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Simulations of Inverted Hexagonal Lipid Structures

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Abstract. Phospholipid molecules are composed of multipolar headgroup and two electrically neutral hydrocarbon tails. The inverted hexagonal phase (H_{II}) belongs to the biologically most significant non-lamellar phospholipid phases in biomembranes. Hence the geometric properties and conditions of transition to the H_{II} phase are nowadays widely studied. In our derivation of the free energy of lipid monolayers we assume that phospholipid molecules are in general anisotropic with respect to the axis perpendicular to the membrane plane. In our model the expression for the phospholipid monolayer free energy consists of two energy contributions: the bending energy which involves also deviatoric term, and the interstitial energy which describes the deformation energy due to stretching of the phospholipid molecule chains. On the basis of the derived expression for the phospholipid monolayer free energy we theoretically predict optimal geometry and physical conditions for the stability of the inverted hexagonal phase.

Key words: inverted hexagonal phase, self-assembly of phospholipids, biomembranes, lamellar lipid phase, non-lamellar lipid phases

Simulacije invertnih heksagonalnih lipidnih struktur

Povzetek. Fosfolipidne molekule sestavljajo multipolna glava in dva nepolarna repa. Invertne heksagonalne faze (H_{II}) spadajo med najpomembnejše fosfolipidne faze v bioloških membranah. Zato so geometrijske lastnosti ter pogoji faznega prehoda v H_{II} fazo v zadnjem času ohranjajo široko zanimanje. V našem teoretičnem modelu H_{II} fosfolipidne faze upoštevamo, da sestavljata prosto energijo fosfolipidne enojne plasti dva prispevka: natezna energija repov fosfolipidnih molekul in upogibna energija fosfolipidne plasti. Pri tem upoštevamo, da so fosfolipidne molekule na splošno anizotropne glede na njihovo vzdolžno glavno os. S pomočjo minimizacije proste energije fosfolipidne enojne plasti teoretično napovemo optimalno geometrijo ter pogoje stabilnosti invertne heksagonalne faze (H_{II}) fosfolipidnih molekul.

Ključne besede: invertne heksagonalne faze, samozdruževanje fosfolipidnih molekul, lamelarna lipidna faza, nelamelarne lipidne faze

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1 Introduction

One of the main components of biological membranes are phospholipids. They have amphiphatic character, i.e. they comprise a polar head group as well as non-polar hydrocarbon chains in one molecule. Such molecules in aqueous solution undergo a self-assembling process and form various structures. Biologically important lipid/water systems are known for their rich polymorphism [1]. The driving force of this process is predominantly the hydrophobic effect where the hydrophilic (polar) surfaces are in contact with water solution while the hydrophobic (non-polar) parts composed of lipid head-groups are hidden from water [2]. The most common and biologically the most relevant phase is the fluid lamellar lipid bilayer phase (L_α), see Fig. 1. The bilayer of lipid molecules represents the basic building block of the plasma membrane, which encloses the cell interior. Nevertheless, non-lamellar model membranes are subject of increasing interest [1, 3, 4, 5], due to their importance in living organisms and due to their promising technical applications such as in drug delivery [6, 7], gene transport and nanotechnology [8]. The bicontinuous cubic phase, inverse hexagonal phase and inverse micellar cubic phase belong to the biologically most relevant non-lamellar mesophases. These mesophases resist excess of water and thus they can be stable under certain conditions in biological systems such as higher temperature and can be observed also in plasma membrane of some bacteria [5, 9].

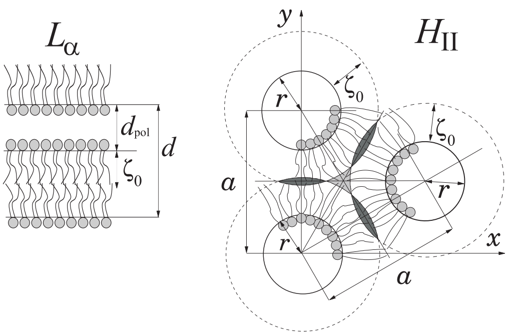

Figure 1. Geometry of lamellar and inverted hexagonal phases. One bilayer and one neighbouring monolayer are depicted for the lamellar phase. Lattice unit of lamellar phase (d-spacing) (d) and distance between the two bilayers (d_{pol}) are denoted. For inverted hexagonal phase three cylinders of radius r at the distance a are depicted. ζ_0 denotes the optimal length of hydrocarbon chains. H_{II} phase requires stretching or compressing some of the hydrocarbon chains with respect to their optimal length ζ_0 as shown schematically.

