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Structural study of the inverted cubic phases of di-dodecyl alkyl-β-D-glucopyranosyl-rac-glycerol

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Abstract. — We present a quantitative study of the bicontinuous cubic phases in the di-dodecyl alkyl-β-D-glucopyranosyl-rac-glycerol (rac-d12:0 β-GlcDAG) lipid-water system. A temperature-composition phase diagram determined using X-ray diffraction shows Ia3d and Pn3/Pn3m cubic phase regions in addition to inverted hexagonal (HII) and several lamellar phase regions. The diffraction data was used to determine the lattice repeat vector, d, and, using suitable models, the lipid monolayer thickness, dL, for all temperatures and compositions in the diagram. The models chosen for the cubic phases were based on lipid bilayers straddling infinitely periodic minimal surfaces (IPMS) as described previously in the literature [1]. Using the structural data derived from the phase diagram, the lyotropic phase transition between the two cubics was modeled by a simple curvature free energy theory. A new result of this theory was an estimate for the Gaussian curvature bending modulus of the lipid monolayer. The model was found to quantitatively describe the phase transition, particularly for phase behavior at low hydration. Our model suggests some universal features should be present in any system that shows non-lamellar phases and we discuss those features with respect to the phase diagrams available in the literature.

1. Introduction (1).

Observations of lipid-water phases that display cubic symmetry are becoming more prevalent. Although their existence has been known for a long time [1-5], it is only within the last half

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(1) Portions of this paper have previously appeared in the Ph.D. dissertation of D. C. Turner, 1990.
decade that it has become apparent that these phases appear in a wide variety of common lipid systems [6, 7], as well as in block co-polymer systems [8-10]. They are commonly found between lamellar and hexagonal phase regions in temperature-composition phase diagrams in a remarkably diverse set of lipid systems, suggesting a universal mechanism of formation or stability. For dual chain lipids, cubic phases fall between the lamellar and the inverted hexagonal (II$_{II}$) phases [11-13], and are probably inverted cubic phases, meaning that the lipid-water interface tends to curl towards the water. It has been suggested that inverted cubic phases will always form between the lamellar and the II$_{II}$ phases in all systems that exhibit the transition [12-14], but that kinetic barriers may curtail their expression in many cases.

While many examples of lipid-water cubic phases can be found in the literature, very little systematic work has been done to quantify the shape of the phase boundaries or the structural dimensions of the observed cubic phases, especially for lipids with two hydrocarbon chains. In particular, complete temperature-composition phase diagrams have been published for only a few systems that have cubic phase regions [5, 6]. Even more importantly, structural dimensions such as lipid length, area per lipid or mean curvature at the interface have rarely been reported. These quantities offer a way to compare the lipid environment in cubic phases to other phases.

In this paper, we describe the temperature-composition phase diagram of di-dodecyl alkyl-β-D-glucopyranosyl-rac-glycerol (hereafter referred to as rac-di-12:0 β-GlcDAG), which forms liquid crystalline cubic phases of two different symmetries: Pn3m/Pn3 and Ia3d [15], along with a lamellar gel phase (L$_{β}$), a lamellar liquid crystalline phase (L$_{α}$) and the inverted hexagonal liquid crystalline (H$_{II}$) phase. This lipid, which consists of a monosaccharide headgroup and two 12-carbon chains, is one of many non-lamellar forming dialkyl sugar lipids that have been synthesized and studied at the University of Alberta [16]. By assuming that the cubic phases can be described as lipid bilayers draped across Infinitely Periodic Minimal Surfaces (IPMS), their structural dimensions have been calculated and compared to the other phases observed. In addition, a simple curvature free energy model is described which gives a qualitative explanation for the location of the individual cubic phases within the phase diagram. This model free energy includes a Gaussian curvature term because the different cubic phases are found to have different Gaussian curvature per lipid molecule. Fitting the model to the experimental data results in a numerical value for the Gaussian curvature modulus in terms of the monolayer bending modulus.

2. Experimental methods.

The lipid, di-12:0 alkyl-β-D-glucopyranosyl-rac-glycerol (rac-di-12:0 β-GlcDAG), was synthesized as described in Mannock et al. [17]. Lipid and deionized water were added gravimetrically to an X-ray capillary (Charles Supper, Natick, Ma.) which was subsequently sealed with 5 minute epoxy. The samples were mixed until uniform with several cycles of freeze-thawing and by moving the lipid from one end of the capillary to the other via a bench top centrifuge; samples were used as quickly as possible thereafter, typically within one week. During data acquisition the capillary was placed in a temperature controlled (± 1 °C) sample holder which was programmed to ramp the temperature from 20 to 85 °C and back in 5 ° increments. The sample was equilibrated at each temperature for 10 minutes and a two minute X-ray exposure was obtained with the Princeton small angle X-ray facility, consisting of a Rigaku Ru-200 X-ray generator coupled to a Silicon Intensified Target (SIT) slow scan TV detector [18, 19]. A two minute exposure was long enough to image even faint diffraction rings in these strongly diffracting samples. The whole sequence of pictures was saved on magnetic tape for subsequent analysis.
At each temperature the diffraction peaks were indexed onto one or more of the following lattices: lamellar, \( H_{II} \) and \( Ia3d \) or \( Pn3m/Pn3 \) cubic lattices. The cubic phases each have a characteristic diffraction signature. The \( Ia3d \) cubic displays a set of reflections which index to \( \sqrt{6}, \sqrt{8}, \sqrt{12}, \sqrt{16}, \sqrt{20}, \sqrt{22}, \sqrt{24}, \sqrt{26}, \ldots \), while the \( Pn3m/Pn3 \) lattices exhibit characteristic reflections which index to \( \sqrt{2}, \sqrt{3}, \sqrt{4}, \sqrt{6}, \sqrt{8}, \sqrt{10}, \sqrt{11}, \sqrt{12}, \sqrt{14}, \sqrt{16}, \ldots \) [15]. \( Pn3m \) and \( Pn3 \) lattices cannot be distinguished by the reciprocal lattice. The \( Pn3m \) cubic showed at least five reflections for indexing, while the first four reflections typically were observed for the \( Ia3d \) cubic. Samples which were near full hydration showed considerable differences in their phase behavior depending upon whether they were being ramped up or down in temperature. This is not unusual for lipid-water systems, which often show hysteretic phase behavior [12]. Some longer time scale measurements were carried out to try to determine the true equilibrium state at the non-overlapping regions in the phase diagram. It was found that the system can remain in an apparently metastable phase for time periods on the order of days or longer. Such behavior makes it quite difficult to discern the true equilibrium phase diagram accurately, and points out the delicate energetic nature of these phase transitions.

