Chapter 9

ESR and Molecular Dynamics

Jack H. Freed

Department of Chemistry, and Chemical Biology, Baker Laborator, Cornell University, Ithaca, New York 14853-1301

Abstract: The development of ESR for the study of spin-relaxation and molecular dynamics of organic radicals and spin labels in fluids is reviewed from a historical perspective.

1.   MOTIONAL NARROWING AND ORGANIC RADICALS

My interest in electron spin relaxation and molecular dynamics began when I was a graduate student with George Fraenkel at Columbia University from 1958-1962. His laboratory was teeming with interest and activity in the area of spin-relaxation, mainly of organic free radicals in liquid solution. Fraenkel had developed a new theory (Stephen and Fraenkel, 1960) which could successfully account for the fact that the measured T_1's from each hyperfine line of semiquinone spectra obtained in his lab were different in magnitude (Schreurs and Fraenkel 1961). Kivelson was completing his theory of unsaturated linewidths, (Kivelson, 1960) that he developed from the seminal Kubo and Tomita theory of lineshapes, (Kubo and Tomita 1954). The Stephen-Fraenkel theory deriving more from the Wangness-Bloch (1953) and Redfield (1957) theories, (more commonly known as Redfield theory today) also incorporated components of Kubo and Tomita theory. In fact, the Stephen-Fraenkel theory of ESR (electron-spin resonance) saturation and the Kivelson theory of unsaturated linewidths were complementary in the insight and understanding they provided into spin-relaxation of organic radicals in solution.
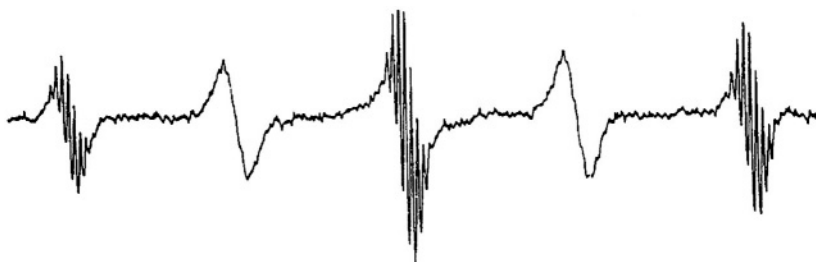
Figure 1. ESR spectrum of *p*-dinitrotetramethylbenzene radical at 20°C showing the alternating linewidth effect. (Magnetic field increases to the right). From Freed and Fraenkel (1962).

In Fraenkel's lab my interest was piqued by the "anomalous alternating linewidth" effect. The electrochemically generated ESR spectrum of the p-dinitrotetramethylbenzene anion showed features that had not been seen before: well-resolved proton shfs appeared on the 1st, 3rd, and 5th lines of the hf splitting from the two equivalent ^{14}N nuclei; but the 2nd and 4th lines were so broad that the proton shfs was completely masked (see Fig. 1). Neither the Kivelson theory of linewidths nor the Stephen-Fraenkel theory of spin-relaxation could explain such a phenomenon. I found that the problem with the earlier theories rested in their improper treatment of multiple or degenerate hf lines which are commonplace for organic radicals. A simple extension of the Kubo-Tomita theory led to the viewpoint that such a multiple hf line must be an "average Lorentzian". I was able to show rigorously from the Redfield theory, that such a multiple hf line must, in general, be a superposition of Lorentzians. For the specific case of the alternating-linewidths of p-dinitrotetramethylbenzene, a particular molecular motional model was needed to complete the explanation. The new theory required out-of-phase correlation of the two ^{14}N hfs, which are assumed to be fluctuating in time, (Freed and Fraenkel, 1962). (One such model would be a rotation of one nitro-group into the benzene plane, thereby increasing its spin-density, while the other is forced to rotate out of the plane, thereby decreasing its spin density, possibly assisted by counterion motions). Such a process would broaden all the hf components except for those arising from nuclear spin configurations in which the two ^{14}N nuclear spin quantum numbers were equal. Work by Bolton and Carrington (1962) at that time on alternating linewidths in durosemiquinone using modified Bloch equations was also consistent with this analysis.

As is often the case in science, the resolution of an "anomaly" led to the formulation of a more generally inclusive theory, in this case the theory of linewidths for organic free radicals. It is, with pleasure, that I note this

theory, published in 1963, (Freed and Fraenkel, 1963) is still accepted today as valid for spectra in the motional narrowing regime. The important improvements to the Freed-Fraenkel theory since then have largely to do with the incorporation of more precise and detailed models of the molecular dynamics into the formulation. One important example of this, was the incorporation of Perrin's model (Perrin, 1934) of anisotropic rotational diffusion into the linewidth theory, and its illustration by reinterpreting a linewidth study on p-dinitrobenzene, (Freed, 1964). This work made clear the utility of ESR for the study of molecular dynamics in liquids.

Another improvement was Fraenkel's (1965) extension of the linewidth theory to include the effects of dynamic frequency shifts, which accompany the fast motional linewidths but are smaller except in special cases, such as for the alternating linewidth effect.

2. DOUBLE RESONANCE AND MOLECULAR DYNAMICS

In 1964, Jim Hyde and Gus Maki first observed ENDOR for organic radicals in liquids, (Hyde and Maki, 1964) (Note ENDOR stands for electron-nuclear double resonance, wherein the ESR signal is partially saturated and the nuclear spins on the radical are irradiated at their NMR frequency). At that time there was no theory for explaining why ENDOR can occur in liquids, and the reason for their successful observation was a mystery. Did it involve spin relaxation and therefore would be relevant for studies of molecular dynamics? Thus it seemed appropriate at that time to undertake a reformulation and generalization of the theory of ESR saturation by analogy to what Fraenkel and I had done with ESR linewidths, and to see if a complete theory would allow for a satisfactory explanation of the Hyde-Maki experiment. This ultimately led to a very general theory of ESR saturation and double resonance which appeared in 1965, (Freed, 1965). This theory showed that any ENDOR effects must be small. [At that time, Jim Hyde came to Cornell to give a lecture. I explained to Jim how I had looked everywhere in "spin-relaxation space", and I still couldn't find effects of more than about a percent. Jim promptly assured me that was about the magnitude of the effects he was seeing! By chopping the NMR frequency, and detecting at the chopping frequency, Jim could get just the difference signal due to the ENDOR]. This formulation and its later extensions have served as the basis of interpreting ESR saturation and ENDOR experiments for motionally-narrowed spectra up to today, (Dorio and Freed, 1979; Kurreck et al, 1988; Möbius et al, 1989).

At Cornell we quantitatively tested the ENDOR theory on several semi-quinones, (Leniart et al, 1975). We showed that aside from explaining the ENDOR enhancements in terms of the spin-relaxation processes and molecular dynamics, a very important feature was the observed linewidths for the NMR transitions of the free radicals. They very nicely complement the information obtained from the ESR linewidths. This is most important for the case of concentration-dependent linewidths. The sources are Heisenberg spin exchange and electron-electron dipolar interactions between colliding radicals. Their very similar effects on ESR linewidths make it very difficult to separate them out, but it is important to do so in order to utilize these interactions to study microscopic molecular diffusion in liquids. However, these two mechanisms have very different relative effects on the NMR vs. the ESR linewidths, so we were able to successfully separate them from our ENDOR studies.

Jim Hyde and I collaborated on aspects of ENDOR. However, our most important collaboration was undoubtedly the development of ELDOR (electron-electron double resonance) in liquids, (Hyde et al, 1968). This came about during a visit to Varian in the spring of 1967, when Jim showed me the very exciting ELDOR spectra he and Jimmy Chien had obtained with a bimodal cavity that Jim had developed. Using the saturation and double resonance theory that I had developed, I could come up with the appropriate theory for the ELDOR experiment. ELDOR, today, has taken on many new configurations, but in all of them it serves as a powerful means of studying spin-relaxation for purposes of exploring both rotational and translational motions in liquids.

3.   SLOW MOTIONAL ESR AND MOLECULAR DYNAMICS

With the theory of ESR linewidths, spin relaxation, saturation, and double resonance well-established by the mid to late 1960's, it could have appeared that all that remained was to apply it to a wide variety of experiments to study molecular dynamics. However, I personally was concerned with some of the theoretical foundations of Wangness-Bloch-Redfield and Kubo-Tomita theory. It appeared to work so well in the motional narrowing region, but it was essentially a perturbation theory, which was ill-defined in the sense that one kept the first non-trivial term in a perturbation expansion, yet there was no way of generating the higher order terms in the expansion. The question remained how to extend Redfield theory to all orders in a systematic fashion as motional rates slow. Using Kubo's method of generalized cumulant expansions in the methodology of

statistical mechanics, (Kubo, 1962, 1963), I was able to provide a formal, general solution. Ordinary cumulant expansions arise in probability theory when one examines statistical averages of exponential functions. Spin relaxation, however, involves ensemble averages of exponentiated spin Hamiltonian super-operators, so one must generalize the conventional probability techniques. Thus, it proved possible to utilize Kubo's generalized cumulant method to develop general theorems about spin-relaxation. For example, one could in principle, calculate the relaxation matrix to all orders in perturbation theory, and I could show that the nth order term involved a particular form of the nth order time correlation function of the molecular motion treated as a random function. Relaxation theory is a long-time limiting theory, but the generalized cumulant method even allowed one to get the finite time corrections. This yielded a general formulation of relaxation theory valid to all orders, (Freed, 1968). Redfield theory provides just the leading or 2nd order term. However, generalized cumulant expansions suffer very severely from the problems of perturbation expansions, viz. each higher order term is much harder to compute than the previous term.

The slow motional ESR problem becomes important when one studies spin-labeled macromolecules, as McConnell was doing at that time. Other efforts on this problem, included that of Kivelson, who had another method that also supplied perturbative corrections to Redfield theory, (Sillescu and Kivelson, 1968), as well as the early work of Korst and Khazanovitch (1964), who had solved the slow-motional problem for the simple case of only a secular perturbation, i.e. a perturbation term in the spin-Hamiltonian that commutes with the main term. With all its complexities, at least the generalized cumulant theory was appropriate for all types of perturbation and all types of motions.

