Towards the development of bioactive packaging

Zvi Hayouka

Institute of Biochemistry, Food Science and Nutrition, Hebrew University of Jerusalem

Food microbial chemistry lab

The Hebrew University of Jerusalem

הפקולטה לחקלאות, מזון וסביבה ע"ש רוברט ה. סמית
The Robert H. Smith Faculty of Agriculture, Food and Environment

Mount Scopus campus

Faculty of Science
The Hebrew University of Jerusalem

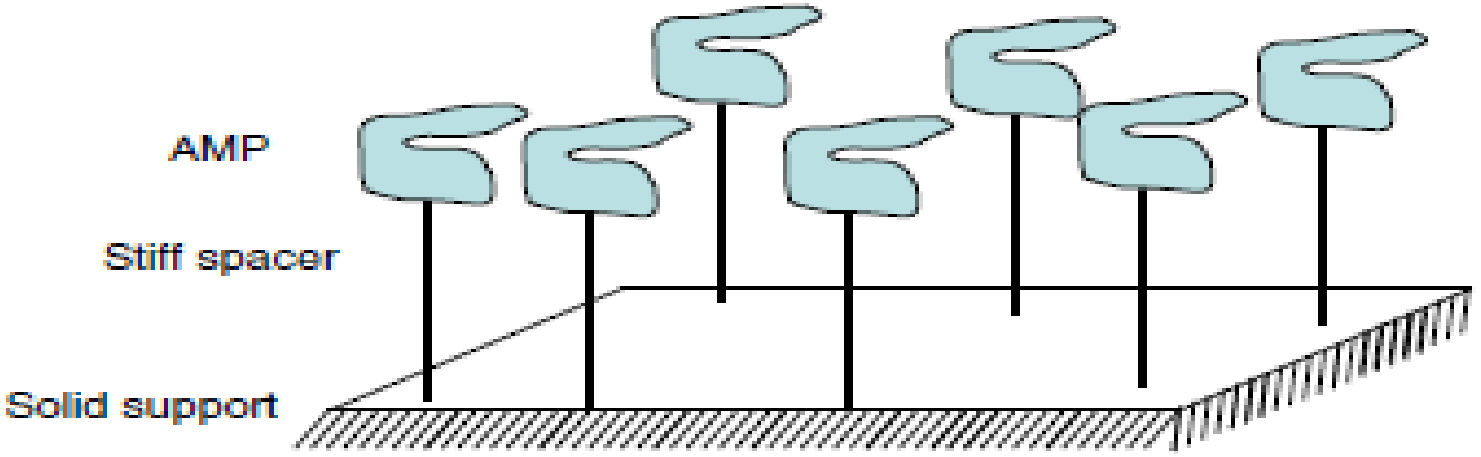
Research goal

Design smart bioactive packaging

Interesting bioactive packaging..

Bioactive packaging

- Slow release of active agents.
- Active agents are immobilized onto the surface materials via covalent linkages to prevent migration to the food.



Designing novel active agents

Antimicrobial agents

- Pathogenic infections represent a persistent threat to human health
- Antibiotic therapy is under severe pressure due to increased antibiotic resistance
- Discovery of new classes of antibiotics is warranted
- One potentially useful classes of antibiotics are the antimicrobial peptides

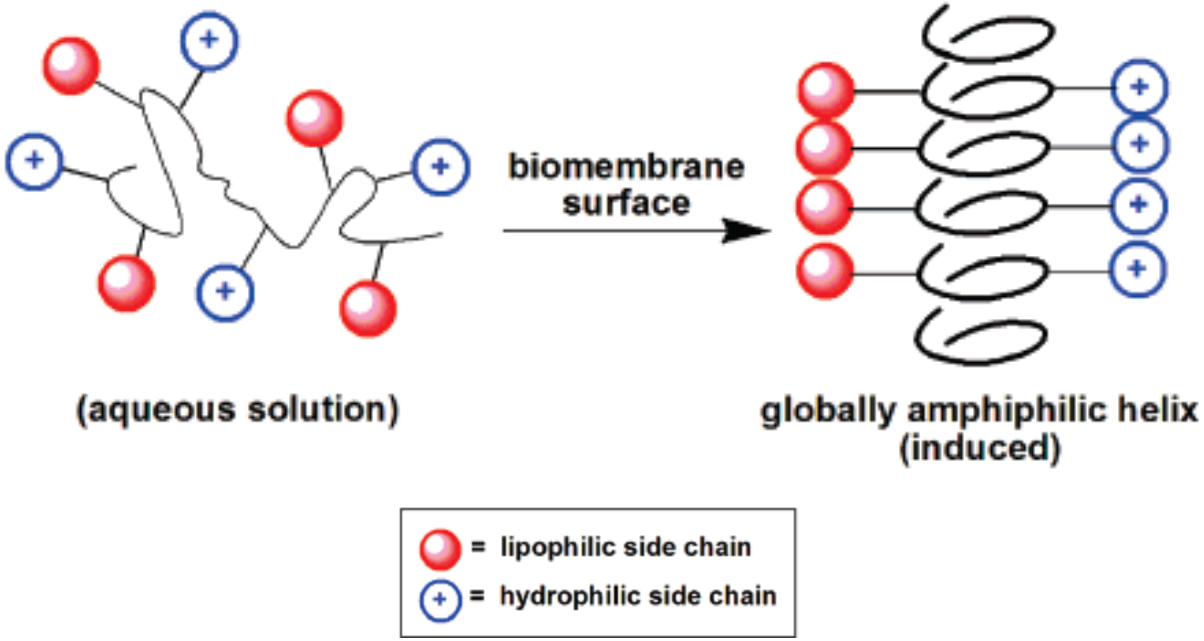
Antimicrobial peptides (AMPs)

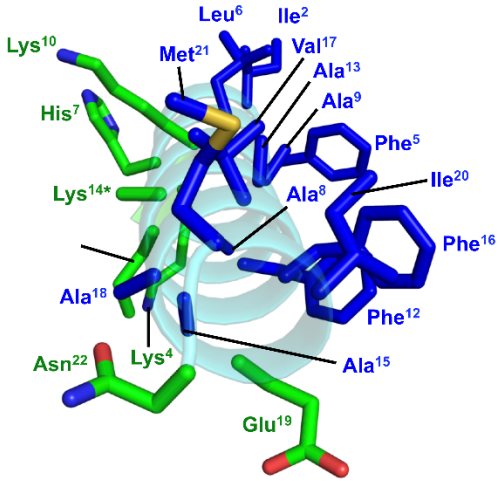
- Produced by eukaryotes as part of the innate immune in response to bacterial infection
- Length of 12 to 50 amino acids
- Most AMP have net positive charge >2 due to excess Arginine or Lysine residues
- Contain ~50% hydrophobic amino acids

AMPs as Inspiration for Development of Novel Antimicrobial Polymers

Antibacterial Activity of AMP

Natural host defense peptides (for eg. Magainin 2, Cecropin A)





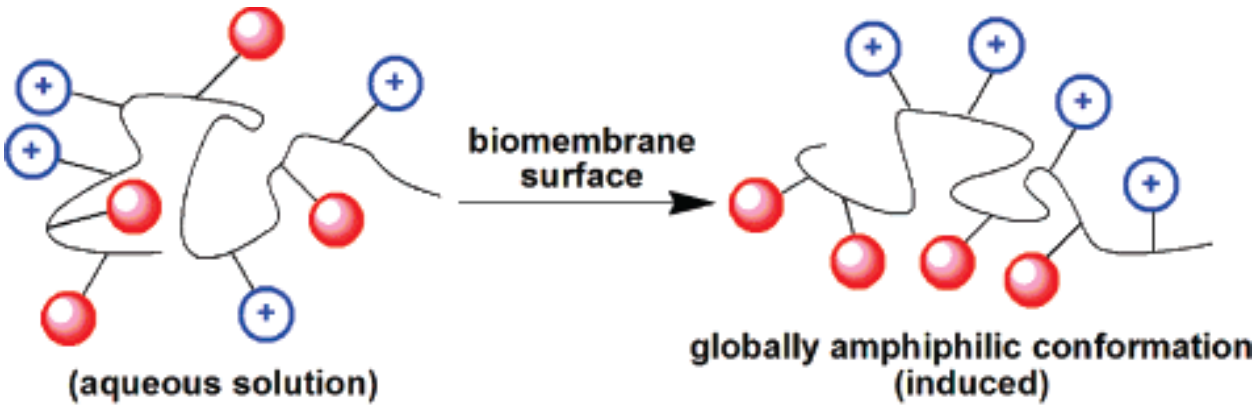
Ala ^{8,13,18}-magainin 2 crystal structure

Do we need defined sequence?

Mowery, B. P. et al. *J. Am. Chem. Soc. 2007,* ***129***, 15474

Random cationic Copolymers: Mimics of Host-Defense Peptides

New Hypothesis (Flexible Oligomers):

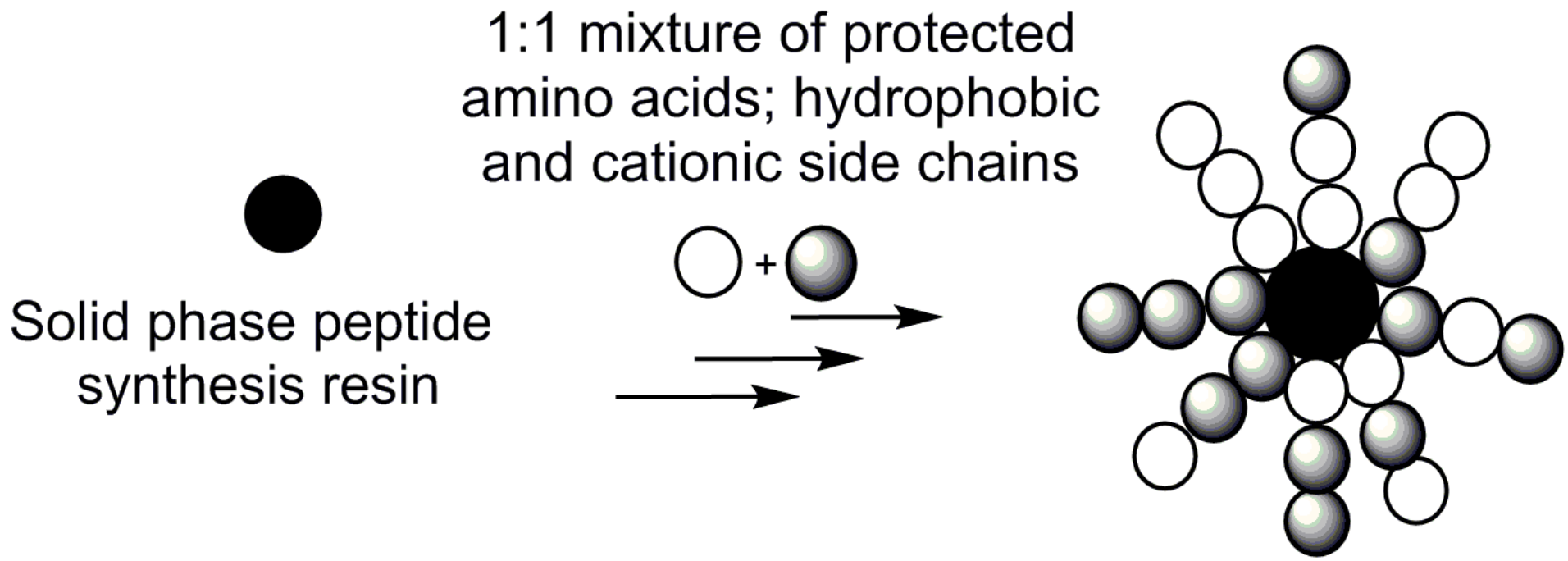


If global amphiphilicity does not require a regular conformation, then sequence control is not necessary.

