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Interactions between proteins bound to biomembranes

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We study a physical model for the interaction between general inclusions bound to fluid membranes that possess finite tension γ, as well as the usual bending rigidity κ. We are motivated by an interest in proteins bound to cell membranes that apply forces to these membranes, due to either entropic or direct chemical interactions. We find an exact analytic solution for the repulsive interaction between two similar circularly symmetric inclusions. This repulsion extends over length scales $\sim\sqrt{\kappa/\gamma}$ and contrasts with the membrane-mediated contact attraction for similar inclusions on tensionless membranes. For noncircularly symmetric inclusions we study the small, algebraically long-ranged, attractive contribution to the force that arises. We discuss the relevance of our results to biological phenomena, such as the budding of caveolae from cell membranes and the striations that are observed on their coats. These, and other, ‘‘gnarly buds’’ may prove fascinating to study further.

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I. INTRODUCTION

A significant proportion of all proteins in a typical eukaryotic cell are membrane proteins. These are found anchored to cell membranes. Many of these carry out tasks such as signal transduction, pore or channel formation, cytoskeletal binding, etc. [1]. Others are involved in endocytosis and exocytosis. In particular, it is now understood that the formation of clathrin coated pits is driven by the controlled geometric aggregation of clathrin which exerts corresponding forces on the cell membrane [2,3]. There also exists a less well understood class of membrane invaginations, known as caveolae, that are less morphologically distinct than clathrin coated pits and resemble aspherical invaginations with a typical size of the order of 100 nm [4]. There is good evidence that caveolae are involved in endocytosis [5] and play an important role in cell signaling [6]. Intriguingly, they are probably also involved in the sensing of shear stress [7,8]. Such stresses would be expected to act on the tension γ of the cell membrane. Thus, the present work may yield predictions for some of the direct physical consequences of surface tension, e.g., on interprotein forces. In Sec. II we discuss some qualitative effects of surface tension although a quantitative analysis of the effect of shear on membrane tension is beyond the scope of the present work. Recent elegant experiments demonstrate that better control of the surface tension may be achieved via micropipette aspiration facilitating studies of the effects of tension on membrane elasticity [9–11] and permeability to water [12].

It has now been shown that caveolins [13], a recently discovered class of membrane-bound proteins, are necessary for the formation of caveolae. These caveolins have a short membrane spanning sequence and N- and C-terminus polypeptide ‘‘tails,’’ totalling about 150 amino acids, *both* found on the cytoplasmic side of the cell membrane. It is thought that these caveolin molecules are typically found in small aggregates of size b (a few nm) containing approximately 15 molecules [14]. A crude thermodynamic (entropic) bound on the bending moment would be $\gtrsim k_B T b$, higher for larger aggregates.

Many theoretical studies have sought to calculate the effect of an adsorbed polymer on flexible, fluid membranes, see, e.g., Refs. [15–19]. One reason for this approach is that the simplified models available from polymer physics allow for a more or less exact computation of the entropic pressure exerted by the polymer on the membrane. Our work represents a generalization of this approach. An anchored polymer is but one example, or model, of a membrane inclusion that exerts a force on the membrane. Any membrane protein that is not perfectly symmetrical across the membrane should exert forces, the distribution of which will be dictated by the precise protein configuration. Furthermore, it can be argued that the physical effect of integral membrane protein can also be described by a well chosen force distribution. Hence there is a need for a general theory on the effect of arbitrary force distributions on a flexible membrane. Our premier interests are large peripheral membrane protein such as caveolin. Our theory is, however, general enough to be applied to any flexible fluid interface with fixed tension, containing embedded impurities.

Much of the previous work on interaction between membrane-bound objects has focused on membranes with vanishing surface tension [15–18], although there have been exceptions [20]. This work exists within the context of an extensive literature on membrane mediated interactions on tensionless membranes, see, e.g., Refs. [21–23], which focussed mainly on Casimir-like forces originating from membrane fluctuations. It was argued that these could be either attractive or repulsive, depending on the temperature and certain details of the model. The interest in tensionless membranes has its origin in the fact that isolated, self-assembled lipid membranes should be tensionless at equilibrium. Importantly, this is not the case for many cell membranes. Other studies include tension as a (shape dependent) parameter determined self-consistently for closed membrane surfaces [24]. A study of the behavior of membranes under constant surface tension may shed light on the physics occurring on

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biological membrane, which are not truly at equilibrium and hence bear substantial tensions [25,26]. Cells commonly adjust their surface tension to a set value via a mechanism known as surface-area regulation [27]. Hence membrane phenomena over sufficiently long time scales effectively occur at constant surface tension. Previous studies of the effects of rodlike objects embedded in fluid membranes [20], adhesive junctions [28], and impurities at gas-liquid interfaces [29,30] share certain similarities with this study.

II. ANALYSIS OF MEMBRANE DEFORMATION AND MEMBRANE-MEDIATED INTERACTIONS

Our aim is to construct a theoretical model for the membrane-mediated interactions between proteins bound to cell membranes. To achieve this we will denote the normal membrane displacement from its average flat state as $u(\mathbf{r})$, where $\mathbf{r}$ is a two-dimensional (2D) vector in the plane of the unperturbed membrane. Motivated by a desire to analyze small membrane deviations, we employ an expansion of the free energy of the membrane in powers and gradients of u. The free energy of a deformed fluid membrane, without inclusions, is well approximated by [31]

$$F_{memb}=\int d^2r\left[\frac{\kappa}{2}[\nabla^2 u(\mathbf{r})]^2+\frac{\gamma}{2}[\boldsymbol{\nabla} u(\mathbf{r})]^2\right], \tag{1}$$

which represents the truncation of an arbitrary expansion at order u^2 (odd orders are excluded by symmetry) and at the second derivatives of u. For a planar membrane, with normal in the $\hat{z}$ direction, the gradient operator is $\boldsymbol{\nabla}=\hat{x}(\partial/\partial x)+\hat{y}(\partial/\partial y)$. The only two parameters that are needed to describe the physics of the membrane are κ, the elastic bending modulus (typically [32] $20k_BT$ for biomembranes) and γ the surface tension (estimated to be [25] 10^{-1} to 10^{-2} pN/nm).

