In vitro testing *of in silico* designed Cationic Antimicrobial Peptides

Natalie Kuljanishvili
Animal Molecular Biology Institute of Agricultural University

Supervisor: Giorgi Zaalishvili, PhD

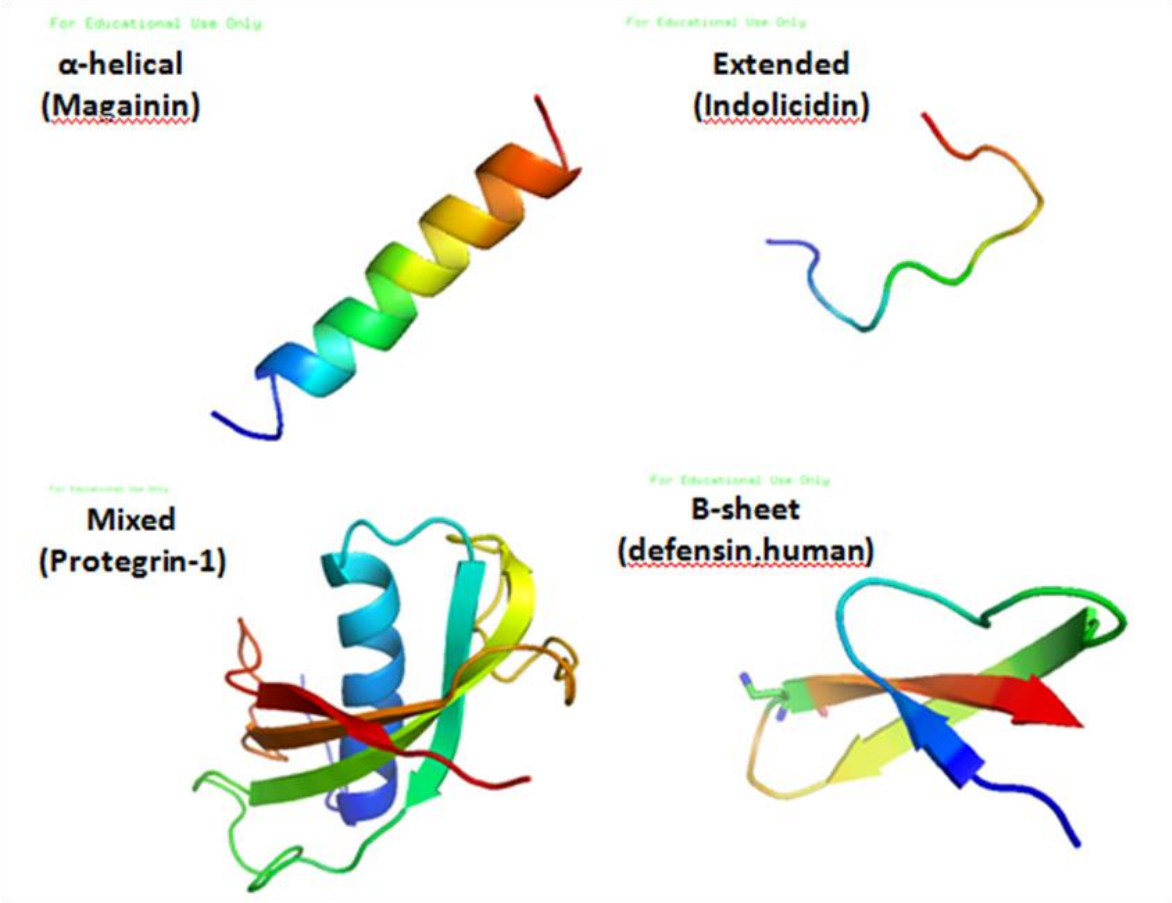
8TH GEORGIAN – GERMAN SCHOOL AND WORKSHOP IN BASIC SCIENCE, August 20 – 24, 2018.

Why antimicrobial peptides?

