Supporting Information

Delineating the Mechanism of Action of a Protease Resistant and Salt Tolerant Synthetic Antimicrobial Peptide against *Pseudomonas aeruginosa*

Gopal Pandit,¹ Tanumoy Sarkar,¹ Vignesh, S. R.,² Swapna Debnath,¹ Priyadarshi Satpati²* and Sunanda Chatterjee¹*

1. Department of Chemistry
   Indian Institute of Technology. Guwahati (IITG)
   Guwahati, Assam-781039
   India.

2. Department of Biosciences and Bioengineering
   Indian Institute of Technology. Guwahati (IITG)
   Guwahati, Assam-781039
   India.
## Contents:

| Figures                                      | Page no. |
|----------------------------------------------|----------|
| Figure S1: MD setup models                   | S4       |
| Figure S2-S8: Analytical HPLC Traces for P31 to P37 | S5-S7    |
| Figure S9-S15: ESI MS for P31 to P37         | S8-S14   |
| Figure S16-S22: $^1$H NMR for P31 to P37     | S15-     |
|                                               | S21      |
| Figure S23: Bar diagram for MIC$_{99\%}$ of P31 to P37 | S22      |
| Figure S24: MIC of P36 against *P. aeruginosa* in the presence of Ca$^{2+}$ and Mg$^{2+}$ ions. | S23      |
| Figure S25: Digital image for hemolytic assay| S24      |
| Figure S26: The bactericidal assay against *P. aeruginosa* in presence of P32 | S25      |
| Figure S27-29: ESI MS for enzymatic action against P36 | S26-     |
|                                               | S28      |
| Figure S30-32: ESI MS for enzymatic action against P4 | S29-     |
|                                               | S31      |
| Figure S33: MIC of P36 against *P. aeruginosa*, post 6 hr. incubation of P36 with the enzyme cocktail | S32      |
| Figure S34: Time kinetics of (A) NPN and (B) PI uptake after addition of 1X and 2X MIC P32 to *P. aeruginosa* cells | S32      |
| Figure S35: CD spectroscopy for P31 to P37   | S33      |
| Figure S36: Live Cell NMR of P36 on *P. aeruginosa* cells | S33      |
Figure S37-S41: Direct and water mediated interaction network of P32/P36 with SDS and DPC in the membrane bound state

Figure S42-43: Residue specific solvent exposure of P32/ P36 in the peptide: membrane mimetic systems.

Figure S44: Peptide-micelle distance during the last 50 ns of MD trajectory

Table S1: MD simulations details
Table S2: MD simulations parameters
Table S3: Sequence of the peptides
Table S4: Physicochemical properties of P32/ P36
Table S5: Structural parameters of P32/P36: SDS complex

References
Materials, Peptide synthesis, purification, characterization, microbial and mammalian cell culture
a) Peptide-Micelle initial models (MD Simulation set-up)

b) Peptide-Micelle MD Simulation Box (Including water and ions)

**Figure S1.** (a) Initial MD setup models (SA, SB, SC): varying relative orientation of P36/P32 and SDS micelle. N- and C- termini are highlighted. (b) Typical MD simulation system (P36 in presence of SDS micelle), solvated with a water box of dimension 100×100×140 Å³, SDS micelle (red- white surface), P36 (sticks), Na⁺ ions (violet sphere), Cl⁻ (green sphere) and water (white transparent surface).
**Figure S2**: Analytical HPLC trace for **P31**.

**Figure S3**: Analytical HPLC trace for **P32**.

**Figure S4**: Analytical HPLC trace for **P33**.
Figure S5: Analytical HPLC trace for P34.

Figure S6: Analytical HPLC trace for P35.
**Figure S7:** Analytical HPLC trace for P36.

