X-ray scattering reveals molecular tilt is an order parameter for the main phase transition in a model biomembrane

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Abstract: Synchrotron diffuse X-ray scattering data reveal a dramatic softening of the molecular tilt modulus K_θ of the model biomembrane composed of DMPC lipids as the temperature is lowered towards the main phase transition temperature T_M. Spontaneous tilt occurs below T_M, suggesting that tilt is a symmetry breaking order parameter. Consistent with this hypothesis it was also shown that a different lipid POPS has no spontaneous tilt below T_M and correspondingly its tilt modulus did not soften as T_M was approached from above. As previously known, the bending modulus K_C of DMPC also softens close to T_M, but unlike the tilt modulus, K_C has a maximum 3 degrees above T_M, which also marks the limit of the well-known anomalous swelling regime. Tilt adds a new perspective to our previous understanding of the main phase transition in lipid bilayers.

Biomembranes in living cells must be flexible, which is one reason that they are nanoscopic materials only a few nanometers thick. Mechanical descriptors are widely recognized to be central for assessing their properties. For transverse flexibility, the bending modulus is the main mechanical descriptor. The Helfrich-Canham (HC) theory[1] is the simplest theoretical framework that incorporates a mechanical modulus for bending. 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 K_C times the membrane curvature squared. Although a recent modification has recently been proposed for gel phase bilayers[2], the HC theory is generally deemed valid for the fluid phase at long length scales.

However, simulations have made it clear that the HC theory is not valid at shorter length scales.[3, 4] A growing consensus is that the continuum theory can be significantly improved by including a molecular tilt degree of freedom. Such a theory was proposed by Hamm and Kozlov (HK).[5] Importantly for biological relevance, it was shown that the HK theory alleviated the concern[6] that the HC theory had predicted an impossibly large activation energy for the biologically essential function of fusion of membranes[7]. Subsequently, it was shown that the HK theory also quantitatively accounts for the observed deviations in the simulated fluctuation spectra.[8] Further development of the tilt theory has been made,[9] including methods for extracting both the tilt modulus and the bending modulus from simulations[10]; this further theory has also passed an additional recent test regarding how the length of the hydrocarbon tails depends on tilt.[11]

For many years my lab has used the traditional HC model for fluctuations to analyze diffuse X-ray scattering in order to obtain the bending modulus K_C, which measures the stiffness of membranes, and the bulk modulus B, which measures the interaction between neighboring membranes in our systems consisting of stacks of membranes. Recently, we have learned how to include the molecular tilt modulus in our analysis of X-ray data.[12] We found that the data are fit better for the tilt-dependent model, and we have reported the first experimental value of the tilt modulus K_θ for bilayers of one type of lipid (DOPC).[13] Re-analysis that includes the tilt modulus is now being reported on earlier X-

ray scattering data collected in this lab.[14] This letter presents results that have the most physics significance.

New results for the tilt modulus of bilayers of the lipid DMPC in the fluid, chain-melted phase are shown in Fig. 1. It is noteworthy that there is good agreement with values recently obtained from simulations, namely, K_θ = 40.2±2 mN/m at T=30ºC from fluctuation spectra using the CHARMM36 all-atom force field[15] and K_θ = 38.8±2 mN/m at T=27ºC by studying buckling with a united atom force field.[16]

