Supporting lipid membranes are useful and important model systems for studying cell membrane properties and membrane-mediated processes [1,2]. Placing a membrane on a flat substrate allows for the application of several different surface sensitive techniques, including atomic force microscopy, x-ray and neutron diffraction, ellipsometry, nuclear magnetic resonance, and others [3]. With the aid of biochemical tools and generic engineering, supported membranes can be functionalized with various membrane-associated proteins [4]. One attractive application of supported membranes is the design of phantom cells exhibiting well-defined adhesive properties and receptor densities [5]. Using advanced imaging techniques, detailed information can be obtained about the structure of the adhesion zone between the receptor-functionalized supported membrane and ligand-containing vesicles that can bind to the supported membrane [6,7]. These studies provide insight into the specific (ligand-receptor) and nonspecific interactions during cell adhesion [8,9]. Understanding these interactions is crucial for the development of drug delivery systems that depend on efficient adhesion between a liposome and the plasma membrane of the target cell.

Adhesion is an immensely complex process involving many physicochemical and biomolecular factors [10]. Many aspects of this process, ranging from the cooperativity in adhesion cluster formation to the influence of stochastic processes such as the ligand-receptor reaction kinetics, have been and continue to be studied theoretically using various models (see, e.g., recent reviews in Refs. [11,12]). In light of this extensive theoretical effort, it is surprising that there is still no satisfactory answer to one of the most fundamental problems associated with adhesion, namely, the characterization of the membrane-mediated interaction between adhesion sites. Detailed knowledge of the strength and range of these interactions is essential for a better understanding of the role they play during the self-assembly of adhesion zones. The lack of theoretical studies of membrane-mediated interactions between adhesion sites is in striking contrast with the extensive literature existing on membrane-mediated interactions between transmembrane proteins. In the latter case, the origin of the interactions is the ability of two proteins to position themselves in a manner that minimizes the total bending elastic energy of the deformed membrane [13]. In addition, the influence of the proteins on the membrane thermal fluctuations leads to membrane-mediated interactions between them, which are analogous to Casimir forces between conducting plates [14]. These interactions fall off with the protein pair separation as $1/r^4$ [13–15] and, therefore, at large distances they are considerably larger than van der Waals and screened electrostatic interactions which decay much faster with $r$. Since adhesion sites between membranes or between a membrane and a surface represent a different type of “constraint” on the shape of the membrane, one can expect Casimir-like interactions to exist between them as well. Below, we explore these interactions for a pair of adhesion sites between a membrane and a flat impenetrable surface and show that these interactions are of effective infinite range.

Consider a membrane of linear size $L$ with bending rigidity $\kappa$ and surface tension $\sigma$, which may also experience a height-dependent harmonic confining potential (whose second derivative with respect to the height is $\gamma$) due to the presence of a nearby flat surface. Let $h(\vec{r})$ be the height function of the membrane, which vanishes at the points where the membrane is attached to the surface and takes positive values everywhere else. The total energy of the membrane is, thus, given by the effective Hamiltonian

$$\mathcal{H} = \int \frac{1}{2} \left[ \kappa (\nabla^2 h)^2 + \sigma (\nabla h)^2 + \gamma h^2 \right] \Phi(h) d^2 \vec{r},$$

(1)

where $\Phi$ represents the hard-wall constraint ($\Phi=1$ for $h \geq 0$, and $\Phi=+\infty$ for $h<0$), and the integration is taken over the cross-sectional (projected) area of the membranes $A_p \sim L^2$. Let us first consider the case where $\sigma=0$ and $\gamma=0$ in Eq. (1). In a previous publication we studied the behavior of a membrane with one attachment site to the surface [16]. We found, quite unexpectedly, that the attachment of the membrane to a flat surface at only single adhesion point does not modify the spectrum of thermal fluctuation of the membrane. The only effect of the attachment is to eliminate the membrane-mediated interactions with conducting plates [14]. These studies provide insight into the specific type of “constraint” on the shape of the membrane, one can expect Casimir-like interactions to exist between them as well.
constant, $T$ is the temperature, and $l$ is some microscopic length scale on the order of the bilayer thickness. The scaling behavior of $\Delta F$ with $L$ can be also obtained by noting that because the single attachment point leaves the spectrum of thermal fluctuation unchanged, the mean height of the membrane above the surface increases as $u(r) = (h(r)) \sim \langle \kappa k_B T \rangle$ with $r$ as the distance from the pinning site [16,17]. Helfrich showed that, as a result of the collisions between the membrane and the surface, there is an effective interaction energy per unit area: $V(r) \sim (\kappa k_B T)^2 / ku(r)^2 \sim (\kappa k_B T)/r^2$ [18]. By integrating this energy density over the projected area of the membrane, one finds that

$$\Delta F = \int V(r) d^2 r = C k_B T \ln \left( \frac{L}{l} \right). \quad (2)$$

