

Název: **Antimicrobial peptides**

Školitel: **Zbyněk Heger**

Datum: **2. 8. 2013**

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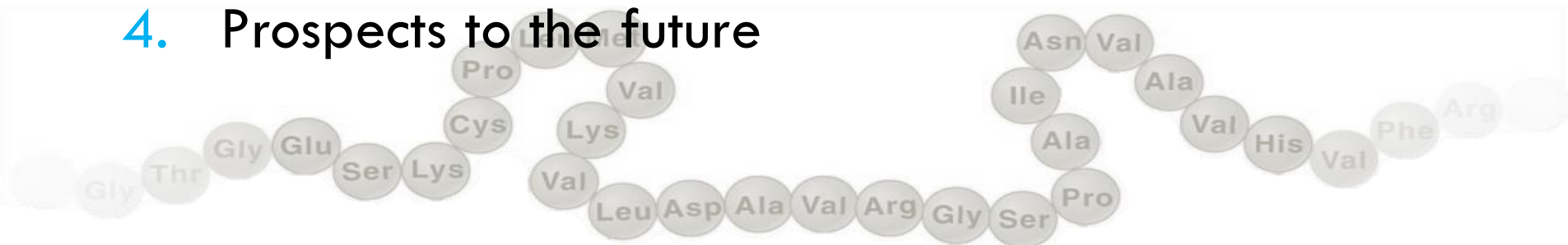
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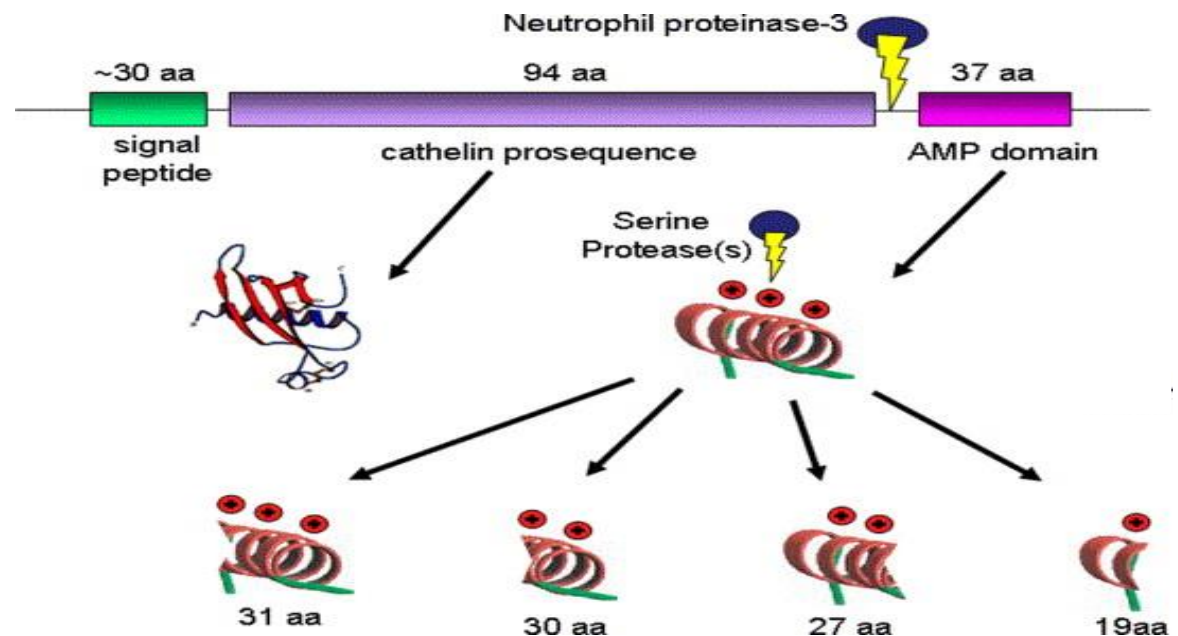
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Antimicrobial Peptides

