Dynamic, yet structured: The cell membrane three decades after the Singer–Nicolson model

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The fluid mosaic membrane model proved to be a very useful hypothesis in explaining many, but certainly not all, phenomena taking place in biological membranes. New experimental data show that the compartmentalization of membrane components can be as important for effective signal transduction as is the fluidity of the membrane. In this work, we pay tribute to the Singer–Nicolson model, which is near its 30th anniversary, honoring its basic features, ''mosaicism'' and ''diffusion,'' which predict the interspersion of proteins and lipids and their ability to undergo dynamic rearrangement via Brownian motion. At the same time, modifications based on quantitative data are proposed, highlighting the often genetically predestined, yet flexible, multilevel structure implementing a vast complexity of cellular functions. This new ''dynamically structured mosaic model'' bears the following characteristics: emphasis is shifted from fluidity to mosaicism, which, in our interpretation, means nonrandom codistribution patterns of specific kinds of membrane proteins forming small-scale clusters at the molecular level and large-scale clusters (groups of clusters, islands) at the submicrometer level. The cohesive forces, which maintain these assemblies as principal elements of the membranes, originate from within a microdomain structure, where lipid–lipid, protein–protein, and protein–lipid interactions, as well as sub- and supramembrane (cytoskeletal, extracellular matrix, other cell) effectors, many of them genetically predestined, play equally important roles. The concept of fluidity in the original model now is interpreted as permissiveness of the architecture to continuous, dynamic restructuring of the molecular- and higher-level clusters according to the needs of the cell and as evoked by the environment.

Scientific dogmas, let alone models, rarely survive more than a quarter of a century without significant modifications. Around its 30th anniversary, the time seems to be ripe for at least a modest modification of an old paradigm. The Singer–Nicolson fluid mosaic membrane model (S-N model) (1) predicts lateral and rotational freedom and random distribution of molecular components in the membrane. Membranes had been considered by the S-N model as ''a two-dimensional oriented solution of integral proteins . . . in the viscous phospholipid bilayer'' (1–3). Now it is known, however, that this freedom of protein (and lipid) mobility is far from being unrestricted. One of the earliest indications of a nonrandom distribution of proteins was provided by the discovery of cocapping (4). The emerging evidence on hierarchically built supramolecular protein complexes (5–7) hindered diffusion of proteins in the membrane (8–10), and the existence of distinct membrane domains termed ''rafts'' (11) also contradicts the S-N model. Therefore, Jacobson *et al*. (2) have correctly stated, ''Most membrane proteins do not enjoy the continuous unrestricted lateral diffusion. . . . Instead, proteins diffuse in a more complicated way that indicates considerable lateral heterogeneity in membrane structure, at least on a nanometer scale.'' The great variety of phospholipid molecular species, the differences in their molecular shapes and physical properties, and their asymmetric distribution in the membrane bilayer all indicate a molecular heterogeneity and the possible formation of membrane microdomains (12).

Advanced Cell Biophysical and Molecular Biological Methodology Provides Quantitative Data on the Static and Dynamic Organization of Membranes

Membrane dynamics, i.e., the ever changing mobility and proximity relationships of lipid and protein molecules in the plasma membrane, have a significant impact on essential cellular processes, such as activation, ligand-receptor recognition, antigen presentation, intercellular interactions (e.g., between target and killer cells), etc. Quantitative measurements of membrane dynamics are possible with fluorescence recovery after photobleaching (13, 14), single-particle tracking techniques (8, 15, 16), and optical trapping by laser tweezers (13, 17, 18). Fluorescence correlation spectroscopy, a method with tradition in the study of reaction kinetics and molecular interactions in solution (19, 20), also has been applied to the study of cellular systems recently (21, 22). The method allows the determination of absolute molecular concentration, mobility, and comobility in small, confocal volume elements of living cells (23). Confocal laser-scanning microscopy (24) at the verge of its resolution limits proved to be successful in determining the uneven cell-surface distribution of various antigens. Scanning near-field optical microscopy (NSOM), a method ideal for assessing localization of membrane proteins at the resolution of several tens of nanometers, also has been gaining space in investigating the cytoplasm membrane (25–28), although, as Edidin (29) points out, ''while NSOM promises much, its application to biology is about where electron microscopy was 40 or 50 years ago.'' These methodologies supported by digital image processing add valuable information to the dynamic data about the spatial distribution and compartmentation of membrane constituents. In general, these approaches provide evidence for the domain-like distribution of lipids and proteins in biological membranes (17, 30, 31).

