Transverse Nuclear Spin Relaxation Studies of Viscoelastic Properties of Membrane Vesicles. I. Theory

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Transverse nuclear spin relaxation measurements employing Carr−Purcell (CP) pulse sequences can provide detailed information on the slow-motional dynamics in biomembranes. In this paper, a comprehensive relaxation model is developed for the analysis of such experiments performed on unilamellar quasi-spherical vesicles. The basis of the model is the stochastic Liouville equation in which two different relaxation processes are considered (i.e., vesicle shape fluctuations and molecular translational diffusion). It is shown that for vesicle radii $R_0 \geq 200$ nm, translational diffusion of the lipid molecules along the vesicle shell is too slow to contribute significantly to transverse spin relaxation in the kHz range, whereas vesicle shape fluctuations constitute the dominant transverse relaxation process. The theory is employed in model calculations for pulse frequency-dependent transverse ^{31}P nuclear spin relaxation rates, $R_{2,\infty}^{CP}(\omega)$, from CP sequences. The analysis reveals that $R_{2,\infty}^{CP}(\omega)$, induced by vesicle fluctuations, depends linearly on ω^{-1} over a wide frequency range in the kHz regime. Notably, within this linear dispersion regime, the bending elastic modulus κ is the only relevant parameter because the magnitude of $R_{2,\infty}^{CP}(\omega)$ does not depend on the size of the vesicle R_0, the effective lateral tension σ, or the viscosity of the surrounding fluid η. On the other hand, R_0, σ, η, and κ determine the frequency at which $R_{2,\infty}^{CP}(\omega)$ levels off to a constant "plateau" value independent of ω. Thus, analysis of the $R_{2,\infty}^{CP}(\omega)$ dispersion profiles is a direct way to determine the bending elastic modulus and other viscoelastic parameters of membrane vesicles.

1. Introduction

Biological membranes exhibit exceptional mechanical properties unequaled by any other soft condensed matter material.[1] A crucial parameter determining the shape fluctuations and functionality of closed biomembranes is the bending elastic modulus κ. Measurements of κ have been performed for membrane vesicles using a variety of methods including video microscopy[2−4] and micropipet[5] and electric deformation techniques[6] as well as nuclear magnetic resonance (NMR) relaxometry in the kHz range.[7,8]

A theoretical basis for the NMR studies of membranes was given by Marqusee et al.[9] They found that the spin−lattice relaxation rate induced by undulations (i.e., out-of-plane fluctuations of a planar membrane) depends linearly on the Larmor frequency, $R_1(\omega_0) \propto \omega_0^{-1}$, which is similar to the dependence in thermotropic liquid crystals with a layered structure.[10] Indeed, such linear dispersion behavior was observed experimentally in the kHz frequency regime for multilamellar stacks of planar membranes[11] and for oligolamellar dispersions of various membrane lipids.[7,8,12,13] Later, however, Halle et al. pointed out that the coupling of the lipid bilayers in the stack might restrict the "free" membrane undulations to the upper MHz regime, where they are hardly observable anymore.[14,15] This argument raised doubts about the ability of NMR relaxometry to determine κ in multilamellar membrane systems.

The objective of the present study is the development of a comprehensive relaxation model for nuclear spins in unilamellar vesicles, where the problem of interbilayer coupling does not exist (see Figure 1). The quasi-spherical shape fluctuations induced in these systems are restricted only by the finite area and volume of the closed bilayer shell.[16] As shown recently within the Redfield limit, the fluctuation modes of these vesicles give rise to nuclear spin relaxation rates with a linear dispersion regime extending far into the kHz range,[17] which provides a direct way to determine the bending elastic modulus and other viscoelastic parameters of membrane vesicles.

Generally, NMR relaxation studies comprise measurements of the nuclear spin relaxation rates in the frequency range between 10^3 and 10^9 Hz.[18] This broad dynamic range is covered by combining standard measurements of the spin−lattice relaxation rate, $R_1(\omega_0)$, in the MHz regime with the field-cycling technique,[19] yielding $R_1(\omega_0)$ at kHz frequencies, or by measuring the dependence of the transverse spin relaxation rate, $R_2^{CP}(\omega)$ on the pulse frequency in a Carr-Purcell multipulse sequence.[11] Notably, the field-cycling method is limited at low Larmor frequencies by internal field effects, which render ambiguous the interpretation of any cutoffs that may appear in the $R_1(\omega_0)$ dispersion profiles. For pulse frequency-dependent transverse

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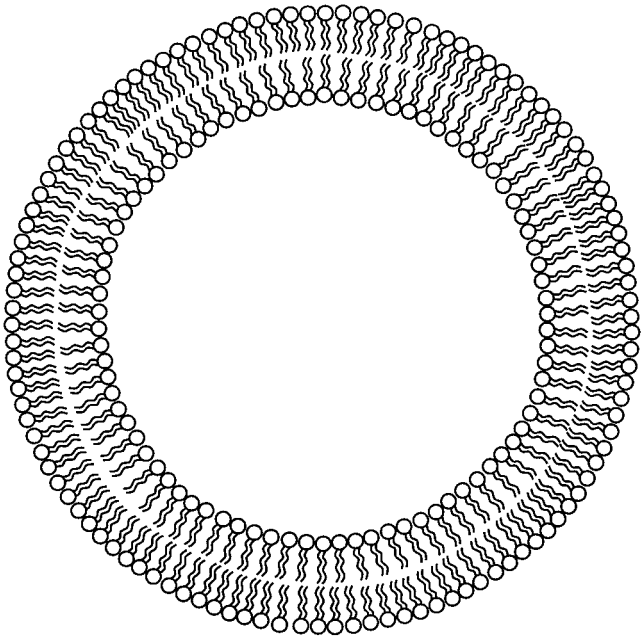

Figure 1. Schematic representation of a unilamellar membrane vesicle. The ^{31}P probe nuclei used in this study are located in the headgroup of the lipid molecules.

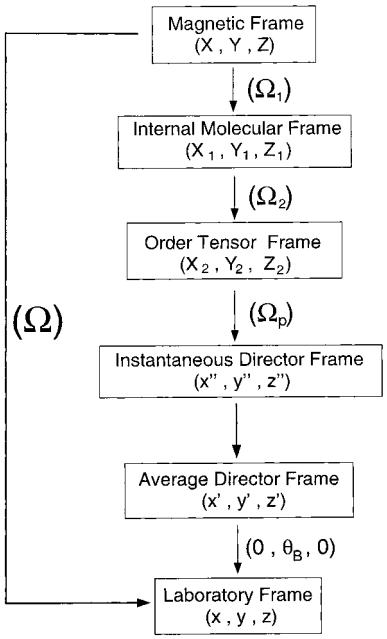

Figure 2. Notation for coordinate systems and Euler transformations used in the transverse ^{31}P nuclear spin relaxation model of unilamellar membrane vesicles.

relaxation studies, such problems do not exist. In the following discussion, we shall therefore develop the methods of analysis specifically for the latter type of experiments.

Vesicle fluctuations are characterized by a broad distribution of thermally activated modes extending over a wide frequency range down to the kHz regime.[16] Consequently, a rigorous analysis of the transverse relaxation induced by these fluctuations is generally hampered by the lack of time-scale separation between the slow elastic modes and the transverse magnetization decay. Under these conditions, the use of a fast-motion theory[17] is no longer justified, and one should resort to a slow-motional approach based on the stochastic Liouville equation.[20,21] Such a formal treatment of the dynamic problem has recently been presented for the specific case of director fluctuations in nematic liquid crystals.[22]

A complete analysis is possible only if analytical solutions of the stochastic Liouville equation are available. We will show that such an analytical solution can be derived if the vesicle fluctuations are described by a multidimensional Gaussian process. As a matter of fact, such a representation can be adopted for the fluctuating membrane normal using a small-angle approximation. In this way, one can derive an exact solution of the transverse magnetization decay in Carr−Purcell sequences.

The paper is organized as follows. In section 2, we introduce the stochastic Liouville equation for the description of the transverse ^{31}P nuclear spin relaxation in unilamellar vesicles. Particular emphasis is given to the analysis of thermally induced vesicle fluctuations associated with the viscoelastic properties of the bilayer membrane. In section 3, explicit expressions are derived for the magnetization decay in Carr−Purcell sequences due to lateral lipid diffusion along the vesicle shell. This process has not yet been addressed under slow-motional conditions. In section 4, the theory is employed in model calculations for the pulse frequency dependence of the transverse ^{31}P nuclear spin relaxation rates. It will be shown that translational diffusion of the lipid molecules is too slow to contribute significantly to transverse relaxation in the kHz range. Rather, thermally excited vesicle fluctuations constitute the dominant transverse relaxation process. Thus, analysis of the dispersion profiles can provide information about the viscoelastic properties of membranes. The final section contains the conclusions of the work, with a discussion of future developments made possible by the new approach.

2. Transverse Relaxation Due to Vesicle Fluctuations

In this section, we develop a transverse NMR relaxation model for ^{31}P nuclei in the headgroup of lipid molecules forming unilamellar vesicles (see Figure 1). Particular emphasis is given to the analysis of thermally excited vesicle fluctuations associated with the viscoelastic properties of the bilayer membrane. In liquid crystal terminology, these collective lipid motions are often referred to as director fluctuations.

