

## 2007 Astellas USA Foundation Award

### Host–Guest Chemistry of the Peptidoglycan

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#### Introduction

The peptidoglycan is the polymer that surrounds, contains, and protects the cytoplasm of bacteria. The structure of this polymer consists of glycan strands cross-linked to each other via oligopeptide stems attached to alternate glycans of the strands. Because the peptidoglycan is a unique structure of bacteria and thus is without a eukaryotic counterpart, small molecule inhibition of the enzymatic events of peptidoglycan biosynthesis coincides with antibacterial activity.<sup>1–3</sup> For this reason, our laboratory has focused on the understanding of key events that involve biological recognition of the peptidoglycan. Examples of these recognition events include the peptidoglycan (and its biosynthetic precursors) acting as a *host* for small molecule binding and the peptidoglycan acting as a *guest* wherein it is recognized by protein macromolecules. Peptidoglycan recognition as a guest occurs during its biosynthesis, during the activation of bacterial resistance pathways, and in the innate immune response of eukaryotes to the presence of bacterial infection. In this Award Address, we emphasize the multidisciplinary approaches that are now being used toward the molecular-level understanding of the host–guest chemistry of the peptidoglycan and with emphasis on the central place of this structure in the continuing pursuit of improved antibacterial chemotherapy.

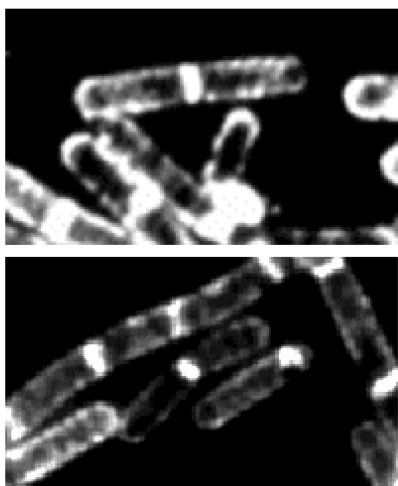
#### Bacterial Peptidoglycan

The peptidoglycan is the exterior surface of the Gram-positive bacterium. In these bacteria, it overlays the cell membrane while leaving a space (termed the “periplasmic” space) to accommodate inter alia the macromolecule proteins of small molecule influx and efflux and of cell envelope biosynthesis. Microscopic images (Figure 1) of the rod-shaped Gram-positive bacterium *Bacillus subtilis*, taken in the presence of sublethal concentrations of a fluorescent derivative of the peptidoglycan-binding antibiotic ramoplanin, show the location of active peptidoglycan synthesis in this bacterium.<sup>4,5</sup> In contrast to the Gram-positive bacterium, the exterior surface of the Gram-negative bacteria is a membrane, underneath which are found sequentially the peptidoglycan, the periplasmic space, and a second (or cellular) membrane. Together, the peptidoglycan and membrane(s) comprise the cell envelope that surrounds the cytoplasm of these bacteria.

Although the evolutionary deletion of peptidoglycan biosynthesis has occurred in most plants, following incorporation of the genome of an endosymbiotic cyanobacterium into that of the plant, expression of peptidoglycan structure still occurs in the plastids of certain mosses.<sup>6</sup> Conversely, while creation of the peptidoglycan polymer appears not to occur in several pathogenic bacterial species that have adapted to living within the cells of host species (the “cell wall-less” bacteria), portions of the peptidoglycan biosynthetic pathway are essential to their viability.<sup>7,8</sup>

The primary role of the peptidoglycan in Gram-positive and Gram-negative bacteria is believed to be structural, and a synonymous term for the peptidoglycan is the bacterial cell wall. The peptidoglycan contains the turgor pressure of the bacterium and provides a scaffold for the many proteins, enzymes, and structural complexes that are located within the cell envelope for communication by the bacterium with its environment.<sup>9</sup> Yet at the same time as the peptidoglycan fulfills this structural role, it undergoes dynamic transformation, structurally accommodating the fundamental processes of bacterial cell growth and division. During cell growth, all of the structural components of the cell envelope must grow in unison. During bacterial cell division, all must separate in unison, without compromise of the integrity of either cell. For these reasons, it is hardly surprising that circumstances leading to the loss of the integrity of the peptidoglycan are potentially lethal circumstances for the bacterium. Indeed, many of the most useful antibiotics used to control bacterial infections (such as the penicillin and cephalosporin  $\beta$ -lactam antibiotics and the vancomycin and teicoplanin glycopeptide antibiotics) use molecular mechanisms that disrupt specific processes involving the peptidoglycan. The uniqueness of the peptidoglycan as a characteristic structure of bacteria explains its value as an antibacterial target. The processes of peptidoglycan biosynthetic growth as a polymer, and eventual separation during bacterial division, demand the orchestrated interplay of specific and structurally complementary enzymes. Accordingly, the integrity of the peptidoglycan structure is monitored by the bacterium, by pathways that remain very poorly understood, as a means of activating the defense systems it possesses to resist these antibiotics. Likewise, because the peptidoglycan is a structure unique to bacteria, its degradation provides several small molecule structures that are used by eukaryotes for immune surveillance against bacterial infection. *All* of these events raise fundamental questions

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**Figure 1.** Visualization of the external peptidoglycan surface of Gram-positive bacteria by the use of fluorescently labeled peptidoglycan-binding antibiotics. These fluorescent microscopy images show rod-shaped *B. subtilis* (the bacterium is approximately 2  $\mu\text{m}$  in length) bacteria stained with fluorescein-labeled ramoplanin. The peptidoglycan is intensely stained at the newly forming division septum and to a lesser extent on the sidewalls and the old pole. These locations coincide with the subcellular locations of lipid II, the key biosynthetic precursor of the peptidoglycan (see Scheme 1). The sidewall staining is suggestive of a helical pattern for peptidoglycan growth during sidewall elongation. This figure is reproduced with permission from Tiyanont, K.; Doan, T.; Lazarus, M. B.; Fang, X.; Rudner, D. Z.; Walker, S. Imaging peptidoglycan biosynthesis in *Bacillus subtilis* with fluorescent antibiotics. *Proc. Natl Acad. Sci. U.S.A.* **2006**, *103*, 11033–11038. Copyright 2006 National Academy of Sciences, U.S.A.

concerning the chemistry of the peptidoglycan. How are the events of its growth, surveillance, and separation accommodated within the structural details of its molecular structure? And how can these events be exploited to the advantage of human and animal health by a molecular-level understanding of its structure as a *host* for antibacterial recognition and as a *guest* for recognition by macromolecules?

