Exploring Molecular Docking Simulations to Investigate the Interaction Between Modified Penicillin Structures and PBP2a in Staphylococcus aureus.

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Abstract

Penicillin-binding proteins (PBPs) play a pivotal role in the mechanism of bacterial resistance, particularly in Staphylococcus aureus. PBP2a, a resistant form of PBP, poses significant challenges in antibiotic resistance due to its mutated structures that affect the binding to antibiotics. This study leverages computational biology and artificial intelligence to compare the binding affinity between the normal PBP and PBP2a to penicillin. As a result, it confirms the risks of these mutations and helps in understanding resistance mechanisms to guide drug design. When utilizing molecular docking and machine learning models, the authors explored structural modifications to penicillin that improve its binding to PBP2a. Our findings suggest that strategic alterations in penicillin's active site significantly enhance its binding potential. Consequently, this research highlights the transformative role of AI in drug discovery and positions computational tools as essential for paving the way for new avenues in drug development. This offers a promising solution to combat antibiotic resistance in pathogenic bacteria.

I. Introduction

i. Penicillin Binding Proteins:

S. aureus, also known as Staphylococcus aureus, is a type of Bacillota and the microbiota of the body (Yang et al., 2024). It is frequently found in the upper respiratory tract and on the skin, it often has a positive effect on catalase and nitrate reduction, and it's a facultative aerobe that can grow in the absence of oxygen (Masalha et al., 2001). On the other hand, Staphylococcus aureus is the leading cause of secondary infections in hospitals and a challenging pathogen in food industries (Rai et al., 2021). As stated in Kluytmans et al., in healthy subjects, about 20% of people are persistent carriers, 60% are intermittent carriers, and approximately 20% rarely carry S. aureus. In fact. S. aureus has gained considerable attention from the medical community due to its involvement in the increasing number of nosocomial and community-acquired infections resulting in nearly half a million hospitalizations and 50,000 deaths each year in the USA alone (Schlecht.,2015). Furthermore, its treatment remains challenging due to the emergence of multi-drug-resistant strains such as MRSA (Rai et al., 2021).

Penicillin Binding Proteins (PBP) are a class of enzymes that are present in virtually all bacteria, especially S. aureus. They are characterized by their affinity for and binding of penicillin (Kumar et al., 2022). PBPs are essential for S aureus cell wall synthesis, and all β-lactam antibiotics (except for tabtoxinine-β-lactam) bind to them. In addition, PBPs are all involved in the final stages of the periplasmic and membrane steps of peptidoglycan biosynthesis, which is the major component of bacterial cell walls. Specifically, bacterial cell wall synthesis is essential to growth, cell division (thus reproduction), and maintaining the cellular structure in

bacteria (Miyachiro et al., 2020). Consequently, bacterial cell wall synthesis is crucial for many aspects that relate to the inhibition of PBPs thus resulting in defects in cell wall structures and irregular growth in cell shape (Tim et al., 2016). One of the most common and important types of PBPs is PBP2. PBP2, which is sensitive to β-lactam antibiotics, is responsible for catalyzing the cross-linking of peptidoglycan strands during cell wall assembly. This transpeptidase activity is crucial for maintaining the structural integrity and rigidity of the bacterial cell wall of S. aureus. PBP2 function is described in **Figure 1,** a schematic representation of the cross-linking process in the *S. aureus* cell wall inhibited by penicillin.

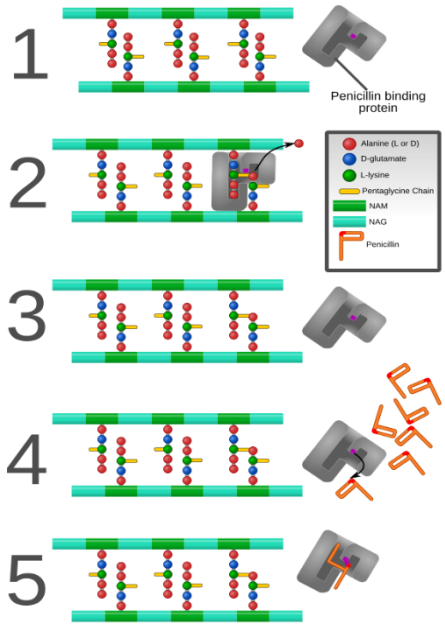


Figure 1: Shows the cross-links in the S. aureus cell wall by penicillin binding protein and subsequent suicide inhibition by penicillin.

ii. PBP2a Mutations

A variant of PBP called PBP2a, specially modified from PBP2, confers resistance to β-lactam antibiotics, saving the cell wall of S. aureus and preventing the inhibition of penicillin. This action allows the turning of routine infections into potentially life-threatening conditions that are significantly harder to treat (Fishovitz., 2014). Furthermore, PBP2a occurs due to the acquisition of the mecA gene, which is typically carried on a mobile genetic element known as the Staphylococcal Cassette Chromosome mec (SCCmec) (Wielders et al., 2002). The SCCmec element facilitates the horizontal transfer of the mecA gene between bacterial strains and species. This process allows the acquisition of new genetic material from other bacterial species or strains (Yuki Uehara, 2022).

