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Hit 'em where it hurts
The growing and structurally diverse family of peptides that target Lipid-II

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Abstract: Era of discovery of lipid II binding antibiotics - In an era where microorganisms develop resistance faster than we can develop new antibiotics, interest in antimicrobial substances produced by competing microorganisms is growing. Microorganisms themselves are an important source of novel antimicrobial substances as they commonly produce toxins to combat other microbes. Screening for novel antimicrobials has lead to the discovery of numerous active compounds in the past decades. In addition to the search for novel antibiotics, understanding their associated mode(s) of action is also of increasing importance. Studying different types of antimicrobial peptides (AMPs) reveals that AMPs have sustained their effectiveness throughout evolution.[1,2] Many AMPs have an amphipathic nature and are positively charged due to the high content of arginine and lysine residues. This gives them the ability to specifically interact with negatively charged membranes and subsequently disrupt the bilayer structure. Several general mechanisms have been assigned for various AMP modes of actions including the carpet model[3,4] and the 'sand-in-the-gearbox' model[5]. However, in order for an AMP to be broadly applicable as a possible therapeutic, it is important that it acts on a specific target, so as to circumvent toxicity and general membrane distortion. In this regard, there are a number of AMPs that are effective at low nanomolar concentrations indicating that they work via a target-specific mechanism rather than via general membrane distortion. This review provides a comprehensive overview of those AMPs that employ such a target, namely the peptidoglycan precursor lipid-II. Dubbed by some as the "bacterial achilles heal," lipid II is a validated antibiotic target. We speculate that future antibacterial agents operating via lipid II-mediated modes of action will play an important role in the battle against pathogenic bacteria by effectively hitting 'em where it hurts!

2. Peptidoglycan Biosynthesis Pathway

The bacterial cell wall is of vital importance for bacteria because it counters the osmotic pressures that arise between the cell's cytosol and its surroundings. Peptidoglycan is synthesized from Lipid II, a building block unique to bacterial cells. Lipid II synthesis starts at the cytosolic side of the membrane where the membrane embedded enzyme MraY links UDP-N-Acetyl-Muramic acid pentapeptide (UDP-MurNAc-pp) to the carrier lipid undecaprenyl phosphate forming lipid-I. Next, the transferase MurG couples UDP-N-Acetyl Glucosamine (UDP-GlcNAc) to the muramoyl moiety of lipid I to form lipid II. Lipid II is then "flipped" to the other side of the bacterial membrane by FtsW after which penicillin binding proteins (PBPs) incorporate lipid II into the growing peptidoglycan network. The residual undecaprenyl pyrophosphate is then dephosphorylated to the monophosphate and flipped back to the cytosolic site to be reused in the lipid II synthesis cycle. (Figure 1, for reviews on the whole process see [6–9])

The bacterial cell wall in Gram-positive bacteria is readily accessible and as such, the bacterial cell wall biosynthesis machinery is a prominent target for different types of antibiotics.[6,7,10] The viability of lipid II as a drug target is clearly demonstrated by vancomycin. Belonging to the glycopeptide class of antibiotics, vancomycin was first isolated from the soil bacteria *Streptomyces orientalis* in 1953 by Eli Lily. The use of vancomycin has significantly increased since the development of penicillin- and methicillin- resistant isolates. Since its introduction into the clinic, vancomycin remains the only antibiotic that targets lipid II and is a last resort treatment against many highly resistant Gram-positive bacteria. During vancomycin's early development phase, researchers were unable to isolate vancomycin resistant bacteria.[11,12] However, after more that 50 years of clinical use vancomycin resistance slowly emerged. Nowadays six different phenotypes of vancomycin resistant bacteria are known, designated as the VanA- to VanG-gene clusters.[12] Bacteria with VanA-, VanB- and VanD-type resistance possess an altered form of lipid wherin the vancomycin-binding D-Ala-D-Ala motif is replaced with D-Ala-D-Lac. Other vancomycin resistant organisms instead employ a D-Ala-D-Ser although this results in less severe resistance. Binding of vancomycin to the D-Ala-D-Ala moiety of lipid