Entropy-driven aggregation in multilamellar membranes

HIROSHI NOGUCHI

Institute for Solid State Physics, University of Tokyo - Kashiwa, Chiba 277-8581, Japan

received 15 May 2013; accepted in final form 10 June 2013
published online 24 June 2013

PACS 87.16.D– Membranes, bilayers, and vesicles
PACS 87.15.A– Theory, modeling, and computer simulation
PACS 82.70.Uv– Surfactants, micellar solutions, vesicles, lamellae, amphiphilic systems,
(hydrophilic and hydrophobic interactions)

Abstract – Membrane-fluctuation–induced attraction between ligand-receptor sites binding neighboring membranes is studied using meshless membrane simulations and the Weil-Farago two-dimensional (2D) lattice model. For the adhesion sites binding two membranes, this entropic interaction is too weak by itself for the adhesion sites to form a large stable domain. However, it is found that this attraction is enhanced sufficiently to induce large domains either when the sites bind three or more neighboring membranes together or have anchorsthatharden surrounding membranes. These effects are understood by the 2D lattice model with minor extensions.

Copyright © EPLA, 2013

Introduction. – Cell adhesion is a fundamental process required for the removal of foreign bodies in immune response, for tissue formation, and for cell motility. It is mediated by specific (ligand-receptor) and non-specific interactions. Recently, lateral interactions between ligand-receptor adhesion sites in membranes have been received growing attention [1–13]. The cooperative aggregation of the adhesion sites yields various patterns of adhesion domains and also morphological changes in cells and liposomes.

Experimentally, lipid membranes supported on a solid substrate are widely used to study immune reaction and protein functions as well as membrane adhesion [1–3]. Several types of anchoring molecules have been developed to control the distance and interactions between the membranes or the membrane and the substrate. In traditional adhesion experiments using the supported membrane, the receptors are immobile on the substrate, while their partners (ligands) are mobile in the fluid membrane. Recent experiments with mobile receptors have revealed that their mobility strengthens the adhesion [2,6]. Diffusion of the receptors can induce a high density of the ligand-receptor bonds in the adhesion domain.

Currently, entropic interactions between (permanently bonded) ligand-receptor sites is a topic of active discussion [7–12]. The membrane height fluctuations yield a repulsive force $f \sim d^{-3}$ between tensionless fluid membranes with a neighboring membrane distance $d$ [14]. Since the adhesion bond holds two membranes close to each other, an effective attraction works between the adhesion sites. If the adhesion sites are aggregated, the rest of the regions of the membranes are allowed to have large height fluctuations. This entropy gain is the source of this attraction. Such an interaction can be considered analogous to hydrophobic or depletion interactions, i.e., entropy loss of water molecules surrounding hydrophobic molecules or polymers surrounding colloids [15,16]. In contrast to these interactions, the membrane interactions are long-range; the potential of the mean forces between the membranes and adhesion sites decays as $\sim r^{-2}$ [9]. Thus, a different type of aggregation behavior can be expected.

Several groups have investigated this entropic interaction theoretically [7,9–12] and via simulations [8,9]. These studies have reported a weak attraction between adhesion sites, which induces small temporal clusters. However, it has been concluded that this force is too weak to form a large cluster by itself, and thus, the researchers have included additional pairwise interactions to investigate the phase separation.

The aim of this letter is to determine the condition required to form a large stable cluster only via this membrane-mediated entropic interaction and to clarify its difference from the typical phase separation generated by a pairwise interaction. We examine two conditions to intensify the attraction: 1) increasing entropy of the height fluctuations by binding more than two membranes and 2) increasing suppression effects on local height fluctuations surrounding the anchoring sites. Although ligand-receptor proteins binding more
than two membranes are not known, ligand-receptor chains connecting several membranes are synthetically producible. Membrane proteins often modify the surrounding membranes [17,18]. Many proteins are surrounded by specific lipids, and proteins with a long hydrophobic core stretch surrounding lipids (hydrophobic mismatch) [19,20]. These effects reduce the flexibility of surrounding lipids and can increase the bending rigidity \( \kappa \) of the surrounding membranes. Since the bending rigidity depends on the membrane thickness \( h \) as \( \kappa \sim h^3 \) [21], the membranes around proteins of positive hydrophobic mismatch have larger bending rigidity.

