Molecular insights into the interactions of GF-17 with the gram-negative and gram-positive bacterial lipid bilayers

Samira Jahangiri | Majid Jafari | Mehdi Arjomand | Faramarz Mehrnejad

1Department of Chemical Engineering, South Tehran Branch, Islamic Azad University, Tehran, Iran
2Computational Nanobiotechnology Laboratory, Department of Life Sciences Engineering, Faculty of New Sciences and Technologies, University of Tehran, Tehran, Iran

Correspondence
Faramarz Mehrnejad, Computational Nanobiotechnology Laboratory, Department of Life Sciences Engineering, Faculty of New Sciences and Technologies, University of Tehran, Tehran 14395-1561, Iran. Email: Mehrnejad@ut.ac.ir

Abstract
The cationic antimicrobial peptide GF-17, a 17-mer-derived peptide from human cathelicidin LL-37, has a significant strength in the killing of the methicillin-resistant Staphylococcus aureus and Escherichia coli strains. Herein, we conducted a series of all-atom molecular dynamics simulations to investigate the ability of GF-17 in perturbing the model membranes of the gram-positive, S. aureus, and gram-negative, E. coli, bacteria. We also explored the contributions of the specific residues in the peptide activity. The molecular dynamics results indicated that the peptide is stabilized on the membrane surface and rapidly binds to the phosphate headgroups of the model membranes through the electrostatic interactions and hydrogen bonds. Furthermore, both polar and nonpolar interactions are energetically favored for the binding with the membrane surface. The research also revealed the important roles of the phenylalanine residues in the early insertion of the peptide into the bacterial model membranes. In addition, the results demonstrated that the central residues Arg23 and Lys25 played a critical role in the binding of GF-17 to both gram-negative and gram-positive model membranes, in excellent agreement with experimental studies. This study emphasizes on the pivotal role of basic residues in prompt association of the peptide on the model membrane surface and on the significance of residues Phe17, Ile24, Phe27, and Val32 in hydrophobic interactions. Therefore, our observations provide insights into the membrane-GF-17 interactions at atomic details that are useful to develop potent antimicrobial peptides targeting multidrug-resistant bacteria.

KEYWORDS
antimicrobial peptide (AMP), cathelicidin LL-37, GF-17, molecular dynamics (MD) simulation

1 | INTRODUCTION

Since the dawn of life, antimicrobial peptides (AMPs) have remained defensive against enveloped viruses, bacteria, fungi, and parasites at host innate immunity in given complex species.1,2 LL-37 is the only member of the human cathelicidin family3 and represents a potential therapeutic compound for its importance in inflammatory suppression, innate and adaptive immunity, wound healing, and apoptosis.2,4 The peptide takes action by disrupting the cell membranes5 using either the toroidal pore formation6,7 or a carpet-like mechanism.5,8 The mechanism of action could be proportional to the type of membrane surface.9 As a consensus view, the peptide readily disrupts the anionic membranes consisting of phosphatidylglycerols (PGs).10,11 With regard to the literatures, LL-37 is capable of suppressing bacterial biofilm formation and growth only at initial stage. On the
one hand, performed biofilms and bacterial adhesion become challenging to treat,\textsuperscript{12,13} and on the other hand, LL\textsubscript{37}, as a natural peptide, is not resistant against proteolytic digestion.\textsuperscript{13,14} Therefore, to improve antiviral, antibacterial, and antibiofilm activity of the human cathelicidin peptide against superbugs, the peptide has been dissected into multiple active, potent, selective, and resistant fragments.\textsuperscript{13-18}

Sieprawska et al\textsuperscript{16} indicated that the truncated peptide belonging to amino acid residues 17 to 37 of LL\textsubscript{37} remains intact against \textit{Staphylococcus aureus}. Nagaoka et al\textsuperscript{19} also demonstrated that the derived peptide, residues 15-32, can neutralize the biological activities of lipopolysaccharides in the gram-negative bacteria. Moreover, a broad spectrum of antimicrobial activities of the amino acid residues 11 to 30 of LL\textsubscript{37} has also been investigated by Braff et al.\textsuperscript{18} In all these studies, the residues 17 to 29 of LL\textsubscript{37} have been considered as the critical core segment for the antimicrobial and anticancer activities.\textsuperscript{5,20-22} In addition, previous NMR studies indicated that Asn30, Leu31, and Val32 are the crucial residues in both antimicrobial and anticancer activities of the truncated peptides.

