Viscoelasticity of two-dimensional fluid membranes under oscillatory tensile loadings

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ABSTRACT Lipid bilayer membranes consisting of two opposite phospholipid monolayers are present in all mammalian cell types, and are largely responsible for the dual solid-fluid behavior of individual cells. Despite numerous studies on the role of in-plane fluidity in membrane deformation, the dynamical viscoelastic nature of lipid bilayers has not yet been fully described. We thus numerically investigate the dynamical viscoelasticity of membranes under small amplitude oscillatory tensile loadings, given in micron-scale membrane surface area. We use hydrodynamic equations of bilayer membranes, obtained by Onsager’s variational principle, wherein the fluid membrane is assumed to be an almost planar bilayer membrane. Simulations are performed for a wide range of oscillatory frequencies and membrane tensions. Our numerical results show that as frequencies increases, membrane characteristics shift from elastic dominant to viscous dominant. Furthermore, such viscous- or elastic-dominant transitions obtained with 1-µm-wide loading profile appear within the range of frequency between 40 Hz and 400 Hz, and almost independently of surface tensions. The transition will shift to lower frequency range as the width of loading profile increases. These numerical results will serve as fundamental knowledge for building a precise continuum membrane model that takes multi-scale dynamics into account, and will provide insight into both passive and active cell dynamics, such as microcirculatory blood flow and cancer metastasis.

SIGNIFICANCE We have explored the dynamical viscoelasticity of a 2D fluid membrane featuring lipid bilayers under oscillatory tensile loadings, by computational and theoretical approach. Our numerical results show that membrane characteristics shift from elastic dominant to viscous dominant when the oscillatory frequency increases. Furthermore, the results obtained with the width of 1-µm-wide loading profile further show that viscous- or elastic-dominant transition appears within the range between 40 Hz and 400 Hz almost independently of surface tension.

INTRODUCTION

Lipid bilayer membranes, consisting of a series of opposing phospholipids arranged in a two-dimensional fluid crystalline assembly with ~5 nm thickness (1), are a common and fundamentally important structure in mammalian cells. Each lipid covers a surface area of approximately 0.7 nm² (= 70 Å²) (2). The membrane structure separates the inside and outside of the cell, and assumes various function-related shapes (3). In addition, membrane mechanical properties affect cell and membrane dynamics, such as active cell migration (4) and endocytosis (5, 6). From a mechanical viewpoint, while phospholipids in membranes can move in the planar direction, their displacement in the thickness direction is restricted, and thus the bilayers can behave as a two-dimensional fluid membrane. Such fluid deformable surfaces exhibit a solid-fluid duality, resulting in unique and complex mechanical characteristics wherein in-plane fluidity and elasticity can emerge simultaneously. Although cell mechanics have been well studied by various experimental strategies such as atomic force microscopy (AFM), micropipette aspiration, and optical tweezers, as reviewed in Ref. (7), an understanding of dynamical viscoelasticity of lipid bilayers under oscillatory loadings is still needed. In human blood flow in particular, red blood cells (RBCs), whose membrane is the main structural component due to lack of a nucleus, are subjected to mechanical stimulation by blood flow, which depends on the heart rate (~1 Hz), and by vessel walls in various organs. Thus, quantitative analysis of the dynamical viscoelasticity of lipid bilayers will provide insights not only into passive cellular flow (8, 9), which is examined in hemorheology (10, 11), but also into active cell dynamics such as those involved in cancer metastasis (12, 13).

In particular in biological materials including the lipid bilayer, which exhibit different responses to stress or strain when applied in different directions, the relationship between the Young’s modulus and shear modulus is not simple. On the other hand, dynamical viscoelasticity, especially as a linear mechanical response of materials, has been characterized by complex moduli representing the tensile or compressive resistance and the shear resistance. Recent experimental techniques have successfully quantified these complex moduli in lipid monolayers or bilayers, assuming a lin-
ear mechanical response of membranes to oscillatory shear strains (14–17). For instance, for different concentration of cholesterol (i.e., DPPC-Chol mixtures), Al-Rekabi and Contrera (14) used AFM to produce a map of the viscoelastic properties of a lipid bilayer composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), which is one of the primary lipids in lung surfactant (18) and is ubiquitous in cell membranes.

Along with these experimental studies, various theoretical frameworks have been proposed to describe fluid membrane dynamics (19–21), and some have been applied to problems regarding the spontaneous conformation of vesicles (22) and human RBCs (23). In these works, lipid bilayers have usually been exploited to describe a continuous elastic membrane (24), considering the scale difference between the micrometer system size and nanometer membrane thickness. Although membrane fluidity has not been fully described, spontaneous curvature has been well explained by an approximation of solid shells that store elastic energy during stretching or bending (25). In terms of soft matter physics, Seifert and Langer (26) successfully described bilayer hydrodynamics for almost planar membranes, where coupling of the membrane dynamics with the surrounding fluid was taken into account by modeling curvature, density-difference elasticity, intermonolayer friction, monolayer two-dimensional (2D) viscosity, and solvent three-dimensional (3D) viscosity. Fournier (27) further extended the results of Seifert and Langer (26) and quantified the effect of membrane tension on the relaxation rate, where the equations describing the dynamics of an almost planar bilayer membrane were derived using Onsager’s variational principle. This principle is an established, unified framework for the dissipative dynamics of a soft matter system (28, 29). It provides hydrodynamical equations pertaining to bilayer membranes by minimizing a Rayleighian consisting of active potential power for dynamical changes and of passive potential power, so-called dissipated power, to resist the change. More recently, Torres-Sánchez et al. (30) proposed new computational methods, that build on Onsager’s formalism and arbitrarily Lagrangian-Eulerian (ALE) formulations. Their methodologies were successfully applied not only to dynamic lipid bilayers, but also to adhesion-independent cell migration (30). Despite these efforts, the dynamical viscoelastic nature of lipid bilayers, especially with regard to tensile loadings, has not yet been fully described. A molecular dynamic (MD) approach has been applied to the oscillatory behavior of the lipid bilayer membrane (31) and to membrane fluctuations of RBCs (32). However, general MD approaches are based on the thermodynamic equilibrium framework, and most of MD studies did not fully describe membrane kinetics in terms of a universal framework of this kind (26, 28, 29).

Therefore, the objective of this study is to clarify the dynamical viscoelasticity of the lipid bilayer under oscillatory tensile loadings using a 2D fluid membrane model following the previous theoretical and numerical study by Fournier (27).

Assuming the linear mechanical responses of the membrane to weak oscillations, a small amplitude oscillatory tensile loadings is considered. Dynamical viscoelasticity is quantified by the complex moduli $E'(\omega) = E'(\omega) + iE''(\omega)$ in an imaginary system. where $i = \sqrt{-1}$ is the imaginary unit, $E'$ is the storage modulus representing elastic component of the stress, and $E''$ is the loss modulus representing the viscous portion. In this study, the metrics is evaluated by scaled mass density and stress in the membrane. Simulations are performed for wide range of loading frequencies $\omega(=2\pi f)$ and surface tensions $\sigma$.

