The plasma membrane as an adaptable fluid mosaic

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ABSTRACT

The fluid mosaic model proposed by Singer and Nicolson established a powerful framework to interrogate biological membranes that has stood the test of time. They proposed that the membrane is a simple fluid, meaning that proteins and lipids are randomly distributed over distances larger than those dictated by direct interactions. Here we present an update to this model that describes a spatially adaptable fluid membrane capable of tuning local composition in response to forces originating outside the membrane plane. This revision is rooted in the thermodynamics of lipid mixtures, draws from recent experimental results, and suggests new modes of membrane function.

KEYWORDS: Phase separation, critical fluctuations, susceptibility, signaling, plasma membranes.

MAIN

In 1972, Singer and Nicolson presented their Fluid Mosaic Model of the cell membrane [1]. This paper presented a diverse set of experimental observations and used them to make thermodynamic arguments to establish a physically plausible and experimentally testable model of the plasma membrane. The two key elements of their presentation were that outward-facing hydrophobic amino acid residues anchor proteins to membranes and that the membrane is a fluid where the majority of proteins and lipids are free to diffuse. These base assertions have been supported time and time again, revolutionizing the types of questions scientists have been able to ask and answer about the role of membranes in a broad range of cellular processes. These foundations have only been reinforced as new methods have enabled even closer views of membrane structure, dynamics, and function.

The fluid mosaic model is firmly grounded in thermodynamics of simple fluids, which unlike crystalline forms of matter, do not exhibit long-range order. Singer and Nicolson argued that since the membrane is fluid, membrane constituents will be a randomly organized mosaic over distances much greater than those characteristic of specific protein-protein and protein-lipid interactions. In the 50 years since its publication, there have been several efforts to update this model to incorporate membrane structure on longer length-scales (e.g. [2–6]). One major development is that we now know that liquid-liquid phase separation can occur in membranes. The presence of this phase transition provides physically rigorous mechanisms for fluid membranes to organize on length-scales greater than those available within a simple fluid.

Inspired by Singer and Nicolson's landmark review, we use this opportunity to describe some newly appreciated aspects of the thermodynamics of fluid membranes and explore how these concepts naturally extend to functional cell membranes. We propose an update to their foundational fluid

mosaic model to include a membrane that is a spatially adaptable fluid, capable of adjusting its local composition in response to interactions with components near the membrane plane. Following Singer and Nicolson's lead, we end by describing the new types of biological questions that can be explored. Our goal is to present a physically rigorous framework rich enough to motivate new questions in the years to come.

A BRIEF OVERVIEW OF THE THERMODYNAMICS OF LIPID MIXTURES

The first observation of macroscopic liquid-liquid phase separation in lipid bilayers came in 2001, when two groups [7,8] independently observed coexisting liquid-ordered (Lo) and liquid-disordered (Ld) phases in bilayer membranes composed of mixtures of lipids containing cholesterol. Since these initial observations, coexisting liquid phases have been observed in many different membrane compositions using a variety of experimental methods (reviewed in [9]). At minimum, Lo-Ld coexistence requires the presence of three components: a high melting temperature lipid, a low melting temperature lipid, and cholesterol or similar sterol [10]. Ternary phase diagrams share the general features shown in Figure 1: at low cholesterol concentrations, there are several regions where a saturated lipid-rich solid phase is present either alone or in coexistence with one or two liquid phases, and at moderate cholesterol concentrations, there is an extended region where two liquid phases coexist. The Lo phase is enriched in cholesterol, saturated lipids, and tends to contain lipids whose chains are more ordered and extended, while the Ld phase is enriched in unsaturated lipids and contains lipids whose chains are more disordered [11]. This region of coexisting liquid phases terminates in a miscibility critical point, where composition fluctuations extend to the micron-scale [12,13]. Beyond the miscibility gap the membrane

