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Potent antiviral activity against HSV-1 and SARS-CoV-2 by antimicrobial peptoids

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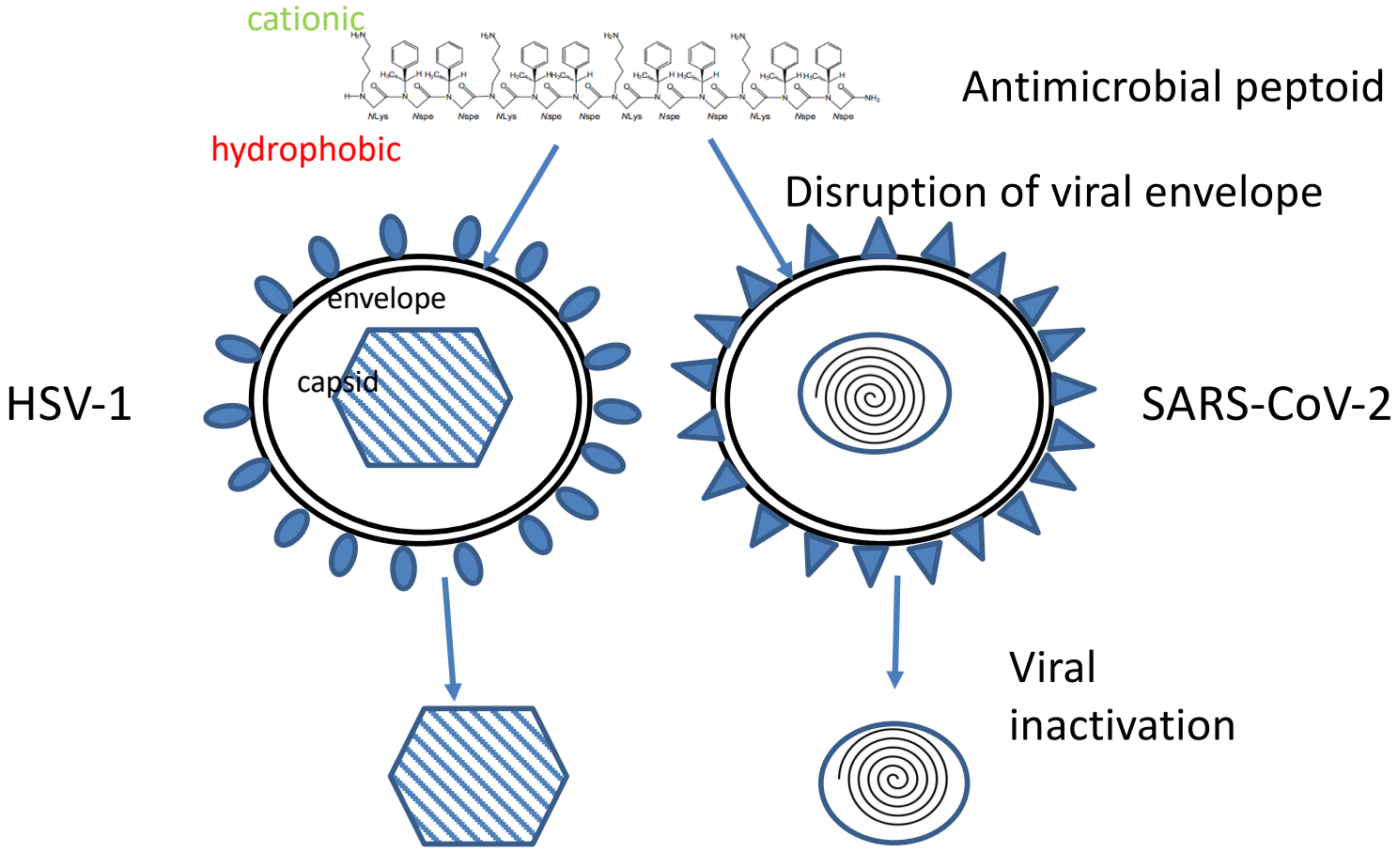
Potent antiviral activity against HSV-1 and SARS-CoV-2 by antimicrobial peptoids



Abstract: Most therapeutic strategies for the development of antiviral agents are designed based on targets specific to each virus. We examined the potential broad-spectrum activity of antimicrobial peptoids (AMPs) against two different viruses. AMPs are mimetics of antimicrobial peptides, which are sequence-specific *N*-substituted glycine oligomers, where their side chains are appended to the backbone amide nitrogens rather than α-carbons. As a result, peptoids are not proteolyzed, and have improved biostability and bioavailability and reduced immunogenicity relative to natural peptides. AMPs exhibit potent in vitro activity against a wide variety of bacteria and fungi *via* disruption of microbial membranes as well intracellular binding to nucleic acids. Thus, we hypothesized that they also exhibit activity against enveloped viruses. HSV-1 is an enveloped DNA virus that causes topical lesions. Incubation of HSV-1 with a panel of AMPs demonstrates variable inactivation of the virus prior to infection of cultured epithelial cells. Peptoids with the best activity exhibited dose- and time-dependent inactivation of HSV-1. Lead compounds inactivate the virus within 30 minutes at μg/ml concentrations. Transmission electron microscopy shows that these compounds remove the viral envelope, similar to what is seen with detergent treatment. We also tested the compound against SARS-CoV-2, an enveloped RNA virus that causes COVID-19. Our results show a dose-dependent inactivation of this virus prior to infection of target cells. Cytotoxicity assays show little toxic effects when applied to the apical surface of well-differentiated air-liquid interface cultures of airway epithelial cells. These results indicate that AMPs are strong candidates for broad-spectrum antiviral agents.

