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NMR-based Structure-Activity Relationship Studies of Antimicrobial Peptides

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A triazolyl bridge enhances antimicrobial activity, while Arg3, Arg7 and Asn11 are key determinants of activity.

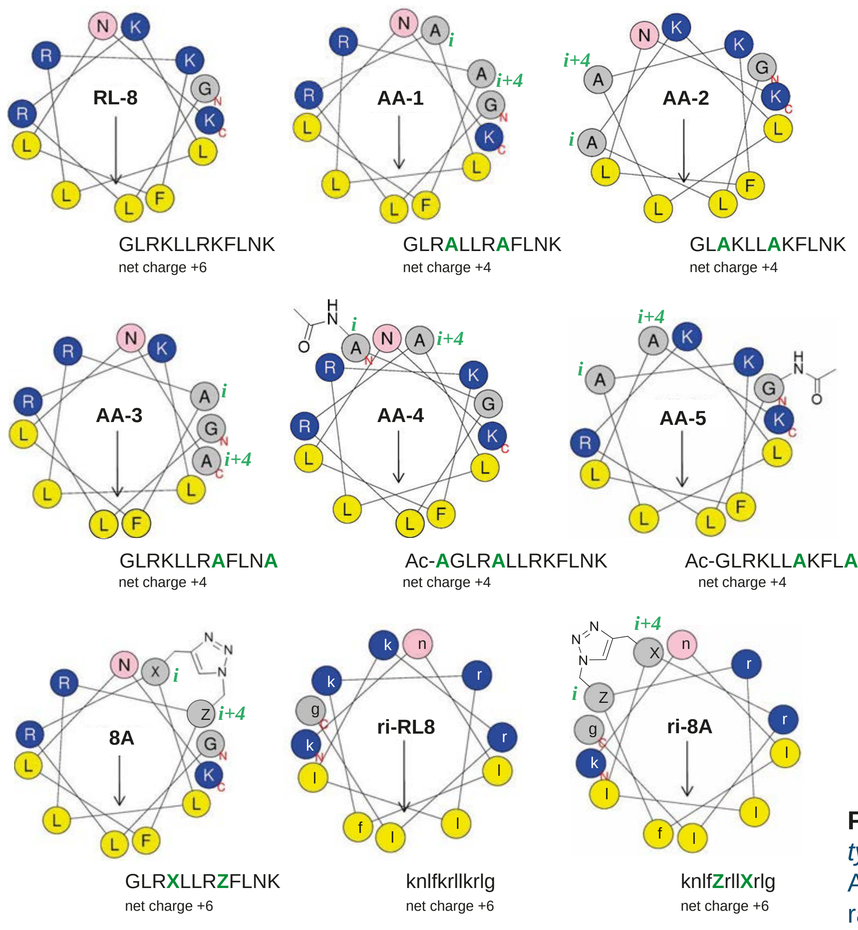


Figure 1. Helical-wheel projections of the designed and analysed peptides.
The N- and C-termini are indicated by red upper-case letters. Amino acids are labelled using the one-letter code, with lower-case letters used for the retro-inverso peptides ri-RL8 and ri-8A. Positively charged residues are highlighted in blue, Asn11 (Asn2 in the retro-inverso peptides) in pink, hydrophobic residues in yellow and all other residues in grey. Acetylation and the triazolyl bridge are shown by their chemical structures. L-Propargylglycine and L-ε-azido-norleucine are denoted X and Z, respectively. Modified residues at positions i and i + 4 are highlighted in green to indicate their spacing within the peptide sequence.

