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Role of Phospholipid Asymmetry in the Stability of Inverted Hexagonal Mesoscopic Phases

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The role of phospholipid asymmetry in the transition from the lamellar (L_{α}) to the inverted hexagonal (H_{II}) phase upon the temperature increase was considered. The equilibrium configuration of the system was determined by the minimum of the free energy including the contribution of the isotropic and deviatoric bending and the interstitial energy of phospholipid monolayers. The shape and local interactions of a single lipid molecule were taken into account. The minimization with respect to the configuration of the lipid layers was performed by a numerical solution of the system of the Euler–Lagrange differential equations and by the Monte Carlo simulated annealing method. At high enough temperature, the lipid molecules attain a shape exhibiting higher intrinsic mean and deviatoric curvatures, which fits better into the H_{II} phase than into the L_{α} phase. Furthermore, the orientational ordering of lipid molecules in the curvature field expressed as the deviatoric bending provides a considerable negative contribution to the free energy, which stabilizes the nonlamellar H_{II} phase. The nucleation configuration for the L_{α}–H_{II} phase transition is tuned by the isotropic and deviatoric bending energies and the interstitial energy.

I. Introduction

In aqueous solution, phospholipids, like other amphiphilic molecules, undergo a self-assembly process, mainly due to the hydrophobic effect. If dissolved in water, phospholipids form structures with a hydrophilic surface directed toward the water, while their hydrophobic chains are shielded on the inside of the aggregates. Within the attained configurations of lipid molecules, different lyotropic mesophases are discerned.^{1–4} The biologically most relevant phase is the fluid lamellar phase (L_α), which has been extensively studied.^{5.6} However, nonlamellar phospholipid phases are increasingly attracting interest due to their importance in cell life regarding transmembrane transport

of matter and membrane fusion.^{7–9} Moreover, applications of nonlamellar phases for interaction with membrane proteins are now being investigated intensively.^{10,11} For instance, in such areas as drug delivery, basic research has led to the assignation of diverse patents based on the inverted hexagonal and other different nonlamellar phases.^{12,13}

Transitions between different phospholipid phases and mechanisms that drive these transitions are of special interest. To interpret the experimental data and to contribute to a better understanding of the underlying mechanisms, theoretical models have been constructed.^{14,15} These include the temperature-dependent change of shape of the phospholipid molecules, expansion of the area of the interface between the phospholipid molecules and the solution, optimal packing of the hydrocarbon tails of phospholipid molecules, and intermonolayer interactions.^{6,14,15}

Physical properties and interactions within the nonlamellar phospholipid systems could be further elucidated from a viewpoint of recent theoretical results regarding the configuration of phospholipid molecules within membranous nanostruc-

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tures.¹⁶⁻¹⁸ Experiments have revealed high anisotropy in the curvature (a large difference between the two principal curvatures) in phospholipid bilayer nanotubules,¹⁹⁻²² torocytic endovesicles of erythrocyte membranes, 23,24 phospholipid bilayer membrane pores,^{25,26} and narrow necks of phospholipid bilayers connecting buds to the mother membrane.¹⁸ To explain the stability of these structures, a unifying theory was proposed based on the in-plane orientational ordering of phospholipid molecules in the local curvature field.¹⁶⁻¹⁸ The curvature field at the given point in the membrane is determined by the neighboring phospholipid molecules. As the phospholipid molecule is due to its structure (two tails and the head), in general asymmetric with respect to the axis pointing normal to the membrane, it could be expected that it would spend, on average, more time in the orientation that is energetically more favorable, that is, has lower energy. Starting from the singlemolecule energy and applying the methods of statistical physics, the free energy of a lipid bilayer was derived.^{18,21,27,28} The bending energy of laterally homogeneous thin shell 29-31 was recovered; however, an additional contribution due to orientational ordering of lipid molecules, that is, the contribution of the deviatoric bending, 32,33 was obtained. 28,21,18 The orientational ordering of anisotropic molecules lowers the free energy of the system; the effect is stronger for stronger anisotropy of molecules and stronger curvature anisotropy.¹⁸

In H_{II} and other nonlamellar phases likewise, there is a high anisotropy in the curvature in all points of the monolayer surfaces (in inverted cylinders, one principal curvature is equal to the inverse negative value of the radius of the cylinder and the other is equal to zero), and it can be expected that in this system, the deviatoric bending is important. In contrast, in the lamellar phase, there should be no deviatoric bending as both principal curvatures of the phospholipid layer are zero and all orientations of the phospholipid molecule are energetically equivalent. It was in our interest to determine the importance of the deviatoric effect in the H_{II} phase and in the $L_{\alpha}-H_{II}$ phase transition regime.

The direct L_{α} -H_{II} phase transition in palmitoyl-oleoylphosphatidylethanolamine (POPE) takes place upon increasing the temperature of the system over 74 °C. Structural data on POPE layers obtained by high-resolution small-angle X-ray diffraction⁶ was taken into account to validate our model. It was assumed that the intrinsic shape of the POPE molecule favors the inverted cylindrical configuration within the lipid monolayer and that the intrinsic anisotropy increases with increasing temperature. The equilibrium configuration of the system was determined by the minimization of the free energy of the lipid monolayers considering the bending and the interstitial energy contributions of the monolayers.

First, we estimated the effect of the ordering of phospholipid molecules by comparing the hypothetical planar, inverted cylindrical, and inverted spherical phases by using a simple model, where the interstitial energy was considered constant for a given phase. Then, the L_{α} and the H_{II} phase were compared by using the interstitial energy based on the stretching of the phospholipid tails. Finally, the formation of the H_{II} phase was simulated by a configuration of two adjacent bilayers with one embedded central cylinder. The obtained configuration promoting the L_{α} - H_{II} transition was compared with the experimentally obtained structural data of POPE.⁶

II. Theory

A. Free Energy of the Phospholipid Monolayer. We consider thin sheet-like formations (monolayers) composed of



Figure 1. A scheme and a corresponding electron density map of the lamellar fluid (L_{α}) phase (left) and of the inverted hexagonal (H_{II}) phase (right). The configurations of the lipid molecules are indicated. In the L_{α} phase, both principal curvatures are equal to zero, while in the H_{II} phase, one of the principal curvatures is equal to zero and the other one is negative. The data for the electron density reconstructions are taken from ref 6. The maps depict the POPE/water structures at 74 °C (compare also Table 1).