The density of the lipid was required for evaluating the lipid volume fraction as a function of temperature. Because the quantity of material necessary to perform these measurements is in excess of 50 mg, the density of the related chiral compound \( 1,2\)-didodecyl-\( \beta \)-D-glucopyranosyl-sn-glycerol, which was in greater supply, was measured under the assumption that the density is largely unaffected by the chiral conformation. The lipid was dispersed in a series of \( D_2O/H_2O \) mixtures with a spread of average densities, and then encased in sealed glass tubes. The mixtures were then equilibrated in a water bath at a set temperature and then in a bench top centrifuge. The temperature of the tube was monitored before and after the centrifugation with a thermocouple to account for possible temperature drift. At any given temperature, lipid sinks when it is of greater density than the solvent, while it floats if it is less dense than the solvent. Carefully bracketing the true lipid density with the \( D_2O/H_2O \) densities can result in a very accurate determination of the lipid density [20]. The density of the lipid aggregate was determined by this method for temperatures between 30 and 75 °C. The empirical fit of density vs. temp is given by \( \rho_L = 1.0371(\pm0.015) - 0.00079(\pm0.0002)T \) [g/ml], where \( T \) is temperature in degrees Celsius. The largest source of error in the measurement is the stability of the sample temperature during the required centrifugation time (\( \pm 3 \) °C).

The volume fraction and \( d \)-spacing are the two parameters necessary for determining the internal dimensions of the phases. By assuming a sharp separation between lipid and water, the internal dimensions of the lamellar and \( H_{II} \) phases were determined by the method outlined by Luzzati [21]. Figure 1 shows a drawing defining the internal dimensions of the lamellar and \( H_{II} \) phases. The water volume fraction \( \phi_W \) was determined from the water mass fraction, \( c_W \), using the density of both the water and lipid, \( \rho_W \) and \( \rho_L \) respectively:

\[
\phi_W = \frac{c_W}{c_W + (1 - c_W)\frac{\rho_W}{\rho_L}}.
\]

Given \( \phi_W \), and the \( d \)-spacing \( d \), the water layer thickness in the lamellar phase is given by \( d_W = \phi_W d \), and the lipid monolayer thickness is \( d_L = 0.5(d-d_W) \). The area per lipid is given by \( a = v_L d_L^{-1} \), where \( v_L \) is the lipid specific volume. Similarly, in the \( H_{II} \) phase, the radius of the water core is \( R_W = d\sqrt{3}/2\pi\phi_W \), the monolayer thickness along the [1, 0] direction is \( d_{H_{II}} = 0.5d - R_W \), and the monolayer thickness along the [1, 1] direction is \( d_{max} = d - \sqrt{3} - R_W \) (see Fig. 1). The area per lipid molecule evaluated at any radius \( R_{pp} \) is given by

\[
a = v_L \frac{2R_{pp}}{R_W^2} \frac{\phi_W}{(1 - \phi_W)}.
\]
Proposed models of the cubic phases based on infinitely periodic minimal surfaces (IPMS) yield corresponding equations for the internal dimensions which will be discussed in the results section.

3. Results.

The temperature and composition diffraction data was compiled into a phase diagram for the rac-di-12:0-β-GlcDAG/water system, shown in figures 2a and b. Figure 2a shows the diagram observed on cooling, while 2b shows the diagram on heating. Table I lists d-spacing vs. concentration and temperature. Knowledge of the exact transition temperatures is limited by the fact that the diffraction patterns were taken every 5 °C. The large hysteretic effect at the wet end of the phase diagram is characteristic of lipid-water dispersions showing non-lamellar phases and it is particularly acute in those systems which show cubic phases [12, 22]. Such behavior implies that the free energy differences between phases is small, particularly with respect to the possibly significant kinetic barriers [13]. All non-lamellar phases are topologically distinct from lamellar phases so the bilayer must be torn apart before it can reform into a non-lamellar phase. Tearing the bilayer exposes the hydrophobic chain region to water which contributes a large unfavorable barrier energy. This also may explain why longer chain lipids appear to show characteristically more hysteretic behavior - their hydrophobic exposure energy is larger [23]. For the rac-di-12:0-β-GlcDAG, the cooling data was judged to be more reliable than the heating data based on the reproducibility of phase boundaries and d-spacings. Some effort was expended to find the true equilibrium phase diagram by observing the behavior of the sample when it was allowed to sit for a long time in the phase diagram regions that do not overlap between heating and cooling. Samples were left for several days in these regions starting from both the heating and the cooling phases. The results are consistent with the true equilibrium phase diagram being a compromise between the heating and cooling diagrams, however, the time scale for the changes observed was too long to determine the equilibrium
Table I. — *D-spacing and phase as a function of composition on cooling. Temperature is in degrees C, d-spacing is in angstroms.*

*Key:* h = HII, la = Lα, lb = lB, d = Pn3m = D cubic, g = lα3d = G cubic.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Water Weight Fraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td>85</td>
<td>h37.6</td>
</tr>
<tr>
<td>80</td>
<td>h37.7</td>
</tr>
<tr>
<td>75</td>
<td>h37.7</td>
</tr>
<tr>
<td>70</td>
<td>h38.0</td>
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<td>65</td>
<td>h38.2</td>
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<td>h38.3</td>
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<td>55</td>
<td>h38.5</td>
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<td>50</td>
<td>h38.8</td>
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<tr>
<td>45</td>
<td>h39.1</td>
</tr>
<tr>
<td>40</td>
<td>h39.2</td>
</tr>
<tr>
<td>35</td>
<td>la + h</td>
</tr>
<tr>
<td>30</td>
<td>lb47.5</td>
</tr>
</tbody>
</table>
Fig. 2. — Temperature-composition phase diagram of rac-di-12:0 β-GlcDAG on a) cooling and on b) heating. The x-axis is percent water by weight in the sample. Symbols: G = gyroid (Ia3d) cubic phase, D = D surface (Pn3m) cubic phase, HII is the inverted hexagonal phase, Lα is the liquid lamellar phase and Lβ is the gel lamellar phase. Data points (not shown) were taken every five degrees between 30 and 85 °C at the water weight fractions shown in Table I. Hatched areas are regions of coexistence. Solid lines indicate excess water boundaries. Phase behavior above 90 °C was not examined.
phase definitively. In many cases no change was observed over at least a week. All of the structural measurements discussed below come from the cooling data unless otherwise noted.

