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11.1 Introduction

In this chapter we will discuss the study of molecular motion by EPR, using both continuous-wave (CW) and pulsed EPR, in particular two-dimensional electron–electron double resonance (2-D-ELDOR). In recent years, the study of protein dynamics using site-directed spin labels (SDSL) by EPR has become an important subject.

The EPR spectra of a paramagnetic probe provide information on the motion of its environment. As compared to NMR, the information obtained from EPR of a spin label offers the following advantages:

- EPR is much more sensitive per spin than NMR.
- The time scale in time-domain experiments of EPR is nanoseconds, whereas it is milliseconds for NMR.
- EPR is capable of focusing on a limited number of spins, as the spin-label spectrum is simple.
- EPR spectra change dramatically with the tumbling motion of the probe, being extremely sensitive to the local fluidity; this is not the usual case in NMR, where nearly complete averaging of the spectra occurs, allowing only residual rotational effects dictated by the T_1 and T_2 relaxation times.
- Fast EPR "snapshots" can be taken with high-frequency EPR, whereas slow EPR "snapshots" are taken with low-frequency EPR. Thus, multifrequency EPR helps one to unravel the complex dynamics of biosystems occurring on different time scales.
- Pulsed EPR provides a tool to distinguish homogeneous broadening, which characterizes the dynamics of the entire environment, from inhomogeneous broadening, which displays the effect of the local structure.

The organization of the chapter is as follows. A historical introduction is provided in Section 11.2, after which some relevant experimental data are described in

Section 11.3 to illustrate the importance of multifrequency EPR in studying motion. The theory of slow motion as studied by EPR is outlined in Section 11.4; this will include discussion of: (i) application of the stochastic Liouville equation (SLE); and (ii) the slowly relaxing local structure (SRLS) model together with the related microscopic ordered-macroscopic disordered (MOMD) model to analyze multifrequency EPR data. In Section 11.5 will be discussed the application of molecular dynamics to the prediction of multifrequency EPR spectra. Some concluding remarks are made in Section 11.5, while Section 11.6 includes the details of literature pertinent to molecular motion, as studied using EPR.

11.2

Historical Background

An account of the historical development of lineshape theory is given by Freed (2005). Historically, the basic theories for magnetic resonance lineshapes mainly for the motional narrowing limit were developed, among others, by Kubo and Tomita (1954), Wangness and Bloch (1953) and Redfield (1957, 1965).

The "anomalous alternating linewidth" effect, wherein the electrochemically generated EPR spectrum of the *p*-dinitrotetramethylbenzene anion showed that well-resolved proton superhyperfine splittings (shfs) appeared on the first, third, and fifth lines of the hf splitting from the two equivalent ¹⁴N nuclei, while the second and fourth lines were so broad that the proton shfs were completely masked, was resolved by the application of a more generally inclusive theory (Freed, 2005). This is the theory of EPR linewidths for organic radicals (Freed and Fraenkel, 1963), and this is still valid today for spectra in the motional-narrowing regime. This theory has been improved since then, with the incorporation of more precise and detailed models of the molecular dynamics into the formulation. An example is the incorporation of anisotropic rotational diffusion into the linewidth theory, and its illustration by reinterpreting a linewidth study on *p*-dinitrobenzene (Freed, 1964).

When Hyde and Maki (1964) first observed the electron nuclear double resonance (ENDOR) of organic radicals in liquids, there was no theory to explain why ENDOR could occur in liquids, and the reason for their successful observation was a mystery. However, a reformulation and generalized theory of EPR saturation, by analogy with the Freed and Fraenkel (1963) theory, led to a general theory of EPR saturation and double resonance (Freed, 1965). To date, this formulation – and its later extensions – serve as the basis for interpreting EPR saturation and ENDOR experiments for motionally narrowed spectra (Leniart, Conner, and Freed, 1975; Dorio and Freed, 1979; Kurreck, Kirste, and Lubitz, 1988; Möbius, Lubitz, and Freed, 1989; Möbius, Lubitz, and Plato, 1989). Using the saturation and double resonance theory, an appropriate theory for electron–electron double resonance (ELDOR) in liquids was developed by Freed (Hyde, Chien, and Freed, 1968) to explain the data obtained by Hyde and Chien. ELDOR serves, among other things, as a powerful means of studying spin relaxation for exploring rotational and translational motions in liquids.

Freed (1968) provided a formal, general formulation of the Redfield theory valid to all orders, using Kubo's method of generalized cumulant expansions in statistical mechanics (Kubo, 1962, 1963). However, in order to study spin-labeled macromolecules, which yield slow-motional EPR spectra, the most powerful method for simulating them is based on the SLE, which only requires the assumption that, for a complete analysis, the motional dynamics can be described by a (general) Markov process. The matrix representation of the SLE, and its solution, then requires computational methods. Such a theory was elucidated by Freed, Bruno, and Polnaszek (1971a) for the relevant cases of *g*-tensor and hyperfine anisotropy, and included saturation phenomena. The slow-motional spectra of peroxylaminedisulfonate (PADS) in ice, which did not show the substantial inhomogeneous broadening of typical spin labels, could be fitted with jump-type reorientations rather than with simple Brownian motion (Goldman et al., 1972). This was an indication that the slow-motional spectra were more sensitive to the microscopic molecular dynamics than were the fast-motional spectra. The SLE approach was also extended to a complete solution of slow-tumbling triplets (Freed, Bruno, and Polnaszek, 1971b), generalizing the findings of Norris and Weissman (1969).

Slow-motional EPR spectra were exploited during the 1970s to acquire new insights into molecular rotational motions in isotropic fluids, in liquid crystals, and in model membranes (Hwang *et al.*, 1975; Polnaszek and Freed, 1975; Lin and Freed, 1979; Smectics, 1979). In addition, the SLE approach was exploited to provide a quantitative theory for the then new phenomena of chemically induced dynamic electron polarization (CIDEP) and chemically induced dynamic nuclear polarization (CIDNP) (Freed and Pederson, 1976). The SLE was also employed to provide a theory for Hyde's saturation-transfer technique, which was useful for studying very slow motions (Hyde and Dalton, 1979; Beth and Robinson, 1989).

These rather tedious slow-motional calculations were challenging, however, and to address this a very efficient method of computing solutions to the SLE was developed during the 1980s, namely the Lanczos algorithm (LA). This drastically reduced the computation time, by at least an order of magnitude, greatly reduced storage requirements, (Moro and Freed, 1981) and also ultimately led to the versions that could be made generally available (see below). The strength of the LA technique is that it takes full advantage of the sparsity of the SLE matrix; also after just a few Lanczos projections it produces a greatly reduced matrix sub-space that includes very effectively what is important to describe the EPR lineshapes. This is partly because the initial or "starting" vector represents essentially the EPR transition moments that include the physics of the EPR experiment. Further, the slowerdecaying eigenvalues of the SLE, which dominate the EPR experiment, are obtained accurately from the small Lanczos sub-space. Unnecessary eigenvalues which are poorly represented in this small sub-space are automatically projected out of the solution when the specific EPR observable-that is, the lineshape-is calculated. Finally, a plaguing problem of the LA approach, namely the accumulation of roundoff error, is overcome because the calculation is terminated before it becomes serious, as one needs only a small sub-space generated by a relatively small number of Lanczos projections. To further enhance the usage of SLE algorithms, very power-

ful methods have been developed for selecting the minimum basis set to represent the SLE (Schneider and Freed, 1989a, 1989b), and for determining reliably that sufficient Lanczos projections have been utilized (Schneider and Freed, 1989a, 1989b). These references provide the most effective computational algorithm to date.

11.3 High-Field Multifrequency CW-EPR Experiments to Unravel Molecular Motion

Figure 11.1 shows the experimental EPR spectra of PDT/toluene at 250 GHz in various motional regimes: motional narrowing, slow motion, and the rigid lattice limit. Figure 11.2 shows a series of simulated multifrequency spectra covering the range of 15 to 2000 GHz for a spin-bearing molecule with a rotational correlation time of 1.7 ns. This shows that a motional process that appears fast at lower



Figure 11.1 EPR spectra of PDT/toluene at 250 GHz in various motional regimes. Motional narrowing (-40°C, -60°C), slow motion (-81°C, -100°C), and rigid limit (-119°C, -129°C).

Figure 11.2 (a) Simulated first-derivative EPR spectra at 9.1 and 250 GHz for a dilute powder containing a cholesterol-like nitroxide (CSL; short vertical lines. The magnetic-field values where CSL absorbs when its x'-, y'-, and z'-axes are parallel to B_0 (Barnes and Freed, 1998); (b) Simulated first-derivative multifrequency EPR spectra for a nitroxide, reorienting with a rotational diffusion constant $R = 10^8 \, \text{s}^{-1}$ (corresponding to rotational correlation time $\tau_R = 1.67 \, \text{ns}$) in the range 15 to 2000 GHz. From this, it is clear that a motional process that appears fast at lower frequencies will appear slow at higher frequencies.



Formation & dissociation of head-to-head dimers by GAsI in DPPC (aligned samples)



Figure 11.3 EPR spectra at 9 and 170 GHz, showing detection of the formation and dissociation of head-to-head dimers by GAsI in aligned samples of DPPC. Channels begin to form above the $L_{\beta}-P_{\beta}$ transition point in spin-labeled gramicidin A in DPPC.

frequencies will seem slow at higher frequencies. Thus, for complex systems, such as proteins or membranes, the slow overall and collective motions will be displayed better at lower frequencies, whereas the fast motions will be more sensitively demonstrated at higher frequencies. An example of monitoring the anisotropy of the motion can be seen in Figure 15.5, which shows how high-frequency (170 GHz) EPR spectra can demonstrate convincingly the anisotropy of motion for molecular rotations about the X-, Y-, and Z-molecular axes (cf. Dzikovski, et al., 2009). An example of the simple detection of a biological process in a model membrane is shown in Figure 11.3, which includes spectra at 9.6 and 170 GHz. Here, the formation and dissociation of head-to-head dimers by spin-labeled gramicidin A (GAsl) in aligned samples of DPPC is observed. Dimer channels of GAsl form above the main transition point in a lipid membrane. Channel formation in the sample manifests as a breaking of Z-ordering of monomers, due to the tilt of the nitroxide moiety upon dimer formation. The conclusion which could be made at 9.6 GHz, after detailed simulation, is very clearly evident from a visual inspection of the spectrum at 170 GHz, namely that the spectral intensity shifts from the Z- to the XY-spectral region and back upon performing a heating and cooling cycle. Another example of how multifrequency EPR distinguishes motion at different



Figure 11.4 An example of how multifrequency EPR distinguishes motion at different temperatures, as exhibited by the EPR spectra of T4 lysozyme spin-labeled at mutant site 131 at 9, 95, 170, and 240GHz at 2, 12, 22, and 32 °C.

temperatures is exhibited by the spectra in Figure 11.4 of T4 lysozyme spin-labeled at mutant site 131 at 9, 95, 170, and 240 GHz at 2, 12, 22, and 32 °C.

11.3.1

Determination of the Axes of Motion from High-Field, High-Frequency (HFHF) EPR Spectra: Orientational Resolution

Since the EPR spectra at millimeter-wave frequencies exhibit very high sensitivity to the *g*-tensor, the spectra provide excellent orientational resolution as compared to that achieved at conventional microwave frequencies (Budil *et al.*, 1989; Earle, Budil, and Freed, 1993; Earle *et al.*, 1997, 1998, and the previous paragraph). This is discussed more in detail in Chapter 15 (see Figure 15.3a and b). Therefore, at 250 GHz for example (Earle, Budil, and Freed, 1993), one can discern about which axis, or axes, the motion occurs in the CW-EPR spectrum. An excellent demonstration of the orientational resolution at 250 GHz in studies utilizing nitroxide spin labels was provided by the data on aligned membranes containing a mixture of headgroups: zwitterionic phosphatidylcholine (PC) and negatively charged phosphatidylserine (PS), using the cholesterol-like spin label (CSL) (Barnes and Freed, 1998). The macroscopic alignment of the membranes further enhanced the orientational resolution at 250 GHz, allowing for an orientation-dependent study (this topic is discussed in greater detail in Chapter 15).

11.3.2

Observation of Motion as a Function of Frequency

It is found that, the higher the EPR frequency, the slower the motion appears for a given diffusion rate, as shown in Figure 11.2. It is found that at the low-frequency end one observes simply the motionally narrowed spectra, which become slowmotional, consistent with the rigid limit (powder-like), at the high-frequency end. This is tantamount to the higher-frequency spectra acting as a faster "snapshot" of the motion (Earle, Budil, and Freed, 1993; Earle *et al.*, 1997), due to the enhanced role of the *g*-tensor (Zeeman) term, linear in the magnetic field, B_0 , in the spin Hamiltonian. This is explained as due to the fact that the condition for motional narrowing, $|H_1(\Omega)|^2 \tau_R^2 << 1$ is no longer valid, rendering the spectra slow motional; here, τ_R is the rotational relaxation time and $H_1(\Omega)$ is the orientation-dependent part of the spin Hamiltonian, with Ω referring to the Euler angles describing the molecular orientation, which increases in magnitude with increasing frequency, ω_0 and magnetic field, B_0 .

11.3.3

Virtues of Multifrequency EPR in Studying Molecular Motion

By exploiting multifrequency EPR spectra, one can decompose complex modes of motions of proteins and DNA according to their different timescales (Liang and Freed, 1999). This also applies to the study of the dynamics of complex fluids, such as glass-forming fluids (Earle *et al.*, 1997) and liquid crystals (Lou *et al.*, 2001). This would result, for example, in "freezing-out" the slow overall tumbling motions in protein spectra at higher frequencies, leaving only the faster internal modes of motion. On the other hand, at lower frequencies one can observe clearly the motions at a slower timescale with the faster motions averaged out. In glass-forming fluids, the faster motions consist of reorientations of the probe molecules, whereas the slower motions relate to the dynamics of the solvent cage (Earle *et al.*, 1997).

