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Probing Elastic and Viscous Properties of Phospholipid Bilayers Using Neutron Spin Echo Spectroscopy

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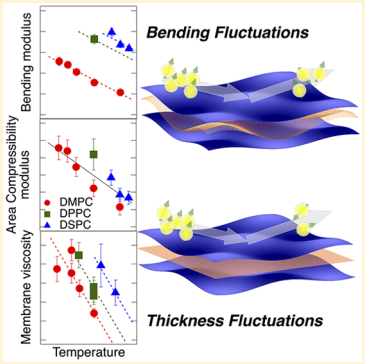
ABSTRACT: The elastic and viscous properties of self-assembled amphiphilic membranes dictate the intricate hierarchy of their structure and dynamics ranging from the diffusion of individual molecules to the large-scale deformation of the membrane. We previously demonstrated that neutron spin echo spectroscopy measurements of model amphiphilic membranes can access the naturally occurring submicrosecond membrane motions, such as bending and thickness fluctuations. Here we show how the experimentally measured fluctuation parameters can be used to determine the inherent membrane properties and demonstrate how membrane viscosity and compressibility modulus are influenced by lipid composition in a series of simple phosphatidylcholine bilayers with different tail lengths as a function of temperature. This approach highlights the interdependence of the bilayer elastic and viscous properties and the collective membrane dynamics and opens new avenues to investigating the mechanical properties of more complex and biologically inspired systems.



Biological membranes are a dynamic platform for transporting various materials across the membrane while also maintaining the physical integrity of the cell. These functions require that biological membranes be rigid enough to protect cell compartmentalization but flexible enough to smoothly deform without breaking the structures. Such distinctive elastic properties of the membranes have motivated a large body of research.[1−3] Theoretical[4−7] and computational[8−11] studies on this topic have advanced our understanding of the underlying physics on the molecular scale and their assemblies, in which the elastic properties dictate the membrane energetics and control the structures and collective membrane fluctuations. Those collective fluctuations, in turn, have been suggested to play an important role in regulating membrane protein functions.[12−15] Moreover, proteins readily diffuse along and redistribute themselves within the membrane, which implies that the membrane viscosity is another key property in maintaining various cell functions.[16,17] Although it is widely accepted that the functionality of biological membranes is ultimately determined by their structure and dynamics, the complex relationship among these properties remains poorly understood.

Given that collective membrane fluctuations are essential for understanding the bilayer physical chemistry,[18] it is imperative to directly measure the corresponding membrane dynamics to validate existing models and to gain insights into systems that are beyond the capabilities of current theory or simulations. Recent neutron spin echo (NSE) experiments have started to probe collective dynamics in lipid and surfactant membranes on the nanoscales and accessed the two primary nanoscale fluctuations in such membranes: bending and thickness fluctuations.[19−26] These observations show that bending fluctuations occur over a wide range of time scales of subnanosecond and longer, while thickness fluctuations are on the order of 100 ns in single-component fluid lipid bilayers.[25] Using existing theories, one can relate the observed fluctuation parameters to the elastic and viscous properties of the membranes.[7,27] The relationship between the height fluctuations measured by NSE and the membrane bending modulus is well-established and has been used to study the effects of lipid tail length and saturation[28] as well as the effects of additives such as cholesterol,[29] proteins,[30] and nanoparticles[31] on the bending modulus. In a recent theoretical work, Bingham et al.[7] established a relation between collective thickness fluctuations and the elastic and viscous properties of the membrane. Experimental results suggest that this theory can be used to interpret the fluctuations measured with NSE.[24]

In this work, we introduce a new analysis method based on the recently developed theory to determine the bilayer elastic and viscous properties and apply this methodology to our previous NSE results[25] combined with additional structure and dynamics measurements for a series of phosphatidylcholine bilayers. The present paper shows direct fits of the experimental relaxation rate for thickness fluctuations to a pseudo-theoretical function with parameters that represent the mechanical properties of the bilayers. The advantage of this approach is

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that we can directly and independently measure the inherent bending and thickness fluctuations in self-assembled membranes and systematically evaluate these parameters without perturbing the systems or adding probe molecules.

