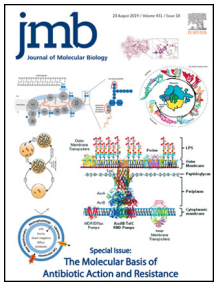

Docking on Lipid II—A Widespread Mechanism for Potent Bactericidal Activities of Antibiotic Peptides

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Abstract

Natural product antibiotics usually target the major biosynthetic pathways of bacterial cells and the search for new targets outside these pathways has proven very difficult. Cell wall biosynthesis maybe the most prominent antibiotic target, and ß-lactams are among the clinically most relevant antibiotics. Among cell wall biosynthesis inhibitors, glycopeptide antibiotics are a second group of important drugs, which bind to the peptidoglycan building block lipid II and prevent the incorporation of the monomeric unit into polymeric cell wall. However, lipid II acts as a docking molecule for many more naturally occurring antibiotics from diverse chemical classes and likely is the most targeted molecule in antibacterial mechanisms. We summarize current knowledge on lipid II binding antibiotics and explain, on the levels of mechanisms and resistance development, why lipid II is such a prominent target, and thus provide insights for the design of new antibiotic drugs.

Introduction

In the past decades, the discovery and development of new antibiotic classes have been largely unsuccessful. Among the many lessons learned from failures was that screening technologies with isolated target molecules were not adequate [1]. In particular, unprecedented targets selected on the basis of mere genomic considerations did not hold the promises made at the beginning of the omics era, leaving us with questions as to what suitable antibiotic targets could be found. Studying the mechanisms of action of new natural product antibiotics coming out of whole cell-based screening, it can be observed that such new antimicrobials almost exclusively interfere with biosynthesis of macromolecules, the classical target pathways of the "old" antibiotics in clinical use. Particularly anti-gram positive natural compounds often turn out to be cell wall biosynthesis inhibitors and the most frequently addressed molecule within this target pathway is the peptidoglycan (PGN) building block lipid II (Fig. 1). Lipid II binding appears a most potent antibiotic mechanism, particularly for antimicrobial peptides of both non-ribosomal (NRPs) origin as well as ribosomally synthesized and postranslationally modified (RiPPs) and even unmodified peptides. Lipid II binders (Fig. 2) can be found among glyco- and lipoglyco-peptides, the teixobactin class and lantibiotics, and they appear most prominently as defensins of fungi, invertebrates and vertebrate species. Particularly the fact that innate immunity of fungi and of virtually every animal species heavily relies on lipid II binding as an antibiotic mechanism for controlling natural flora and antagonizing potential pathogens demonstrates the relevance of this target and prompts important questions for antibiotic drug discovery and development: (i) what makes lipid II binding such a powerful antibiotic mechanism that evolution of high affinity binders occurred in so many different chemical classes of molecules? (ii) why is lipid II binding still an effective mechanism in spite of "permanent application" particularly in innate immunity and what are the requirements and possible mechanisms for developing resistance against lipid II-binding antibiotics? Adequate

