A microscopic model calculation of the phase diagram of ternary mixtures of cholesterol and saturated and unsaturated lipids

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We review a recent calculation in which a microscopic model was employed to describe a mixture of a saturated lipid, an unsaturated lipid, and cholesterol. The model was solved within the self-consistent field approximation. It is capable of producing several classes of phase diagram, but only one of them shows a liquid, liquid coexistence region. The lipids in the cholesterol-rich liquid are more ordered than those in the cholesterol-poor liquid. Within this model, coexistence of two liquids in the ternary system is intimately tied to such coexistence in the binary cholesterol, saturated lipid system.

1. INTRODUCTION

The importance of lipid rafts is clearly indicated by the very large number of experimental studies devoted to them. Remarkably, very few theoretical studies have addressed the subject. Underlying almost all of them is the assumption, which we share, that such rafts are a consequence of the equilibrium behavior of membranes composed of lipids and cholesterol. Of these studies, half are phenomenological in character (1, 2), as opposed to more microscopic treatments based upon molecular models and statistical mechanics (3, 4). The behavior of a membrane of lipids and cholesterol is sufficiently complex that it does not, as yet, lend itself to detailed computational simulation over time scales sufficient to investigate raft formation and structure (5).

We have tried (6) to explore a middle course between that of phenomenology, whose strength *and* weakness is the generality of its description, and detailed, computational, modeling whose strength *and* weakness is its specificity. We employ, below, a molecular model which concentrates on the hydrophobic interior of a bilayer membrane, one which describes lipid chains reasonably well, but which ignores almost completely the interaction of headgroups. The approach, therefore, highlights the importance of packing effects. Because the chains are described well, one can determine the degree of chain ordering in any particular phase. The model describes a ternary mixture of saturated and unsaturated lipids and cholesterol, and considers only *local* binary interactions between them. Furthermore, the model is solved within self-consistent field theory which ignores the correlations between entities.

Given these limitations we can explore the conditions under which two liquid phases can coexist, of which one would represent the "raft" domain, and the other the sea in which the raft floats. In a ternary mixture of cholesterol, saturated and unsaturated lipids we find the following. If one ignores the interactions between the cholesterol and the lipids, so that the cholesterol simply takes up space in the bilayer interior, then one finds only a saturated-lipid rich gel phase below the main chain transition of the saturated lipid. The gel phase coexists with a *single* liquid phase. There is no liquid-liquid coexistence. If one now turns on an interaction between cholesterol and lipids which causes the former to help order the latter, one finds that the gel phase becomes swollen with cholesterol, a reasonable result, but not one observed experimentally. If one now considers the interaction between cholesterols as well, one finds in addition to the gel phase *two* liquid phases, one which is cholesterol-rich and relatively well-ordered, the other cholesterol poor and poorly ordered. Furthermore, these two liquid phases extend all the way to the binary cholesterol, saturated lipid system, as in the experimental work of Vist and Davis (7). The phase diagram which we have calculated is that of a system of a saturated lipid with two tails of sixteen carbons, C16:0, an unsaturated lipid with two monounsaturated tails of eighteen carbons, C18:1, and cholesterol. It is shown in Fig. 1, and is quite similar

to those which has been reported experimentally for the system sphingomyelin, palmitoyloleoylsphatidylcholine (POPC), and cholesterol (8–10).

We will not emphasize the detailed procedures by which our results have been obtained. Descriptions of them are readily available (11–14). Rather, in the following, we will try to convey the character of what has been done so that the reader can better appreciate what the calculation has produced, and better judge its merits as well as its limitations.

