Statistical Mechanics of Membrane Adhesion by Reversible Molecular Bonds

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(Received 11 October 1994)

We present a simple statistical-mechanical model for membranes adhering by thermally fluctuating, reversible molecular bonds. The free energy of the molecular bonds, which interact through the membrane tension, can be related to that of the one-component two-dimensional Coulomb plasma in the grand-canonical ensemble. Using the known properties of the Coulomb plasma, we compute the macroscopic membrane-membrane adhesive potential. We find that, unlike existing theories of membrane adhesion, thermal fluctuations *assist* adhesion and that the weak-adhesion potential has an unexpectedly long, temperature-controlled range.

PACS numbers: 87.22.Fy, 34.20.Cf, 82.65.Dp, 87.22.Bt

The nature of the interactions between surfactant-bilayer membranes controls, in part, the phase behavior of amphiphilic systems [1]. Membrane-membrane forces consequently have been well studied, in particular, for one-component model bilayers for which it was established that the dominant attractive force is the van der Waals interaction while electrostatic repulsion and hydration forces produce repulsion [2]. It was also shown [3] that thermally induced membrane-membrane collisions play an important role. They generate an effective repulsion, the Helfrich force [4], which competes with the van der Waals force.

Membrane-membrane forces have also been measured [5] for biological membranes. Adhesion in biological membranes is not due to the van der Waals attraction but instead to the formation of weak, but highly specific, molecular bonds between complementary pairs of proteins [denoted by "lock" (L) and "key" (K)] imbedded in the two membranes [6]. Bonded L and K pairs, referred to below as "LK molecules," have binding energies which are only of order $(1-10)k_BT$ [7]. Thermal fluctuations between the bonded and unbonded states are thus important and in this Letter we will show how, for a simple model, these fluctuations produce a long-range *attraction* between the membranes whose strength exceeds that of the repulsion generated by thermal collisions.

Our model is illustrated in Fig. 1. The spacing between LK molecules is, under typical conditions, large compared to molecular dimensions, so we will neglect direct protein-protein forces. LK molecules do, however, interact indirectly through the elastic force they exert on the membranes [8], which can produce a membrane-mediated long-range coupling [9]. We will assume the two membranes to be under a tension γ, as is true for white blood cells (WBC's) [10] and for adhering cells in general [11]. In that case, the membrane-mediated elastic coupling produces a logarithmic pair potential between LK molecules [see Eq. (8)]. Nonadhesive membrane glycoproteins ("glycocalix") squeezed between the membranes provide a long-range osmotic repulsive pressure Π [12]. We will assume Π to be constant. Finally, the L and K molecules are assumed to be mobile inside the membranes [13].

The statistical mechanics of the model can be examined by a mapping of its partition function onto that of the grand-canonical partition function of the one-component two-dimensional (2D) Coulomb plasma (OCP). Using this mapping, we find the following results for the adhesion potential energy. For LK binding energies E_B of order k_BT (weak binding), the potential is smooth and has a temperature controlled range $\varsigma = (2k_BT/\pi\gamma)^{1/2}$ which is large compared to the microscopic range of protein-protein forces. It exceeds the thermal collision repulsive force and it does not vanish in the limit of zero binding energy. In the strong binding limit, with E_B large compared to k_BT, there is a freezing transition from a 2D liquid to a 2D solid for membrane spacings of order ζ, followed by a sudden loss of adhesion for spacings of order $\Delta h_f \propto (E_B/\gamma)^{1/2}$. Examples of calculated adhesion potentials are shown in Fig. 2.

The Hamiltonian of our model is the sum of an elastic energy for the membrane-membrane spacing and an osmotic repulsive energy

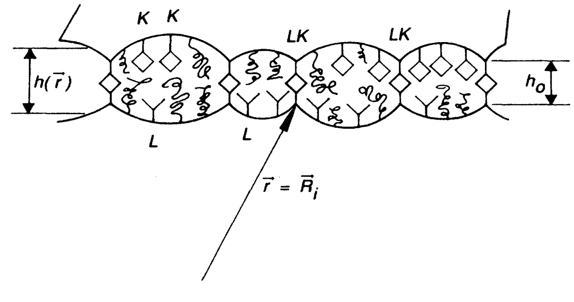

FIG. 1. Membrane adhesion by lock-and-key molecules. The intermembrane spacing is $h(\mathbf{r})$. Mobile lock (L) and key (K) molecules can form weak, specific chemical bonds (LK) at sites $\mathbf{R}_i$. They impose a local membrane spacing h_0. Nonadhesive glycoproteins (wiggly lines) produce a long-range repulsion.

