Dynamics of lipid bilayers from comparative analysis of $^2$H and $^{13}$C nuclear magnetic resonance relaxation data as a function of frequency and temperature

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(Received 8 April 1997; accepted 10 September 1997)

Analysis of the nuclear spin relaxation rates of lipid membranes provides a powerful means of studying the dynamics of these important biological representatives of soft matter. Here, temperature- and frequency-dependent $^2$H and $^{13}$C nuclear magnetic resonance (NMR) relaxation rates for vesicles and multilamellar dispersions of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) in the liquid–crystalline state have been fitted simultaneously to various dynamic models for different positions of the acyl chains. The data include $^2$H $R_{1Z}$ rates (Zeeman order of electric quadrupolar interaction) acquired at 12 external magnetic field strengths from 0.382 to 14.6 T, corresponding to a frequency range from $\omega_B/2\pi=2.50–95.3$ MHz; and $^2$H $R_{1Q}$ rates (quadrupolar order of electric quadrupolar interaction) at 15.3, 46.1, and 76.8 MHz. Moreover, $^{13}$C $R_{1Z}$ data (Zeeman order of magnetic dipolar interaction) for DMPC are included at six magnetic field strengths, ranging from 1.40 to 17.6 T, thereby enabling extension of the frequency range to effectively $(\omega_Q+\omega_{Q})/2\pi=938.7$ MHz. Use of the generalized approach allows formulation of noncollective segmental and molecular diffusion models, as well as collective director fluctuation models, which were tested by fitting the $^2$H $R_{1Z}$ data at different frequencies and temperatures (30 °C and 50 °C). The corresponding $^{13}$C relaxation rates were predicted theoretically and compared to experiment, thus allowing one to unify the $^{13}$C and $^2$H NMR data for bilayer lipids in the fluid state. A further new aspect is that the spectral densities of motion have been explicitly calculated from the $^2$H $R_{1Z}$ and $R_{1Q}$ data at 40 °C. We conclude that the relaxation in fluid membrane bilayers is governed predominantly by relatively slow motions, which modulate the residual coupling remaining from faster local motions (order fluctuations). Only the molecular diffusion model, including an additional slow motional process, and the membrane deformation model describing three-dimensional collective fluctuations fit the $^2$H NMR data and predict the $^{13}$C NMR data in the MHz range. Orientational correlation functions have been calculated, which emphasizes the importance of NMR relaxation as a unique tool for investigating the dynamics of lipid bilayers and biological membranes. © 1997 American Institute of Physics.

I. INTRODUCTION

Lipid bilayers comprise the essential structural matrix of biological membranes and encompass a rich hierarchy of motions, including noncollective and collective reorientations of segments, molecules, and domains.1–10 The underlying common feature of various approaches aimed at describing reorientations in bilayer lipids is the calculation, numerical or analytical, of the correlation functions due to segmental, molecular, and collective motions. In the case of NMR spectroscopy, the second-rank correlation functions and their Fourier transforms, the irreducible spectral densities of motion, are of interest.11 Nuclear spin relaxation theory8,11–14 relates the irreducible spectral densities corresponding to the fluctuating second-rank couplings to various macroscopic relaxation observables, including $R_{1Z}$, $R_{1Q}$, and $R_2$. Fitting the theoretical spectral densities to experimental NMR lineshapes and relaxation rates15 provides a valuable tool for investigating the dynamics of anisotropic liquid–crystalline systems in relation to their material properties, and in the case of lipid membranes,16 to their biological roles. Yet no general consensus has been achieved with regard to the predominant mechanisms of nuclear spin relaxation in lipid bilayers.

To critically test various dynamical models for lipid bilayers, one needs to study the dependence of the relaxation on the (i) segmental ordering, (ii) bilayer orientation, (iii) frequency (magnetic field strength), and (iv) temperature.17 As a rule, faster segmental reorientations modulate the static coupling tensor corresponding to the quadrupolar or dipolar interactions, whereas slower motions can further modulate the residual coupling. In the latter case, the order fluctuations can be due either to noncollective molecular reorientations within the mean field of the bilayer (potential of mean torque), or alternatively, to collective thermal excitations of the bilayer treated as a continuous medium.18 For macroscopically aligned bilayers, the study of the relaxation rates as a function of the sample orientation at different frequencies allows one to calculate explicitly the spectral densities
of motion in the frame associated with the normal to the bilayer surface (director). A combined analysis of the frequency and temperature dependence of the relaxation rates can give important new knowledge about the predominant mechanisms of nuclear spin relaxation and their characteristic time scales. This information can then yield an enhanced understanding of the equilibrium and dynamical properties of lipid bilayers, which in turn is essential with regard to characterization of the forces leading to the self-organization of lipids and the biological activities of membranes.

Current formulations of dynamical models for lipid bilayers include discrete jumps, rotational diffusion, and collective models describing a continuous spectrum of random fluctuations. More recently, Brownian and molecular dynamics (MD) simulations of lipid assemblies have been carried out. For disaturated phosphatidylcholines in the liquid-crystalline state the $^2$H NMR relaxation rates are proportional to the square of the observed order parameter, which reflects a reorientation of the residual coupling tensor left over from faster segmental motions, as opposed to modulation of the static electric field gradient (EFG) tensor due to local dynamics. This observation points to an interpretation in which the relaxation of the bilayer hydrocarbon region is largely governed by order fluctuations due to slower motions. By applying the methods of statistical mechanics, one can obtain analytical closed-form expressions for the correlation functions for various types of stochastic motions, e.g., noncollective molecular reorientations due to rotational diffusion in the presence of a restoring potential. Another possibility is to consider collective excitations of the bilayer, e.g., as described by a flexible surface model involving two-dimensional thermal director fluctuations (splay collective fluctuations of the normal to a flexible surface), which pertains to a smectic-like picture of lipid membranes. Alternatively, formulation of the collective lipid dynamics involving a three-dimensional membrane deformation model (splay, twist, and bend deformations) can be considered, which corresponds to a nematic-like picture. At first glance, the former appears more realistic for quasi-two-dimensional objects such as membranes, and in this regard the three-dimensional collective fluctuation model, in which the finite thickness of the bilayer is included, has been criticized. Consideration of collective mechanisms of relaxation has also been regarded as unnecessary for computer simulations of lipid membranes. In other work, the approach has been to fit separately the angular- and frequency-dependent relaxation data for individual segment positions of the lipid acyl chains, which has led in some cases to a large discrepancy of the fitting parameters for the same model. Moreover, the analysis of the dependence of the relaxation on bilayer orientation in fluid lipid membranes has not shown a dramatic change of the relaxation times with the angle between the bilayer normal and the magnetic field, making it difficult to fit the data uniquely. Proton field-cycling NMR techniques have not made it possible to investigate the dynamics of individual segments; instead, the relaxation arising from the cumulative effect of all the protons has been observed.

A consistent approach should involve a simultaneous analysis of the broadest possible range of relaxation data for the various acyl chain positions (segments) taken at different orientations of the specimen, frequencies (magnetic field strengths), and temperatures. Here the authors have further addressed the question: which motions, collective or noncollective, predominantly contribute to the $^2$H and $^{13}$C NMR relaxation rates of liquid-crystalline phospholipids in the MHz regime? A relatively broad spectrum of frequencies has been investigated for vesicles and multilamellar dispersions of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), ranging from $\omega_{0}/2\pi = 2.50$ MHz to an effective value of $(\omega_{c} + \omega_{0})/2\pi = 938.7$ MHz corresponding to $^{13}$C relaxation. Segments and molecular diffusion models as well as director fluctuation models have been fitted simultaneously to the $^2$H $R_{1Z}$ relaxation data for positions C3 and C7 of the DMPC acyl chains at different temperatures, and the corresponding $^{13}$C $R_{1Z}$ values have been theoretically calculated and compared to experiment. For the first time such a complete range of relaxation data involving a rather broad frequency range, different chain positions, mechanisms of nuclear relaxation, and temperatures has been analyzed, which has made it possible to unify the $^2$H and $^{13}$C NMR data for lipid bilayers. The results emphasize the importance of NMR relaxation techniques in providing new knowledge of the structural dynamics of biological lipid systems.

II. GENERAL THEORETICAL BACKGROUND

The theory of NMR relaxation gives relationships between the spectral densities of motion and the observable spin-relaxation rates. Two types of orientationally dependent coupling mechanisms of spin relaxation are considered here: the quadrupolar interaction of the deuteron ($^2$H) quadrupolar moment with the electric field gradient (EFG) of its chemical bond, and the magnetic dipolar interaction between a carbon ($^{13}$C) nuclear spin and its directly bonded proton ($^1$H). In general, the relaxation is due to fluctuations of the perturbing Hamiltonian that reflect the nature of the motions predominantly governing the spin relaxation. By applying time-dependent perturbation theory, expressions for the different spin-relaxation rates can be derived. In what follows, we shall employ a unified notation for the dipolar and quadrupolar interactions, which is closely related to that adopted by H"aberlen and by Spiess. However, it differs slightly from the classical notation used in the text by Abragam.

A. Unified representation of the coupling Hamiltonian for magnetic dipolar and electric quadrupolar interactions

In terms of an irreducible representation for magnetic dipolar and electric quadrupolar interactions, the Hamiltonian may be expressed as:

$$\hat{H}_A = C_\lambda \sum_{m=-2}^{2} (-1)^m T^{2}_{-m} V^{2}_{m} \hat{V}_{m}^{2}.$$

(2.1)
a) segmental diffusion
b) molecular diffusion
c) collective director fluctuations

FIG. 1. Models used to characterize dynamics of bilayer lipids: (a) segmental diffusion model; (b) molecular diffusion model; (c) collective director fluctuations described by the flexible surface model or membrane deformation model. Various intermediate frames are used to treat the relaxation of the $^{13}$C or $^2$H nuclei of a lipid segment, due to magnetic dipolar or electric interaction model. Various intermediate frames are used to treat the relaxation of fluctuations described by the flexible surface model or membrane deformation model;

As a rule, the fluctuating orientationally dependent EFG tensor $V_{m}^{(2)}$ can be expressed in terms of the rotational transformation of the irreducible components within the principal axis system (PAS) to the laboratory frame by

$$V_{m}^{(2)}_{\text{lab}} = \sum_{s=-2}^{2} V_{s}^{(2)\text{PAS}} D_{sm}^{(2)}(\Omega_{PL}).$$

Clearly, the time dependence is contained in the Euler angles $\Omega_{PL}$ describing the orientation of the PAS associated with a particular C–H bond and, therefore, in the corresponding Wigner rotation elements $D_{sm}^{(2)}(\Omega_{PL})$. The irreducible components of the coupling tensor, corresponding to its principal values, $V_{m}^{(2)\text{PAS}}$, can be written as

$$V_{0}^{(2)\text{PAS}} = \sqrt{\frac{7}{2}} V_{ZZ} = \sqrt{\frac{7}{2}} \delta_{\lambda}, \quad (2.4a)$$
$$V_{\pm 1}^{(2)\text{PAS}} = 0, \quad (2.4b)$$
$$V_{\pm 2}^{(2)\text{PAS}} = \frac{1}{2} (V_{XX} - V_{YY}) = - \frac{1}{2} \delta_{\lambda} \eta_{\lambda}, \quad (2.4c)$$

where $V_{ii}$ are the principal values of the coupling tensor $V_{m}$ that is diagonal in a Cartesian basis. The coupling parameters for the magnetic dipolar interaction and the electric quadrupolar interaction are the following: dipolar coupling, $C_{D} = -2 \gamma_{1} T_{S}$; $\delta_{D} = (r_{1} S_{1})$, and $\eta_{D} = 0$; quadrupolar coupling, $C_{Q} = e Q / 2 h$, $\delta_{Q} = e q$, and $\eta_{Q} \neq 0$, in general. Here $r_{1} S_{1}$ is the distance between the $^{13}$C and $^2$H nuclei, which is time averaged in the case of $\delta_{D}$; $\gamma_{1}$ and $\gamma_{S}$ are the corresponding magnetogyric ratios; $Q$ is the quadrupolar moment; and $e q$ is the electric field gradient at the nucleus.

