Measurements of Modulated Lamellar $P_{\beta'}$ Phases of Interacting Lipid Membranes

Daniel C. Wack[a] and Watt W. Webb

School of Applied and Engineering Physics, Cornell University, Ithaca, New York 14853-2501

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A diffraction study of lattice constants in the $P_{\beta'}$ ("rippled") phase of hydrated lecithins suggests that the lamellar modulation arises from a delicate interplay between the energies associated with membrane curvature and with hydration, as modified by interactions between membranes. Our results imply the existence of a Lifshitz point in the global phase diagram of the lecithin-water system.

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We report here the results of a systematic high-resolution x-ray diffraction study of the modulated ("rippled," or $P_{\beta'}$) membrane phase of hydrated multilamellar diacylphosphatidylcholines (lecithin).[1] Our data for the dependence of the mean membrane thickness $\bar{d}_l$ and ripple wave vector Q_r on the hydrocarbon chain length N_c and the degree of hydration combine with existing thermodynamic data to yield a simple physical mechanism for modulated-membrane-phase stability. The data support a phenomenological model of structural phase transitions in interacting membranes recently proposed by Goldstein and Leibler.[2] Hydration interactions, which are supposed to dominate only at small intermembrane spacings,[3,4] play a central role throughout the $P_{\beta'}$ phase. The systematic trends imply the existence of a Lifshitz multicritical point[5] within the global lecithin phase diagram, which appears to be accessible by variation of N_c and water composition.

Figure 1 displays the schematic phase boundaries of lecithin in the vicinity of the $P_{\beta'}$ phase. At full hydration the $P_{\beta'}$ phase appears intermediate to the high-temperature, fluid phase L_α and the low-temperature, solidlike phase $L_{\beta'}$. Latent heats ΔH_m at the upper or "main" transition T_m are proportional to chain length N_c, while those at the lower or "pretransition" T_p are independent of N_c.[6,7] This trend suggests that the pretransition is dominated by membrane surface energetics. As the water content is decreased, the phase terminates at a third triple point T_3, which is found at water volume fraction $\phi_w \simeq 0.18$.[8] Previous x-ray diffraction studies[1,9] of the $P_{\beta'}$ phase have indicated that membrane stacking and rippling periodicities show the symmetry of a 2D monoclinic lattice, with constants insensitive to temperature but strongly dependent on ϕ_ω. In this study we measured diffraction patterns of structures found along isothermal paths through the single-phase region, as indicated by the dashed line in Fig. 1. We varied hydrocarbon chain lengths over the range $N_c = 12$ to 19, to obtain a set of structural measurements in states of comparable hydration interaction.

Powder diffraction samples were prepared by our mixing weighed amounts of distilled water and synthetic lecithin and inserting them in a 1.0-mm quartz x-ray capillary, which was then sealed. Measured weight fractions of water c_w and lipid c_l were converted to volume fraction of water by $\phi_w = c_w v_w/(c_w v_w + c_l v_l)$, where we assume that the partial specific volume of water v_w is equivalent to the bulk value, and we take the partial specific volume of lipid v_l for each N_c in the $P_{\beta'}$ phase from the measurements of Nagle and Wilkinson.[6] The capillaries were equilibrated in an evacuated cell whose temperature was controlled to $\pm 0.1\,^\circ$C by thermoelectric elements. High-resolution synchrotron x-ray diffraction experiments were performed on the A3 line at the Cornell High Energy Synchrotron Source with a horizontally focused 8.3-keV beam. With film recording, the width of the experimental resolution function was $\simeq 7\times10^{-4}$ Å^{-1}, and with a linear position-sensitive detector, it was $\simeq 2\times10^{-3}$ Å^{-1}. Lattice constants were