Restrictions in the lateral mobility of both lipid and protein components were studied extensively by using the fluorescence-recovery-after-photobleaching technique, measuring the diffusion of fluorescently labeled membrane components from nonbleached areas into a small, bleached spot. Lateral diffusion parameters of MHC molecules (9) were highly dependent on the bleached-spot size. Because diffusion rate in a lipid bilayer is expected to be independent of this size, one plausible explanation is the mosaic-like domain structure of the biological membranes that restrict the barrier-free path of proteins and can be partly responsible for the clustered arrangements of membrane proteins.

Abbreviations: S-N model, Singer–Nicolson fluid mosaic membrane model; FRET, fluorescence resonance energy transfer; TCR, T cell antigen receptor.

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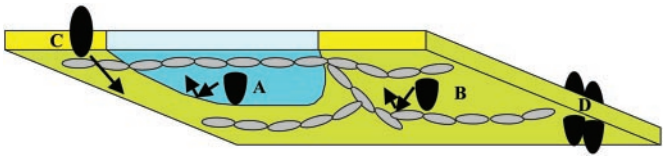

Fig. 1. Proteins experience different types of restrictions to translational diffusion in the plasma membrane. The view of the membrane is shown from beneath. A, Proteins showing preferential accumulation in a lipid microdomain may be confined to the area of the microdomain if the activation energy of passing the domain barrier is larger than the kinetic energy of the protein. The extent to which passing a domain barrier is prohibited is determined by the preference of the protein for the lipid environment: if the protein interacts preferentially and avidly with lipids of the microdomain, it may be reluctant to leave. B and C, The cytoskeleton is also important in restricting free, lateral diffusion of membrane proteins. Proteins whose intracellular domain is long are unable to pass through a fence composed of a filament of the cytoskeleton (B), whereas proteins with a short intracellular domain are free to move across such a fence (C). D, Associations of proteins experience more viscous force; therefore, their translational diffusion rate is usually smaller than that of monomeric proteins.

Single-particle tracking follows the "random walk" of a gold particle fixed to a cell-surface protein. For the transferrin receptor and E-cadherin, a barrier-free path of ≈400 nm was determined (8), whereas for wild-type and cytoplasmatically truncated mutant class I MHC, ≈600 and ≈1,700 nm were measured, respectively (13), indicating that, for membrane-spanning proteins, the barrier-free path is in the same range and that barriers to the "free" diffusion are present 2–3 nm below the membrane bilayer. As shown in Fig. 1, cytoskeletal elements and intracytosolic molecules with functional connections to cell-surface receptors have the capacity either to slow down or to completely stop the lateral motion of transmembrane proteins.

Fluorescence Resonance Energy Transfer (FRET) Highlights Nanometer-Scale Associations in the Cell Membrane

It has been suggested that membrane proteins can be codistributed nonrandomly, and this nonrandom yet dynamic codistribution pattern even may be controlled genetically (32–35). This assumption has been supported by a significant amount of experimental data (36, 37). A major asset in studying these codistributions was the application of FRET to cellular systems. In FRET, an excited fluorescent dye (donor) donates energy to an acceptor dye if the separation distance between them is 1–10 nm. In the 1970s, cell-surface lectins were the first to be investigated by FRET (38, 39). Since the early 1980s, more systematic and better established data acquisition and evaluation methods of FRET were introduced in flow cytometry (35, 40). A quantitative method was introduced in digital fluorescence microscopy that exploited the differences in photobleaching kinetics of donor dyes in the presence and absence of acceptors (36, 41). Recently, a new method, based on systematic and normalized FRET measurements between individual receptor pairs yielding a receptor map by triangulation, has been introduced to describe the exact two-dimensional topology of receptor clusters (7, 37). The homo- and heteroassociations of MHC class I and II (42), the IL-2 receptor α-subunit and intercellular adhesion molecule 1 (43–45), MHC molecules and transferrin receptors (46), CD4 and CD8 antigens (47, 48), the T cell antigen receptor (TCR)/CD3 complex (49), tetraspan molecules (CD53, CD81, CD82) and CD20 with MHC class I and class II (50), the three subunits of the multisubunit IL-2 receptor (37), the tumor necrosis factor receptor (51), Fas (CD95; ref. 52), and many others have been analyzed in detail. Many receptors were found to be oligomers preassembled without ligand binding, including tumor necrosis factor receptor, Fas, and TCR. Results for the IL-2 receptor indicated that its subunits are preassembled in T cells and do not require cytokine-induced aggregation. This colocalization was modulated significantly by binding of relevant ILs. In addition, there is evidence that the IL-15 receptor α-subunit, which shares the β- and γ-subunits with IL-2Rα, also can form preassembled supramolecular structures with IL-2Rβ and the "common" γ-chain (53–55). These data (37) have challenged the frequently applied paradigm that multisubunit receptors are assembled under the influence of their specific ligands and support an alternative model in which preassembled receptor clusters facilitate faster and stronger biological responses, because there is no need for lateral diffusion of receptors to associate.

