Protein Dynamics by NMR Spin Relaxation: The Slowly Relaxing Local Structure Perspective

Eva Meirovitch
Bar-Ilan University, Ramat-Gan, Israel

Antonino Polimeno
University of Padova, Padova, Italy

&

Jack H. Freed
Cornell University, Ithaca, NY, USA

1 INTRODUCTION

Protein dynamics by NMR has been reviewed extensively in recent years.[1–4] These surveys show convincingly that information on structure has to be complemented by information on motion to properly characterize proteins and understand their function. The timescale accessible by NMR extends from picoseconds to days. Heteronuclear NMR spin relaxation applies to the picoseconds to nanoseconds regime, with the slow limit determined by the global tumbling of the protein, and the rate for restricted internal motion of the probe (typically a small protein-attached spin-bearing moiety) being faster.

We present in this article the slowly relaxing local structure (SRLS) approach,[5–7] which we developed for analyzing NMR spin relaxation in proteins.[8–10] This method is also suitable to treat (in the appropriate limit) polycrystalline protein samples,[11] and analyze residual dipolar couplings (RDCs) from proteins. SRLS is a mesoscopic stochastic approach to rotational motions in liquids.[5] So far, it has been implemented as a two-body (protein and probe) coupled-rotator theory. With SRLS, one solves a Smoluchowki equation within the scope of tensorial descriptions of the two rotators, and of the local ordering at the site of the motion of the probe. The solution of the Smoluchowski equation yields the spectral densities that enter the expressions for the experimentally measured relaxation parameters.

The motion of the probe is restricted by its immediate anisotropic protein surroundings. SRLS treats this motion by analogy with the classical treatment of restricted motions in liquids.[12–15] One may consider it as the extension of these approaches, which also accounts for the overall motion of the protein and for the dynamical coupling between these two dynamic modes. The main features of established standard approaches are recovered in the limit where the two rotators have significantly different timescales. However, the tensorial properties are rarely simple for proteins in this limit.

The traditional method for analyzing NMR spin relaxation in proteins is model-free (MF).[16,17] The MF perspective is different. Assuming that the dynamic complexity of proteins warrants only the simplest description, MF devises analytical spectral densities that implicitly treat only the simplest tensorial properties and ignore mode-coupling. Since the former are not simple in actual cases, the MF parameters absorb the unaccounted for features, thereby becoming physically vague composites. This is detrimental to the physical picture even when mode-coupling is not important (by mode-coupling, we mean effects from statistical interdependence of the various motions).

The objective of this article is to present the approach to spin relaxation in proteins, as represented by two-body Smoluchowski SRLS. The reader is referred to our previous work, which shows that SRLS has provided new insights into protein dynamics, yet at a level that is in keeping with the limited experimental data. Here we highlight the physical clarity, consistency, and generality of the results. In contrast, we delineate the serious limitations of the MF approach.

2 THE SLOWLY RELAXING LOCAL STRUCTURE

2.1 Diffusion-Restricted Local Motion with the Global Motion Frozen

Nordio and Busolin[12] and Freed and coworkers[13] treated diffusive rotational reorientation of an axial probe in a uniaxial liquid crystal. These developments can also be viewed as treatments of diffusion-restricted local motions in proteins, with the global motion frozen. They are general in allowing for an arbitrary tilt between the local ordering/local diffusion and magnetic frames and also for magnetic tensors of arbitrary symmetry and orientation. Polnaszek and Freed[18] extended the development of Ref. 13 by allowing rhombic local molecular ordering.

In the theories developed in Refs 12, 13, one solves the rotational diffusion equation for the probability density $P(\Omega, t)$ for the orientation of the probe:

$$\partial P(\Omega, t)/\partial t = -\Gamma_\Omega P(\Omega, t)$$

where

$$-\Gamma_\Omega = R\nabla_\Omega{}^2 P(\Omega, t) - (R/k_B T)(\sin\beta)^{-1}\partial/\partial\beta[\sin\beta \mathrm{T} P(\Omega, t)] \quad (1)$$

Equation (1) is appropriately referred to as a *Smoluchowski equation*. Here Γ_Ω is the Smoluchowski operator, R is the isotropic rotational diffusion coefficient, ∇^2_Ω is the rotational diffusion operator in the Euler angles $\Omega \to \alpha, \beta, \gamma$, and $\mathbf{T}$ is the restoring torque. The latter is equal to $-\partial U/\partial\beta$ in the

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This article was published in the *Encyclopedia of Magnetic Resonance* in 2011 by John Wiley & Sons, Ltd.
DOI: 10.1002/9780470034590.emrstm1243

case of an axial restoring potential, e.g., $U \cong 3/2\, c_0^2 \cos^2\beta$ (c_0^2 is in units of $k_B T$). One diagonalizes the representation of the operator Γ_Ω, typically using the normalized forms of the Wigner rotation matrix elements $D^L_{KM}(\Omega)$ as a convenient basis set, to obtain the eigenfunctions and eigenvalues of Γ_Ω. Then the time correlation functions (TCFs) of these normalized $D^L_{KM}(\Omega)$ (as well as their cross-correlation functions with $D^{L'}_{K'M'}(\Omega)$ where in general $L' \neq L$, $K' \neq K$, and/or $M' \neq M$) may be expressed in terms of these eigenfunctions and eigenvalues. Their Fourier transforms yield the spectral densities from which the magnetic resonance relaxation parameters, such as T_1, T_2, and heteronuclear NOE, are calculated.

These TCFs are generally found to be a sum of exponential decays, where the decay constants are the respective eigenvalues and the weighting factor of each decaying exponential gives the relative importance of that eigenfunction in the TCF. The general expressions for rhombic $\boldsymbol{R}$ tensor and rhombic potential $U(\Omega)$, which replace the respective quantities in the Γ_Ω of equation (1), are given in Ref. 18. Again, the TCFs for the $D^L_{KM}(\Omega)$ are found to be sums of exponential decays determined by the eigenfunctions and eigenvalues of the more general Smoluchowski operator Γ_Ω.

2.2 Diffusion-Restricted Local Motion Decoupled from the Global Motion

A simple limit of the two-body Smoluchowski SRLS model describes a probe that reorients rapidly in a slowly moving "cage", which exerts on it a potential of mean torque (POMT).[19] In the present context, the cage represents the protein and the probe represents the spin-bearing moiety. In the simple limit, it is assumed that (i) the motions of the probe and the cage occur on very different timescales, (ii) the properties of the second-rank tensors involved are very simple, and (iii) the local ordering is weak.

