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The Fluid–Mosaic Model of Cell Membranes: Some General Principles

Garth L. Nicolson
Department of Molecular Pathology,
The Institute for Molecular Medicine,
Huntington Beach, California 92647, USA
gnicolson@immed.org

Abstract
In 1972 the Fluid–Mosaic Membrane (FMM) model was proposed as a nanometer-scale representation of cell membranes. It was based on some general principles, such as thermodynamic considerations, intercalation of globular, amphipathic proteins and glycoproteins into a glycerolphospholipid bilayer, independent protein and lipid dynamics, cooperativity, trans-membrane linkages and other characteristics. Earlier models proposed trimolecular structures (proteins/phospholipids/proteins) or sheets of repeating globular lipoprotein units. These earlier models were flawed, because they did not allow discrete membrane domains, autonomous proteins and lipids, or individual lateral dynamics. The FMM model was also consistent with membrane asymmetry, cis- and trans-membrane linkages and associations of components into dynamic multi-molecular complexes/domains. The FMM has remained useful for conceptualizing the basic organizational principles and properties of biological membranes. Additional important information has also been incorporated into the FMM, such as membrane-associated cytoskeletal assemblies, extracellular matrix interactions, transmembrane controls, and specialized lipid-protein domains. The presence of dense, structured membrane domains has reduced significantly the extent of fluid-lipid membrane areas, and the FMM model is now considered to be more mosaic and dense than the original 1972 proposal.

Key Word and Phrases
Biomembrane Model, Membrane Domains, Extracellular Matrix, Lipid Rafts, Membrane Structure, Membrane Dynamics, Cytoskeletal Interactions, FMM, Fluid–Mosaic Membrane, GPI, glycosylphosphatidylinositol.

1. The Fluid–Mosaic Membrane (FMM) and other Models of Biomembranes

Biological membranes are unique in their structures, compositions, dynamics and functions; however, there are some general structural and dynamic principles that govern the organization of all cellular membranes. These principles should be present in any schematic representation of biomembrane structure. Thus, the Fluid–Mosaic Membrane (FMM) model was introduced in 1972 as a simplified, general framework for the basic organization and dynamics of biomembranes. It was never proposed as the detailed explanation for every possible biomembrane [1]-[4]. Since its introduction, the FMM model has been shown to be the most practical and suitable nanometer-scale representation of cellular membrane structure and dynamics compared to other membrane models. With time, however, periodic updating has been required to update the FMM model as new data became available [2]-[5]. The updated FMM model still retains the most significant elements of cell membrane structure, such as membrane continuity, cooperativity, and asymmetry, as well as important aspects of membrane dynamics and adaptation [1]-[7].

Recently we proposed that there are a few general principles that should apply to essentially all cellular membranes [5], [6]. These general principles should be represented in any all-purpose model of biomembranes [5], [6], [8]. Some examples of some general principles of membrane structure, organization and dynamics have been presented previously [5], [6].

Principle 1, Essentially all biomembranes are held together by noncovalent forces driven mainly by hydrophobic interactions and van der Walls forces and to a much lesser degree by covalent bonds

and ionic and other interactions.

As stated in the above general principle, biomembranes in aqueous solutions are basically macromolecular structures that are bound together predominantly by hydrophobic interactions and van der Walls forces, but also to some degree by ionic interactions and a small number of covalent bonds [1]-[7]. At the nanometer organizational level these considerations should result in the creation of models that represent dynamic barrier matrix structures of amphipathic lipid and protein components associated into largely noncovalently-bound structures that show various degrees of lateral and rotational mobilities [1]-[7].

Principle 2, Glycerolphospholipid bilayers provide the basic matrix and continuity of biomembranes, and amphipathic integral membrane proteins and glycoproteins are intercalated asymmetrically into the lipid bilayer matrix.

The basic barrier structure of biological membranes is a bilayer lipid matrix of glycerolphospholipids. This was implied by the results of Langmuir [9], who experimented with oil layers on aqueous surfaces. By collecting the lipid components of red blood cells Gorter and Grendel [10] were able to estimate that these cells are surrounded by two layers of membrane lipids. Cell membrane capacitance experiments also suggested that cell membranes are approximately 4 nm thick, which is generally consistent with biomembrane lipids being present in a lipid bilayer format [11]. Some historical representations of cell membranes proposed that biomembranes were basically a phospholipid bilayer matrix plus some membrane proteins [1]-[3], [7], [11], [12].

By the middle of the last century there were basically three different competing models that represented how membrane proteins and lipids were organized to form biomembranes (Fig. 1). First, Danielli and Davson [13] proposed that cell membranes were basically glycerolphospholipid bilayers that interacted with compressed or beta-sheet-structured proteins via the hydrophilic head groups of membrane phospholipids and amino acids [13] (Fig. 1A). Robertson [14] used transmission electron microscopy to visualize this structure in osmium-fixed cells embedded in polymer resins and transversely thin-sectioned. His images revealed tri-molecular layers of osmium-stained membrane components. This visual representation of biomembranes was promoted as support for the basic organization of biomembranes as trimolecular or layered structures of protein/lipid/protein units (*the Unit Membrane*) (Fig. 1A) [14].

A completely different biomembrane model was proposed by Benson [15] and separately by Vanderkooi and Green [16]. This model was based on a monolayer of repeating subunits of lipoproteins and did not incorporate a phospholipid bilayer into its structure (Fig. 1B). The different variations of the *Trilayer* model or the *Unit Membrane* [13], [14] and the *Lipoprotein Subunit* or *Protein Crystal* [15], [16] models of biomembrane structure were not consistent with all of the available contemporaneous data on biological membranes [1]-[12]. Of the proposals for biomembrane structure and organization, only the FMM model has fulfilled all of the known basic properties and characteristics of cellular membranes that were available in the late-20th century (Fig. 1C) [1]-[12].