Electron Microscopy and Scanning-Force Microscopy Reveal Submicrometer Clusters of Membrane Receptors

FRET measurements detect molecular associations in the 1- to 10-nm range. Combined application of electron and scanning-force microscopy made possible the discovery of a new, higher hierarchical level of receptor clustering in lymphoid cells (36). The distribution of ImmunoGold labels attached to receptors showed a nonrandom pattern, differing from the Poisson distribution assumed for randomly scattered molecules. The method also was used under near-physiological conditions, where the gold particles were detected with scanning-force microscopy by using tapping mode on hydrated samples (36). The observation that nanometer-scale islets of MHC class I molecules in the cell membrane are organized into micrometer-sized "island groups" was extended to MHC class II (56) and to the IL-2 receptor α-subunit and the transferrin receptor (24). Sequential application of different-sized gold labels targeted to MHC classes I and II also revealed successively that positive FRET data did not necessarily mean that each receptor was homo- or heteroassociated in a large population. The degree of association for MHC class II to MHC class I was 66%, but only 25% of MHC class I molecules were in the molecular vicinity of MHC class II (56).

Besides establishing the nonrandom distribution of receptors, ImmunoGold labeling also provided for estimating the average size of higher-order molecular clusters (or island groups) (24). These were in the range of 400–800 nm for various receptors, in good correlation with mean barrier-free paths reported earlier (8, 13). Confocal laser-scanning microscopy of fluorescently labeled, live, and fixed cells followed by surface reconstruction and spatial autocorrelation analysis confirmed the existence of such receptor clusters with sizes essentially equal to those deduced from electron microscopy (24).

Lipid Rafts: The Functional Equivalent of Receptor Islands?

Numerous studies directed at the plasma membrane have provided evidence for the existence of distinct domains in the submicron range (24–26, 36, 56–60). Paralleling these observations, the term "lipid rafts" was coined based on studies of epithelial cell polarity and gained widespread popularity in the past years (11). Biochemical analysis suggested that rafts consist of cholesterol and sphingolipids in the exoplasmic leaflet of the lipid bilayer and cholesterol and phospholipids with saturated fatty acids in the endoplasmic leaflet (61). They are surrounded by more fluid membrane domains, which are abundant in unsaturated fatty acids (62). Polyunsaturated phosphatidylcholines and phosphatidylethanolamines (63) including ethanolamine plasmalogens were detected in rafts by quantitative electrospray ionization and mass spectrometry (64), probably reflecting a regulatory mechanism that offsets the rigidifying effect of cholesterol (63). In addition, 18:1 and 22:6 phosphatidylethanolamines and ethanolamine plasmalogens are prone to form nonbilayer phases (65, 66), and the association of soluble proteins (such as G proteins) to membranes depends on the propensity of reverse hexagonal-phase areas (67). This observation highlights the possible role of lipid rafts in signal-

transduction processes. Isolating lipid rafts as detergent-resistant membrane domains, followed by Western blotting, indicated that rafts indeed are efficient concentrators of various proteins, many of them active in cell signaling. Among these were glycosylphosphatidylinositol-anchored proteins (68), cholesterol-linked and palmitoylated proteins such as Hedgehog (69), Src-family kinases and α-subunits of heterotrimeric G proteins (70), cytokine receptors (71), and integrins (72).

