Influence of bilayer fluctuations on the steric interactions between polymer-grafted bilayers

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Influence of bilayer fluctuations on the steric interactions between polymer-grafted bilayers

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We propose a mechanism, through which the low membrane bending rigidity changes the steric polymer repulsion between polymer-grafted bilayers. We calculate the energy of a polymer chain trapped between two thermally undulating surfaces. The proposed treatment is in terms of de Gennes' theory of fluctuations in liquid crystals, later developed by Helfrich for membranes. We show that the confinement energy of the polymer increases due to membrane fluctuations. Also, the repulsive forces between soft polymer-grafted bilayers can extend further from the surface than the height of the polymer layer.

1. Introduction

Lipid bilayers are 'soft' and less ordered than the materials usually studied in condensed matter physics. Their physical and material properties are determined by the interplay between intermolecular weak forces, hydrophobic effects and thermal agitation.

Spontaneous movements of red blood cells (known as flicker phenomenon) were first observed as early as 1890 [1]. Now, it is known that the mechanism of the flicker effect is a simple physical one. It is due to the undulations of the plasma membrane with wavelength comparable to the cell size. The observed large amplitude is due to the fluidity of the membrane and the fact that the curvature elastic energy is comparable to kT at room temperature. The fluctuations are favoured by the entropy associated with the large number of accessible undulational configurations.

The theoretical treatment of membrane thermal undulations is closely related to and based on the curvature elasticity of the membrane. The cases of undulations around the planar [2–4] and spherical states [5–11] are both considered. Shape fluctuations of giant vesicles have also been observed [7–11].

The thermal undulations have been shown to induce repulsive undulation forces between fluid bilayers [2–4, 12, 13]. This is an interaction, characteristic only to soft surfaces. It is the result of the mutual steric hindrance of undulating membranes in close proximity. Helfrich was the first one to derive the energy density, associated with

these fluctuations as [2]

$$F_f = \frac{3\pi^2}{128} \frac{(kT)^2}{k_c(2h)^2},$$ (1)

where $2h$ is the distance between bilayers. This energy was measured by McIntosh et al. [13].

Recently, experimental studies of the repulsive pressures between bilayers, containing lipids with polymer covalently attached to their heads (polymer-lipids) were initiated [14–16]. The interest is determined by the stabilizing activity of the polymer in these polymer-grafted bilayers. For instance, the incorporation of polymer-lipids into liposomes enhances their blood circulation time, thus increasing their potential to be used as an effective intravenous liposome drug delivery system [15, 17–21].

The behaviour of polymer chains grafted to solid surfaces was first studied by de Gennes [22]. According to him, two concentration regimes can be identified for the polymer chains at the surface, and the physical characteristics of the polymer system are different in these two regimes. When the grafting density is low, the chains form separate 'mushrooms', each with a size $R_F \cong aN^{3/5}$, where N is the degree of polymerization and a is the monomer size. (In polymer theories the term 'monomer' is used for the repeat unit in the polymerized chain. It is referred to as 'mer' in materials science.) Such a situation is shown in figure 1. When the grafting level is high, the chains overlap laterally to form a continuous 'brush' and the polymer system can now be considered a semidilute solution. The brush regime is shown in figure 2. Brushes have been described by scaling [22–26] and mean-field [27–30] theories.

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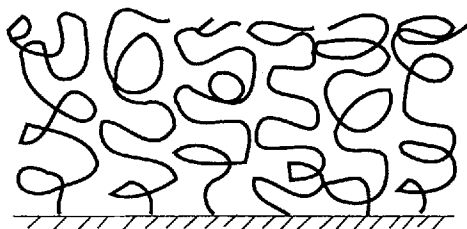Figure 1. Schematic diagram showing the mushroom regime.

Figure 2. Schematic diagram showing a brush.

If compressed, the polymer chain in both regimes (mushrooms and brush) will respond with a spring-like force, enhancing the repulsive properties of the surface of grafting.

The question that we will address in this paper is whether the fluidity (or the undulations) of the bilayer will change the steric repulsive forces between two polymer-grafted bilayers.

2. The mushroom regime

2.1. Bending rigidity

First, let us discuss the effect of the polymer mushrooms on the bending rigidity of the membrane.