This was convincingly demonstrated in the case of proteins by Barnes et al. (1999), using the 9 and 250 GHz CW-EPR spectra of spin-labeled mutants of the stable protein T4 lysozyme in aqueous solution. At 250 GHz, the overall rotation was too slow to significantly affect the spectrum, so that it was satisfactorily described by the simpler MOMD model (Meirovitch, Nayeem, and Freed, 1984; as discussed in Section 11.3.4.2.1), because the overall motion was perceived to be so slow at 250 GHz that it corresponded to the rigid limit, and a good resolution of the internal dynamics was achieved. Using the internal motion parameters so obtained at 250 GHz, the 9 GHz lineshape data were fitted to the SRLS model (as described in Section 11.3.4.2.1) to successfully obtain the rates for the global dynamics. In this manner, with multifrequency data, the two types of motion were separated and the spectral resolution of these motions was significantly enhanced. The details of the SRLS model as applied to protein dynamics are illustrated in Figure 11.5. Very recently, an extensive multifrequency study on spin-labeled T4L covering four frequencies showed how simultaneous quantitative fits could be obtained with the SRLS model (Zhang et al., 2010).



Figure 11.5 Protein dynamics of spin-labeled protein (left), showing three types of motion: spin-label reorientation; side-chain fluctuations; and global tumbling. The SRLS model is illustrated, including relevant motional parameters (Liang and Freed, 1999).

The same multifrequency approach was applied to a study of the dynamic structure of model membranes using an end-chain lipid (Lou, Ge, and Freed, 2001), where the 250 GHz data were exploited in terms of the MOMD model relating to just the internal dynamics and ordering of the ends of the acyl-chains, whereas the 9 GHz spectra are affected by both the internal and overall motions, and analyzed in terms of the SRLS model. It should be pointed out here that if the 250 GHz spectra are not taken into account, then the 9 GHz spectra, which provide only limited resolution to the dynamics, could be fitted to the simpler MOMD model. However, the dynamic and ordering parameters obtained must be interpreted as a composite of both the internal and overall motions, with no obvious way of separating them (Lou, Ge, and Freed, 2001).

11.3.4

Stochastic Liouville Equation (SLE) to Describe Slow-Motional EPR Spectra

A quantitative treatment of slow-motional EPR is accomplished by solving the SLE, using the combined spin and orientational distribution function, $\rho(\Omega,t)$, which is composed of both the spin density matrix, $\rho(t)$, and the orientational distribution function, $P(\Omega,t)$, governed by the differential equation:

$$\frac{\partial \rho(\Omega, T)}{\partial t} = -i[\hat{H}, \rho] - \hat{\Gamma}_{\Omega} \rho(\Omega, t), \qquad (11.1)$$

where $\hat{\Gamma}$ is the operator for the rotational diffusion, and Ω again represents the Euler angles between the fluctuating molecular frame and the laboratory frame.

It should be noted that: (i) the normal density matrix is obtained by averaging $\rho(\Omega,t)$ over all Ω : $\rho(t) = \langle \rho(\Omega,t) \rangle_{\Omega}$; and (ii) $\rho(\Omega,t)$ reduces to $P(\Omega,t)$ when there are no electron or nuclear spins: S = I = 0. Schneider and Freed (1989a) describe the details of calculating slow-motional EPR lineshapes for a nitroxide radical in solution, and Misra (2007) describes similar details for an electron spin ($S = \frac{1}{2}$) coupled to two nuclei with arbitrary spins, whereas Zerbetto *et al.* (2007) describe details for a doubly spin-labeled system.

11.3.4.1 Calculation of Slow-Motion Spectrum

The EPR lineshape function: This is expressed as follows:

$$I(\omega) = \left\langle \left\langle \upsilon \left[i(\omega - \omega_0) \hat{I} + (\Gamma_{\Omega} - i\hat{H}^x) \right]^{-1} \middle| \upsilon \right\rangle \right\rangle,$$
(11.2)

where $|v\rangle$ is the "starting vector", which contains the information that: (i) the spectrum is an isotropic average over all orientations (except, of course for macroscopically aligned fluids, e.g. liquid crystals), which implies that only the components of $|v\rangle$ which contain $D_{0,0}^0 = 1$ are nonzero; and (ii) only the components of $|v\rangle$ which correspond to $p_s = 1$; $m'_1 = m_1$, (where p^s refers to the coherence order of the electron spins cf. below) excited by the radiation, are nonzero:

$$|\nu\rangle = \phi_{0,0}^{0}(\Omega) \times |p_{s}| = 1, p_{I} = 0, m_{I} >>,$$
 (11.3)

with $m_I = +1$, 0, -1 for ¹⁴N.

Finally, the spectrum is calculated to be;

$$I(\boldsymbol{\omega}) = \frac{1}{\pi} \operatorname{Re}\left\{\sum_{j=1}^{N} \frac{C_j^2}{\lambda_j + i(\boldsymbol{\omega} - \boldsymbol{\omega}_0)}\right\},\tag{11.4}$$

where λ_j is the *j*th complex eigenvalue of the SL operator, and C_j is the component of the *j*th eigenvector along the direction of the starting vector $|\nu\gg$. The real part of λ_j ; Re λ_j corresponds to a T_2^{-1} -like decay and Im λ_j ; to the resonant frequency of the jth "mode." Equation 11.2 yields the Redfield motional narrowing result (Redfield, 1965) for fast motions, whereas it yields the rigidlimit (solid-like) powder pattern in the very slow motion limit.

The Stochastic Liouville Equation Using the superoperator notation for the spin Hamiltonian, H^x , the SLE (Equation 11.1 above) can be expressed as

can be expressed as

$$\frac{\partial \rho(\Omega, T)}{\partial t} = -i[\hat{H}, \rho] - \hat{\Gamma}_{\Omega}\rho(\Omega, t) = [i\hat{H}^{*} - \hat{\Gamma}_{\Omega}]\rho(\Omega, t)$$
(11.5)

The last equality of Equation 11.5 defines the superoperator form, H^x , by relating it to the second term of Equation 11.5. [For more details, see Schneider and Freed

(1989a,b) and Misra (2007).] The spin Hamiltonian in Equation 11.5, which consists of hyperfine and Zeeman terms for S = 1/2, can be expressed as:

$$\hat{H} = \sum_{\substack{\text{sum}\\\text{over}\\\text{indices}}} \hat{A}_{\mu,\ell}^{(\ell,m)} D_{mm'}^{\ell}(\Omega_{LG}) F_{\mu,G}^{(\ell,m')^*}, \tag{11.6}$$

In Equation 11.6, $\hat{A}_{\mu,\ell}^{(\ell,m)}$ is the irreducible spin tensor with spin operators quantized in the laboratory (L) frame in which the *z*-axis is along the external magnetic field, B_0 ; $\hat{F}_{\mu,G}^{(\ell,m')*}$ are molecular functions quantized in the molecular (G) frame, which is fixed in the molecular frame; and $D_{m,m'}^{\ell}(\Omega_{LG})$ are the Wigner rotation coefficients, which effect transformations of the matrix elements between the L and G frames (for more detail, see Section 11.3.4.1.8).

Simple Anisotropic Tumbling with Axial Symmetry In the simplest model of rotational diffusion such as the motions of rods or discs, one can express the rate of change of the orientational distribution function as follows:

$$\frac{\partial P(\Omega, t)}{\partial t} = -\nabla_{\Omega} \cdot R \cdot \nabla_{\Omega} P(\Omega, t) = -\Gamma_{\Omega} P(\Omega, t), \qquad (11.7)$$

where *R* is the rotational diffusion tensor, diagonal in the appropriate molecular frame. The solution of this diffusion equation has well-defined eigenfunctions, which are for the case of axial symmetry:

$$\Phi_{M,K}^{L}(\Omega) = \sqrt{\frac{2L+1}{8\pi^2}} D_{M,K}^{L}(\Omega), \qquad (11.8)$$

These are normalized Wigner rotation coefficients, whose eigenvalues are given by the damping rates:

$$E_{M,K}^{L} = R_{\perp}L(L+1) + (R_{\parallel} - R_{\perp})K^{2}, \qquad (11.9)$$

where $R_{xx} = R_{yy} = R_{\perp}$ and $R_{zz} = R_{\parallel}$. These can be generalized to nonaxial diffusion (Freed, 1994).

Matrix Representation of the SLE Operator $(i\hat{H}^x - \hat{\Gamma})$ First, the matrix elements of the SLE operator must be expressed in a convenient orthonormal basis set, which is in the direct-product space of the orientational and spin functions, as follows:

$$|\sigma >> = \Phi_{M,K}^{L}(\Omega) \otimes (|S, m_{s}| >< S, m_{s}'|) \otimes (|I, m_{I}| >< I, m_{I}'|),$$
(11.10)

For the nitroxides being considered here, $S = \frac{1}{2}$, and I = 1 (¹⁴N) or $I = \frac{1}{2}$ (¹⁵N) (with this understanding, the spin indices *S* and *I* will be dropped hereafter). For ease of considering coherence order, modified spin magnetic quantum numbers will now be used, as follows:

$$p^{s} = m_{s} - m_{s}; \quad q^{s} = m_{s} + m_{s'}$$
 (11.11)

Here, p^s defines the coherence order: $p^s = 0$ corresponds to the diagonal elements of the density matrix, whereas $p^s = \pm 1$ corresponds to off-diagonal matrix elements between which the radiation field induces transitions.

Basis Sets of the SL Operator: Lanczos Algorithm The basis set required to represent the SL operator is rather large, of dimension *N*, which requires rather exorbitant times to diagonalize the SL matrix. One can achieve order-of-magnitude and even greater reduction in computation time by employing the LA, given that the SL matrix is sparse. This is achieved by providing an objective criterion to determine when a sufficient sub-space, with dimension $n_s \ll N$, has been generated by exploiting the starting vector $|v \rangle >$ to select out a small sub-set of vectors, known as Lanczos vectors, which span the sub-space required to calculate the EPR lineshape. This subspace is then projected out, and the SL matrix is converted to tri-diagonal form, which is easily diagonalized, or else solved by the method of continued fractions (Schneider and Freed, 1989a,b). In this manner, a greatly reduced number of multiplications is required. In a modified form, the LA can be used to provide an objective method to prune the original basis set to go from *N* to a minimum N_{min} needed to represent the relevant eigenvectors.

Diffusion in Anisotropic Media In an anisotropic medium, such as liquid crystals or membranes (or in the presence of side-chain motion in proteins), the orientational distribution of the spin probe is not isotropic. In that case, its equilibrium distribution, $P_{eq}(\Omega)$, can be derived from an orientational potential energy, $U(\Omega)$, which is the potential of mean torque experienced by it:

$$P_{eq}(\Omega) = \frac{\exp[-U(\Omega)/k_B T]}{\int d\Omega \exp[-U(\Omega)/k_B T]},$$
(11.12)

where k_B is Boltzmann's constant and T is the temperature.

The diffusion operator then becomes:

$$\hat{\Gamma}_{\Omega} = \nabla_{\Omega} \cdot R \cdot \left[\nabla_{\Omega} + \frac{1}{k_{B}T} (\nabla_{\Omega} \cdot U) \right] P(\Omega, t).$$
(11.13)

Equation 11.13 is known as a Smoluchowski equation. It has the property that for any initial $P(\Omega,0)$, $\lim_{t\to\infty} P(\Omega, t) = P_{eq}(\Omega)$; in other words, $P_{eq}(\Omega)$ is an eigenfunction of $\hat{\Gamma}_{\Omega}$ with the zero eigenvalue. $\hat{\Gamma}_{\Omega}$, as given by Equation 11.12, is nonsymmetric but can be converted into the symmetric form by the following transformation:

$$\widetilde{\Gamma}_{\Omega} = P_{eq}^{-1/2}(\Omega) \widehat{\Gamma}_{\Omega}(\Omega) P_{eq}^{1/2}(\Omega), \qquad (11.14)$$

which yields:

$$\tilde{\Gamma}_{\Omega} = [\nabla_{\Omega} - (\nabla_{\Omega}U)/2k_{B}T] \cdot R \cdot [\nabla_{\Omega} + (\nabla_{\Omega}U)/2k_{B}T].$$
(11.15)

The diffusion Equation 11.7 may be solved for $\tilde{P}(\Omega, t) = P_{eq}^{-1/2}(\Omega)P(\Omega, t)$. The symmetric matrix $\tilde{\Gamma}_{\Omega}$ can be diagonalized after calculating its matrix elements explicitly in the basis formed by the functions $\Phi_{K,M}^{L}(\Omega)$. The new SL operator becomes:

$$i\hat{H}^{x}-\tilde{\Gamma}_{\Omega}, \tag{11.16}$$

for which the new starting vector is:

$$|\tilde{\upsilon}\rangle\rangle = P_{eq}^{1/2}(\Omega) |\upsilon\rangle\rangle, \tag{11.17}$$

Finally, the slow-motional EPR lineshape is given by

$$I(\boldsymbol{\omega}) = \pi^{-1} \left\langle \left\langle \tilde{\boldsymbol{\upsilon}} \middle| \left[i(\boldsymbol{\omega} - \boldsymbol{\omega}_0) \hat{I} + (\tilde{\Gamma}_{\Omega} - i\hat{H}^*) \right]^{-1} \middle| \tilde{\boldsymbol{\upsilon}} \right\rangle \right\rangle,$$
(11.18)

where H^x is defined in Equation 11.2. Equation 11.18 is solved using the same procedure as before, by diagonalization of this SL operator and using the original basis set. For more details see Schneider and Freed (1989a,b) and Misra (2007).

The Potential Function, $U(\Omega)$, and the Ordering Tensor S The potential energy operator, $U(\Omega)$, can be expanded in terms of the Wigner rotation matrix elements $D_{M,K}^{L}(\Omega)$ as follows:

$$-U(\Omega)/k_{B}T = \sum_{L,M,K} c_{M,K}^{L} D_{M,K}^{L}(\Omega).$$
(11.19)

The resulting ordering tensor elements can be obtained by using $P_{eq}(\Omega)$ as follows:

$$S_{0} = \langle D_{0,0}^{2}(\Omega) \rangle = \int d\Omega P_{eq}(\Omega) D_{0,0}^{2}(\Omega);$$

$$S_{2} = \langle D_{0,2}^{2}(\Omega) + D_{0,-2}^{2}(\Omega) \rangle.$$
(11.20)

It should be noted that only S_0 and S_2 are utilized in most cases of interest here.

