

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 Satpati^{2*} and Sunanda Chatterjee^{1*}

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)

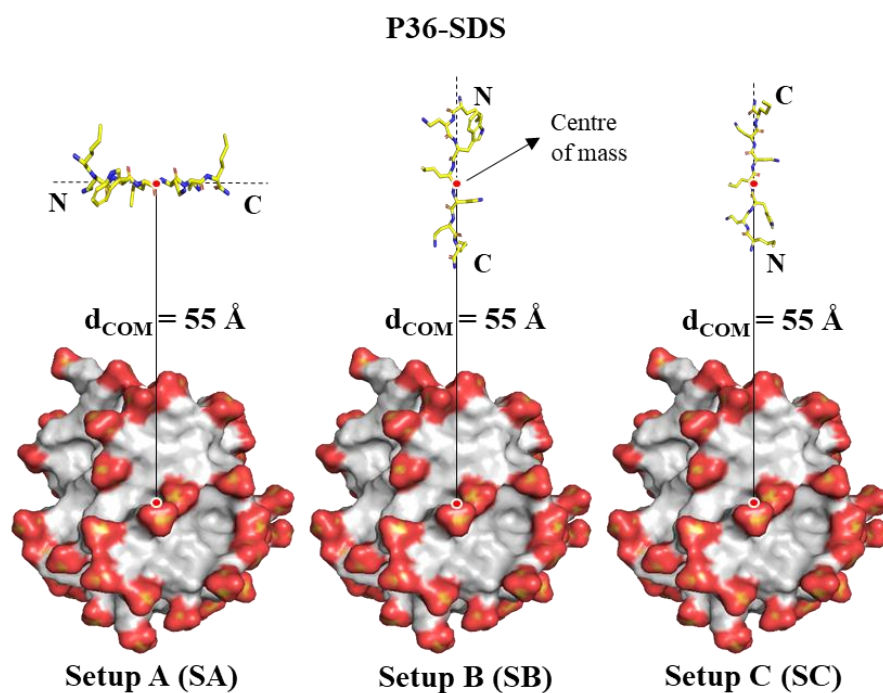
Guwahati, Assam-781039

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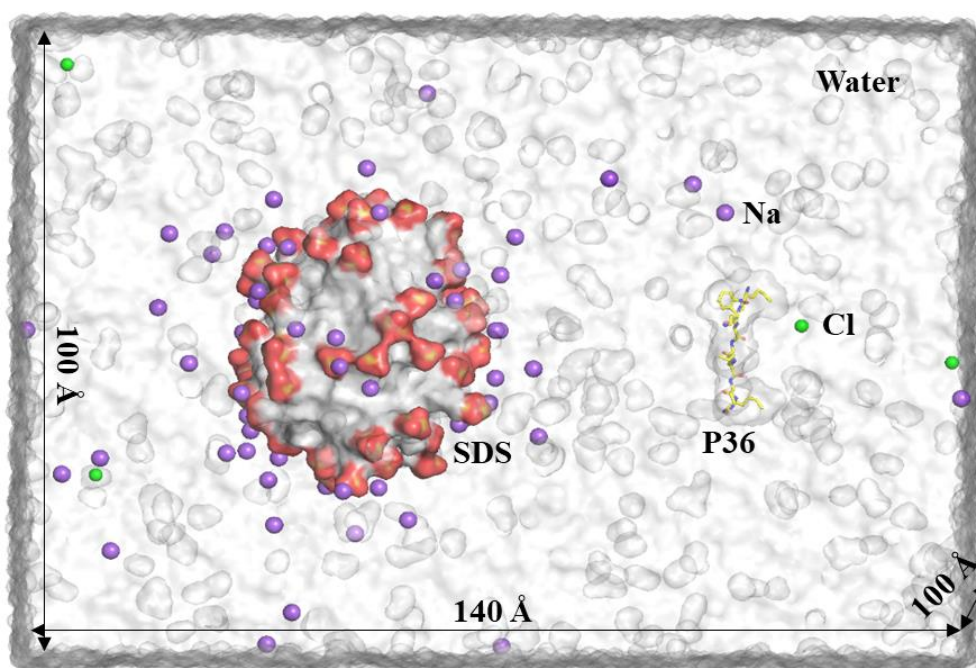
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a) Peptide-Micelle initial models (MD Simulation set-up)



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 $100 \times 100 \times 140 \text{ \AA}^3$, SDS micelle (red- white surface), P36 (sticks), Na^+ ions (violet sphere), Cl^- (green sphere) and water (white transparent surface).

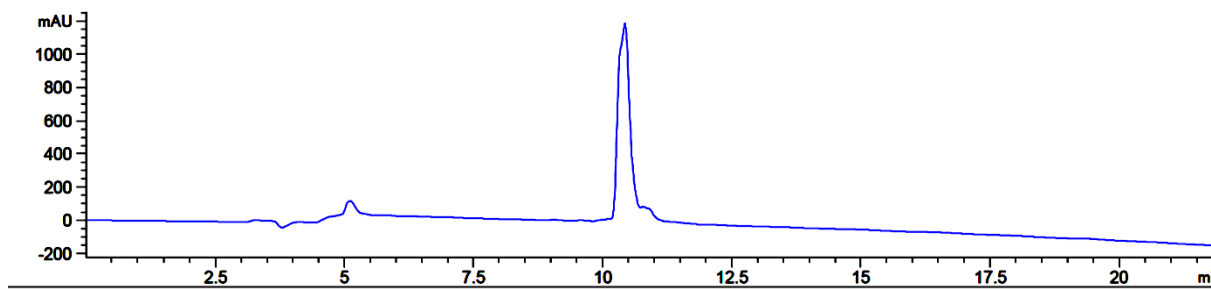


Figure S2: Analytical HPLC trace for **P31**.

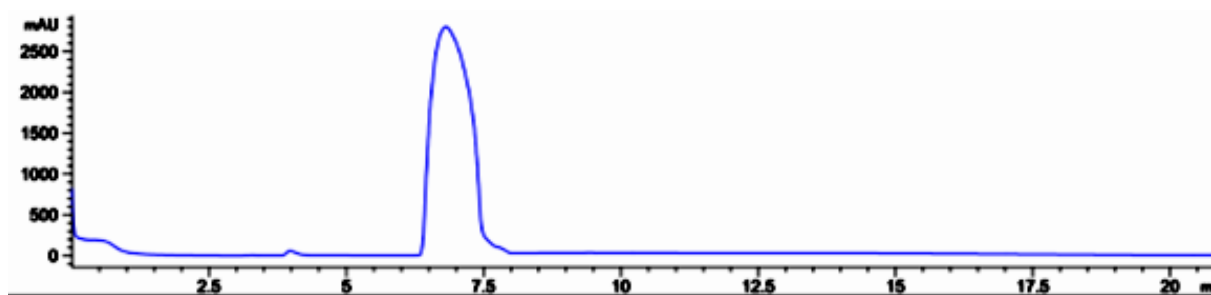


Figure S3: Analytical HPLC trace for **P32**.

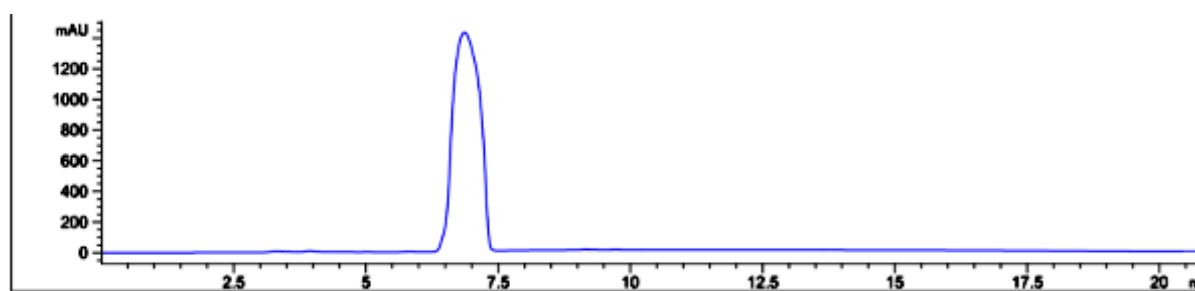


Figure S4: Analytical HPLC trace for **P33**.

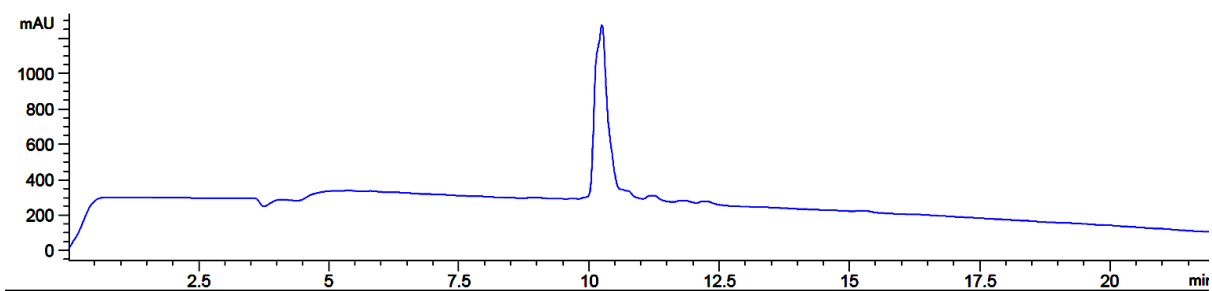


Figure S5: Analytical HPLC trace for **P34**.

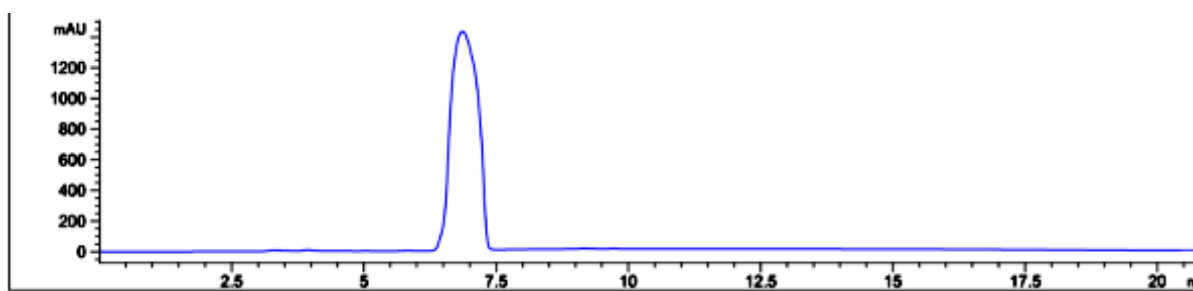


Figure S6: Analytical HPLC trace for **P35**.

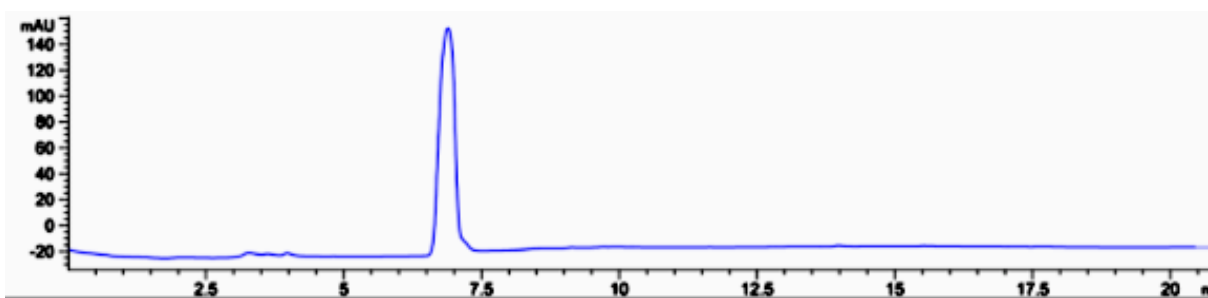


Figure S7: Analytical HPLC trace for **P36**.

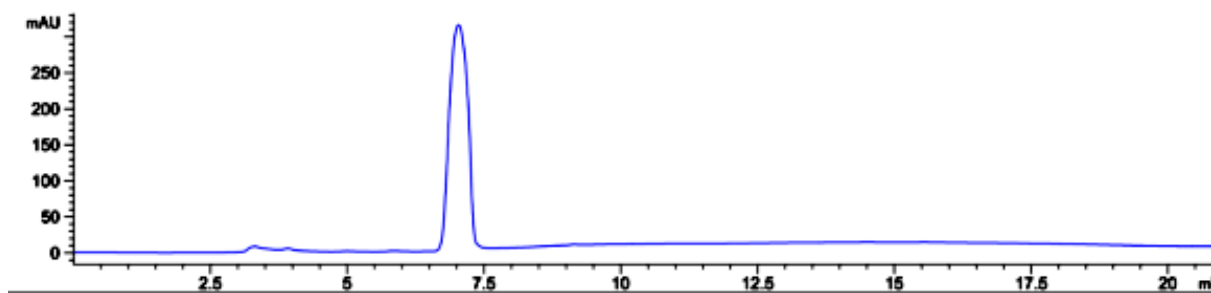


Figure S8: Analytical HPLC trace for **P37**.

Sample Name	GP-31-1	Position	P2-F1	Instrument Name	Instrument 1	User Name	
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Data Filename	GP-31-1.d	ACQ Method	ESI ALS 100-2000.m	Comment		Acquired Time	5/14/2019 5:15:37 PM

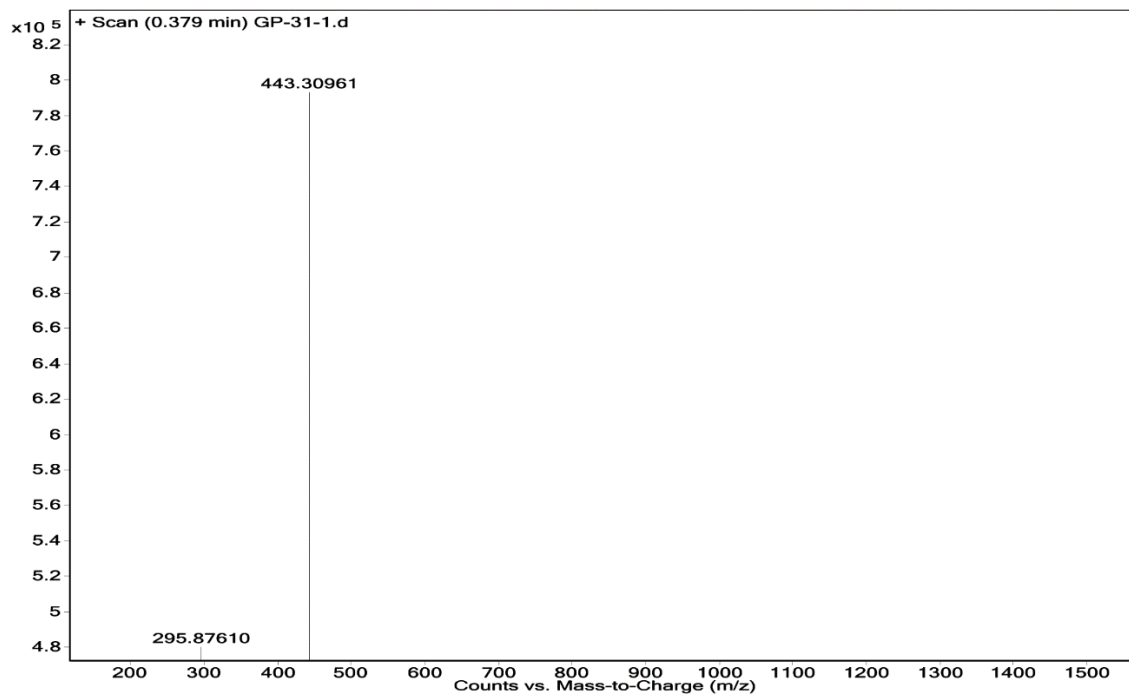


Figure S9. ESI-MS of **P31**. Calc. $(M+H)^+$ for $C_{44}H_{76}N_{12}O_7 = 885.5993$ Da ; Obs. $(M+2H)^{2+} = 443.3096$ Da, $(M+3H)^{3+} = 295.8761$ Da.

