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Review

The Fluid—Mosaic Model of Membrane Structure: Still relevant to understanding the structure, function and dynamics of biological membranes after more than 40 years☆,☆☆

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ABSTRACT

In 1972 the Fluid—Mosaic Membrane Model of membrane structure was proposed based on thermodynamic principals of organization of membrane lipids and proteins and available evidence of asymmetry and lateral mobility within the membrane matrix [S. J. Singer and G. L. Nicolson, Science 175 (1972) 720–731]. After over 40 years, this basic model of the cell membrane remains relevant for describing the basic nano-structures of a variety of intracellular and cellular membranes of plant and animal cells and lower forms of life. In the intervening years, however, new information has documented the importance and roles of specialized membrane domains, such as lipid rafts and protein/glycoprotein complexes, in describing the macrostructure, dynamics and functions of cellular membranes as well as the roles of membrane-associated cytoskeletal fences and extracellular matrix structures in limiting the lateral diffusion and range of motion of membrane components. These newer data build on the foundation of the original model and add new layers of complexity and hierarchy, but the concepts described in the original model are still applicable today. In updated versions of the model more emphasis has been placed on the mosaic nature of the macrostructure of cellular membranes where many protein and lipid components are limited in their rotational and lateral motilities in the membrane plane, especially in their natural states where lipid–lipid, protein–protein and lipid–protein interactions as well as cell–matrix, cell–cell and intracellular membrane-associated protein and cytoskeletal interactions are important in restraining the lateral motility and range of motion of particular membrane components. The formation of specialized membrane domains and the presence of tightly packed integral membrane protein complexes due to membrane-associated fences, fenceposts and other structures are considered very important in describing membrane dynamics and architecture. These structures along with membrane-associated cytoskeletal and extracellular structures maintain the long-range, non-random mosaic macro-organization of membranes, while smaller membrane nano- and submicro-sized domains, such as lipid rafts and protein complexes, are important in maintaining specialized membrane structures that are in cooperative dynamic flux in a crowded membrane plane. This article is part of a Special Issue entitled: Membrane structure and function: Relevance in the cell's physiology, pathology and therapy.

Contents

Abbreviations: BAR, the Bin/amphiphysin/Rvs family of lipid-binding molecules; CIC, chloride channel gene superfamily; EGF, epidermal growth factor; F-MMM, Fluid—Mosaic Membrane Model; FRAP, fluorescent recovery after photobleaching; GPI, glycosylphosphatidylinositol-anchored; N-WASP, neural Wiskott–Aldrich syndrome protein; OLT, optical laser trapping; PC, phosphatidylcholine; SPT, single-particle tracking

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1. Introduction: The Fluid—Mosaic Membrane model

When the Fluid—Mosaic Membrane Model (F-MMM) of biological membrane structure was first introduced in 1972, it was envisioned as a basic framework model for cell membranes that could explain existing data on membrane proteins and lipid structures and their dynamics and help plan and predict future experimental outcomes [1]. At the time the accepted model for cellular membrane structure was the Unit Membrane Model of Robertson [2–4] or Davidson–Danielli Tri-Layer (protein–lipid–protein) Model of membrane structure [5]. The tri-layer membrane model was based on the lipid bilayer proposal of Gorter and Grendel [6], with added unfolded protein sheets on either side of a lipid bilayer. Later some trans-membrane protein components were added to reconcile observations on intramembranous particles, such as those found by Pinto da Silva and Branton, who freeze fractured cell membranes with surface bound ferritin markers [7], and the discovery of trans-membrane proteins (review: [8]) However, the basic Unit model has remained a tri-layer structure with most proteins present in extended forms (beta configurations) bound to the lipid bilayer by electrostatic and other hydrophilic forces [4].

An alternative to the tri-layer models for membrane structure was proposed at the time based on repeating subunits of lipoproteins without a supporting lipid bilayer matrix [9,10]. Both the Unit Membrane [2–4] and Subunit Membrane [9,10] Models had certain limitations in explaining existing data on membrane structure [1,8]. These earlier membrane models, with the exception of the F-MMM, also did not take into account the ability of components in membranes to rapidly move laterally and dynamically and change their topographic distributions, which was an important aspect of the F-MMM [1].

As first proposed, the F-MMM depicted biological membranes as a matrix made up of a mostly fluid bilayer of phospholipids with mobile globular integral membrane proteins and glycoproteins that were intercalated into the fluid lipid bilayer (Fig. 1, 1972) [1]. Confirmations of the bilayer structure of membrane phospholipids and their lateral motion in the membrane plane have been the subjects of a number of reviews over the years [11–22]. For example, Edidin [17] reviewed the history of membrane lipid structural proposals over the last century and concluded that there has been overwhelming support that membrane phospholipids were indeed present as a bilayer structure. The proposal that intrinsic or integral membrane proteins existed as globular structures that were inserted into a fluid lipid environment was based on structural and spectroscopic analyses as well as physical measurements of protein rotation and motion in the membrane plane [1,8,23–28]. Other methods also indicated that (at least some) membrane proteins were capable of rapid rotational and lateral movements [1,17,27–39]. (Only a few examples are given here).

Although the F-MMM has been cited as the most successful general model of biological membranes [40], it suffered from being accepted literally as a 'one model fits all' for every cellular membrane under all conditions. Thus the criticisms came, mostly after 20 years or more, that the F-MMM did not provide adequate explanations for every cell membrane structure, especially those recently discovered, such as lipid rafts, nor could it adequately explain the dynamics of all membrane components [41–45]. Considering the vast amounts of new data on membranes that have been published since 1972, these criticisms were valid and understandable. However, I hope to demonstrate that the F-MMM actually evolved from the original model in 1972, and within a few years alternative forms (or cartoons) of the F-MMM did take into account many of the criticisms that came later. As mentioned, most of the revisions to the F-MMM occurred within a few years after its initial introduction [30,31], whereas most of the criticisms came decades after the original proposal. In this review I have used only a few examples to make this point.

It is rare that scientific models are not modified from their original forms to reflect new observations or data that were not anticipated when the models were first proposed. That is also the case here. Nonetheless, it is now widely accepted (including this author) that there were limitations of the F-MMM as originally proposed in explaining the mosaic or domain structures present in membranes, especially those membranes found in specialized tissues and cells. Thus I have re-termed the model as the 'Fluid—Mosaic Membrane Model' to highlight the important role of mosaic, aggregate and domain structures in membranes and the restraints on lateral mobility of many if not most membrane protein components. This designation was done not to revise history or justify claims that were never part of the original model; it was done simply to make the model more consistent with newer information that was not available in 1972. It also takes into account a more macro-structural view of cellular membranes as apposed to the more limited submicro-structural view of the F-MMM as originally conceived [1].

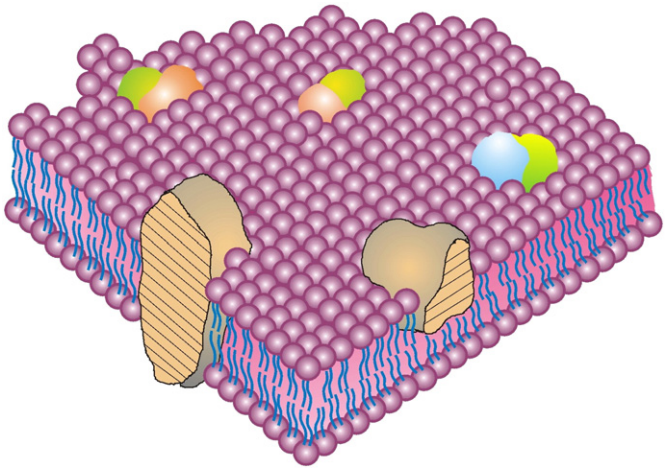

Fig. 1. The Fluid—Mosaic Membrane Model of biological membrane structure, as originally proposed in 1972. In this cross-sectional submicro- or nano-sized structural view of a cell membrane the solid bodies with stippled cut surfaces represent globular integral membrane proteins, which at intermediate range are randomly distributed in the plane of the membrane. At short range, some integral membrane proteins form specific integral protein complexes, as shown in the figure. The figure represents integral proteins in a completely fluid bilayer lipid phase, and it does not contain other membrane-associated structures or membrane domains of different compostions.
From Singer and Nicolson [1].

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Within a few years after its introduction, it had become obvious that the original F-MMM needed to be modified or augmented to reflect the emerging data on extracellular and intracellular mechanisms that can affect the lateral distributions and movements of plasma membrane components, and especially those that limit the mobility of many membrane integral proteins and glycoproteins [12,30]. Thus 1976 elaborations of the basic F-MMM (new cartoons!) included the interactions of extracellular matrix and membrane-associated cytoskeletal components with cell membranes and their potential influence on the mobility and distribution of trans-membrane glycoproteins as well as the possibility that less mobile lipid–protein or lipid–lipid domains might exist in membranes as frozen or semi-frozen islands of less mobile lipids in a sea of fluid phospholipids (Fig. 2, 1976 [30]). As will be discussed below, the hypothesis that trans-membrane interactions with membrane structures exist and influence their dynamics was important in explaining the controls over membrane structure, component mobility, and importantly function. Indeed, the more recent discoveries of lipid rafts and specialized membrane domains, membrane-associated 'fences' and membrane 'fenceposts,' among other membrane structures, and their possible functions in controlling and restraining membrane component distribution continue this trend.