We employ one of the solvent-free meshless membrane models [22,23] to tackle this problem. Since we study large-scale membrane fluctuations, the detailed structures of the bilayer are negligible, so that the membrane is considered as a curved surface. To discretize the membrane, mesh membrane methods such as the square mesh method used in [8] are also available. Here, we choose meshless membranes to avoid the influence of the mesh structures on the cluster structures. In our meshless model, one membrane particle represents a patch of the bilayer membrane and a membrane can be spontaneously formed. We also use the Weil-Farago two-dimensional (2D) lattice model [10] to understand the membrane-mediated interactions as effective potentials.

Methods. –

Meshless membrane simulation. In this study, we consider \( N_{lay} \) layers of quasi-planar fluid membranes. Each membrane is represented by a self-assembled one-layer sheet of \( N_{mb} \) particles. These membranes are bound by \( N_{bond} \) permanently bonded adhesion sites, which are represented by a linear chain of harmonic bonds using a harmonic potential \( U_{bond} = \sum_{i < j} (k_{bond}/2)(r_{i,j} - b_{bond})^2 \), where \( r_{i,j} \) denotes the distance between \( i \)-th and \( (i + 1) \)-th particles and these two particles belong to neighboring membranes (see fig. 1(a)).

Since the details of the meshless membrane model are described in refs. [23–25], we briefly explain the model here. The particles interact with each other via the potential \( U = \varepsilon(U_{rep} + U_{att}) + U_\alpha + U_{bond} \), which consists of a soft-core excluded-volume potential \( U_{rep} \) with diameter \( \sigma \), an attractive potential \( U_{att} \), a curvature potential \( U_\alpha \), and a bond potential \( U_{bond} \). In order to ensure that the interactions between neighbor membranes are only a short-range repulsion, the membrane particles interact with the particles in different membranes only via the repulsive potential \( U_{rep} \), while in each membrane these three membrane potentials \( (U_{rep}, U_{att}, U_\alpha) \) are taken over all contained particles.

The excluded-volume potential is given by \( U_{rep} = \sum_{i<j} \exp(-20(r_{i,j}/\sigma - 1) + 0.126f_{cut}(r_{i,j}/\sigma)) \). The interaction is smoothly cut off by a \( C^\infty \) cutoff function [23]

\[
f_{cut}(s) = \exp \left[ A \left( 1 + \frac{1}{(s/s_{cut})^{12} - 1} \right) \right] \theta(s_{cut} - s),
\]

where \( A = 1 \), \( s_{cut} = 1.2 \), and \( \theta(s) \) denotes the unit step function. The potential \( U_{att} \) is a function of the local density of particles, \( \rho_i = \sum_j f_{cut}(r_{i,j}/\sigma) \), with the parameters \( s_{half} = 1.8, s_{cut} = 2.1, \) and \( A = \ln(2)((s_{cut}/s_{half})^{12} - 1) \). Here, \( \rho_i \) denotes the number of particles in a sphere whose radius is approximately \( r_{att} = s_{half}\sigma \). The potential \( U_{att} \) is given by \( U_{att} = \sum_i \frac{0.25 \ln(1 + \exp(-4(r_{i}/\rho^*)}}{C} - C \), where \( C = 0.25 \ln(1 + \exp(4\rho^*)) \). This body potential acts as a pair potential \( U_{att} \sim -\rho_i \) with the cutoff at \( \rho_i \approx \rho^* \), and it can stabilize the fluid phase of membranes over a wide range of parameter sets.

