# Chapter 19

# Recent Advances in Modeling Membrane β-Barrel Proteins Using Molecular Dynamics Simulations: From Their Lipid Environments to Their Assemblies

**Anna L. Duncan, Ya Gao, Evert Haanappel, Wonpil Im, and Matthieu Chavent**

## Abstract

Spurred by advances in AI-driven modeling and experimental methods, molecular dynamics simulations are now acting as a platform to integrate these different approaches. This combination of methods is especially useful to understand β-barrel proteins from the molecular level, e.g., identifying specific interactions with lipids or small molecules, up to assemblies comprised of hundreds of proteins and thousands of lipids. In this minireview, we will discuss recent advances, mainly from the last 5 years, in modeling β-barrel proteins and their assemblies. These approaches require specific kinds of modeling and potentially different model resolutions that we will first describe in Subheading 1. We will then focus on different aspects of β-barrel protein modeling: how different types of molecules can diffuse through β-barrel proteins (Subheading 2); how lipids can interact with these proteins (Subheading 3); how β-barrel proteins can interact with membrane partners (Subheading 4) or periplasmic extensions and partners (Subheading 5) to form large assemblies.

**Key words** Molecular dynamics, Multiscale modeling, Outer membrane proteins, Protein-protein interactions, Protein-lipid interactions

## Abbreviations

| | |
|---|---|
| AA | All-atom |
| AI | Artificial intelligence |
| CG | Coarse grained |
| CIP | Ciprofloxacin |
| LOS | Lipooligosaccharide |
| LPS | Lipopolysaccharide |
| Lpt | Lipopolysaccharide transport |
| MD | Molecular dynamics |
| ML | Machine learning |
| MSM | Markov state model |
| NMR | Nuclear magnetic resonance |

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OM Outer membrane
OMP Outer membrane protein
PG Peptidoglycan
PNA Peptide nucleic acid
TASS Temperature accelerated sliced sampling

## 1 Introduction

β-barrel proteins are made of anti-parallel ß-sheets arranged in a cylindrical shape with a hydrophilic interior and a hydrophobic exterior. This confers interesting biophysical properties to this class of proteins, which can be modeled using molecular dynamics (MD) simulations at different scales, enabling us to capture and explain in particular the capability to span biological membranes and interact with protein partners, while allowing the diffusion of small molecules through their interior. Several decades of progresses have culminated in modeling in silico the complexity of biological membranes [1–3]. This is especially true for modeling bacterial membranes [4–7]. In this minireview, we will showcase recent advances, mainly obtained during the last 5 years, in modeling β-barrel proteins from understanding local interactions with small molecules, surrounding lipids, and protein partners to studying large protein assemblies. We will also show how methodological advances have helped to model β-barrel proteins and their interactions with intracellular partners extending the canonical 2D scope (i.e., in the membrane plane) to a fully 3D landscape.

## 2 Methodological Development to Model β-Barrel Proteins

Before presenting applications, it is important to first describe the methodological context. Methodological advances were driven by improvements both in computational power [8–10] and in experimental techniques capable of determining the structure of very large membrane protein assemblies [11–14]. It is currently possible to model both very large assemblies composed of millions of particles and very specific interactions between few atoms. To switch from one type of modeling to the other, it is often required to adapt the resolution of the model to the given size of the system [15]. Two widely used resolutions in MD simulations are atomistic (or all-atom, AA) and coarse-grained (CG) resolutions. The former allows modeling all the atoms and molecular interactions, while the latter groups several atoms (typically four heavy atoms, *see* **Notes 1** and **2**) into one bead, which considerably reduces the complexity of the model [16]. One of the main force fields (*see* **Notes 1** and **2**) used in CG-MD simulations is the so-called MARTINI force field

[17]. In comparison with CG-MD simulations, the diversity of the force fields for atomistic simulations is more pronounced [18] and researchers need to assess which force field is the most suited to their needs [19–22]. It is possible to switch from one type of resolution to the other using dedicated tools [23–25]. It is worth mentioning that artificial intelligence (AI) approaches are now clearly impacting the development of force fields for MD simulations [26, 27]. For interested readers who want to get started using MD simulations for their research, accessible tutorials and guides are available to model lipid membranes [28] and membrane proteins [29, 30].

