Double-mode relaxation of highly deformed anisotropic vesicles

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Lipid vesicles are known to undergo complex conformational transitions, but it remains challenging to systematically characterize nonequilibrium membrane dynamics in flow. Here, we report the direct observation of anisotropic vesicle relaxation from highly deformed shapes using a Stokes trap. Vesicle shape relaxation is described by two distinct characteristic timescales governed by the bending modulus and membrane tension. Interestingly, the fast double-mode timescale is found to depend on vesicle deflation or reduced volume. Experimental results are well described by a viscoelastic model of a deformed membrane. Overall, these results show that vesicle relaxation is governed by an interplay between membrane elastic moduli, surface tension, and vesicle deflation.

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Membrane-bound vesicles are ubiquitous in biological systems \([1,2]\) and drug delivery applications \([3]\). Phospholipid vesicles are often used as model systems to study the mechanical properties of living cells \([4-10]\). In addition, synthetic vesicles serve as triggered-release agents \([11]\) or encapsulants in detergents and fabric softeners \([12]\). In many cases, structure-property relations underlie the functional behavior of these materials. Despite recent progress, however, the nonequilibrium shape dynamics of highly deflated vesicles is not yet fully understood \([13-18]\).

Vesicles undergo a wide array of stretching dynamics in flow depending on the flow type and equilibrium vesicle shape \([19-24]\). In shear flow, vesicles exhibit tumbling, tank-treading, and membrane trembling behavior that depends on the flow strength and viscosity ratio \([18,19,25]\). In extensional flow, vesicles with nonspherical equilibrium shapes exhibit a wide array of conformational transitions, including a tubular-to-symmetric dumbbell transition for highly deflated vesicles \([20,23,26,27]\), a spheroidal-to-asymmetric dumbbell transition for moderately deflated vesicles \([22,23,26]\), and a nearly spherical-to-ellipsoidal transition for weakly deflated vesicles \([23,28-31]\). Such deformable membrane behavior is naturally exploited in biological systems; for example, red blood cells readily adopt biconcave disk shapes \([32]\), enabling large reversible deformation while traversing thin capillaries during circulation.

Vesicle relaxation following deformation is critically important for shape dynamics and reversible elastic behavior \([6]\). Prior work has focused on the near-equilibrium relaxation of quasispherical vesicles following small deformations, induced by relatively weak forces using optical tweezers \([30]\) or electrodeformation \([33]\). Kanttsler et al. \([20]\) observed the relaxation of a weakly deformed tubular-shaped vesicle, albeit only for a small ensemble size. Broadly, fundamental studies of shape relaxation for freely suspended vesicles following large nonlinear deformations are challenging due to the need for precise flow control and manipulation without using micropipettes or direct physical contact of membranes.

Here, we report the direct observation of anisotropic vesicle relaxation following large nonlinear deformations in extensional flow. Vesicles with nonspherical shapes at equilibrium are deformed in precisely controlled flows using a Stokes trap \([34-37]\), followed by relaxation under quiescent conditions. Remarkably, our results show that highly deformed, freely suspended anisotropic vesicles relax by a double-mode exponential pathway governed by two distinct and well-separated timescales corresponding to characteristic bending and surface tension timescales.

Giant unilamellar vesicles (GUVs) are prepared from a mixture of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 0.12 mol % of the fluorescent lipid 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) in 100 mM sucrose buffer using an electroformation method (Supplemental Material \([38]\)) \([23,39]\). Following electroformation, GUVs are slightly deflated by introducing a higher osmolarity sucrose solution to the outer fluid. Deflated vesicles are described by a reduced volume \(v = (3V\sqrt{4\pi})/A^{3/2}\), where \(V\) and \(A\) are the equilibrium vesicle volume and surface area, respectively, determined by revolution of the membrane contour, as previously described \([22,23]\). In this way, \(v\) is a measure of vesicle anisotropy, such that \(v = 1\) corresponds to a perfectly spherical shape.

Prior to vesicle stretching experiments, we determined the average bending modulus of quasispherical vesicles to be \(\kappa = 22.3 k_BT\) using fluctuation spectroscopy, as previously described \([23,40,41]\). In all subsequent experiments, vesicles are deformed in the bending-dominated regime, such that no area stretching of the membrane occurs in the initial stretching step prior to vesicle relaxation (Supplemental Material \([38]\)). The crossover tension from the bending to the area stretching...
Here, vesicles are deformed in extensional flow using strain of deflated vesicles with reduced volumes. Large nonlinear deformation for the broad range of reduced volumes reveals that vesicle shape relaxes via two stages: an initial fast retraction step, where the length of thin tether rapidly shortens, followed by a slow relaxation step in which the vesicle eventually relaxes back to its equilibrium shape. The transient relaxation trajectory reveals that vesicle shape relaxes via two stages: an initial fast retraction step, where the length of thin tether rapidly shortens, followed by a slow relaxation step in which the vesicle returns to an equilibrium shape.