Sample Name	SAMPLE 42	Position	P2-E3	Instrument Name	Instrument 1	User Name	
Inj Vol	20	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	GP-32.d	ACQ Method	ESI ALS 100-3000.m	Comment		Acquired Time	5/13/2019 6:26:48 PM

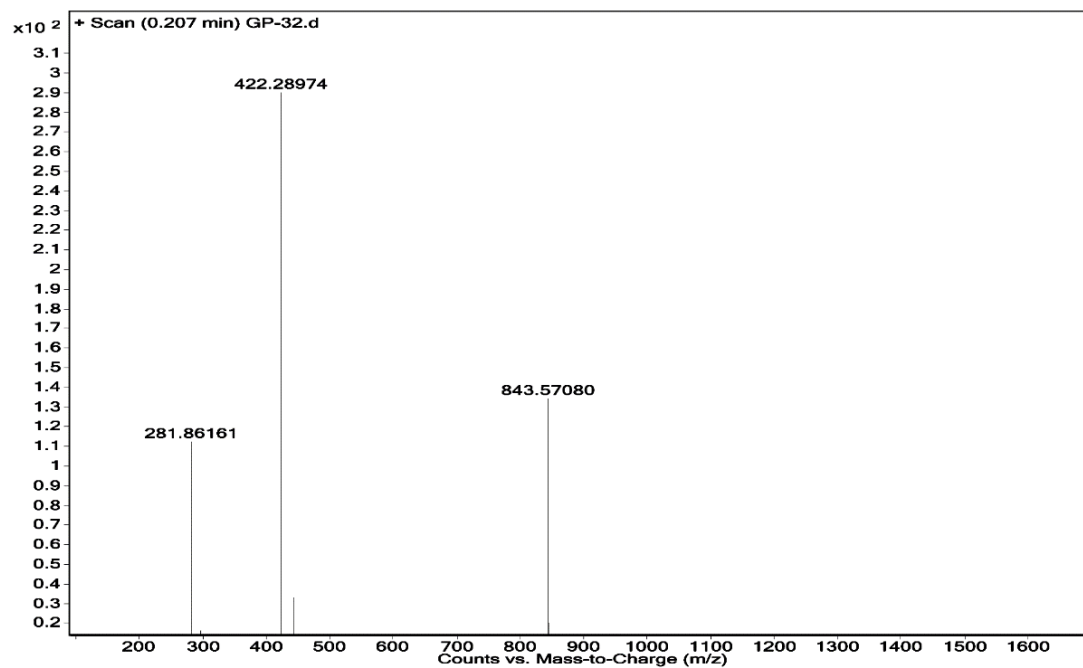


Figure S10: ESI-MS of **P32**. Calc. $(M+H)^+$ for $C_{41}H_{70}N_{12}O_7 = 843.5524$ Da; Obs. $(M+H)^+ = 843.5708$ Da, $(M+2H)^{2+} = 422.2897$ Da, $(M+3H)^{3+} = 281.8616$ Da.

Sample Name	SAMPLE 43	Position	P2-E4	Instrument Name	Instrument 1	User Name	
Inj Vol	20	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	GP-33.d	ACQ Method	ESI ALS 100-3000.m	Comment		Acquired Time	5/13/2019 6:28:39 PM

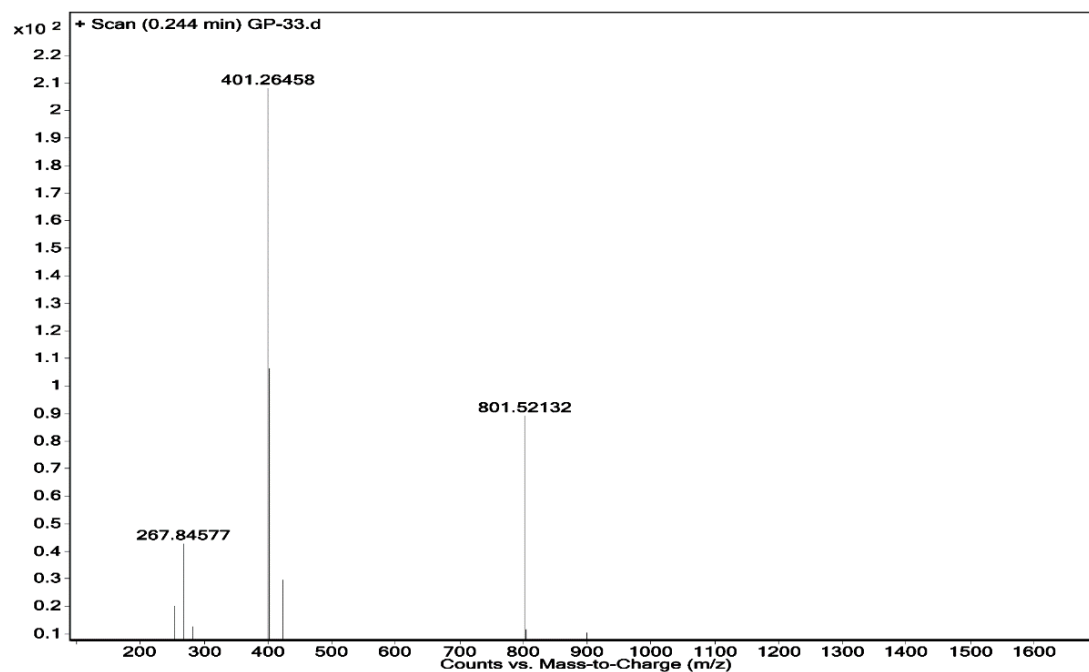


Figure S11: ESI-MS of **P33**. Calc. $(M+H)^+$ for $C_{38}H_{64}N_{12}O_7 = 801.5054$ Da; Obs. $(M+H)^+ = 801.5213$ Da, $(M+2H)^{2+} = 401.2645$ Da, $(M+3H)^{3+} = 267.8457$ Da.

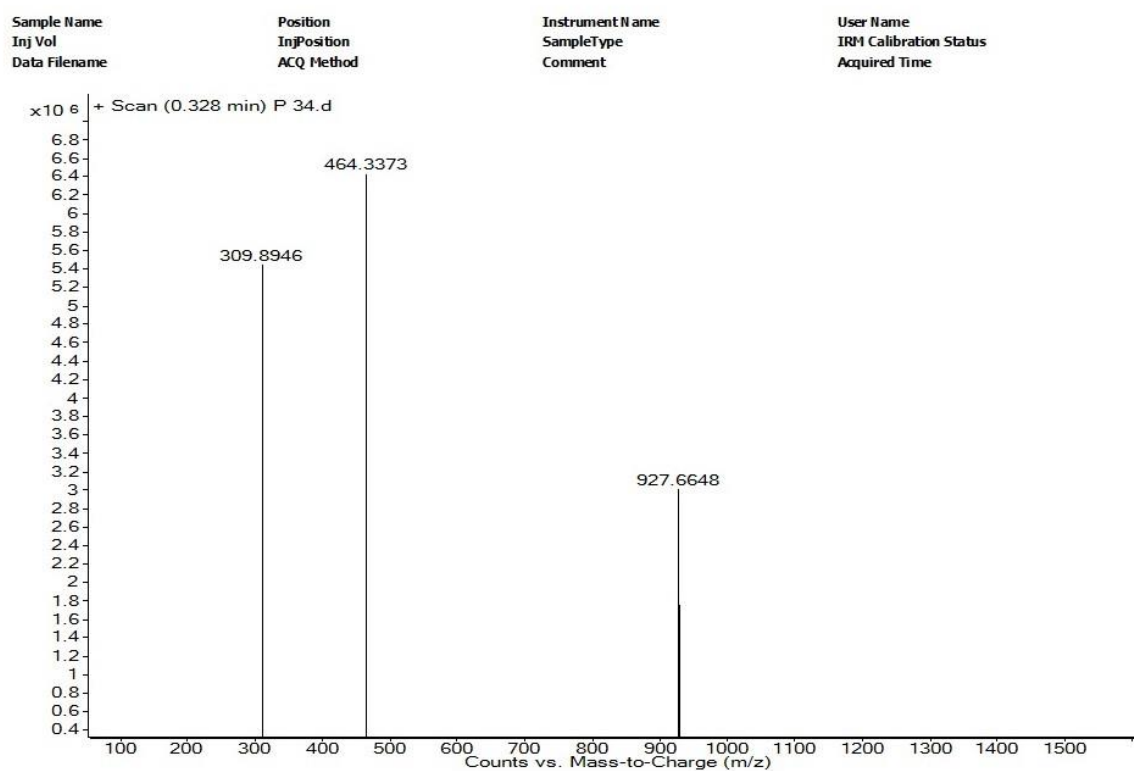


Figure S12: ESI-MS of **P34**. Calc. $(M+H)^+$ for $C_{47}H_{82}N_{12}O_7 = 927.6502$ Da; Obs. $(M+H)^+ = 927.6648$ Da, $(M+2H)^{2+} = 464.3373$ Da, $(M+3H)^{3+} = 309.8946$ Da.

Sample Name	SAMPLE 44	Position	P2-E5	Instrument Name	Instrument 1	User Name	
Inj Vol	20	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	GP-34.d	ACQ Method	ESI ALS 100-3000.m	Comment		Acquired Time	5/13/2019 6:30:29 PM

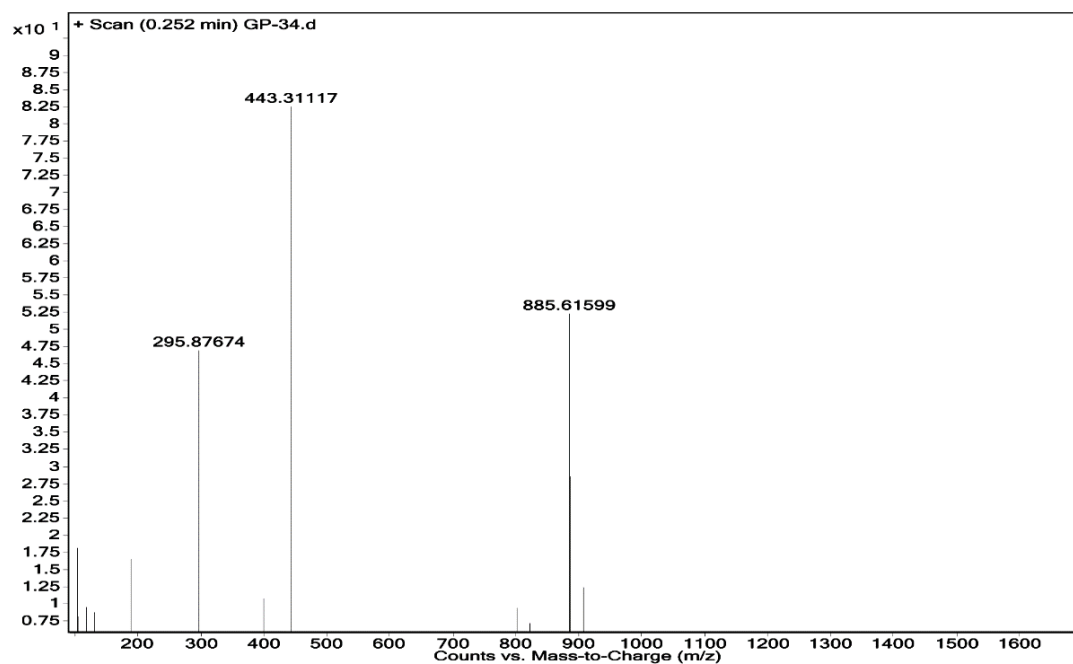


Figure S13: ESI-MS of **P35**. Calc. $(M+H)^+$ for $C_{44}H_{76}N_{12}O_7 = 885.5993$ Da; Obs. $(M+H)^+ = 885.6159$ Da, $(M+2H)^{2+} = 443.3112$ Da, $(M+3H)^{3+} = 295.8767$ Da.

Sample Name	GP-35-1	Position	P2-F5	Instrument Name	Instrument 1	User Name	
Inj Vol	20	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	GP-35-1.d	ACQ Method	ESI ALS 100-2000.m	Comment		Acquired Time	5/14/2019 5:23:03 PM

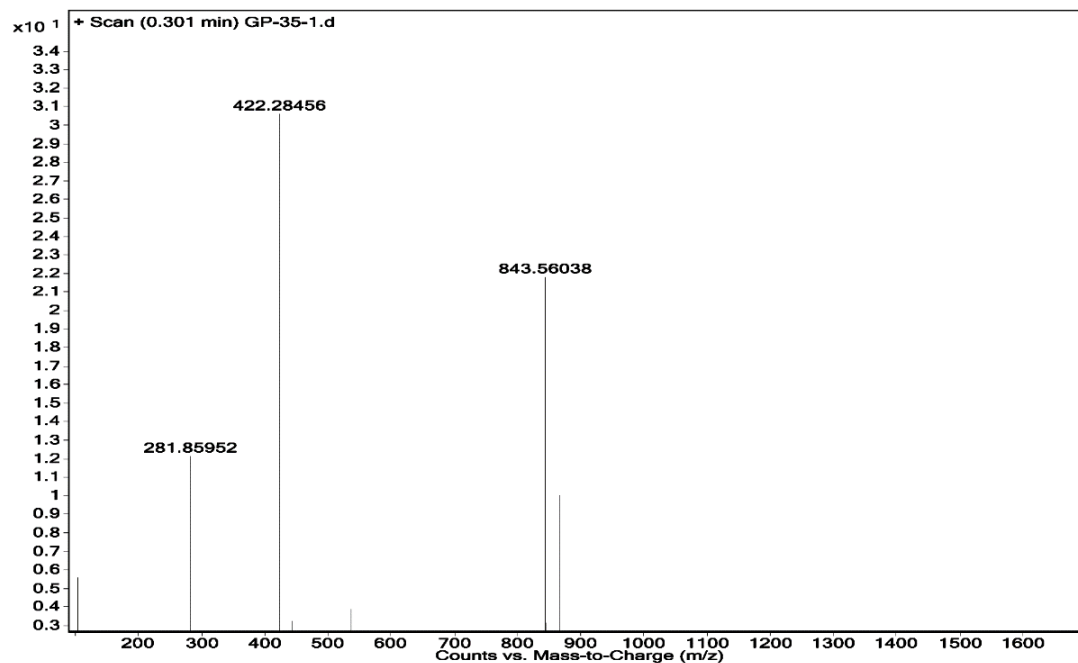


Figure S14: ESI-MS of **P36**. Calc. $(M+H)^+$ for $C_{41}H_{70}N_{12}O_7 = 843.5524$ Da; Obs. $(M+H)^+ = 843.5604$ Da, $(M+2H)^{2+} = 422.2845$ Da, $(M+3H)^{3+} = 281.8595$ Da.

Sample Name	GP-36-1	Position	P2-F6	Instrument Name	Instrument 1	User Name	
Inj Vol	20	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	GP-36-1.d	ACQ Method	ESI ALS 100-2000.m	Comment		Acquired Time	5/14/2019 5:24:52 PM

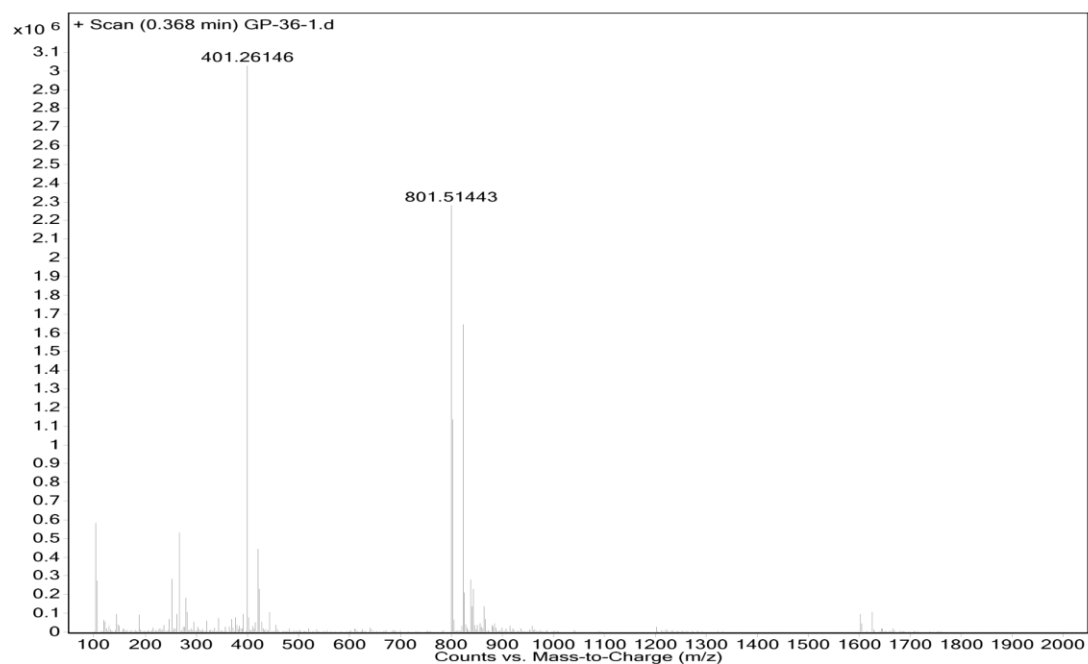


Figure S15: ESI-MS of **P37**. Calc. $(M+H)^+$ for $C_{38}H_{64}N_{12}O_7 = 801.5054$ Da; Obs. $(M+H)^+ = 801.5143$ Da, $(M+2H)^{2+} = 401.2614$ Da.