**METHODS**

Model of lipid bilayer membrane

Following the previous theoretical and numerical study by Fournier (27), we consider a lipid bilayer membrane made of only one lipid type in an unbounded flow field. The membrane shape is therefore characterized by the height $z = h(r, t)$ from the plane at $z = 0$ to the membrane mid-surface, where $r$ is the membrane coordinate projected onto the x-y plane. Thus, the membrane coordinate is expressed as $R = (r, h(r))$. Two monolayers in the membrane possess a lipid mass density $n^\pm(r, t)$ in deformed state $h(r, t)$, where superscripts “+” and “−” represent upper monolayer ($z > h$) and lower ($z < h$) monolayer, respectively. Using a mass density $n_0$ in the tensionless state as the reference, the membrane state can be described by its shape (or height) $h(r, t)$ and scaled mass density $\rho^\pm(r, t)$ as:

$$\rho^\pm(r, t) = \frac{n^\pm(r, t) - n_0}{n_0}.$$  (1)

We also consider the 3D solvent velocities $V^\alpha_\pm(R, t)$ ($\alpha = x, y, z$) on either side of the membrane, and the 2D lipid velocities $v^\pm_i(r, t), (i = x, y)$ in both monolayers. A schematic of the 2D fluid membrane is shown in Fig. 1.

The bulk solvent is assumed to be an incompressible fluid, and hence $V^\alpha_\pm$ satisfies the following mass conservation law:

$$\partial_\alpha V^\alpha_\pm = 0,$$  (2)

where $\partial_\alpha = \partial / \partial R_\alpha$. The lipid mass conservation equation is $\partial_t n^\pm + \partial_i (n^\pm v^\pm_i) = 0$, where $\partial_i = \partial / \partial r_i$. The first order approximation leads the following equation:

$$\partial_t \rho^\pm + \partial_i v^\pm_i = 0.$$  (3)

The Einstein summation convention where repeating the same index twice in a single term implies summation over all possible values of that index is adopted. Considering no-slip boundary conditions on the membrane surface, the external fluid must satisfy the following equations:

$$V^\alpha_\pm|_{z=h} = v^\pm_i,$$  (4)

$$V^\alpha_\pm|_{z=h} = \partial_i h.$$  (5)
Figure 1: Geometrical description of the membrane shape \( h(\mathbf{r}, t) \) and membrane coordinate \( \mathbf{R}(\mathbf{r}, h(\mathbf{r})) \) with scaled densities \( \rho^\pm \), monolayer velocities \( \mathbf{v}^\pm \), bulk solvent velocities \( \mathbf{V}^\pm \), and the surface distance away from the membrane mid-surface \( e \).

Equation (4) gives

\[
\partial_t V_i = \partial_t V_i^\pm \bigg|_{z=h} + \frac{\partial V_i}{\partial z} \bigg|_{z=h} \partial_t h, \tag{6}
\]

and the first term of the right-hand side is equal to \(-\partial V_i^\pm / \partial z\bigg|_{z=h}\) due to Eq. (2). The first-order approximation as well as the Stokes approximation allows us to neglect the second term in Eq. (6). Then, Eq. (3) gives \( \rho^\pm \) in Eq. (1) as:

\[
\rho^\pm = \int \frac{\partial V_i^\pm}{\partial z} \bigg|_{z=h} dt. \tag{7}
\]

Note that the upper and lower monolayers satisfy \( \rho^+ + \rho^- = 0 \) (27).

**Rayleighian and power components**

In the Stokes approximation (i.e., a problem simply satisfies all forces balance without all inertial effects), the dynamical equations for the motion of the membrane in the bulk solvent can be given by minimizing the total Rayleighian of the system with respect to all the dynamical variables (28, 33, 34):

\[
\mathcal{R} = \frac{1}{2} W + \mathcal{H} = \mathcal{P}_b^\pm + \mathcal{P}_s^\pm + \mathcal{P}_l^\pm + \mathcal{H}_{int} + \mathcal{H}_{ext}, \tag{8}
\]

where \( W/2 = (\mathcal{P}_b^+ + \mathcal{P}_s^+ + \mathcal{P}_l^+) \) is the resistive power against dynamical changes, the so-called dissipated power, which consists of three sources (29): \( \mathcal{P}_b \), the viscous dissipation in the bulk solvent above and below the membrane; \( \mathcal{P}_s \), the viscous dissipation in the lipid fluids of the two monolayers due to 2D viscosity; and \( \mathcal{P}_l \), the dissipation associated with the intermonolayer friction (26, 35). Moreover, \( \mathcal{H} = \mathcal{H}_{int} + \mathcal{H}_{ext} \) is the driving power for dynamical changes and consists of the intrinsic elastic power \( \mathcal{H}_{int} \) and external elastic power \( \mathcal{H}_{ext} \).

As described in Ref. (27), the three dissipated power sources in Eq. (9) can be written as:

\[
\mathcal{P}_b^\pm = \int_{B^\pm} d\mathbf{r} \eta D_{ab} D_{ab}^\pm,
\]

\[
\mathcal{P}_s^\pm = \int d\mathbf{r} \left( \eta_2 d_{ij}^s d_{ij}^s + \frac{\lambda_2}{2} d_{ii}^s d_{jj}^s \right),
\]

\[
\mathcal{P}_l^\pm = \int d\mathbf{r} \frac{b}{2} (v^+ - v^-)^2,
\]

where \( B^\pm \) is the volume defined by \( z > h \) or \( z < h \), \( D_{ab}^\pm(\mathbf{r}) = \left( \partial_v^+ V_i^+ + \partial_v^- V_i^- \right) / 2 \) is the rate-of-deformation tensor in the bulk solvent, \( d_{ij}^s(\mathbf{r}, s) = \left( \partial_v^s V_i^s + \partial_v^s V_i^s \right) / 2 \) is the rate-of-deformation tensor in the monolayer fluids, \( \eta \) is the bulk solvent viscosity, \( \eta_2 \) is the 2D viscosity, \( \lambda_2 \) is the dilatational viscosity, and \( b \) is the intermonolayer friction coefficient.