*Principle 3***,** Biomembrane components usually maximize compatible molecular interactions, such as hydrophobic matching of proteins and lipids, in order to approach the lowest free energy state.

The concept that non-covalent hydrophobic forces are the principle driving force resulting in the matching of hydrophobic and hydrophilic regions of integral membrane components along with the exclusion of water from the hydrophobic regions of biomembranes turns out to be the most likely reasons for membrane stability in aqueous media [1]-[9], [17]-[21]. Thus, when amphipathic membrane proteins are newly synthesized on intracellular membranes, they are folded and intercalated asymmetrically into a lipid bilayer matrix to form new membrane [19]-[22]. This newly created membrane structure is held together primarily due to the hydrophobic effect and van der Waals forces, allowing biomembranes to resist stresses, such as mechanical forces, and approach the lowest free energy state [1]-[4], [17]-[19], [21], [23].

Principle 4, Biomembranes are generally organized or segregated into various domains or regions of different compositions, rotational and lateral mobilities, lifetimes and functions.

Biomembrane lipids, and in particular membrane glycerolphospholipids, can exist in different phase states—in their simplest forms as solid gel and fluid phases, or more properly as solid-

ordered, liquid-ordered, or liquid-disordered states. One of the important characteristics of biomembranes is the presence of different, distinct membrane regions of diverse lipid and protein compositions. In addition, the spatial arrangements, rotational and lateral mobilities and lifetimes of components of these diverse membrane regions are also different from the bulk or average properties of membranes [21]-[26]. These different characteristics also extend to the inner and outer leaflets of the lipid bilayer, and this type of complex structure forms the basis for the existence of different, compositionally distinct, lipid and protein membrane regions that have been termed membrane domains [24]-[26].

Membrane domains are distinct membrane regions that differ in composition and physical properties from bulk biomembranes. Membrane domains are also considered to be functionally important in the determining the regional properties of membranes and many of the unique organ and system activities of specialized cells [23]-[27]. They were originally conceived as ordered structures in a generally fluid membrane that formed as a consequence of the lateral segregation and association of specific lipids and some unique proteins. Moreover, these domain structures differed from surrounding membrane in their molecular compositions, physical properties, lifetimes, and importantly their functions [26], [27]. Although membrane domains were not specifically mentioned in the original publication on the FMM model [1], they were proposed shortly thereafter in my subsequent reviews on membrane structure and organization [2].

Membrane domains are dynamic lipid and usually lipid-protein structures that can form, associate, disassociate or disperse over time [3]-[6], [18], [21]-[26]. Although the concept of membrane domains with specialized lipid and protein compositions began initially with the simple concept of small, dynamic microdomains (*'lipid rafts'*) that are involved in certain forms of cell signaling [27], [28], more recently this concept has been expanded to include much larger, less dynamic membrane domains that are involved in quite different functions, such as linking these membranes to other intracellular or extracellular structures [2]-[6], [24], [25]. At the extreme culmination of this concept, or at the maximum size extreme, there are particular domains on specialized cells, such as epithelial cell-extracellular matrix-linked basal domains. These large, less dynamic domains can involve sizable fractions of a cell surface membrane and are essentially membranes immobilized by extracellular matrix and intracellular cytoskeletal interactions that result in physically and functionally polarized cells [29]. Using atomic force microscopy there is now evidence for the physical existence of membrane domains [24], [25]. Evidence for the existence of functional membrane domains or specialized cell membrane regions in specialized cells has existed for some time [24]-[27].

2. Current Views of the FMM Model and Some Other Characteristics

Since it was proposed in 1972, the FMM model has obviously required certain updates or refinements as new data became available. Major updates were published by us in 1976 [2] and 2014 [3], and minor updates in 2021 [4], 2022 [5] and 2023 [6]. There were also a number of updates of the FMM model published by others [7], [8], [12], [18], [21]-[27], [30] to list a few—some additional updates will be discussed below).

Any replacement for the FMM model would likely be very similar to the original basic FMM model, but more complex [4], [5], in order to be consistent with available data. First, we know that there are large numbers of unique membrane proteins/glycoproteins and lipids/glycolipids that are present in each type of cellular membrane [12], [18], [20], [21], and this provides a diversity of possible structures, dynamics and molecular interactions that are observed in different membranes at above nanoscale distances [4], [7], [12], [17]-[19], [23]-[28]. The unique structures and organizations of specific cell membranes are not usually features found in non-specific or general models of biomembranes. However, there are some basic principles that are important in any general representation of biomembrane structure and organization. That such general principles might relate to how membranes interact with other cellular and extracellular components will be considered later in this brief review.

Importantly, we realize that biomembranes are much more complex than the simple, stylized, basic models suggest [5], [6]. For example, membrane lipids comprise several classes that contain glycerolphospholipids, sphingolipids, sterols, and other lipids [18], [20]-[23], [27], [28]. Due to

their cooperative nature and hydrophobic matching properties various combinations of these lipids at physiological temperatures organize nonrandomly into lipid bilayers and then by lateral segregation form dynamic nanometer to micrometer-sized lipid assemblages or domains of more or less defined compositions [23]-[28]. As described above, such lipid domains usually possess properties different from the bulk membrane lipids. In addition, some ordered-lipid domains can occur basically as islands within regions of or surrounded by disordered fluid-phase disordered-lipids. Under physiological conditions these membrane lipids form into various sized lipid domains that can exist as dynamic solid-lipid or ordered-lipid domains within zones of disordered-fluid or ordered-fluid state lipids [21], [23]-[28]. Such reorganizations of lipids and proteins in membranes can also change the local thickness of the membrane, which can vary slightly depending on the matching of lipid fatty acids and integral membrane proteins [30].

