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Review

Physical properties of lipid bilayer membranes: relevance to membrane biological functions★✪

Witold K. Subczynski[1, 2]✉ and Anna Wisniewska[1]

[1]*Biophysics Department, Institute of Molecular Biology, Jagiellonian University, Kraków, Poland;* [2]*Biophysics Research Institute, Medical College of Wisconsin, Milwaukee, Wisconsin, U.S.A.*

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Over the last 25 years one of us (WKS) has been investigating physical properties of lipid bilayer membranes. In 1991 a group led by WKS was organized into the Laboratory of Structure and Dynamics of Biological Membranes, the effective member of which is AW. Using mainly the electron paramagnetic resonance (EPR) spin-labeling method, we obtained unexpected results, which are significant for the better understanding of the functioning of biological membranes. We have developed a new pulse EPR spin-labeling method for the detection of membrane domains and evaluation of lipid exchange rates. This review will be focused on our main results which can be summarized as follows: (1) Unsaturation of alkyl chains greatly reduces the ordering and rigidifying effects of cholesterol although the unsaturation alone gives only minor fluidizing effects, as observed by order and reorientational motion, and rather significant rigidifying effects, as observed by translational motion of probe molecules; (2)

★75th Anniversary of Membrane Lipid Bilayer Concept.

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✉Corresponding author: Witold K. Subczynski, Biophysics Department, Institute of Molecular Biology, Jagiellonian University, Al. A. Mickiewicza 3, 31-120 Kraków, Poland; Tel. (48 12) 634 1305; fax: (48 12) 633 6907; e-mail: subczyn@mol.uj.edu.pl

Abbreviations: EPR, electron paramagnetic resonance; PC, phosphatidylcholine; DXPC (where X = L, M, P, S, B, O, E), dilauroyl-, dimyristoyl-, dipalmitoyl-, distearoyl-, dibehenoyl-, dioleoyl-, dielaidoylphosphatidylcholine, respectively; EYPC, egg-yolk phosphatidylcholine; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; BR, bacteriorhodopsin; IFV, influenza virus; SASL, stearic acid spin label; 16-SASL, 16-doxylstearic acid spin label; SLOT domain, slow oxygen transport domain; DOT method, method of discrimination by oxygen transport; $T_{1,}$ spin-lattice relaxation time.

Fluid-phase model membranes and cell plasma membranes are not barriers to oxygen and nitric oxide transport; (3) Polar carotenoids can regulate membrane fluidity in a way similar to cholesterol; (4) Formation of effective hydrophobic barriers to the permeation of small polar molecules across membranes requires alkyl chain unsaturation and/or the presence of cholesterol; (5) Fluid-phase micro-immiscibility takes place in *cis*-unsaturated phosphatidylcholine–cholesterol membranes and induces the formation of cholesterol-rich domains; (6) In membranes containing high concentrations of transmembrane proteins a new lipid domain is formed, with lipids trapped within aggregates of proteins, in which the lipid dynamics is diminished to the level of gel-phase.

For 75 years the lipid bilayer has been assumed to provide the structural basis of the biological membrane [1]. The old models [1, 2] treated this bilayer as a rigid lipid structure which provided the construction to attach proteins in a fairly structureless manner. The lipid bilayer also represented the fundamental permeability barrier to the passage of polar molecules into or out of a cell. The fluid-mosaic model imparted a certain degree of fluidity to the membrane. An essential point of this model is that the membrane proteins are floating in a sea of excess lipid molecules organized in a lipid bilayer [3]. Fluidity is perhaps the most obvious physical feature of a membrane and is an essential requirement for its biological viability [4]. The membrane as a many-component system should also possess a certain lateral lipid organization on many different length and time scales. It is important to recognize these dynamic structures and to characterize them in a quantitative manner in order to better understand different membrane functions.

Our investigations were carried out using the electron paramagnetic resonance (EPR) spin-labeling method with phospholipid, fatty acid, and sterol-type spin labels. The monitoring group (the nitroxide free-radical moiety) of spin labels can be located at various depths in the membrane and in different membrane domains. Thus, the local information obtained using EPR spectroscopy enables us to study the three-dimensional dynamic organization of the membrane. Conventional EPR with phospholipid and fatty acid spin labels has been used to study the alkyl chain order and motion [5, 6–8], and with spin-labeled sterol analogues to study cholesterol motion [6, 7, 9]. Saturation-recovery EPR with dual probes was also utilized. Bimolecular collision rates between paramagnetic molecules and nitroxide spin labels have been evaluated from the spin-lattice relaxation time (T_1) of spin labels [10–15]. Since T_1 of spin labels (10^{-6}–10^{-5} s) is much longer than their rotational correlation times (10^{-10}–10^{-9} s), membrane dynamics in longer time-space scales can be obtained. Our results also provide information by which the quality of a membrane simulation may be assessed [16–18].

