

# Modeling and simulation of bacterial outer membranes and interactions with membrane proteins

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The outer membrane (OM) of Gram-negative bacteria is composed of phospholipids in the periplasmic leaflet and lipopolysaccharides (LPS) in the external leaflet, along with  $\beta$ -barrel OM proteins (OMPs) and lipidated periplasmic lipoproteins. As a defensive barrier to toxic compounds, an LPS molecule has high antigenic diversity and unique combination of OM-anchored lipid A with core oligosaccharides and O-antigen polysaccharides, creating dynamic protein–LPS and LPS–LPS interactions. Here, we review recent efforts on modeling and simulation of native-like bacterial OMs to explore structures, dynamics, and interactions of different OM components and their roles in transportation of ions, substrates, and antibiotics across the OM and accessibility of monoclonal antibodies (mAbs) to surface epitopes. Simulation studies attempting to provide insight into the structural basis for LPS transport and OMP insertion in the bacterial OM are also highlighted.

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LPS is vital for both the structural and functional integrity of Gram-negative bacteria. It is composed of a phylogenetically conserved lipid A, a (inner and outer) core oligosaccharide, and highly diverse O-antigen polysaccharides with various lengths of repeating units that determine bacteria's antigenic diversity [5,6]. Most widely studied *Escherichia coli* OM has LPS molecules covering nearly three quarters of the OM surface per cell [7]. The bacterial growth conditions influence the phenotypic expression of surface determinants such as rough (absence of O-antigen polysaccharides) to smooth LPS (presence of O-antigen polysaccharides) [4]. For example, *E. coli* is reported to have five known core structures (R1-R4 and K12) and more than 180 O-antigen serotypes (Figure 1) [8\*]. Clearly, the heterogeneity of core structures as well as O-antigen unit length and sequence diversity can create dynamic protein–LPS and LPS–LPS interactions. Understanding these interactions at the molecular level can provide insight into how bacteria restrict antibodies' access and increase antibiotic resistance, as well as how the permeability of the OM is affected even in different serological groups of the same species [9,10].

Advances in modeling and simulation of complex membrane models along with increasing computational resources and growing experimental data have made it possible to explore structural properties and dynamics of different OM components and their roles in transportation of ions, substrates, and antibiotics across the OM, as well as importance of protein–LPS interactions in mAb accessibility [11–13]. In this review, we highlight such modeling and simulation efforts together with computational studies of LPS transport and OMP insertion in the bacterial OM.

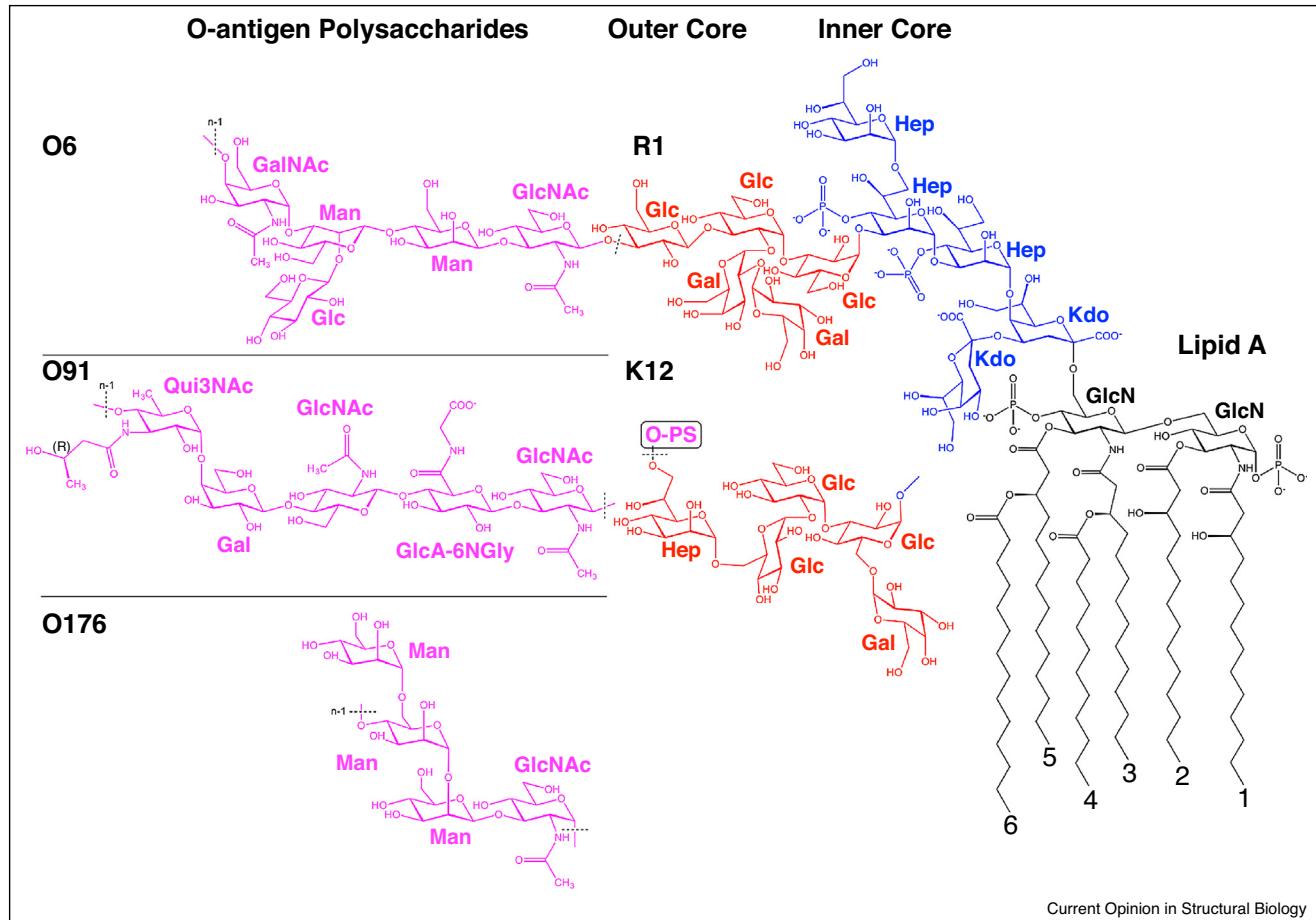
## Structure, dynamics, and flexibility of LPS in the OM

