Nuclear-spin relaxation induced by shape fluctuations in membrane vesicles

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Nuclear-spin relaxation rates resulting from shape fluctuations of *unilamellar quasispherical vesicles* are calculated. We show that in the kHz range these fluctuations yield—in contrast to previous conclusions on planar membranes – a relaxation rate proportional to the inverse Larmor frequency and provide direct information on the bending rigidity of membranes.

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Lipid vesicles represent simple model systems for the study of structural and dynamical properties of biomembranes [1]. A crucial parameter determining the shape fluctuations and functionality of a closed membrane is the bending elastic energy and the associated modulus κ. Measurements of κ have been performed using a variety of methods including video microscopy [2–4], micropipet [5], and electric-field deformation techniques [6], as well as nuclear magnetic resonance (NMR) relaxometry [7,8]. A theoretical basis for the NMR studies was given by Marqusee *et al.* [9] who calculated the nuclear-spin relaxation rate due to out-of-plane fluctuations, i.e., undulations, of a planar membrane. They found that the spin-lattice relaxation induced by planar membrane undulations depends linearly on the Larmor frequency, $T_1^{-1} \propto \omega^{-1}$, similarly as in thermotropic liquid crystals with a layered structure [10]. Although the spectrum of collective orientational fluctuations extends over a wide dynamic range, the relative contribution of this mechanism to the total relaxation rate is expected to be greatest at kHz frequencies where the effects of fast molecular motions are negligible. In fact, the linear dispersion behavior was experimentally observed in the kHz frequency regime for multilamellar stacks of planar membranes [11] and for oligolamellar dispersions of various lipids [7,8,12,13]. Later, however, doubts were raised about the capability of NMR to determine κ in such systems as the intermembrane coupling might limit the free membrane undulations to the upper MHz regime, where they are hardly observable anymore [14,15].

In this paper, we consider the relaxation of nuclear spins in *unilamellar quasispherical vesicles* where the problem of interbilayer interactions does not exist. Thermally excited fluctuations are restricted only by the finite area and volume of the closed bilayer shell. We show that the fluctuation modes characteristic of such vesicles give rise to spin relaxation with a linear dispersion regime extending far into the kHz range. This opens a direct way to determine the bending elastic modulus and some other viscoelastic properties of membrane vesicles. The new approach is well suited to supplement video microscopy techniques by extending the accessible frequency range of fluctuations. This might be particularly interesting in view of the conjectured dependence of the bending elastic modulus on the wavelength of the elastic deformations [16].

NMR relaxometry generally comprises the nuclear-spin-relaxation measurements in the frequency range between 10^3 and 10^9 Hz [17,18]. This wide dynamic range is covered either by combining standard measurements of the spin-lattice relaxation time (T_1) in the high-MHz regime with the field-cycling technique [17], or by measuring the dependence of the transverse spin-relaxation time on the pulse spacing in the Carr-Purcell-Meiboom-Gill echo experiment (T_{2E}^{CP}) [11]. Various nuclei, such as ^{1}H, ^{2}H, ^{13}C, and ^{31}P, have been used as NMR probes in lipid membranes. In all cases, the spin-relaxation results from the time-dependent interaction of the probe nucleus with the local electric or magnetic fields. The relaxation is directly related to the intensity and frequency of the thermal motions of the spin-bearing molecule. Quantitatively, the relaxation rates are determined by the strength of the nuclear interaction and by the spectral density functions $J(\omega)$ that are Fourier transforms of the correlation functions related to molecular motions. Fast internal and local reorientations of lipid molecules dominate the relaxation in the MHz regime. These motions only partly average the anisotropic nuclear-spin interactions because of orientational order of the molecules in the lipid bilayer. The residual anisotropy is then modulated by slow collective lipid motions, associated with fluctuations in the shape of the vesicles. Exactly these latter motions and their contribution to nuclear-spin relaxation will be discussed in this paper.

