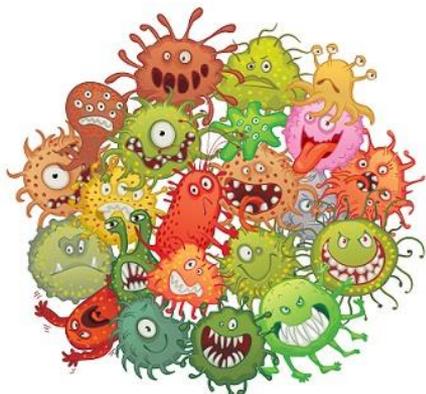




**POLITECNICO
DI TORINO**



Antimicrobial modifications of synthetic polymers

Valeria Chiono

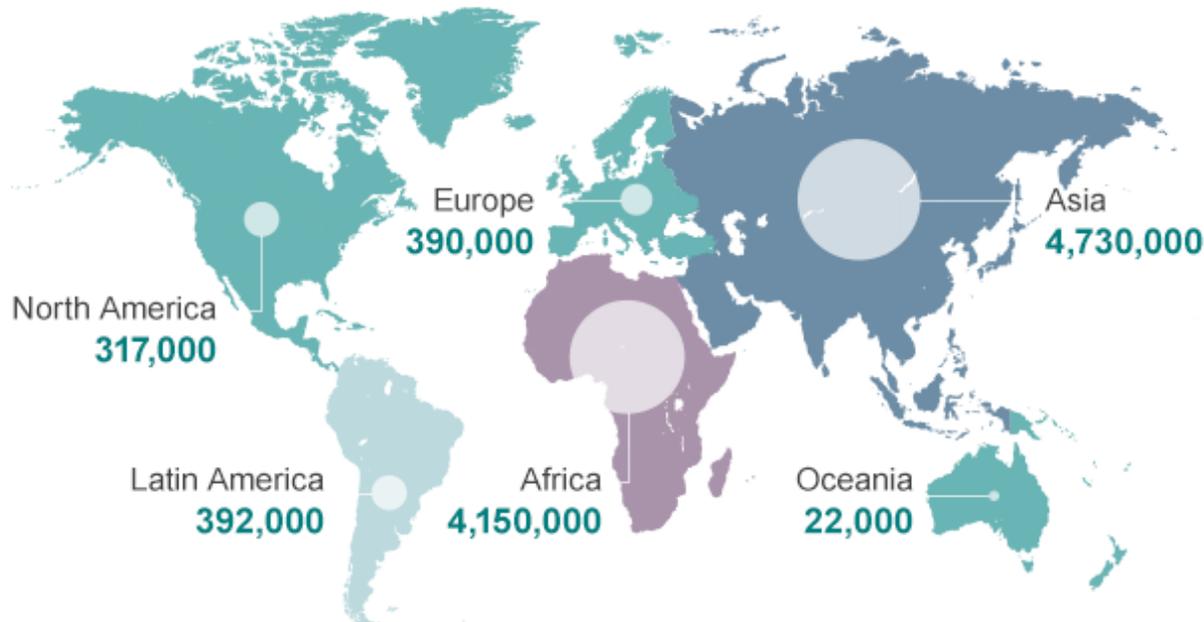
Department of Mechanical and Aerospace Engineering, Politecnico di Torino,
Corso Duca degli Abruzzi 24, 10129 Turin, Italy; valeria.chiono@polito.it

ANTIMICROBIAL POLYMERS

Antimicrobial polymers are needed to avoid bacterial infection, as an alternative to the use of antibiotics, which abuse is the cause of superbug development.

Superbugs, resistant to antimicrobials, are estimated to account for **700,000 deaths** each year.

Deaths attributable to antimicrobial resistance every year by 2050



Source: Review on Antimicrobial Resistance 2014

Antibacterial polymers reduce the extent of attachment and proliferation of bacteria and can be classified as:

- (i) Antibiofouling polymers
- (ii) **Bactericidal polymers**

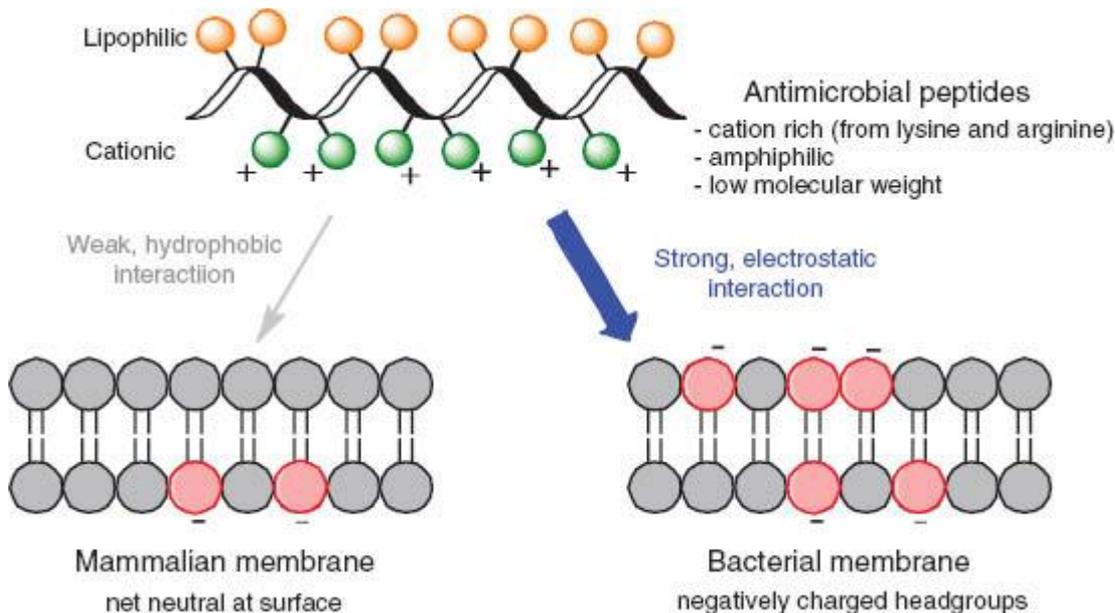
STRATEGIES FOR BACTERICIDAL POLYMERS

1. Polymers that mimic antimicrobial peptides
2. Polymers that interfere with quorum sensing communication in biofilm

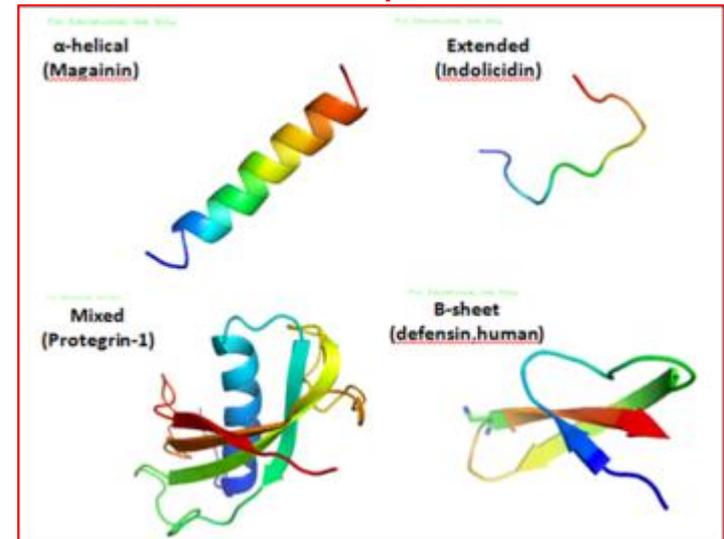
INNOVATIVE STRATEGIES (I)

Polymers that mimic antimicrobial peptides (AMPs)

- The AMPs are biologically active molecules produced by wide variety of organisms as an essential component of their innate immune response.
- The primary role of the AMPs is host defense by exerting cytotoxicity on the invading pathogenic microorganisms
- Currently, more than 2,000 AMPs have been reported in antimicrobial peptide database (<http://aps.unmc.edu/AP/main.php/>).



Examples

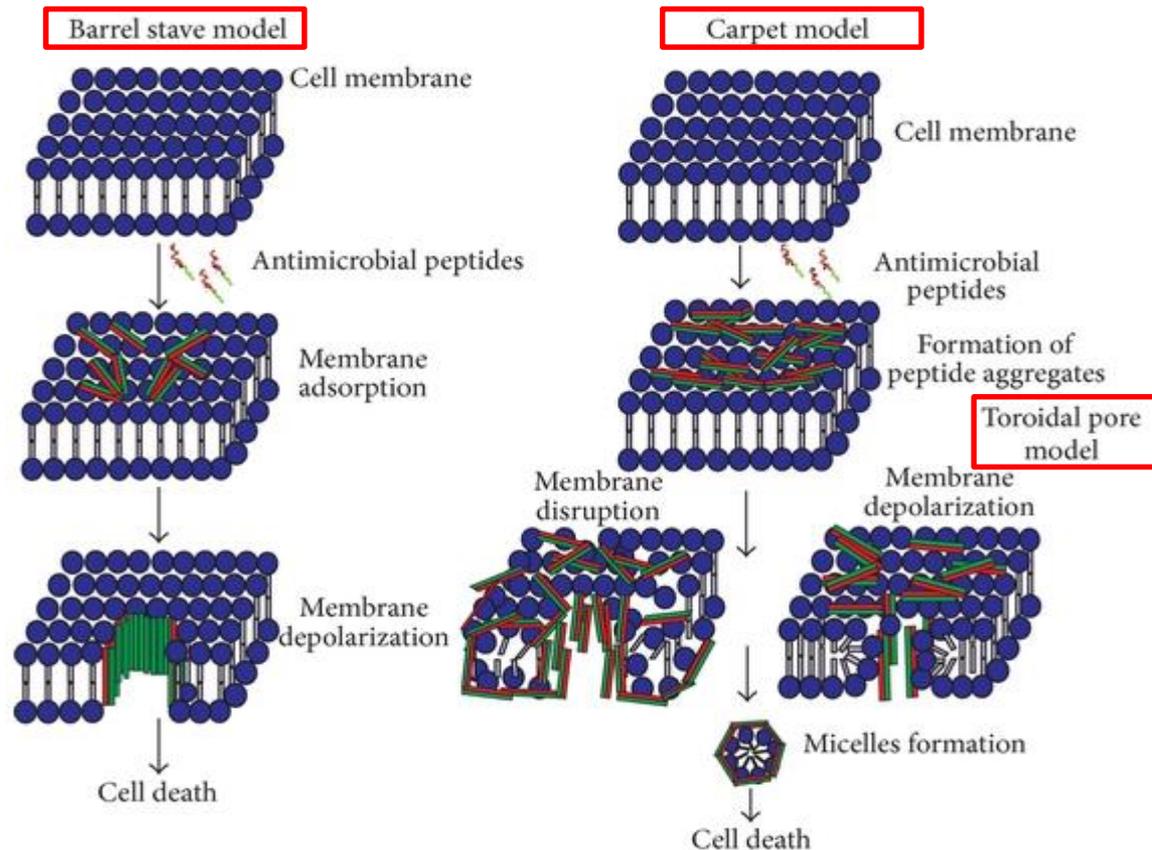


INNOVATIVE STRATEGIES (I)

Polymers that mimic antimicrobial peptides (AMPs)

