In Silico Antibiofilm Activity of A Sulphated Polysaccharide Ulvan on Biofilm Inducing Proteins of Staphylococcus Aureus

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**Abstract:** Biofilms formed by Staphylococcus aureus pose significant challenges in both clinical and industrial settings due to their resistance to antibiotics and immune responses. This study investigates the potential of Ulvan, a sulphated polysaccharide derived from green seaweed, as an anti-biofilm agent against S. aureus. Using in silico molecular docking, we explored the interactions between Ulvan and key biofilm-inducing proteins, Surface protein G (SasG) and Biofilm-associated protein (Bap). The binding affinity between Ulvan and SasG was found to be -6.4 kcal/mol, with interactions including four van der Waals bonds, five hydrogen bonds, and one π-anion interaction. Ulvan’s interaction with Bap exhibited a stronger binding affinity of -7.9 kcal/mol, characterized by seven van der Waals interactions, nine hydrogen bonds, and one carbon-hydrogen bond. These interactions suggest that Ulvan can significantly disrupt the structural integrity and functional roles of these proteins, potentially inhibiting biofilm formation and maintenance. The findings indicate that Ulvan may serve as a potent anti-biofilm agent, offering a novel approach to managing S. aureus infections. Future research should focus on validating these in silico results through in vitro and in vivo studies, as well as exploring the synergistic effects of Ulvan with existing antibiotics. This study underscores the potential of harnessing natural polysaccharides like Ulvan to combat biofilm-associated infections, addressing an urgent need for innovative antimicrobial strategies.