The cationic peptide GF\textsubscript{17}, the residues 17 to 32 of LL\textsubscript{37}, has been identified by Wang et al. through the NMR-based structure studies.\textsuperscript{15} GF\textsubscript{17} has the potential to suppress bacterial adhesion, biofilm growth, and performed biofilms.\textsuperscript{13,14} The peptide also is able to kill gram-negative bacteria such as \textit{Escherichia coli} K-12.\textsuperscript{23} Compared with the human LL\textsubscript{37}, GF\textsubscript{17} is highly active against the methicillin-resistant \textit{S. aureus} strains (USA200, USA300, USA400, MU50, UAMS-1, and Newman) in vitro and in vivo.\textsuperscript{13,14,23} The peptide is also active against the human immunodeficiency virus type 1\textsuperscript{13} and cancer cells.\textsuperscript{15} GF\textsubscript{17} is composed of the nonpolar residues Phe17, Ile20, Val21, Ile24, Phe27, Leu28, Leu31, and Val32 on the hydrophobic side opposite the charged and polar residues Lys18, Arg19, Gln22, Asp26, Arg29, and Asn30 (as numbered in LL\textsubscript{37}) (Figure 1A). The cationic residues Arg23 and Lys25 residing on hydrophobic and hydrophilic interface are important for direct interactions with the bacterial membranes.\textsuperscript{23} All in all, previous studies have shown that GF\textsubscript{17} rapidly binds to PG and induces anionic lipids clustering in the gram-positive bacteria,\textsuperscript{23,24} whereas the gram-negative model membranes [containing a myriad of phosphatidylethanolamines (PEs) and varying amounts of PGs] are less involved in the electrostatic interactions with the cationic residues of GF\textsubscript{17}.\textsuperscript{9}

Molecular dynamics (MD) simulation enables us to follow and characterize dynamics, structure, and the mechanisms of interaction of biomolecules with atomic details.\textsuperscript{25-27} MD computer simulations have also been used to provide a new direction toward de novo protein and peptide design.\textsuperscript{28-30} This approach allows AMPs to be computationally re-embedded into biomembranes, and the molecular interactions with surrounding lipid molecules to be investigated. A number of recent lipid-peptide interaction studies have identified specific residues which bind to distinct sites in lipid bilayers. Therefore, in this study, MD was applied to indicate the contribution of each amino acid in the early insertion of GF\textsubscript{17} into the bacterial model membranes. The results emphasize on the pivotal role of
the basic residues particularly Arg23 and Arg29 in making large contribution to the strong electrostatic interactions between the peptide and lipid headgroups, so that the arginine substitutions in making mutations did not present remarkable results in the peptide activity.23

Herein, we used 2 models of the bacterial membrane in the different ratios of palmitoyl-oleoyl-PG/palmitoyl-oleoyl-PE to investigate the capability of GF-17 in disrupting the bacterial model membranes and all interactions between the peptide and the gram-negative and gram-positive bacterial membranes from a molecular perspective. In general, there are 3 primary objectives of this research: (a) To observe the behavior of the peptide toward different composition of lipid bilayers in the bacterial model membranes. (b) To investigate the contribution of the polar and nonpolar interactions to the membrane binding of the peptide. (c) To calculate the contribution of each residue to the peptide adsorption and penetration into the different lipid bilayer composition. The results from this investigation would provide a clear-cut clue to de novo peptide design in the killing of the methicillin-resistant S. aureus and E. coli strains.

