

Molecular Modeling of Structures and Interaction of Short Peptides and Sortase Family Protein of Enterococcus Faecalis: Basis for Developing Peptide-Based Therapeutics Against Multidrug Resistant Strains

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The Enterococcus faecalis (*E. faecalis*) infection starts with initial adhesion to a host cell or abiotic surface by multiple adhesions on its cell membrane. The pathogenicity is due to virulence factors SrtA, SrtC, EbpA, EbpB, EbpC, and Aggregation Substance. *E. faecalis* developed resistance to the majority of standard therapies. Additionally, a notable key feature of *E. faecalis* is its ability to form biofilm in vivo. *E. faecalis* strains show resistance to aminoglycosides and β -lactam antibiotics with different degree of susceptibility. Sortases (SrtA and SrtC) are enzymes spatially localized at the septal region in majority of gram-positive bacteria during the cell cycle, which in-turn plays an important role in proper assembling of adhesive surface proteins and pilus on cell membrane. The studies have also proved that the both SrtA and SrtC were focally localized in *E. faecalis* and essential for efficient bacterial colonization and biofilm formation on the host tissue surfaces. Using homology modeling and protein-peptide flexible docking methods, we report here the detailed interaction between peptides and EfSrt (Q836L7) enzyme. Plausible binding modes between EfSrt and the selected short biofilm active peptides were revealed from protein-peptide flexible docking. The simulation data further revealed critical residues at the complex interface and provided more details about the interactions between the peptides and EfSrt. The flexible docking simulations showed that the peptide-EfSrt binding was achieved through hydrogen bonding, hydrophobic, and van der Waals interaction. The strength of interactions between peptide-EfSrt complexes were calculated using standard energy calculations involving non-bonded interactions like electrostatic, van der Waals, and hydrogen bonds.

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1Molecular modeling of structures and interaction of short peptides and 2Sortase family protein of *Enterococcus faecalis*: Basis for developing peptide- 3based therapeutics against multidrug resistant strains

4

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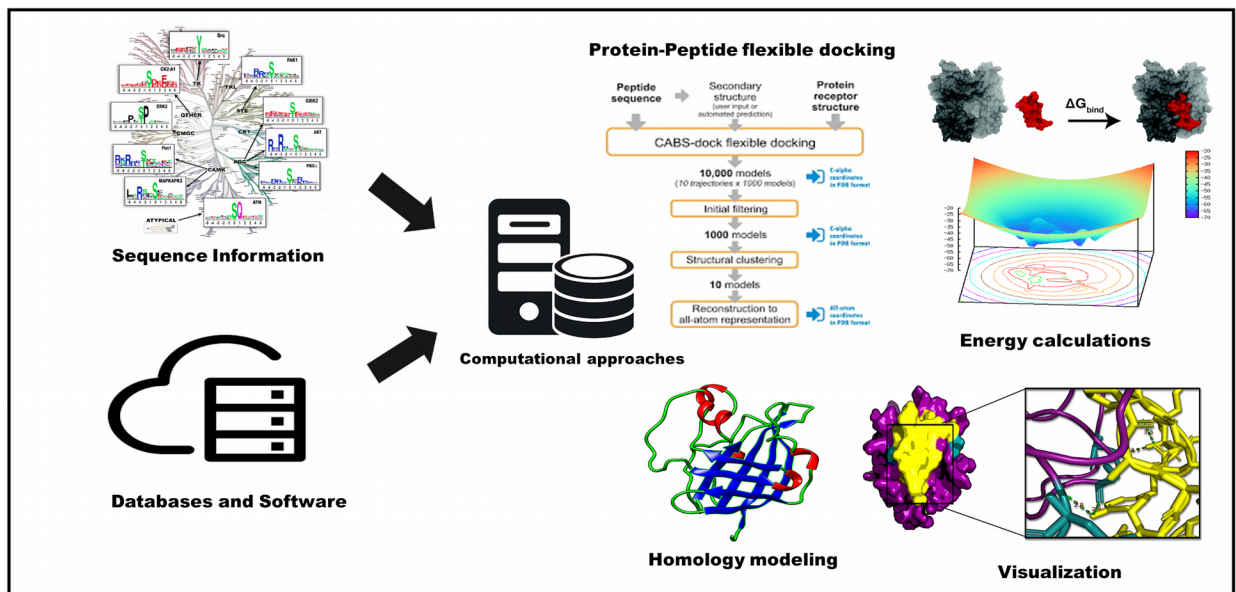
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11

12Graphical abstract



13

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2

15Abstract

16The *Enterococcus faecalis* (*E. faecalis*) infection starts with initial adhesion to a host cell or
17abiotic surface by multiple adhesions on its cell membrane. The pathogenicity is due to virulence
18factors SrtA, SrtC, EbpA, EbpB, EbpC, and Aggregation Substance. *E. faecalis* developed
19resistance to the majority of standard therapies. Additionally, a notable key feature of *E. faecalis*
20is its ability to form biofilm *in vivo*. *E. faecalis* strains show resistance to aminoglycosides and β -
21lactam antibiotics with different degree of susceptibility. Sortases (SrtA and SrtC) are enzymes
22spatially localized at the septal region in majority of gram-positive bacteria during the cell cycle,
23which in-turn plays an important role in proper assembling of adhesive surface proteins and pilus
24on cell membrane. The studies have also proved that the both SrtA and SrtC were focally
25localized in *E. faecalis* and essential for efficient bacterial colonization and biofilm formation on
26the host tissue surfaces Using homology modeling and protein-peptide flexible docking methods,
27we report here the detailed interaction between peptides and *Ef*Srt (Q836L7) enzyme. Plausible
28binding modes between *Ef*Srt and the selected short biofilm active peptides were revealed from
29protein-peptide flexible docking. The simulation data further revealed critical residues at the
30complex interface and provided more details about the interactions between the peptides and
31*Ef*Srt. The flexible docking simulations showed that the peptide-*Ef*Srt binding was achieved
32through hydrogen bonding, hydrophobic, and van der Waals interaction. The strength of
33interactions between peptide-*Ef*Srt complexes were calculated using standard energy calculations
34involving non-bonded interactions like electrostatic, van der Waals, and hydrogen bonds.

35**Keywords:** Drug resistance, *E. faecalis*, Biofilm, Sortase enzymes, protein-peptide docking,
36standard energy calculations.

37

38Introduction

39*Enterococcus faecalis* (*E. faecalis*) is a gram positive commensal opportunistic pathogen,
40responsible for various hospital acquired infections ¹. *E. faecalis* strains can overcome several
41harsh environments by forming biofilms on host tissues and abiotic surfaces. Furthermore, *E.*
42*faecalis* harbors virulence proteins on its cell membrane and an antimicrobial peptide sensing
43system to protect them against antibiotics and cationic antimicrobial peptides, respectively ².
44*E. faecalis* causes over 18,000 deaths a year, making it an important pathogen to be studied. Over
45the past three decades, various research groups have studied *E. faecalis* strains intensively to
46explore its interaction with hosts, determine the basis of pathogenesis, and virulence proteins
47localization to find effective treatment strategies ³. However, currently there is no effective drug
48available for its prevention and infections still exist. *E. faecalis* is also capable of forming
49biofilms and responsible for various biofilm-associated infections such as Urinary Tract Infection
50(UTI), surgical wound infection, and nosocomial bacteremia ⁴. The mature biofilm showed high
51tolerance to antibiotics than the planktonic bacteria, even at higher concentrations of 10–1000
52times ⁵. The *E. faecalis* infection starts with initial adhesion to a host cell or abiotic surface by
53multiple adhesions on its cell membrane. The pathogenicity is due to virulence factors SrtA,
54SrtC, EbpA, EbpB, EbpC, and Aggregation Substance ⁶. *E. faecalis* developed resistance to the
55majority of standard therapies ⁷. Additionally, a notable key feature of *E. faecalis* is its ability to
56form biofilm ⁸. *E. faecalis* strains show resistance to aminoglycosides and β -lactam antibiotics
57with different degree of susceptibility ⁹. It shows moderate resistance to former due to its low
58permeability through the cell wall (aminoglycoside molecules are larger) and intrinsic resistance
59to later due to over-expression of penicillin binding proteins. The antibiotics available today
60were not effective against Multiple Drug Resistance (MDR) enterococcal infections such

61endocarditis or bacteremia along with neutropenia ¹⁰. The antibiotics combinations such as
62ciprofloxacin with ampicillin, novobiocin with doxycycline, and penicillin with vancomycin
63were used to treat enterococcal infections ¹¹, but efficiency of this treatment remains doubtful.

64 Sortases (SrtA and SrtC) are enzymes spatially localized at the septal region in majority
65of gram-positive bacteria during the cell cycle, which in-turn plays an important role in proper
66assembling of adhesive surface proteins and pilus on cell membrane ¹². The studies have also
67proved that the both SrtA and SrtC were focally localized in *E. faecalis* and essential for efficient
68bacterial colonization and biofilm formation on the host tissue surfaces ¹³. The Sortase mutants
69produce defective pili and found to be less virulent than the wild type strain ¹⁴. The Spatial
70localization of virulence factors and Sortase mediated pilus assembly in *Enterococcus faecalis*
71was illustrated in **Figure 1**.

72Recent studies on antimicrobials suggest that peptides as possible drug candidates and to
73overcome drug resistance in *E. faecalis*. Moreover, studies have also shown that this species can
74rapidly acquire resistance even to cationic antimicrobial peptides such as β -beta defensins ¹⁵.
75Multiple peptide resistance Factors (mprF1 and mprF2) are integral membrane proteins
76responsible for developing resistance against cationic antimicrobial proteins in the majority of
77the gram-positive bacteria. Studies on *E. faecalis* suggested that mprF2 is essential for the
78aminoacylation of phosphatidylglycerol (PG) and synthesis of Lys-PG, Ala-PG, and Arg-PG
79variants. Whereas, mprF1 does not play a role in aminoacylation of PG. Furthermore, the
80aminoacylation of PG by mprF2 increases resistance against cationic antimicrobial peptides ¹⁵.
81The strain *E. faecalis* OG1RF circumvent antimicrobial peptides using MprF (Multiple peptide
82resistance Factors) protein assisted Antimicrobial peptide sensing system by altering the net
83surface charge of bacterial cell membrane to repel incoming antimicrobial peptides ¹⁶.

84 The peptides targeting Sortase family proteins were identified as potential therapeutics to
85 kill MDR bacterial strains, which is recently an emerging field in the drug discovery process ¹⁷.
86 Hence in this study we performed protein-peptide docking to identify potential biofilm active
87 peptides that can bind to Sortase family protein thereby inhibiting its function. The screened
88 peptides can be further used for designing novel peptide-based therapeutics against MDR *E.*
89 *faecalis* infection.

