Atomistic and coarse grain simulations of the cell envelope of Gram-negative bacteria: what have we learned?

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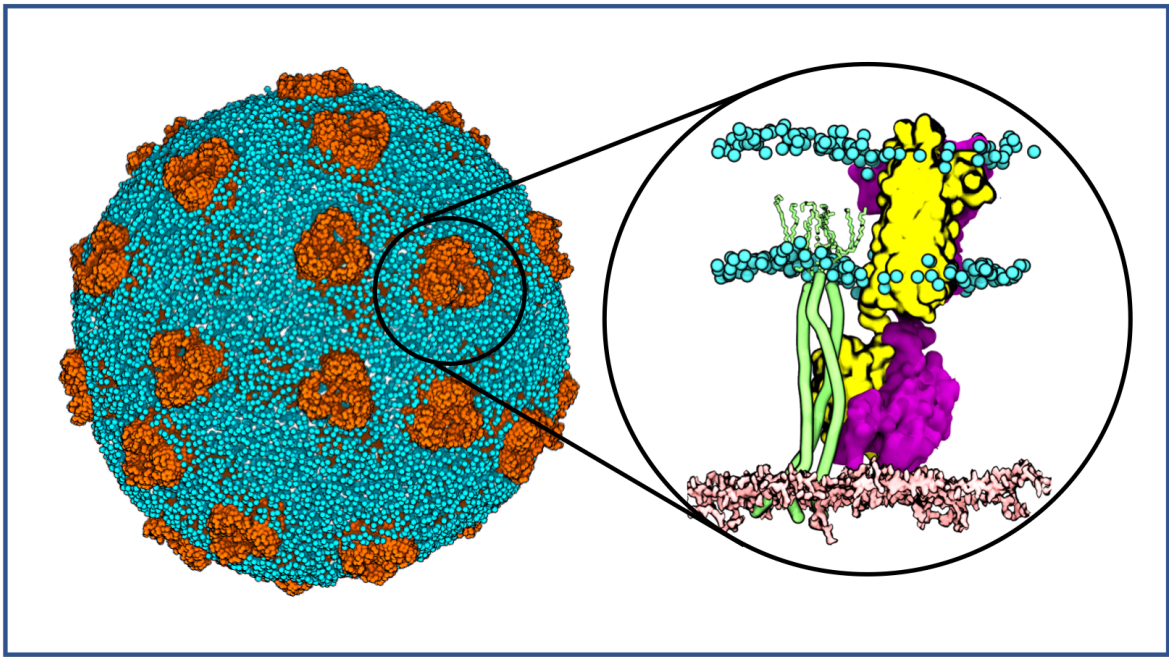
Conspectus

Bacterial membranes, and those of Gram-negative bacteria in particular, are some of the most biochemically diverse membranes known. They incorporate a wide range of lipid types and proteins of varying sizes, architectures and functions. While simpler biological membranes have been the focus of a myriad simulation studies over the years that have yielded invaluable details to complement, and often to direct ongoing experimental studies, simulations of complex bacterial membranes have been slower to emerge. However, the last few years have seen tremendous activity in this area, leading to advances such as the development of atomistic and coarse-grain models of the lipopolysaccharide (LPS) component of the outer membrane that are compatible with widely used simulation codes. In this Account we review our contributions to the field of molecular simulations of the bacterial cell envelope, including the development of models of both membranes and the cell wall of Gram-negative bacteria, with a predominant focus on *E. coli.* At the atomistic level, simulations of chemically accurate models of both membranes have revealed the tightly cross-linked nature of the LPS headgroups and have shown that penetration of solutes through these regions is not as straightforward as the route through phospholipids. The energetic differences between the two routes have been calculated. Simulations of native outer membrane proteins in LPS-containing membranes have shown that the conformational dynamics of the proteins is not only slower in LPS, but it is also different compared to in simpler models of phospholipid bilayers. These, chemically more complex and consequently biologically more relevant models are leading to details of conformational dynamics that were previously inaccessible from simulations. Coarse-grain models have enabled simulations of multi-protein systems on timescales of microseconds, leading to insights into not only the rates of protein and lipid diffusion, but also into the trends in their respective directions of flow. We find that the motion of LPS molecules is highly correlated with each other, but also with outer membrane proteins embedded within the membrane. We have shown that the two leaflets of the outer membrane exhibit communication, whereby regions of low disorder in one leaflet correspond to regions of high disorder in the other. The cell wall remains a comparatively neglected component, although models of the *E. coli* peptidoglycan are now emerging, particularly at the atomistic level. Our simulations of Braun's lipoprotein have shown that bending and tilting of this protein affords a degree of variability in the gap between the cell wall and the OM. The non-covalent interactions with the cell wall of proteins such as OmpA can further influence the width of this gap by extension or contraction of their linker domains. Overall we have shown that the dynamics of proteins, lipids and other molecular species within the outer membrane cannot be approximated using simpler phospholipid bilayers, if one is addressing questions regarding the *in vivo* behaviour of Gram-negative bacteria. These membranes have their own unique chemical characteristics which cannot be decoupled from their biological functions.



Conspectus graphic

Introduction

Bacterial cells are surrounded by cell membranes. In Gram-negative bacteria the cell envelope comprises two membranes separated by the periplasmic space, which contains a thin cell wall (Figure 1). The membranes provide protection by controlling the movement of molecules between the cell and the external environment, and by providing a physical barrier that mechanically encloses the internal contents of the cells from the environment[1]. Over recent years it has become apparent that the lipids constituting the membranes have important roles in modulating (i) the activity of membrane proteins, (ii) membrane curvature and (iii) membrane thickness. Consequently, building accurate computational models of these lipids is essential to gaining a molecular-level understanding of the biological importance of the chemical composition of lipids that comprise membranes. In the following account we review our own contribution to the field of molecular simulations of the cell envelope of Gram-negative bacteria over the past decade in the context of related studies and the overall development of the field.