In addition, the continuous evolution and enhancement of PBP2a reflects how selection pressure from β-lactam antibiotics drives the evolution of this resistance mechanism, allowing bacteria with SCCmec to thrive under antibiotic pressure and spread more effectively.

Due to its different structure from normal PBPs, PBP2a is resistant to antibiotics, specifically methicillin and penicillin. PBP2a is produced by Methicillin-resistant Staphylococcus aureus (MRSA), which is known to be extremely dangerous as they are harder to treat due to its antibiotic resistance and can develop further life-threatening conditions. Furthermore, 28% of community-acquired S. aureus strains are resistant to antibiotics, ensuring MRSA threat (Wielders et al.). It is stated by Rai et al. that people with MRSA are 64% more likely to die than people with MSSA, a normal non-mutated, infection. MRSA also accounts for >60% of S. aureus clinical isolates in Japan, Singapore, and Taiwan, >50% in Italy and Portugal, and 34% in the United States, as stated in Lim et al. Thus, MRSA complexities and life-threatening lead to an increase in the importance of the fields of analyzing and comparing PBP2a interactions, willing to dramatically increase their binding affinity with antibiotics.

iii. Artificial Intelligence Tools

Artificial Intelligence tools are transforming research across disciplines by enhancing data analysis, image processing, and predictive modeling. Their automation and simulations improve the efficiency of high-throughput experiments and personalized medicine. Tools like AutoDock and AlphaFold exemplify this transformation.

AutoDock was developed to provide an automated procedure for predicting the interaction of ligands (small molecules) with macromolecular targets. AutoDock accelerates the process of identifying potential drug candidates and provides detailed insights into the binding mechanisms and affinities between molecules and proteins, designing more effective and specific drugs. Moreover, it reduces the need for extensive experimental screening, saving both time and resources in the early stages of drug development (Morris et al. 2001). After researchers input 3D structures of ligands and target proteins, AutoDock uses scoring functions to evaluate the binding affinity between ligands and proteins. These functions are part of the AutoDock software and are integral to predicting the strength of interactions (Morris et al. 2001).

With a database of the structures of numerous proteins, AlphaFold is designed to predict the 3D structures of proteins based on their amino acid sequences. AlphaFold significantly advances the field of structural biology by providing highly accurate predictions of protein structures, which were previously difficult to achieve without experimental methods. Thus, AlphaFold accelerates research and drug discovery, reducing the time and cost associated with experimental structure determination.

iv. Our Objectives

This study aims to investigate how structural differences between PBP2 and PBP2a influence their binding affinity with penicillin antibiotics, and how the authors could increase the binding affinity between PBP2a and penicillin.

After modeling our parameters, the authors will be utilizing AI and computational biology tools to predict the pockets of PBP2 and PBP2a, thus predicting and discussing the differences in their binding affinities to the penicillin, seeking for comparison.

Our objective is to provide insights into enhancing antibiotics and drug design by altering penicillin structure to enhance its binding affinity to PBP2a and increase antibiotic efficiency.

II. Literature Review

This chapter establishes a background idea by providing a comprehensive overview of academic and practitioner literature. It discusses the effects of mutations on various diseases and harnesses AI in disease analysis. Each paragraph in Subsections i and ii references a specific paper, showing a concise summary. Additionally, subsection iii highlights our unique contributions.

i. Impact of Genetic Mutations on Disease

According to Farinha et al. (2022), the mutation that causes cystic fibrosis (CF), an autosomal recessive disease that mainly affects Caucasians, occurs in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Encoded by this gene, chloride, and bicarbonate channel are essential for ion transport in epithelial cells. The most common mutation, F508del, significantly impairs CFTR function, increasing the risk of infection and causing respiratory problems such as decreased mucus clearance. More than 2,100 CFTR mutations are categorized in this review according to how they affect the synthesis, folding, and function of proteins.

With a focus on precision medicine, it highlights the importance of understanding these many pathways to develop personalized therapeutics such as pharmacological correctors and chaperones. Patient results in CF therapy can be significantly improved by tailoring medications to each patient's specific genetic profile.

Justin et al. (2023) investigate genetic modifiers of Sickle Cell Disease (SCD) and their impact on disease severity. It identifies important genetic variants by analyzing 571 papers, particularly those that affect fetal hemoglobin (HbF) levels, like BCL11A, HBS1L-MYB, and HBG2 variants. It is shown that variants are related to a lower risk of complications such as kidney disease and stroke. However, because of uneven study designs, a large number of observed connections are not validated. To enhance further studies on SCD genetic modifiers, the report emphasizes the importance of uniform methods and the importance of genetic knowledge in developing customized therapy to improve SCD patient outcomes.

The role of GBA1 gene mutations in Parkinson's disease (PD) was examined in the study (Jenny et al., 2019). GBA1, the gene encoding glucocerebrosidase, is mutated in Gaucher disease and is linked to an increased risk of Parkinson's disease. The study looks at the molecular mechanisms that GBA1 mutations that impact lysosomal function use to enhance α-synuclein buildup. Part of the procedure involves looking at genetic and biochemical data on GBA1 mutations and how they impact lysosomal activity. Results show that glucocerebrosidase activity is lowered by GBA1 mutations, disrupting lysosomal homeostasis and elevating α-synuclein, an essential PD characteristic. The study also looks into potential therapeutic techniques for improving lysosomal function and slowing the progression of Parkinson's disease, such as gene therapy and enzyme replacement.