Characterizing LPS structure and dynamics as well as physicochemical properties of the OM is crucial for understanding its biological roles, including the agonistic/antagonistic action on the host innate immune response. Lipid A, also known as endotoxin or pathogen associated molecular patterns, carries toxic properties of LPS and acts as a potent activator of the host innate immune system mainly via the TLR4/MD-2 receptor complex [14]. Lipid A possesses an archetypal structure of a  $\beta$ -(1 → 6)-linked d-GlcN disaccharide (Figure 1) that is acylated with four to eight fatty acids of different lengths, and there exist complex chemical substitutions

## Introduction

The Gram-negative bacterial OM is unique in biology with a well-pronounced asymmetric bilayer with LPS in the outer leaflet and phospholipids in the inner leaflet [1,2]. Exchange of molecular species across the OM is ensured by various  $\beta$ -barrel OMPs and periplasmic lipoproteins. The OM separates the periplasm from the external environment and functions as a selective barrier that prevents the entry of toxic molecules such as antibiotics and bile salts into the bacteria, which is crucial for survival of bacteria in diverse and hostile environments, but causes significant threats to the public health [3,4].

Figure 1



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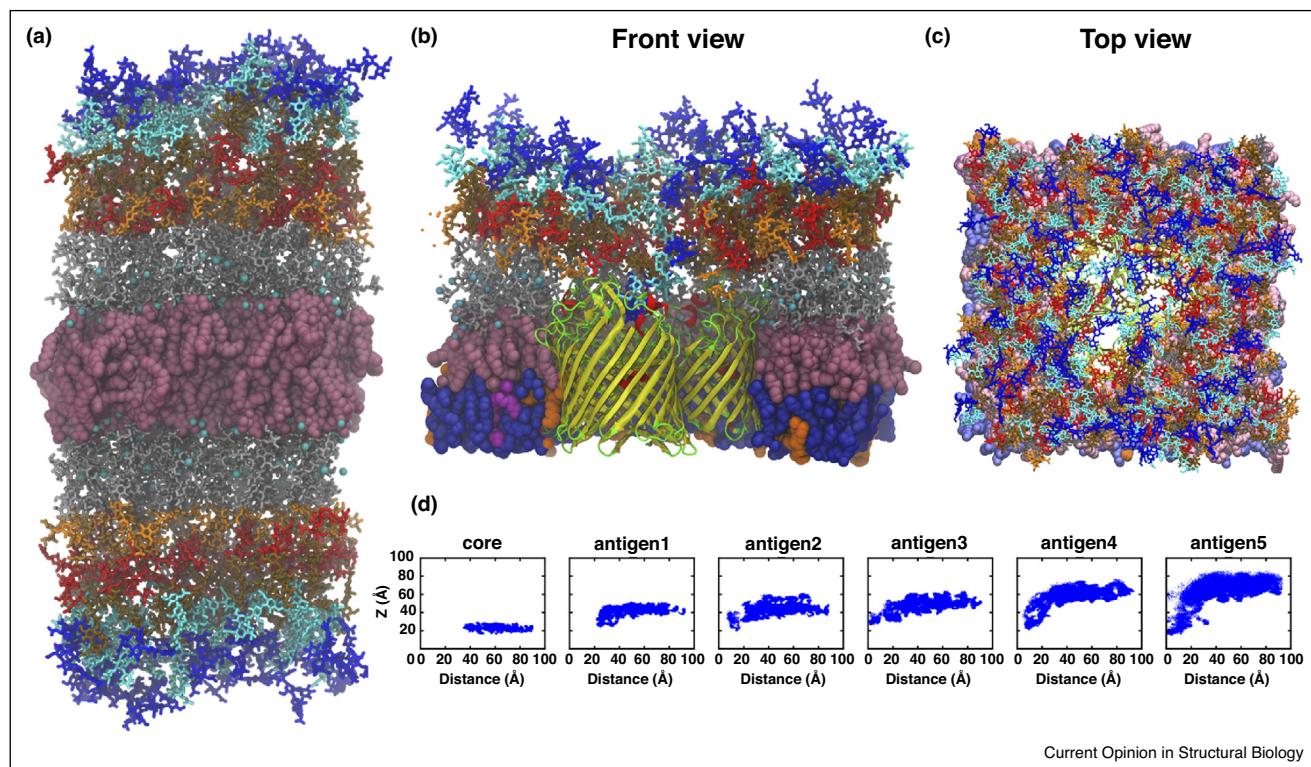
Chemical structures of *E. coli* R1.06 LPS along with K12 core and two other O91 and O176 antigen structures (Kdo: 2-keto-3-deoxyoctulosonate; Hep: l-glycero-D-manno heptose; Man: D-mannose; Glc: D-glucose; Gal: D-galactose; GlcN: D-glucosamine; GlcNAc: N-acetyl-D-glucosamine; GalNAc: N-acetyl-D-galactosamine; GlcA-6NGly: D-Glucoronate-6-N-Glycine; Qui3NAc: D-Quinovose-(R)-3-hydroxybutyramido).

in lipid A from certain bacterial species [15]. How these variations affect the packing, rigidity, and permeability of LPS bilayers and their interactions with OMPs and small molecules is an interesting and emerging research topic.

Kim *et al.* [16] recently investigated the bilayer properties of 21 distinct lipid A types from 12 different bacterial species (having different acyl chain number ( $N_{\text{CHAIN}}$ ) and length ( $L_{\text{CHAIN}}$ ), and various chemical modifications) using all-atom molecular dynamics (MD) simulations and the CHARMM force field (FF) [17–21]. The area per lipid A increases as a function of  $N_{\text{CHAIN}}$  and the membrane thickness increases as a function of  $L_{\text{CHAIN}}$ . Influence of neutralizing ion type such as  $\text{Ca}^{2+}$ ,  $\text{K}^+$ , and  $\text{Na}^+$  on the stability and the integrity of lipid A bilayers appears to be minimal. However, the residence time of  $\text{Ca}^{2+}$  ions near the lipid A headgroups is longer than those of  $\text{K}^+$  and  $\text{Na}^+$  ion types, which is well correlated with lower lateral diffusion and higher compressibility of lipid A in  $\text{Ca}^{2+}$  neutralized systems.

The effects of temperature, nature of cations, and chain numbers on the physicochemical properties of lipid A bilayers were also investigated using MD simulations with the GROMOS, GLYCAM, and AMBER FFs [22–25].

Wu *et al.* performed the MD simulation studies on *E. coli* LPS-only (Figure 2a) and OM-like bilayers (with and without OMPs (Figure 2b and c)) using different components of LPS, such as lipid A only, lipid A + R1-core (LPS0), LPS0 + 5 O6 antigen repeating units, and LPS0 + 10 O6 antigen repeating units [26,27]. Additions of LPS components indeed influence both LPS structures and its overall bilayer properties. Analyses of LPS-only simulations show that the area per lipid A increases, and bilayer order decreases as more LPS components are added, indicating slightly looser lipid A packing in the smooth LPS membrane compared to the rough LPS systems. Interestingly, more than 50% of the  $\text{Ca}^{2+}$  coordination sites are occupied by water molecules in the lipid A head

**Figure 2**

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LPS structures and flexibility. (a) *E. coli* LPS5 (lipid A + R1 core + 5?units of O6 antigen), and (b) front and (c) top views of an OM with LPS5 and OmpF trimer. Lipid A is represented as pink spheres, core sugars as gray stick models, and O-antigen polysaccharides as stick models with antigen1 (orange), antigen2 (red), antigen3 (ochre), antigen4 (cyan), and antigen5 (blue). The inner leaflet contains phospholipids with phosphatidylethanolamine (blue spheres), phosphatidylglycerol (orange spheres), and cardiolipin (magenta spheres) in a ratio of 75:20:5.  $\text{Ca}^{2+}$  ions are represented as cyan small spheres. (d) 2D scatter plots of the centers of mass of core and O-antigen repeating units along the z axis and the distance from the OmpF trimer center in (b). The OM hydrophobic core is centered at z = 0.