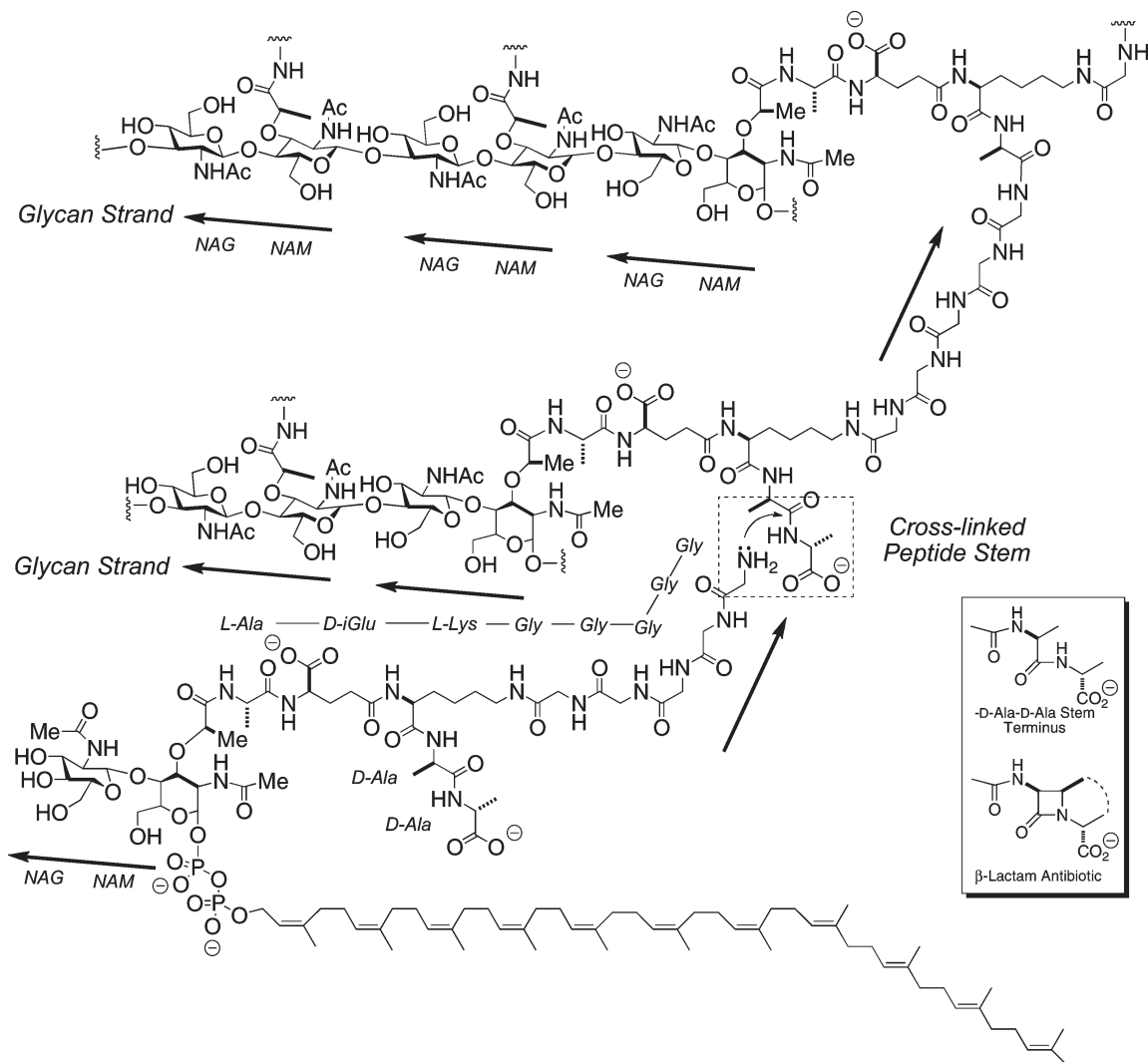
### Structure of the Peptidoglycan

The basic bonding arrangement of the bacterial cell wall polymer has been known for some time and is reflected by the term *peptidoglycan*.<sup>10</sup> The peptidoglycan is a polymer consisting of glycan strands, synthesized by the transglycosylase activity of bifunctional enzymes. The glycan strands are subsequently cross-linked by the conjoining of oligopeptide stems attached to alternate glycans of the strand. The process achieving this cross-linking is a transpeptidation reaction, catalyzed by a separate domain of these same bifunctional peptidoglycan-biosynthesizing enzymes.<sup>11–14</sup> The glycan structure is substantially identical for both Gram-negative and Gram-positive bacteria and consists of repeating units of a disaccharide composed of *N*-acetyl-2-amino-2-deoxyglucosamine (NAG<sup>a</sup>) in  $\beta$ -1,4-glycosidic linkage to the saccharide *N*-acetylmuramic acid (NAM). NAM, a derivative of NAG, has an additional ether-linked O-3 lactyl substituent. The carboxylate of the O-3 lactyl group is further substituted by an oligopeptide. The terminus of this oligopeptide is a repeat

of the *D*-amino acid *D*-alanine. This -*D*-Ala-*D*-Ala stem terminus is key to the cross-linking chemistry of the transpeptidation. During transpeptidation, the penultimate *D*-Ala terminus of the one glycan strand acylates the amine terminus of a stem peptide provided by an adjacent glycan strand, with departure of the ultimate -*D*-Ala as a leaving group. With the important exception of some new antibiotic-resistant Gram-positive bacteria,<sup>15</sup> the use of a -*D*-Ala-*D*-Ala stem terminus for the transpeptidase-catalyzed cross-linking of glycan strands in cell wall biosynthesis is common to both Gram-positive and Gram-negative bacteria. Although there are strong similarities in the structures of the rest of the oligopeptide stems of the Gram-negative and Gram-positive bacteria, significant variations in the composition, length, and stereochemistry of the oligopeptide structure are encountered.<sup>16</sup> These variations reflect both innate differences in biosynthesis and resistance adaptations.<sup>17–20</sup> Indeed, there is no better evidence of how much more there is to understand concerning the peptidoglycan than the recent discovery that *D*-amino acids *other* than *D*-Ala are important modulators of its structure.<sup>21,22</sup>

A sense of the biosynthetic complexity of these events is shown in Scheme 1, which shows the key structures involved in the biosynthesis and cross-linking of three glycan strands of the cell wall of the Gram-positive bacterium *Staphylococcus aureus*. The structure in the lower left of this chart is the key biosynthetic precursor for cell wall biosynthesis, lipid II. In lipid II of *S. aureus*, the peptide stem contains additionally a -Gly<sub>5</sub> “bridge” pentapeptide.<sup>23–26</sup> The amine terminus of this pentaglycine bridge is the nucleophile acceptor in cross-linking to the *D*-alanil acyl donor. Lipid II itself is assembled in the cytoplasm and inner leaflet of the bacterial cell membrane by a series of enzymatic reactions.<sup>27–29</sup> In the final steps of its biosynthesis, the hydrophobic undecaprenol segment of lipid II embeds into the inner leaflet of the cell membrane, exposing the hydrophilic NAG–NAM disaccharide to the cytoplasm. Sequential assembly of the oligopeptide stem and bridge structures on the NAM saccharide completes the lipid II structure. Lipid II is then translocated to the outer leaflet of the cell membrane by a poorly characterized lipid translocase.<sup>30–35</sup> Following this translocation, lengthening of the glycan strand occurs by transglycosylase catalysis<sup>36–39</sup> wherein one molecule of lipid II acts as a NAG–NAM donor, releasing the undecaprenylpyrophosphate for return to the cytoplasm and reuse in lipid II (and in lipid A, an intermediate used in the biosynthesis of the lipopolysaccharides of the exterior membrane of Gram-negative bacteria) synthesis.<sup>40–43</sup> The result of transglycosylase catalysis is pairwise NAG–NAM lengthening of the glycan strands.<sup>38,44</sup> As previously noted, the transglycosylase and transpeptidase enzymatic activities of peptidoglycan biosynthesis are catalyzed by separate and by spatially distanced domains of a single enzyme structure.<sup>13,45</sup> These enzymes are called the high-molecular-weight penicillin-binding proteins (HMW PBPs), wherein their name reflects their preeminence as the molecular target of the  $\beta$ -lactam (which include the penicillin and cephalosporin) antibiotics. The  $\beta$ -lactams engage in the functionally irreversible acylation of the active site serine of the PBP transpeptidase domain. As a result, further catalysis of the transpeptidation event of peptidoglycan synthesis is blocked.<sup>46–48</sup> The inability of the bacterium to complete this final event of cell wall biosynthesis either arrests bacterial growth or is bactericidal to the bacterium, depending on the structure of the  $\beta$ -lactam and the particular PBP enzymes

<sup>a</sup> Abbreviations: NAG, *N*-acetyl-2-amino-2-deoxyglucosamine; NAM, *N*-acetylmuramic acid; PBP, penicillin binding protein; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococci; PGRP, peptidoglycan recognition protein.

**Scheme 1.** Cross-Linking Event in Peptidoglycan Biosynthesis by the Gram-Positive Bacterium *S. aureus*<sup>a</sup>

<sup>a</sup> The structure of the immediate biosynthetic precursor for the peptidoglycan of *S. aureus*, lipid II-Gly<sub>5</sub> is shown as the bottom structure. The cross-linking event is highlighted within the hashed-edged box shown in the center right. This stem cross-linking event, wherein the amine terminus of the pentaglycine displaces the D-alanine terminus on the stem of an adjacent glycan strand, is catalyzed by the transpeptidase domain of a high molecular mass PBP enzyme. This reaction proceeds through an acyl-enzyme intermediate (not depicted). Lipid II-Gly<sub>5</sub> participation in transpeptidase cross-linking is shown only for the purpose of illustration. Peptide stem cross-linking almost certainly occurs subsequent to lipid II-Gly<sub>5</sub>-dependent transglycosylase-catalyzed elongation of the glycan strand. The inset box (lower right) shows the  $\beta$ -lactam antibiotic structure as a mimetic of a conformation of the -D-Ala-D-Ala stem used in the cross-linking.

