Adhesion of Membranes via Anchored Stickers

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Interacting membranes are studied which experience generic repulsive and specific attractive forces. The latter are mediated by anchored stickers, i.e., by anchored molecules with adhesive segments. This mechanism which underlies the adhesion of cell membranes can be studied in systems consisting of lipid bilayers with anchored polymers. It is shown that flexible membranes can adhere only by stickers if the sticker concentration exceeds a certain tension-dependent threshold. If the multicomponent membranes undergo phase separation, their adhesion is dominated by the sticker-rich domains. [S0031-9007(96)00932-5]

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The adhesion of membranes is governed by a variety of *generic* interactions arising from various intermolecular forces such as electrostatic or van der Waals forces [1]. If the membranes carry electric charges of the same sign, the electrostatic forces give a *repulsive* contribution to the intermembrane interactions. On the other hand, two identical membranes which carry no electric charges and have identical composition experience *attractive* van der Waals interactions. This attraction is renormalized by thermally excited shape fluctuations which leads to an unbinding transition from a bound state at low temperatures to an unbound state at high temperatures [2,3]. For two membranes which differ in their composition or for a membrane interacting with a rigid wall, the van der Waals interactions can be attractive or repulsive.

In addition to these generic forces, the membranes can interact via *specific* forces mediated by macromolecules or polymers. I will focus here on polymers which are anchored in one membrane and have another "sticky" segment by which they can interact with the other membrane. Such macromolecules will be referred to as anchored stickers. They lead to bonds or bridges between the two membranes (so-called *trans* interactions) which may consist of a single sticker, of two adhering stickers, or of two stickers which are connected by an additional linker molecule. Prominent examples from biology are cell adhesion molecules or receptor/ligand pairs which are anchored in cell membranes [4]. In the absence of such molecules, cell membranes effectively repel one another [5].

Cell membranes are rather complex systems containing a huge number of different types of molecules. In order to understand the interplay between generic repulsion and specific attraction from a physical point of view, it is desirable to consider simplified model systems such as lipid bilayers which repel each other, e.g., by electrostatic forces and which contain only one type (or a small number of different types) of stickers. Possible candidates for artificial stickers are linear polymers which are anchored by a lipid molecule attached to one of its ends or by a hydrophobic segment which fits into the lipid bilayer. In this Letter, I will consider such model membranes which do not adhere in the absence of the stickers.

If the stickers consist of very flexible polymers, they can bend back and form arches on a single membrane as shown in Fig. 1(a). For a given separation of the membranes, the configurational entropy of such an arch state is larger than the entropy of a bridge state. Therefore, most stickers will form arches and only a small fraction of these stickers will actually form bridges. Since the entropy of the polymer in the arch state attains its maximum in the limit of large separations, these arches lead to a repulsive contribution to the polymer-induced interaction between

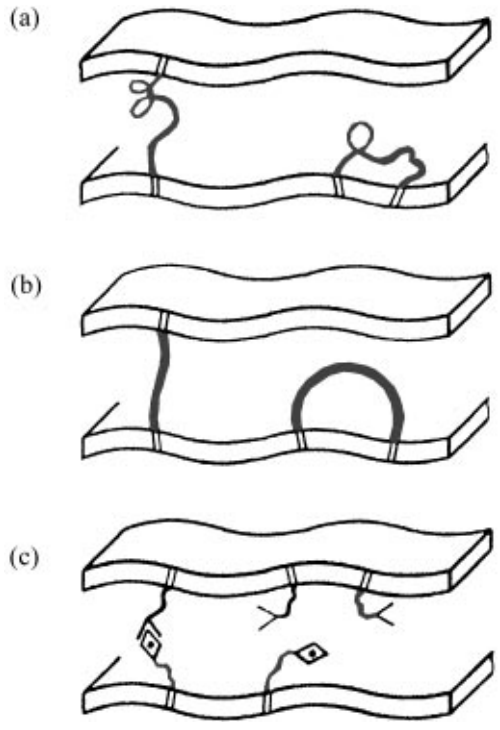

FIG. 1. Pairs of membranes with stickers which can form bridges (left) or arches (right). In (a) and (b) the stickers consist of flexible and rigid polymers, respectively. In (c) the bridges are formed from two different types of stickers.

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the membranes which can dominate the attractive contribution arising from the bridges.

In order to suppress the formation of arches, one may use stickers which consist of rigid polymers; compare Fig. 1(b): If the bending rigidity of the polymers is sufficiently large, arches become unlikely since the increase in their bending energy dominates over the gain in their entropy. Arches can be avoided completely if the bridges consist of two different stickers forming a receptor/ligand bond and if each type of sticker is present only in one membrane; see Fig. 1(c). The latter situation corresponds to heterophilic bonds between cell membranes but will be more difficult to control in artificial membrane-polymer systems.