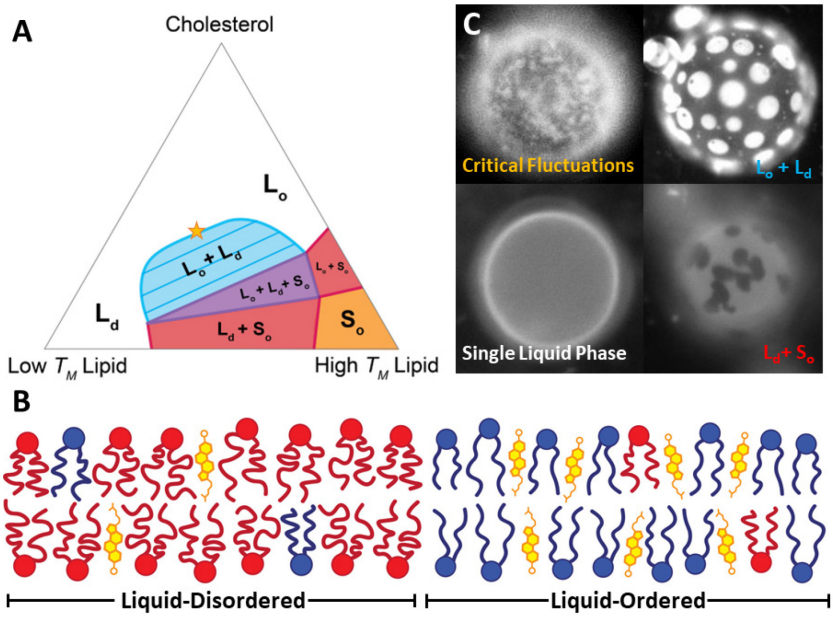

Figure 1: Phase behavior of ternary bilayers. (A) A qualitative phase diagram for ternary lipid bilayers includes regions for solid (So) phase and liquid-solid phase coexistence at low cholesterol concentrations. At moderate cholesterol concentrations, a broad region supports coexistence of the saturated lipid-rich liquid-ordered (Lo) phase and the unsaturated lipid-rich liquid-disordered (Ld) phase. The region terminates at a critical point (star). Beyond the coexistence lines (white regions in diagram), the membrane exists in a single fluid phase. (B) Schematic representation of Ld and Lo phases in model membranes. The Ld phase contains lipids with more disordered tails and is enriched in unsaturated lipids. The Lo phase contains lipids with more ordered tails and is enriched in saturated lipids. (C) Images of phase behavior in GUVs. (upper left) micron-scale composition fluctuation for a composition near the critical point. (upper right) vesicles with coexisting Ld and Lo phases. (lower left) Vesicle in a single liquid phase. (lower right) Vesicle containing solid (dark) and Ld (bright) phases. Vesicles prepared and imaged as in [11] and phase diagram is a schematic reproduction of one presented in [13].

is in a single liquid phase, but numerous studies document structure within this single phase when using spectroscopic experimental methods sensitive to heterogeneity on scales on or below tens of nanometers (e.g. [14–16]).

Many physical models have been proposed to describe the heterogeneity present in single phase membranes composed of lipid mixtures, but most draw from the observation that the membrane can macroscopically phase separate under some set of thermodynamic conditions (reviewed in [17]). The miscibility gap terminates in a critical point (star in Figure 1A), and a consequence of this topography is that single phase membranes with compositions in the vicinity of this upper phase boundary will have high compositional susceptibility. In thermodynamics, a susceptibility describes how much an extensive property of the system is dependent on changes in its intensive conjugate force [18]. One common example is compressibility, which describes how the volume of a three-dimensional system changes when a pressure is applied. Another example is magnetic susceptibility, which describes how much a material will magnetize when placed within a magnetic field. The compositional susceptibility describes how much local composition changes in response to a force that biases that composition.

Single phase membranes with high compositional susceptibility exhibit two related behaviors that can be observed experimentally. The first behavior is that weak, thermal forces give rise to spontaneous, dynamic composition fluctuations with composition and physical properties that resemble Lo or Ld phases. Single phase membranes with compositions very close to the critical point contain micron-scale fluctuations because the compositional susceptibility is very large (as in Figure 1C, top left). The compositional susceptibility is smaller for compositions away from the critical point, but remains large enough to produce nanoscale fluctuations [9,19]. The second hallmark of a membrane with high compositional susceptibly is that large and long-lived domains can be induced by external forces. Here, the compositional contrast of induced domains most closely resemble Lo or Ld phases close to the critical point. Away from the critical point, the contrast of an induced domain depends on both the magnitude of the force biasing composition and on the magnitude of the compositional susceptibility. Several experimental examples of induced domains in model membranes are highlighted in Figure 2A-C [20–22]. In this way, a single phase but highly susceptible membrane can be considered a spatially adaptable fluid, capable of adjusting local composition in response to its interactions with its environment.

