

Crystal Structure of the Membrane-bound Bifunctional Transglycosylase PBP1b from *Escherichia Coli*

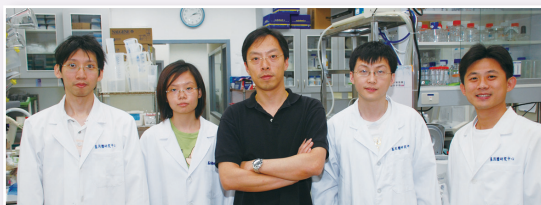
*Drug-resistant bacteria have caused serious medical problems in recent years, and the need for new antibacterial agents is undisputed. Transglycosylase, a multidomain membrane protein essential for cell wall synthesis, is an excellent target for the development of new antibiotics. Here, we determined the X-ray crystal structure of the bifunctional transglycosylase penicillin-binding protein 1b (PBP1b) from *Escherichia coli* in complex with its inhibitor moenomycin to 2.16-Å resolution. In addition to the transglycosylase and transpeptidase domains, our structure provides a complete visualization of this important antibacterial target, and reveals a domain for protein–protein interaction and a transmembrane helix domain essential for substrate binding, enzymatic activity, and membrane orientation.*

Beamlines

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From left to right: M.-T. Sung, C.-Y. Huang, C. Ma, L.-Y. Chou and Y.-T. Lai.

In the last decade, the prevalence and occasional outbreaks of drug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE), have posed appalling hurdles in the treatment of bacterial infections. New antibacterial agents are, as a result, in desperate demand to combat these pernicious antibiotic-resistant problems that can otherwise cause life-or-death struggles.

Bacteria cell wall is a mesh-like structure of cross-linked peptidoglycan, which is essential to scaffold the cytoplasmic membrane and to maintain structural integrity of the cell. Cell wall synthesis at the membrane surface is mainly carried out by the membrane-bound enzymes, transpeptidases and transglycosylases, and inhibitors of the transpeptidase are among the most popular antibiotics in clinical use today.

Escherichia coli PBP1b is a bifunctional transglycosylase, also known as peptidoglycan glycosyltransferase or murein synthase. It contains a transmembrane (TM) helix, 2 enzymatic domains [transglycosylase (TG) and transpeptidase (TP)], and a domain composed of ~100 aa residues between TM and TG with unknown structure and functionality (Fig. 1B). For >50 years, TP has been the main target for 2 most important classes of antibiotics: β -lactams (e.g., penicillin and methicillin) and glycopeptides (e.g., vancomycin). Not too long after they were introduced, resistant bacteria had emerged rapidly and caused serious medical problems. In contrast, resistant strains against moenomycin, the only natural inhibitor to TG from *Streptomyces*, have rarely been found. The development of new antibiotics against TG has been highly anticipated, and not until recently have the molecular structures of TG been available, even with the TM structure undefined.

Two crystal structures of transglycosylase, a bifunctional transglycosylase from *S. aureus* (referred to as SaPBP2) and a transglycosylase domain from *Aquifex aeolicus* (referred to as AaPGT), have been determined recently with their TM domain or TM and TP domains removed, respectively.^[1-3] These structures revealed critical interac-

tions between protein and moenomycin and served as good platforms for antibiotic development. We have previously demonstrated that the TM helix domain is important for the binding between *E. coli* PBP1b and moenomycin. In addition, we found that the full-length PBP1b also showed a substantially higher TG enzymatic activity than a TM truncated counterpart. Therefore, in this study, our purified full-length PBP1b possessing a similar enzymatic activity [k_{cat} is $3.14 \pm 0.236 \text{ s}^{-1}$, K_m is $18.3 \pm 4.05 \text{ }\mu\text{M}$ and k_{cat}/K_m is $(1.74 \pm 0.3) \times 10^5 \text{ M}^{-1}\text{s}^{-1}$] to previous studies was chosen for structure determination by X-ray crystallography.

The crystal structure of *E. coli* PBP1b in complex with moenomycin was solved at 2.16-Å resolution (Fig. 1A). Our protein construct includes amino acid residues 58–804, containing TM, an unknown domain, TG, and TP domains. By using a multiwavelength anomalous dispersion (MAD) approach with crystals from selenomethionine-labeled proteins, the phase information was obtained to generate a protein electron density map. The structure was built from residues 66–800, except 2 loop regions with absent electron density (residues 249–267 and 399–406), and was refined to good quality with R_{work} and R_{free} values of 20.6% and 25.1%, respectively.

