Finite-size effects in biomimetic smectic films

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(Received 8 March 2004; published 20 December 2004)

Thin stacks of lipid multibilayers supported on rigid silicon and mica substrates are found to exhibit finite-size effects. Using neutron diffraction we find that the repeat spacing (d) of stacks containing up to a few tens of bilayers depends on their thickness (D), with d increasing with decreasing D. Differences in d are larger in the low-temperature $L_{\beta'}$ phase consisting of rigid bilayers than in the high-temperature L_α phase where the bilayers are more flexible. Various scenarios that may be responsible for this counterintuitive observation are discussed.

Thin films supported on solid substrates are ubiquitous in scientific and technological applications. The interfaces of such films are known to induce novel types of behavior in these systems, unlike those seen in the bulk. It has been possible in recent years to probe directly the structure of the interfacial region, and the atoms in a semiconductor passivation layer were found to be "locked-in" to those of the substrate [1]. Epitaxial strain, due to the mismatch between the lattice parameters in the thin film and the substrate, has been shown to substantially increase the critical temperature of superconducting thin films [2]. The interfaces are also known to influence the glass transition temperature (T_g) in thin polymer films, and the distribution of T_g as a function of the depth from the surface has recently been determined [3]. In comparison, smectic films consisting of a stack of fluid layers aligned parallel to the interfaces seem, at first, much simpler systems. However, interesting surface ordering phenomena, such as layer-by-layer freezing and the induction of new phases, have been observed in them [4–6], and have been attributed to the suppression of thermal fluctuations of the smectic layers by the interfaces [7].

Lipids such as 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) are amphiphilic molecules that self-assemble in water to form bilayers. Above the chain melting transition temperature (T_m), these bilayers have liquidlike in-plane order, and hence are rather flexible. Below T_m, they develop a higher degree of in-plane positional order [8,9] and become very rigid. Stacks of such bilayers form the L_α and $L_{\beta'}$ (gel) phases, respectively, above and below T_m, with quasi-long-range positional order in the direction parallel to the bilayer normal [10]. Thermal undulations of the bilayers result in an effective interbilayer repulsion, which accounts for the very large repeat spacing d found in the L_α phase of surfactant systems with very flexible bilayers [11,12].

The influence of the interfaces on fluid lipid multibilayer stacks has over the years attracted much attention [13–18]. A rigid interface has been shown to quench thermal undulations in nearby bilayers and reduce the interbilayer repulsion, leading to lower values of d [14]. However, a recent theoretical study finds that the influence of the substrate is confined to just a few bilayers close to it [18], in agreement with earlier experimental results [16,17]. Interestingly, this study also suggests the possibility of having a larger d in the interfacial region when the surface tension is sufficiently small. However, we would expect these effects to be negligible in the $L_{\beta'}$ phase, due to the enhanced bending rigidity of the bilayers.

We have carried out neutron diffraction experiments on DMPC multilayer stacks of different thicknesses, deposited on silicon and mica substrates. The experiments were done at 84% relative humidity (RH), in order that the L_α phase transfoms directly into the $L_{\beta'}$ phase below T_m, and does not go through the ripple phase normally observed at higher hydrations [8]. The temperature dependence of d was determined from the diffraction data for stacks of different thicknesses. Surprisingly, at a given temperature, d is found to be larger in thinner films, with the differences being more pronounced in the $L_{\beta'}$ phase.

DMPC was purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. It was dissolved in chloroform, and an appropriate amount of the solution was sprayed evenly onto a substrate of dimensions $6.0 \times 2.5 \times 0.1$ cm^3 using an artist's airbrush. After evaporation in air, residual solvent was removed by placing the sample in vacuum for 2 h. The average number of bilayers (N) in the film was estimated from the amount of DMPC deposited and the area of the substrate, assuming an area per lipid of 60 Å^2 [10,19,20]. The values of N for the samples studied are approximately 8, 18, 2400, and 8500.