A last key observation is that the membrane is molecularly thin, therefore it can be considered a two dimensional (2D) fluid under many relevant circumstances [23]. The dimensionality of a physical system has profound impacts on the shape of the phase boundary and on how the compositional susceptibility varies with thermodynamic parameters such as temperature and composition [24,25]. 2D systems such as membranes exhibit a particularly broad region of composition space where the susceptibility remains large [19,21], suggesting that membranes can act as adaptable fluids over a broad range of conditions. In principle, compositional susceptibility arising from the Lo-Ld phase transition could couple to other types of membrane-mediated interactions. These could include membrane asymmetry or membrane curvature (as in Figure 2C) and could further enhance the ability of membranes to organize its local composition in response to external cues.

THE PLASMA MEMBRANE AS AN ADAPTABLE FLUID

The thermodynamics of model membranes described above can predict key properties of cell membrane organization if cell membranes are in single phase but have a high compositional

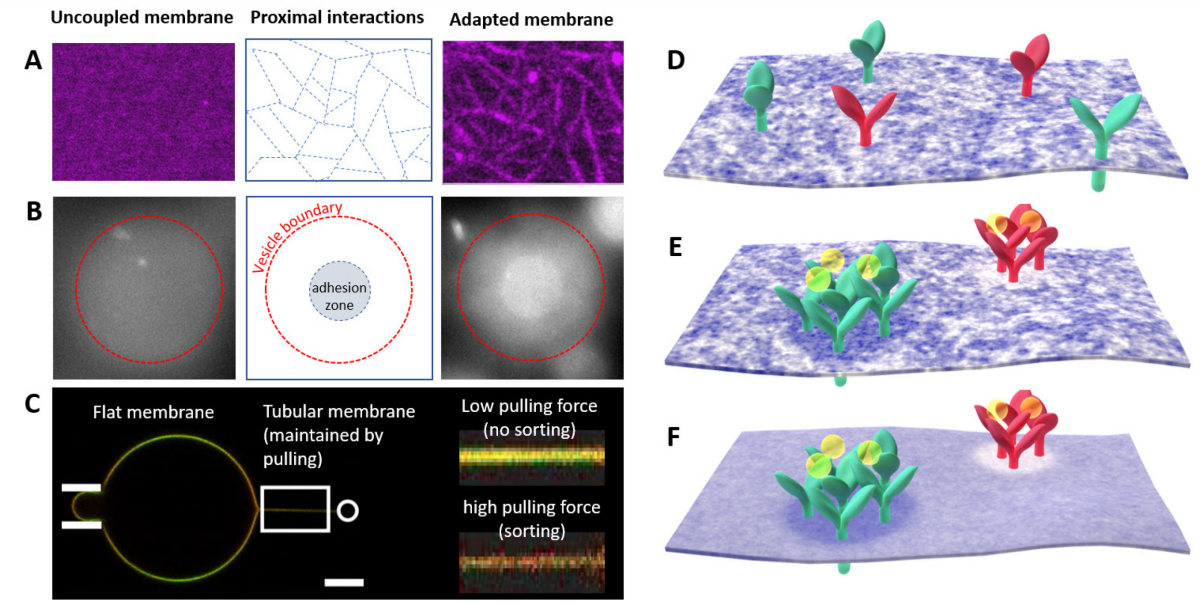

Figure 2: Induced domains in adaptable membranes. A-C. Examples of induced domains in adaptable model membranes. A. Induced domain structure within a planar supported membrane bound to a reconstituted actin meshwork. (Left) A planar supported membrane without actin coupling is in a single liquid phase. A subset of lipids bind to proximal actin (center) leading to the induction of domains that follow the actin structure (right). Adapted from [20]. B. Induced domain in a giant vesicle adhered to a supported membrane. (Left) the dorsal surface of a vesicle is in a single liquid phase. The ventral surface binds to a supported membrane (not shown), recruiting a subset of lipids to the adhesion zone (center) leading to the induction of an extended domain at the site of adhesion (right). Adapted from [21]. C. Induced domain in a tether pulled from a spherical vesicle membrane. (left) a giant vesicle is held by a pipette (denoted as parallel white lines) and a tether is pulled using a bead (demoted as a white circle). (right top) at a low pulling force, two fluorescent lipid analogs are present at equal levels in the cylindrical tether. (right bottom) the relative concentration of two probes change when the tether is pulled further, producing a tubule that is more narrow. Adapted from [22]. D-F. Schematic representation of spontaneous nanoscopic and induced domains in an adaptable membrane. D. Spontaneous, nanoscopic structure is present in snapshots of an adaptable membrane with high compositional susceptibility. E. Domains extend when membrane resident proteins are clustered by external interactions, here represented as yellow spherical ligands. F. Extended induced domains at clustered proteins persist when the membrane composition is averaged over time producing local environments that resemble Lo or Ld membrane phases.