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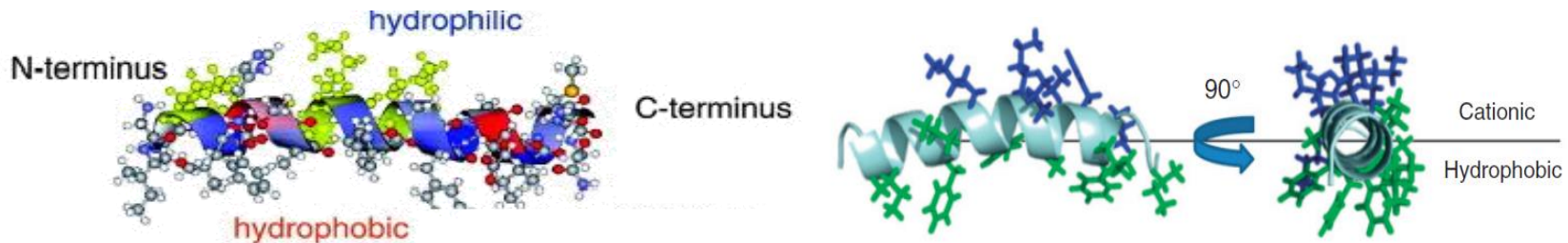
- Ubiquitous in nature,
- described in bacteria, fungi, plants and all vertebrates,
- Important part of mammals immune system,
- nowadays known more than 1000 representatives,
- cationic and anionic peptides.



General Structure of Cationic Peptides

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- Peptides having less than 40 amino acids,
- lysine and arginine residues,
- high content of hydrophobic domains residues,
- lack of information about the structure of most of peptides.



Secondary structures of antimicrobial peptides



β - stranded



α - helical



Extended

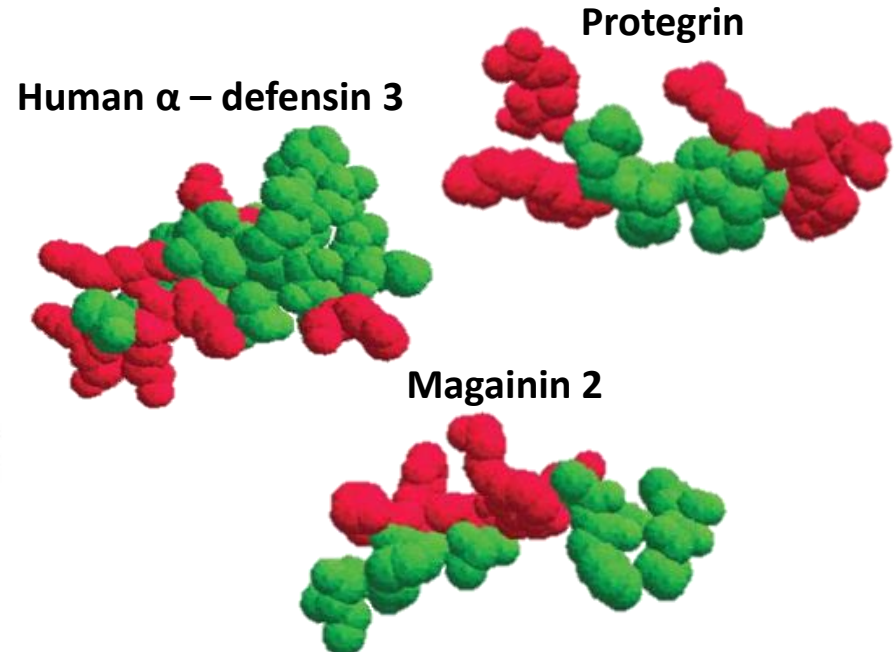
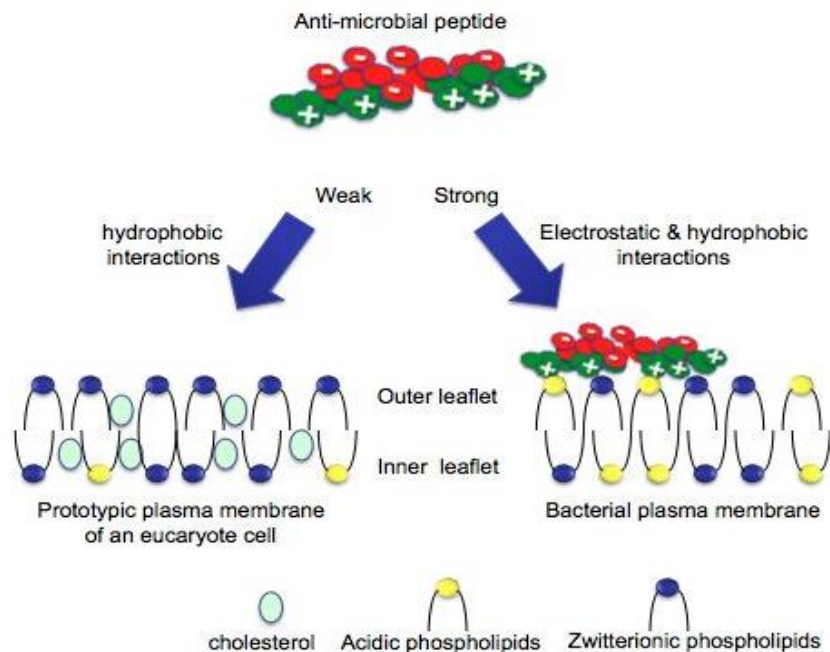