group and core regions, indicating the importance of water and  $\text{Ca}^{2+}$  in stabilizing and maintaining the integrity of the OM structure. This is consistent with other studies, indicating that  $\text{Ca}^{2+}$  ions favor lamellar arrangements of smooth and rough LPS membranes at temperature ranges of 283–343 K and physiological water content [22,25].

In terms of the dynamic behavior of LPS components, lipid A and core sugars are relatively immobile (compared to phospholipids), and show little translation (during the simulation timescales), independent to the core type (R1 or K12), length of O-antigen (0, 5, or 10 repeating units), and LPS compositions (homogeneous or heterogeneous) [26,27,28<sup>••</sup>]. This is consistent with other studies, suggesting an order of magnitude slower diffusion for lipid A compared to phospholipids [29]. The rigidity and low mobility of lipid A and core sugars are mainly attributed to the divalent ( $\text{Ca}^{2+}$ ) ion-mediated cross-linking electrostatic interaction networks with negatively charged  $\text{PO}_4^{2-}$  and  $\text{COO}^-$  groups in the lipid A and core regions. In contrast, O-antigen polysaccharide units are flexible,

with the flexibility sequentially increasing from the O-antigen unit attached to the outer core to the farthest residues. The lateral conformational space also increases along the membrane normal (z-axis) (Figure 2d), depending on its local environment such as densely packed (homogeneous bilayers) versus mixed LPS (heterogeneous bilayers) environments. Good agreement between the effective inter-proton distance values obtained from NMR experiments on the O6-antigen polysaccharide in solution and those calculated from the MD simulations of the LPS-only bilayers suggests similar flexibility of O6 antigen repeating units (at the disaccharide level) in both simulation and solution, giving confidence in the LPS model [26,27,28<sup>••</sup>].

To understand passive water permeation across a lipid A membrane, Wei *et al.* used a cavity insertion Widom method combined with MD simulation for *E. coli* lipid A (hexa-acyl chains) and its tetra-acyl chain analogue at 298 K [30]. This study illustrated a hexagonal compact packing for hexa-acyl chains (in ordered gel-like bilayers) in contrast to less ordered ripple structures for tetra-acyl

chains. This study also shows that a free energy barrier for water permeation in *E. coli* lipid A ( $\sim 5.5$  kcal/mol) is higher than that in tetra-acyl chains lipid A ( $\sim 4.8$  kcal/mol). Pigget *et al.* investigated the electroporation mechanism of the *E. coli* and *Staphylococcus aureus* membranes using MD simulations by applying different electric field strengths [29]. The asymmetric *E. coli* OM (composed of *RdI* LPS in the outer leaflet and phospholipids in the inner leaflet) appears to be more resistant to poration than the symmetric membrane (composed of phospholipids) of *S. aureus*. This arises from immobility of LPS molecules caused by cation-mediated tight cross-linking and strong LPS-LPS hydrogen-bonding networks.

### Structures, dynamics, and interactions of OMPs in the OMs

Changes in LPS composition due to environmental and growth conditions may affect local environments of embedded OMPs and influence OMP-LPS and OMP-OMP interactions. Several MD simulations of OMPs in OMs have been performed to explore how the properties of the OM could affect OMPs or vice versa. For example, Wu *et al.* simulated the *E. coli* OM with outer membrane phospholipase A (OmpLA) [27]. Interestingly, the OM without OmpLA shows a hydrophobic thickness of  $\sim 25$  Å compared to the corresponding phospholipid bilayer ( $\sim 28$  Å). The elucidated reduction of the hydrophobic thickness was in good agreement with the average hydrophobic thickness of  $\sim 24$  Å of known OMP structures [31]. The OM thickness near OmpLA becomes  $\sim 20$  Å because of membrane thinning due to specific OmpLA-OM interactions (i.e., hydrophobic matching) and is similar to that of a dilauroyl-phosphatidylcholine (DLPC) bilayer (where OmpLA folds better than in thicker bilayers [32,33]). Interactions between LPS (headgroups and core oligosaccharide) and OmpLA loops restrict movement of loops, which may be biologically relevant, but is absent in the model system with OmpLA inserted in a DLPC bilayer [27].

