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Review

Formation and regulation of lipid microdomains in cell membranes: Theory, modeling, and speculation

Jun Fan [a], Maria Sammalkorpi [a,1], Mikko Haataja [b,*]

[a] Department of Mechanical and Aerospace Engineering, Princeton University, Princeton, NJ 08544, USA
[b] Department of Mechanical and Aerospace Engineering, The Princeton Institute for the Science and Technology of Materials (PRISM), The Program in Applied and Computational Mathematics (PACM), Princeton University, Princeton, NJ 08544, USA

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ABSTRACT

Compositional lipid microdomains ("lipid rafts") in plasma membranes are believed to be important components of many cellular processes. The biophysical mechanisms by which cells regulate the size, lifetime, and spatial localization of these domains are rather poorly understood at the moment. Over the years, experimental studies of raft formation have inspired several phenomenological theories and speculations incorporating a wide variety of thermodynamic assumptions regarding lipid–lipid and lipid–protein interactions, and the potential role of active cellular processes on membrane structure. Here we critically review and discuss these theories, models, and speculations, and present our view on future directions.

1. Introduction

The plasma membrane (PM) enveloping mammalian cells is a lipid bilayer composed primarily of thousands of types of lipids and membrane proteins. Besides being the physical boundary of cell, the PM also functions as a selective sieve through which matter and information pass. Although the initial hypothesis regarding the PM microstructure endowed it with properties of those corresponding to a two-dimensional, spatially homogeneous mixture of lipids and proteins (the so-called fluid mosaic model) [1], it is now well-established that the PM is far from featureless and has a "patchy" microstructure with spatially organized regions of structure and function, both in terms of lipids and proteins; for an overview, see Ref. [2]. In terms of lipids, the heterogeneous membrane is believed to consist of a mixture of a dispersed "lipid raft" phase, enriched in cholesterol, raft-associated proteins, and saturated lipids (such as sphingolipids), and the "non-raft" matrix phase [3,4]; in model membranes, the lipid raft phase is typically associated with a so-called "liquid-ordered" (l_o) phase, while the non-raft phase has been identified as the "liquid-disordered" (l_d) phase based on differences in the short-ranged lipid translational and conformational order. The rafts have been implicated in a number of important cellular processes including signal transduction, membrane trafficking, and protein sorting [4–6]; in addition, viral entry, assembly, and budding are also facilitated by the raft domains [7].

Although lipid rafts have not been directly observed in vivo, there exists compelling, albeit indirect, evidence to support the existence of the rafts [8–12]. The consensus is that the rafts in vivo are highly dynamic microdomains with characteristic linear dimensions in the order of 20–200 nm [8–12], with life times ranging from 10^{-2} s [13] to 10^{3} s [10]. These microdomains often contain proteins, and their spatial distribution may depend on the coupling of these proteins to the cytoskeleton [11,12,14]. Very recently, advances in experimental techniques have allowed for the first time non-invasive, in vivo imaging of single diffusing lipid molecules and proteins with unprecedented spatial resolution within the membrane [15]. The biophysical picture emerging from the experiments of Eggeling et al. is that of local "trapping" of raft-associated lipids (i.e., sphingolipids) in regions of $\sim$20 nm in linear dimension with typical trapping times of $\sim 10^{-2}$ s [15]; it is tempting to associate these regions with the raft domains. Such techniques are expected to yield in the near future detailed quantitative information about the dynamics of isolated tagged lipids and lipid clusters at the nanoscale in in vivo membranes.

In contrast to in vivo membranes, phase separation and l_o/l_d phase coexistence have been observed in model membranes, such as monolayers [16], bilayers on supported substrate [17], and giant unilamellar vesicles (GUVs) [16,18]. Most notably, in comparison to in vivo membranes, the raft domain size in model membranes

* Corresponding author. Fax: +1 609 258 5877.
E-mail address: mhaataja@princeton.edu (M. Haataja).
[1] Formerly M. Huhtala.