90 **Materials and methods**

91 **Computational platform**

92 All the computational simulations were carried out on the Linux Mint 18.3 Cinnamon 64-bit
93 platform in Lenovo G50-45 workstation on AMD A8-6410 APU @ 2.00GHz processor. All the
94 software and tools used in this study were open source platforms or free to use for academic
95 purposes.

96 **Primary and secondary structural analysis**

97 The sequence of *E. faecalis* Sortase family protein with accession ID Q836L7 was retrieved from
98 UNIPROT database ¹⁸ and its basic sequence information were calculated using ExPASy
99 ProtParam ¹⁹. The physicochemical properties of *EfSrt* including number of amino acid,
100 molecular weight (Mwt), amino acid composition, theoretical isoelectric point, aliphatic index,
101 *in vitro*/*in vivo* half-life instability index, and grand average of hydropathicity (GRAVY) were
102 theoretically calculated. The short antibiofilm peptides of length 10-30 were obtained from
103 BaAMPs database ²⁰ and their physico chemical characteristics were calculated using the inbuilt
104 module of BaAMPs database and used for tertiary structure modeling, docking analysis, and
105 energy calculations.

106

107

108Tertiary structure modelling

109The lack of crystallography structural data of *E. faecalis* Sortase family protein (Q836L7)
110remained as a bottleneck. Hence we performed homology modeling to depict the 3D structure ²¹.
111The obtained FASTA sequence was used as input to the PSI-BLAST with default parameters to
112find suitable the template for performing the homology modeling ²². The best template was
113selected from suggested templates based on high percentage of sequence identity, query
114coverage, and valid E-value. The homology modeling was performed using a standalone tool
115MODELLER v9.21 ²³. The results were ranked based on Discrete Optimized Protein Energy
116(DOPE) score, the models with least DOPE score were selected and administered to model
117validation ²⁴. The modeled 3D structure was refined using Galaxyrefine web server for better
118quality ²⁵. The refined 3D coordinates were analyzed for dihedral angles distribution using
119RAMPAGE web server ²⁶. Further, refined structure was validated using ProSA web server
120which provides an overall quality score for a modeled structure based on C α positions ²⁷. The
121reliability of modeled protein was assessed using the Superpose 1.0 web by superimposing the
122modeled structure of *EfSrt* with template structure and Root Mean Square Deviation (RMSD)
123was calculated ²⁸. The tertiary structure of ligand peptides was designed using CABS-dock ²⁹.
124Finally, the tertiary structure of *EfSrt* and peptides were visualized using Chimera v1.13.1 ³⁰.

125Protein-peptide flexible docking

126Sortase family proteins (SrtA,B, and C) play an important role in initial attachment of planktonic
127bacterial cells, and subsequent biofilm formation ³¹. In *E. faecalis*, the cell wall anchoring of
128virulence factors such as aggregation substance and pili were facilitated by Sortase enzymes.
129Therefore, Sortase family protein (Q836L7) was considered as the docking receptor and the

130antibiofilm active peptides were used as ligands. Finally, the receptor protein and peptides were
 131docked using CABS-dock standalone ³². In CABS-dock the modeled three dimensional structure
 132was used as receptor protein and peptide sequences along with secondary structure data was used
 133as ligand peptides ³³. The CABS-dock performs simulation search for the binding site allowing
 134for full flexibility of the peptide and small fluctuations of the receptor backbone. The CABS-
 135dock protocol consists of the following steps (i) Generating random structures, (ii) Simulation of
 136binding and docking, (iii) Selection of the final representative models, and (iv) Reconstruction of
 137the final models. When protein-peptide docking was performed using CABS-dock with default
 138settings, the structure of the peptide was kept as fully flexible and the structure of the protein
 139receptor was maintained near the initial conformation using soft distance restraints. The soft
 140distance restraints allow small fluctuations of the receptor backbone (1 Å) and large fluctuations
 141of the side chains. Based on the RMSD values of the cluster the top 10 complexes were sorted.
 142The Gibbs free energy (ΔG) and Dissociation constant (K_d) were used to explain the binding
 143strength or potential of the drug-protein complex during drug screening and therapeutics
 144development ³⁴. Therefore, ΔG and K_d of protein-peptide complexes were predicted using the
 145PRODIGY web server at 37°C ³⁵. The K_d value of protein-peptide complexes were calculated
 146using ΔG value obtained from PRODIGY using following equation,

$$147 \Delta G = RT \times \ln K_d \quad (1)$$

148where, R, ΔG , and T are the ideal gas constant, gibbs free energy, and temperature (Kelvin),
 149respectively. The binding energy was calculated as follows,

$$150 \Delta G = - 0.09459 \times IC_{\text{charged/charged}} - 0.10007 \times IC_{\text{charged/apolar}} + 0.19577 \times IC_{\text{polar/polar}} - 0.22671 \times IC_{\text{polar/apolar}} + \\ 151 0.18681 \times \%NIS_{\text{apolar}} + 0.3810 \times \%NIS_{\text{charged}} - 15.9433 \quad (2)$$

152where, IC_{XY} represents interfacial contacts in terms of physicochemical properties and %NIS
 153represents percentage of non-interacting surfaces in terms of physicochemical properties. The
 154interfacial contacts of protein-peptide complexes were analyzed using COCOMAPS ³⁶ and
 155PPCheck ³⁷ web server and visualized using Chimera v1.13.1.

156Non bonded Energy calculation of Protein-peptide complexes

157The strength of interactions between protein-protein complexes were calculated using standard
 158energy calculations involving non-bonded interactions like electrostatic, van der Waals, and
 159hydrogen bonds. The hydrogen atoms of protein-peptide complexes were fixed geometrically
 160and then hydrogen bond energy was calculated as follows,

$$161E = q_1q_2 [1/r(ON) + 1/r(CH) - 1/r(OH) - 1/r(CN)] * 332 * 4.184 \text{ kJ/mol} \quad (3)$$

162where q_1 and q_2 are partial atomic charges, $r()$ is the inter-atomic distance between the
 163corresponding atoms. The van der Waals interaction energies are calculated using equation (4)

$$164E = 4.184 (E_i E_j) \times [((R_i + R_j)/r)^{12} - 2((R_i + R_j)/r)^6] \text{ KJ/mol} \quad (4)$$

165where R is the Van der Waals radius for an atom, E is the van der Waals well depth, r is the
 166distance between the atoms. The electrostatic interaction energies for favourable as well as non
 167favourable interactions are calculated according to Coulomb's law by considering the
 168interprotomer charged atomic pairs at $\leq 10 \text{ \AA}$.

169Results and Discussion

170The antimicrobial peptides were identified as potential alternative therapy to treat MDR bacterial
 171infections. In the past two decades we have identified hundreds of peptides from natural sources
 172and studied their biological activity both *in vivo* and *in vitro* ³⁸. The studies on antibacterial
 173peptides showed that there is a relationship between structure and functions of these peptides ³⁹.
 174For example, Members of the defensin family are highly similar in protein sequence but they

175show differential antimicrobial activity ⁴⁰. Hence it is important to depict the structure-function
176relationship of these defensin peptides. The cationic antimicrobial peptide Human β -beta
177defensin 2 disrupts the localization pattern of membrane protein SrtA and SecA in *E. faecalis* ⁴¹,
178⁴². The studies have also proved that the both SrtA and SrtC were focally localized in *E. faecalis*
179and essential for efficient bacterial colonization and biofilm formation on the host tissue surfaces
180and was identified as an attractive drug target ⁴³. We performed protein-peptide flexible docking
181to identify potential biofilm active peptides that can bind to Sortase family protein thereby
182inhibiting its function. Also the identified peptide binding *Ef*Srt residues can be considered as
183potential target sites for the development of potential peptide based therapeutics against biofilm
184associated infections. Therefore, in this study biofilm active peptides collected from literature
185sources were screened to investigate its binding mechanism with *E. faecalis* SrtA.

186The primary sequence information of query sequence Q836L7 was theoretically calculated using
187ExPasy ProtParam suggests that the protein has molecular weight (32025.32) and found to be
188basic (theoretical PI of 9.57), stable (Instability Index < 40), hydrophilic (negative GRAVY
189value) in nature, and thermostable (higher AI value). Additionally, the half-life was theoretically
190calculated to be about 30 hours (*in vitro*) in mammalian reticulocytes, >20 hours (*in vivo*) in
191yeast, and >10 hours (*in vivo*) in *E. coli*. The secondary structural analysis demonstrated the
192presence of 7.7% helix, 39.4% sheet, 20.6% turn, and 32.3% coil and secondary structure view
193of modeled structure was illustrated in **Figure 1a**. The physicochemical properties of the peptide
194ligands were theoretically and depicted in **Table 1**. The length of the peptide ligands ranges from
19510-30 AA and showed diverse net charge variation. However, lack of crystallography structural
196data of *E. faecalis* Sortase family protein (Q836L7) remained as a bottleneck. The PSI-BLAST
197analysis yielded crystal structure of Sortase C-1 from *Streptococcus pneumoniae* (PDB ID:

1982w1j.1) with sequence similarity (45.50 %) and sequence coverage (70%) as template for *EfSrt* ⁴⁴.
199The template structure 2w1j.1 was found to be monomer with resolution of 1.24 Å. The
200homology modeling was performed using MODELLER v9.21 and the best crude models were
201selected based on DOPE scores and subjected for structural refinement. The selected crude
202model was refined using Galaxy Refine web server and validated using PROSA and RAMPAGE
203web servers. The Z-score of the refined model was found to be -5.92 as compared to -6.87 of
204crude model which indicated that the structural refinement using Galaxy Refine web server
205improved the model quality to a greater extent. The Ramachandran plot analysis of refined
206structure using RAMPAGE web server showed that 146 (95.4%) residues were found in the
207favored region with 3.3% (5) and 1.3% (2) residues in allowed region and outlier region,
208respectively. The superposition of template structure and refined model structure was performed
209using a superpose web server and RMSD was calculated as 1.31 Å and illustrated in **Figure 1b**.
210The protein-peptide flexible docking was performed using CABS dock standalone package. For
211docking analysis, the refined model of *EfSrt* was used as receptor and peptides sequences along
212with secondary structure information was used as ligand. The CABS dock tool ranks the best
213protein-peptides based on cluster density, average RMSD, and max RMSD. The binding strength
214or potential of the best complexes obtained from CABS dock were further evaluated based on the
215Gibbs free energy (ΔG) and Dissociation constant (K_d) and given in **Table 2**. The ΔG value of
216peptide-protein complexes ranges from -10.9 to -7.1 kcal mol⁻¹ and complexes with lowest ΔG
217values were selected for energy calculations and post docking interaction analysis. The hydrogen
218bond interaction profile, atom details, distance of peptide-protein complexes were provided in
219**Table 3**.

220Alpha-Defensin-3 is a short peptide of 30 amino acids and molecular weight of 3489.533 Da has
221three antiparallel beta sheets, covering over 60% of the peptide structure reported to be a role
222player in innate immunity ⁴⁵. Alpha-Defensin-3 has highly stabilized structure due to the
223presence of three disulfide bridges.

