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Broad spectrum activity of antimicrobial peptoids

Chaired by DR. ALFREDO BERZAL-HERRANZ;
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pharmaceuticals MDPI

Gill Diamond[1,*], Erika Figgins[1], Denny Gao[1], Annelise Barron[2] and Kent Kirschenbaum[3]

[1] Department of Oral Immunology and infectious Diseases, University of Louisville School of Dentistry, Louisville, KY USA;
[2] Department of Bioengineering, Stanford University, Palo Alto, CA USA;
[3] Department of Chemistry, New York University, New York, NY USA.

* Corresponding author: gill.diamond@Louisville.edu

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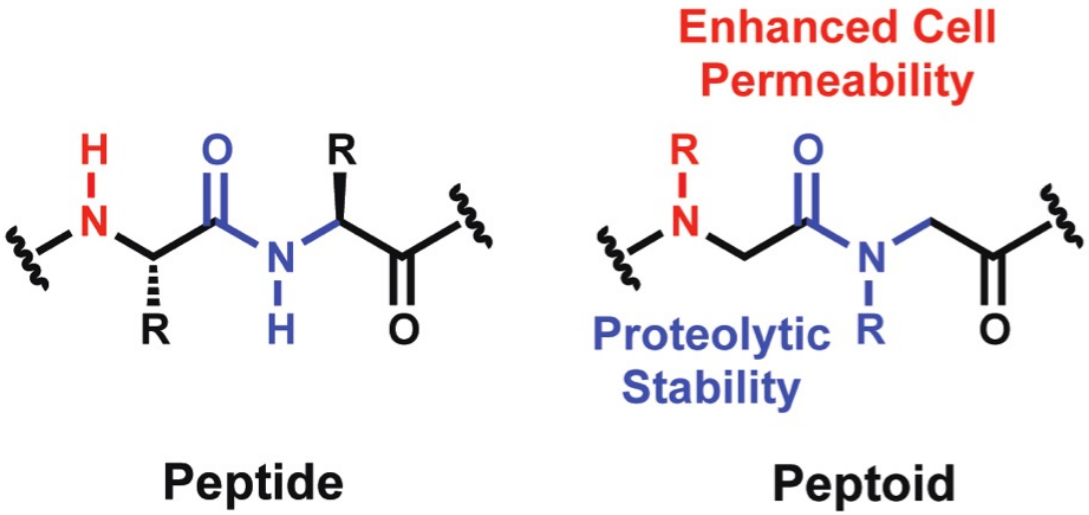
Broad spectrum activity of
antimicrobial peptoids

Peptoids versus Peptides



Abstract:
Antimicrobial peptides (AMPs) are naturally occurring host defense molecules, representing an evolutionarily ancient innate immune mechanism against pathogenic infection. As such, many of these predominantly cationic and amphipathic peptides have been examined for their potential as anti-infective agents. AMP families such as the defensins and cathelicidins exhibit broad-spectrum antimicrobial activity against a wide variety of bacteria, fungi and viruses, predominantly by disruption of the microbial membrane. Due to this physical mechanism, development of resistance by the pathogen is rare. Thus, they represent a great potential for a new type of anti-infective agent. However, due to a variety of reasons, including protease sensitivity and poor bioavailability, they have not been developed into actual therapeutics. To circumvent these issues, we have examined the potential for small molecule mimetics of AMPs, which would be protease resistant, and have better bioavailability. We previously demonstrated activity of one such class of mimetics, sequence-specific *N*-substituted glycine oligomers, or peptoids, against the human viral pathogen Herpes Simplex Virus-1 (HSV-1), as well as some bacteria. Here we compare the activity, both in vitro and in vivo, of select peptoids against bacteria, fungi and viruses, to begin to study the structure/activity relationship with a broad spectrum of microbial pathogens. Our results show that some peptoid structures are more active against one type of pathogen than another. However, at least two of the tested peptoids exhibit potent activity against Gram+ bacteria, Gram- bacteria, fungi and viruses. Our result suggest that these molecules can be developed into potent broad-spectrum antimicrobial agents.

