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Living on the edge: Simulations of bacterial outer-membrane proteins

Anna Pavlova [a], Hyea Hwang [b], Karl Lundquist [a], Curtis Balusek [a], James C. Gumbart [a,*]

[a] School of Physics, Georgia Institute of Technology, Atlanta, GA 30332, United States
[b] School of Materials Science and Engineering, Georgia Institute of Technology, Atlanta, GA 30332, United States

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ABSTRACT

Gram-negative bacteria are distinguished in part by a second, outer membrane surrounding them. This membrane is distinct from others, possessing an outer leaflet composed not of typical phospholipids but rather large, highly charged molecules known as lipopolysaccharides. Therefore, modeling the structure and dynamics of proteins embedded in the outer membrane requires careful consideration of their native environment. In this review, we examine how simulations of such outer-membrane proteins have evolved over the last two decades, culminating most recently in detailed, highly accurate atomistic models of the outer membrane. We also draw attention to how the simulations have coupled with experiments to produce novel insights unattainable through a single approach. This article is part of a Special Issue entitled: Membrane Proteins edited by J.C. Gumbart and Sergei Noskov.

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1. Introduction

Membrane proteins present a number of unique challenges when attempting to study them using molecular dynamics (MD) simulation techniques. First and foremost is the proper accounting of their native environment. Membranes themselves have a number of properties that make them difficult to model accurately such as complex phase behavior, high heterogeneity, and sensitive electrostatics [1]. Nonetheless, the significant insights to be gained from simulating membrane proteins meant that the simulations would be attempted regardless of any required approximations, evidenced by their long history [2–8]. In the last few years, numerous advancements in lipid force fields have led to simulations that can match their experimental counterparts to a high degree of accuracy [9–12].

Although membrane-protein simulations have become routine, the vast majority are of α-helical integral membrane proteins, with structures of β-barrel proteins comprising less than 20% of all known membrane-protein structures (estimated from Membrane Proteins of Known 3D Structure database; http://blanco.biomol.uci.edu/mpstruc/). β-barrel membrane proteins are almost exclusively found in the outer membrane (OM) of Gram-negative bacteria. One likely reason for their structure is to evade recognition by the Sec apparatus when crossing the inner membrane [13], thus avoiding premature insertion; additionally, β barrels are extremely stable by comparison to other membrane proteins [14].

It should not be too surprising that the OM engenders an entirely different form of protein as it is unlike any other membrane. While it has typical phospholipids on its inner leaflet, the outer leaflet is composed of lipopolysaccharides (LPS). LPS have a core lipid A, a lipid-like molecule with (usually) six acyl chains joined through glucosamine sugars, followed by both a core oligosaccharide region and then a variable length polysaccharide termed the O-antigen [15]. Functional differences between the OM and typical membranes include an incredibly low diffusion rate (100× less [16]), a smaller hydrophobic thickness, and significant number of bound divalent, Ca^{2+} and Mg^{2+}, ions at the outer leaflet, due to a large number of phosphate groups.

In this paper, we review simulations of OM proteins (OMP), highlighting those that have taken into account the native OM environment, as well as simulations of the OM itself. We focus on a few classes of proteins, namely gated channels that import substrates, secretion systems that export substrates, and proteins involved in OM assembly (see Fig. 1). Although porins (both specific and non-specific) comprise a large fraction of OM proteins, they are reviewed in detail in this issue [17] and are only briefly touched upon here. Additionally, a more general review of bacterial-membrane-protein (both inner and outer membrane) simulations has been published recently [18].

2. Pure OM

The OM serves as a molecular sieve that permits the passage of small hydrophilic molecules and excludes potentially harmful enzymes or other large molecules. This sieving property of the OM is due to the presence of a few major proteins called porins. The atomic structure of the first bacterial porin from *Rhodobacter capsulatus* was elucidated in 1991 by Weiss et al. [19] and the dynamics of porins were first explored using MD simulations in 1994 by Björkstén et al. [20]. However, the solvent molecules were only incorporated implicitly in early studies

* Corresponding author.
E-mail address: gumbart@physics.gatech.edu (J.C. Gumbart).

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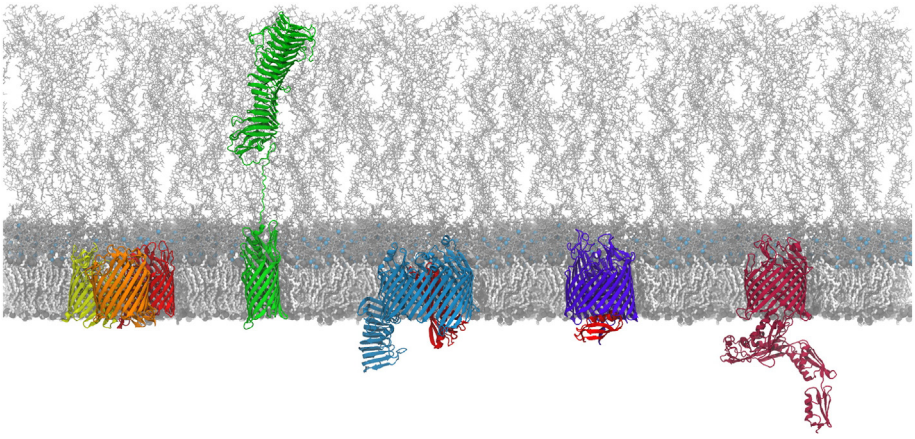

Fig. 1. Exemplary membrane proteins against an OM background. The hydrophobic core of the membrane is shown in white, the oligosaccharide region in dark grey with associated divalent ions in blue, and the O-antigen region above in light gray. From left to right are an OmpF trimer (PDB 2ZFG), the full-length autotransporter pertactin (modeled), LptD/E (PDB 4Q35), BtuB with the C-terminal part of TonB (red) attached (PDB 2GSK), and BamA (PDB 4K3B).