TABLE 1: Geometrical Parameters of the L_{α} and the H_{II} Phases of the POPE/Water Structures at $T = 74^{\circ}C^{\alpha}$

	$L_{\alpha}(74 \ ^{\circ}C)$	$H_{II}(74 \ ^{\circ}C)$
<i>d</i> , <i>a</i> [nm]	4.99	7.24
$d_{\rm pol}, r[\rm nm]$	2.5	2.67
$\zeta_0(\zeta_{\min}, \zeta_{\max})[nm]$	1.47	1.13 (0.95,1.51)
$a_0[nm^2]$	0.65	0.65
Н	0	0.187

 a The structural parameters are defined in Figure 3 from Rappolt et al. 6

lipid molecules. The equilibrium configuration of the system is given by the shape of the surfaces representing the monolayers (Figure 1).

The shape of the monolayer at any point can be described by the two principal curvatures C_1 and C_2 and the direction of the principal axes system. In the case of planar geometry (Figure 1), curvatures of all normal cuts including both principal curvatures are zero ($C_1 = C_2 = 0$), and all choices of the principal axes system are equivalent. The inverted hexagonal (H_{II}) phase (Figure 1) can be roughly described as composed of cylinders, in which the head groups of phospholipid molecules are directed toward the center of the cylinder ($C_1 = 0, C_2 < 0$) while in the inverted micellar (M_{II}) phase ($C_1 = C_2 < 0$).

The equilibrium configuration is determined by the minimum of the free energy of the system F

$$F = \min \tag{1}$$

at given constraints, which are relevant in the particular situation studied. We find that the observed configurations of the system correspond to the equilibrium states. Within this framework, it remains to be determined which contributions to the free energy and which constraints are the most relevant for the system. The free energy of lipid molecules in inverted hexagonal and lamellar phases may be expressed in terms of bending, interstitial, hydration, and van der Waals energies.¹⁴ It was shown, though,¹⁴ that the van der Waals energy and the hydration energy are much less important in the L_{α} to H_{II} transition than the bending and the interstitial contributions in the excess water conditions. For instance, the energy of membrane unbinding in the L_{α}-H_{II} transition

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Figure 2. Schematic representation of different intrinsic shapes of some isotropic and anisotropic lipids and detergents. Front and side views are shown.

is an order of magnitude lower than the bending energy.³⁴ Therefore, in the following, only the bending and the interstitial energy contributions are considered.

1. *Bending Energy.* The lipid molecule (the membrane constituent) is composed of carbohydrate tails and a head group. Its configuration in the layer can be described as a prolate-like structure, whereby the molecules are closely packed as to form a sheet-like continuum. A local curvature can be ascribed to this continuum at each chosen point. As there is no apparent evidence for the symmetry of this structure with respect to the axis perpendicular to the membrane, it is generally considered anisotropic.

It is assumed that some membrane constituent, due to its structure and local interactions, energetically prefers a local geometry that is described by the two intrinsic principal curvatures C_{1m} and C_{2m} .^{16,17} The intrinsic principal curvatures in general differ from each other (Figure 2) ($C_{1m} \neq C_{2m}$), meaning that the constituent is intrinsically anisotropic. In a special case when $C_{1m} = C_{2m}$, the constituent is isotropic. Isotropic constituents favoring zero curvature ($C_{1m} = C_{2m} = 0$) will form a planar monolayer, while the constituents with an inverted conical shape ($C_{1m} = 0, C_{2m} < 0$) will form an inverted hexagonal structure.³⁵ The intrinsic curvatures account for the shape of the molecule and local interactions of the molecule with its surroundings, including the hydration effects.¹⁴

The bending energy derives from the mismatch between the intrinsic and the actual membrane curvatures.²⁸ Previous models^{14,36} considered the mismatch between the intrinsic mean curvature and the actual mean curvature. Here, we have taken into account also the mismatch between the intrinsic and the actual curvature deviator and the orientation of the anisotropic molecule within the monolayer, which affects the energy of the molecule. It is assumed that a molecule will attain orientation corresponding to the lowest energy, and consequently, orientational ordering within the monolayer will take place. The ordering is enhanced by direct interactions between the molecules. The membrane lipid asymmetry is thereby assumed to influence the equilibrium configuration and the macroscopic features of the lipid system.¹⁸

To derive the bending energy, the monolayer area is divided into small patches of constant curvature, which however contain a large number of molecules; therefore, the methods of the statistical physics can be applied. We consider that all phospholipid molecules are equal and independent and are subject to the curvature field. The lattice statistics approach is used, drawing an analogy from the problem of the noninteracting magnetic dipoles in the external magnetic field,³⁷ the curvature



Figure 3. Geometry of the lamellar packing of monolayers and that of the hexagonal packing of inverted cylinders. One bilayer and one neighboring monolayer are depicted for the lamellar phase. The lattice repeat distance given in the polar extension (including water and headgroups) is defined as d_{pol} . Three cylinders of radius *r* are within a distance of *a* to each other. The parameter ζ_0 denotes the equilibrium length of hydrocarbon chains. As the distance between the cylinders is not constant, stretching or compressing of some hydrocarbon chains (shown by shading) is required in order to fill the voids.

deviator taking the role of the external magnetic field.¹⁸ The direct interactions between phospholipid molecules are taken into account by considering that the molecules, which are oriented in such a way that their orientational energy in the curvature field is lower, exhibit more favorable packing.¹⁸ Therefore, the energy of any molecule in the patch is lower if there are more like-oriented molecules in the patch.¹⁸ Considering all of the above, the bending energy of the monolayer is

$$F_{\rm b} = \int_{A} \frac{n_0 \xi}{2} ((H - H_{\rm m})^2 + D^2 + D_{\rm m}^2) dA - n_0 kT \int_{A} \ln \left(2 \cosh \left(\frac{\xi (1 + (\tilde{k}/kT)) D_{\rm m} D}{kT} \right) \right) dA$$
(2)

where $H = (C_1 + C_2)/2$ is the local mean curvature of the monolayer, $D = |C_1 - C_2|/2$ is the local curvature deviator, $H_m = (C_{1m} + C_{2m})/2$ is the intrinsic mean curvature, $D_m = |C_{1m} - C_{2m}|/2$ is the intrinsic curvature deviator, n_0 is the area density of the lipid molecules, ξ is the constant describing the strength of the interaction between a single lipid molecule and the surrounding membrane continuum, k is the Boltzmann constant, T is temperature, \tilde{k} is the constant describing the direct interaction between lipid molecules,¹⁸ and dA is the area element of the monolayer surface.