Slika 2. Primerjava geometrije lamelarne in invertne heksagonalne fosfolipidne faze. V primeru lamelarne faze (leva slika) sta prikazani ena dvojna ter ena enojna fosfolipidna plast. Prikazani sta tudi mrežna konstanta (d) ter razdalja med površinama dveh sosednjih dvojnih plasti (d_{pol}). V shematskem prikazu invertne heksagonalne faze je prikazana razdalja med geometrijskima osema dveh sosednjih cilindrov (a). Polmer posameznega cilindra (približno v nevtralni ravnini enojne plasti) je označen z r. Simbol ζ_0 označuje optimalno dolžino repov lipidnih molekul. V invertni heksagonalni fazi so repi lipidnih molekul v splošnem raztegnjeni ali skrčeni glede na optimalno dolžno ζ_0.

2 Inverted Hexagonal Phase

Lipids in the inverted hexagonal phase are self-assembled in long tubes arranged in a hexagonal lattice. Fig. 1 shows the geometry of the H_{II} phase: two neighbouring tubes with diameter r are located at the distance a. The phospholipid chains point outwards from the cylinder surface defined as the pivotal plane while the headgroups form polar nanotubes filled with aqueous solution. The geometry of H_{II} phase is described by the mean and Gaussian curvatures [1]:

$$H = \frac{C_1 + C_2}{2} \quad \text{and} \quad K = C_1 C_2, \tag{1}$$

where C_1 and C_2 are the principal curvatures of the plane. However, for highly curved structures it is more suitable to use curvature deviator D [10]:

$$D = |C_1 - C_2|/2. \tag{2}$$

The invariants H, K and D are connected through the relation $H^2 = D^2 + K$.

It can be seen in Fig. 1 that all lipid tails in hexagonal lattice do not have the same length. There are triangular regions (called voids) between neighboring tubes that are considerably energetically expensive, because the lipid chains in these regions need to stretch beyond the average length ζ_0. In theoretical studies of stability of inverted hexagonal phases it is therefore necessary to take into account an energy term which accounts for the stretching of the hydrocarbon chains in void regions [11, 12, 13, 14].

3 Energy

In general, solving the stability conditions for different lipid phases is a problem of defining the free energy of the system and its minimization. Thus we consider for the total free energy of the hexagonal phase two energy contributions: the energy of local bending and the interstitial energy (voids filling energy). Starting from a single molecule energy and applying the methods of statistical physics, the free energy of a lipid monolayer (bilayer) was derived [10]. The local bending energy of laterally homogeneous monolayer (bilayer) [15] was recovered, however, an additional contribution due to orientational ordering of lipid molecules - i.e. the contribution of the deviatoric bending was obtained [10]. The orientational ordering of anisotropic phospholipids lowers the free energy of the system; the effect is more pronounced for larger anisotropy of lipid molecules and stronger membrane curvature anisotropy [10]. We proposed the interstitial energy dependent on the stretching of the lipid chains in order to fill the voids in the lattice. The stretching of chains can be expressed with the term of the harmonic spring model [16]:

$$f_{\text{d}} = \tau(\zeta - \zeta_0)^2, \tag{3}$$

where ζ and ζ_0 are the actual and the reference lengths of lipid chains, respectively. Introducing the geometrical properties of H_{II} phase into this term, we can define the interstitial energy of one lipid cylinder in H_{II} phase. From summating the bending energy and interstitial energy contribution we can define the total free energy per lipid molecule in inverted hexagonal phase [17]:

$$
\begin{aligned}
F &= \frac{\xi}{2}\left((H-H_m)^2+D^2+D_m^2\right)- \\
&- kT\ln\left(2\cosh\left(\frac{(1+\frac{\tilde{k}}{kT})\xi D_m D}{kT}\right)\right)+ \\
&+ \frac{6}{\pi}\tau\left(\frac{a^2\sqrt{3}}{12}-a(r+\zeta_0)\ln\sqrt{3}+\frac{\pi}{6}(r+\zeta_0)^2\right),
\end{aligned}
\tag{4}
$$

where a and r are geometric parameters described in Fig. 1, T is thermodynamic temperature, k is Boltzmann constant, ξ and $\tilde{k}$ are constants described in next section and H_m and D_m are mean intrinsic curvature and curvature deviator, respectively [10].

