Entropy-driven aggregation of adhesion sites of supported membranes

N. Weil and O. Farago

Regular Article

Entropy-driven aggregation of adhesion sites of supported membranes

N. Weil and O. Farago[a]

Department of Biomedical Engineering, Ben Gurion University, Be'er Sheva 84105, Israel

Abstract. We study, by means of mean-field calculations and Monte Carlo simulations of a lattice gas model, the distribution of adhesion sites of a bilayer membrane and a supporting flat surface. Our model accounts for the many-body character of the attractive interactions between adhesion points induced by the membrane thermal fluctuations. We show that while the fluctuation-mediated interactions alone are not sufficient to allow the formation of aggregation domains, they greatly reduce the strength of the direct interactions required to facilitate cluster formation. Specifically, for adhesion molecules interacting via a short-range attractive potential, the strength of the direct interactions required for aggregation is reduced by about a factor of two to below the thermal energy $k_B T$.

1 Introduction

Adhesion between two membranes or between a membrane and another surface is an important topic for its ubiquitous occurrence in biological and biophysical processes. This process, during which two interfaces attract each other, can in principle be facilitated by non-specific attractive interactions (*e.g.*, Coulomb and van der Waals interactions). Cell adhesion, however, is usually caused by highly specific receptor molecules located at the outside of the plasma membrane of the cell, that can bind to specific ligands on the opposite surface [1,2]. Typically, the area density of receptors is rather low which does not lead to efficient bio-adhesion. However, when facing a surface with enough ligands, the receptors may cluster into highly concentrated adhesion domains to establish much stronger binding [3,4]. Formation of adhesion clusters occurs in many biological processes [5], including the binding of white blood cells to pathogens [6], cadherin-mediated adhesion of neighboring cells [7], and focal adhesion of cells to the extracellular matrix [8]. Much insight into these bio-adhesion processes has been gained from experimental studies of biomimetic membranes with receptors molecules that interact with surfaces covered by ligands [9–14].

Generally speaking, adhesion-induced domain formation requires some attractive intermolecular interactions between the receptor-ligand pairs. These interactions include both *direct* and *membrane-mediated* contributions. The former are typically described by pairwise interactions which are infinitely repulsive at very small molecular separations and attractive at somewhat longer (but still

[a] e-mail: ofarago@bgu.ac.il

finite) distances [15]. The effect of the direct interactions between adhesion bonds can be studied in the framework of the thoroughly researched lattice gas model [16]. Specifically, there exists a critical value ϵ_c of the strength of the attractive part of the intermolecular pair potential above which the system may phase-separate into domains with high and low concentrations of adhesion bonds.

Much less is known about the indirect interactions between adhesion sites which are mediated by the membrane thermal fluctuations. The entropic origin of these interactions can be easily understood as follows: Consider two adhesion bonds between two membranes or between a membrane and a surface. The adhesion points restrict the thermal height fluctuations of the membrane in their vicinity. This entropy loss can be minimized if the two adhesion bonds are brought to the same place, in which case the membrane becomes pinned at only one point and not two. The membrane fluctuations, thus, induce an attractive potential of mean force between the adhesion points.

In a previous publication, we analyzed the membrane-mediated interactions between two adhesion bonds of a bilayer membrane and a supporting surface [17]. We found that for "point-like" adhesion molecules whose size is comparable to or smaller than the thickness of the membrane ($l \sim 4$–5 nm), the potential of mean force is an infinitely long-range attractive potential that grows logarithmically with the pair distance r:

$$U(r) = 2k_B T \ln\left(\frac{r}{l}\right). \tag{1}$$

Equation (1) holds for *tensionless* membranes, which will be the case discussed in the following. When surface tension is applied, this form holds for pair separations

$r \ll \xi_\sigma = \sqrt{\kappa/\sigma}$, where κ and σ denote the membrane bending rigidity and surface tension, respectively. For $r \gg \xi_\sigma$, $U(r)$ is decreased by a factor of 2 [17]. We leave the discussion of stressed membranes to a future publication.