2. Interstitial Energy. In the lamellar phase L_{α} , the monolayers in contact with water are closely packed, and there are no voids (Figures 1 and 3). In other systems, as for example in the inverted hexagonal phase or in the inverted micellar phase, the distance between two adjacent monolayers varies over the monolayer surface. Some of the lipid tails have more space, while others are squeezed with respect to an average length (Figure 3). To avoid water pockets, the hydrocarbon tails of lipid molecules have to stretch accordingly. We note that in a hypothetical system of closely packed inverted micelles, the contribution of the void-filling energy is much larger than that in a system of inverted cylinders, as close packing is more impaired in three dimensions.

In a first approximation, the void-filling energy contribution is considered as a constant for a given simple configuration (cylinders or spheres)¹⁴ 16578 J. Phys. Chem. B, Vol. 112, No. 51, 2008

$$F_{\rm v}^{\rm H} = \chi \tag{3}$$

where the constant χ is different for different configurations. By using this simple model, we obtain a rough estimate of the energy contributions corresponding to different hypothetical phases.

In a more detailed description of adjacent monolayers, it was found that the deformation of a single lipid molecule is subject to Hooke's Law, so that the void-filling energy contribution is³⁸

$$F_{\rm v} = \tau n_0 \int_A \left(\zeta - \zeta_0\right)^2 \mathrm{d}A \tag{4}$$

where ζ is the length of the lipid molecule, ζ_0 is the reference length of the molecule, and τ is the stretching modulus of the lipid molecule.

Considering the geometry of the hexagonal phase (Figure 3), the length of the hydrocarbon tail may be expressed as

$$\zeta = \frac{a}{2\cos\varphi} - r \tag{5}$$

where *a* is the unit cell parameter defined in Figure 3, *r* is the radius of the water pore from the center of the pore to the pivotal plane,³⁹ and φ is the angle between the *y*-axis and a given position of the lipid molecule. Equation 5 is valid for 1/6 of the area of one cylinder, that is, the angle φ is defined in the range of $\varphi \in [-\pi/6, \pi/6]$. Considering the hexagonal symmetry and definition of the interstitial energy (eq 4), the average interstitial energy per lipid molecule in the inverted hexagonal phase can be expressed as

$$F_{v}^{H} = \frac{6}{\pi} \tau \left(\frac{a^{2}\sqrt{3}}{12} - a(r+\zeta_{0}) \ln \sqrt{3} + \frac{\pi}{6} (r+\zeta_{0})^{2} \right) \quad (6)$$

3. Determination of the Equilibrium Configuration of the *Planar, Inverted Cylindrical, and Inverted Spherical Systems.* The planar, inverted cylindrical, and inverted spherical systems were described as surfaces with constant principal curvatures. In the planar system H = D = 0, in the inverted cylindrical system $H = D = -1/2r_{cyl}$, where r_{cyl} is the radius of the cylinder, while in the inverted spherical system $H = -1/r_{sph}$, where r_{sph} is the radius of the sphere and D = 0. The minimal value of the free energy of a unit patch of the lipid monolayer with respect to the mean curvature H was calculated by using eqs 2 and 6 and the model constants given in the section entitled "Estimation of Model Constants".

B. Lamellar to Inverted Hexagonal Phase Transition. The temperature affects the configuration of phospholipid molecules in the head group and in the tail regions.³⁵ The motion of the hydrocarbon chain increases with increasing temperature; consequently, there is an increase of the relative number of the "gauche" C–C bond configurations which require more space in the lateral (in-plane) direction of the monolayer. Since there are two tails, they effectively spread in the direction of their alignment, thereby increasing also the anisotropy of the tail region.⁴⁰ In fact, a simple molecular-wedge-shaped model, which was applied to interpret experimental X-ray data in the inverted hexagonal phase of a PE/water system, demonstrates clearly that the wedge angle increases monotonously as a function of temperature.⁴⁰



Figure 4. Parametrization of the pivotal surface of two adjacent bilayers with an embedded cylinder (for details, see text). There is another closure on the right side of the central cylinder.

In order to describe the $L_{\alpha}-H_{II}$ phase transition, we follow a simple scenario, in which a configuration is imagined consisting of an inverted central phospholipid cylinder embedded in two adjacent lipid bilayers. The two inner lamellar monolayers are fused, exhibiting bulb-like closures on both sides of the cylinder, while the outer monolayer leaflets follow the contours of the cylinder and of the bulb-like closures (Figure 4). The chosen configuration is considered the smallest unit to study the nucleation process of the H_{II} hexagonal phase in the L_{α} phase and is based on experimental results.^{6,40}

To estimate the overall optimum configuration (the shape of the neighboring closures, the radius of the central cylinder, and the shapes of the neighboring enclosing monolayers), the minimum of the free energy of the system was determined. For this, an original approach was developed, which combines the solution of the Euler–Lagrange differential equations and the Monte Carlo simulated annealing numerical method, as described below.

As the temperature increases, $D_{\rm m}$ and $|H_{\rm m}|$ change, thereby affecting the equilibrium configuration. The nucleation configuration is considered as stable when the free energy per molecule of the configuration with the bulb-like closure is lower than the free energy per molecule of the all-planar configuration.

