Revealing the Antibiofilm Activity of a Cyanobacterial Polyketide Anabaenopeptin on Inhibiting Biofilm Inducing Proteins of Streptococcus Mutans and Enterococcus Faecalis

R Madhumitha1 , H.Lokesh1,a)

1Madhu Dental Centre, Chennai, Tamil Nadu, India

Corresponding Author: a)[lakshmi18jogeswari@gmail.com](mailto:lakshmi18jogeswari@gmail.com)

**Abstract:** This study investigates the molecular interactions of Anabaenopeptin, a cyclic peptide isolated from cyanobacteria, with two bacterial proteins: VicK-like protein (CovS) and ABC transporter ATP-binding protein. Molecular docking simulations were employed to elucidate the binding affinities and interaction profiles of Anabaenopeptin with these target proteins. The results revealed a binding affinity of -7.5 kcal/mol between Anabaenopeptin and CovS, with interactions including Van der Waals forces, conventional hydrogen bonds, carbon-hydrogen bonds, alkyl bonds, and π-alkyl bonds. A stronger binding affinity of -8.2 kcal/mol was observed between Anabaenopeptin and the ABC transporter ATP-binding protein, characterized by a complex network of interactions including Van der Waals forces, π-sigma bonds, π-alkyl bonds, and alkyl interactions. The stronger affinity and diverse interaction profile with the ABC transporter protein suggest that Anabaenopeptin may have a more pronounced effect on cellular transport processes compared to its interaction with CovS. These findings provide valuable insights into the potential mechanisms of action of Anabaenopeptin and its possible role in modulating bacterial signal transduction and transport mechanisms. The study highlights the potential of Anabaenopeptin as a lead compound for the development of novel antimicrobial agents targeting crucial bacterial proteins. However, further experimental validation, including in vitro and in vivo assays, is necessary to confirm these computational predictions and fully elucidate the biological activities of Anabaenopeptin. This research contributes to our understanding of cyclic peptides as potential antibacterial agents and provides a foundation for future structure-activity relationship studies aimed at enhancing the specificity and potency of Anabaenopeptin-based compounds.