Cell membranes and other biomembranes are typically in a *fluid* state. Therefore, the model membranes considered here are also taken to be fluid which implies that both the lipid molecules and the anchored stickers are *mobile* and can move along the membranes (the case of immobile stickers will be discussed briefly at the end). In general, two stickers within the same membrane will exhibit different types of *cis* interactions which can be repulsive or attractive. I will first discuss the case in which these interactions are effectively repulsive (arising, e.g., from electric charges) or in which the attractive components of these interactions are overcome by the entropy of mixing. In this case, each membrane consisting of a mixture of lipids and anchored stickers is in a homogeneous state, i.e., in a state with uniform composition.

The simplest adhesion geometry is provided by one fluid membrane, say 1, with anchored stickers which interacts with another membrane or a rigid wall, say 2, without stickers. The lateral size of such a sticker is denoted by $a_\parallel$, the mean separation of their anchors by $\xi_{\rm an}$. The sticker concentration is measured by the area fraction $X_1 \equiv (a_\parallel/\xi_{\rm an})^2$.

A systematic theory for such a system must include (i) the degrees of freedom related to the shape of the membranes and (ii) those related to the positions of the stickers. The displacements of the two membranes will be described by the displacement fields $l_1(x)$ and $l_2(x)$ which depend on the lateral coordinate x; their local separation is then given by $l \equiv l_2 - l_1$. The positions of the stickers within membrane 1 which point towards the other membrane are described by the density field $n_1(x)$. The effective Hamiltonian for these variables has the generic form

$$\mathcal{H}\{l, n_1\} = \mathcal{H}_{\rm el}\{l\} + \mathcal{H}_1\{n_1\} + \mathcal{H}_{12}\{l, n_1\}. \quad (1)$$

If the two membranes have bending rigidites κ_1 and κ_2 and if they experience lateral tensions Σ_1 and Σ_2, the elastic term $\mathcal{H}_{\rm el}$ in (1) is given by [1]

$$\mathcal{H}_{\rm el}\{l\} = \int d^2x \{\tfrac{1}{2}\kappa(\nabla^2 l)^2 + \tfrac{1}{2}\Sigma(\nabla l)^2\}, \quad (2)$$

with $\kappa \equiv \kappa_1\kappa_2/(\kappa_1 + \kappa_2)$ and $\Sigma \equiv \Sigma_1\Sigma_2/(\Sigma_1 + \Sigma_2)$.

The second term $\mathcal{H}_1$ in (1) contains the cis interactions between the stickers whereas the last term $\mathcal{H}_{12}$ embodies the sticker-mediated trans interactions between the membranes. In order to discuss the latter term, it is convenient to discretize space into a two-dimensional lattice with lattice constant $a_\parallel$ and lattice sites i. The sticker positions are now described by occupation numbers $n_{1i} = 0, 1$ and the membrane separation by $l = l_i$. In terms of these variables, the interaction term $\mathcal{H}_{12}$ has the form

$$\mathcal{H}_{12}\{l, n_1\} = \sum_i a_\parallel^2 \{(1 - n_{1i})V_0(l_i) + n_{1i}V_1(l_i)\}. \quad (3)$$

Thus, in the absence and presence of a sticker, the interaction potential is given by $V_0(l)$ and $V_1(l)$, respectively. As mentioned, $V_0(l)$ is taken to be purely repulsive. In contrast, the one-sticker potential $V_1(l)$ is characterized by a potential well of depth $|U_1|$ and of range l_1.

Since the stickers are mobile, all degrees of freedom are in thermal equilibrium and one may perform an annealed average of the partition function over the sticker positions. In the dilute limit of small sticker concentration X_1, one may ignore the *cis* interactions and thus the correlations between the stickers. Likewise, these correlations are negligible in the dense limit with $X_1 \lesssim 1$. In these cases, the annealed average over the sticker positions for a given configuration of the separation field l leads to the effective sticker potential $V(l)$ as given by

$$e^{-\overline{V}(l)} \equiv (1 - X_1)e^{-\overline{V}_0(l)} + X_1 e^{-\overline{V}_1(l)}, \quad (4)$$

with $\overline{V} \equiv a_\parallel^2 V/T$ etc. where T denotes the temperature in energy units (i.e., the Boltzmann constant k_B has been absorbed into the symbol T). It follows from (4) that the effective potential well contained in $V(l)$ has the depth $|U|$ as given by

$$a_\parallel^2|U|/T \approx X_1(\exp[a_\parallel^2|U_1|/T] - 1) \quad (5)$$

for small sticker concentration X_1.

