

Supplemental information

The mechanism underlying toxicity of a venom peptide against insects reveals how ants are master at disrupting membranes

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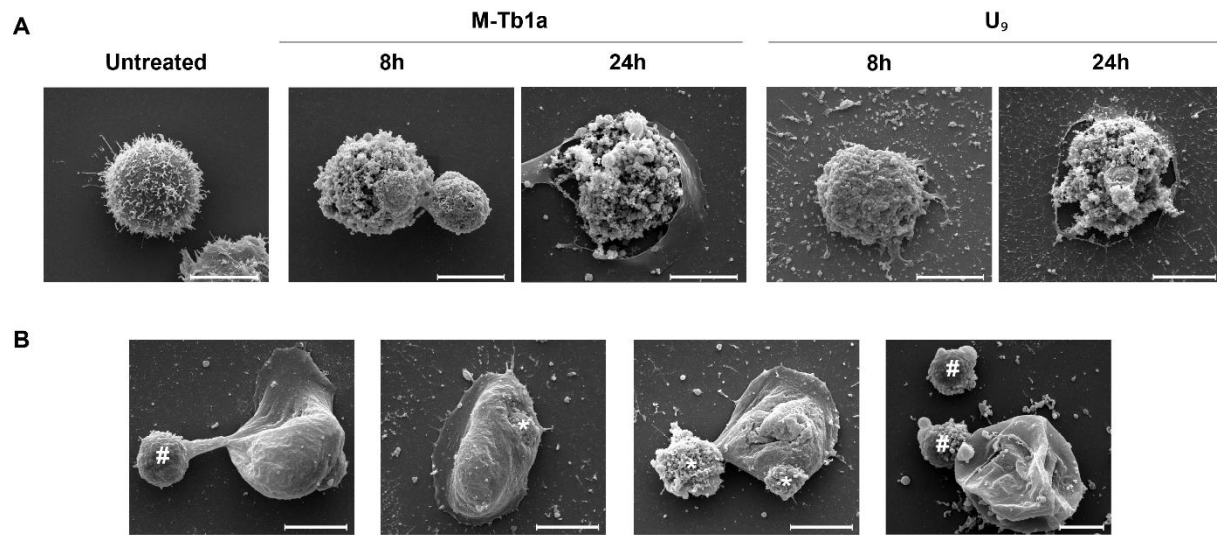


Figure S1 - Lysis of S2 cells and expulsion of intracellular contents after exposure to M-Tb1a and U₉, Related to Figure 5. A. Cell lysis after exposure to either peptide at LC₅₀ for 8h and 24h. **B.** S2 cells after exposure to U₉ at 18 μ M for 24h. #: intracellular organelles with membrane preservation; *: intracellular content without membrane. Scale bar: 5 μ m.

