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Review

Experimentally determined tilt and bending moduli of single-component lipid bilayers

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ABSTRACT

Values of the bending modulus K_C and the tilt modulus K_θ are reported for single component lipid bilayers. The lipids studied have the common names DOPC, DMPC, diC22:1PC, SOPC, POPC, diPhyPC, DLPC, DPPC, DHPC and DEPC, listed in the order of number of samples examined. The experimental method, thus far the only one that measures the tilt modulus of lipid bilayers, first obtains diffuse X-ray scattering data from oriented stacks of bilayers. The values of the moduli emerge from fitting the data to the accepted tilt-dependent continuum model for the free energy of a single bilayer, further enhanced by interactions between bilayers in the stack. The results indicate the broad trend that the tilt modulus for these PC lipids is smaller the closer the temperature is to the main transition temperature. Another trend is that inclusion of tilt raises the value of the bending modulus more for lipids with smaller values of the tilt modulus. Values of both moduli are compared to recent literature values obtained from simulations and values of the bending modulus are compared to the literature values obtained by other experimental methods.

Contents

1. Introduction

It is widely recognized that an especially important property of biomembranes is their flexibility that allows for biological function. For this, the bending modulus K_C is the most important measure of resistance to bending. Along with spontaneous curvature and the Gaussian modulus, it provides the basic, lowest-order, long length scale description of the curvature properties of membranes, as embodied in the Helfrich-Canham theory (Helfrich, 1973), which is the simplest theoretical framework that incorporates the bending modulus. For symmetric lipid bilayers of fixed topology this is a one parameter continuum theory in which the curvature energy is proportional to the bending modulus, which is lipid specific, times the membrane curvature squared. Although a recent modification has recently been proposed for gel phase bilayers (Diggins et al., 2015), the Helfrich-Canham (HC) theory is generally deemed valid for the fluid phase at long length scales.

In contrast, simulations have made it clear that the HC theory is not valid at shorter length scales (Goetz et al., 1999; Lindahl and Edholm, 2000). A growing consensus is that the continuum theory can be significantly improved by including a molecular tilt degree of freedom, even for the fluid phase above the chain melting phase transition and (Hamm and Kozlov, 2000) (HK) proposed such a theory. It has long been known that chains in phosphatidylcholine lipids undergo cooperative, spontaneous tilt in the gel phase. Of course, the average tilt is zero in the fluid phase, but individual

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chains may tilt locally. The HK theory incorporates this with a tilt field and an appropriate addition to the theoretical free energy. Importantly for biological relevance, it was shown (Kozlovsky and Kozlov, 2002) that the HK theory alleviated a major theoretical concern, namely, that the HC theory had predicted an impossibly large activation energy for the fusion of membranes (Siegel, 1999). Subsequently, it was shown that the HK theory also quantitatively accounts for the observed deviations in the simulated fluctuation spectra (May et al., 2007). Further development of the tilt theory has been made (Watson et al., 2011), including methods for extracting both the tilt modulus K_θ and the bending modulus K_C from simulations (Watson et al., 2012); this further theory has also passed an additional recent test regarding how the length of the hydrocarbon tails depends on the actual tilt of individual chains (Kopelevich and Nagle, 2015).

While it has been clear that incorporating both bending and tilt is important for theory and simulations, experimentally measuring the accompanying tilt modulus has lagged. It appears to be impossible to measure K_θ by the classical methods that have been developed to measure K_C because those measurements are on length scales in the plane of the membrane that are too large to detect tilt (Nagle et al., 2015). In contrast, diffuse x-ray scattering obtains transverse flexibility information at a shorter in-plane length scale. Recently, it has been shown how to obtain the value of the tilt modulus, as well as of the bending modulus, from diffuse x-ray scattering, and results have been presented for DOPC bilayers (Jablin et al., 2014). At this time, this is the only experimental method that has been advanced for obtaining values of the tilt modulus of lipid bilayers.

Diffuse x-ray scattering data for many lipid bilayers have been obtained in this lab since 2003. These data had previously been analyzed based on the HC theory and values of K_C had been reported in a series of papers, each of which focused on one or a few lipids or on lipid bilayers with an additive. The present paper reports the results of using the tilt-dependent theory to re-analyze some of those data. All of the results in this paper are for single component lipid bilayers in the fluid phase, mostly at T = 30°C except for two lipids that have higher phase transition temperatures. The effect of temperature and effects of additives, e.g. cholesterol, will be presented elsewhere. In addition to providing the first experimental values of the tilt modulus K_θ for many bilayers in addition to DOPC, this study reports revised x-ray values for the bending modulus K_C that are then compared to literature values obtained by classical methods.