The TCF $C(t)$ obtained by solving the appropriate Smoluchowski equation comprises three terms.[19] They represent effects of the slow protein motion, the reorientation of the probe with respect to the POMT, and a negative cross-term, which represents their statistical interdependence from the point of view of the probe. By analogy with the quantum mechanical model of the motion of a low-mass particle relative to a heavy particle, this was also called a *Born–Oppenheimer approximation*[19] (see also Ref. 20).

The Fourier transform of $C(t)$ is given by Meirovitch *et al*[10] as

$$j(\omega) = \frac{(S_0^2)^2 \tau_m}{(1+\omega^2 \tau_m{}^2)} + \frac{(1-(S_0^2)^2)\tau)}{(1+\omega^2\tau^2)} \qquad (2)$$

where S_0^2 is the axial order parameter defined in terms of a Legendre polynomial of rank 2, $\tau_m = 1/(6R^C)$ is the correlation time for the slow reorientation of the protein, $\tau = 1/(6R^L)$ is the correlation time for the faster ($\tau \ll \tau_m$) reorientation of the probe, and R^C and R^L are rotational rate constants. The local ordering/local diffusion and magnetic frames are taken as the same, implying that $j(\omega)$ of equation (2) is given by $j_{KK}(\omega)$, $K = 0$ (K is the order of the rank 2 local ordering tensor). The measurable spectral density $J(\omega)$, in terms of which the experimental relaxation parameters are defined, is equal in this case to $j_{00}(\omega)$.[14,15]

Note that the form of $J(\omega)$ is simple both because of large timescale separation and because the symmetry-related and geometry-related properties of the second-rank tensors involved are simple. Finally, the eigenfunctions of the diffusion operator of the probe are the same as the (Wigner) eigenfunctions of the freely diffusing probe. This is an approximation valid in the limit where the POMT is weak and the ratio R^C/R^L is small.[9,10]

When a spherical particle reorients rapidly in the presence of a strong axial POMT, simple eigenfunctions are obtained by solving a simple diffusion equation.[13,18] The solution yields a "renormalized" correlation time $\tau_{ren} \sim 2\tau/c_0^2$.[18] In the limit of a strong axial POMT and small ratio R^C/R^L, the full SRLS solution features a dominant local motional correlation time which agrees with τ_{ren}[9,10,21] and has eigenfunctions given in Ref. 18. In this limit, equation (2), with τ replaced by τ_{ren}, is a good approximation to the SRLS spectral density.[9] A convenient summary of the original derivation[19] of equation (2) is presented in Ref. 10. It shows that this equation is a reasonable result for all magnitudes of the axial POMT.

2.3 Diffusion-Restricted Local Motion Coupled to the Global Motion

The full two-body Smoluchowski SRLS theory as applied to NMR spin relaxation in proteins is outlined in Refs 8–10. A brief summary is given below. The SRLS frames are shown in Figure 1(a); the magnetic tensors apply to N–H bond dynamics. LF is the space-fixed laboratory frame with its Z-axis parallel to the external magnetic field. M1F is the principal axis system (PAS) of the global diffusion tensor $\boldsymbol{R}^{\mathbf{C}}$. VF is the local director. The M1F and VF frames are fixed in the protein. The OF frame is the PAS of the local ordering tensor $\boldsymbol{S}_{\mathbf{I}}$. M2F is the PAS of the local diffusion tensor. $\boldsymbol{R}^{\mathbf{L}}$, DF is the PAS of the magnetic ^{15}N– ^{1}H dipolar tensor. CF is the PAS of the ^{15}N chemical shift anisotropy (CSA) tensor. OF, M2F, DF, and CF are fixed in the probe.

The Euler angles Ω_{M1F-VF}, Ω_{OF-M2F}, Ω_{OF-DF}, and Ω_{DF-CF} are time-independent. The time-dependent Euler angles Ω_{LF-M1F} are modulated by the global motion. The distributed Euler angles Ω_{VF-OF} are associated with the local ordering. They enter the POMT and the equilibrium probability distribution function P_{eq}. The time-dependent Euler angles Ω_{VF-M2F} are modulated by the local motion.

For describing the local motion, we use a relative (probe versus protein) coordinate scheme; that is, $\Omega_{M1F-OF}(t) = \Omega_{LF-OF}(t) - \Omega_{LF-M1F}(t)$.[9,10,22] Each axial rotator when uncoupled is associated with three decay rates, $\tau_K^{-1} = 6R_\perp + K^2(R_{||} - R_\perp)$, $K = 0$, 1, 2, where R stands for either R^C or R^L.[14,15] The two rotators are coupled by the POMT, $U(\Omega_{VF-OF})$.[6,7] The diffusion equation for the coupled system is given by Meirovitch *et al*.[9,10]:

$$\frac{\partial}{\partial t} P(X,t) = -\hat{\Gamma} P(X,t) \qquad (3)$$

where X is a set of coordinates completely describing the system. One has

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This article was published in the *Encyclopedia of Magnetic Resonance* in 2011 by John Wiley & Sons, Ltd.
DOI: 10.1002/9780470034590.emrstm1243

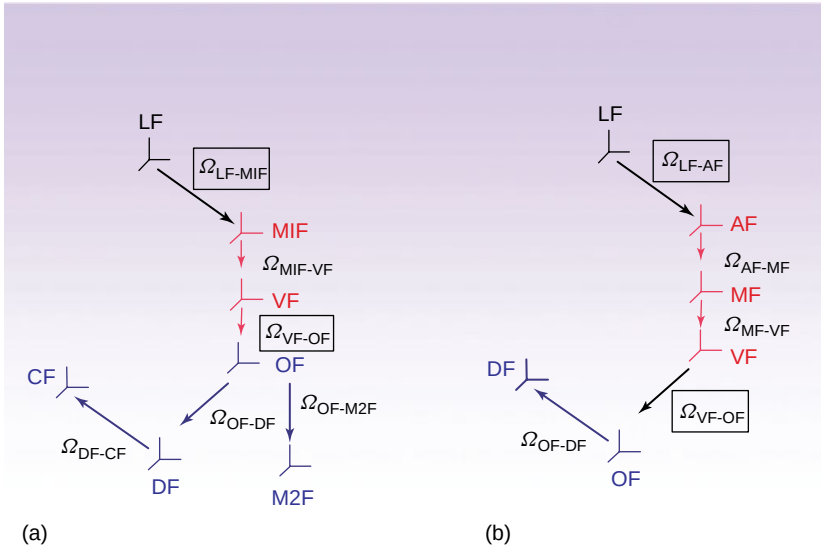

Figure 1 (a) SRLS reference frames for spin relaxation analysis. LF—laboratory frame, with its Z-axis along the external magnetic field; M1F—PAS of the global diffusion tensor; VF—local director frame; OF—PAS of the local ordering tensor, $\mathbf{S}_{\text{l}}$; M2F—PAS of the local diffusion tensor; DF—PAS of the ^{15}N–^{1}H dipolar tensor; CF—PAS of the ^{15}N chemical shift anisotropy (CSA) tensor. The frames M1F and VF (colored red) are fixed in the protein. The frames OF, M2F, DF, and CF (colored blue) are fixed in the probe. The boxed Euler angles are time-dependent/distributed. (b) SRLS reference frames for RDC analysis. As in part (a), except that the frames M1F and M2F are omitted and the frames AF—PAS of the global ordering tensor $\mathbf{S}_{\text{g}}$ and MF—molecular frame of the 3-D structure, are added