**Keywords:** Anabaenopeptin, molecular docking, CovS, ABC transporter, antimicrobial agents

# Introduction

Biofilms are complex microbial communities adhering to surfaces and encased in a self-produced extracellular matrix. These structures pose significant challenges in medical and industrial settings due to their enhanced resistance to antimicrobial agents and host immune responses [(Ramsundar et al., 2023; Rieshy et al., 2023; Singh et al., 2023)](https://paperpile.com/c/Y7GNFP/7WztW+SbKMa+DjuDn). Among the various microorganisms capable of forming biofilms, Streptococcus mutans and Enterococcus faecalis are of particular concern in oral and systemic infections. Streptococcus mutans, a gram-positive facultative anaerobe, is a primary etiological agent of dental caries [(Pavithra et al., 2023; Shenoy et al., 2023; Thomas & Jain, 2023)](https://paperpile.com/c/Y7GNFP/e0ZuB+B3ENe+upG41). Its ability to form biofilms on tooth surfaces contributes significantly to its virulence and persistence in the oral cavity [(Lemos et al., 2019)](https://paperpile.com/c/Y7GNFP/Ydwg). Similarly, Enterococcus faecalis, another gram-positive bacterium, is implicated in various nosocomial infections and is notorious for its ability to form biofilms on medical devices and tissues [(Ch’ng et al., 2019)](https://paperpile.com/c/Y7GNFP/qUxU). The biofilm-forming capabilities of these organisms present a formidable challenge in treatment and prevention strategies [(Chokkattu et al., 2023; Dharman et al., 2023; Govindaraj & Shanmugam, 2023)](https://paperpile.com/c/Y7GNFP/oezTf+LZ4a2+QEJkW).The process of biofilm formation is intricately regulated by various proteins and signaling molecules [(Gandhi et al., 2021; Katyal et al., 2023; Priyadharshini et al., 2023)](https://paperpile.com/c/Y7GNFP/A8XnU+nMrO2+UwpDA). In S. mutans, key proteins involved in biofilm formation include glucosyltransferases (GtfB, GtfC, and GtfD), which synthesize extracellular polysaccharides, and antigen I/II (also known as SpaP or P1), which mediates initial adherence to tooth surfaces [(Bowen & Koo, 2011)](https://paperpile.com/c/Y7GNFP/pCU0). For E. faecalis, important biofilm-inducing proteins include aggregation substance (AS), enterococcal surface protein (Esp), and gelatinase (GelE) [(Mohamed & Huang, 2007)](https://paperpile.com/c/Y7GNFP/159W). Given the increasing prevalence of antibiotic resistance and the limitations of current treatment options, there is a pressing need for novel approaches to combat biofilm-associated infections [(Janani et al., 2021; Kachhara et al., 2021; Subramanian et al., 2023)](https://paperpile.com/c/Y7GNFP/qzdlE+9Wo5L+6DblU). Natural products have emerged as a promising source of anti-biofilm compounds, with marine and freshwater organisms providing a vast reservoir of bioactive molecules [(Pérez et al., 2016)](https://paperpile.com/c/Y7GNFP/uhCl).Cyanobacteria, in particular, have garnered attention for their diverse array of secondary metabolites with potential therapeutic applications [(Doshi et al., 2023; Lampl et al., 2023; Pandiyan et al., 2023)](https://paperpile.com/c/Y7GNFP/UQ8Be+6syOx+XKVYQ). These photosynthetic prokaryotes produce a wide range of bioactive compounds, including peptides, polyketides, and alkaloids, many of which exhibit antimicrobial and anti-biofilm properties [(Vijayakumar & Menakha, 2015)](https://paperpile.com/c/Y7GNFP/rzsg). Anabaenopeptins, a class of cyclic peptides produced by various cyanobacterial genera, have shown promise in several biological activities, including protease inhibition and antimicrobial effects [(Spoof et al., 2015)](https://paperpile.com/c/Y7GNFP/fhN4). While the antibacterial properties of anabaenopeptins have been documented, their potential as anti-biofilm agents, particularly against S. mutans and E. faecalis, remains largely unexplored [(Rajeshkumar & Lakshmi, 2021; Sivakumar et al., 2021)](https://paperpile.com/c/Y7GNFP/LPrw+a6hE).In recent years, in silico molecular docking studies have emerged as valuable tools in drug discovery and development. These computational approaches allow for the rapid screening of large compound libraries against specific protein targets, providing insights into potential molecular interactions and mechanisms of action [(Kitchen et al., 2004)](https://paperpile.com/c/Y7GNFP/U7ch). By simulating the binding of ligands to protein targets, molecular docking can predict the likelihood of a compound's efficacy and guide further experimental investigations. The application of in silico techniques to study the interactions between anabaenopeptins and biofilm-inducing proteins of S. mutans and E. faecalis represents a novel approach in the search for effective anti-biofilm agents. This computational strategy can offer valuable insights into the potential mechanisms by which anabaenopeptins might inhibit biofilm formation in these clinically relevant pathogens.

# Materials and Methods

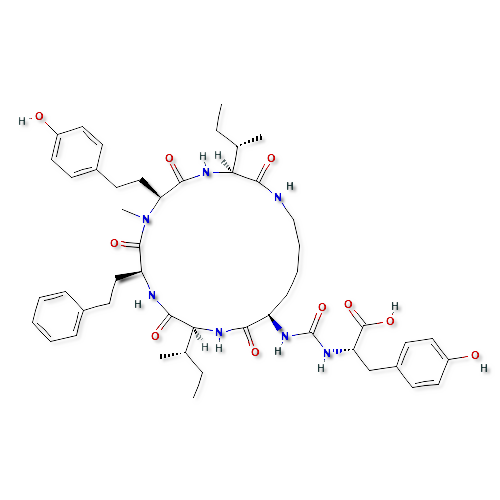
Anabaenopeptin MM913 (C49H67N7O10) is a cyanobacterial polyketide with a molecular weight of 914.1 g/mol. In this study, Anabaenopeptin was used as the ligand, with its structure (PubChem CID: 50924070) sourced from PubChem, NCBI, NIH. The research focused on two key biofilm-inducing proteins: the Putative histidine kinase CovS; VicK-like protein (PDB: 4I5S) from Streptococcus mutans [(Wang et al., 2013)](https://paperpile.com/c/Y7GNFP/4DJq) and the ABC transporter ATP-binding protein (PDB: 7OCY) from Enterococcus faecalis [(Meier et al., 2023)](https://paperpile.com/c/Y7GNFP/3I6W). The molecular structures of these proteins were obtained from the RCSB PDB. Both structures were processed to remove unnecessary 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 (Almatrafi et al., 2024). Molecular docking was carried out using the virtual screening software PyRx-Python Prescription 0.8 and AutoDock Vina as the docking engine [(Akshatha et al., 2021; Dallakyan & Olson, 2015; Trott & Olson, 2010)](https://paperpile.com/c/Y7GNFP/pPBR+hCmy+zJIW). The grid center and dimensions coordinates were adjusted and recorded in Table 1. The optimal binding model was determined based on the lowest binding affinity, and the interactions between Anabaenopeptin and the target proteins were visualized and analyzed using BIOVIA Discovery Studio Visualizer 2024(Saadh et al., 2024).