Mowery, B. P. et al. *J. Am. Chem. Soc. 2007,* ***129***, 15474

New approach for the synthesis of cationic polymers

The “mixture” approach: Synthesis of sequence-random peptide mixtures

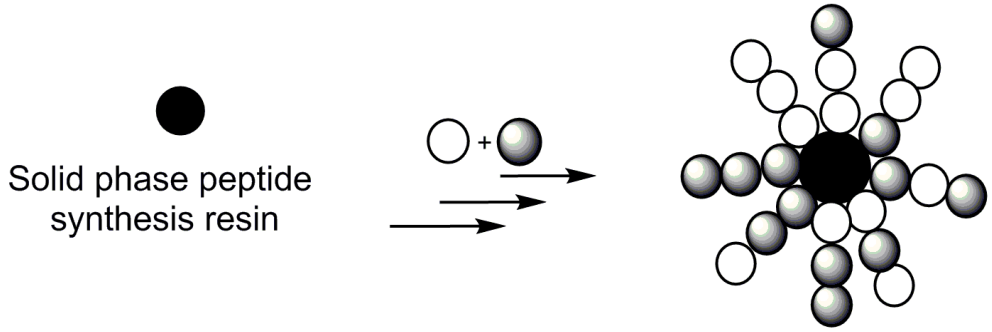


Multiple sequences on each bead

The mixture approach

Studying the effects of:

- Composition
- Length
- Molar ratio
- Stereochemistry



On the antimicrobial and hemolytic activity

The mixture approach

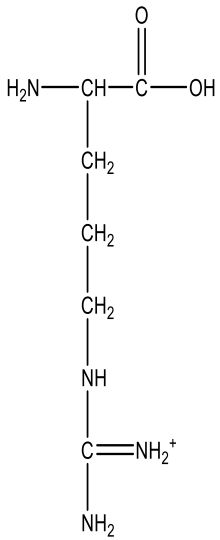
Studying the effect of:

- Composition
- Length
- Molar ratio
- Stereochemistry

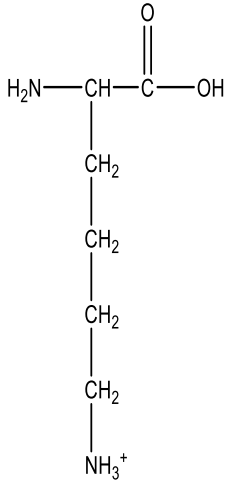
On the antimicrobial and hemolytic activity