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is much larger, typically comparable to the vesicle size. This brings out the fundamental question, namely why do model membranes exhibit large, persistent l_o domains, while in living cells the microstructure displays much smaller spatial features and correspondingly shorter feature lifetimes? One important difference between model membrane systems and a living cell is that model membranes are not affected by active cellular processes, such as vesicle trafficking [19] or rapid lipid flip-flopping between two leaflets, which are critical in maintaining the asymmetric lipid distribution across the bilayer [20]. Furthermore, model membranes typically neither contain proteins nor are supported by a cytoskeleton. Therefore, the corresponding molecular interactions are also absent. Interestingly, it has been demonstrated in both model membrane systems [21] and plasma membrane spheres [22] that the cholera toxin B (CTB)-mediated cross-linking of the ganglioside GM_1, a minor membrane component, can induce the formation of large-scale ($\sim\mu$m) coexisting liquid phases from a "featureless", single-phase membrane. As discussed very recently by Lingwood et al. [23], these observations highlight the critical role that protein–lipid interactions may play in inducing the formation and clustering of raft domains in vivo. Furthermore, very recent work by Kaiser et al. [24] demonstrates that significant differences exist between the structure of the l_o domains observed in model membranes and the less-ordered structure of the raft domains found in multicomponent plasma membranes.

While the evidence for the existence of rafts in vivo is reasonably convincing in our view, the biophysical origins of the microdomain formation remain under lively debate. Resolving the raft formation mechanism in the PM is important, as it would provide novel insights into the cell's ability to regulate the size, lifetime, and spatial localization of these domains. However, the challenges in developing physically and biologically-based models for the membrane microstructure and its dynamics are multifaceted. First, molecular scale interactions between lipids and proteins are fundamentally responsible for microstructure formation, and the collective dynamics of the lipids and proteins, coupled to exterior solvent flow fields, facilitate large-scale reorganization of the membrane. Thus, while the raft domain organization fundamentally originates from molecular level phenomena, its effects are amplified at mesoscopic length and time scales where the rafts operate. Second, the spatially-extended nature of the membrane "patchiness" together with dynamic membrane processes, due to both thermodynamic fluctuations and non-equilibrium cellular events (such as endo- and exocytosis), make direct atomistic simulations of membrane processes of rather limited value due to stringent restrictions on accessible length and time scales. To date, the most extensive simulations with atomistic detail relevant for raft studies have been carried out by Niemelä et al. [25] and Risselada and Marrink [26]. The former study focused on the structural properties of the l_o and l_d phases in a ternary lipid system via molecular dynamics simulations extending up to 100 ns, while the latter employed a coarse-grained molecular model to elucidate the early-time formation kinetics of phase separated lipid domains in a ternary lipid system. From a biological perspective, these simulations focused on rather simple model membranes, and the biological and biophysical complexity of real membranes precludes extensive theoretical studies at the molecular scale due to computational limitations. Thus, the existing models for membrane microstructure formation are phenomenological by nature, and attempt to incorporate simple-yet-generic biophysical mechanisms for raft formation and regulation in the form of mathematical descriptions of the membrane patchiness. In more technical terms, these models are not tailored to resolve the properties of the system at the single molecule level; instead, they provide mathematically and computationally tractable, effective descriptions of the system behavior across mesoscopic length and time scales. The appealing feature of such models is that they provide a means to understand general membrane properties, among them membrane microstructure formation, for a broad class of cell membranes. It is also important to stress that despite their phenomenological nature, these models yield quantitative predictions, amenable to experimental verification (or, rather, falsification); we return to this point in the concluding remarks.

In this review, our goal is to critically discuss theoretical and computational approaches that have been employed to study raft domain structure and dynamics. The focus is on phenomenological mesoscale models, whose predictions and properties will be confronted with existing experimental data. Where applicable, estimates will be given for the characteristic linear dimensions and lifetimes of the raft domains for representative model parameter values. In our view, once the general physical mechanisms governing raft formation have been established, more microscopic models can be employed to assess and quantify system-specific properties.

The rest of the paper is organized as follows: as will be discussed shortly, the existing mesoscale models for raft formation, shown schematically in Fig. 1, generally fall into two categories – those invoking thermodynamic equilibrium and those allowing for non-equilibrium effects. Hence, we will use this characteristic as the organizing principle, and first discuss the equilibrium models in Section 2, before turning to the non-equilibrium ones in Section 3. Finally, concluding remarks and future directions will be presented in Section 4.