For the special case of $\eta_{D} = 0$, the components of the irreducible electric field gradient in the laboratory frame are, according to Eq. (2.3),

$$V_{m}^{(2)\text{lab}} = V_{0}^{(2)\text{PAS}} D_{sm}^{(2)}(\Omega_{PL}).$$

The latter corresponds to the magnetic dipolar coupling or to an axially symmetric electric field gradient in the case of the electric quadrupolar interaction, which should be a reasonable assumption for an aliphatic C–$^2$H bond ($sp^{3}$ hybridization), for example. In Eq. (2.5) the Euler angles $\Omega_{PL}$ describe the orientation of the PAS with respect to the laboratory frame. By using Eq. (2.5), the expression for the Hamiltonian, Eq. (2.1), can be rewritten as

$$\hat{H}_{\lambda} = C_{\lambda} V_{0}^{(2)\text{PAS}} \sum_{m=-2}^{2} (-1)^{m} D_{sm}^{(2)}(\Omega_{PL}) T_{m}^{(2)\text{lab}}.$$

All of the time dependence and orientational information is contained in the Wigner rotation matrix elements $D_{sm}^{(2)}(\Omega_{PL}) \rightarrow D_{sm}^{(2)}(\Omega_{PL}, t)$. The closure property of the rotation group can then be used to express the Wigner elements $D_{sm}^{(2)}(\Omega_{PL})$ as a result of different subtransformations. This allows one to include and examine the effects of local segmental, molecular, or collective motions on the spectral den-
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...and, therefore, on the dependence of the relaxation rates on the bilayer orientation, ordering, and frequency (magnetic field strength); cf. Fig. 1.  

B. Equation of motion for the density matrix in the interaction picture

To describe the relaxation of the average magnetization of a statistical ensemble of nuclear spins in the presence of a time-dependent perturbing Hamiltonian, Eq. (2.1), it is convenient to consider the evolution of the density matrix in the interaction picture,

$$\frac{\partial \rho'}{\partial t} = -i[\hat{H}'(t) - \langle \hat{H}' \rangle, \rho'] .$$

(2.7)

In the above expression $\rho$ is the density matrix, the prime ('$') implies the corresponding operator in the interaction picture, the angular brackets denote the time averaging, and the square brackets designate the commutator. Neglecting the correlation between the fluctuating coupling Hamiltonian and the density matrix, one can further write, up to first order, that

$$\frac{\partial \rho'}{\partial t} = -\int_0^\infty [\hat{H}'(t) - \langle \hat{H}' \rangle, [\hat{H}'(t-\tau) - \langle \hat{H}' \rangle, \rho']] d\tau .$$

(2.8)

It can be shown from the transformation properties of the spin operators $\hat{I}_Z$, $\hat{I}_x$, $\hat{S}_Z$, and $\hat{S}_x$, that, in the interaction picture, the irreducible components of the spin operators $T_{m}^{(2)\text{lab}}$ have the form

$$T_{m}^{(2)\text{lab}}(t)' = \sum_q T_{m,q}^{(2)} e^{-i\omega_{m,q} t} .$$

(2.9)

Here $\omega_{m,q}$ are the characteristic frequencies arising from the transformation to the interaction picture, which can be expressed, in general, as a linear combination of the resonance frequencies of the relaxing spin(s). The spin operators $T_{m,q}^{(2)}$ contain the pairwise products of $\hat{I}_Z$, $\hat{I}_x$, $\hat{S}_Z$, and $\hat{S}_x$, cf. Eqs. (2.2), and are related to the irreducible components of the $T_{m,q}^{(2)}$ operators by

$$T_{m}^{(2)\text{lab}} = \sum_q T_{m,q}^{(2)} .$$

(2.10)

Substituting the Hamiltonian, Eq. (2.1), into Eq. (2.8) and taking the ensemble average, one finds that

$$\frac{\partial \rho'}{\partial t} = -\frac{1}{2} (C_{\lambda} V_0^{(2)\text{PAS}})^2 \sum_{m,q} \int_{-\infty}^{\infty} G_m(\tau) e^{-i\omega_{m,q}\tau}$$

$$\times [T_{m,q}^{(2)} [T_{m,q}' \rho']] d\tau .$$

(2.11)

The correlation functions $G_m(\tau)$ describe the dynamics of a particular C–H bond, associated with the PAS, and are given by

$$G_m(\tau) = \langle (V_{m}^{(2)\text{lab}}(t + \tau) - \langle V_{m}^{(2)\text{lab}} \rangle)^*$$

$$\times (V_{m}^{(2)\text{lab}}(t) - \langle V_{m}^{(2)\text{lab}} \rangle) / (V_0^{(2)\text{PAS}})^2 \rangle .$$

(2.12)

Note that for the special case of $\eta_{\lambda} = 0$, corresponding to an axially symmetric EFG, Eq. (2.12) reduces to

$$G_m(\tau) = \langle (D_{0,m}^{(2)}(\Omega_{PL}, t + \tau) - \langle D_{0,m}^{(2)}(\Omega_{PL}) \rangle)^*$$

$$\times (D_{0,m}^{(2)}(\Omega_{PL}, t) - \langle D_{0,m}^{(2)}(\Omega_{PL}) \rangle) \rangle .$$

Finally, introducing the irreducible spectral densities of motion as the Fourier transforms of the orientational correlation functions, one obtains that

$$\frac{\partial \rho'}{\partial t} = -\frac{1}{2} (C_{\lambda} V_0^{(2)\text{PAS}})^2 \sum_{m,q} J_m(\omega_{m,q})$$

$$\times [T_{m,q}^{(2)} [T_{m,q}' \rho']] ,$$

(2.13)

where

$$J_m(\omega_{m,q}) = \int_{-\infty}^{\infty} G_m(\tau) e^{-i\omega_{m,q}\tau} d\tau .$$

(2.14)

Equation (2.13) can then be used to calculate the relaxation times for the average (observable) value of any operator $\hat{A}$ by using the relation

$$\langle \hat{A} \rangle = \text{Tr}(\rho' \hat{A}) .$$

(2.15)

According to Eq. (2.13), in general, the observable relaxation rates may be written as a linear combination of the irreducible spectral densities of motion in a spherical basis. The coefficients of this expansion may be calculated from the double commutators, and reflect the specific nature of the interaction. All the frequency and orientational information is, therefore, contained in the irreducible spectral densities of motion.

C. Macroscopic relaxation observables

Use of Eq. (2.13) makes it possible to obtain relationships between the observable spin relaxation rates and the spectral densities of the motions that predominantly contribute to the relaxation. Transforming the irreducible spin operators $T_{m,q}^{(2)\text{lab}}$, Eqs. (2.2), into the interaction picture and computing the double commutators in Eq. (2.13), one obtains for the relaxation rate of the Zeeman order, viz. $\langle \hat{I}_Z \rangle$, in the case of the quadrupolar interaction that

$$R_{1Z}(^2\text{H}) = \frac{1}{4 \pi^2} \langle Q \rangle^2 \left[ J_1(\omega_D) + 4 J_2(2\omega_D) \right] .$$

(2.16)

Here $\omega_D = e^2 q Q / \hbar = 170$ kHz is the static quadrupolar constant, and $\omega_D$ is the deuterium Larmor frequency. Likewise, the relaxation of the quadrupolar order of the quadrupolar interaction, i.e. relaxation of the quantity $\langle \hat{I}_Z^2 - 3(I(1))^2 \rangle$, is given by

$$R_{1Q}(^2\text{H}) = \frac{1}{4 \pi^2} \langle Q \rangle^2 J_1(\omega_D) .$$

(2.17)

Finally, the expression for the Zeeman relaxation rate of the $^{13}$C spin under conditions of proton decoupling becomes

$$R_{1Z}(^{13}\text{C}) = \frac{1}{4} N_H \pi^2 \langle Q \rangle^2 \left[ J_1(\omega_H - \omega_C) + \frac{1}{2} J_1(\omega_C) + J_2(\omega_H + \omega_C) \right] ,$$

(2.18)

i.e., in the absence of cross-correlation between the two neighboring protons, and with the same definition of the spectral densities $J_m(\omega)$.
\[ \chi_D = (\gamma_H \gamma_e \hbar/2 \pi^2) (r_{CH}^3) \] is the static dipolar coupling constant averaged over faster vibrational motions; and the ratio of the Larmor frequencies of the proton and carbon is \( \omega_{13C}/\omega_c = 3.975 \). The vibrationally averaged distance between the \( ^{13}C \) and \(^1H\) nuclei in a C–H bond is assumed to be \( r_{CH} = 1.14 \text{ Å} \). 53, 54

The above formulas provide a basis for testing different dynamical models for the description of both the \( ^{13}C \) and \( ^2H \) NMR data involving different types of relaxation mechanisms. It should be noted that in vesicles or multilamellar dispersions, orientational averaging of the relaxation results in vanishing of the projection index \( m \) on account of the spherical symmetry; 55 there is no unique direction so that \( J_m(\omega) \to \langle J_m(\omega) \rangle \). In this case, as follows from Eqs. (2.16)–(2.18), if one knows the \( R_{1Z} \) and \( R_{1Q} \) relaxation rates at a given deuterium Larmor frequency \( \omega_D \), one can obtain not only the value of the spectral density \( J(\omega_D) \) but also the value of \( J(2\omega_D) \). This enables one to extend the effective frequency range by a factor of 2. Moreover, due to the presence of terms such as \( J(\omega_D + \omega_c) \), the \( ^{13}C \) relaxation rates include the spectral densities at much higher frequencies than in the case of the \( ^2H \) relaxation rates. 53, 56, 57 For example, a model fitted to the \( ^2H \) \( R_{1Z} \) relaxation rates within the range 2.50–95.3 MHz should be able to predict the \( ^{13}C \) relaxation data at substantially higher effective frequencies, up to \( (\omega_D + \omega_c)/2 \pi \) MHz. Of course, one could start by fitting \( ^{13}C \) \( R_{1Z} \) data to a particular model and then try to predict the \( ^2H \) relaxation rates, or fit the observable quantities simultaneously. Either approach is subject to the data availability and data reduction involved. In the present paper the first approach has been chosen. Namely, first the \( ^2H \) relaxation data have been fitted to various models, including noncollective segmental diffusion and molecular diffusion models and collective two-dimensional and three-dimensional director fluctuation models. Then the \( ^{13}C \) \( R_{1Z} \) relaxation rates have been calculated and compared to the experimental values at six different frequencies for positions C3 and C7 of the phospholipid acyl chains.

We turn now to the mathematical and physical description of the various motional models. The noncollective models are formulated in terms of the full treatment of rotational diffusion in the presence of a potential of mean torque (full diffusion model), as well as using a strong-collisional (symmetric top) approximation that simplifies the final results (simplified diffusion model). By fitting the models to the relaxation data, it is shown that the two approaches yield a comparable quality of the fits; thus, the spectral densities given by the noncollective models are not strongly sensitive to the form of the orienting potential within the time and frequency scales considered. 18

III. NONCOLLECTIVE DIFFUSION MODELS FOR ROTATIONS OF MEMBRANE CONSTITUENTS

A. Segmental diffusion model

According to Eqs. (2.3) and (2.6), all the orientational and dynamic information is contained in the Wigner rotation matrix elements \( D_{nm}^{(2)}(\Omega_{PL}) \). One can, however, further separate the time-dependent part (containing the dynamic information) from the time-independent part (containing the information about the average structure, including the average orientations of bonds, domains, and the specimen as a whole) in the \( D_{nm}^{(2)}(\Omega_{PL}) \) by introducing various intermediate transformations (Fig. 1). In general, the matrix elements \( D_{nm}^{(2)}(\Omega_{PL}) \) can be represented as a sum of the products of the corresponding Wigner rotation matrices according to the well-known closure property of the rotation group. 39 In the simplest case of segmental isomerizations modeled as diffusion, there are three transformations involved; cf. part (a) of Fig. 1. First, the fixed transformation from the principal axis system (PAS) to the intermediate system, associated with the corresponding lipid chain segment, is described by the Euler angles \( \Omega_{PI} \). For the case of methane groups, the \( z \) axis of the internal frame is taken as the normal to the H–C–H plane, and, therefore, it may be assumed that \( \beta_{PI} = 90^\circ \). Second, the time-dependent transformation corresponding to the small-step diffusive motion of the segment with respect to the bilayer director frame is described by the Euler angles \( \Omega_{DP}(\ell) \). The final rotational transformation involves the fixed angles \( \Omega_{DL} \) (not shown), which describe the orientation of the average director \( \mathbf{n}_0 \) of the specimen as a whole with respect to the laboratory frame, associated with the external magnetic field \( \mathbf{B}_0 \).

