J Mater Sci (2007) 42:8904–8910
DOI 10.1007/s10853-007-1720-9

An lower bound on receptor density for stable cell adhesion due to thermal undulations

Yuan Lin · L. B. Freund

Received: 16 December 2006 / Accepted: 7 March 2007 / Published online: 17 July 2007

Abstract The adhesion of a living cell to an extracellular matrix surface is effected through the bonding of receptor molecules in the cell membrane to compatible ligand molecules on the surface. In a series of experiments on adhesion of cells to a substrate surface with a controlled density of ligand binding sites, Arnold et al. (ChemPhysChem 5:383, 2004) showed that tight cell adhesions could form only if the areal density of binding sites on the substrate was higher than some critical value. Furthermore, this critical value was consistent across the four cell types examined in the experiments. For ligand density below the critical level, on the other hand, virtually no adhesions formed. In this article, we examine the competition between thermal undulations of the cell membrane and its adhesion to the substrate. In particular, we show that thermal undulations destabilize membrane bonding to the substrate unless the bond spacing is below a certain level. By following this line of reasoning in the context of classical statistical mechanics, we obtain an estimate of the critical value of spacing which is in reasonable agreement with the observations.

Introduction

One of the most extensively studied topics in modern biophysics is cell adhesion, a phenomenon central to cell motility [1], endocytosis [2], and other biological processes. For cell-to-cell or cell-to-extracellular matrix adhesion to occur, binding proteins (integrins or ligands) within the cell membrane must aggregate into compact clusters in order to bond specifically with molecules outside the cell. These adhesion patches can continue to grow by recruiting more membrane bound adhesion molecules from the vicinity [3–5]. This occurs naturally because the bonding of a molecule in the membrane lowers the entropy of the binder distribution locally, giving rise to an osmotic pressure gradient which, in turn, induces binder transport into the region. As is shown in [6], cell adhesion is influenced by numerous factors including binding affinity between integrins and ligands, the concentration of binding molecules, the properties of the bonding surfaces, and so on.

Arnold and coworkers [7] have reported results of experiments which revealed remarkable aspects of adhesion at a size scale between the dimensions of the individual bonds (a few nanometers) and the dimensions of a tight adhesion patch (a few microns). They deposited gold nanodots periodically on a polyethylene glycol based substrate, chosen because it had no bonding affinity for cell membranes. One binding molecule, an RGD ligand, was then attached to each dot; a dot size of 8 nm or less—a dimension below the diameter of a ligand—was chosen in order to limit the number of molecules per dot to just one. By controlling the spacing of the ligands in this way, it was possible to observe the influence of density of ligands on cell adhesion. No feature of the system other than the spacing was modified from experiment to experiment. It was reported in [7] that a critical spacing between dots exists for a class of cell types. For dots spaced more closely together than this critical spacing, tight cell adhesion patches formed. On the other hand, for spacings larger than this critical value, almost no bonds between the cell and the substrate were formed.

Y. Lin · L. B. Freund (✉)
Division of Engineering, Brown University, 184 Hope Street, Providence, RI 02912, USA
e-mail: freund@brown.edu

Motivated by this striking evidence, we examine the relationship between the natural thermal undulations of a cell wall immersed in a heat bath and the spacing of binding sites necessary to effect adhesion to the substrate surface. In particular, we show that the magnitude of membrane fluctuations strongly depends on the average binding energy density. Comparison of this fluctuation magnitude at a reference point to the width of the energy well, which is introduced to describe the binding interaction, defines a natural value of spacing between binding sites. For binding site spacing smaller than this critical value, stable adhesion in the statistical sense can occur. For binding site spacing larger than this critical value, however, any bonds formed are unstable because thermal undulation can ultimately overcome the bond resistance so the surfaces will not adhere.

The plan of this paper is as follows. In the following section, a one-dimensional configuration is considered; this relatively simple situation provides a convenient vehicle for introducing the concepts and mathematical strategy involved in the approach. Two models where adhesion is treated either as being localized at discrete binding sites or continuous across the interface are presented, and their asymptotic behaviors as the membrane size becomes much larger than the spacing between binding sites are examined. The analysis is generalized to the more realistic two-dimensional configuration in this section after that, and comparisons between the theoretical prediction and experimental observations are presented. Concluding remarks are summarized in the final section.

