Universal Journal of Fluid Mechanics **9 (2021), 35-50**
www.papersciences.com **EU Academy of Sciences (EUAS) e-Symposium**

The Singer-Nicolson Fluid-Mosaic Membrane Model and Membrane Lipid Replacement

Garth L. Nicolson
Department of Molecular Pathology
The Institute for Molecular Medicine
16371 Gothard Street H
Huntington Beach, California 92647, USA
gnicolson@immed.org

Abstract
The original Fluid-Mosaic Membrane Model of cellular and intracellular biomembrane structure was based primarily on thermodynamic principals and the available data on biomembrane dynamics at the time of its presentation [*Singer S.J. and Nicolson G.L.*,'The Fluid Mosaic Model of the structure of cell membranes', *Science*, **175** (1972), 720-731]. Approximately 50 years later this basic model remains relevant for describing the fundamental, simplified structural characteristics of biomembranes and some of their primary dynamic properties. However, plasma and intracellular membranes are certainly more complex than the original scheme that was proposed, and concepts of membrane hierarchy, fluidity, intra- and extracellular membrane connections, specialized membrane domains and different aspects of membrane dynamics and electrical properties have evolved over the intervening years. For example, the importance of specialized membrane domains, such as lipid rafts and phospholipid-protein complexes, peripheral, cytoskeletal and extracellular membrane connections and channels and their importance in determining the macrostructure and especially the dynamics of biomembranes and their roles in signaling and homeostasis have become much more complicated. In addition, the roles of membrane-associated cytoskeletal structures and extracellular matrix in limiting the mobility and range of motion of membrane components have added new layers of complexity and hierarchy to the original model. These dynamic structures lend themselves to new concepts in biomembrane medicine, and one of these, Membrane Lipid Replacement, has been developed to naturally modify membrane glycerolphospholipid composition and membrane protein-lipid interactions, and in the process repair and restore disease- and aging-associated damage to intracellular and plasma membranes. The use of Membrane Lipid Replacement with specific oral glycerolphospholipids has resulted in significant improvements in clinically important symptoms, such as fatigue, pain, and neurological symptoms.

Key Word and Phrases
Biomembranes, Extracellular Matrix, Lipid Rafts, Biomembrane-associated Cytoskeleton, Biomembrane Asymmetry, Biomembrane Dynamics, Biomembrane Lipid Exchange.

1. Introduction

In 1972 the Fluid-Mosaic Membrane Model of biological membrane structure was presented as a basic framework model for interpreting existing 1970s data on membrane proteins and lipids, and their dynamic properties [1]. The accepted model at the time for cellular membrane structure was a static tri-layer model consisting of a protein-lipid-protein matrix, as proposed by Danielli and Davson [2]. This model was later refined slightly and re-presented by Robertson as the Unit Membrane Model [3]. All of the biomembrane models during this era were based on the lipid bilayer membrane proposal of Gorter and Grendel [4], which turned out to be correct. However, the accepted biomembrane models of the time were all based on hydrophilic interactions between lipid headgroups and charged amino acids in membrane proteins. To account for the amounts of proteins and glycoproteins found in biomembranes Roberson [5] proposed that membrane proteins must also be unfolded beta-sheets that are present on each side of a lipid bilayer. A few trans-membrane proteins were added later by Robertson [5] to reconcile observations on biomembrane

ion transport and freeze-fractured electron microscopic images of globules within cell membranes [6]. All of these models required electrostatic and other forces to hold biomembrane components together, which turned out to be completely incorrect based on membrane properties. For example, biomembranes cannot be disrupted by interference with ionic or electrostatic forces [1]. Alternatively, to correct for the fact that ionic/electrostatic forces do not hold biomembrane components together, a membrane model based on adjacent units of lipoproteins, called the Lipoprotein Subunit Membrane Model, was proposed [7], [8]. This model lacked a lipid bilayer matrix, but as stated above the presence of phospholipid bilayer matrix in biological membranes precludes this type of model [4].

The Fluid-Mosaic Membrane Model [1] has survived over time, because it was based on the natural tendency of membrane glycerophospholipids to form into bilayers in aqueous solutions and membrane proteins to intercalate into the lipid bilayers. The amphipathic structures of integral, globular membrane proteins allow their intercalation into the hydrophobic matrix of a biomembrane lipid bilayer. This model incorporated the different categories of membrane proteins (hydrophobic integral or intrinsic proteins and hydrophilic peripheral or extrinsic proteins) and the ability of both lipids and some protein components in membranes to move laterally in the membrane plane and change their topographic distributions over time (Fig. 1) [1]. The bilayer structures of membrane phospholipids and their lateral motions in the bilayer plane were also consistent with existing data on model phospholipid bilayers (reviewed in [9]-[15]).

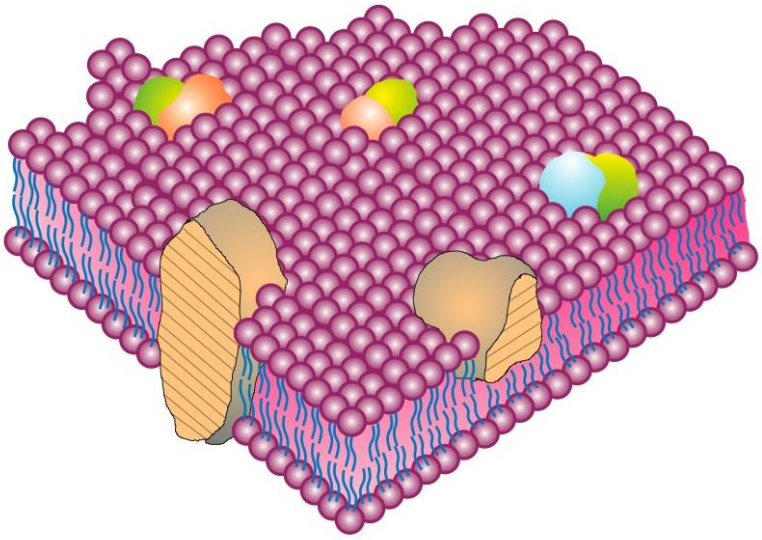

Fig. 1. The Singer-Nicolson Fluid—Mosaic Membrane Model of cell membrane structure as proposed in 1972. In this view of a cell membrane the solid bodies with stippled cut surfaces represent globular integral membrane proteins randomly distributed in the plane of the membrane. Some integral membrane proteins form specific integral protein complexes, as shown in the figure. Integral proteins are represented in a fluid lipid bilayer. The model does not contain other membrane-associated structures or membrane domains. (from Nicolson [10] with permission).

The history of biomembrane lipid structural models over the last century have been reviewed by Edidin [12]. He concluded that most, if not all, cellular membranes must contain their lipids in a phospholipid bilayer matrix. In contrast to other biomembrane models, the Fluid-Mosaic Membrane Model proposed that integral or intrinsic proteins must exist as globular structures imbedded into a phospholipid bilayer due to hydrophobic interactions [1]. In addition, peripheral or extrinsic membrane proteins can also be present, but they are not intercalated into the phospholipid matrix due to hydrophobic forces. The Fluid-Mosaic Membrane Model was found to be consistent with and supported by overwhelming evidence published over the intervening years [9]-[19].

The basic structure of the Fluid-Mosaic Membrane Model of biomembranes has been shown to be remarkably consistent with data published since the early 1960s [9]-[21]. But it was inevitable

that the original model would not be able to explain all of the data published after 1972. Thus the Fluid-Mosaic Membrane Model has necessarily evolved from the original proposal [1], and the adjustments that were incorporated into the model that took into account many of the issues that have been raised about the 1972 model subsequent to its publication [9], [10]. Most of these criticisms came decades after the original model and did not question the fundamental structural principles expressed in the original 1972 model [19], [22]-[27].

It is now widely accepted that there are some limitations in the Fluid-Mosaic Model as originally proposed in 1972 [1]. For example, after the model was first presented, specialized lipid domains (lipid rafts) were discovered, often in those plasma membranes found in specialized tissues and cells. Early modifications (1976) of the basic Fluid-Mosaic Membrane Model included the interactions of extracellular matrix with the exterior surfaces of cells and membrane-associated cytoskeletal components with interior surfaces of cell membranes and their influences on the mobility and distribution of trans-membrane glycoproteins [9]. Also, the possibility that less mobile lipid-protein or lipid-lipid domains might exist in biomembranes as frozen or semi-frozen islands in a sea of fluid phospholipids was also proposed [9]. As will be discussed the next sections, the hypothesis that trans-membrane interactions with membrane-associated structures inside and outside of cells can influence membrane dynamics was an important concept in explaining the structure, mobility, and function of biomembranes [9], [10], [28]. The discoveries of lipid rafts and other specialized membrane domains, including membrane-associated 'fences' and membrane 'fenceposts' that restrain mobility and their possible functions in controlling membrane protein distribution and mobility, have extended the evolution of the Fluid-Mosaic Membrane Model [10], [12], [23]-[37].

