Simulations of outer membrane channels and their permeability

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ABSTRACT

Channels in the outer membrane of Gram-negative bacteria provide essential pathways for the controlled and unidirectional transport of ions, nutrients and metabolites into the cell. At the same time the outer membrane serves as a physical barrier for the penetration of noxious substances such as antibiotics into the bacteria. Most antibiotics have to pass through these membrane channels to either reach cytoplasmic bound targets or to further cross the hydrophobic inner membrane. Considering the pharmaceutical significance of antibiotics, understanding the functional role and mechanism of these channels is of fundamental importance in developing strategies to design new drugs with enhanced permeation abilities. Due to the biological complexity of membrane channels and experimental limitations, computer simulations have proven to be a powerful tool to investigate the structure, dynamics and interactions of membrane channels. Considerable progress has been made in computer simulations of membrane channels during the last decade. The goal of this review is to provide an overview of the computational techniques and their roles in modeling the transport across outer membrane channels. A special emphasis is put on all-atom molecular dynamics simulations employed to better understand the transport of molecules. Moreover, recent molecular simulations of ion, substrate and antibiotics translocation through membrane pores are briefly summarized.

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1. Introduction

The use of molecular dynamics (MD) simulations based on Newton's second law has become an established research approach in molecular biology. MD simulations have proven to be an extremely valuable tool for understanding the function of membrane channels at the molecular level. In 1981, a first MD simulation of a membrane channel was reported [1]. Since then, MD simulations have, for example, been applied to the study of ion channels [2,3,4], transporters [5,6] and other membrane channels [7]. In recent years, this field has attracted an enormous interest in modeling and understanding the common priniciples underlying in the function of these proteins [8,9]. In the future such comprehensive knowledge at the molecular level will certainly lead to a vast amount of applications in biotechnology and pharmaceutical research.

One particularly important class of membrane channels is the class of outer membrane (OM) channels present in Gram-negative bacteria. These pores consist of rather rigid β-barrel sheets which form channels to selectively regulate the entry and exit of nutrients, substrates and xenobiotics in and out of the bacterial cell. In Fig. 1 OM proteins of various sizes from Gram-negative bacteria are shown. Functionally, they can be classified as non-specific diffusion channels or porins, e.g., OmpF, specific channels, e.g., NanC, CymA and OprD, active transporters, e.g., BtuB, and OM domains of efflux channels, e.g., TolC. In this review we limited our discussion to porins and specific channels which mainly regulate the entry of substances into the periplasm of bacteria. It is believed that most antibiotics and in particular small hydrophilic compounds penetrate the OM through these channels to reach the cytoplasmic targets [11]. From the pharmaceutical perspective, these membrane channels are crucial for drug delivery and their importance is highlighted by the susceptibility of the respective microorganism to antibiotics. Understanding the molecular mechanism(s) behind the selective permeability of substrates and antibiotics is of great medical importance to combat antibiotic resistance. Such understanding at the molecular level will prove valuable in developing strategies for rational design of new antibiotics [12,13].

Over the years, various techniques have been successfully employed to understand the structural and functional properties of bacterial OM channels [14] such as atomic-force microscopy [15], bilayer electrophysiological measurements [16,17], NMR [18,19], mass spectroscopy [20], X-ray crystallography [21,22] and MD simulations [23–25]. Yet, it is difficult to identify the key factors involved in the substrate preference, and the interactions of the pores with antibiotics are poorly understood due to their complexity and experimental limitations. Most importantly, at the single molecule level electrophysiology experiments have proven to be an important tool for characterizing the biophysical properties of these channels. This technique has been widely applied to the study of ion, substrates and antibiotics transport across these channels [26–30]. On the other hand, X-ray crystallography provides a wealth of structural information about the bacterial OM channels and their interactions with substrates and antibiotics [29–31]. All-atom MD simulations have the advantage of connecting the static picture of
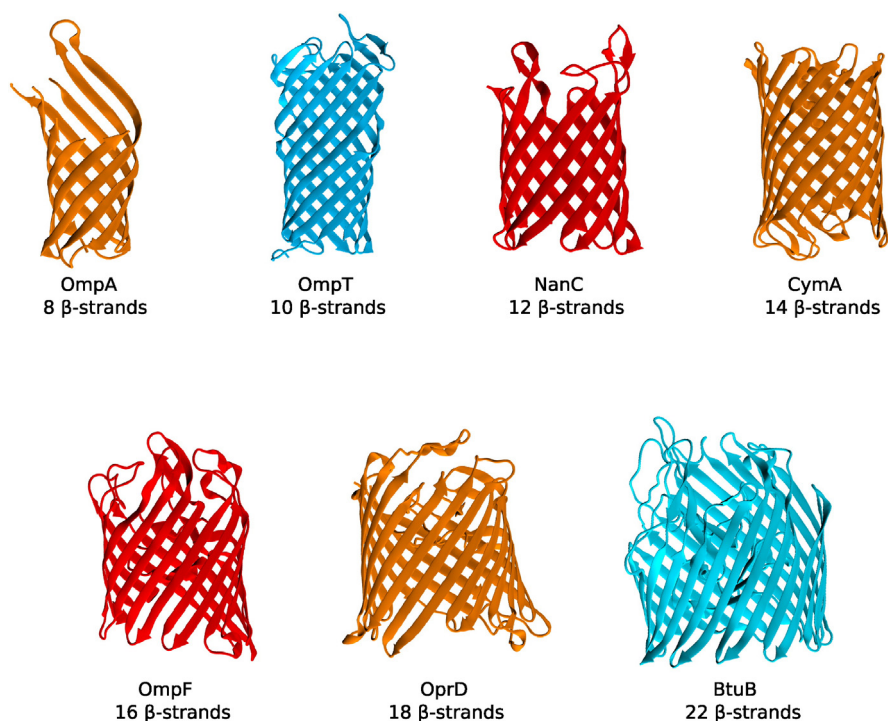
Fig. 1. Outer membrane channels of Gram-negative bacteria with different sizes ranging from 8 β-strands to 22 β-strands. The channels shown are OmpA (pdb code: 1QJP), OmpT (pdb code: 1I78), NanC (pdb code: 2WJQ), OmpF (pdb code: 2ZFG) and BtuB (pdb code: 2GSK) from *E. coli*; CymA (pdb code: 4V3G) from *K. oxytoca*; OrpD (pdb code: 3SY7) from *P. aeruginosa*. The figure was generated using VMD [10].