$$X = (\Omega_{\text{VF-OF}}, \Omega_{\text{LF-VF}})$$

$$\begin{aligned}\hat{\Gamma} = {} & \hat{J}(\Omega_{\text{VF-OF}})\boldsymbol{R}^{\text{L}}P_{\text{eq}}\hat{J}(\Omega_{\text{VF-OF}})P_{\text{eq}}^{-1} \\ & + [\hat{J}(\Omega_{\text{VF-OF}}) - \hat{J}(\Omega_{\text{LF-VF}})]\boldsymbol{R}^{\text{C}}P_{\text{eq}}[\hat{J}(\Omega_{\text{VF-OF}}) \\ & - \hat{J}(\Omega_{\text{LF-VF}})]P_{\text{eq}}^{-1}\end{aligned} \quad (4)$$

where $\hat{J}(\Omega_{\text{VF-OF}})$ and $\hat{J}(\Omega_{\text{LF-VF}})$ are the infinitesimal rotation operators for the probe and the protein, respectively. Note that $\Omega_{\text{LF-VF}} = \Omega_{\text{LF-M1F}} + \Omega_{\text{M1F-VF}}$. That is, $\Omega_{\text{LF-VF}}$ represents the combined effects of rotations by both sets of Euler angles on the right of this equation, where $\Omega_{\text{M1F-VF}}$ is time-independent.

The Boltzmann distribution is $P_{\text{eq}} = \exp[-U(\Omega_{\text{VF-OF}})/k_{\text{B}}T]/\langle\exp[-U(\Omega_{\text{VF-OF}})/k_{\text{B}}T]\rangle$. In general, the potential $U(\Omega_{\text{VF-OF}})$ is expanded in the full basis set of the Wigner rotation matrix elements. When only the $L = 2$ terms are preserved, one has[9,10,22]

$$\begin{aligned}u(\Omega_{\text{VF-OF}}) = \frac{U(\Omega_{\text{VF-OF}})}{k_{\text{B}}T} \approx {} & -c_0^2 D_{0,0}^2(\Omega_{\text{VF-OF}}) \\ & - c_2^2[D_{0,2}^2(\Omega_{\text{VF-OF}}) + D_{0,-2}^2(\Omega_{\text{VF-OF}})]\end{aligned} \quad (5)$$

The coefficient c_0^2 evaluates the strength of the POMF, and c_2^2 its nonaxiality. Expansion terms corresponding to $L = 4$, $K = 0, 2, 4$, (c_0^4, c_2^4, and c_4^4) are included in our most recent computational scheme.[22] They allow a more detailed modeling, in particular diffusion within two wells with less frequent jumps between them.[6,23] More general multipotential-well models can be included by adding appropriate terms in the expansion of $U(\Omega_{\text{VF-OF}})$. This is relevant for dynamics that are more complex.

The local order parameters are defined as[9,10,22]

$$\begin{aligned}&\langle D_{0m}^2(\Omega_{\text{VF-OF}})\rangle \\ &\quad = \frac{\int \text{d}\Omega_{\text{VF-OF}} D_{0m}^2(\Omega_{\text{VF-OF}})\exp[-u(\Omega_{\text{VF-OF}})]}{\int \text{d}\Omega_{\text{VF-OF}}\exp[-u(\Omega_{\text{VF-OF}})]}\end{aligned} \quad (6)$$

We assume that at least threefold symmetry prevails around the local director and at least twofold symmetry prevails around the Z-axis of the local ordering frame. In this case, only $S_{\text{l}0}^2 \equiv \langle D_{00}^2(\Omega_{\text{VF-OF}})\rangle$ and $S_{\text{l}2}^2 \equiv \langle D_{02}^2(\Omega_{\text{VF-OF}}) + D_{0-2}^2(\Omega_{\text{VF-OF}})\rangle$ survive.[7] The Saupe scheme order parameters relate to $S_{\text{l}0}^2$ and $S_{\text{l}2}^2$ as $S_{\text{l},xx} = (\sqrt{3/2}S_{\text{l}2}^2 - S_{\text{l}0}^2)/2$, $S_{\text{l},yy} = -(\sqrt{3/2}S_{\text{l}2}^2 + S_{\text{l}0}^2)/2$, and $S_{\text{l},zz} = S_{\text{l}0}^2$.[14,15]

Equation (3) is solved to yield the SRLS TCFs, which lead by Fourier transformation to the spectral densities $j_{K,K'}(\omega) = \sum_i \frac{c_{K,K',i}\tau_i}{1+\omega^2\tau_i^2}$.[9,10,22] In practice, a finite number of terms is sufficient for numerical convergence of the solution. The $j_{K,K'}(\omega)$ functions are assembled into the measurable spectral densities according to the local geometry.[15] For N–H bond dynamics, the relevant measurable spectral densities are J^{DD} for the ^{15}N– ^{1}H dipolar interaction and J^{CC} for the ^{15}N CSA interaction. J^{DD} depends on the Euler angles $\Omega_{\text{OF-DF}}$. $J^{\text{CC}}(\omega)$ is calculated from $J^{\text{DD}}(\omega)$; it depends on the Euler angles $\Omega_{\text{OF-DF}}$ and $\Omega_{\text{DF-CF}}$.

Cross-correlated spin relaxation, featuring $J^{\text{XY}}(\omega)$, is treated in complete analogy with autocorrelated spin relaxation.[15] Thus, the calculation of $J^{\text{DD}}(\omega)$ ($J^{\text{CC}}(\omega)$) from the $J_{\text{kk}'}(\omega)$ functions is based on the Wigner rotation $R(\Omega_{\text{OF-DF}})(R(\Omega_{\text{OF-CF}}))$, whereas the calculation of $J^{\text{DC}}(\omega)$ from the $J_{\text{kk}'}(\omega)$ functions is based on the Wigner rotation $R(\Omega_{\text{OF-DF}})$ followed by the Wigner rotation $R(\Omega_{\text{DF-CF}})$.

