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Membrane sorting via the extracellular matrix

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ABSTRACT

We consider the coupling between a membrane and the extracellular matrix. Computer simulations demonstrate that the latter coupling is able to sort lipids. It is assumed that membranes are elastic manifolds, and that this manifold is disrupted by the extracellular matrix. For a solid-supported membrane with an actin network on top, regions of positive curvature are induced below the actin fibers. A similar mechanism is conceivable by assuming that the proteins which connect the cytoskeleton to the membrane induce local membrane curvature. The regions of non-zero curvature exist irrespective of any phase transition the lipids themselves may undergo. For lipids that prefer certain curvature, the extracellular matrix thus provides a spatial template for the resulting lateral domain structure of the membrane.

1. Introduction

Ever since the lipid raft hypothesis has been established [1], the lateral organization of membranes has been intensely studied. Much has been learned from model membranes, in which the number of lipid species is strongly reduced compared to their biological counterparts, enabling detailed and systematic investigations [2,3]. The hope is that a good understanding of the model system will also provide valuable insight into biological membranes.

Investigations of model membranes have established one fact beyond any doubt, namely the occurrence of phase transitions in these systems. For example, ternary membrane mixtures containing saturated lipids, unsaturated lipids, and cholesterol, demix into two fluid phases upon lowering the temperature [4]. Furthermore, in single component membranes, there exists the main transition, between a phase where the lipid tails are ordered, and one where the tails are disordered [5,6]. Consequently, it is tempting to assume that phase transitions play a key role in biological membranes as well [7,8]. Of course, a minimal condition for this hypothesis is that all biological membranes operate at conditions that are close to phase transitions. Given the enormous diversity in membrane compositions between cells, different "body" temperatures between species, coupling of the membrane to active processes in the cell cortex [9], an intriguing mechanism must have evolved to keep the membrane "tuned" to the vicinity of a phase transition. While such a mechanism may well exist, its details remain elusive to this day.

The purpose of this paper is to highlight that phase transitions are not the only means to bring about lateral organization in biological membranes. In our view, a key difference between biological and model membranes is the presence of an extracellular matrix in the former: A biological membrane is not free, but instead intricately connected to its environment, for example to the cytoskeleton network. As we will show for a very simple model, the mere connection to the environment is already sufficient to induce lateral organization. Our model is inspired by a recent experiment of a (model) membrane "sandwiched" between a substrate and an actin network [10]. This experiment revealed a lateral domain structure in the membrane that was strongly correlated to the actin fibers. We will show here how the interplay between the substrate, the actin network, and the membrane elastic properties already provides a "template" for this structure, i.e. completely independent of any phase transition the lipids may exhibit. Next, we consider how such a mechanism could manifest itself in situations where a substrate is absent, but where the proteins that connect the cytoskeleton to the membrane induce local membrane curvature. Our proposed mechanisms are in line with recent studies that also indicate the importance of the cytoskeleton in bringing about lateral organization, such as the formation of protein–lipid complexes [11], and GPI-anchored protein clusters [12]. In addition, the coupling to the cytoskeleton induces spatial confinement, which affects the spectrum of membrane height fluctuations [13–15], as well as protein diffusion [16,17].

2. Model and method

Our membrane model is defined on a two-dimensional (2D) $L \times L$ periodic square lattice. To describe the out-of-plane height deformations, each lattice site i is given a real number h_i to denote the local membrane height (Monge representation). We consider a membrane that strongly interacts with its environment. This interaction, for

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instance with the solid substrate or the cytoskeleton network, will typically constrain the membrane height fluctuations. In general, the free energy of the system is given by

$$\mathcal{H} = \frac{\kappa a^2}{2}\sum_i \left(\nabla^2 h_i\right)^2 + \mathcal{H}_{\text{env}}, \tag{1}$$

where a is the lattice spacing, and the sum is over all lattice sites. The first term in Eq. (1) is the elastic energy of the membrane, given in lowest order of the Helfrich expansion with κ being the bending modulus [18]. On the lattice, the Laplacian is expressed using the standard finite-difference expression [19]. The second term $\mathcal{H}_{\text{env}}$ describes the membrane–environment interaction, and needs to be defined explicitly for the case of interest.

We perform Monte Carlo (MC) to simulate Eq. (1). The MC move is to update the height of a randomly selected lattice site. To this end, we propose a new height for the chosen site and accept it with the Metropolis probability, $P_{\text{acc}} = \min[1, e^{-\beta\Delta\mathcal{H}}]$, where $\beta = 1/k_{\text{B}}T$ with k_{B} as the Boltzmann constant, T as the temperature, and $\Delta\mathcal{H}$ as the free energy difference computed according to Eq. (1). For a free energy $\mathcal{H}$ that is quadratic in the height, $\sim \sum_i (A_i h_i^2 + B_i h_i)$, one can optimally propose the new height to the chosen site i from a Gaussian distribution with mean $-B_i/2A_i$ and variance $k_{\text{B}}T/2A_i$ [20], which is how the present simulations are performed.

3. Results

3.1. Membrane "sandwiched" between a solid substrate and an actin network

We first consider a solid-supported membrane bound to an actin network, which resembles the situation addressed experimentally in Ref. [10]. In this case, the interaction potential in Eq. (1) is given by

$$\mathcal{H}_{\text{env}} = \mathcal{H}_{\text{sub}} + \mathcal{H}_{\text{act}}, \tag{2}$$

where the first term describes the interaction of the membrane with the solid support, and the second term describes the influence of the actin network.

