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## Preface

Biological membranes are the essential structuring elements of all living cells. The core elements of a membrane are a lipid bilayer and integral and peripheral proteins. While the lipid bilayer constitutes the hydrophobic barrier that prevents arbitrary exchange of solutes, the transmembrane proteins allow the regulated exchange of solutes or they transduce signals from one side of the membrane to the other. Many enzymatic reactions take place at the membrane–water interface. Specific lipid–protein interactions are important for the stable integration and activity of integral and peripheral membrane proteins. The unique structure of the lipid bilayer requires specific surface properties of integral and peripheral proteins so that the proteins can function. Protein surfaces exposed to the fatty core of the membrane are typically hydrophobic, while protein surfaces exposed to the aqueous space usually are composed of polar amino acid residues. The polar–apolar interface of the lipid bilayer is formed by the glycerol backbone and by the polar head group of the various phospholipid species and therefore an important region for lipid–protein interactions. The fatty acyl chains of the lipids can vary a lot in length and degree of unsaturation and the membranes may contain cholesterol, sphingolipids, etc. This has consequences, e.g., for membrane thickness and fluidity. Furthermore, the complex membrane composition often leads to the formation of micro-domains with distinct physicochemical properties.

To gain detailed insight into membrane properties, it is therefore of great importance to understand the complex nature of the interactions of membrane proteins with lipids. This volume provides a selection of protocols to examine protein–lipid interactions, membrane and membrane protein structure, how membrane proteins affect lipids, and how they are in turn affected by the lipid bilayer and lipid properties.

Numerous methodologies have been developed in the past, each with its own advantages and limitations. The methods described here are all actively used, complementary, and necessary to obtain comprehensive information about membrane structure and function. The method of choice is determined by the information that is sought, but is dependent on the properties of the sample, the available quantity, and the required sensitivity. Label-free approaches described in this book include methodologies like quartz-crystal microbalances with dissipation, surface plasmon resonance, isothermal titration calorimetry, and differential scanning calorimetry. These are useful methods, e.g., to monitor binding events and to obtain the free energies, enthalpies, and entropies of protein–lipid interactions. Imaging techniques like electron microscopy and atomic force microscopy are used to examine the structure and organization of protein–lipid complexes in membranes. Atomic force spectroscopy allows the probing of mechanical properties of macromolecules, e.g., the force necessary to unfold a single protein in a lipid environment. Neutron scattering is an emerging technique to study the structure of protein–lipid complexes, which in combination with deuteration of either lipids or proteins allows resolving the inner structure of big and dynamic lipid–protein complexes. The secondary structure of native and nonnative proteins in lipid membranes can conveniently be monitored by circular dichroism spectroscopy and synchrotron radiation circular dichroism spectroscopy. The development of the latter greatly extended the recordable wavelength range, strengthening structural investigations. Secondary structure and in addition the orientation and order parameters of membrane proteins in lipid bilayers can be obtained from infrared-spectroscopic methods. In combination with isotope editing methods, these are also used to determine changes in local protein conformation.

The specificity and selectivity of protein interactions with lipid species is efficiently investigated by combining the labeling of lipids and proteins with either fluorescence or electron paramagnetic resonance spectroscopy, which are both very sensitive techniques to examine the protein–lipid interface. These methods are applied with great success to probe the topology of peptides and proteins in membranes. Fluorescence quenching is a fast and reliable technique to determine the location of fluorescent amino acids, especially tryptophan residues in lipid bilayers. Förster energy transfer is a highly sensitive fluorescence method that is useful to measure distances up to 10 nm and able to detect direct binding as well as deviations from homogeneity of the lipid distribution around a protein. Fluorescence methodologies have also been successfully applied, e.g., to track single molecules like transmembrane proteins in planar supported membranes. Single particle tracking allows imaging and tracking single fluorescent molecules with good spatial and temporal resolution. Particle association and dissociation events can be monitored. Fluorescence correlation spectroscopy uses the time correlation of temporal fluctuations of fluorescence, which are detected in a focal volume, to explore dynamic events with high temporal resolution and statistical accuracy. Lipid-spin labeling and electron paramagnetic resonance (EPR also called electron spin resonance, ESR) spectroscopy have been highly successful in the determination of the protein-solvating lipid shell, that is, the stoichiometry of the lipid interactions with integral membrane proteins. Since the technique allows the estimation of mobile and protein-immobilized lipids and their exchange rates, it has also been very successful in probing the lipid-selectivity of transmembrane proteins. In combination with site-directed mutagenesis, EPR of spin-labeled proteins has become a powerful tool to examine protein structure and dynamics, even in complex systems that are not accessible with other approaches. Nuclear magnetic resonance (NMR) spectroscopy, combined with isotopic labeling, is widely used to probe the structure and dynamics of proteins either in solution or in a lipid environment. Solution-phase NMR is performed with detergent-solubilized membrane proteins and usually yields high-resolution structures. Solid-state NMR is performed either as magic angle spinning (MAS) NMR to obtain highly resolved protein structures with spectra resembling fast isotropically tumbling proteins in solution or with oriented samples. When applied to oriented samples, NMR gives valuable information on the dynamics and orientation of lipids and proteins in membranes and allows determining, e.g., peptide orientation, lipid-order parameters, lipid-phases, etc. Lipid–protein interactions have been investigated in small, large, and giant vesicles, in supported lipid bilayers, in lipid monolayers, with short-chain lipid micelles, or even with single lipids. More recently lipid nanodiscs have been developed, which are disc-like fragments of lipid bilayers that are stabilized by two amphipathic helical proteins. Nanodiscs have been shown to be a robust means for stabilizing and investigating protein–lipid interactions and nanodisc-applications are reviewed here. The last chapter in this book is dedicated to a range of molecular dynamics simulation approaches to lipid–protein interactions, including both atomistically detailed and coarse-grain methods. Lipid–protein interactions are heavily investigated and while it is clear that not all methods can be described in a single book, the present volume covers a wide range of the methods typically used in this area of research. I thank all contributing authors for providing interesting and highly valuable methods, insights, and reviews for this volume of *Methods in Molecular Biology*.



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