(for example, the Gram-negative bacterium *Neisseria gonorrhoeae* has 4, while the Gram-positive bacterium *Bacillus subtilis* has at least 16) which are inactivated.<sup>49</sup> The key mimicry used by the  $\beta$ -lactam in this deception is with the -D-Ala-D-Ala peptidoglycan stem terminus, as first suggested by Tipper and Strominger.<sup>50–53</sup> During normal peptidoglycan biosynthesis, the active site serine of the PBP transpeptidase domain is acylated by the -D-Ala-D-Ala stem, with departure of the terminal -D-Ala as a leaving group. Interception of the -D-alanylacyl-enzyme by the amino terminus of an amino acid from an adjoining peptidoglycan strand cross-links the two strands. The Gram-positive bacterium *S. aureus* uses the amine of the N-terminal glycine of a pentaglycine bridge extension added to the  $\epsilon$ -amine of the L-lysine of the oligopeptide stem of the NAM saccharide. In other bacteria (such as the Gram-negative bacterium *E. coli*) a bridge extension is not used, and *meso*-diaminopimelic acid is used instead of L-lysine. The  $\beta$ -lactams, acting as -D-Ala-D-Ala mimetics

(Scheme 1), efficiently acylate the same active site serine used during catalytic transpeptidation, but because the  $\beta$ -lactam introduces a steric impediment not found in -D-Ala-D-Ala, acyl transfer to the amine provided by a neighboring glycan strand is not possible.<sup>54–57</sup> The catalytic activity of the transpeptidase domain of the PBP is thus inactivated.

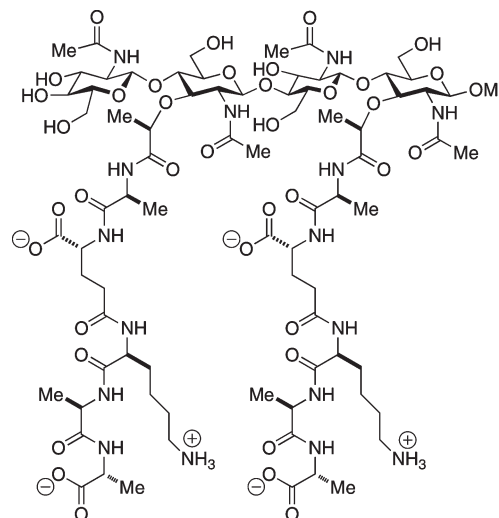
Scheme 1 leaves absent any sense of a higher order structure to the peptidoglycan polymer. The three-dimensional structure of the peptidoglycan is not known. As the last major biopolymer with an unknown three-dimensional structure, it is hardly surprising that a full breadth of experimental methodology has been applied toward this objective. The key unanswered questions regarding the three-dimensional structure of the peptidoglycan include the length of the glycan strands, the mechanism by which glycan length is controlled,<sup>58</sup> the orientations of the glycan strands as they straddle the transpeptidase domain of the PBP for cross-linking, the spatial orientation of the interconnected stems to the glycan

strands,<sup>26</sup> and the spatial orientation of the entire peptidoglycan with respect to the cell membrane.<sup>59–61</sup> This structural problem may be further divided to encompass possible structural differences between the “static” regions of the peptidoglycan, where the cell wall growth does not occur or is completed, and the “dynamic” regions where the peptidoglycan is growing for eventual septation into a daughter cell. Moreover, it is now known that the biosynthesis of the cell envelope components is coordinated by cytoskeletal assemblies within the cytoplasm of the bacterium.<sup>12,62–69</sup> With respect to the peptidoglycan as a component of the cell envelope, the remarkable structural similarities within the PBP family of enzymes<sup>45</sup> suggest commonality (of at least some structural features) among all of the bacteria. Nonetheless, bacteria exemplify such diversity of shape (including spherical, rod, curved, and spiral morphologies<sup>70–72</sup>) that variation in the peptidoglycan structure may be needed to accommodate these shapes.<sup>73</sup> The merits of several limiting possibilities to the three-dimensional peptidoglycan have been extensively discussed<sup>45,59,60</sup> and have stimulated the creation of sophisticated peptidoglycan models that valiantly attempt to reconcile current knowledge concerning peptidoglycan strand interconnection, constituent stoichiometry, extent of cross-linking, shape, growth, and division.<sup>26,61,74–76</sup>

A quantitative sense of the dimensions of the peptidoglycan polymer is available from microscopy. The peptidoglycan of the Gram-negative rod-shaped bacterium *E. coli* forms a remarkably consistent (in terms of depth) shell around the entire bacterium, as seen by cryoelectron tomography.<sup>77</sup> The depth of the *E. coli* peptidoglycan is approximately 6.4 nm, while the depth of the peptidoglycan of a second rod-shaped Gram-negative bacterium, *Pseudomonas aeruginosa*, is less (2.4 nm) by cryoelectron microscopy.<sup>78,79</sup> In these images the peptidoglycan is visualized as closely apposing the outer membrane of these two bacteria and above a periplasmic space. Microscopy studies of other Gram-negative bacteria suggest that a more central location of the peptidoglycan within the periplasmic space is also possible.<sup>80–82</sup> In contrast to the relatively thin peptidoglycan dimensions seen for Gram-negative bacteria, the depth of the mature peptidoglycan exoskeleton of the Gram-positive *S. aureus* cocci is greater (19 nm), and the peptidoglycan depth is even greater at the cross-wall formed during *S. aureus* cell division.<sup>83,84</sup> Atomic force microscopy of the *S. aureus* peptidoglycan<sup>85</sup> and of germinating Gram-positive spores<sup>86</sup> shows the surface of the Gram-positive peptidoglycan exoskeleton as a porous network of peptidoglycan strands.

Molecular-level experimental evidence concerning the three-dimensional structure of the peptidoglycan is being sought. A notable contribution from our own laboratory was the NMR determination of the solution structure of a single [NAG–NAM]<sub>2</sub> peptidoglycan strand (Scheme 2).<sup>87,88</sup> This tetrasaccharide was sufficiently small ( $M_r = 1930$ ) to be within the reach of synthesis but yet sufficiently large to demonstrate the innate conformational preference of an isolated peptidoglycan strand. The observed conformation for this strand was a right-handed helical glycan exhibiting 3-fold rotational periodicity for its peptide stem. It is most curious that several proteins that interact directly with the peptidoglycan via binding domains (such as the Braun lipoprotein of *Escherichia coli*,<sup>89</sup> the Omp porins of Gram-negative bacteria,<sup>90–92</sup> and the TolC channel protein of efflux assemblies<sup>93–95</sup>) oligomerize with 3-fold symmetry. The relevance of the 3-fold rotational periodicity seen for the

**Scheme 2.** Synthetic [NAG–NAM]<sub>2</sub> Mimetic of the Non-Cross-Linked Peptidoglycan of the Cell Wall, Used for the NMR Determination of the Solution Conformation of a Peptidoglycan Strand

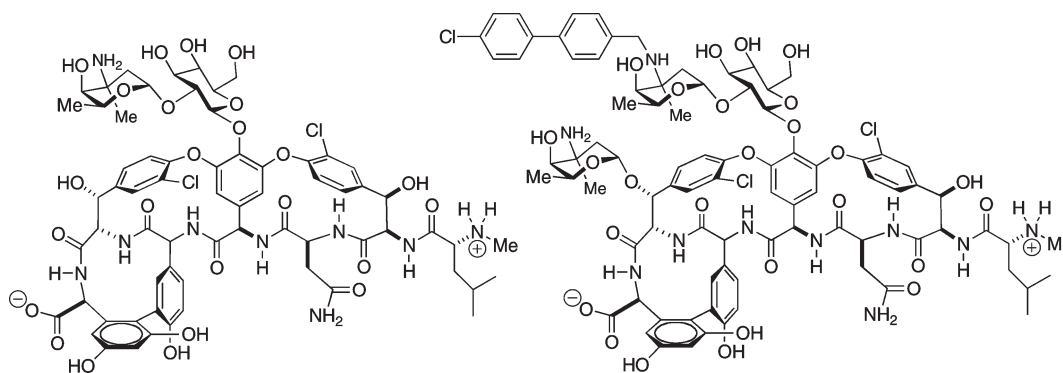


peptidoglycan stems for the structure of Scheme 2, with respect to biological recognition of the uncross-linked peptidoglycan structure, is attested to by a breadth of experimental studies involving both bacterial and eukaryotic proteins, as discussed below.