The Spin Hamiltonian Operator This can be expressed as:

$$\hat{H} = \sum_{\mu=g,A} \sum_{l=0,2} \sum_{m=-\ell}^{\ell} \sum_{m'=-\ell}^{l} \sum_{m'=-\ell}^{l} A_{\mu,\ell}^{(\ell,m)} D_{mm'}^{\ell}(\Omega_{LM}) D_{m'm'}^{\ell}(\Omega_{MG}) F_{\mu,G}^{(l,m')^*}.$$
(11.21)

In Equation 11.21, the tensor elements $\hat{A}_{\mu,\ell}^{(\ell,m)}$ are constituted by the external magnetic field B_0 , and the spin operators **S**, *I*, *S* · *I*, expressed in the laboratory frame, whereas the tensor elements $F_{\mu,G}^{(\ell,m')}$ are constituted by the diagonal matrix elements g_{xx} , $g_{\gamma\gamma}$, g_{zz} , A_{xx} , $A_{\gamma\gamma}$, A_{zz} of the *g*- and *A*-matrices expressed in the *g*-matrix frame. The reference frames: L, M, and G in Equation 11.20 are defined next.

Reference Frames Used in the MOMD Model to Define the Orientation of a Spin Probe to Study its Structural and Dynamic Properties The various frames used for this purpose are defined as follows, and illustrated in Figure 11.6:

- Lab frame (*L*): This is defined with respect to the external magnetic field, whose direction is used as its *z*-axis.
- Director frame (D): The director, n̂, parallel to the membrane normal, defines this frame, which is tilted relative to the magnetic field by the angle ψ, and is obtained by transformation by the set of Euler angles Ψ_{L→D} from LF to DF.



Figure 11.6 Reference frames used to define the orientation of a sample to study its structural and dynamic properties. (i) Lab frame (LF), defined with respect to the external magnetic field, whose direction is used as its *z*-axis; (ii) Director frame (DF), defined by the director, \hat{n} , of the membrane, tilted relative to the magnetic field by the angle ψ , and obtained by the transformation by the set of Euler angles $\Psi_{L \to D}$ from LF to DF; (iii) Molecular frame (MF), fixed within the molecule; (iv) g-tensor frame (GF), the principal-axes frame of the g-tensor of the paramagnetic ion, and is obtained by the transformation by the set of Euler angles $\Phi_{M\rightarrow G}$ from MF to GF; (v) A-tensor frame, defined by the principal-axes of the A-tensor, obtained by the transformation by the set of Euler angles Ω_A from GF to AF.

- Molecular frame (*M*): This is defined by the principal axes of the molecular diffusion tensor (or molecular ordering frame), and is fixed within the molecule.
- **g-Tensor frame (G):** This is the principal-axis frame of the g-matrix, and is obtained by transformation by the set of Euler angles $\Phi_{M\to G}$ from MF to GF.
- A-tensor frame: This is defined by the principal-axes of the A-tensor, and is obtained by the transformation by the set of Euler angles Ω_A from GF to AF.

In order to define the orientation of the spin-label, the typical molecular magnetic tensor in irreducible tensor notation is transformed from the MF to LF frame as follows:

$$F_{\mu,\ell}^{(2,m)^{*}} = \sum_{m',m'',m''} F_{\mu,m}^{(2,m'')^{*}} D_{m,m'}^{2} (\Psi_{L\to D}) D_{m',m''}^{2} (\Omega_{D\to M}) D_{m',m''}^{2} (\Phi_{M\to G}),$$
(11.22)

where $\Psi_{L \to D} = (0, \psi, 0)$ is a sufficient set of transformation Euler angles, from LF to DF.



Figure 11.7 X-band CW-EPR spectra for the NO radical, shown as a function of the director tilt (ψ) in the second and subsequent plots from the top in descending order, for $\psi = 90^{\circ}$, 75°, 60°, 45°, 30°, 15°, 0°, respectively, along with its MOMD spectrum displayed at the top.

Figure 11.7 shows a simulated X-band CW-EPR spectrum for the nitroxide radical as a function of the director tilt (ψ), along with its MOMD spectrum (as discussed in Section 11.3.4.2.2).

11.3.4.2 MOMD and SRLS Models

Slow-Motional EPR Lineshape This is calculated using Equation 11.1.

MOMD This is applicable, for example, to membrane vesicles or a very slowly tumbling protein with internal (side-chain) motion (Meirovitch, Nayeem, and Freed, 1984). In order to take into account random macroscopic disorder, for the case when there exists microscopic order, one should take an average of the spectra from all orientations, ψ , which define the transformation angles $\Psi_{L\rightarrow D}$ that appear in Equation 11.23, to obtain the composite MOMD spectrum, as follows:

$$I(\omega) = \int I(\omega, \psi) \sin \psi \, d\psi \tag{11.23}$$

By definition, this spectrum is inhomogeneously broadened, but it happens in a characteristic manner, which depends on the ordering potential, or equivalently upon the ordering tensor S-for example, that given by Equation 11.21.

Diffusion Operator This operator used in the SLE, as described by Equations 11.7 and 11.13, based on the assumption of over-damped motions inherent in

Smoluchowski equations as well as axially symmetric diffusion, is expressed as follows (see Schneider and Freed, 1989a; Misra, 2007 for more details):

$$\hat{\Gamma}_{\Omega} = \hat{\Gamma}(\Omega_{LM}, \Omega_{LC}) = \hat{\Gamma}^{0}(\Omega_{LM}) + \hat{F}^{C}(\Omega_{LC}) + \hat{F}^{0}(-\Omega_{C'M}) + \hat{F}^{C}(-\Omega_{C'M}), \quad (11.24)$$

where,

$$\hat{\Gamma}^{\circ}(\Omega_{LM}) = R^{\circ}_{\perp} \hat{J}^{\circ 2} + (R^{0}_{\parallel} - R^{0}_{\perp}) \hat{J}^{\circ 2}_{z}; \qquad (11.24a)$$

$$\hat{\Gamma}^{c}(\Omega_{LC}) = R^{c}_{\perp} \hat{J}^{c2} + (R^{c}_{\parallel} - R^{c}_{\perp}) \hat{J}^{c2}_{z};$$
(11.24b)

$$F^{\circ}(-\Omega_{C'M}) = \frac{1}{2} \Big[R^{\circ}_{\perp} (\hat{J}^{\circ 2} u(\Omega_{C'M}) + (R^{0}_{\parallel} - R^{0}_{\perp}) \hat{J}^{\circ 2}_{z} u(\Omega_{C'M})) \Big] \\ - \frac{1}{4} \Big[R^{\circ}_{\perp} (\hat{J}^{\circ}_{+} u(\Omega_{C'M}) \hat{J}^{\circ}_{-} u(\Omega_{C'M}) + R^{0}_{\parallel} (\hat{J}^{\circ 2}_{z} u(\Omega_{C'M}))^{2} \Big]; \qquad (11.24c)$$

$$F^{c}(-\Omega_{C'M}) = \frac{1}{2} \Big[R^{c}_{\perp} (\hat{J}^{c2} u(\Omega_{C'M}) + (R^{c}_{\parallel} - R^{c}_{\perp}) \hat{J}^{c2}_{z} u(\Omega_{C'M})) \Big] - \frac{1}{4} \Big[R^{c}_{\perp} (\hat{J}^{c}_{+} u(\Omega_{C'M}) \hat{J}^{c}_{-} u(\Omega_{C'M}) + R^{c}_{\parallel} (\hat{J}^{c2}_{z} u(\Omega_{C'M}))^{2} \Big].$$
(11.24d)

Equations 11.24 implies Γ_{Ω} depends on several quantities, as follows:

$$\hat{\Gamma}_{\Omega} = \hat{\Gamma}(R^o_{\perp}, R^o_{\parallel}, R^c_{\perp}, R^c_{\parallel}, c^2_0, c^2_2).$$

Internal-Ordering Potential The explicit form of this potential, to be used in Equations 11.24c and d, is

$$u(\Omega_{C'M}) \equiv -U(\Omega_{C'M})/k_BT$$

= $c_0^2 D_{00}^2(\Omega_{C'M}) + c_2^2 \left[D_{02}^2(\Omega_{C'M}) + D_{0-2}^2(\Omega_{C'M}) \right].$

Slowly Relaxing Local Structure (SRLS) Model With the enhanced resolution offered by the HF-HF EPR, more sophisticated models of molecular reorientation have been proposed to fit these EPR spectra. Now, the many-body problem of dealing with the microscopic details of fluids is approximated by a set of collective degrees of freedom which represent the main effects of the solvent on a rotating solute. These collective variables are modeled as a loose solvent "cage," which is considered to be relaxing slowly and within which the solute is assumed to be reorienting more rapidly. This so-called slowly relaxing local structure (SRLS) is obtained by generalizing the MOMD model by allowing the Euler angles $\Psi_{{\scriptscriptstyle L}
ightarrow {\scriptscriptstyle D}}$ to fluctuate in time due to some slow overall process; this may be a slow tumbling of the vesicle or an overall rotation of the protein. The enhanced sensitivity of very high frequency (VHF) EPR to rotational dynamics was exploited successfully to explore the details of the dynamic solvent cage in a 250 GHz EPR study of the dynamics of several nitroxide spin probes dissolved in the glass-forming solvent ortho-terphenyl (OTP) (Earle et al., 1997). As shown in Figure 11.8, the SRLS model adequately fits the model-sensitive regions of the 250GHz spectra, leading to a coherent picture of the dynamics. The rotational diffusion tensors of the various probes appropriately conform to the simple expectation that the diffusion constant becomes larger as the probe becomes smaller. The relaxation rate of the cage is





terphenyl solvent (solid line) experiment. The dashed line indicates the SRLS model, and the dashed-dotted line simple Brownian diffusion (Earle *et al.*, 1997).

found to be the slowest, and independent of the particular probe, consistent with the fact that the cage relaxation involves primarily the movement of the OTP solvent molecules. Further, it was possible to estimate the magnitude and directionality of the cage-orienting potential. In addition, the dynamics affected the slow-motional EPR spectra in a nonlinear manner. This enables one to discern between two limiting cases: (i) a homogeneous liquid characterized by a complex motional dynamics, such as described by the SRLS model; and (ii) an inhomogeneous liquid characterized by a distribution of simple relaxation times, for example, Brownian tumbling, with (ii) shown to be incompatible with the 250 GHz spectra.

The SLE remains valid in this augmented SRLS model, since the combined system of solute plus cage is represented by collective Markovian equations. The Lanczos projections then effectively determine the extent to which the cage variables are needed to analyze the EPR spectrum (Polimeno and Freed, 1995). The various details applicable to the consideration of a membrane vesicle are shown in Figure 11.5, together with details of the various rotational motions, and restricted internal motion, as well as the orientation of the *g*-tensor frame. It should be noted that in the limit when $R_{\perp}^{c} \rightarrow 0$, the SRLS model becomes the MOMD model.

The coordinate systems used in considering the SRLS model are shown in Figure 11.6. These are: (i) Laboratory (LF); (ii) Global diffusion (CF), obtained via transformation by the set of Euler angles $\Omega_{LC}(t)$ from LF; (iii) Internal director (C'F), obtained by transformation by the set of Euler angles $\Omega_{CC'}$ from CF; (iv) Internal diffusion (MF), obtained by transformation by the set of Euler angles $\Omega_{CM}(t)$ from C'F; and (v) Magnetic-tensor frame (GF), obtained by transformation by the set of Euler angles Ω_{MG} from MF.

According to an earlier version of the SRLS model (Freed, 1977; Meirovitch *et al.*, 2010 review), the spectral density is derived to be:

$$J_{K,M}(\omega) = \frac{\kappa(K, M)\tau_{R}}{1+\omega^{2}\tau_{R}^{2}} + \frac{1}{5}[5\kappa(0, M)]^{2}\delta_{K,0}\langle |S_{t}|^{2}\rangle \times \left[\frac{\tau_{x}}{1+\omega^{2}\tau_{x}^{2}} - \frac{\tau_{R}'}{1+\omega^{2}\tau_{R}'^{2}}\right],$$

where $\tau_R^{\prime 2} = \tau_R^{-1} + \tau_x^{-1} \cong \tau_R^{-1} >> \tau_x^{-1}$. For an isotropic medium $\kappa(K, M) = 1/5$, which is equivalent to what is known as the model-free form in NMR (Meirovitch *et al.*, 2010 review).

SRLS lineshape function: The dependencies of this are expressed as follows:

$$I(\omega)_{SRLS} = I(R_{\perp}^{o}, R_{\parallel}^{o}, R_{\perp}^{c}, R_{\parallel}^{c}, c_{0}^{2}, c_{2}^{2}, \beta_{MG}, \omega).$$

11.4

Pulsed EPR Study of Molecular Motion

A major drawback of CW-EPR for relaxation studies is its inability to extract homogeneous line broadening reliably from inhomogeneously broadened EPR spectra, such as those obtained with nitroxide spin labels. This homogeneous line broadening is due to the motional modulation of the hyperfine and *g*-tensors, as well as that from the other spin-relaxation processes. The inhomogeneous broadening, which is typically due to the undesirable effects of unresolved proton superhyperfine splitting and local ordering in the MOMD model, obscures the homogeneous line broadening. However, by using pulsed EPR–specifically electron spin echo (ESE)–the inhomogeneous broadening can be canceled and the homogeneous linewidths, i.e. the inverse of T_2 , determined. ESE spectrometers of this type have been constructed by Stillman *et al.* (1980) and in much refined form by Borbat, Crepeau, and Freed (1997) and reviewed by Freed (2000).

It emerges that there occurs a homogeneous T_2 minimum as a function of temperature, an example being that observed by Brown (1974), Stillman, Schwartz, and Freed (1980) and Millhauser and Freed (1984). This occurs because T_2 depends differently on the rotational correlation time ($T_R = 1/6R$), for fast and slow motions. Specifically, for fast motion, T_2 exhibits the well-known inverse dependence on correlation time to a positive (usually fractional) power. A detailed explanation of this in terms of the SLE was provided by Schwartz, Stillman, and Freed (1982). In the slow-motional regime, the T_2 is affected in two limiting cases as follows. First, in the strong jump reorientation limit, each jump causes a large change in the

resonant frequency, which leads to an uncertainty in lifetime broadening, and T_2 becomes equal to the correlation time (Mason and Freed, 1974). On the other hand, in the limit of simple Brownian motion (the infinitesimal jump limit), T_2 is roughly proportional to the square-root of the correlation time, as interpreted by Kivelson and Lee (1982) in an heuristic manner.

