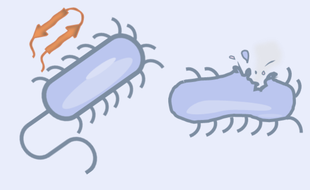
SAR Study of Antimicrobial Peptide with Cross-Kingdom Activity

Aparna Palakkurussi Rathessan [1], Fereshteh Ghazisaeedi [2,3], Krithika Unmesh [5], Pascal-Kolja Bingül [6], Johannes Kupke [2], Suvrat Chowdhary [1], Dennis Hanke[2,3], Maria Andrea Mroginski [5], Marcus Fulde [2,3,4] and Beate Koksch [1]

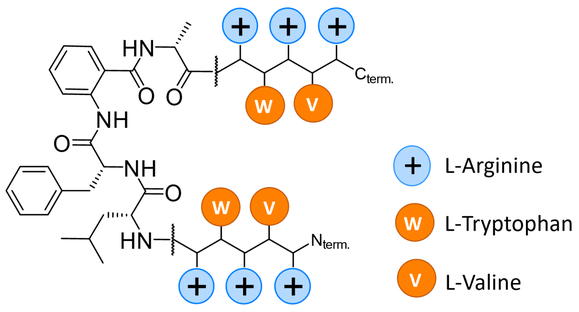
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Research Aim

Antimicrobial resistance (AMR) is a critical global health concern that threatens the effective prevention and treatment of an ever-increasing range of infections caused by microbial pathogens. Antimicrobial peptides (AMPs) are recognized as potential lead structures for developing next-generation therapeutics. We aim to enhance the immense potential of AMPs through conformational tuning and differential fluorination on two different turn-like peptide architectures, SAJO and C7.

SAJO System



SAJO-2 [1,2,3]

N-	*N-terminal strand*	*β-turn motif*	*C-terminal strand*	*-C*

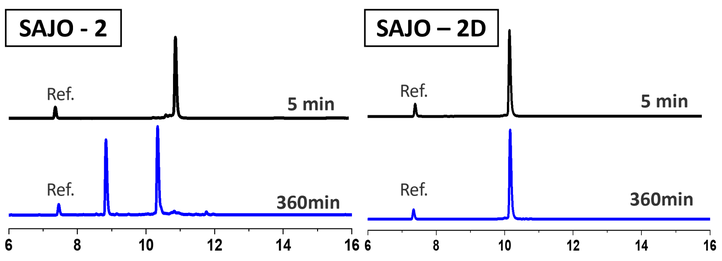
N_{term}-Arg-Val-Arg-Trp-Arg---[D-Leu-D-Phe-[2]Abz-D-Ala]---Arg-Trp-Arg-Val-Arg-C_{term}

Arg-Val-Arg-Trp-Arg	SAJO-2	Arg-Trp-Arg-Val-Arg
D-Arg-Val-D-Arg-Trp-D-Arg	SAJO-D	D-Arg-Trp-D-Arg-Val-D-Arg
Arg-Val-D-Arg-Trp-Arg	SAJO-1D	Arg-Trp-D-Arg-Val-Arg
D-Arg-Val-D-Arg-Trp-Arg	SAJO-2D	Arg-Trp-D-Arg-Val-D-Arg
Arg-Pfp-D-Arg-Trp-Arg	SAJO-PfpGly-1D	Arg-Trp-D-Arg-Pfp-Arg

peptides (serial dilutions, start: 1024 µg/mL) *incubated with bacteria culture (2 × 10^6 CFU/mL), pH 7.4; 37 °C (24 h).*

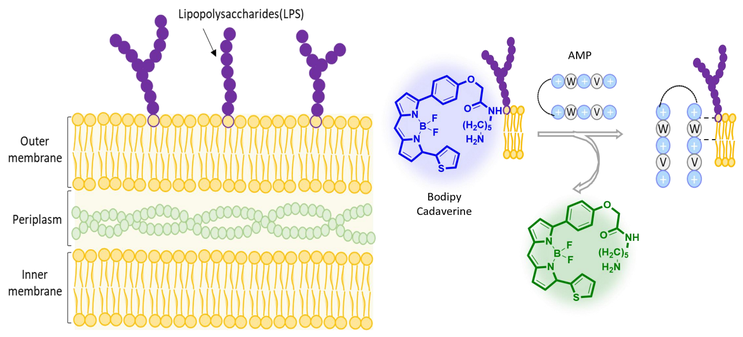
Antimicrobial Peptide	*S.* Typhimurium ATCC 14028	*E. coli* ATCC 25922	*S. aureus* ATCC 29213	*P. aeruginosa* ATCC 27853	*C. albicans* IMT 9655
SAJO - 2	32	**16**	64	64	**32**
SAJO - D	32	**16**	256	512	**32**
SAJO - 1D	32	32	128	1024	**32**
SAJO - 2D	**16**	32	256	1024	**32**
SAJO - PfpGly-1D	32	32	64	512	**32**