Before performing the actual MD simulations, it is first necessary to build the simulation system, with all its molecular components (proteins, lipids, ligands, ions, and water molecules) properly placed. There now exist dedicated tools streamlining the process of model creation. One example is CHARMM-GUI *Membrane Builder*, a webserver allowing the creation of a complete membrane system both in AA and in CG resolution [31–34]. Notably, CHARMM-GUI Membrane Builder supports various lipopolysaccharide (LPS) models from many different Gram-negative bacteria [32]. To model membrane systems with the MARTINI CG force field, one can also use a Python script called *insane* (an acronym for INSert membrANE) to model either lipid membranes or membrane protein assemblies [35]. MemProtMD is a database containing thousands of membrane proteins, extracted from the Protein Data Bank (PDB), embedded into a model membrane [36]. Simulations and the subsequent analysis results can be conveniently accessed through a web browser, enabling users to explore interactive 3D visualizations of the assembled bilayer, as well as 2D visualizations depicting lipid contact data and membrane protein topology. To specifically model outer membrane proteins (OMP) from Gram-negative bacteria, Baltoumas and colleagues have developed an automated pipeline to insert OMP models into LPS-containing membranes [37].

Once the calculations for MD simulations are completed using one of the various simulation codes [38], one needs to visualize and analyze the results. This task has become increasingly difficult due to the size and the complexity of the models. Specific tools and methodologies are now available to render the visualization and the analysis of membrane systems within reach of the majority of researchers [28, 39].

## 3 Through the Pore: From Channels to Nanopores

In bacteria and mitochondria, β-barrel proteins situated in the OM constitute the passage point for numerous molecules. MD simulations at AA resolution allow the study of how these molecules can

interact with protein loops and diffuse through the pore. Hereafter, we present a selection of recently modeled molecules, ranging from few-atom molecules (ions and small compounds) to oligonucleotides and peptides (*see* Fig. 1).

Extensive AA-MD simulations were used in combination with a Markov state model (MSM) to explore the dynamics of the L3 internal loop in the *E. coli* OmpF protein [40]. These simulations help to distinguish between open and closed states of OmpF. In this latter state, electrophysiology simulations revealed a significant reduction of ionic currents. AA-MD simulations were also used to study permeation paths of potassium and chloride ions in VDAC channels of *Saccharomyces cerevisiae* [41]. The ion permeation properties may be influenced by surrounding OM lipids. Using AA-MD simulations, Lee et al. have shown how the outer core and O-antigens of LPS may sterically occlude the channel entrance and decrease the diffusion constants of ions approaching the OccK5 protein (also known as OpdH) from *Pseudomonas aeruginosa* [42]. MD simulations were also used in combination with solid-state NMR to study the permeability of AlkL from *Pseudomonas putida*, a minimalistic OMP, for hydrophobic molecules such as carvone or octane [43]. In this work, the authors proposed the release of hydrophobic compounds in the membrane after a diffusion through extracellular loops. Contrary to hydrophilic compounds, here the hydrophobic compounds do not seem to traverse the pore. This lateral diffusion model was also proposed for the FadL channel of *P. putida* for the uptake of monoaromatic hydrocarbons (MAH) such as benzene or toluene [44].

