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Sensing a little friction

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Lipid membranes are central to the architecture of living cells, providing protection from the external environment as well as internal compartmentalization necessary for the biochemical reactions that sustain life. Their primary role as a simple barrier belies the chemical complexity of these multifunctional materials. Modern lipidomics techniques have uncovered a remarkably rich set of lipid building blocks that endow cells with precise control over the properties of their membranes (1,2) and the ability to change these properties in space and time. This natural lipid diversity presents a challenge for scientists seeking to understand the connections between membrane composition and function.

One successful strategy for delineating the role of different lipids is a bottom-up approach, in which simplified model membranes are formed from one or a few lipids, and the resulting membrane thoroughly characterized. By systematically changing composition, the influence of individual lipids can be teased apart. Robust methods have been developed for measuring a slew of structural and elastic membrane properties, including thickness and area per molecule (3), lateral compressibility and bending rigidity (4), and lipid spontaneous curvature (5), to name a few. An extensive library of data for these properties, compiled for an ever-growing number of lipids, informs theory and simulations and enables predictions for the behaviors of more complicated mixtures. Key to the success of this approach is the ability to obtain a consistent set of measurements for a large variety of structurally different lipids, a goal that is not always achievable within the constraints of available techniques.

A notable example is the relative paucity of experimental data for membrane viscous properties. These include the coefficient of interleaflet friction b, which quantifies the drag (or resistance to flow) when the two leaflets slide past each other. As a dynamic property, b has been challenging to determine historically since it requires measuring either equilibrium or nonequilibrium membrane motions at the correct length and timescales. Current estimates of b are from studies of nonequilibrium dynamics of rapidly pulling a tether from a GUV (6), measuring equilibrium membrane fluctuation dynamics with video microscopy (7) or neutron spin echo (NSE) spectroscopy (8), or measuring lipid diffusion in supported lipid bilayers (9). These methods each require appropriate data analysis frameworks that describe how the relaxation time of the measured dynamic processes depends on b. Given the challenging nature of the experiments and the data analysis, it is not surprising that current reports often provide at best an order-of-magnitude estimate for b, with values for the same lipid in different studies differing by as much as two orders-of-magnitude.

In this issue of *Biophysical Journal*, Anthony et al. expand the use of a relatively new experimental method for determining b to show that the method is sensitive to small changes in b in different model membranes and, in turn, that b depends on the lipid composition of the membrane. The authors take advantage of shear stress-driven "tank-treading" that occurs in supported bilayers formed in microfluidic channels when they are subjected to a shear stress from a bulk liquid flowing over the membrane. The flow causes a rolling motion in which the free membrane leaflet in contact with the liquid slides over the stationary leaflet in contact with the solid surface of the channel, reminiscent of the moving treads of a tracked vehicle. In the specially designed microfluidic channels, the sliding velocity of the bilayer is determined by b.

The authors first perform a series of benchmarking experiments looking at the effects of applied shear stress on the measured b value as well as how different fluorescent tracer lipids affected the results. Importantly, they show that the extracted values of b for a given lipid are highly reproducible and independent of the applied shear stress above the main transition temperature of the lipid. Meanwhile, the

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different tracer lipids affected the measured b values by up to 17% when the fluorescent dye was incorporated into the lipid tail versus the headgroup, even at a low loading of only 0.8 mol %. The authors speculate that the differences were due to the disrupted tail packing from the bulky fluorescent molecule in BODIPY C12 but, even more importantly, these control studies already point to the sensitivity of the method and how much the interleaflet friction depends on lipid chemistry and membrane composition.

Having proven the sensitivity and reproducibility of their method, the authors go on to report the first comparison of b values for different model lipid membranes measured with a single technique. They show that b varies with lipid composition following a general trend that DOPC $<$ POPC $\sim$ DLPC $<$ DOPC +30% chol $<<$ DLPC +30% chol. While the result that b depends on lipid composition was expected, the careful and systematic studies performed by the authors are a first and essential step toward understanding how the interleaflet interactions affect biomembrane properties.

The shear-driven tank-treading method used by Anthony et al. offers several advantages over other methods for measuring b. The most notable are that the method is reproducible and sensitive, allowing the authors to quantify differences in b in saturated and unsaturated lipid membranes, with and without cholesterol, for the first time. The data interpretation also is comparatively straightforward, and the method measures steady-state membrane motions that are microns in length scale and accessible with an optical microscope, making the method adaptable and readily adoptable in other laboratories.

