JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Communication
pubs.acs.org/JACS

Determination of Biomembrane Bending Moduli in Fully Atomistic Simulations

Zachary A. Levine,[†,‡] Richard M. Venable,[§] Max C. Watson,[∥] Michael G. Lerner,[⊥] Joan-Emma Shea,[†,‡] Richard W. Pastor,[§] and Frank L. H. Brown*[,†,‡]

[†]Department of Physics and [‡]Department of Chemistry and Biochemistry, University of California, Santa Barbara, California 93106, United States

[§]Laboratory of Computational Biology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892, United States

[∥]Center for Neutron Research, National Institute of Standards and Technology, Gaithersburg, Maryland 20899, United States

[⊥]Department of Physics and Astronomy, Earlham College, Richmond, Indiana 47374, United States

Supporting Information

ABSTRACT: The bilayer bending modulus (K_c) is one of the most important physical constants characterizing lipid membranes, but precisely measuring it is a challenge, both experimentally and computationally. Experimental measurements on chemically identical bilayers often differ depending upon the techniques employed, and robust simulation results have previously been limited to coarse-grained models (at varying levels of resolution). This Communication demonstrates the extraction of K_c from fully atomistic molecular dynamics simulations for three different single-component lipid bilayers (DPPC, DOPC, and DOPE). The results agree quantitatively with experiments that measure thermal shape fluctuations in giant unilamellar vesicles. Lipid tilt, twist, and compression moduli are also reported.

The primary constituents of most cellular membranes are phospholipids, which self-assemble into fluid bilayer structures and provide a quasi-two-dimensional environment for the remaining membrane components. The underlying fluid nature of the membrane is required to allow rapid changes of the bilayer shape and efficient lateral motion of membrane components, and to incorporate new membrane components or jettison old ones with minimal restructuring. Furthermore, the fluid membrane surface naturally flows and heals itself in response to applied perturbations and molecular motions.

As a consequence of the fluid nature of lipid bilayers, the long length-scale biophysics of these systems is considerably simpler than would naively be expected for a generic thin elastic sheet.[1,2] In fact, the energetic costs associated with large-scale shape deformations of homogeneous lipid bilayers with identical leaflets and constant surface topology are completely characterized by a single constant, K_c, the bilayer bending modulus. K_c serves to distinguish chemically disparate bilayers in many common experiments on model membranes and retains an important role in the behavior of more complex biomembrane systems.

Considerable effort has been dedicated to the experimental measurement of K_c in varied membrane systems via an array of techniques.[3−10] Unfortunately, different experiments yield substantially different values of K_c for chemically identical membranes; this is a longstanding, well-known and unexplained puzzle[3,4] that highlights the considerable challenges involved in quantitatively characterizing lipid bilayers. It might be expected that molecular simulations could help interpret experiment and elucidate, if not fully resolve, the discrepancies between competing measurements, but this has not previously been possible for two reasons. First, fully atomistic models suitable for quantitative comparison to experiment are computationally expensive; the small membranes that can be studied are difficult to interpret using approaches developed in the context coarse-grained models and substantially larger box sizes (see below). Second, lipid force fields have been notoriously difficult to parametrize;[11,12] only recently have fully atomistic potentials been developed that appear to show close correspondence with experiment. This communication exploits recent theoretical developments[13] and a state-of-the-art force field[14] to overcome these limitiations and directly calculate K_c for simulated all-atom bilayers.

The traditional approach[15−17] to extract K_c from membrane simulations involves the application of Helfrich−Canham (HC) theory,[1,2] which models the membrane as a thin, structureless and homogeneous fluid sheet. Simulations are run under conditions of vanishing surface tension and constant temperature for a membrane that spans the square xy plane of a periodic simulation box. The overall bilayer shape is monitored throughout the simulation and is quantified through the "height field", $h(x,y) = h(\mathbf{r})$, which indicates the z-direction displacement of the bilayer from a reference plane. The power spectrum of the height fluctuations is predicted by HC to be[18]

$$\langle |h_q|^2 \rangle = \frac{k_B T}{K_c q^4} \tag{1}$$

where q is the wavevector, T is temperature, and k_B is Boltzmann's constant. Fitting the simulated power spectrum to eq 1 yields K_c. This approach is valid in the limit of $q \to 0$, but