Keywords: Antibiotics; antivirals; antifungals; peptide mimetics

Antimicrobial peptides

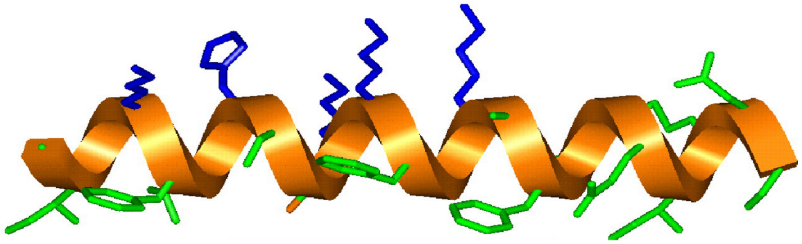
- Short, generally cationic, broad-spectrum antimicrobial proteins
- Found at mucosal surfaces
  - Skin secretions in fish and amphibians
  - Oral cavity, trachea, small intestine, female reproductive tract in mammals
- Found in myeloid cells
  - Neutrophils, alveolar macrophages

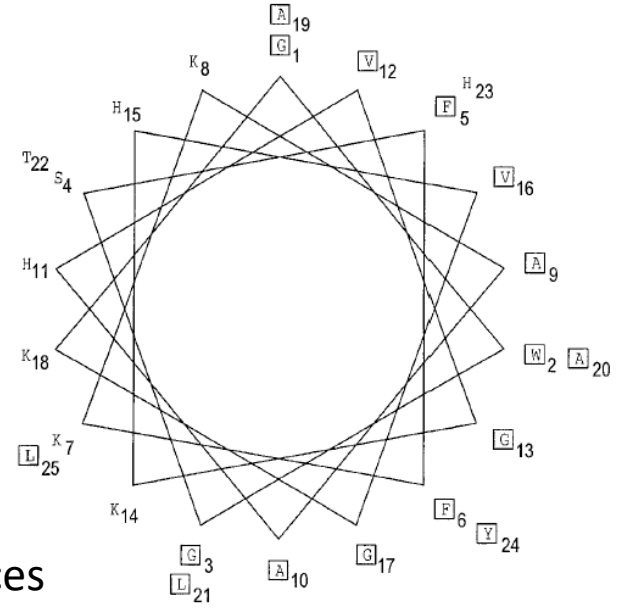
Types of AMPs

- Linear
  - Amphipathic α-helical
- Cysteine-rich
  - β-sheet
- Peptides with specific amino acids
  - Rich in His, Pro or Trp

Linear peptides

- Magainin (frog skin)
- Pleurocidin (fish skin)
- Cecropin (insect)
- Protegrin (pig leukocytes)
- LL-37 (human cells)

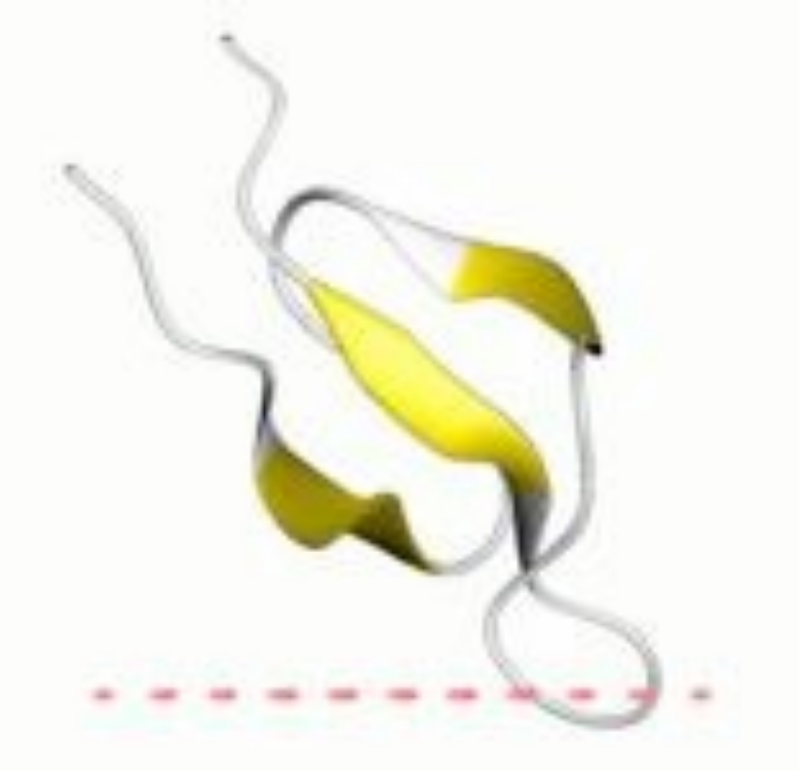




Form cationic amphipathic α-helices

Cysteine-rich peptides

- α-defensins
  - PMNs, small intestine
- β-defensins
  - Epithelial cells, some blood cells
- θ-defensins
  - Rhesus monkey PMNs



