In Silico Evaluation of Nostophycin as an Antibiofilm Agent Against Biofilm-Inducing Proteins of Streptococcus Mutans

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**Abstract:**Biofilms formed by Streptococcus mutans are significant contributors to dental caries, posing challenges due to their resistance to conventional treatments. This study explores the potential of Nostophycin, a cyanobacterial polyketide, as an anti-biofilm agent targeting two key biofilm-inducing proteins: Glucan-binding protein C (GbpC) and Glucosyltransferase-I (GtfB). Using in silico molecular docking, we evaluated the interactions between Nostophycin and these proteins. Nostophycin exhibited a strong binding affinity to GbpC with a binding energy of -9.6 kcal/mol, forming multiple interactions including three van der Waals bonds, seven hydrogen bonds, and two π-π-stacked interactions. These interactions suggest that Nostophycin can significantly disrupt the function of GbpC, inhibiting the adhesion and accumulation of S. mutans within the biofilm matrix. Similarly, Nostophycin showed a binding affinity of -8.5 kcal/mol to GtfB, forming interactions such as five van der Waals bonds and two hydrogen bonds. By inhibiting GtfB, Nostophycin can impede glucan synthesis, weakening the biofilm structure. These findings highlight the potential of Nostophycin to prevent biofilm formation and destabilize existing biofilms. Future studies should validate these results in vitro and in vivo to confirm Nostophycin's efficacy and explore its application in dental care. This research underscores the potential of natural products like Nostophycin in developing innovative anti-biofilm strategies to combat dental caries and other biofilm-associated infections.