11.4.1 T₂-Type Field-Swept 2D ESE

It is obviously not possible to extract much information on motional dynamics from a single value of T_2 . In the fast-motional regime one observes different T_2 's for the different hf lines. In the slow-motional regime, one can study the variation of T_2 across the spectrum to obtain information on motional models. This would be superior to studying the CW lineshape of a slow-motional EPR experiment, in that the T_2 relates solely to the dynamic processes. This advantage is dispelled in the regime of very slow motions, however, where "solid-state" relaxation processes, such as spin diffusion, become dominant in T_2 . In one approach, the homogeneous T_2 can be measured by using pulsed EPR, wherein the magnetic field is swept and the spin echo is collected from weak, i.e. highly selective, microwave pulses (Millhauser and Freed, 1984). The Fourier transform of these signals in the echodecay time, τ , provides a 2-D spectrum in which the homogeneous lineshape appears along the frequency axis, while the EPR lineshape essentially appears along the field axis. This is shown for tempone in 85% glycerol/H₂O at -75 °C in Figure 11.9. Thus, the homogeneous T_2 variation across the spectrum can be studied, and explained quite successfully by a Brownian reorientational model. Subsequently, it was found that the patterns of T_2 variation across the spectrum, when plotted in a normalized contour fashion, could be used to distinguish the model of rotation and also the degree of rotational anisotropy (see Figure 11.9). This technique found further application to spin labels in oriented model membranes and to labeled proteins, as well as to slow motions on surfaces (Millhauser et al., 1987; Freed, 1987).

11.4.2 Magnetization Transfer by Field-Swept 2-D-ESE

This technique, which can be used to determine the magnetization transfer rates across the EPR spectrum, is performed in the same manner as a T_2 -type 2-D-ESE experiment, but where a stimulated echo sequence $\pi/2-\pi/2-\pi/2$ replaces the echo sequence $\pi/2-\pi$, and the time *T* is stepped out between the second and third pulses (Schwartz, Millhauser, and Freed, 1986). The variation with *T* is governed by two exponential decays according to the approximate theory; one is in T_1 , and the other is in T_A , an effective magnetization transfer time (for $T_A \ll T_1$). The spin-bearing molecules irradiated by the first two $\pi/2$ pulses are shifted by slow-rotational reorientations to frequencies outside the irradiated region, and are therefore not detected by the third $\pi/2$ pulse. Thus, this magnetization transfer process leads to

2.1000

3285.0



2.1000

3285.0

3190.0



3237.5

GAUSS

(a) taken along the width = 0 axis; (c) The analogous contours for cholesterol in *n*-butylbenzene at -135 °C. These show the different contour patterns from the near-spherical tempone compared to that from the cigar-shaped cholestane (Millhauser and Freed, 1984).

3237.5

GAUSS

3190.0

a more rapid decay of the stimulated echo as a function of *T*. A Brownian rotation model also predicts a T_A variation across the spectrum. Dramatic variations of T_A across the spectrum for NO₂ adsorbed onto crushed vycor, attributed to very anisotropic rotational motion on the surface (Freed, 1987). There was found an enhanced T_A for the spectral regimes corresponding to *x*- and *z*-molecular axes being parallel to the magnetic field, implying more rapid rotation about the *y*-axis, which is parallel to the line connecting to the two oxygen atoms. This motional anisotropy was clearly visible from the 2-D contours, without requiring further detailed analysis.

11.4.3 Stepped-Field Spin-Echo ELDOR

This is a more informative method of studying magnetization transfer. In this technique, an alternative to using two microwave frequencies, the magnetic field is stepped out during the time between the first inverting π pulse and the detecting $\pi/2-\pi$ spin-echo sequence (Hornak and Freed, 1983; Dzuba *et al.*, 1984). The comprehensive theory of spin relaxation in ESE for fast and slow motions includes both longitudinal and cross-relaxation in liquids (Schwartz, 1984; Schwartz, Millhauser, and Freed, 1986). In ELDOR, one observes the transitions out of a certain spectral region, and the spectral region to which the transition is made.

11.4.4

2-D Fourier Transform EPR

Two-dimensional (2-D) NMR was first developed by Richard Ernst and coworkers in 1976 (Aue and Ernst, 1976), and this led in 1979 to the study of magnetization transfer. In 2D NMR one uses nonselective radiofrequency (rf) pulses to successfully irradiate the entire spectrum and to collect the data shortly after pulse application. This process introduced coherences simultaneously to all spectral components, and enabled the observation of coherence transfer between these components. Ernst and Jeener subsequently showed how magnetization transfer could also be studied in this manner (Jeener et al., 1979). Nonetheless, it took another ten years for 2-D-EPR to incorporate these ideas (Gorcester and Freed, 1986), for the simple reason that the EPR experiment is much more difficult to carry out. In the case of EPR, microwaves are used rather than rf used in NMR. As the EPR relaxation times are orders of magnitudes faster, pulse widths are required that are orders of magnitude shorter, and the spectral bandwidths that must be orders of magnitude wider. Consequently, it proved necessary to develop modern FT techniques in EPR as a requirement for developing 2-D-EPR. Modern FT-ESR appeared at Bowman's laboratory in Argonne (Angerhofer, Massoth, and Bowman, 1988), at Dinse's laboratory in Dortmund, Germany (Dobbert, Prisner, and Dinse, 1986), at Lebdev's laboratory in Moscow (Panferov et al., 1984), and in Freed's laboratory at Cornell (Eliav et al., 1984; Gorcester and Freed, 1986; Freed, 1986). The 2-D FT-EPR experiments conducted at Cornell consisted of a 2-D-ESE experiment, appropriately called spin-echo-correlated spectroscopy (SECSY) which utilizes two $\pi/2$ pulses, and a free induction decay (FID)-based 2-D-exchange experiment which utilizes three $\pi/2$ pulses, now referred to as 2-D-ELDOR (Gorcester and Freed, 1986). With SECSY, it was possible to obtain homogeneous T_2^{-1} values from the whole spectrum simultaneously from an (inhomogeneously broadened) EPR signal. In contrast, the (first) FT-based 2-D-ELDOR experiment exhibited crosspeak development that had resulted from a Heisenberg spin-exchange. To make the technique of 2-D-FT-EPR generally applicable, sophisticated phase-cycling was introduced on the technical side, whilst on the theoretical side a full analysis was developed for the fast-motional 2-D spectra, taking into account the generation of

cross-peaks by the Heisenberg exchange (HE) and electron-nuclear dipolar (END) terms. Additional studies involved how to distinguish between the respective contributions to enable quantitative measurements could be made of HE in an anisotropic fluid (Gorcester and Freed, 1988), and of END terms in a liquid crystal (Gorcester, Ranavare, and Freed, 1989). The measurement of END terms led to sophisticated insights being acquired into molecular dynamics in ordered fluids, that could not be obtained with CW-EPR. The measurement of rates of chemical exchange in a semi-quinone system was also demonstrated, by using 2-D-ELDOR (Angerhofer, Massoth, and Bowman, 1988).

Subsequently, 2-D-FT-EPR was further developed to address the slow-motion regime by introducing further improvements. This was accomplished by increasing the spectral coverage to 250 MHz, enhancing the data-acquisition rates, significantly reducing the spectrometer dead times (Patyal et al., 1990a in the case of SECSY:ESR, and Patyal et al., 1990bin the case of 2-D-ELDOR), and developing the general theory for the quantitative analysis of 2-D spectra (Lee, Budil, and Freed, 1994b). Complex fluids could then be studied in detail, including phospholipid membrane vesicles (Lee et al., 1994a; Crepeau et al., 1994), liquid crystalline solutions (Sastry et al., 1996a, 1996b), and liquid-crystalline polymers (Xu et al., 1996). The dead times here were reduced to approximately 50-60 ns. Simultaneous fits of 2-D-ELDOR data at several mixing times, T_m , provided a third dimension in that one could monitor how the cross-peaks would grow in relation to the auto peaks with increasing mixing time, as shown in Figure 11.10 (Costa-Filho, Shimoyama, and Freed, 2003a) for a liquid-crystalline phase of lipid vesicles, and in Figure 11.11 (Costa-Filho, Shimoyama, and Freed, 2003b) for the effects of the peptide gramicidin A of lipid vesicles. This information provides quantitative information on the nuclear spin-flip-inducing processes of both HE, which are related to translational diffusion, and the intramolecular electron-nuclear dipolar interaction, which is related to tumbling motions. In those studies further technical improvements had brought the dead-times down to 25-30 ns. The more recent work of Chiang et al. (2007) on lipid-cholesterol mixtures of varying compositions and temperatures is a tour de force that illustrates the great power of 2D-ELDOR in extracting all the available dynamical information for complex systems. It was aided by an improved method of 2D-ELDOR data analysis called the "full Sc." method (Chiang and Freed, 2006).

11.4.4.1 Lineshapes of the Auto and Cross-Peaks: Homogeneous (HB) and Inhomogeneous Broadening (IB)

Two types of line shape can be obtained from the correlation spectroscopy (COSY) and 2-D-ELDOR data. Depending on the coherence pathway, one can obtain either the FID-like signal (S_{c+}), which is sometimes referred to as the "anti-echo," or the echo-like (S_c) signal, wherein there is refocusing of the inhomogeneous broadening terms in the spin Hamiltonian, which leads to their cancellation in the echo that is formed. The echo-like S_c . 2-D signal can be Fourier transformed and rearranged to obtain the homogeneous broadening along one frequency dimension, and essentially the CW spectrum along the other frequency dimension.



Figure 11.10 2-D-ELDOR signals at 17.3 GHz versus mixing time, T_m , of 16-PC in liquid-crystalline phase from pure lipid vesicles (left column) compared with 16 PC

in liquid-ordered phase from 1:1 ratio lipid to cholesterol (right column) at 50°C (Costa-Filho, Shimoyama, and Freed, 2003a).

sion. In this way, one obtains the 2-D-SECSY format from the COSY format by this transformation. In the case of 2-D-ELDOR, the same transformation provides the HB for the auto peaks in the 2-D-ELDOR S_c spectrum, whereas the cross-peaks are affected by any differences in the IB that exist between the two spectral lines



Figure 11.11 2-D-ELDOR signals at 17.3 G, showing the effect of peptide gramicidin A (GA) on the dynamic structure of a lipid membrane containing (end chain) nitroxidelabeled lipid (16-PC) at 75 °C. (a) Pure lipid, mixing time, $T_m = 400$ ns; (b–d) 1:1 lipid to GA with $T_m = 400$ ns, 50 ns, and 1.6 µs, respectively (Costa-Filho *et al.*, 2003b).

connected by the particular cross-peak. The information so obtained from the auto and cross-peaks can be further exploited to study spin relaxation in detail. The S_c format is particularly useful for studying ultra-slow motions, e.g. macromolecules in viscous media (Saxena and Freed, 1997). On the other hand, the FID-like S_{c+} 2-D spectra contain the full effects of inhomogeneous broadening.

11.4.5 MOMD and SRLS Models and 2-D-ELDOR

From the above discussion, it is clear that the dynamics and structure of complex fluids can be studied in great detail by exploiting the S_c . 2-D-ELDOR spectra. This enables one to study the microscopic alignment in lipid membranes, which gives rise to a superposition of the "single crystal-like" spectra obtained for each orientation of the membrane normal with respect to the static magnetic field. This orientational alignment is provided by the microscopic structure that typically characterizes complex fluids, about which the molecular tumbling occurs. Membrane vesicles exhibit "powder-like" spectra, as they possess membrane components at all angles with respect to the magnetic field, which is referred to as MOMD. The local ordering determines the IB and the information on dynamics can be obtained from the S_c spectra, which yields homogeneous T_2 -values, as well as the development of the cross peaks with mixing time. [See Crepeau *et al.* (1994); Patyal, Crepeau, and Freed (1997) and Chiang *et al.* (2007), who studied several different nitroxide spin labels in phospholipid membrane vesicles to obtain accurate dynamics and ordering parameters in the context of MOMD.]

2-D-ELDOR is extremely sensitive to the properties of membrane vesicles, with the data acquired showing dramatic changes in the membranes' properties. Moreover, such changes can even be detected visually from the spectral patterns by a simple inspection; an example is seen in Figure 11.10, which shows the 2-D-ELDOR contour plots as a function of the mixing time, T_m , for the spin-labeled lipid, 1-palmitoyl-2-(16-doxyl stearoyl) phosphatidylcholine (16-PC) in pure lipid vesicles, in a standard liquid-crystalline phase and also for a lipid-cholesterol mixture in 1:1 ratio, in a "liquid-ordered" (LO) phase (Ge *et al.*, 1999). The qualitative difference in the spectra indicate that the LO phase exhibits a significantly greater ordering than the liquid crystalline phase, due to its increased microscopic ordering. In addition, the LO phase exhibits a much slower development of cross-peaks as a function of T_m , due to a restricted range of orientational motion as a result of the presence of microscopic ordering (Costa-Filho, Shimoyama, and Freed, 2003a).

In addition to the complex inhomogeneous lineshapes that are caused by MOMD (the theory of which was provided by Meirovitch, Nayeem, and Freed, 1984), there exists another often-encountered source of IB. This, specifically, is the slowmotional regime that does not average out the rigid limit line shapes completely, as the motions are too slow. This problem is dealt with effectively in the MOMD theory. As the slow-motional spectra have a comparable time scale to that for molecular dynamics, they provide a greater insight into the microscopic details of the molecular dynamics. As with complex fluids, it was found that a more sophisticated model than the MOMD model-specifically, the SRLS model as described above-was needed to analyze the 2-D-ELDOR spectra in order to achieve a reasonably good agreement with experiment. The SRLS model was tested in studies on a macroscopically aligned liquid crystal solvent, called 40,8 (Sastry et al., 1996a, 1996b). This solvent exhibits many phases as a function of temperature, including isotropic, nematic, liquid-like smectic A, solid-like smectic B, and crystalline phases. This model, in addition to using the macroscopic liquid crystalline-orienting potential, has provided consistently better fits than were obtained with the simpler MOMD model, and does not include any local structure. Hence, one could, using the macroscopically aligned samples, obtain very extensive relaxation, dynamic, and structural information which includes virtually all of the parameters obtainable from any EPR experiments on spin relaxation in a complex fluid! These ten parameters are as follows: the two-term (asymmetric) macroscopic ordering potential in the liquid crystalline phases; the axially symmetric diffusion tensor for the probe; its two-term orienting potential in the local structure or cage; the relaxation rate for the cage; the residual homogeneous T_2^{-1} due to processes other than the reorientational modulation of the ¹⁴N dipolar and g-tensors; the residual (Gaussian) inhomogeneous broadening not due to the specific slow-motional contributions from the ¹⁴N hf- and g-tensors; and the overall T_1 for the electron spins.