We have studied the effects of cholesterol, polar carotenoids and integral membrane proteins on the structure and dynamics of saturated and unsaturated phosphatidylcholine (PC) membranes. As saturated PCs we have used dilauroyl-, dimyristoyl-, dipalmitoyl-, distearoyl-, dibehenoyl-PC (DLPC, DMPC, DPPC, DSPC, DBPC, respectively). As unsaturated PCs we have used dioleoyl-, dielaidoyl-, 1-palmitoyl-2-oleoyl-, and egg-yolk-PC (DOPC, DEPC, POPC and EYPC, respectively).

ARE UNSATURATED MEMBRANES MORE FLUID THAN SATURATED ONES?

We have intensively studied saturated and unsaturated PC–cholesterol membranes with special attention paid to the fluidizing effect of *cis*-unsaturated alkyl chains. For the conclusions to be valid, the properties of saturated and unsaturated fluid-phase membranes were compared at the same temperatures. In saturated PC membranes the alkyl chain order in-

creases and the alkyl chain reorientational motion decreases when the length of the PC alkyl chain increases [5–7]. These differences are rather small and decrease when the comparison is made well above the main phase-transition temperature. A similar dependence was observed for sterol-type spin labels [9, 19], however, the effects observed were stronger than those for stearic acid spin labels (SASLs).

Introduction of unsaturation into the PC alkyl chains has only a minor effect on the order and reorientational motion of membrane alkyl chains. For example, the order parameter measured for SASLs in EYPC membranes is greater than those in DLPC and DMPC membranes and smaller than those in DPPC, DSPC and DBPC membranes. Similarly, the rate of reorientational motion of 16-doxylstearic acid spin label (16-SASL) in EYPC membranes lies between those in DMPC and DPPC membranes [5–7, 20, 21]. Additionally, the measured phenomena which depend on translational diffusion always indicate slower motion in unsaturated membranes. This was shown for transport of small hydrophobic molecules as oxygen (M_r = 32) [12, 14, 20] and a membrane soluble square planar copper complex (M_r = 394) [13]. The T_1 for a given lipid spin label is always greater in unsaturated *versus* saturated membranes, showing slower Brownian translational motion of the free-radical fragment [14, 21, 22]. Other evidence comes from measurements of the collision frequency between SASL pairs, which is always greater in saturated than in unsaturated PC membranes [21, 22]. This is true for homogeneous pairs for which collision depends primarily on the lateral diffusion of SASL, as well as for inhomogeneous pairs for which collision requires a vertical fluctuation of the nitroxide toward the membrane surface. Also fluorescence photobleaching experiments indicated that lateral diffusion of a fluorescently labeled phospholipid was larger in DMPC than in DOPC bilayer [5]. These observations contradict the general view of the effect of the introduction of unsaturation into the alkyl chain on membrane fluidity, i.e., that unsaturation increases the fluidity [23, 24].

These dual effects on membrane fluidity introduced by unsaturated alkyl chains can be explained by the structure of the double bond and packing defects created in the lipid bilayer. The presence of a *cis* double bond will introduce a bend of 30° at that bond in the unsaturated alkyl chain. This would create some nonconformability between chains and increase chain disorder. Also the presence of either *cis* or *trans* double bonds in the alkyl chain would reduce the dynamics of the chain around the rigid double bonds.

SASL's order parameter plotted as a function of mole fraction of cholesterol at the same temperature in fluid phase PC membranes with saturated alkyl chains of 14–22 carbons is nearly the same. This occurs in a wide range of temperatures and cholesterol mole fractions [5]. The ordering effect of cholesterol in saturated membranes is very strong. Cholesterol also strongly reduces the reorientational motion of SASLs and sterol-type spin labels [9]. Unsaturated alkyl chains greatly reduce these effects of cholesterol [5, 9], which allowed us to make the following final conclusion. The "fluidizing" effect of unsaturated chains observed in biological membranes seems to manifest itself by moderating the "rigidifying" effect of cholesterol (or other membrane modifiers).

DO CAROTENOIDS REGULATE MEMBRANE FLUIDITY?