For rhombic local ordering and an axial (e.g., dipolar) magnetic interaction, six distinct pairs, $K, K' = (0, 0)$, (1,1), (2,2), (0,2), (−1, 1), and (−2, 2), have to be considered. The

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This article was published in the *Encyclopedia of Magnetic Resonance* in 2011 by John Wiley & Sons, Ltd.
DOI: 10.1002/9780470034590.emrstm1243

explicit expression for $J^{DD}(\omega)$ is

$$\begin{aligned} J^{DD}(\omega) = {} & (d^2_{00}(\beta_{OF-DF}))^2 j_{00}(\omega) + 2(d^2_{10}(\beta_{OF-DF}))^2 j_{11}(\omega) \\ & + 2(d^2_{20}(\beta_{OF-DF}))^2 j_{22}(\omega) \\ & + 4d^2_{00}(\beta_{OF-DF}) d^2_{20}(\beta_{OF-DF}) j_{02}(\omega) \\ & + 2d^2_{-10}(\beta_{OF-DF}) d^2_{10}(\beta_{OF-DF}) j_{-11}(\omega) \\ & + 2d^2_{-20}(\beta_{OF-DF}) d^2_{20}(\beta_{OF-DF}) j_{-22}(\omega) \end{aligned} \quad (7)$$

The ^{15}N relaxation parameters T_1, T_2, and ^{15}N–{^{1}H} NOE, are calculated as a function of $J^{DD}(0)$, $J^{DD}(\omega_H)$, $J^{DD}(\omega_N)$, $J^{DD}(\omega_H - \omega_N)$, $J^{DD}(\omega_H + \omega_N)$, $J^{CC}(0)$, $J^{CC}(\omega_H)$ (where ω_H and ω_N are the Larmor frequencies of the ^{1}H and ^{15}N nuclei, respectively,) and the magnetic interactions, using standard expressions for spin relaxation.[24,25] The cross-correlated relaxation rates associated with N– H bond dynamics, i.e., η_z and η_{xy},[26] feature the measurable spectral density obtained as outlined above, and the ^{15}N– ^{1}H dipolar/^{15}N CSA magnetic interaction cross-term.

For ^{2}H relaxation in $^{13}CDH_2$ methyl groups, one should use the ^{2}H quadrupolar tensor frame, QF, in Figure 1, and replace "DF" with "QF" in equation (7).[27–29] The measurable spectral densities are $J^{QQ}(0)$, $J^{QQ}(\omega_D)$, and $J^{QQ}(2\omega_D)$(where ω_D is the Larmor frequency of the ^{2}H nucleus).[30] Together with the magnitude of the quadrupolar interaction, they determine the experimental relaxation rates, typically ^{2}H T_1 and T_2, but also ^{2}H double-quantum, quadrupole order and transverse antiphase magnetization relaxation rates,[31] according to standard expressions for NMR spin relaxation.[30,31]

In addition to the enhancements to the POMF mentioned above, our most recent fitting scheme for SRLS[22] allows separating the local ordering and the local diffusion frames, and also allows for rhombic local ($\boldsymbol{R}^L$) and global ($\boldsymbol{R}^C$) diffusion tensors. Importantly, the SRLS program has been integrated with a hydrodynamics-based approach for calculating anisotropic $\boldsymbol{R}^C$ tensors.[32] The programming language C++ has been used, the code parallelized, and object-oriented programming used. These features brought about a 10-fold increase in efficiency relative to the fitting scheme developed in Ref. 9. We call this software package C++OPPS (COupled Protein Probe Smoluchowski).[22] It is available at the website http://www.chimica/unipd.it/licc/software.html.

SRLS constitutes a useful theoretical/computational tool for analyzing bio-macromolecular dynamics. Clearly, it is not practical to use it in its most general form in a given calculation. The parameter combination appropriate for analyzing given experimental data is determined by requiring both good correspondence between theory and experiment and physical relevance of the results. For example, using 6-data-point ^{15}N relaxation datasets acquired at two magnetic fields, we found that allowing R^L, c^2_0, c^2_2, and β_{OF-DF} to vary is an appropriate approach.[10,22]

In the limit of large timescale separation and strong POMTs, and in the (hypothetical) limit where τ of a small probe is practically the same as τ_m, inertial aspects of the probe motion could become important. In these cases, a full Fokker–Planck–Kramers (FPK) treatment, also developed in Ref. 5, is advisable. The implementation of FPK SRLS to NMR spin relaxation in proteins is under way.

2.4 SRLS Applications

We have studied ^{15}N–^{1}H amide group dynamics[8–10,21,22,33–39] and $^{13}CDH_2$ methyl dynamics.[10,27–29] Details appear in the respective references; here we only summarize our main conclusions. The important factors that affect the analyses include the asymmetry of the POMT, the fact that in the presence of a POMT the eigenfunctions of the (axial) local motional diffusion operator are no longer simple, and the possibility of mode-coupling. For amide bonds located in well-structured regions of the protein, the dominant factor is the asymmetry of the POMT. For amide bonds located in mobile domains and flexible loops, all the factors mentioned above are important. For methyl dynamics, mode-coupling is typically unimportant, but the other factors are important.

N– H bonds reorient primarily around the C^α_{i-1}–C^α_i axis with picosecond correlation times when located in well-structured regions, and nanosecond correlation times when located in mobile domains or flexible loops. In the former case, the local ordering around C^α_{i-1}– C^α_i is strong with large rhombicity; in the latter case, it is strong with moderate rhombicity.[8–10,21,22,33–39]

The local ordering at methyl sites in proteins is weak and rhombic, with the main local ordering axis parallel to the C–CH_3 bond. The local motion is typically fast relative to the global motion. Variations in the form of the rhombic local potential constitute the main factor determining the diversity of the experimental data.[10,27–29]

Activation energies for the local motion have been obtained for N– H bonds[37] and methyl groups[29] of entire proteins. This is not a trivial achievement.

3 DECOUPLING PROTEIN DYNAMICS: THE MODEL-FREE APPROACH

3.1 Model-free

Here it is assumed that the global and local motions are statistically independent.[16,17] On the basis of this assumption, the total TCF, $C(t)$, is factored into the product $C^C(t) \times C^L(t)$, with $C^C(t)$ ($C^L(t)$) denoting the TCF for global (local) motion. For spherical proteins, one has $C^C(t) = \exp(-t/\tau_m)$. The function $C^L(t)$ is devised on the basis of the theory of moments. It is exact at times zero and infinity, at short (picosecond) times it decays with an effective rate constant $1/\tau_e$ to a plateau, and in the nanosecond regime it decays exponentially to zero with rate constant $1/\tau_m$.[16] The Fourier transform of $C(t)$ is given by

$$J(\omega) = \frac{S^2\tau_m}{(1+\omega^2{\tau_m}^2)} + \frac{(1-S^2)\tau_e^{|}}{[1+\omega^2(\tau_e^{|})^2]} \quad (8)$$

where $1/\tau_e^{|} = 1/\tau_m + 1/\tau_e$, and $1/\tau_e^{|} \sim 1/\tau_e$ by virtue of τ_m, ns $\gg \tau_e$, ps.

