PREFACE

Integral membrane proteins constitute a significant portion of the entire proteome in different organisms. They mediate a wide range of signal recognition and communication processes across the cell membranes. These proteins are one of the most important classes of drug targets, and more than half of the currently marketed drugs are aimed at membrane proteins. In spite of their crucial physiological roles, structural characterization, especially high-resolution structure determination, of membrane proteins lags significantly behind that of soluble proteins. There are numerous challenges encountered at every step in the process of membrane protein crystallography such as recombinant protein expression, homogenous purification, and crystallization. These two volumes (volume 556 & 557) of *Methods in Enzymology* aim to provide a comprehensive coverage of various steps involved in the process of membrane protein structural characterization through general protocols and case examples.

The very first step in the process of structural studies of membrane proteins is their recombinant expression in heterologous hosts for large-scale protein production. In Section I of Volume 556, we provide a collection of chapters that describe either a step-by-step protocol or a general overview for recombinant expression of various types of membrane proteins in different expression hosts. These chapters cover recent advances in conventional *E. coli*-based expression of membrane proteins, yeast-based expression systems for large-scale production of eukaryotic membrane proteins, and cell culture-based membrane protein overexpression in insect cells and mammalian cells. Furthermore, several chapters in this section also discuss relatively uncommon but promising strategies for expressing membrane proteins, e.g., *Drosophila melanogaster*, *Xenopus* oocytes, and *Wolinella succinogenes*.

Biochemical and functional characterization of recombinant membrane proteins is important to ensure their native-like behavior before structural studies can be undertaken. Section II of Volume 556 encompasses several chapters that cover various methods for characterizing membrane proteins such as reconstitution in lipid environment, cross-linking, fluorescence, and spectroscopy-based approaches to investigate conformational changes and surface plasmon resonance-based strategies to study ligand–protein interactions.

Once the recombinant membrane protein expression has been established and functional characterization reveals native-like properties, the next steps are to solubilize and purify them efficiently in functional forms. In Section I of Volume 557, we present a collection of chapters that provide generally applicable protocols and discussions on efficient solubilization and purification of recombinant membrane proteins. One of the major challenges in membrane protein crystallization is their conformational flexibility and limited polar surface area to make crystal contacts. In Section II of Volume 557, three chapters describe general protocols and successful examples of generating antibody fragments against membrane proteins using phage display technology to address these challenges.

Although crystallography of membrane proteins provides high-resolution structural information, it yields only a static snapshot of the protein architecture. Therefore, dynamic studies of membrane proteins are highly invaluable to obtain a complete understanding of their function. Chapters 13–16 in Section III of Volume 557 highlight various biophysical approaches such as electron paramagnetic resonance and nuclear magnetic resonance methodologies that yield dynamic insights into the structure and function of membrane proteins.

One of the major goals in membrane protein structural studies is their crystallization for high-resolution structure determination by X-ray crystallography. Chapters 17–22 in Section IV of Volume 557 present streamlined protocols and discussions on various methods for protein crystallization including a case example of protein crystallography by X-ray free-electron laser, the latest development in the area of protein crystallography.

Similar to dynamic methodologies, computational approaches can also provide extremely valuable insights into membrane protein functions. In Section V of Volume 557, authors present case examples of computational approaches that were applied to better understand three different classes of membrane proteins.

Overall, these two volumes provide an extensive and unique coverage of various aspects in membrane protein studies and they will be extremely useful to researchers engaged in the area of membrane proteins.

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