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Universality in Interacting Membranes: The Effect of Cosurfactants on the Interfacial Rigidity

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We report on synchrotron x-ray scattering studies of multimembrane systems in the fluid L_α phase. By embedding cosurfactant molecules in a model biological membrane (di-myristoyl-phosphotidyl-choline) system, we dramatically reduce the bending rigidity of the fluid bilayer. This effect leads to a crossover from a microscopically driven to a *fluctuation-induced universal regime for intermembrane interactions*. Studies of a homologous cosurfactant series, with varying hydrocarbon chain length between 5 and 12, allow us to attribute this reduction in k_c to the *thinning* of the membrane.

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The understanding of the surface properties of both fluid and more ordered membranes has recently attracted much experimental and theoretical attention.[1-10] This focus of attention stems primarily from the inherent interest in elucidating the statistical physics of two-dimensional fluctuating surfaces. In some sense the physical properties of fluid membranes are unique because they have negligible surface tension. Consequently, their free energy is governed by their geometrical shape and its fluctuations. The rigidity k_c associated with the restoring force to layer bending is then the important modulus which in many cases will determine the physical state of the membrane. From a biophysical viewpoint, the physical nature of a membrane surface will have a profound influence on membrane-membrane interactions, which influence processes such as cell-cell contact.

In usual multilayer systems consisting of stacks of alternating membranes (composed of a single type of lipid) and water, the bending modulus has been measured[7] to be about $25k_BT$ in the fluid L_α phase. This implies that these rigid interfaces with $k_c \gg k_BT$ are flat and possess macroscopic persistence lengths[9] of order microns so that thermal fluctuations on significantly smaller length scales are not important.[8]

On the other hand, in the L_α phase of some ternary and quaternary systems[1,2,6] very large dilutions with intermembrane distances of ~ 1000 Å are possible. Recent synchrotron-based studies[1,2] have shown that the thermodynamic stability of these phases is due to an effectively long-range repulsive interaction (which we refer to as undulation forces) arising from the mutual hindrance of fluctuating membranes with a very small bending rigidity $k_c \approx k_BT$. Since this interaction[5] scales as k_c^{-1}, it completely overwhelms the attractive van der Waals (vdW) interaction and stabilizes the membranes at large separations.

The main difference between the L_α phases of these two systems lies in the nature of the bilayer film, which for the dilute systems usually contains cosurfactant in addition to surfactant molecules. Additionally, in many instances the dilute lamellar phases are found in the vicinity of the random microemulsion phase (with a similar interface) whose stability is thought[9] to be associated with a small $k_c \approx k_BT$. The cosurfactant thus appears to be the crucial ingredient; but, prior to this work, the precise mechanism for the reduction of the interfacial rigidity has remained elusive.

To elucidate the microscopic mechanisms that control the interfacial bending modulus, we embedded cosurfactant (pentanol) molecules in the biological multimembrane system of di-myristoyl-phosphotidyl-choline (DMPC) and water. The DMPC-water system consists of uncharged lipid molecules stacked in double layers separated by water, leading to the L_α phase which exhibits a large bending modulus $k_c \approx 25k_BT$.[3,5,7] This multilayered structure can only be diluted up to a limiting interlayer separation of 25 Å, beyond which the L_α phase coexists with pure water.[3,10] We find, upon addition of pentanol molecules, that once a ratio of about 2 cosurfactant to surfactant molecules is exceeded, we are able to dilute the mixed membrane system up to very large separations $d \gtrsim 200$ Å. This large dilution range is associated with a dramatic reduction in $k_c \approx k_BT$ which in turn leads to the onset of a large repulsive undulation interaction between layers,[5] completely overwhelming the vdW attraction.

To further demonstrate the significance of these results, we have measured k_c using synchrotron x-ray scattering, in a series of ternary systems of sodium dodecyl sulphate (SDS) and cosurfactant membranes in water, as a function of the cosurfactant chain length for a homologous series between pentanol (C_5OH) and dodecanol ($C_{12}OH$). We find that k_c is in fact a strong nonlinear function of the cosurfactant chain length and that the primary effect of replacing longer-chain surfactants by shorter-chain cosurfactants in a mixed membrane system is the *thinning of the membrane* which leads to a dramatic reduction in k_c.

The experiments were carried out at the National Synchrotron Light Source at Brookhaven on the Exxon beam line X-10A. The spectrometer configuration details were discussed previously.[1,2] We used a double-bounce Si(111) monochromator and a triple-bounce Si(111) analyzer crystal set at 8 keV in the nondispersive mode to obtain a longitudinal resolution of $dq_{\parallel}=0.00008$ Å^{-1} (HWHM). Tight slits set the out-of-plane resolution at $dq_{\perp}=0.001$ Å^{-1} (HWHM). The samples were sealed in standard 1.5-mm capillaries which resulted in randomly oriented lamellar domains.

