



1. Introduction

Venomomics, the largescale study of venom composition has revolutionized the discovery of bioactive compounds with therapeutic potential. Among venom-derived molecules, antimicrobial peptides (AMPs) from venom containing organisms has emerged as promising candidates in the fight against multidrug-resistant pathogens.

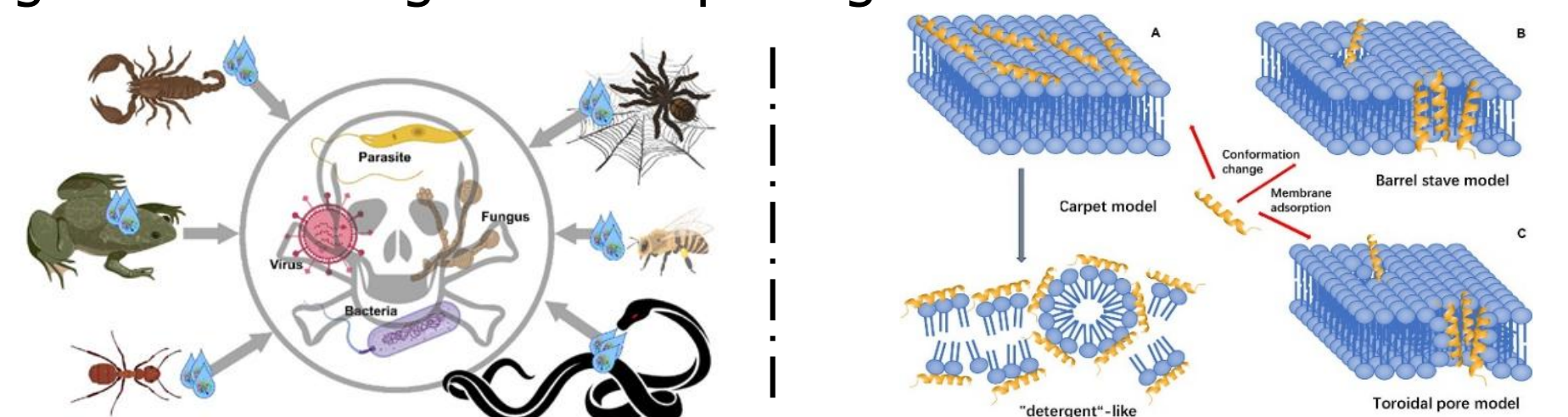


Figure 1: Antimicrobials from venomous organisms

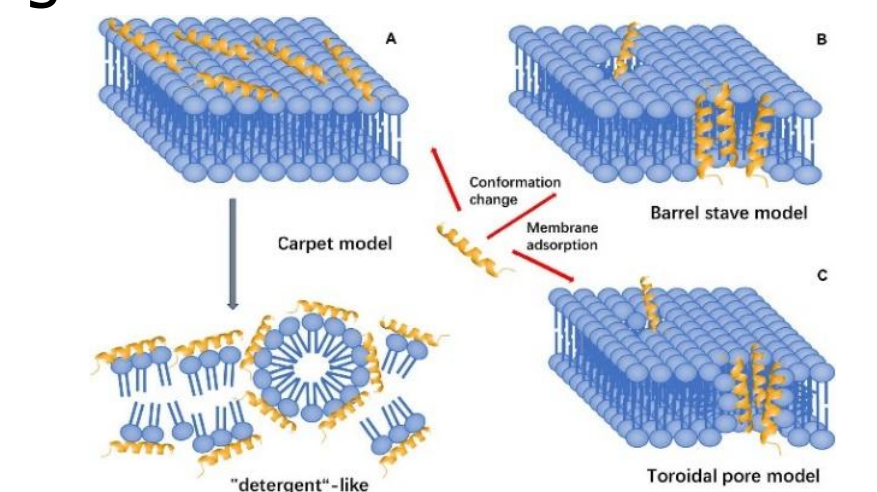


Figure 2: Antimicrobial peptides modes of action

A study revealed that AMPs from the venom of a Trinidad Thick-tailed scorpion, *Tityus trinitatis*, showed broad spectrum efficacy, however their clinical potential is hindered by their high cytotoxicity, notably hemolytic activity.