Principle 5, The interactions of membrane lipids and proteins are driven by hydrophobic matching of their hydrophobic surfaces, resulting in molecular sorting that can give rise to various dynamic membrane domains of differing compositions, component mobilities, lipid-lipid and lipid-protein interactions and lifetimes.

Similar to the diversity of membrane lipids in biomembranes, membrane proteins/glycoproteins can be assigned to different categories or groups based on their amino acid sequences, amphipathic and other properties, and functions [1], [3], [17], [20]. In the FMM model proteins and glycoproteins were divided to three simple categories: integral, peripheral [1]-[3], [17] and (proposed subsequently) membrane-associated [2], [3]. Integral or intrinsic membrane proteins were considered to be globular, amphipathic proteins that intercalate into the membrane lipid bilayer matrix and are largely stabilized by hydrophobic forces. Their exposed hydrophobic surfaces directly interact with phospholipid fatty acid hydrophobic tails or other hydrophobic moieties. Fig. 3C shows the original FMM model where only integral membrane proteins are shown without lipid sorting or hydrophobic matching [3], [4].

In the FMM model integral membrane proteins penetrate into the membrane lipid bilayer hydrophobic matrix to various degrees, from completely spanning the membrane to barely intruding into the lipid bilayer. This degree of lipid penetration depends on the extent of protein hydrophobic surfaces and the ability to form matching hydrophobic lipid interactions [1]-[4], [18], [20]. It was also proposed that bilayer thickness, intrinsic lipid curvature, elastic compression and other properties of the lipid bilayer can vary around membrane proteins according to the nature of the proteins and surrounding lipids and that this can modify membrane protein function [30], [31]. This suggests that under certain conditions the lipid bilayer can be an allosteric regulator of membrane function [31].

In contrast to integral membrane proteins, peripheral membrane proteins were never proposed to be essential to membrane integrity [1], [2]. They were proposed to be attached or linked to membranes mainly by electrostatic bonds or other forces [1]-[4], [17], and in some cases linked to membranes via covalent bonds with phospholipids (reviewed elsewhere [26], [27]). Peripheral membrane proteins were thus removable from membranes without demolishing basic membrane structure and continuity [1]-[3], [17]. They have been identified as important components in providing membrane integrity, curvature, scaffolding, and other properties that are important to membrane stability, elasticity and rigidity as well as cell shape and cell physical characteristics [3]-[6], [32], [33]. In addition, peripheral membrane proteins can also serve as attachment points or receptors for enzymes and signaling complexes [2-6].

The different natures of lipid and protein interactions are not only structurally and organizationally significant, but they are functionally important as well. Many membrane proteins are modulated in their functional activities by surrounding and sometimes tightly bound lipids. Some examples are ion channels, membrane receptors, enzymes and other membrane proteins [4], [18], [21], [30], [31]). Removal of these specific, often tightly bound, lipids usually results in loss of function.

Principle 6, The lateral mobilities of certain cell membrane proteins may, under certain conditions, be controlled by membrane-associated components, such as cytoskeletal elements, extracellular matrix or stromal components.

It became necessary within a few years after the original FMM model to add another cellular membrane protein category: membrane-associated proteins [2]. Membrane-associated proteins were not considered integral or essential to membrane structure, but they were deemed to be important in controlling the stability and mobility of certain membrane domains or individual membrane proteins. They are also thought to be important in integrating or binding plasma membranes with other intracellular (or extracellular) structures [3]. Membrane-associated proteins can be globular in structure, but they are not generally amphipathic or tightly associated with the hydrophobic lipid matrix region of membranes, with some exceptions [34]. They are thought to be attached to membranes mainly by weak electrostatic forces [34]. Membrane-associated proteins are thought to be transiently associated with membranes through direct interactions with integral membrane proteins or specific membrane lipids [1]-[4], [34], [35].

The membrane-associated protein category was proposed to explain the presence of cytoplasmic polymeric protein structures that link cell membranes to other intracellular membranes and cytoplasmic components [2], [3], [4]. Often these polymeric structures, such as cell cytoskeletal elements, can immobilize large regions of plasma or other membranes. At the extracellular surface specific attachment components can also connect plasma membranes to extracellular matrix and stromal elements [2]-[6]. The first example of membrane-associated components at the cytoplasmic membrane surface were actin-containing cytoskeletal elements [2], [3]. Other examples are membrane structures at specific membrane synaptic regions in neurons and muscle, as well as in cilia and other structures in other cell types. In neuronal synapses neurotransmitter release and the binding of neurotransmitters to postsynaptic receptors are dependent on membrane lipid and protein dynamic interactions and the involvement of membrane-associated cytoskeletal elements [35].

Membrane-associated proteins are also involved in controlling membrane and cell shape and structural integrity, including cellular rigidity and tension and the regulation of trans-membrane protein dynamics [2]-[6]. They can also provide links to other intracellular and extracellular proteins, glycoproteins and polysaccharides [2]-[6]. Under certain circumstances they are also involved in pathogenic processes, such as the formation and release of exosomes that are involved in cancer metastasis and immune regulation [36].

The lateral movements of integral membrane proteins can vary from essentially little mobility over a time span of minutes (or only rotational movements within the time frame of seconds) to a variety of lateral mobility rates [37], [38]. These lateral movements can be random or nonrandom and can occur along apparent routes, as if such movements were controlled by forces other than those within cell membranes themselves [38]. There are also a number of restraining interactions that can be dynamic and potentially limit protein/glycoprotein lateral mobilities and direct the lateral displays of components on membrane surfaces [5], [6], [37], [38].