The solid-state NMR spectrum of the isolated peptidoglycan polymer of *E. coli* (a single molecule having an approximate molecular mass of  $3 \times 10^9$  Da) shows remarkable simplicity.<sup>96</sup> Spin diffusion NMR spectra of the intact <sup>13</sup>C-glycine-labeled *S. aureus* peptidoglycan is most consistent with spatial proximity between the pentaglycyl bridge and the glycan strand, interpreted as requiring that all of the peptide stems lie parallel in a plane perpendicular to the glycan.<sup>97</sup> Given the ability of the peptidoglycan to accommodate extraordinary variation within the peptide stem,<sup>18,25</sup> as evidenced by the facile adaptation by *S. aureus* to the use of both short and long peptide bridges,<sup>98,99</sup> expectations for a single consensus three-dimensional peptidoglycan structure may be unreasonable. Perhaps the most promising future efforts toward this objective are those examining the direct interaction of the peptidoglycan with bacterial hyperstructure. For example, substantial recent progress has been made toward the molecular interaction of the peptidoglycan with the stator protein of the flagellum hyperstructure, a protein that possesses a well-defined peptidoglycan-binding domain<sup>100–103</sup> in order to anchor the flagellum hyperstructure to the cell wall.<sup>104–106</sup>

### Peptidoglycan as Host

Given the necessity of peptidoglycan integrity to bacterial viability, it is not surprising that the peptidoglycan is a target for antibiotics. Indeed, the clinical development of new antibiotics having the peptidoglycan as their molecular target remains no less compelling an objective today than it was at the dawn of the 20th century antibiotic era. Antibiotic recognition of the peptidoglycan occurs at the early stages of peptidoglycan biosynthesis, as well as by the polymeric peptidoglycan. A particularly important point for antibiotic recognition of the peptidoglycan occurs at the PBP-dependent events of transglycosylation and transpeptidation.<sup>40,107</sup>

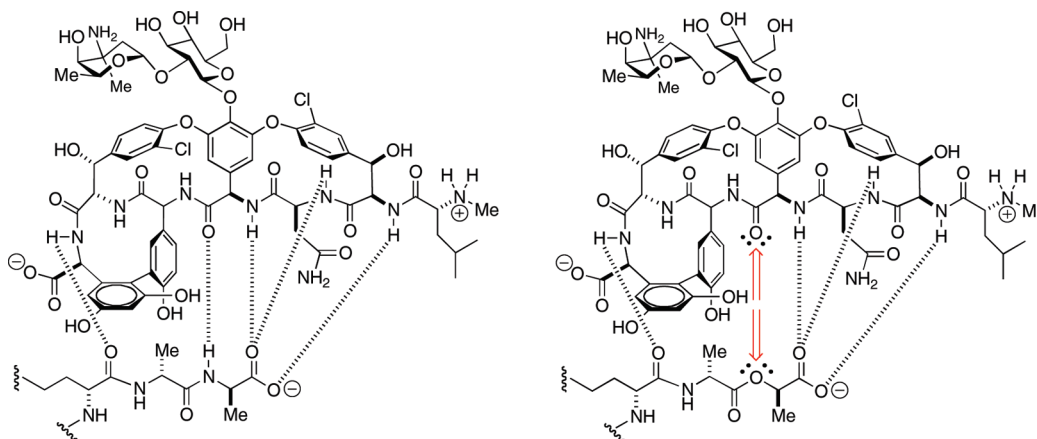
**Scheme 3.** Structures of Vancomycin (Left) and Oritavancin (Right)<sup>152</sup>

As briefly discussed above, the chemotherapeutic value of the  $\beta$ -lactam antibiotics rests on their ability to function as mimetics of the unique -D-Ala-D-Ala motif used during this transpeptidation reaction (additional aspects of the relationship between the  $\beta$ -lactams and the peptidoglycan are covered in the section on the peptidoglycan as guest). For clarity of focus, our discussion on the peptidoglycan as a host structure is restricted to the single example of vancomycin as a chemotherapeutically important example of noncovalent peptidoglycan recognition. Numerous examples of antibiotics other than the glycopeptides (as exemplified by vancomycin and teicoplanin) exploiting noncovalent peptidoglycan host-guest recognition are known and include the closely related (but mechanistically separate) lipoglycopeptides (exemplified by oritavancin and telavancin),<sup>108</sup> the lipoglycopeptides (exemplified by ramoplanin),<sup>109–111</sup> the mannopeptimycins,<sup>112,113</sup> and the peptide-derived lantibiotics (exemplified by nisin and clausin).<sup>114–118</sup> Of these antibiotic classes, the molecular recognition mechanism of the vancomycin class of antibiotics (Scheme 3) against Gram-positive pathogens is the best understood.<sup>119–122</sup> Vancomycin recognizes primarily the acyl-D-Ala-D-Ala motif at the peptide stem terminus of the NAM residue of lipid II, the key biosynthetic intermediate in peptidoglycan biosynthesis. Consequently, this aspect of the molecular mechanism of vancomycin is exerted largely through steric interference by the bound antibiotic at the transglycosylase stage of peptidoglycan biosynthesis.<sup>123</sup> The complex formed between the -D-Ala-D-Ala motif and vancomycin is noncovalent and is characterized by strong electrostatic (including both ion pair and hydrogen-bond) interactions.<sup>124–127</sup> The thermodynamics of vancomycin binding in solution with the [NAG-NAM]<sub>2</sub> cell wall mimetic shown in Scheme 2 correspond to a 1.0  $\mu$ M dissociation constant ( $\Delta G^\circ = -34.5$  kJ mol<sup>-1</sup>) that is dominated by favorable enthalpy ( $\Delta H^\circ = -41.2$  kJ mol<sup>-1</sup>) at a relatively small entropic cost ( $T\Delta S^\circ = -6.7$  kJ mol<sup>-1</sup>).<sup>128</sup> Although this in vitro dissociation constant is notably greater than the nanomolar benchmark that customarily defines strong biological interaction, the effective concentration of the -D-Ala-D-Ala motif encountered by vancomycin as it engages the peptidoglycan surface of Gram-positive bacteria is high. The supramolecular organization of the vancomycin-*N*-acetyl-D-Ala-D-Ala dipeptide complex formed in vitro shows back-to-back and side-to-side intermolecular contact between the vancomycin molecules of the complex.<sup>129–131</sup> These solid-state structures were preceded by the observation that vancomycin poorly self-associates to form a dimer in solution, in the absence of peptidoglycan structure.<sup>119,132–135</sup> Covalent vancomycin dimers with strong antibiotic activity are known.<sup>136</sup> Exploitation of the vancomycin-peptidoglycan complex for the purpose of delivering a second antibacterial agent, representing

the concept of hybrid antibacterial design,<sup>137</sup> also has been demonstrated by a covalently linked vancomycin-cephalosporin hybrid with potent Gram-positive activity.<sup>138,139</sup>

NMR analyses of vancomycin (and its derivatives) bound to the intact peptidoglycan of *S. aureus* indicate that the pentaglycine bridge that is unique to *S. aureus* further contributes to the binding interaction.<sup>140,141</sup> A recent (and surprising) structure-activity development within the vancomycin class is the discovery that the interactions of vancomycin and of oritavancin, a semisynthetic lipoglycopeptide derivative of vancomycin (Scheme 3),<sup>142–147</sup> are fundamentally different. Oritavancin exhibits much faster bactericidal activity against methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant enterococci (VRE) than does vancomycin.<sup>143</sup> Solid-state NMR evaluation of oritavancin derivatives<sup>148,149</sup> indicates that oritavancin has two separate binding interactions, one using the -D-Ala-D-Ala and the other the pentaglycine bridge motifs. Whereas both vancomycin and oritavancin have comparable inhibition of the transglycosylase step of peptidoglycan biosynthesis, as a result of the pentaglycine interaction oritavancin is the better transpeptidase inhibitor.<sup>149</sup> Using <sup>19</sup>F REDOR NMR of a fluorinated oritavancin derivative, Kim et al. observe oritavancin to preferentially bind to the mature, as distinct from the nascent, peptidoglycan of *S. aureus* protoplast membranes.<sup>150</sup> Direct NMR comparison of vancomycin and oritavancin binding to whole cells of a second Gram-positive pathogen (*Enterococcus faecium*) that does not use a pentaglycine bridge further suggests the value of a secondary binding site interaction with respect to antibacterial potency.<sup>123</sup> Glycopeptide binding to the *E. faecium* peptidoglycan occurs only to nascent peptidoglycan and further correlates to preferential transpeptidase inhibition, in contrast with preferential transglycosylase inhibition by vancomycin.<sup>123</sup> Independent evidence supporting different binding interactions for oritavancin compared to vancomycin, using the peptidoglycan structures of both methicillin-resistant *S. aureus* and vancomycin-resistant *Enterococcus faecalis*, is also provided by transmission electron microscopy.<sup>151</sup> With both the MRSA and VRE strains, oritavancin showed enhanced affinity for binding to the peptidoglycan at the septum, the eventual point of cell lysis.<sup>151</sup> The importance of further understanding of the structure-activity basis for peptidoglycan-glycopeptide host-guest chemistry, toward improvement in the breadth and potency of these (and other) antibacterials, cannot be overstated.