crystallography with the kinetic information obtained from electrophysiological experiments. The combined approach of X-ray crystallography, electrophysiology and computational experiments has already allowed for an improved understanding of OM channel permeation features at the molecular level and their success is highlighted, for example, in recent publications [30,32–34]. However, the importance of such findings at the single molecule level in non-native environments have to be tested in a native cellular environment where multiple factors are involved and work together leading to the complex process of membrane permeation.

What can be learned from simulations of OM channels? Computer experiments of membrane pores can simplify the picture of complex details involved in the transport of molecules which are missing from experiments and offer a possible explanation to experimental findings. To this extend, molecular simulations are sometimes coined as "computational microscope" [35]. Modeling of OM channels can be performed with the aim to study topics such as (i) ion transport and kinetic properties, (ii) the role of binding sites, constriction regions or selectivity filters located inside the channels, (iii) structural features important for substrate preference or selectivity of the channel, (iv) quantification of the interactions between proteins, water molecules, ions or other solute molecules, (v) the rate-limiting steps involved in the permeation of molecules, (vi) the energetics and kinetics of antibiotic permeation, (vii) the dynamics of channels in different environments, (viii) effects of extracellular or lumen loops (gating-like behavior) on transport and (ix) gross physicochemical properties required for the permeation of molecules. In the past, several groups employed MD simulations to address the above issues in OM channels such as OmpG [36], OmpA [37], OmpF [38–40], OmpC [41,42], FecA [43], PorB [44]. OprP [33,45–47], OprO [32], OprD [48–50] and CarO [51]. The current state of the art includes simulations of OM channels with complex lipopolysacchardies (LPS)-phospholipid membrane environment [52–54] and microsecond time scales [50]. However, till now, most simulation studies have focused on the transport of ions [55] and less have tried [24,28,30,34] to understand substrate translocation events that are important for the physiological function of most bacterial membrane channels. As a

starting point, it is a sensible approach to investigate ion transport across these channels to apprehend the fundamental principles assisting or hindering transport. Due to the availability of computational power, new numerical algorithms, molecular structures of different bacterial channels as well as microbiological data on the uptake of substrates and antibiotics, it is, however, now very timely to model and unravel the structural elements involved in the uptake of more complex substrates.

This article tries to provide an overview of simulations aimed at bacterial OM channels with an emphasis on the transport of bulky molecules. In a first step, we review the theoretical and computational methods widely used to investigate the structure, dynamics and function of OM channels. Moreover, we focus on the usage of all-atom MD simulations with a special emphasis is placed on potential applications and practical limitations of the methods. Subsequently, we briefly discuss issues of computational system preparation. In a third step, we then discuss simulation studies in this area and highlight how in most cases modeling is used to complement experimental measurements and to improve the understanding of the channel functionality. In a further step, we recap simulations performed to investigate the transport of antibiotics through OM channels using free-energy calculations. Finally, the importance of further developments in this field to design virtual screening methods and their part in rational drug discovery is briefly addressed.

2. Simulation approaches

Among the large number of computational methods to study the membrane channels, most of them can be divided into three different categories [55]: at the continuum level, the Poisson-Nernst-Planck model [56–60]; Brownian Dynamics (BD) [61–63], in which the environment is typically represented by continuum parameters; and all-atom molecular dynamics (MD) [64–66] which is a fully microscopic description with all atoms treated explicitly. Furthermore, to some extend these methods can be combined with each other so some limitations associated with a particular approach can be overcome. In this