Procaryota, which appeared in the early stages of biological evolution when oxygen was not present in the Earth's atmosphere, in contrast to Eucaryota, do not contain cholesterol in their membranes. Instead, they contain carotenoids or other terpenoids [25]. It is already accepted that cholesterol is a major membrane compound which regulates mem-

brane fluidity in Eucaryota [26]. The hypothesis that carotenoids serve as cholesterol equivalents in some Procaryota was postulated by Rohmer *et al.* [25].

In our papers we demonstrated that the effects of polar carotenoids (dihydroxycarotenoids such as lutein, zeaxanthin, violaxanthin) on the structure and dynamics of lipid bilayer membranes are in many aspects similar to the effects of cholesterol [6, 7, 27]. Both increase order and decrease the alkyl chain motion in fluid-phase membranes, and disorder lipids in gel-phase membranes. Both are known to broaden the gel-to-fluid phase transition and increase the mobility of polar headgroups. As a rule, the presence of unsaturated alkyl chains moderates the effect of polar carotenoids and cholesterol [5, 7]. In saturated membranes 10 mol% of polar carotenoids exerts an effect similar to that of 15–20 mol% of cholesterol. This is due to the fact that the molecule of cholesterol is located in one leaflet of the bilayer, while one carotenoid molecule influences both leaflets. The ordering effect of cholesterol does not depend on the bilayer thickness [5], whereas the relation between the length of the carotenoid molecule and the thickness of the membrane is a significant factor in determining the effect of polar carotenoids on membrane properties [7, 27]. To manifest those effects the rigid rod-shaped carotenoid molecule must possess two polar groups at the ends of the hydrophobic "bar". Non-polar carotenoids such as β-carotene practically do not affect physical properties of the membrane [27, 28].

Membranes of extreme halophiles [29] and thermophilic bacteria [30] contain a fairly large amount of polar carotenoids. These bacteria, which live in extreme conditions, should possess stable membranes that provide a high barrier to non-specific penetration of small molecules. Incorporation of polar carotenoids into these membranes serves this purpose well. Carotenoids stabilize both halves of the lipid bilayer like transmembrane "rivets" and increase membrane rigidity by ordering the alkyl chains of lipids. They also raise the membrane hydrophobic barrier for polar molecules and ions [27] and the rigidity barrier for small non-polar molecules [20, 31]. Our results thus provide significant support for Rohmer's *et al.* [25] hypothesis that polar carotenoids regulate the membrane properties of Procaryota in a manner similar to cholesterol in Eucaryota.

IS THE LIPID BILAYER MEMBRANE A BARRIER TO OXYGEN TRANSPORT?

We have studied the transport of oxygen within and across model membranes and the lipid portion of biological membranes [8, 10, 12, 14, 18, 20, 32–38]. Our method is based on measurements of bimolecular collision rates between molecular oxygen and nitroxide spin labels in the membrane by observing either the T_1 or the EPR linewidth [39]. The collision rate is proportional to the product of the local diffusion coefficient and the local concentration of oxygen at the place in the membrane where the nitroxide moiety is located. In this way the profiles of the oxygen diffusion-concentration product across the membrane could be obtained.

All fluid-phase model membranes [12, 14, 20] and biological membranes [35, 36] show similar, bell-shaped profiles, with the oxygen diffusion-concentration product in the membrane center severalfold greater than that in and near the headgroup region. Membrane modifiers affect oxygen transport[1] within the lipid bilayer differently in different membrane regions. Cholesterol significantly de-

[1] Here, the word „transport" is used in its basic physical sense indicating the product of the (local) translational diffusion coefficient and the (local) concentration of oxygen in the membrane.

creases oxygen transport in the polar headgroup region and in the hydrocarbon region near the polar headgroups and increases it in the membrane center [12, 14]. This indicates that the major barrier for oxygen permeability across the membrane is located in and near the headgroup region. The extended membranous systems may, in principle, serve as pathways for intracellular oxygen transport, parallel to the membrane surface. Oxygen transport across the cytosol seems to be less effective. This hypothesis was formulated by Skulachev [40] for the transport of small hydrophobic solutes within the cell.

Polar carotenoids decrease the oxygen diffusion-concentration product in saturated and unsaturated membranes [20]. The effect is strongest in the membrane center and negligible in the headgroup region.