2. Theoretical models for lipid microdomain formation: equilibrium phenomena

There are several theoretical scenarios, which argue that (1) the presence of lipid microdomains is an inherent property of the membrane in thermodynamic equilibrium, and (2) that the structure and dynamics of the microdomains are controlled by thermodynamic forces alone. We begin by discussing the most recently proposed scenario in this category. A possible explanation for the discrepancy between the experimental results in vitro and in vivo, i.e., the large-scale phase separation vs. nanoscale rafts, put forth by Veatch et al., is that the temperature of the in vivo system is above the critical temperature at which phase separation commences [27]. Very recent experiments on giant plasma membrane vesicles (GPMVs), which directly form from the components of a cell membrane and contain the PM lipids and proteins, indicate that the critical temperature for these GPMVs indeed is lower than the physiological temperature [27]. In this picture, raft domains are simply manifestations of transient compositional fluctuations driven by thermal noise, and the characteristic domain size is related to the static correlation length ξ in the system, which diverges as the critical point is approached. A schematic cartoon of this scenario is presented in Fig. 1A. Furthermore, by extrapolating the measured correlation length value to higher temperatures, an estimate for the typical domain size of $L \approx 20$ nm at 37 °C was presented [27], well in line with the consensus domain size scale for rafts. On the other hand, as diffusive lipid transport facilitates the formation and dissociation of these nanoscale domains in thermal equilibrium, their typical life times τ can be estimated from $\tau \approx L^2/D \approx 10^{-4}$ s, where $D = 10^{-12}$ m^2/s denotes the typical lipid diffusivity. We note that the magnitude of τ is rather small in comparison with the reported experimental estimates for raft lifetimes, and it is difficult to rationalize how such small domains can be spatially localized for much longer than a fraction of a millisecond within this picture. It should also be pointed out that, in contrast to GPMVs, chemically untreated plasma membrane preparations from the same cell source do not phase separate into coexisting l_o/l_d domains at comparable temperatures [24]; the underlying

