Diffusion of liquid domains in lipid bilayer membranes

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We report diffusion coefficients of micron-scale liquid domains in giant unilamellar vesicles of phospholipids and cholesterol. The trajectory of each domain is tracked, and the mean square displacement grows linearly in time as expected for Brownian motion. We study domain diffusion as a function of composition and temperature, and measure how diffusion depends on domain size.

In complex biological membranes, objects. Lastly, the membrane and its surroundings have molecules, which limits continuum approaches to large curvature. Secondly, the membrane is composed of macroobjects. Unlike in three dimensional (3D) diffusion, the size of the diffusing object is not the only length-scale that enters into the problem. For example, the membrane has finite thickness, a finite surface area, and often a nonzero curvature. Secondly, the membrane is composed of macromolecules, which limits continuum approaches to large objects. Lastly, the membrane and its surroundings have different viscosities. In complex biological membranes, additional length-scales may be important, such as the distance between membrane proteins or the size of corraals created by the actin cytoskeleton.

In this Letter, we directly measure diffusion of liquid domains in giant unilamellar vesicles (GUVs) of radius ≃ 20 µm as in Figure 1. These domains are micron-scale, circular, span the lipid bilayer, and undergo Brownian motion. By measuring diffusion of bilayer domains over a wide parameter range of more than one decade in domain radii and three decades in 2D membrane viscosities, we probe the two limiting models of Saffman-Delbrück and Hughes et al. We find a cross-over between the two models which would not have been predicted from previous monolayer results. In the cases where our data are well fit by the Saffman-Delbrück equation, we are able to extract viscosities of lipid phases and diffusion activation energies.

Domains move in a background phase with two dimensional (2D) membrane viscosity (η’’). The diffusion coefficient of a membrane inclusion was originally described by Saffman and Delbrück:

\[ D(r) = \frac{k_B T}{4\pi \eta''} \left[ \log \left( \frac{\eta''}{\eta_{\text{w}} r} \right) - \gamma + \frac{1}{2} \right], \]

where \( r \) is the radius of the inclusion, \( \gamma = 0.5772 \) and we have chosen boundary conditions appropriate for liquid domains in a liquid membrane to yield the factor of 1/2. A key parameter in the hydrodynamics of this system is the lengthscale \( \lambda_0 \) defined by the ratio of the membrane \( \eta'' \) to the 3D bulk viscosity of water (\( \eta_\text{w} \)) such that \( \lambda_0 = \eta''/\eta_\text{w} \).

FIG. 1: Fluorescence microscopy phase diagram of DOPC/DPPC/cholesterol and vesicle images at 20°C. Semi-quantitative dashed tie-lines cross the Lα-Lα coexistence region [3]. Some vesicles studied have a continuous Lα (bright) phase (a-b) whereas others have a continuous Lα (dark) phase (c-d). One composition (e) has a continuous dark Lα phase which may contain both Lα and gel (Sb) phase lipids [10]. Vesicle compositions are shown as mol% DOPC/DPPC/Chol.

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opposite limit of $\lambda_0 < r$ \cite{5}:

$$D(r) = \frac{k_BT}{16\eta_w r}. \quad (2)$$

It is important to notice that for $\lambda_0 < r$ the diffusion coefficient is more strongly dependent on the inclusion’s radius, but is independent of the membrane viscosity. This case was verified experimentally through observations of micron-scale domains in monolayers \cite{11}. Other theoretical work has addressed different inclusion shapes as well as large domains \cite{12}.

Spherical giant unilamellar vesicles (GUVs; 30-100$\mu$m diameter) are made by electroformation \cite{13} of a ternary mixture of cholesterol with phospholipids of both high (DPPC; di(16:0)PC) and low (DOPC; di(18:1)PC) melting temperatures. Materials and methods have been described previously \cite{10}. The vesicle membranes are initially uniform at high temperature, and phase separate into two liquid phases when vesicle suspensions are placed on a pre-cooled microscope stage. The less viscous $L_o$ phase is labeled by fluorescent dye (Texas Red-DPPE). The composition and viscosity of the two phases depend on the composition and temperature of the entire vesicle. With time, domains coalesce, allowing us to probe a range of domain sizes at constant temperature.