susceptibility. In this state, cell membranes would be expected to exhibit the same behaviors observed in model membranes. Namely, small, transient composition fluctuations that resemble Lo and Ld phases would occur spontaneously (Figure 2D), and larger and more long-lived domains could be induced by external forces (Figure 2E,F). Indeed, several lines of experimental evidence identify these key features in isolated and intact cell plasma membranes.

A first line of evidence supporting the view that cell plasma membranes are in a single liquid but highly susceptible phase at growth temperature comes from measurements in isolated plasma membrane vesicles. Isolated plasma membranes can contain coexisting Lo and Ld phases [26], but past studies exclusively report macroscopic phase transition temperatures well below growth temperatures for a broad range of cell types, with the majority of studies reporting that transition temperatures are 15-30°C below growth temperature (e.g. [26–29]). In one example, membrane composition changed in a

way to maintain transition temperature 17°C below growth temperature within a zebrafish cell line capable of adapting to growth over a range of temperatures [30], suggesting that this state is biologically tuned. In addition, isolated plasma membranes exhibit both hallmarks of having a high compositional susceptibility. First, micron-sized composition fluctuations are reported in isolated vesicles very close to the transition temperature [27] and multiple studies document nanometer-sized lateral heterogeneity in vesicles at higher temperature [28,31,32]. Second, large domains are induced when external forces template domain structure [21]. These observations indicate that isolated plasma membranes have high compositional susceptibility, analogous to purified model membranes with compositions close to a phase boundary with a critical point.

Experimental evidence in intact cells also support the view that intact cell plasma membranes are in a single liquid but highly susceptible phase at growth temperature. Transient and nano-scale structure is well documented in intact cell membranes (e.g. [33–37]), suggesting that these membranes have a high compositional susceptibility. Most intact cell membranes do not contain macroscopic domains even at low temperatures [19,38], although a bilayer phase resembling the Lo phase can be isolated in intact cells exposed to low temperature and certain detergents [4,39]. This suggests that intact cell plasma membranes have compositions that are capable of phase separation upon modest changes in thermodynamic parameters.

Additional studies suggest that membrane domains can be induced in intact cell plasma membranes when proteins are clustered by ligands or antibodies. There is long-standing evidence that domains defined by the presence of clustered immune receptors have protein and lipid compositions that differ from the membrane as a whole (e.g. [40]). Early work relied on extraction of proteins that associate with clustered receptors using detergents or cavitation, although these methods introduce well characterized artifacts that impede the interpretation of experimental results [41]. More recent studies have used new probes and high resolution fluorescence imaging methodologies to characterize domains in intact membranes and reproduce many features of past models [36,42–47]. Specifically, membrane domains at receptor clusters have compositions and physical properties reminiscent of the Lo phase in isolated vesicles and emerge in response to receptor clustering. Plasma membrane domains stabilized by protein clustering are not limited to transmembrane immune receptors. Clustering GPI linked proteins or GM1 lipids on the extracellular face of the plasma membrane can also alter the composition and dynamics of membrane lipids and proteins [48,49]. These domains span membrane leaflets and again are reminiscent of the Lo phase in vesicles. While these examples report domains that resemble the Lo phase, it is also possible to induce a domain that resembles the Ld phase. This has been observed upon clustering an Ld preferring transmembrane anchor [42].

Based on experiments in model membranes and cells, as well as thermodynamic properties of membranes, we propose that the plasma membrane is a spatially adaptable fluid mosaic (Fig 2D-F). This membrane is in a single phase but has a high susceptibility to variations in composition, allowing for the spontaneous assembly of dynamic nanoscopic domains driven by weak thermal forces. The high compositional susceptibility also enables membranes to adapt their local composition when outside forces act on the membrane plane, for example when a subset of components are clustered by soluble ligands or antibodies. These scaffolding interactions provide the forces required to stabilize an extended and long-lived membrane domain.