The curvature potential is given by \( U_\alpha = \sum_i k_{\alpha,i}\alpha_{pl}(r_i) \), where \( k_{\alpha,i} = k_{\alpha}^{bond} \) and \( k_{\alpha,i} = k_{\alpha}^{norm} \) for the adhesion sites (membrane particles bonded with neighboring membranes) and normal (unbonded) membrane particles, respectively. The shape parameter aplanarity \( \alpha_{pl} \) is defined as

\[
\alpha_{pl} = \frac{9\lambda_1 \lambda_2 \lambda_3}{(\lambda_1 + \lambda_2 + \lambda_3)(\lambda_1 \lambda_2 + \lambda_2 \lambda_3 + \lambda_3 \lambda_1)},
\]

where \( \lambda_1, \lambda_2, \) and \( \lambda_3 \) are the eigenvalues of the weighted gyration tensor, \( a_{\alpha \beta} = \sum_{i,j} (\alpha_{i,j} - \alpha_{G})(\beta_{i,j} - \beta_{G})w_{mb}(r_{i,j}) \), where \( \alpha, \beta = x, y, z \) and \( r_{G} = \sum_i r_i w_{mb}(r_{i,j})/\sum_j w_{mb}(r_{i,j}) \). The aplanarity \( \alpha_{pl} \) represents the degree of deviation from a plane, and it is
membranes \((N_{\text{lay}} = 2)\). The anchoring sites harden the surrounding membranes at \(\kappa_t > 1\).

2D lattice model. The potential of the mean force between two adhesion sites with distance \(r\) in tensionless membranes is derived by Farago as \(\Phi(r) = 2k_B T \ln (r/l)\), where \(l\) denotes the membrane thickness [9]. However, the sum of this potential interaction between all pairs of the adhesion sites yields too strong an attraction, since the pair interaction can be shielded by other sites. Weil and Farago proposed a 2D lattice model to take into account the multibody nature of the interaction [10]. Since local membrane height fluctuations are mainly suppressed by the nearest adhesion site, the entropy of the membrane segment can be expressed by a function of the distance \(d_i^{\text{min}}\) to the nearest site. The entropy of the height fluctuations are expressed as an effective potential,

\[
U_{2d} = \sum_{i=1}^{N_{2d}} u_i (1 - s_i),
\]

\[
u_i = \frac{(N_{\text{lay}} - 1) k_B T}{\pi} \left( \frac{l_0}{d_i^{\text{min}}} \right)^2,
\]

for a triangular lattice with unit lattice length \(l_0\), and \(N_{2d}\) denotes the number of lattice sites. For the adhesion sites and the unbonded sites \(s_i = 1\) and \(s_i = 0\), respectively, so that the integration is essentially taken over the unbonded lattice sites. In order to treat multilamellar membranes, we slightly extend the Weil-Farago model by adding a factor \(N_{\text{lay}} - 1\) in eq. (5). Thus, the height differences between neighboring membranes are assumed to independently fluctuate. We use the Metropolis Monte Carlo method to obtain the equilibrium states for the membranes with an almost-square shape \(L_x = 149 l_0\) and \(L_y = 86 \sqrt{3} l_0\) with \(N_{2d} = 25628\).

Double membranes. – First, we investigate interactions between the adhesion sites binding two membranes \((N_{\text{lay}} = 2)\), and we confirm the conclusions of previous studies [8–10]. The adhesion sites are distributed throughout the membranes and their small clusters are temporarily formed but do not grow into large stable clusters (see fig. 1). Even if a simulation is started from a large cluster, it gradually dissolves into the mixed state.