There are limitations with any experimental method, and the authors are careful to mention caveats. They note that their results were extremely sensitive to coverslip preparation, suggesting that well-established and careful protocols are needed in other laboratories interested in implementing similar methods. The measurements are made with supported lipid bilayers, which are inherently asymmetric, and it is not clear how this asymmetry affects the measured b values. The study was also limited to lipids that are fluid at ambient temperature due to a lack of temperature control in the experimental setup. Improvements planned by the authors will further expand the impact of the method by enabling measurements of b as a function of temperature and for a wider range of lipids and their mixtures. Interestingly, the lack of temperature control led to the serendipitous discovery of an anomalous gelation of DMPC membranes close to their melting transition temperature. Because pressures within the microfluidic device are orders of magnitude lower than pressures known to increase the main chain transition temperature of saturated lipids, it is likely that the observed gel phase is induced by the applied shear stress. Although not the main focus of the current study, these flow effects on lipid phase behavior are fascinating and worthy of further study in their own right.

The main results of Anthony et al. provide an intriguing glimpse into the range of influence lipid composition imparts on interleaflet friction. As biophysicists, we are encouraged to wonder what comes next? For example, will experimental measurements verify the finding from simulations that interleaflet friction is strongly influenced by lipid chain length mismatch? Interdigitation occurs to some extent in all phospholipid bilayers due to the tilt of the glycerol backbone and resulting differences in the penetration depths of *sn*-1 and *sn*-2 chains, but it can be especially pronounced in bilayers composed of mixed chain lipids. A straightforward test would be to measure b for a series of lipids with identical total number of acyl chain carbons, but differing degree of chain asymmetry (i.e., 16:0/16:0, 14:0/18:0, 12:0/20:0). Using an analogous set of lipids in coarse-grained simulations, den Otter and Shkulipa found that b more than doubled with increasing chain mismatch (10), whereas increasing the length or degree of unsaturation of lipids with nominally identical chains resulted in much more modest changes in b. In biological membranes, a similar role might be played by sphingolipids, some of which exhibit an unusually large difference in the length of their sphingosine and N-acyl chains. Temperature control that was unavailable in the present studies will clearly be required for such measurements.

It will also be informative to measure b in compositionally asymmetric bilayers. This is crucial for a deeper understanding of the dynamics of shape-shifting cell membranes and how energy is dissipated during biological processes that change membrane curvature (e.g., tubulation, endo- and exocytosis, cell motility, and division). Interleaflet friction is also one aspect of transbilayer coupling, in that it reflects the strength of interactions occurring at the bilayer midplane. Measurements of b in asymmetric bilayers may therefore lead to a better understanding of raft phenomena, as lateral lipid organization within each leaflet (11,12) as well as domain registration (13–15) can be strongly influenced by cross-leaflet interactions.

More generally, having accurate values of b is essential for understanding any dynamic process that requires the membrane leaflets to slide past one another. The value of b determines not only the energy associated with the interleaflet drag, but also dictates the timescales of the associated motions and becomes especially important at the length scale of the membrane itself. Even thermal bending fluctuations at the nanometer length scale and nanosecond timescale require the bilayer leaflets to move past one another, as first recognized by Evans and Young (6) as well as Seifert and Langer (16). At the nanometer length scale, the description of the membrane as a thin structureless sheet no longer holds, and bending the membrane requires that one leaflet expands and the other contracts so that the lipids have to locally rearrange to accommodate the shape change. Interleaflet friction is

so important in these local shape changes, where the very definition of the crossover wavenumber depends on the value of b.

The value of b determines the relaxation rate of the membrane fluctuations measured at the nanometer length scale and nanosecond timescale, as well as the predicted relative amplitudes of the different modes (17). Knowing how b changes with lipid structure and membrane composition may have important implications for understanding differences in reported values of membrane bending moduli obtained from measurements of the time average amplitude of membrane fluctuations (x-ray scattering or flicker spectroscopy) compared with the effective bending modulus obtained from measurements of the relaxation rate of these membrane fluctuations in the time domain (NSE or dynamic light scattering). For example, Anthony et al. showed that adding cholesterol to DOPC lipid membranes increases the interleaflet friction by upward of 40%, which could affect the relaxation dynamics of cholesterol-containing membranes, such as those recently measured with NSE (18). Having precise and reproducible estimates for b is crucial for understanding membrane dynamics and mechanics, especially in the time domain.

With implications for processes ranging from raft formation to dynamic cell shape changes, Anthony et al. unveil how an important, and often overlooked, dynamic property markedly depends on lipid structure and membrane composition. Who knew a little membrane friction could have big biological consequences?

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