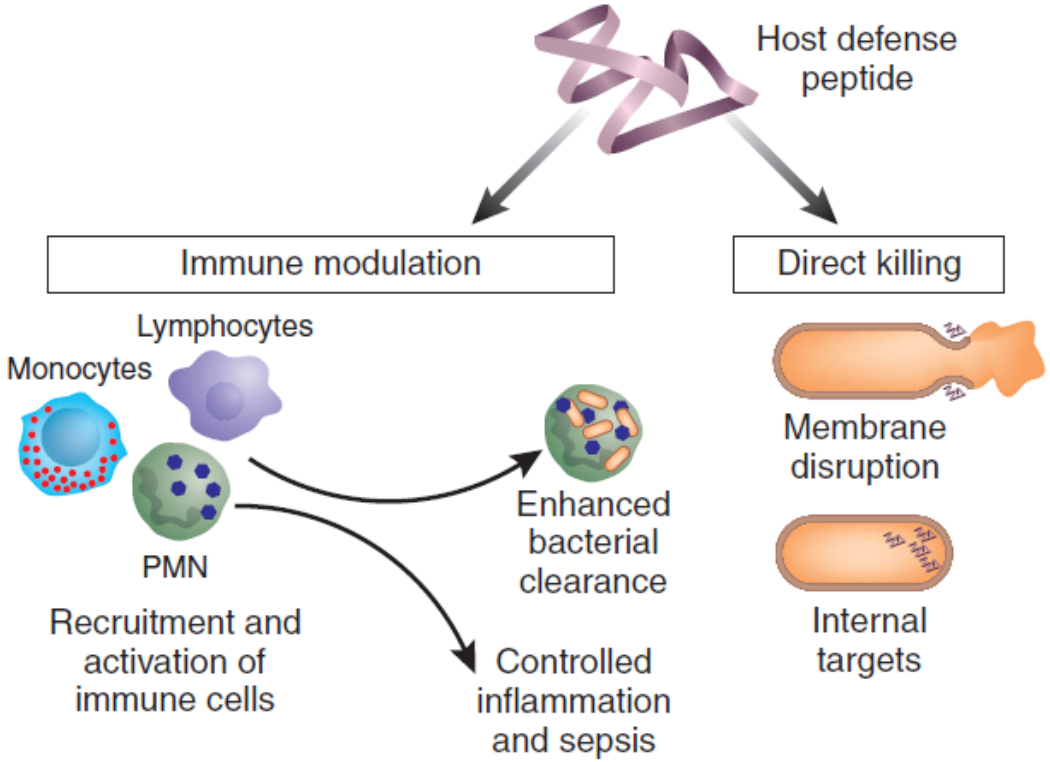
- Presented in prokaryotes, archaea and eukaryotes
- Broad spectrum of targeted organisms
- Mainly targets polyanionic and/or hydrophobic molecules
- Broad spectrum of antimicrobial characteristics, similar physical properties and evolutional success

Natural cationic antimicrobial peptides share similar physical properties:

- Small size (12-50 amino acids long)
- Cationicity (overall charge +2 to +9)
- Hydrophobicity
- Amphipathic design



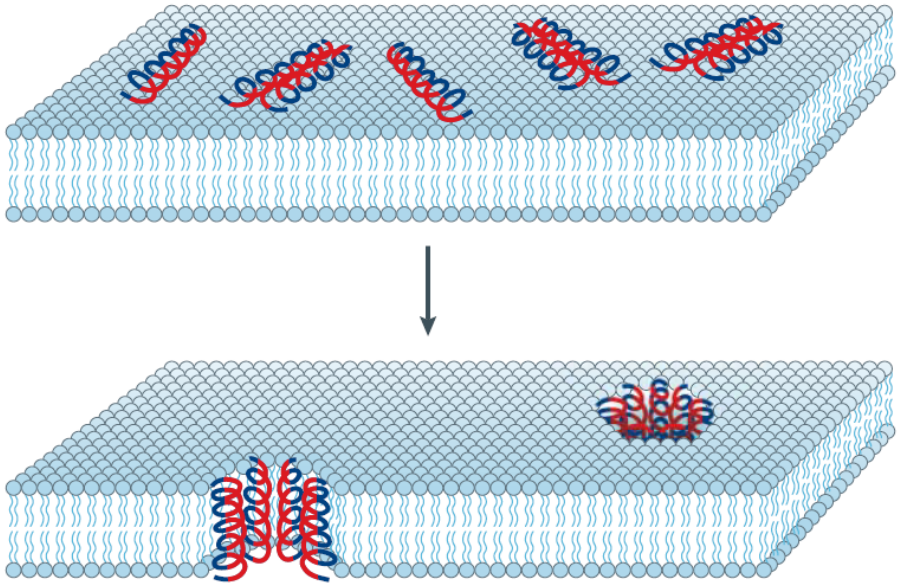
Mechanisms of actions of Cationic Antimicrobial Peptides (CAMPs)