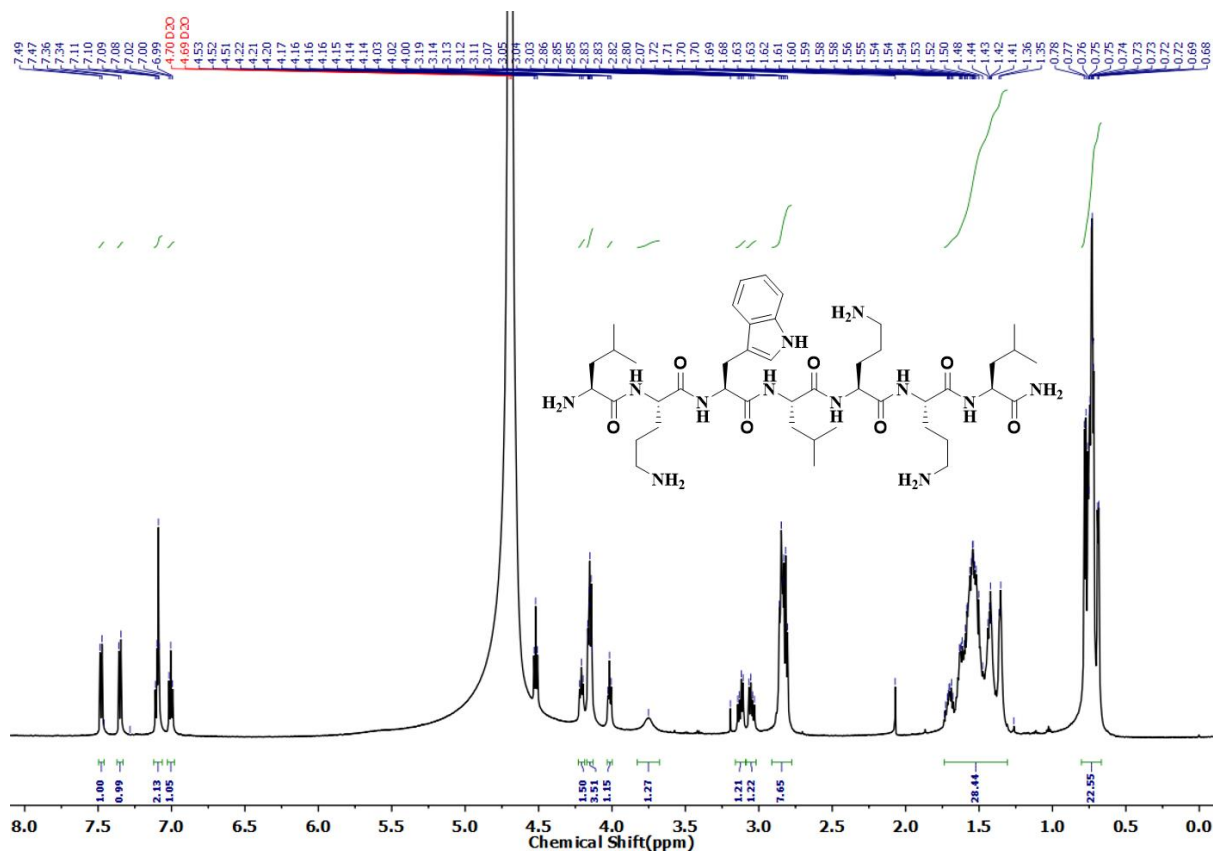


Figure S16: ^1H NMR of **P31** in D_2O at room temperature. ^1H NMR (600 MHz, D_2O). 0.8-0.96 (22H, Leu), 1.19-1.87 (28H, Leu and Orn), 2.9-3.3 (8H, m, Orn and Trp), 3.7-4.7 (7 αH , 1 merged with water signal), 7.1- 7.6 (5H, Trp).

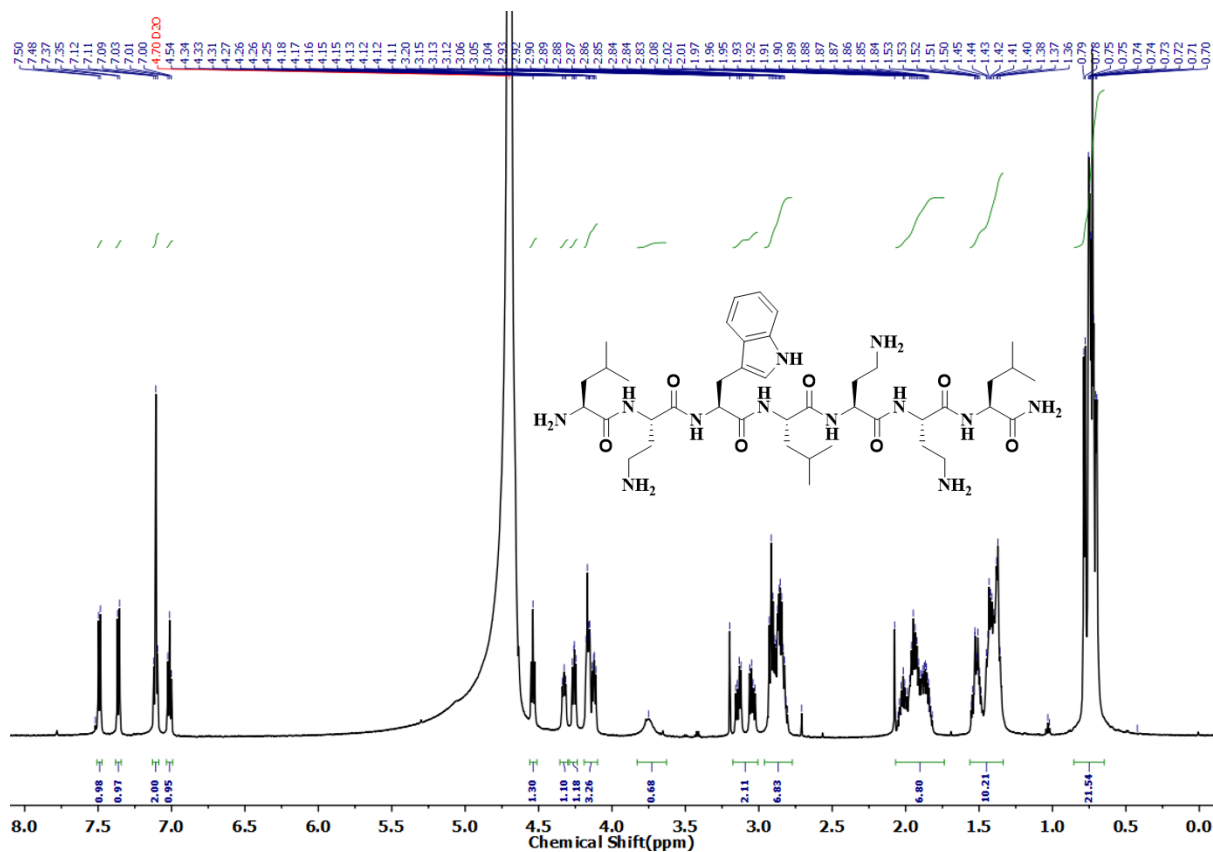


Figure S17: ^1H NMR of **P32** in D_2O at room temperature. ^1H NMR (600 MHz, D_2O). 0.8-0.96 (22H, Leu), 1.19-1.59 (10H, Leu and Dab), 1.79-2.14 (7H, m, Dab), 3.7-4.6 (7 αH , 1 merged with water signal), 6.98- 7.56 (5H, Trp).

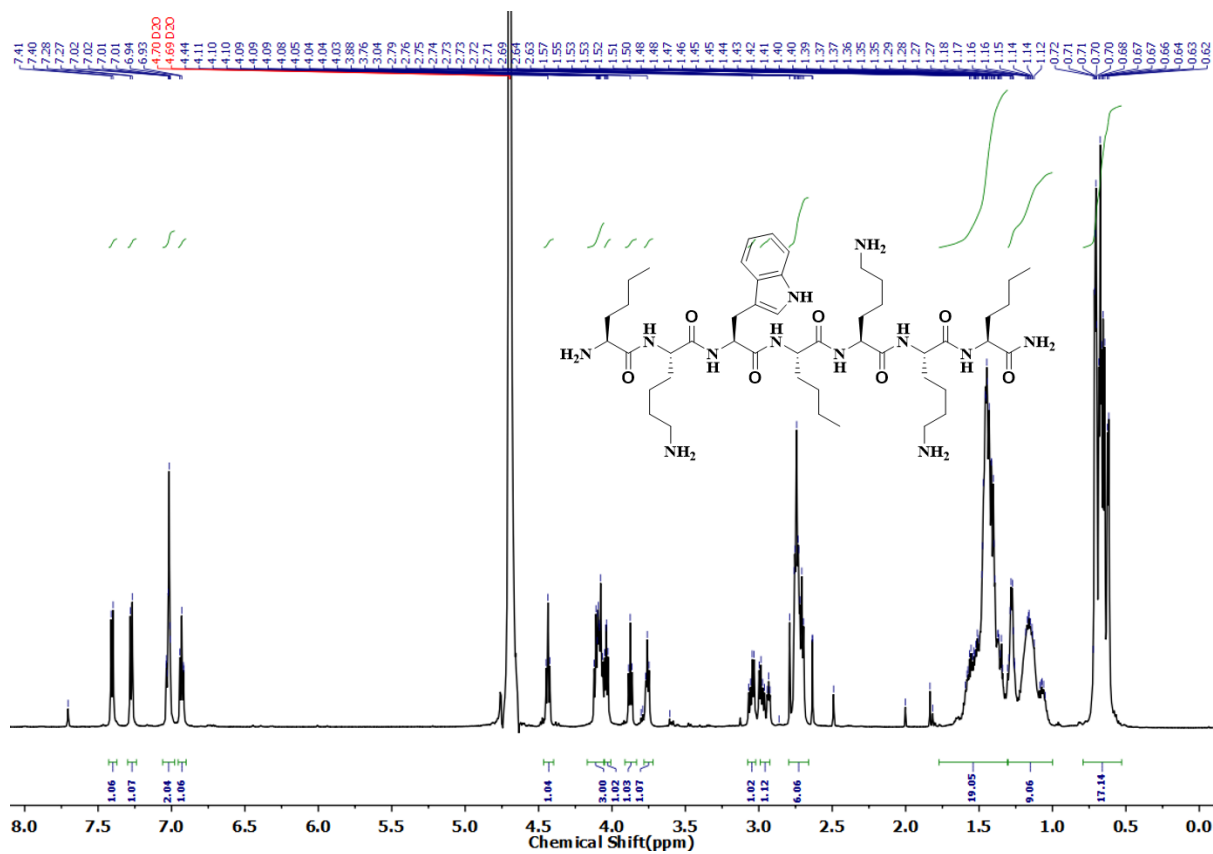


Figure S19: ^1H NMR of **P34** in D_2O at room temperature. ^1H NMR (600 MHz, D_2O). 0.59-0.74 (17H, Nle, Orn), 1.03-1.60 (28H, Nle and Orn), 2.68-3.07 (8H, m, Orn and Trp), 3.74-4.45 (7 αH), 6.92- 7.41 (5H, Trp).

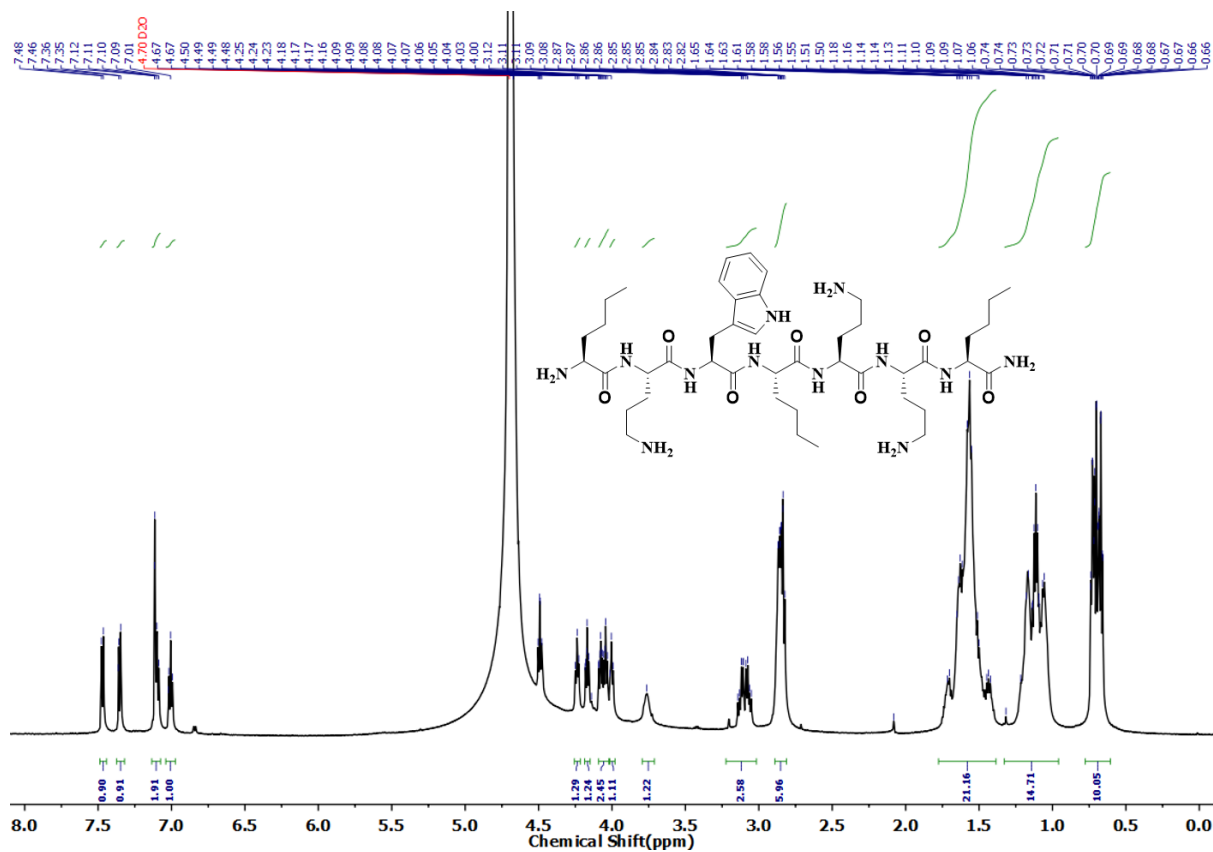


Figure S20: ¹H NMR of **P35** in D₂O at room temperature. ¹H NMR (600 MHz, D₂O). 0.6-1.28 (24H, Nle, Orn), 1.33-1.81 (21H, Nle and Orn), 2.74-3.19 (8H, m, Orn and Trp), 3.67-4.55 (7 αH, 1 merged with water signal), 6.97- 7.52 (5H, Trp).