The overall fold of the TG domain in our structure, in complex with moenomycin, is highly similar to the 2 available transglycosylase structures from SaPBP2 and AaPGT. The RMSD is 1.53 Å for 145 Ca atoms between TG domains from *E. coli* PBP1b and SaPBP2, and 1.46 Å for 143 Ca atoms between *E. coli* PBP1b and AaPGT. However, the residues involved in potential interactions with moenomycin (defined with distance cutoff of 3.2 Å) showed similarities and differences in these transglycosylase structures (Fig. 2 B and C). The resemblance between our structure and SaPBP2 may explain the observation that transglycosylases from *E. coli* and *S. aureus* share comparable binding affinity to moenomycin. In addition, the interacting residues of the TG domain around the E ring, the F ring, the phosphate group, and the carboxylate group of moenomycin are more conserved than the interacting residues with the remaining parts (Fig. 2A). The conserved interacting residues in the binding pocket of transglycosylases can be considered as the most critical region to be studied in the process of antibiotic design. Our result is in agreement with the previous findings to define the minimal pharmacophore in moenomycin, in which the EF-ring phosphoglycerate portion together with either the C or the D ring forms critical interactions with proteins.^[3] The crystal structure of *E. coli* PBP1b represents a structural platform of transglycosylase, in particular for Gram-negative bacterial pathogens, for the development of antibiotics.

In addition to the TM, TG, and TP domains that are commonly found in bifunctional transglycosylases, an unexpected domain was observed in our crystal structure (Fig. 1A). This domain, comprising residues 109–200, folds with a 5-antiparallel-stranded β -sheet ($\beta 2$ – $\beta 6$) and 1 α -helix ($\alpha 1$) and forms more interactions with the TP domain (with buried surface area of 630.17 Å²) and less interactions with the TG domain (313.01 Å²). In comparison with the structure of SaPBP2, which shows no direct interactions between the TG and TP domain, addition of this extra domain makes *E. coli* PBP1b a more compact structure. By using Dali search, this domain was found to be structurally homologous to domains in UvrB (RMSD is 1.8 Å for 82 Ca atoms, with 24% sequence identity) and TRCF (transcription-coupled repair factor) (RMSD is 1.6 Å for 82 Ca atoms, with 14% sequence identity). Based on the highly similar fold, we referred to this domain as UB2H (UvrB domain 2 homolog) domain.

The presence of the TM helix (residues 66–96) in our structure allowed us to postulate the orientation of the *E. coli* PBP1b molecule in lipid bilayers. It is commonly accepted that tryptophan and tyrosine residues have a higher frequency to be found at the lipid–water interface

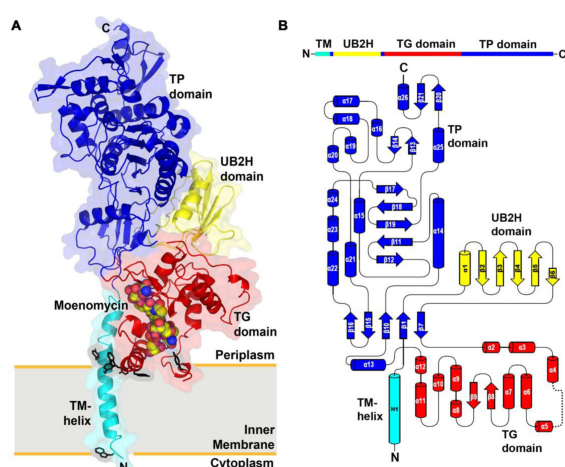


Fig. 1: (A) The crystal structure of PBP1b is represented as a ribbon diagram. The TM, UB2H, TG, and TP domains are color coded in cyan, yellow, red, and blue, respectively. Moenomycin is represented as van der Waals spheres. Tryptophan and tyrosine residues located near the water–membrane interfaces are shown in black sticks. The proposed membrane location is indicated by a gray rectangle. (B) The 1D and 2D topology of *E. coli* PBP1b are color-coded as in (A).