The question is how these biochemically identified, detergent-resistant membrane domains correspond to the observed confinement of membrane proteins. As Jacobson and Dietrich (73) pointed out, ". . . the nature of the *in vivo* correlate of such detergent-resistant membranes remains enigmatic. In principle, microscopy should be able to determine whether the postulated rafts exist." Recently, it has been shown that indeed microscopic equivalents of rafts, or, rather, aggregates of rafts, can be detected by using high-resolution confocal microscopy and that their disassembly by depletion or *in situ* complexation of cholesterol not only destroys their morphology (24) but also impairs their signaling capabilities; for example, in T cells, it hinders Stat5 and Stat3 phosphorylation via the IL-2 receptor (71). Based on the notion that rafts essentially are membrane units formed from transport vesicles fusing to the membrane, one would expect their size to be very small (59). In photonic force microscopic experiments, it was determined that raft size is <50 nm in diameter (74), representing $\approx$3,500 sphingolipid molecules. This indicates that membrane patches observed in fluorescence microscopy, bearing raft marker proteins and/or lipids, probably are aggregates of these basic building blocks. In some instances, these larger aggregates are not observed in resting cells (75, 76) and can be seen only upon crosslinking the "unit rafts" (75). In other cases, cells in their native state present surface patches of submicrometer size, identifiable as rafts based on their composition (24, 77).

Rafts Are Dynamic Structures Reshaped as Function Requires

A few years ago, little was known about the stability and lifespan of lipid rafts in living cells. It appears now that, compared with the relatively stable nature of phases in artificial bilayers, lipid rafts in cells are relatively short-lived. As Edidin points out in a recent review (78), ". . . domains are now thought to be smaller and less stable then they were in 1992." Lipid probes with saturated chains on average spend 13 ms in one domain (79); the average lifetime of stable domains is found to be on the scale of tens of seconds (10). Pulsed EPR measurements indicate a very fast exchange rate between protein-rich and bulk domains in the membrane, reaching residency times as low as 15 μs (80). This possibility of rapidly changing composition and location in the membrane, as well as the ability to form aggregates of various sizes, easily can account for the dynamic regulatory role lipid rafts play in various signaling processes. Such a function has been established for receptor tyrosine kinases (27) and for immune receptors such as the TCR (81) and the IgE receptor (82, 83).

The first step in immunoreceptor signaling is represented by ligand-dependent receptor aggregation, followed by receptor phosphorylation by tyrosine kinases of the Src family. Lipid rafts have been identified as platforms wherein signal transduction molecules may interact with the aggregated immunoreceptors. Multichain immune recognition receptors such as TCR (84) and FcεRI (83) use common mechanisms by which lipid rafts assist in the initiation of signaling.

The onset of T cell activation is associated with the formation of the so-called "immunological synapse" (85) between T cells and the cells that they are recognizing. This synapse starts with the initial clustering of receptors and promotes the centralized accumulation of TCRs that has been termed the "central supramolecular activating complex" with a corresponding peripheral ring, consisting of lymphocyte function-associated antigen 1 (LFA-1) ligands (86). The surface components of antigen-presenting cells are also integral to these clusters; MHC–peptide complexes are found in the central supramolecular activating complex, whereas intercellular adhesion molecule 1, the LFA-1 counterreceptor, is concentrated in the peripheral supramolecular activating complex. Initially, the TCRs are not necessarily engaged in the center, but as cell–cell interaction develops, they translocate from the periphery into the center of the synapse (87, 88). According to the serial triggering model, in this manner, very few peptide-presenting MHC molecules can activate a high number of TCRs (89). It appears important in terms of the interaction that palmitoylation of the membrane-proximal cysteines of CD4 and the association of CD4 with Lck contribute to the enrichment of CD4 in lipid rafts (90) and that, furthermore, K^+ channels also reside in rafts in the molecular vicinity of TCR (91). The structure of the CTL–target cell contact is similar to that observed between T cell and antigen-presenting cell, a ring of adhesion proteins surrounding the inner signal molecule domain. Lytic granule secretion occurs in a separate domain within the adhesion ring (92). As for the spatiotemporal coordination of signaling by the high-affinity IgE receptor FcεRI, lipid rafts first concentrate Lyn protein kinases while excluding the FcεRIs from these domains. Upon crosslinking FcεRIs by the antigen, the receptors rapidly translocate into the lipid rafts followed by their phosphorylation and the subsequent recruitment of Syk and PLCγ into these domains (93). The latter process also involves accumulation of actin cytoskeleton to the active domains (82).