To include the work done by the pressure (normal force per unit area) $f(\mathbf{r})$ exerted by one or more inclusions we exploit the fact that, for small deviations, the work done is merely the surface integral of the product of pressure and distance. The displacement has no effect on the pressure field at this order. Thus the total free energy, including the effect of inclusions is

$$F=\int d^2r\left[\frac{\kappa}{2}[\nabla^2 u(\mathbf{r})]^2+\frac{\gamma}{2}[\boldsymbol{\nabla} u(\mathbf{r})]^2-f(\mathbf{r})u(\mathbf{r})\right], \tag{2}$$

where the term involving $f(\mathbf{r})$ is chosen to have a minus sign to ensure that the membrane displacement has the desired sign. At thermodynamic equilibrium the free energy F must reach its minimum value, at which point $u(\mathbf{r})$ satisfies the Euler-Lagrange equation

$$\mathcal{L}_1\mathcal{L}_2 u(\mathbf{r})-\frac{f(\mathbf{r})}{\kappa}=0 \tag{3}$$

with $\mathcal{L}_1=\nabla^2$, $\mathcal{L}_2=\nabla^2-k^2$, and $k^2=\gamma/\kappa$ indicating an intrinsic length scale $k^{-1}\approx 30$ nm (with perhaps 6 nm$\lesssim k^{-1}\lesssim$100 nm). We solve this under the boundary condition $\lim_{\mathbf{r}\to\infty}\boldsymbol{\nabla}u(\mathbf{r})=0$ and that $\lim_{\mathbf{r}\to 0}u(\mathbf{r})$ exists. Since the commutator $[\mathcal{L}_1,\mathcal{L}_2]=0$ it can be shown that

$$u(\mathbf{r})=\frac{1}{\gamma}\int[G_1(\mathbf{r},\mathbf{r}')-G_2(\mathbf{r},\mathbf{r}')]f(\mathbf{r}')d^2\mathbf{r}', \tag{4}$$

where G_1 and G_2 are the Green's functions corresponding to the self-adjoint problems determined by $\mathcal{L}_1$ and $\mathcal{L}_2$ (and their boundary conditions), respectively. We now seek to find the Green's functions of these self-adjoint operators.

For $\mathcal{L}_1$ we solve

$$\mathcal{L}_1 G_1(\mathbf{r},\mathbf{r}')=\nabla^2 G_1(\mathbf{r},\mathbf{r}')=-\delta(\mathbf{r}-\mathbf{r}'). \tag{5}$$

The only boundary conditions compatible with this equation are Von Neumann conditions at infinity, i.e., $\lim_{\mathbf{r}-\mathbf{r}'\to\infty}\boldsymbol{\nabla}G_1(\mathbf{r}-\mathbf{r}')=0$. This corresponds to the membrane being asymptotically flat and yields the well known Green's function of Poisson's equation in two-dimensions,

$$G_1(\mathbf{r},\mathbf{r}')=-\frac{1}{2\pi}\ln A|\mathbf{r}-\mathbf{r}'|, \tag{6}$$

where A is an arbitrary constant with dimensions of (length)$^{-1}$. We choose $A=k$ for convenience, this choice corresponds only to a definition of the zero of the free energy.

For $\mathcal{L}_2$ we must solve

$$(\nabla^2-k^2)G_2(\mathbf{r},\mathbf{r}')=-\delta(\mathbf{r}-\mathbf{r}'), \tag{7}$$

subject to the boundary conditions specified above. This self-adjoint problem has linearly independent solutions $K_0(k|\mathbf{r}-\mathbf{r}'|)$ and $I_0(k|\mathbf{r}-\mathbf{r}'|)$. These are, respectively, the zeroth order modified Bessel functions of the first and second kind. The function $I_0(x)$ diverges as $x\to\infty$, therefore we write the Green's function as

$$G_2(\mathbf{r}-\mathbf{r}')=\frac{1}{2\pi}K_0(k|\mathbf{r}-\mathbf{r}'|). \tag{8}$$

This gives us the solution of Eq. (3), subject to the stated boundary conditions as

$$u(\mathbf{r})=\int G(\mathbf{r},\mathbf{r}')f(\mathbf{r}')d^2\mathbf{r}' \tag{9}$$

with the Green's function

$$G(\mathbf{r},\mathbf{r}')=-\frac{1}{2\pi\gamma}[K_0(k|\mathbf{r}-\mathbf{r}'|)+\ln k|\mathbf{r}-\mathbf{r}'|]. \tag{10}$$

Equations (9) and (10) represent a solution for the equilibrium membrane displacement $u(\mathbf{r})$ due to an arbitrary force distribution. The formal analysis is concluded in the Appendix, where we show that this solution is unique.

A. Interaction potential between two proteins

Consider a fluid membrane with two, not necessarily identical, inclusions bound to it. Denote the pressure fields acting on the membrane surface due to each inclusion by

$\psi^{(1)}$ and $\psi^{(2)}$. Without loss of generality we may let $\psi^{(1)}$ be centered at the origin and let the vector $\mathbf{r}$ joining the centers of the pressure distributions lie along the x axis. Mathematically, this means that we write the total pressure distribution as

$$f(\mathbf{r}') = \psi^{(1)}(\mathbf{r}') + \psi^{(2)}(\mathbf{r}' - \mathbf{r}), \tag{11}$$

where $\psi^{(2)}(\mathbf{r}' - \mathbf{r})$ is the pressure distribution of the second inclusion centered at $\mathbf{r}$, rather that the origin. Hence the free energy at equilibrium is given by $F = F^{(1)}_{self} + F^{(2)}_{self} + \Phi(\mathbf{r})$, where $F^{(i)}_{self}$ is the self-energy of the ith inclusion and $\Phi(\mathbf{r})$ is the interaction energy. We are interested only in $\Phi(\mathbf{r})$ as this is what determines the physics of interest to us here. By direct analysis or analogy with electrostatics this may be expressed as

$$\Phi(\mathbf{r}) = -\int d^2\mathbf{r}' \int d^2\mathbf{r}'' \psi^{(1)}(\mathbf{r}')\psi^{(2)}(\mathbf{r}'')G(\mathbf{r} - \mathbf{r}' + \mathbf{r}''), \tag{12}$$

where $G(\mathbf{r})$ is the real space Green's function.

