Vincent Kim

Professor: Nikki Lewis

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Compare and contrast mode of action of penicillin and vancomycin: Why penicillin is still an effective antibiotic today

Abstract

Penicillin is a group of antibiotics that contains β-lactam, which prevents peptidoglycan crosslinking and indirectly bursts bacterial cell walls. It is widely used today against many infections caused by *staphylococci* and *streptococci* bacteria. Although antibiotics were effective at treating disease in the early development of these treatments, the late 20th century has seen an increase in antibiotic resistance. However, penicillin-derived antibiotics are still used today through generations and we see fewer cases of resistance to this antibiotic. Understanding the interactions between penicillin and bacterial proteins would be useful for studies on counteracting antibiotic resistance. Other antibiotic called Vancomycin was compared with penicillin because vancomycin resistance is arising in late 20th century like Vancomycin-resistant Enterococcus. Computational methods were used to propose interactions between 6I1E and comparable ligands to understand what the mode of action of penicillin is. It was found that SER294 likely interacts with the carboxylic acid functional group. Additionally, assessment of vancomycin resistance provided a case study for understanding how resistance happens. Comparison of interactions between ligands and residue suggested that GLN67 and ALA88 were the key residues and mutations from Δ110 to 115 showed the significant loss of activity against

substrate. This paper highlighted that each antibiotic reacts with hydrogen bond interaction between ligand and residues. In penicillin, amoxicillin and carbenicillin interacted through hydrogen bond. In vancomycin, it likely interacts through hydrogen bonding in D-Ala-D-Ala. Further steps would be choosing antibiotics that work through the same function as penicillin and comparing the structural differences and ligand interactions.

Introduction

Antibiotics are known as antibacterial substances that kill, destroy, or slow down the growth of bacteria which used to treat diseases. The modern-day antibiotic began to be used in 1936 and still used powerful, life-saving medications for people. There are multiple types of antibiotics such as penicillin, tetracyclines and vancomycin.

Antibiotics work against bacteria in three ways: attacking the cell wall of the bacteria, interfering the bacteria reproduction, or blocking protein production in bacteria. In the cell wall of bacteria, penicillin prevents the bacteria synthesizing a molecule that needs to survive in the human body. It kills bacteria through binding of the beta-lactam ring to DD-transpeptidase. Another antibiotic tetracycline blocks the bacteria's growth and reproduction. When antibiotics had been prescribed for the first time, they worked efficiently so that most bacteria were killed or stopped growing. However, antibiotics have been used for a long time with frequently prescribed bacteria that were able to gain resistance by reducing the effectiveness of drugs and chemicals that are designed to prevent infections. This process is called antibiotic resistance. The cause of antibiotic resistance was coming from using antibiotics to patients and development of immunology. Every time a patient takes antibiotics, sensitive bacteria are killed but some resistant bacteria are leftover and grow. Therefore, as different kinds of antibiotics are produced,

the bacteria evolve at the same time to get a resistance. The further study of antibiotic resistance research is going on with different types of antibiotics (NIH, 2020).

Fortunately, one of the antibiotics that is still effective is penicillin, which is the first antibiotic discovered by Alexander Fleming in 1928. The basic chemical structure consists of four-membered β-lactam ring, which is essential for structural moiety for penicillin antibacterial activity, thiazolidine ring, 6-aminopenicillanic acid side chain, and N-acyl group. If the β-lactam ring alternates its ring structure, it forms penicilloic acid and antibacterial activity is lost. Therefore, it is important to maintain the β-lactam ring. The side chain varies derivatives of penicillin: penicillin V, penicillin G, and so on. The function of penicillin is to inhibit bacterial cell wall synthesis stage 3 and interact with penicillin binding protein to lead bacterial lysis. The PBP which is called penicillin binding protein has binding affinity of the target site which allows penicillin to bind more tightly to bacteria. There are different types of PBPs (1a, 1b, 2, 3, 3a/3x, 4, 5/6, 7). In this study, the penicillin binding protein 3 was chosen out of all PBPs because the *Pseudomonas aeruginosa* cell growth only occurred when protein was expressed on integrated plasmid. In addition, the deletion of PBP3 caused the defect in cell division (Chen, Zhang, et al. 2017).

In this study, PBP ID 6I1E (Bellini, et al. 2019) which contains crystal structure of penicillin binding protein 3 from *Pseudomonas aeruginosa* with amoxicillin was selected as the structural and AXL ligand was selected to do re-docking. The PDB ID 3OCL (Sainsbury, et al. 2010) which contains crystal structure of penicillin binding protein 3 from *Pseudomonas aeruginosa* with carbenicillin was selected as the structural model and CB9 ligand was selected to do cross-docking. 2D structures of penicillin, amoxicillin, and carbenicillin represented the functional group comparison (Figure 1).

To know the penicillin's effectiveness as an antibiotic, another antibiotic was compared with penicillin. The vancomycin was chosen because it has the same function with penicillin that inhibits cell wall synthesis stage 3. Vancomycin is an antiamphoteric (react both acid and base) glycopeptide antibiotic produced by *Amycolatopsis orientalis*. It is important to treat the threat of *Clostridium difficile* colitis, but antibiotic resistance occurred to certain gram-positive bacteria that were once susceptible to vancomycin. It consists of the structure of the seven-membered peptide chain, which were three phenylglycine systems, two chlorinated tyrosine, aspartic acid, and N-methyl leucine (Figure 2). The vancomycin structure has been related to hydrogen bonding between antibiotic and D-alanyl-D-alanine carboxyl terminus, which provides the mode of the vancomycin.