**Figure S8:** Analytical HPLC trace for P37.
Figure S9. ESI-MS of P31. Calc. (M+H)$^+$ for C$_{44}$H$_{76}$N$_{12}$O$_7$ = 885.5993 Da; Obs. (M+2H)$^{2+}$ = 443.3096 Da, (M+3H)$^{3+}$ = 295.8761 Da.
Figure S10: ESI-MS of P32. Calc. (M+H)$^+$ for C$_{41}$H$_{70}$N$_{12}$O$_7$ = 843.5524 Da; Obs. (M+H)$^+$=843.5708 Da, (M+2H)$^{2+}$= 422.2897 Da, (M+3H)$^{3+}$ = 281.8616 Da.
Figure S11: ESI-MS of P33. Calc. (M+H)$^+$ for C$_{38}$H$_{64}$N$_{12}$O$_7$: = 801.5054 Da; Obs. (M+H)$^+$ = 801.5213 Da, (M+2H)$^{2+}$ = 401.2645 Da, (M+3H)$^{3+}$ = 267.8457 Da.
Figure S12: ESI-MS of P34. Calc. (M+H)$^+$ for C$_{47}$H$_{82}$Ni$_{12}$O$_7$ = 927.6502 Da; Obs. (M+H)$^+$ = 927.6648 Da, (M+2H)$^{2+}$ = 464.3373 Da, (M+3H)$^{3+}$ = 309.8946 Da.
Figure S13: ESI-MS of P35. Calc. (M+H)^+ for C_{44}H_{76}N_{12}O_{7}= 885.5993 Da; Obs. (M+H)^+ = 885.6159 Da, (M+2H)^2+ = 443.3112 Da, (M+3H)^3+ = 295.8767 Da.
Figure S14: ESI-MS of P36. Calc. (M+H)$^+$ for C$_{41}$H$_{70}$N$_{12}$O$_7$ = 843.5524 Da; Obs. (M+H)$^+$ = 843.5604 Da, (M+2H)$^{2+}$ = 422.2845 Da, (M+3H)$^{3+}$ = 281.8595 Da.
Figure S15: ESI-MS of P37. Calc. (M+H)$^+$ for C$_{38}$H$_{64}$N$_{12}$O$_7$: 801.5054 Da; Obs. (M+H)$^+$ = 801.5143 Da, (M+2H)$^{2+}$ = 401.2614 Da.
Figure S16: $^1$H NMR of P31 in D$_2$O at room temperature. $^1$H NMR (600 MHz, D$_2$O). 0.8-0.96 (22H, Leu), 1.19-1.87 (28H, Leu and Orn), 2.9-3.3 (8H, m, Orn and Trp), 3.7-4.7 (7 αH, 1 merged with water signal), 7.1-7.6 (5H, Trp).
**Figure S17:** $^1$H NMR of P32 in D$_2$O at room temperature. $^1$H NMR (600 MHz, D$_2$O). 0.8-0.96 (22H, Leu), 1.19-1.59 (10H, Leu and Dab), 1.79-2.14 (7H, m, Dab), 3.7-4.6 (7 αH, 1 merged with water signal), 6.98- 7.56 (5H, Trp).
Figure S18: $^1$H NMR of P33 in D$_2$O at room temperature. $^1$H NMR (600 MHz, D$_2$O). 0.8-0.96 (22H, Leu), 1.29-1.61 (10H, Leu and Dap), 2.99-3.4 (10H, m, Dab and Trp), 3.7-4.6 (7 αH, 1 merged with water signal), 6.98-7.58 (5H, Trp).
**Figure S19:** $^1$H NMR of P34 in D$_2$O at room temperature. $^1$H NMR (600 MHz, D$_2$O). 0.59-0.74 (17H, Nle, Orn), 1.03-1.60 (28H, Nle and Orn), 2.68-3.07 (8H, m, Orn and Trp), 3.74-4.45 (7 $\alpha$H), 6.92-7.41 (5H, Trp).
Figure S20: $^1$H NMR of P35 in D$_2$O at room temperature. $^1$H NMR (600 MHz, D$_2$O). 0.6-1.28 (24H, Nle, Orn), 1.33-1.81 (21H, Nle and Orn), 2.74-3.19 (8H, m, Orn and Trp), 3.67-4.55 (7 $\alpha$H, 1 merged with water signal), 6.97-7.52 (5H, Trp).
Figure S21: $^1$H NMR of P36 in D$_2$O at room temperature. $^1$H NMR (600 MHz, D$_2$O). 0.58-1.31 (23H, Nle, Dab), 1.41-2.14 (14H, Nle and Dab), 1.79-2.14 (8H, m, Dab and Trp), 3.7-4.6 (7 αH, 1 merged with water signal), 6.98-7.56 (5H, Trp).
Figure S22: $^1$H NMR of P37 in D$_2$O at room temperature. $^1$H NMR (600 MHz, D$_2$O). 0.57-1.24 (28H, Nle, Dap), 1.44-1.74 (7H, Nle and Dap), 2.99-3.42 (10H, m, Dap and Trp), 3.7-4.6 (7 $\alpha$H, 3 merged with water signal), 6.98- 7.58 (5H, Trp).
Figure S23: The bar plots represent MIC$_{99\%}$ of P31 to P37 in presence of 10 mM phosphate buffer. The micro broth dilution assay was performed in increasing concentration of respective peptides and killing percentage was calculated, after overnight incubation. (Upper Panel: against *P. aeruginosa*; middle: against *S. aureus*; lower: against *K. pneumoniae*). Control
experiment was done in presence of 100 μM polymixin B and other readings were normalized against it.