Keywords: 3 to 5 keywords separated by semi colons

Introduction

- Traditional therapeutic strategies to treat pathogenic viruses involve designing drugs to target specific viral enzymes
  - Acyclovir for Herpes Simplex Virus (HSV)-1
    - Inactivates HSV-specific DNA polymerases
  - Remdesivir for Ebola (and SARS-CoV-2)
    - Inactivates RNA-dependent RNA polymerase
- Problems with this approach
  - Resistance
  - Different drugs for each virus

Introduction

- New approach for antiviral drug development
- Design a drug that targets the overall structure
  - No resistance development
  - Can target multiple viruses

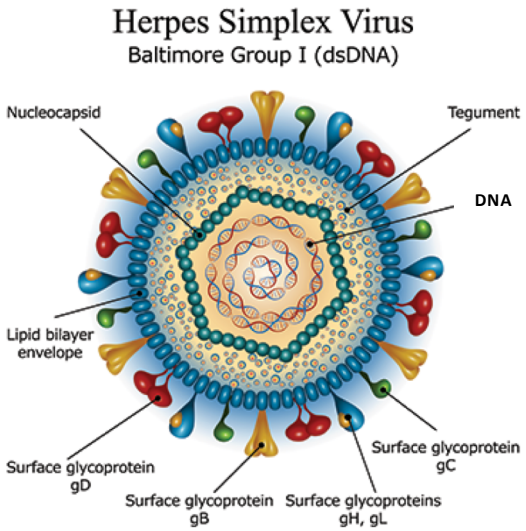
Introduction

- Viral structures:
  - Enveloped vs. non-enveloped
  - RNA vs. DNA

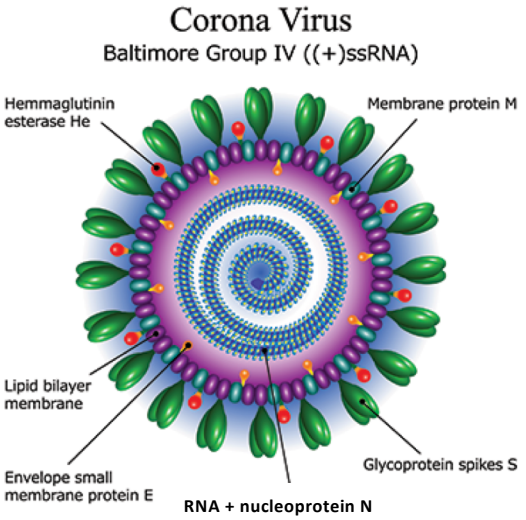
- Goal: Design an antiviral drug based on antimicrobial peptides to target the viral envelope

Introduction

- HSV-1
  - Enveloped DNA virus



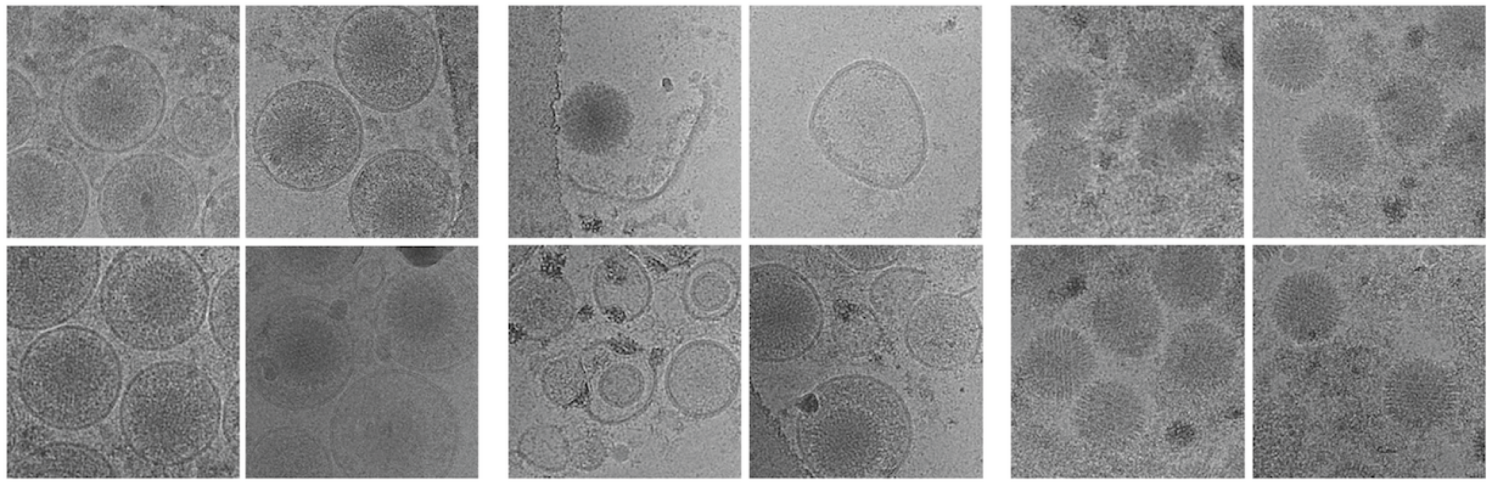
- SARS-CoV-2
  - Enveloped RNA virus



Introduction

- Antimicrobial peptides
- Broad-spectrum antimicrobial agents
- Cationic, amphipathic peptides
- Important components of innate immunity
- Mechanism of action is through membrane disruption
- Defensins, cathelicidins, protegrins, magainins

Human cathelicidin LL-37 inactivates KSVH by disrupting the viral envelope



Brice et al., Antiviral Res., 158:25, 2018

Introduction

Can we use antimicrobial peptides as antiviral therapeutics?