A recent attempt to repeat the classic experiment of Frye and Edidin (94) with currently available technologies provided interesting data underlining the dynamism of membrane domains. Cells labeled with different fluorescent antibodies were fused with each other. Near-field scanning optical microscopic and parallel FRET studies revealed that intermixing of micrometer-scale protein clusters started right after cell fusion, but there was a delay of about 20 min in the intermixing of nanometer-scale protein associations (28), which clearly indicates the hierarchy of protein associations (Fig. 2). Although these experiments corroborate the existence of protein clusters, they emphasize their dynamism, which may be important for rapidly reshuffling protein interactions.

Static and Dynamic Factors Organizing Membrane Domains

The physical and chemical forces giving rise to membrane domains are under intensive investigation (2, 57). One presumes that several intracellular and extracellular constraints and forces influence the size and distribution of these clusters, one of them being the cholesterol content of the membrane area in question (11, 95), and changing the cholesterol composition of the cell membrane alters the association pattern and signaling properties of various molecules (71, 95).

Recently, it was proposed that lipids tend to adopt a superlattice distribution in fluid-mixed bilayers and distribution of phospholipids in these structures is determined by the molecular shape and the charge of the head group (96, 97). These superlattice structures do not cover the whole membrane area; rather, they are in dynamic equilibrium with areas in which lipids are distributed randomly. The presence of proteins can modify these structures by depleting or attracting certain lipid species because of similarities or differences in molecular shapes. However, fatty acid and polar head group composition of phospholipids (96), as well as the thermotropic and lamellar- to nonlamellar-phase transitions, are controlled precisely in a way that overall fluidity is reached below body temperature. Molecular architecture of certain phospholipids (98) and proportion of nonbilayer-forming lipids (66) may contribute to this phenomenon. Remarkably, the liquid-ordered- to liquid-disordered-phase transition temperature of rafts proper is 13–15°C above (99) the main transition

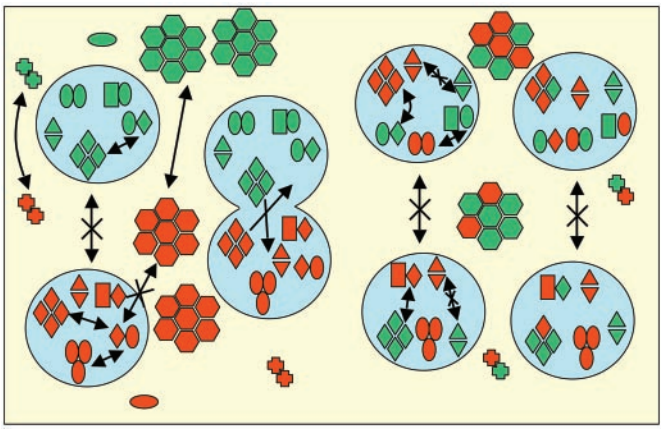

Fig. 2. Dynamics in the hierarchical association of membrane proteins. Imaging of nanometer- and micrometer-sized protein clusters give an overview of the hierarchical association of membrane proteins. Two cell samples previously labeled with different fluorescent antibodies (green and red symbols) were fused. Lipid rafts (blue circles) are known to accumulate a specific set of proteins. Micrometer-sized protein clusters exchanged components with each other, but this process respected lipid microdomain barriers: proteins known to be in different membrane microdomains never intermixed with each other. After a lag period of ≈20 min, intermixing of nanometer-sized protein clusters also took place. However, this process was not as widespread as the intermixing of micrometer-sized clusters, because some proteins (e.g., MHC class II) did not show a significant ability to move from one nanometer-sized cluster to another.

