Part I

Introduction and Background

1 Motivation and Outline

In simple terms a biological membrane consists of two layers of fat that slide on each other – hence membranes are called lipid bilayers. Biological membranes include more than just the cell membrane. Within the cell, there are several structures that are bound by membranes; these structures are called organelles and include the nucleus, mitochondria, endoplasmic reticulum, Gogli apparatus, and lysomes.

1.1 Why Membranes?

Why biological membranes? Membranes and the macromolecules embedded in them perform extremely important biological functions. They form selectively permeable barriers that enclose cells and organelles within the cell. The cell membrane is the target of physical, chemical, and biological agents such as thermal and mechanical stress, toxins, hormones, viruses, and microbes.

Biological membranes exhibit remarkable properties. Embedded in the membrane are protein macromolecules that perform crucial functions for a living cell. For example, ion channels are subnanosized pores formed out of proteins in the membrane that selectively open and close and allow ions to flow into the cell. It is known that almost 25 percent of genes code for membrane proteins. Also, more than 50 percent of available drugs target membrane proteins [369]. Cytoskeletal filaments and sterols, such as cholesterol, give structural stability to the membrane. Antimicrobial drugs bind to specific sites in the membrane of bacterial cells and induce pores in the membrane that compromise the integrity of the membrane and lead to bacterial cell death. Applying a voltage across a membrane causes the membrane to spontaneously form pores – this process is called electroporation and is crucial for drug delivery mechanisms. A membrane has several moving parts that comprise lipids and macromolecules that perform a variety of biological tasks. For example, the assembly of new cellular membranes commonly results from old membranes in which membrane-bound enzymes construct new lipid molecules. These new lipid molecules then either diffuse into the old membrane or form vesicles which can merge with other membranes via vesicle fusion. Vesicle fusion requires the coordination of several proteins and macromolecules as biological membranes do not spontaneously fuse. The process of vesicle fusion and cellular membrane assembly is still an active area of research.

Why artificial membranes? Given the importance and remarkable properties of biological membranes, there is strong motivation to build artificial membranes that mimic them. An artificial membrane allows us to target specific structures in biological membranes and study their properties to elucidate the structural and dynamic properties of cell membranes in a controlled environment. More importantly, one can build synthetic biological devices out of precisely engineered artificial membranes that achieve remarkable properties. For example, the moving parts of the artificial membrane can be precisely engineered to design a supersensitive biosensor that can detect as low as femtomolar concentrations of target molecules. Apart from the challenge of mimicking a biological membrane, another challenge encountered with artificial cell membranes is the bioelectronic interface. Charged ions carry information in biological membranes, whereas electrons carry information in electrical devices. So how should a bioelectronic interface be built to interface ions and electrons?

1.2 Guided Tour of the Book

This book provides a comprehensive treatment of how to engineer artificial membranes: their design, construction, experimental studies, and mathematical models. The book consists of three interrelated parts. The schematic organization and interdependencies of the three parts are illustrated in Figure 1.1.

Part I is an elementary primer on biochemistry for applied mathematics and engineering readers. The background is important for understanding how engineered artificial membranes are constructed and mathematically modeled. The description includes lipids, peptides and proteins, ion channels, chemical components of engineered artificial membranes, and the bioelectronic interface. Additionally, the relation between cell membranes and the types of engineered artificial membranes present in the literature is described.

Part II discusses methods for constructing engineered artificial membranes and devices built out of these engineered artificial membranes. In Chapter 4 the formation process of engineered artificial membranes constructed using the solvent-exchange technique is presented. Additionally, information is provided on how to insert peptide and protein macromolecules of interest, and how to grow cells on the membrane surface. Chapter 5 provides a comprehensive presentation of the ion-channel switch (ICS) biosensor which is composed of an engineered membrane and is designed to detect specific analyte species.[1] A schematic of the ICS biosensor is provided in Figure 1.2. The ICS biosensor is composed of millions of fully functioning nanomachines embedded in an engineered tethered membrane. The nanomachines are designed to bind with specific analyte species such as proteins, hormones, polypeptides, microorganisms, oligonucleotides, DNA segments, polymers, and viruses in cluttered electrolyte

[1] Throughout this book, the term *analyte* denotes the target molecules that we wish to detect. The analyte is typically administered in an electrolyte solution; this is called the analyte solution. We are interested in estimating the concentration of analyte in the solution.