Some concepts of cell membrane structure produced a few years after the Fluid-Mosaic Membrane Model were much less homogeneous looking than the original model (for example see [9]). They contained additional information not included in the original model, such as protein and lipid aggregations and their segregation into membrane domains, interactions with cytoskeletal and extracellular matrix, among other features (some of these are shown in [9]). Newer revisions of the Fluid-Mosaic Membrane Model have retained all of the basic elements found in the original model [10]. However, in the newer models of biomembranes the arrangements of components into more compact structures and domains with less membrane lipid bilayer in between domains maximizes the mosaic nature of these structures [10].

2. Some Principles of Biomembrane Structure

An important concept of biomembrane structure is that hydrophobic interactions are especially important in maintaining a stable structure [14], [38]. Hydrophobic molecules self-associate to exclude water interactions, whereas hydrophilic molecules interact with the aqueous ionic environments. Membrane phospholipids self-assemble to form bilayers due to the energy provided by the hydrophobic effect and van der Waals forces [39]. Biomembrane integral globular proteins interact with membrane lipids, mainly their acyl tails, due to hydrophobic forces and much less to hydrophilic interactions between the lipid head groups and protein hydrophilic groups [15], [39], [40]. As originally proposed [1], [14], the basic nano-scale structural organization of biomembranes has remained relatively consistent with current evidence [9], [10], [15], [18], [21]-[24], [40]-[45], with some modifications [26], [27], [32], [34], [35], [40], [45]. This will be briefly discussed.

Biomembranes are dynamic structures that are quite susceptible to mechanical and other forms of membrane deformation. For example, biomembranes deform when confronted with forces less than those driven by the hydrophobic effect and van der Waals forces [46]. Membrane deformation depends on the energies of lipid tilt and splay, which in turn are dependent on lipid fatty acid composition [46]. Lipids that support positive spontaneous curvature can reverse the effects of lipids that support negative spontaneous curvature, and this may be important in biomembrane fusion and other dynamic biomembrane interactions [47].

In order for the components of membranes to assemble into non-leaky structures that characterize biomembranes integral membrane proteins and adjacent glycerolphospholipids must adjust to each other's hydrophobic structures. It was proposed that the Fluid-Mosaic Membrane

Model required some refinements to account for this important property [40]. In addition, the lateral sorting of phospholipids and proteins was thought to be due to interactions between their hydrophobic structural regions and to a lesser degree on the interactions between their hydrophilic parts of their structures. This sorting tends to prevent mismatches between lipids and proteins and favors certain interactions that maximize complimentary forces between these components [48]. This concept was incorporated into the Mattress Model of biomembranes that describes how variations in the hydrophobic parts of lipids and proteins drive associations or hydrophobic matching between different types of membrane components to prevent membrane distortions [48].

Membrane distortions, such as deformation, curvature, compression and expansion, are driven by different forces and components within biomembranes [46]-[51]. For example, certain soluble proteins can bind to membranes and cause deformation. Examples are proteins that contain BAR (Bin/amphiphysin/Rvs) domains that form crescent-shaped α-helical bundles that bind to membranes via electrostatic and hydrophobic interactions. BAR proteins can generate membrane curvature by scaffolding to the inner surface of a biomembrane, causing it to bend to the curvature of the protein [52]. When inserted into a biomembrane some proteins alter their shape by undergoing folding transitions to form α-helices that wedge between membrane components, thus deforming the membrane and resulting in membrane curvature [53].

3. Integral and Peripheral Biomembrane Proteins

In the original presentation of the Fluid-Mosaic Membrane Model it was important to distinguish between the integral (or intrinsic) proteins that are tightly bound to membranes by mainly hydrophobic forces and intercalated into the membrane lipid hydrophobic matrix and peripheral (or extrinsic) proteins that are loosely bound by electrostatic or other non-hydrophobic interactions [1]. There are numerous examples of both types of biomembrane proteins, and this is discussed in more detail in other publications [14]-[17]. Integral membrane proteins are critical in defining the basic membrane nano-scale structure of biomembranes. Peripheral membrane proteins also have an important role, but not necessarily in maintaining basic biomembrane nano-scale structure. Peripheral membrane proteins appear to be more important in providing protein attachment sites, scaffolding, tethering or providing membrane-supporting structures, membrane curvature-preserving structures, and attachment points for soluble enzymes and signaling molecules.

Peripheral (or extrinsic) membrane proteins were originally defined operationally as proteins that could be removed from biomembranes without destroying the basic nano-scale membrane structure [1], [13]. It was an operational definition to help explain the roles of different membrane proteins in defining basic biomembrane structure. Peripheral membrane proteins do not have to be strictly globular in structure, and certainly not amphipathic, and by definition they can also include proteins with extensive β-sheet structures that bind to membranes mainly by ionic and other non-hydrophobic forces [3], [5].

4. Cytoskeletal and Extracellular Matrix Biomembrane Interactions

It was apparent a few years after the publication of the Fluid-Mosaic Membrane Model [1] that another class of membrane proteins was needed in addition to integral and peripheral membrane proteins. This new class of membrane-associated proteins was not strictly related to biomembrane structure. But they were proteins that affected biomembrane properties and function. They include cytoskeletal and signaling proteins that interact at the inner membrane surface and certain glycoproteins and glycosaminoglycans that bind to and stabilize biomembranes at their outer surfaces [9], [10]. They function as parts of adhesion structures at cell surfaces, and cell motility traction points or stabilizing points within cells. These protein components are membrane-associated but not directly involved in the integral nano-scale structure of cell membranes. Thus biomembrane structure is not dependent on their presence; however, they are important in maintaining membrane function and dynamics. They are also important in various cellular activities, such as cell adhesion, motility and spreading, endocytosis, exocytosis and many other important processes [9], [10].

Cytoskeletal and extracellular matrix membrane-associated interactions can alter cell membrane macrostructure by causing reductions or restrictions in cell movement or mobility and by causing global movements of membrane glycoprotein and lipid domains by tethering these complexes to intracellular or extracellular structures [9], [10]. This can involve cell membrane-associated actin-containing cytoskeletal components that are essential in moving or restraining trans-membrane integral membrane proteins through intermediate peripheral membrane proteins and other components [9], [10].

By 1976 there were several examples of restriction of mobility of integral membrane components and involvement of the cytoskeleton in translocation of membrane components [9]. For example, during antigen capping on lymphoid cells certain initially mobile cell surface antigens, even if they were present in small mobile clusters, were found to be trapped into large, immobile trans-membrane complexes in a temperature- and energy-dependent process involving cytoskeletal elements [65]-[67]. This process eventually resulted in cytoskeletal-mediated endocytosis of some of the large macromolecular membrane complexes (receptor-rich domains or "receptor patches") [68]. We now know that in lymphoid cells antigen clustering, antigen domain formation, internalization, acidification of the resulting endosomes, degradation in endosomes, and membrane recycling are all part of the normal lymphocyte activation process [68].

The structures that mediate trans-membrane linkages between clusters of integral membrane receptors and the cytoskeleton were ultimately found to be much more complex than first thought [9]. They are now known to involve multiple membrane peripheral proteins, lipid-protein-receptor domains, and enzymes that assemble into submembrane plaques or supramolecular structures that secure the membrane to cytoskeletal elements [69]-[71].

The mobility of integral membrane components can also be restricted by cell-cell and cell-matrix interactions. When cells are bound to other cells or to extracellular matrix, at least some of their membrane receptors are immobilized in the process [72]-[74]. Cell adhesion complexes that are immobilized by matrix interactions are capable in some cells of communicating signals that are transmitted through a dynamically assembled actin-containing cytoskeleton to generate mechanical forces that can move cells or resist exterior mechanical forces [75], [76]. This system of highly specialized glycoproteins and proteins (extracellular matrix, integral membrane proteins, peripheral membrane proteins, adaptor proteins, cytoskeletal elements, among other components) may be necessary to convert biochemical signals into mechanical forces that are important in cellular behavior. Along with intracellular signaling pathways, these molecular-mechanical pathways have been proposed to be important regulators of cell function [76].

The original proposals on the role of membrane-associated cytoskeletal elements and their influence on biomembrane structure and dynamics have proved to be relatively simplistic [9]. This complex system is slowly being dissected and its multiple subcomponents identified [71], [77]-[82]. For example, we now know that certain membrane-interacting components, such as septins, GTPases, and other components, can form higher-order structures, such as bundles, filaments and even ring structures that bind to actin filaments and microtubules [83]-[85]. Although membrane peripheral proteins have been identified in cytoskeletal interactions [86], membrane lipids are also important. Indeed, specialized phospholipids may regulate interactions between certain membrane lipid domains, such as lipid rafts (specialized lipid domains), and phosphatidylinositol isoform-binding proteins [87].