A direct relevance of peptidoglycan-vancomycin host-guest chemistry to the understanding of the resistance mechanisms against the vancomycin antibacterial class is already proven. Several of the resistance mechanisms against

**Scheme 4.** Hydrogen-Bonding Interactions in the -D-Ala-D-Ala Peptidoglycan–Vancomycin Host–Guest Complex (Left) Compared to the -D-Ala-D-Lac Peptidoglycan–Vancomycin Host–Guest Complex (Right)<sup>a</sup>

<sup>a</sup> The loss of a key hydrogen bond, and its replacement by unfavorable non-bonding repulsive interactions, is indicated by the red arrows in the -D-Ala-D-Lac depsipeptide complex.<sup>161</sup>

vancomycin correlate directly to lowering the effectiveness of its complex with the peptidoglycan.<sup>153–155</sup> One mechanism is the thickening of the cell wall, resulting in greater propensity for nonproductive complex formation on the bacterial surface while simultaneously limiting the access of vancomycin to the site of cell wall biosynthesis at the base of the cell wall.<sup>156–159</sup> Two other mechanisms involve modification of the peptide stem. Replacement of the acyl-D-Ala-D-Ala stem terminus with a depsipeptide (ester-containing) acyl-D-Ala-D-Lac terminus abolishes the central hydrogen bond between the vancomycin amide carbonyl and the acyl-D-Ala-D-Ala amide NH<sup>160</sup> while concomitantly introducing unfavorable non-bonding interactions (Scheme 4).<sup>161–163</sup> As a result, the affinity of vancomycin for the peptidoglycan decreases by 3 orders of magnitude, matching the decrease in antibiotic efficacy.<sup>164</sup> This vancomycin resistance mechanism is now common in the enterococci and has been recently observed in *S. aureus*. The biosynthetic enzymatic activities that are necessary to accomplish this change in peptide stem structure may have originated by adaptation of the self-resistance mechanism used by the microorganisms that biosynthesize these glycopeptides.<sup>165</sup> A third resistance mechanism, used by the enterococci, involves peptidoglycan cross-linking to the acyl–enzyme formed from the stem acyl-L-lysine-D-Ala amide bond rather than the acyl-D-Ala-D-Ala amide bond. This cross-linking is catalyzed by an L,D-transpeptidase enzyme<sup>166</sup> rather than the D,D-transpeptidase activity of the PBPs. This change in acyl donor evades the consequence of the vancomycin complexation and results in high-level resistance of the enterococci to both the  $\beta$ -lactam and the glycopeptide classes of antibiotics.<sup>167</sup>

As important as the events of molecular recognition are to antibiotic activity, it is now generally recognized that the antibiotic recognition event (whether the antibiotic is a  $\beta$ -lactam (peptidoglycan targeted), a glycopeptide (peptidoglycan targeted), an aminoglycoside (ribosome targeted) or other antibiotic/target pairing) serves primarily as *initiating* events. Antibiotic activity results from a much further downstream disruption of homeostasis within the metabolic pathways of the bacterium. These disruptions compromise the ability of the bacterium to grow (antibiotic is bacteriostatic) or to survive (bactericidal). At the molecular level, however, we presently have almost no understanding as to *how* an

antibiotic recognition event generates a signal, how this signal is propagated, and how this propagation ultimately results in the loss of homeostasis for a particular pathway(s). Moreover, we further suffer in this ignorance, since it is likely that the molecular events of signal propagation leading to cell disruption may also coincide with the molecular events leading to expression of mutations allowing resistance or to the expression of a resistance response. An exciting recent development is recognition that the bacterial SOS response and in particular the generation of reactive oxygen species are central events to the mechanism of action of many antibiotics, including that of vancomycin.<sup>168–173</sup>

A final example of the application of peptidoglycan host–guest chemistry is the visualization of the peptidoglycan surface. Peptidoglycan biosynthesis is coordinated with the biosynthesis of the other components of the cell envelope.<sup>12,65,174–182</sup> The ability of the peptidoglycan to recognize fluorescent conjugates of vancomycin and of ramoplanin (see Figure 1) has allowed visualization of the pattern of peptidoglycan biosynthesis<sup>5,12,21,174,176,183</sup> and correlation of antibiotic location with relative resistance.<sup>159</sup> Complementary fluorescence microscopy images suggest a helical pattern for cell envelope assembly in rod-shaped Gram-positive (such as *B. subtilis*) and Gram-negative (such as *E. coli*) bacteria, as also visualized from the perspective of the membrane lipids<sup>184,185</sup> and of the chemoreceptor proteins.<sup>186</sup>

### Peptidoglycan as Guest

A preponderance of research effort on the peptidoglycan has focused on the structural relationship between the  $\beta$ -lactam antibiotics and the acyl-D-Ala-D-Ala stem structure<sup>46,47,187</sup> in relation to  $\beta$ -lactam inactivation of the transpeptidation/carboxypeptidase activities of the PBPs.<sup>13</sup> While this focus is by no means misplaced, a richer perspective on the guest chemistry of the peptidoglycan is now evident. Peptidoglycan recognition embraces not just the PBPs of cell wall biosynthesis but also a sweeping breadth of bacterial enzymes that monitor peptidoglycan integrity (to detect the presence of antibiotics that would compromise peptidoglycan integrity) and for activating resistance mechanisms and for controlling peptidoglycan degradation (during septation and for some bacteria the recycling of old peptidoglycan into new peptidoglycan). *All* of these events are now understood to be

highly regulated and interdependent in ways that we are only now beginning to understand. Moreover, the detection of several of the structural components of the peptidoglycan by protein recognition is a key mechanism for eukaryotic activation of innate immunity in response to bacterial infection. An evaluation of the full interplay of these processes is beyond the scope of this Award Address. Our emphasis here is the growing appreciation of the value of chemical synthesis of discrete peptidoglycan structures (“muropeptides”) as a prerequisite to the understanding of the specific roles played by the key proteins and enzymes of these processes.

The value of peptidoglycan of defined structure to elucidation of the extraordinary breadth of its host–guest chemistry is proven in terms of understanding its biosynthesis,<sup>37,188–192</sup> its recognition by enzymes,<sup>193</sup> and its immune recognition.<sup>194–202</sup> These examples include peptidoglycan structures obtained by both chemoenzymatic and total synthesis. Our laboratory has used the total synthesis of peptidoglycan structures to address a diversity of important problems concerning peptidoglycan host–guest chemistry. These problems include the substrate specificity of the ampD amidase of peptidoglycan recycling, the structural basis for peptidoglycan binding by the peptidoglycan recognition proteins of innate immunity, the structural basis for peptidoglycan recognition by a phage endolysin, and peptidoglycan recognition by a PBP.