2. THE MODEL AND ITS SELF-CONSISTENT FIELD SOLUTION

2.1. Theory of the Disordered Liquid

It is useful to first recall the earlier theory upon which our own treatment is a logical extension. Let us consider the problem of calculating the area per headgroup in the disordered liquid phase of a bilayer which is comprised of a single kind of lipid. The problem is somewhat analogous to calculating the volume per atom of liquid Argon. The latter is a difficult problem due to the competition between the long-range, attractive, van der Waals interactions, and the short-range, hard-core, repulsive interactions. The former is even more difficult due to the vastly greater number of internal degrees of freedom of the lipid molecule compared to an Argon atom. The approach to this problem taken by Ben-Shaul et al. (11, 14) was as follows. One knows that the effect of the competing interactions is to produce in the bilayer an interior which is very much like an incompressible liquid. So why not replace the original system of interacting lipids with another system of *non-interacting* lipids, but one in which the density in the interior of the bilayer is constrained to be constant? This amounts to replacing the interacting, unconstrained, system by one which is non-interacting, but constrained. The Hamiltonian of the new, non-interacting, system is very simple, of course:

$$H = \sum_{\gamma=1}^{n} \sum_{k=1}^{N} h_{\gamma,k}, \tag{1}$$

where n is the number of lipid tails, N is the number of segments in each chain, and $h_{\gamma,k}$ is the Hamiltonian of a single chain, γ, which now only contains the energy of gauche bonds at segment k. The problem looks easy, but there is a difficulty which we encounter when we try to calculate the Helmholtz free energy

$$F(T, n, A, V) = Tr' P' [H + k_B T \ln P'], \tag{2}$$

where A is the area of the membrane and V its volume. Here,

$$P' = \exp[-\beta H]/Q', \tag{3}$$

is the probability to observe a configuration of energy H, $\beta = 1/k_BT$, and

$$Q' = Tr' \exp[-\beta H], \tag{4}$$

is the partition function of the system. The prime indicates that *only chain configurations which contribute a uniform core density are to be considered in the trace.* This is the difficulty, but it is easily surmounted by going to a different ensemble, one in which the local density is *not* specified, but its conjugate, essentially the local pressure, is. To do this we define a local volume fraction $\hat{\Phi}(z)$,

$$\hat{\Phi}(z) = \frac{1}{A}\sum_{\gamma=1}^{n}\hat{\phi}_\gamma(z), \tag{5}$$

$$\hat{\phi}_\gamma(z) = \sum_{k=1}^{N}\nu_k\delta(z - z_{\gamma,k}), \tag{6}$$

$$\frac{A}{V}\int dz\hat{\Phi}(z) = \frac{n\sum\nu_k}{V} = 1, \tag{7}$$

where z is the coordinate normal to the bilayer surface and the ν_k are the volumes of the k'th segment. In Eq. 7, the hydrophobic core of volume V consists only of the sum of its monomeric volumes. This *incompressibility constraint* requires the non-interacting system of lipid chains to occupy a liquid-like slab of fixed density n/V. Under this constraint, the free energy of Eq. 2 reduces to a natural function of two extensive quantities, $F(T, n, A, V) \to F(T, n, A)$.

Now the probability of a configuration in an external field, $\Pi(z)$, can be written

$$P = \frac{1}{Q}\exp[-\beta H - \frac{A}{\nu_0}\int dz\Pi(z)\hat{\Phi}(z)], \tag{8}$$

$$Q = Tr\ \exp[-\beta H - \frac{A}{\nu_0}\int dz\Pi(z)\hat{\Phi}(z)], \tag{9}$$

where ν_0 is any convenient molecular volume, one which has simply been introduced to make the field $\Pi(z)$ dimensionless. The free energy in this ensemble, which is simply the Legendre transform with respect to A of $F(T, n, A)$, is

$$G(T, n, \Pi) = F(T, n, A) + \frac{Ak_BT}{\nu_0}\int \Pi(z)dz, \tag{10}$$

and is given by

$$G = -k_BT\log Q, \tag{11}$$

$$= Tr\ P[H + \frac{Ak_BT}{\nu_0}\int dz\Pi(z)\hat{\Phi}(z) + k_BT\ln P]. \tag{12}$$