224The Alpha-Defensin-3-*EfSrt* complex showed ΔG and K_d values of $-10.9 \text{ kcal mol}^{-1}$ and 2.00E^{-08}
225M, respectively. CABS dock results suggested that peptide Alpha-Defensin-3 had better
226interactions with *EfSrt* than other peptides with cluster size (24.4929), average RMSD (4.61358),
227and Maximum RMSD (35.4444). The Alpha-Defensin-3 forms five hydrogen bonds with *EfSrt*
228residues at binding interface. The Alpha-Defensin-3 residues ASP1, TYR3, CYS9 were actively
229involved in hydrogen bonding of average bond length of 2.77 \AA ($N=5$) with *EfSrt* residues. The
230atom OH of TYR3 and atom N of ASP 1 were identified as functionally important atoms of
231Alpha-Defensin-3 peptide for *EfSrt* binding. The hydrophobic interactions play an important role
232in peptide-protein binding. Alpha-Defensin-3 forms hydrophobic interactions with residues
233LEU134 (5.46 \AA), LEU134 (6.70 \AA), and LEU201 (5.06 \AA). The peptide residues ILE6, ALA8,
234and ALA8 were actively involved in hydrophobic interaction with LEU residues at Alpha-
235Defensin-3-*EfSrt* interface. Previous studies on structure activity relationship of defensin
236peptides suggested that conserved CYS amino acids and associated disulfide bridges were related
237to its antibacterial activity. The disulfide bonding State and connectivity in the Alpha-Defensin-3
238was calculated using the DISULFIND as (2,9) and (4,19). Here we noticed that CYS9 forms
239disulfide bond with CYS2 and forms hydrogen bond with HIS 202 (2.66 \AA) residue of *EfSrt*. The
240hydrogen bond interactions between the Alpha-Defensin-3-*EfSrt* complex was illustrated in
241**Figure 3d**. The hydrogen bond interaction by disulfide bonding CYS residue may be a unique
242feature for defensin family peptide and this might be preliminary *in silico* evidence for

243contribution of disulfide bridges forming CYS residues towards antibacterial activity of defensin
244family peptides. Similarly, the residue CYS 15 of a cationic defensin peptide HBD2 forms
245hydrogen bond of length 3.32 Å with LYS96 residue of *EfSrt*. HBD2 residue CYS 15 also forms
246disulfide bonds with CYS30 as shown in **Figure 3a**. The post docking analysis identified four
247hydrophobic interactions ILE14-TYR98, 24TYR-TYR98, VAL18-TYR139, and PHE19-
248TYR139.

249Pleurocidin is a 2.7 kDa peptide with 25 amino acids which belongs to a family of alpha helical
250cationic AMP containing amphipathic alpha-helical conformation ⁴⁶. This has a broad spectrum
251antimicrobial activity against Gram positive and Gram negative bacteria with no cytotoxicity
252toward mammalian cells and low hemolytic activity ⁴⁶. The action mechanism of Pleurocidin is
253translocating strong membrane and pore formation ability with amphipathic helix which reacts
254with both neutral and acidic anionic phospholipid membranes. Pleurocidin can inhibit nucleic
255acid and synthesis of protein without the damage of cytoplasmic membranes of *Escherichia coli*
256at low concentration and at high concentration can potentially kill by causing membrane
257leakages and causing pore channels ⁴⁷. Pleurocidin shows high activity against biofilms *in vitro*
258⁴⁸. The Pleurocidin-*EfSrt* complex showed ΔG and K_d values of $-10.7 \text{ kcal mol}^{-1}$ and $3.00\text{E}^{-08} \text{ M}$,
259respectively. The Pleurocidin residues TYR24, VAL16, TYR24, THR22, THR24 were actively
260involved in hydrogen bonding interaction with residues at Pleurocidin-*EfSrt* interface as
261illustrated in **Figure 3c**. The results coincide with previous findings that the antimicrobial
262activity of pleurocidin is retained in a C-terminal 12-amino acid fragment ⁴⁹. The CABS dock
263results suggested that Pleurocidin-*EfSrt* had cluster size (56.1134), average RMSD (1.81775),
264and Maximum RMSD (22.0803). The Pleurocidin forms five hydrogen bonds with *EfSrt* residues
265ASP82 (2.58 Å), THR196 (2.61 Å), THR196 (3.43 Å), ARG224 (2.7 Å) at binding interface. The

266Pleurocidin residues ALA9, ALA10, TYR24, LEU25, PHE5 were actively involved in
267hydrophobic interaction with *EfSrt* interface. Pleurocidin forms hydrophobic interactions with
268residues PHE84 (5.29 Å), LEU134 (6.99 Å), ILE203 (6.79 Å), ILE220 (6.29 Å), PHE84 (4.81
269Å) at *EfSrt* interface.

270

271Chrysopsin-1, an amphipathic α -helical AMP found in the gill cells of red sea bream. Molecular
272weight of Chrysopsin-1 is 2892.79 and its hydrophobicity is 48% with a 25-residue peptide. It
273is a cationic AMP with the capability of broad spectrum bactericidal activity against both gram-
274positive and gram-negative bacteria ⁵⁰. The peptide has broad range activity against bacteria but
275is more hemolytic compared to other antimicrobial peptides such as Magainin ⁵¹. It is a bioactive
276peptide that is noted by their unique amino acid compositions such as arginine/lysine-rich
277peptides. However, histidine-rich bioactive peptides such as Chrysopsin-1 are found rarely ⁵².
278Chrysopsin-1 had a significantly lethal effect on *S. mutans* biofilm by inhibiting the bioactivity
279of lipopolysaccharide ⁵⁰. Three dimensional representation of the best Chrysopsin-1-*EfSrt*
280complex was illustrated in **Figure 3b**. CABS dock cluster size, average RMSD, and max RMSD
281were found to be 25.2909, 8.46153, and 30.1433 respectively. The post docking analysis suggests
282that the peptide chrysopsin-1 shows a high-binding affinity with *EfSrt* interface. It forms four
283hydrogen bond interactions with *EfSrt* interface residues SER (2.75), GLU100 (2.89), HIS102
284(3.22), ASP95 (2.98) as illustrated in **Table 3** and has an ΔG and K_d values of $-10.1 \text{ kcal mol}^{-1}$
285and $7.10 \times 10^{-8} \text{ M}$, respectively. The chrysopsin-1 forms hydrophobic interactions with *EfSrt*
286interface residues ALA124, LEU125, LEU126, LEU127, and LEU156. The Chrysopsin-1
287residues ARG23 and ARG24 were identified as potential residues responsible for Chrysopsin-1-
288*EfSrt* complex binding.

289The strength of interactions between peptide-protein complexes were calculated using standard
290energy calculations involving non-bonded interactions like electrostatic, van der Waals, and
291hydrogen bonds. The van der Waals energy, electrostatic energy, hydrogen bond energy, and total
292stabilizing energy of top four peptide-protein complexes were calculated and presented in **Table**
293**4**. The negative values in energy calculation of top scored complexes shows a good affinity for
294*EfSrt*. The total stabilization energy calculation results coincide well with predicted ΔG and K_d
295values for all top scored complexes. The *EfSrt* interfacial residues forming hydrogen bonds with
296peptide ligands were illustrated in **Figure 4**. From standard energy calculation it is evident that
297van der Waals interactions play an important role in peptide-protein complex formation. In all
298four peptide-protein complexes studied, the van der Waals interactions contribute most to the
299binding energy. The results suggest that hydrogen bonds, hydrophobic interactions, and van der
300Waals interactions helps in molecular recognition by providing specificity and directionality to
301the protein-peptide complex formation.

302**Conclusion**

303This study was performed to identify an effective peptide against *EfSrt* enzyme using protein-
304peptide flexible docking approach. Detailed inspection on molecular interaction of peptides
305towards *EfSrt* enzyme suggests potential residues responsible for peptide-*EfSrt* enzyme complex
306formation. Furthermore, we have noticed disulfide bond forming cysteine residues of peptides
307Alpha-Defensin-3 and HBD2 forms hydrogen bonds with *EfSrt* enzyme and responsible for
308peptide-*EfSrt* enzyme complex formation. similarly, C-terminal 12-amino acids of peptide
309pleurocidin plays an important role in hydrogen bonding and hydrophobic interactions with *EfSrt*
310enzyme. The results provide valuable information at the atomic level for the good binding
311affinity. In all four peptide-protein complexes studied, the van der Waals interactions contribute

312most to the binding energy. The results suggest that hydrogen bonds, hydrophobic interactions,
 313and van der Waals helps in molecular recognition by providing specificity and directionality to
 314the protein-peptide complex formation. However, the peptides identified in this study is the
 315outcome of an *in silico* protein-peptide flexible docking approach; therefore, it is crucial to prove
 316the proposed hypothesis through experimental validation in both *in vivo* and *in vitro* conditions
 317to prove the efficacy and safety of the identified peptides which may involve the purification of
 318peptides and *EfSrt* enzyme followed by the crystallization of protein-peptide complex.

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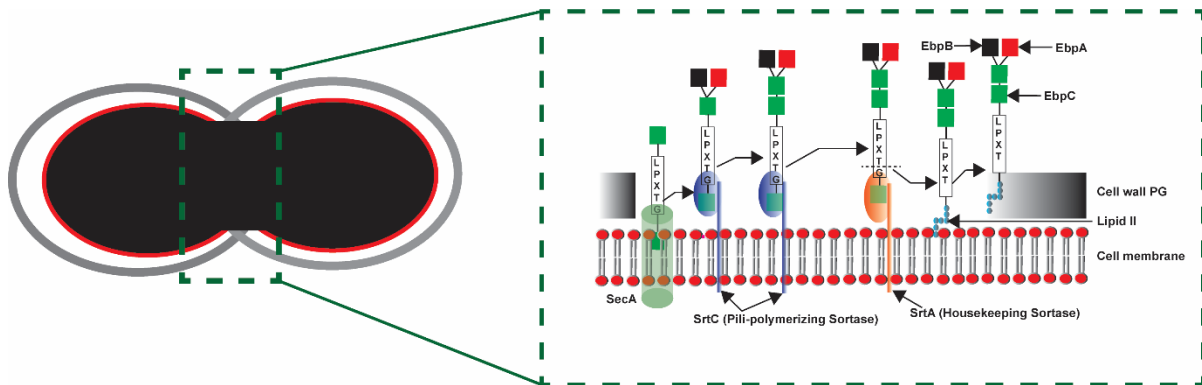
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Figures