It is possible to transport even larger hydrophilic compounds like amino acids through the pore of a β-barrel protein. Samsudin and Khalid performed steered MD and umbrella sampling simulations (*see* **Note 3**) to determine the permeation pathway of arginine through the OprD channel from *P. aeruginosa* [45]. MD simulations also suggested that the arginine is surrounded by a shell of water molecules during the translocation through the pore. The binding of the peptide substrate ARRA to *E. coli* OmpT was recently explored by combining AA-MD simulations and umbrella sampling [46]. The translocation of even larger peptides like protamine, a 32-amino-acid-long polycationic peptide, through the CymA channel from *Klebsiella oxytoca* has been studied [47]. MD simulations were also used to study the interaction of *E. coli* BtuB with a peptide nucleic acid (PNA) covalently linked to vitamin $B_{12}$ [48]. PNA is a synthetic DNA analog with a peptide-like backbone that can strongly bind to nucleic acids essential for bacterial growth. This can thus constitute an interesting antibacterial strategy. The transport of vitamin $B_{12}$ alone through *E. coli* BtuB was also explored by combining steered MD, umbrella sampling, and Gaussian force-simulated annealing [49].

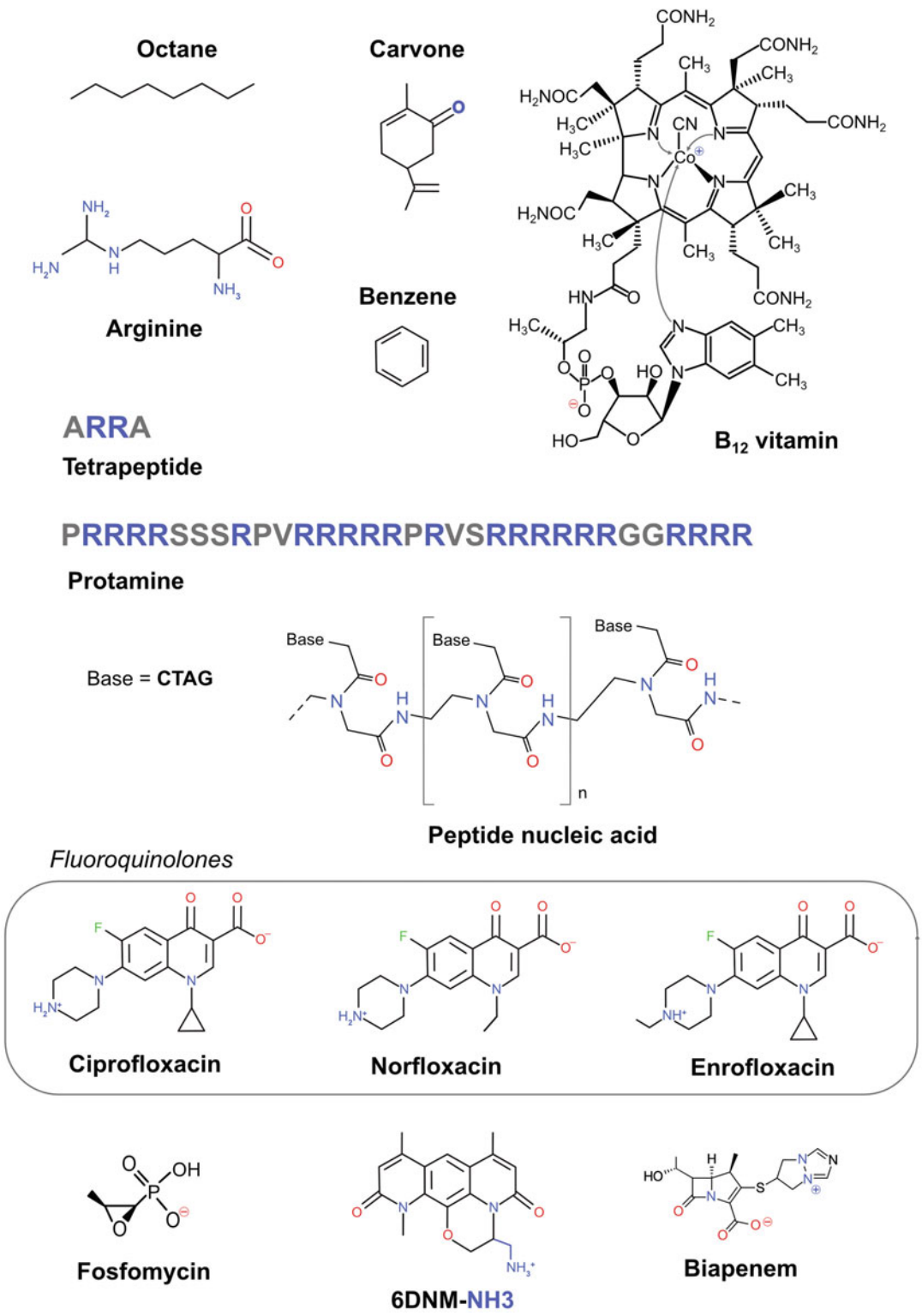


**Fig. 1** Subset of small molecules and peptides, presented in Subheading 3, interacting with β-barrel proteins studied by multiscale molecular dynamics simulations

Due to their crucial role in the uptake of nutrients, β-barrel proteins are the target of numerous molecules that can potentially block this uptake and thereby act as antimicrobial drug candidates. MD simulations can give valuable insight into understanding how these molecules affect the structures and functions of β-barrel proteins. Fluoroquinolones are a class of broad-spectrum antibiotics. The permeation of ciprofloxacin (CIP), through OmpF, was studied using a temperature-accelerated sliced sampling (TASS) approach to characterize the two potential permeation pathways—the orientation of CIP inside the pore and its interactions with water molecules [50, 51]. The effect of divalent ions on the diffusivity of norfloxacin was investigated by AA-MD simulations and tested across several OM channels: OmpF and OmpC from *E. coli* and Omp35 and Omp36 from *Enterobacter aerogenes* [52]. Enrofloxacin and CIP permeation pathways across OmpC were also explored [53, 54]. Fosfomycin is a small phosphonic acid antibiotic discovered in *Streptomyces* strains. The fosfomycin permeability across the *E. coli* OmpF was investigated by AA-MD simulations including free energy and applied field techniques [55]. Fosfomycin