Vancomycin targets the peptidoglycan cell wall of Gram-positive bacteria by binding to the D-alanine-D-alanine residue of peptidoglycan precursor and preventing their crosslinking (Holliday and Hornby). This leads to cell lysis. However, in past decades, the vancomycin resistance was raised by nine types of operons that replaced D-Ala-D-Ala terminus of peptidoglycan to D-Ala-D-lactate specifically (Van A, Van B, Van D, and Van M) or D-Ala-D-serine specifically (van C, Van E, Van G, Van L, Van N). For example, many papers have been published that the switching of D-Ala-D-Ala to D-Ala-D-Lac results in loss of hydrogen bonding interaction and loss of 1,000-fold reduction of vancomycin-binding affinity. The cause of D-Ala-D-Lac comes from transposable element Tn1546 also called jumping genes of three structural Van H, Van A, and VanX (Reynolds, et al. 1994). Transposable element is a repetitive DNA sequence that can change the position within the genome. It can create or reverse DNA mutations. There is also an effect of the D-Ala-D-Ser precursor that reduces the binding affinity (Lebreton, et al. 2011).

In this study, the PBP ID 4MUR which contains crystal structure of vancomycin resistance D, D-dipeptidase/D, D-pentapeptides VanXYc D59s mutant was selected which does not contain D-Ala-D-Ala residue. 4MUT which contains vancomycin resistance D, D-dipeptidase/D, D-pentapeptides Van XY_c D59S mutant in complex with DAL (D-Alanine) was selected. The PDB ID 4OAK which contains vancomycin resistance D, D-dipeptidase/D, D-pentapeptides Van XY_c D59S mutant in complex with D-Alanine-D-Alanine and copper (II) with (DAL) was selected (Figure 3) (Mezaine-Cherif, Stogios, et al. 2014). A comparison of the 4MUT and 4OAK structures would show where differences in protein-ligand interactions occur, and provide information about the purpose for the second D-Ala residue. The D59S mutation means that aspartic acid amino acid has been switched to serine at residue position 59. The wild type protein structure was not successfully crystallized and, therefore, unavailable. Furthermore, the single location mutation changes K_{cat}, K_m, and K_{cat}/K_m values, and understanding how other changes in the active site of D-ala-D-ala influence resistance is important.

This paper discusses the mechanism of the action of penicillin by examining the derivatives amoxicillin and carbenicillin with computational methods. In addition, the mechanism for vancomycin resistance was evaluated by exploring the structure of C terminal end of D-Ala-D-Ala. Finally, it brings up a reason why penicillin is still an effective antibiotic in comparison to vancomycin.

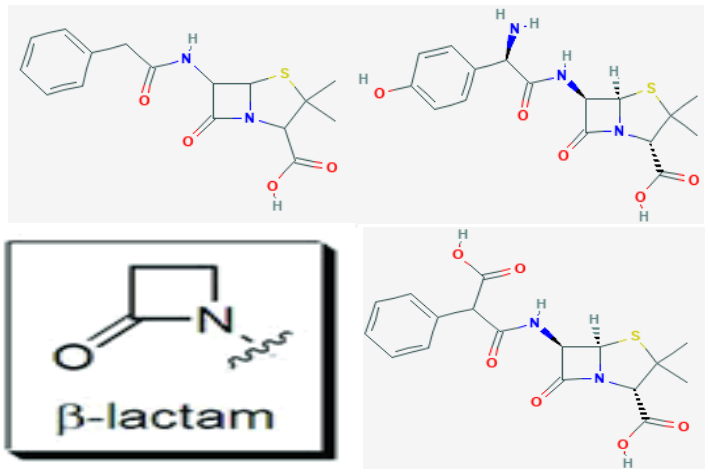

Figure 1. 2D structures of antibiotics. From left to right on the top, structure of penicillin and amoxicillin. From left to right on the bottom, ß-lactam ring, and structure of carbenicillin.

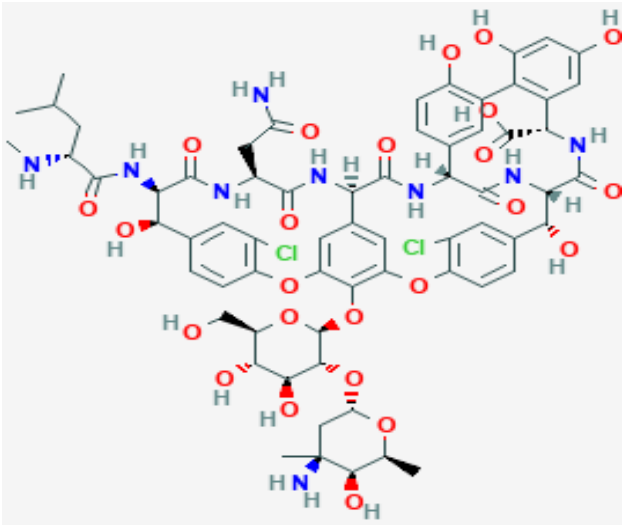

Figure 2. 2D structure of vancomycin

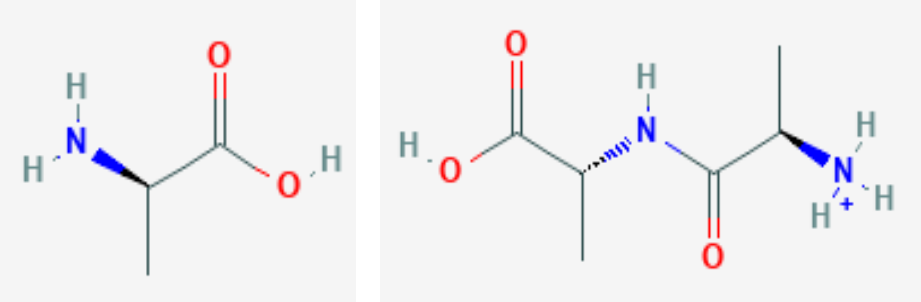

Figure 3. 2D structure of D-Alaine (left) and D-Ala-D-Ala (right). DAL (D-Alanine), NH2 is interaction with the GLU153 and Zinc. D-Ala-D-Ala, NH2 Interact with ASP102. Oxygen double bond interact with ALA88.

