Stop Building a Cell Wall

This report features the work of Che Ma and his co-workers published in Proc. Natl. Acad. Sci. USA ***109****, 6496 (2012).*

Most bacteria, such as *E. coli*, have an extra cell wall outside the cell surface.[1] This cell wall is essential to hold the shape of a bacterium, and prevents it from bursting due to osmotic pressure in an aqueous environment. This wall is made of peptide and glycan, a long and complicated sugar chain. The unit of the cell wall is a peptidoglycan containing a disaccharide -- GluNAc and MurNAc -- and a peptide stemming from the carboxyl group of MurNAc. Two enzymes on the membrane of the cell surface -- transglycosylase (TG) and transpeptidase (TP) -- harden the cell wall by cross-linking these glycans and peptides together respectively.

Discovered in 1928, penicillin inhibits the transpeptidase and disrupts the cell-wall synthesis, thus killing bacteria. Since then, various antibiotics, including ampicillin, that target the synthesis of cell walls have been discovered and developed. Most antibiotics target and inhibit transpeptidase, including beta-lactams (penicillin and methicillin), glycopeptides (vancomycin and teicoplanin) and glycolipopeptides. Those belonging to the β-lactam antibiotics are digestible with β-lactamase in bacteria. The misuse of β-lactam antibiotics unintentionally promotes the survival of bacteria harboring the gene of β-lactamase, which in turn renders the antibiotics useless. A similar situation occurs for other common antibiotics. Antibiotic resistance has hence become a major threat to public health.

Scientists now turn their attention to another enzyme involved in cell-wall synthesis, transglycosylase, and hope to find new antibiotics to target it. Transglycosylase takes the precursor of peptidoglycan, Lipid II, and cross-links the disaccharide together, forming a continuous (-GlcNAc-MurNAc-)$_n$ backbone. Transpeptidase then cross links the peptides stemming from MurNac with a neighboring peptidoglycan chain into a mesh of peptidoglycan network. In 2009, Dr. Che Ma's group solved the structure of bifunctional transglycosylase PBP1b from *E. coli*; this enzyme has both transglycosylase and transpeptidase in the same protein.[2] In 2012, his group published the structures of transglycosylase in a complex with lipid II and other chemical compounds from *Staphylococcus aureus* (SaMGT) (Fig. 1).[3] This work used beamlines **13B1** and **13C1** at NSRRC, Taiwan, and **BL44XU** at SPring-8, Japan.

Transglycosylase contains an acceptor site and a donor site. The acceptor site binds disaccharide monomer lipid II and the donor site to bind another lipid II and accommodate the growing glycan chain. Inhibitors, such as vancomycin, antibiotics ramoplanin and manno-peptimycin, target the lipid II binding of transglycosylase. Only moenomycin inhibits the donor site, but its poor pharmacokinetic properties confine its use to animals (Fig. 2).

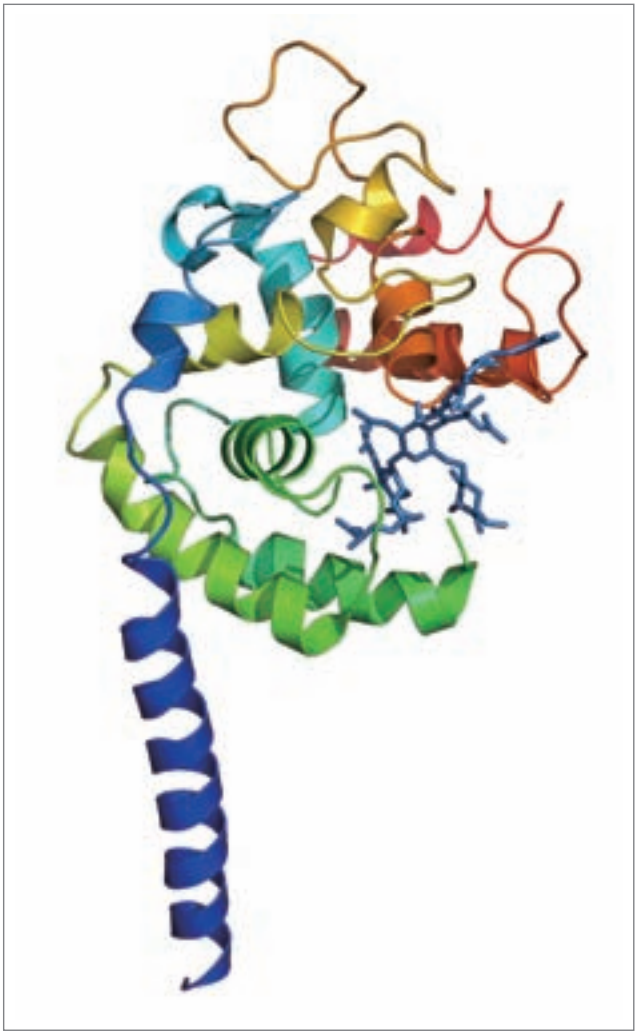

Fig. 1: Overall structure of Staphylococcus aureus transglycosylase in complex with moenomycin (cyna). A transmembrane helix (dark blue) anchor SaMGT onto membrane and is important for the catalytic activity of SaMGT, similar to what the authors found in *E. coli* PBP1b.

