Evaluating the Antibiofilm Activity of a Cyanobacterial Polyketide Anabaenopeptilide 90A on Streptococcus Mutans

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**Abstract:** Anabaenopeptilide 90A, a cyclic depsipeptide derived from Anabaena cyanobacteria, shows promise as an antibiofilm agent against Streptococcus mutans, a primary etiological agent of dental caries. This study employs molecular docking to investigate the interactions of Anabaenopeptilide 90A with key biofilm-associated proteins, Glucan-binding protein C (GbpC) and Glucosyltransferase-I (GtfB). Molecular docking simulations revealed a strong binding affinity of Anabaenopeptilide 90A with GbpC (-8.7 kcal/mol) and GtfB (-7.2 kcal/mol). The interactions between Anabaenopeptilide 90A and GbpC involved hydrogen bonds with TYR392, ASP408, SER347, and TRP45, along with π-π stacked interactions with TRP351. Similarly, Anabaenopeptilide 90A formed a salt-bridge, hydrogen bonds, carbon-hydrogen bonds, and π-alkyl interactions with GtfB, indicating potential inhibition of enzymatic glucan synthesis. Targeting GbpC and GtfB highlights Anabaenopeptilide 90A's dual mechanism against S. mutans biofilms, potentially disrupting matrix formation and bacterial adherence. Such multifaceted targeting is crucial for combating biofilm-associated dental diseases and reducing antibiotic resistance. This study underscores the therapeutic potential of Anabaenopeptilide 90A and provides structural insights into its interactions with biofilm-associated proteins. Experimental validation of these findings could pave the way for developing Anabaenopeptilide 90A as a novel therapeutic agent for preventing dental caries and managing oral health.