**Figure S24.** MIC of **P36** against *P. aeruginosa* in the presence of Ca^{2+} and Mg^{2+} ions.
Figure S25: Above: Digital image represent hemolytic assay for P32 and P36 against human RBC at different peptide concentration (25, 50, 100, 200 μM). Bottom: Bar diagram showing
the % hemolysis for $\textbf{P32}$ (black) and $\textbf{P36}$ (red) against different concentrations of the peptides. Buffer and Triton-X 100 treated as negative and positive control.

**Figure S26:** The bactericidal assay against $\textit{P. aeruginosa}$ in presence of $\textbf{P32}$. The images depict the CFU/ml on nutrient agar plates at different time point.
Figure S27: Control ESI MS of P36 for enzymatic action study. Calc. (M+H)+ for \( \text{C}_{41}\text{H}_{70}\text{N}_{12}\text{O}_{7}\) = 843.5524 Da; Obs. (M+H)\(^+\) = 843.5526 Da, (M+2H)\(^2+\) = 422.2809 Da, (M+3H)\(^3+\) = 281.8555 Da.
Figure S28: ESI MS of P36 in presence of enzyme mixture (30 min. approximately). Calc. (M+H)$^+$ for C$_{41}$H$_{70}$N$_{12}$O$_7$ = 843.5524 Da; Obs. (M+H)$^+$ = 843.5598 Da, (M+2H)$^{2+}$ = 422.2846 Da, (M+3H)$^{3+}$ = 281.8580 Da.
Figure S29: ESI MS of P36 in presence of enzyme mixture (6 hr. approximately). Calc. (M+H)$^+$ for C₄₁H₇₀N₁₂O₇= 843.5524 Da; Obs. (M+H)$^+$ = 843.5604 Da, (M+2H)$^{2+}$ = 422.2845 Da, (M+3H)$^{3+}$ = 281.8595 Da.
Figure S30: Control ESI MS of P4 for enzymatic action study. Calc. (M+H)^+ for C_{47}H_{82}N_{12}O_{7}= 927.6502 Da; Obs. (M+H)^+ = 927.6586 Da, (M+2H)^{2+} = 464.3339 Da, (M+3H)^{3+} = 309.8918 Da.
Figure S31: ESI MS of P4 in presence of enzyme mixture (30 min. approximately). Calc. (M+H)⁺ for C₄₇H₈₂Ni₂O₇= 927.6502 Da.