For the case that we have two inclusions with circular symmetry we are able to determine the interaction potential exactly. Consider two circularly symmetric inclusions, i.e., $\psi^{(i)}(\mathbf{r}') = \psi^{(i)}(|\mathbf{r}'|) = \psi^{(i)}(r')$ such that $\psi^{(i)}(r') = 0$ for $r' > b$, where b is some maximum radius of the inclusion. Also each pressure field is such that no average force acts $[\int \psi^{(i)}(\mathbf{r}')d^2\mathbf{r}' = 0]$. For inclusions that are not anchored to external structures, such as the cytoskeleton, this condition must be satisfied on general grounds. Indeed Newton's third law requires that any average force applied to the membrane by an inclusion must be equal and opposite to the reaction force applied by the inclusion to some external structure. If there is no external structure for this reaction force to act against then there can be no average force on the membrane. We assume that the pressure fields do not overlap so that $r > 2b$. Under these assumptions the logarithmic term in Eq. (10) gives a vanishing contribution to the integral Eq. (12) due to Gauss theorem. It corresponds to the electric field due to two circularly symmetric charged disks in two-dimensions, each carrying no average charge and thus with zero field in the region outside both disks. This analogy will be revisited when we discuss noncircularly symmetric inclusions in Sec. IV below. The potential between two circularly symmetric inclusions is therefore given by

$$\Phi(\mathbf{r}) = \frac{1}{2\pi\gamma}\int_0^b r'dr' \int_0^b r''dr'' \psi^{(1)}(r')\psi^{(2)}(r'') \times \int_0^{2\pi} d\theta' \int_0^{2\pi} d\theta'' K_0(k|\mathbf{r} - (\mathbf{r}' - \mathbf{r}'')|), \tag{13}$$

the upper limits of integration are both b since $\psi(r') = 0$ for $r > b$. We make use of the identities

$$\int_0^{2\pi} K_0(k|\mathbf{r} - (\mathbf{r}' - \mathbf{r}'')|)d\theta'' = 2\pi K_0(kr)I_0(k|\mathbf{r}' - \mathbf{r}''|) \tag{14}$$

valid for $r > |\mathbf{r}' - \mathbf{r}''|$, and

$$\int_0^{2\pi} I_0(k|\mathbf{r}' - \mathbf{r}''|)d\theta' = 2\pi I_0(kr')I_0(kr'') \tag{15}$$

valid for $r' \neq r''$. Thus we are able to derive an exact analytical form for the interaction potential between nonoverlapping $(r > 2b)$ circularly symmetric inclusions

$$\Phi(\mathbf{r}) = \frac{1}{2\pi\gamma}\zeta^{(1)}\zeta^{(2)}K_0(kr), \tag{16}$$

where $\zeta^{(i)} = 2\pi\int_0^\infty r'\psi^{(i)}(r')I_0(kr')dr'$ characterizes the strength of the ith membrane-inclusion coupling. By employing the expansion of $I_0(kr')$ the quantity $\zeta^{(i)}$ can be thought of as a series of moments of the force distribution. The radial force is as usual given by the derivative of the potential $f_r = -\partial\Phi/\partial r$. The interaction is everywhere *repulsive*. Some previous studies have reported attractive interactions between polymer chains grafted on tensionless membranes [15,16]. In these studies the position $u(0)$ of the chain grafting point was fixed, effectively by a Lagrange force that then acted to ensure that there was no average force, although this condition was not identified explicitly by these authors. In the case of vanishing tension it was found that there was no interaction between inclusions unless they ''touch,'' i.e., their force fields overlap. One can understand this as follows. For tensionless membranes the membrane deformation under the force field is dictated by the minimization of an energy similar to Eq. (2) but with $\gamma = 0$. The membrane deformation outside the extent of the force field simply minimizes the curvature energy, hence has a zero mean curvature and does not contribute to the total energy. As two inclusions approach each other, the total energy does not change until one force field couples to the membrane deformation directly under the other force field, since the deformation energy of any piece of membrane that it not in direct contact with a force field is zero. There is no characteristic length scale k^{-1} to give the interactions' finite range when $\gamma = 0$. In consequence, inclusions interact only when the two force fields overlap. In the case of grafted ideal polymer chains the energy is decreased when the force fields overlap because they both want to deform the membrane in the same direction, resulting in an attraction. We note that the interaction between overlapping membrane inclusions is very sensitive to the direct physical, rather than membrane mediated, interactions between them, unlike Eq. (16) which is universal. Were we to blindly apply our formalism to the case of overlapping force distributions we are also able to recover a regime where the interactions are attractive. In the $\gamma \rightarrow 0$ limit Eq. (16) gives vanishing interactions, entirely consistant with these earlier results for tensionless membranes.

As mentioned in the Introduction, recent experiments on the effect of shear forces on living cells provide a crude control on membrane tension. In future studies we plan to calculate the precise effect of this on, e.g., surface phase equilibria and budding phenomena.

III. MICROPHASE SEPARATION AT THE SURFACE OF CAVEOLAR INVAGINATION

Membrane-mediated interactions between membrane-bound inclusions have been extensively studied theoretically [21] with, however, relatively little discussion of how experiments could easily support these predictions. Although presumably long range, these interactions are expected to be quite small, and are probably often dominated by stronger, (bio)chemically specific, short range phenomena. Long range interactions can, however, profoundly influence the phase behavior of certain membrane proteins even if stronger, short range interactions, are present. As we emphasize below, studies of such surface phases might provide an indirect probe of the membrane-mediated interactions of interest here. This might be of particular relevance for the study of the phase behavior of the protein caveolin, which is found at high density on certain membrane invaginations called caveolae (see Sec. I).

One peculiar feature of the caveolae bulbs is their texture. Distinct striations are seen at the surface of these buds [4] (see also Fig. 13.48 in Ref. [1]). These are now thought to correspond with the organization and alignment of caveolin oligomers on the membrane [33]. The observation of these surface stripes is intriguing, and the reader may find it interesting that radially symmetrical oligomers can give rise to nonsymmetrical surface phases. We discuss this below, arguing that the stripe phase may be a signature of the membrane-mediated repulsion between protein aggregates, such as those calculated in Sec. II. Indeed, studying the phase behavior of membrane inclusions may provide one of the best ways to test theory against experiment in this field. Molecular dissection of the caveolin protein has shown that caveolin oligomers strongly attract each other through contacts of specific protein sequences [6] (a short range attraction). It is well known that a solution of particles interacting via hard core repulsion and a short range attraction undergoes a gas-liquid phase separation. This results in large dense regions (the liquid) coexisting with less dense regions (the gas). The caveolin oligomer being anchored to the cell membrane, there exists an additional membrane-mediated, longer range repulsion between between them, as demonstrated in Sec. II, which allows for a more complex phase behavior [45]. It has been recently argued in the light of computer simulations [34–36] that a long range repulsion should break the gas and liquid phases into microdomains. The short range attraction still locally drives a gas-liquid phase separation, but large aggregates are costly because of the long range repulsion. The microdomains are circular liquid islands at low density, circular gas regions at high density, and stripes otherwise.