**Keywords:** Anabaenopeptilide 90A; Streptococcus mutans; Molecular docking; Antibiofilm

# Introduction

Biofilms are complex communities of microorganisms that adhere to surfaces and are embedded within a self-produced extracellular matrix[(Ramsundar et al., 2023; Rieshy et al., 2023; S. Singh et al., 2023)](https://paperpile.com/c/7qnS0v/gm5F3+wGPig+xaOD9). These structures pose significant challenges in clinical settings due to their high resistance to antibiotics and the immune system[(Pavithra et al., 2023; Shenoy et al., 2023; Thomas & Jain, 2023)](https://paperpile.com/c/7qnS0v/9yCOZ+vw1YX+nBA2A). One of the primary contributors to dental biofilms is Streptococcus mutans, a bacterium implicated in the formation of dental caries. S. mutans utilizes a biofilm mode of growth to thrive in the oral cavity, facilitating its role in tooth decay by producing acids that demineralize the tooth enamel [(Bowen et al., 2018)](https://paperpile.com/c/7qnS0v/zt96). After examination of the age and gender distribution of patients undergoing tooth extraction due to dental caries at revealed a higher prevalence in males and individuals aged 31-40. The results highlight the importance of raising awareness about the causes of tooth loss for prevention [(Pratha et al., 2020)](https://paperpile.com/c/7qnS0v/kYTH). A review analyzed the genotypic diversity of *Streptococcus mutans* in relation to Early Childhood Caries (ECC). It concluded that genetic polymorphisms and mutations vary between children with and without ECC [(Ravikumar et al., 2021)](https://paperpile.com/c/7qnS0v/iivt). A study compared the antibacterial effectiveness of chlorhexidine and pomegranate peel extract (PPE) oral rinses against *S. mutans*, *Lactobacilli*, and *Veillonella* in patients with advanced dental caries, showing that PPE was effective but less so than chlorhexidine in reducing *S. mutans* levels [(Jacob et al., 2021)](https://paperpile.com/c/7qnS0v/83qV). The search for new antibiofilm agents has led researchers to explore natural products, particularly those derived from cyanobacteria[(Doshi et al., 2023; Lampl et al., 2023; Pandiyan et al., 2023)](https://paperpile.com/c/7qnS0v/ctbKm+I4DeO+5KnUX). Cyanobacteria are prolific producers of bioactive compounds with diverse biological activities, including antibacterial, antifungal, antiviral, and anticancer properties [(R. K. Singh et al., 2011)](https://paperpile.com/c/7qnS0v/563L). Among these compounds, polyketides have garnered attention for their potent biological activities. Anabaenopeptilide 90A, a polyketide isolated from the cyanobacterium Anabaena, has shown promising potential as an antibiofilm agent[(Janani et al., 2021; Kachhara et al., 2021; Subramanian et al., 2023)](https://paperpile.com/c/7qnS0v/NKNJI+iFwWC+6f3a7).Anabaenopeptilide 90A is a cyclic depsipeptide known for its unique structure and bioactivity. Previous studies have highlighted its antifungal and cytotoxic properties [(Vestola et al., 2014)](https://paperpile.com/c/7qnS0v/h2Ij). However, its antibiofilm activity, particularly against S. mutans, remains underexplored. Understanding the mechanisms by which Anabaenopeptilide 90A inhibits biofilm formation could pave the way for novel therapeutic strategies to combat dental caries[(Gandhi et al., 2021; Katyal et al., 2023; Priyadharshini et al., 2023)](https://paperpile.com/c/7qnS0v/kxgEy+BRqyW+pNgHr).The study of biofilm inhibition requires detailed insights into the interactions between bioactive compounds and bacterial biofilm components[(Chokkattu et al., 2023; Dharman et al., 2023; Govindaraj & Shanmugam, 2023)](https://paperpile.com/c/7qnS0v/Tnh2k+wLbez+UTzjP). In silico approaches, such as molecular docking, have become invaluable tools in this regard. Molecular docking allows researchers to predict the binding affinity and specificity of small molecules to target proteins, providing a detailed understanding of potential inhibitory mechanisms [(Morris & Lim-Wilby, 2008)](https://paperpile.com/c/7qnS0v/hzBj). By simulating the interaction of Anabaenopeptilide 90A with key proteins involved in S. mutans biofilm formation, we can identify potential molecular targets and elucidate the compound’s mode of action[(Rajeshkumar & Lakshmi, 2021; Sivakumar et al., 2021)](https://paperpile.com/c/7qnS0v/JXb1T+fXUfu).Key proteins involved in S. mutans biofilm formation include glucosyltransferases (Gtfs), which are responsible for the synthesis of extracellular polysaccharides that form the biofilm matrix [(Bowen & Koo, 2011)](https://paperpile.com/c/7qnS0v/6hGK). Gtfs facilitate the adherence of S. mutans to tooth surfaces and contribute to the structural integrity of the biofilm. Inhibiting Gtf activity could disrupt biofilm formation and reduce the cariogenic potential of S. mutans. Another critical protein is sortase A, an enzyme that anchors surface proteins to the bacterial cell wall, playing a crucial role in adhesion and biofilm maturation [(Kavanaugh & Horswill, 2016)](https://paperpile.com/c/7qnS0v/KxxI).The interaction of Anabaenopeptilide 90A with these target proteins can be investigated using molecular docking studies. By predicting the binding affinity and mapping the binding sites, we can gain insights into how Anabaenopeptilide 90A may inhibit the activity of Gtfs and sortase A. Previous studies have demonstrated the efficacy of similar approaches in identifying potential antibiofilm agents and understanding their mechanisms of action [(Deepasree & Subhashree, 2023)](https://paperpile.com/c/7qnS0v/BezF).In addition to molecular docking, molecular dynamics (MD) simulations can provide further insights into the stability and dynamics of the protein-ligand complexes. MD simulations allow us to observe the behavior of the complex over time, providing a more comprehensive understanding of the interactions and potential inhibitory effects [(Hollingsworth & Dror, 2018)](https://paperpile.com/c/7qnS0v/89Ko). Combining molecular docking and MD simulations offers a robust framework for evaluating the antibiofilm activity of Anabaenopeptilide 90A.The significance of this study lies in its potential to identify a novel antibiofilm agent against S. mutans. Dental caries remains a prevalent and costly oral health issue worldwide, and the development of new therapeutic strategies is essential. Natural products like Anabaenopeptilide 90A offer a promising avenue for the discovery of new antibiofilm compounds with the potential to enhance oral health.In conclusion, this study aims to evaluate the antibiofilm activity of Anabaenopeptilide 90A against S. mutans using an in-silico approach. By leveraging molecular docking and MD simulations, we seek to elucidate the interactions between Anabaenopeptilide 90A and key proteins involved in biofilm formation. The findings from this study could provide valuable insights into the potential of Anabaenopeptilide 90A as a novel therapeutic agent for the prevention and treatment of dental caries.

