Chapter 1
What Is So Unique About Biomembrane Organization and Dynamics?

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Abstract Biological membranes are complex *quasi* two-dimensional, supramolecular assemblies of a diverse variety of lipids, proteins and carbohydrates, that compartmentalize living matter into cells and subcellular structures. Membranes are held together by the hydrophobic effect, which is an entropy-driven process originating from strong attractive forces between water molecules. Membrane organization and dynamics are characterized by the absence of intermolecular connectivity among its constituent units, thermodynamically controlled (spontaneous) self assembly, and inherent dynamics characterized by a gradient. Membrane phenomena display a wide range of spatiotemporal scales, thereby making it challenging for experiments and simulations alike. We envision that unraveling the spatiotemporal complexity of biological membranes would enable us to build a more robust membrane model, which would help in addressing unresolved issues in human health and disease.

1.1 Cellular Membranes as Identity Markers

A long time back, biochemists used to think that a living cell is a bag full of enzymes. In reality, eukaryotic cells are characterized by a number of compartments separated from each other and the cytoplasm by thin membranes (see Fig. 1.1). The composition, organization and physical dimension of the intracellular organelle membranes exhibit a lot of variation. The outermost membrane in eukaryotic cells is termed the plasma membrane, which separates the interior of the cell from the outer milieu and provides the cell its unique identity. Cellular organization is therefore characterized by morphological compartmentalization

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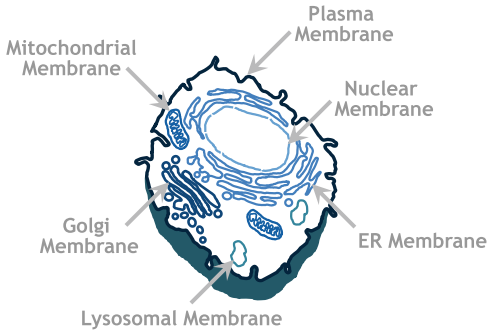

Fig. 1.1 A schematic representation of a eukaryotic cell showing the plasma membrane and membrane bound organelles

offered by the membrane. Membranes compartmentalize living matter into cells and subcellular structures. Cells require some mechanism to prevent dissipation (diffusing away) of their genetic information (contained mainly in the nucleus) and therefore "*compartmentalization has long been recognized as a physical prerequisite for Darwinian evolution*" [1]. Importantly, the membrane is the first organelle in a cell to sense any stress or stimuli [2].

In physical terms, membranes can be described as a complex anisotropic fluid that are deformable and can therefore be treated as soft matter [3, 4]. In molecular terms, this means membranes are optimally fluid to be able to carry out their function while maintaining their characteristic selective barrier properties. Membranes present themselves to macromolecules as highly structured interfaces on which important biochemical processes are carried out and catalyzed. For this reason, the structure and molecular organization of membranes are crucial for membrane function.

Biological membranes are complex *quasi* two-dimensional, supramolecular assemblies of a diverse variety of lipids, proteins and carbohydrates (see Fig. 1.2a). Membranes of eukaryotic cells contain thousands of diverse lipid types [6, 7]. Membranes provide an identity to the cell and its organelles, and represent an ideal milieu for the proper function of membrane proteins. Cells are densely packed with membranes. In fact, ~35% of the dry weight of a cell is that of its membranes. The human body is composed of $\sim 10^{14}$ cells which correspond to a total membrane surface area of ~3 km^2. Contrary to textbook descriptions [8], cellular membranes are often crowded [9, 10] with a high protein density (typically ~25,000 proteins/μm^2; [11]). Even a number of years back it was postulated that there could be only a few lipid molecules separating two protein molecules in a biological membrane [12]. Taken together, the model of biological membranes is evolving into one where "*the membrane resembles a cobble-stone pavement, with the proteins organized in patches that are surrounded by lipidic rims, rather than icebergs floating in a sea of lipids*" [10].

The actin cytoskeletal network underlying the membrane was initially not considered to be an active part of the membrane. However, this has changed in recent years. A number of observations using sophisticated microscopic techniques have established the notion of actin cytoskeleton dependent dynamics of molecules

