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REVIEW

Model architectures for bacterial membranes

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Abstract
The complex composition of bacterial membranes has a significant impact on the understanding of pathogen function and their development towards antibiotic resistance. In addition to the inherent complexity and biosafety risks of studying biological pathogen membranes, the continual rise of antibiotic resistance and its significant economical and clinical consequences has motivated the development of numerous in vitro model membrane systems with tuneable compositions, geometries, and sizes. Approaches discussed in this review include liposomes, solid-supported bilayers, and computational simulations which have been used to explore various processes including drug-membrane interactions, lipid-protein interactions, host–pathogen interactions, and structure-induced bacterial pathogenesis. The advantages, limitations, and applicable analytical tools of all architectures are summarised with a perspective for future research efforts in architectural improvement and elucidation of resistance development strategies and membrane-targeting antibiotic mechanisms.

Keywords Model membrane · Lipids · Membrane · Biophysics · Bacteria

Introduction

All organisms rely on the presence of biological membranes acting as barriers between the inside and outside cellular environments. The functionality of such membranes is dictated by the types of lipids and other molecules that make up their often highly complex structure (Watson 2015; Guidotti 1972).

The "ESKAPE" pathogens, a faction of Gram-negative (GN) and Gram-positive (GP) bacteria, are responsible for the majority of nosocomial infections and are deemed a great threat to global healthcare because of their multidrug resistance (MDR) (Boucher et al. 2009; Mar et al. 2017; Pendleton et al. 2013; Rice 2010; Santajit and Indrawattana 2016; Ventola 2015). MDR bacterial pathogens can overexpress intrinsic resistance markers via adaptive mutations and acquire various foreign resistance factors through gene transfer processes (Gould and Bal 2013; Ventola 2015; Chilambi et al. 2018; Fernández and Hancock 2012; Prestinaci et al. 2015; Jiang et al. 2019a). This makes them resistant to even the most effective antimicrobial medications, rendering once treatable infections untreatable (Mar et al. 2017; Renwick et al. 2016). Antimicrobial resistance has resulted in significant economic damage due to increased patient morbidity and mortality (Boucher et al. 2009; Ventola 2015; Renwick et al. 2016; Dutescu and Hillier 2021; D'Andrea et al. 2019; Tacconelli et al. 2018). Given the lack of success in marketing novel therapeutic antimicrobial agents including teixobactins, antimicrobial nanomaterials, and micro-engineered biomolecules (Mulani et al. 2019; Makabenta et al. 2021; Fatima et al. 2021; Mantravadi et al. 2019; Charbonneau et al. 2020; Hussein et al. 2020), current research has been devoted to sourcing natural antimicrobial products due to their chemical diversity and reported effectiveness as narrow- or broad-spectrum antibiotics (Hutchings et al. 2019; Quinto et al. 2019; Ghrairi et al. 2019). However, further research is required to ensure their clinical utility and to develop a better understanding of their mechanism of action. This highlights the critical requirement to understand the mechanisms behind pathogen resistance development and antimicrobial action.

The bacterial lipid membrane of MDR pathogens plays a significant part in the resistance development towards membrane-targeting antibiotics (polymyxins, β-lactams, glycopeptides, and lipopeptides), which typically penetrate the cell membrane to facilitate cellular entry of medication,

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or directly disrupt the cell membranes structural integrity to facilitate cell lysis (Kapoor et al. 2017; Epand et al. 2016; Tenover 2006; Dias and Rauter 2019). The membrane lipid profile can dictate the effectiveness of antibiotics and drug-efflux proteins that mediate the expulsion of antibiotics from the bacterium. Pathogen adaptation mechanisms alter the native lipid composition which facilitates structural modifications, including changes in membrane fluidity, organisation, and packing, that circumvents the effects of antibiotics and evades host immune attack (Jiang et al. 2019a, 2019b; Dadhich and Kapoor 2020; Han et al. 2018; Maifiah et al. 2016; Mishra et al. 2012). The unique structure of the membrane in GN bacteria is the primary reason for their rapid resistance development compared to GP bacteria (Breijyeh et al. 2020; Ghai and Ghai 2018). The lipid asymmetry, rigidity, and biochemistry of the LPS molecules in the membrane provide a considerable defensive barrier against numerous antibiotics (Breijyeh et al. 2020; Delcour 2009; Vasoo et al. 2015). Changes in the lipophilic composition and membrane structure can also influence various membrane-associated processes such as protein-lipid electrostatic interactions, ligand-binding, cell-to-cell communication, transport, and protein folding, translocation, and function (Corradi et al. 2019; Collinson 2019; Lin and Weibel 2016; Martens et al. 2019, 2016; Norimatsu et al. 2017; Du et al. 2018).

The bacterial lipid membrane is a viable target for novel antibiotic treatments as the lipophilic composition is crucial to antibiotic efficacy, and targeting the lipid membrane rather than biochemical pathways can prolong antibiotic resistance development (Dias and Rauter 2019; Lam et al. 2016). A better understanding of the bacterial lipid membrane and its interactions with antibiotics is thus imperative for subsequent antibiotic research and development efforts.

However, systematic studies of the bacterial cell membrane structure and its processes are difficult to perform when studying live bacterial cells due to the nanometre dimensions of their membranes as well as their high level of complexity (Behuria et al. 2020). Bacteria also possess a cell wall that requires removal prior to investigating membrane-mediated activities (Brown et al. 2010; Veron et al. 2008). The inherent complexity of biological bacterial cell membranes which contain numerous peptides, sugars, membrane proteins, lipids, and carbohydrates makes systematic investigations difficult (Andersson et al. 2018a; Castellana and Cremer 2006). Pathogenic bacteria especially pose unique investigatory challenges due to rigorous biosafety protocols (Behuria et al. 2020). An alternate method to analyse membrane-associated processes is to purify the bacterial membrane; however, the isolation process requires expensive instrumentation which is difficult to perform in common laboratories (Qing et al. 2019). Due to these limitations, progressions in the understanding of the organisation, structure, and processes that occur in biological bacterial membranes have been driven primarily through research on in vitro model membrane systems (Strahl and Errington 2017).

A variety of different model systems have been designed to mimic biological membranes in a controlled environment with only the most essential components (Salehi-Reyhani et al. 2017). Model membranes were developed as an accessible experimental platform to analyse membrane structure and function in an environment that replicates the fundamental environmental and physiochemical properties of biological membranes, whilst reducing their innate complexity (Andersson et al. 2018a, 2020, 2018b; Andersson and Köper 2016; Chan and Boxer 2007; Jackman et al. 2012; Siontorou et al. 2017). Model membrane systems are computationally modelled, free-standing, or solid-supported bilayer structures composed of various lipophilic compounds and proteins (Chan and Boxer 2007; Siontorou et al. 2017).

They enable the use of numerous microscopic, spectroscopic, electrochemical, reflectometric, and algorithmic analytical techniques often inaccessible when studying live cells (Wiebalck et al. 2016; Zieleniecki et al. 2016). The analytical techniques can, for example, reveal the mechanism of action surrounding membrane-targeting antibiotics (Peetla et al. 2009; Knobloch et al. 2015). Numerous model membrane systems have been designed to investigate membrane-drug interactions (Hollmann et al. 2018); however, few mimic bacterial membranes or the architecture of the ESKAPE pathogens.

Here, we provide an overview of the structure and lipophilic composition of GN and GP bacterial membranes and current membrane modelling systems for these structures, including liposomes, solid-supported bilayers, and computational simulations.

Bacterial membranes

Lipids in bacterial membranes serve as important structural and functional constituents and have important roles in membrane organisation, cell recognition, membrane fluidity, energy storage, direct modulation, membrane stability, cell signalling, and membrane formation (Solntceva et al. 2020; Carvalho and Caramujo 2018; Willdigg and Helmann 2021). To perform such complex and diverse functions, bacterial membranes are composed of approximately equivalent proportions of lipids and proteins and are complex structures with a high degree of organisation and variation between bacterial species and their GN and GP classifications (Strahl and Errington 2017; Epand and Epand 2009a; Sohlenkamp and Geiger 2016).

GN and GP bacterial lipid membranes are predominantly formed by phospholipids which are composed of a phosphate group, 2–4 hydrophobic fatty acid units, a variable

hydrophilic head group, and a glycerol moiety (Sohlenkamp and Geiger 2016; Alagumuthu et al. 2019; Fahy et al. 2011). Phospholipids are organised in a classical bilayer described by the fluid-mosaic model (Singer and Nicolson 1972). The model has since been refined to accommodate the presence of lipid domains and cytoskeletal proteins that restrict and sectionalise lipid and protein diffusion (Strahl and Errington 2017; Meer et al. 2008; Barák and Muchová 2013). Both GN and GP bacteria contain a large variety of straight or branched, saturated, or unsaturated carboxylic acids with long aliphatic chains, known as fatty acids, that serve as essential building blocks for multiple lipophilic compounds (Carvalho and Caramujo 2018; Cronan and Thomas 2009). Numerous glycolipids, which are composed of a carbohydrate attached by a glycosidic bond containing 1–2 fatty acid units, are also typical constituents in the membranes of GN and GP bacteria (Bertani and Ruiz 2018; Reichmann and Gründling 2011). In addition to the aforementioned common lipid species, bacteria can also possess species-specific lipids (Solntceva et al. 2020).

Within bacterial species of different and the same Gram types, the lipid membrane contains a high degree of structural, chemical, and functional variability whereby numerous lipid molecular variants are present that differ in size, number, chemical composition, and isomeric form (Strahl and Errington 2017; Sohlenkamp and Geiger 2016; May and Grabowicz 2018; Rahman et al. 2000). Pathogens can also readily acquire multiple exogenous lipophilic bodies which generate substantial variation between pathogen strains and species (Jiang et al. 2019a; Jasim et al. 2018). The key lipid species present in the ESKAPE pathogens has been studied extensively (Table 1) (Sohlenkamp and Geiger 2016).

GN bacterial membranes consist of two lipid bilayers separated by a viscous, protein-enriched aqueous periplasmic space and a thin peptidoglycan (murein) wall (Fig. 1) (Kapoor et al. 2017; Barák and Muchová 2013; Silhavy et al. 2010). The inner membrane (IM) is comprised of an asymmetric phospholipid bilayer that encases the cytosol and harbours membrane proteins responsible for transport, energy production, protein secretion, and lipid biosynthesis (Silhavy et al. 2010; Bogdanov et al. 2020). The murein wall is responsible for protecting the bacterium against osmotic and mechanical stresses and maintaining bacterium shape (Kapoor et al. 2017; Silhavy et al. 2010). The outer membrane (OM) is attached to the murein wall via lipoproteins (Silhavy et al. 2010). The OM is an asymmetric lipid bilayer surrounding the periplasmic space (Kapoor et al. 2017; Paulowski et al. 2020). The proximal leaflet is comprised of phospholipids, whilst the distal leaflet is predominantly comprised of LPS which functions as a protective barrier (Silhavy et al. 2010; Cian et al. 2020). LPS is a glycolipid constructed of three distinct parts: lipid A (hydrophobic domain), the oligosaccharide core (hydrophilic domain), and the O-antigen (outmost polysaccharide domain) (Raetz and Whitfield 2002; Wang and Quinn 2010). The structure of LPS differs significantly between GN bacterial species due to survival adaptations in response to changes in environmental stimuli including pH, temperature, specific ion concentrations, osmolality, and toxins (including antibiotics) (Li et al. 2012; Needham and Trent 2013; Trent et al. 2006;

Table 1 Diversity of membrane lipid species documented for the ESKAPE pathogens

Bacterial species	Major membrane lipid species	References
E. faecium	PG, CL, Lysyl-PG, GP-DGDAG, Type I LTA, FA	Mishra et al. 2012; Theilacker et al. 2012)
S. aureus	PG, CL, Lyso-PG, GPL, Lysyl-PG, Type I LTA, FA	Epand and Epand 2009a; Song et al. 2020; Schneewind and Missiakas 2014; Kilelee et al. 2010; Malanovic and Lohner 2016; Oku et al. 2004; White and Frerman 1967)
K. pneumoniae	PG, PE, CL, SL, PC, Lysyl-PG, Lyso-PE, PI, PA, Lyso-PA, Lyso-PC, LPS, FA	Epand and Epand 2009a; Jasim et al. 2018; Vinogradov et al. 2002; Hobby et al. 2019)
A. baumannii	PE, PG, CL, Lyso-PE, Acyl-PG, PA, MLCL, PE-OH, CL-OH, MLCL-OH, LPS, FA	Jiang et al. 2019a; Unno et al. 2017; Jiang et al. 2020; Lopalco et al. 2017)
P. aeruginosa	PG, CL, PE, PC, OL, Alanyl-PG, RL, LPS, FA	Epand and Epand 2009a; Malanovic and Lohner 2016; Chao et al. 2010; Lam et al. 2011; Klein et al. 2009; Lewenza et al. 2011; Pramanik et al. 1990; Wilderman et al. 2002; Soberón-Chávez et al. 2005)
Enterobacter species[†] (*E. cloacae*, *E. hormaechei*, *and E. aerogenes)*	PG, PE, CL, LPS, FA	Epand and Epand 2009a; Bøse and Gjerde 1980; Gill and Suisted 1978; Kämpfer et al. 2015; Davin-Regli et al. 2019; Epand and Epand 2009b; Epand et al. 2010)

[†]As there are 22 species found in the *Enterobacter* genus, only common species described in nosocomial infections were analysed and lipid compositions are assumed to be similar between each (same genus)(Davin-Regli et al. 2019; Epand et al. 2010; Villegas and Quinn 2002)

[*]See Supplementary Information (Sects. 1 and 2) for bacterial and lipid species acronym definitions, respectively

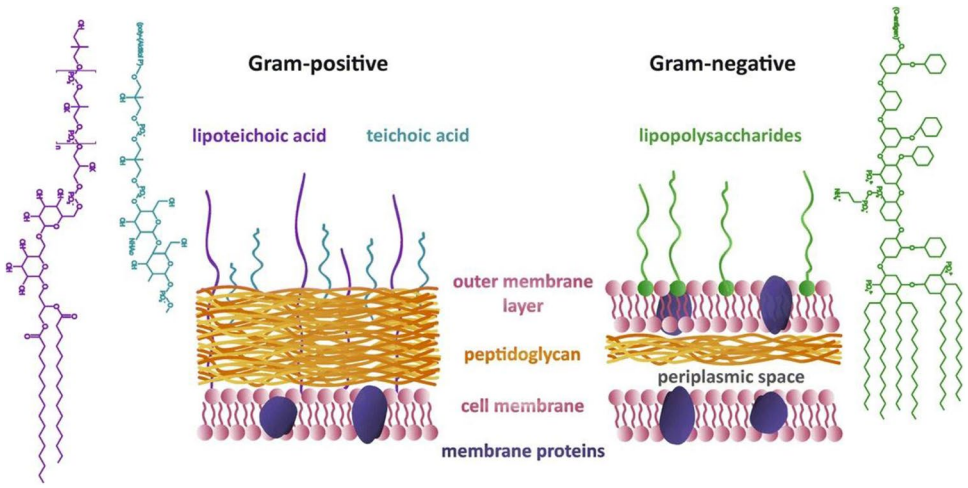

Fig. 1 Schematic depiction of the key structural differences in the cell walls of GN and GP bacteria (used with permission from (Pajerski et al. 2019))

Simpson and Trent 2019). Biochemical modifications to LPS domains or selective LPS production abandonment (specific to *A. baumannii* only) have been found to allow GN bacterial pathogens to evade host-immune attack, increase pathogenesis, and develop antimicrobial resistance (Needham and Trent 2013; Trent et al. 2006; Simpson and Trent 2019; Maldonado et al. 2016; Moffatt et al. 2010; Pelletier et al. 2013), for example, LPS modification adaptation strategies adopted by GN bacteria to protect themselves from cationic antimicrobials such as polymyxins include hydroxylation, dephosphorylation, palmitoylation, phosphatidylethanolamine addition, and 4-amino-4-deoxy-L-arabinose (L-Ara4N) addition to the lipid A portion (Dortet et al. 2020; Olaitan et al. 2014). The most common and effective modification to LPS in GN bacterial pathogens is the addition of L-Ara4N via cationic substitution of the 4'-phosphate group on the lipid A moiety (Olaitan et al. 2014; Nikaido 2003). This modification reduces the net charge of lipid A which, consequently, decreases the degree of electrostatic repulsion experienced between neighbouring LPS molecules. The incorporation of these cationic constituents results in a net positive charge of LPS upon biosynthesis which, inevitably, repulses cationic antimicrobials (Dortet et al. 2020; Olaitan et al. 2014). This repulsion results in antimicrobial resistance as the membrane has developed protection against OM disruption. In addition, murein lipoproteins and β-barrel proteins are present in the OM for murein wall anchoring and small (anions, maltodextrins, and maltose) and large molecule (antibiotics, vitamins and chelates) diffusion or transport (Silhavy et al. 2010).

The OM and LPS leaflets are absent in most GP bacteria which, in GN bacteria, are crucial in providing an additional stabilising layer around the bacterium and protect the bacterium from environmental hazards (Malanovic and Lohner 2016; Silhavy et al. 2010). To compensate for the OM deficit and withstand the osmotic and mechanical pressures exerted on the plasma membrane, GP bacteria are surrounded by a murein wall that is notably thicker (40–80 nm) in GP bacteria than those found in GN bacteria (7–8 nm) (Kapoor et al. 2017; Epand and Epand 2009a; Barák and Muchová 2013; Malanovic and Lohner 2016; Silhavy et al. 2010). Teichoic acids, including LTA, thread through the murein layers to anchor the murein wall to the membrane and regulate cell envelope function and structure (Malanovic and Lohner 2016; Silhavy et al. 2010). LTA is an alditol phosphate polymer linked by a glycolipid anchor that secures it to the lipid membrane (Solntceva et al. 2020; Percy and Gründling 2014). The structure of LTA varies significantly between GP bacterial species whereby there are five types of LTA (types I–V) that differ in core structure and glycolipid anchor (Percy and Gründling 2014; Shiraishi et al. 2013). Similarly to LPS in GN bacteria, biochemical modifications to the LTA backbone structure have been found to illicit antimicrobial resistance in GP bacterial pathogens (Percy and Gründling 2014; Gutmann et al. 1996; Saar-Dover et al. 2012). For example, the D-alanylation of LTA mediated by the *dlt* operon and/or incorporation of L-lysine in PG via the *mprF* gene can lead to an enhanced resistance against cationic antimicrobials (Percy and Gründling 2014; Saar-Dover et al. 2012; Abachin et al. 2002; Peschel et al. 1999; Reichmann et al. 2013). The modification increases the overall net positive surface charge of the membrane and reduces the binding affinity of cationic antimicrobials (Percy and Gründling 2014; Abachin et al. 2002; Peschel et al. 1999). However, other pathways may also be involved in resistance development. The addition of D-alanine, for example, also changes the conformation of LTA resulting in an increase in cell wall density and cell surface rigidity (Percy and Gründling 2014; Saar-Dover et al. 2012). This leads then to a reduction in the permeation of cationic antimicrobials through the cell. The membranes of GP bacteria are comprised of a single asymmetric phospholipid bilayer

that encases the cytosol (Silhavy et al. 2010; Rosado et al. 2015; Jones et al. 2008). As there is no OM in GP bacteria to harbour extracellular proteins, GP bacteria are decorated with numerous proteins bound via peptide anchors, covalent interactions, lipid anchors, or non-covalent interactions to the membrane, murein wall, and/or teichoic acids that perform functions analogous to those found in GN bacteria (Malanovic and Lohner 2016; Silhavy et al. 2010; Scott and Barnett 2006).

Model membrane systems

Various model membrane systems have been established. Here, we focus on systems that specifically mimic microbial membranes.

Liposomes

Liposomes are spherical-shaped vesicles ranging from nano- to micrometre diameters that are comprised of one or more phospholipid bilayers that encase an aqueous core (Siontorou et al. 2017; Akbarzadeh et al. 2013). Liposome structures are categorised according to their lamellar structure and vesicular size: unilamellar vesicles (ULV) can be small (SUV, 0.02–0.04 µm), medium (MUV, 0.04–0.08 µm), large (LUV, 0.1–1 µm), and giant (GUV, > 1 µm) (Siontorou et al. 2017; Akbarzadeh et al. 2013; Šturm and Poklar Ulrih 2021). Oligolamellar vesicles (OLV) are > 0.5 µm and can contain 2–5 concentrically arranged bilayers, multilamellar vesicles (MLV) are > 0.7 µm and can contain concentrically arranged 5–25 bilayers, and multivesicular vesicles (MVV) are 1–100 µm and can contain one or more non-concentrically arranged internal bilayers (Fig. 2) (Akbarzadeh et al. 2013; Navas et al. 2005; Giuliano et al. 2021; Mu et al. 2018). Liposomes are easily formed via numerous methods as reviewed elsewhere (Siontorou et al. 2017; Akbarzadeh et al. 2013; Šturm and Poklar Ulrih 2021). Liposome properties can differ depending on the method of preparation, size, lipophilic composition, surface charge, and functionalisation which allows for a considerable degree of customisation (Gabizon et al. 1998; Sherratt and Mason 2018; Fan et al. 2007; Bozzuto and Molinari 2015; Riaz et al. 2018; Sakai-Kato et al. 2019).