**Keywords:** Ulvan, Staphylococcus aureus, biofilm, molecular docking, anti-biofilm agent

# Introduction

Biofilms, complex assemblies of microorganisms attached to surfaces and encased in a self-produced matrix, present significant challenges in both medical and industrial settings. Among the myriads of pathogens capable of forming biofilms, Staphylococcus aureus stands out due to its prevalence and the severity of infections it can cause[(Ramsundar et al., 2023; Rieshy et al., 2023; Singh et al., 2023)](https://paperpile.com/c/31AFth/1BXmC+LX1fr+3kKMr). Biofilm-associated infections are notoriously difficult to treat because the biofilm matrix provides a protective environment that enhances bacterial resistance to antibiotics and the host immune response [(Costerton et al., 1999)](https://paperpile.com/c/31AFth/gnNr). The Garlic-Lemon (Ga-Li) mixture showed antimicrobial efficacy comparable to 3% sodium hypochlorite in reducing microbial load in root canals of teeth with asymptomatic apical periodontitis, indicating its potential as a biocompatible alternative for endodontic irrigation [(Siddique et al., 2020)](https://paperpile.com/c/31AFth/RVe5). The antimicrobial effects of clove and cinnamon oils on clinically isolated resistant strains of Pseudomonas aeruginosa, E. coli, and Klebsiella pneumoniae, given their proven ability to reduce the virulence of drug-resistant bacteria and the growing concern of antibiotic resistance resulting from misuse [(Anandhi et al., 2022)](https://paperpile.com/c/31AFth/hX50). The selenium nanoparticles of *Capparis decidua* showed notable antibacterial effects against *E. coli* and *Lactobacillus* species [(Sneka & Santhakumar, 2021)](https://paperpile.com/c/31AFth/mkWy). Addressing this challenge requires innovative approaches, including the use of novel anti-biofilm agents[(Rajeshkumar & Lakshmi, 2021; Sivakumar et al., 2021)](https://paperpile.com/c/31AFth/acJtn+q7akf).One promising candidate in this regard is Ulvan, a sulphated polysaccharide derived from green seaweed, particularly from the genus Ulva. Ulvan has garnered attention due to its diverse biological activities, which include antioxidant, antiviral, and antibacterial properties [(Lahaye & Robic, 2007)](https://paperpile.com/c/31AFth/Twba). The sulphated groups in Ulvan are believed to play a critical role in its bioactivity, including its potential to disrupt biofilm formation[(Chokkattu et al., 2023; Dharman et al., 2023; Govindaraj & Shanmugam, 2023)](https://paperpile.com/c/31AFth/uSJMD+bnxmj+JKOp5).The ability of Staphylococcus aureus to form biofilms is primarily mediated by a variety of proteins and polysaccharides that facilitate adhesion to surfaces and the accumulation of biofilm matrix[(Gandhi et al., 2021; Katyal et al., 2023; Priyadharshini et al., 2023)](https://paperpile.com/c/31AFth/rAECp+jsSK7+HgWdl). Key proteins involved in this process include clumping factors (ClfA and ClfB), fibronectin-binding proteins (FnBPA and FnBPB), and biofilm-associated protein (Bap) [(Otto, 2013)](https://paperpile.com/c/31AFth/ONII). These proteins enable S. aureus to adhere to host tissues and implanted medical devices, initiating biofilm formation and leading to persistent infections.In silico molecular docking is a computational technique that allows researchers to predict the interaction between a small molecule and a target protein at the atomic level[(Janani et al., 2021; Kachhara et al., 2021; Subramanian et al., 2023)](https://paperpile.com/c/31AFth/2ElBl+dGkev+9n1wA). This method has become invaluable in the early stages of drug discovery, particularly for screening large libraries of compounds and understanding the molecular basis of ligand-protein interactions [(Pagadala et al., 2017)](https://paperpile.com/c/31AFth/cRmh). By simulating the docking of Ulvan with biofilm-inducing proteins of S. aureus, researchers can identify potential binding sites and predict the efficacy of Ulvan as an anti-biofilm agent[(Doshi et al., 2023; Lampl et al., 2023; Pandiyan et al., 2023)](https://paperpile.com/c/31AFth/fVfjG+IwUvI+iEzgT).The primary aim of this study is to explore the antibiofilm potential of Ulvan against Staphylococcus aureus using in silico molecular docking techniques. Specifically, we seek to:Identify and characterize the binding interactions between Ulvan and key biofilm-inducing proteins of S. aureus. Evaluate the potential inhibitory effects of Ulvan on biofilm formation by assessing its binding affinity and stability with target proteins. Provide a molecular basis for the development of Ulvan as a novel anti-biofilm agent. Previous studies have demonstrated the anti-biofilm activity of various polysaccharides, including those derived from marine sources. For instance, alginate and carrageenan have shown promise in inhibiting biofilm formation and disrupting established biofilms [(Zammuto et al., 2022)](https://paperpile.com/c/31AFth/Ozas). However, the antibiofilm activity of Ulvan, specifically against S. aureus, remains underexplored[(Pavithra et al., 2023; Shenoy et al., 2023; Thomas & Jain, 2023)](https://paperpile.com/c/31AFth/Kyd8V+3vjCw+x1LKv).Investigating Ulvan's interaction with biofilm-inducing proteins of S. aureus is crucial, given the urgent need for new strategies to combat biofilm-associated infections. The outcomes of this study could pave the way for the development of Ulvan-based therapies, potentially reducing the reliance on traditional antibiotics and mitigating the risk of antibiotic resistance[(Ramsundar et al., 2023; Rieshy et al., 2023; Singh et al., 2023)](https://paperpile.com/c/31AFth/1BXmC+LX1fr+3kKMr).To achieve our objectives, we will employ a combination of computational tools and techniques. The primary steps involved in this in silico study include:Protein and Ligand Preparation: The three-dimensional structures of biofilm-inducing proteins will be obtained from the Protein Data Bank (PDB). The structure of Ulvan will be modeled using cheminformatics tools.Molecular Docking: The docking studies will be performed using software such as AutoDock Vina, which allows for the prediction of binding affinity and identification of key interaction residues.The docking results will be analyzed to determine the binding energy and interaction patterns. The stability of the Ulvan-protein complexes will be further validated using molecular dynamics simulations.This study is expected to provide valuable insights into the potential of Ulvan as an anti-biofilm agent against Staphylococcus aureus. By elucidating the molecular interactions between Ulvan and biofilm-inducing proteins, we aim to contribute to the broader effort of developing effective anti-biofilm strategies. Ultimately, this research could lead to innovative treatments that improve patient outcomes and address the growing problem of biofilm-associated infections.