Because the chains are noninteracting, the probability of a given configuration of all chains is simply the product of the probabilities of each chain to be found in their individual configuration, $P = cP_1^n$, where c is an uninteresting constant, and

$$P_1 = \frac{1}{Q_1}\exp[-\beta\sum_{k=1}^{N} h_k - \frac{1}{\nu_0}\int dz\Pi(z)\hat{\phi}(z)], \tag{13}$$

$$Q_1 = Tr\exp[-\beta\sum_{k=1}^{N} h_k - \frac{1}{\nu_0}\int dz\Pi(z)\hat{\phi}(z)], \tag{14}$$

and h_k contains the energy of the gauche bonds of the single chain. The field $\Pi(z)$ is then determined by requiring that the ensemble-average, local volume fraction, $<\hat{\Phi}(z)>$, be a prescribed constant value at *all* z,

$$<\hat{\Phi}(z)> = \frac{\beta\nu_0}{A}\frac{\delta G}{\delta\Pi(z)} = 1. \tag{15}$$

One sees from Eq. 14 that Q_1 is the partition function of a single chain in the external field $\Pi(z)$. So the central assumption of Ben-Shaul et al. has reduced the many chain problem to that of calculating the one-chain partition function in an external field. The calculation of this partition function is the essential problem in this method, and its difficulty depends on how realistic a description of the chains one takes. Ben Shaul et al. take Flory's Rotational Isomeric States Model (15) in which each bond between CH_2 groups can take one of three configurations; gauche-plus, gauche-minus, or trans. For m independent bonds, this is only 3^m configurations. However one also has to specify an origin and *direction* of the chain. This leads to many more configurations. Typically one enumerates on the order of 10^7 chain configurations. Any configurations which intersect themselves, or which break the planar boundary between the bilayer and its surrounding water, are discarded. The remaining contributions to the partition function are weighted by the field $\Pi(z)$, and from the partition function one calculates the density. The field $\Pi(z)$ is adjusted until the density is uniform inside the core of the bilayer.

The field $\Pi(z)$ accounts, in an average way, for the local intermolecular repulsions that are needed to keep the chain at constant density at each z. Thus, the replacement of the multichain partition function by one of non-interacting, but constrained chains, is in essence a mean-field approximation of the effect of the intermolecular repulsions, one which employs the field, $\Pi(z)$, conjugate to the local density. The van der Waals attractions hold the hydrophobic liquid core together. Since the density is homogeneous, the contribution of the van der Waals interactions to the free energy is simply a constant, which we ignore.

Utilizing the field, $\Pi(z)$, one calculates the single chain partition function, eq. 14, and the Helmholtz free energy

per chain, which is the Legendre transform of eq. 12,

$$f(T,a) = g - \int dz \Pi(z)\phi(z) \equiv F(T,a)/n, \tag{16}$$

where $g \equiv G/n$ and $a \equiv A/n$ are the Gibbs-like free energy and the area per chain, respectively.

The surface tension of the bilayer is $\sigma = \partial f/\partial a$, which vanishes at a minimum of $f(T,a)$. We require the system to maintain zero tension since as we expect the real system does so. There *is* a minimum in this model because at large areas per chain, the chains must have many gauche bonds in order to fill the space, and these gauche bonds cost energy. At small values of a, the chains must be tightly packed, with the consequence that there are few gauche bonds, and little entropy. The minimum occurs at the optimum trade off of these two effects. The area per chain one obtains from this is a bit large compared to experiment, so Ben Shaul et al. also include the effect of the repulsive interaction between water and the hydrophobic chains. They take this contribution to the free energy per chain to be $\sigma_0 a$ with σ_0 the usual oil, water interfacial tension. This term shifts the minimum to smaller a and one finds the minimum to occur at $0.64nm^2$ for a *two-chain* lipid, which is in satisfactory agreement with experiment.

