Petrov E, Rohde PR, et al. Flying-patch patch-clamp study of G22E-MscL mutant under high hydrostatic pressure. Biophys J. 2011;100(7):1635–41.
Schumann U, Edwards MD, et al. YbdG in *Escherichia coli* is a threshold-setting mechanosensitive channel with MscM activity. Proc Natl Acad Sci. 2010;107(28):12664–9.

Membrane Anchor

▶ Glycosylphosphatidylinositol

Membrane Asymmetry

▶ Lipid Bilayer Asymmetry

Membrane Dynamics

▶ NMR of Lipids

Membrane Fluidity

Boris Dzikovski and Jack Freed
National Biomedical Center for Advanced ESR Technology, Department of Chemistry and Chemical Biology, Baker Laboratory, Cornell University, Ithaca, NY, USA

Synonyms

Mobility of membrane components

Definition

The variety of anisotropic motions which contribute to the mobility of components in the biological membrane

Introduction

In 1972, Singer and Nicolson (Singer and Nicolson 1972) suggested the so-called fluid mosaic model of the biological membrane (Fig. 1). This useful hypothesis explained many phenomena occurring in model and biological membranes. According to this model, membrane proteins and other membrane-embedded compounds are suspended in a two-dimensional fluid formed by phospholipids. This fluid state of membrane lipids is critical for membrane function. It allows, for example, free diffusion and equal distribution of new cell-synthesized lipids and proteins; lateral diffusion of proteins and other molecules in signaling events and other membrane reactions; membrane fusion, that is, fusion of vesicles with organelles; separation of membranes during cell division; etc.

Membrane fluidity, which describes the ease of movement for molecules in the membrane environment, is a general concept that lacks a precise definition. It is much broader than the strict physical definition of fluidity as the reciprocal of viscosity in the case of isotropic liquids. In general, "membrane fluidity" implies various anisotropic motions, which contribute to the mobility of components of a membrane.

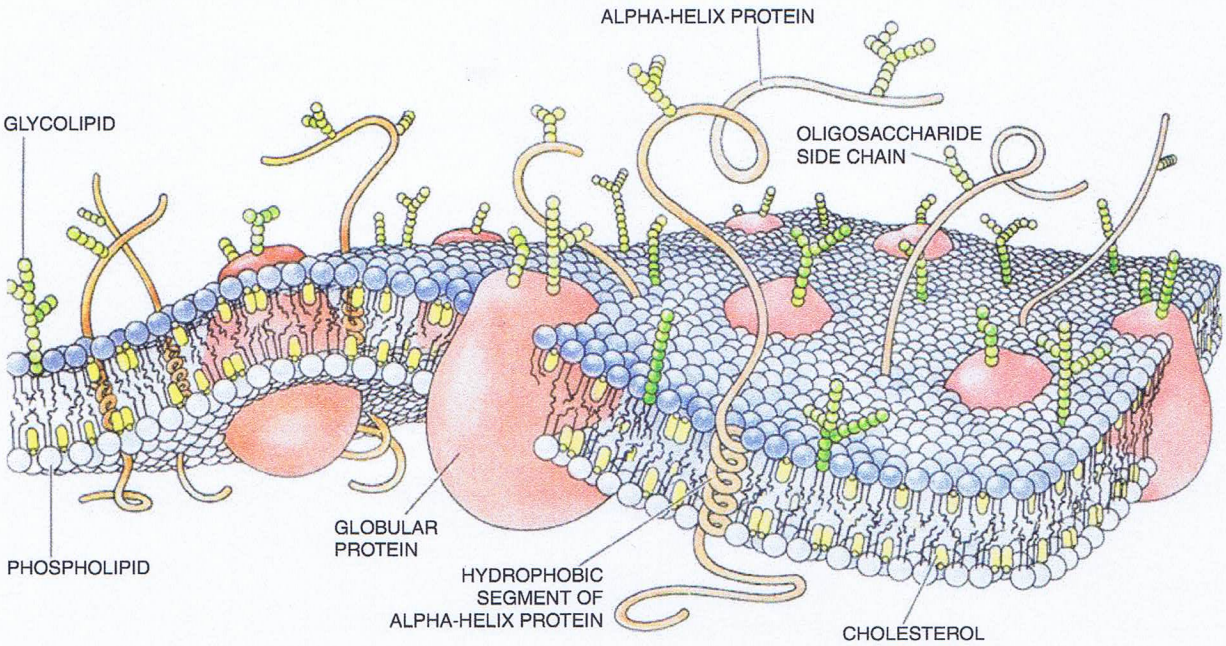
The lipid membrane, as a whole, shows a unique combination of fluidity and rigidity. In terms of the solubility and diffusion of small nonpolar molecules, the membrane behaves very much like an oil drop. In contrast, the translational diffusion constants of lipids and proteins in membranes are characteristic of media with the viscosity over two orders of magnitude greater than that of oil such as hexadecane. Also, in most cases, the membrane represents an impermeable barrier for ions and other hydrophilic compounds.

