PREFACE

Membrane transport is fundamental in living cells. However, limited molecular details on function and structure left us with more open questions than answers on this enigmatic biological process. Since solving the first structure of a membrane protein in 1985, X-ray crystallography and single particle cryo-electron microscopy have yielded constantly increasing number of atomic and near-atomic structures, however their amount lingered behind expectations. Similar, dynamical methods like NMR, EPR and single-molecule FRET faced multiple experimental challenges, e.g., the low amount of expressed membrane proteins and the lack of adequate membrane-mimicking environment. In addition, functional descriptions of active and passive membrane transport processes essential to complement and interpret structure and dynamics are likewise difficult to obtain. Undoubtedly, working with membrane transport proteins remains challenging. The overwhelming struggles that might be encountered at each step have figuratively stigmatized this fascinating research field.

New approaches in structure-function methodology and membrane protein handling have recently surfaced, raising hope that we will enter a new era of unprecedented molecular detail in membrane transport. This includes new aspects in membrane protein expression, in thermo-stabilization, in providing more native membrane environment as well as new technical advances in obtaining molecular and functional information.

In this regard, we are proud to present in this volume of Methods in Enzymology a timely overview on cutting-edge techniques that have advanced the structure-function toolbox of membrane transporters and channels. The individual chapters describe innovations from expression over structure determination to dynamical characterization providing creative experimental solutions to typical problems when working with membrane proteins. Hence, this volume is a compilation of knowledge of long-standing membrane protein experts, who have contributed for years with their work to the field of membrane transport.

Stefan Raunser and co-workers begin the volume with a chapter describing a method to study membrane proteins by single particle analysis in a nearly native lipid bilayer using lipid nanodiscs. In case studies, they recount cryo-EM-specific benefits and challenges of this method focusing on membrane proteins.

The second chapter, by Klaus Fendler and colleagues, introduces an electrophysiology method, the Solid Supported Membrane method, for a cell-free functional characterization of electrogenic transporters and channels. The chapter provides in "hands-on" format detailed information on sample and buffer preparation as well as the interpretation of the results.

The groups of Poul Nissen and Jens Frauenfeld report on a complementary method to prepare bilayer nanoparticles using saposin A, a protein that is involved in lipid transport, as flexible and adaptive scaffold protein. The chapter by Dirk Slotboom and co-workers focuses on single-molecule imaging of liposome-reconstituted membrane transporters. Here membrane-reconstituted transporters with fluorescent labels are used to visualize the transport-associated dynamics by total internal reflection fluorescence microscopy at the single-molecule level.

The fifth chapter by members of the group of David Drew covers the important issue of screening for overexpression condition of membrane proteins. In a high-throughput setup in *Escherichia coli* the authors document the advantages of their "one-shot expression approach" approach on several well-known transport proteins.

Eric Geertsma and colleagues take a different approach on the same problem, the overexpression of membrane proteins in in *Escherichia coli*. Their procedure involves transcriptional fusions of small additional RNA combined with a tuneable promoter adjusting the membrane protein expression rate to the downstream folding capacity.

The following chapter by Joseph Mindell and Christopher Mulligan is an outstanding review of the very successful techniques that exploit the chemistry of cysteine to test the structure and mechanism not only of membrane proteins. The authors describe numerous applications reaching from cross-linking and labelling for monitoring conformational changes, to cross-linking strategies coupled to structural studies.

Cysteine modifications are also an important tool in Christos Pliotas's chapter; this chapter provides a detailed description of pulsed-EPR methodology using mechanosensitive ion channels as model to investigate oligomerization, conformation, and the effect of lipids on the protein.

The last chapter offers insights into strategies from Christopher Tate's lab for the thermo-stabilization of a GPCR–mini-G protein complex. This chapter summarizes theoretical and practical aspects of the methodology used for stabilizing this complex and provides examples of thermo-stability shifts.

We are convinced that your research will be stimulated by these new approaches and hope that you will enjoy this collection.

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