Samples were taken in an air-tight aluminum cannister, shown in Fig. 1, for the diffraction experiments. The relative humidity inside the chamber was maintained at ~84% by placing a saturated KCl/D$_2$O solution [21], while the sample temperature was controlled to an accuracy of ±0.2 °C using a circulating water bath. Equilibrium was ascertained by two or more successive scans exhibiting the same d. Thin samples took only a few minutes to equilibrate, whereas very thick ones took several hours. Data were taken on the N5 triple-axis spectrometer located at the NRU reactor (Chalk River Laboratories, Canada) using neutrons of wavelength 2.37 Å, obtained from the (002) reflection of a pyrolytic-graphite monochromator. The Bragg peak positions were determined by fitting a Gaussian and an exponential back-
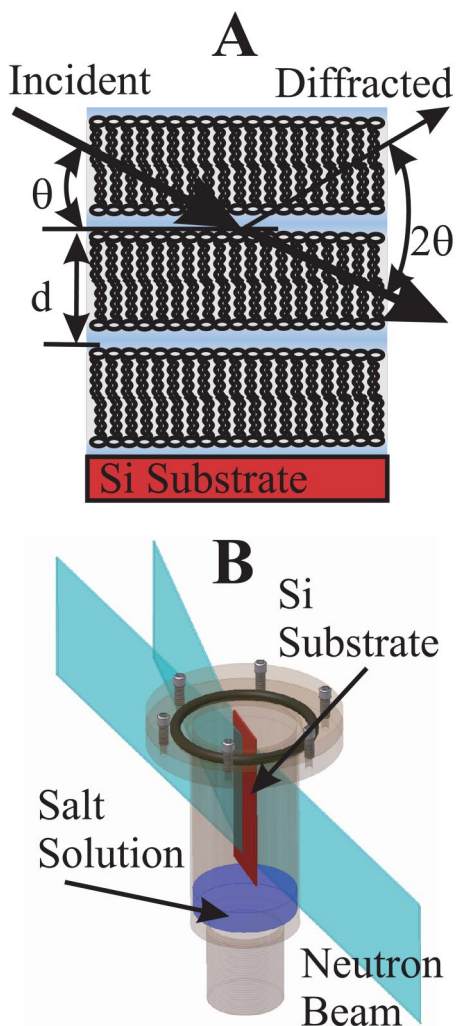
FIG. 1. Sample cell used for neutron diffraction. The sample assembly consists of a substrate made out of a single crystal of silicon, or mica, on which the sample is aligned, and an aluminum block that holds the sample in the appropriate geometry. Temperature gradients are minimized by isolating the sample from the outside environment using a thermal jacket and a massive aluminum block.
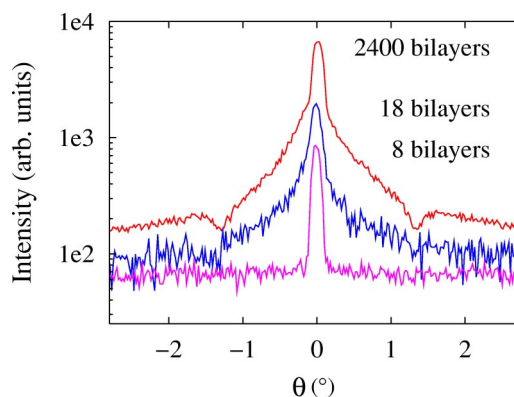

FIG. 2. Rocking curves of the first-order Bragg reflection for the samples on silicon substrates with $N \sim 8$ (a), 18 (b), and 2400 (c). The small dips in intensity at around $\pm 1.25°$ in (c) are due to absorption of the incident and diffracted beams by the sample. The width of the rocking curve is inversely proportional to the degree of orientation of the bilayers parallel to the substrate.

ground over the entire range of the scattering vector (q) accessed by the experiment, resulting in an accuracy in d of about ± 0.2 Å. Alignment of the bilayers on the substrate was checked by measuring the rocking curves of the different samples (Fig. 2). The sharp peaks in the figure, and the absence of any other features, indicate the very high degree of orientation, especially in the case of thin samples, of the bilayers with respect to the substrate.

Independent estimates of the average thickness of all thin films were obtained from the widths of the diffraction peaks. Reflections from very thick samples were found to be resolution-limited, and hence were assumed to represent the instrumental resolution. Taking the diffraction peaks to be Gaussians, the number of bilayers (N) of very thin samples is estimated to be in the range 15–20. For the thicker samples, we get N in the range 25–35. The corresponding values obtained from the amount of lipid deposited on the substrate

are 8 and 18, respectively. Hence we can take the lipid to be spread reasonably uniformly on the substrate.