Equation (8) is valid rigorously for a "frozen" protein, with $\tau_m = \infty$.[16] Hence it is valid approximately for $\tau_m \gg \tau_e$, the condition that underlies the assumption of statistical independence. TCFs for restricted local motions are typically multiexponential (section "Diffusion-Restricted Local Motion with the Global Motion Frozen" and Refs 12–15). Only

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This article was published in the *Encyclopedia of Magnetic Resonance* in 2011 by John Wiley & Sons, Ltd.
DOI: 10.1002/9780470034590.emrstm1243

in limiting cases may a single decay constant be used as an approximation. For wobble-in-a-cone in a square-well potential, the cone vertex angle has to be smaller than 50°.[40] For wobble-in-a-cone in a cosine-squared potential, the threshold is 15°.[9] For diffusive local motion in a strong axial potential, the dimensionless coefficient c_0^2 must be larger than 10 and the timescale separation larger than 100 for a single correlation time given by $2\tau/c_0^2$ to be valid.[9]

The parameter τ_e is defined as the area of the exact TCF for internal motion divided by $(1 - S^2)$.[16] This is a mathematical definition. As pointed out above, model-related parameters are limited by the range of physical validity of the respective model. MF mathematical parameters are not limited.

The parameter S^2 is set equal to $C^L(\infty) \equiv \sum_{m=0,\pm1,\pm2} \langle |Y_{2m}(\theta, \varphi)| \rangle^2$, where Y_{2m} are the spherical harmonics of Brink and Satchler.[41] The angles θ and φ define the orientation of a rhombic local ordering frame relative to a uniaxial local director. In MF, the local ordering frame is implicitly the same as the axial magnetic frames. A physical frame cannot be both axial and rhombic.

S^2 is considered as a measure of the amplitude of the local motion.[16,42] This interpretation, which is appropriate in the limit when the local motion is so fast that its effects (to the spectrum and spin relaxation) are completely averaged out,[7] prompted the utilization of S^2 to calculate conformational entropy.[3,4] The physical meaning of the latter quantity is thus problematic outside the limit where $S^2 \to (S_0^2)^2 = \langle P_2(\cos\theta) \rangle^2$, θ is small, and $\tau_e \to 0$.

The MF spectral density[16] is formally the same as equation (2), which was obtained by solving the appropriate Smoluchowski equation.[19] In particular, S^2 is formally analogous to $(S_0^2)^2$, and τ_e is formally analogous to τ for small S^2 and to τ_{ren} for large S^2 (on a 0–1 scale).[9,10] Actual spectral densities do not usually fulfill the validity conditions of equation (2); hence, equation (8) is typically used outside of its range of physical validity.

A comment on how MF addresses cross-correlated relaxation is in order. As pointed out above, the MF formula represents $j_{00}(\omega)$. Hence, for autocorrelated relaxation, MF has to take $J^{CC}(\omega) = J^{DD}(\omega) = J(\omega)$. Similarly, for cross-correlated relaxation, MF can only offer $J^{XY}(\omega) = (d_{00}^2) j_{00}(\omega)$ as measurable spectral density,[43] with the terms containing $j_{11}(\omega)$ and $j_{22}(\omega)$ omitted. This will be appropriate if $R_{||}^L \gg R_\perp^L$, rendering $j_{00}(\omega)$ much larger than $j_{11}(\omega)$ and $j_{22}(\omega)$. This condition has been properly stated (in a somewhat different form) in Ref. 43.

3.2 Extended MF

Besides a fast local motional term with correlation time τ_f and squared order parameter S_f^2, the EMF spectral density also includes a slow local motional term with correlation time τ_s and squared order parameter S_s^2.[44] The correlation time τ_s is about 10 times smaller than τ_m for small loops, and comparable to τ_m for mobile domains and large loops. The EMF formula has also been derived on the basis of the theory of moments.[16]

Lin and Freed[45] developed an extension of equation (2) for weak rhombic ordering and axial diffusion (equation (B6) of Ref. 45). For a 90° tilt between the axial magnetic frame and the main local ordering axis, the measurable spectral density is (within a good approximation) formally equivalent to the EMF spectral density. However, the spectral density developed in reference 45 includes general properties of the magnetic and ordering tensors, rendering it physically very different from the EMF spectral density. Thus, τ_f and τ_s become $\tau_{||}^L = 1/6R_{||}^L$ and $\tau_\perp^L = 1/6R_\perp^L$, with $R_{||}^L$ and $R_\perp^L$ representing the components of the axial local diffusion tensor, and S_f and S_s can be expressed as functions of S_0^2 and S_2^2, the components of the rhombic local ordering tensor.[45] The SRLS parameters have been obtained by solving the appropriate Smoluchowski equation, whereas the EMF parameters have been derived from a mathematical ansatz for the spectral density. Equation (B6) of Ref. 45 is only the case for small R^C/R^L and weak ordering. Therefore, the EMF formula should not be used when $\tau_s \sim \tau_m$ and when S_f and S_s do not comply with weak local ordering (in principle, it should not be used when $\tau_s \sim \tau_m$ is associated with a small probe; as noted, this is a hypothetical case).

3.3 Methyl Dynamics

As pointed out in section "Diffusion-Restricted Local Motion Coupled to the Global Motion", in our use of SRLS we treat methyl dynamics in much the same way as we treat N–H bond dynamics. The complexity of the former process is accounted for by low tensor symmetry, in particular rhombic local ordering. MF treats methyl dynamics[16,42] by reinterpreting equation (8) to represent two local motions: rotation around the C–CH_3 axis described by Woessner's model,[46] and axial fluctuations of the C–CH_3 axis[16,42]. The former motion is associated with $P_2(\cos 110.5^\circ)^2 = 0.1$ as squared order parameter, the latter with S_{axis}^2, and S^2 is set equal to $0.1 \times S_{axis}^2$. The effective correlation time τ_e represents both local motions.[16,42]

Inspection of Woessner's theory[46] shows that this model does not include an order parameter. The trigonometric expression $P_2(\cos 110.5^\circ) = (d_{00}^2(110.5^\circ)^2$ is the coefficient of the $j_{00}(\omega)$-containing term of the measurable spectral density (the latter comprises all three terms, $j_{KK}(\omega)$, $K = 0, 1, 2$, given that the (axial) local diffusion and magnetic tensor frames are tilted). The correlation time $\tau_\perp$ represents in Woessner's model the isotropic global tumbling, whereas the correlation time $\tau_{||}$ represents the local motion. The condition that $\tau_\perp \gg \tau_{||}$ has to be fulfilled. In the MF description of methyl dynamics, one has $\tau_\perp = \tau_{||} = \tau_e$, with τ_m representing the global motion. This scenario is inconsistent from a physical point of view.