Figure 2 shows characteristic frequencies (reciprocal of characteristic times) of different kinds of molecular motions in the membrane in comparison to frequency ranges in which various spectroscopic techniques are sensitive to molecular motion (Gennis 1989).

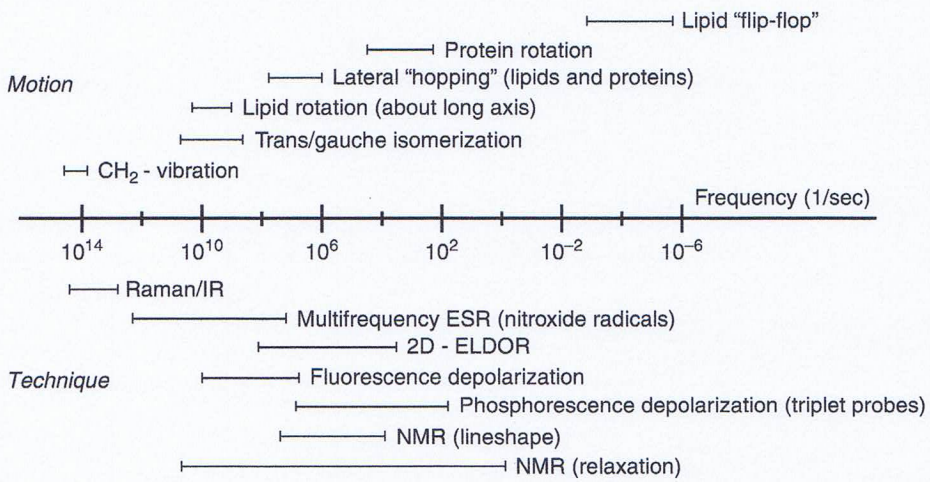
Physical Parameters Associated with Membrane Fluidity

Diffusion Constants

Diffusion is the random movement of a particle due to exchange of thermal energy with its environment. Membrane lipids and proteins participate in highly anisotropic translational and rotational diffusion motion. Translational diffusion in the plane of the membrane is described by the mean square lateral displacement after a time Δt: $\langle r^2 \rangle = 4D_T \Delta t$. Lipid



Membrane Fluidity, Fig. 1 Singer-Nicolson model of fluid membrane (From Bretscher (1985))



Membrane Fluidity, Fig. 2 The characteristic frequencies of molecular motions of membrane proteins and lipids compared with the frequency ranges in which various spectroscopic techniques are sensitive to molecular motion (Modified from (Gennis 1989))

lateral diffusion coefficients in fluid phase bilayers are typically in the range $D_T \sim 10^{-8}$ to 10^{-7} cm^2/s (Marsh 1990).

Methods for measuring translational diffusion coefficients for lipids in membranes can be classified into two distinct categories. In the first one, which corresponds to short-range diffusion measurements, D is obtained from determination of frequencies of bimolecular collisions within the membrane, through fluorescence quenching, ESR, etc. In the second one, which corresponds to long-range diffusion measurements, D is usually determined from the time required to fill a defined region of the membrane. The most popular fluorescent technique for studying

long-term diffusion is *fluorescence recovery after photobleaching (FRAP)* (Gennis 1989) which can measure translational diffusion coefficients in the range from about 10^{-7} to 10^{-12} cm^2/s. Among magnetic resonance techniques for measuring long-range diffusion are dynamic imaging of diffusion by ESR (DID-ESR) (Freed 1994), which has been used to measure $10^{-5} > D_T > 10^{-10}$ cm^2/s, and *pulse field gradient (PFG)* NMR spectroscopy (Price 1997).