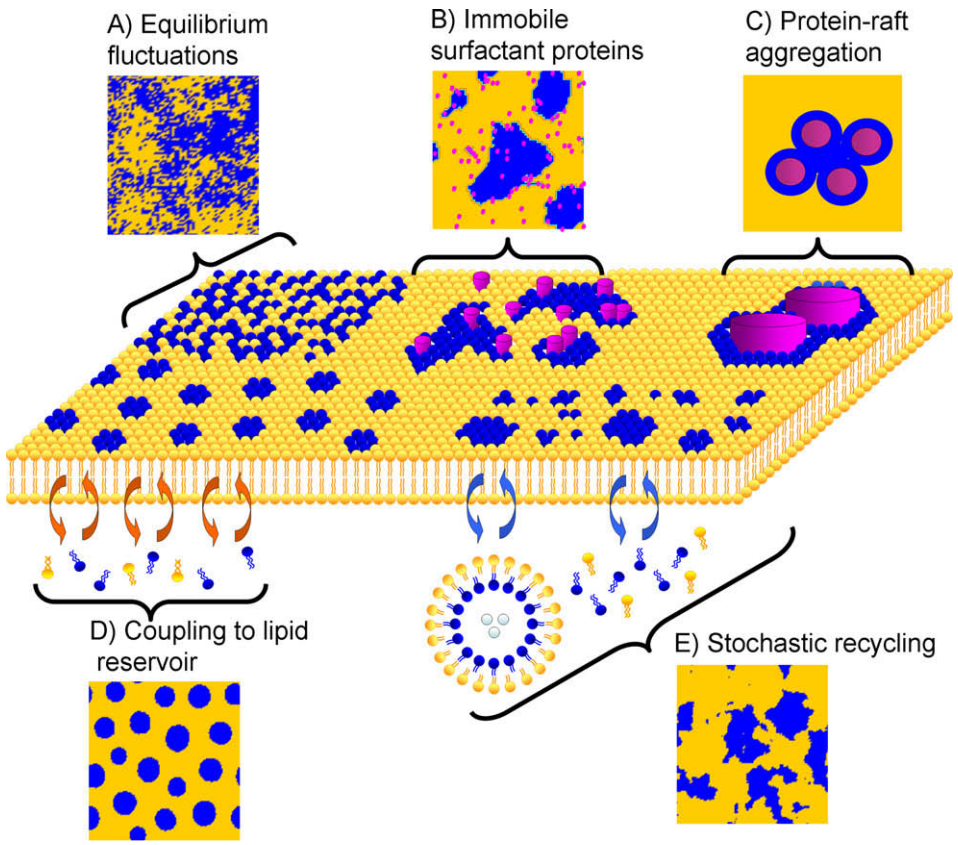

Fig. 1. The cartoon summarizes the existing theoretical raft formation mechanisms. The top row presents the three theoretical scenarios, which base the presence of lipid microdomains on thermal equilibrium processes and thermodynamic forces, while the bottom row displays two non-equilibrium scenarios in which active lipid recycling is the key process to controlling domain formation. More specifically, scenario A corresponds to the plasma membrane exhibiting thermal equilibrium fluctuations near the critical temperature, which results in the formation of small, short lifetime raft domains (blue patches) and non-raft domains (yellow patches). In scenario B, immobile surfactant membrane proteins (purple cylinders) pin compositional lipid interfaces and induce finite-sized rafts. In scenario C, the protein-raft interaction stabilizes rafts. Here, the protein-raft clusters (purple cylinders surrounded by blue raft phase) result from short range attraction, which induces clustering, and long range repulsion, which prevents the formation of large clusters. Scenario D in turn corresponds to a situation in which the membrane is coupled to a lipid reservoir, and this coupling suppresses the formation of large domains. Finally, in scenario E, vesicular and non-vesicular lipid recycling suppresses macroscopic phase separation and results in micro-scale rafts with a broad size distribution. The snapshots, except for C which is a schematic cartoon, correspond to simulations using extensions of the model introduced in Ref. [51].

reasons for this discrepancy are not completely understood at the moment. Care must therefore be exercised in assessing the biological relevance of these critical fluctuations observed in GPMVs. Additionally, as will be discussed in more detail below, the attractive interaction of these raft domains with mobile raft-associated membrane proteins could lead to a large-scale clustering of the proteins and the raft-associated lipids. Furthermore, GPMVs lack a cytoskeleton and active cellular processes, and thus cannot fully represent real cells. While appealing, these observations need further experimental verification with intact cells to verify the speculation of equilibrium fluctuations as the origins of microdomain formation.

Following a somewhat different line of reasoning, Yethiraj and Weisshaar [28] invoked the presence of membrane proteins as a possible means to control the size and spatial localization of raft domains; such a possibility has also been suggested by Swamy et al. [29] based on experimental evidence. In Yethiraj and Weisshaar's model, the membrane contains immobile proteins, which have a favorable energetic interaction with the compositional domain walls, and hence tend to locally pin these interfaces. A cartoon representation of this model for microdomain formation is presented in Fig. 1B. In this model, at sufficiently high temperatures, lipid phase separation occurs only at the microscale, while a decrease in temperature eventually will induce macroscopic phase separation. Put in another way, the presence of these "surfactant" proteins effectively reduces the critical temperature of the system, and it was hypothesized that although phase separation may occur in pure immiscible lipid system at physiological temperatures (such as the in vitro model membrane systems), it does not occur in vivo due to the presence of these immobile membrane proteins. Furthermore, the raft domain size is determined by the interplay between line tension (promoting formation of large domains) and protein areal density and interaction strength with compositional domain walls (increase in both tends to lead to a decrease in raft domain size). The characteristic raft domain size can be estimated by balancing the line tension force for a circular domain of radius L with the pinning force due to the proteins with the result $L \approx \sigma\xi L_p/\epsilon$. Here, σ denotes the line tension between the compositional domains, L_p denotes the average separation between the proteins, and ϵ denotes the interaction strength between the proteins and the compositional interface. Upon employing physically reasonable values $\sigma \approx 10^{-12}$ N, $\xi \approx 5$ nm, and $\epsilon \approx k_B T$, we obtain $L \approx 5L_p$, that is, the typical raft size is expected to be on the order of the average separation between the proteins. Hence, a rather high areal density ($\sim 10^4/\mu m^2$) of immobile surfactant proteins is required to sustain stable raft domains at the nanoscale within this scenario. With regard to dynamics, Fan et al. have recently shown that once formed, the nanoscale rafts persist over macroscopic time scales due to pinning of compositional domain boundaries by the immobile membrane proteins [30]. On the other hand, by exploiting results from other studies with a similar model [31], it can be argued that allowing the surfactant proteins to

diffuse would lead to both a smaller decrease in the critical temperature and faster relaxation times for raft domains. Therefore, on the whole, this scenario is appealing, but there remain questions to be answered regarding the actual decrease in the critical temperature due to interactions with mobile proteins, and the required areal density of such proteins for the stabilization of nanoscale raft domains.