A one-dimensional model

The phenomenon of thermal undulations of a membrane has been studied by many authors, for example [8] and [9], in the context of identifying the persistence length of a membrane. Other situations in which the membrane and its substrate interact have also been considered; see for example [10] and [11]. The thermal fluctuations of fluid membranes in the presence of periodic confining potentials were considered by Gov and Safran [12] and the undulation spectra membrane were examined by Merath and Seifert [13].

As a basis for arriving at the essential result in the simplest way, consider a nominally flat membrane as shown in Fig. 1. The membrane surface is immersed in a thermal bath and is positioned near a substrate with potential binding sites. For the one-dimensional model, deflection is assumed to vary in only one direction, along which spatial position is identified by the x coordinate. The potential binding sites are identified by the circular symbols on the membrane in the figure. Denote the total number of binding sites by N_b. For simplicity, assume that

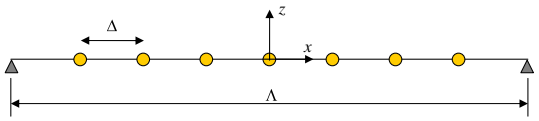

Fig. 1 Schematic diagram of an elastic membrane with length Λ. Potential binding sites are uniformly distributed along the membrane with regular spacing Δ. The membrane is constrained against transverse deflection at both ends

N_b is an odd number and that the binding sites are distributed along the membrane at equal intervals of Δ; see Fig. 1. The extent of the membrane in the x direction is Λ, so $\Lambda = (N_b + 1)\Delta$. The reference plane of the membrane coincides with $-\Lambda/2 \le x \le \Lambda/2$, $z = 0$ as shown in Fig. 1.

In order to describe adhesion, the interaction between the membrane and the substrate at potential binding sites must be prescribed. Here, adhesion is represented by means of a potential energy well, with the depth of the well corresponding to the energy reduction achieved when bonding occurs in the absence of other physical effects. In other words, it is the chemical potential of bonding. The width of the potential well represents the compliance of the bond. We believe this is a realistic description of bonding when the molecules involved in adhesion can be relatively large and compliant. As suggested in Lin et al. [14], a convenient choice for the shape of such a well is

$$u_b(q) = -kT\ln\left[\left(e^{\frac{C_b}{kT}} - 1\right)e^{-\frac{\Omega}{2kT}q^2} + 1\right] \tag{1}$$

where u_b is the bonding interaction energy, q is the deflection of membrane in the z direction at this binding site and kT is the thermal energy unit. C_b is the depth of the energy well at deflection $q = 0$ and Ω is the curvature of well at the same point. Notice that, in the one-dimensional model, C_b represents the total energy reduction achieved in bonding at the site across a unit thickness in the y (out-of-plane) direction in Fig. 1, and has the physical dimensions force × length. The physical units of Ω are force/length and, as has been pointed out in Lin et al. [14], the interaction potential shown in (1) can be approximated by a harmonic potential

$$u_b(q) \approx \frac{1}{2}\Omega q^2 - C_b \tag{2}$$

within the well itself. Figure 2 shows the comparison between the full potential and the harmonic approximation, where the solid line corresponds to (1) for $C_b = 10$ kT and $\Omega = 20$ kT/nm^2; the dashed line represents the harmonic potential (2) for the same parameters. As is evident form the figure, these two potentials are nearly identical when the deflection q is deep in the well, and only when q