3.3.1 Assessment of the MF Approach

SRLS is more general than MF, yielding the latter in simple limits.[9,10] The key elements that have been found to be important with the SRLS model in actual cases are not represented in MF. Consequently, the oversimplified MF formulae absorb the unaccounted for effects and become parameterizing entities with a much vaguer physical meaning. We call this process "force-fitting". The best-fit parameters emerging from the corresponding SRLS and MF data-fitting processes have been compared.[9,10] Large quantitative differences and qualitatively different trends were obtained; in some cases, functional dynamics were undetected or misinterpreted.[8–10,21,22,27–29,33–39]

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This article was published in the *Encyclopedia of Magnetic Resonance* in 2011 by John Wiley & Sons, Ltd.
DOI: 10.1002/9780470034590.emrstm1243

The separation of the contributions of the global and local motion variables is treated in a general manner in Appendix B of Ref. 20. In this context, one might contemplate enhanced descriptions of the local motion contribution. However, experience[12–15] shows that, even in the limit of frozen global motion, the description of the local motion is not simple.

It may be concluded that MF often fails to extract properly the important information on structural dynamics inherent in the experimental data. In some cases, conceptual misunderstandings arise because the physical parameters are not well defined. For example, cross-correlation such as encountered in the ^{15}N–^{1}H moiety signifies simultaneous modulation of two different magnetic interactions (in this case dipolar ^{15}N–^{1}H and ^{15}N CSA) by the same dynamic process. Some MF articles present cross-correlation in this context as a phenomenon involving two different mechanisms.[47]

3.4 Additional Decoupled Protein Dynamics Developments

Halle and Wennerström developed equation (8) in the context of ^{2}H relaxation of water in heterogeneous systems.[48] Rhombic magnetic tensors are allowed for. This generalization applies only in the extreme motional narrowing limit for the local motion (equation (137) of Chapter VIII of Ref. 24). In a recent paper, Halle developed a number of MF TCFs that feature intricate composites.[49] On the basis of mathematical arguments, some are considered valid in the mode-coupling regime. Comments on SRLS, to which we responded in Ref. 50, were made.

Brainard and Szabo developed a model that may be considered the generalization of the MF description of methyl dynamics, with separate correlation times assigned to the two local motions.[51] It is indicated that factorization of the generalized order parameter requires that both local motions be in the extreme motional narrowing limit. Lipari and Szabo[52] used the TCF of Ref. 40, which represents the $C^{\mathrm{L}}_{K=0}(t)$ component of the wobble-in-a-cone in a square-well potential model. The pertinent order parameter is shown in Ref. 53 to be given by $S_0^2 = \langle P_2(\cos\theta)\rangle = C^{\mathrm{L}}_{K=0}(\infty)$. In Ref. 54, Padé approximants are developed for $C^{\mathrm{L}}_K(t)$, $K = 0$, 1, and 2. Validity limits are determined, and analytical expressions for $1/\tau_K$, and $j_K(0)$ are derived.[54]

These developments represent established standard treatments of a physical model, which in its simplest form yields $C^{\mathrm{L}}(t)$ of equation (8). The established standard perspective was replaced in Refs 16, 17 by the MF point of view.

4 MODELS FOR LOCAL PROTEIN MOTIONS

The $C^{\mathrm{L}}_{K=0}(t)$ TCF of wobble-in-a-cone in a square-well potential was obtained in Ref. 40 by solving the appropriate Smoluchowski equation. Wang and Pecora treated wobble-in-a-cone for a rhombic probability distribution of probe orientations. Numerical solutions, given in terms of Legendre polynomials of noninteger degree, were obtained.[55]

Experimental ^{13}C relaxation data from methionine methyl groups of dihydrofolate reductase could be reproduced with Woessner's model[46] combined with asymmetric (but not with axial) fluctuations of the S–$^{13}CH_3$ group.[56] Concerted motions along a lysine side chain were treated.[57] Internal motions in proteins were treated by Wallach,[58] Woessner,[46,59] Daragan and Mayo,[60] LeMaster,[61] Korzhnev *et al*.,[62] Atkinson and Kieffer,[63] and others.

The 3-D Gaussian axial fluctuations (3-D GAF) model[64] provides an analytical description of anisotropic peptide plane motion around C^{α}_{i-1}–C^{α}_{i}. Harmonic local fluctuations are calculated with molecular dynamics (MD) simulations. Experimental ^{15}N and ^{13}C′ spin relaxation data from 76% of the peptide planes pertaining to rigid parts of the ubiquitin backbone were properly reproduced.[65] 3-D GAF was not designed to treat motions slower than approximately 10 ps. Nevertheless, it is often used to treat local motions comparable to the global motion, and even slower than it.[66]

5 ADDITIONAL DEVELOPMENTS INVOLVING SRLS

5.1 Molecular Dynamics

NMR-related order parameters have been derived from MD trajectories according to the MF paradigm.[10] The common method utilizes the assumption that $C(t) = C^{\mathrm{C}}(t) \times C^{\mathrm{L}}(t)$. The global motion is eliminated from the MD trajectory; subsequently, S^2 is set equal to the plateau value of $C^{\mathrm{L}}(t)$. This method applies when $C(t)$ features a plateau value.[42] Another common method is to calculate $S^2 \equiv \sum_{m=0,\pm1,\pm2} \langle |Y_{2\mathrm{m}}(\theta,\varphi)|\rangle^2$.[16] This is accomplished using a simple formula valid for strong axial local ordering in the extreme motional narrowing limit for the local motion.[67] Yet, this formula is being used more generally, even in the presence of nanosecond local motions. In some cases, it is considered "exact".[68]

A detailed discussion of spin-relaxation-related parameters obtained with MD and comparison with their MF counterparts appears in Appendix B of Ref. 10. It includes the few cases in which more elaborate $C^{\mathrm{L}}(t)$ functions were used, methods for determining $C^{\mathrm{C}}(t)$ with MD simulations were suggested, and $C(t)$ was derived directly from the MD trajectory. All of these developments rely in one way or the other on the MF paradigm.

5.2 Polycrystalline Powder Samples of Internally Mobile Proteins

^{2}H, ^{13}C, and ^{15}N NMR have been used to study protein dynamics in the solid state.[10] We developed the microscopic order macroscopic disorder (MOMD) approach for analyzing electron spin resonance (ESR) motional lineshapes from membranes and proteins.[11] MOMD represents the SRLS limit for a protein whose overall motion is frozen. It was applied successfully to liposomes, proteins, and DNA fragments.[10] It can be adapted relatively easily to NMR applications by including in the formalism the appropriate excitation pulse schemes and magnetic interactions. This will make it possible to study NMR spin relaxation from proteins in solution and in the solid state with SRLS.