In 1969, I spent a sabbatic with Kubo in Tokyo. He called my attention to two of his papers, wherein he had congealed, in a largely heuristic sense, his ideas on stochastic Liouville equations, (Kubo, 1969a,b). I realized how I could incorporate an approach based on the stochastic Liouville equation (SLE) to develop a complete analysis of the ESR slow-motional problem. In order to use the SLE one had to assume that the motional dynamics could be described statistically by a Markov process. More serious in practical terms was the need to take the matrix representations of the SLE and solve for the ESR spectrum on a computer, which could not be done in Tokyo at that time and had to be done at Cornell. The theory was worked out for all relevant cases of g-tensor and hyperfine anisotropy, and it was extended to include saturation phenomena, (Freed et al, 1971a).
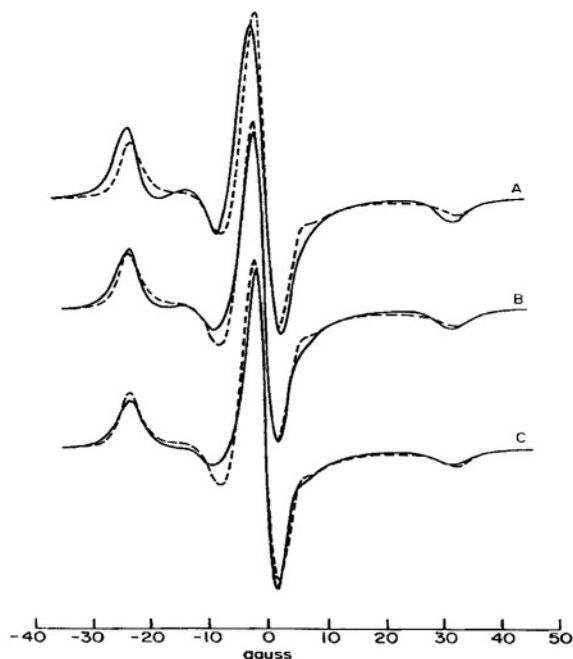
Figure 2. Slow motional ESR spectrum from peroxylamine disulfonate anion in frozen D_2O at T = -60°C. The dashed line is the experimental spectrum, and the solid lines are calculated for a particular microscopic model of rotational reorientation but with differing anisotropy of the rotational diffusion tensor: A) isotropic; B) moderate anisotropy; C) larger anisotropy. From Goldman et al (1972).

At Cornell, we successfully completed experiments using peroxylamine-disulfonate (PADS) in ice. These gave lovely slow-motional spectra with none of the inhomogeneous broadening of typical spin-labels (see Fig. 2). An important discovery was that we could fit these spectra significantly better if we assumed jump-type reorientations rather than simple Brownian motion, (Goldman et al, 1972). Thus, the slow motional spectra were proving to be more sensitive to the microscopic molecular dynamics than are the fast motional spectra. It was also possible to extend the SLE approach to a complete solution of slow-tumbling triplets, (Freed et al, 1971b) generalizing earlier work of Norris and Weissman (1969). In addition both Gordon and Lynden-Bell made early contributions to the slow motional problem, (Gordon and Messenger, 1972).

Work continued on slow-motional ESR through the 1970's utilizing the spectra to obtain new insights into molecular rotational motions in ordinary isotropic fluids, in liquid crystals, and in model membranes, (Hwang et al, 1975; Polnaszek and Freed, 1975). The SLE approach was also utilized to

provide a quantitative theory for the then new phenomena of chemically-induced dynamic spin polarization: CIDEP and CIDNP (Freed and Pederson, 1976). The great challenge was in carrying out the rather tedious slow-motional simulations.

Also, in that period, Dalton and Robinson, (Hyde and Dalton, 1979; Beth and Robinson, 1989) managed to employ the SLE to provide a theory for Hyde's new saturation transfer technique, which is useful for studying very slow motions.

During that period it was also possible to improve on the formulation of the SLE, to study more thoroughly its range of validity, and to extend its range of applicability. Nevertheless, these initial efforts have successfully withstood the tests of time. Clearly, the most important accomplishment was the development in the 1980's of a very efficient method of computing solutions to the SLE that drastically reduced the computation time and storage requirements and ultimately led to versions that could be made generally available, (Moro and Freed, 1981).

Working with Giorgio Moro, we uncovered material about the Method of Moments. This is a formal procedure for projecting out a sub-space, known as a Krylov space, starting from a real symmetric or Hermitian matrix and an initial vector, each of dimension n. This sub-space will, in general, be of dimension m lower than n (i.e. m < n), and in the representation of the basis vectors obtained, the m-dimensional approximation to the original matrix will be in tridiagonal form. The practical implementation for computation involved a specific algorithm, known as the Lanczos Algorithm (LA), which, however was known to suffer badly from computer round-off error. Another problem confronted us in that the LA had been developed for real-symmetric or Hermitian matrices, but not for the complex symmetric matrices (which are non-Hermitian) that one generates with the SLE. The available theorems no longer necessarily applied, including a guarantee that the matrix is diagonalizable. We were not especially troubled by the latter fact, since we could diagonalize SLE matrices by standard, but slow, methods. However, the complex symmetric arithmetic could (and does) increase the problem of computer round-off error.

Despite these concerns, we found the LA succeeded admirably for numerically solving the SLE, (Moro and Freed, 1981). It reduced computation time by at least an order of magnitude, and it also greatly reduced storage requirements. Why does the LA work so well? First of all, it takes full advantage of the sparsity of the SLE matrix. Secondly, after just a few Lanczos projections it produces a sub-space that very effectively includes what is important for the ESR experiments. This is partly because the initial vector is a kind of statement of the physics of the ESR experiment. It essentially represents the ESR transition moments. Thirdly, the ESR

experiment is dominated by the slowly decaying eigenvalues of the SLE, and these are accurately obtained from the small sub-space approximation. But what about the eigenvalues that are poorly represented? They are automatically projected out of the solution when one calculates the specific ESR observable, viz. the lineshape. And what about the round-off error? Since we only need a small sub-space generated by a relatively small number of Lanczos projections, the calculation is terminated before the round-off error becomes serious. Important for the execution of these programs, (Schneider and Freed 1989a) are very powerful methods we developed for selecting the minimum basis set to represent the SLE, and for reliably determining when sufficient Lanczos projections have been utilized, (Schneider and Freed, 1989b).

Thus the Lanczos algorithm provides both a conceptually insightful approach as well as an extremely powerful computational algorithm for the SLE. It has since been possible to show the connection of the LA with other mathematical methodologies, but none other lends itself so effectively to a computational algorithm, (Schneider and Freed, 1989b). The most instructive connection is that to the Mori method, well-known in statistical mechanics.

4. HIGH FIELD ESR AND MOLECULAR DYNAMICS

With the advent of high field far-infrared (FIR) ESR, with its enhanced resolution to motional dynamics, we have found it desirable to use enhanced models for molecular reorientation to fit these spectra. We can now dispense with the old and worn jump models of diffusion. Instead we approximate the many-body problem of dealing with the microscopic details of the fluid by a set of collective degrees of freedom that represent the main effects of the solvent on a rotating solute. These collective variables are taken as a loose solvent "cage" that is slowly relaxing. The solute is then reorienting more rapidly in this cage. This is called the slowly relaxing local structure (SRLS) model. Since we approximate the combined system of solute plus cage by Markovian equations, the SLE remains valid in this augmented form. Then the Lanczos projections effectively determine the extent to which the cage variables are needed to interpret the ESR spectrum, (Polimeno and Freed, 1995).

In addition to the greater sensitivity of FIR-ESR (e.g. 250 GHz) to the details of the molecular motions in fluids, another virtue of FIR-ESR (e.g. 250 GHz) over ESR at conventional microwave frequencies is the excellent orientational resolution it provides for studies utilizing nitroxide spin labels

(Budil et al, 1989; Earle et al, 1993, 1997, 1998). As a result, at 250 GHz, once motion is discernible in the spectrum, one can discern about which axis (or axes) the motion occurs, (Earle et al, 1993).
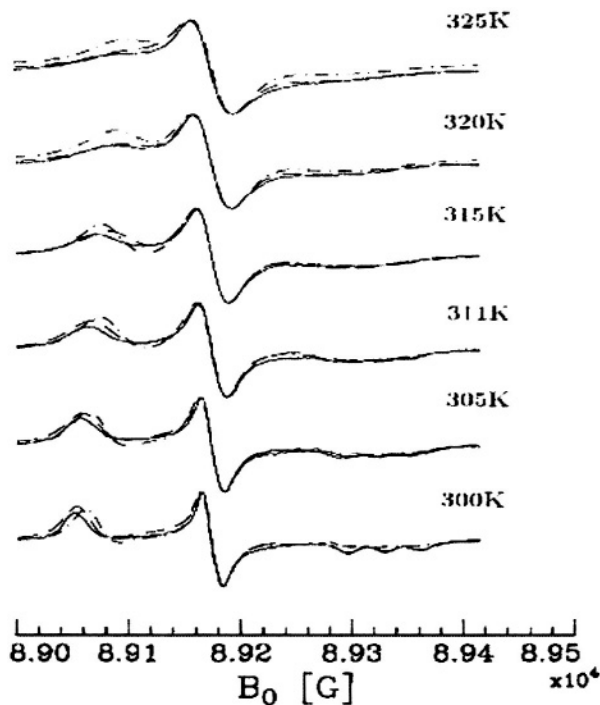

Figure 3. Comparison of two models for fitting effects of rotational diffusion on 250 GHz electron spin resonance spectra of spin probe of a cholesterol-like nitroxide (CSL) in ortho-terphenyl solvent. *(Solid line)* Experiment, *(dashed line)* the SRLS model, and *(dashed-dotted line)* simple Brownian diffusion (Earle et al, 1997).