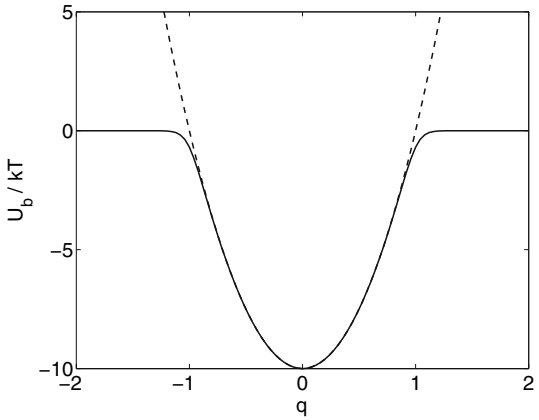

Fig. 2 Shape of the energy well as described in (1) (*solid line*), and its comparison with the harmonic approximation (*dashed line*)

approaches the rim of the well does the quadratic potential begin to deviate significantly from the full potential. The width of the well δ can be estimated from (2) as

$$\delta = \sqrt{\frac{2C_b}{\Omega}}. \tag{3}$$

Of course, one can proceed by using (1) to describe the effect of adhesion, as was done in Lin et al. [14]. However, in the current study we mainly focus on the conditions where stable adhesions are formed. In this case, the deflection magnitude of the membrane at each binding point is expected to be less than the well width δ come on and substantially less for a high probability of bonding. Consequently, use of the harmonic approximation of interaction energy should yield reasonably accurate results. Furthermore, using (2) instead of (1) can greatly simplify the analysis, the benefits of which will become evident in the analysis described next. Based on these considerations, (2) is chosen here to describe the interaction energy at each binding point.

Assume that the membrane is constrained against transverse deflection at $x = \pm\Lambda/2$ (see Fig. 1) but is otherwise unconstrained except at the binding sites. A truncated Fourier series approximation of the transverse deflection $h(x)$ can be written as

$$h(x) = \delta \sum_{n=1,3,5\ldots}^{N_{odd}} a_n \cos\left(\frac{n\pi x}{\Lambda}\right) \tag{4}$$

in which N_{odd} is an odd number; the number of modes being considered is then $(N_{odd} + 1)/2$. The coefficients a_n represent a set of non-dimensional random variables describing the response of the membrane to thermal excitation. Suppose the transverse deflection amplitude is small enough so that the energy of deformation arises only from bending. If the elastic bending stiffness of the membrane is denoted by C_e then the elastic bending energy U_e of the membrane is

$$U_e = \frac{1}{2} C_e \int_{-\Lambda/2}^{\Lambda/2} h''(x)^2 dx. \tag{5}$$

Notice that the physical dimensions of C_e are force × length^2. Assuming that the binding potential is the same for all potential binding sites, the total binding energy is

$$U_b = \sum_{i=1}^{N_b} u_b(h_i). \tag{6}$$

Here $h_i = \delta \sum_{n=1,3,5\ldots}^{N_{odd}} a_n \cos(n\pi x_i/\Lambda)$ is the deflection of the membrane at binding point $x_i = i \times \Delta - \Lambda/2$. In the framework of statistical mechanics, the partition function Z of the system is

$$Z = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\cdots\int_{-\infty}^{\infty} e^{-(U_e+U_b)/kT} da_1 da_3 \ldots da_{N_{odd}}. \tag{7}$$

Following substitution of the expressions for U_e and U_b into (7), the partition function becomes

$$Z = e^{N_b C_b/kT} \int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\cdots\int_{-\infty}^{\infty} e^{-\mathbf{a}^T\cdot\mathbf{Q}\cdot\mathbf{a}} da_1 da_3 \ldots da_{N_{odd}} \tag{8}$$

in which the vector $\mathbf{a}$ is defined as $\mathbf{a} = [a_1, a_3, \ldots, a_{N_{odd}}]^T$. The components of the matrix $\mathbf{Q}$ are

$$Q_{mn} = c_b \sum_{i=1}^{N_b} \cos\left(\frac{n\pi x_i}{\Lambda}\right)\cos\left(\frac{m\pi x_i}{\Lambda}\right) + c_e \frac{n^4}{\lambda^3}\delta_{mn}, \tag{9}$$

where $c_e = (\pi^4/4)(C_e/\delta kT)$, $\lambda = \Lambda/\delta$ and $c_b = (C_b/kT) = (\Omega\delta^2/2kT)$. Obviously $\mathbf{Q}$ is a real, symmetric matrix. Furthermore, because $U_e + U_b$ is a positive definite function of the random variables, the matrix $\mathbf{Q}$ can be diagonalized by a proper basis transformation, that is,

$$\overline{\mathbf{Q}} = \mathbf{A}^T \cdot \mathbf{Q} \cdot \mathbf{A}. \tag{10}$$