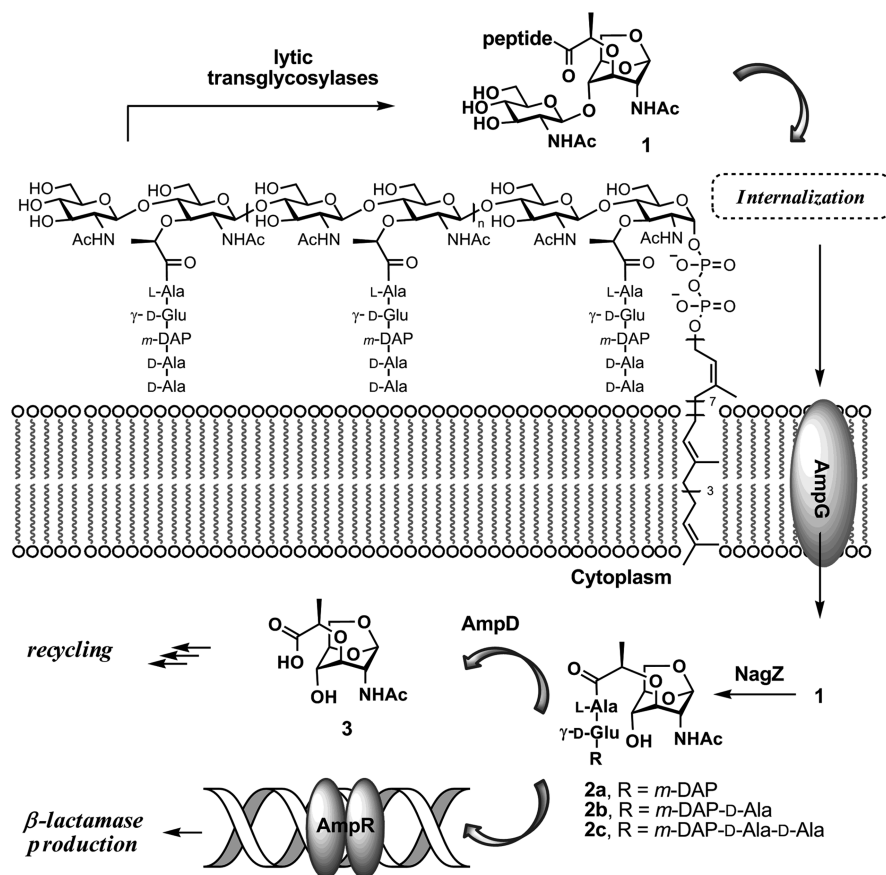
An emerging area is the signaling processes by which antibiotics induce defensive and virulence responses by bacteria. The integrity of the peptidoglycan structure is monitored continuously by the bacterium, and we know little of how this monitoring is done. In many Gram-negative bacterial pathogens,  $\beta$ -lactamase induction as a resistance mechanism occurs through a muropeptide-signaling pathway.<sup>203–205</sup> The  $\beta$ -lactamases are defensive enzymes that recognize  $\beta$ -lactam antibiotics as substrates and catalyze the hydrolytic opening of the  $\beta$ -lactam ring. This destruction of the pivotal structural feature of the  $\beta$ -lactams, used for PBP inactivation, abolishes their antibiotic activity. An antagonist of this signaling could have great value by preserving  $\beta$ -lactam activity against these bacteria, by suppression of  $\beta$ -lactamase expression. Although key events in the pathway are known, how these events interconnect remains largely unknown. Clearly, the pivotal event is initial detection of the  $\beta$ -lactam antibiotic. In resistant Gram-positive bacteria,  $\beta$ -lactam recognition occurs by acylation of a serine residue located in a surface-exposed transmembrane signaling protein.<sup>206–213</sup> The triggering event in  $\beta$ -lactam recognition by Gram-negative bacteria now appears also to involve serine acylation but here of specific members of the PBP family. The PBP enzyme family is divided between the high-molecular-mass enzymes (having both transglycosylase and transpeptidase activity) and the low-molecular-mass enzymes (having primarily carboxypeptidase activity, catalyzed by domains with very strong structural homology to the transpeptidase domain of the high- $M_r$  PBPs). At least one functional high- $M_r$  PBP enzyme is essential for bacterial viability while the low- $M_r$  PBPs, although having ancillary roles relating to shape<sup>214–216</sup> and septation,<sup>217</sup> are regarded as noncritical, notwithstanding their evolutionary conservation.<sup>218</sup> In the Gram-negative bacterium *Pseudomonas aeruginosa*,  $\beta$ -lactam acylation of a specific low- $M_r$  PBP senses the presence of  $\beta$ -lactam antibiotics.<sup>219</sup> Structural analysis of the cognate *Haemophilus influenzae* PBP suggests a conformational translocation involving a specific phenylalanine (a residue that is not found in other low- $M_r$  PBPs) in signaling.<sup>220</sup> The next step in signal

transduction is unknown. Loss of the activity of this PBP, however, ultimately coincides with a change in the steady-state muropeptide catabolite levels governed by a muropeptide recycling pathway and interpreted to result in derepression of the  $\beta$ -lactamase gene.

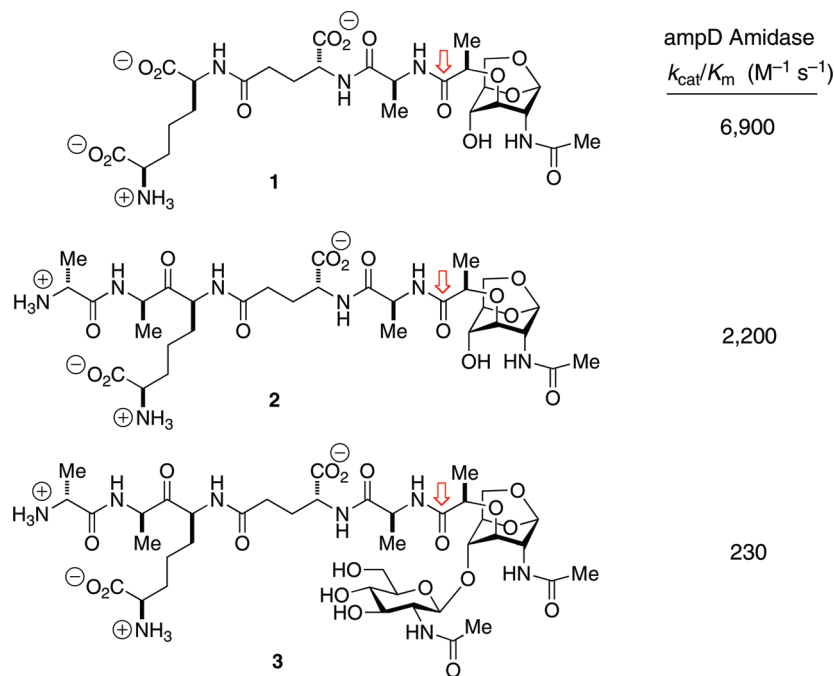
As we now understand the Gram-negative peptidoglycan-recycling pathway that regulates  $\beta$ -lactamase expression, recycling of the glycan strands of the peptidoglycan gives a steady-state muropeptide pool.<sup>221,222</sup> The initial step in muropeptide recycling is catalyzed by a family of enzymes found in the periplasm, the lytic transglycosylases, that cleave the  $\beta$ -(1,4) NAG–NAM disaccharide bond to give muropeptides having as a terminus the unusual bicyclic 1,6-anhydromuramamide saccharide. The mechanism for formation of the 1,6-anhydromuramamide by these enzymes is intramolecular interception by the C-6 alcohol of an incipient oxocarbenium cation species.<sup>223,224</sup> While the presence of anhydromuramic termini in endogenous Gram-negative muropeptides is long recognized, the circumstances governing their appearance are unknown. The 1,6-anhydromuramamide muropeptides enter the cytoplasm from the periplasmic space by passage through the proton-motive driven AmpG transporter.<sup>225,226</sup> Within the cytoplasm, the terminal 1,6-anhydromuramamide is separated from the glycan by the NagZ glycosylase.<sup>227,228</sup> A 1,6-anhydromuramamide structure is believed to bind to the AmpR transcription factor to derepress  $\beta$ -lactamase expression.<sup>229</sup> The final step in this portion of the pathway for peptidoglycan recycling is hydrolytic cleavage of the lactyl amide of 1,6-anhydromuramamide, catalyzed by AmpD amidase and giving the 1,6-anhydromuramic monosaccharide and the free peptide stem as products. A schematic overview of these events is shown in Scheme 5.

Seminal studies on this pathway used the 1,6-anhydroMurNAc-L-Ala-D-iGln-mA<sub>2</sub>pm muropeptide (a variant of structure **1** of Scheme 6 with D-iGln in place of D-iGlu), isolated from bacterium following deletion of the *ampD* gene.<sup>230</sup> The value of a more complete set of 1,6-anhydroMurNAc muropeptides, in order to clarify key structural questions within this pathway, was evident to us. Two examples of such questions include the confirmation of the *endo*- and *exo*-glycosylase preferences within the lytic transglycosylase family of enzymes<sup>231</sup> and correlation of the substrate specificity of AmpD with the structure recognized by the AmpR transcription factor. Accordingly, adaptation of outstanding synthetic precedent<sup>232</sup> provided the set of muropeptides shown in Scheme 6.<sup>233</sup> Evaluation of this set with the AmpD enzyme led to two conclusions.<sup>234</sup> The first conclusion (comparing **1** and **2** to **3**) strongly suggests that AmpD functions in the recycling pathway after NagZ, as previously believed.<sup>235</sup> The second conclusion, comparing the very similar  $k_{\text{cat}}/K_m$  values for **1** and **2**, is that the substrate specificity of this enzyme correlates more closely to the presence of a (1,6-anhydromuramyl)alaninyl peptide than to the peptide structure beyond the alanine residue. Since muramyl peptide **2** accumulates following exposure of the bacterium to  $\beta$ -lactams and is only slightly less capable as a substrate for AmpD, the structure of the AmpR ligand used in derepressing the  $\beta$ -lactamase gene remains open. Clearly, a detailed understanding of the interplay within the NagZ–AmpD–AmpR nexus will be critical to identifying opportunities for understanding the role of muropeptides in intracellular communication between prokaryotes<sup>236–238</sup> and in developing new means for antibiotic management and discovery against emerging Gram-negative pathogens.<sup>239,240</sup> A sense of these

**Scheme 5.** Peptidoglycan Fragmentation Leads to NAG–NAM Segments, Which Are Transported across Plasma Membrane To Initiate the Recycling of These Segments and Gene Induction for Production of the  $\beta$ -Lactamase Enzyme. Reprinted with permission from ref 234. Copyright 2009 American Chemical Society.