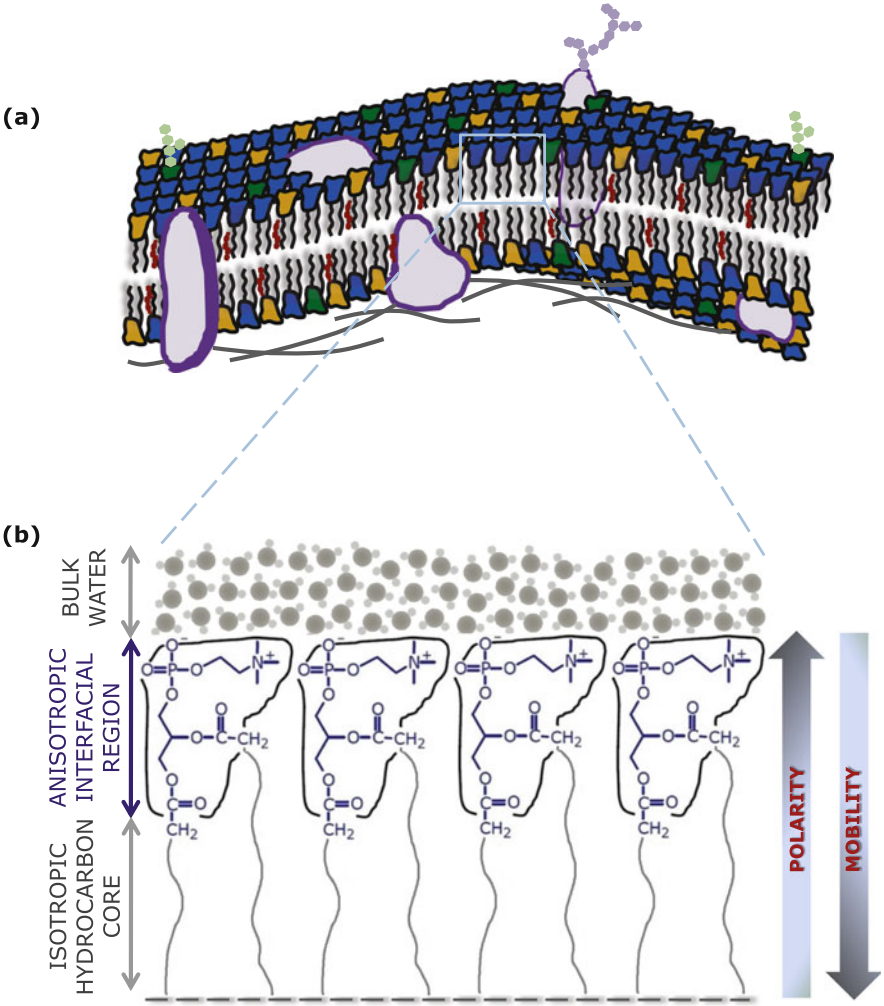

Fig. 1.2 (**a**) A schematic representation of a membrane bilayer showing the major components of biological membranes: lipids, proteins and carbohydrates. The actin cytoskeleton is also shown since emerging evidence suggests that it is coupled to the membrane (see Sect. 1.1). Eukaryotic membranes consist of phospholipids, sterols and sphingolipids. The predominant phospholipids are zwitterionic (shown in *blue*) phosphatidylcholine and phosphatidylethanolamine; and negatively charged (shown in *mustard*) phosphatidylglycerol and phosphatidylserine. Negatively charged lipids are key players in lipid-protein interactions and are known to modulate membrane insertion, translocation and subsequent function of membrane proteins. Cholesterol, the predominant sterol in eukaryotic membranes, is shown in *maroon* and sphingolipids in *green*. Cholesterol is a functionally relevant lipid in terms of its role in the organization, dynamics and function of biological membranes. Integral and peripheral membrane proteins are shown in *purple*. The underlying actin cytoskeleton, depicted in *gray*, imparts structural integrity and induces dynamic compartmentalization with its meshwork underneath the membrane, thereby leading to membrane domains. (**b**) An enlarged representation of one-half of the bilayer (highlighted by a *light blue box*) shows the intrinsically anisotropic nature of biological membranes. The *dotted line* at the bottom indicates the center of the bilayer. The anisotropy along the membrane z-axis compartmentalizes the membrane leaflet into two regions characterized by differential dynamics, as reported by

in cell membranes [13–15], and have led to the 'anchored protein picket model' of membranes [16]. In addition, a model involving cross-talk between membrane cholesterol and actin cytoskeleton is emerging based on observations such as destabilization of the cortical actin cytoskeleton due to depletion of plasma membrane cholesterol ([17, 18]; Sarkar P, Kumar GA, Shrivastava S, Chattopadhyay A, unpublished observations). This implies that the membrane components (lipids, proteins, carbohydrates and the underlying cytoskeleton) must interact with each other in order to provide much needed functionality to the membrane.