Rotational diffusion is characterized by the mean square angular deviation during the time interval Δt: $\langle\theta^2\rangle = 6D_R\Delta t$. Highly anisotropic motion, typical for lipid molecules in the membrane, is usually described by two rotational diffusion coefficients $D_{R\parallel}$ and $D_{R\perp}$, which correspond to diffusion about the long diffusion axis and perpendicular to it, respectively. The diffusion coefficients are related to corresponding rotational correlation times measured by NMR, ESR, fluorescent depolarization, etc., as: $\tau_{R\parallel} = 1/6D_{R\parallel}$; $\tau_{R\perp} = 1/6D_{R\perp}$. For fluid phase bilayers, the typical rotational diffusion coefficients are of the order of $D_{R\parallel} \sim (1\text{–}4)\cdot 10^8$ s^{-1} and $D_{R\perp} \sim (1\text{–}4)\cdot 10^7$ s^{-1} (Marsh 1990) This example can help in visualizing lipid motion in the membrane: for an area per lipid of 60 Å^2 with $D_{R\parallel}$ and D_T of $4\cdot 10^8$ s^{-1} and $1\cdot 10^{-8}$ cm^2/s, respectively, one can see that a lipid molecule travels a distance of approximately its width, while rotating once around its long axis. A value of D_T of 10^{-8} cm^2/s, typically measured for lipids in biomembranes, corresponds to a net distance traversed of about 2 μm in 1 s.

Flip-Flop Diffusion

Lipid molecules, in principle, can exchange between the two monolayers of the bilayer. For polar lipids, it is an extremely slow process with characteristic times of hours or even days (Homan and Pownall 1988). For membrane proteins, no appreciable flip-flop mobility has yet been observed, in good accord with the fact that inner and outer leaflets of natural membranes are usually asymmetric with respect to their protein and lipid composition. On the other hand, cholesterol has a relatively high rate of spontaneous flipping between two membrane leaflets ($t_{½} \sim 1$ s) (Lange et al. 1981).

Order Parameters

The membrane lipid layer is an ordered liquid crystal phase with the preferred orientation of the lipid molecules perpendicular to the membrane plane. By definition, if θ is the angle of the long molecular axis with respect to the bilayer normal, the order parameter S, which is a measure of the orientation distribution, is given as the average of $P_2(\cos\theta)$, the second Legendre polynomial: $S = \langle P_2(\cos\theta)\rangle = \frac{1}{2}\langle 3\cos^2\theta - 1\rangle$. One can see that S varies between $-1/2$ and 1. These limiting cases have the following meaning: when S = 1, all molecules are exactly perpendicular to the membrane plane. When S = $-1/2$, $\theta = 90°$, and all molecules have their long axis parallel to the membrane surface. The case of S = 0 usually corresponds to a random distribution of molecular axes relative to the membrane normal. In energy terms, the rotation of the molecular long axis in the liquid crystal is restricted within an orienting potential that is simply approximated as $U(\theta) = \lambda \cdot \cos^2\theta$, where λ is the strength of the potential. The ordering of the lipid chain relative to the bilayer normal can then be expressed as:

$$S_{zz} = \tfrac{1}{2}\langle 3\cos^2\theta - 1\rangle = \frac{1}{2}\frac{\int_0^\pi (3\cos^2\theta - 1)\exp\left[-\frac{U(\theta)}{kT}\right]\sin\theta d\theta}{\int_0^\pi \exp\left[-\frac{U(\theta)}{kT}\right]\sin\theta d\theta}.$$

A variety of physical techniques allow for determination of order parameters, separately (NMR), or simultaneously with mobility parameters (fluorescence depolarization, fluorescence correlation spectroscopy, ESR).

Permeability

The passive permeability of lipid membranes is another fluidity related parameter. For many nonpolar molecules, the predominant permeation pathway is solubility-diffusion, a combination of partitioning and diffusion across the bilayer, both of which depend on lipid fluidity.