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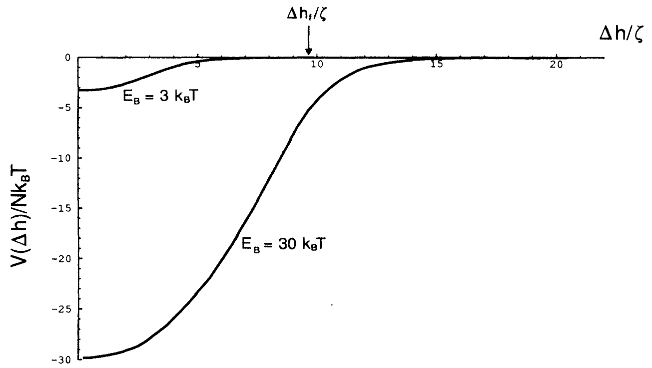

FIG. 2. Adhesion potential energy $V(\bar{h})/k_BTN$ for weak ($E_B = 3k_BT$) and strong ($E_B = 30k_BT$) binding. N is the number of lock (or key) molecules in a layer. The membrane spacing Δh is measured from the spacing h_0 imposed by a lock-and-key assembly and is in units of $\varsigma = (2k_BT/\pi\gamma)^{1/2}$. The fracture spacing Δh_f for $E_B = 30k_BT$ is indicated.

$$H = \frac{1}{2}\int_{\text{disk}} [\gamma(\nabla h)^2 + \kappa(\nabla^2 h)^2]d^2r - \Pi \int_{\text{disk}} h(\mathbf{r})d^2r\,, \tag{1}$$

with $h(\mathbf{r})$ the position-dependent intermembrane spacing and κ the Helfrich bending energy [4]. We will assume circular membranes of area A, flat at the edges. The bottom membrane contains N indistinguishable L molecules, and the top membrane contains N indistinguishable K molecules. A thermally fluctuating fraction $\phi = M/N$ is combined into LK molecules. At the sites $\mathbf{R}_i$ ($i = 1,2,\ldots,M$) of the LK molecules, $h(\mathbf{r})$ is assumed fixed: $h(\mathbf{r} = \mathbf{R}_i) = h_0$ with h_0 the size of an LK molecule.

The free energy of the model is computed in two steps. We first fix (i) the mean intermembrane spacing at $\langle h\rangle = \bar{h}$ and (ii) the number of LK molecules at M and compute the free energy per particle $f(\phi,\bar{h})$ at fixed ϕ and $\langle h\rangle = \bar{h}$. From Eq. (1) it follows that

$$f(\phi,\bar{h}) = -\frac{k_BT}{M}\ln\Bigg(\frac{1}{M!}\int\prod_{i=1}^{M} d^2\mathbf{R}_i \times \int_c D[h]e^{-\frac{1}{2}\beta\int_{\text{disk}}[\gamma(\nabla h)^2+\kappa(\nabla^2 h)^2]d^2r}\Bigg) + \text{const}. \tag{2}$$

The functional integral in Eq. (2) over the spacing profile $h(\mathbf{r})$ is constrained by the conditions $h(\mathbf{r} = \mathbf{R}_i) = h_0$ and by the imposition of a mean spacing $\langle h\rangle = \bar{h}$. We will not include any membrane-membrane collisions and later check when this assumption holds.

The second step is to calculate the total free energy allowing ϕ and $\bar{h}$ to vary. This step can be performed straightforwardly in the thermodynamic limit $N \to \infty$, $A \to \infty$ with $\rho = A/N$ fixed using the method of steepest descent. The result is that the full free energy must be the minimum of

$$\frac{F(\phi,\bar{h}) - F_0}{\rho A} = k_BT\{\phi\ln\phi + 2(1-\phi)\ln(1-\phi)\} + \phi f_{\text{ex}}(\phi,\bar{h}) - \rho^{-1}\Pi\bar{h} - E_B\phi\,, \tag{3}$$

with respect to ϕ and $\bar{h}$ (we absorbed a constant of order k_BT in a redefinition of E_B). Here, $f_{\text{ex}}(\phi,\bar{h})$ is the difference between $f(\phi,\bar{h})$ and the free energy per particle if there is no elastic coupling.