2. Experimental

All the lipids studied had been obtained from Avanti Polar Lipids (Alabaster, Alabama), 1,2-dilauroyl-*sn-glycero*-3-phosphocholine (DLPC), 1,2-dimyristoyl-*sn-glycero*-3-phosphocholine (DMPC), 1,2-dipalmitoyl-*sn-glycero*-3-phosphocholine (DPPC), 1-palmitoyl-2-oleoyl-*sn-glycero*-3-phosphocholine (POPC), 1-stearoyl-2-oleoyl-*sn-glycero*-3-phosphocholine (SOPC), 1,2-dioleoyl-*sn-glycero*-3-phosphocholine (DOPC), 1,2-dierucoyl-*sn-glycero*-3-phosphocholine (diC22:1PC), 1,2-dielaidoyl-*sn-glycero*-3-phosphocholine (DEPC), 1,2-diphytanoyl-*sn-glycero*-3-phosphocholine (diPhyPC), 1,2-di-*O*-hexadecyl-*sn-glycero*-3-phosphocholine (DHPC). Oriented stacks consisting of approximately 2000 bilayers had been prepared using the rock-and-roll method (Tristram-Nagle, 2007).

The method for obtaining diffuse x-ray scattering data has been previously described (Kucerka et al., 2005a; Liu, 2003; Jablin, 2015). Briefly, oriented stacks of approximately 2000 bilayers supported on a flat Si substrate are rotated in the x-ray beam to provide cumulative intensity for all incident angles on a CCD detector. An example of the CCD image is shown in Fig. 1. All data re-analyzed in this paper had been obtained at the CHESS synchrotron.

The mechanical moduli were evaluated using a fitting program (NFIT, (Liu, 2003; Jablin, 2015)) that obtained the smallest χ^2 in an

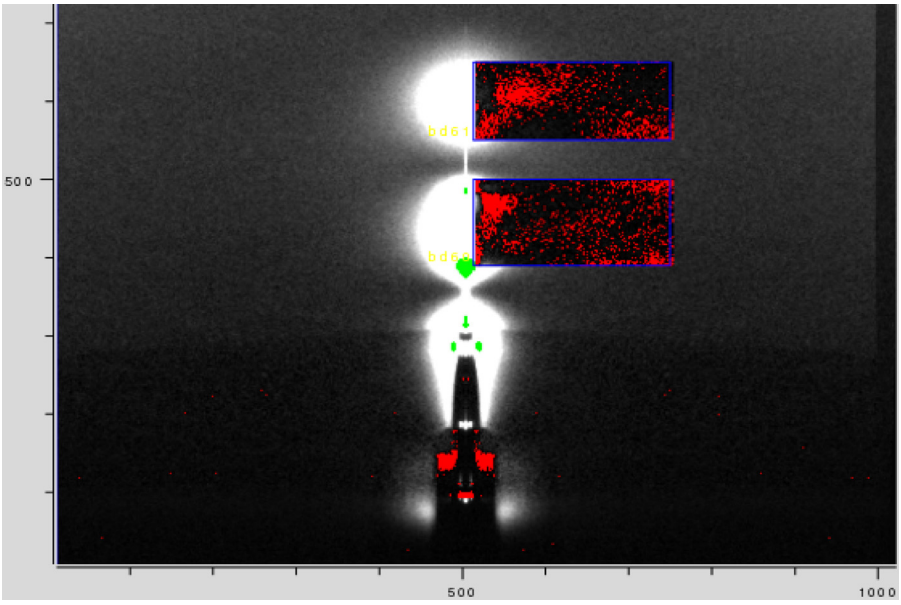

Fig. 1. Rendition of the background subtracted, symmetrized, scattering intensity with an overlay of the fitted residuals in the two rectangles lightly outlined in blue. The largest intensities (above 4000) are shown by green pixels. Red pixels indicate negative values. The diffuse scattering is shown by a grayscale from black (zero) to full white (above 200). The strongly attenuated direct beam is near the small white dot located near the bottom at pixel (500,80). Less strongly attenuated are the h = 1 order at (500,185) and the h = 2 order at (500,290) near which there are three green spots due to its unattenuated tails; both these orders appear in what we define as the first lobe of diffuse scattering. The second lobe contains two green regions at larger values of pz; these are smeared h = 3 and h = 4 orders. The data were fit in the two rectangular boxes outlined in blue and the portrayed intensities in those boxes are the residuals to the fit, with values typically less than 1% of the average intensity. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

