Reply to "Hydrocarbon chain conformation in the H_{II} phase"

Dear Sir:

Gruner takes issue with wording in my paper (Scherer, 1989*b*) which attempted to describe, in what are admittedly too few words, the essence of his theory of "intrinsic curvature," as applied to the structure of the inverted hexagonal phase. He objects that his theory does not depend on assumptions of the state of interdigitation of chains and that he expects that interdigitation does occur (Tate and Gruner, 1987, Fig. 1).

My statement that "an underlying assumption of his hypothesis is that the CH_2 chains of the lipids around a hydrophilic tube are not interdigitated, thereby leading to a strain in those chains that are pointed in the *b* lattice direction" was prompted by the following texts:

"We assume that, for a given lipid, temperature, and geometry, the lipid tails have a well-defined mean length that is the result of the number of gauche rotameric carbon bonds excited in the hydrocarbon. For example, in the H_{II} phase (Fig. 1), we take this length to be approximately equal to $d_{H_{II}}$ (from Gruner, 1985)." The illustration in his Fig. 1 depicts some interdigitation of chain ends.

"Since the corners of the hexagons are further from the central water core than the sides, the lipid must have a distribution of lengths ranging from $d_{H_{II}}$ to d_{max} if it is to fill the cell . . . The distribution of lengths is possible since the lipids are not rigid molecules above T_c, the chain melt temperature. Above this temperature, many gauche rotamers exist within the acyl chain, allowing the chains to exist in many possible configurations, providing the distribution of lengths necessary for the H_{II} phase (from Tate and Gruner, 1987)." In Fig. 1 of this paper, they state that "the H_{II} phase consists of cylinders of water (radius R_w) on a hexagonal lattice surrounded by the lipid. The lipids must take on lengths from $d_{H_{II}}$ to d_{max} if they are to fill the lattice . . . In a real system there would, of course, be more interdigitation of the chain ends than is shown in this cartoon." The cartoon shows no interdigitation of chain ends.

While I admit that the intrinsic curvature hypothesis does not make assumptions about interdigitation, Gruner's chain packing arguments do restrict chain interdigitation. Gruner's early work assumes that the mean distribution of chain lengths falls at the value of $d_{H_{II}}$. Tate and Gruner (1987) assume that the mean distribution of chain lengths must fall between $d_{H_{II}}$ and d_{max}, where $d_{H_{II}}$ represents the average chain length for the "relaxed" state and d_{max} represents the average length for the maximally "stressed" state. They only allow the chain ends to interdigitate. In my analysis (1989*b*), the "mean fluid chain length," $d\star$, determined from the average hydrocarbon chain volume ($V_{hc} = Sd\star$), has values at maximum hydration of 10.3 Å for C_{12} and 19.2 Å for C_{20} chains. These values are quite close to the values for *b*, the shortest distance between the hydrophobic/hydrophilic boundary (H/hc) and the central point between three adjacent tubes. *b* is similar to d_{max}, but not equivalent because of the boundary difference. $d\star$ is substantially larger than *c*, the distance between the H/hc boundary and the midpoint between adjacent tubes (which is analogous to $d_{H_{II}}$), and requires a chain overlap length of 6 Å for DDPE (62% of $d\star$) and 12 Å for DAPE (65% of $d\star$) at maximum hydration. Thus, the calculated (average maximum) overlap suggests more than an interdigitation of chain ends. At lower hydrations $d\star$ becomes larger than *b*, indicating interdigitation of the central region between tubes as well as between adjacent tubes.

In his letter, Gruner claims that "the model presented by Scherer is an extreme case in which the radial chain length variation is reduced. This necessarily results in large variations of the local hydrocarbon density or in sideways deformation of the chains, as may be seen by considering the overlap volumes of adjacent H_{II} tubes in Fig. 4 of Scherer (1989)." The model which I presented *only* depends on the position of the H/hc boundary and straightforward volumetric calculations. The magnitude of $d\star$ is obtained directly from knowledge of the level of hydration and the partial specific volume of lipid. My interpretation of the value of $d\star$ as indicating longer mean chain lengths does narrow the distribution of mean chain (radial) lengths. It follows that the variation in "sideways" deformations would be reduced because longer interdigitated chains between adjacent tubes need fewer gauche conformations. Thus, the difference between the number of gauche conformations in chains in the *b* and *c* lattice directions is reduced. Gruner's model requires that chains in the *c* lattice direction have more gauche bonds than those in the *b* lattice direction.