**Keywords:**Nostophycin, Streptococcus mutans, biofilm, molecular docking, anti-biofilm agent

# Introduction

Biofilms are complex microbial communities embedded within a self-produced extracellular matrix, adhering to both biotic and abiotic surfaces [(Ramsundar et al., 2023; Rieshy et al., 2023; Singh et al., 2023)](https://paperpile.com/c/5QA48N/FcJP5+asdqN+pt3m0). These structures confer significant advantages to the resident microorganisms, including enhanced resistance to antibiotics and immune responses, which complicates treatment strategies and contributes to chronic infections [(Flemming et al., 2016)](https://paperpile.com/c/5QA48N/NtHv). One of the primary pathogens implicated in biofilm-related dental diseases is Streptococcus mutans, a major causative agent of dental caries [(Bowen et al., 2018)](https://paperpile.com/c/5QA48N/QcXt). *Helicobacter pylori* was detected in 70% of children with severe carious lesions and was associated with higher caries status. Cavitated carious lesions may serve as a reservoir for *H. pylori*, contributing to increased caries severity [(Sruthi et al., 2023)](https://paperpile.com/c/5QA48N/u4t7). For example, dental caries remains highly prevalent among 5- to 12-year-old school children in Chandigarh, with 46.9% affected [(Prabakar et al., 2020)](https://paperpile.com/c/5QA48N/yd1O). A systematic review revealed significant genetic polymorphism among S. mutans strains, indicating variations between caries-active and caries-free children [(Ravikumar et al., 2021)](https://paperpile.com/c/5QA48N/sKBa). The ability of S. mutans to form robust biofilms is facilitated by specific proteins, notably Glucan-binding protein C (GbpC) and Glucosyltransferase-I (GtfB). Targeting these proteins could offer a promising approach to mitigating biofilm formation and subsequent dental pathologies [(Pavithra et al., 2023; Shenoy, Maiti, et al., 2023; Thomas & Jain, 2023)](https://paperpile.com/c/5QA48N/ppuU+5hox+Ta5c).S. mutans initiates biofilm formation by adhering to the tooth surface and producing extracellular polysaccharides (EPS), which serve as a scaffold for further microbial colonization and biofilm maturation [(Koo et al., 2013)](https://paperpile.com/c/5QA48N/lfwR). GbpC and GtfB are critical to this process. GbpC binds to glucans, facilitating the accumulation of S. mutans on tooth surfaces, while GtfB synthesizes insoluble glucans from sucrose, providing a matrix that anchors bacterial cells [(Banas & Vickerman, 2003)](https://paperpile.com/c/5QA48N/cD0k).Natural products have been a rich source of antimicrobial agents, and cyanobacteria have gained attention for their diverse bioactive compounds [(Burja et al., 2001)](https://paperpile.com/c/5QA48N/arqc). Nostophycin, a polyketide derived from cyanobacteria, has exhibited promising antimicrobial properties [(Fewer et al., 2011)](https://paperpile.com/c/5QA48N/cRwX). Polyketides are a class of secondary metabolites known for their structural diversity and potent biological activities, including antibacterial, antifungal, and anticancer effects [(Weissman & Leadlay, 2005)](https://paperpile.com/c/5QA48N/5vyq). Nostophycin’s unique chemical structure and bioactivity suggest potential as an antibiofilm agent, warranting further investigation into its interactions with key biofilm-inducing proteins of S. mutans.In silico molecular docking is a powerful tool used to predict the binding affinity and interaction patterns between small molecules and target proteins [(Pagadala et al., 2017)](https://paperpile.com/c/5QA48N/v8XD). This computational approach enables the identification of potential inhibitory compounds by simulating their interaction with protein targets at the molecular level [(Doshi et al., 2023; Lampl et al., 2023; Pandiyan et al., 2023)](https://paperpile.com/c/5QA48N/VPs4G+rRKOp+yZEYw). In this study, we employed molecular docking to explore the antibiofilm activity of Nostophycin against GbpC (PDB: 6CAM) and GtfB (PDB: 8FKL) of S. mutans. The objective was to elucidate the binding mechanisms and predict the efficacy of Nostophycin in disrupting biofilm formation.GbpC is a surface-associated protein that plays a pivotal role in the adherence and accumulation of S. mutans within the dental biofilm [(Ajdić et al., 2002)](https://paperpile.com/c/5QA48N/Kees). The binding of GbpC to glucans, synthesized by glucosyltransferases, facilitates the cohesion and stabilization of the biofilm matrix. Targeting GbpC could disrupt this crucial interaction, thereby hindering biofilm formation and maintenance.GtfB is an enzyme that catalyzes the synthesis of insoluble glucans from dietary sucrose, contributing to the EPS matrix of the biofilm [(Hanada & Kuramitsu, 1989)](https://paperpile.com/c/5QA48N/K7Ya). These glucans enhance the adhesion of S. mutans to tooth surfaces and provide a scaffold for biofilm architecture. Inhibiting GtfB activity would reduce glucan synthesis, weakening the biofilm structure and impairing the bacterium's ability to colonize and persist on the tooth surface.The primary aim of this study is to investigate the potential of Nostophycin as an anti-biofilm agent against Streptococcus mutans through in silico molecular docking studies. Specifically, we seek to:Determine the binding affinity and interaction patterns between Nostophycin and the biofilm-inducing proteins GbpC and GtfB.Evaluate the potential inhibitory effects of Nostophycin on biofilm formation by assessing its binding stability with these target proteins.Provide a molecular basis for the development of Nostophycin as a novel therapeutic agent for the prevention and treatment of dental cariesTo achieve these objectives, we employed the following methodological steps:The three-dimensional structures of GbpC (PDB: 6CAM) and GtfB (PDB: 8FKL) were obtained from the Protein Data Bank. The molecular structure of Nostophycin was prepared using cheminformatics tools(Saadh et al., 2024).The docking studies were performed using AutoDock Vina, which provides predictions of binding affinity and identifies key interaction residues [(Dallakyan & Olson, 2015; Morris et al., 2009; Morris & Lim-Wilby, 2008; Trott & Olson, 2010)](https://paperpile.com/c/5QA48N/iUel+dp74+IG8E+pcii).Analysis and Validation: The docking results were analyzed to determine the binding energy and interaction patterns, followed by validation using molecular dynamics simulations to assess the stability of the Nostophycin-protein complexes (Almatrafi et al., 2024).