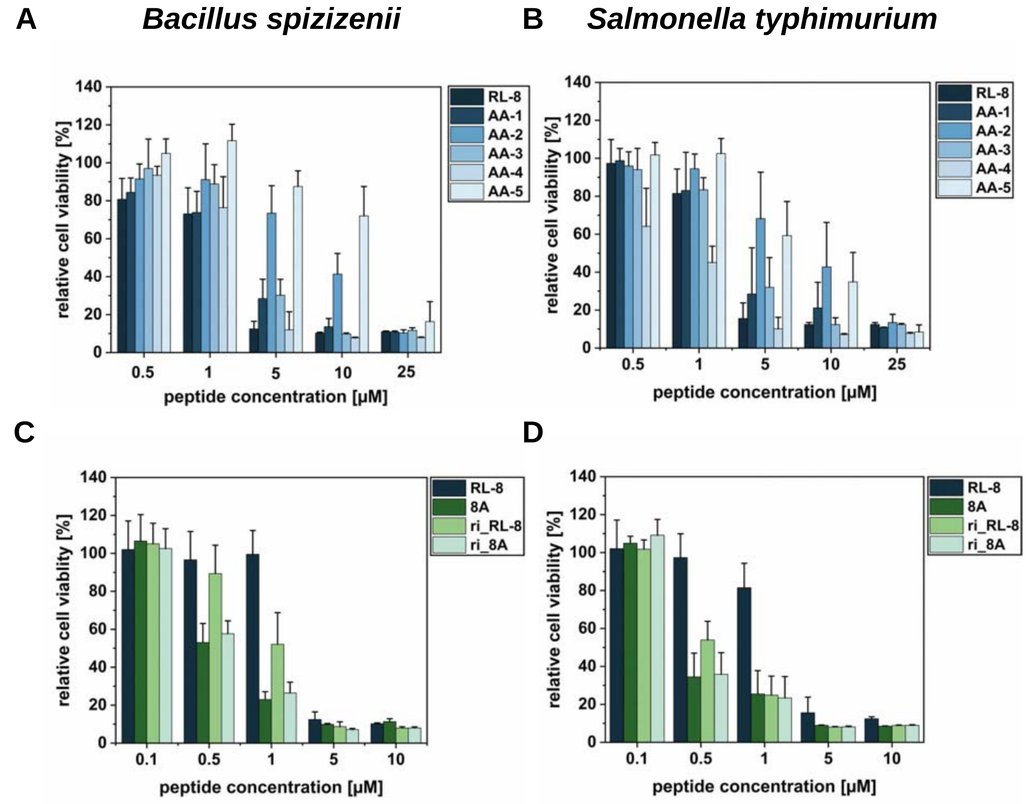


Figure 2. Antimicrobial activity of the analysed peptides. Relative cell viability of *B. spizizenii* (**A**, **C**) and *S. typhimurium* (**B**, **D**) was determined at increasing peptide concentrations. Panels **A–B** show RL-8 and AA-1 to AA-5, whereas panels **C–D** compare RL-8, 8A, ri-RL-8 and ri-8A. Note the different peptide concentration ranges used in the two sets of experiments.

The molecular surface of peptide 8A presents favourable features for membrane interaction.

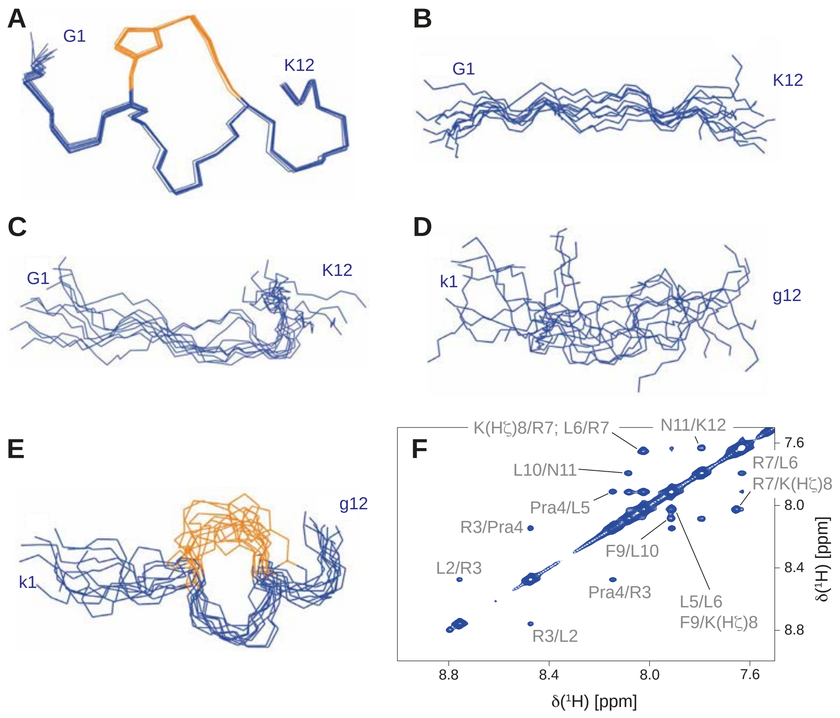


Figure 3. NMR-derived structures of the analysed peptides.
A–E Conformational ensembles of the ten lowest-energy structures of 8A (**A**), RL-8 (**B**), AA-2 (**C**), ri-RL-8 (**D**) and ri-8A (**E**). Peptide backbones are shown in blue and triazolyl bridges in orange. N- and C-termini are labelled by residue and position.
F Amide–amide region of the 2D $^{1}H,^{1}H$-NOESY spectrum of 8A. Amino acids are labelled using the one-letter code; Pra, L-propargylglycine.