GOOD

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

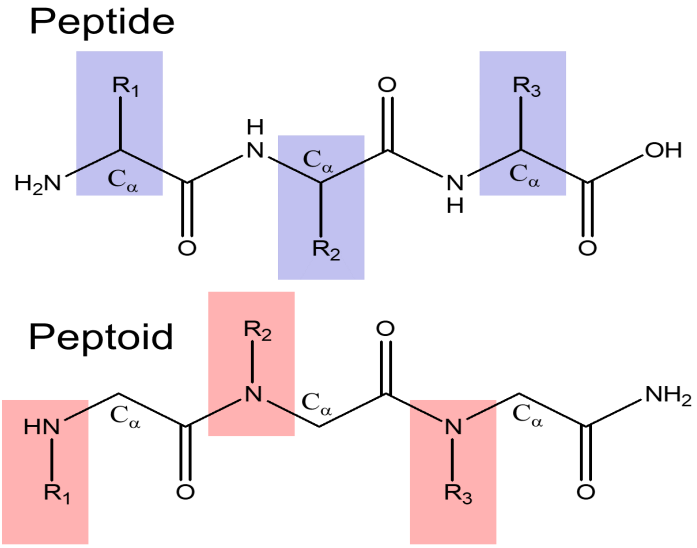
BAD

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

Introduction

Design novel antiviral molecules based on antimicrobial peptides

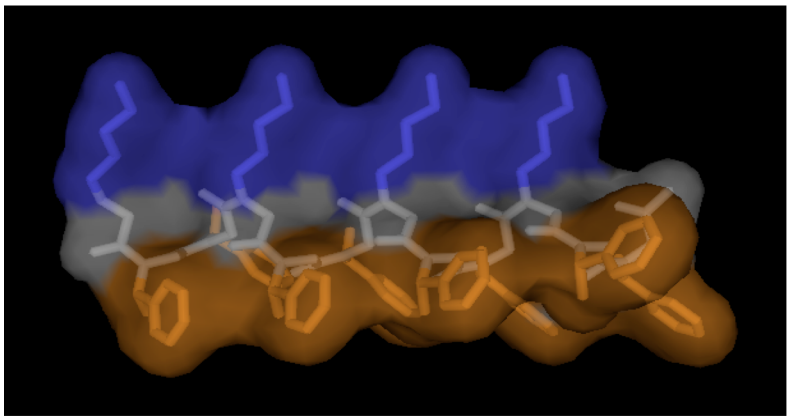
- β-peptoids: *N*-substituted glycine polymers
- Resistant to proteases
- Inexpensive to synthesize
- Peptoid helices: α-chiral side chains stabilize helical structures , ~ 3 residues per turn, a helical pitch of 6 - 6.7 Å^2

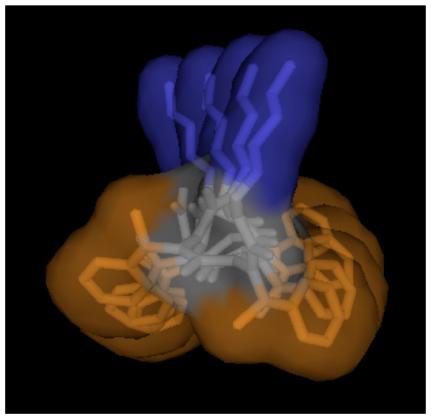


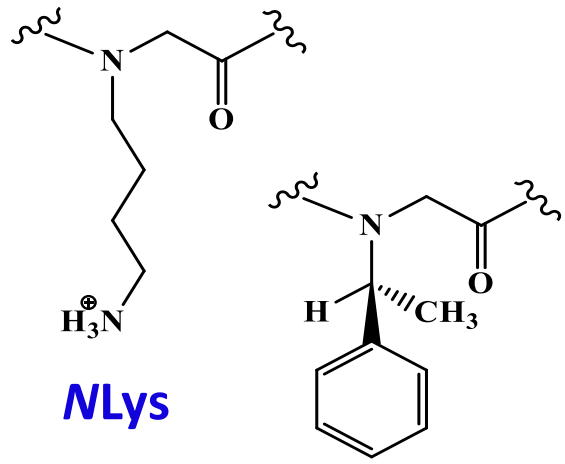
Synthetic, non-natural peptoid mimics of natural AMPs:

Short, sequence-specific, helical oligo-N-substituted glycines

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





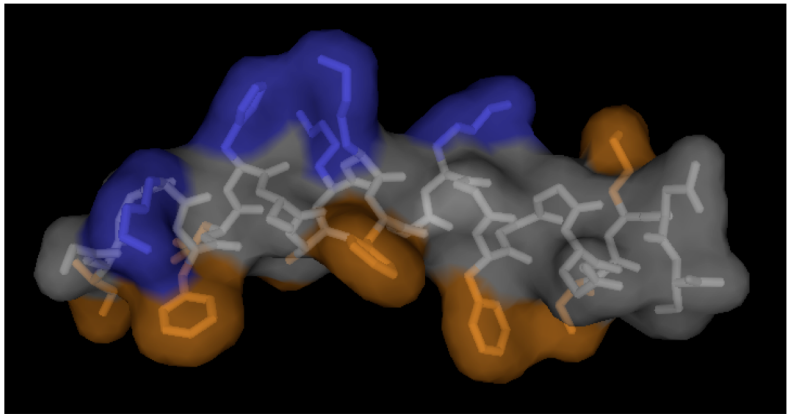


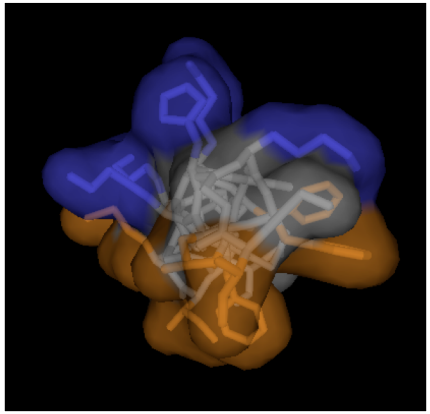
***N*Lys**

***N*spe**

(phenylethylgycine)

Magainin-2: GIGKFLHSAKKFGKAFVGEIMNS–NH$_2$



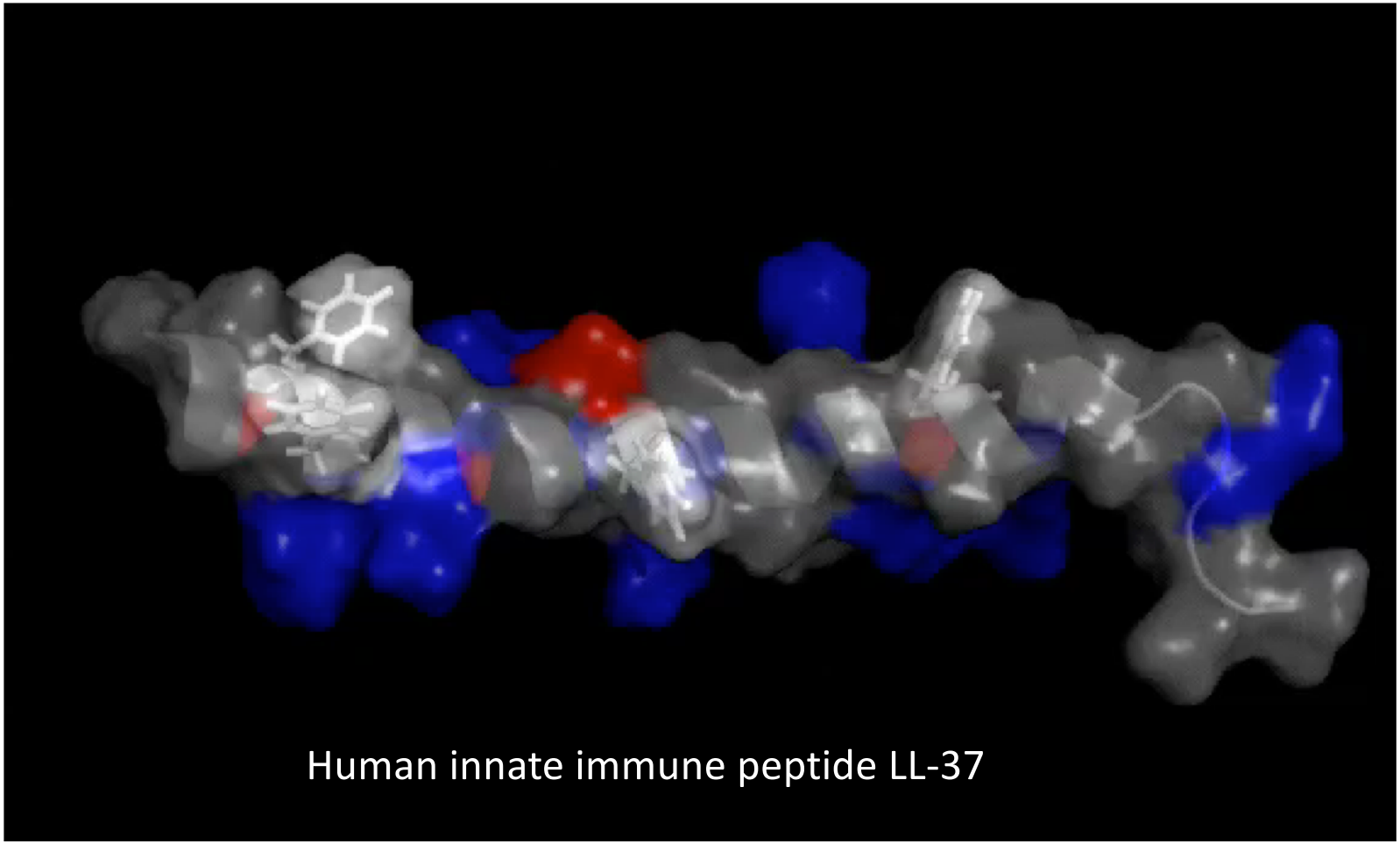


cationic

hydrophobic

(Pexiganan)