When a small piece of the membrane is bent, the bending elastic energy per unit area, g_c is given, according to Helfrich [31] by the expression

$$g_c = \frac{k_c}{2}(c_1 + c_2)^2 + \bar{k}_c c_1 c_2, \qquad (2)$$

where c_1 and c_2 are the principal curvatures in a given point and $c_1 c_2$ is the Gaussian curvature. k_c and $\bar{k}_c$ are the elastic constants for bending and saddle (or Gaussian) bending, respectively. The bending rigidity coefficients can be obtained by calculating the energy density change upon cylindrical $(c_1 = c, c_2 = 0)$ and spherical $(c_1 = c_2 = c)$ deformation

$$g_{cyl} = \tfrac{1}{2}k_c c^2 \qquad (3)$$

and

$$g_{sph} = (2k_c + \bar{k}_c)c^2. \qquad (4)$$

From these two equations one finds k_c and $\bar{k}_c$.

Using these relations we now predict the influence of the polymer in the mushroom regime on the bending rigidity of the membrane. We first suppose that the free energy of the mixed bilayer is the sum of the elastic energies of the phospholipid membrane and the grafted polymer:

$$\left.\begin{array}{l} g'_{cyl} = g_{cyl} + g_{cyl_{polymer}} \\[2mm] g'_{sph} = g_{sph} + g_{sph_{polymer}}. \end{array}\right\} \qquad (5)$$

and

This means that the elastic coefficients of the two components will sum up:

and

$$\left.\begin{array}{l} k'_c = k_c + k_{c_{polymer}} \\[2mm] \bar{k}'_c = \bar{k}_c + \bar{k}_{c_{polymer}}. \end{array}\right\} \qquad (6)$$

For both relations (5) and (6) the prime refers to the characteristics of the composite bilayer (lipid membrane and grafted polymer). The assumption is based on the fact that the volume fraction of the polymer in the aqueous surface layer is much less than one and the bilayer bathing solution is not much changed in the presence of the grafted mushrooms. Then it is reasonable to accept that the chemical potential of the lipid molecules is not affected much by the presence of the polymer.

We assume that in the liquid bilayer the lipids (and the polymer-grafted lipids in particular) easily diffuse in the plane of the bilayer and eventually 'collide' with each other.

The simplest way of modelling the case of low polymer surface coverage is as a van der Waals gas confined to a two-dimensional surface. This is a gas of mushrooms, moving in the plane of the bilayer. When in contact, the polymer coils will not interpenetrate each other, they will act like hard spheres.

The potential of interactions is assumed to be

$$U = \infty \quad \text{for} \quad z < R_F \qquad (7)$$

where R_F is the Flory radius, a measure of the size of a polymer coil in solution and

$$U = 0 \quad \text{for} \quad z > R_F. \qquad (8)$$

This is justified by the fact that the energy density, causing coil deformations is much higher than the pressure of this gas. To prove this, let us do a crude calculation: In the dilute solution, the pressure is

$$\Pi_{dilute} = \frac{kT}{R^2} \qquad (9)$$

where R is the average distance between coils. $R \gg R_F$.

The pressure, needed to deform a coil, is of the order of [32]

$$\Pi = \frac{kT}{\xi^2} \qquad (10)$$

where ξ is a characteristic length and $\xi < R_F$. Obviously $R \gg \xi$ and $\Pi_{dilute} \ll \Pi$.

Let us calculate the chemical potential μ of one mushroom of this gas. The free energy of the van der Waals gas of mushrooms is given by [33]

$$F = -XkT\left[\ln\left(n_q \frac{(S - XR_F^2)}{X}\right) + 1\right]. \qquad (11)$$

Here S is the area of the bilayer and X is the number of

mushrooms, confined to one side of it. Obviously, $X = S/D^2$, where D is the distance between the mushroom grafting points. R_F^2 is the area per one mushroom. $(S - XR_F^2)$ then is the unoccupied, accessible area. n_q is the quantum concentration, which can be easily shown to be much higher than the mushroom surface concentration.

$$\mu = \frac{\partial F}{\partial X} = kT \left(\frac{S}{S - XR_F^2} - \ln \left(n_q \frac{(S - XR_F^2)}{X} \right) - 1 \right)$$

$$= kT \left(\frac{R_F^2}{D^2} - \ln \left(n_q D^2 (1 - R_F^2/D^2) \right) \right) \qquad (12)$$

for $R_F^2 \ll D^2$.