Looped

Chemical Properties

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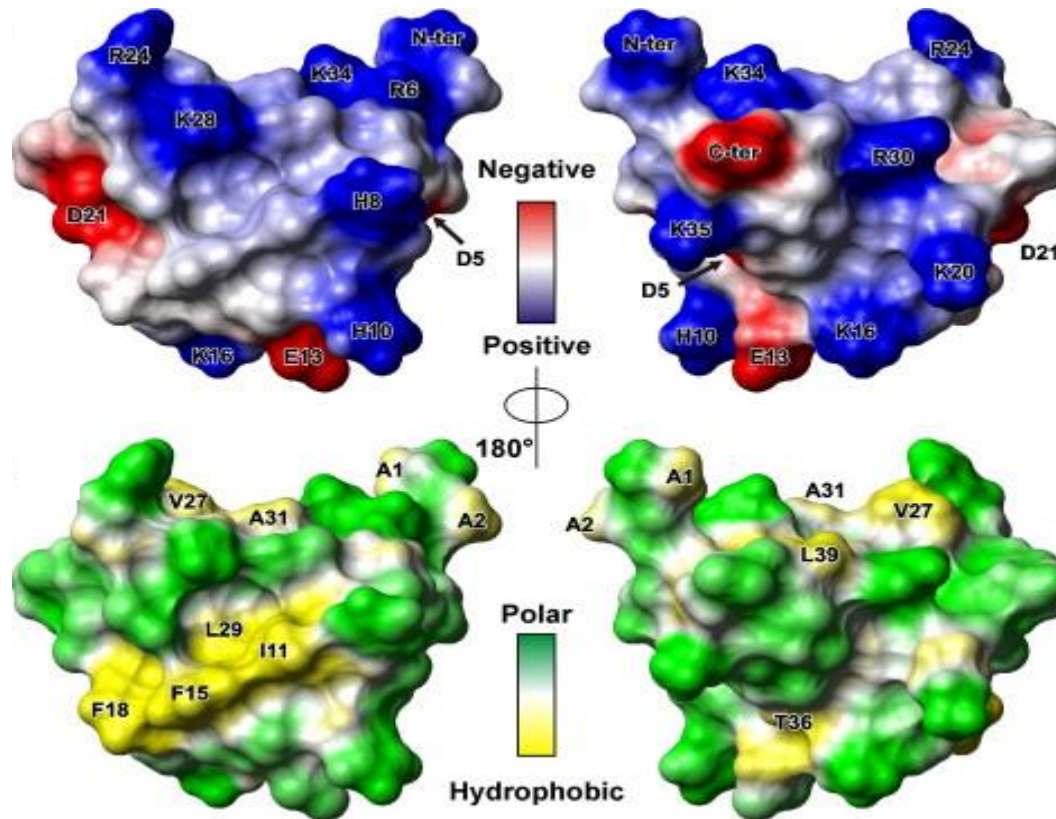
- Positive charge - interaction with the bacterial membrane,
- distinction of microbial cells, based on the composition of the bacterial membrane rich on phospholipids.