An equation very similar to Eq. (2) has been previously derived in Ref. [17] in the context of self-assembly of membrane junctions. In that reference, the mean-field free energy per adhesion site was found to have a logarithmic dependence on the mean distance between sites. This result has been interpreted as a renormalization of the temperature downward. Our finding that $C = 2$ implies that the attachment free energy (2) exactly cancels the mixing entropy term of the adhesion sites and, therefore, the renormalized temperature $T_r = 0$. The inability of this simple mean-field calculation to predict whether the adhesion sites tend to aggregate ($T_r < 0$) or segregate ($T_r > 0$) emphasizes the need for a more detailed analysis of the membrane-mediated interactions that also take into account their many-body nature. We leave most of the discussion of many-body effects to a future publication and focus here on the pair-correlation function between adhesion sites.

The attachment free energy (2) is distributed within a volume $V \sim L^2 \Delta_0$, where $\Delta_0 = u(L) - L \langle k_B T / \kappa \rangle$ is the mean height of the membrane. It, therefore, seems reasonable to speculate that disjoining pressure between the membrane and the surface scales as

$$P \sim \Delta F / V \sim \frac{\kappa k_B T}{L} \ln (L/l). \quad (3)$$

The pressure between the membrane and the underlying surface is not uniform, however, and rather decreases with $r$ because points on the manifold that are closer to the attachment site tend to collide more frequently with the surface. Defining the distance-dependent pressure $P(r)$, the mean disjoining pressure can be calculated as $P \sim (L^2)^{1/2} P(r) dr$, which can be reconciled with Eq. (3) by assuming that $P(r) \sim (\kappa k_B T / L^2)$. Since the pressure is caused by collisions between the membrane and the surface, one may conclude that the probability density that the membrane comes into contact with the surface at distance $r$ from the attachment point has the same scaling behavior as $P(r)$,

$$\Pi[h(r) = 0] \sim P(\tilde{r}) \sim \frac{1}{r^2}. \quad (4)$$

Let us now turn to the problem of a supported membrane with two adhesion points. Let $\tilde{r} = \tilde{r}_0$ denote the position of the second adhesion point fixed at the origin, $\tilde{r} = 0$. The potential of mean force between the adhesion sites is defined as $\phi(\tilde{r}_0) = -k_B T \ln g(\tilde{r}_0)$, where $g(z)$ is the pair distribution function expressed as a function of the coordinate of the second adhesion site. By definition, $g(z)$ is also equal to $\Pi[h(\tilde{r}_0) = 0]$, the probability density that a configuration with an adhesion site at $\tilde{r} = 0$ makes contact with the surface at $\tilde{r}_0$ as well. We thus conclude that $g(\tilde{r}_0) = \Pi[h(\tilde{r}_0) = 0]$, and together with Eq. (4), we arrive at the following scaling result for the pair-correlation function:

$$g(\tilde{r}_0) \sim 1/r^2. \quad (5)$$

From this we find that the potential of mean force between the two adhesion sites is an infinitely long range attractive potential that grows logarithmically with the pair distance $r_0$:

$\phi(\tilde{r}_0) = -k_B T \ln g(\tilde{r}_0) = 2k_B T \ln (r_0/\tilde{r}_0)$.