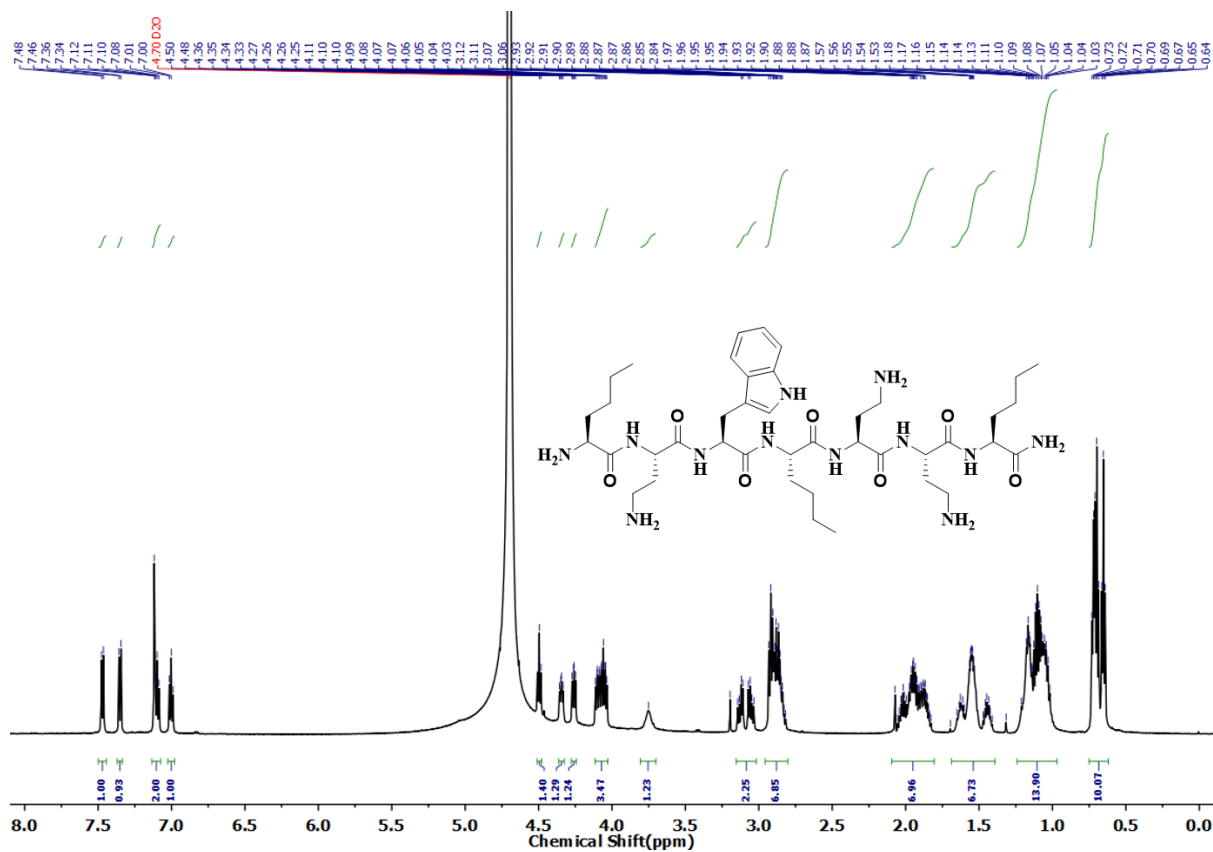


Figure S21: ^1H NMR of **P36** in D_2O at room temperature. ^1H NMR (600 MHz, D_2O). 0.58-1.31 (23H, Nle, Dab), 1.41-2.14 (14H, Nle and Dab), 1.79-2.14 (8H, m, Dab and Trp), 3.7-4.6 (7 αH , 1 merged with water signal), 6.98- 7.56 (5H, Trp).

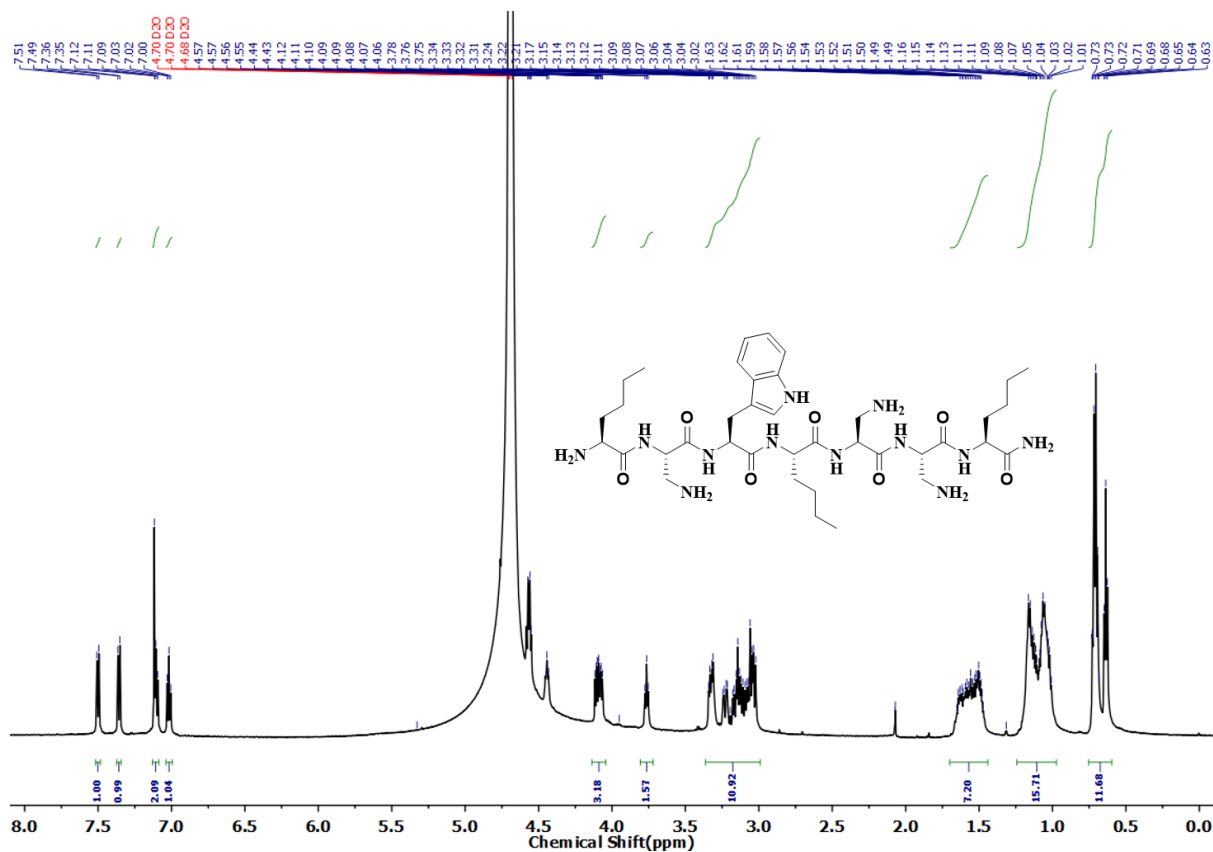


Figure S22: ^1H NMR of **P37** in D_2O at room temperature. ^1H NMR (600 MHz, D_2O). 0.57-1.24 (28H, Nle, Dap), 1.44-1.74 (7H, Nle and Dap), 2.99-3.42 (10H, m, Dap and Trp), 3.7-4.6 (7 αH , 3 merged with water signal), 6.98- 7.58 (5H, Trp).

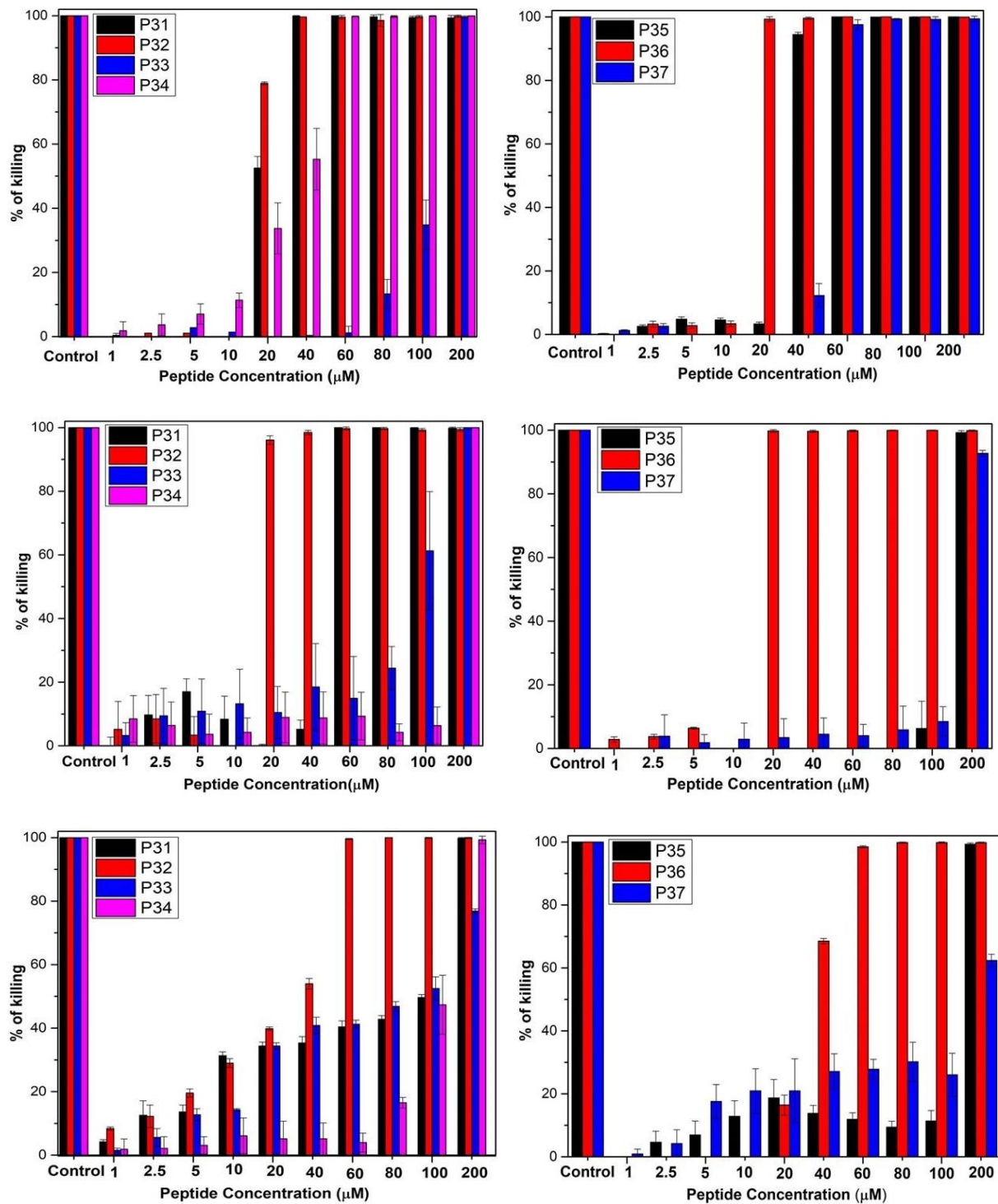


Figure S23: The bar plots represent MIC_{99%} of P31 to P37 in presence of 10 mM phosphate buffer. The micro broth dilution assay was performed in increasing concentration of respective peptides and killing percentage was calculated, after overnight incubation. (Upper Panel: against *P. aeruginosa*; 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.

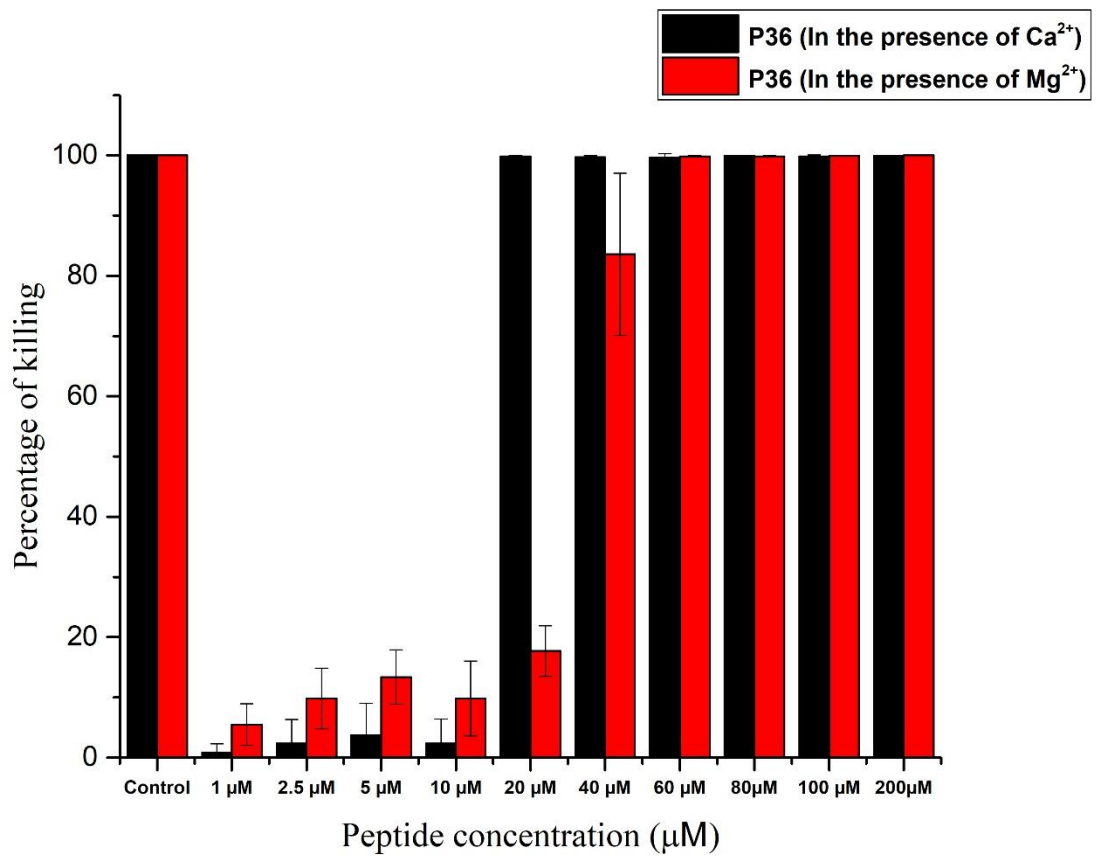


Figure S24. MIC of P36 against *P. aeruginosa* in the presence of Ca^{2+} and Mg^{2+} ions.