In a 250-GHz ESR study of the dynamics of several nitroxide spin probes dissolved in the glass-forming solvent ortho-terphenyl (OTP), we demonstrated how the enhanced sensitivity to rotational dynamics of the slow-motional spectra could be utilized to explore details of the dynamic solvent cage, (Earle et al, 1997). The SRLS model adequately fits the model-sensitive regions of the 250-GHz spectra (cf. Figure 3) and leads to a coherent picture of the dynamics: The rotational diffusion tensors of the various probes exhibit simple behavior such that the smaller the probe is the larger the diffusion coefficient. The cage relaxation rate is the slowest, but it is independent of the particular probe. This interesting observation appears reasonable when one considers that the cage relaxation involves just the movement of the OTP solvent molecules. In addition, the magnitude and

directionality of the cage-orienting potential could be obtained. As expected, only probes comparable to or larger than the OTP molecules experience substantial potentials, of 2-4 kT. It was possible to show that the nonlinear way in which the dynamics affects the slow-motional ESR spectra allows one to distinguish between two limiting cases. The first is that of a homogeneous liquid, but with a complex motional dynamics, (e.g. the SRLS model that was used). The second is that of an inhomogeneous liquid with a distribution of simple relaxation times (e.g. Brownian tumbling). The latter was shown to be incompatible with the 250-GHz spectra.
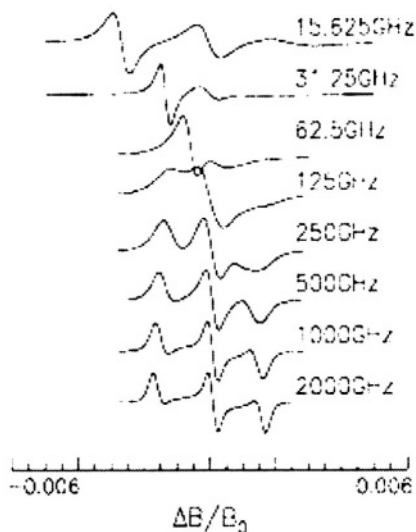

Figure 4. Simulation of derivative electron spin resonance spectra for a nitroxide, reorienting with a rotational diffusion coefficient $R = 10^8$ s^{-1} (corresponding to rotational correlation time $\tau_R = 1.67$ ns) for a wide range of frequencies.

Another virtue of FIR ESR is the fact that the higher the ESR frequency, the slower the motion appears to be for a given diffusion rate. This is illustrated in Figure 4, where I show simulated spectra corresponding to the same motional rate but for different ESR frequencies, ranging from 15 GHz to 2 THz. At the low frequency end, one observes simple motionally narrowed spectra, whereas at the high frequency end, the spectra are very slow motional, almost at the rigid limit. Thus we see that the higher-frequency ESR spectra act as a faster "snapshot" of the dynamic, (Earle et al, 1993, 1997). This is because of the increased role of the g-tensor term, which is linear in magnetic field, B_o in the spin-Hamiltonian. As the orientation-dependent part of the spin-Hamiltonian, $H_1(\Omega)$ increases in magnitude with increasing frequency, ω_o and B_o , the motional-narrowing

condition $\left|H_1(\Omega)\right|^2 \tau_R^2 \ll 1$ fails, (where τ_R is the rotational relaxation time) and the spectra become slow motional.

This snapshot feature suggests a multifrequency ESR approach to the study of the dynamics of complex fluids, such as glass-forming fluids and liquid crystals, as well as to the complex modes of motion of proteins and DNA, which should enable one to decompose the different modes according to their different timescales (Liang and Freed, 1999). For example, in the case of proteins, the higher frequency ESR spectra should "freeze-out" the slow overall tumbling motions, leaving only the faster internal modes of motion, whereas ESR performed at lower frequencies is sensitive to the motions on a slower timescale. In glass-forming fluids, as we have seen, the faster motions consist of reorientations of probe molecules, whereas the slower motions relate to the dynamics of the solvent cage.

Figure 5. (left) Protein Dynamics of Spin-labeled Protein: There are three kind of motions, spin-label reorientation, side chain fluctuations and global tumbling. (right) The SRLS model is illustrated including relevant motional parameters (Liang and Freed, 1999; Liang et al, 2000).

The virtues of such a multifrequency approach were demonstrated in a study, using 9- and 250-GHz spectrometers, on spin-labeled mutants of the soluble protein T4 lysozyme in aqueous solution (Barnes et al, 1999). In the fast timescale of the 250-GHz ESR experiment, the overall rotation was too slow to significantly affect the spectrum, so that it could satisfactorily be

described by the simple MOMD (microscopic order but macroscopic
disorder) model (Meirovitch et al 1984), wherein the overall motion is so
slow that it corresponds to the rigid limit (see below), which yielded good
spectral resolution for the internal dynamics. Then, by fixing the internal
motional parameters at the values obtained from the 250-GHz data, the
SRLS fits to the 9-GHz line shapes successfully yielded the rates for the
global dynamics. Thus the two types of motion were separated, and spectral
resolution to these motions was significantly enhanced. The SRLS model as
it applies to protein dynamics is shown in Figure 5.
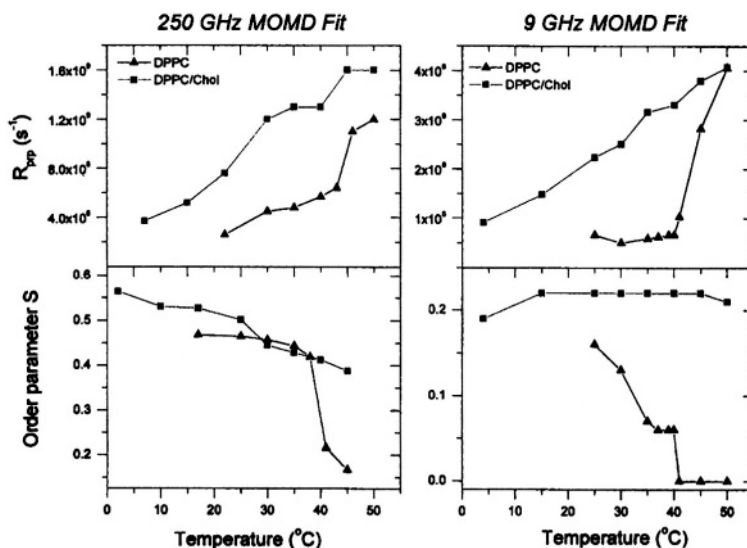
Figure 6. Rotational Diffusion rates and Order parameters of 16PC in lipid membranes (with
and without cholesterol) from 250 GHz and 9 GHz ESR spectra obtained from their
respective MOMD fits (Lou et al, 2001). At 250 GHz these reflect just the internal motions; at
9 GHz they are a composite of internal and overall motions.

This same multifrequency approach was applied to a study of the
dynamic structure of model membranes using an end-chain labeled lipid,
(Lou et al, 2001). It was found that the results at 250 GHz could be
interpreted in terms of the MOMD model relating to just the internal
dynamics and ordering of the ends of the acyl-chains, with the slower overall
lipid dynamics frozen-out on the time-scale of the 250 GHz experiment, (cf
Figure 6). The 9 GHz spectra, however, are affected by both the internal and
overall motions, so they were analyzed in terms of the SRLS model, which
explicitly includes both types of motion, using the parameters for the internal
dynamics obtained from the analysis of the 250 GHz spectra. It is worth
noting, however, that if the 250 GHz spectra are ignored, then the 9 GHz
spectra, with their limited resolution to dynamics, could be fit to a simple

MOMD model, but the dynamic and ordering parameters obtained must be interpreted as a composite of both the internal and overall motions, (cf Figure 6) with no obvious way of separating them.
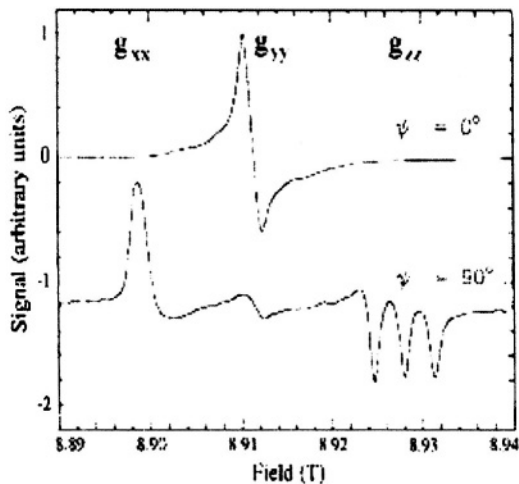

Figure 7. 250-GHz derivative electron spin resonance spectra from cholesterol-like nitroxide in aligned PC-rich membrane with the membrane normal parallel ($\Psi = 0°$) and perpendicular ($\Psi = 90°$) to the magnetic field, (Barnes and Freed, 1998).

A striking demonstration of the excellent orientational resolution at 250 GHz in studies utilizing nitroxide spin labels was provided by a study on macroscopically 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 further enhanced the orientational resolution at 250 GHz and permitted an orientation-dependent study, (cf Figure 7).