We can interpret the function $F(\phi,\bar{h})$ as the mean-field free energy of a reactive mixture of L's and K's in chemical equilibrium with their reaction product, the LK molecules, so $L + K \Leftrightarrow LK$. The equilibrium constant of the reaction is proportional to $e^{\beta E_B}$. The elastic free energy $f_{\text{ex}}(\phi,\bar{h})$ represents a "correlation energy" between the products causing deviations from simple chemical equilibrium theory. Our problem is reduced to computing $f_{\text{ex}}(\phi,\bar{h})$.

To gain qualitative insight, consider Eq. (3) in the regime of large $\bar{h}$ when the LK fraction ϕ must be small. To estimate $f_{\text{ex}}(\phi,\bar{h})$, note that the elastic deformation surrounding a point perturbation in an elastic sheet is proportional to $\ln(r)$. The elastic self-energy of the dimple of height $\Delta h = \bar{h} - h_0$ surrounding an LK molecule is then of order $\gamma\Delta h^2\ln(R/\xi)$ with R a large distance cutoff and ξ a small distance cutoff. The free energy reduces to

$$\frac{F(\phi,\bar{h}) - F_0}{\rho A} \propto k_BT\phi\Big\{-(\beta E_B - 2) + \ln\phi + \gamma\Delta h^2\ln(R/\xi)\Big\} - \rho^{-1}\Pi\bar{h}\,. \tag{4}$$

For R, we must take the nearest-neighbor distance $R_{\text{nn}} \propto \phi^{-1/2}$. The elastic term in Eq. (4) is then of the same mathematical form as the entropic term. It effectively lowers the thermal energy k_BT by an amount of order $\gamma\Delta h^2$. When $\gamma\Delta h^2$ exceeds a critical value of order k_BT, the effective temperature becomes negative so we must expect a freezing transition around spacings of order $\varsigma \propto \sqrt{k_BT/\gamma}$. Next, when $\gamma\Delta h^2$ exceeds a critical value of order E_B, the free energy is minimized by $\phi = 0$. Adhesion is lost since there are no assemblies left, so we should expect fracture around a spacing $\Delta h_f \propto \sqrt{E_B/\gamma}$.

To compute $f_{\text{ex}}(\phi,\bar{h})$, we minimize the elastic energy in Eq. (1) with respect to the profile $h(\mathbf{r})$, under the constraints $h(\mathbf{r} = \mathbf{R}_i) = h_0$ and $\langle h\rangle = \bar{h}$. Using the Lagrange multipliers μ_i to fix the membrane spacing at the sites of LK molecules and the Lagrange multiplier ν to fix the mean spacing, one finds that the profile $h_c(\mathbf{r})$ which minimizes $f_{\text{ex}}(\phi,\bar{h})$ obeys

$$(\gamma\nabla^2 - \kappa\nabla^4)h_c = -\nu + \sum_{i=1}^{N}\mu_i\delta(r - \mathbf{R}_i)\,. \tag{5}$$

Using Gauss' law and the boundary condition of flat edges, it follows from Eq. (5) that

$$\nu = \frac{\sum_{i=1}^{M}\mu_i}{A}\,. \tag{6}$$

The solution of Eq. (5) is

$$h_c(\mathbf{r}) \cong \langle h\rangle_{\{\mathbf{R}_i\}} + (1/2\pi\gamma)\sum_{i=1}^{M}\mu_i \ln\Big(|\mathbf{r} - \mathbf{R}_i|/\xi\Big) - A(\nu/4\pi\gamma)\Big[2\ln R/\xi) - 1 + (r/R)^2\Big], \quad (7)$$

with $\xi = \sqrt{\kappa/\gamma}$ and with $\langle h\rangle_{\{\mathbf{R}_i\}}$ an integration constant. Equation (7) is valid only outside a set of disks of radius ξ surrounding each *LK* molecule (of order 100 Å for WBC's). Formally, $h_c(\mathbf{r})$ is proportional to the 2D electrostatic potential of a set of M "charges" μ_i placed on a disk with a uniform neutralizing background charge density proportional to ν.