Here $\mathbf{A}$ is a normalized basis transformation matrix and $\overline{\mathbf{Q}}$ is a positive definite diagonal matrix. The transformed random variables spanning configuration space are given in terms of the transformation matrix by $\overline{\mathbf{a}} = \mathbf{A}^T\mathbf{a}$. Then the integral in (8) reduces to a product of one dimensional Gaussian integrals. Hence, the partition function can be evaluated exactly as

$$Z = e^{N_b c_b} \prod_{n=1,3,5\ldots}^{N_{odd}} \left[\frac{\pi}{\overline{Q}_{nn}}\right]^{1/2}. \tag{11}$$

The thermal average or expectation value of any physical quantity, say g, then can be calculated by

$$\langle g \rangle = \frac{1}{Z}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\cdots\int_{-\infty}^{\infty} g(a_1, a_3, \ldots, a_{N_{odd}}) \times e^{-(U_e+U_b)/kT} da_1 da_3 \ldots da_{N_{odd}}. \tag{12}$$

In the context of stability of adhesion, the expectation values of the magnitude of membrane deflection at each binding points are of particular interest. Here, we focus on circumstances only at the binding point at $x = 0$, although any other particular site may be examined in the same way. The standard deviation σ in the deflection at that point is readily calculated as

$$\sigma = \delta\sqrt{\left\langle \left(\sum_{n=1,3,5\ldots}^{N_{odd}} a_n\right)^2 \right\rangle}$$

by means of (12), and the normalized result is

$$\frac{\sigma^2}{\delta^2} = \sum_{n=1,3,5\ldots}^{N_{odd}} \frac{\overline{P}_{nn}}{2\overline{Q}_{nn}} \tag{13}$$

where the matrix $\overline{\mathbf{P}}$ is defined as $\overline{P} = \mathbf{A}^T\mathbf{P}\mathbf{A}$, and $\mathbf{P}$ is a matrix with each component equal to 1. The ratio σ/δ compares the standard deviation of fluctuations in the membrane deflection at the binding point to the width of the energy well. For adhesion to be stable, this quantity should be less than 1, and perhaps substantially less. If the criterion for stable adhesion is given in the form

$$\sigma \le \sigma_{cr} \tag{14}$$

then the smaller the value of σ_{cr}, the higher the confidence one has that the adhesion will remain stable. For example, if the distribution of membrane deflection at the binding point is assumed to be Gaussian, then choosing $\sigma_{cr}/\delta = 1$ gives us about 68% confidence level that the adhesion will remain stable, whereas the confidence level increases to about 95% if one chooses $\sigma_{cr}/\delta = 1/2$. The dependence of the ratio σ/δ on other parameters, such as binding energy C_b or spacing between two sites Δ, for example, can be studied numerically on the basis of (13), as described by Lin et al. [14]. With the parameters chosen as $\lambda = 100$, $N_b = 41$, $c_e = 10^4$ and $c_b = 6$, Fig. 3 shows the sensitivity of σ/δ to the number of modes included in the calculation. In the figure, the solid line represents the result of using the harmonic potential (2) and the diamond symbols indicate results obtained by using the interaction potential defined in (1). It is clear that these two potentials yield nearly identical results, as expected. From simple analysis, it can be shown that the difference between the predictions obtained by using these two potentials is a power series in the dimensionless factor e^{-c_b}. If c_b is relatively large, which is the case for real cell adhesions where the binding energy per bond is about $C_b = 10$ kT, then this difference becomes very small.