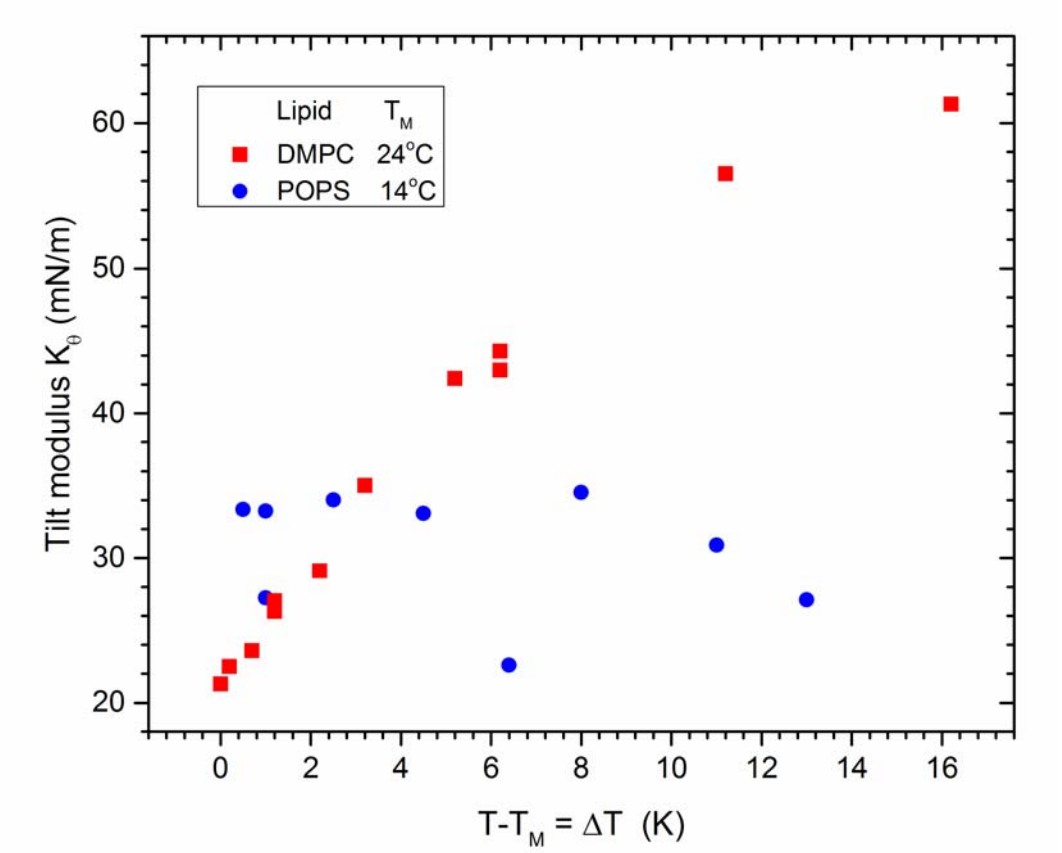

Figure 1: Tilt modulus K_θ versus temperature for DMPC and POPS bilayers with phase transition temperatures T_M shown in the legend. Results were obtained from analysis of diffuse X-ray scattering data.[12, 13]

However, the most striking aspect of Fig. 1, not yet investigated by simulations, is the rapid decrease in K_θ as the main transition in DMPC is approached by lowering temperature within the fluid phase. This opens a new perspective for the main phase

transition. In critical phenomena spontaneous symmetry breaking of a quantity below the critical point identifies an order parameter and a modulus (the inverse susceptibility) of that order parameter then vanishes at a critical point. It is well known that there is spontaneous tilt in the gel phase.[17] Although the main transition is into the ripple phase, recent structural work[18] shows that the hydrocarbon chains in the DMPC ripple phase also have spontaneous tilt (along with other interesting features not comprehended by existing theory). The fact that the transition is ultimately first order could just mean that the usual thermodynamic trajectory only goes close to but not exactly through a critical point. It is therefore a reasonable hypothesis that tilt is an order parameter for the main phase transition in DMPC.

This hypothesis has been further tested by examining a different lipid that does not have spontaneous tilt below the main transition. As is well understood, ordered lipid hydrocarbon chains spontaneously tilt when the head group steric area is larger than the lateral area of parallel ordered chains because cooperative tilting maximizes the cohesive van der Waals energy between parallel chains.[19] Phosphatidylserine (PS) lipids have smaller headgroup volumes than phosphatidylcholine (PC) lipids,[20] so the chains in such lipids are less likely to tilt. For this study we used wide angle x-ray scattering shown in Fig. 2 to verify that the hydrocarbon chains of the POPS lipid are untilted below the main phase transition temperature at $T_M = 14°C$. The electrostatic repulsion of the singly charged POPS lipids leads to large repeat spacings (D~150-190 Å) in samples consisting of stacks of bilayers, and this provides enough diffuse scattering intensity for analysis even with the smaller intensity provided by an in-house rotating anode instead of the CHESS synchrotron that was used to obtain the DMPC results. Figure 1 shows that the tilt modulus of POPS has little temperature dependence upon approaching the transition. This is consistent with the hypothesis that the tilt modulus can exhibit critical behavior when it is a symmetry breaking order parameter in the low temperature phase, but that it is unlikely to when tilt symmetry is not broken.