Received: August 2, 2014
Published: September 9, 2014

   dx.doi.org/10.1021/ja507910r | J. Am. Chem. Soc. 2014, 136, 13582−13585

fails for small systems when bilayer thickness is comparable to the box size and the "structureless thin sheet" assumption of HC breaks down. This failure is explicitly demonstrated in Figure S6 for the simulations reported in this study; the traditional methodology is not presently suited to fully atomistic membrane models since computational limitations preclude the use of simulation boxes large enough to render eq 1 valid.

Disagreements between eq 1 and simulated spectra outside the low q limit were originally attributed to lipid protrusions,[15] but recent studies[19,20] have demonstrated that lipid tilting[21] is the primary culprit at wavelengths comparable to and somewhat larger than bilayer thickness (protrusions and other microscopic fluctuations do contribute to the spectra at shorter wavelengths). This observation motivates an alternate approach to determine K_c *in silico* based on the analysis of thermal fluctuations in lipid orientation,[13] where the effects of lipid tilting decouple from the lipid orientation energetics associated with K_c. A theoretical justification, implementation details, and validation in the context of coarse-grained simulations for this method can be found elsewhere;[13,19] below, we include only enough details to outline how the methodology is applied to the fully atomic simulations of this study. Detailed practical instructions for the analysis are included in the Supporting Information (SI).

The unit vector $\mathbb{n}_j^{(\alpha)}$ (α takes the value 1 or 2 for upper and lower bilayer leaflets, respectively), which defines the orientation of lipid j, points from the midpoint between the lipid headgroup phosphorus and glycerol backbone carbon C2, to the midpoint between the terminal methyl carbons of the lipid tails (Figure 1); alternate microscopic definitions for $\mathbb{n}_j^{(\alpha)}$

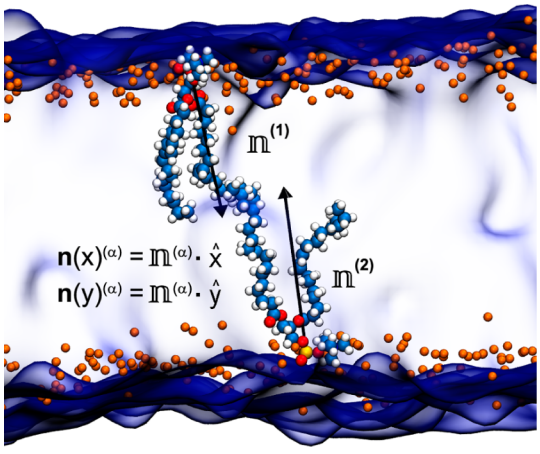

Figure 1. Lipid orientation vectors $\mathbb{n}_j^{(\alpha)}$ are defined from the midpoint between lipid headgroup phosphorus and glycerol C2, to the midpoint between terminal methyl groups in the tail region. Atom positions are from a simulation snapshot, with carbon atoms colored blue, hydrogen white, oxygen red, and phosphorus yellow/orange. Surrounding lipids are represented by a blue, semitransparent surface that outlines the aqueous-membrane interface.

are analyzed in the SI. The molecular orientations are translated into a two-dimensional orientation vector field by first projecting $\mathbb{n}_j^{(\alpha)}$ onto the xy plane and then mapping the projected $\boldsymbol{n}_j^{(\alpha)}$ onto a two-dimensional real space grid,[19] which yields $\boldsymbol{n}(\boldsymbol{r})^{(\alpha)}$. Fast Fourier transformations then yield $\boldsymbol{n}_q^{(\alpha)}$, from which the related bilayer quantity $\hat{\boldsymbol{n}}_q = {}^1/_2[\boldsymbol{n}_q^{(1)} - \boldsymbol{n}_q^{(2)}]$ follows. $\hat{\boldsymbol{n}}_q$ is decomposed into longitudinal and transverse components via $\hat{\boldsymbol{n}}_q^{\parallel} = {}^1/_q[\boldsymbol{q}\cdot\hat{\boldsymbol{n}}_q]$ and $\hat{\boldsymbol{n}}_q^{\perp} = {}^1/_q[\boldsymbol{q}\times\hat{\boldsymbol{n}}_q]\cdot\hat{z}$ to allow comparison to the theoretical predictions:[13]

$$\langle|\hat{n}_q^{\parallel}|^2\rangle = \frac{k_B T}{K_c q^2}, \quad \langle|\hat{n}_q^{\perp}|^2\rangle = \frac{k_B T}{K_\theta + K_{tw} q^2} \tag{2}$$

where K_θ and K_{tw} are the lipid tilt and twist moduli, respectively.