Natural Roles of AMPs

- Antimicrobial defense of surfaces
  - magainins on amphibian skin
  - β-defensins on mammalian epithelium
- Oxygen-independent antimicrobial activity of phagocytic cells
  - α-defensins in PMNs
- Chemotactic agents for innate immune defense cells
  - β-defensins, LL-37

Antimicrobial Peptides as Therapeutics

GOOD

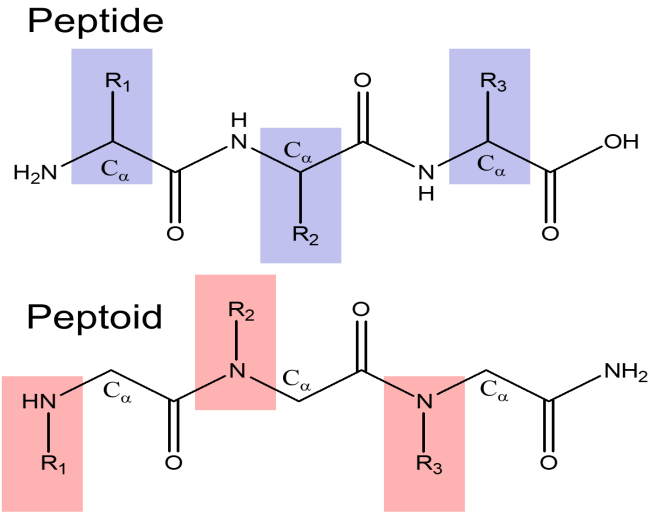
- Naturally occurring
- Broad-spectrum antimicrobials
- Little resistance
- Low antigenicity

BAD

- Protease sensitive
- Expensive to produce and purify
- Often are inactivated by other proteins

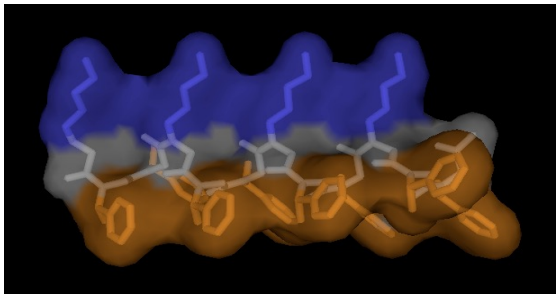
Design novel antimicrobial molecules based on AMPs

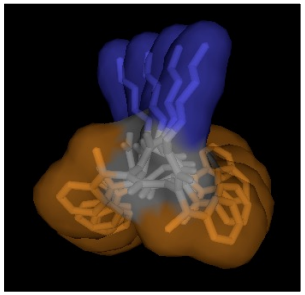
- Peptoids: *N*-substituted glycine polymers
- Resistant to proteases
- Inexpensive to synthesize
- Peptoid helices: α-chiral side chains stabilize helical structures , ~ 3 residues per turn, a helical pitch of 6 - 6.7 Å^2

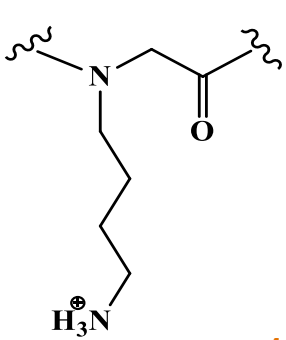


Synthetic, non-natural peptoid mimics of natural AMPs:
Short, sequence-specific, helical oligo-N-substituted glycines

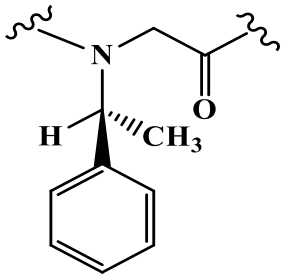
Peptoid **1**: H–(*N*Lys–*N*spe–*N*spe)$_4$–NH$_2$