Liposomes have been constructed to mimic the OM, IM, and cytoplasmic space of various non-pathogenic and pathogenic bacteria (Table 2) (Behuria et al. 2020; Bogdanov et al. 2020; Paulowski et al. 2020; Tuerkova et al. 2020; Dombach et al. 2020; Jamasbi et al. 2014; Kumagai et al. 2019; Pérez-Peinado et al. 2018; Malishev et al. 2018; Kahveci et al. 2016; Lopes et al. 2012; Cheng et al. 2011; Marín-Menéndez et al. 2017; Fernandez et al. 2011; Domenech et al. 2009; Pinheiro et al. 2013; D'Errico et al. 2010; Furusato et al. 2018; Kiss et al. 2021; Jiménez et al. 2011; Sikder et al. 2019; Kubiak et al. 2011; Mohanan et al. 2020; Ruhr and Sahl 1985; Bharatiya et al. 2021).

Often GUVs or LUVs are used that contain either bacterial lipid extracts (> 4 lipid species), or synthetic lipids

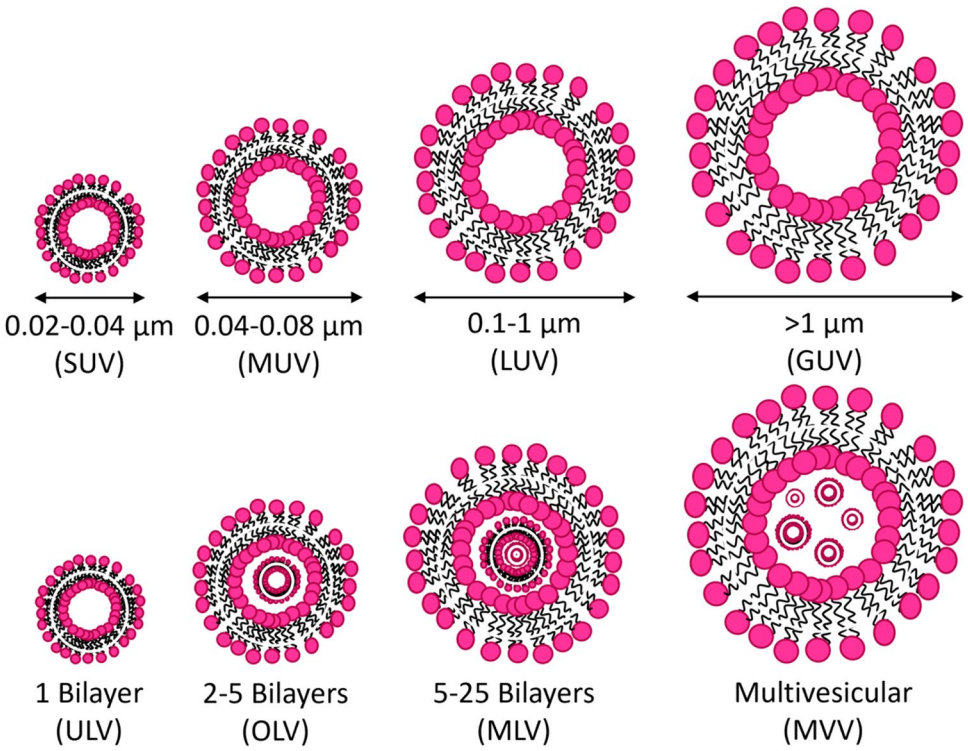

Fig. 2 Schematic representation of different sizes (top) and lamellar structures (bottom) of liposomes

Table 2 Summary of cited liposome models, the lipid source, the lipid species utilised, and their corresponding research outcomes

Model type	Reference	Lipid source	Lipid species	Research outcomes
GUV	Behuria et al. 2020)	*E. coli* polar lipid extract (DH5α)	PE, PG, CL	Development of a facile, inexpensive, and reproducible method for producing bacterial GUVs
	Furusato et al. 2018)	Purchased synthesised lipids	POPC, POPG, Rhod-DOPE	Formation of membrane-associated proteins using a cell-free protein synthesis system inside GUVs
	Jiménez et al. 2011)	*E. coli* lipid extract (JM600)	Unspecified lipid content from the extracts	Incorporation of soluble proto-ring proteins into GUVs for probing of divisome component interactions
	Kubiak et al. 2011)	Purchased *E. coli* B (ATCC 11,303) polar lipid extracts, *E. coli* (O55:B5) LPS extracts, *E. coli* (EH-100) LPS extracts, *E. coli* (J5) LPS extracts, *E. coli* (F583) LPS, and lipid A extracts and synthesised lipids	Extracted: PE, PG, CL, S-LPS, FITC-LPS, Ra-LPS, Rc-LPS, Rd-LPS, MPLA Synthesised: Rhod-DHPE	Development of novel protocol for formation of GUVs composed of LPS species and *E. coli* extracts
	Mohanan et al. 2020)	*E. coli* B (ATCC 11,303) polar lipid extracts and purchased synthesised lipids	Extracted: PE, PG, CL Synthesised: DOPG, Lysyl-PG, TOCL	Development of GUV-based GN and GP bacterial membrane vesicles
	Saliba et al. 2014)	Purchased porcine brain extract, *S. cerevisiae* (yeast) extract, *P. cirerri* (yeast) extract, *and* synthesised lipids	Extracted: PIP, SL (PHS including phosphate forms and phytocer) Synthesised: DAG, POPC, TOCL, DOPS, DOPG, POPE, DOPA, DOPI (including phosphate forms), SL (dihydrocer, cer (including phosphate and fluorescent forms), SO (including phosphate and florescent forms), DHS (including phosphate and fluorescent forms)), PEG350-PE, PEG2000-PE, ATTO647N-DMPE, NBD-PG, bodipy FL-PE Unknown source: ES	Systematic characterisation of protein-lipid interactions using a microarray of liposomes
	Turner et al. 2015)	Purchased synthesised lipids	DOPE, DOPG, TOCL, Lysyl-PG	Analysis of *C. botulinum* toxin type A using culture and liposomal methods to assess loss of sterility
	Paulowski et al. 2020)	LPS extracts from *P. mirabilis* (R_{45}). Purchased *E. coli* lipid extracts and synthesised lipids	Extracted: PE, PG, R-LPS Synthesised: CL, Rhod-DHPE, NBD-PE, FITC-PE	Demonstrate experimental methods to model the asymmetry of GN bacteria. The model's usability was assessed for lipid domain analysis and peptide and protein interaction by characterising lipid flip-flop and phase behaviour
LUV	Sikder et al. 2019)	Purchased synthesised lipids	DPPC, DPPG, DPPE	Programmable supramolecular assembly of π-amphiphile(s) for determination of interactions with bacteria and membrane mimicking liposomes

Table 2 (continued)

Model type	Reference	Lipid source	Lipid species	Research outcomes
	Som and Tew 2008)	Purchased *E. coli* B (ATCC 11,303) total lipid extracts and synthesised lipids	Extracted: PE, PG, CL, unspecified lipid content, Egg-Lyso-PC Synthesised: DOPE, DOPC, DOPG, DOPS	Use of a variety of lipid and lipid extract combinations to show that lipid structure and type could be more important than headgroup charge for determining membrane selectivity towards multiple antimicrobial oligomers
	Samuel and Gillmor 2016)	Purchased synthesised lipids	DOPG, DOPC, DPPC, DPPG	Examination of kinetics, behaviours and potential mechanisms of the NA-CATH peptide using SUVs
	Sborgi et al. 2016)	Purchased *E. coli* B (ATCC 11,303) polar lipid extract, porcine brain total lipid extract and synthesised lipids	Extracted: PG, PE, CL, PA, PS, PI, PC, unspecified lipid content Synthesised: DMPC	Determination that gasdermin D is the direct and final executor of pyroptotic cell death using liposome-inserted gasdermin D
	Carrasco-López et al. 2011)	Purchased *E. coli* B (ATCC 11,303) lipid extract (unspecified)	Polar (PE, PG, CL) or total (PE, PG, CL, unspecified lipid content)	Investigate the activation mechanism of AmpD peptidoglycan amidase to represent the regulatory processes that occur for other intracellular members of the amidase_2 family
	Sasaki et al. 2019)	Purchased *E. coli* B (ATCC 11,303) polar lipid extract and synthesised lipids	Extracted: PG, PE, CL Synthesised: DAG	Determination that YidC accelerates MPIase-dependent membrane protein integration
	Cheng et al. 2014)	Purchased synthesised lipids	POPC, POPG, POPE	Mechanistic contributions of membrane depolarisation in *S. aureus* towards the bactericidal activity of ramoplanin
	Lombardi et al. 2017)	Purchased bovine heart CL extract and synthesised lipids	Extracted: CL Synthesised: DOPE, DOPG, DPPE, DPPG, NBD-PE, Rhod-PE, 5-SLPC, 14-SLPC	Perturbation of lipid membranes by myxinidin mutant WMR due to anionic lipid segregation
	Zhang et al. 2014)	Purchased synthesised lipids	DMPC, DMPG, TOCL	Using cardiolipin in liposomes to show that changes in membrane lipid composition can allow bacteria to become resistant to daptomycin
	Domenech et al. 2009)	Purchased bovine heart CL extracts and synthesised lipids	Extracted: CL Synthesised: POPC, DPPG, POPG, POPE	Investigate the effect of vancomycin and oritavancin on the permeability and organisation of phospholipids in bacterial membrane models
	Fernandez et al. 2011)	Purchased synthesised lipids	DMPC, DMPG, d-DPMC, d-DMPG	Investigate the drug-membrane interactions between the synthetic antimicrobial peptide P5 and bacterial and human membrane models using solid-state NMR and circular dichroism
	Marín-Menéndez et al. 2017)	Purchased synthesised lipids	POPC, PG, CL	Develop bacterial model membranes to investigate the drug-membrane interactions and delivery mechanism of oligonucleotide therapeutics

Table 2 (continued)

Model type	Reference	Lipid source	Lipid species	Research outcomes
	Lopes et al. 2012)	Purchased *E. coli* B (ATCC 11,303) total lipid extracts	PE, PG, CL, unspecified lipid content	Generate model membranes that represent *Y. kristensenii* and *P. mirabilis* to determine differences in lipid phase transitions with variations in lipid composition ratios
	Jamasbi et al. 2014)	Purchased synthesised lipids	POPE, POPG	Investigate and compare the cytosolic and antimicrobial mechanism of action of the lytic peptide, melittin, between prokaryotic and eukaryotic model membranes
	Tuerkova et al. 2020)	Purchased synthesised lipids	POPC, POPG	Investigate the mechanism of action regarding pore formation induced by kinked helical antimicrobial peptides via fluorescence leakage assays
SUV	Kiss et al. 2021)	Purchased *E. coli* (EH100) LPS extracts and synthesised lipids	Extracted: Ra-LPS Synthesised: DMPC	Facile development of synthetic bacterial membrane models through the step-by-step construction of SUVs
	Brian Chia et al. 2011)	Purchased *E. coli* B (ATCC 11,303) total lipid extract, bovine brain total lipid extract, and synthesised lipids	Extracted: PG, PE, CL, unspecified lipid content Synthesised: DMPC, DMPG	Investigation of peptide selectivity using vesicles to show that natural lipid extracts compare better to MIC values than synthetic lipids
	Bharatiya et al. 2021)	Purchased *B. subtilis* LTA extracts and synthesised lipids	Extracted: LTA Synthesised: DPPG, DPPE, TMCL	Investigate how different compositional variations of LTA alter the structural integrity and stability in model GP membranes
	Bogdanov et al. 2020)	Lipid extracts from *E.coli* strains W3110, W3899, EH150, UE54, BKT12, AL95, AT2033, and *Y. pseudotuberculosis* (O:1b IP32953). Purchased *E. coli* B (ATCC 11,303) polar lipid extracts and purchased plus in-house synthesised lipids	Extracted: PE, PG, CL, PS, Lyso-PE, *N*-acyl-PE, PA, CDP-DAG Synthesised: DPPE, DPPS, TNP-PE, DNP-PE, TNP-LPE, TNP-LPS, TNP-PS, DFDNP-LPE, DFDNP-LPS, DFDNP-PE, DFDNP-PS	To determine how phospholipids are distributed in the IM of GN bacteria and how different phospholipid species influences the distribution and regulation of phospholipid species across the leaflets. The phospholipid asymmetry is discussed in the context of bacterial growth, phospholipid synthesis and translocation, and adjustments in the physical and chemical properties of the membrane
	Cheng et al. 2011)	Purchased bovine heart CL extract and synthesised lipids	Extracted: CL Synthesised: POPG, POPC, POPE	Investigate how the lipid composition in GP and GN bacterial models influence the drug-membrane interactions between various cationic antimicrobial peptides
MLV	D'Errico et al. 2010)	LPS extracts from *B. cenocepacia* ET-12 (LMG 16,656), *B. multivorans* (C1576), *A. tumefaciens* (TT111) and *S. enterica* (minnesota R595). Purchased synthesised lipids	Extracted: R-LPS, S-LPS, Re-LPS Synthesised: DOPE	Characterisation of liposome formation based on initial LPS molecular structure
	Pinheiro et al. 2013)	Purchased synthesised lipids	DMPG, DPPE, DPPG	Investigate the drug-membrane interactions between Rifabutin and bacterial and human membrane models using wide- and small-angle X-ray scattering

Table 2 (continued)

Model type	Reference	Lipid source	Lipid species	Research outcomes
	Kumagai et al. 2019)	Extracted LPS from *P. aeruginosa* (PA01) and purchased synthesised lipids	Extracted: LPS Synthesised: POPE, POPG, TOCL, DOTAP	Generate model GN and GP membranes to test the function of newly synthesised antimicrobial peptides. The antimicrobials were tested to assess the drug-membrane interactions and killing efficiency
LUV and GUV	Kahveci et al. 2016)	Purchased bovine heart CL extract and synthesised lipids	Extracted: CL Synthesised: DOPE, DOPG	Analyse the interactions between mammalian and bacterial membrane models and conjugated fluorophores. The models were used to assess fluorophore-lipid binding affinity for the selective cell recognition
SUV and GUV	Malishev et al. 2018)	Purchased bovine heart CL extract and synthesised lipids	Extracted: CL Synthesised: DOPE, DOPG	Investigate the differences in protein-membrane interactions of amyloid protein, TasA, between mimic bacterial and eukaryotic cell membranes
SUV and LUV	Pérez-Peinado et al. 2018)	Purchased *E. coli* B (ATCC 11,303) polar lipid extract and synthesised lipids	Extracted: PE, PG, CL Synthesised: POPC, POPG	Determine the mechanism of action of the antimicrobial peptides, crotalicidin, and its fragment, on the Om of GN bacteria. Liposome models specifically were used to analyse preferential binding and the degree of membrane disruption
Unspecified liposome type	Su et al. 2011)	*E. coli* (WBB06) LPS extract and purchased synthesised lipids	Extracted: Re-LPS Synthesised: POPE, POPG, DEPE	Determination of Gram selectivity among β-hairpin AMPs using LPS-based model systems
	Hancock and Nikaido 1978)	*P. aeruginosa* (PAO1) LPS and lipid extracts, and *S. typhimurium* (LT2M1) LPS and lipid extracts	Unspecified lipid content, R-LPS, S-LPS	Develop an improved method to separate the OM and IM of *P. aeruginosa.* Saccharide retention between liposomes and proteoliposomes was also investigated to compare exclusion limits between *P. aeruginosa* and enteric bacteria, *S. enterica*
	Ruhr and Sahl 1985)	*S. cohnii* (22), *B. subtilis* (W23), *M. luteus* (ATCC 4698) and soybean lipid extracts	Unspecified lipid content, Soy-PC	To determine the effect of the peptide antimicrobial, nisin, on the membrane potential and transport processes of GP bacteria
	Dombach et al. 2020)	Purchased *E. coli* B (ATCC 11,303) polar lipid extract	PE, PG, CL	Investigate the mechanism of action of a small molecule found in macrophages, JD1, that declines the survival and/or growth of GN bacteria

*See Supplementary Information (Sect. 1 and 2) for bacterial and lipid species acronym definitions, respectively

determined by the user (< 3 lipid species) asymmetrically arranged in a bilayer. Liposome formation using bacterial lipid extracts provide a more biologically attune system as various lipid species and their native molecular variants are inherently incorporated. Under an artificially user-defined composition, the inner and outer leaflets for GP liposome models commonly contain PG, lysyl-PG, and CL, whilst GN liposome models commonly contain PE, PG, and CL and uncommonly LPS. Liposome models have been utilised to investigate basic structural (lipid domain architecture, rigidity, diffusion, and lateral organisation) and rheological (constriction, shrinkage, and invagination) membrane properties. In addition, protein and peptide-lipid interactions (Saliba et al. 2014; Su et al. 2011), lipid composition-dependent uptake, release, and molecule function (i.e. membrane-targeting antibiotics) (Kilelee et al. 2010; Som and Tew 2008; Brian Chia et al. 2011), pore formation (Samuel and Gillmor 2016; Sborgi et al. 2016), and protein activity (Carrasco-López et al. 2011; Sasaki et al. 2019) have been explored.

Liposome models have been developed for the ESKAPE pathogens and have been used to investigate host–pathogen interactions, membrane permeability, and the effect of membrane composition on antimicrobial susceptibility (Turner et al. 2015; Cheng et al. 2014; Lombardi et al. 2017; Zhang et al. 2014; Hancock and Nikaido 1978; Ciesielski et al. 2013; Lee et al. 1992; Mitchell et al. 2016). Liposomes from synthetic PC and PG lipids and *S. aureus* lipid extracts were used to determine the effects of lipid acyl chain branching on antimicrobial peptide activity (Mitchell et al. 2016). This was achieved by measuring efflux kinetics of the encapsulated fluorescent dye carboxyfluorescein, mediated by the model peptide δ-lysin. Liposomes composed of anteiso-branched isomers were less susceptible to peptide-induced perturbations than liposomes containing iso-branched isomers. In addition, liposomes made from *S. aureus* extracts were more resistant to peptide-induced perturbation than liposomes composed of synthetic lipids, most likely due to the additional increased fraction of anteiso-branched fatty acids.

In a different approach, the association of LPS extracted from *K. pneumoniae* with eukaryotic lipids has been investigated with respect to host immunodetection strategies (Ciesielski et al. 2013). This was achieved by analysing liposome-liposome interactions between pathogen membrane model liposomes containing LPS and PC and host membrane model liposomes containing PC, SL, and cholesterol. LPS preferentially segregated in ordered SL/cholesterol rich domains which was linked to the evolutionary drive for eukaryotic cells to generate, within such domains, a sensory protein for bacterial detection. The permeability of various carbapenems via porins in proteoliposomes reconstituted from lipids extracted from the OM of susceptible and resistant strains *E. cloacae* has also been studied (Lee et al. 1992). Carbapenem permeability and efficacy was highly dependent on the lipophilic constitution of the OM and the amount and type of porins present.

While liposomes are very useful systems to study, they pose some challenges for detailed biophysical studies. Lipid composition is often difficult to control (Rideau et al. 2018; Weinberger et al. 2013). Methods to enhance compositional complexity have been developed (Göpfrich et al. 2019; Pautot et al. 2003); however, they can inhibit surface property analysis (Rideau et al. 2018). The metastable structure of liposomes and their susceptibility to lipophilic, oxidative, and hydrolytic degradation offers poor long-term stability (Akbarzadeh et al. 2013; Nkanga et al. 2019). Additionally, lipids often have relatively high phase transition temperatures which impede liposome formation (Eeman and Deleu 2010; Vestergaard et al, 2008). Finally, despite existing stabilisation methods (Schmid et al. 2015), protein reconstitution in liposomes still remains a challenge (Chan and Boxer 2007; Siontorou et al. 2017).

Solid-supported bilayers:

Solid-supported bilayer lipid membranes (sBLMs) consist of a lipid bilayer that is placed onto a solid substrate either via direct contact, via separation by a polymer cushion, or allowed to float directly above a covalently-bound self-assembled monolayer or a supported bilayer (Fig. 3) (Andersson and Köper 2016; Belegrinou et al. 2011; Sackmann 1996; Foglia et al. 2015). Tethered bilayer lipid membranes (tBLMs) are sBLMs with the proximal bilayer leaflet covalently linked to the substrate though thiolipid, oligopeptide, alkane- and aromatic-thiol, polymer, or protein anchors (Andersson and Köper 2016; Andersson et al. 2018b; Jackman et al. 2012; Li et al. 2015; Köper 2007). sBLMs and tBLMs have good electrical sealing properties, are air-stable, and can be formed via Langmuir transfer, vesicle fusion, or solvent-exchange techniques (Andersson et al. 2020; Jackman et al. 2012; Girard-Egrot and Maniti 2021; Kurniawan et al. 2018; Richter et al. 2003).