Cell membranes are not autonomous and separate from other intracellular membranes and organelles. They are continuously interacting with other cellular structures-receiving signals, directing contacts, sending instructions, maintaining cellular polarity and mechanical properties, while undergoing constant turnover of their constituent components. Thus they can be considered as completely integrated mechanostructures.

5. Biomembrane Protein-Protein and Lipid-Lipid Interactions

The majority of biomembrane proteins and glycoproteins are not likely to be isolated components or complexes floating freely in a fluid lipid environment (as in Fig. 1 [1]). Functionally they are often assembled into macromolecular complexes, for example, to initiate signaling process that are important in a variety of cellular functions. As their cellular and

biochemical functions have been elucidated over the years, it has become much clearer how super-molecular complexes of membrane proteins and glycoproteins (domains) perform a variety of cellular functions.

To demonstrate their functional activities biomembrane proteins have been cloned, sequenced and functionally expressed [15]-[17]. In such studies single membrane components can be studied, but usually membrane protein and glycoprotein complexes are not made up of single proteins or glycoproteins [88]-[90].

Biomembrane integral protein-protein interactions, which can be driven by ligand binding, are involved in the dynamic formation of trans-membrane signaling complexes. Eventually the complexes can become activated for recruitment of additional peripheral proteins at the inner cell membrane surface to form supramolecular trans-membrane structures that are competent for cellular signaling [76], [90]-[92]. For example, upon binding of cell surface trans-membrane integrin receptors to their ligand, the integrin heterodimers are thought to undergo a 'bending' conformational change that allows recruitment of submembrane plaque proteins that, in turn, directly or indirectly bind to actin complexes linked to the cytoskeleton [78]. Subsequently a potentially larger group of other signaling molecules and enzymes can be bound to the submembrane supramolecular complexes, leading to the formation of stable, trans-membrane super-complexes [78], [92].

There are a variety of cell membrane glycoprotein-protein oligomeric complexes that are involved in cell-cell interactions and the formation of specialized super-structures between adjacent cells in tissues. These will not be discussed here, but the reader can find additional examples elsewhere (reviewed in [10]).

As discussed above, membrane lipids are asymmetrically arranged in cell membranes (reviewed in [20], [21], [54]-[56], [58], [60]). They are also unevenly distributed in the membrane plane (reviewed in [30], [44], [62], [93]-[96]). 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 animal cell membranes is particularly important in the formation of membrane lipid domains [21], [62], [95]-[97]. Lipid-lipid interaction studies using mixtures of membrane phospholipids, cholesterol and sphingomyelin have shown the importance of domain structures in model lipid membranes [62], [98], [99].

Cholesterol is particularly important in cell membrane lipid organization [62], [96]-[99]. This is thought to be due, in part, to cholesterol's "schizophrenic" affinity for fluid and solid phases of membrane lipid bilayers [96]. Cholesterol partitions into liquid ordered and disorderd phases to roughly the same extent and changes the properties of different lipid phases or lipid domains [95].

In plasma membranes sphingolipids are also important in the formation of more-ordered membrane lipid domains [35], [94]. Sphingomyelins and phosphatidylcholines constitute more than one-half of plasma membrane phospholipids and form the main partners for cholesterol interactions [94], [100]. Sphingomyelins and cholesterol are critically important in the separation and formation of ordered lipid domains (lipid rafts) that are generally surrounded by liquid phase lipids [20], [94], [101].

The formation of more ordered lipid phases in plasma membranes is generally important in membrane domain formation and, in particular, the lipid raft hypothesis [20], [35], [44], [45]. The formation of membrane lipid domains or rafts is now thought to be a dynamic and reversible process that confers functional change at the outer surface of plasma membranes [30], [35], [45], [102]-[104]. Lipid domain formation appears to be driven by multiple forces, such as hydrogen bonding, hydrophobic entropic forces, charge pairing and van der Waals forces [62], [101].

Lipid domains or rafts in plasma membranes contain specific lipids, integral proteins and peripheral proteins, and they can develop into platforms for signal transduction and other cellular functions [35], [104]-[106]. Plasma membrane lipid rafts are now thought to constitute functional, dynamic nano-sized domains of diameter <300 nm (most are ~10-200 nm in diameter). They are characterized by increased amounts of cholesterol and sphingolipids [31], [37], [45], [107]. Cell membrane lipid rafts were found to be much smaller than the specialized lipid domains found in artificial membrane bilayers, and their boundary lipids were found to exchange rapidly (every 10-

100 nsec) with lipids in the bulk cell membrane. They also tended to exclude unsaturated phospholipids and cholesterol from their boundaries [108].

Under certain conditions integral and peripheral membrane proteins may be sequestered into plasma membrane lipid rafts [30], [104], [106], [109]. Neuman et al. [106] have discussed the properties of lipid rafts (and other membrane domains) that make them biologically important, such as their involvement in cellular processes: endocytosis, signal transduction, cell death, among other events. Lipid rafts are dynamic structures, and the lipids in these domains can quickly exchange with lipids in the bulk fluid membrane as well as other rafts. Neuman et al. speculated that there may be different turnover rates for each raft constituent. A spectrum of submicro- or nano-domains may exist in biomembranes that contain different lipid and protein compositions, physical characteristics and functions [106]. There may be a limited number of lipid compositions or combinations that can form lipid domains, and these domains appear to be non-randomly distributed. These domains tend to adopt a superlattice structure in biomembranes [110].

6. Biomembrane Lipid-Protein Interactions

Integral membrane proteins can move and interact with different membrane domains, but they must also directly interact with membrane phospholipids in order to produce a stable, intact plasma membrane. Portions of their structures must directly interact with the acyl side chains of phospholipid fatty acids or the hydrophobic portions of other membrane lipids in order to be stabilized in the membrane. This is accomplished by hydrophobic matching between the hydrophobic lipid bilayer core and a stretch or combination of hydrophobic amino acids in an integral membrane protein [15], [16], [21], [43], [48], [111].

The concept of hydrophobic matching between the hydrophobic core of a biomembrane lipid bilayer and hydrophobic stretches of amino acids in integral membrane proteins was essential for understanding the formation and stability of biomembranes [96], [111]-[114]. If the hydrophobic portions within this structure are mismatched, an elastic distortion of the lipid matrix around the integral membrane protein(s) can occur [48], [96], [114]. If hydrophobic matching of particular phospholipids or boundary lipids adjacent to integral membrane proteins does not occur, there will be an energy penalty that results in an elastic distortion of the lipid matrix [96], [111]-[114]. If this distortion is large enough, the integral protein may undergo a conformational change, and this could potentially cause effects on protein function or alter the integrity of the biomembrane. It could also affect protein-protein interactions and result in integral protein aggregation in the membrane plane [114].

A range of integral protein interactions with lipids occurs in biomembranes, and these are apparently controlled by the coherence lengths of the interactions between integral membrane proteins and their boundary lipids. If large enough, this can result in capillary-condensation and wetting around the integral protein [43], [115]. Other interactions, such as electrostatic interactions between charged amino acids and phospholipids, can complicate this picture, and under certain circumstances electrostatic interactions might even overcome hydrophobic matching [114]. Lipid preference for certain integral membrane proteins can result in capillary condensation, and when this occurs around two or more integral membrane proteins, it can result in wetting and the formation of a capillary condensate between adjacent proteins. This can produce a lipid-mediated force that drives the formation and stabilization of the integral protein complex [96], [114], [115].

The hydrophobic matching principle may also be an essential property in the formation of specialized lipid domains or rafts (see previous section), and it could also be an important mechanism for selective partitioning of integral proteins into specialized membrane lipid domains. To be sequestered into a lipid domain an integral protein's hydrophobic structure must match up with the hydrophobic thickness of the domain [115]. If the hydrophobic portions of this structure are mismatched, there will be an elastic distortion of the boundary lipid matrix around the integral membrane protein. Without hydrophobic matching of particular boundary lipids immediately adjacent to particular membrane proteins, there will be an energy penalty that causes an elastic distortion of the boundary lipid matrix [96], [114]. If the energy penalty is large enough, the integral protein may undergo a conformational change, and this could potentially cause effects on

protein function. It can also result in the exclusion of certain lipids, such as cholesterol, from the boundary lipid layer due to unfavorable membrane protein hydrophobic matching [96], [114].