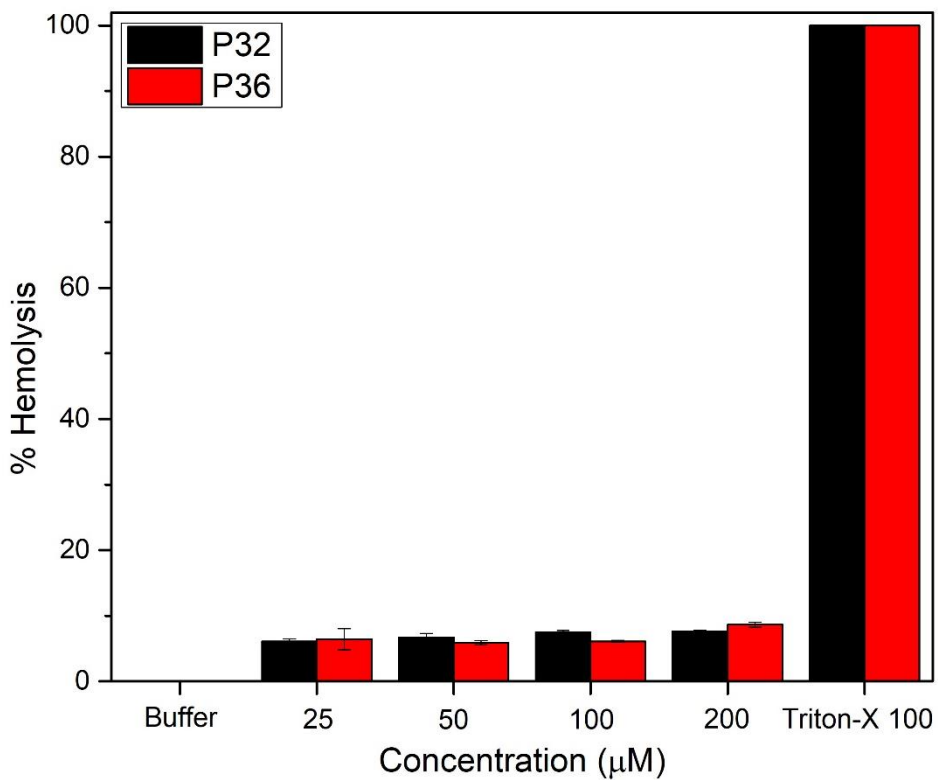
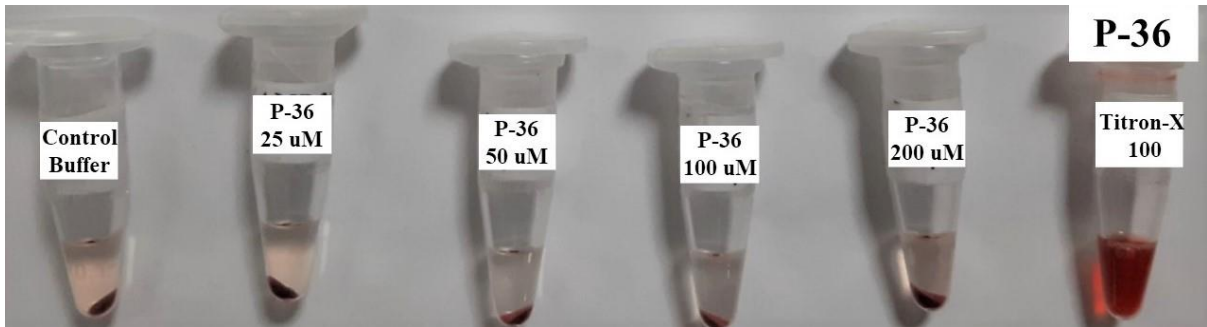
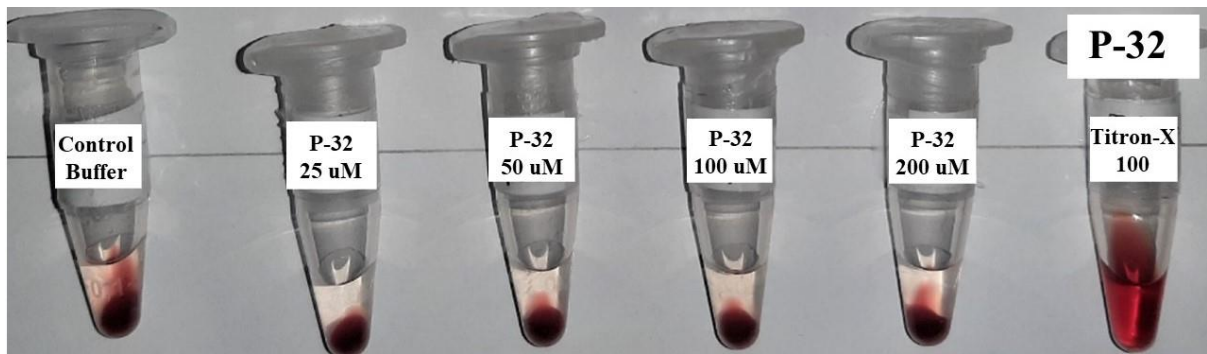


Figure S25: Above: Digital image represent hemolytic assay for **P32** and **P36** against human RBC at different peptide concentration (25, 50, 100, 200 µM). Bottom: Bar diagram showing

the % hemolysis for **P32** (black) and **P36** (red) against different concentrations of the peptides. Buffer and Triton-X 100 treated as negative and positive control.

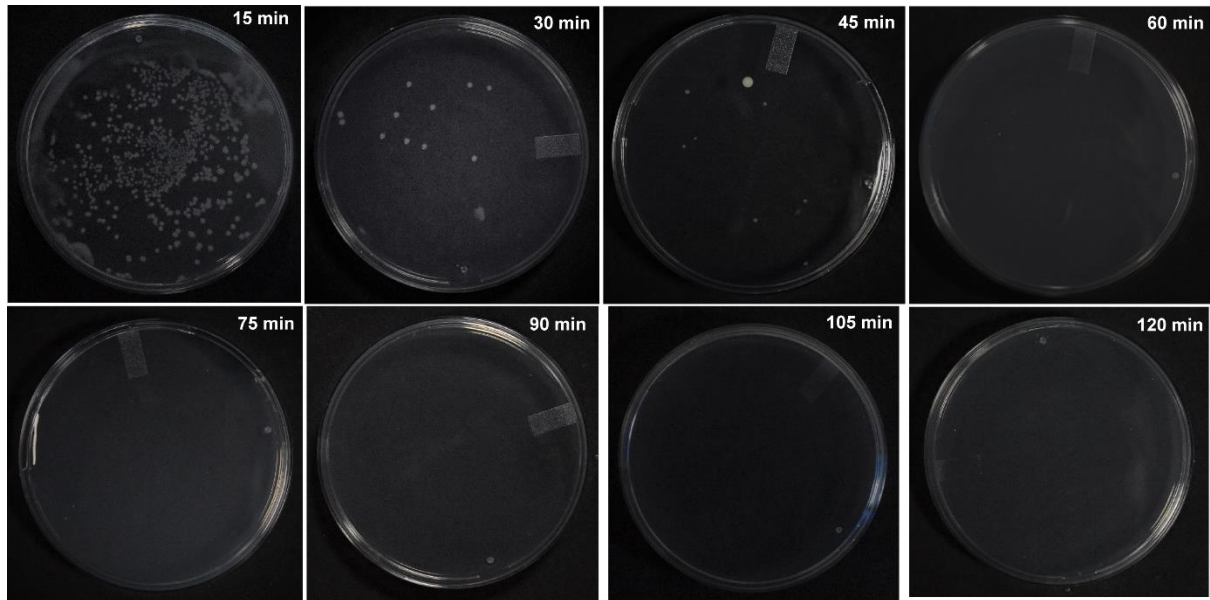


Figure S26: The bactericidal assay against *P. aeruginosa* in presence of **P32**. The images depict the CFU/ml on nutrient agar plates at different time point.

Sample Name	amp-35	Position	P1-C2	Instrument Name	Instrument 1	User Name	
Inj Vol	20	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	GP-AMP-35.d	ACQ Method	ESI ALS 100-2000.m	Comment		Acquired Time	1/23/2021 1:13:52 P

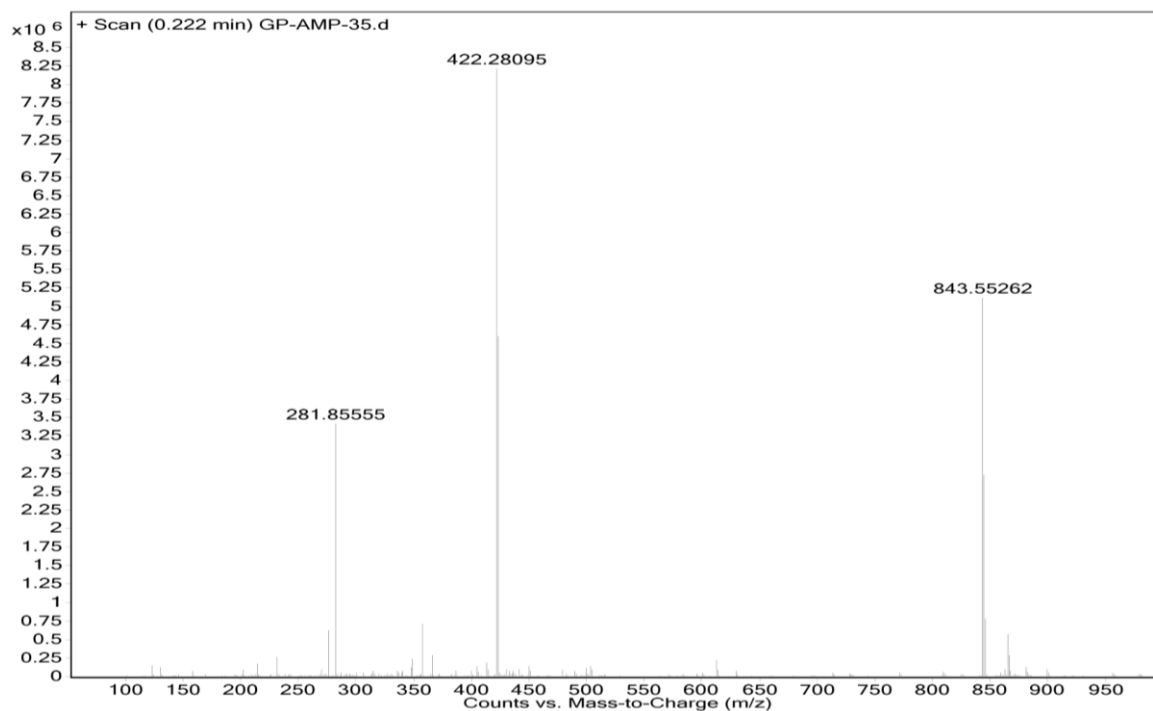


Figure S27: Control ESI MS of **P36** for enzymatic action study. Calc. $(M+H)^+$ for $C_{41}H_{70}N_{12}O_7 = 843.5524$ Da; Obs. $(M+H)^+ = 843.5526$ Da, $(M+2H)^{2+} = 422.2809$ Da, $(M+3H)^{3+} = 281.8555$ Da.

Sample Name	AMP-35-ALL	Position	P1-C6	Instrument Name	Instrument 1	User Name	
Inj Vol	20	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	GP-AMP-35+TRY+CHY+PR	ACQ Method	ESI ALS 100-2000.m	Comment		Acquired Time	1/23/2021 1:42:43 PM

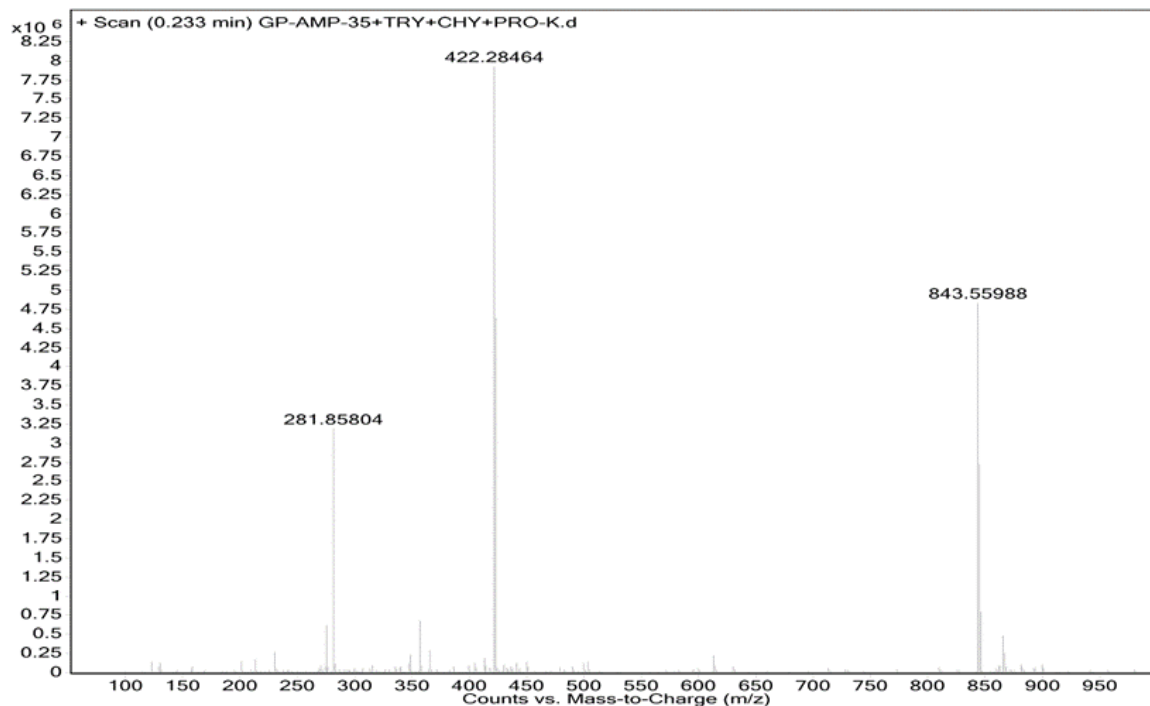


Figure S28: ESI MS of **P36** in presence of enzyme mixture (30 min. approximately). Calc. $(M+H)^+$ for $C_{41}H_{70}N_{12}O_7 = 843.5524$ Da; Obs. $(M+H)^+ = 843.5598$ Da, $(M+2H)^{2+} = 422.2846$ Da, $(M+3H)^{3+} = 281.8580$ Da.

Sample Name	AMP-35 ALL INC	Position	P1-D5	Instrument Name	Instrument 1	User Name	
Inj Vol	20	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	GP-AMP-35+TRY+CHY+PR	ACQ Method	ESI ALS 100-2000.m	Comment		Acquired Time	1/23/2021 2:53:53 PM

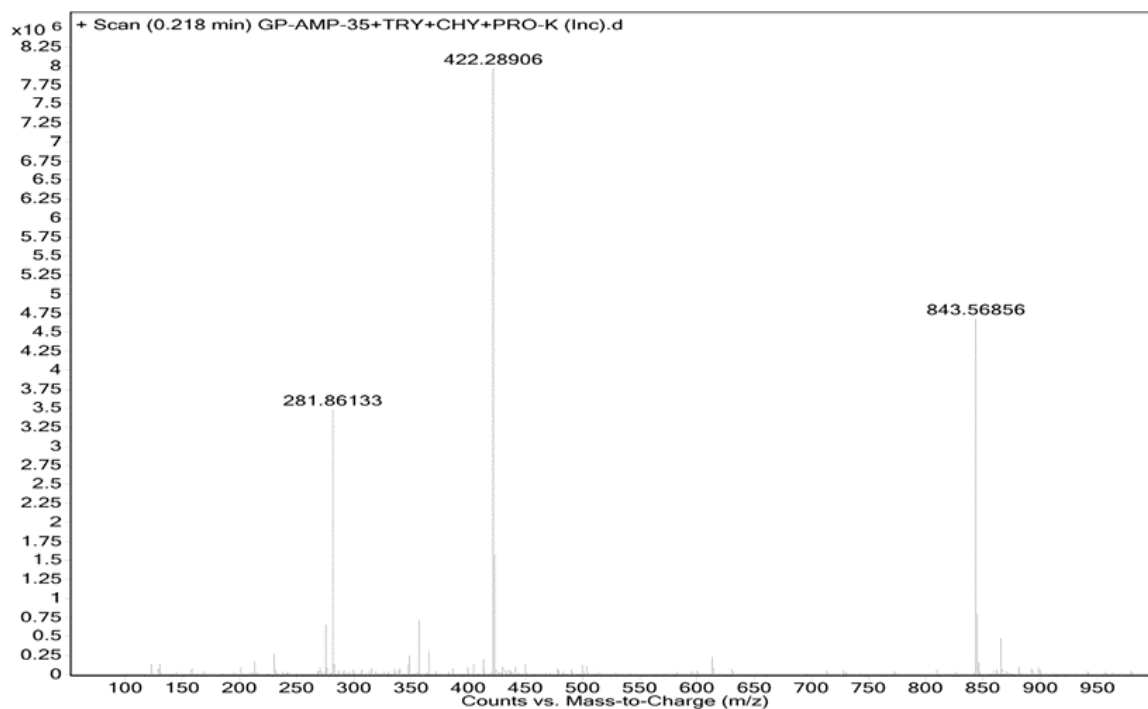


Figure S29: ESI MS of **P36** in presence of enzyme mixture (6 hr. approximately). Calc. $(M+H)^+$ for $C_{41}H_{70}N_{12}O_7 = 843.5524$ Da; Obs. $(M+H)^+ = 843.5604$ Da, $(M+2H)^{2+} = 422.2845$ Da, $(M+3H)^{3+} = 281.8595$ Da.

