Preface

Structural biology of integral membrane proteins has been in the limelight ever since the first 7 Å resolution three-dimensional structure of bacteriorhodopsin was determined by electron crystallography and published in 1975. Since then, there have been incredible advances in our ability to express any membrane protein in heterologous expression systems and purify them in a functional form suitable for crystallization. Some membrane proteins have proven to be amenable for structural analysis. We now have a wealth of information on the structure and function of bacterial ion channels, transporters, respiratory complexes, and photosynthetic assemblies, which has led to the award of a number of Nobel Prizes, highlighting the importance of these proteins in biology and the difficulty in determining their structures. Nevertheless, structure determination of mammalian membrane proteins has proven much more difficult, but in the last five years there have been dramatic advances in our understanding of why these proteins are more difficult than their bacterial counterparts. This has been demonstrated most graphically with the structure determination of G-protein-coupled receptors (GPCRs), where a series of complementary and generic engineering and crystallization methodologies have been developed in different laboratories around the world, making it possible to determine the structure of any GPCR provided that enough authentically folded receptors can be expressed.

Expression of many integral membrane proteins remains challenging. Human membrane proteins often require molecular chaperones to fold correctly in a process that may take hours, and the proteins may be far less stable than their bacterial homologues. Thus the challenge of producing milligrams of correctly folded protein remains. This volume addresses many of the problems associated with producing membrane proteins and more importantly how to purify them in a functional form using stabilizing detergents and detergent mimetics, allowing subsequent biophysical and structural analyses. Every membrane protein behaves in its own unique fashion, with quirks and peccadilloes enough to make each protein a challenge to express, purify, and crystallize. Thus the more tools we have in our toolbox of protocols for handling membrane proteins, the greater chance we have of making even the most wayward membrane protein behave.

Structural biology of membrane proteins is entering a new era. Electron cryo-microscopy was recently used for the first structure determination of an integral membrane protein to 3.4 Å resolution by single particle analysis; in the next few years as technology develops, this will become easier and promises the possibility of determining structures of any protein over about 250 kDa in size without the need for crystallization. The X-ray free electron laser has shown how high-resolution structures can be determined from micron-sized crystals of membrane proteins using only a few hundred micrograms of purified protein. New developments in electron diffraction of sub-micron crystals also show great promise for future structural analyses. Structure-based drug design for GPCRs is a reality, with multiple structures being determined of a single receptor bound to different drug candidates. However, if you cannot express and purify your membrane protein of interest in a biologically relevant state, then these great advances are superfluous. Thus, there will always be the need for improvements in expression systems and for careful biochemical analysis of the proteins produced.

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