Understanding the interactions between Nostophycin and biofilm-inducing proteins of S. mutans can offer valuable insights into the development of new strategies for preventing and treating biofilm-associated dental diseases [(Janani et al., 2021; Kachhara et al., 2021; Subramanian et al., 2023)](https://paperpile.com/c/5QA48N/G6PWF+K5dFV+b5BWo). If Nostophycin demonstrates strong binding affinity and effective inhibition of GbpC and GtfB, it could be developed into a potent anti-biofilm agent, providing an alternative to traditional antimicrobial treatments and addressing the growing issue of antibiotic resistance [(Shenoy, Ahmed, et al., 2022; Shenoy, Maiti, et al., 2023; Shenoy, Rajaraman, et al., 2022)](https://paperpile.com/c/5QA48N/5hox+kQ846+gngMW).This study aims to explore the antibiofilm potential of Nostophycin against Streptococcus mutans through in silico molecular docking. By targeting the biofilm-inducing proteins GbpC and GtfB, Nostophycin could disrupt biofilm formation and offer a novel approach to managing dental caries. The findings of this research may pave the way for new therapeutic strategies that leverage natural products to combat biofilm-associated infections.

# Materials and Methods

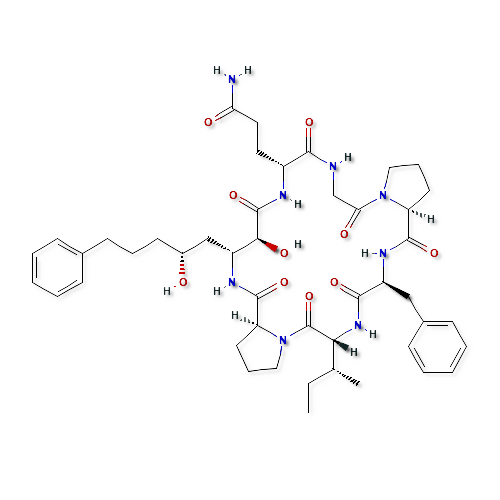
Nostophycin (C46H64N8O10) is a cyanobacterial polyketide with a molecular weight of 889 g/mol. For this study, Nostophycin was used as the ligand, with its structure obtained from PubChem (PubChem CID: 10724374) provided by the National Library of Medicine, NCBI, and NIH. Two key biofilm-inducing marker proteins, Glucan-binding protein C (GbpC) (PDB: 6CAM) [(Mieher et al., 2018)](https://paperpile.com/c/5QA48N/J0VA) and Glucosyltransferase-I (GtfB) (PDB: 8FKL) [(Schormann et al., 2023)](https://paperpile.com/c/5QA48N/9tsk) from Streptococcus mutans, were selected for the study. The molecular structures of these proteins were retrieved from the RCSB Protein Data Bank. The structures were processed to remove unwanted ligands, chains, and water molecules, and polar charges were added using BIOVIA Discovery Studio Visualizer 2024 (v24.1.0.23298) by Dassault Systems Biovia Corp. Molecular docking was conducted between Nostophycin and the biofilm-inducing proteins GbpC and GtfB using PyRx-Python Prescription 0.8 with AutoDock Vina as the docking engine (14,17–20). The grid center and dimensions were adjusted, and the coordinates were recorded and summarized in Table 1. The best-fit model, based on the lowest binding affinity, was derived, and the bond interactions between Nostophycin and the proteins were visualized, analyzed, and documented using BIOVIA Discovery Studio Visualizer 2024.