Yet another plausible approach to explain raft formation is to invoke protein–lipid interactions as the key factor which may stabilize the highly dynamic rafts, or even induce aggregation of larger lipid rafts [6,13,32,33]. Membrane protein aggregation has been reported for various types of proteins [34–39] and is thought to result from a competition between a short range attraction, such as homophilic interaction [38] or depletion effect [39], and a long range repulsion, which may be due to steric [38] or electrostatic [39] repulsion. The speculation is that these proteins are accompanied by a band of lipids surrounding them [6,13,32,33], and the aggregation of proteins would result in a concomitant lipid aggregate; see Fig. 1C. In this scenario, the spatial localization and size of the raft would thus be dictated by the proteins. To date, there have been few quantitative theoretical studies of protein aggregation, or its relation to lipid microdomain formation. In Ref. [38], a model which phenomenologically incorporates both short-ranged attractive and long-ranged repulsive interactions was shown to provide a quantitative match with experiments regarding protein aggregate size and aggregation dynamics. Along the same lines, a similar model was proposed in Refs. [40,41] to account for the aggregation of mobile proteins into finite-sized clusters. The implication from this line of reasoning is that the raft domains have effectively been demoted to the role of passive spectators, and are at the mercy of the proteins with which they are associated. In reality, the state of affairs is likely to be more complicated than this. To this end, consider an isolated protein, which attracts a band of raft-associated lipids due to, e.g., hydrophobic mismatch or membrane curvature effects, and further assume that the system is at a temperature above its critical temperature at which spontaneous lipid phase separation takes place. Physically, the width of the band in this case is proportional to the spatial correlation length of the system, which diverges as the temperature approaches the critical point. Furthermore, there exists an attractive interaction between the proteins, mediated by the lipids, which extends across the width of the lipid band; that is, two proteins whose bands overlap spatially attract each other [42]. Therefore, for sufficiently large correlation lengths (larger than the scale over which, say, repulsive steric interactions operate) and large interaction energies, the proteins would aggregate into larger and larger mesoscale clusters. The implication of this line of reasoning is that local variations in the spatial correlation length via, e.g., variations in the local lipid composition, could lead to spatially targeted protein (and thus lipid) aggregation. To test this idea of protein mediated lipid raft aggregation, one simply needs to vary the temperature while monitoring the clustering of proteins. In particular, at lower temperatures (where the correlation length increases rapidly), clustering of proteins should be readily observed.

Similar line of reasoning can also be employed to qualitatively understand how CTB-mediated cross-linking of the ganglioside GM_1 can induce the formation of large-scale ($\sim\mu m$) coexisting liquid phases from a single-phase membrane in both model membrane systems [21] and plasma membrane spheres [22]. Given that the CTB particles have a favorable interaction with raft forming lipids (i.e., GM_1), the addition of CTB to the PM will in general promote the formation of the raft phase. More quantitatively, we expect that the addition of CTB will both shift the phase boundaries at fixed temperature as well as shift the critical temperature to a higher value at fixed lipid concentration. In simple mean-field theory, the shift in the critical temperature can be estimated to be proportional to $\varepsilon^2\rho_{CTB}$, where ε denotes the protein-lipid interaction strength and ρ_{CTB} denotes the areal CTB density. While these arguments lead to qualitative predictions consistent with experimental observations, more quantitative studies of protein–lipid and lipid–lipid interactions are required to provide a better understanding of the thermodynamics of complex, multicomponent lipid/protein systems.

