Membrane Viscosity Determined from Shear-Driven Flow in Giant Vesicles

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The viscosity of lipid bilayer membranes plays an important role in determining the diffusion constant of embedded proteins and the dynamics of membrane deformations, yet it has historically proven very difficult to measure. Here we introduce a new method based on quantification of the large-scale circulation patterns induced inside vesicles adhered to a solid surface and subjected to simple shear flow in a microfluidic device. Particle Image Velocimetry based on spinning disk confocal imaging of tracer particles inside and outside of the vesicle, and tracking of phase-separated membrane domains are used to reconstruct the full three-dimensional flow pattern induced by the shear. These measurements show excellent agreement with the predictions of a recent theoretical analysis, and allow direct determination of the membrane viscosity.

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Ever since the work of Saffman and Delbrück on the dynamics of inclusions in biological membranes [1] it has been recognized that lipid bilayers can be viewed as ultrathin fluid layers endowed with a surface viscosity. Along with that of the surrounding fluid, this viscosity plays an important role in determining the translational and rotational diffusion constants of inclusions within the membrane [2]. A body of theoretical work [3, 4] suggests that nonequilibrium dynamics of vesicles in external flows [5] can also be sensitive to the value of this viscosity [6]. As the membrane viscosity $\eta_m$ can be expressed as $d \times \eta$, where $d$ is the membrane thickness and $\eta$ is the bilayer fluid viscosity, the nanometric scale of $d$ renders $\eta_m$ very small. Not surprisingly, it has proven difficult to measure $\eta_m$: ingenious techniques that have been developed include measurements of the motion of membrane-bound microspheres [7], diffusion constants of domains in multicomponent membranes [8, 9], and observation of fluctuation dynamics in membranes near a critical point [10, 11]. Further afield, monolayers admit additional experimental techniques, including methods based on surface rheology [12] and microrheology methods such as observing dynamics of submerged optically trapped [13] or membrane-bound [14] microspheres. Rheological experiments have the advantage of being able to detect non-Newtonian behavior [15].

Interest in membrane dynamics also extends to flows within vesicles, especially in plant science, as the plant vacuole is contained within the vacuolar membrane (or tonoplast), which can comprise some of the largest lipid vesicles known: in internodal cells of the aquatic plant Chara corallina these can be cylinders 1 mm in diameter and up to 10 cm long [16]. This tonoplast is subject to continuous hydrodynamic shear through the action of cytoplasmic streaming, motion of the cytoplasm surrounding the vacuole [17]. Because of its potential role in transport [18] there is great interest in the three-dimensional characteristics of such shear-induced flows [19] and the role played by the intervening tonoplast [20].

A key development in the study of membrane fluid dynamics was the conceptually simple experiment of Vézy, et al. [21] (see also [22]) in which a vesicle was adhered to a solid surface and subjected to a simple shear flow. The flow induced in the membrane took the form of two vortices, rather than the simple overturning flow that would occur in a hemispherical droplet of one fluid in the background of an immiscible second fluid, without the membrane [23, 24]. This difference is attributable to the incompressibility of the membrane, which restricts...
the flow field to one that is two-dimensionally divergence free, i.e. area conserving, on the vesicle surface [24].

Since viscosity is the coefficient of proportionality between force per unit area on a surface and the adjacent shear rate, it is natural to ask whether the experimental setup of Vézy, et al. [21] suggests a means to study membrane fluid mechanics in detail. To this end, we describe here a method that quantifies the flows set up by shear of adherent vesicles, and, through a recent calculation [24], provides a means of determining membrane viscosity. The method uses Particle Image Velocimetry (PIV) to measure the three-dimensional flows inside and outside vesicles, and particle tracking to monitor the shear-induced movement of phase-separated domains within the membrane, in a microfluidic environment.

Figure 1 shows the experimental setup: a vesicle of radius $R$, typically in the range of $10 - 40 \mu m$, adheres to the surface of a microfluidic chamber in the presence of a flow with shear rate $\dot{\gamma}$. The chamber, typically 2 mm wide and 200 $\mu m$ deep, is made from polydimethylsiloxane (PDMS) by soft lithography and sealed with a glass coverslip that has been treated to promote vesicle adhesion. Vesicles were produced by standard methods of electroformation [26] in 100 mM sucrose with or without 0.5 $\mu m$ microspheres (Invitrogen). We chose lipid compositions to obtain two substantially different membrane viscosities. One composition gives primarily liquid-ordered ($L_o$) vesicles with a small fraction of liquid-disordered ($L_d$) phase at room temperature ($\sim 23^\circ C$): 40 mol% cholesterol (Sigma-Aldrich, MO, USA), 55% DPPC (dipalmitoylphosphatidylcholine), and 5% DiPhyPC (diphytanoylphosphatidylcholine). DPPC, DOPC and DiPhyPC were purchased from Avanti Polar Lipids (Alabama, USA) and used without further purification. Vesicles containing primarily $L_o$ phase with a small fraction of gel domains were made from 85% DOPC and 15% DPPC. The $L_d$ phases were labeled with 0.5% TexasRed-DPPE (Invitrogen). Coverslips were cleaned aggressively in NaOH and soaked in a solution of 0.001% polylysine for 30 minutes for use with $L_d$ phase vesicles, or in 0.0005% polyethyleneimine for 5 minutes for use with $L_o$ phase vesicles. Vesicles were gently osmotically deflated by diluting into 130 mM glucose and 10 mM HEPES shortly before loading into the chamber.