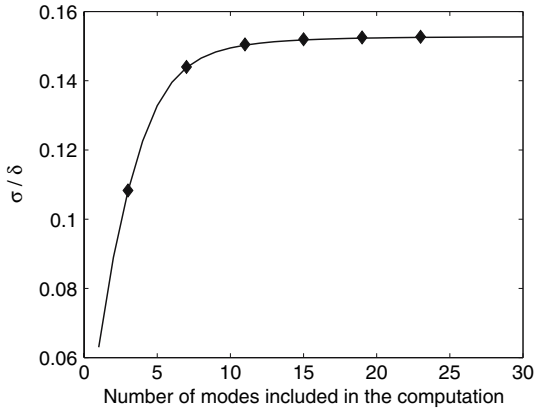

Fig. 3 Dependence of the value σ/δ on the number of modes included in the computation. The *solid line* represents the computational results obtained by using the harmonic interaction potential (2) and the *diamond symbols* correspond to results obtained by using the potential given in (1)

The numerical results also show that only the first few deformation modes, corresponding to relatively large wavelengths, contribute significantly to the value of σ/δ. This outcome can be understood by realizing that the bending energy associated with any mode, identified by wavenumber n, is proportional to n^4, as indicated by (5), so the magnitude of this mode a_n necessarily decreases rapidly with increasing n because the energy cost becomes increasingly high. This suggests that the discrete bonding assumption can be relaxed and that the behavior can be captured by means of a model based on continuous bonding. In other words, at the scale of long wavelengths the spacing Δ between binding points becomes relatively small so that, effectively, adhesion is possible "continuously" along the membrane.

Therefore, we re-examine the question on the basis of an assumption that the adhesion is continuous along the membrane and that the density of interaction potential for adhesion is still given by (2). Now, u_b represents the adhesion energy per unit length. In this case C_b has the physical units of force and Ω has the dimensions force/length^2. The total elastic energy is still given by (5) and the total binding energy now takes the form

$$U_b = \int_{-\Lambda/2}^{\Lambda/2} u_b dx. \tag{15}$$

The partition function Z, defined in (7), becomes

$$Z = \sqrt{\pi} e^{c_b \lambda} \prod_{n=1,3,5\ldots}^{N_{odd}} \left[\frac{c_b \lambda}{2} + \frac{c_e n^4}{\lambda^3} \right]^{-1/2}. \quad (16)$$

where $c_b = C_b \delta / kT$. The non-dimensional parameters λ and c_e have the same forms as previously. Similarly, the standard deviation σ of membrane fluctuations at point $x = 0$ is evaluated from (12) to become

$$\frac{\sigma^2}{\delta^2} = \sum_{n=1,3,5\ldots}^{N_{odd}} \left[c_b \lambda + \frac{2 c_e n^4}{\lambda^3} \right]^{-1}. \quad (17)$$

Typically, the size of a cluster of molecular bonds that makes up a focal adhesion is in the micrometer range whereas the width of the energy well is in the nanometer range, at most. Consequently, the value of λ is at least on the order of 100 and usually larger. Taking the fact that λ is large and letting $N_{odd} \to \infty$, the summation of the infinite series appearing in (17) is convergent and the result is

$$\frac{\sigma^2}{\delta^2} = \frac{\pi}{8} \left[\frac{2}{c_e c_b^3} \right]^{1/4}. \quad (18)$$

This is an extremely simple result because the length of the membrane λ drops out, and σ/δ is determined solely by the two dimensionless parameters c_e and c_b, the bending modulus of the membrane and the adhesion energy density. To ascertain the validity of (18), a comparison with results of the discrete model was considered. On the basis of $\lambda = 100$, $c_e = 10^4$ as the values of key system parameters and $\sigma_{cr}/\delta = 1$ as the criterion for stable adhesion, the critical adhesion energy density, computed from the discrete model (13) and normalized by the prediction from continuous model (18), is plotted in Fig. 4 against the number of binding points N_b within the membrane. Clearly, the discrete model result approaches that of the continuous model asymptotically as N_b increases. Therefore, the continuous adhesion model appears to be applicable to real biological systems where hundreds of binding sites can be distributed in close proximity to each other along the membrane.

Generalization to a two-dimensional system

In this section, the foregoing analysis is extended to the more realistic two dimensional configurations. In this case, consider a nominally flat, square membrane with size $\Lambda \times \Lambda$ which is immersed in a thermal bath and which is positioned near a substrate with potential binding sites. The transverse deflection of the membrane h (x,y) in this instance depends on two independent variables x and y; for this reason, the model is identified as being two-dimensional. The potential binding sites are indicated by the small circular symbols on the membrane shown in Fig. 5. As is evident from the diagram, these sites are arranged in a square pattern with spacing $\Delta << \Lambda$ in both the x and y directions of a rectangular coordinate frame in the nominal plane of the membrane.