The structure of the phase diagram (Fig. 2a) is very similar to the phase diagram of glycerol-monoleate (GMO) determined by Hyde and co-workers [5]. There is a low temperature lamellar gel phase \( (L_g) \) uniformly across the composition range up to 30 to 35 °C. Above 75 to 80 °C, but below 90 °C, there is an \( H_{II} \) phase which gradually displaces the other phases at the dry end until there is a direct transition to the \( L_g \) phase. The region above 90 °C was not examined. The middle portion of the diagram shows two different cubic phases and a lamellar liquid crystalline phase. The cubic phase with \( Pn3m/Pn3 \) symmetry (labelled D in the figure; the origin of this nomenclature is explained below) is the high temperature and high water content phase, while the \( Ia3d \) phase (labelled G) is the low temperature, low water cubic. This cubic does not exist at excess water on cooling, but does exist at high water contents (up to 38 %) on heating (Fig. 2b). The authors know of no instances where the \( Ia3d \) cubic is found as an equilibrium excess water phase for any single component system. At low temperatures the \( L_\alpha \) phase exists as a bubble within the \( Ia3d \) cubic region. This is an interesting feature which means that as one travels along isothermally toward lower water concentration the cubic phase is re-entrant. A re-entrant \( H_{II} \) phase has recently been observed for the DOPE-water system [24]. Re-entrant phases imply a very delicate free energy balance between the two phases throughout the whole region (see [24]).

The location of the excess water lines (dotted) in the phase diagram were determined by examining the change in \( d \)-spacing with water concentration. The point where the \( d \)-spacing stops changing with added water is the excess water point, or point of full hydration. Additional water simply pools as bulk water. Due to the lack of low water concentration points for the \( Pn3m/Pn3 \) cubic phase, the \( d \)-spacings for all temperatures were plotted against concentration and the best line was drawn which separated all samples below excess with those above excess (Fig. 3). This will give the excess water point as a function of temperature provided that the change in the excess water point is well fit by a linear function of \( d \)-spacing to lowest order, which is justified to within the precision of the measurements. The \( H_{II} \) and the \( L_g \) phase regions have enough low concentration points to determine the excess water through the usual method at each temperature (Fig. 4; see [25]).

Models of the cubic phase unit cells were based on Infinitely Periodic Minimal Surfaces (IPMS) as originally suggested by Scriven [26]. Infinitely Periodic Minimal Surfaces (IPMS) are mathematical surfaces that are periodic in three dimensions which have zero mean curvature at every point on the surface. Apart from the trivial example of an infinite plane, this means that they are saddle surfaces which can be connected together smoothly to form an infinite periodic array. Several IPMS were discovered by Schwarz and his students in the 19\(^{th} \) century [27]. These include his primitive cubic P surface and his tetragonal D surface. More recently, Schoen discovered 13 more IPMS by using a computerized numerical search [28], and others have been discovered since. This article will primarily be concerned with Schwarz's P and D surfaces along with Schoen's Gyroid (hereafter called the G surface).

The mean curvature at any point on a surface is defined as the average of its two principle curvatures, \( c_1 \) and \( c_2 \), which are the inverses of the principle radii of curvature \( R_1 \) and \( R_2 \) (see [29] for a discussion). The mean curvature, \( c \), is thus defined as:

\[
c = \frac{1}{2} \left( \frac{1}{R_1} + \frac{1}{R_2} \right).
\]  

(3)

IPMS have the property that \( c = 0 \) everywhere.

In addition to mean curvature, surfaces can also have Gaussian curvature, \( g \), which is defined as the product of the two principle curvatures: \( g = c_1 c_2 \). The Gaussian curvature is a
Fig. 3. — Determination of the excess water composition for the D surface (Pn3m) cubic phase of rac-
di-12:0 β-GlcDAG lipid as a function of d-spacing at excess water, determined on cooling. Diamonds
eq 50 °C, filled triangles = 55 °C, open triangles = 60 °C, filled squares = 65 °C, and open squares
= 70 °C. The dotted lines delimit a band indicating the most probable location of the excess water
composition. The circled point is in coexistence with the G surface (Ia3d) cubic phase so its water
content is not precisely known.

topological invariant of the surface, meaning that it is independent of the representation of
the surface [30], whereas the sign of the mean curvature is dependent on the orientation of the
surface. The integral of the Gaussian curvature over a closed surface is a topological constant
of the surface. The relationship is given by the Gauss-Bonnet theorem:

\[ \int_S gdS = 2\pi \chi, \]  

where the integral is over the whole surface, S, and \( \chi \) is an integer called the Euler characteristic
of the surface [30]. This relationship also holds for open surfaces which are bounded by geodesic
curves. The Euler characteristic is related to the genus of the surface, \( p \), by \( \chi = 2(1 - p) \) for
a closed surface. The genus of a surface is a count of the number of holes (or handles) in the
Fig. 4. — Determination of the excess water compositions as a function of temperature for rac-di-12:0 β-GlcDAG in a) the HII phase and b) the Lβ phase. In a) triangle = 75 °C, filled circle = 80 °C, and open circle = 85 °C. In b) data is at 30 °C. All data obtained upon cooling.

We can define an Euler characteristic per unit cell, χ, and also a surface area per unit cell, A0. These are given in table II for the three surfaces [14]: here A0 is given as a dimensionless fraction of the unit cell cubic lattice size. For example, if a is the cubic unit cell repeat, then the true area per unit cell is: A0a2.
Table II. — Constants for the P, D and G minimal surfaces, from reference [40].

<table>
<thead>
<tr>
<th>Surface</th>
<th>$\chi$</th>
<th>$A_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>-2</td>
<td>1.919</td>
</tr>
<tr>
<td>P</td>
<td>-4</td>
<td>2.345</td>
</tr>
<tr>
<td>G</td>
<td>-8</td>
<td>3.091</td>
</tr>
</tbody>
</table>

Fig. 5. — Lipid and water as they are believed to organized onto IPMS: a) Schwarz’s P surface and b) Schwarz’s D surface. The hatching of dots indicates the two distinct but congruent water compartments. The lipid tails are arranged to lie (on average) on the minimal surface, with the headgroups displaced from the surface. Figure drawn by Dr. M. W. Tate.