area of the CCD that comprised approximately 50,000 CCD pixels as shown in Fig. 1; in reciprocal space this generally ranged from about 0.2 to 0.6 $Å^{-1}$ in the vertical direction along the average bilayer normal and from about 0.01 to 0.3 $Å^{-1}$ in the horizontal in-plane direction. There are many input parameters in NFIT. These include obvious experimental conditions, as well as less obvious ones like beam divergence and energy dispersion. Input parameters also include details of the sample geometry and the repeat spacing (D) of bilayers in the stack. A full list of input parameters is provided in Supplementary material along with their uncertainties and how much those uncertainties affect the values of the bending and tilt moduli obtained from NFIT. The uncertainties in most input parameters affect the values of the moduli relatively little compared to differences obtained from nominally identical samples. An exception is that two different treatments of the background gave differences in K_C by about 10% for some lipids, and even larger differences for K_θ also occurred. Reported values in this paper are averages from the two treatments. The tilt-dependent analysis has been described briefly (Jablin et al., 2014) and also in much more detail (Jablin, 2015).

Typically, data were obtained for several values of the lamellar repeat D spacing of the stack of bilayers; D varied as the hydration level varied. However, the results in this report only include the best exposure from one D spacing per sample because the moduli, in accordance with theory, do not depend systematically upon D, as was previously shown for the tilt-independent bending modulus (Pan et al., 2008). The analysis also must fit a bulk B modulus. The value of B clearly depends upon D (Pan et al., 2008) because B involves the interaction between neighboring bilayers which becomes exponentially stronger as D decreases (Petrache et al., 1998). Interestingly, the value of B was very nearly the same for a given D whether the tilt-dependent or the tilt-independent analysis is used. The criteria for the chosen exposure for each sample were (i) the D spacing was large enough to have substantial diffuse scattering for accurate analysis, (ii) the sample was not noticeably flooded with excess water which can occur when trying to achieve maximal hydration, and (iii) choosing a location on the sample where it was well oriented. It was advantageous to hydrate samples to near their fully hydrated level, defined as the value of D obtained in unoriented multilamellar vesicles (MLV) dispersed in excess water, because there was then more diffuse scattering; however, the other criteria sometimes motivated reporting results from a smaller value of D.

3. Results

Table 1 presents the main results of this paper. Column 1 of Table 1 lists the single component lipids for which diffuse x-ray scattering data are available. Column 2 lists the number of independent samples. The different samples of most lipids were examined on different synchrotron runs. A notable exception was that 14 samples of DOPC were examined on the same run; these had the same standard deviation in the values of the moduli as samples from different runs, indicating that sample variation, not experimental condition, was primarily correlated with uncertainties in the values of the moduli. The third column shows the temperature. The next column gives the range of lamellar repeat D spacings analyzed for the different samples of each lipid. Although the maximum D in this range is typically close to the fully hydrated value, it is smaller for some lipids.

The column labeled K_θ in Table 1 shows the mean values of the tilt modulus weighted for the N samples for each lipid. (Most weights were unity, but a few less optimal samples were assigned smaller weights.) The column labeled K_C^{td} in Table 1 shows the mean value of the bending modulus obtained by allowing the tilt modulus K_θ to fit; this is called the tilt-dependent (^{td}) value of K_C. The column labeled K_C^{ti} in Table 1 gives the value of the bending modulus obtained by fixing K_θ to a large value; this suppresses consideration of the tilt degree of freedom so these are called the tilt-independent (^{ti}) values of K_C. The uncertainties shown in Table 1 are the standard errors of the mean for those lipids for which we had more than one sample. The uncertainty assigned to lipids for which there was only one sample was set close to the maximum standard deviation found for the other lipids. The standard deviations obtained from all samples of the same lipid can be thought of as arising from two sources. First, is the uncertainty obtained from the systematic fitting protocol. This is rather small compared to the second uncertainty which comes from different samples of the same lipid. We have tried to identify an experimental cause for this sample variation and to eliminate it, but without success. Fig. 2 shows variations in the values of K_C^{td} and K_θ for all DOPC samples.