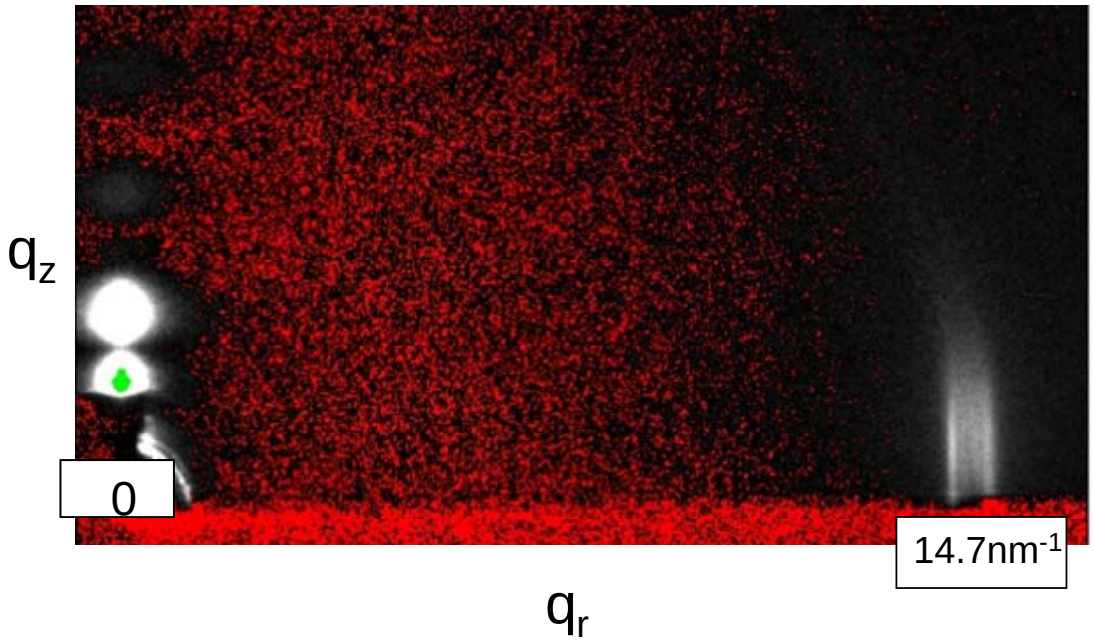

Figure 2. Grazing incidence background subtracted scattering intensity from oriented bilayers of POPS at T = 5°C. The beam is located at 0, lamellar diffraction from the stack of membranes appears along the q_r= 0 meridian. Wide angle scattering from aligned hydrocarbon chains is centered at q_r = 1.47 Å^{-1} and q_z=0, with negative q_z intensity cut off by the substrate. The non-zero extent in the q_z direction is due to the finite thickness of the chain region. Lipids with tilted chains exhibit additional wide angle peaks not centered on the q_z = 0 equator.[21]

Figure 3 shows that the new tilt-dependent analysis continues to find that the bending modulus K_C also decreases as the main transition is approached from the fluid phase,[22] although not as dramatically as the tilt modulus. A decrease in K_C as T_M is approached has been correlated with an anomalous swelling in the repeat D spacing of bilayers in a stack.[23] When tilt is included in the analysis, the maximum in K_C occurs at a lower temperature (27 C) than previously (30 C) when the tilt independent analysis was used. This new maximum actually agrees better with the onset of the anomalous swelling

regime.[22] In contrast to DMPC, K_C for POPS (not shown) does not exhibit a maximum, but slowly increases as T approaches T_M; gradual stiffening with reducing temperature is the ordinary behavior that one would expect *a priori* for conventional materials.