Membrane lipid boundary effects can also cause changes in protein-protein interactions that could result in membrane integral protein aggregation in the membrane plane. By examining the rotational diffusion rates of rhodopsin in reconstituted bilayer membranes, Kusumi and Hyde [108] were able to relate specific phospholipid acyl chain-lengths to the state of rhodopsin movement and aggregation. They found that hydrophobic mismatch between proteins and lipids is unfavorable energetically, but the mismatching may be minimized by the transient formation of protein-protein complexes.

Another important property of biomembranes is the tendency of certain lipids to induce curvature stress and the ability of certain membrane peripheral proteins to overcome this stress [53]. This property has some similarities to hydrophobic matching, and the binding of integral proteins to particular lipids could change the conformation of nearby integral proteins, for example, to open or close membrane channels [113]. Alternatively, the binding of peripheral membrane proteins directly to the lipid head groups could decrease or promote lipid curvature as the lipids conform to the protein shape [53].

In addition to hydrophobic matching of proteins and lipids in cellular membranes, there are additional physical considerations, such as lateral pressure forces, lateral lipid composition and phases, membrane curvature, ionic interactions, among others, that must be considered to produce an overall tensionless biomembrane structure [43], [96], [112], [113], [115].

7. Restrictions on Biomembrane Lateral Mobility

Most integral membrane proteins are not completely free to laterally move in a fluid lipid matrix, as originally proposed [1]. In fact, there are subtle restrictions on the lateral movements of most integral membrane proteins, and at least some lipids, in biomembranes [9], [10], [26], [27]. Restrictions on the lateral movements of integral membrane proteins have been shown to be due to: (*a*) extracellular matrix, which can immobilize biomembrane components, including trans-membrane components, (*b*) specialized membrane domains, such as lipid rafts, (*c*) the formation of large, supramolecular protein complexes and (*d*) peripheral membrane barriers at the inner membrane surface, such as membrane-associated corrals or cytoskeletal barriers [10], [22], [23], [26]-[28], [41], [69].

Various methods have been used to study the dynamics of cell surface integral membrane proteins. Jacobson et al. [23] have placed the lateral movements of membrane proteins into four main categories: (*a*) transient confinement by obstacle protein clusters (also called membrane 'fenceposts' or 'pickets'); (*b*) transient confinement by a cytoskeletal meshwork into defined domains (or 'corrals'); (*c*) directed motion by direct or indirect attachment to the cytoskeleton; and (*d*) free, random diffusion in the fluid parts of thesmembrane. A slightly different list of the categories of lateral movements of biomembrane components has been proposed: (*a*) free diffusion; (*b*) movement limited by meshwork barriers ('fences' or 'corrals'); and (*c*) movement limited by traps and domains (such as lipid rafts) [36]. The original description of integral membrane proteins freely diffusing in the membrane plane (Fig. 1) pertains to only one of these categories. Thus over time biomembranes have become more complicated, and this more accurately reflects the complex nature of biomembranes and cells.

Biomembrane dynamics can obviously change with time, and this may vary depending on the cell type. For example, it is thought that a substantial portion of integral membrane proteins, even on free cells like blood leukocytes, are confined to small membrane domains by lattices and corrals. Although they may be able to rotate in the plane of the membrane and move small distances, apparently they are not completely free to diffuse large distances in the membrane plane under normal circumstances. Thus integral membrane proteins may be restricted to certain surface regions in biomembranes [10], [26], [27]. Integral membrane proteins can also escape from one domain to an adjacent domain, and even escape such domains altogether, and this may be related to the sizes and types of membrane-associated cytoplasmic structures, cytoskeletal and extracellular interactions, and the abilities of biomembrane components to undergo formation of membrane complexes [26], [27].

The approximate surface areas that define plasma membrane receptor domains have been estimated to vary from 0.04 to 0.24 μm^2, and the approximate transit times of membrane receptors in these membrane domains can range from approximately 3 to 30 sec [27]-[29]. Overall, cell membrane domains have been estimated to range in diameter from 2-300 nm. For example, actin-cytoskeletal 'fence' domains have been found in the diameter range of 40-300 nm, lipid raft domains in the diameter range of 2-20 nm, and dynamic integral membrane protein complexes in domain diameters of 3-10 nm [27]. The different types and sizes of cell membrane domains may allow cells to deploy various levels of signal selection and complexity.

8. Biomembrane Hierarchical Organization

Multi-dimensional plasma membrane structures exist so that cells can quickly respond to a multitude of intracellular and extracellular signals. Kusumi et al. [26], [27] have proposed that plasma membranes are organized into dynamic hierarchical structures. Within these hierarchical structures membrane components may be limited in their diffusion rates, which are generally 5-50-times slower than when the same components are reconstituted into artificial phospholipid membranes. Macroscopic diffusion rates in cell membranes can also be increased more than 20-times through disruption of membrane-associated cytoskeletal networks that may normally restrict their mobility [27].

That membrane-associated cytoskeletal networks can restrict the mobility of trans-membrane integral proteins compared to their free lateral diffusion was discussed previously [9], [10], [22], [23], [41], [116]. Indeed, cytoskeletal-disrupting drugs have been known for some time to change the rates of integral membrane protein lateral diffusion [10], [66], [117]. The impedance of mobility of membrane components was found to be directly related in many cell types to cytoskeletal 'fencing' and the formation of cytoskeletal 'corrals' (Fig. 2) [10], [23], [26]-[28].

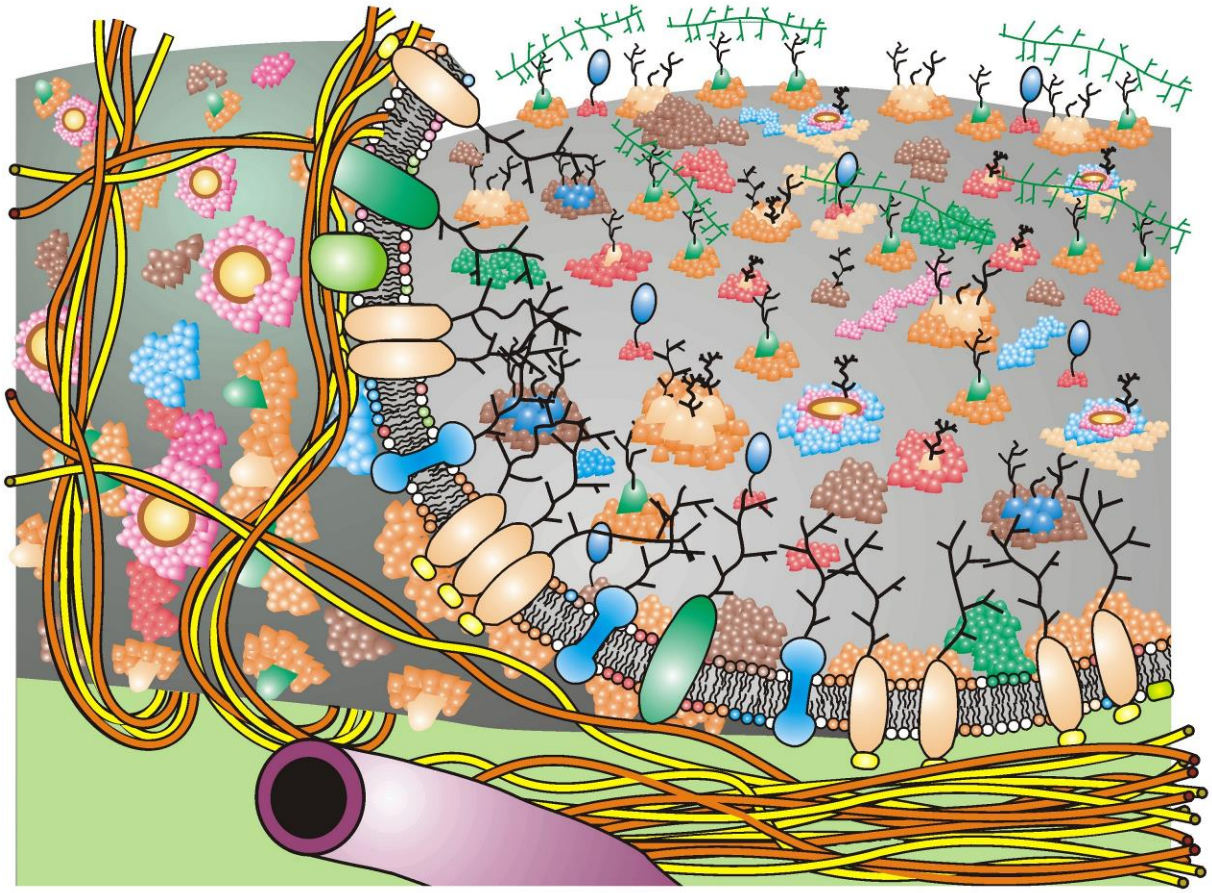

Fig. 2. An updated Fluid-Mosaic Membrane Model representation that contains membrane domain structures and membrane-associated cytoskeletal and extracellular structures. The cell membrane has been

pealed back to the left to reveal the cytoplasmic membrane surface and cell membrane-associated cytoskeletal elements that form barriers ('corrals') that limit the lateral motions of some of the integral membrane proteins. In addition, membrane-associated cytoskeletal structures are directly interacting with integral membrane proteins at the inner membrane surface along with matrix components at the outer surface. Although this diagram presents possible mechanisms of integral membrane protein mobility restraint, it does not accurately represent the sizes and structures of integral membrane proteins, lipid domains or membrane-associated cytoskeletal structures. (from Nicolson [10] with permission).