Table 1
Mean moduli for the N samples of the lipid bilayers studied at the shown temperature T. The tilt-dependent bending modulus K_C^{td} and the tilt-independent bending modulus K_C^{ti} are given in units of kT and their ratio is shown in the third to last column. Uncertainties in the moduli are the standard error of the mean for N > 1 and for N = 1 they were estimated from standard deviations of other lipids. Previously published results for the tilt-independent bending modulus are shown in the penultimate column as K_C^{lit} and the ratio with the K_C^{ti} obtained in this paper is shown in the last column.

Lipid	N samples	T (°C)	D (Å)	K_θ (mN/m)	K_C^{td}/kT	K_C^{ti}/kT	K_C^{td}/K_C^{ti}	K_C^{lit}/kT	K_C^{ti}/K_C^{lit}
DOPC	26	30	60.9–64.9	89 ± 4	19.4 ± 0.7	16.3 ± 0.5	1.19	18.2 ± 2.7[a]	0.89
DMPC	13	30	60.6–63.2	44 ± 2	24.6 ± 1.0	15.6 ± 0.6	1.58	15.8 ± 2.4[a]	0.99
diC22:1PC	4	30	66.9–69.0	58 ± 5	46.2 ± 1.2	28.3 ± 1.3	1.63	30.6 ± 1.6[a]	0.93
POPC	4	30	62.0–64.3	73 ± 12	25.7 ± 2.1	19.2 ± 1.2	1.34	20.3[b]	0.95
SOPC	3	30	62.9–66.9	60 ± 5	24.6 ± 2.9	18.5 ± 1.9	1.33	21.1 ± 1.6[a]	0.86
diPhyPC	3	30	61.8–63.4	109 ± 18	17.4 ± 1.8	14.9 ± 1.3	1.17	12.4 ± 1.5[c]	1.20
DLPC	2	30	61.2–62.9	55 ± 5	20.4 ± 1.4	14.3 ± 0.7	1.43	13.2[d]	1.09
DPPC	2	50	64–67	43 ± 4	27.5 ± 3.4	18.3 ± 3.1	1.50	15.0 ± 1.6[e]	1.22
DHPC(ether)	1	48	66.5	38 ± 16	30.6 ± 4.5	19.1 ± 3.1	1.61	12.4 ± 1.7[e]	1.53
DEPC(trans18:1)	1	30	71	57 ± 16	21.2 ± 4.5	17.0 ± 3.1	1.25	–	–

References are
[a] Nagle (2013).
[b] Kucerka et al. (2005b).
[c] Tristram-Nagle et al. (2010).
[d] Kucerka et al. (2005a).
[e] Guler et al. (2009).

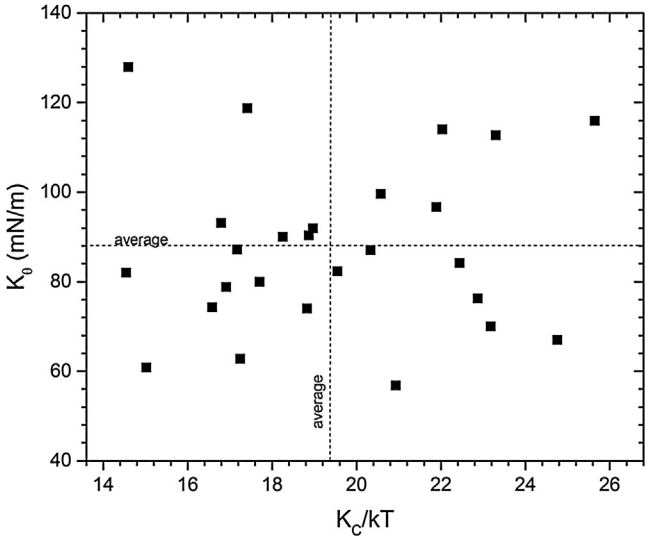

Fig. 2. Results for the tilt K_θ and bending K_C^{td} moduli for the 26 samples of DOPC.