The cluster size is almost independent of the mean density of adhesion sites \(\phi = N_{\text{bond}} / N_{\text{mb}}\). The radial distribution function \(g(r)\) multiplied by \(\phi\) for the distance \(r\) projected in the \(xy\)-plane is shown in fig. 2. When all of the particles at distance \(r\) are adhesion sites and the projected membrane density is uniform, \(\phi g(r) = 1\). With increasing \(\phi\), the number of contacted particle pairs increases by only 10%, while the density at \(r/l_0 \gg 1\) linearly increases. Thus, the clusters do not grow, and instead the excess amount of adhesion sites dissolve in isolation or form other small clusters. The pair interactions of adhesion sites are shielded by other surrounding sites, and the local density in the clusters is saturated.
The Weil-Farago 2D lattice model [10] reproduces our simulation results well (compare the solid and dashed lines in fig. 2). In particular, the heights of the first peak of the simulation and the model coincide. In the lattice model, $g(r)$ exhibits slightly slower decays, which are likely caused by the difference in the unit area: $a_0 = 1.1l_0^2$ and $a_0 = (\sqrt{3}/2)l_0^2$ for the meshless and lattice models, respectively. If the two-body potential $\Phi(r_{ij})$ [9] is instead employed, all adhesion sites assemble into one cluster at $N_{\text{bond}} \geq 4$. Thus, the multibodiness of the interactions is crucial to understand this clustering.

**Three or more membranes.** – In order to produce a large stable cluster, the bending entropy of membranes is enhanced by the addition of more layers of membranes. For triple membranes ($N_{\text{lay}} = 3$), the adhesion sites form a single large domain, whose shape shows large fluctuations (see figs. 3(a) and (a')). A few sites often leave the domain but soon return before moving far away, since an isolated site will further suppress the membrane fluctuations of the larger area. With increasing $N_{\text{lay}}$, the domain becomes more compact and circular (see figs. 3(b) and (b')).

The distribution of the number ratio $P_{\text{bond}} = n_{\text{bond}}(r)/n_{\text{all}}(r)$ of bonded membrane particles in the middle layer of the membranes is shown in fig. 4(a), where $n_{\text{bond}}(r)$ and $n_{\text{all}}(r)$ are the number of bonded and all particles, respectively, at a distance $r$ from the center of the domain. The number ratio is uniform in the middle of the domain. Interestingly, many unbonded membrane particles still remain in the domain even at $N_{\text{lay}} = 8$ ($P_{\text{bond}} = 0.58$).

The $N_{\text{lay}}$ dependences of two shape parameters are shown in fig. 5. The radius of gyration $R_g$ is normalized by $R_g^0 = \sqrt{a_0 N_{\text{bond}}}/2\pi$ for a densely packed circular domain. The shape deviation from a circular disk is calculated as $\alpha_c = (\nu_1 - \nu_2)/(\nu_1 + \nu_2)$, where $\nu_1$ and $\nu_2$ denote two eigenvalues of the gyration tensor of the adhesions sites. Although the domain shape becomes circular, its size does not approach $R_g/R_g^0 = 1$ but 1.3. This saturation is caused by the existence of the unbonded particles in the domain.
The domain shapes at $\phi = 0.0039$ and $\phi = 0.016$ ($N_{\text{bond}} = 100, 400$) are also compared in fig. 5. The normalized size $R_g/R_0^d$ coincides very well. Smaller domains have slightly larger values of $\alpha$, since the same amplitude of the boundary fluctuation yields larger effects on the whole shape for a smaller domain. At $\phi = 0.0039$, $P_{\text{bond}}$ also exhibits a distribution similar to that at $\phi = 0.016$ (data not shown). Thus, the domain formation is not sensitive to the domain size, at least when the domain is sufficiently smaller than the membrane area.

A single domain is formed when $N_{\text{lay}} \geq 3$ also in the 2D lattice model (see figs. 3–5). The 2D domains are more compact and they comprise a lesser number of unbonded sites than the domains in the meshless membrane simulations. In the lipid bilayer membranes, the height fluctuations are governed by molecular protrusions smaller in length than the membrane thickness [21]. This protrusion is taken into account in the meshless membrane model but not in the lattice model. The absence of the protrusion likely causes the reduction in the domain size. Except for this domain size difference, the lattice model reproduces the domain properties very well. Thus, the addition of the membrane layers can be simply interpreted as a linear entropy increase of the membrane height fluctuations.

**Double membranes with membrane-hardening anchors.** Membrane proteins often modify the structure of surrounding membranes and form an annular shell of specific ligands [17,18]. Here, we simply consider the effect of the anchor proteins on the main quantity being examined in this study, i.e., the bending rigidity $\kappa$. The anchors of ligands or receptors suppress the bending fluctuations of the surrounding membranes.