To gain a quantitative understanding of how stripes may form on caveolae we study the stability of an homogenous (2D) solution of oligomers of area $s_b = \pi b^2$ interacting via a potential $V(\mathbf{r})$, which include a short range (the particle size b) attractive exponential interaction and the longer range membrane mediated repulsion of Eq. (16): $V(r) = -E_a e^{-r/b} + E_r K_0(kr)$, where E_a and E_r are, respectively, the strengths of the attraction and the repulsion. We look at small perturbations $\delta\phi$ [with the conservation rule $\int dS\,\delta\phi(r)=0$] around the average surface coverage ϕ_0. The free energy includes the pair interaction and the translation entropy of the inclusions, for which we use the gas-on-a-lattice model. Expanding the free energy,

$$F = \int \frac{dS}{s_b}[\phi \ln \phi + (1-\phi)\ln(1-\phi)] + \frac{1}{2}\int \frac{dS dS'}{s_b^2}\phi(r)V(|r-r'|)\phi(r'),$$

$$\delta F = \int \frac{dS}{s_b}\frac{\delta\phi^2}{2\phi_0(1-\phi_0)} + \frac{1}{2}\int \frac{dS dS'}{s_b^2}\delta\phi(r)V(|r-r'|)\delta\phi(r'). \tag{17}$$

The Fourier transform $\delta\phi(r) = S\int[d^2q/(2\pi)^2]\delta\phi_q e^{i\mathbf{q}\cdot\mathbf{r}}$ allows us to investigate the formation of structures. It leads to

$$\delta F = \frac{1}{2}\frac{S^2}{s_b}\int \frac{d^2q}{(2\pi)^2}\mathcal{V}_q|\delta\phi_q|^2$$

with

$$\mathcal{V}_q = \frac{T}{\phi_0(1-\phi_0)} + \frac{1}{s_b}V_q. \tag{18}$$

If V_q, the Fourier transform of $V(\mathbf{r})$, is sufficiently negative (attractive) for a given mode q then $\mathcal{V}_q<0$ and the mode is unstable. Substituting the expression for $V(\mathbf{r})$, we obtain

$$\mathcal{V}_q = 2k_BT\left[\frac{1}{2\phi_0(1-\phi_0)} + \left(-E_a\frac{1}{[1+(bq)^2]^{3/2}} + E_r\frac{1}{k^2+q^2}\right)\right]. \tag{19}$$

In the absence of long range repulsion, the most unstable mode is always $q=0$ (macrophase separation), and the liquid-gas transition is observed provided $2E_a\phi_0(1-\phi_0) > k_BT$. Because of the membrane-mediated long range repulsion, the function $\mathcal{V}_q$ presents a minimum if $E_r > \frac{3}{2}E_a(kb)^4 \sim 10^{-3}E_a$ (for the typical numbers $b \simeq 5$ nm and $k^{-1} \sim 30$ nm). One sees that although the strength of membrane-mediated interaction [Eq. (16)] is expected to be quite small: $E_r \simeq 10^{-2}k_BT$ (see Sec. V A or Ref. [37] for a more detailed analysis), it is of much longer range, and should be competent to produce a well ordered phase.

The phase separation occurs preferentially for a mode given by $\partial\mathcal{V}_q/\partial q = 0$, and periodic arrays of dense and dilute regions are observed. In the limit $kb \ll 1$ the structure has a typical size $2\pi/q^*$ which is independent of the range of the repulsion: $q^*b = (2E_r/3E_a)^{1/4} \simeq \frac{1}{4}$. It thus defines dense stripes of width about five particle diameters, which agrees quite well with the experimental observations. The existence of a stripe phase at the surface of the biological structures known as caveolae is thus quantitatively consistent with the existence of a membrane-mediated repulsion between proteins described by Eq. (16).

This analysis does not help us to discriminate between circular domains and stripes. Computer simulation have clearly shown that this transition indeed exists for high enough surface coverage [35,36].

Note that the formation of striped mesostructures in membrane has been predicted on the basis of curvature effects only [38,39]. However, it can be shown that their existence at the surface of invagination of such large curvature as the caveolae ($R \sim 50$ nm) is inconsistent with the existence of invaginations with a well defined size.

IV. FAR-FIELD INTERACTIONS OF NONCIRCULARLY SYMMETRIC INCLUSIONS

If the force distribution of the inclusions is not circularly symmetric then no general analytic solution to Eq. (12) exists. However, we can still proceed by examining the far-field interactions of slightly asymmetric inclusions. In order to do this we will parametrize the asymmetry of the sth inclusion by

$$D_{ij}^{(s)} = \int d^2\mathbf{r}'\, r_i' r_j' \,\psi^{(s)}(\mathbf{r}') = \begin{pmatrix} c+\epsilon^{(s)} & 0 \\ 0 & c-\epsilon^{(s)} \end{pmatrix}, \quad (20)$$

where i and j are Cartesian indices, c controls the average (isotropic) magnitude, and ϵ controls the anisotropy of a slightly "elliptical" force distribution, extended in the x direction and contracted in the y direction (for $\epsilon > 0$). This force distribution can be rotated by the usual rotation matrix $R(\theta)$ to obtain the effective moment $R_{im}(\theta^{(s)}) R_{jn}(\theta^{(s)}) D_{mn}^{(s)}$ of an "elliptical" force distribution rotated by an angle $\theta^{(s)}$ relative to the x axis. This expression involves two rotations, one for each factor of $\mathbf{r}'$ in Eq. (20).

It is important to first note is that the interaction can be separated into two terms, one that is an integral involving $K_0(k|\mathbf{r}-\mathbf{r}'+\mathbf{r}''|)$ and the other that is an integral involving $\ln k|\mathbf{r}-\mathbf{r}'+\mathbf{r}''|$. The first of these terms gives a contribution to the interaction potential that is dominated by a term like Eq. (16), with corrections due to the asymmetry that are smaller (and also asymptotically exponentially short ranged). This conclusion is independent of the precise choice of asymmetry, provided only that it is small. The contribution from the second term is more interesting and can be shown to give rise to a small but *long ranged* correction $\delta\Phi$ to the interaction potential. This can be best understood by way of multipolelike expansion of $\ln k|\mathbf{r}-\tilde{\mathbf{r}}|$, where $|\tilde{\mathbf{r}}| \ll |\mathbf{r}|$ and we choose the direction of $\mathbf{r}=r\hat{x}$ to define the x axis (the 1 direction), without loss of generality. Thus $\tilde{r}_1$ and $\tilde{r}_2$ are the x and y components of $\tilde{r}$ and

$$\ln k|\mathbf{r}-\tilde{\mathbf{r}}| = \ln kr - \frac{\tilde{r}_1}{r} + \frac{\tilde{r}_2^2-\tilde{r}_1^2}{2r^2} - \frac{\tilde{r}_1^3 - 3\tilde{r}_1\tilde{r}_2^2}{3r^3} + \frac{6\tilde{r}_1^2\tilde{r}_2^2 - \tilde{r}_1^4 - \tilde{r}_2^4}{4r^4} + O\left(\frac{1}{r^5}\right). \quad (21)$$