The temperature dependence of d as a function of the number of lipid bilayers (N) supported on silicon substrates, is shown in Fig. 3. All samples exhibit an abrupt increase in d at around 29 °C, which can be identified as the chain melting $(L_{\beta'} \rightarrow L_{\alpha})$ transition of the lipid [8]. The L_{α} and $L_{\beta'}$ phases coexist over ~ 1 °C for the $N \sim 2400$ and 8500 samples, probably due to the presence of temperature/humidity gradients in the cell. In contrast, the transition in thinner samples is gradual, as would be expected from the rounding of the first-order transition in finite-sized systems [22]. The surprising result, however, is the dependence of d on the thickness of very thin samples. The spacings of the two thicker samples with $N \sim 2400$ and 8500 are, within experimental error, identical over the entire range of temperatures studied. On the other hand, the $N \sim 20$ sample has a slightly larger spacing than the two thicker ones in both the L_{α} and $L_{\beta'}$ phases. These differences in d are much more
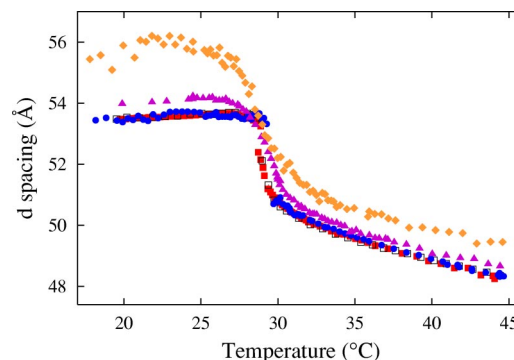

FIG. 3. Temperature dependence at 84% RH of the d-spacing of DMPC multilayers with $N \sim 8500$ (●), 2400 (heating: ■, cooling: □), 18 (▲), and 8 (◆), supported on silicon. The abrupt change at ~ 29 °C is due to the $L_{\alpha} \rightarrow L_{\beta'}$ transition, which is shifted to higher temperatures at lower RH [8]. Note the increase in d with decreasing N, which is more prominent in $L_{\beta'}$ phase.
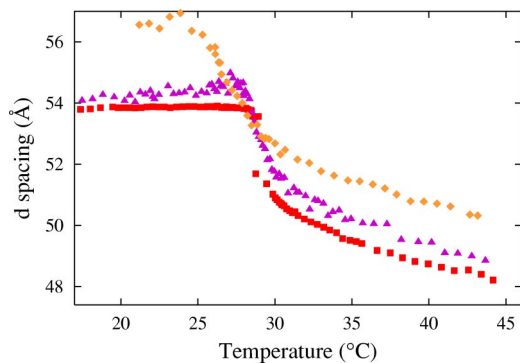
FIG. 4. Temperature dependence at 84% RH of the d-spacing of DMPC multilayers with N$\sim$2400 (■), 18 (▲), and 8 (♦), supported on mica. The thickest sample has the same spacing as the thick samples on silicon. However, the two thinner samples have slightly larger spacings than the corresponding samples on silicon. Presently, we do not know if the differences between the silicon and mica supported bilayers are genuine or due to small differences in the sample thickness.

pronounced in the thinner sample with N$\sim$8. Thus the data presented in Fig. 3 clearly show a systematic increase of the spacing with decreasing N, in both the $L_{\beta'}$ and L_α phases. Of note is that the magnitude of the increase in d in the $L_{\beta'}$ phase is almost twice that in the L_α. Experiments carried out on mica supported multibilayers gave rise to results similar to those observed from silicon supported stacks (Fig. 4). As far as we are aware, this increase in d with decreasing N has not been previously observed in substrate supported bilayer stacks, and points to the existence of finite-size effects in these and possibly other related systems.

Although there is some degree of uncertainty in the thickness of the different films, due to the various approximations involved in the estimates, the above discussion shows that our ranking of the samples based on their thickness is indisputable. Therefore, the increasing trend of d with decreasing N is unambiguous even though we cannot deduce the precise functional dependence of d on N from the present data. The observed increase in d can arise from an increase in the bilayer thickness d_B, from the thickening of the water layer separating adjacent bilayers d_W, or from a combination of both. Below we discuss mechanisms that can lead to an increase in either of these parameters in the present context [23].