MOMD treats diffusive motion. Jump-type motions, which often occur in solids, can be implemented by devising the

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This article was published in the *Encyclopedia of Magnetic Resonance* in 2011 by John Wiley & Sons, Ltd.
DOI: 10.1002/9780470034590.emrstm1243

appropriate Markov operators. Efforts to obtain high-resolution dynamic NMR lineshapes from polycrystalline protein samples are in progress.[69,70]

5.3 Residual Dipolar Couplings

SRLS applies to both isotropic[5,8–10] and anisotropic[6] solvents. The SRLS frame scheme relevant for RDC analysis is shown in Figure 1(b). The diffusion frames M1F and M2F of Figure 1(a) are not relevant here; on the other hand, the global ordering frame AF and the molecular frame MF are relevant.

The contribution of the dipolar interaction between two the nuclei i and j to the spin Hamiltonian is given by the following expression[14,15,18]:

$$H_{ij,\mathrm{DF}} = \sum_{m,k} \langle D^2_{m,k}(\Omega_{\mathrm{LF-DF}})\rangle F^{(2,k)*}_{ij,\mathrm{DF}} T^{(2,m)}_{ij,\mathrm{LF}} \qquad (9)$$

where $F^{(2,k)}_{ij,\mathrm{DF}}$ denotes the components of the magnetic dipolar tensor in the DF frame, and $T^{(2,m)}_{L}$ denotes the components of the relevant spin operators in the LF frame. $D^2_{m,k}$ are the Wigner rotation matrix elements.

For rigid proteins, $\Omega_{\mathrm{LF-DF}}$ is given by the sum $\Omega_{\mathrm{LF-AF}} + \Omega_{\mathrm{AF-DF}}$. That is, $\Omega_{\mathrm{LF-DF}}$ represents the combined effects of rotations by both sets of Euler angles on the right of this equation, where $\Omega_{\mathrm{AF-DF}}$ is time independent. Averaging the appropriate trigonometric functions over the Euler angles $\Omega_{\mathrm{LF-AF}}$ yields the global order parameters. The Euler angles $\Omega_{\mathrm{AF-DF}}$ provide the structural/geometric information of interest in the various structure-related applications of the RDC.

The POMT associated with the global ordering ($\mathrm{POMT_g}$), $u(\Omega_{\mathrm{LF-AF}})$, is expanded in the full basis set of the Wigner rotation matrix elements. With only the lowest order, $L = 2$, terms preserved, one has[6,7,18]

$$u(\Omega_{\mathrm{LF-AF}}) = \frac{U(\Omega_{\mathrm{LF-AF}})}{k_{\mathrm{B}}T} \approx -a^2_0 D^2_{0,0}(\Omega_{\mathrm{LF-AF}}) - a^2_2[D^2_{0,2}(\Omega_{\mathrm{LF-AF}}) + D^2_{0,-2}(\Omega_{\mathrm{LF-AF}})] \qquad (10)$$

with a^2_0 and a^2_2 denoting the axial and rhombic potential coefficients.

The global order parameters are defined as

$$\langle D^2_{0m}(\Omega_{\mathrm{LF-AF}})\rangle = \frac{\int \mathrm{d}\Omega_{\mathrm{LF-AF}} D^2_{0m}(\Omega_{\mathrm{LF-AF}}) \exp[-u(\Omega_{\mathrm{LF-AF}})]}{\int \mathrm{d}\Omega_{\mathrm{LF-AF}} \exp[-u(\Omega_{\mathrm{LF-AF}})]} \qquad (11)$$

For at least threefold symmetry around the LC director and at least twofold symmetry around the Z-axis of the global ordering frame, only $S^2_{\mathrm{g0}} \equiv \langle D^2_{00}(\Omega_{\mathrm{LF-AF}})\rangle$ and $S^2_{\mathrm{g2}} \equiv \langle D^2_{02}(\Omega_{\mathrm{LF-AF}}) + D^2_{0-2}(\Omega_{\mathrm{LF-AF}})\rangle$ survive.[14,15]

The RDC between the nuclei i and j is given by

$$\mathrm{RDC}_{ij} = \left(\frac{\mu_0}{4\pi}\right)\gamma_i\gamma_j h/(4\pi^2 {r_{ij}}^3)[S^2_0 P_2(\cos\beta_{\mathrm{AF-DF}}) + \left(\frac{3}{2}\right)^{1/2}(S^2_2 \sin^2(\beta_{\mathrm{AF-DF}})\cos(2\alpha_{\mathrm{AF-DF}})] \qquad (12)$$

where μ_0 is the permeability of vacuum, γ_i and γ_j are the magnetogyric ratios of the nuclei i and j, h is the Planck constant, and r_{ij} is the distance between the nuclei i and j. Methods for determining the global diffusion tensor, $\boldsymbol{A}$, have been developed.[71]

In the presence of local motion, $\Omega_{\mathrm{LF-DF}}$ is given by the sum $\Omega_{\mathrm{LF-AF}} + \Omega_{\mathrm{AF-MF}} + \Omega_{\mathrm{MF-VF}} + \Omega_{\mathrm{VF-OF}} + \Omega_{\mathrm{OF-DF}}$ (Figure1b). That is, $\Omega_{\mathrm{LF-DF}}$ represents the combined effects of rotations by all sets of Euler angles comprising this sum, where $\Omega_{\mathrm{AF-MF}}$, $\Omega_{\mathrm{MF-VF}}$, and $\Omega_{\mathrm{OF-DF}}$ are time independent. It is assumed that the $\mathrm{POMT_g}$ and the POMT (equation (5)) are uncorrelated.[72] Hence, one may average separately over $\Omega_{\mathrm{LF-AF}}$ to obtain S^2_{g0} and S^2_{g2}, and over $\Omega_{\mathrm{VF-OF}}$ to obtain S^2_{l0} and S^2_{l2}. The SRLS approach treats the local ordering by analogy with the global ordering. Thus, one has $\boldsymbol{S}_{\mathrm{g}}$, $u(\Omega_{\mathrm{LF-AF}})$, and $P_{\mathrm{eq,g}}$ for the global ordering and $\boldsymbol{S}_{\mathbf{l}}$, $u(\Omega_{\mathrm{VF-OF}})$, and $P_{\mathrm{eq,l}}$ for the local ordering.[10]