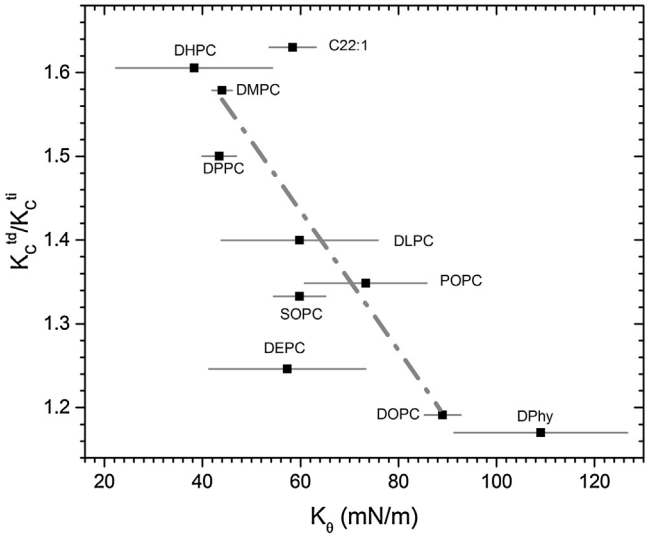

Fig. 3. Ratio of tilt-dependent bending modulus K_C^{td} to tilt-independent bending modulus K_C^{ti} versus tilt modulus K_θ for the 10 lipids studied. The dash-dot line suggests a correlation.

The third to last column in Table 1 shows the ratio of the average tilt-dependent bending modulus K_C^{td} to the average tilt-independent bending modulus K_C^{ti}. Uncertainties are not assigned to these ratios because the two values are correlated to an uncertain degree. Fig. 3 plots this ratio versus the tilt modulus for all our studied lipids. The results for the most studied lipid bilayers indicate that the smaller average tilt modulus of DMPC increases the tilt-dependent bending modulus K_C^{td} more compared to the tilt-independent bending modulus K_C^{ti} than does the larger average tilt modulus of DOPC. Of the other lipids, only the deviation of C22:1 is substantially inconsistent with the general trend indicated in Fig. 3.

The next to last column in Table 1 shows previously published values of tilt-independent K_C from this lab and the last column shows the ratio with the new tilt-independent values. Some deviations from unity occur partly because additional samples are now averaged. Other differences may be attributed to the new NFIT program and the associated protocol that treat coherence and domain sizes somewhat differently (Jablin, 2015).

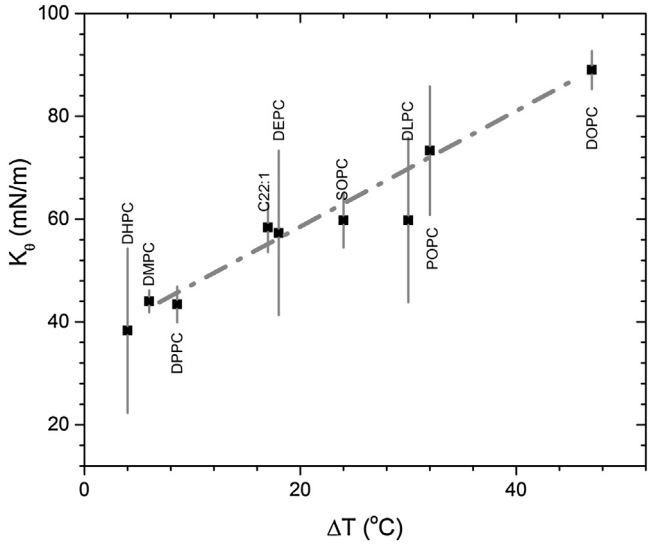

Fig. 4. Tilt modulus Kθ versus temperature difference ΔT between the experimental temperature and the main chain melting transition temperature T_M of each of 9 lipids with known T_M. A correlation is suggested by the dash-dot line.

Fig. 4 shows the values of the tilt modulus versus the difference ΔT between the measured temperature and temperature T_M of the main transition from the fluid phase into the rippled or gel phase. As in Fig. 3, there is a strong difference for our most studied DOPC and DMPC lipids. The dot-dash line in Fig. 4 indicates a strong correlation for all the lipids with phosphocholine (PC) headgroups. This leads one to predict that the tilt modulus should decrease as the main transition is approached for each lipid. A subsequent paper will present data that confirm this prediction for DMPC.