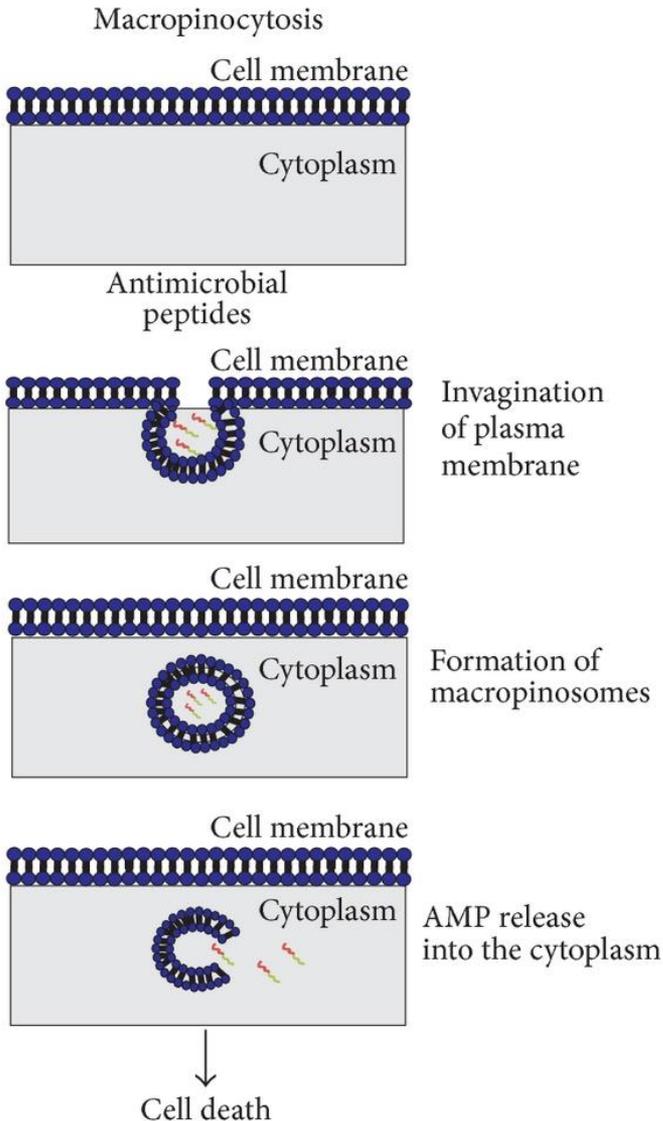
Proposed mechanisms of actions of AMPs:

(a) **Membrane acting peptides** exploit energy (ATP)-independent mechanism: it includes barrel stave model, carpet model, and toroidal pore model.



INNOVATIVE STRATEGIES (I)

Polymers that mimic antimicrobial peptides (AMPs)



Proposed mechanisms of actions of AMPs:
(b) **Non-membrane acting peptides** exploit energy (ATP)-dependent mechanism: it includes **macropinocytosis**

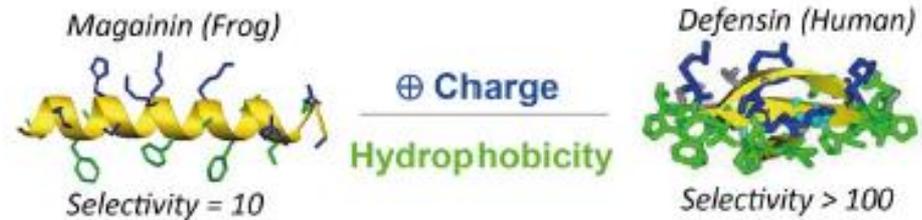
- **AMPs have a bactericidal effect and do not have a specific mechanism reducing the development of bacterial resistance**
- **Antibiotics have specific intracellular targets**

INNOVATIVE STRATEGIES (I)

Polymers that mimic antimicrobial peptides (AMPs)

Advantages of AMPs:

- Broad spectrum activity
- Rapid onset of killing
- Selective for bacteria vs. cells
- Low level of induced resistance
- Broad anti-inflammatory activity



$$\text{Selectivity} = \text{HC}_{50} / \text{MIC}_{90}$$

Disadvantages of AMPs:

- Potential local toxicity
- Haemolytic activity
- Susceptibility to proteolysis
- pH sensitivity
- Sensitization and allergy after repeated applications
- Costs for the synthesis

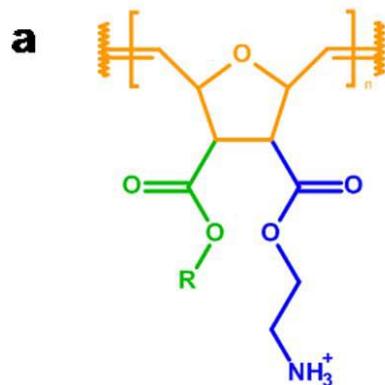
Only few AMPs are in clinical trials.

AMP-mimics polymers have excellent antimicrobial activity, cell selectivity, low resistance formation potential, easy availability, resistance to degradation.

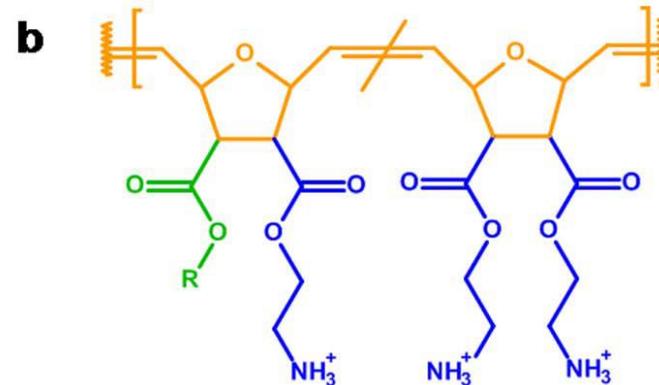
INNOVATIVE STRATEGIES (I)

Polymers that mimic antimicrobial peptides (AMPs)

Poly(oxonorbornene)-based synthetic mimics of antimicrobial peptides (SMAMPs)



- Active against Gram-negative *E. coli* and Gram-positive *Staphylococcus aureus*
- Non-cell selective



- New improved copolymer with increased cell compatibility due to superior hydrophilicity (additional diamine repeating unit)

a) SMAMPs with tunable antimicrobial activity and cell-compatibility can be obtained by varying the hydrophobic groups (green, R = methyl to hexyl) and hydrophilic groups (blue) that are attached to the poly(oxonorbornene backbone) (orange) [1].

b) Structure of the new SMAMP copolymers [2].