When investigating the effects of the peptide gramicidin A (GA) on the dynamic structure of model membranes, the changes in 2-D-ELDOR spectra-when as compared to those in the CW-EPR spectra-were found to be more dramatic, thus demonstrating the much greater sensitivity of 2-D-FT-EPR to molecular dynamics (Patyal, Crepeau, and Freed, 1997). It emerges that in these studies, performed at 9.3 GHz and with dead times of 50-60 ns, one could only be used to study the bulk lipids and not the boundary lipids that coated the peptide, evidence from which was provided in the CW-EPR spectra, albeit at very limited resolution. This problem was overcome, however, by invoking the higher-frequency, 17.3 GHz, 2-D-ELDOR, with an increased signal-to-noise ratio (SNR) and reduced dead times (~25-30 ns) to demonstrate the presence of two components (Costa-Filho *et al.*, 2003b). These were: (i) the bulk component, as reported by Patyal, Crepeau, and Freed (1997), which exhibited relatively fast dynamics; and (ii) the boundary lipid, which grows in as the GA is added, and whose 2-D-ELDOR spectrum is undoubtedly that of a more slowly reorienting lipid, as expected. Moreover, these spectra could be simulated with a model of bending the end-chain of the lipid as it coated the GA. Such details of the dynamic structure of complex membrane systems can only be obtained using 2-D-ELDOR. The recent studies of membrane systems (Chiang et al., 2007) carry even further the capabilities of 2-D-ELDOR.

11.4.6

Extension of 2-D-ELDOR to Higher Frequencies

Just as in CW-EPR described earlier in this chapter, one can hope to perform multifrequency 2-D-ELDOR studies. That this is feasible was demonstrated by Hofbauer *et al.* (2004) and Earle *et al.* (2005) at 95 GHz. We show in Fig. 11.12 just such an example. The challenges here are the much greater spectral bandwidths to irradiate and the much shorter T_2 decays. In addition, these higher frequency spectra with greater orientational resolution pose a much greater challenge to their theoretical simulation in the slow-motional regime. However, a new computational algorithm has very recently been developed (Chiang and Freed, 2011) which promises to overcome these difficulties.

11.5

Simulation of Multifrequency EPR Spectra Using More Atomistic Detail Including Molecular Dynamics and Stochastic Trajectories

11.5.1

Augmented SLE

Improved modeling has been used for the stochastic modeling of the side-chain dynamics of spin-labeled proteins, an example being MTSSL (1-oxyl-2,2,5,5-tetramethyl- Δ^3 -pyrroline-3-(methyl)methanethiosulfonate spin label) linked to poly α -helix domain (Tombolato, Ferrarini, and Freed, 2006a). The features of this model are described briefly as follows. Here, one considers stable conformers as



Figure 11.12 2D-ELDOR spectra of Gramicidin A spin label (GASI) in aligned DPPC membranes at 7 °C: with the director parallel to the applied field ($\psi = 0^{\circ}$) with mixing times (a) 50 and (b) 200ns,

respectively. The spectral extent is approximately 350 MHz, corresponding to $\pm 6 \text{ mT}$ ($\pm 60 \text{ G}$). The B₁ in this case is about 1.7 mT (17 G).

determined from quantum mechanical calculations and utilizes estimates of the chain dynamics. The simplifying assumption is made that conformers with low barriers exhibit a fast exchange, while those with high barriers exhibit no exchange in the EPR time scale. There are no free parameters used in these calculations. The modified SLE used here is:

$$\frac{\partial \rho(\Omega_{D}, t)}{\partial t} = -i\overline{L(\Omega_{D})}\rho(\Omega_{D}, t) - \Gamma_{\Omega}\rho(\Omega_{D}, t) - [T_{2}^{-1}(\Omega_{D}) + \Gamma(\Omega_{D})]\rho(\Omega_{D}, t),$$
(11.25)

where $L(\Omega_D)$ is the Liouville superoperator with the magnetic tensors partially averaged out by chain dynamics, $\Gamma(\Omega_D)$ is the diffusion operator for overall protein tumbling, and $T_2^{-1}(\Omega_D)$ is the linewidth contribution from chain dynamics, as calculated using Redfield theory (Redfield, 1965), i.e., motional narrowing theory. The torsional energy profiles were obtained using quantum mechanics, taking into account the constraints imposed by the local environment. Torsional motions about each of the five dihedral angles were taken as independent. The details of the potential energy provide the description of the system in terms of the significant rotamers undergoing conformational jumps and the librations which occur about the minima of the side-chain torsional potentials. The approximate diffusive treatment of the dynamics used provides a reasonable account of energetic and frictional features of the tether. This analysis enables one to estimate the amplitude and time-scale of the chain motions. Then, by Equation 11.25, it is possible to derive some general considerations on the effect of the tether dynamics on EPR spectra. This approach is thus based on a simple model, which leads to an easier interpretation of the determining factors of conformational dynamics of the side chain. The theory contains many realistic features, predicting some general results on the geometry and kinetics of this dynamics. These results have been exploited to directly introduce the dynamics of the nitroxide on lineshape analysis, for example, to interpret the EPR spectra of mutants of T4 lysozyme (Tombolato, Ferrarini, and Freed, 2006b). Another approach for obtaining detailed simulations is to employ either Monte-Carlo simulations (Sale et al., 2002, 2005), which are not covered here, or MD simulations using dynamic trajectories. General procedures for simulating the EPR spectra of nitroxide spin labels from MD and stochastic trajectories are described below.

11.5.2

MD Simulations Using Trajectories

The EPR spectrum may be calculated from a time evolution of the transverse magnetization, which is determined from the time dependence of the spin Hamiltonian. In order to take into account the dynamics of the spin label, one needs to consider appropriate models, such as the SLE technique described by Schneider and Freed (1989a), as outlined above. The dynamics of the electron–nuclear spin-coupled system of the spin label is treated quantum mechanically, whereas the rotational dynamics of the spin label can be treated classically. When one deals directly with the probability density to take into account the dynamic stochastic processes, the coupled classical-quantum evolution is described by the SLE, as discussed above. However, another approach was developed more recently by Sezer, Freed, and Roux (2008a, 2008b; Sezer, Freed, and Roux, 2009), and used successfully to simulate EPR spectra. This involves the use of dynamic trajectories with explicit realizations of the process in the time domain.

11.5.3 Use of Dynamic Trajectories to Simulate Multifrequency EPR Spectra

The simulation of EPR spectra using trajectories has been carried out in the past, for example by Saunders and Johnson (1968), Pederson (1972), and Robinson, Slutsky, and Auteri (1992). It has been claimed that this approach has the following advantages:

- It is possible to generate trajectories for more complicated stochastic models that can be treated by the SLE formalism, as suggested by Hakansson, Persson, and Westlund (2002) and by Persson *et al.* (2002). But, the SLE approach does allow for a wide range of sophisticated models (Meirovitch *et al.*, 2010), and is orders of magnitude faster (Sezer *et al.*, 2008a).
- It is possible to simulate EPR spectra directly from atomistic MD trajectories, without invoking any stochastic model (Eviatar, van der Heide, and Levine, 1995; Steinhoff and Hubbell, 1996; Hakansson *et al.*, 2001; Stoica, 2004; and Beier and Steinhoff, 2006), although in much of this work the quantum spin dynamics has been overly simplified (Sezer *et al.*, 2008a). A major disadvantage is the much greater computation time by orders of magnitude for computing EPR spectra by trajectories vs. use of the SLE (Sezer *et al.*, 2008a).

High-frequency EPR provides an increased sensitivity to dynamics on the subnanosecond time scale, and therefore provides experimental spectra which can be exploited to establish a tighter connection with MD simulations.

The treatment of Sezer, Freed, and Roux (2008a) will be detailed here (albeit only in outline due to restricted space) to provide a description of the procedure of using MD trajectories to simulate multifrequency EPR spectra. In general, many, long trajectories are required for the convergence of spectra (Robinson, Slutsky, and Auteri, 1992; Hakansson et al., 2001; Stoica, 2004; Eviatar, van der Heide, and Levine, 1995). Alternatively, it is possible to use MD trajectories to estimate the parameters of a preselected stochastic dynamic model, and then to use these parameters either by solving the SLE (Budil et al., 2006) or by generating trajectories (Steinhoff and Hubbell, 1996; Beier and Steinhoff, 2006). Previously, spectra from more sophisticated rotational dynamical models, such as MOMD and SRLS (as discussed above), were not simulated by applying the trajectory-based approach. Only simple isotropic diffusion (Pederson, 1972; Robinson, Slutsky, and Auteri, 1992) or isotropic diffusion in a cone (Fedchenia, Westlund, and Cegrell, 1993) were taken into account, by employing rotational diffusion trajectories to simulate the EPR spectra. The trajectory-based approach was not exploited to its full potential, due to a lack of any rigorous formalism to simulate the trajectories for anisotropic diffusion.

An efficient numerical integrator to generate trajectories for sophisticated anisotropic rotational diffusion models, such as MOMD and SRLS, was developed to achieve this. In the practical algorithm, the gap between the small time steps at which the snapshots along the MD trajectories are available, and the longer time steps required for numerical propagation of the stochastic or quantal dynamics is

accounted for by using time-averaging procedures. One describes the quantal spin dynamics in relation to the numerical propagation of the relevant part of the density matrix in Hilbert space, and the classical anisotropic Brownian diffusion in a potential, so as to develop an accurate and efficient numerical integrator for general rotational diffusion. Finally, one can compare the spectra for free and restricted rotational diffusion models simulated by using the developed timedomain integrators with the SLE. To this end, one utilizes time-averaging arguments to bridge the gap between the various integration time steps. Multifrequency spectra may then be simulated by using rotational diffusion or MD trajectories.

11.5.4

Numerical Integrators

11.5.4.1 Integration of the Quantal Spin Dynamics

Since the CW and FID spectra are formally equivalent (Abragam, 1961), the discussion here will be based on terms of the latter, which is numerically more appropriate.

The Spin Hamiltonian and the Interaction Picture The case of the nitroxide spin probe, which consists of an unpaired electron spin $S = \frac{1}{2}$ and a ¹⁴N nucleus (I = 1), will be considered. (The case of 15N follows in a similar manner.) In units of angular frequency, using the standard notation, the spin Hamiltonian of a nitroxide is

$$H(t) = \gamma_e(B \cdot G(t) \cdot S + I \cdot A(t) \cdot S), \qquad (11.26)$$

where γ_i is the electronic gyromagnetic ratio, A is the hyperfine tensor (in units of magnetic field) and $G(t) \equiv g(t)/g_c$, with g_c being the free-electron *g*-factor. (Note this is the same Hamiltonian as in Equations 11.4 and 11.5.) Typically, the G and A tensors are diagonal in the same coordinate frame, referred to as N. They are also time-dependent due to the motion of the frame N with respect to the laboratory frame, L, (this explicitly time dependence is only implicit in Equations 11.4 and 11.5.) defined with respect to the external magnetic field **B**: $(0,0,B_0)$, in which the electronic spin is quantized, so that all the vector and tensor components in Equation 11.26 are defined with respect to L. The nuclear Zeeman and quadrupolar interactions are neglected here, but they can be easily included if required; the coupling with other spins is also ignored. The electronic and nuclear spins localized on a single spin label are described by the state vector $|\psi(t)\rangle$, the dynamics of which is governed by the spin Hamiltonian via the Schrödinger equation. The spin Hamiltonian given by Equation 11.26 can be broken into two parts: (i) a large and timeindependent part H; and (ii) the remaining time-dependent part, which is denoted as V(t). One can express

$$H \equiv \gamma_e G_0 B_0 S_z = \omega_0 S_z; \quad G_0 \equiv \frac{1}{3} Tr\{G\}$$

The state vector oscillates with the Larmor frequency ω_0 in the absence of V(t), whereas in its presence the instantaneous frequency of precession fluctuates around ω_0 by a time-dependent modulation, much smaller than ω_0 .

It is more convenient to transform to the coordinate frame rotating at the Larmor frequency, i.e. what is referred to as the "interaction picture," so that one does not have to use extremely small integration steps required to resolve the fast oscillations at ω_0 . To go into the interaction picture, one transforms the state vector and the operators as follows:

$$|\psi'(t)\rangle \equiv e^{iHt} |\psi(t)\rangle, V'(t) \equiv e^{iHt}V(t)e^{-iHt},$$

which transforms the Schrödinger equation to

$$|\dot{\psi}'(t)\rangle = -iV'(t)\psi'(t), \qquad (11.27)$$

where the dot indicates a derivative with respect to time. The spin operators are transformed as

$$S'_{z} = S_{z}, S'_{+} = S_{+}e^{+i\omega_{0}t}, S'_{-} = S_{+}e^{-i\omega_{0}t}$$
(11.28)

Finally, the time-dependent part of the Hamiltonian becomes, in the interaction picture,

$$V'(t) = V_z(t) + \sum_{\kappa = \pm} V_\kappa(t) e^{i\kappa\omega_0 t},$$
(11.29)

where the operators $V_v(t) \equiv (b_v(t) + a_v(t))S_v$, $v = z, \pm$, are defined as follows;

$$a_{z}(t) \equiv \gamma_{e} \sum_{i=x,y,z} A_{iz}(t)I_{i},$$

$$a_{\pm}(t) = \gamma_{e} \sum_{i} \frac{1}{2} (A_{ix}(t) \mp iA_{iy}(t))I_{i},$$

which act only on the nuclear spin state, and the scalars

$$b_z(t) \equiv \gamma_e B_0 G'_{zz}(t),$$

$$b_{\pm}(t) \equiv \gamma_e B_0 \frac{1}{2} (G'_{zx}(t) \mp i G'_{zy}(t))$$

are expressed in terms of the traceless tensor:

$$G'(t) = G(t) - G_0 E,$$

where *E* denotes the identity matrix in the electronic space.