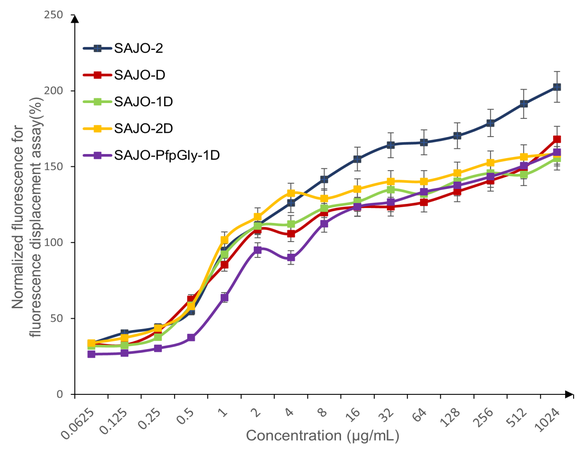
Enzymatic digestion



Digestion profile of SAJO-2 and SAJO-2D by *α-chymotrypsin*

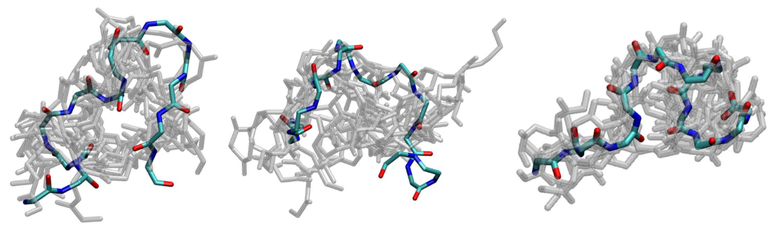
LPS Binding Affinity Studies





The peptides displace Bodipy Cadaverine dye from the lipid A, which dequenches the dye and enhances the fluorescence signal

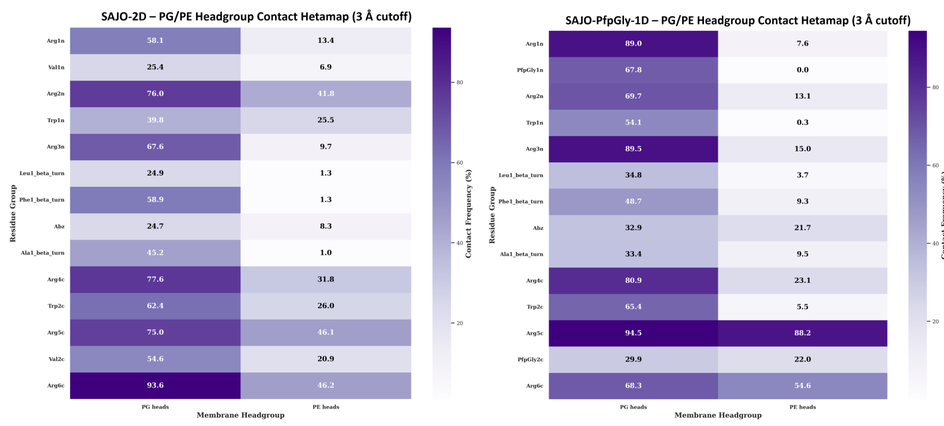
Molecular Dynamics Simulations: In Solution



SAJO-2D **SAJO-PfpGly-1D** **SAJO-PfpGly**

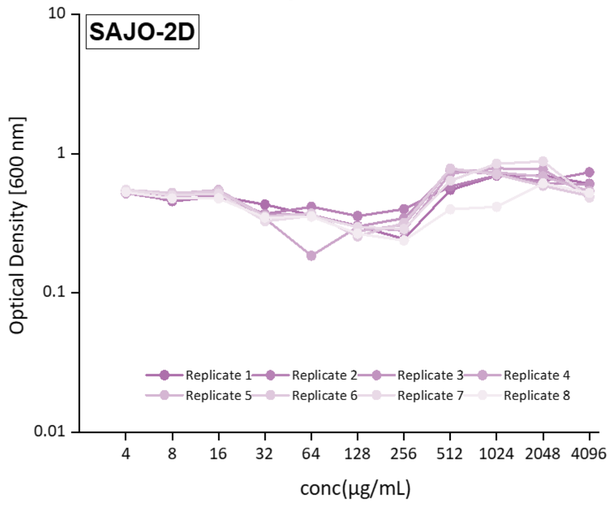
Simulations performed in aqueous solution reveals D-modified fluorinated analogue leads to a more open structure and a markedly broader conformational ensemble

Molecular Dynamics Simulations: POPG/POPE Bilayers



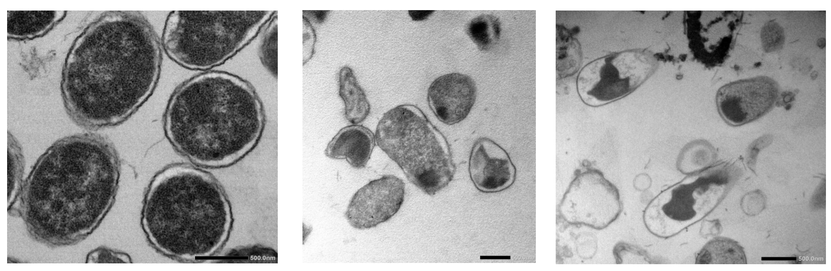
SAJO-2D shows stronger membrane contacts, through its C-terminal residues, and displays enhanced POPG selectivity. SAJO-PfpGly-1D displays the strongest minimal POPE interaction profile, followed by tryptophan and the PfpGly unit.