[1] K. Lienkamp et al. *J. Am. Chem. Soc.* 2008; 130: 9836.

[2] Ali Al-Ahmad et al. *PLOS One* 2013; 8: e73812

INNOVATIVE STRATEGIES (I)

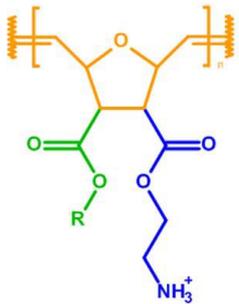
Polymers that mimic antimicrobial peptides (AMPs)

Poly(oxonorbornene)-based synthetic mimics of antimicrobial peptides (SMAMPs)

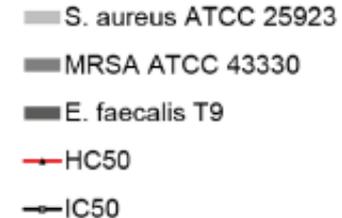
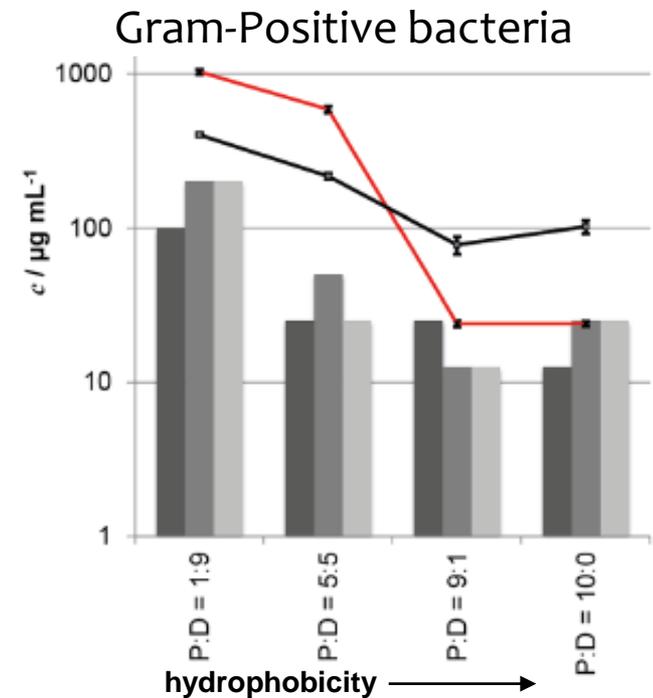
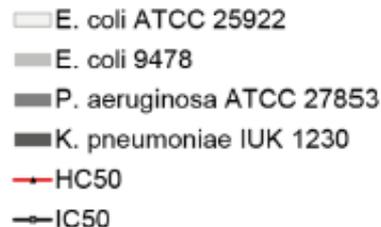
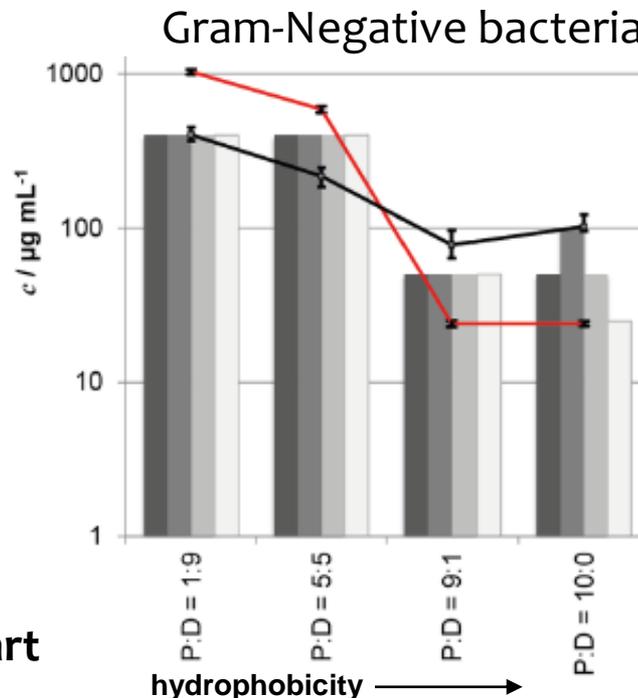
Antimicrobial activity (MIC) and biocompatibility (HC50 and IC50)

Tested SMAMP:

a



P: propyl (R)
D: hydrophilic part



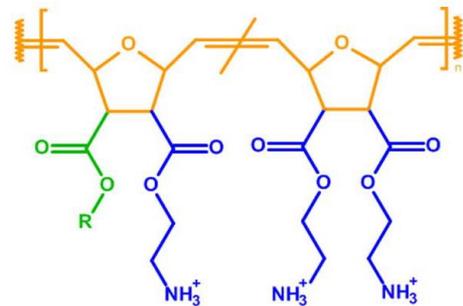
INNOVATIVE STRATEGIES (I)

Polymers that mimic antimicrobial peptides (AMPs)

Poly(oxonorbornene)-based synthetic mimics of antimicrobial peptides (SMAMPs)