To get a basic idea about the effect of vesicle fluctuations on nuclear-spin relaxation, we adopt a hydrodynamical model, developed by Milner and Safran [19] on the basis of Helfrich's theory of the elasticity of lipid bilayers [20]. In this model, a vesicle is considered as a quasispherical shell of radius R_0 with constant volume $V=4\pi R_0^3/3$ and fixed area A. These assumptions are justified as the energy needed to compress the fluid inside or outside the vesicle or to change the area per lipid molecule is much larger than the energy needed to bend the bilayer. It is assumed that the vesicle is flaccid, i.e., the area A is larger than the area of the spherical shell with volume V, and that the difference can be expressed in terms of the dimensionless excess area Δ that depends on the vesicle preparation and on the temperature,

$$A=(4\pi+\Delta)R_0^2. \tag{1}$$

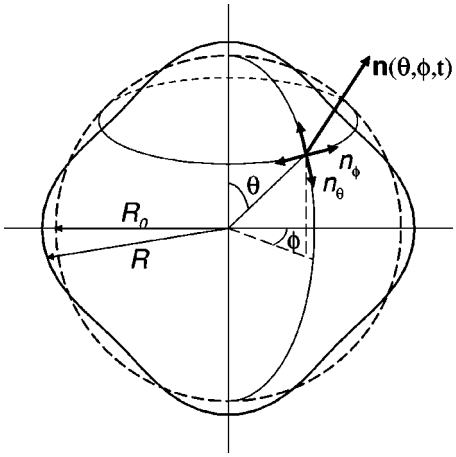

FIG. 1. Schematic presentation of a quasispherical vesicle: $\mathbf{n}(\theta,\phi,t)$ is the local normal, R_0 is the radius of a sphere with volume V, and R includes the time-dependent radial displacement.

For a quantitative modeling of the fluctuations, the vesicle is described by a slightly deformed spherical surface as

$$R(\theta,\phi)=R_0[1+u(\theta,\phi)], \tag{2}$$

where θ and ϕ denote the polar and azimuthal angles of the positional vector on the sphere and $u(\theta,\phi)$ represents the instantaneous displacement of the vesicle surface from its spherical conformation. It is convenient to expand $u(\theta,\phi)$ in a series of spherical harmonics $Y_{l,m}(\theta,\phi)$,

$$u(\theta,\phi)=\sum_l \sum_{m=-l}^{l} u_{l,m} Y_{l,m}(\theta,\phi). \tag{3}$$

The indices l and m specify the normal modes in spherical geometry that are discrete in contrast to the planar case where the undulations are expressed in terms of plane waves with a continuous distribution of in-plane wave vectors $\mathbf{q}_\perp$.

The displacement of the membrane $u(\theta,\phi)$ generates a curvature and consequently a deflection of the local membrane normal away from its average orientation in the radial direction (Fig. 1). The unit vector $\mathbf{n}=(n_r,n_\theta,n_\phi)$, representing the instantaneous direction of the local membrane normal, corresponds to the well-known director in thermotropic liquid crystals. If the amplitudes of fluctuations are small and only the leading terms up to the first order in $u(\theta,\phi)$ are retained, the components n_θ and n_ϕ of the normal can be expressed in terms of the radial displacements through

$$n_\theta=-\frac{\partial u(\theta,\phi)}{\partial\theta}, \quad n_\phi=-\frac{1}{\sin\theta}\frac{\partial u(\theta,\phi)}{\partial\phi}. \tag{4}$$

Since the orientational fluctuations of the normal are equivalent to collective orientational fluctuations of the lipid molecules, their effect on the spin relaxation is described by the spectral density function [9,15,21,22]

$$J(\omega)=\mathrm{Re}\int_{-\infty}^{+\infty}[\langle n_\theta(0)n_\theta^*(t)\rangle+\langle n_\phi(0)n_\phi^*(t)\rangle]\mathrm{e}^{-i\omega t}dt. \tag{5}$$

Here again, the small amplitude approximation was used. To calculate the correlation functions in Eq. (5), n_θ and n_ϕ are expressed in terms of the membrane displacements [Eq. (4)] and in the next step in terms of a superposition of spherical waves with amplitudes $u_{l,m}(t)$. For symmetry reasons the correlation functions do not depend on the position on the sphere. Taking into account the orthogonality of spherical harmonics, we obtain $J(\omega)$ as a sum of contributions of individual excitation modes characterized by the number l:

$$J(\omega)=\frac{1}{4\pi}\sum_{l=2}^{l_{\max}} l(l+1)(2l+1) \times\mathrm{Re}\int_{-\infty}^{+\infty}\langle u_{l,m}(0)u_{l,m}^*(t)\rangle\mathrm{e}^{-i\omega t}dt. \tag{6}$$

The upper limit of summation, $l_{\max}$, is determined by the size of the molecules, $l_{\max}\approx\pi R_0/a$, where a is the average distance between neighboring molecules in the lateral direction. The lower limit is $l=2$ since the $l=0$ and $l=1$ modes do not contribute to the relaxation. They represent a uniform radial displacement and a vesicle translation, respectively. The correlation function in Eq. (6) does not depend on m [19], therefore the summation over m has been performed directly.