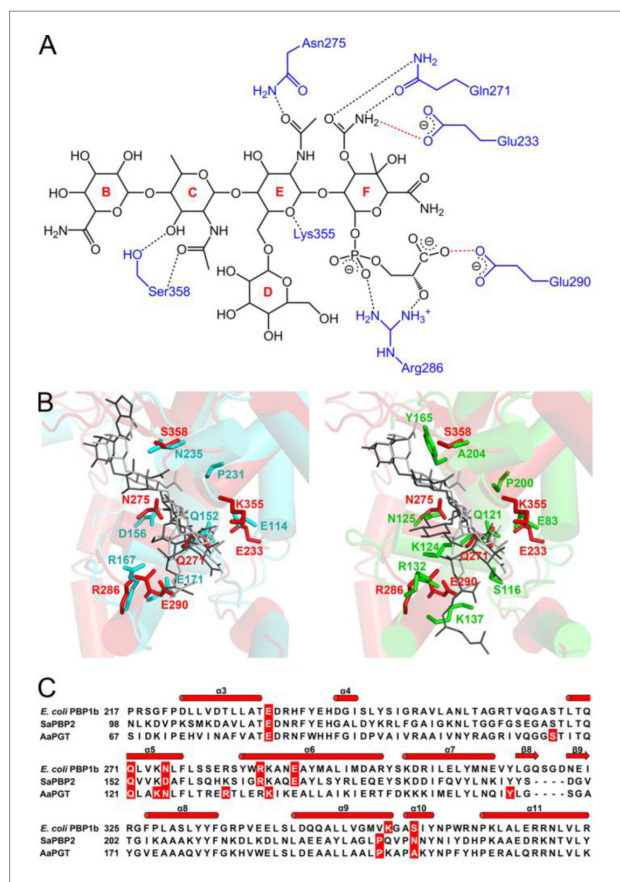


Fig. 2: (A) The potential hydrogen-bonding interactions (distance cutoff 3.2 Å) between *E. coli* PBP1b and moenomycin are shown as dashed lines in black. The interactions between the putative active sites (E233 and E290) and moenomycin are shown as dashed lines in red. (B) Comparison of moenomycin-binding modes between *E. coli* PBP1b and SaBP2 (Left); between *E. coli* PBP1b and AaPGT (Right). TG and residues of *E. coli* PBP1b, SaBP2, and AaPGT are shown in red, cyan, and green, respectively. Moenomycin are shown in light gray (for *E. coli* PBP1b) and dark gray (for SaBP2 and AaPGT). (C) Sequences of TG from *E. coli* PBP1b, SaBP2, and AaPGT are aligned according to their secondary structure elements. Residues forming the potential interaction with moenomycin are shaded in red.

in membrane proteins. We examined all plausible tryptophan and tyrosine residues in the TG domain and TM helix and found a plane consisting of tryptophan and tyrosine residues that might be associated with lipids (Fig. 1A). As a result, the established membrane orientation made the bottom of the TG domain partially embedded in lipid bilayers. Also, based on this model, the C terminus of the TM helix (residues 88–96; ~2 helical turns) is not embedded in the membrane.

To further test the validity of this membrane orientation model, we performed molecular dynamics (MD) simulations. In the MD simulations, the proposed orientation of *E. coli* PBP1b in lipid bilayers was observed to be energetically stable. The stable orientation recurred in different MD simulations strengthened our proposed model.

The *E. coli* TP domain closely resembles the corresponding region in the SaBP2 structure; however, the relative orientation between the TG and TP domains are dissimilar between our structure and SaBP2 (Fig. 3A).^[1,4] Despite the discrepancy, we considered all different orientations plausible because of the possibly inherent flexibility of a hinge region.^[4] Different crystal structures can simply represent different structural states of the bifunctional transglycosylases. The changes in the relative orienta-

tion of SaBP2 had been proposed to be correlated to the regulation of TG activity.^[4]

We have previously observed that the binding affinity of moenomycin to *E. coli* PBP1b is TM domain dependent. However, no direct interaction between moenomycin and the TM helix was observed in our crystal structure. Furthermore, removal of the TM helix does not affect the structure of TG domain in the binding site, when comparing our structure and SaBP2 in their moenomycin binding pockets (Fig. 2A). We therefore suggest that the TM helix simply stabilizes the protein–membrane interaction, and the resulting orientation limits the interaction between PBP1b and moenomycin or lipid II in the membrane in a 2D lateral diffusion fashion. Removal of TM may destabilize the protein–membrane interaction, thus affecting moenomycin or lipid II binding to TG. Indeed, stable protein–membrane interaction has been reported recently to be crucial for the normal function of some membrane proteins, and hence it has been suggested to be a target for drug discovery.