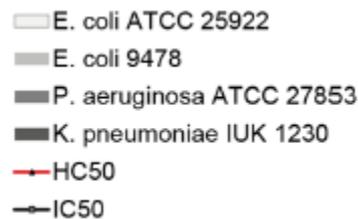
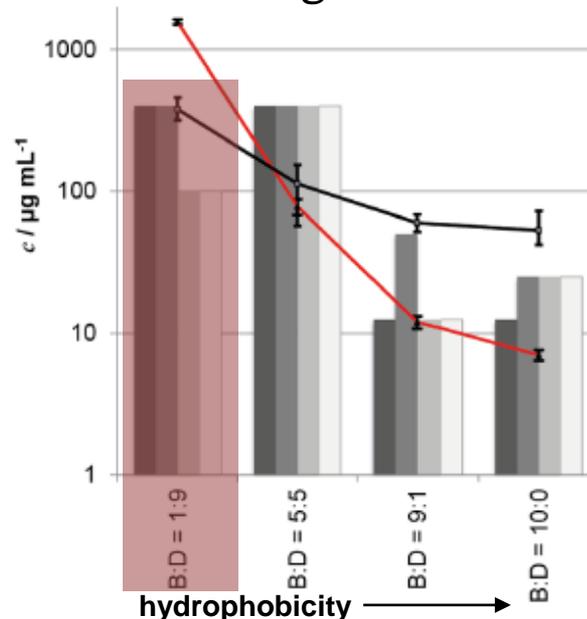
Antimicrobial activity (MIC) and biocompatibility (HC50 and IC50)

Tested SMAMP:

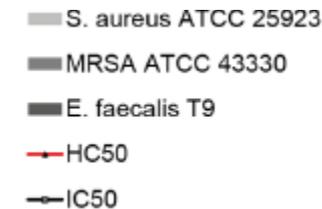
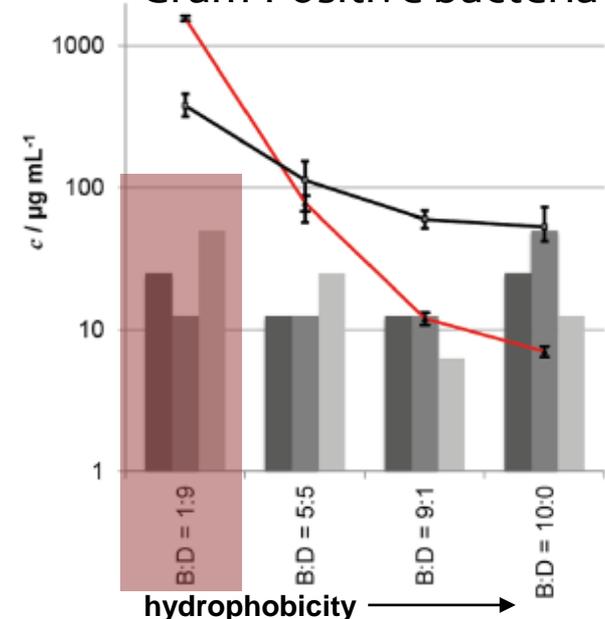


B: butyl (R)
D: hydrophilic part

Gram-Negative bacteria



Gram-Positive bacteria



INNOVATIVE STRATEGIES (I)

Polymers that mimic antimicrobial peptides (AMPs)

Poly(oxonorbornene)-based synthetic mimics of antimicrobial peptides (SMAMPs)

Transmission electron micrographs of bacteria stained, embedded in a Durcopan matrix, and microtomed.

Untreated bacteria:

- dense structures
- continuous cell envelopes

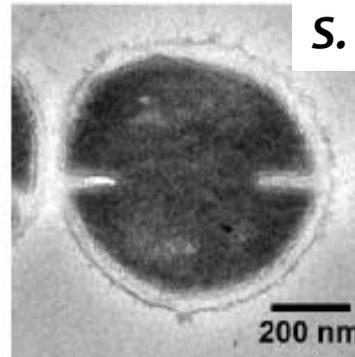
Treated *S. Aureus*:

- fuzzy peptidoglycan layer
- discontinuous membranes
- less dense cell structure

Treated *E. Coli*:

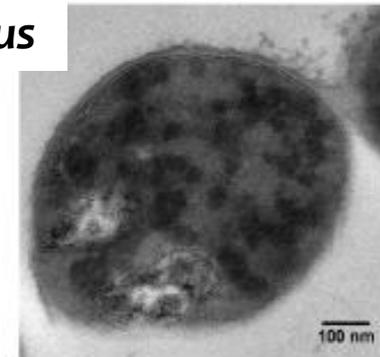
- Several concave notches
- Significant loss of cell content
- Disintegrated cell envelopes

Untreated

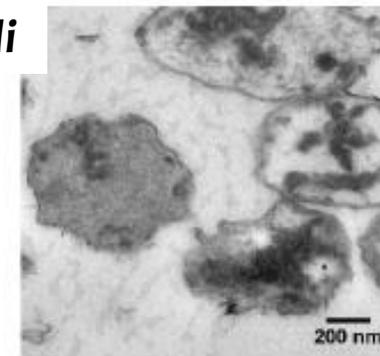
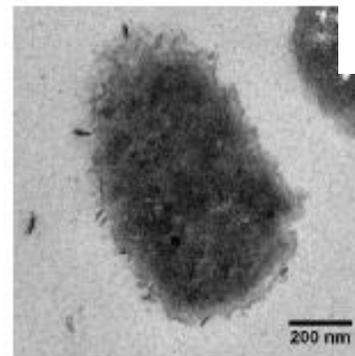


S. aureus

Incubation with SMAMPs
(P:D = 10:0, 100 µg/mL, 4 hours, at 37°C).