We tested the validity of Eq. (5) by using constant surface-tension (frame tension) Monte Carlo (MC) simulations of a coarse-grained implicit solvent bilayer model. The details of the model (which is suitable for simulations of bilayer membranes at large spatial and temporal scales) and simulations can be found in Ref. [16], where we discuss the problem of a membrane with one attachment point to the surface. In the present work, we have a flat surface and 2000 coarse-grained lipids that form a square bilayer patch of linear size $L$. Each lipid is represented by a short string consisting of one head bead and two tail beads. The lipids reside on one side of the surface. Two lipids are attached to surface at their head beads. The location of one of these head beads is fixed at the origin, while the second head bead is allowed to diffuse on the flat surface. By sampling the position of the latter bead, the pair distribution function can be computed and compared with the power-law distribution [Eq. (5)]. There is, however, a problem with this seemingly straightforward strategy. The simulation time must be much longer than both (i) the typical relaxation time of the longest bending mode and (ii) the typical diffusion time across the membrane of the mobile adhesion point. Unfortunately, both these characteristic times grow very rapidly with $L$, in a way that makes the application of the standard Metropolis MC algorithm impractical. To overcome this problem we used two “tricks”: the relaxation times of the thermal bending modes were reduced by applying the recently proposed “mode excitation MC” (MEMC) scheme [19]. The MEMC scheme utilizes collective update moves that lead to fast excitation and relaxation of the long-wavelength modes. The problem arising from the slow diffusion of the mobile adhesion point was solved by identifying the unpinned lipid whose head group is located closest to the surface and introducing a new MC move that attempts to place this lipid on the surface while lifting the mobile pinned lipid away from the surface. More precisely, if the height of the closest unpinned head group to the surface is $h_0 > 0$, the move attempt consists of a simultaneous change in the heights of all the beads of both the unpinned and pinned lipids—the former are reduced by $h_0$, while the latter are increased by $h_0$. One can easily verify
that for this new type of MC move, detailed balance is satisfied by the conventional Metropolis acceptance rule: $p(\text{old} \rightarrow \text{new}) = \min[1, \exp(-\Delta E/k_BT)]$, where $\Delta E$ is the energy change caused by the move attempt. The new move allows the mobile pinning site to “jump” from one place on the membrane to another and enables efficient sampling of the pair-correlation function $g(\hat{r})$ within a reasonable simulation time.

Our results, which are shown in Fig. 1, agree very well with the functional form predicted in Eq. (5). The slope of the straight line on the log-log plot is equal to $-2$. Deviations from the power-law behavior $g(\hat{r}) \sim 1/\hat{r}^2$ can be observed at small $(r/L < 0.05)$ and large $(r/L > 0.5)$ distances only. At small separations, the molecular nature of the lipids becomes important and the radial pair distribution function is dominated by the depletion shells around the lipids. At large distances, the mobile adhesion site reaches the center of the square membrane where it becomes attracted not only by the fixed adhesion site but also by its periodic images. In this regime, the many-body nature of the membrane-mediated potential must be taken into account.

Let us now consider the case of a membrane which is also subjected to lateral surface tension $\sigma > 0$ [see Eq. (1)]. For a stressed membrane, one can define the “surface-tension crossover length,” $\xi_{\sigma} \sim \sqrt{\sigma/\sigma}$, which marks a crossover between two regimes. On length scales much smaller than $\xi_{\sigma}$, the thermal fluctuations are governed by the bending elasticity of the membrane while the surface-tension term in Eq. (1) is negligible. Therefore, for $r \ll \xi_{\sigma}$, one can expect Eq. (5) for the pair-correlation function to hold. On length scales much larger than $\xi_{\sigma}$, the surface-tension term in Eq. (1) becomes dominant, which leads to the suppression of the long-wavelength thermal fluctuations. Consequently, for $r \gg \xi_{\sigma}$, the decay of the pair-correlation function should be slower than that predicted by Eq. (5). The behavior of $g(\hat{r})$ in this regime can be derived using the following argument: let $h(\hat{r})$ be the height function of the stressed membrane. Define the function $H(\hat{r})$ such that $\kappa(\nabla^2 H)^2 = \sigma(\nabla h)^2$. The manifold depicted by the function $H$ represents a nonstressed bilayer membrane, and from Eq. (4) we have that $\Pi[H(\hat{r})=0] \sim 1/\hat{r}^2$. At large $\hat{r}$, the two functions can be related by the simple scaling relation $\kappa H^2/\hat{r}^4 \sim \sigma h^2/\hat{r}^2$, i.e., $h(\hat{r}) \sim (\xi_{\sigma}/\hat{r}) H(\hat{r})$ [20]. This scaling relationship implies that the probability density $\Pi[h(\hat{r})=0] \sim (r/\xi_{\sigma}) \Pi[H(\hat{r})=0]$. As previously noted, the function $\Pi[H(\hat{r})=0]$ is, in fact, the pair distribution function, which leads us to conclude that in the surface-tension dominated regime, $r \gg \xi_{\sigma}$, $g(\hat{r}) \sim (r/\xi_{\sigma})^1/\hat{r}^2 \sim 1/\hat{r}$. The potential of mean force, $\phi(\hat{r}) = -k_BT \ln[g(\hat{r})] \approx k_BT \ln(r)$, simply decreases to half of the value of $\phi(\hat{r})$ in nonstressed membranes. Notice that this form is independent of $\sigma$ whose magnitude influences only the half of the crossover length $\xi_{\sigma}$ between the two scaling regimes. Figure 2 shows our simulation results for a membrane with surface tension $\sigma = 3.6k_BT/l^2$, where the microscopic length scale $l$ is taken as the length of the three-bead model lipid ($l \sim 2$ nm; the linear size of the membrane patch in our simulations $L \sim 12.5l$). The two scaling regimes with $g(\hat{r}) \sim 1/\hat{r}^2$ for small $r$ and $g(\hat{r}) \sim 1/r$ for large $r$ can be clearly seen. In a previous paper we measured the bending rigidity of the membrane and found $\kappa \sim 8k_BT$ [19]. This gives $\xi_{\sigma} \sim 1.5l \sim 0.12L$, which is consistent with Fig. 2 where the crossover between the scaling regimes takes place around $r \sim 0.25L$.