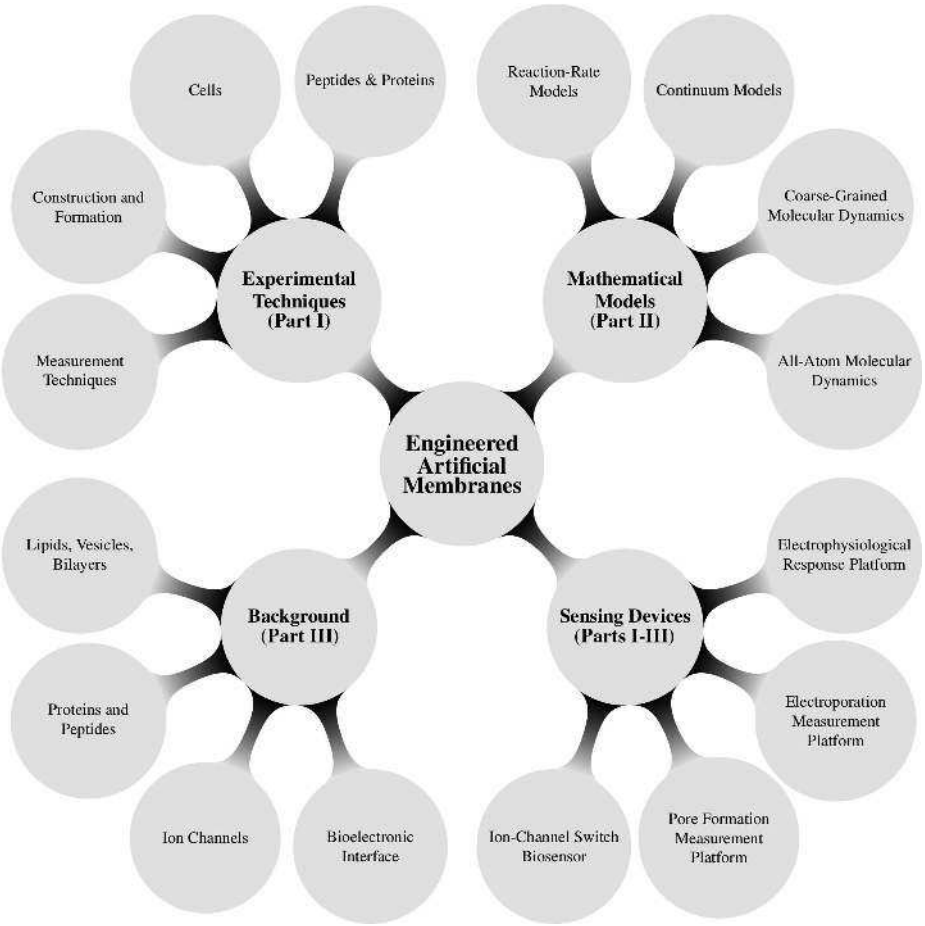

Figure 1.1 Concept chart of the topics presented in this book. Part I presents a biochemistry primer for engineered artificial membranes. Part II focuses on experimental techniques and Part III describes mathematical models of engineered artificial membranes. Parts II and III discuss the four sensing devices, namely, the ion-channel switch biosensor, the pore formation measurement platform, the electroporation measurement platform, and the electrophysiological response platform.

environments. If the analyte is present, these nanomachines will bind with the analyte, causing a decrease in the conductance of the membrane. At a macroscopic level, the conductance change allows the ICS biosensor to detect femtomolar (1×10^{-15} molar) concentrations of target analyte species. Chapter 6 presents the formation and operation of the physiochemical engineered membrane platforms (pore formation measurement platform [PFMP], electroporation measurement platform [EMP], and electrophysiological response platform [ERP]). Chapter 7 presents several experimental techniques for measuring the structural and functional properties of engineered membranes using electrochemical and optical techniques.