Gold is the most commonly utilised substrate material for sBLMs and tBLMs due to its stability, facile functionalisation, and versatility in surface analysis techniques (Andersson and Köper 2016). However, other substrates including mercury, quartz, glass, aluminium oxide, indium tin oxide, silicon oxide, sapphire, mica, silver, and titanium oxide can also be utilised (Andersson et al. 2018b; Girard-Egrot and Maniti 2021; Clifton et al. 2020; Giess et al. 2004).

Surface sensitive techniques such as surface plasmon resonance, ellipsometry, neutron or X-ray reflectometry, atomic force microscopy, electrochemical impedance spectroscopy, quartz crystal microbalance with dissipation monitoring, and infrared reflection absorption spectroscopy are well-suited methods of surface analysis for these planar systems

in aqueous solution (Ferhan et al. 2017; Wittenberg et al. 2014; Steltenkamp et al. 2006).

While these membrane systems commonly have simple lipid compositions, increased biological accuracy can be achieved in both sBLMs and tBLMs by customising the lipid composition to change membrane electrical sealing and structural properties (Andersson and Köper 2016; Andersson et al. 2018b; Girard-Egrot and Maniti 2021). tBLMs can also change the aforementioned membrane properties and facilitate protein incorporation by customising the tethering type, composition, and density. The OM and IM of various non-pathogenic and pathogenic bacteria have been modelled using both tBLMs and sBLMs (Table 3) (Paulowski et al. 2020; Pérez-Peinado et al. 2018; Weiss et al. 2010; Clifton et al. 2013; Paracini et al. 2018; Hughes et al. 2019; Dodd et al. 2008; Michel et al. 2017; Adhyapak et al. 2020; Nakatani et al. 2019; Hoiles and Krishnamurthy 2015; Schneck et al. 2009; Lee et al. 2020; Nedelkovski et al. 2013; Niu et al. 2017; Sharma et al. 2020; McGillivray et al. 2009).

These architectures often contain a limited number (1–4) of synthetic lipid species; however, they can also contain bacterial lipid extracts (> 4 lipid species) asymmetrically arranged in a bilayer. Unlike user-defined systems which are limited to the number and type of lipid species and their associated molecular variations incorporated, architectures formed from bacterial lipid extracts generate increasingly accurate biological models as various lipid species and their native molecular variants are inherently incorporated. Under user-defined compositions, the inner and outer leaflets of architectures modelling GN and GP bacteria commonly contain one molecular variation of PC. Few architectures have been developed where the inner and outer leaflets contain the most common lipid species or analogues thereof for GN (PE, PG and CL) and GP (PG, CL, and lysyl-PG) bacteria. For sBLM and tBLM systems, lysyl-PG is often substituted with DOTAP as it is more affordable for the increased quantities required to generate the architectures (Dupuy et al. 2018; Li and Smith 2019). Few architectures modelling the membrane of GN or GP bacteria have also been developed to

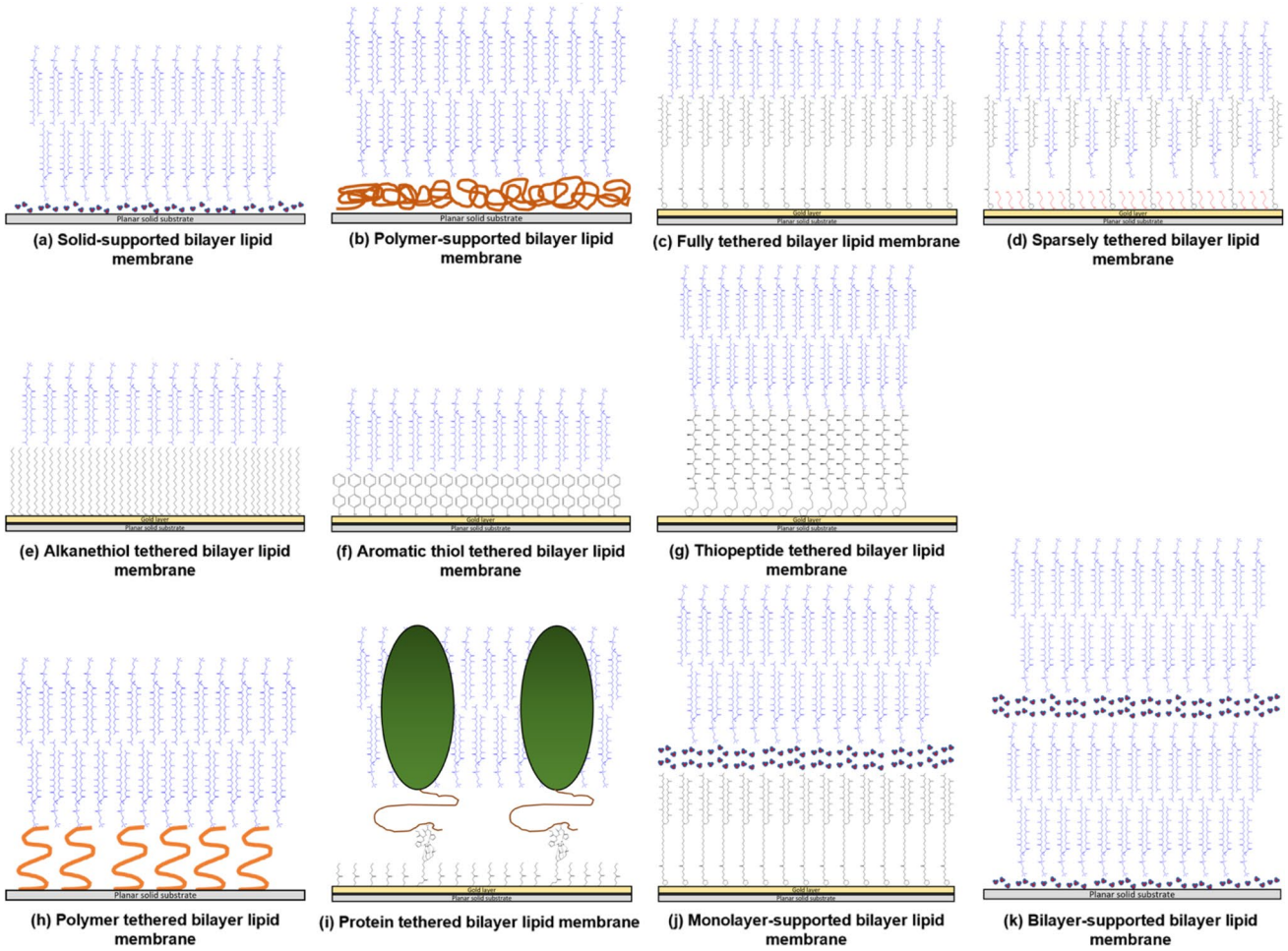

Fig. 3 Schematic representation of various solid supported model membrane architectures. Please see text for details

contain LPS (Andersson et al. 2018a; Clifton et al. 2015; Hsia et al. 2016; Thomas et al. 1999) or murein (Spencelayh et al. 2006). The model architectures have been utilised to investigate general structural (thickness, roughness, and lipid density) and electrical membrane properties. In addition, the mechanism of interaction between antibiotic compounds and membrane constituents (Chilambi et al. 2018; Dupuy et al. 2018; Li and Smith 2019), lipid-protein interactions (Mirandela et al. 2019), ion transport (Maccarini et al. 2017), and redox-active enzyme function and characterisation (Jeuken et al. 2006, 2005) have been explored.

Limited architectures have been generated to model the ESKAPE pathogens and investigate electrochemical and structural changes with lipophilic composition (Jiang et al. 2019b; Mohamed et al. 2021; Zang et al. 2021). Recently, a tBLM for *A. baumannii* has been developed to model the OM in the presence and absence of exogenously incorporated omega-3 polyunsaturated fatty acid (PUFA) and docosahexaenoic acid (DHA) (Zang et al. 2021). Both tBLMs generated were asymmetrical and were constructed from lipid samples extracted from *A. baumannii* actively growing in the presence or absence of DHA. The tBLMs were used to determine whether DHA incorporation disrupted the function of efflux system AdeB due to impaired proton motive force retention from induced ion leakage. Both tBLM models were electrochemically similar therefore suggesting that AdeB dysfunction was not due to the membrane's ability to maintain a proton motive force upon DHA incorporation. sBLM models for *S. aureus* have been developed to assess how upregulation in CL biosynthesis in daptomycin-resistant strains decreases antibiotic susceptibility (Jiang et al. 2019b). PG, lysyl-PG and CL in different concentration ratios were used to mimic resistant and susceptible strains. The daptomycin-resistant strain membrane was found to be thicker than the susceptible strain. The structural changes resulted in concentration-dependent changes in daptomycin interaction. At low daptomycin concentrations, the susceptible strain exhibited decreases in lipid volume whilst high concentrations induced considerable membrane penetration and disruption. In contrast, the resistant-strain exhibited only slight lipid volume reductions for all daptomycin concentrations analysed. This demonstrated that lipid-induced structural modifications can impair daptomycin efficacy.

Both sBLM and tBLM systems possess limitations unique to each architecture. sBLM systems can be unstable due to no linkage between the lipid bilayer and the substrate (Andersson and Köper 2016; Andersson et al. 2018b; Girard-Egrot and Maniti 2021). As a result, measurements requiring days or weeks are difficult to achieve. Direct bilayer-substrate contact can also create an insufficient amount of space for bilayer-spanning protein incorporation (Castellana and Cremer 2006; Andersson and Köper 2016; Alghalayini et al. 2019; Tamm and McConnell 1985). Protein-substrate contact induces denaturation or impaired function which hinders functional, electrical, or structural studies (Alghalayini et al. 2019; Tanaka and Sackmann 2005). Membrane structural and electrical properties are also subject to substrate topology, whereby any substrate imperfections will cause defects in the bilayer and hinder its resistance towards current transfer (Andersson and Köper 2016; Andersson et al. 2018b; Girard-Egrot and Maniti 2021). Using a polymer cushion to support the bilayer can partially reduce substrate topological effects, maintain bilayer fluidity, and prevent substrate-protein contact (Andersson and Köper 2016; Andersson et al. 2018b; Belegrinou et al. 2011). However, polymer cushion swelling behaviour, assembly, thickness, and morphology are difficult to control which dampens the electrical qualities of the lipid bilayer (Naumann et al. 2001, 2002). tBLMs were generated to circumvent all aforementioned limitations of sBLMs. However, the disadvantage of increased stability and electrical sealing in tBLM systems is decreased lateral lipid mobility (Andersson et al. 2018b). Depending upon the application, there are also disadvantages to using different types of tethers (Jackman et al. 2012). Similarly to liposomes, consideration of the lipid phase transition temperature can be crucial to successful lipid incorporation and architecture formation (Eeman and Deleu 2010; Vestergaard and d., Hamada, T., Takagi, M., 2008).

Computational modelling

Despite the progress made in developing sophisticated experimental techniques that can directly investigate live bacterial cells and reveal complex lateral membrane organisation processes (Deleu et al. 2014; Lyman et al. 2018; Nickels et al. 2015), analysing the molecular details surrounding membrane organisation still proves difficult (Maity et al. 2015; Marrink et al. 2019). Molecular dynamics (MD) techniques can serve as a "computational microscope" whereby interactions between all constituents in the system can be analysed at an atomistic level (Marrink et al. 2019; Ingólfsson et al. 2016). The quality of the set of parameters that dictate particle interaction, known as the force field (FF), is crucial to the success of an MD simulation (MacKerell 2004). In biomolecular simulations, numerous FFs have been employed: implicit, supra-coarse-grain, coarse-grain, and all-atom (Marrink et al. 2019; Mori et al. 2016). All FFs are similar regarding their main approximations and function; however, the level of resolution between each is distinctive (Fig. 4) (MacKerell 2004). The highest level of resolution is full atomistic detail which is the most commonly utilised model for complex membrane systems. These include bacterial membranes, organelle membranes, plasma membranes and viral envelopes, protein folding, drug-membrane interactions, protein–ligand complex stability, protein–protein

Table 3 Summary of cited solid supported bilayer models, the lipid source, the lipid species utilised and their corresponding research outcomes

Model type	Reference	Lipid source	Lipid species	Research outcomes
tBLM	Andersson et al. 2018a)	Purchased synthesised lipids and *E. coli* (J5) LPS extracts	Extracted: Rc-LPS Synthesised: DPhyPC, d-DPhyPC	Generate a model membrane that mimics the OM of GN bacteria. Structural and electrical properties were investigated with respect to the influence of divalent ions and antibiotics
	Weiss et al. 2010)	Purchased *E. coli* B (ATCC 11,303) polar lipid extracts	PE, PG, CL	Develop an assay to assess the activity of cytochrome bo_3 in response to the substrate, ubiquinol-10, in the presence of multiple different inhibitors
	Nakatani et al. 2019)	Purchased *E. coli* B (ATCC 11,303) polar lipid extracts	PE, PG, CL	Develop a model bacterial architecture to analyse the catalytic behaviour of Type-II NADH:quinone oxidoreductase in the presence of various the substrates (quinone, quinone analogues and NADH) and inhibitors (phenothiazines)
	Hoiles and Krishnamurthy 2015)	Purchased synthesised lipids	POPG, Ether-DPhyPC, DPGE	Investigate pore formation dynamics and reaction-mechanism of the antimicrobial peptide, peptidyl-glycine leucine-carboxyamide, in archaebacterial model membranes
	Nedelkovski et al. 2013)	Purchased synthesised lipids	DPhyPC	Generate a biomimetic bacterial membrane architecture that produces enhanced infrared signals to better analyse the photoexcitation mechanism of photosynthetic reaction centres in *R. sphaeroides*
	Niu et al. 2017)	Purchased synthesised lipids and LPS extract from *S. enterica* (minnesota R595)	Extracted: Lipid A Synthesised: DPhyPC, DPhyPG	Investigate the molecular mechanism, interactions, and impact of the antimicrobial peptide, V4, on the electrical and mechanical properties of bacterial membrane models
	McGillivray et al. 2009)	Purchased synthesised lipids	DPhyPC	Develop a model bacterial membrane to analyse the structural and electrical properties and lipid-protein interactions of α-hemolysin channels derived from *S. aureus*

Table 3 (continued)

Model type	Reference	Lipid source	Lipid species	Research outcomes
	Hsia et al. 2016)	Purchased synthesised lipids and *E. coli* (JC8031) lipid extracts	Extracted: unspecified lipid content from the extracts Synthesised: DOPC, PEG5000-PE	Develop a model membrane of the OM of GN bacteria. The formation of the membrane was characterised kinetically and acoustically to assess surface coverage, vesicle rupture and architecture mass. Properties including membrane diffusivity, mobility, viscoelasticity and lipid and protein symmetry were also investigated. Changes in membrane properties, mass and kinetics were also investigated in the presence of antibiotics
	Thomas et al. 1999)	Purchased synthesised lipids and *E. coli* (K12 D31m4) lipid A extracts	Extracted: DPLA Synthesised: DMPC, Biotin-PE	Investigate and identify the sequestering effectiveness and neutralisation mechanism between LPS and polymyxin B compared to polymyxin B synthetic peptide mimics
	Spencelayh et al. 2006)	*E. coli* JM1100 (pPER3) and purchased egg lipid extracts	Egg-PC, unspecified lipid content from the *E. coli* extracts	Generate a biomimetic bacterial membrane that facilitates the in vitro synthesis of peptidoglycan using native precursors. The binding behaviour between different antibiotics and the peptidoglycan precursors
	Mirandela et al. 2019)	Purchased synthesised lipids and *E. coli* B (ATCC 11,303) polar lipid extracts	Extracted: PG, PE, CL Synthesised: POPC	Investigate how the lipid-protein interaction between a mimetic GN lipid bilayer and an ammonium transporter protein native to *E. coli* affects transporter activity
	Maccarini et al. 2017)	Purchased synthesised lipids	DMPC, GDPE, DPEPC, DOPC, DOPE, DMPA, cholesterol	Develop a procedure to optimise the cell-free production of and incorporation of a porin from *P. aeruginosa* in a functional conformation
	Jeuken et al. 2006)	Purchased *E. coli* B (ATCC 11,303) polar lipid extracts	PG, PE, CL	Characterise the function and structure of redox-active enzyme, cytochrome bo_3, derived from *E. coli*
	Jeuken et al. 2005)	Purchased *E. coli* B (ATCC 11,303) polar lipid extracts and egg lipid extracts. *B. subtilis* (3G18/pBSD1200) lipid extracts	PG, PE, CL, Lysine-Acyl-PG, egg-PC, unspecified lipid content from the *B. subtilis* extracts	Electrochemically characterise the function of redox-active membrane protein, succinate menaquinone oxidoreductase, native to *B. subtilis*
	Dupuy et al. 2018)	Purchased synthesised lipids and *E. coli* (O111:B4) LPS extracts	Extracted: S-LPS Synthesised: POPE, POPG, TOCL, POPC, DOTAP, KDO2, DLPG	Develop model GP and GN bacterial membranes to elude the biophysical interaction mechanism between the antimicrobial peptide Colistin and different lipid compositions

Table 3 (continued)

Model type	Reference	Lipid source	Lipid species	Research outcomes
	Hughes et al. 2019)	Purchased synthesised lipids and *E. coli* (EH100) LPS extracts	Extracted: Ra-LPS Synthesised: d-DPPC	Collect biophysical information and investigate the physical properties of the OM of GN bacteria using model membranes and computational simulations
	Mohamed et al. 2021)	Lipid extracts *from P. aeruginosa* (PA14), *A. baumannii* (LAC-4) and *E. cloacae* (ATCC 13,407) and purchased synthesised lipids	Extracted: unspecified lipid content but LPS was detected and quantified Synthesised: PEG5000-POPC, PEG5000-DHPE	Generate OM model bilayers of three GN ESKAPE pathogens and investigate the model's biophysical characteristics, and drug-membrane interactions with various antimicrobial compounds
sBLM	Adhyapak et al. 2020)	*M. smegmatis* (mc^2155) lipid extracts	PA, PE, PG and PI (including lyso forms); CL; DAG (including meromycolyl forms); SfL; DAT; GPepL; MA (including alpha and keto forms); PIM (including mono-acylated forms); TAT; MG; MPM; TDM; MB (including carboxy, cell-bound iron-loaded, monodeoxy, dideoxy and hybrid forms); MQ; PDIM; Ac2SGL; TG; DG; PCA (including hydroxy forms); CET; GPD; MCA; MPanA; MpenA; MSA; MCSA; L5P	Investigate the membrane lipid domain architecture, fluidity, packing, dynamics, synthesis regulation and lateral organisation in protein-free membrane models of mycobacteria
	Schneck et al. 2009)	*S. enterica* (R60 and R595) lipid extracts	Lipid A, Ra-LPS-Ra, Re-LPS	Model the influences of different LPS mutations on the mechanical properties and intermembrane interactions in the presence and absence of divalent ions using GN bacterial OM models
	Lee et al. 2020)	*E. coli* BL21 (K-12 MG1655) total lipid extracts	PE, PG, PA	Investigate the impact of the antimicrobial peptide, maculatin 1.1, on the mechanical properties of lipid domains in bacterial membrane models simulating exponential and stationary growth phases
	Sharma et al. 2020)	Purchased *E. coli* B (ATCC 11,303) total lipid extracts and *E. coli* (O111:B4) LPS extracts and synthesised lipids	Extracted: S-LPS, PE, CL, PG, unspecified lipid species Synthesised: POPE, ATTO488-DMPE, ATTO647N-DMPE	Generate a model membrane that mimics the OM and IM of *E. coli*. Membrane lipid diffusiveness, fluidity, packing, and mobility was analysed with respect to the transport of the antimicrobial thymol
	Clifton et al. 2015)	Purchased *E. coli* (EH100) LPS extracts and synthesised lipids	Extracted: Ra-LPS, Synthesised: DPPC, d-DPPC	Generate an asymmetric model membrane that mimics the IM and OM of *E. coli*
	Li and Smith 2019)	Purchased synthesised lipids	POPG, DOTAP, TOCL, POPE, TopFluor-PE, TopFluor-TOCL	Develop model GP and GN asymmetric bacterial IMs. Lipid diffusion dynamics was investigated in the presence and absence of antimicrobial peptide binding

Table 3 (continued)

Model type	Reference	Lipid source	Lipid species	Research outcomes
	Michel et al. 2017)	Purchased synthesised lipids and LPS extract from *S. enterica* (minnesota R595)	Extracted: Re-LPS Synthesised: SOPE, SOPG, TOCL, d-POPG, d-POPE	Develop and characterise a model GN asymmetrical bacterial IMs to antimicrobial plasticins
	Paulowski et al. 2020)	LPS extracts from *P. mirabilis* (R_{45}). Purchased *E. coli* lipid extracts and synthesised lipids	Extracted: PE, PG, R-LPS Synthesised: CL, Rhod-DHPE, NBD-PE, FITC-PE	Demonstrate experimental methods to model the asymmetry of GN bacteria. The model's usability was assessed for lipid domain analysis and peptide and protein interaction by characterising lipid flip-flop and phase behaviour
	Dodd et al. 2008)	Purchased synthesised lipids and E. coli (BL21(DE3)) lipid extracts	Extracted: unspecified lipid content Synthesised: Egg-PC, TRF-DHPE, NBD-PC	Generate sBLMs that contain mixtures of native E. coli lipids with Egg-PC with the intention of generating a simple model membrane for the study of drug-membrane interactions and numerous process that occur in bacterial membranes. The structural properties of the generated sBLMs were assessed using various surface sensitive analytical techniques
	Clifton et al. 2013)	Lipid A and LPS extracts from E. coli strains F583, EH100 and J5. Purchased synthesised lipids	Extracted: lipid A, Ra-LPS, Rc-LPS Synthesised: DPPC, d-DPPC	Develop a facile two-step approach to modelling the OM of GN bacteria. Via neutron reflectometry, the lipid distribution and coverage between leaflets, and membrane stability and structure were analysed
	Pérez-Peinado et al. 2018)	Purchased *E. coli* B (ATCC 11,303) polar lipid extract and synthesised lipids	Extracted: PE, PG, CL Synthesised: POPC, POPG	Determine the mechanism of action of the antimicrobial peptides, crotalicidin and its fragment, on the Om of GN bacteria. sBLM models specifically were used to analyse the membrane permeabilisation mechanism
sBLM and tBLM	Chilambi et al. 2018)	Purchased synthesised lipids and *E. faecalis* OG1RF (wild type), EFC3C and EFC3Py (resistant strains) extracts	Extracted: unspecified lipid species from extracts, various FAs Synthesised: DPDEPC, GPDE, DOPC, POPG	Investigate the antimicrobial mechanism of antimicrobial conjugated oligoelectrolytes through changes in the fatty acid, genetic and uptake profiles between wild type and resistant strains of *E. faecalis*
	Paracini et al. 2018)	Purchased synthesised lipids and LPS extract from E. *coli* (EH100)	Extracted: Ra-LPS Synthesised: d-DPPC	Investigate how the physical structure of the lipid OM of GN bacteria influences the drug-membrane interactions of polymyxin B

*See Supplementary Information (Sect. 1 and 2) for bacterial and lipid species acronym definitions, respectively

interaction modulators, lipid domain formation and behaviour, membrane curvature sensing and formation, membrane remodelling events, and lipid-protein binding site identification and binding strength (Matamoros-Recio et al. 2021; Bennett and Tieleman 2013; Chan et al. 2015; Kabedev et al. 2021; Khan et al. 2019; Lazim et al. 2020; Liu et al. 2021; Parkin et al. 2015; Reddy and Sansom 2016; Singharoy and Schulten 2017). Full atomistic detail significantly expands the predictive power of molecular dynamics simulations. To enhance the spatiotemporal range of MD simulations and decrease system complexity, the lower resolution level FFs can be utilised (Mori et al. 2016; Liu et al. 2021).