1.2 What Holds the Membrane Together?

The physical principle underlying the formation of membranes is the hydrophobic effect [19–21]. The hydrophobic effect describes how an aqueous medium deals with non-polar substances. The driving force behind the hydrophobic effect is essentially entropic in nature and has its genesis in the strong attractive forces between water molecules that must be disrupted to accommodate hydrophobic moieties in it and the entropic cost of incorporating a non-polar molecule in water. This effect should not be confused with the force of interaction among two non-polar (hydrophobic) molecules which plays a very minor role in hydrophobic effect. The hydrophobic effect serves as a common mechanism responsible for formation of other organized membrane-mimetic molecular assemblies, such as micelles and reverse micelles, and folding of globular proteins.

1.3 Unique Features of Biomembrane Organization and Dynamics

A unique feature of membrane organization, different from other macromolecular assemblies prevalent in biology, is that there is no intermolecular connectivity (and the implied information content) among membrane constituents (lack of a sequence). The fundamental paradigm in the protein world, a specific sequential

Fig. 1.2 (continued) spectroscopic techniques (such as ESR, NMR, fluorescence) and molecular dynamics simulations. The anisotropic membrane interface (shown in *blue*) ensures a dynamic segregation between the bulk aqueous phase and the isotropic hydrocarbon-like core of the membrane (shown in *gray*). Both the bulk aqueous phase and the hydrocarbon-like core of the membrane are characterized by fast and isotropic solvent relaxation. The membrane interface is uniquely characterized by a functionally relevant chemically heterogeneous environment, slow solvent relaxation, and limited water penetration (interfacial water). This inherent membrane anisotropy is reflected in polarity and mobility gradients (shown as *shaded gray arrows*) along the membrane normal (perpendicular to the plane of the membrane). See text for more details. Adapted and modified with permission from [5]. Copyright (2011) American Chemical Society

arrangement of the constituent units (amino acids), is therefore not a deciding factor in self assembly of lipids in biological membranes. Instead, self assembly of lipids into membranes is a consequence of the amphipathic nature of lipid molecules. The prominent dogma of molecular biology, *i.e.*, sequence dictating function, is therefore absent in biological membranes. Viewed from another perspective, the lack of intermolecular connectivity (sequence) provides the membrane its inherent dynamic nature. This makes the study of biological membranes unique and challenging.

The interfacial region (see Fig. 1.2b) is the most important part of the membrane, in terms of physicochemical characteristics and function [5, 22]. The membrane interface exhibits distinct motional and dielectric characteristics [23] different from the bulk aqueous phase and the more isotropic hydrocarbon-like deeper regions of the membrane. In a chemical sense, the membrane interface plays a crucial role in substrate recognition and activity of membrane-active enzymes [24]. The reduced probability (due to geometrical constraints) of energetically favorable hydrogen bonding induces dynamic confinement of water molecules at the membrane interface [25]. The membrane interface displays slow rates of solvent relaxation [5, 22, 26–29], participates in intermolecular charge interactions [30] and hydrogen bonding mediated by the polar lipid headgroup [31, 32].

As mentioned above, a unique feature of membranes is their inherent dynamics, characterized by a gradient along the bilayer normal (z-axis) (see Fig. 1.2b) [5, 33]. While the center of the membrane bilayer is nearly isotropic, the upper portion, only a few angstroms away toward the membrane surface, is highly ordered [5, 22]. As a consequence of this organization, properties such as polarity, segmental mobility (collectively termed as membrane fluidity), ability to form hydrogen bonds, and extent of solvent penetration vary in a depth-dependent manner in the membrane [34]. In addition, biological membranes display a gradient of environmental heterogeneity along the bilayer normal [33]. Taken together, absence of intermolecular connectivity (sequence), thermodynamically controlled (spontaneous) self assembly, and inherent dynamics characterized by a gradient, represent the essential aspects of membrane organization and dynamics.

1.4 Spatiotemporal Scales of Membrane Phenomena

Cellular events at the membrane span a wide range of spatiotemporal scales (see Fig. 1.3) [35–37]. An important aspect of the cell membrane is its dynamics that span a very large range of time scales, which supports a wide variety of biological processes, necessary for cellular function. The corresponding length scales also cover several orders of magnitude. Monitoring membrane dynamics with all its complexities continues to be challenging in contemporary membrane biophysics. Membrane probes offer the possibility of measuring membrane dynamics at various spatiotemporal resolutions, depending on the experimental approach chosen [38]. However, it is not possible to address problems in these spatiotemporal scales using any single technique and phenomena-dependent approaches are necessary.

