Domain Formation in the Plasma Membrane: Roles of Nonequilibrium Lipid Transport and Membrane Proteins

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Compositional lipid domains (''lipid rafts'') in plasma membranes are believed to be important components of many cellular processes. The mechanisms by which cells regulate the sizes and lifetimes of these spatially extended domains are poorly understood at the moment. Here we show that the competition between phase separation in an immiscible lipid system and active cellular lipid transport processes naturally leads to the formation of such domains. Furthermore, we demonstrate that local interactions with immobile membrane proteins can spatially localize the rafts and lead to further clustering.

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The plasma membrane (PM) enveloping mammalian cells is a bilayer consisting primarily of several ($\sim 10^3$) types of lipids and proteins. Cholesterol has a higher affinity for saturated lipids, and is thought to participate in the formation of phase-separated ''liquid-ordered'' (LO) domains enriched in saturated lipids and cholesterol, embedded in a ''liquid-disordered'' (LD) matrix. *Lipid rafts* are LO compositional domains on the exoplasmic leaflet of the PM that are believed to participate in the control of transmembrane signal transduction, membrane trafficking, cytoskeletal composition, and viral budding [1–4]. While evidence for their presence in living cells is mostly indirect, the consensus is that the rafts have a typical radius $R \sim 20$–200 nm and that they are highly dynamic entities with estimated lifetimes ranging from $\sim 10^{-3}$ [5] to 10^2 s [6].

In comparison to *in vivo* cells, a simpler and more controlled way of studying LO domains of raft composition in bilayers exists *in vitro*, using giant unilamellar vesicles (GUVs) [7–10], supported bilayers [11,12], or freestanding bilayer films [13]. Many interesting observations of the phase separation and coexistence of LO/LD and other phases of lipid mixtures in GUVs have been made in recent years [7–9]. These studies have revealed the presence of LO domains which typically grow large enough to be observed using optical means in contrast to the much smaller rafts *in vivo*. It is precisely this discrepancy in the domain sizes between the *in vivo* and *in vitro* systems that we wish to address in this Letter.

We begin by noting that the relative simplicity of these *in vitro* systems, which is one of their most appealing features, is also their main drawback. For example, the interaction of lipids or cholesterol with membrane proteins [5,14] or the effects of cross-linking of proteins [15], both of which have been proposed to control raft formation and aggregation, are difficult to ascertain. Furthermore, the roles of more complex, nonequilibrium cellular influences cannot be easily assessed (see, e.g., Ref. [16] for a lucid discussion). A case in point is the exchange of lipids (henceforth called ''recycling'') between the two membrane leaflets [17] or with the interior of the cell [18,19]. Below, we argue that both nonequilibrium vesicular and nonvesicular lipid transport events between the cell interior and the membrane, in conjunction with phase separation driven by thermodynamics, conspire to produce rafts of finite size with a broad size distribution. We also demonstrate how protein clusters may spatially localize the rafts, as well as promote the formation of extended raft aggregates.

Model.—From the perspective of the exoplasmic leaflet, the combination of nonvesicular and vesicular lipid trafficking events effectively leads to a dynamic spatial rearrangement of the lipids while maintaining a relatively stable average composition across the cell membrane as a whole in steady state—see Fig. 1. Rather than trying to model all of the possible transport events explicitly, here we assume that they simply give rise to local fluctuations in the average lipid composition for patches of the PM whose linear dimensions are smaller than a characteristic length scale (''recycling length'') $\ell \lesssim L_{\mathrm{cell}}$, where L_{cell} denotes the linear dimension of a cell. That is, the lipid composition is nonconserved at spatial scales $\ll \ell$. This approximation provides us with a robust and physically based means to investigate the importance of cellular lipid transport processes on raft formation kinetics. As shown below, determining a precise value for ℓ is not critical as the raft properties are only weakly dependent on it.