**Table 1.** The grid centre and dimension parameters set for VicK-like protein and ABC transporter ATP-binding protein

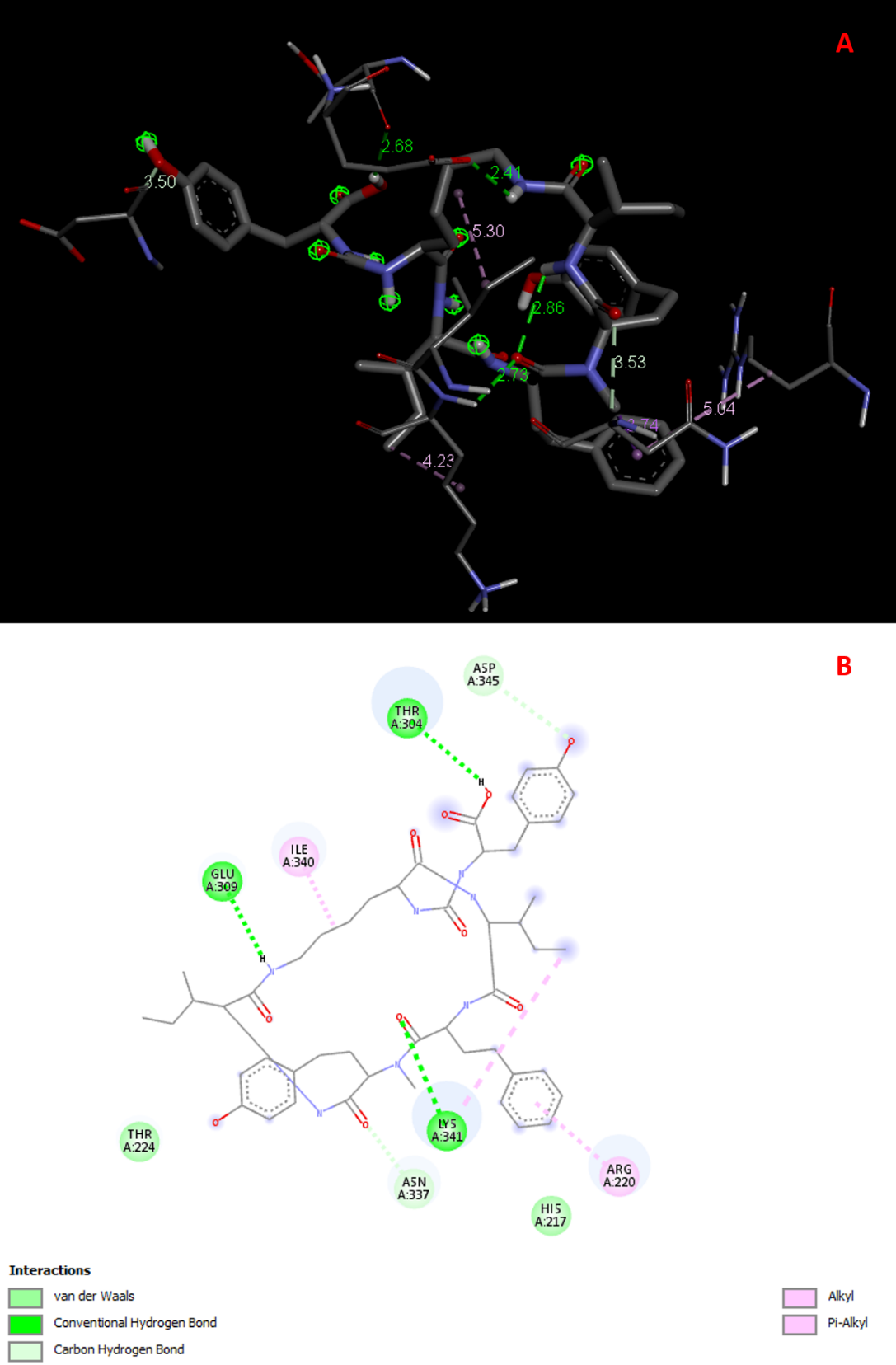
|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Grid Centre** | |  | **Dimensions (Å)** | | |
| Protein | PDB | X | Y | Z | X | Y | Z |
| VicK-like protein (CovS) | 4I5S | 26.99 | 63.26 | 5.90 | 140.82 | 74.95 | 157.09 |
| ABC transporter ATP-binding protein | 7OCY | 151.54 | 146.75 | 134.85 | 85.10 | 93.57 | 154.14 |