We probe five lipid compositions in the ternary system of DOPC/DPPC/Chol, as in Figure 1. In previous microscopy and $^2$H NMR measurements, we established that vesicles with these five compositions separate into a liquid-ordered ($L_o$) phase rich in the saturated lipid DPPC and a liquid crystalline ($L_a$) phase rich in the unsaturated lipid DOPC \cite{10,14}. Two of the five compositions contain a continuous bright $L_o$ phase (Figure 1a-b), and two contain a continuous dark $L_o$ phase (Figure 1c-d). One composition falls within a three phase region (Figure 1e). The presence of three phases is clear in $^2$H NMR experiments (manuscript in preparation), but is difficult to detect by microscopy. We probe the viscosity of the continuous phase by tracking domains of the minority phase.

Membrane domains are identified by an image processing program written in Matlab (Figure 2). A Gaussian filter is applied to images before thresholding, to identify features in the size range of domains. Almost no domains are lost by this algorithm. Domains are accepted if: a) the diameter falls between a minimum (2 pixel) and maximum value; b) the shape is circular, such that all points in the domain perimeter lie within 20% of the mean domain radius; and c) the center of mass lies within a circle defined by 0.8 of the vesicle radius. Since all domains are round (shape fluctuations are minimal), these criteria discriminate against occasional problems arising from image analysis filtering (for example, two domains very close to each other will not be accepted).

A separate program tracks domain trajectories with logic similar to existing codes, i.e. by matching a domain with the nearest feature in the next image \cite{15}. Average diffusion is subtracted to yield unbiased domain motion. Matching generates no false positives but does not have a perfect success rate. We therefore divide each movie (typically 100 frames at 0.34 s/frame) into 20 sets of 5 frames over which most domains are tracked successfully. Domain size does not change over this period. The average of vertical and horizontal mean square displacements (MSD) is linear with time $t$ and fit to $<x^2> = 2D(rt)$ as expected for diffusion. Over five frames, the MSD is $\leq 0.03 \mu m^2$ which is much smaller than the particle separation, and we see no effect of domain packing.

Figure 3 shows diffusion coefficients as a function of domain size. The data has been culled to report only sets in which wide ranges of domain sizes are observed for any fixed temperature and composition. The dashed lines in Figure 3 show the asymptotic 1/$r$ behavior given in Eq. 2. Within error, all data fall on or below this theoretical upper bound in diffusion coefficient. Eq. 2 is independent of membrane viscosity and holds when membrane viscosity is low, or domain radius is large i.e. when $\lambda_0 \ll r$ \cite{3}. For the low viscosity $L_o$ phase (e.g. Figure 3f), it can be seen that the conditions of low membrane viscosity and large domain radius are met through most of the temperature range, because most data fall along the dashed line.

All data below the dashed line in Figure 3 correspond to membranes with high viscosity, notably $L_o$ phases at low temperatures. We have chosen to fit our data to the Saffman-Delbrück equation, which should hold when membrane viscosity is high. It is clear that this set of data does not have a $D(r) \sim r^{-1}$ dependence, and we find instead reasonable fits to Eq. 1 with a single fitting parameter ($\eta''$). Since our domain radii are limited to $\geq 0.5 \mu m$ by our optical resolution and $\leq 10 \mu m$ by our vesicle diameters, we cannot prove that Eq. 1 is the only
expression that could fit our data. Nevertheless, fitting to Eq. $1$ allows us to extract $\eta''$ for high viscosity membranes.

There have been multiple experiments designed to test the logarithmic form of Eq. $1$ [7, 11, 16], and its range of applicability is still controversial. For example, recent work asserts that individual proteins diffuse with a stronger size dependence, as $D(r) \sim r^{-1}$ [16], due to a break-down of the continuum approximation of the membrane for small inclusions [16, 17]. Here we explore domain radii well within the continuum limit ($r \gg r_{\text{single molecule}} \sim 0.5\text{nm}$).

Our results can be extrapolated to estimate the diffusion rate of raft domains in cell membranes. Lipid rafts are reported to have diameters of 10-100nm [18]. This length-scale falls within the regime where the Saffman-Delbrück equation should apply. We calculate diffusion coefficients for 10-100nm domains in our system to be between $3 \times 10^{-3}$ and $1.5 \times 10^{-1}\text{nm}^2/\text{sec}$. These values differ from those extrapolated from single molecule measurements using the Saffman-Delbrück equation [1]. Given the suggestion that single molecule measurements do not probe the continuum limit, it may be more valid to estimate raft diffusion coefficients by extrapolating down from large domains rather than up from single molecules.