In our approach, the ''impulses'' due to lipid trafficking events accumulate and conspire to provide an effective stochastic noise term for the evolution equation of the local

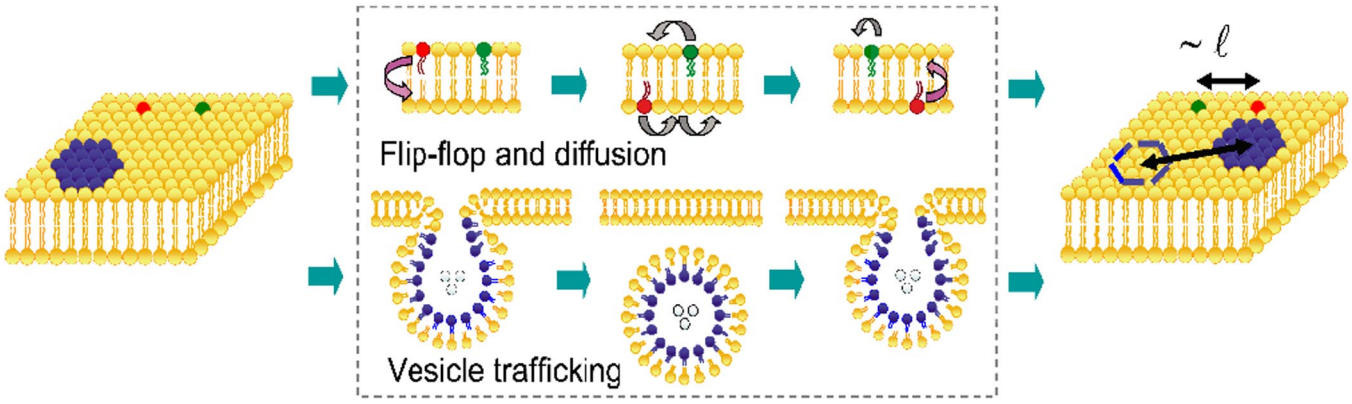

FIG. 1 (color online). Schematic presentation of the nonequilibrium lipid transport processes considered here. Both protein-facilitated flip-flop events (upper pathway) and vesicle trafficking events between the plasma membrane and internal organelles (lower pathway) lead to an effective rearrangement of the lipid composition over a scale $\sim \ell$ in the exoplasmic leaflet.

composition. This noise term has the following properties: (1) Near T_c, it is translationally invariant, white noise; (2) because of conservation of the lipid composition at large scales, the noise term integrates to zero; (3) the redistribution of lipids occurs at rate H and is restricted to spatial scales $\lesssim \ell$. We thus propose the following (dimensionless) stochastic, nonlinear diffusion equation for the local composition within the exoplasmic leaflet in the presence of recycling:

$$\frac{\partial c(\mathbf{r},\tau)}{\partial \tau} = \nabla^2[-\nabla^2 c - c + c^3 + g(\mathbf{r})] + \eta(\mathbf{r},\tau), \quad (1)$$

where η denotes a stochastic Gaussian noise with mean $\langle \eta \rangle = 0$ and correlator $\langle \eta(\mathbf{r},\tau_1)\eta(\mathbf{r}',\tau_2)\rangle = -H^2/(2\pi)\nabla^2 K_0(|\mathbf{r}-\mathbf{r}'|/\ell)\delta(\tau_1-\tau_2)$; here, $K_0(x)$ denotes a modified Bessel function of the second kind of the zeroth order. In Eq. (1), length and time are measured in units of the mean-field correlation length and characteristic relaxation time of the system, respectively, both of which diverge as the critical point is approached. Furthermore, $c = -1(+1)$ represents the LO raft (LD) phase. In the absence of the stochastic recycling term, the binary system equilibrates via phase-separation processes, wherein the raft domains continually coarsen to reduce excess interfacial energy. Equations of this kind, i.e., without the recycling term, have been employed in the past to investigate both phase behavior and phase-separation dynamics in spherical GUVs [20–22] and other geometries [23–25], with additional coupling terms between the local membrane curvature or deformation and $c(\mathbf{r},\tau)$. The term $g(\mathbf{r})$ describes a short-ranged interaction of lipids with membrane proteins such that $g = 0$ away from a protein, while $g > 0$ ($g < 0$) denoting an attractive (repulsive) protein-lipid raft interaction in the vicinity of a protein. In Fourier representation, the noise correlator becomes $\langle \eta(\mathbf{q},\tau_1)\eta(\mathbf{q}',\tau_2)\rangle = \frac{H^2q^2\ell^2}{1+q^2\ell^2}\delta(\mathbf{q}+\mathbf{q}')\delta(\tau_1-\tau_2)$. The composition is nonconserved at spatial scales $\lesssim \ell$ and asymptotically conserved with an effective "temperature" $T_{\text{eff}} = H^2\ell^2/2$. Furthermore, by equating the fluctuations in the average composition within a region of size $\ll \ell^2$ due to recycling to those in a discrete lipid system where lipids are removed or added to the leaflet at rate p, it can be shown that p is related to H via $p = 3H^2D(T_c - T)^2/(4A_{\text{lipid}}T_c^2)$ [26]. Here D and A_{lipid} denote the lipid diffusivity and area per lipid, respectively.