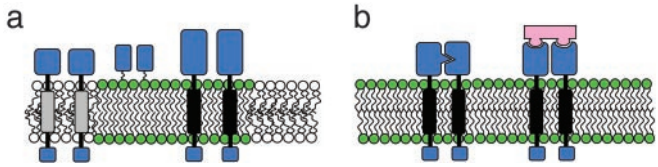

Fig. 3. Association of proteins can be induced by selective accumulation of proteins in distinct lipid microdomains (*a*) or by specific protein–protein interactions (*b*). (*a*) The membrane contains lipid microdomains with distinct lipid compositions. These membrane areas harbor different sets of proteins. Green lipid molecules preferentially accumulate proteins whose transmembrane domain is displayed in black and also proteins that are attached to the extracellular leaflet of the membrane (glycosylphosphatidylinositol-anchored proteins). The mechanism for the selective accumulation of proteins in a given lipid environment can be explained by a preference of proteins for the chemical (hydrophobicity) or physical (membrane thickness, microviscosity) properties of the lipid microdomain. Nanometer-sized protein associations can be considered a lipid-mediated interaction in this case. (*b*) Specific protein–protein interactions mediated by transmembrane proteins or ligands binding to them also may be responsible for the generation of protein associations.

temperature of membranes (100) because of the high cholesterol and sphingomyelin content. Under pathological conditions such as neurodegenerative diseases, this balance is upset.

Lipid rafts often are looked at as structures originating solely from lipid–lipid interactions. One should not, however, overlook the fact that proteins and protein–lipid interactions could be equally important in the formation, maintenance, and dynamics of these domains. In fact, one can imagine the basic unit of a raft as a single protein molecule surrounded by lipids that are especially suitable as its environment regarding polarity and steric complementarity. In addition to the classic investigations (101), where very precise predictions were given about the membrane-spanning α-helices, recent data also suggest that the exact composition and nature of α-helical structures will influence heavily how and in what phospholipid environment this polypeptide chain can be hosted, if at all (102). This alone would be enough to account for protein patterns in cell membranes that are predestined genetically, as we have predicted decades ago (32). The primary and secondary structure of proteins newly synthesized, together with the sorting capabilities in vesicular transport, can easily determine which proteins will be embedded into a certain lipid environment and which other proteins will be their immediate neighbors. For example, it has been demonstrated that the transmembrane domain of CD40 (103) and influenza hemagglutinin (104) determines their partitioning into lipid rafts.

On a similar note but looking at the dynamic side, changes in the structure of proteins [a most evident example being those occurring after their interaction with other proteins or those generated by changes in membrane potential (105)], as well as changes in the constitution or thickness of the various lipid regions, can easily cause reshuffling of the components of lipid rafts so that the best steric/energetic stability is achieved again (106).

The strength of interaction between proteins and their immediate lipid environment is well characterized when lymphoid cells with labeled MHC molecules are fused (28). MHC class II molecules from the two cells did not intermix even after 80 min and maintained their monomeric behavior. It was proposed that the small size of the microdomain allowed such a strong interaction with the membrane-spanning parts of the protein molecule that the cohesive forces prevented the fusion of these microdomains. Interestingly, class II molecules did intermix with class I molecules of the other cell. MHC class I probably was accommodated in larger rafts, which could incorporate the small rafts of class II molecules in their entirety.

As for the assembly and maintenance of protein clusters, the internalization-recycling machinery is one important candidate "force": proteins are recruited to sites of endosome formation, which gives rise to protein associations (107, 108). S—S bonds between integral membrane proteins were implicated in the case of human killer cell-activating receptors expressed in the plasma membrane of natural killer cells as multimeric complexes (109). Partitioning of covalently linked, saturated acyl chains into liquid-ordered phase domains is likely to be an important mechanism for targeting proteins to rafts, whereas prenylated proteins tend to be excluded from there (110). Thus, even without direct protein–protein interactions, preferential accumulation of certain proteins in a lipid domain may induce homo- or heteroassociation as well as formation of micrometer-sized protein clusters (Fig. 3).

Do We Need a New Paradigm?