Figure 3: Trinidad Thick-tailed scorpion, *Tityus trinitatis*

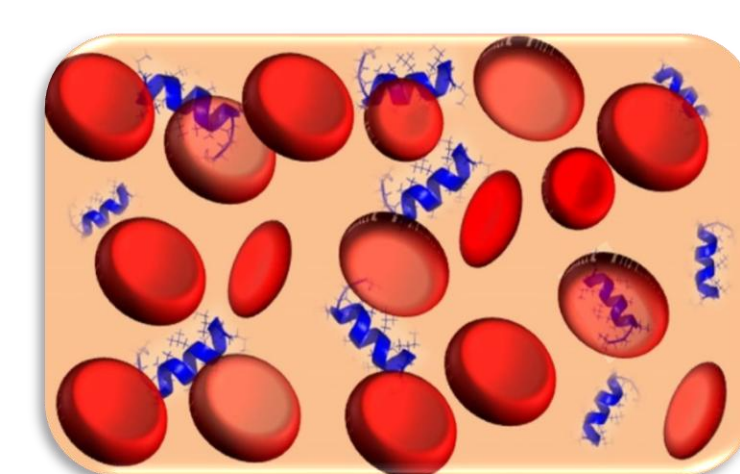
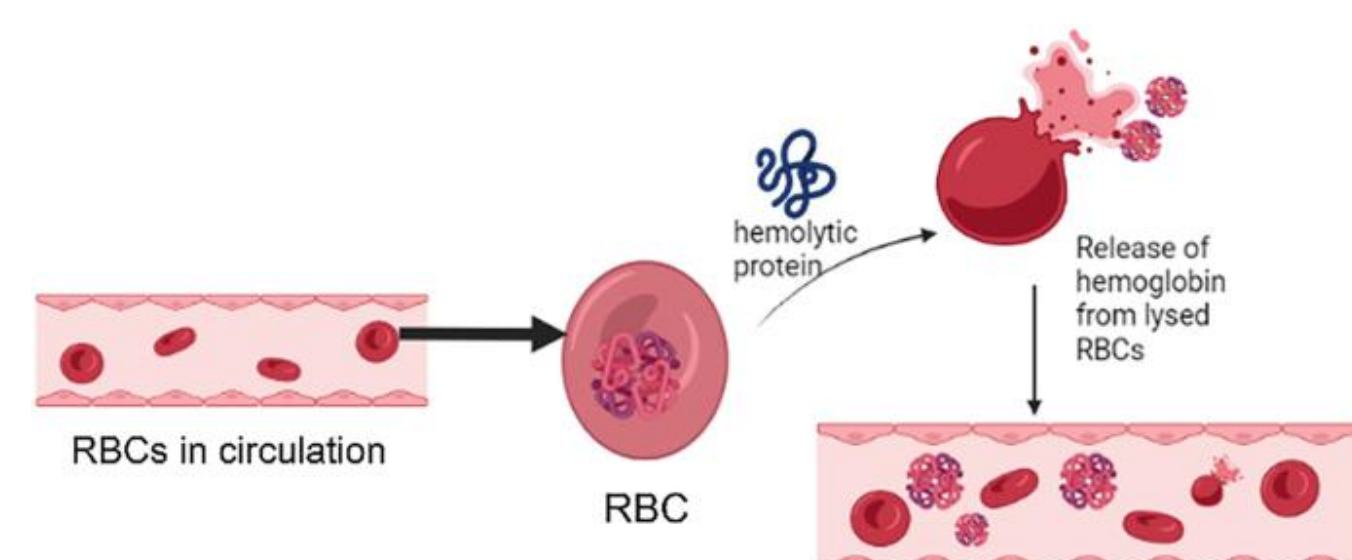
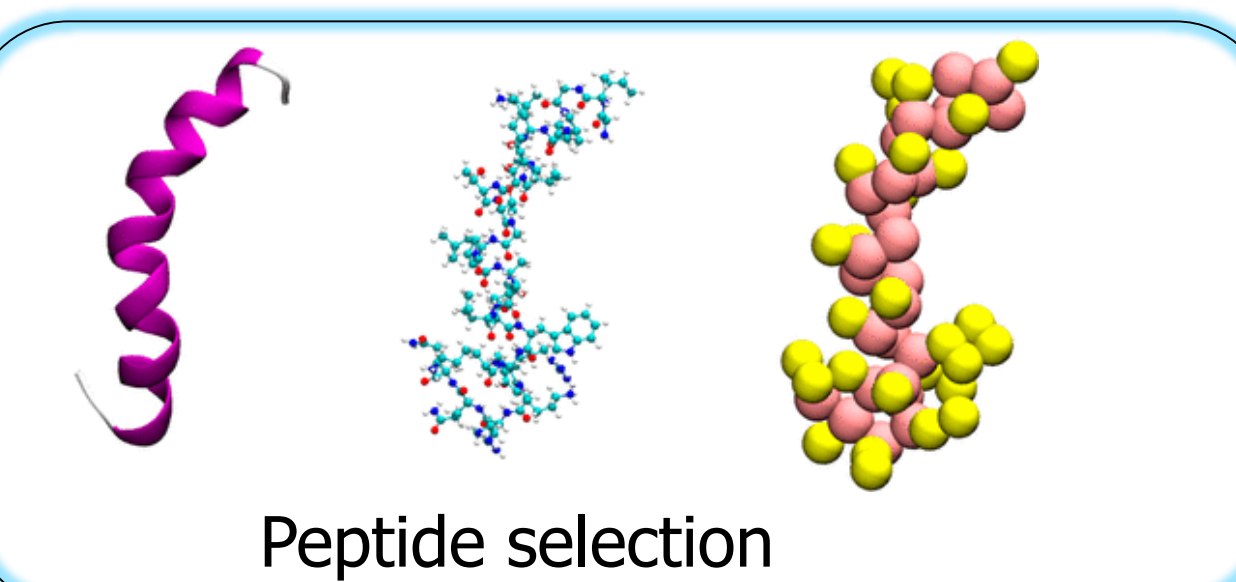