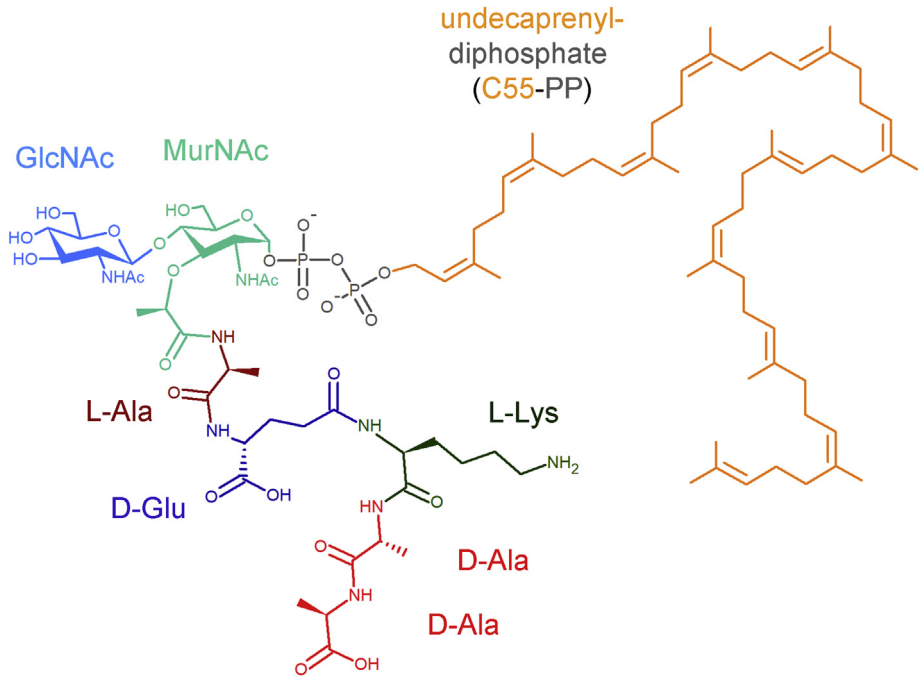

Fig. 1. General structure of lipid II (bactoprenyl-diphosphate-GlcNAc-MurNAc-pentapeptide). The molecule depicted here contains L-lysine at position three of the stempeptide, which is characteristic for most gram-positive bacteria. Gram-negative bacteria often carry diaminopimelic acid at this position. Further variants and modifications are known (reviewed elsewhere) [2].

answers to these questions may help to better understand principles that govern antibiotic killing mechanisms and contribute to finding and developing new antimicrobial drugs. Here, we first give an overview on the classes of molecules for which lipid II binding has been described so far and then elaborate molecular and cellular aspects associated with lipid II-binding and possible resistance development.

Antibiotic Peptide Classes Targeting Lipid II

Glyco- and Lipoglyco-peptides

Glycopeptide antibiotics are natural or semisynthetic strongly glycosylated RiPPs widely used to treat infections with gram-positive pathogens such as methicillin-resistant *Staphylococcus aureus* [3]. These glycopeptide antibiotics share a structural composition with a relatively conserved heptapeptide core carrying (amino) sugar moieties as substituents. Bacteria belonging to the order of the actinomycetales produce a variety of glycopeptide antibiotics, which differ in the composition of the heptapeptide core as well as in the type and number of sugar components. Further variations include halogenations or the attachment of hydrophobic substituents characteristic for the lipoglycopeptides.

Vancomycin, the first member of the class of glycopeptide antibiotics, was clinically approved in 1958 in the United States and is still a key compound for the treatment of infections caused by gram-positive pathogens. As for all glycopeptide antibiotics, its aglycone cavity represents the biologically active component binding to the D-alanine–D-alanine terminus of lipid II with high affinity eventually preventing its availability for the penicillin-binding proteins (PBPs) [4]. Consequently, the incorporation of the PGN building block into the PGN meshwork through transglycosylation and transpeptidation is abrogated. The application of vancomycin is often limited due to evolved resistance mechanisms. As such, some bacteria produce an alternative version of lipid II characterized by the presence of a D-alanine–D-lactate terminus, which drastically reduces the affinity of the antibiotic for the cell wall precursor, or bacteria gradually adapt to the antibiotic often through background mutations stabilizing phenotypical stress response mechanisms (see below). To overcome glycopeptide resistance, vancomycin has been further developed and variants such as telavancin, dalbavancin and oritavancin were clinically approved in 2009 and 2014, respectively [5]. These, as well as the naturally occurring teicoplanin (approved 1988 in Europe), are characterized by additional structural moieties that promote