Summarizing, cell membranes are known to be dynamic, flexible structures that can be distorted, deformed, compressed or expanded by different forces [3], [4], [18], [19], [31], [38]. They are also known to contain heterogeneous domains or regions that have proved to be more mosaic than fluid. Membrane proteins play and important role in controlling membrane shape and function. Indeed, certain peripheral membrane proteins can bind to membranes and cause membrane deformation and produce membrane distortion and curvature as a result of flexing and bending of membranes to fit peripheral protein structures [31]-[33]. In contrast to the direct interactions of peripheral membrane proteins on membranes, membrane-associated proteins are thought to act indirectly on membranes, usually through intermediate protein or lipid attachments [3], [4], [7], [34]. Cell membrane-associated proteins/glycoproteins can be present in the cell cytoplasm or in the extracellular space between cells or between cells and stroma. By stabilizing cell membranes, membrane-associated proteins can modulate the dynamic properties of membrane proteins and glycoproteins and attenuate or stimulate their lateral movements [2]-[5], [36], [38]-[40]. Membrane-associated proteins can also restrict certain membrane components to specific membrane domains, or limit the dynamics of the domains themselves [4]-[6], [38]-[40]. They can also be involved in energy-dependent directional movements of membrane complexes [2]-[6], [36]-[41]. There are examples of membrane-associated polymeric actin-containing protein complexes that are involved in energy-dependent directional movements of cell membrane domains and

membrane components, and they are involved in multiple activities, such as cell motility, endo- and exocytosis, cell shape changes, among other phenomenon [36]-[41].

3. Versions of the FMM Model that Go By Other Names

Over the last 50 years new biomembrane models that are actually just versions of the FMM model have been offered as replacements for the original model [42]-[45]. It was also suggested that there is no basis for a general nanometer scale model of cell membranes [46]. We have disagreed with this notion [5], [6]. The FMM model still represents the best textbook nanometer-scale model for the basic structure, organization and dynamics of cell membranes, irrespective of the evolving array of diverse properties and functions of different cellular membranes [1]-[7]. Although there are shortcomings in the 1972 FMM model (mostly due to new discoveries that could not have been foreseen over 50 years ago) [4]-[6], [18], [37], [41]-[49], the failure of the FMM model to specifically explain all of the intricacies of cellular membranes and their dynamic properties is not a sufficient reason to discard the FMM model. For example, the FMM model could never foresee the extreme range of lateral mobilities and compositions of various membrane components and other properties [2]-[6], [24], [27], [30], [38], [42]-[49]. Such considerations do not detract significantly from the FMM model and do not eliminate it as the accepted basic nano-scale model for cellular membrane structure and organization [3], [4], [6].

Principle 7, Biomembranes are asymmetric in the distributions of membrane proteins, glycoproteins and peripheral and membrane-associated proteins as well as phospholipids on inner and outer membrane leaflets.

Membrane asymmetry was mentioned (and supported with actual data) and incorporated into the 1972 FMM model [1]. The possibility of widespread flip-flop of membrane components was considered unlikely for most components by estimating the amount of free energy required to flip amphipathic membrane components from one side of a lipid bilayer to the other side [1], [2], [17]. Every cell membrane examined thus far has been found to be asymmetric in the display of membrane components on the interior and exterior membrane surfaces [1]-[7], [17], [50].

The basic features of the FMM model at the nanoscale level have been recognized as remarkably consistent with most experimental findings over the last 50 years [1]-[6]. Although a few recent publications have added some details on membrane structure and dynamics, the basic nanoscale features of the FMM model have not changed significantly over the years [1]-[7], [38], [42]-[49], [51]. The addition of new data on membrane dynamics has added essential new and useful information. Thus the wide-ranging variations found in the dynamics of cell membrane components, such as in the lateral mobilities of various protein and lipid constituents, and the additions of membrane domains and other specialized structures have added important new information on membrane structure and dynamics [2]-[6], [37]-[40], [42]-[49]. Some cell membrane features, such as membrane mosaic complexes (densely displayed multi-component complexes), specialized membrane domains (lipid rafts and other domains), membrane-associated components (cytoskeletal elements, and other structures), have been added to the FMM model [2]-[7], [18], [21], [23]-[28], [37]-[49]. Therefore, the FMM model has undergone various updates over the years since its introduction. This has resulted in an updated nanoscale model that is certainly more complex, less homogeneous, and linked to other structures, resulting in a more densely packed (more mosaic) structure than the original 1972 proposal [2]-[6].

Principle 8, Cellular membranes are more compact (mosaic), complicated and have a more multi-component organization than originally envisioned in 1972, and this restricts the dynamics of proteins and lipids and some domains.

We are left with the concept of a hierarchical, yet dynamic cell membrane organization that is more mosaic and more diverse in its component mobilities than any of the 1970s notions of cell membrane structure and organization [3]-[7], [45], [48], [49]. This recent hierarchical scheme of cell membrane organization is definitely more layered and complex in its structural features in order to account for recent data on the presence of macromolecular structures that act as circumscribed barriers to protein and lipid lateral mobilities and limits to membrane component displays [4]-[7], [38], [45], [48]-[49].

4. Membrane Domains, Vlustering of Membrane Components and the FMM Model

In the original FMM model the presence of oligomeric protein/glycoprotein structures in cell membranes was presented but not discussed in any detail [1], as were the possible roles of specialized lipid-protein regions within membranes [2]-[5], [7], [38], [42], [45], [48]. At the early stages of the evolution of membrane models, speculation was more prevalent than actual acquisition of critical data. However, many of the giant gaps in our fundamental knowledge of biomembrane structure and dynamics began to slowly close with the development of newer experimental procedures that allowed membranes to be studied dynamically at higher resolutions [38], [48], [49].