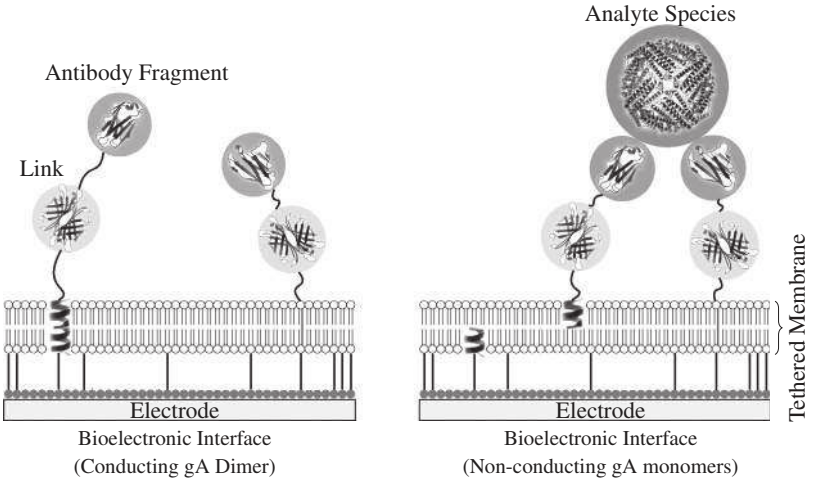

Figure 1.2 Schematic of the ion-channel switch (ICS) biosensor. The ICS biosensor is composed of mobile gramicidin (gA) monomers attached to antibody fragment receptors through a streptavidin-biotin linkage, tethered gA monomers, and tethered-membrane-spanning lipids which are connected to antibody fragment receptors through a streptavidin-biotin linkage. The antibody fragment receptor is designed to bind to a specific analyte species in the analyte solution. When the antibody fragment receptors bind with the analyte species, this causes the population of conducting gA dimers in the ICS biosensor to decrease, which results in an overall decrease in the conductance of the tethered membrane.

Part III deals with mathematical and computer simulation models of engineered membranes. The aim is to use dynamic models to relate the structure of an engineered membrane to its function. The mathematical and simulation models that we develop account for the moving components in membranes, the behavior of macromolecules in the membrane, transport phenomena in the analyte solution, and the bioelectronic interface. We use engineered membranes (hardware) in combination with mathematical models (software) to go from structure to function. The hardware (Part II) allows us to consider various components in their natural state (as they interact with other components), whereas the software (Part III) then zooms into specific subparts in isolation. This combination of hardware (ex vivo and in vitro) and software (in silico) is a unique feature of this book. Conceptually, Part II of the book can be viewed as a biomimetic hardware approximation to real-life membranes. Part III then constructs mathematical models for the devices in Part II and can be viewed as a second-level approximation to real-life membranes.

The models used in Part III include all-atom molecular dynamics, coarse-grained molecular dynamics, continuum theory, and reaction-rate theory. The time and spatial scales associated with each of these models are illustrated in Figure 1.3. These multi-time-scale models allow us to predict the response of engineered artificial membranes from the microscopic (molecular scale) to the macroscopic (experimentally measurable device) scale. Throughout Part III, discussion of the numerical methods used to evaluate all the models are provided to ensure that the reader can repeat all the