# Materials and Methods

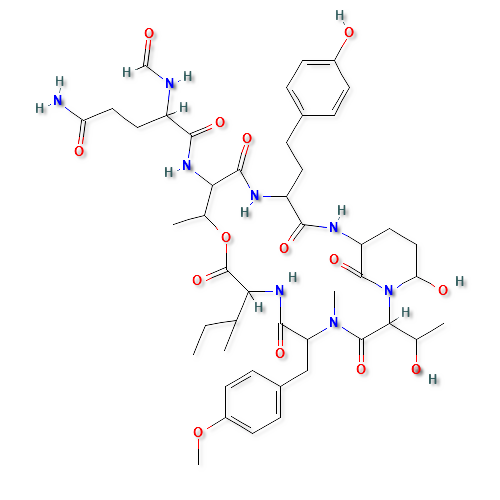
Anabaenopeptilide 90A (C₄₆H₆₄N₈O₁₄) is a cyanobacterial polyketide with a molecular weight of 953 g/mol (Almatrafi et al., 2024). In this study, Anabaenopeptilide 90A serves as the ligand, with its structure (PubChem CID: 23724540) obtained from PubChem, a database provided by the National Library of Medicine, NCBI, and NIH. (Saadh et al., 2024)The research focused on two key biofilm-inducing proteins: Glucan-binding Protein C (GbpC) (PDB: 6CAM) [(Mieher et al., 2018)](https://paperpile.com/c/7qnS0v/Da7b) and Glucosyltransferase-I (GtfB) (PDB: 8FKL) [(Schormann et al., 2023)](https://paperpile.com/c/7qnS0v/L3B1) from Streptococcus mutans. Their molecular structures were retrieved from the Protein Data Bank (RCSB PDB). These structures were visualized, and non-essential ligands, chains, and water molecules were removed, while polar charges were added using BIOVIA Discovery Studio Visualizer 2024 (v24.1.0.23298), developed by Dassault Systèmes Biovia Corp.Molecular docking simulations were conducted to explore interactions between Anabaenopeptilide 90A and the biofilm-inducing proteins GbpC and GtfB. The docking simulations were performed using PyRx-Python Prescription 0.8 with AutoDock Vina as the molecular docking engine [(Akshatha et al., 2021; Dallakyan & Olson, 2015; Trott & Olson, 2010)](https://paperpile.com/c/7qnS0v/ufnN+txkQ+zXLF). The grid center and dimensions were adjusted and documented, as detailed in Table 1. The optimal model was selected based on the lowest binding affinity. The interactions between the ligand and the proteins were visualized, analyzed, and recorded using BIOVIA Discovery Studio Visualizer 2024.