Figure 4: AMPs with hemolytic effect on human erythrocytes

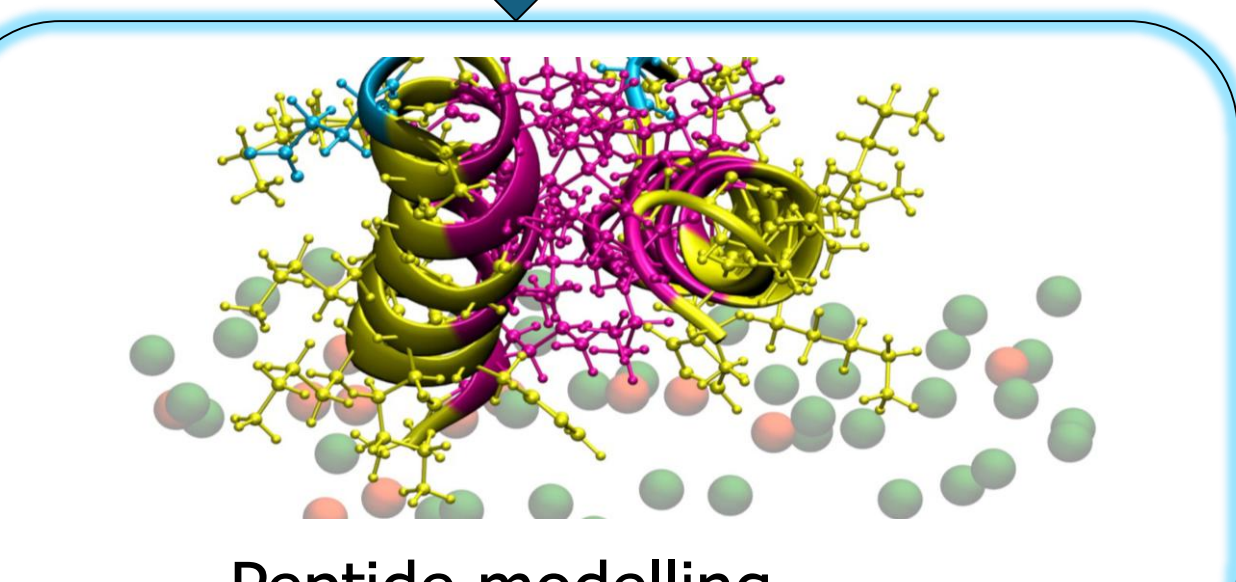
This study **aims** to engineer and optimize an (AMP) **Ttap-3**, derived from the venom of the Trinidad Thick-tailed scorpion, *Tityus trinitatis*, to enhance its antimicrobial activity while minimizing hemolytic effects.