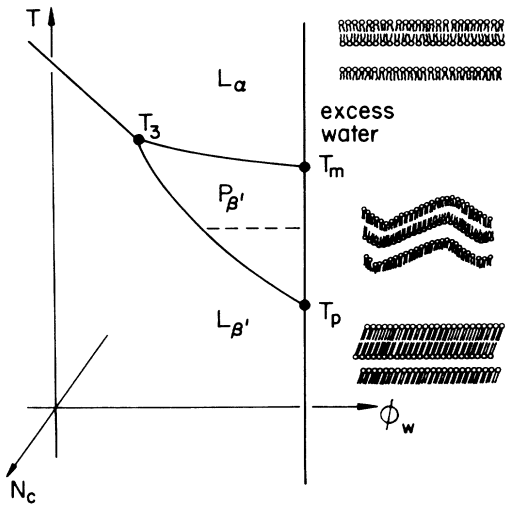

FIG. 1. Schematic lecithin-water phase diagram near full hydration. The triple point T_3 ($P_{\beta'} \leftrightarrow L_\alpha + L_{\beta'}$) occurs at $\phi_w \simeq 0.18 \pm 0.01$. Dashed line represents the isothermal paths explored in this study, midway between the triple points T_m and T_p. For hydrocarbon chain lengths $N_c = 12$ to 19, these temperatures were −7.0, 7.0, 18.0, 28.0, 39.0, 45.5, 53.0, and 59.1 °C, respectively. Drawings at right suggest the physical state of the phospholipid bilayer in each fully hydrated phase.

derived by a nonlinear least-squares fit of a 2D monoclinic formula to the observed positions. A full description of the experimental details and additional results will be published elsewhere.[10]

All $P_{\beta'}$ samples showed a "low-angle" ($|Q| < 0.6$ Å^{-1}) powder pattern which could be indexed on a 2D monoclinic lattice with constants d_s, λ_r, and θ_m being the stacking distance, ripple wavelength, and monoclinic angle, respectively. For $\phi_w \lesssim 0.30$, powder samples showed 20 to 25 resolution-limited peaks, but at higher water contents increased disorder both weakened higher-order peaks and smeared closely spaced one. Lattice-constant data are shown only for those samples having at least six resolved and unambiguously indexed peaks. When the position of the ripple wave vector $Q_r(l) \equiv 2\pi l/\lambda_r$ did not overlap other peaks, harmonics $l = 2$ to 4 were visible, and showed structure factors greater in magnitude than the fundamental. The structural implications of this feature will be discussed elsewhere.[10] For all N_c, θ_m was $\simeq 98°$ for $\phi_w \lesssim 0.25$ but decreased towards 90° as ϕ_w increased. To represent the total repeat spacing normal to the *average* layer position, we form the quantity $d_l = d_s \sin\theta_m$. By use of the calculated volume fraction ϕ_w, this total spacing can be decomposed into mean lipid and mean water layer thicknesses, $\bar{d}_l$ and $\bar{d}_w$. Finally, using the volume per lipid molecule V_l, we can obtain the mean projected area per head group at the aqueous interface: $\bar{A}_l = 2V_l/\bar{d}_l$. We found that $\bar{d}_w$ increased linearly with ϕ_w for all N_c up to the highest water contents explored ($\phi_w \simeq 0.38$). We present the structural analysis as a function of the water thickness $\bar{d}_w$ to compare states of equal hydration interaction strength.

Figure 2 summarizes our measurements of the structural properties of modulated lecithin membranes. The first two panels show that, for all N_c, the mean membrane thickness $\bar{d}_l$ *increases* as the separation decreases, with the area per head group $\bar{A}_l$ following inversely. At a fixed membrane separation, while $\bar{A}_l$ is independent of N_c, $\bar{d}_l$ increases with N_c at the rate of 0.20 nm per CH_2 pair. The cross section normal to the chain axis of a pair of hydrocarbon chains packed in a hexagonal array is $\simeq 0.40$ nm^2, and the increment along the chain axis per CH_2 pair is $\simeq 0.25$ nm. The regular increase of $\bar{d}_l$ at fixed $\bar{A}_l$ implies that chain conformation and molecular tilt are constant. The values of $\bar{d}_l$ and $\bar{A}_l$ imply extended chains tilted at $\simeq 37°$ with respect to the mean membrane normal.