The work of this research group shows that the transmembrane helix of transglycosylase not only anchors the protein to the cell membrane, as previously thought, but also contributes a hydrophobic interaction to the binding of substrate Lipid II in SaMGT and antibiotic moenomycin in *E. coli* PBP1b. By comparing four structures of SaMGT bindings with lipid II analog 3, NBD-lipid II as a native substrate, moenomycin as antibiotic and SaMGT alone, these scientists concluded that residues K140 and R148, instead of the previously thought E156 at a donor site, stabilize the pyrophosphate-leaving group of lipid II. E100 in the acceptor sites acts as a general base for 4-OH of GlcNAc to facilitate the transglycosylation reaction (Fig. 3). It is important to deprotonate 4-OH of GlcNAc in lipid II. On inverting the position of 4-OH at the GlcNAc group of Lipid II to form GalNAc of analog 3, it binds more tightly with G130, but cannot serve as a substrate for E100. This property makes analog 3 a prospective candidate to develop improved Lipid II substrate-based antibiotics to inhibit transglycosylase.

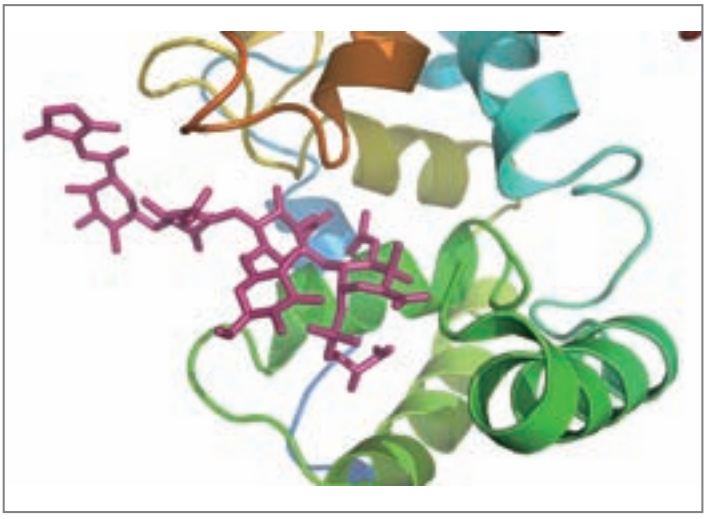

Fig. 2: A close look of binding pocket of SaMGT with moenomycin (pink).

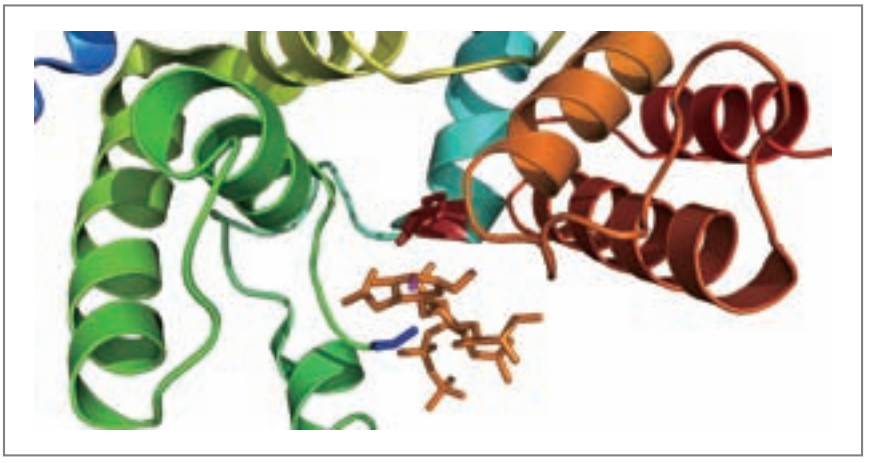

Fig. 3: Binding of Lipid II analog 3 (orange) with SaMGT. E100 from SaMGT is depicted in red, G130 in blue and 4-OH of GalNAc in pink. This structure emphasizes the importance of E100 in deprotonating 4-OH of GlcNAc and provides a good template to design better antibiotics.

References

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3. C.-Y. Huang, H.-W. Shih, L.-Y. Lin, Y.-W. Tien, T.-J.R. Cheng, W.-C. Cheng, C.-H. Wong, and C. Ma, Proc. Natl. Acad. Sci. USA **109**, 6496 (2012).