In this paper we consider the same system as in ref. [17], but instead of two adhesion points we look at a membrane which is pinned to a flat impenetrable surface at multiple sites. To analyze the aggregation behavior of the adhesion sites we first need to generalize eq. (1) and write down the fluctuation-induced interaction energy as a function of the coordinates of the adhesion sites $U(\boldsymbol{r}_1, \boldsymbol{r}_2, \boldsymbol{r}_3, \ldots, \boldsymbol{r}_N)$. This is a non-trivial problem since the fluctuation-mediated interaction is a many-body potential which cannot be expressed as the sum of two body terms of the form in eq. (1). The many-body nature of $U(\boldsymbol{r}_1, \boldsymbol{r}_2, \boldsymbol{r}_3, \ldots, \boldsymbol{r}_N)$ is best illustrated by an example: Consider a cluster of two adhesion points located close to each other at $\boldsymbol{r}_1 \simeq \boldsymbol{r}_2$, and a third distant adhesion point located at $\boldsymbol{r}_3$. Having a single adhesion point at $\boldsymbol{r}_1$ or $\boldsymbol{r}_2$ instead of the two-point cluster will not result in any change in the spectrum of membrane thermal fluctuations. Therefore, the third point is attracted to the two-point cluster by the same potential of mean force (1) to which it is attracted to one adhesion point, and not by a potential which is twice larger than potential (1), which would be the case if $U(\boldsymbol{r}_1, \boldsymbol{r}_2, \boldsymbol{r}_3, \ldots, \boldsymbol{r}_N)$ is the sum of pair interactions. What is the exact form of $U(\boldsymbol{r}_1, \boldsymbol{r}_2, \boldsymbol{r}_3, \ldots, \boldsymbol{r}_N)$ is still an open question that needs to be resolved for the fluctuation-induced domain formation to be understood. Several approximations to this problem which avoid direct many-body calculations have been proposed. Weikl and Lipowsky introduced a mean-field theory in which the effect of the pinning points is represented by a *homogeneous* attractive interaction between the fluctuating membrane and the underlying surface (or between the membrane and another membrane) [18]. They concluded that the homogeneous fluctuation-induced potential alone cannot facilitate the formation of adhesion zones, but it greatly reduces ϵ_c, the critical strength of the direct interactions between the adhesion bonds above which the formation of adhesion clusters is possible. A very similar conclusion has been recently reached by Speck *et al.* using rigorous statistical mechanical methods [19]. However, in their model the hard-wall interaction between the membrane and the surface has been replaced with a harmonic confining potential.

In this work we present a more accurate approach to the problem which employs a non-additive many-body potential $U(\boldsymbol{r}_1, \boldsymbol{r}_2, \boldsymbol{r}_3, \ldots, \boldsymbol{r}_N)$. The derivation of the potential $U(\boldsymbol{r}_1, \boldsymbol{r}_2, \boldsymbol{r}_3, \ldots, \boldsymbol{r}_N)$, which is based on our previous statistical mechanical studies of the membrane thermal fluctuations with one [20] and two [17] adhesion points, is presented in sect. 2. The idea is somewhat different than the one previously used by others. As in refs. [18, 19], we integrate out the membrane degrees of freedom and map the problem onto the lattice gas model where the *occupied* sites represent the adhesion bonds. But unlike in refs. [18,19], the interaction energy $U(\boldsymbol{r}_1, \boldsymbol{r}_2, \boldsymbol{r}_3, \ldots, \boldsymbol{r}_N)$ is not expressed as the sum of two body interactions between

Fig. 1. Schematic of the system under investigation, consisting of a membrane which is pinned to a flat impenetrable surface at multiple adhesion points. The adhesion points can diffuse freely on the surface, and they may cluster to increase the conformational entropy of the fluctuating membrane.

nearest-neighbor lattice sites. Instead, it is calculated from the *empty* sites that represent the unpinned sections of the membrane. The energy assigned with each empty site represents the loss of entropy due to the reduction of the membrane fluctuations in the area around that site. We argue that the extent by which the membrane fluctuations are locally restricted depends mainly on the distance to the closest pinning point. Therefore, the calculation of $U(\boldsymbol{r}_1, \boldsymbol{r}_2, \boldsymbol{r}_3, \ldots, \boldsymbol{r}_N)$ for a given distribution of pinning sites involves the division of the lattice into Voronoi cells and summing their contributions to the free energy. In sect. 3 we present a mean-field analysis of our model, and in sect. 4 we present the results of Monte Carlo lattice simulations. We find, in agreement with other theoretical studies, that, while the fluctuation-mediated interactions alone are not sufficient to allow the formation of aggregation clusters, they greatly reduce the strength of the residual (direct) interactions between adhesion points which is required to facilitate cluster formation. More specifically, we find that the strength of the short-range cohesive energy that allows condensation is reduced by about a factor of two and falls below the thermal energy $k_B T$. In sect. 5 we summarize and discuss our results.

