Preface

The importance of membrane proteins is clear from their prevalence in the genome (*ca.* 25% of genes encode membrane proteins[1]). They also constitute ~50% of current drug targets, and have key roles in a wide range of cellular functions, including transport, signalling and cell–cell interactions. Despite their physiological and pharmaceutical importance, membrane proteins constitute <1% of known protein structures. Thus, there are currently less than 200 distinct high-resolution membrane protein structures, of which just over half consist of bundles of hydrophobic transmembrane (TM) α-helices. For membrane proteins, interaction with lipids is essential for protein function; bilayer properties, such as hydrophobic thickness or lipid composition, can affect membrane protein activity.[2] Although often crystallized as membrane protein–detergent complexes, in most cases only a few tightly bound lipid molecules remain.[3] Thus, the crystal structure rarely contains explicit information on where the protein is located in the bilayer.

In addition to the proteins in membranes, the lipid bilayer environment is a complex two-dimensional liquid crystalline system. Furthermore, it is difficult to map details of protein–membrane interactions using experimental techniques. This makes biomembranes an ideal subject for computer simulations. However, due to the size of most membrane proteins and the simulation timescales involved, it is only recently that simulations have enabled prediction of biological properties to be made. Atomistic-detail (AT) approaches still generally fall short of timescales for, *e.g.*, helix aggregation/folding, but recent, more extended simulations have revealed aspects of functionally relevant local motions and of interactions with lipid molecules. New developments in coarse-grained (CG) simulations potentially can probe multi-microsecond dynamics of extremely large systems. Furthermore, the combination of CG and AT simulations in multi-scale approaches offers the possibility of combining efficient sampling with accurate modelling of key local interactions.

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This book reviews recent progress in simulations of biomembranes, ranging from fundamental methodologies and advances in modelling of lipid bilayers through to applications to a number of transport and signalling proteins.

In Chapter 1, Peter Tieleman provides an introduction to the methodologies and parameters that are commonly used in molecular dynamics simulations of biomembranes. He begins by discussing force fields that are commonly used and how parameters are developed before going on to highlight some of the problems that exist in trying to simulate a greater variety of compounds and their interaction with the membrane. A central issue to performing simulations of membrane proteins embedded within a lipid bilayer is the initial setup. The various options for this are discussed, followed by what is probably the biggest problem not only for membrane simulations, but also for molecular dynamics (MD) simulations in general: the sampling problem. Although there are problems, we can also be reassured that there are several methods available that can improve sampling to the point that the results are both reliable and reproducible. The chapter then focuses on three main methodological areas that are of particular importance for membrane simulations: pressure coupling, electrostatics and periodicity. In fact, a lot of the parameter choices made for pressure coupling and electrostatics stem from the fact that the membrane is nearly always treated as a periodic system. Finally, the chapter ends with an indication of the future developments in this area, including continued work in force field development. For example, the inclusion of polarizable lipids is something that is going to appear with increasing frequency.

Chapter 2, by Ollila and Vattulainen, illustrates very nicely how detailed application of the principles laid out in Chapter 1 can provide detailed information, some of which is often not accessible very easily by experimental techniques. They focus in particular on the lateral pressure profiles that exist within biomembranes and discuss how they can be influenced by various factors, including lipid composition, the presence of sterols and anaesthetics. They also demonstrate that the lateral pressure profile exhibits large variations as one moves along the bilayer normal and that as a consequence it is not surprising that membrane proteins, such as the mechanosensitive channel (MscL), are able to exploit this to great mechanistic effect. Although much of the work on lateral pressure profiles has been performed with atomistic molecular dynamics, Ollila and Vattulainen also discuss contributions from coarse-grained molecular dynamics simulations which, provided that care is taken with respect to the parameterization, offer the advantage of larger systems being run for longer periods of time.

Coarse-grained simulations are also making an increasing impact in molecular dynamics simulations of membrane proteins and peptides. The focus of Chapter 3, by Rouse, Carpenter and Sansom, is to provide a brief introduction to the general methodology and to highlight some recent test cases. In particular, it addresses how CG simulations may be used to explore (i) the interactions of α-helices with a lipid bilayer and (ii) the interactions of transmembrane α-helices with one another within a lipid bilayer. The latter is of relevance both

to modelling of membrane protein folding and to signalling across membranes by changes in helix oligomerization and/or packing.

In Chapter 4, Orsi and Essex provide an overview of how atomistic and coarse-grained simulations have contributed to our understanding of passive permeation of small molecules and drugs. Such information is of enormous benefit to the pharmaceutical industry, where advance knowledge of the permeation properties of a candidate drug molecule can potentially make huge savings by avoiding the synthesis of candidate molecules that have bad permeation characteristics. After describing some of the main experimental techniques and various solubility–diffusion models, the authors describe how the '*z*-constraint method' in particular has been used to good effect to calculate permeability coefficients of small molecules. The chapter also discusses a relatively new area of interest, that of nanomaterials, such as fullerenes, and their interaction with membranes, before concluding that computational power is now at a point where significant progress in this area can be expected.

In Chapter 5, Martin and Jakob Ulmschneider provide a comprehensive discussion of the progress made in using implicit membrane models to study peptide folding. Implicit models have the advantage that sampling times can be significantly expanded compared with atomistic representations. Indeed, for the well-studied model peptide WALP, they argue that by most commonly used metrics used to evaluate conformational exploration, the speed-up is somewhere between a factor of 50 and 100. Although there are certain limitations to the implicit model (for example, the lack of bilayer deformation), the authors demonstrate that these models can be extremely useful for addressing certain questions and can match experimentally derived data very well indeed. Although currently limited to small peptides, there is no doubt that future developments in this area will ultimately allow us to make progress in *ab initio* predictions of membrane proteins.