5.   SPIN-ECHOES AND MOLECULAR DYNAMICS

A major weakness of cw ESR for relaxation studies is the problem of extracting reliable homogeneous line broadening from inhomogeneously broadened ESR spectra such as from nitroxide spin labels. This homogeneous line broadening is the contribution to the linewidth that arises from the motional modulation of the hyperfine and g-tensors as well as the other spin-relaxation processes. It is obscured by the inhomogeneous broadening, which is due, for example, to the unresolved proton superhyperfine interactions. This was a particular problem for our work on

macroscopically aligned samples of liquid crystals, because small amounts of misalignment could appear as extra inhomogeneous broadening, which varies for the different ^{14}N hf lines, thereby being easily mistaken for the homogeneous broadening with its well-known variation with hf line. However, by means of electron-spin echoes, one can cancel inhomogeneous broadening and obtain the homogeneous widths, which are the inverse of T_2.
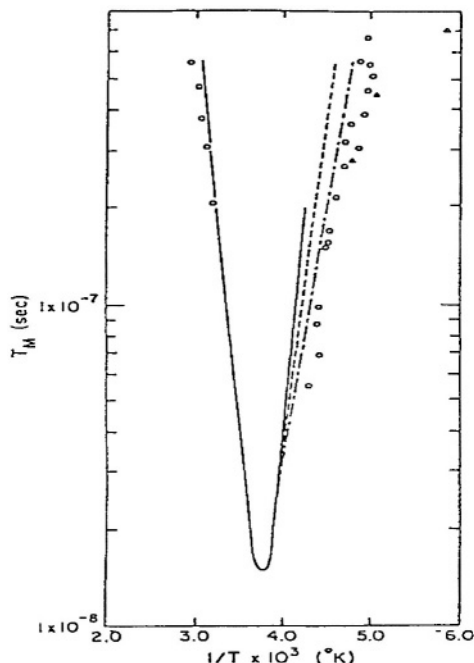

Figure 8. A graph of T_2 (or T_M) vs. inverse temperature for the spin probe tempone in 85% glycerol/$H_2$0. Experimental data are shown as circles (from Stillman et al, 1980) and triangles (from Millhauser and Freed, 1984). The lines show predictions for T_2 for Brownian and Jump models. Today with much improved spectrometers (Borbat et al, 1997; Freed, 2000) it is now possible to cover the whole range of T_2 including the T_2 minimum of ca. 14 ns.

After constructing an electron-spin echo (ESE) spectrometer, we conducted initial ESR experiments on fluids. In particular, we were able to explore T_2's for fast through slow motions for the system of PD-Tempone in glycerol-water solvent (see Fig. 8), (Stillman et al 1980). For fast motion, T_2 has the well-known inverse dependence on correlation time, but for slow motion the homogeneous T_2 depends on the correlation time to a positive, usually fractional, power. Thus there is a T_2 minimum not generally appreciated (but also observed by Ian Brown, 1974). We were able to offer a coherent explanation in terms of the SLE, and this led to a comprehensive theory for spin-relaxation and ESE, (Schwartz et al, 1982). What then is the

interpretation for T_2 in the slow-motional regime? In the limit of strong jump reorientation each jump leads to a large change in resonant frequency thereby leading to an uncertainty in lifetime broadening. Thus, in this limit, T_2 equals the correlation time (as noted earlier by Mason and myself in Mason and Freed, 1974). In the limit of simple Brownian motion, T_2 is roughly proportional to the half power of the correlation time. A heuristic interpretation of this is due to Kivelson and Lee, (1982).

A disturbing limitation of ESR measurements of T_2 is that one just obtains a single parameter from which to extract information on motional dynamics. Of course, in the fast motional regime one may study the variation of T_2 with hf line. For the slow motional regime, initial theory and experience did show that the measured T_2 displays some variation across the spectrum. It seemed reasonable to suppose that by studying this variation, sufficient information could be obtained from which to infer details of motional models. This would be analogous to studying the full lineshape of a slow-motional ESR experiment, but it would have the big advantage that the homogeneous T_2 relates solely to the dynamical processes. This advantage is, however, limited by the fact that when molecular motions are slow enough, then solid-state relaxation processes, such as spin diffusion, take over. However, one can explore slower processes by studying the homogeneous T_2 rather than the near-rigid-limit cw-ESR spectra.

Initial ESE experiments of this type were performed by sweeping the magnetic field and collecting the spin echo from weak, or highly selective, microwave pulses, (Millhauser and Freed, 1984). When Fourier-Transformed in the echo delay time, τ this led to a 2D-ESR spectrum in which the homogeneous lineshape is plotted along the frequency axis, and essentially the ESR lineshape appears along the field axis (see Fig. 9). This 2D spectrum thus effectively supplies the homogeneous T_2 variation across the ESR spectrum. For the case of Tempone in glycerol-water, we found a substantial variation in the T_2. Our theoretical analysis showed that a Brownian reorientational model could quite successfully explain this variation. This is because, given the $\cos^2\theta$ type of angular dependence of the hf and g-tensor interaction terms, the variation in these terms with a small change in angle, θ depends significantly on the value of θ, hence on the position in the very slow-motional spectrum. On the other hand, a strong jump diffusion model leads to a uniform T_2 across the spectrum, since for this case $T_2 = \tau_R$, the mean rotational jump time, as already mentioned. In fact, we found that the patterns of T_2 variation across the spectrum, plotted in a normalized contour fashion could themselves be utilized to distinguish the model of motion, and the degree of rotational anisotropy (see Fig. 9). This method was then extended to spin labels in oriented model membranes and to labeled proteins, to slow motions on surfaces.
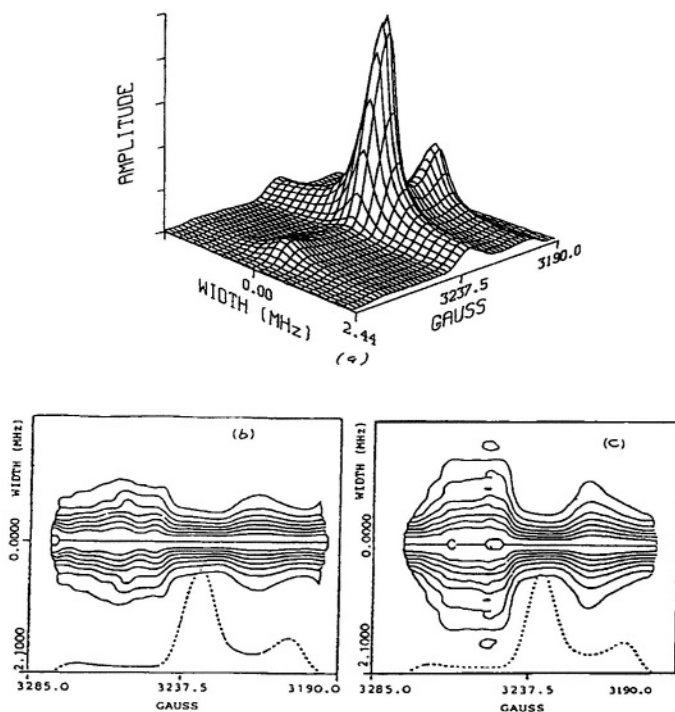
Figure 9. Fig. 9a shows the 2D-ESE spectrum of tempone in 85% glycerol/H_2O at -75°C. Slices along the width axis provide the homogeneous lineshape for the different magnetic field positions of the ESR spectrum. Fig. 9b shows the normalized contours for Fig. 9a as well as the spectral slice from Fig. 9a taken along the width = 0 MHz axis. Fig. 9c provides the analogous contours for cholestane in n-butylbenzene at -135°C. These show the different contour patterns from the nearly spherical tempone vs. that from the cigar-shaped cholestane. From Millhauser and Freed (1986).

Next, a field-swept 2D-ESE experiment, from which one obtains the magnetization transfer rates across the ESR spectrum, was performed in a manner analogous to the T_2-type 2D-ESE experiment, except that a stimulated echo sequence: $\pi/2$ - $\pi/2$ - $\pi/2$, replaces the spin-echo sequence $\pi/2$ - π, and one steps out the time T between the second and third pulses, (Schwartz et al, 1986). Here theory showed that as a function of T there are at least two exponential decays: one is in T_1 and the second (to a reasonable approximation) is in T_A. an effective magnetization transfer time (for the relevant case of $T_A \ll T_1$). The slow rotational reorientations shift spin-bearing molecules irradiated by the first two $\pi/2$ pulses to frequencies outside the irradiated region. Thus they are not detected after the third $\pi/2$ pulse. This magnetization transfer process, thus leads to a more rapid decay of the stimulated echo as a function of T. A Brownian rotation model will

also give a T_A variation across the spectrum, because the effectiveness of rotation taking the spins out of the irradiated region depends upon angle, θ through the $\cos^2\theta$ dependence of the magnetic tensor terms. We obtained dramatic variation of T_A across the spectrum for NO_2 adsorbed on crushed vycor, which could be attributed to very anisotropic rotational motion on the surface. For this case there is an enhanced T_A for the spectral regimes corresponding to x and z molecular axes being parallel to the magnetic field, which clearly implies more rapid rotation about the y-axis (which is parallel to the line connecting the two Oxygen atoms). This motional anisotropy is clearly visible from the 2D-contours without the need for detailed spectral analysis (see Fig. 10).
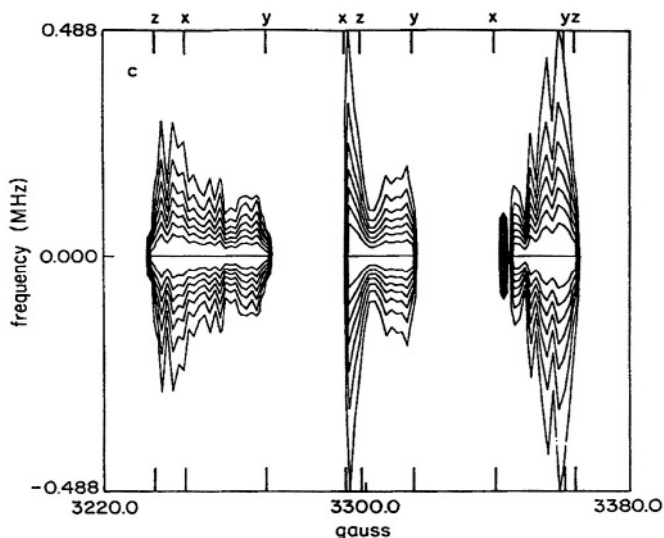

Figure 10. 2D-ESE contours from the stimulated echo sequence for NO_2 adsorbed on vycor at 35°K showing rates of magnetization transfer. It shows relatively rapid rotation about the molecular y axis (i.e. the axis parallel to the oxygen-oxygen internuclear vector). From Schwartz et al (1986).