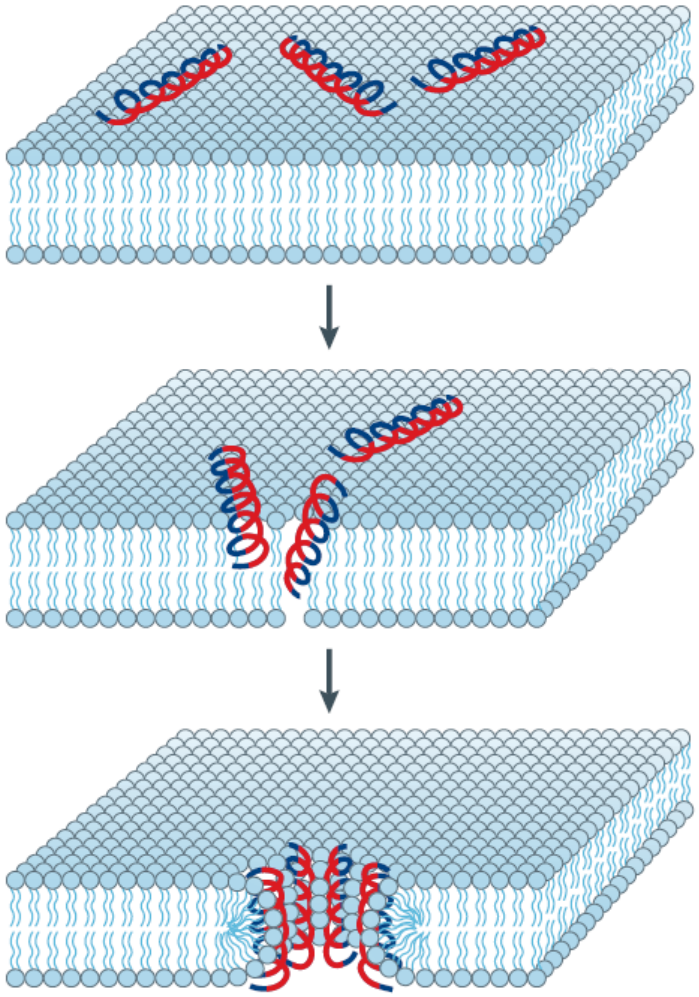
Directly acting CAMPs can be divided as:

- Membrane active
- Intracellularly active

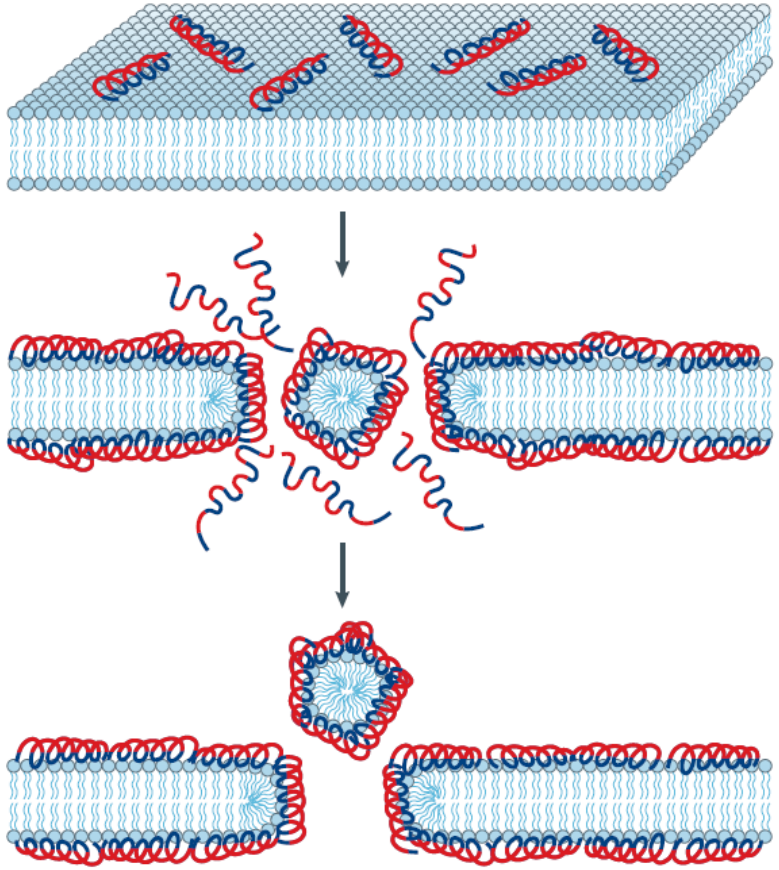
Membrane active Cationic Antimicrobial Peptides