***N*Lys**

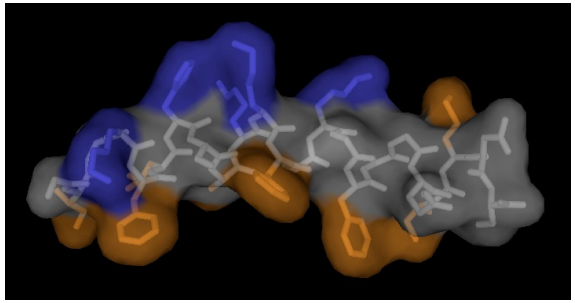


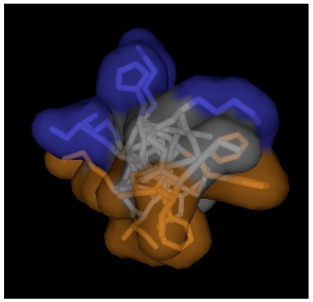
***N*spe**
(phenylethylgycine)

cationic

hydrophobic

Magainin-2: GIGKFLHSAKKFGKAFVGEIMNS–NH$_2$





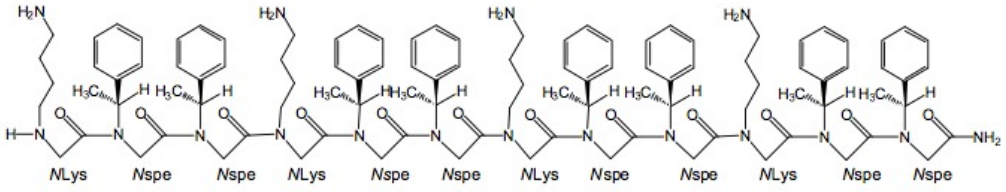
(Pexiganan)

Chongsiriwatana, N.P. et al. *PNAS*, **2008,** 105: 2794-2799.

Designing an antimicrobial peptoid based on LL-37

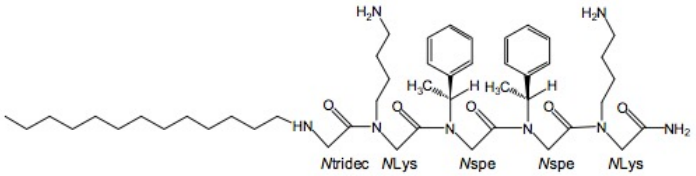
Peptoid 1
(MW 1819)

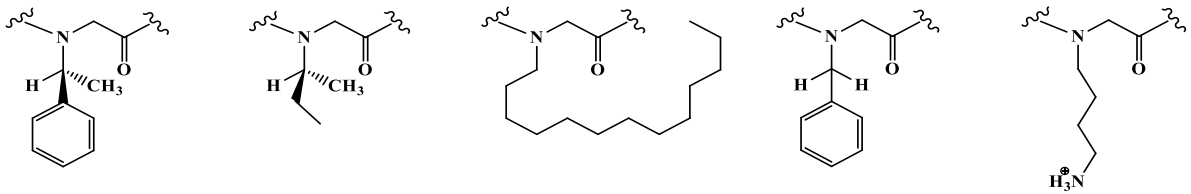
(MXB1)



Peptoid 1-C13$_{4mer}$
(MW 835)

(MXB5)



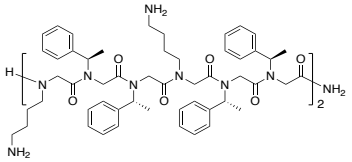


***N*spe** ***N*ssb** ***N*tridec** ***N*pm** ***N*Lys**

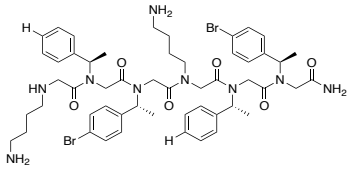
Chongsiriwatana, N.P. et al. **2011**, *Antimicrob. Agents Chemother.* 55: 417-420.