A characteristic transient relaxation trajectory for a highly deformed vesicle ($\nu = 0.5$) is shown in Fig. 1(b), with the time series of images shown in Fig. 1(a). Prior to flow cessation, the vesicle deforms into a symmetric dumbbell with a long, thin tether connecting the two spherical ends [Fig. 1(a)]. Following flow cessation, the vesicle eventually relaxes back to its equilibrium shape. The transient relaxation trajectory reveals that vesicle shape relaxes via two stages: an initial fast retraction step, where the length of thin tether rapidly shortens, followed by a slow relaxation step in which the vesicle returns to an equilibrium shape.

A series of characteristic relaxation trajectories for vesicles with different reduced volumes $\nu$ is shown in Fig. 1(c) (Supplemental Material [38], Fig. S4), where the vesicle aspect ratio is defined by $L(t)/L_0 - 1$, and $L(t)$ is the time-dependent vesicle extension along the elongational axis. In all cases, our results show that the vesicle relaxation trajectories can be described by a double-mode exponential decay across a wide range of reduced volumes [Fig. 1(c)]:

$$\frac{L(t)}{L_0} - 1 = A \exp(-t/\tau_1) + B \exp(-t/\tau_2),$$

where $\tau_1$ and $\tau_2$ are the fast and slow relaxation times, respectively, and $A$ and $B$ are numerical constants. Repeated relaxation experiments on the same vesicle show nearly identical relaxation trajectories (Supplemental Material [38], Fig. S2). Overall, these observations for quasispherical vesicles are well described by a single exponential decay, as shown in Fig. S1 [38].
fast double-mode relaxation time $\tau_1/\tau_{bend}$ is a function of the vesicle reduced volume $\nu$, as shown in Fig. 2(a). In particular, the normalized fast retraction time $\tau_1/\tau_{bend}$ decreases as the reduced volume increases, which is consistent with the fact that vesicles with smaller reduced volumes have larger surface area to volume ratios and hence larger degrees of membrane flappiness. In Fig. 2, error bars for reduced volume arise from measurement uncertainty in the vesicle equivalent radius $R$, propagated from vesicle surface area $A$ and volume $V$, as previously described [23].

Following the initial fast relaxation step, the vesicle membrane transitions to a slow relaxation process described by a second timescale $\tau_2$. Interestingly, the numerical values of $\tau_2$ are on the order of the characteristic surface tension timescale $t_{surf} = \mu L_{trans}/\sigma_0$ (Supplemental Material [38], Fig. S7), where $\sigma_0$ is the ensemble-averaged equilibrium tension [23] and $L_{trans}$ is the vesicle stretch at the crossover time between the fast and slow regimes, defined as $t_c = C_1 t_2/\tau_1$ from Eq. (1), where $C = \ln B/A$. In particular, our results show that $\tau_2$ is within an order of magnitude of $t_{surf}$, which is consistent with the relatively broad distribution of membrane tensions known to result from generating vesicles using electroformation [22,23,33]. Here, the membrane tension for any single vesicle may vary by an order of magnitude around the mean value $\sigma_0$ for the ensemble. Overall, the slow mode described by $t_{surf}$ is analogous to the relaxation of Newtonian fluid drops following deformation in flow, where a constant surface tension drives the drop back to its equilibrium spherical shape [47].

Interestingly, our results further show that the vesicle aspect ratio at the transition between the fast and slow relaxation phases ($L_{trans}/L_0 - 1$) is a function of reduced volume [Fig. 2(b)]. These results suggest that the bending modulus $\kappa$ may have a functional dependence on the reduced volume $\nu$. Broadly, these findings might have origins in more subtle aspects of membrane mechanics of deflated anisotropic vesicles. Deflated vesicles are known to exhibit an imbalance in lipid density in the two leaflets of the membrane that induces a spontaneous curvature generation [48]. Such modification of the spontaneous curvature and high lateral diffusion of individual lipid molecules in the bilayer for vesicles with smaller reduced volumes may reduce the energy requirements for thermal fluctuations, thereby resulting in a dependence of bending modulus on the degree of osmotic deflation, similar to the observed decrease in bending modulus accompanied by enhanced thermal fluctuations in DOPC bilayers by insertion of an external molecule [49]. Finally, relaxation of highly deformed vesicles with a dumbbell shape involves fluid flow through the thin tether connecting the bulbs. Resistance to flow through the thin tether introduces an additional timescale $\mu L_{max}^2 R_t/\sigma_0 r_i^2$, where $R_t$ is the radius of the spherical bulb consuming the thin tether, and $r_i$ is the tether radius at the beginning of relaxation. For our experiments, this timescale is several orders of magnitude larger than $\tau_1$ and $\tau_2$, suggesting that bending fluctuations and surface tension drive the conformational relaxation of deformed vesicles.