Barrel-stave model, Brogden *et al*



Toroidal pore model, Brogden *et al.*

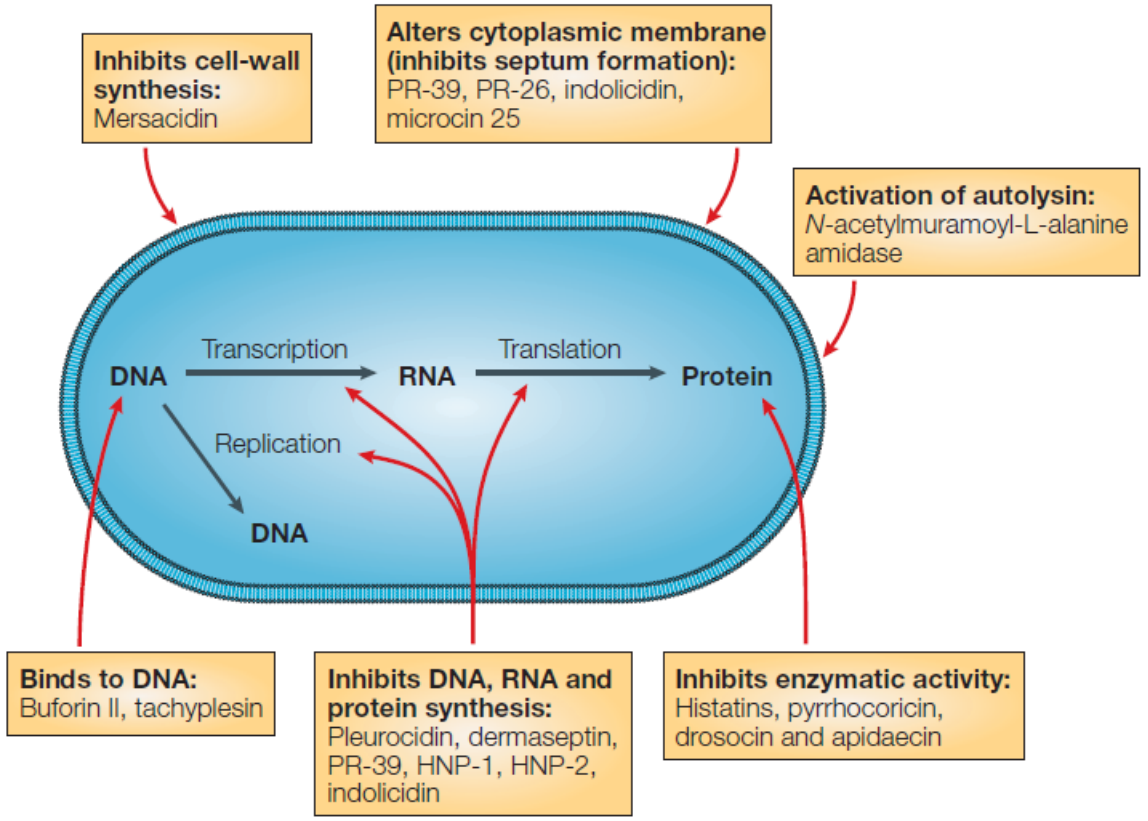


Carpet model, Brogden *et al*

Cationic Antimicrobial Peptides with intracellular targets

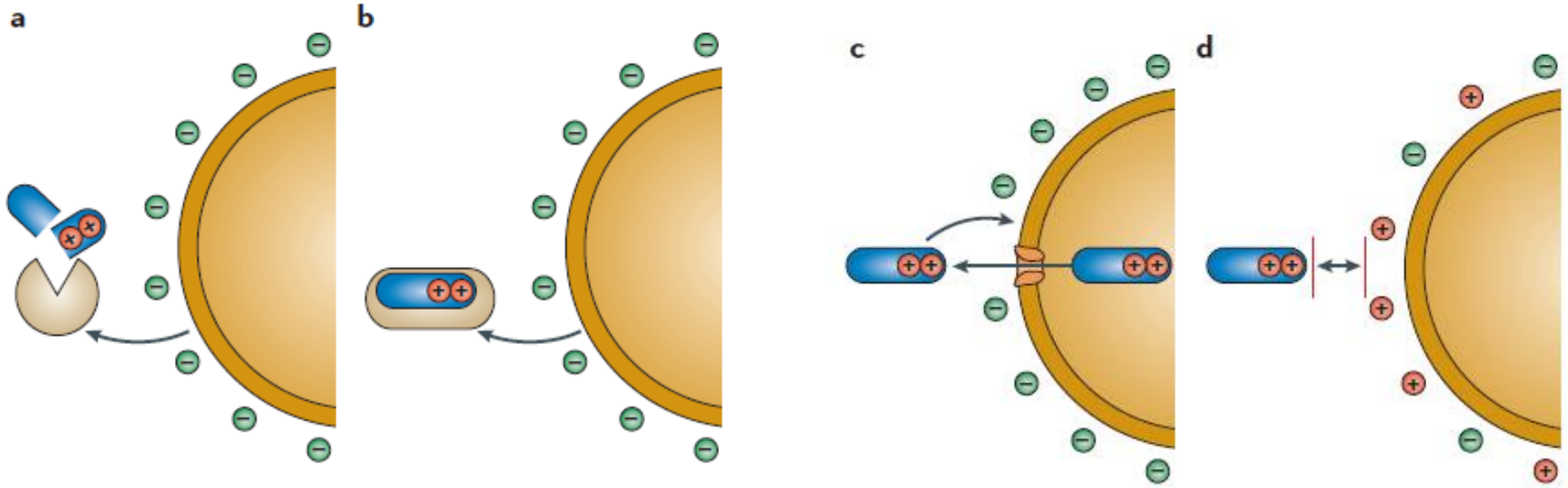
Cationic AMPs that act on:

- Nucleic acids
- Protein synthesis
- Translation and protein folding



Brogden *et al.*

Resistance against Cationic Antimicrobial Peptides



a. Degradation of CAMPs by bacterial proteases
b. Proteins secreted from bacteria prevent CAMPs from reaching bacterial cell membrane
c. Some CAMPs can be actively extruded from bacterial cells
d. Bacteria change net anionic charge of their envelope

Andreas Peschel *et al*

During the project - 27 synthetic Cationic Antimicrobial Peptide

20 CAMPs designed against gram-negative bacteria:

LCAPL1-LCAPL19, LCAPLD

- 13 amino acid
- C-terminal amidation
- One peptide containing D-amino acids

7 CAMPs designed against gram-positive bacteria:

LifeTein-AMP-1S - 8S

- 13 amino acid
- C-terminal amidation
- One peptide containing D-amino acids

Linear CAMPs were designed in Laboratory of Bioinformatics; I. Beritashvili Center of Experimental Biomedicine, by using semi-supervised machine-learning approach with a density-based clustering algorithm. The suggested algorithm is available on https://dbaasp.org

In vitro testing of *In silico* designed CAMPs

- ✓**Antibacterial activity (MICs)**
- ✓**Hemolytic activity**
- ✓**Protease resistance**

Antimicrobial activity

MIC – Minimal Inhibitory Concentration

Antimicrobial activity is affected by:

- Type of the target organism
- *Existence* of Ions in the medium
- Amino acid composition of the peptides