Using the generalized approach of Brown, 18 treatment of the segmental isomerizations in terms of rotational diffusion gives the following result for the spectral densities, referred to as the full segmental diffusion model (full model I), 16, 25

\[
J_m(\omega) = \sum_r \sum_n \left[ D_{nr}^{(2)}(\Omega_{PI}) - \frac{m_0}{6} \left( D_{nr}^{(2)}(\Omega_{PI}) + D_{nr}^{(2)}(\Omega_{PI}) \right) \right]^2 \\
\times \left( |D_{nr}^{(2)}(\Omega_{ID})|^2 - |D_{nr}^{(2)}(\Omega_{ID})|^2 \delta_{\alpha\beta} \delta_{\alpha0} \right) \\
\times D_{nm}^{(2)}(\Omega_{DL})^2. \tag{3.1}
\]

Here the mean-squared reorientational amplitudes are denoted by \( \langle |D_{nr}^{(2)}(\Omega_{ID})|^2 \rangle = \langle |D_{nr}^{(2)}(\Omega_{ID})|^2 \delta_{\alpha\beta} \delta_{\alpha0} \rangle \). For a noncollective diffusion model, the correlation functions and spectral densities refer to an individual segment within the potential of the mean torque of the bilayer, and are independent of the size of the system. This is analogous to considering a canonical ensemble in which the angular brackets \( \langle \rangle \) refer to a time or ensemble average (ergodic hypothesis) \textit{(vide infra)}. The reduced spectral densities \( j_{rn}^{(2)}(\Omega_{ID}, \omega) \) are Lorentzians with correlation times \( \tau_{rn} \),

\[
j_{rn}^{(2)}(\Omega_{ID}, \omega) = \frac{2\tau_{rn}}{1 + (\omega \tau_{rn})^2}. \tag{3.2}
\]

For the general case of anisotropic rotational diffusion, the expression for the correlation times \( \tau_{rn} \) is

\[
\frac{1}{\tau_{rn}} = \left[ \alpha_{rn} + (\eta_{rn} - 1)r_{rn}^2 \right] D_{\perp}. \tag{3.3}
\]

where the parameters \( \alpha_{rn} \) and \( \eta_{rn} \) arise from the solution of the generalized diffusion equation in the presence of a potential of
mean torque,\textsuperscript{22,23,25} and $\eta_D = D_1/D_2$ is the anisotropy of the rotational diffusion tensor $D$. However, it has been shown that the $^2$H NMR relaxation is not strongly sensitive to the form of the restoring potential.$\textsuperscript{15,18,25,26}$

For the purposes of illustration, let us use the strong-collisional approximation\textsuperscript{22} [referred to as the simplified segmental diffusion model (I)] to express the results in closed form. Neglecting the potential one has $\alpha_{rr} \to 0$, so that $\tau_{rr} \to \tau_r$, which are independent of the third rotational Euler angle $\gamma_{ID}$ (the symmetric top limit), leading to

$$j^{(2)}_{rr} (\Omega_{ID}, \omega) \to j^{(2)}_{rr} (\Omega_{ID}, \omega) \equiv j_r (\omega).$$

(3.4)

Hence there are three different reduced spectral densities ($r = 0, 1, 2$). Moreover, for the case of systems such as vesicles and random multilamellar dispersions\textsuperscript{35} that possess spherical symmetry, one can average the last transformation in Eq. (3.1), leading to vanishing of the projection index $m$; thus $|D_{nm}^{(2)} (\Omega_{DL})|^2 \to |D_{nm}^{(2)} (\Omega_{ID})|^3 = \frac{1}{2}$. Now the $j_r (\omega)$ are independent of the index $n$, so that one can further use the identity $\sum_n |D_{nm}^{(2)} (\Omega_{ID})|^2 = 1$ to simplify Eq. (3.1) for the case of an axially symmetric residual coupling tensor ($\eta_k = 0$). This yields

$$J (\omega) = \frac{1}{2} \left[ |D_{00}^{(2)} (\Omega_{PD})|^2 \left( 1 - |D_{00}^{(2)} (\Omega_{ID})|^2 \right) j_0 (\omega) + 2 |D_{10}^{(2)} (\Omega_{PD})|^2 j_1 (\omega) + 2 |D_{20}^{(2)} (\Omega_{PD})|^2 j_2 (\omega) \right].$$

(3.5)

Let us now assume that $\beta_{PD} = 90^\circ$ (the angle between the C–H bond and the $z$ axis of the internal segmental frame), which is appropriate for methylene groups, leading to

$$J (\omega) = \frac{1}{2} \left( 1 - |D_{00}^{(2)} (\Omega_{ID})|^2 \right) j_0 (\omega) + 3 j_2 (\omega).$$

(3.6)

The value of the second-rank order parameter $D_{00}^{(2)} (\Omega_{ID})$ is found from the experimentally determined $^2$H NMR residual quadrupolar splitting,

$$\Delta \nu_Q = \frac{2}{5} \chi \eta S_{CD}.$$  

(3.7)

Here the observed order parameter $S_{CD}$ is given by

$$S_{CD} = S^{(2)} = \langle D_{00}^{(2)} (\Omega_{PD}) \rangle = D_{00}^{(2)} (\Omega_{PD})/D_{00}^{(2)} (\Omega_{ID}) = - \frac{1}{2} |D_{00}^{(2)} (\Omega_{ID})|,$$

(3.8)

which may be readily obtained by using the closure property and applying axial averaging about the macroscopic bilayer normal (director). Thus, given a symmetric top approximation in which $\beta_{PD} = 90^\circ$, the orientationally averaged spectral density in the case of segmental motions can be written as a weighted sum of two Lorentzians,

$$J (\omega) = \frac{1}{2 \pi} \left[ 1 - 4 S_{CD}^2 j_0 (\omega) + 3 j_2 (\omega) \right].$$

(3.9)

It is noteworthy that the above formula includes two fitting parameters, namely $\tau_r$ and $\tau_t$.

In the more complete treatment, the mean-squared reorientational amplitudes $\langle |D_{nm}^{(2)} (\Omega_{ID})|^2 - |D_{nm}^{(2)} (\Omega_{DL})|^2 \rangle^2 \times \delta_{m0} \delta_{n0}$ are expressed in terms of the potential $U (\beta_{ID})$ using the Boltzmann distribution,

$$\langle |D_{nm}^{(2)} (\Omega_{ID})|^2 \rangle^2 \times \delta_{m0} \delta_{n0} = \frac{\int_{0}^{\infty} \exp (- U (\beta_{ID})/kT) \sin \beta_{ID} d \beta_{ID}}{\int_{0}^{\infty} \sin \beta_{ID} d \beta_{ID}}.$$  

(3.10)

Alternatively, the mean-squared moduli $\langle |D_{nm}^{(2)} (\Omega_{ID})|^2 \rangle$ can be obtained using a Clebsch–Gordon series expansion of the order parameters $D_{j q n}^{(2)} (\Omega_{ID})$, where $j = 1, \ldots, 4$. Assuming a potential of mean torque, i.e., $U (\beta_{ID}) = - \lambda_1 (\cos \beta_{ID})$, where $\lambda_1$ is taken as positive and $\lambda_2 (\cos \beta_{ID})$ is a Legendre polynomial, and using Eq. (3.3) for the relaxation times $\tau_{rr}$, one has again two effective fitting parameters, viz. $D_1$ and $D_\perp$. Both the simplified and full segmental diffusion models will be used, as discussed later.

### B. Molecular diffusion model

So far only the local motions of the separate segments have been treated, and the flexible molecule as a whole has not been explicitly taken into account. Certainly motion of the molecule does occur, but one can expect that it takes place on a longer time scale. The molecular motions can be considered within the mean-field picture as small-step reorientations in the presence of a potential of mean torque; an average moment of inertia tensor for the entire molecule is assumed. The transformations needed to describe the spectral densities are shown in part (b) of Fig. 1. The first set of Euler angles $\beta_{PD}(t)$ pertain to a transformation of the static EFG tensor from the PAS to the intermediate frame; the second fixed set $\Omega_{IM}$ transforms the residual EFG tensor to the molecular frame; the third set $\Omega_{MD}(t)$ corresponds to the time-dependent orientation of the molecule with respect to the bilayer normal (director); and finally, $\Omega_{DL}$ (not shown) performs the fourth transformation from the fixed director frame to the laboratory frame.\textsuperscript{18,25} In calculating the spectral densities, one approach is to assume that the faster local segmental motions and slower molecular motions are statistically independent. One can then take the above statistical independence or time scale separation into consideration, and use a simple product approximation for the relevant second-rank order parameters, that is to say, $S^{(2)} = S_{t}^{(2)} S_{s}^{(2)}$, where $S_{t}^{(2)} = \langle D_{00}^{(2)} (\Omega_{PD}) \rangle D_{00}^{(2)} (\Omega_{IM}) = \langle D_{00}^{(2)} (\Omega_{PM}) \rangle$ and $S_{s}^{(2)} = \langle D_{00}^{(2)} (\Omega_{MD}) \rangle$. The generalized approach\textsuperscript{18} then gives

$$J_{m} (\omega) = \langle |D_{nm}^{(2)} (\Omega_{ID})|^2 \rangle^2 \sum_n |D_{nm}^{(2)} (\Omega_{IM}) - \eta D_{nm}^{(2)} (\Omega_{ID})| \frac{1}{\sqrt{6}} \left[ |D_{2m}^{(2)} (\Omega_{IM}) + D_{2m}^{(2)} (\Omega_{IM})| \right]^2$$

$$\times \left( \langle |D_{qm}^{(2)} (\Omega_{MD})|^2 \rangle^2 - \langle |D_{qm}^{(2)} (\Omega_{MD})|^2 \rangle^2 \delta_{q0} \delta_{m0} \right) |D_{nm}^{(2)} (\Omega_{DL})|^2.$$

(3.11)
which is referred to as the full molecular diffusion model (full model II). In Eq. (3.11), \( \eta_{\text{eff}}^f \) is the asymmetry parameter of the residual EFG tensor that is modulated by faster segmental motions; \( \langle D_{\text{qnn}}^{(2)}(\Omega_{MD}) \rangle \) - \( \langle D_{\text{qnn}}^{(2)}(\Omega_{MD}) \rangle \delta_{q0} \), \( \delta_{q0} \) denotes the mean-squared reorientational amplitudes; and \( j_0(\omega) \) are the reduced spectral densities for molecular diffusion with correlation times given by \( 1/\tau_{q0} = [\alpha_{qnn} + (\eta_D - 1) q^2]D_q \). Note that the overall motion of the lipid molecule on the spectral density. Therefore, the orientation of the internal enthalpic amplitudes; and \( \tilde{\sigma}_f \) denotes the observed order parameter determined from the \( ^{2}H \) NMR quadrupolar splitting. Eq. (3.7), is given for the case of \( \eta_{\text{eff}}^f = 0 \) by

\[
S_{\text{CD}}^{(2)} = \langle D_{\text{qnn}}^{(2)}(\Omega_{MD}) \rangle D_{\text{qnn}}^{(2)}(\Omega_{MD}) \frac{1}{S_{\text{CD}}^{(2)}} \left[ \left( 1 - S_{\text{CD}}^{(2)} \right) j_0(\omega) + 3j_2(\omega) \right].
\]

Therefore, the orientation of the residual coupling tensor with respect to the lipid axis can have an appreciable effect on the spectral density. Note that the overall motion of a lipid molecule (segment) can be decomposed into two motions: rocking in the presence of the potential, the \( j_0(\omega) \) term, and rocking plus rotation about the longer molecular (segmental) axis, i.e. wobbling, as included in the \( j_1(\omega) \) terms. The simplified molecular diffusion model thus includes a total of three fitting parameters, viz. \( S_1^{(2)} = \langle D_{\text{qnn}}^{(2)}(\Omega_{MD}) \rangle \), \( \gamma_0 \), and \( \gamma_2 \). In the full treatment of the molecular diffusion model (II), the mean-squared reorientational amplitudes \( \langle D_{\text{qnn}}^{(2)}(\Omega_{MD}) \rangle \delta_{q0} \delta_{q0} \) are given analogously to Eq. (3.10) for the segmental diffusion model (I), with a total of three fitting parameters, viz. \( \gamma_1 \), \( D_1 \), and \( D_2 \). The cases of both \( j = 1 \) (odd potential) and \( j = 2 \) (even potential of the Maier–Saupe form), as well as both the simplified and complete treatment of the molecular diffusion, will be used and discussed later.

### C. Comparison of diffusion models

At this juncture, one can draw a general conclusion about such noncollective diffusion models. Namely, they yield expressions for the spectral density as a sum of Lorentzians, corresponding to a discrete spectrum of correlation times. The correlation functions in the presence of orientational averaging (vesicles, multilamellar dispersions) are given, in the case of the segmental and molecular diffusion models, respectively, by the Fourier transforms of Eqs. (3.1) and (3.11). One obtains for the simplified segmental diffusion model (I) in the case of \( \beta_{\text{MD}} = 90^\circ \) that

\[
G(\tau) = \frac{1}{I_0} \left[ 1 - 4S_{\text{CD}}^{(2)} e^{-\pi/\tau_0} + 3e^{-\pi/\tau_2} \right],
\]

and for the simplified molecular diffusion model (II), in the case of \( \beta_{\text{MD}} = 90^\circ \), that

\[
G(\tau) = \frac{1}{5} S_{\text{CD}}^{(2)} \left[ (1 - S_{\text{CD}}^{(2)} e^{-\pi/\tau_0} + 3e^{-\pi/\tau_2} \right].
\]
times \(\tau_c\), are related to the diffusion tensor of the molecule (segment) and the mean-field potential in which the molecule (segment) undergoes hindered rotational diffusion.