Methods

Preparation of initial structure models

UCSF CHIMERA (Pettersen, et al. 2004) is a program used to and analyze molecular structures. The software includes functions for sequence alignments, structural visualization of protein-ligand interactions, electronic density mapping, and visualization of docking results. For this study, CHIMERA was used mostly for the assessment of docking results and structural differences for the proteins of interest. The PDB file for 6I1E was downloaded from the RCSB website. The 6I1E and amoxicillin (ligand ID AXL) were separated and saved as individual PDB files. Hydrogen atoms were added to both the protein and the AXL ligand. A second PDB structure, 3OCL, that contained a different penicillin derivative, carbenicillin (ligand ID CB9) was download from the RCSB website. This ligand served as a secondary control test for the 6I1E structure model to assess the ability of the docking program to identify binding modes for other penicillin-like compounds.

Docking procedure

AutoDock Tools 1.5.6 (ADT) (Morris, et al. 2009) was used to add charges to the ligand and protein structure models. The charged PDB files were saved as PDBQT files and used as the starting structures for conducting docking with AutoDock Vina (Trott and Olson, 2010). The grid parameters were also set using ADT. The grid box was set to cover most of the protein to identify the potential location of the ligand binding site. AutoDock Vina was used to screen for binding modes of AXL and CB9 within the 6I1E structure model.

Redocking and cross docking procedure

Re-docking shows the ability of the docking program to reproduce the position of a co-crystallized ligand within its native crystal structure. This test determines if an appropriate search area was set for docking. In this study, the docking of AXL into the 6I1E structure model was the re-docking step. Cross-docking allows for the testing of other ligands with known crystal structure orientations within the selected structure model. The CB9 ligand from 3OCL was used for this test, which determines if the structure model and docking parameters will work for ligand other than the co-crystalized amoxicillin natively found within 6I1E.

For all docking, grid boxes were identified for the search space within the binding cavity. The grid box provides limitations to the docking software to make finding the lowest energy docked pose faster and easier. For redocking, the grid box was set to 38 Å by 38 Å by 38 Å, with the center at (-7.541, 34.097, -17.167), and grid spacing of 1.0 Å, which yielded 59,319 grid points and a total grid volume of 54,872 $Å^3$. For cross docking, the grid box for docking of CB9 was set to 34 Å by 30 Å by 32 Å, the center at (-7.541, 34.097, -17.167), and grid spacing of 1.0 Å, which yielded 35,805 grid points and a total grid volume of 54,872 $Å^3$. The two grid boxes were used because each box represented for searching the binding cavity of the ligand.

Re-docking and cross docking analysis

The docked poses of both AXL and CB9 were compared to the original PBP3 crystal structures of 6I1E and 3OCL, respectively. The PDB structures were superimposed with 6I1E as the reference for the process so that the comparison could be made in the same coordinate space. This also meant that the similar grid boxes could be used for docking of both ligands relative to the 6I1E structure model (Figure 4).

When evaluating the docked poses, the calculated free energy of binding provides an estimate of how well a ligand binds to the receptor. In principle, the higher the negative number, the greater binding affinity of a ligand for the receptor. There were nine poses generated by AutoDock Vina for AXL and CB9, each with a free energy of binding value measured in kilocalories per mole (kcal/mol). The Ligand Explorer tool on the RCSB website (https://www.rcsb.org/pdb/) was used to identify the ligand and residue interactions between AXL and 6I1E. The ARG489, TYR409, SER485, and SER294 residues potentially interacted with AXL based on this assessment. Two types of protein-ligand interactions were identified: direct and indirect interactions. Direct interactions occurred between a residue and the ligand without a water molecule in between and indirect interactions occurred between a residue and a ligand with water serving as the connection for the interaction.

To analyze the ligand from different crystal structure vs original crystal structure, the potential ligand CB9 from 3OCL was compared with 6I1E. The Ligand Explore tool on RCSB was used to identify the ligand and residue interaction between CB9 and 6I1E. The TYR409, ASN351, SER294, SER349, SER485, THR87, and GLY533 residues potentially interacted with CB9 based on the assessment.

Structural difference visualization procedure

The development of vancomycin resistance was used as a way to identify the effect of structural change the development of bacteria cell walls. The PDB structures used for structural assessment were 4MUR, 4OAK, and 4MUT, which were all developed by the same group for the same research study. The PDB structures were downloaded from the RCSB website. The 4OAK structure contained D-Ala-D-Ala residues, which allowed for visualization of the key differences that arise from the absence of D-Ala-D-Ala in 4MUR. Next, 4OAK and 4MUT were compared to find the key differences between absence and presence of a single D-Ala residue to see whether the different types of bond or residue were involved.