Sample Name	P4	Position	P1-C7	Instrument Name	Instrument 1	User Name	
Inj Vol	20	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	GP-AMP-P4.d	ACQ Method	ESI ALS 100-2000.m	Comment		Acquired Time	1/23/2021 1:55:59 PM

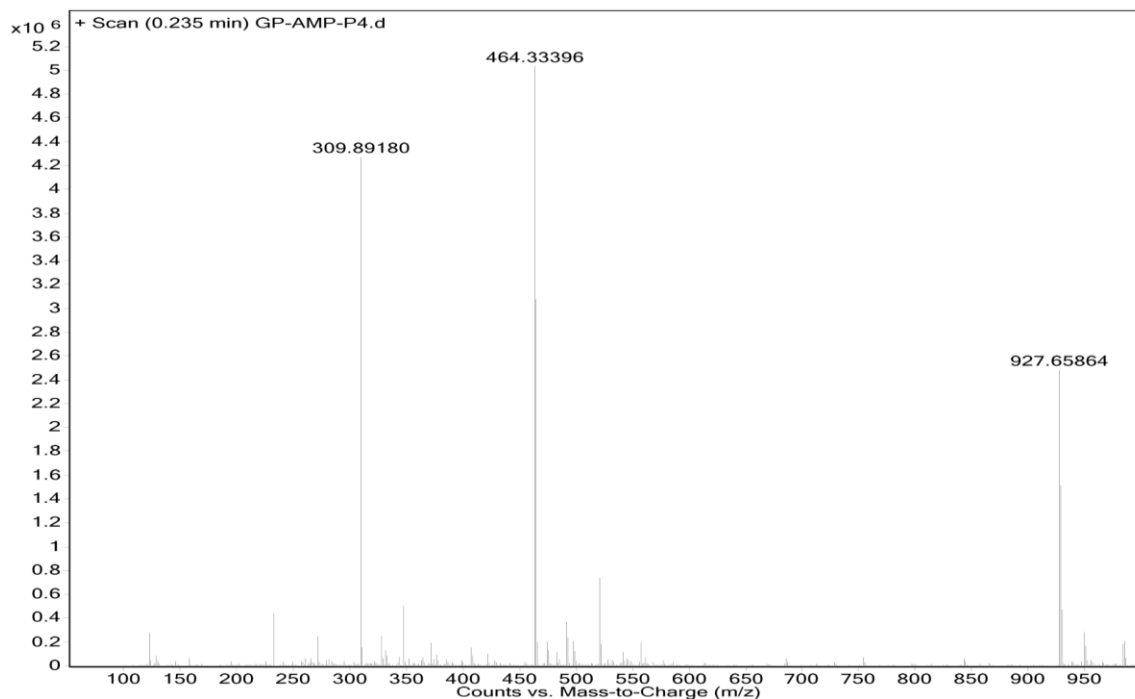


Figure S30: Control ESI MS of **P4** for enzymatic action study. Calc. $(M+H)^+$ for $C_{47}H_{82}N_{12}O_7 = 927.6502$ Da; Obs. $(M+H)^+ = 927.6586$ Da, $(M+2H)^{2+} = 464.3339$ Da, $(M+3H)^{3+} = 309.8918$ Da.

Sample Name	P4 ALL	Position	P1-C11	Instrument Name	Instrument 1	User Name	
Inj Vol	20	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	GP-AMP- P4+TRY+CHY+PR	ACQ Method	ESI ALS 100-2000.m	Comment		Acquired Time	1/23/2021 2:21:14 PM

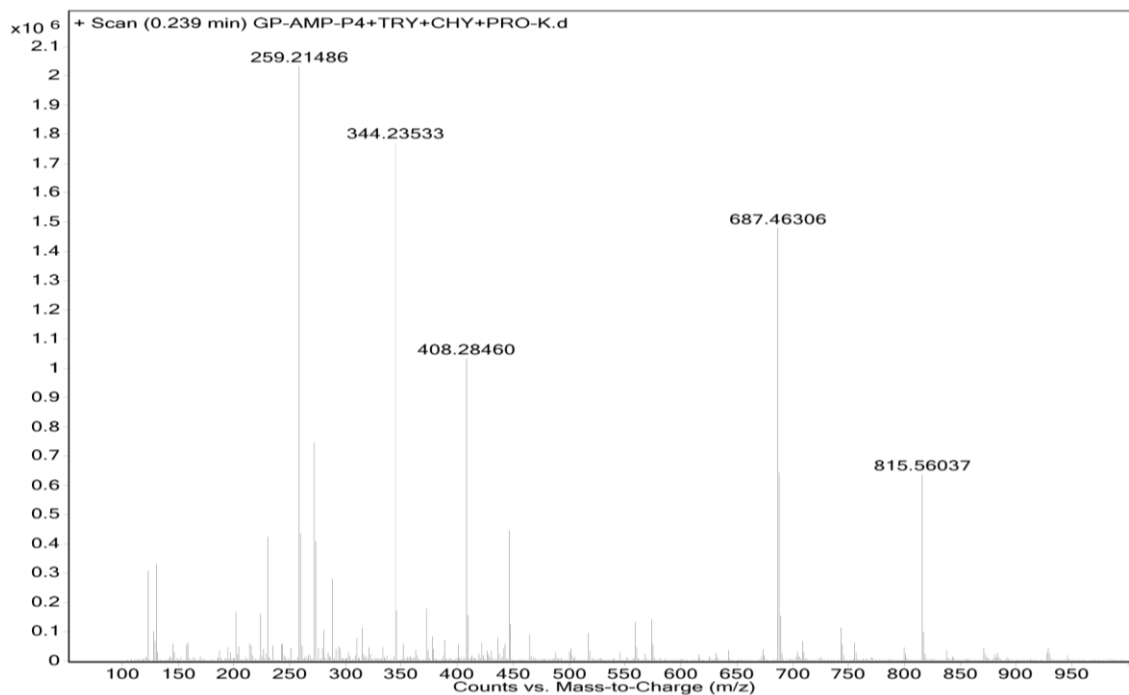


Figure S31: ESI MS of **P4** in presence of enzyme mixture (30 min. approximately). Calc. $(M+H)^+$ for $C_{47}H_{82}N_{12}O_7 = 927.6502$ Da.

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

Sample Name	P4 ALL	Position	P1-D9	Instrument Name	Instrument 1	User Name	
Inj Vol	20	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	GP-AMP- P4+TRY+CHY+PR	ACQ Method	ESI ALS 100-2000.m	Comment		Acquired Time	1/23/2021 3:12:57 PM

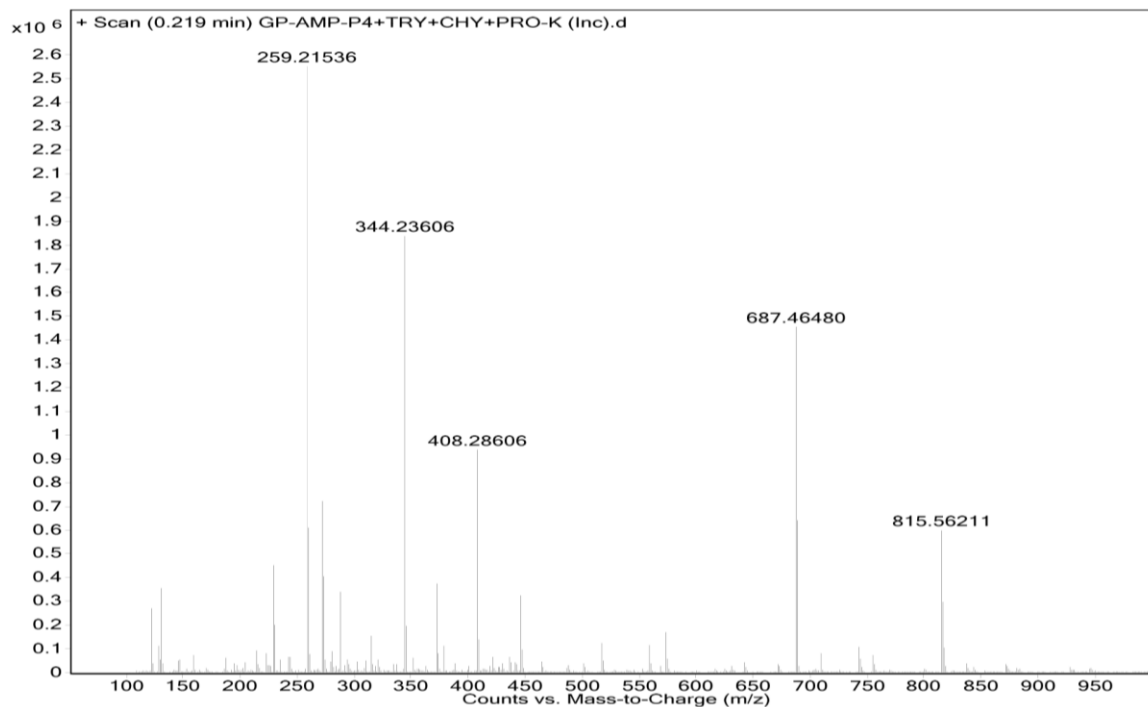


Figure S32: ESI MS of **P4** in presence of enzyme mixture (6 hr. approximately). Calc.

$(M+H)^+$ for $C_{47}H_{82}N_{12}O_7 = 927.6502$ Da.

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

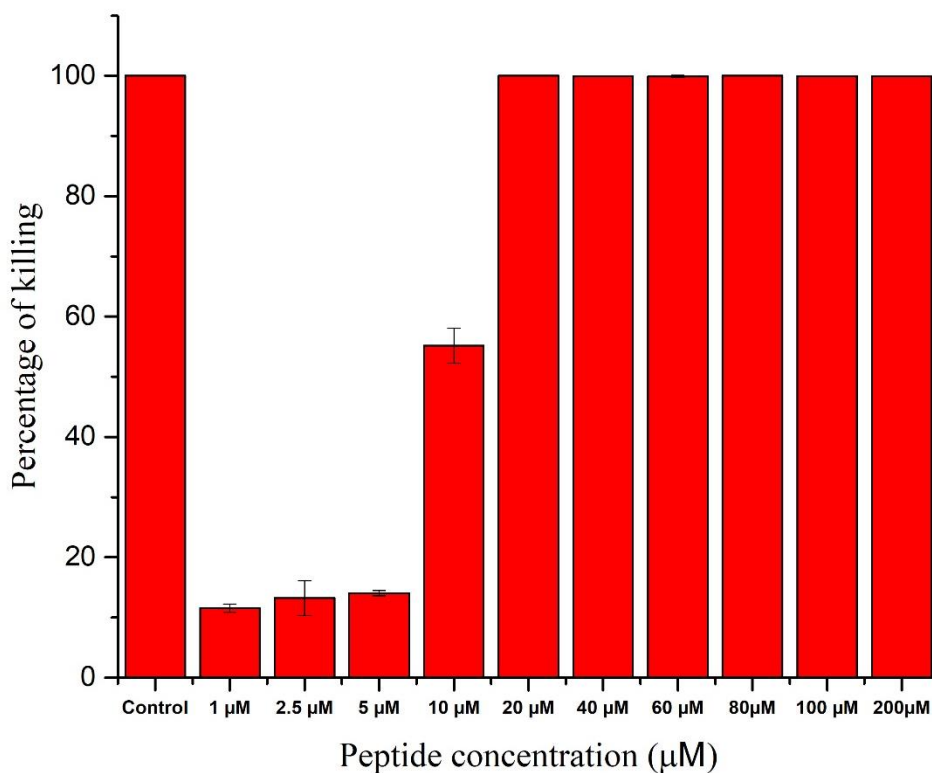


Figure S33. MIC of **P36** against *P. aeruginosa*, post 6 hr. incubation of **P36** with the enzyme cocktail (Mixture of Chymotrypsin, Proteinase-K and Trypsin in the ratio 1:1:1). Experiment was performed in triplicates.

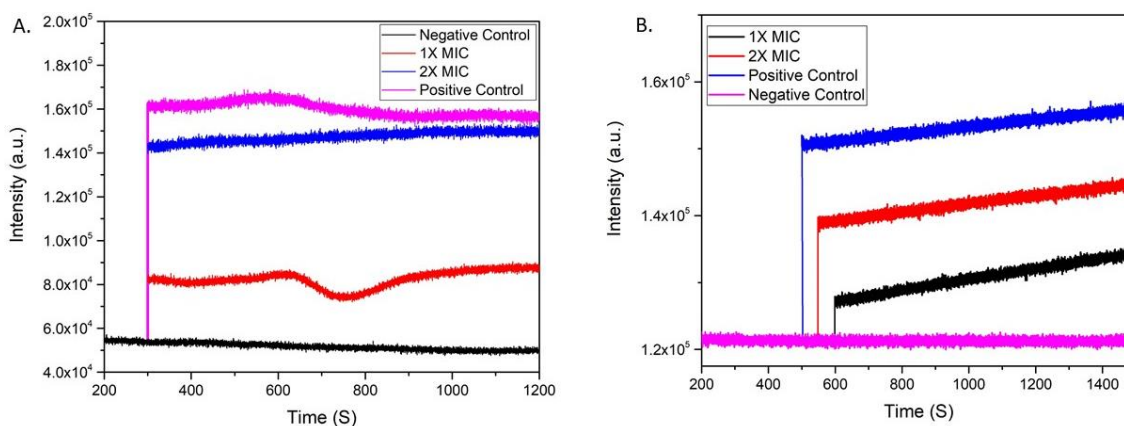


Figure S34. Time kinetics of (A) NPN and (B) PI uptake after addition of 1X and 2X MIC **P32** to *P. aeruginosa* cells, which indicate the outer and inner membrane permeability respectively. All the experiments were performed in triplets.

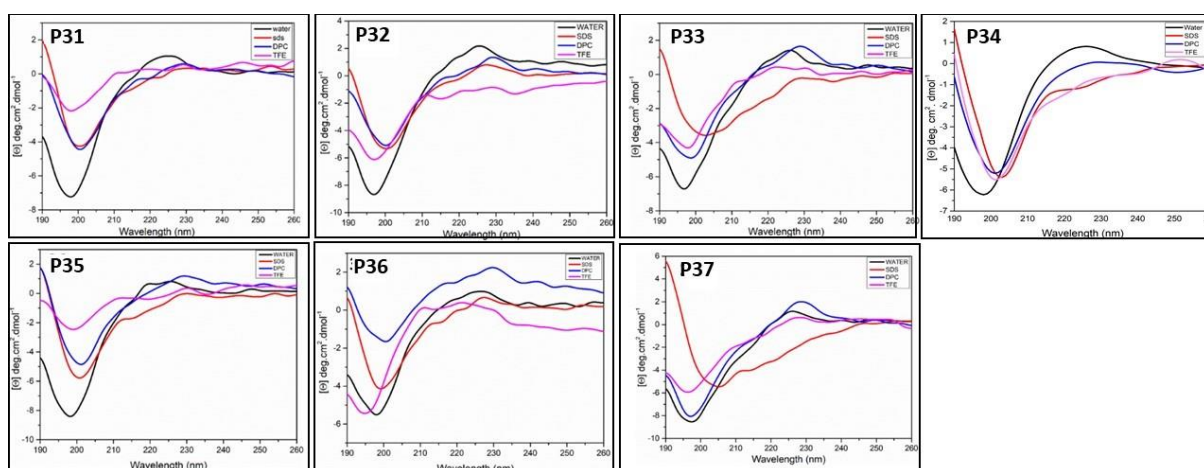


Figure S35: CD spectral study of P31 to P37 in presence of different membrane mimetic environments: SDS mimics the bacterial membrane and DPC mimics the mammalian membrane. 50% TFE promotes the alpha helical propensity.

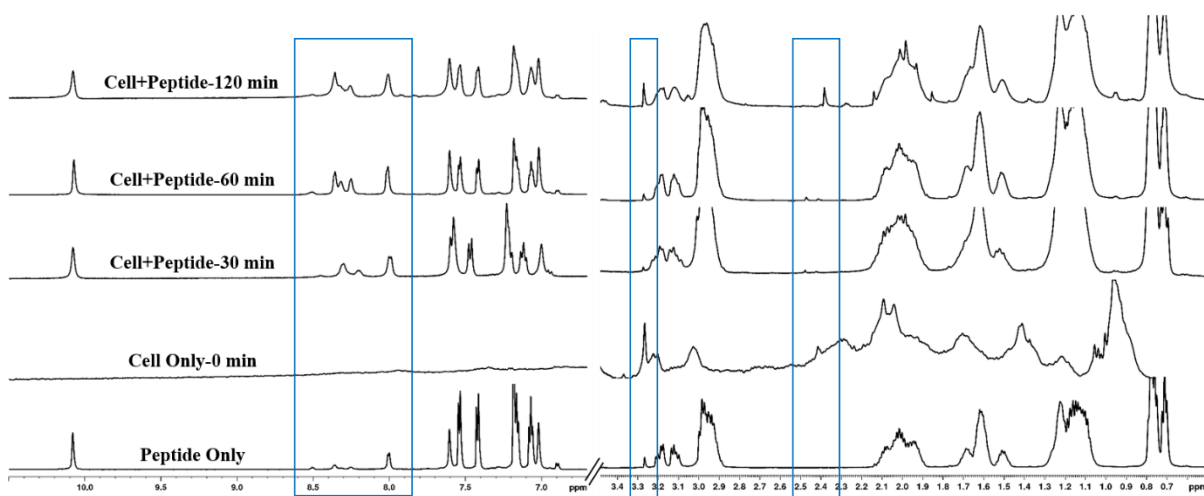


Figure S36: Live cell NMR spectroscopy of P36 in the presence of *P. aeruginosa* cells. Partial stacked plots of 1D ^1H NMR spectra at different times of incubation of P36 with *P. aeruginosa*. The blue boxes highlight the newly appeared spectral lines over the time interval.