1. Determination of the Pivotal Map of the Closure by Minimization of the Monolayer Bending Energy. The surface of the monolayer forming a closure is described by the radius vector $\mathbf{r} = (x, y, z(x))$ (Figure 4), from where the mean and the Gaussian curvatures are $2H = (\partial^2 z/\partial x^2)/(1 + (\partial z/\partial x)^2)^{3/2}$ and C_1C_2 = 0. The surface is given in terms of the arclength *l*, so that sin $\psi = dz/dl$ and cos $\psi = dx/dl$. Considering the above, the mean curvature is expressed as $2H = 2D = d\psi/dl$, while the area element is dA = dl dy. Due to symmetry, only the part of the closure above on one side of the *x*-axis is considered in determination of the shape of the closure.

The coordinates, the area, the area element and the bending energy are written in dimensionless form. Normalizing the length by an arbitrary z_0 (in our case, we set $z_0 = 1$ nm) gives dimensionless curvatures $h = z_0H$, $d = z_0D$, $h_m = z_0H_m$, $d_m = z_0D_m$ and dimensionless arclength $\tilde{l} = l/z_0$. The area element is normalized to yz_0 . The bending energy is normalized to $n_0\xi y/2z_0$

$$f_{\rm b} = \int (h - h_{\rm m})^2 \mathrm{d}\tilde{l} + \int (d^2 + d_{\rm m}^2) \mathrm{d}\tilde{l} - \kappa \int \ln(2\cosh((1 + \tilde{k}/kT)\vartheta 2d_{\rm m}d)) \mathrm{d}\tilde{l}$$
(7)

where

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$$\kappa = 1/\vartheta = \frac{2kTz_0^2}{\xi} \tag{8}$$

To minimize the bending energy (eq 7), a functional

$$L = \left(\frac{1}{2}\frac{\mathrm{d}\psi}{\mathrm{d}\tilde{l}} - h_{\mathrm{m}}\right)^{2} + \frac{1}{4}\left(\frac{\mathrm{d}\psi}{\mathrm{d}\tilde{l}}\right)^{2} - \kappa \ln(2\cosh((1 + \tilde{k}/kT)\vartheta 2d_{\mathrm{m}}d)) - \lambda\left(\cos\psi - \frac{\mathrm{d}x}{\mathrm{d}\tilde{l}}\right) - \nu\left(\sin\psi - \frac{\mathrm{d}z}{\mathrm{d}\tilde{l}}\right)$$
(9)

where λ and ν are Lagrange multipliers, is minimized by solving a system of Euler–Lagrange equations

$$\frac{\partial L}{\partial \psi} - \frac{\mathrm{d}}{\mathrm{d}\tilde{l}} \left(\frac{\partial L}{\partial \psi_{\tilde{l}}} \right) = 0 \tag{10}$$

$$\frac{\partial L}{\partial x} - \frac{\mathrm{d}}{\mathrm{d}\tilde{l}} \left(\frac{\partial L}{\partial x_{\tilde{l}}} \right) = 0 \tag{11}$$

$$\frac{\partial L}{\partial z} - \frac{\mathrm{d}}{\mathrm{d}\tilde{l}} \left(\frac{\partial L}{\partial z_{\tilde{l}}} \right) = 0 \tag{12}$$

where $\psi_{\tilde{l}} = d\psi/d\tilde{l}$, $x_{\tilde{l}} = dx/d\tilde{l}$, and $z_{\tilde{l}} = dz/d\tilde{l}$. By introducing the variable

$$Y = x \frac{d\psi}{d\tilde{l}}$$
(13)

a system of equations (eqs 10-12) yields

$$\frac{\mathrm{d}\mathbf{Y}}{\mathrm{d}\tilde{l}} = \frac{\mathbf{Y}}{x}\cos\psi + \frac{(\lambda\sin\psi - \nu\cos\psi)x}{1 - [\kappa\vartheta^2 d_{\mathrm{m}}^2/\cosh^2(\vartheta d_{\mathrm{m}}\mathbf{Y}/x)]} \quad (14)$$

 $\lambda = \text{const}$ $\nu = \text{const}$ (15)

The system of eqs 14 and 15 was solved numerically by using the Merson method to yield the equilibrium contour map of the pivotal plane of the bulb-like closure as shown in Figure 4.

2. Determination of the Equilibrium Configuration of the Lamellar to Inverted Hexagonal Transition by the Monte Carlo Simulated Annealing Method. The configuration of monolayers adjacent to the central cylinder representing a nucleation line for the L_{α} -H_{II} phase transition is described by the radius of the central cylinder and a set of *N* angles ψ_i , i = 1, 2,..., N, describing the bulb-like closure and the surrounding monolayers (Figure 4). The boundary conditions were introduced to reflect connections within the different parts of the system. Only the smallest unit was considered. Due to symmetry, this unit includes a quarter cylinder, half of a bulb-like closure, and one neighboring monolayer.

The minimization of the free energy of the system was performed by the Monte Carlo simulated annealing sampling strategy.⁴¹ Within this approach, it is assumed that the system satisfies the ergodicity requirement, that is, any local minimum is accessible from any other minimum after a finite number of random sampling steps.⁴² In contrast to the conventional Metropolis Monte Carlo method, all values of the angles ψ_i

together with the radius of the central cylinder r were updated in each step.⁴¹ After a step, the total free energy of the system was evaluated by the Metropolis criterion⁴² and compared with the free energy of the previously accepted state. To find a global minimum in the multivariational space, the simulation was started at sufficiently high temperature according to the Metropolis criterion, while according to the cooling schedule, the temperature was decreased after each step until it reached the zero value.

The configuration of the system composed of the shape of the bulb-like closure determined by solving the Euler–Lagrange equations, the radius of the central cylinder determined by the maximal value of the *z* coordinate of this bulb-like closure, and two adjacent flat monolayers sandwiching the bulb-like closure and the cylinder were taken as a first approximation in the procedure of the energy minimization by the Monte Carlo simulated annealing method. This choice of the initial configuration considerably increased the speed of a rather timeconsuming Monte Carlo simulated annealing method.

