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Review

Physical mechanisms of micro- and nanodomain formation in multicomponent lipid membranes

Friederike Schmid

Institute of Physics, Johannes Gutenberg University, 55099 Mainz, Germany

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ABSTRACT

This article summarizes a variety of physical mechanisms proposed in the literature, which can generate micro- and nanodomains in multicomponent lipid bilayers and biomembranes. It mainly focusses on lipid-driven mechanisms that do not involve direct protein-protein interactions. Specifically, it considers (i) equilibrium mechanisms based on lipid-lipid phase separation such as critical cluster formation close to critical points, and multiple domain formation in curved geometries, (ii) equilibrium mechanisms that stabilize two-dimensional microemulsions, such as the effect of linactants and the effect of curvature-composition coupling in bilayers and monolayers, and (iii) non-equilibrium mechanisms induced by the interaction of a biomembrane with the cellular environment, such as membrane recycling and the pinning effects of the cytoplasm. Theoretical predictions are discussed together with simulations and experiments. The presentation is guided by the theory of phase transitions and critical phenomena, and the appendix summarizes the mathematical background in a concise way within the framework of the Ginzburg-Landau theory. This article is part of a Special Issue entitled: Lipid order/lipid defects and lipid-control of protein activity edited by Dirk Schneider.

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1. Introduction

Ever since Simons and Ikonen first coined the term "rafts" to describe certain lateral inhomogeneities in lipid membranes [1], the question whether rafts exist in vivo and why they form has been the subject of a lively and often controversial debate in the biophysics community [2–17]. Even the meaning of the word "raft" has long remained vague, until in 2006 the participants of a Keystone Symposium on Lipid Rafts and Cell function have formulated a "consensus definition" [18]: *"Membrane rafts are small (10–200 nm), heterogeneous, highly dynamic, sterol- and sphingolipid-enriched domains that compartmentalize cellular processes. Small rafts can sometimes be stabilized to form larger platforms through protein-protein and protein-lipid interactions."* This description now serves as a reference for the identification of raft-like structures, even though not all structures that have been associated with rafts fulfill all criteria. For example, "rafts" do not always contain sphingolipids [19,20].

The raft concept is supported by increasing experimental evidence – e.g., from tracking of lipid diffusion [21], or from super-resolution microscopy [22–24] – that lipid membranes are heterogeneous on a nanometer scale [25]. Nano- and microdomains in membranes have been observed in a large range of organisms, including prokaryotic cells [19,20], single-cell organisms [26], and plant cells [27]. Motivated by the observation that sphingolipids (sphingomyelins, glycosphingolipids, ceramides) – which participate in cellular signalling – tend to enrich in raft domains [28–30], it has been speculated that rafts may serve a biological purpose in cell-cell recognition and signal transduction [28,31]. On the other hand, raft-like structures were also observed in prokaryotic membranes which do not contain sphingolipids [19].

One class of lipids which seems to be very prominently involved in raft formation is the sterol class [32,33]. With few exceptions [34], higher sterols such as cholesterol and ergosterol are typically enriched in rafts. Recently, LaRocca et al. performed a systematic substitution study on prokaryotic membranes, and reported that domain formation was suppressed if cholesterol was depleted or substituted with the wrong sterol [20]. Since the cholesterol molecule with its rigid structure has an ordering effect on the acyl chains of the surrounding lipids [35–37], the apparently dominant role of cholesterol suggests an interpretation of rafts in terms of a local nucleation of ordered cholesterol-rich "liquid ordered" (*lo*) domains in a "liquid disordered" (*ld*) sea of cholesterol-poor phase [33]. "Inverse domains" have also been observed [38], where highly disordered domains with a high content of polyunsaturated fatty acids segregate from a cholesterol-rich environment.