Figure 2(c) displays the modulation wave vector $Q_r \equiv 2\pi/\lambda_r$. We find that near full hydration, Q_r decreases slowly with $\bar{d}_w$, but it also scales inversely with N_c, and thus inversely with the membrane thickness $\bar{d}_l$. To identify the effects of membrane hydration, we eliminate the variations with $\bar{d}_l$ be defining a reduced modulation wave vector $\tilde{Q}_r \equiv 2\pi\bar{d}_l/\lambda_r$. The values of $\tilde{Q}_r$ for each N_c plotted in Fig. 2(d) with use of the data of Figs. 2(a) and 2(c) can almost be reduced to a single curve, within

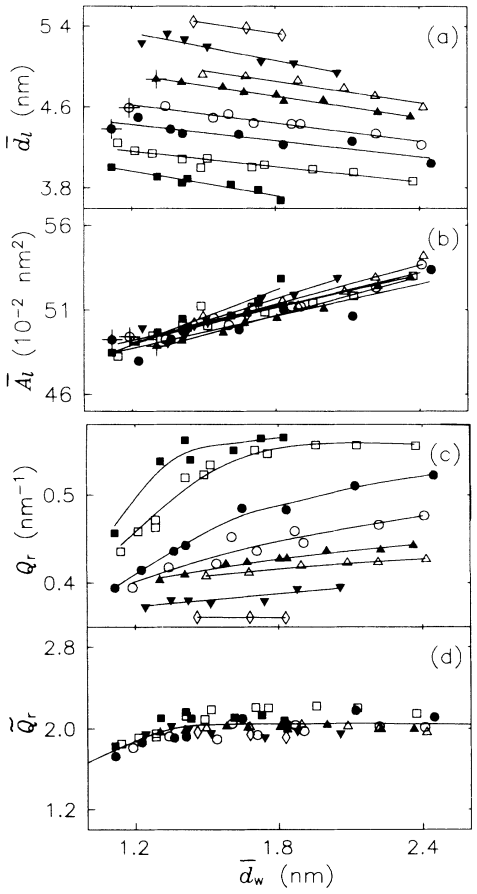

FIG. 2. $P_{\beta'}$-phase structural data for $N_c = 12$ to 19, shown sequentially from the lowest to highest curve in (a), plotted as a function of water thickness $\bar{d}_w$ at fixed temperature. (a) Average bilayer thickness $\bar{d}_l$; linear fit superimposed. (b) Average projected area per lipid molecule $\bar{A}_l$. (c) Modulation wavelength λ_r ($\equiv 2\pi/Q_r$), fitted by smoothing splines. (d) Reduced modulation wave vector $\tilde{Q}_r$, with smoothing curve.

the overall accuracy. The limiting values of $\tilde{Q}_r \equiv \tilde{Q}_0$ at large $\bar{d}_w$ presumably represent the effects of hydration of individual membranes, while the slopes at small $\bar{d}_w$ reveal intermembrane hydration interactions.

By combining our measurements of $\bar{d}_l$ with previous measurements of d_l in the L_α phase,[4,8,11] we can estimate the fractional change $\Delta\psi_m$ in membrane thickness at T_m near full hydration; i.e., $\Delta\psi_m \equiv [\bar{d}_l(P_{\beta'}) - d_l(L_\alpha)]/d_l(L_\alpha)$. Figure 3 presents $\Delta\psi_m$ as well as the latent heats ΔH_m at T_m. As a function of N_c, both properties extrapolate to zero at roughly $N_c^* = 9$–10, similar to an approach to a critical point. At the simplest level, this can be attributed to the pinning of chain ends to head groups, which prevents a portion $\sim N_c^*$ of the hydrocarbon chain from participating fully in the bulk hydrocarbon-chain melting transition.[12] Thus, by variation of chain length, the melting transition in membranes can be tuned between the quasi-2D regime $N_c \lesssim N_c^*$, in which melting is dom-

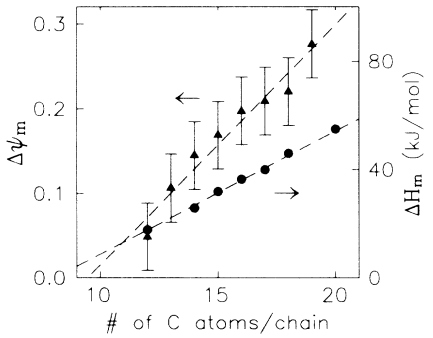

FIG. 3. Fractional change in membrane thickness $\Delta\psi_m$ and latent heats ΔH_m at T_m. Data for $d_l(L_\alpha)$ from Ref. 11, and for ΔH_m from Ref. 7.