Assuming a small level of interdigitation between the lipids, and considering a small area of the membrane mid-surface \( dS = \left[ 1 + (\nabla h)^2 / 2 \right] d\mathbf{r} + O(h^4) \) and curvature \( c = \nabla^2 h + O(h^3) \), the density fields are essentially uncoupled. Hence, the internal elastic power of the membrane can be written as described in Ref. (26):

\[
\mathcal{H}_{int} = \int_S dS \left[ \frac{\sigma}{2} (\nabla^2 h)^2 + \frac{\kappa}{2} \left( \nabla^2 h \right)^2 + \frac{k}{2} \left( \rho^+ + e\nabla^2 h \right)^2 + \frac{k}{2} \left( \rho^- - e\nabla^2 h \right)^2 \right], \tag{13}
\]

where \( \sigma \) is the membrane tension, \( \kappa \) is the membrane bending rigidity, \( k \) is the monolayer stretching coefficient, and \( e \) is the surface distance away from the membrane mid-surface [see Fig. 1].

The external elastic energy representing oscillatory loading and loading profile are defined as:

\[
\mathcal{H}_{ext} = \int_{S_p} d\mathbf{r} \rho p(\mathbf{r}, t), \tag{14}
\]

\[
p(\mathbf{r}, t) = p_0 \exp \left( -\frac{12|\mathbf{r}|^2}{w^2} \right) \sin(\omega t), \tag{15}
\]

where \( p_0 \) is the loading amplitude, and \( w \) is the width of the loading profile characterized by the Gauss function. The integration is performed in the area \( S_p \), which is the projection onto the reference plane. Considering the previous micropipette aspiration test in blood granulocytes (36), where experiments were carried out with pipet sizes of 2–2.75 \( \mu \)m and suction pressures of \( \geq 1 \) Pa, the loading amplitude \( p_0 \) and the width of the loading profile \( w \) was set as \( p_0 = 0.5 \) Pa and \( w = 1 \) \( \mu \)m, respectively. The loading area is corresponding to or smaller than scan sizes in the AFM experiment (\( \geq 2.0-\mu \)m square) (14). Since the average wall shear stress in precapillary arterioles of normal human was estimated as 1.7 to
21.1 Pa (37), the loading amplitude \( p_0 \) is slightly smaller than physiological relevant stress in microcirculation. The scale of force applied area \( w \) corresponds to gaps in the endothelial barrier (\( \sim 1 \mu m \)) during the initial stages of transmigration of cancer cells (38), and could be found, e.g., in RBC-platelet or -microparticle hydrodynamic interactions (39, 40). Representative snapshots of extending membranes are shown in Figs. 2(a) and 2(b). We also confirm that the given amplitude of oscillatory tensile loadings is small, and hence the problem is linear mechanical responses of the membrane to weak oscillations, see panel (c) of Fig. 2.

Considering the Stokes approximation and differentiation of aforementioned Rayleighian (9), we have a dynamical equation of the membrane. The precise derivation of the equation is described in Appendix.

**Dynamical equations and methodology**

The Fourier transforms of surface and bulk quantities in the \((x, y)\) plane are defined by:

\[
\begin{align*}
\hat{f}(r, t) &= \int \frac{d \mathbf{q}}{(2\pi)^2} \hat{f}(\mathbf{q}, t)e^{i\mathbf{q} \cdot \mathbf{r}}, \\
\hat{g}(r, z, t) &= \int \frac{d \mathbf{q}}{(2\pi)^2} \hat{g}(\mathbf{q}, z, t)e^{i\mathbf{q} \cdot \mathbf{r}},
\end{align*}
\]

where \( \mathbf{q} \) is a wave-vector in the semi-Fourier space. Thus, we have \( h(\mathbf{q}, t) \) and \( \rho(\mathbf{q}, t) \) by Eq. (16), and \( \tilde{V}(\mathbf{q}, z, t) \) by Eq. (17). The caret symbols “\(^\hat{\cdot}\)” are omitted below.

Assuming the axial symmetry with respect to the \( z \)-axis \((x = y = 0)\), we obtain the linear time-evolution equation of \( h(\mathbf{q}, t) \) and \( \rho(\mathbf{q}, t) \) as:

\[
\partial_t \begin{pmatrix} \hat{h} \\ \hat{\rho} \end{pmatrix} = -\mathbf{M}(\mathbf{q}) \begin{pmatrix} \hat{h} \\ \hat{\rho} \end{pmatrix} + \begin{pmatrix} \pi p_0 w^2 \\ 0 \end{pmatrix} \exp \left( -\frac{w^2 q^2}{48\eta} \sin (\omega t) \right),
\]

where \( \mathbf{M}(\mathbf{q}) \) is the dynamical matrix:

\[
\mathbf{M}(\mathbf{q}) = \begin{pmatrix} \sigma q + k q^3 \\ \frac{4\eta q}{4q k e^2} \\ -\frac{k e q}{4q^2} \\ -\frac{b + \eta q + \eta s q}{2(b + \eta q + \eta s q^2)} \end{pmatrix}.
\]

Standard parameter values are used in this study following (27): \( \kappa = 2k e^2 \) is the effective bending rigidity at fixed lipid densities (26), \( \kappa = 10^{-19} \) J, \( k = 0.1 \) J/m², \( e = 1.0 \) nm, \( b = 10^9 \) J/s/m⁴, \( \eta = 10^{-3} \) J/s/m³, and \( \eta_s (= \eta_2 + \lambda_2/2) \) is the surface viscosity (= \( 10^{-9} \) J/s/m³) (41–45).

The explicit fourth-order Runge-Kutta method is used for the time integration. Owing to the axial symmetry assumption, the Fourier transform leads to Hankel transform, and we have

\[
\begin{pmatrix} h(r, t) \\ \rho(r, t) \end{pmatrix} = \frac{1}{2\pi} \int_0^{\infty} dq \begin{pmatrix} h(q, t) \\ \rho(q, t) \end{pmatrix} J_0(qr)q,
\]

where \( \tau_{zz} \) is the normal stress acting on the membrane, and \( J_0 \) is the Bessel function of the first kind. In particular, the form of \( \tau_{zz} \) in Fourier space is:

\[
\tau_{zz}(q) = 2\eta q \partial_t h(q) \pm \frac{\pi p_0 w^2}{24} \exp \left( -\frac{w^2 q^2}{48} \right) \sin (\omega t).
\]

The first term of the right-hand side represents the \((z, z)\) component of the liquid stress tensor \( T_{ij} \equiv -P \delta_{ij} + \eta \partial_t \eta_{ij} + \partial_t v_j \), evaluated for the upper and lower monolayers, and \( P \) is a one of the Lagrange multiplier fields (see Appendix) corresponding to the hydrostatic pressure, and \( \delta_{ij} \) is the Kronecker delta.