Several MD models simulating the OM and IM of bacteria have been constructed at both the atomistic and coarse-grained levels of resolution (Table 4). (Bogdanov et al. 2020; Tuerkova et al. 2020; Hughes et al. 2019; Balusek and Gumbart 2016; Baltoumas et al. 2019; Gao et al. 2020; Kholina et al. 2020; Li and Guo 2013; Abellón-Ruiz et al. 2017; Berglund et al. 2015; Hsu et al. 2017a, 2017b; Ma et al. 2017a, 2017b, 2015; Mehmood et al. 2016; Orekhov et al. 2018; Shearer et al. 2019; Shearer and Khalid 2018; Rice and Wereszczynski 2018; Patel et al. 2016; Piggot et al. 2011; Carpenter et al. 2016; Fleming et al. 2016; Wu et al. 2013, 2014a; Duay et al. 2019; Khondker et al. 2019; Pandit and Klauda 2012; Pothula et al. 2016; Shahane et al. 2019).

These models often contain 2 or more different lipid species asymmetrically arranged in a bilayer, with the outer and inner leaflets composed primarily of LPS (restricted to the outer leaflet) and/or a mixture of PE, PG and sometimes CL. To compensate for the significant variation in the constituents of the phospholipids and LPS between bacterial strains and species, a range of different phospholipid and LPS fragments and variants have been parametrised for use in MD programs (Lee et al. 2018; Wu et al. 2014b). The models have been utilised to characterise and explore various membrane channels and bacterial membrane properties including divalent cation binding, density, diffusion, packing, rigidity, and average area per lipid. In addition, lipid changes between bacterial growth cycles (Khakbaz and Klauda 2015; Lim and Klauda 2011), effects of mechanical and oxidative stressors (Hwang et al. 2018), molecule permeation and partitioning (Jin et al. 2021; Hsu et al. 2016), and the lipophilic influence on membrane protein function and packing (Khalid et al. 2015; Patel et al. 2017) have also been explored.

Bacterial membranes modelling the ESKAPE pathogens have also been simulated to investigate drug-membrane interactions, lipid-protein interactions, and structural changes associated with bacterial pathogenesis (Zang et al. 2021; Piggot et al. 2011; Lee et al. 2017; Ocampo-Ibáñez et al. 2020; Alkhalifa et al. 2020; Lins and Straatsma 2001; Yu and Klauda 2018; Kirschner et al. 2012; Dias et al. 2014; Oosten and Harroun 2016; Chakraborty et al. 2020; Kim et al. 2016). Models for *A. baumannii* containing the OM/IM spanning AdeB RND drug-efflux complex in the presence and absence of incorporated host-derived PUFAs, arachidonic acid, and DHA have been developed within the coarse-grained FF to investigate PUFA-mediated antibiotic susceptibility (Zang et al. 2021). All three simulated membranes were asymmetrical, contained three different lipid species notably PG, CL, and PE and 2–7 molecular variations of each. PUFA incorporation was shown to morphologically disrupt AdeB, resulting in impaired efflux function and presented a potential weakness in *A. baumannii's* MDR capacity. Chakraborty et al. (2020) also explored various drug-membrane-dependent interactions of two antimicrobial peptides, battacin analogues octapeptide 17 and pentapeptide 30, with the IM of *S. aureus* using an atomistic FF (Chakraborty et al. 2020). The IM was an asymmetric three-component mixture predominately of PG, lysine-PG, DPG, and CL. Kim et al. (2016) modelled homogenous bilayers from 12 pathogenic bacterial species, including *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa*, using an atomistic FF to investigate atomistic-scale similarities and differences in

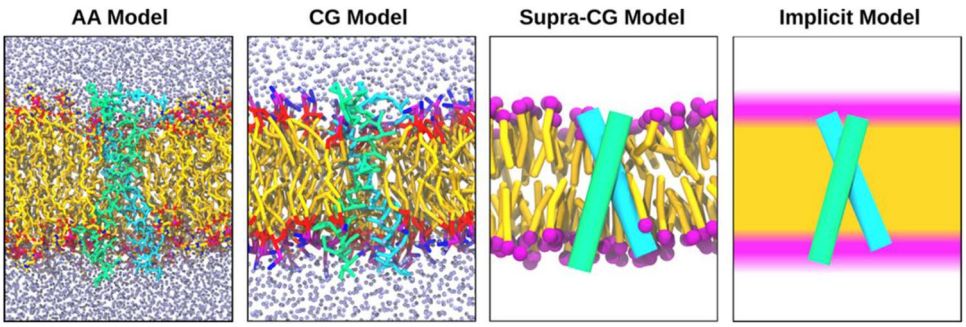

Fig. 4 Schematic representation of different resolutions in molecular dynamics simulations of lipid membranes. All atom (AA) resolution explicitly considers all atoms. Coarse-grain (CG) resolution considers small atom groups and their associated hydrogens. Supra-CG resolution represents solvents implicitly and proteins and lipids as qualitative few-bead models. Implicit resolution further integrates out lipid molecules. (modified with permission from (Marrink et al. 2019))

Table 4 Summary of cited computational models, the bacterial species modelled, and the lipid species utilised and their corresponding research outcomes

Model type	Reference	Modelled bacterial species	Lipid species	Research outcomes
Atomistic (all-atom)	Balusek and Gumbart 2016)	_	POPE, Ra-LPS modelled from E. coli strain K-12	Investigate transport protein-LPS interactions and its effect on Ca^{2+} binding for vitamin transport in GN bacteria
	Duay et al. 2019)	_	POPE, POPG	Determine how Zn ions and pH affect the binding of the antimicrobial peptide, ClavA, to a membrane
	Khondker et al. 2019)	_	POPC, POPS, DMPS	Investigate how the molecular density of a bilayer plays a significant role in the interactions of antimicrobial drugs with the membrane
	Pandit and Klauda 2012)	*E. coli*	POPE, POPG, PMPE, multiple molecular variations of PE and PG that mimic the main constituents of *E. coli* strains K12 LM 3118 and K12 NBRC 3301, PDSPE, PDSPC	Introduce cyclic moieties into the membrane to obtain a more realistic model
	Shahane et al. 2019)	_	POPE, POPG	Determine how membrane composition influences the interaction with various antimicrobial peptides
	Khakbaz and Klauda 2015)	*E. coli*	POPE, PPPE, OSPE, PMPE, QMPE, PSPG, PMPG	Simulated parameters of complex membrane composition and compared how they differ significantly from simpler models
	Lim and Klauda 2011)	*C. trachomatis*	DPhyPC, 13-MpPPC, 14-MpPPC, DPPC, DMPE, DOPE, DOPG, SLPC, PPPE, DSPE, DLPE, POPE, cholesterol	Determine how increased lipid chain branching affects bilayer properties such as elastic modulus and chain order
	Jin et al. 2021)	_	DOPC, POPE, POPG	Demonstrates the interaction of model membranes with various native and non-native small molecules used in quorum sensing
	Lee et al. 2017)	*P. aeruginosa, E. coli*	PPPE, PVPG, PVCL, R-LPS, S-LPS	Investigate how the composition of a membrane influences its interaction with an OM protein
	Ocampo-Ibáñez et al. 2020)	*P. aeruginosa and K. pneumoniae*	POPE, PMCL, POPG	Investigates how the interactions between the membrane and the cationic antimicrobial peptide, CecD, depends on the membrane composition
	Alkhalifa et al. 2020)	*E. coli, S. aureus*	POPC, DLPG, DLPE, TMCL	Determines how the membrane composition influences membrane interaction with various quaternary ammonium compounds
	Piggot et al. 2011)	*E. coli, S. aureus*	LPS, Lysyl-DPPG, POPE, POPG, DMPG, DPPE, CL	Demonstrates how membranes of various lipid composition show different electroporation properties
	Lins and Straatsma 2001)	*P. aeruginosa (PAO1)*	PE, R-LPS	Detailed description of the construction of an LPS membrane

Table 4 (continued)

Model type	Reference	Modelled bacterial species	Lipid species	Research outcomes
	Yu and Klauda 2018)	*P. aeruginosa (PAO1)*	POPE, POPG, YOPE, PMSPG, PMSPE, DPPE, YOPG, DPPG	Description of a simulation using the CHARMM (Chemistry at Harvard Macromolecular Mechanics) FF to simulate in IM of *P. aeruginosa*
	Hwang et al. 2018)	*E. coli*	POPE, POPG, PMPE, QMPE, PMPG, PSPG, OSPE, Ra-LPS	Mechanical properties of the membrane are influenced by both the cell wall as well as the OM
	Bogdanov et al. 2020)	_	DOPE, DOPG, TOCL, FDNB-PE	Elucidate the mechanism behind the inability of 1,5-difluoro-2,4-dinitobenzene to be able to cross-link PE based on phospholipid location in GN bacterial model membranes
	Piggot et al. 2013)	*E. coli*	POPC, PVPE, PVPG, PVCL, Rd-LPS	Model a transporter protein FecA, native to *E. coli* to identify various LPS-protein interactions and determine how it affects the conformational dynamics of FecA
	Kirschner et al. 2012)	*P. aeruginosa (PAO1)*	R-LPS, DPPE	Extend the GLYCAM06 FF to incorporate a new set of parameters that expands the number of monosaccharides that can be added to LPS and, consequently, improve the structure reproduction and membrane permeability for GN bacterial membrane models
	Wu et al. 2013)	*E. coli*	R-LPS, S-LPS	To build and model each LPS constituent based on chemical and spectroscopy investigations. Each consistent in LPS was used to gain insight on LPS properties, LPS molecule dynamics and LPS structure within an LPS bilayer. The addition of the O-antigen was also implemented to investigate how the O-antigen chain heterogeneity influenced membrane dynamins, structure, and properties. Simulations of the O-antigen were validated via NMR
	Dias et al. 2014)	*P. aeruginosa*	DPPE, R-LPS	Investigate how the chemical remodelling of LPS affects the electrostatic properties and structural dynamics of the OM of GN pathogen *P. aeruginosa*
	Wu et al. 2014a)	*E. coli*	PPPE, PVCL, PVPG, R-LPS	Investigate the structural properties the E. coli OM and any protein-lipid interactions experienced between the OM and phospholipase A

Table 4 (continued)

Model type	Reference	Modelled bacterial species	Lipid species	Research outcomes
	Carpenter et al. 2016)	*E. coli*	Re-LPS, PE, PG, CL	Determine the free energy of permeation of ethane, benzene, hexane, ethanol, water, and acetic acid through an OM model of *E. coli*
	Fleming et al. 2016)	*E. coli*	R-LPS, PPPE, PVPG, PVCL	Investigate the conformation flexibility of transmembrane transporter protein, BamA, to determine how membrane interactions with the polypeptide transport-associated domain influence conformation dynamics
	Patel et al. 2016)	*E. coli*	PPPE, PVPG, PVCL, DMPC, R-LPS and S-LPS modelled from the LPS structure of *E. coli* strain K12	Investigate the impact of how structural differences in various LPS molecules affect the function, dynamics, and structure of the transport protein OmpF. In addition, the importance of protein-LPS interactions was investigated to determine ion permeability and pore access behaviour in different LPS environments
	Rice and Wereszczynski 2018)	*S. enterica*	POPE, LPS (8 different variations both modified and unmodified)	Generate symmetric GN bacterial OMs to determine how the key lipid A differences in *S. enterica* alter bacterial virulence via changes in membrane properties
	Hughes et al. 2019)	_	DPPC, R-LPS modelled from the LPS structure of *E. coli* strain K12	Investigate the physical properties and biophysical behaviour of the GN bacterial OM including the lateral packing, lipid asymmetry, bilayer density and lipid profile. The results from the simulation were compared to experimental models to determine the degree of agreeability between the methods
	Li and Guo 2013)	_	DOPE, DOPG	Investigate drug-membrane interactions to comprehend the mechanism of action of the antimicrobial EO-OPE-1 (C3)
	Gao et al. 2020)	_	DPPG, PSPG, PVPG, R-LPS and S-LPS were modelled from the LPS structure of *E. coli*	Determine changes in membrane structural properties and lipid-membrane interactions upon the incorporation of enterobacterial common antigen glycoconjugates
Course-grain	Ma et al. 2017b)	*H. pylori, P. gingivalis, B. fragilis, B. pertussis, C. trachomatis, C. jejuni, N. meningitidis, and S. enterica*	Lipid A variants from each species analysed, DPPE	To investigate how the molecular profile of lipid A significantly affects the biophysical properties of the membrane such as phase transition temperatures
	Ma et al. 2015)	_	R-LPS, S-LPS, DPPE, various polysaccharides	Simulate a full GN bacterial membrane with an OM, peptidoglycan later and an IM

Table 4 (continued)

Model type	Reference	Modelled bacterial species	Lipid species	Research outcomes
	Oosten and Harroun 2016)	*P. aeruginosa*	R-LPS, POPE	An optimised simulation for a full LPS membrane
	Hsu et al. 2016)	_	POPE, Re-LPS, Ra-LPS	Investigate how the interaction of fullerenes with membrane is dependent on the membrane composition, especially the LPS structure
	Shearer et al. 2020)	*E. coli*	POPE, POPG, CL, Re-LPS	To test numerous simulation methods to determine the best protocol for lipid convergence. This is tested by quantifying the potential of mean force for LPS and phospholipid extraction from model GN bacterial IM and OM bilayers, and lateral mixing of LPS and phospholipids within model GN bacterial IM and OM bilayers
	Shearer et al. 2019)	*E. coli*	Re-LPS, Ra-LPS, DPPC, POPG, POPE, S-LPS, S-LPS-PE	To investigate protein-lipid interactions influenced by the amount of LPS, lipid mobility and protein composition on the function of six native proteins in *E. coli*
	Berglund et al. 2015)	*E. coli*	Re-LPS, PVCL, PVPE, PVPG	Investigate the mechanisms of interaction between the antimicrobial peptide, polymyxin B1, with the OM and IM of *E. coli*
	Ma et al. 2017a)	*E. coli*	DPPE, POPG, CL, Lipid A alone, Lipid A attached to its core oligosaccharides	Determine the structural properties of Lipid A with and without its core oligosaccharides, and investigate the stepwise oligomerisation process of OmpF monomers into more complex dimer and trimer structures
	Hsu et al. 2017b)	_	Ra-LPS, Re-LPS, POPG, POPE, CL	Generate a new feature for CHARMM-GUI *Martini Maker* via simulating micelle, nanodisc, vesicle, and bilayer systems in the absence and presence of membrane proteins to allow users to model complex bacterial OMs containing LPS
	Orekhov et al. 2018)	_	DPPE, POPC, POPE, POPG, Ra-LPS modelled from *P. aeruginosa strain PAO1*	Investigate the solvation behaviour of substituted polycationic metallophthalocyanines, which can result in photodynamic inactivation of GN and GP bacteria, in model bacterial membranes. The models were further utilised in investigating the molecular structure of substituted polycationic metallophthalo-cyanines, and their interactions with the membrane

Table 4 (continued)

Model type	Reference	Modelled bacterial species	Lipid species	Research outcomes
	Mehmood et al. 2016)	*E. coli*	POPE, POPG, CL	Determine which phospholipids specifically bind to the ATP-binding cassette transporter McjD in different phospholipid membrane compositions, and investigate how they impact the function and stability of the transporter
	Shearer and Khalid 2018)	_	POPE, POPG, CL, LPS	Investigate the differences in membrane dynamics and structure between symmetrical and asymmetrical GN bacterial membranes in the presence and absence of transmembrane proteins
	Hsu et al. 2017a)	*E. coli (K12)*	POPE, PVPG, CL, Re-LPS	Construct a model IM and OM of E. coli decorated with various native membrane proteins and connected by the transmembrane multi-drug efflux protein complex AcrBZ-ToIC. The model was used to investigate membrane curvature based, lipid diffusion, protein and lipid movement, lipid flow, lipid movement and protein-lipid interactions
	Kholina et al. 2020)	_	POPG, POPE	Determine how various cationic antiseptics interact with model membranes by monitoring membrane structural changes
	Tuerkova et al. 2020)	_	POPC, POPS, POPG	Determine how kinks in helical antimicrobial peptides affects membrane pore formation
Atomistic (all-atom) and course-grain	Abellón-Ruiz et al. 2017)	_	Re-LPS, POPE	Characterise and analyse the functional mechanism, structure, and lipid membrane interactions of the GN OM lipoprotein MlaA
	Baltoumas et al. 2019)	_	LPS (modelled from *P. aeruginosa* and *E. coli*), Lipid A (modelled from *E. coli*, *P. aeruginosa*, *H. pylori*, *N. meningitidis*), POPC, DOPC, DSPC, DPPC, DMPC, DLPC, POPE, DOPE, DSPE, DPPE, DMPE, DLPE, POPS, DOPS, DSPS, DPPS, DMPS, DLPS, POPG, DOPG, DSPG, DPPG, DMPG, DLPG, CL (both mono-and di-anionic forms)	Comparing the versatility and abilities of the program GNOMM (Gram-Negative Outer Membrane Modeler) in constructing and analysing the complex OM of GN bacteria across four different FFs

*See Supplementary Information (Sect. 1 and 2) for bacterial and lipid species acronym definitions, respectively

membrane properties induced by the structural variations in LPS (Kim et al. 2016).

Molecular dynamic simulations can provide a detailed picture of membrane structure, yet they sometimes limited by the high complexity of biological membrane systems. For comprehensive reviews of the analytical limitations of MD simulations, see Marrink et al. (2019) (Marrink et al. 2019) and Goossens and Winter (2018). (Goossens and Winter 2018) Developments in the field are however very promising.

Outlook

The membrane models used to mimic pathogenic bacterial membranes and the techniques used to analyse them have provided useful information on the lateral organisation of these adaptable quasi two-dimensional architectures during resistance development. Each architecture possesses individual advantages and limitations when investigating drug-membrane interactions, lipid-protein interactions, host–pathogen interactions, and structure-induced bacterial pathogenesis. As in vitro modelling systems advance, the quest for increased realism has not ceased. Key challenges include observing and incorporating complex membrane proteins such as drug-efflux proteins, connecting theoretical and experimental results, and incorporating more complex lipophilic assemblies. Current model systems are created utilising well-defined lipid mixtures, and whilst simplification is necessary for specific membrane-mediated interaction analyses, oversimplification provides an insufficient understanding of complex bacterial membrane systems and processes. By incorporating more complex compositions (proteins and lipids), insights into essential pathogen resistance development processes, membrane-targeting antimicrobial mechanisms, and generating fully artificial architectures that safely captures numerous essential pathogenic biological features can be made to help combat the devastating consequences of antibiotic resistance.

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Declarations

Conflict of interest The authors declare no competing interests.