4 Model Constants

The minimal value of the free energy of a unit patch of the lipid monolayer dependent on the mean principal curvature H_m was calculated by using Eq. (4) while the model constants are given in Table 1. The value of the interaction constant was estimated from monolayer bending constant $\xi = 2k_c a_0$, where for POPE $k_c = 11kT$ it is the bending constant [18] and $a_0 = 0.65 \cdot 10^{-18}\mathrm{m}^2$ is the area per phospholipid molecule [19]. The reference length of the phospholipid tails ζ_0 (Fig. 1) was taken to be 1.30 nm [19]. In calculation of the interstitial energy, the lipid stretching modulus τ was taken to be $0.95\ kT\ \mathrm{nm}^{-2}$ and $9.5\ kT\ \mathrm{nm}^{-2}$ (see [16]). For the sake of simplicity it was assumed that the molecules favor cylindrical geometry, i.e. $|H_\mathrm{m}| = D_\mathrm{m}$, which represents the wedge-shape model of the lipid molecule. The effect of the temperature was simulated by increasing the intrinsic curvatures $|H_\mathrm{m}|$ and D_m with increasing temperature which is consistent with increased spreading of the phospholipid tails while the headgroup extensions in POPE remain relatively unchanged. The range of the intrinsic curvatures was taken to be from 0 to $0.4\ \mathrm{nm}^{-1}$, corresponding to curvature radii down to 1 nm. To study the effect of the deviatoric bending, also the hypothetical case where the molecules are isotropic ($D_\mathrm{m} = 0$) was considered.

Table 1. Geometrical parameters of the L$_\alpha$ and the H$_{II}$ phases at $T = 74°$ C. The structural parameters are defined in Fig. 1. The experimental values are taken from [19].
Tabela 1. Geometrijski parametr L$_\alpha$ in H$_{II}$ faze pri temperaturi $T = 74°$ C. Pomen strukturnih prametrov je podan na sl. 1

	L$_\alpha$(74°C)	H$_{II}$(74°C)
$d, a[\mathrm{nm}]$	4.99	7.24
$d_\mathrm{pol}, r[\mathrm{nm}]$	2.5	2.67
$\zeta_0(\zeta_\mathrm{min}, \zeta_\mathrm{max})[\mathrm{nm}]$	1.47	1.13 (0.95,1.51)
$a_0[\mathrm{nm}^2]$	0.65	0.65
$-H[\mathrm{nm}^{-1}]$	0	0.187

5 Results

To show stability of the inverted hexagonal phase, we compare two lipid phases: planar and cylindrical. The systems were described as surfaces with constant principal curvatures. In the planar system (L$_\alpha$), $H = D = 0$ and in the inverted cylindrical system (H$_{II}$), $H = -D = -1/2r$, where r is the radius of the cylinder. 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 Eq. (4), where for L$_\alpha$ phase the interstitial energy term is omitted. Fig. 2 shows the free energy per lipid molecule F/n_0A in dependence on the intrinsic mean curvature H_m for the L$_\alpha$ and the H$_{II}$ phase. We can compare the total free energy per molecule for anisotropic and isotropic phospholipid molecules in dependance on the mean intrinsic curvature H_m. It can be seen in Fig. 2 that there are two curves corresponding to the inverted hexagonal phase with different stiffness constants τ and one curve corresponding to the lamellar phase. For stiff hydrocarbon chains (high values of τ) the lamellar phase has lower energy than the inverted hexagonal phase, while for decreasing τ, the inverted hexagonal phase is energetically more favorable than the lamellar phase for high

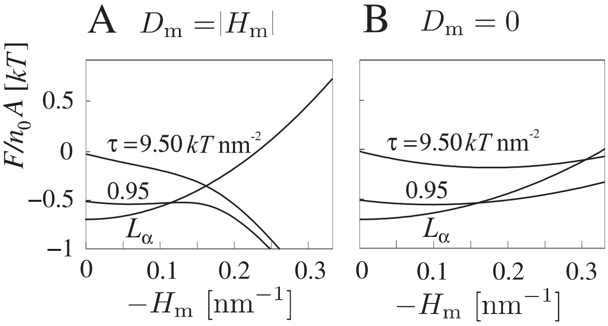

Figure 2. Free energy per lipid molecule F/n_0A consisting of the bending and the interstitial contributions in dependence on the intrinsic mean curvature of lipid molecules $|H_\mathrm{m}|$ in the L$_\alpha$ and H$_\mathrm{II}$ phase for various values of stiffness of hydrocarbon chains τ for (A) $|H_\mathrm{m}| = D_\mathrm{m}$ and (B) $D_\mathrm{m} = 0$. See Eq. (4). $\tilde{k}/kT = 1$.
Slika 2. Izračunana prosta energija na lipidno molekulo (F/n_0A) v odvisnosti spontane povprečne ukrivljenosti lipidnih molekul $|H_\mathrm{m}|$ v L$_\alpha$ in H$_\mathrm{II}$ lipidni fazi za različne vrednosti konstante τ ter $\tilde{k}/kT = 1$.

enough $|H_\mathrm{m}|$. Isotropic lipid molecules in the inverted hexagonal phase also exhibit the lowest energy for less stiff hydrocarbon chains. By comparing Figs. 2A and 2B, it is important to point out that the anisotropy of phospholipid molecules evokes a steeper increase in the absolute value of the energy difference between the lamellar and the inverted hexagonal phases with temperature and therefore promotes and stabilizes the H$_{II}$ phase profoundly.