The High-Field (HF) Approximation In the interaction picture–or the rotating frame in the present case – the fast-varying term responsible for Larmor precession has been removed in the effective Hamiltonian. This means that $|\psi'(t)\rangle$ varies on a time scale which is now much longer than the Larmor precession time scale. However, there are parts of the Hamiltonian which oscillate at the Larmor frequency; these are the terms containing $e^{i\kappa\omega_0 t}$ in Equation 11.29, which average out the effect of the slowly varying coefficients $V_{\pm}(t)$, that depend in turn on the magnetic tensors G(t) and A(t). Then, in order to calculate the slowly varying quantity, the transverse magnetization in this frame, one can consider only the slowly varying part $V_z(t)$ of Equation 11.29. This leads to the high-field approximation,

derived as the zeroth-order term in the expansion of the Schrödinger Equation 11.27 in powers of $\varepsilon = 1/\omega_0$, by seeking a solution of Equation 11.27 in the form:

$$|\dot{\psi}'(t)\rangle = |\psi^0(t)\rangle + \varepsilon \sum_{\kappa=\pm} |\psi^\kappa(t)\rangle e^{i\kappa t/\varepsilon},$$

where both $|\psi^0(t)\rangle$ and $|\psi^{\kappa}(t)\rangle$ are slowly varying. Proceeding in this fashion, one tries to derive an equation of motion for $|\psi^0(t)\rangle$. Finally, one obtains an equation for the slowly varying part of the state vector, correct to the first order in ε :

$$\left|\dot{\psi}^{0}(t)\right\rangle = -iH_{S}(t)\left|\psi^{0}(t)\right\rangle,\tag{11.30}$$

where the effective slow Hamiltonian is as follows:

$$H_{S}(t) \equiv V_{z}(t) + \mathcal{E}[V_{+}(t), V_{-}(t)], \qquad (11.31)$$

In carrying out further analysis of $H_s(t)$, neglecting the terms which depend on $1/\omega_0$ as justified in the high-field approximation, and retaining only the V_z part of the Hamiltonian, one is led to the effective Hamiltonian:

$$H_{HF}(t) = \gamma_e \left(B_0 G_{zz'}(t) + I \cdot a(t) \cdot S_z \right); a_i(t) \equiv A_{iz}(t).$$
(11.32)

It should be noted that an equivalent form of Equation 11.32 is the starting point for the SLE analysis of slow motion for the unsaturated line shapes (Freed, Bruno, and Polnaszek, 1971a; Polnaszek and Freed, 1975; Meirovitch, Nayeem, and Freed, 1984; Schneider and Freed, 1989a, b; Polimeno and Freed, 1993; Polimeno and Freed, 1995; Budil *et al.*, 1996; Liang and Freed, 1999). In the HF approximation, the contribution of spin flips to the decay of transverse magnetization has been neglected; this is due to ignoring the terms in S_{\pm} in the spin Hamiltonian. It emerges that the slow Hamiltonian, given by Equation 11.31 and its lowestorder approximation, expressed by Equation 11.32, are diagonal in the electronic Hilbert space, and do not allow spin flips. As a consequence, these Hamiltonians are not suitable to describe the arrival to equilibrium of the longitudinal magnetization, which is effected by these spin flips. Thus, in order to treat the phenomena which cause T_1 relaxation, it would be necessary to consider fast dynamics at the time scale of the Larmor precession.

The HF approximation decouples the spin dynamics of the $m_s = \frac{1}{2}(+)$ and

 $m_s = -\frac{1}{2}(-)$ sectors of the Hilbert space, seen by introducing the state vector $|\psi'(t)\rangle = \begin{pmatrix} |\psi'^+(t)\rangle \\ |\psi'^-(t)\rangle \end{pmatrix}$, and the Hamiltonian, given by Equation 11.32 in Equation 11.30, obtaining

$$\begin{pmatrix} |\dot{\psi}^{\prime+}(t)\rangle \\ |\dot{\psi}^{\prime-}(t)\rangle \end{pmatrix} = -i \begin{pmatrix} H_{HF}^{++}(t) & 0 \\ 0 & H_{HF}^{--}(t) \end{pmatrix} \begin{pmatrix} |\psi^{\prime+}(t)\rangle \\ |\psi^{\prime-}(t)\rangle \end{pmatrix},$$
(11.33)

where the slow state vector $|\psi^{0}\rangle$ was replaced by the state vector $|\psi'\rangle$ in the interaction picture. In view of Equation 11.33, numerical integration of the quantum dynamics is achieved by keeping track of the temporal development of the two parts $|\psi'^{\pm}\rangle$ separately, following the short-time propagation scheme, as follows:

$$\left|\psi^{\prime\pm}(t+\Delta t)\right\rangle = e^{\pm i\Delta t H_{HF}^{++}(t)} \left|\psi^{\prime\pm}(t)\right\rangle,\tag{11.34}$$

where the equivalence $H_{HF}^{--} = -H_{HF}^{++}$, valid in the HF approximation, was used. It is noted that the quantum integrator summarized by Equation 11.34 was also used by Eviatar, van der Heide, and Levine (1995), with their vectors *P* and *Q* corresponding to $|\psi'^{\pm}\rangle$.

Calculation of the Spectrum by the Use of Reduced Density Operator The CW spectrum, the object of the present simulation, is the Fourier–Laplace transform of the transverse magnetization $M_+ = M_x + iM_y$:

$$\tilde{M}_+(\omega) = \int e^{-i\omega t} M_+(t) dt,$$

where $M_+(t) = \langle \hat{M}_+(t) \rangle$ is the quantum-mechanical expectation value of the operator $\hat{M}_+(t) \propto \hat{S}_+$. It is noted, using Equation 11.28 that

$$M_{+}(t) = \left\langle \psi'(t) \left| \hat{M}_{+}' \right| \psi'(t) \right\rangle = e^{i\omega_{0}t} \left\langle \psi'(t) \left| \hat{M}_{+} \right| \psi'(t) \right\rangle$$

which shows that equivalently one can use the Schrödinger picture by sandwiching the operator \hat{M}_+ with the state vector $\langle \psi'(t) \rangle$ in the interaction picture and simply shifting the spectrum by the Larmor frequency by multiplying with $e^{i\omega_0 t}$. Accordingly, one gets

$$\tilde{M}_{+}(\omega+\omega_{0}) = \int_{0}^{\infty} e^{i\omega_{0}t} \left\langle \psi'(t) \middle| \hat{M}_{+} \middle| \psi'(t) \right\rangle dt = \int_{0}^{\infty} e^{i\omega_{0}t} \left\langle \psi'^{+}(t) \middle| \hat{M}_{+} \middle| \psi'^{-}(t) \right\rangle dt, \quad (11.35)$$

where the last equality has been written in view of S_+ , proportional to \hat{M}_+ , being a raising operator. Defining the reduced density matrix:

$$\rho'^{++}(t) \equiv |\psi'^{-}(t)\rangle \langle \psi'^{+}(t)|, \qquad (11.36)$$

one obtains

$$\langle \psi'(t) | M_+ | \psi'(t) \rangle = Tr \{ M_+ \rho'^{-+}(t) \}.$$

It should be noted here that, in the HF approximation, the time evolution of ρ'^{-+} is independent of the time evolution of the other sectors of the reduced spin density matrix (ρ'^{++} , ρ'^{+-} , and ρ'^{--}), defined analogously to ρ'^{-+} as in Equation 11.36. It follows from the short-time propagator described by Equation 11.34 and the definition of ρ'^{-+} , Equation 11.36, that the short-term dynamics of ρ'^{-+} is:

$$\rho'^{-+}(t+\Delta t) = e^{i\Delta t H_{HF}^{++}(t)} \rho'^{-+}(t) e^{i\Delta t H_{HF}^{++}(t)}$$
(11.37)

It is also noted that the same matrix acts on both sides of ρ'^{-+} in the above equation; this is different from the evolution of the full density matrix in the Hilbert space.

Equation 11.37 is the key expression for the integrator for the relevant sector of the quantum spin dynamics being developed here. In order to evaluate this

efficiently, one needs to quickly calculate the matrix in the exponential part of Equation 11.37:

$$e^{i\Delta t \hat{H}_{HF}^{++}(t)} = e^{i\Delta t (1/2)\gamma_{e} (B_{0}G'_{2Z}(t) + a(t) \cdot I)}.$$
(11.38)

at each step. It is easy to take into account the first term in the second parenthesis in Equation 11.38, as it amounts to a simple, time-dependent phase factor. However, the second term in this parenthesis is more complicated to evaluate. The straightforward method to evaluate is by first diagonalizing the matrix $a(t) \cdot I$ in the nuclear space by a similarity transformation, exponentiating its eigenvalues, and then applying the inverse similarity transformation. Sezer, Freed, Roux (2008a) discuss a more efficient alternative by invoking the relation between the nuclear spin matrices and the three-dimensional (3-D) representation of the rotation group, so that

$$\hat{N} = \sum_{i} n_i \hat{I}_i,$$

where $\hat{N} = (n_x, n_y, n_z)$ is a unit vector that satisfies the exponential expansion:

$$e^{-i\theta\hat{N}} = E_I - i(\sin\theta)\hat{N} - (1 - \cos\theta)\hat{N}^2, \qquad (11.39)$$

where E_I denotes the identity operator in the 3-D Hilbert space of the nuclear spin. As a consequence, one can avoid solving the eigenvalue problem of $a(t) \cdot I$ at each time step, and instead calculate the magnitude a and the direction n of the vector a(t). One needs to use the angle $\theta = \gamma_e \Delta t \frac{1}{2}a$ and the unit vector n to construct the short-term propagator (Equation 11.39), as shown explicitly by Sezer, Freed, Roux (2008a), who used the following equations:

$$\operatorname{Re}(e^{-i\theta\hat{N}}) = I_{3} + \begin{pmatrix} c_{\theta} \left[n_{z}^{2} + \left(\frac{1}{2}\right) \left(n_{x}^{2} + n_{\gamma}^{2}\right) \right] & \left(\frac{1}{\sqrt{2}}\right) \left[s_{\theta}n_{\gamma} + c_{\theta}n_{z}n_{x} \right] & c_{\theta} \left(\frac{1}{2}\right) \left(n_{x}^{2} - n_{\gamma}^{2}\right) \\ \left(\frac{1}{\sqrt{2}}\right) \left[-s_{\theta}n_{\gamma} + c_{\theta}n_{z}n_{x} \right] & c_{\theta} \cdot \left(n_{x}^{2} + n_{\gamma}^{2}\right) & \left(\frac{1}{\sqrt{2}}\right) \left[s_{\theta}n_{\gamma} - c_{\theta}n_{z}n_{x} \right] \\ c_{\theta} \left(\frac{1}{2}\right) \left(n_{x}^{2} - n_{\gamma}^{2}\right) & \left(\frac{1}{\sqrt{2}}\right) \left[-s_{\theta}n_{\gamma} - c_{\theta}n_{z}n_{x} \right] & c_{\theta} \left[n_{z}^{2} + \left(\frac{1}{2}\right) \left(n_{x}^{2} + n_{\gamma}^{2}\right)\right] \end{pmatrix}$$

and

$$\operatorname{Im}(e^{-i\theta N}) = \begin{pmatrix} s_{\theta}n_{z} & \left(\frac{1}{\sqrt{2}}\right) [s_{\theta}n_{x} - c_{\theta}n_{z}n_{\gamma}] & -c_{\theta}n_{x}n_{\gamma} \\ \left(\frac{1}{\sqrt{2}}\right) [s_{\theta}n_{x} + c_{\theta}n_{z}n_{\gamma}] & 0 & \left(\frac{1}{\sqrt{2}}\right) [s_{\theta}n_{x} + c_{\theta}n_{z}n_{\gamma}] \\ c_{\theta}n_{x}n_{\gamma} & \left(\frac{1}{\sqrt{2}}\right) [-s_{\theta}n_{\gamma} - c_{\theta}n_{z}n_{x}] & -s_{\theta}n_{z} \end{pmatrix}$$

Sezer, Freed, and Roux (2008a) make the argument that it is preferable to work with the density matrix, ρ'^{-+} , rather than using the state vector $|\psi'^{\pm}\rangle$, since the former represents the ensemble average of all the state vectors consistent with the macroscopic initial condition, when calculating the FID after a $\pi/2$ (90°) pulse

applied at time t = 0, which renders $M_+(t = 0^+) = 1$, which does not uniquely determine the state vector $|\psi(0^+)\rangle$. Thus, if one used the state vector, an additional averaging over all the possible starting state vectors that give the correct initial magnetization is required. One, therefore, eliminates the sampling noise associated with averaging over a finite number of initial state vectors by propagating the density matrix rather than using the state vector. This justifies the extra computational cost of propagating a 3×3 matrix as compared with a 3×1 vector.

Equilibrium and Time-Dependent Density Matrix Starting with the decoupled initial conditions, one can express the density operator in terms of the average Hamiltonian:

$$\rho^{eq} = \rho(0) \propto \exp(-\hbar \hat{H}/k_B T) \cong a(\hat{E} - bS_z), \qquad (11.40)$$

where \hat{E} is the identity operator in Hilbert space and a and b are scaler coefficient. In writing the last term, the fact that the sample is equilibrated under the influence of a constant magnetic field, and the average Hamiltonian is less than 1% of k_BT so that the exponential can be expanded to the first order only, have been taken into account. At a later time, given that \hat{E} commutes with the Hamiltonian, one can write the density matrix in the form:

$$\rho(t) \cong a(\hat{E} + \sigma(t)).$$

It should also be noted that \hat{E} does not affect the expectation value of the magnetization, since $Tr\{\hat{M}\hat{E}\}=0$, as \hat{M} is proportional to \hat{S} . Thus, one need only keep track of $\sigma(t)$, which is, in fact, the only relevant part of the density matrix. It is further noted from Equation 11.40 that $\sigma(0) = \sigma^{eq} \propto S_z$. After applying the 90° pulse, $\sigma(0^+) \propto S_\gamma$, which means that $\sigma^{-+}(0^+) \propto E_I$.

11.5.4.2 Generation of Stochastic Trajectories for Rotational Diffusion

In this section, an explanation is provided of how to develop an efficient numerical integrator for the rotational Brownian diffusion of a body-fixed frame (B) with respect to a space fixed frame (S). If there exists an ordering potential $U(\Omega)$, it can be parameterized by using the Euler angles, Ω ; here, $\Omega = (\alpha, \beta, \gamma)$ describes the instantaneous orientation of B with respect to S. The basic model considered here forms the basis for more sophisticated motional models such as MOMD and SRLS.