Lovering et al.^[1,4] have elegantly proposed that the moenomycin molecule in the binding site of transglycosylase structurally mimics lipid IV, the dimerized peptidoglycan from 2 molecules of lipid II, and suggested a mechanism of peptidoglycan elongation where the grow-

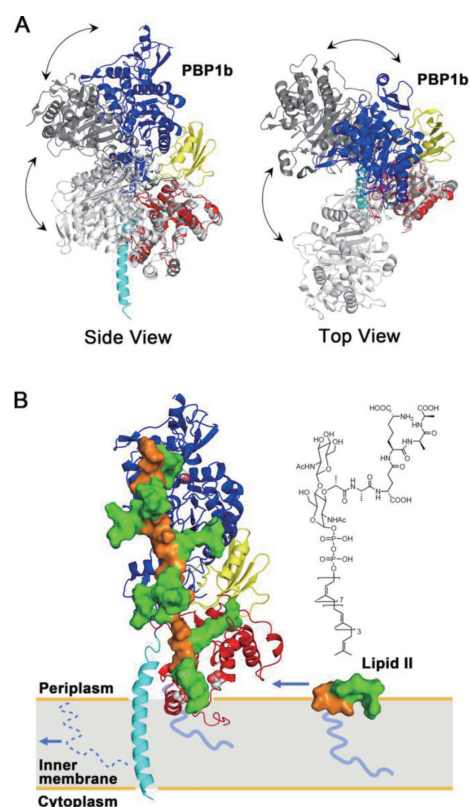


Fig. 3: (A) *E. coli* PBP1b and two *SaBP2* conformers were indicated by colored, light gray (PDB ID code 3DWK) and dark gray (PDB ID code 2OLV), respectively. The TG domain of *SaBP2* was superimposed onto the TG of *E. coli* PBP1b. Side view (Left) and top view (Right) of the comparison reveals possible flexibility of a hinge region between TG and TP domains. (B) Active sites of TG and TP were shown by van der Waals spheres. The disaccharide, pentapeptide, and lipid tail of lipid II or peptidoglycan were shown in orange surface, green surface, and blue line, respectively. A single strand of the proposed peptidoglycan model was docked onto the structure of *E. coli* PBP1b, with lipid IV portion replacing moenomycin. The incoming lipid II, of which the chemical structure is shown on top, diffuses in the plane of the membrane. After the TG reaction, the lipid moiety of this lipid II is kept as the membrane anchor, whereas the original lipid tail (shown as dotted line) is recycled. The polymerized peptidoglycan grows perpendicularly to the membrane and toward the TP domain, where the cross-linking reaction of the pentapeptides takes place.

ing glycan chain acts as an acceptor for the nucleophilic attack with lipid II as a donor. Recently, the architecture of peptidoglycan has been modeled based on the NMR structure of a lipid IV derivative. Using the proposed peptidoglycan model, we docked a single strand of the peptidoglycan onto the structure of *E. coli* PBP1b, with the lipid IV portion replacing moenomycin (Fig. 3B). We noted that the distance (65.8 Å) between the active-site residues of the TG and TP domains in our structure corresponds well to the distance (67.1 Å) between the reaction sites on the peptidoglycan. In this model, the surface of PBP1b in contact with peptidoglycan is largely composed of loops, which are possibly flexible and capable of accommodating the polymerizing peptidoglycan. Therefore, the membrane orientation of PBP1b established by the transmembrane helix implies that its product, peptidoglycan, can be synthesized perpendicularly to the membrane surface. In contrast to the conventional views that cell wall consists of layers of cross-linked peptidoglycans with their glycan backbones lying parallel to the membrane surface, our structure and model suggest the possibility of vertical orientation of peptidoglycans at the membrane surface, at least when they are initially synthesized.

Experimental Station

Protein crystallography end station

Publications

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