

Preface

The past few years have seen exciting advances in the field of membrane protein structural biology. Although membrane proteins still constitute a small fraction of the total number of solved protein structures (see Hartmut Michel's and Stephen White's summaries at <http://www.mpibp-frankfurt.mpg.de/michel/public/memprotstruct.html>, http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html), an exponential increase of membrane protein structures has been observed¹ and is anticipated to continue. This book addresses a number of issues pertaining to membrane protein structural biology. The first section describes approaches for expression and purification of membrane proteins, and a general introduction to detergents. The major challenge in this field is with eukaryotic membrane proteins, which is reflected by the contributions in this section. The second section addresses selected methods for structure determination of membrane proteins, such as solution and solid-state nuclear magnetic resonance, atomic force microscopy, electron microscopy including single particle analysis and lipidic cubic phase crystallization. The final section highlights some of the recently solved membrane protein structures.

The content of the individual chapters varies from basic introduction to very detailed description of a particular theme. Therefore, the experienced membrane protein biochemist, as well as the novice, will find useful information. While we have endeavored to provide a balanced overview, not all topics could be covered. Clearly, this book is not an exhaustive reference for all concepts relating to membrane protein structural biology. However, the following paragraphs give some further information with key references on topics not addressed by this book.

Heterologous expression and purification of membrane proteins have been covered by a number of reviews.²⁻⁵ Here, the reader will find information on both prokaryotic and eukaryotic membrane proteins. Until recently, all structures of eukaryotic membrane proteins, for example the visual pigment rhodopsin,^{6,7} had been solved with material from natural sources, reflecting the difficulty of producing functional eukaryotic membrane proteins in heterologous expression systems. The new structure of a recombinant mammalian voltage-gated potassium channel, produced in the yeast *Pichia pastoris*, is therefore exciting.⁸

Structure determination of any membrane protein necessitates that the purified protein retain its native fold and full functionality. Progress on refolding of membrane proteins, deposited as aggregates in the cytoplasm of *Escherichia coli*, has been demonstrated for seven-helix G-protein-coupled receptors^{9,10} and bacterial outer membrane proteins (for reviews, see Refs. 11 and 12). Likewise, cell-free *in vitro* expression has been reported for some membrane proteins,¹³⁻¹⁵ and the potential usefulness of this method for generating functional membrane protein for structural studies is under investigation.

Overproduction of correctly folded membrane proteins is intimately linked to efficient membrane insertion. Great advances have been made in understanding the mechanism of membrane protein insertion into membranes, but a rational approach to predicting good ‘overexpressors’ from their amino acid sequences is still lacking. It is simply fascinating to see how the expression levels of closely related proteins, such as seven-helix G-protein-coupled receptors, vary in a particular host and under particular growth conditions (see for example Ref. 16). Two recent topics warrant highlighting: (a) Elegant work combining theory and biochemical experiments provides the basic features of how transmembrane helices are recognized by the endoplasmic reticulum translocon (‘biological’ hydrophobicity scale^{17,18}). (b) Successful recombinant overproduction of membrane proteins appears to be linked to avoidance of stress responses in the host cell¹⁹ and can be related to the differential expression of genes involved in membrane protein secretion and cellular physiology.²⁰ This implies that simple approaches for increasing recombinant membrane protein yields may be unsuccessful, emphasizing the benefit of considering a ‘whole-cell’ approach for developing general strategies to obtain high levels of functional membrane proteins, especially of eukaryotic targets.

The process of membrane protein crystallization usually starts with the extraction of the target from membranes by using detergents, followed by purification. However, the yields of purified membrane proteins can be low, which has limited the number of crystallization parameters to be explored. An obvious (but expensive) solution to this problem is to conserve protein by using nanoliter-pipetting robots (see for example Ref. 21). Likewise, smaller crystals can now be analyzed due to improvements in synchrotron beam lines. Another avenue for improving the chance of successful crystallization is to employ Fv or Fab antibody fragments for co-crystallization. This approach relies on expanding the hydrophilic surface of a membrane protein, facilitating formation of crystal contacts – the reader is referred to^{22–26} for more information. Along the same lines, complexes between an integral membrane protein with little hydrophilic surface, and its soluble partner, may be more amenable to successful crystallization than the membrane protein alone. The recent structure of the mammalian voltage-gated potassium channel, Kv1.2-β2, illustrates this concept nicely.⁸ For the general principles of protein crystallization, the reader is referred to Refs. 27 and 28.

Mass spectrometry has found increasing use for the characterization and analysis of integral membrane proteins, despite the unfavorable spectrometer response caused by detergent and lipid molecules associated with membrane proteins. The reader is referred to the following publications for recent advances in this field.^{29–33}

Another spectroscopic method, utilizing spin labeling for electron paramagnetic resonance experiments, has provided dynamic structural information on membrane proteins, in contrast to static views obtained from X-ray crystal structures. This technique has been applied, for example, to channels,^{34,35} seven-helix G-protein-coupled receptors³⁶ and bacterial outer membrane transporters.³⁷

This book presents a selected number of recent membrane protein structures, but many other exciting structures could not be included here. Among these are ATP-binding-cassette transporters,^{38,39} major-facilitator superfamily transporters,^{40,41} the sodium/proton antiporter from *E. coli*,⁴² a bacterial homolog of a neurotransmitter transporter,⁴³ bacterial and mammalian potassium channels,^{8,26,44–46} chloride

channels/transporters,^{25,47} plant light harvesting complexes^{48,49} and cytochrome b6f.^{50,51} A complete list of all membrane protein structures can be found at the web sites listed in the first paragraph.

Many integral membrane proteins, including eukaryotic members, can already be produced in quantities sufficient to consider structural and functional analyses. Further technological advances will continue to promote the exponential growth of membrane protein structures. Likewise, protocols to assess the dynamics and functions of membrane proteins continue to be developed, especially in the fields of solution and solid-state nuclear magnetic resonance. Most importantly, the dedication and enthusiasm of researchers, combined with good biochemistry, will propel membrane protein structural biology to new levels.

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October 2005

Acknowledgment

The research of the editors is supported by the Intramural Research Program of the NIH, National Institute of Diabetes and Digestive and Kidney Diseases.

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