References

Watson H (2015) Biological membranes. Essays Biochem 59:43–69. https://doi.org/10.1042/bse0590043

Guidotti G (1972) The composition of biological membranes. Arch Intern Med 129(2):194–201. https://doi.org/10.1001/archinte.1972.00320020038003

Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, Scheld M, Spellberg B, Bartlett J (2009) Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis 48(1):1–12. https://doi.org/10.1086/595011

Del Mar CB, Scott AM, Glasziou PP, Hoffmann T, van Driel ML, Beller E, Phillips SM, Dartnell J (2017) Reducing antibiotic prescribing in Australian general practice: time for a national strategy. Med J Aust 207(9):401–406. https://doi.org/10.5694/mja17.00574

Pendleton JN, Gorman SP, Gilmore BF (2013) Clinical relevance of the ESKAPE pathogens. Expert Rev Anti Infect Ther 11(3):297–308. https://doi.org/10.1586/eri.13.12

Rice LB (2010) Progress and challenges in implementing the research on ESKAPE pathogens. Infect Control Hosp Epidemiol 31(S1):S7–S10. https://doi.org/10.1086/655995

Santajit, S.; Indrawattana, N., Mechanisms of antimicrobial resistance in ESKAPE pathogens. *BioMed research international* **2016,** *2016*. https://doi.org/10.1155/2016/2475067

Ventola CL (2015) The antibiotic resistance crisis: part 1: causes and threats. Pharmacy and Therapeutics 40(4):277

Chilambi GS, Gao IH, Yoon BK, Park S, Kawakami LM, Ravikumar V, Chan-Park MB, Cho N-J, Bazan GC, Kline KA (2018) Membrane adaptation limitations in Enterococcus faecalis underlie sensitivity and the inability to develop significant resistance to conjugated oligoelectrolytes. RSC Adv 8(19):10284–10293. https://doi.org/10.1039/C7RA11823F

Gould IM, Bal AM (2013) New antibiotic agents in the pipeline and how they can help overcome microbial resistance. Virulence 4(2):185–191. https://doi.org/10.4161/viru.22507

Fernández L, Hancock RE (2012) Adaptive and mutational resistance: role of porins and efflux pumps in drug resistance. Clin Microbiol Rev 25(4):661–681. https://doi.org/10.1128/cmr.00043-12

Prestinaci F, Pezzotti P, Pantosti A (2015) Antimicrobial resistance: a global multifaceted phenomenon. Pathogens and Global Health 109(7):309–318. https://doi.org/10.1179/2047773215Y.0000000030

Jiang, J.-H.; Hassan, K. A.; Begg, S. L.; Rupasinghe, T. W.; Naidu, V.; Pederick, V. G.; Khorvash, M.; Whittall, J. J.; Paton, J. C.; Paulsen, I. T., Identification of novel Acinetobacter baumannii host fatty acid stress adaptation strategies. *Mbio* **2019,** *10* (1). https://doi.org/10.1128/mBio.02056-18

Renwick, M. J.; Simpkin, V.; Mossialos, E.; Organization, W. H., *Targeting innovation in antibiotic drug discovery and development: The need for a One Health–One Europe–One World Framework*. World Health Organization. Regional Office for Europe: 2016

Dutescu IA, Hillier SA (2021) Encouraging the Development of New Antibiotics: Are Financial Incentives the Right Way Forward? A Systematic Review and Case Study. Infect Drug Resist 14:415. https://doi.org/10.2147/IDR.S287792

D'Andrea, M. M.; Fraziano, M.; Thaller, M. C.; Rossolini, G. M., The urgent need for novel antimicrobial agents and strategies to fight antibiotic resistance. Multidisciplinary Digital Publishing Institute: 2019. https://doi.org/10.3390/antibiotics8040254

Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, Pulcini C, Kahlmeter G, Kluytmans J, Carmeli Y (2018) Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 18(3):318–327. https://doi.org/10.1016/s1473-3099(17)30753-3

Mulani MS, Kamble EE, Kumkar SN, Tawre MS, Pardesi KR (2019) Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. Front Microbiol 10:539. https://doi.org/10.3389/fmicb.2019.00539

Makabenta JMV, Nabawy A, Li C-H, Schmidt-Malan S, Patel R, Rotello VM (2021) Nanomaterial-based therapeutics for antibiotic-resistant bacterial infections. Nat Rev Microbiol 19(1):23–36. https://doi.org/10.1038/s41579-020-0420-1

Fatima F, Siddiqui S, Khan WA (2021) Nanoparticles as novel emerging therapeutic antibacterial agents in the antibiotics resistant era. Biol Trace Elem Res 199(7):2552–2564. https://doi.org/10.1007/s12011-020-02394-3

Mantravadi PK, Kalesh KA, Dobson RC, Hudson AO, Parthasarathy A (2019) The quest for novel antimicrobial compounds: emerging trends in research, development, and technologies. Antibiotics 8(1):8. https://doi.org/10.3390/antibiotics8010008

Charbonneau MR, Isabella VM, Li N, Kurtz CB (2020) Developing a new class of engineered live bacterial therapeutics to treat human diseases. Nat Commun 11(1):1–11. https://doi.org/10.1038/s41467-020-15508-1

Hussein M, Karas JA, Schneider-Futschik EK, Chen F, Swarbrick J, Paulin OK, Hoyer D, Baker M, Zhu Y, Li J (2020) The killing mechanism of teixobactin against methicillin-resistant Staphylococcus aureus: an untargeted metabolomics study. Msystems 5(3):e00077-e120. https://doi.org/10.1128/mSystems.00077-20

Hutchings MI, Truman AW, Wilkinson B (2019) Antibiotics: past, present and future. Curr Opin Microbiol 51:72–80. https://doi.org/10.1016/j.mib.2019.10.008

Quinto EJ, Caro I, Villalobos-Delgado LH, Mateo J, De-Mateo-Silleras B, Redondo-Del-Río MP (2019) Food Safety through Natural Antimicrobials. Antibiotics 8(4):208. https://doi.org/10.3390/antibiotics8040208

Ghrairi, T.; Jaraud, S.; Alves, A.; Fleury, Y.; El Salabi, A.; Chouchani, C., New insights into and updates on antimicrobial agents from natural products. Hindawi: 2019. https://doi.org/10.1155/2019/7079864

Kapoor G, Saigal S, Elongavan A (2017) Action and resistance mechanisms of antibiotics: A guide for clinicians. J Anaesthesiol Clin Pharmacol 33(3):300. https://doi.org/10.4103/joacp.JOACP_349_15

Epand RM, Walker C, Epand RF, Magarvey NA (2016) Molecular mechanisms of membrane targeting antibiotics. Biochimica et Biophysica Acta (BBA)-Biomembranes 1858(5):980–987. https://doi.org/10.1016/j.bbamem.2015.10.018

Tenover FC (2006) Mechanisms of antimicrobial resistance in bacteria. Am J Med 119(6):S3–S10. https://doi.org/10.1016/j.amjmed.2006.03.011

Dias C, Rauter AP (2019) Membrane-targeting antibiotics: recent developments outside the peptide space. Future Med Chem 11(3):211–228. https://doi.org/10.4155/fmc-2018-0254

Dadhich R, Kapoor S (2020) Various Facets of Pathogenic Lipids in Infectious Diseases: Exploring Virulent Lipid-Host Interactome and Their Druggability. J Membr Biol 253(5):399–423. https://doi.org/10.1007/s00232-020-00135-0

Han, M.-L.; Zhu, Y.; Creek, D. J.; Lin, Y.-W.; Anderson, D.; Shen, H.-H.; Tsuji, B.; Gutu, A. D.; Moskowitz, S. M.; Velkov, T., Alterations of metabolic and lipid profiles in polymyxin-resistant Pseudomonas aeruginosa. *Antimicrobial agents and chemotherapy* **2018,** *62* (6). https://doi.org/10.1128/AAC.02656-17

Jiang J-H, Bhuiyan MS, Shen H-H, Cameron DR, Rupasinghe TW, Wu C-M, Le Brun AP, Kostoulias X, Domene C, Fulcher AJ (2019b) Antibiotic resistance and host immune evasion in Staphylococcus aureus mediated by a metabolic adaptation. Proc Natl Acad Sci 116(9):3722–3727. https://doi.org/10.1073/pnas.1812066116

Maifiah MHM, Cheah S-E, Johnson MD, Han M-L, Boyce JD, Thamlikitkul V, Forrest A, Kaye KS, Hertzog P, Purcell AW (2016) Global metabolic analyses identify key differences in metabolite levels between polymyxin-susceptible and polymyxin-resistant Acinetobacter baumannii. Sci Rep 6(1):1–17. https://doi.org/10.1038/srep22287

Mishra NN, Bayer AS, Tran TT, Shamoo Y, Mileykovskaya E, Dowhan W, Guan Z, Arias CA (2012) Daptomycin resistance in enterococci is associated with distinct alterations of cell membrane phospholipid content. PloS one 7(8):e43958. https://doi.org/10.1371/journal.pone.0043958

Breijyeh Z, Jubeh B, Karaman R (2020) Resistance of Gram-negative bacteria to current antibacterial agents and approaches to resolve it. Molecules 25(6):1340. https://doi.org/10.3390/molecules25061340

Ghai I, Ghai S (2018) Understanding antibiotic resistance via outer membrane permeability. Infect Drug Resist 11:523. https://doi.org/10.2147/idr.s156995

Delcour AH (2009) Outer membrane permeability and antibiotic resistance. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics 1794(5):808–816. https://doi.org/10.1016/j.bbapap.2008.11.005

Vasoo, S.; Barreto, J. N.; Tosh, P. K. In *Emerging issues in gram-negative bacterial resistance: an update for the practicing clinician*, Mayo Clinic Proceedings, Elsevier: 2015, 395–403. https://doi.org/10.1016/j.mayocp.2014.12.002

Corradi V, Sejdiu BI, Mesa-Galloso H, Abdizadeh H, Noskov SY, Marrink SJ, Tieleman DP (2019) Emerging diversity in lipid–protein interactions. Chem Rev 119(9):5775–5848. https://doi.org/10.1021/acs.chemrev.8b00451

Collinson I (2019) The dynamic ATP-Driven mechanism of bacterial protein translocation and the critical role of phospholipids. Front Microbiol 10:1217. https://doi.org/10.3389/fmicb.2019.01217

Lin T-Y, Weibel DB (2016) Organization and function of anionic phospholipids in bacteria. Appl Microbiol Biotechnol 100(10):4255–4267. https://doi.org/10.1007/s00253-016-7468-x

Martens C, Shekhar M, Lau AM, Tajkhorshid E, Politis A (2019) Integrating hydrogen–deuterium exchange mass spectrometry with molecular dynamics simulations to probe lipid-modulated conformational changes in membrane proteins. Nat Protoc 14(11):3183–3204. https://doi.org/10.1038/s41596-019-0219-6

Martens C, Stein RA, Masureel M, Roth A, Mishra S, Dawaliby R, Konijnenberg A, Sobott F, Govaerts C, Mchaourab HS (2016) Lipids modulate the conformational dynamics of a secondary multidrug transporter. Nat Struct Mol Biol 23(8):744. https://doi.org/10.1038/nsmb.3262

Norimatsu Y, Hasegawa K, Shimizu N, Toyoshima C (2017) Protein–phospholipid interplay revealed with crystals of a calcium pump. Nature 545(7653):193–198. https://doi.org/10.1038/nature22357

Du D, Wang-Kan X, Neuberger A, van Veen HW, Pos KM, Piddock LJ, Luisi BF (2018) Multidrug efflux pumps: structure, function and regulation. Nat Rev Microbiol 16(9):523–539. https://doi.org/10.1038/s41579-018-0048-6

Lam SJ, O'Brien-Simpson NM, Pantarat N, Sulistio A, Wong EH, Chen Y-Y, Lenzo JC, Holden JA, Blencowe A, Reynolds EC (2016)

Combating multidrug-resistant Gram-negative bacteria with structurally nanoengineered antimicrobial peptide polymers. Nat Microbiol 1(11):1–11. https://doi.org/10.1038/nmicrobiol.2016.162

Behuria, H.; Pal, N.; Munda, R.; Sahu, S., Preparation of Giant Unilamellar Vesicles (GUVS) from Bacterial Polar Lipid Extract: Developing a Prokaryotic Model Membrane System. In *Biotechnology for Sustainable Utilization of Bioresources*, Astral International Pvt. Ltd: New Delhi, 2020, 309-320

Brown S, Meredith T, Swoboda J, Walker S (2010) Staphylococcus aureus and Bacillus subtilis W23 make polyribitol wall teichoic acids using different enzymatic pathways. Chem Biol 17(10):1101–1110. https://doi.org/10.1016/j.chembiol.2010.07.017

Veron W, Orange N, Feuilloley MG, Lesouhaitier O (2008) Natriuretic peptides modify Pseudomonas fluorescens cytotoxicity by regulating cyclic nucleotides and modifying LPS structure. BMC Microbiol 8(1):1–11. https://doi.org/10.1186/1471-2180-8-114

Andersson J, Fuller MA, Wood K, Holt SA, Köper I (2018a) A tethered bilayer lipid membrane that mimics microbial membranes. Phys Chem Chem Phys 20(18):12958–12969. https://doi.org/10.1039/C8CP01346B

Castellana ET, Cremer PS (2006) Solid supported lipid bilayers: From biophysical studies to sensor design. Surf Sci Rep 61(10):429–444. https://doi.org/10.1016/j.surfrep.2006.06.001

Qing G, Gong N, Chen X, Chen J, Zhang H, Wang Y, Wang R, Zhang S, Zhang Z, Zhao X (2019) Natural and engineered bacterial outer membrane vesicles. Biophysics Reports 5(4):184–198. https://doi.org/10.1007/s41048-019-00095-6

Strahl H, Errington J (2017) Bacterial membranes: structure, domains, and function. Annu Rev Microbiol 71:519–538. https://doi.org/10.1146/annurev-micro-102215-095630

Salehi-Reyhani A, Ces O, Elani Y (2017) Artificial cell mimics as simplified models for the study of cell biology. Exp Biol Med 242(13):1309–1317. https://doi.org/10.1177/1535370217711441

Andersson J, Bilotto P, Mears LL, Fossati S, Ramach U, Köper I, Valtiner M, Knoll W (2020) Solid-supported lipid bilayers–A versatile tool for the structural and functional characterization of membrane proteins. Methods 180:56–68. https://doi.org/10.1016/j.ymeth.2020.09.005

Andersson J, Köper I (2016) Tethered and polymer supported bilayer lipid membranes: structure and function. Membranes 6(2):30. https://doi.org/10.3390/membranes6020030

Andersson J, Köper I, Knoll W (2018b) Tethered membrane architectures—design and applications. Front Mater 5:55. https://doi.org/10.3389/fmats.2018.00055

Chan Y-HM, Boxer SG (2007) Model membrane systems and their applications. Curr Opin Chem Biol 11(6):581–587. https://doi.org/10.1016/j.cbpa.2007.09.020

Jackman JA, Knoll W, Cho N-J (2012) Biotechnology applications of tethered lipid bilayer membranes. Materials 5(12):2637–2657. https://doi.org/10.3390/ma5122637

Siontorou CG, Nikoleli G-P, Nikolelis DP, Karapetis SK (2017) Artificial lipid membranes: Past, present, and future. Membranes 7(3):38. https://doi.org/10.3390/membranes7030038

Wiebalck S, Kozuch J, Forbrig E, Tzschucke CC, Jeuken LJ, Hildebrandt P (2016) Monitoring the transmembrane proton gradient generated by cytochrome bo 3 in tethered bilayer lipid membranes using SEIRA spectroscopy. J Phys Chem B 120(9):2249–2256. https://doi.org/10.1021/acs.jpcb.6b01435

Zieleniecki JL, Nagarajan Y, Waters S, Rongala J, Thompson V, Hrmova M, Köper I (2016) Cell-free synthesis of a functional membrane transporter into a tethered bilayer lipid membrane. Langmuir 32(10):2445–2449. https://doi.org/10.1021/acs.langmuir.5b04059

Peetla C, Stine A, Labhasetwar V (2009) Biophysical interactions with model lipid membranes: applications in drug discovery and drug delivery. Mol Pharm 6(5):1264–1276. https://doi.org/10.1021/mp9000662

Knobloch J, Suhendro DK, Zieleniecki JL, Shapter JG, Köper I (2015) Membrane–drug interactions studied using model membrane systems. Saudi J Biol Sci 22(6):714–718. https://doi.org/10.1016/j.sjbs.2015.03.007

Hollmann A, Martinez M, Maturana P, Semorile LC, Maffia PC (2018) Antimicrobial peptides: interaction with model and biological membranes and synergism with chemical antibiotics. Front Chem 6:204. https://doi.org/10.3389/fchem.2018.00204

Solntceva V, Kostrzewa M, Larrouy-Maumus G (2020) Detection of species-specific lipids by routine MALDI TOF mass spectrometry to unlock the challenges of microbial identification and antimicrobial susceptibility testing. Front Cell Infect Microbiol 10:914. https://doi.org/10.3389/fcimb.2020.621452

De Carvalho CC, Caramujo MJ (2018) The various roles of fatty acids. Molecules 23(10):2583. https://doi.org/10.3390/molecules23102583

Willdigg JR, Helmann JD (2021) Mini Review: Bacterial Membrane Composition and Its Modulation in Response to Stress. Front Mol Biosci 8:338. https://doi.org/10.3389/fmolb.2021.634438

Epand RM, Epand RF (2009) Lipid domains in bacterial membranes and the action of antimicrobial agents. Biochimica et Biophysica Acta (BBA)-Biomembranes 1788(1):289–294. https://doi.org/10.1016/j.bbamem.2008.08.023

Sohlenkamp C, Geiger O (2016) Bacterial membrane lipids: diversity in structures and pathways. FEMS Microbiol Rev 40(1):133–159. https://doi.org/10.1093/femsre/fuv008

Alagumuthu M, Dahiya D, Nigam PS (2019) Phospholipid—the dynamic structure between living and non-living world; a much obligatory supramolecule for present and future [J]. AIMS Mol Sci 6(1):1–19. https://doi.org/10.3934/molsci.2019.1.1

Fahy E, Cotter D, Sud M, Subramaniam S (2011) Lipid classification, structures and tools. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids 1811(11):637–647. https://doi.org/10.1016/j.bbalip.2011.06.009

Singer SJ, Nicolson GL (1972) The fluid mosaic model of the structure of cell membranes. Science 175(4023):720–731. https://doi.org/10.1126/science.175.4023.720

Van Meer G, Voelker DR, Feigenson GW (2008) Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol 9(2):112–124. https://doi.org/10.1038/nrm2330

Barák I, Muchová K (2013) The role of lipid domains in bacterial cell processes. Int J Mol Sci 14(2):4050–4065. https://doi.org/10.3390/ijms14024050

Cronan JE, Thomas J (2009) Bacterial fatty acid synthesis and its relationships with polyketide synthetic pathways. Methods Enzymol 459:395–433. https://doi.org/10.1016/s0076-6879(09)04617-5

Bertani, B.; Ruiz, N., Function and biogenesis of lipopolysaccharides. *EcoSal Plus* **2018,** *8* (1). https://doi.org/10.1128/ecosalplus.esp-0001-2018

Reichmann NT, Gründling A (2011) Location, synthesis and function of glycolipids and polyglycerolphosphate lipoteichoic acid in Gram-positive bacteria of the phylum Firmicutes. FEMS Microbiol Lett 319(2):97–105. https://doi.org/10.1111/j.1574-6968.2011.02260.x

May KL, Grabowicz M (2018) The bacterial outer membrane is an evolving antibiotic barrier. Proc Natl Acad Sci 115(36):8852–8854. https://doi.org/10.1073/pnas.1812779115

Rahman MM, Kolli VK, Kahler CM, Shih G, Stephens DS, Carlson RW (2000) The membrane phospholipids of Neisseria meningitidis and Neisseria gonorrhoeae as characterized by fast atom bombardment mass spectrometry. Microbiology 146(8):1901–1911. https://doi.org/10.1099/00221287-146-8-1901

Jasim R, Han M-L, Zhu Y, Hu X, Hussein MH, Lin Y-W, Zhou QT, Dong CYD, Li J, Velkov T (2018) Lipidomic analysis of the outer membrane vesicles from paired polymyxin-susceptible and-resistant Klebsiella pneumoniae clinical isolates. Int J Mol Sci 19(8):2356. https://doi.org/10.3390/ijms19082356

Theilacker C, Kropec A, Hammer F, Sava I, Wobser D, Sakinc T, Codée JD, Hogendorf WF, van der Marel GA, Huebner J (2012) Protection against Staphylococcus aureus by antibody to the polyglycerolphosphate backbone of heterologous lipoteichoic acid. J Infect Dis 205(7):1076–1085. https://doi.org/10.1093/infdis/jis022

Song H-S, Choi T-R, Han Y-H, Park Y-L, Park JY, Yang S-Y, Bhatia SK, Gurav R, Kim Y-G, Kim J-S (2020) Increased resistance of a methicillin-resistant Staphylococcus aureus Δ agr mutant with modified control in fatty acid metabolism. AMB Express 10(1):1–10. https://doi.org/10.1186/s13568-020-01000-y