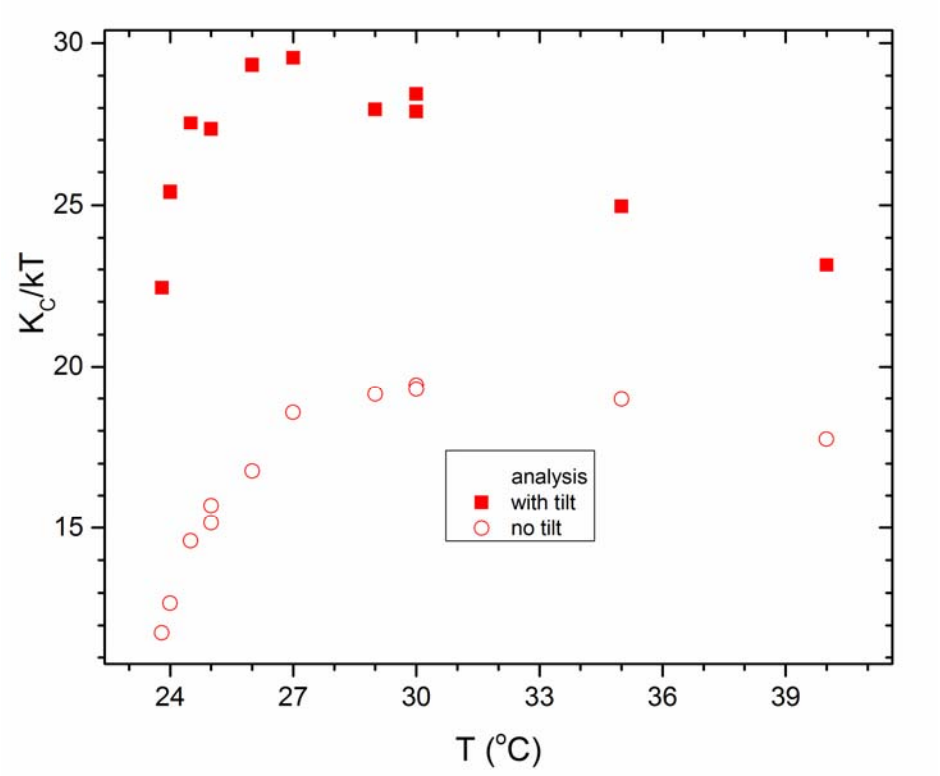

Figure 3: Bending modulus K_C versus temperature for DMPC bilayers with and without tilt included in the analysis of the diffuse X-ray scattering data.[13]

The older tilt-independent analysis essentially assumed that the tilt modulus was infinite. Allowing a finite tilt modulus in the analysis increases the obtained values of K_C as is shown in Fig. 3. This is roughly analogous to having two springs in series: decreasing the tilt spring constant requires an increase in the bending spring constant in order to retain the same overall stiffness. However, this analogy is not perfect because, compared to K_C, smaller K_θ softens preferentially at smaller length scales; this is fortunate because otherwise, separate values for the two moduli could not be extracted from the experimental data. The increase in the value of K_C when tilt is included is greater for DMPC than for the DOPC lipid because K_θ is smaller for DMPC than for DOPC.[13] Values obtained from the new tilt-dependent analysis for ten types of lipids

measured at 30 °C are reported elsewhere where they are compared to values obtained from other methods and from simulations.[14] The new analysis reduces the previous differences[24] between the X-ray values of K_C and the generally larger values obtained by classical experimental methods that examine the shapes of giant unilamellar vesicles on large length scales where the Helfrich-Canham model should be accurate.[25] In contrast, neutron spin-echo (NSE), like the x-ray method, measures at smaller length scales than the classical methods. NSE values of K_C/kT for DMPC range from 15[26, 27] to about 20[28-30]. These agree better with the no tilt values in Fig. 3 than with the new tilt dependent values. A similar comparison also holds for the POPC lipid.[28, 31] This is not surprising as all the NSE analyses use the original Helfrich-Canham model; it would be interesting to have those analyses repeated using the tilt dependent model.

Let us return to the main theme of this paper, namely, the hypothesis that tilt is an order parameter whose modulus decreases dramatically as the main transition is approached for lipids that have spontaneous breaking of tilt symmetry below the main transition. Fig. 4 shows the values of the tilt modulus for nine PC lipids versus the temperature difference ΔT between the temperature T of measurement and the main transition temperature T_M of that lipid. The systematic trend fully supports the hypothesis.

It is well recognized that the main phase transition in single-component lipid bilayers like DMPC is a first order transition. However, it is well recognized in the field of critical phenomena that first order transitions may occur in the vicinity of a critical point in which case critical phenomena are still evident. For example, in fluids, there could be a physical constraint on the pressure that makes the temperature trajectory pass close the critical point while intersecting the line of first order transitions that lead to the critical point but along a different thermodynamic trajectory. Then, instead of the modulus coupled to the order parameter going to zero, the modulus becomes small but remains non-zero at the ultimate first order transition temperature. A more specific explanation is that it has been widely recognized for a long time that the main transition in lipid bilayers is primarily driven by a cooperative collapse of the disordered hydrocarbon lipid chains into the all-trans aligned state, as evidenced by the large transition enthalpy.[32] This chain freezing transition could occur at a higher T than the temperature of vanishing K_θ. It may

be noted that a toy model of that chain melting transition indicates an unusual sort of order parameter and critical point.[33, 34] That toy model (which does not include tilt) also shows how other parameters can cause the system to avoid its critical point under the usual experimental conditions and undergo a first order transition instead.[35]