The partitioning of plasma membranes into specific domains in order to limit the dynamics of their integral membrane protein components, at least part of the time, to circumscribed areas can create relatively stable membrane zones (domains) of increased receptor densities. This is thought to be important in receiving and processing extracellular signals by cells. Also, extracellular networks or lattices can potentially partition the plasma membrane into non-stabilized and stabilized membrane domains [10], [34]. This could be important in tissue organization and function. In addition, the trapping of mobile integral membrane proteins into corrals constructed of cytoskeletal fencing (or extracellular lattices) may be dependent on the state of protein complex formation (size of integral protein complexes). Some integral protein monomers have been found to escape from such corrals, but in their oligomeric complex form they may not be able to escape at the same rates [28]. Thus membrane corrals (or extracellular lattices) may selectively limit free diffusion of membrane complexes in the membrane plane, and at the same time they may present increased receptor densities in specific membrane domains [27].

Cell membrane dynamic compartmentalization may result in the enhanced presentation of specific components in specialized domains. This may be important in signal transduction, cell activation, cell differentiation, cell identification, and other complex plasma membrane events. By changing the range of movements, distributions and collision rates of various cellular receptors, their display and ability to associate into higher order complexes can be controlled. Thus it has been proposed that plasma membranes possess hierarchical architecture consisting of various membrane domains or compartments.These include: cytoskeletal limited domains or corrals formed by cytoskeletal fences, fenceposts or pickets; lipid raft domains; and dynamic, oligomeric integral membrane protein domains that may or may not be linked to the cytoskeleton, among other possibilities (Fig. 2). The basic nano-scale membrane unit in such schemes would still be a Fluid-Mosaic Membrane [26], [27].

9. Biomembrane Lipid Replacement

When biomembrane phospholipids are damaged, they must be repaired or replaced to return cellular functions to normal [118]-[120]. The replacement of damaged membrane phospholipids with undamaged, functional membrane phospholipids is essential for maintaining a variety of cellular and tissue functions and for general health [118], [119]. Membrane phospholipids that form the plasma membrane and intracellular membranes (*a*) provide separation of enzymatic and chemical reactions into discrete cellular compartments or organelles, (*b*) are essential for the function of membrane enzymes, (*c*) are an important energy storage reservoir, and (*d*) provide precursors for bioactive molecules that function in signal transduction and molecular recognition pathways [118]-[120]. Membrane phospholipids are thus essential for general health, and they must be constantly repaired or replaced in order to maintain membrane functions.

To maintain healthy cellular membranes we normally utilize an appropriate diet rich in membrane precursors, such as phospholipids and other metabolites [118]-[120]. This can be difficult to achieve, especially during chronic illnesses, so dietary supplements containing membrane phospholipids have been used to maintain biomembrane health. This presents its own problem, since these molecules are susceptible to oxidation and deterioration, and in unprotected form they have limited half-lives. When orally ingested, membrane phospholipids can be degraded or modified within the gastrointestinal system and absorbed; or they can be taken in as intact molecules without degradation or modification [118]-[121]. When present in the gastrointestinal system, they can be absorbed as small phospholipid globules and micelles with their constituents undegraded [119]. The process overall is very efficient, and over 90% of ingested phospholipids

are absorbed and transported into the blood within hours [122]. While in the blood circulation, membrane phospholipids are usually associated with carrier molecules, such as lipoproteins, or cell membranes of blood cells. However, when they are present in excess amounts compared to fasting levels, they can also be present in small phospholipid globules or micelles. Eventually they are transported to tissues and cells where they are transferred by direct membrane contact, endocytosis or by carrier and transport proteins into cells. Once inside cells, membrane phospholipids they are moved by various mechanisms, including concentration gradients, to various cellular and organelle membranes and compartments [118], [119].

Membrane phospholipids, such as glycerolphospholipids, are enzymatically modified during and after their delivery by exchange of their head groups and fatty acid side chains to reflect the specific compositions of their destination membranes. Once they have been partitioned into their destination membrane sites, glycerolphospholipids and other membrane lipids can be further modified enzymatically to reflect the changing functional needs of organelles and cells. The entire process of uptake, transport, replacement and removal is driven generally by a mass action process, such that when membrane phospholipids are in great excess, they have an advantage in being able to reach their final cellular destinations without significant degradation or free radical modification. The reverse of this process, the removal of damaged, oxidized phospholipids, also appears to be a mass action process [119].

10. Biomembrane Lipid Replacement in Aging and Chronic Illnesses

The most common therapeutic use of membrane phospholipids is to treat fatigue [119], [121]. Fatigue is the most common complaint of patients seeking general medical care, and it is associated with aging and most if not all chronic medical conditions [123]. Fatigue is considered a complex, multi-dimensional sensation that is not well understood, but it is perceived to be associated with a loss of overall energy, mental or physical tiredness, a feeling of exhaustion or diminished endurance, and an inability to perform even simple tasks without exertion. In aged individuals and in chronic diseases fatigue is present due to a variety of causes [123]. Moderate to severe fatigue has been directly related to loss of mitochondrial function and diminished production of mitochondrial ATP [124].

In chronic fatigue syndrome or myalgic encephalomyelitis (CFS/ME) fatigue is the most obvious symptom [125], [126]. In fact, in many chronic diseases, aging, cancers and other conditions, chronic fatigue is often the most common symptom found. In contrast, mild fatigue not related to mitochondrial function can also be found in depression and in some psychiatric conditions [125].

Membrane Lipid Replacement or the oral use of protected glycerolphosholipids has been successfully used for reducing fatigue in patients with chronic fatigue and other chronic illnesses, such as CFS/ME, fibromyalgia, and other fatiguing illnesses [118], [119], [121]. In our cross-over clinical study on the effects of oral NTFactor®, a mixture of glycerolphospholipids, pre- and probiotics and other ingredients, was to treat chronic fatigue in moderately to severely fatigued, aged subjects (61-77 years-old). There was good correspondence between the reductions in fatigue and improvements in mitochondrial function [127]. This study indicated that aged individuals with moderate to severe chronic fatigue benefited significantly from taking the oral glycerolphospholipid supplement, and their mitochondrial function, as measured by the inner mitochondrial inner membrane trans-membrane potential, was similar to much younger subjects, but only if they continued to take the oral supplement [127]. Similar positive results on the effects of the glycerolphospholipid supplement NTFactor® on reducing fatigue (reductions of 35-43%) were found in patients with chronic fatigue syndrome (CFS/ME), fibromyalgia, Gulf War illness, chronic Lyme disease and various cancers (reductions in fatigue of 26-43%) [118], [119], [121], [125], [127].

Membrane Lipid Replacement to resolve other symptoms, such as pain, peripheral neuropathy and gastrointestinal symptoms [128], [129]. To reduce these symptoms ~~required~~ higher minimum doses of glycerolphospholipid supplement NTFactor Lipids®, usually above 5 g per day [128], [129]. For example, we have found that large reductions in pain and other symptoms required 6 g per day of oral membrane glycerolphospholipids to be effective, and many patients gradually

moved to higher daily oral doses of membrane phospholipids. We are now examining the possible mechanism(s) whereby membrane glycerolphospholipids alter nerve membrane depolarization and other important properties involved in pain transmission.