# Materials and Methods

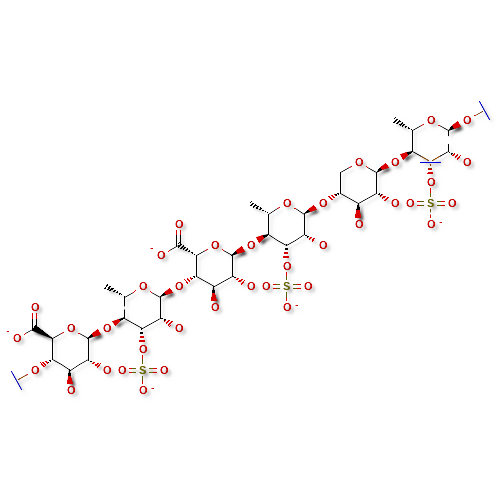
Ulvan, a cell wall sulfated polysaccharide found in green seaweeds, was used as the ligand in this study. The structure of Ulvan (PubChem CID: 405234592) was obtained from PubChem (National Library of Medicine, NCBI, NIH). Two key biofilm-inducing marker proteins, Surface protein G (SasG) (PDB: 4WVE) [(Gruszka et al., 2015)](https://paperpile.com/c/31AFth/zJO8) and Biofilm-associated surface protein (Bap) (PDB: 7C7R) [(Ma et al., 2021)](https://paperpile.com/c/31AFth/xopt) from Staphylococcus aureus, were selected for the study. The structures of these proteins were retrieved from the RCSB Protein Data Bank. The protein structures were visualized, and unnecessary 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 was performed with Ulvan and the biofilm-inducing proteins SasG and Bap using PyRx-Python Prescription 0.8 and AutoDock Vina (molecular docking engine) [(Akshatha et al., 2021; Dallakyan & Olson, 2015; Morris et al., 2009; Trott & Olson, 2010)](https://paperpile.com/c/31AFth/P1oh+4jeC+lMV4+5KKj). The grid center and dimension coordinates were adjusted, recorded, and tabulated in Table 1. The best-fit models were determined based on the lowest binding affinities, and the interactions between Ulvan and the proteins were visualized, interpreted, and recorded using BIOVIA Discovery Studio Visualizer 2024.

**Table 1.** The grid centre and dimension parameters set for Surface protein G (SasG) and Biofilm-associated surface protein (Bap)

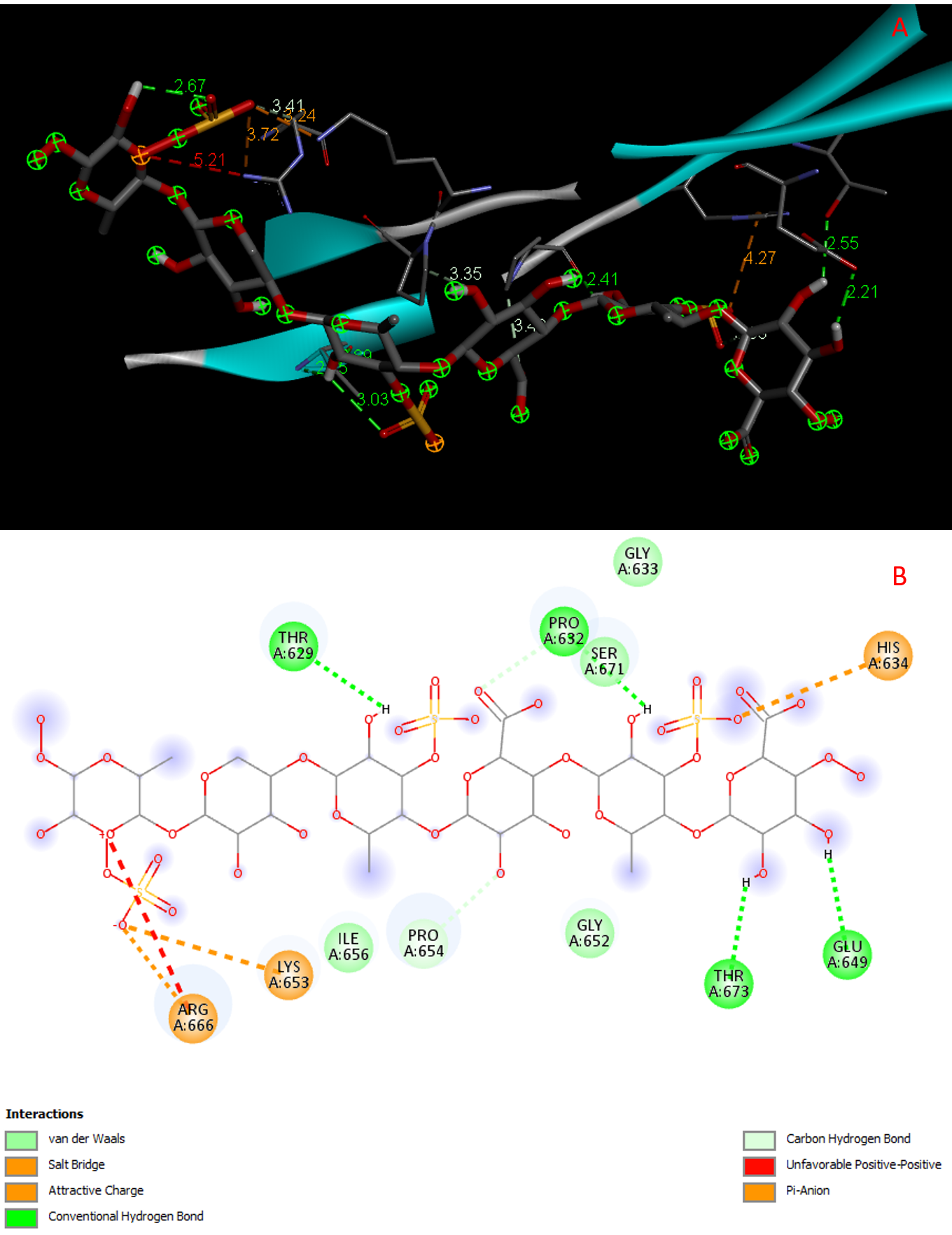
|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Grid Centre** | |  | **Dimensions (Å)** | | |
| Protein | PDB | X | Y | Z | X | Y | Z |
| Surface protein G (SasG) | 4WVE | 1.45 | -14.79 | 2.54 | 82.38 | 111.06 | 210.56 |
| Biofilm-associated surface protein (Bap) | 7C7R | 154.13 | 47.53 | 11.50 | 87.80 | 76.46 | 76.79 |