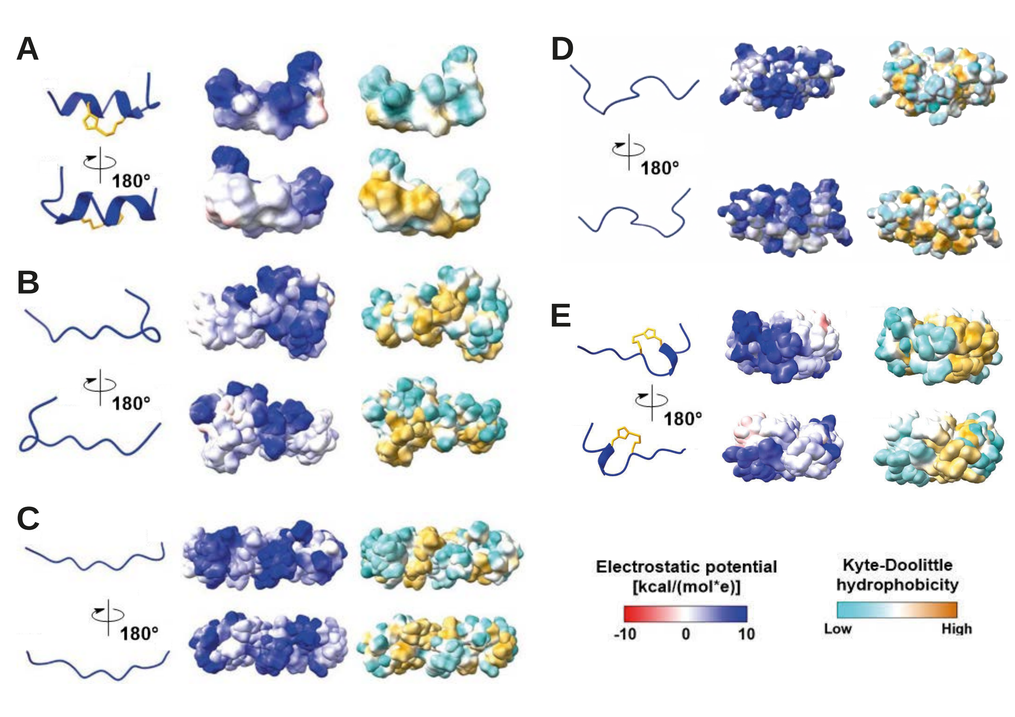


Figure 4. Surface properties of the NMR-derived peptide structures.
A–E Electrostatic surface potential and Kyte–Doolittle hydrophobicity of 8A (**A**), AA-2 (**B**), RL-8 (**C**), ri-RL-8 (**D**) and ri-8A (**E**). Surface orientations correspond to the representative structures shown on the left; colour scales are indicated at the bottom right.

CD validates the NMR-derived structures, while chemical modification improves serum stability and micelle binding promotes α-helicity.

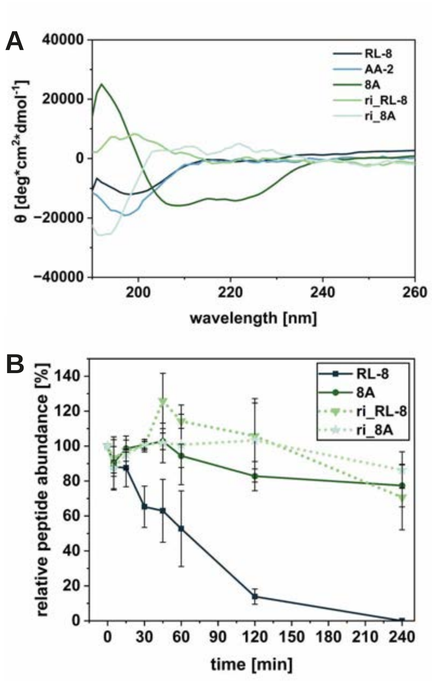


Figure 5. CD spectroscopy and serum stability of the analysed peptides.
A CD spectra of the indicated peptides recorded in H_2O.
B Stability of RL-8, 8A, ri-RL-8 and ri-8A in human serum.