Phase State and Membrane Fluidity

A remarkable property of lipid bilayers is their structural phase transitions (thermotropic polymorphism). For example, fully hydrated pure diacylphosphatidylcholines exhibit one fluid phase, L_α, and three crystalline phases, P_β, L'_β, and L_c (Gennis 1989) (Table 1). Because of the high degree of disorder caused by defects, the P_β and L'_β phases are usually

Membrane Fluidity, Table 1 Translational diffusion coefficients of lipids and order parameters in some membrane phases

Phase	D_T	S[a]
Liquid-crystalline (L_α)	10^{-8} cm^2/s	0–0.2
Gel (L_β)	10^{-11} cm^2/s	0.2–0.9
Gel (P_β)	Similar to L_β, but the bilayer is rippled	
Gel (L'_β)	The same as L_β, but the chains are tilted 32°	
Liquid-ordered (L_o)	10^{-8} cm^2/s	0.2–0.9

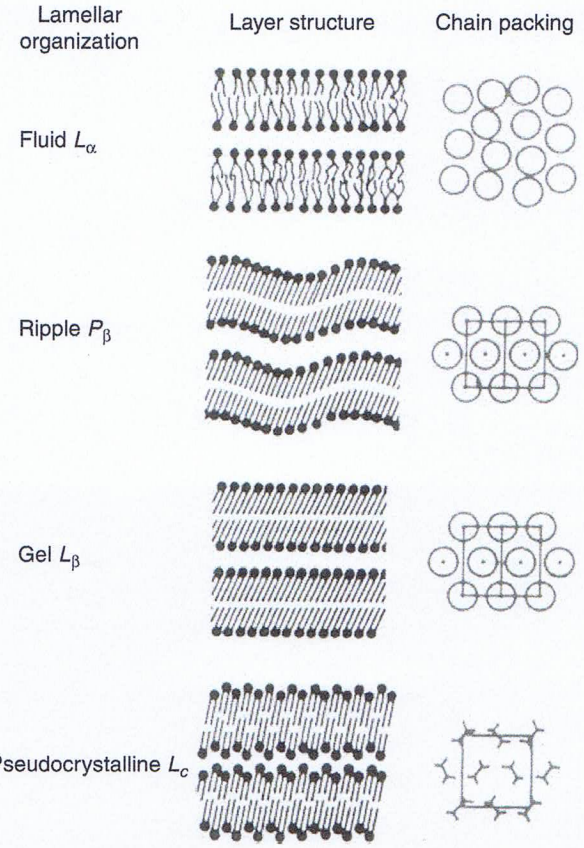
[a]Measured by NMR

called gel phases. The P_β phase is sometimes called a "ripple phase" because the surface of the bilayer is rippled and presents a wave-like appearance in electron micrographs (Fig. 3). Depending on the nature of the lipid and presence of additional components (cholesterol, etc.), the P_β phase may be present or absent in the phase diagram, and a tilted gel L'_β could be replaced by the L_β phase, having similar physical properties, but no tilt of the hydrocarbon chains.

In the gel phase, lipid chains are usually well aligned with little rotation around the C-C bonds which are predominantly in the *trans* position. The lipid chains are tightly packed, the chain ordering is high, the bilayer thickness is maximal, and the surface area per lipid headgroup is relatively small. The physiological importance of gel-like phases is limited.

At the "main transition" temperature, T_m the gel phase undergoes a transition to the L_α (liquid crystal) phase. At the transition point, the surface area increases, and the bilayer thickness and chain order decrease. In the fluid phase, hydrocarbon chains tend to contain a larger number of gauche isomers (Yeagle 2005). At this transition, the DSC (differential scan calorimetry) shows a sharp peak in the heat capacity occurring over a narrow temperature range. The transition between L'_β and P_β phases also can be detected by DSC and is called the pretransition. The transition between L_c and L'_β phases is in most cases hard to observe due to typical supercooling of the L'_β phase. Depending on the membrane composition, hydration, and temperature, a number of 2D and 3D nonlamellar lipid phases are possible, including the well-studied hexagonal (H_I and H_{II}) and cubic phases.

Natural biomembranes contain a complex mixture of various phospholipids with cholesterol and sphingomyelins. In general, they exist in the fluid phase. Maintaining membrane fluidity seems to be



Membrane Fluidity, Fig. 3 Organization of the lamellar bilayer phases of DPPC in the fluid (L_α), ripple (P_β), gel (L'_β), and pseudocrystalline (L_c) states. A top view of the packing of the hydrocarbon chains is shown in the last column (From (Jain 1988))

extremely important for the survival of the cell and the whole organism. It is well known for model membranes that a decrease in the chain length or the introduction of unsaturation into the hydrocarbon chain causes a decrease in the main transition temperature. Consistent with this observation, microorganisms, plants, and animals (poikilotherms or hibernating mammals) are acclimated to low temperatures by altering their membrane lipid composition, increasing the degree of lipid unsaturation or decreasing the average chain length (McElhaney 1984).