Peptoids tested

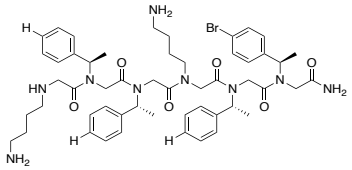
MXB-1



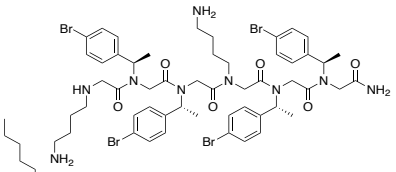
MXB-2



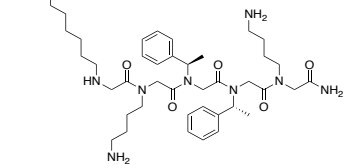
MXB-3



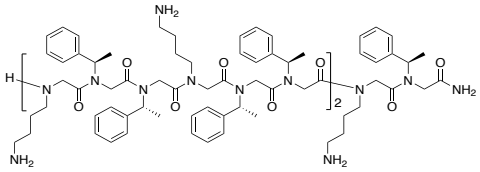
MXB-4



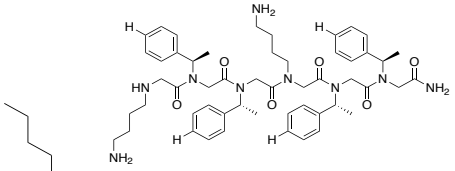
MXB-5



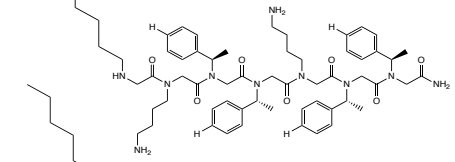
MXB-6



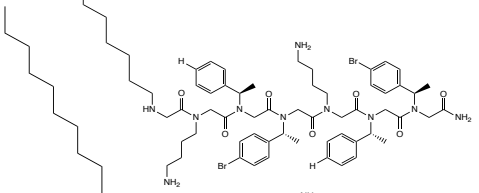
MXB-7



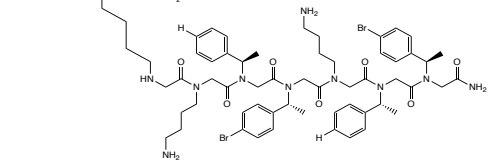
MXB-8



MXB-9



MXB-10



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Results and discussion

Activity of peptoids against bacteria

Minimal Inhibitory Concentrations (µg/ml)			
	P. gingivalis	*T. forsythia*	*S. sputigena*
MXB1	2	8	1
MXB2	2	4	
MXB3	16 z		16
MXB4	2		>64
MXB5	2	8-16	>64
MXB6	2		16
MXB7	64		0.25
MXB8	2	4	>64
MXB9	8-16	64	4
MXB10	16		>64

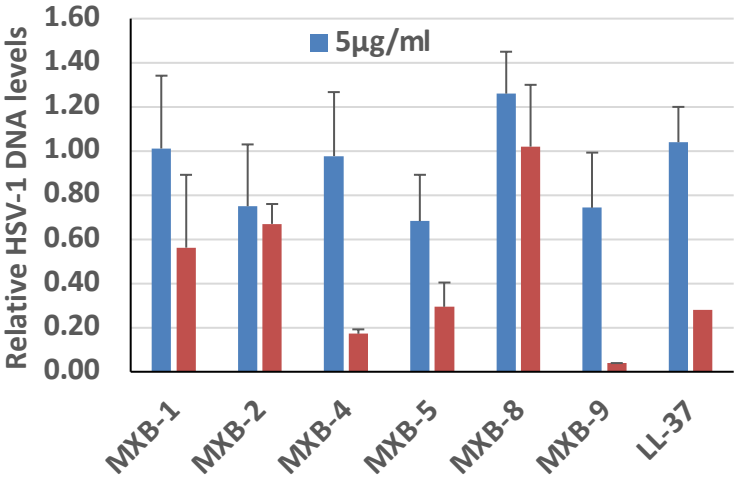
	Minimal Bactericidal Concentrations (µg/ml)		
	P. gingivalis	*T. forsythia*	*S. sputigena*
MXB1	4	32	1
MXB2	4	16	
MXB3	32		32
MXB4	2		>64
MXB5	2-4	32	>64
MXB6	4		32
MXB7	64		0.5
MXB8	4	16	>64
MXB9	8-16	>64	8
MXB10	32		64