Patel *et al.* recently examined the influence of different LPS environments (homogeneous and mixed-LPS compositions) on the structure and dynamics of outer membrane protein F (OmpF) porin and also their effects on the accessibility of mAbs to surface epitopes of OmpF using all-atom MD simulations (Figure 2b and c) [28<sup>••</sup>]. The interaction patterns between OmpF residues and LPS explain the limited accessibility of mAbs to certain epitope residues shielded by LPS. This is consistent with experimental data, showing that shortening the LPS core sugars (rfa *E. coli*-K-12 mutants) sequentially increase the number of mAbs that can recognize OmpF surface epitopes. Interestingly, LPS molecules with O-antigen polysaccharides, which could be more biologically relevant compared to rough LPS environments, more efficiently occlude access of mAbs to epitope sites (Figure 2b and c).

Khalid and co-workers reported MD simulations of the TonB-dependent transporter FecA from *E. coli*, and autotransporter Hia from *Haemophilus influenzae* in asymmetric LPS-containing membranes [34,35]. Both simulations prove the significance of including LPS in stabilizing functionally important loops compared to symmetric phospholipid membrane systems. Recently, Lee *et al.* performed MD simulations to investigate the structural properties, dynamics, and interactions of OprH in the OMs of *E. coli* and *Pseudomonas aeruginosa* species. The interaction pattern analysis between OprH and LPS and pair-wise RMSD calculations for protein loops show that interaction patterns and conformational dynamics of loops vary with LPS compositions. In both OM environments, calculated interaction patterns identified key residues for OprH-LPS interactions, which are consistent with the experimental data [36].

Several simulation studies have been performed to provide insight into the mechanisms of OMP and LPS insertion to the OM. The  $\beta$ -barrel assembly machine (BAM) is a multisubunit protein complex that catalyzes the insertion and folding of OMPs to the OM [37–39]. The central core of BAM is BamA that is a  $\beta$ -barrel protein embedded in the OM with a long periplasmic POTRA domain (composed of five globular subdomains). There are four other associated periplasmic lipoproteins (BamB/C/D/E) [40,41]. Crystallographic studies along with equilibrium MD simulations of BamA in a gel-like dimyristoyl-phosphatidylethanolamine (DMPE) bilayer proposed a destabilization in membranes due to hydrophobic thinning along the barrel exterior and lateral gate opening between 1st and 16th strands as a mechanism of the insertion of nascent  $\beta$ -barrel proteins into the OM [42<sup>•</sup>]. A similar mechanism of lateral gate opening was hypothesized for the passage of the LPS molecule to the OM via LptD  $\beta$ -barrel. Dong *et al.* [43<sup>••</sup>] performed MD simulations of a LptD/LptE complex in DMPE:DMPG (dimyristoyl-phosphatidylglycerol) bilayers at pressure below  $-65$  bar and showed a separation of the lateral gate. Another 100-ns equilibrium simulation in *E. coli* K12 LPS:POPE bilayers, performed by Botos *et al.* [44], indicated the role of conserved proline residues for lateral gate opening in LptD/LptE complex which may facilitate substrate passage. These findings were supported by experimental studies that either crosslinking of the lateral gate or mutation of the proline residues near the lateral gate to alanine proved to be harmful for bacterial growth.

Reports on variable conformations and dynamics of the POTRA domain suggest the idea of conformational cycling for the BAM complex, which is believed to facilitate the insertion of client proteins into the OM [45]. To elucidate the conformational dynamics of the POTRA domain, Fleming *et al.* [46] explored conformational dynamics of the POTRA domain in full length BamA embedded in an *E. coli* OM using MD simulations

at 310 K. Interactions of the POTRA domain with the periplasmic surface appear to modulate its conformational flexibility. Interestingly, orientations of the membrane-bound POTRA domain are competent to interact with BamB and BamD. This further supports the involvement of selection of different POTRA domain conformations in the mechanism of BAM-facilitated insertion of OM  $\beta$ -barrel proteins. Notably, though the authors observed  $\sim 4 \text{ \AA}$  thinning of the OM near BamA, a lateral gate opening between 1st and 16th strands was not observed in  $\sim 5\text{-}\mu\text{s}$  total simulation time unlike earlier simulations in a gel-like DMPE bilayer [42<sup>•</sup>].

Altogether these studies provide atomistic details signifying the importance of including native-like bacterial OMs for OMPs' structure and dynamics in the OM, shielding its epitopes from immune recognition, and other biological functions. In addition, these studies also point out a key limitation of OMP simulations in phospholipid bilayers, which cannot incorporate genuine OMP-LPS (core and O-antigen sugars) interactions. However, building a LPS molecule is not trivial due to the complexity of carbohydrate linkages. In this context, *LPS Modeler* (<http://www.charmm-gui.org/input/lps>) in CHARMM-GUI, [47] is notable as it simplifies generation of structural models of various LPS molecules. Currently, *LPS Modeler* supports LPS structures of 14 bacteria species with 32 lipid A types, 49 core types, and 222 O-antigen types. *LPS Modeler* is expected to open enormous future opportunities for building and assembly of OMPs in a specific bacterial OM, based on the procedure in CHARMM-GUI *Membrane Builder* [48,49] developed by Im and co-workers.