2 The many-body fluctuation-mediated potential

We consider the system shown schematically in fig. 1 consisting of a fluctuating membrane of linear size L which is pinned to a flat impenetrable surface at several sites. The free-energy cost of a single adhesion point is given by [21,20]

$$F_1 = k_B T \ln \left(\frac{L^2}{l^2} \right) = 2 k_B T \ln \left(\frac{L}{l} \right). \qquad (2)$$

This result has been derived in ref. [20] by noting that pinning the membrane to the surface at one point does not modify the membrane spectrum of thermal fluctuations. It does, however, eliminate the membrane translational degree of freedom by enforcing the global minimum of the membrane height function to be located at the point of contact with the surface. A different interpretation to eq. (2) is that between the membrane and the surface there is an effective interaction free energy per unit area of the form

$$V(r) = \frac{k_B T}{\pi r^2}, \qquad (3)$$

that represents the entropy cost due to the reduction of the membrane fluctuations at distance r from the adhesion

site [17]. The attachment free energy is then derived by integrating the free-energy density $U(r)$ over the projected area of the membrane

$$F_1 = \int_l^L 2\pi r V(r) \mathrm{d}r. \qquad (4)$$

The free-energy density (3) can likewise be employed to derive the fluctuation-mediated pair potential (1). However, when we have two adhesion points, the distance r in eq. (3) should be the distance of the unit membrane area to the closest adhesion point. With this choice of r, let us now consider a square membrane ($-L/2 \leq x, y \leq L/2$) with two adhesion points located at $(x, y) = (\pm r_0/2, 0)$. The attachment free energy is calculated by integrating $V(r)$ over the projected area of the membrane (excluding a region of size l around the adhesion points):

$$F_2 = 4 \int_0^{L/2} \mathrm{d}y \left[\int_0^{(r_0-l)/2} \mathrm{d}x \frac{k_B T}{\pi \left[y^2 + (x - r_0/2)^2 \right]} \right.$$
$$\left. + \int_{(r_0+l)/2}^{L/2} \mathrm{d}x \frac{k_B T}{\pi \left[y^2 + (x - r_0/2)^2 \right]} \right]. \qquad (5)$$

Integrating over y yields

$$F_2 = \frac{4 k_B T}{\pi} \left[\int_0^{(r_0-l)/2} \frac{\mathrm{d}x}{|x - r_0/2|} \tan^{-1} \left(\frac{L}{2|x - r_0/2|} \right) \right.$$
$$\left. + \int_{(r_0+l)/2}^{L/2} \frac{\mathrm{d}x}{|x - r_0/2|} \tan^{-1} \left(\frac{L}{2|x - r_0/2|} \right) \right]. \qquad (6)$$

Assuming that $l < r_0 \ll L$, the inverse tangent function in eq. (6) can be approximated by the constant value of $\pi/2$ over most of the integration range (except near the boundaries of the membrane $|x| \sim L/2$ which, nevertheless, does not influence the dependence of the result on r_0). With this approximation, one gets

$$F_2(r_0, L) \simeq 2 k_B T \ln \left(\frac{L}{l} \right) + 2 k_B T \ln \left(\frac{r_0}{l} \right)$$
$$= F_1(L) + U(r_0). \qquad (7)$$

The first term in eq. (7) is the free-energy cost of a single adhesion site (2), which is the expected value when the two adhesion points coincide ($r_0 \simeq l$) to form a single cluster. The second term, which represents the additional free-energy cost associated with the separation of the adhesion points, is identified as the fluctuation-induced pair potential, in agreement with eq. (1).

We now wish to generalize eqs. (5) and (7), and propose that the many-body fluctuation-mediated potential is given by

$$U(\boldsymbol{r}_1, \boldsymbol{r}_2, \boldsymbol{r}_3, \ldots, \boldsymbol{r}_N) = F_N(\boldsymbol{r}_1, \boldsymbol{r}_2, \boldsymbol{r}_3, \ldots, \boldsymbol{r}_N, L) - F_1(L). \qquad (8)$$

The attachment free energy F_N of N adhesion points is calculated by integrating the free-energy density

$$F_N = \int \frac{k_B T}{\pi d^2(\boldsymbol{r}, \boldsymbol{r}_1, \boldsymbol{r}_2, \boldsymbol{r}_3, \ldots, \boldsymbol{r}_N)} \mathrm{d}\boldsymbol{r}, \qquad (9)$$

where d is the distance of the membrane unit area to the nearest adhesion point

$$d = \min_{i=1 \ldots N} \left(|\boldsymbol{r} - \boldsymbol{r}_i| \right), \qquad (10)$$