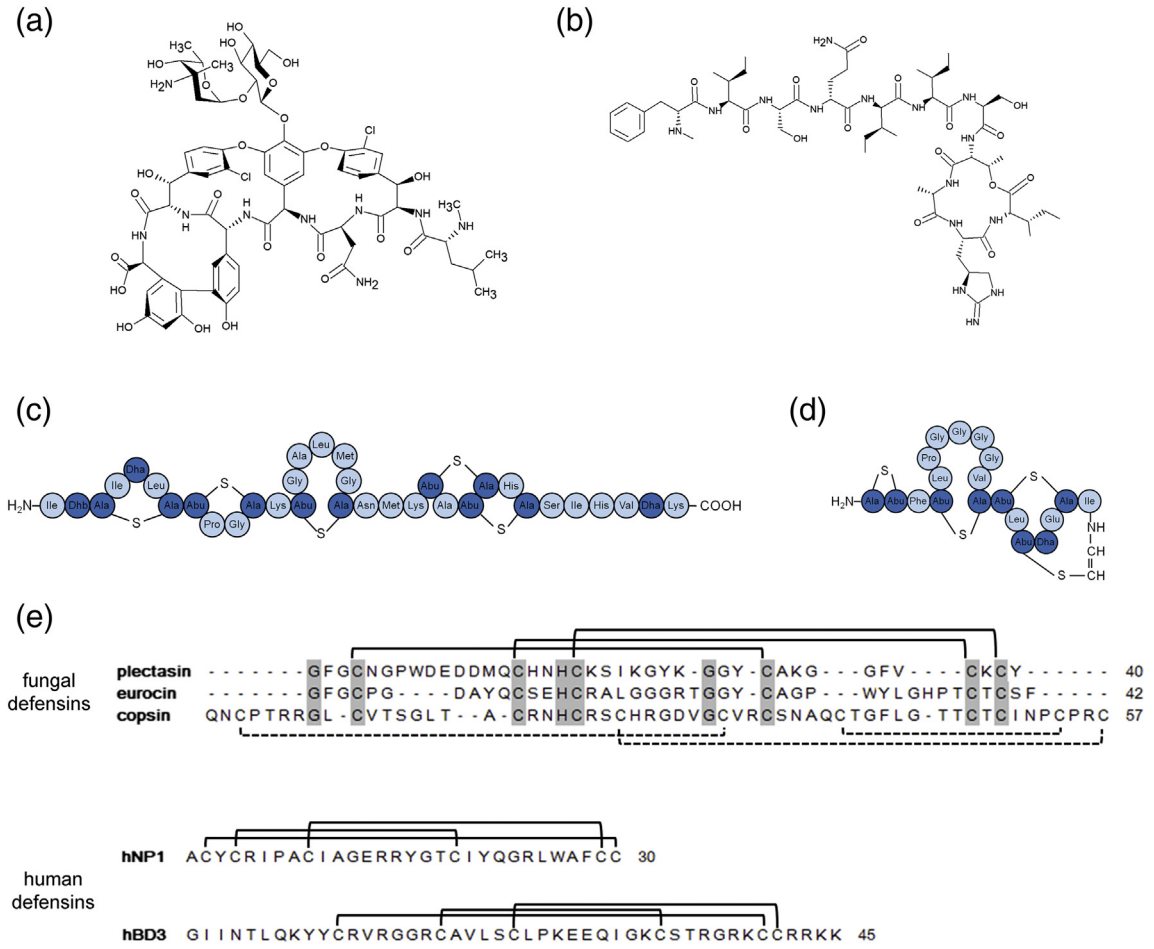

Fig. 2. Structures of the prominent lipid II binders vancomycin (A), teixobactin (B) and schematic illustration of nisin (C) and mersacidin (D) as well as primary structures of selected lipid II-binding defensins (E). Unmodified amino acids in panels C and D are depicted as light-blues circles, and post-translationally modified amino acids are depicted in dark blue. Disulfide bond connectivity in panel E is indicated with solid lines, and additional disulfide bonds present in copsin but not in plectasin and eurocin are indicated with dashed lines.

membrane anchoring of the antibiotics. In telavancin, dalbavancin and teicoplanin, fatty acid components confer a lipophilic character to the molecules while oritavancin contains a lipophilic 4′-chlorobiphenyl methyl side chain. It has been shown that membrane anchoring facilitates target binding by increasing the local concentration of the drug at the membrane. Furthermore, a facilitated dimerization may contribute to the increased potency. Due to the integration into the cell membrane, these compounds likely effect membrane integrity, and indeed, perturbation of the membrane potential by oritavancin and telavancin has been observed [6,7]. The development of the lipoglycopeptides led to drugs with beneficial pharmacokinetics, less side effects and/or increased potency. However, mutants resistant also to lipoglycopeptides appeared in the clinic. Nevertheless, pathogens susceptible to lipoglycopeptides include vancomycin-resistant enterococci (VRE), vancomycin-resistant *S. aureus* and vancomycin-intermediate *S. aureus* (VISA) strains. This, however, cannot entirely be attributed to membrane anchoring of the compounds as also other modifications appear to be involved. While VanA-mediated VREs are usually also resistant against teicoplanin, telavancin and dalbavancin, oritavancin is active against VanA and VanB-mediated VRE. For this compound, additional crucial binding sites within the lipid II molecule (i.e., the crossbridge and the D-iso-glutamine in position 2 of the stem peptide) have been identified [8–10].

Depsipeptides

Ramoplanin has been developed into clinical phase III with a focus on *Clostridioides difficile* but has not been approved so far. It is strongly amphipathic and contains a short acyl side chain,

which is important for membrane anchoring and activity; for review on this compound, see, for example, Ref. [11]. Ramoplanin was shown to bind lipid II with extraordinary affinity [12] and it may also be translocated across the membrane, and inhibition of intracellular lipid I- and lipid II-consuming reactions may occur (e.g., MurG and FemXAB). Dimerization of ramoplanin [12] seems a prerequisite for binding to the pyrophosphate moiety of lipid II and the analysis of its crystal structure confirmed the formation of dimers and identified possible interactions with lipid II [13]. The availability of a total synthesis protocol and ramoplanin analogues [14,15] and of the related compound enduracidin [16] will be useful for further analysis of structure–function relationships. More cyclic depsipeptides inhibiting the transglycosylation step can be found in the plusbacin and katanosin groups of natural compounds. Katanosin B appears structurally identical to lysobactin and was chemically synthesized [17,18]. Plusbacin A3 and Katanosin B were discussed to block cell wall biosynthesis at the level of lipid II binding [19], although formal evidence for lipid II binding and molecular details of the interaction have not been reported.