2 | COMPUTATIONAL METHODS

2.1 | Setup of molecular dynamics simulation

The peptide was first found at the AMP Database (APD ID: AP00708)31 and the initial structure obtained from Protein Data Bank (PDB ID: 2LSM).23 A blend of palmitoyl-oleoyl-PG and palmitoyl-oleoyl-PE lipids with ratios of 3:1 and 1:3 for S. aureus and E. coli, respectively, were used based on the previous same studies.32 The starting coordinates and topologies of the lipid bilayers were created using the CHARMM-GUI web site.33 To provide well equilibrated systems, all pure bilayers were simulated for 150 ns and the last snapshots of the systems were used as the initial coordinates for each peptide-bilayer simulation.34 GF-17 is an amphipathic peptide with symmetrical hydrophobic and hydrophilic faces. To increase the precision and accuracy of the results, both hydrophobic and hydrophilic faces of GF-17 were placed approximately 3 nm above the model membranes, and then each system was simulated for 300 ns (Figure 1B). The peptide was placed in the parallel orientation to the surface of the model membranes as previously observed; the human cathelicidin peptide lies parallel to the surface of the bacterial models of the membrane.5-7,35

All MD simulations were carried out using the GROMACS 5.1.2 package.36 The CHARMM force field37 was applied to the peptide and all model membrane. To neutralize the systems, the appropriate numbers of the sodium and chloride ions were added to each simulation box. In all simulation systems, periodic boundary conditions were used along all simulations box axes and the transferable intermolecular potential 3 point (TIP3P) water model38 was applied to solvate the systems. The LINCS algorithm39 was used to constrain all covalent bonds. Simulations were conducted using a 1.2-nm distance cutoff for the van der Waals interactions and short-range electrostatic interactions. The Particle Mesh Ewald algorithm40 was considered for calculation of the long-range electrostatic interactions. All systems were energy minimized using the steepest descent algorithm and then were equilibrated using the NVT ensemble (constant number of particles, volume, and temperature) for 300 ps. Each system then gradually was equilibrated by the NPT ensemble (constant number of particles, pressure, and temperature), and the temperature was maintained at 323 K using the Nose-Hoover algorithm temperature.41,42 During the NPT equilibration, the pressure was maintained at 1 bar by the Parrinello-Rahman barostat.43 Six independent MD simulations were performed in the current study and further information for each simulation system is given in Table 1.

2.2 | Calculation of the binding free energy

Calculation of the binding free energy would provide insight into the polar and nonpolar interactions between the peptide and the bacterial model membranes. The g_mmpbsa tool was used to calculate the binding free energy by the molecular mechanic Poisson–Boltzmann surface area (MM-PBSA) method.44 The total binding free energy (ΔG) is obtained by summing up the difference of polar interaction free energy (ΔGpolar) and the difference of nonpolar interaction free energy (ΔGnonpolar) and can be expressed as

\[ \Delta G_{\text{total}} = \Delta G_{\text{polar}} + \Delta G_{\text{nonpolar}} \]  \hfill (1)

where \( \Delta G_{\text{polar}} \) is the polar binding energy and can be calculated by summing up the difference of electrostatic interaction energy (\( \Delta G_{\text{elec}} \)) and the difference of polar solvation energy (\( \Delta G_{\text{ps}} \)).

\[ \Delta G_{\text{polar}} = \Delta G_{\text{elec}} + \Delta G_{\text{ps}} \]  \hfill (2)

\( \Delta G_{\text{nonpolar}} \) is the nonpolar binding energy and can be obtained by summing up the difference of van der Waals interaction energy (\( \Delta G_{\text{vdw}} \)) and the difference of nonpolar solvation energy (\( \Delta G_{\text{ns}} \)).

\[ \Delta G_{\text{nonpolar}} = \Delta G_{\text{vdw}} + \Delta G_{\text{ns}} \]  \hfill (3)
The energy calculations were performed for the last 30 ns of all MD simulations. The length of the simulation steps was 0.2 ns, ie, 151 snapshots were used for the energy calculations.