Larsson and co-workers proposed that lipid monolayers are draped on either side of the minimal surface with the terminus of the lipid hydrocarbon chains lying on the surface [1]. Several low resolution X-ray reconstructions of the Pn3m/Pn3 cubic phase are consistent with a model of this type [4, 32, 33]. The remaining space in the structure is divided into two congruent interpenetrating compartments for the P and D surface models, or two mirror image compartments for the G surface. In inverted lipid-water structures, water fills these compartments. Figure 5 shows an artist’s rendering of the proposed structure for a lipid bilayer draped on the P and D surfaces. The two distinct water and lipid compartments are frequently called bicontinuous, although these structures are actually quadracontinuous because each lipid monolayer is itself a distinct continuous volume.

While the mean curvature at the end of the lipid hydrocarbon chains is zero (they are lying on the minimal surface), the curvature at the lipid headgroup is non-zero because the headgroup is displaced away from the minimal surface by the length of the lipid [34]. Although the shape of the headgroup surface can be arbitrary, two different surfaces represent the extremes: the constant thickness surface and the constant mean curvature surface. For the constant thickness surface the monolayer thickness is a constant throughout the structure while the mean headgroup curvature varies [14]. For the surface of constant mean curvature, curvature at the headgroup remains constant while the lipid monolayer thickness varies. Anderson has examined the surfaces of constant mean curvature numerically for both the P and D surfaces [31].
The constant mean curvature and constant thickness surfaces represent two energetic extremes if hydrocarbon packing and bending energy are the two main terms in the free energy expansion [14]. In this case, the constant thickness surface will minimize the packing free energy along with having a relatively large bending energy, while the constant mean curvature surface will have a large packing free energy (because the lipid packing environment is not uniform) along with minimal bending energy. The situation has been described as frustration; the constant thickness surface is curvature frustrated while the constant mean curvature surface is packing frustrated [14, 35-38]. The true surface is probably some compromise. Anderson and co-workers have shown, however, that the constant mean curvature surface is nearly coincident with the constant thickness surface [31, 14]. In light of this result, this paper will assume that the structure is a constant thickness surface for all further discussions because the calculation of internal dimensions becomes much simpler. This approximation is acceptable because measurement errors are large enough to preclude experimentally distinguishing between the cases.

Given the constant thickness model described above and the assumption of distinct water and lipid regions within the unit cell, the internal structural dimensions can be calculated and compared to the dimensions in the lamellar and \( \text{H}_{\text{II}} \) phases. The diffraction signature of the D surface is consistent with the Pn3m space group (\( Q^{224} \) in the International Tables for X-ray Crystallography nomenclature; [39]), the diffraction from the P surface is consistent with the Im3m (\( Q^{229} \)) space group and the diffraction from the G surface is consistent with the Ia3d (\( Q^{230} \)) space group. From the unit cell basis vector (d-spacing), \( d \), and the volume fraction of lipid, \( \phi_L \), we can calculate the lipid length \( l \), the mean curvature \( c \) at the headgroup and the area per lipid at the headgroup \( a_0 \). Each of these values can be compared directly to their counterparts in the lamellar and \( \text{H}_{\text{II}} \) phases.

The lipid length is determined by solving the following equation (combine Eq's (2), (6) and (7) of Ref. [40]):

\[
\phi_L = 2A_0 \left( \frac{l}{d} \right) + \frac{4\pi}{3} \chi \left( \frac{l}{d} \right)^3,
\]

where \( A_0 \) and \( \chi \) are the constants for the minimal surface given in table II. The area at the headgroup integrated over a single monolayer within the unit cell, \( A_l \), is given by [14]:

\[
A_l = A_0 d^2 + 2\pi \chi l^2.
\]

This equation can be divided by the volume of lipid in a monolayer, \( \phi_L d^3/2 \), and multiplied by the lipid specific volume, \( v_L \), to get the area per lipid at the headgroup \( a_0 \). Finally, the mean curvature at the headgroup, \( < c_I > \), averaged over the unit cell is given by [14]:

\[
< c_I > = \frac{-2\pi \chi l}{A_I}
\]

The sign of the curvature is taken to be positive when the lipid headgroup surface bends toward the water. Practically, this means that the cross-sectional area per lipid molecule increases from the head to the tail, as is the case for the \( \text{H}_{\text{II}} \) phase. For comparison, the mean curvature at the headgroup in the \( \text{H}_{\text{II}} \) phase is simply given by \( c_I = 1/2R_W \), where \( R_W \) is the radius of the water core (Eq. (3)). The area per lipid and mean curvature can be evaluated at any point along the lipid length by adjusting the value of \( l \) in the above equations.

Internal dimensions for the lamellar and \( \text{H}_{\text{II}} \) phase were calculated with the equations in the methods section. Weight fractions for all phases were converted to volume fractions using equation (1) before all calculations were done.
Fig. 6. — Plot of lipid length as a function of temperature for several phases and water compositions in the cooling phase diagram of rac-di-12:0 β-GlcDAG, showing both $d_{\text{HII}}$ and $d_{\text{max}}$ (see Fig. 1) for the HII phase. The lines are a fit to $d_{\text{HII}}$ and $d_{\text{max}}$ extrapolated to the low temperature regime. For the cubic phases, the mean lipid length is shown. Open squares = Excess water HII, filled circles = lamellar at 22.4% water, filled squares = excess water Pn3m. Filled triangles, open circles, and open diamonds represent Ia3d at 16, 27.5, and 31.2% water respectively.

Figure 6 shows a plot of the lipid length for the rac-di-12:0 β-GlcDAG + water system at all temperatures and compositions; both $d_{\text{HII}}$ and $d_{\text{max}}$ are shown for the HII phase. For the phases which exist at excess water only the lipid lengths at full hydration are given. The length measurement is rather crude because the $d$-spacing measured for the cubic phases (particularly the G surface) is only reproducible to $\pm 1.5 \, \text{Å}$, which, with the weight fraction known to $\pm 0.01$, gives a measurement error of $\pm 0.8 \, \text{Å}$ in length. At this resolution, the lipid length is roughly continuous across the D to G cubic transition. A more precise measurement may show a small jump as the transition is crossed which may be energetically important. At this resolution, however, lipid length does not distinguish among the different cubic phases.