3. Estimation of Model Constants. In order to determine the free energy of the configuration of monolayers, the values of constants were estimated. The value of interaction constant ξ was estimated from monolayer bending constant $\xi = 2k_c a_0$, where for POPE, $k_c = 11kT$ is the bending constant⁴³ and $a_0 =$ $0.65 \times 10^{-18} \text{ m}^2$ is the area per phosholipid molecule.⁶ The reference length of the phospholipid tail ζ_0 (Figure 3) was taken to be 1.30 nm.⁶ In estimation of the interstitial energy, the lipid stretching modulus τ was taken to be in the range from 0.95kTto $95kT \text{ nm}^{-2}$.³⁸ It was taken for simplicity that the molecules favor the cylindrical geometry, that is, $|H_m| = D_m$. The effect of the temperature was simulated by increasing the intrinsic curvatures $|H_m|$ and D_m with increasing temperature, which is consistent with the spreading of the phospholipid tails while the head groups in POPE are rather small.⁴⁰ The range of the intrinsic curvatures was taken to be 0-0.4 nm⁻¹, corresponding to the curvature radii down to 1 nm. To study the effect of the deviatoric bending, also the hypothetical case where the molecules are isotropic $(D_m = 0)$ was considered.

III. Results

A. Comparison between Planar, Cylindrical, and Spherical Systems. To explain the effect of the individual contributions to the free energy, we first determine the equilibrium configuration obtained by minimization of the monolayer bending energy (eq 2) only. Three different geometries are compared, planar (corresponding to the lamellar L_{α} phase), inverted spherical (corresponding to the inverted micellar M_{II} phase), and inverted cylindrical (corresponding to the inverted hexagonal H_{II} phase). The lamellar phase composed of molecules which favor a flat surface ($H_m = 0$) yields a negative normalized free energy due to the constant term $-\ln 2$, accounting for (in principle) two orientational states of the molecules (Figure 5). This term is present in all configurations and can be considered as a reference value of the free energy per molecule.

Figure 5 shows the equilibrium bending energy per lipid molecule (F_b/n_0A) $(|H_m| = D_m)$ as a function of the intrinsic mean curvature H_m (A). A hypothetical case without the deviatoric effect $(D_m = 0)$ is also given for comparison (B). For small $|H_m| = D_m$, the bending energy increases with increasing $|H_m|$ in all three geometries (panel A). In the M_{II} and L_{α} phases which are isotropic with respect to the curvature (D = 0), there is no orientational ordering of the molecules, and the bending energy monotonously increases also for larger $|H_m|$ $= D_m$. In the H_{II} phase however, the nonzero values of both the



Figure 5. The equilibrium bending energy per lipid molecule F_b/n_0A dependent on the intrinsic mean curvature H_m for the L_{α} , M_{II} , and H_{II} phases. (A) A system composed of anisotropic molecules ($D_m = |H_m|$); (B) a system composed of isotropic molecules ($D_m = 0$). See eq 2.

intrinsic curvature deviator D_m and the curvature deviator D give rise to a negative energy contribution of the deviatoric bending. Therefore, the equilibrium free energy reaches a maximum upon an increase of D_m (which, for this particular choice of molecules, is equal to $|H_m|$) but then decreases at a certain threshold, and the H_{II} phase becomes energetically the most favorable. Summing up, for small $|H_m| = D_m$, the M_{II} phase has the lowest bending energy, while at larger $|H_m| = D_m$, the H_{II} phase becomes the most favorable due to the orientational ordering of phospholipid molecules. The effect is stronger for higher constant \tilde{k} describing the direct interaction between phospholipid tails (Figure 5A).

Figure 5 B shows that for isotropic molecules, the M_{II} phase is always favored, that is, the calculated energy per lipid $F_b/$ n_0A in the M_{II} phase is equal to the reference value and is the smallest compared to the energy of the L_{α} and the H_{II} phase. We note that for isotropic molecules, there can be no energy lowering due to the orientational ordering of the molecules since all orientations of the molecules are energetically equivalent.

The deviatoric bending of anisotropic molecules thus alone explains the stability of the H_{II} phase at higher temperatures. At lower temperatures, the M_{II} phase is energetically favored except for $|H_m| = D_m = 0$, where the L_{α} phase is the stable phase. At small $|H_m| = D_m$ however, the equilibrium radii of the simulated M_{II} phase are so large that this case would correspond to flat membrane systems. For some intermediate $|H_m| = D_m$, the simulated M_{II} phase consists of aggregated micelles of the same size, which are actually not observed.⁴⁴

To obtain a better description of the system also in the intermediate range of $|H_{\rm m}| = D_{\rm m}$, we include the effect of void-filling energy by using a simple model, where the void-filling energy is considered constant for a given geometry (eq 3). Figure 6 shows the minimal free energy $F/n_0A = F_b/n_0A + F_v/n_0A$, dependent on the intrinsic mean curvature $H_{\rm m}$ for the L_{α}, H_{II}, and M_{II} phases. Since the energy contribution of voids is smaller in the system of close-packed inverted cylinders than that in the system of close-packed inverted spheres, the value of the void-filling energy per lipid molecule χ was taken to be lower for cylinders than that for spheres. It was estimated from the results of Kozlov et al.,¹⁴ that χ should be of the order of kT,¹⁴ therefore, we took for the H_{II} phase $\chi = 1kT$ and for the M_{II} phase $\chi = 2kT$.

In Figure 6A and B, the curves corresponding to the H_{II} and M_{II} phases from Figure 5 are shifted up for different constants χ , respectively, and an overall picture is now more realistic. As a consequence, it can be seen in Figure 6A and B that for small $D_{\rm m} = |H_{\rm m}|$, the L_{α} phase is energetically the most favorable since it requires no void-filling energy. For anisotropic molecules



Figure 6. The equilibrium free energy per lipid molecule F/n_0A consisting of the contribution of bending and of a constant for the void filling per lipid molecule, dependent on the intrinsic mean curvature $|H_m|$ for the L_{α}, H_{II}, and M_{II} phases. (A) $D_m = |H_m|$; (B) $D_m = 0$. See eqs 2 and 3, respectively; $\bar{k}/kT = 1$.



Figure 7. Free energy per lipid molecule F/n_0A consisting of the bending and the interstitial contributions, dependent on the intrinsic mean curvature $|H_m|$ in the L_{α} and H_{II} phases for various stiffnesses of hydrocarbon chains τ for (A) $|H_m| = D_m$ and (B) $D_m = 0$. See eqs 2 and 6, respectively; $\tilde{k}/kT = 1$.