Let us start by introducing several reference systems of axes that are necessary to separate the various types of motions. First, we define the laboratory frame (LF), $(\vec{x}, \vec{y}, \vec{z})$, in which the z axis is oriented along the static magnetic field (see Figure 2). Then, we introduce the average director frame (ADF), $(\vec{x}', \vec{y}', \vec{z}')$, where the z' axis specifies the average molecular alignment at each point on the sphere. Obviously, the z' axis is collinear with the local normal to the vesicle surface (see Figure 1). By assuming rotational isotropy of the orientational properties about the z' axis, the orthogonal directions are arbitrarily chosen. In addition, we introduce the instantaneous director frame (IDF), $(\vec{x}'', \vec{y}'', \vec{z}'')$, where the z'' axis specifies the instantaneous director fluctuating about the z' axis such that

$$\overline{n_{x'}} = \overline{n_{y'}} = 0 \tag{1}$$

Here, $\vec{n} = (n_{x'}, n_{y'}, n_{z'})$ is the representation of the instantaneous director as a unit vector in the ADF (see Figure 2).

To account for the local motions, two molecular coordinate systems are introduced, namely, the order tensor frame (OTF), $(\vec{X}_2, \vec{Y}_2, \vec{Z}_2)$, and an internal molecular frame (IMF), $(\vec{X}_1, \vec{Y}_1, \vec{Z}_1)$ (see Figure 2). For simplicity, we assume that the order tensor and the rotational diffusion tensor of the lipid molecule are collinear and axially symmetric along $\vec{Z}_2$. Finally, we have to consider the magnetic frame (MF), $(\vec{X}, \vec{Y}, \vec{Z})$, in which the chemical shift tensor of the ^{31}P nucleus is diagonal (see Figure 2).

Spin Hamiltonian. The spin Hamiltonian, $H(\Omega)$, representing Zeeman and chemical shift (CS) interactions in the LF, may be written as

$$H(\Omega) = -\hbar\gamma B_0 I_z + \sum_{l=0}^{2}\sum_{m=-l}^{+l} (-1)^m F_{l,-m}^{Lab}(\Omega)\, T_{l,m} \quad (2)$$

where γ, B_0, and I_z are the gyromagnetic ratio of the ^{31}P nucleus, the static magnetic field, and the z component of the nuclear spin operator, **I**, respectively. $F_{l,m}^{Lab}(\Omega)$ and $T_{l,m}$ denote LF spatial and spin operators, respectively. Ω is a set of Euler angles specifying the transformation from the MF to the LF (see Figure 2). The nonzero spin operators of the CS interactions are given by $T_{0,0} = -(1/\sqrt{3})\hbar B_0 I_z$, $T_{1,\pm1} = \pm(1/\sqrt{2})\hbar B_0 I_{\pm1}$, $T_{2,0} = \sqrt{2/3}\hbar B_0 I_z$, and $T_{2,\pm1} = \pm(1/\sqrt{2})\hbar B_0 I_{\pm1}$, where $I_{\pm1} = I_x \pm iI_y$ denote the nuclear spin−shift operators.

We now assume that the experimental observations refer to a time window in which longitudinal relaxation has negligible effects. Thus, one can apply the secular approximation and neglect all terms containing the spin operators I_x and I_y. This treatment leads to

$$H(\Omega) = -\hbar\gamma B_0 I_z - \frac{1}{\sqrt{3}}\hbar B_0 F_{0,0}^{Lab}(\Omega) I_z + \frac{2}{3}\hbar B_0 F_{2,0}^{Lab}(\Omega) I_z \quad (3)$$

where the tensorial components describing the symmetry and the magnitude of the CS interations transform under rotation from the MF to the LF according to

$$F_{l,m}^{Lab}(\Omega) = \sum_{n=-l}^{+l} D_{n,m}^{l}(\Omega) F_{l,n}^{Mag} \quad (4)$$

The nonzero spatial operators, $F_{l,n}^{Mag}$, in the MF are given by $F_{0,0}^{Mag} = -\sqrt{3}\gamma\sigma_{iso}$, $F_{2,0}^{Mag} = (\sqrt{6}/3)\gamma\Delta\sigma$, and $F_{2,\pm2}^{Mag} = (1/3)\gamma\eta\Delta\sigma$, where $\sigma_{iso} = (1/3)(\sigma_{XX} + \sigma_{YY} + \sigma_{ZZ})$ is the isotropic chemical shift, $\Delta\sigma = \sigma_{ZZ} - (1/2)(\sigma_{XX} + \sigma_{YY})$ specifies the anisotropy of the chemical shift tensor, and $\eta = (3/2)(\sigma_{XX} - \sigma_{YY})/\Delta\sigma$ is the biaxiality parameter. By substituting these tensorial components in eq 4, one obtains

$$H(\Omega) = -\hbar\gamma B_0(1-\sigma_{iso}) I_z + \frac{2}{3}\hbar\gamma B_0 \Delta\sigma P_2(\vec{u}_B\cdot\vec{u}_{CS}) I_z + \sqrt{\frac{2}{27}}\hbar\gamma B_0 \Delta\sigma\eta[D_{-2,0}^2(\Omega) + D_{2,0}^2(\Omega)] I_z \quad (5)$$

where $\vec{u}_B \equiv \vec{z}$ and $\vec{u}_{CS} \equiv \vec{Z}$.

We now assume fast internal and overall molecular motions of the lipid molecules and describe the effects of these motions according to the standard Redfield theory.[23] Consequently, the above Hamiltonian can be averaged over the equilibrium distributions of the intramolecular and intermolecular degrees of freedom, Ω_1 and Ω_p (see Figure 2). Assuming a "nematic-like" distribution of the OTF with respect to the IDF, the averaged tensor elements $\langle D_{m,0}^2(\Omega)\rangle_{\Omega_1,\Omega_p}$ can be written as

$$\langle D_{m,0}^2(\Omega)\rangle_{\Omega_1,\Omega_p} = S_{MOL}\phi_m(\Omega_2) P_2(\vec{z}''\cdot\vec{u}_B) \qquad m = 0, \pm2 \quad (6)$$

where

$$S_{MOL} = \langle P_2(\vec{z}''\cdot\vec{Z}_2)\rangle_{\Omega_p} \quad (7)$$

denotes the orientational order parameter of the Z_2 axis with respect to the IDF, which is often referred to as the molecular order parameter. The functions $\phi_m(\Omega_2)$ are given by

$$\phi_m(\Omega_2) = \sum_{m'=-2}^{2} \langle D_{m,m'}^2(\Omega_1)\rangle_{\Omega_1} D_{m',0}^2(\Omega_2) \quad (8)$$

where $\langle D_{m,m'}^2(\Omega_1)\rangle_{\Omega_1}$ represent "internal" order parameters and $D_{m',0}^2(\Omega_2)$ are pure geometrical factors for the transformation from the IMF to the OTF (see Figure 2).

In the limit of small-angle fluctuations of the IDF with respect to the ADF, the so-called linear approximation can be applied to the expansion of the kernel $P_2(\vec{z}''\cdot\vec{u}_B)$ appearing in eq 6. Thus, one obtains

$$P_2(\vec{z}''\cdot\vec{u}_B) \simeq P_2(\cos\theta_B) + \sqrt{6} d_{0,1}^2(\theta_B)\, n_{x'}(\vec{r}_p) \quad (9)$$

where θ_B denotes the angle between the external field and the local average director (see Figure 2), $d_{0,1}^2(\theta_B) = \sqrt{3}/2 \sin\theta_B \cos\theta_B$ is the reduced Wigner function, and $n_{x'}(\vec{r}_p)$ is the transverse component of the fluctuating director at the position $\vec{r}_p$ of the probe molecule. Considering eqs 6−9, the averaged Hamiltonian, $H \equiv \langle H(\Omega)\rangle_{\Omega_1,\Omega_p}$, can be written as

$$H = H_0 + H_{CS} \quad (10)$$

$$H_0/\hbar = \omega_0 I_z \qquad H_{CS}/\hbar = (\overline{\omega_{CS}} + \Delta\omega_{CS}(\mathbf{q})) I_z \quad (11)$$

where ω_0 and $\overline{\omega_{CS}}$ are characteristic frequencies given by

$$\omega_0 = -\gamma B_0(1-\sigma_{iso}) \qquad \overline{\omega_{CS}} = f\gamma B_0 \Delta\sigma S_{MOL} P_2(\cos\theta_B) \quad (12)$$

$$f = \frac{2}{3}\phi_0(\Omega_2) + \sqrt{\frac{2}{27}}\eta[\phi_{-2}(\Omega_2) + \phi_2(\Omega_2)] \quad (13)$$

and

$$\Delta\omega_{CS}(\mathbf{q}) = \Delta\omega'_{CS} n_{x'}(\vec{r}_p) \qquad \Delta\omega'_{CS} = \sqrt{6} f\gamma B_0 \Delta\sigma S_{MOL} d_{0,1}^2(\theta_B) \quad (14)$$

is the magnitude (in frequency units) of the CS interactions modulated by the coupling of the spin system to the local director field.