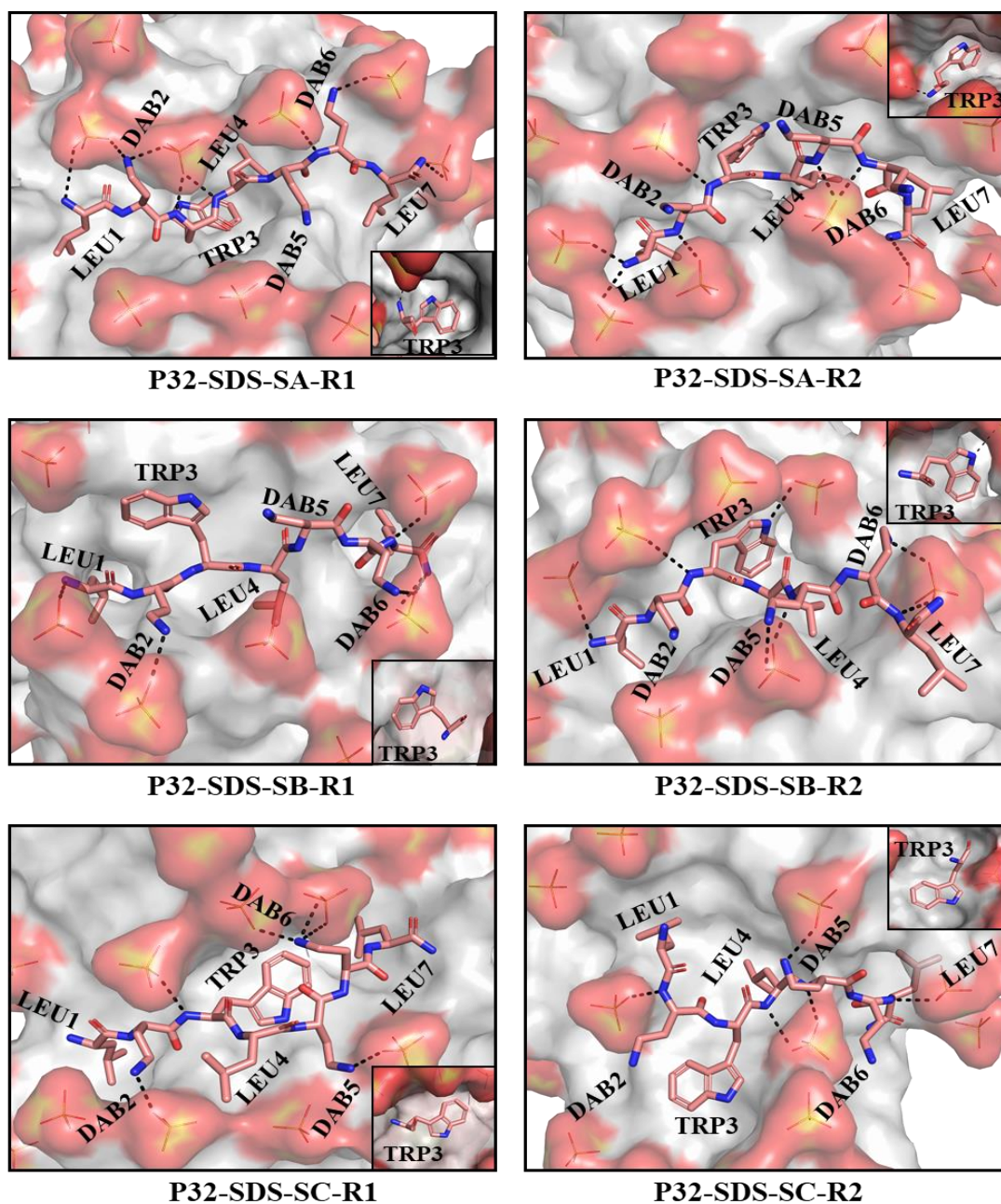


Figure S37. P32-SDS direct interactions are portrayed from the final structure of the P32: SDS micelle MD simulation after 50 ns. Positively charged tips of the peptide (N-terminal, side-chains of Dab2, Dab5 and Dab6) interact directly with sulphates of SDS. P32 is represented in salmon coloured sticks (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).

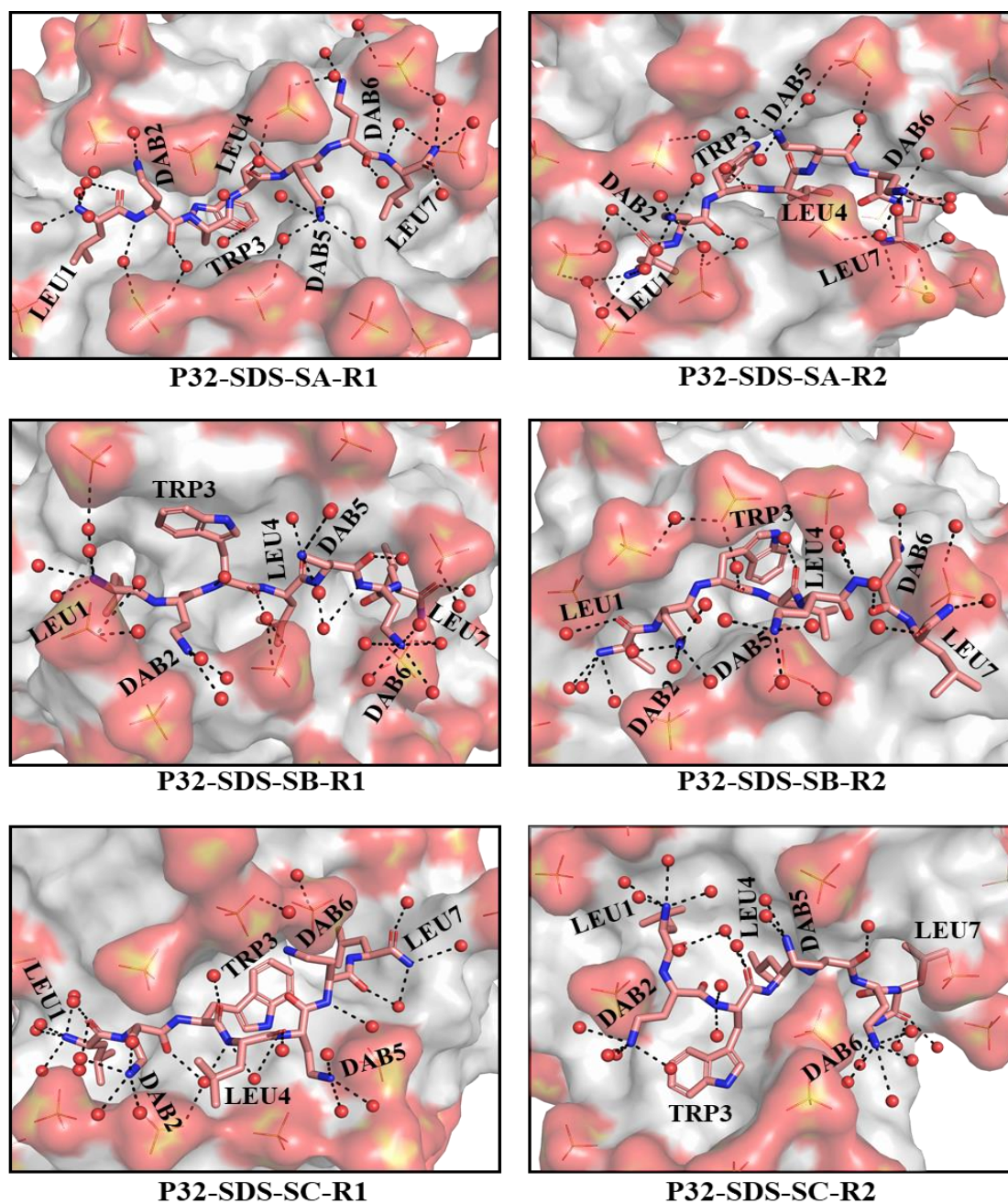


Figure S38. P32 water interactions observed from the final MD structure of the P32: SDS complex after 50 ns. Waters interact with the positively charged tips of the peptide (N-terminal, side-chain of Dab2, Dab5 and Dab6). Water mediated interactions between peptide positive charges and the negatively 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).

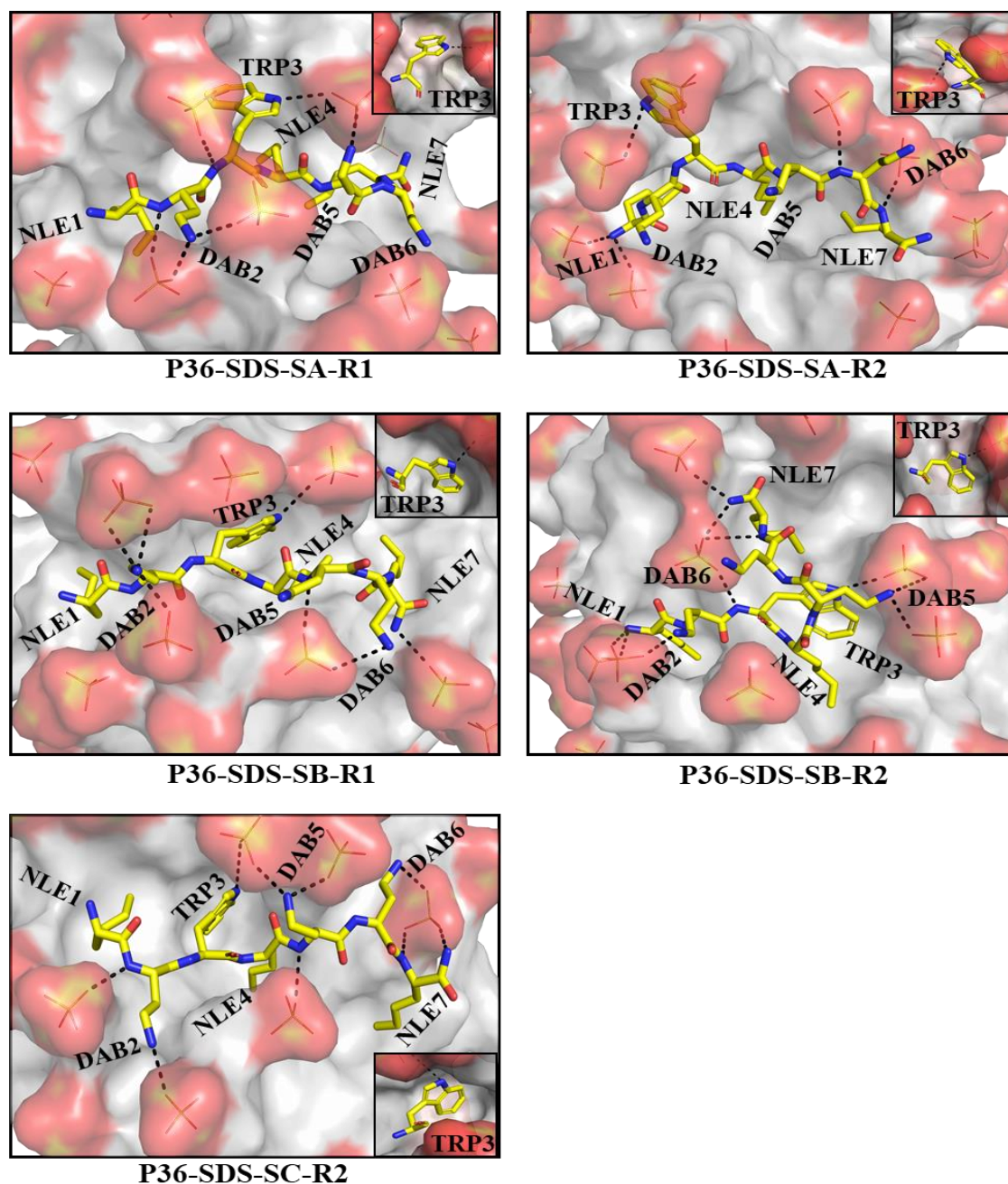


Figure S39. P36-SDS direct interactions are portrayed from the final structure of the P36: SDS micelle MD simulation after 50 ns. Positively charged tips of the peptide (N-terminal, side-chains of Dab2, Dab5 and Dab6) interact directly with sulphates of SDS. P36 is represented in yellow coloured sticks (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).

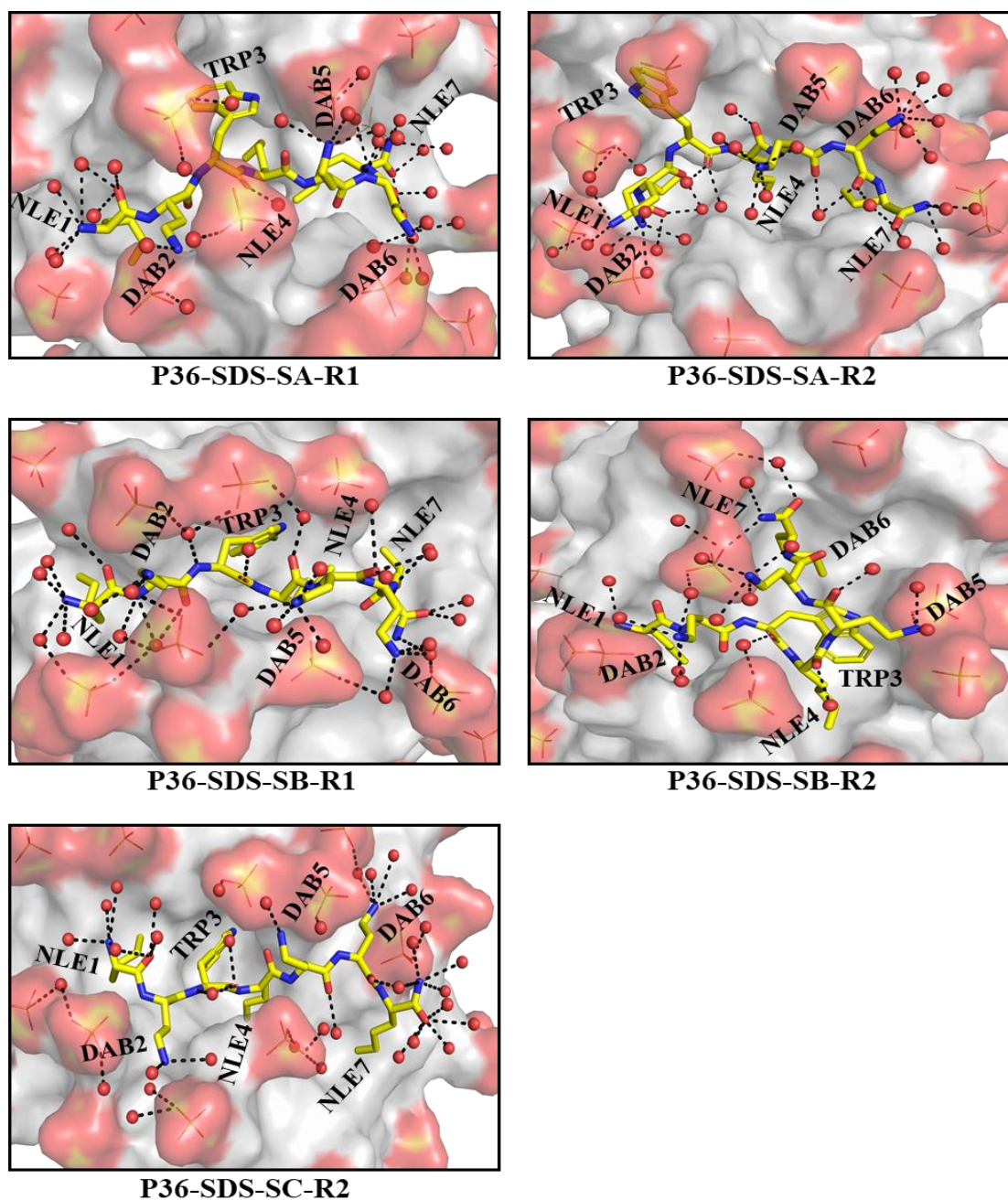
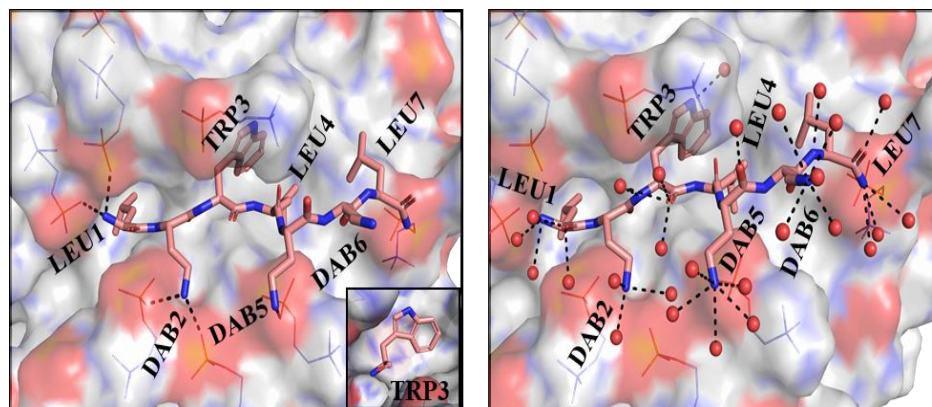
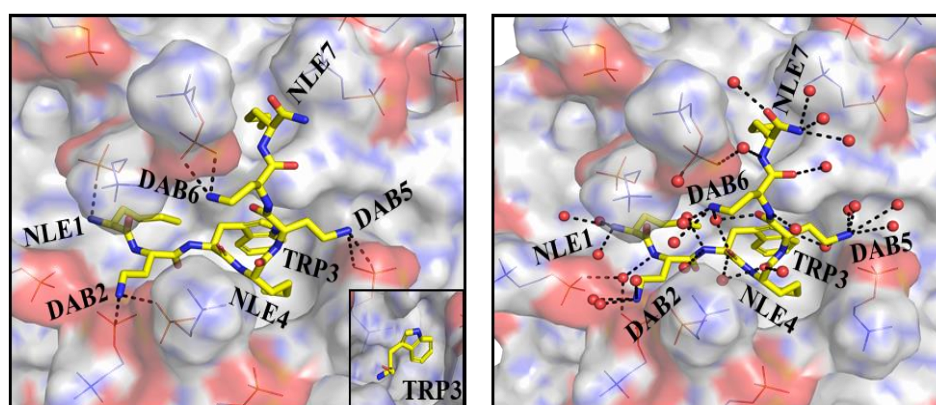