The role of the major histocompatibility complex (MHC) locus in autoimmune and infectious diseases is shown in the study (Matzaraki et al., 2017). The study examines the previous 50 years of genetic relationships between MHC variations and illness vulnerability. The methodology is centered on fine-mapping MHC variations, imputation, and large-scale genetic screens. The findings highlight the important role that MHC variations play in controlling immune responses and determining a person's vulnerability to viral diseases like HIV and autoimmune disorders like rheumatoid arthritis. Furthermore, the ability to identify MHC variations has improved because of sophisticated genomic mapping technologies like the Immunochip platform. This work advances our knowledge of immune-mediated illness mechanisms and opens the door to novel treatment approaches that target pathways connected to MHC.

Shah et al. (2017) highlight the role of the major histocompatibility complex (MHC) locus in autoimmune and infectious diseases. The study looks at the genetic associations between MHC variants and susceptibility to sickness over the preceding 50 years. The process is based on large-scale genetic screening, imputation, and fine mapping of MHC variants. The results highlight the critical role that MHC variants play in regulating immune responses and identifying an individual's susceptibility to autoimmune diseases such as rheumatoid arthritis and viral diseases like HIV. Furthermore, sophisticated genomic mapping tools such as the Immunochip platform have improved the ability to find changes in MHC. This study contributes to our understanding of immune-mediated disease mechanisms and makes new therapeutic strategies that target MHC-related pathways possible.

ii. Harnessing Artificial Intelligence in Diseases Analyzing

Tran et al., (2021) examine the use of deep learning (DL) in oncology, emphasizing DL's capacity to evaluate complex cancer biology through the use of many data types, including transcriptomic, methylation, and genomic data. To do that, they examined the impact of mutations on myofibrillar aggregation and employed deep-learning models trained on genomic and clinical data to predict mutation-driven cancer pathways. Regarding that, research indicates that mutations, especially those about sarcomere-related genes, significantly impact the course of the disease by impairing the structure and function of myofibrils. Additionally, it emphasizes how these mutations in the pathophysiology of heart failure, which are bolstered by AI's increasing ability to detect mutation patterns, enhance treatment care by customizing therapy options based on mutation profiles.

As shown in Nature (2021), AI was used to predict the structure of over 20,000 human proteins and nearly all known proteins from 20 model organisms, totaling around 365,000 predictions. AlphaFold was created by the DeepMind team, who trained it on evolutionary history, DNA sequences, and known protein forms. The data and source code were made available to the public. Compared to conventional approaches that took months or years to predict protein structures, AlphaFold can do it in minutes to hours. Furthermore, it illustrated how biomedical research has impacted our understanding of diseases at the cellular level, from the course of the disease to the effectiveness of treatment.

Utilizing developments in population frequency data and protein structure prediction, AlphaMissense is a deep learning model presented in Cheng et al. (2023) that was created to forecast the pathogenicity of missense mutations in the human proteome. To predict variant effects without relying on human-curated annotations, the model integrates structural context from AlphaFold, unsupervised protein language modeling, and fine-tuning on population frequency data. With 90% accuracy, AlphaMissense delivers state-of-the-art performance in predicting the pathogenicity of missense variants on the ClinVar dataset, classifying 32% of variants as likely pathogenic and 57% as likely benign.

The aim of Rajesh et al. (2023) is to test for diabetic retinopathy (DR), a major cause of blindness, using AI algorithms. It emphasizes the necessity of yearly eye exams for diabetics and the advancement of AI to control the volume of screenings. Fundus photo-tagged data sets are used to train AI algorithms. Its training data is divided into training, validation, and testing splits. The models are assessed according to how well they perform in comparison to human graders. Several AI systems have demonstrated >85% sensitivity and specificity in identifying referable DR, including IDx-DR, EyeArt, and AEYE-DS. These algorithms are approved for clinical use by the food and drug administration (FDA) after undergoing prospective validation. Cost-effectiveness, equity and bias, and medicolegal issues are covered in the study when applying AI algorithms to clinical settings.

Using chest CT images, Ortiz et al, (2022) attempted to create deep-learning models to forecast COVID-19 results.

It created a cutting-edge algorithm to translate 3D CT volumes into 2D images. Furthermore, a multitask model for segmenting pulmonary lesions in lungs infected with COVID-19 was developed in this study.

Moreover, it facilitated clinical decision-making and enhanced mortality risk assessment by merging CT characteristics with clinical metadata. With an AUC of 0.80, the CT and clinical data model outperformed the clinical data model alone in terms of prediction accuracy.

iii. Our Innovation

Our study seeks to highlight the existing knowledge of the impact of mutations on different diseases, as they cause dramatic consequences, as shown in subsequence i. We also seek to present how various AI tools, which are existing, were used in analyzing diseases, as shown in subsequence ii. Our study seeks to make noteworthy original contributions in the following ways:

- To model the 3D structure and identify the binding pockets to penicillin in PBP2 and PBP2a using computational biology tools
- To measure and compare the binding affinities between penicillin and both proteins by conducting molecular docking simulations using AutoDock.
- To increase the binding affinity between penicillin and PBP2a, by altering penicillin 3D structure, seeking for enhancements in antibiotics designs.