The adaptive fluid mosaic model draws heavily on equilibrium arguments and experimental findings in model membranes, some of which might not translate directly to cell membranes. In cells, there are numerous non-equilibrium processes that likely impact membrane structure and function. For example, membrane components can bind directly or indirectly to motors that expend energy to drive molecular transport [50], and proteins within membranes contribute to enzymatic cycles that produce or expend energy [51]. Plasma membranes are also asymmetric in their membrane composition. This non-equilibrium state is actively maintained, and this is expected, among other things, to influence the conditions needed for phase separation [52]. Moreover, the lipids and proteins that reside within the plasma membrane are actively transported to and from intracellular pools [53]. Gradients in electric potential across cell membranes are also actively maintained, and large and rapid changes in the membrane potential often accompany signaling processes. Together, the important differences between cell and model membranes suggest that the perspectives gained from model membranes may only apply in certain limits. In most cases, the nature of these limits have yet to be discovered.

FUNCTION WITHIN AN ADAPTABLE FLUID MOSIAC

The enormous impact of Singer and Nicolson's fluid mosaic model came from the framework it provided that allowed researchers to ask and answer new biological questions. Inspired by their approach, we close this mini-review by speculating on what knowledge gaps can be informed by the new perspective that cell plasma membranes can act as an adaptable fluid.

Receptor clustering as a means to initiate signaling responses: There are numerous examples of signaling systems where a signal is initiated after a membrane receptor is engaged and clustered by an extracellular ligand. In some instances, receptor engagement leads to conformational changes within receptors that transmit information. In other cases, clustering receptors brings regulatory domains into close proximity, allowing them to act on neighboring receptors to initiate a signal. Both of these examples are well understood within a simple fluid mosaic model. There are other cases where a simple fluid mosaic model is not sufficient to describe cellular behavior, for example when immune receptors are clustered with antibodies that do not engage their native binding site, or when GPI-linked proteins that do not have transmembrane domains engage with clustering ligands. In these cases it is hard to understand how a cell might sense the organization of membrane proteins since simply being in close proximity does not directly impact receptor interactions with other membrane components unless they are directly bound. In an adaptive fluid, interactions between receptors and other membrane components can change in response to receptor clustering because the membrane composition locally adjusts to enrich or deplete certain components.

For the case of immune signaling systems, the ability to locally adjust membrane composition could impact signaling functions. Tyrosine phosphorylation at the immune cell plasma membrane is regulated through a competition between transmembrane phosphatases that prefer Ld domains and inner leaflet peripheral kinases that typically prefer Lo domains. In the absence of clustering ligands or antibodies, protein concentrations and kinetic rates are established such that phosphatases dominate, leading to lower levels of phosphorylated membrane proteins [54]. Upon clustering receptors, the adaptable membrane undergoes organization of its membrane components to stabilize an extended membrane domain that enriches kinases and depletes phosphatases. This local shift in the kinase-phosphatase balance could lead to enhanced tyrosine phosphorylation local to clustered receptors, aiding in the propagation of signaling responses. Aspects of this picture have been probed experimentally. In B cells, it has been shown that tyrosine phosphorylation can be localized to stabilized domains induced by

clustering GM1 lipids bound to cholera toxin B subunit [42]. In more recent work, it was found that the magnitude of BCR tyrosine phosphorylation is modulated through perturbations that impact compositional susceptibility in both B and T cell immune signaling systems [45]. These results suggest that even though intact cell members differ in important ways from model membranes, considering the membrane as a fluid capable of organizing its local environment in response to cellular cues provides tools to interpret results in cells.

An actin cortex templates structure in an adaptive membrane. The cortical cytoskeleton provides structural stability to the cell and contributes to the organization and dynamics of plasma membrane components. Within a simple fluid, the cortical cytoskeleton can act to pin membrane components or restrict the motions of proteins with bulky cytoplasmic domains [55,56], but is not expected to significantly impact the motions of other components. This is because diffusion in simple 2D liquids is space filling, meaning that Brownian motion should allow proteins to efficiently find paths through porous obstacles [19]. In contrast, a cortical cytoskeleton proximal to an adaptable fluid membrane could impact the organization and dynamics of proteins that do not directly bind to the cortex, by inducing membrane domains [19]. Domains induced through coupling an adaptable membrane to actin could provide additional barriers to diffusion for components that partition into a different phase.