Writing $h(\mathbf{r}) = h_c(\mathbf{r}) + h'(\mathbf{r})$, performing the functional integral over $h'(\mathbf{r})$ in Eq. (2), and using Eq. (7) gives an effective potential energy $V(\{\mathbf{R}_{i=1,\dots,M}\}) = V_c(\{\mathbf{R}_{i=1,\dots,M}\}) + V_T(\{\mathbf{R}_{i=1,\dots,M}\})$ for the set of M charges which is the sum of an enthalpic term with long-range repulsive forces between the *LK* molecules [due to the elastic energy of the deformation profile Eq. (7)] and an entropic term with long-range attractive forces [due to thermal fluctuations of the membrane shape around Eq. (7)]:

$$V_c(\{\mathbf{R}_{i=1,\dots,M}\}) \approx -\frac{e^2}{2}\sum_{i>j}\ln\left(\frac{\mathbf{R}_{i,j}}{R}\right)^2 + \frac{Me^2}{2}\sum_{i=1}^{M}\left(\frac{\mathbf{R}_i}{R}\right)^2 + \frac{Me^2}{2}\ln(R/\xi) - \frac{3M^2e^2}{8}, \quad (8a)$$

$$V_T(\{\mathbf{R}_{i=1,\dots,M}\}) \approx k_BT\left(\frac{M}{2}\ln\left[\frac{\ln(R/\xi)}{\beta\gamma\xi^2}\right] + \sum_{i\neq j}\ln\left[\frac{\ln(|\mathbf{R}_{i,j}|/\xi)}{\beta\gamma\xi^2}\right] + \cdots\right). \quad (8b)$$

In Eq. (8a), $e = \nu/\sqrt{2\pi\gamma\rho\phi}$ is the effective charge, $\rho = N/A$ the concentration of adhesion molecules, and $\mathbf{R}_{i,j} = \mathbf{R}_i - \mathbf{R}_j$. We neglected in Eq. (8a) fluctuations of the μ_i parameters and set the μ_i equal to $\nu A/M$ [see Eq. (6)], with ν to be determined self-consistently later. Monte Carlo simulation of the general case [14] indicates that this "mean-field" assumption is valid provided the normalized density $\rho\xi^2$ is small. In Eq. (8b) we neglected three-body interaction terms and beyond.

The potential $V_c(\{\mathbf{R}_{i=1,\dots,M}\})$ is identical to the electrostatic energy of an OCP of M particles of charge e in a uniform neutralizing background [15]. If $\Gamma = e^2/k_BT$ is large compared to $\ln^{-1}(1/\xi^2\rho\phi)$ then the entropic force between the *LK* molecules is small compared to the enthalpic force. Up to an additive constant, $f_{\text{ex}}(\phi,\overline{h})$ is then well approximated by the excess canonical free energy per particle of the OCP. From the extensiveness of the OCP free energy [16] and from scaling arguments [17], it is known that

$$f_{\text{ex}}(\phi,\overline{h})/k_BT = -\frac{\Gamma}{4}\ln(\pi\rho\phi\xi^2) + \Gamma g(\Gamma). \quad (9)$$

The function $g(\Gamma)$ has the limiting behavior [18]

$$g(\Gamma) = \begin{cases} \frac{1}{4}\{\ln(2/\Gamma) + 1 - 2C\}, & \Gamma \to 0, \quad (10a) \\ -0.374 + (\ln\Gamma - 0.262)/\Gamma, & \Gamma \to \infty, \quad (10b) \end{cases}$$

With $C = 0.577$ Euler's constant. In the small Γ limit, the assemblies form a fluid "plasma" with $(\Gamma/\rho\phi)^{1/2}$ acting as a screening length. With increasing Γ, we reach a freezing point at $\Gamma = \Gamma_c$ (with [19] $16\sqrt{3\pi} > \Gamma_c > 2$). Beyond Γ_c, the *LK* molecules form a triangular Wigner crystal with, possibly, an intervening hexatic phase just below Γ_c. The equation of state is, for any Γ, that of an ideal gas with an effective temperature $T_{\text{eff}} = T - e^2/4k_B$ that is below the real temperature [19].

To establish the self-consistency condition on the magnitude of the charge e, we insert Eq. (5) into Eq. (1) and use the constraints and Gauss' law to show that the internal energy equals $\frac{1}{2}A\nu(\overline{h} - h_0)$. Using the relationship between free energy and internal energy and the definition of Γ, it follows that

$$\Delta h/\varsigma = \Gamma^{1/2}\left\{-\frac{\ln(\pi\rho\phi\xi^2)}{2} + 2g(\Gamma) + \Gamma g'(\Gamma)\right\}, \quad (11)$$

with $\varsigma = (2k_BT/\pi\gamma)^{1/2}$ the characteristic length scale encountered below Eq. (4), and with $\Delta h \equiv \overline{h} - h_0$ the mean membrane spacing measured from h_0.