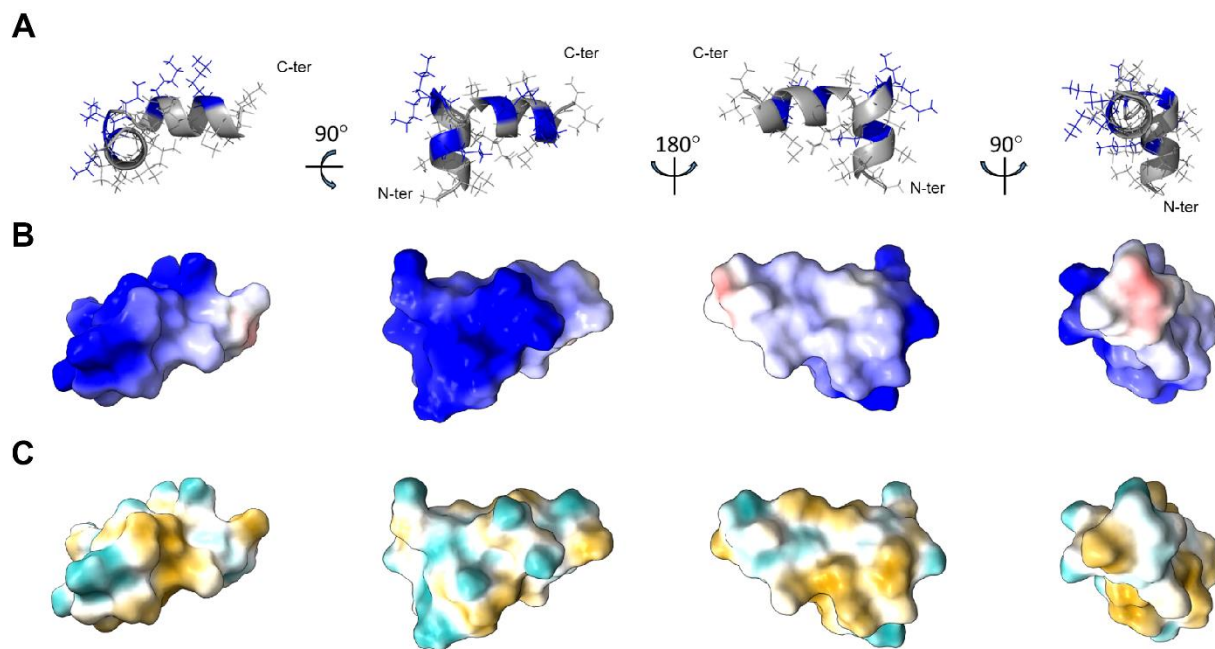


Figure S2 - Solution structure of the U₉ peptide, Related to Figure 10. **A.** Cartoon representation of the lowest-energy solution structure of U₉ along different axes. **B.** Surface coloring ranges from red for the negative potential to white to blue for the positive potential. **C.** Surface coloring ranges from dark goldenrod for the most hydrophobic potentials, through white, to dark cyan for the most hydrophilic potentials.

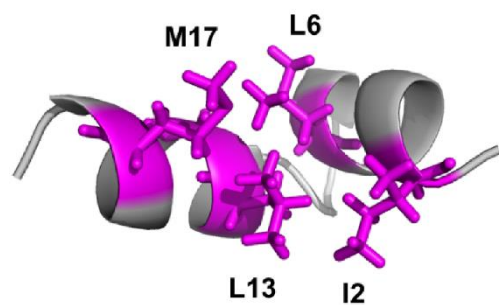
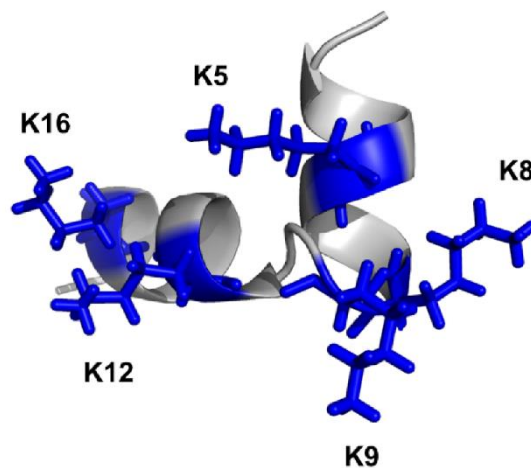
A**B**

Figure S3 - Distribution of amphiphilic and hydrophobic residues of the U₉ peptide, Related to Figure 10. A. Evidence of the amphiphilic structure of U₉ due to the formation of a hydrophobic core on the concave side of the HTH motif. **B.** Distribution of lysine residues on the convex side of the HTH motif.