# Results and Discussion

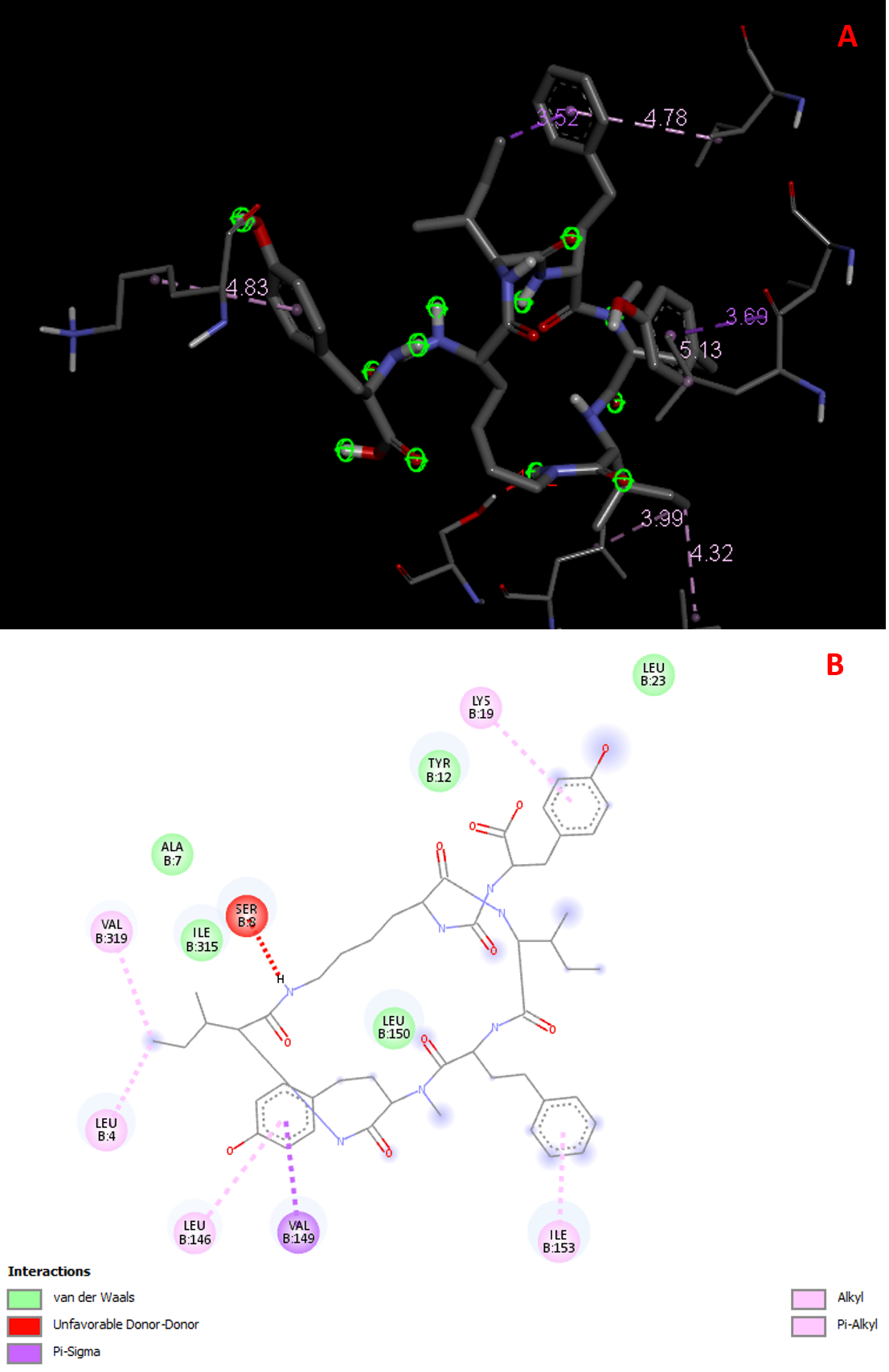
The molecular structure of Anabaenopeptin is shown in Figure 1. The lowest binding affinity between Anabaenopeptin and the VicK-like protein (CovS) was -7.5 kcal/mol (Table 2). Molecular docking revealed that Anabaenopeptin interacts with CovS through two Van der Waals interactions (THR224; HIS217), three conventional hydrogen bonds (LYS341; GLU309; THR304), two carbon-hydrogen bonds (ASN337; ASP345), one alkyl bond (ARG220), and two π-alkyl bonds (LYS341; ILE340) (Fig. 2 and Table 4). For the ABC transporter ATP-binding protein, the lowest binding affinity obtained with Anabaenopeptin was -8.2 kcal/mol (Table 3). The interactions observed include five Van der Waals interactions (LEU23; TYR12; ALA7; ILE315; LEU150), one unfavorable donor-donor interaction (SER8), one π-sigma bond (VAL149), three π-alkyl bonds (ILE153; LYS9; LEU146), and two alkyl interactions (LEU4; VAL319) (Fig. 3 and Table 5).



