putting Eq. (B9) in Eq. (B8) and eliminating redundant indices that arise in the Kronecker delta functions we get to the final expression

$$\begin{aligned}
& \langle j_1, m_1, k_1 | \otimes \langle j_2, m_2, k_2 | \delta f P_{\text{eq}}^{1/2} \rangle \\
&= \delta_{j_1,2} \delta_{m_1,m'} \delta_{k_1,m} \sqrt{\frac{4\pi}{(4)!}} \sum_{\ell=0}^{\ell_{\max}} (-1)^{\ell} \\
&\quad \times \sqrt{\frac{(2\ell+4)!}{(2\ell+1)!}} C(\ell, k_2; \ell+2, m_1 - k_2 | 2, m_1) \\
&\quad \times \frac{r_{BC}^{\ell}}{r_{AB}^{\ell+3}} Y_{m_2}^{\ell}(\Omega_{AC}^2) Y_{m_1-k_2}^{\ell+2}(\Omega_{BC}) \sqrt{\frac{(2j_2+1)}{8\pi^2}} \frac{8\pi^2}{2j_2+1} \\
&\quad \times \sum_{J=|j_2-\ell|}^{j_2+\ell} \varepsilon_0^J C(J, 0, \ell, m_2 | j_2, m_2) C(J, 0, \ell, k_2 | j_2, k_2). \quad (\text{B10})
\end{aligned}$$

The denominator of Eq. (B1) can be solved either with a discrete approach, using Wigner functions properties as demonstrate above, or by numerical integration.

- ¹ L. H. Greene, T. E. Lewis, S. Addou, A. Cuff, T. Dallman, M. Dibley, O. Redfern, F. Pearl, R. Nambudiry, A. Reid, I. Sillitoe, C. Yeats, J. M. Thornton, and C. A. Orengo, *Nucleic Acids Res.* **35**, D291 (2007).
- ² W. P. Kelly and M. P. H. Stumpf, *Curr. Opin. Biotechnol.* **19**, 396 (2008).
- ³ P. Tompa and M. Fuxreiter, *Trends Biochem. Sci.* **33**, 2 (2008).
- ⁴ A. G. Palmer and F. Massi, *Chem. Rev. (Washington, D.C.)* **106**, 1700 (2006).
- ⁵ A. Polimeno and J. H. Freed, *Adv. Chem. Phys.* **83**, 89 (1993).
- ⁶ A. Polimeno and J. H. Freed, *J. Phys. Chem.* **99**, 10995 (1995).
- ⁷ Y. E. Ryabov and D. Fushman, *J. Am. Chem. Soc.* **129**, 3315 (2007).
- ⁸ G. M. Clore, A. Szabo, A. Bax, L. E. Kay, P. C. Driscoll, and A. M. Gronenborn, *J. Am. Chem. Soc.* **112**, 4989 (1990).
- ⁹ J. L. Baber, A. Szabo, and N. Tjandra, *J. Am. Chem. Soc.* **123**, 3953 (2001).
- ¹⁰ K. Chen and N. Tjandra, *J. Am. Chem. Soc.* **130**, 12745 (2008).
- ¹¹ V. Wong, D. A. Case, and A. Szabo, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 11016 (2009).
- ¹² V. Tugarinov, Z. C. Liang, Y. E. Shapiro, J. H. Freed, and E. Meirovitch, *J. Am. Chem. Soc.* **123**, 3055 (2001).
- ¹³ E. Meirovitch, A. Polimeno, and J. H. Freed, *J. Phys. Chem. B* **110**, 20615 (2006).
- ¹⁴ E. Meirovitch, Y. E. Shapiro, A. Polimeno, and J. H. Freed, *J. Phys. Chem. A* **110**, 8366 (2006).
- ¹⁵ V. Tugarinov, Y. E. Shapiro, Z. C. Liang, J. H. Freed, and E. Meirovitch, *J. Mol. Biol.* **315**, 155 (2002).
- ¹⁶ Y. E. Shapiro and E. Meirovitch, *J. Phys. Chem. B* **113**, 7003 (2009).
- ¹⁷ *Solution NMR of Paramagnetic Molecules*, Applications to Metallobiomolecules and Models, Vol. 2, edited by I. Bertini, C. Luchinat, and G. Parigi (Elsevier, Tokyo, 2001).
- ¹⁸ G. Otting, *J. Biomol. NMR* **42**, 1 (2008).
- ¹⁹ T. D. Zhuang, H. S. Lee, B. Imperiali, and J. H. Prestegard, *Protein Sci.* **17**, 1220 (2008).
- ²⁰ J. Iwahara, C. Tang, and G. M. Clore, *J. Magn. Reson.* **184**, 185 (2007).
- ²¹ J. Iwahara, C. D. Schwieters, and G. M. Clore, *J. Am. Chem. Soc.* **126**, 5879 (2004).
- ²² J. L. Battiste and G. Wagner, *Biochemistry* **39**, 5355 (2000).
- ²³ M. A. MacNaughtan, M. Kamar, G. Alvarez-Manilla, A. Venot, J. Glushka, J. M. Pierce, and J. H. Prestegard, *J. Mol. Biol.* **366**, 1266 (2007).
- ²⁴ J. R. Gillespie and D. Shortle, *J. Mol. Biol.* **268**, 158 (1997).
- ²⁵ J. R. Gillespie and D. Shortle, *J. Mol. Biol.* **268**, 170 (1997).
- ²⁶ J. Iwahara, C. D. Schwieters, and G. M. Clore, *J. Am. Chem. Soc.* **126**, 12800 (2004).
- ²⁷ C. Eichmüller and N. R. Skrynnikov, *J. Biomol. NMR* **37**, 79 (2007).
- ²⁸ X. Wang, S. Srisailam, A. A. Yee, A. Lemak, C. Arrowsmith, J. H. Prestegard, and F. Tian, *J. Biomol. NMR* **39**, 53 (2007).
- ²⁹ Y. Xue, I. S. Podkorytov, D. K. Rao, N. Benjamin, H. L. Sun, and N. R. Skrynnikov, *Protein Sci.* **18**, 1401 (2009).
- ³⁰ I. Bertini, C. Luchinat, and G. Parigi, *Prog. Nucl. Magn. Reson. Spectrosc.* **40**, 249 (2002).