Teixobactin is a recently discovered 1242.47-Da depsipeptide produced by *Eleftheria terrae*. The isolation of this ß-proteobacterium was enabled by the use of an isolation chip designed to grow and isolate bacteria otherwise considered to be "unculturable" [20]. Teixobactin is highly potent against a variety of gram-positive pathogens, including *Mycobacterium tuberculosis*, *C. difficile* and antibiotic-resistant strains including methicillin-resistant *S. aureus*, VISA and VRE, and is currently in preclinical development in phase IIB. It was shown that teixobactin binds not only lipid II but also undecaprenyl pyrophosphate and the wall teichoic acid (WTA) precursor lipid III. Binding to WTA precursors likely accounts for the rapid bacteriolytic effect of teixobactin as WTA are important for the proper localization of autolysins thus preventing uncontrolled autolysis. Indeed, the bacteriolytic effect of the compound was shown to depend on the major autolysin Atl in *S. aureus*, which has been shown to delocalize upon teixobaction incubation [21]. Target binding of teixobactin primarily depends on the pyrophosphate sugar moiety of the lipid carrier and accordingly, teixobactin also binds lipid II variants containing D-Ala–D-Lac and D-Ala–D-Ser termini in line with its activity against VRE and vancomyin-resistant *S. aureus*. Remarkably, no teixobactin-resistant strain could be obtained in the laboratory when *S. aureus* or *M. tuberculosis* was plated on media containing a low dose (4 × MIC) of the compound. Likewise, serial passaging of *S. aureus* in the presence of sub-MIC levels did not produce resistant mutants [20], and no resistant mutant has been described in the literature so far.

Lantibiotics

Lantibiotics are a most prominent class of ribosomally-made and post-translationally modified peptides (RiPPs), characterized by potent antibiotic activities [22]. Structurally, the distinctive features are thioether-based intramolecular rings, introduced through lanthionine (Lan) and methyl-lanthionine (Me-Lan) residues, as well as dehydro amino acids (dehydro-alanine, Dha, and dehydro-butyrine, Dhb) which stem from site-specific dehydration of Ser and Thr side chains. Following Ser and Thr dehydration, the SH-group of Cys residues is enzymatically added to some of the Dha and Dhb side chains resulting in Lan- and Me-Lan-based thioether bridges [23]. Notably, with respect to molecular mechanisms, lantibiotics were the first lipid II-binding compounds that were studied with purified and full-length lipid II.

The by far best-studied lantibiotic peptide is nisin, which was described as early as 1928 [24] and has gained a remarkable history as an efficacious and safe food preservative since the 1960s [25]. Initially, its mode of action was controversial. A first report described nisin as a membrane disruptive agent that would rapidly release UV-absorbing material form treated cells [26], whereas Reisinger and colleagues [27] used bacterial membrane vesicles and reported inhibition of the membrane-bound steps of PGN biosynthesis as the primary mode of action of nisin. In 1985 it was shown, based on killing kinetics and other whole-cell assays, that rapid pore formation leads to immediate killing of staphylococci [28] and that membrane poration requires a trans-negative membrane potential [29]. However, it was not before 1998, when purified lipid II became available in the course of mode-of-action studies on the lantibiotic mersacidin [30], that the controversies could be resolved such that an integrated model for nisin action could be proposed [31]. In this study, lipid II-doped artificial liposomes were used to demonstrate that the cell wall precursor plays a decisive role in the rapid pore formation process. This model was subsequently verified [32] and described for many other lantibiotics with elongated structures, the so-called class A lantibiotics (e.g., nisin, epidermin, subtilin and related molecules with the nisin-type lipid II binding motif) [32–34]. Simultaneously, the globular lantibiotic mersacidin and related lantibiotics were shown to also bind to lipid II, however, without producing a major impact on membrane integrity [30].

The lipid II-dependent pore formation by nisin has been characterized in detail. Initially, a complex is formed, which, in planar bilayers, produces a transient pore of 2 nm in diameter [35] and which was postulated to contain eight nisin plus four lipid II molecules [36]. However, it appears that the pore complex keeps growing in size by recruitment of

virtually thousands of both nisin and lipid II molecules leading to massive lipid rearrangements and membrane damage [37,38]. Particularly interesting is a group of two-peptide lantibiotics, of which lacticin 3147 is the prototype. In this case, one peptide with a mersacidin-binding motif forms a complex with lipid II, which is then able to recruit a second peptide that inserts into the membrane for efficient pore formation [39]. Meanwhile, there is also structural information available, which clearly shows the pyrophosphate linkage group in lipid II, and the conserved ring motifs in the nisin-type and mersacidin-type of lantibiotics are the primary interaction sites [40–42].