The order parameter profile of the chains, which is essentially the angle between CH_2 planes and the bilayer normal, can be calculated because one knows the equilibrium probability of each configuration of the chains. The order parameter can be measured by nuclear magnetic resonance. There is good agreement between theory and experiment.

A deficiency of this theory which must be addressed in order to apply it to the phenomenon of rafts is that it is not capable of addressing the issue of how local chain order can be affected by the interactions with cholesterol, an interaction which probably is crucial in distinguishing liquid-ordered phases from liquid-disordered phases. If one cannot produce two such liquid phases, one will certainly not be able to describe the existence of rafts as a coexistence phenomena. Another issue related to interactions and local order is the lack of the possibility of describing a gel phase, in this theory. To address these shortcomings, we extended (12) the theory as described in the following section.

2.2. The Description of Orientational Order

It is not difficult to understand why the theory presented above does not produce a gel phase. In describing packing effects, only a local volume fraction, Eq. 5, was introduced. As a consequence, all information is lost about the local orientation of bonds between adjacent acyl groups in a chain. In other words, the theory as presented only

requires that the density in the interior be uniform. It does not give greater weight to configurations which are more ordered than average and which could therefore more easily fulfill the constraints of packing.

To remedy this defect, we specify the local orientation of the chain by the normal to the plane determined by the k'th CH_2 group

$$\mathbf{u}_k = \frac{\mathbf{r}_{k-1} - \mathbf{r}_{k+1}}{|\mathbf{r}_{k-1} - \mathbf{r}_{k+1}|}, \qquad k = 1...N-1, \tag{17}$$

where the $\mathbf{r}_k$ are the position vectors of the k'th segment in the chain. In analogy to the number densities, Eqs. 5 and 6, we define the *bond* densities

$$\hat{\Xi}(z) = \frac{1}{A}\sum_{\gamma=1}^{n} \hat{\xi}_\sigma(z), \tag{18}$$

$$\hat{\xi}(z) = \sum_{k=1}^{N-1} \nu(k)\delta(z - z_k) g(\mathbf{u}\cdot\mathbf{c}) \tag{19}$$

which tells us how well these local bonds are oriented with respect to the bilayer normal $\mathbf{c}$. We have defined

$$g(\mathbf{u}\cdot\mathbf{c}) \equiv (m+1/2)(\mathbf{u}\cdot\mathbf{c})^{2m} \tag{20}$$

For large m, $g \approx m\exp(-m\theta^2)$ where θ is the angle between the two unit vectors. We found $m = 18$ to be reasonable. Note that $g(\mathbf{u}\cdot\mathbf{c})$ is unity if the bond vector $\mathbf{u}$ is aligned with the bilayer, and falls exponentially with the angle between the two vectors.

To express the fact that it is energetically favorable for bonds which are in the same local region to be aligned with one another, and with the bilayer normal, one adds to the system's Hamiltonian a simple interaction

$$V(\mathbf{u},\mathbf{u}') = -(J/\nu_0) g(\mathbf{u}\cdot\mathbf{c}) g(\mathbf{u}'\cdot\mathbf{c}), \tag{21}$$

This interaction favors neighboring bond orientations which are aligned with one another and with the bilayer normal, with the strength of the interaction falling exponentially if either bond deviates from the normal. This interaction between bonds is *not* rotationally invariant. We do not expect it to be because the environment is not rotationally invariant as the bilayer normal provides a particular direction in space.

The extension of the probability distribution function, Eq. 13,14, is immediate (12)

$$P_1 = \frac{1}{Q_1}\exp\{-\beta\sum_{k=1}^{N} h_k - \frac{1}{\nu_0}\int dz[\Pi(z)\hat{\phi}(z) + B(z)\hat{\xi}(z)]\}, \tag{22}$$

$$Q_1 = Tr\exp\{-\beta\sum_{k=1}^{N} h_k - \frac{1}{\nu_0}\int dz[\Pi(z)\hat{\phi}(z) + B(z)\hat{\xi}(z)]. \tag{23}$$