Schneewind O, Missiakas D (2014) Lipoteichoic acids, phosphate-containing polymers in the envelope of gram-positive bacteria. J Bacteriol 196(6):1133–1142. https://doi.org/10.1128/JB.01155-13

Kilelee E, Pokorny A, Yeaman MR, Bayer AS (2010) Lysyl-phosphatidylglycerol attenuates membrane perturbation rather than surface association of the cationic antimicrobial peptide 6W-RP-1 in a model membrane system: implications for daptomycin resistance. Antimicrob Agents Chemother 54(10):4476–4479. https://doi.org/10.1128/AAC.00191-10

Malanovic N, Lohner K (2016) Gram-positive bacterial cell envelopes: The impact on the activity of antimicrobial peptides. Biochimica et Biophysica Acta (BBA)-Biomembranes 1858(5):936–946. https://doi.org/10.1016/j.bbamem.2015.11.004

Oku Y, Kurokawa K, Ichihashi N, Sekimizu K (2004) Characterization of the Staphylococcus aureus mprF gene, involved in lysinylation of phosphatidylglycerol. Microbiology 150(1):45–51. https://doi.org/10.1099/mic.0.26706-0

White DC, Frerman FE (1967) Extraction, characterization, and cellular localization of the lipids of Staphylococcus aureus. J Bacteriol 94(6):1854–1867. https://doi.org/10.1128/jb.94.6.1854-1867.1967

Vinogradov E, Frirdich E, MacLean LL, Perry MB, Petersen BO, Duus JØ, Whitfield C (2002) Structures of lipopolysaccharides from Klebsiella pneumoniae: Elucidation of the structure of the linkage region between core and polysaccharide O chain and identification of the residues at the non-reducing termini of the Ochains. J Biol Chem 277(28):25070–25081. https://doi.org/10.1074/jbc.m202683200

Hobby CR, Herndon JL, Morrow CA, Peters RE, Symes SJ, Giles DK (2019) Exogenous fatty acids alter phospholipid composition, membrane permeability, capacity for biofilm formation, and antimicrobial peptide susceptibility in Klebsiella pneumoniae. Microbiologyopen 8(2):e00635. https://doi.org/10.1002/mbo3.635

Unno, Y.; Sato, Y.; Nishida, S.; Nakano, A.; Nakano, R.; Ubagai, T.; Ono, Y., Acinetobacter baumannii Lipopolysaccharide Influences Adipokine Expression in 3T3-L1 Adipocytes. *Mediators of inflammation* **2017,** *2017*. https://doi.org/10.1155/2017/9039302

Jiang X, Yang K, Yuan B, Han M, Zhu Y, Roberts KD, Patil NA, Li J, Gong B, Hancock RE (2020) Molecular dynamics simulations informed by membrane lipidomics reveal the structure–interaction relationship of polymyxins with the lipid A-based outer membrane of Acinetobacter baumannii. J Antimicrob Chemother 75(12):3534–3543. https://doi.org/10.1093/jac/dkaa376

Lopalco P, Stahl J, Annese C, Averhoff B, Corcelli A (2017) Identification of unique cardiolipin and monolysocardiolipin species in Acinetobacter baumannii. Sci Rep 7(1):1–12. https://doi.org/10.1038/s41598-017-03214-w

Chao J, Wolfaardt GM, Arts MT (2010) Characterization of Pseudomonas aeruginosa fatty acid profiles in biofilms and batch planktonic cultures. Can J Microbiol 56(12):1028–1039. https://doi.org/10.1139/w10-093

Lam JS, Taylor VL, Islam ST, Hao Y, Kocíncová D (2011) Genetic and functional diversity of Pseudomonas aeruginosa lipopolysaccharide. Front Microbiol 2:118. https://doi.org/10.3389/fmicb.2011.00118

Klein S, Lorenzo C, Hoffmann S, Walther JM, Storbeck S, Piekarski T, Tindall BJ, Wray V, Nimtz M, Moser J (2009) Adaptation of Pseudomonas aeruginosa to various conditions includes tRNA-dependent formation of alanyl-phosphatidylglycerol. Mol Microbiol 71(3):551–565. https://doi.org/10.1111/j.1365-2958.2008.06562.x

Lewenza S, Falsafi R, Bains M, Rohs P, Stupak J, Sprott GD, Hancock RE (2011) The olsA gene mediates the synthesis of an ornithine lipid in Pseudomonas aeruginosa during growth under phosphate-limiting conditions, but is not involved in antimicrobial peptide susceptibility. FEMS Microbiol Lett 320(2):95–102. https://doi.org/10.1111/j.1574-6968.2011.02295.x

Pramanik B, Zechman J, Das P, Bartner P (1990) Bacterial phospholipid analysis by fast atom bombardment mass spectrometry. Biomed Environ Mass Spectrom 19(3):164–170. https://doi.org/10.1002/bms.1200190312

Wilderman PJ, Vasil AI, Martin WE, Murphy RC, Vasil ML (2002) Pseudomonas aeruginosa synthesizes phosphatidylcholine by use of the phosphatidylcholine synthase pathway. J Bacteriol 184(17):4792–4799. https://doi.org/10.1128/jb.184.17.4792-4799.2002

Soberón-Chávez G, Lépine F, Déziel E (2005) Production of rhamnolipids by Pseudomonas aeruginosa. Appl Microbiol Biotechnol 68(6):718–725. https://doi.org/10.1007/s00253-005-0150-3

Bøse B, Gjerde J (1980) Fatty acid patterns in the classification of some representatives of the families Enterobacteriaceae and Vibrionaceae. Microbiology 116(1):41–49. https://doi.org/10.1099/00221287-116-1-41

Gill C, Suisted J (1978) The effects of temperature and growth rate on the proportion of unsaturated fatty acids in bacterial lipids. Microbiology 104(1):31–36. https://doi.org/10.1099/00221287-104-1-31

Kämpfer P, McInroy JA, Glaeser SP (2015) Enterobacter muelleri sp. nov., isolated from the rhizosphere of Zea mays. Int J Syst Evol Microbiol 65(Pt_11):4093–4099. https://doi.org/10.1099/ijsem.0.000547

Davin-Regli A, Lavigne J-P, Pagès J-M (2019) Enterobacter spp.: update on taxonomy, clinical aspects, and emerging antimicrobial resistance. Clin Microbiol Rev 32(4):e00002-19. https://doi.org/10.1128/cmr.00002-19

Epand RM, Epand RF (2009b) Domains in bacterial membranes and the action of antimicrobial agents. Mol BioSyst 5(6):580–587. https://doi.org/10.1016/j.bbamem.2008.08.023

Epand RM, Epand RF, Arnusch CJ, Papahadjopoulos-Sternberg B, Wang G, Shai Y (2010) Lipid clustering by three homologous arginine-rich antimicrobial peptides is insensitive to amino acid arrangement and induced secondary structure. Biochimica et Biophysica Acta (BBA)-Biomembranes 1798(6):1272–1280. https://doi.org/10.1016/j.bbamem.2010.03.012

Villegas, M. V.; Quinn, J. P., Enterobacter species. *Antimicrobial therapy and vaccines. Maryland: Apple Trees Productions LLC* **2002**, 255–63.

Silhavy TJ, Kahne D, Walker S (2010) The bacterial cell envelope. Cold Spring Harbor Perspect Biol 2(5):a000414. https://doi.org/10.1101/cshperspect.a000414

Bogdanov M, Pyrshev K, Yesylevskyy S, Ryabichko S, Boiko V, Ivanchenko P, Kiyamova R, Guan Z, Ramseyer C, Dowhan W

(2020) Phospholipid distribution in the cytoplasmic membrane of Gram-negative bacteria is highly asymmetric, dynamic, and cell shape-dependent. Sci Adv 6(23):eaaz6333. https://doi.org/10.1126/sciadv.aaz6333

Paulowski L, Donoghue A, Nehls C, Groth S, Koistinen M, Hagge SO, Böhling A, Winterhalter M, Gutsmann T (2020) The beauty of asymmetric membranes: Reconstitution of the outer membrane of Gram-negative bacteria. Front Cell Dev Biol 8:586. https://doi.org/10.3389/fcell.2020.00586

Cian, M.; Giordano, N.; Mettlach, J.; Minor, K.; Dalebroux, Z., Separation of the Cell Envelope for Gram-negative Bacteria into Inner and Outer Membrane Fractions with Technical Adjustments for Acinetobacter baumannii. *Journal of Visualized Experiments: Jove* **2020,** (158). https://doi.org/10.3791/60517

Raetz CR, Whitfield C (2002) Lipopolysaccharide endotoxins. Annu Rev Biochem 71(1):635–700. https://doi.org/10.1146/annurev.biochem.71.110601.135414

Wang, X.; Quinn, P. J., Endotoxins: lipopolysaccharides of gram-negative bacteria. In *Endotoxins: structure, function and recognition*, Springer: 2010; pp 3–25. https://doi.org/10.1007/978-90-481-9078-2_1

Li Y, Powell DA, Shaffer SA, Rasko DA, Pelletier MR, Leszyk JD, Scott AJ, Masoudi A, Goodlett DR, Wang X (2012) LPS remodeling is an evolved survival strategy for bacteria. Proc Natl Acad Sci 109(22):8716–8721. https://doi.org/10.1073/pnas.1202908109

Needham BD, Trent MS (2013) Fortifying the barrier: the impact of lipid A remodelling on bacterial pathogenesis. Nat Rev Microbiol 11(7):467–481. https://doi.org/10.1038/nrmicro3047

Trent MS, Stead CM, Tran AX, Hankins JV (2006) Diversity of Endotoxin and Its Impact on Pathogenesis. J Endotoxin Res 12(4):205–223. https://doi.org/10.1179/096805106x118825

Simpson BW, Trent MS (2019) Pushing the envelope: LPS modifications and their consequences. Nat Rev Microbiol 17(7):403–416. https://doi.org/10.1038/s41579-019-0201-x

Maldonado RF, Sá-Correia I, Valvano MA (2016) Lipopolysaccharide modification in Gram-negative bacteria during chronic infection. FEMS Microbiol Rev 40(4):480–493. https://doi.org/10.1093/femsre/fuw007

Moffatt, J. H.; Harper, M.; Harrison, P.; Hale, J. D.; Vinogradov, E.; Seemann, T.; Henry, R.; Crane, B.; St. Michael, F.; Cox, A. D., Colistin resistance in Acinetobacter baumannii is mediated by complete loss of lipopolysaccharide production. *Antimicrob Agents Chemother* **2010,** *54* (12), 4971-4977. https://doi.org/10.1128/aac.00834-10

Pelletier MR, Casella LG, Jones JW, Adams MD, Zurawski DV, Hazlett KR, Doi Y, Ernst RK (2013) Unique structural modifications are present in the lipopolysaccharide from colistin-resistant strains of Acinetobacter baumannii. Antimicrob Agents Chemother 57(10):4831–4840. https://doi.org/10.1128/aac.00865-13

Dortet L, Broda A, Bernabeu S, Glupczynski Y, Bogaerts P, Bonnin R, Naas T, Filloux A, Larrouy-Maumus G (2020) Optimization of the MALDIxin test for the rapid identification of colistin resistance in Klebsiella pneumoniae using MALDI-TOF MS. J Antimicrob Chemother 75(1):110–116. https://doi.org/10.1093/jac/dkz405

Olaitan AO, Morand S, Rolain J-M (2014) Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front Microbiol 5:643. https://doi.org/10.3389/fmicb.2014.00643

Nikaido H (2003) Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 67(4):593–656. https://doi.org/10.1128/mmbr.67.4.593-656.2003

Pajerski W, Ochonska D, Brzychczy-Wloch M, Indyka P, Jarosz M, Golda-Cepa M, Sojka Z, Kotarba A (2019) Attachment efficiency of gold nanoparticles by Gram-positive and Gram-negative bacterial strains governed by surface charges. J Nanopart Res 21(8):1–12. https://doi.org/10.1007/s11051-019-4617-z

Percy MG, Gründling A (2014) Lipoteichoic acid synthesis and function in gram-positive bacteria. Annu Rev Microbiol 68:81–100. https://doi.org/10.1146/annurev-micro-091213-112949

Shiraishi T, Yokota S-I, Morita N, Fukiya S, Tomita S, Tanaka N, Okada S, Yokota A (2013) Characterization of a Lactobacillus gasseri JCM 1131T lipoteichoic acid with a novel glycolipid anchor structure. Appl Environ Microbiol 79(10):3315–3318. https://doi.org/10.1128/AEM.00243-13

Gutmann L, Al-Obeid S, Billot-Klein D, Ebnet E, Fischer W (1996) Penicillin tolerance and modification of lipoteichoic acid associated with expression of vancomycin resistance in VanB-type Enterococcus faecium D366. Antimicrob Agents Chemother 40(1):257–259. https://doi.org/10.1128/AAC.40.1.257

Saar-Dover, R.; Bitler, A.; Nezer, R.; Shmuel-Galia, L.; Firon, A.; Shimoni, E.; Trieu-Cuot, P.; Shai, Y., D-alanylation of lipoteichoic acids confers resistance to cationic peptides in group B streptococcus by increasing the cell wall density. **2012**. https://doi.org/10.1371/journal.ppat.1002891

Abachin E, Poyart C, Pellegrini E, Milohanic E, Fiedler F, Berche P, Trieu-Cuot P (2002) Formation of d-alanyl-lipoteichoic acid is required for adhesion and virulence of Listeria monocytogenes. Mol Microbiol 43(1):1–14. https://doi.org/10.1046/j.1365-2958.2002.02723.x

Peschel A, Otto M, Jack RW, Kalbacher H, Jung G, Götz F (1999) Inactivation of the dlt Operon inStaphylococcus aureus Confers Sensitivity to Defensins, Protegrins, and Other Antimicrobial Peptides. J Biol Chem 274(13):8405–8410. https://doi.org/10.1074/jbc.274.13.8405

Reichmann NT, Cassona CP, Gründling A (2013) Revised mechanism of D-alanine incorporation into cell wall polymers in Gram-positive bacteria. Microbiology 159(Pt 9):1868. https://doi.org/10.1099/mic.0.069898-0

Rosado H, Turner RD, Foster SJ, Taylor PW (2015) Impact of the β-lactam resistance modifier (−)-epicatechin gallate on the non-random distribution of phospholipids across the cytoplasmic membrane of Staphylococcus aureus. Int J Mol Sci 16(8):16710–16727. https://doi.org/10.3390/ijms160816710

Jones T, Yeaman MR, Sakoulas G, Yang S-J, Proctor RA, Sahl H-G, Schrenzel J, Xiong YQ, Bayer AS (2008) Failures in clinical treatment of Staphylococcus aureus infection with daptomycin are associated with alterations in surface charge, membrane phospholipid asymmetry, and drug binding. Antimicrob Agents Chemother 52(1):269–278. https://doi.org/10.1128/aac.00719-07

Scott JR, Barnett TC (2006) Surface proteins of gram-positive bacteria and how they get there. Annu Rev Microbiol 60:397–423. https://doi.org/10.1146/annurev.micro.60.080805.142256

Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, Samiei M, Kouhi M, Nejati-Koshki K (2013) Liposome: classification, preparation, and applications. Nanoscale Res Lett 8(1):1–9. https://doi.org/10.1186/1556-276X-8-102

Šturm L, Poklar Ulrih N (2021) Basic Methods for Preparation of Liposomes and Studying Their Interactions with Different Compounds, with the Emphasis on Polyphenols. Int J Mol Sci 22(12):6547. https://doi.org/10.3390/ijms22126547

Navas BP, Lohner K, Deutsch G, Sevcsik E, Riske K, Dimova R, Garidel P, Pabst G (2005) Composition dependence of vesicle morphology and mixing properties in a bacterial model membrane system. Biochimica et Biophysica Acta (BBA)-Biomembranes 1716(1):40–48. https://doi.org/10.1016/j.bbamem.2005.08.003

Giuliano CB, Cvjetan N, Ayache J, Walde PJ (2021) Multivesicular Vesicles: Preparation and Applications. ChemSystemsChem 3(2):e2000049. https://doi.org/10.1002/syst.202000049

Mu H, Wang Y, Chu Y, Jiang Y, Hua H, Chu L, Wang K, Wang A, Liu W, Li Y (2018) Multivesicular liposomes for sustained release of

bevacizumab in treating laser-induced choroidal neovascularization. Drug Delivery 25(1):1372–1383. https://doi.org/10.1080/10717544.2018.1474967

Gabizon A, Goren D, Cohen R, Barenholz Y (1998) Development of liposomal anthracyclines: from basics to clinical applications. J Control Release 53(1–3):275–279. https://doi.org/10.1016/s0168-3659(97)00261-7

Sherratt SC, Mason RP (2018) Eicosapentaenoic acid and docosahexaenoic acid have distinct membrane locations and lipid interactions as determined by X-ray diffraction. Chem Phys Lipid 212:73–79. https://doi.org/10.1016/j.chemphyslip.2018.01.002

Fan M, Xu S, Xia S, Zhang X (2007) Effect of different preparation methods on physicochemical properties of salidroside liposomes. J Agric Food Chem 55(8):3089–3095. https://doi.org/10.1021/jf062935q

Bozzuto G, Molinari A (2015) Liposomes as nanomedical devices. Int J Nanomed 10:975. https://doi.org/10.2147/ijn.s68861

Riaz MK, Riaz MA, Zhang X, Lin C, Wong KH, Chen X, Zhang G, Lu A, Yang Z (2018) Surface functionalization and targeting strategies of liposomes in solid tumor therapy: A review. Int J Mol Sci 19(1):195. https://doi.org/10.3390/ijms19010195

Sakai-Kato K, Yoshida K, Izutsu K-I (2019) Effect of surface charge on the size-dependent cellular internalization of liposomes. Chem Phys Lipids 224:104726. https://doi.org/10.1016/j.chemphyslip.2019.01.004

Tuerkova A, Kabelka I, Králová T, Sukeník L, Pokorná Š, Hof M, Vácha R (2020) Effect of helical kink in antimicrobial peptides on membrane pore formation. Elife 9:e47946. https://doi.org/10.7554/eLife.47946

Dombach JL, Quintana JL, Nagy TA, Wan C, Crooks AL, Yu H, Su C-C, Yu EW, Shen J, Detweiler CS (2020) A small molecule that mitigates bacterial infection disrupts Gram-negative cell membranes and is inhibited by cholesterol and neutral lipids. PLoS pathogens 16(12):e1009119. https://doi.org/10.1371/journal.ppat.1009119

Jamasbi E, Batinovic S, Sharples RA, Sani M-A, Robins-Browne RM, Wade JD, Separovic F, Hossain MA (2014) Melittin peptides exhibit different activity on different cells and model membranes. Amino Acids 46(12):2759–2766. https://doi.org/10.1007/s00726-014-1833-9

Kumagai A, Dupuy FG, Arsov Z, Elhady Y, Moody D, Ernst RK, Deslouches B, Montelaro RC, Di YP, Tristram-Nagle S (2019) Elastic behavior of model membranes with antimicrobial peptides depends on lipid specificity and d-enantiomers. Soft Matter 15(8):1860–1868. https://doi.org/10.1039/c8sm02180e

Pérez-Peinado C, Dias SA, Domingues MM, Benfield AH, Freire JM, Rádis-Baptista G, Gaspar D, Castanho MA, Craik DJ, Henriques ST (2018) Mechanisms of bacterial membrane permeabilization by crotalicidin (Ctn) and its fragment Ctn (15–34), antimicrobial peptides from rattlesnake venom. J Biol Chem 293(5):1536–1549. https://doi.org/10.1074/jbc.RA117.000125

Malishev R, Abbasi R, Jelinek R, Chai L (2018) Bacterial model membranes reshape fibrillation of a functional amyloid protein. Biochemistry 57(35):5230–5238. https://doi.org/10.1021/acs.biochem.8b00002

Kahveci Z, Vázquez-Guilló R, Mira A, Martinez L, Falcó A, Mallavia R, Mateo CR (2016) Selective recognition and imaging of bacterial model membranes over mammalian ones by using cationic conjugated polyelectrolytes. Analyst 141(22):6287–6296. https://doi.org/10.1039/c6an01427e

Lopes SC, Neves CS, Eaton P, Gameiro P (2012) Improved model systems for bacterial membranes from differing species: the importance of varying composition in PE/PG/cardiolipin ternary mixtures. Mol Membr Biol 29(6):207–217. 152. https://doi.org/10.3109/09687688.2012.700491

Cheng JT, Hale JD, Elliott M, Hancock RE, Straus SK (2011) The importance of bacterial membrane composition in the structure and function of aurein 2.2 and selected variants. Biochimica Et Biophysica Acta (BBA)-Biomembranes 1808(3):622–633. https://doi.org/10.1016/j.bbamem.2010.11.025

Marín-Menéndez A, Montis C, Díaz-Calvo T, Carta D, Hatzixanthis K, Morris CJ, McArthur M, Berti D (2017) Antimicrobial nanoplexes meet model bacterial membranes: the key role of Cardiolipin. Sci Rep 7(1):1–13. https://doi.org/10.1038/srep41242