Because $\Lambda >> \Delta$, we expect the continuum bonding model to provide an accurate prediction on the basis of the analysis presented in the preceding section. As above, assume the binding energy density u_b to be given by (2).

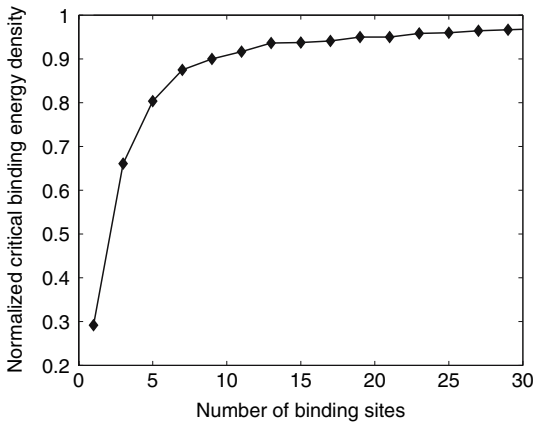

Fig. 4 Dependence of the critical adhesion energy density from the discrete bonding model, normalized by the corresponding value implied by the continuous bonding model, on the number of binding points N_b along the membrane

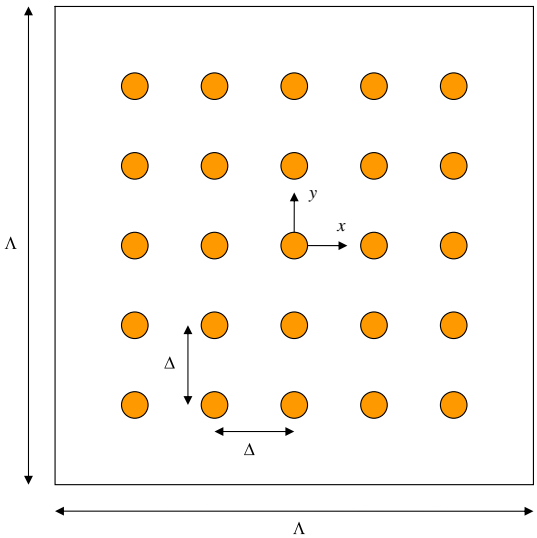

Fig. 5 Sketch of a portion of a large membrane. The *circle symbols* represent the binding sites on the substrate, and these sites are arranged in a regular square array with spacing Δ. The extent of the membrane in the plane is $\Lambda \times \Lambda$, where $\Lambda >> \Delta$

Notice that, in this case, C_b and Ω have the dimensions force/length and force/length^3, respectively. If the depth of the potential well representing bond interaction energy at any discrete site is Γ_b, then

$$C_b = \frac{\Gamma_b}{\Delta^2} \tag{19}$$

in the present instance.

We proceed in a manner similar to that followed for the one-dimensional case. The membrane is constrained against transverse deflection at its edges and the deflection $h(x,y)$ is represented by the truncated Fourier series

$$h(x,y) = \delta \sum_{m=1,3,5\ldots}^{N_{odd}} \sum_{n=1,3,5\ldots}^{N_{odd}} a_{mn} \cos\left(\frac{m\pi x}{\Lambda}\right) \cos\left(\frac{n\pi y}{\Lambda}\right). \tag{20}$$

As before, the parameters a_{mn} represent a set of random variables spanning the available configuration space of the system. The total bending energy is

$$U_e = \frac{1}{2} C_e \int_{-\Lambda/2}^{\Lambda/2} \int_{-\Lambda/2}^{\Lambda/2} \left(\frac{\partial^2 h}{\partial x^2} + \frac{\partial^2 h}{\partial y^2}\right)^2 dx\, dy \tag{21}$$

where the bending modulus C_e has the physical dimensions force × length. The total adhesion energy U_b is

$$U_b = \int_{-\Lambda/2}^{\Lambda/2} \int_{-\Lambda/2}^{\Lambda/2} u_b dx\, dy. \tag{22}$$