(Figure 6A) at a certain threshold $D_m = |H_m|$, the H_{II} phase becomes energetically the most favorable due to the orientational ordering of the lipid molecules. In the isotropic case (Figure 6B), all curves monotonously increase; however, the curve corresponding to the L_{α} phase increases faster, and therefore, it will eventually cross with the curve corresponding to the H_{II} phase. However, the value of $|H_m|$ where the intersection would take place will be very high (out of range given in Figure 6, where the maximal value of 0.3 nm⁻¹ already corresponds to cylinders with a radius of 1.67 nm).

In short, the effects shown in Figure 6A indicate that in the simple model where the interstitial energy is taken to be constant within a phase, an increase of $D_{\rm m} = |H_{\rm m}|$, which is caused by the increase of temperature, can induce the transformation from L_{α} to the H_{II} lipid phase. Taking into account the interstitial energy for small $|H_{\rm m}|$ (lower temperature) renders the L_{α} phase energetically the most favorable, while at a certain threshold $D_{\rm m} = |H_{\rm m}|$ (higher temperature), the H_{II} phase becomes energetically the most favorable.

B. A More Detailed Comparison between Planar and Cylindrical Systems. Having eliminated the M_{II} phase due to high packing frustration (Figure 6), in the following, we compare only the L_{α} and the H_{II} phases by using an improved model for the void filling energy, where stretching of the lipid tails in the actual hexagonal geometry is taken into account (Figure 3 and eq 6). Figure 7 shows the free energy per lipid molecule F/n_0A , dependent on the intrinsic mean curvature H_m for the L_{α} and the H_{II} phase. An optimal configuration is calculated by minimization of the monolayer free energy composed of the bending and the void-filling contributions. Three different values of the lipid stretching modulus τ were considered. Lipids



Figure 8. Structural parameters of the H_{II} phase for the case $D_m = |H_m|$. (A) The optimal unit cell parameter *a* and (B) the optimal water core radius *r* (core center to polar/apolar interface) are plotted versus the mean curvature H_m for different lipid chain rigidities τ . The two horizontal dashed lines mark the realistic values for *a* and *r*, respectively (Table 1). For definitions of *a* and *r*, see Figure 3; $\tilde{k}/kT = 1$.

exhibiting high compliance are described by low values of τ , while lipids which are rather stiff and exhibit poor compliance are described by high values of τ . It is noteworthy that longerchain lipids have higher compliance than shorter ones and therefore promote more easily the formation of the inverted hexagonal phase.⁴ For both cases ($D_m = |H_m|$) (Figure 7A) and ($D_m = 0$) (Figure 7B), the L_{α} phase is energetically the most favorable for small $|H_m|$. For larger values of the intrinsic curvature $|H_m|$, the H_{II} phase is energetically the most favorable.

Increasing the value of the stretching modulus τ considerably increases the free energy (Figure 7A and B). With increasing τ , the void-filling energy becomes more important relative to the bending energy. As there is no void-filling contribution to the free energy in the L_{α} phase, increasing of τ increases the free energy of the H_{II} phase with respect to the free energy in the L_{α} phase. Accordingly, for smaller τ , the H_{II} phase is preferred already at smaller |*H*_m| (Figure 7 B).

To promote and stabilize the H_{II} phase with increasing temperature, the energy difference between the L_{α} and the H_{II} phases should be larger than the energy of thermal shape fluctuations. It is not likely that the system will remain at high energy; rather, the configuration will adjust such that the free energy is minimized. Further, it is important to stress that the anisotropy of the phospholipid molecules contributes to a steeper increase of the absolute value of the energy difference between the L_{α} and the H_{II} phases with temperature (compare panels A and B in Figure 7) and therefore promotes and stabilizes the H_{II} phase profoundly.

Figure 8 shows the dependence of the unit parameter *a* (panel A) and of the effective radius of the pivotal surface in the H_{II} phase *r* (panel B) on the intrinsic mean curvature H_m for anisotropic molecules ($D_m = |H_m|$). The increase of $|H_m|$ causes a decrease of both *a* and *r*.

It is instructive to compare the given plots with experimental data (Figure 8, dashed lines). First, it teaches us that realistic stretching moduli most probably lie between 0 and 20 kT nm⁻² (for large enough τ , e.g. $\tau = 95 kT$ nm⁻² no realistic dimensions of the H_{II} lattice can be achieved). Second, the range of realistic intrinsic curvatures $-H_{\rm m}$ lies probably in the range of 0.1–0.2 nm⁻¹. Note that this comes close to the value of the mean curvature -H of the POPE/water system (Table 1).

C. Configuration at the Lamellar to Inverted Hexagonal Transition. We calculated the equilibrium configuration of the system in which an inverted central phospholipid cylinder is embedded in two adjacent lipid bilayers. In Figure 9,

snapshots of the equilibrium configurations for anisotropic molecules $D_m = |H_m|$ and different values of model constants are displayed. From left to right, the intrinsic lipid curvature is increased. The top row presents the L_{\alpha} phase. Below, from top to bottom, the stretching modulus of the lipids is increased. The value of the equilibrium free energy *f* is given for the L_{\alpha} phase, while the values of the difference between the energy of the H_{II} phase and the L_{\alpha} phase $\Delta f = f_{H_{II}} - f_{L_\alpha}$ are depicted for the H_{II} phase. The radius of the central cylinder *r* is given for all configurations.

The configuration with the cylinder and bulb-like closures becomes energetically more favorable than the L_{α} phase at high enough values of $D_m = |H_m|$. This is in agreement with the fact that the formation of the H_{II} phase is promoted with increasing temperature.⁶ For higher $D_m = |H_m|$, the energy difference Δf decreases, thereby favoring the configuration with the cylinder. The difference between the configurations C and F in Figure 9 is -0.55kT per lipid molecule in favor of the configuration with the cylinder, which is considerable.