Both longitudinal and cross-relaxation in liquids were included in the comprehensive theory of spin relaxation in ESE for fast and slow motions, (Schwartz, 1984; Schwartz et al, 1986). A major motivation for the analysis of cross-relaxation was the spin-echo ELDOR experiment we performed in the very viscous regime for PD-Tempone in glycerol/water, (Hornak and Freed, 1983). Instead of using two microwave frequencies, the magnetic field was stepped out during the time between the first inverting π pulse and the detecting $\pi/2$ - π spin echo sequence. [This technique had been independently developed by Tsvetkov and co-workers (Dzuba et al, 1984)]. Using the theory we showed that a substantial orientation-independent

nuclear-spin-flip rate could explain the ELDOR experiment, (Schwartz, 1984; Schwartz et al, 1986).

Clearly the most informative method of studying magnetization transfer is by ELDOR. One observes not only the transitions out of a certain spectral region but also the spectral region to which the transition is made. This was the basic idea of the stepped field spin-echo ELDOR experiment. One could attempt, by a combination of sweeping one or both frequencies of an ELDOR experiment and/or sweeping the field and the field jump, to perform a 2D experiment as a function of the pumping and observing frequencies. (Tsvetkov and his co-workers did, in fact, develop the use of two microwave sources, wherein they swept one of them, (Dzuba and Tsvetkov, 1988). This requires a resonator with a low enough Q that it could sustain two separated frequencies. But once this is the case, another more general and more elegant method suggests itself, which removes the need for field sweeping and stepping, and it only requires one microwave source. It does require collection of the free-induction decay or the echo decay after the last pulse, but it could be obtained very rapidly.

6.   TWO-DIMENSIONAL FOURIER TRANSFORM ESR

In 1976 Richard Ernst and co-workers published the first 2D-NMR experiments, (Aue et al, 1976) that used Fourier Transform (FT) methods with their multiplex advantage for collecting the whole spectrum simultaneously. This also means the successful irradiation of the whole spectrum with a single non-selective rf pulse, and the ability to collect data shortly after such a pulse. Further, the non-selective pulse from a single rf source introduces coherence simultaneously to all spectral components enabling the observation of coherence transfer between these components. In 1979, Ernst and Jeener showed how magnetization transfer could also be studied in this manner, (Jeener et al, 1979).

Why were these ideas not incorporated into ESR until 1986? Clearly the ESR experiment is much more difficult than the comparable NMR one. In ESR we use microwave rather than rf technology. Relaxation times are orders of magnitude faster, pulse widths need to be orders of magnitude shorter, and spectral bandwidths are orders of magnitude wider.

Clearly, it was necessary to develop modern FT techniques in ESR as a prerequisite to developing the ESR analogues to 2D-NMR. Modern FT techniques appeared almost the same time in Bowman's lab in Argonne, (Angerhofer et al, 1988) Dinse's lab in Dortmund, Germany, (Dobbert et al, 1986) Lebedev's lab in Moscow, (Panferov et al, 1984) and my own lab in

the 1984-1986 period. My motivation was partly the hope of performing modern 2D-FT-ESR experiments and partly the hope of studying the spectra from transient radicals. In fact, in 1984, we succeeded in obtaining the free induction decay (FID) of a transient photogenerated electron from Rb/THF solutions, thereby distinguishing its spectrum from the stable solvated electron, (Eliav et al, 1984). In another experiment, we showed that the B_1 microwave fields in the rotating frame need not be much larger than the spectral bandwidth to obtain reasonable coverage in an FT-ESR experiment, (Hornak and Freed, 1986). One merely has to accept a rotation of the spins into the rotating x'-y' plane instead of precisely along the x' axis (for a B_1, along the y' axis). Then quadrature detection plus standard phase corrections yielded the pure absorption from the FID. Also we showed the advantages of utilizing a loop-gap resonator which can supply large B_1 fields, but with low Q's to reduce resonator ringing and thereby spectrometer dead time after the pulse.

After introducing a digitizing oscilloscope and a home-built quadrature detector, we were able to obtain good FID's and FT spectra from fast motional nitroxides with a total spectral width of 90 MHz, and this immediately led to the first two-dimensional FT-ESR experiments on the fast motional nitroxide system. They consisted of a 2D-ESE experiment, appropriately called a SECSY (spin-echo correlated spectroscopy) experiment and an FID-based 2D-exchange experiment, which we now call 2D-ELDOR, (Gorcester and Freed, 1986). This first FT-based 2D-ELDOR experiment showed cross-peak development resulting from Heisenberg spin-exchange (see Fig. 11). The SECSY experiment showed how the homogeneous T_2 values from all the hf lines could be obtained simultaneously from an inhomogeneously broadened ESR signal. Thus 2D-FT-ESR became a reality.

There were still a number of major challenges to make 2D-FT-ESR generally applicable. Sophisticated phase cycling was added and a full theoretical analysis for the fast motional 2D spectra in terms of how Heisenberg exchange (HE) and electron-nuclear dipolar (END) terms generate the cross peaks, was developed. We showed how their respective contributions could be readily distinguished. This led to quantitative measurements of HE in an isotropic fluid, (Gorcester and Freed, 1988) and of END terms in a liquid crystal, (Gorcester et al, 1989). This latter study could be utilized to provide sophisticated insights on molecular dynamics in ordered fluids in a way that cw-ESR linewidths could not. Also, Bowman showed how 2D-ELDOR could be used to measure rates of chemical exchange in a semi-quinone system, (Angerhofer et al, 1988).
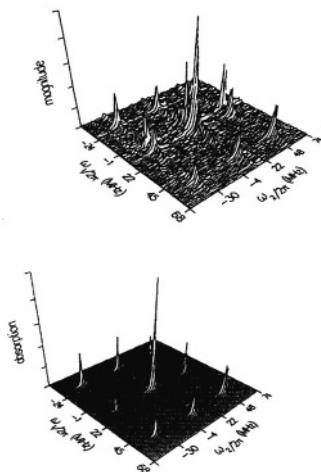
Figure 11. 2D- ELDOR spectrum of solution of PD-Tempone in **toluene-d₈** at 21°C, a) raw data; b) after analysis by linear predictive methods. The cross-peaks are due to Heisenberg spin exchange. From Gorcester and Freed, (1988).

With additional improvements to the 2D-FT-ESR spectrometer, which increased the spectral coverage to about 250 MHz, and greatly increased our data acquisition rates and significantly reduced spectrometer dead-times, it became possible to extend 2D-FT-ESR to the slow motional regime, (Patyal et al, 1990). These developments necessitated more subtle instrumental and filtering improvements before we could fully benefit from the increased signal-to-noise that was achieved. Also, a general theory for these experiments was needed for their interpretation. When developed, we could demonstrate the good agreement between theory and experiment.

It then became possible to perform detailed studies on complex fluids, (Lee et al, 1994; Crepeau et al, 1994). These include phospholipid membrane vesicles (cf. Figure 12) (Lee et al, 1994a; Crepeau et al, 1994), liquid crystalline solutions (Sastry et al, 1996a, b) and liquid crystalline polymers, (Xu et al, 1996). A key feature was dead times of ca 50-60 ns. The detailed theory (Lee et al, 1994b) enabled quantitative analysis of these 2D spectra. In the case of 2D-ELDOR, simultaneous fits of experiments at several mixing times, T_m, provided in effect, a third dimension. One can watch how the cross peaks grow in relative to the auto peaks with increasing mixing time, (cf. Figures 13 and 14). This supplies quantitative information on the nuclear-spin-flip-inducing processes of both HE, which reports on translational diffusion, and the intramolecular electron-nuclear dipolar interaction, which reports on the tumbling motions.
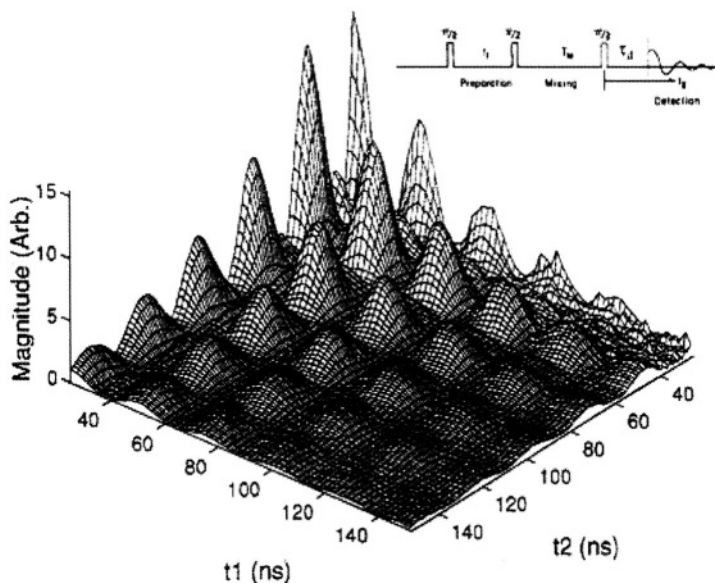
Figure 12. 2D-ELDOR at 17.3 GHz. The time domain S_{c-} spectrum showing ESR timescale; from phospholipid that is end chain labeled with nitroxide (16-PC) in lipid vesicles, (Borbat et al, 1997). *(Inset)* Pulse sequence.