The formal treatment of such a coupling is achieved by introducing the multidimensional stochastic variable **q**. Let us suppose that the surface of the vesicle is divided into microscopical domains that are compatible with the collective nature of the local director. Thus, we obtain a homogeneous division of the distorted surface into discrete surface elements characterized by the coordinates $\vec{r}_j$. The array **q** of relevant stochastic variables is then built as

$$\mathbf{q} = (..., n_{x'}(\vec{r}_j), n_{y'}(\vec{r}_j), ...) \quad (15)$$

where $n_{x'}(\vec{r}_j)$ and $n_{y'}(\vec{r}_j)$ are the transverse components of the local director field in the jth microdomain.

By introducing a vector **v** having the same dimensions as **q**, with null entries except for an unitary value corresponding to the selected director field component, one can rewrite eq 14 as

$$\Delta\omega_{CS}(\mathbf{q}) = \Delta\omega'_{CS}\mathbf{v}^{\dagger}\cdot\mathbf{q} \quad (16)$$

Equation 16 clearly shows that the modulated Hamiltonian is treated within the linear approximation, implying that the expansion of the Hamiltonian in terms of transverse components

of the local director field is restricted to the lowest order. It must be emphasized that such an approximation holds if (i) the angular amplitudes of the fluctuations are small ($n_{z'}(\vec{r}_p) \simeq 1$ and $n_{x'}(\vec{r}_p)$, $n_{y'}(\vec{r}_p) \simeq 0$) and if (ii) only noncanonical geometries are considered (i.e., $\theta_B \neq 0°, 90°$). Notice that we implicitly neglect the effects of translational diffusion of the lipid molecule across the vesicle's surface;[24,25] otherwise, the probe position, $\vec{r}_p$, has to be included among the stochastic variables. Consequently, in the following, θ_B is considered to be a fixed parameter.

Slow-Motional Analysis. The basis of the slow-motional analysis of the coupled spin and director dynamics is the stochastic Liouville equation[20,21]

$$\frac{\partial}{\partial t}\rho(\mathbf{q}, t) = -[(i/\hbar)H^X_{CS} + \Gamma_{\mathbf{q}} + R]\rho(\mathbf{q}, t) \quad (17)$$

which describes the time dependence of the spin-density matrix $\rho(\mathbf{q}, t)$ under the influence of the Hamiltonian superoperator H^X_{CS} and the evolution operator $\Gamma_{\mathbf{q}}$ of the stochastic variables $\mathbf{q}$ used to describe the director fluctuations. Spin relaxation due to fast molecular motions is considered by the Redfield operator, R, acting on the spin degrees of freedom only. Recently, a formal treatment of the director dynamics under slow-motional conditions has been described for an $I = 1$ spin system exhibiting quadrupolar interactions.[22] The same procedure has been followed in the present case referring to an $I = {}^1/_2$ nucleus with anisotropic CS interactions in a Carr−Purcell−Meiboom−Gill (CP) sequence (i.e., $({}^{\pi}/_2)_x - [\tau - (\pi)_y - \tau]_n$).[26] Generally, the signal intensity of the nth echo in a CP sequence can be described by

$$M_y(2n\tau)/M_{eq} = Re\{G_{CP}(2n\tau)\} \exp(-2n\tau/T_2^0) \quad (18)$$

where $(T_2^0)^{-1}$ is the contribution from the fast molecular motions,

$$G_{CP}(2n\tau) = \int d\mathbf{q} \cdots U(\tau)^* U(\tau)^* U(\tau)\, p_{eq}(\mathbf{q}) \quad (19)$$

is the characteristic function, and

$$U(t) = \exp\{-i\Delta\omega_{CS}(\mathbf{q})t - \Gamma_{\mathbf{q}}t\} \quad (20)$$

is the evolution operator.

For the analysis of the CP experiment, we introduce a specific transverse relaxation rate, $R^{CP}_{2,n}(\tau)$, on the basis of the relative decrease of the magnetization during cycle n:

$$R^{CP}_{2,n}(\tau) = -\frac{1}{2\tau}\ln\frac{M_y(2n\tau)}{M_y(2(n-1)\tau)} \quad (21)$$

In describing the director fluctuations by a multidimensional Gaussian process,[22,27] $R^{CP}_{2,n}(\tau)$ can be expressed as a linear combination of the functions $g(2j\tau)$, with $j = 0, 1, ..., n$ where

$$g(t) = (\Delta\omega'_{CS})^2 \int_0^t dt' \int_0^{t'} dt'' \, \overline{n_{x'}(\vec{r}_p)_0 n_{x'}(\vec{r}_p)_{t''}} \quad (22)$$

is the double time integral of the autocorrelation function, $\overline{n_{x'}(\vec{r}_p)_0 n_{x'}(\vec{r}_p)_t}$, of the transverse component of the local director field. Let us assume that there exists a suitable representation of the director field in terms of normal modes associated with a transformation of the multidimensional stochastic variable (i.e., $\mathbf{q} \rightarrow \tilde{\mathbf{q}}$). In that case, the time autocorrelation function can be decomposed into a sum of independent terms according to

$$\overline{n_{x'}(\vec{r}_p)_0 n_{x'}(\vec{r}_p)_t} = \sum_j \sigma_j^2 e^{-t/\tau_j} \quad (23)$$

where σ_j^2 and τ_j are the mean-square amplitudes and relaxation times of the normal modes j. The search for such a normal mode representation is equivalent to the search for a representation in which the elastic free energy for the whole vesicle can be written (exactly or approximately) as a sum of uncoupled terms. This search will be demonstrated in the next section.

Generally, the dependence of $R^{CP}_{2,n}(\tau)$ on the number of cycles in the CP sequence is rather weak. Consequently, one can interpret the experimental data in terms of an asymptotic relaxation rate for an infinite sequence, $R^{CP}_{2,\infty}(\tau) = \lim_{n\to\infty} R^{CP}_{2,n}(\tau)$. For such an asymptotic relaxation rate, the following compact result has been obtained:[22]

$$R^{CP}_{2,\infty}(\tau) = (T_2^0)^{-1} + (\Delta\omega'_{CS})^2 \sum_j \sigma_j^2 \tau_j [1 - (\tau_j/\tau)\tanh(\tau/\tau_j)] \quad (24)$$

where $(T_2^0)^{-1}$ is the contribution from the fast molecular motions. This result implies[22] that one can analyze the measured transverse relaxation rate as a superposition of independent contributions evaluated according to the Luz−Meiboom equation,[28] which was originally derived within the Redfield limit. It should be mentioned that such an extension of the Luz−Meiboom equation to the slow motion regime of director fluctuations was conjectured in the past from the analysis of the dispersion behavior of the experimental transverse relaxation rates.[7,8,13]

Finally, let us introduce the observed splitting, $\Delta\nu_{obs}$, that is defined as the full width of the experimental ^{31}P NMR powder spectrum. Because both the molecular and the collective motions contribute to the averaging of the CS interactions, one can write

$$\Delta\nu_{obs} = \Delta\nu S_{MOL} S_{ODF}$$
$$\Delta\nu \equiv \frac{1}{2\pi}|\overline{\omega_{CS}}(90°) - \overline{\omega_{CS}}(0°)|_{S_{MOL}=1} = \frac{3}{4\pi} f|\gamma B_0 \Delta\sigma| \quad (25)$$

where S_{ODF} is the order parameter of the director fluctuations:

$$S_{ODF} = \frac{1}{2}(3\overline{n_{z'}^2} - 1) = 1 - \frac{3}{2}(\overline{n_{x'}^2} + \overline{n_{y'}^2}) \equiv 1 - 3\sum_j \sigma_j^2 \quad (26)$$

Evidently, S_{ODF} characterizes the alignment of the local fluctuating director with respect to the average director. By expressing the factor $\Delta\omega'_{CS}$ in eq 14 in terms of the observed splitting, $\Delta\nu_{obs}$, the transverse ^{31}P spin relaxation rate in a CP sequence for a particular orientation θ_B can be written as

$$R^{CP}_{2,\infty}(\tau) = \frac{1}{T_2^0} + 16\pi^2\left(\frac{\Delta\nu_{obs}}{S_{ODF}}\right)^2 \sin^2\theta_B \cos^2\theta_B \sum_j \sigma_j^2 \tau_j \times [1 - (\tau_j/\tau)\tanh(\tau/\tau_j)] \quad (27)$$

Notably, this analytical expression is the same as that evaluated previously[7,8] according to the Luz−Meiboom equation,[28] which was originally derived within the Redfield limit.