Figure 1 shows a typical intermediate scattering function, $I(q, t)/I(q, 0)$, measured using NSE, where q and t correspond to wavenumber transfer and Fourier time, respectively. The solid lines in Figure 1 are fits to a single-membrane fluctuation model proposed by Zilman and Granek: $I(q, t)/I(q, 0) = \exp[-(\Gamma t)^{2/3}]$, where Γ is the relaxation rate.[27] Specifically, the relaxation rate for bending fluctuations, Γ_b, is given by the expression $\Gamma_b = 0.025\gamma\sqrt{\frac{k_BT}{\tilde{\kappa}}}\frac{k_BT}{\eta}q^3$, where $\tilde{\kappa}$ is the effective bending modulus, η is the solvent viscosity, k_B is the Boltzmann constant, T is the temperature, and γ accounts for the orientational averaging between the membrane plaquettes and the scattered neutrons. When $\tilde{\kappa} \gg k_BT$, as in the present lipid bilayers, $\gamma = 1$ is applied.[27,32] The inset to Figure 1 shows Γ following a q^3 dependence, as expected from the above expression for Γ_b. The slope of the curve is proportional to the effective bending modulus, $\tilde{\kappa}$. Accounting for the intermonolayer frictions,[5,6] Watson and Brown[33] showed that the effective bending modulus measured in NSE can be related to the intrinsic bending modulus, κ, by $\tilde{\kappa} = \kappa + 2h^2k_m$, in which h denotes the height of the neutral surface from the bilayer midplane and k_m is the monolayer area compressibility modulus. k_m is defined as $k_m = 12\kappa_m/h_c^2$, where κ_m is the monolayer bending modulus and h_c is the monolayer hydrocarbon thickness.[34] The monolayer parameters can be reexpressed in terms of the bilayer parameters as $\kappa_m = \kappa/2$, yielding $\tilde{\kappa} = \{1 + 48(h/2h_c)^2\}\kappa$. The height of the neutral surface cannot be measured experimentally and remains a topic of discussion in literature with values of $h/2h_c$ ranging from 0.25 to 0.6.[10,11,30,35−37] In the past, we have used a value of $h/2h_c \approx 0.6$ to fit NSE data; however, this value puts the neutral surface within the headgroup region of the bilayer, and h is generally assumed to be close to the interface between the hydrophilic headgroup and hydrophobic tail.[38−45] Accordingly, here we use a value of $h/2h_c = 0.5$ to analyze the data.

Accepting these refinements, Γ_b can be rewritten as

$$\frac{\Gamma_b}{q^3} = 0.0069\sqrt{\frac{k_BT}{\kappa}}\frac{k_BT}{\eta} \quad (1)$$

The corresponding values of κ for the lipid bilayers are shown in Figure 2a. κ increases with decreasing temperature in each lipid bilayer as well as with increasing lipid tail length. It has already been well established in the literature that κ increases quadratically with bilayer thickness.[1,28,46]

Given that bending fluctuations cause one leaflet to compress and the other leaflet to stretch, one can use the thin elastic sheet model that predicts a direct relationship between κ and the bilayer area compressibility modulus K_A, as $K_A = \beta\kappa/(2h_c)^2$.[34] The coupling constant β depends on the degree of coupling between the two leaflets: $\beta = 12$ when the two monolayers are fully coupled, while $\beta = 48$ when the monolayers are completely uncoupled. Rawicz et al.[46] proposed a value of $\beta = 24$ using a polymer brush model that has been shown to hold true for a number of fluid lipid bilayers.[47] Accordingly, we have adopted a value of $\beta = 24$ in this paper, and calculated K_A by combining h_c values measured using small-angle neutron scattering (SANS) and κ values from NSE. Figure 2b shows the resulting values for the three different phospholipid tail lengths, dimyristoyl-, dipalmitoyl-, and distearoyl-phosphocholine (DMPC, DPPC, and DSPC). The values of K_A are independent of the lipid tail length, as expected,[46] and decrease with increasing temperature. The linear fit provided K_A (N/m) $= -0.0037(7)T + 1.68(7)$.