translocation was also studied by AA-MD simulations for OrpO and OrpP from *P. aeruginosa* [56]. Recently an extensive set of AA-MD simulations and free energy calculations were performed to better understand how the introduction of a primary amine might enhance the permeation of antibiotics through OM channels [57]. The amine may enhance permeation by allowing the molecule to align its dipole with the electric field inside the porin's lumen, and it also establishes favorable electrostatic interactions with charged residues as the molecule moves through the pore. While these studies were all performed using AA-MD simulations, the recent developments of the MARTINI force field [17], especially aimed toward modeling small molecules [58], may allow researchers to use this less expensive approach to study antibiotic interactions with β-barrel proteins. Recent work explored how to use CG-MD simulations to investigate the interaction of the *P. aeruginosa* OccD3 porin with different carbapenems [59].

β-barrel proteins can be also engineered [60] and redesigned to serve as synthetic nanopores [61] to create, e.g., biomimetic membranes for water filtration [62] or to sequence nucleic acids [63] or proteins [64, 65]. MD simulations can help to better characterize these new molecules and their interactions with different solutes [61]. Two main classes of β-barrel proteins were studied using multiscale MD simulations: bacterial channels and pore-forming toxins (this is elaborated in Subheading 4). In the first category, one can cite the investigation of cyclodextrin and ions transport through the ΔCymA nanopore, a mutant of the protein without the 15 N-terminal residues, from *K. oxytoca* [66, 67]. MD simulations were also used to probe the stability of de novo design of a β-hairpin and its nanopore assembly in a membrane. This assembly formed a

pore allowing the detection of a single polypeptide chain [68]. In the second category, the biophysical properties of several nanopores based on pore-forming toxin were investigated. The α-hemolysin was studied to assess its ability to recognize homopeptides [69] and its ionic transport and selectivity [70, 71]. For the aerolysin nanopore, translocation of different poly-arginine peptides [72], nucleotide discrimination [73], and detection of posttranslational modifications [74] were investigated. Ion conductance of both bacterial channels and pore-forming toxin nanopores was investigated using steric exclusion model and AA-MD simulations [75]. For interested readers, a recent perspective has summarized advances in understanding key issues in molecular simulations of antibiotic translocation and in the development of nanopore sensors [76].