**Table 1.** The grid centre and dimension parameters set for MCP-1 and NFκB

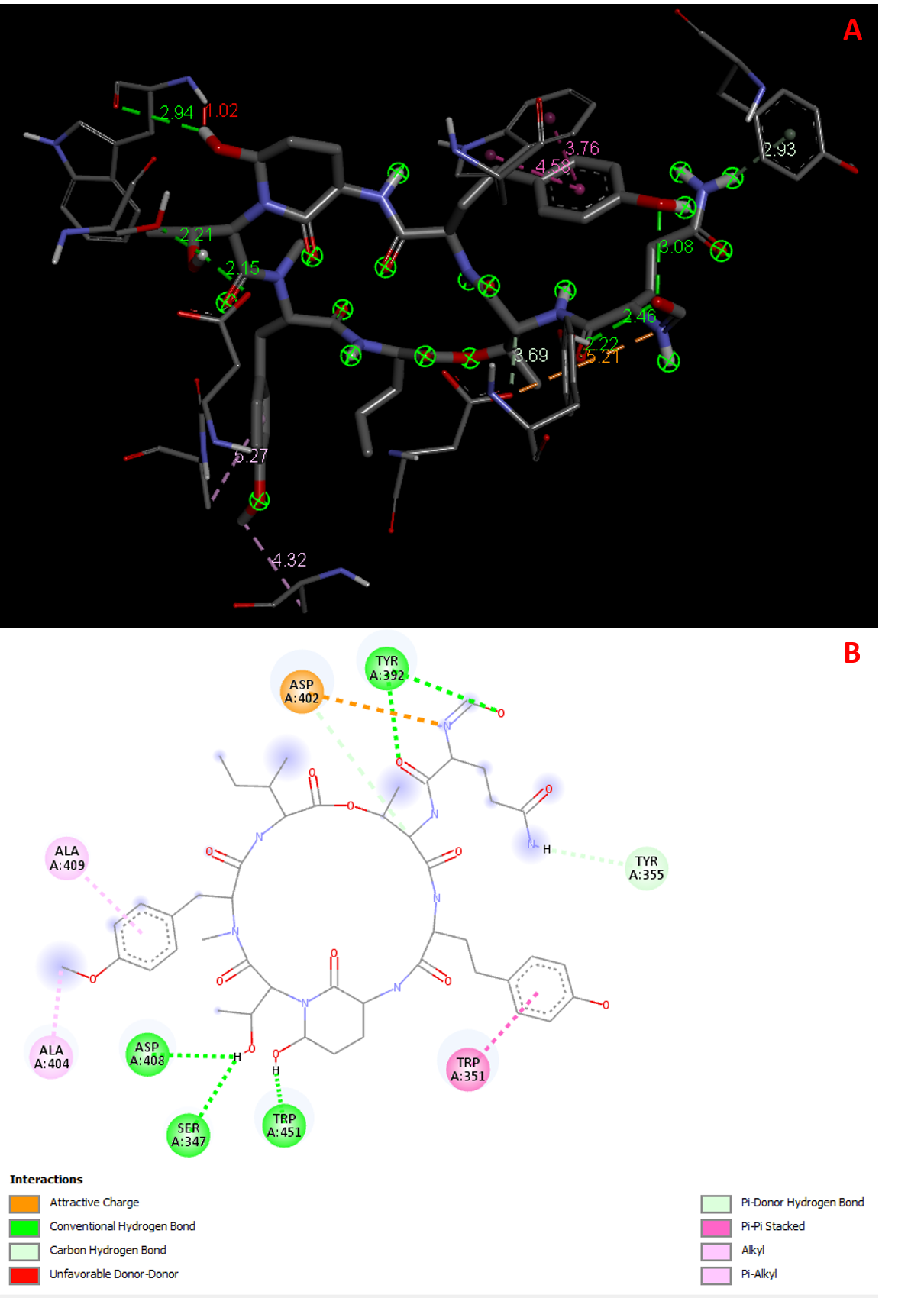
|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Grid Centre** | |  | **Dimensions (Å)** | | |
| **Protein** | **PDB** | **X** | **Y** | **Z** | **X** | **Y** | **Z** |
| Glucan-binding protein C (GbpC) | 6CAM | 238.90 | -25.33 | 11.64 | 111.07 | 68.25 | 88.98 |
| Glucosyltransferase-I (GtfB) | 8FKL | -14.07 | -20.37 | 19.24 | 97.85 | 85.25 | 100.25 |