The radius of the central cylinder decreases with increasing $|H_{\rm m}|$ as the molecules with higher $|H_{\rm m}|$ favor larger curvature exhibited in smaller cylinder radii. The radius of the central cylinder decreases with increasing stiffness of the lipids (increasing τ) (Figure 9 D–G, E–H, and F–I), which means that its insertion into the lamellar phase becomes less disturbing for adjacent lipid layers, that is, approaching a coplanar configuration. For high enough τ , the increasing intrinsic curvatures of the lipids do not have a considerable effect on the radius of the central cylinder, and the configuration is determined mostly by the stretching energy of the phospholipid chains, while the length of the phospholipid chains is as close as possible to its intrinsic optimal value. For larger $|H_m|$ and small τ , the radius r is determined mainly by the bending energy, which is the lowest when r comes close to $|1/H_{\rm m}|$. With the increasing contribution of the interstitial energy, the shape of the curved monolayers is mainly determined by avoiding the stretching of the lipid tails.

To resume, when the free energy of the presented configuration is calculated and compared to the energy of the pure lamellar system, the configurations of anisotropic molecules with high enough intrinsic curvatures and low enough stretching modula have a lower energy with respect to the L_{α} phase and will promote the formation of the H_{II} phase.

The "transition point" from the L_{α} to the H_{II} phase occurs in the nucleation model for the configurations which have at a given $|H_m|$ the same energy as the L_{α} (Figure 10). By comparing the values of $|H_{\rm m}|$ corresponding to the nucleation transition (Figure 10) and the values corresponding to the $L_{\alpha}{-}H_{II}$ transition (Figure 7), we can see that for $\tau = 0.95 kT/nm^2$, the $L_{\alpha}-H_{II}$ transition takes place for smaller $|H_m|$ (lower temperature) than the nucleation transition, which is not realistic. Note also, that the simulated cylinder radius at 3.49 nm is far too large compared to experimental data.^{6,40} For larger τ (e.g., $\tau =$ 9.5kT/nm²) however, the respective $|H_{\rm m}|$ values for both transitions are close to 0.155 nm⁻¹. The L_{α} -H_{II} transition takes place at slightly higher $|H_m|$ (temperature) than the nucleation transition. Also, the given value of r = 2.47 nm is in very good agreement with experimental data. For POPE, nucleation radii of 2.3-2.5 nm were estimated on the basis of structural X-ray data.^{6,45} As the decrease of the free energy with increasing $|H_m|$ is more pronounced in the all-hexagonal phase (Figure 7) than in the nucleation configuration (Figure 9), the values of τ at around $9.5kT/nm^2$ would correspond to stabilization of the H_{II} phase at higher temperatures, the transition involving a previous



Figure 9. Configuration of the lipid monolayers adjacent to a central cylinder representing a nucleation line in the $L_{\alpha}-H_{II}$ transition for different intrinsic curvatures of the lipid molecules H_m and different stretching modula of the phospholipid tails τ . It is considered that $(D_m = |H_m|)$. The free energy per lipid molecule and the radius of the central cylinder are given for each configuration; $\tilde{k}/kT = 1$.

$\tau = 0.95 \ kT \mathrm{nm}^{-2}$	$\tau = 9.5 \ kT \mathrm{nm}^{-2}$	$\tau = 95 \ kT \mathrm{nm}^{-2}$
r = 3.49 nm $H_{\rm m} = -0.143 \text{ nm}^{-1}$ 10 n	m $r = 2.47 \text{ nm}$ m $H_{\rm m} = -0.155 \text{ nm}^{-1}$	r = 1.49 nm $H_{\rm m}$ =-0.208 nm ⁻¹

Figure 10. Different nucleation configurations for different stretching modula of phospholipid tails τ . For all configurations $\Delta f = 0$; $\tilde{k}/kT = 1$.

nucleation configuration. For large τ (e.g., $\tau = 95kT/\text{nm}^2$), the nucleation transition takes place at $|H_{\rm m}| = 0.208 \text{ nm}^{-1}$ (Figure 10), well before the L_{α} -H_{II} transition at $|H_{\rm m}| = 0.25 \text{ nm}^{-1}$ (Figure 7). The two transitions are rather far apart, which would correspond to a stable nucleation configuration within a larger temperature interval. This is not very probable, and a too small cylinder radius ($\approx 1.1 \text{ nm}$) confirms the exclusion of this scenario.

IV. Discussion

It was previously found by Kozlov and colleagues that the bending and the interstitial energies have the largest effect on the determination of the stable inverted hexagonal phase, whereby the spontaneous curvature which determines the bending contribution partly describes the hydration of the lipid monolayer.14 In previous works, the elastic energy of the bending of isotropic thin shells was used as a basis.²⁹⁻³¹ Within the energy of the bending of isotropic thin shells,²⁹⁻³¹ the quadratic expansion of the bending energy with respect to the two principal curvatures was applied. The bending energy was expressed by two invariants of the curvature tensor, the mean curvature H and the Gaussian curvature $K = C_1 C_2$.²⁹⁻³¹ The quadratic approximation proved insufficient in describing the system of lipid monolayers in the vicinity of phase transitions; therefore, terms of higher order in the mean and the Gaussian curvatures were included.^{15,34} However, these terms are not transparent with respect to physical meaning. Also, two additional constants are necessary in this model for which the values are unknown.

In our description, we pursue the model proposed by Kozlov et al.¹⁴ The intrinsic curvatures which determine the

bending contribution in our model describe the shape of the molecule and all of the local interactions with its surroundings. However, we improved the description of the bending in a different way. Instead of introducing the higher-order terms in the curvature tensor invariants,^{15,34} we have chosen a different tensor as a basis of our model. It is considered in our model that the surrounding molecules create the curvature field into which the chosen molecule must adapt. The singlemolecule energy expresses the energy which is spent by adjusting the molecule into its location in the monolayer, taking into account that there exists a monolayer into which the molecule would fit perfectly, that is, without spending any energy. The curvature of this ideal monolayer represents the intrinsic shape of the molecule given by the principal curvatures C_{1m} and C_{2m} . To obtain the expression for the single-molecule energy, an expansion in terms of invariants of the "mismatch" tensor M is performed⁴⁶

$$E = \frac{k_1}{2} (\operatorname{Tr}(\underline{M}))^2 + k_2 \operatorname{Det}(\underline{M})$$
(16)

where k_1 and k_2 are constants

$$\underline{M} = \underline{R}\underline{C}_{\mathrm{m}}\underline{R}^{-1} - \underline{C}$$
(17)