Finally, the fourth type of model relates the raft formation and stabilization in the PM to membrane curvature effects. In model membranes, it has been observed that phase separating compositional domains are often associated with regions of spatially-varying membrane curvature, due to differences in the preferred curvature of the different lipids forming the multicomponent membrane. Furthermore, in some cases, the domains form regular droplet phases while in others the domains coarsen before budding off [43]. Physically, both the coarsening and budding processes are driven by the line tension, which exists between the compositional domains, while they are counteracted by membrane tension, which tries to minimize the overall membrane area. There are two important characteristic lengths associated with the system, namely the so-called "invagination length" [44–46] $\xi_L = \kappa/\sigma$, where $\kappa \approx 10^{-19}$ J denotes the bending rigidity and σ denotes the line tension, and $\xi_M = (\kappa/\lambda)^{1/2}$, where $\lambda \approx 10^{-7}$ N/m denotes the (lateral) membrane tension. Physically, away from the critical point, we expect the budding process to occur once the compositional domain size exceeds $\xi_L \approx 100$ nm, while compositional patterning is expected to occur at the scale given by $\xi_M \approx 1$ μm; compositional variations at length scales smaller than ξ_M lead to a decrease in the total energy of the system, while such variations at larger scales are energetically too costly [47]. Again, the characteristic domain lifetime can be estimated from $\tau \approx \xi_M{}^2/D \approx 1$ s. It is noteworthy that ξ_L increases rapidly as the critical point is approached, while ξ_M is relatively insensitive to temperature variations. Therefore, we expect compositional pattern formation to take place in the vicinity of the critical point with a characteristic length scale given by ξ_M, whereas away from the critical point, the domains coarsen until budding off of the membrane at size scale given by ξ_L. It is noteworthy that both characteristic length scales ξ_M and ξ_L are on the order of 100 nm or greater, and thus significantly larger than the putative raft domains in vivo. It is thus reasonable to assert that membrane curvature effects play only a secondary role in stabilizing the raft domains at the nanoscale.

3. Theoretical models for lipid microdomain formation: non-equilibrium phenomena

Having reviewed theoretical scenarios for raft formation in thermal equilibrium, we now turn to a discussion of theoretical models which invoke non-equilibrium cellular processes to explain the formation and regulation of lipid microdomains. The fundamental hypothesis underlying all of these models is that cells maintain a non-equilibrium lipid composition via lipid recycling to and from the membrane either by coupling to a lipid reservoir (see Fig. 1D) or by vesicular and non-vesicular lipid transport events (see Fig. 1E); the main differences reside in the details of how these active lipid transport processes are modeled. Within this general framework, it is the interplay between active lipid transport processes and lipid thermodynamics, which endows the raft domains with their mechanical and biophysical properties.

We begin by noting that considering the membrane as a mixture of multiple species, which phase separate at temperatures below the critical temperature, several non-equilibrium phenomena-based theories for microdomain formation in PM have been formulated. More precisely, the competition between phase separation and lipid recycling in a multiple species system, at a temperature

below the critical temperature, has been found to dominate the microstructure formation in the models of Refs. [48–53]. In Ref. [48], Foret proposed a model in which the main contributors to microdomain formation were lipid phase separation and coupling to a lipid reservoir, which introduces a non-equilibrium factor to the model. In this model, schematically shown in Fig. 1D, lipids are locally "generated" at a constant rate everywhere along the membrane and removed at a rate proportional to their local concentration. The former process, combined with the tendency of the lipids to phase separate in equilibrium, creates large raft domains while the latter one suppresses the domains from growing. Consequently, finite-sized rafts emerge, and the raft size depends on the competition between thermodynamics, diffusion, and the lipid exchange rate. The appealing feature of the model is that it gives rise to finite-sized raft domains over a broad parameter range. However, there are two issues with the model predictions: First, the model produces rafts which are of the same characteristic size throughout the system, while in vivo the size distribution is broad [8–12]. Second, once the rafts form, they are static, whereas in living cells the rafts are dynamic entities with different life spans expected for different raft sizes.