So far, however, only linear contributions of the orthogonal components of the fluctuating director have been employed in the analysis (see eq 16). Consequently, the theory cannot account for orientations in which the local vesicle normal is either parallel or perpendicular to the magnetic field (i.e., $\theta_B = 0°$, 90°). In such cases, consideration of the bilinear terms of the

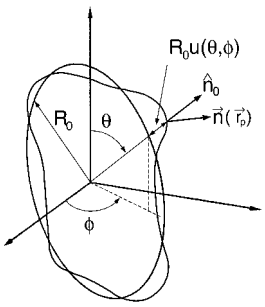

Figure 3. Schematic representation of fluctuations of quasi-spherical vesicles. θ and ϕ denote the polar and azimuthal angles, respectively, of the considered surface point with respect to an arbitrary reference system. $R_0u(\theta, \phi)$ characterizes the radial deviation from the spherical conformation.

director field would be required. Studies along these lines are currently in progress.

Fluctuations of Quasi-Spherical Vesicles. The model of quasi-spherical fluctuations was developed by Milner and Safran[16] on the basis of Helfrich's theory of the elasticity of lipid bilayers[29] and the hydrodynamic approach of Schneider et al.[30] In this model, a vesicle is considered to be a quasi-spherical closed shell of radius R_0 characterized by a fixed volume $V = 4\pi R_0^3/3$ and a fixed area A. The vesicle is assumed to be flaccid, a fact that may be described by the dimensionless excess area Δ:[31]

$$A = (4\pi + \Delta)R_0^2 \tag{28}$$

Apart from the bending rigidity of the membrane, the excess area is an important parameter characterizing the shape and the thermal fluctuations of a vesicle. In fact, Δ specifies the deviation of the shape of the vesicle from a sphere of the same volume. Note that in the case of quasi-spherical vesicle fluctuations the excess area should be small.

For quantitative modeling of the fluctuations, the vesicle is described by a slightly deformed spherical surface as (see Figure 3)

$$R(\theta, \phi) = R_0(1 + u(\theta, \phi)) \tag{29}$$

where $u(\theta, \phi)$ is a dimensionless parameter characterizing the radial deviation from the spherical conformation. θ and ϕ denote the polar and azimuthal angle of the considered surface point with respect to an arbitrary reference system (see Figure 3). The positional-dependent function $u(\theta, \phi)$ is now expanded in terms of the spherical harmonics $Y_{l,m}(\theta, \phi)$ according to

$$u(\theta, \phi) = \sum_{l} \sum_{m=-l}^{+l} u_{l,m} Y_{l,m}(\theta, \phi) \tag{30}$$

Using this expansion, the total elastic free energy of the vesicle can be written as a sum of independent contributions that are quadratic in the components $u_{l,m}$.[16,30] This expansion clearly shows that the spherical harmonics represent normal modes for the description of the thermal fluctuations of quasi-spherical vesicles. Consequently, the time autocorrelation function of the corresponding stochastic variables $u_{l,m}$ can be expressed as

$$\overline{u_{l,m}(0)\, u_{l',m'}(t)} = \delta_{l,l'}\delta_{m,m'}\, \overline{u_{l,m}^2} e^{-t/\tau_l} \tag{31}$$

where the mean-square amplitude and relaxation time for each mode are given by[16, 30]

$$\overline{u_{l,m}^2} = \frac{k_B T}{\kappa} \frac{1}{(l+2)(l-1)(l^2+l+\sigma)} \tag{32}$$

$$\tau_l = \frac{\eta R_0^3}{\kappa} \frac{(2l+1)(2l^2+2l-1)}{(l-1)l(l+1)(l+2)(l^2+l+\sigma)} \tag{33}$$

respectively. Here, η is the viscosity of the surrounding fluid, κ is the bending elastic modulus of the membrane, and σ is the dimensionless effective lateral tension that is related to the excess area Δ by the implicit equation[4,31]

$$\Delta = \frac{k_B T}{2\kappa} \sum_{l=2}^{l_{max}} \frac{2l+1}{l^2+l+\sigma} \tag{34}$$

According to Milner and Safran, the summation over l in eq 34 is extended from $l = 2$ to $l_{max} \simeq \pi R_0/a$, where a is the average intermolecular distance.[16]

We now derive an expression for the time autocorrelation function of the transverse components of the local director field, which is required for the analysis of the CP multipulse experiment (eq 24). Let us first introduce the ADF in spherical geometry:

$$(\vec{x}', \vec{y}', \vec{z}') \rightarrow (\hat{\theta}, \hat{\phi}, \hat{n}_0) \tag{35}$$

$(\hat{\theta}, \hat{\phi}, \hat{n}_0)$ denotes a set of orthogonal positional-dependent unit vectors in spherical coordinates. The unit vector $\hat{n}_0$ specifies the radial direction at each point on the surface that is coincident with the local average director (see Figure 3). Thus, the local instantaneous director can be written as $\vec{n}(\vec{r}_p) = (n_\theta, n_\phi, n_{n_0})$, where n_θ and n_ϕ represent the transverse components of the local director field. To calculate the relevant correlation function defined in eq 23, n_θ and n_ϕ are expressed, to lowest order, in terms of the radial displacement $u(\theta, \phi)$ by

$$n_\theta \simeq -\nabla_\theta u(\theta, \phi) = -\frac{\partial u(\theta, \phi)}{\partial \theta}$$
$$n_\phi \simeq -\nabla_\phi u(\theta, \phi) = -\frac{1}{\sin\theta}\frac{\partial u(\theta, \phi)}{\partial \phi} \tag{36}$$

Note that the expressions in eq 36 are obtained from the exact relations

$$n_{n_0} = (1+u)M^{-1} \qquad n_\theta = n_\theta^0 M^{-1} \qquad n_\phi = n_\phi^0 M^{-1} \tag{37}$$

$$n_\theta^0 = -\nabla_\theta u(\theta, \phi) \qquad n_\phi^0 = -\nabla_\phi u(\theta, \phi) \tag{38}$$

$$M = [(1+u)^2 + (n_\theta^0)^2 + (n_\phi^0)^2]^{1/2} \tag{39}$$

by expanding the function M into powers of u, n_θ^0, and n_ϕ^0 and by retaining only the leading terms under the condition $|u(\theta, \phi)| \ll 1$. Therefore,

$$n_\theta \simeq n_\theta^0 \qquad n_\phi \simeq n_\phi^0 \tag{40}$$

Evidently, eq 36 is exact only in the limit $|u(\theta, \phi)| \rightarrow 0$. This means that the transverse components of the local director field are calculated using a small-angle approximation for the quasi-spherical fluctuations, which is consistent with the linear approximation for the modulated spin Hamiltonian (eq 16).

The partial derivatives in eq 36 are now evaluated by converting the components of the gradient into components of the angular momentum operator that refer to the chosen system of axes[32] (see Figure 3):

$$\nabla_\theta = \frac{\mathrm{i}}{\sin\theta}\hat{J}_z \qquad \nabla_\phi = \frac{1}{2}[\mathrm{e}^{-\mathrm{i}\phi}\hat{J}_+ - \mathrm{e}^{\mathrm{i}\phi}\hat{J}_-] \quad (41)$$

$\hat{J}_\pm = \hat{J}_x \pm \mathrm{i}\hat{J}_y$ are the shift operators, and the action on the spherical harmonics is given by $\hat{J}_z Y_{l,m}(\theta, \phi) = mY_{l,m}(\theta, \phi)$ and $\hat{J}_\pm Y_{l,m}(\theta, \phi) = [l(l+1) - m(m \pm 1)]^{1/2} Y_{l,m\pm1}(\theta,\phi)$. Evidently, the transverse components of the director field, n_θ and n_ϕ, which are approximated according to eq 36, can be expressed as linear combinations of the radial displacements $u_{l,m}$ with pure geometrical time-independent coefficients. For $|u(\theta, \phi)| \ll 1$, this treatment ensures the Gaussian nature of the fluctuation process, which is strictly required for the slow-motional treatment of director fluctuations in a CP multipulse experiment.[22]

Evaluation of the partial derivatives and calculation of the time autocorrelation functions for n_θ and n_ϕ using the condition of independent modes (eq 31) finally yields

$$\overline{n_\theta(\vec{r}_\mathrm{p})_0\, n_\theta(\vec{r}_\mathrm{p})_t} \equiv \overline{n_\phi(\vec{r}_\mathrm{p})_0\, n_\phi(\vec{r}_\mathrm{p})_t} = \frac{1}{8\pi}\sum_{l=2}^{l_{\max}} l(l+1)(2l+1)\,\overline{u_{l,m}(0)u_{l,m}(t)} \quad (42)$$

where the dependence on the surface position is lost because of the statistical equivalence of all points on the sphere. The determination of the rank-dependent multiplier factors, appearing in eq 42, is conveniently achieved by a normalized averaging over the sphere using the orthonormality properties of the spherical harmonics.[32,33] Thus, we have established the following assignments

$$\sigma_j^2 \rightarrow \sigma_l^2 \equiv \frac{1}{8\pi} l(l+1)(2l+1)\overline{u_{l,m}^2} \qquad \tau_j \rightarrow \tau_l \quad (43)$$

where $\overline{u_{l,m}^2}$ and τ_l are given in eqs 32 and 33. The expressions for σ_j^2 and τ_j can now be inserted in eq 27, yielding an analytical expression for the transverse relaxation rate, $R_{2,\infty}^{\mathrm{CP}}(\tau)$, in a CP multipulse sequence. Finally, by inserting σ_j^2 into eq 26, one obtains the order parameter, S_{ODF}, of the director fluctuations, which is necessary for a self-consistent analysis of the CP experiment. In the next section, we briefly discuss the limiting case of quasi-spherical fluctuations for $R_0 \rightarrow \infty$ (i.e., fluctuations of flat membranes).