In addition, more detailed treatments have been proposed \(18,24,41\) which include contributions from other motions, e.g., vesicle tumbling and internal motions, by adding them in the resulting expression for the observable relaxation rates, assuming statistically independent processes. For example, neglecting cross-correlations, the Zeeman (spin-lattice) relaxation rate can be expressed as\(^{25}\)

\[
R_{1Z} = R_{1Z}^{\text{int}} + R_{1Z}^{\text{mol}} + R_{1Z}^{\text{slow}}.
\]  

Here \(R_{1Z}^{\text{int}}\) is due to the faster internal motions, e.g., as given by the segmental diffusion model (I), and \(R_{1Z}^{\text{mol}}\) corresponds to the molecular diffusion model (II), Eq. (3.11). The third term \(R_{1Z}^{\text{slow}}\) accounts for additional slow motional processes such as vesicle tumbling or lipid lateral diffusion, and is described by a single Lorentzian with correlation time \(\tau_c\) that can be calculated from the spectral linewidths. The effect of including these terms in the noncollective models will be discussed in the subsequent sections of this paper.

IV. COLLECTIVE MODELS FOR BILAYER FLUCTUATIONS

Up to now we have taken into account only noncollective diffusion-type models for individual constituents of the lipid membrane. Let us go a step up in the hierarchy of motions, and examine collective fluctuations such as thermal excitations of the domains within the bilayer. Given a continuous spectrum of such excitations that undergo damped, first-order relaxation, one can formally write the spectral density as

\[
J(\omega) = \int_{\tau_{\text{min}}}^{\tau_{\text{max}}} W(\tau_c) \frac{2\tau_c}{1 + (\omega \tau_c)^2} d\tau_c,
\]  

where \(\tau_{\text{max}}\) and \(\tau_{\text{min}}\) are the upper and lower cutoffs for the distribution of correlation times.

The transformations used to describe the collective excitations, formulated within a continuum approximation as direct

\[
\langle D_{0\pm 1}^{(2)} (\Omega_{\text{ND}}, \tau + \tau) D_{0\pm 1}^{(2)} (\Omega_{\text{ND}}, \tau) \rangle \approx \frac{1}{2} \langle \delta n^\ast (r, t + \tau) \cdot \delta n (r, t) \rangle
\]

where the vector \(\delta n (r, t)\) indicates the deviation of the bilayer normal\(^{61}\) from its equilibrium position at point \(r\) and at time \(t\).

It is convenient to write the correlation function for director fluctuations, spatially averaged over the liquid–crystalline sample, in terms of the corresponding Fourier transform. Assuming first-order relaxation of the excitation modes, \(\delta n (q, t)\) with correlation times \(\tau_q\), and using Parseval’s theorem, one can express the correlation function as an integral over the \(q\) space, corresponding to the wave vectors of the thermal fluctuations,\(^{18,26,59}\)

\[
\langle \delta n^\ast (r, t + \tau) \cdot \delta n (r, t) \rangle
\]

\[
= \int_{q_{\text{min}}}^{q_{\text{max}}=\infty} \langle \delta n (q, t) \rangle^2 e^{-|q|^2 \tau_q} d^2 q.
\]  

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Here $d$ is the dimensionality of the space that depends on the number of deformation modes considered by the model, and $\mathbf{\delta n}(\mathbf{q}, t)$ is the Fourier transform of $\mathbf{\delta n}(\mathbf{r}, t)$, given by

$$\mathbf{\delta n}(\mathbf{q}, t) = \frac{1}{(2\pi)^d} \int \mathbf{\delta n}(\mathbf{r}, t) e^{-i\mathbf{q} \cdot \mathbf{r}} d^d\mathbf{r}. \quad (4.6)$$

The choice of the zero lower limit in Eq. (4.5) corresponds to neglecting the edge effects (a sample of infinite size), while the infinite upper limit neglects the cutoff for the wave vectors reciprocal to the average segmental dimensions. These limits are assumed to fall outside of the frequency range of interest, and thus do not appreciably influence the measured relaxation rates.

Now the deformation free energy of a liquid crystal can be expressed in the $\mathbf{q}$ space as a bilinear form in the wave vectors $\mathbf{q}$,

$$F = F_0 + \frac{1}{2V} \sum_{\mathbf{q}} \sum_{i=1,2} \sum_{j=1}^d K_i \mathbf{\delta n}_\alpha^2(\mathbf{q}) q_j^2. \quad (4.7)$$

The $K_i$ are the elastic constants in each direction of the wave vector components $q_i$. $\mathbf{\delta n}_\alpha(\mathbf{q})$ is the absolute value of the deviation of the normal from its average position in the Fourier space, $\alpha$ is a polarization of an excitation corresponding to two uncoupled modes, i.e., splay plus bend and twist plus bend, and $V$ is the sample volume. Replacing the time averaging by ensemble averaging (ergodic hypothesis) in Eq. (4.5) and applying the equipartition theorem to the modes in $\mathbf{q}$ space, where the deformation energy is quadratic, we find the mean-squared amplitudes,

$$\langle |\mathbf{\delta n}_\alpha(\mathbf{q}, t)|^2 \rangle = \frac{kT}{E(\mathbf{q})}, \quad (4.8)$$

where

$$E(\mathbf{q}) = \sum_{i=1}^d K_i q_i^2. \quad (4.9)$$

The correlation times are given by

$$\tau_q = \frac{\eta}{E(\mathbf{q})}, \quad (4.10)$$

where $\eta$ is the bilayer viscosity; it is assumed that the correlation times do not depend on the polarization of an excitation.

Substituting Eqs. (4.4), (4.5), (4.8), and (4.10) into Eq. (4.3), one obtains for the spectral density that

$$J_m(\omega) = \frac{3}{2} \left|\langle D^{(2)}_{m0}(\Omega_{PN})\rangle\right|^2 \frac{1}{(2\pi)^d} \int_{-\infty}^{\infty} \frac{kT}{E(\mathbf{q})} e^{-|q|/\eta} e^{-i\omega \tau} d^d\mathbf{q} d\tau \times \sum_{n=\pm 1} |D^{(2)}_{nm}(\Omega_{DL})|^2. \quad (4.11)$$

It should be emphasized that the angular dependence, contained in the terms $|D^{(2)}_{m\pm 1}(\Omega_{DL})|^2$, reflects the small-angle approximation used to derive Eq. (4.11). Next, assuming that the integral over $\mathbf{q}$ with infinite limits exists at all $\tau$, one can exchange the order of integration with respect to $\mathbf{q}$ and $\tau$, yielding

$$J_m(\omega) = \frac{3\omega}{2} \left|\langle D^{(2)}_{m0}(\Omega_{PN})\rangle\right|^2 \frac{1}{(2\pi)^d} \int_{-\infty}^{\infty} \frac{kT}{E(\mathbf{q})} \frac{1}{|q|^2} e^{-|q|/\eta} e^{-i\omega \tau} d^d\mathbf{q} d\tau \times \sum_{n=\pm 1} |D^{(2)}_{nm}(\Omega_{DL})|^2. \quad (4.12)$$

By making the substitution $q_i \rightarrow \omega^{1/2} q_i'$, where $i = 1, \ldots, d$, we obtain as a final result that

$$J_m(\omega) = \frac{3\omega}{2} \left|\langle D^{(2)}_{m0}(\Omega_{PN})\rangle\right|^2 \frac{kT}{(2\pi)^d} \frac{1}{\omega^{1-(d/2)}} \times \int_{-\infty}^{\infty} \frac{1}{|E(\mathbf{q}')/\eta|^2} e^{-|q'|/\eta} \frac{1}{|q'|^2} e^{-i\omega \tau} d^d\mathbf{q}' d\tau \times \sum_{n=\pm 1} |D^{(2)}_{nm}(\Omega_{DL})|^2. \quad (4.13)$$

Clearly, the integral in Eq. (4.13) is independent of frequency, given that the integration is performed over the infinite $\mathbf{q}'$ space.

Equation (4.13) may be applied to a broad range of physical systems. It is noteworthy that the characteristic frequency dependence does not change even if the elastic constants are different for the various deformation modes, since Eq. (4.13) is a general result for any bilinear form of the free energy. The frequency dependence of the NMR relaxation rates can thus be indicative of the types of collective fluctuations that occur in a liquid crystal. The dimensionality $d$ corresponds to the deformation types that can occur in the system. For example, the case of $d = 1$ corresponds to undulations of one-dimensional systems, including polymeric chains and biopolymers such as deoxyribonucleic acid (DNA). Equation (4.13) yields in the one-dimensional case an $\omega^{-3/2}$ dependence and can be compared to earlier analogous conclusions. The case of $d = 2$, including splay deformation modes only (twist modes are neglected due to the relatively large values of the corresponding elastic constants), pertains to a smecticlike picture of a lipid bilayer and is referred to herein as the flexible surface model (model III). In the case of vesicles and multimembrane dispersions (powder-type samples), the projection index $m$ vanishes due to vesicle tumbling and/or translationally induced rotations of the lipids over the curved membrane surface, and Eq. (4.13) leads to

$$J(\omega) = \frac{S_{CD}^2}{\omega} \frac{D'}{\omega}. \quad (4.14)$$

Finally, a three-dimensional treatment ($d = 3$) corresponds to a smecticlike picture of the lipid bilayer interior, including splay, twist, and bend modes, and is designated as the
membrane deformation model (IV). Equation (4.13) yields then an $\omega^{-1/2}$ dependence, and the expression for the spectral density can be simply rewritten as

$$J(\omega) = \frac{S_{CD}^2}{\omega} \frac{D}{\sqrt{\omega}}. \quad (4.15)$$

Analogously to the molecular diffusion model (II), the expressions for the spectral density given by the collective fluctuation models, Eqs. (4.14) and (4.15), are scaled by the order parameter squared, which makes it possible to describe simultaneously the relaxation data from different segments along the acyl chains. The fitting coefficients $D'$ and $D$ correspond to the integral in Eq. (4.13), and contain the macroscopic parameters characterizing the membrane such as the viscosity $\eta$ and elasticity constant $K$. In the case of the simplest one-elastic approximation for $d = 2$, the explicit form for the coefficient $D'$ is

$$D' = \frac{3 \sqrt{2}}{5} \left( \frac{kT}{2KS_s^r} \right), \quad (4.16)$$

whereas the expression for $D$ becomes

$$D = \frac{3}{5} \left( \frac{kT}{\pi K^{3/2}S_s^{(r)^2}} \right) \sqrt{\frac{\eta}{2}}. \quad (4.17)$$

As described above, the correlation functions for the flexible surface model (2-D director fluctuations; model III) and the membrane deformation model (3-D director fluctuations; model IV) are the Fourier transform partners of the

<table>
<thead>
<tr>
<th>TABLE II: Summary of $^2$H NMR relaxation data for 1,2[3',3',13'-2H] DMPC vesicles and C3 acyl segment of DMPC-d$_{54}$ multilamellar dispersions in the liquid crystalline state at different frequencies and temperatures.</th>
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<td><strong>Frequency/ MHz</strong></td>
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<td>153.6</td>
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<td>190.5</td>
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</table>

$a$Data at 30 °C and 50 °C are for vesicles of 1,2[3',3',13'-2H] DMPC; the data at 40 °C refer to the C3 segment of multilamellar dispersions of DMPC-d$_{54}$ (perdeuterated acyl chains) in the liquid crystalline phase (Ref. 67). Phospholipids were synthesized and fatty acids were $^2$H labelled and characterized following standard procedures (Refs. 37, 89–91). $^2$H NMR studies were carried out as described in Refs. 66, 67. The standard deviations (S.D.) correspond to the random errors from the nonlinear regression fits.

$b$Estimated values interpolated from data at 30 °C and 50 °C assuming an Arrhenius activation dependence.

$c$Values of spectral densities at 40 °C obtained using Eqs. (2.16) and (2.17). In some cases, e.g., at 46.13 MHz and 95.25 MHz, Eqs. (2.16) and (2.17) have been used to calculate the approximate values of the spectral densities at twice the Larmor frequency.
TABLE II. Summary of \(^2\)H NMR relaxation data for 1,2[\(^7\)\(^\dagger\),\(^7\)\(^\dagger\),\(^2\)H] DMPC vesicles and C7 acyl segment of DMPC-\(d_{54}\) multilamellar dispersions in the liquid crystalline state at different frequencies and temperatures.