**Analysis of dynamical viscoelasticity**

We assume a linear mechanical response of the membrane to weak oscillatory strains \( \varepsilon(t) = \varepsilon_0 \exp (i\omega t) \), and evaluate a stress on the membrane \( \Sigma(t) = \Sigma_0 \exp (i(\omega t + \delta)) \) as:

\[
\Sigma(t) = E^\prime(\omega) \varepsilon(t),
\]

where \( \delta \) is the phase difference between the tensile strain \( \varepsilon(t) \) and stress \( \sigma(t) \). \( E^\prime(\omega) \) can be decomposed into two components:

\[
E^\prime(\omega) = \frac{\Sigma(t)}{\varepsilon(t)} = \frac{\Sigma_0}{\varepsilon_0} (\cos \delta + i \sin \delta) = E'(\omega) + iE''(\omega),
\]

where the real part \( E'(\omega) = (\Sigma_0/\varepsilon_0) \cos \delta \) is the storage modulus representing the elastic component of the stress, and the imaginary part \( E''(\omega) = (\Sigma_0/\varepsilon_0) \sin \delta \) is the loss modulus representing the viscous part (46, 47). We also define the loss tangent as:

\[
\tan \delta = \frac{E''}{E'}.
\]

Although various experimental techniques have been proposed to measure the dynamical viscosity of the membrane, it is still challenge to tackle the lipid dynamics during deformation. Optical tweezer experiments showed that a membrane shape corresponding to membrane strain of 1,2-Dioleoyl-sn-glycerol-3-phosphocholine (DOPC) giant unilamellar vesicles acts as a reporter of applied forces (48). In this study, instead of introducing membrane area strain, we can directly track the lipid molecular density, which is basis for dynamical models including lipid tilt near molecular inclusions or physicochemical interaction of lipids. In this study, therefore the strain \( \varepsilon(t) \) and stress \( \Sigma(t) \) are evaluated by the scaled mass density in upper monolayer \( \rho^+(t) \) in Eqs. (1) and (7) (or mean scaled
mass density, $\rho = (\rho^+ - \rho^-)/2$) and the normal stress acting on the membrane in upper monolayer $\tau_{zz}^*(t)$ in Eq. (21), respectively. Since applied loading amplitude $p_0$ is still small, and since it is practically difficult to quantify the dynamical viscoelasticity of whole membrane, aforementioned scaled density and normal stress are evaluated at the center of the membrane $r = 0$ (i.e., $\rho^+|_{r=0}$ and $\tau_{zz}^+|_{r=0}$), where the amplitudes are maximized.

**RESULTS**

First, we investigate the membrane behavior under oscillatory tensile loadings with a specific frequency $f$ for infinitesimally small surface tension $\sigma$ ($= 10^{-5} N/m$), which can be assumed as $\sigma = 0 N/m$. Figures 3(a) and 3(b) show one cycle of $h$, mean scaled density $\rho$, i.e., $\rho = (\rho^+ - \rho^-)/2$, corresponding to the upper monolayer scaled density $\rho^+$, normal stress on the upper monolayer along the $z$-direction $\tau_{zz}^+$, and external loads $\rho_{ext}$ at the center of the membrane $r = 0$ for different loading frequencies $f$ ($= 10^2$ Hz and $10^3$ Hz) after they have fully developed, where these values are normalized by each amplitude $\chi_{max}$, and shifted so that each baseline is the mean value $\chi_m$. Since $h|_{r=0}$ and density $\rho|_{r=0}$ drift as time passes, we use data after they have fully saturated to quantify the phase differences among these values. At relatively low frequency $f = 10^2$ Hz, there is no significant phase differences between $\tau_{zz}^+|_{r=0}$ and $\rho_{ext}|_{r=0}$ and between $h|_{r=0}$ and $\rho|_{r=0}$, where the later values ($h|_{r=0}$ and $\rho|_{r=0}$) are late for the other two ($\tau_{zz}^+|_{r=0}$ and $\rho_{ext}|_{r=0}$) [Fig. 2(a)]. More higher frequency condition ($f = 10^3$ Hz) causes apparent phase differences among those values, where the stress $\tau_{zz}^+$, and scaled density $\rho|_{r=0}$ start to be later for the external load $\rho_{ext}|_{r=0}$ and height $h|_{r=0}$, respectively [Fig. 2(b)].

We also investigate Lissajous-Bowditch plots (49, 50) of $\tau_{zz}^+|_{r=0}$ versus $\rho|_{r=0}$, following the classical way to analyze nonlinear oscillations. Following Figs. 2(a) and 2(b), these values are normalized by each amplitude, and shifted so that each baseline is the mean value [see Fig. 2(c)]. We recall that an elastic solid would correspond to a straight line deviated at $\pi/4$ from $y$-axis, a Newtonian fluid to a perfect circle, and viscoelastic materials under small amplitude of oscillatory loading to an ellipse whose major axis deviates at $\pi/4$ from $y$-axis. Nonlinear responses can generally lead to a more complex shape, which itself is a characterization of the nonlinear viscoelasticity of the suspension (49), as usually observed under large amplitude of oscillatory loadings. The result for the membrane are shown in Fig. 2(c), where the Lissajous plots are asymptotic from the ellipsoidal shape to the circle one as $f$ increases. The result indicates that given amplitude is small enough to assume linear mechanical response of the membrane to oscillatory tensile loadings.

Figure 3(a) and (b) show a series of representative snapshots of the deformed membrane during loading cycle $T (=1/f = 10^{-2} s)$, where color contours represent the mean scaled density $\rho$, and the stress in the tensile direction $\tau_{zz}^+$. At the

Figure 2: (a, b) Time history of the calculated values $\chi$; membrane height $h|_{r=0}$ (red solid line), mean scaled density $\rho|_{r=0}$ (blue dashed line), membrane stress $\tau_{zz}^+|_{r=0}$ (green solid line), and external load $\rho_{ext}|_{r=0}$ (black dashed line) at the center of the membrane $r = 0$ during a period $T$, obtained with (a) $f = 10^2$ Hz and (b) $f = 10^3$ Hz, respectively. Those values are normalized by the each amplitude $\chi_{max}$, and shifted so that each baseline is the mean value $\chi_m$. (c) Lissajous-Bowditch plots of normalized membrane stress $[\tau_{zz}^+|_{r=0} - (\tau_{zz}^+|_{r=0})_{0}] / (\tau_{zz}^+|_{r=0})_{0}$ vs. the normal stress acting $[\rho|_{r=0} - (\rho|_{r=0})_{0}] / (\rho|_{r=0})_{0}$ for different $f (= 10^2$ and $10^3$ Hz). The results are obtained with infinitesimally small surface tension $\sigma \approx 0$. 
phase when the height of the membrane \( h\lbrack r=0 \rbrack \) at \( r = 0 \) is the maximum (\( t = 0 \)), \( \tau_{zz}^{\ast}\lbrack r=0 \rbrack \) is large, while the density responds late [Figs. 3(a) and 3(b)]. Figure 3(c) shows one cycle (0.95 \( \leq t \leq 1 \)) of \( h\lbrack r=0 \), \( \rho\lbrack r=0 \), \( \tau_{zz}^{\ast}\lbrack r=0 \), and \( p_{\text{ext}}\lbrack r=0 \) after they have fully developed.