# Results and Discussion

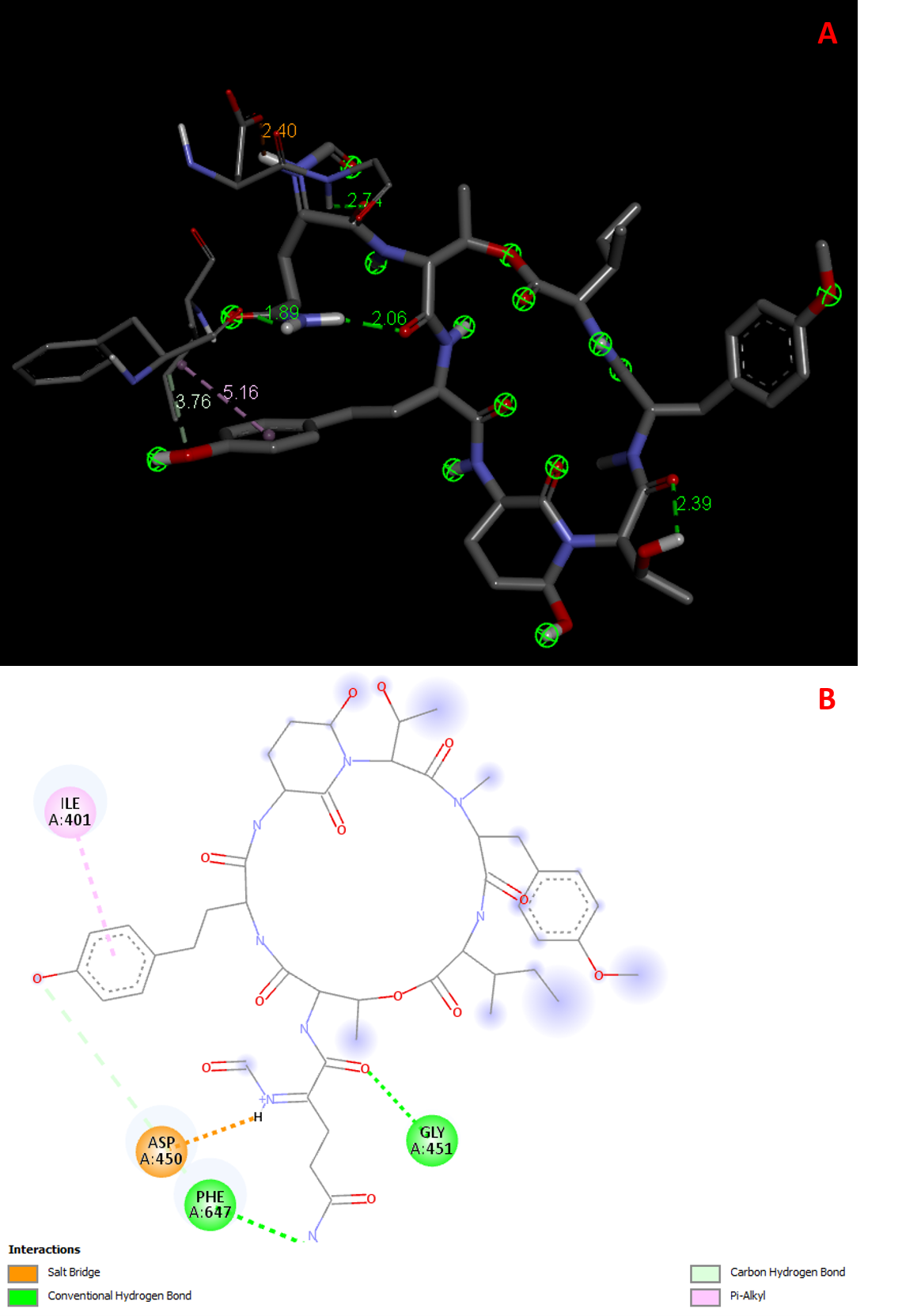
The molecular structure of Anabaenopeptilide 90A is illustrated in Figure 1. The lowest binding affinity observed between Anabaenopeptilide 90A and Glucan-binding Protein C (GbpC) was -8.7 kcal/mol (Table 2). Molecular docking results revealed that Anabaenopeptilide 90A interacts with GbpC through various interactions: one attractive charge with ASP402, five hydrogen bonds with residues TYR392, ASP408, SER347, and TRP45, and one carbon-hydrogen bond also with ASP402. Additionally, the interactions include one unfavorable donor-donor interaction with TRP451, one π-donor hydrogen bond with TYR355, two π-π stacked interactions with TRP351, one alkyl interaction with ALA409, and one π-alkyl interaction with ALA404 (Fig. 2 and Table 4).The binding affinity between Anabaenopeptilide 90A and Glucosyltransferase-I (GtfB) was -7.2 kcal/mol (Table 3). The interactions in this case include one salt bridge, two hydrogen bonds, one carbon-hydrogen bond, and one π-alkyl interaction (Fig. 3 and Table 5).



**Figure 1**. Molecular structure of a cyanobacterial polyketide Nostocyclophane D



**Figure 2.** Molecular interactions between the ligand Anabaenopeptilide 90A and Glucan-binding protein C (GbpC) showing one attractive charge, five hydrogen bonds, one carbon-hydrogen bond, one unfavourable donor-donor, one π-donor hydrogen, two π-π stacked, one alkyl and one π-alkyl interactions; A) Three-dimensional view, B) Two-dimensional view.