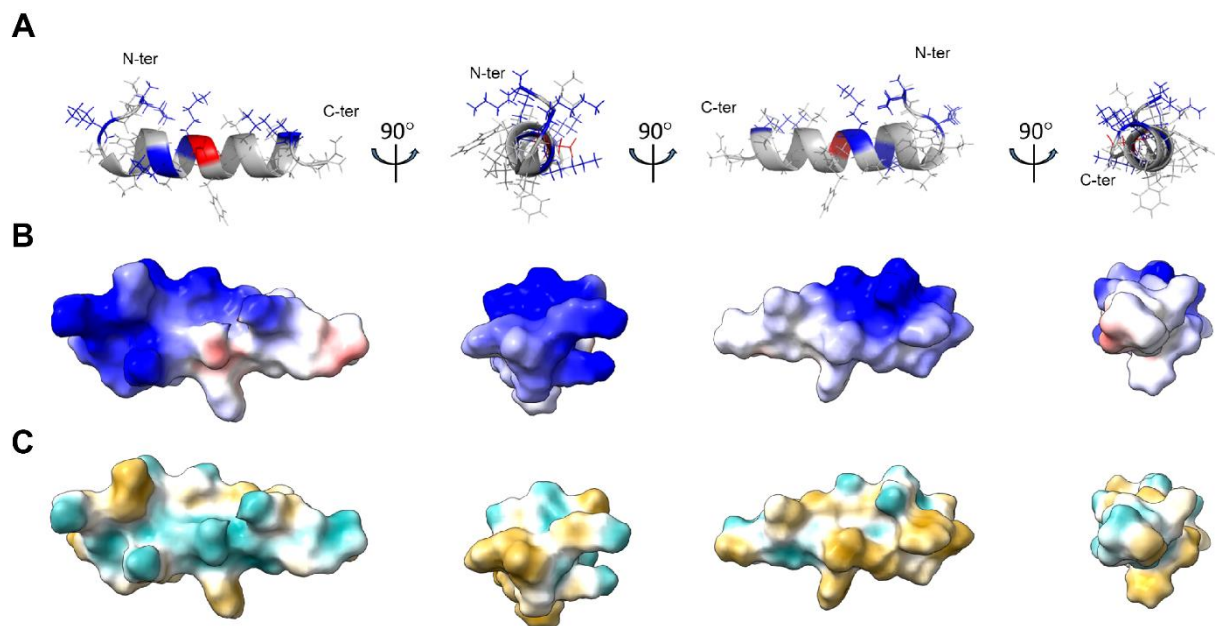


Figure S4 - Solution structure of the M-Tb1a peptide, Related to Figure 10. **A.** Cartoon representation of the lowest-energy solution structure of the M-Tb1a along different axes. **B.** The surface coloring ranges from red for the negative potential to white to blue for the positive potential. **C.** Surface coloring ranges from dark goldenrod for the most hydrophobic potentials, through white, to dark cyan for the most hydrophilic potentials.