Considering Eq. (23), the storage modulus \( E' \) and loss modulus \( E'' \) can be quantified using \( \rho^{\ast}\lbrack r=0 \) and \( \tau_{zz}^{\ast}\lbrack r=0 \), and the results are shown in Figs. 4(a) and 4(b), respectively. Previous experimental data of DPPC bilayers using AFM by Al-Rekabi and Contera (14) are also plotted, where \( E' = 63\pm15.3 \text{ MPa} \), and \( E'' = 89\pm20.0 \text{ MPa} \) for \( f = 150-420 \text{ kHz} \).

Our numerical results show that both storage modulus and loss modulus increase with frequency \( f \) (Fig. 4). Especially at the highest membrane tension \( \sigma = 10^{-3} \text{ N/m} \), both values agree well with previous experimental results (14). We evaluate the calculated \( E' \) and \( E'' \) based on a power law \( y = \beta x^{\alpha} \). At the highest \( \sigma \) (\( = 10^{-3} \text{ N/m} \)), the increase of \( E' \) for relatively low \( f \) (\( \leq 10^{3} \text{ Hz} \)) can be well approximated as \( E' \propto f^{0.055} \), while \( E'' \) for full range of \( f (10^{0} \text{ Hz} \leq f \leq 10^{10} \text{ Hz}) \) that we investigated can be well approximated as \( E'' \propto f^{0.86} \). Note that the mean value and standard deviation of \( \alpha \) for different \( \sigma \) were \( 0.103 \pm 0.059 \text{ Pa} \) in \( E' \) and \( 0.753 \pm 0.251 \text{ Pa} \) in \( E'' \). These results indicate that lipid bilayer membrane characterized by fluid membrane cannot be modeled as Maxwell materials (Appendix), where \( E' \propto f^{2} \) and \( E'' \propto f \) for low \( f \).

To investigate the effects of frequency \( f \) and membrane tension \( \sigma \), the phase differences among \( h\lbrack r=0 \), \( \rho\lbrack r=0 \), \( \tau_{zz}^{\ast}\lbrack r=0 \), and \( p_{\text{ext}}\lbrack r=0 \) as a function of frequency \( f \) are shown in Figs. 5(a)–(c). The phase difference \( \delta_{h,p} \) between \( h\lbrack r=0 \) and \( p_{\text{ext}}\lbrack r=0 \) is shown in Fig. 5(a). The result shows that \( \delta_{h,p} \) gradually reaches \( \pi/2 \) as the frequency \( f \) increases and as the membrane tension \( \sigma \) decreases [Fig. 5(a)].

On the other hand, the phase difference \( \delta_{\rho,p} \) between \( \rho\lbrack r=0 \) and \( p_{\text{ext}}\lbrack r=0 \) gradually reaches \( \pi \) as the frequency \( f \) increases and as the membrane tension \( \sigma \) decreases [Fig. 5(b)]. In comparison with \( \delta_{h,p} \) [Fig. 5(a)], the effect of the membrane tension \( \sigma \) on the phase difference \( \delta_{\rho,p} \) is relatively small.

Similar to \( \delta_{h,p} \), the phase difference \( \delta_{\rho,\tau_{zz}^{\ast}} \) between \( \tau_{zz}^{\ast}\lbrack r=0 \) and \( \rho\lbrack r=0 \) reaches \( \pi/2 \) as the frequency \( f \) increases and as the membrane tension \( \sigma \) decreases [Fig. 5(c)]. Especially for higher frequencies \( f \geq 10^{5} \text{ Hz} \), \( \delta_{\rho,\tau_{zz}^{\ast}} \) are uniformly close to \( \pi/2 \) independently of \( \sigma \) [Fig. 5(c)].

Considering the specific value of \( \delta_{\rho,\tau_{zz}^{\ast}} = \pi/4 \), i.e., the loss tangent \( \tan (\delta_{\rho,\tau_{zz}^{\ast}}) = 1 \), the membrane characteristic shifts from elastic-dominant (i.e., \( \delta_{\rho,\tau_{zz}^{\ast}} < \pi/4 \) and \( \tan (\delta_{\rho,\tau_{zz}^{\ast}}) < 1 \)) to viscous dominant (i.e., \( \delta_{\rho,\tau_{zz}^{\ast}} > \pi/4 \) and \( \tan (\delta_{\rho,\tau_{zz}^{\ast}}) > 1 \)) as the frequency \( f \) increases [Fig. 5(c)]. Furthermore, the transition appears within the range of 40 Hz \( \leq f \leq 400 \text{ Hz} \) for all \( \sigma \) that we investigated [Fig. 5(c)].

We also investigated the effect of the width of loading profile \( w \) on viscoelastic metrics. The results of \( E', E'' \), and \( \delta_{\rho,\tau_{zz}^{\ast}} \) at \( \sigma = 0 \) for \( 10^{5} \text{ Hz} \), \( f \leq 10^{5} \text{ Hz} \), obtained with different width of loading profile \( w = 0.5 \text{, and } 2 \text{ \mu m} \) are superposed on Fig. 4 and Fig. 5(c). The results are shown in figure 6. \( E' \) tends to increase as \( w \) decreases [Fig. 6(a)] while \( E'' \) remains almost the same [Fig. 6(b)]. In consequence, the phase difference \( \delta_{\rho,\tau_{zz}^{\ast}} \) increases with \( w \) [Fig. 6(c)]. Since small \( w \) leads large curvature, resulting in large internal elastic energy (or power) of the membrane and \( E' \). The results indicate that apparent fluidity of a 2D fluid membrane becomes greater with larger loading profile \( w \).

**DISCUSSION**

Recent experimental techniques have made it possible to measure the dynamical viscoelasticity of the lipid bilayer, where complex “shear" moduli, \( G' (\omega) + iG'' (\omega) \), are quantified assuming a linear mechanical response of the membrane to oscillatory shear strain (15–17). These attempts have shown that monolayers of liquid-condensed dipalmitoylphosphatidylcholine (LC-DPPC) tend to behave as viscous dominant (\( G' < G'' \)) as frequency increases, especially for \( f (\leq 10^{5} \text{ Hz} \) (15, 17). It is also known that complex shear moduli of a phospholipid bilayer composed of 1,2-dimyristoyl-sn-glycero-3-phospho-choline (DMPC) are affected by membrane states or temperatures, wherein a viscous-dominant (\( G'' > G' \)) state is uniformly present at the liquid-gel transition temperature (\( = 23.5 \text{ °C} \)) independently of frequency, while at a liquid phase temperature (\( = 20.1 \text{ °C} \)) or gel phase temperature (\( = 25.8 \text{ °C} \)), the viscous-dominant state occurs at low frequencies (\( f (\leq 10^{3} \text{ Hz} \) and the elastic-dominant (\( G' > G'' \)) state occurs at high frequencies (\( f (> 10^{5} \text{ Hz} \) (16). Applying tensile loadings using AFM, Al-Rekabi and Contera (14) showed the effect of cholesterol concentration on the dynamical viscoelasticity at relatively high frequencies (\( \sim 150–420 \text{ kHz} \)) of a lipid bilayer composed DPPC-Chol mixture. Despite these insights, the dynamical viscoelastic characteristics of a bilayer composed of pure phospholipid (e.g., DPPC) has not yet been fully described, especially under tensile loadings. We therefore tackle this issue by model analysis following a previous theoretical and numerical study by Fournier (27), and quantify the dynamical viscoelasticity of an almost planar bilayer membrane under oscillatory tensile loadings. We obtained hydrodynamical equations of bilayer membranes using Onsager’s variational principle, which is an established unified framework for the dissipative dynamics of soft matter systems (28, 29).