In addition, the line shapes of the auto and cross peaks are particularly informative. In fact, there are two types of line shapes provided by the COSY (correlation spectroscopy) and 2D-ELDOR experiments. They arise because the experiment provides two types of 2D spectrum, depending on the coherence pathway: One is FID-like (sometimes referred to as the anti-echo) and the other is echo-like, i.e. there is a refocusing of the inhomogeneous broadening (IB) terms in the spin-Hamiltonian leading to their cancellation in the echo formation. The echo-like (or S_{c-}) 2D signal can in fact be transformed to provide just the homogeneous broadening (HB) along one frequency dimension, ω_1, whereas the other frequency dimension, ω_2, provides essentially the cw spectrum. This transformation takes one from the COSY to the SECSY format. In the 2D-ELDOR spectrum, this same transformation will yield the HB for the auto peaks, but the cross-peaks will be affected by any differences in the IB existing between the two spectral lines connected by that cross peak. Thus, the 2D-ELDOR S_{c-} spectrum provides detailed information on spin relaxation via the cross-peak development and the HB of the auto peaks, whereas the differences in IB show up in the cross peaks. The FID-like S_{c+} 2D spectra include the full effects of inhomogeneous broadening. The 2D-SECSY format is particularly useful for ultraslow motions, for example macromolecules in viscous media, (Saxena and Freed, 1997).
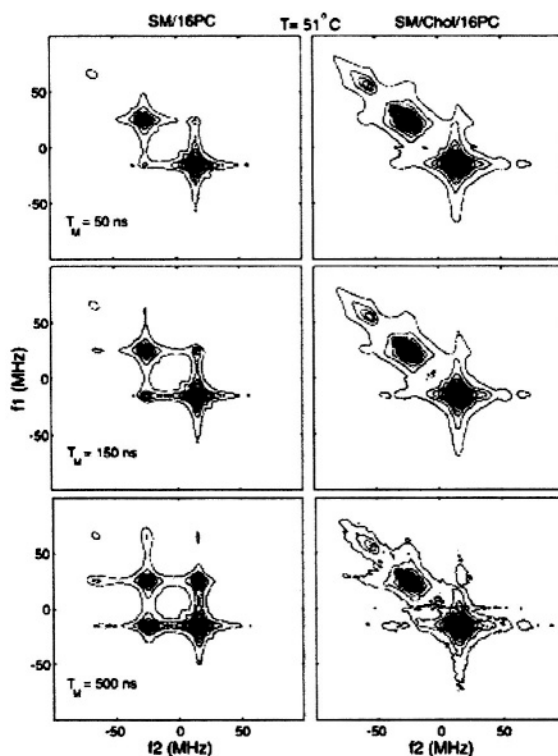
Figure 13. 2D-ELDOR at 17.3 GHz vs mixing time, T_m, of 16-PC in liquid crystalline phase from pure lipid vesicles *(left side)* compared with 16 PC in liquid-ordered phase from 1:1 ratio lipid to cholesterol *(right side)* at 50°C, (Costa-Filho et al, 2003a).

We showed that taken together, the S_{c-} and S_{c+} 2D-ELDOR spectra are especially useful for the study of the dynamics and structure of complex fluids. This is because complex fluids typically show a microscopic structure, such that molecular tumbling occurs with respect to this structure, which provides the local orientational alignment. This can be readily appreciated in the case of lipid membranes. If they are macroscopically aligned, then one would observe the different "single-crystal-like" spectra obtained for each orientation of the membrane normal with respect to the constant magnetic field. Membrane vesicles, however, simultaneously have membrane components at all angles with respect to the magnetic field, and they thereby provide "powder-like" spectra that is referred to as microscopically ordered, but macroscopically disordered. The extent of the local ordering is thus reflected in the IB, and details about the aligning fields can be obtained from the differences of the IB for the different hyperfine (hf) lines. At the same time, the S_{c-} spectra permit one to obtain dynamics from the homogeneous T_2's and the development of the cross peaks with mixing

time. Such a program was carried out (Patyal et al, 1994; Crepeau et al, 1994) using several different nitroxide spin labels in phospholipid membrane vesicles to obtain accurate dynamics and ordering parameters.
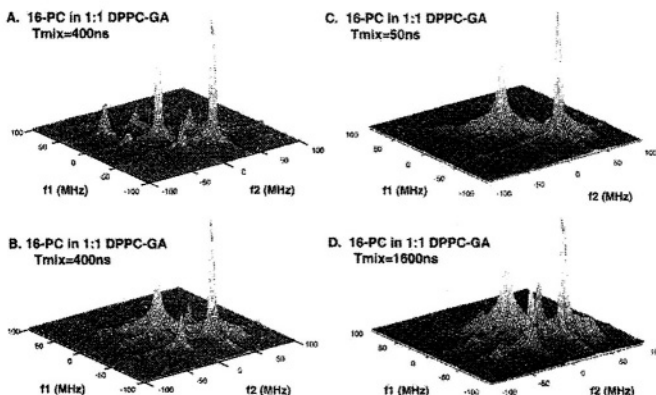

Figure 14. 2D-ELDOR at 17.3 GHz showing effect of peptide gramicidin A (GA) on dynamic structure of lipid membrane containing (end chain) nitroxide labeled lipid (16-PC) at 75°C. *(A)* Pure lipid, mixing time, $T_m = 400$ ns. *(B, C, D)* 1:1 lipid to GA with $T_m = 400$ ns, 50 ns, and 1.6 μs, respectively (Costa-Filho et al, 2003b).

In general, one finds that the 2D-ELDOR spectra from membrane vesicles show more dramatic changes as the membrane properties are varied. This can even enable simple interpretations of these spectra just in terms of pattern recognition. For example, in Figure 13, 2D-ELDOR contour plots as a function of mixing time, T_m, are shown for the spin-labeled lipid, 1-palmitoyl-2-(16-doxyl stearoyl) phosphatidylcholine (16-PC) in pure lipid vesicles and for a lipid-cholesterol mixture in the ratio 1:1. The former is in the standard liquid crystalline phase, whereas the latter is in a "liquid-ordered" LO phase, (Ge et al, 1999). The spectra are qualitatively different, emphasizing that the LO phase exhibits significantly greater ordering than the liquid crystalline phase at 51°C. The increased microscopic ordering leads to increased IB affecting the spectra from the LO phase. In addition, the restriction of the range of orientational motion, due to the microscopic ordering in the LO phase, shows up as a much slower development of cross peaks vs T_m, (Costa-Filho et al, 2003a).

In addition to the microscopic ordering but macroscopic disorder (MOMD) (Meirovitch et al, 1984) that gives rise to complex inhomogeneous line shapes, these spectra are often in the slow-motional regime, i.e. the motions are too slow to provide complete averaging of the rigid-limit line shapes. This is another source of IB that is effectively dealt with in the theory for MOMD spectra. These slow motional spectra provide more

insight into the microscopic details of the molecular dynamics because their timescales are comparable. It was found that for complex fluids, a more sophisticated model than the MOMD model, i.e. the SRLS model referred to above, was needed to analyze the 2D-ELDOR spectra in order to achieve reasonably good agreement with experiment.

We used studies on a macroscopically aligned liquid crystal solvent called 4O,8 to test the applicability of the SRLS model, (Sastry et al 1996a, b). This is a liquid crystal that exhibits many phases as a function of temperature, including isotropic, nematic, liquid-like smectic A, solid-like smectic B, and crystalline phases. We found consistently better fits using a SRLS model (in addition to the macroscopic liquid crystalline orienting potential) than with the standard simpler model that does not include any local structure. These studies demonstrated the very extensive relaxation, dynamic, and structural information that one can obtain from 2D-ELDOR experiments performed as a function of mixing time. In all, 10 such parameters could be effectively extracted. They include the two-term (asymmetric) macroscopic orienting 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 ^{14}N dipolar and g-tensors, the residual (Gaussian) inhomogeneous broadening not due to the specific slow-motional contributions from the ^{14}N hf and g-tensors, and the overall T_1 for the electron-spins. These constitute virtually all the parameters that one can hope to obtain from any ESR experiment(s) on spin relaxation in a complex fluid!

The virtues of the improved 2D-FT-ESR technology were further demonstrated in studies of the effect of the peptide, gramicidin A (GA), on the dynamic structure of model membranes. Earlier studies that showed that the changes in the 2D-ELDOR spectra on adding GA were much more dramatic than the changes in the cw-ESR spectra, emphasizing the much greater sensitivity of the former to molecular dynamics, (Patyal et al, 1997). However, these studies, performed at 9.3 GHz with a τ_d of 50-60 ns, related just to the bulk lipids. They showed no clear indications of the so-called boundary lipids that coat the peptide. Evidence for the boundary lipid exists in cw-ESR spectra but is of very limited resolution. More recently using 17.3-GHz 2D-ELDOR with its increased SNR and decreased dead times (τ_d ~ 25-30 ns), we have been able to obtain 2D-ELDOR spectra (Costa-Filho et al, 2003b) that show the presence of two components, viz the bulk lipid component, previously seen by Patyal et al, (1997) which shows relatively fast dynamics, and a second, the presumed boundary lipid, which grows in as GA is added (cf. Figure 14). Its 2D-ELDOR spectrum is clearly that of a

more slowly reorienting lipid, as expected. In addition, simulations of these spectra are consistent with a dynamic bending of the end-chain of the lipid as it coats the GA. This level of detail of the dynamic structure of complex membrane systems is not likely achievable by other means.

7.   PROSPECTUS

At present the modern methods of ESR have rendered it a powerful technique for studying molecular dynamics in a wide variety of chemical, physical, and biological systems. In NMR, molecular motions in fluids lead to nearly complete averaging of the motion-dependent terms in the spin Hamiltonian, so only their residual effects, reflected in the T_1 and T_2, report on dynamics. In ESR, however, there are often dramatic lineshape variations resulting from the molecular motions, which are particularly sensitive to the microscopic details of the dynamics. This feature is significantly enhanced in the multi-frequency approach, as we have seen. 2D-ELDOR provides unique features in resolving homogeneous from inhomogeneous broadening, clearly distinguishing cross-relaxation processes, as well as T_1's, all of which are valuable for studying molecular dynamics.

A key future development would be to extend 2D-ELDOR to higher frequencies and then to perform multifrequency studies of molecular dynamics by this powerful method. A coherent pulsed high-power spectrometer at 95 GHz has recently been developed to address this objective, (Hofbauer et al, 2003). Another challenge continues to be the development of spin labels with more limited flexibility and well-defined conformations, especially with regard to the study of protein dynamics, (Columbus and Hubbell, 2002). This would reduce effects of the internal motions of the spin label's tether that otherwise can interfere with extracting the more relevant features of the molecular dynamics.