and the integration is carried over the projected area of the membrane except for small regions of size l near each adhesion point. What we essentially argue here is that the extent by which the membrane thermal fluctuations are limited at each spot depends almost exclusively on the distance to the nearest adhesion point. All the other adhesion points are effectively screened. This suggestion is supported not only by the above example, eqs. (5)-(7), but also by our observation from recent computer simulations presented in ref. [17]. In that paper, we determined the pair potential (1) by using Monte Carlo simulations of a coarse-grained bilayer model with two adhesion points. Our simulations results were in excellent agreement with eq. (1) despite the fact that each adhesion point interacts not only with the other adhesion point but also with its infinite array of periodic images. It is unlikely that the periodic images have such a negligible contribution on $U(r)$ for all values of r unless they are simply screened. In other words, the local fluctuation behavior of the membrane is governed by the distance to the nearest adhesion point while the spatial distribution of the other, more distant, points is irrelevant. Thus, calculating F_N for a given distribution of adhesion points involves the construction of the 2D Voronoi diagram of the configuration, and summation of the free-energy contributions coming from each Voronoi cell, S_i

$$F_N = \sum_{i=1}^N \int \mathrm{d}S_i \frac{k_B T}{\pi r^2}, \qquad (11)$$

where r is the distance to the adhesion point which is located inside the Voronoi cell.

3 Mean-field theory

To study the formation of adhesion clusters in supported membranes, we consider a lattice of N_s sites with lattice spacing set equal to l (the cut-off microscopic length scale) of which $N < N_s$ sites are occupied by adhesion points. For each spatial configuration of the system, the energy is written as the sum of two terms

$$E = E_{\mathrm{LG}} + F_N. \qquad (12)$$

For the first term in eq. (12) representing the direct interaction between the adhesion points, we take the lattice gas energy with nearest-neighbor interactions

$$E_{\mathrm{LG}} = \sum_{\langle i,j \rangle} -\epsilon s_i s_j, \qquad (13)$$

where $s_i = 1$ for an occupied site, $s_i = 0$ for a vacant site, and ϵ is the site-site interaction energy. The second term

in eq. (12), which represents the attachment free energy, is given by the discrete form of eq. (11)

$$F_N = \sum_{i=1}^{N_s} \frac{k_B T}{\pi} \left(\frac{l}{r}\right)^2 (1 - s_i), \qquad (14)$$

where r is the distance from a vacant lattice site to the nearest occupied lattice site.

The phase behavior of the system can be analyzed through mean-field theory. Let us assume that the adhesion points form $N_c < N$ clusters. The free energy of system includes three contributions: i) the mixing entropy of the adhesion clusters, ii) the energy E_{LG} of the direct interactions between the adhesion points, and iii) the attachment free energy F_N. The first free-energy contribution is given by

$$\frac{F_{\mathrm{mix}}}{k_B T} = N_c \left[\ln\left(\frac{N_c}{N_s}\right) - 1\right] + \frac{1}{2} c \left(\frac{N_c^2}{N_s}\right), \qquad (15)$$

where c is the second virial coefficient. On average, each cluster consists of (N/N_c) adhesion points; and if we assume that it has a roughly circular shape, then $c \simeq 4(N/N_c)$. Denoting the number densities of the adhesion points by $\phi = N/N_s$, and of the clusters by $\phi^* = N_c/N_s \leq \phi$, the free energy of mixing per lattice site is given by

$$\frac{F_{\mathrm{mix}}}{N_s k_B T} = \phi^* \left[\ln\left(\phi^*\right) - 1\right] + 2\phi\phi^*. \qquad (16)$$

The second contribution to the free energy is due to the direct interactions between the adhesion points. The ground state of the interaction energy E_{LG} is achieved when a single circular adhesion domain with minimal surface is formed. If we set the ground state as the reference energy, the energy of an ensemble of clusters can be estimated as being proportional to the total length of the domain boundaries. For N_c circular clusters of size (N/N_c) we have

$$\frac{E_{\mathrm{LG}}}{N_s k_B T} = \lambda \frac{N_c}{N_s} \sqrt{\frac{N}{N_c}} = \lambda \sqrt{\phi\phi^*}, \qquad (17)$$

where λ, the associated dimensionless line tension, is proportional to the interaction energy ϵ in eq. (13) and B, the mean number of nearest-neighbor vacant sites per occupied site on the boundary of a cluster ($B \to 1$ for very large clusters).

$$\lambda = 2\sqrt{\pi}B\epsilon. \qquad (18)$$

The sum of free-energy contributions (16) and (17) constitutes the total free-energy density (per lattice site) of a 2D lattice gas of clusters

$$\frac{F_{\mathrm{LG}}}{N_s k_B T} = \phi^* \ln(\phi^*) - \phi^* + 2\phi\phi^* + \lambda\sqrt{\phi\phi^*}. \qquad (19)$$