III. Modeling the Parameters

The authors used the National Center for Biotechnology Information's (NCBI) GenBank database, which includes more than 38941654 reported protein sequences for the S. aureus, for the amino acid sequences of PBP2 and PBP2a. Then, AlphaFold was utilized for the 3D structure of both proteins, which are shown in **Figure 2** and **Figure 3**, and also for the PDB file format of them, **which are shown in Additional File 1 and Additional File 2**.

AutoDock tools (ADT) were utilized to prepare the PDB file format, by adding all necessary hydrogen atoms hydrogen atoms, especially polar hydrogens, since they are important for accurate molecular interactions. In addition, the authors assigned charges and, on the other hand, removed unrequired water molecules that are modified. In the end, it resulted in transforming this file from the PDB format into the PDBQT format, **shown in Additional Files 3 and Additional Files 4**, which is used in AutoDock simulations.

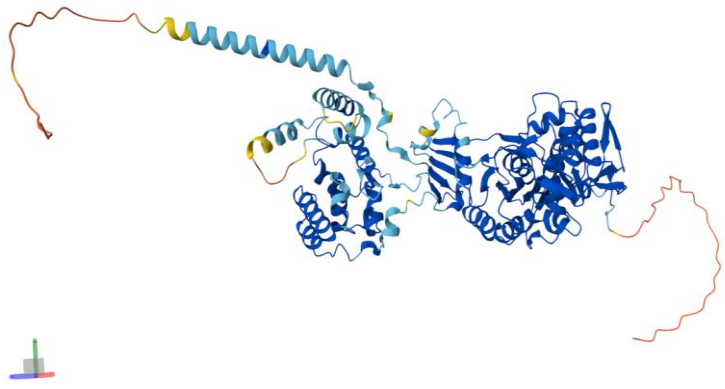


Figure 2: Shows the 3D structure of PBP2.

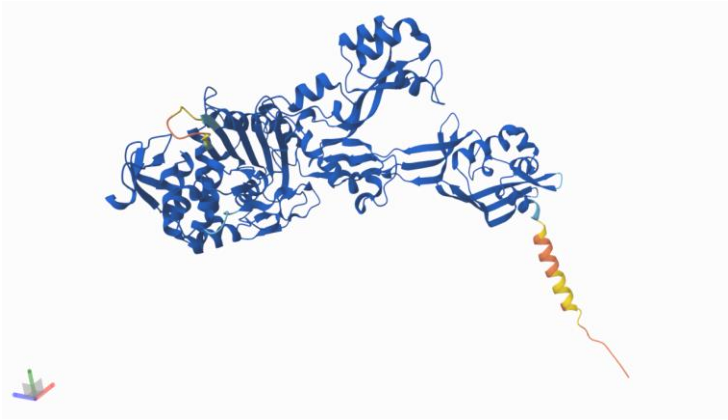


Figure 3: Shows the 3D structure of PBP2a.

Furthermore, CASTp tools were utilized to visualize the pockets or the docking sites of both proteins, with a probe radius set to default, **which are shown in Figure 3 and Figure 4.**

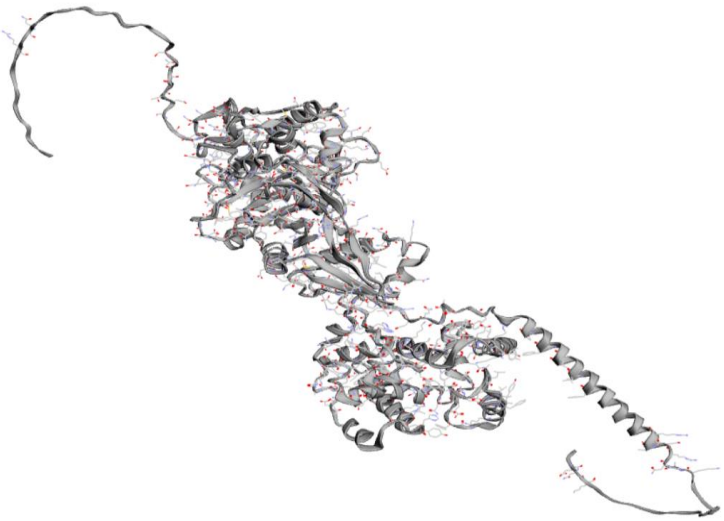


Figure 3: Shows the pockets of PBP2.