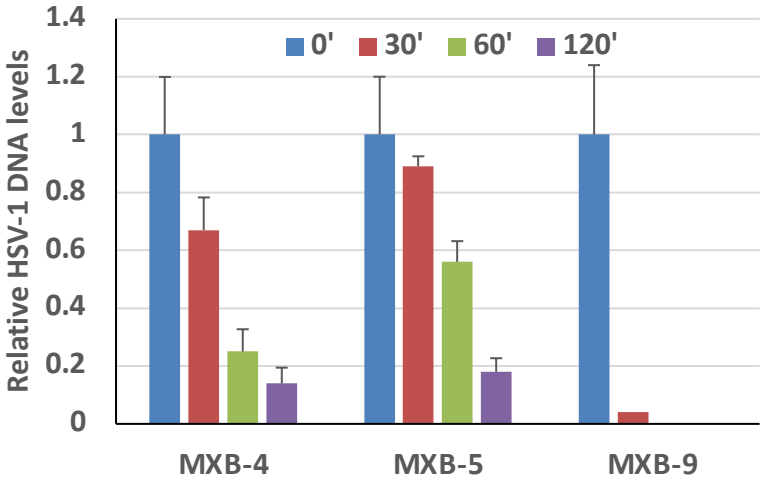
Activity of peptoids against fungi

	C. AURIS STRAIN 381		C. AURIS STRAIN 382	
PEPTOID	MIC	MFC	MIC	MFC
MXB-01	> 64	> 64	> 64	> 64
MXB-02	> 64	> 64	> 64	> 64
MXB-03	> 64	> 64	> 64	> 64
MXB-04	8	64	16	> 64
MXB-05	8	32	8	64
MXB-06	> 64	> 64	> 64	> 64
MXB-07	> 64	> 64	> 64	> 64
MXB-08	16	64	16	64
MXB-09	> 64	64	> 64	> 64
MXB-10	64	> 64	32	> 64

	MUCOR CIRCINELLOIDES	
PEPTOID	MIC	MFC
MXB-01	16	32
MXB-02	16	32
MXB-03		
MXB-04	8	8
MXB-05	4	4
MXB-06		
MXB-07		
MXB-08	8	8
MXB-09	32	32
MXB-10	2	2

Activity of peptoids against viruses- HSV-1





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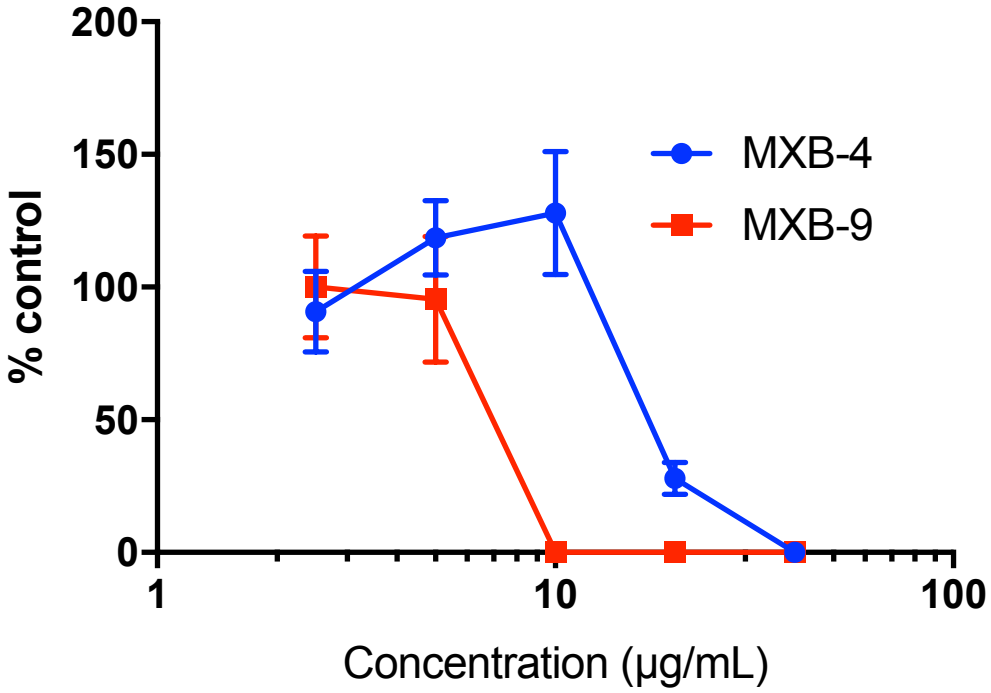
Activity of peptoids against viruses- SARS-CoV-2