Results

2D structure comparison penicillin, amoxicillin, and carbenicillin

Amoxicillin and carbenicillin are derivatives of penicillin. When the 2D structures are compared, the amoxicillin has an OH group at the end of a benzyl ring, which is also called a phenol ring and amine group. The phenol group has the ability to disable the linkage of bacterial enzymes that are needed for bacterial cell wall synthesis. Carbenicillin has a carboxylic acid group and benzene ring instead of the amine and phenol groups. The carboxylic acid group serves as an electron withdrawing group and makes the compound more acidic because of less nucleophilic of amide oxygen. This characteristic helped to interact with D, D-dipeptide substrate with PBP-Ser complex for cross-linked peptide (University of York).

Re-docking and cross docking component

Docked poses for AXL and CB9 showed similarity to the original co-crystallized ligands which implied successful re-docking and cross docking. Also, the docking software was able to

reproduce the original ligand orientation. For the redocking, the affinity was -7.3 kcal/mol. The second mode was used to represent redocking because it had a pose of re-docked ligand closest to the original ligand although first pose had the highest ligand affinity (Figure 4). Cross docking showed the affinity binding was -6.6 kcal/mol. The fourth mode was used to represent cross docking with the same reason as docking (Figure 5).

The docked poses for the control ligands suggested that binding interactions occur between the ligands and SER294. The SER294 directly interacted at the end of the hydroxyl group of both ligands, which makes it a key residue. Structurally, the carboxylic acid of the thiazolidine group is important. It is usually deprotonated and stays negatively charged. The negative charge allows it to bind to the positively charged amino acid within the active site of the transpeptidase enzyme. Although the serine is not a positively charged amino acid, it possesses polarity, which allows it to interact with the carboxylic acid (Inglis, et al. 2009). Transpeptidase is the enzyme that drives cell wall synthesis in bacteria and penicillin binds to inhibit it. The re-docking and cross-docking results agree with the mode of action amoxicillin and carbenicillin use to inhibit bacterial cell wall synthesis. Therefore, the docked result suggested that the interaction carboxylic acid functional group and hydroxyl group from serine serves as a key point of mechanism and leads to find out how penicillin works in bacteria cell.

Structural difference visualization analysis

The comparison of the 4MUR and 4OAK structures showed the vancomycin affinity of D-Ala-D-Ala residue (Figure 6). The presence of D-Ala-D-Ala residue gives high affinity precursor for vancomycin binding. 4OAK is characterized by six bond interactions. The six-hydrogen bond interaction were GLN67, ALA88, SER93, GLU153, ASP102, and PHE86. These residues directly interacted with DAL ligand. The residues HIS156 and HIS95 interacted with

the copper (II) ion serving as a source of binding stability. This led to more folding and more catalytic efficiency. The carboxylates of D-Ala-D-Ala occupied with the active site by interaction. Copper (II) ion served as cofactor for the adjusting chemical reaction response (Figure 7).

The comparing 4OAK and 4MUT showed the difference of the single D-Ala residue. It had five bond interactions on 4MUT (Figure 8). The residues were involved SER93, GLN67, GLU153, and ALA88. All four ligand are directly interacted. The HIS156 and HIS95 were involved with zinc (II) ions. The zinc (II) ion served as cofactor. In both structures' residues, the GLN67, ALA88, SER93, and GLU153 were in common. To tell that these residues serve as key residue in 4OAK and 4MUT, the mutation on these amino acids were performed to know substrate-binding affinity (Mezaine-Cherif, et al. 2014).

Structural analysis of 4MUT, 4MUR, and 4OAK

GLN67 and ALA88 were in common for D-Ala and interacted with ligand. The two out of four residues were reported as key residues by doing missense mutations, where single nucleotide changes caused the codons to code different amino acid. The GLU153 was changed to alanine. However, there was no change in binding. However, it was known that GLU153 had the role of leaving amino group of D-Ala after hydrolysis on 4MUT. The SER93 was not analyzed that the data was not given. The first mutation was happened changing GLN67 to serine. In other words, the nonpolar amino acid was converted to polar amino acid. The K_m (mM) increased from 0.61 ± 0.18 (mM) to 1.23 ± 0.24 (mM) which showed less affinity to the binding to the ligand. In this case, the polarity affected the binding cavity specifically hydrogen bond. The second mutation was happened changing ALA88 to aspartate. In other words, nonpolar amino acid converted to polar amino acid. The K_m (mM) increased from 1.28 ± 0.20 (mM) to 13.80 ± 4.5

(mM). The polarity also affected the binding cavity to decrease the substrate binding. These suggests that the ALA88 and GLN67 were key residue for the maintaining interaction with ligand. Also, VanXYc Δ110 to 115 mutants was done to see whether these residues were key residues or not. The result was that the mutation caused to lose the binding activity to substrate. These structural analyses come from previous paper (Mezaine-Cherif, et al. 2014).