**Figure 1.** Molecular structure of a cyanobacterial polyketide Anabaenopeptin MM913



**Figure 2.** Molecular interactions between the ligand Anabaenopeptin and VicK-like protein (CovS) showing two Van der Waals interactions (THR224; HIS217), three conventional hydrogen bonds (LYS341; GLU309; THR304), two carbon-hydrogen bonds (ASN337; ASP345), one alkyl bond (ARG220), two π-alkyl bond (LYS341; ILE340) interactions; A) Three-dimensional view, B) Two-dimensional view.



**Figure 3.** Molecular interactions between the ligand Anabaenopeptin and ABC transporter ATP-binding protein showing five Van der Waals interactions (LEU23; TYR12; ALA7; ILE315; LEU150), one unfavourable donor-donor interaction (SER8), one π-sigma bond (VAL149), three π-alkyl bonds (ILE153; LYS9; LEU146), and two alkyl interactions (LEU4; VAL319); A) Three-dimensional view, B) Two-dimensional view.

**Table 2.** The table retrieved after molecular docking between Anabaenopeptin and VicK-like protein (CovS) showing binding affinity.

|  |  |  |  |
| --- | --- | --- | --- |
| **Ligand** | **Binding Affinity** | **rmsd/ub** | **rmsd/lb** |
| 4i5s\_50924070\_uff\_E=26910723590.36\_mmff94\_E=177.74 | -7.5 | 0 | 0 |
| 4i5s\_50924070\_uff\_E=26910723590.36\_mmff94\_E=177.74 | -7.5 | 9.295 | 3.311 |
| 4i5s\_50924070\_uff\_E=26910723590.36\_mmff94\_E=177.74 | -7.2 | 9.356 | 3.105 |
| 4i5s\_50924070\_uff\_E=26910723590.36\_mmff94\_E=177.74 | -7 | 29.978 | 24.071 |
| 4i5s\_50924070\_uff\_E=26910723590.36\_mmff94\_E=177.74 | -6.9 | 29.443 | 22.627 |
| 4i5s\_50924070\_uff\_E=26910723590.36\_mmff94\_E=177.74 | -6.9 | 29.109 | 22.686 |
| 4i5s\_50924070\_uff\_E=26910723590.36\_mmff94\_E=177.74 | -6.9 | 9.668 | 5.354 |
| 4i5s\_50924070\_uff\_E=26910723590.36\_mmff94\_E=177.74 | -6.8 | 62.465 | 57.304 |
| 4i5s\_50924070\_uff\_E=26910723590.36\_mmff94\_E=177.74 | -6.7 | 9.161 | 3.35 |