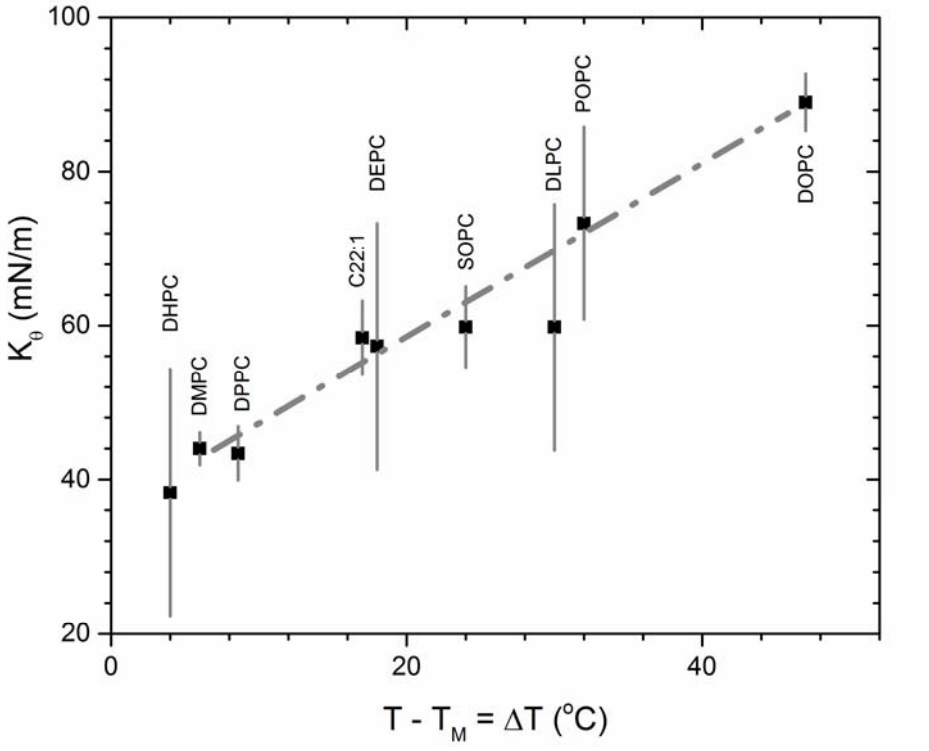

Figure 4. Tilt modulus K_θ versus temperature difference ΔT between the experimental temperature and the main chain melting transition temperature T_M of each of 9 lipids with known T_M and with the same large PC headgroup that induces spontaneous tilt in gel phases.[14]

There was some previous experimental evidence that the main transition in DMPC, while definitely first order, was in the vicinity of a critical point. The thermal coefficient of volume expansion $(\partial V/\partial T)_P$ increased as T approached T_M from above,[36] but volume is rather weakly coupled to the driving forces for the transition, so the data were not as dramatic as they are for K_θ in Fig. 1. Nevertheless, it would be surprising that the criticality signal for DMPC evidenced in Fig. 1 is so strong if the main driver of

the transition is only chain melting. Of course, the result in this paper reveals that chain tilting is also intimately involved in the main transition in DMPC.

This brings one to theoretical modeling. The above discussion suggests that a model should definitely involve chain melting, with its area dependent order parameter, for both DMPC and POPS. For DMPC it should now also involve chain tilting, which would have an XY/planar order parameter with "spin" dimension two and spatial dimension two in the parlance of critical phenomenology.[37] But there is even more. While the low temperature gel phase has spontaneous tilt and frozen chains, DMPC only enters this phase 10 degrees below the main transition. The intervening phase between the fluid phase and the gel phase is the unusual ripple phase in which the membrane has a well-defined out-of-plane asymmetric ripple; that defines yet a third order parameter that becomes non-zero below the main transition and then vanishes again 10° lower in the gel phase. Interestingly, lipids like POPS that do not have tilt in their low temperature phase also do not have the intervening ripple phase, which suggests that there is coupling between tilting and rippling. What is needed for PC lipids like DMPC is a grand unified theory that accommodates this rich and varied phase behavior of and that includes the newly discovered softening of the tilt modulus reported in this paper as well as the recently discovered staggered monolayer structure of the DMPC ripple phase.[18] Perhaps previously proposed Landau-Ginzburg continuum models[38-41] may lead the way.

Comparing the values of simulated tilt moduli with experiment provides another test to validate simulations. On the other hand, experimental evaluation of tilt moduli is relatively difficult, with only one known method at present, so validated simulations are likely to be more efficient for generating data for many lipid bilayers under varying conditions, such as the temperature dependence near the main phase transition of DMPC bilayers focused on in this report. In conclusion, the inclusion of tilt in the analysis of both simulations and experiment is providing new insight into the mechanical and physical behavior of lipid bilayers.

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