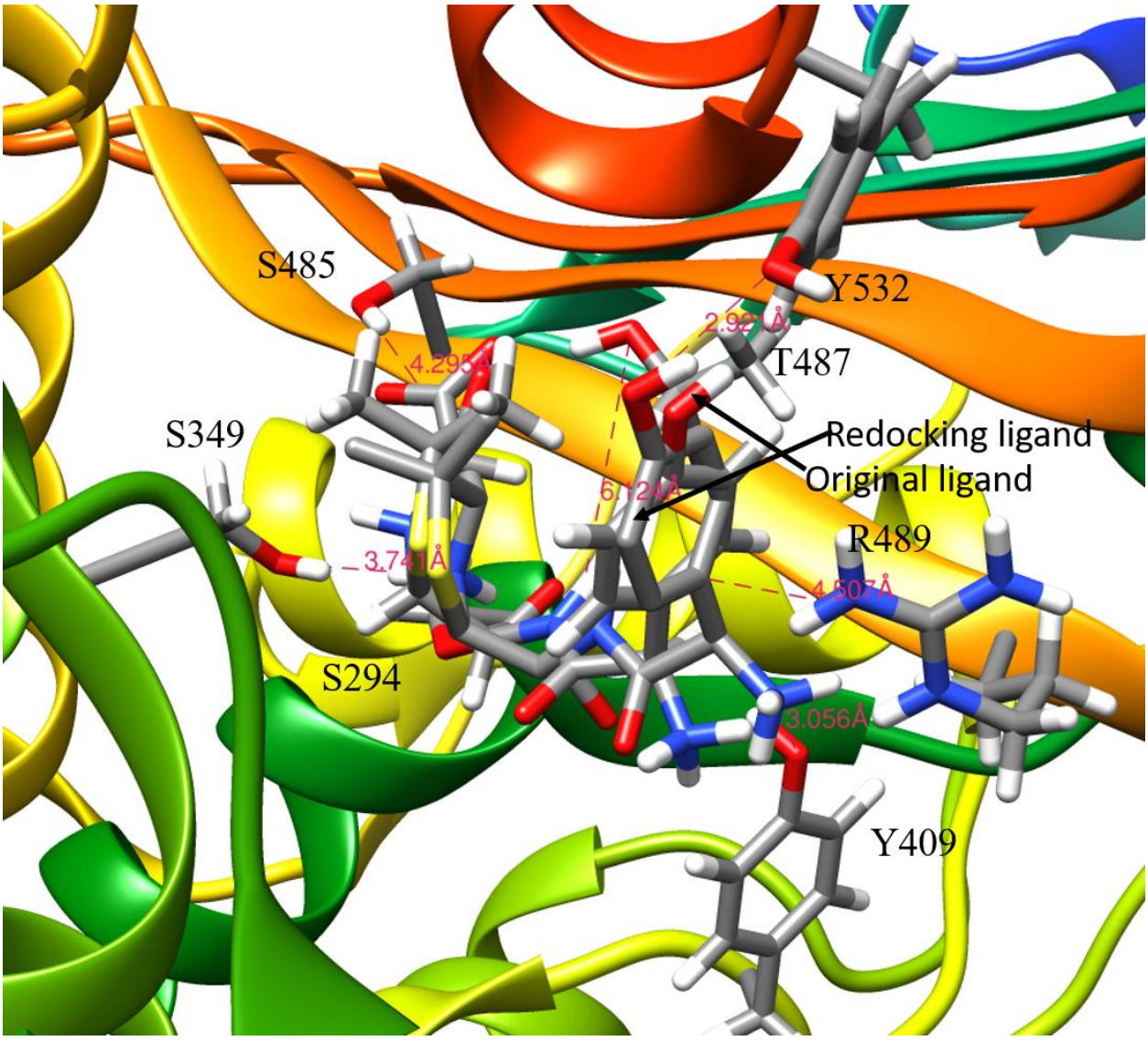

Figure 4. AXL ligand from 6I1E macromolecule redocking. Residues that are involved with AXL ligand were labeled. AXL of original and redocked ligand were labeled as arrow. Atom-specific coloring: dark gray = carbon, red = oxygen, blue = nitrogen, white = hydrogen bond