The area per lipid at the headgroup divided by the volume per lipid is shown in figure 7. The value of $a_0/v_L$ in both of the cubic phases lies in between the large value of the $L_\alpha$ phase and the
small value of the II\textsubscript{II} phase. As in the case of the lipid length, \( a_0/v_L \) is continuous across the D to G transition within error (± 0.002 Å\(^{-1}\)). Interestingly, \( a_0/v_L \) is very nearly independent of temperature for the D cubic phase at excess water. This is precisely the situation observed in the II\textsubscript{II} phase of DOPE at excess water [25]. The cause of this phenomena is unknown; however, it would be interesting to see if it is a universal characteristic of all inverted non-lamellar phases.
The parameter of most interest is the mean curvature at the headgroup surface, $<c_l>$. This is an average of the monolayer curvature over each point in the unit cell. Like the area and length, the curvature of the cubic phases lies between the zero curvature lamellar phase and the high curvature $\Pi_{II}$. Figure 8 shows the inverse of the mean curvature, $<c_l>^{-1}$. The inverse of the lamellar mean curvature would lie at infinity on this plot. The strong temperature and concentration dependence of the monolayer curvature in non-lamellar phases is displayed clearly in this plot. This behavior has been described previously for the $\Pi_{II}$ phase as a geometric multiplication of the small change in lipid length [25]. Said another way, the geometry of certain phases requires small changes in lipid length to lead to large changes in...
curvature. At the phase boundary, the G surface cubic has a larger curvature than the D surface cubic. Therefore, in the region of phase coexistence, the monolayer curvature is the most direct way to distinguish among the cubic phases. In some sense, it could be considered the major component of an order parameter for the transition. In fact, in the discussion section, a simple curvature free energy theory is shown to predict semi-quantitatively the observed transition from the D surface to the G surface cubic.

In general, cubic phases show intermediate behavior intermediate to the lamellar and the $H_{11}$ phase in terms of area per lipid, mean curvature and lipid length. The average lipid length is roughly 10% shorter than the lamellar lipid length while still being between the maximum and minimum lengths found in the $H_{11}$ phase. As a caution, the reader is again reminded that the quantities calculated for the cubic phases are all based on the constant lipid length model, in which a monolayer of constant thickness is laid down on each side of the minimal surface. In reality, the thickness of the monolayer probably varies within the unit cell. This would be similar to the situation in the $H_{11}$ monolayer where the lipid hydrocarbon must pack anisotropically [41]. So, properly, the lipid length determined above may be better represented as a mean length. In any case, the curvature, area and length of the lipid all suggest that the reason the cubic phases fall between the lamellar and $H_{11}$ phases in the phase diagram is because these structural parameters are all suitable intermediates to those of the lamellar and $H_{11}$ phases. This is a qualitative explanation of the major features in the observed lamellar-cubic-$H_{11}$ phase diagram. In the discussion section below we stop grouping all the cubic phases together and describe a simple model of the phase transitions between them.

4. Discussion.

The driving forces responsible for the formation of inverted cubic phases are not quantitatively understood. As described above, it has been suggested that, under certain conditions, they are a better compromise between curvature and packing frustration than the lamellar and $H_{11}$ geometries [14, 36-38]. The stability criteria of one of the cubic phases over the others has only recently received attention in the literature [42, 43]. Except for coexistence regions, one cubic is always stable with respect to the other two and they are found in particular regions of the phase diagram. Typically, the G surface cubic is found at the dry end of the phase diagram and the D and P surface cubics occupy the well hydrated end.

The one internal characteristic which clearly distinguishes between the different cubic structures is the mean curvature at the lipid water interface. In this section we write a simple curvature free energy model for the cubic phases which includes both mean curvature and Gaussian curvature terms. Within this formulation all other contributions to the free energy, such as chain packing, are ignored, or assumed to change minimally across the phase transition. This assumption may be particularly dangerous if there were a considerable packing free energy contribution. Despite this, the curvature model is found to give the proper qualitative flavor of the transition in the sense that it predicts the location of each cubic in the phase diagram with respect to the others. Future refinements that include a packing free energy term will undoubtedly improve the quantitative accuracy of the theory. Such a term will be difficult to measure, however, due to the poor precision of the lipid length measurement.

A complete curvature free energy for a lipid monolayer given to second order in the curvature can be written as [35, 44, 45]:

$$f_c = 2K(c_l - c_0)^2 + \tilde{K}g,$$

where $f_c$ is the free energy per unit area, $c_0$ is the spontaneous curvature, $c_l$ is the mean curvature, $g$ is the Gaussian curvature and $K$ and $\tilde{K}$ are the elastic bending constants for
mean and Gaussian curvature, respectively. Ideally, the spontaneous curvature, \( c_0 \), is the curvature that the monolayer would assume if it were free from all other constraints. It is also sometimes termed the intrinsic curvature of the monolayer. As it stands, this equation has three unknowns: \( K \), \( c_0 \) and \( \tilde{K} \), and two quantities that can be determined from the diffraction data: \( c_I \) and \( g \). Since the free energy only needs to be compared on a relative scale, an overall scale factor can be factored out, eliminating one of the unknowns. Dividing through by \( 2K \) results in the scaled free energy

\[
f_c/2K = (c_I - c_0)^2 + rg, \tag{9}
\]

where \( r \) has been defined as \( r \equiv \tilde{K}/2K \). One is also allowed to arbitrarily decide what surface within the monolayer the mean and Gaussian curvature are defined with respect to. For example, they can be defined at the headgroup surface, or somewhere within the monolayer. Rand and co-workers suggest that the “neutral” or “pivotal” plane is the appropriate surface at which to define curvature. For the \( \Pi \) phase, the area per lipid at the pivotal plane surface experiences the minimum change with dehydration [46]. For this paper, we will consider the position of the “pivotal” plane to be an invariant parameter of the material, although that has not yet been proven to be the case. The curvature model will be examined with the curvature defined to both the headgroup surface and the pivotal plane surface.

For any monolayer, we will define the radius to the headgroup surface as \( R_W \) and the radius to the pivotal plane as \( R_{pp} \). In the free energy expression (9), the curvature, \( c_I \), can be defined to either the surface at \( R_W \) or the surface at \( R_{pp} \). In each case, the spontaneous curvature of the monolayer, \( c_0 \), will also be defined with respect to the same surface. Gruner and co-workers have shown that the spontaneous curvature in the \( \Pi \) is nearly identical to the monolayer curvature expressed at full hydration [46, 47]. Therefore, if the internal dimensions of the \( \Pi \) phase unit cell are known, we can say that the spontaneous curvature, \( c_0 \), is \( 1/R_W \) when curvature is measured at the monolayer surface, or \( 1/R_{pp} \) when curvature is measured at the pivotal plane. Since there are several points in the phase diagram where the \( \Pi \) phase exists at full hydration the spontaneous curvature can be determined as a function of temperature in that region. Extrapolating linearly downward in temperature into the cubic region gives the spontaneous curvature of the monolayer as a function of temperature, \( c_0(T) \). This approximation is made out of necessity because the spontaneous curvature has only been determined in excess water \( \Pi \) phases [46, 47]. Given \( c_0(T) \), only \( r \) needs to be evaluated in order to calculate the scaled free energy of the different cubic phases. The discussion that follows shows how \( r \) can be determined from the point of full hydration, or excess water point, of the \( \Pi \) surface cubic phase.