Recent data that do not fit the S-N model can be summarized as follows: (*i*) nonrandom codistribution patterns of receptors in the plasma membrane at different hierarchical levels; (*ii*) quasipermanent molecular contacts to cytoskeletal elements and signal-transducing molecules; (*iii*) much shorter barrier-free path than expected for unrestricted diffusion; (*iv*) domain structure of the lipid components of membranes has the capacity to segregate or colocalize membrane proteins; (*v*) participation of integral membrane proteins in the maintenance of membrane domains suggests that proteins are as important as structural elements as lipids; and (*vi*) dynamic reorganization of protein elements in membrane domains allows for streamlined cellular responses and is restricted by protein–lipid and protein–protein interactions.

Thus, the straightforward application of the S-N model as a frame of events is impossible without introducing a new concept. This new concept has the following attributes emphasizing the colocalization, comobility, and nonrandom codistribution of a significant number of cell-surface molecules: (*i*) the mobility of

the cell-surface (transmembrane, glycosylphosphatidylinositol-anchored, or any other type of) proteins is restricted by lipid-domain segregation and the length of the free diffusion pathway covered without bumping into boundaries; (*ii*) membrane proteins may colocalize with each other on the 1- to 10-nm scale in a homologous or heterologous fashion, making the mosaicism of the S-N model prevalent; (*iii*) a second hierarchical level of protein clustering ranging to several hundred nanometers can be observed for many membrane proteins; (*iv*) proteins or protein clusters frequently are accommodated by lipid rafts organized by weak or strong interactions above, inside, or below the cell membrane; (*v*) some receptor types (e.g., tumor necrosis factor and IL receptors) are in a preassembled supramolecular formation even in the absence of their physiological ligands and may form a tighter formation upon ligand binding; (*vi*) ligand-evoked receptor aggregations (e.g., epidermal growth factor receptor family) are distinctly different from preformed oligomers, yet may serve as equally important amplifying factors of transmembrane signaling; (*vii*) the smallest microdomains can be considered modules that accommodate membrane proteins either alone or in functional oligomers preassembled from subunits, and these can be the building units of larger signaling domains (such as those formed in the immunological synapse); (*viii*) the α-helical membrane-spanning parts of transmembrane proteins are matched in length and shape by the aliphatic side chains of lipids constituting the membrane domains that preferentially accommodate them; (*ix*) the localization of proteins in different lipid regions of the plasma membrane can be determined genetically because the amino acid sequence of the transmembrane domain and sequence-dependent covalent modifications define the possible, specific lipid–protein interactions; (*x*) proteins are likely to have an equally important role in determining the constituents, structure, and dynamics of membrane domains; (*xi*) whereas artificial lipid bilayers tend to spontaneously form segregated structures, the dynamics and specificity in the living cell membrane are provided by specific protein–protein and protein–lipid interactions, as well as the targeted and sensitively regulated nature of vesicular transport processes; and (*xii*) identification of the origin and characteristics of microdomains and receptor assemblies therein may help us understand the immediate past and future of cells, their activation state, and reactivity. Such signals may carry diagnostic, prognostic, or even therapeutic values if these nonrandom receptor patterns can be linked to diseases affecting the different states and/or altered genetic material of the cell.

In light of the above attributes, we must understand that biological details are far more complicated than the resolving power of a simple model, which describes generalized, uniform behavior of molecules in the membrane. The S-N model is valid, and free diffusion can occur within domain borders, where molecular interactions do not interfere. This means that the emphasis must be shifted from the fluidity to the mosaicism of the S-N model. Mosaicism can restrict free diffusion through one of the following ways: (*i*) lipid domain structure, (*ii*) cytoskeletal or other cytosolic interactions, or (*iii*) homo- and heteroassociations with other integral proteins. These interactions have the capacity to increase the lifetime of an intermolecular encounter, thereby increasing the possibilities for bi- or multilateral interactions, which sometimes simply are called receptor crosstalk. Hence, the overall mobility of molecular elements of the membrane can be accepted with the above restrictions, making the membrane a heavily compartmentalized, quasi-two-dimensional structure, which is more mosaic-like than fluid. In this two-dimensional plane, diffusion, intermolecular forces, the ever changing membrane potential, and extracellular influences can dynamically generate and destroy supramolecular structures. We propose that this new model of the cell membrane be called the dynamically structured mosaic model.

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