In this study, viscoelastic metrics \( E' (\omega) = E' (\omega) + iE'' (\omega) \) as a response to tensile loadings were introduced, and quantified by the time difference between the scaled mass density \( \rho \) and normal stress acting on the membrane \( \tau_{zz} \). Our numerical results show that membrane characteristics shift from elastic-dominant to viscous dominant (\( E'' > E' \)) when the frequency \( f \) increases (Fig. 5), which are consistent with previous experimental measurements in DPPC bilayers (14) and in cells (51). However, this is counter to the tendency in complex shear moduli (\( G'' > G' \)), especially those obtained with a DMPC bilayer at liquid-phase and gel-phase.
Figure 3: (a, b) Representative snapshots of extending membrane under tensile loadings for \( f = 10^2 \text{ Hz} \), where color contours represent (a) the mean scaled density \( \rho \) and (b) the membrane stress \( \tau_{zz}^+ \). (c) Distribution of the calculated values \( \chi \) at each time period (\( t = 0, T/4, T/2, \) and \( 3T/4 \)) for \( f = 10^2 \text{ Hz} \). The results are obtained with \( \sigma = 0 \).

temperatures (16). Such viscoelastic behavior of the membrane under tensile loadings cannot be estimated by the well-known Maxwell materials, where complex moduli can be estimated as \( E' \propto f^2 \) and \( E'' \propto f \) for low \( f \). Our numerical results further show that such viscous- or elastic-dominant transitions appear within the range of 40 Hz \( \leq f \leq 400 \text{ Hz} \) at 1-\( \mu \text{m-width} \) \( (w = 1 \mu \text{m}) \) of loading profile for all surface tensions \( \sigma \) that we investigated \( (0 \leq \sigma \leq 10^{-3} \text{ N/m}) \). The transition will shift to lower frequency range as \( w \) increases (Fig. 6). These numerical results especially at \( w = 1 \mu \text{m} \) suggest that RBC membranes behave almost as elastic sheets under a physiological human heart beat, which is close to 1 Hz, and that viscous characteristics emerge for high frequencies \( O(f) \geq 10^1 \text{ Hz} \), which may happen in artificial blood pumps. Therefore, the knowledge would be helpful to design novel artificial blood pump to avoid the risk of rupture of RBCs, the so-called hemolysis, due to high frequency-dependent viscous stress on the RBC membrane. In more recently, the use of microbubbles coated by a biocompatible shell (e.g., lipid bilayer) as the ultrasound contrast agent have attracted attentions not only for echocardiography but also for the detection of the tumor and other therapeutic purposes (52), where the range of microbubble sizes appropriate for clinical use corresponds to resonance frequencies on the order of 1–10 MHz. However, dynamical viscoelasticity of the shell has been still debated. Our numerical results will gain insights into its design, in terms of mechanical properties to adequately adapt with surrounding solvent with high oscillatory frequencies.

To understand the formation mechanism of viscous- or elastic-dominant transition, we calculate the relaxation rates, which correspond to the eigenvalues \( \gamma_i(q) \) \( (i = 1 \text{ or } 2) \) of the dynamical matrix \( \mathbf{M}(q) \) in Eq. (19) as a function of wave-vector \( q \) for different surface tensions \( \sigma \) [see Fig. 7 in Appendix]. For lower loading frequencies \( f < 10^2 \text{ Hz} \), \( \gamma_i \) for \( q > 10^6 \approx 1/w \) are higher than \( f \). This indicates that the membrane exhibits fast relaxation or well-follow to loadings, resulting in small phase differences. While for higher loading frequencies \( f > 10^2 \text{ Hz} \), \( \gamma_i \) lower than \( f \) appears for \( q > 1/w \). Hence, the membrane cannot relax the scaled density \( \rho \), whose relaxation is represented by \( \gamma_2 \), under such high loading frequencies. Consequently, the phase difference \( \delta_{\rho,\tau_{zz}} \) increases. The results may be able to explain previous experimental data about viscoelastic property of cells. Rigato et al. (51) experimentally studied the rheological behavior of 3T3 fibroblast cells, which is a mouse fibroblast-like cell line, in a vast frequency range by treating them with four different drugs. They quantified viscoelastic properties with a frequency-dependent complex shear moduli \((G' \text{ and } G'')\). Although the magnitudes of the modulus were different from those of the metrics in our study \((E' \text{ and } E'')\), they also exhibited viscous- or elastic-dominant transition appears at the specific frequency. Furthermore, the transition frequency was lower in cells with disrupted actin or reduced prestress \((28 \text{ kHz} \text{ and } 56 \text{ kHz}, \text{ respectively})\); compared to 84 kHz for
Figure 4: Storage modulus (a) $E'$ and (b) loss modulus $E''$ as a function of frequency $f$ for different membrane tensions $\sigma$. Experimental data by Al-Rekabi and Contera (14) are also plotted. Power laws obtained with the largest $\sigma = 10^{-3}$ N/m$^2$ for $E'$ in relatively small $f \leq 10^3$ Hz and for $E''$ in whole rage of $f$ are also shown as dash-dot lines.

Figure 5: Phase difference (a) $\delta_{h,p}$ between $h|_{r=0}$ and $p_{ext}|_{r=0}$, (b) $\delta_{\rho,p}$ between $\rho|_{r=0}$ and $p_{ext}|_{r=0}$, and (c) $\delta_{\tau^+,\tau^+}$ between $\tau^+|_{r=0}$ and $\rho|_{r=0}$ as a function of frequency $f$ for different surface tensions $\sigma$. Dashed and dash-dot lines represent specific phase difference values of 0.5 and 0.25, respectively.
untreated cells) (51). The experimental results are consistent with that our numerical results obtained with larger surface tensions $\sigma$ [Fig. 5(c)]. More recent theoretical study by Hang et al. (53) proposed a self-similar hierarchical model, which is in broad agreement with all existing experimentally measured $G'$ and $G''$ including (51). Despite these efforts, the authors acknowledged that there is still no consensus on the formation mechanism of the change (54). Our numerical results of $\delta_{p_{xz}}$ [Fig. 5(c)] and the relaxation rates [Fig. 7] may give one explanation for the problem. Although some similarities are found in our metrics $E^*$ and complex shear moduli $G^*$, rigorous experimental measurements are required to relate with each other, which is a future study.