Use of Quaternions to Treat Rotational Dynamics The kinematics of rotations required here can be conveniently treated by using quaternions (Lynden-Bell and Stone, 1989), rather than Euler angles. The components of the quaternion for the orientation of B with respect to S being given in terms of the Euler angles $\Omega = \{\alpha, \beta, \gamma\}$ are calculated as follows (Lynden-Bell and Stone, 1989):

$$q_{0} = \cos(\beta/2)\cos((\gamma + \alpha)/2), q_{1} = \sin(\beta/2)\sin((\gamma - \alpha)/2), q_{2} = \sin(\beta/2)\cos((\gamma - \alpha)/2), q_{3} = \cos(\beta/2)\sin((\gamma + \alpha)/2),$$
(11.41)

In terms of the components of the quaternion, the 3×3 rotation matrix emerges as (Biedenharn and Louck, 1981):

$$R = \begin{pmatrix} q_0^2 + q_1^2 - q_2^2 - q_3^2 & 2q_1q_2 - 2q_0q_3 & 2q_1q_3 + 2q_0q_2 \\ 2q_1q_2 + 2q_0q_3 & q_0^2 - q_1^2 + q_2^2 - q_3^2 & 2q_2q_3 - 2q_0q_1 \\ 2q_1q_3 - 2q_0q_2 & 2q_2q_3 + 2q_0q_1 & q_0^2 - q_1^2 - q_2^2 + q_3^2 \end{pmatrix}.$$
 (11.42)

The bottom row of Equation 11.42 contains just the components of the vector z of the stationary coordinate system with respect to the axes of B, which are denoted, for later use, as:

$$X \equiv R_{zx} = (z)_{x'}; \quad Y \equiv R_{zy} = (z)_{y'}; \quad Z \equiv R_{zz} = (z)_{z'}.$$
(11.43)

The orientation of B with respect to S is described by the 2×2 unitary matrix, which can be expanded in terms of the Pauli spin matrices σ_1 , σ_2 , σ_3 and the 2 × 2 identity matrix σ_0 (not to be confused with σ , the density matrix used above), as follows:

$$Q = \begin{pmatrix} q_0 - iq_3 & -q_2 - iq_1 \\ q_2 - iq_1 & q_0 + iq_3 \end{pmatrix} = q_0 \sigma_0 - i \sum_{i=1,2,3} q_i \sigma_i,$$
(11.44)

characterized by unit determinant:

$$q_0^2 + q_1^2 + q_2^2 + q_3^2 = 1. (11.45)$$

The components of the quaternion corresponding to the transformation relating B to S are the real numbers q_i . Q becomes time-dependent when there is motion of B with respect to S, described by the equation of motion:

$$\frac{d}{dt}Q(t) = W(t)Q(t), \quad \text{where } W(t) = -i\frac{1}{2}\sum_{i}w_{i}(t)\sigma_{i}, \qquad (11.46)$$

where $\omega(t)$ is the instantaneous angular velocity of B. Q(t), as given by Equation 11.46, can be numerically integrated to generate the time series of Q, in the same way as achieved by Fedchenia, Westlund, and Cegrell (1993) in their rigorous treatment of isotropic rotational diffusion restricted to a conical region.

When considering anisotropic diffusion, one needs to work with the components of $\boldsymbol{\omega}$ with respect to B, denoted as $\tilde{\boldsymbol{\omega}}_{i'}$, rather than with respect to S, as in the treatment of isotropic rotational diffusion described above. The equation of motion of *Q* becomes:

$$\frac{d}{dt}Q(t) = Q(t)\tilde{W}(t), \quad \tilde{W}(t) = -i\frac{1}{2}\sum_{i}\tilde{w}_{i}(t)\sigma_{i}.$$
(11.47)

It should be noted that, in Equation 11.47, the components of the angular velocity of the rotating frame are with respect to the stationary frame, whereas in Equation 11.46 they are with respect to the body-fixed frame. Integration of Equation 11.47 yields:

$$Q(t + \Delta t) = Q(t)e^{\Delta t \overline{W}(t)},$$
(11.48)

which preserves the determinant of *Q*, and in turn the normalization of the quaternion, as given by Equation 11.45. Now, in close analogy to the evaluation of

Equation 11.38 using Equation 11.39, one can exponentiate the matrix in Equation 11.48 calculating only the trigonometric functions:

$$\exp\left(-i\sum_{i}\frac{\tilde{\omega}_{i}\Delta t}{2}\sigma_{i}\right) = \cos\theta\sigma_{0} - i\sin\theta\sum_{i}u_{i}\sigma_{i}$$
$$= \begin{pmatrix}\cos\theta - iu_{z}\sin\theta & -(u_{y} + iu_{x})\sin\theta\\(u_{y} - iu_{x})\sin\theta & \cos\theta + iu_{z}\sin\theta\end{pmatrix}.$$
(11.49)

In Equation 11.49, θ and $u = (u_x, u_y, u_z)$ denote, respectively, the magnitude and the direction of the vector $\tilde{\omega}(t)\Delta t/2$. The propagation of the quaternion $Q_{\rm SB}$ describing the orientation of the coordinate system B with respect to the system S, is described by Equations 11.48 and 11.49, provided that one knows the physics of the orientational dynamics to determine how $\tilde{\omega}(t)$ changes with time. This is described in the following subsection.

Consideration of Anisotropic Brownian Diffusion in an External Potential Here, one takes into account the rotational diffusion in the presence of a potential $U(\Omega)$ (cf. Equation 11.19). Hereafter, the tilde over ω will be dropped, and it will be assumed that all the vector and tensor components are taken with respect to the coordinate system B. The components of the instantaneous angular velocity $\omega(t)$ in B follow the equation of motion (Kalmykov, 2001 and Coffey, Kalmykov, and Waldron, 2004):

$$\boldsymbol{\omega}(t) = -\mathbf{D}\boldsymbol{\nabla}\boldsymbol{u}(\boldsymbol{\Omega}(t)) + \boldsymbol{\xi}(t), \qquad (11.50)$$

in the limit of high friction, so that inertial terms are neglected. In Equation 11.50, the first term on the right-hand side corresponds to the systematic torque due to the potential $u(\Omega) \equiv U(\Omega)/k_BT$, whereas the second term, $\boldsymbol{\xi}(t)$, is the random torque that leads to the orientational diffusion. The other symbols in Equation 11.50 are

D, the rotational diffusion tensor, which is diagonal in B, and $\nabla = \left(\frac{\partial}{\partial \phi_x}, \frac{\partial}{\partial \phi_y}, \frac{\partial}{\partial \phi_z}\right)$, with ϕ_i being the angle of rotation around the *i*th axis of B. The components of

the random torque satisfy the conditions (Kalmykov, 2001; Coffey, Kalmykov, and Waldron, 2004):

$$\mathbf{E}\{\xi_{i}(t)\} = 0, \quad \mathbf{E}\{\xi_{i}(t_{1}), \xi_{j}(t_{2})\} = 2D_{ii}\delta_{ij}\delta(t_{1}-t_{2}), \tag{11.51}$$

where **E** denotes the expectation value over the Gaussian probability density of $\boldsymbol{\xi}$. Here, D_{ii} are the components of **D** with respect to B. The conditions in Equation 11.51 are valid only when the components of $\boldsymbol{\xi}$ are expressed in the coordinate frame in which the diffusion tensor is diagonal, which is the only frame in which the components of the diffusion tensor, and therefore, the intensities of the random torque decouple. When the diffusion tensor is isotropic this is naturally true in any coordinate system, including the space-fixed frame, so that it is possible, in this case, to exclusively express all the vector components with respect to S. However, for the general anisotropic case, one has to work with the components of the diffusion tensor is so that, as noted above, it is imperative to use

Equation 11.47 instead of Equation 11.46 for the equation of motion for the quaternion.

One can use the angular momentum operator *J* to describe the torque $-\nabla u(\Omega)$ (Polimeno and Freed, 1993; Kalmykov, 2001 and Coffey, Kalmykov, and Waldron, 2004):

$$-\nabla u(\Omega) = -iJu(\Omega),$$

which can be written in the component form as

$$\omega_i(t) = -iD_{ii}J_iu(\Omega(t)) + \xi_i(t), \qquad (11.52)$$

where the partial differential operators corresponding to the components J_i expressed in B are (Hakansson, Persson, and Westlund, 2002):

$$J_z = -i\frac{\partial}{\partial\gamma}; \quad J_{\pm} = e^{\pm i\gamma} \left[-i\cot\beta\frac{\partial}{\partial\gamma} \pm \frac{\partial}{\partial\beta} + \frac{i}{\sin\beta}\frac{\partial}{\partial\alpha} \right]; \quad J_{\pm} = J_x \pm iJ_\gamma.$$

In order to facilitate operating with J_i on the potential, it is convenient to express the latter as an expansion over the eigenfunctions of J_i (Polnaszek *et al.*, 1973, Meirovitch, Nayeem, and Freed, 1984; Polimeno and Freed, 1993, 1995):

$$u(\Omega) = -\sum_{j,m} c_j^m D_{0m}^j(\Omega)$$

The Wigner functions:

 $D_{nm}^{j}(\Omega) = e^{-in\alpha} d_{nm}^{j} e^{-im\gamma}$

are the eigenfunctions of J_z :

$$J_z D_{nm}^j(\Omega) = -m D_{nm}^j(\Omega).$$
(11.53)

They also have the property that

$$J_{\pm}D_{nm}^{j}(\Omega) = -\sqrt{j(j+1) - m(m\pm 1)}D_{nm\pm 1}^{j}(\Omega).$$
(11.54)

With the use of Equations 11.53 and 11.54, the problem of differentiation of the potential transforms to straightforward algebraic manipulation of the components of the quaternion.

Finally, three specific forms of the potential used by Sezer, Freed, and Roux (2008a), are listed below:

i) for the potential $u(\Omega) = -c_0^2 D_{00}^2(\Omega) = -c_0^2 \frac{1}{2}(3Z^2 - 1)$, which favors those orientations for which *z*- and *z'*-axes are either parallel or antiparallel (*Z* = ±1):

$$-iJ_{x}u = -i\sqrt{\frac{3}{2}}c_{0}^{2}[D_{01}^{2} + D_{0-1}^{2}] = -3c_{0}^{2}YZ;$$

$$-iJ_{y}u = -\sqrt{\frac{3}{2}}c_{0}^{2}[D_{01}^{2} - D_{0-1}^{2}] = 3c_{0}^{2}XZ; \quad -iJ_{z}u = 0$$
(11.55)

(It is noted that the primed axes refer to those in the body-fixed frame B.)

(11.57)

ii) for the potential $u(\Omega) = -c_2^2 [D_{02}^2(\Omega) + D_{0-2}^2(\Omega)] = -c_2^2 \frac{\sqrt{6}}{2} (X^2 - Y^2)$, which favors orientations in which the *z*-axis is parallel or antiparallel to $x' (X = \pm 1)$ and disfavors orientations in which the *z*-axis is parallel or antiparallel to $y' (Y = \pm 1)$:

$$-iJ_{x}u = -ic_{2}^{2}[D_{0-1}^{2} + D_{01}^{2}] = -\sqrt{6}c_{2}^{2}YZ; \quad -iJ_{y}u = -c_{2}^{2}[D_{0-1}^{2} - D_{01}^{2}] = -\sqrt{6}c_{2}^{2}XZ;$$
$$-iJ_{z}u = -2ic_{2}^{2}[D_{02}^{2} + D_{0-2}^{2}] = 2\sqrt{6}c_{2}^{2}XY$$

iii) from (i) and (ii), one has for the general potential:

$$u(\Omega) = -c_0^2 D_{00}^2 - c_2^2 [D_{02}^2(\Omega) - D_{0-2}^2(\Omega)], \qquad (11.56)$$

$$-iJ_x u = \left(-3c_0^2 - \sqrt{6}c_2^2\right) YZ; \quad -iJ_y u = \left(3c_0^2 - \sqrt{6}c_2^2\right) XZ; \quad -iJ_z u = 2\sqrt{6}c_2^2 XY,$$

where *X*, *Y*, and *Z* are defined in Equation 11.43.

Equation 11.52 can be numerically integrated by generating three random numbers $N_i(t)$ with Gaussian distribution of zero mean and unit standard deviation, taking into account the statistical properties of the random term in Equation 11.52, as described by Equation 11.51, so that

$$\frac{\omega_i(t)\Delta t}{2} = -iJ_i u(\Omega(t)) \frac{D_{ii}(t)\Delta t}{2} + \sqrt{\frac{D_{ii}(t)\Delta t}{2}} N_i(t), \qquad (11.58)$$

which is the necessary input to calculate propagation of the quaternion Q_{SB} using Equations 11.48 and 11.49, which describe the orientation of the coordinate system B with respect to the system S.

Spherical Grid to Incorporate the Initial Conditions for Rotational Diffusion These can be generated as random orientations of B with respect to S, weighted by the Boltzmann factor $\exp(-u(\Omega)/k_BT)$. Ponti (1999) has shown that systematically covering the surface of a sphere with a homogeneously distributed grid is much more efficient than a random choice. To this end, it has been demonstrated convincingly that distributing the points along a spiral that twists from the north pole to the south pole provides the most efficient grid with a high convergence rate (Ponti, 1999). Accordingly, the spherical polar coordinates of the points along the spiral are: $\theta_i = arc \cos(s_i)$; $\phi_i = \sqrt{\pi N} arc \sin(s_i)$, where $s_i \in (-1, 1)$; $i = 1, \ldots, N$, parameterizes the spiral and N is the number of points on the spiral. It is further noted that since the potentials $u(\Omega)$ used here are proportional to the Wigner functions $D_{0m}^{i}(\Omega)$, as given by Equation 11.57, which are independent of the Euler angle α , the initial conditions for the Euler angles are chosen as $\alpha = 0$, $\beta = \theta_i$, $\gamma = \phi_i$; the corresponding quaternion is calculated by using Equation 11.41.