In the meshless membrane model, the locally large bending rigidity is given by a large value of $k_B/\alpha \propto k_B^{\text{bond}}$ of the bonded membrane particles. In the limit $\kappa = k_B^{\text{bond}}/k_B^{\text{norm}} \to \infty$, the neighboring membrane at a distance $r<3\sigma$ from the adhesion sites becomes completely flat. This local flattening induces a stable domain formation even at $N_{\text{lay}} = 2$. With increasing $\kappa$, the domain radius decreases (see fig. 6(a)). In the domain, the hardened membrane areas are overlapped, so that total hardened area is reduced by the domain formation.

In order to take this effect into account, the potential, given by eq. (5), in the 2D lattice model is modified as

$$u_i = \begin{cases} \frac{k_B T}{\pi} \left( \frac{l_0}{R_{\text{min}} - r_{AO}} \right)^2, & (d_i^{\text{min}} \geq r_{AO} + l_0), \\ \frac{k_B T}{\pi}, & (d_i^{\text{min}} < r_{AO} + l_0). \end{cases} \tag{6}$$

The surrounding membrane sites at the distance $r < r_{AO}$ are flat and have no bending fluctuations. This treatment is similar to the Asakura-Oosaka theory for the depletion interaction [15,16].

As the height fluctuations of several neighbor sites are suppressed at $r_{AO}/l_0 \gtrsim 2$, a single domain is formed (see fig. 6(b)). At large values of $r_{AO}$, the domain radius is saturated to $R_g/R_0^d = 2$, which is considerably larger than the value $R_g/R_0^d = 1.3$ of the meshless simulations. Thus, the omission of the protrusions works differently from the case of the $N_{\text{lay}}$ increase. It reduces the effective attractions. Since the protrusion is also suppressed at large values of $\kappa$, the surrounding unbonded membrane particles lose more entropy than in the case of the 2D lattice representation.

**Summary and discussions.** We have revealed that the clustering of the adhesion sites binding neighboring membranes can be caused only by entropic interactions via membrane height fluctuations. The reduction in the membrane fluctuations due to close contact yields an attractive interaction between the adhesion sites. For binding between two membranes, this interaction is too weak to form a large stable domain and increasing the number of the adhesion sites does not lead to cluster growth. In order to overcome this problem, we extend the system to enhance this attraction in two ways: 1) The number of membrane layers is increased; when three or more membranes are bound, the adhesion sites form one large domain. 2) Adhesion anchors that harden surrounding membranes are employed. This induces a depletion type of attraction between the adhesion sites, since the areas of the surrounding membranes are overlapped in the

![Fig. 6: (Colour on-line) Variation in the radius of gyration $R_g$ of the adhesion sites with the membrane-hardening anchors at $N_{\text{lay}} = 2$ for $\phi = 0.0039$ (□, △) and $\phi = 0.016$ (○, ×). (a) Dependence on the ratio of bending rigidity $\kappa$ in the meshless membrane simulation. (b) Dependence on the depletion radius $r_{AO}$ in the 2D depletion lattice model (eq. (6)). Snapshots at $\kappa = 8$ and $r_{AO} = 5l_0$ are shown in the insets of (a) and (b), respectively.](image-url)
clusters. Both conditions intensify the entropy gain by the cluster formation and their effects can be explained by the extended 2D lattice models.

Although ligand-receptor pairs bind only two membranes in living cells, ligand-receptor chains connecting several membranes are synthetically producible. Our predictions on clustering in multimembrane systems can be experimentally examined using supporting membranes. On the other hand, the latter depletion-like interactions may play a role in biomembranes and this type of domain formation can be induced not only in adhesion anchor proteins but also in other membrane-associated proteins. It is reported that the adhesion sites are accumulated on fixed membrane boundary [11]. The adhesion sites and other proteins can likely form a domain at a specific region in a plasma membrane via interactions with the cytoskeleton.