Additional related reviews may be found elsewhere, (Freed, 1998, 2000, 2002; Borbat et al, 2001).

8.   ACKNOWLEDGEMENTS:

9.   GLOSSARY OF ABBREVIATIONS

CIDEP: Chemically-induced Dynamic Electron Spin Polarization
CIDNP: Chemically-induced Dynamic Nuclear Spin Polarization
COSY: Correlation Spectroscopy
CSL: Cholestane Spin Label
ELDOR: Electron-Electron Double Resonance
END: Electron-Nuclear Dipolar Interaction
ENDOR: Electron-Nuclear Double Resonance
ESE: Electron Spin Echoes
ESR: Electron Spin Resonance
FID: Free Induction Decay
FIR: Far Infrared
FT: Fourier Transform
GA: Gramicidin A
HB: Homogeneous Broadening
HE: Heisenberg Spin Exchange
IB: Inhomogeneous Broadening
LA: Lanczos Algorithm
LO: Liquid Ordered
MOMD: Microscopic Order with Macroscopic Disorder
NMR: Nuclear Magnetic Resonance
OTP: Ortho-Terphenyl
SECSY: Spin Echo Correlation Spectroscopy
SLE: Stochastic Liouville Equation
SNR: Signal-to-Noise Ratio
SRLS: Slowly Relaxing Local Structure

10.   REFERENCES

Angerhofer, A., Massoth, R.J. and Bowman, M.K. (1988) Fourier transform EPR measurement of homogeneous electron transfer rates. *Israel J. Chem.* **28,** 227-238.

Aue, W.P., Bartholdi and Ernst, R.R. (1976) Two-dimensional Spectroscopy application to nuclear magnetic resonance. *J. Chem. Phys.* **64,** 2229-2246.

Barnes, J.P. and Freed, J.H. (1998) Dynamics and ordering in mixed model membranes of DMPC and DMPS: A 250 GHz ESR study. *Biophys. J.* **75**, 2532-2546.

Barnes, J., Liang, Z., Mchaourab, H., Freed, J.H. and Hubbell, W.L. (1999) A multi-frequency ESR study of T4 lysozyme dynamics. *Biophys. J.* **76**, 3298-3306.

Beth, A.H. and Robinson, B.H. (1989) Nitrogen-15 and deuterium substituted spin labels for studies of very slow rotational motion. *Biol. Magn. Res.* **8**, 179-254.

Bloch, F., (1956) Dynamical theory of nuclear induction II. *Phys. Rev.* **102,** 104-135.

Bolton, J.R. and Carrington, A. (1962) Line width alternation in the electron spin resonance spectrum of the durosemiquinone cation. *Mol. Phys.* **5**, 161-167.

Borbat, P.P, Crepeau, R.H., and Freed, J.H. (1997) Multifrequency two-dimensional Fourier transform ESR: An X/Ku band spectrometer. *J. Magn. Res.* **127**, 155-167.

Borbat, P.P., Costa-Filho, A.J., Earle, K.A., Moscicki, J.K. and Freed, J.H. (2001) Electron spin resonance in studies of membranes and proteins. *Science* **291**, 266-269.

Brown, I.M. (1974) Electron spin-echo envelope decays and molecular motion: Rotational and translational diffusion. *J. Chem. Phys.* **60**, 4930-4938.

Budil, D.E., Earle, K.A., Lynch, W.B., and Freed, J.H. (1989) Electron paramagnetic resonance at 1 millimeter wavelengths. In *Advanced EPR Applications in Biology and Biochemistry,* ed. A. Hoff, Elsevier, Amsterdam, **8**, 307-340.

Columbus, L. and Hubbell, W.L. (2002) A new spin on protein dynamics. *Trends in Biochem. Sciences* **27**, 288-295.

Costa-Filho, A.J., Shimoyama, Y. and Freed, J.H. (2003a) A 2D-ELDOR study of the liquid ordered phase in multilamellar vesicle membranes, *Biophys. J.* (in press).

Costa-Filho, A.J., Crepeau, R.H., Borbat, P.P., Ge, M. and Freed, J.H. (2003b) Lipid-Gramicidin interactions: Dynamic structure of the boundary lipid by 2D-ELDOR, *Biophys. J.* (in press).

Crepeau, R.H., Saxena, S.K. Lee, S., Patyal, B.R. and Freed, J.H. (1994) Studies on lipid membranes by two dimensional-fourier transform ESR: Enhancement of resolution to ordering and dynamics. *Biophys. J.* **66**, 1489-1504.

Dobbert, O., Prisner, T. and Dinse, K.P. (1986) Single-channel quadrature FT ESR. *J. Magn. Res.* **70**, 173-175.

Dorio, M. and Freed, J.H. (eds) (1979) *Multiple Electron Resonance Spectroscopy;* Möbius, K., Lubitz, W. and Freed, J.H. (1989) Liquid-state ENDOR and triple resonance. In *Advanced EPR, Applications in Biology and Biochemistry,* (Hoff, A.J., ed.) pp. 441-499, Elsevier, Amsterdam, The Netherlands

Dzuba, S.A., Maryasov, A.G., Salikhov, K.M. and Tsetkov, Yu. D. (1984) Superslow rotations of nitroxide radicals studied by pulse EPR spectroscopy. *J. Magn. Reson..* **58**, 95-117.

Dzuba , S.A. and Tsvetkov, Yu.D. (1988) Magnetization transfer in pulsed EPR of ^{15}N nitroxides: reorientational motion model of molecules in glassy liquids. *Chem. Phys.* **120**, 291-298.

Earle, K.A., Budil, D.E. and Freed, J.H. (1993) 250 GHz EPR of nitroxides in the slow-motional regime: Models of rotational diffusion. *J. Phys. Chem.* **97**, 13289-13297.

Earle, K.A., Moscicki, J., Polimeno, A. and Freed, J.H. (1997) A 250 GHz ESR study of *o*-terphenyl: Dynamic cage effects above T_c. *J. Chem. Phys.* **106**, 9996-10015.

Earle, K.A., Moscicki, J, Polimeno, A. and Freed, J.H., (1998) Response to "Comment on 'A 250 GHz ESR study of *o*-terphenyl dynamic cage effects above T_c. *J. Chem. Phys.* **109**, 10525-26.

Eliav. U. and Freed, J.H. (1984) The oscillatory nature of polarization evolution in CIDEP. *J. Phys. Chem.* **88**, 1277-1280.

Fraenkel, G.K. (1965) Static and dynamic frequency shifts in electron spin resonance. *J. Chem. Phys.* **42**, 4275-4298.

Freed, J.H. (1964) Anisotropic rotational diffusion and electron spin resonance line-widths. *J. Chem. Phys.* **41**, 2077-2083.

Freed, J.H. (1965) Theory of saturation and double resonance effects in ESR spectra. *J. Chem. Phys.* **43**, 2312-2332.

Freed, J.H. (1968) Generalized cumulant expansions and spin-relaxation theory. *J. Chem. Phys.* **49**, 376-391.

Freed, J.H. (1998) Linewidths, lineshapes, and spin relaxation in the one and two-dimensional ESR of organic radicals and spin-labels. In *Foundations of Modern EPR* (G. Eaton, S. Eaton, and K. Salikhov, eds.) Ch. I.7, pp 658-683. World Scientific, NJ.

Freed, J.H. (2000) New technologies in electron spin resonance. *Ann. Rev. of Phys. Chem.* **51**, 655-689.

Freed, J.H. (2002) Modern ESR methods in studies of the dynamic structure of proteins and membranes. In *EPR in the 21st. Century,* (Kawamori, A., Yamauchi, J. and Ohta, H. eds. Elsevier Science, Amsterdam, Netherlands), 719-730.

Freed, J.H. and Fraenkel, G.K.(1962) Anomalous alternating linewidths in ESR spectra. *J. Chem. Phys.* **37**, 1156-1157.

Freed, J.H. and Fraenkel, G.K. (1963) Theory of linewidths in electron spin resonance spectra. *J. Chem. Phys.* **39**, 326-348.

Freed, J.H., Bruno, G.V. and Polnaszek, C. (1971a) ESR lineshapes and saturation in the slow motional region. *J. Phys. Chem.* **75**, 3385-3399.

Freed, J.H., Bruno, G.V. and Polnaszek, C.F. (1971b) ESR lineshapes for triplets undergoing slow rotational reorientation. *J. Chem. Phys.* **55**, 5270-5281.

Freed, J.H. and Pederson, J.B. (1976) The theory of chemically-induced dynamic spin polarization. *Adv. Magn. Res.* **8**, 1-84.

Ge, M., Field, K.A., Aneja, R, Holowka, D., Baird, B., and Freed, J.H. (1999) ESR characterization of liquid ordered phase of detergent resistant membranes from RBL-2H3 cells. *Biophys. J.,* **77**, 925-933.

Goldman, S.A., Bruno, G.V., Polnaszek, C.F. and Freed, J.H. (1972) An ESR study of anisotropic rotational reorientation and slow tumbling in liquid and frozen media. *J. Chem. Phys.* **56***, 716-735.

Gorcester, J. and Freed, J.H. (1986) Two dimensional fourier transform ESR spectroscopy. *J. Chem. Phys.* **85**, 5375-5377.

Gorcester, J. and Freed, J.H. (1988) Two-dimensional fourier transform ESR corrrelation spectroscopy. *J. Chem. Phys.* **88**, 4678-4693.

Gorcester, J., Rananavare, S.R. and Freed, J.H. (1989) Two dimensional ELDOR and electron spin echo study of solute dynamics in smectics. *J. Chem. Phys.* **90,** 5764-5786.

Gordon, R.G. and Messenger, T. (1972). Magnetic resonance line shapes in slowly tumbling molecules. In *Electron Spin Relaxation in Liquids,* (Muus, L.T. and Atkins, P.W. Eds.) Plenum, New York.