## 4 β-Barrel Proteins in Their *Local* Environment

Lipids play fundamental roles in the folding [77, 78] and stability [79] of β-barrel proteins and in anchoring OMPs within the OM. MD simulations are uniquely capable of identifying and assessing specific protein-lipid interactions [80] as well as allowing the characterization of lipid binding sites [81].

Modeling of bacterial OM lipids has recently seen much progress, both at the atomistic scale [82, 83] and at CG resolution [33, 84, 85], enabling a wealth of new studies, in particular on OMP-LPS interactions (*see* Fig. 2a), which are challenging to study experimentally. AA-MD simulations have identified the role of calcium in specific OMP-LPS interactions and identified binding sites on *E. cloacae* OmpE36 that were in good agreement with those observed in crystal structures [90]. LPS has further been shown to have a unique interaction fingerprint with a diverse array of *E. coli* OMPs (OmpA, FhuA, OmpF, EstA, BtuB, and OmpX) [91]. Beyond the structural level, recent studies have shown that LPS plays a role in regulating OMP function. As an example, AA-MD simulations have shown that interactions of LPS with OprH affect the structure and dynamics of its extracellular loops [92]. As mentioned previously, AA-MD simulations of Occk5 demonstrated that LPS modulates ion transport through this OMP by hindering ion accessibility to the pore [42]. MD simulations in tandem with NMR spectroscopy showed that LPS interactions with the Ail protein change pathogen membrane properties, which confers enhanced resistance to the plague-causing bacterium *Yersinia pestis* [93]. MD simulations have also been used to uncover how LPS interactions with OmpD drive effective immunization with this OMP from *S. typhimurium* but not *S. enteritidis*, despite only the single amino acid difference between the OmpD homologs