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



Human innate immune peptide LL-37

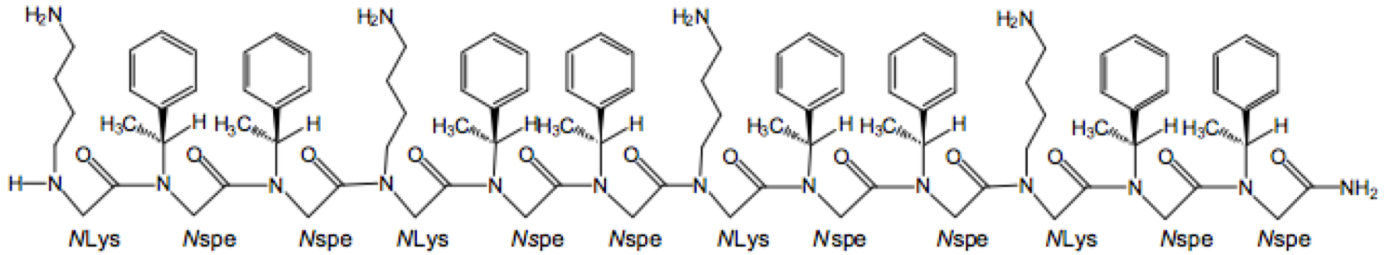
LL-37 is the single human ***cathelicidin*** innate immune peptide, and exhibits potent antiviral activity against enveloped viruses

G. Wang, *J. Biol. Chem.* (2008) Vol. 283, No. 47, pp. 32631-32643.

Designing an antimicrobial peptoid based on LL-37

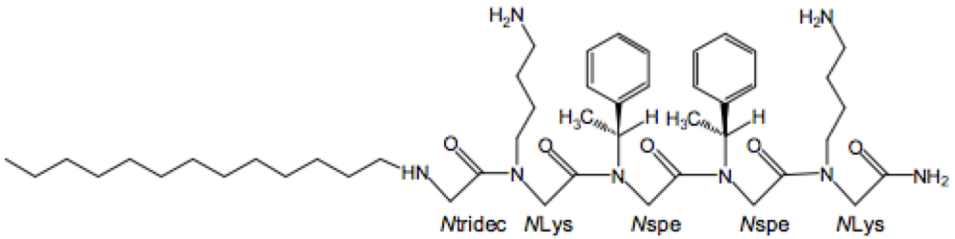
Peptoid 1
(MW 1819)

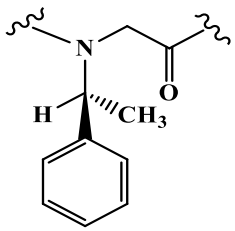
(MXB1)



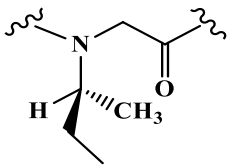
Peptoid 1-C13$_{4mer}$
(MW 835)

(MXB5)

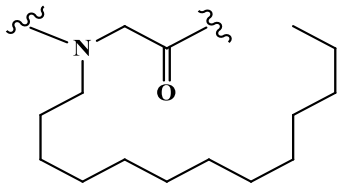




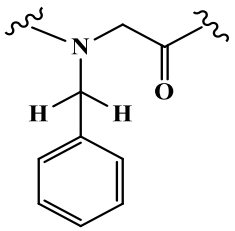
***N*spe**



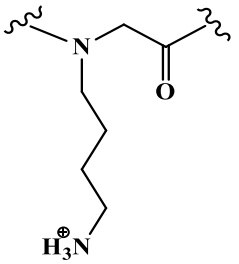
***N*ssb**



***N*tridec**



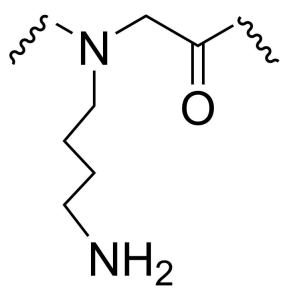
***N*pm**



***N*Lys**

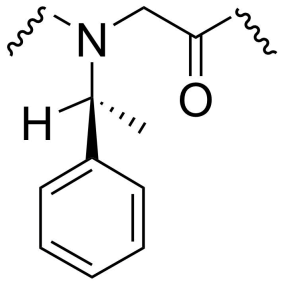
Chongsiriwatana, N.P. et al. **2011**, *Antimicrob. Agents Chemother.* 55: 417-420.