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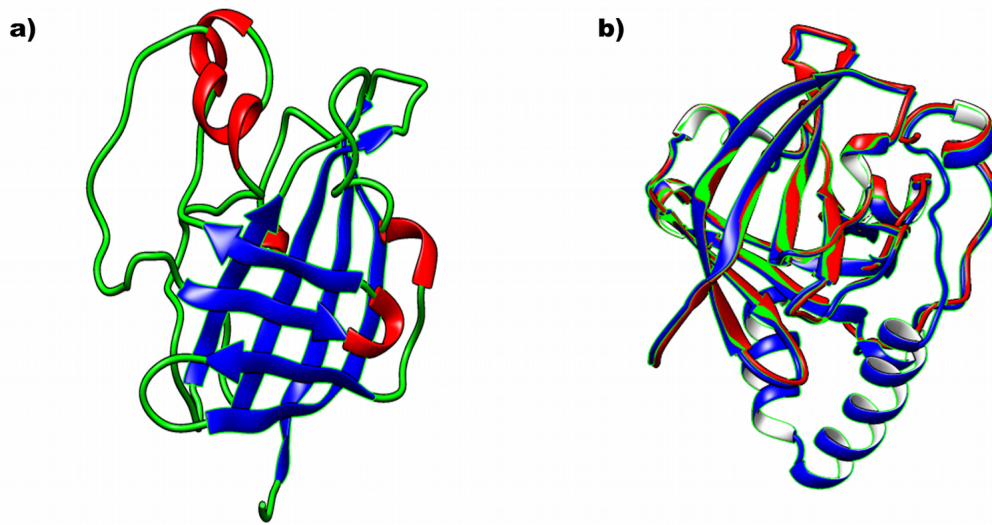


455**Figure 1.** Spatial localization of virulence factors and Sortase mediated pilus assembly in *E.*
 456*faecalis*. Endocarditis and biofilm associated pili virulence proteins (EbpA, EbpB, EbpC),
 457Sortases (SrtA, SrtC), Peptidoglycan (PG), and universally conserved protein conducting channel
 458(SecA).

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463 **Figure 2.** Homology modeling and its structural validation. a) secondary structure of Srt
464 displaying helix (red), beta sheets (blue), and loops (green), b) Superimposition of *Ef*Srt and
465 template structure.

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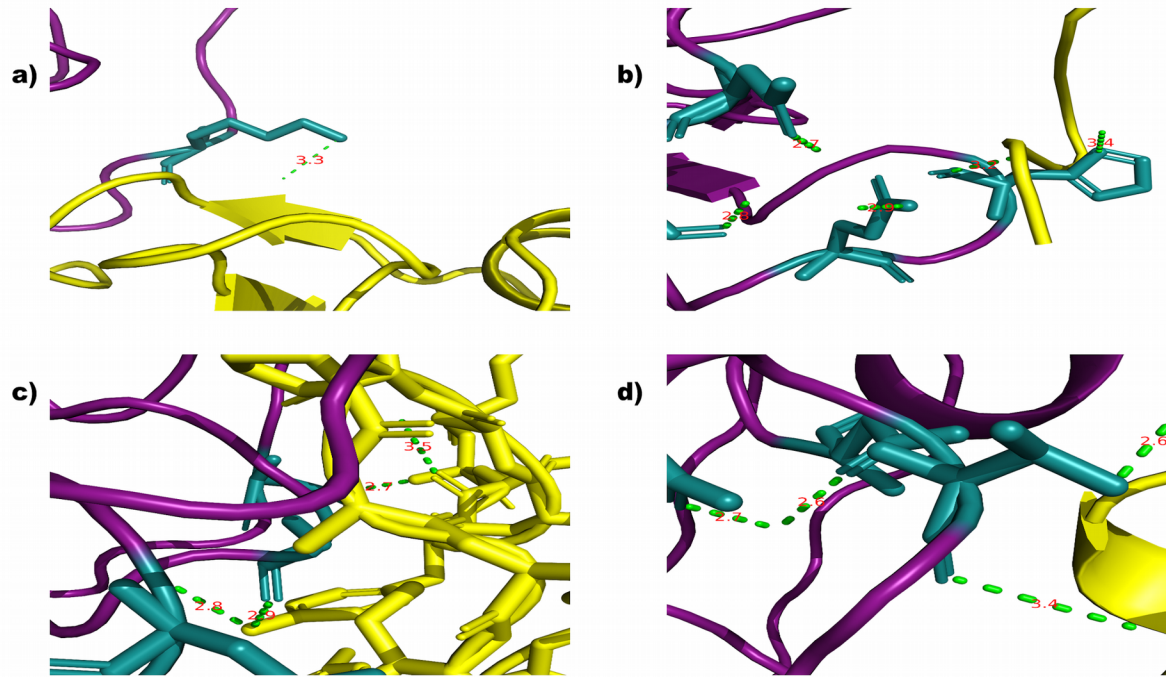
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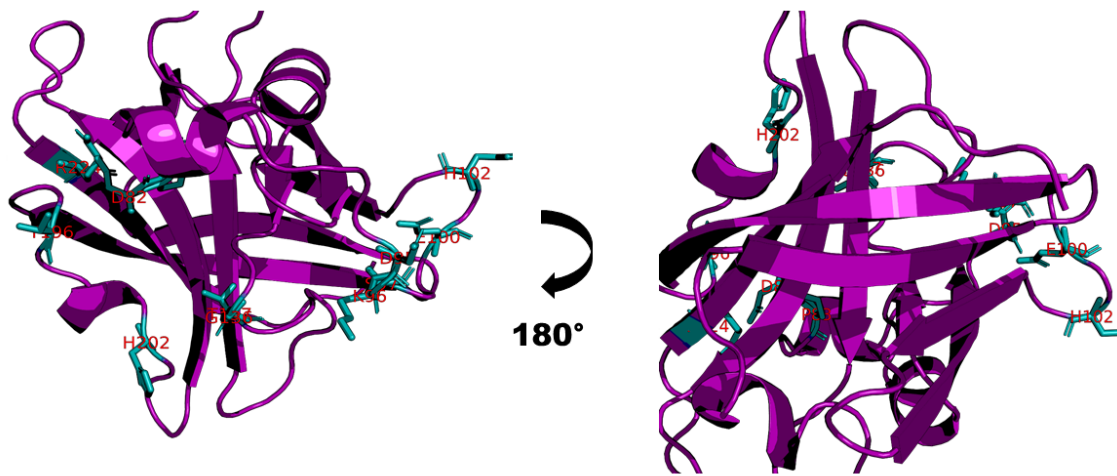


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476 **Figure 3.** Hydrogen bond interactions between *EfSrt* and top scored peptides. a) HBD2, b)
477 Chrysopsin-1, c) Pleurocidin, and d) Alpha-Defensin-3.

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481**Figure 4.** 3D structure of *EfSrt* (colored in deep purple), on two faces (rotation of 180°).

482Residues forming hydrogen bonds with peptide ligands are highlighted as sticks and were

483colored in cyan.

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Tables

496**Table 1.** Physicochemical characteristics of peptides used in this study

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Peptides	Size	NetCharge @5	NetCharge @7	Isoelectric Point	Molecular Weight	Hydrophobicity (CCS)	Hydrophobic Mom (CCS)
Lactoferricin (17-30)	14	6.038	5.945	12.263	1922.044	-1.907	1.175
Magainin-I	23	4.119	3.217	10.803	2408.308	-0.378	3.415
Histatin 5	24	12.009	6.657	10.892	3034.519	-4.679	1.436
Pleurocidin	25	6.946	4.695	10.866	2709.47	-0.532	2.147
Chrysopsin-1	25	8.915	5.937	12.813	2890.662	0.24	2.345
BMAP-27	26	11.007	10.215	12.843	3224.047	-0.342	3.554
Melittin B	26	5.038	4.975	12.546	2845.743	-0.015	3.041
BMAP-28	27	7.038	6.975	12.526	3072.932	0.463	3.76
SMAP-29	29	10.007	9.215	3254.036	3254.036	-0.083	3.545
Alpha-Defensin-3	30	1.222	0.853	7.906	2425.85	0.119	1.86

498

499**Table 2.** Binding energy, Dissociation constant, and cluster properties of peptides against Srt protein of *E. faecalis*

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Protein-peptide complex	ΔG (kcal mol ⁻¹)	Kd (M) at 37.0 °C	Cluster property		
			cluster density	average rmsd	max rmsd
<i>Ef</i> Srt-Chrysopsin 1	-10.1	7.10E ⁻⁰⁸	25.2909	8.46153	30.1433
<i>Ef</i> Srt-Magainin I	-9	4.40E ⁻⁰⁷	26.1185	4.51788	26.6159
<i>Ef</i> Srt-Histatin 5	-7.7	4.50E ⁻⁰⁶	20.4049	6.71407	21.3364
<i>Ef</i> Srt-Alpha-Defensin-3	-10.9	2.00E ⁻⁰⁸	24.4929	4.61358	35.4444
<i>Ef</i> Srt-BMAP 27	-7.1	5.90E ⁻⁰⁶	38.6465	3.77784	30.4999
<i>Ef</i> Srt-HBD2	-9.1	1.70E ⁻⁰⁶	38.9539	5.53975	28.7022
<i>Ef</i> Srt-Melittin B	-8.7	7.20E ⁻⁰⁷	38.8961	2.00534	6.30558
<i>Ef</i> Srt-Pleurocidin	-10.7	3.00E ⁻⁰⁸	56.1134	1.81775	22.0803
<i>Ef</i> Srt-SMAP-29	-8.1	1.90E ⁻⁰⁶	36.5661	3.06294	20.8314

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511 **Table 3.** Hydrogen bond interactions between the top scored peptides and *E. faecalis* Srt protein

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Peptides	Sortase Chain:A				Peptides Chain:B				Bond type and distance	
	Residue Number	Amino Acid	Chain ID	Interacting atoms	Residue Number	Amino Acid	Chain ID	Interacting atoms	Type of H-Bond	Distance (D-A) Å

Chrysopsin-1	97	SER	A	O	24	ARG	B	NH2	BS	2.75
	100	GLU	A	OE2	24	ARG	B	NH1	SS	2.89
	102	HIS	A	O	23	ARG	B	NE	BS	3.22
	95	ASP	A	OD1	24	ARG	B	NE	SS	2.98
Alpha-defensin 3	95	ASP	A	OD1	1	ASP	B	N	SB	2.74
	137	THR	A	O	3	TYR	B	OH	BS	2.83
	136	GLY	A	O	3	TYR	B	OH	SM	2.89
	202	HIS	A	ND1	9	CIS	B	O	SM	2.66
	95	ASP	A	OD1	1	ASP	B	N	MS	2.74
Pleurocidin	224	ARG	A	NH1	24	TYR	B	OH	SS	2.7
	196	THR	A	OG1	16	VAL	B	O	SM	2.61
	82	ASP	A	OD1	24	TYR	B	OH	SS	2.58
	196	THR	A	O	22	THR	B	OG1	SM	3.43
HBD2	96	LYS	A	NZ	15	CYS	B	O	SM	3.32