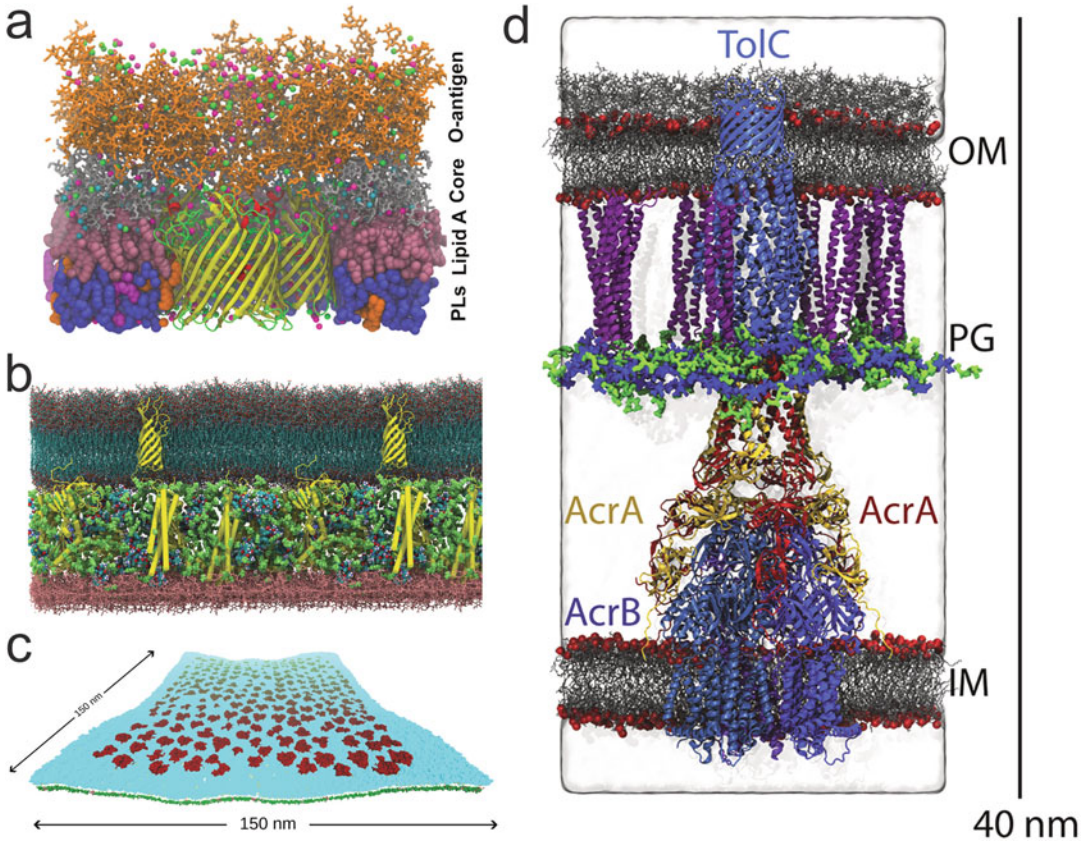


**Fig. 2** Illustrative examples of systems presented in Subheadings 3, 4, and 5. (**a**) AA modeling of the OmpF protein surrounded by LPS [86]. (**b**) AA model of OM and periplasmic space containing proteins and osmolytes [87]. (**c**) CG modeling of an OMP island measuring 150 $nm^2$ along the membrane plane [88]. OMPs are shown in red and are embedded in an outer membrane model containing 100% RaLPS in the outer leaflet, and a 90:5:5 ratio of POPE/POPG/cardiolipin in the inner leaflet. The RaLPS core region is shown in cyan, RaLPS phosphates are shown in yellow, and RaLPS acyl chains are shown in white. POPE, POPG, and cardiolipin lipids are shown in green, white, and pink, respectively. (**d**) AA modeling of the AcrAB-TolC multidrug efflux pump spanning the whole cell envelope [89] from the inner membrane (IM) through the peptidoglycan (PG) and up to the outer membrane (OM)

[94]. The interactions of surface-exposed loops with lipooligosaccharide (LOS) in a protein sequence dependent manner may alter the binding of antibodies to β-barrel proteins as seen for PorB from *N. meningitidis* [95]. MD simulations coupled with advanced mass spectrometry techniques have proposed how LPS is inserted by the LPS transport protein LptDE from *K. pneumoniae* [96] and helped to describe the mechanisms of deacylation of LPS by the β-barrel protein, LpxR, from *S. typhimurium* [97]. AA and CG MD simulations illustrated how thermodynamics drives membrane association of numerous lipoproteins, including the *E. coli* BAM complex, LptE, and CusC [98].

Multiscale simulations have also been used to show how other lipids affect β-barrel protein function. For instance, phosphatidylethanolamine of the mitochondrial OM influences the anion selectivity of VDAC and thereby regulates its function [99], while CG-MD simulations highlighted how ceramide lipids can bind to VDAC2 to trigger mitochondrial apoptosis, thus acting as a tumor-suppressing lipid [100]. Integrating MD and Brownian dynamics and electric field simulations with biochemical data has resulted in the ability to model protein complexes, such as the complex formed by the mitochondrial VDAC1 and hexokinase II, which would have been extremely challenging to achieve solely by experimental means, due to the interaction of hexokinase II with the membrane [101]. Phosphatidylglycerol binding to OmpF is sensitive to pH, and MD simulations mimicking different pH levels uncovered lipid interaction patterns suggesting that the lipid interactions attenuate *E. coli* OmpF channel closure [102].