Variants with *N*-terminal alkylation, *para*-bromination of *N*spe



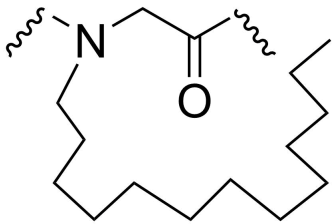
***N*Lys**

l-(4-aminobutyl)glycine



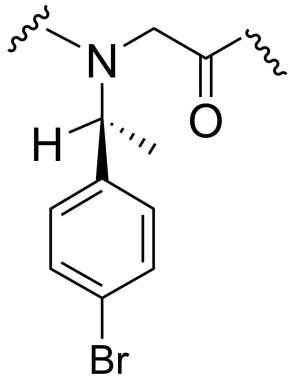
***N*spe**

N-(S)-(1-phenylethyl)glycine



***N*tridec**

N-(tridecyl)glycine



***para-bromo N*spe**

(S)-(-)-1-(4-bromophenylethyll)glycine

Håvard Jenssen
LBNL Molecular Foundry

Variants with *N*-terminal alkylation or *para*-bromination of *N*spe

Peptoid Sequences		
MXB1 (Peptoid 1)	H-(*N*Lys-*N*spe-*N*spe)$_4$-NH$_2$	12mer
MXB2	H-(*N*Lys-*N*spe-*N*spe(p-Br))$_2$-NH$_2$	6mer
MXB3	H-*N*Lys-*N*spe-*N*spe-*N*Lys-*N*spe-*N*spe(p-Br)-NH$_2$	6mer
MXB4	H-(*N*Lys-*N*spe(p-Br)-*N*spe(p-Br))$_2$-NH$_2$	6mer
MXB5 (C13-4mer)	H-*N*tridec-*N*Lys-*N*spe-*N*spe-*N*Lys-NH$_2$	5mer
MXB6 (1-11mer)	H-(*N*Lys-*N*spe-*N*spe)$_3$-*N*Lys-*N*spe-NH$_2$	11mer
MXB7	H-(*N*Lys-*N*spe-*N*spe)$_2$-NH$_2$ (neg. control)	6mer
MXB8	H-*N*dec-(*N*Lys-*N*spe-*N*spe)$_2$-NH$_2$	7mer
MXB9	H-*N*dec-(*N*Lys-*N*spe-*N*spe(p-Br))$_2$-NH$_2$	7mer
MXB10	H-*N*tridec-(*N*Lys-*N*spe-*N*spe(p-Br))$_2$-NH$_2$	7mer

Molchanova, N. et al., 2020 Sci. Rep. 10:14805

Results and discussion

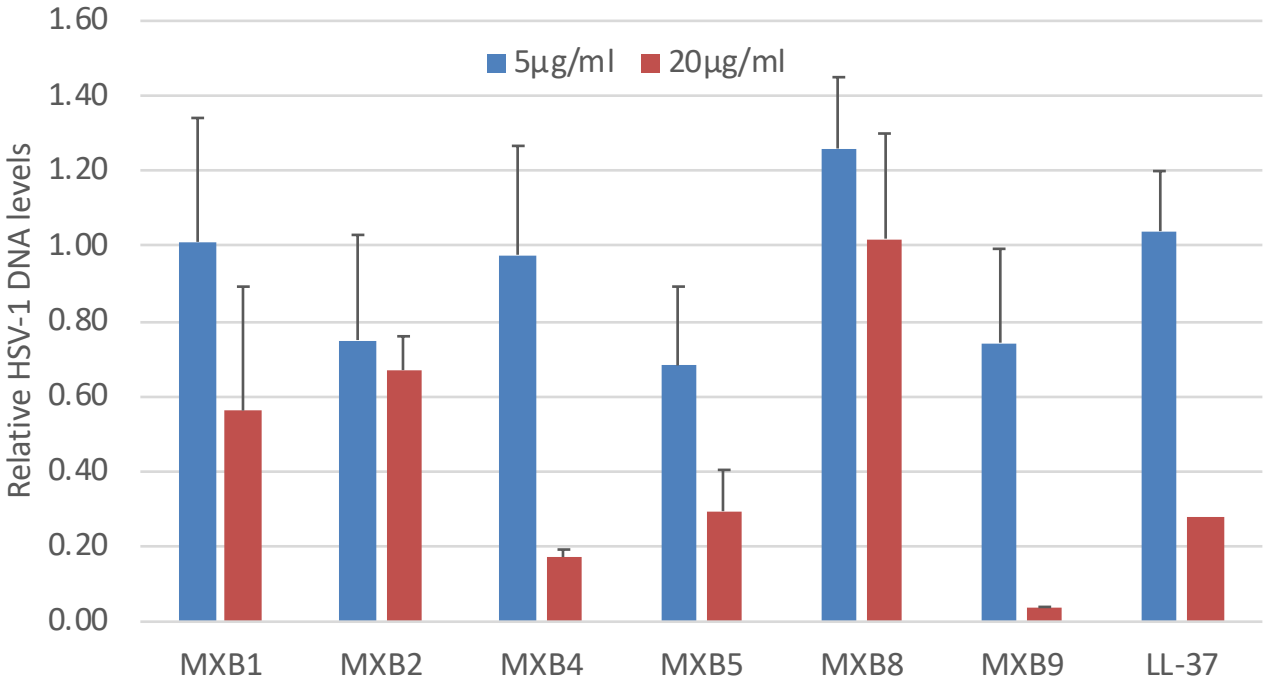
Quantify antiviral activity of antimicrobial peptoids

Assay:

- Incubate HSV-1 in the presence of peptoid
- Infect oral keratinocyte cell line OKF6/TERT-1
- Quantify HSV-1 genomic DNA after 24 hours