To find the appropriate free energy for comparison among the cubic phases, take the average of equation (9) over the area of the unit cell. The result is

\[
< f/2K > = \frac{1}{A_i} \int_{A_i} (f/2K) dA = \frac{c_i^2}{A_i} - 2c_0 < c_i > + c_0^2 + r \int_{A_i} g dA = \frac{c_i^2}{A_i} - 2c_0 < c_i > + c_0^2 + \frac{2gX}{A_i}, \tag{10}
\]

where the Gauss-Bonnet theorem (Eq. (4)) has been used to evaluate the average of the Gaussian curvature. The mean value of the curvature can be evaluated with the aid of equation (7), and the second moment of the mean curvature, \( < c_i^2 > \), can be written as,

\[
< c_i^2 > = \frac{X}{A_i} H_a \left( \frac{\partial^2 \chi}{d^2 A_0} \right), \tag{11}
\]
where the function $H_2(t)$ can be calculated from the following expansion [14, 48]:

$$H_2(t) = \frac{1}{2} \sum_{n=0}^{\infty} (0.959445t)^{n+1} S_{n+2}. \quad (12)$$

The first 8 values for $S_n$ have been computed: $S_1 = -4\pi$, $S_2 = 100.294$, $S_3 = -865.936$, $S_4 = 7.8225 \times 10^3$, $S_5 = -7.28926 \times 10^4$, $S_6 = 6.9552 \times 10^5$, $S_7 = -6.76558 \times 10^6$ and $S_8 = 6.68197 \times 10^7$. The first 6 terms of this expression converge to within 1% for $\phi_L < 0.73$ [14].

The free energy (Eq. (10)) is a function of the mean curvature and the Gaussian curvature, which can be written as functions of any two of the following three variables: $\phi_L$, $d$ and $l$. Our curvature approximation does not contain any terms which depend strongly on the length of the lipid, such as a hydrocarbon packing free energy, so the lipid length will be considered a constant in the following calculation. This approximation can be crudely justified by the observation that the lipid length remains approximately constant across the cubic-cubic phase transition boundary (Fig. 6). Given this caveat, the minimum of the free energy with respect to $\phi_L$ should occur at the excess water point. Since $\phi_L$ and $d$-spacing are mathematically related along the excess water boundary line, the minimization can be done equally well with respect to $d$. The simple dependence of the free energy on $d$ makes this easier to carry out analytically. Since the excess water point is known for the D surface cubic (Fig. 3), the last free parameter in the free energy, $r$, can be determined. Once the free energy is known, it can be used to predict the D to G surface phase transition and compared with the observed phase transition.

Using equations (7) and (11), the free energy equation (Eq. (10)) can be rewritten as:

$$f/2K = \frac{\chi}{A_\nu} H_2 \left( \frac{r^2 \chi}{d^2 A_0} \right) + \frac{2\pi \chi}{A_\nu} (2c_0 l' + r) + c_0^2 \quad (13)$$

where the subscript $l'$ indicates the effective monolayer thickness when the curvature is defined to a surface other than the headgroup surface, as in the case of the pivotal plane.

Equation (13) above refers to a free energy per unit area. For comparison among different cubic phases, we would like to calculate the free energy per lipid molecule, $\mu$. This is easily accomplished by multiplying equation (13) by the total area in the unit cell, $2A_\nu$, and dividing by the number of lipid molecules in the unit cell, $\phi_L d^2/v_L$, where $v_L$ is the specific volume of a lipid molecule which we assume to be the same for the different phases. We thus obtain:

$$\mu = \frac{2v_L}{\phi_L d^3} \left[ \chi H_2 \left( \frac{r^2 \chi}{d^2 A_0} \right) + 2\pi \chi (2c_0 l' + r) + c_0^2 A_\nu \right]. \quad (14)$$

Minimizing equation (14) with respect to $d$ (for constant $l$), results in

$$\left( \phi_L d^3 \right) \left[ \frac{\partial H_2}{\partial d} + 4\pi \chi c_0 \frac{\partial l'}{\partial d} + c_0^2 \frac{\partial A_\nu}{\partial d} \right] - \frac{\partial (\phi_L d^3)}{\partial d} \left[ \chi H_2 + 2\pi \chi (2c_0 l' + r) + c_0^2 A_\nu \right] = 0, \quad (15)$$

which can be rearranged to read

$$r = \frac{\phi_L d^3}{2\pi \chi} \left[ \frac{\partial (\phi_L d^3)}{\partial d} \right]^{-1} \left[ \frac{\partial H_2}{\partial d} + 4\pi \chi c_0 \frac{\partial l'}{\partial d} + c_0^2 \frac{\partial A_\nu}{\partial d} \right] - \frac{H_2}{2\pi} - 2c_0 l' - \frac{c_0^2 A_\nu}{2\pi \chi} \quad (16)$$
Equation (16) relates the ratio of the Gaussian bending constant to mean-curvature bending constant, \( r \), to the equilibrium unit cell size, \( d \). As mentioned above we could have also found an equivalent relation in terms of the volume fraction of lipid, \( \phi_L \), in the excess water region (where \( d \) or \( \phi_L \) is free to vary). From this equation, and the extrapolated spontaneous curvature, we can determine \( r \) from the experimental data.

The derivatives appearing in equation (16) can be evaluated using equations (5), (6), and (12) to be,

\[
\frac{\partial (\phi_L d^3)}{\partial d} = 4A_0 / d, \quad (17)
\]

\[
\frac{\partial A_1}{\partial d} = 2A_0 d + 4\pi \chi l' \frac{\partial l'}{\partial d}, \quad (18)
\]

and

\[
\frac{\partial H_2}{\partial d} = d^{-1} \left( \frac{\partial l'}{\partial d} - \frac{l'}{d} \right) H'_2 \quad (19)
\]

with

\[
H'_2 = \frac{l'^2 \chi}{d^2 A_0} = \frac{d}{2l'} \sum_{n=0}^{\infty} (2n + 2)(0.959445 \frac{l'^2 \chi}{d^2 A_0})^{n+1} S_{n+2}. \quad (20)
\]

These equations are constructed so they can be evaluated for any effective monolayer thickness \( l' \), as in the case when the curvature is defined to the pivotal plane rather than the lipid headgroup surface.