3 RESULTS AND DISCUSSION
3.1 Role of each GF-17 residue in the membrane activity

As previously mentioned, GF-17 refers to the residues 17-32 of the human cathelicidin LL-37 and adopts an amphipathic helical structure in the presence of the sodium dodecyl sulfate (SDS) micelles.23 According to the experimental studies, both Arg23 and Lys25 are the crucial residues for the antimicrobial activity of GF-17 against the gram-negative bacteria, as these residues directly expose to the bacterial membranes. In the central helix of the human cathelicidin peptide (GF-17 fragment), direct arginine-PG-lipid contacts were observed for Arg23 by solution NMR spectroscopy.45

To determine the role of the residues in the activities of GF-17, we calculated the radial distribution functions between the Arg23 and Lys25 side chains and the phosphorus atoms of the model membranes (Figure 2A,B). In the S. aureus model membrane (Figure 2A), Arg23 showed 2 peaks at approximately 0.3 and 0.4 nm (dashed lines), suggesting the distance for the occurrence of hydrogen bonds and electrostatic interactions, respectively, between the side chains of residues and the phosphorus atoms. Aforementioned, in the case of S. aureus model membrane direct Arg23-PG-lipid contacts were observed by solution NMR spectroscopy.45 These 2 peaks were also observed for Arg23 and Lys25 with the phosphorus atoms in the E. coli simulation system, which are indicating the hydrogen bond formation as well as electrostatic interactions (Figure 2B). All radial distribution function analyses in excellent agreement with the previous experimental studies suggest that the cationic residues of GF-17 directly interact with the bacterial model membranes.

Regarding previous experimental studies, the side chains of the phenylalanine residues (Phe17 and Phe27) and the interfacial basic residues directly interact with the PG lipid molecules.9 Therefore, the radial distribution functions between the phenylalanine side chains and the hydrophobic acyl chains of 2 bacterial membranes was also calculated (Figure 2C,D). As can be seen, the phenylalanine residues show a peak at approximately 0.5 nm away from phosphate groups, which indicates that hydrophobic interactions are occurring between the peptide side chains and the acyl chains of the bacterial membranes (dashed lines). Taken together, these results
confirmed insertion of the phenylalanine residues into the hydrophobic core of the bacterial model membranes.

### 3.2 Adsorption and insertion of GF-17 onto the model membranes

To investigate the insertion depth of the total peptide into all bacterial model membranes, we also calculated the distance between the center of mass (COM) of the peptide backbone and the lipid bilayers (Figure 3). An early capture of the peptide from its hydrophilic side was observed by the headgroups of *S. aureus* membrane (Figure 3A). As can be seen, approximately after 45 ns of the simulation time, GF-17 was adsorbed on the phosphate headgroups and stabilized to the end of simulation time. This is probably due to the formation of salt bridges between the cationic residues and the phosphorus atoms that will be discussed in the next sections. Such a surface localization has also been observed in the previous studies.\(^5\) In the MD3 simulation with the hydrophobic side of the peptide facing the *S. aureus* model membrane, the peptide adsorbed on the membrane after approximately 100 ns of simulation time, which takes a longer time than MD2 because of the initial disposition of the peptide in MD2 and MD3. Referring to the discovered direct arginine-PG-lipid contacts by NMR experiments,\(^4\) the electrostatic interactions are the main reason for the potent association of the selected basic residues with the anionic PG lipids. These interactions lead to a prompt and deep association of the peptide with the *S. aureus* model membrane containing scores of the PG lipids. In addition, in MD5 and MD6 simulations, the peptide bound to the phosphorus atoms of the headgroups at the end of simulation time; however, we observed the peptide attachment to the membrane surface much earlier in MD2 and MD3. This low affinity for early adsorption can be related to the large number of zwitterionic lipid molecules (the palmitoyl-oleoyl-PE membrane) in the *E. coli* model membrane.