All-atom simulations of the OMP-OM systems generally are limited in terms of the simulation time and the system size, as they are computationally expensive and LPS diffusion is an order of magnitude slower than phospholipids. To overcome these limitations, efforts have been made to developing coarse-grained (CG) LPS models [50]. For example, a few CG LPS models have been developed as extensions of the Martini FF [51,52<sup>•</sup>]. One of these models has been incorporated into *Martini Maker* (<http://www.charmm-gui.org/input/martini>) in CHARMM-GUI [53], providing a quick access to build and simulate CG OM systems in bilayer, micelle, and vesicle environments. Models built from efficient computational methods [54<sup>••</sup>] and tools like CHARMM-GUI will be useful in understanding longer timescale dynamic processes of larger systems representing a biological realism (Figure 3).

## Permeation through the bacterial OM and challenges

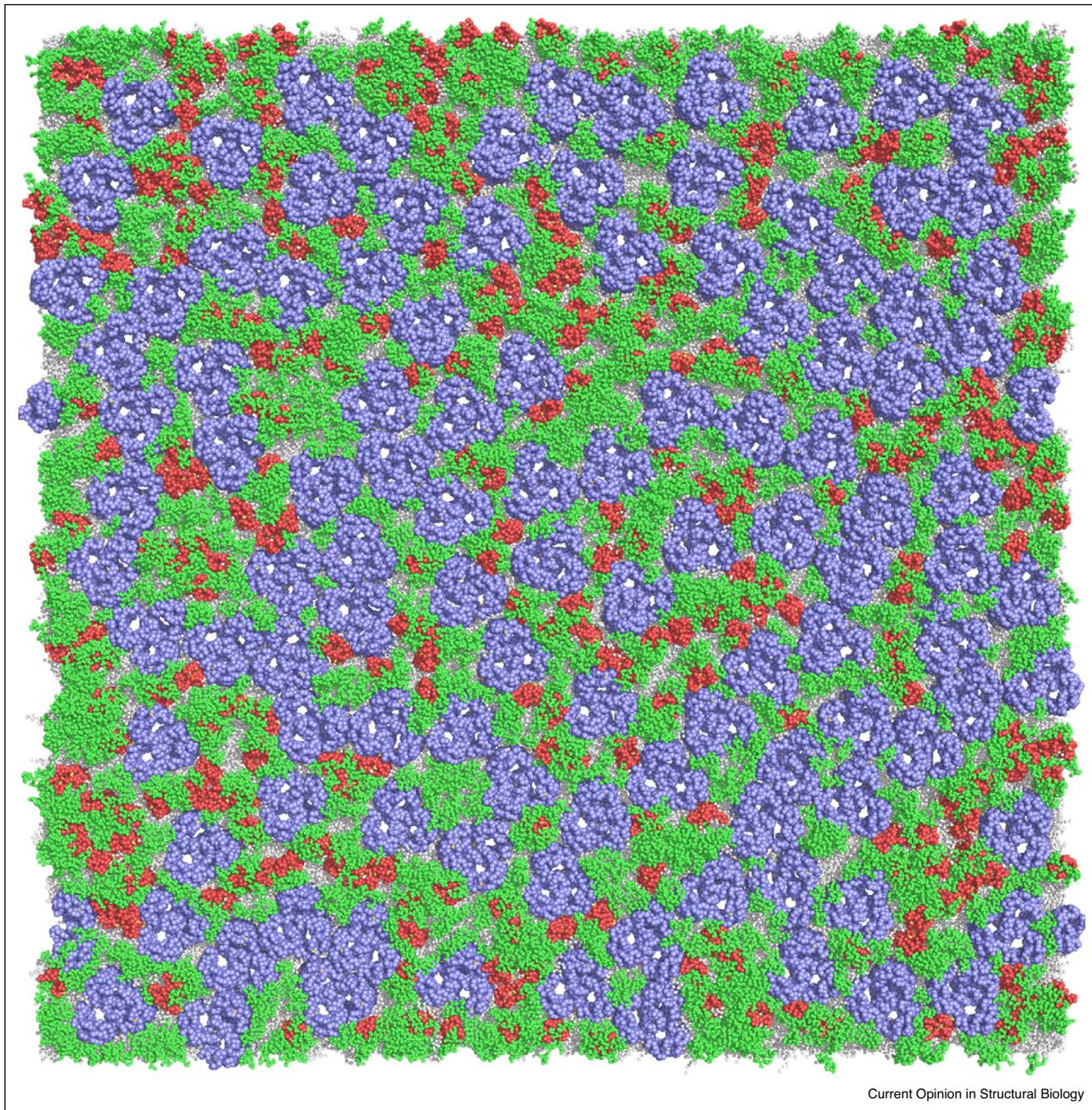
General porins and/or substrate-specific channels in the OM ensure passive diffusion of small molecules less than 600 Da, and bulky molecules are translocated across the