Composition activity study

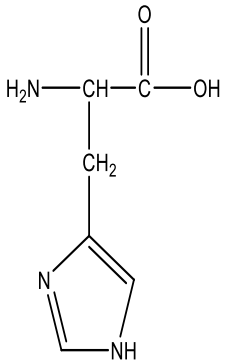
R
Arginine



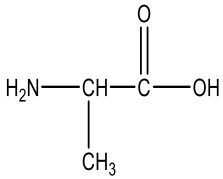
K
Lysine



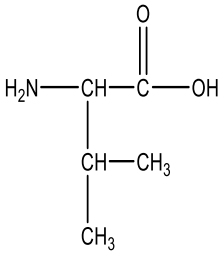
H
Histidine



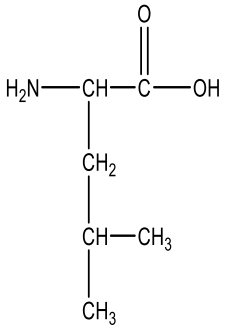
A
Alanine



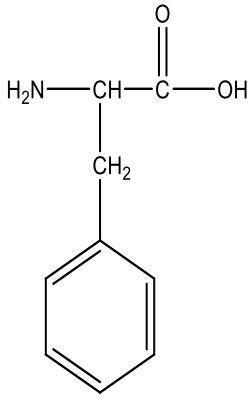
V
Valine



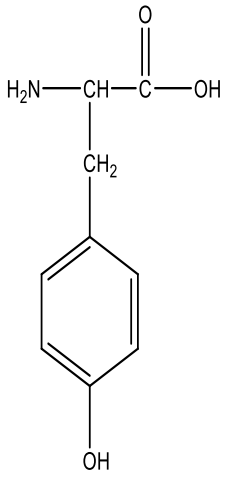
L
Leucine



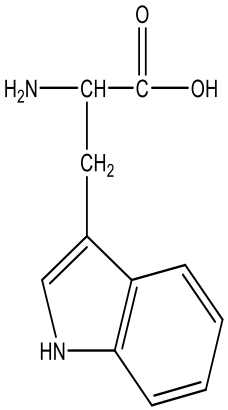
F
Phenylalanine



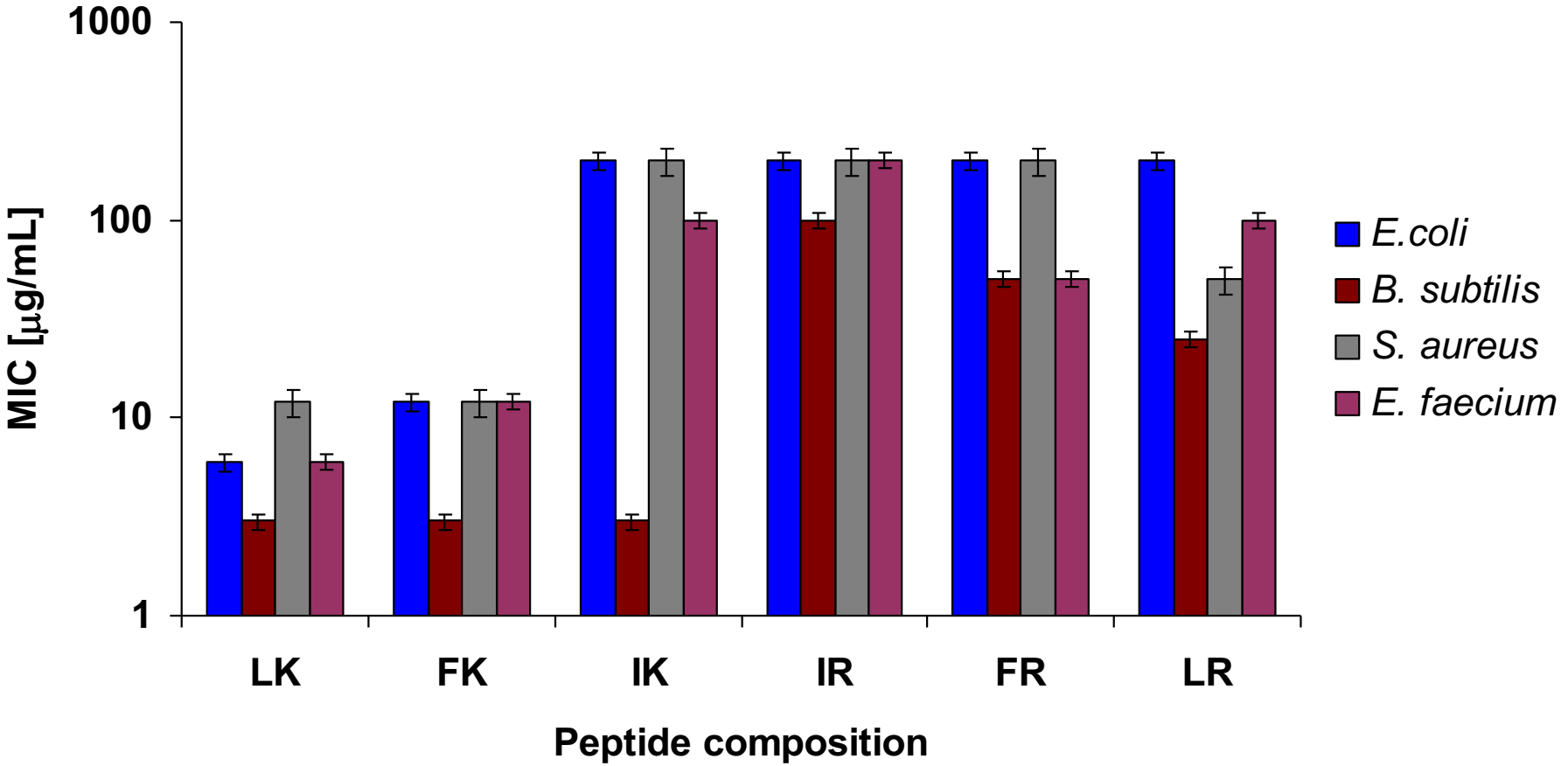
Y
Tyrosine



W
Tryptophan

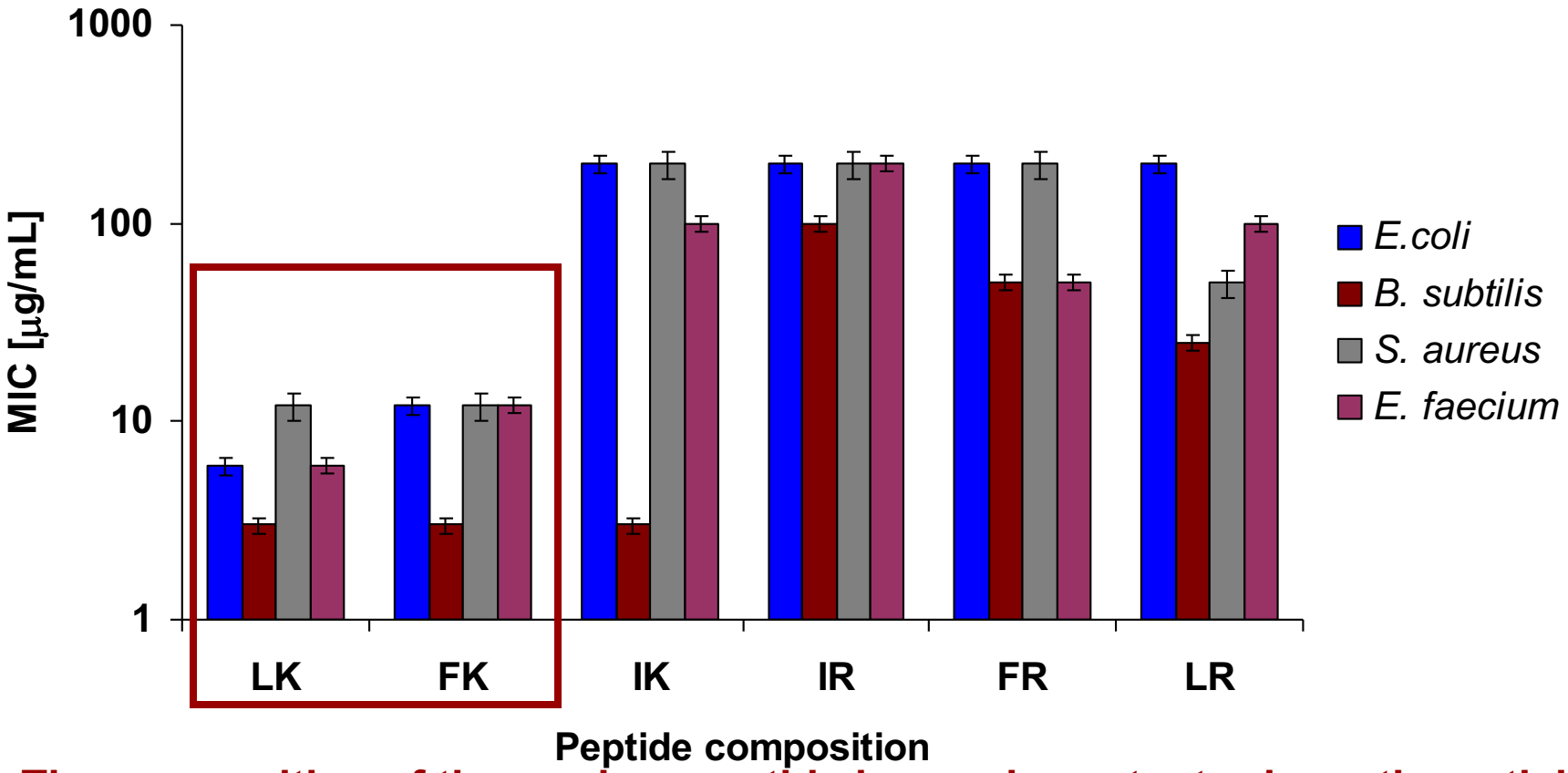


1:1 Leu-Lys or Phe-Lys 20-mer pools display good antimicrobial activity



Hayouka Z. et al. *J. Am. Chem. Soc*. 2013, **135**, 11748

1:1 Leu-Lys or Phe-Lys 20-mer pools display good antimicrobial activity

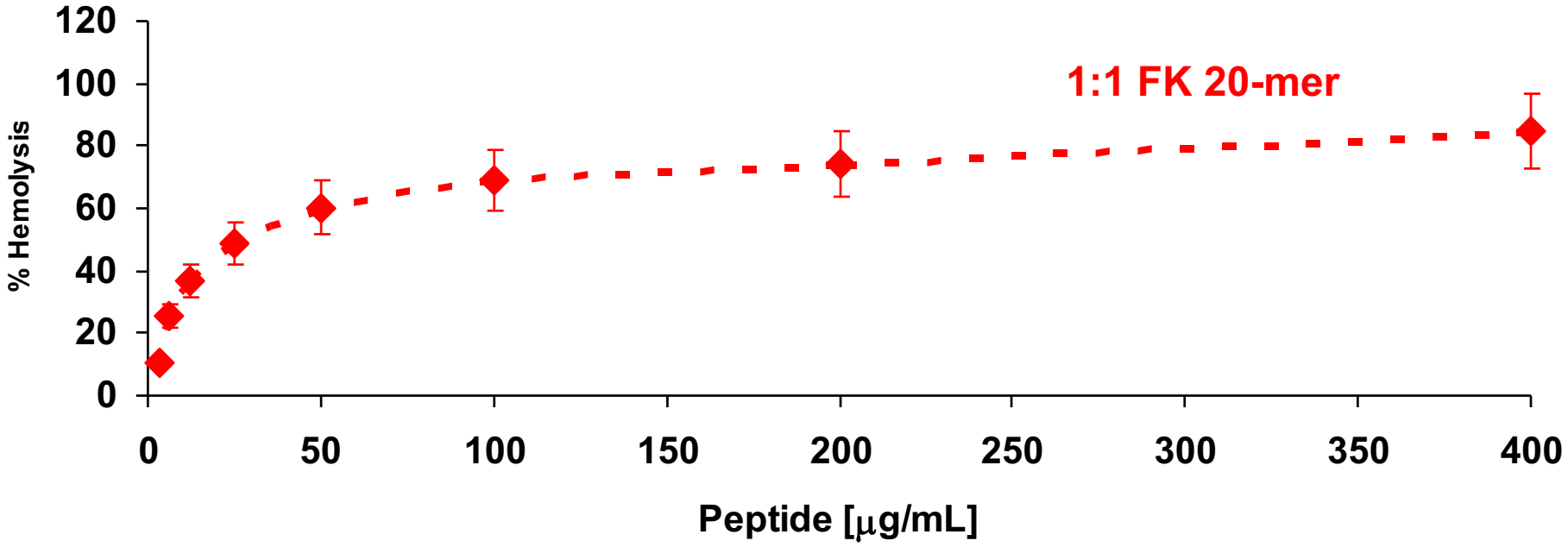


The composition of the random peptide has an important role on the activity

Phe-Lys random pools were selected for further study

Hayouka Z. et al. *J. Am. Chem. Soc*. 2013, **135**, 11748

1:1 Phe-Lys 20-mer pools were the most active but also very hemolytic



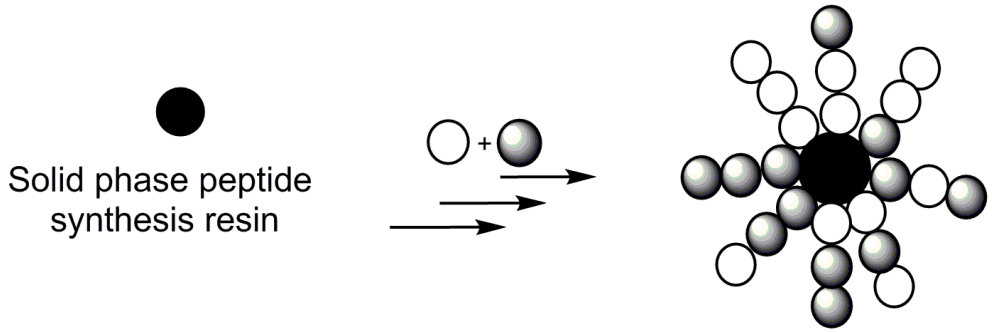
1:1 Phe-Lys 20-mer pool displayed low selectivity

Hayouka Z. et al. *J. Am. Chem. Soc*. 2013, **135**, 11748

The mixture approach

Studying the effect of:

- Composition
- Length
- Molar ratio
- Stereochemistry



On the antimicrobial and hemolytic activity

1:1 Phe-Lys 20-mer pools displayed potent antimicrobial activity

The mixture approach

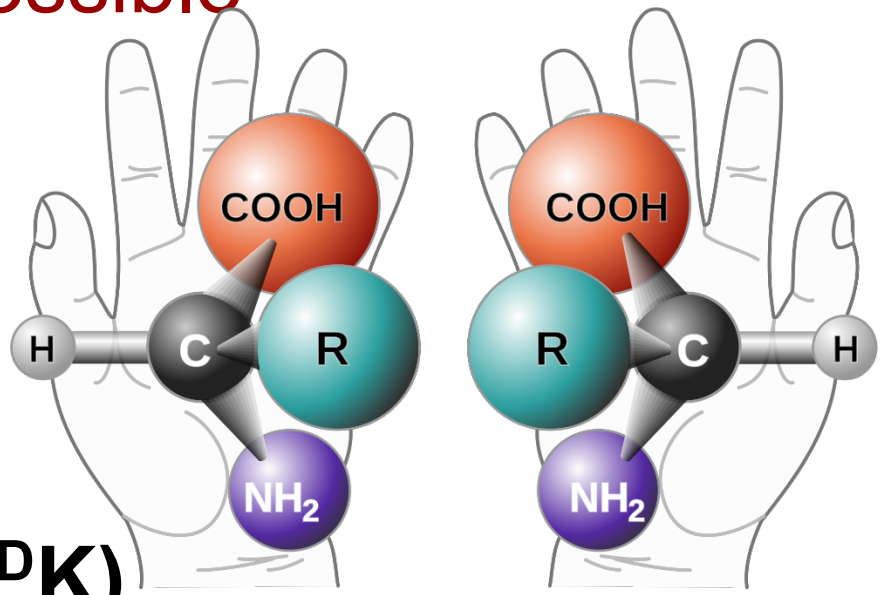
Studying the effect of:

- Composition
- Length
- Molar ratio
- Stereochemistry

On the antimicrobial and hemolytic activity

Studying the effect of stereochemistry on the random pools activity

- We synthesized all the possible stereoisomers pools:



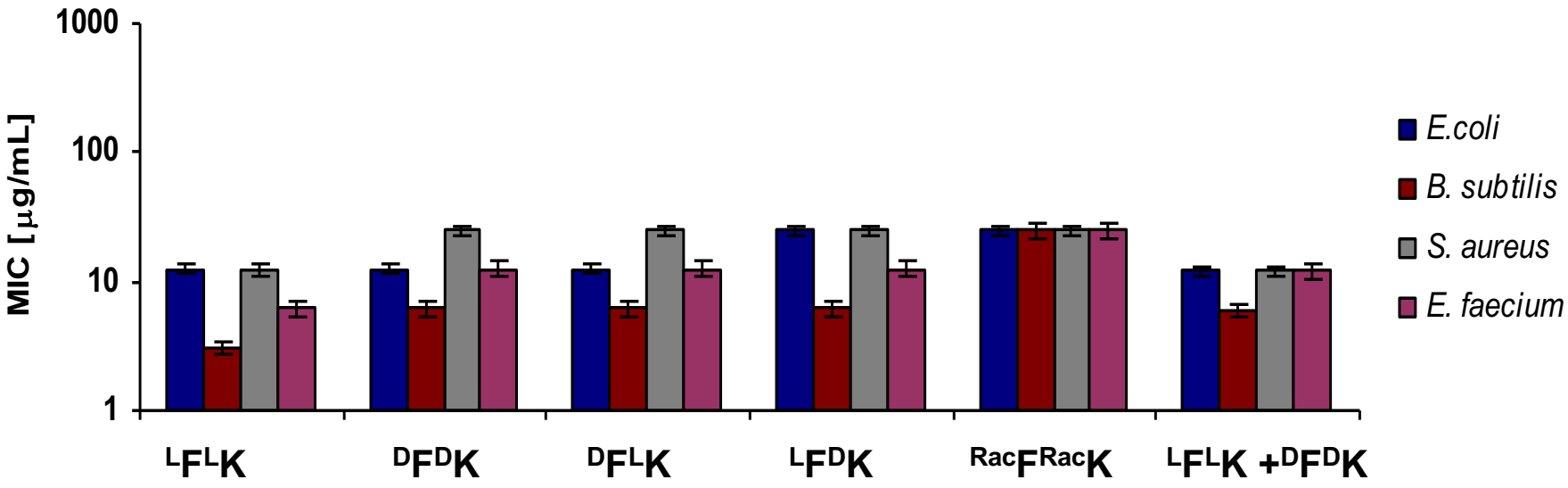
L-Homochiral **(FK)**

D-Homochiral **(^{D}F^{D}K)**

Heterochiral **(^{D}FK and F^{D}K)**

Random stereoisomers **(FK Rac)**

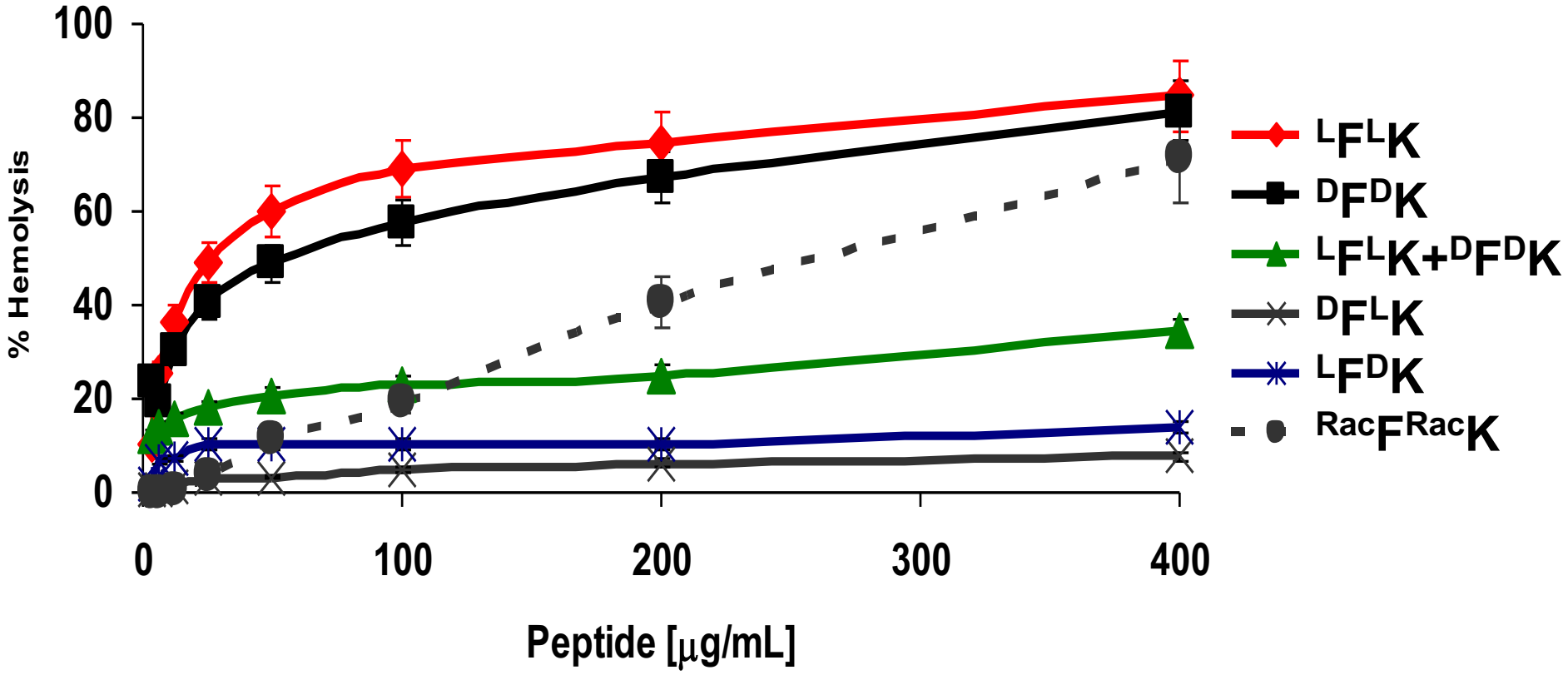
Stereochemistry has minor effect on the antimicrobial activity



The stereochemistry of the random peptide pools did not effect on the antimicrobial activity

Hayouka Z. et al. *J. Am. Chem. Soc*. 2013, **135**, 11748

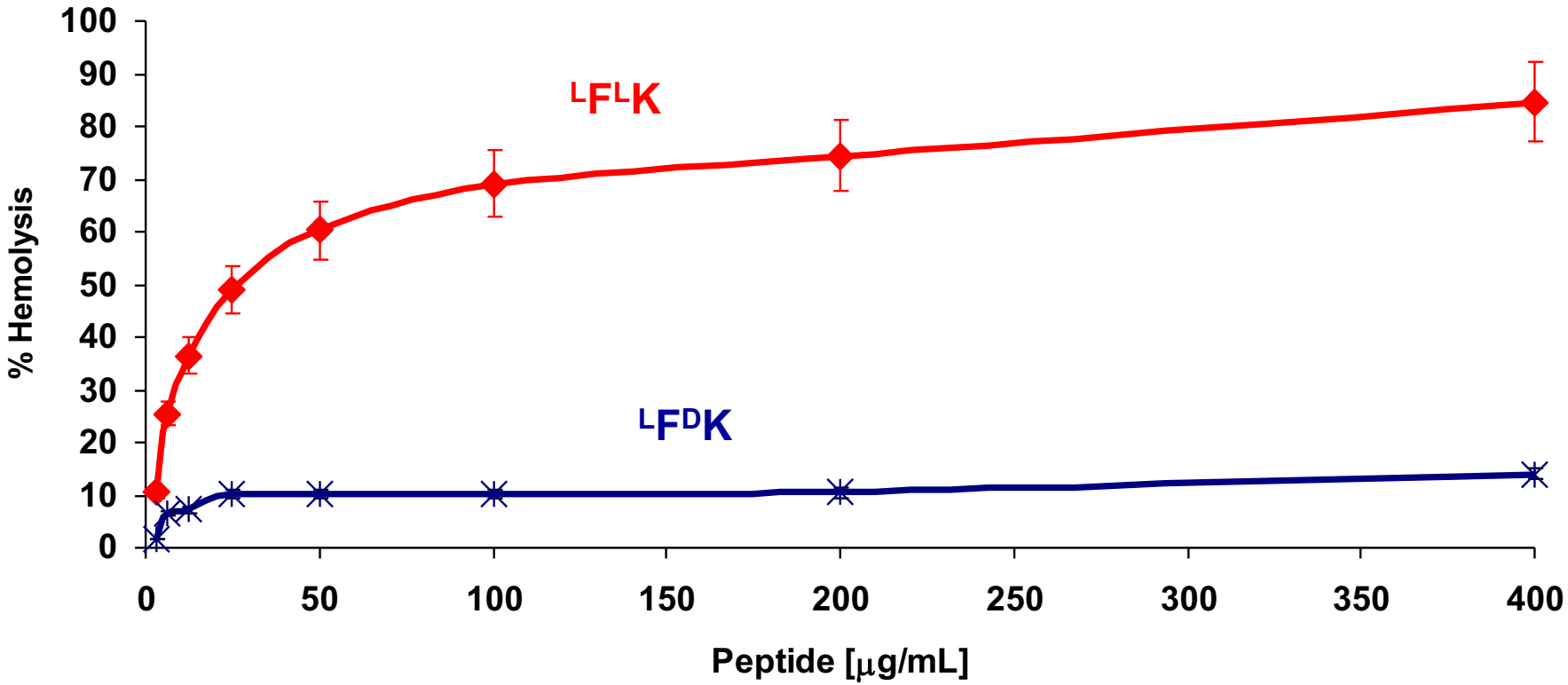
Heterochiral peptide pools display lower hemolytic activity



The heterochiral random pools are selective and active compounds

Hayouka Z. et al. *J. Am. Chem. Soc*. 2013, **135**, 11748

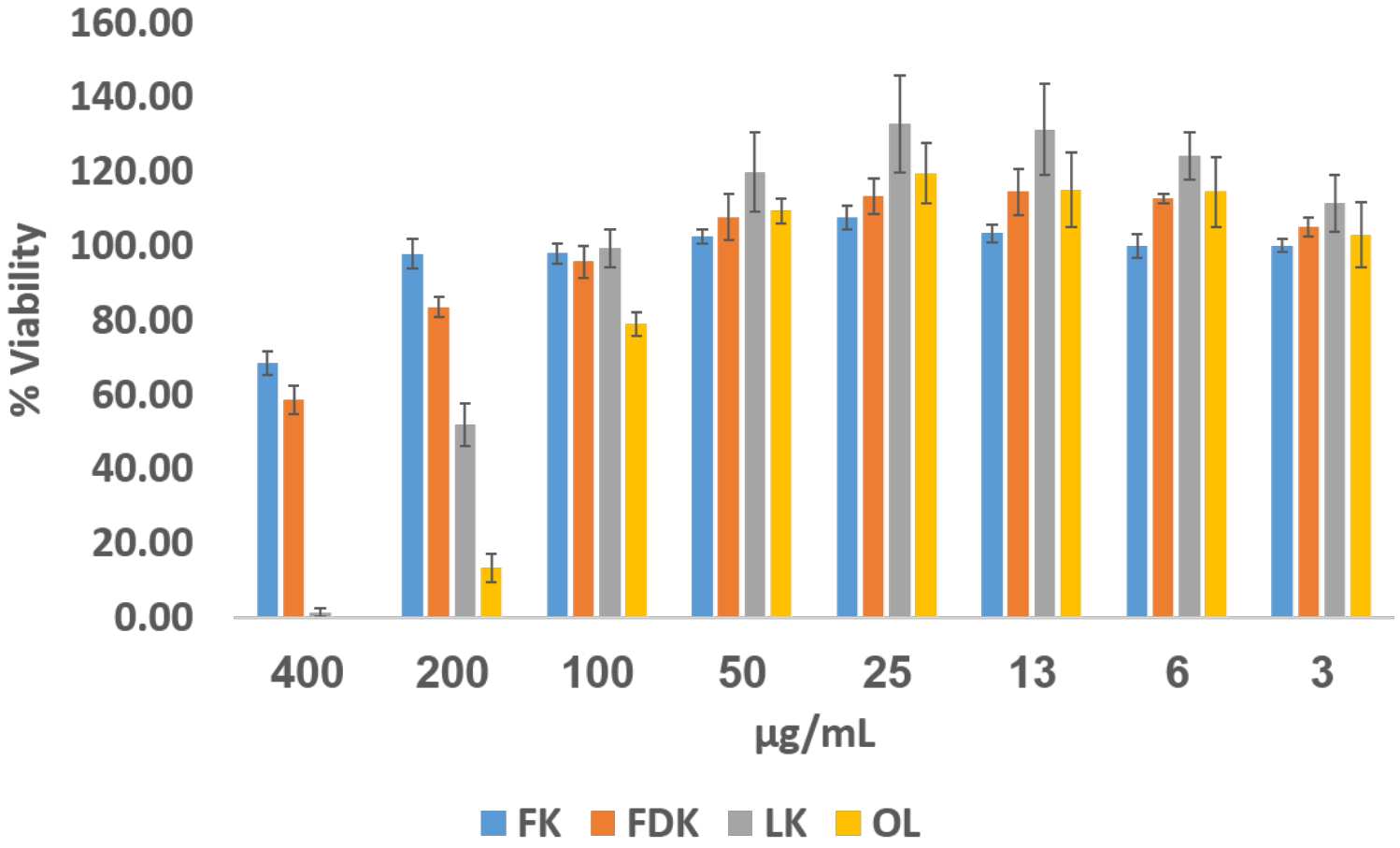
The heterochiral mixture is very selective