The contribution of the dipolar interaction between the nuclei i and j to the spin Hamiltonian is given by[10]

$$H_{ij,\mathrm{DF}} = \sum_{p,q,r,s} \langle D^2_{0,p}(\Omega_{\mathrm{LF-AF}})\rangle D^2_{p,q}(\Omega_{\mathrm{AF-MF}}) D^2_{q,r}(\Omega_{\mathrm{MF-VF}}) \times \langle D^2_{r,s}(\Omega_{\mathrm{VF-OF}})\rangle D^2_{s,0}(\Omega_{\mathrm{OF-DF}}) F^{(2,0)*}_{ij,\mathrm{DF}} T^{(2,0)}_{ij,\mathrm{LF}} \qquad (13)$$

This expression represents the RDC between the nuclei i and j for uncorrelated external and internal potentials. It is a product of seven terms. The first term, specific to a given LC medium, yields the global order parameters, S^2_{g0} and S^2_{g2}. The second term, also specific to a given LC medium, describes the orientation of the AF frame with respect to the MF frame. The third term represents the structural/geometric information of interest in the various structure-related applications of the RDC. The fourth term yields the local order parameters S^2_{l0} and S^2_{l2}. The fifth term describes the relative orientation of the local ordering and dipolar frames.

6 FUTURE DIRECTIONS

SRLS, based upon a two-body rotational Smoluchowski equation, has served as a working model providing an insightful picture of protein dynamics.[8–10,21,22,27–29,33–39] It can be improved in several ways. For example, three-body Smoluchowski SRLS can incorporate domain or loop motion in addition to local-probe motion and protein tumbling. An approach based on FPK SRLS, which treats cases where the overdamped diffusion limit is exceeded, can also be implemented.

It will be useful to compare the results of SRLS analyses with the results of MD simulations. Once the SRLS analysis is completed, one can readily compute the relevant TCFs of the $D^2_{KK'}(\Omega)$ from the best-fit parameters (MF can only provide force-fitted $D^2_{00}(\Omega)$ functions). Progress on how such functions may be obtained from MD simulations is illustrated in Refs 73, 74. Complex ESR lineshapes that agree very well with experiment and have also been successfully analyzed by SRLS are shown in those articles. Similar techniques should be applicable to the reproduction of the relevant NMR relaxation parameters.

In its present implementation, SRLS does not treat explicitly correlated N–H bond motions along the polypeptide chain. More advanced modeling, such as developed in Refs 75 (for ESR) and 76 (for NMR), is required. Efforts along these lines are under way.

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This article was published in the *Encyclopedia of Magnetic Resonance* in 2011 by John Wiley & Sons, Ltd.
DOI: 10.1002/9780470034590.emrstm1243

7 CONCLUSIONS

Experimental NMR spin relaxation data from proteins comprise unique information on local potentials, local ordering, conformational distributions, global and local motional rates, associated activation energies, mode-coupling, and features of local geometry. This information can be extracted insightfully with stochastic models. In many cases, the Smoluchowski implementation of SRLS provides an appropriate tool for accomplishing this. One can extend the scope of SRLS to study structural dynamics of proteins as outlined in the section "Future Directions".

The SRLS approach is a comprehensive one. It can treat in a consistent manner spin relaxation, polycrystalline lineshapes, and RDCs.

8 RELATED ARTICLES

Structure and Dynamics of Disordered Proteins

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This article was published in the *Encyclopedia of Magnetic Resonance* in 2011 by John Wiley & Sons, Ltd.
DOI: 10.1002/9780470034590.emrstm1243

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Acknowledgments

This work was supported by the Israel Science Foundation (Grant No. 347/07 to E. M.), the Binational Science Foundation (Grant No. 2 006 050 to E. M. and J. H. F.), the German-Israeli Science Foundation for Scientific Research and Development (Grant No. 928-190.0/2006 to E. M. and Christian Griesinger of Max Planck Institute, Göttingen, Germany), and the Damadian Center for Magnetic Resonance at Bar-Ilan University, Israel. This work was also supported by the National Center for Research Resources of the National Institutes of Health (Grant No. P41-RR016 292 to J. H. F.). A. P. acknowledges support provided by the Italian Ministero dell'Istruzione, Universitàe Ricerca (MIUR Grant No. PRIN2006_2006033728), and by the University of Padova (Grant "Progetto Strategico" HELIOS 2009).

Biographical Sketches

Eva Meirovitch. *b*. 1944. BSc, 1966, Chemistry, The Hebrew University, Jerusalem, Israel. MSc, 1968, Physical Chemistry, The Hebrew University, Jerusalem, Israel (thesis supervisor Professor Chava Lifshitz). PhD, 1975. Magnetic Resonance, The Weizmann Institute of Science, Rehovot, Israel (supervisors Professor Zeev Luz and Dr Jozef Gilboa). Weizmann Post-Doctoral Fellow, 1977–1980, Cornell University, Ithaca, NY (with Professor Jack H. Freed). Senior Scientist and Associate Professor, Magnetic Resonance, 1980–1986; Solar Energy Project, 1986–1989, The Weizmann Institute of Science, Rehovot, Israel. Manager of NMR project, 1989–1993, Biosym Technologies, San-Diego, CA, USA. Visiting Scientist, 1993–1996, The Supercomputer Computations Research Institute, Florida State University, Tallahassee, FL, USA. Associate Professor, 1996–2008; Professor, 2008–present, Bar-Ilan University, Ramat-Gan, Israel.

Antonino Polimeno. *b*. 1963. MS (Laurea) 1987, Chemistry, University of Padova, Padova, Italy; PhD 1990, Chemistry, University of Padova, Padova, Italy (thesis supervisor Professor P.-L. Nordio). Post-Doctoral Fellow, 1900–1991, Cornell University, Ithaca, NY (with Professor Jack H. Freed). Assistant Professor, 1991–2000, University of Padova, Padova, Italy. Visiting scientist, Cornell University, Ithaca, NY, 1994–1995. Associate Professor, 2000–2010; Professor, 2010–present, University of Padova, Padova, Italy.

Jack H. Freed. *b*. 1938. B. Eng., 1958, Chemical Engineering, Yale University, USA; MS, 1959, PhD 1962, Physical Chemistry, Columbia University, USA. NSF Post-Doctoral Fellow, 1962–1963, Cambridge University, UK. Assistant Professor 1963–1967; Associate Professor 1967–1973; Professor 1973–2007; Frank and Robert Laughlin Professor of Physical Chemistry 2007–present, Cornell University, Ithaca, NY, USA. Director of National Center for Advanced ESR Technology (ACERT) 2001–present, Cornell University, Ithaca, NY, USA; President of International ESR Society, 2008–present.

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This article was published in the *Encyclopedia of Magnetic Resonance* in 2011 by John Wiley & Sons, Ltd.
DOI: 10.1002/9780470034590.emrstm1243