Thus models of cell membranes produced a few years after the F-MMM were much less homogeneous looking than the original F-MMM depiction (for example, Fig. 2, 1976 [30]), and they contained additional information not included in the original model, such as protein and lipid aggregations and segregation into domains, cytoskeletal interactions, and extracellular matrix interactions. However, the revised F-MMM cartoons still contain all of the basic elements of the original F-MMM. These newer concepts of membrane regulation and hierarchy will be discussed later in this review, but this contribution should not be considered an exhaustive discussion of the topic.

In addition to intracellular and extracellular influences on plasma membrane dynamics, in certain membranes the packing of components into very compact structures and domains maximized the mosaic nature of such membranes. For example, viruses, cell junctions, adhesion sites, lipid rafts, mitochondrial inner membranes and other compact membranous structures possess limited lateral macro-mobility of specific membrane components while still exhibiting the basic microstructure of the F-MMM. This will be considered in later sections of this review. Due to the vast literature on various cellular membranes that could not be carefully considered in a single review it has been necessary here to concentrate on cell or plasma membrane structure and function.

2. Thermodynamic considerations

As Singer described in his personal memoir on the history of membrane models [46], the landmark article by Kauzmann [47] on the concept of hydrophobic interactions and their importance in the thermodynamics of protein structure played a critically important role in the development of the F-MMM. The propensity of hydrophobic structures to self-associate to exclude water interactions (driven entropically by water exclusion) and the propensity of hydrophilic structures to interact with the aqueous environment form the thermodynamic basis for the formation and stability of biological membranes. Thus membrane lipids, mainly phospholipids, self-assemble with their hydrophobic tails excluding water to form bilayers due to the energy provided by the van der Waals forces and the hydrophobic effect [49]. Membrane proteins (at least the integral or intrinsic proteins, see later sections) were proposed to be globular in structure, not extended β-sheet-protein structures as proposed in other membrane models [2–5], and their interactions with membrane lipids were due mainly to hydrophobic forces and much less due to hydrophilic interactions between the lipid head groups and hydrophilic groups on proteins. This concept did not preclude or diminish the significance of hydrophilic interactions between membrane lipids and integral membrane globular proteins. It simply described the relative importance of hydrophobic interactions in determining the basic microstructure of cell membranes.

As globular structures, integral membrane proteins were also proposed to be amphipathic with their hydrophobic domains embedded in the hydrophobic interior of the lipid bilayer and one or two hydrophilic domains protruding from the hydrophobic portion of the lipid bilayer into the surrounding aqueous environment [1,17,23–25,27–31,46–50]. This type of basic microstructural protein molecular organization, as proposed in the F-MMM [1] and previous publications [23,24,49], remains completely consistent with current available evidence [1,12,17,23–25,27–31,40–54]. (Only a few of the many publications that support this basic proposal are cited here). Of course, there are also hydrophilic

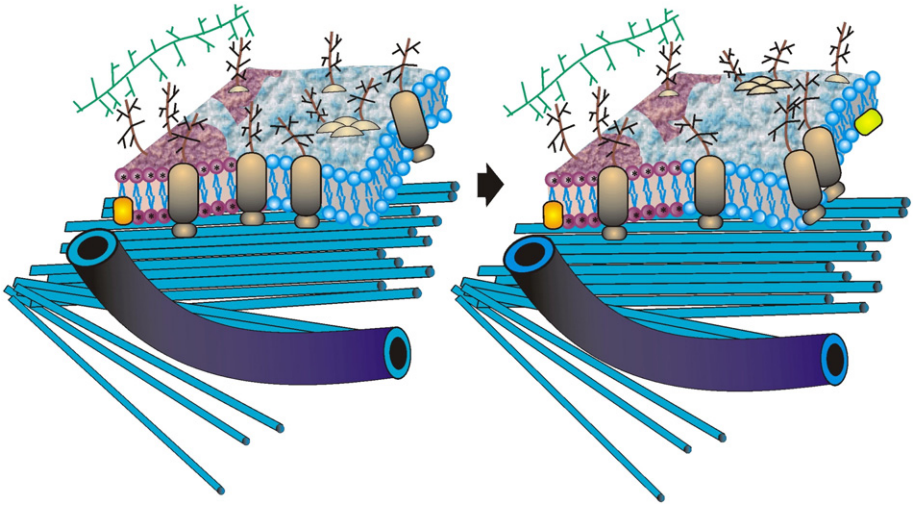

Fig. 2. A modified schematic version of the Fluid—Mosaic Membrane Model of biological membrane structure, as proposed in 1976. In this version, different snapshots in time are represented by the two panels. Some integral membrane glycoproteins are relatively free to diffuse laterally in the membrane plane within a fluid lipid region or domain, whereas others are "anchored" or relatively impeded by a cytoskeletal assemblage or an ordered or solid lipid phase. In this scheme, an integral membrane glycoprotein complex is also being displaced by membrane-associated cytoskeletal components in an energy-dependent process. Although this figure suggests some possible integral membrane protein and lipid mobility restraint mechanisms, it does not accurately present the sizes or structures of integral membrane proteins, cytoskeletal structures, polysaccharides, lipids, submicro- or nano-sized domains or membrane-associated cytoskeletal structures or their crowding in the membrane.
From Nicolson [30].

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ion channels in membranes that are formed by assemblages of transmembrane globular proteins [46,50–52]. The importance of these in maintaining cellular ionic balance and electrical properties of membranes will not be discussed here.

Similar to integral membrane proteins, membrane lipids, mainly glycerophospholipids, were proposed in the F-MMM to be arranged in a bilayer [1], as originally advanced by Gorter and Grendel [6], to prevent the hydrophobic portions of their structures from exposure to aqueous environments [1,16–18,21,22,30,31,41,47–49,55]. There are hundreds of different types of lipids within cells, and most of these are membrane lipids, suggesting that each type of lipid may play a role in determining membrane function, structure, topology and dynamics [56–59]. There are many excellent reviews on the properties of membrane lipids and their roles in specialized membrane domains and dynamics [16–19,22,23,56–69], and thus this important topic will not be discussed in detail here. Structures, such as lipid rafts and their role in membrane dynamics and other specialized membrane structures, will be considered in another section of this review.

Glycerophospholipid membrane bilayers deform when confronted with forces less than those driven by the hydrophobic effect [48]. Elastic membrane deformation, such as curvature elasticity, depends on the energies of lipid tilt and splay, which in turn are dependent on lipid composition [70,71]. Thus lipids that support positive spontaneous curvature can reverse the effects of lipids that support negative spontaneous curvature to maintain membrane form, and this difference may be important in membrane fusion, fission and other membrane–membrane interactions.

Another thermodynamic consideration of the F-MMM was that since the free energy required to flip membrane lipids and proteins across the hydrophobic membrane interior would be substantial, cell membrane flip-flop that could result in symmetric structures should be exceptionally low [1,49]. Without significant flip-flop, cell membrane inner surfaces should be different in protein and lipid composition from their outer surfaces, a point raised and discussed by many reviewers [1,8,12,27,30,31,40,41,49,50]. This will be considered further in the next section.

To accommodate various lipid–protein interactions so that different lipids and proteins could adjust to each other's hydrophobic structures Israelachvili proposed that the F-MMM needed to be refined to account for these differences [41]. Elaborating further, Mouritsen and Bloom suggested that sorting of lipids and proteins based on the interactions of hydrophobic regions (and to a lesser degree their hydrophilic interactions) of these different classes of molecules prevented mismatches between lipids and proteins [69]. They called this the Mattress Model of lipid–protein interactions in membranes, and their purpose was to describe how variations in the hydrophobic parts of lipids and proteins drive associations or hydrophobic matching between these different classes of membrane components to prevent membrane distortions [69]. This will be considered again in a later section.

What is new on the thermodynamics of membranes since the F-MMM was introduced is mainly a consideration of the forces (and molecules) that govern membrane deformation, curvature, compression and expansion [68–75]. For example, proteins that contain BAR (Bin/amphiphysin/Rvs) domains that form crescent-shaped α-helical bundles that bind to membranes via electrostatic and hydrophobic interactions are believed to generate membrane curvature by scaffolding to the surface of the membrane causing it to bend to the curvature of the protein [76]. Alternatively, there are also proteins that when inserted into a membrane can alter their shape by undergoing folding transitions to form α-helices that wedge membrane components, deform the membrane and cause curvature by this deformation [77]. These events are likely to be important in the formation of highly specialized membrane vesicles, tubes, spikes and other membrane structures but will not be discussed in detail here.

3. Asymmetry of membranes

The asymmetric nature of cell membranes was known for some time before the F-MMM was published [1,4,8,12], and in fact, Bretscher proposed that this was one of the five major principles that govern membrane structure [12]. For example, it was known that phospholipids and proteins are asymmetrically distributed between the inner and outer membrane leaflets, and there was little 'flip-flop' from one side to the other, as shown by phospholipase digestion, radiolabelling, magnetic resonance studies and electron microscopy labeling experiments [16,30,31,39,40,78–87]. Thus cell membranes maintain their asymmetry, and for good reason—they must be capable of maintaining an appropriate asymmetry between inner and outer membrane enzymes, receptors, phospholipids, oligosaccharides, proteins and other structures. Thus maintaining differences between the inside and the outside of cells to facilitate the appropriate display of receptors, adhesion molecules, signaling systems, scaffolding structures and other molecules on opposite membrane surfaces is probably a logical structural requirement.