**Table 1.** The grid centre and dimension parameters set for Glucan-binding protein C (GbpC) and Glucosyltransferase-I (GtfB)

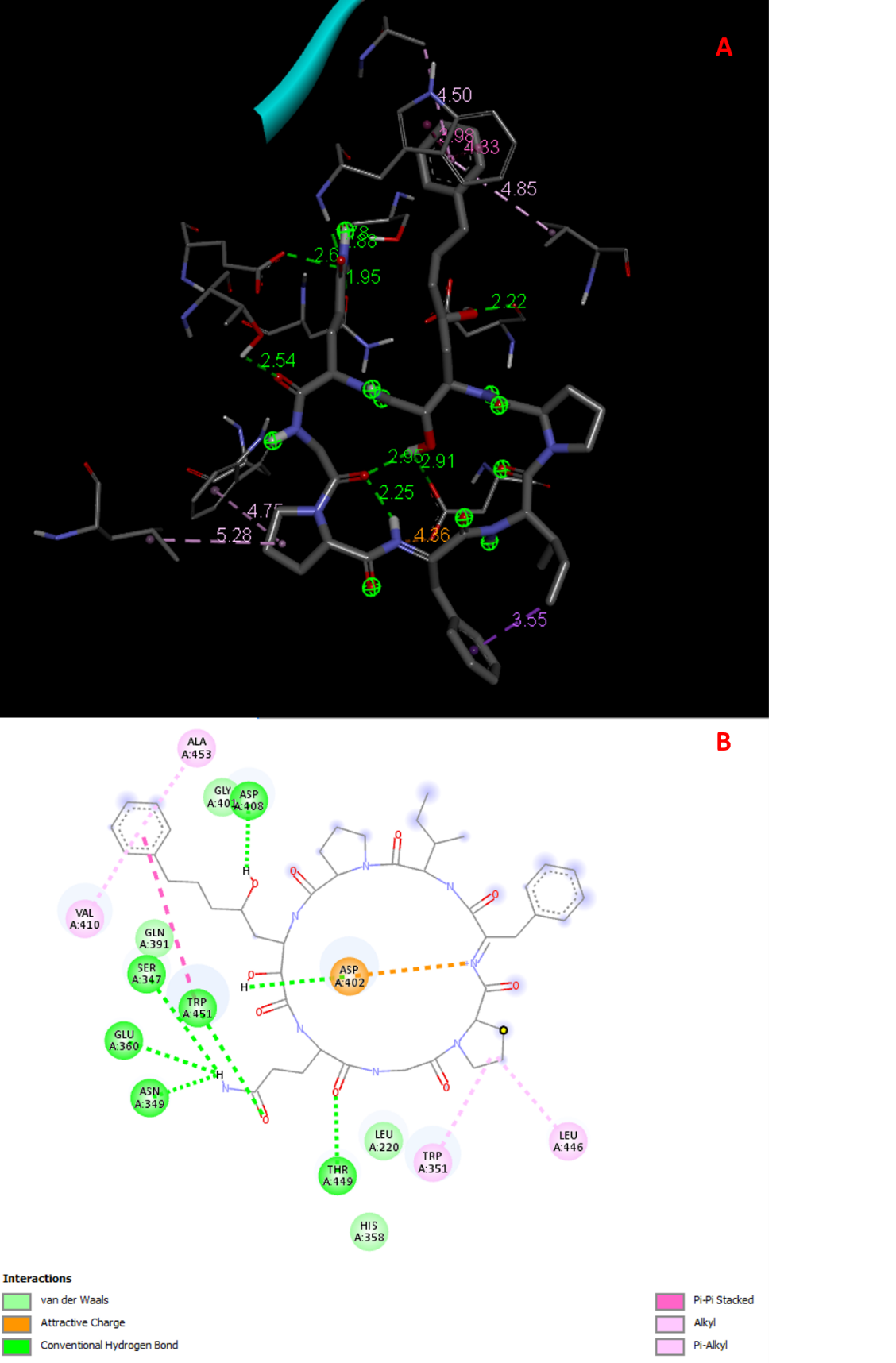
|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Grid Centre** | |  | **Dimensions (Å)** | | |
| Protein | PDB | X | Y | Z | X | Y | Z |
| Glucan-binding protein C (GbpC) | 6CAM | 235.49 | -24.96 | 8.07 | 95.38 | 75.53 | 79.65 |
| Glucosyltransferase-I (GtfB) | 8FKL | -10.82 | -21.33 | 17.5 | 79.4 | 96.49 | 88.57 |