To determine the structural stability after the peptide adsorption on the lipid bilayers, the root mean square deviation (RMSD) of the C\(\alpha\) atoms was also calculated.\(^6\) In the MD7 simulation, the peptide nearly reached a stable structure in aqueous solution after approximately 90 ns of the simulation time.\(^7\) Figure 3 indicates that, compared with the control (MD7), the peptide nearly reached a stable structure in the MD2, MD3, and MD5 simulations after approximately 50 ns of simulation time because of its direct interaction with the headgroup of the membranes.
Accordingly, the distance between the center of mass of the peptide and phosphate groups did not significantly change and nearly reached a plateau over the entire simulation time.

These results are also in agreement with experimental studies, that is, GF-17 assumes a helical structure upon contact with hydrophobic ambient. The RMSD analysis of the MD6 simulation revealed that GF-17 nearly reached a stable structure after adsorption on the membrane surface, over the last 25 ns of simulation time. This was indicated by the distance analysis, where the peptide reached the phosphorus atoms and remained in this region over the last simulation time steps. It is also important to point out the RMSD and distance analyses of the peptide demonstrated such stability over the membrane after extending simulation time about more 50 ns for the MD6 simulation (see Supporting Information Figure S1 in supplementary materials).

### 3.3 Step by step penetration of GF-17 into the model membranes

The poor hydrophobicity of GF-17 excludes its binding to the bacterial membranes but not human cell membranes containing the zwitterionic phosphocholine headgroup. In the MD3 and MD6 simulations, Lys18 and Arg19 rapidly bound to the membrane surface through the hydrogen bonds and the electrostatic interactions (Figure 4A,B, and Supplementary Information Figure S2) and caused the rest of the peptide to adsorb on the membrane from its hydrophilic side (Figure 4C,D), although it initially was positioned from the hydrophobic side facing the model membrane (Figure 1B). With increasing simulation time, the peptide rotated toward the hydrophobic face, the phenylalanine residues passed the phosphate headgroups, while repeatedly the basic residues pushed them back (Figure 4E). In MD2 and MD5 simulations, the adsorption and insertion of the peptide...
on the model membranes were the same as the MD3 and MD6 simulations. Previous NMR studies revealed that Phe17 and Phe27 act as an anchor for GF-17 in complex with the PG micelles. Li et al. also demonstrated that Leu28, Arg29, Asn30, Leu31, and Val32 are the key residues for the antimicrobial and anticancer activity of the truncated peptide using NMR studies. As previously mentioned, Phe17 and Phe27 passed the phosphate atoms during the simulation time (Figure 4F), and Leu28 entered the hydrophobic regions of the bilayer (Figure 4G). With increasing simulation time, Asn30, Leu31, and Val32 also passed the phosphorus atoms (Figures 4H,I), while they were pushed back by the cationic residues for the strong electrostatic interactions and hydrogen bonds between the positively charged side chains and negatively charged phosphate headgroups of the model membranes (Figure 4J). In other words, the basic residues electrostatically interact with the phosphorus atoms and impede the penetration of the hydrophobic residues into the hydrophobic core of the membrane. Taken together the phenylalanine, asparagine, valine, and leucine residues repeatedly passed the phosphorus atoms, but they were ruled out by the arginine and lysine residues. Consistent with the NMR studies, these results all indicate the importance of Phe17, Phe27, Leu28, Asp30, Leu31, and Val32 in the early insertion and membrane activity of the peptide. In general, our results illustrate the significance of the cationic residues in the early adsorption and the hydrophobic residues in the insertion of the peptide, independent of the initial disposition.

3.4 | Membrane structure changes

3.4.1 | Order parameter of the model membranes

The deuterium order parameter indicates the effect of GF-17 on the structure of the bacterial model membranes (Figure 5). Previous experimental studies of the human cathelicidin peptide in complex with the zwitterionic and anionic membranes, revealed an overall decrease of the order parameter indicating an increase in the membrane disordering. Our order analysis demonstrated that, compared with the pure bilayers, the ordering of both acyl chains was reduced in all simulations, when the peptide was in complex with the bilayers. Therefore, GF-17 showed the same behavior on the membrane ordering as its parent LL-37.