I do not agree with the statement that reducing the radial chain length distribution necessarily results in large variations in the chain density. Consider a lamellar system with fluid chains. Increase the splay of the chains (by changing the curvature at the H/hc boundary), but keep the mean chain length the same. This would create a gradient of chain density from normal, close to the head group, to very low at the ends of the chains. Interdigitating chains from lipids in the adjacent tube would allow the chain density to move back toward the normal state. Splay in the *b* lattice direction also creates a decrease in density. In this case, the density decrease is counteracted by the presence of chains from lipids in two other tubes. These are only qualitative arguments and I agree completely with Gruner that a detailed study of hydrocarbon packing in this system using statistical mechanics should be attempted.

One may question whether the longer mean chain lengths implied by the approximation that $d\star \approx b$ for fully hydrated lipids are consistent with the observed fluidity of the chains. The mean fluid chain length, $d\star$, for the H_{II} phase of DAPE at 99°C has very nearly the same value (19.2 Å) as the average hydrocarbon chain length $d_{hc}/2$ for the lamellar phase at 95°C (calculated from the data of Seddon et al., 1984, using the procedures given by Scherer, 1989*a*).[1] Thus, lengthening the

[1] It may be easily shown that when the *d* spacing for the L_α phase equals the *d* spacing ($a\sqrt{3}/2$) for the H_{II} phase (equal water content) and the partial specific volume of the lipids are equal (equal hydrocarbon volume or same temperature), the following identities hold for structural parameters of the L_α and H_{II} phases: $S(L_\alpha) = S(H_{II}$ as defined by Scherer, 1989*b*); $d_{hc}/2(L_\alpha) = d\star\ (H_{II})$; and $d_H\ (L_\alpha) = d_H/2(H_{II}) = R_c$, the radius of the hydrophilic cylinder.

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radial distribution of chain lengths for the H_{II} phase to values of the order of b should not adversely affect the chain fluidity in the H_{II} phase. Because the lengths of fully extended C_{12} and C_{20} chains are ~15 and 25 Å, chains with lengths $d\star$ still contain enough gauche rotamers to be fluid. It should be noted that the enthalpy of the transition from L_α to H_{II} is very small (Seddon et al., 1983) which indicates that the differences in the number of gauche rotamers for these two phases, at the same temperature, are not large.

Gruner objects to my statement which disclaims the importance of stress because of the small effect that adding small amounts of tetradecane to DOPE has on the observed dimensions (lattice spacings or tube radii) because he believes it inferred that they had drawn a correlation between stress and tube radii. No such inference was intended. Gruner (1985, Fig. 1) considered chains with lengths $< d_{H_{II}}$ to be "nearly relaxed," and those which extend into the central region between three tubes as "stressed." However, if the mean chain length is only half the length of a fully extended chain or 70% of the mean chain length in the L_α phase at the same temperature, the chains between adjacent tubes would appear to be in a more disordered or higher energy state relative to the more normally extended chains in the b lattice direction. Addition of alkane into the central region between tubes should enlarge the lattice and permit the highly disordered chains between adjacent tubes to extend to more normal fluid chain lengths. Because adding small amounts of alkane expands the lattice only slightly (Gruner et al., 1986), it seemed reasonable to propose that the energy of the chains between tubes is lowered by interdigitation, thereby minimizing stress throughout the hydrocarbon matrix.

Gruner points out that "the spontaneous curvature and the amount of water available control the tube diameter." I agree with this statement. However, determining the consequences of changes in "spontaneous curvature" quantitatively is difficult because "spontaneous curvature" is not a function of a single variable, but depends on both head group and hydrocarbon chain volume with the latter quantity depending on chain length and temperature.

Gruner's objection to my statement that "the reduced strain hypothesis has been invoked to explain observed increases in tube radii from incorporation of relatively longer chain lipids . . ." is valid. I should have added . . . with small amounts of alkane. In this case, not only is the L_α–H_{II} transition temperature, T_{bh}, lowered, but the tube radii increase as well.