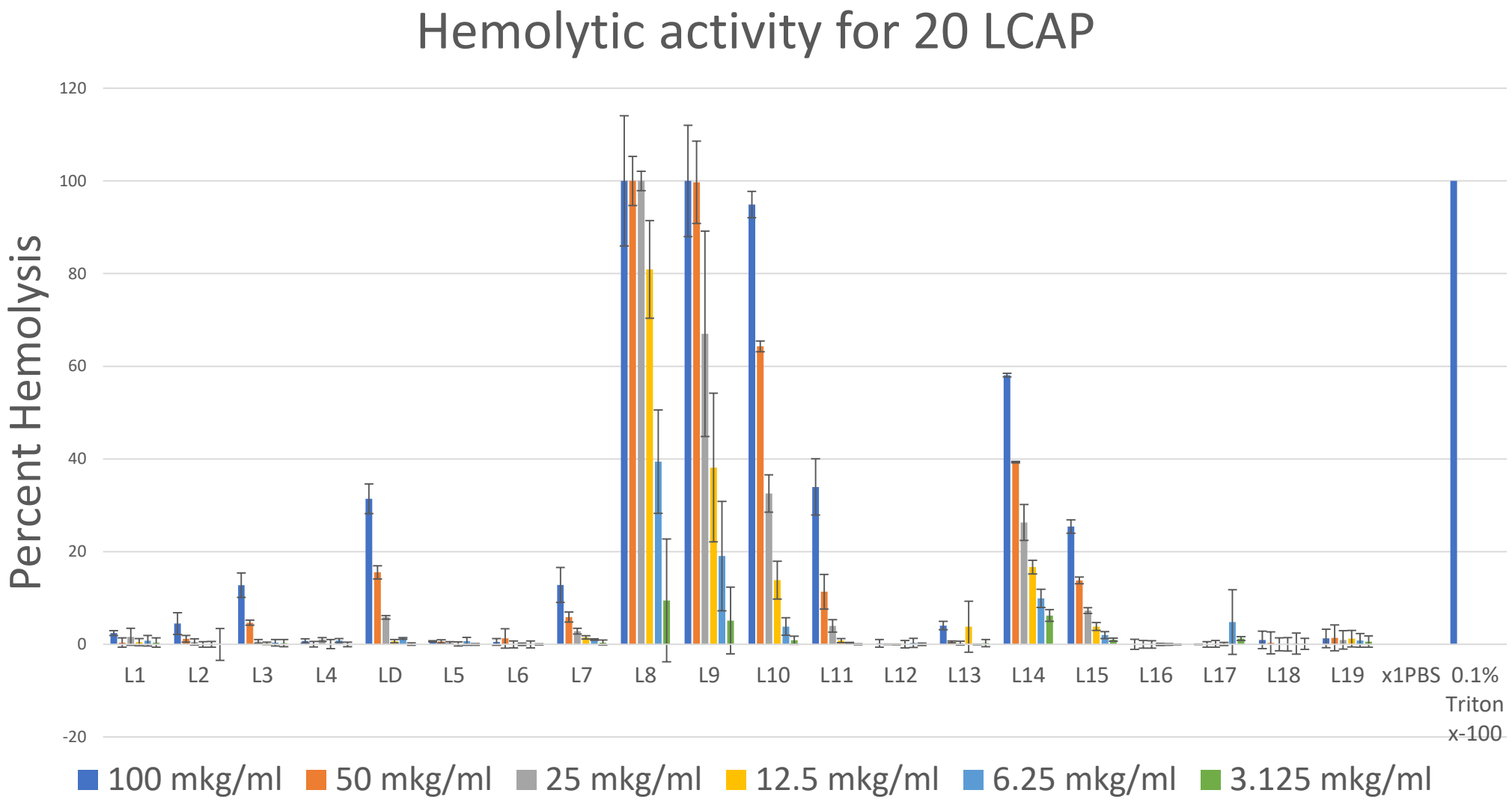
Peptide	MIC (µg/ml)					
	E. coli DH5-α		*E. coli* ATCC 25922		*S. aureus* ATCC 25923	
	LB	LB (NaCl supplemented)	LB	LB (NaCl supplemented)	TSB	MHB
LCAPL1	> 3.125	> 12.5	> 3.125	> 25-12.5	>100	>50
LCAPL2	> 3.125	> 6.25	> 3.125	> 12.5-6.25	>100	>25
LCAPL3	> 6.25	> 12.5	> 12.5	> 25	≥100	>25
LCAPL4	> 12.5	> 25	> 6.25	> 25	≥100	>50
LCAPL5	> 6.25	> 50-25	> 12.5	> 100	>100	>100
LCAPL6	> 6.25	> 50-25	> 6.25	> 25	>100	>100
LCAPL7	> 6.25	> 25	> 3.125	> 12.5-6.25	≥100	>25
LCAPL8	> 3.125	< 3.125	> 3.125	> 3.125	≤3.125	≤3.125
LCAPL9	> 12.5	≤ 3.125	> 25-12.5	> 6.25-3.125	≤3.125	≤3.125
LCAPL10	> 3.125	> 6.25	> 6.25	> 6.25-3.125	≥12.5	>3.125
LCAPL11	> 100	> 100	> 50-25	> 50	≥100	>50
LCAPL12	> 100	> 100	> 50	> 100	>100	>100
LCAPL13	> 6.25	> 25	> 3.125	> 12.5	>100	>100
LCAPL14	> 6.25	> 6.25	> 25	> 6.25	>100	>25
LCAPL15	> 3.125	> 3.125	> 3.125	> 3.125	>50	>12.5
LCAPL16	> 100	> 100	> 100	> 100	>100	>100
LCAPL17	> 100	> 100	> 100	> 100	>100	>100
LCAPL18	> 100	> 100	> 25	> 100	>100	>100
LCALL19	> 100	> 100	> 100	> 100	>100	>100
LCAPLD	≤ 3.125	> 6.25-3.125	> 3.125	> 12.5	>100	>50