The self consistent equations which determine the two unknown fields, $\Pi(z)$ and $B(z)$, are now

$$< \hat{\Phi}(z) > \; = \; 1, \tag{24}$$

$$< \hat{\Xi}(z) > \; = \; -\frac{k_B T B(z)}{J}. \tag{25}$$

3. NOTES ON PURE AND MIXED LIPID SYSTEMS

The first result that emerges from this extension is that one finds, for a system of lipids with two saturated chains, C16:0, a first-order main chain transition from a disordered liquid phase to a more ordered one, which we identify with the gel phase. Again the distinction between more and less ordered follows from examination of the calculated chain order parameters. The interaction strength, J, is fixed so that the transition temperature occurs at that measured (16) for DPPC, 315K . The area per head group in the fluid phase at 323K is calculated to be 67.0 $\mathrm{\AA}^2$, compared with the experimental value (17) of 64.0 $\mathrm{\AA}^2$, while that in the gel phase at 293K is calculated to be 49.9 $\mathrm{\AA}^2$, compared to the experimental value (17) of 47.9 $\mathrm{\AA}^2$. At the transition, the average number of gauche bonds is calculated to be reduced from 4.3 in the liquid to 2.1 in the gel phase.

It is straightforward to repeat the calculation for a system consisting of a mono-unsaturated lipid, C18:1. Nothing in the Hamiltonian, or the interactions, changes at all. In particular, the strength, J, of the interaction of Eq. 21 remains the same. What does change is the ensemble of configurations of the chains, for they all now have a single double-bond in them which causes a kink at its location. The consequence is that there are fewer configurations which can take advantage of the interaction between chains of Eq. 21 which lowers the interaction energy between chains which are well aligned with one another and the bilayer normal. As a consequence, one finds for this system that the main chain transition now occurs at a temperature below zero degrees centigrade, in agreement with experiment. This is very nice, because the one parameter we could play with, the strength of the interaction, had already been set by the transition temperature of the saturated system. So there was nothing to adjust in order to get the main chain temperature of the unsaturated system correct. Nonetheless the theory gets it right.

The phase diagram of a mixture of the saturated and unsaturated chains is easily obtained (12), and is shown in the left-hand panel of Fig. 1(a). One obtains a liquid phase above the first-order, main chain transition of the saturated lipid, and coexistence between liquid and gel phases below it.

4. NOTES ON THE TERNARY SYSTEM

The last component to be introduced in order to make this a ternary system is cholesterol (6). One knows where all the atoms in the cholesterol molecule are, just as one knows where all the atoms are in the lipid chains. Thus one can easily take into account the contribution of cholesterol to the volume of the bilayer interior which is constrained to be constant. One also has to consider the binary interactions between cholesterol and the lipids, and between the cholesterol themselves. We have chosen the same kind of interaction between these pairs as between lipid chains; that is we have chosen an interaction which favors *local* alignment of the elements in the binary pair with one another and with the normal to the bilayer normal. We only have to identify what we mean by the alignment of cholesterol. The alignment of its small acyl tail is defined as for the lipid chains. For the rigid part of the cholesterol, we introduce a unit vector, $\mathbf{u}_c$, which extends from the third to the 17'th carbon in the molecule, using the conventional labeling, (see the inset to Fig. 2), and a second normal perpendicular to the planes of the rings. All interactions can now be written

$$V_{\sigma,\sigma'} = -(J_{\sigma,\sigma'}/\nu_0) g(\mathbf{u}_\sigma \cdot \mathbf{c}) g(\mathbf{u}_{\sigma'} \cdot \mathbf{c}), \tag{26}$$

where σ is an index taking the values, s,u, and c denoting saturated, unsaturated, and cholesterol, respectively. The strengths of the interactions between lipid segments, $J_{s,s}$, $J_{s,u}$, and $J_{u,u}$ are taken to be identical, $J_{l,l}$, because we believe the lipids chains are distinguished by their configurations, not by their interaction strengths. With the addition of cholesterol, there are additional interactions. We take the strengths of the interactions between cholesterol and both lipids to be identical also, and for the same reason as above: $J_{s,c} = J_{u,c} \equiv J_{l,c}$. Lastly, there is an interaction between cholesterols, of strength $J_{c,c}$. Thus there are now two strengths which can be varied, the strength of the cholesterol lipid interaction, $J_{l,c}$, and the strength of the cholesterol, cholesterol interaction, $J_{c,c}$. We have systematically explored the effect of these two interactions, with the following results.