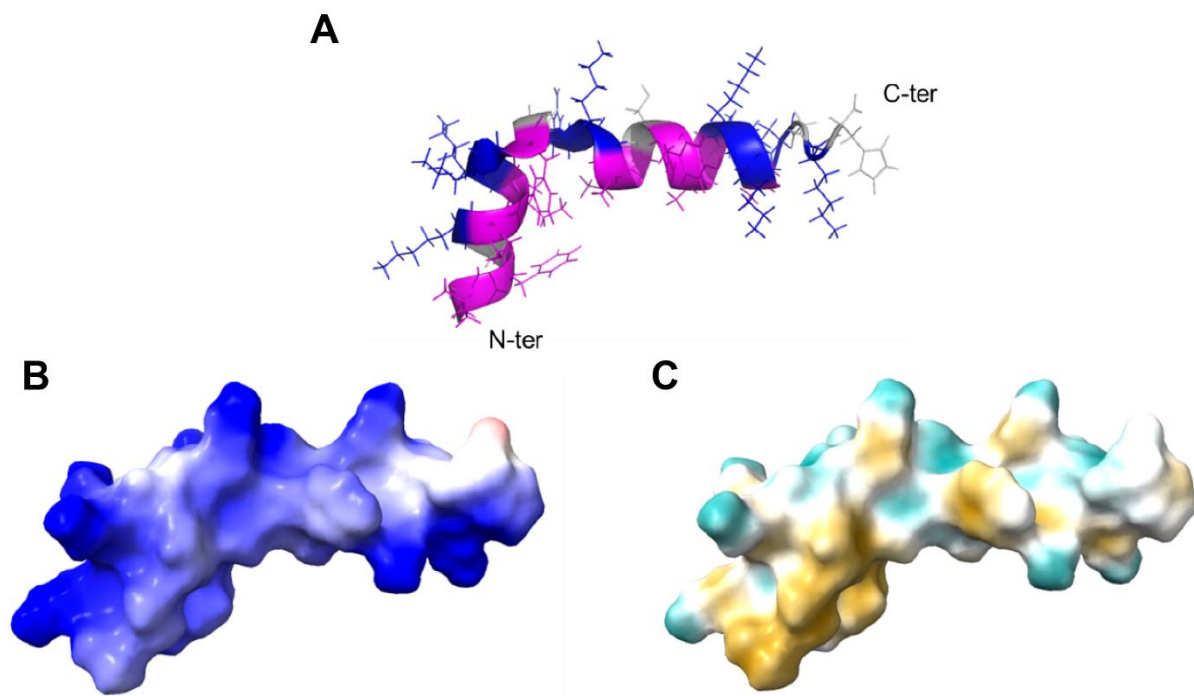


Figure S5 - Solution structure of the Lt2a peptide, Related to Table 1. **A.** Cartoon representation of the lowest-energy solution structure of Lt2a in 100 mM SDS-d25 (PDB 2G9P) determined by Dubovskii et al.^[S1]. Hydrophobic residues are colored in magenta and basic residues in blue. **B.** Surface coloring ranges from red for the negative potential to white to blue for the positive potential. **C.** Surface coloring ranges from dark goldenrod for the most hydrophobic potentials, through white, to dark cyan for the most hydrophilic potentials.

M-Tb1a **KIKIPWGKVKDFLVGGMKAV**-NH₂

U₉ **GIVTKLIKK-GVKLGLKMAI**-NH₂
Lt2a **GLFGKLIKKFGRKAI SYAVKKARGKH**
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Figure S6 - Amino acid sequences of the M-Tb1a, U₉ and Lt2a peptides, Related to Table 1. Hydrophobic residues are written in magenta, basic residues in blue and acidic residues in red. Residues involved in a hinge are framed.

Table S1 - Experimental restraints and structural statistics of U₉, Related to Figure 10. Structural statistics are calculated for all fifteen of the lowest-energy structures.

NMR restraints		
<i>Distance restraints</i>		
Total NOE		346
Unambiguous		260
Ambiguous		86
Hydrogen bonds		9
<i>Dihedral angle restraints</i>		30
Structural statistics		
<i>Average violations per structure</i>		
NOEs $\geq 0.5\text{\AA}$		0
NOEs $\geq 0.3\text{\AA}$		0
Hydrogen bonds $\geq 0.3\text{\AA}$		0
Dihedrals ≥ 10		0
<i>Average pairwise RMSD (\AA)</i>	backbone atoms	heavy atoms
U ₉ -MYRTX-Tb1a	0.45 \pm 0.11	0.94 \pm 0.17
<i>Ramachandran analysis</i>		
Most favored region (%)		79
Allowed region (%)		21
Generously allowed (%)		0
Disallowed (%)		0
<i>Energies (kcal.mol⁻¹)^a</i>		
Total		-481.0 \pm 7.2
Van der Walls		-168.9 \pm 3.1
Electrostatic		-533.9 \pm 4.9
Residual NOE		14.8 \pm 3.7

a: values are given as mean \pm standard deviation (n=15)

Table S2 - Experimental restraints and structural statistics of the M-Tb1a, Related to Figure 10. Structural statistics are calculated for all fifteen of the lowest-energy structures.

NMR restraints		
<i>Distance restraints</i>		
Total NOE		308
Unambiguous		286
Ambiguous		22
Hydrogen bonds		14
Structural statistics		
<i>Average violations per structure</i>		
NOEs $\geq 0.5\text{\AA}$		0
NOEs $\geq 0.3\text{\AA}$		0
Hydrogen bonds $\geq 0.3\text{\AA}$		0
<i>Average pairwise RMSD (\AA)</i>	backbone atoms	heavy atoms
M-MYRTX-Tb1a	0.68 ± 0.21	1.40 ± 0.31
<i>Ramachandran analysis</i>		
Most favored region (%)		68
Allowed region (%)		25
Generously allowed (%)		0
Disallowed (%)		7
<i>Energies (kcal.mol⁻¹)^a</i>		
Total		-454.9 ± 16.3
Van der Walls		-161.5 ± 3.8
Electrostatic		-605.9 ± 11.8
Residual NOE		11.7 ± 3.0

a: values are given as mean \pm standard deviation (n=15)

References

- [S1]. Dubovskii, P. V., Volynsky, P.E., Polyansky, A.A., Chupin, V. V., Efremov, R.G., and Arseniev, A.S. (2006). Spatial structure and activity mechanism of a novel spider antimicrobial peptide. *Biochemistry* 45, 10759–10767. 10.1021/bi060635w.