11.5.4.3 Testing the Integrators: Generation of Trajectories for Typical Stochastic Models of Spin-Label Dynamics

Using the framework of rotational dynamics described above, one can generate trajectories for typical stochastic models of the spin-label dynamics, for example, Brownian rotational diffusion (BD), MOMD (Meirovitch, Nayeem, and Freed,

1984), and SRLS (Polimeno and Freed, 1993, 1995). These are schematically represented as follows:

BD:
$$L \rightarrow$$
 free (an)isotropic diffusion $\rightarrow M$ (fixed) $\rightarrow N$;
MOMD: $L \rightarrow$ powder $\rightarrow D \rightarrow$ restricted (an)isotropic diffusion
 $\rightarrow M$ (fixed) $\rightarrow N$;
SRLS: $L \rightarrow$ free isotropic diffusion $\rightarrow D \rightarrow$ restricted (an)isotropic
diffusion $\rightarrow M$ (fixed) $\rightarrow N$

Here, M and D refer to the body-fixed frame (B) and the stationary frame (S), respectively, as defined above. In the MOMD and SRLS models, the molecular frame M can diffuse with respect to the director frame D, which can itself be either randomly oriented in the MOMD model, or it can undergo free isotropic diffusion with respect to L in the SRLS model. In considering the diffusive motion of D with respect to L, D plays the role of the body-fixed frame B, whereas L plays the role of the stationary frame S. The intermediate director frame is skipped in the BD model, as there is no external ordering potential. L is identified with S, and M with B when simulating this model by using the formalism described in Section 11.5.3.2. In a given model, the initial conditions for each of the diffusion parts are chosen from the points distributed on a spherical grid.

Broadening of Spectral Lines due to Additional Relaxation Mechanisms To date, these have not been accounted for in the simulation, although they can be included phenomenologically in the form of Lorentzian and Gaussian relaxation times. To take into account Lorentzian broadening with relaxation time constant T_L , one multiplies the magnetization $M_+(t)$ by e^{-t/T_L} . On the other hand, one convolutes the spectral line with a Gaussian to take into account Gaussian broadening, that is multiply $M_+(t)$ by $e^{-t^2/8T_G^2}$, where T_G is the derivative peak-to-peak linewidth of the Gaussian, since convolution in the frequency domain is equivalent to multiplication in the time domain. Finally, since the trajectories are of some finite duration T, the resultant appearance of high frequencies in the Fourier transform can be suppressed by multiplying by the Hamming window (Ernst, Bodenhausen, and Wokaun, 1987):

$$h_T(t) = 0.54 + 0.46\cos(\pi t/T)$$

Thus, taking into account all these considerations, a derivative-mode absorption spectrum is calculated as

$$\frac{d\tilde{M}_{+}(\omega)}{d\omega} = \operatorname{Im} \int_{0}^{T} dt \, t e^{-i\omega t} h_{T}(t) e^{-t/T_{L}} e^{-t^{2}/8T_{G}^{2}} M_{+}(t).$$
(11.59)

Illustrative Examples (BD and MOMD Models) Using $B_0 = 0.34$ T, and the following values for the nitroxide magnetic tensors: $g^N = \text{diag}(2.008\,09, 2.005\,85, 2.002\,02)$ and $A^N = \text{diag}(6.2, 4.3, 36.9)$ G, the time domain spectra, as simulated by the trajectory-based approach described above for the BD model with the isotropic diffusion are shown in Figure 11.13, which also shows, for comparison, the spectra simulated by using the SLE-based approach developed by Freed and coworkers



Figure 11.13 Spectra of isotropic free diffusion for various diffusion rates in units of 10^6 s^{-1} (indicated next to each spectrum), simulated by using the trajectory-based approach (dashed lines) and the SLE

(continuous lines). The magnetic-tensor components are $g^N = \text{diag}(2.008\,09, 2.005\,85, 2.002\,02)$ and $A^N = \text{diag}(6.2, 4.3, 36.9);$ $B_0 = 0.34 \text{T}$ (Sezer, 2008a).

over motional regimes from slow ($D = 1 \times 10^6 \, {
m s}^{-1}$; correlation time $\tau = 167 \, {
m ns}$) to fast ($D = 100 \times 10^6 \,\text{s}^{-1}$; correlation time $\tau = 1.67 \,\text{ns}$). [For relating D to τ , it is noted that the correlation time τ is inversely proportional to $D(\tau = 1/(6D))$. There is excellent agreement between the trajectory-based and SLE-based approaches over the whole motional regime. In Figure 11.14 is shown the effect of the anisotropy of the diffusion tensor for both the trajectory-based and SLE-based approaches, using the same values of the nitroxide magnetic tensors as those used for Figure 11.13 and, again, the agreement between the two is excellent. It should be noted from Figure 11.14, that a fast rotational diffusion about the nitroxide *z*-axis ($D_{zz} > D_{yy} > D_{xx}$), as shown in the top spectrum in Figure 11.14, does not mix the larger A_{zz} component with the smaller components A_{xx} and A_{yy} , unlike that in the fast rotation about the x- and y-axes as seen in the bottom two spectra of Figure 11.14. In other words, the resulting spectrum is more slow-like in the former case, as compared to that for the latter two, for which the averaging of Azz is more efficient. Figure 11.15 displays the effect of an ordering potential on the spectra, using the same values of the nitroxide magnetic tensors as those used for Figure 11.13, for two cases:

• The upper spectrum in Figure 11.15 is simulated for the potential $u(\Omega) = -c_0^2 D_{00}^2(\Omega) = -c_0^2 \frac{1}{2}(3Z^2 - 1)$, which favors those orientations for which

538 11 Molecular Motions



Figure 11.14 Simulated time-domain (dashed) and frequency-domain (continuous) spectra of anisotropic-free diffusion. The components of the diffusion tensor, $10 \times 10^6 \text{ s}^{-1}$, $30 \times 10^6 \text{ s}^{-1}$, and $100 \times 10^6 \text{ s}^{-1}$,

were assigned in the order indicated in the plot. The magnetic tensors are given in the caption to Figure 11.12; $B_0 = 0.34$ T (Sezer, 2008a).





nonzero coefficient is indicated next to the spectrum. $D = 30 \times 10^{-6} \text{ s}^{-1}$. The magnetic tensors are given in the caption to Figure 11.12; $B_0 = 0.34 \text{ T}$ (Sezer, 2008a).

the *z*- and *z*'-axes are either parallel or antiparallel ($Z = \pm 1$), given by Equation 11.55 above with $c_0^2 = 2.0$.

• The lower spectrum in Figure 11.15 is simulated for the potential $u(\Omega) = -c_2^2[D_{02}^2(\Omega) + D_{0-2}^2(\Omega)] = -c_2^2 \frac{\sqrt{6}}{2}(X^2 - Y^2)$, which favors orientations in which the *z*-axis is parallel or antiparallel to $x'(X = \pm 1)$ and disfavors orienta-

Model	B ₀ (T)	stpN	∆t (ns)	freN	rst <i>N</i>	$T_G^{-1}(G)$
BD	0.34	800	1.0	1600	800	1.0
MOMD	0.34	2000	0.4	3200	1600	1.0

 Table 11.1
 Parameters used in the simulation of spectra shown in Figures 11.13–11.15.

tions in which the *z*-axis is parallel or antiparallel to $y'(Y = \pm 1)$, given by Equation 11.56 above with $c_2^2 = 2.0$.

The value of $D = 30 \times 10^6 \text{ s}^{-1}$, describing isotropic diffusion, was used for both the simulations. Again, excellent agreement was found between the trajectory-based and SLE-based approaches (see also Beth *et al.*, 2008).

Discussion of Simulation Parameters (Figures 11.13-11.15) The details of these parameters, which are listed in Table 11.1, are as follows. The duration of each trajectory is the product of "stpN" and Δt , which are, respectively, the number of simulation steps over which each stochastic trajectory lasted and the integration time step. "freN" is the number of spherical grid points used for free diffusion of M (BD) and the random distribution of D (MOMD) with respect to L, whereas "rstN" is the number of spherical grid points used for restricted diffusion of M with respect to *D* (MOMD). In the case of BD, since this restricted diffusion is not present, "rstN" indicates the number of independent trajectories initiated from each of the "freN" spherical grid points. The final column of Table 11.1 lists the value of the inhomogeneous Gaussian broadening introduced in the spectra by hand. It should be noted that the integration time step Δt (see Table 11.1) used to simulate the spectra in Figures 11.13 and 11.14 is much smaller than all but one of the correlation times mentioned above (1.67 ns) for the correlation time scales (1.67 ns to 167 ns) of the rotational diffusion. It should, then, be sufficient to follow the dynamics, except for 1.67 ns for $D = 100 \times 10^6 \text{ s}^{-1}$. However, the excellent agreement of these results in all cases with those calculated using the SLE approach shows that, even in this case, the integration time step is sufficient. In order to ensure adequate resolution of the gradient of the potential energy a smaller integration step was chosen for the two MOMD models, for simulations using MD and SLE procedures (see also DeSensi et al., 2008).

With regards to the times of computation, at least a 1000-fold longer computer time was required when using stochastic trajectories than when using SLE. Consequently, the use of trajectories is worthwhile only when the dynamics cannot be treated with the SLE approach, at which time the MD simulations should be used. Otherwise, when simulations of spectra based on BD, MOMD, and SRLS models are required, the SLE method should be the method of choice, on the basis of its greater efficiency.

Combination of MD and Stochastic Trajectories In order that the experimental spectra are realistically reproduced by simulations, it is necessary to introduce the effect of the rotational diffusive dynamics, in addition to the dynamics of the spin

labels present in the MD trajectories, to sample the slower global macromolecular dynamics, for example, the tumbling of a protein in solution. This is accomplished by allowing the coordinate system *M*, which is attached to the macromolecule, to undergo isotropic or anisotropic rotational diffusion with respect to the laboratory-fixed coordinate frame *L*, as shown below:

$$L \rightarrow \text{rotational diffusion} \rightarrow M \rightarrow \text{MD trajectories} \rightarrow N$$
 (11.60)

In this procedure, MD trajectories provide the dynamics of the coordinate frame N with respect to M, while the use of time-domain formalism developed above generates the dynamics of M with respect to L. Sezer, Freed, and Roux (2008b) illustrate the methodology to combine MD with stochastic trajectories to a spin labeled, polyanaline α -helix in explicit solvent by specifically resolving some formal issues related to the application of such a stochastic/MD trajectory-based approach, in which stochastic trajectories were used to take into account the tumbling dynamics which are slow and poorly sampled in atomistic MD simulations. Three methodological prerequisites were resolved:

- An accurate and efficient numerical scheme for propagating the quantum dynamics of the spins, achieved by working with the reduced density matrix in Hilbert space.
- An accurate and efficient numerical scheme for the treatment of rotational Brownian diffusion.
- The general case of restricted anisotropic diffusion, treated by using quaternions instead of Euler angles to parameterize the relative orientation of two coordinate systems, to which fits naturally the familiar restricting potential, written as a sum of a few spherical harmonics.

The time averaging of the magnetic tensors was also considered to bridge the gap between the fast time scale of the MD trajectories and the slow time scale of the quantum propagation. To this end, averaging time windows appropriate for the simulations of spectra at different magnetic fields were estimated.

It should be noted that, although MD and stochastic trajectories are used together, as proposed by Sezer, Freed, and Roux (2008a), the demands on the number and duration of the MD trajectories are largely unrealistic for routine MD simulations of solvated spin-labeled proteins. An alternative is to build stochastic, discrete-state Markov-chain models from the MD trajectories (Sezer *et al.*, 2008c) and then use them to simulate the EPR spectra. This follows the scheme:

 $L \rightarrow$ rotational diffusion $\rightarrow M \rightarrow$ Markov chain $\rightarrow N$.

For this model, the time-domain integrators and the time averaging arguments proposed here remain equally valid.

A comparison with, and a relevant review of, other reports on MD simulations is provided by Sezer, Freed, and Roux (2008a). For the developments in MD simulations to spin-labeled proteins, which are beyond the scope of this chapter, the reader is referred (in addition to the above citations), to the publications by Sezer, Freed, and Roux (2008a, 2008b, 2008c) and Sezer *et al.* (2009).

11.6 Concluding Remarks

As compared with CW-EPR, pulsed EPR has proven itself to be a much more powerful technique for the study molecular dynamics in a large variety of chemical, physical, and biological systems. However, whereas NMR enables the study of residual effects of motion-dependent terms – as reflected in the values of T_1 and T_2 in the spin Hamiltonian-that are completely averaged out by the molecular motion, dramatic lineshape variations are often found in CW-EPR spectra, which are particularly sensitive to the molecular motions. Moreover, these features are significantly enhanced when a multifrequency approach is adopted. Further, by using 2-D-ELDOR, molecular dynamics can be studied in much greater detail. Likewise, it is possible uniquely to resolve homogeneous from inhomogeneous broadening, and to clearly distinguish among cross-relaxation processes, in addition to determining T_1 values. As is clear from the above discussions, it is desirable to extend 2-D-ELDOR to higher frequencies in order to perform multifrequency studies that will provide even more detailed information on molecular motion than has hitherto been attained. To this end, Hofbauer et al. (2004) have developed a coherent pulsed high-power spectrometer operating at 95 GHz. It is also important to develop spin labels with a more limited flexibility and well-defined conformations, particularly with regards to the study of protein dynamics (Columbus and Hubbell, 2002). This is necessary in order to reduce the effects of the internal motions of the spin label's tether, which interferes when identifying the more relevant features of molecular dynamics from the data acquired. In recent developments involving the study of molecular motion by EPR, molecular-dynamics simulations using stochastic trajectories have been successfully applied to simulate the EPR spectra of nitroxide spin labels, an example being the analysis of the sidechain dynamics of spin-labeled proteins.

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Pertinent Literature

Many reviews have been produced on molecular motion studied with EPR, the most notable being that of Freed (2005), which has provided the bulk of the material included in this chapter. Other related reviews include those by Freed (1998, 2000, 2002) and by Borbat *et al.* (2001). The details of the solution of slow-motion EPR spectra for a nitroxide radical ($S = \frac{1}{2}$, I = 1) are described by Schneider and Freed (1989a,b), while those for an electron spin ($S = \frac{1}{2}$) coupled to two nuclear

spins with arbitrary spins are provided by Misra (2007) and the two nitroxide case is given by Zerbetto *et al.* (2007). With regards to molecular dynamics simulations, the article of Sezer, Freed, and Roux (2008a) incorporates a host of information, much of which is included in the theory presented in this chapter. The additional papers by Sezer *et al.* (2008b, 2008c, 2009) describe further how this procedure is applied to calculate EPR spectra of spin-labeled proteins from MD simulations.

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- 544 11 Molecular Motions
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