Planar Membrane Fluctuations. For the quantitative description of quasi-planar membrane fluctuations, one can expand the local displacement from the average plane according to

$$u(\vec{r}_\perp) = \sum_{\vec{q}_\perp} u_{\mathrm{q}_\perp} \exp(-\mathrm{i}\vec{q}_\perp \cdot \vec{r}_\perp) \quad (44)$$

where $\vec{r}_\perp$ is the positional vector on the surface and $\vec{q}_\perp$ are in-plane wavevectors in 2-D reciprocal space. The familiar equipartition theorem yields for the mean-square amplitude of each mode[34]

$$\overline{u_{q_\perp}^2} = \frac{k_\mathrm{B}T}{A(\kappa q_\perp^4 + \sigma' q_\perp^2)} \quad (45)$$

where $A = \lambda_\mathrm{l} \times \lambda_\mathrm{l}$ characterizes the dimension of the flat membrane, κ is the bending elastic modulus of the membrane, λ_l is the long wavelength cutoff, and $\sigma' q_\perp^2$ accounts for possible lateral tension.[34] The transverse components $n_{x'}(\vec{r}_\perp)$ and $n_{y'}(\vec{r}_\perp)$ of the instantaneous local normal are calculated as in the case of the quasi-spherical fluctuations according to

$$n_{x'}(\vec{r}_\perp) = -\nabla_{x'} u(\vec{r}_\perp)\; n_{y'}(\vec{r}_\perp) = -\nabla_{y'} u(\vec{r}_\perp) \quad (46)$$

Evaluation of the partial derivatives associated with the components of the gradient and calculation of the time autocorrelation function of $n_{x'}(\vec{r}_\perp)$, using the condition of independent normal modes leads to the following assignment (see eq 24):

$$\sigma_j^2 \rightarrow \sigma_{q_\perp}^2 \equiv \frac{q_x^2 k_\mathrm{B}T}{A(\kappa q_\perp^4 + \sigma' q_\perp^2)} \quad (47)$$

For the mode relaxation time $\tau_{q_\perp}$ of quasi-planar membrane fluctuations, two different models exist. If the relaxation time of the modes is determined by the bending rigidity of the membrane and by the viscous damping of the surrounding fluid, the dispersion relation is given by[34]

$$\tau_j \rightarrow \tau_{q_\perp} \equiv 4\eta/(\kappa q_\perp^3) \quad (48)$$

where η denotes the viscosity of the fluid. For the "internal" dissipative mechanism, a somewhat different dispersion relation is predicted:[9]

$$\tau_j \rightarrow \tau_{q_\perp} \equiv \eta_{\mathrm{eff}} d/(\kappa q_\perp^2) \quad (49)$$

η_{eff} is the effective bending viscosity of the membrane, and d is the membrane thickness. Finally, by converting the summation over $\vec{q}_\perp$ into an integral, the asymptotic relaxation rate, $R_{2,\infty}^{\mathrm{CP}}(\omega)$, defined in eq 24 results in

$$R_{2,\infty}^{\mathrm{CP}}(\tau) = (T_2^0)^{-1} + (\Delta\omega'_{\mathrm{CS}})^2 \frac{k_\mathrm{B}T}{4\pi}\int_{q_{\perp l}}^{q_{\perp c}} \mathrm{d}q_\perp \frac{q_\perp}{\kappa q_\perp^2 + \sigma'} \times \tau_{q_\perp}[1 - (\tau_{q_\perp}/\tau)\tanh(\tau/\tau_{q_\perp})] \quad (50)$$

where $q_{\perp l} = 2\pi/\lambda_l$, $q_{\perp c} = 2\pi/\lambda_\mathrm{c}$, and λ_c is the short wavelength cutoff.

3. Transverse Relaxation Due to Translational Lipid Diffusion

General Considerations. Consideration of the translational diffusion of the lipid molecules as an additional relaxation mechanism requires reformulation of the slow-motional model starting from the modulated spin Hamiltonian given in eq 11. The positional coordinates of the microdomain, $\vec{r}_\mathrm{p}$, in which the probe molecule is located now become stochastic variables whose dynamics can be modeled as a diffusive random walk among the various sites (i.e., microdomains of the discretized sample).

First, the stochastic nature of $\vec{r}_\mathrm{p}$ leads to a modulation of the angle $\theta_\mathrm{B}(\vec{r}_\mathrm{p})$ between the external magnetic field and the local average director. Evidently, in the case of macroscopically oriented samples such as planar membranes, the angle θ_B is positionally invariant. Second, the selecting vector $\mathbf{v}(\vec{r}_\mathrm{p})$ in eq 16 now is stochastically modulated. Consequently, the time evolution of the density matrix, $\rho(\mathbf{q}, \vec{r}_\mathrm{p}, t)$, has to be described in terms of two sets of variables, $\vec{r}_\mathrm{p}$ and $\mathbf{q}$. If the translational dynamics and the director fluctuations are independent processes (i.e., if $p_{\mathrm{eq}}(\vec{r}_\mathrm{p}, \mathbf{q}) \equiv p_{\mathrm{eq}}(\vec{r}_\mathrm{p}) p_{\mathrm{eq}}(\mathbf{q})$) and if the global diffusion matrix is factorized into two blocks, the stochastic operator $\Gamma_{\vec{r}_\mathrm{p},\mathbf{q}}$ can be written as $\Gamma_{\vec{r}_\mathrm{p},\mathbf{q}} = \Gamma_{\vec{r}_\mathrm{p}} + \Gamma_\mathbf{q}$. Under these conditions, the evolution operator of eq 20 assumes the generalized form

$$U(t) = \exp\{-[i\overline{\omega_{CS}}[\theta_B(\vec{r}_p)] + i\Delta\omega'_{CS}[\theta_B(\vec{r}_p)]\,\mathbf{v}(\vec{r}_p)^{\dagger}\cdot\mathbf{q} + \Gamma_{\vec{r}_p} + \Gamma_{\mathbf{q}}]t\} \quad (51)$$

and the new characteristic function replacing eq 19 results in

$$G_{CP}(2n\tau) = \int d\vec{r}_p \int d\mathbf{q}...U(\tau)^*\, U(\tau)^*\, U(\tau)\, p_{eq}(\vec{r}_p)\, p_{eq}(\mathbf{q}) \quad (52)$$

In principle, the formal solution of eq 52 for the studied membrane vesicles is possible.[22] Such a comprehensive treatment would start with the convenient change of the positional stochastic variable according to $\vec{r}_p \rightarrow \Omega_B \equiv (\alpha_B(\vec{r}_p), \beta_B(\vec{r}_p), \gamma_B(\vec{r}_p))$, where Ω_B represents a set of Euler angles characterizing the orientation of the ADF relative to the LF with the obvious equivalence $\beta_B(\vec{r}_p) \equiv \theta_B(\vec{r}_p)$. The dynamics of the variables Ω_B can then be modeled as a virtual small-angle diffusion process of a rigid sphere, often referred to as translationally induced rotations.[24] The corresponding rotational diffusion coefficient, D^{rot}, is related to the translational diffusion coefficient, D^{transl}, of the probe molecule by[24]

$$D^{rot} = \frac{D^{transl}}{R_0^2} \quad (53)$$

where R_0 denotes the average radius of the vesicle. In principle, the tumbling of the whole vesicle has to be considered by adding a tumbling diffusion coefficient, D^{tumbl}. However, such a correction can be neglected if the linear extension of the probe molecule, l_p, is much smaller than the radius of the vesicle. This is verified by expressing D^{transl} and D^{tumbl} according to the well-known Stokes−Einstein and Stokes−Einstein−Debye relations (i.e., $D^{transl} = k_BT/(6\pi\eta l_p)$ and $D^{tumbl} = k_BT/(8\pi\eta R_0^3)$) and by assuming comparable viscosities η for the tumbling process of the whole vesicle and for the translational motion of the probe across the shell of the vesicle.

Translationally Induced Rotations. For clarity, the solution of the coupled model involving both types of stochastic variables, Ω_B and $\mathbf{q}$, is not attempted here. Rather, in the following, we present a slow-motional description of the transverse relaxation rate, $R_{2,n}^{CP}(\tau)$, defined in eq 21 for the case of translationally induced rotations along the nondistorted spherical surface. It is our aim to specify the dispersion behavior of $R_{2,n}^{CP}(\tau)$ under the assumption that these rotations represent the unique transverse relaxation process and that director fluctuations can be neglected.