The calculated complex moduli obtained with the specific surface tension $\sigma = 10^{-3}$ N/m agree well with those in DPPC bilayers (14). Although surface tension depends on various conditions, e.g., temperature and cholesterol concentration, the orders of magnitude that we investigated ($0 \leq \sigma \leq 10^{-3}$ N/m) cover the physiologically relevant surface tension not only of a bilayer composed of pure phospholipid but also of some cell membranes such as lined structures. For instance, the interfacial tension of a lipid bilayer was numerically estimated in the range of $6 \times 10^{-3}$ to 0.2 N/m (55, 56). By applying a pressure difference on both sides of the membrane and the measuring of its curvature, the interfacial tension of lipid bilayers is identified as $(3.4 \pm 0.6) \times 10^{-3}$ N/m, with variation depending on the electrolyte composition (57): 1.623 $\times 10^{-3}$ N/m and 4.715 $\times 10^{-3}$ N/m for pure lecithin and pure cholesterol membranes, respectively (58). Using AFM, the lateral tension of pore-spanning lipid bilayers composed of N,N-dimethyl-N,N-dioctadecylammonium bromide (DODAB) was estimated as $1.0 \times 10^{-3}$ N/m in the fluid state, and as $5.0 \times 10^{-3}$ N/m in the gel state (59). Considering these studies, the order of magnitude of surface tension of lipid bilayers in the fluid state can be estimated as $O(\sigma) = 10^{-3}$ N/m. Hence, our numerical results for complex moduli and the phase differences obtained with $\sigma = 1$ mN/m potentially represent the dynamical viscoelasticity of a lipid bilayer membrane in the fluid state and at fixed temperature.

Although we considered a homogeneous lipid bilayer membrane, cell membranes in vivo contain non-negligible numbers of various proteins, e.g., cholesterol and phosphatidylcholine, and are embedded with various functional molecules such as peptides, proteins, and polysaccharides. Previous studies have revealed that changes to the local mechanical properties of the cell membrane regulate the propagation of forces in cells (4), thus modulating a variety of membrane-related dynamics such as membrane trafficking, endocytosis, actin assembly, cell signaling, and protein function (5, 6). Mechanical forces propagating through the membrane are also central to the propagation of the action potential in neurons (60) and affect the activity of membrane proteins such as ion channels (61, 62). Moreover, it has been reported that mechanical properties can modulate a membrane’s interface with its surrounding liquid and selectively control ionic ad-

Figure 6: (a) $E'$, (b) $E''$, and (c) $\delta_{p_{xz}}$ at $\sigma = 0$ in $10^1 \leq f \leq 10^3$ Hz for different width of loading profile $w$ ($= 0.5$, and $2 \mu m$).
sorption and condensation (63). Hence, it would be interesting to study how these factors, which may be roughly represented by stretching or bending stiffness in our membrane model, change dynamical viscoelasticity of the membrane. Furthermore, we considered fixed surface viscosity \( \eta_s \approx 10^{-9} \text{ J-s/m}^2 \) (41–45). The contribution of \( \eta_s \) to \( E' \) and \( E'' \) will be reported in a future study as well as other membrane parameters including stretching or bending stiffness.

Our numerical results and quantitative model analysis of the dynamical viscoelasticity of lipid bilayers will be helpful to build more rigorous continuum membrane models that consider multi-scale dynamics, and to gain insights not only into passive cellular flow, e.g., stable cell configuration (8, 9) and hemorheology (10, 11), but also into active cell migration such as in cancer metastasis (38).

**CONCLUSIONS**

We have explored the dynamical viscoelasticity of a 2D fluid membrane featuring lipid bilayers under oscillatory tensile loadings. Following a previous theoretical and numerical study by Fournier (27), we have used hydrodynamical equations of bilayer membranes, obtained with Onsager’s variational principle (28, 29). Our numerical results show that membrane characteristics shift from elastic dominant to viscous dominant (\( E' < E'' \)) when the frequency \( f \) increases. Calculated complex moduli obtained with a specific surface tension (\( \sigma = 10^{-3} \text{ N/m} \)) agree well with those in a previous experimental work using DPPC bilayers, especially at high frequencies (14). Our numerical results obtained with the width of loading profile \( w = 1 \mu\text{m} \) further show that viscous- or elastic-dominant transition appears within the range 40 Hz \( \leq f \leq 400 \text{ Hz} \) almost independently of surface tension \( \sigma \). The transition will shift to lower frequency range as the width of loading profile increases. These numerical results provide fundamental knowledge to build more rigorous continuum membrane models that consider multi-scale dynamics, and yield insight into characteristic cell dynamics at various time scales.

**APPENDIX**

**Derivation of the dynamical equation**

Taking into account the constraints described as (2)–(5), and introducing the Lagrange multiplier fields \( P^\pm (r), \xi^\pm (r), \mu_i^\pm (r), \) and \( \gamma^\pm (r) \), we take an extremum for the functional \( R^* \)

\[
R^* = \sum_{\varepsilon=+} \int_B \left[ \eta D_\alpha D^\varepsilon_{\alpha} - P^\varepsilon \partial_\alpha V^\varepsilon_{\alpha} \right] + \sum_{\varepsilon=+} \int_B \left[ \eta d_i d^\varepsilon_i J_{ij} + \frac{\lambda_2}{2} d^\varepsilon_{ij} d^\varepsilon_{ji} + \xi^\varepsilon (\rho^\varepsilon + \partial_i v_i^\varepsilon) + \mu_i^\varepsilon (v_i^\varepsilon - V_i^\varepsilon) + \gamma^\varepsilon (h - V_i^\varepsilon) \right] + k (\rho^\varepsilon + \varepsilon e \nabla^2 h) \dot{\rho}^\varepsilon + \varepsilon k e \nabla^2 (\rho^\varepsilon + \varepsilon e \nabla^2 h) \dot{h} + \int_B \left[ \frac{b}{2} (v^+ - v^-)^2 + (k \nabla^4 h - \sigma \nabla^2 h + p_0 \exp \left( -12 |r|^2 / w^2 \right) ) \dot{h} \right].
\]