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<th>Frequency/ MHz</th>
<th>(T_{1Z}/\text{ms at 30 }^\circ\text{C} \pm \text{S.D.}^a)</th>
<th>(T_{1Z}/\text{ms at 50 }^\circ\text{C} \pm \text{S.D.}^a)</th>
<th>Preexponential factor, A/s</th>
<th>Activation energy, (E_a/\text{kJ mol}^{-1} \pm \text{S.D.}^a)</th>
<th>(T_{1Z}/\text{ms at 40 }^\circ\text{C} \pm \text{S.D.}^a)</th>
<th>(T_{1\omega}/\text{ms at 40 }^\circ\text{C} \pm \text{S.D.}^a)</th>
<th>(J(\omega)/10^{-11} \text{s at 40 }^\circ\text{C}^d)</th>
<th>(J(2\omega)/10^{-11} \text{s at 40 }^\circ\text{C}^d)</th>
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\(^a\) Data at 30 °C and 50 °C refer to vesicles of 1,2[\(^7\)\(^\dagger\),\(^7\)\(^\dagger\),\(^2\)H] DMPC; the data at 40 °C correspond to the C7 segment of multilamellar dispersions of DMPC-\(d_{54}\) (perdeuterated acyl chains) in the liquid crystalline phase (Ref. 67). The standard deviations (S.D.) reflect the random error from the nonlinear regression fits.

\(^b\) Values interpolated from data at 30 °C and 50 °C given an Arrhenius activation dependence.

\(^c\) Estimated values from interpolating the data at neighboring frequencies.

\(^d\) Values of spectral densities at 40 °C calculated using Eqs. (2.16) and (2.17). In some cases, e.g., at 46.13 and 95.25 MHz, Eqs. (2.16) and (2.17) have been used to obtain the approximate values of the spectral densities at double the Larmor frequency.

The above corresponds to the asymptotic behavior of the correlation function for model IV can be written as

\[
G(\tau) = \frac{S_{CD}^2}{\sqrt{2\pi}} \frac{D}{\sqrt{\tau}}
\]

V. ANALYSIS OF EXPERIMENTAL NMR RELAXATION DATA

To investigate the contributions of different types of motions to the observable NMR relaxation rates, \(^2\)H \(R_{1Z}\) data for vesicles of DMPC specifically deuterated at positions C3 and C7 of the lipid acyl chains\(^66\) and \(^2\)H \(R_{1Z}\) and \(R_{1\omega}\) data for DMPC-\(d_{54}\) multilamellar dispersions\(^67\) have been analyzed in conjunction with natural abundance \(^1\)C NMR \(R_{1Z}\) rates for DMPC vesicles (this work).\(^68\) For the multilamellar dispersions the inversion recovery method followed by the quadrupolar echo\(^5,69\) was utilized for the \(^2\)H \(R_{1Z}\) measurements, and the broadband Jeener–Broekaert sequence\(^67,70,71\) was used for \(^2\)H \(R_{1\omega}\) measurements. The \(^2\)H \(R_{1Z}\) data were acquired at 12 external magnetic field strengths, ranging...
from 0.382 to 14.6 T, corresponding to a frequency range from \( \omega_0/2\pi = 2.50 - 95.3 \) MHz. In addition, \( ^2\text{H} \) \( R_{1Q} \) data were measured at 15.3, 46.1, and 76.8 MHz. The \( ^2\text{H} \) NMR data are summarized in Tables I and II. From the values of the \( ^2\text{H} \) NMR quadrupolar splittings for multilamellar dispersions of DMPC-d5 with perdeuterated acyl chains,\(^67,72 \) one can determine the orientational order parameters, \( S_{CD} \). For the C3 and C7 segments, these are, respectively, 0.209 and 0.190 at 30 °C; 0.200 and 0.186 at 40 °C; and 0.181 and 0.168 at 50 °C.\(^67,72 \) By contrast, in vesicles, due to the fast motional averaging on the NMR scale the residual quadrupolar splittings are averaged to zero. The \( ^1\text{C} \) \( R_{1Z} \) relaxation rates (Zeeman order of magnetic dipolar interaction) were measured by using the inversion recovery technique under conditions of proton decoupling at six external magnetic field strengths, from 1.40 to 17.6 T, corresponding to the range 15.0–188.7 MHz and are summarized in Table III. It should be noted that the \( ^1\text{C} \) inversion recovery curves can be described satisfactorily by a single relaxation time, which allows one to neglect the possible influences of cross-correlations involving the protons in methylene groups.\(^52 \)

Theoretical spectral densities for the various models, given by Eqs. (3.1), (3.9), (3.11), (3.15), (4.14), and (4.15), were fit simultaneously to the \( ^2\text{H} \) \( R_{1Z} \) data corresponding to the C3 and C7 acyl chain segments, using the Levenberg–Marquardt algorithm, and then tested for their ability to predict the experimental \( ^1\text{C} \) \( R_{1Z} \) data by using Eq. (2.18). The fits were weighted by the inverse standard deviation squared.\(^73 \) As mentioned earlier, due to the isomorphism of the coupling Hamiltonians, both the quadrupolar (\(^2\text{H} \)) and dipolar (\(^1\text{C} \)) relaxation rates for a given segment can be expressed in terms of the same spectral densities of motion. Both the simplified and complete diffusion models were used, including potentials of mean torque having odd or even parity, and the effect of including vesicle tumbling in the noncollective model, cf. Eq. (3.18), was studied.\(^24,66 \) In the latter case, the \( ^2\text{H} \) and \( ^1\text{C} \) \( R_{1Z} \) data for segments C3 and C7 were fit simultaneously by using Eq. (3.18) with an odd potential of mean torque, i.e., \( U(\beta_MD) = -\lambda_1 P_1(\cos \beta_M) \).

An additional aspect is that the values of the orientationally averaged spectral densities of motion \( J(\omega) \) were calculated at different frequencies from the \( ^2\text{H} \) \( R_{1Z} \) and \( R_{1Q} \) data at 40 °C. As follows from the expressions for \( R_{1Z} \) and \( R_{1Q} \), Eqs. (2.16) and (2.17), one can determine not only the value of the spectral density at a given Larmor frequency \( \omega_0 \), but also its value at 2\( \omega_0 \). This extends the effective frequency range up to 191 MHz in the case of \(^2\text{H} \) NMR. All the available \( R_{1Q} \) data\(^67 \) have been measured at 40 °C. Thus, to obtain additional \( R_{1Z} \) values at 40 °C, the 30 °C and 50 °C data were interpolated by assuming an Arrhenius temperature dependence, which is obeyed within acceptable accuracy for the \(^2\text{H} \) NMR relaxation rates.\(^3 \) This involves some propagation of errors, but may still be used for illustration of the experimental frequency dispersion of the spectral densities and relaxation rates at 40 °C. From the results of fitting the relaxation data, theoretical correlation functions were calculated for the noncollective molecular diffusion and 3-D collective fluctuation models, by Fourier transforming Eq. (3.11) and using Eq. (4.18).

VI. RESULTS

A. Data analysis for segment C3 of DMPC in the liquid–crystalline state at 30 °C

A relatively broad range of frequency- and temperature-dependent relaxation data for lipid bilayers in the liquid crystalline state has been considered in this research. As can be seen from Fig. 2, the experimental \(^2\text{H} \) \( R_{1Z} \) relaxation rates decrease smoothly and monotonically with increasing frequency over the entire range studied.\(^66 \) This characteristic frequency dependence of the relaxation appears to be a general feature of the dynamics of lipid bilayers in the liquid crystalline state.\(^6,18,55,66 \) The NMR frequency range presently available does not allow one to detect any leveling off of the \( R_{1Z} \) relaxation rates at either low or high frequencies, e.g., as seen in the case of surfactant micelles.\(^74 \) In addition, Fig. 2 shows a comparison of the theoretical fits to the simplified segmental diffusion model (I) and the molecular diffusion model (II). Both an isotropic and anisotropic rotational diffusion tensor has been considered in each case. First, the diffusion asymmetry parameter has been held fixed at a value of \( \eta_D = 1 \) (isotropic diffusion), which in the simplified treat-

<table>
<thead>
<tr>
<th>TABLE III. Summary of natural abundance (^1\text{C} ) NMR relaxation data for vesicles of DMPC in the liquid crystalline state.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Frequency/MHz</strong></td>
</tr>
<tr>
<td>15.04</td>
</tr>
<tr>
<td>25.15</td>
</tr>
<tr>
<td>45.29</td>
</tr>
<tr>
<td>90.50</td>
</tr>
<tr>
<td>150.8</td>
</tr>
<tr>
<td>188.7</td>
</tr>
</tbody>
</table>

\(^a \) Data are from Ref. 68; the 150.8 and 188.7 MHz data were measured using a Varian Unity 600 spectrometer (Lund University) and a Bruker AMX 750 spectrometer (University of Munich), respectively.

\(^b \) The unresolved \((\text{CH}_2)_n\) peak in the \(^1\text{C} \) NMR spectra corresponds to segments C4-C11 of the DMPC acyl chains.
It can be seen that the data are not described by a single Lorentzian, neither in the case of segmental diffusion, nor in the isotropic and anisotropic cases; cf. Fig. 2. The anisotropic molecular diffusion model (II) in its simplified form fits the data fairly well, except at the lower frequencies (cf. the text).

FIG. 2. Theoretical fits of 2H spin-lattice relaxation rates ($R_{1\text{Z}}$) for vesicles of 1,2[3’,3’-2H] DMPC i.e. deuterated at the C3 segment of both acyl chains, as a function of frequency (magnetic field strength), in the liquid crystalline state at 30°C (O). The 2H $R_{1\text{Z}}$ data (Table I) are fitted to a simplified isotropic segmental diffusion model (…); fitting parameters: $D_1 = D_{1\text{i}} = 1.48 \pm 0.04 \times 10^8\text{s}^{-1}$; a simplified anisotropic segmental diffusion model (…; $D_1 = 3.49 \pm 1.42 \times 10^8\text{s}^{-1}$; $D_{1\text{i}} = 1.75 \pm 0.04 \times 10^8\text{s}^{-1}$); a simplified isotropic molecular diffusion model (…); $D_1 = D_{3\text{i}} = 1.86 \pm 0.33 \times 10^8\text{s}^{-1}$; $S_{1\text{i}}^2 = 0.80 \pm 0.02$); and a simplified anisotropic molecular diffusion model (…; $D_1 = 2.53 \pm 0.28 \times 10^8\text{s}^{-1}$; $D_{3\text{i}} = 5.31 \pm 0.42 \times 10^8\text{s}^{-1}$; $S_{1\text{i}}^2 = 0.715 \pm 0.015$). Note that the 2H $R_{1\text{Z}}$ relaxation rates of the lipid acyl chain segments cannot be fit by either an isotropic or anisotropic segmental diffusion model (I), or by an isotropic molecular diffusion model (II) having a single Lorentzian. However, the anisotropic molecular diffusion model (II) in its simplified form fits the data fairly well, except at the lower frequencies (cf. the text).

Data points, but rather predictions of the experimental 13C $R_{1\text{Z}}$ data using the 2H $R_{1\text{Z}}$ fitting parameters. For heuristic purposes, the simplified molecular diffusion model has been used, Eq. (3.12); it is shown later that the full treatment gives essentially the same results. As follows from Eq. (3.12), the number of terms in the spectral density depends on the orientation of the residual coupling tensor given by the value of $\beta_{IM}$. First one should note that the choice of $\beta_{IM} = 0°$ yields one Lorentzian and does not fit the frequency-dependent data at all (Fig. 3). Another assumption would be a perpendicular orientation of the residual coupling tensor with respect to the long molecular axis, i.e. $\beta_{IM} = 90°$. This leads, in the symmetric top approximation, to a sum of two Lorentzians, cf. Eq. (3.15), and yields satisfactory fits to the data; cf. Fig. 3. An intermediate value of $\beta_{IM} = 45°$ gives a sum of three Lorentzians, and results in equal quality of the fit to the 2H $R_{1\text{Z}}$ data, but a worse prediction of the 13C $R_{1\text{Z}}$ data at higher frequencies. It is noteworthy that letting $\beta_{IM}$ vary as a free parameter yields $\beta_{IM} = 80.2°$, which implies an orientation of the residual coupling tensor nearly perpendicular to the molecular long axis. However, the low-frequency dispersion is incompletely described by the molecular diffusion model (II), which may be due to the presence of additional types of motions. For example, the inclusion of vesicle tumbling or translational lipid diffusion over their curved surfaces (which are stochastically equivalent) in the spectral density of motion may account for part of the low-frequency dispersion (<10 MHz) (vide infra).

Table IV. 2H NMR spectral linewidths for vesicles of 1,2[3’,3’-2H] DMPC and 1,2[7’,7’-2H] DMPC in the liquid–crystalline state.

<table>
<thead>
<tr>
<th>C3 segment</th>
<th>C7 segment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linewidth/Hz</td>
<td>30°C</td>
</tr>
<tr>
<td>Correlation time,a</td>
<td>4.34</td>
</tr>
<tr>
<td>$\tau_{1\text{Z}}$/10⁻¹ s</td>
<td></td>
</tr>
</tbody>
</table>

aMeasured as full widths at half-height assuming a Lorentzian lineshape (Refs. 66, 92).

bThe 2H NMR spectra of DMPC vesicles were obtained at 61.4 MHz. Correlation times were calculated using the relation $R_2(\text{H}) = \frac{1}{2\pi \chi_0^2 \rho(0)} = \frac{1}{2\pi \chi_0^2 \rho(0)}$.
are shown to the simplified anisotropic molecular diffusion model.