# Results

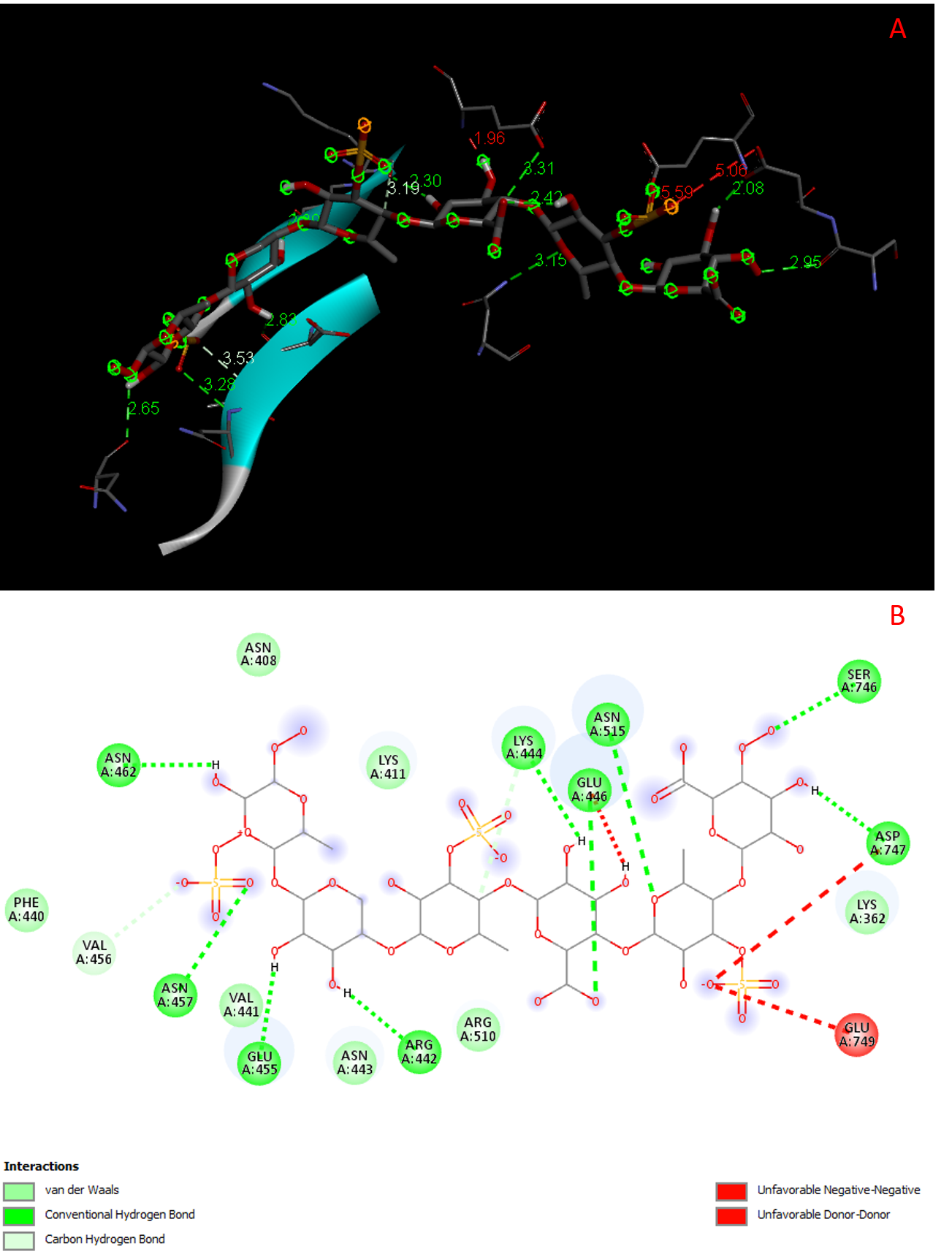
The molecular structure of Ulvan is shown in Figure 1. The lowest binding affinity between Ulvan and Surface protein G (SasG) was -6.4 kcal/mol (Table 2). Molecular docking revealed that Ulvan interacts with SasG through four van der Waals interactions (GLY633; SER671; GLY652; ILE656), five conventional hydrogen bonds (two with THR629; PRO632; THR673; GLU649), three carbon-hydrogen bonds (PRO632; PRO654; ARG666), one unfavorable positive-positive interaction (ARG666), one salt bridge (LYS633), one attractive charge (ARG666), and one π-anion interaction (HIS634) (Fig. 2 and Table 4).The binding affinity between Ulvan and Biofilm-associated surface protein (Bap) was -7.9 kcal/mol (Table 3). The interactions include seven van der Waals contacts (ASN408; LYS411; LYS362; ARG510; ASN443; VAL441; PHE440), nine conventional hydrogen bonds (SER746; ASP747; GLU446; ASN515; LYS444; ARG442; GLU455; ASN457; ASN462), two unfavorable positive-positive interactions (ASP747; GLU749), one unfavorable donor-donor interaction (GLU446), and one carbon-hydrogen bond (VAL456) (Fig. 3 and Table 5).