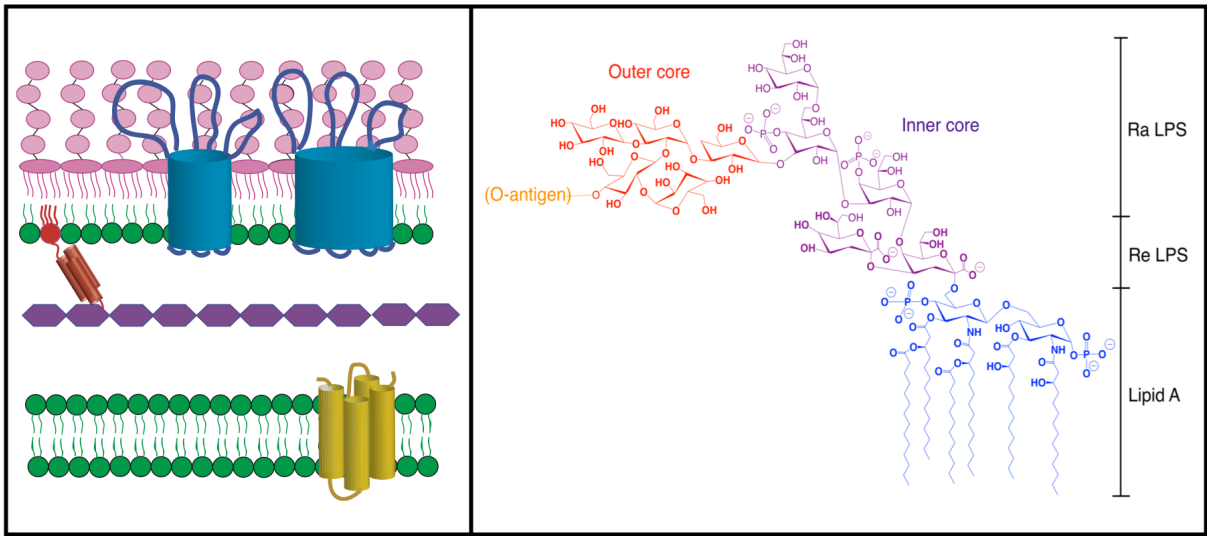


Figure 1. A schematic diagram of the cell envelope of Gram-negative bacteria (left). The LPS molecules are pink, phospholipids are green, peptidoglycan is purple, outer membrane proteins are blue, inner membrane proteins are yellow, and Braun's lipoprotein is red. The chemical structure of LPS from E. coli (right).

The outer membrane (OM) of Gram-negative bacteria is arguably the most complex bacterial membrane in terms of structure and composition. The lipid composition is asymmetric; the outer leaflet is composed of lipopolysaccharide (LPS) molecules, whereas the inner leaflet contains a mixture of phospholipids. The precise chemical structure of LPS is dependent upon the bacterial species, strain and even stage of the lifecycle. In general, it consists of lipid A molecule, a core oligosaccharide with an inner and outer core region, and an O-specific polysaccharide antigen chain[1]. The lipid A component comprises a phosphorylated, 1-6 linked glucosamine dimer bound to lipid acyl chains that are anchored into the hydrophobic region of the outer membrane. The number of tails of the lipid A moiety vary from 4 to 7, across bacterial species; for example in *E .coli* there are 6 tails in lipid A, compared to 5 in *P. aeruginosa*[1]. Covalently linked to lipid A is the core portion of the molecule, which is further divided into the inner and outer core regions. The inner core is attached to lipid A and contains a high proportion of rare sugars such as 2-keto-3-deoxyoctulosonate (Kdo) and L-

glycero-D-manno-heptose[2]. In contrast, the outer core is composed of sugars such as hexoses and hexosamines (Figure 1).

Electroporation of *E. coli* membranes.

Motivated by a desire to understand the process of electroporation of bacterial membranes, in 2011, we reported the first atomistic simulations of *E. coli* LPS. Our work built on earlier studies of Straatsma and colleagues who had reported a model of the *P. aeruginosa* OM with an LPS-containing outer leaflet and an inner leaflet with an extremely simplified phospholipid composition[3]. We employed our own GROMOS based force field, to simulate an outer leaflet comprising Rd_1 LPS molecules and a mixture of phospholipids in the inner leaflet[4]. We note that the Im group were the first to report an atomistic OM model in which all LPS molecules included the O-antigen[5]. The length of Rd_1 LPS is intermediate between Re and Ra LPS. We employed our model to explore the molecular mechanisms of electroporation in the *E. coli* outer and inner membranes, and the cell membrane of *S. aureus*, the latter two are symmetrical in terms of their lipid compositions. Simulations of electroporation of simple symmetric and asymmetric phospholipid bilayers have previously been reported[6-8], but to our knowledge our simulations were the first of representative bacterial membranes[4]. Electroporation of the OM occurred when movement of one or two water molecules towards the membrane core was accompanied by movement of a phospholipid headgroup (never LPS) towards the core (Figure 2). The defect was formed in the inner leaflet irrespective of the direction of the electric field. These water molecules were able to penetrate further into the membrane core, where they formed single-file water channels. Most of the water molecules were located in the inner leaflet where they were stabilized through hydrogen-bonding with the phospholipid head groups. Where full poration was observed, the inner leaflet phospholipids moved into the membrane core to interact with water molecules.

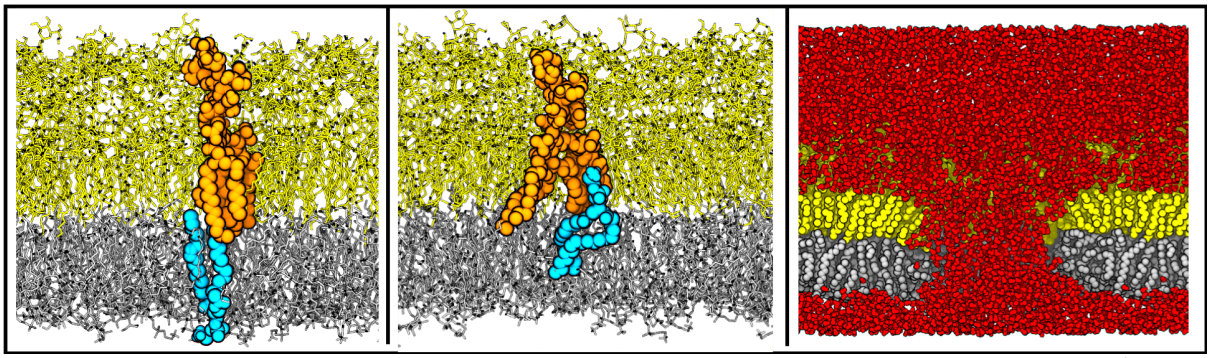


Figure 2. Atomistic simulations of electroporation of the E. coli outer membrane[4]. Left: initial system with one LPS (orange) and one phospholipid (cyan) highlighted, middle: the headgroup of the highlighted phospholipid has moved into the core of the membrane, right; a fully porated membrane. Water is red, phospholipids are grey and LPS molecules yellow.

This was often enabled through flip-flopping of the phospholipids, such that they became embedded within the LPS molecules of the outer leaflet. Only once a stable, phospholipid-lined pore was formed and continued to expand, did the LPS molecules move to line the pore. This mechanism of electroporation highlights a potential route through which defects in the outer leaflet may be overcome; the movement of phospholipids of the inner leaflet into the outer leaflet, to plug any defects such as depletion of LPS.