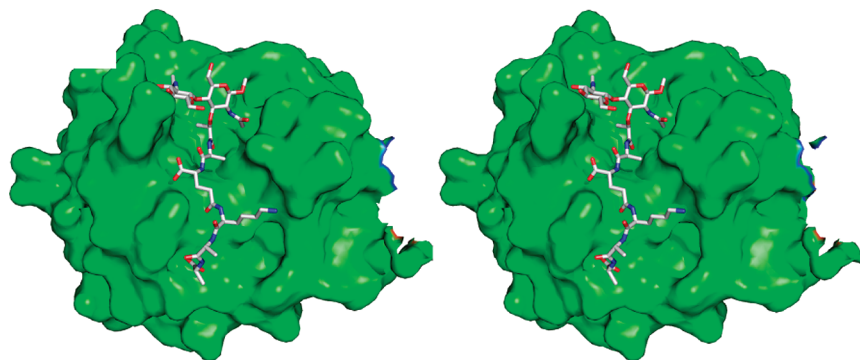
**Scheme 6.** Substrate Specificity, Measured by  $k_{\text{cat}}/K_m$ , for *Citrobacter freundii* AmpD Amidase-Catalyzed Amide Bond Hydrolysis (Cleavage Point Indicated by the Arrows) of Three Anhydromuramyl Muropeptide Structures



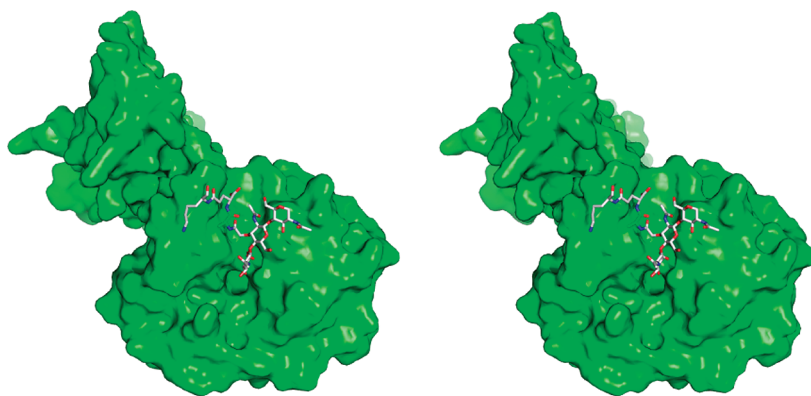
latter opportunities is evidenced by the observation of antibacterial synergy between NagZ inhibitors and the  $\beta$ -lactams.<sup>241–243</sup>

The peptidoglycan structure is used not only in bacteria-to-bacteria communication but by eukaryotes engaging in bacterial symbiosis<sup>244,245</sup> and by eukaryotes in the immunological





**Figure 2.** Computational stereoview of the  $[\text{NAG-NAM}]_2$  structure (see Scheme 2) in complex with the human PGRP-1 peptidoglycan recognition protein, based on an X-ray structure of a smaller synthetic fragment bound to the protein (PDB code 2EAX).<sup>273</sup> The approximate diameter of the protein is 4 nm. The perspective shown for the protein orients the peptidoglycan cleft from top to bottom.



**Figure 3.** Catalytic module of the Cpl-1 endolysin in complex with peptidoglycan (PDB code 2J8G).<sup>276</sup> A comparison of this protein structure with those of Figures 2 and 4 emphasizes the evolutionary convergence of different protein motifs for the purpose of peptidoglycan binding.

detection of, and response to, bacterial infection. Mammals possess a complex and interdependent array of peptidoglycan-binding proteins for innate immunity, including proteins involved in immune activation<sup>246–249</sup> and proteins (some are enzymes) that are also intrinsically bactericidal (peptidoglycan recognition proteins, PGRPs) by a vancomycin-like mechanism of peptidoglycan adherence.<sup>250–257</sup> An understanding of the structural specificity of this large family of proteins is relevant to the mechanisms by which bacterial pathogens are recognized<sup>196,258,259</sup> and how some evade immune surveillance<sup>260–263</sup> and for the purpose of identifying adjuvants for immune stimulation.<sup>199,202,264</sup> In insects, muropeptide recognition by the insect PGRPs appears to be the exclusive mechanism by which bacterial infection is detected.<sup>265</sup> Some insect PGRPs have enzymatic activity, while others are protein receptors for the muropeptide. Some are specific for lysine-containing muropeptides (lysine is found in the peptide stem of many Gram-positive bacteria), while others are specific for diaminopimelate-containing muropeptides (as are found in Gram-negative and rod-shaped Gram-positive bacteria).<sup>266</sup> Muropeptide binding to the insect PGRP results in PGRP heterodimerization, culminating in an innate immune response synthesis of antimicrobial defensive peptides.

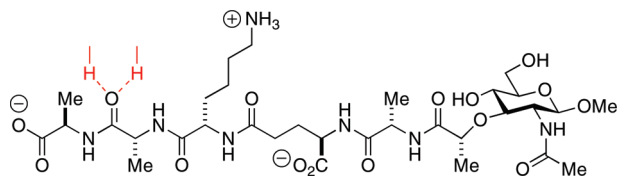
The mechanism of PGRP signaling in humans is different from that of insects. Human muropeptide recognition by PGRPs is one component of a much more complex innate immune response to bacteria (other bacteria-derived structure also contributes to human recognition of bacteria, including the lipopolysaccharides, the cell-wall protein derived lipopep-

tides, mannan saccharides, and proteins of the flagellum). Human PGRP interaction with bacteria is directly bactericidal<sup>253</sup> in a zinc-dependent process.<sup>267</sup> The structural basis for peptidoglycan recognition by the PGRPs has been studied using synthetic peptidoglycan fragments<sup>195</sup> and by the crystal structure determination of uncomplexed PGRPs<sup>198,268,269</sup> and peptidoglycan-complexed insect PGRPs<sup>270,271</sup> and human PGRPs.<sup>272</sup> These structures indicate that these PGRP use both the glycan and peptide stem substructures of the muropeptides in recognition. In collaboration with the Mariuzza group at the University of Maryland, we determined the structure of human PGRP-1 $\beta$  complexed with a NAG-NAM-L-Ala- $\gamma$ -D-Glu-L-Lys-D-Ala-D-Ala muropeptide.<sup>273</sup> The bound glycan conformation of this complex is virtually identical to the conformation of the  $[\text{NAG-NAM}]_2$  peptidoglycan (structure of Scheme 2) seen in solution.<sup>88</sup> Indeed, the occurrence of this same glycan conformation in peptidoglycan complexes with a phage endolysin and with a PBP (discussed below), and by others with yet other peptidoglycan-recognizing enzymes,<sup>44,274</sup> suggests that this conformation of the peptidoglycan may be the rule and not the exception. A computational model using the  $[\text{NAG-NAM}]_2$  structure bound to this PGRP, based on crystallographic data of a partial peptidoglycan structure seen in the electron density, is shown in Figure 2. In contrast to the glycan conformation, a different peptide stem substructure is seen as a result of a steric imposition of a different conformation on the lactyl amide of the NAM. These observations are consistent with the PGRP itself acting as a steric impediment for completion of cell wall synthesis while simultaneously stabilizing a peptide stem

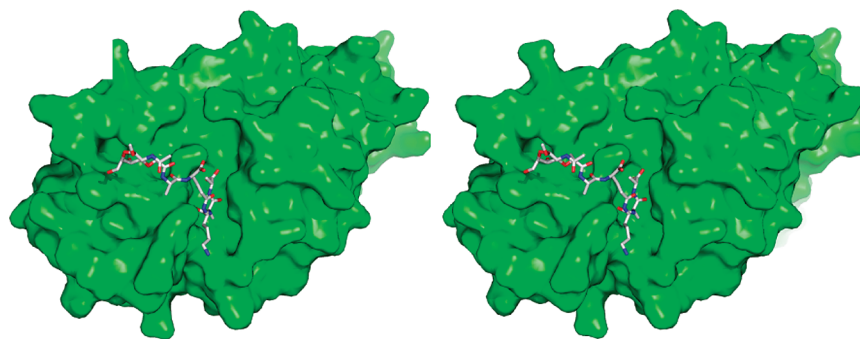
conformation that is unproductive for transpeptidase-dependent cross-linking.