The chemical potential μ is a function of the distance between points of grafting, which depends on the curvature. The energy density change upon bending then will be

$$g = (\mu(D_o) + \mu(D_i) - 2\mu(D))/D^2, \qquad (13)$$

where D_o and D_i are the distances between polymer grafting points for the outer and inner monolayer. D is this distance for a planar bilayer. Now we have to find the relationship between D, D_o and D_i for both cylindrical and spherical deformation.

2.1.1. Cylindrical deformation

In this case,

$$\frac{D_o^2}{D^2} = \frac{R + d/2}{R} \qquad (14)$$

and

$$\frac{D_i^2}{D^2} = \frac{R}{R + d/2}, \qquad (15)$$

where d is the thickness of the membrane. Then

$$D_o^2 = D^2 \left(1 + \frac{dc}{2} \right) \qquad (16)$$

and

$$D_i^2 = D^2 \left(1 + \frac{dc}{2} \right). \qquad (17)$$

The substitution of (17) and (16) into (13) gives

$$g_{cyl} = \frac{kT}{D^2} \left(\frac{R_F^2}{D^2} + \frac{1}{4} \right) d^2 c^2 \qquad (18)$$

and from (3)

$$k_{c_{polymer}} = \frac{kT}{D^2} \left(2 \frac{R_F^2}{D^2} + \frac{1}{2} \right) d^2, \qquad (19)$$

or the mushrooms increase the bending rigidity of the membrane. To make an estimate, let us consider a polymer with molecular weight 2000 Da ($R_F \cong 35$ Å). We will use the following values: $d = 50$ Å for the lipid bilayer thickness, $D^2 = A/M$, where $A = 70$ Å is the area of a lipid molecule and $M = 3$ per cent is the fraction of polymer-

lipids in the lipid mixture. (For these parameters $M = 3$ per cent will be a concentration within the mushroom regime.) This gives $k_{c_{polymer}} \propto 0.5kT$, i.e. the increase of the membrane bending rigidity due to the grafted mushrooms is small.

2.1.2. Spherical deformation

In case of spherical deformation,

$$\frac{D_o^2}{D^2} = \frac{(R + d/2)^2}{R^2}, \qquad (20)$$

$$\frac{D_i^2}{D^2} = \frac{R^2}{(R + d/2)^2} \qquad (21)$$

$$D_o^2 = D^2 \left(1 + dc + \frac{d^2 c^2}{4} \right), \qquad (22)$$

and

$$D_i^2 = D^2 \left(1 - dc + \frac{d^2 c^2}{4} \right). \qquad (23)$$

From (23), (22) and (13) we obtain

$$g_{sph} = \frac{kT}{D^2} \left(3 \frac{R_F^2}{D^2} + \frac{1}{2} \right) d^2 c^2, \qquad (24)$$

or from (4)

$$\bar{k}_{c_{polymer}} = -\frac{kT}{D^2} \left(\frac{R_F^2}{D^2} + \frac{1}{2} \right) d^2 \qquad (25)$$

We obtained $\bar{k}_{c_{polymer}} < 0$. It is of the order of kT. In case the $\bar{k}_{c_{polymer}}$ exceeds the gaussian rigidity of the 'pure' lipid bilayer we may expect instabilities in the planar bilayer phase: the incorporation of polymer-lipids into lipid bilayers can lead to the appearance of 'rippled phases' having zero mean curvature and non-zero gaussian curvature.

2.2. Undulation-induced polymer repulsion

As discussed in the introduction, the mushrooms extend at a distance

$$L = R_F \cong aN^{3/5}. \qquad (26)$$

If the surfaces do not fluctuate, the interactions will be zero for $h > R_F$, where h is half the average distance between the two bilayers.

Now we will consider the effect of the undulating membranes on bilayer interactive properties for $h > R_F$ and $h < R_F$.

2.2.1. $h > R_F$

We can assume that the outer edge of the mushrooms define a surface. This surface will 'follow' the undulations of the surface of grafting. Two such effective surfaces then will experience repulsive forces of the Helfrich's type

$$F_f = \frac{3\pi^2}{128} \frac{(kT)^2}{k_c'(2h - 2R_F)^2},$$ (27)

where h is half the average distance between the bilayers and k_c' is the rigidity of the membrane. Due to the membrane undulations the repulsive forces between the mushroom-covered bilayers will extend further from the surface than the average mushroom size, i.e. an 'undulation tail' will appear.

Although the analysis, presented here is simple and somewhat naive, we suggest that equation (27) gives an estimate of the average undulation energy density for $h > R_F$. Note that it does not apply for h equal to or very close to R_F.