Additionally, we have developed a method to estimate the membrane oxygen permeability coefficient (P_M) using the profiles of the oxygen diffusion-concentration product across the membrane [12]. Creation of fast-decaying oxygen gradients is not necessary. Values of P_M for pure fluid-phase lipid bilayer membranes are about two times greater than the oxygen permeability coefficient of a water layer of the same thickness. Cholesterol at high concentration decreases the value of P_M of model membranes 3- to 5-fold and polar carotenoids at 10 mol% twofold [12, 14, 20, 31]. The P_M measured for the lipid bilayer portion of the Chinese hamster ovary plasma membrane is about two times lower than that of an appropriate water layer [35] while that of the thylakoid membrane is about the same as that of a water layer [36]. The smallest P_M is observed for lipid domains crowded with integral membrane proteins. The lipid domain of the purple membrane showed P_M about 6 to 10 times smaller than that of a fluid-phase lipid bilayer [37]. The interior of integral membrane proteins is practically not permeable to oxygen [41]. Based on these permeability coefficients the possible oxygen concentration differences across the cell plasma membrane, the mitochondrial membrane, and the thylakoid membrane at physiological conditions during oxygen consumption or production were estimated as 0.012 μM, 0.12 μM, and 1 μM, respectively [14, 36]. The overall conclusion from these studies is that membranes are not barriers to oxygen transport.

Because both oxygen and nitric oxide (NO) are paramagnetic and have similar physical properties, a similar approach can be used to study NO transport within and across the lipid bilayer membrane [42]. Data obtained by us [43, 44] give a solid base for the conclusion that fluid-phase membranes are not barriers to NO transport either.

For the understanding of chemical reactions involving oxygen and/or NO, the most important parameter is the product of their local diffusion coefficient and local concentration. Spin-label oximetry and spin-label NO-metry allow measurements of these products in restricted domains such as membranes. The product mentioned above is thus of fundamental interest in itself, and separation into component factors, diffusion and concentration, is not necessary. For oxygen, important chemical reactions occurring within the membrane include lipid peroxidation as well as the formation of reactive oxygen species. In membranes, NO is protected against reactions with water soluble compounds, which limit its lifetime [45]. Membranes could be thus considered as short-time storage environments for NO within the cell [43] that supplement the long time NO-buffering provided by plasma *S*-nitrosothiols [46, 47]. It can be inferred, however, that reactions of NO with oxygen will proceed more readily in the membrane than in aqueous phase. Here we should consider two cases. At low NO concentrations the nitrosyldioxyl radical is formed in a first- order reaction with respect to NO. However, the nitrosyldioxyl radical in the membrane cannot be stabilized by hydrogen bonding with water. Thus at low concentrations NO dissolved in membranes may be more stable than in the aqueous phase [48]. At high NO concen-

trations the second-order reaction (with respect to NO) of NO with oxygen is dramatically accelerated within the hydrophobic region of membranes, and the hydrophobic compartments of the cell should be considered as important sites for NO disappearance [49].

HYDROPHOBIC BARRIER OF LIPID BILAYER MEMBRANES FORMED BY ALKYL CHAIN UNSATURATION AND CHOLESTEROL

One of the most fundamental properties of biological membranes is that they act as barriers to the permeation of polar molecules. This barrier effect is largely due to the hydrophobicity of the membrane interior. We have undertaken a comprehensive investigation of the profiles of hydrophobic barriers of model membranes consisting of various PCs and different membrane modifiers. The detailed profiles of these hydrophobic barriers may provide a basis for the understanding of membrane permeability for both polar and non-polar molecules. In addition, such information is necessary for the study of lateral transport and chemical reactions involving small molecules within the membrane. Hydrophobicity in the membrane is largely determined by the extent of water penetration into the membrane since dehydration abolishes the hydrophobic gradients in lipid bilayers [50].