**Table 3.** The table retrieved after molecular docking between Anabaenopeptin and ABC transporter ATP-binding protein showing binding affinity.

|  |  |  |  |
| --- | --- | --- | --- |
| **Ligand** | **Binding Affinity** | **rmsd/ub** | **rmsd/lb** |
| 7ocy\_50924070\_uff\_E=26910723590.36\_mmff94\_E=177.74 | -8.2 | 0 | 0 |
| 7ocy\_50924070\_uff\_E=26910723590.36\_mmff94\_E=177.74 | -8 | 5.363 | 3.134 |
| 7ocy\_50924070\_uff\_E=26910723590.36\_mmff94\_E=177.74 | -7.8 | 2.312 | 1.732 |
| 7ocy\_50924070\_uff\_E=26910723590.36\_mmff94\_E=177.74 | -7.7 | 40.018 | 35.043 |
| 7ocy\_50924070\_uff\_E=26910723590.36\_mmff94\_E=177.74 | -7.6 | 9.681 | 2.531 |
| 7ocy\_50924070\_uff\_E=26910723590.36\_mmff94\_E=177.74 | -7.5 | 7.418 | 4.336 |
| 7ocy\_50924070\_uff\_E=26910723590.36\_mmff94\_E=177.74 | -7.5 | 10.059 | 3.392 |
| 7ocy\_50924070\_uff\_E=26910723590.36\_mmff94\_E=177.74 | -7.5 | 41.005 | 35.881 |
| 7ocy\_50924070\_uff\_E=26910723590.36\_mmff94\_E=177.74 | -7.4 | 10.646 | 2.737 |

**Table 4.** The table showing bond interactions and its length between Anabaenopeptin and VicK-like protein (CovS) showing amino acid residues involves in it

|  |  |  |  |
| --- | --- | --- | --- |
| **Bond Interactions** | **No. of bonds** | **Bond length (Å)** | **Amino acid residue** |
| Van der Waals interactions | 2 |  | THR224  HIS217 |
| Conventional hydrogen bonds | 3 | 2.73 | LYS341 |
| 2.41 | GLU309 |
| 2.68 | THR304 |
| Carbon-hydrogen bonds | 2 | 3.53 | ASN337 |
| 3.50 | ASP345 |
| Alkyl | 1 | 5.04 | ARG220 |
| π-alkyl | 2 | 4.23 | LYS341 |
|  |  | 5.30 | ILE340 |
| Total number of interactions | 10 |  |  |

**Table 5.** The table showing bond interactions and its length between Anabaenopeptin and ABC transporter ATP-binding protein showing amino acid residues involves in it

|  |  |  |  |
| --- | --- | --- | --- |
| **Bond Interactions** | **No. of bonds** | **Bond length (Å)** | **Amino acid residue** |
| Van der Waals interactions | 5 |  | LEU23  TYR12  ALA7  ILE315  LEU150 |
| Unfavourable donor-donor | 1 | 1.52 | SER8 |
| π-sigma | 1 | 3.69 | VAL149 |
| π-alkyl | 3 | 4.78 | ILE153 |
| 4.83 | LYS19 |
| 5.13 | LEU146 |
| Alkyl | 2 | 3.99 | LEU4 |
| 4.32 | VAL319 |
| Total number of interactions | 12 |  |  |