Chemical Properties

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Aurelin

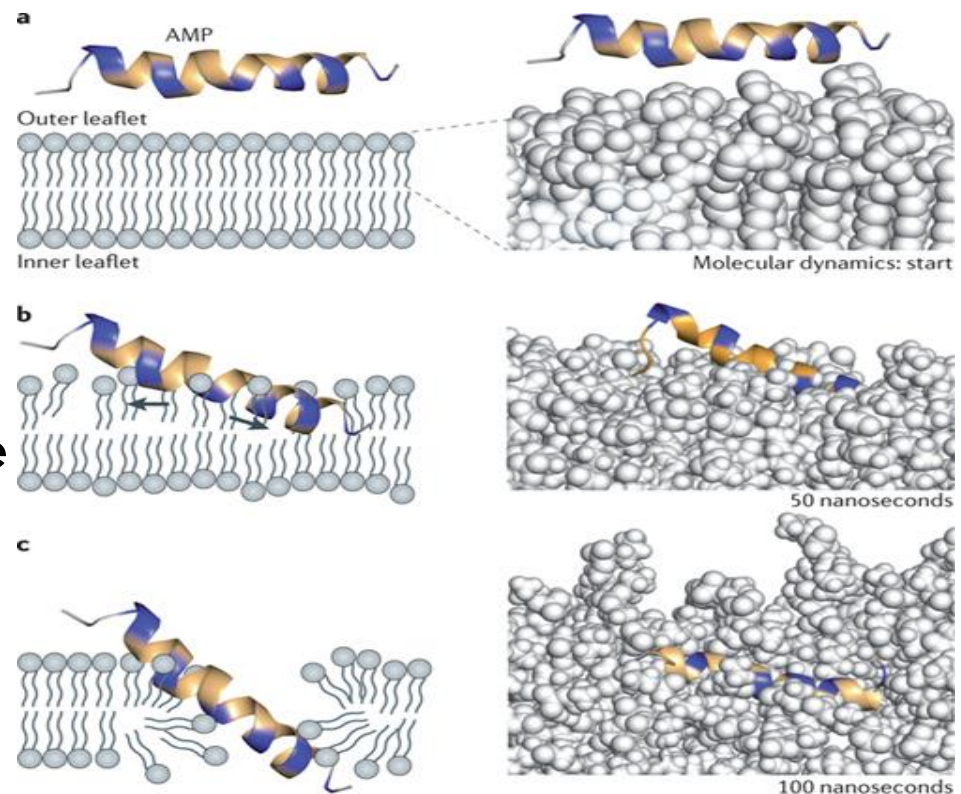


Shenkarev et al. (2012): Recombinant expression and solution structure of antimicrobial peptide aurelin from jellyfish *Aurelia aurita*. *Biochemical and Biophysical Research Communications*: 429 (63-69).

Mechanisms of Action

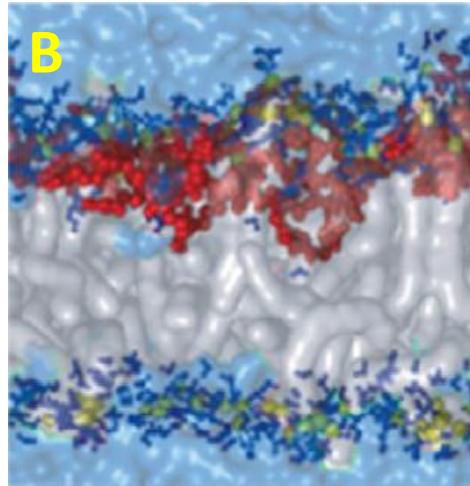
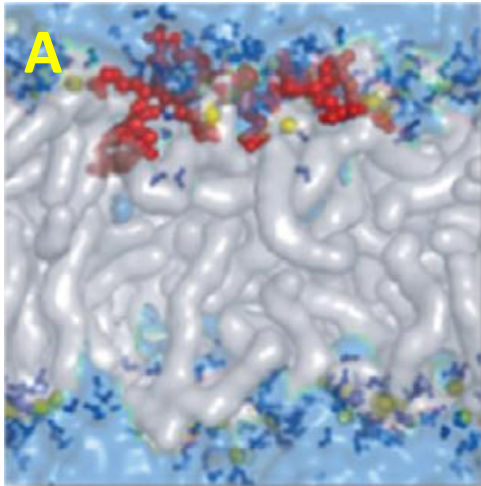
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- Can kill Gram negative and Gram positive bacteria (including resistant strains), mycobacteria, viruses and fungi,
- described also influence on cancerous cells,
- electrostatic interaction with membrane,
- depolarization of membrane and cell death,
- all factors – elusive.