Cholesterol and Membrane Fluidity. Cholesterol, which is an important constituent of cell membranes, plays a crucial role in maintaining membrane fluidity. It effectively inhibits the transition to the gel phase. Even though some plasma membranes, such as nerve myelin membranes, contain a high concentration of lipids that form gel phase bilayers, the

presence of cholesterol keeps these membranes in a fluid phase. However, interaction with the rigid cholesterol ring affects hydrocarbon chains of lipids in the liquid crystal phase (L_α) and leads to formation of a new phase, the liquid-ordered (L_o) phase (Quinn and Wolf 2009). The phase is well characterized by a variety of physical methods and does not exist in pure lipids or their mixtures. In the liquid-ordered phase, the long axis rotation and lateral diffusion rates are similar to the L_α phase, but the acyl chains are predominantly in an all-trans conformation, and hence, the order parameters are similar to the L_β phase (Table 1). Recently, the cholesterol-rich L_o phase has been strongly associated with microdomains in live cells – the so-called lipid rafts (Lingwood and Simons 2010).

Fluidity Versus Mosaicity: Beyond the Singer-Nicolson Model

The Singer-Nicolson model of the membrane played a very important role in understanding membrane structure and function. However, many properties of biomembranes are not consistent with the model. In recent years, a growing consensus points at more complex membrane structure, which can be characterized as "dynamically structured fluid mosaic." Compared to the original fluid mosaic model, the emphasis has shifted from fluidity to mosaicity. Experimental observations have led to the "membrane microdomain" concept, describing compartmentalization/organization of membrane components into nonrandom, well-defined patterns.

One observation, which is inconsistent with the simple fluid mosaic model, is the reduced diffusion coefficients of membrane molecules in the plasma membrane compared to model membranes and biomembranes deprived of their cytoskeleton (blebs). Another observation is the oligomerization-induced slowing of diffusion (Kusumi et al. 2005). It manifests itself in much greater effect of diffusant size on the translational diffusion rate than predicted by the theory of Saffman-Delbrück (SD) based on the Singer-Nicolson model (Saffman and Delbrück 1975). If a transmembrane protein is approximated as a rigid cylinder of radius r and height h, floating in a two-dimensional liquid of viscosity η with matching thickness (h) surrounded by an aqueous medium of viscosity η_l, the SD theory gives the following expression for its translational diffusion coefficient D_T:

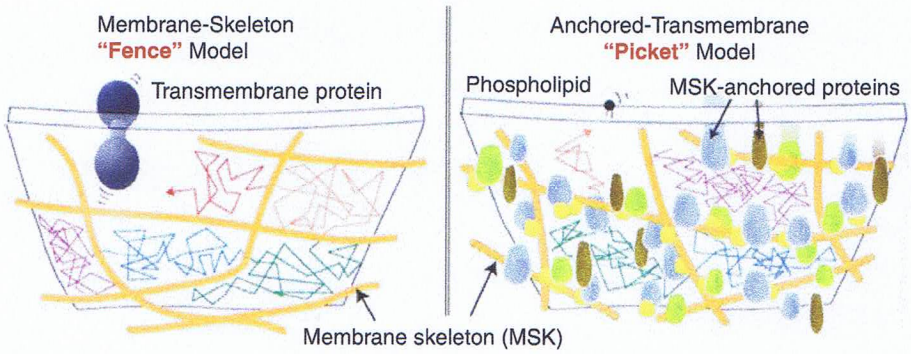
$$D_T = \frac{k_B T}{4\pi\eta h}\left(\ln\frac{\eta h}{\eta_1 r} - \gamma\right),$$

where γ is the Euler constant ($\approx$0.577216). This formula predicts a very weak dependence of translational diffusion on the size of diffusant. For example, for a tenfold increase in the protein radius, from 5 to 50 Å, the theory predicts for a 50 Å-thick bilayer a decrease in the diffusion rate by a factor of 1.57. Although this insensitivity looks somehow counterintuitive, the Saffman-Delbrück formula gives good results for proteins incorporated into model membranes.