Indicating on differences in the mechanism of action

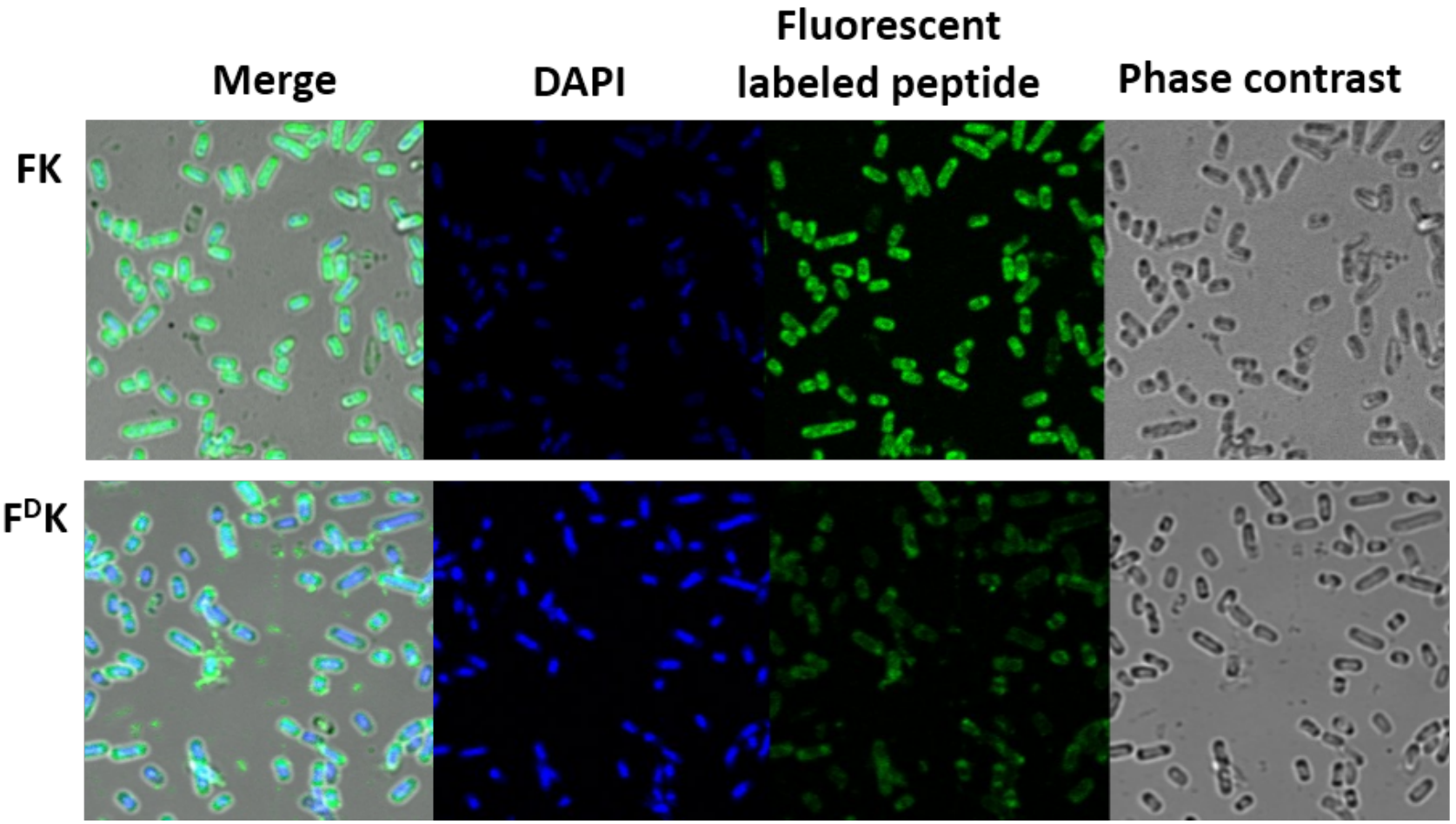
Hayouka Z. et al. *J. Am. Chem. Soc*. 2013, **135**, 11748

Cytotoxicity of random peptide mixtures



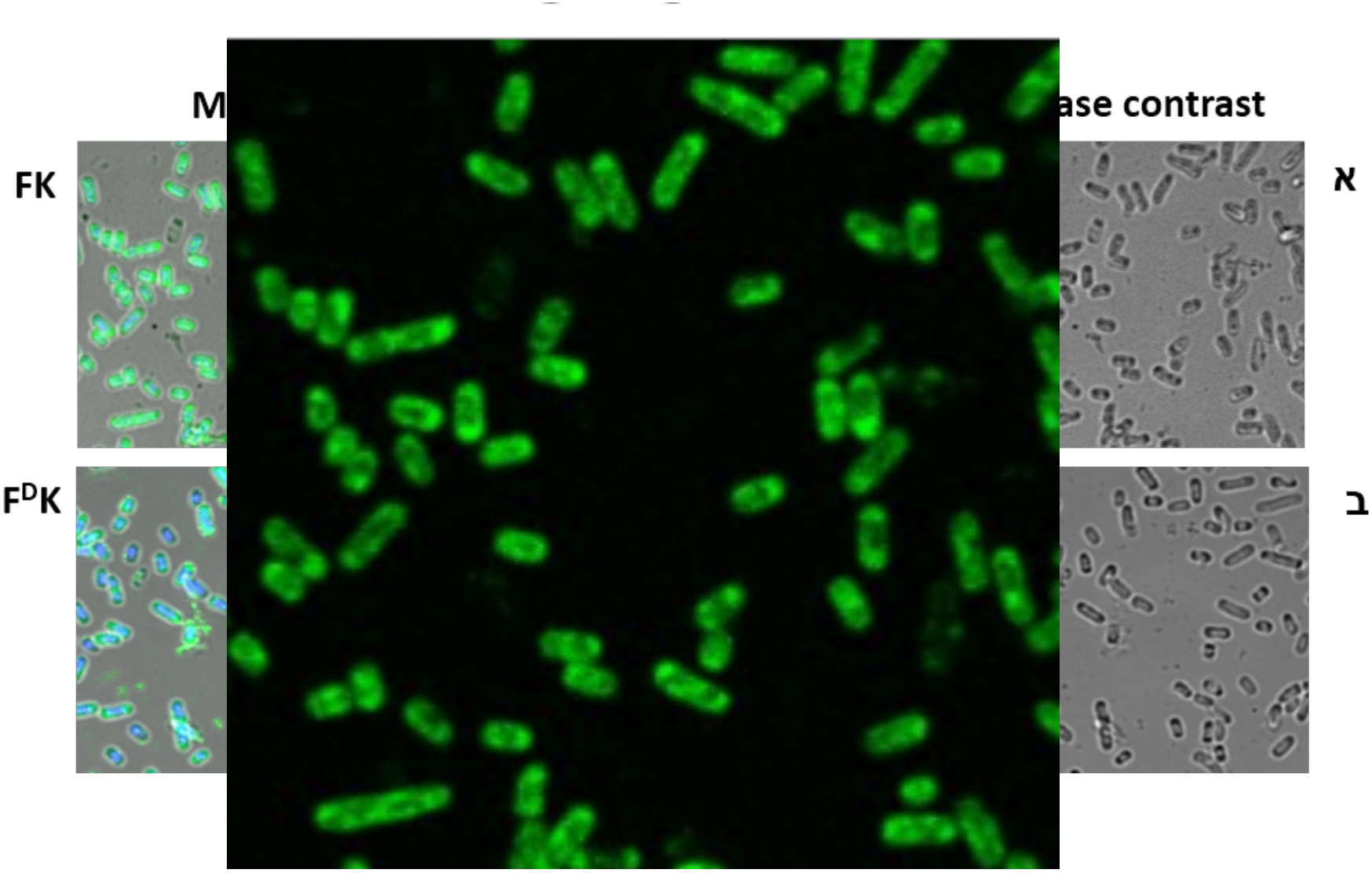
Random peptides mixtures are not toxic to Caco-2 cells

Random peptide mixtures penetrate bacterial cells



45 min. incubation,
50 µg/mL

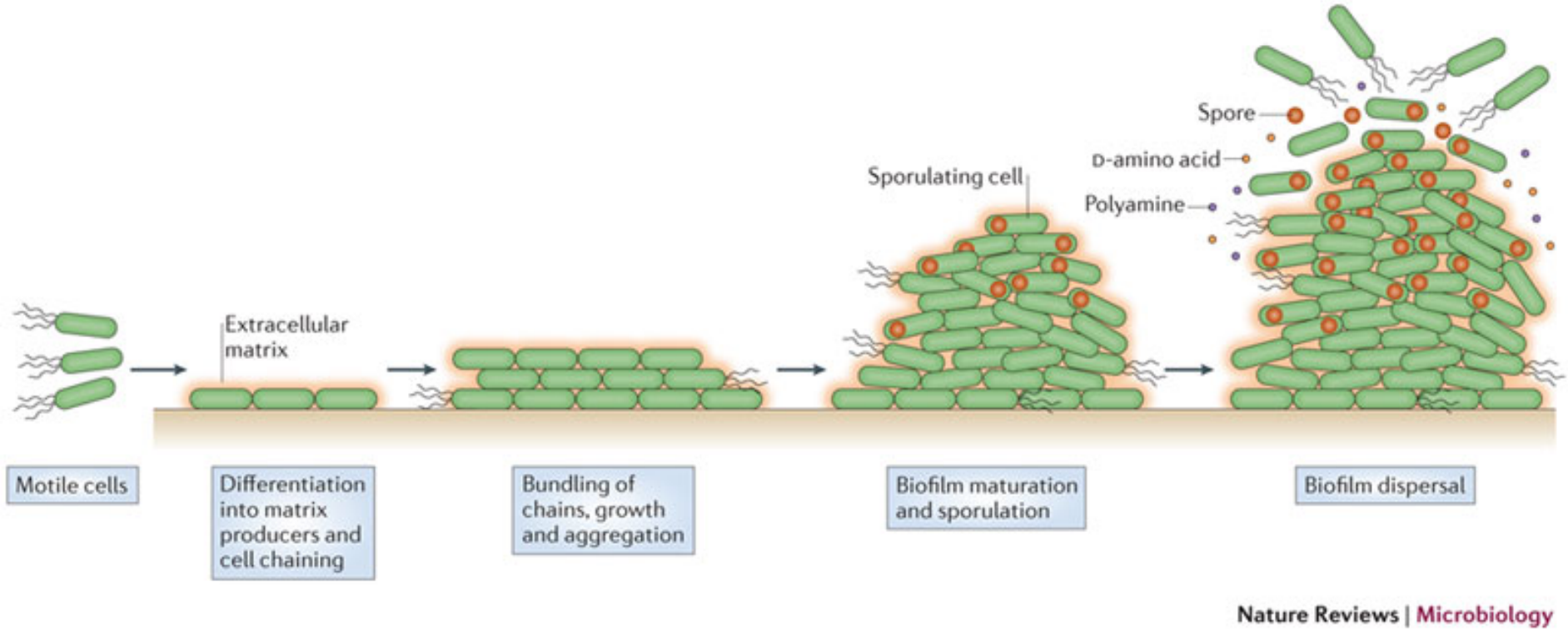
Random peptide mixtures penetrate bacterial cells



Do our compounds inhibit biofilm formation or eradicate mature biofilm?