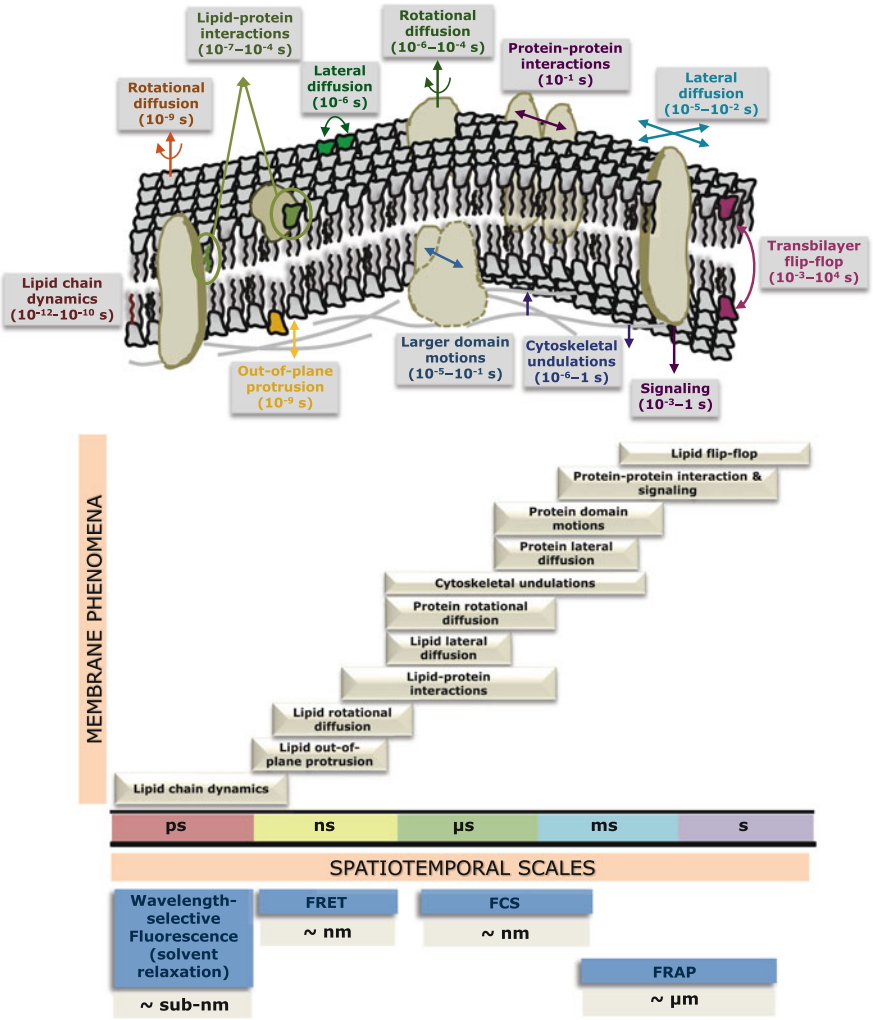

Fig. 1.3 Range of spatiotemporal scales relevant in biological membrane phenomena. Predominant membrane-associated processes are shown, with the corresponding time scales increasing *from left to right*. As shown in the figure, the range of time scales for membrane phenomena could span more than ten orders of magnitude, whereas the spatial scale ranges cover over three orders of magnitude. It is not possible to resolve these spatiotemporal scales simultaneously using any single technique. It is therefore crucial to select techniques with spatiotemporal scales comparable to that of the specific phenomenon probed. In this respect, fluorescence-based approaches (shown at the *bottom of the figure*) appear suitable since many membrane-associated processes can be addressed by fluorescence spectroscopic and microscopic techniques. Adapted and modified from [35]

Among various techniques used, fluorescence-based approaches offer certain advantages due to their enhanced sensitivity, minimal perturbation, multiplicity of measurable parameters, and suitable time scales that allow the analysis of several

membrane phenomena [39]. With the advent of confocal microscopy and organelle-specific probes, it has now become possible to explore lateral [40] and rotational [41] dynamics of specific organelle membranes such as Golgi membranes.

1.5 Future Perspectives: What Lies Ahead

Unraveling the spatiotemporal complexity of biological membranes appears to hold the key to understanding the molecular basis of diseases that pose a threat to mankind. This constitutes a major challenging area of research in the post-genomic era, particularly keeping in mind the fact that more than 50% of current drug targets in all clinical areas are membrane proteins [42]. In addition, the biological membrane plays a crucial role in amyloidogenic diseases that are associated with protein aggregation [43]. Membrane lipid mediated pathogen entry into host cells offers another potential avenue for developing novel therapeutic strategies to effectively tackle intracellular pathogenesis [44]. Tissue-specific and age-dependent drug efficacy represents another promising development in human health [45]. The articles that follow in this monograph will address several important topics in membrane biology with focus on membrane lipids and proteins using experimental and simulation approaches.

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