The critical advantage of eq 2 over eq 1 is that eq 2 is derived within a framework that explicitly accounts for lipid tilting, yet the quantity $\langle|\hat{n}_q^{\parallel}|^2\rangle$ depends solely on K_c. Generalizing eq 1 to account for lipid tilt/twist at the same level of theory underlying eq 2 yields[13,19,20]

$$\langle|h_q|^2\rangle = k_B T\left(\frac{1}{K_c q^4} + \frac{1}{K_\theta q^2}\right) \tag{3}$$

which explicitly demonstrates the contribution of lipid tilting to the height spectra at large q. In principle, one could attempt to extract K_c from simulated height spectra by comparing to eq 3; however, this procedure introduces substantial errors and uncertainties relative to analysis based on $\langle|\hat{n}_q^{\parallel}|^2\rangle$ (see SI). An alternate orientation-based aproach for estimating K_c in multicomponent membranes has also been proposed.[22] As shown in the SI, K_c values obtained via this methodology lie well outside the statistical errors of the present approach, but appear accurate to within 20% or less for the lipids considered in this study. Hence, while suitable for analyzing trends associated with changes in lipid composition or phase, the methodology of ref 22 is not suited to precise quantitative determination of K_c.

All-atom molecular dynamics simulations were carried out with the CHARMM package[23] on fully hydrated dipalmitoylphosphatidylcholine (DPPC) membranes at 323 K, and dioleoylphosphatidylcholine (DOPC) and dioleoylphosphatidylethanolamine (DOPE) membranes at 298 K. These particular lipids were chosen because they have been extensively characterized within the CHARMM36 force field.[14] Experimentally, DOPE is in the inverse hexagonal (H_{II}) phase under these conditions[24] and the DOPE bilayer should be considered metastable. Simulating under metastable conditions allows for direct comparison to the experimental H_{II} K_c data under comparable thermodynamic conditions (see below) and also allows for a direct comparison to DOPC at identical conditions. Each system contained 648 lipids and was run for at least 100 ns (DPPC, 110 ns; DOPC, 170 ns; DOPE, 140 ns). Lipid orientation vectors were sampled every 0.5 ns, and the power spectra indicated in eq 2 were obtained by averaging over this sampling after dropping the first 10 ns. See SI for further details.

The relevant power spectra are plotted in Figure 2, and the extracted physical constants (K_c, K_θ, and K_{tw}) are listed in Table 1. The simple functional form associated with the longitudinal orientation spectra (eq 2) suggests that $\langle|\hat{n}_q^{\parallel}|^2\rangle q^2$ should be constant over the regime where the underlying theory is valid; this regime extends over multiple wavevectors within the simulation box, clearly indicating that 648 lipid systems are sufficiently large to provide converged results for the lipid orientation spectra. In contrast, the traditional approach applied to the height spectra is obviously not converged (Figure S6), highlighting the utility of the lipid orientation approach. Microscopic noise (e.g., protrusions) contributes to both types of spectra, which is why the simulation results strongly