The normal modes involved in Eq. (6) are overdamped since a strong dissipation arises from viscous damping of the bilayer by the surrounding fluid. In this process, the restoring force of the membrane is balanced by the viscous resistance of the water. According to Schneider *et al.* [23] and Milner and Safran [19], the time correlation function of the radial membrane displacements $u_{l,m}$ can be expressed as

$$\langle u_{l,m}(0)u_{l,m}^*(t)\rangle=\langle|u_{l,m}|^2\rangle e^{-t/\tau_l}, \tag{7}$$

where

$$\langle|u_{l,m}|^2\rangle=\frac{k_BT}{\kappa}\frac{1}{(l+2)(l-1)(l^2+l+\sigma)} \tag{8}$$

and

$$\tau_l=\frac{\eta R_0^3}{\kappa}\frac{(2l+1)(2l^2+2l-1)}{l(l+1)(l+2)(l-1)(l^2+l+\sigma)}. \tag{9}$$

Here, η is the viscosity of water and σ denotes an effective lateral tension related to the excess area Δ. It is interesting to note that Eq. (8), though evaluated for a simple model, does not change for the more elaborated models of vesicle fluctuations [1]. Taking into account that lateral molecular diffusion is much slower than collective membrane dynamics, $J(\omega)$ is calculated by inserting Eqs. (7)–(8) into Eq. (6) and integrating over the time [24]. This leads to $J(\omega)$

$$J(\omega)=\frac{k_BT}{4\pi\kappa}\sum_{l=2}^{l_{\max}}\frac{l(l+1)(2l+1)}{(l^2+l-2)(l^2+l+\sigma)}\frac{2\tau_l}{(1+\omega^2\tau_l^2)}. \tag{10}$$

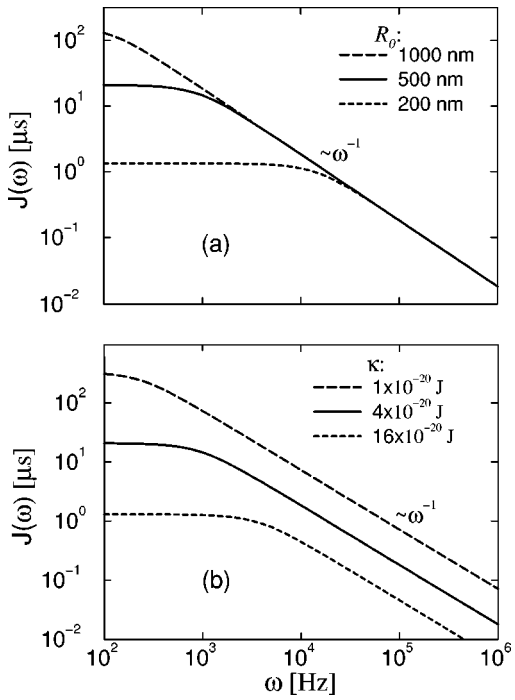

FIG. 2. Frequency dependence of the spectral density function $J(\omega)$: (a) for three different radii R_0 of the vesicle and $\kappa=4\times10^{-20}$ J; (b) for three different values of the bending elastic modulus κ and $R_0=500$ nm. Other parameters used in the plot are: $\eta=6.5\times10^{-4}$ Ns/m^2, $\sigma=0$, and $T=313$ K.

In Fig. 2, the frequency dependence of the spectral density function, given by Eq. (10), is depicted for different vesicle sizes (upper panel) and for different values of κ (lower panel). One sees that $J(\omega)$—and consequently the nuclear spin relaxation rates—depend linearly on ω^{-1} over a wide frequency range. Notably, within this linear dispersion regime, the magnitude of $J(\omega)$ is independent of the size of the vesicle R_0, the effective lateral tension σ, and the viscosity of the surrounding medium η, leaving the bending elastic modulus κ as the only relevant parameter. On the other hand, R_0, σ, η, and κ determine the frequency at which $J(\omega)$ levels off to assume a constant ''plateau'' value independent of ω. Our results show that for vesicles with a radius $R_0\geqslant\sim0.4$ μm, the linear dispersion extends down to $\omega\sim10^4$ Hz regardless of the small variations in viscosity and lateral tension that might occur in practice.

A linear dependence of $J(\omega)$ was predicted earlier for relaxation due to undulations of a free planar membrane [9]. It was also pointed out that in this case $J(\omega)$ only depends 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, since this requires knowledge of the compression modulus B, which is extremely scarce. According to a previous conjecture, B might be large enough to restrict the linear dispersion regime of $J(\omega)$ to MHz frequencies [14]. Evidently, *unilamellar vesicles* represent better-defined model systems, since the low cutoff frequency depends entirely on the relaxation time of the slowest mode as long as the magnetic damping is negligible.