The domain formations in bound membranes are different from those of typical phase separation by a pairwise interaction. When two types of molecules are phase-separated in a binary fluid, a small fraction of either type of molecules dissolves in the other phase, and their fractions rapidly decrease with decreasing temperature. In the case of the membrane adhesion sites, the competition between the entropies in the perpendicular direction (height fluctuations) and in the horizontal directions (mixing of the adhesion sites) determines the phase behavior, so that it does not directly depend on the temperature (it may indirectly be affected by a change in the membrane properties). While a large amount of unbonded membrane particles dissolves in circular domains, no adhesions sites dissolve in the membranes even from largely deformed domains. This asymmetry is caused by the long-range height correlation of the membranes. Such stable domains involving other lipids and proteins may form a good platform for biological functions.

***

The author would like to thank G. GOMPPER and H. SHIBA for informative discussions. The numerical calculations were partly carried out on SGI Altix ICE 8400EX at ISSP Supercomputer Center, University of Tokyo. This work is supported by KAKENHI (25400425) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

REFERENCES

[1] Tanaka M. and Sackmann E., Nature, 437 (2005) 656.
[2] Smith A.-S. and Sackmann E., ChemPhysChem, 10 (2009) 66.
[3] Achalkumar A. S., Bushby R. J. and Evans S. D., Soft Matter, 6 (2010) 6036.
[4] Weikl T. R., Asfaw M., Krobath B. R. H. and Lipowsky R., Soft Matter, 5 (2009) 3213.
[5] Kaizuka Y. and Groves J. T., Biophys. J., 86 (2004) 905.
[6] Smith A.-S., Sengupta K., Goennenwein S., Seifert U. and Sackmann E., Proc. Natl. Acad. Sci. U.S.A., 105 (2008) 6906.
[7] Bruinsma R., Goulain M. and Pincus P., Biophys. J., 67 (1994) 746.
[8] Krobath H., Schütz G. J., Lipowsky R. and Weikl T. R., EPL, 78 (2007) 38003.
[9] Farago O., Phys. Rev. E, 81 (2010) 050902.
[10] Weil N. and Farago O., Eur. Phys. J. E, 33 (2010) 81.
[11] Weil N. and Farago O., Phys. Rev. E, 84 (2011) 051907.
[12] Speck T., Phys. Rev. E, 83 (2011) 050901.
[13] Speck T. and Vink R. L. C., Phys. Rev. E, 86 (2012) 031923.
[14] Helfrich W., Z. Naturforsch., 28c (1973) 693.
[15] Asakura S. and Oosawa F., J. Chem. Phys., 22 (1954) 1255.
[16] Lekkerkerker H. N. W. and Tuitner R., Colloids and the Depletion Interaction (Springer, Dordrecht) 2011.
[17] Lee A. G., Biochim. Biophys. Acta, 1666 (2004) 62.
[18] Contreras F.-X., Ernst A. M., Wieland F. and Brügger B., Cold Spring Harb. Perspect. Biol., 3 (2011) a004705.
[19] de Meyer F. J., Venturoli M. and Smit B., Biophys. J., 95 (2008) 1851.
[20] Neder J., Nielara P., West B. and Schmid F., New. J. Phys., 14 (2012) 125017.
[21] Goetz R., Gompper G. and Lipowsky R., Phys. Rev. Lett., 82 (1999) 221.
[22] Noguchi H., J. Phys. Soc. Jpn., 78 (2009) 041007.
[23] Noguchi H. and Gompper G., Phys. Rev. E, 73 (2006) 021903.
[24] Noguchi H. and Gompper G., J. Chem. Phys., 125 (2006) 164908.
[25] Shiba H. and Noguchi H., Phys. Rev. E, 84 (2011) 031926.
[26] Feller S. E., Zhang Y., Pastor R. W. and Brooks B. R., J. Chem. Phys., 103 (1995) 4613.
[27] Noguchi H., Soft Matter, 8 (2012) 3146.