As is well appreciated by now[1,2,11,12] three-dimensional structures whose densities are periodic in only one direction, such as the lyotropic L_α phase, are marginally stable to thermal fluctuations which destroy the long-range order and replace the δ-function bilayer stacking structure factor peaks at $(0,0,q_m=mq_0=m2\pi/d)$ (d denotes interlayer spacing and $m=1,2,\ldots$ is the harmonic number) by weaker algebraic singularities whose asymptotic form is described by power laws: $I(0,0,q_z)\sim|q_z-q_m|^{-2+\eta_m}$ and $I(q_\rho,0,q_m)\sim q_\rho^{-4+2\eta_m}$. q_ρ and q_z are components of the wave vector parallel and normal to the layers and

$$\eta_m=m^2q_0^2k_BT/[8\pi(BK)^{1/2}]\,, \qquad (1)$$

is the exponent which describes the algebraic decay of layer correlations, where B and K are the bulk moduli for layer compression (erg/cm^3) and layer curvature (erg/cm). K is related to the bending rigidity for a single bilayer: $K=k_c/d$. This power-law behavior has been recently confirmed in a series of quaternary and ternary lyotropic L_α phases.[1,2] While the asymptotic forms for $I(q)$ are simple, to quantitatively analyze the data we fit with the exact expression for the structure factor:

$$I(q)\sim\int dz\int d\rho\, S(z,\rho)e^{-R^2\pi/L^2}\times(\sin qR/qR)e^{-iq_mz}\,. \qquad (2)$$

Here,

$$S(z,\rho)\sim(1/\rho)^{2\eta_m}\exp\{-\eta_m[2\gamma+E_1(\rho^2/4\lambda z)]\}$$

is the layer-layer correlation function,[11] where $R^2=z^2+\rho^2$, γ is Euler's constant, $E_1(x)$ is the exponential integral function, and $\lambda=(K/B)^{1/2}$. In Eq. (2), the exponential term incorporates a finite-size effect because of the observed finite lamellar domain sizes typically between ~2000 and ~10000 Å (L^3 is the domain volume). The precise steps leading to Eq. (2) have been discussed previously.[1,2] The analysis consists of simultaneous fits of Eq. (2) (convoluted with the resolution function) to either 2 or 3 harmonics (depending on whether the third harmonic is observable), where the scaling of η_m $(=m^2\eta_1)$ is incorporated. The solid lines shown in Fig. 1 are the results of typical fits which give a satisfactory description of the scattering and allow us to obtain an accurate measurement of $\eta_1(d)$.

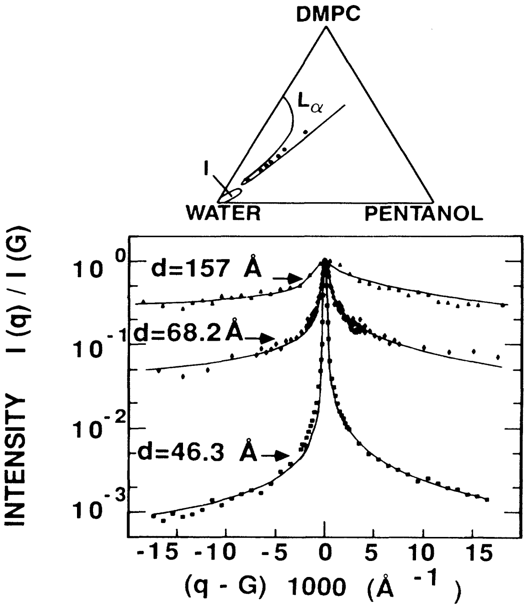

FIG. 1. Top: Schematic phase diagram of the ternary DMPC-pentanol-water system studied along the water dilution line (mixtures studied are indicated as dots). Bottom: Profile for the first harmonic $(G=q_0)$ plotted on semilog scale for three mixtures (with $w=0.38$, 0.58, and 0.80; w=water weight %) along the water dilution line for the DMPC-pentanol-water system. The solid lines are fits by Eq. (2) where all peak intensities are normalized to unity.