Analytical arguments.—In the limit $\ell \to \infty$, the noise correlator becomes $\langle \eta(\mathbf{q},\tau_1)\eta(\mathbf{q}',\tau_2)\rangle = H^2\delta(\mathbf{q}+\mathbf{q}')\delta(\tau_1-\tau_2)(1-\delta_{\mathbf{q},0})$, where the term $(1-\delta_{\mathbf{q},0})$ ensures that the average composition remains constant globally. If we now relax this constraint, due to the local conservation law inherent in Eq. (1) and the nonconserved nature of the noise term, the Fourier component $\hat{c}(\mathbf{q}=\mathbf{0},\tau)$ exhibits a temporal random walk. Consequently, $\lim_{\tau\to\infty}\langle|\hat{c}(\mathbf{q})|^2\rangle \propto \tau \to \infty$ as $\mathbf{q} \to 0$. This implies that the compositional domains become self-similar and display algebraic correlations [27], leading to a broad domain size distribution [28]. In the opposite limit $\ell \to 0$ with $H\ell = \text{const} \leq (H\ell)_{\text{crit}} = 2.1 \pm 0.4$, on the other hand, the model describes an equilibrium system which continuously coarsens. Thus, sufficiently rapid and/or long-ranged lipid exchange processes are required to counteract coarsening.

The physical picture that emerges is thus as follows. In the coarsening regime observed *in vitro*, that is, for $H\ell < (H\ell)_{\text{crit}}$, the system equilibrates via continuous coarsening of the raft domains, and stochastic fluctuations simply renormalize the surface tension [29]. In the noncoarsening regime $[H\ell > (H\ell)_{\text{crit}}]$, on the other hand, we expect any domain whose linear dimension $L \gg \ell$ to fragment as $T_{\text{eff}} > T_c$. Hence, phase separation at scales $\gg \ell$ is suppressed. For spatial scales below ℓ, however, we expect domains to coarsen and fragment in a highly dynamic fashion due to the competition between line tension (which promotes coarsening) and lipid trafficking events (which promote fragmentation), leading to a broad domain size distribution. These arguments are indeed confirmed by the simulations discussed next.

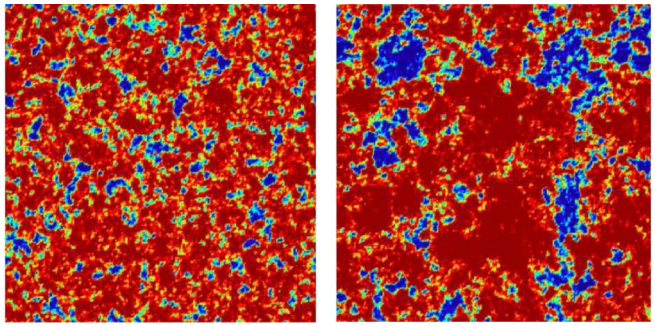

FIG. 2 (color online). Snapshots of domain structures in steady state under recycling with recycling length $\ell = 4$ (left) and $\ell = 1280$ (right). Notice the presence of finite-sized lipid rafts within the liquid-disordered phase.