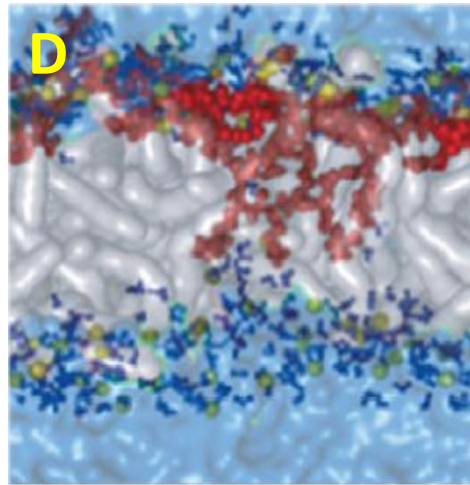
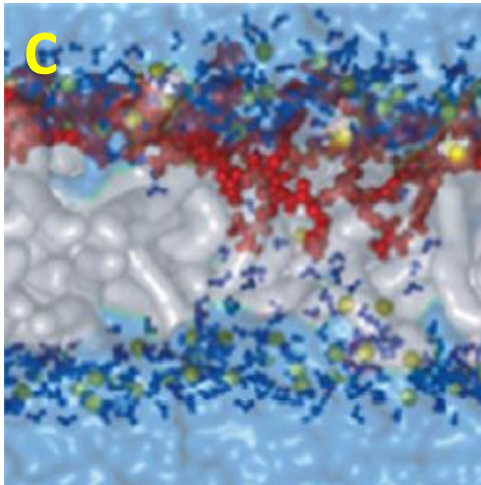
Mechanisms of Action

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A) Conformational changes and arrangement on the membrane