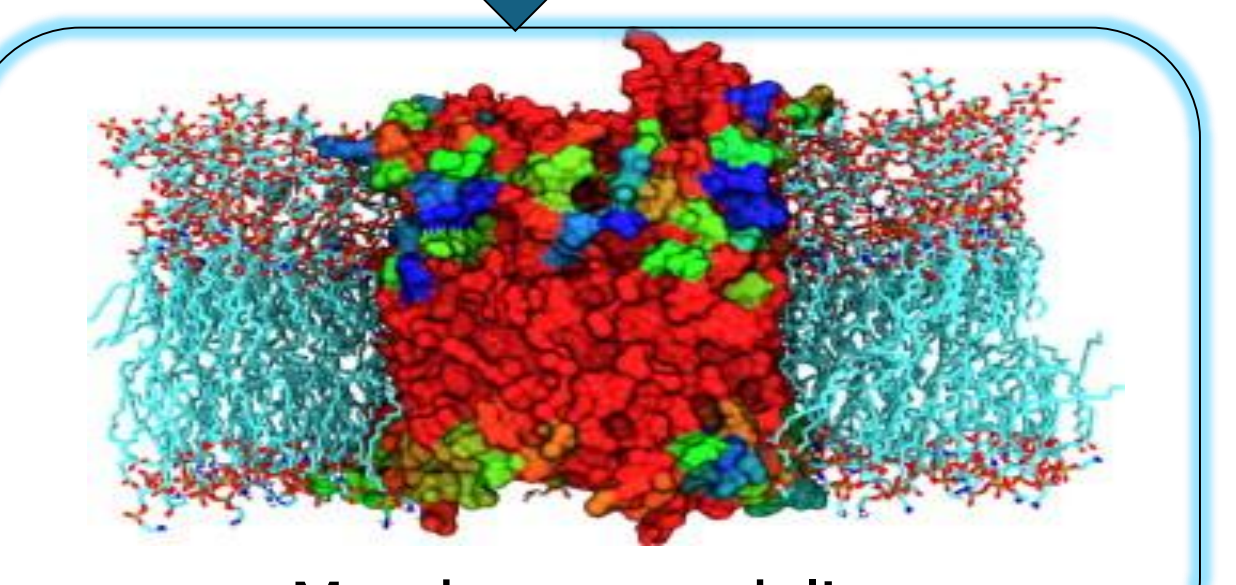
2. Experimental Design



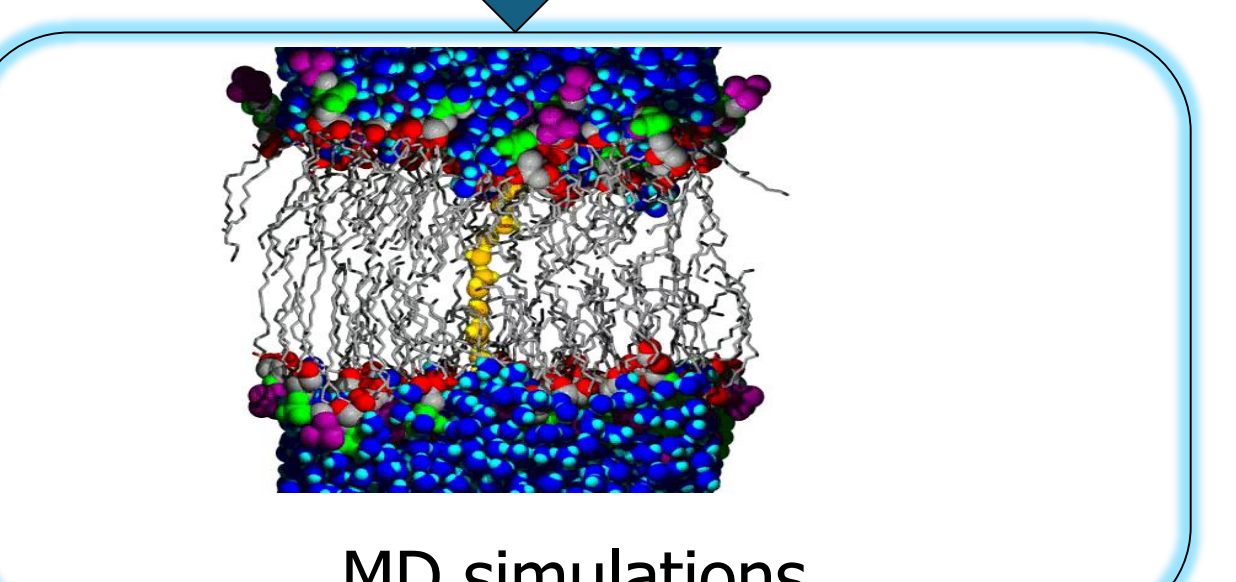
Peptide selection



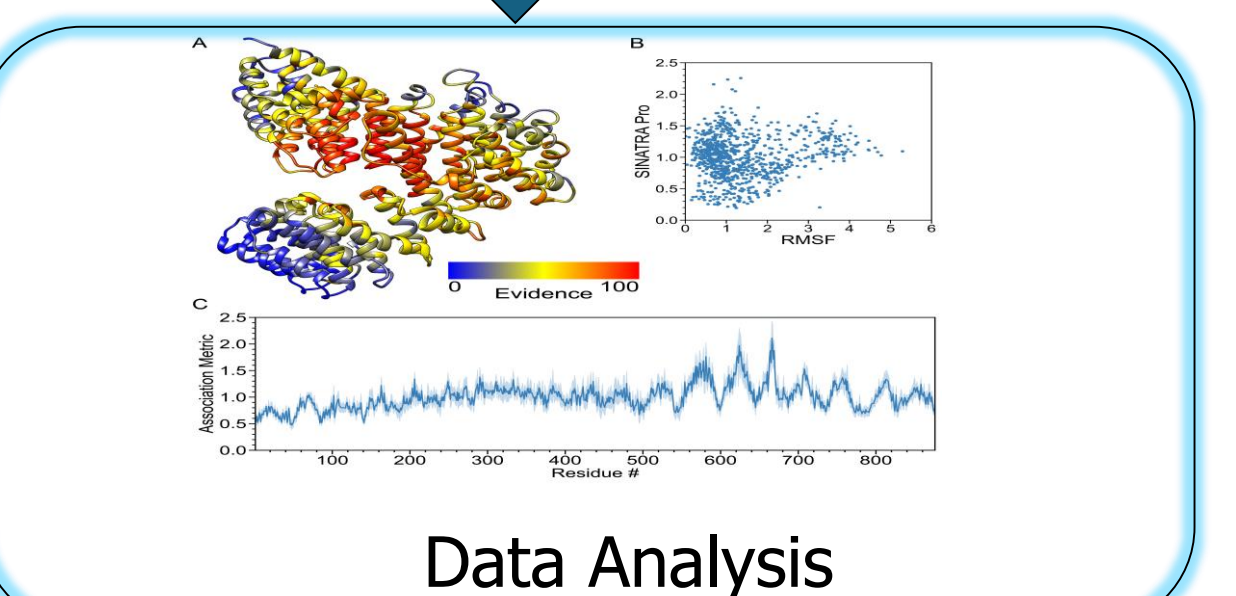
Peptide modelling



Membrane modeling



MD simulations



Data Analysis

3. Results and Discussion

1 Minimum Inhibitory Concentration and Hemolytic Activity of Peptides *in-vitro*

Table 1: MIC and hemolytic concentrations of isolated peptides

Peptide	Hemolytic activity $\mu\text{g/ml}$	MIC $\mu\text{g/ml}$	Sequence	AMP
Ttap-1	18	3.13	FLGSLFSIGSKLLPGVFKLFSRKKQ	0.96