On the contrary, for the plasma membrane, the effect of oligomerization on the diffusion rate is much stronger than predicted by the formula. For example, for linked couples of GFP (green fluorescent protein) – E-cadherin – oligomerization with an aggregation number between 2 and 10 slows down the diffusion up to 40 times (Iino et al. 2001). Also, single-particle tracking experiments carried out by Kusumi and coauthors showed that in natural biomembranes, the diffusion does not follow usual Brownian patterns but consists of a series of random Brownian walks within confined areas (compartments) followed by longer-distance hops between compartment (see trajectory in Fig. 4). The compartment size varies depending on the cell nature but is relatively insensitive to the diffusant, showing the same size for transmembrane proteins and phospholipids. For example, in the case of normal rat kidney epithelial (NRK) cells, the average compartment size is 230 nm with an average residency time within the compartment of $\sim$ 11 ns (Kusumi et al. 2005).

The hop-diffusion pattern cannot be found in liposomes or membrane blebs. In these membranes, the membrane molecules show simple Brownian diffusion with a single diffusion coefficient (Fujiwara et al. 2002).

Because the assumption of simple Brownian diffusion breaks down, the diffusion in biomembranes cannot be described by a single diffusion coefficient. For instance, FRAP experiments in the plasma membrane showed that the observed translational diffusion



Membrane Fluidity, Fig. 4 Membrane skeleton fence and anchored transmembrane picket model (From (Ritchie et al. 2003))

rates depend on the size of the initial photobleached spot, which is also inconsistent with a simple Singer-Nicolson model.

Accumulating evidence clearly points at involvement of the cell cytoskeleton in the compartmentalization of the membrane, in particular the fine cytoskeleton filaments formed by actin in most eukaryotic cells or spectrin in mammalian red blood cells. However, single-particle tracking experiments show the same patterns of hop-diffusion for lipid molecules located in the extracellular leaflet of the plasma membrane. How can the membrane skeleton, which is located only on the cytoplasmic surface of the membrane, suppress the motion of lipids on the extracellular side?

To reconcile this apparent contradiction, the *membrane skeleton fence and anchored transmembrane picket model* was proposed (Ritchie et al. 2003). According to this model, transmembrane proteins anchored to and lined up along the membrane skeleton (fence) effectively act as a row of posts for the fence against the free diffusion of lipids (Fig. 4). This model is consistent with the observation that the hop rate of transmembrane proteins increases after the partial removal of the cytoplasmic domain of transmembrane proteins but is not affected by the removal of the major fraction of the extracellular domains of transmembrane proteins or extracellular matrix. Within the compartment borders, membrane molecules undergo simple Brownian diffusion. In a sense, the Singer-Nicolson model is adequate for dimensions of about 10 × 10 nm, the special scale of the original cartoon depicted by the authors in 1972. However, beyond such distances, simple extensions of the fluid mosaic model fail and a substantial paradigm shift are required from a two-dimensional continuum fluid to the compartmentalized fluid.

Summary

Membrane fluidity is a general concept. It implies various anisotropic motions, which contribute to the mobility of components of a membrane. The so-called fluid mosaic model of the biological membrane (Singer and Nicolson 1972) assumes that membrane proteins and other membrane-embedded compounds are suspended in a two-dimensional fluid formed by phospholipids. This useful model explained many phenomena occurring in model and biological membranes. However, in recent years, a growing consensus points at more complex membrane structure, which can be characterized as "dynamically structured fluid mosaic" with the cell cytoskeleton playing crucial role in the compartmentalization of the membrane.

Cross-References

- EPR Spectroscopy: General Principles
- Functional Roles of Lipids in Membranes
- Fluorescence and FRET in Membranes
- Interspin Distance Determination by EPR
- Lipid Flip-Flop
- Lipid Lateral Diffusion
- NMR
- Phase Transitions and Phase Behavior of Lipids
- Pulsed Field Gradient NMR
- Single Particle Tracking

References

Bretscher MS. The molecules of the cell membrane. Sci Am. 1985;253:100–8.