Defensins

Defensins are a structurally defined class of antimicrobial peptides that are produced by virtually all eukaryotic organisms [43]. Defensins of vertebrates, invertebrates, plants and fungi share a conserved core structure consisting of an alpha-helical domain linked via three or four disulfide bridges to a two-stranded antiparallel beta-sheet, forming the so-called cysteine-stabilized alpha-helix beta-sheet (CS) motif (concisely reviewed elsewhere) [44,45]. While the plant defensins appear to primarily antagonize fungi, all others have broad-spectrum antibacterial activities. Plectasin is the prototype peptide for fungal defensins that act specifically and with high potency against gram-positive bacteria [46]. Its activity relies on high-affinity binding of lipid II, which is based on four hydrogen bonds between the pyrophosphate moiety and plectasin in addition to salt bridge formation between the Glu-side chain of lipid II with both His18 and the free the N-terminus of plectasin [47]. Several other fungal defensins, for example, eurocin [48] and copsin [49], have similar lipid II binding properties. Moreover, some invertebrate defensins such as the oyster defensin family *cg*-def are strong lipid II binders [50]. These peptides seem to share a lipid II-binding motif with many others defensins from fungi and invertebrates, which can be identified in genome sequences [45]. In contrast, vertebrate peptides of the alpha- and beta-defensin classes have broad-spectrum activity against gram-positive and gram-negative bacteria. The anti-gram-negative activity obviously involves interactions with lipopolysaccharide (LPS) and destabilization of the outer-membrane lipopolysaccharide layer, although molecular details and cellular downstream effects of such bactericidal activity still remain poorly understood. Killing of gram-positive bacteria, however, is clearly based on lipid II binding, which has been shown for human defensins hNP1 [51] and hBD3 [51,52]. It is interesting to note though that retention of anti-gram-negative activity appears to go along with reduction of affinity for lipid II by 1 order of magnitude as compared to plectasin [45,52].

Bacteriocins

Often, the term “bacteriocin” is broadly used for antimicrobial proteins and peptides, and the heavily modified lantibiotics are occasionally included in this group as class I bacteriocins, mainly with respect to potential applications [25]. However, in earlier definitions, the term bacteriocins refers to unmodified antibacterial proteins and peptides with very narrow spectra of activity resulting from specific bacteriocin receptors in the cell envelope of a given bacterial species or even subspecies [53]. Among such bacteriocins, lactococcin 972 sticks out in that its bactericidal activity is based on specific binding of lipid II [54]. Lipid II interactions may be enabled by an unusual ß-sandwich structure comprising two three-stranded antiparallel ß-sheets with the C-terminal strand closing a loop formed by the sheets of the bacteriocin [55].

Other Peptides

Not all lipid II binding peptides can be classified into the above-mentioned groups. As such, two calcium dependent lipopeptides were recently discovered by soil microbiome screening. The compounds were termed malacidins (for metagenomic acidic lipopeptide antibiotic), and malacidin A showed remarkable activity against a set of gram positive pathogens [56].

A similar activity spectrum was reported for siamycin I. This ribosomally synthesized post-translationally modified peptide belongs to the group of lasso peptides, and as a first member of this group, lipid II binding was recently shown for this compound [57].

Why Is Lipid II Such an Effective Target?

The PGN biosynthesis machinery is an important target pathway for numerous clinically important antibiotics (Fig. 3), as PGN is absent in eukaryotes but essential for the vast majority of pathogenic bacteria. Within this pathway, most antimicrobials either block the biosynthesis of lipid II or prevent its incorporation into the PGN network. Lipid II is the first PGN building block that appears on the outside of the cytoplasmic membrane, making it readily accessible also for bulky molecules such as the glycopeptide antibiotics and defensins. As a direct consequence, sequestration of lipid II causes PGN biosynthesis to stop, as the building blocks are no longer available for the cell wall biosynthesis machinery. Likewise, blocking the enzymatic activities in the PGN biosynthesis machinery (e.g., through the inactivation of PBPs by ß-lactams) prevents the integration of PGN precursors. However, secondary cellular consequences of antibiotics targeting the PGN pathway are multifaceted as lipid II clearly