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516 **Table 4.** Energy profile of top scored protein-peptide complexes

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Protein-peptide complex	Hydrogen Bond Energy	Electrostatic Energy	Van der Waals Energy	Total Stabilizing Energy
Chrysopsin 1	-16.99	-40.32	-181.45	-238.76
Alpha-Defensin-3	-17.05	-40.04	-193.32	-250.41
Pleurocidin	-14.13	-14.12	-173.9	-202.15
Human Beta Defensin 2	-10.47	-46.73	-193.47	-250.67

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1 **Molecular modeling of structures and interaction of short peptides and Sortase**
2 **family protein of *Enterococcus faecalis*: Basis for developing peptide-based**
3 **therapeutics against multidrug resistant strains**

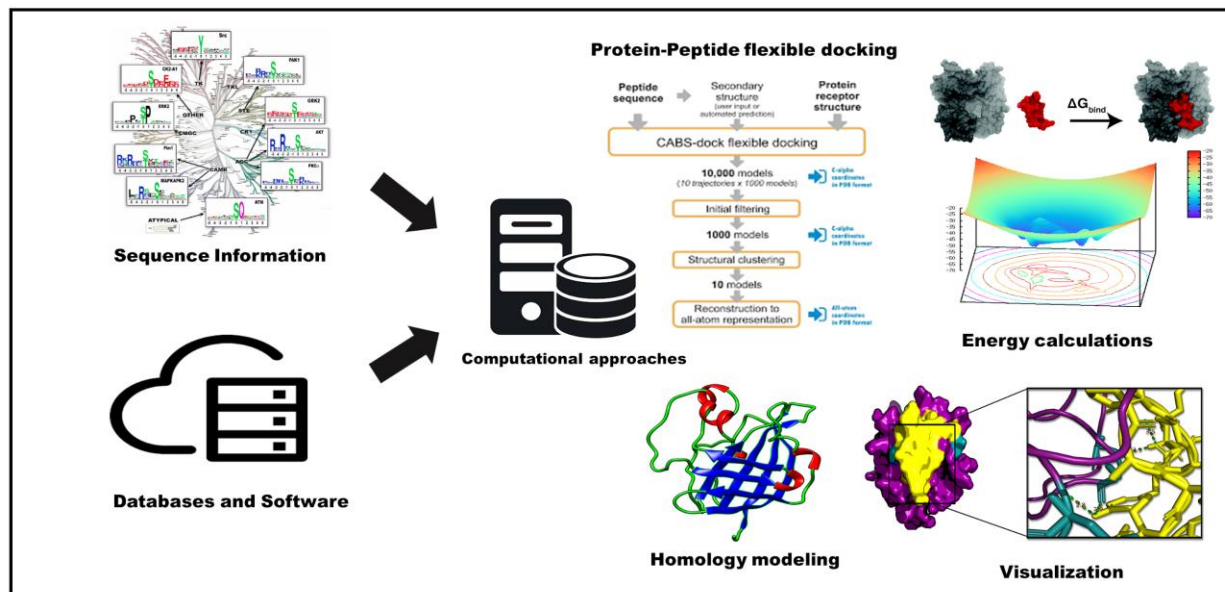
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5 Muthusaravanan Sivaramakrishnan ^{a,b}, Kumaravel Kandaswamy ^b, Cashlin Anna Suveetha Gnana
6 Rajan ^a, Janamitra Arjun ^a, Rejoe Raymond ^a and Ram Kothandan ^{*a}.

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11

12 **Graphical abstract**



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14

15 **Abstract**

16 The *Enterococcus faecalis* (*E. faecalis*) infection starts with initial adhesion to a host cell or abiotic
17 surface by multiple adhesions on its cell membrane. The pathogenicity is due to virulence factors
18 SrtA, SrtC, EbpA, EbpB, EbpC, and Aggregation Substance. *E. faecalis* developed resistance to
19 the majority of standard therapies. Additionally, a notable key feature of *E. faecalis* is its ability to
20 form biofilm *in vivo*. *E. faecalis* strains show resistance to aminoglycosides and β -lactam
21 antibiotics with different degree of susceptibility. Sortases (SrtA and SrtC) are enzymes spatially
22 localized at the septal region in majority of gram-positive bacteria during the cell cycle, which in-
23 turn plays an important role in proper assembling of adhesive surface proteins and pilus on cell
24 membrane. The studies have also proved that the both SrtA and SrtC were focally localized in *E.*
25 *faecalis* and essential for efficient bacterial colonization and biofilm formation on the host tissue
26 surfaces Using homology modeling and protein-peptide flexible docking methods, we report here
27 the detailed interaction between peptides and *Ef*Srt (Q836L7) enzyme. Plausible binding modes
28 between *Ef*Srt and the selected short biofilm active peptides were revealed from protein-peptide
29 flexible docking. The simulation data further revealed critical residues at the complex interface
30 and provided more details about the interactions between the peptides and *Ef*Srt. The flexible
31 docking simulations showed that the peptide-*Ef*Srt binding was achieved through hydrogen
32 bonding, hydrophobic, and van der Waals interaction. The strength of interactions between
33 peptide-*Ef*Srt complexes were calculated using standard energy calculations involving non-bonded
34 interactions like electrostatic, van der Waals, and hydrogen bonds.

35 **Keywords:** Drug resistance, *E. faecalis*, Biofilm, Sortase enzymes, protein-peptide docking,
36 standard energy calculations.

37

38 **Introduction**

39 *Enterococcus faecalis* (*E. faecalis*) is a gram positive commensal opportunistic pathogen,
40 responsible for various hospital acquired infections ¹. *E. faecalis* strains can overcome several
41 harsh environments by forming biofilms on host tissues and abiotic surfaces. Furthermore, *E.*
42 *faecalis* harbors virulence proteins on its cell membrane and an antimicrobial peptide sensing
43 system to protect them against antibiotics and cationic antimicrobial peptides, respectively ².
44 *E. faecalis* causes over 18,000 deaths a year, making it an important pathogen to be studied. Over
45 the past three decades, various research groups have studied *E. faecalis* strains intensively to
46 explore its interaction with hosts, determine the basis of pathogenesis, and virulence proteins
47 localization to find effective treatment strategies ³. However, currently there is no effective drug
48 available for its prevention and infections still exist. *E. faecalis* is also capable of forming biofilms
49 and responsible for various biofilm-associated infections such as Urinary Tract Infection (UTI),
50 surgical wound infection, and nosocomial bacteremia ⁴. The mature biofilm showed high tolerance
51 to antibiotics than the planktonic bacteria, even at higher concentrations of 10–1000 times ⁵. The
52 *E. faecalis* infection starts with initial adhesion to a host cell or abiotic surface by multiple
53 adhesions on its cell membrane. The pathogenicity is due to virulence factors SrtA, SrtC, EbpA,
54 EbpB, EbpC, and Aggregation Substance ⁶. *E. faecalis* developed resistance to the majority of
55 standard therapies ⁷. Additionally, a notable key feature of *E. faecalis* is its ability to form biofilm
56 ⁸. *E. faecalis* strains show resistance to aminoglycosides and β -lactam antibiotics with different
57 degree of susceptibility ⁹. It shows moderate resistance to former due to its low permeability
58 through the cell wall (aminoglycoside molecules are larger) and intrinsic resistance to later due to
59 over-expression of penicillin binding proteins. The antibiotics available today were not effective
60 against Multiple Drug Resistance (MDR) enterococcal infections such endocarditis or bacteremia

61 along with neutropenia ¹⁰. The antibiotics combinations such as ciprofloxacin with ampicillin,
62 novobiocin with doxycycline, and penicillin with vancomycin were used to treat enterococcal
63 infections ¹¹, but efficiency of this treatment remains doubtful.

64 Sortases (SrtA and SrtC) are enzymes spatially localized at the septal region in majority of
65 gram-positive bacteria during the cell cycle, which in-turn plays an important role in proper
66 assembling of adhesive surface proteins and pilus on cell membrane ¹². The studies have also
67 proved that the both SrtA and SrtC were focally localized in *E. faecalis* and essential for efficient
68 bacterial colonization and biofilm formation on the host tissue surfaces ¹³. The Sortase mutants
69 produce defective pili and found to be less virulent than the wild type strain ¹⁴. The Spatial
70 localization of virulence factors and Sortase mediated pilus assembly in *Enterococcus faecalis* was
71 illustrated in **Figure 1**.

72 Recent studies on antimicrobials suggest that peptides as possible drug candidates and to overcome
73 drug resistance in *E. faecalis*. Moreover, studies have also shown that this species can rapidly
74 acquire resistance even to cationic antimicrobial peptides such as β -beta defensins ¹⁵. Multiple
75 peptide resistance Factors (mprF1 and mprF2) are integral membrane proteins responsible for
76 developing resistance against cationic antimicrobial proteins in the majority of the gram-positive
77 bacteria. Studies on *E. faecalis* suggested that mprF2 is essential for the aminoacylation of
78 phosphatidylglycerol (PG) and synthesis of Lys-PG, Ala-PG, and Arg-PG variants. Whereas,
79 mprF1 does not play a role in aminoacylation of PG. Furthermore, the aminoacylation of PG by
80 mprF2 increases resistance against cationic antimicrobial peptides ¹⁵. The strain *E. faecalis*
81 OG1RF circumvent antimicrobial peptides using MprF (Multiple peptide resistance Factors)
82 protein assisted Antimicrobial peptide sensing system by altering the net surface charge of
83 bacterial cell membrane to repel incoming antimicrobial peptides ¹⁶.

84 The peptides targeting Sortase family proteins were identified as potential therapeutics to
85 kill MDR bacterial strains, which is recently an emerging field in the drug discovery process ¹⁷.
86 Hence in this study we performed protein-peptide docking to identify potential biofilm active
87 peptides that can bind to Sortase family protein thereby inhibiting its function. The screened
88 peptides can be further used for designing novel peptide-based therapeutics against MDR *E.*
89 *faecalis* infection.

90 **Materials and methods**

91 **Computational platform**

92 All the computational simulations were carried out on the Linux Mint 18.3 Cinnamon 64-bit
93 platform in Lenovo G50-45 workstation on AMD A8-6410 APU @ 2.00GHz processor. All the
94 software and tools used in this study were open source platforms or free to use for academic
95 purposes.

96 **Primary and secondary structural analysis**

97 The sequence of *E. faecalis* Sortase family protein with accession ID Q836L7 was retrieved from
98 UNIPROT database ¹⁸ and its basic sequence information were calculated using ExPASy
99 ProtParam ¹⁹. The physicochemical properties of *EfSrt* including number of amino acid, molecular
100 weight (Mwt), amino acid composition, theoretical isoelectric point, aliphatic index, *in vitro/in*
101 *vivo* half-life instability index, and grand average of hydropathicity (GRAVY) were theoretically
102 calculated. The short antibiofilm peptides of length 10-30 were obtained from BaAMPs database
103 ²⁰ and their physico chemical characteristics were calculated using the inbuilt module of BaAMPs
104 database and used for tertiary structure modeling, docking analysis, and energy calculations.