# Results and Discussion

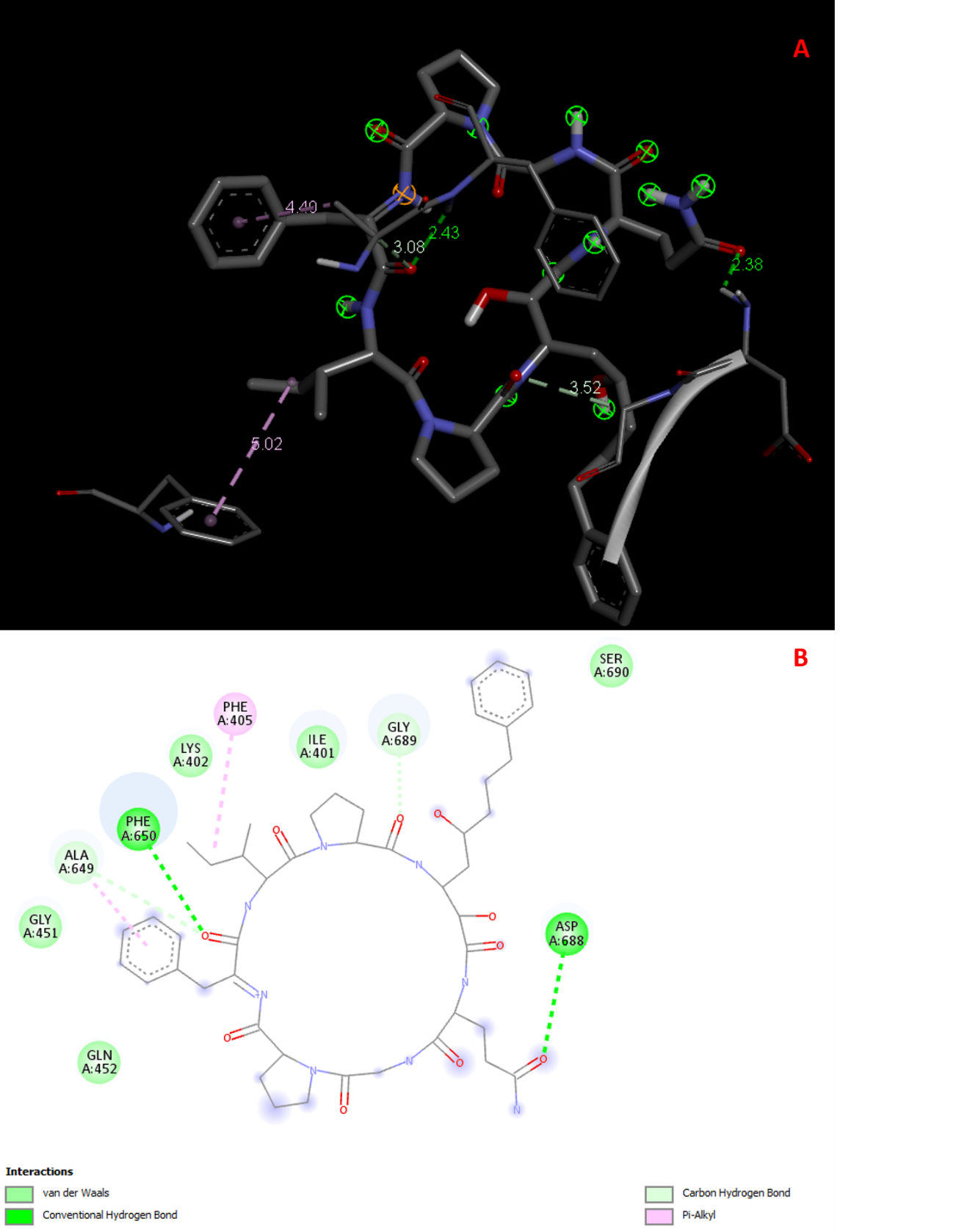
The molecular structure of Nostophycin is illustrated in Figure 1. The molecular docking results revealed that the lowest binding affinity between Nostophycin and Glucan-binding protein C (GbpC) was -9.6 kcal/mol (Table 2). Nostophycin interacted with GbpC through three van der Waals interactions (GLY401, LEU220, GLN391), seven conventional hydrogen bonds (ASP408, ASP402, THR449, TRP451, SER347, GLU360, ASN349), three π-alkyl interactions (TRP351, ALA453, VAL410), two π-π-stacked interactions (both with TRP451), one attractive charge (ASP402), and one alkyl interaction (LEU446) (Fig. 2 and Table 4). The binding affinity between Nostophycin and Glucosyltransferase-I (GtfB) was -8.5 kcal/mol (Table 3). The interactions with GtfB included five van der Waals interactions (SER690, ILE401, LYS402, GLY451, GLN452), two conventional hydrogen bonds (PHE650, ASP688), two carbon-hydrogen bonds (GLU689, ALA649), and two π-alkyl interactions (PHE405, ALA649) (Fig. 3 and Table 5).