815.5603 corresponds to (M+H)⁺ of the fragment LKWLKK

408.2846 corresponds to (M+2H)²⁺ of the fragment LKWLKK

687.46306 corresponds to (M+H)⁺ of the fragment LKWLK

344.2353 corresponds to (M+2H)²⁺ of the fragment LKWLK

258.2148 corresponds to (M+H)⁺ of the fragment LK

Others small peaks seen corresponds to several other fragments of the peptide P4: LKWLKKL-NH₂
**Figure S32:** ESI MS of P4 in presence of enzyme mixture (6 hr. approximately). Calc. (M+H)^+ for C_{47}H_{82}N_{12}O_{7} = 927.6502 Da.

815.5603 corresponds to (M+H)^+ of the fragment LKWKK

408.2846 corresponds to (M+2H)^2+ of the fragment LKWKK

687.46306 corresponds to (M+H)^+ of the fragment LKWKL

344.2353 corresponds to (M+2H)^2+ of the fragment LKWKL

258.2148 corresponds to (M+H)^+ of the fragment LK

Others small peaks seen corresponds to several other fragments of the peptide P4: LKWKLKL-NH₂
**Figure S33.** MIC of P36 against *P. aeruginosa*, post 6 hr. incubation of P36 with the enzyme cocktail (Mixture of Chymotrypsin, Proteinase-K and Trypsin in the ratio 1:1:1). Experiment was performed in triplicates.

**Figure S34.** Time kinetics of (A) NPN and (B) PI uptake after addition of 1X and 2X MIC P32 to *P. aeruginosa* cells, which indicate the outer and inner membrane permeability respectively. All the experiments were performed in triplicets.
**Figure S35:** CD spectral study of P31 to P37 in presence of different membrane mimetic environments: SDS mimics the bacterial membrane and DPC mimics the mammalian membrane. 50% TFE promotes the alpha helical propensity.

**Figure S36:** Live cell NMR spectroscopy of P36 in the presence of *P. aeruginosa* cells. Partial stacked plots of 1D $^1$H NMR spectra at different times of incubation of P36 with *P. aeruginosa*. The blue boxes highlight the newly appeared spectral lines over the time interval.
**Figure S37. P32-SDS direct interactions** are portrayed from the final structure of the P32: SDS micelle MD simulation after 50 ns. Positively charged tips of the peptide (N-terminal, side-chains of Dab2, Dab5 and Dab6) interact directly with sulphates of SDS. P32 is represented in salmon coloured sticks (Nitrogen: blue, Oxygen: red). SDS is shown in surface representation and sulphates of SDS interacting with P32 are shown in sticks form (Sulphur: yellow, Oxygen: red). The local environment of Trp3 is shown explicitly in a solid box. (SA, SB, SC) represent distinctly different simulation setups, (R1, R2) indicate two independent
replicas for each simulation setup. Hydrogens are not shown for clarity. Broken black lines represent the interactions (heavy atom distances ≤ 3.4 Å).

**Figure S38.** P32 water interactions observed from the final MD structure of the P32: SDS complex after 50 ns. Waters interact with the positively charged tips of the peptide (N-terminal, side-chain of Dab2, Dab5 and Dab6). Water mediated interactions between peptide positive charges and the negatively charged sulphates of SDS are frequently observed. Leu1 and Leu4 have less solvent exposure than Leu7 (Figure S33). P32 is represented in salmon coloured sticks (Nitrogen: blue, Oxygen: red) and oxygens of water are shown in red spheres. SDS is
shown in surface representation and sulphates of SDS interacting with water are shown in sticks form (Sulphur: yellow, Oxygen: red). Hydrogens are not shown for clarity. Broken black lines represent the interactions (heavy atom distances ≤ 3.4 Å).

Figure S39. P36-SDS direct interactions are portrayed from the final structure of the P36: SDS micelle MD simulation after 50 ns. Positively charged tips of the peptide (N-terminal, side-chains of Dab2, Dab5 and Dab6) interact directly with sulphates of SDS. P36 is represented in yellow coloured sticks (Nitrogen: blue, Oxygen: red). SDS is shown in surface representation and sulphates of SDS interacting with P36 are shown in sticks form (Sulphur:
yellow, Oxygen: red). The local environment of Trp3 is shown explicitly in a solid box. (SA, SB, SC) represent distinctly different simulation setups, (R1, R2) indicate two independent replicas for each simulation setup. Hydrogens are not shown for clarity. Broken black lines represent the interactions (heavy atom distances ≤ 3.4 Å).