105

106

107 **Tertiary structure modelling**

108 The lack of crystallography structural data of *E. faecalis* Sortase family protein (Q836L7)
109 remained as a bottleneck. Hence we performed homology modeling to depict the 3D structure ²¹.
110 The obtained FASTA sequence was used as input to the PSI-BLAST with default parameters to
111 find suitable the template for performing the homology modeling ²². The best template was
112 selected from suggested templates based on high percentage of sequence identity, query coverage,
113 and valid E-value. The homology modeling was performed using a standalone tool MODELLER
114 v9.21 ²³. The results were ranked based on Discrete Optimized Protein Energy (DOPE) score, the
115 models with least DOPE score were selected and administered to model validation ²⁴. The modeled
116 3D structure was refined using Galaxyrefine web server for better quality ²⁵. The refined 3D
117 coordinates were analyzed for dihedral angles distribution using RAMPAGE web server ²⁶.
118 Further, refined structure was validated using ProSA web server which provides an overall quality
119 score for a modeled structure based on C α positions ²⁷. The reliability of modeled protein was
120 assessed using the Superpose 1.0 web by superimposing the modeled structure of *EfSrt* with
121 template structure and Root Mean Square Deviation (RMSD) was calculated ²⁸. The tertiary
122 structure of ligand peptides was designed using CABS-dock ²⁹. Finally, the tertiary structure of
123 *EfSrt* and peptides were visualized using Chimera v1.13.1 ³⁰.

124 **Protein-peptide flexible docking**

125 Sortase family proteins (SrtA,B, and C) play an important role in initial attachment of planktonic
126 bacterial cells, and subsequent biofilm formation ³¹. In *E. faecalis*, the cell wall anchoring of
127 virulence factors such as aggregation substance and pili were facilitated by Sortase enzymes.
128 Therefore, Sortase family protein (Q836L7) was considered as the docking receptor and the
129 antibiofilm active peptides were used as ligands. Finally, the receptor protein and peptides were

130 docked using CABS-dock standalone³². In CABS-dock the modeled three dimensional structure
131 was used as receptor protein and peptide sequences along with secondary structure data was used
132 as ligand peptides³³. The CABS-dock performs simulation search for the binding site allowing for
133 full flexibility of the peptide and small fluctuations of the receptor backbone. The CABS-dock
134 protocol consists of the following steps (i) Generating random structures, (ii) Simulation of binding
135 and docking, (iii) Selection of the final representative models, and (iv) Reconstruction of the final
136 models. When protein-peptide docking was performed using CABS-dock with default settings, the
137 structure of the peptide was kept as fully flexible and the structure of the protein receptor was
138 maintained near the initial conformation using soft distance restraints. The soft distance restraints
139 allow small fluctuations of the receptor backbone (1 Å) and large fluctuations of the side chains.
140 Based on the RMSD values of the cluster the top 10 complexes were sorted. The Gibbs free energy
141 (ΔG) and Dissociation constant (K_d) were used to explain the binding strength or potential of the
142 drug-protein complex during drug screening and therapeutics development³⁴. Therefore, ΔG and
143 K_d of protein-peptide complexes were predicted using the PRODIGY web server at 37°C³⁵. The
144 K_d value of protein-peptide complexes were calculated using ΔG value obtained from PRODIGY
145 using following equation,

$$146 \quad \Delta G = RT \times \ln K_d \quad (1)$$

147 where, R, ΔG , and T are the ideal gas constant, gibbs free energy, and temperature (Kelvin),
148 respectively. The binding energy was calculated as follows,

$$149 \quad \Delta G = -0.09459 \times IC_{\text{charged/charged}} - 0.10007 \times IC_{\text{charged/apolar}} + 0.19577 \times IC_{\text{polar/polar}} - 0.22671 \times IC_{\text{polar/apolar}} \\ 150 \quad + 0.18681 \times \%NIS_{\text{apolar}} + 0.3810 \times \%NIS_{\text{charged}} - 15.9433 \quad (2)$$

151 where, $IC_{X/Y}$ represents interfacial contacts in terms of physicochemical properties and %NIS
152 represents percentage of non-interacting surfaces in terms of physicochemical properties. The

153 interfacial contacts of protein-peptide complexes were analyzed using COCOMAPS³⁶ and
154 PPCheck³⁷ web server and visualized using Chimera v1.13.1.

155 **Non bonded Energy calculation of Protein-peptide complexes**

156 The strength of interactions between protein-protein complexes were calculated using standard
157 energy calculations involving non-bonded interactions like electrostatic, van der Waals, and
158 hydrogen bonds. The hydrogen atoms of protein-peptide complexes were fixed geometrically and
159 then hydrogen bond energy was calculated as follows,

$$160 \quad E = q_1q_2 [1/r(\text{ON}) + 1/r(\text{CH}) - 1/r(\text{OH}) - 1/r(\text{CN})] * 332 * 4.184 \text{ kJ/mol} \quad (3)$$

161 where q_1 and q_2 are partial atomic charges, $r()$ is the inter-atomic distance between the
162 corresponding atoms. The van der Waals interaction energies are calculated using equation (4)

$$163 \quad E = 4.184 (E_i E_j) \times [((R_i + R_j)/r)^{12} - 2((R_i + R_j)/r)^6] \text{ KJ/mol} \quad (4)$$

164 where R is the Van der Waals radius for an atom, E is the van der Waals well depth, r is the distance
165 between the atoms. The electrostatic interaction energies for favourable as well as non favourable
166 interactions are calculated according to Coulomb's law by considering the interprotomer charged
167 atomic pairs at $\leq 10 \text{ \AA}$.

168 **Results and Discussion**

169 The antimicrobial peptides were identified as potential alternative therapy to treat MDR bacterial
170 infections. In the past two decades we have identified hundreds of peptides from natural sources
171 and studied their biological activity both *in vivo* and *in vitro*³⁸. The studies on antibacterial
172 peptides showed that there is a relationship between structure and functions of these peptides³⁹.
173 For example, Members of the defensin family are highly similar in protein sequence but they show
174 differential antimicrobial activity⁴⁰. Hence it is important to depict the structure-function
175 relationship of these defensin peptides. The cationic antimicrobial peptide Human β -beta defensin

176 2 disrupts the localization pattern of membrane protein SrtA and SecA in *E. faecalis*^{41, 42}. The
177 studies have also proved that the both SrtA and SrtC were focally localized in *E. faecalis* and
178 essential for efficient bacterial colonization and biofilm formation on the host tissue surfaces and
179 was identified as an attractive drug target⁴³. We performed protein-peptide flexible docking to
180 identify potential biofilm active peptides that can bind to Sortase family protein thereby inhibiting
181 its function. Also the identified peptide binding *EfSrt* residues can be considered as potential target
182 sites for the development of potential peptide based therapeutics against biofilm associated
183 infections. Therefore, in this study biofilm active peptides collected from literature sources were
184 screened to investigate its binding mechanism with *E. faecalis* SrtA.

185 The primary sequence information of query sequence Q836L7 was theoretically calculated using
186 ExPasy ProtParam suggests that the protein has molecular weight (32025.32) and found to be basic
187 (theoretical PI of 9.57), stable (Instability Index < 40), hydrophilic (negative GRAVY value) in
188 nature, and thermostable (higher AI value). Additionally, the half-life was theoretically calculated
189 to be about 30 hours (*in vitro*) in mammalian reticulocytes, >20 hours (*in vivo*) in yeast, and >10
190 hours (*in vivo*) in *E. coli*. The secondary structural analysis demonstrated the presence of 7.7%
191 helix, 39.4% sheet, 20.6% turn, and 32.3% coil and secondary structure view of modeled structure
192 was illustrated in **Figure 1a**. The physicochemical properties of the peptide ligands were
193 theoretically and depicted in **Table 1**. The length of the peptide ligands ranges from 10-30 AA and
194 showed diverse net charge variation. However, lack of crystallography structural data of *E. faecalis*
195 Sortase family protein (Q836L7) remained as a bottleneck. The PSI-BLAST analysis yielded
196 crystal structure of Sortase C-1 from *Streptococcus pneumoniae* (PDB ID: 2w1j.1) with sequence
197 similarity (45.50 %) and sequence coverage (70%) as template for *EfSrt*⁴⁴. The template structure
198 2w1j.1 was found to be monomer with resolution of 1.24 Å. The homology modeling was

199 performed using MODELLER v9.21 and the best crude models were selected based on DOPE
200 scores and subjected for structural refinement. The selected crude model was refined using Galaxy
201 Refine web server and validated using PROSA and RAMPAGE web servers. The Z-score of the
202 refined model was found to be -5.92 as compared to -6.87 of crude model which indicated that the
203 structural refinement using Galaxy Refine web server improved the model quality to a greater
204 extent. The Ramachandran plot analysis of refined structure using RAMPAGE web server showed
205 that 146 (95.4%) residues were found in the favored region with 3.3% (5) and 1.3% (2) residues
206 in allowed region and outlier region, respectively. The superposition of template structure and
207 refined model structure was performed using a superpose web server and RMSD was calculated
208 as 1.31 Å and illustrated in **Figure 1b**.

209 The protein-peptide flexible docking was performed using CABS dock standalone package. For
210 docking analysis, the refined model of *EfSrt* was used as receptor and peptides sequences along
211 with secondary structure information was used as ligand. The CABS dock tool ranks the best
212 protein-peptides based on cluster density, average RMSD, and max RMSD. The binding strength
213 or potential of the best complexes obtained from CABS dock were further evaluated based on the
214 Gibbs free energy (ΔG) and Dissociation constant (K_d) and given in **Table 2**. The ΔG value of
215 peptide-protein complexes ranges from -10.9 to -7.1 kcal mol⁻¹ and complexes with lowest ΔG values
216 were selected for energy calculations and post docking interaction analysis. The hydrogen bond
217 interaction profile, atom details, distance of peptide-protein complexes were provided in **Table 3**.
218 Alpha-Defensin-3 is a short peptide of 30 amino acids and molecular weight of 3489.533 Da has
219 three antiparallel beta sheets, covering over 60% of the peptide structure reported to be a role
220 player in innate immunity⁴⁵. Alpha-Defensin-3 has highly stabilized structure due to the presence
221 of three disulfide bridges.