Results and discussion

Antimicrobial peptoids exhibit potent antiviral activity against HSV-1



Results and discussion

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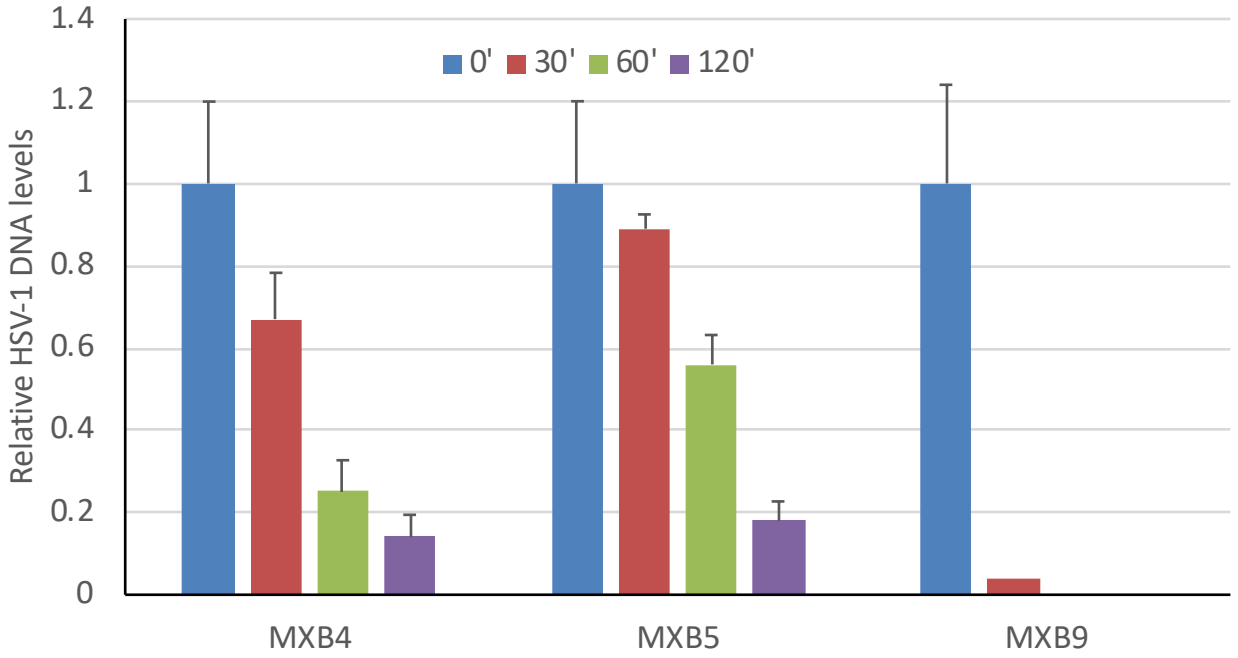
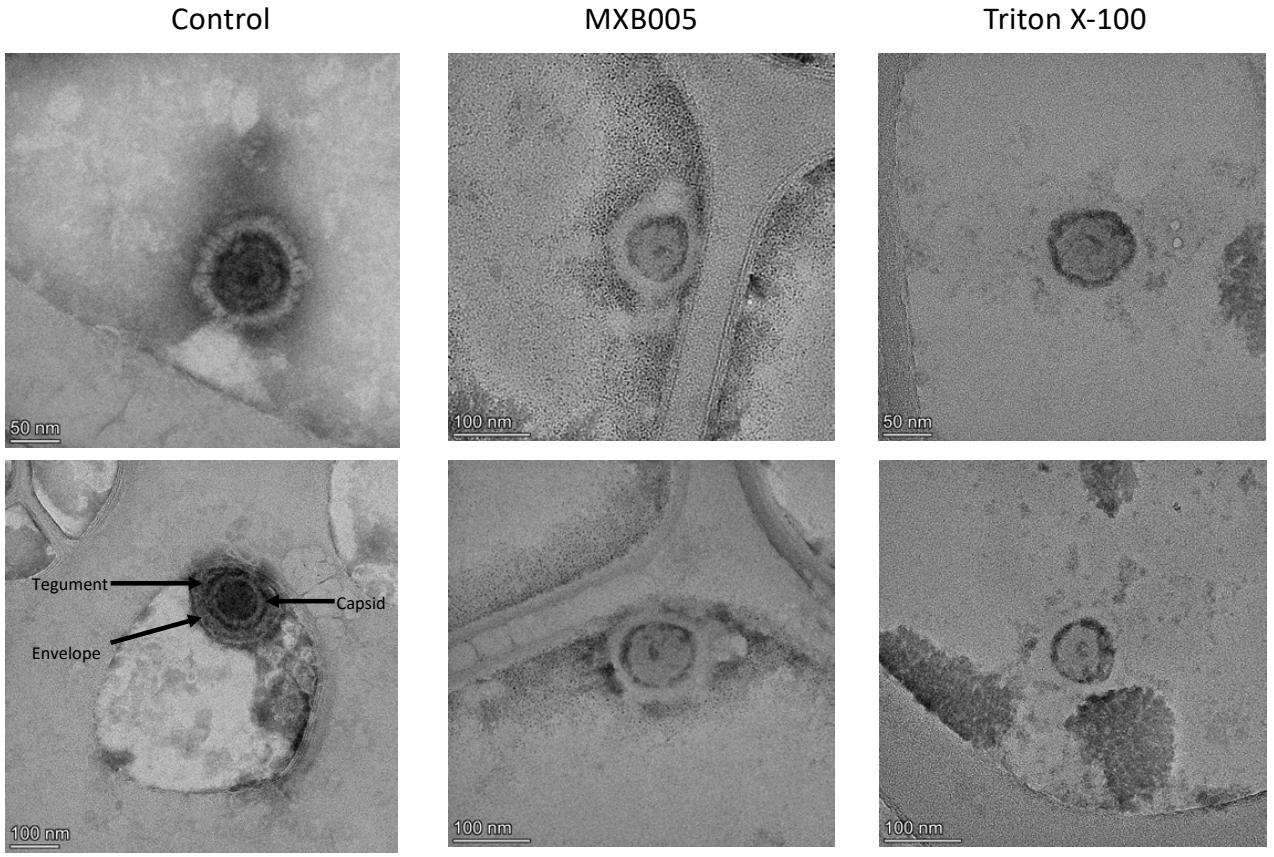