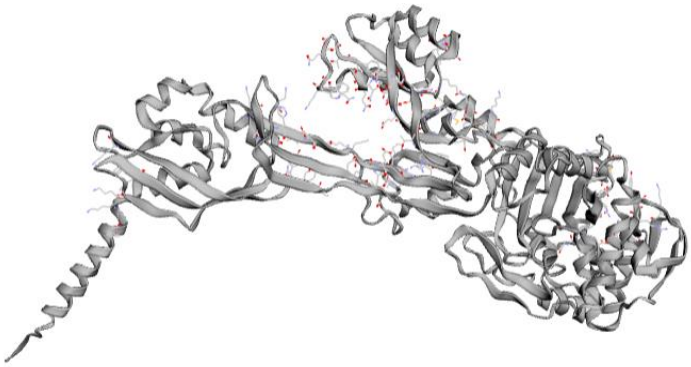


Figure 4: Shows the pockets of PBP2a

NCBI PubChem was also used for utilizing the structure of penicillin and the open form penicillin G was chosen, also called benzylpenicillin, which is the one used as an antibiotic. Penicillin G SMILES form was also utilized:

$$CC1(C)SC([NH2+]C1C([O-])=O)C(NC(=O)Cc1ccccc1)C=O$$

The 2D structure of penicillin G is also **shown in Figure 5.**

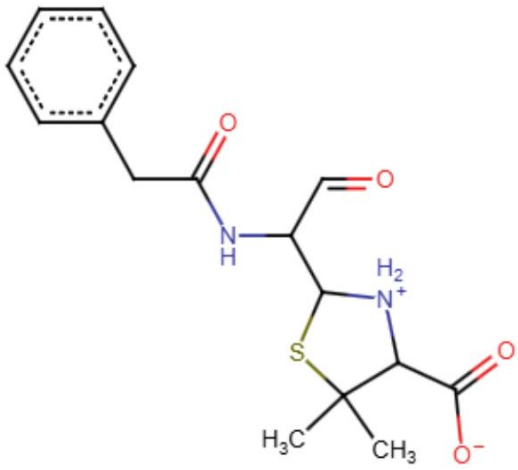


Figure 5: Shows the 2D structure of penicillin G.

IV. AutoDock Analysis

AutoDock was utilized, as it is the most efficient tool, to measure the affinity of the binding sites with the highest volume between the ligand and the protein. The last version of AutoDock, AutoDock GPU, was downloaded from the Center for Computational Structural Biology and the prepared PDBQT files was used as an input. The affinity between the two proteins, PBP2 and PBP2a, and penicillin were calculated. With the addition of negatives, it resulted in higher affinity. As a result, the authors managed to show the affinities in relation to the number of bindings, with the nucleic acids, in the highest volume binding sites.

Figure 6 shows the calculated affinity in (kcal/mol) between PBP2 and penicillin. With the average of −6.218 kcal/mol, and reaching -7.0 kcal/mol, it shows high binding affinities between the ligand and the protein.

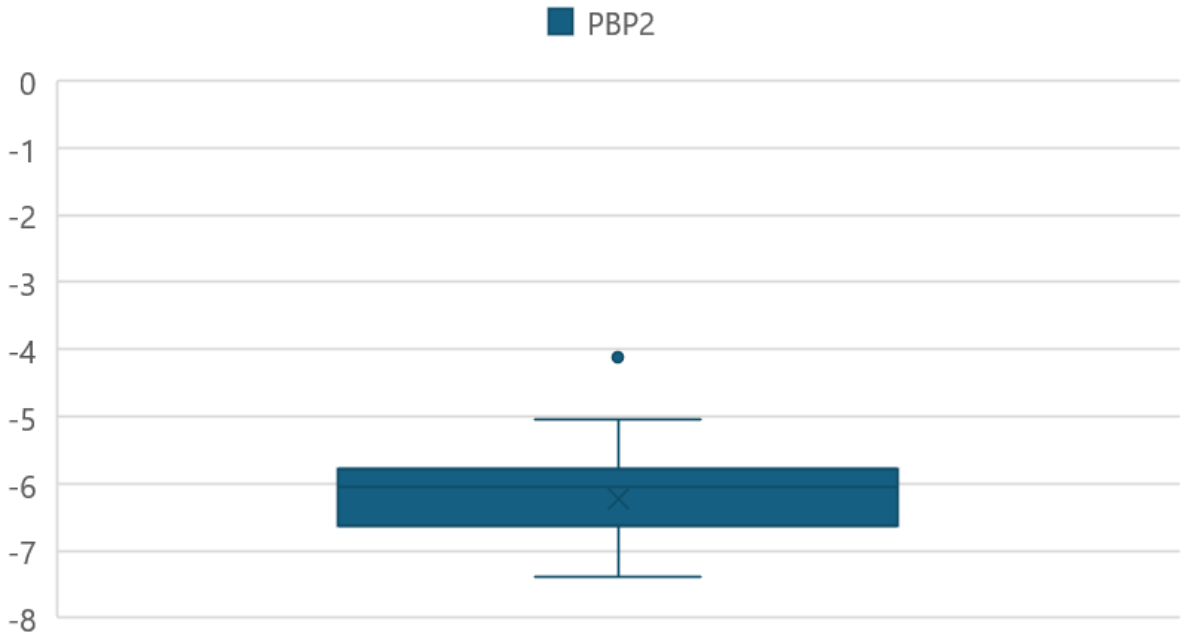


Figure 6: Shows the calculated affinities of PBP2 and the number of bindings.

Figure 7 shows the calculated affinity in (kcal/mol) between PBP2a and penicillin, which shows low affinities mostly ranging between -3.9 and -4.8 kcal/mol. It has an average of -4.425 kcal/mol, and it almost does not exceed -5.0 kcal/mol.