B) Critical concentration – vertical orientation of peptide

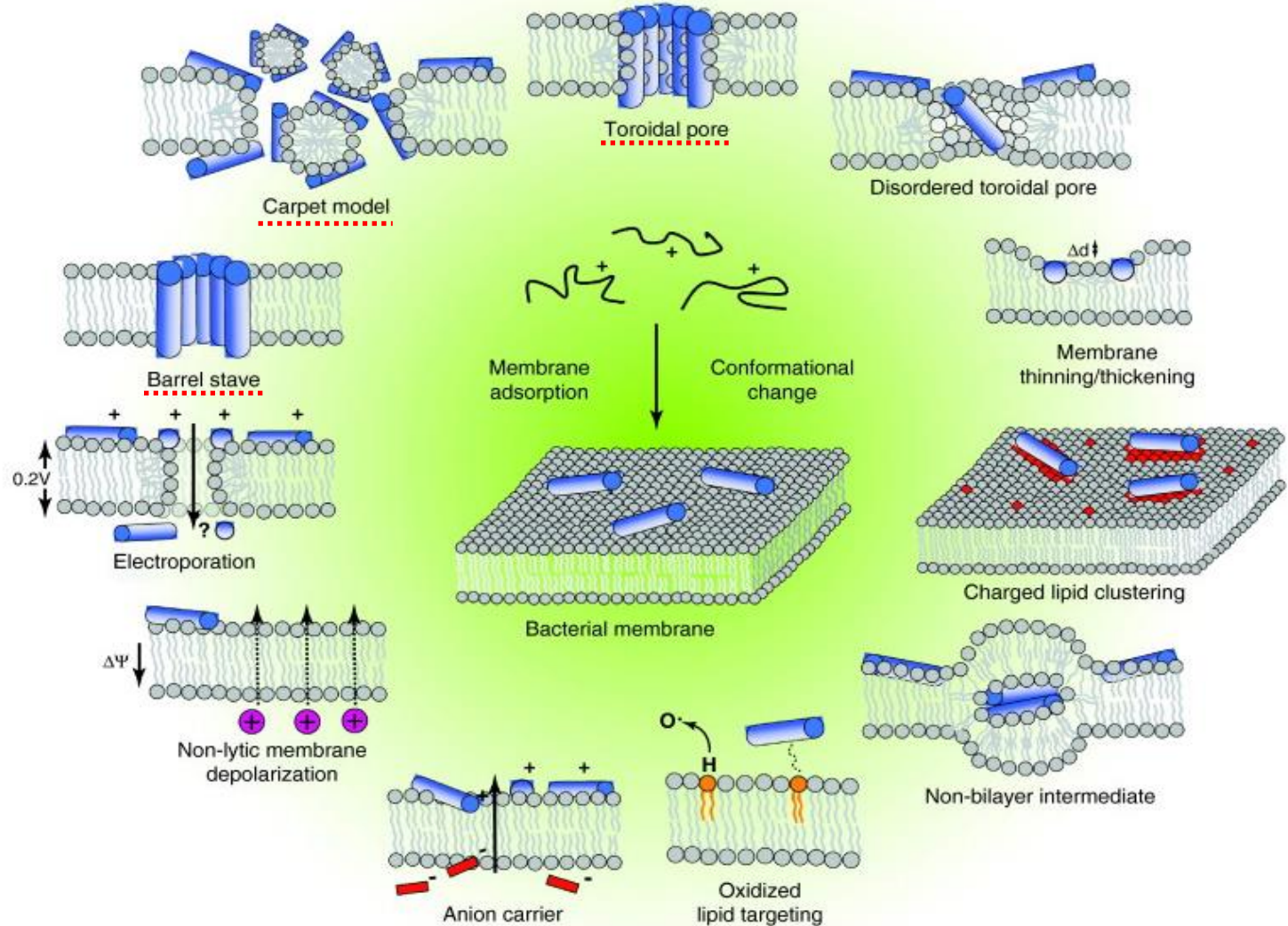


C) Penetrating into hydrophilic part of cell membrane

D) Forming of pores in membrane

Mechanisms of Action

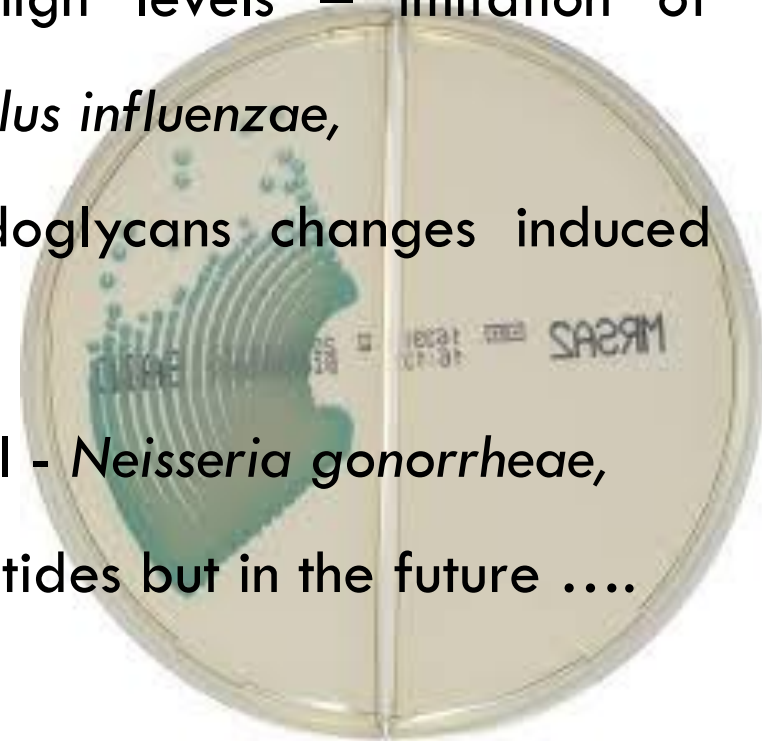
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Possible Resistance