In the absence of lateral tension, two membranes which interact via an attractive, short-ranged potential well with depth $|U|$ and range l_1 undergo an unbinding transition at the critical value $|U| = |U_*| = u_* T^2/\kappa l_1^2$. Such a transition was first predicted from functional renormalization applied to the interaction potential [2]. The dimensionless coefficient u_* has the value $u_* \simeq 0.2$ as determined by Monte Carlo simulations [6]. In the present context, the potential strength $|U|$ is proportional to X_1 as in (5) which implies the critical sticker concentration

$$X_{1*} \simeq u_*(T/\kappa)(a_\parallel/l_1)^2/(\exp[a_\parallel^2|U_1|/T] - 1) \quad (6)$$

for tensionless membranes where $a_\parallel$ denotes the lateral size of the stickers as before. For phospholipid bilayers with $\kappa \simeq 10^{-19}$ J, this expression leads to the estimate

$X_{1*} \lesssim 10^{-2}$ at room temperature. Thus, the critical value of X_1 is found to lie within the dilute regime which justifies the use of (4).

The sticker concentration must exceed the threshold value X_{1*} as given by (6) in order to induce adhesion of the two membranes. On the other hand, if the concentration X_1 approaches the critical value X_{1*} from above, the membranes undergo a continuous unbinding transition and their mean separation $\langle l \rangle$ diverges as $\langle l \rangle \sim l_1/\epsilon$ with $\epsilon \equiv (X_1 - X_{1*})/X_{1*}$ as follows from previous work using functional renormalization [2] and Monte Carlo simulations [6].

Now, assume that the membranes experience lateral tensions which act to suppress their shape fluctuations [7,8]. In this situation, the phase diagram depends on two dimensionless variables: the rescaled potential depth $u \equiv |U|\kappa l_1^2/T^2$ and the rescaled tension $s \equiv \Sigma l_1^2/T$. For small s, the singular part of the free energy F per unit area exhibits the scaling form $F(u - u_*, s) = b^{-2}F[b^{\lambda_u}(u - u_*), b^{\lambda_s}s]$ with $\lambda_u = 1$ and $\lambda_s = 2$ [9,10]. This implies that the phase boundary is given by $u = u_c(s) \approx u_* - c_*\sqrt{s}$ or by

$$|U_c| \approx |U_*|\left(1 - c_*\sqrt{\Sigma l_1^2/T}\right) \quad \text{for small } \Sigma\,, \qquad (7)$$

with a dimensionless coefficient c_* of order one [11].

For large s, on the other hand, the shape fluctuations are strongly suppressed, and the critical value of $u \sim |U|$ goes to zero. In this limit, one may apply functional renormalization up to first order in the interaction potential $V(l)$. This method has been previously used in the context of wetting [12] and for protrusion forces [13], and its results have been confirmed by Monte Carlo simulations [13,14]. For the square well potential considered here, functional renormalization predicts the phase boundary $u = u_c(s) \sim s\exp[-2\sqrt{2\pi s}\,]$ or

$$|U_c| \sim (T\Sigma/\kappa)\exp[-2l_1/l_\Sigma] \quad \text{for large } \Sigma\,, \qquad (8)$$

where the new length scale $l_\Sigma \equiv (T/2\Sigma)^{1/2}$ has been introduced. The latter scale governs the membrane roughness in the presence of lateral tension. The relation (8) implies that $|U_c|$ decays rapidly to zero as soon as $l_\Sigma < 2l_1$ or $\Sigma > \Sigma_* = s_*T/l_1^2$ with $s_* \equiv 1/8\pi \simeq 0.04$ [15].

It now follows from (7) and (5) that the adhesion threshold X_{1c} decreases with increasing tension as

$$X_{1c} \approx X_{1*}\left(1 - c_*'\sqrt{\Sigma/\Sigma_*}\right), \qquad (9)$$

with the coefficient $c_*' \equiv c_*\sqrt{s_*}/u_* \simeq c_*$. Since c_* is of order one, this relation should be applicable up to $\Sigma \simeq \Sigma_*$ where it matches with the large tension behavior as given by (8) with $|U_c| \sim X_{1c}$ [16].