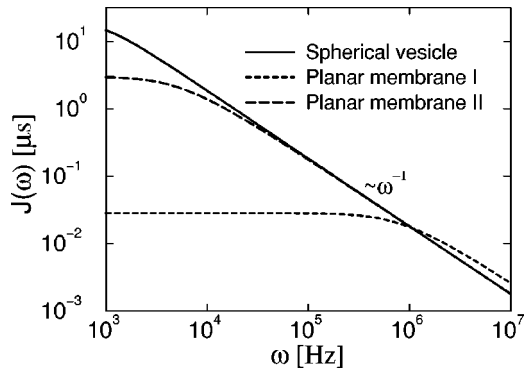

FIG. 3. Frequency dependence of the spectral density function $J(\omega)$ for a quasispherical vesicle (solid line), a freely undulating planar membrane with $\tau_{q_\perp}=\eta d/\kappa q_\perp^2$ (dotted line), and a freely undulating planar membrane with $\tau_{q_\perp}=4\eta/\kappa q_\perp^3$ (dashed line). The long wavelength cutoff of the planar membrane fluctuations is $2R_0$ in both cases. Other parameters used in the plot are: $R_0=500$ nm, $\kappa=4\times10^{-20}$ J, $\eta=6.5\times10^{-4}$ Ns/m^2, $\sigma=0$, $d=4$ nm, and $T=313$ K.

Nevertheless, it is interesting to compare the $J(\omega)$ 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 simplicity, λ_l was set equal to the diameter of the vesicle. The comparison is presented in Fig. 3. The two curves, referring to planar fluctuations, differ in the dispersion relation for the mode relaxation time $\tau_{q_\perp}$. First, $J(\omega)$ was calculated for $\tau_{q_\perp}=\eta d/\kappa q_\perp^2$, where d denotes the thickness of the membrane. 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]. The resulting $J(\omega)$ turns out to be frequency independent (''plateau'' value) in the whole kHz range (dotted line). Second, $J(\omega)$ was calculated using $\tau_{q_\perp}=4\eta/\kappa q_\perp^3$, that has been derived by taking into account the hydrodynamic interaction of the bilayer membrane with the surrounding medium [25,26]. With this dispersion relation, $J(\omega)$ levels off at a cutoff frequency that is at least two orders of magnitude smaller than in the first case (dashed line). Notably, in the linear regime, the spectral density function for planar fluctuations, derived with $\tau_{q_\perp}=4\eta/\kappa q_\perp^3$, assumes the simple form

$$J(\omega)=\frac{k_BT}{6\kappa\omega}, \tag{11}$$

which is identical with that obtained for quasispherical fluctuations in the same frequency range (solid line).

Finally, it should be noted that the kHz frequency range of calculated $J(\omega)$ is well accessible by present NMR techniques. The method of detergent dialysis enables preparation of unilamellar vesicle dispersions with a narrow distribution of vesicle sizes [27]. The proportionality coefficient between the measured relaxation rate and $J(\omega)$ can exactly be determined by measuring simultaneously the relaxation rate and the splitting of the NMR spectrum [28]. This coefficient depends also on the position of the probe nucleus in the

vesicle, a procedure has been developed to extract separately the relaxation rate for particular orientations [29]. In principle, one might expect that lateral translational diffusion of lipid molecules could average the relaxation rates of different parts of the vesicle. In reality, however, the diffusion with a coefficient of $D \approx 5 \times 10^{-12}$ m^2/s is too slow for such an effect in vesicles larger than a few hundred nanometers [30,31]. It is also too slow to contribute significantly to the relaxation process in the kHz range [27]. For the same reason, contributions from Brownian motion of the vesicle as a whole can be neglected.

On the basis of the above arguments we can be reasonably sure to predict that the nuclear-spin-relaxation rates from *unilamellar quasispherical vesicles* are governed by shape fluctuations for at least a few frequency decades in the kHz range. 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 could be relevant to studies of the modulation of the membrane rigidity by different membrane constituents, such as cholesterol or proteins. In addition, the new technique could provide the experimental basis for a crucial test of molecular-dynamics simulations of bilayer membranes in the mesoscopic range [32]. Finally, we showed that even in planar membranes, NMR relaxometry might give a notion on the dispersion relation for the mode relaxation time, since it drastically affects the low-frequency cutoff of the spectral density function.

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