**Figure 1.** Molecular structure of a sulphated polysaccharide Ulvan



**Figure 2.** Molecular interactions between the ligand Ulvan and Surface protein G (SasG) showing four van der Waals interactions (GLY633; SER671; GLY652; ILE656), five conventional hydrogen bonds (two THR629; PRO632; THR673; GLU649), three carbon-hydrogen bonds (PRO632; PRO654; ARG666), one unfavourable positive-positive interaction (ARG666), one salt bridge (LYS633), one attractive charge (ARG666), and one π-anion interactions (HIS634); A) Three-dimensional view, B) Two-dimensional view.



**Figure 3.** Molecular interactions between the ligand Ulvan and Biofilm-associated surface protein (Bap) showing include seven van der Waals interactions (ASN408; LYS411; LYS362; ARG510; ASN443; VAL441; PHE440), nine conventional hydrogen bonds (SER746; ASP747; GLU446; ASN515; LYS444; ARG442; G;U455; ASN457; ASN462), two unfavourable positive-positive interaction (ASP747; GLU749), one unfavourable donor-donor interaction (GLU446), and one carbon-hydrogen bond interaction (VAL456); A) Three-dimensional view, B) Two-dimensional view.

**Table 2.** The table retrieved after molecular docking between Ulvan and Surface protein G (SasG) showing binding affinity.

|  |  |  |  |
| --- | --- | --- | --- |
| **Ligand** | **Binding Affinity** | **rmsd/ub** | **rmsd/lb** |
| 4wve\_A\_405234592\_ghemical\_E=8567.53 | -6.4 | 0 | 0 |
| 4wve\_A\_405234592\_ghemical\_E=8567.53 | -6.3 | 1.943 | 2.484 |
| 4wve\_A\_405234592\_ghemical\_E=8567.53 | -6.2 | 1.44 | 1.914 |
| 4wve\_A\_405234592\_ghemical\_E=8567.53 | -5.7 | 2.884 | 17.608 |
| 4wve\_A\_405234592\_ghemical\_E=8567.53 | -5.6 | 16.845 | 25.885 |
| 4wve\_A\_405234592\_ghemical\_E=8567.53 | -5.6 | 1.64 | 2.441 |
| 4wve\_A\_405234592\_ghemical\_E=8567.53 | -5.5 | 11.839 | 22.736 |
| 4wve\_A\_405234592\_ghemical\_E=8567.53 | -5.4 | 9.691 | 17.39 |
| 4wve\_A\_405234592\_ghemical\_E=8567.53 | -5.4 | 54.714 | 64.019 |