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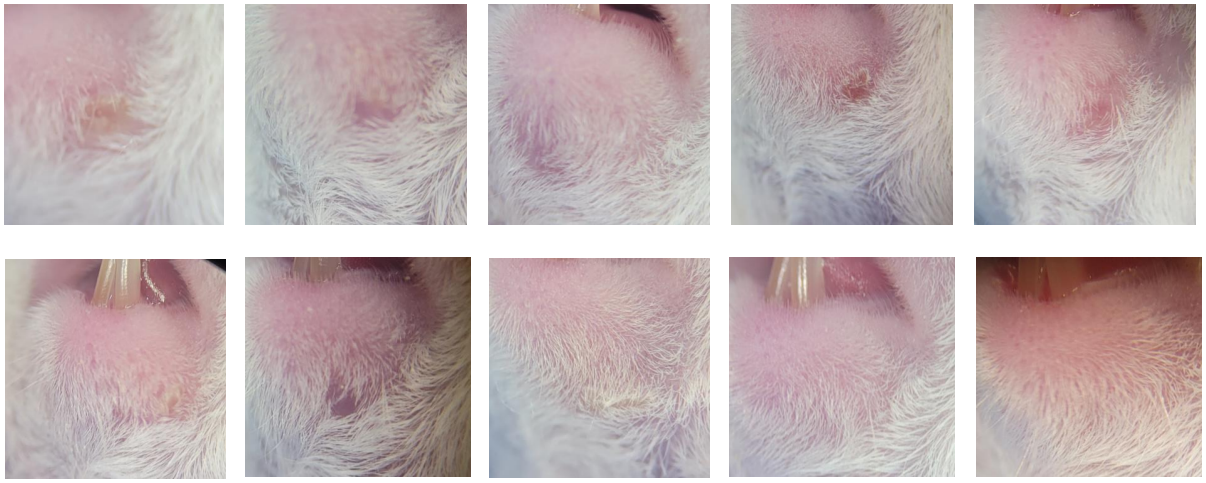
In vivo activity of peptoids against HSV-1

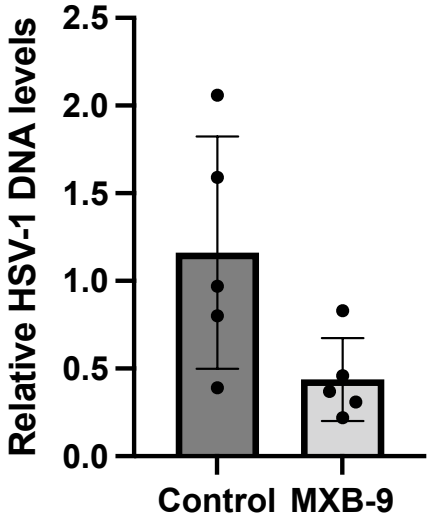
Daily Topical Treatment of 50µl Peptoid in CM Cellulose at Day 2 After Infection, Harvested at Day 5 Ameliorates the Lesion and Decreases Viral DNA in the Trigeminal Nerve