Gruner takes issue with two of my conclusions: one "encompassed in Eq. 14" and a second "in the prediction of a central vacuum void." My analysis did not "predict" a central void at zero hydration. However, it did show that the presence of a central void could account for the 2 Å discrepancy between the height of the PE headgroup in the lamellar crystalline phase (≈6 Å) and the calculated tube radius of the dehydrated H_{II} phase (≈8 Å). The computed "void" volume is only 20 $Å^3$ per lipid compared with a total lipid volume of 1,000 $Å^3$ and should not be entropically expensive. If any water does remain in the "void" region, it amounts to less than one water molecule per lipid and does not greatly affect observed lattice spacing. Whether or not a small amount of water occupies or does not occupy the "void" region is unimportant to the calculation of the size of the "void" volume or the tube radius. The assumption of no radial disorder in the head groups seemed reasonable and, as stated in the paper, was prompted by the obvious presence of dipole/dipole interactions and lateral hydrogen bonding, which are known to exist in crystalline (tightly packed) PE headgroups.

Contrary to Gruner's statement, Eq. 14 did not follow from any comparisons between b and the $d_{hc}/2$ distance of the L_α phase. Rather, it followed from the observation that the calculated values of $d\star$ (H_{II} phase) for the fully hydrated states of DDPE and DAPE are quite close to the calculated lattice distance b. These two distances need not be the same, and are clearly not so at reduced states of hydration. I should also note that this relationship, which seems to hold approximately for C_{12} and C_{20} chains of saturated lipids, should be tested further by comparing the values of $d\star$ and b for saturated PE lipids of intermediate chain length.

It should be noted that my Eq. 14, which uses the approximation that $d\star \approx b$, implies some basic relationships between structural parameters for all H_{II} phases at maximum hydration. For example it follows that

$$V_H/V_{hc} = R_c/2d\star \approx 0.7021. \quad (1)$$

Increasing the hydrocarbon volume by addition of alkane (at constant temperature) should increase the value of $d\star$, which requires that R_c and the hydration per lipid increase. Increasing the average head group volume by addition of PC (which would also increase the average headgroup hydration) should require an addition of alkane to maintain the balance indicated by Eq. 1. Dehydration obviously decreases the value of the ratio in Eq. 1.

The change in structural parameters with temperature was not considered in my earlier paper. However, Tate and Gruner (1989) have recently measured the hydration and temperature dependence of the H_{II} lattice of DOPE and DOPE/DOPC mixtures. They use the Luzzati boundary in their analysis of the x-ray data and find that the volume per lipid V_l divided by the lipid surface area is approximately constant and has values of 25.4, 26.0, and 25.0 Å at temperatures of 10, 50, and 90°C, respectively. The analogous ratio, using the H/hc boundary, is $V_{hc}/S = d\star$. Using their data, the value of $d\star$ decreases only slightly from 16.72 to 16.69 to 16.04 Å at the same temperature points. Thus, increasing the temperature increases the surface area with very little effect on $d\star$. Over the same increase in temperature, the value of R_c decreases from 27.6 to 23.1 to 20.5 Å. The exact values of the ratio in Eq. 1 are 0.824 Å at 10°C, 0.693 Å at 50°C, and 0.638 at 90°C. Thus, the range of values for this ratio fall on either side of the approximate value given in Eq. 1. The ratio is higher when $d\star$ is less than b, and lower when $d\star$ is greater than b (b has values of 17.5, 16.6, and 15.6 Å at 10, 50, and 90°C). c, which is analogous to Gruner's $d_{H_{II}}$, has values ranging from 11.5 to 11.3 to 10.7 Å. The values of $d\star$ are closer to the values for b than for c, at all temperatures.

One might speculate why the behavior of $d\star$ with temperature is so much different than the behavior of $d_{hc}/2$ for lamellar phases. The DMPC L_α phase shows increases in the surface area of 5 $Å^2$, and decreases in the bilayer thickness of the order of 3 Å, over a temperature range of 20 to 30°C (Janiak et al., 1976, 1979). Both effects are attributable to an increase in the number of gauche rotamers. In the DOPE H_{II} phase, the interdigitated chains need not compress as much as in the lamellar case

because the increase in volume can be accommodated by small increases in S. The calculated change in S at the H/hc boundary ranges from 57.2 Å^2 at 10°C to 63.9 Å^2 at 90°C. The value of S for DOPE in the lamellar phase at 2°C is 66.3 Å^2 and the value for $d_{hc}/2$ is 14.3 Å (calculated from the data of Tate and Gruner, 1988, using the method of Scherer, 1989*a*). The longer average fluid chain lengths (by 2 Å) and smaller surface areas (by 9 Å^2) of the H_{II} phase are consistent with the notion that smaller surface areas produce longer chains. Unlike the surface areas calculated for the H_{II} phase using the Luzzati boundary (Tate and Gruner, 1989), the values of S assuming the H/hc boundary are directly comparable with the lamellar phase.

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