E. coli



INNOVATIVE STRATEGIES (I)

Polymers that mimic antimicrobial peptides (AMPs)

Structurally nanoengineered antimicrobial peptide polymers (SNAPPs)

nature
microbiology

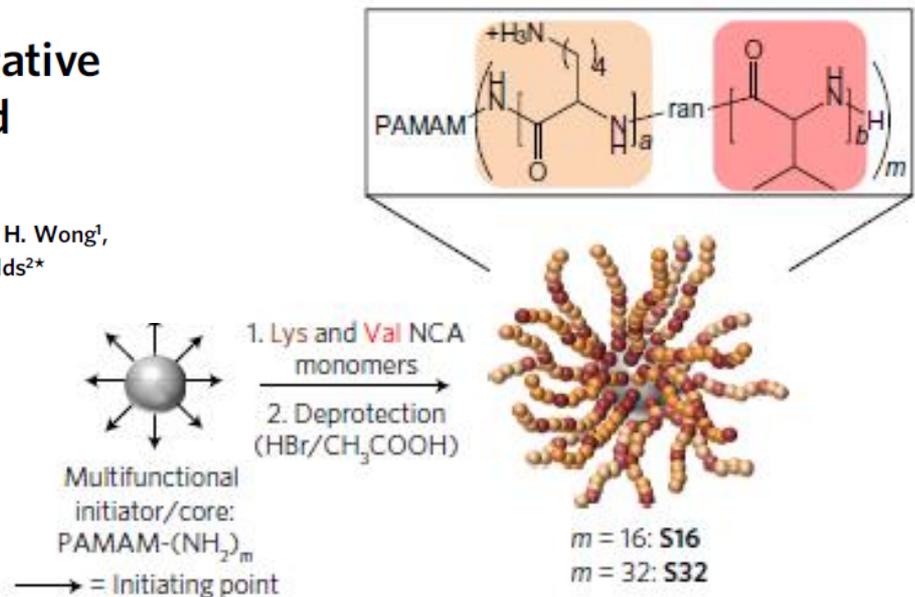
ARTICLES

PUBLISHED: 12 SEPTEMBER 2016 | ARTICLE NUMBER: 16162 | DOI: 10.1038/NMICROBIOL.2016.162

Combating multidrug-resistant Gram-negative bacteria with structurally nanoengineered antimicrobial peptide polymers

Shu J. Lam¹, Neil M. O'Brien-Simpson², Namfon Pantarat², Adrian Sulistio¹, Edgar H. H. Wong¹, Yu-Yen Chen², Jason C. Lenzo², James A. Holden², Anton Blencowe^{1,3}, Eric C. Reynolds^{2*} and Greg G. Qiao^{1*}

- Poly(amido amine) (PAMAM) dendrimer core with 16 and 32 peripheral primary amines
- Synthesis via ring-opening polymerization of lysine and valine N-carboxyanhydrides (NCAs), initiated from the terminal amines of PAMAM dendrimers.
- Two nanostructures with 16- and 32-arm formed (S16 and S32)



Hydrodynamic diameter (D_H):
S16 = 7.7 nm
S32 = 13.5 nm

Lysine to valine ratio: 2:1
30 residues per arm

INNOVATIVE STRATEGIES (I)

Polymers that mimic antimicrobial peptides (AMPs)

Structurally nanoengineered antimicrobial peptide polymers (SNAPPs)

Minimum bactericidal concentration (MBC)

Table 1 | Antimicrobial activity of SNAPPs and other peptides against a range of Gram-negative pathogens.

Antimicrobial type	Code/name	Medium	MBC* (μM)					
			<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>A. baumannii</i>	CMDR <i>P. aeruginosa</i>	CMDR <i>A. baumannii</i>
SNAPP	S16	MHB	0.72 ± 0.06	1.42 ± 0.08	1.54 ± 0.08	0.85 ± 0.05	1.38 ± 0.03	1.61 ± 0.23
		MEM	0.17 ± 0.01	0.07 ± 0.04	0.19 ± 0.05	0.05^\dagger	0.08^\dagger	0.05 ± 0.01
	S32	MHB	0.72 ± 0.54	0.97 ± 0.05	0.83 ± 0.14	0.79 ± 0.02	1.00^\dagger	0.85 ± 0.03
		MEM	0.05 ± 0.01	0.02^\dagger	0.08 ± 0.02	0.02^\dagger	0.03 ± 0.01	0.03 ± 0.01
AMP	Ovispirin [‡]	MHB	8.39 ± 0.44	95.49 ± 9.73	11.49 ± 4.86	2.21 ± 0.88	Not tested	Not tested
	Magainin II [‡]	MHB	47.85 ± 6.08	55.96 ± 2.84	154.59 ± 9.32	19.87 ± 3.24	Not tested	Not tested
	Melittin [‡]	MHB	33.71 ± 5.18	29.37 ± 8.24	109.25 ± 20.43	0.91 ± 0.09	Not tested	Not tested

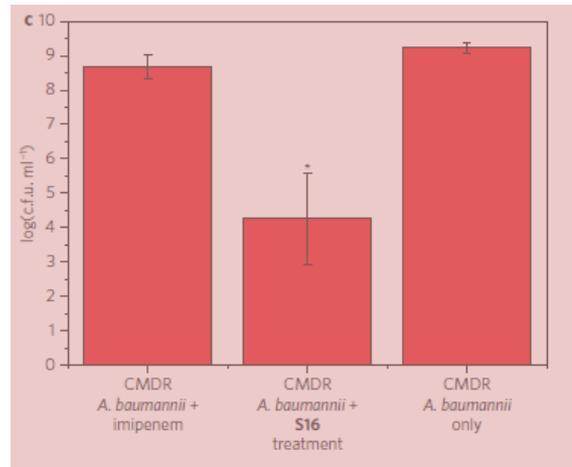
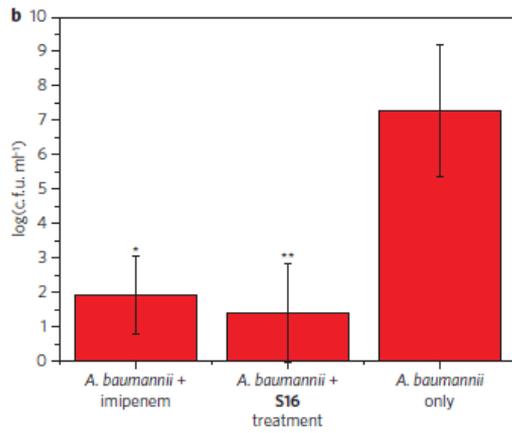
- MBC of SNAPPs for gram-negative species < 1.61 μM
- SNAPP antimicrobial activity not selective for certain gram-negative bacteria while AMPs were selective.
- Effective also against colistin-resistant and multidrug resistant (CMDR) bacteria
- Resistant mechanisms were not developed after 24 days sub-MBC administration
- Efficacy was due to the star architecture with amphiphilic and charged arms (similar linear peptides showed CMCs 40-fold higher): high concentration of charges and aggregation at the bacterial membrane leading to its disruption
- Biocompatibility of SNAPPs: not-inducing hemolysis at 100xMBC concentration; biocompatible with two types of mammalian cells (human embryonic kidney cells and rat hepatoma cells)