As can be seen, the ordering of the hydrophilic tails in the S. aureus model membrane was slightly higher than that of the E. coli membrane, in agreement with the previous NMR experiments. The order results also demonstrated a higher ordering for the C1 and C2 atoms of the sn2 acyl chains in the S. aureus model membrane. With regard to the distance analyses, the peptide laid deeper in the phosphate headgroup regions of the S. aureus model membrane. Therefore, the initial segment of the acyl chains in the S. aureus model membrane would be more restricted by GF-17 than that of in the E. coli membrane. The same decrease of order in both starting orientation of GF-17 indicated that the initial orientation of the peptide could not significantly affect the effectiveness of the peptide on the model membranes.

3.4.2 | Density distributions of all atoms in the model membranes

We calculated the density distributions of atoms along the bilayer normal direction to evaluate the effects of the peptide on cohesion and structure of the lipid bilayers. As
demonstrated in Figure 6, the density profile of the peptide has overlapped with that of the headgroup regions, indicating the surface localization of the peptide on the models of the membrane. The peptide localized deeper in the MD2 simulation than others, in compliance with the distance analyses for the MD2 simulation, the peptide evenly distributed in the headgroup regions of the model membranes (Figure 6A). Furthermore, the distribution of the peptide in the hydrophobic regions of the S. aureus model membrane was larger than that of

FIGURE 5  Deuterium order parameter of the hydrophobic tails for (A) the S. aureus model membrane and (B) the E. coli model membrane. The filled magenta and red lines represent the ordering of the acyl chains for the pure bilayers and the hydrophilic side of GF-17 facing the bilayer model, respectively. The dashed cyan lines show the ordering of the hydrophobic tails for the hydrophobic side of the peptide facing the model membrane.

FIGURE 6  Mass density profiles of the membrane components in (A) MD2, (B) MD3, (C) MD5, and (D) MD6 simulations.
the *E. coli* membrane, indicating a slightly deep insertion of the peptide into the hydrophobic tails. Compared with the lower leaflet, a decrease was observed in the density profile of the *S. aureus* model membrane upper leaflet (dashed lines in Figure 6A,B). In contrast, in case of the *E. coli* membrane, the density distribution of upper leaflet was slightly higher than that of lower leaflet (Dashed lines in Figure 6C,D). These results all indicated that the peptide affects the upper leaflet of both model membranes during the entire simulation time.

### 3.5 | Binding free energy

#### 3.5.1 | Binding free energy of the total peptide

The binding free energy for the entire peptide was calculated to determine the roles of all interactions in early adsorption of GF-17 on the model membranes. As can be observed in Figure 7A, the peptide was favored to interact with the model membranes through both polar and nonpolar interactions, particularly the electrostatic interactions portrayed outstandingly in the peptide binding.

We refer to the hydrophobic interactions as the secondary interactions along the electrostatic ones, in accordance with previous results proposed by Wang et al.\textsuperscript{23,24,45} as well as similar MD studies stating the importance of electrostatic interactions in immediate peptide–membrane binding and hydrophobic interactions in subsequent insertion.\textsuperscript{32,47-49} As indicated in Figure 7A, the polar energies in the case of *S. aureus* were markedly stronger than that of *E. coli* because the existing a large number of PG lipid molecules in the *S. aureus* model membrane. Therefore, this composition of the membrane has the higher net charge and causes the peptide to adsorb strongly on the *S. aureus* model membrane. The total binding free energy in the MD2, MD3, MD5, and MD6 simulations was $-8030.2$, $-8545.4$, $-4687.1$, and $-3936.6$ KJ/mol, respectively, confirming a strong binding of GF-17 to the *S. aureus* model membrane (Figure 7A). A previous experimental study indicated that GF-17 is stronger in the killing of *S. aureus* than *E. coli*.\textsuperscript{23} It could be due to the peptide’s potency of making strong interactions with the negatively charged membranes.