**Table 3.** The table retrieved after molecular docking between Ulvan and Biofilm-associated surface protein (Bap) showing binding affinity.

|  |  |  |  |
| --- | --- | --- | --- |
| **Ligand** | **Binding Affinity** | **rmsd/ub** | **rmsd/lb** |
| 7c7r\_A\_405234592\_ghemical\_E=8567.53 | -7.9 | 0 | 0 |
| 7c7r\_A\_405234592\_ghemical\_E=8567.53 | -7.7 | 2.221 | 1.607 |
| 7c7r\_A\_405234592\_ghemical\_E=8567.53 | -7.6 | 2.244 | 1.66 |
| 7c7r\_A\_405234592\_ghemical\_E=8567.53 | -7.5 | 20.129 | 14.312 |
| 7c7r\_A\_405234592\_ghemical\_E=8567.53 | -7.5 | 42.987 | 39.447 |
| 7c7r\_A\_405234592\_ghemical\_E=8567.53 | -7.5 | 42.4 | 34.828 |
| 7c7r\_A\_405234592\_ghemical\_E=8567.53 | -7.4 | 2.589 | 1.638 |
| 7c7r\_A\_405234592\_ghemical\_E=8567.53 | -7.4 | 44.294 | 40.118 |
| 7c7r\_A\_405234592\_ghemical\_E=8567.53 | -7.4 | 19.573 | 9.324 |

**Table 4.** The table showing bond interactions and its length between Ulvan and Surface protein G (SasG) showing amino acid residues involves in it

|  |  |  |  |
| --- | --- | --- | --- |
| **Bond Interactions** | **No. of bonds** | **Bond length (Å)** | **Amino acid residue** |
| Van der Waals interactions | 4 |  | GLY633  SER671  GLY652 |
| Conventional hydrogen bonds | 5 | 2.75 | THR629 |
| 2.89 | THR629 |
| 2.41 | PRO632 |
| 2.55 | THR673 |
| 2.21 | GLU649 |
| Carbon-hydrogen bond | 3 | 3.49 | PRO632 |
| 3.35 | PRO654 |
| 3.41 | ARG666 |
| Unfavourable positive-positive | 1 | 5.21 | ARG666 |
| Salt bridge | 1 | 3.24 | LYS633 |
| Attractive charge | 1 | 3.72 | ARG666 |
| π-anion | 1 | 4.27 | HIS634 |
| Total number of interactions | 16 |  |  |

# Discussion

The investigation of Ulvan, a sulphated polysaccharide, against Staphylococcus aureus biofilm-inducing proteins through in silico molecular docking has provided significant insights into its potential as an anti-biofilm agent. The findings demonstrate promising interactions between Ulvan and key proteins involved in biofilm formation, suggesting that Ulvan could disrupt biofilm integrity and prevent S. aureus from establishing persistent infections.The molecular docking results revealed that Ulvan interacts with Surface protein G (SasG) with a binding affinity of -6.4 kcal/mol (Table 2). This interaction involves multiple types of bonds, which play crucial roles in stabilizing the Ulvan-SasG complex (Figure 2 and Table 4). Specifically, four van der Waals interactions (GLY633, SER671, GLY652, ILE656) and five conventional hydrogen bonds (two THR629, PRO632, THR673, GLU649) highlight the strong affinity between Ulvan and SasG. Additionally, three carbon-hydrogen bonds (PRO632, PRO654, ARG666) and one salt bridge (LYS633) further stabilize the complex.Interestingly, the presence of an unfavorable positive-positive interaction (ARG666) and an attractive charge (ARG666) suggests that Ulvan’s binding to SasG might induce conformational changes in the protein(Saadh et al., 2024). The π-anion interaction with HIS634 also indicates a significant non-covalent interaction contributing to the binding affinity (Almatrafi et al., 2024). The diverse nature of these interactions underscores the potential of Ulvan to interfere with the structural integrity of SasG, potentially inhibiting its role in biofilm formation.Ulvan exhibited an even stronger interaction with the Biofilm-associated surface protein (Bap), with a binding affinity of -7.9 kcal/mol (Table 3). This higher affinity suggests that Ulvan is particularly effective against Bap, a critical protein in the formation and maintenance of S. aureus biofilms. The docking results showed a complex network of interactions, including seven van der Waals interactions (ASN408, LYS411, LYS362, ARG510, ASN443, VAL441, PHE440) and nine conventional hydrogen bonds (SER746, ASP747, GLU446, ASN515, LYS444, ARG442, GLU455, ASN457, ASN462) (Figure 3 and Table 5).The strong hydrogen bonding network suggests that Ulvan can form a highly stable complex with Bap, potentially disrupting its function. However, the presence of two unfavorable positive-positive interactions (ASP747, GLU749) and one unfavorable donor-donor interaction (GLU446) indicates that while the overall binding affinity is high, there are areas of electrostatic repulsion that might affect the binding dynamics. The single carbon-hydrogen bond interaction (VAL456) further stabilizes the complex but to a lesser extent compared to the hydrogen bonds and van der Waals interactions.The extensive interactions between Ulvan and these biofilm-inducing proteins suggest multiple mechanisms by which Ulvan could exert its anti-biofilm effects.