Let us first rewrite the modulated component of the spin Hamiltonian according to

$$\overline{\omega_{CS}} = \omega'_0 D^2_{0,0}(\Omega_B) \qquad \omega'_0 = f\gamma B_0 \Delta\sigma S_{MOL} \equiv \frac{4\pi}{3}|\Delta\nu S_{MOL}| \quad (54)$$

where $D^2_{0,0}(\Omega_B) \equiv P_2(\cos\theta_B)$ is a Wigner function. Then, the evolution operator (eq 51) takes the form

$$U(t) = e^{(-i\omega'_0 D^2_{0,0}(\Omega_B)t - \Gamma_{\Omega_B}t)} \quad (55)$$

where Γ_{Ω_B} is a Smoluchowsky operator for the free rotational motion of a rigid body[35]

$$\Gamma_{\Omega_B} = \hat{\mathbf{J}}^{Tr}\cdot\mathbf{D}^{rot}\cdot\hat{\mathbf{J}} \quad (56)$$

and $\hat{\mathbf{J}}$ is the vectorial angular momentum operator with Cartesian components referring to the ADF. The rotational diffusion tensor, $\mathbf{D}^{rot}$, is assumed to be spherical, and the unique coefficient, D^{rot}, has been specified in eq 53.

Equation 52 can be rewritten in terms of the Hermitian scalar product over the space of the Euler angles:

$$G_{CP}(2n\tau) = \langle p_{eq}(\Omega_B)^{1/2}|...e^{-\tilde{O}^{\dagger}\tau}\, e^{-\tilde{O}^{\dagger}\tau}\, e^{-\tilde{O}\tau}|p_{eq}(\Omega_B)^{1/2}\rangle \quad (57)$$

The operator $\tilde{O}$ is defined as

$$\tilde{O} \equiv i\omega'_0 D^2_{0,0}(\Omega_B) + \Gamma_{\Omega_B} \quad (58)$$

and $p_{eq}(\Omega_B) = 1/8\pi^2$ according to the isotropic orientational distribution of the ADF. Note that the usual braket notation is adopted here for the Hermitian scalar product that is explicitly given by

$$\langle f(\Omega_B)|g(\Omega_B)\rangle \equiv \int_0^{2\pi} d\alpha_B \int_0^{\pi} d\beta_B \sin\beta_B \int_0^{2\pi} d\gamma_B \times f(\alpha_B, \beta_B, \gamma_B)^*\, g(\alpha_B, \beta_B, \gamma_B) \quad (59)$$

Equation 57 is conveniently converted into matrix form by expanding the scalar product into the orthonormal set of eigenfunctions of Γ_{Ω_B} (i.e., the normalized Wigner functions).[35] Using $|J, m, k\rangle \equiv [(2J+1)/8\pi^2]^{1/2} D^J_{m,k}(\Omega_B)$, the orthonormality condition for the basis functions can be written as $\langle J', m', k'|J, m, k\rangle = \delta_{J,J'}\,\delta_{m,m'}\,\delta_{k,k'}$ and $p_{eq}(\Omega_B)^{1/2} \equiv |0, 0, 0\rangle$. It is easily verified that the matrix representation of the operators $\tilde{O}$ and $\tilde{O}^{\dagger}$ in this basis is block diagonal with respect to the projection numbers m and k. The same is true for the corresponding exponential operators that have the same symmetry. It is therefore convenient to operate directly within the subset of functions $|J\rangle \equiv |J, 0, 0\rangle$. The matrix representation of $\tilde{O}$ is then

$$\tilde{O}_{J,J'} = D^{rot}J(J+1)\,\delta_{J,J'} + i\omega'_0\left(\frac{2J'+1}{2J+1}\right)^{1/2} C(J', 2, J; 0, 0)^2 \quad (60)$$

where the Clebsch−Gordan coefficients $C(J', 2, J; 0, 0)$ are not equal to zero only if J and J' are both even or both odd.[33]

Let us now introduce the following τ-dependent complex symmetric matrix

$$\mathbf{Q}(\tau) = e^{-\tilde{\mathbf{O}}\tau}\, e^{-\tilde{\mathbf{O}}^{\dagger}\tau}\, e^{-\tilde{\mathbf{O}}^{\dagger}\tau}\, e^{-\tilde{\mathbf{O}}\tau} \quad (61)$$

and the corresponding Hermitian matrix

$$\mathbf{V}(\tau) = e^{-\tilde{\mathbf{O}}^{\dagger}\tau}\, e^{-\tilde{\mathbf{O}}\tau} \quad (62)$$

in which $\mathbf{Q}(\tau) = \mathbf{V}(\tau)^{Tr}\cdot\mathbf{V}(\tau)$. Then, the scalar product defined in eq 57 can be written as

$$n \text{ even:} \qquad G_{CP}(2n\tau) = [\mathbf{Q}(\tau)^{n/2}]_{0,0} \quad (63)$$

$$n \text{ odd:} \qquad G_{CP}(2n\tau) = [\mathbf{V}(\tau)\cdot\mathbf{Q}(\tau)^{(n-1)/2}]_{0,0} \quad (64)$$

The elements of the matrixes $\mathbf{Q}(\tau)$ and $\mathbf{V}(\tau)$ are conveniently evaluated by diagonalizing the matrix $\tilde{\mathbf{O}}$ according to

$$\Lambda = \mathbf{C}^{-1}\cdot\tilde{\mathbf{O}}\cdot\mathbf{C} \quad (65)$$

where $\mathbf{C}^{-1} \equiv \mathbf{C}^{Tr}$ (because of the symmetry of $\tilde{\mathbf{O}}$) and $\lambda_i = \Lambda_{i,i}$ denotes the ith complex eigenvalue of Λ. Substituting $\exp(-\tilde{\mathbf{O}}\tau) \equiv \mathbf{C}\cdot\exp(-\Lambda\tau)\cdot\mathbf{C}^{Tr}$ and $\exp(-\tilde{\mathbf{O}}^{\dagger}\tau) \equiv \mathbf{C}^*\cdot\exp(-\Lambda^*\tau)\cdot[\mathbf{C}^{Tr}]^*$ into eqs 61 and 62 yields explicit expressions for the requested matrix elements

$$Q(\tau)_{i,j} = \sum_{m,m',m''} C_{i,m} C_{j,m''} M_{m,m'} M_{m'',m'} \, e^{-(\lambda_m + 2\lambda^*_{m'} + \lambda_{m''})\tau} \quad (66)$$

$$V(\tau)_{i,j} = \sum_{m,m'} C^*_{i,m} \, C_{j,m'} M_{m',m} \, e^{-(\lambda^*_m + \lambda_{m'})\tau} \quad (67)$$

where $\mathbf{M} \equiv \mathbf{M}^\dagger = \mathbf{C}^{\mathrm{Tr}} \cdot \mathbf{C}^*$.

We can now diagonalize the matrix $\mathbf{Q}(\tau)$ for each value of τ according to

$$\mathbf{W}(\tau) = \mathbf{T}(\tau)^{-1} \cdot \mathbf{Q}(\tau) \cdot \mathbf{T}(\tau) \quad (68)$$

where $\mathbf{T}(\tau)^{-1} \equiv \mathbf{T}(\tau)^{\mathrm{Tr}}$ and $\mathbf{W}(\tau)$ is the diagonal matrix of the eigenvalues. The powers of $\mathbf{Q}(\tau)$ appearing in eqs 63 and 64 are then given by $\mathbf{Q}(\tau)^m = \mathbf{T}(\tau) \cdot \mathbf{W}(\tau)^m \cdot \mathbf{T}(\tau)^{\mathrm{Tr}}$. Knowing the echo intensities (i.e., $Re\{G_{\mathrm{CP}}(2n\tau)\}$), the transverse relaxation rates, $R^{\mathrm{CP}}_{2,n}(\tau)$, are readily evaluated using eq 21. Finally, by choosing n to be sufficiently large such that convergence is achieved for all values of the pulse spacing, the asymptotic limit, $R^{\mathrm{CP}}_{2,\infty}(\tau)$, can be calculated. Clearly, any parametric dependence of $R^{\mathrm{CP}}_{2,\infty}(\tau)$ on θ_{B} is now lost, in contrast to the result obtained for director fluctuations (see eq 27). It should be noted that the above-described procedure is analogous to that employed by Freed et al.[36]

4. Model Calculations for Membrane Vesicles

In the following discussion, the main features of the present NMR relaxation model will be demonstrated for the specific case of ^{31}P nuclei located in the headgroup of lipid molecules forming unilamellar vesicles. It should be emphasized, however, that the slow-motional theory is rather general and can be applied to other probe nuclei provided that they exhibit localized magnetic interactions. First, we calculate transverse ^{31}P nuclear spin relaxation rates, $R^{\mathrm{CP}}_{2,\infty}(\omega)$, under the assumption that fluctuations of the quasi-spherical vesicles constitute the dominant transverse relaxation process. Here, ω denotes the pulse frequency, which is defined as the inverse pulse spacing in the CP sequence. The results are then compared with those obtained for planar membrane fluctuations with different dispersion relations for the damping times of the modes. Finally, we present model calculations for $R^{\mathrm{CP}}_{2,\infty}(\omega)$ assuming that translationally induced rotations of the lipid molecules constitute the major relaxation process.