There are also questions from the FMM model about the states of aggregation and dispersion of some membrane components [2]-[4], [38]. That cell membrane proteins/glycoproteins can exist in normally dispersed or micro-clustered distributions was discussed early on [3], [4], [7], but the role of clustering of membrane components or the formation of ultra-dense, specialized membrane regions has only recently become considered as a determinant of membrane function [47]-[49], [51]. At an early stage in the examination of the state of dispersion/clustering of membrane components it was known that similar components on red blood cell membranes from different species could show quite different topographic distributions (micro-clusters or completely dispersed), but the functional reasons for this remained elusive [52], [53].

The notion that the formation of stable micro-clusters of proteins/glycoproteins in plasma membranes is a rather common event has been confirmed using new technologies developed to study the localization, clustering and dynamics of single cell surface molecules at nanometer resolutions [48], [51], [54]-[56]. Using live cells and fluorescent-particle tracking/fluorescence microscopy the associations of membrane proteins and their abilities to cluster at high spatial and temporal resolutions have now been possible [48], [54[, [55]. Such procedures have led to investigators to conclude that some cell surface receptors are normally present in small nanoclusters but have the ability to undergo further clustering. The resulting larger aggregations of receptors turn out to be important in providing the greatest receptor signaling efficiencies for cells after the binding of specific signaling ligands [54], [55].

With time, the basic nanoscale structure and organization found in cell membrane models has evolved from the 1972 FMM model with the incorporated of new information (for example, see [4]-[7], [43], [45], [49]). Newer models of biomembrane structure contain additional membrane features, such as membrane lipid signaling domains (often called lipid rafts), and components with exceptionally different dynamic properties from bulk membrane components. Importantly, in the newer versions of the FMM model the displays of protein components has resulted in increased membrane protein densities and clusters of tightly bound membrane components (thus more mosaic and less fluid in organization [3]-[5], [45]).

The addition of domains of varying sizes, compositions and mobilities to membrane models has added additioinal functional significance to the FMM model [2]-[7], [25], [28], [44], [49], [51], [57]. These revised models are consistent with estimates of the spatial distributions of membrane proteins in living cell membranes in applied electrical fields [54], [55]. The dynamic issues and the topical distributions of membrane components have functional consequences. For example, when cell signaling is initiated by the binding of specific ligands, some of the involved structures can form into specific regulatory and mechanical platforms (due to clustering) that subsequently become linked to various intra- and extracellular components, including the cytoskeleton and other structures. This complex sequence of events is necessary for signals to be transmitted into a cell's interior [3]-[7], [38]-[40], [48], [49], [51], [56].

In the 1972 FMM model cell membrane components were portrayed as basically randomly distributed and unrestrained in their lateral movements [1]. We have known for almost 50 years that this is not an accurate portrayal of most membrane components [2]-[7], [38]-[40], [42]-[49], [51], [54], [55]. However, in contrast to the statements of some authors [42]-[46], certain membrane properties, including the variable lateral mobilities of membrane components and domain formation, have been integrated into updated FMM models for some time [2]-[7].

In the FMM model membrane lipids are particularly important and form the matrix of most cellular membranes [1]-[6]. Cellular membranes are known to contain hundreds of different lipid

types, and most of these minor lipids are present in minute concentrations [11], [18], [21], [57]. We still do not know the function of these minor lipids in membrane activities. Most membrane lipids were originally thought to be capable of free lateral motion [1], but shortly after the FMM model was introduced, we had to conclude that many membrane components are mostly restrained in their lateral mobilities, especially if they are present in non-fluid lipid domains [12], [18], [21], [23], [57].

The functional consequences of the presence of low abundance membrane lipids in various cellular membranes remains unknown. As briefly described above, in the bulk membrane lipids the hydrophobic phospholipid fatty acid tails of specific phospholipids can interact with the hydrophobic surfaces of certain membrane proteins, and to a lesser degree there can also be some secondary hydrophilic interactions between protein ionic and zwitterionic amino acids and the charged head groups of specific phospholipids. Such interactions can be used to stabilize membrane domains and prevent their disassociation into freely mobile membrane components [18]-[21], [27], [30], [35], [43].

Principle 9, Specific membrane lipids present in specialized lipid and lipid-protein domains are a common feature of biomembrane structure and function.

Some membrane lipids, such as sphingolipids and other phospholipids, are quite important in the formation of specialized membrane lipid mosaic-ordered domains [21], [26]-[28], [47], [57]-[63]. There are different types of membrane lipid domains, but one type of lipid domain forms from phosphatidylcholine segregating with sphingomyelins and cholesterol into specialized mosaic-ordered lipid domains or lipid rafts [18], [24]-[28], [47], [48], [60]. Lipid domains/rafts in cell membranes are generally bordered by liquid-phase lipids. Therefore, these lipid domains have the ability to undergo some lateral movement in a liquid-phase lipid membrane environment [61-65]. Membrane lipid domains/rafts can also recruit additional lipids and proteins into their structures, and this appears to be important, at least in some instances, for their physiological functions [43], [44], [47], [60]-[65].

The lipids within mosaic-ordered lipid domains are not completely immobilized and restrained. These lipids still maintain some rotational and lateral mobilities as well as the abilities to exchange with bulk lipids. Indeed, even lipids in mosaic-ordered domains are able to slowly exchange with some surrounding bulk membrane lipids as well as with lipids in other mosaic-ordered lipid domains [43], [58], [61]-[65]. The lipid mosaic-ordered domains, such as lipid rafts, are usually less than 300 nm in diameter. Although most lipid mosaic-ordered domains are approximately 10-200 nm in diameter, some are slightly larger [47], [62], [64], [65]. Moreover, the sizes of lipid mosaic-ordered domains are not fixed. They can undergo domain-domain clustering, in some cases induced by proteins and protein-lipid interactions, resulting in an increase in domain diameters to micrometer size ranges, or >300 nm in diameter [47], [64], [65].