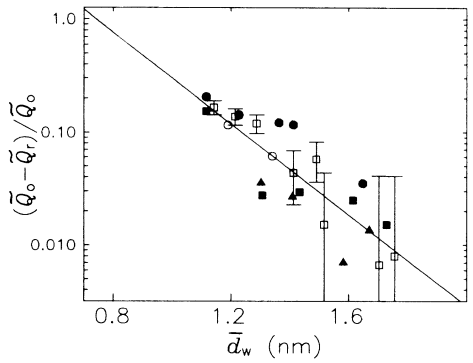

FIG. 4. Fractional change, for $N_c \leq 16$, of reduced modulation wave vector, $(\tilde{Q}_0-\tilde{Q}_r)/\tilde{Q}_0$, relative to its value at full hydration $\tilde{Q}_0$; solid line is an exponential fit, with slope $\lambda_h = 0.23 \pm 0.03$ nm. Error bars are shown on particularly complete data for $N_c = 13$, and are dominated by the uncertainty in $\tilde{Q}_0 (\simeq 0.1$ nm).

inated by translational disordering, and the 3D limit $N_c \gtrsim N_c^*$, dominated by chain conformational disorder.

Goldstein and Leibler have recently proposed a phenomenological model for the lamellar phases of interacting lipid membranes.[2] It is a continuum theory based on a scalar order parameter ψ proportional to the membrane thickness: $\psi \equiv [d_l(T) - d_0]/d_0$, where d_0 is a reference thickness, taken as that of the fluid phase. The model combines a Landau theory of intramembrane melting transitions and a continuum model of molecular forces between membranes, which are dominated by hydration interactions. The Hamiltonian for an isolated membrane has the form

$$H_0[\psi] = \int d^2x \{ \tfrac{1}{2}\Sigma(\nabla\psi)^2 + \tfrac{1}{2}K(\nabla^2\psi)^2 + \tfrac{1}{2}a_2(T-T_0)\psi^2 + \tfrac{1}{3}a_3\psi^3 + \tfrac{1}{4}a_4\psi^4 \} .$$

T_0 is the critical temperature of a uniform system in the absence of a cubic term. For values of the phenomenological coefficients $a_3 \neq 0$ and Σ sufficiently negative, the model exhibits three phases: $L_\alpha[\psi(x) \equiv 0]$ and $L_{\beta'}$ $[\psi(x) = \text{const} > 0]$ which are planar phases, and at intermediate temperatures, a modulated phase $P_{\beta'}$ $[\psi = \psi(x)]$. The model predicts that at the $P_{\beta'} \leftrightarrow L_\alpha$ transition $Q_r \equiv (|\Sigma|/2K)^{1/2} > 0$, except when $\Sigma = a_2 = a_3 = 0$, which corresponds to a *Lifshitz point*. At this point the wavelength of the modulation diverges.[5]

The continuum model for molecular interactions between neutral membranes includes contributions of van der Waals attraction and the "hydration" repulsion. Measurements on L_α and $L_{\beta'}$ membranes[3,4] show that the hydration interaction $V_h(d_w)$ is roughly exponential in form: $V_h(d_w) = H \exp(-d_w/\lambda_h)$, where the decay length $\lambda_h \simeq 0.25$ nm, and H is constant of order 0.1–1.0 J/m^2. The geometrical relation $d_l/d_w = (1-\phi_w)/\phi_w$ displays the model's coupling between interactions, which are dependent on ϕ_w, and ψ, which is dependent on d_l. We will not show that this model is consistent with our structural analysis of the $P_{\beta'}$ phase.