The binary DMPC-water system is a model biological membrane system which has been extensively studied.[3,5,7,10] To understand the effect of cosurfactant molecules on the phase behavior, we extended the binary system to the ternary DMPC-pentanol-water system. We show at the top of Fig. 1 the region of stability for the L_α phase at $T=25\,^\circ$C. Along the DMPC-water line, the L_α phase is stable up to a maximum dilution of 40% water (by weight) which corresponds to a maximum interlayer separation $(d-\delta)$ of 25 Å (δ denotes membrane thickness). This corresponds to the spacing for which the vdW attraction equals the hydration repulsion.[10] What is striking is that for a cosurfactant/surfactant ratio between two and four, the L_α phase is stable over a much larger water dilution range with the maximum dilution corresponding to a multimembrane system with more than 80% water by weight.

To elucidate the nature of the interlayer interactions in this dilute "almost unbound" region of stability for the L_α phase, we measured the power-law exponent η for seven samples ($w=0.38$, 0.54, 0.58, 0.64, 0.67, 0.71, and 0.80; w equals water weight %) along the water dilution line shown as dots at the top of Fig. 1. We show in Fig. 1, for three samples along the dilution, longitudinal scans through the first harmonic of $I(q)$ plotted on a logarithmic scale versus $q-q_0$, where the peak intensities are normalized to unity and the solid lines are the results of fits by Eq. (2). From the data it is qualitatively clear that the ratio of the tail to peak scattering which measures η is increasing quite dramatically as d increases. We compare this behavior to that observed for systems where undulation interactions dominate.[1,2] The undulation energy derived from the Helfrich theory is given by[5]

$$F_{\rm und/A} = 0.33[(k_BT)^2/k_c(d-\delta)^2]\,.$$

This leads to a simple prediction of a *universal function*[1,2] for η_1 which characterizes the algebraic decay of layer correlations:

$$\eta_1 = \tfrac{4}{3}(1-\delta/d)^2\,. \qquad (3)$$

We plot in Fig. 2(a) the results for η_1 vs $(1-\delta/d)^2$ for the DMPC-pentanol-water system as solid squares, together with our previously obtained data for the SDS-pentanol membranes diluted respectively with (i) dodecane[1] (open squares) and (ii) brine[2] (open circles). The theoretical prediction for η_1 is drawn as a solid line. *The universal behavior is now clear and is in remarkable agreement with the prediction of the Helfrich theory*[5] *for multimembranes where undulation forces dominate.* Thus, the embedding of cosurfactants in an initially rigid interface reduces k_c, leading to a strongly fluctuating regime where the intermembrane forces arise from the mutual repulsion of undulating membranes.

To elucidate the microscopic effect responsible for such a dramatic reduction of k_c, we studied a homologous series of cosurfactants with varying chain lengths from pentanol (C_5OH) to dodecanol ($C_{12}OH$). For the SDS-alcohol series which consists of negatively charged bilayer membranes separated by water layers, the electrostatic interactions are long range[13] and significantly larger than all other forces such as hydration, van der Waals, and undulations.[2,5,10] Consequently, the power-law exponent η is dominated by electrostatic forces and gives a direct measurement of k_c.[14] In this case the interlayer electrostatic free energy can be calculated exactly from the one-dimensional Poisson-Boltzmann equation and one readily derives η_1,[2,13]

$$\eta_1^{\rm elec}(d) = (k_BTL_e/2gk_c)^{0.5}[(1-\delta/d)^{1.5}/d^{0.5}]\,, \qquad (4)$$

where $g = [1-3(D/d_w)+6(D/d_w)^2+\cdots]$. Here, $d_w = d-\delta$, $L_e = \pi e^2/\epsilon k_BT \sim 22$ Å, and $D = a/L_e$ [a is the surface area per (charged) polar head: one negative charge per SDS molecule, the alcohols are neutral, and all surface charges are assumed to be dissociated].

To verify this distance dependence of $N_1^{\rm elec}(d)$ we measured $I(q)$ for the SDS-hexanol (C_6OH) charged membrane system as we separate the layers through a water dilution over the range of stability of the L_α phase. The results for η_1 are shown as open triangles in Fig. 2(b). The solid line is the prediction of Eq. (4), where the value for $k_c(=Kd)$ (which varies smoothly between $1k_BT$ and $2k_BT$) is obtained from the measured values of $\lambda[=(K/B)^{1/2}]$ and $\eta \sim 1/(KB)^{1/2}$.[14] This behavior was also verified recently[2] for the SDS-pentanol-water dilution system. We stress the large difference in behavior in $\eta_1(d)$ over the dilution range between the electrostatically stabilized dilute membranes [Fig. 2(b)] and those stabilized [Fig. 2(a)] by entropically induced undulation forces: While in the former case η_1 changes by less than 20%, it varies by about an order of magnitude in the latter systems. To our knowledge, previous work on dilute L_α phases in ternary surfactant-alcohol-water systems consist of charged surfactants where the dilution is achieved through repulsive electrostatic interactions.[15] The uncharged DMPC-pentanol-water system exhibits a dilute L_α phase because of repulsive undulation forces.