Of particular interest around the time of publication of the F-MMM was the repeated finding of asymmetric distributions of various phospholipids between the inner and outer leaflets of cell membranes [12,56,59,78,85–89]. At the simplest level one can imagine that the enrichment of amine- and serine-containing phospholipids found on the cytoplasmic surface and choline-containing phospholipids and sphingomyelins on the outer surface of the cell membrane (which in turn, creates increased affinity of cholesterol in the outer bilayer leaflet) might have some advantage in terms of membrane associations of cytoplasmic proteins and maintenance of enzymatic activities. However, it is now known that there is a cost to pay for not maintaining appropriate cell membrane asymmetry, and it is not just the appropriate display of enzymes, receptors and other functional components of membranes. Disruption of the normal membrane asymmetry is generally associated with cell activation (activation of cell adhesion, aggregation, apoptosis, recognition by phagocytic cells, etc.), and it can also be associated with pathologic conditions [89].

Cell membrane lipid asymmetry may also guide membrane curvature and other aspects of membrane structure [59,69,73,74,87,88]. The compositional differences between the inner and outer leaflets of the cell membrane lipid bilayer suggest that the outer leaflet is curvature neutral, whereas the inner leaflet may have a preference for negative curvature, or as Zimmerberg and Gawrich state in their review, at the inner surface the polar interface has a smaller lateral area than the hydrocarbon chain region and thus drives a net curvature to minimize total curvature energy of the bilayer [73].

It follows that a number of lipid transporters that have been discovered are important in maintaining lipid asymmetry, such as cytofacially-directed, ATP-dependent transporters ('flippases') and exofacially-directed, ATP-independent transporters ('floppases'), but there are also bidirectional, ATP-independent transporters ('scramblases') [88–90]. The existence of several of these phospholipid transporters in maintaining the proper phospholipid asymmetries in the cell membrane suggests that maintenance of membrane asymmetry is functionally essential for cells [88–90].

Membrane integral protein asymmetry, on the other hand, is easier to explain (but certainly no less complex) and is probably initiated at the time of protein synthesis [50,51,91,92]. The asymmetries of integral membrane proteins are likely formed during the initial insertion of the polypeptide chains into the membrane mediated by translocons, molecular gatekeepers that allow newly synthesized polypeptide chains to pass across or directly integrate into the lipid portions of the membrane [91,92]. Thus in contrast to phospholipids, integral membrane protein asymmetry does not have to be actively maintained after initial biosynthesis. The energy required to flip integral globular membrane proteins across a hydrophobic barrier would be enormous, and thus this would be an unlikely and uncommon event [1,49].

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4. Three classes of membrane proteins and membrane-associated proteins

When the F-MMM was first proposed, it was important to distinguish between the integral (or intrinsic) proteins that were tightly bound to membranes by mainly hydrophobic forces and intercalated into the membrane hydrophobic matrix as apposed to peripheral (or extrinsic) proteins that were loosely bound by electrostatic or other non-hydrophobic interactions to hydrophilic regions of membranes [1,27,49]. There are numerous examples of both types of membrane proteins, and this has been discussed in more detail elsewhere [27,50–53,84,87,91]. Up to now I have mainly discussed integral membrane proteins and their importance in defining basic cell membrane microstructure; however, peripheral membrane proteins also have an important role, but not necessarily in maintaining the basic microstructures of membranes. They appear to be more important in providing enzyme activities, protein attachment sites, scaffolding, tethering or membrane-supporting structures, membrane curvature-preserving components and attachment points for soluble enzymes and signaling molecules.

Peripheral (or extrinsic) membrane proteins were originally operationally defined as proteins that could be removed from cell membranes without destroying the basic F-MMM microstructure [1,49]. This was an operational not an exact definition to help explain the roles of different membrane proteins in defining the basic microstructure of cell membranes. Peripheral membrane proteins do not have to be strictly globular in structure, and they would include the Robinson proteins with extensive β-sheet structures that bind to membranes mainly by ionic and other interactions [3,4].

In 1976 I proposed a new class of membrane proteins (membrane-associated proteins) to the mix [30], but these are not really membrane proteins at all. They are cytoskeletal and associated signaling proteins at the inner membrane surface and certain glycoproteins and linked glycosaminoglycans at the outer membrane surface (Fig. 2, 1976) [30]. These membrane-associated components are thought to be involved in stabilizing cell membranes (and thus cells) and immobilizing membrane components outside the cell to the extracellular matrix or across the membrane to cytoskeletal networks inside cells where they can function as parts of adhesion structures or cell motility traction points. Thus these components are membrane-associated but not involved in the integral microstructure of cell membranes, and cell membrane microstructure is not dependent on their presence. However, that does not mean that they are not important in maintaining membrane function and dynamics, because they are especially important in events such as cell–matrix and cell–cell adhesion and its stabilization, cell motility and spreading, endocytosis, exocytosis and many other important cell membrane and cellular activities.

As with peripheral membrane proteins, membrane-associated components should be removable from cell membranes without disruption of the phospholipid and globular protein membrane microstructure. Some properties of membrane-associated cytoskeletal components and extracellular matrix components will be discussed later in this review.

5. Cis and trans-membrane control

Shortly after the F-MMM was first published [1], it was apparent that there were cytoplasmic as well as extracellular influences over cell membrane structure and dynamics, and not all cell membrane components were found to be freely mobile in the membrane plane. Using antibody, lectin and drug treatments as well as protein/glycoprotein crosslinking and distribution studies it was apparent that many membrane glycoproteins and proteins were not completely free to rapidly roam in the plane of the membrane, or at least their mobilities and distributions were subject to local control within the plane of the membrane (cis-membrane control) or across the membrane (trans-membrane control) [93].

This was first studied with red blood cell membrane ghosts using antibodies against external antigens or against a protein, spectrin, known to be an inner membrane peripheral protein [94]. The major erythrocyte sialoglycoprotein was followed in its distribution using an electron microscopic label [95]. Perturbation of outer membrane surface blood groups (cis) or inner membrane surface spectrin (trans) by antibodies caused aggregation or clustering of the trans-membrane sialoglycoprotein [95,96]. Similarly, binding and perturbation of outer membrane glycoproteins was found to cause a trans-membrane organizational change at the inner surface as seen by an increase in chemical crosslinking of inner membrane components using bifunctional crosslinking reagents [97].

As early as 1973 it was apparent that certain specialized cell membranes were highly differentiated along their surfaces in terms of the nonrandom display and mobility of cell surface components and the restriction of some membrane components to specific regions of cells. In addition, this type of highly differentiated macrostructure had the capacity to change quickly given the appropriate signal(s). For example, highly specialized cells, such as mammalian spermatozoa, are surrounded not only by a continuous plasma membrane but also one that is highly differentiated in terms of the distribution of cell surface components [98]. Studying hamster sperm we found that the distribution of sialoglycoproteins was very different from head to midsection to tail, indicating that sperm membrane sialoglycoproteins were not entirely mobile and capable of freely intermixing in the plane of the membrane [99]. It was hypothesized that trans-membrane restraints maintained segregation of some membrane glycoprotein components and prevented their free mobility [99]. However, after the sperm interacted with an ovum and fertilization occurred, this situation could quickly change [100].

Sperm integral membrane components can also be restrained in their mobility by cis interactions occurring at the outer surface [101]. For example, PH-20 is a sperm surface protein that is involved in sperm–egg adhesion and is anchored to the membrane via binding to outer surface phosphatidylinositol. Such glycosylphosphatidylinositol (GPI)-anchored components can be highly mobile in the membrane plane [99]. In contrast, sperm PH-20 lateral mobility was found to be highly restricted [101]. However, when sperm underwent acrosome reaction in preparation for fertilization, the PH-20 was found to be freely diffusing and capable of rapid lateral mobility. Interestingly, the mobility of an unattached probe in the lipid bilayer suggested that the general state of fluidity of the membrane was not responsible for restraining the mobility of PH-20, suggesting that other types of cis interactions, such as that found in localized lipid rafts, might be responsibility for the low mobility of PH-20 in sperm membranes before fertilization [101]. The formation and dynamics of lipid domains and rafts and their restraints on membrane mobility will be discussed in a later section.

6. Membrane-associated cytoskeletal and extracellular matrix interactions

There are a number of situations where trans-membrane controls can alter the macrostructure of cell membranes. For example, trans-membrane controls could cause either a reduction or restriction in freedom of lateral movement or mobility or cause global movements of membrane glycoprotein aggregates and lipid domains by tethering these complexes to cellular actin-containing fibers and in some cases indirectly to microtubular structures (reviews: [30,31,33–39,102–111]). This later situation can occur when cell membrane-associated actin-containing cytoskeletal components are involved in moving or restraining trans-membrane integral membrane proteins through intermediate peripheral membrane proteins and other components (Fig. 3, 1976 [30]). At the time this cartoon (Fig. 3) was drawn, there were already examples of restriction of mobility of integral membrane components. During antigen capping where initially mobile cell surface

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antigens, even if present in small mobile clusters [112], were found to be trapped into large, relatively immobile macromolecular trans-membrane complexes in a temperature- and energy-dependent process involving cytoskeletal elements [113–115]. This process eventually resulted in cytoskeletal-mediated endocytosis of some but not all of the large macromolecular complexes [116]. We now know in lymphoid cells that antigen clustering, domain formation, internalization, acidification of the resulting endosomes, degradation and membrane recycling are all part of the normal activation process [117]. This process also dynamically rearranges and changes the composition of cell membranes. In addition, the organizational structures that mediate trans-membrane linkages between clusters of integral membrane receptors and the cytoskeleton were much more complex than the simplistic cartoons of the 1970s, and they are now thought to involve multiple membrane peripheral proteins, lipid–protein domains, and enzymes that assemble into submembrane plaques or supramolecular structures that connect the membrane to a complex cytoskeletal system [103,104,108,109,118,119].