We first ask what is the phase diagram if the strongest interaction of the three is that between lipids themselves, so that the cholesterol interactions are weak, and cholesterol affects the system only through its volume and the constraint that the density in the interior of the bilayer be constant. Fig. 2 shows the result for the limiting case in which $J_{l,c} = J_{c,c} = 0$. The only aligning interaction is between lipids, and its strength is again set so that the main chain transition of the saturated lipid is that of DDPC.

One sees that there is a gel phase, and only one liquid phase. Thus there can be no liquid, liquid coexistence.

We reasoned next that perhaps the cholesterol, lipid interaction should be enhanced. This would have the effect

of making the lipids more ordered, and might bring about a phase separation between the more ordered lipids near the cholesterol and the disordered lipids. Therefore we increased the strength, $J_{l,c}$, of the cholesterol, lipid interaction, while keeping the cholesterol, cholesterol interaction, $J_{c,c}$, small. The possibility which we desired did not come about due to this change in interaction strength. Instead we found that the gel phase became swollen with cholesterol. Further if the cholesterol, lipid interaction became too strong, the main chain temperature increased with cholesterol composition, rather than decreased. All of these are understandable effects of the increased cholesterol, lipid interaction, but do not seem to be observed in experiment.

We therefore turned on the interactions between cholesterol such that the cholesterols would, at reasonable temperatures, phase separate from the lipids even in the binary cholesterol, saturated lipid system. The phase diagram in Fig. 1 is obtained this way with $J_{c,c} = 0.73 J_{l,l}$, and $J_{l.c} = 0.78 J_{l,l}$. One sees that there are now two liquid phases; one is cholesterol-rich, the other cholesterol poor. In the former, the lipids are relatively well ordered, while in the cholesterol-poor phase, the lipids are not so well ordered. The region in which these two liquids coexist would be the regions in which rafts would occur. The order parameters of the three phases which coexist at 300 K are shown in the inset.

The origin of the two liquid phases in this model is clear. They arise from the tendency of cholesterol to phase separate from the lipids, creating a cholesterol-rich liquid, the ordered liquid, and a cholesterol-poor one, the disordered liquid. At temperatures above the main chain transition, a coexistence region between the two liquids extends across the ternary diagram from the cholesterol, saturated-lipid binary axis, to the cholesterol, unsaturated lipid binary axis, as shown in Fig. 3.

As the temperature is increased from 320 K, the liquid-liquid coexistence region eventually detaches from the binary cholesterol, unsaturated-lipid axis at a critical point, and recedes toward the cholesterol, saturated-lipid axis, vanishing there at a critical point. As the temperature is decreased from 320 K, the gel phase appears at 315 K. As the temperature is lowered, the region of liquid disordered phase on the binary cholesterol, saturated-lipid axis decreases. When the two coexistence regions, gel, liquid disordered, and liquid ordered, liquid disordered, just touch one another at a critical tie line, a three phase coexistence region begins and grows with further decrease of temperature. Note that in this model, the gel phase does nothing of significance other than to take up space in the ternary diagram.

5. DISCUSSION

The phase diagram obtained in our calculation for the ternary system of a saturated lipid, and unsaturated lipid, and cholesterol, is the first obtained from a microscopic model. It has the nice feature of displaying a region of coexistence between two liquid phases, one rich in cholesterol and relatively well-ordered saturated lipids, the other rich in relatively poorly-ordered unsaturated lipids. This region of coexistence would be the locus of "rafts". It resembles the phase diagram observed experimentally for the system sphingomyelin, POPC, and cholesterol(8–10).