The idea of a surface, defined by the outer edge of the mushrooms applies only if the bilayer surface is densely covered by mushrooms. If the polymer-lipid concentration is very low, however, a mushroom, grafted to one surface can be compressed directly against the apposing surface: i.e. the mushrooms grafted on apposing surfaces can interdigitate. For this limiting case we propose undulation energy density of the kind

$$F_f = \frac{3\pi^2}{128} \frac{(kT)^2}{k_c'(2h - R_F)^2}$$ (28)

for $2h > R_F$.

2.2.2. $h < R_F$

We calculate the confinement energy of a mushroom, trapped between two thermally undulating surfaces. The proposed treatment is in terms of de Gennes' theory [24] of fluctuations in liquid crystals, later developed by Helfrich [2] for membranes.

In our calculations we assume that the membrane is under no tension and fluctuates around its planar state. The polymer is assumed to affect the membrane undulations by changing its bending rigidity. The fluctuations of the bilayer change the confinement energy of the polymer.

We will assume that two mushrooms grafted on opposite bilayers are compressed against each other (see figure 3), i.e. the compression will be described by half the distance between the bilayers which will be denoted here by h'.

The confinement energy of the polymer mushroom is [32]

$$F_{msh} = kTN \left(\frac{a}{h'}\right)^{5/3}.$$ (29)

If the bilayers fluctuate h' can be written as

$$h' = h + u(x, y, t),$$ (30)

where h is half the average distance between the surfaces and $u(x, y, t)$ is a small displacement from the planar state (see figure 4). Then we can present the confinement free energy in the form

$$F(h')_{msh} = F(h)_{msh} + \Delta F_{msh}.$$ (31)

We will be interested in ΔF_{msh}, which is due to the thermal fluctuations of the membrane.

Expanding ΔF_{msh} for $u \ll h$ we get

$$\Delta F_{msh} = kTN \left(\frac{a}{h}\right)^{5/3} \left(-\frac{5u}{3h} + \frac{20u^2}{9h^2}\right).$$ (32)

Let us adopt periodic boundary conditions: we divide the membrane into squares with size R and assume that $u(x, y, t) = u(x + iR, y + jR, t)$ where i and j are integers. Let there be n mushrooms on area R^2. The undulation energy averaged over this area is

$$\Delta \bar{F}_{msh} = n \int \frac{\Delta F}{R^2} dx\, dy = n \frac{kTN}{R^2} \left(\frac{a}{h}\right)^{5/3}$$

$$\times \int \left(-\frac{5u(x, y, t)}{3h} + \frac{20u^2(x, y, t)}{9h^2}\right) dx\, dy.$$ (33)
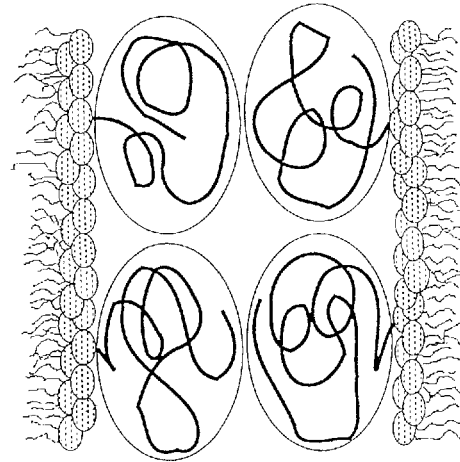

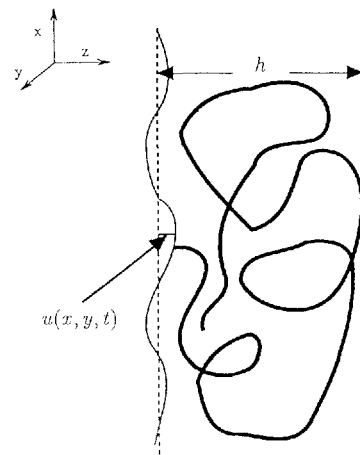Figure 3. Mushrooms, compressed between two lipid surfaces.

Figure 4. Schematic diagram showing a mushroom grafted on an undulating surface.