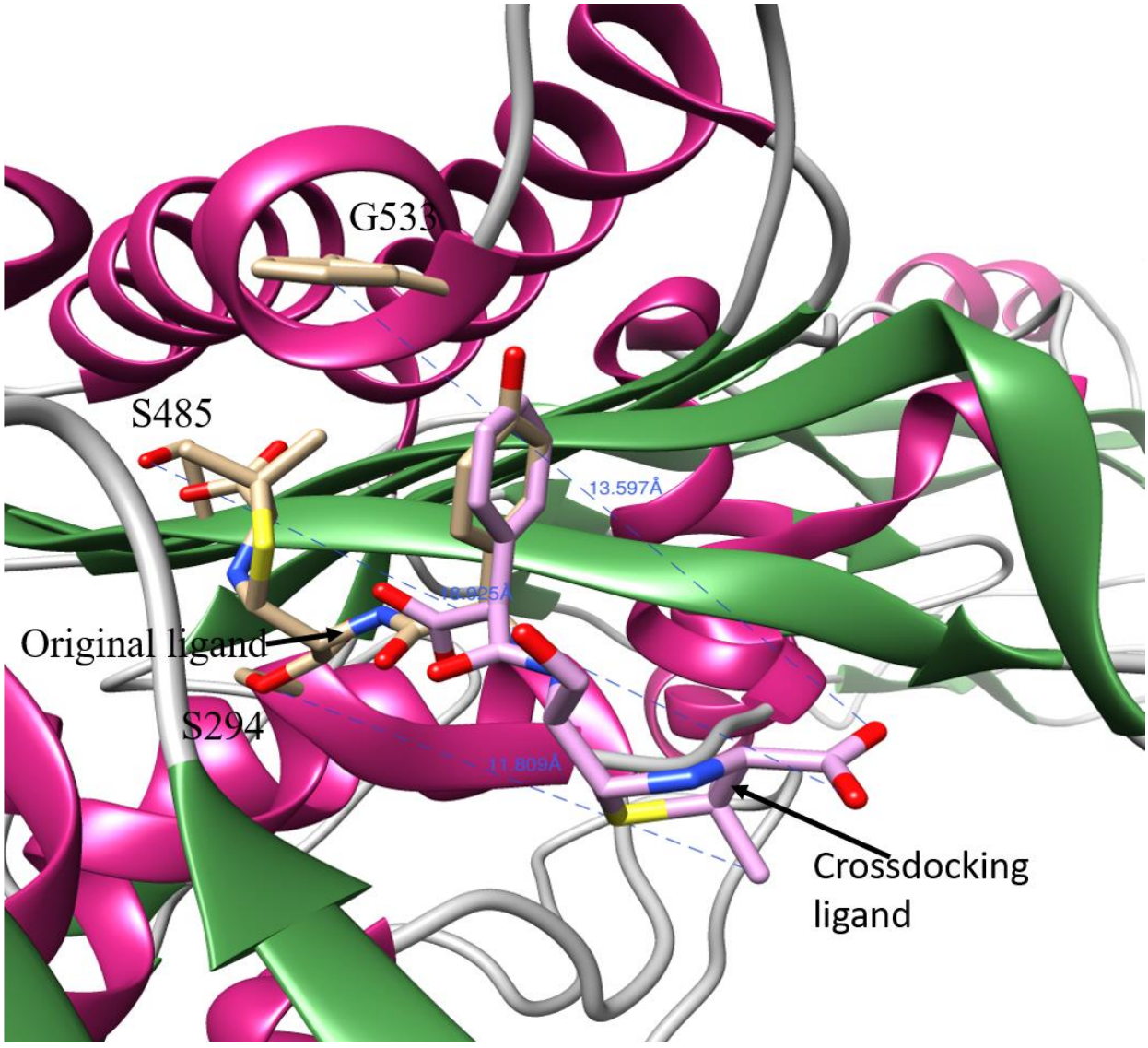

Figure 5. Crossdocking CB9 ligand with 6I1E residues. Predicted CB9 ligand (pink) cross dock and comparing with AXL ligand (light brown). Residues that are involved with with CB9 ligand were labeled. Atom-specific coloring: pink/light brown = carbon, red = oxygen pink, blue = nitrogen, yellow = sulfur, white = hydrogen. Distance was labeled.

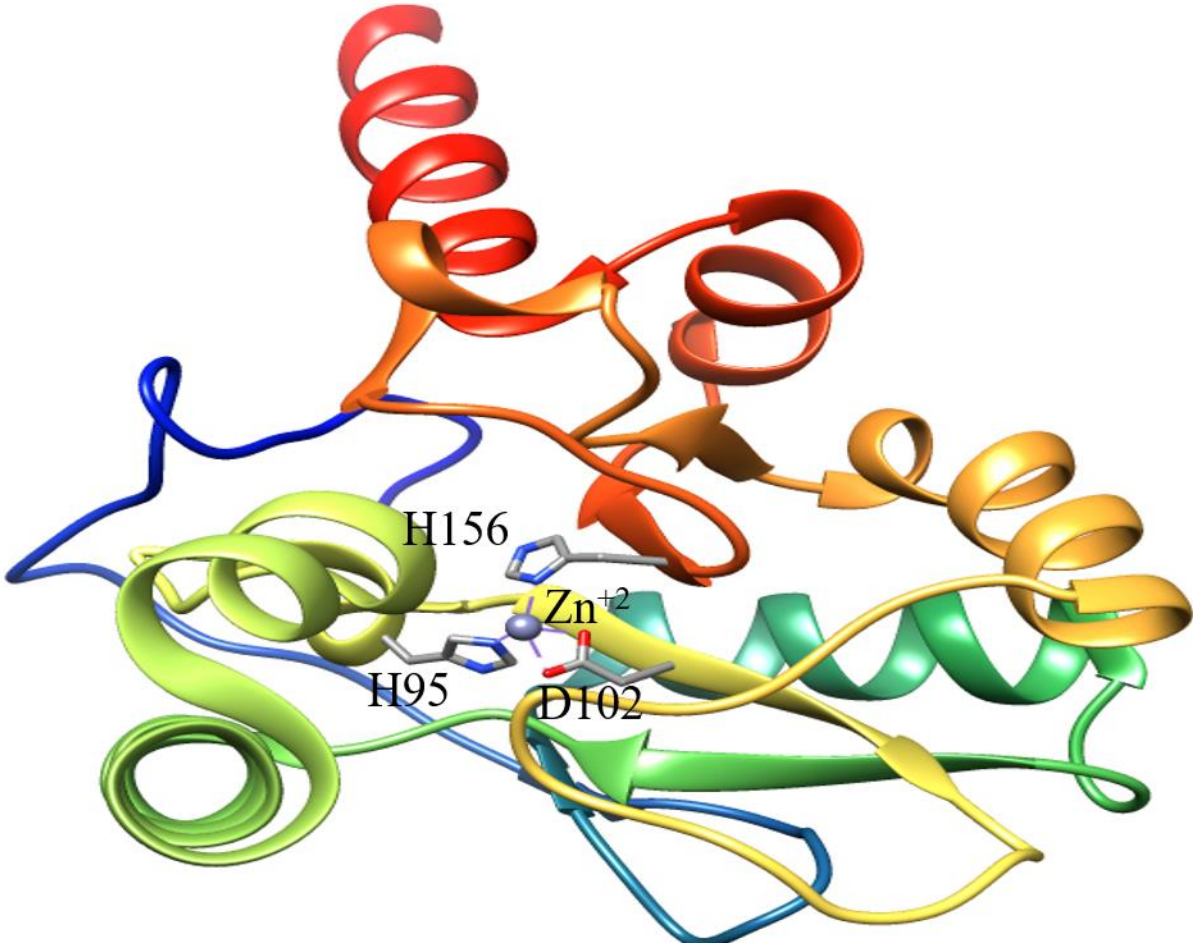

Figure 6. 4MUR without D-Ala-D-Ala. HIS156, HIS95, and ASP102 involved in with Zinc (II) for interaction. Atom-specific coloring: gray = carbon, red = oxygen, blue = nitrogen, white = hydrogen.