**Figure S40.** P36 water interactions are observed from the final MD structure of the P36: SDS complex after 50 ns. Waters interact with the positively charged tips of the peptide (N-terminal, side-chain of Dab2, Dab5 and Dab6). Water mediated interactions between peptide positive charges and the negatively charged sulphates of SDS are frequently observed. Nle1 and Nle4
had less solvent exposure than Nle7 (Figure S34). P36 is represented in yellow coloured sticks (Nitrogen: blue, Oxygen: red) and oxygens of water are shown in red spheres. SDS is shown in surface representation and sulphates of SDS interacting with water are shown in sticks form (Sulphur: yellow, Oxygen: red). Hydrogens are not shown for clarity. Broken black lines represent the interactions (distances ≤ 3.4 Å).

Figure S41. P32(P36): DPC direct and water mediated interactions. Representative MD snapshot (after 50 ns trajectory). Positively charged tips of the peptide (N-terminal, side-chains of Dab2, Dab5 and Dab6) interact directly with phosphates of DPC and waters. Water mediated interactions between peptide positive charges and the negatively charged phosphates of DPC are frequently observed. The local environment of Trp3 is shown explicitly in a solid box. (SA) represent distinct simulation setup and (R1) indicate independent run for the simulation setup. (a) Left image: P32-DPC direct interaction. Right Image: P32-DPC water interactions. Leu1 and Leu4 have less solvent exposure than Leu7 (Figure S33). P32 is represented in salmon coloured sticks (Nitrogen: blue, Oxygen: red). (b) Left image: P36-DPC direct interaction.
Right Image: P36-DPC water interactions. Nle1 and Nle7 have less solvent exposure than Nle4. (Figure S34). P36 is represented in yellow coloured sticks (Nitrogen: blue, Oxygen: red). DPC is shown in surface representation and phosphates of DPC interacting with P32(P36) are shown in sticks form (Carbon: blue, Phosphorus: orange, Oxygen: red) and oxygens of water are shown in red spheres. Hydrogens are not shown for clarity. Broken black lines represent the interactions (heavy atom distances ≤ 3.4 Å).
Figure S42. Trajectory averaged (last 30 ns) residue-wise solvent exposure (SASA in Å²) of P32 sidechain are shown in the yellow net-plot with contours of constant solvent exposure (increases as one goes away from the centre).

Figure S43. Trajectory averaged (last 30 ns*) residue-wise solvent exposure (SASA in Å²) of P36 sidechain are shown in the yellow net-plot with contours of constant solvent exposure (increases as one goes away from the centre). [*except P36-DPC-SA-R1 (last 5ns)]
Figure S44. Peptide- Micelle distance as a function of time. $d_{\text{COM}}$ is the distance between the centre of mass of peptide and the centre of mass of micelle (SDS/DPC). For the peptide- SDS simulations (Setup: SA, SB, and SC of Figure S1. a), temporally averaged $d_{\text{COM}}$ (averaging over 2 independent MD runs, see Table S1) is plotted. (a) P32-micelle (SDS/DPC) $d_{\text{COM}}$ plot. (b) P36-micelle (SDS/DPC) $d_{\text{COM}}$ plot. The black horizontal line is the averaged $d_{\text{COM}}$ obtained from the last 30 ns of the 50ns trajectory that corresponds to stable peptide: SDS complex. 14.6Å(14.9Å) for stable P32(P36): SDS micelle.
**Supplementary Tables**