## 5 Biogenesis and Large Assemblies of β-Barrel Proteins

β-barrel proteins are composed of a cylindrical arrangement of antiparallel β-sheets with hydrophobic residues tending to face the barrel's exterior. This structure allows specific features and constraints in term of protein folding, biogenesis, and interactions with membrane protein partners. Recently, de novo design was applied to engineer custom β-barrel proteins [103]. Multiscale (AA and CG) MD simulations are especially useful to gain insights into the biogenesis of these proteins, their folding, and the formation of larger membrane protein assemblies.

The biogenesis of OMPs in Gram-negative bacteria is mediated by the β-barrel assembly machinery (BAM) composed of five components called Bam A–E [104, 105]. The opening of BamA releasing newly folded β-barrel proteins into the OM, called lateral gating, was studied by AA-MD simulations [106]. Membrane thinning and distortion near this BamA's lateral gate were observed in simulations [107, 108]. The conformational plasticity of BAM was explored by Cryo-EM and MD simulations [109]. This study demonstrated that plasticity of the barrel domain of BamA is essential for the function of BAM. The BamA plasticity was also studied in the context of its interaction with its substrate EspP by Cryo-EM [110] and MD simulations [111] allowing a better characterization of the sequential conformational dynamics of BAM during the late stages of OMP assembly. Due to its central role of OMP biogenesis, BamA has been a target for the development of novel antibiotics. One of them, dynobactin A, identified by computational approaches, specifically targets lateral gating [112]. This BamA-antibiotic interaction was recently investigated by MD simulations [113]. Recently, a combination of cryo-electron microscopy, X-ray

crystallography, native mass spectroscopy, in vivo experiments, and MD simulations was also used to decipher the association of Darobactin with BamA [114].

After the incorporation into the OM, β-barrel proteins can form large molecular assemblies with restricted diffusion, so-called islands [115]. CG-MD simulations suggested that island formation was driven by protein-protein interactions [115, 116]. Due to limitations of this CG modeling to depict realistic diffusion of LPS, these initial CG-MD simulations were performed without LPS molecules. Recently, however, these models were further extended to take into account the role of LPS in mediating protein-protein interactions [88] (*see* Fig. 2c). These large supramolecular assemblies of membrane proteins and lipids may affect the rigidity of the membrane as seen by CG-MD simulations [117]. Assemblies of other types of proteins, such as pore-forming proteins [118, 119], may even drastically affect the membrane organization. While individual pore-forming proteins are soluble, the assembly of multiple copies of these proteins at the membrane may lead to the formation of a large β-barrel pore. Assembly, interactions with lipids, and membrane pore formation by pore-forming proteins were investigated using both AA- and CG-MD simulations. Pneumolysin prepore interaction with lipids and intermediate steps leading to a complete pore were investigated by multiscale simulations [120]. Using a similar multiscale approach, the creation of a pore by gasdermin proteins has also been characterized [121–123].

## 6 Beyond the Membrane Plane

β-barrel proteins can also extend outside the membrane plane both via intra- and extracellular domains and interactions with protein partners [124, 125]. Here we will focus our attention on periplasmic domains. These may modulate β-barrel proteins functions, help transferring substrates, or favor interactions with other layers of the bacterial envelope such as the PG. The BamA subunit contains a large periplasmic domain composed of five globular polypeptide transport-associated (POTRA) motifs linked in tandem and numbered 1–5 from the N-terminal end [126]. Atomistic MD simulations have proposed a model of POTRA interacting with the phospholipids not only via hydrogen bonds but also via hydrophobic interactions engaged with tryptophan residues [127]. These interactions may favor different POTRA structural configurations by drifting at the membrane surface. Some of these conformations may be compatible with binding to BamB and BamD. CryoEM and AA-MD simulations of the BAM machinery (containing BamA +POTRA and BamB-E) in a reconstituted nanodiscs revealed membrane deformations [108]. The lipopolysaccharide transport (Lpt)

machinery transfers LPS molecules into the OM [128]. The release of LPS molecules may also occur via a lateral gating mechanism as seen for BamA (*see* Subheading 4). AA-MD simulations have suggested that the periplasmic domain of LptDE is highly dynamic [129] and that the LPS substrate helps the opening of the lateral gate [96, 129].