[20–22]. Later MD simulations of porins became more realistic because they were performed with explicit ions and solvent molecules, although they still used a symmetric phospholipid bilayer membrane [23–25].

The OM possesses a strictly asymmetric nature with respect to its lipid composition by having LPS in the outer leaflet and a mixture of phospholipids in the inner leaflet. The emergence of models that incorporate the heterogeneity and asymmetry of the lipid components has been one of the most important aspects of bacterial membrane study [26]. The electrostatic properties of lipid membranes are of profound importance as they are directly associated with membrane potential and, consequently, with numerous membrane-mediated biological processes such as voltage-dependent gating of porin channels and binding of charged molecules [27]. Fig. 2 shows the electrostatic potential maps and profiles of symmetric and asymmetric membranes calculated from MD simulation by the authors. The symmetric-bilayer system consists of 75% POPE and 25% POPG, and the asymmetric-bilayer system has an inner leaflet of POPE lipids and an outer leaflet composed of LPS molecules. The simulations were performed over a period of 100 ns for each system with the molecular dynamics package NAMD 2.9 [28] and the CHARMM36 force field [9], using the last 70 ns for analysis. The potential difference between bulk water and the membrane was calculated as 705 mV and 722 mV for symmetric and asymmetric membranes, respectively, showing good agreement with a recent MD study on a symmetric DPPC bilayer, which found a difference of 0.7 V [29]. The major differences in the electrostatic potential between the LPS-containing and phospholipid membranes are primarily due to differences in their outer leaflet composition; the LPS-containing membrane has a broad peak in the middle (acyl chains) and an additional peak for the outer core of the LPS molecule. The negatively charged head groups of the LPS are neutralized by bound Ca^{2+} ions (see Fig. 2C). The differences emphasize the importance of the accurate modeling of OM, because incorporating the electrostatic asymmetry of the bacterial OM will alter the forces involved in membrane transport phenomena, affecting, e.g., the development of novel antibacterial drugs.

Along with refinements in biological detail, force fields released in the last decade enabled development of a new generation of LPS-containing OM models. The first MD simulation work to incorporate the asymmetry of the bacterial OM in *Pseudomonas aeruginosa*, reported by Straatsma et al. [30], was performed with the AMBER96 [31] and the GLYCAM93 [32] force fields to represent the lipid and carbohydrate molecules, respectively. These force field parameters were tested in several simulations of the rough (containing O-antigen) and smooth (lacking O-antigen) LPS membranes in *P. aeruginosa* [33,34] and in *Escherichia coli* [35]. The simulations of rough LPS membrane of *P. aeruginosa* that used an updated GLYCAM06 force field showed good agreement with neutron diffraction measurements [36]. In addition, GROMOS force field parameters for the Lipid A portion of LPS from *P. aeruginosa* as well as LPS (LPS + inner core) from *E. coli* were developed and used in several studies [37,38]. Piggot et al. [38] performed MD simulation of electroporation of two complex membranes, from Gram-negative (*E. coli*) and Gram-positive (*Staphylococcus aureus*) bacteria, using the GROMOS53A6 force field [39]. Although Piggot et al. successfully retained the chemical complexity of membranes by considering the details of their lipidic components, the order parameters obtained for their membranes were higher than the experimental values [38,40].

More recently, the LPS in *E. coli* was parameterized using the CHARMM36 force field [9] by Wu et al. [41,42], and their resulting structure agreed well with that from simulations using GLYCAM06 force field [36]. The results from Wu et al. also showed a better agreement with experimental DMPC order parameters [40] compared to those using GROMOS53A6 force field [39]. Wu et al. performed MD simulations of

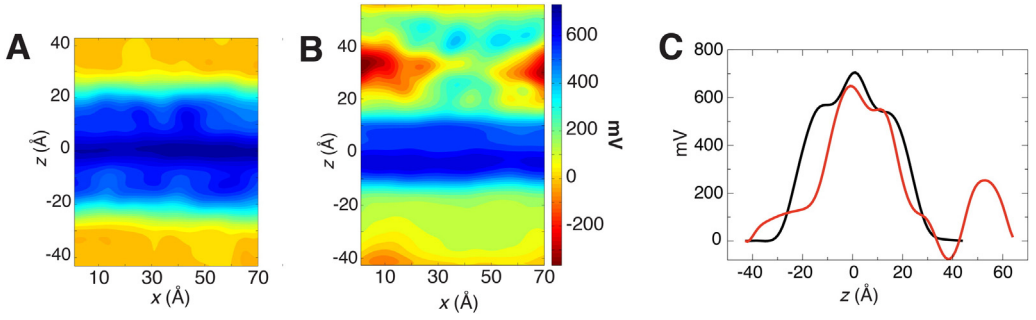

Fig. 2. Membrane electrostatic potential. (A,B) Electrostatic potential mapped onto a two-dimensional slice along the xy plane of (A) a symmetric phospholipid membrane and (B) an asymmetric LPS-containing outer membrane. (C) Electrostatic potential profile along the z axis (black: symmetric phospholipid membrane, red: asymmetric OM).

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