FIG. 3. Theoretical fits of $^2$H correlation times, describes the low-frequency data within the fluctuation model, which considers a broader spectrum of dispersion of the relaxation rates. In contrast, the 3-D collective molecular diffusion model to account for the low-frequency dis-

slower motions need to be included in the noncollective model. (•), part (a), and prediction of $^{13}$C $R_{1Z}$ natural abundance data corresponding to the C3 segment of vesicles of DMPC (•), part (b), in the liquid crystalline state at 30 °C. Fits of the $^2$H and $^{13}$C $R_{1Z}$ data (Tables I and III) are shown to the simplified anisotropic molecular diffusion model (II) as a function of the orientation of the residual coupling tensor with respect to the intermediate frame: $\beta_{IM}=0^\circ$ (---; fitting parameters: $D_1=1.86\pm0.33\times10^8\text{ s}^{-1}$, $S_2^{(1)}=0.430\pm0.014$, $\beta_{IM}=45^\circ$ (-----; $D_1=3.68\pm0.29\times10^8\text{ s}^{-1}$, $D_2=1.36\pm0.08\times10^8\text{ s}^{-1}$, $S_2^{(1)}=0.658\pm0.010$, $\beta_{IM}=90^\circ$ (----; $D_1=2.53\pm0.28\times10^7\text{ s}^{-1}$, $D_2=5.31\pm0.42\times10^6\text{ s}^{-1}$, $S_2^{(1)}=0.715\pm0.015$), and $\beta_{IM}$ being a free parameter [---; $\beta_{IM}$ (optimal) = 80.2° $\pm22.5^\circ$, $D_1=2.23\pm0.38\times10^7\text{ s}^{-1}$, $D_2=7.62\pm0.16\times10^6\text{ s}^{-1}$, $S_2^{(1)}=0.735\pm0.028$]. The one-Lorentzian model with $\beta_{IM}$ fixed at 0° is clearly unsuccessful in fitting the $^2$H $R_{1Z}$ data, part (a), whereas the three-Lorentzian model with $\beta_{IM}$ held constant at 45° fails to predict the $^{13}$C data at higher frequencies, part (b). The simplified two-Lorentzian molecular diffusion model ($\beta_{IM}=90^\circ$) fits the data almost equivalently to the three-

Lorentzian model having $\beta_{IM}$ as a free parameter. Consequently, the choice of an perpendicular orientation of the residual coupling tensor with respect to the long molecular axis appears plausible.

bly well in the frequency range $>10\text{ MHz}$. Additional slower motions need to be included in the noncollective molecular diffusion model to account for the low-frequency dispersion of the relaxation rates. In contrast, the 3-D collective fluctuation model, which considers a broader spectrum of correlation times, describes the low-frequency data within the applicable accuracy without the need for more fitting parameters.

B. Simultaneous data analysis for segments C3 and C7 of DMPC at different temperatures

Moreover, simultaneous fitting of the relaxation rates corresponding to different segments at various temperatures can give further insights regarding the applicability of the various dynamical models.

Table I and III accounts for the data to within the applicable experimental accuracy.

FIG. 4. Theoretical fits of $^2$H $R_{1Z}$ data for vesicles of 1,2[3',3'-$^2$H] DMPC (•), part (a), and the prediction of $^{13}$C $R_{1Z}$ natural abundance data corresponding to the C3 segment of vesicles of DMPC (•), part (b), in the liquid crystalline state at 30 °C. The $^2$H and $^{13}$C $R_{1Z}$ data (Tables I and III) are fitted to the flexible surface model (2-D collective fluctuations; ---; fitting parameter $D'=0.557\pm0.039$) and the membrane deformation model (3-D collective fluctuations; ---; $D=2.51\pm0.02\times10^{-5}\text{ s}^{-1/2}$). The 2-D flexible surface model (III) fails to describe the $^2$H NMR data and predict the $^{13}$C NMR data, whereas the 3-D membrane deformation model (IV) accounts for the data to within the applicable experimental accuracy.
correlation times.\textsuperscript{18,75} Theoretical fits of the four dynamic models to the experimental \textsuperscript{2}H spin-lattice relaxation rates for both segments C3 and C7 of the DMPC acyl chains at 30 °C are shown in parts (a) and (b) of Fig. 5; the fitting parameters are summarized in Table V. Comparison of Figs. 3 and 5 reveals that the full diffusion models fit the data essentially the same as their simplified formulations given in terms of two Lorentzians (symmetric top approximation), Eqs. (3.9) and (3.15). The quality of the fits does not depend appreciably on the parity of the potential of mean torque, as pointed out by Trouard and co-workers.\textsuperscript{25} i.e. \( U(\beta_{MD}) = -\lambda_1 P_1(\cos \beta_{MD}) \) versus \( U(\beta_{MD}) = -\lambda_2 P_2(\cos \beta_{MD}) \), which results in only slightly different values for the fitting parameters: cf. Table V. Thus, the noncollective diffusion models are relatively insensitive to the explicit form of the orienting potential.\textsuperscript{18} Parts (a) and (b) of Fig. 5 shows that the models corresponding to segmental motions and two-dimensional director fluctuations fail to describe the \textsuperscript{2}H R\textsubscript{1Z} relaxation rates for the C3 and C7 segments of DMPC, thus reinforcing the conclusions of Sec. VI A. At higher frequencies, the segmental diffusion model (I) predicts a nearly constant value for the relaxation rates; while the flexible surface model (2-D director fluctuations; model III) does not fit the data at all. In contrast, the collective membrane deformation model (3-D director fluctuations; model IV) follows well the general behavior of the experimental values and requires only a single fitting parameter; cf. Eq. (4.15). The abilities of each model to predict theoretically the experimental \textsuperscript{13}C relaxation rates are presented in parts (c) and (d) of Fig. 5 for segments C3 and C7, the latter corresponding to the \((\text{CH}_2)_n\) resonance of DMPC, respectively. Again, the molecular diffusion model (II) and the membrane deformation model (IV) best predict the \textsuperscript{13}C R\textsubscript{1Z} data. Figure 6 shows a similar comparison of the fits to the four models at 50 °C, for segments C3 and C7 of DMPC vesicles. Here the membrane deformation model (IV) fits the \textsuperscript{2}H NMR data less satisfactorily than in Fig. 5, whereas the molecular diffusion model (II) predicts \textsuperscript{13}C R\textsubscript{1Z} values that are too low at the higher frequencies.

Yet another important observation is that according to the fitting results in Table V, the temperature behavior of the parameters given by the molecular diffusion model is physically unrealistic, since the values of the diffusion coefficients \( D_1 \) and \( D_4 \) decrease as temperature increases. Moreover, the molecular diffusion model (II) results in an increase of the molecular ordering with temperature as given by the slow order parameter \( S^2(2) = \langle D^{(2)}(\Omega_{MD}) \rangle \); cf. Table V. Therefore, even though the molecular diffusion model (II) and the membrane deformation model (IV) appear to describe the data equally well in the frequency range \( >10 \text{ MHz} \), the former gives an unrealistic temperature behavior of the fitting parameters. The same final fitting parameters have been obtained by using vastly different starting values, and, therefore, this behavior does not reflect local minima in the values of chi-squared.

To address the undesirable nonphysical temperature dependence of the fitting parameters, the influence of additional slow motions, e.g., due to vesicle rotational diffusion (tumbling), was included in the noncollective molecular diffusion model, assuming an odd potential of mean torque. Figure 7 shows the results of simultaneous fits of the full model (II) to the \textsuperscript{2}H and \textsuperscript{13}C R\textsubscript{1Z} data for segments C3 and C7 of the DMPC acyl chains. One approach is to directly estimate the contribution from such slower motions by using the experimentally measured parameters reported in the literature.\textsuperscript{66} For instance, taking the values for the coefficient of translational self-diffusion\textsuperscript{76,77} \( D_T = 3 \times 10^{-12} \text{ m}^2 \text{ s}^{-1} \); viscosity of water \( \eta = 797.7 \mu \text{ Pa s} \); \( T = 303 \text{ K} \); and the vesicle radius \( r_{ves} = 12.5 \text{ nm} \), the rotational correlation time for vesicle tumbling can be calculated from the Einstein–Stokes expression \( 1/\tau_c = 3kT/4\pi \eta r_{ves} + 6D_T/r_{ves}^2 \), and is equal to 1.32 \times 10^{-6} \text{ s} \). For the case of unrestricted isotropic diffusion, the contribution from vesicle tumbling is estimated to be \( R_{1Z} = 21.0 \text{ s}^{-1} \) at 2.50 MHz, the lowest value studied.\textsuperscript{78} At higher frequencies, even smaller values are found. Therefore, according to this approach the vesicle tumbling has only a minimal contribution to the R\textsubscript{1Z} relaxation rates, as calculated from the literature data, even at low frequencies where the experimental \textsuperscript{2}H R\textsubscript{1Z} values are about 200 s\textsuperscript{-1}, cf. Fig. 2. An alternative approach\textsuperscript{24} is to directly calculate the effective correlation time \( \tau_c \) for slow motions, such as vesicle tumbling, by using the relation between the spectral linewidth
### TABLE V. Summary of fitting parameters used in dynamical models.a

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C3 segment</th>
<th>C7 segment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 °C</td>
<td>40 °C</td>
</tr>
<tr>
<td>$S_{CD}^b$</td>
<td>0.209</td>
<td>0.200</td>
</tr>
<tr>
<td>$\eta_s$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$\sigma_0^r$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Segmental diffusion $D_s/10^5$ s$^{-1}$</td>
<td>1.75</td>
<td>2.25</td>
</tr>
<tr>
<td>(odd potential), $\lambda_1 P_1 (\cos \beta_{MD})$</td>
<td>±0.04</td>
<td>±0.60</td>
</tr>
<tr>
<td>(even potential), $\lambda_2 P_2 (\cos \beta_{MD})$</td>
<td>±0.13</td>
<td>±0.51</td>
</tr>
<tr>
<td>$\lambda_1$</td>
<td>±0.14</td>
<td>±0.19</td>
</tr>
<tr>
<td>$S_s^{(2)} (D_{s0}^2 (\Omega_{MD}))^d$</td>
<td>0.707</td>
<td>0.832</td>
</tr>
<tr>
<td>Molecular diffusion $D_{MD}/10^5$ s$^{-1}$</td>
<td>6.88</td>
<td>4.95</td>
</tr>
<tr>
<td>(odd potential), $\lambda_1 P_1 (\cos \beta_{MD})$</td>
<td>±0.13</td>
<td>±0.51</td>
</tr>
<tr>
<td>$\lambda_2$</td>
<td>±0.51</td>
<td>±0.157</td>
</tr>
<tr>
<td>$S_s^{(2)} (D_{s0}^2 (\Omega_{MD}))^d$</td>
<td>0.711</td>
<td>0.832</td>
</tr>
<tr>
<td>Molecular diffusion plus vesicle tumbling (odd potential), $D_{s0}/10^9$ s$^{-1}$</td>
<td>8.11</td>
<td>3.94</td>
</tr>
<tr>
<td>$\lambda_1$</td>
<td>±0.58</td>
<td>±0.166</td>
</tr>
<tr>
<td>$S_s^{(2)} (D_{s0}^2 (\Omega_{MD}))^d$</td>
<td>0.691</td>
<td>0.624</td>
</tr>
<tr>
<td>Flexible surface $D/10^5$ s$^{-1}$</td>
<td>4.11</td>
<td>0.17</td>
</tr>
<tr>
<td>$R_{MD} / \text{s}^{-1} e$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Membrane deformation $D/10^{-5}$ s$^{-1}$</td>
<td>2.51</td>
<td>1.88</td>
</tr>
<tr>
<td>(3-D director fluctuations)</td>
<td>±0.02</td>
<td>±0.05</td>
</tr>
</tbody>
</table>

aAll curve fitting has been done by using the Levenberg–Marquardt algorithm. The fits have been statistically weighted by the inverse square of the standard deviation (Ref. 73).
bValues obtained from quadrupolar splittings of experimental $^2$H NMR spectra using Eq. (3.7) (Refs. 67, 72).
cParameter held constant at this value.
dValues obtained using Eq. (3.10).
eAverage correlation times from Table IV corresponding to an additional slow motional process.
and the transverse relaxation time given by the formula
\[ R_2(\text{H}) = \frac{9}{8}\pi^2 \chi^2 \omega^2 J(0) = \frac{9}{8}\pi^2 \chi_Q^2 \Delta^2 \tau_c \] (Table IV). This yields substantially shorter correlation times \( \tau_c \) than those obtained from the literature values for vesicle tumbling; cf. Table V, and results in a larger contribution to the deuterium spin-lattice relaxation rates at lower frequencies.24

By using the latter approach,24 Eq. (3.18) was used to fit both the \(^2\text{H}\) and \(^{13}\text{C}\) \( R_{1Z} \) data; the fitting parameters are summarized in Table V. The value of \( R_{1Z}^{\text{fast}} \), corresponding to fast internal motions, could not be determined within an applicable accuracy from the fit, and, therefore it has been held fixed at zero.25 As can be seen from Fig. 7 and Table V, in addition to the improved quality of the fit at frequencies <10 MHz by including additional slow motions, e.g., vesicle tumbling, the physically unrealistic temperature dependence of the diffusion coefficients and segmental ordering is eliminated. Thus, inclusion of the \(^2\text{H}\) NMR lineshape data for DMPC vesicles (Table IV) improves the physical consistency of the noncollective molecular diffusion model.24 On the other hand, the relatively large discrepancy between the vesicle tumbling correlation times obtained from the literature values and those determined from the lineshapes (Table IV) makes it difficult to conclude whether the relaxation at low frequencies is indeed due to the vesicle tumbling, or to some other slow motional process.