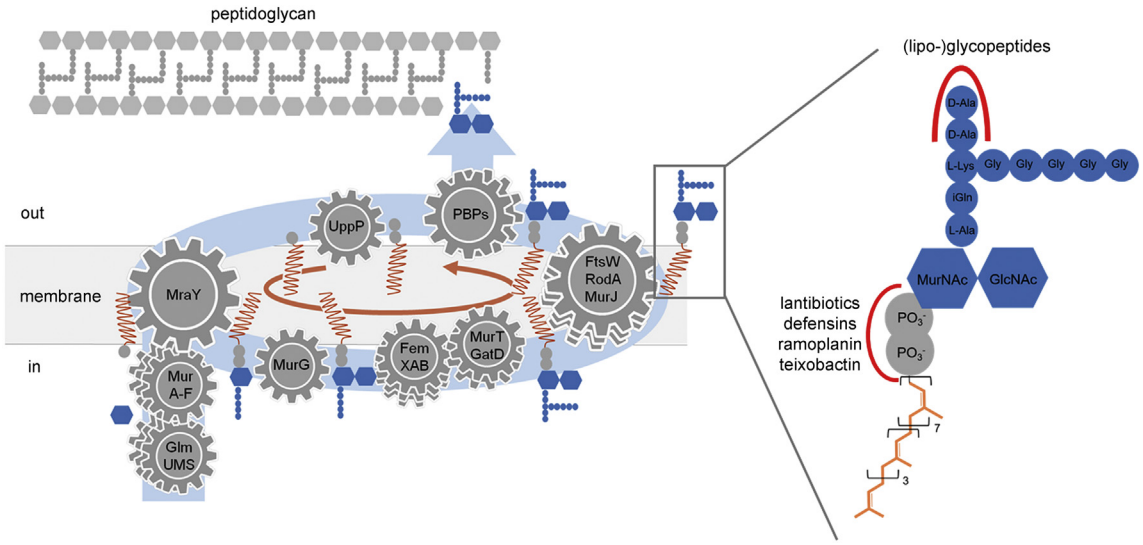

Fig. 3. Schematic illustration of the core PGN biosynthesis machinery of *S. aureus*. The central PGN building block lipid II is boxed and expanded. Minimal binding motifs of prominent lipid II binders are indicated. Additional binding sites are discussed in the text.

appears to be much more than a mere PGN building block. A complex membrane-associated network of protein–protein and protein–lipid interaction exists within the PGN biosynthesis machinery to ensure the optimal order of events during cell growth and cell division and lipid II is discussed to be involved in the orchestration of these processes [58]. It has, for instance, been shown that lipid II is the signal molecule of the eukaryotic-like serine/threonine kinase PknB in *S. aureus* [59] and M. tuberculosis [73]. By sensing the availability of lipid II, this kinase likely contributes to integrative fine-tuning of cell envelope biosynthetic pathways and cell division [59]. In addition, lipid II directly affects the spatial organization of proteins, which is an important factor for their optimal biological activity. The localization of PBPs is dependent on substrate (i.e., lipid II) availability [60,61]. In *S. aureus*, it has been shown that proper localization of PBP2 is no longer possible when the active site of the PBP is blocked by ß-lactams, when substrate availability is limited due to the inhibition of lipid II biosynthesis or when lipid II is sequestered by vancomycin [60]. Likewise, membrane-bound filaments of the bacterial actin homolog MreB organize PGN biosynthesis machineries at the sidewalls of *Bacillus subtilis*, and membrane association of MreB filaments was shown to depend on cellular levels of lipid II [62]. Accordingly, depletion of the PGN precursor leads to the disassembly of MreB filaments and consequently to disintegrated PGN biosynthesis. In untreated cells, lipid II levels are relatively low, and importantly, antibiotics affect these levels to a different extent. A comprehensive study is lacking, but it has been shown that the lipid II-binding antibiotics vancomycin, ramoplanin and lysobactin lead to increased lipid II levels [63]. In contrast, antibiotics affecting lipid II biosynthesis (e.g., D-cycloserine, fosfomycin) are expected to decrease the cellular levels of lipid II. Furthermore, interfering with the incorporation of the PGN building block likely affects also earlier bactoprenol-coupled precursors that are shared with other biosynthetic pathways such as the WTA biosynthesis machinery. In summary, the cellular downstream effects of binding or interfering with lipid II are complex and apparently trigger irreversible physical damage to the cells such as delocalization of spatially-organized enzymes and other components of the cell wall and divisome machineries. Although not fully understood, such cellular damages go well beyond the stresses which bacterial cells are equipped to counteract and cannot be easily repaired. It appears an attractive hypothesis, that for bactericidal activities, such irreversible physical damage needs to follow primary target inhibition and that such damage can best be achieved by targeting the spatio-temporal organization of biosynthetic machineries of macromolecules, as we find it for most natural product antibiotics. In the case of lipid II, such damage is particular strong since sequestration of the building block can be combined with membrane damage that virtually impacts on all other cellular processes that are organized in or at the membrane. This, together with the fact that lipid II is readily accessible on the outside of the cell, may well explain why lipid II can be considered the bacterial “Achille's heel” [64].