It is convenient to analyse the displacement in terms of Fourier components

$$u = \sum_q u_q(t)\exp(-iqr) \tag{34}$$

where

$$r = (x, y) \quad \text{and} \quad q = (q_x, q_y) = (n_x 2\pi/R, n_y 2\pi/R).$$

Then

$$\Delta\bar{F}_{msh} = n\frac{kTN}{R^2}\left(\frac{a}{h}\right)^{5/3}\left[\frac{-5}{3h}\sum_q u_q(t)\int_0^R \exp(-iq_x x)\,dx\right.$$

$$\times \int_0^R \exp(-iq_y y)\,dy + \frac{20}{9h^2}\sum_q\sum_{q'} u_q(t)u_{q'}(t)$$

$$\left.\times \int_{R^2}(\exp - [i(q + q')r]\,dr\right]. \tag{35}$$

The first term in the sum is zero and

$$\Delta\bar{F}_{msh} = \frac{20}{9}\frac{R^2}{R^2}\frac{n}{h^2}kTN\left(\frac{a}{h}\right)^{5/3}$$

$$\times \sum_q\sum_{q'} u_q(t)u_{q'}(t)\delta(q + q') \tag{36}$$

$$= \frac{20}{9}\frac{n}{h^2}kTN\left(\frac{a}{h}\right)^{5/3}\sum_q u_q(t)u_{-q}(t). \tag{37}$$

Taking into account that the complex conjugate u_q^* is equal to u_{-q} (because u is real) and averaging over time we get

$$\Delta\bar{F}_{msh} = \frac{20}{9}\frac{n}{h^2}kTN\left(\frac{a}{h}\right)^{5/3}\sum_q |u_q^-|^2, \tag{38}$$

where $|u_q^-|^2$ is the mean square displacement of the membrane, derived by Helfrich [2] on the basis of his formula for the curvature elastic energy and the assumption of independent modes of membrane fluctuations

$$|u_q^-|^2 = \frac{kT}{R^2 k_c'(q_x^2 + q_y^2)^2}. \tag{39}$$

Using this expression, for the mean free undulation energy we get

$$\Delta\bar{F}_{msh} = \frac{20}{9}\frac{n}{h^2}\frac{(kT)^2}{k_c}\frac{N}{R^2}\left(\frac{a}{h}\right)^{5/3}\sum_{q_x}\sum_{q_y}\frac{1}{(q_x^2 + q_y^2)^2}. \tag{40}$$

Taking into account that

$$\sum_{q_x}\sum_{q_y}\propto\frac{R^2}{(2\pi)^2}\frac{2\pi}{4}\int q\,dq \tag{41}$$

we have

$$\Delta\bar{F}_{msh} = \frac{20}{9}\frac{n}{h^2}\frac{(kT)^2N}{(2\pi)k_c}\left(\frac{a}{h}\right)^{5/3}\frac{1}{4}\int\frac{dq}{q^3} \tag{42}$$

$$= \frac{20}{9}\frac{(kT)^2N}{(2\pi)^3 k_c}\left(\frac{a}{h}\right)^{5/3}\frac{1}{8}\left(\frac{r_{max}^2 - r_{min}^2}{h^2}\right), \tag{43}$$

where $q_{max} = 2\pi/r_{min}$ and $q_{min} = 2\pi/r_{max}$ are the two cut-off frequencies. It is natural to choose r_{min} to be the square root of the area A per lipid molecule in the bilayer, much less than r_{max}. Then the free energy increase per one mushroom due to the undulations will be

$$\Delta F_{msh} = \frac{5}{18}\frac{(kT)^2N}{(2\pi)^3 k_c}\left(\frac{a}{h}\right)^{5/3}\frac{r_{max}^2}{h^2}. \tag{44}$$

In order to estimate the effect we write the free energy per mushroom in the form

$$F_{msh}(h') = F_{msh}(h)(1 + \delta_{fluct}^{msh}) \tag{45}$$

where

$$\delta_{fluct}^{msh} = \frac{5}{18}\frac{kT}{k_c}\frac{1}{(2\pi)^3}\frac{r_{max}^2}{h^2}, \tag{46}$$