The effective monolayer thickness at the pivotal plane, \( l' \), can be related to the true lipid monolayer thickness, \( l \). By definition, the pivotal plane surface divides the lipid volume into two regions: a fraction \( p \) contained between the pivotal plane and the chain termini and a fraction \( (1 - p) \) between the plane and the headgroups. We can, therefore, define an effective volume fraction

\[
\phi'_L = p \phi_L, \quad (21)
\]

which is the fraction of the total lipid volume enclosed by the pivotal plane. With this, the effective monolayer thickness at the pivotal plane can be determined by substituting the relation for \( \phi \) from equation (5) into equation (21) and getting:

\[
2A_0 d^2 l' + \frac{4\pi}{3} \chi l'^3 = p(2A_0 d^2 l + \frac{4\pi}{3} \chi l^3). \quad (22)
\]

Differentiating this equation determines the derivative of \( l' \) with respect to \( d \) to be

\[
\frac{\partial l'}{\partial d} = \frac{4A_0 d (p l' - l')}{2A_0 d^2 + 4\pi \chi l'^2}. \quad (23)
\]

The equations derived above can now be used to relate the free energy of each of the cubic phases to one another.

Evaluating these equations at the excess water point for the D surface results in a value for \( r \), the ratio of the Gaussian bending constant to the mean curvature bending constant. As an example, we can calculate \( r \) at 45 °C. For the D surface cubic the excess water point is \( \phi_L = 0.643 \) and the \( d \)-spacing is \( d = 91.5 \) Å, leading to \( l = 16.5 \) Å (Eq. (5)). If the curvature is defined with respect to the headgroup surface then the extrapolated water core radius for the \( H_II \) phase is \( R_W = 18.0 \) Å (Fig. 8), and, therefore, the spontaneous curvature is \( \sigma_0 = 36^{-1} \) Å\(^{-1}\). Plugging these values into equation (16) results in \( r = \tilde{K}/2K = -0.227 \). Following the same procedure, \( r \) was calculated for all temperatures between 45 and 70 °C and is shown in figure 9.
Over this temperature range, \( r \) changes from \(-0.227\) to \(-0.057\). With the curvature defined to the headgroup surface, this theory has two problems: first, the temperature dependence should be explicitly contained in the temperature dependent value of the spontaneous curvature, \( c_0 \), not \( r \), and second, the sign of \( K \) would cross zero near 76 °C. The Gaussian curvature for these surfaces is negative because the principle curvatures are in opposite directions. A positive value of \( K \) means that the free energy is reduced at a saddle point, or to say it another way, the surface is unstable to saddle type fluctuations. This is physically unreasonable because the surface can arbitrarily add saddle curvature, reducing the free energy, while holding the mean curvature constant. If this were the case, the minimal surfaces with very large negative Euler characteristics (\( \chi \)) would be the stable phases. Since this is not observed it is expected that \( K \) should be negative, and therefore the free energy is stable against saddle fluctuations. The theory just described fails in the experimentally observed temperature region. Failure of the theory may mean that the form of the free energy expansion for curvature may be invalid. For example, it may require higher order terms. Since the radius of curvature of these surfaces \( (c_l^{-1}) \) is of the same order of magnitude as the lipid monolayer thickness the surface to which
the curvature is defined is arbitrary. By locating that surface at the pivotal plane described by Rand and co-workers [46] we find that the results improve considerably.

For the HII phase, the pivotal plane is determined by finding the point within the monolayer where the area per lipid experiences the smallest variation with water composition. This plane is found to be very roughly near the middle of the monolayer and is defined by the fraction of the lipid monolayer excluded from within one side of the surface. For example, in the HII phase, the surface encloses the water in the central tube and a fraction 1 - p of the lipid volume. The same prescription will work for the cubic phases, although it may be more difficult to visualize. The minimum area variation is a soft function of location within the monolayer, so the surface can only be determined to ± (3 to 4) Å. For our calculation, we will arbitrarily pick the location that includes 50% of the lipid volume, which is near the center of the monolayer. As an example, we calculate r at 45 °C for the curvature defined at the pivotal plane. The new effective lipid fraction, φ' L, is one half of the true lipid fraction, so φ' L = φL/2 = 0.3215. At d = 91.5 Å this gives an effective monolayer half-thickness (distance from bilayer mid-plane to pivotal plane) of l' = 7.8 Å. The spontaneous curvature at the pivotal plane can be determined from the radius at the half volume fraction radius (Rpp) for the HII phase and extrapolating to 45 °C. This procedure yields a value of c0' = 1/2Rpp = 53.42−1 Å−1. Plugging these values into equation (16) results in r = −0.146. Figure 9 shows a plot of r as a function of temperature overlaid on the plot where curvature is defined to the headgroup. The value ranges from a maximum of −0.132 to a minimum of −0.146, with an average of −0.139. The small temperature dependence is more reasonable and the value of r is consistent with the results of a recent calculation of the ratio for di-block polymer systems [45, 49]. Physically, this is a sensible value because the resulting Gaussian free energy is the same order of magnitude as the mean curvature free energy.

Given the values of r deduced above, the free energy can be calculated as a function of composition for both the D and G surface cubics to see if the model predicts the observed phase transition. The monolayer thickness, l, can be held constant at the excess water D surface value while the composition is varied and the free energy is calculated.

Using the curvature form defined to the pivotal plane, the free energy per lipid from equation (14) was determined for the both the D and G surface cubics for constant monolayer thickness as a function of water composition. The resulting curves are shown in figure 10 at both 45 °C and 60 °C, with the value of r taken to be r = −0.139. The excess water line shows the experimentally measured excess water point as a function of temperature. The similarity of the two free energy curves displays the close topological relationship between the surfaces. Nevertheless, the curves behave almost exactly as they would be expected to behave: the G surface has the lowest free energy in the dry end of the composition region, while the D surface is favored at the wet end. This is the same pattern that is observed experimentally: the G surface appears at the dry end of the phase diagram and the D surface is found at the wet end.