Biofilm formation

Structured aggregation of surface-attached microorganisms encased in an extracellular matrix



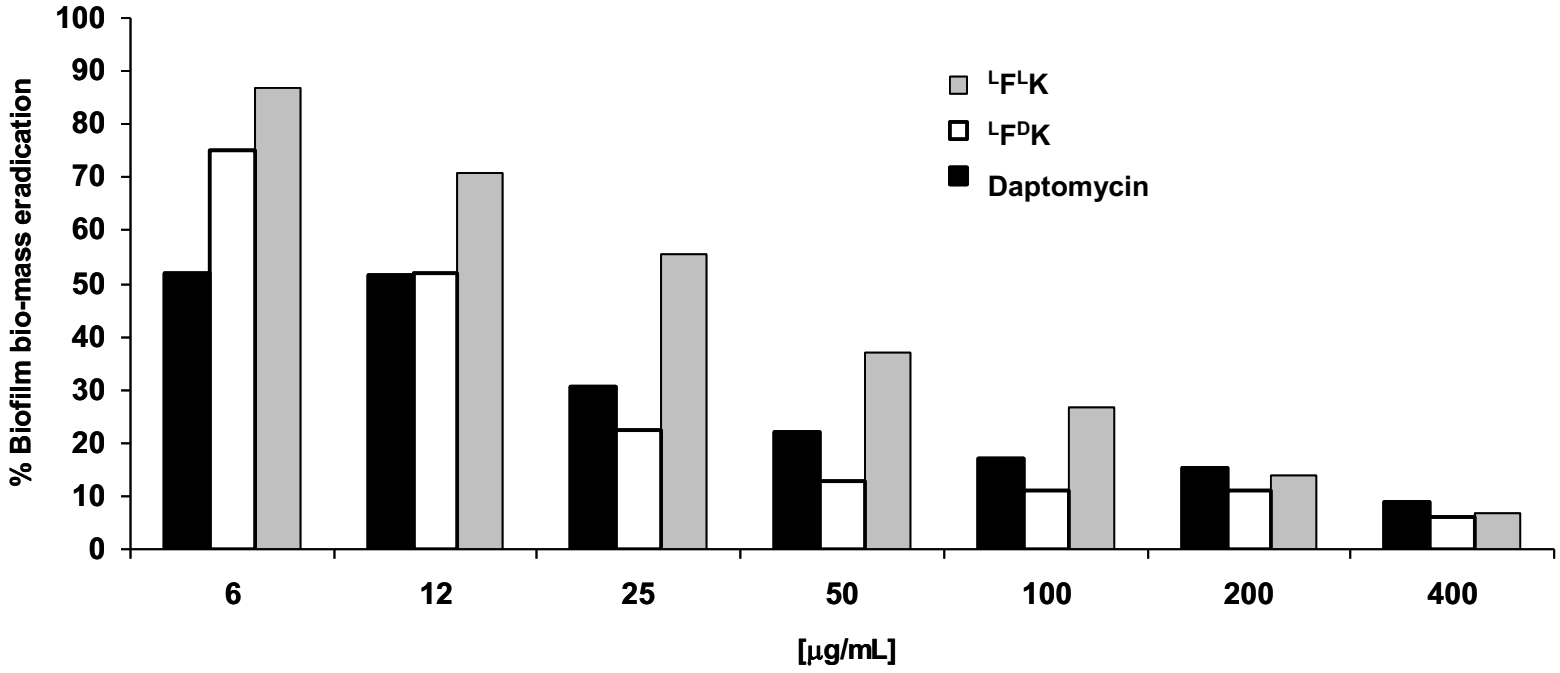
The dynamic process of biofilm formation

Biofilm formation

- Bacterial cells within biofilms are less susceptible to conventional antibiotics

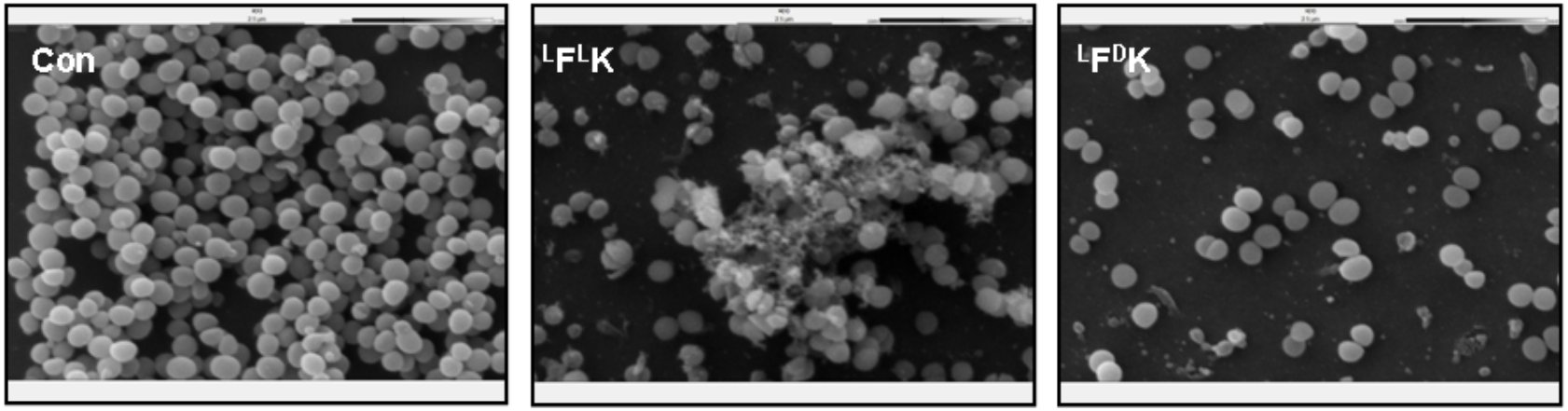
- Methicilin-resistant Staphylococcus aureus (MRSA) is one of the most serious biofilm-forming pathogens that cause complications ranging from minor to life threatening infections

FK random mixture eradicates mature biofilm complex



Hayouka et. el. *Chem Commun*, 2016, 52(44), 7102-5.

SEM of FK activity towards MRSA biofilm



Scan electron microscopy representative images of MRSA biofilm formation on glass surfaces treated with or without 100μg/mL LFLK or LFDK from triplicate.
Images shown were taken at magnification 10,000x.The selected images were chosen as the best representatives of the amount of biofilm on the glass surfaces.

Hayouka et. el. *Chem Commun*, 2016, 52(44), 7102-5.

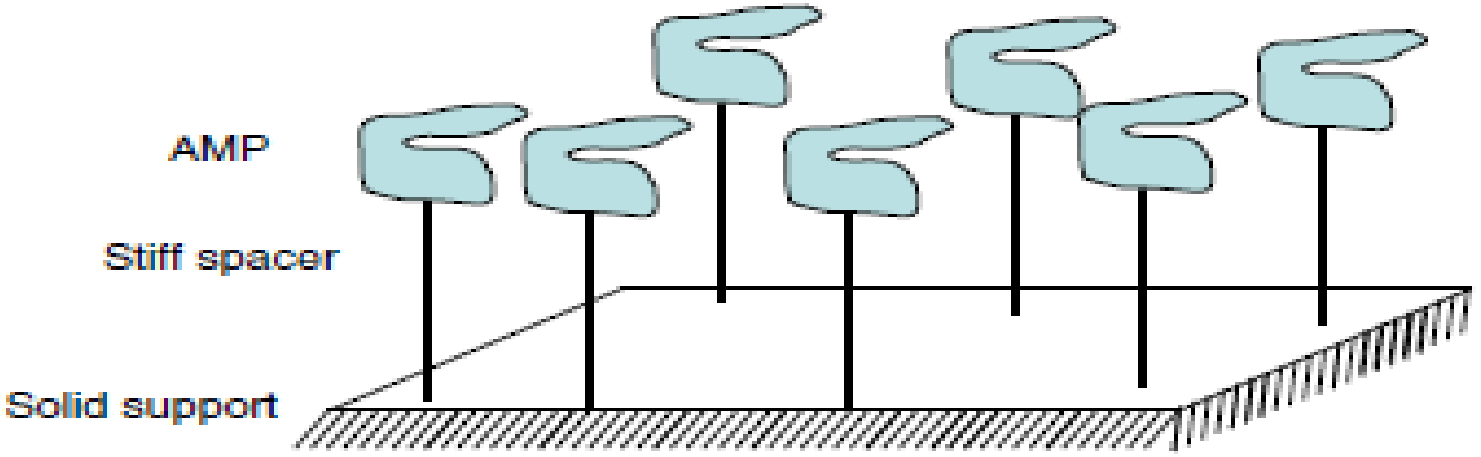
Conclusions

- New method to synthesize random peptide pools
- Random peptides showed good antimicrobial and antibiofilm activity, with MIC in the low μg/mL range
- Heterochiral peptide pools display broad antimicrobial activity and high selectivity
- Impossible to develop resistance towards mixture of peptides

Design smart bioactive packaging

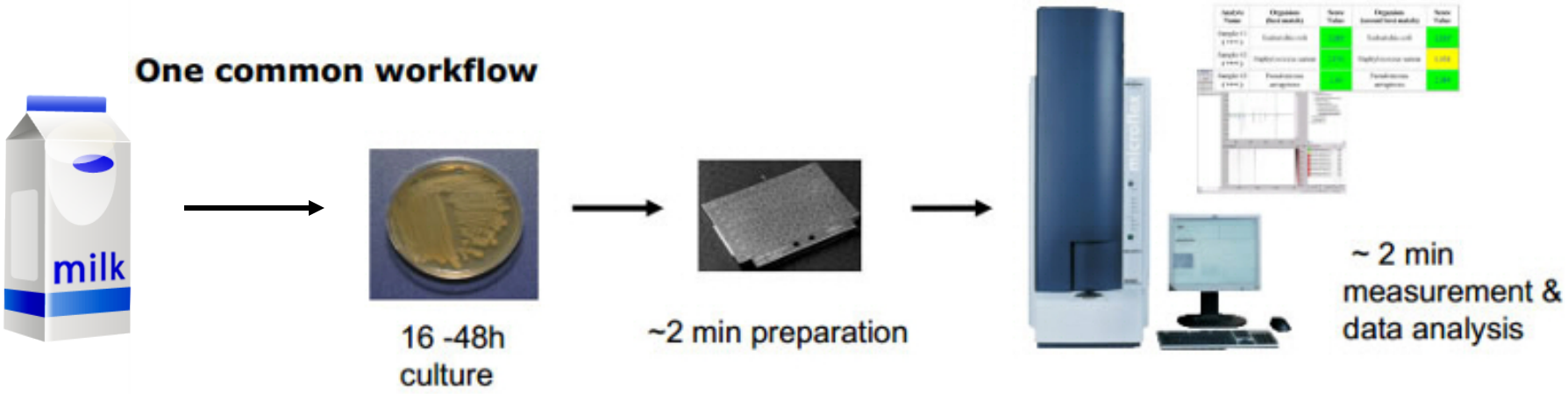
Bioactive packaging

- Most of them are based on slow release of antimicrobial agents.
- Active agents are immobilized onto the surface materials via covalent linkages to prevent migration to the food.