Figure S40. P36 water interactions are observed from the final MD structure of the P36: SDS complex after 50 ns. Waters interact with the positively charged tips of the peptide (N-terminal, side-chain of Dab2, Dab5 and Dab6). Water mediated interactions between peptide positive charges and the negatively 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).



a) P32-DPC-SA-R1



b) P36-DPC-SA-R1

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).

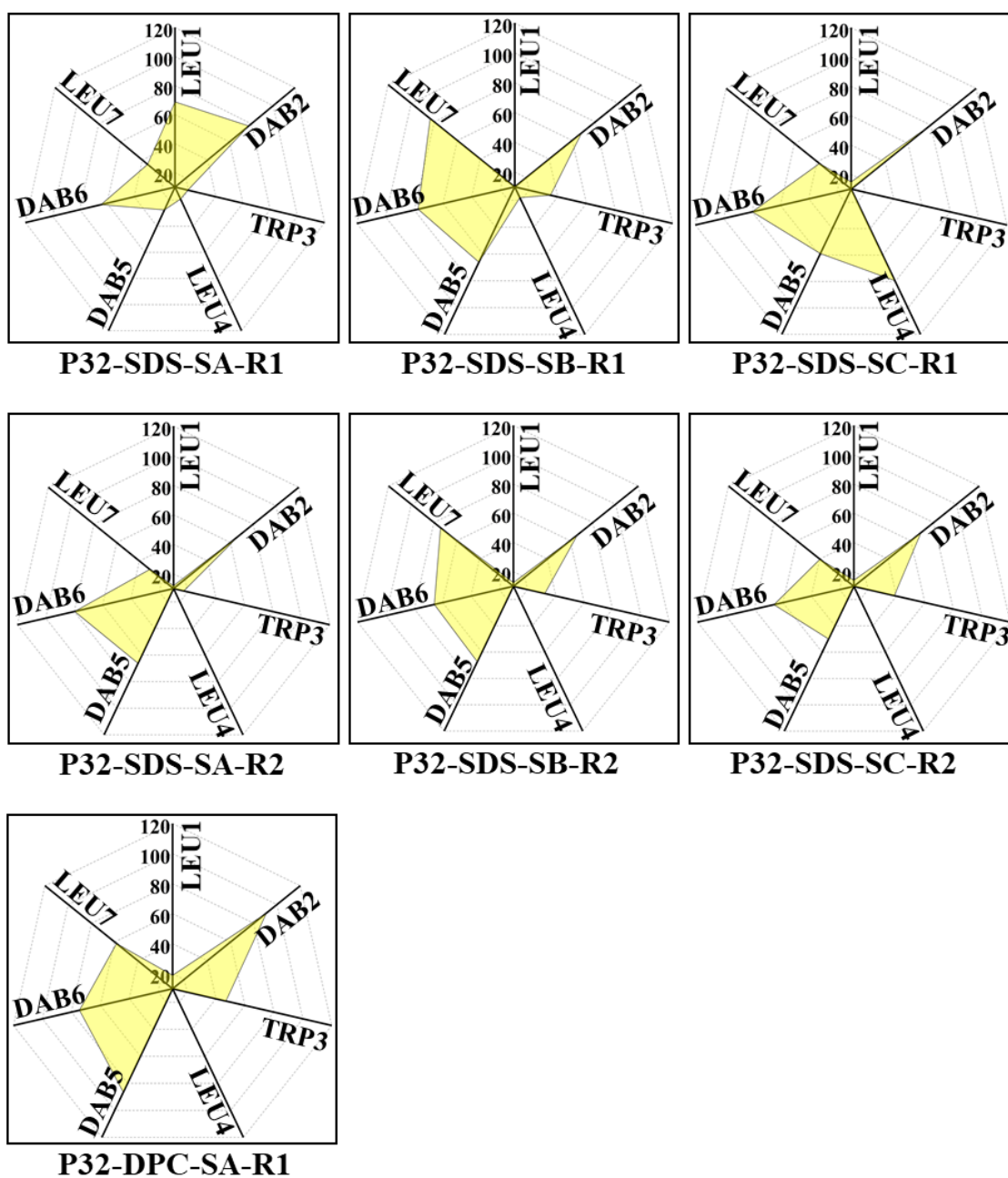


Figure S42. Trajectory averaged (last 30 ns) residue-wise solvent exposure (SASA in \AA^2) of P32 sidechain are shown in the yellow net-plot with contours of constant solvent exposure (increases as one goes away from the centre).

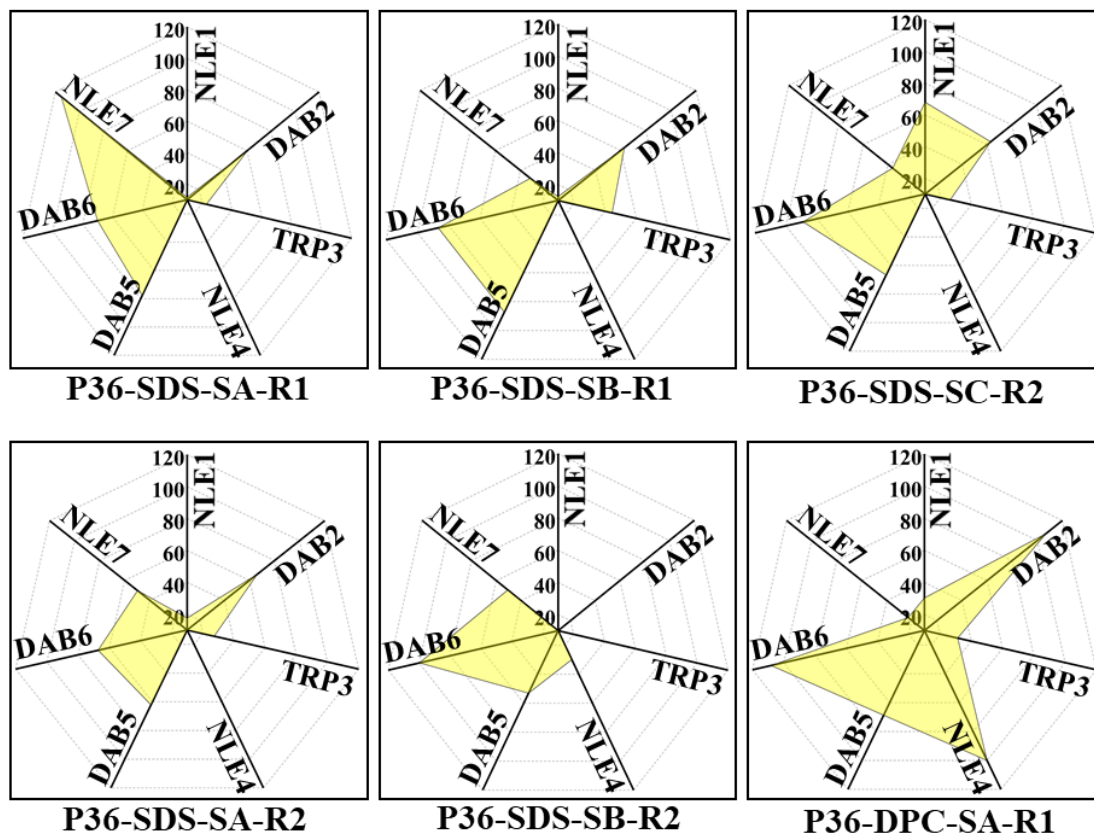


Figure S43. Trajectory averaged (last 30 ns*) residue-wise solvent exposure (SASA in \AA^2) of P36 sidechain are shown in the yellow net-plot with contours of constant solvent exposure (increases as one goes away from the centre). [*except P36-DPC-SA-R1 (last 5ns)]

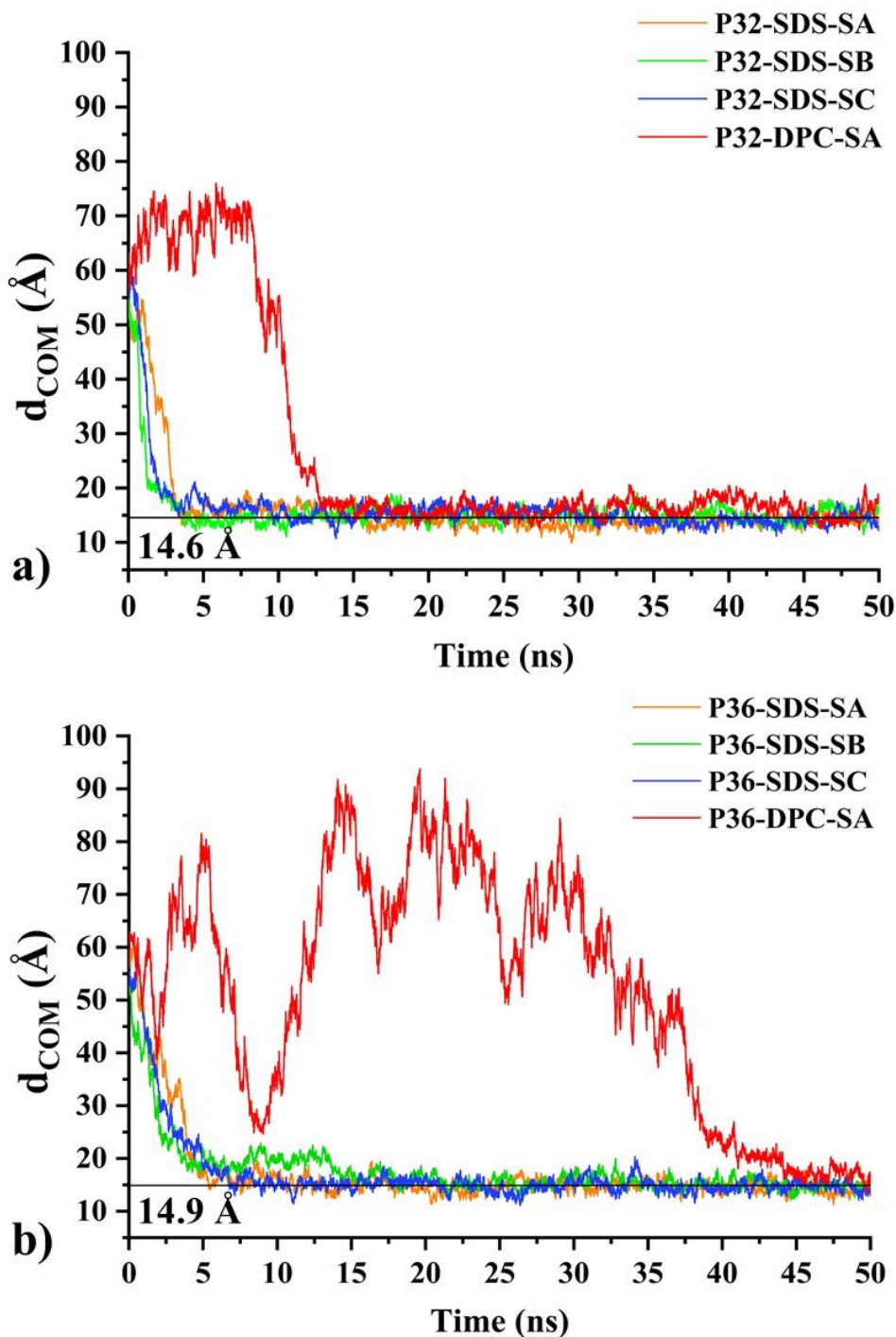


Figure S44. Peptide- Micelle distance as a function of time. d_{COM} is the distance between the centre of mass of peptide and the centre of mass of micelle (SDS/DPC). For the peptide- SDS simulations (Setup: SA, SB, and SC of Figure S1. a), temporally averaged d_{COM} (averaging over 2 independent MD runs, see Table S1) is plotted. (a) P32-micelle (SDS/DPC) d_{COM} plot. (b) P36-micelle (SDS/DPC) d_{COM} plot. The black horizontal line is the averaged d_{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 (\AA^3)	No of Molecules	Simulation Time (ns)
1	Free SDS	1 (R1)	100×100×100	SDS – 60 SOL – 31861 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 Na – 60	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 Na – 60	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-CONH ₂
2	P31	LOrnWLOrnOrnL-CONH ₂
3	P32	LDabWLDabDabL-CONH ₂
4	P33	LDapWLDapDapL-CONH ₂
5	P34	NleKWNleKKNle-CONH ₂
6	P35	NleOrnWNleOrnOrnNle-CONH ₂
7	P36	NleDabWNleDabDabNle-CONH ₂
8	P37	NleDapWNleKKNle-CONH ₂

Table S4: Physicochemical properties of P31-37.

Peptide code	No. of AA	Net charges	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 (M+3H) ³⁺ =295.8761	2.006
P32	7	4	Dab	843.5524	(M+H) ⁺ =843.5708 (M+2H) ²⁺ =422.2897 (M+3H) ³⁺ =281.8616	6.814
P33	7	4	Dap	801.5054	(M+H) ⁺ =801.5213 (M+2H) ²⁺ =401.2645 (M+3H) ³⁺ =267.8457	6.951
P34	7	4	Nle	927.6502	(M+H) ⁺ =927.6502 (M+2H) ²⁺ =464.3373 (M+3H) ³⁺ =309.8946	7.6
P35	7	4	Orn, Nle	885.5993	(M+H) ⁺ =885.6159 (M+2H) ²⁺ =443.3112 (M+3H) ³⁺ =295.8767	6.86
P36	7	4	Dab, Nle	843.5524	(M+H) ⁺ =843.5604 (M+2H) ²⁺ =422.2845 (M+3H) ³⁺ =281.8595	7.003
P37	7	4	Dap, Nle	801.5054	(M+H) ⁺ =801.5143 (M+2H) ²⁺ =401.2614	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	Micelle Radius (Å)	Peptide Secondary Structures		
		AVG±SD	AVG±SD	Coil (%)	Bend (%)	Turn (%)
1	Free SDS*	0.176±0.07	21.64±0.32			
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 $N \cdot \log(N)$ 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 to 100% 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.