Functional membrane lipid domains or rafts, such as those involved in cell signaling, usually contain some peripheral and integral membrane proteins as well as lipid-linked proteins [60]-[62]. These mixed lipid-protein domains are not static in composition or distribution [58]-[62]. They can undergo further changes in lipid and/or protein composition, and these changes/additions can convert passive domains/rafts into active, functional membrane platforms that can initiate signal transduction. These different trans-membrane-coupled, mixed protein-lipid domains/rafts are thought to initiate various cellular events, such as immune signaling, host-pathogen interactions and cell death programs [61]-[65].

5. The Concept of Trans-Membrane Control

When the FMM model was first presented in 1972, there was little to no information in the literature on membrane interactions with intracellular and extracellular components [1]. Although membrane interactions were assumed to be important in the attachment of cells to extracellular matrix and stroma as well as for maintenance of cell structure, integrity and movement, the precise membrane molecular linkages were yet to be determined. Within a few years after the introduction of the FMM model it was apparent that intracellular and extracellular connections through cell membranes could alter cell membrane dynamics and trans-membrane control mechanisms [2]-[6], [66], [67]. The existence of specialized membrane domains and their dynamic interactions with

membrane-associated structures was published after the original FMM model was introduced [1]. However, by 1976 new membrane attachments, such as cytoskeleton-trans-membrane linkages, were recognized as important in immobilizing membrane domains as well as directional movements of domains using energy-dependent cytoskeletal assemblages [2], [3]. The roles of cytoskeletal and extracellular elements in membrane properties are now well-accepted, and they are now known to be essential in cell movements, maintenance of cell polarity and preservation of tissue mechanics and organization [2]-[6], [37]-[41], [48]-[49], [68]-[71].

Of the textbook models of cell membranes, most of them present cell membranes as independent structures that are not linked to cytoplasmic components inside the cell and to extracellular components outside the cell [2], [3]. At least for plasma membranes, we know that they are fully integrated with intracellular and extracellular elements, and this is particularly true for cells within tissues and organs. Cell shape, orientation, stabilization, adhesion, movement, communication and other attributes are influenced or controlled by membrane-cytoskeletal and membrane-extracellular matrix linkages [2]-[6], [36], [39]-[41], [68]-[72]. Membrane distortion, domain formation, clustering, submembrane plaque assembly, endocytosis, exocytosis, and other features of cells are all impacted by various intra- and extracellular linkages [3]-[7], [36], [39]-[41], [68]-[71], [73]-[76].

Principle 10, Cell membranes have specialized domains that are involved in dynamic trans-membrane extracellular and intracellular linkages that are important in a variety of cellular properties, such as cell signaling, communication, endocytosis, exocytosis, and other features of normal cell physiology.

In well-evolved species with multiple tissues and organs cell signaling events are vital for homeostasis of the organism, and they are especially important for circulating cells. There are a variety of cell signaling mechanisms that are linked to various cell types, including those signals mediated by soluble ions, endocrines, hormones, lipids, peptides and released membrane vesicles (exosomes) and membrane fragments [36], [77], [78]. Most cell signals, such as various soluble and membrane-bound signaling molecules, arrive at a plasma membrane surface of a specific cell type and attach to specific receptors. Next, receptor conformational and aggregational changes result in membrane clusters that provide information that can be transmitted across the membrane to the inner membrane surface where additional component clustering can occur. Eventually, inner membrane surface proteins or cytoplasmic proteins and enzymes can attach to the aggregated structure at the inner membrane surface to form super-sized complexes that can actively transmit signals to a cell's interior [3]-[6], [39]-[41], [49], [68]-[71].

On the exterior membrane surfaces of cells various membrane protein-lipid domains (or alternatively small lipid rafts) appear to be important in the initial stages of trans-membrane signaling [26]-[28], [61]-[65]. This is particularly relevant for membrane domains that contain glycosylphosphatidylinositol (GPI)-linked proteins [26], [27], [61]-[65], [79]-[81]. The formation and dynamic movements of receptors and their trans-membrane linkages play important roles in trans-membrane signaling pathways [60]-[65]. This is but one example of dynamic cell membrane processes that may be initiated by the formation of nanoclusters within membrane domains or the coalescence of small domains into larger structures that constitute signaling platforms [3]-[6], [64]-[69], [80]-[84].

6. Hierarchical Membrane Structure and its Importance in Membrane Dynamics

In addition to the organization of cell membranes into domains and their regulation by trans-membrane events, there are even larger protein-rich domains on the exterior and interior surfaces of most tissue plasma membranes that are important in tissue organization and other properties [7], [49]. In some cell membranes these larger multi-protein structures provide barriers to the free movements of other integral membrane proteins and thus are important in the maintenance of very large domains that appear to be important in certain cellular activities [7], [38], [48], [49]. For example, some cell membrane components found in these specialized membrane domains appear to have a variety of restrictions on the free rotational and lateral mobilities of trans-membrane and inner membrane proteins [38], [48]. These mobility restrictions can affect residence times within specific domains or membrane regions [7], [37], [38], [48], [49]. In addition, differences in the

mobilities and distributions of specific membrane proteins may be affected by local domain compositions, spatial variabilities, trans-membrane linkages and membrane obstacles that limit free mobility of cell membrane components [3]-[7], [37], [48], [49], [68]-[71].

The presence of particular membrane proteins and lipids, the existence of peripheral and membrane-associated proteins as well as various linkages to extracellular and intracellular structures and other factors may be collectively important in regulating and controlling membrane organization and dynamics [2]-[7], [37]-[40], [45]-[49]. The consequences of this type of regulation and control in membranes are to fine tune signals into and out of particular cells and change their physiological states.