The impact of divalent cations and slow diffusion of LPS

The molecular basis of the permeability of the OM has been a focus of membrane studies for many years, driven in part by the desire to understand mechanisms of antibiotic resistance[1]. The role of ions in maintaining the structural integrity of the OM was suggested as early as 1965[9]. But more detailed studies confirming the tight-crosslinking of LPS headgroups by cations and the precise nature of the ions came much later. The first simulations to show binding of divalent cations to the phosphorylated sugars of LPS was reported by Lins & Straatsma from simulations that were state-of-the-art at the time, but are rather short by current standards[3]. Encouragingly longer simulations from at least three other groups have provided independent verification and additional insights[5,10,11]. The combined NMR and simulation study reported by Im and co-workers revealed the core sugars of LPS to be structurally more rigid than the O-antigen sugars, and suggested this is a consequence of the calcium-mediated cross-linking of the former[5]. Our simulations, performed alongside the experimental work of the Lakey group showed that replacing divalent cations with monovalent cations in a bilayer composed of LPS in one leaflet and phosphatidylcholine (PC) phospholipids in the other leaflet leads to breakdown of the bilayer[11]. In these simulations, the asymmetric bilayer was stable for 500 ns when Ca^{2+} ions were cross-linking the lipid A headgroups of Re LPS. However when the Ca^{2+} ions were replaced by twice the number of Na^{+} ions, within just 200 ns water molecules were observed to penetrate the bilayer. PC lipids had inserted into the outer leaflet and both LPS and PC lipid head groups had begun to move into the bilayer core. The experiments were performed by using EDTA to remove the divalent cations, leading to bilayer disruption. Thus, in agreement with previous work[9,12], the role of divalent cations in cross-linking the headgroups of lipid A within LPS is firmly established. The tight cross-linking of the LPS headgroups contributes to the slow diffusion rate of LPS, which has been reported to be approximately one order of magnitude slower than phospholipids[4]. Simulation studies have shown that the reduced diffusion rate of LPS slows down permeation of molecules across the outer membrane[13]. We encountered the effect of slow diffusing LPS when considering permeation of the antimicrobial peptide polymyxin B1 (PMB1). We observed PM1 insertion into a symmetric inner membrane model within 100 ns, but no penetration into an Re LPS-containing model of the outer membrane[14]. The lack of penetration within atomistic simulation timescales was the combined effect of slow diffusion of LPS and the strong electrostatic interactions between the LPS phosphate and hydroxyl groups and the amino groups of PMB1 (Figure 3).

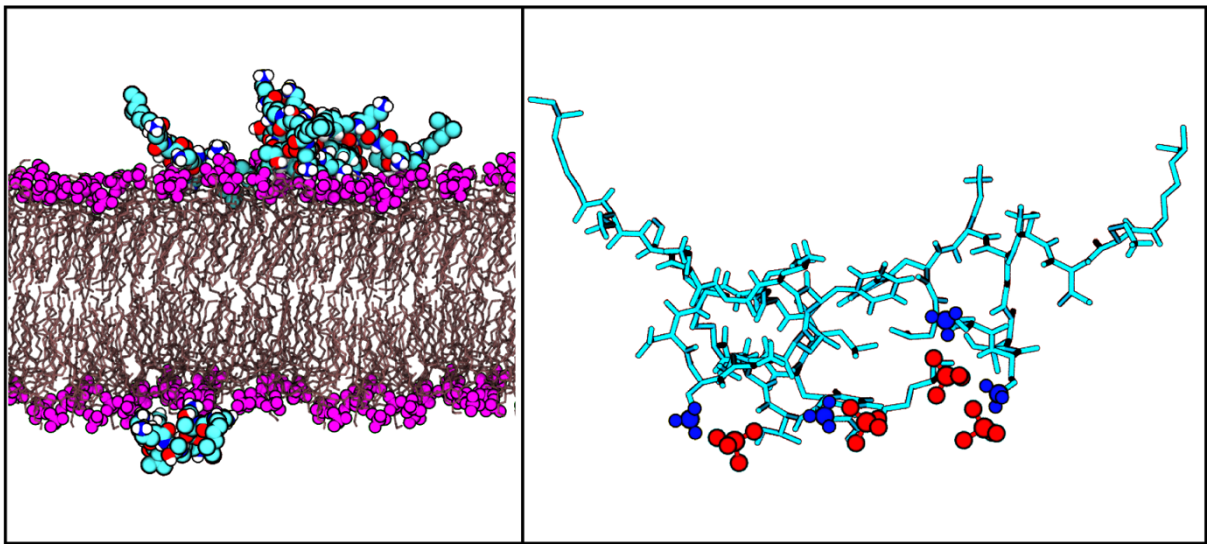


Figure 3. Polymyxin B1 did not penetrate into an Re LPS bilayer in atomistic simulations [14]. The panel on the right shows typical electrostatic interactions between polymyxin B1 and LPS, red spheres represent phosphate moieties of Re LPS and blue spheres show NH_3^+ groups on the lipopeptide.

Similar observations were subsequently reported by Santos *et al*[15]. Given the mechanism of action of PMB1 is still debated, it is unclear whether the lack of tail penetration in the simulations is an equilibrium property of PMB1 and LPS-containing simulations, or an artefact of simulations that are too short. This poses somewhat of a conundrum for simulation studies; we have appropriately detailed models of the OM when we incorporate LPS, but the consequent slower dynamics necessitates running much longer simulations.

Development of coarse grained LPS model

To overcome some of these timescale limitations, we developed a coarse-grain (CG) force-field of LPS based on the popular MARTINI force-field[16]. In order to accurately reproduce the physical properties of LPS fine-grained simulations, we developed a freely available code: PyCGTool[17]. This program uses fine-grained trajectories to determine appropriate bond lengths and angles for the course-grain counterparts. Our code can be used to parameterise any coarse-grain system if the user provides the mapping and fine-grained trajectories. Given the earlier described limitations of studying PMB1 penetration into the OM with atomistic models, we employed our CG membrane model to study this system, in the process also parameterising a CG model of PMB1 using PyCGTool[18]. The CG simulations showed strong membrane-binding of PMB1, in agreement with our atomistic study. Insertion of PMB1 into the core of the Re-LPS membrane was observed, albeit only once as the benzyl and isobutyl groups of the peptide penetrated the phosphate interface of the. Localised glass-like behaviour of the membrane upon the peptide binding was observed, this agrees qualitatively with both our atomistic simulations and those of Santos *et al*, in which LPS-containing membranes thickened upon binding PMB1, suggesting increased acyl-chain order[15]. Thus our CG models are able to provide biologically relevant insights into the behaviour of macromolecules, such as antimicrobials when they interact with the OM of Gram-negative bacteria. In order to facilitate others in setting up and running CG simulations of LPS-containing flat bilayers, spherical vesicles, micelles and nanodiscs we have worked alongside the Im and Marrink groups to

provide this extra functionality to the Im group's very popular web-based CHARMM GUI server[19].

Different and slower dynamics in LPS.

There are still relatively few studies of native OM proteins (OMPs) in LPS-containing membranes. Given there are often multiple charged residues located in the loops of these proteins, it is reasonable to expect they will interact strongly with the highly anionic LPS molecules in the outer leaflet. We have performed atomistic simulations of a number of OMPs in LPS-containing membranes, in studies that reveal that the dynamics, especially of the long extracellular loops are not only slower, but also different in LPS compared to phospholipids. Here, we describe our work on three OMPs; FecA, Hia and OmpA.