The mobility of integral cell membrane components can also be restricted by cell–cell and cell–matrix interactions. Many years ago we noticed that when cells were aggregated, their surface components at the zones of cell–cell interactions became immobilized over time, possibly due to close membrane glycoprotein interactions or ligand-binding and multiple receptor interactions between adjacent cells [120]. Also, when cells are bound to an extracellular matrix, it is well known that at least some of their membrane matrix receptors are immobilized in the process. For example, Kobialka and colleagues have studied this by analyzing the effects of glycosaminoglycans on a receptor (H1) that normally does not bind to matrix components and cycles between the plasma membrane and endosomes [121]. By modifying the receptor to include sequence tags of 10–17 amino acids encoding a glycosaminoglycan attachment site, they were able to convert the H1 receptor into a glycosaminoglycan-binding receptor. They found that the distribution of the modified receptor was altered due to its matrix immobilization, and in the process endocytosis was inhibited [121].

Among the examples of extracellular matrix anchoring of integral cell membrane receptors, the membrane polarity and matrix restrictions of epithelial cell receptors stand out. When this was studied in culture, integral membrane receptors for extracellular matrix components tended to be located at the basolateral surface, whereas some cell surface components were found mainly at the apical surface. However, not all integral membrane matrix receptors appeared to be immobilized to the basolateral surface on epithelial cells. Salas et al. found that although there was segregation (and restriction of mobility) of most basolateral glycoproteins, there were also mobile fractions that were not anchored to matrix or cytoskeletal structures and were apparently free to migrate to other domains of the epithelial cell membrane [122]. The role of lipid segregation in epithelial cells into specialized domains will be discussed later in this review.

Trans-membrane adhesion complexes that are immobilized by matrix interactions are now known, at least in some cells, to communicate signals that are transmitted through an assembled actin-containing cytoskeleton to generate mechanical forces that can move cells or resist exterior mechanical stresses, such as external fluid flow [123,124]. Thus from the extracellular matrix to integral membrane proteins to peripheral membrane proteins to adaptor proteins to cytoskeletal elements, this serial system of highly specialized glycoprotein and

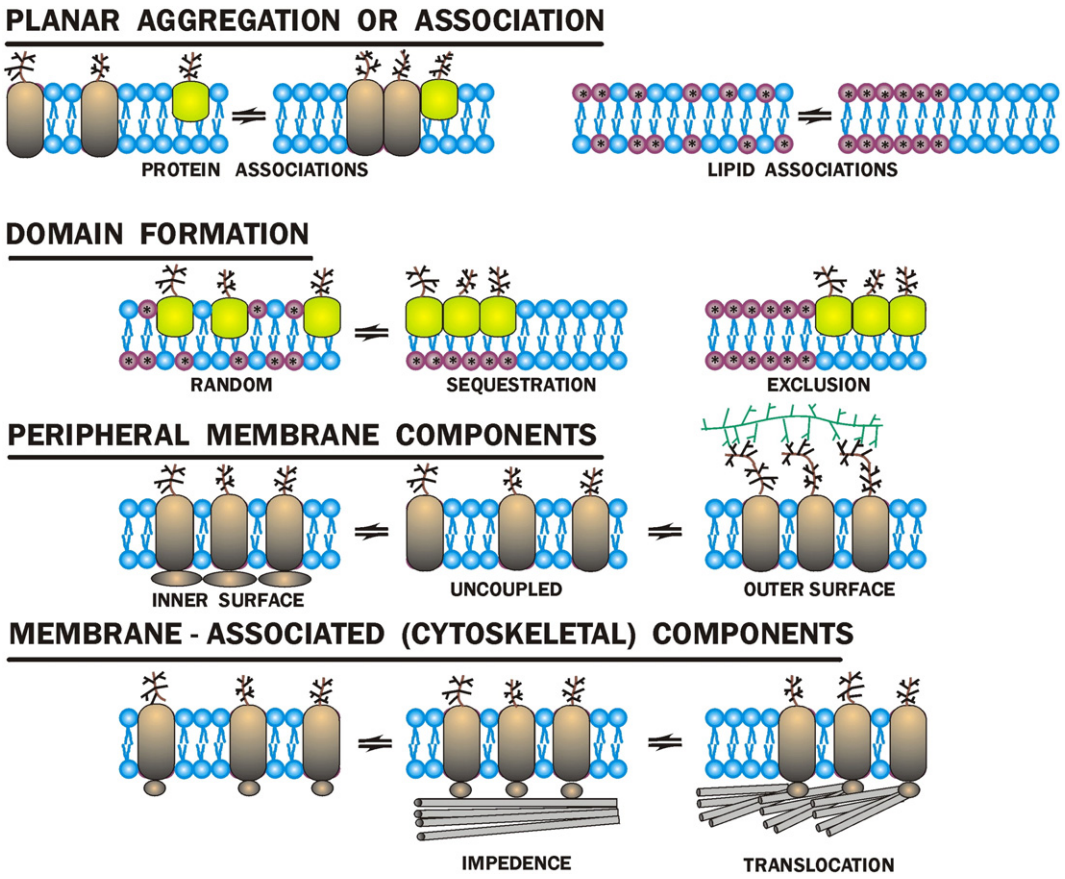

Fig. 3. Some proposed mobility-restraining mechanisms that could potentially affect the rates of lateral diffusion and mobility of membrane glycoproteins and phospholipids in the membrane plane, as envisioned in 1976. In this scheme, different mechanisms (glycoprotein complexes, membrane domains, cytoskeletal interactions, extracellular matrix interactions, peripheral membrane protein interactions, lipid–lipid interactions, etc.) can restrain the lateral movements of membrane components and affect their macromolecular structures and distributions. Although this figure suggests some possible integral membrane protein and lipid mobility restraint mechanisms, it does not accurately present the sizes or structures of integral membrane proteins, cytoskeletal structures, polysaccharides, lipids, nano-domains or membrane-associated cytoskeletal structures or their crowding in the membrane.
From Nicolson [30].

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protein network probably evolved to convert biochemical signals into mechanical forces that are important in cellular behavior. Such mechanical-molecular pathways were proposed by Roca-Cusach et al. along with better-known biochemical pathways to be key regulators of cell function [123].

What has changed from the earlier proposals on cell membrane-associated cytoskeletal elements and their influence on membrane structure and dynamics is that these complex systems are now being carefully dissected and their multiple subcomponents identified [102–111,118,123,125–127]. For example, we now know that certain membrane-interacting components, such as septins, GTPases, and other components, are involved in cytokinesis and can form higher-order bundles, filaments and ring structures that bind to actin filaments and microtubules [127–130]. Septin aggregates or filaments do not contribute to the generation of contractile forces of the cytoskeleton, but they probably form scaffolds at the cell membrane inner surface that interact with and form a link to cytoskeletal elements [130]. In addition, they may also be involved in forming diffusion barriers in cell membranes that result in compartmentalization of membrane proteins to specific cell membrane domains [131].

Other membrane-associated structures may be involved in the removal or trafficking of cell membrane components or their movements to other organelles or compartments within cells. For example, it had been known for some time that directed transport of vesicles formed through endocytosis and other processes makes up a system of small vesicle targeting through secretory and endocytic pathways. These specialized intracellular vesicles are characterized by the presence of 60–100 nm electron-dense coats on the surface of the vesicles [132,133]. Subsequently the electron-dense coats were isolated and found to be composed of a lattice-like shell comprised mainly of the protein clathrin [134]. Clathrins along with other adaptor proteins that form the localized electron-dense coats ('patches') were found bound to aggregated trans-membrane proteins and linked to the cytoskeletal system where the newly formed endocytic vesicles were being pulled away from donor membrane. Eventually the small, coated endosome vesicles were transported to acceptor organelles or other sites within cells [135]. For this delivery system to be specific, there had to be several unique membrane recruiting and signaling proteins that were essential for sorting and delivery of the coated vesicles to the appropriate intracellular site [136]. This constituted only one of many examples of the role of endocytosis in cellular physiology and immunology, topics that are too complex to be carefully assessed here. Thus the reader is directed to some recent reviews on this specific topic [135,137–143].

Although cell membrane peripheral proteins have been identified as important in cytoskeletal interactions [144], such as those that bind to actin-binding proteins as briefly introduced above (see also [145,146]), membrane lipids are also important [146,147]. Specifically, phosphoinositides may regulate interactions between specialized membrane lipid domains through lipid rafts using GPI isoform-binding proteins, such as unfolded N-WASP (neural Wiskott–Aldrich syndrome protein that is now thought to be widely expressed), that in turn binds to protein Arp2/3 and actin [147].

Future studies will ultimately confirm at the molecular level what we now strongly suspect—that cells are completely integrated mechanostructures—and cell membranes are not autonomous and separate from other intracellular membranes and organelles. They are continuously interacting with other cellular structures and receiving signals, directing contacts and sending instructions, maintaining cellular polarity and mechanical properties, while undergoing constant turnover of their constituents [144,146,148–150].

Cells also shed plasma membranes (exocytosis), and this is done, in part, to signal other cells and initiate important physiological processes. These important topics will not be discussed here, and there are several recent reviews that are available on these topics [138,151–153].

7. Protein–protein interactions within membranes

Most cell membrane proteins and glycoproteins are not isolated components or complexes floating in a fluid lipid environment (as envisioned in Fig. 1, 1972 [1]). They can be assembled into macromolecular complexes (the formation of such complexes can also be part of a signaling mechanism) that take part in a variety of cooperative cellular functions, including ion and metabolite transport, cellular recognition, enzyme activation and other signaling events, cell adhesion, movement, etc. Although this was acknowledged in the original description of the F-MMM [1], over the years it has taken on additional importance in describing the interactive relationships of cell membrane glycoprotein complexes and inner membrane peripheral protein components. As their cellular and biochemical functions have been elucidated over the years, it has become much clearer how multimeric complexes of cell membrane proteins and glycoproteins perform the variety of actions attributed to them.