Principle 11, Cell membrane components exhibit different lateral mobilities and distributions that appear to be functionally important and related to interactions with various membrane structures, barriers and domains. These interactions may be involved in maintaining the physiological states of cells.

The functional importance of different topographical distributions and lateral mobilities of integral membrane glycoproteins and lipids in cell membranes has been the subject of several investigations that seek to understand how information can be passed between cells in order to maintain organ and tissue homeostasis [3]-[7], [23]-[25], [37], [45], [49], [51], [56]-[58], [67]-[69], [75], [81]. The various ways in which membrane signaling and cell physiological maintenance are regulated may be determined by membrane dynamics. Examples of this in various cell types can be observed by following the different categories of lateral movements of specific cell surface receptors. Previously various cell membrane protein dynamics were portrayed as: (*a*) random movements or free diffusion in fluid membrane regions; (*b*) transient movements confined by membrane obstacles, such as protein clusters or aggregations of membrane proteins; (*c*) transient movements that are constrained by structural domains or by barriers (sometimes called ‘corrals’) composed of cytoskeletal elements and their membrane attachment molecules at the inner cell membrane surface; or (*d*) directed movements due to trans-membrane protein attachment to and contraction of a membrane-associated cytoskeletal system (reviewed in [4]-[6]).

What has been added to previous or earlier versions of the FMM model in order to explain the complex dynamics of membrane components is the addition of mosaic structures or formation of dense domains of integral membrane proteins. This is consistent with observations that some integral membrane proteins appear to be localized within dense mosaic structures. Thus some membrane proteins appear to be inherently clustered in their distributions on the surfaces of cells. Inside these mosaic structures integral membrane proteins appear to be relatively immobilized or their movements restricted, and thus they are not freely mobile and capable of movement into other membrane domains or membrane regions [4]-[6], [32], [51]. The consequence of this is the existence of particular membrane domains with essentially immobilized components that are incapable of exchange with similar components outside of their own domains.

There is also an alternative to the essentially permanent localization of certain membrane proteins within specific membrane domains. This is observed for certain cell membrane proteins (or other components) where cell surface localization may be only partially confined over time to particular membrane domains, even those domains with protein structural barriers [4], [5], [7], [49]. Thus intermittently mobile integral proteins and some lipids may be able to move from one domain to other domains.

Many integral membrane proteins may have their amino acid sequences folded or arranged to recognize self, and this allows them to aggregate into stable clusters. Alternatively, they may undergo dynamic changes in clustering and dispersion. Eventually this can result in the formation of super-sized mosaic structures in membranes or their disappearance over time [3]-[6], [43], [51]. Super-sized membrane mosaic structures may be important in initiating the internalizing of membranes into endosomes, or releasing membrane domains in extracellular vesicles or exosomes [31], [69], [73], [74]. The movements of membrane lipids and proteins/glycoproteins between adjacent membrane domains may be limited by the strength and extent of their interactions with similar or different domain components, much of which may be driven by hydrophobic matching and the fluidity or lack of fluidity in the surrounding lipid matrix [25],[38],[54],[55].

Principle 12, Cell membranes are organized at multiple levels. The first or basic level is represented by a lipid bilayer plus intercalated, amphipathic proteins. The next level is represented by membrane domains that contains specific mixtures of lipids and integral and peripheral membrane protein. The next level is the complex domain level which additionally contains various complexes and membrane-associated cytoskeletal elements and/or extracellular matrix components. Finally, at the hierarchical level all of the above can exist plus additional structural or barrier elements that produce additional restrictions on component mobilities.

Although cell membranes are dynamic structures, they are also well regulated and organized into various less dynamic domains. Thus cell membranes may be characterized by their component compositions, sizes, interactions, dynamics, and linkages to other membrane and membrane-associated structures (such as cytoskeletal and matrix elements). Importantly membrane components are organized into specific domains that can be unlinked or linked to other membrane-associated structures. For example, protein components can exist in small lipid domains or rafts, or larger mixed protein-lipid domains, or in rather large, complex lipid-glycoprotein-membrane-associated cytoskeletal domains that are linked to other structures inside or outside cells [27], [28], [36], [38]-[40], [43], [49], [60]-[65], [68]-[71].

Although the first membrane domains that were enriched in isolates were small lipid domains enriched in sphingolipids and cholesterol [24]-[28], there are other possibilities of membrane domains with compositions different from bulk cell membrane compositions [25], [28]. Membrane domains are thought to exist in a variety of sizes and compositions [25], [27]. The approximate diameters of commonly found membrane domains have been estimated [4], [5], [49]. The smallest domains seen in cell membranes have been nano- or meso-sized domains where their diameters can vary from 2–300 nm. The smaller more lipid domains, such as 'lipid rafts,' are usually 2–20 nm in diameter, whereas simple protein-lipid complexes of one to a few integral membrane proteins surrounded by specific, tightly-bound lipids can vary from 3-10 nm in diameter. Some integral membrane proteins can also aggregate into much larger membrane complexes or specialized domains that can be over one-hundred-times larger. There are also a variety of complex, lipid-protein-actin-containing cytoskeletal-linked domains. For example, the 'fence' domains possess diameters that vary from 40–300 nm. Finally, there are also micro- and submicrometer-sized mixed domains that are characterized by having surface areas that can vary from 0.04-0.24 μm^2 [7], [49]. The diameters and areas of membrane domains can vary widely and their range may only be a transient characteristic.

Thus, membrane domains can show extreme variations in size and surface area, as has been documented with fluorescence microscopy using fluorescent-labeled antibodies or fluorescent-labeled lipids. By using intact antibodies membrane components can also be aggregated into extremely large polar aggregations, as has been observed using fluorescent-labeled antibodies [1]-[6].