The TonB dependent transporters are a family of OMPs composed of twenty two-stranded beta-barrels. Comparative simulations of the TonB-dependent transporter FecA in an asymmetric Rd_1 LPS-containing membrane versus a phospholipid bilayer revealed LPS impacts significantly upon the loop dynamics via two distinct mechanisms[20]. Short-lived, but frequently formed non-specific hydrogen-bonding interactions alter the local fluctuations in loop movement, whilst the bulky LPS molecules provide steric resistance to large-scale conformational rearrangements of the loops. Similarly, altered loop dynamics in phospholipids compared to LPS were later reported for another TonB-dependent transporter, BtuB[21]. OmpA is a multi-domain protein composed of an eight-stranded barrel at the N-terminal domain, and a periplasm-residing, soluble, C-terminal domain. The structure of the former has been determined, while the structure of the C-terminal domain of homologues from other bacteria have been determined, but not from *E. coli.* Simulation studies of the OmpA barrel in a simple model membrane containing only phospholipids showed a high degree of flexibility of the extracellular loops compared to the periplasmic turns[22]. We simulated a model of the full-length OmpA as a homodimer based on work by the Robinson group[23], in an Ra LPS-containing *E. coli* membrane model[24]. Our simulations showed that the extracellular loops were noticeably less mobile in our OM model, owing to multiple interactions between basic residues in the loops, with anionic moieties of LPS. We identified specific hydrogen bonds and salt bridges between LPS and the loops of OmpA, that are involved in the dimerization interface, whilst other loops involved in the dimerization interface did not interact with the LPS to any appreciable extent. We have also shown that LPS can bind to distinct extracellular protein domains, as demonstrated in our work on the autotransporter Hia from *Haemophilus influenza*[25]. Hia is comprised of a beta-barrel containing three alpha-helices which extend out of the barrel. Atomistic simulations of Hia in an asymmetric OM model revealed a group of lysine residues formed a small positively charged patch on the helical extracellular domain. These residues formed salt bridges with sugar and phosphate moieties of the surrounding LPS molecules, which may help to anchor Hia within the outer leaflet (Figure 4). The highly anionic nature of LPS enables a single lysine in the protein to interact with multiple anionic moieties of LPS. Taken together our simulations of FecA, OmpA and Hia and those of Gumbart on BtuB[21] and Im on OprH[26] and OmpLA[27] indicate that protein-LPS interactions, may have crucial structural and functional consequences for OMPs with varying topologies.

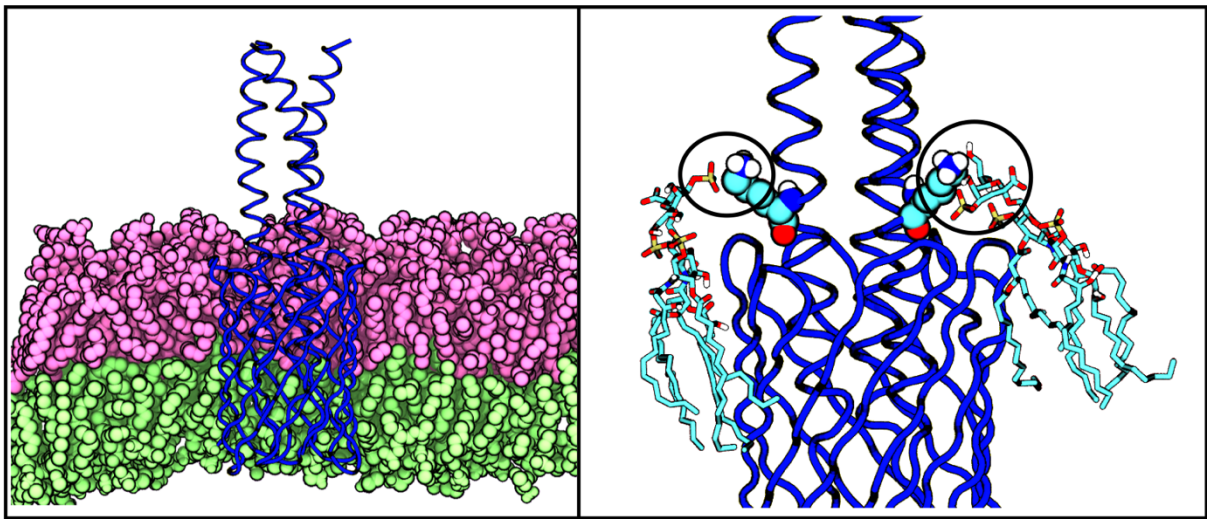


Figure 4. Atomistic simulations of Hia in an LPS-containing membrane revealed the role of LPS-lysine residues in orientating the protein within the outer membrane. We hypothesise that these lysine residues of Hia play a similar role to the 'bands' of aromatics residues on the outer surface of other OMPs that are thought to anchor those proteins in the outer membrane.

Asymmetric energetic barriers for permeation of small molecules.
It is important to quantify the energetic differences between the two membranes of *E.coli* for permeation of small molecules. In order to do this, we employed umbrella sampling with the weighted histogram analysis method (WHAM) to calculate potential of mean force profiles for permeation of benzene, hexane, ethane, acetic acid, and ethanol across our OM model[4,28]. Our simulations revealed a marked difference in the energetic barriers presented by the headgroups of LPS compared to the headgroups of the phospholipids of the inner leaflet. The barrier at the LPS headgroup region was most pronounced for hexane, which is likely a consequence of the longer hydrophobic length of hexane compared to benzene and ethane. The maximum free energy in the LPS headgroup regions was calculated as ~6 kcal/mol for hexane, <5 kcal/mol for benzene, and ~3 kcal/mol for ethane. Interestingly no appreciable barriers to the entry of any of the hydrophobic solutes into the phospholipid head groups were observed. Previously free-energy calculations of small solute permeation through symmetrical phospholipid bilayers reported small aliphatic chains such as propane to have similar barriers through the headgroup region of phosphatidylethanolamine lipid headgroups as benzene[29,30]. Inspection of the windows corresponding to these regions revealed that the lack of barriers in our simulations were a consequence of the high mobility of the phospholipids, which rearrange locally, such that the hydrophobic solutes become exposed to parts of the lipid tail regions, rather than the headgroups. The faster diffusion of the inner leaflet phospholipids we observed in the asymmetric OM compared to the inner membrane facilitates this process[4]. It was noted that the previously reported simulations were too short to observe these rearrangements. Thus, our simulations provided the first reported energetics of small molecule permeation across the OM, but also a note of caution regarding the ever-present problem of simulation timescales.