**Figure 3.** Molecular interactions between the ligand Anabaenopeptilide 90A and Glucosyltransferase-I (GtfB) showing one salt-bridge, two hydrogen bonds, one carbon-hydrogen bond, and one π-alkyl interactions; A) Three-dimensional view, B) Two-dimensional view.

**Table 2.** The table retrieved after molecular docking between Anabaenopeptilide 90A and Glucan-binding protein C (GbpC)

|  |  |  |  |
| --- | --- | --- | --- |
| **Ligand** | **Binding Affinity** | **rmsd/ub** | rmsd/lb |
| 6cam-1\_23724540\_uff\_E=2296.33\_uff\_E=1507.82 | -8.7 | 0 | 0 |
| 6cam-1\_23724540\_uff\_E=2296.33\_uff\_E=1507.82 | -8.5 | 3.048 | 2.207 |
| 6cam-1\_23724540\_uff\_E=2296.33\_uff\_E=1507.82 | -7.7 | 2.026 | 1.175 |
| 6cam-1\_23724540\_uff\_E=2296.33\_uff\_E=1507.82 | -7.7 | 10.261 | 3.891 |
| 6cam-1\_23724540\_uff\_E=2296.33\_uff\_E=1507.82 | -7.6 | 25.876 | 23.588 |
| 6cam-1\_23724540\_uff\_E=2296.33\_uff\_E=1507.82 | -7.4 | 23.119 | 20.238 |
| 6cam-1\_23724540\_uff\_E=2296.33\_uff\_E=1507.82 | -7.4 | 2.721 | 1.723 |
| 6cam-1\_23724540\_uff\_E=2296.33\_uff\_E=1507.82 | -7.2 | 4.484 | 3.133 |
| 6cam-1\_23724540\_uff\_E=2296.33\_uff\_E=1507.82 | -7.2 | 10.684 | 4.073 |

**Table 3.** The table retrieved after molecular docking between Anabaenopeptilide 90A and Glucosyltransferase-I (GtfB) showing binding affinity.

|  |  |  |  |
| --- | --- | --- | --- |
| **Ligand** | **Binding Affinity** | **rmsd/ub** | **rmsd/lb** |
| 8fkl-1\_23724540\_uff\_E=2296.33\_uff\_E=1507.82 | -7.2 | 0 | 0 |
| 8fkl-1\_23724540\_uff\_E=2296.33\_uff\_E=1507.82 | -7.1 | 8.632 | 3.21 |
| 8fkl-1\_23724540\_uff\_E=2296.33\_uff\_E=1507.82 | -7.1 | 31.443 | 24.435 |
| 8fkl-1\_23724540\_uff\_E=2296.33\_uff\_E=1507.82 | -6.9 | 9.349 | 4.516 |
| 8fkl-1\_23724540\_uff\_E=2296.33\_uff\_E=1507.82 | -6.8 | 8.851 | 2.986 |
| 8fkl-1\_23724540\_uff\_E=2296.33\_uff\_E=1507.82 | -6.7 | 9.149 | 3.902 |
| 8fkl-1\_23724540\_uff\_E=2296.33\_uff\_E=1507.82 | -6.7 | 28.655 | 23.291 |
| 8fkl-1\_23724540\_uff\_E=2296.33\_uff\_E=1507.82 | -6.6 | 59.092 | 52.807 |
| 8fkl-1\_23724540\_uff\_E=2296.33\_uff\_E=1507.82 | -6.6 | 43.666 | 37.657 |

**Table 4.** The table showing bond interactions and its length between Anabaenopeptilide 90A and Glucosyltransferase-I (GtfB) showing amino acid residues involves in it

|  |  |  |  |
| --- | --- | --- | --- |
| **Bond Interactions** | **No. of bonds** | **Bond length (Å)** | **Amino acid residue** |
| Salt Bridge | 1 | 2.4 | ASP450 |
| Conventional Hydrogen Bond | 2 | 1.89 | PHE647 |
| 2.74 | GLY451 |
| Carbon Hydrogen Bond | 1 | 3.76 | PHE647 |
| Pi-Alkyl | 1 | 5.16 | ILE401 |
| Total number of interactions | 5 |  |  |