Viscoelastic models are widely used to characterize the rheological properties of soft materials such as polymers, tissues, and spherical cell aggregates [50–52]. Although bulk rheological measurements of vesicles could be used to
determine the dynamic elastic and viscous modulus of these materials, such experiments are challenging due to sample polydispersity. Here, we present a simple mechanical model which describes the viscoelastic properties of single isolated vesicles over a wide range of frequencies and large deformations. In particular, the double-mode relaxation behavior of a vesicle membrane can be described by a viscoelastic model consisting of two Maxwell elements in a parallel arrangement with moduli ($E_1$, $E_2$) and viscosities ($\eta_1$, $\eta_2$) (Fig. 3 and Supplemental Material [38]). Experimental data on vesicle relaxation is well described by the two-mode viscoelastic model, as shown in Fig. 3(a), thereby enabling determination of the model parameters $E_1$, $E_2$, $\eta_1$, and $\eta_2$. Using the model and parameters determined by fitting to experimental data, the frequency-dependent complex shear modulus $G'(\omega)$ can be determined. In particular, the storage modulus $G'(\omega)$ and the loss modulus $G''(\omega)$ can be obtained for single vesicles (Supplemental Material [38]) [53]:

$$G'(\omega) = \frac{E_1(\omega \eta_1^2)}{1 + (\omega E_1)^2} + \frac{E_2(\omega \eta_2^2)}{1 + (\omega E_2)^2},$$

$$G''(\omega) = \frac{E_1(\omega \eta_1^2)}{1 + (\omega E_1)^2} + \frac{E_2(\omega \eta_2^2)}{1 + (\omega E_2)^2},$$

where $\omega$ is the deformation frequency.

Plots of $G'(\omega)$ and $G''(\omega)$ for vesicles with different reduced volumes are shown in Fig. 3(b). Results from model predictions show that the elastic modulus $G'(\omega)$ increases with frequency and becomes larger than the viscous modulus $G''(\omega)$ at a crossover frequency corresponding to the fast timescale $\tau_1$, which is a signature of a transition from fluid to solidlike behavior. Moreover, the model predicts an approximate plateau modulus $G_0 \approx 10^{-4}$ Pa [Fig. 3(b)], which has not been previously reported for lipid vesicles in the literature.

The parameters from the micromechanical model can be related to membrane physical properties. Interestingly, the model yields a value of $\eta_1 \approx 10^{-3}$ Pa s, which is order-of-magnitude consistent with the solution viscosity in our experiments. The values of $\eta_2$ obtained from the model range between $10^{-4}$ and $10^{-3}$ Pa s for different vesicles in the ensemble. Assuming a membrane thickness of 5 nm, values of $\eta_2$ correspond to a membrane viscosity of $10^{-13}$–$10^{-12}$ Pa s m, which are three orders of magnitude lower than the membrane viscosity of spherical DOPC lipid vesicles measured from rotational and translational diffusion probes [54]. However, our work focuses on nonspherical deflated vesicles, which are qualitatively different than spherical vesicles. In particular, the availability of large excess area and higher lateral diffusion of individual lipid molecules in the bilayer membrane are consistent with a decrease in the membrane viscosity. From this perspective, we posit that the values of $\eta_1$ and $\eta_2$ from the micromechanical model correspond to the solution viscosity and membrane viscosity, respectively.

We further consider the moduli $E_1$ and $E_2$ obtained from applying the micromechanical model to our experimental data. The value of $E_1$ was determined to be $\approx 10^{-14}$ Pa, whereas the value of $E_2$ ranges between $10^{-4}$ and $10^{-3}$ Pa. For a typical vesicle size of $R = 10$ $\mu$m, we can estimate the membrane bending modulus from the relation $\kappa \approx E_1R^3$, yielding $\kappa \approx 10^{-19}$ J, which is in good agreement with the bending modulus measured for DOPC lipid vesicles in our work ($\kappa = 22.3 k_BT$) [23]. Similarly, the surface tension $\sigma \approx E_2R$ of vesicles varies between $10^{-3}$ and $10^{-5}$ N/m, which is consistent with the values determined at equilibrium using fluctuation spectroscopy [23]. While a complete viscoelastic model would relate the physical parameters $E_1$, $E_2$, $\eta_1$, $\eta_2$ to bending modulus, tension, and medium viscosity, the broad variety of equilibrium shapes (e.g., tubular, discoid, spheroid) of deflated vesicles generally complicates analytical modeling, as the base state is perturbed far from a spherical geometry [52,55]. Numerical simulations could be used to characterize the dynamic frequency-dependent rheological properties of lipid vesicles and polymersomes following high deformation.

In this Rapid Communication, we directly observe the relaxation of highly deformed vesicles in quiescent solution. Our results show that vesicles dissipate stress via two distinct and well-separated timescales, with a fast and a slow timescale attributed to the relaxation of bending fluctuation modes and surface tension–dominated modes, respectively. Broadly speaking, these results show how the interplay between vesicle reduced volume, bending forces, and surface tension yields a complex relaxation behavior that has not been previously observed in tethers extruded from quasispherical vesicles [56,57]. These results highlight the use of the
Stokes trap in observing the dynamic behavior of vesicle shape relaxation following deformation in strong flows. This methodology of combining gentle flow-based trapping with fluorescence microscopy to induce large membrane deformation will open new avenues in understanding the dynamics of other membrane-bound particles such as polymersomes, capsules, and living cells without the need for micropipettes or external manipulation of membranes.

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