By cloning, sequencing and functionally expressing integral membrane proteins further insights into their activities and structure–function relationships have been possible [25,50–53]. In these studies single membrane components can be involved, but usually membrane protein and glycoprotein complexes (not single protein units and often dynamically controlled) perform a variety of essential tasks for cells [154–156]. For example, the insulin receptor consists of a heterotetrameric complex that activates an intracellular tyrosine kinase domain in one of the protein subunits. Then the receptor undergoes a series of intramolecular trans-phosphorylation reactions in which one subunit of the complex phosphorylates an adjacent subunit of the complex in order to initiate the signaling process [157].

Protein–protein interactions, which can also be driven by ligand binding, are involved in the dynamic formation of glycoprotein trans-membrane signaling complexes at the cell surface. These interactions may also involve multimeric complexes that exist before ligand binding. Eventually the complexes can become activated for cis recruitment of additional components and then trans recruitment of peripheral proteins at the inner cell membrane surface to form supramolecular trans-membrane structures that are competent for cell signaling [158]. Well-studied examples of this are the interactions of cell surface receptors with extracellular matrix components, such as the interactions of the appropriate matrix ligands with cell surface integrins. Integrins are heterodimeric trans-membrane cell surface glycoprotein receptors that lack enzymatic activity [159]. Upon binding their ligand, the integrin heterodimers are thought to undergo a 'bending' conformational change that allows their recruitment of submembrane plaque proteins that, in turn, directly or indirectly bind to actin, and thus link the submembrane complex to the cytoskeleton [160,161]. The protein–protein interactions do not stop at this point, as a potentially larger group of other signaling molecules and enzymes can now be bound to the submembrane, supramolecular complex that forms, leading to the formation of stable focal adhesion complexes. The process is more complex than can be easily described here, so the reader is referred to more appropriate reviews for additional detail [159–161].

As primitive organisms evolved, their cell membranes became more complex, and as Dias proposed, this more complex type of plasma membrane structure likely paralleled the appearance of differentiated tissues and organs [162]. For example, the CIC gene family of ion channel proteins evolved to express an unusually wide variety of functions, with some members of this family possessing gated chloride channel activities and others possessing secondary chloride transporter or chloride–proton exchange activities [163]. This type of evolution-driven heterogeneity of cell membrane integral proteins into families with similar but distinct functions and structures is quite obvious to researchers who study integral membrane proteins [50,51,53].

An example of the types of membrane protein–protein (or protein–glycoprotein) complexes that are commonly found in various cell membranes is the major protein/glycoprotein complex of the erythrocyte membrane [84,154–167]. This cell membrane is unique in its physical

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properties, since it can resist fluid stresses that would tear most cells apart [165,168], but its basic microstructure still corresponds to the basic principles of the F-MMM. In the case of the erythrocyte membrane, it derives its elastic properties from an underlying network of inner membrane-associated peripheral proteins composed of spectrin and other proteins that are transiently linked to (or at least surrounding) transmembrane glycoprotein complexes [166–170]. Even as earlier models of the red blood cell membrane were being re-evaluated (for example, compare Fig. 7 of Ref. [30] drawn in 1976 with the 2008 Fig. 5 of ref. [166]), the basic principles of transmembrane glycoprotein complexes interacting with peripheral membrane proteins and cytoskeletal components were amply apparent at about the time of the F-MMM proposal [30,31,84]. In the case of the erythrocyte inner membrane spectrin [94,97,165,171] and its associated proteins [165–170], this complex appears to interact with asymmetrically distributed phospholipids as well as trans-membrane glycoproteins [166,167,172]. This will be discussed again, below.

There are a variety of cell membrane glycoprotein–protein complexes that are involved in cell–cell interactions and the formation of specialized structures between adjacent cells in tissues. For example, epithelial cells are coupled into polarized tissues and have multiple, complex junctional structures that link them and provide molecular seals, and in some cases they can also transfer solutes and signals from cell to cell while providing structural support via cytoskeletal linkages [173–179]. These structures generally have features in common. For example, interactions through the cytoskeleton systems and plasma membrane complexes in adjacent cells result to an integrated network throughout similar cells and tissues [173,177,178]. Cellular junctions also control vital communication pathways and ion linkages between cells [173,174,176,178], and they can seal off tissues from environmental contamination [175,176]. The junctional complexes are built with structural subunits that are assembled by protein–protein interactions in the lipid membrane environment, and in some cases, such as gap junctions, forming pore structures that allow cell-to-cell flow of ions and signaling molecules [174,177,178].

8. Protein–lipid interactions within membranes

Membrane integral proteins (or globular glycoproteins with their saccharide portions facing the eventual exterior cell surface) must interact with membrane lipids in a bilayer configuration to assemble into an intact plasma membrane. Thus portions of their structures must directly interact with the acyl portions of membrane phospholipids or hydrophobic portions of other membrane lipids. This is accomplished by hydrophobic matching between the hydrophobic lipid bilayer acyl core of boundary phospholipids and a stretch or combination of hydrophobic amino acids displayed by integral membrane proteins and glycoproteins [28,41,55,59,69,180–182].

In actual biomembranes there are additional considerations, such as lateral pressure forces, lateral lipid composition and phase, curvature, and charge interactions, that must be taken into account to produce an overall tensionless structure [28,55,59,69,180,182]. The role and formation of different lipid phases and domains in cell membranes [62–64] and their effects on integral protein distribution will be considered later in this review.

The concept of hydrophobic matching between the hydrophobic core of the boundary lipids in the lipid bilayer and hydrophobic stretches of amino acids in integral membrane proteins was essential for understanding the formation of a stable cell membrane structure [169,182–185]. If the hydrophobic portions of this structure are mismatched, there will be an elastic distortion of the lipid matrix around the integral membrane protein [69,182,183]. In order to produce an appropriate structure hydrophobic matching of particular lipids immediately near particular membrane proteins ('boundary lipids') must be accomplished, or there will be an energy penalty that causes an elastic distortion of the boundary lipid matrix immediately around the integral protein [183,184]. If the energy penalty is large enough, the integral protein may undergo a conformational change, and this was proposed to potentially cause effects on protein function [183,184]. This can also result in the exclusion of certain lipids, such as cholesterol, from the boundary lipid layer due to unfavorable membrane protein hydrophobic matching [185–187].

Lipid boundary effects can also affect protein-protein interactions and result in membrane integral protein aggregation in the membrane plane [183,185]. This was shown in the experiments of Kusumi and Hyde [188] where the rotational diffusion rates and states of aggregation of rhodopsin in reconstituted bilayer membranes were related to specific PC acyl chain-lengths. When PC acyl chain hydrophobic matching with rhodopsin occurred, such as in membranes made with C-16 PC, rhodopsin existed mostly as monomers with rotational diffusion rates similar to those found in intact disk membranes. However, when the PC acyl chain lengths were longer or shorter than C-16, rotational diffusion rates were significantly less, indicating the formation of transient protein dimers and oligomers with reduced rotational motions [188]. The results were interpreted as follows: hydrophobic mismatch is so unfavorable energetically that hydrophobic mismatching between proteins and lipids is minimized by transient formation of protein-protein complexes in the membrane plane [185,188].

Other interactions between proteins and lipids, such as electrostatic interactions between charged amino acids and phospholipids, complicate this picture, and Mouritsen and colleagues have proposed that under certain circumstances electrostatic interactions could even overcome or overrule hydrophobic matching [59,182,183]. Gil et al. [28] have proposed that lipid preference for certain integral proteins results in capillary condensation, and if this occurs around two or more integral membrane proteins, it gives rise to wetting and the formation of a capillary condensate between adjacent integral proteins, which in turn leads to a lipid-mediated joining force that drives the formation and stabilization of integral protein oligomeric complexes.

The hydrophobic matching principle may be especially important in the formation of specialized lipid domains or rafts (see next section) where enrichment in cholesterol and sphingolipids occurs, and this could be an important mechanism for selective partitioning of integral proteins into these specialized membrane regions. In this case an integral protein's hydrophobic structure must match up with the hydrophobic thickness of the specialized lipid domain to be sequestered into the domain [185,189].

Another property important in lipid–protein interactions is the propensity of some lipids to induce curvature stress and the ability of certain membrane peripheral proteins to overcome this stress [76,77]. This property is similar to hydrophobic matching, but the binding of integral proteins to particular lipids could shift the conformation of nearby integral proteins, for example, to open or close membrane channels [181]. Alternatively, the binding of peripheral membrane proteins directly to the lipid head groups could decrease or promote lipid curvature as discussed previously [77,182]. The concept of trans-bilayer stress and the mechanisms that membrane proteins use to adapt to this stress to form non-lamellar phases are important determinants in protein–lipid interactions [59].

9. Lipid–lipid interactions within membranes

As mentioned in Section 3, it has been known for some time that membrane lipids are asymmetrically arranged in cell membranes [78,85–90,190]. In addition, they are also unevenly and dynamically distributed in the membrane plane (examples [191–194]); this was also discussed and extensively referenced in previous reviews [11–18,21,22,30,38–40]. Certain lipids change the fluidity, dynamics and lateral structures of cell membranes, such as cholesterol, which as the only sterol present and the single most abundant lipid in biomembranes is particularly important in the formation of membrane domains [22,59–62,68,69,182,183,191]. Lipid–lipid in vitro interaction studies using mixtures of membrane phospholipids,

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cholesterol and sphingomyelin (in a 62:1:1 mixture) have shown that at any one time cholesterol and sphingomyelin form 1:1 dimers, although their energies of interaction are similar [192,194,195].