There is long-standing evidence that membrane domains and the cortical cytoskeleton are intertwined (e.g. [6,57]), although the exact mechanisms and functional impacts have been hard to pin down. If membrane domains somehow played a role in regulating cortical actin assembly and stability, for example by impacting the localization of kinases and phosphatases involved in phosphatidylinositol metabolism [58], then it would follow that perturbations of membrane phase behavior would feed back onto cortical actin, impacting its structure and function.

Actin has a well-documented role in tuning the magnitude of signaling responses in immune cells [59–62]. This past work often attributes actin's role, at least in part, as impeding the rate at which receptors can interact with regulatory elements. An adaptable membrane provides new mechanisms whereby an actin cortex could play regulatory roles, by tuning the location and properties of induced domains. This could take the form of inhibiting domains if the network is sufficiently dense or by facilitating the organization of large domains that amplify signals [60–62]. In B cells, actin depletion leads to the activation of receptors even in the absence of clustering ligands [59], and it is intriguing to speculate that this may be due to changes in the domains induced when a susceptible membrane interacts with actin scaffolds.

Regulation of individual proteins by an adaptive fluid: The functions of numerous membrane proteins depend on the composition of their embedding membrane. In some cases this is due to direct interactions between specific lipids, but in other cases this arises because functional states of proteins are stabilized by specific membrane material properties. A protein embedded in an adaptive fluid membrane will be able to adjust its local membrane environment to more closely resemble a Lo or Ld phase without a large energy cost. For proteins with transmembrane regions that take on multiple confirmations, an adaptive membrane could change the energy landscape associated with transformation between states [63], or enable spatial organization of activity. This concept has been considered theoretically for the case of an ion channel embedded in an adaptive membrane, where it was found that changing the compositional susceptibility through tuning of the phase transition temperature could lead to robust changes in protein function and dynamics [64].

We imagine that biochemical networks containing membrane anchored proteins with specific phase preferences will also be impacted by an adaptive membrane. A protein's ability to adjust its local environment will impact how it interacts with other membrane components, with proteins that favor the same environment having effective attractive interactions and proteins that favor different environments having effective repulsive interactions [65,66]. For proteins whose function is regulated through interactions with other membrane components, this could mean that their activity can be modulated by changing the compositional susceptibility of the membrane as a whole.

An adaptive fluid can facilitate the condensation of cytoplasmic components: Recent excitement about the role of protein and/or nucleic acid condensation in the cytoplasm and nucleus has led to the discovery that similar condensation likely happens at membranes [67]. How might an adaptive membrane contribute to this process? For one, membrane regions in contact with protein droplets are expected to be extended domains whose composition will depend on the phase preference of the interacting proteins. In this way, protein droplets could template membrane structures which could in turn sort membrane components that do not directly bind to the droplets themselves. If protein droplets couple to membranes through lipid post-translational modifications such as palmitoylation, we would expect them to stabilize a domain that resembles the Lo phase. This may be the case at the neuronal post-synapse where proteins such as PSD95 are present in the post-synaptic density and also can be palmitoylated [68]. An adaptive membrane can also aid in the assembly of droplets at membranes by lowering the energy cost to clustering components that anchor droplets to membranes [69]. This might provide a means to allow droplet assembly to be sensitive to the thermodynamic state of both membrane and soluble protein components. If this were the case, one could envision signaling pathways whose sensitivity could be tuned by both membrane properties and the availability and activity of scaffolding components, allowing cells to tune function along multiple dimensions simultaneously.

CONCLUSIONS

In conclusion, here we present an update to the fluid mosaic model where the membrane is not a simple fluid, but one capable of adapting composition locally in response to interactions beyond the membrane plane. In this revised model, the membrane can retain its fluid nature while allowing structure over length-scales much larger than those governing interactions between proteins or between proteins and lipids. Importantly, this spatially adaptable fluid mosaic model is grounded in thermodynamics, and is possible because membranes can contain coexisting liquid phases. This model is also grounded in experiments suggesting that cell plasma membranes are in a single liquid phase but exhibit hallmarks of having a high compositional susceptibility, namely that small and dynamic domains can form spontaneously and that domains with Lo or Ld-like local composition can be induced in response to external forces. The protein mosaic in turn navigates this rich compositional landscape, whose topography is determined by the susceptibility of the membrane and the nature of interactions acting on the membrane plane. Most importantly, this spatially adaptable fluid mosaic model suggests new classes of questions that we hope will spur even more ideas and revisions in the years to come.

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