Fernandez DI, Sani M-A, Gehman JD, Hahm K-S, Separovic F (2011) Interactions of a synthetic Leu–Lys-rich antimicrobial peptide with phospholipid bilayers. Eur Biophys J 40(4):471–480. https://doi.org/10.1007/s00249-010-0660-5

Domenech O, Francius G, Tulkens PM, Van Bambeke F, Dufrêne Y, Mingeot-Leclercq M-P (2009) Interactions of oritavancin, a new lipoglycopeptide derived from vancomycin, with phospholipid bilayers: effect on membrane permeability and nanoscale lipid membrane organization. Biochimica et Biophysica Acta (BBA)-Biomembranes 1788(9):1832–1840. https://doi.org/10.1016/j.bbamem.2009.05.003

Pinheiro M, Nunes CU, Caio JM, Moiteiro C, Lúcio M, Brezesinski G, Reis S (2013) The influence of rifabutin on human and bacterial membrane models: Implications for its mechanism of action. J Phys Chem B 117(20):6187–6193. https://doi.org/10.1021/jp403073v

D'Errico G, Silipo A, Mangiapia G, Vitiello G, Radulescu A, Molinaro A, Lanzetta R, Paduano L (2010) Characterization of liposomes formed by lipopolysaccharides from Burkholderia cenocepacia, Burkholderia multivorans and Agrobacterium tumefaciens: from the molecular structure to the aggregate architecture. Phys Chem Chem Phys 12(41):13574–13585. https://doi.org/10.1039/C0CP00066C

Furusato T, Horie F, Matsubayashi HT, Amikura K, Kuruma Y, Ueda T (2018) De novo synthesis of basal bacterial cell division proteins FtsZ, FtsA, and ZipA inside giant vesicles. ACS Synth Biol 7(4):953–961. https://doi.org/10.1021/acssynbio.7b00350

Kiss B, Bozó T, Mudra D, Tordai H, Herényi L, Kellermayer M (2021) Development, structure and mechanics of a synthetic E. coli outer membrane model. Nanoscale Adv 3(3):755–766. https://doi.org/10.1039/D0NA00977F

Jiménez M, Martos A, Vicente M, Rivas G (2011) Reconstitution and organization of Escherichia coli proto-ring elements (FtsZ and FtsA) inside giant unilamellar vesicles obtained from bacterial inner membranes. J Biol Chem 286(13):11236–11241. https://doi.org/10.1074/jbc.m110.194365

Sikder A, Sarkar J, Barman R, Ghosh S (2019) Directional Supramolecular Assembly of π-Amphiphiles with Tunable Surface Functionality and Impact on the Antimicrobial Activity. J Phys Chem B 123(33):7169–7177. https://doi.org/10.1021/acs.jpcb.9b05193

Kubiak J, Brewer J, Hansen S, Bagatolli LA (2011) Lipid lateral organization on giant unilamellar vesicles containing lipopolysaccharides. Biophys J 100(4):978–986. https://doi.org/10.1016/j.bpj.2011.01.012

Mohanan G, Nair KS, Nampoothiri KM, Bajaj H (2020) Engineering bio-mimicking functional vesicles with multiple compartments for quantifying molecular transport. Chem Sci 11(18):4669–4679. https://doi.org/10.1039/D0SC00084A

Ruhr E, Sahl H-G (1985) Mode of action of the peptide antibiotic nisin and influence on the membrane potential of whole cells and on cytoplasmic and artificial membrane vesicles. Antimicrob Agents Chemother 27(5):841–845. https://doi.org/10.1128/aac.27.5.841

Bharatiya B, Wang G, Rogers SE, Pedersen JS, Mann S, Briscoe WH (2021) Mixed liposomes containing gram-positive bacteria

lipids: Lipoteichoic acid (LTA) induced structural changes. Colloids Surf B 199:111551. https://doi.org/10.1016/j.colsurfb.2020.111551

Saliba A-E, Vonkova I, Ceschia S, Findlay GM, Maeda K, Tischer C, Deghou S, Van Noort V, Bork P, Pawson T (2014) A quantitative liposome microarray to systematically characterize protein-lipid interactions. Nat Methods 11(1):47–50. https://doi.org/10.1038/nmeth.2734

Turner M, Singhrao SK, Dennison SR, Morton LHG, Crean S (2015) Challenging the Clostridium botulinum toxin type A (BoNT/A) with a selection of microorganisms by culture methods and extended storage of used vials to assess the loss of sterility. J Dent Appl 2(5):223–228

Som A, Tew GN (2008) Influence of lipid composition on membrane activity of antimicrobial phenylene ethynylene oligomers. J Phys Chem B 112(11):3495–3502. https://doi.org/10.1021/jp077487j

Samuel R, Gillmor S (2016) Membrane phase characteristics control NA-CATH activity. Biochimica et Biophysica Acta (BBA)-Biomembranes 1858(9):1974–1982. https://doi.org/10.1016/j.bbamem.2016.05.015

Sborgi L, Rühl S, Mulvihill E, Pipercevic J, Heilig R, Stahlberg H, Farady CJ, Müller DJ, Broz P, Hiller S (2016) GSDMD membrane pore formation constitutes the mechanism of pyroptotic cell death. EMBO J 35(16):1766–1778. https://doi.org/10.15252/embj.201694696

Carrasco-López C, Rojas-Altuve A, Zhang W, Hesek D, Lee M, Barbe S, André I, Ferrer P, Silva-Martin N, Castro GR (2011) Crystal structures of bacterial peptidoglycan amidase AmpD and an unprecedented activation mechanism. J Biol Chem 286(36):31714–31722. https://doi.org/10.1074/jbc.M111.264366

Sasaki M, Nishikawa H, Suzuki S, Moser M, Huber M, Sawasato K, Matsubayashi HT, Kumazaki K, Tsukazaki T, Kuruma Y (2019) The bacterial protein YidC accelerates MPIase-dependent integration of membrane proteins. J Biol Chem 294(49):18898–18908. https://doi.org/10.1074/jbc.ra119.011248

Cheng M, Huang JX, Ramu S, Butler MS, Cooper MA (2014) Ramoplanin at bactericidal concentrations induces bacterial membrane depolarization in Staphylococcus aureus. Antimicrob Agents Chemother 58(11):6819–6827. https://doi.org/10.1128/AAC.00061-14

Lombardi L, Stellato MI, Oliva R, Falanga A, Galdiero M, Petraccone L, D'Errico G, De Santis A, Galdiero S, Del Vecchio P (2017) Antimicrobial peptides at work: interaction of myxinidin and its mutant WMR with lipid bilayers mimicking the P. aeruginosa and E. coli membranes. Sci Rep 7(1):1–15. https://doi.org/10.1038/srep44425

Zhang T, Muraih JK, Tishbi N, Herskowitz J, Victor RL, Silverman J, Uwumarenogie S, Taylor SD, Palmer M, Mintzer E (2014) Cardiolipin prevents membrane translocation and permeabilization by daptomycin. J Biol Chem 289(17):11584–11591. https://doi.org/10.1074/jbc.m114.554444

Brian Chia C, Gong Y, Bowie JH, Zuegg J, Cooper MA (2011) Membrane binding and perturbation studies of the antimicrobial peptides caerin, citropin, and maculatin. Pept Sci 96(2):147–157. https://doi.org/10.1002/bip.21438

Su Y, Waring AJ, Ruchala P, Hong M (2011) Structures of β-hairpin antimicrobial protegrin peptides in lipopolysaccharide membranes: mechanism of gram selectivity obtained from solid-state nuclear magnetic resonance. Biochemistry 50(12):2072–2083. https://doi.org/10.1021/bi101975v

Hancock R, Nikaido H (1978) Outer membranes of gram-negative bacteria. XIX. Isolation from Pseudomonas aeruginosa PAO1 and use in reconstitution and definition of the permeability barrier. J Bacteriol 136(1):381–390. https://doi.org/10.1128/jb.136.1.381-390.1978

Ciesielski F, Griffin DC, Rittig M, Moriyón I, Bonev BB (2013) Interactions of lipopolysaccharide with lipid membranes, raft models—A solid state NMR study. Biochimica et Biophysica Acta (BBA)-Biomembranes 1828(8):1731–1742. https://doi.org/10.1016/j.bbamem.2013.03.029

Lee E-H, Collatz E, Trias J, Gutmann L (1992) Diffusion of β-lactam antibiotics into proteoliposomes reconstituted with outer membranes of isogenic imipenem-susceptible and-resistant strains of Enterobacter cloacae. Microbiology 138(11):2347–2351. https://doi.org/10.1099/00221287-138-11-2347

Mitchell NJ, Seaton P, Pokorny A (2016) Branched phospholipids render lipid vesicles more susceptible to membrane-active peptides. Biochimica et Biophysica Acta (BBA)-Biomembranes 1858(5):988–994. https://doi.org/10.1016/j.bbamem.2015.10.014

Rideau E, Dimova R, Schwille P, Wurm FR, Landfester K (2018) Liposomes and polymersomes: a comparative review towards cell mimicking. Chem Soc Rev 47(23):8572–8610. https://doi.org/10.1039/C8CS00162F

Weinberger A, Tsai F-C, Koenderink GH, Schmidt TF, Itri R, Meier W, Schmatko T, Schröder A, Marques C (2013) Gel-assisted formation of giant unilamellar vesicles. Biophys J 105(1):154–164. https://doi.org/10.1016/j.bpj.2013.05.024

Göpfrich K, Haller B, Staufer O, Dreher Y, Mersdorf U, Platzman I, Spatz JP (2019) One-pot assembly of complex giant unilamellar vesicle-based synthetic cells. ACS Synth Biol 8(5):937–947. https://doi.org/10.1021/acssynbio.9b00034

Pautot S, Frisken BJ, Weitz D (2003) Engineering asymmetric vesicles. Proc Natl Acad Sci 100(19):10718–10721. https://doi.org/10.1073/pnas.1931005100

Nkanga, C. I.; Bapolisi, A. M.; Okafor, N. I.; Krause, R. W. M., General perception of liposomes: formation, manufacturing and applications. *Liposomes-advances and perspectives* **2019**. https://doi.org/10.5772/intechopen.84255

Eeman M, Deleu M (2010) From biological membranes to biomimetic model membranes. Biotechnol Agron Soc Environ 14(4):719–736.

Vestergaard MD, Hamada T, Takagi M (2008) Using model membranes for the study of amyloid beta: lipid interactions and neurotoxicity. Biotechnol Bioeng 99(4):753–763. https://doi.org/10.1002/bit.21731

Schmid EM, Richmond DL, Fletcher DA (2015) Reconstitution of proteins on electroformed giant unilamellar vesicles. Methods Cell Biol 128:319–338. https://doi.org/10.1016/bs.mcb.2015.02.004

Belegrinou S, Menon S, Dobrunz D, Meier W (2011) Solid-supported polymeric membranes. Soft Matter 7(6):2202–2210. https://doi.org/10.1039/C0SM01163K

Sackmann E (1996) Supported membranes: scientific and practical applications. Science 271(5245):43–48. https://doi.org/10.1126/science.271.5245.43

Foglia F, Lawrence M, Barlow D (2015) Studies of model biological and bio-mimetic membrane structure: reflectivity vs diffraction, a critical comparison. Curr Opin Colloid Interface Sci 20(4):235–243. https://doi.org/10.1016/j.cocis.2015.08.001

Li C, Wang M, Ferguson M, Zhan W (2015) Phospholipid/aromatic thiol hybrid bilayers. Langmuir 31(18):5228–5234. https://doi.org/10.1021/acs.langmuir.5b00476

Köper I (2007) Insulating tethered bilayer lipid membranes to study membrane proteins. Mol BioSyst 3(10):651–657. https://doi.org/10.1039/B707168J

Girard-Egrot AP, Maniti O (2021) Why Do Tethered-Bilayer Lipid Membranes Suit for Functional Membrane Protein Reincorporation? Appl Sci 11(11):4876. https://doi.org/10.3390/app11114876

Kurniawan J, de Ventrici Souza JOF, Dang AT, Liu GY, Kuhl TL (2018) Preparation and characterization of solid-supported lipid bilayers formed by Langmuir-Blodgett deposition: a tutorial. Langmuir 34(51):15622–15639. https://doi.org/10.1021/acs.langmuir.8b03504

Richter RP, Him JLK, Brisson A (2003) Supported lipid membranes. Mater Today 6(11):32–37. https://doi.org/10.1016/S1369-7021(03)01129-5

Clifton LA, Campbell RA, Sebastiani F, Campos-Terán J, Gonzalez-Martinez JF, Björklund S, Sotres J, Cárdenas M (2020) Design and use of model membranes to study biomolecular interactions using complementary surface-sensitive techniques. Adv Colloid Interface Sci 277:102118. https://doi.org/10.1016/j.cis.2020.102118

Giess F, Friedrich MG, Heberle J, Naumann RL, Knoll W (2004) The protein-tethered lipid bilayer: A novel mimic of the biological membrane. Biophys J 87(5):3213–3220. https://doi.org/10.1529/biophysj.104.046169

Ferhan AR, Jackman JA, Cho N-J (2017) Probing Spatial Proximity of Supported Lipid Bilayers to Silica Surfaces by Localized Surface Plasmon Resonance Sensing. Anal Chem 89(7):4301–4308. https://doi.org/10.1021/acs.analchem.7b00370

Wittenberg NJ, Wootla B, Jordan LR, Denic A, Warrington AE, Oh S-H, Rodriguez M (2014) Applications of SPR for the characterization of molecules important in the pathogenesis and treatment of neurodegenerative diseases. Expert Rev Neurother 14(4):449–463. https://doi.org/10.1586/14737175.2014.896199

Steltenkamp S, Müller MM, Deserno M, Hennesthal C, Steinem C, Janshoff A (2006) Mechanical Properties of Pore-Spanning Lipid Bilayers Probed by Atomic Force Microscopy. Biophys J 91(1):217–226. https://doi.org/10.1529/biophysj.106.081398

Weiss SA, Bushby RJ, Evans SD, Jeuken LJ (2010) A study of cytochrome bo3 in a tethered bilayer lipid membrane. Biochimica et Biophysica Acta (BBA)-Bioenergetics 1797(12):1917–1923. https://doi.org/10.1016/j.bbabio.2010.01.012

Clifton LA, Skoda MW, Daulton EL, Hughes AV, Le Brun AP, Lakey JH, Holt SA (2013) Asymmetric phospholipid: lipopolysaccharide bilayers; a Gram-negative bacterial outer membrane mimic. J R Soc Interface 10(89):20130810. https://doi.org/10.1098/rsif.2013.0810

Paracini N, Clifton LA, Skoda MW, Lakey JH (2018) Liquid crystalline bacterial outer membranes are critical for antibiotic susceptibility. Proc Natl Acad Sci 115(32):E7587–E7594. https://doi.org/10.1073/pnas.1803975115

Hughes AV, Patel DS, Widmalm G, Klauda JB, Clifton LA, Im W (2019) Physical properties of bacterial outer membrane models: neutron reflectometry & molecular simulation. Biophys J 116(6):1095–1104. https://doi.org/10.1016/j.bpj.2019.02.001

Dodd CE, Johnson BR, Jeuken LJ, Bugg TD, Bushby RJ, Evans SD, Native E (2008) coli inner membrane incorporation in solid-supported lipid bilayer membranes. Biointerphases 3(2):FA59–FA67. https://doi.org/10.1116/1.2896113

Michel J, Wang Y, Kiesel I, Gerelli Y, Rosilio V (2017) Disruption of asymmetric lipid bilayer models mimicking the outer membrane of gram-negative bacteria by an active plasticin. Langmuir 33(41):11028–11039. https://doi.org/10.1021/acs.langmuir.7b02864

Adhyapak P, Srivatsav AT, Mishra M, Singh A, Narayan R, Kapoor S (2020) Dynamical organization of compositionally distinct inner and outer membrane lipids of mycobacteria. Biophys J 118(6):1279–1291. https://doi.org/10.1016/j.bpj.2020.01.027

Nakatani Y, Shimaki Y, Dutta D, Muench SP, Ireton K, Cook GM, Jeuken LJ (2019) Unprecedented properties of phenothiazines unraveled by a NDH-2 bioelectrochemical assay platform. J Am Chem Soc 142(3):1311–1320. https://doi.org/10.1021/jacs.9b10254

Hoiles W, Krishnamurthy V (2015) Dynamic modeling of antimicrobial pore formation in engineered tethered membranes. IEEE Trans Mol Biol Multi-Scale Commun 1(3):265–276. https://doi.org/10.1109/TMBMC.2016.2537299

Schneck E, Oliveira RG, Rehfeldt F, Demé B, Brandenburg K, Seydel U, Tanaka M (2009) Mechanical properties of interacting lipopolysaccharide membranes from bacteria mutants studied by specular and off-specular neutron scattering. Phys Rev E 80(4):041929. https://doi.org/10.1103/PhysRevE.80.041929

Lee, T.-H.; Hofferek, V.; Sani, M.-a.; Separovic, F.; Reid, G.; Aguilar, M. I., The Impact of Antibacterial Peptides on Bacterial Lipid Membranes Depends on Stage of Growth. *Faraday Discussions* **2020**. https://doi.org/10.1039/D0FD00052C

Nedelkovski V, Schwaighofer A, Wraight CA, Nowak C, Naumann RL (2013) Surface-enhanced infrared absorption spectroscopy (SEIRAS) of light-activated photosynthetic reaction centers from Rhodobacter sphaeroides reconstituted in a biomimetic membrane system. J Phys Chem C 117(32):16357–16363. https://doi.org/10.1021/jp4056347

Niu L, Wohland T, Knoll W, Köper I (2017) Interaction of a synthetic antimicrobial peptide with a model bilayer platform mimicking bacterial membranes. Biointerphases 12(4):04E404. https://doi.org/10.1116/1.5001020

Sharma P, Parthasarathi S, Patil N, Waskar M, Raut JS, Puranik M, Ayappa KG, Basu JK (2020) Assessing barriers for antimicrobial penetration in complex asymmetric bacterial membranes: A case study with thymol. Langmuir 36(30):8800–8814. https://doi.org/10.1021/acs.langmuir.0c01124

McGillivray DJ, Valincius G, Heinrich F, Robertson JW, Vanderah DJ, Febo-Ayala W, Ignatjev I, Lösche M, Kasianowicz JJ (2009) Structure of functional Staphylococcus aureus α-hemolysin channels in tethered bilayer lipid membranes. Biophys J 96(4):1547–1553. https://doi.org/10.1016/j.bpj.2008.11.020

Dupuy FG, Pagano I, Andenoro K, Peralta MF, Elhady Y, Heinrich F, Tristram-Nagle S (2018) Selective interaction of colistin with lipid model membranes. Biophys J 114(4):919–928. https://doi.org/10.1016/j.bpj.2017.12.027

Li X, Smith AW (2019) Quantifying Lipid Mobility and Peptide Binding for Gram-Negative and Gram-Positive Model Supported Lipid Bilayers. J Phys Chem B 123(49):10433–10440. https://doi.org/10.1021/acs.jpcb.9b09709

Clifton LA, Holt SA, Hughes AV, Daulton EL, Arunmanee W, Heinrich F, Khalid S, Jefferies D, Charlton TR, Webster JR (2015) An accurate in vitro model of the E. coli envelope. Angew Chem Int Ed 54(41):11952–11955. https://doi.org/10.1002/anie.201504287

Hsia C-Y, Chen L, Singh RR, DeLisa MP, Daniel S (2016) A molecularly complete planar bacterial outer membrane platform. Sci Rep 6(1):1–14. https://doi.org/10.1038/srep32715

Thomas CJ, Surolia N, Surolia A (1999) Surface plasmon resonance studies resolve the enigmatic endotoxin neutralizing activity of polymyxin B. J Biol Chem 274(42):29624–29627. https://doi.org/10.1074/jbc.274.42.29624

Spencelayh MJ, Cheng Y, Bushby RJ, Bugg TD, Li JJ, Henderson PJ, O'Reilly J, Evans SD (2006) Antibiotic action and peptidoglycan formation on tethered lipid bilayer membranes. Angewandte Chemie 118(13):2165–2170. https://doi.org/10.1002/ange.200504035

Mirandela GD, Tamburrino G, Hoskisson PA, Zachariae U, Javelle A (2019) The lipid environment determines the activity of the Escherichia coli ammonium transporter AmtB. FASEB J 33(2):1989–1999. https://doi.org/10.1096/fj.201800782r

Maccarini M, Gayet L, Alcaraz J-P, Liguori L, Stidder B, Watkins EB, Lenormand J-L, Martin DK (2017) Functional characterization of cell-free expressed OprF porin from Pseudomonas aeruginosa stably incorporated in tethered lipid bilayers. Langmuir 33(38):9988–9996. https://doi.org/10.1021/acs.langmuir.7b01731

Jeuken LJ, Connell SD, Henderson PJ, Gennis RB, Evans SD, Bushby RJ (2006) Redox enzymes in tethered membranes. J Am Chem Soc 128(5):1711–1716. https://doi.org/10.1021/ja056972u