References

1. *Singer S.J. and Nicolson G.L.,* 'The Fluid Mosaic Model of the structure of cell membranes', *Science, **175** (1972), 720-731.*
2. *Danielli J.F. and Davson H.,* 'A contribution to the theory of permeability of thin films', *J. Cell Comp. Physiol., **5** (1935), 495-508.*
3. *Robertson J.D.,* 'The ultrastructure of cell membranes and their derivatives', *Biochem. Soc. Symp., **16** (1959), 3-43.*
4. *Gorter E. and Grendel F.,* 'On bimolecular layers of lipoids on the chyromocytes of the blood', *J. Exp. Med., **41** (1925), 439-443.*
5. *Robertson J.D.,* 'The molecular structure and contact relationships of cell membranes', *Prog. Biophys. Biophysical Chem., **10** (1960), 343-418.*
6. *Pinto da Silva P. and Branton D.,* 'Membrane splitting in freeze-etching. Covalently bound ferritin as a membrane marker.' *J. Cell Biol., **45** (1979), 598-605.*
7. *Benson A.A.,* 'On the orientation of lipids in chloroplast and cell membranes', *J. Am. Oil Chem. Soc., **43** (1966), 265-270.*
8. *Green D.E., Allmann D.W., Bachmann E., Baum H., Kopaczyk K., Korman E.F, et al.,* 'Formation of membranes by repeating units', *Arch. Biochem. Biophys., **119** (1987). 312-335.*
9. *Nicolson G.L.,* 'Transmembrane control of the receptors on normal and tumor cells. I. Cytoplasmic influence over cell surface surface components', *Biochim. Biophys. Acta, **457** (1976), 57-108.*
10. *Nicolson G.L.,* 'The Fluid-Mosaic Model of Membrane Structure: still relevant to understanding the structure, function and dynamics of biological membranes after more than 40 years', *Biochim. Biophys. Acta, **1838** (1914), 1451-1466.*
11. *Nicolson G.L., Ji T. and Poste G.,* 'Dynamic Aspects of Cell Surface Organization.' In: The dynamics of cell membrane organization', *Poste G. and Nicolson G.L.,* Eds., Elsevier, New York, *1977,* pp. 1-73.
12. *Edidin M.,* 'Lipids on the frontier: a quarter century of cell-membrane bilayers', *Nat. Rev. Mol. Cell Biol., **4** (2003), 414-418.*
13. *Singer S.J.,* 'The molecular organization of membranes', In: *Structure and Function of Biological Membranes*', *Rothfield L.I.,* Ed., Academic Press, New York, *1971,* pp. 145-222.
14. *Singer S.J.,* 'The molecular organization of membranes', *Annu. Rev. Biochem., **43** (1974), 805-833.*
15. *Singer S.J.,* 'The structure and insertion of integral proteins in membranes', *Annu. Rev. Cell Biol., **6** (1990), 247-296.*
16. *von Heijne G.,* 'Transcending the impenetrable: how proteins come to terms with membranes', *Biochim. Biophys. Acta, **947** (1988), 307-333.*
17. *von Heijne G.,* 'Membrane-protein topology', *Nat. Rev. Molec. Biol., **7** (2006), 909-918.*
18. *Bretscher MS.,* 'Membrane structure: some general principals', *Science, **181** (1973), 622-829.*
19. *de Weer P.,* 'A century of thinking about cell membranes', *Annu. Rev. Physiol., **62** (2000), 919-926.*
20. *van Meer G., Voelker D.R. and Feigenson G.W.,* 'Membrane lipids. Where they are and how they behave', *Nat. Rev. Mol. Cell Biol., **9** (2008), 112-124.*
21. *Bagatolli L.A., Ipsen J.H., Simonsen A.C. and Mouritsen O.G.,* 'An outlook on the organization of lipids in membranes: searching for a realistic connection with the organization of biological membranes', *Prog. Lipid Res., **49** (2010), 378-389.*
22. *Zhang F., Lee G.M. and Jacobson K.,* 'Protein lateral mobility as a reflection of membrane microstructure', *Bioessays, **15** (1993), 579-588.*
23. *Jacobson K., Sheets E.D., Simson R.*. 'Revisiting the fluid mosaic model of membranes', *Science, **268** (1995), 1441-1442.*
24. *Verbe G., Szöllosi J., Matkó J., Nagy P., Farkas T., Vigh L., et al.,* 'Dynamic, yet structured: The cell membrane three decades after the Singer-Nicolson model', *Proc. Natl. Acad. Sci. USA, **100** (2003), 8053-8058.*
25. *Winiewska A., Draus J. and Subczynski W.K.,*'Is the fluid-mosaic model of biological membranes fully relevant? Studies on lipid organization in model and biological membranes', *Cell Mol. Biol. Lett., **8** (2003), 147-159.*
26. *Kusumi A., Suzuki K.G., Kasai R.S., Ritchie K. and Fujiwara T.K.,* 'Hierarchical mesoscale domain organization of the plasma membrane', *Trends Biochem. Sci., **36** (2011), 604-615.*
27. *Kusumi A., Fujiwara T.K., Chadda R., Xie M., Tsunoyama T.A., Kalay Z., et al.,* 'Dynamic organizing

principals of the plasma membrane that regulate signal transduction: commemorating the fortieth anniversary of Singer and Nicolson's fluid-mosaic model', *Annu. Rev. Cell Dev. Biol*., ***28*** *(2012), 215-250.*

28. *Kusumi A. and Sako Y.,* 'Cell surface organization by the membrane skeleton', *Curr. Opin. Cell Biol.*, ***8*** *(1996), 566-574.*
29. *Kusumi A., Sako Y. and Yamamoto Y.M.,* 'Confined lateral diffusion of membrane receptors as studied by single particle tracking (nanovid microscopy). Effects of calcium-induced differentiation in cultured epithelial cells', *Biophys. J*., ***65*** *(1993), 2021-2040.*
30. *Jacobson K., Mouritsen O.G. and Anderson R.G.W.,* 'Lipid rafts: at a crossroad between cell biology and physics', *Nat. Cell Biol*., ***9*** *(2007), 7-14.*
31. *Simons K. and Toomre D.,* 'Lipid rafts and signal transduction', *Nat. Rev. Mol. Cell Biol*., ***1*** *(2000), 31-39.*
32. *Lavi Y, Edidin M.A. and Gheber L.A.,* 'Dynamic patches of membrane proteins', *Biophys. J*., ***93*** *(2007), L35-L37.*
33. *Escribá P.V., Gonzáles-Ros J.M., Goñi F.M., Kinnunen P.K.J., Vigh L., Sánchez-Magraner L., et al.,* 'Membranes: a meeting point for lipids, proteins and therapies', *J. Cell Mol. Med*., ***12*** *(2008), 829-875.*
34. *Lajoie P., Goetz J.G., Dennis J.W. and Nabi I.R.* 'Lattices, rafts and scaffolds: domain regulation of receptor signaling at the plasma membrane', *J. Cell Biol*., ***185*** *(2009), 381-385.*
35. *Lingwood D. and Simons K.,* 'Lipid rafts as a membrane-organizing principle', *Science*, ***327*** *(2010), 46-50.*
36. *He H.-T. and Marguet D.,* 'Detecting nanodomains in living cell membranes by fluorescence correlation spectroscopy', *Annu. Rev. Phys. Chem*., ***62*** *(2011). 417-436.*
37. *Quinn PJ.,* 'A lipid matrix model of membrane raft structure'. *Prog. Lipid Res*., ***49*** *(2010), 390-406.*
38. *Kauzmann W.,* 'Some factors in the interpretation of protein denaturing', *Adv. Protein Chem*., ***14*** *(1959), 1-63.*
39. *Cramer W.A., Engelman D.M., von Heijne G. and Rees D.C.,* 'Forces involved in the assembly and stabilization of membrane proteins', *FASEB J*., ***6*** *(1992), 3397-3402.*
40. *Israelachvili J.N.* Refinement of the Fluid-Mosaic Model of membrane structure', *Biochim. Biophys. Acta*, ***469*** *(1977), 221-225.*
41. *Jacobson K., Ishihara A. and Inman R.,* 'Lateral diffusion of proteins in membranes', *Annu. Rev. Physiol.*, ***49 (****1987), 163-175.*
42. *Watts A.,* 'Membrane structure and dynamics', *Curr. Opin. Cell Biol*., ***1*** *(1989), 691-700.*
43. *Gil T., Ipsen J.H., Mouritsen O.G., Sabra M.C., Sperotto M.M. and Zuckermann M.J.*. 'Theoretical analysis of protein organization in lipid membranes', *Biochim. Biophys. Acta*, ***1376*** *(1998), 245-266.*
44. *Simons K. and Vaz W.L.,* 'Model systems, lipid rafts, and cell membranes', *Annu. Rev. Biophys. Biomol. Struct.*, ***33*** *(2004), 269-295.*
45. *Simons K. and Sampaio JL.,* 'Membrane organization and lipid rafts', *Cold Spring Harb. Perspect. Biol*., ***3*** *(2010), a004697.*
46. *Zimmerberg J. and Gawrich J.K.,* 'The physical chemistry of biological membranes', *Nat. Chem. Biol.*, ***11*** *(2006), 564-567.*
47. *McMahon H.T. and Gallop JL.,* 'Membrane curvature and mechanisms of dynamic cell membrane modeling', *Nature*, ***438*** *(2005), 590-596.*
48. *Mouritsen O.G. and Bloom M.,* 'Mattress model of lipid-protein interactions in membranes', *Biophys. J*. 1984, ***46*** *(1984), 141-153.*
49. *Chernomordik LV. and Kozlov M.M.,* 'Protein-lipid interplay in fusion and fission of biological membranes', *Annu. Rev. Biochem*., ***72*** *(2003), 175-207.*
50. *Zimmerberg J. and Kozlov M.M.,* 'How proteins produce cellular membrane curvature', *Nat. Rev. Mol. Cell Biol*., ***7*** *(2006), 9-19.*
51. *Baumgart T., Capraro B.R., Zhu C. and Das S.L.,* 'Theromodynamics and mechanics of membrane curvature generation and sensing by proteins and lipids', *Annu. Rev. Phys. Chem*., ***62*** *(2011), 483-506.*
52. *Frost A., Unger V.M. and De Camilli P.,* 'The BAR domain superfamily: membrane-molding macromolecules', *Cell*, ***137*** *(2009), 191-196.*
53. *Antonny B.,* 'Membrane deformation by protein coats', *Curr. Opin. Cell Biol*., ***18*** *(2006), 386-394.*
54. *Rothman J.E. and Lenard J.,* 'Membrane asymmetry', *Science*, ***195*** *(1977), 743-753.*
55. *Ftemadi A.-H.,* 'Membrane asymmetry: a survey and critical appraisal of the methodology. II. Methods for assessing the unequal distribution of lipids', *Biochim. Biophys. Acta*, ***604*** *(1980). 423-475.*
56. *Stoeckenius W. and Engelman D.M.,* 'Current models for the structure of biological membranes', *J. Cell Biol*., ***42*** *(1969), 613-646.*