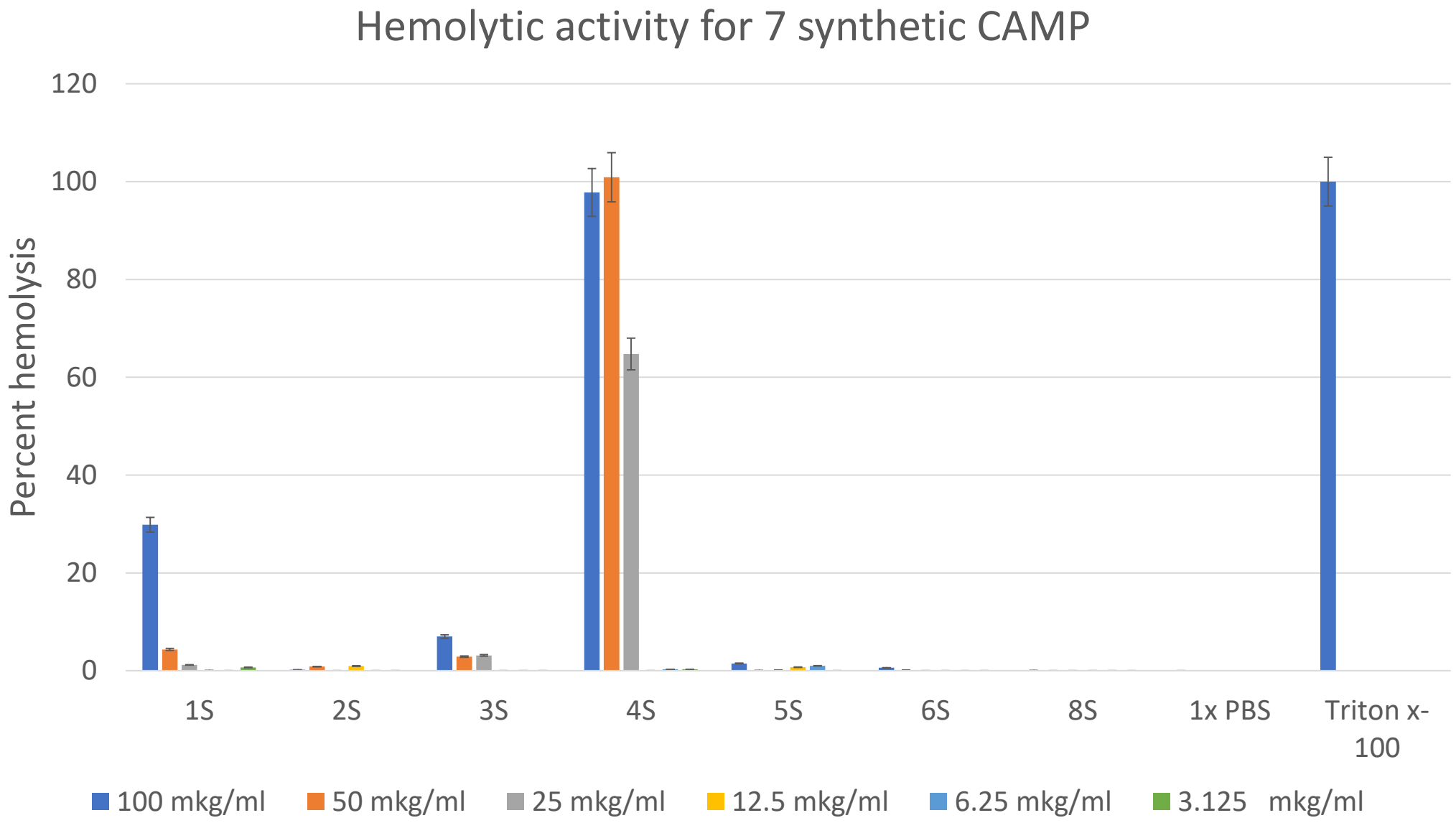
Table 1. MICs for synthetic Linear Cationic Antimicrobial Peptides designed for *E.coli*

Antimicrobial activity

	MIC (µg/ml)		
	S. aureus ATCC 25924	*E. coli* ATCC 25922	
Peptide	MHB	LB	LB (NaCl supplemented)
LifeTein-AMP-1S	>50	>3.125	>6.25
LifeTein-AMP-2S	>100	>25	>100
LifeTein-AMP-3S	>25	>3.125	>12.5
LifeTein-AMP-4S	≤3.125	<3.125	<3.125
LifeTein-AMP-5S	>12.5	<3.125	>25
LifeTein-AMP-6S	>100	>6.25	>100
LifeTein-AMP-8S	≤3.125	<3.125	<3.125