The conditions of no overall force $\int d^2\mathbf{r}'\,\psi(\mathbf{r}')=0$ and no overall moment $\int d^2\mathbf{r}'\,\mathbf{r}'\,\psi(\mathbf{r}')=0$ for each inclusion mean that the first nonvanishing contribution to $\delta\Phi \sim 1/r^4$, the classical result for electrostatic quadrupole-quadrupole interactions in two dimensions. Using $\tilde{\mathbf{r}}=\mathbf{r}'-\mathbf{r}''$ in Eq. (21) to leading order in $1/r$ we have [46]

$$\delta\Phi = \frac{1}{4\pi\gamma}\int d^2\mathbf{r}' \int d^2\mathbf{r}''\,\psi^{(1)}(\mathbf{r}')\,\psi^{(2)}(\mathbf{r}'')\ln k|\mathbf{r}-\mathbf{r}'+\mathbf{r}''| = \frac{-3\epsilon^{(1)}\epsilon^{(2)}}{2\pi\gamma r^4}\cos 2(\theta^{(1)}+\theta^{(2)}). \quad (22)$$

This result demonstrates that elliptical inclusions attract if arranged so that $\theta^{(1)}+\theta^{(2)}=n\pi$ with n an integer, as reported in an earlier study of rodlike inclusions [20]. This condition represents a degenerate family of orientations in which the orientation of the quadrupoles has reflectional symmetry about the midplane. This perturbative interaction is long-ranged, scaling like $1/r^4$. Interestingly, this dominates the exponentially short ranged repulsion from Eq. (16) for large enough separations.

There is a straightforward way to understand the appearance of an algebraic potential for anisotropic inclusions. The physics of Eq. (5) is that of the Poisson equation in which the force distribution is analogous to the electrostatic charge. The potential due to a point charge in two-dimensions is therefore Eq. (6). Gauss law tells us that there is no field outside a circularly symmetric charge distribution that has no overall charge (imbalance). This condition is analogous to our requirement here that there be no overall force $\int d^2\mathbf{r}' f(\mathbf{r}')=0$ by Newton's law. If there is no field the potential is constant (zero without loss of generality) and no forces act on the inclusions. However, if we relax the condition that the force (charge) distribution is circularly symmetric there is no longer a simple symmetry argument that the radial and azimuthal components of the field must be zero. Indeed they are not. As is usual in multipole expansions in electrostatics an algebraic potential results. It is here analogous to the quadrupole-quadrupole interaction in electrostatics, since there are no dipole moments $\int d^2\mathbf{r}'\,\mathbf{r}'\rangle f(\mathbf{r}')=0$. This is due to the fact that there can be no external first force moment (torque) on the membrane if it is not anchored to any external structure against which the moment can act.

The physical consequences of these interactions are potentially significant. In spite of the fact that the dominant interaction is repulsive, the surface concentration of membrane inclusions may often be maintained at fairly high surface fractions by the regulatory mechanisms of the living cell. The composition of the surface coats of caveolae may be further enriched in several important membrane components, including cholesterol and proteins in the caveolin family, as well as others [6,13]. This means that there is an effective surface pressure, driven by a chemical potential difference that forces the inclusions to partially overcome their repulsion. In this situation the attractive $O(\epsilon^{(1)}\epsilon^{(2)})$ interactions might become significant, leading to an in plane anisotropic phase separation such as is observed in model systems [34–36,40] and on the coats of caveolae [4]. For caveolae the typical interactions seem too weak [37] to be solely responsible for the stripe morphology, which may rather be dominated by specific attractions, see Sec. III [33]. Finally, the existence of an attractive interaction that can arise from fluctuations in the inclusion force distribution (shape), as parametrized by ϵ, suggests the possibility of an attractive, fluctuation driven force reminiscent of van der Waals forces. This mechanism should extend to systems such as wetting droplets at gas-liquid interfaces. These can potentially exert much stronger forces on the interface, resulting in a more exaggerated effect (see Sec. V B).

V. ORIGIN OF THE FORCE DISTRIBUTION

Up to this point we have merely postulated the existence of a force distribution $\psi(\mathbf{r}')$ due to a membrane bound inclusion. This force can arise from direct mechanical effects, such as due to the geometry and shape of the inclusion, e.g., the protein clathrin, or entropic effects due to the asymmetric anchoring of, e.g., flexible hydrophilic polymers, to a membrane. The budding of caveolae is now thought to be driven by the protein caveolin which is known to form small oligomers resembling a number of polypeptide chains extending only from the cytoplasmic side of the membrane [14,41].

We have in mind that we have demonstrated that interactions should exist in general, but have not yet addressed the question of how big they might typically be. We are particularly interested in any models that can be chosen to be broadly comparable with membrane proteins. In this section we will present two examples of models for the origin of a force distribution arising from anchored polymer chains. This approach represents the application of a simple, and hence rather "idealized," theory drawn from polymer physics. Its exact quantitative applicability to polypeptide chains is almost certainly limited to a small subset of peptide sequences that either resemble a diffuse random coil or a dynamic, dense hydrophobic globule. Nonetheless, in the absence of any detailed microscopic information on a specific inclusion's configuration and stability the approaches that we outline below probably represent the best chance for us to obtain a rough idea of the likely scale of the effects that we have described in this paper. It is quite possible that the forces exerted by membrane proteins with significant well defined tertiary structure may be somewhat higher than the estimates that we will present below. This encourages us to consider our estimates as approximate lower bounds on the scale that these effects might reach.

In polymer physics terminology the two models that we consider for the force applied by a membrane-bound polymer will correspond to the two limits of "good" and "poor" solvent conditions, respectively [42]. We will discuss below how these models might apply to caveolin homo-oligomers. Other recent studies of the physics of flexible polymers grafted onto tensionless fluid membranes [15–18] undoubtedly share similar motivation. One feature that these earlier studies all have in common is that there is only an attraction for polymer force distributions that overlap one-another on the surface of the membrane and, when this is the case, the interaction is *attractive*. This is opposite in sign to the interactions that we predict on membranes that bear tension, see Eq. (16). Furthermore our interactions are extended, with ranges $\sim k^{-1}$.