The idea that lipid-cholesterol bilayers may demix into "liquid ordered" and "liquid disordered" states had been put forward already in 1987 by Ipsen et al. [39] based on experimental data by Vist et al. [40] (see Section 2). In contrast to equilibrium phase separated domains, however, lipid rafts are small and transient structures. The question why such domains should form has intrigued scientists for some time. In vivo, membranes are filled with proteins and lipid domains are typically correlated with protein clusters. Therefore, it has been argued that the observed membrane heterogeneities could be driven by protein-protein interactions alone [41]. If "raft proteins" associate with "raft lipids" such as cholesterol, it seems conceivable that a protein cluster could nucleate a liquid ordered *lo* lipid domain in its vicinity. On the other hand, recent experiments by Sevcsik et al. [17] have shown that immobilized lipid anchored raft proteins do not seem to have a measurable effect on the membrane environment. This suggests that the formation of lipid domains is primarily driven by the lipids and not by the membrane proteins.

Indeed, nanostructures and microstructures are also observed in pure model lipid bilayers. One prominent example in one-component bilayers is the modulated "ripple phase" $P_{\beta'}$, which generically emerges in the transition region between the fluid phase L_α and a tilted gel phase $L_{\beta'}$ [42–47] (see Section 2). Here and throughout, the term "modulated" refers to periodic or quasi-periodic patterns – in this case striped patterns with periodicities of the order of 10 nm. In multicomponent lipid bilayers, experimental evidence for the existence of nanoscopic cholesterol-rich domains has been provided by Förster resonance electron transfer (FRET) and electron spin resonance (ESR) experiments [48,49], neutron scattering methods [33,50-53], interferometric scattering microscopy [24], and atomic force microscopy [54,55]. Micron-size domain patterns were observed in multicomponent giant unilamellar vesicles [56–64]. We will discuss these observations in more detail further below.

On the side of theoretical membrane science, a number of mechanisms have been proposed that can generate micro- and nanostructures in lipid bilayers. The purpose of the present paper is to give an overview over these mechanisms and to explain and discuss some prominent examples. The review mainly focusses on lipid driven mechanisms that do not involve direct protein-protein interactions. Since virtually all of these mechanisms are based on the phase behavior of membranes in one way or another, the discussion will be guided by the theory of phase transitions and critical phenomena. To make it accessible for a general audience while still giving mathematical background, the mathematical aspects of the discussion are presented separately in the appendix.

This overview is far from complete. For further information on different aspects of the topic, the reader is referred to other recent reviews, e.g. by Fan et al. [65] (focussing on nonequilibrium mechanisms), Palmieri et al. [66] (focussing on mechanisms based on line active molecules), Lipowsky [67] (focussing on membrane shapes and membrane remodeling), and Komura and Andelman [68] (focussing on phase separation, phase separation dynamics and on microemulsions). In particular, the present article only touches on the issue of multiscale computer simulations of heterogeneous lipid bilayers, which is a challenge in itself and has been discussed in several recent review articles [69–73].

The remaining article is organized as follows: In the next section, Section 2, we briefly discuss the phase behavior of lipid membranes, focussing on lipid-lipid phase separation. Section 3 considers mechanisms of domain formation in pure membranes which are based on incomplete phase separation. This includes the formation of critical clusters just above a critical demixing point (Section 3.1) as well as the appearance of multidomain structures on curved vesicles that emerge in order to minimize the total bending energy (Section 3.2). In Section 4, we review physical mechanisms that generate equilibrium two-dimensional microemulsions, e.g., due to line active agents (Section 4.1), or due to lipid curvature induced elastic interactions (Sections 4.2 and 4.3). Section 5 reviews domain-stabilizing mechanisms that rely on an interaction between the membrane and its environment, such as membrane recycling (Section 5.1), the presence of pinning sites (Section 5.2), or other interactions that influence the phase separation kinetics (Section 5.3). We summarize and conclude in Section 6. Finally, Appendix A provides a unified mathematical description of the domain-forming mechanisms discussed in the main text within the framework of the Ginzburg-Landau theory.

The following abbreviations will be used in the text:

- AFM (atomic force microscopy)
- NMR (nuclear magnetic resonance)
- FRET (Förster resonance electron transfer)
- ESR (electron spin resonance)
- GUV (giant unilamellar vesicle)
- GMPV (giant plasma membrane vesicle)
- DPPC (dipalmitoylphosphatidylcholine)
- DMPC (dimiristoylphosphatidylcholine)

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