INNOVATIVE STRATEGIES (I)

Polymers that mimic antimicrobial peptides (AMPs)

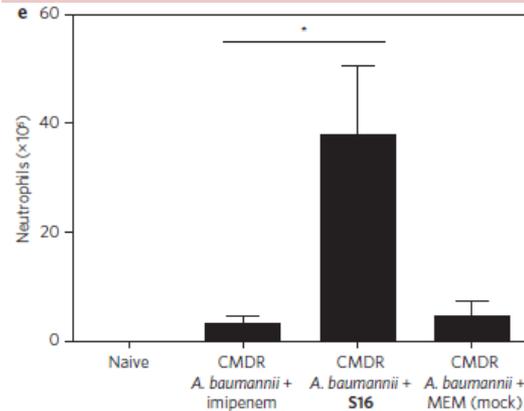
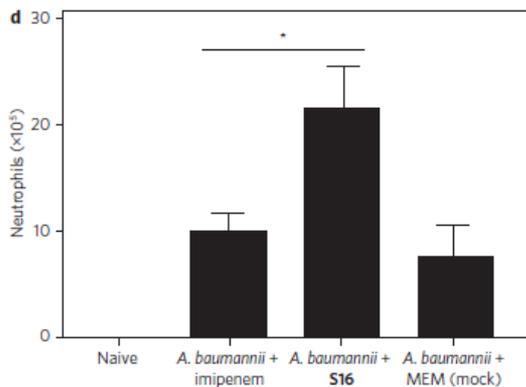
Structurally nanoengineered antimicrobial peptide polymers (SNAPPs)

In vivo efficacy of SNAPPs in mouse peritoneal model



S16 caused:

- 99% eradication of bacteria in blood;
- > 5-Log CFU reduction of bacteria in the peritoneal cavity
- > 3-Log CFU reduction in spleen
- **Recruitment of neutrophils able to release antimicrobial peptides**



First example of polymer able to treat CMDR gram-negative bacteria infections.

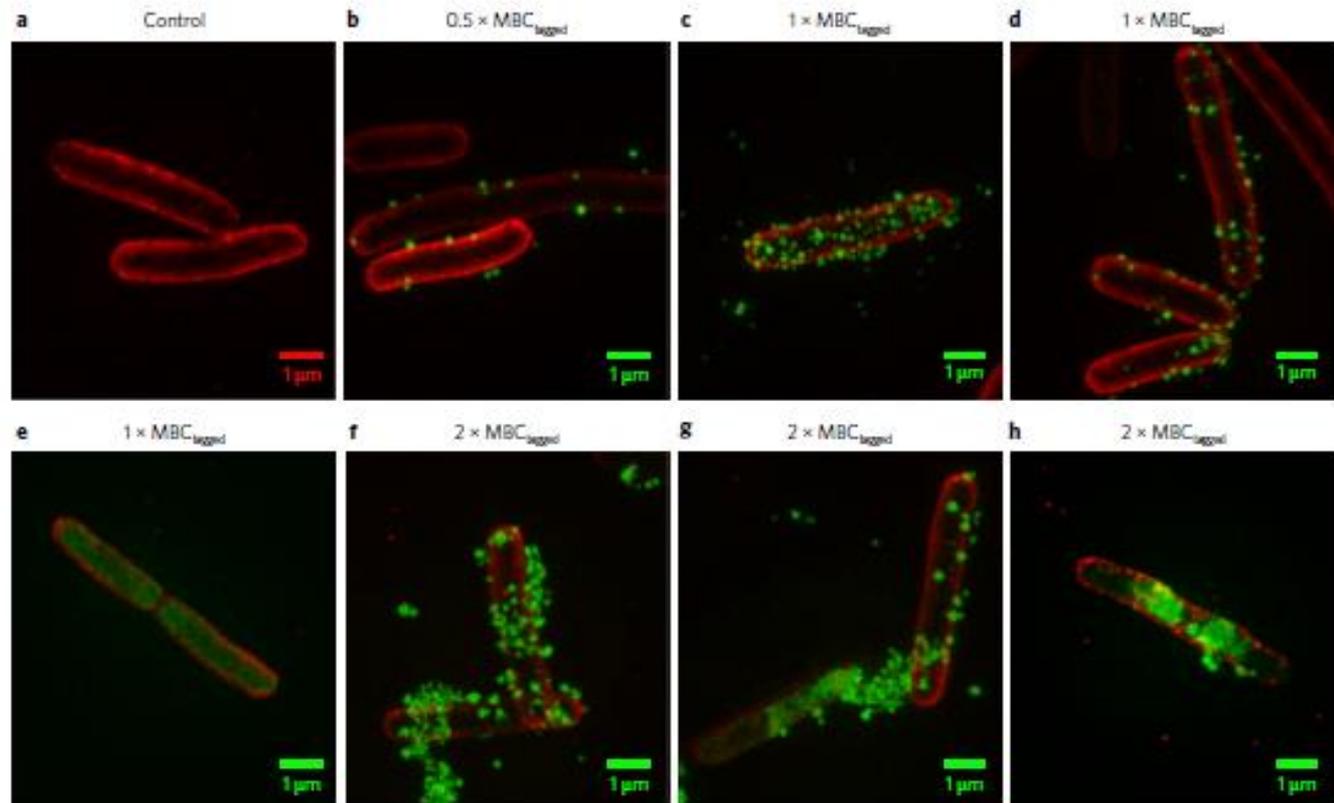
INNOVATIVE STRATEGIES (I)