A. Anchored polymers and polymer aggregates: Good solvent conditions

Motivated by possible biophysical relevance we investigate the effect of flexible polymer chains anchored to a small patch of membrane, of radius a. We assume that the chains are in a good solvent, i.e., that they are found in extended, hydrated random coil configurations. The chains form a hemisphere of outer radius $b>a$, see Fig. 1. This model may be used to treat polymer homo-oligomers made up of a general number Q of chains. As such, it shares many features with those that are known for caveolin homo-oligomers [14].

Within the "corona" of the polymer hemisphere, at radial distances $b<r<a$ from the center of the polymer aggregate, there exists a characteristic correlation length $\xi(r)$ for the polymer chains which crudely represents the spatial distance between collisions between the segments of a chain with other chains or the membrane [42]. The conservation of number of chains implies [43] $\frac{1}{2}4\pi r^2 \simeq Q\xi(r)^2$ for $a<r<b$. This is since the surface area of a hemisphere of radius r is filled by close packed blobs, up to a constant of order unity. From this one may immediately deduce the scaling of the correlation length $\xi(r)=(2\pi/Q)^{1/2}r$. The work done in generating each blob is k_BT, independent of the blob size. Thus we may write the pressure in this region as the energy per blob divided by the volume of a blob [43]

$$f(\mathbf{r})=\frac{k_BT}{\xi(r)^3}=\left(\frac{Q}{2\pi}\right)^{3/2}\frac{k_BT}{r^3} \tag{23}$$

for $a<r<b$. For $r>b$ the pressure is here assumed to be zero [47]. The pressure in the core binding region is assumed to be constant and must involve a total force equal and opposite to that applied by the corona so that no average force acts, as required by Newton's second law.

$$f(r)=\begin{cases} E_o a^{-3} & \text{if} \quad 0<r<a \\ \dfrac{-E_o}{2(1-a/b)}r^{-3} & \text{if} \quad a<r<b \\ 0 & \text{if} \quad r>b, \end{cases} \tag{24}$$

where $E_o=f(0)a^3=2k_BT(1-a/b)(Q/2\pi)^{3/2}$ is a characteristic energy related to the force applied at the center of the aggregate. This model represents one approximate framework for the understanding of forces applied by membrane biopolymers, see Fig 1. It possesses several important fea-

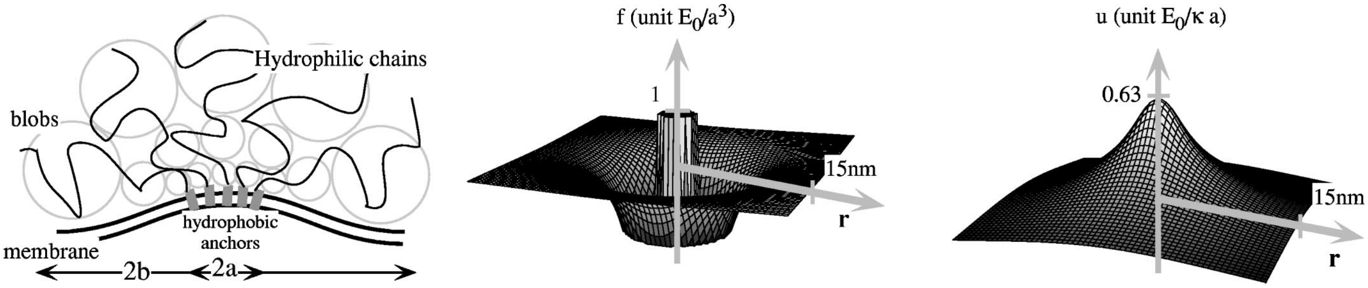

FIG. 1. Sketch of the form of the anchored polymer aggregate in good solvent conditions, when the polymer configurations are somewhat extended from the membrane. Our model for the force distribution $f(\mathbf{r}')$ is given by Eq. (24) and represents the force per area applied to the membrane by the polymers. The membrane is pushed down by the "corona" of the grafted polymers out to $r=b=5$ nm and is pulled upwards by the anchored "core" inside $r'=a=2$ nm as a result. For aggregates residing on the cytoplasmic face of the membrane, including caveolin homo-oligomers the cell interior would be above the membrane. The resulting deformation $u(\mathbf{r}')$ is shown out to $r'=15$ nm for the following values of the parameter values: $\gamma=10^{-4}$ J/m^{-2}, $\kappa=20k_BT$, and $Q=15$. The force and deformation can be expressed in terms of the characteristic energy E_o, see Eq. (24).

tures: It involves no average force being applied to the membrane. It involves no average first force moment by symmetry. The first nonvanishing moment is the second, corresponding to a bending moment with magnitude controlled by $\int r^2 f(r) d^2 r \simeq E_o a$ with the characteristic energy scale $E_o \gtrsim k_BT$ that is entropic in origin.

Models similar to this have been proposed elsewhere [15–18] although, as emphasized previously, the physical behavior of these inclusions on fluid membranes that are under tension is rather different.

We now proceed to give an estimate of the membrane deformation and interaction potentials due to membrane-bound biopolymers within the good solvent polymer brush model described above.

For biological membranes primarily composed of a bilayer of phospholipids the tension and rigidites are typically [32,25]

$$\kappa=10k_BT\text{–}40k_BT, \quad \kappa_{bio}=20k_BT,$$

$$\gamma=10^{-5}\text{–}10^{-3}\ \text{J/m}^2, \quad \gamma_{bio}=10^{-4}\ \text{J/m}^2, \tag{25}$$

where x_{bio} refers to what we would take as a single "typical" value for biological membranes. From this we obtain the length scale

$$k^{-1}=6\ \text{nm–}100\ \text{nm}, \quad k_{bio}^{-1}=30\ \text{nm}, \tag{26}$$

which may crudely be identified with the range of the interactions.

1. Membrane deformation due to the inclusion

The deformation at $r=0$ is

$$u_{brush}(r=0)=\frac{E_0}{\kappa}\alpha \tag{27}$$

in a frame where $u=0$ at infinity. This therefore represents the total magnitude of the normal deviation of the membrane expressed in terms of a dimensionless ratio E_0/κ and a characteristic length α.