**Table S1.** Peptide (P32/P36) in the presence and absence of micelle (SDS/DPC) is considered for MD simulations. Initial structural model (see Figure S27.a), the number of independent replicas (varying the initial velocities), box size, composition and post-equilibrated MD run-length. A total of 810 ns of production MD was performed.
| S. No | System (Simulation Setup A/B/C) | No of replicas (replica label) | Box Size ($\text{Å}^3$) | No of Molecules | Simulation Time (ns) |
|-------|---------------------------------|-------------------------------|--------------------------|----------------|---------------------|
| 1     | Free SDS                        | 1 (R1)                        | 100×100×100              | SDS – 60 SOL – 31681 Na – 60 | 5                   |
| 2     | Free DPC                        | 1 (R1)                        | 100×100×100              | DPC – 60 SOL – 31684 | 5                   |
| 3     | Free P32                        | 1 (R1)                        | 60×60×60                 | Peptide – 1 SOL – 6979 Cl – 4 | 50                  |
| 4     | P32-SDS (SA)                    | 2 (R1, R2)                    | 100×100×140              | Peptide – 1 SDS – 60 SOL – 47606 Cl – 4 | 50,50               |
| 5     | P32-SDS (SB)                    | 2 (R1, R2)                    |                            |                                | 50,50               |
| 6     | P32-SDS (SC)                    | 2 (R1, R2)                    |                            |                                | 50,50               |
| 7     | P32-DPC (SA)                    | 1 (R1)                        | 100×100×140              | Peptide – 1 DPC – 60 SOL – 47364 Cl – 4 | 50                  |
| 8     | Free P36                        | 1 (R1)                        | 60×60×60                 | Peptide – 1 SOL – 6978 Cl – 4 | 50                  |
| 9     | P36-SDS (SA)                    | 2 (R1, R2)                    | 100×100×140              | Peptide – 1 SDS – 60 SOL – 47597 Cl – 4 | 50,50               |
| 10    | P36-SDS (SB)                    | 2 (R1, R2)                    |                            |                                | 50,50               |
| 11    | P36-SDS (SC)                    | 2 (R1, R2)                    |                            |                                | 50,50               |
| 12    | P36-DPC (SA)                    | 1 (R1)                        | 100×100×140              | Peptide – 1 DPC – 60 SOL – 47355 Cl – 4 | 50                  |
Table S2. Parameters used in MD simulations.

| Molecular Dynamics Parameters (NPT ensemble) |
|---------------------------------------------|
| Integrator, time step                       | Leap-frog algorithm, 2 fs |
| Hydrogen Bonds Constraint Algorithm         | LINCS$^1$ |
| Long range electrostatics, short range electrostatic cut-off | Particle Mesh Ewald (PME)$^2$, 12 Å |
| Short range van der Waals cut-off            | 12 Å |
| Boundary Conditions                         | Periodic Boundary condition |
| Temperature control, coupling constant      | Velocity rescaling algorithm$^3$, 0.1 ps |
| Pressure control, coupling constant         | Parrinello-Rahman algorithm$^4$, 2.0 ps |
| Temperature                                 | 310 K |
| Pressure                                    | 1 bar |

Table S3: Sequence of the Peptides P31-37

| Sl. NO | Peptide name | Peptide sequence |
|--------|--------------|------------------|
| 1      | P4           | LKWLKKL-CNH₂      |
| 2      | P31          | LOrnWLOrnOrnL-CNH₂|
| 3      | P32          | LDabWLĐabĐabL-CNH₂|
| 4      | P33          | LDapWLĐapĐapL-CNH₂|
| 5      | P34          | NleKWNLěKKNle-CNH₂|
| 6      | P35          | NleOrnWNleOrnOrnNle-CNH₂|
| 7      | P36          | NleĐabWNleĐabĐabNle-CNH₂|
| 8      | P37          | NleĐapWNleKKNle-CNH₂|
**Table S4:** Physicochemical properties of P31-37.