dx.doi.org/10.1021/ja507910r | J. Am. Chem. Soc. 2014, 136, 13582−13585

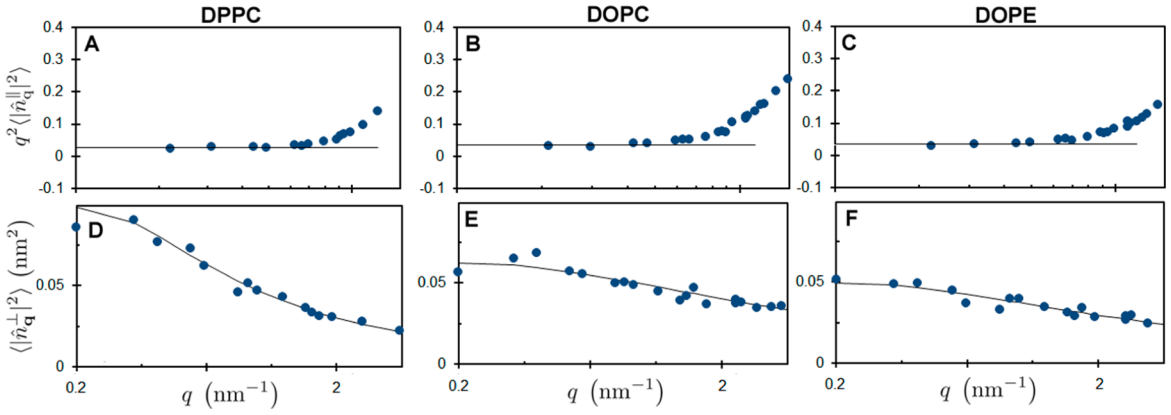

Figure 2. (A–C) Power spectra of longitudinal lipid orientation weighted by q^2. The horizontal black lines represent the best fit to eq 2 over the smallest four wave vectors in the simulations; the plateau region extends over at least the smallest four wave vectors for all three simulations, clearly indicating well-converged values of K_c with system size. (D–F) Power spectra of transverse lipid orientation and the associated best-fit curves to eq 2.

Table 1. Summary of Calculated Bilayer Bending, Tilt and Twist Moduli, Area Compressibility Moduli, and Area Per Lipid[a]

	temp (°C)	K_c ($\times 10^{-20}$ J)	K_θ ($\times 10^{-20}$ J/nm^2)	K_{tw} ($\times 10^{-20}$ J)	K_A (dyn/cm)	area per lipid (Å^2)
DPPC	50	15.6 ± 0.5	4.9 ± 0.2	2.0 ± 0.1	210 ± 15	62.9 ± 0.1
DOPC	24.9	11.4 ± 0.3	6.6 ± 0.2	0.9 ± 0.1	290 ± 20	68.9 ± 0.1
DOPE	24.9	11.4 ± 0.3	8.3 ± 0.4	1.4 ± 0.1	250 ± 50	62.3 ± 0.1

[a]Standard errors were calculated by comparing K_c in 10 ns blocks (Tables S1–S3 and Figures S2–S4).

diverge from theoretical expectations at the higher q wave vectors of the boxes.

The bending modulus for all-atom DPPC membranes compares well with experimental measurements that track thermal shape fluctuations of giant unilamellar vesicles (K_c = 15.0 × 10^{-20} J at 49.4 °C).[3,9] Indeed, the two numbers are nearly identical within the statistical errors of our sampling. (We also note that this value is identical to that previously obtained for MARTINI DPPC.[13]) The bending modulus for all-atom DOPC membranes is similarly in excellent agreement with vesicle fluctuation experiments (K_c = 10.9 × 10^{-20} J at 23 °C).[4,25] DOPE vesicle fluctuation measurements are not available; the experimental numbers available for DOPE involve measurements of monolayer moduli in the H_{II} phase with added tetradecane,[3,26] suggesting $K_c \approx 10 \times 10^{-20}$ J at 22 °C for a bilayer, a value within 14% of the present simulation

As noted above, it is well known that different experimental techniques yield inconsistent values for K_c.[3,4] For example, numbers obtained via thermal fluctuations of vesicles are typically 1.5–2.5 times larger than those obtained via X-ray scattering or micropipette aspiration.[4] For DOPC, the one system we consider that has been studied by all three experimental methods, the factor is approximately 1.4.[4] Given the uncertain experimental landscape and limited catalog of lipids examined in this study, it is premature to make any broad claims regarding global agreement/disagreement between simulation and experiment in the determination of K_c for lipid bilayer membranes. However, among all experimental techniques found in the literature, analysis of thermal vesicle fluctuations would certainly seem to be the technique most closely related to the present simulations. The vesicle fluctuation experiments and our simulations both involve the tracking of thermal fluctuations in single bilayers absent of any external perturbations. In this sense, it is reassuring that the simulations agree well with this class of measurements.