δ_{fluct}^{msh} is a measure of the ratio $\Delta F^{msh}/F^{msh}(h)$. We see that it depends on the cut-off radius r_{max}. For membranes of size L the cut-off radius $r_{max} = L$ and δ_{fluct}^{msh} is inversely proportional to the bending rigidity. If L is large, however, the amplitude of undulational modes can become very large according to (39) and can exceed h. For such large systems we will choose the cut-off r_{max} in the following way: following Helfrich [2] we will demand that the average mean square displacement u^2 does not exceed $h^2/6$. The mean square displacement is

$$u^2 = \sum_q |u_q^-|^2, \tag{47}$$

where $|u_q^-|^2$ is given by equation (39). Then u^2 will depend on the cut-off radius r_{max} as

$$u^2 = \frac{h^2}{6} = \frac{1}{8}\frac{kT}{k_c}\frac{r_{max}^2}{(2\pi)^3}, \tag{48}$$

or

$$r_{max}^2 = 8\pi(2\pi)^3\frac{h^2}{6}\frac{k_c}{kT}, \tag{49}$$

which gives

$$\delta_{fluct} = \frac{\Delta F}{F_0} = 0{\cdot}37, \tag{50}$$

i.e. for large (infinite) systems the undulations can increase the steric repulsion by 37 per cent.

At the end we would like to note that de Gennes's equation (29) applies only for polymers, strongly compressed with respect to their random coil size (32). The analysis presented in this section is based on equation (29) and so it also applies to strongly compressed mushrooms. As discussed in § 2.2.1. equation (27) applies only for $h > R_F$. One may expect a regime of crossover (a smooth transition) between the two analytical predictions (27) and (44). It will not be discussed here in detail. The treatment of the two limiting cases presented in this paper however reveals the physics of the polymer-

grafted bilayer system and can give an estimate of the magnitude of the effects.

3. The brush regime

3.1. Bending rigidity

In the brush regime polymer chains interact laterally. These lateral steric interactions strongly influence the bending rigidity of the membrane. The bending rigidity of the brush-covered bilayer is again the sum (even in the semidilute solution the volume fraction of the polymer is much less than one and the bilayer bathing solution is not much changed in the presence of the grafted polymer)

$$k_c' = k_c + k_{c_{polymer}}. \tag{51}$$

$k_{c_{polymer}}$ for brushes was calculated by Milner and Witten [35] to be

$$k_{c_{brush}} = \frac{9}{64} \left(\frac{12}{\pi^2}\right)^{1/3} kTN^3 \left(\frac{a}{D}\right)^{14/3}. \tag{52}$$

This shows that the brush induces large, nonlinear increase of the bending rigidity of the membrane. A thick dense brush can increase the rigidity of a membrane by orders of magnitude and suppress the undulations.

3.2. Undulation-induced polymer repulsion

The extension of the brush from the surface depends not only on the degree of polymerization N as in the case of mushrooms, but also on the grafting density. The bigger the density, the thicker the brush. The brush height L is calculated by Milner et al. [27,28],

$$L = \left(\frac{12}{\pi^2}\right)^{1/3} N \frac{a^{5/3}}{D^{2/3}}. \tag{53}$$

Again we will consider the two cases: $h > L$ and $h < L$.

3.2.1. $h > L$

We write the repulsive forces due to the undulations in analogy to the mushroom case

$$F_f = \frac{3\pi^2}{128} \frac{(kT)^2}{k_c'(2h - 2L)^2}, \tag{54}$$

where L is the brush height and k_c' is given by (51) and (52). Due to the high values of the bending rigidity of the brush-covered membrane these forces are expected to be much weaker than in the mushroom regime. Again, equation (54) does not apply for h equal to or very close to R_F.

3.2.2. $h < L$

We will work in terms of the mean field theory of Milner, Witten and Cates [27–30], which gives the most adequate description of the brush so far. The free energy

of the compressed brush in terms of the mean-field theory is

$$F_{MWC}(h) = kT \left(\frac{\pi^2}{12}\right)^{1/3} N \frac{a^{4/3}}{D^{4/3}} \left(\frac{L}{2h'} + \frac{h'^2}{2L^2} - \frac{h'^5}{10L^5}\right), \tag{55}$$

where L is given by (53).

For brushes grafted on soft surfaces, half the distance between the bilayers can be written again as

$$h' = h + u(x, y, t) \tag{56}$$

and

$$F(h')_{MWC} = F(h)_{MWC} + \Delta F_{MWC}, \tag{57}$$

where ΔF_{MWC} is due to the thermal undulations of the membrane.

In analogy to the mushroom case for the free energy of one chain we get

$$\Delta F_{MWC}(h) = \left(\frac{\pi^2}{12}\right)^{1/3} N \frac{(kT)^2}{k_c'} \frac{a^{4/3}}{D^{4/3}} \left(\frac{L}{2h} + \frac{h^2}{2L^2} - \frac{h^5}{L^5}\right)$$
$$\times \frac{1}{(2\pi)^3} \frac{1}{8} \left(\frac{r_{max}^2}{h^2}\right). \tag{58}$$

Again, one may expect a regime of crossover (a smooth transition) between the two analytical predictions (54) and (58).