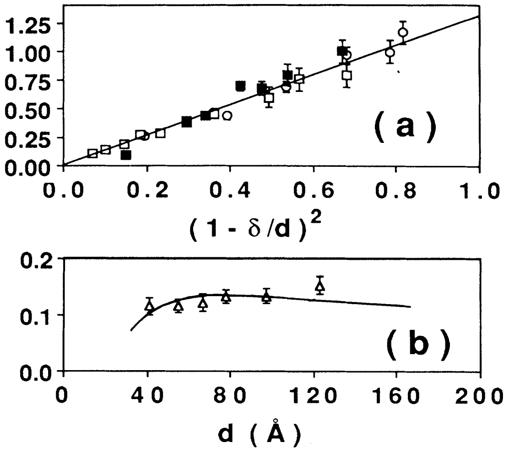

FIG. 2. (a) Power-law exponent η as a function of $(1-\delta/d)^2$ for three dilution systems. The solid squares are for the DMPC-pentanol-water system while the open circles and squares are for previously studied (Refs. 1 and 2) SDS-pentanol membranes along brine and oil dilution lines, respectively. The solid line is the prediction of the Helfrich theory [Eq. (3)]. (b) η as a function of the intermembrane distance d for the SDS-hexanol water dilution system. The solid line is the prediction of the Poisson-Boltzmann equation for long-range electrostatic forces [Eq. (4)].

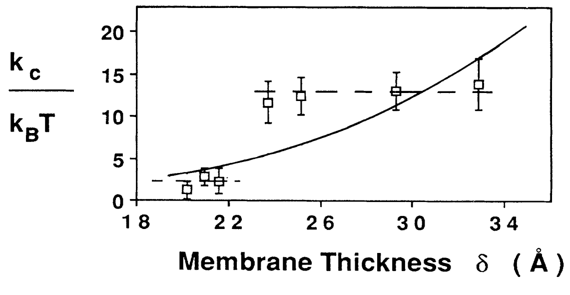

FIG. 3. k_c as a function of the membrane thickness for the SDS-cosurfactant-water mixtures for seven varying cosurfactant chain lengths. The solid line follows a δ^3 law which may be expected from elasticity theory (Ref. 16). The dashed line indicates discontinuous behavior as discussed in the text.

Thus, for the SDS-alcohol system, Eq. (4) accurately describes the power-law exponent measured by x rays. Alternatively, by measuring η and inverting Eq. (4) we obtain $k_c \sim 1/\eta^2$. We plot in Fig. 3 k_c as a function of the membrane thickness for the seven homologous samples studied. In each case, the cosurfactant/surfactant/water weight ratio was 0.21/0.15/0.64 and *the principal purpose of changing the cosurfactant tail length was to change the membrane (cosurfactant plus surfactant) thickness.* As we see, while k_c/k_BT is between 1 and 3 for the first three samples (C_5OH, C_6OH, C_7OH) the data show an abrupt increase around $\delta \approx 23$ Å towards a significantly larger value for the rigidity for the latter four samples (C_8OH, C_9OH, $C_{10}OH$, $C_{12}OH$).

Harmonic spring models and scaling arguments[16] lead to simple predictions of a power-law dependence of the bending rigidity on the thickness of the membrane: $k_c \sim \delta^p$ with p between 2 and 3. The solid line in Fig. 3 follows a δ^3 dependence which when extrapolated to $\delta \approx 35$ Å (corresponding to the thickness for the pure DMPC[3] multimembrane in water) gives a value of about $20k_BT$ consistent with typical values obtained by other workers.[7] We stress that while our data show the expected increase of k_c with δ, the behavior appears to be discontinuous (shown as a dashed line in Fig. 3) rather than power law.

Although we do not have an explanation for the apparent kink observed in the data, our experimental results give a strong indication of an unambiguous trend in the correlation of k_c with the thickness of the membrane. This supports our contention that the primary effect of replacing surfactants with shorter chain cosurfactants in a mixed system is the thinning of the interface which in turn leads to a reduction of the rigidity modulus k_c. In this case then repulsive undulation forces completely overwhelm the vdW attraction and one crosses over from the classical microscopic regime for "bound membranes" to the floppy "almost unbound" regime for fluctuating dilute membranes.

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