It is not without its faults, however. In particular, the regions of phase coexistence in the binary system of saturated lipid and cholesterol are too wide, and occur at a concentration of cholesterol which is much higher than that observed by Vist and Davis(7). However, this may only be due to the form of the interaction we have taken, Eq. 21, between cholesterol and lipid which falls exponentially with the local angle between cholesterol and lipid. Indeed a recent calculation(19) which is similar in spirit to that described here but utilizes an interaction in which the ordering effect of cholesterol on the chains remains strong over a much larger range of angles obtains a coexistence region in which the cholesterol concentration is much smaller, and is in reasonable agreement with experiment. Our calculation also utilizes the rotational isomeric description of the chains due to Flory (15). More accurate configurations generated from molecular dynamics simulations can be used instead (18). Perhaps more important is that mean-field calculations, such as ours and others (19), ignore the correlation between lipid and cholesterol; i.e. that the rough and smooth faces of the cholesterol ring structure presumably interact rather differently with the chains. Just such a preferential interaction of the saturated lipid with the smooth face of cholesterol was observed in simulations (5). Furthermore, our calculation has largely neglected the effects of headgroups. The headgroups may play an important role in packing with cholesterol, whose hydrophilic volume is proportionally small.

Irrespective of these shortcomings, the calculation teaches us several things. First, it indicates that the main chain transition, and the gel phase, are almost certainly irrelevant to the phenomenon of rafts. The presence of a gel phase and its associated three-phase triangle, of course, take up room in the Gibbs triangle, thereby limiting the region in which rafts could exist. But this is a negative role. The irrelevance of the gel phase is also indicated by the observation of liquid-liquid coexistence in some systems well above any main chain transition (20). Second, if one believes that binary interactions are sufficient to describe the system, then our calculation indicates that liquid-liquid coexistence in the ternary system is intimately tied to its existence in at least one of the binary systems. In retrospect, this is not difficult to understand. Suppose that in a ternary system of S, U, and C, the interaction between S and C were the most repulsive. Then the addition of U to the system, with its weaker interactions with the other two

components, can not cause phase separation to occur at a higher temperature. Thus the separation occurs first in the binary system. There is experimental evidence that phase separation does extend out to the binary systems(8–10), and evidence that it does not (20). Third, in our model, the basic mechanism of the phase separation is simply that cholesterol prefers an environment of itself to that of lipids. If this be true, then one expects tie lines between the two liquid phases which are relatively vertical, as seen in Fig. 3. There is some evidence for this (22). On the other hand, there is also evidence against it (20, 21). If experiment ultimately concludes that the predictions of our model calculation are not correct, one has to consider what additions are needed to the basic model we have presented. Fortunately, it is likely that the efficacy of trial, additional, effects can be judged by utilization of the techniques we have presented here.

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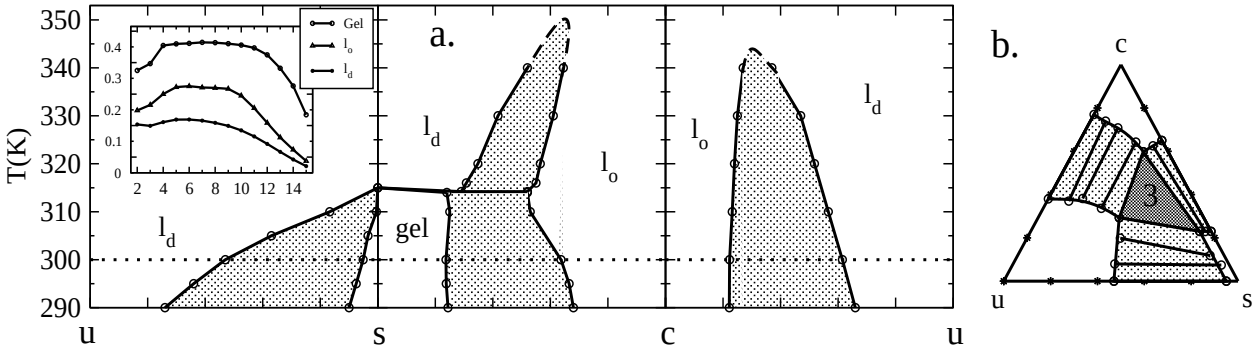