# Discussion

The molecular structure of Anabaenopeptin, as depicted in figure 1, provides valuable insights into its potential interactions with various proteins. This cyclic peptide, originally isolated from cyanobacteria, has garnered significant attention due to its diverse biological activities and potential therapeutic applications [(Welker & von Döhren, 2006)](https://paperpile.com/c/Y7GNFP/b9xJ). The recent molecular docking studies focusing on Anabaenopeptin's interactions with VicK-like protein (CovS) and ABC transporter ATP-binding protein offer intriguing results that warrant further discussion.The interaction between Anabaenopeptin and CovS, a histidine kinase involved in bacterial two-component signal transduction systems, demonstrates a binding affinity of -7.5 kcal/mol (Table 2). This relatively strong binding suggests a potential inhibitory effect on CovS function, which could have implications for bacterial virulence and survival [(Stock et al., 2000)](https://paperpile.com/c/Y7GNFP/u2fK). The molecular docking results, as illustrated in Fig. 2 and detailed in Table 4, reveal a complex network of interactions between Anabaenopeptin and CovS. The presence of two Van der Waals interactions (THR224 and HIS217) contributes to the overall stability of the ligand-protein complex. Additionally, the three conventional hydrogen bonds formed with LYS341, GLU309, and THR304 play a crucial role in anchoring Anabaenopeptin within the binding pocket of CovS [(Bissantz et al., 2010)](https://paperpile.com/c/Y7GNFP/RwKo).The carbon-hydrogen bonds involving ASN337 and ASP345, although weaker than conventional hydrogen bonds, further stabilize the complex. The alkyl bond with ARG220 and the two π-alkyl bonds with LYS341 and ILE340 provide hydrophobic interactions that are essential for ligand recognition and binding [(Salentin et al., 2015)](https://paperpile.com/c/Y7GNFP/YSYT). These diverse interactions collectively contribute to the observed binding affinity and suggest that Anabaenopeptin could potentially modulate CovS activity, thereby affecting bacterial signal transduction pathways.Interestingly, the molecular docking studies also revealed a stronger interaction between Anabaenopeptin and the ABC transporter ATP-binding protein, with a binding affinity of -8.2 kcal/mol (Table 3). ABC transporters are integral membrane proteins that play crucial roles in various cellular processes, including nutrient uptake and drug efflux [(Locher, 2009)](https://paperpile.com/c/Y7GNFP/yyuM). The higher binding affinity observed with this protein suggests that Anabaenopeptin may have a more pronounced effect on ABC transporter function compared to CovS.The interaction profile between Anabaenopeptin and the ABC transporter ATP-binding protein, as shown in Fig. 3 and detailed in Table 5, reveals a complex network of bonds. The five Van der Waals interactions involving LEU23, TYR12, ALA7, ILE315, and LEU150 provide a foundation for ligand recognition. The presence of an unfavorable donor-donor interaction with SER8 is noteworthy, as it may introduce some instability in the complex. However, this potential destabilization appears to be offset by the numerous favorable interactions observed [(Du et al., 2016)](https://paperpile.com/c/Y7GNFP/TjYB).The π-sigma bond with VAL149 and the three π-alkyl bonds involving ILE153, LYS9, and LEU146 contribute significantly to the stability of the complex through hydrophobic interactions. These interactions, along with the two alkyl interactions involving LEU4 and VAL319, play a crucial role in positioning Anabaenopeptin within the binding pocket of the ABC transporter ATP-binding protein [(Berman et al., 2000)](https://paperpile.com/c/Y7GNFP/GKpf).The stronger binding affinity and diverse interaction profile observed between Anabaenopeptin and the ABC transporter ATP-binding protein suggest that this cyclic peptide may have a more pronounced effect on ABC transporter function. This could potentially lead to alterations in cellular transport processes, which may have implications for bacterial survival and drug resistance.In conclusion, the molecular docking studies of Anabaenopeptin with CovS and ABC transporter ATP-binding protein provide valuable insights into the potential mechanisms of action of this cyclic peptide. The observed interactions and binding affinities suggest that Anabaenopeptin may have multifaceted effects on bacterial cellular processes, potentially modulating both signal transduction pathways and transport mechanisms. These findings open up new avenues for research into the development of novel antimicrobial strategies targeting these crucial bacterial proteins.Further experimental studies, including in vitro and in vivo assays, are necessary to validate these computational predictions and elucidate the full spectrum of Anabaenopeptin's biological activities. Additionally, structure-activity relationship studies could provide valuable information for the design of Anabaenopeptin analogues with enhanced specificity and potency against bacterial targets.

# Conclusion

In conclusion, molecular docking studies of Anabaenopeptin with CovS and ABC transporter ATP-binding protein reveal promising interactions, suggesting potential antimicrobial activity. The stronger affinity for the ABC transporter protein indicates a possible impact on bacterial transport mechanisms. These findings open new avenues for antimicrobial research, but require further experimental validation. Overall, this study provides a foundation for developing novel antibacterial agents based on the Anabaenopeptin structure.

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