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Natural Products at Work: Structural Insights into Inhibition of the Bacterial Membrane Protein MraY

*Stefan Koppermann and Christian Ducho**

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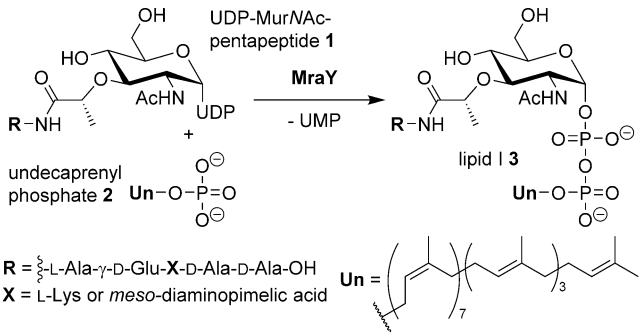
Bacterial resistance towards established antibiotics continues to emerge and represents an increasing threat to human health.[1] Consequently, there is a significant need for novel antibacterial agents with either new or previously unexploited modes of action. Since the development of penicillins in the 1940s, the formation of the bacterial cell wall (i.e. peptidoglycan biosynthesis) has served as a useful source for antimicrobial drug targets.[2] However, nearly all established antibiotics that block peptidoglycan biosynthesis target the late extracellular steps. In contrast, the early intracellular or membrane-associated steps of peptidoglycan formation have only found limited attention in the development of antimicrobial drugs.

One key step in the intracellular section of peptidoglycan biosynthesis is the association of the soluble cytosolic precursor UDP-Mur*N*Ac pentapeptide ("Park's nucleotide", **1**) with the cellular membrane. The reaction of **1** with the lipophilic membrane anchor undecaprenyl phosphate (**2**) affords the membrane-associated intermediate lipid I (**3**, Scheme 1). This reaction is catalyzed by the integral membrane protein MraY (translocase I), which is discussed as a potential novel drug target for antibacterial agents.[3] The MraY enzyme is a member of the PNPT (polyprenyl-phosphate *N*-acetylhexosamine 1-phosphate transferase) superfamily that also plays an important role in protein glycosylation. MraY is inhibited by naturally occurring uridine-derived secondary metabolites commonly referred to as "nucleoside antibiotics", including structurally distinct subclasses such as muraymycins, caprazamycins, liposidomycins, capuramycins, and mureidomycins. Their potential as MraY inhibitors results in nucleoside antibiotics and their synthetic analogues being promising lead structures for drug development.[4]



Scheme 1. Reaction catalyzed by the bacterial membrane protein MraY. UDP = uridine diphosphate, UMP = uridine monophosphate.

A topology model predicted MraY to be comprised of ten transmembrane helices and five cytoplasmic loops forming the active site.[5] Mechanistic studies on MraY, including site-directed mutagenesis, were also reported, but have not yet furnished a universally accepted molecular mechanism of MraY catalysis.[6] MraY homologues from different bacteria were heterologously overexpressed, including methods for cell-free expression of the protein.[7] A remarkable breakthrough was achieved in 2013 when the first X-ray crystal structure of an MraY homologue, in this case from the extremophile *Aquifex aeolicus*, was reported by Lee and co-workers, which confirmed several key features of the topology model.[8] However, this X-ray crystal structure has not yet provided any insights into MraY inhibition by nucleoside antibiotics, thus limiting its impact on the development of MraY inhibitors.

This current state-of-the-art has been significantly advanced by another recent publication of Lee and co-workers.[9] They have now obtained the first X-ray crystal structure of MraY (again from *Aquifex aeolicus*) in complex with an inhibitor, in this case the natural product muraymycin D2 (MD2, **4**; Figure 1), at 2.95 Å resolution. Just like the ligand-free apoenzyme, the MraY–MD2 complex crystallized as a dimer, with each monomer consisting of ten transmembrane helices (TM1–10) and five intracellular loops (A–E). The active site of MraY is located at the cytosolic side of the cellular membrane (Figure 1 A,B). Previous studies had revealed three amino acid residues (Asp117, Asp118, and Asp265) to be crucial for catalytic activity.[6] These residues are highly conserved within the PNPT superfamily of enzymes, but MD2 does not interact with any of them. Instead, the MraY protein undergoes significant conformational changes upon ligand binding, with several residues (i.e.