While measuring "hydrophobicity", we take advantage of solvent effects on the hyperfine interaction of spin labels [51]. The z component of the hyperfine interaction correlates with the dielectric constant ε of the bulk solvent. Because of that, local hydrophobicity in the membrane is often compared with that of the bulk solvent [19, 50, 52, 53]. We have shown that saturated PC membranes exhibit low hydrophobicity across the membrane, and at the membrane center, where hydrophobicity is the highest, it is comparable to that of propanol and octanol, which have dielectric constants of 10–20 [53]. Introduction of a double bond to the alkyl chain increases hydrophobicity at all locations in the membrane. This effect is considerably greater for the *cis* configuration, for which hydrophobicity in the membrane center increases to the level of dipropylamine (ε = 3) [8, 53]. Incorporation of cholesterol (30 mol%) increases polarity (water penetration) of the polar headgroup region and the near-surface region of hydrocarbon chains. It also sharply increases hydrophobicity from the 9^{th}–10^{th} carbon inward in PC membranes to the level of hexane (ε = 2) [53]. This suggests that the lipid portion of the biological membrane can be a significant hydrophobic barrier only when it includes unsaturated alkyl chains and/or cholesterol. Polar carotenoids (10 mol%) also increase hydrophobicity of the hydrocarbon core of the lipid bilayer to the level of pure hexane at the center of unsaturated PC membranes [27]. Transmembrane peptides affect membrane hydrophobicity differently depending on their structure. Incorporation of gramicidin decreases hydrophobicity in both the polar headgroup region and the hydrocarbon region of the PC bilayer. At the gramicidin/PC interface the hydrophobicity profile becomes very flat and is identical for saturated and unsaturated membranes [54]. In contrast, a transmembrane α-helical peptide, Ac-$K_2L_{24}K_2$-amide (L_{24}) (9 mol%), increases hydrophobicity of the center of PC membranes to the level of pure hexane [8]. The results presented above correlate well with permeability data for water [55] and amino acids [56], and ion penetration into the bilayer [53, 54, 57].

Permeability coefficients of small nonelectrolytes correlate well with oil/water partition coefficients (Overton's rule [58], see also discussion in [35]). In discussions of the mechanism by which small nonelectrolytes permeate across membranes, particular attention has been directed toward the location of the permeation barriers in the lipid bilayer, and the physical nature of the rate-limiting barrier [14, 59, 60]. We have previously

shown that the major resistance to permeation of molecular oxygen across the membrane is located at the polar headgroup region and the near-surface region in the hydrocarbon phase [12, 14]. This suggests that the locations of the permeation barriers are different for polar and non-polar molecules: for polar molecules, the major permeation resistance is the hydrophobic barrier in the membrane center, while for non-polar molecules, the resistance is the rigidity barrier near the membrane surface.

The role of cholesterol in the plasma membrane is a long-standing puzzle. It has been proposed that the mixture of saturated and unsaturated alkyl chains with cholesterol provides a fluidity buffer [5, 61]. Present results suggest that cholesterol has several important functions in the plasma membrane, specific to this membrane. Since cellular plasma membranes face the cell's external environment, the membrane barriers must be very high to block non-specific permeation of small molecules. Incorporation of cholesterol into the membrane serves this purpose well because cholesterol simultaneously raises hydrophobic barriers for polar molecules and increases rigidity barriers for non-polar molecules.

SIZE, STRUCTURE AND DYNAMICS OF MEMBRANE DOMAINS

Cells build various structures and arrays of proteins and lipids within and around the plasma membrane, which are essential for the proper function of these molecules and the plasma membrane [62–64]. The size of a membrane domain may range from the scale of several molecules (molecular clusters) up to several microns [65–67]. The domains may be formed and disintegrated continually with lifetimes ranging from about 10 ns for cholesterol clusters in unsaturated PC membranes [13, 68] or seconds for clathrin-coated pits [69], up to hours for cell adhesion structures [70]. Additionally, in longer-lasting domains, the constituent molecules may be changing all the time, just like in micelles, whose constituent molecules move in and out continuously [63].

The specific goal of our research was to gain insight into the organization and dynamics of cholesterol-rich [13] and protein-rich domains [8, 37]. We were also concerned with molecular exchange between domains in the membrane, the exchange of lipids between a domain and the bulk phase in particular [38].

A series of studies on the effects of cholesterol on PC membranes was carried out [5, 9, 13, 14, 53, 68]. Cholesterol and unsaturated PC molecules are not easily miscible due to steric nonconformability between the fused ring structure of cholesterol and the rigid 30^{o} bend at the *cis* double bond in unsaturated alkyl chains. This leads to the formation of cholesterol-rich domains (cholesterol clusters) which are forming and dispersing continuously. These cholesterol-rich domains are small (several molecules) and/or have short lifetimes (1 to 100 ns).

We found that the transmembrane α-helical peptide L_{24} is highly miscible in a POPC membrane even at high concentration [8]. This peptide was designed so that the central polyleucine segment formed a stable α-helix which partitioned into the hydrophobic core of lipid bilayers with the charged terminal lysine residues anchoring the ends of the peptide to the bilayer surfaces [71]. Since 16–18 molecules of PC are required to surround a transmembrane α-helical peptide [72], L_{24} must form L_{24}-rich regions at L_{24}/POPC ratio of 1/10 instantaneously, but such regions must be formed and dispersed rapidly at a time scale shorter than 0.1 μs (T_1 of spin labels in the presence of oxygen). This miscibility is likely due to the 4 lysine groups. Their positive charges may also be responsible for preventing the aggregation of L_{24}. The structural simplicity of L_{24} and its well-characterized behaviour in lipid bilayers make it an excellent model compound for fundamental studies of the effects of the transmembrane portions of

natural membrane proteins on the organization and dynamics of the host lipid bilayer.