**Table 5.** The table showing bond interactions and its length between Anabaenopeptilide 90A and Glucan-binding protein C (GbpC) showing amino acid residues involves in it

|  |  |  |  |
| --- | --- | --- | --- |
| **Bond Interactions** | **No. of bonds** | **Bond length (Å)** | **Amino acid residue** |
| Attractive Charge | 1 | 5.21 | ASP402 |
| Conventional Hydrogen Bond | 5 | 2.15 | ASP408 |
| 2.21 | SER347 |
| 2.94 | TRP451 |
| 2.22 | TYR392 |
| 2.46 | TYR392 |
| Carbon Hydrogen Bond | 1 | 3.69 | ASP402 |
| Unfavourable Donor-Donor | 1 | 1.02 | TRP451 |
| Pi-Donor Hydrogen Bond | 1 | 2.93 | TYR355 |
| Pi-Pi Stacked | 2 | 3.76 | TRP351 |
| 4.58 | TRP351 |
| Alkyl | 1 | 5.27 | ALA409 |
| Pi-Alkyl | 1 | 4.32 | ALA404 |
| Total number of interactions | 13 |  |  |

# Discussion

Anabaenopeptilide 90A, a cyclic depsipeptide isolated from Anabaena cyanobacteria, has shown significant potential as an antibiofilm agent against Streptococcus mutans, a major contributor to dental caries. This study employed molecular docking to investigate the interactions between Anabaenopeptilide 90A and two crucial proteins involved in biofilm formation: Glucan-binding protein C (GbpC) and Glucosyltransferase-I (GtfB).The binding affinity of Anabaenopeptilide 90A with GbpC was determined to be -8.7 kcal/mol, indicating a strong interaction between the compound and the protein. GbpC is known for its role in binding to glucans, contributing to the structural integrity of S. mutans biofilms [(Ahn et al., 2010)](https://paperpile.com/c/7qnS0v/gNzN). Molecular docking revealed multiple interactions between Anabaenopeptilide 90A and GbpC, including hydrogen bonds with TYR392, ASP408, SER347, and TRP45, as well as π-π stacked interactions with TRP351. These interactions suggest that Anabaenopeptilide 90A may interfere with GbpC's ability to bind glucans, thereby disrupting biofilm formation and reducing the adherence of S. mutans to tooth surfaces.Furthermore, Anabaenopeptilide 90A exhibited a binding affinity of -7.2 kcal/mol with GtfB, a critical enzyme responsible for synthesizing glucans from sucrose within the biofilm matrix [(Koo et al., 2010)](https://paperpile.com/c/7qnS0v/sG2h). The interactions observed in the docking study included a salt-bridge, hydrogen bonds, carbon-hydrogen bonds, and π-alkyl interactions. These interactions suggest that Anabaenopeptilide 90A may inhibit the enzymatic activity of GtfB, thereby reducing the production of glucans essential for biofilm formation.The ability of Anabaenopeptilide 90A to target both GbpC and GtfB highlights its potential as a multifaceted antibiofilm agent. Targeting multiple proteins involved in biofilm formation is advantageous as it reduces the likelihood of resistance development and enhances therapeutic efficacy [(R. K. Singh et al., 2011)](https://paperpile.com/c/7qnS0v/563L). Moreover, the specificity of interactions observed in the molecular docking study provides insights into the structural features of Anabaenopeptilide 90A that contribute to its antibiofilm activity.While molecular docking provides valuable predictions of ligand-protein interactions, it is essential to validate these findings through experimental studies. In vitro assays can confirm whether Anabaenopeptilide 90A effectively inhibits biofilm formation, disrupts bacterial adherence, and attenuates virulence factors associated with S. mutans pathogenicity [(Bowen et al., 2018)](https://paperpile.com/c/7qnS0v/zt96).

# Conclusion

In conclusion, the molecular docking study of Anabaenopeptilide 90A with GbpC and GtfB provides mechanistic insights into its potential as an antibiofilm agent against Streptococcus mutans. The strong binding affinities and specific interactions observed support further investigation of Anabaenopeptilide 90A for its therapeutic potential in combating dental caries. Future research should focus on experimental validation and optimization of Anabaenopeptilide 90A to enhance its efficacy and develop novel strategies for oral health management.

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