To estimate the effect again we write the free energy per polymer in the brush regime in the form

$$F_{MWC}(h') = F_{MWC}(h)(1 + \delta_{fluct}^{br}). \tag{59}$$

This can be done easily for high compressions, when $(h/L)^5$ is negligible with respect to $(h/L)^2$. In this case, from (55) and (58) we obtain

$$\delta_{fluct}^{br} \cong \frac{1}{8} \frac{kT}{k_c'} \frac{1}{(2\pi)^3} \frac{r_{max}^2}{h^2}, \tag{60}$$

where k_c' is the rigidity of the brush-covered lipid bilayer.

For large systems using (49) we find

$$\delta_{fluct}^{br} = \frac{\Delta F}{F_0} = 0.16. \tag{61}$$

For small finite systems (systems with size $L < r_{max}$, where r_{max} is given by (49)) the cut-off radius is the size of the system L. The relative free energy increase due to the thermal undulations δ_{fluct}^{br} is given by (60) with $L = r_{max}$ and will depend on the bending rigidity of the membrane.

4. Discussion

We propose a mechanism, through which the low bending rigidity may change the steric polymer repulsion between soft polymer-coated surfaces.

In terms of the proposed model we find:

(i) The interactions between two soft polymer-grafted bilayers extend further than twice the average

polymer extension. For membrane separations larger than twice the height of the polymer layer they are given by (27) and (54) for mushrooms and brushes respectively. The dependence of these forces is inversely proportional to the membrane bending rigidity, which is increased by the presence of the polymer. This increase is much smaller for mushrooms than for brushes. A thick dense brush can suppress the membrane fluctuations and greatly reduce the 'undulation tail'.

(ii) The relative confinement energy increase due to membrane undulations does not depend on k_c for compressed mushrooms and brushes for large (infinite) systems.

(iii) The relative free energy increase due to membrane undulations is 37 per cent for compressed mushrooms and 16 per cent for compressed brushes for large (infinite) systems.

The results (ii) and (iii) follow from our assumption for the cut-off radius (see (49)): modes, which lead to displacements larger than the separation between the bilayers, are excluded from the analysis.

For small finite systems (systems with size $L < r_{max}$, where r_{max} is given by (49)), the cut-off radius will be the size of the system. Then the relative free energy contribution due to membrane undulations for compressed mushrooms and brushes will be given by (46) and (60), respectively, with $L = r_{max}$ and will depend on the bending rigidity of the membrane.

We would like to point out that R_F or L gives the average extension of the polymer (in the mushroom and brush regime, respectively) from the bilayer surface. We can expect random fluctuations in the height of the polymer layer (particularly in the case of mushrooms). The major contributor to the non-uniform polymer layer height in a real system, however, is the inherent polydispersity of the commercially available polymers (data, kindly provided by Union Carbide, show that this polydispersity is close to gaussian). The effect of polydispersity on the interactive properties is considered elsewhere (A. Kenworthy, K. Hristova, D. Needham and T. J. McIntosh, *Biophysical Journal* (submitted)). One may expect that the non-uniform polymer layer height changes the undulational contribution to polymer repulsion, especially for $h > R_F$ (equations (27) and (54)). We assume, however, that the presented analysis gives an estimate of the average polymer repulsion due to bilayer undulations. Also, we can assume that the effect of polydispersity and the effect of bilayer fluctuations, as considered here under the assumption of monodisperse polymer chains with molecular weight equal to the value for which the gaussian distribution of molecular weights has its peak, are

independent and the respective free energies sum up. This is a simple model, and it is yet to be tested.

Based on our calculations, we suggest that the energy density of polymer steric repulsion will be different for lipid bilayers above and below the gel to liquid crystalline phase transition (assuming that all other system parameters and the polymer-solvent interactions in particular do not change). Experimentally, this can be tested measuring the repulsive pressures between polymer-grafted bilayers below and above the phase transition as a function of the separation between bilayers. This should be done with unsupported bilayers. A method of choice then may be the osmotic stress method [13, 14, 36–42] in which the separation distance between two adjacent bilayer surfaces in a multilamellar liposomal suspension is measured as a function of applied osmotic pressure using X-ray diffraction.

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