Larger simulation systems: correlated lipid motion.
The development of coarse-grain models of LPS and easy-to-use methods for constructing LPS-containing systems, such as sMARTINI-maker as part of CHARMM

GUI have enabled the simulation of larger systems than previously have been practical with atomistic methods[19,31]. Simulations of systems composed of multiple proteins and biologically relevant mixtures of lipids are crucial for providing a better understanding of global membrane characteristics such as curvature and deformation, diffusion of proteins and lipids, protein aggregation, and lipid sorting. Coarse grained models are ideal for such studies largely for two reasons: firstly, fewer calculations are required to achieve a certain timescale given multiple atoms are combined into larger particles and thus there are fewer interaction sites, and secondly the flatter potential energy surfaces that result from the reduced dimensionality give an additional speed-up. Our first simulations of large systems were of spherical vesicles containing multiple copy numbers of the trimeric porin OmpF and various lipids to mimic the bacterial OM environment[32]. At that time we nor others had yet to develop a coarse grain model of LPS, and thus DLPC lipids were used in the outer leaflet as their tail lengths are almost identical to those of LPS. Local membrane thinning around OmpF due to hydrophobic mismatch was found to drive long-range communication resulting in OmpF clustering. The OmpF proteins were shown to affect lipid motion as far as 6 nm from their outer surface, a substantially longer ranging effect than previously reported from simulations of flat bilayers[33]. Given we used vesicles in our simulations, we also circumvented the issue of any potential periodicity effects on the diffusion rates. These simulations exemplified how localised membrane perturbation around proteins can have far reaching effects, indeed when one considers the crowded nature of the outer membrane, then these effects are likely to cause perturbations across the entire membrane. Thus, while coupled motion of protein and annular lipids have been demonstrated in the past[33,34], we showed a substantially larger distance over which protein and lipid displacement are correlated. It is notable that while there have been a number of coarse-grain simulations of large bacterial membrane systems with simplified OM models, there are fewer such studies of larger systems incorporating LPS[32,33,35,36] . We performed simulations of the *E. coli* cell envelope in which the TolC/AcrABZ efflux pump spanned both membranes and the periplasmic space, the latter was approximated as a water-filled region separating the two membranes (Figure 5)[37]. The results of these simulations revealed stark differences in the motion of LPS compared to phospholipids. Whilst it had previously been shown that the motion of LPS is restricted compared to phospholipids, our simulations revealed that LPS motion within the outer leaflet is also highly correlated. Indeed LPS molecules are correlated with each other, but also with the proteins within the membrane: they all move in the same direction. The phospholipids of the inner leaflet and the inner membrane display far less correlation in their motion. Furthermore, enrichment of specific lipid types around AcrBZ and AqpZ, two proteins of the inner membrane, was reported. Similar lipid 'fingerprints' of membrane proteins have been reported from coarse-grain, large scale simulations of other membrane proteins, most notably from Tieleman and co-workers[38]. We recently showed communication between the two leaflets of the outer membrane, in which regions of high lipid disorder in the outer leaflet were directly above regions of high order in the inner leaflet[39]. Thus, studies of larger simulations enabled by CG models, are opening up new channels of lipid-protein interactions; these studies are still very much in their infancy and not yet *de rigour* within the biomolecular simulation community, but show enormous promise.

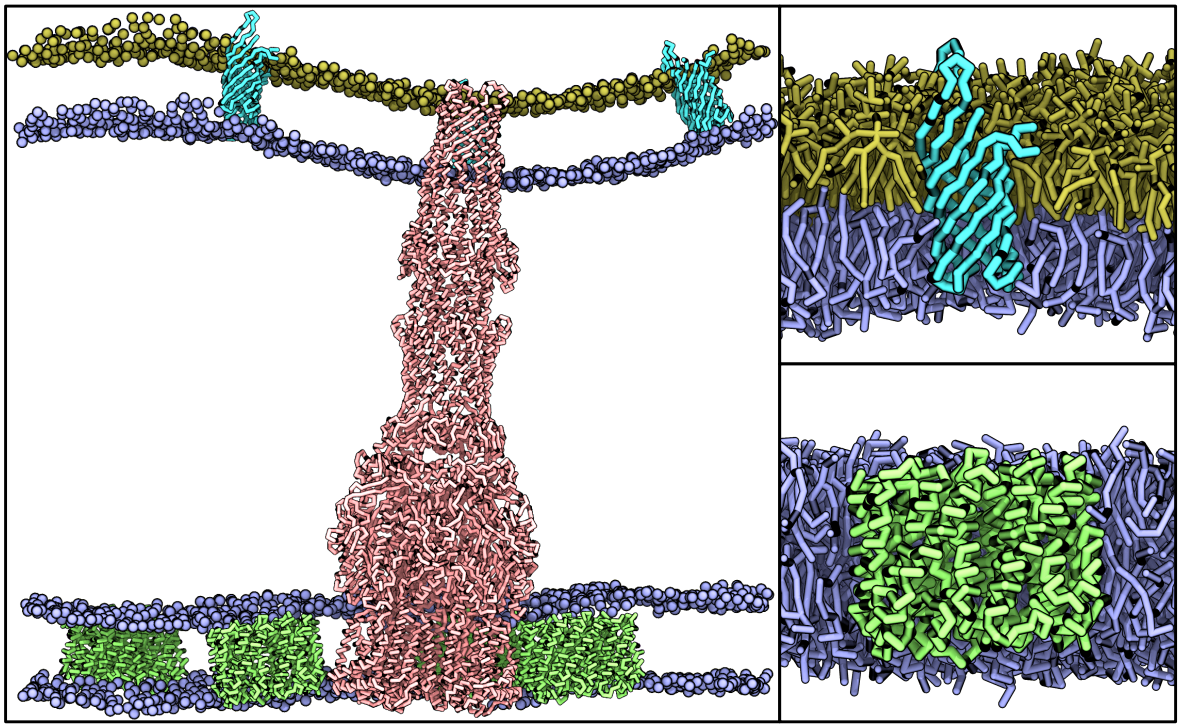


Figure 5. Two views of a simulation system incorporating both the inner and outer membranes of E. coli reported by Hsu et al[37]. LPS is gold, phospholipids are purple, AqpZ is green, OmpA is cyan, and the TolC-AcrABZ efflux pump is pink.

The cell wall.

In Gram-negative bacteria the cell wall is located in the periplasm, between the inner and outer membranes. The cell wall is composed of a network of peptide and sugar molecules commonly known as peptidoglycan. The peptidoglycan mesh is covalently linked to the OM via Braun's lipoprotein (BLP) and non-covalently to both membranes via certain integral membrane proteins. Earlier simulation studies of the cell wall by Gumbart et al. focused on attempting to characterise the physical properties of peptidoglycan such as elasticity, pore size, and thickness[40]. Pioneering experimental studies in which atomic force microscopy is used to image the intact cell wall by Foster and Hobbs have revealed that the composition and architecture of the cell wall is far more complex than the textbook picture of fairly ordered layers[41,42]. While the AFM experiments provide details of the cell wall, the relatively faster motion of the proteins associated with peptidoglycan impede their detection by AFM, and therefore details of how the cell wall is positioned with respect to the proteins and other components of the cell envelope remain sparse. BLP is anchored to the OM via a lipidated N-terminus and is covalently bound to the peptide chain of the peptidoglycan through its C-terminus. Previously it had been reported that the length of BLP has a direct influence on the distance between the cell wall layer and the OM of *E. coli*[43]. To explore this, we developed atomistic parameters for peptidoglycan from *E. coli* and used these to study interactions of the cell wall with full length OmpA in the presence of BLP (Figure 6)[44,45]. Our simulations showed that the distance between the cell wall and the OM is not as simple as just being a function of the BLP length. In the simulations, BLP was able to tilt and bend with respect to the OM, effectively shifting the cell wall closer to the membrane. This smaller gap facilitated initial binding of OmpA to the cell wall. Dimerisation of OmpA, eliminated this dependency of OmpA on BLP, as the OmpA C-