[*] S. Koppermann, Prof. C. Ducho
Department of Pharmacy, Pharmaceutical and Medicinal Chemistry
Saarland University, Campus C2 3, 66123 Saarbrücken (Germany)
E-mail: christian.ducho@uni-saarland.de
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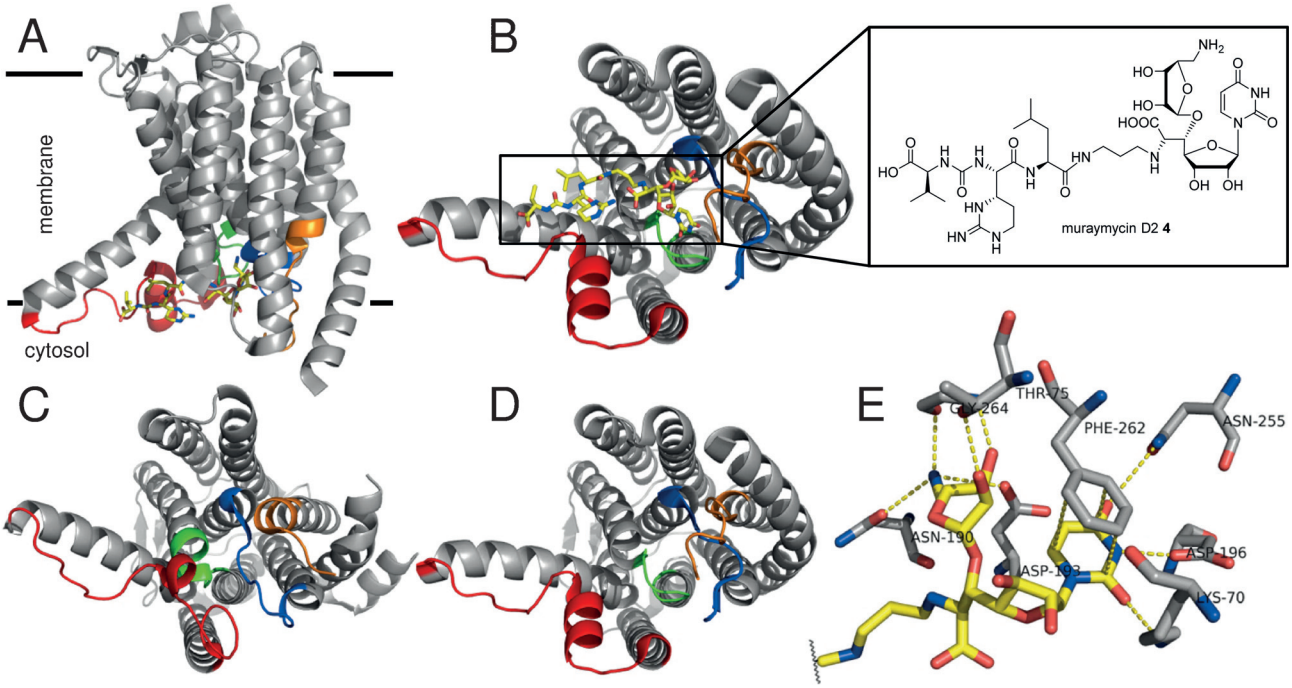

Figure 1. A) X-ray crystal structure of MraY in complex with muraymycin D2 (MD2, **4**; PDB 5CKR)[9] and its schematically represented position in the cellular membrane. The backbone of the MD2 ligand is shown in yellow. B) View of the MraY–MD2 complex from the cytosolic side of the protein as well as the structure of MD2. C) X-ray crystal structure of the ligand-free apoprotein (PDB 4J72),[8] view from the cytosolic side. D) MraY–MD2 complex[9] without the ligand, view from the cytosolic side. To bind MD2, MraY undergoes significant conformational changes, which are illustrated by direct comparison of (C) and (D). Parts of the protein which move long distances upon ligand binding are highlighted in color. E) Example of molecular insight into the MraY–MD2 complex: interactions of the 5′-*O*-aminoribosylated uridine-derived core structure of MD2 with the protein.

the uracil-binding moieties) moving distances of 5–17 Å (Figure 1 C,D). The rearranged loops C and D contribute to the formation of a pocket to bind the uracil base and the 5′-*O*-aminoribosyl moiety of MD2. Helix TM9 shows a kink and is, therefore, divided into two parts: TM9a and TM9b. TM9b rotates away from the active site, thereby inducing a conformational shift of loop E which leads to an opened structure for binding of the peptide part of MD2. A video produced by the Lee group (available online together with their publication)[9] impressively illustrates these conformational rearrangements. This conformational plasticity of MraY underlines that insights into MraY inhibition can hardly be derived from the structure of the ligand-free apoprotein.[8] Furthermore, it should be noted that the X-ray crystal structure of the MraY–MD2 complex also reveals very detailed molecular insights into the interaction of the inhibitor with the protein (Figure 1 E).

Lee and co-workers also performed mutagenesis studies and isothermal titration calorimetry (ITC) experiments with the resultant mutants to further elucidate MD2 binding affinities. Remarkably, some mutations (e.g. Asp193Asn) resulted in functional proteins with low or no affinity for MD2. This suggests that the natural substrate **1** and MD2 **4** employ different modes of MraY binding, even though MD2 acts as a competitive inhibitor. Overall, it was demonstrated that MD2 showed no interaction with several catalytically crucial residues and that the 5′-*O*-aminoribosyl moiety of MD2 does not act as a pyrophosphate mimic. Although Mg^{2+} (as a potential phosphate-binding entity) is essential for the catalytic activity of MraY, it turned out to have no effect on MD2 binding. In the development of inhibitors of PNPT enzymes such as MraY, the molecular imitation of pyrophosphates represents a severe challenge to medicinal chemistry with respect to cellular uptake and target affinity. The discussed structure of the MraY–MD2 complex strikingly demonstrates how nature has overcome this hurdle.

In conclusion, the recently published first X-ray crystal structure of MraY (from *Aquifex aeolicus*) in complex with the nucleoside antibiotic muraymycin D2[9] represents a milestone in research on this bacterial membrane protein and its naturally occurring inhibitors. Both the revealed conformational plasticity of MraY and the molecular details of the MraY–MD2 interaction will enable a much more rational future design of MraY inhibitors. Thus, it can be expected that the development of MraY-inhibiting antibacterial drug candidates will greatly benefit from these recent advances.

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