Preface

The plasma membrane is the gateway through which cells sense and respond to their microenvironment. Critical to this process are cell surface proteins that span (transmembrane) or are anchored/embedded in the plasma membrane. Cell surface proteins perform diverse functions, including nutrient and ion transport, intra- and intercellular communication, receptor signaling, and enzymatic reactions. Altogether, the collection of proteins that reside at the cell surface (i.e., surfaceome) facilitates interactions with pathogens, binding of chemical messengers, and transmission of signaling cascades, and it is required for cell migration, adhesion, and survival. Surfaceome content, including protein identity and modifications, differs among cell types and is dynamic during development and disease states. For these reasons, and the fact that cell surface proteins are accessible, the surfaceome is a rich source of drug and immunotherapy targets and contains unique markers that can be used to identify cell types, disease states, and cellular phenotypes. Despite their critical functions in health and disease, cell surface proteins have historically been understudied in most cell and tissue types. This is due, in part, to the challenges posed by their relatively low abundance when compared to intracellular proteins, their hydrophobic nature, and the difficulty in biophysically purifying plasma membrane proteins without contamination from intracellular membrane components. Moreover, high-quality antibodies are currently available for a limited subset of cell surface proteins.

Considering these challenges, the development and dissemination of modern methods and technologies that enable the study of cell surface proteins will undoubtedly advance a broad range of research efforts, including our understanding of cellular differentiation and development, host-pathogen interactions, and metastatic processes, and will lead to the development of new treatments for disease. In this volume of Methods in Molecular Biology: The Surfacome, we have assembled 19 chapters that cover a variety of methods ranging from molecular and cellular biology to proteomics to bioinformatics. The overall aim of this edition is to provide state-of-the-art techniques and tools to assess the surfaceome content, modifications, and function. The volume does not include standard approaches extensively reviewed elsewhere, nor does it include methods to analyze lipids and glycans, which are key components of the plasma membrane and worthy of separate volumes dedicated to their study. While most of the methods described in this volume are generally applicable to any cell type, some chapters focus on specific cell types and/or specific molecule classes of interest. These latter chapters are designed to illustrate the application of these procedures and protocols in defined systems, but the approaches should be applicable across a broad range of cells. Altogether, we hope this collection of methods will facilitate the study of cell surface protein biology and function and lead to the discovery of new drug and immunotherapy targets for treating disease and new immunophenotyping markers for studying cellular function, differentiation, and disease. The chapters are arranged in four parts, beginning with discovery-based and then targeted strategies for cataloging surfaceome content, moving to functional assays for specific protein and cell types, and ending with computational approaches.

Part I focuses on discovery-based approaches for cataloging surfaceome content and includes methods to analyze the surfaceome of bacteria, avian embryos, and mammalian systems. Chapters in this part focus on modern proteomic methods that offer the ability to

specifically target cell surface proteins with limited interference from intracellular membrane proteins. These include surface membrane protein enrichment techniques, using proteases to "shave" proteins from the surface of bacteria to identify surface-exposed proteins, and exploiting the avian system to study developmental changes in cell surface proteins, including bioinformatics-based techniques to translate to human orthologs. Subsequent chapters describe the Cell Surface Capture Technology, a targeted analytical approach to specifically identify cell surface N-glycoproteins, the use of iron oxide nanoparticles to enrich plasma membrane proteins, and methods to profile secreted proteins and exosomes in cell culture, a topic that has recently gained attention across a variety of research disciplines.

Part II focuses on targeted approaches to analyze the surfaceome. The chapters in this part include methods to overexpress specific targets in Sf9 cells and an approach to generate bispecific antibodies that are valuable for targeting cancer and somatic cells. Also included is a tutorial chapter on flow cytometry and its application to immunophenotyping to assist novices in their pursuit of surface proteins. The last chapter in this part provides an example of how ELISA and flow cytometry are applied to detecting the G protein-coupled receptor CXCR4, a strategy particularly valuable for investigators interested in G proteins and in drug repurposing.

Part III focuses on cell-based functional analyses. This part begins with a review on voltage-dependent sodium channels and methods for high content electrophysiological analyses. Methods are then described for the evaluation of vascular endothelial cell functions and approaches to study signal transduction of surface receptor tyrosine kinase in neurons. A comprehensive analysis of cell polarity, using retinal pigmented epithelium as a model system, is then described, including techniques for immunostaining for apical and basolateral membrane markers, polarized cytokine secretion, fluid transport, phagocytosis, and identification of plasma membrane proteins through cell surface capturing technologies as described in the first part. This part finishes with a description of methods that take advantage of extracellular matrix components to capture mesenchymal stromal cells under flow, model disease states, and ultimately analyze cell-matrix interactions through the use of 3D microtissues.

Part IV focuses on computational approaches in surfaceome studies and describes a new web-based platform, Targets-search, that incorporates information from a variety of sources including the Cell Surface Protein Atlas and online drug databases, to facilitate identification of surface proteins that are informative for a particular cell type or disease and known drugs that interact with these proteins.

In closing, we would like to thank Springer for its support, dedication to this project, and patience in developing this book. We also wish to especially thank all of the authors for their time, energy, and valuable contributions. With their efforts, we have assembled what we hope will be a valuable resource for those research laboratories working to advance the study of surface protein biology.

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