For a potential range $l_1 \simeq 3$ nm, one has $\Sigma_* \simeq 0.02$ mJ/m^2 at room temperature. For lipid bilayers in aqueous solution, the lateral tension has been measured by different methods. For lipid vesicles which are aspirated by micropipettes, the lateral tension can be controlled by the suction pressure and can be varied from $\Sigma \simeq 10^{-4}$ mJ/m^2 at initial aspiration up to the tension of rupture, $\Sigma_{\max}$, which is of the order of a few mJ/m^2 [17]. For vesicles which are filled with a sugar solution and which stick to a solid wall because of gravity, lateral tensions in the range 10^{-3} mJ/m$^2 \lesssim \Sigma \lesssim 10^{-1}$ mJ/m^2 have been obtained from an analysis of the shape fluctuations [18]. Furthermore, bunches of adhering bilayers exhibit effective contact angles from which lateral tensions in the range 3×10^{-6} mJ/m$^2 \lesssim \Sigma \lesssim 10^{-3}$ mJ/m^2 have been deduced [19]. A rather new experimental method is provided by optical tweezers [20] by which one presumably applies a lateral tension below 10^{-3} mJ/m^2 onto the bilayers [21]. Therefore, the low-tension regime with $\Sigma < \Sigma_* \simeq 0.02$ mJ/m^2 is accessible for lipid bilayer systems.

For the square well potential, functional renormalization predicts universal critical behavior along the whole transition line $u = u_c(s)$ with $s > 0$. Along this line, the mean separation is found to behave as $\langle l \rangle \sim l_1 \ln[1/\epsilon]/\epsilon$ with $\epsilon = (X_1 - X_{1c})/X_{1c}$ which should be compared with $\langle l \rangle \sim l_1/\epsilon$ for $s = 0$. This similarity is, however, restricted to the behavior of $\langle l \rangle$. The membrane roughness, for example, behaves as $\sim\langle l \rangle \sim 1/\epsilon$ for $s = 0$ but as $\sim\sqrt{\langle l \rangle} \sim 1/\sqrt{\epsilon}$ for $s > 0$.

So far, I have assumed that the membrane consisting of a mixture of lipids and anchored stickers is in the homogeneous one-phase region for which one may ignore the *cis* interactions of the stickers within the same membrane. If the latter interactions are effectively attractive, the stickers may form clusters or domains which lead to new adhesion phenomena. Thus, let us assume that the mixed bilayer has been prepared in a two-phase region in which a sticker-poor phase, say α, coexists with a sticker-rich phase, say β. If such a membrane undergoes phase separation, one has α domains with a small sticker concentration $X_1 = X_\alpha$ and β domains with a larger sticker concentration $X_1 = X_\beta$. When this membrane interacts with another membrane without stickers, the effective sticker attraction $|U|$ is now given by $|U_\alpha| \sim X_\alpha$ and $|U_\beta| \sim X_\beta$, respectively.

In general, both interaction strengths will differ from each other and may lie above or below the adhesion threshold $|U_c|$. This leads to different mean separations $\langle l \rangle_\alpha$ and $\langle l \rangle_\beta$ for the different membrane domains. One interesting case is obtained if $|U_\alpha| < |U_c|$ but $|U_\beta| > |U_c|$. In the latter situation, the interacting membranes only adhere via the β domains. This resembles the focal adhesions which are often observed for cell membranes.

A systematic theory for the interplay of domain formation and adhesion should start from a model as given by (1)–(3) with $\mathcal{H}_1\{n_1\} = \sum W_{ij}n_{1i}n_{1j} - \mu_1\sum n_{1i}$. Attractive *cis* interactions between two stickers at i and j are described by $W_{ij} < 0$. Starting with such a model, one

may calculate the concentrations X_α and X_β in terms of the interaction parameters [22]. In addition, one may study the effect of composition fluctuations which are important if the mixed membrane were close to a critical point.

It is straightforward to generalize the above theory to two fluid membranes which *both* contain stickers. If the bonds or bridges between the membranes are formed by two adhering stickers, the effective strength $|U|$ of the sticker-mediated attraction is proportional to X_1X_2 where X_1 and X_2 are the sticker concentrations in membranes 1 and 2, respectively. Likewise, if these bridges consist of two stickers connected by one linker molecule, the effective strength $|U|$ is proportional to $X_1X_2p(1-p)$ where p is the fraction of stickers with a bound linker molecule. Phase separation within the interacting membranes now leads to three possible values $|U_{\alpha\alpha}|$, $|U_{\alpha\beta}|$, and $|U_{\beta\beta}|$ for the sticker-mediated attraction.

Finally, let us briefly consider the adhesion induced by *immobile* stickers. Examples are provided (i) by membranes which are attached to solid surfaces via stickers which are grafted onto these surfaces, (ii) by polymerized membranes with stickers, and (iii) by cell adhesion molecules which are attached to the cytoskeleton within the cell. In these systems, the disorder in the sticker positions is quenched or frozen which represents a marginal and irrelevant perturbation at the unbinding transition of fluid and polymerized membranes, respectively [9]. The critical concentration X_c of immobile stickers will again exhibit an adhesion threshold as found here for mobile ones. On the other hand, new critical behavior is expected for tensionless fluid membranes as has been recently found for the analogous system of one-dimensional strings with quenched random interactions [23] but this remains to be studied.

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