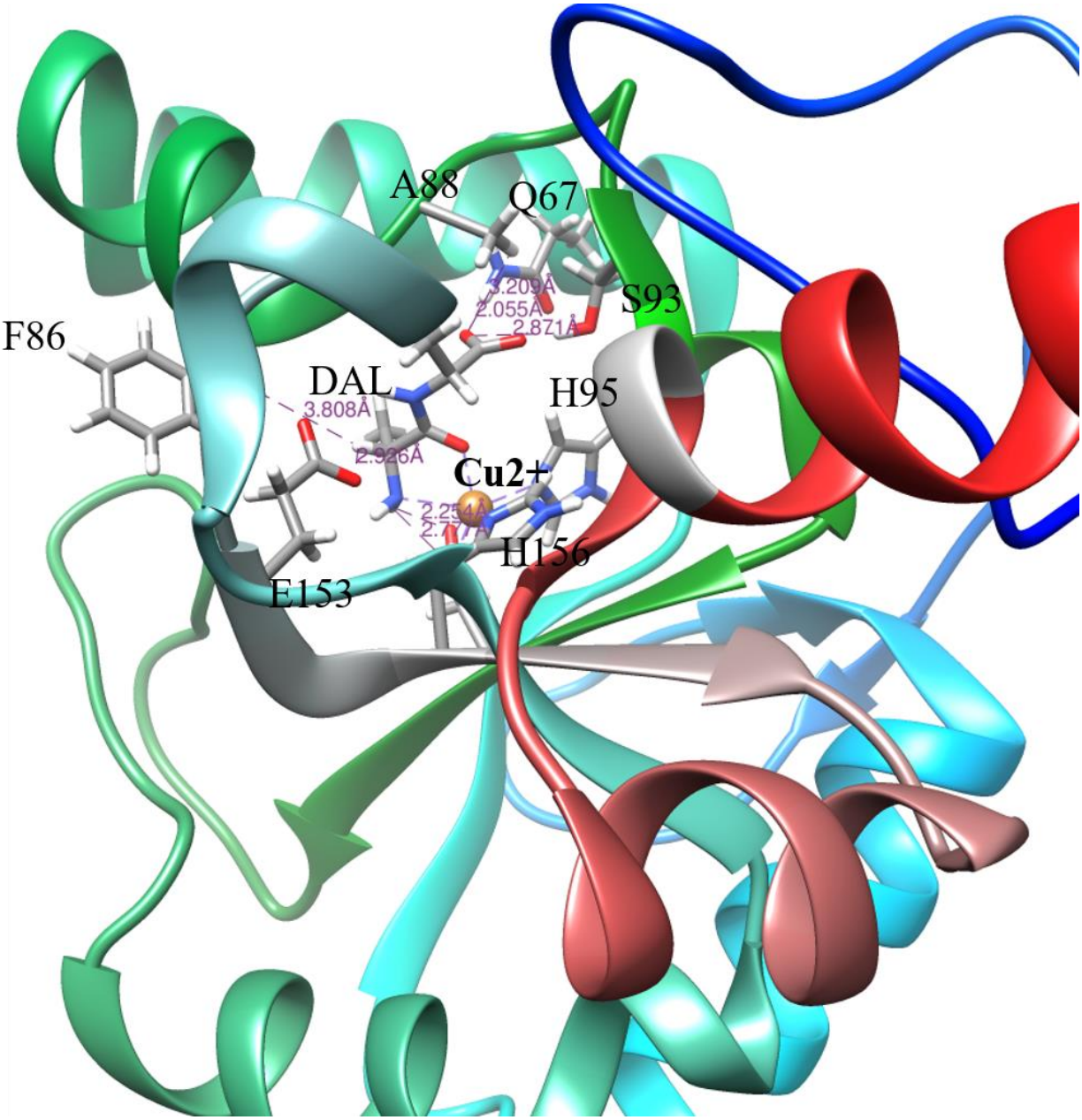

Figure 7. 4OAK with D-Ala-D-Ala. ALA88 and GLN67 are key residue interaction with D-Ala-D-Ala. Other residues are involved in D-Ala-D-Ala indirectly through hydrogen bond. Atom-specific coloring: gray = carbon, red = oxygen, blue = nitrogen, white = hydrogen