Our analysis of the culled data set in Figure 3 shows that high viscosity membranes produce data that fall well below the dashed line in Figure 3 and that fit Eq. $1$ reasonably well. We conclude that any remaining unplotted data that fall well below the dashed line should also fit Eq. $1$. We use this data to yield a size-independent $D_0$ using:

$$D(r) = D_0 \left[ \log \left( \frac{a_0}{r} \right) - 0.0772 \right] ,$$

with $D_0 = \frac{k_B T}{4\pi \eta''}$.

Figure 4 shows a plot of the resulting $D_0$ and 2D membrane viscosity vs. temperature ($T$). We find that the $L_o$ phase viscosities for membranes of 1:1 DOPC/DPPC + 30%Chol and 1:2 DOPC/DPPC + 30%Chol are similar, suggesting that $L_o$ viscosities are not highly composition dependent. In contrast, viscosities for membranes of 1:4 DOPC/DPPC + 20%Chol are much larger, consistent with these membranes falling within the three phase region in Figure 4.

At $22^\circ C$, we find 2D membrane viscosities for the $L_o$ phase of $10^{-8} \leq \eta''(\text{Ns/m}) \leq 5 \times 10^{-7}$. Surface shear rheometry finds monolayer viscosities on the order of $10^{-6}$ to $10^{-5}(\text{Ns/m})$ only in liquid condensed phases [19], which is consistent with tight packing of lipids in bilayer $L_o$ phases. In contrast, the 2D membrane viscosity of the $L_o$ phase is low, and results in $D \sim r^{-1}$. In monolayers, the same $D \sim r^{-1}$ dependence is found for solid domains diffusing across a liquid background [11].

In the membrane literature, a 3D membrane viscosity, $\eta_{3D}$, is defined as $\eta_{3D} \simeq \eta''/h$, where $h$ is the bilayer thickness. Assuming $h = 3.3\text{nm}$, we find $3 \leq \eta_{3D}(\text{Pa}s) \leq 150$, on the order of [16, 20] or greater than [21] published values for model membranes. However, the relation between $\eta_{3D}$ and $\eta''$ is not exact, because lipids anchored to the interface differ from a thin homogeneous layer. Indeed, the lipid headgroups often determine the membrane viscosity [7]. This is not always appreciated, and may be a source of ambiguity in discussions of the Saffman-Delbrück model [16].
Figure 1 demonstrates that domains diffuse in membranes of high viscosity via an activated process. If the 2D membrane viscosity ($\eta''$) were independent of T, we would expect $D_0(T) \sim T$. Instead, we find a better fit for $\log(D_0(T)) \sim -T^{-1}$, consistent with an activation energy $E_0$ for diffusion such that $D_0 \sim \exp(-E_0/k_B T)$. The data in Figure 4 follow Arrhenius behavior even though a gel phase emerges at low temperature for some mixtures. Composition of the $L_o$ phase varies only slightly with temperature $10^4$. Activation energies for individual lipids $[22, 23, 24]$ have been attributed to the energy required to hop into an available free volume $[7, 24]$. Larger particles such as protein aggregates yield lower apparent activation energies $[17]$. Fig. 4 lists activation energies for domain diffusing in $L_o$ phases. We find activation energies greater than those reported for single molecules in similar membranes, including DPPC/Chol membranes at high temperature (30-80 kJ/mol) $[22]$, as well as $L_o$ lipids in phase separated DOPC/DPPC/Chol membranes at low temperature ($\sim 80$ kJ/mol) $[25]$.

In summary, we present a simple method for quantifying the movement of domains in membranes containing coexisting liquid phases. We find that domains diffuse via Brownian motion, and that diffusion rates are described by different models under different experimental conditions. At high temperatures and in membranes with a continuous $L_o$ phase, membrane viscosity is low, diffusion constants are independent of membrane properties, and domains diffuse with a radial dependence of $D \sim 1/r$. In membranes with a higher viscosity continuous phase, domain movement does depend on membrane physical properties and the radial dependence can be fit by a Saffman-Delbrück model with $D \sim \log(1/r)$. For these membranes, we determine 2D viscosities and report activation energies for domain diffusion.

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