Hofbauer, W., Earle, K.A., Dunnam, C. and Freed, J.H. *High-Power Pulsed Spectrometer at 95 GHz for 2D-FT-ESR* (in preparation).

Hornak, J.P. and Freed, J.H. (1983) ELDOR spin echoes and slow motions. *Chem. Phys. Lett.* **101**, 115-119.

Hornak, J.P. and Freed, J.H. (1986) Spectral rotation in pulsed ESR spectroscopy. *J. Magn. Reson.* **67**, 501-518.

Hwang, J.S., Mason, R.P., Hwang, L.P. and Freed, J.H. ((1975) ESR studies of anisotropic rotational reorientation and slow tumbling in liquid and frozen media III: PD-tempone and an analysis of fluctuating torques. *J. Phys. Chem.* **79**, 489-511.

Hyde, J.S. and Maki, A.H. (1964) ENDOR of a free radical in solution. *J. Chem. Phys.* **40**, 3117-3118.

Hyde, J.S., Chien, J.C.W. and Freed, J.H. (1968) Electron-electron double resonance of free radicals in solution. *J. Chem. Phys.* **48**, 4211-4226.

Hyde, J.S. and Dalton, L.R. (1979) Saturation-Transfer spectroscopy. In *Spin Labeling II. Theory and Applications,* (Berliner, L.J. ed.) pp 3-70, Academic Press, NY.

Jeener, J., Meier, B.H., Bachmann, P. and Ernst, R.R. (1979) Investigation of exchange processes by two-dimensional NMR spectroscopy. *J. Chem. Phys.* **71**, 4546-4593.

Kivelson, D. (1960) Theory of ESR linewidths of free radicals. *J. Chem. Phys.* **33**, 1094-1106.

Kivelson, D. and Lee, S. (1982) Theory of ESR parallel-edge lines of slowly tumbling molecules. *J. Chem. Phys.* **76**, 5746-5754.

Korst, N.N. and Khazanovitch, T.N. (1964) Relaxation and shape of paramagnetic resonance lines in highly viscous media. *Soviet Phys. JETP,* **18**, 1049-1055.

Kubo, R. (1962) Generalized cumulant expansion method. *J. Phys. Soc. Japan,* **17**, 1100-1120.

Kubo, R. (1963) Stochastic Liouville equations. *J. Math.Phys.***4***,* 174-183.

Kubo, R. (1969a). A stochastic theory of line shape. In *Advan. Chem. Phys.,* (Schuler, K. E., ed). **15**, 101-127, Wiley, NY.

Kubo, R. (1969b) Stochastic theories of randomly modulated systems. *J. Phys. Soc. Japan,* **26**, Supplement 1-5.

Kubo, R. and Tomita, K. (1954) A general theory of magnetic resonance absorption. *J. Phys. Soc. Japan* **9**, 888-919.

Kurreck, H., Kirste, B. and Lubitz, W. (1988) Electron nuclear double resonance spectroscopy of radicals in solution. VCH Verlagsgesellschaft, Weinheim, Germany.

Lee, S., Patyal, B.R., Saxena, S.H., Crepeau, R.H., and Freed, J.H. (1994a) Two-dimensional fourier transform ESR in complex fluids. *Chem. Phys. Lett.* **221**, 397-406.

Lee, S., Budil, D.E., and Freed, J.H. (1994b) Theory of Two-Dimensional Fourier Transform ESR for Ordered and Viscous Fluids. *J. Chem. Phys.* **101**, 5529-5558.

Leniart, D.S., Connor, H.D. and Freed, J.H. (1975) An ESR and ENDOR study of spin-relaxation of semi quinones in liquid solution. *J. Chem. Phys.* **63**, 165-199.

Liang, Z.C. and Freed, J.H. (1999) As assessment of the applicability of multifrequency ESR to study the complex dynamics of biomolecules. *J. Phys. Chem.B* **103**, 6384-6396.

Lou, Y., Ge, M. and Freed, J.H., (2001) A Multifrequency ESR Study of the Complex Dynamics of Membranes, *J. Phys. Chem. B* **105**, 11053-11056.

Mason, R.P. and Freed, J.H. (1974) Estimating microsecond rotational correlation times from lifetime broadening of nitroxide ESR spectra. *J. Phys. Chem.* **78**, 1321-1323.

Meirovitch, E., Nayeem, A. and Freed, J.H. (1984) An analysis of protein-lipid interactions based on model simulations of ESR spectra. *J. Phys. Chem.* **88**, 3454-3465.

Millhauser, G.L. and Freed, J.H. (1984) Two dimensional electron-spin-echoes and slow motions. *J. Chem. Phys.* **81**, 37-48.

Möbius, K., Lubitz, W. and Plato, M., (1989) Liquid State ENDOR and triple resonance. In *Advanced EPR, Applications in Biology and Biochemistry* (Hoff, A.J. ed.), pp. 441-449, Elsevier, Amsterdam.

Moro, G. and Freed, J.H. (1981) The efficient computation of magnetic resonance spectra and related correlation functions from stochastic Liouville Equation. *J. Phys. Chem.* **84**, 2837-2840.

Norris, J.R. and Weissman, S.I. (1969) Studies of rotational diffusion through the electron-electron dipolar interaction. *J. Phys. Chem.* **73**, 3119-3124.

Panferov, Y.F., Grinberg, O.Y., Dubinskii, A.A. and Lebedev, Y.S. (1984) ESR fourier transform spectroscopy with correction for nonuniformity of phase distortion. *Dokl. Phys. Chem.* **278**, 888-890.

Patyal, B.R., Crepeau, R.H., Gamliel, D. and Freed, J.H. (1990) Two dimensional fourier transform ESR in the slow motional and rigid limits: SECSY:ESR, Two-dimensional fourier transform ESR in the slow motional and rigid limits: 2D-ELDOR. *Chem. Phys. Lett.* **175**, 445-452 ,453-460.

Patyal, B., Crepeau, R.H. and Freed, J.H. (1997) Lipid-Gramicidin interactions using two-dimensional fourier-transform electron spin resonance. *Biophys. J.,* **73**, 2201-2220.

Perrin, F. (1934) Mouvement brownien d'un ellipsoïde, 1: Dispersion diélectrique pour des molécules ellipsoïde. *J. Phys. Radium* **5**, 497-511.

Polimeno, A., and Freed, J.H. (1995) Slow motional ESR in complex fluids: The slowly relaxing local structure model of solvent cage effects. *J. Phys. Chem.* **99**, 10995-11006

Polnaszek, C.F. and Freed, J.H. (1975) ESR Studies of anisotropic ordering, spin relaxation, and slow tumbling in liquid crystalline solvents. *J. Phys. Chem.* **79**, 2283-2306.

Redfield, A.G. (1965) The theory of relaxation processes. *Adv. Magn. Res.* **1**, 1-32.

Sastry, V.S.S., Polimeno, A., Crepeau, R.H. and Freed, J.H. (1996a) Studies of spin relaxation and molecular dynamics in liquid crystals by two dimensional fourier transform ESR: I. Cholestane in butoxy benzylidene-octylaniline and dynamic cage effects. *J. Chem. Phys.* **105**, 5753-5772.

Sastry, V.S.S., Polimeno, A., Crepeau, R.H. and Freed, J.H. (1996b) Studies of spin relaxation and molecular dynamics in liquid crystals by two dimensional fourier transform ESR: II PD-tempone in butoxy benzylidene-octylaniline and dynamic cage effects. *J. Chem. Phys.* **105**, 5773-5791.

Saxena, S.K. and Freed, J.H. (1997) Two dimensional ESR and slow motions. *J. Phys. Chem. A,* **101**, 7998-8008.

Schreurs, J.W.H. and Fraenkel, G.K. (1961) Anomalous relaxation of hyperfine components in electron spin resonance. *J. Chem. Phys.* **34**, 756-768 .

Schneider, D.J. and Freed, J.H. (1989a) Calculating Slow Motional Magnetic Resonance Spectra: A User's Guide, in *Spin Labeling- Theory and Applications, Vol. III, Biol. Magn. Res,.* **8**, 1-76

Schneider, D.J. and Freed, J.H. (1989b) Spin relaxation and motional dynamics, in *Lasers, Molecules and Methods,* J.O. Hirschfelder, R.E. Wyatt, and R.D. Coalson, Eds. *Adv. Chem. Phys.* **73**, 387-528.

Schwartz, L.J., (1984). *Molecular Reorientation and Time Domain ESR,* Ph.D. Thesis, Cornell University.

Schwartz, L.J., Stillman, A.J. and Freed, J.H. (1982) Analysis of electron-spin echoes by spectral representation of the Stochastic-Liouville equation. *J. Chem. Phys.* **77**, 5410-5425.

Schwartz, L.J., Millhauser, G.L. and Freed, J.H. (1986) Two dimensional electron spin echoes: Magnetization transfer and molecular dynamics. *Chem. Phys. Lett.* **127**, 60-66.

Sillescu, H. and Kivelson, D. (1968) Theory of spin-lattice relaxation in classical liquids. *J. Chem. Phys.* **48**, 3493-3505.

Stephen, M.J. and Fraenkel, G.K. (1960) Theory of saturation in electron spin resonance spectra. *J. Chem. Phys.* **32**, 1435-1444.

Stillman, A.E., Schwartz, L.J. and Freed, J.H. (1980) Direct determination of rotational correlation time by electron-spin echoes. *J. Chem. Phys.* **73**, 3502-3503.

Wangness, R.K. and Bloch, F. (1953) The dynamical theory of nuclear induction. *Phys. Rev.,* **102**, 728-739

Xu, D., Crepeau, R.H., Ober, C.K. and Freed, J.H. (1996) Molecular dynamics of a liquid crystalline polymer studied by two-dimensional fourier transform and cw-ESR. *J. Phys. Chem.* **100**, 15873-15885.