Lipid II Modifications and Resistance Development

Generally, lipid II is a highly conserved molecule that does not allow for easy modification. However,

the often communicated statement that resistance to lipid II-binders cannot develop because target modifications are not possible, must be taken with caution. A detailed description of the known lipid II modifications is given elsewhere [2], and only general schemes relating to antibiotic resistance are elaborated here.

The transport of short saccharide units across membranes is usually achieved by coupling sugar moieties to a poly-isoprenoid linker, and such carrier systems are found throughout the living world. The molecular design of such a carrier includes the formation of a sugar–pyrophosphate–isoprenoid linkage unit, which provides the primary interaction site for the majority of lipid II-binding antibiotics and thus represents an almost universal target, which cannot be modified. Also, the basic PGN building block, the disaccharide-pentapeptide moiety, is conserved as such, and in Eubacteria comprises an *N*-acetylmuramic acid–*N*-acetyl-glucosamine disaccharide. In contrast, a number of modifications can be found for the pentapeptide side chain, which may have defined impact on the activity of specific antibiotics. However, such modification cannot be achieved rapidly through single-point mutations as in the case of protein targets; rather, they typically require the acquisition of genetic elements that allow for the respective modification reactions or even for an entire modification pathway. Moreover, these genetic elements tend not to be easily expressed in the new host bacterium, since the modified lipid II molecule needs to be compatible as a substrate with the entire cell wall building machinery, for example, with the transglycosylation and transpeptidation enzymes catalyzing the polymerization of the building block. Thus, substantial compensatory mutations are necessary to stabilize the new resistance phenotype and the establishment of, for example, vancomycin-resistant enterococci as described below, is a very rare event. The wide distribution of such resistant strains is due to clonal spread rather than new genetic events.

Much more often observed, however, are resistance phenotypes that develop gradually and that may be even reversible, since they result from the capacity of bacteria to sense and counteract cell wall stresses. Typically, antibiotic peptides are cationic and amphiphilic, and first contacts with target microbes involve interactions with negatively charged components of the cell wall. Strikingly, when peptide antibiotics, rather than being cationic, are overall negatively charged such as the lantibiotic mersacidin or daptomycin, they need to form a complex with Ca^{2+} for potent activity. The importance of charge-mediated interactions of peptide antibiotics with the cell envelope is reflected by effective resistance mechanisms based on mostly reversible reduction of the overall charge of the cell wall polymers such as LPS, and WTA or lipoteichoic acids [2].

Many of such charge modifications do not primarily affect the binding affinity of the antibiotics for the specific target site on lipid II but rather reduce accumulation of the positively charged antibiotic in the cell wall mesh, yielding moderate, but clinically relevant reduction of bacterial sensitivity. VISA strains instead are characterized by a thickened and less crosslinked cell wall. This leads to an increased accumulation of the antibiotic within the cell wall, thereby preventing its access to the lipid II within the membrane [65]. Interestingly, two *S. aureus* isolates were recently obtained from a patient that received long-term therapy with dalbavancin. These strains showed a thickened cell wall and were resistant against dalbavancin and teicoplanin but susceptible toward vancomycin [66].

High-level resistance can be achieved when the Glu residue in position 2 of the lipid II stem peptide is modified into a Gln. The amidation reaction is catalyzed by the bi-enzyme complex MurT/GatD [67]. The resulting Gln–lipid II has reduced affinity for plectasin and related defensins, which were reported to form hydrogen bonds to the Glu residue [47]. MurT/GatD is frequently found among pathogenic gram-positive bacteria, and thus, amidation of the stem peptide may be regarded as effective immune escape mechanism. Analysis of conditional MurT/GatD mutants demonstrated the importance of Gln residues for optimal growth rate, drug resistance and sensitivity of the staphylococcal PGN to the host defense factor lysozyme [68] and also shows that lipid II-handling enzymes, possibly in a co-evolutionary process, have been adapted to prefer, or even require the amidated building block.

The most prominent and clinically relevant modification of lipid II is the exchange of the terminal D-Ala to a D-lactate residue (or D-serine), as first described for VanA-type resistant enterococci (e.g., Ref. [69]). These strains have acquired genetic elements mediating the production of an alternative D-Ala–D-lactate dipeptide, which is then added by the cytosolic MurF-ligase to the emerging soluble cell wall building block before it is linked to bactoprenol-P. The genetic information originates most probably from glycopeptide-producing streptomycetes, which use the D-Ala–D-lactate version of lipid II for self-protection. Unlike all other lipid II binding antibiotics discovered so far, which target the pyrophosphate group, vancomycin and related glycopeptide antibiotics interrupt cell-wall biosynthesis in gram-positive bacteria by binding primarily to the D-Ala–D-Ala dipeptide terminus of lipid II. In this case, one of the five hydrogen bonds usually formed between the vancomycin molecule and the D-Ala–D-Ala terminus cannot form. Together with the resulting repulsive interaction between the two oxygen atoms in the lactate and the vancomycin molecule, this leads to a 1000-fold loss in the affinity of vancomycin for lipid II [70].