While K_c is well studied in the membrane biophysics literature, the tilt and twist moduli, K_θ and K_{tw}, are considered far less often. Experimentally, the DOPE tilt modulus was estimated to be $K_\theta \approx 8 \times 10^{-20}$ J/nm^2 based on analysis of the L_α-to-H_{II} phase transition.[21] This measurement represents the only published experimental lipid tilt modulus. Experimental measurements of K_{tw} appear to be unavailable. The values reported in Table 1 for K_θ and K_{tw} are similar to those obtained from coarse-grained lipid models at various levels of resolution[13,19,20] and fall within the ranges suggested by theoretical estimates.[21,27] (Interestingly, the tilt modulus for MARTINI DPPC[13,20] appears to be approximately twice as large as the fully atomic result found here.) As previously observed for coarse-grained lipid models,[13,20] the values of K_θ and K_{tw} depend upon the precise definition of the molecular $\mathfrak{n}_j^{(\alpha)}$ vectors applied in the analysis, though differences in K_c upon redefinition of the vector are similar to the statistical errors reported in Table 1 (Tables S4 and S5). Values of the isothermal area compressibility modulus (K_A) as determined via an analysis of box fluctuations are also reported in Table 1, and are in good agreement with experiments.[7,28] We note that alternate methods for determining the tilt modulus from simulations exist in the literature.[19,20,22]

This Communication demonstrates the extraction of bending moduli from all-atom simulations of lipid bilayers. The reported computations involve 648 lipids, ~20,000 waters, and simulation durations of ~100 ns, which are readily accomplished using modern computing clusters. Furthermore, these system sizes are intentionally conservative to demonstrate convergence of the data. The plots in Figure 2 suggest convergence at wavelengths comparable to half the current box sizes (one-fourth the current number of lipids), implying that smaller simulations could be used to accurately predict the reported moduli. For example, as shown in the SI, a simulation of 288 DPPC lipids yields K_c = (15.1 ± 0.4) × 10^{-20} J. It is

dx.doi.org/10.1021/ja507910r | J. Am. Chem. Soc. 2014, 136, 13582–13585

hoped that simulations of this nature will help illuminate the nature of experimental inconsistencies in the measurement of K_c and will serve as a standard tool in the refinement of molecular force fields. The bilayer bending modulus is among the most important biophysical properties of membranes, and it is critical to quantify this number as precisely as possible in both experiments and detailed simulations.

■ ASSOCIATED CONTENT

Supporting Information

Molecular dynamics simulation details; simulation analysis details; modulus standard error obtained through block averages; membrane modulus values using alternate definitions for $\mathfrak{m}^{(\alpha)}$; calculation of membrane area per lipid and K_A; alternate analysis methods and a test of box size dependence. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

flbrown@chem.ucsb.edu

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank John Nagle and Evan Evans for helpful discussions regarding this work. Partial financial support was provided by NSF grants CHE-1153096 and MCB-1158577, and the intramural research program at the NIH, National Heart, Lung and Blood Institute. We also acknowledge support from the Center for Scientific Computing at the CNSI and MRL (an NSF MRSEC) under grants DMR-1121053 and NSF CNS-0960316, in addition to the computational capabilities of the National Institutes of Health, Bethesda, MD (NHLBI LoBoS cluster) and Texas Advanced Computing Center (TACC) at the University of Texas at Austin.