The third contribution of the attachment free energy can be estimated as follows. The clusters form N_c Voronoi cells, each of which has on average an area of $A_{\mathrm{Vor}} = (N_s/N_c)l^2$. The attachment free energy of each Voroni cell is given an equation similar to eq. (2) for the attachment

free energy of one adhesion point, but with A_{Vor} instead of the total membrane area L^2. Thus

$$F_N = N_c \left[k_B T \ln\left(\frac{N_s}{N_c}\right)\right], \qquad (20)$$

and the attachment free-energy density is given by

$$\frac{F_N}{N_s k_B T} = -\phi^* \ln(\phi^*), \qquad (21)$$

which eliminates the first term in the lattice gas free-energy density (eq. (19)), yielding

$$\frac{F}{N_s k_B T} = \frac{F_{\mathrm{LG}}}{N_s k_B T} + \frac{F_N}{N_s k_B T} = -\phi^* + 2\phi\phi^* + \lambda\sqrt{\phi\phi^*}. \qquad (22)$$

We consider a low density of adhesion sites $\phi \ll 1$, which also implies a low number density of adhesion clusters since $\phi^* \leq \phi$. By minimizing the free-energy density we obtain the equilibrium value of the ϕ^* for the lattice gas problem (eq. (19)) and for the adhesion points of a fluctuating supported membrane (eq. (22)). In both cases, the system undergoes a first-order phase transition at $\lambda_1(\phi)$ from the gas phase ($\phi^* = \phi$) to a condensed phase consisting of only a few clusters ($\phi^* \sim 0$). Also, in both cases the free energy reaches a maximum at intermediate densities ($0 < \phi^* < \phi$). This free-energy barrier for condensation disappears at the spinodal point $\lambda_2(\phi) > \lambda_1(\phi)$. For the lattice gas problem we find

$$\lambda_1^{\mathrm{LG}} = 1 - 2\phi - \ln(\phi)$$
$$\lambda_2^{\mathrm{LG}} = -4\phi - 2\ln(\phi), \qquad (23)$$

while for the adhesion points of fluctuating membranes we have

$$\lambda_1 = 1 - 2\phi$$
$$\lambda_2 = 2 - 4\phi = 2\lambda_1. \qquad (24)$$

The results of eqs. (23) and (24) are summarized in fig. 2A and B, respectively. The important points in the results are that: i) $\lambda_1 > 0$, which means that the fluctuation-induced interactions alone are not sufficient to induce aggregation of adhesion domains, but ii) they greatly reduce the strength of the direct interactions required to facilitate cluster formation since $\lambda_1 < \lambda_1^{\mathrm{LG}}$ (and also $\lambda_2 < \lambda_2^{\mathrm{LG}}$). In the following section we support these conclusions with MC simulations. We show that for adhesion points of fluctuating membranes, the site-site cohesive energy ϵ for the onset of aggregation falls below the thermal energy $k_B T$.

4 Computer simulations

To further investigate the aggregation behavior of adhesion points, we performed Monte Carlo (MC) simulations of our lattice gas model with the total configurational energy given by the sum of direct (eq. (13)) and fluctuation-induced (eq. (14)) interactions. We used a 120×138 triangular lattice (that has an aspect ratio very close to 1) with
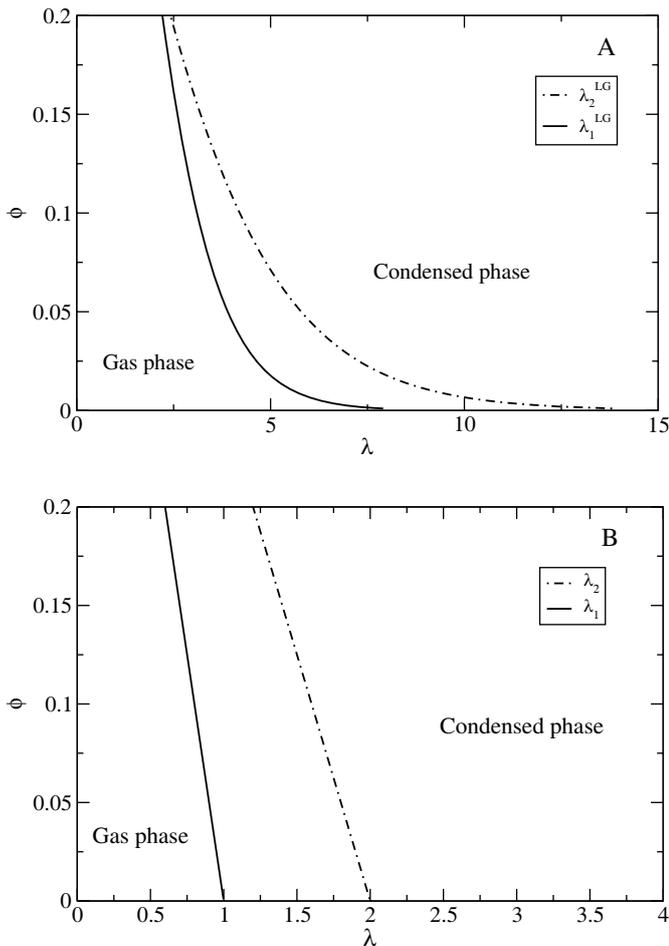
Fig. 2. The phase diagram of the adhesion sites calculated within the mean-field approximation. (A) Equation (23) for the lattice gas model. (B) Equation (24) for adhesion points of fluctuating membranes. λ_1 and λ_2 represent the first-order transition and spinodal lines, respectively.

