

## Preface

Membrane proteins are known to be key molecules in cellular communications, from signal transduction to ion exchanges or transport of metabolites and other molecules. They also participate in the synthesis of ATP, by generating the proton gradient necessary for the rotatory motor of ATP-synthetase to function and to catalyze ATP formation from ADP and inorganic phosphate. Membrane proteins are necessary for the import of soluble or membrane proteins from the cytosol, where they are synthesized into various compartments such as the mitochondrial matrix or outer and inner mitochondrial membranes. Living organisms have also designed efficient machineries that protect cells from toxic elements. Bacteria or eukaryotic cells have, in their membranes, efflux pumps that will clean the cell. The efflux of toxic elements also has drastic consequences for the efficiency of drugs that may find difficulties in penetrating the cell in order to be active. In contrast to soluble proteins, membrane proteins are embedded in a medium which is organized continuously from the atomic level (at the nanoscale) to the micron range. However, the mesoscopic organization of membranes influences, through long-range effects, the properties of the molecules that are embedded in the membranes. Therefore, an understanding of the function of membrane-integrated molecular machineries necessitates a description of the proteins on the atomic level, their various conformations, their specialized organization, as well as their dynamics within the membrane.

Despite attracting great interest, membrane proteins are still difficult to study at the molecular level. Indeed, they are difficult to produce, to extract from their natural environment, and to purify in a native conformation. However, during the past decade efforts have been stepped up worldwide such that several new structures have been resolved at high resolution and their details published within the past two to three years. All of these structures have opened a wide field of discussion about the function and the topology of membrane proteins, their interactions with lipids, the need for such interactions, interactions with ligands or cofactors, and a large number of functional mechanisms could be postulated. At the same time, it has also become clear from the results of many studies that, even with very high-resolution structures, the atomic details were insufficient to understand the function. Further information was needed on the identification and characterization of different conformations, on the dynamics that are necessary for

conformational changes, on how membrane proteins are inserted in their natural environment, and on how they are organized within the membrane. Although, crystallography represents an extremely powerful method by which to describe the atomic structures of proteins, an ensemble of complementary biophysical approaches is essential in order to fully describe the structure–function relationships of proteins in general, and of membrane proteins in particular.

This book will serve as a cutting-edge resource for the biophysical methods that are – or soon will be – the major techniques used in the field. Each chapter is dedicated to a specific approach, describing the method involved, highlighting the experimental procedure and/or the basic principles, and offering an up-to-date understanding of what is measured, what can be deduced from the measurements, as well as the limitations of each procedure. This comprehensive reference book will be helpful to junior scientists whose target is to solve structure–function problems associated with membrane proteins, and will surely guide them in their experimental choices. Indeed, this book will also serve as a resource for anybody who is interested in membranes.

Following a general introduction to membrane protein structures and X-ray crystallography, the book is divided in five sections. Part I (the Introduction) is dedicated to structural approaches, while in Part II, Chapter 2 describes several aspects of electron microscopy either on single particles or on two-dimensional and tubular crystals, and Chapter 3 illustrates the current possibilities of NMR, and their future. Part III is centered on molecular interactions and the study of large molecular assemblies, with Chapter 4 illustrating how analytical ultracentrifugation can be used to address the study of membrane proteins solubilized in detergent micelles. Chapter 5 discusses how surface plasmon resonance – a well-known method used to study molecular interaction with soluble proteins – can also be adapted to membrane proteins. Molecular interactions and the topology of large assemblies of membrane proteins, either in reconstituted systems or in natural membranes, can also be studied by using atomic force microscopy, as shown in Chapter 6. Part IV is focused on dynamics, either by computational or experimental approaches. Here, Chapter 7 illustrates the possibilities of molecular dynamic calculations, while Chapter 8 describes how transport pathways can be followed by free energy calculations and Chapter 9 highlights the power of neutron scattering for studying membrane protein in their natural environment. Part V focuses on spectroscopies of various types. For example, circular dichroism can be extended to membrane proteins, as shown in Chapter 10, whilst infrared or Raman spectroscopy is able to probe either global folding properties or very fine local information, as demonstrated in Chapters 11 and 12, respectively. Finally, Part VI is devoted to functional approaches in whole cells, wherein Chapter 13 explains the possibilities offered by FRET or BRET experiments.