#### 3.5.2 | Binding free energy of each residue of GF-17

We also computed the contributions of each residue to the total binding free energy to better understand the role of each amino acid in the binding of the peptide to the model membranes (Figure 7B). Consistent with experiments,\textsuperscript{23} Lys18, Arg19, Arg23, Lys25, and Arg29 favored the total binding energy in all simulations. As previously demonstrated, they are important residues in the interactions of GF-17 with the bacterial components. The values of the total binding free energy for these basic residues in the *E. coli* model membrane were less than that of the *S. aureus* membrane. Our results are in good agreement with previous experimental studies, reporting that the value of the energy contributions for Arg23 is higher than other basic residues in the MD2 and MD3 simulations.\textsuperscript{24} Therefore, this indicates the significant role of the residue in the killing of the *S. aureus* strains.
The interaction energy of the negatively charged Asp26 in all simulations was unfavorable because of the presence of repulsive forces between the phosphorus atom of the membranes and the negatively charged side chain (Figure 7B). Consequently, in all simulations, the peptide rotated toward its hydrophobic side, when associated with the membrane. Probably, Asp26 is the reason for the peptide rotation on the membrane surfaces.

Previous experimental studies revealed that the phenylalanine residues of an engineered AMP can significantly increase the activity of the peptide versus pathogens. In this study, the Phe17 and Phe27 residues had their considerable contributions (ΔG < −10 Kj/mol) to the total binding free energy in the most of simulations. Figure 7C illustrates the energy contribution of each hydrophobic residue consistent with previous results. As can be seen, the other hydrophobic residues of GF17 also had significant contributions in total interactions of the peptide with the model membranes.

### 3.5.3 Salt bridge's contributions to the binding free energy

According to the Table 2, the calculated electrostatic energies of the salt bridge formations demonstrate much higher values for interactions between the basic residues and the headgroups in S. aureus than E.coli because of the scores of the negatively charged PG lipids in the S. aureus membrane. As a result, the peptide strongly bound the model membranes in MD2 and MD3 containing a myriad of the PG vesicles. As the distance analyses previously confirmed, the peptide was stabilized on the surface of the membranes in MD2 and MD3 steadier than in MD5 and MD6.

### 4 CONCLUSIONS

The central helix of LL-37 is considered the critical segment for the antimicrobial and anticancer activity of the human cathelicidin peptide. The residues 17-32 of LL-37 constitutes symmetric amphipathic configuration and its poor hydrophobicity increases the peptide activity against pathogens and cancer cells, while making a decrease in human cell cytotoxicity. To establish an insight into the molecular interactions of the cationic and hydrophobic residues, we studied the truncated human cathelicidin peptide GF-17 in complex with the S. aureus and E. coli models of the membrane. The distance and density analyses demonstrated a surface localization of GF-17 in complex with the bacterial model membranes. The density distributions analysis also revealed a stronger penetration of GF-17 into the hydrophobic regions of the S. aureus model membranes. In addition, we demonstrated the significance of the basic residues in the early adsorption and surface-localization of GF-17 on the membrane surface as well as preventing the hydrophobic residues from entering the hydrophobic core of the membrane. Moreover, both polar and nonpolar interactions indicated to favor the peptide binding with the bacterial model membrane and order analysis revealed an increase in the membrane disordering upon the peptide adsorption regardless of the initial orientation of the peptide toward the model membranes. The MD simulation results clarify the primary importance of electrostatic interactions as driving forces in the GF-17 binding and the secondary importance of hydrophobic interactions in the deep penetration of the peptide into the hydrophobic core of the bacterial model membranes. On the basis of our findings, GF-17 can be considered as a good candidate for new antimicrobial drugs development.

### CONFLICTS OF INTEREST

The authors declare that they have no conflict of interests.

### ORCID

Faramarz Mehrnejad [http://orcid.org/0000-0002-2991-3079]

### REFERENCES


**SUPPORTING INFORMATION**

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