| Peptide code | No. of AA | Net charge(s) | Non-standard AAs | Theoretical MW (Da.) (M+H)^+ | Observed MW (Da.) | Retention Time (min.) |
|--------------|-----------|---------------|------------------|-------------------------------|------------------|-----------------------|
| P4 (control) | 7         | 4             | -                | 927.6502                      | (M+H)^+ = 927.6504 | 8.58                  |
| P31          | 7         | 4             | Orn              | 885.5993                      | (M+2H)^{2+} = 443.3096 | 2.006                 |
| P32          | 7         | 4             | Dab              | 843.5524                      | (M+H)^+ = 843.5708 | 6.814                 |
| P33          | 7         | 4             | Dap              | 801.5054                      | (M+H)^+ = 801.5213 | 6.951                 |
| P34          | 7         | 4             | Nle              | 927.6502                      | (M+H)^+ = 927.6502 | 7.6                   |
| P35          | 7         | 4             | Orn, Nle         | 885.5993                      | (M+H)^+ = 885.6159 | 6.86                  |
| P36          | 7         | 4             | Dab, Nle         | 843.5524                      | (M+H)^+ = 843.5604 | 7.003                 |
| P37          | 7         | 4             | Dap, Nle         | 801.5054                      | (M+H)^+ = 801.5143 | 7.034                 |
Table S5. **Structural parameters.** Data averaged from the last 30 ns of the 50 ns MD trajectory, except * & # systems. (* & # are calculated from the last 1 ns and 5 ns respectively).

| S. No | Simulation Systems     | Eccentricity AVG±SD | Micelle Radius (Å) AVG±SD | Peptide Secondary Structures |
|-------|------------------------|----------------------|---------------------------|------------------------------|
|       |                        |                      |                           | Coil (%) | Bend (%) | Turn (%) |
| 1     | Free SDS*              | 0.176±0.07           | 21.64±0.32                | 94      | 5       | 1        |
| 2     | Free DPC*              | 0.105±0.04           | 22.66±0.18                |         |         |          |
| 3     | Free P32-R1            |                      |                           | 94      | 5       | 1        |
| 4     | P32-SDS-SA-R1          | 0.151±0.06           | 21.66±0.28                | 91      | 9       | -        |
| 5     | P32-SDS-SA-R2          | 0.150±0.05           | 21.67±0.26                | 92      | 8       | -        |
| 6     | P32-SDS-SB-R1          | 0.170±0.06           | 21.66±0.29                | 97      | 3       | -        |
| 7     | P32-SDS-SB-R2          | 0.153±0.06           | 21.55±0.29                | 100     | -       | -        |
| 8     | P32-SDS-SC-R1          | 0.136±0.05           | 21.55±0.26                | 81      | 19      | -        |
| 9     | P32-SDS-SC-R2          | 0.165±0.06           | 21.72±0.32                | 83      | 16      | 1        |
| 10    | P32-DPC-SA-R1          | 0.104±0.04           | 22.92±0.20                | 100     | -       | -        |
| 11    | Free P36-R1            |                      |                           | 93      | 7       | -        |
| 12    | P36-SDS-SA-R1          | 0.169±0.06           | 22.20±0.86                | 97      | 2       | 1        |
| 13    | P36-SDS-SA-R2          | 0.157±0.06           | 21.71±0.31                | 98      | 2       | -        |
| 14    | P36-SDS-SB-R1          | 0.148±0.06           | 21.50±0.28                | 86      | 13      | 1        |
| 15    | P36-SDS-SB-R2          | 0.154±0.06           | 21.48±0.35                | 73      | 23      | 4        |
| 16    | P36-SDS-SC-R1          | 0.143±0.06           | 21.45±0.34                | 85      | 8       | 7        |
| 17    | P36-SDS-SC-R2          | 0.156±0.06           | 21.69±0.30                | 94      | 6       | -        |
| 18    | P36-DPC-SA-R1#         | 0.105±0.04           | 22.79±0.22                | 73      | 27      | -        |
References:

1. Hess, Berk, et al. LINCS: a linear constraint solver for molecular simulations. *Journal of computational Chem.* 1997, 18, 1463-1472.