Quantitatively, the model requires some subtle improvement. The minimum of the D curve roughly matches the experimental excess water point, and it would match exactly if the weakly temperature dependent value of r were used instead of the average over all temperatures. The common tangent construction can be used to determine the predicted coexistence region for the D and G surface cubics. Unfortunately, the two curves have exactly the same minimum so the tangent common to both curves connects their minima, which are separated by 0.5 to 1 wt. %. The width of the coexistence region for the experimental data is about 1 to 2 % (Fig. 2), in reasonable agreement. However, the experimental phase diagram has a thin region of D surface cubic below the excess water line while the model predicts coexistence up to and including the excess water point for the D surface. That means that the D surface can never exist at lower free energy than the G surface, even when bulk water is available, and therefore
should never exist as a single phase. The G surface cubic should always be coexisting with the D surface cubic at high water contents because there is no reason to prefer one or the other since their free energies would be identical. Something must break the degeneracy because the D surface cubic is experimentally observed as a single phase in excess water.

This problem is compounded when the third of the common IPMS cubics, the P surface, is added to the plot. This phase is observed with much less frequency than the other two phases, and is usually found at the cool, wet end of the phase diagram. It was not observed for the rac-di-12:0-β-GlcDAG lipid system. As can be seen in figure 11, the minimum of the P surface free energy occurs at higher water content than either the D or G surface, in qualitative agreement with the usual conditions for its formation. Once again, however, the minimum free energy is degenerate to the D and G surface minima. This again implies that all of the surfaces will be equally preferred at high water contents, in contrast to the known experimental data.

All the features described above can be seen directly from the free energy function, equation (14), upon close inspection. In this curvature elasticity theory, the difference among various cubic structures is characterized by only two topological parameters: $\chi$ and $A_0$. With some algebra, one can show that the free energy, equation (14), can be brought into a single scaling form for all three cubic phases:

$$\mu = t^{-3} v L M(t)$$

(24)
where the scaling variable is defined as $t = \frac{l^2 \chi}{d^2 A_0}$.

The single scaling function implies that all 3 cubic structures have the same free energy minimum, at some value of $t^*$ ($t^* < 0$ because $\chi < 0$). Because the topological constants $\chi$ and $A_0$ are different for cubics, the minimum free energy will occur at different values of $d$ or $\phi_L$. In fact, with the aid of equation (5), it is possible to see which cubic phase appears at the dry end and which one appears at the wet end of the phase diagram as we can show with the following argument. At the free energy minimum, $l/d^* = (t^* A_0/\chi)^{1/2}$. Using equation (5), we obtain the lipid volume fraction at the minimum to be,

$$\phi_L^* = 2A_0(t^* A_0/\chi)^{1/2} + \frac{4\pi}{3} \chi(t^* A_0/\chi)^{3/2} = \text{const} \times A_0^{3/2} |\chi|^{-1/2}$$

(25)

From table II, we can see easily that $(\phi_L^*)_G > (\phi_L^*)_D > (\phi_L^*)_P$. This, together with the concaveness of the free energy curves, implies the sequence $G \rightarrow D \rightarrow P$, as the water content is increased.

The degeneracy of the free energy minima for the different cubic structures is likely a consequence of keeping only up to quadratic terms in the curvature expansion. Higher order curvature terms and/or thermal fluctuations [50] can break this degeneracy. Furthermore, small contributions from other possible free energy terms, such as a hydrocarbon packing en-
ergy or microscopic (non-continuum) interactions, will also break the degeneracy predicted by the simple curvature model. At present, a general expression for packing energy remains elusive. In addition, one would like to compare the overall free energy of the cubic phases with both the lamellar and H_{II} phases in order to predict the full phase diagram. However, this may require going beyond the simple curvature theory presented in this paper to include the additional free energy contributions. Nevertheless, in spite of its inherent approximations, the simple curvature theory is in qualitative agreement with the experiments. We believe that is captures most of the essential physics involved in the phase transition. The deficiencies can only be considered as motivation for further study in this area.

5. Conclusion.

A description of the delicate energetics which control the phase behavior of lyotropic liquid crystal systems, such as lipid-water dispersions, requires quantitative knowledge of the structures one is trying to understand. Given a simple model for the distribution of matter within the phase, the important structural parameters, such as monolayer thickness, water thickness, monolayer curvature, and area per molecule, can be determined using X-ray diffraction to measure the lattice repeat spacing and knowing the composition, as was shown by Luzzati [21]. By assuming that the cubic structures are described by lipid monolayers draped across infinitely periodic minimal surfaces, we have calculated the structural dimensions for the rac-di-12:0 β-GlcDAG + water system over all regions of the phase diagram. This allows not just for comparisons between the cubic phases but also for comparison with the dimensions of the lamellar and H_{II} phases. Thus, this system provides a quantitative test for any theory which claims to plausibly describe the lyotropic phase transitions among these phases.

One may expect that any theory of the lyotropic phase transitions to non-lamellar phases must be very general, since the topology of the rac-di-12:0 β-GlcDAG phase diagram is very similar to the monoelaidin + water phase diagram [5]. The phenomenological curvature model described above is very general in its application yet is a powerful tool for qualitatively (or semi-quantitatively) predicting phase transitions among the cubic phases. It is surprising that such a simple model, given only to second order in curvature, is useful for determining phase boundaries and coexistence regions, particularly because the physics was derived for the bending of thin sheets and applied to the bending of thick sheets (monolayers). It is not known over what range of curvature this free energy expansion is valid. It is obvious, however, that the notion of the pivotal plane is crucial to the its success in the model above.

One of the most important results of the theory is an estimate of the Gaussian curvature bending modulus for the lipid monolayer. The sign of the modulus is consistent with the expectation that the monolayer must be stable against saddle type fluctuations, that is, fluctuations where the mean curvature remains constant but the Gaussian curvature decreases without limit. The negative sign of the modulus ensures an increase in free energy for any decrease in Gaussian curvature. The magnitude of the modulus tells us that the free energy is distributed roughly equally between mean curvature energy and Gaussian curvature energy, and therefore, Gaussian bending is an important component of any description of phase transitions among cubic phases.

Finally, the model is incomplete as it stands. A second "competing" term must be added to the free energy for the theory to quantitatively describe transitions among the cubics. Ideally, that term should be chosen to allow free energy comparison of the cubics with lamellar and H_{II} phases, which cannot be described by only a curvature free energy. Kirk, Gruner and co-workers have suggested that the free energy of packing lipid hydrocarbon chains is the other term which must be considered along with monolayer curvature [35, 51, 52]. Certainly
the packing environment for a lipid changes greatly between the lamellar and non-lamellar phases, yet, no simple quantitative measure of the packing free energy has been developed, although theoretical estimates are beginning to be considered [53]. It is heartening to note, therefore, that among some of the non-lamellar phases, curvature alone can successfully be used to approximately map out a great deal of the interesting phase behavior.

References