Following a similar line of reasoning, Gómez et al. [49] proposed a more detailed model for a mixture of saturated and unsaturated lipids, as well as cholesterol. The model, shown schematically in Fig. 1D, is based on the idea that the raft distribution and dynamics could be controlled by spatial variations in cholesterol alone, as lipid rafts are enriched in saturated lipids and cholesterol. Therefore, only cholesterol is recycled in this model, and the recycling is performed in a similar manner as in Foret's model: cholesterol molecules are constantly "incorporated" into the membrane everywhere with a constant rate, and released back in to the reservoir at a rate proportional to the local cholesterol concentration. The results are also qualitatively similar to Foret's model: the model predicts uniformly distributed, stationary rafts of the same characteristic size. As the authors noted, however, inclusion of thermal fluctuations in the model equations results in fluctuating raft domains with a narrow size distribution. Typical raft domain sizes were reported to be on the order of 40–100 nm (and thus consistent with experimental estimates) when recycling rates of 1–400/s were imposed. Finally, it is interesting to note that although Foret's and Gómez et al.'s models were framed as having incorporated non-equilibrium phenomena, they reduce to an effective equilibrium model for a block copolymer system [54], and the resulting raft size can be identified with the effective block size in thermal equilibrium.

Currently, two fundamentally non-equilibrium models have been proposed: first, Turner et al. formulated a model to describe the distribution of rafts with different sizes in the cell membrane via a master equation approach [50]. The main assumption of this model is that lipid patches with raft domain composition are constantly transported into the cell membrane. The smaller raft domains can associate into larger ones, and larger domains can either dissociate into smaller ones or be removed from the membrane altogether. The rates of raft generation, removal, association, and dissociation determine the size distribution of rafts in the system. The resulting size distribution is broad, consistent with experimental observations [8–12]. Furthermore, in the physically realistic limit of a large line tension between the raft and non-raft phases, small domains form and disappear with a characteristic diffusion time scale $\sim 10^{-5}$ s due to domain coalescence events, while large domains disappear with a rate dictated by the recycling rate. Upon choosing a recycling rate in the range 10^{-2}–10^{2}/s, the average raft domain size was found to vary from approximately 8 nm to 50 nm, implying that the characteristic time scale for the disappearance of large domains is between 0.01 s and 100 s for this range of recycling rates. Thus, the characteristic domain size is consistent with experimental estimates, and the raft domain lifetimes in this model span many orders of magnitude, again in agreement with experiments. On the other hand, this model is not capable of predicting the morphology of the rafts, and cannot assess the role of proteins on lateral organization of the membrane.

More recently, Fan et al. proposed a spatially-extended continuum model [51], where the competition between phase separation driven domain coarsening and recycling due to vesicular and non-vesicular lipid trafficking events results in a broad raft domain size distribution. This model also introduced the so-called "recycling length", which is the typical distance over which lipid re-distribution takes place along the membrane. For a typical recycling rate 1/s and recycling length 1 μm, the characteristic domain size was found to be on the order of 40 nm. Furthermore, the simulated rafts were found to be highly dynamic entities with varying life times with larger rafts persisting longer than smaller rafts, which is consistent with experimental results [10,13]. The models of Refs. [50,51] are presented by the cartoon in Fig. 1E. The non-equilibrium model of Ref. [51] was further expanded in Ref. [52] to incorporate the effects of compartmentalization on raft formation and dynamics with the outcome that lipid diffusion barriers can fragment raft aggregates while immobile membrane proteins may also affect the spatial distribution of the rafts.

Finally, it should also be mentioned that Gheber and Edidin [53] studied membrane patchiness in a single-component lipid system in the presence of lipid recycling, which adds and removes lipids to and from the membrane constantly in time and stochastically in space. The lipids in their system form raft-like patches due to the presence of lateral diffusion barriers, and the compartment size directly controls the raft domain size while lipid diffusion across the barrier controls the raft lifetime. It is interesting to note that while Gheber and Edidin conclude that diffusion barriers are required for microdomain formation in single-component systems, Fan et al. have very recently shown [30] that microdomain formation does occur for fast enough recycling also in the absence of diffusion barriers in multicomponent systems.

4. Concluding remarks and future perspectives

In this review, we have critically discussed existing theoretical models developed to explain lipid microdomain formation processes in the PM. More specifically, we have focused on effective mesoscale models, which can describe microstructural organization of the PM in terms of the local lipid content across several length and time scales, ranging from the molecular up to the cellular scales; while informative, current state-of-the-art molecular simulations are unfortunately too restricted in both accessible spatial and temporal scales to be of practical relevance in deciphering the origins of the collective processes controlling the formation of lipid microdomains. Both thermal equilibrium and non-equilibrium models explaining the formation of rafts have been proposed, and there are also variations as to whether the lipid mixture is assumed to be below or above its critical temperature. The common feature amongst the models is that they all give rise to finite-sized raft domains, and the key differences between the models are in the details of how these finite-sized domains are sustained. Generally speaking, models invoking thermal equilibrium are appealing as they imply that the cell may be able to take advantage of the raft domains "for free" as transient, fluctuating domains would always be present at physiologically relevant temperatures. However, it is difficult to envision how raft domains could be spatially targeted within this framework, apart from condensation around immobile membrane proteins. On the other hand, models based on non-equilibrium lipid transport suggest that cells may actively target the spatial extent and localization of the raft domains by anisotropic

