Preface

Biological membranes play a central role in cell structure, shape, and functions. While the plasma membrane surrounds the cell and separates its interior from the extracellular environment, a number of intracellular membranes compartmentalize the cell interior into organelles having different functions. Being selectively permeable to ions and organic molecules, cell membranes primarily control the exchange of substances and nutriments in and out of cells. They are also involved in a variety of cellular processes, such as cell adhesion and signaling. Biological membranes of eukaryotic cells are essentially built up by a lipid bilayer, constituted of a large number of different lipids, where a large variety of proteins are inserted. Glycerophospholipids are the main structural components of cellular membranes, either the plasma membrane or membranes of organelles. Together with the other types of lipids, including sphingolipids and cholesterol, these amphipathic molecules spontaneously self-assemble into lipid bilayers so that their hydrophobic tails are isolated from the surrounding polar medium and their more hydrophilic head groups are exposed to the extracellular medium and the cytosol. Initially, according to the "fluid mosaic model," biological membranes and, notably, the plasma membrane were simply perceived as a lipidic "sea," in which membrane proteins were freely diffusing. Nowadays, it is well recognized that this simplistic picture is inappropriate and that biological membranes are highly heterogeneous systems in which embedded molecules show complex diffusion patterns. In fact, due to their high structural and chemical diversity and their highly heterogeneous spatiotemporal distribution, lipids play a key role in membrane functions by regulating the conformational state and functions of proteins inserted into or associated with the membranes. Lipids also directly participate in signaling pathways and constitute direct molecular receptors of a number of external agents. An important consequence of the diversity and dynamics of membrane lipids is the formation of transient but highly ordered lipid domains, often referred to as membrane "rafts" that allow tight spatial packing of important proteins. These domains are thought to function as signaling platforms in a variety of important biological functions. Although the membrane properties and functions are of key importance, their investigation proved to be difficult due to their intrinsic characteristics.

Indeed, the depth of the membrane bilayer is only 4–5 nm, which is two orders of magnitude below the resolution of optical microscopes, precluding any direct visualization of membranes in the "z" direction. Similarly, the highly dynamic nature as well as the small size of lipid "rafts" (below 100 nm) precluded, up to now, their direct observation. Another key issue is the highly anisotropic structure of the bilayer, which generates steep gradients at the nanometer scale. For instance, in phospholipids characterized by polar head groups and highly apolar aliphatic chains, there are steep gradients of polarity and hydration between the membrane surface and its interior. Moreover, the asymmetric distribution of charges at the membrane level generates a number of static electric fields with different localizations, often classified as surface (Ψs), dipole (Ψd), and transmembrane (Ψt) potentials. Another issue of key importance in biomembranes is their asymmetric lipidic distribution between the inner and outer leaflets, especially in the plasma membrane, since negatively charged phospholipids, like phosphatidylserine, are mainly distributed in the inner leaflet while sphingolipids are distributed in the outer leaflet. In addition, to ensure the efficient absorption of external molecules needed for cell metabolism, the plasma membrane continuously undergoes endocytosis, a process in which the plasma membrane engulfs the extracellular content through small vesicles that detach into the cytoplasm. As a consequence, it is considered that the whole membrane surface of fibroblasts is internalized every 16 minutes. Taking into account these various aspects, it is obvious that the design of membrane probes and instrumentation for characterizing membrane properties and functions is particularly challenging. Nevertheless, due to the consistent efforts of a large scientific community and the decisive impact of fluorescence-based techniques, remarkable progress has been made in the last decades in the understanding of membrane characteristics and functions.

In this context, this book illustrates some of these major advances through a collection of review articles written by invited authors who are experts in the subjects of membranes and fluorescence. A number of these authors have presented their work at the 12th International Conference on Methods and Applications of Fluorescence (MAF-12), held in Strasbourg, France, in September 2011. Since their inception in 1989, the biennial MAF conferences have become the largest meetings dedicated to studies concerning fluorescence, being highly interdisciplinary and covering areas including physics, chemistry, nanotechnology, biology, and medicine. The MAF-12 conference has attracted more than 400 attendees from 40 countries worldwide.

This book is organized into three parts. The first part deals with membrane probes and model membranes. Two "historical" membrane probes, namely, Laurdan- and NBD-labeled lipids, are presented, with a discussion on both their early and present applications. The two-color 3-hydroxychromone probes are also described, with a focus on the importance of their precise location and orientation in the membrane for their applications in sensing membrane potentials, lipid domains, and apoptosis. Three review articles on membrane models, such as lipid vesicles and supported bilayers, are also presented. Model membranes with controlled composition and environment continue to play a central role in understanding the

basic physicochemical properties of biological membranes. These review articles focus specifically on the use of FRET to probe the lateral heterogeneity of membranes and lipid–protein interactions and the use of time-resolved fluorescence techniques to monitor hydration and mobility changes across the bilayer. The second part describes the use of fluorescent-based microscopy techniques, and notably of advanced quantitative and high-resolution techniques, to explore the properties of biological membranes. This part illustrates the key advances that have been made in the understanding of membrane organization, dynamics, and interactions using techniques such as wide-field microscopy; multiphoton microscopy; fluorescence lifetime imaging microscopy (FLIM); fluorescence correlation spectroscopy (FCS), spot variation FCS, and FCS combined with stimulated emission depletion (STED) microscopy; polarization-resolved fluorescence microscopy; and near-field optical nanoscopy. The last part focuses on the investigation by these techniques of membrane proteins and, notably, membrane receptors that play a central role in a number of signaling pathways and are, thus, largely targeted in therapeutic strategies. Two review articles discuss the strategies that can be used to explore either the biophysical properties of a hypothetical membrane receptor or the oligomerization of G-protein-coupled receptors (GPCR). Two additional review articles focus on the characterization of serotonin 1A and TNF receptors. Finally, the last review article deals with the assembly of retroviral HIV-1 particles at the plasma membrane level.

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