A complementary perspective for macromolecule recognition of the peptidoglycan structure is provided by the structure of this same [NAG–NAM]<sub>2</sub> peptidoglycan strand bound to a catalytically inactive (E94Q) mutant of the pneumococcal phage Cpl-1 lysozyme, solved in collaboration with the Hermoso laboratory at the Instituto de Química-Física Rocasolano, CSIC in Madrid. The Cpl-1 lysozyme is an endolysin that catalyzes hydrolytic cleavage of the peptidoglycan at the NAG–NAM glycosidic bond, functioning in the final cell lysis step of the phage reproductive cycle.<sup>275</sup> Cpl-1 has a two-domain structure, with one domain anchoring the enzyme to

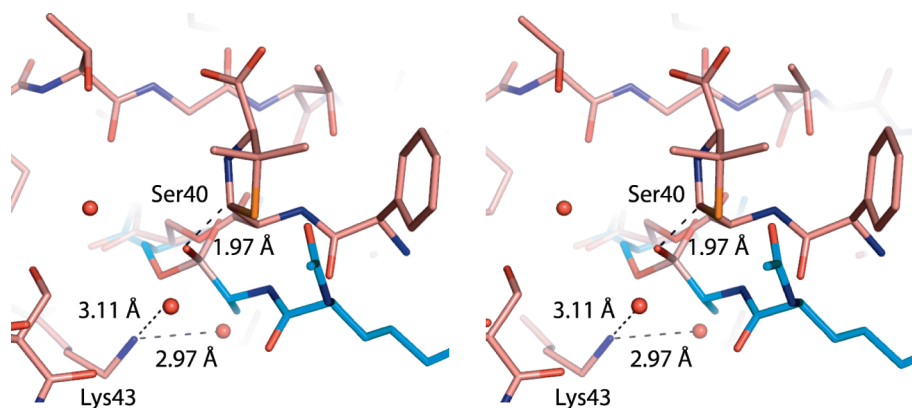
**Scheme 7.** Structure of the Synthetic Peptidoglycan<sup>87</sup> Used in the Determination of the Crystal Structure of a Michaelis Complex-like Structure with *E. coli* PBP6<sup>a</sup>



<sup>a</sup>The red-colored hydrogen bonds drawn to the (scissile) amide carbonyl of the penultimate D-Ala residue indicates its occupancy of the oxyanion hole of the active site.



**Figure 4.** Complex of *E. coli* PBP6 (catalytic domain of monomer B, PDB code 3ITB) with the peptidoglycan structure that is shown in Scheme 7.<sup>57</sup>



**Figure 5.** Stereoview of the PBP6 acyl–enzyme complex compared to that of the deacylation transition state. A PBP5 transition-state analogue structure (PDB code 1Z6F) is superimposed onto the PBP6 structure with ampicillin (PDB code 3ITA), showing active site residues 39, 40, 43, 108, and 210. The transition-state analogue and the catalytic serine of PBP5 are shown in blue, whereas ampicillin and PBP6 are shown in pink. Water molecules are represented as red spheres. Key distances are displayed as dashed lines. The amine group of Lys43 engages in hydrogen bonds with two water molecules in the PBP6 acyl–enzyme complex. The distance between the  $\beta$ -lactam thiazolidine bridging carbon atom of ampicillin and the boronic acid oxygen of the superimposed transition state analogue is only 1.97 Å. This carbon presents a steric impediment to hydrolytic deacylation.<sup>47</sup> Reprinted with permission from ref 57. Copyright 2009 American Chemical Society.

the cell wall and the second domain containing the active site. Five saccharides of the peptidoglycan flank the active site during catalysis. The [NAG–NAM]<sub>2</sub> tetrasaccharide is bound by the inactive E94Q mutant (Figure 3) in positions +1 to +3 (NAG–NAM–NAG, with the trailing NAM disordered) of the active site.<sup>276</sup> Again, this bound conformation is *identical* to the conformation encountered for the glycan strand in solution. The existence of a *common* peptidoglycan recognition pattern, encompassing peptidoglycan recognition by the PBP glycosyltransferase domains (which are related to the phage  $\lambda$ -lysozyme), the PGRPs, and the Cpl-1 endolysin, is implied.

Recent experiments suggest inclusion of the transpeptidase domain of the PBPs in this list. In collaboration with the Shoichet laboratory at the University of California, San Francisco a peptidoglycan segment (Scheme 7) occupied the active site of the *E. coli* PBP6 low-*M<sub>r</sub>* PBP when diffused into the crystalline enzyme at a catalytically suboptimal pH (Figure 4).<sup>57</sup> This peptidoglycan segment has the -D-Ala-D-Ala motif recognized by this PBP for its catalysis. In the resulting PBP6 complex, the carbonyl of the scissile D-Ala fully engages the catalytic residues of the active site. This complex strongly resembles our expectations for the Michaelis complex (preacylation complex) of a peptidoglycan with this enzyme. The inability of the crystalline PBP6 to process this peptidoglycan as a substrate likely reflects a combination of factors, including the suboptimal pH used for crystallization

relative to catalysis and the intrinsic limitation of this peptidoglycan as a substrate of PBP6 ( $k_{\text{cat}}/K_m = 20 \text{ M}^{-1} \text{ s}^{-1}$ ). A comparison of the structure of Figure 4 with the crystal structure of a penicillin-inactivated PBP5 carboxypeptidase was especially rewarding (Figure 5). In the PBP5 acyl-enzyme, the steric clash between the bridging carbon atom (between the N and S atoms of the thiazolidine ring of the ring-opened penicillin) and the position otherwise required for the catalytic water molecule (a clash anticipated previously by Silvaggi et al.<sup>47</sup>) adequately explains the molecular mechanism for  $\beta$ -lactam inactivation of the PBPs. The anticipation that other peptidoglycan-binding proteins recognize the peptidoglycan in the same conformation as is found in solution for a single peptidoglycan strand is supported by yet other recent experimental<sup>44,277</sup> and computational studies.<sup>278</sup>

### Host–Guest Chemistry of the Peptidoglycan

The definition of the bacterial peptidoglycan as an amorphous polymer, tasked with the simple (albeit essential) role of a structural scaffold and barrier, is inadequate. The solitary glycan strand in solution has an ordered conformation that is directly relevant to a host of biological roles, and it is now reasonable to believe that this conformation will continue to have relevance to the host–guest chemistry of the peptidoglycan. While the conformations of the peptidoglycan polymer is likely different in ways we cannot yet anticipate, experiments where the peptidoglycan serves as host for antibiotics (as exemplified by vancomycin) support the relevance of in vitro study of the peptidoglycan structure. Likewise, the chemical synthesis of peptidoglycan fragments having defined structure has opened a vast realm to more precisely explore peptidoglycan recognition by macromolecules. The peptidoglycan is one of the last remaining biological polymers of unknown three-dimensional structure. An extraordinary diversity of opportunities, seen in terms of the recognition chemistry of the peptidoglycan structure, are now coming within experimental reach.

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### Biographies

**Jed F. Fisher** has engaged almost the full breadth of biological chemistry (including research on antibiotics, lipids, peptides, saccharides, drug candidates, coenzymes, and enzymes) in the course of a career embracing bioorganic mechanism and medicinal chemistry. The enigma of the peptidoglycan engages this entire breadth.

**Shahriar Mobashery** received his undergraduate and graduate degrees from the University of Southern California and the University of Chicago, respectively. Following a short post-doctoral stay at the Rockefeller University, he served on the faculty at Wayne State University before relocating to the University of Notre Dame in 2003. He thrives on multidisciplinary research encompassing organic chemistry, biochemistry, molecular biology, microbiology, and computation. He has had the good fortune of working with outstanding researchers in these disciplines, within and outside his own research group.

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