Measurements were made on a Zeiss Cell Observer spinning disk confocal microscope with an electron-multipled CCD camera (Evoke, Photometrics; 512 $\times$ 512 pixels), using an NA 1.4/63X oil-immersion objective. Flows were controlled by a syringe pump (PHD2000, Harvard Apparatus) and quantified by measuring far upstream from vesicles the speed of microspheres as a function of height above the coverslip. Shear rates were typically in the range $1 \leq \dot{\gamma} \leq 6$ s$^{-1}$. PIV was done with Matlab by adapting standard code [27] to track small dilute tracers by finding the time-averaged velocity field [29]. For 3D reconstruction, movies were recorded at $\sim$30 frames per second at intervals of 2-3 $\mu m$ throughout and above vesicles containing microspheres (Figure 2b), giving 2D velocity field slices (Fig. 2a). From a stack of such slices a 3D velocity field was determined from the incompressibility relation. Figure 21 shows a representative example of such streamlines.

To understand the flows set up in and around the vesicle we distill the essential results of a recent calculation [24]. Assume that the vesicle is a hemispherical cap of radius $R$ and origin $x = y = z = 0$, adhered to the plane $z = 0$, and let $(r, \theta, \phi)$ be spherical polar coordinates centered at the origin. Given the fluid viscosity $\eta_-$ inside the vesicle ($r < R$), the membrane viscosity $\eta_+$, and the external fluid viscosity $\eta_m$ ($r > R$), we wish to find three velocity fields: $u^-$ inside the vesicle, the 2D flow
The three velocities must be continuous across the membrane, \( \nabla \cdot \mathbf{u} = 0 \). The two external flows obey the unforced Stokes equations and incompressibility, \( \hat{\gamma} \mathbf{x} \), would be continuous at the interface, but a membrane would be continuous at the interface, but a membrane as a result of the membrane involving its Gaussian curvature \( K = R^{-2} \). If \( e_{\parallel} = e_{\theta} \), the boundary condition is

\[
\eta_m \left( \hat{\nabla}^2 \mathbf{u}^m + K \mathbf{u}^m \right) + 2 \left[ \eta_+ e_{\parallel}^+ - \eta_0 e_{\parallel} \right]_{r=R} = \hat{\nabla} \Pi. \tag{1}
\]

For the case \( \eta_+ = \eta_- \), the interior flows that emerge from this calculation match closely those seen in experiment. Cross-sectional profiles shown in Fig. 2 at various elevations above the surface agree with the experimental profiles, with significant counterflow both inside and outside the vesicle near its lateral edges, and over much of lowest cross section. The observed geometry of the internal streamlines (Fig. 2d) follows that predicted theoretically (Fig. 2a), and the maximum downstream membrane speed is observed at the vesicle apex, as predicted. The fluid motion external to the membrane and the orbiting of domains within the membrane (Figs. 3a, b) are both in agreement with theory. In addition to the circulating motion of the membrane domains we have observed over long periods of time their gradual migration to the two vortex centers on either side of the vesicle midline, leaving a depleted region at the apex (Fig. 3b). This appears to be an example of the motion across streamlines described by Bretherton [22]. Note also the existence of closed streamlines outside the vesicle, as predicted [25].

By plotting the downstream velocity as a function of \( z \) through the vesicle apex (Fig. 4), a direct quantitative comparison can be made between theory and experiment. The discontinuity in the derivative of the fluid velocity at the membrane, set by the gradient of the membrane tension through Eq. (1) is clearly seen in the downstream velocity as a function of \( z \) through the vesicle apex (Fig. 4). This provides perhaps the first direct measurement of tension gradients within bilayer membranes under shear. For the vesicles composed primarily of \( L_o \) phase the fluid velocity within the vesicle is significantly lower than for \( L_d \) vesicles as a direct consequence of the greater dissipation in the former, as discussed further below. Returning to the domain tracking in Figs. 1c & d, we observe smaller lateral thermal fluctuations in the \( L_o \) vesicle due to its greater membrane viscosity.

An important empirical result of the calculation [25] is that the speed \( v_0 = |\mathbf{u}^m| \) of the membrane at the apex of the vesicle has a simple dependence on \( r_{\pm} \equiv \eta_m/R_{\Pi} \), the non-dimensional form of the ‘Saffman-Delbrück’ length \( r_{\pm} \equiv \eta_m/\eta_{\pm} \) [31]: \( R_{\Pi}/v_0 = A r_+ / r_- + B r_+ + C \), where \( A, B, C \) are known constants. When the inner and

\[
\eta_0 \left( \hat{\nabla}^2 \mathbf{u}^0 + K \mathbf{u}^0 \right) + 2 \left[ \eta_+ e_{\parallel}^+ - \eta_0 e_{\parallel} \right]_{r=R} = \hat{\nabla} \Pi. \tag{1}
\]
Values at the lower end would change the vibrations near a compositional critical point in membranes ordered lipid phases and from studying dynamic fluctuations previously [7, 8] have ranged from 2 to 3 different shear rates to confirm linearity of fluid shear. Detailed analysis of those flow fields for low-viscosity membranes confirms quantitatively a theoretically predicted geometric velocity attenuation effect, and it reveals the scale of membrane viscosity necessary to significantly affect shear-driven flows. The combination of techniques described here may prove useful in the study of more complex systems involving membranes under shear, such as those found in large eukaryotic cells and perhaps in contexts within developmental biology, where the membranes may be more tightly coupled to cytoskeletal filaments and the internal cellular rheology may be non-Newtonian.

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