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- Production of proteases – degradation of peptides - *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*,
- Expression of fosfatidylcholin in high levels – imitation of mammal cell membranes - *Haemophilus influenzae*,
- mutation of *mur B* gene – peptidoglycans changes induced resistance - *Staphylococcus aureus*,
- reverse exclusion of peptide from cell - *Neisseria gonorrhoeae*,
- Mechanisms working only at few peptides but in the future



Apidaecins

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- Small, looped, proline-rich peptides composed of 18-20 amino acids,
- many isoforms produced by adult insects, *Hymenoptera* order,
- consist of two regions, the conserved - the general antibacterial capacity, variable - the antibacterial spectrum,
- the most prominent components of the honey bee humoral defense against microbial invasion.



Apidaecins

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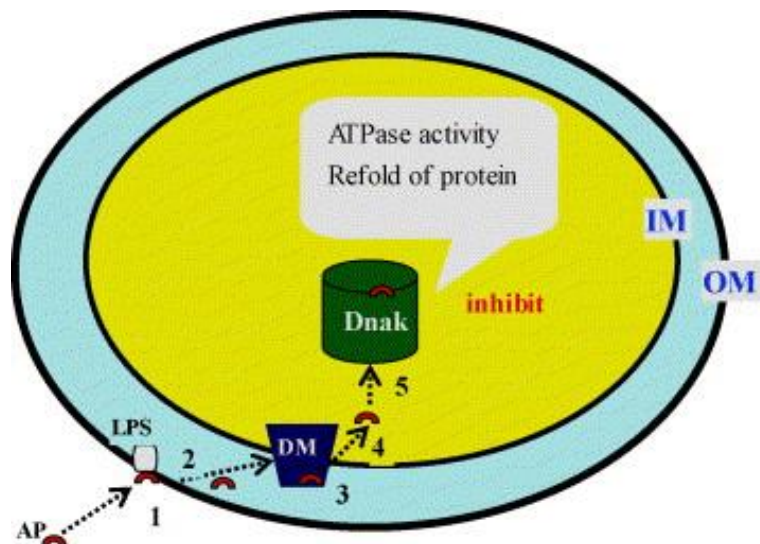
Resources	Isoforms	Peptides sequences	MH+
Honey bee	HbIa	GNNRPVYIPQPRPPHPRI	2109.46
	HbIb	GNNRPVYIPQPRPPHPRL	2109.46
	HbII	GNNRPIYIPQPRPPHPRL	2123.48
	HbIII	GNNRPIYISQPRPPHPRL ***** ** *****	2099.42 (n.a.)
Bumble bee	Bb + A	ANRPVYIPPPRPPHPRL	1978.36
	Bb - A	-NRPVYIPPPRPPHPRL *****	1907.28
Cicada killer	Ck P	NRPTYVPPRPPHPRL	1894.22
	Ck A	NRPTYVPAPRPPHPRL ***** *****	1869.19
Bald-faced hornet	Ho+	GKPRPQQVP-PRPPHPRL	1958.33
	Ho-	--RPQQVP-PRPPHPRL ***** *****	1675.99 (n.a.)
Yellow jackets and German wasps	Yj + S	SNKPRPQQVP-PRPPHPRL	2102.46
	Yj - S	-NKPRPQQVP-PRPPHPRL ***** *****	2015.38
<i>C. disparis</i>	Cd1+	GKPNRPRPAPIQ-PRPPHPRL	2282.72
	Cd1-	---NRPRPAPIQ-PRPPHPRL	2000.38
	Cd2+	GKPNKPRPAPIK-PRPPHPRL	2254.75
	Cd2-	---NKPRPAPIK-PRPPHPRL	1972.4 (n.a.)
	Cd3+	GKPSKPRPAPIK-PRPPHPRL	2227.72
	Cd3-	---SKPRPAPIK-PRPPHPRL ***** *****	1945.38
Conserved sequence of all the isoforms		RP PRPPHPR	

Adopted from Li et al. (2006) Apidaecin-type peptides: Biodiversity, structure–function relationships and mode of action. *Peptides*: 27, 2350-2359.

Properties of Apidaecins

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- Active against a wide range of Gram-negative bacteria,
- penetrate into cell interior through outer and inner bacteria membrane (elusive),
- target molecule – probably bacterial HSP 70 DnaK,
- acting specifically on a bacterial protein and ATPase activity.