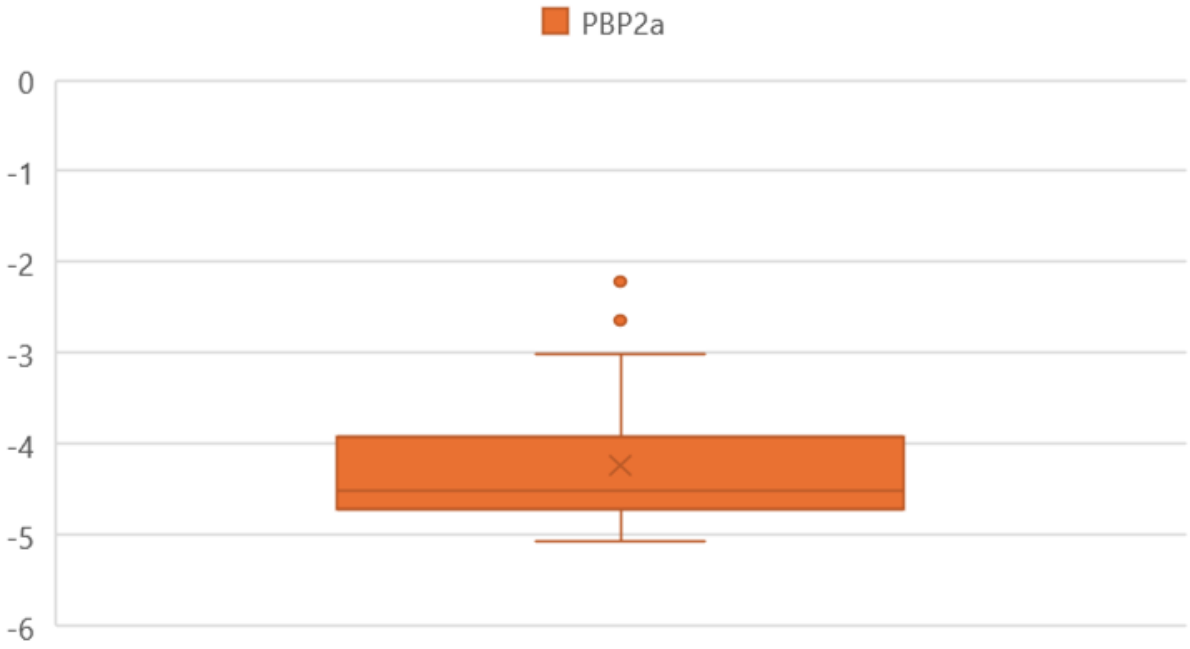


Figure 7: Shows the calculated affinities of PBP2a and the number of bindings.

Through the figures, it is clearly shown that there is a big difference in the affinity between PBP2 and PBP2a in binding with penicillin. This simulation asserts the dramatic effect of PBP2a mutations on medicine efficiency, ensuring the need of a new efficient treatment, with high binding affinity.

Figure 8 combines the binding affinity of both PBP2 and PBPP2a, highlighting the differences and provide a comparative analysis of their binding affinities with penicillin.

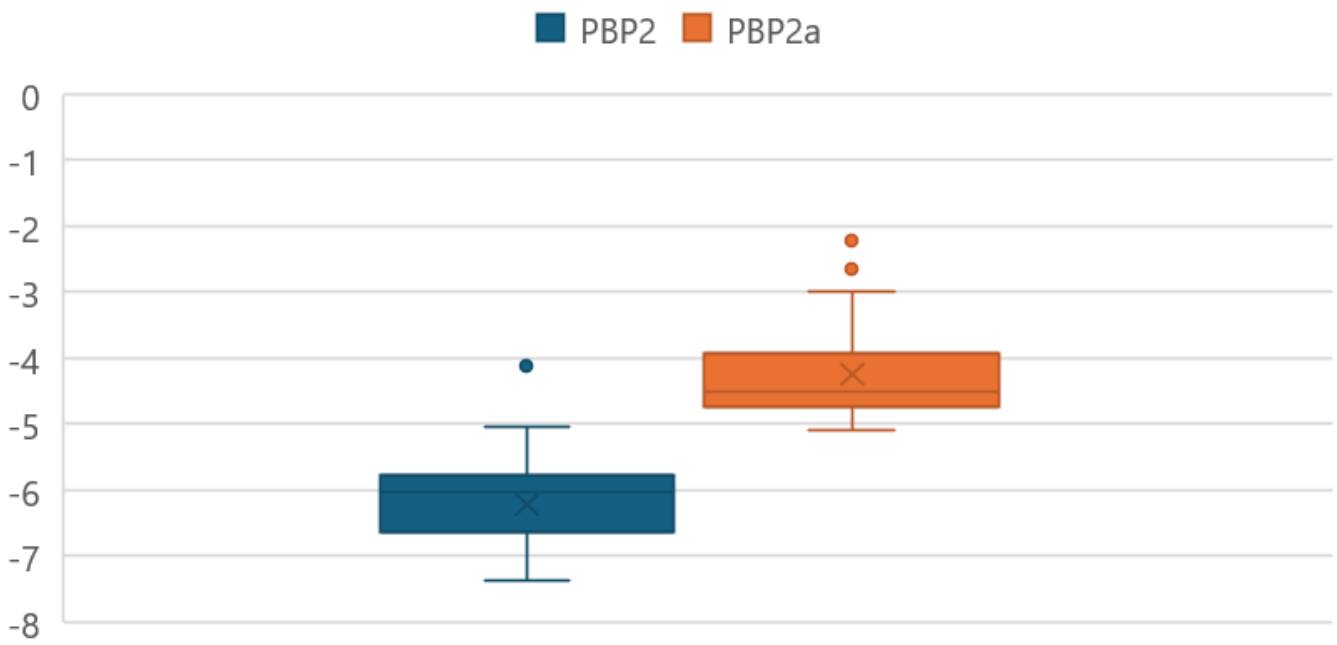


Figure 8: Shows the difference in the calculated binding affinities of PBP2 and PBP2a., with the more negative, the more the binding affinity becomes.

V. Penicillin Structure Modifications

In this section, the authors investigate the impact of structural modifications on penicillin to observe their effects on the binding affinity with PBP2a. The alterations are designed to target specifically the most active sites within PBP2a, with the goal of enhancing binding affinity and potentially circumventing resistance mechanisms. Understanding these interactions at a molecular level will help guide the development of more effective β-lactam antibiotics and provide insights into overcoming antibiotic resistance mechanisms. The open form penicillin G was described in the previous section, and its affinity with the most active site within PBP2a is described in **Figure 11 Series 1**.

Over 25 penicillin different modifications were tested, ranging from significant structural modifications to more subtle changes to the open form of penicillin G. Only two with the highest increase in affinity were chosen.

The first modified structure is different in having a thiazolidine ring instead of benzene ring. In addition, changes in the side chains and the functional groups are made, **as shown in Figure 9**. In **Figure 11 Series 2**, comes the calculated binding affinity within the same binding site as the original form of penicillin. It is shown to have slightly less affinity, except for one period where the affinity increased from 4.6 to 5.4 kcal/mol. Its SMILES form is also provided:

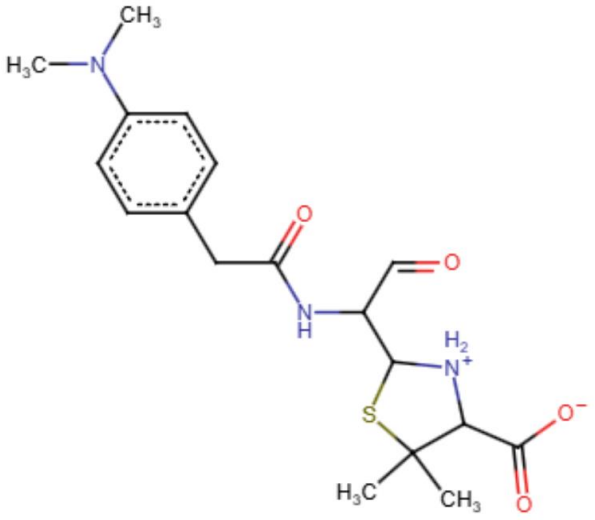


Figure 9: Shows the 2D structure of the first modified penicillin structure.

$$CN(C)c1ccc(CC(=O)NC(C=O)C2[NH2+]C(C([O-])=O)C(C)(C)S2)cc1$$

The SMILES form of the original penicillin is also repeated for clarity:

$$CC1(C)SC([NH2+]C1C([O-]) = O)C(NC(= O)Cc1ccccc1)C = O$$

The second modified structure of penicillin is the greatest example that small changes can dramatically affect the results. As only an as extra carboxyl group was added, **as shown in figure 10**, it dramatically affected the binding affinity with PBP2a, **as shown Figure 11 Series 3**.

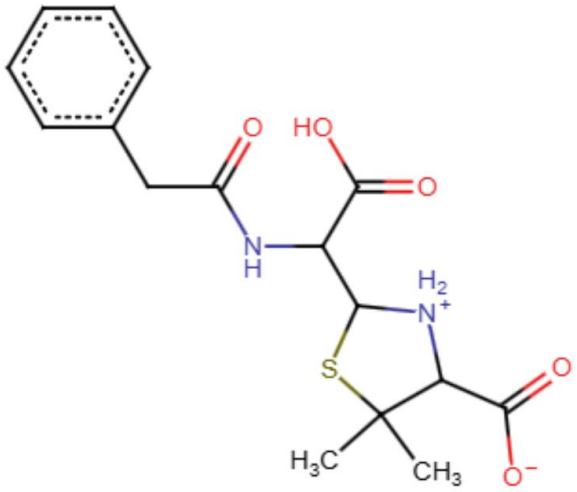


Figure 10: shows the 2D structure of the second modified penicillin structure.

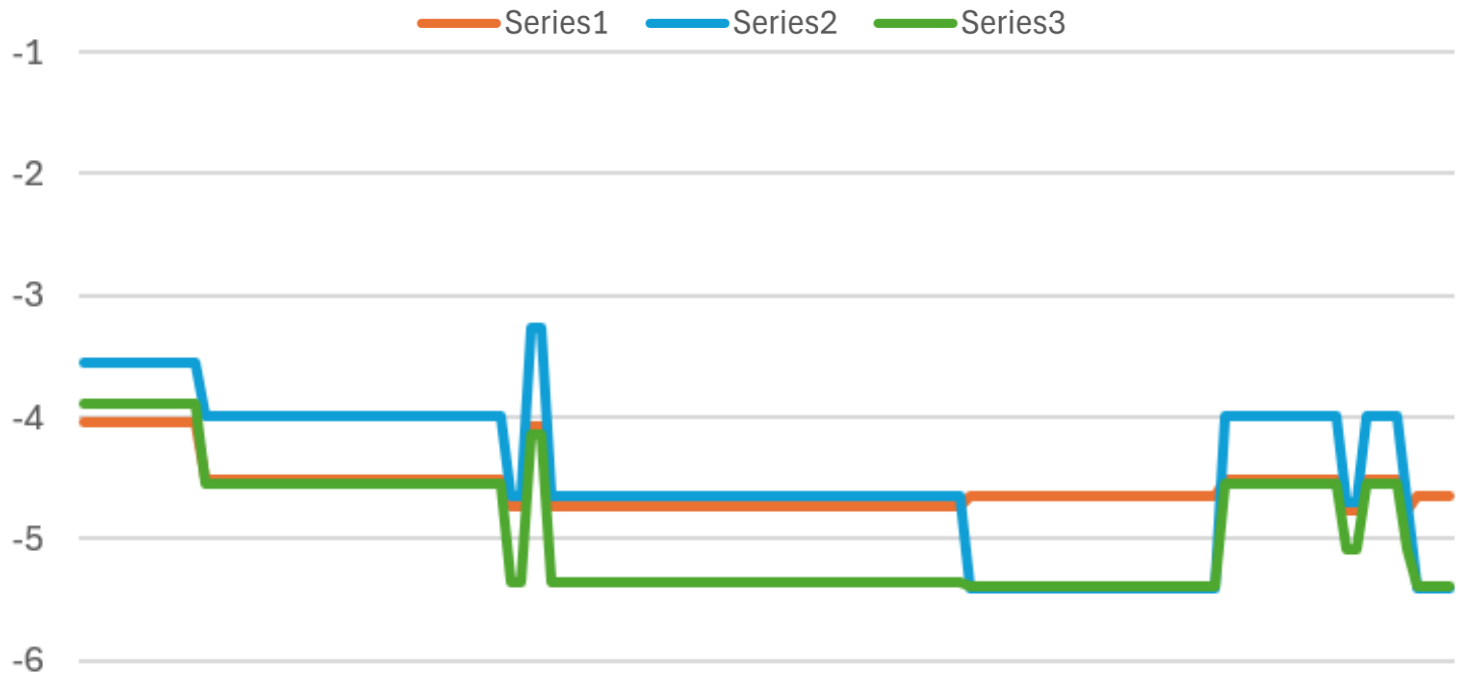


Figure 11: Shows the 3 forms of penicillin in binding with PBP2a, where Series 1 is the original form, Series 2 is the first modified form, Series 3 is the second modified form.

VI. Discussion

Our analysis supports the theory that structural differences between PBP2 and PBP2a significantly influence their binding affinity with penicillin antibiotics. Through our simulations, the authors demonstrated that PBP2a exhibits much lower affinity for penicillin compared to PBP2, due to its altered structure. This perspective validates the resistance mechanism in MRSA. Our results should be taken into account when numerically comparing the binding affinity of both proteins to penicillin, showing the effect of their structural differences and how they play a crucial role in antibiotic resistance.

While other research has focused on the development of new antibiotics, our research focused on enhancing current antibiotics structure. By altering the structure of penicillin, the authors observed an increase in the binding affinity to PBP2a leading to a suggestion that structural alterations of antibiotics could overcome this resistance, further decreasing MRSA risks. In addition, by challenging the idea that PBP2a's resistance is entirely insurmountable, it suggests and opens new avenues for designing beta-lactam antibiotics that would potentially bypass this resistance.

Moreover, our results were constrained by the influence of the computational methods and the selected parameters that affect the accuracy of the binding predictions, which may not capture all relevant interactions or conformational changes that occur in real biological systems. Furthermore, the simulations ignored the microenvironmental effect around the interaction between the ligand and the protein, like other cellular components or varying environmental conditions.

Despite these limitations, the findings remain valid for addressing our research question regarding the impact of structural differences on antibiotic binding affinities.

They contribute to a deeper understanding of resistance mechanisms in MRSA and can inform future research directions. constituting the fundamental mechanisms underlying

In conclusion, while our research examines the structural differences between PBP2 and PBP2a that influence their binding affinity with penicillin, further research is necessary to validate these findings in experimental settings, incorporating different microenvironmental effects. Future studies should also consider exploring a wider range modified antibiotics to identify compounds that may effectively inhibit PBP2a. Additionally, these studies could investigate alternative pathways for overcoming PBP2a resistance, such as using combination therapies that enhance the efficacy of existing antibiotics. As shown in the authors' study, the potential of artificial intelligence offers transformative potential in drug design, with the ability to accelerate the discovery of novel therapeutics and optimize binding affinity to overcome challenges like antibiotic resistance.

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