For parameters that might be typical of caveolin homo-oligomers, $a=2$ nm and $b=5$ nm, we find

$$\alpha(k^{-1}=6\ \text{nm})=0.7\ \text{nm},$$

$$\alpha(k^{-1}=30\ \text{nm})=1.5\ \text{nm}, \quad \alpha(k^{-1}=100\ \text{nm})=2\ \text{nm}, \tag{28}$$

much the same as for slightly larger inclusions with $a=1$ nm and $b=10$ nm,

$$\alpha(k^{-1}=6\ \text{nm})=1.3\ \text{nm},$$

$$\alpha(k^{-1}=30\ \text{nm})=2.7\ \text{nm}, \quad \alpha(k^{-1}=100\ \text{nm})=4\ \text{nm}. \tag{29}$$

2. Membrane mediated interaction energy between inclusions

The interaction energy is expressed as

$$\Phi_{brush}=\frac{E_0^2}{\kappa}\beta, \tag{30}$$

which is the product of an energy E_0^2/κ, typically of the order of k_BT, and a dimensionless number β. For $a=2$ nm and $b=5$ nm,

$$\beta(k^{-1}=6\ \text{nm})=3\times10^{-2},$$

$$\beta(k^{-1}=30\ \text{nm})=10^{-3}, \quad \beta(k^{-1}=100\ \text{nm})=10^{-4}, \tag{31}$$

while for $a=1$ nm and $b=10$ nm we find instead

$$\beta(k^{-1}=6\ \ \text{nm})=0.13,$$

$$\beta(k^{-1}=30\ \ \text{nm})=5\times10^{-3},$$

$$\beta(k^{-1}=100\ \ \text{nm})=5\times10^{-4}. \tag{32}$$

B. Poor solvent conditions

If the polymer chains are more hydrophobic, and the solvent conditions are poor, the polymer chains may collapse

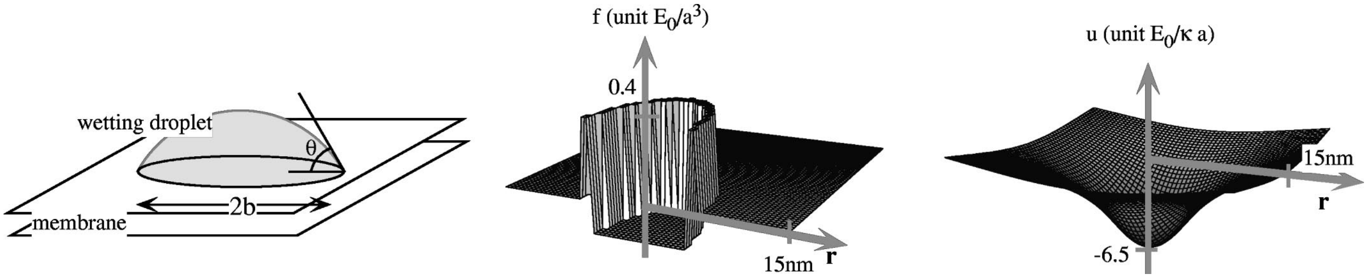

FIG. 2. Sketch of the shape of the anchored aggregate in poor solvent conditions, when the polymer forms a droplet on the membrane that is assumed to largely exclude water. Our model for the force distribution $f(\mathbf{r}')$ is given by Eq. (35). The membrane is now pulled upwards by the resolved Young's force on the contact line at $r'=b=5$ nm and is pushed downwards by the Laplace hydrostatic pressure for $r'<b$ as a result. The resulting deformation $u(\mathbf{r}')$ is shown out to $r'=15$ nm for the same total integrated force $\int d^2r'|f(\mathbf{r}')|$ shown in Fig. 1, for comparison. All the other parameters' values are also as given in the caption of Fig. 1. Even for the same integrated absolute force the membrane deformation is an order of magnitude larger than shown in Fig. 1, as well as having opposite sign. The enhanced effect is due to the concentration of the forces at the exterior of the aggregate.

into a tight, roughly hemispherical, region from which water is largely exuded. This represents the natural opposite limit to the good solvent polymer model considered above. In the poor solvent case we propose to model the inclusion as if it were a fluid droplet that partially ''wets'' the membrane surface [44]. At any point on the circumference of the drop the forces per unit length (due to the surface energy) are in equilibrium. Resolving these forces perpendicular to the membrane we find a total force acting upward

$$F=2\pi\sigma\sin\theta, \tag{33}$$

where b is the radius of the drop, σ is the energy cost per unit area of producing the interface between the inclusion and the external poor solvent, typically water, and θ is the contact angle given by Young's law [44]. Since we are in equilibrium this force must be balanced by the force acting on the interface between the drop (inclusion) and the membrane for $r<b$. To find the corresponding pressure we need only consider the partial wetting droplet as if it formed part of a larger sphere. The pressure is constant everywhere inside the sphere and is given by the Laplace law,

$$P=\frac{\partial E}{\partial V}=\frac{2\sigma}{R}, \tag{34}$$

where $R=b/\sin\theta$ is the radius of the sphere. The radius R can therefore be related to the volume of the drop (and b) by simple geometry. Hence we may write the pressure distribution as

$$f(\mathbf{r})=\frac{\sigma}{R}[b\,\delta(r-b)-2\Theta(b-r)], \tag{35}$$

where $\Theta(x)$ is the Heaviside unit step function.

This model represents a different possible physical origin for the pressure distribution that might be valid, e.g., for surface-anchored hydrophobic polymers in the poor solvent regime, when little water penetrates the protein chains, see Fig. 2. It also preserves the same features as the good solvent polymer model: It involves no average applied force or first force moment and has a bending moment $\int r^2 f(r)d^2r \simeq E_o b$ that is enhanced by the localization of part of the force distribution around the exterior circumference $r=b$ of the inclusion's footprint on the membrane. The characteristic energy scale is now chemical in origin, as it is controlled by chemical parameters such as σ. It is difficult to give a quantitative scale for this, although typical oil-water interfacial tensions of $\sigma_{ow}\gtrsim 3\times10^{-2}$ J m^{-2} suggest $E_o\gtrsim\sigma_{ow}b^2 \approx 180k_BT$ with $b\approx5$ nm. This large energy suggests thermodynamically ''strong'' interactions.