Ttap-2	31	25	IFGMIPSLIGGLISAFK	0.94
Ttap-3	95	50	FFSLIPSLIGGLVSAIK	0.87

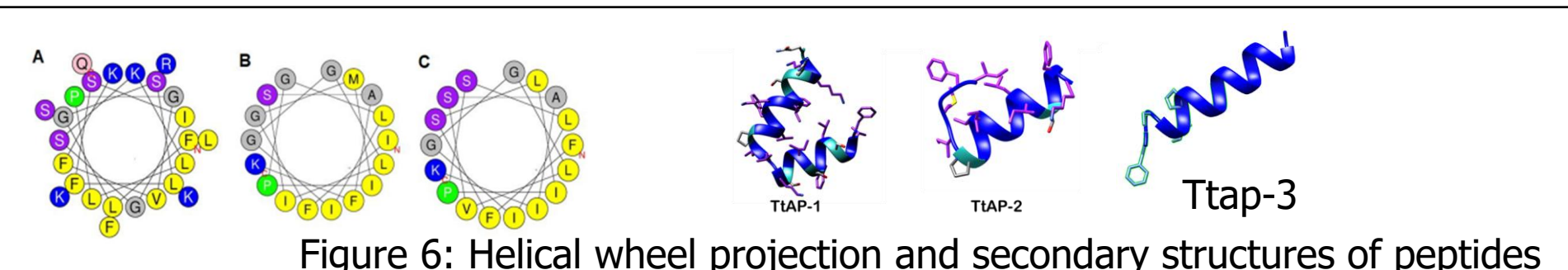


Figure 6: Helical wheel projection and secondary structures of peptides

A two dimensional representation of helix structure with corresponding secondary structures

2 Confirmation of Ttap-3 Activity from *in-vitro* Studies using Computations

Ttap-3: FFSLIPLIGGLVSAIK

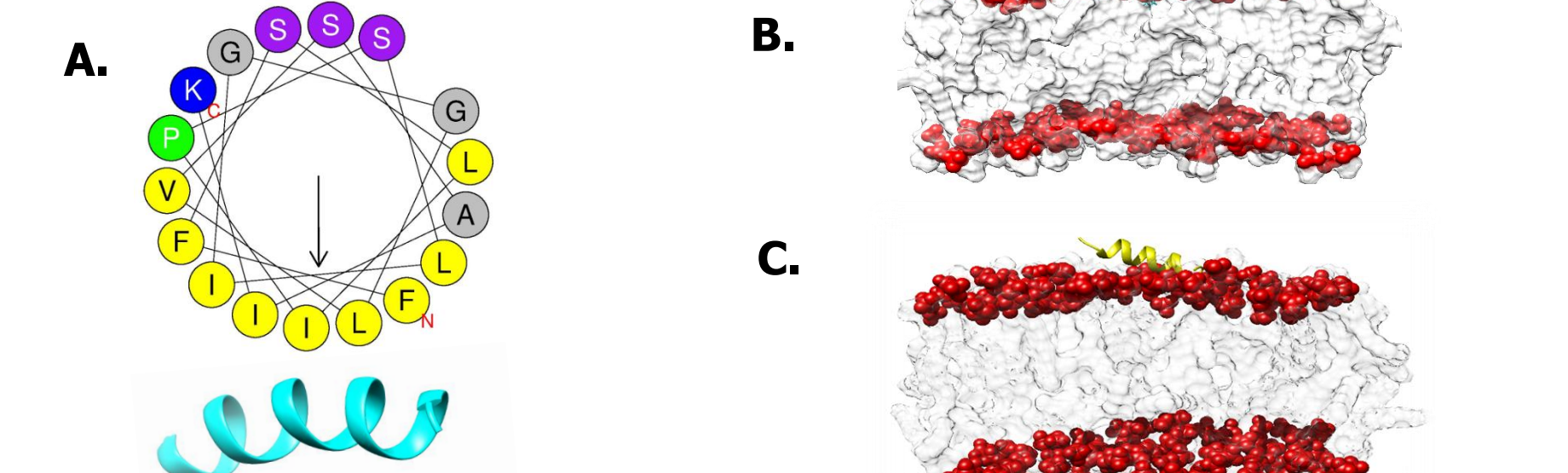


Figure 7: A) Hydrophobicity distribution and secondary structure B) Mammalian simulation C) Bacterial simulation