■ REFERENCES

(1) Helfrich, W. *Z. Naturforsch. C: J. Biosci.* **1973**, *C28*, 693.
(2) Canham, P. B. *J. Theor. Biol.* **1970**, *26*, 61.
(3) Marsh, D. *Chem. Phys. Lipids* **2006**, *144*, 146.
(4) Nagle, J. F. *Faraday Discuss.* **2013**, *161*, 11.
(5) Boal, D. H. *Mechanics of the cell*; Cambridge University Press: Cambridge, UK, 2002.
(6) Liu, Y. F.; Nagle, J. F. *Phys. Rev. E* **2004**, *69*, No. 040901.
(7) Rawicz, W.; Olbrich, K. C.; McIntosh, T.; Needham, D.; Evans, E. *Biophys. J.* **2000**, *79*, 328.
(8) Tian, A. W.; Capraro, B. R.; Esposito, C.; Baumgart, T. *Biophys. J.* **2009**, *97*, 1636.
(9) Fernandezpuente, L.; Bivas, I.; Mitov, M. D.; Meleard, P. *Europhys. Lett.* **1994**, *28*, 181.
(10) Lee, C. H.; Lin, W. C.; Wang, J. P. *Phys. Rev. E* **2001**, *64*, No. 020901.
(11) Pastor, R. W.; MacKerell, A. D. *J. Phys. Chem. Lett.* **2011**, *2*, 1526.
(12) Klauda, J. B.; Venable, R. M.; MacKerell, A. D.; Pastor, R. W. *Comput. Model. Membr. Bilayers* **2008**, *60*, 1.
(13) Watson, M. C.; Brandt, E. G.; Welch, P. M.; Brown, F. L. H. *Phys. Rev. Lett.* **2012**, *109*, No. 028102.
(14) Klauda, J. B.; Venable, R. M.; Freites, J. A.; O'Connor, J. W.; Tobias, D. J.; Mondragon-Ramirez, C.; Vorobyov, I.; MacKerell, A. D.; Pastor, R. W. *J. Phys. Chem. B* **2010**, *114*, 7830.
(15) Goetz, R.; Gompper, G.; Lipowsky, R. *Phys. Rev. Lett.* **1999**, *82*, 221.
(16) Marrink, S. J.; Mark, A. E. *J. Phys. Chem. B* **2001**, *105*, 6122.
(17) Lindahl, E.; Edholm, O. *Biophys. J.* **2000**, *79*, 426.
(18) Safran, S. A. *Statistical Thermodynamics of Surfaces, Interfaces and Membranes*; Westview Press: Boulder, CO, 1994.
(19) Watson, M. C.; Penev, E. S.; Welch, P. M.; Brown, F. L. H. *J. Chem. Phys.* **2011**, *135*, No. 244701.
(20) May, E. R.; Narang, A.; Kopelevich, D. I. *Phys. Rev. E* **2007**, *76*, No. 021913.
(21) Hamm, M.; Kozlov, M. M. *Eur. Phys. J. E* **2000**, *3*, 323.
(22) Khelashvili, G.; Kollmitzer, B.; Heftberger, P.; Pabst, G.; Harries, D. *J. Chem. Theory Comput.* **2013**, *9*, 3866.
(23) Brooks, B. R.; Brooks, C. L.; Mackerell, A. D.; Nilsson, L.; Petrella, R. J.; Roux, B.; Won, Y.; Archontis, G.; Bartels, C.; Boresch, S.; Caflisch, A.; Caves, L.; Cui, Q.; Dinner, A. R.; Feig, M.; Fischer, S.; Gao, J.; Hodoscek, M.; Im, W.; Kuczera, K.; Lazaridis, T.; Ma, J.; Ovchinnikov, V.; Paci, E.; Pastor, R. W.; Post, C. B.; Pu, J. Z.; Schaefer, M.; Tidor, B.; Venable, R. M.; Woodcock, H. L.; Wu, X.; Yang, W.; York, D. M.; Karplus, M. *J. Comput. Chem.* **2009**, *30*, 1545.
(24) Gawrisch, K.; Parsegian, V. A.; Hajduk, D. A.; Tate, M. W.; Gruner, S. M.; Fuller, N. L.; Rand, R. P. *Biochemistry* **1992**, *31*, 2856.
(25) Gracia, R. S.; Bezlyepkina, N.; Knorr, R. L.; Lipowsky, R.; Dimova, R. *Soft Matter* **2010**, *6*, 1472.
(26) Fuller, N.; Rand, R. P. *Biophys. J.* **2001**, *81*, 243.
(27) Kessel, A.; Ben-Tal, N.; May, S. *Biophys. J.* **2001**, *81*, 643.
(28) Evans, E.; Rawicz, W.; Smith, B. A. *Faraday Discuss.* **2013**, *161*, 591.