 \underline{R} is the rotation matrix

$$\underline{R} = \begin{bmatrix} \cos \omega & -\sin \omega \\ \sin \omega & \cos \omega \end{bmatrix}$$
(18)

 \underline{C} is the diagonalized curvature tensor

$$\underline{C} = \begin{bmatrix} C_1 & 0\\ 0 & C_2 \end{bmatrix} \tag{19}$$

and \underline{C}_{m} is the diagonalized intrinsic curvature tensor

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$$\underline{C}_{\rm m} = \begin{bmatrix} C_{\rm 1m} & 0\\ 0 & C_{\rm 2m} \end{bmatrix} \tag{20}$$

It is taken into account that the principal axes of the respective systems are rotated in the plane of the membrane with respect to each other for an angle ω . In statistical averaging, it is considered that there are only two possible states (orientations) of the molecule, that is, the state with the highest energy and the state with the lowest energy.¹⁸ The procedure gives an expression which depends on the monolayer curvatures, more precisely, on the invariants of the curvature tensor, H and D. The "choice" of the invariants is therefore a result of the derivation starting from the mismatch tensor rather than of an a priori expansion in terms of the invariants of the curvature tensor. The derivation of the Landau energy of the thin laterally isotropic elastic shell²⁹ is based on the expansion of the energy in terms of the invariants of the strain tensor, while mathematical elaboration and further assumptions lead to the expression in terms of the curvature tensor invariants H and K. A bold step forward was taken by Helfrich,³¹ who directly expanded the energy in terms of the curvature tensor invariants H and K and, in this way, included the spontaneous curvature into the description in a simple and transparent way. Here, in starting from the single-molecule energy and the mismatch tensor, the Helfrich expression is recovered; however, an additional term corresponding to the orientational ordering of the molecules is obtained. It turns out to depend on the curvature tensor invariant D, which was not used in the derivations and expressions of Landau and Helfrich.

The invariants H, D, and K are connected through the relation

$$H^2 = D^2 + K \tag{21}$$

Therefore, in principle, the energy can be expressed by either set of two invariants (H and K in the Landau-Helfrich expressions or H and D in the expressions used in this work). Having found that the isotropic Landau–Helfrich free energy³¹ does not describe well the system in the vicinity of the phase transition, we find that both, including the terms of higher order in H in K and using another set of invariants (H and D), represent an improvement. However, considering the orientational ordering which leads to the invariants H and D, besides providing the relevant physical mechanism, also avoids introduction of additional constants for which the values are not known. The constants in our model comprised in the parameter ξ can be estimated directly from previous experimental data on phospholipid systems. The proposed mechanism and the corresponding formalism was proven advantageous also in description of other highly curved systems including membraneous structures^{16,27,47,48} and inorganic materials.^{17,49,50}

Models based on the isotropic elasticity described the L_{α} -H_{II} phase transition by showing that at a certain temperature, the free energy of the system is lowered as it converts from the L_{α} phase to the H_{II} phase.^{51,52} However, the energy difference was found to be <0.1*kT*.¹⁴ Our results pursue the general conclusions of the previous models; however, the obtained energy difference becomes much larger at elevated temperatures if the orientational ordering of anisotropic molecules on highly curved surfaces of the H_{II} phase is taken into account (Figure 7). This energy difference is sufficient for the stability of a single cylinder within the lamellar stack and therefore supports previously suggested nucleation models, which are based on line defects.^{6,40,53,54}

It is noteworthy that both the deviatoric contribution as well as the stretching energy of the chains are necessary to explain the L_{α} – H_{II} phase transition for the model constants estimated from experimental data.⁶ In a first approach, the effect of the stretching of the chains was considered to be constant (as in ref 14), while in a second step, we improved the model by introducing the conformational free energy of the individual hydrocarbon chains in the hexagonal lattice (the stretching energy of the hydrocarbon chains). Assuming solely the isotropic bending and the stretching energy of the chains leads to quite high intrinsic curvatures of the phospholipid molecules in comparison to the curvature of the cylinders in the stable inverted hexagonal phase, whereas neglecting the stretching energy of the chains does not predict the stability of the lamellar phase for low intrinsic curvatures of the lipids, as observed in real lyotropic mesophases.⁴

In this work, we have considered the transition from the L_{α} to the H_{II} phase (the heating transition). However, the experimental data on the cooling transition show a considerable hysteresis since the transition temperature of the L_{α} -H_{II} phase conversion lies typically 5-10 °C higher than the minimum temperature at which the fully developed H_{II} phase remains stable.55 On the basis of the presented results, we cannot explain the hysteresis since, for that, we would need a separate scenario based on the experimental data and the corresponding model to describe how a fragment of the planar configuration starts to form between the cylinders. The heating and the cooling transitions do not involve the path through the same states of the system. Such analysis of hysteresis is out of the scope of the presented work but will be considered in the future. Further improvements of our models for the L_{α} -H_{II} phase regime will include an energy term for the contact regions of the opposed lipid chains (methyl trough region) and will allow slight deviations in the roundness of the polar interface in the H_{II} phase.⁵⁶ Further, temperature dependencies and the role of chain saturation shall be analyzed in greater detail. The above will not only refine the prediction of the stability of the nonlamellar mesoscopic lipid phases but also be useful for the reconstruction of precise phase diagrams.

V. Conclusion

Our results indicate that the deviatoric bending can explain the stability of the H_{II} phase at higher temperatures. However, for the L_{α} - H_{II} transition, tuning of the deviatoric bending energy by the isotropic bending energy and the interstitial energy is needed. We show that with increasing absolute values of intrinsic curvatures (which were assumed to increase with increasing temperature), the L_{α} - H_{II} phase transition, which is observed in experiments,^{6,40,57} takes place beyond a certain threshold temperature. Finally, by using our model, we could reproduce realistic structures in good agreement with experimental results.^{6,40} To conclude, we have shown that the deviatoric bending plays an important role in the stability of the H_{II} phase and in the L_{α} - H_{II} phase transition.

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