For a nonstressed membrane experiencing a harmonic confining potential [$\gamma > 0$ in Eq. (1)], the “harmonic confinement crossover length,” $\xi_{\gamma} \sim \sqrt{(\kappa/\gamma)}$, can be defined which marks the transition between two scaling regimes. For $r \ll \xi_{\gamma}$, the thermal fluctuations are dominated by the bending-rigidity term in the Hamiltonian and, therefore, $g(\hat{r}) \sim 1/\hat{r}^2$. For $r \gg \xi_{\gamma}$, the fluctuation spectrum is dominated by the harmonic confinement term. Since this term is a local one, the influence of the adhesion site is screened and pair-correlation function saturates to a constant value, $g(\hat{r}) \sim r^3$, which means that fluctuation-mediated force between the adhesion sites vanishes [21]. To verify these predictions, we added an energy term for each lipid in our model, $E=(1/2)\gamma r^2 h^2$, which is proportional to the square of the height $h$ of the lipid. The variable $\gamma$ is related to $\gamma$ in Eq. (1) by $2\gamma/a = \gamma$, where $a$ is the cross-sectional area per lipid and the factor of 2 is due to the two leaflets of the membranes. Here, we take $\gamma = 0.072$, which together with the previously computed value $\gamma = 0.15l^2$ [19] gives $\gamma = 0.096k_BT/l^2$. This yields, $\xi_{\gamma} \sim 3l \sim 0.25L$. The results, presented in Fig. 3, show the two scaling regimes for small and large $r$, where the crossover between them occurs at $r \sim 0.3L$.

To conclude, we have calculated the membrane-mediated...
interactions between two adhesion sites of a bilayer membrane and a supporting flat surface. We found that the potential of mean force is an infinitely long range attractive potential that grows logarithmically with the pair distance \( r \): \( \phi(\tilde{r})/k_BT = c \ln r \). The constant \( c \) takes three possible values depending on which term in Eq. (1) dominates the effective Hamiltonian of the system: \( c=2 \) in the bending-rigidity dominated regime (which always prevails at small pair separations), \( c=1 \) in the surface-tension dominated regime, and \( c=0 \) in the regime where the dominant term is of the harmonic confinement. It is important to note that even when \( c=2 \) at all pair separations [i.e., for \( \gamma=0 \) and \( \sigma=0 \) in Eq. (1)], the membrane-mediated attractive potential is not strong enough to bind the pair of adhesion sites. The pair-correlation function in this case, \( g(\tilde{r}) \sim 1/r^2 \), and the mean pair separation increases with the size of the system:

\[
\langle r \rangle = \int_0^1 r^2 g(r)dr/\int_0^1 rrg(r)dr \sim L/\ln L.
\]

This, however, does not render the fluctuation-mediated interactions between adhesion sites unimportant. These interactions may very well provide a powerful aggregation mechanism of receptor-ligand binding domains. In order to correctly analyze the aggregation behavior of an ensemble of adhesion sites, one must take into account the many-body nature of the fluctuation-induced interactions between them. Such a study is currently underway.

This work was supported by the Israel Science Foundation (Grant No. 946/08).

[20] A more careful analysis [see, e.g., W. Helfrich and R.-M. Serruss, Nuovo Cimento D 3, 137 (1984)] shows that there is a logarithmic correction to this scaling relation. At large pair distances \( H(r) \sim r \), while \( h(r) \sim \ln(r) \).
[21] This argument also holds for potentials of the form \( V = (1/2)\gamma(h-h^*)^2 \) as well. In this case, the screening takes place on length scales much larger than (twice) the “healing length” of each adhesion point, which is the typical length from the adhesion site at which the membrane returns to the equilibrium spacing, \( h=h^* \), from the surface.