FIG. 1: Binary phase diagrams of the C16:0, C18:1, cholesterol system. The saturated lipid-cholesterol mixture has a triple point very near the main-chain transition temperature, so that the gel, l_d coexistence region is very narrow. Dashed lines are extrapolations. The ternary mixture at $T = 300K$ is shown in Fig. 1b. Inset: order parameters of saturated tails in the three coexisting phases at 300K.

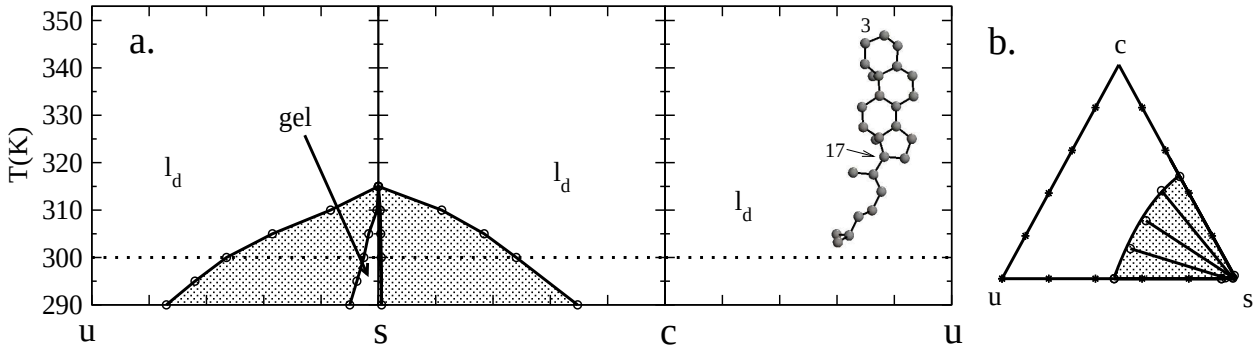

FIG. 2: Calculated phase diagrams of the three binary mixtures of cholesterol (c), saturated (s), and unsaturated (u) lipids in temperature-composition space for $J_{ll}(m+1/2)^2/k_BT^* = 1.44$ and $J_{lc} = J_{cc} = 0.0$. These binary diagrams form the sides of the Gibbs prism, a cut through which at 300K produces the Gibbs triangle shown in Fig. 1b. Regions of two-phase coexistence are shaded, and some tie lines are shown. Inset: One of the configurations of our model cholesterol.

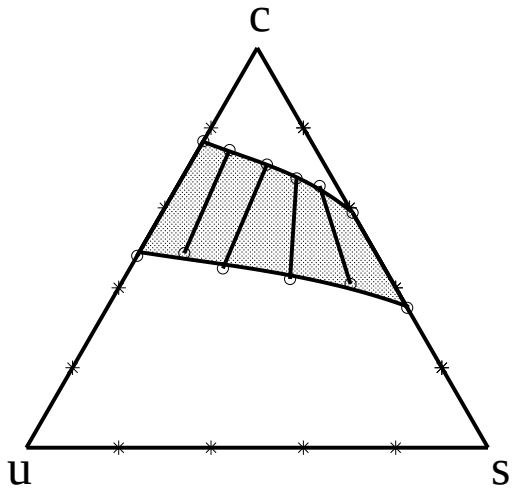

FIG. 3: Phase diagram of the ternary mixture at T=320K. Interactions are the same as in Fig. 1.