Lip

HSV alone

MXB-9 50 µg/ml

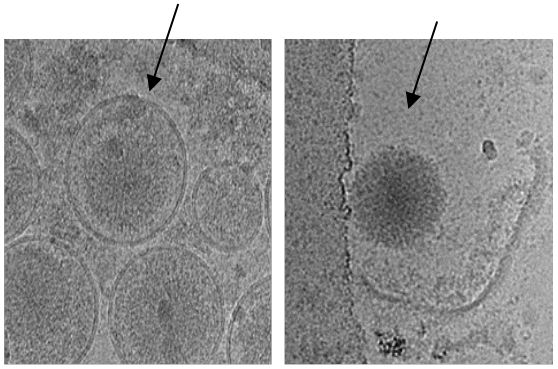




Trigeminal nerve

Mechanism of action

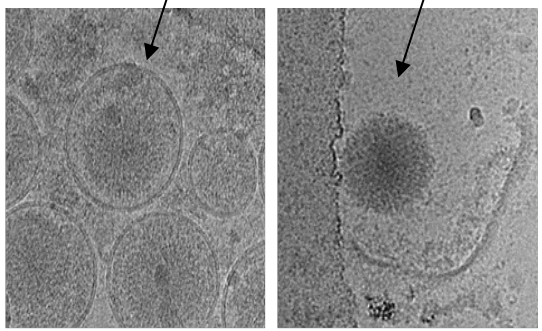
- AMPs disrupt microbial membranes



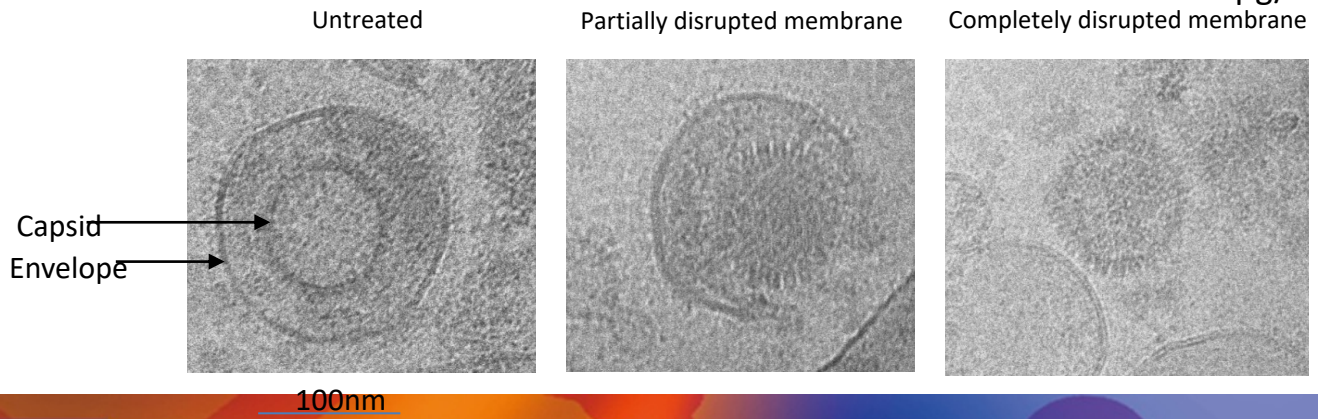
Control 20µg/ml LL-37

Mechanism of action

- AMPs disrupt microbial membranes
- Peptoids appear to do the same

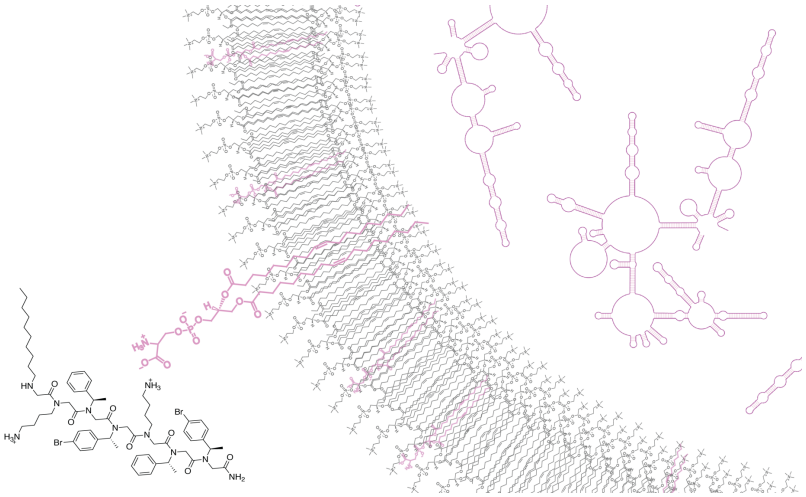


Control 20µg/ml LL-37



Mechanism of action

- AMPs disrupt microbial membranes
- Peptoids appear to do the same
- Key factor is an initial electrostatic interaction with negatively charged membranes



Comparison of activity

	Porphyromonas gingivalis	*Selenomonas sputigena*	*Candida albicans*	*Candida auris*	HSV-1	SARS-CoV-2
MXB1						
MXB2						
MXB3						
MXB4						
MXB5						
MXB6						
MXB7						
MXB8						
MXB9						
MXB10						

Summary

- Peptoids exhibit potent antimicrobial activity
- Activity is broad-spectrum across kingdoms of microbes
- Different peptoids are active against different microbes
- Some structures appear more broad-spectrum than others
- Activity appears to be against microbial membranes
  - Preliminary data suggest no development of resistance

Conclusions

- Antimicrobial peptoids can be developed as novel antimicrobial agents to treat a variety of infections
- Especially important in viral infections, where secondary bacterial and/or fungal infections occur

Acknowledgments

U*of*L

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