Principle 13, Cell membranes are characterized by dynamic changes in domain mobility, area, composition and structure. The assembly and disassembly of various domains may allow cells to react to microenvironmental signals, and also transmit signals received at the cell surface to intracellular targets.

A significantly more complex view of cell membrane structure and organization (hierarchical organization) was proposed by Kusumi et al. to explain the complex organization of membranes and the rapid and selective responses of cells to specific extracellular signals [5], [49]. If cell receptors are limited in number or expressed in dispersed distributions, they might not be functional if receptor clustering is a requirement to initiate signaling. It may be more efficient to have various receptors pre-positioned on the cell surface in more concentrated arrays within signaling domains so that they can rapidly aggregate into supramolecular clusters that are competent for trans-membrane signaling [49]. The presence of molecular 'fences' or membrane protein barriers on the inner cell membrane surface are thought to limit the range of lateral motions and densities of integral trans-membrane protein components. This could create more stable, local membrane domains with higher receptor densities. Thus the pre-positioning of receptors in more densely arranged domains is likely to make them more proficient at ligand binding and clustering

without the requirement of extensive long-range rearrangements, thereby increasing the efficiency and efficacy of signaling [49].

Different types of membrane domains and other membrane structures may have evolved in order for cells and tissues to adapt to the requirements of large numbers of different extracellular signals and cell surface specific receptors. In addition to the dynamic membrane domains involved in cell signaling pathways, other situations may require less dynamic, more stable, and more mosaic membrane structures. For example, the adhesion ligands and receptors involved in tissue cell-cell interactions that govern properties like cell polarity and tissue organization do not require highly efficient interactions. This type of cell-cell interaction can occur slowly and may use more mosaic, less mobile membrane structures that are linked to membrane-associated cytoskeletal structures at the inner cell membrane surface. At the outer cell membrane surface linkages to extracellular matrix, extracellular elements in pericellular spaces or receptors on adjacent tissue cells do not require rapid and efficient interactions. There are numerous examples of cell junctions and matrix interactions that limit specific membrane component mobility and maintain cells within tissues and preserve tissue mechanical properties. These cell-cell and cell-matrix linkages form integrated tissue networks that are important in organizing cells within tissues and maintaining tensile forces and mechanical viscoelastic responses of cells and tissues [82]-[84].

The FMM model has undergone numerous changes over time as more elements and properties were added to the basic model. Over time, the basic model has changed so that higher densities of membrane components (more mosaic) were represented in basic cell membrane structure and organization [3], [4], [6], [49], [51]. The general principles and basic nanoscale description of a cell membrane, however, has not substantially changed from the 1972 model [1]-[6]. Various models and schemes for membrane structure have been presented since the FMM model (for example [44]-[46]), but the most basic, important elements of the original 1972 model have survived over the last 50+ years [5], [6]. Although the FMM model may not be able to address all of the properties found in specific membranes and may not answer every new question, it has provided us with a reasonable and functional nanoscale picture of how cell membranes are organized and the importance of this organization in membrane dynamics and function.

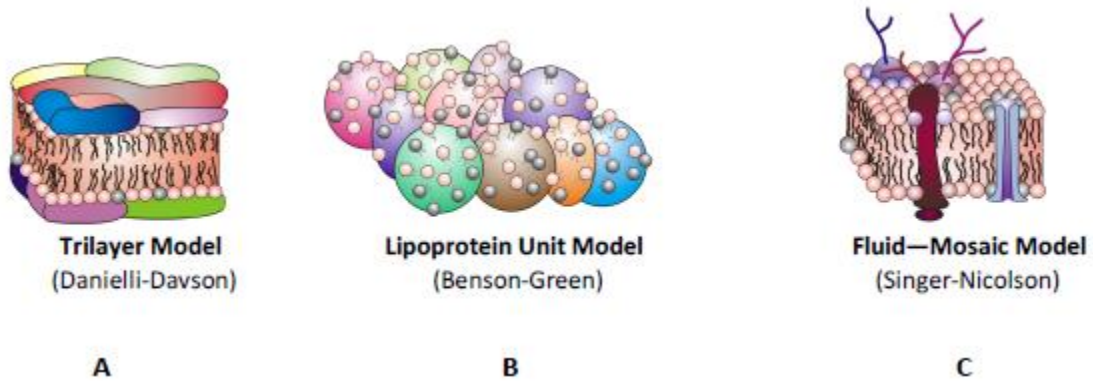

Figure 1. The three major models for cell membrane structure proposed in the last century. (**A**) The Trilayer Model of cell membrane structure. Proposed by Danielli and Davson [13] and later by Robertson [14] the Trilayer Model proposed that beta-sheet membrane proteins are attached by ionic groups to a lipid bilayer. (**B**) The Lipoprotein Subunit Model of cell membrane structure. Proposed by Benson [15] and separately by Vanderkooi and Green [16] the Lipoprotein Subunit Model of cell membrane structure did not have a lipid bilayer and instead proposed that cell membranes were composed of repeating lipoprotein subunits. (**C**) The Singer-Nicolson Fluid—Mosaic Membrane Model of cell membrane structure as proposed in 1972 [1]. In this view of a cell membrane globular integral membrane proteins are intercalated to various degrees into a lipid bilayer. Some integral membrane proteins form specific integral protein complexes, as shown in the figure. The model does not contain peripheral membrane proteins, other membrane-associated structures, or membrane domains and does not accurately represent the density of membrane protein components (modified from Nicolson and Ferreira [6]).

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Conflicts of Interest
G.L.N. is a part-time consultant to Nutritional Therapeutics, Inc. of New York and Naturally Plus, Inc. of Taiwan. No other possible conflicts of interest are reported.

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