Mouritsen has discussed the role of cholesterol in regulating membrane organization as a compromise between cholesterol's "schizophrenic" affinity for fluid and solid phases of the lipid membrane matrix [182]. He and his colleagues proposed a new type of membrane phase, the liquid-ordered phase (to distinguish it from the liquid-disordered or fluid phase), which along with a lipid solid phase results from a compromise between cholesterol's affinities for various lipid phases [196]. Indeed, cholesterol partitions into liquid ordered and disordered phases to roughly the same extent, indicating that cholesterol does not have a strong preference for any of these phases and interacts similarly with lipids in multiple phases [22].

Moreover, cholesterol (and possibly other lipids) may play an important role in the sorting of membrane proteins and lipids through hydrophobic matching. By modifying the thickness of the hydrophobic cores of membranes certain integral proteins may be partitioned away from certain cellular membranes into other cellular membranes. For example, differences in composition found between Golgi membranes and plasma membranes, which are initially derived from membranes like Golgi, may be caused by such hydrophobic match-sorting [193]. This may be aided by the differential partitioning of cholesterol, which tends to segregate away from phospholipids with unsaturated acyl chains into membrane domains containing phospholipids with saturated acyl chains where it can form more transient, stable complexes [109,186–188].

In addition to cholesterol, sphingolipids are also important in the formation of less fluid lipid membrane domains [197,199]. Sphingomyelins and phosphatidylcholines constitute more than 50% of plasma membrane phospholipids and form the main interaction partners for cholesterol in cell membranes [199,200]. In model membranes sphingomyelins and cholesterol are critically important in the formation of liquid-ordered phases or domains that are generally surrounded by a liquid lipid phase [22,199].

The different lipid phases found in plasma membranes appear to be especially important in membrane domain formation and the lipid raft hypothesis [16–18,59,67,68,197,200,201]. The concept of specialized lipid domains or lipid rafts arose from studies in epithelial cells where the sorting of lipids into polarized membrane domains were studied by differential detergent extraction of the apical and basolateral membranes [202]. van Meer and Simons also showed that fluorescent precursors of lipids destined for apical sites were sorted intracellularly from basolateral lipids and deposited into the apical epithelial cell membrane [203].

The formation of membrane lipid nano-sized domains is now thought to be a dynamic and reversible process that can quickly change [67,68,182,197,204–207]. Lipid domain formation appears to be driven by multiple forces: hydrogen bonding, hydrophobic entropic forces, charge pairing and van der Waals forces [66,195,198]. When these interactions drive specific lipids into transient membrane meso-sized domains in cell membranes, the rather small structures that are formed are called lipid rafts [16–18,63,64,66–68,103,197,204–208].

Lipid domains, rafts and their formation in cell membranes have been the subjects of a number of recent reviews, and I won't attempt to duplicate these excellent contributions [16–18,63–69,197,197,200,205–214]. In addition, specialized structures in plasma membranes, such as rafts that contain specific lipids, integral proteins or even peripheral proteins, can constitute compartmentalized signaling platforms for signal transduction and other cellular functions [67,103,197,200,206–214]. Although there remain technical limitations that still impede investigations into the exact structural relationships between lipid rafts and the membranes from which they are derived [210–214], most investigators consider plasma membrane lipid rafts to constitute functional, dynamic submicro- or nano-sized domains (<300 nm, most ~10–200 nm) that are characterized by the enrichments of cholesterol and sphingolipids [18,63,64,67–69,207–221]. These specialized lipid domains or rafts form and dissipate rather quickly, with half-lives in the range of 10–20 ms. They are also much smaller than the typical ordered-liquid domains found in artificial membranes, and until recently their dynamics were not completely understood [213,214,221,222]. It is thought that small, unstable lipid rafts containing cholesterol and sphingolipids undergo dynamic changes which result in larger signaling rafts that are characterized by clustering and stabilization of raft molecules [214,221,222].

Technical limitations in time and space scales that are inherent with magnetic resonance techniques previously caused some confusion as to the actual rates of exchange of raft boundary lipids with the bulk membrane lipids (this has been carefully discussed by Kusumi et al. [214]). We now know that almost all boundary lipids exchange very rapidly (every 10–100 ns) with the lipids in the bulk membrane, and the presence of trans-membrane proteins increases boundary residency times. Similar to the case of integral membrane proteins, there is also a strong tendency to exclude cholesterol and unsaturated phospholipids from boundary lipids [214].

In addition to the possibility that integral membrane proteins may be sequestered into lipid rafts [206,207,210,212], lipid-linked peripheral proteins can also be caught up in lipid domains [223]. For example, GPI-anchored proteins in the plasma membrane can be incorporated into lipid rafts [223,224]. This apparently also occurs as an active process involving actin-containing cytoskeletal elements that draw small nano-sized clusters of GPI-anchored proteins into larger lipid domains of <450 nm [224]. Once in the larger domains, the GPI-anchored proteins can undergo further diffusion ("hop diffusion") between other actin-regulated domains with an average dwell time per domain of 1–3 ms [225]. In addition to being dependent on GPI for anchorage, the lipid clusters are also dependent on cholesterol, Src kinases, and caveolin [226]. In the case of the Thy-1 GPI-anchored protein/lipid raft, a complex of the trans-membrane Src kinase, along with another integral membrane protein (carboxyl-terminal Src kinase-binding protein) appears to be the trans-membrane link to the actin-cytoskeleton [227].

In their recent review on the subject of lipid rafts, Neumann et al. discussed the biological importance of lipid rafts and domains to cells [212]. The confinement of cell membrane constituents to lipid rafts or domains can signal critical cellular processes, such as endocytosis, signal transduction, cell death and other events. Since the lipids in these domains can exchange rapidly with lipids in the bulk fluid membrane as well as other rafts, the raft or lipid domain environment is very dynamic. They speculate that there may be different turnover (or hop-over) rates for each raft constituent, and it is likely that a spectrum of submicro- or nano-sized domains exists in cell membranes that contain different lipid (and protein) compositions, physical characteristics and functions [212]. Of course, none of this could have been appreciated when the F-MMM was first published [1].

The more limited mobility of lipids in specialized domains or rafts and in islands or in boundary lipids around integral membrane proteins has resulted in updated proposals on membrane structural models that limit the fraction of completely free diffusing lipids and proteins in biomembranes [17,19,22,44,59,69,228,229]. Edidin [17] (courtesy of P.K.J. Kinnunen) and Escribá et al. [228] have produced new cartoons of cell membrane structure that show specialized lipid submicrodomains around integral membrane proteins and glycoproteins and severe heterogeneity in topographic distribution and asymmetry of different membrane lipids (Fig. 4).

10. Different forms of mobility restriction in membranes

Although the original F-MMM proposed that integral membrane proteins are intercalated into a fluid lipid matrix and thus free to move laterally in the membrane plane [1], we now know that there are restrictions on the lateral mobilities of most integral membrane

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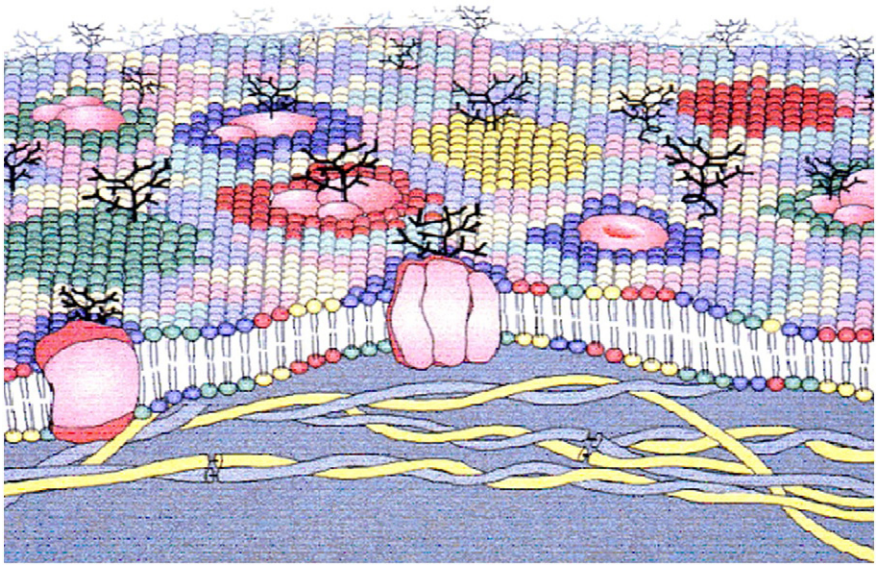

Fig. 4. A schematic illustration of a modification of the Fluid—Mosaic Membrane Model, as envisioned by Escribá and colleagues in 2008. Different lipids are indicated in various colors forming specialized domains around integral membrane proteins and gylcoproteins as well as being asymmetrically distributed across the membrane.
From Escribá et al. [228].

proteins and at least some to most lipids in the plasma membrane. In fact, this was an important aspect in cartoons published shortly (4 years) after the original F-MMM was presented [30,31]. There it was proposed that restriction of mobility could be accomplished by cis- and trans-membrane controls as well as by sequestration of integral membrane proteins into less mobile membrane domains (see Section 5 [30,31,93]). However, it took decades before these notions of mobility restraint could be carefully dissected and fully appreciated, and when they were examined in more detail, the restraint systems took on a slightly different appearance. The reason for the delay was mainly technical, and new instruments and techniques were needed to follow the lateral movements of membrane constituents in more detail [20,42–45,205,208,210,214,218,223,225,228–230].