and/or inhomogeneous lipid trafficking. This concept of active targeting brings forth interesting prospects of externally manipulating the cellular processes dependent on the presence of rafts.

The existing models for explaining PM lipid microdomain formation incorporate processes and mechanisms that have a varying physical and biological basis, and the fundamental question that must be answered experimentally is, which mechanism(s) is (are) the operational ones in intact cells? The current factors that have been employed to differentiate between the performances of the currently existing models are the capability of the model to reproduce the experimentally reported (I) characteristic raft domain size, (II) broad domain size distribution, and (III) broad lifetime distribution. Furthermore, recent experiments demonstrating that the CTB-mediated cross-linking of the ganglioside GM_1 can induce the formation of large-scale ($\sim\mu m$) coexisting liquid phases from a single-phase membrane in both model membrane systems [21] and plasma membrane spheres [22] suggest that lipid–protein interactions may play a crucial role in the formation and regulation of rafts. The issue here is, however, that the existing experimental evidence is indirect and it is not always clear how the measured quantity relates to the fundamental properties of the microdomains. For example, extracting the raft size from the fluctuation spectra of trapped particles in an optical tweezer is based on assumptions relating the size of a raft to its diffusivity within the membrane. More generally speaking, tagged proteins or lipids are almost exclusively employed as reporters of the underlying structure and dynamics of the membrane, and it is critical to relate the dynamics of these reporter particles to those of the membrane. In this regard, microscopic simulations can be exploited to establish these "missing" links, and thus provide means to interpret experimental data more quantitatively.

With regard to extracting the formation mechanism of microdomains in vivo from experiments, we believe that the required experimental data will be available very soon with the recent emergence of far-field fluorescence techniques, with acronyms such as STORM, F-PALM, and STED, with which single or multiple diffusing lipid molecules or proteins can be tracked in a real cell membrane under in vivo conditions with unprecedented spatial resolution; for a very recent review of the topic, see Ref. [55]. For example, stimulated emission depletion far-field fluorescence nanoscopy (STED) allows examination of details with a spatial resolution of 30 nm and a temporal resolution of less than millisecond [15]. It is conceivable that such techniques will yield, in the near future, detailed quantitative information about the dynamics of isolated tagged lipids and collective dynamics of lipid clusters at the nanoscale in in vivo membranes. Progress in this direction would be very timely and fruitful, as it has been very recently demonstrated by Fan et al. [30] that the existing theoretical models display quantitatively distinct signatures in their collective dynamic behavior of lipid clusters. More specifically, Fan et al. have developed a method based on combining the spatial and temporal fluctuation spectra of multiple tagged lipids, which is able to differentiate between the theoretical mechanisms discussed in this review, and thus providing the means to resolve the long-standing issue of raft formation and regulation mechanism.

Once the overall controlling mechanism for the membrane microdomain formation has been established, the next step from the modeling point of view involves unraveling the system-specific, molecular scale details of the dynamics and structure of the rafts. In this endeavor, more microscopic methods than the ones reviewed here, come into play. For example, a very recent MD simulation concludes that the diffusional dynamics of lipids at the molecular level is strongly coupled and displays collective "flows" [56]; such collective dynamics will affect many molecular mechanisms of cellular processes, including membrane fusion and pore formation, as well as raft dynamics. More broadly speaking, what is required to address biophysically and biologically relevant questions is the development of new theoretical and computational strategies for studying dynamical phenomena in multicomponent membranes, including active, non-equilibrium cellular processes, that span large length and time scales, and the interaction of membrane proteins and their environment. On the long term, it is expected that such an endeavor would facilitate the emergence of an improved, quantitative understanding of membrane structure and dynamics over multiple length and time scales.

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