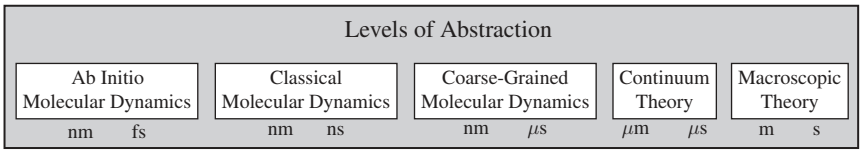

Figure 1.3 Time and spatial scales associated with engineered tethered-membrane models presented in this book.

computations in this book. Additionally, methods to visualize all-atom molecular dynamics and coarse-grained molecular dynamics models of engineered artificial membranes are also provided. Chapter 8 begins with a detailed study of the application of reaction-rate theory to study important membrane properties. This includes electrochemical impedance spectroscopy, the most widely used method for measuring membrane properties, and surface reaction dynamics for the interaction of molecules with the membrane surface. Chapter 9 introduces reaction-rate models for the ICS biosensor, and black-box models of the ICS biosensor. Chapter 10 provides continuum theories useful for modeling the advection-diffusion processes in ICS biosensors and the PFMP. These allow important biological parameters to be estimated such as analyte concentration and diffusion from the measured current response from the engineered membrane platforms. Chapter 11 presents a detailed analysis of the process of electroporation in engineered membranes. Additionally, using extensive experimental measurements, we illustrate how the dynamic models can be used to measure the dynamics of embedded ion channels and cells grown on the surface of the engineered membrane. Chapter 13 illustrates how the ERP with mesoscopic models can be used to estimate the electrophysiological response of ion channels and cells grown on the surface of engineered tethered membranes. Chapter 14 introduces how coarse-grained molecular dynamics can be used to compute important biological parameters of engineered membranes, and how these can be related to experimental measurements. Chapter 14 also presents a case study of how coarse-grained molecular dynamics together with a continuum model can be used to understand the effect of antimicrobial drugs on the membrane. At the most fundamental level is all-atom molecular dynamics. Chapter 15 introduces how molecular dynamics can be used to gain important insight into the statics and dynamics of engineered membranes. Molecular dynamics simulations are used to estimate essential parameters of artificial membranes, understand aqueous pore formation in membranes, and model switching mechanisms of gramicidin channels (which is a crucial part of the ICS biosensor). An interesting aspect of Chapters 14 and 15 is that they use molecular dynamics simulation to understand the structure-function relationship of synthetic biological devices; traditionally molecular dynamics has been used for studying biological molecules. Chapter 16 closes with a complete overview of the atom-to-device model of engineered tethered membranes constructed in Part III of this book.

Case studies. This book contains several important experimental and modeling case studies that provide a wealth of insight on how artificial cell membranes operate. For example, using measurements from the ICS and dynamic models, we estimate the concentration of the molecule species streptavidin thyroid stimulating hormone, ferritin,

and human chorionic gonadotrophin in solution and whole blood. Using experimental results from the PFMP we study the pore-formation dynamics of the protein toxin α-hemolysin [397] from *Staphylococcus aureus*, and the pore-formation dynamics of the antimicrobial peptide peptidyl-glycylleucine-carboxyamide (PGLa). Using results from the EMP important insights are gained into the effect the bioelectronic interface, archaebacterial, *Escherichia coli*, and *Saccharomyces cerevisiae* lipids, electrolyte concentration, and cholesterol content have on the process of electroporation. Using the dynamics and experimental measurements from the ERP we study the electrophysiological response of the voltage-gated sodium ion channel, and skeletal myoblast cells.

Finally, this book has three appendices. Appendix A is a short elementary review of partial differential equations (including construction of nondimensionalized models) that are used to model membranes. Appendix B provides details for performing coarse-grained molecular dynamics and all-atom molecular dynamics simulations of engineered artificial membranes. Appendix C provides numerical methods for solving the continuum models, including the physical constants, of engineered artificial membranes presented in the book.

Color Figures. High-resolution colored figures for the book can be downloaded from the website of the book at www.cambridge.org/engineered-artificial-membranes