**Figure 1.** Molecular structure of a cyanobacterial polyketide Nostophycin



**Figure 2.** Molecular interactions between the ligand Nostophycin and Glucan-binding protein C (GbpC) showing three van der Waals interactions (GLY401; Ley220; GLN391), seven conventional hydrogen bonds (ASP408; ASP402; THR449; TRP451; SER347; GLU360; ASN349), three π-alkyl interactions (TRP351; ALA453; VAL410), two π-π-stacked interactions (two for TRP451), one attractive charge (ASP402), and one alkyl interactions (LEU446); A) Three-dimensional view, B) Two-dimensional view.



**Figure 3.** Molecular interactions between the ligand Nostophycin and Glucosyltransferase-I (GtfB) showing include five van der Waals interactions (SER690; ILE401; LYS402; GLY451; GLN452), two conventional hydrogen bonds (PHE650; ASP688), two carbon-hydrogen bonds (GLU689; ALA649), and two π-alkyl interactions (PHE405; ALA649); A) Three-dimensional view, B) Two-dimensional view.

**Table 2.** The table retrieved after molecular docking between Nostophycin and Glucan-binding protein C (GbpC) showing binding affinity.

|  |  |  |  |
| --- | --- | --- | --- |
| **Ligand** | **Binding Affinity** | **rmsd/ub** | **rmsd/lb** |
| 6cam-1\_10724374\_uff\_E=1975.62 | -9.6 | 0 | 0 |
| 6cam-1\_10724374\_uff\_E=1975.62 | -9.6 | 2.497 | 1.479 |
| Table 2: continued | | | |
| 6cam-1\_10724374\_uff\_E=1975.62 | -9.4 | 7.527 | 2.06 |
| 6cam-1\_10724374\_uff\_E=1975.62 | -9.4 | 11.58 | 2.61 |
| 6cam-1\_10724374\_uff\_E=1975.62 | -9 | 25.919 | 21.592 |
| 6cam-1\_10724374\_uff\_E=1975.62 | -8.9 | 11.368 | 4.623 |
| 6cam-1\_10724374\_uff\_E=1975.62 | -8.9 | 7.581 | 2.881 |
| 6cam-1\_10724374\_uff\_E=1975.62 | -8.9 | 11.239 | 4.026 |
| 6cam-1\_10724374\_uff\_E=1975.62 | -8.8 | 8.442 | 4.963 |