Taking an extremum for \( R^* \) with respect to the fields \( V_i^\pm, \dot{V}_i^\pm, \dot{h}, v_i^\pm, P^\pm, \xi^\pm, \mu_i^\pm, \) and \( \gamma^\pm \) yields

\[
\frac{\partial R^*}{\partial V_i^\pm (r, 0)} = 0 \rightarrow \mp \eta (\partial_i V_i^\pm + \partial_t V_i^\pm) - \mu_i^\pm = 0, \\
\frac{\partial R^*}{\partial \dot{V}_i^\pm (r, 0)} = 0 \rightarrow \mp 2 \eta \partial_t V_i^\pm + P^\pm - \gamma^\pm = 0, \\
\frac{\partial R^*}{\partial \dot{h}} = 0 \rightarrow \xi^\pm + k (\rho^\pm + \varepsilon \nabla^2 h) = 0, \\
\frac{\partial R^*}{\partial \dot{h}} = 0 \rightarrow \gamma^\pm + k \nabla^4 h - \sigma \nabla^2 h + k e \nabla^2 (\rho^\pm - \rho^-) + p_0 \exp \left( -12 |r|^2 / w^2 \right) = 0, \\
\frac{\partial R^*}{\partial v_i^\pm} = 0 \rightarrow \eta_2 \partial_j \partial_i v_i^\pm - (\eta_2 + \lambda_2) \partial_i \partial_j v_i^\pm - \partial_t \eta^\pm + \mu_i^\pm + b (v_i^\pm - v_i^-) = 0.
\]
Further description about the derivation of the membrane hydrodynamic equations in Eqs. (18) and (19) are referred to the work by Ref. (27).

**Linear Maxwell materials**

The constitutive equations for the Maxwell materials, which are represented by a linear combination of the two types of material responses: a dashpot (viscous fluid) and a spring (elastic solid), can be written as:

\[
\Sigma(t) + \frac{\eta_d}{E_s} \dot{\varepsilon}(t) = \eta_d \dot{\varepsilon}(t),
\]

where \(\Sigma(t)\) and \(\varepsilon(t)\) are the total stress and strain at time \(t\), \(E_s\) is the elastic constant of the material, and \(\eta_d\) is the viscosity of fluid. Consider periodic strain \(\varepsilon(t) = \varepsilon_0 \exp(i\omega t)\) and its response \(\Sigma(t) = \Sigma_0 \exp(i\omega t + \delta)\), their time derivations are written as:

\[
\dot{\varepsilon}(t) = i\omega \varepsilon_0 \exp(i\omega t) = i\omega \varepsilon(t),
\]

\[
\dot{\Sigma}(t) = i\omega \Sigma_0 \exp(i\omega t + \delta) = i\omega \Sigma(t),
\]

Substituting these equations into Eq. (31), we have

\[
\dot{\Sigma}(t) + \frac{i\omega \eta_d}{E_s} \Sigma(t) = i\omega \eta_d \dot{\varepsilon}(t),
\]

\[
\Rightarrow \dot{\Sigma}(t) = \left(\frac{(\omega \mathcal{T})^2 E_s}{1 + (\omega \mathcal{T})^2} + i\frac{\omega \mathcal{T} E_s}{1 + (\omega \mathcal{T})^2}\right) \varepsilon(t),
\]

\[
= \left(\frac{(\omega \mathcal{T})^2 E_s}{1 + (\omega \mathcal{T})^2}\right) \dot{\varepsilon}(t) + \frac{\eta_d}{1 + (\omega \mathcal{T})^2} \dot{\varepsilon}(t),
\]

where we have introduced the Maxwell relaxation time \(\mathcal{T} = \eta_d / E_s\) (46). Equations (34) and (35) demonstrate that the Maxwell model exhibits stress response both in and out of phase with the applied deformation. Comparing the expressions between Eq. (22) and Eq. (34), we conclude that complex modulus \(E' = E'(\omega) + iE''(\omega)\) are defined as:

\[
E'(\omega) = \frac{(\omega \mathcal{T})^2 E_s}{1 + (\omega \mathcal{T})^2},
\]

\[
E''(\omega) = \frac{\omega \mathcal{T} E_s}{1 + (\omega \mathcal{T})^2}.
\]

We also conclude that the stress response can be interpreted in terms of a frequency-dependent elastic modulus \(\tilde{E}\) and viscosity \(\tilde{\eta}\) as:

\[
\tilde{E}(\omega) = E'(\omega) = \frac{(\omega \mathcal{T})^2 E_s}{1 + (\omega \mathcal{T})^2},
\]

\[
\tilde{\eta}(\omega) = \frac{1}{1 + (\omega \mathcal{T})^2} \eta_d.
\]

At short times \((\omega \mathcal{T} \gg 1)\), the Maxwell model behaves like a solid with the elastic modulus \(\tilde{E}(\omega) \approx E_s\), while at long times \((\omega \mathcal{T} \ll 1)\) it behaves as a viscous fluid with the viscosity \(\tilde{\eta}(\omega) \approx \eta_d\). The crossover between the two regimes occurs when the time scale of deformation is similar to the time scale of relaxation, \(\omega^{-1} \sim \mathcal{T}\).

The relaxation rates

The relaxation rates, which correspond to the eigenvalues \(\gamma_i(q) (i = 1 \text{ or } 2)\) of the dynamical matrix \(M(q)\) in Eq. (19), is described as a function of wave-vector \(q\) for different surface tensions \(\sigma\) as shown in Fig. 7, where \(\gamma_i(q)\) are obtained with the standard values given below Eq. (19). The values of \(\gamma_1\) increase with \(\sigma\) at relatively low \(q\) and collapse on a single curve for high \(q\). The values of \(\gamma_2\) are much lower than \(\gamma_1\) and follow almost common line except for infinitesimally small \(\sigma (\approx 0)\). It is known that \(\gamma_1\) corresponds to the relaxation of \(h\) at fixed \(\rho\), while \(\gamma_2\) to the relaxation of \(\rho\) at fixed \(h\) (27). Considering the scale of loading area \(w = 1 \mu m\), let us take a representative wave-length as \(\lambda = 2\pi/q = 1 \mu m\), corresponding to \(q \geq 10^6 \text{ m}^{-1}\). The values of \(\gamma_1\) collapses for over \(10^{-7} \text{ Hz}\), while \(\gamma_2\) around \(10^5 \text{ Hz}\) independently of \(\sigma\).

![Figure 7: The eigenvalues of the dynamical matrix \(M(q)\) in Eq. (19) as a function of wave-vector \(q\) for different surface tensions \(\sigma\): (solid lines) \(\gamma_1(q)\) and (dashed lines) \(\gamma_2(q)\). The results are obtained with the standard values given in the text. Dash-dot line represent the eigenvalues of 100 Hz.](image)

**AUTHOR CONTRIBUTIONS**

N.T., N.Y., and S.W. designed the research. M.S., and N.T. carried out simulations, analyzed the data. N.T., and N.Y. wrote the article and edited revised manuscript. M.S., N.T., and N.Y. interpreted results of simulations. N.T. prepared figures. M.S., N.T., N.Y., and S.W. approved final version of manuscript.

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DECLARATION OF INTERESTS
The authors declare no competing interests.

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