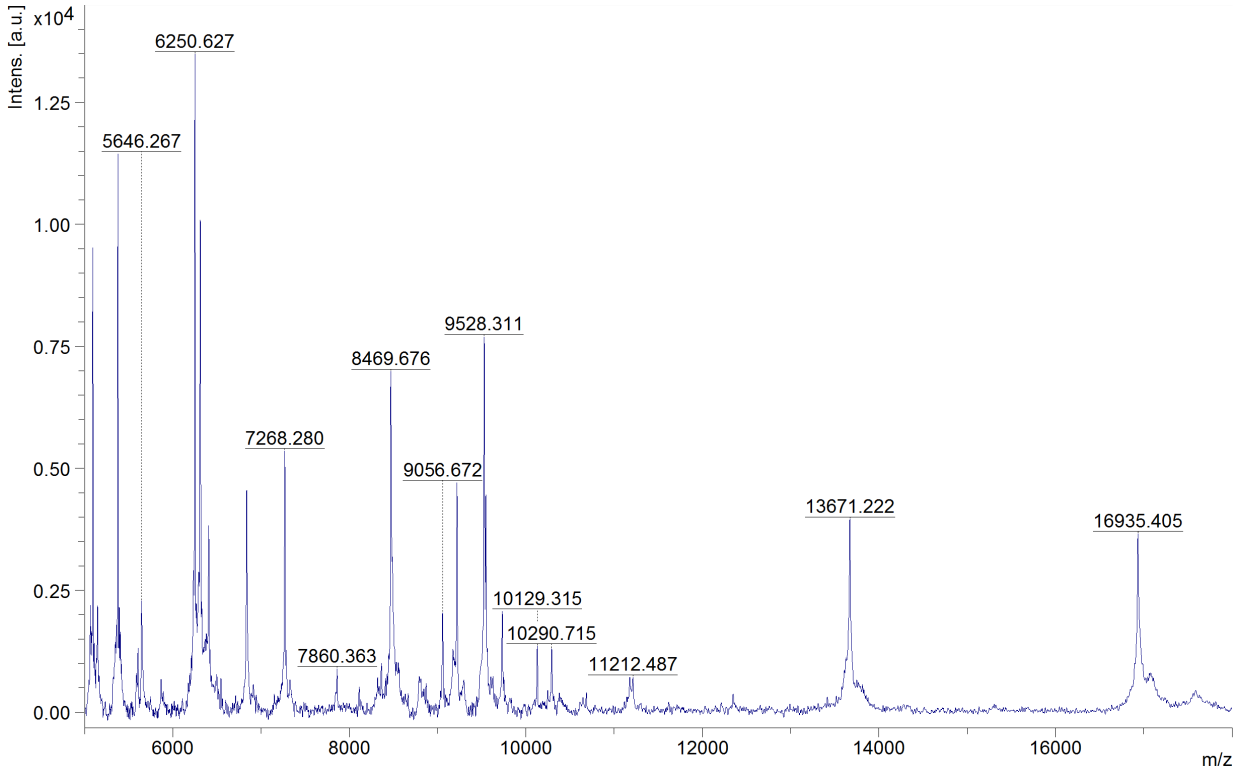
Defining the causes of food spoilage

Identifying microorganisms using MALDI TOF



- MALDI TOF (Bruker) Biotyper measures highly abundant proteins that are found in all microorganisms
- The characteristic patterns are used to reliably and accurately identify a particular microorganism down to the species level.

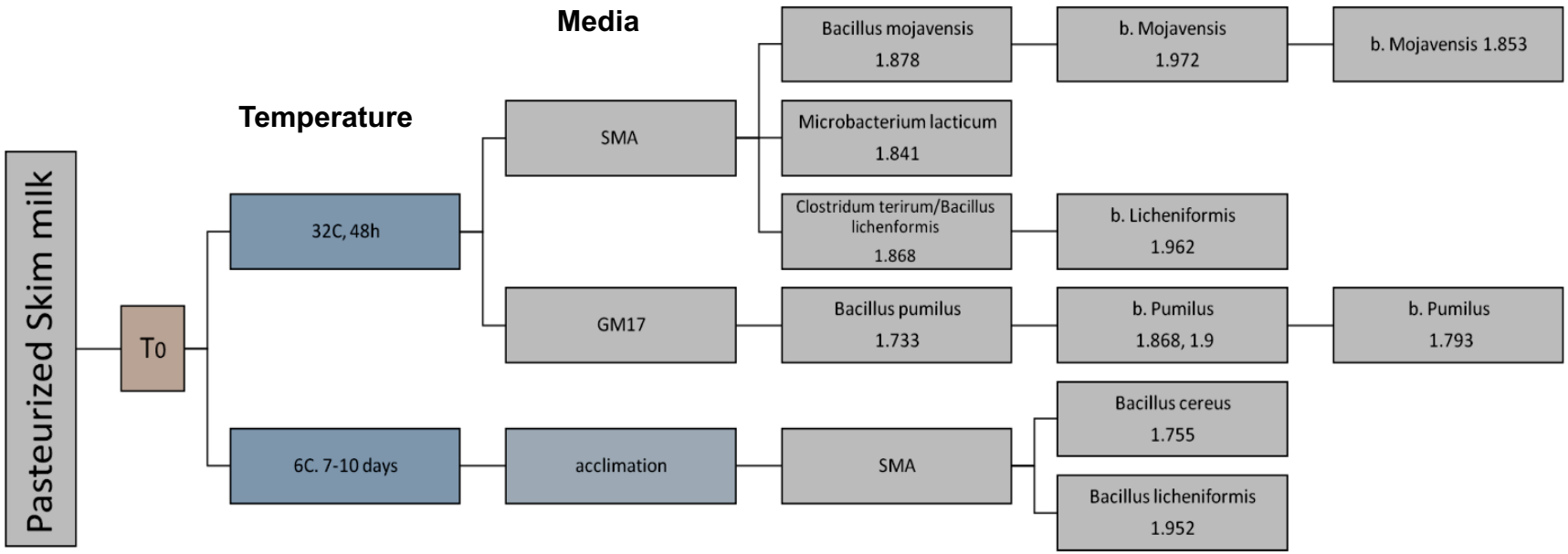
Identifying microorganisms using MALDI TOF



Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (++)	Escherichia coli MB11464_1 CHB	2.204	562
2 (++)	Escherichia coli DSM 682 DSM	2.144	562
3 (++)	Escherichia coli DH5alpha BRL	2.074	562
4 (++)	Escherichia coli ATCC 35218 CHB	2.063	562

Characterizing milk spoilage bacteria

We used milk that was kept in room temperature and in 4°C for few days and identify the bacteria using the Biotyper.



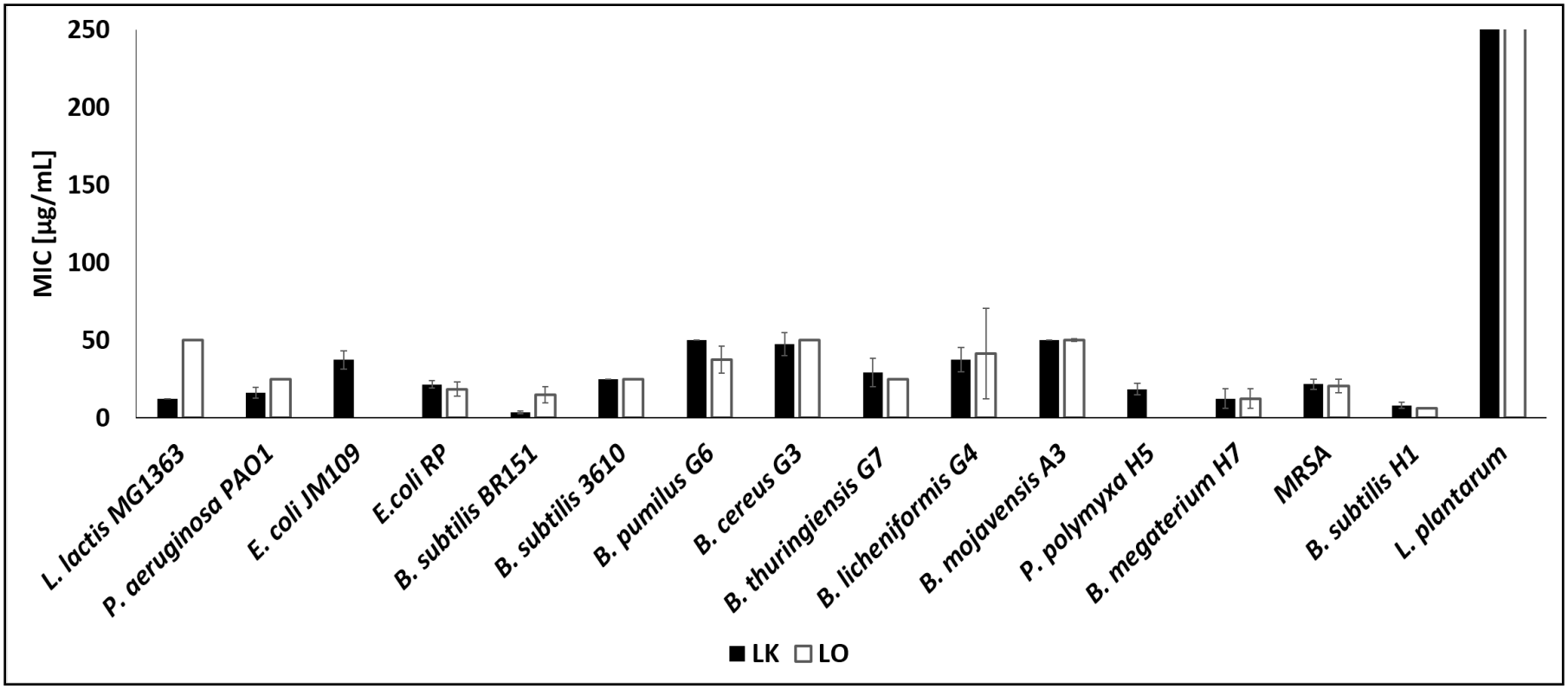
Isolated and identified bacteria

Room temperature storage			
Mesophilic bacteria		**Psychrotrophic bacteria**	
Bacillus cereus	**2.366**	***Bacillus licheniformis***	**2.242**
Bacillus licheniformis	**1.904**	***Bacillus massiliensis***	**1.986**
Paenibacillus polymyxa/poriare	**2.148**	***Bacillus cereus***	**2.22**
Micrococcus luteus	**2.336**	***Paenibacillus polymyxa***	**1.94**
Bacillus thuringiensis	**2.144**	***Bacillus thuringiensis***	**2.162**
Microbacterium lactium	**1.903**	***Pseudomonass rhodesiae***	**2.183**