222 The Alpha-Defensin-3-*EfSrt* complex showed ΔG and K_d values of $-10.9 \text{ kcal mol}^{-1}$ and 2.00E^{-08}
223 M, respectively. CABS dock results suggested that peptide Alpha-Defensin-3 had better
224 interactions with *EfSrt* than other peptides with cluster size (24.4929), average RMSD (4.61358),
225 and Maximum RMSD (35.4444). The Alpha-Defensin-3 forms five hydrogen bonds with *EfSrt*
226 residues at binding interface. The Alpha-Defensin-3 residues ASP1, TYR3, CYS9 were actively
227 involved in hydrogen bonding of average bond length of 2.77 \AA ($N=5$) with *EfSrt* residues. The
228 atom OH of TYR3 and atom N of ASP 1 were identified as functionally important atoms of Alpha-
229 Defensin-3 peptide for *EfSrt* binding. The hydrophobic interactions play an important role in
230 peptide-protein binding. Alpha-Defensin-3 forms hydrophobic interactions with residues LEU134
231 (5.46 \AA), LEU134 (6.70 \AA), and LEU201 (5.06 \AA). The peptide residues ILE6, ALA8, and ALA8
232 were actively involved in hydrophobic interaction with LEU residues at Alpha-Defensin-3-*EfSrt*
233 interface. Previous studies on structure activity relationship of defensin peptides suggested that
234 conserved CYS amino acids and associated disulfide bridges were related to its antibacterial
235 activity. The disulfide bonding State and connectivity in the Alpha-Defensin-3 was calculated
236 using the DISULFIND as (2,9) and (4,19). Here we noticed that CYS9 forms disulfide bond with
237 CYS2 and forms hydrogen bond with HIS 202 (2.66 \AA) residue of *EfSrt*. The hydrogen bond
238 interactions between the Alpha-Defensin-3-*EfSrt* complex was illustrated in **Figure 3d**. The
239 hydrogen bond interaction by disulfide bonding CYS residue may be a unique feature for defensin
240 family peptide and this might be preliminary *in silico* evidence for contribution of disulfide bridges
241 forming CYS residues towards antibacterial activity of defensin family peptides. Similarly, the
242 residue CYS 15 of a cationic defensin peptide HBD2 forms hydrogen bond of length 3.32 \AA with
243 LYS96 residue of *EfSrt*. HBD2 residue CYS 15 also forms disulfide bonds with CYS30 as shown

244 in **Figure 3a**. The post docking analysis identified four hydrophobic interactions ILE14-TYR98,
245 24TYR-TYR98, VAL18-TYR139, and PHE19-TYR139.

246 Pleurocidin is a 2.7 kDa peptide with 25 amino acids which belongs to a family of alpha helical
247 cationic AMP containing amphipathic alpha-helical conformation ⁴⁶. This has a broad spectrum
248 antimicrobial activity against Gram positive and Gram negative bacteria with no cytotoxicity
249 toward mammalian cells and low hemolytic activity ⁴⁶. The action mechanism of Pleurocidin is
250 translocating strong membrane and pore formation ability with amphipathic helix which reacts
251 with both neutral and acidic anionic phospholipid membranes. Pleurocidin can inhibit nucleic acid
252 and synthesis of protein without the damage of cytoplasmic membranes of *Escherichia coli* at low
253 concentration and at high concentration can potentially kill by causing membrane leakages and
254 causing pore channels ⁴⁷. Pleurocidin shows high activity against biofilms *in vitro* ⁴⁸. The
255 Pleurocidin-*EfSrt* complex showed ΔG and K_d values of $-10.7 \text{ kcal mol}^{-1}$ and $3.00\text{E}^{-08} \text{ M}$,
256 respectively. The Pleurocidin residues TYR24, VAL16, TYR24, THR22, THR24 were actively
257 involved in hydrogen bonding interaction with residues at Pleurocidin-*EfSrt* interface as illustrated
258 in **Figure 3c**. The results coincide with previous findings that the antimicrobial activity of
259 pleurocidin is retained in a C-terminal 12-amino acid fragment ⁴⁹. The CABS dock results
260 suggested that Pleurocidin-*EfSrt* had cluster size (56.1134), average RMSD (1.81775), and
261 Maximum RMSD (22.0803). The Pleurocidin forms five hydrogen bonds with *EfSrt* residues
262 ASP82 (2.58 Å), THR196 (2.61 Å), THR196 (3.43 Å), ARG224 (2.7 Å) at binding interface. The
263 Pleurocidin residues ALA9, ALA10, TYR24, LEU25, PHE5 were actively involved in
264 hydrophobic interaction with *EfSrt* interface. Pleurocidin forms hydrophobic interactions with
265 residues PHE84 (5.29 Å), LEU134 (6.99 Å), ILE203 (6.79 Å), ILE220 (6.29 Å), PHE84 (4.81 Å)
266 at *EfSrt* interface.

267

268 Chrysopsin-1, an amphipathic α -helical AMP found in the gill cells of red sea bream. Molecular
269 weight of Chrysopsin-1 is 2892.79 and its hydrophobicity is 48% with a 25-residue peptide. It is
270 a cationic AMP with the capability of broad spectrum bactericidal activity against both gram-
271 positive and gram-negative bacteria ⁵⁰. The peptide has broad range activity against bacteria but is
272 more hemolytic compared to other antimicrobial peptides such as Magainin ⁵¹. It is a bioactive
273 peptide that is noted by their unique amino acid compositions such as arginine/lysine-rich peptides.
274 However, histidine-rich bioactive peptides such as Chrysopsin-1 are found rarely ⁵².
275 Chrysopsin-1 had a significantly lethal effect on *S. mutans* biofilm by inhibiting the bioactivity
276 of lipopolysaccharide ⁵⁰. Three dimensional representation of the best Chrysopsin-1-*Ef*Srt
277 complex was illustrated in **Figure 3b**. CABS dock cluster size, average RMSD, and max RMSD
278 were found to be 25.2909, 8.46153, and 30.1433 respectively. The post docking analysis suggests
279 that the peptide chrysopsin-1 shows a high-binding affinity with *Ef*Srt interface. It forms four
280 hydrogen bond interactions with *Ef*Srt interface residues SER (2.75), GLU100 (2.89), HIS102
281 (3.22), ASP95 (2.98) as illustrated in **Table 3** and has an ΔG and K_d values of $-10.1 \text{ kcal mol}^{-1}$
282 and $7.10\text{E}^{-08} \text{ M}$, respectively. The chrysopsin-1 forms hydrophobic interactions with *Ef*Srt
283 interface residues ALA124, LEU125, LEU126, LEU127, and LEU156. The Chrysopsin-1
284 residues ARG23 and ARG24 were identified as potential residues responsible for Chrysopsin-1-
285 *Ef*Srt complex binding.

286 The strength of interactions between peptide-protein complexes were calculated using standard
287 energy calculations involving non-bonded interactions like electrostatic, van der Waals, and
288 hydrogen bonds. The van der Waals energy, electrostatic energy, hydrogen bond energy, and total
289 stabilizing energy of top four peptide-protein complexes were calculated and presented in **Table**

290 4. The negative values in energy calculation of top scored complexes shows a good affinity for
291 *EfSrt*. The total stabilization energy calculation results coincide well with predicted ΔG and K_d
292 values for all top scored complexes. The *EfSrt* interfacial residues forming hydrogen bonds with
293 peptide ligands were illustrated in **Figure 4**. From standard energy calculation it is evident that
294 van der Waals interactions play an important role in peptide-protein complex formation. In all four
295 peptide-protein complexes studied, the van der Waals interactions contribute most to the binding
296 energy. The results suggest that hydrogen bonds, hydrophobic interactions, and van der Waals
297 interactions helps in molecular recognition by providing specificity and directionality to the
298 protein-peptide complex formation.

299 **Conclusion**

300 This study was performed to identify an effective peptide against *EfSrt* enzyme using protein-
301 peptide flexible docking approach. Detailed inspection on molecular interaction of peptides
302 towards *EfSrt* enzyme suggests potential residues responsible for peptide-*EfSrt* enzyme complex
303 formation. Furthermore, we have noticed disulfide bond forming cysteine residues of peptides
304 Alpha-Defensin-3 and HBD2 forms hydrogen bonds with *EfSrt* enzyme and responsible for
305 peptide-*EfSrt* enzyme complex formation. similarly, C-terminal 12-amino acids of peptide
306 pleurocidin plays an important role in hydrogen bonding and hydrophobic interactions with *EfSrt*
307 enzyme. The results provide valuable information at the atomic level for the good binding affinity.
308 In all four peptide-protein complexes studied, the van der Waals interactions contribute most to
309 the binding energy. The results suggest that hydrogen bonds, hydrophobic interactions, and van
310 der Waals helps in molecular recognition by providing specificity and directionality to the protein-
311 peptide complex formation. However, the peptides identified in this study is the outcome of an in
312 silico protein-peptide flexible docking approach; therefore, it is crucial to prove the proposed

313 hypothesis through experimental validation in both *in vivo* and *in vitro* conditions to prove the
314 efficacy and safety of the identified peptides which may involve the purification of peptides and
315 *EfSrt* enzyme followed by the crystallization of protein-peptide complex.

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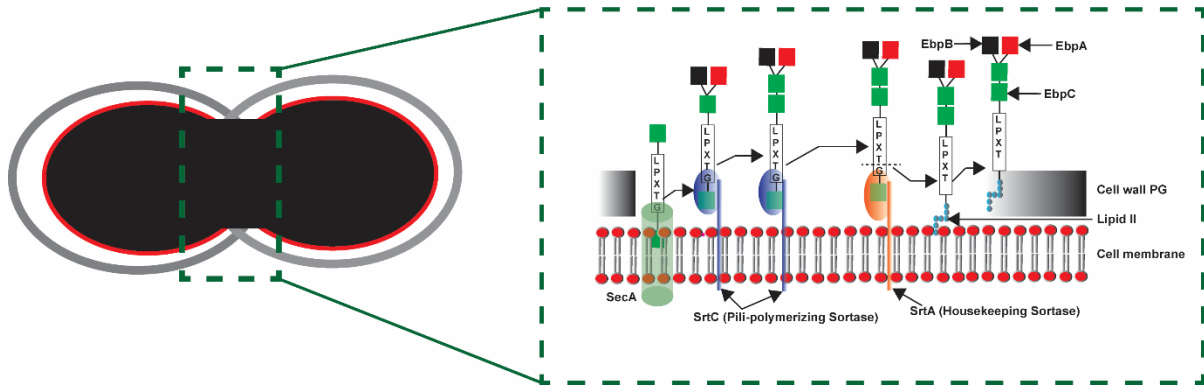
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444

445

Figures

446



447

448 **Figure 1.** Spatial localization of virulence factors and Sortase mediated pilus assembly in *E.*

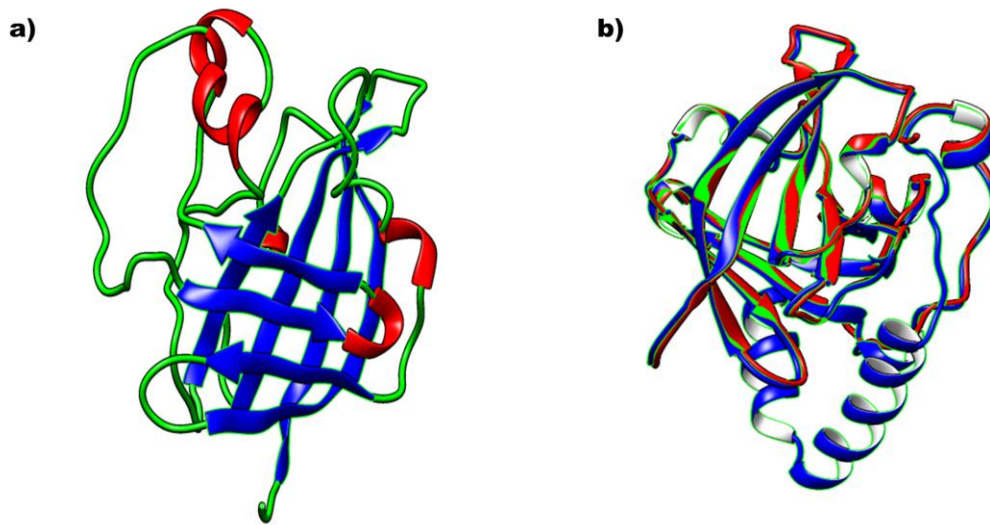
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454

455 **Figure 2.** Homology modeling and its structural validation. a) secondary structure of Srt displaying
456 helix (red), beta sheets (blue), and loops (green), b) Superimposition of *Ef*Srt and template
457 structure.