C. Orientationally averaged spectral densities of motion for segments C3 and C7 of DMPC

As mentioned above, given the values of \( R_{1Z} \) and \( R_{1Q} \), one can directly calculate the values of the spectral densities of motion \( J(\omega) \) and \( J(2\omega) \) by using Eqs. (2.16) and (2.17) in the case of spherical orientational averaging (vesicles, multilamellar dispersions). As a new aspect of the current research, the values of the spectral densities have been explicitly calculated from the \(^2\text{H}\) \( R_{1Z} \) and \( R_{1Q} \) data for DMPC at 40 °C within the frequency range of \( \omega_{1Z}2\pi = 7.50 \text{ MHz} \) to \( \omega_{1Z}2\pi = 191 \text{ MHz} \) (Fig. 8). Although some propagation of errors is involved, the principles of the approach are illustrated. The data correspond well to the theoretical spectral densities previously obtained from fitting the \(^2\text{H}\) \( R_{1Z} \) experimental values directly, albeit with less data reduction (Table V). Here again, the molecular diffusion model (II) and the membrane deformation model (3-D director fluctuations; model IV) best describe the spectral densities of motion calculated from the experimental \(^2\text{H}\) \( R_{1Z} \) and \( R_{1Q} \) rates. Due to the significant data reduction involved in obtaining the values of \( J(\omega) \) and \( J(2\omega) \), the above results cannot be considered as an independent conclusion. However, this approach can be followed up in future studies.

FIG. 6. Summary of results of fitting the \(^2\text{H}\) \( R_{1Z} \) relaxation rates of 1,2[\(^3\text{H},\text{H}\)] DMPC and 1,2[\(^7\text{H},\text{H}\)] DMPC vesicles (■), parts (a) and (b), and prediction of \(^{13}\text{C}\) \( R_{1Z} \) natural abundance data corresponding to the C3 and C7 segments of DMPC vesicles (■), parts (c) and (d), in the liquid crystalline state at 50 °C. Full segmental diffusion model (---); full molecular diffusion model (-----); 2-D flexible surface model (-----); and the 3-D membrane deformation model (-----). The data are presented in Tables I–III, and the fitting parameters are included in Table V. As in Fig. 5, the molecular diffusion model (II) and membrane deformation model (3-D fluctuations; model IV) fit the \(^2\text{H}\) \( R_{1Z} \) data and predict the \(^{13}\text{C}\) \( R_{1Z} \) data more accurately than the segmental diffusion model (I) and the flexible surface model (2-D fluctuations; model III).

FIG. 7. The effect of including an additional slow motional term on fits of the full molecular diffusion model (II) to the \(^2\text{H}\) and \(^{13}\text{C}\) \( R_{1Z} \) relaxation dispersion for vesicles of DMPC in the liquid crystalline state at 30 °C (■) and 50 °C (■). The \(^2\text{H}\) \( R_{1Z} \) relaxation rates for 1,2[\(^3\text{H},\text{H}\)] DMPC and 1,2[\(^7\text{H},\text{H}\)] DMPC are shown in parts (a) and (b), and predictions of \(^{13}\text{C}\) \( R_{1Z} \) natural abundance data corresponding to the C3 and C7 segments in parts (c) and (d). The data are included in Tables I–IV, and the effective correlation times and fitting parameters are given in Tables IV and V. Inclusion of an additional slow motion, e.g., due to the vesicle tumbling in the noncollective molecular diffusion model, improves the quality of the fit and eliminates the nonphysical dependence of the fitting parameters as a function of temperature (cf. the text).
D. Correlation functions for noncollective and collective models

Finally, the orientationally averaged theoretical correlation functions obtained from the results of fitting the various dynamical models to NMR observables are presented in Fig. 9. The full molecular diffusion model (II) yields a relatively slow decay rate of the correlation function, which is a consequence of the relatively small molecular diffusion coefficients; cf. Table V. By contrast, the correlation functions for the membrane deformation model (3-D director fluctuations; model IV) exhibit a relatively fast decay within the first 100 ps, which is the result of a $\tau^{-1/2}$ dependence due to the broad continuous spectrum of excitations considered by the model; Eq. (4.18). Although the molecular diffusion model and the 3-D director fluctuation model yield spectral densities that describe the $^{13}$C and $^2$H $R_{1Z}$ data as a function of frequency comparably well, as can be seen from Fig. 9, their correlation functions (the Fourier transforms of the spectral densities) are rather different. Nonetheless, the curves approach each other at times longer than $10^{-9}$ s, i.e., in the time scale reciprocal to the frequency range in which the models are fitted. Since the value of $R_{1Z}$ corresponding to fast internal motions cannot be determined unambiguously from the fits, it is impossible to quantify any initial decay of the correlation function in the case of the noncollective molecular diffusion model (II). The correlation functions given by the membrane deformation model (IV) are comparable to the results of recent molecular dynamics simulations, which also show a significant decay within several tens of picoseconds.\textsuperscript{33}

VII. DISCUSSION

In the present work we have investigated the major features of the nuclear spin relaxation in lipid membranes by considering a broad spectrum of frequency- and temperature-dependent $^2$H and $^{13}$C NMR relaxation data in the MHz range. A representative phospholipid, DMPC, has been studied in the liquid crystalline phase, including different positions in the acyl chains for both unilamellar vesicles and multilamellar dispersions. In the case of $^2$H and $^{13}$C NMR,
the nuclear spin–relaxation is due to orientational fluctuations of the coupling tensor corresponding to the quadrupolar or dipolar interactions, respectively. These fluctuations can originate from relatively fast local motions, such as trans-gauche rotational isomerizations, and low-frequency torsional oscillations of the lipid acyl chains, glycerol backbone, and polar head groups. Alternatively, slower motions may be important, including hindered rotational diffusion of the flexible lipid molecules within the bilayer, and collective deformations of the membrane treated as a continuous medium. Dynamical models involving noncollective and collective fluctuations have been treated in closed form, and used to fit the $^2$H and $^{13}$C $R_{1Z}$ relaxation rates of DMPC bilayers as a function of frequency (magnetic field strength). An important aspect is that one can unify the independent $^2$H and $^{13}$C $R_{1Z}$ data for lipid bilayers in the liquid–crystalline state in terms of the corresponding spectral densities of motion. Thus, knowledge of the $^2$H $R_{1Z}$ relaxation rates enables one to predict the $^{13}$C $R_{1Z}$ relaxation rates for the same acyl chain segment, and vice versa, in terms of an appropriate motional model.

A. Model-free aspects

A general conclusion is that the frequency dependence of the $^2$H and $^{13}$C $R_{1Z}$ relaxation rates does not originate from local motions alone, which modulate the static coupling tensor. Rather, the predominant contribution in the MHz range arises from slower motions that modulate the residual coupling tensor remaining or left over from the local motions. It is important to recognize that the mean-squared amplitudes and correlation times of the motions that predominantly influence the relaxation and associated spectral densities may, in fact, correspond to physically different types of motion. For example, the relaxation can include a contribution from fast motions of a local nature that preaverage the coupling tensor to residual values, which, in turn, are further modulated by additional slower motions of larger amplitude. The contributions of these faster and slower motions to the observed NMR relaxation rates can be inherently different; they both influence the relaxation, but in characteristically different ways. Namely, the mean-squared amplitudes reflect the preaveraging due to fast local motions, and include the further averaging over the slower time scale; whereas the motional correlation times are determined by the slower motions alone within the MHz frequency range. Hence, the local motions affect the relaxation through their influence on the residual coupling parameters, corresponding to the motional mean-squared amplitudes, and not on the correlation times, as previously discussed. Moreover, the local mean-squared amplitudes can vary as a function of position in the lipid molecule as described by the well-known order parameter ($S_{CD}$) profiles along the acyl chains; whereas the correlation times due to the slow motions are most likely similar or identical for the different segment positions of the phospholipid molecules within the bilayer. As a consequence, the variation in the relaxation rates along the acyl chains is governed mainly via the mean-squared amplitudes due to local motions, such as trans-gauche acyl chain isomerizations, which reflect the equilibrium properties on a short time scale. On the other hand, the correlation times are due to the slower motions, which manifest the dynamics over the longer time scale.

B. Physical features of noncollective and collective models for lipid membrane dynamics

Herein the following question has been addressed: what is the nature of the motions that predominantly govern the nuclear spin relaxation of membrane lipid bilayers in the MHz range? Four different motional models have been considered: (i) a formulation of local motions in terms of a segmental diffusion model (alternatively, jump models for rotational isomerizations can be considered) (model I); and three formulations for the slower motions, viz. (ii) a molecular diffusion model for noncollective motions of the flexible lipids relative to the potential of mean torque of the bilayer (mean-field picture; model II), (iii) a simple flexible surface model for collective 2-D fluctuations of the membrane (smecticlike picture; model III), and finally (iv) a membrane deformation model for collective 3-D fluctuations of the bilayer (nematiclike picture; model IV). The diffusion models are characterized by a discrete spectrum of correlation times, whereas the collective models encompass a continuum of elastic bilayer disturbances. Both the noncollective molecular diffusion model (II) and the three-dimensional membrane deformation model (IV) describe the relaxation data for particular acyl chain positions (segments) at a given temperature, whereas the segmental diffusion model (I) and the two-dimensional flexible surface model (III) do not fit the data at all. In addition, the asymptotic behavior of the correlation functions for the C3 and C7 acyl chain segments has been obtained for both the noncollective molecular diffusion model (II) and alternatively the 3-D collective fluctuation model (IV). In the case of either model II or model IV, the rather small contribution to the spectral density from faster segmental motions is consistent with the notion that the local microviscosity of the bilayer hydrocarbon interior is comparable to that of a liquid n-paraffin, such as n-hexadecane.

First let us consider the noncollective models that give rise to a sum of Lorentzians, corresponding to a discrete spectrum of correlation times, due to treatment of the segmental isomerizations or molecular reorientations as a diffusion process. As discussed above, the segmental diffusion model (I) can be essentially ruled out based on the results of the fits to the present data. This indicates the need for a dynamical model in which both internal motions and overall motion are explicitly treated. One possibility is to consider a molecular diffusion model (II) for the reorientations of the lipid molecules within the potential of mean torque of the bilayer. Such model is formally analogous to the segmental diffusion model in which the residual coupling parameters replace the static values; collective modes are not treated explicitly, but rather a potential of mean torque (mean-field picture) is introduced. The flexible lipid mol-
molecules are assumed to reorient analogously to a rigid rotor, such that the moments of inertia are averaged over the time scale of the faster segmental motions. Although the degree of entanglement of the chains may in fact vary rather significantly as a function of depth in the bilayer, an effective or average rotational diffusion tensor for the entire molecule is assumed. The noncollective diffusion model is analogous to the dynamic cage model of Freed and co-workers, in which the various chain modes are considered in terms of restricted local diffusion of the acyl segments, together with the overall rotational diffusion of the lipids within the bilayer. As a rule, the molecular diffusion model (II) is able to fit the major features of the relaxation to a good degree of approximation.