Simulation results.—Finite differencing was adopted for both spatial and temporal derivatives in the discretized version of Eq. (1). The system size is $L \times L$, where $L = 256$ with periodic boundary conditions; the results discussed in this Letter did not display any detectable finite-size effects. The initial composition was taken to be homogenous. A dimensionless time step $\Delta\tau = 0.005$ and grid spacing $\Delta x = 1$ were employed in all simulations; we verified that the simulation results have converged with respect to both of these choices. Finally, the noise term was constructed in Fourier representation.

Let us now focus on the noncoarsening regime, i.e., $H\ell \gtrsim 2.1$. Figure 2 displays typical domain structures in steady state obtained by numerically solving Eq. (1) with two different recycling lengths ℓ for a fixed lipid raft area fraction $\phi = 0.15$ and recycling rate $H = 1.41$. The snapshots reveal two important characteristic features of the model. First, the raft domains have irregular shapes. Second, systems with larger ℓ sustain larger raft domains in the noncoarsening regime. Furthermore, careful observations of time-dependent domain morphologies reveal that small raft domains have a very short lifetime while larger domains persist much longer.

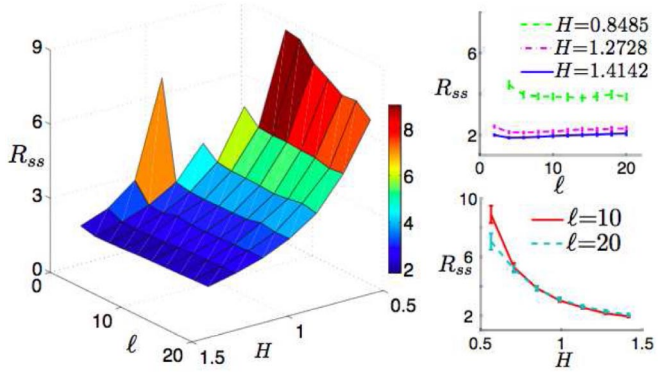

FIG. 3 (color online). Steady-state raft size vs H and ℓ. The two frames on the right display slices through the data at fixed H and ℓ, respectively.

To quantify these observations, we have computed the mean domain size $R(t) \equiv 2\phi L^2/L_B(t)$, where $L_B(t)$ denotes the total interfacial length in the system at time t, as a function of time and the domain area distribution in steady state. In the noncoarsening regime, $R(t)$ saturates at a value dependent on H and ℓ after a crossover period; that is, $\lim_{t\to\infty} R(t) \equiv R_{ss} = R_{ss}(H, \ell)$. The steady-state mean domain size R_{ss} is shown in Fig. 3 for several values of ℓ and H for $\phi = 0.15$. In general, decreasing H at fixed ℓ leads to a monotonically increasing R_{ss}, while varying ℓ at fixed H displays nonmonotonic behavior. In particular, for $0 \leq \ell < \ell^*$, the system coarsens indefinitely and thus $R_{ss} \to \infty$. Upon increasing ℓ beyond ℓ^*, R_{ss} saturates at a value dependent on H and ℓ and initially decreases as coarsening is suppressed. Upon further increasing ℓ, R_{ss} starts to increase as larger and larger domains appear in the system. Taking $D = 10^{-13}$ m^2/s [30], $T_c = 310$ K, $T_c - T = 1$ K, and $A_{\text{lipid}} = 10^{-18}$ m^2 [16], a recycling rate of $p = 0.2$ s^{-1} (2 s^{-1}) would lead to an average raft domain size $R_{ss} \approx 63$ nm (18 nm) for 36 nm $< \ell \lesssim 200$ nm, in good accord with experimental estimates [6].

The steady-state domain area distribution $P(A)$ in turn is displayed in Fig. 4 for the raft area fraction $\phi = 1/4$ and $H = 1.41$. This figure shows that ℓ essentially provides an infrared cutoff for $P(A)$; that is, the formation of domains larger than $\sim\ell^2$ is strongly suppressed. Note also that $P(A) \sim A^{-\alpha}$ for $1 < A \ll \ell^2$, where $\alpha \approx 1.6 \pm 0.1$. We have verified that α is independent of H when coarsening is suppressed. Thus, the scaling of the average domain area in terms of ℓ is given by $\langle A \rangle \sim \ell^{2(2-\alpha)}$, which implies that