57. *Pomorski T.S., Hrafnsdottir S., Devaux P.F, and van Meer G.,* 'Lipid distribution and transport across cellular membranes', *Semin. Cell Dev. Biol*., ***12*** *(2001), 139-148.*
58. *Daleke D.L.,* 'Regulation of transbilayer plasma membrane phospholipid asymmetry', *J. Lipid Res.*, ***44*** *(2003), 233-242.*
59. *Sharom F.J.,* 'Flipping and flopping—lipids on the move', *IUBMB Life.*, ***63*** *(2011), 736-746.*
60. *Quinn P.J.,* 'Plasma membrane phospholipid asymmetry', *Subcell. Biochem*., ***36*** *(2002), 39-60.*
61. *Brown D.A. and London E.,* 'Structure of ordered lipid domains in biological membranes', *J. Membr. Biol*., ***164*** *(1998), 103-114.*
62. *Ohvo-Rekilä H., Ramstedt B., Leppimäki P. and Slotte J.P.,* 'Cholesterol interactions with phospholipids in membranes', *Prog. Lipid Res*., ***41*** *(2002), 66-97.*
63. *Silvius J.R.,* 'Partitioning of membrane molecules between raft and non-raft domains: insights from model-membrane studies', *Biochim. Biophys. Acta*, ***1746*** *(2005), 193-202.*
64. *von Heijne G.,* 'Recent advances in the understanding of membrane protein assembly and structure', *Quart. Rev. Biophys*., ***32*** *(2000), 285-307.*
65. *Edidin M. and Weiss A.,* 'Antigen cap formation in cultured fibroblasts: a reflection of membrane fluidity and cell motility', *Proc. Natl. Acad. Sci. USA*, ***69*** *(1972), 2456-2459.*
66. *Poste G., Papahadjopoulos D. and Nicolson G.L.,* 'Local anesthetics affect transmembrane cytoskeletal control of mobility and distribution of cell surface receptors', *Proc. Natl. Acad. Sci. USA*, ***72*** *(1975), 4430-4434.*
67. *Bourguigon L.Y. and Singer S.J.,* 'Transmembrane interactions and the mechanism of capping of surface receptors by their specific ligands', *Proc. Natl. Acad. Sci. USA*, ***74*** *(1977), 5031-5035.*
68. *Unanue E.R., Perkins W.D. and Karnovsky M.J.,* 'Ligand-induced movement of lymphocyte membrane macromolecules. I. Analysis by immunofluorescence and ultrastructural radioautography', *J. Exp. Med*., ***136*** *(1972), 885-906.*
69. *Geiger B., Yehuda-Levenberg S. and Bershadsky A.D.,* 'Molecular interactions in the submembrane plaque of cell-cell and cell-matrix adhesions', *Acta Anat. (Basel)*, ***154*** *(1995), 42-62.*
70. *Fuchs E. and Cleveland D.W.,* 'A structural scaffolding of intermediate filaments in health and disease', *Science*, ***279*** *(1998), 1897-1907.*
71. *Chichili C.R. and Rogers W.,* 'Cytoskeleton-membrane interactions in membrane raft structure', *Cell Mol. Life Sci*., ***66*** *(2009), 2319-2328.*
72. *Nicolson G.L. and Poste G.,* 'Lectin-mediated agglutination of murine lymphoma cells. Cell surface deformability and reversibility of agglutination by saccharides', *Biochim. Biophys. Acta*, ***554*** *(1979), 520-531.*
73. *Salas P.J., Vega-Salas D.E., Hochman J., Rodriguez-Boulan E. and Edidin M.,* 'Selective anchoring in the specific plasma membrane domain: a role in epithelial cell polarity', *J. Cell Biol*., ***107*** *(1988), 2363-2376.*
74. *Kobialka S., Beuret N., Ben-Tekya H. and Spiess M.,* 'Gycosaminoglycan chains affect exocytic and endocytic protein traffic', *Traffic*, ***10*** *(2009), 1845-1855.*
75. *Geiger B.,* 'Membrane-cytoskeletal interaction', *Biochim. Biophys. Acta*, ***737*** *(1983), 305-341.*
76. *Roca-Cusach P., Iskratsch T. and Sheetz M.P.,* 'Finding the weakest link-exploring integrin-mediated mechanical molecular pathways', *J. Cell Sci*., ***125*** *(2012), 3025-3038.*
77. *Geiger B. and Bershadsky A.,* 'Assembly and mechanosensory function of focal contacts', *Curr. Opin. Cell Biol*., ***13*** *(2001), 584-592.*
78. *Geiger B., Bershadsky A., Pankov R. and Yamada K.M.,* 'Transmembrane extracellular matrix-cytoskeleton crosstalk', *Nat. Rev. Mol. Cell Biol*., ***2*** *(2001), 793-805.*
79. *Sheetz M.P.,* 'Cell control by membrane-cytoskeleton adhesion', *Nat. Rev. Mol. Cell Biol*., ***2*** *(2001), 392-396.*
80. *Parsons J.T., Horwitz A.R. and Schwartz M.A.,* 'Cell adhesion. Integrating cytoskeletal dynamics and cellular tension', *Nat. Rev. Mol. Cell Biol*., ***11*** *(2010), 633-643.*
81. *Anitei M. and Hoflack B.,* 'Bridging membrane and cytoskeleton dynamics in the secretory and endocytic pathways', *Nat. Cell Biol*., ***14*** *(2011), 11-19.*
82. *Jaqaman K. and Grinstein S.,* 'Regulation from within: the cytoskeleton in transmembrane signaling', *Trends Cell Biol*., ***22*** *(2012), 515-526.*
83. *Weirich C.S., Erzberger J.P. and Barral Y.,* 'The septin family of GTPases: architecture and dynamics', *Nat. Rev. Mol. Cell Biol*., ***9*** *(2008), 478-489.*
84. *Hagiwara A., Taaka Y., Hikawa R., Morone N., et al.,* 'Submembranous septins as relatively stable components of actin-based membrane skeleton', *Cytoskeleton*, ***68*** *(2011), 512-525.*
85. *Mostowy S. and Cossart P.,* 'Septins: the fourth component of the cytoskeleton', *Nat. Rev. Mol. Cell Biol*., ***13*** *(2012), 183-194.*