2. Tom, D.; York, D.; Pedersen. L. Particle mesh Ewald: An N· log (N) method for Ewald sums in large systems. *J. Chem. Phys.* 1993, 98, 10089-10092.

3. Giovanni, B.; Donadio, D.; Parrinello. M. Canonical sampling through velocity rescaling. *J. Chem. Phys.* 2007, 126, 014101.

4. Michele, P.; Rahman. A. Polymorphic transitions in single crystals: A new molecular dynamics method. *J. Appl. Phys.* 1981, 52, 7182-7190.

Materials: All of the protected amino acids, coupling reagents such as PyBOP, HOBT and piperidine, were obtained from GL Biochem (Shanghai, China). Solvents including dimethylformamide (DMF) and acetonitrile were purchased from Merck. Glyceraldehyde, Poly-L-lysine solution, polymyxin B, and calcein were purchased from Sigma-Aldrich Co. (Saint Louis, MO). Acetic anhydride was obtained from department of chemistry, IIT Guwahati. All bacterial media components were purchased from MTCC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG) were obtained from Avanti Polar Lipids (Alabaster, AL). 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), Dulbecco’s modified Eagle medium, and dimethyl sulfoxide were purchased from Sigma-Aldrich. Streptomycin sulfate was procured from Invitrogen.

Bacterial and Fungal Strains and Reagents: Common laboratory bacterial strain *P. aeruginosa* (MTCC 2488), *K. pneumoniae* (MTCC 443) and *S. aureus* (MTCC 96) were obtained from the microbial type culture collection and gene bank, IMTECH, Chandigarh, India.
**Peptide Synthesis.**

Peptides P31-37 were synthesized by standard Fmoc-based solid-phase peptide synthesis method on MBHA-Rink amide resin (loading 0.7 mmol/g). For each amino-acid attachment, 2.5 equiv. of Fmoc amino acids, 2.5 equiv. of coupling reagent (PyBOP), 2.5 equiv of HOBT, and 5 equiv. of base (DIPEA) were used. For avoiding incomplete reaction, coupling cycles were repeated, followed by capping with 7:2:1 DMF, acetic anhydride, and pyridine. Fmoc deprotection was performed with 20% piperidine in DMF. The final peptide was cleaved from the resin using a cleavage cocktail (96% TFA, 2.5% TIS, and 1.5% H2O) for 2 h. The crude peptide was precipitated by cold diethyl ether followed by centrifugation to get the crude solid peptide.

**Peptide Purification and Characterization.**

Crude peptides were purified by reverse-phase high-performance liquid chromatography (Thermo Scientific Dionex Ultimate 3000) on a semi preparative Biobasic 8 column using binary {CH3CN-H2O (5–100%)} solvent system at a flow rate of 5 mL/min using dual UV detection at 214 and 280 nm. Purity of the peptides were confirmed using a Thermo Scientific Dionex Ultimate 3000 analytical HPLC system and a Thermo Scientific BioBasic 18 analytical column. A flow rate of 1 ml/min and a linear gradient of 10 to100% CH3CN-H2O was used. The purified peptides were characterized by mass spectrometry on Agilent-Q-TOF 6500 instrument in electrospray ionization positive mode, equipped with Mass Hunter workstation software. The purified peptides were further characterized by 1H-NMR spectroscopy on a Bruker Ascend Aeon 600 MHz spectrometer at 298 K in D2O solvent. Peptide stock solution was prepared either in water, in sodium phosphate buffer (pH 7.4), or both and filter-sterilized unless stated otherwise.
Microbial Culture.

For antimicrobial activity assay, *P. aeruginosa*, *K. pneumoniae* and *S. aureus* purchased from MTCC, Chandigarh, were grown in nutrient broth and maintained at 310 K under shaking conditions.

Cell Culture

Human normal embryonic kidney (HEK-293) cells were purchased from the National Centre for Cell Science (NCCS, Pune, India). The cell line was maintained in DMEM supplemented with 10% (v/v) fetal bovine serum and 1% penicillin and streptomycin at 310 K in humidified air containing 5% CO₂.