OM by TonB-dependent active transporters. These porins/channels/transporters also represent potential targets for antibiotics [55,56]. Ion selectivity and conductivity of channels have been a prime focus of several experimental and computational studies including a careful computational study reported by Im *et al.* and others for OmpF ion selectivity and conductance [57–59]. In addition, molecular mechanism of antibiotics permeation through these channels and the key interactions of the pores with antibiotics were also explored using equilibrium, steered, and metadynamics MD simulations [60<sup>••</sup>]. However, molecular packing and rigidity of asymmetric bacterial OMs was not considered in most of these studies. This is important as a recent simulation study with O-antigen polysaccharides shows restricted ion movement in the outer leaflet due to increased hindrance to the ion permeation pathways by molecular crowding of LPS. Therefore, different LPS environment could affect ion (and potentially other solutes) diffusion into porins/channels/transporters (Fig. 4), but not their basic functioning [28<sup>••</sup>]. This is also supported by a recent report by Khalid and co-workers who used all atom MD simulations to show less energetically favorable environment for permeation of small molecules through LPS outer leaflet as compared to phospholipids inner leaflet in a model of the *E. coli* OM [61].

Polymyxin B1 (PMB1) is a known antimicrobial peptide that permeates across the bacterial OM by self-promoted uptake. Using all-atoms MD simulations, Berglund *et al.* explored interactions of PMB1 with an asymmetric Re-LPS OM model and a symmetric lipid A membrane [62]. Although penetration of PMB1 into the acyl tails region of the bilayers was not observed within the simulation time ( $\sim 1.5 \mu\text{s}$ ), insertion of  $\alpha,\gamma$ -diamino butyric acid (DAB) residues between the LPS sugar head groups was evident in this study. DAB has been suggested to be primarily responsible for the initial binding of PMB1 to the negatively charged phosphates of LPS. In addition, the observed aggregation of PMB1 in the OM models was suggested as the plausible first step in pore forming or self-regulated translocation of PMB1. Similarly, 300-ns simulations show that strong electrostatic binding of bH1 antimicrobial peptide dendrimer via 2,3-diaminopropionic acid to LPS membrane is the driving force to facilitate penetration [63].

Increasing antibiotic resistant pathogens have been threatening the public health. The situation is worrisome with very slow pace in development of new class of antibiotics drugs due to (i) insufficient experimental and computational knowledge of physicochemical properties of the OMs from different bacterial species, which governs permeability of OMs, and (ii) inadequate chemical diversity and lack of studies that correlate physicochemical properties of compounds that permeate through these OMs. In addition, biological reality is that the cell