terminal domain bound to the cell wall even without BLP present, perhaps due to the stronger electrostatic interactions with the OmpA dimer. Experimental studies have shown that the dimeric interface is largely localised in the C-terminal domain[23], suggesting a possible role of the dimer in improving cell wall binding. Our simulations revealed that once bound to the cell wall, the length of the linker connecting the membrane embedded N-terminal ß-barrel domain and the peptidoglycan bound C-terminal domain of OmpA to be highly adaptable, which is potentially important in providing a flexible mechanical support for the cell wall. We observe cell wall undulations when the OmpA dimer is bound to the cell wall, in the absence of BLP. When BLP is present there are substantially fewer undulations. We note that OmpA mutants lacking BLP have been shown to survive but produce an increased number of vesicles than a normal cell[46] thus the undulations observed in our simulations may well represent a step in vesicle formation.

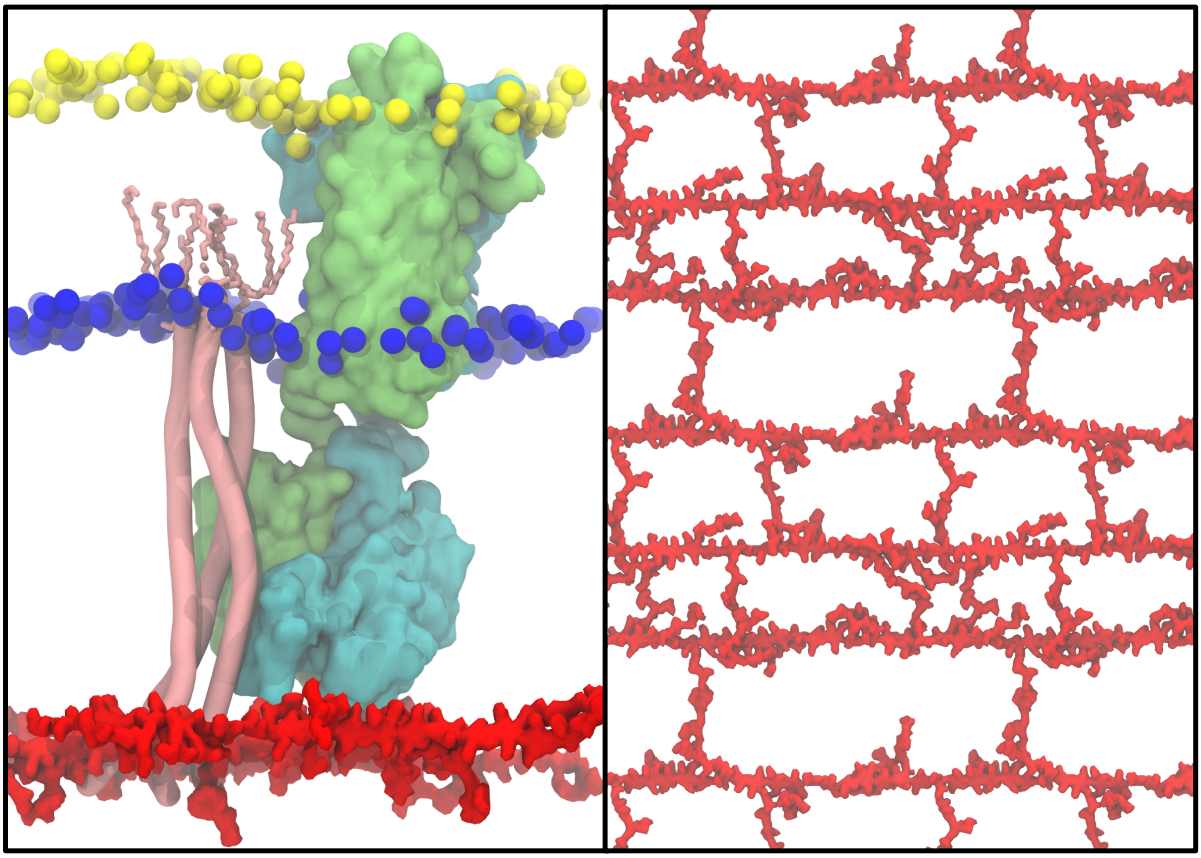


Figure 6. Snapshots of the simulations reported in Samsudin et al[44]. The components shown are peptidoglycan cell wall (red), OmpA dimer (cyan and green), BLP (pink) phosphorus atoms from LPS (yellow) and phosphorus atoms from phospholipids (blue). The panel on the left highlights interactions between these proteins and peptidoglycan, while the panel on the right shows the network of strands and cross-links within the peptidoglycan model.

Summary and outlook

In summary, the development of both atomistic and coarse-grained models of LPS have broadened the scope of molecular simulations of bacterial membranes, particularly when both levels of granularity are used to study different aspects of the

same system; the details of specific interactions are provided by the atomistic simulations whereas properties such as diffusion and local membrane biophysics are provided by coarse-grain models. We have learned that the loops of native OMPs form specific interactions with LPS, which are largely electrostatic in nature. These interactions slow down and alter the conformational lability of the loops and thus cannot be ignored when asking questions about *in vivo* behavior. Divalent cations tightly cross-link the lipid A headgroups, which leads to slow diffusion of LPS even in the absence of proteins. In this regard we note here that the structural rigidity of the OM of Gram-negative bacteria is similar to the OM of mycobacteria; the moieties of the latter (inner leaflet) are largely covalent bonded to each other and also to the cell wall[47]. In terms of larger systems; our simulations have revealed insights into the coupled nature of protein and lipid motion. We have shown that despite the vast differences between the mobilities of the two leaflets of the outer membrane, there is communication between them. The extent and nature of the routes of communication between the leaflets and the biological need for this communication is an area for future work. We have learned that the OmpA dimer is able to form persistent interactions that link the OM to the cell wall even in the absence of BLP. We are continuing our studies to explore whether this is a general property of multidomain OMPs that extend into the periplasm, bearing in mind that disrupting any potential interactions of membrane-anchored proteins with the cell wall may provide a target for novel antibiotics.

Looking ahead, atomistic simulations will continue to be invaluable for studying the nuances of molecular interactions such as those involved in protein-drug recognition and protein-protein interactions, with larger systems becoming tractable due to availability of greater computational power. Likewise, coarse grain methods will undoubtedly be used to characterise increasingly larger systems which incorporate multiple other cell envelope components and also extend to bacteria-host interactions. Given the wide range of (i) bacterial membrane protein and lipid structures and (ii) time and length scales involved in the biologically relevant phenomena of these structures, moving forward, multidisciplinary studies will be essential for future studies. The slow movement of LPS compared to phospholipids is likely to necessitate using enhanced sampling methods, particularly if we aim to bridge the gaps that currently exist between experimental and simulation studies. To study enzymatic process that occur within the bacterial membranes, the quantum level of detail will be an essential addition to our current toolkit. Thus in future, we will endeavour to develop protocols for truly multiscale simulations in our pursuit of the ‘virtual cell envelope’.