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

|  |  |  |  |
| --- | --- | --- | --- |
| **Ligand** | **Binding Affinity** | **rmsd/ub** | **rmsd/lb** |
| 8fkl-1\_10724374\_uff\_E=1975.62 | -8.5 | 0 | 0 |
| 8fkl-1\_10724374\_uff\_E=1975.62 | -8.3 | 3.455 | 1.839 |
| 8fkl-1\_10724374\_uff\_E=1975.62 | -8.2 | 11.837 | 4.955 |
| 8fkl-1\_10724374\_uff\_E=1975.62 | -8 | 30.086 | 23.224 |
| 8fkl-1\_10724374\_uff\_E=1975.62 | -8 | 11.926 | 5.741 |
| 8fkl-1\_10724374\_uff\_E=1975.62 | -7.8 | 12.439 | 5.361 |
| 8fkl-1\_10724374\_uff\_E=1975.62 | -7.7 | 29.594 | 25.012 |
| 8fkl-1\_10724374\_uff\_E=1975.62 | -7.7 | 12.985 | 5.999 |
| 8fkl-1\_10724374\_uff\_E=1975.62 | -7.6 | 40.778 | 36.293 |

**Table 4.** The table showing bond interactions and its length between Nostophycin and Glucan-binding protein C (GbpC) showing amino acid residues involves in it

|  |  |  |  |
| --- | --- | --- | --- |
| **Bond Interactions** | **No. of bonds** | **Bond length (Å)** | **Amino acid residue** |
| Van der Waals interactions | 3 |  | GLY401  LEU220  GLN391 |
| Conventional hydrogen bonds | 7 | 2.22 | ASP408 |
| 2.91 | ASP402 |
| 2.54 | THR449 |
| 1.78 | TRP451 |
| 2.88 | SER347 |
| 2.63 | GLU360 |
| 1.95 | ASN349 |
| Attractive charge | 1 | 4.36 | ASP402 |
| π- π Stacked | 2 | 4.33 | TRP451 |
| 3.98 | TRP451 |
| π-alkyl | 3 | 4.75 | TRP351 |
| 4.50 | ALA453 |
| 4.85 | VAL410 |
| Total number of interactions | 16 |  |  |

**Table 5**. The table showing bond interactions and its length between Nostophycin and Glucosyltransferase-I (GtfB) showing amino acid residues involves in it

|  |  |  |  |
| --- | --- | --- | --- |
| **Bond Interactions** | **No. of bonds** | **Bond length (Å)** | **Amino acid residue** |
| Van der Waals interactions | 5 |  | SER690  ILE401  LYS402  GLY451  GLN452 |
| Conventional hydrogen bonds | 2 | 2.43 | PHE650 |
|  |  | 2.38 | ASP688 |
| π-alkyl | 2 | 5.02 | PHE405 |
|  |  | 4.40 | ALA649 |
| Carbon-hydrogen bond | 2 | 3.52 | GLY689 |
|  |  | 3.08 | ALA649 |
| Total number of interactions | 11 |  |  |