**Table 5.** The table showing bond interactions and its length Ulvan and Biofilm-associated surface protein (Bap) showing amino acid residues involves in it

|  |  |  |  |
| --- | --- | --- | --- |
| Bond Interactions | No. of bonds | Bond length (Å) | Amino acid residue |
| Van der Waals interactions | 7 |  | ASN408  LYS411  LYS362  ARG510  ASN443  VAL441  PHE440 |
| Conventional hydrogen bonds | 9 | 2.95 | SER746 |
|  |  | 2.08 | ASP747 |
| 3.31 | GLU446 |
| 3.15 | ASN515 |
| 2.30 | LYS444 |
| 2.39 | ARG442 |
| 2.83 | GLU455 |
| 3.28 | ASN457 |
| 2.65 | ASN462 |
| Carbon-hydrogen bond | 1 | 3.53 | VAL456 |
| Unfavourable positive-positive | 2 | 5.06 | ASP747 |
| 5.59 | GLU749 |
| Unfavourable donor-donor | 1 | 1.96 | GLU446 |
| Total number of interactions | 20 |  |  |

The binding of Ulvan to SasG and Bap likely hinders these proteins' ability to mediate cell adhesion and biofilm matrix formation. This inhibition could prevent the initial stages of biofilm development, reducing the establishment of S. aureus biofilms on surfaces.Moreover, the structural modifications induced by Ulvan binding might disrupt the stability of existing biofilms, making the bacterial cells more susceptible to antibiotic treatment and immune clearance [(Costerton et al., 1999)](https://paperpile.com/c/31AFth/gnNr). The multi-faceted interaction profile of Ulvan, involving hydrogen bonds, van der Waals forces, and other non-covalent interactions, indicates that it can effectively target and disrupt the biofilm formation machinery of S. aureus.The results of this study provide a strong rationale for further exploration of Ulvan as an anti-biofilm agent. Given its natural origin and the complexity of its interactions with biofilm-inducing proteins, Ulvan could offer a novel approach to managing S. aureus infections, particularly those associated with biofilms. Future research should focus on validating these in silico findings through in vitro and in vivo studies, assessing the efficacy of Ulvan in preventing and disrupting biofilms in clinical settings [(Zammuto et al., 2022)](https://paperpile.com/c/31AFth/Ozas).Additionally, exploring the synergistic effects of Ulvan with existing antibiotics could enhance the therapeutic outcomes against S. aureus biofilm-associated infections. Understanding the molecular basis of Ulvan’s interactions with other biofilm-related proteins could also uncover broader applications for this polysaccharide in combating biofilms formed by other pathogenic bacteria.

# Conclusion

This study has highlighted the potential of Ulvan as a powerful anti-biofilm agent against Staphylococcus aureus, with strong binding affinities to key biofilm-inducing proteins SasG and Bap. The diverse and stable interactions formed between Ulvan and these proteins suggest significant disruption of biofilm formation and maintenance mechanisms. These findings pave the way for the development of novel anti-biofilm strategies incorporating Ulvan, addressing the critical need for new approaches to manage biofilm-associated infections.

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