Freed JH. Field gradient ESR and molecular diffusion in model membranes. Annu Rev Biophys Biomol Struct. 1994;23:1–25.
Fujiwara T, Ritchie K, Murakoshi H, Jacobson K, Kusumi A. Phospholipids undergo hop diffusion in compartmentalized cell membrane. J Cell Biol. 2002;157:1071–81.
Gennis RB. Biomembranes. Molecular structure and function. New York/Berlin/Heidelberg/London/Paris/Tokyo: Springer; 1989.
Homan R, Pownall HJ. Transbilayer diffusion of phospholipids: dependence on headgroup structure and acyl chain length. Biochim Biophys Acta. 1988;938:155–66.
Iino R, Koyama I, Kusumi A. Single molecule imaging of green fluorescent proteins in living cells: E-cadherin forms oligomers on the free cell surface. Biophys J. 2001;80:2667–77.
Jain MK. Introduction to biological membranes. New York: Wiley; 1988.
Kusumi A, Nakada C, Ritchie K, Murase K, Suzuki K, Murakoshi H, Kasai RS, Kondo J, Fujiwara T. Paradigm shift of the plasma membrane concept from the two-dimensional continuum fluid to the partitioned fluid: high-speed single-molecule tracking of membrane molecules. Annu Rev Biophys Biomol Struct. 2005;34:351–78.
Lange Y, Dolde J, Steck TL. The rate of transmembrane movement of cholesterol in the human erythrocyte. J Biol Chem. 1981;256:5321–3.
Lingwood D, Simons K. Lipid rafts as a membrane-organizing principle. Science. 2010;327:46–50.
Marsh D. CRC handbook of lipid bilayers. Boca Raton/Ann Arbor/Boston: CRC Press; 1990.
McElhaney RN. The relationship between membrane fluidity and phase state and the ability of bacteria and mycoplasmas to grow and survive at different temperatures. Biomembranes. 1984;12:249–76.
Price WS. Pulsed field gradient nuclear magnetic resonance as a tool for studying translational diffusion: part 1. Basic theory. Concepts Magn Reson. 1997;9:299–336.
Quinn PJ, Wolf C. The liquid-ordered phase in membranes. Biochim Biophys Acta. 2009;1788:33–46.
Ritchie K, Iino R, Fujiwara T, Murase K, Kusumi A. The fence and picket structure of the plasma membrane of live cells as revealed by single molecule techniques. Mol Membr Biol. 2003;20:13–8.
Saffman PG, Delbrück M. Brownian motion in biological membranes. PNAS. 1975;72:3111–3.
Singer SJ, Nicolson GL. The fluid mosaic model of the structure of cell membranes. Science. 1972;175:720–31.
Yeagle P. The structure of biological membranes. Boca Raton/Ann Arbor/London: CRC Press; 2005.

Membrane Ionic and Capacitative Currents

▶ Bioelectricity, Ionic Basis of Membrane Potentials and Propagation of Voltage Signals

Membrane Lipid Electrostatics

Gregor Cevc
The Advanced Treatments Institute, Gauting, Germany

Introduction

Ionizable polar residue(s) on lipid membrane headgroups can gain net charges in polar solvents, such as water, dependent on the aggregated lipids as well as the dissolved proton concentration (see the Table 1). Ions also interact with lipid charges and thus affect membrane properties. The right panel of Fig. 1 illustrates, for example, sodium chloride effects on some phase transitions in fully hydrated zwitterionic phosphatidylcholine bilayers. The left panel reveals charge-driven accumulation of sodium counterions near the corresponding anionic phosphatidylglycerol bilayers. Electro-osmotic phenomena involving living organism membranes are more complex but fundamentally similar.

Membrane-Solvent Interactions

Even the simplest boundary devoid of charges perturbs proximal solvent. Hydrogen bonds or their restrictions orient solvent molecules and enforce local density and polarization oscillations. The quasi-exponential envelope of the positive oscillations decays on a length scale of $\lambda_W \sim 0.1$ nm. The short range of solvation allows spatial distribution and thermal motions of lipid polar residues to average-out the oscillatory solvent structure effects, which are consequently unobservable near lipid bilayers (Cevc 1990), unlike near hard, smooth surfaces. The small value of λ_W also helps efficient screening of lipid partial charges by the atomic partial charges on each solvent molecule. Consequently, the polar moieties orientation and motion, partial charges density distribution, and the restricted, quasi-exponentially changing, solvent-accessible space within each lipid-solvent interphase together govern membrane lipid electrostatics and solvation.