The standard deviation σ of membrane fluctuations at point $x = 0$, $y = 0$ can then be evaluated by means of (12) as

$$\frac{\sigma^2}{\delta^2} = \sum_{m=1,3,5\ldots}^{N_{odd}} \sum_{n=1,3,5\ldots}^{N_{odd}} \left[\frac{c_b \lambda^2}{2} + \frac{c_e (m^2+n^2)^2}{\lambda^2}\right]^{-1} \tag{23}$$

where

$$c_e = \frac{\pi^4}{4}\frac{C_e}{kT}, \quad \lambda = \frac{\Lambda}{\delta}, \quad c_b = \frac{C_b \delta^2}{kT} = \frac{\Omega \delta^4}{2kT} \tag{24}$$

are dimensionless variables similar to those defined for the one-dimensional case. For sufficiently large values of N_{odd}, the value of the double sum in (23) can be approximated accurately by a double integral. The double series is convergent so there is no disadvantage in allowing the total number of modes N_{odd} to become large. Accounting for the fact that the increments in both m and n in the series is 2, the integral approximation to the double series is

$$\frac{\sigma^2}{\delta^2} \approx \frac{1}{4}\frac{\lambda^2}{c_e} \int_0^{N_{odd}+1} \int_0^{N_{odd}+1} \left[\frac{c_b}{2c_e}\lambda^4 + (x^2+y^2)^2\right]^{-1} dx\, dy. \tag{25}$$

From the form of the integrand, it is clear that N_{odd} must be substantially larger than $\lambda\,(c_b/2c_e)^{1/4}$ for the approximation $N_{odd} \to \infty$ to be valid. In terms of the model, the values of c_e and c_b are of the same order so the restriction implies that the number of modes taken into account must be large enough so that the shortest wavelength included will be less than Δ, a reasonable expectation. For $N_{odd} \to \infty$, the double integral is readily evaluated by elementary methods, with the result that

$$\frac{\sigma^2}{\delta^2} = \frac{\pi^2}{32}\sqrt{\frac{2}{c_e c_b}}. \tag{26}$$

In order to draw quantitative inferences from (26) concerning the critical value of bond site spacing below which adhesion is stable, assume that the bending modulus of the membrane is C_e = 20 kT, a typical value for bilayer lipid membranes bilayer lipid membranes [8]. Also, the chemical potential of a single bond is assumed to have the value Γ_b = 10 kT, a typical chemical bonding energy for an integrin-RGD ligand pair [15]. For these parameter values, the dependence of the critical spacing Δ_{cr}/δ on σ/δ is illustrated in Fig. 6. It is evident from the figure that the value of a Δ_{cr} implied by the model depends quite strongly on the value of σ/δ that is taken to represent adhesion. As noted above, for a Gaussian probability distribution, a variance of δ^2 implies a 68% probability that the membrane is within the adhesion well, while a variance of $1/4\delta^2$ implies a 95% likelihood of the bond having been completed.

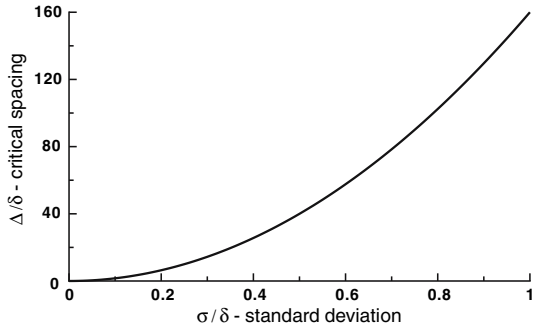

Fig. 6 Dependence of the normalized spacing between membrane binding sites on the normalized standard deviation in membrane fluctuation at a particular binding site within the adhesion energy well, illustrated for membrane bending stiffness C_e = 20 kT and chemical potential Γ_b = 10 kT