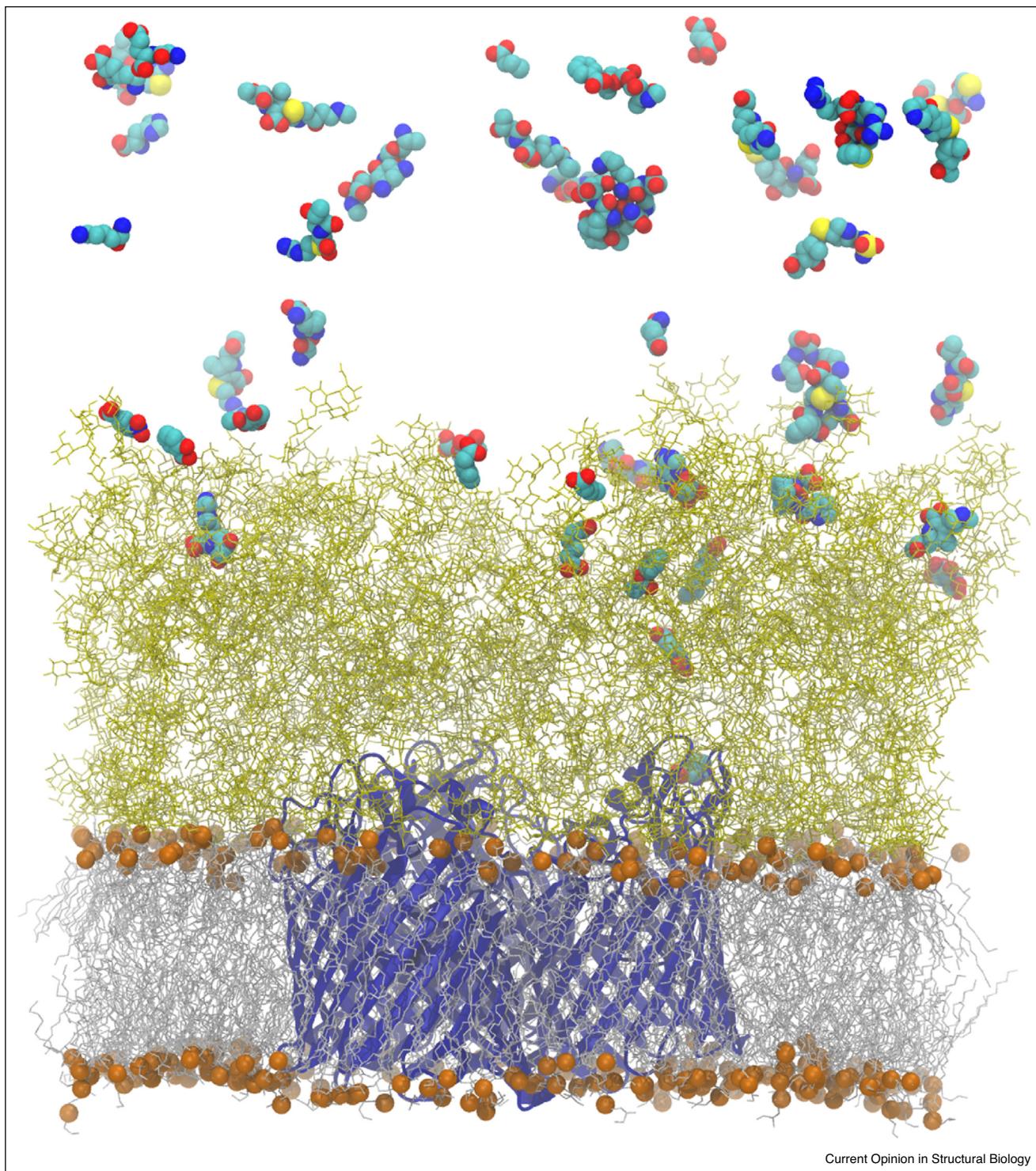
Figure 3



A top view of a crowding OM coarse-grained model using the Martini FF. The system consists of 114?OmpF trimer (4.7%), 282?OmpA (11.6%), and 2034?LPS (83.7%) in the upper leaflet, and 3629?phosphatidylethanolamine (75.1%), 963?phosphatidylglycerol (19.9%), and 240?cardiolipin (5%) in the bottom leaflet. OmpFs are colored in blue, OmpAs in red, LPS sugars in green and lipid A and phospholipids in white. The system size is ~991 Å × 991 Å.

membranes of Gram-negative bacteria also have at least one efflux pump (e.g., AcrAB-TolC of *E. coli*), which expels variety of antimicrobial agents and diminishes their effectiveness [64]. Simulations in native-like OM

rather than in symmetric phospholipid bilayers could facilitate greater insights into the permeability properties across the OM, which could lead to discovery of new antimicrobial agents and vaccine development.

**Figure 4**

A snapshot from an all-atom simulation of OmpF in an LPS5 OM (in [Figure 2b](#)) with selected 51?small molecules representation from amino acids, sugars and antibiotics (space-filling models). All core and O-antigen sugars are represented by yellow lines.

## Conclusions

Recent advances in modeling and simulation of complex bacterial OMs have made it possible to explore the structures, dynamics, and interactions of the OM and the OMPs in the OM. Molecular-level insights gained from these studies indicate importance of incorporating LPS molecules, and suggest their roles in transportation of ions and substrates across the OM and epitope accessibility to mAbs. This is also important as significant efforts are currently made towards how permeability properties change for the OMs from different bacterial species and how these properties correlate with physicochemical properties of compounds that permeate through these OMs. Tools like *LPS Modeler* in conjunction with *Membrane Builder* in CHARMM-GUI will be indispensable towards the computational modeling of OMPs in asymmetric LPS/phospholipids bilayers. CG modeling of bacterial OMs is also expected to be useful to overcome system size limitation and slow diffusivity of LPS, which will in turn provide molecular level insight into long timescale dynamic processes. Altogether molecular modeling and simulation allow exploration of complex and crowded realistic biological membranes with enormous future opportunities to study the interaction mechanism between the bacterial OM and OMPs and to facilitate the discovery of new antibiotic drugs.

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