4. Discussion

There is an important connection between the tilt modulus and the bending modulus regarding the overall flexibility of membranes. Inclusion of tilt in the tilt-dependent analysis always increases the value of K_C^{td} compared to the tilt-independent value K_C^{ti}, as shown in the third to last column of Table 1. This is expected because a reduction in either modulus softens a membrane overall. The tilt-independent fit assumes an infinite value of K_θ. Allowing K_θ to fit in the tilt-dependent analysis therefore increases K_C in order to achieve the same overall softness. (However, note that, compared to K_C, K_θ softens preferentially at smaller length scales – otherwise, separate values for the two moduli could not be extracted from the experimental data and the goodness of the fit would not be better for the tilt-dependent fit.) It then follows that the increase in K_C should generally be greater for a lipid that has a smaller K_θ than for one with a larger K_θ. Fig. 3 generally confirms this, especially for DOPC and DMPC for which we have the best statistics. Fig. 3 shows that the ratio of tilt-independent to tilt-dependent values of K_C for other lipids generally follows the trend, with the most notable exception being C22:1 which forms the thickest bilayers (Kucerka et al., 2005b).

As a tilt degree of freedom makes membranes more flexible at the short length scale appropriate for important properties like fusion, it should be of interest to learn what affects the tilt modulus. Unexpectedly, the strongest correlation found here for the PC lipids is the proximity ΔT to the main fluid disordered to ordered phase transition temperature shown in Fig. 4. This is reminiscent of early biomembrane studies that reported optimal growth of various organisms occurred above but near the chain melting transition (Steim et al., 1969), although one should not push this connection too far as biomembranes are complex

mixtures of many lipids and proteins. The fraction of unsaturated double bonds in the hydrocarbon chains would have seemed, *a priori*, to provide a useful correlation, and it does, in so far as double bonds lower the phase transition temperature. However, both C22:1 and DOPC have higher fractions of double bonds than SOPC and POPC, but of these four lipids, the former pair have widely different values of K_θ and the latter pair have intermediate values at T = 30 °C. One might also have guessed *a priori* that the tilt modulus would be correlated with the thickness of the bilayer, but our best studied DOPC and DMPC have nearly equal thicknesses (Nagle and Tristram-Nagle, 2000), but much different values of K_θ. The result that K_θ is strongly correlated with ΔT leads one to expect that the tilt modulus should decrease as the main transition is approached for each lipid; a subsequent paper will report results that strongly confirm this expectation for DMPC.

The idea that the tilt modulus increases with increasing temperature seems counter-intuitive, so it is reassuring that there is a theoretical equation that predicts the same direction (Watson et al., 2011),

$$K_\theta = 2\gamma_{ow} - B/A^2, \quad (1)$$

where γ_{ow} is oil-water surface tension (typically ~50 mN/m) and B/A^2 was subtracted to take into account the repulsive interaction between headgroups, where A is the area/lipid and B is a positive constant. According to Eq. (1), K_θ would increase with increasing temperature because area A increases. It is interesting to see whether Eq. (1) agrees with our data. Fig. 5 shows values of K_θ when B is fixed to provide agreement with the experimental values for DMPC and experimental values of A are used. Disagreement for DOPC is especially strong. Because the repulsive interaction between headgroups becomes infinite for A smaller than the A ~48 Å^2 that occurs in the gel phase of PC lipids (Tristram-Nagle et al., 2002), Fig. 5 also shows values of K_θ for a modified formula where A is replaced by (A –48 Å^2). Disagreement is then alleviated for DOPC and DPhyPC, although agreement is poorer for three other lipids. Neither equation correlates as well as Fig. 4.

Table 2 compares values of the moduli from recent atomistic simulations with X-ray values. Three of the papers used the analysis method developed by Brown's group (Watson et al., 2012).