Ttap 3 peptide simulated against bacteria and mammalian membranes reveals membrane destruction confirming *in vitro* assay

3 Hemolytic Status and Antimicrobial Class of Engineered Peptides

Table 2: Engineered peptides from Ttap-3

PEPTIDE	CLASS	HEMOLYSIS
FFSLIPSLIGGLVSAIK	AMP	ACTIVE
KIASVLGGILSPILSFF	AMP	NOT ACTIVE
FFALIR	AMP	NOT ACTIVE
PGSLIPSLIGGLVSAIK	AMP	NOT ACTIVE

Ttap 3 peptide showed poor hemolytic profile compared to engineered peptides that demonstrated good hemolytic profile with all peptides remaining antimicrobial in nature

4 Membrane Simulation of Engineered Peptides

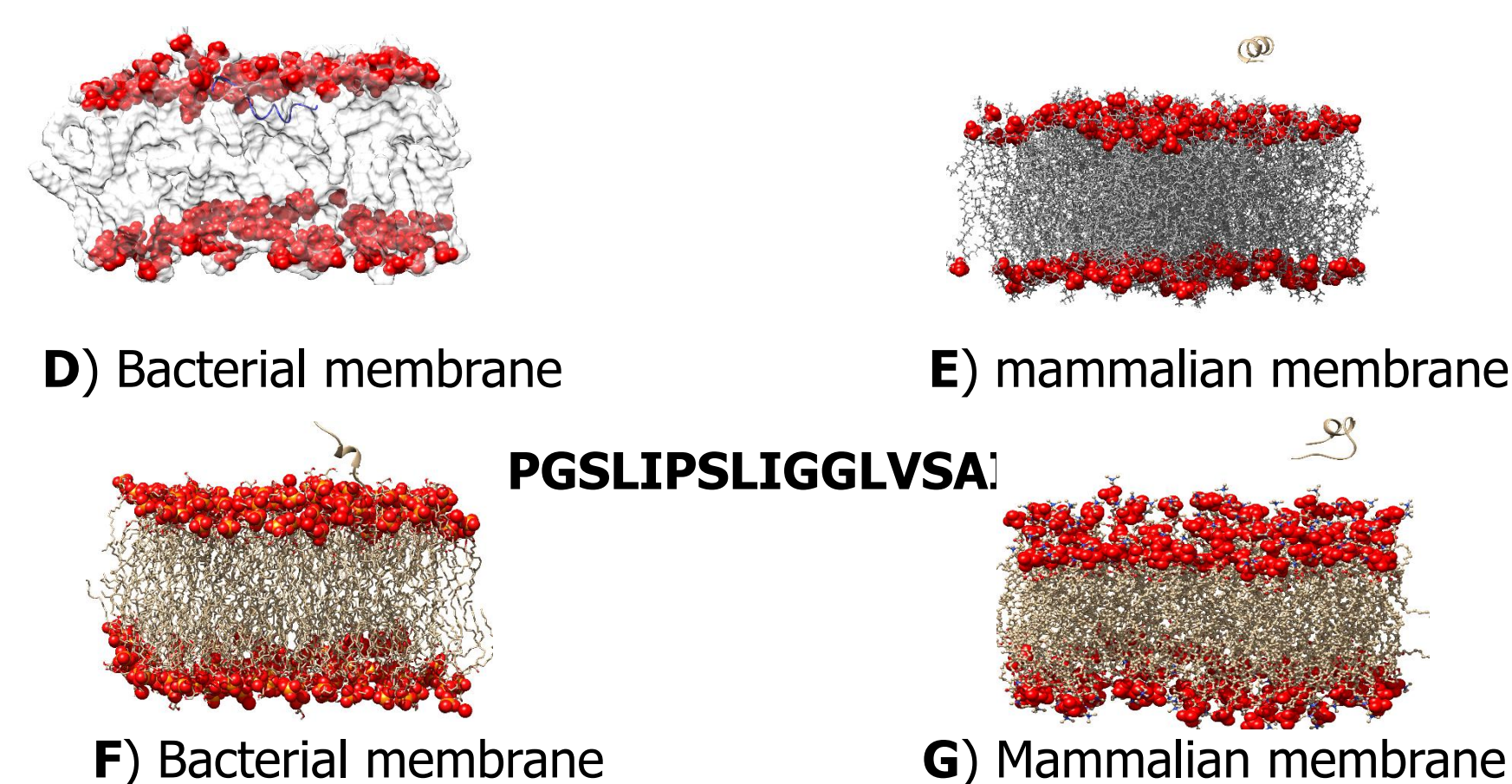
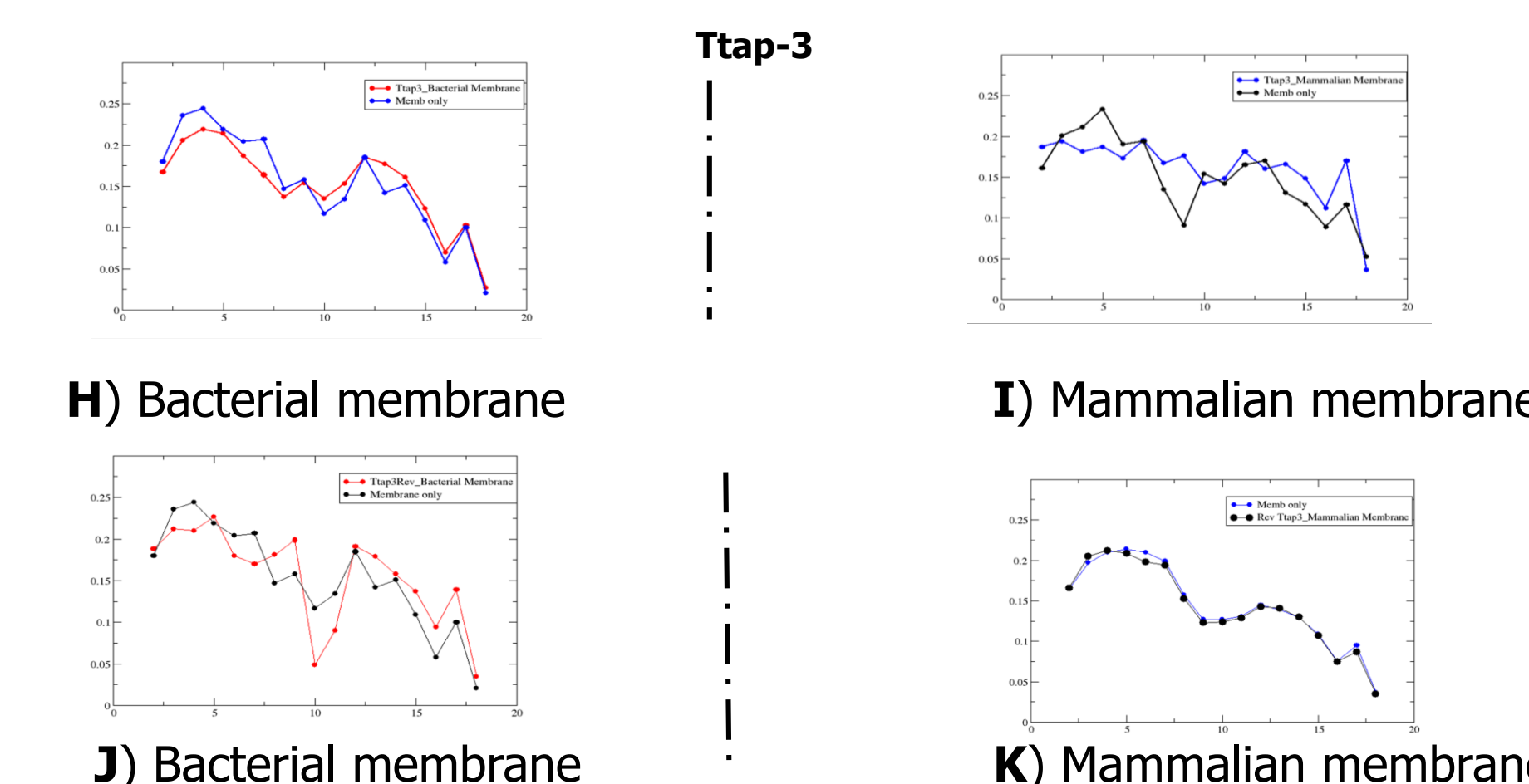


Figure 8: Engineered Peptide against specific membranes

Simulations revealed engineered peptides interacted with bacteria membrane with no significant interaction with mammalian membrane

5 Lipid Bilayer Structural Dynamics Simulations



Ttap 3-membrane simulations resulted in the disorientation of lipid components of both membranes whereas Reversed Ttap 3 led to lipid deviation for only bacteria membrane