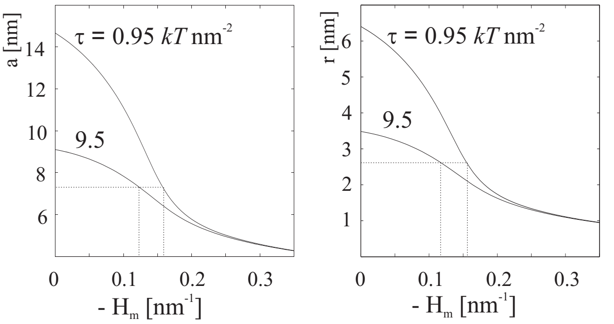

Figure 3. Structural parameters of the H$_{II}$ phase for the case where $D_\mathrm{m} = |H_\mathrm{m}|$. (A) The optimal unit cell parameter a and (B) the optimal pivotal plane radius r (core center to polar/apolar interface) are plotted versus the absolute value of the mean curvature H_m for different lipid chain rigidities τ. For definitions of a and r see Fig. 1. $\tilde{k}/kT = 1$. The dashed line shows experimental results from [19, 20].
Slika 2. Izračunane ravnovesne vrednosti strukturnih parametrov H$_{II}$ faze v odvisnosti od $D_\mathrm{m} = |H_\mathrm{m}|$ za različne vrednosti τ in $\tilde{k}/kT = 1$. Definiciji parametrov a in r sta podani na sl. 1.

For a more detailed study of H$_{II}$ phase we constructed graphs of structural parameters of the H$_{II}$ phase. Fig. 3 shows the dependence of the cylinder radius r and of the distance between the centers of the lipid cylinders a, respectively, (Fig. 1), on the intrinsic curvature $|H_m|$ for two values of constant τ of anisotropic lipid molecules. The H$_{II}$ phase is composed of lipid cylinders with small radius r and small separation a for lipids of large mean intrinsic curvatures. Decreasing the absolute value of the mean intrinsic curvature $|H_m|$ increases both the radius of the H$_{II}$ cylinders and the lattice length. However, cylinders of large radii increases the void space and the corresponding stretching of hydrocarbon chains.

It is instructive to compare the given plots with experimental data (Fig. 3, dashed lines) where the radius of the cylinder $r = 2.67$ nm and lattice distance $a = 7.24$ nm for the transition temperature 74° C [19, 20]. First, it teaches us that realistic value of the stretching moduli τ most probably lie between 0 and 20 kT nm^{-2}. Second, the range of realistic intrinsic mean curvatures $-H_\mathrm{m}$ lies probably in the range of $0.1 - 0.2$ nm^{-1}. The effect of interstitial energy in stabilization of the hexagonal phase is obvious if the value of τ is large enough. Large diameter of the lipid cylinder r produces larger voids that are energetically unfavorable. One can also see that there is a proportional dependence between r and a, (Fig. 4). The equation of the proportional line is similar to the rough estimation observed $a = 2r + 2\zeta_0$, where $\zeta_0 = 1.3\mathrm{nm}$, see Fig. 1.

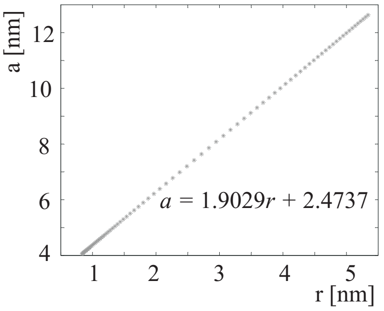

Figure 4. Calculated dependence of the cylinder radius (r) on the lattice constant a corresponding to the minimal free energy of the system, $\tau = 9.5kT[\mathrm{nm}^{-}2]$.
Slika 4. Izračunana odvisnost radija cilindra (r) od mrežne konstante a, ki ustreza minimalni energiji sistema, $\tau = 9.5kT[\mathrm{nm}^{-}2]$.

6 Conclusion

Stability of the inverted hexagonal phase depends on the energy balance between different contributions to the system free energy, hence the main problem in theoretical description of the lamellar to inverted hexagonal phase transition and explanation of the stability of the H$_{II}$ lipid phase consists in finding the proper expression for the free energy of the system. Most of the contemporary theoretical models of the free energy of the inverted hexagonal phase have shown that in addition to the bending energy term, it is necessary to consider also the energy term, which depends on the dimension of the 'voids' in the hexagonal lattice, the so-called interstitial energy [12, 13, 14, 21]. We followed this assumption and took into account the interstitial energy which in our model is expressed by the stretching energy of phospholipid chains. In spite of some simplifications introduced in our theoretical description, results of our simulations are in good agreement with experimental results [19, 20]. Our results also show that deviatoric anisotropic bending plays an important role in stability of the inverted hexagonal phase.

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