When biomembrane dynamics began to be more precisely examined several years after the publication of the F-MMM, the lateral movements of integral membrane proteins did not fit the free lateral diffusion found for proteins like rhodopsin or integral membrane proteins reconstituted into artificial fluid lipid bilayers [20,34,109]. In fact, most integral membrane proteins had diffusion coefficients that were ten to one hundred-fold lower than rhodopsin or freely diffusing membrane proteins [20,34,109,214]. Moreover, when rotational diffusion coefficients were measured, many membrane integral proteins were only capable of rapid rotations after their cytoskeletal interactions were broken [26,231]. In some cases restrictions on integral membrane protein lateral movements were found under conditions where rotational mobilities were apparently unrestricted, suggesting that there were also indirect restraints on lateral mobility [109,232].

Restrictions on the lateral mobility of integral membrane proteins have been linked to extracellular restrictions, such as binding to extracellular matrix, to the formation of specialized membrane nanodomains, such as lipid rafts, and to the formation of large supramolecular protein complexes or domains where high protein concentrations decrease lateral diffusion rates [34,212,214,233]. The membrane-associated cytoskeletal and peripheral membrane protein barriers to free lateral movements of integral membrane proteins have been likened to inner membrane surface corrals where membrane proteins can move freely within a corral or skeletal fencework, but they only rarely cross over to adjacent corrals [34,35,43,109,234,218] (see Fig. 5). Membrane-associated cytoskeletal systems have been known for some time to be dynamically associated with immobilization or directed movements of trans-membrane integral proteins (see Section 6 for references). However, this type of domain restriction by cytoskeletal fencework is a relatively new concept [35,36,43,44,109,185]. In addition, membrane integral protein components themselves may be involved in the dynamic formation of nano-sized domains by initiating cis interactions at the extracellular surface resulting in macromolecular complexes [236].

Using various methods, such as fluorescent recovery after photobleaching (FRAP), single-particle tracking (SPT), optical laser trapping (OLT), fluorescence correlation spectroscopy (FCS), Förster resonance energy transfer and other techniques the various modes of integral membrane protein/glycoprotein lateral movements have now been revealed [34,36,39,43,44,109,162,185,222,237]. For example, Jacobson et al. [43] proposed that the lateral movements of integral membrane proteins could be placed into four different modes. Reviewing the results from three of the optical methods listed above

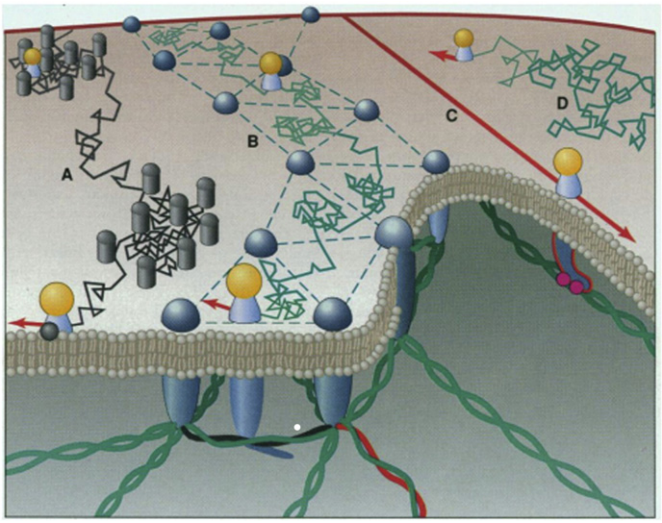

Fig. 5. Modes of integral membrane protein lateral mobility at the cell surface as envisioned by Jacobson and colleagues in 1995. Integral membrane protein lateral movements are described as: (A) transient confinement by obstacle clusters; (B) transient confinement by the cytoskeleton (fences); (C) directed motion by direct attachment to the cytoskeleton; and (D) free, random diffusion in the membrane plane.
From Jacobson et al. [43].

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(FRAP, SPT and OLT), Jacobson and colleagues described integral membrane protein lateral movement modes as: (*i*) transient confinement by obstacle clusters (also called fencepost clusters or pickets [109,238]) (Fig. 5, mode A); (*ii*) transient confinement by the cytoskeletal meshwork to defined areas (by cytoskeletal fences or corrals formed by fencepost integral membrane proteins attached to cytoskeletal elements or fences [109,238,239]) (Fig. 5, mode B); (*iii*) directed motion by direct or indirect attachment to the cytoskeleton (Fig. 5, mode C); and (*iv*) free, random diffusion in the fluid membrane (Fig. 5, mode D) [43]. A similar but slightly different list of integral membrane protein lateral movement modes was produced by He and Marguet [237]; they characterized the modes of lateral movement as: (*i*) free diffusion; (*ii*) movement limited by meshwork barriers (such as fences or corrals); and (*iii*) movement limited by traps and domains (such as lipid rafts). In any case, it is obvious that the original F-MMM description of integral membrane proteins freely diffusing in the membrane plane pertains to only one of these modes of lateral mobility.

The actual situation in cell membranes is now considered much more complicated than originally envisioned in 1972 [1]. A substantial proportion of integral membrane proteins are now considered confined, at least transiently, to small membrane nano- and micro-sized domains, lattices and corrals and are not freely diffusing in the membrane plane. Even with the appearance of impediments to integral membrane protein lateral movements, the transient times in various compartments or domains suggest that these impediments to free lateral diffusion are temporary. Thus integral proteins can escape from one domain to an adjacent domain and even escape domains altogether that limit lateral mobility, and this may be related to the sizes of their cytoplasmic structures, the natures of their cytoskeletal and extracellular interactions, and their abilities to dynamically undergo protein complex formation through cis interactions.

In addition, within the lipid environment of the cell membrane Somerharju et al. [229] proposed that there are a limited number of allowed lipid compositions or combinations that form lipid domains, and the lipid components within these domains are not randomly distributed; they tend to adopt a structured superlattice network [229].

Using keratinocytes as a cellular model Kusumi et al. [238] have estimated the approximate sizes of plasma membrane micro- and submicro-sized domains. They can vary in size from 0.04 to 0.24 μm^2, and the approximate transit times of membrane receptors in the membrane domains can range from 3 to 30 s. Overall, they propose that membrane nano-meso-sized domains are in the range of 2–300 nm diameter, with actin-cytoskeletal fence domains [239] in the range of 40–300 nm, lipid raft domains in the range of 2–20 nm, and dynamic integral membrane protein domains (dimmers/oligomers and greater complexes of integral membrane proteins and membrane-associated proteins [185]) in the range of 3–10 nm in diameter [109]. These different types of cell membrane nano-, submicro- and micro-sized domains and motility controls likely evolved for a reason, and it is probably due to the many different intra- and extracellular signals and membrane properties necessary for cellular function.

11. Hierarchical membrane structures and the Fluid—Mosaic Membrane

The plasma membrane forms a dynamic multi-dimensional architecture that can quickly respond to intracellular (and extracellular) events. In order to accomplish this Kusumi and colleagues have proposed that plasma membranes are organized into dynamic hierarchical structures [109,185]. Within these hierarchical structures membrane components (macroscopically) diffuse from 5- to 50-times slower than when the same components are reconstituted into artificial membranes without membrane-associated cytoskeletal or other structures. Conversely, the macroscopic diffusion rates can also be increased (by 20-fold) through disruption of membrane-associated cytoskeletal networks [109].

The notion of membrane-associated cytoskeletal networks impeding the mobility of trans-membrane integral proteins is not new and was discussed in many previous reviews (see Sections 5 and 6 and [30,31,34,36,125,127]). For example, cytoskeletal-disrupting drugs have been known for some time to change integral membrane protein dynamics (reviews: [30,31,114,240]). Using drugs or substances that augment or dissociate cytoskeletal networks Tsuji and Ohnishi showed that the impedance of lateral mobility of an integral membrane protein was dependent on the state of aggregation of membrane-associated cytoskeletal elements [241]. In addition, in cells where the membrane-associated cytoskeletal network was disrupted due to mutation, the microscopic diffusion rate of a trans-membrane integral protein was found to be 50-times faster than on normal cells [242]. Importantly, what is new is that the impedance of mobility (or lateral diffusion) of many membrane components can now be directly related in many cell types to cytoskeletal fencing and the formation of cytoskeletal corrals (see Fig. 5) [109,185,243]. This type of cytoskeletal control may be more complex than originally envisioned, and there are apparently different types of membrane-associated cytoskeletal structures and membrane interactions, some of which could be less susceptible to cytoskeletal-disrupting drugs. These controls could be important in regulating the lateral mobilities and distributions of membrane submicro- and micro-sized domains.

Partitioning plasma membranes and their integral membrane proteins into fenced corrals or other obstacle restraints creates relatively stable membrane zones of high densities for certain components. This can also be created under certain conditions by the formation of integral membrane protein complexes tethered directly to the cytoskeleton, membrane-associated scaffolds, or to integral membrane proteins or glycolipids sequestered into specific lipid domains [30,228]. Such properties might be necessary to facilitate further interactions, such as component clustering into supramolecular complexes, or to present enriched cell surface domains to other cells or to ligands in the extracellular environment [30,244]. Extracellular networks or lattices can also partition the plasma membrane into domains [243]. Moreover, the trapping of integral membrane proteins inside corrals constructed of cytoskeletal fencing/fenceposts (or extracellular lattices) appears to be dependent on the state of integral membrane protein complex formation. Some integral protein monomers can escape from corrals but their oligomeric complexes cannot, or at least they cannot escape at the same rates [243]. Thus the corrals (or extracellular lattices) may selectively limit integral membrane protein oligomeric complexes from freely diffusing in the membrane plane and limit them to spending most of their time in specific cell membrane domains.