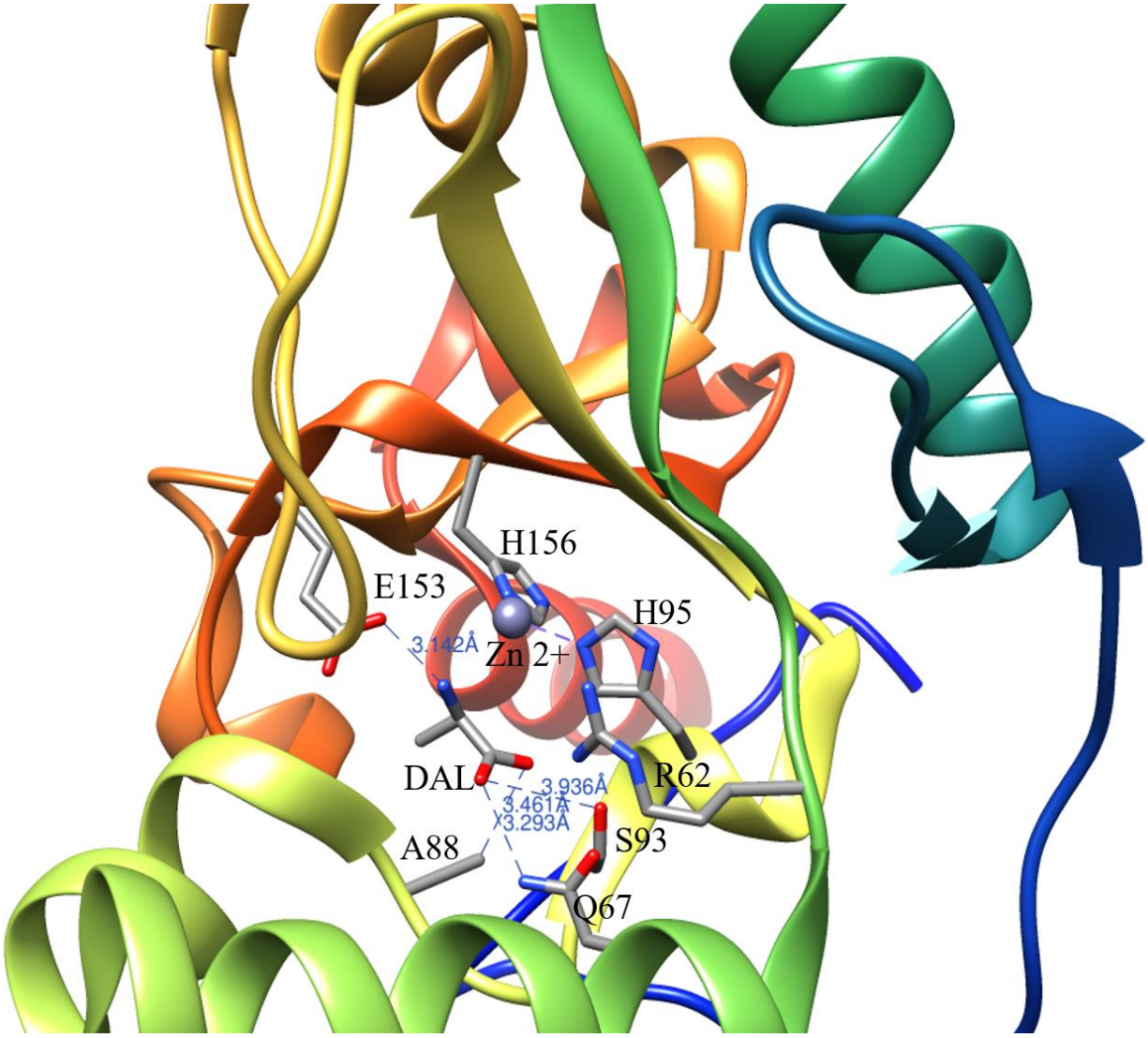

Figure 8. 4MUT with D-Ala. ALA88 and GLN67 are key residue interaction with D-Ala-D-Ala. Other residues are involved in D-Ala-D-Ala indirectly through hydrogen bond. Atom-specific coloring: gray = carbon, red = oxygen, blue = nitrogen, white = hydrogen

Discussion

Why penicillin is still effective antibiotic?

Antibiotic mode of action brought up difference in the resistance. The mechanism of the action of penicillin was binding to the transpeptidase enzyme and breaks the cross-link of the peptidoglycan cell wall in bacteria using penicillin binding protein. This referred that penicillin indirectly attacked the peptidoglycan cell wall and it required PBP as an intermediate step. Although PBPs within bacteria can undergo point mutations, a difference in the effect of the mutations occurs because vancomycin binds directly to the protein while penicillin does not (Hackbarth, et al.1995). For redock and cross-dock result, it was known that the penicillin binding protein is required for penicillin derivative antibiotics to bind to transpeptidase enzyme.

For vancomycin resistance mode of action, it likely interacted with hydrophobic residue because changed to polar residue affected the lowered affinity. This suggested that vancomycin has the interaction through hydrogen bond, gains the strength of dipeptide bonding, and directly binds to C terminal of D-Ala-D-Ala to do transpeptidase activity.

Conclusion

Based on these studies, it was able to define the predicted mechanism of action of amoxicillin and carbenicillin, which supported similar mechanism of penicillin does. In addition, the vancomycin-resistant mutant structure gave an idea of how vancomycin resistance interacts with ligand and residue. The structure provides information about the mechanism of action of vancomycin. However, I did not find specific evidence of why residue mutant has higher rates compared to penicillin binding protein.

Two directions of future work can be done. The first one is to choose the macromolecule that contains antibiotic instead of vancomycin to compare with penicillin. The vancomycin antibiotic was so large that the docking was complicated to do so. Second, since the computational method explained the interaction with residue and ligand on both antibiotics, the future work that could be done is to do the experiment as wet lab as in vitro whether these results were qualified in experimental setting. These future works can bring insights into the molecular basis why penicillin is still effective antibiotics in today.

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