86. *Schwarz U.S. and Gardel M.L.,* 'United we stand: integrating the actin cytoskeleton and cell-matrix adhesions in cellular mechanotransduction', *J. Cell Sci*., ***125*** *(2012), 3051-3060.*
87. *Janmey P.A. and Lindberg U.,* 'Cytoskeletal regulation: rich in lipids', *Nat. Rev. Mol. Cell Biol*., ***5*** *(2004), 658-666.*
88. *Hollenberg M.D.,* 'Structure-activity relationships for transmembrane signaling: the receptor's turn', *FASEB J*., ***5*** *(1991), 178-186.*
89. *Jordan J.D., Landau E.M. and Iyengar R.,* 'Signaling networks: the origins of cellular multitasking', *Cell*, ***103*** *(2000), 193-200.*
90. *Cho W.* 'Building signaling complexes at the membrane', *Sci. STKE*, ***2006*** *(2006), pe7.*
91. *Damjanovich S., Gáspár R. Jr. and Pieri C.,* 'Dynamic receptor superstructures at the plasma membrane', *Q. Rev. Biophys*., ***30*** *(1997), 67-106.*
92. *Campbell I.D. and Humphries M.J.,* 'Integrin structure, activation and interactions', *Cold Spring Harb. Perspect. Biol., **3** (2011), a004994.*
93. *Somerharju P., Virtanen J.A. and Cheng K.H.,* 'Lateral organization of membrane lipids. The superlattice view', *Biochim. Biophys. Acta*, ***1440*** *(1999), 32-48.*
94. Ramstedt B. and Slotte JP., 'Sphingolipids and the formation of sterol-enriched ordered membrane domains', *Biochim. Biophys. Acta*, ***1758*** *(2006), 1945-1956.*
95. *Lindblom G. and Orädd G.,* 'Lipid lateral diffusion and membrane heterogeneity', *Biochim. Biophys. Acta*, ***1788*** *(2009), 234-244.*
96. *Mouritsen O.G.,* 'Model answers to lipid membrane questions', *Cold Spring Harb. Perspect. Biol., **3** (2011), a004622.*
97. *Quinn P.J.,* 'Lipid-lipid interactions in bilayer membranes: married couples and casual liaisons', *Prog. Lipid Res*., ***31*** *(2012), 179-198.*
98. *Aittoniemi J., Niemela P.S., Hyvonen M.T., Karttunen M. and Vattulainen I.,* 'Insight into the putative specific interactions between cholesterol, sphingomyelin, and palmitoyl-oleoyl phosphatidylcholine', *Biophys. J*., ***92*** *(2007), 1125–1137.*
99. *Zhang Z., Bhide S.Y. and Berkowitz M.L.,* 'Molecular dynamics simulations of bilayers containing mixtures of sphingomyelin with cholesterol and phosphatidylcholine with cholesterol', *J. Phys. Chem. B, **111** (2007), 12888–12897.*
100. *Lingwood D., Kiser H.-J., Levental I. and Simons K.,* 'Lipid rafts as functional heterogeneity in cell membranes', *Biochem. Soc. Trans*., ***37*** *(2009), 955-560.*
101. *Quinn P.J. and Wolf C.,* 'The liquid-ordered phase in membranes', *Biochim. Biophys. Acta*, ***1788*** *(2009), 33-46.*
102. *Simons K. and Ikonen E.,* 'Functional rafts in cell membranes', *Nature*, ***387*** *(1997), 569-572.*
103. *Mayor S. and Rao M.,* 'Rafts; scale-dependent, active lipid organization at the cell surface', *Traffic*, ***5*** *(2004), 231-240.*
104. *Simons K. and Gerl M.J.,* 'Revitalizing membrane rafts: new tools and insights', *Nat. Rev. Mol. Cell Biol*., ***11*** *(2010), 688-699.*
105. *Sengupta P., Baird B. and Holowka D.,* 'Lipid rafts, fluid/fluid phase separation, and their relevance to plasma membrane structure and function', *Semin. Cell Dev. Biol*., ***18*** *(2007), 583-590.*
106. *Neumann A.K., Itano M.S. and Jacobson K.,* 'Understanding lipid rafts and other related membrane domains', *F1000 Biol. Rep*., ***2*** *(2010), 31-36.*
107. *Suzuki K.G.,* 'Lipid rafts generate digital-like signal transduction in cell plasma membranes', *Biotechnol. J*., ***7*** *(2012), 753-761.*
108. *Kusumi A. and Hyde J.S.,* 'Spin-label saturation-transfer electron spin resonance detection of transient association of rhodopsin in reconstituted membranes', *Biochemistry*, ***21*** *(1982), 5978-5983.*
109. *Sharma P., Varma R., Sarsij R.C., Gousett K., Krishnamoorthy G., et al.,* 'Nanoscale organization of multiple GPI-anchored proteins in living cell membranes', *Cell*, ***20*** *(2004), 577-589.*
110. *Somerharju P., Virtanen J.A., Cheng K.H. and Hermansson M.,* 'The superlattice model of lateral organization of membranes and its implications on membrane lipid homeostasis', *Biochim. Biophys. Acta*, ***1788*** *(2009), 12-23.*
111. *Israelachvili J.N., Marcelja S. and Hom R.G.,* 'Physical principals of membrane organization', *Q. Rev. Biophys*., ***13*** *(1980), 121-200.*
112. *Dumas F., Lebrun M.C. and Tocanne J.-F.,* 'Is the protein/lipid hydrophobic matching principle relevant to membrane organization and functions?', *FEBS Lett*., ***458*** *(1999), 271-277.*
113. *Andersen O.S. and Koeppe R.E.I.,* 'Bilayer thickness and membrane protein function: an energetic perspective', *Annu. Rev. Biophys. Biomol. Struct*., ***36*** *(2007), 107-130.*
114. *Mouritsen O.G.,* 'Lipids, curvature and nano-medicine', *Eur. J. Lipid Sci. Technol*., ***113*** *(2007), 1174-1187.*

115. *Gill T., Sabra M.C., Ipsen J.H. and Mouritsen O.G.,* 'Wetting and capillary condensation as a means of protein organization in membranes', *Biophys. J., **73** (1997), 1728-1741.*
116. *Edidin M., Kuo S.C. and Sheetz M.P.,* 'Lateral movements of membrane glycoproteins restricted by dynamic cytoplasmic barriers', *Science, **254** (1991), 1379-1382.*
117. *Doherty G.J. and McMahon H.T.,* 'Mediation, modulation, and consequences of membrane-cytoskeletal interactions', *Annu. Rev. Biophys., **37** (2008), 65-95.*
118. *Nicolson G.L.,* 'Membrane Lipid Replacement: clinical studies using a natural medicine approach to restoring membrane function and improving health', *Intern. J. Clin. Med., **7** (2016), 133-143.*
119. *Nicolson G.L. and Ash M.E.,* 'Membrane Lipid Replacement for chronic illnesses, aging and cancer using oral glycerolphospholipid formulations with fructooligosaccharides to restore phospholipid function in cellular membranes, organelles, cells and tissues', *Biochim. Biophys. Acta Biomembr., **1859** (2017), 1704-1724.*
120. *Küllenberg D., Taylor L.A., Schneider M. and Massing U.,* 'Health effects of dietary phospholipids', *Lipids Health Dis., **11** (2012), e3.*
121. *Nicolson G.L., Rosenblatt S., Ferreira de Mattos G., Settineri R., et al.,* 'Clinical uses of Membrane Lipid Replacement supplements in restoring membrane function and reducing fatigue in chronic diseases and cancer', *Discoveries, **4** (2016), e54.*
122. *Zierenberg O, and Grundy S.M.,* 'Intestinal absorption of polyenephosphatidylcholine in man', *J. Lipid Res., **23** (1982), 1136-1142.*
123. *Kroenke K., Wood D.R., Mangelsdorff A.D., Meiero N.J. and Powell J.B.,* 'Chronic fatigue in primary care. Prevalence, patient characteristics, and outcome', *J. Am. Med. Assoc., **260** (1988), 929-934.*
124. *Filler K., Lyon D., Bennett J., McCain N., Elswick R., et al.,* 'Association of mitochondrial dysfunction and fatigue: a review of the literature', *BBA Clin., **1** (2014), 12-23.*
125. *Nicolson G.L.*, 'Mitochondrial dysfunction and chronic disease: treatment with natural supplements', *Integr. Med., **13** (2014), 35-43.*
126. *Komaroff A.L. and Buchwald D.,* 'Symptoms and signs of chronic fatigue syndrome', *Rev. Infect. Dis., **13** (1991), S8-S11.*
127. *Agadjanyan M., Vasilevko V., Ghochikyan A., Berns P., Kesslak P., Settineri R.A. and Nicolson G.L.,* 'Nutritional supplement (NTFactor) restores mitochondrial function and reduces moderately severe fatigue in aged subjects', *J. Chronic Fatigue Syndr., **11** (2003), 23-36.*
128. *Nicolson G.L. and Breeding P.C.,* 'Membrane Lipid Replacement for reduction of pain, fatigue, gastrointestinal and other symptoms in patients with peripheral pain: case reports', *Case Rep. Rev., **1** (2020), 1-3.*
129. *Nicolson G.L., Breeding P.C.. Settineri R. and Ferreira de Mattos G.,* 'Aging and chronic illnesses: Membrane Lipid Replacement for restoring mitochondrial function and reducing fatigue, pain, and other symptoms in aged individuals', *Bioactive Comp. Health Dis., **3** (2020), 194-203.*