We have also studied the molecular organization of protein-rich domains in reconstituted bacteriorhodopsin (BR) membranes [37]. Our results indicate the presence of a specific lipid domain which only appears at high BR/PC ratio (1/40) at which BR exists mainly as trimers and oligomers of trimers. These domains had a slow oxygen transport rate and thus were termed SLOT domains. They were thought to be protein-rich domains in which lipids are sandwiched either between two protein molecules or between a protein and boundary lipids. The alkyl chain motion of these lipids is suppressed to the level of a gel-phase membrane. The lifetime of such trapped-lipid domain must be greater than the rotational correlation time of BR trimers or oligomers of trimers, which was over 100 μs [37]. The rate of the exchange of lipids between a SLOT domain and bulk region was not measured with the T_1 technique, and thus has to be greater than the spin-lattice relaxation rate (about 10^6 s^{-1}).

Further, we investigated membrane domains in the influenza virus (IFV) envelope membrane, the simplest paradigm for the study of biomembranes [38]. It is highly similar in lipid composition to the plasma membrane of the host animal cell [73], contains a high concentration of cholesterol [74] and two major viral transmembrane glycoproteins, hemagglutinin and neuraminidase [75]. They both have a single transmembrane α-helix and exist as trimers and tetramers, respectively [76, 77]. The two-component saturation recovery signal of a fatty acid spin label incorporated in the IFV membrane was observed indicating the presence of a SLOT domain. In this investigation we encountered a case in which the exchange of lipids between domains had to be explicitly included in the analysis, thus enabling us to obtain the lipid exchange rates between a SLOT domain and the bulk domain. Since the oxygen transport rate in a SLOT domain is much lower than that expected for cholesterol-rich domains [12, 14] or domains containing single (non-associated) transmembrane α-helices [8], the presence of clustered transmembrane proteins in the domain is essential to explain such low rate of oxygen transport in the domain [37]. Furthermore, the oxygen transport rate in a SLOT domain is even lower than in the purple membrane, where BR molecules are aggregated [37]. All this suggests that the SLOT domain in IFV membranes may be a raft domain rich in cholesterol and stabilized by the presence of clustered proteins. Judging from the ratio of the inbound *versus* the outbound rates of the lipid in the SLOT domain, the SLOT domain as a whole may occupy about one-third of the membrane, which is a substantial area of the IFV membrane. We believe this is the first case where a SLOT domain has been found in biological membranes.

CONCLUDING REMARKS

Seventy-five years ago the basic structure of the biological membrane as a lipid bilayer was established [1], but the investigation of the physical properties of lipid bilayers still gives significant information for the understanding of membrane organization and dynamics, and thus its biological function. What is new in our methods which allowed us to obtain the results described above? In our work molecular oxygen was used as a probe to study the three-dimensional molecular organization and dynamics in membranes. Molecular oxygen has a unique characteristic as a membrane probe; its small size and appropriate hydrophobicity allow it to enter the small vacant pockets that are transiently formed in the membrane. Therefore, the bimolecular collision rate between oxygen and nitroxide spin labels placed in the membrane is sensitive to the dynamics of *gauche-trans* isomerization of lipid alkyl chains and to the structural nonconformability of neighboring lipids [10, 12, 14]. Molecular oxygen makes a

particularly useful probe to study the molecular organization of protein-rich membranes. The DOT method (the method of discrimination by oxygen transport) developed by us for the analysis of saturation recovery EPR spin labeling data using molecular oxygen as a probe may be successfully used in the studies of SLOT domains, the protein-stabilized cholesterol-rich raft domains in particular, and the exchange of lipids between domains [8, 37, 38].

Our investigations indicate that the presently accepted fluid-mosaic model proposed by Singer & Nicolson [3] is not applicable or may even be misleading in understanding the diffusion-solubility characteristics of solutes in a membrane, in which most lipid molecules are in contact with one or two protein molecules and protein association is a common occurrence. This model has to be greatly modified to better describe the structure and function of biological membranes.

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