Conclusions—Can We Make Better Use of Lipid II-Binding Antibiotics?

Lipid II clearly is the most targeted molecule in microbial interactions in natural environments. Its highly conserved molecular design, its availability on the outside of the cytoplasmic membrane and the antibiotic potency that can be achieved through combining inhibition of PGN biosynthesis with various degrees of membrane damage and subsequent cellular downstream effects make it unique among antibiotic targets. In contrast, among therapeutically used antibiotics, it is only the glyco- and lipoglycopeptides that are lifesaving second-line antibiotics, applied particularly for infections with otherwise resistant gram-positive bacteria. Obviously, due to their physico-chemical properties, lipid II-binding compounds are not the easiest molecules to develop for clinical use [71]. Generally, lipid II binders are comparatively large molecules with molecular masses ranging from 1242 for teixobactin to approximately 5000 for some defensins, and natural compounds of this size tend to be more difficult to modify chemically than small synthetic compounds, not to mention total synthesis. The peptidic nature of the compounds, particularly for the ribosomally-made peptides, further restricts the options for chemical modifications. However, it is the amphiphilic nature with overall cationic properties of lantibiotics and defensins that is a major challenge for clinical development, since it is often associated with toxicity. Membrane active compounds are easily suspected of causing unspecific toxic effects; however, the approval of the above-mentioned glyco-lipopeptides telavancin, dalbavancin and oritavancin, as well as daptomycin with its unique mode-of-action, clearly shows that such issues can be successfully addressed, for example, by optimizing dosing schemes [71]. These compounds also demonstrate that it is a promising concept to combine membrane modulating activities with specific targeting of bacterial membranes through lipid II binding for improving potency and overcoming resistance.

The size of lipid II-binding antibiotics poses yet another problem, in that such compounds are too big to pass through porins in the outer membrane (OM) of gram-negative bacteria, a feature that makes a large section of prokaryotic life intrinsically resistant to lipid II binders. Given the fact that antimicrobial peptides—whether or not lipid II targetin—represent such a successful antibiotic principle in nature, it is even tempting to speculate that the evolution of an OM may have been driven by such compounds. Currently, there are no lipid II binders known that would be able to cross the OM; however, it has been well established that some classes of unmodified cationic antimicrobial peptides can pass the OM in a process termed “self-promoted uptake” (for review, see Hancock and Sahl [44]). Also, the cationic antimicrobial peptide polymyxin B is well known to destabilize the LPS layer of the OM such that larger molecules can reach the periplasmic space. Therefore, it appears not impossible to engineer molecules that can pass the OM and eventually target lipid II.

Rational development of Lipid II binders toward antibiotic drugs could be greatly supported if more structural information on the interactions with antibiotics would be available. So far, neither solution nor crystal structures of any antibiotic with full-length lipid II could be solved. Obviously, the long bactoprenol chain is detrimental for obtaining both suitable crystals and NMR spectra. The structural information available for lipid II-binding lantibiotics [40,41] has been obtained with truncated C15–lipid II, containing only three isoprenoid units. These studies provide important information on the interaction of the respective conserved motifs in the nisin-like and mersacidin-like lantibiotics with the pyrophosphate linkage unit. However, further interactions with the bactoprenol chain should take place to explain the membrane disruptive aggregation behavior of the nisin-type molecules.

The pioneering studies on vancomycin binding to lipid II were performed with the soluble stem peptide of Lipid II [72]. However, as in the case of oritavancin [8–10], the interaction of glycopeptides with naturally occurring lipid II, including, for example, the amidated Glu-2 and a pentaglycine bridge attached to Lys-3 as found in staphylococci, is much more complex and can explain the efficacy of these glycopeptide variants toward otherwise resistant strains. Therefore, with advances in structure determination technologies such as solid-state NMR, we may expect better information on the interaction of lipid II binders with their target, which could eventually enable rational design strategies and thus improve chances for development of clinically usable drugs targeting this unique and most relevant bacterial structure.

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PGN, peptidoglycan; PBPs, penicillin-binding proteins; VRE, vancomycin-resistant enterococci; VISA, vancomycin-intermediate *S. aureus*; WTA, wall teichoic acid; LPS, lipopolysaccharide; OM, outer membrane.

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