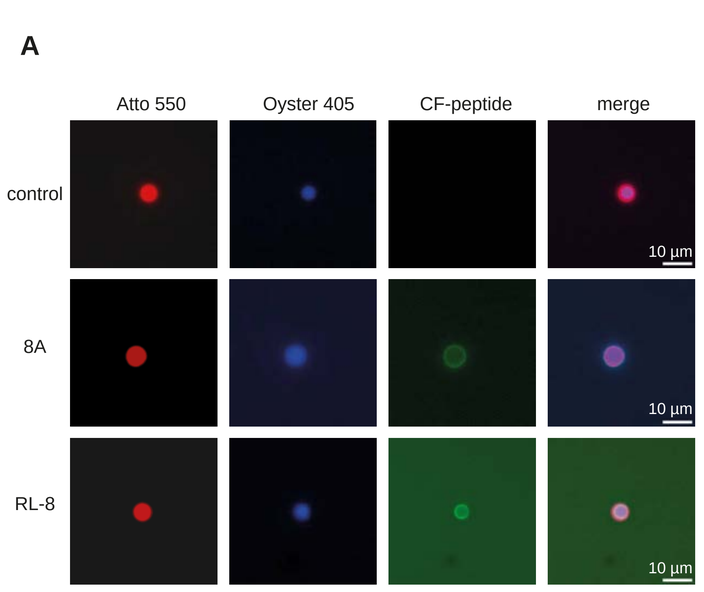
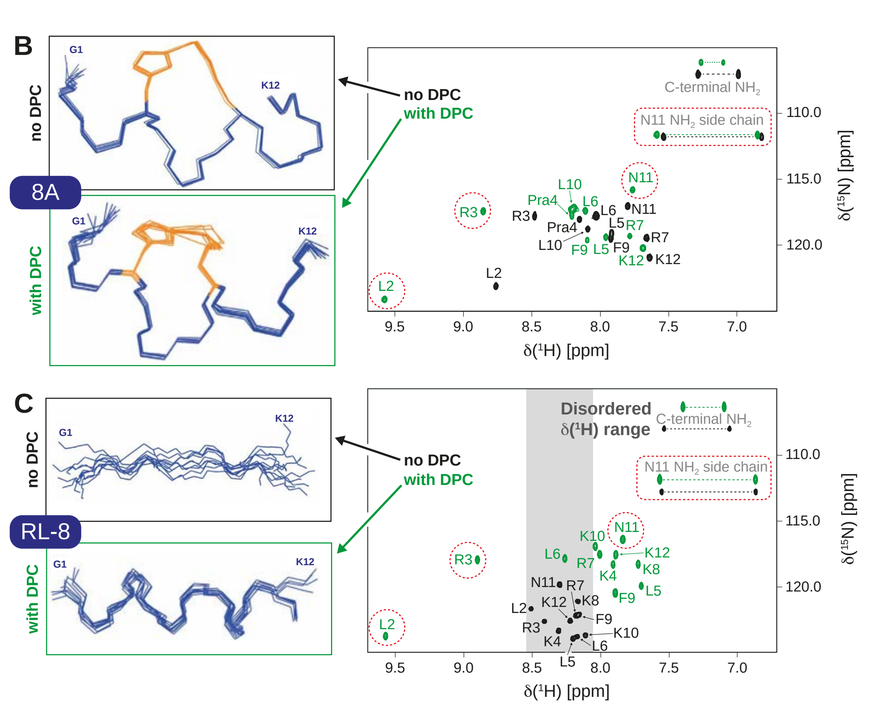


Figure 6. Membrane interactions of peptides 8A and RL-8.
A Fluorescence microscopy images of GUVs without peptide (control, top row), with 8A (middle row) and with RL-8 (bottom row).
B–C NMR-derived structures and $^{1}H,^{15}N$-HSQC spectra of 8A (**B**) and RL-8 (**C**) in H_2O in the absence and presence of DPC micelles.



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University of Cologne
DFG Deutsche Forschungsgemeinschaft German Research Foundation
FULBRIGHT Germany
EMBO
HFSP International Human Frontier Science Program Organization