Biographical Information

Syma Khalid is Professor of Computational Biophysics at the University of Southampton. She received her MChem in Chemistry and PhD in Computational Chemistry from the University of Warwick. Her postdoctoral work was conducted in the Department of Biochemistry at Oxford University. Since 2007 she has been at the University of Southampton leading a research group focused on the structure-function-dynamics relationships of the bacterial cell envelope.

Thomas Piggot studied Biochemistry at the University of Bristol before completing a Masters in Bioinformatics at the University of York and returning to Bristol for a PhD

in Molecular Modelling. He did his postdoctoral work at the University of Southampton. Currently he works for the UK Ministry of Defence where his research interests range from applying DNA sequencing in biological detection, to using molecular simulations for biodefence.

Firdaus Samsudin is a postdoctoral research fellow at the University of Southampton. He utilizes molecular dynamics simulation to understand how bacteria protect themselves with their complex cell envelope. Firdaus completed a BSc in Biotechnology at Imperial College London and received a DPhil in Biochemistry from the University of Oxford.

References

(1) Nikaido, H. Molecular basis of bacterial outer membrane permeability revisited. *Microbiol. Molec. Biol. Rev.* **2003**, *67*, 593-656.

(2) Erridge, C.; Bennett-Guerrero, E.; Poxton, I. R. Structure and function of lipopolysaccharides. *Microbes Infect* **2002**, *4*, 837-851.

(3) Lins, R. D.; Straatsma, T. P. Computer simulation of the rough lipopolysaccharide membrane of *Pseudomonas aeruginosa*. *Biophys. J.* **2001**, *81*, 1037-1046.

(4) Piggot, T. J.; Holdbrook, D. A.; Khalid, S. Electroporation of the E. coli and S. Aureus membranes: molecular dynamics simulations of complex bacterial membranes. *The journal of physical chemistry. B* **2011**, *115*, 13381-13388.

(5) Wu, E. L.; Engstrom, O.; Jo, S.; Stuhlsatz, D.; Yeom, M. S.; Klauda, J. B.; Widmalm, G.; Im, W. Molecular dynamics and NMR spectroscopy studies of E. coli lipopolysaccharide structure and dynamics. *Biophys J* **2013**, *105*, 1444-1455.

(6) Vernier, P. T.; Ziegler, M. J.; Sun, Y. H.; Chang, W. V.; Gundersen, M. A.; Tieleman, D. P. Nanopore formation and phosphatidylserine externalization in a phospholipid bilayer at high transmembrane potential. *Journal of the American Chemical Society* **2006**, *128*, 6288-6289.

(7) Tarek, M. Membrane electroporation: a molecular dynamics simulation. *Biophys. J.* **2005**, *88*, 4045-4053.

(8) Levine, Z. A.; Wu, Y. H.; Ziegler, M. J.; Tieleman, D. P.; Vernier, P. T. Electroporation Sensitivity of Oxidized Phospholipid Bilayers. *Biophys J* **2009**, *96*, 41a-41a.

(9) Leive, L. Release of lipopolysaccharide by EDTA treatment of E. coli. *Biochemical and biophysical research communications* **1965**, *21*, 290-296.

(10) Lam, N. H.; Ma, Z.; Ha, B. Y. Electrostatic modification of the lipopolysaccharide layer: competing effects of divalent cations and polycationic or polyanionic molecules. *Soft Matter* **2014**, *10*, 7528-7544.

(11) Clifton, L. A.; Holt, S. A.; Hughes, A. V.; Daulton, E. L.; Arunmanee, W.; Heinrich, F.; Khalid, S.; Jefferies, D.; Charlton, T. R.; Webster, J. R.; Kinane, C. J.; Lakey, J. H. An Accurate In Vitro Model of the E. coli Envelope. *Angewandte Chemie* **2015**.

(12) Pontes, F. J.; Rusu, V. H.; Soares, T. A.; Lins, R. D. The Effect of Temperature, Cations, and Number of Acyl Chains on the Lamellar to Non-Lamellar Transition in Lipid-A Membranes: A Microscopic View. *J Chem Theory Comput* **2012**, *8*, 3830-3838.

(13) Soares, T. A.; Straatsma, T. P. Assessment of the convergence of molecular dynamics simulations of lipopolysaccharide membranes. *Mol Simulat* **2008**, *34*, 295-307.

(14) Berglund, N. A.; Piggot, T. J.; Jefferies, D.; Sessions, R. B.; Bond, P. J.; Khalid, S. Interaction of the antimicrobial peptide polymyxin B1 with both membranes of E. coli: a molecular dynamics study. *PLoS computational biology* **2015**, *11*, e1004180.

(15) Santos, D. E. S.; Pol-Fachin, L.; Lins, R. D.; Soares, T. A. Polymyxin Binding to the Bacterial Outer Membrane Reveals Cation Displacement and Increasing Membrane Curvature in Susceptible but Not in Resistant Lipopolysaccharide Chemotypes. *J Chem Inf Model* **2017**, *57*, 2181-2193.

(16) Hsu, P. C.; Jefferies, D.; Khalid, S. Molecular Dynamics Simulations Predict the Pathways via Which Pristine Fullerenes Penetrate Bacterial Membranes. *The journal of physical chemistry. B* **2016**, *120*, 11170-11179.

(17) Graham, J. A.; Essex, J. W.; Khalid, S. PyCGTOOL: Automated Generation of Coarse-Grained Molecular Dynamics Models from Atomistic Trajectories. *J Chem Inf Model* **2017**, *57*, 650-656.

(18) Jefferies, D.; Hsu, P. C.; Khalid, S. Through the Lipopolysaccharide Glass: A Potent Antimicrobial Peptide Induces Phase Changes in Membranes. *Biochemistry* **2017**, *56*, 1672-1679.

(19) Hsu, P. C.; Bruininks, B. M. H.; Jefferies, D.; de Souza, P. C. T.; Lee, J.; Patel, D. S.; Marrink, S. J.; Qi, Y. F.; Khalid, S.; Im, W. CHARMM-GUI Martini Maker for modeling and simulation of complex bacterial membranes with lipopolysaccharides. *Journal of Computational Chemistry* **2017**, *38*, 2354-2363.

(20) Piggot, T. J.; Holdbrook, D. A.; Khalid, S. Conformational dynamics and membrane interactions of the E. coli outer membrane protein FecA: a molecular dynamics simulation study. *Biochimica et biophysica acta* **2013**, *1828*, 284-293.

(21) Balusek, C.; Gumbart, J. C. Role of the Native Outer-Membrane Environment on the Transporter BtuB. *Biophys J* **2016**, *111*, 1409-1417.