Adaptive Laboratory Evolution with *E. coli*



Rapid bacterial adaptation to AMPs

Transmission Electron Microscopy

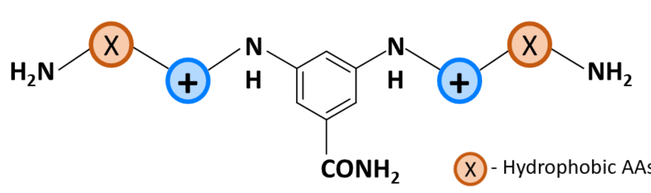


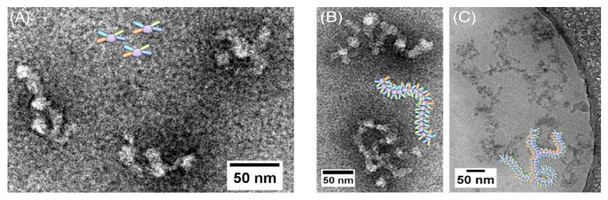
E. Coli (Control) | SAJO-2D 2 x MIC (24h) | SAJO-PfpGly-1D 2 x MIC (24h)

Extensive disruption to cells by SAJO analogues

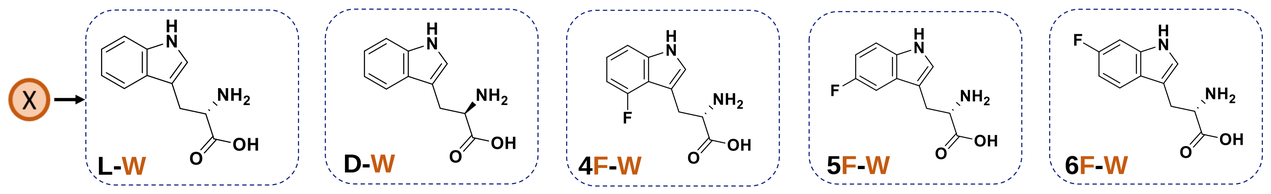
Read full publication [4]

C7 System

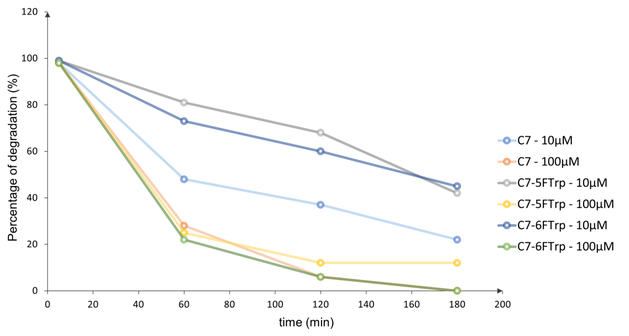




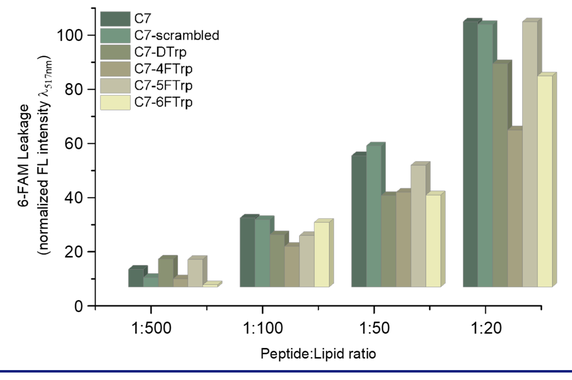
(A) monomers, (B) oligomers and (C) multimers [5]



Enzymatic Digestion



POPE/POPG Liposome Leakage Assay



Outlook

- SAJO peptides will be investigated for their efficiency in targeting yeast and hyphal forms of *Candida* species.
- Further antimicrobial and toxicity studies will be performed on the C7 peptidomimetic system

Acknowledgements

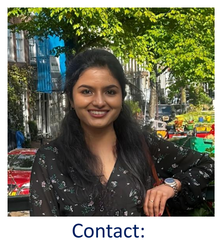
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[3] S. Chowdhary, A. P. Rathessan, T. Pelzer, M. Fulde, B. Koksch, “Peptide With Antimicrobial Activity”, int. patent, EP4375289A1
[4] A. P. Rathessan, F. Ghazisaeedi, K. Unmesh, P. Bingul, J. Kupke, S. Chowdhary, D. Hanke, M. A. Mroginski, M. Fulde, B. Koksch, *Biochemistry*, 2026, 65 (12): 1944-1956
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