Table 1 summarizes the parameters used in the calculations. Values for the translational diffusion coefficient[37] and the bending elastic modulus[31] are adopted from the literature. The value for η corresponds to the viscosity of water. For the effective lateral tension, values of $0 < \sigma < 100$ are reported.[31] For simplicity, a value of $\sigma = 0$ has been assumed.

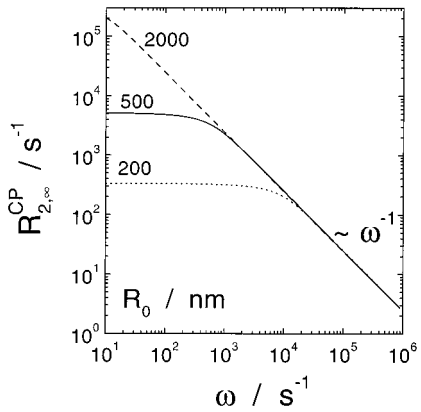

Figure 4. Dependence of the transverse ^{31}P nuclear spin relaxation rate $R^{\mathrm{CP}}_{2,\infty}(\omega)$ on the pulse frequency ω in a Carr−Purcell sequence for fluctuations of quasi-spherical vesicles. The dispersion profiles refer to three different vesicle radii: $R_0 = 200$ nm (···), $R_0 = 500$ nm (—), and $R_0 = 2000$ nm (− − −). All other parameters used in the plot are listed in Table 1.

Vesicle Fluctuations. Let us first discuss the results obtained for fluctuations of quasi-spherical vesicles. In Figure 4, the pulse frequency dependence of the transverse ^{31}P nuclear spin relaxation rate, $R^{\mathrm{CP}}_{2,\infty}(\omega)$, is depicted for three different vesicle sizes. The underlying parameters are listed in Table 1. Note that $R^{\mathrm{CP}}_{2,\infty}(\omega)$ depends linearly on ω^{-1} over a wide frequency range. Within this linear dispersion regime, the magnitude of $R^{\mathrm{CP}}_{2,\infty}(\omega)$ is independent of the size of the vesicle R_0 (see Figure 4), the effective lateral tension σ (see Figure 5), and the viscosity of the surrounding fluid η (see Figure 6). The bending elastic modulus κ is thus, apart from the strength of the nuclear interactions, the relevant parameter determining the relaxation rate. Other parameters such as R_0, σ, and η together with κ determine the frequency at which $R^{\mathrm{CP}}_{2,\infty}(\omega)$ levels off to a constant "plateau" value that is independent of ω.

Our model calculations show that for vesicles with a radius of $R_0 \geq 240$ nm the linear dispersion extends down to 10^4 Hz regardless of the variation of the viscosity and lateral tension within reasonable limits (5×10^{-4} to 5×10^{-3} Pa s for η and $\sigma < 100$). Thus, the bending elastic modulus κ can reliably be

TABLE 1: Standard Parameter Set Used in Model Calculations of Pulse Frequency-Dependent Transverse Nuclear Spin Relaxation Rates from Carr−Purcell Pulse Sequences[a]

		relaxation process		
parameters		quasi-spherical fluctuations	quasi-planar fluctuations	translationally induced rotations
residual magnetic anisotropy[b]	$\Delta\nu S_{\mathrm{MOL}}$	5 kHz	5 kHz	5 kHz
vesicle radius	R_0	500 nm		500 nm
translational diffusion coefficient[c]	D^{transl}			5×10^{-12} m^2 s^{-1}
bending elastic modulus[d]	κ	4×10^{-20} J	4×10^{-20}J	
thermal energy[e]	$k_{\mathrm{B}}T$	4.3×10^{-21} J	4.3×10^{-21} J	
bilayer thickness	d		4 nm	
viscosity[f]	$\eta(\eta_{\mathrm{eff}})$	6.5×10^{-4} Pa s	6.5×10^{-4} Pa s	
lateral tension[g]	$\sigma(\sigma')$	0	0	
intermolecular distance[h]	a	1 nm		
short wavelength cutoff[i]	λ_{c}		1 nm	
long wavelength cutoff[i]	λ_{l}		1 mm	
average director orientation[j]	θ_{B}	45°	45°	

[a] Parameters refer to lipid molecules in membrane vesicles and characterize different relaxation processes. [b] Residual magnetic anisotropy modulated by slow lipid motions. [c] Adapted from the literature.[37] [d] Selected from literature data.[31] [e] $T = 313$ K. [f] Viscosity of water. [g] Values of $0 < \sigma < 100$ are reported in the literature.[31] [h] See eq 34. [i] See eq 50. [j] θ_{B} denotes the angle between the average director and the external magnetic field.

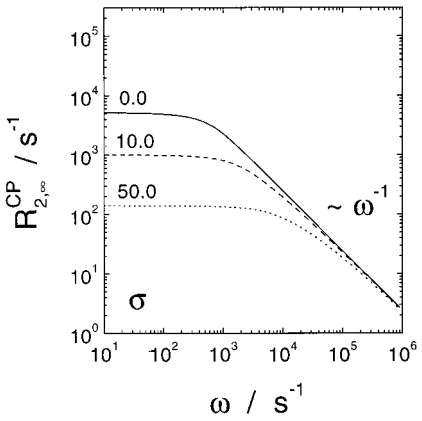

Figure 5. Dependence of the transverse ^{31}P nuclear spin relaxation rate $R_{2,\infty}^{CP}(\omega)$ on the pulse frequency ω in a Carr−Purcell sequence for fluctuations of quasi-spherical vesicles. The dispersion profiles refer to three different values of the effective lateral tension: $\sigma = 50$ (···), $\sigma = 10$ (− − −), and $\sigma = 0$ (—). All other parameters used in the plot are listed in Table 1.

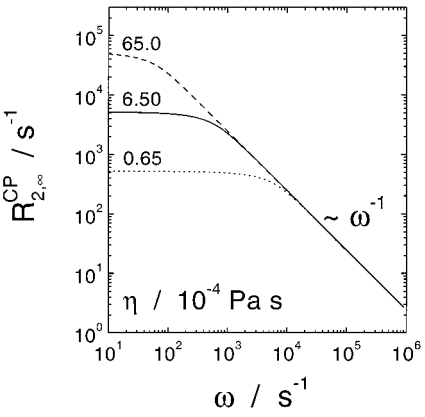

Figure 6. Dependence of the transverse ^{31}P nuclear spin relaxation rate $R_{2,\infty}^{CP}(\omega)$ on the pulse frequency ω in a Carr−Purcell sequence for fluctuations of quasi-spherical vesicles. The dispersion profiles refer to three different viscosities: $\eta = 0.65\times10^{-4}$ Pa s (···), $\eta = 6.50\times10^{-4}$ Pa s (—), and $\eta = 65.0\times10^{-4}$ Pa s (− − −). All other parameters used in the plot are listed in Table 1.

extracted from the slope of $R_{2,\infty}^{CP}(\omega)$ versus ω^{-1}, as demonstrated in Figure 7. Evidently, the new approach is well-suited to supplement video microscopy techniques[31] by extending the frequency range of fluctuations accessible to experimental observations, which might be particularly interesting in view of the conjectured dependence of the bending elastic modulus on the wavelength of the elastic deformations.[38]

It should be pointed out that η and σ have a distinct effect on the low-frequency cutoff of the $R_{2,\infty}^{CP}(\omega)$ dispersion profile. Note that for $\sigma > 10$ the transition from the linear to the plateau regime extends over several decades in ω (see Figure 5). Thus, a careful analysis of the low-frequency cutoff of the $R_{2,\infty}^{CP}(\omega)$ dispersion curve can provide reliable values for the lateral tension σ.

A linear dispersion regime was predicted earlier for longitudinal and transverse spin relaxation due to undulations of a free planar membrane.[9,11] It was also pointed out that in this case $R_1(\omega_0)$ and $R_{2,\infty}^{CP}(\omega)$ depend only on the bending elastic modulus κ. In reality, however, it is difficult to estimate the conditions under which free-membrane undulations occur in a multilamellar stack because this requires knowledge of the compression modulus B, which is extremely rare. According to a previous conjecture, B might be large enough to restrict

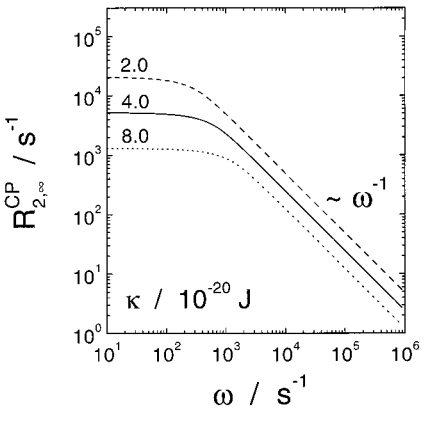

Figure 7. Dependence of the transverse ^{31}P nuclear spin relaxation rate $R_{2,\infty}^{CP}(\omega)$ on the pulse frequency ω in a Carr−Purcell sequence for fluctuations of quasi-spherical vesicles. The dispersion profiles refer to three different bending elastic moduli: $\kappa = 8.0\times10^{-20}$ J (···), $\kappa = 4.0\times10^{-20}$ J (—), and $\kappa = 2.0\times10^{-20}$ J (− − −). All other parameters used in the plot are listed in Table 1.