Table 2. Antimicrobial activity (MICs) for synthetic Linear Cationic Antimicrobial Peptides designed for *S. aureus*


Hemolytic activity for 20 LCAP
Percent Hemolysis
120
100
80
60
40
20
0
-20
L1
L2
L3
L4
LD
L5
L6
L7
L8
L9
L10
L11
L12
L13
L14
L15
L16
L17
L18
L19
x1PBS
0.1% Triton x-100
100 mkg/ml
50 mkg/ml
25 mkg/ml
12.5 mkg/ml
6.25 mkg/ml
3.125 mkg/ml

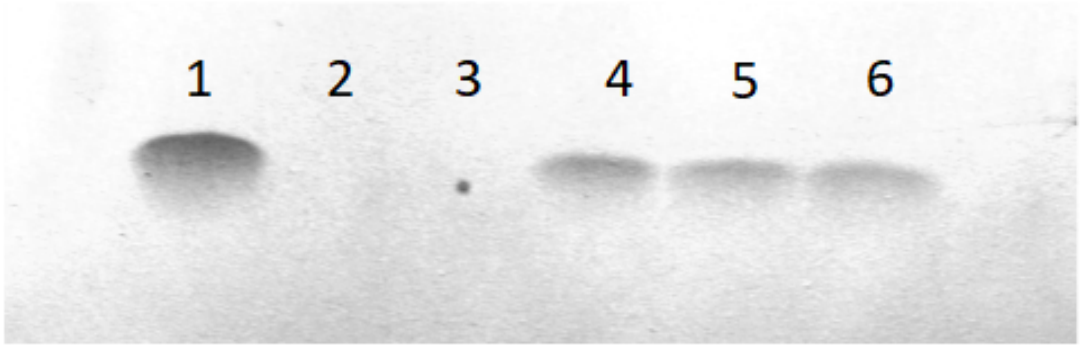

Hemolytic activity for 7 synthetic CAMP
Percent hemolysis
120
100
80
60
40
20
0
1S
2S
3S
4S
5S
6S
8S
1x PBS
Triton x-100
100 mkg/ml
50 mkg/ml
25 mkg/ml
12.5 mkg/ml
6.25 mkg/ml
3.125 mkg/ml

Resistance to proteolysis

Tricine-SDS-PAGE

10µg of peptides were digested by Proteinase K at a molar ratio of 500:1 and 1000:1, respectively. The final concentration of peptides in a reaction mix was 200µg/ml. After 16h incubation at 37°C, protease resistance was analyzed by Tricine-SDS-PAGE, using 4%, 10% and 16% gels.

Results:



Picture 1. Analysis of protease resistance of the designed peptides by Tricine-SDS-PAGE. Lane 1, LCAPL1; Lane 2, LCAPL1 plus Proeinase K (Molar ratio 500:1); Lane 3, LCAPL1 plus Proteinase K (Molar ratio 1000:1);
Lane 4, LCAPLD; Lane 5, LCAPLD plus Proteinase K (Molar ratio 500:1); Lane 6, LCAPLD plus Proteinase K (Molar ratio 1000:1).

	Peptide to α-Chymotrypsin Molar ratio		Peptide to Proteinase K Molar ratio	
	1000:1	500:1	1000:1	500:1
BSA	+	+	+	+
LCAPL1	+		+	+
LCAPLD	-	-	-	-
LCAPL2	+		+	
LCAPL3	+		+	
LCAPL4	+		partial +	partial +
LCAPL5	+		partial +	partial +
LCAPL6	+		+	
LCAPL7	+		partial +	+
LCAPL8	+		+	
LCAPL9	+		+	
LCAPL10	+		+	
LCAPL11	+		partial +	+
LCAPL12	+		partial +	partial +
LCAPL13	+		partial +	+
LCAPL14	partial +	partial +	partial +	+
LCAPL15	+		+	
LCAPL16	+		+	
LCAPL17	+		+	
LCAPL18	+		+	
LCAPL19	+		+	
LeifTein-AMP-1S	+		+	
LeifTein-AMP-2S	+		+	
LeifTein-AMP-3S	+		partial +	+
LeifTein-AMP-4S	+		partial +	+
LeifTein-AMP-5S	+		-	partial +
LeifTein-AMP-6S	+		partial +	+
LeifTein-AMP-8S	-	-	-	-

Conclusions

- The predictive tool works
- The antimicrobial activity of the CAMPs is affected by several factors
- There might be a correlation between high hemolytic and high antimicrobial activity
- Peptides are more resistant to Proteinase K than against α-Chymotrypsin