To compare the model results to experimental results, it is necessary to choose a value for δ, the half width of the energy well representing interaction between bonding elements. Suppose that we adopt the value of $\delta \approx 1\,\text{nm}$, a large value relative to atomic dimensions, in order to reflect the compliance of the adhesion molecules. From a statistical point of view, the value of σ/δ should be less than 1, and perhaps substantially less, in order for the behavior to correspond to a stable adhesion. If we assume that the critical values of spacing Δ_{cr} lies within the range for which the value of σ/δ varies from 1/2 to 1 then, on the basis of (26), the implied range of critical spacing is from 40 nm to 160 nm. This range includes spacings comparable to the critical spacing range 58–73 nm observed by Arnold et al. [7]. It is noted that the spacing in the experiments referred to a regular hexagonal array of binding sites. From simple geometrical considerations and the assumption that bond energy per unit area is the same for either square or hexagonal arrays of binding sites, the critical spacing for a hexagonal array falls within the range 43–172 nm.

Finally, we note that the assumption that the outer boundaries of the square membrane are constrained against transverse deflection is somewhat arbitrary. Instead, if it is assumed that the edges are subjected to periodic boundary conditions, rather than conditions of vanishing deflection, then the final result corresponding to (26) is modified slightly, taking the form

$$\frac{\sigma^2}{\delta^2} = \frac{\pi^2}{8}\sqrt{\frac{2}{c_e c_b}}. \tag{27}$$

In this case, the critical spacing is implied to be in the range 20–80 nm for a square array of binding sites, and in the range 21–86 nm for a hexagonal array. Again, these estimates correspond to the full range $1/2 < \sigma/\delta < 1$ for the standard deviation in fluctuation of in transverse deflection for the representative bond site of the membrane.

Conclusions

In this article the stability of adhesive contact between a compliant cell membrane and a substrate has been considered. The binding sites have been assumed to be periodically distributed over the substrate surface. The membrane undergoes continual thermal fluctuations due to its immersion in a large heat bath, and these fluctuations are necessarily suppressed to some degree in the process of adhesion. The competition between thermal fluctuations and formation of adhesive bonds has been examined within the framework of classical statistical mechanics. Mathematical models for adhesion have been examined for two cases, one in which adhesion sites are widely spaced and therefore discrete and a second in which adhesion site spacing is small enough so that adhesion can be viewed as continuous over the membrane surface. The equivalence between these two cases has been demonstrated under circumstances for which the size of the membrane being considered is much larger than the spacing between binding sites.

It has been found that background thermal undulations set an upper limit on bond site spacing for stable adhesions to form, in a statistical sense. A contact region with adhesions sites space more widely than this upper bound will likely not adhere; if bonds are formed, their state is unstable and they will be overcome by thermal fluctuations. An estimate of the critical spacing based on reasonable values of system parameters is in good agreement with experimental observations that have been reported on the actual spacing for a number of cell types.

Acknowledgments The research support of the National Science Foundation through the Brown University MRSEC Program (DMR-0079964) is gratefully acknowledged.

References

1. Harris AK, Wild P, Stopak D (1980) Science 208:177
2. Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD (1989) Molecular biology of the cell, 2nd edn. Garland Publishing, New York and London
3. Boulbitch A, Guttenberg Z, Sackmann E (2001) Biophys J 81:2743
4. Freund LB, Lin Y (2004) J Mech Phys Solids 52:2455
5. Shenoy VB, Freund LB (2005) Proc Natl Acad Sci USA 102:3213
6. Hersel U, Dahmen C, Kessler H (2003) Biomaterials 24:4385
7. Arnold M, Cavalcanti-Adam EA, Glass R, Blümmel J, Eck W, Kantlehner M, Kessler H, Spatz JP (2004) ChemPhysChem 5:383
8. Boal D (2002) Mechanics of the cell. Cambridge University Press, Cambridge
9. de Gennes PG, Taupin C (1982) J Phys Chem 86:2294
10. Gov N, Zilman AG, Safran S (2003) Phys Rev Lett 90:228101
11. Lin LC-L, Brown FL (2004) Biophys J 86:764
12. Gov N, Safran SA (2004) Phys Rev E 69:011101
13. Merath R-J, Seifert U (2006) Phys Rev E 73:010401
14. Lin Y, Inamdar M, Freund LB (2007) J Mech Phys Solids 55: in press
15. Goennenwein S, Tanaka M, Hu B, Moroder L, Sackmann E (2003) Biophys J 85:646