The Khalid lab has used AA-MD simulations to study how the dimerization of OmpA protein through its cytoplasmic C-terminal domain may maintain a distance between the PG layer and the OM hence limiting the PG distortion [130]. AA-MD simulations also suggested that OmpA interactions with the PG layer are facilitated by tripartite contacts between Braun's lipoprotein, the PG layer, and the OmpA C-terminal domain [131]. The crowded OM and periplasm were also modeled using AA-MD simulations to study the travel of the antibiotic polymyxin B1 through the periplasm [87]. These simulations revealed that polymyxin B1 forms both transient and long-lived interactions with proteins, osmolytes, lipids of the OM, and the cell wall and is rarely uncomplexed when in the periplasm (*see* Fig. 2b).

Recent works have also started modeling how trans-envelope processes, such as mechanical stress sensing and metabolite efflux, are coordinated across the three envelope layers of Gram-negative bacterial cells, i.e., IM PG OM [89, 132] (*see* Fig. 2d).

## 7 Conclusion

With their β-barrel core, β-barrel proteins may be seen as less flexible than other types of proteins [133, 134]. However, based on recent advances coupling multiscale MD simulations with other experimental approaches, it is now clear that their intrinsic flexibility coupled to their diffusion in the membrane and their interactions with membrane peripheral partners play an important role in their function.

Here, we have given an overview of recent advances in MD simulations to decipher β-barrel protein dynamics at different scales: from the flexibility of extracellular loops and their interactions with lipids and small molecules to large assemblies of proteins and diffusion of molecules in different regions of the cell envelope. We are now moving toward very large and complex models to create digital twins of the bacterial cell envelope to develop new antibiotics or membranes composed of nanopores to design new biotechnological tools.

With recent advances in AI and experiments integrated to MD simulations, it will become increasingly feasible to model very complex biophysical mechanisms involving β-barrel proteins and shed new lights onto their function.

## 8 Notes

1. The term "force field" refers to a set of potential energy terms and specific implementation details, including parameter values, to calculate intra- and inter-molecular forces between atoms and particles (for CG systems, see below) [38]. Different force fields are optimized for specific types of molecules such as proteins [135], lipids [136] or DNA [137]. Thus, it is important to use the most appropriate force field for a given molecular system.
2. The goal of coarse grained (CG) mapping is to provide accelerated calculations by reducing the effective number of particles and using longer time scales, compared to AA resolution. In general, CG mapping requires a bottom-up strategy [138] by grouping several atoms together into a single bead and by assigning their overall chemical features (polarity, hydrophobicity, charge) and center of mass to that bead. For the MARTINI force field, the CG mapping originally consisted of grouping typically four heavy atoms (i.e., not including the hydrogen atoms) into one bead. With the development of the third version of MARTINI [139], it is now possible to select smaller bead types representing two to three atoms. Numerous tools exist that streamline CG mapping, such as PyCGTOOL [140], Auto-MARTINI [141], Swarm-CG [142, 143], CGCompiler [144], or MAD [145]. Recently, the use of machine learning (ML) has helped to automatically design new CG models for proteins [27, 146, 147].
3. The two approaches *known as steered molecular dynamics (SMD) simulations and umbrella sampling (US)* belong to the enhanced sampling methods category that allow sampling of larger portions of the configuration space of complex systems in a given amount of simulation time [148]. Steered molecular dynamics emulates atomic force microscopy experiments by introducing a fictitious 3D particle moving at constant velocity and connected to a molecule by a harmonic spring. It is often used to study folding/unfolding of proteins or ligand binding [149, 150]. Umbrella sampling allows exploration of one specific path by biasing the simulation along one (or more-dimensional) reaction coordinate to calculate energy barriers between different states [151].

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