16S rRNA sequencing showed similar results

Stren Tal and Hayouka Zvi. *Submitted*

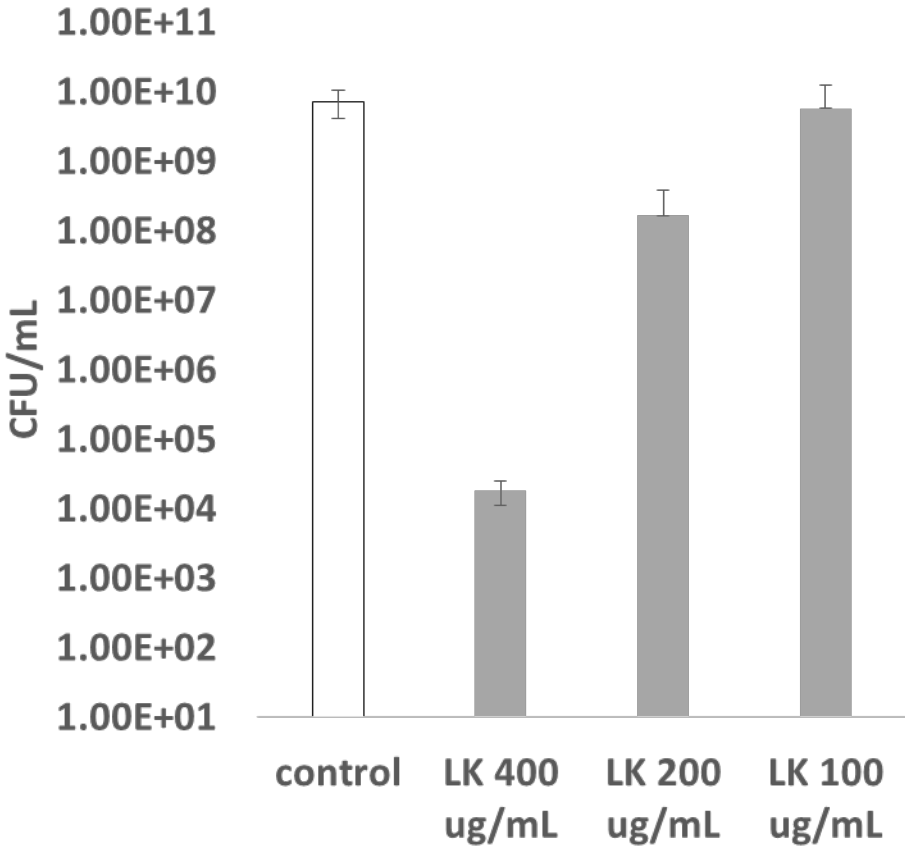
Antimicrobial activity of our active agents towards isolated milk bacteria



Our random peptide mixture is able to inhibit most of the isolated bacteria from milk very efficiently

Stren Tal and Hayouka Zvi. *Submitted*

Characterizing the antimicrobial activity of our compounds in UHT milk

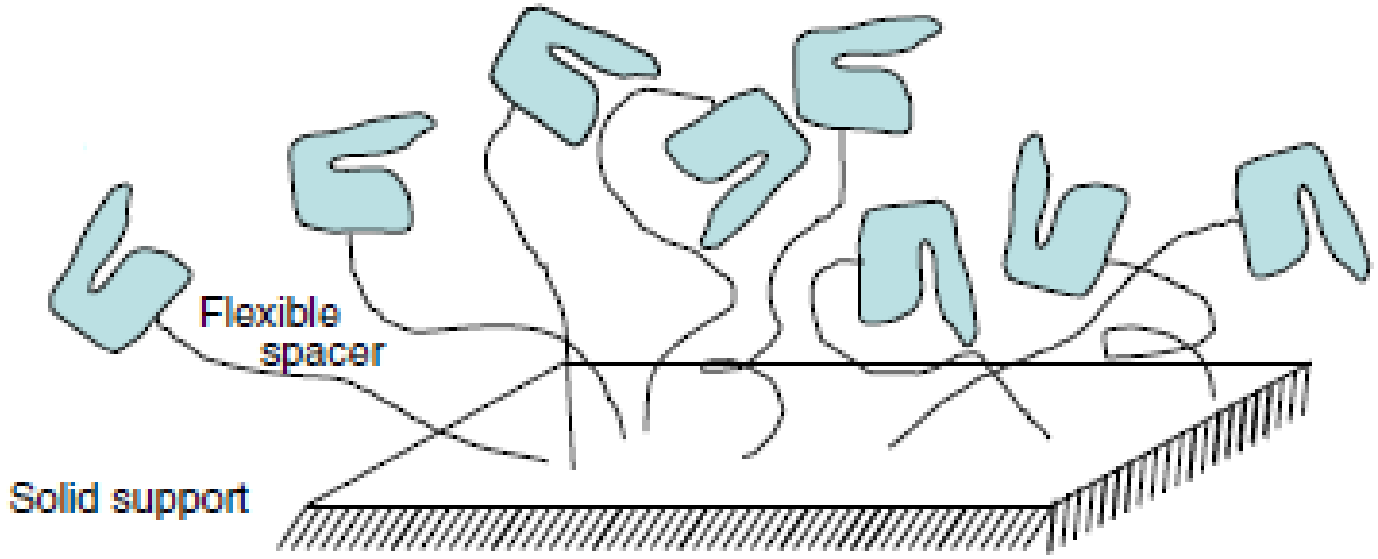


Our random peptide mixture showed 6 log reduction of *B. subtilis* in UHT milk

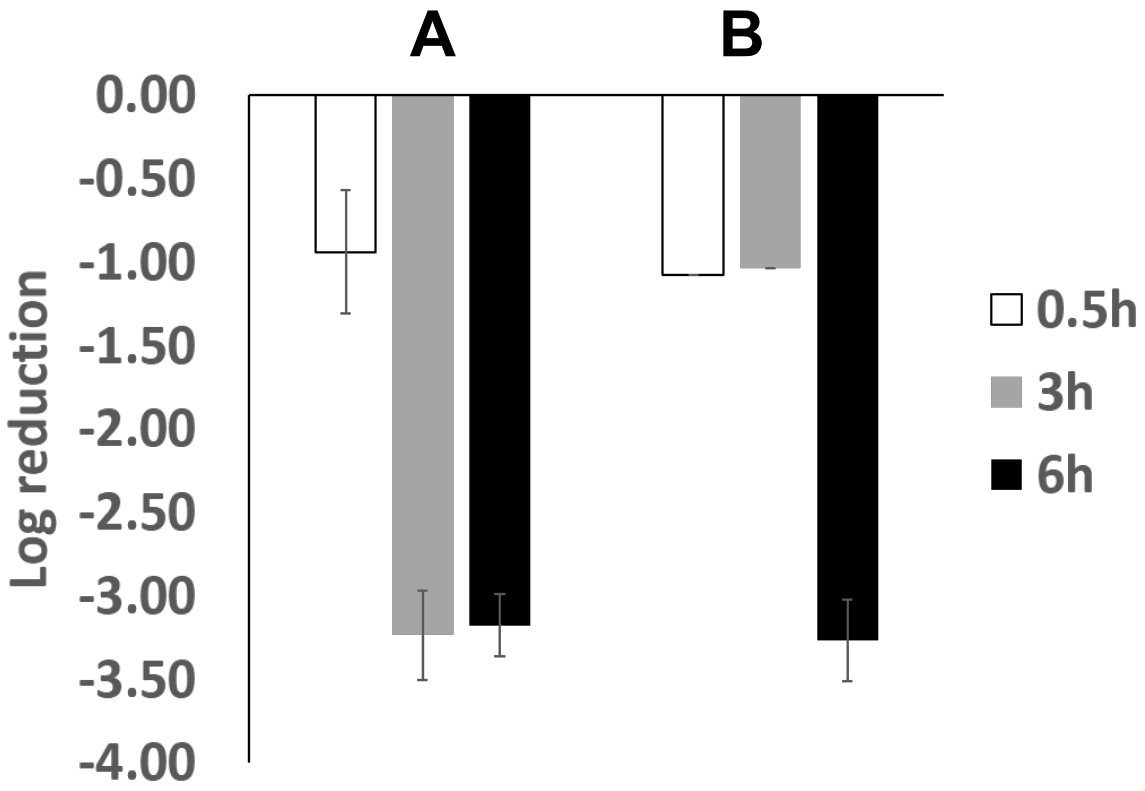
Immobilization of the active agents

Immobilization the active agents

We are currently designing new chemical approaches to immobilize our active agents on several surfaces.



Preliminary results of our bioactive surfaces



Our bioactive surfaces are active towards gram negative and positive bacteria

Other projects

- Food preservations
- Edible coating
- Water treatment
- Crop protection
- Foodborne bacterial Quorum sensing inhibitors
- Inhibiting vital protein-protein interaction in bacterial cells

Acknowledgment

The group:

Irena Peri Ph.D
Einav Malach PhD
Vinayak Ghate PhD
Rachel Bochnik-Tamir
Tal Stren
Carmit Ginesin
Avishag Yehuda
Ronen Bostan
Shiri Topman
Eyal Krieger
Yael Palman
Shani Kornhauser

German-Israeli Foundation for Scientific Research and Development

State of Israel Ministry of Health משרד הבריאות, ישראל

Yissum Hebrew University Technology Transfer

ActInPak

Thank you for your attention!

Acknowledgment

The group:

Irena Peri Ph.D
Einav Malach PhD
Vinayak Ghate PhD
Rachel Bochnik-Tamir
Tal Stren
Carmit Ginesin
Avishag Yehuda
Ronen Bostan
Shiri Topman
Eyal Krieger
Yael Palman
Shani Kornhauser

משרד החקלאות ופיתוח הכפר

GIF German-Israeli Foundation for Scientific Research and Development

State of Israel Ministry of Health משרד הבריאות, ישראל

Yissum Hebrew University Technology Transfer

ActInPak

Thank you for your attention!