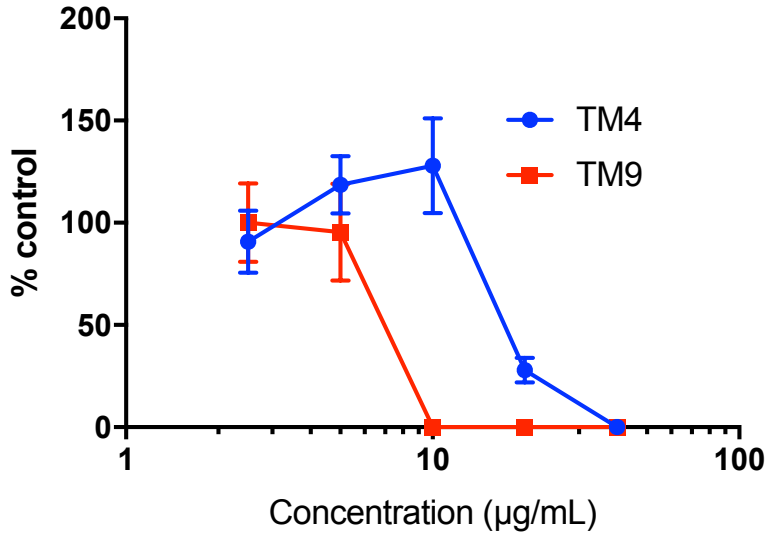
Results and discussion

Antimicrobial peptoids disrupt the HSV-1 viral envelope



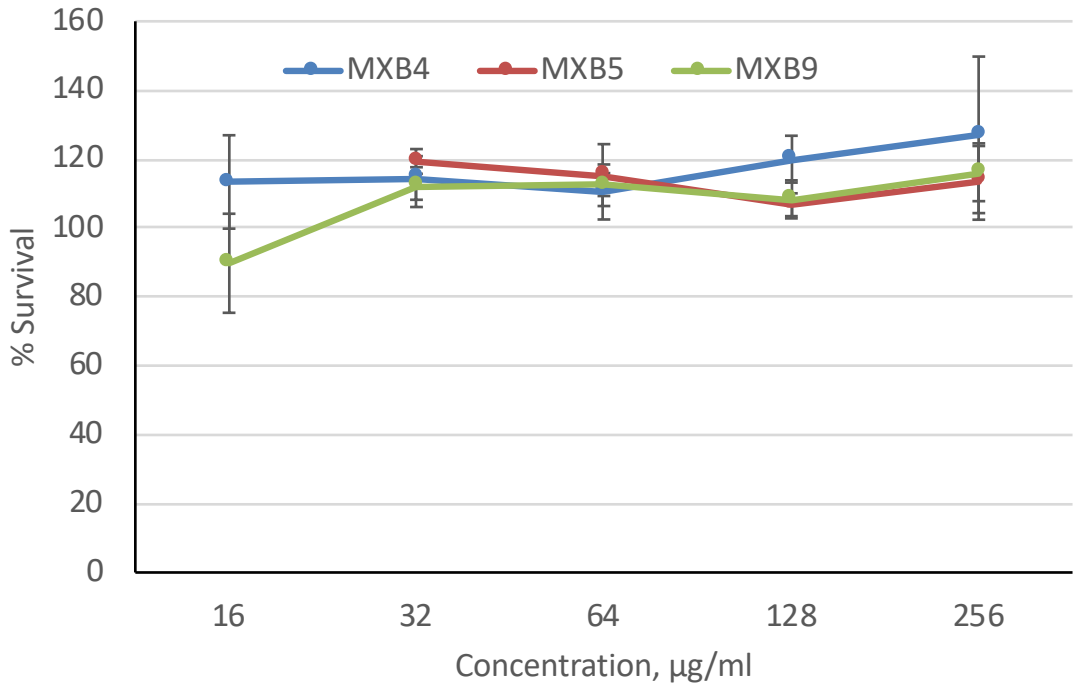
Results and discussion

Antimicrobial peptoids inactivate SARS-CoV-2



Results and discussion

Antimicrobial peptoids are not cytotoxic



Conclusions

- Antimicrobial peptoids exhibit potent *in vitro* activity against two different types of enveloped viruses
- No *in vitro* cytotoxicity is observed in differentiated cell cultures
- As with antimicrobial peptides, the activity appears to be through disruption of the viral envelope
- Thus, these peptoids can be developed as broad-spectrum antiviral agents, to treat viral infections including COVID-19

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