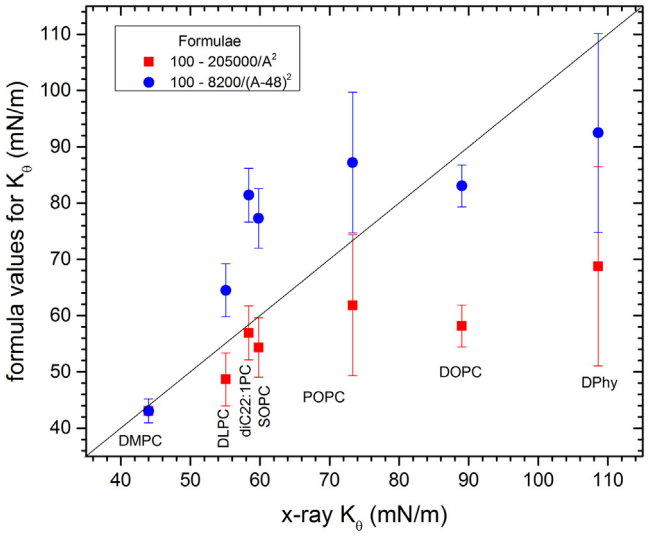

Fig. 5. Estimates of tilt modulus from Eq. (1) (red squares) and from a modified equation (blue circles) compared to experimental values. The solid line represents perfect agreement. References for experimental values for the areas A are in the caption to Table 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Comparison of the values of the tilt-dependent X-ray moduli with those from simulations, where UA connotes united atom and CH36 connotes version 36 of CHARMM.

Lipid	Method	T (°C)	K_θ (mN/m)	K_C/kT	Reference
DMPC	UA	27	56	39	Watson et al. (2012)
	UA/buckles	27	39 ± 2	24.9 ± 1.0	Wang and Deserno (2016)
	CH36	30	40.2	29.2 ± 1.2	Venable et al. (2015)
	X-ray	30	43 ± 2	25.1 ± 1.0	This paper
DOPC	CH36	24.9	66 ± 2	27.7 ± 0.7	Levine et al. (2014)
	CH36	25	64.0	28.7 ± 1.0	Venable et al. (2015)
	X-ray	30	95 ± 7	19.8 ± 1.4	Jablin et al. (2014)
	X-ray	30	89 ± 4	19.4 ± 0.7	This paper
	X-ray	25	84	20.6	Using Pan et al. (2008)
DPPC	CH36	50	49 ± 2	35.0 ± 1.1	Levine et al. (2014)
	CH36	50	48.8	35.4 ± 0.9	Venable et al. (2015)
	X-ray	50	44 ± 16	28.8 ± 4.5	This paper
POPC	CH36	30	55.2	31.6 ± 1.0	Venable et al. (2015)
	X-ray	30	69 ± 17	24.6 ± 2.6	This paper

An exception (Wang and Deserno, 2016) analyzed membrane buckling. For DMPC the two latest simulations agree well with each other (especially when the temperature difference is considered as both moduli rapidly decrease with decreasing temperature in this range), and there is satisfactory agreement with the X-ray results. For DOPC the X-ray values are significantly larger for K_θ and significantly smaller for K_C. An *a prior* reconciliation might be based on Fig. 3, which shows that, for different lipids, a smaller average K_θ increases K_C more. However, Fig. 2 shows that there is no correlation between K_θ and K_C for individual samples of DOPC. Another reconciliation of the DOPC values could be based on the temperature difference between the simulations and the experiments. Indeed, there are DOPC X-ray data at 15 °C and 45 °C (Pan et al., 2008) that have been reanalyzed, and interpolation to T = 25 °C gives values 6% smaller for K_θ and 6% larger for K_C. While this is in the right direction, it falls short, only decreasing K_θ to 84 mN/m and increasing K_C/kT to 20.6. It might also be noted that K_θ ≈80 mN/m can be gleaned (Nagle et al., 2015) from a real space simulation analysis (Khelashvili and Harries, 2013). For DPPC and POPC, the X-ray uncertainties for K_θ are quite large, so there is no disagreement with simulations in Table 2. The X-ray value for K_C for DPPC is a bit smaller, like DMPC. For POPC the difference divided by the average is 0.25, roughly midway between the similar normalized difference for DMPC (0.15)and DOPC (0.39), suggesting that chain unsaturation may be involved. It may also be noted that the Supplementary material of (Levine et al., 2014) report 18% differences in K_θ that depend upon how the headgroup end of the molecular tilt was defined; possibly larger differences might occur if the tail end of chains were reconsidered as was done for DMPC only by (Wang and Deserno, 2016), and this might alleviate the discrepancy for lipids with unsaturated chains. In contrast to simulations, the x-ray analysis is based only upon the continuum model which is impervious to such molecular distinctions, so differences with simulation results due to atomistic tilt definition should be explored further by simulators.