In Chapter 6, Yin, Arkhipov and Schulten provide an excellent example of the application of a multi-scale approach. Through a systematic study of the interaction of BAR domains with membranes, we are guided through four different levels of treatment, starting from atomistic molecular dynamics and all the way up to a continuum representation based on elastic theory. Some of their findings could only really have been obtained by using this approach due to the sheer size and timescale of these systems. For example, it appears that the lattice arrangements of these BAR domains is critical in determining the end result in terms of membrane curvature. The authors allude to the fact that multi-scale approaches will be an increasing necessity if we are to make progress properly in the growing field of systems biology.

Ion channels are an important class of membrane proteins, from a both a biological and a pharmacological perspective, and have been the focus of a wide range of computation studies. In Chapter 7, Robertson, Jogini and Roux describe how continuum electrostatics calculations can be used to provide valuable insights into the relationship between structure and biological function in potassium channels. These studies focus on a careful treatment of electrostatics both within the transmembrane region and also in those regions

of some K^+ channel protein pores which project beyond the lipid bilayer. They also discuss how the voltage difference across a biological membrane results in a field which is focused on the voltage-sensing elements of Kv channels.

Apparently related to potassium channels, at least in the transmembrane domain, are the ionotropic glutamate receptors. This family of receptors mediates nearly all of the fast synaptic neurotransmission in the brain and consequently is of interest in terms of both understanding memory and learning, and also the pathology of many neurological disorders and conditions. In Chapter 8, Vijayan, Iorga and Biggin discuss how computational methods have contributed to our understanding of this important class of proteins. In addition to summarizing the work performed so far, they indicate the areas for future study. In particular, the recent tetrameric structure opens up the field to a whole range of questions.

In Chapter 9, we move to the outer membrane of Gram negative bacteria as Khalid and Baaden describe how molecular dynamics simulations have contributed to our understanding of beta barrel proteins. Even in the context of so-called simple barrels, MD studies (on OmpA) have shown that underlying motions may play a prominent role in controlling the nature of the pattern of water flux through these channels *via* modulation of conformation of the salt bridges. This once again highlights the intimate nature of the relationship between structure, dynamics and function, as inferred by Ollila and Vattulainen in Chapter 2. Khalid and Baaden also draw out attention to the fact that despite possessing relatively simple architecture, some of these proteins appear to be more than just holes in the membrane. Autotransporters exploit a barrel (formed by a C-terminal domain within the autotransporter itself) to secrete themselves out of the cell. The precise mechanism of this process is still unclear and it is likely that MD will help to improve models of secretion for these proteins. It also appears that the barrel proteins serve as suitable scaffolds for common enzymatic classes such as phospholipases (OMPLAA) and proteases (OmpT). Often the key residues for these reactions are located on loops which are presumably highly flexible and particularly prone to lattice forces in X-ray crystallography. Thus MD can offer a complementary approach to the study of these proteins. The chapter ends with a discussion of how barrel proteins are also being increasingly exploited in technological applications such as biosensors. One approach is to recognize analytes by the 'signature' single-channel signal they give. MD simulations can be used to study the key interactions and to predict, *in silico*, mutations that might lead to better discriminatory power.

Chapter 10 by Emad Tajkhorshid and co-workers illustrates how large increases in computer power and recent progress in membrane protein X-ray crystallography can give insight into the mechanisms of active transport. Using large-scale molecular dynamics simulations, they have shown that although simulation of the complete cycle of a membrane transporter is still some way off, these calculations can be used to capture successfully many of the key steps involved in active transport. This approach is particularly complementary to the study of these systems where experiments are still difficult to design in the absence of structural knowledge of the full pathway of translocation.

Simulations can now be used to explore the possible exploitation of biomembranes in bionanotechnology. One such application is described in Chapter 11, where Wallace and Sansom review the use of both atomistic and coarse-grained simulations to explore the interactions between carbon nanotubes (CNTs) and model biomembranes. Issues of parameterization of CNTs for simulations are of special importance, and are likely to be an area of future refinement of methodologies. Simulations have been used to characterize the interactions of CNTs with detergent and lipid molecules, and also with simple lipid bilayers. Once embedded within a bilayer, CNTs may form transbilayer pores. Simulations have been used to explore the behaviour of water and ions in such pores, and to explore their potential as 'nanosyringes' for injection across cell membranes.

In summary, the studies reviewed in this book show that MD simulations and related computational studies are now a mainstream component of studying the biophysics and function of membranes and their proteins. The evidence for this is provided in part by the increasing use of simulation studies to aid the functional analysis of membrane protein structures, *e.g.* in characterizing the specificity mechanisms of transmembrane pores.[4]

From a simulation perspective, it is evident that multi-scale simulation approaches will become increasingly important, carefully matching the granularity of the simulation to that of the biological problem being examined. We will see the continued importance of methodological developments for membrane simulations, and of especial importance will be careful parameterization of a wider range of lipids. Given these developments, simulation studies in the future will address a number of key problems in membrane biology (*e.g.* transport, signalling, fusion) with increasing emphasis on improved biological realism and complexity. In this fashion, membrane simulations will have a major impact on fundamental biomedical science (*i.e.* mechanistic understanding of membrane cell biology), and on more 'applied' areas such as pharmacology and bionanotechnology of membranes.

References

1. J. Nilsson, B. Persson and G. von Heijne, *Proteins Struct. Funct. Bioinf.*, 2005, **60**, 606.
2. C. Hunte, *Biochem. Soc. Trans.*, 2005, **33**, 938.
3. H. Palsdottir and C. Hunte, *Biochim. Biophys. Acta*, 2004, **1666**, 2.
4. J. S. Hub and B. L. de Groot, *Proc. Natl. Acad. Sci. USA*, 2008, **105**, 1198.

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