- ³¹M. Gueron, J. Magn. Reson. **19**, 58 (1975).
- ³²A. J. Vega and D. Fiat, *Mol. Phys.* **31**, 347 (1976).
- ³³D. A. Varshalovich, A. N. Moskalev, and V. K. Khersonskii, *Quantum Theory of Angular Momentum*, 1st ed. (World Scientific, Singapore, 1988).
- ³⁴B. J. Berne, in *Physical Chemistry: An Advanced Treatise*, edited by H. Eyring, O. Henderson, and W. Jost (Academic, New York, 1971), Vol. 8B.
- ³⁵C. F. Polnaszek, G. V. Bruno, and J. H. Freed, *J. Chem. Phys.* **58**, 3185 (1973).
- ³⁶G. Moro and J. H. Freed, *J. Chem. Phys.* **75**, 3157 (1981).
- ³⁷G. Moro and J. H. Freed, *J. Chem. Phys.* **74**, 3757 (1981).
- ³⁸D. J. Schneider and J. H. Freed, *Adv. Chem. Phys.* **73**, 387 (1989).
- ³⁹P. Z. Qin, K. Hideg, J. Feigon, and W. L. Hubbell, *Biochemistry* **42**, 6772 (2003).
- ⁴⁰A. Tarantola, *Inverse Problem Theory and Methods for Model Parameter Estimation* (Society for Industrial and Applied Mathematics, Philadelphia, 2005).
- ⁴¹M. W. F. Fischer, J. A. Losonczi, J. L. Weaver, and J. H. Prestegard, *Biochemistry* **38**, 9013 (1999); I. Bertini, P. Kursula, C. Luchinat, G. Parigi, J. Vahokoski, M. Wilmanns, and J. Yuan, *J. Am. Chem. Soc.* **131**, 5134 (2009); E. B. Bertelsen, L. Chang, J. E. Gestwicki, and E. R. P. Zuiderweg, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 8471 (2009).
- ⁴²M. W. Clarkson, M. Lei, E. Z. Eisenmesser, W. Labeikovsky, A. Redfield, and D. Kern, *J. Biomol. NMR* **45**, 217 (2009).
- ⁴³I. Bertini, G. Cavallaro, M. Cosenza, R. Kummerle, C. Luchinat, M. Piccioli, and L. Poggi, *J. Biomol. NMR* **23**, 115 (2002).
- ⁴⁴A. Polimeno, G. J. Moro, and J. H. Freed, *J. Chem. Phys.* **104**, 1090 (1996); D. Frezzato, A. Polimeno, A. Ferrarini, and G. J. Moro, *Theor. Chem. Acc.* **117**, 1017 (2007).
- ⁴⁵W. A. Wegener, R. M. Dowben, and V. J. Koester, *J. Chem. Phys.* **73**, 4086 (1980).
- ⁴⁶S. C. Harvey, P. Mellado, and J. G. Delatorre, *J. Chem. Phys.* **78**, 2081 (1983).
- ⁴⁷G. Moro, *Chem. Phys.* **118**, 181 (1987).
- ⁴⁸H. M. Ådland and A. Mikkelsen, *J. Chem. Phys.* **118**, 3376 (2003).
- ⁴⁹B. Halle, *J. Chem. Phys.* **119**, 12372 (2003).
- ⁵⁰F. Capozzi, F. Casadei, and C. Luchinat, *J. Biol. Inorg. Chem.* **11**, 949 (2006).
- ⁵¹I. Bertini, C. Del Bianco, I. Gelis, N. Katsaros, C. Luchinat, G. Parigi, M. Peana, A. Provenzani, and M. A. Zoroddu, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 6841 (2004).
- ⁵²G. A. Rabinovich, M. A. Toscanol, S. S. Jackson, and G. R. Vasta, *Curr. Opin. Struct. Biol.* **17**, 513 (2007).