1. Membrane deformation due to the inclusion

For $b=10$ nm, $\sigma=40$ dyn/cm [48] the membrane's normal deformation at $r=0$ is

$$u_0(k^{-1}=9\ \text{nm})=37\ \text{nm},$$

$$u_0(k^{-1}=30\ \text{nm})=65\ \text{nm},\quad u_0(k^{-1}=90\ \text{nm})=92\ \text{nm} \tag{36}$$

for

$$b=100\ \text{nm},\ \ \sigma=40\ \text{dyn/cm},$$

$$u_0(k^{-1}=9\ \text{nm})=2700\ \text{nm},$$

$$u_0(k^{-1}=30\ \text{nm})=15\ \mu\text{m},\ \ u_0(k^{-1}=90\ \text{nm})=37\ \mu\text{m}. \tag{37}$$

2. Membrane mediated interaction energy between inclusions

The interaction potential energy has a simple analytical form:

$$\Phi=\frac{1}{2\pi\gamma}\zeta^2K_0(kr)=\frac{E_b^2}{2\pi\kappa}(kb)^2K_0(kr)\beta^2, \tag{38}$$

with

$$\beta=\frac{2\pi}{(kb)^2}\left(I_0(kb)-\frac{2}{kb}I_1(kb)\right). \tag{39}$$

For $b=10$ nm and $\sigma=40$ dyn/cm the energy scale E_0 [simply the potential Φ divided by $K_0(kr)k_BT$] is

$$E_0(k^{-1}=9\ \text{nm})=25,\quad E_0(k^{-1}=30\ \text{nm})=2,$$
$$E_0(k^{-1}=90\ \text{nm})=0.2 \tag{40}$$

for $b=100$ nm$\sigma=40$ dyn/cm,

$$E_0(k^{-1}=9\ \text{nm})=10^9,$$
$$E_0(k^{-1}=30\ \text{nm})=3\times10^6,\quad E_0(k^{-1}=90\ \text{nm})=2\times10^5. \tag{41}$$

VI. CONCLUSIONS

We show that a model for the interactions between inclusions bound to fluid membranes can be solved exactly for circularly symmetric inclusions bound to membranes that are under tension. We argue that proteins bound to cell membranes can apply no average force, or first moment of force, to the membrane unless they are also anchored to an external structure, such as the cytoskeleton. By this we mean that the total of any and all forces that the membrane protein acts to apply downwards against the membrane must exactly equal the magnitude of similar forces applied upwards. This is a consequence of Newton's laws of motion.

We proposed idealized models for the origin of these forces due to either entropic or direct chemical interactions. The interactions between two circularly symmetric inclusions are *repulsive* and are asymptotically exponentially short ranged with a typical extent on biological membranes that is of the order of $k^{-1}\approx 30$ nm. This result contrasts with the attraction predicted to appear between similar inclusions on tensionless membranes. For non circularly symmetric inclusions we predict an additional algebraically long ranged attractive contribution.

We discuss how competing attractive and repulsive interactions are known to sometimes produce a stripelike morphology. These phases are reminiscent of the stripes observed on the surface of caveloae, which we refer to as ''gnarly buds,'' and we discuss how a similar lateral phase separation could occur in these systems with typical length scales comparable to the ranges of the interactions.

We believe that the results discussed here may have wider applications in understanding biological phenomena, including lateral phase separation in phospholipid membranes and endocytotic budding. Indeed, a detailed theoretical analysis of the formation of caveolae invaginations on model phospholipid membranes utilizes many of the results derived here [37].

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APPENDIX: UNIQUENESS OF THE SOLUTION FOR THE EQUILIBRIUM MEMBRANE DISPLACEMENT DUE TO AN ARBITRARY FORCE DISTRIBUTION

Equations (9) and (10) permit the calculation of the equilibrium membrane displacement due to an arbitrary force distribution. This solution is unique, as may be demonstrated as follows.

We may add to our solution any solution of the homogeneous version of Eq. (3) without changing the result. Let $u_1(\mathbf{r})$ satisfy

$$\nabla^2(\nabla^2-k^2)u_1(\mathbf{r})=0 \tag{A1}$$

and the boundary conditions imposed. Multiplying by $u_1(\mathbf{r})$ and integrating we have

$$\int u_1(\mathbf{r})\nabla^2(\nabla^2-k^2)u_1(\mathbf{r})d^2\mathbf{r}=0. \tag{A2}$$

Integrating by parts and noting that the integrated terms are zero by virtue of the boundary conditions we obtain

$$\int \{[\nabla^2 u_1(\mathbf{r})]^2+k^2[\boldsymbol{\nabla} u_1(\mathbf{r})]^2\}d^2\mathbf{r}=0. \tag{A3}$$

The integrand is everywhere positive so we must have $\nabla^2 u_1(\mathbf{r})=0$ and $\nabla u_1(\mathbf{r})=0$ everywhere. Thus the only admissible solution of the homogeneous equation is $u(\mathbf{r})=\text{const}$. This corresponds only to a redefinition of the zero of the displacement and so without loss of generality we take this constant to be zero. This freedom of choice is a consequence of the translational symmetry of the problem in the direction perpendicular to the planar membrane.

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[45] A number of other factors, including variation in the membrane composition, may influence the phase behavior of these objects.

[46] From $\delta\Phi=(1/4\pi\gamma r^4)\int d^2\mathbf{r}'\int d^2\mathbf{r}''\psi^{(1)}(\mathbf{r}')\psi^{(2)}(\mathbf{r}'')\times\frac{3}{2}[4x'x''y'y''-(x'^2-y'^2)(x''^2-y''^2)]$ hence $\delta\Phi=1/4\pi\gamma r^4 R_{im}(\theta^{(1)})R_{jn}(\theta^{(1)})D_{mn}(\epsilon^{(1)})R_{kp}(\theta^{(2)})R_{lq}(\theta^{(2)})\times D_{pq}(\epsilon^{(2)})\mathcal{G}_{ijkl}$ with the interactions defined by the kernal $\mathcal{G}_{ijkl}=\frac{3}{2}[4\delta_{i1}\delta_{j2}\delta_{k1}\delta_{l2}-(\delta_{i1}\delta_{j1}-\delta_{i2}\delta_{j2})(\delta_{k1}\delta_{l1}-\delta_{k2}\delta_{l2})]$ and the result follows by contracting over all indices.

[47] In nature there will often be a smooth crossover around $r=b$. Nonetheless, we consider our approximation to be as good as any alternative and insist that, at large distances, the pressure must ultimately vanish due to the finite lengths of the polymer chains.

[48] The wetting drop is characterized by a surface tension, which for highly hydrophobic chains approaches the oil-water tension $\sigma=10$ dyn/cm–40 dyn/cm$=2.5$–$10k_BT/\text{nm}^2$, and a curvature radius for the surface of the drop $R=1$–100 nm. These combine to give a pressure $f_b=\sigma/R=2.5\times10^{-2}-10k_BT/\text{nm}^3$. We arbitrarily choose $b=R/2$ to give an idea of the scales and define the energy unit $E_b=f_bb^3=\sigma R^3/8$, so $E_b=0.3k_BT$–10^4k_BT. With $\sigma_{bio}=40$ dyn/cm$=10k_BT/\text{nm}^2$ and $R_{bio}=10$ nm we obtain $f_{b,bio}=1k_BT/\text{nm}^3$ and $E_{b,bio}=125k_BT$. We give only the results for $\sigma=40$ dyn/cm and for $\kappa=20k_BT$ for compactness. With these values $k^{-1}=9$–90 nm.