Additional thermal fluctuations can be measured by applying one of the advantages of neutron scattering; i.e. replacing H atoms with D dramatically alters the scattering contrast without significantly modifying the physicochemical properties of the systems.[48] When we exchange H for D in the lipid tails, the scattering contrast of the tails can be matched to the surrounding solvent, making the tails "invisible" to the neutrons and thus highlighting the scattering from the lipid headgroups. Since the scattering contrast is predominantly from the lipid headgroups, we now emphasize the dynamics normal to the plane of the membrane. As shown in Figure 3, the data display an underlying q^3 dependence of Γ, characteristic of the bending fluctuations discussed above; however, there is also a distinct peak in the data at $q \approx 1.0$ nm^{-1}. The peak indicates an enhancement in the dynamics at the length scale of the bilayer thickness, which we assign to the bilayer thickness fluctuations.[21−26]

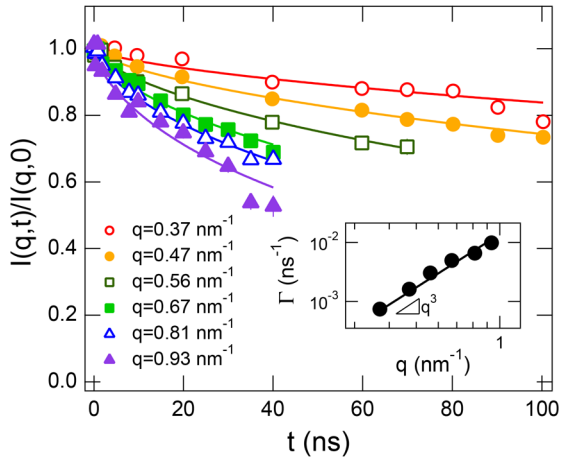

Figure 1. Intermediate scattering functions measured by NSE for protiated DMPC in D_2O at $T = 338.15$ K. Inset indicates the q dependence of the relaxation rate Γ ($\propto q^3$) shown as a solid line. Error bars represent ±1 standard deviation throughout the paper.

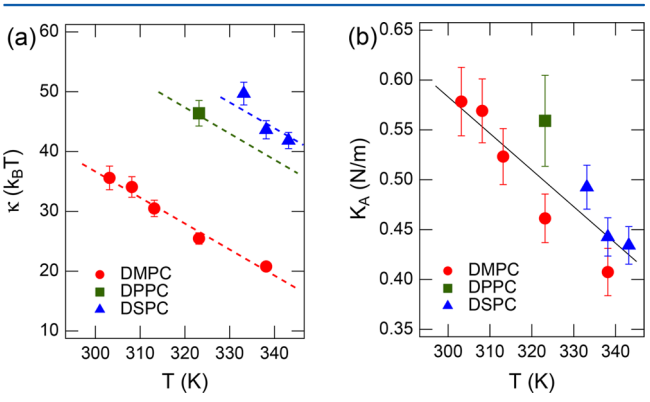

Figure 2. (a) Temperature variation of the bending modulus κ for DMPC, DPPC, and DSPC. The dashed lines guide the eyes. (b) Temperature dependence of the area compressibility modulus K_A. The solid line indicates the result of a linear fit of the data.

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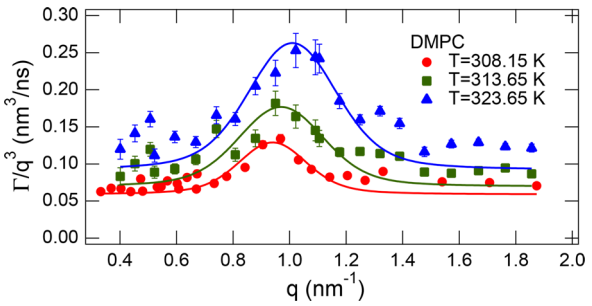

Figure 3. Normalized relaxation rate Γ/q^3 for tail-deuterated DMPC vesicles at different temperatures. The lines indicate the result of the fit with only one fit parameter μ to a convolution product between eq 5 and Gaussian distribution function representing experimental wavelength distribution.