To examine the molecular diffusion model (II) in more detail, simultaneous analysis of the data for different segments and temperatures has been carried out. The results show that if only the contribution from the noncollective molecular diffusion is considered, then a physically unrealistic temperature dependence of the fitting parameters is obtained, such as decreased diffusion rates and increased ordering with temperature. This is independent of the choice of the potential of mean torque, or whether an additional constant term due to fast internal motions is included. However, by including a contribution from an additional slow motional process such as vesicle tumbling or lipid lateral diffusion, one can eliminate this undesirable temperature behavior of the fitting parameters, and improve the fit at frequencies < 10 MHz. In addition, the quality of the fits to the molecular diffusion model does not depend strongly on the potential parity, i.e., whether an odd potential, \( U(\beta_{MD}) = -\lambda_1 P_1(\cos \beta_{MD}) \), or even potential, \( U(\beta_{MD}) = -\lambda_2 P_2(\cos \beta_{MD}) \), is used, or whether the strong collisional (symmetric top) approximation is employed. It follows that the model is relatively insensitive to the form of the orienting potential, as concluded earlier. The molecular diffusion model allows one to obtain dynamical information about the motion of lipid molecules from the results of fitting the NMR relaxation data. For instance, the values of the diffusion rates corresponding to the hindered rocking in the presence of a potential \( D_l \) are of the order of \( 10^{-1} \) s\(^{-1}\). The unrestricted rotation about the longer molecular axes is considerably faster, by almost two orders of magnitude \( D_t \) is of the order of \( 10^0 \) s\(^{-1}\). Also, the magnitude of the slow order parameter of about 0.7 would mean a relatively strong average orientation of the molecule with respect to the director axis, which is consistent with earlier work.

Let us next turn to the collective fluctuation models, which formally approximate the bilayer as a continuous medium having director excitation modes that decay as first-order relaxation processes. In the case of the flexible surface model (2-D director fluctuations; model III) splay deformations are principally involved that yield an \( \omega^{-1} \) frequency dependence. On the other hand, the membrane deformation model (3-D director fluctuations; model IV) formally considers three deformation modes, i.e., splay, twist, and bend, and is analogous to the treatment of a nematic liquid crystal, which results in an \( \omega^{-1/2} \) dependence.

Whereas the diffusion models yield an infinite but discrete spectrum of correlation times, corresponding to the eigenvalues of the diffusion operator, the collective models are given by an integral expression, Eq. (4.1), which encompasses a continuous distribution of correlation times. The distribution of correlation times for the various \( q \) modes \( W(\tau_q) \rightarrow W(\tau_q) \) can be obtained for the case of 3-D director fluctuations by assuming a single elastic constant \( K \) and expressing the wave vector amplitudes \( q \) in terms of the correlation times \( \tau_q \); cf. Eqs. (4.8)–(4.10). By using Eq. (4.1) it can be seen that \( W(\tau_q) \) has a \( \tau_q^{-3/2} \) dependence. The divergence of \( W(\tau_q) \) at short correlation times can be explained by the assumption of infinite upper limits for the director excitation modes, as implicitly used in applying the equipartition theorem to the thermal excitation amplitudes, Eq. (4.8). However, including the cutoffs would require the use of a Fourier series expansion, instead of an integral as in Eq. (4.5), which would make it mathematically difficult to obtain results in closed form. The \( \omega^{-1/2} \) dependence obtained within the MHz frequency range may be a special case of a model described by a more complicated, yet continuous, distribution of correlation times. On the other hand, inclusion of an additional contribution from slow motions such as molecular diffusion or vesicle tumbling in the membrane deformation model, which encompasses an effectively infinite continuous spectrum of correlation times, would possibly contradict the neglect of a low-frequency cutoff. This could introduce difficulties with regard to statistical independence or the separation of time scales.

One should note that the ability of the membrane deformation model to describe the relaxation dispersion of the \( ^2 \)H and \( ^1 \)C NMR data for the different acyl segments of lipid bilayers at different temperatures is of possible biophysical significance with regard to lipid membrane dynamics. For the case of a fluctuating bilayer, one can, in general, distinguish between (i) the free membrane limit, where the wavelengths of collective fluctuations are less than the separation between the bilayers (nematiclike picture), and (ii) the coupled membrane limit, where the wavelengths are large in comparison to the membrane thickness or bilayer separation (smecticlike picture). The former case may explain the existence of protrusion forces between the lamellae acting over short distances due to entropic confinement, whereas the latter may represent larger-wavelength undulations giving rise to interbilayer repulsions. The membrane deformation model (3-D director fluctuations; model IV) would correspond to the free membrane limit in which the fluctuations of individual bilayers are essentially uncoupled. As one example, fluctuations in the cross-sectional areas of lipids in each of the two monolayers could be correlated in a manner leading to collective deformations of the bilayer. On the other hand, the flexible surface model (2-D director fluctuations; model III) would encompass the longer-wavelength fluctuations. The different membrane coupling regimes and their influences on the spectral densities of motion have been recently studied in more detail by Halle and co-workers.
bilayer coupling are unlikely. Moreover, it has been found experimentally that in the mid-MHz range vesicles and multilamellar dispersions have nearly the same \(^2\)H and \(^{13}\)C R\(_{1Z}\) rates, which means that coupling between the bilayers does not appreciably affect the spin-lattice relaxation within this frequency range.\(^5,6\) An upper bound to the frequency range of surface deformations can be estimated theoretically by using the results of Halle and Gustafsson.\(^65\) Assuming a coefficient for the surface translational diffusion of the lipid molecules of \(D_1 \approx D_T = 3 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}\) and that the area per lipid \(^2\)H is \(a^2 = 65 \text{ Å}^2\), by substituting these values in Eq. (7.2) of Ref. 65 one obtains a value for the upper frequency cutoff of the transverse director modes of \(\omega_a = \pi^2 D_1/a^2 = \pi^2 (3 \times 10^{-12} \text{ m}^2 \text{ s}^{-1})/65 \times 10^{-20} \text{ m}^2 = 4.6 \times 10^7 \text{ rad s}^{-1} = 7.2 \text{ MHz}\). This implies that the weakly coupled regime corresponding to an \(\omega^{-1}\) dependence [cf. Eq. (7.22) of Ref. 65] occurs at frequencies much less than 7.2 MHz, which is below the frequency range investigated in this paper. Other regimes of strong membrane couplings\(^65\) fall into the kHz range and even below. Thus, our available frequency range does not allow one to further distinguish among the different membrane coupling regimes in the low- to mid-MHz range. Since the membrane deformation model is able to describe the \(^2\)H and \(^{13}\)C R\(_{1Z}\) dispersion in the MHz range, the nematickeye picture may be more appropriate for the description of lipid membrane dynamics than the smecticlike picture. This implies that, although membranes are two-dimensional structures over very large length scales,\(^28,41,65\) alternative formulations that have appeared in the literature. For example, a detailed model including smectolicorder fluctuations, molecular rotations, lateral diffusion, and translationally induced rotations has been fitted to the experimental proton (\(^1\)H) relaxation times of DMPC by using magnetic field-cycling techniques, as pioneered by Noack.\(^41\) Assuming that the collective fluctuations have an \(\omega^{-1}\) dependence (2-D director fluctuations), rather than an \(\omega^{-1/2}\) dependence (3-D director fluctuations), a very good fit has been obtained over a very broad frequency range from 100 Hz to 300 MHz. The results have led to the conclusion that collective fluctuations are detectable, but only at low frequencies (<100 kHz), so that the dispersion in the MHz range is governed by the various diffusional processes.\(^41\) Still, it seems unclear whether the excellent fit proves indeed the correctness of the physical picture involving several types of motions, or just reflects the influence of a relatively large number of fitting parameters (a total of 11 at a given temperature, after a substantial parameter reduction by using literature values for diffusion coefficients, characteristic reorientation times, etc). Moreover, the treatment of different motions in the expressions for the spin relaxation rates represents, to our understanding, a problem that may transcend a simple addition of Lorentzians: one may also need to consider cross-correlations.\(^60,85\) A somewhat analogous treatment of molecular diffusional motions has also been used by Halle,\(^24\) in which a six-parameter model was used to fit experimental \(^2\)H R\(_{1Z}\) data for segment C3 of DMPC as a function of frequency from 2.50 to 61.4 MHz.\(^66\) Again, it has been suggested that the relaxation in phospholipid bilayers is predominantly governed by hindered rotational diffusion of the flexible lipid molecules in the mid-MHz range, albeit with an additional contribution from vesicle tumbling in the low-MHz range. But it is difficult to say whether the low-frequency dispersion is due entirely to the vesicle tumbling or to another slow motional processes, such as lipid lateral diffusion, since the vesicle tumbling correlation times calculated from the NMR spectral linewidths\(^24\) differ substantially from those obtained using the Stokes–Einstein equation.\(^86\) Equivalently, the order parameters, obtained from the vesicle \(^2\)H NMR lineshapes by assuming the Stokes–Einstein equation for vesicle rotational diffusion, are about two-fold smaller than in the case of the corresponding lipid multilamellar dispersions,\(^86\) cf. Table IV. Therefore, further research including more extensive experimental measurements is needed to resolve the longstanding issue regarding the general applicability of noncollective versus collective models,\(^18\) for the nuclear spin relaxation of lipid bilayers in the MHz range.

C. Further discussion and comparison with the results of molecular dynamics simulations

The issue of whether a noncollective molecular diffusion model or a collective model for bilayer excitations is appropriate for explaining the dispersion of the nuclear spin relaxation rates of lipid bilayers in the MHz range has been debated in previous work.\(^4–6,18,24,41,42\) For example, the simple membrane deformation model (3-D collective fluctuations; model IV) is favored, since it accounts for the \(^2\)H and \(^{13}\)C R\(_{1Z}\) relaxation data with a single adjustable parameter (the reduced chi-squared\(^73\) is smallest). On the other hand, considerations of physical plausibility may favor either the molecular diffusion model (II), or the membrane deformation model (IV).
An additional important point involves the correspondence of the experimental NMR results to the force fields that account for the equilibrium and dynamical properties of membrane lipid bilayers in computer models. In this regard, the NMR relaxation rates of lipid bilayers comprise one of the primary experimental observables that are simulated using molecular dynamics. Consequently, it is worthwhile to emphasize the relationship between the NMR data analysis, molecular dynamics, and Langevin dynamics simulations. It is noteworthy that the decay rates of the acyl chain correlation functions given by the membrane deformation model (3-D director fluctuations) are in fact rather similar to those obtained from molecular dynamics simulations. Clearly, the extrapolated behavior of the correlation functions at times < 10^{-10} s corresponds to much faster motions than predominantly contribute to NMR relaxation in the presently available MHz frequency range. In future molecular dynamics simulations, it would be interesting to study in more detail the transition between the fast decaying part of the correlation function resulting from faster noncollective segmental motions, and the slowly decaying tail corresponding to slower noncollective molecular or possibly collective motions. It should also be remarked that the bilayer correlation functions (Fig. 9) have been obtained by comparing different dynamic models treated in mathematically closed forms to the experimental NMR results. This makes it possible to consider the NMR relaxation data analysis as a useful tool that can help reveal the major features of the motions over time scales >> 10^{-10} s as an adjunct to molecular dynamics simulations. The correlation functions can then be directly tested by fitting the experimentally measured NMR relaxation rates and can be extrapolated to much longer times.

In conclusion, the results of a unified analysis of the frequency- and temperature-dependent 2H and 13C NMR relaxation data indicate the observed relaxation rates of lipid bilayers reflect a broad spectrum of correlation times. Individual segmental and molecular reorientations alone cannot describe the low-frequency dispersion of the relaxation rates; further slower motions such as vesicle tumbling or lipid lateral diffusion may need to be included, thus widening the spectrum of the correlation times considered by the model. On the other hand, the values of some fitting parameters do not correspond well to other measurements obtained from alternative experimental techniques, which can make it difficult to establish a clear physical picture. In contrast, the membrane deformation model describing three-dimensional collective fluctuations, which encompasses an effectively infinite continuous range of the correlation times and includes a single effective fitting parameter, can account for the spin relaxation in lipid bilayers over the whole MHz frequency range studied in the present work.

ACKNOWLEDGMENTS

We are grateful to Axel Bothner-By and P. K. Mishra of Carnegie-Mellon University for their help in obtaining the 95.25 MHz 2H NMR relaxation data, Göran Carlström and Vladimir Denisov of Lund University for assistance in acquiring the 150.8 MHz 13C NMR relaxation data, and Klaus Beyer and Thomas Huber of the University of Munich for the 188.7 MHz 13C NMR relaxation data. We also thank Ulf Henriksen, Stephan Moltke, Ulf Olsson, Richard Pastor, W. Ron Salzman, J. Michael Schurr, Olle Söderman, and Håkan Wennerström for helpful discussions. This work was supported by grants from the U.S. National Institutes of Health, the U.S. National Science Foundation, and the Swedish Natural Science Research Council.

32 Abbreviations used: 2-D, two dimensional; 3-D, three dimensional; EFG,
electric field gradient; DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine; lab, laboratory frame; MD, molecular dynamics; NMR, nuclear magnetic resonance; PAS, principal axis system.


50. Note that the transformations involved in expressing the components of the electric field gradient tensor within the laboratory frame $V_{2114}^{\text{lab}}$ in terms of the principal values $v_{2114}^{\text{PAS}}$ are active; however, the Euler angles are conventionally defined in terms of passive rotation of the coordinate system (Ref. 49).


61. A typographical error is present in Ref. 18, Eq. (4.13), which should correspond to Eq. (4.4) of the present paper.