Chain length as a scaling field.—The vanishing of both ΔH_m and $\Delta\psi_m$ at a characteristic value $N_c = N_c^*$ (Fig. 3) is consistent with the appearance of a critical point at $T_m(N_c^*)$ in the scaling field of the parameter N_c which represents the membrane thickness. Extrapolation of known values of $T_m(N_c)$ to N_c^* yields 255 K, which is roughly equal to the critical temperature $T_0 \simeq 260$ K indicated by our fitting the model to experimental values of T_m, T_p, and T_3 at fixed N_c. Thus we find that the "mobile" hydrocarbon chain length $N_c - N_c^*$ is an important scaling field in membrane phase behavior.

Coupling of interactions to thickness.—The increase in $\bar{d}_l$ with dehydration [Fig. 2(a)], which parallels behavior observed in L_α and $L_{\beta'}$ phases,[4] is consistent with the model's coupling of hydration interactions to the membrane thickness.[13] Although the order parameter does not distinguish between thickness changes due to tilt and chain conformation, tilt is implicated by our data for $\bar{A}_l$ [Fig. 2(b)].

Membrane curvature energy.—The term $K(\nabla^2\psi)^2$ behaves *like* membrane curvature energy. Continuum elastic theories for the dependence of membrane rigidity on thickness predict $K \propto (d_l)^y$, $2 \leq y \leq 3$,[14,15] in which case $Q_r = (|\Sigma|/2K)^{1/2} \propto 1/(\bar{d}_l)^{y/2}$. The scaling of Q_r with membrane thickness [Figs. 2(c) and 2(d)] strongly suggests that membrane curvature energy plays a fundamental role in the modulation energetics.

Coupling of Q_r with $\bar{d}_w$ through intermembrane interactions.—A generalized theory for hydration forces between *modulated* membranes shows that interactions effectively contribute a positive term to Σ: $\Sigma \rightarrow \Sigma_0 + H_1 \exp(-\bar{d}_w/\lambda_h)$.[2] Thus the fractional shift of the reduced modulation wave vector from its value $\tilde{Q}_0$ at full hydration, $(\tilde{Q}_0 - \tilde{Q}_r)/\tilde{Q}_0$, should vary as $\exp(-\bar{d}_w/\lambda_h)$. An exponential fit (Fig. 4) yields a decay length of 0.23 ± 0.03 nm, consistent with the values of λ_h measured in L_α and $L_{\beta'}$ phases of lecithin.[3,4] Extrapolation

to $\tilde{Q}_r = 0$, where Σ would also vanish, defines a membrane separation $\bar{d}_w^* \simeq 0.7 \pm 0.2$ nm, which is equal to the measured membrane separation at the triple point T_3, within the experimental uncertainty.[8]

Evidence for a Lifshitz point.—The implied accessibility of the state $\Sigma = 0$ within the global phase diagram (N_c, T, ϕ_w) for lecithin, together with the scaling of ΔH_m and $\Delta\psi_w$ shown in Fig. 3, suggests that a line of triple points $T_3(N_c, \phi_w)$ ends at a Lifshitz point at $N_c^* \simeq 9$ and $\phi_w \simeq 0.18$. The systematic variations in Q_r that we observe for $N_c > N_c^*$ can then be attributed to the *proximity* of the Lifshitz point. Structural measurements near the triple point T_3 as a function of N_c (possibly with mixtures of chain lengths to make N_c quasicontinuous) would test the proposal of this multicritical point.

A complete microscopic description of the structural modulation is a difficult problem, whether experimentally or theoretically undertaken, especially if structural defects are significant. The important conclusion of our work is that *many aspects of the global behavior of the $P_{\beta'}$ phase of lecithin can be understood without reference to the details of the modulated membrane structure.*[10] The systematic structural analysis presented here provides a basis for further microscopic theoretical approaches.

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[a]Present address: GE Co., French Rd., Utica, NY 13503.

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