

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 $\tilde{\kappa} = \kappa + 2h^2k_m$ takes the place of κ in the precursor (8) to equation 1 in Chakraborty et al. (5). However, $2h^2k_m$ 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.

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^aDepartment of Physics, Carnegie Mellon University, Pittsburgh, PA 15213; ^bDepartment of Physics and Astronomy, University of British Columbia, Vancouver, BC V6T1Z1, Canada; ^cLaboratoire Physico-Chimie Curie, Institut Curie, PSL Research University, CNRS UMR168, 75005 Paris, France; ^dDepartment of Chemistry, University of Pennsylvania, Philadelphia, PA 19104; and ^eDepartment of Theory and Bio-Systems, Max Planck Institute of Colloids and Interfaces, 14424 Potsdam, Germany

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

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