6 RMSD Analysis and Hydrogen Bonding Pattern Evaluation

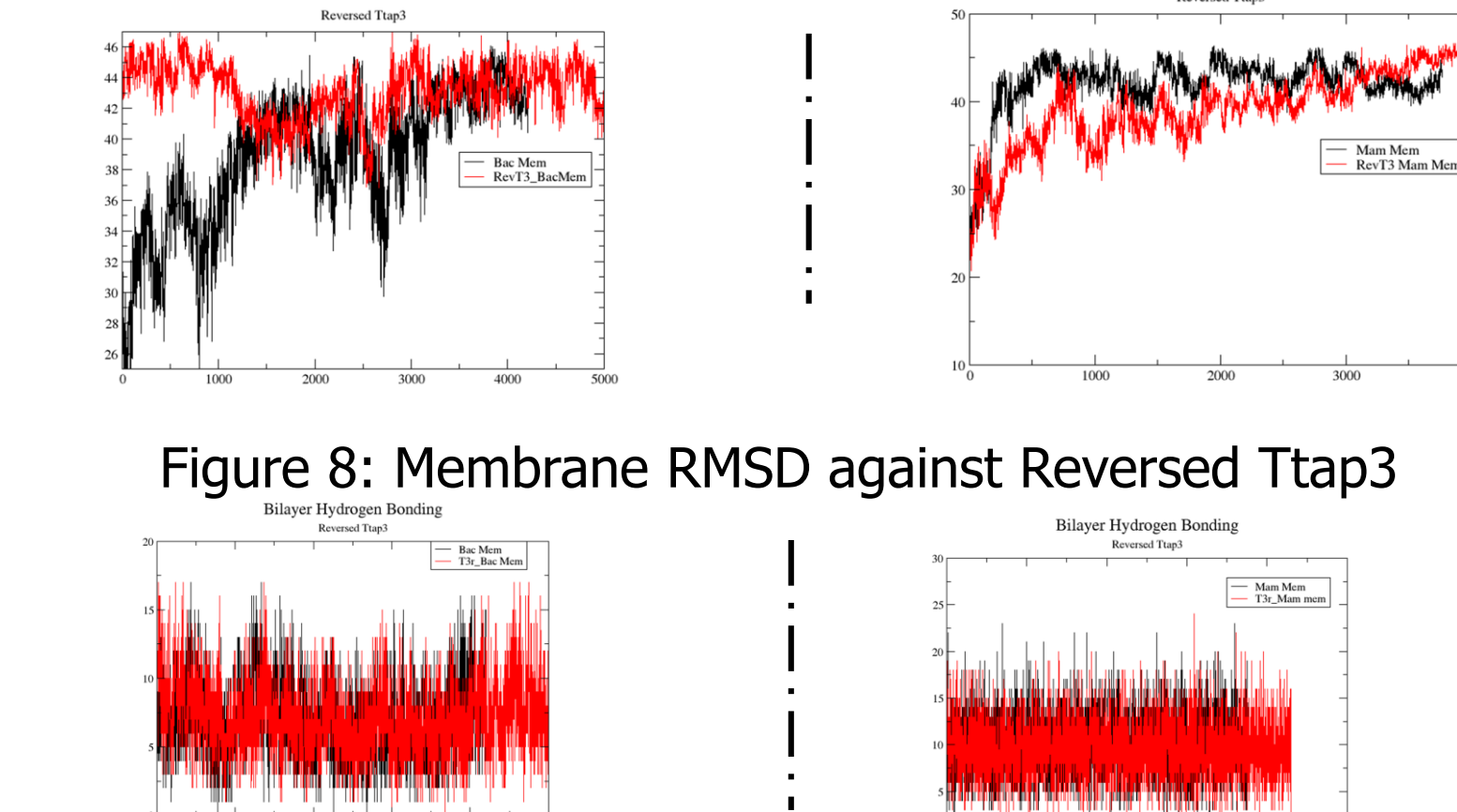


Figure 8: Membrane RMSD against Reversed Ttap3

Figure 9: Hydrogen Bonding Pattern in lipid bilayer

Engineered peptide resulted in a high bacteria membrane RMSD with poor hydrogen bond patterns

4. Conclusion

- Through computational engineering and molecular dynamics simulations, analogs of Ttap 3 peptide was successfully designed with reduced hemolytic profile.
- By introducing targeted mutations and analyzing membrane interaction profiles, engineered peptides retained antimicrobial potency with diminished interaction with human erythrocyte, paving way for more safer and effective antimicrobial agents.

5. References

- Mechkarska, M., Cuning, T.S., Taggart, M.G., Ternan, N.G., Leprince, J., Coquet, L., Jouenne, T., Tena-Garcés, J., Calvete, J.J. and Conlon, J.M., 2023. Identification of an antimicrobial peptide from the venom of the Trinidad thick-tailed scorpion *Tityus trinitatis* with potent activity against ESKAPE pathogens and *Clostridioides difficile*. *Antibiotics*, 12(9), p.1404.
- de Oliveira, A.N., Soares, A.M. and Da Silva, S.L., 2023. Why to study peptides from venomous and poisonous animals?. *International Journal of Peptide Research and Therapeutics*, 29(5), p.76.