The observed behavior can be accounted for if a sufficiently strong repulsive van der Waals interaction exists between the lipid/substrate and lipid/water vapor interfaces of the stack, leading to a larger d_W by incorporating more water from the atmosphere. The resulting disjoining pressure is given by $P=A/(6\pi D^3)$, where D is the total multibilayer stack thickness and A is the Hamakar constant [24]. A can be estimated from the dielectric properties of the three media involved, namely substrate, lipid stack, and vapor phase. For the silicon–lipid–water vapor system, A is $\sim-1.6\times10^{-19}$ J [25], whereas it is $\sim-1.0\times10^{-20}$ J for the mica substrate. The negative values of A are indicative of the repulsive nature of the interaction and arise from the fact that the refractive index of the multibilayer stack, composed of lipid and

water, is intermediate between those of the substrate and the water vapor. Since we do not observe any difference in the behavior of films supported on the two different substrates (i.e., silicon and mica), in spite of large differences in the corresponding values of A, van der Waals interactions cannot be responsible for the observed behavior. This conclusion is further supported by the fact that even for the thinnest stack with $D\sim1000$ Å, the repulsive van der Waals pressure is many orders of magnitude smaller than the effective osmotic pressure at 84% RH [26], whereas a much larger decrease in the osmotic pressure is required to produce the observed change in d.

Another mechanism that might be invoked is surface ordering similar to that seen in some thermotropic smectic films [4–7]. This can result in the formation of a few $L_{\beta'}$ bilayers near the interface even at temperatures much above T_m. In thin samples, the coexistence of $L_{\beta'}$ and L_α phases can give rise to an effective d-spacing intermediate to those of the two pure phases. In thick stacks, the contribution from the interfacial regions will be negligible, and the d measured should correspond to that in the bulk. Hence, this mechanism can account for a larger spacing of very thin films near the chain melting transition in the L_α phase, but cannot explain (a) the larger d of thin stacks in the L_α phase even at $\sim$15 °C above T_m, and (b) the fact that the differences in d between the thin and thick bilayer stacks are larger in the $L_{\beta'}$ phase.

Gao and Golubović [18] have recently shown that the interfacial region in a lipid film could have, on an average, either a smaller or a larger d_W compared to the bulk, depending on whether the reduced surface tension ($\bar{\gamma}=\gamma/\sqrt{\kappa B}$) is larger or smaller than a critical value $\bar{\gamma}^*(\approx0.6)$. Here κ and B are the bilayer bending rigidity and the compression modulus of the stack, respectively, and $\gamma_s=\sqrt{\kappa B}$ is the characteristic smectic surface tension scale [27]. The higher spacing results when the bilayers at the interface are more vulnerable to thermal undulations than those in the bulk. While such a situation cannot be ruled out in the L_α phase, it is very unlikely that this effect will be dominant in the $L_{\beta'}$ phase, where the bilayers are very rigid. Therefore, the larger differences in d observed in the $L_{\beta'}$ phase cannot easily be reconciled with the theory of Gao and Golubović [18].

The observed dependence of d on D is also consistent with an increase in the average bilayer thickness d_B in either or both interfacial regions. In the $L_{\beta'}$ phase, it might arise from a lowering of the molecular tilt, whereas in the L_α phase, a decrease in the degree of chain disorder can be envisaged. It is, however, difficult to understand how the influence of the substrate would propagate beyond the bilayer(s) at the interface(s) in the presence of aqueous layers separating them. However, at the low humidities used in this study, it is possible that the water molecules in these thin layers are highly ordered [28] and hence help in establishing long-range *trans*-bilayer correlations of the molecular conformation. In principle, high-resolution x-ray or neutron reflectometry experiments on samples of uniform thickness may be used to verify whether or not a thickening of the bilayers is contributing to the observed dependence of d on D. However, in practice, the small changes involved and the fact that d would most likely vary across the interfacial re-

gion would make a definitive conclusion difficult.

In conclusion, neutron-diffraction experiments on lipid multilayers aligned on solid substrates show the existence of finite-size effects, which lead to an increase in the repeat spacing of very thin stacks. Comparable results are obtained from samples supported on both silicon and mica substrates, indicating that these effects are essentially insensitive to the type of substrate used. The mechanism responsible for the observed behavior is presently not clear. We hope that these counterintuitive and unexpected observations will stimulate further theoretical as well as experimental work on the structure of very thin lipid films.

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