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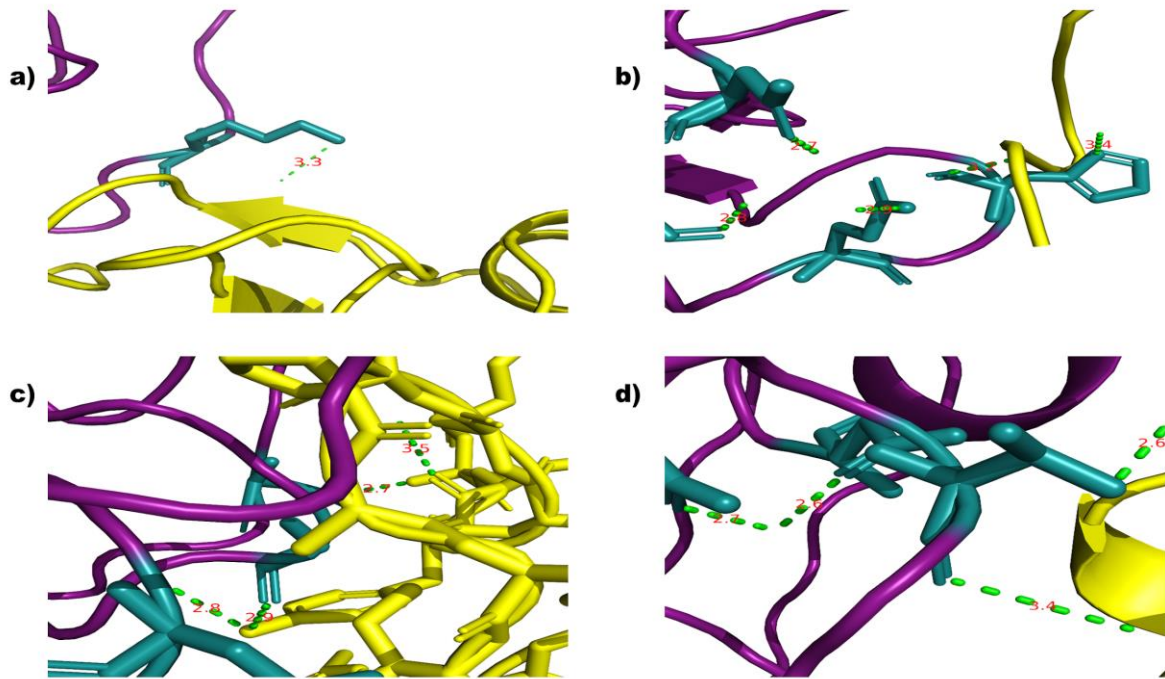
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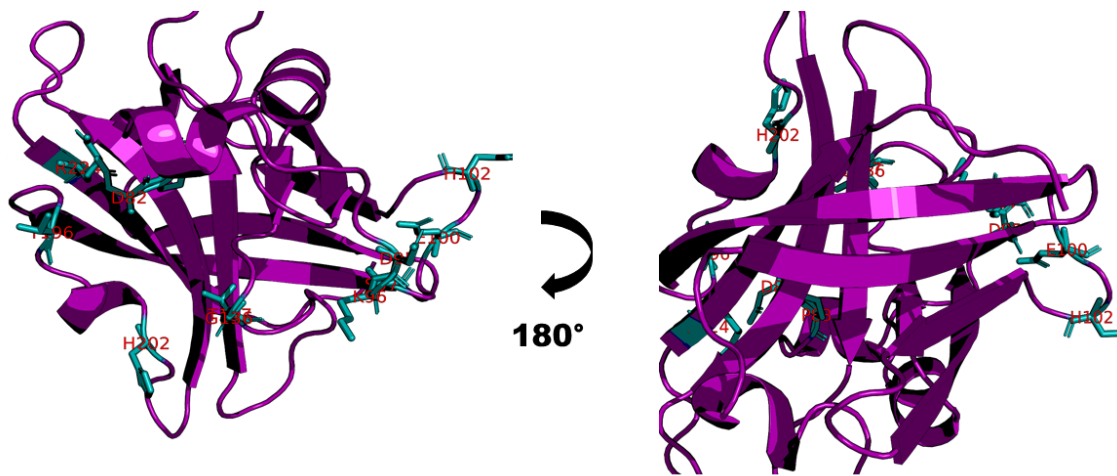
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468 **Figure 3.** Hydrogen bond interactions between *E/Srt* and top scored peptides. a) HBD2, b)

469 Chrysopsin-1, c) Pleurocidin, and d) Alpha-Defensin-3.

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473 **Figure 4.** 3D structure of *EfSrt* (colored in deep purple), on two faces (rotation of 180°). Residues
474 forming hydrogen bonds with peptide ligands are highlighted as sticks and were colored in cyan.

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Tables487 **Table 1.** Physicochemical characteristics of peptides used in this study

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Peptides	Size	NetCharge @5	NetCharge @7	Isoelectric Point	Molecular Weight	Hydrophobicity (CCS)	Hydrophobic Mom (CCS)
Lactoferricin (17-30)	14	6.038	5.945	12.263	1922.044	-1.907	1.175
Magainin-I	23	4.119	3.217	10.803	2408.308	-0.378	3.415
Histatin 5	24	12.009	6.657	10.892	3034.519	-4.679	1.436
Pleurocidin	25	6.946	4.695	10.866	2709.47	-0.532	2.147
Chrysopsin-1	25	8.915	5.937	12.813	2890.662	0.24	2.345
BMAP-27	26	11.007	10.215	12.843	3224.047	-0.342	3.554
Melittin B	26	5.038	4.975	12.546	2845.743	-0.015	3.041
BMAP-28	27	7.038	6.975	12.526	3072.932	0.463	3.76
SMAP-29	29	10.007	9.215	3254.036	3254.036	-0.083	3.545
Alpha-Defensin-3	30	1.222	0.853	7.906	2425.85	0.119	1.86

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490 **Table 2.** Binding energy, Dissociation constant, and cluster properties of peptides against Srt protein of *E. faecalis*

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Protein-peptide complex	ΔG (kcal mol ⁻¹)	Kd (M) at 37.0 °C	Cluster property		
			cluster density	average rmsd	max rmsd
<i>Ef</i> Srt-Chrysopsin 1	-10.1	7.10E ⁻⁰⁸	25.2909	8.46153	30.1433
<i>Ef</i> Srt-Magainin I	-9	4.40E ⁻⁰⁷	26.1185	4.51788	26.6159
<i>Ef</i> Srt-Histatin 5	-7.7	4.50E ⁻⁰⁶	20.4049	6.71407	21.3364
<i>Ef</i> Srt-Alpha-Defensin-3	-10.9	2.00E ⁻⁰⁸	24.4929	4.61358	35.4444
<i>Ef</i> Srt-BMAP 27	-7.1	5.90E ⁻⁰⁶	38.6465	3.77784	30.4999
<i>Ef</i> Srt-HBD2	-9.1	1.70E ⁻⁰⁶	38.9539	5.53975	28.7022
<i>Ef</i> Srt-Melittin B	-8.7	7.20E ⁻⁰⁷	38.8961	2.00534	6.30558
<i>Ef</i> Srt-Pleurocidin	-10.7	3.00E ⁻⁰⁸	56.1134	1.81775	22.0803
<i>Ef</i> Srt-SMAP-29	-8.1	1.90E ⁻⁰⁶	36.5661	3.06294	20.8314

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502 **Table 3.** Hydrogen bond interactions between the top scored peptides and *E. faecalis* Srt protein

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Peptides	Sortase Chain:A				Peptides Chain:B				Bond type and distance	
	Residue Number	Amino Acid	Chain ID	Interacting atoms	Residue Number	Amino Acid	Chain ID	Interacting atoms	Type of H-Bond	Distance (D-A) Å
Chrysopsin-1	97	SER	A	O	24	ARG	B	NH2	BS	2.75
	100	GLU	A	OE2	24	ARG	B	NH1	SS	2.89
	102	HIS	A	O	23	ARG	B	NE	BS	3.22
	95	ASP	A	OD1	24	ARG	B	NE	SS	2.98
Alpha-defensin 3	95	ASP	A	OD1	1	ASP	B	N	SB	2.74
	137	THR	A	O	3	TYR	B	OH	BS	2.83
	136	GLY	A	O	3	TYR	B	OH	SM	2.89
	202	HIS	A	ND1	9	CIS	B	O	SM	2.66
	95	ASP	A	OD1	1	ASP	B	N	MS	2.74
Pleurocidin	224	ARG	A	NH1	24	TYR	B	OH	SS	2.7
	196	THR	A	OG1	16	VAL	B	O	SM	2.61
	82	ASP	A	OD1	24	TYR	B	OH	SS	2.58
	196	THR	A	O	22	THR	B	OG1	SM	3.43
HBD2	96	LYS	A	NZ	15	CYS	B	O	SM	3.32

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507 **Table 4.** Energy profile of top scored protein-peptide complexes

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Protein-peptide complex	Hydrogen Bond Energy	Electrostatic Energy	Van der Waals Energy	Total Stabilizing Energy
Chrysopsin 1	-16.99	-40.32	-181.45	-238.76
Alpha-Defensin-3	-17.05	-40.04	-193.32	-250.41
Pleurocidin	-14.13	-14.12	-173.9	-202.15
Human Beta Defensin 2	-10.47	-46.73	-193.47	-250.67

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