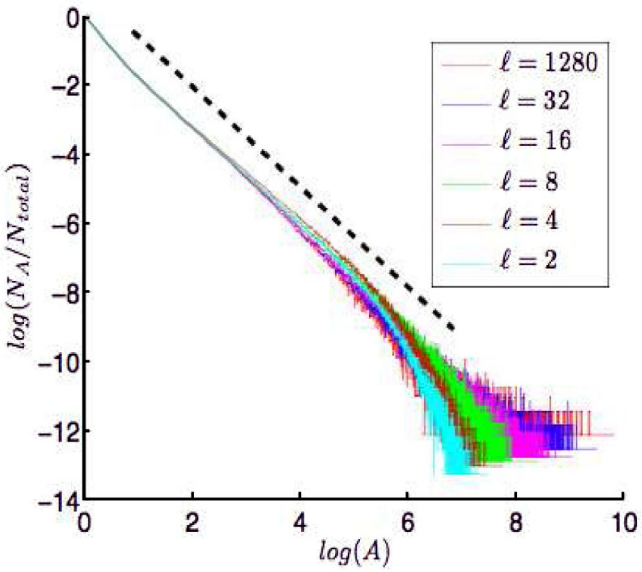

FIG. 4 (color online). Domain size distribution for lipid raft area fraction $\phi = 1/4$ and $H = 1.41$ for several values of the recycling length ℓ. N_A denotes the number of domains of size A. The dashed line has a slope of -1.6 and serves as a guide to the eye.

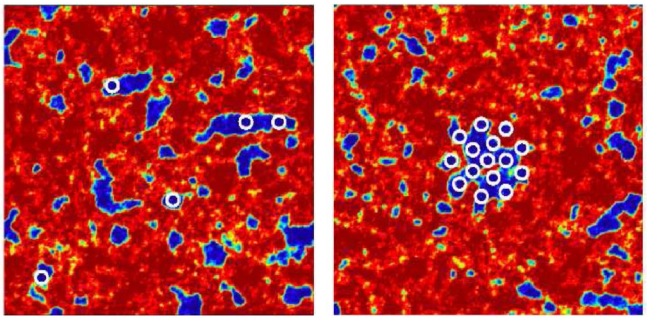

FIG. 5 (color online). Spatial localization and aggregation of lipid rafts due to membrane proteins (indicated by white circles). Left panel: raft distribution around five randomly distributed proteins. Right panel: extended raft domain in the presence a protein cluster.

$R_{ss} \sim \ell^{2-\alpha} = \ell^{0.4}$. Therefore, R_{ss} is only weakly dependent on ℓ.

Finally, the presence of immobile membrane proteins may give rise to relatively stable and long-lived rafts in their vicinity—see Fig. 5. Here, to illustrate this phenomenon, we have set the local protein-composition interaction to favor the lipid raft phase ($g = 0.5$ within a disk of radius 10 around a protein and $g = 0$ elsewhere); the other parameters employed were $\phi = 0.15$, $H = 0.707$, and $\ell = 8$. Upon letting the proteins cluster, an extended raft domain forms around the cluster, implying that the clustering of proteins may indeed contribute to the aggregation of rafts, as discussed in Refs. [1,3]; such clusters of proteins in cholesterol-rich domains have also been observed in recent experiments in the cytoplasmic leaflet of the PM [31].

Discussion.—We have argued that cellular and interlayer lipid transport coupled to a tendency to phase separate can give rise to a steady state in which the compositional domains attain a finite size in the exoplasmic leaflet of the PM. We predict that sufficiently rapid recycling is required to produce finite-sized raft domains with a broad size distribution [19]; we have also observed indications that the corresponding raft domain lifetime distribution is broad, offering a plausible explanation for the wide range of lifetimes reported in the literature [5,6]. Finally, we find that proteins may spatially localize the rafts, and the clustering of proteins may contribute to the aggregation of rafts as reviewed in Refs. [1,3]. In closing, our hope is that the work presented here will catalyze new theoretical and experimental investigations of the coupling between raft formation via domain growth and cellular lipid transport processes.

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