A needless but interesting controversy

John F. Nagle[a,1], Evan A. Evans[b], Patricia Bassereau[c], Tobias Baumgart[d], Stephanie Tristram-Nagle[a], and Rumiana Dimova[e]

A decade ago, four independent groups reported the unanticipated and still surprising result that the bending modulus κ of bilayers of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), unlike other standard lipids, does not increase with addition of cholesterol. This was shown from tube pulling (1, 2) shape analysis (SA) and electro-deformation (ED) of giant vesicles (3), and X-ray diffuse scattering (XDS) fluctuations in bilayer stacks (4). However, a recent PNAS paper (5) based on neutron spin echo (NSE) and NMR relaxation (NMRR) claims that the bending modulus of DOPC increases threefold for cholesterol mole fractions of 50%. Thus, it would appear that this is a controversial topic for understanding membrane mechanics.

This controversy is needless because NSE and NMRR measure something different from the classical Helfrich bending modulus. These methods sense relaxation, which is the τ-dependent decay of time-averaged $\langle A(t+\tau)A(t)\rangle$ for the quantities A measured by these techniques. Viscosity slows down such decays while not changing $\langle A(t)A(t)\rangle$, so these methods enrich our perspective on the cholesterol story. However, they do not measure the time-averaged mean-square fluctuations $\langle A(t)A(t)\rangle$ with $\tau = 0$ that determine the bending modulus. Tube pulling and ED are static $\tau = 0$ methods. In addition, contrary to claims by Chakraborty et al. (5), the XDS method has a photon picosecond timescale so it too effectively measures at $\tau = 0$. In contrast, it is well recognized that viscosity affects the NSE nanosecond timescale (6).

Chakraborty et al. (5) also state that its shorter length-scale methods are more appropriate than longer length-scale SA and ED methods, but the opposite is true because the Helfrich bending modulus is defined as the first approximation that applies to longer length scales. At shorter length scales of NSE and XDS, molecular tilt plays a role (7), and that is not included in the analysis by Chakraborty et al. (5).

The NSE timescale has been accommodated in theory (6) that shows that a dynamical bending modulus $\tilde{\kappa} = \kappa + 2h^2 k_m$ takes the place of κ in the precursor (8) to equation **1** in Chakraborty et al. (5). However, $2h^2 k_m$ is an order-of-magnitude larger than the bending modulus κ, so how this term is handled is crucial for obtaining NSE values of κ. Instead, with the same assumptions made by Chakraborty et al. (5) for the location h of the neutral plane, the measured area compressibility modulus $2k_m$ (9) fully accounts for the cholesterol-induced increase in the NSE value of $\tilde{\kappa}$ without any increase in κ. While this might suggest that NSE has nothing to add to this field, we constructively suggest that an important contribution could be the experimental determination of h not measured previously.

Instead, Chakraborty et al. (5) use the polymer-brush model to relate $2k_m$ to κ. This model works well for bilayers without rigid cholesterol rings (10), but the model proposed by Pan et al. (4) is better suited for high cholesterol. Insistence that the polymer-brush model works for all systems appeals to the desire for universal theory, but imposing that desire prevents deeper understanding of membrane mechanics as revealed by surprising phenomena such as the effect of cholesterol on the bending modulus of DOPC bilayers.

Acknowledgments

We acknowledge R. Lipowsky, P. Vlahovska, H. Faizi, and M. Deserno for helpful discussions.

1 B. Sorre *et al.*, Curvature-driven lipid sorting needs proximity to a demixing point and is aided by proteins. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 5622–5626 (2009).

2 A. Tian, B. R. Capraro, C. Esposito, T. Baumgart, Bending stiffness depends on curvature of ternary lipid mixture tubular membranes. *Biophys. J.* **97**, 1636–1646 (2009).

[a]Department of Physics, Carnegie Mellon University, Pittsburgh, PA 15213; [b]Department of Physics and Astronomy, University of British Columbia, Vancouver, BC V6T1Z1, Canada; [c]Laboratoire Physico-Chimie Curie, Institut Curie, PSL Research University, CNRS UMR168, 75005 Paris, France; [d]Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104; and [e]Department of Theory and Bio-Systems, Max Planck Institute of Colloids and Interfaces, 14424 Potsdam, Germany

Author contributions: J.F.N., E.A.E., P.B., T.B., S.T.-N., and R.D. performed research; and J.F.N. and R.D. wrote the paper.

The authors declare no competing interest.

[1]To whom correspondence may be addressed. Email: nagle@cmu.edu.

Published May 5, 2021.

3 R. S. Gracia, N. Bezlyepkina, R. L. Knorr, R. Lipowsky, R. Dimova, Effect of cholesterol on the rigidity of saturated and unsaturated membranes: Fluctuation and electrodeformation analysis of giant vesicles. *Soft Matter* **6**, 1472–1482 (2010).
4 J. Pan, S. Tristram-Nagle, J. F. Nagle, Effect of cholesterol on structural and mechanical properties of membranes depends on lipid chain saturation. *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* **80**, 021931 (2009).
5 S. Chakraborty *et al.*, How cholesterol stiffens unsaturated lipid membranes. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 21896–21905 (2020).
6 M. C. Watson, Y. Peng, Y. Zheng, F. L. H. Brown, The intermediate scattering function for lipid bilayer membranes: From nanometers to microns. *J. Chem. Phys.* **135**, 194701 (2011).
7 M. Hamm, M. M. Kozlov, Elastic energy of tilt and bending of fluid membranes. *Eur. Phys. J. E* **3**, 323–335 (2000).
8 M. Nagao, E. G. Kelley, R. Ashkar, R. Bradbury, P. D. Butler, Probing elastic and viscous properties of phospholipid bilayers using neutron spin echo spectroscopy. *J. Phys. Chem. Lett.* **8**, 4679–4684 (2017).
9 W. Rawicz, B. A. Smith, T. J. McIntosh, S. A. Simon, E. Evans, Elasticity, strength, and water permeability of bilayers that contain raft microdomain-forming lipids. *Biophys. J.* **94**, 4725–4736 (2008).
10 W. Rawicz, K. C. Olbrich, T. McIntosh, D. Needham, E. Evans, Effect of chain length and unsaturation on elasticity of lipid bilayers. *Biophys. J.* **79**, 328–339 (2000).