# Discussion

The study of Nostophycin, a cyanobacterial polyketide, as an antibiofilm agent against Streptococcus mutans through in silico molecular docking has provided compelling insights into its potential efficacy. The docking results indicate significant interactions between Nostophycin and two critical biofilm-inducing proteins, Glucan-binding protein C (GbpC) and Glucosyltransferase-I (GtfB), suggesting that Nostophycin could effectively disrupt biofilm formation and stability.The molecular docking results showed that Nostophycin exhibits a strong binding affinity to GbpC, with a binding energy of -9.6 kcal/mol (Table 2). This high affinity indicates a robust interaction, which is crucial for the potential inhibition of GbpC’s function in biofilm formation. The detailed interaction analysis revealed that Nostophycin forms three van der Waals interactions (GLY401, LEY220, GLN391) and seven conventional hydrogen bonds (ASP408, ASP402, THR449, TRP451, SER347, GLU360, ASN349). These hydrogen bonds are particularly significant as they contribute to the stability of the Nostophycin-GbpC complex [(Gandhi et al., 2021; Katyal et al., 2023; Priyadharshini et al., 2023)](https://paperpile.com/c/5QA48N/ck75y+Xyqiq+mvAQY).Additionally, the presence of three π-alkyl interactions (TRP351, ALA453, VAL410) and two π-π-stacked interactions with TRP451 suggests that aromatic and hydrophobic interactions play a vital role in the binding mechanism (Figure 2 and Table 4). The attractive charge interaction with ASP402 and the alkyl interaction with LEU446 further stabilize the complex, highlighting the multifaceted nature of the interaction between Nostophycin and GbpC.The inhibition of GbpC by Nostophycin could prevent the adhesion of S. mutans to the tooth surface, thereby disrupting the initial stages of biofilm formation. GbpC is known to bind glucans, facilitating the accumulation of S. mutans within the biofilm matrix [(Banas & Vickerman, 2003)](https://paperpile.com/c/5QA48N/cD0k). By interfering with GbpC, Nostophycin may reduce the bacterial load on tooth surfaces, thereby mitigating the progression of dental caries.Nostophycin also showed a significant binding affinity to Glucosyltransferase-I (GtfB), with a binding energy of -8.5 kcal/mol (Table 3). This interaction is characterized by five van der Waals interactions (SER690, ILE401, LYS402, GLY451, GLN452) and two conventional hydrogen bonds (PHE650, ASP688). The formation of hydrogen bonds with residues like ASP688 and PHE650 is essential for the stabilization of the Nostophycin-GtfB complex, which could inhibit the enzyme’s activity [(Chokkattu et al., 2023; Dharman et al., 2023; Govindaraj & Shanmugam, 2023)](https://paperpile.com/c/5QA48N/cGLFM+KVww7+9SUE7).The docking results also identified two carbon-hydrogen bonds (GLU689, ALA649) and two π-alkyl interactions (PHE405, ALA649), further supporting the stability and specificity of the interaction (Figure 3 and Table 5). These interactions suggest that Nostophycin can effectively bind to GtfB, potentially hindering its glucosyltransferase activity [(Rajeshkumar & Lakshmi, 2021; Sivakumar et al., 2021)](https://paperpile.com/c/5QA48N/StnR3+TkJGG). GtfB is crucial for synthesizing insoluble glucans from sucrose, which form the backbone of the EPS matrix in S. mutans biofilms [(Hanada & Kuramitsu, 1989)](https://paperpile.com/c/5QA48N/K7Ya). By inhibiting GtfB, Nostophycin may reduce the structural integrity of the biofilm, making it more susceptible to mechanical removal and antimicrobial agents.The molecular interactions between Nostophycin and the biofilm-inducing proteins GbpC and GtfB suggest multiple mechanisms by which Nostophycin could exert its anti-biofilm effects. The strong binding affinities and diverse interactions, including hydrogen bonds, van der Waals forces, π-alkyl, and π-π-stacked interactions, indicate that Nostophycin can effectively disrupt the function of these proteins.By targeting GbpC, Nostophycin may prevent the initial adhesion of S. mutans to tooth surfaces, thereby inhibiting biofilm formation at its earliest stages. In contrast, the inhibition of GtfB could impede the synthesis of the glucan matrix, essential for the structural stability and maturation of the biofilm [(Maheshwaran et al., 2024; Merchant et al., 2025; Shenoy, Rohinikumar, et al., 2023)](https://paperpile.com/c/5QA48N/zbkmY+VuKQe+PdGsg). These dual mechanisms highlight the potential of Nostophycin to both prevent biofilm formation and destabilize existing biofilms, offering a comprehensive approach to managing S. mutans infections.The in silico findings of this study provide a strong foundation for further research into Nostophycin as an anti-biofilm agent. Future work should focus on validating these results through in vitro and in vivo studies, assessing the efficacy of Nostophycin in disrupting S. mutans biofilms under realistic conditions. Additionally, exploring the synergistic effects of Nostophycin with existing antimicrobial agents could enhance its therapeutic potential and help overcome the challenges associated with biofilm-associated infections.

# Conclusion

This study demonstrates the potential of Nostophycin as a powerful anti-biofilm agent against Streptococcus mutans. The strong binding affinities and diverse interactions with GbpC and GtfB suggest that Nostophycin can effectively disrupt biofilm formation and stability. These findings pave the way for developing novel therapeutic strategies leveraging natural products to combat biofilm-associated infections, addressing the critical need for innovative antimicrobial approaches.

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