What is the function of limiting the mobility of integral membrane proteins to corrals formed by membrane-associated cytoskeleton fences, fenceposts or extracellular lattices? This question is more difficult to answer. It was proposed that membrane compartmentalization could be important in signal transduction, cell activation, cell differentiation and other complex membrane events by changing the range of movements, distributions and collision rates of various cellular receptors and thus affecting their display [109,185]. Kusumi et al. [109] list a number of potential examples of this, some of which were discussed above and in other sections of this review.

The most intriguing example used by Kusumi et al. to explain the possible function of cytoskeletal corrals was that they can potentially increase the local concentrations of membrane receptors and their local rates of biomolecular collisions without affecting the overall rates of cell surface collisions [109,185]. Thus they can create spatial variations in membrane reaction rates without affecting overall cellular reaction rates. In support of this hypothesis, the rate of EGF receptor bimolecular complex formation, a necessary step in EGF receptor activation, increased with an increase in the density of actin-cytoskeletal corrals at the inner plasma membrane surface [245]. Similar spatial variations were also proposed for peripheral membrane components and membrane-associated enzymes [109].

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Kusumi et al. [109,185] have proposed that the plasma membrane hierarchical architecture consists of membrane domains or compartments: (*i*) actin-cytoskeletal limited domains or corrals formed by cytoskeletal fences anchored to trans-membrane proteins (fenceposts or pickets); (*ii*) lipid raft domains that can also contain GPI-linked peripheral proteins (see Section 9); (*iii*) dynamic, oligomeric integral membrane protein domains that may or may not be linked to the cytoskeleton. Finally, the basic nano-scale membrane feature would be a fluid—mosaic membrane.

The function of the plasma membrane hierarchical architecture would be to: (a) provide a mechanism to enhance cell membrane collision/reaction rates; (b) regulate spatial variations in duration of membrane molecular collisions; and (c) partition the entire plasma membrane into specialized domains [109,185].

12. The revised Fluid—Mosaic Membrane Model

After more than 40 years one would expect that the F-MMM would need extensive revision. In fact, the basic nano-scale model proposed in 1972 (Fig. 1) [1] does not require extensive revision beyond the versions published in 1976 (Fig. 2) [30,31], but it does require revision that takes into consideration the data published in the last four decades and some of the misconceptions inherent in the 1970s era models [1,30,31]. Thus I have attempted to present a modified F-MMM that takes into account many of the numerous contributions that have been made since the 1970s (Fig. 6). This model incorporates recent information on membrane domains, lipid rafts and cytoskeletal fencing that were unknown in the 1970s. The literature abounds with membrane models, but it should be clear that for basic membrane structure at the nano-structural level the F-MMM has been severely tested and found worthy, if not completely accurate at higher levels of organization where crowding and specialized domain formation are important structural considerations [17,41–45,59–61,66–69,109,110,181,185,200,201,205–212,220,228–239]. Indeed, membrane crowding may dominate cellular membrane structure, and most membrane models do not take this into consideration. Yeagle [246] discussed the issue of membrane crowding and concluded that most models do not take this into account. In fact, most membranes are very likely crowded structures, with integral membrane proteins separated by only a few layers of phospholipid molecules [246].

In their article Verbe et al. [44] listed a number of structural shortcomings of the original 1972 F-MMM proposal. (I will not attempt here to discuss all of the functional shortcomings of membrane models) Some of these criticisms were actually addressed in the 1976 version of the F-MMM (Fig. 2) [30,31], and I have attempted to overcome most of the remainder of these in a revised F-MMM (Fig. 6). Obviously many of these criticisms cannot be easily addressed in any cartoon model of the plasma membrane, especially properties that are dynamic and cannot be presented in a static medium, but I have attempted to incorporate most of the important criticisms of the F-MMM into a single figure (Fig. 6). In this figure different types of interactions occur with integral membrane proteins and glycoproteins, membrane lipids, and membrane-associated cytoskeletal systems and extracellular matrix components. These non-random interactions are important in controlling the mobilities, distributions and aggregation states of membrane components. Since the cell membrane is a dynamic structure, changes in the interactions of membrane components are especially important in determining functional properties. In time, this picture will undoubtedly become more and more complex, just as the original concepts of membrane structure have evolved into more and more complex structures.

An important aspect of the revised F-MMM was discussed by Bagatolli et al. [59]. That is the concept that molecules in biological membranes exist as non-uniform, non-random cooperative elements in thermodynamic equilibrium phases with compositional fluctuations. Membrane molecules can exist in domains of small-scale nano-sized structures to domains of micrometer size or more in the membrane plane. This concept was completely ignored in the original F-MMM, but it is vitally important to our understanding of non-random membrane cooperative interactions, the dynamics of membrane components and the reversible formations of membrane domains. In Fig. 6 this is

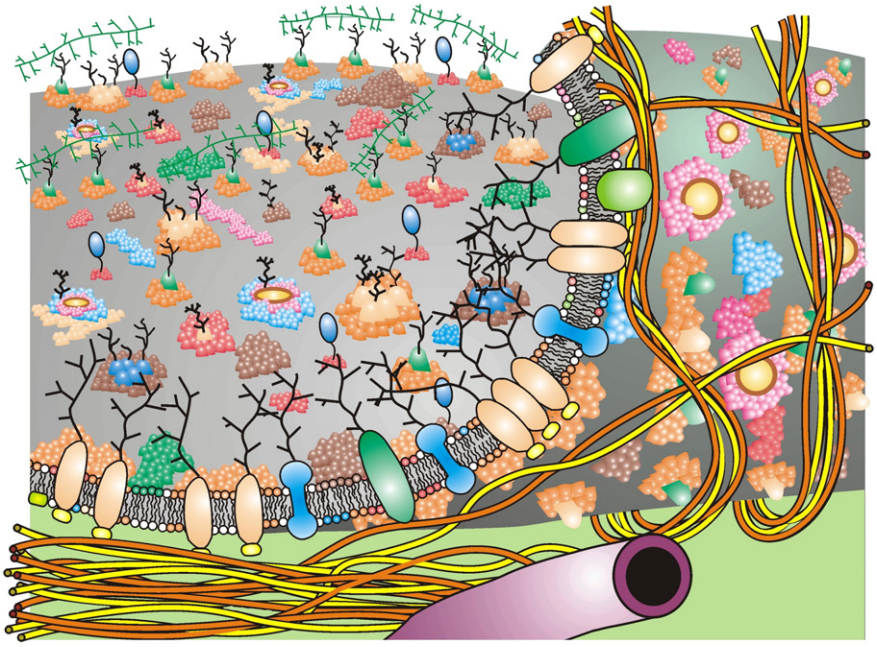

Fig. 6. An updated Fluid—Mosaic Membrane Model that contains information on membrane domain structures and membrane-associated cytoskeletal and extracellular structures. Different integral proteins, glycoproteins, lipids and oligosaccharides are represented by different colors, and where the membrane has been peeled-up to view the inner membrane surface cytoskeletal fencing is apparent that restricts the lateral diffusion of some but not all trans-membrane glycoproteins. Other lateral diffusion restriction mechanisms are also represented, such as lipid domains, integral membrane glycoprotein complex formation (seen in the membrane cut-away), polysaccharide–glycoprotein associations (at the far top left) and direct or indirect attachment of inner surface membrane domains to cytoskeletal elements (at lower left). Although this figure suggests some possible integral membrane protein and lipid mobility restraint mechanisms, it does not accurately present the sizes or structures of integral membrane proteins, cytoskeletal structures, polysaccharides, lipids, submicro- or nano-sized domains or membrane-associated cytoskeletal structures or their crowding in the membrane.

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schematically represented by molecules (within domains) of different colors, but such colors or distinctions are illusions that bear no resemblance to real membranes, nor is there any way to represent the compositional fluctuations and dynamics within the colored domains of Fig. 6.

13. Future directions

Future investigations on cell membranes will likely involve membrane modifications to improve health or correct membrane dysfunctions in transport, signaling, biosynthesis, remodeling and other membrane functions. In this context membrane lipid modifications by dietary supplements and drugs to improve function and correct defects in membrane structure, enzymatics, signaling and domain formation will be important as well as the development of new membrane-active agents to manipulate and correct pathological alterations of membrane components and their activities and dynamics [247,248].

The last few decades of research on cellular membranes has concentrated on composition, function and dynamics. Future work will likely concentrate on answering additional questions on the thermodynamics and physical explanations concerning the relationships between the structure and functional activities of membrane components.

Cellular membranes are not autonomous cellular structures, because they are linked in several ways throughout the cellular interior through cytoplasmic systems, including cytoskeletal networks, signal transduction systems, transport systems, and other structural, enzymatic and communication networks. In tissues membranes are also linked outside the cell to extracellular matrix, other cells and to interstitial protein structures. Thus cellular membranes are fully integrated structures within tissues, and plasma membranes must at the same time be sensitive and reactive to environmental changes and signals. This is probably why membranes have evolved to become such complex, dynamic structures. They have to quickly and selectively respond to a number of quite different signals from inside and outside cells. How they do this by subtle changes in membrane structure, dynamics and organization will continue to intrigue investigators for some time.

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