(22) Khalid, S.; Bond, P. J.; Carpenter, T.; Sansom, M. S. OmpA: gating and dynamics via molecular dynamics simulations. *Biochimica et biophysica acta* **2008**, *1778*, 1871-1880.

(23) Marcoux, J.; Politis, A.; Rinehart, D.; Marshall, D. P.; Wallace, M. I.; Tamm, L. K.; Robinson, C. V. Mass spectrometry defines the C-terminal dimerization domain and enables modeling of the structure of full-length OmpA. *Structure* **2014**, *22*, 781-790.

(24) Ortiz-Suarez, M. L.; Samsudin, F.; Piggot, T. J.; Bond, P. J.; Khalid, S. Full-Length OmpA: Structure, Function, and Membrane Interactions Predicted by Molecular Dynamics Simulations. *Biophys J* **2016**, *111*, 1692-1702.

(25) Holdbrook, D. A.; Piggot, T. J.; Sansom, M. S.; Khalid, S. Stability and membrane interactions of an autotransport protein: MD simulations of the Hia translocator domain in a complex membrane environment. *Biochimica et biophysica acta* **2013**, *1828*, 715-723.

(26) Lee, J.; Patel, D. S.; Kucharska, I.; Tamm, L. K.; Im, W. Refinement of OprH-LPS Interactions by Molecular Simulations. *Biophys J* **2017**, *112*, 346-355.

(27) Wu, E. L.; Fleming, P. J.; Klauda, J. B.; Fleming, K. G.; Im, W. A Molecular Dynamics Simulation Study of Outer Membrane Phospholipase a (OMPLA) Structure and Dynamics in an Asymmetric Lipopolysaccharide Membrane. *Biophys J* **2014**, *106*, 656a-656a.

(28) Carpenter, T. S.; Parkin, J.; Khalid, S. The Free Energy of Small Solute Permeation through the Escherichia coli Outer Membrane Has a Distinctly Asymmetric Profile. *J Phys Chem Lett* **2016**, *7*, 3446-3451.

(29) Bemporad, D.; Luttmann, C.; Essex, J. W. Computer simulation of small molecule permeation across a lipid bilayer: dependence on bilayer properties and solute volume, size, and cross-sectional area. *Biophys J* **2004**, *87*, 1-13.

(30) Wennberg, C. L.; van der Spoel, D.; Hub, J. S. Large influence of cholesterol on solute partitioning into lipid membranes. *Journal of the American Chemical Society* **2012**, *134*, 5351-5361.

(31) Kumar, R.; Iyer, V. G.; Im, W. CHARMM-GUI: A graphical user interface for the CHARMM users. *Abstr Pap Am Chem S* **2007**, *233*, 273-273.

(32) Holdbrook, D. A.; Huber, R. G.; Piggot, T. J.; Bond, P. J.; Khalid, S. Dynamics of Crowded Vesicles: Local and Global Responses to Membrane Composition. *PloS one* **2016**, *11*, e0156963.

(33) Goose, J. E.; Sansom, M. S. Reduced lateral mobility of lipids and proteins in crowded membranes. *PLoS computational biology* **2013**, *9*, e1003033.

(34) Niemela, P. S.; Miettinen, M. S.; Monticelli, L.; Hammaren, H.; Bjelkmar, P.; Murtola, T.; Lindahl, E.; Vattulainen, I. Membrane proteins diffuse as dynamic complexes with lipids. *Journal of the American Chemical Society* **2010**, *132*, 7574-7575.

(35) Fowler, P. W.; Helie, J.; Duncan, A.; Chavent, M.; Koldso, H.; Sansom, M. S. Membrane stiffness is modified by integral membrane proteins. *Soft Matter* **2016**, *12*, 7792-7803.

(36) Chavent, M.; Duncan, A. L.; Rassam, P.; Birkholz, O.; Helie, J.; Reddy, T.; Beliaev, D.; Hambly, B.; Piehler, J.; Kleanthous, C.; Sansom, M. S. P. How nanoscale protein interactions determine the mesoscale dynamic organisation of bacterial outer membrane proteins. *Nat Commun* **2018**, *9*, 2846.

(37) Hsu, P. C.; Samsudin, F.; Shearer, J.; Khalid, S. It Is Complicated: Curvature, Diffusion, and Lipid Sorting within the Two Membranes of Escherichia coli. *J Phys Chem Lett* **2017**, *8*, 5513-5518.

(38) Corradi, V.; Mendez-Villuendas, E.; Ingolfsson, H. I.; Gu, R. X.; Siuda, I.; Melo, M. N.; Moussatova, A.; DeGagne, L. J.; Sejdiu, B. I.; Singh, G.; Wassenaar, T. A.; Magnero, K. D.; Marrink, S. J.; Tieleman, D. P. Lipid-Protein Interactions Are Unique Fingerprints for Membrane Proteins. *Acs Central Sci* **2018**, *4*, 709-717.

(39) Shearer, J.; Khalid, S. Communication between the leaflets of asymmetric membranes revealed from coarse-grain molecular dynamics simulations. *Sci Rep* **2018**, *8*, 1805.

(40) Gumbart, J. C.; Beeby, M.; Jensen, G. J.; Roux, B. Escherichia coli peptidoglycan structure and mechanics as predicted by atomic-scale simulations. *PLoS computational biology* **2014**, *10*, e1003475.

(41) Turner, R. D.; Hobbs, J. K.; Foster, S. J. Atomic Force Microscopy Analysis of Bacterial Cell Wall Peptidoglycan Architecture. *Methods in molecular biology* **2016**, *1440*, 3-9.

(42) Turner, R. D.; Mesnage, S.; Hobbs, J. K.; Foster, S. J. Molecular imaging of glycan chains couples cell-wall polysaccharide architecture to bacterial cell morphology. *Nat Commun* **2018**, *9*, 1263.

(43) Cohen, E. J.; Ferreira, J. L.; Ladinsky, M. S.; Beeby, M.; Hughes, K. T. Nanoscale-length control of the flagellar driveshaft requires hitting the tethered outer membrane. *Science* **2017**, *356*, 197-200.

(44) Samsudin, F.; Boags, A.; Piggot, T. J.; Khalid, S. Braun's Lipoprotein Facilitates OmpA Interaction with the Escherichia coli Cell Wall. *Biophys J* **2017**, *113*, 1496-1504.

(45) Samsudin, F.; Ortiz-Suarez, M. L.; Piggot, T. J.; Bond, P. J.; Khalid, S. OmpA: A Flexible Clamp for Bacterial Cell Wall Attachment. *Structure* **2016**, *24*, 2227-2235.

(46) Asmar, A. T.; Collet, J. F. Lpp, the Braun lipoprotein, turns 50-major achievements and remaining issues. *FEMS Microbiol Lett* **2018**, *365*.

(47) Chiaradia, L.; Lefebvre, C.; Parra, J.; Marcoux, J.; Burlet-Schiltz, O.; Etienne, G.; Tropis, M.; Daffe, M. Dissecting the mycobacterial cell envelope and defining the composition of the native mycomembrane. *Sci Rep* **2017**, *7*, 12807.