We will express our results in terms of the adhesive potential $V(\overline{h}) = F(\overline{h}) + \Pi\overline{h} - F_0$, with $F(\overline{h})$ the minimum of $F(\phi,\overline{h})$ with respect to ϕ for fixed $\overline{h}$. This potential has the property that $dV(\overline{h})/d\overline{h} = \Pi$ for a free energy minimum, so the adhesive force per unit area derived from $V(\overline{h})$ equals the repulsive osmotic pressure. We will discuss the results separately for the strong-binding regime, with $E_B \gg k_BT$, and the weak-binding regime, with $E_B \ll k_BT$.

For strong binding the *LK* assembly fraction ϕ is close to 1 for $\Delta h < \Delta h_f$ and close to 0 for $\Delta h > \Delta h_f$ with

$$\Delta h_f \cong \sqrt{\frac{2E_B}{\pi\gamma}[-\ln(\pi\rho\xi^2) + 4g(\infty)]} \quad (12)$$

(assuming $\rho\xi^2 \ll 1$). For $\Delta h < \Delta h_f$, the adhesion potential is

$$\frac{V(\overline{h})}{\rho A} \cong -E_B + \frac{\pi}{2} \times \frac{-\frac{1}{4}\ln(\pi\rho\xi^2) + g(\Gamma_{\Delta h})}{[-\frac{1}{2}\ln(\pi\rho\xi^2) + 2g(\Gamma_{\Delta h}) + \Gamma_{\Delta h}g'(\Gamma_{\Delta h})]^2}\gamma\Delta h^2 \quad (\Delta h < \Delta h_f), \quad (13)$$

with $\Gamma_{\Delta h}$ the solution of Eq. (11) for $\phi = 1$. The potential Eq. (13) is nearly harmonic with a spring constant per molecule of order the membrane tension γ. For small Δh, the *LK*'s are in the liquid state [20]. The freezing transition occurs when $\Gamma_{\Delta h} = \Gamma_c$, which happens when Δh is of order ζ. Increasing Δh further, we reach Δh_f where ϕ

rapidly drops from to an exponentially small value. Adhesion is nearly lost but there remains a weak attractive potential,

$$\frac{V(\bar{h})}{\rho A} \approx -2k_B T e^{[\beta E_B + 4g(\infty) - \ln(\pi\rho\xi^2)]/2} e^{-\Delta h/\varsigma} \qquad (\Delta h > \Delta h_f). \qquad (14)$$

In the weak-binding regime, the potential energy $V(\bar{h})$ crosses over smoothly from approximately harmonic to exponentially decaying with no sudden loss of adhesion. For $\Delta h \gg \zeta$, Eq. (14) is recovered. The range of the potential is ζ. Interestingly, adhesion persists when we allow a weakly negative E_B. This means that the entropic part of the free energy actually favors adhesion (at least in the absence of collisions). To see why, consider again the L's and K's as a reactive mixture in chemical equilibrium with their reaction product, the LK molecules. Even if the LK binding energy is negative, there is still a modest concentration of LK molecules for entropic reasons. Preventing LK formation by increasing the membrane spacing decreases this entropic contribution to the free energy, leading to an adhesive force.

Collisions between the thermally fluctuating membranes were not included in our calculation. They increase the free energy per unit area by an amount of order k_BT/ξ_p^2, with ξ_p the typical distance between collisions ("patch size"). For tense membranes with a mean spacing of $\bar{h} + h_0$, the patch size is [21] $\xi_p \approx a\exp(\alpha[(\bar{h} + \Delta h)/\zeta]^2)$ with α a constant and a the microscopic cutoff. Collisions can be neglected if [22] k_BT/ξ_p^2 is small compared to $V(\bar{h})/A$. In the strong-binding regime, this condition is satisfied provided $E_B\rho a^2/k_BT \gg \exp[-2\alpha(h_0/\varsigma)^2]$, and in the weak-binding regime if $\rho a^2 \gg \exp[-2\alpha(h_0/\varsigma)^2]$. If these conditions are not satisfied, then $V(\bar{h})/A$ exceeds k_BT/ξ_p^2 only for Δh large compared to ζ.