Fig. 3. Initial configurations of the simulations in which (A) the sites are randomly distributed on the lattice, and (B) put in a single compact cluster. Representative equilibrium configurations of (C-D) our model (eqs. (13) and (14)) and (E-F) the standard lattice gas model (eq. (13) only) for $\phi = 0.1$ and $\epsilon = 1k_BT$. Configurations (C) and (E) evolved from the initial state (A), while (D) and (F) evolved from (B).

periodic boundary conditions. We simulated the system at two different densities $\phi = N/N_s = 0.05$ and $\phi = 0.1$, and for various values of ϵ ranging from 0 to $3k_BT$. For comparison, we also simulated the standard lattice gas model (for which the configurational energy is given by eq. (13), without the fluctuation-mediated free energy eq. (14)). For each density ϕ and for each value of ϵ, we performed 8-16 independent runs starting from different initial configurations where the points are either randomly distributed on the lattice (as in fig. 3A) or put in a single cluster (see fig. 3B). The system was then equilibrated until the distribution of points in all the independent runs looks similar (see *e.g.*, fig. 3C *vs.* D, and fig. 3E *vs.* F). Equilibrium time for the different samples ranges from 3.6×10^5 to 10^6 MC time units, where each MC time unit consists of N single-particle move attempts. For the adhesion points problem, each particle was displaced to a randomly chosen nearest-neighbor lattice site, which enabled us to employ an efficient algorithm to update the Voronoi diagram needed for calculating the fluctuation-mediated free energy (14). For the standard lattice gas model, each move attempt con-

sisted of randomly selecting a particle and moving it to the nearest vacant point in a randomly chosen lattice direction [22]. After the first stage of equilibration, the simulations were continued for 3×10^5 MC time units during which data was collected every third MC time unit.

To examine the occurrence of a phase transition from a gas to a condensed phase, we measured the average number of clusters in the system (where a cluster is defined as a set of neighboring occupied sites), and the mean value of the energy of direct interactions between sites, $\langle E_{\rm LG} \rangle$ (see eq. (13)). Our results are summarized in fig. 4A (for $\phi = 0.05$) and B (for $\phi = 0.1$). For each ϕ we measured these quantities both for the standard lattice gas model (open symbols and dash-dotted lines in fig. 4) and for adhesion points which also interact via the fluctuation-mediated free energy eq. (14) (solid symbols and solid lines in fig. 4). The number of clusters is denoted by squares (values on the right y-axis of the figures), while $\langle E_{\rm LG} \rangle$ is represented by circles (values on the left y-axis).

The gas phase is characterized by a large number of small clusters, some of which may be of the size of a single
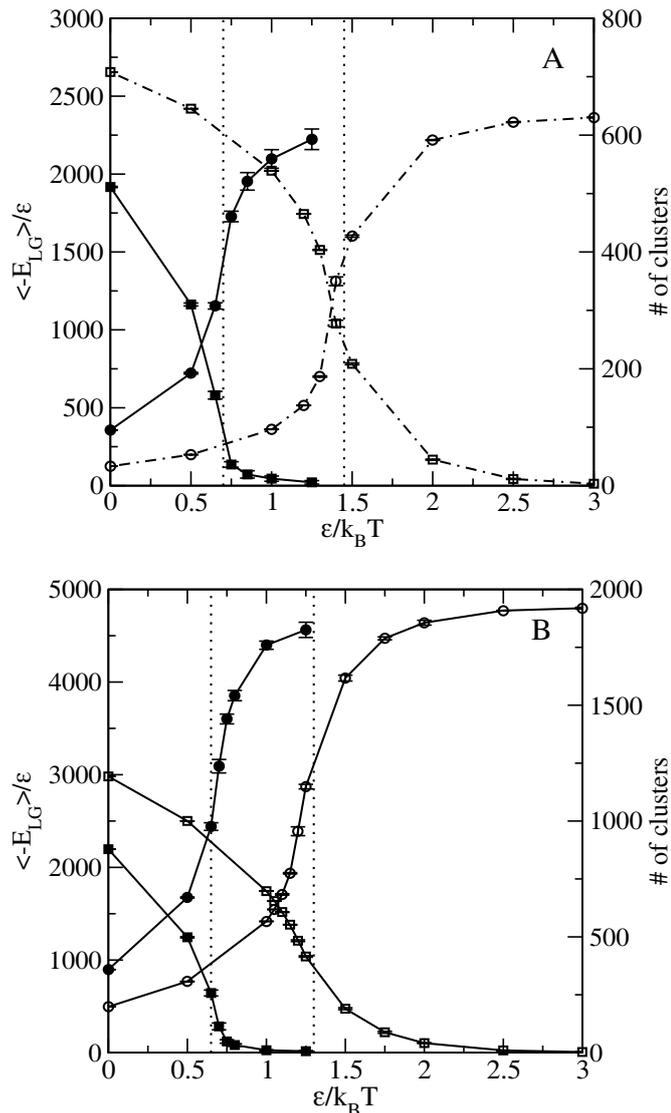
Fig. 4. Left y-axis: the energy of direct interactions between sites, $\langle E_{\mathrm{LG}} \rangle$ (eq. (13)), as a function of ϵ, for $\phi = 0.05$ (A) and $\phi = 0.1$ (B). Solid circles: results for our model for adhesion points. Open circles: results for the standard lattice gas model. Right y-axis: the number of clusters as a function of ϵ, for $\phi = 0.05$ (A) and $\phi = 0.1$ (B). Solid squares: results for our model for adhesion points. Open squares: results for the standard lattice gas model.

site. Furthermore, since each occupied site has a relatively small number of neighboring occupied sites, the mean configurational energy $\langle -E_{\mathrm{LG}} \rangle$ is relatively low. Conversely, when the sites form large clusters in the condensed phase, $\langle -E_{\mathrm{LG}} \rangle$ is high, and the total number of clusters decreases (and in many cases, especially for large values of ϵ, we simply observe only a single cluster in our system). Figure 4 exhibits an abrupt, clearly first-order, transition from a gas phase with a large number of clusters and small $\langle -E_{\mathrm{LG}} \rangle$ to a condensed state with a small number of clusters and large $\langle -E_{\mathrm{LG}} \rangle$. The estimated values of ϵ at the transition are (see vertical lines in fig. 4): $\epsilon_t \simeq 0.7 k_B T$

($\phi = 0.05$) and $\epsilon_t \simeq 0.65 k_B T$ ($\phi = 0.1$). In comparison (see also fig. 4), for the standard lattice gas model, the transition values are roughly twice larger than these values: $\epsilon_t^{\mathrm{LG}} \simeq 1.45 k_B T$ ($\phi = 0.05$) and $\epsilon_t^{\mathrm{LG}} \sim 1.3 k_B T$ ($\phi = 0.1$). Figure 3C-F exhibit typical equilibrium configurations of the system at $\phi = 0.1$ and $\epsilon = 1 k_B T$. For the lattice gas model $\epsilon_t^{\mathrm{LG}} > 1 k_B T$, and at equilibrium the system is in the gas phase (figs. 3E and F). When the fluctuation-induced interactions eq. (14) are introduced, ϵ_t falls below $1 k_B T$ and the system is in the condensed phase where most of the particles belong to one large cluster (figs. 3C and D).

Our computational results, which show that the fluctuation-mediated interactions reduce the strength of ϵ_t, are in a qualitative agreement with the mean-field theory prediction (sect. 3). To make a quantitative comparison between the theory and the simulations, one needs to estimate the parameter B appearing in eq. (18). Several reasons make such an estimation difficult and inaccurate: First, our non-standard mean-field theory is based on the assumption that the clusters are circular and roughly have the same size, which is quite a crude approximation. Second, tracing the precise location of ϵ_t in fig. 4 is largely inaccurate because of the finite size of the system that makes the transition look like a crossover. To reduce the large uncertainties associated with the determination of ϵ_t, one can look at the difference between the value of this quantity in our model and for the standard lattice gas model. Using

$$\lambda_1^{\mathrm{LG}} - \lambda_1 = 2\sqrt{\pi} B \left(\epsilon_t^{\mathrm{LG}} - \epsilon_t \right), \qquad (25)$$

for $\phi = 0.1$, we find $B \simeq 1$, as indeed expected for large clusters.

5 Summary and discussion

In this paper we studied the aggregation behavior of adhesion points between a fluctuating membrane and a supporting surface. We demonstrated that the problem can be mapped onto a lattice gas model with two types of molecular interactions: i) direct site-site pair interactions and ii) Casimir-like interactions which are mediated by the membrane thermal fluctuations. The fluctuation-mediated interactions, which are inherently of many-body character, are calculated in our model by summing over the vacant rather than the occupied sites of the lattice. Each vacant site represents a small unit area of the fluctuating membrane, and the fluctuation-mediated potential expresses the local free-energy cost due to the restriction imposed by the adhesion points on the membrane thermal fluctuations. This free-energy cost depends mainly on the distance between the vacant sites and the nearest occupied site. Therefore, such a many-body potential is calculated by determining the Voronoi diagram for each lattice configuration, which can be quite easily implemented in MC simulations.

We used mean-field calculations and MC computer simulations to investigate the phase behavior of a lattice

gas of adhesion sites at low densities. We showed that upon increasing the strength of the site-site interactions ϵ, the system undergoes a first-order phase transition into a condensed state. The fluctuation-induced interactions lower the value of ϵ at the phase transition to below the thermal energy $k_B T$. This result suggests that fluctuation-mediated effects play a central role in the formation of adhesion domains in biomimetic and biological membranes.

References

1. M.C. Beckerle (Editor), *Cell Adhesion* (Oxford University Press, Oxford, 2001).
2. D.A. Lauffenburger, J. Linderman, *Receptors: Models for binding, Trafficking, and Signaling* (Oxford University Press, Oxford, 1995).
3. A.-S. Smith, U. Seifert, Soft Matter **3**, 275 (2007).
4. T.R. Weikl, M. Asfaw, H. Krobath, B. Różycki, R. Lipowsky, Soft Matter **5**, 3213 (2009).
5. P.F. Lenne, A. Nicolas, Soft Matter **5**, 2841 (2009).
6. V. Naggli, Int. J. Biochem. Biochem Cell Biol. **35**, 1619 (2003).
7. K. Giehl, A. Menke, Front. Biosci. **13**, 3975 (2008).
8. B. Geiger, A. Bershadsky, R. Pankov, K.M. Yamada, Nat. Rev. Mol. Cell Biol. **2**, 793 (2001).
9. J. Salafsky, J.T. Groves, S.G. Boxer, Biochemistry **35**, 14773 (1996).
10. A. Kloboucek, A. Behirsch, J. Faix, E. Sackmann, Biophys. J. **77**, 2311 (1999).
11. D. Cuvelier, P. Nassoy, Phys. Rev. Lett. **93**, 228101 (2004).
12. M. Tanaka, E. Sackmann, Nature **437**, 656 (2005).
13. M. Lamblet, B. Delord, L. Johannes, D. van Effenterre, P. Bassereau, Eur. Phys. J. E **26**, 205 (2008).
14. K. Sengupta, L. Limozin, Phys. Rev. Lett. **104**, 088101 (2010).
15. J. Israelachvili, *Intermolecular and Surface Forces* (Academic Press, London, 1985).
16. B. Simon, *The Statistical Mechanics of Lattice Gases I* (Princeton University Press, Princeton, 1993).
17. O. Farago, Phys. Rev. E **81**, 050902(R) (2010).
18. T.R. Weikl, R. Lipowsky, in *Advances in Planar Lipid Bilayers and Liposomes*, edited by A.L. Liu, Vol. **5** (Elsevier, Amsterdam, 2006).
19. T. Speck, E. Reister, U. Seifert, preprint: cond-mat:1004.2696 (2010).
20. O. Farago, Phys. Rev. E **78**, 051919 (2008).
21. R. Bruinsma, M. Goulian, P. Pincus, Biophys. J. **67**, 746 (1994).
22. Such MC moves greatly reduce the rejection probability due to excluded-volume interaction. One can easily verify that for these moves, detailed balance is satisfied by the conventional Metropolis acceptance rule $p(\text{old} \rightarrow \text{new}) = \min(1, \exp(-\Delta E / k_B T))$, where ΔE is the energy change caused by the move attempt.