In the past, we fit the peak in the data with a Lorentz function to phenomenologically explain the enhanced dynamics[21−26]

$$\frac{\Gamma}{q^3} = \frac{\Gamma_b}{q^3} + \frac{(\tau_{TF}q_0^3)^{-1}}{1 + (q - q_0)^2\xi^2} \quad (2)$$

in which the first term describes the underlying bending dynamics, quantified using protiated lipid bilayers, and the second term empirically fits the thickness fluctuation peak. The two important parameters we extracted from the Lorentz function were the relaxation time τ_{TF} and the fluctuation amplitude $\Delta h_c = 2h_c(q_0\xi)^{-1}$, with ξ^{-1} being the half width at half-maximum of the Lorentzian.[23,49,50] Additionally, q_0 corresponds to the peak location of the Lorentz function, which is equivalent to a dip location in the form factor of the bilayers measured by SANS.

Here we modify our description of the thickness fluctuations and represent the experimentally observed amplitude and relaxation time in terms of the elastic and viscous parameters of the membrane. Statistical mechanics predict a relationship between the area compressibility modulus K_A and the fractional change in area $\sigma_A = \Delta A/A$ as[8,51]

$$K_A = \frac{k_BT}{\sigma_A^2 A_0} \quad (3)$$

where A and A_0 are the unit area of the membrane and the area per molecule, respectively. Assuming the bilayer volume compressibility is negligible ($V = Ah_c$, $\Delta V/V = \Delta A/A + \Delta h_c/h_c \approx 0$), σ_A is compensated for by a corresponding change in thickness $\sigma_h = \Delta h_c/h_c$, that is, $\sigma_h^2 = \sigma_A^2$. Therefore, the thickness fluctuation amplitude can be expressed in terms of K_A as $\sigma_h = \sqrt{k_BT/(K_AA_0)}$.

Bingham, Smye, and Olmsted[7] proposed that the peristaltic mode (thickness fluctuations) relates to K_A and is damped by the viscosities of the solvent, η, and membrane, μ. When the wavelengths of the mode are shorter than the Saffman−Delbrück length,[52] represented by μ/η, the in-plane monolayer viscosity dominates and the damping is independent of the wavelength. In general, this condition is satisfied in pure lipid bilayers, and μ is expressed as[7]

$$\mu \approx K_A\tau_{TF} \quad (4)$$

These refinements lead to a new expression of the Lorentzian using the bilayers' elastic and viscous parameters as

$$\frac{\Gamma}{q^3} = \frac{\Gamma_b}{q^3} + \frac{K_Ak_BT}{\mu q_0^3k_BT + 4\mu q_0K_AA_0(q - q_0)^2} \quad (5)$$

In this equation, K_A is calculated from κ determined by NSE experiments with protiated lipid bilayers, assuming that K_A is the same for protiated and tail deuterated lipids within the experimental uncertainty. A_0 is expressed as $A_0 = V_L/(h_c + h_h)$, where V_L and h_h represent the lipid volume and the headgroup thickness, respectively. V_L can be determined from density measurements to find the specific molecular volume[53] or calculated from equations for the lipid volume available in literature.[54] The headgroup thickness is a parameter that is not yet well-defined but varies from (0.8 to 1) nm in literature.[55] In this paper, h_h = 1 nm was assumed as a typical value.[55−57] In addition, h_c and q_0 are determined by SANS; therefore, all of the parameters in eq 5 including the value of A_0 are known except for the membrane viscosity μ. This refined analysis method now defines the peak shape in Γ/q^3 in terms of the structural and elastic parameters. In the process, the number of fit parameters also has been reduced from two to one, which is particularly useful for temperatures near the phase-transition boundary where the NSE data at the peak location are noisy.

Representative fit results are given in Figure 3, showing that the present treatment describes the data well. It is important to note that in this fitting procedure we used the convolution product between eq 5 and a Gaussian distribution function (with the full width at half-maximum, typically ∼13%, calculated using equations developed in SANS data treatment[58]) to account for the effects of the finite instrumental q resolution.

The temperature dependence of μ is shown in Figure 4 for DMPC, DPPC, and DSPC. The membrane viscosity decreases with increasing temperature while also increasing with increasing tail length. The estimated values of μ are on the order of 10 nPa·s·m. Interestingly, the value of μ for these three lipids is about the same ($\mu \approx 100$ nPa·s·m) at $T \approx T_m$, which suggests a strong coupling between the bilayer phase behavior and the lipid motion. Values reported for lipid membrane viscosity in literature span several orders of magnitude, ranging from (∼2 to 3) nPa·s·m from fluorescence probe measurements,[45] falling ball viscometry,[59] domain motions,[60−63] and tether formation[64] to ∼10 nPa·s·m based on diffusion of tracer particles[65] to ∼700 nPa·s·m based on red blood cell recovery times from micropipette aspiration.[66,67] Our values for μ fall in the middle of this wide range, and, perhaps more importantly, they are determined without incorporating fluorescent probes or tracer particles.