Application of our results to bioadhesion requires knowledge of the binding energies of adhesive proteins such as the selectin [8] and integrin [23] molecules. Although these have not yet been measured directly, on the basis of macroscopic studies it is believed that selectin is weak binding and integrin strong binding [24]. Selectin-and integrin-based adhesion thus may exhibit the two types of potentials shown in Fig. 2. If we use the measured membrane tension of WBC's [12] in the weak-binding range $\varsigma = (2k_BT/\pi\gamma)^{1/2}$, we find a range of order 100 Å. The strong-binding range Δh_f should be even longer. Experimental studies [9] of the interaction range of strong-binding LK molecules report values only of the order of 10 Å. A possible origin for the discrepancy lies in the fact that current experiments so far are performed on lipid layers attached to solid substrates, which surpresses membrane deformability—a key ingredient of our model.

We would like to thank P. Olague and C. Safinya for helpful discussions. We also would like to thank the Institute of Theoretical Physics for support and hospitality.

[1] S. Leibler, in *Statistical Mechanics of Membranes and Surfaces,* Jerusalem Winter School, edited by D.R. Nelson, T. Piran, and S. Weinberg (World Scientific, Signapore, 1989).

[2] J.N. Israelachvili, *Intermolecular and Surface Forces* (Academic, Orlando, FL, 1985).

[3] C.R. Safinya, D. Roux, G.S. Smith, S.K. Sinha, P. Dimon, N. Clark, and A-M. Bellocq, Phys. Rev. Lett. **57**, 2718 (1986).

[4] W. Helfrich, Z. Naturforsch. **33a**, 305 (1978).

[5] E. Evans, Biophys. J. **48**, 175 (1985); **48**, 185 (1985).

[6] N. Sharon and H. Lis, Sci. Am. **268**, 74 (1993); L.A. Lasky, Science **258**, 964 (1992).

[7] E-L Florin, V. Moy, and H. Gaub, Science **264**, 415 (1994), and references therein. The commonly used biotin-streptavidin LK pair has a considerably larger E_B than the selectin-glycoprotein bonds considered here.

[8] R. Abney, J. Braun, and J. Owicki, Biophys. J. **52**, 441 (1987).

[9] R. Bruinsma, M. Goulian, and P. Pincus, Biophys. J. **67**, 746 (1994).

[10] E. Evans and A. Yeung, Biophys. J. **52**, 551 (1987).

[11] U. Seifert and R. Lipowsky, Phys. Rev. A **42**, 4768 (1990).

[12] G.I. Bell, Science **200**, 618 (1978); G.I. Bell, M. Dembo, and P. Bongrand, Biophys. J. **45**, 1051 (1984).

[13] The glycoprotein ligands of selectin adhesion molecules are mobile; see L.A. Lasky, M.S. Singer, D. Dowbenko, Y. Imai, W. Henzel, C. Fennie, S. Watson, and S. Rosen, in *Cold Spring Harbor Symposia on Quantitative Biology* (Cold Spring Harbor Lab. Press, Plainview, NY, 1992), Vol. LVII.

[14] D. Zuckerman and R. Bruinsma (to be published).

[15] R. Sari, D. Merlini, and R. Calinon, J. Phys. A **9**, 1539 (1976).

[16] R. Sari and D. Merlini, J. Stat. Phys. **14**, 91 (1976).

[17] E. Hauge and P. Hemmer, Phys. Norv. **5**, 209 (1971).

[18] A. Alastuey and B. Jancovici, J. Phys. **42**, 1 (1981).

[19] The lower bound on Γ_c follows from the exact solution at $\Gamma = 2$, while the upper bound is the Kosterlitz-Thouless limit using the bare shear modulus of Ref. [20].

[20] For $\Delta h/\zeta \ll \ln^{-1/2}(1/\xi^2\rho\phi_0)$ the entropic force [Eq. (8b)] between the assemblies becomes the dominant long-range interaction. We have not explored this regime.

[21] M.E. Fisher and D.S. Fisher, Phys. Rev. B **25**, 3192 (1982).

[22] This argument does not include excess collisions near the adhesion sites (see Ref. [9]) which reduce the effective LK binding energy.

[23] Integrin molecules are believed to be attached to the cytoskeleton which restricts their mobility. See E. Luna, Science **258**, 955 (1992).

[24] M. Lawrence and T. Springer, Cell **65**, 59 (1991).