Values of the tilt-independent X-ray bending modulus K_C have previously been compared to literature values obtained from giant unilamellar vesicles using the classical shape analysis (SA) or aspiration pipette membrane mechanics (MM) (Nagle, 2013). Fig. 3 emphasizes that the X-ray values are now larger when the tilt-dependent analysis is employed. Table 3 enables a comparison of these new values to literature values obtained using other methods. Previously, the tilt-independent X-ray values of K_C had

Table 3
K_C/kT values of the bending modulus obtained for some single component bilayers using the new tilt-dependent X-ray method compared to literature values obtained using micromechanical manipulation (MM), shape analysis (SA) and tethers.

Method Lipid	X-ray	MM	SA	Tethers	SA/ X-ray
DOPC	19.4 ± 0.7	19.1 ± 2.2[a]	26.5 ± 2.7[d]	16 ± 2[j]	1.37 ± 0.18
			24 ± 2[e]	23 ± 5[k]	1.23 ± 0.14
SOPC	24.6 ± 2.9	20.2 ± 1.3[a]	30.4 ± 1.7[b,f]	26.8 ± 1.8[l]	1.24 ± 0.19
		30 ± 1.6[b]	29.4 ± 6.1[g]		1.20 ± 0.36
		36.9 ± 1.2[c]	37.9 ± 0.8[h]		1.54 ± 0.15
DMPC	25.1 ± 1.0	13.4 ± 1.4[a]	31.1 ± 1.9[i]		1.24 ± 0.12
diC22:1PC	46.2 ± 1.2	27.3 ± 3.4[a]			

Literature values have been adjusted to 30 °C (Nagle, 2013).
[a] Rawicz et al. (2000).
[b] Vitkova et al. (2006).
[c] Henriksen and Ipsen (2004).
[d] Gracia et al. (2010).
[e] Shchelokovskyy et al. (2011).
[f] Meleard et al. (1998).
[g] Pecreaux et al. (2004).
[h] Henriksen and Ipsen (2002).
[i] Meleard et al. (1997).
[j] Sorre et al. (2009).
[k] Tian et al. (2009).
[l] Heinrich and Waugh (1996).

agreed with the earliest MM values (Rawicz et al., 2000). The new X-ray values still agree well for DOPC and borderline well for SOPC, but there are now large differences for DMPC and diC22:1PC. A few MM results for SOPC from other labs continue to be larger even than the new tilt-dependent X-ray results in Table 3. The SA values of K_C tend to be larger than the new X-ray values as indicated in the last column in Table 3 which shows the ratios of those values. Previously, using the tilt-dependent X-ray values the ratio was much larger for DMPC than for DOPC. Now, the ratios in Table 3 appear not to depend significantly upon the lipid. It should be reiterated that the SA method is definitely not affected by tilt and, while the MM method could be marginally affected by tilt as it spans many decades of length scales, only the shortest decade is affected by tilt (Nagle et al., 2015), so other explanations have to be sought for residual systematic differences with x-ray results. One obvious difference is that the classical methods study GUV samples whereas x-ray samples are multilamellar arrays which have interactions between adjacent bilayers which are accounted for by the bulk B modulus. Although this is only a harmonic approximation, it is consistent with the corresponding level of approximation in the HK single membrane continuum model.

Many researchers interested only in the overall flexibility of a membrane may not be prepared to consider both a bending modulus and now a tilt modulus. In that case, the older tilt-independent X-ray values would actually give more appropriate measures of overall flexibility in the sense that they represent a kind of average softness due to the true tilt-dependent bending modulus and the tilt modulus. However, even the tilt-independent values of the bending modulus are too large to overcome the energy crisis for the stalk model of membrane fusion (Siegel, 1999), so both moduli should be considered. In this regard, it is interesting to compare our two best studied lipids. DMPC and DOPC have nearly the same thickness (Nagle and Tristram-Nagle, 2000) and Table 1 indicates only slightly larger tilt-independent values of K_C^{ti} for DOPC, suggesting that there should not be much difference in bilayer properties that depend upon bending. However, the tilt-dependent results for K_C^{td} indicate that DMPC should be stiffer than DOPC for properties that depend upon bending at large length scales and less stiff than DOPC for smaller length scale properties that depend upon the tilt modulus.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemphyslip.2017.04.006.

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