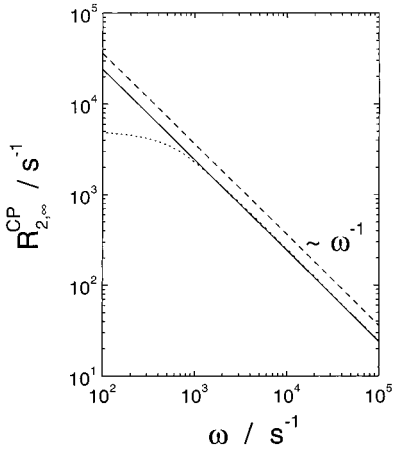

Figure 8. Dependence of the transverse ^{31}P nuclear spin relaxation rate $R_{2,\infty}^{CP}(\omega)$ on the pulse frequency ω in a Carr−Purcell sequence for collective orientational fluctuations. The dispersion profiles refer to three different hydrodynamic models: fluctuations of quasi-spherical vesicles (···), quasi-planar fluctuations with $\tau_{q_\perp} = 4\eta/\kappa q_\perp^3$ (—), and quasi-planar fluctuations with $\tau_{q\perp} = \eta_{eff} d/\kappa q_\perp^2$ (− − −). All three model calculations are based on the same parameters listed in Table 1.

the linear dispersion of $R_{2,\infty}^{CP}(\omega)$ to the upper MHz regime.[14] Evidently, unilamellar vesicles represent better-defined model systems because the low-frequency cutoff depends entirely on the parameters R_0, η, σ, and κ.

Nevertheless, it is interesting to compare $R_{2,\infty}^{CP}(\omega)$ dispersion profiles calculated for unilamellar vesicles with those obtained for planar fluctuations with a given long wavelength cutoff $\lambda_l = 2\pi/q_{\perp l}$. For clarity, λ_l was set equal to 1 mm. The comparison is presented in Figure 8. Note that the same viscoelastic parameters have been used in all three model calculations (see Table 1). The two dispersion profiles, referring to planar fluctuations, differ in the dispersion relation for the damping times of the elastic modes, $\tau_{q\perp}$. First, $R_{2,\infty}^{CP}(\omega)$ was calculated using $\tau_{q_\perp} = 4\eta/\kappa q_\perp^3$ (see eq 48), where a strong dissipation arising from the viscous damping of the surrounding fluid has been taken into account.[34] The resulting $R_{2,\infty}^{CP}(\omega)$ dispersion profile (solid line) is linearly dependent on ω^{-1} over the whole kHz range. Interestingly, the absolute values of $R_{2,\infty}^{CP}(\omega)$ are identical to those obtained for quasi-spherical fluctuations in the linear dispersion regime (dotted line).

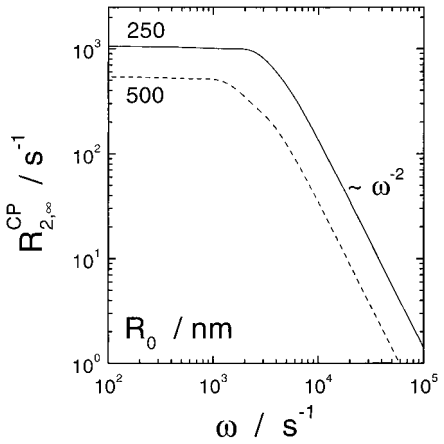

Figure 9. Dependence of the transverse ^{31}P nuclear spin relaxation rate $R_{2,\infty}^{CP}(\omega)$ on the pulse frequency ω in a Carr−Purcell sequence for translational lipid diffusion. The dispersion profiles refer to two different vesicle radii: $R_0 = 500$ nm (− − −) and $R_0 = 250$ nm (—). All other parameters used in the plot are listed in Table 1.

Second, $R_{2,\infty}^{CP}(\omega)$ was calculated for $\tau_{q_\perp} = \eta_{eff} d/\kappa q_\perp^2$ according to eq 49. Such a dependence of $\tau_{q_\perp}$ on $q_\perp$ is characteristic of thermotropic liquid crystals but has also been used for bilayer membranes.[9,11,14] With this dispersion relation, the dashed relaxation curve is obtained. One sees that the absolute values of $R_{2,\infty}^{CP}(\omega)$ are now increased by a factor of $^3/_2$. This result might be relevant to the assessment of bending elastic moduli that have been extracted on the basis of a planar fluctuation model.[7,8]

Lateral Lipid Diffusion. In principle, one might expect that translationally induced rotations of lipid molecules contribute to the transverse relaxation process. The vesicle radii studied experimentally[39] ($R_0 = 240$ nm and $R_0 = 425$ nm) display effective rotational diffusion correlation times, $R_0^2/6D^{transl}$, of approximately 2−6 ms. These values place the lateral diffusion process in the slow-motional regime, where a characteristic frequency dependence is expected.

Figure 9 shows calculated $R_{2,\infty}^{CP}(\omega)$ dispersion profiles for two different radii ($R_0 = 250$ nm and $R_0 = 500$ nm) assuming that translationally induced rotations represent the unique transverse relaxation process. The other parameters employed in the calculations are summarized in Table 1. Clearly, both relaxation curves exhibit a characteristic dispersion with a plateau at low frequencies and an inverse square dependence, $R_{2,\infty}^{CP}(\omega) \propto \omega^{-2}$, at higher frequencies. The fact that this is *not* observed experimentally indicates that, at least for vesicles with $R_0 \geq 200$ nm, lateral diffusion does not represent the major spin relaxation mechanism.[39] Also, experimental nuclear spin relaxation rates, $R_2^{CP}(\omega)$, for vesicles with distinct radii are found to be virtually indistinguishable over a wide range of pulse frequencies,[39] in contrast to the present calculations. This result is further evidence of some other mechanism dominating the transverse spin relaxation.

Evidently, translational lipid diffusion is too slow to contribute significantly to transverse relaxation in the kHz range.[40] This result is demonstrated in Figure 10, where we compare $R_{2,\infty}^{CP}(\omega)$ dispersion profiles for vesicle fluctuations (solid line) and lateral lipid diffusion (dotted line). The relaxation curves refer to a vesicle radius of $R_0 = 500$ nm and a residual magnetic anisotropy of 5 kHz. All other parameters underlying the calculations have been adopted from the literature (see Table 1). One sees that lateral diffusion across the vesicle shell makes only a minor contribution to the transverse relaxation rates, in contrast to previous suggestions.[43,44] The same is true for contributions from the tumbling motion of the vesicle as a whole.

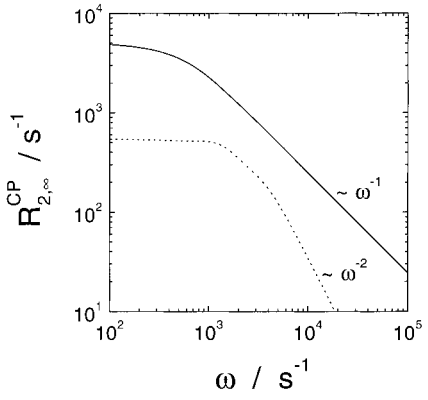

Figure 10. Dependence of the transverse ^{31}P nuclear spin relaxation rate $R_{2,\infty}^{CP}(\omega)$ on the pulse frequency ω in a Carr−Purcell sequence for two different relaxation mechanisms: translationally induced rotations (•••) and vesicle shape fluctuations (—). The dispersion profiles refer to the same vesicle radius of $R_0 = 500$ nm. All other parameters used in the plot are listed in Table 1.

5. Conclusions

A slow-motional approach to the analysis of transverse nuclear spin relaxation experiments has been presented for unilamellar, quasi-spherical membrane vesicles. Two relaxation mechanisms have been investigated: vesicle shape fluctuations and lateral diffusion of molecules along the curved surface of the sphere. It has been shown that even in the case of medium-sized vesicles (i.e., for radii $R_0 \geq 200$ nm) translational diffusion of the lipid molecules is too slow to contribute significantly to transverse spin relaxation in the kHz range. Rather, thermally excited vesicle fluctuations constitute the dominant transverse relaxation process. It turns out that an analytical solution of the stochastic Liouville equation is obtained if the vesicle fluctuations are modeled as a multidimensional Gaussian process.[22]

This theory has been employed to describe the pulse frequency dependence of transverse nuclear spin relaxation rates in CP sequences.[11,26] A detailed analysis reveals that the relaxation rates should be governed by quasi-spherical vesicle fluctuations for at least a few decades in the kHz range.[39] Values for the bending elastic modulus of the membrane might easily be extracted from the linear dispersion regime, where κ is the only adjustable parameter. This result could be relevant to studies of the modulation of membrane rigidity by different membrane constituents such as cholesterol or proteins.

In addition, the new approach could be used to evaluate reliable values of the effective lateral tension σ, which is difficult to determine with other techniques. Finally, the transverse NMR relaxation method might provide information about the dispersion relation governing the damping times of the elastic modes because the dispersion relation significantly affects the observable dispersion profiles.

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