Jeuken LJ, Connell SD, Nurnabi M, O'Reilly J, Henderson PJ, Evans SD, Bushby RJ (2005) Direct electrochemical interaction

between a modified gold electrode and a bacterial membrane extract. Langmuir 21(4):1481–1488. https://doi.org/10.1021/la047732f

Mohamed Z, Shin J-H, Ghosh S, Sharma AK, Pinnock F, Bint E Naser Farnush S, Dörr T, Daniel S (2021) Clinically relevant bacterial outer membrane models for antibiotic screening applications. ACS Infect Dis 7(9):2707–2722. https://doi.org/10.1021/acsinfecdis.1c00217

Zang M, MacDermott-Opeskin H, Adams FG, Naidu V, Waters JK, Carey AB, Ashenden A, McLean KT, Brazel EB, Jiang J-H, Panizza A, Trappetti C, Paton JC, Peleg AY, Köper I, Paulsen IT, Hassan KA, O'Mara ML, Eijkelkamp BA (2021) The Membrane Composition Defines the Spatial Organization and Function of a Major Acinetobacter baumannii Drug Efflux System. mBio 12(3):1–6. https://doi.org/10.1128/mBio.01070-21

Alghalayini A, Garcia A, Berry T, Cranfield CG (2019) The use of tethered bilayer lipid membranes to identify the mechanisms of antimicrobial peptide interactions with lipid bilayers. Antibiotics 8(1):12. https://doi.org/10.3390/antibiotics8010012

Tamm LK, McConnell HM (1985) Supported phospholipid bilayers. Biophys J 47(1):105–113. https://doi.org/10.1016/s0006-3495(85)83882-0

Tanaka M, Sackmann E (2005) Polymer-supported membranes as models of the cell surface. Nature 437(7059):656–663. https://doi.org/10.1038/nature04164

Naumann C, Knoll W, Frank C (2001) Hindered Diffusion in Polymer-Tethered Membranes: A Monolayer Study at the Air− Water Interface. Biomacromol 2(4):1097–1103. https://doi.org/10.1021/bm010022t

Naumann CA, Prucker O, Lehmann T, Rühe J, Knoll W, Frank CW (2002) The polymer-supported phospholipid bilayer: Tethering as a new approach to substrate− membrane stabilization. Biomacromol 3(1):27–35. https://doi.org/10.1021/bm0100211

Deleu M, Crowet J-M, Nasir MN, Lins L (2014) Complementary biophysical tools to investigate lipid specificity in the interaction between bioactive molecules and the plasma membrane: A review. Biochimica et Biophysica Acta (BBA)-Biomembranes 1838(12):3171–3190. https://doi.org/10.1016/j.bbamem.2014.08.023

Lyman E, Hsieh C-L, Eggeling C (2018) From dynamics to membrane organization: experimental breakthroughs occasion a "modeling manifesto." Biophys J 115(4):595–604. https://doi.org/10.1016/j.bpj.2018.07.012

Nickels JD, Smith JC, Cheng X (2015) Lateral organization, bilayer asymmetry, and inter-leaflet coupling of biological membranes. Chem Phys Lipid 192:87–99. https://doi.org/10.1016/j.chemphyslip.2015.07.012

Maity PC, Yang J, Klaesener K, Reth M (2015) The nanoscale organization of the B lymphocyte membrane. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research 1853(4):830–840. https://doi.org/10.1016/j.bbamcr.2014.11.010

Marrink SJ, Corradi V, Souza PC, Ingólfsson HI, Tieleman DP, Sansom MS (2019) Computational modeling of realistic cell membranes. Chem Rev 119(9):6184–6226. https://doi.org/10.1021/acs.chemrev.8b00460

Ingólfsson HI, Arnarez C, Periole X, Marrink SJ (2016) Computational 'microscopy'of cellular membranes. J Cell Sci 129(2):257–268. https://doi.org/10.1242/jcs.176040

MacKerell AD Jr (2004) Empirical force fields for biological macromolecules: overview and issues. J Comput Chem 25(13):1584–1604. https://doi.org/10.1002/jcc.20082

Mori T, Miyashita N, Im W, Feig M, Sugita Y (2016) Molecular dynamics simulations of biological membranes and membrane proteins using enhanced conformational sampling algorithms. Biochimica et Biophysica Acta (BBA)-Biomembranes 1858(7):1635–1651. https://doi.org/10.1016/j.bbamem.2015.12.032

Matamoros-Recio A, Franco-Gonzalez JF, Forgione RE, Torres-Mozas A, Silipo A, Martín-Santamaría S (2021) Understanding the Antibacterial Resistance: Computational Explorations in Bacterial Membranes. ACS Omega 6(9):6041–6054. https://doi.org/10.1021/acsomega.0c05590

Bennett WD, Tieleman DP (2013) Computer simulations of lipid membrane domains. Biochimica et Biophysica Acta (BBA)-Biomembranes 1828(8):1765–1776. https://doi.org/10.1016/j.bbamem.2013.03.004

Chan C, Wen H, Lu L, Fan J (2015) Multiscale molecular dynamics simulations of membrane remodeling by Bin/Amphiphysin/Rvs family proteins. Chinese Physics B 25(1):018707. https://doi.org/10.1088/1674-1056/25/1/018707

Kabedev A, Hossain S, Hubert M, Larsson P, Bergström CA (2021) Molecular dynamics simulations reveal membrane interactions for poorly water-soluble drugs: impact of bile solubilization and drug aggregation. J Pharm Sci 110(1):176–185. https://doi.org/10.1016/j.xphs.2020.10.061

Khan SH, Prakash A, Pandey P, Lynn AM, Islam A, Hassan MI, Ahmad F (2019) Protein folding: Molecular dynamics simulations and in vitro studies for probing mechanism of urea-and guanidinium chloride-induced unfolding of horse cytochrome-c. Int J Biol Macromol 122:695–704. https://doi.org/10.1016/j.ijbiomac.2018.10.186

Lazim R, Suh D, Choi S (2020) Advances in molecular dynamics simulations and enhanced sampling methods for the study of protein systems. Int J Mol Sci 21(17):6339. https://doi.org/10.3390/ijms21176339

Liu Y, de Vries AH, Pezeshkian W, Marrink SJ (2021) Capturing Membrane Phase Separation by Dual Resolution Molecular Dynamics Simulations. J Chem Theory Comput 17(9):5876–5884. https://doi.org/10.1021/acs.jctc.1c00151

Parkin J, Chavent M, Khalid S (2015) Molecular simulations of Gram-negative bacterial membranes: a vignette of some recent successes. Biophys J 109(3):461–468. https://doi.org/10.1016/j.bpj.2015.06.050

Reddy T, Sansom MS (2016) Computational virology: from the inside out. Biochimica et Biophysica Acta (BBA)-Biomembranes 1858(7):1610–1618. https://doi.org/10.1016/j.bbamem.2016.02.007

Singharoy A, Schulten K (2017) Atom-Resolved View of a Cell Organelle on a Computational Microscope. Biophys J 112(3):176a. https://doi.org/10.1016/j.bpj.2016.11.973

Balusek C, Gumbart JC (2016) Role of the native outer-membrane environment on the transporter BtuB. Biophys J 111(7):1409–1417. https://doi.org/10.1016/j.bpj.2016.08.033

Baltoumas FA, Hamodrakas SJ, Iconomidou VA (2019) The gram-negative outer membrane modeler: Automated building of lipopolysaccharide-rich bacterial outer membranes in four force fields. J Comput Chem 40(18):1727–1734. https://doi.org/10.1002/jcc.25823

Gao Y, Lee J, Widmalm G, Im W (2020) Modeling and Simulation of Bacterial Outer Membranes with Lipopolysaccharides and Enterobacterial Common Antigen. J Phys Chem B 124(28):5948–5956. https://doi.org/10.1021/acs.jpcb.0c03353

Kholina EG, Kovalenko IB, Bozdaganyan ME, Strakhovskaya MG, Orekhov PS (2020) Cationic antiseptics facilitate pore formation in model bacterial membranes. J Phys Chem B 124(39):8593–8600. https://doi.org/10.1021/acs.jpcb.0c07212

Li Y, Guo H (2013) Atomistic simulations of an antimicrobial molecule interacting with a model bacterial membrane. Theoret Chem Acc 132(1):1–8. https://doi.org/10.1007/s00214-012-1303-y

Abellón-Ruiz J, Kaptan SS, Baslé A, Claudi B, Bumann D, Kleinekathöfer U, van den Berg B (2017) Structural basis for maintenance of bacterial outer membrane lipid asymmetry. Nat Microbiol 2(12):1616–1623. https://doi.org/10.1038/s41564-017-0046-x

Berglund NA, Piggot TJ, Jefferies D, Sessions RB, Bond PJ, Khalid S (2015) Interaction of the antimicrobial peptide polymyxin B1 with both membranes of E. coli: a molecular dynamics study. PLoS Comput Biol 11(4):e1004180. https://doi.org/10.1371/journal.pcbi.1004180

Hsu P-C, Samsudin F, Shearer J, Khalid S (2017a) It is complicated: curvature, diffusion, and lipid sorting within the two membranes of Escherichia coli. J Phys Chem Lett 8(22):5513–5518. https://doi.org/10.1021/acs.jpclett.7b02432

Hsu, P. C.; Bruininks, B. M.; Jefferies, D.; Cesar Telles de Souza, P.; Lee, J.; Patel, D. S.; Marrink, S. J.; Qi, Y.; Khalid, S.; Im, W., CHARMM-GUI Martini Maker for modeling and simulation of complex bacterial membranes with lipopolysaccharides. Wiley Online Library: 2017. https://doi.org/10.1002/jcc.24895

Ma H, Khan A, Nangia S (2017a) Dynamics of OmpF trimer formation in the bacterial outer membrane of Escherichia coli. Langmuir 34(19):5623–5634. https://doi.org/10.1021/acs.langmuir.7b02653

Mehmood S, Corradi V, Choudhury HG, Hussain R, Becker P, Axford D, Zirah S, Rebuffat S, Tieleman DP, Robinson CV (2016) Structural and functional basis for lipid synergy on the activity of the antibacterial peptide ABC transporter McjD. J Biol Chem 291(41):21656–21668. https://doi.org/10.1074/jbc.M116.732107

Orekhov PS, Kholina EG, Bozdaganyan ME, Nesterenko AM, Kovalenko IB, Strakhovskaya MG (2018) Molecular mechanism of uptake of cationic photoantimicrobial phthalocyanine across bacterial membranes revealed by molecular dynamics simulations. J Phys Chem B 122(14):3711–3722. https://doi.org/10.1021/acs.jpcb.7b11707

Shearer J, Jefferies D, Khalid S (2019) Outer membrane proteins OmpA, FhuA, OmpF, EstA, BtuB, and OmpX have unique lipopolysaccharide fingerprints. J Chem Theory Comput 15(4):2608–2619. https://doi.org/10.1021/acs.jctc.8b01059

Shearer J, Khalid S (2018) Communication between the leaflets of asymmetric membranes revealed from coarse-grain molecular dynamics simulations. Sci Rep 8(1):1–6. https://doi.org/10.1038/s41598-018-20227-1

Rice A, Wereszczynski J (2018) Atomistic scale effects of lipopolysaccharide modifications on bacterial outer membrane defenses. Biophys J 114(6):1389–1399. https://doi.org/10.1016/j.bpj.2018.02.006

Patel DS, Re S, Wu EL, Qi Y, Klebba PE, Widmalm G, Yeom MS, Sugita Y, Im W (2016) Dynamics and interactions of OmpF and LPS: influence on pore accessibility and ion permeability. Biophys J 110(4):930–938. https://doi.org/10.1016/j.bpj.2016.01.002

Piggot TJ, Holdbrook DA, Khalid S (2011) Electroporation of the E. coli and S. aureus membranes: molecular dynamics simulations of complex bacterial membranes. J Phys Chem B 115(45):13381–13388. https://doi.org/10.1021/jp207013v

Carpenter TS, Parkin J, Khalid S (2016) The free energy of small solute permeation through the Escherichia coli outer membrane has a distinctly asymmetric profile. J Phys Chem Lett 7(17):3446–3451. https://doi.org/10.1021/acs.jpclett.6b01399

Fleming PJ, Patel DS, Wu EL, Qi Y, Yeom MS, Sousa MC, Fleming KG, Im W (2016) BamA POTRA domain interacts with a native lipid membrane surface. Biophys J 110(12):2698–2709. https://doi.org/10.1016/j.bpj.2016.05.010

Wu EL, Engström O, Jo S, Stuhlsatz D, Yeom MS, Klauda JB, Widmalm G, Im W (2013) Molecular dynamics and NMR spectroscopy studies of E. coli lipopolysaccharide structure and dynamics. Biophys J 105(6):1444–1455. https://doi.org/10.1016/j.bpj.2013.08.002

Wu EL, Fleming PJ, Yeom MS, Widmalm G, Klauda JB, Fleming KG, Im W (2014) E. coli outer membrane and interactions with OmpLA. Biophys J 106(11):2493–2502. https://doi.org/10.1016/j.bpj.2014.04.024

Duay SS, Sharma G, Prabhakar R, Angeles-Boza AM, May ER (2019) Molecular dynamics investigation into the effect of zinc (II) on the structure and membrane interactions of the antimicrobial peptide Clavanin A. J Phys Chem B 123(15):3163–3176. https://doi.org/10.1021/acs.jpcb.8b11496

Khondker A, Dhaliwal AK, Saem S, Mahmood A, Fradin C, Moran-Mirabal J, Rheinstädter MC (2019) Membrane charge and lipid packing determine polymyxin-induced membrane damage. Commun Biol 2(1):1–11. https://doi.org/10.1038/s42003-019-0297-6

Ma H, Cummins DD, Edelstein NB, Gomez J, Khan A, Llewellyn MD, Picudella T, Willsey SR, Nangia S (2017b) Modeling diversity in structures of bacterial outer membrane lipids. J Chem Theory Comput 13(2):811–824. https://doi.org/10.1021/acs.jctc.6b00856

Ma H, Irudayanathan FJ, Jiang W, Nangia S (2015) Simulating Gram-negative bacterial outer membrane: a coarse grain model. J Phys Chem B 119(46):14668–14682. https://doi.org/10.1021/acs.jpcb.5b07122

Pandit KR, Klauda JB (2012) Membrane models of E. coli containing cyclic moieties in the aliphatic lipid chain. Biochimica et Biophysica Acta (BBA)-Biomembranes 1818(5):1205–1210. https://doi.org/10.1016/j.bbamem.2012.01.009

Pothula KR, Solano CJ, Kleinekathöfer U (2016) Simulations of outer membrane channels and their permeability. Biochimica et Biophysica Acta (BBA)-Biomembranes 1858(7):1760–1771. https://doi.org/10.1016/j.bbamem.2015.12.020

Shahane G, Ding W, Palaiokostas M, Azevedo HS, Orsi M (2019) Interaction of antimicrobial lipopeptides with bacterial lipid bilayers. J Membr Biol 252(4):317–329. https://doi.org/10.1007/s00232-019-00068-3

Khakbaz P, Klauda JB (2015) Probing the importance of lipid diversity in cell membranes via molecular simulation. Chem Phys Lipid 192:12–22. https://doi.org/10.1016/j.chemphyslip.2015.08.003

Lim JB, Klauda JB (2011) Lipid chain branching at the iso-and anteiso-positions in complex chlamydia membranes: A molecular dynamics study. Biochimica et Biophysica Acta (BBA)-Biomembranes 1808(1):323–331. https://doi.org/10.1016/j.bbamem.2010.07.036

Jin T, Patel SJ, Van Lehn RC (2021) Molecular simulations of lipid membrane partitioning and translocation by bacterial quorum sensing modulators. Plos one 16(2):e0246187. https://doi.org/10.1371/journal.pone.0246187

Lee J, Patel DS, Kucharska I, Tamm LK, Im W (2017) Refinement of OprH-LPS interactions by molecular simulations. Biophys J 112(2):346–355. https://doi.org/10.1016/j.bpj.2016.12.006

Ocampo-Ibáñez ID, Liscano Y, Rivera-Sánchez SP, Oñate-Garzón J, Lugo-Guevara AD, Flórez-Elvira LJ, Lesmes MC (2020) A Novel Cecropin D-Derived Short Cationic Antimicrobial Peptide Exhibits Antibacterial Activity Against Wild-Type and Multidrug-Resistant Strains of Klebsiella pneumoniae and Pseudomonas aeruginosa. Evol Bioinforma 16:1176934320936266. https://doi.org/10.1177/1176934320936266

Alkhalifa S, Jennings MC, Granata D, Klein M, Wuest WM, Minbiole KP, Carnevale V (2020) Analysis of the Destabilization of Bacterial Membranes by Quaternary Ammonium Compounds: A Combined Experimental and Computational Study. Chembiochem 21(10):1510. https://doi.org/10.1002/cbic.201900698

Lins RD, Straatsma T (2001) Computer simulation of the rough lipopolysaccharide membrane of Pseudomonas aeruginosa. Biophys J 81(2):1037–1046. https://doi.org/10.1016/S0006-3495(01)75761-X

Yu Y, Klauda JB (2018) Modeling Pseudomonas aeruginosa inner plasma membrane in planktonic and biofilm modes. J Chem Phys 149(21):215102. https://doi.org/10.1063/1.5052629

Hwang H, Paracini N, Parks JM, Lakey JH, Gumbart JC (2018) Distribution of mechanical stress in the Escherichia coli cell envelope. Biochimica et Biophysica Acta (BBA)-Biomembranes 1860(12):2566–2575. https://doi.org/10.1016/j.bbamem.2018.09.020

Piggot TJ, Holdbrook DA, Khalid S (2013) Conformational dynamics and membrane interactions of the E coli outer membrane protein FecA: a molecular dynamics simulation study. Biochimica et Biophysica Acta (BBA)-Biomembranes 1828(2):284–293. https://doi.org/10.1016/j.bbamem.2012.08.021

Kirschner KN, Lins RD, Maass A, Soares TA (2012) A glycam-based force field for simulations of lipopolysaccharide membranes: parametrization and validation. J Chem Theory Comput 8(11):4719–4731. https://doi.org/10.1021/ct300534j

Dias RP, da Hora GC, Ramstedt M, Soares TA (2014) Outer membrane remodeling: the structural dynamics and electrostatics of rough lipopolysaccharide chemotypes. J Chem Theory Comput 10(6):2488–2497. https://doi.org/10.1021/ct500075h

Van Oosten B, Harroun TA (2016) A MARTINI extension for Pseudomonas aeruginosa PAO1 lipopolysaccharide. J Mol Graph Model 63:125–133. https://doi.org/10.1016/j.jmgm.2015.12.002

Hsu P-C, Jefferies D, Khalid S (2016) Molecular dynamics simulations predict the pathways via which pristine fullerenes penetrate bacterial membranes. J Phys Chem B 120(43):11170–11179. https://doi.org/10.1021/acs.jpcb.6b06615

Shearer J, Marzinek JK, Bond PJ, Khalid S (2020) Molecular dynamics simulations of bacterial outer membrane lipid extraction: Adequate sampling? J Chem Phys 153(4):044122. https://doi.org/10.1063/5.0017734

Lee J, Patel DS, Ståhle J, Park S-J, Kern NR, Kim S, Lee J, Cheng X, Valvano MA, Holst O (2018) CHARMM-GUI membrane builder for complex biological membrane simulations with glycolipids and lipoglycans. J Chem Theory Comput 15(1):775–786. https://doi.org/10.1021/acs.jctc.8b01066

Wu, E. L.; Cheng, X.; Jo, S.; Rui, H.; Song, K. C.; Dávila-Contreras, E. M.; Qi, Y.; Lee, J.; Monje-Galvan, V.; Venable, R. M., CHARMM-GUI membrane builder toward realistic biological membrane simulations. Wiley Online Library: 2014. https://doi.org/10.1002/jcc.23702

Khalid S, Berglund NA, Holdbrook DA, Leung YM, Parkin J (2015) The membranes of Gram-negative bacteria: progress in molecular modelling and simulation. Biochem Soc Trans 43(2):162–167. https://doi.org/10.1042/bst20140262

Patel DS, Qi Y, Im W (2017) Modeling and simulation of bacterial outer membranes and interactions with membrane proteins. Curr Opin Struct Biol 43:131–140. https://doi.org/10.1016/j.sbi.2017.01.003

Chakraborty, A.; Kobzev, E.; Chan, J.; de Zoysa, G. H.; Sarojini, V.; Piggot, T. J.; Allison, J. R., Molecular Dynamics Simulation of the Interaction of Two Linear Battacin Analogs with Model Gram-Positive and Gram-Negative Bacterial Cell Membranes. *ACS Omega* **2020**. https://doi.org/10.1021/acsomega.0c04752

Kim S, Patel DS, Park S, Slusky J, Klauda JB, Widmalm G, Im W (2016) Bilayer properties of lipid A from various Gram-negative bacteria. Biophys J 111(8):1750–1760. https://doi.org/10.1016/j.bpj.2016.09.001

Goossens K, De Winter H (2018) Molecular dynamics simulations of membrane proteins: An overview. J Chem Inf Model 58(11):2193–2202. https://doi.org/10.1021/acs.jcim.8b00639