AP - Apidacein

LPS – Lipospolyacharide

DM – Docking molecule

IM – Inner membrane

OM – Outer membrane

Our Prospects to the Future

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- Isolation of peptides from honey bees (maybe next two weeks),
- experiments with its antimicrobial (but also antiviral) properties,
- acquiring of peptide from genetically modified bacteria,
- influence of mutagens on bacteria – obtaining of random mutations (maybe with better attributes),
- synthesis of peptides – peptide synthesizer (depends on machine),
- ... transporters or who knows ? 😊



Conclusion

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- Animal toxins contain many type of peptides.
- Most of them exert very interesting properties against different pathogens.
- Bacterial strains have evolved ways to adapt or become resistant to the currently available antibiotics, thus Apidaecins can be used as new candidates of peptide antibiotics lethal mainly to Gram-negative bacteria.
- There exists also potential to destroy viral capsules. Big potential in the treatment of HIV, attributed to melittin.

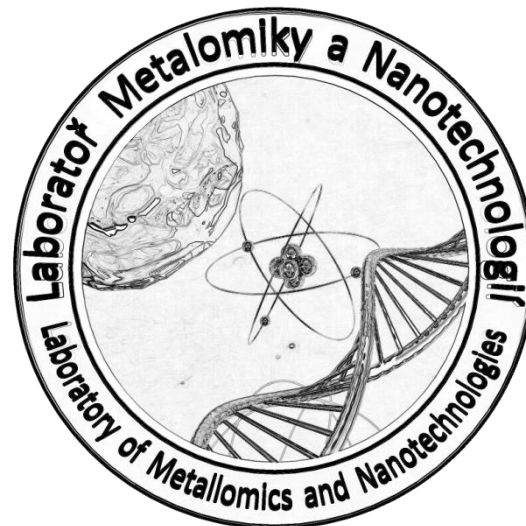
Acknowledgment

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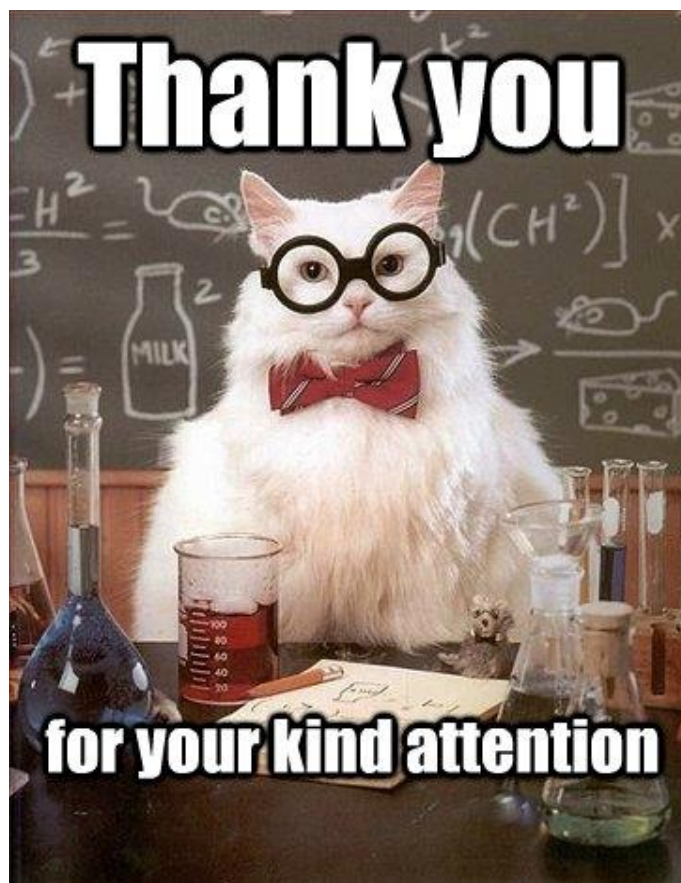
Prof. Ing. René Kizek, Ph.D.

and

Colleagues from



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ



Reg.č.projektu: CZ.1.07/2.4.00/31.0023

Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu