Membrane proteins represent almost 40% of all proteins, but only a small number of their structures have been determined. Alone or associated with other proteins, membrane proteins play several roles in the cells. They are involved in signal transduction, ion exchanges, transport of metabolites, molecules or proteins. Cellular communications are controlled or regulated by membrane proteins. Indeed, they are involved in communications between cells, outside/inside cell exchanges, cytosolic traffic among different organelles as well as cytosol/organelles exchanges.

Only a few functional classes of membrane proteins have been structurally characterized and mostly are transporters working alone. Membrane proteins are difficult to study mostly because they are often poorly abundant and thus difficult to purify in amounts compatible with structural studies. Heterologous overexpression of recombinant membrane protein is a strategy that has permitted the study of several membrane protein structures at an atomic level. However, membrane proteins are located in an hydrophobic environment such as the cellular bilayers, and their functions often involve hydrophilic contacts with lipids, resulting in the paradox that membrane proteins need lipids to work but they also need detergent addition to be purified. When proteins are associated in complexes in a functional way, their stabilization is often difficult in purification protocols and requires numerous trial and error steps.

Determination of a structure is a crucial step but never solves the functional question. Indeed, activation or inactivation of membrane proteins involves numerous factors such as ligand binding, phosphorylation of specific residues, and posttranslational modifications. From a pharmacological point of view, ligands induce or block a functional response that may involve either a single protein or a cascade of several proteins mixing membranous and soluble ones. This assembly of proteins can form stable or dynamic interacting complexes. In the cellular environment, these complexes are probably quite easy to form if one considers on one hand the protein concentrations inside the cytosol or the membranes and on the other hand the relative proximity of organelles in these cells. A fundamental aim of structural biology is to move from understanding structure and dynamics to controlling molecular function.

This book describes major techniques used in the field of membrane protein structure determination. It is divided into five sections describing different techniques used to solve atomic structure either from purified membrane proteins or in silico. It also describes techniques that permit the capture of atomic scale pictures of membrane proteins in their lipid and protein environment to make “movies” from different instant pictures that will describe membrane protein functioning. It presents techniques scaling up from atomic to molecular that will render protein complexes in membrane of organelles and cells.

The first section presents various strategies to purify membrane proteins since getting pure and homogenous material is a significant hurdle. Chapter 1 describes some techniques to characterize membrane protein preparations such as detergent content. Chapter 2 is devoted to the specific case of the adenosine nucleotide transporter (ANT), which a natural abundance in the inner membrane of mitochondria has permitted its three-dimensional structure determination, whereas structure–function relationships have been studied using
mutants over expressed in yeast. Chapter 3 focuses on the importance of overexpression when membrane proteins are not naturally abundant. The specific case of a bacterial expression system used for a small mitochondrial membrane protein, the translocase TSPO is presented. Chapter 4 highlights the difficulties encountered for large membrane proteins overexpression; it describes a different expression system used for the specific case of an ABC transporter.

The second section presents various strategies to get three-dimensional crystals and solve the structure by X-ray diffraction. Chapter 5 describes the various steps of membrane protein crystallography in two different approaches that are vapor diffusion and lipidic phases. Chapter 6 discusses the gain for a membrane protein family to solve the atomic structure of one of its members. Chapter 7 analyzes what can be learned about the function of a single protein from its various atomic structures through the example of the sarcoplasmic calcium pump (SERCA-ATPase). Chapter 8 presents recent progress in the study of a membrane protein with high potential as a pharmaceutical target, the G protein-coupled receptor (GPCR) family.

The third section presents the various possibilities to gain structural information for a membrane protein using electron microscopy observations. Chapter 9 uses the insect aquaporin AQPcic to go from its characterization in situ to its homotetrameric structure of purified protein reconstituted in membrane. Chapter 10 describes two-dimensional crystal formation and basic electron microscopy image analysis of membrane proteins. Chapter 11 presents a specific combination of cryo-electron tomography and single particle analysis of membrane protein embedded in stacked lipid bilayers. Chapter 12 describes, step-by-step, the process of electron tomography of mitochondria containing numerous membrane proteins. Chapter 13 is devoted to molecular modeling processes that permit to reach atomic structure of membrane protein conformation, combining its electron microscope derived map and atomic structure from a different conformation.

The fourth section presents recent advances in nuclear magnetic resonance (NMR) to study membrane proteins and lipids. Chapter 14 goes through the various strategies that are available to solve atomic structure or protein–protein and protein–ligand interactions using different NMR approaches. Chapter 15 is devoted to the analysis of what can be learned from the structure of membrane protein fragments in regard to the overall protein. Chapter 16 used the peculiar example of the phospholamban to show by NMR analysis the structural dynamic of regulation of a membrane protein by a smaller interacting membrane protein. Chapter 17 describes step-by-step detergent solubilized membrane protein structure determination by solution-state NMR. Chapter 18 presents how solid-state NMR is a powerful tool to study lipid structure and dynamics in a membrane environment.

The fifth section presents molecular modeling strategies that can be used either to get membrane protein structures or to move from atomic structure to dynamic understanding of a molecular functioning mechanism. Chapter 19 goes through the various possibilities to build and to analyze membrane protein models. Chapter 20 describes step by step how to build a three-dimensional model of a membrane protein. Chapter 21 presents molecular dynamics of membrane peptides and proteins in their lipid environment. Chapter 22 further describes membrane protein dynamics, presenting increasing time scale ranging from femtoseconds to seconds. Chapter 23 shows synergy between experimental data and computational modeling to delineate the ligand binding pocket of a GPCR, a step toward a rational for drug design.

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