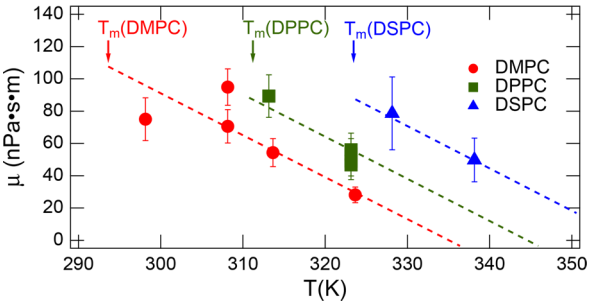

Figure 4. Temperature dependence of μ for DMPC, DPPC, and DSPC bilayers. The arrows indicate T_m for each tail-deuterated lipid bilayer.[25] The dashed lines are guides to the eye.

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In summary, we show here that NSE measurements of the equilibrium membrane fluctuations can be used to determine the elastic and viscous properties of a lipid bilayer, namely, κ or K_A and μ with minimal perturbation to the system. We note that this analysis is based on Helfrich−Canham theory and does not account for the molecular tilt degree of freedom. Recent simulation and diffuse X-ray scattering results have shown that incorporating the tilt modulus, K_θ, more accurately describes the dynamics on small length scales.[10,68−71] These small length scales are also accessible by NSE, suggesting that NSE may provide an additional experimental method for determining K_θ, and it would be interesting to consider the effects of molecular tilt in future NSE data analysis.

Finally, the same membrane properties measured here that govern the equilibrium thermal fluctuations also influence the complex viscoelastic response of lipid bilayers to non-equilibrium deformations. Rahimi and Arroyo suggested that the viscoelastic response of membranes is determined by the membrane elasticity, viscosity, and interleaflet friction, that is, κ or K_A, μ, and β.[72] These parameters are potentially accessible by independently measuring both the collective bending and thickness fluctuations using NSE as described here. Because both fluctuations are controlled by κ or K_A, it may be possible to estimate these two parameters independently and therefore extract the coupling constant β. However, an independent determination of K_A from these fluctuations is currently limited by the quality of the experimental data. Alternatively, β could be estimated by combining our NSE methods with another technique to measure κ or K_A. Because it is well known that gelation,[73] degree of saturation of the lipid tail[74] or mixing lipids with cholesterol[47] can significantly modulate interleaflet coupling, the type of experiments described here can potentially be applied to a wide variety of lipid membranes to gain insights into the dynamics and elastic properties of lipid bilayers. We hope that future NSE experiments will provide a more detailed understanding of the interplay between membranes' thermal fluctuations and mechanical properties, which is key to resolving the complex relationships between the structure, dynamics, and functions in biologically relevant systems.

METHODS

The experimental data were collected at the NSE spectrometers on NG5-NSE and NGA-NSE at National Institute of Standards and Technology (NIST)[75−77] and on IN15 at the Institut Laue Langevin (ILL). Complementary structural measurements were performed on the SANS instruments at the NG3 and NG7 beamlines at NIST[78,79] and on D22 at ILL. Three phospholipids with different tail lengths, DMPC, DPPC, and DSPC, were studied.[25] The samples were prepared using a standard extrusion method that yielded ∼100 nm diameter unilamellar vesicles. Measurements were performed at a series of temperatures above the melting transition temperature, T_m, of each lipid sample, ensuring that the membranes were in the fluid phase. To highlight either the bending or thickness fluctuations, two scattering contrast schemes were employed. Protiated lipids in D_2O were used to measure bending fluctuations, while tail-deuterated lipids in D_2O were used to observe thickness fluctuations. The SANS measurements were performed at (10 or 20) mg/mL, while NSE measurements were done at 100 mg/mL.

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Notes

The authors declare no competing financial interest.

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