Solid-supported membranes are separated from the substrate by an ultra-thin hydration layer typically 1 nm thick. Consequently, the membrane–substrate interaction is a strong one [21–24]. It can be expressed as a superposition of repulsive hydration (steric) and attractive van der Waals forces [25]. This typically results in a membrane–substrate interaction featuring a minimum some distance above the support. We expand up to quadratic order around the minimum, leading to

$$\mathcal{H}_{\text{sub}} = \frac{\alpha a^2}{2}\sum_i h_i^2, \tag{3}$$

where α is the strength of the harmonic potential [26,27]. For simplicity, the minimum of the harmonic potential is set to $h = 0$, which we take as the reference from which the membrane height variables h_i are measured. We emphasize that by using a harmonic potential, the free energy Eq. (1) remains quadratic in h_i and so we can use the Gaussian distribution method of Ref. [20] to optimally propose new height variables during the MC simulations.

Next, we describe the effect of the actin term $\mathcal{H}_{\text{act}}$. In the experiment of Ref. [10], an actin network is deposited on top of the supported membrane, i.e. the membrane is "sandwiched" between the substrate and the actin network. In experiments [10,28], actin is bound to the membrane via cross-linker molecules, such as streptavidin, referred to as *pinning sites* in what follows. The pinning sites are immobilized obstacles randomly distributed along the actin fibers. In line with previous simulations [10,28,29], we represent the actin network by a Voronoi diagram obtained from a set of random points. The thickness of the actin fibers is one lattice site, the typical compartment size is chosen to be ~ 100 nm. The resulting Voronoi diagram is then superimposed on the lattice of height variables. Next, we place the pinning sites, at randomly selected points along the edges of the Voronoi diagram. Once put in place, the pinning sites remain fixed, i.e. they cannot diffuse along the actin fibers. We assume that the effect of a pinning site is to locally push the membrane down, i.e. away from the reference height $h = 0$ toward negative values. We incorporate this effect into our simulations by fixing the height variable at each pinning site to a negative value $h_{\text{P}} < 0$ (for simplicity, the same value h_{P} is used for all the pinning sites). During the simulations, MC moves are thus *not* applied to pinning sites. Since Eq. (3) is a quadratic expansion, our analysis is restricted to small values of h_{P}. An extreme upper bound is the thickness of the hydration layer ~10 Å, which is the maximum distance the membrane can be pushed down, and where Eq. (3) certainly breaks down. For this reason, in the analysis to be presented, we restrict h_{P} to several Å at most.

We simulate a system of size $L = 400$ with lattice spacing $a = 2$ nm. For the presented results, we use a typical value $\beta\kappa = 70$ for the bending rigidity [27,30]. At room temperature, $T = 300$ K, this corresponds to $\kappa = 2.9 \times 10^{-19}$ Nm, which is close to the value used in Ref. [10]. Fig. 1 shows a snapshot of the membrane, color-coded according to the thermally averaged height (left) and curvature (right). In both cases 25% of the actin network is covered by pinning sites. Results are presented for two values of the strength of the harmonic potential, $\beta\alpha a^4 = 2$ (a) and $\beta\alpha a^4 = 4$ (b). The reported values of α in literature cover quite a wide range [20,26,27]. Our results use values comparable to $k_{\text{B}}T$ [20]. The deviation from the reference height at the pinning sites is set as $h_{\text{P}} = -6$ Å. The simulations ran for 4×10^6 sweeps, after having been equilibrated for 4×10^5 sweeps (each sweep is L^2 attempted MC moves).

As can be seen from height and curvature profiles, the actin pattern is clearly "pressed" onto the membrane. In particular, in the curvature snapshots, one can see that along the actin fibers an on-average positive curvature has been induced. This effect persists even at low fractions of pinning sites. To quantify this, we measured the cross correlation between the curvature snapshot $\mathbf{c}$ and the actin network $\mathbf{a}$ using the Pearson correlation coefficient (PCC). The advantage of using this quantity is that it can also be measured in experiments via fluorescence spectroscopy [10]. The PCC is defined as

$$\text{PCC} = \frac{\sum_i (\mathbf{c}_i - \bar{\mathbf{c}})(\mathbf{a}_i - \bar{\mathbf{a}})}{\sqrt{\sum_i (\mathbf{c}_i - \bar{\mathbf{c}})^2}\sqrt{\sum_i (\mathbf{a}_i - \bar{\mathbf{a}})^2}}, \tag{4}$$

where $\bar{\mathbf{c}}$ and $\bar{\mathbf{a}}$ are the mean "pixel" values of the curvature and actin images, and the sum over all lattice sites (for the actin image, $\mathbf{a}_i$ is zero everywhere except at sites that intersect with an edge of the Voronoi network, for which $\mathbf{a}_i = 1$). A positive (negative) value of PCC means that the curvature is positive (negative) on average underneath the actin fibers, while a value of zero means that there is no correlation.

Fig. 2(a) shows how PCC varies with the pinning fraction, using $h_{\text{P}} = -6$ Å. The PCC initially increases linearly from zero with the pinning fraction. In the linear regime, the pinning sites are isolated from each other. Each pinning site thus contributes to the PCC by the same amount, which explains the linear increase. At larger pinning fractions, the pinning sites are no longer isolated, i.e. their "regions of influence" begin to overlap, which explains the downward curvature in the data. The effect of the pinning height h_{P} is shown in Fig. 2(b) for a pinning fraction of 40%: By pushing the membrane further down, PCC increases.

For a solid-supported membrane with an actin network "on-top", these results suggest a mechanism for lateral domain formation in membranes that does not require any phase separation between lipids. The pinning sites along the actin fibers locally push the membrane down, leading to non-zero average curvature below the fibers. Consider now a lipid mixture, with one of the lipid species preferring regions of, say, positive curvature (the coupling between membrane composition