Polymers that mimic antimicrobial peptides (AMPs)

Structurally nanoengineered antimicrobial peptide polymers (SNAPPs)

Mechanism of action of SNAPPs against gram-negative bacteria

3D structured illumination microscopy



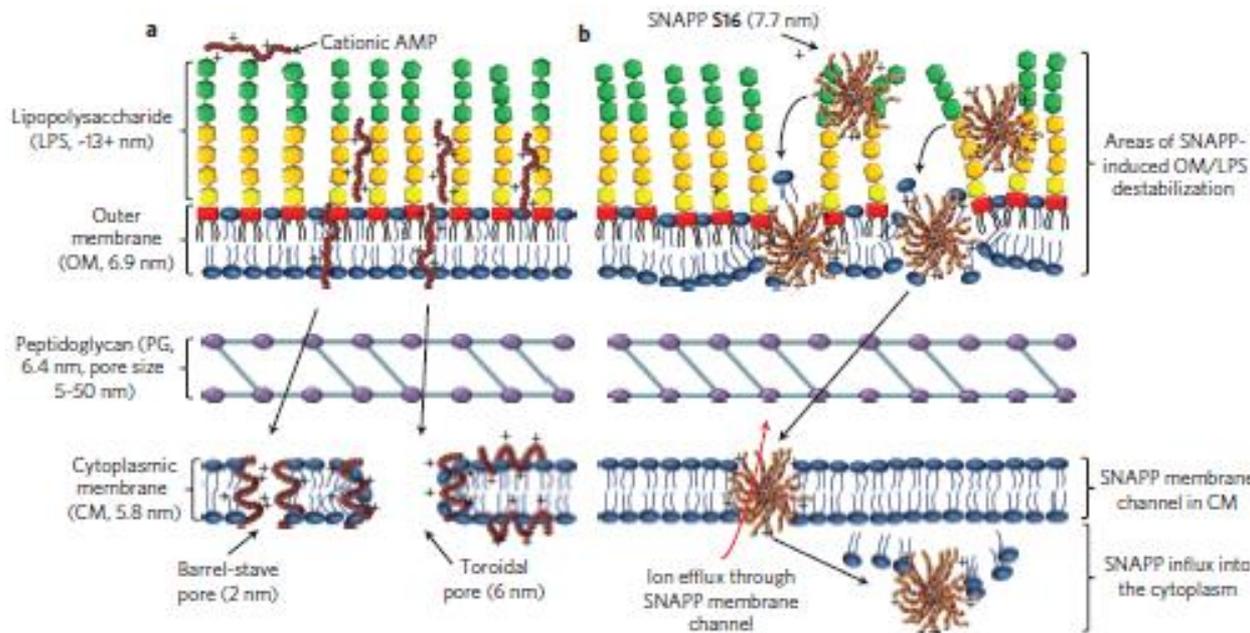
E. Coli in red
SNAPP in green

INNOVATIVE STRATEGIES (I)

Polymers that mimic antimicrobial peptides (AMPs)

Structurally nanoengineered antimicrobial peptide polymers (SNAPPs)

Mechanism of action of SNAPPs against gram-negative bacteria



- ❖ SNAPPs interact with OM, PG and CM layers and kill the cell by fragmenting or destabilising the OM and disrupting CM (unregulated ion movement occurs)
- ❖ Additional induction of apoptotic death pathway lysing the cell

- ❑ AMPs kill bacteria by multiple mechanisms but have one major mechanism to kill bacteria
- ❑ SNAPPs kill bacterial by multiple mechanisms, hence bacteria cannot acquire resistance against them

INNOVATIVE STRATEGIES (II)

Polymers that interfere with quorum sensing (QS)

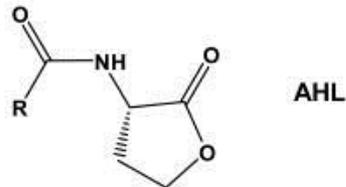
- ❑ **Quorum sensing (QS)** is the complex process by which micro-organisms present within biofilms communicate each other.
- ❑ QS is mediated by small signaling molecules, called **autoinducers**, secreted by microorganisms, that allows bacteria to detect their local population density and to coordinate their gene expression.
- ❑ When autoinducers exceed a concentration threshold, bacteria respond by modulating their functions.
- ❑ Autoinducers comprise:
 - (i) **Oligopeptides** in Gram-positive bacteria
 - (ii) **N-acyl homoserine lactone (acyl-HSL)** in Gram-negative bacteria
 - (iii) **Autoinducer-2 (AI-2)** present in both types.

INNOVATIVE STRATEGIES (II)

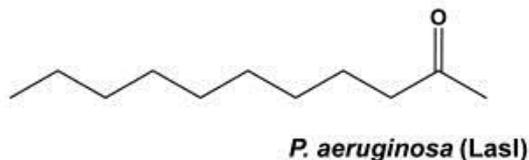
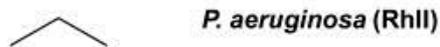
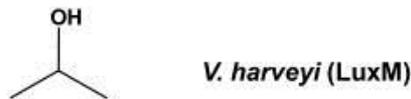
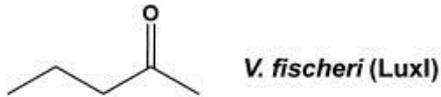
Polymers that interfere with quorum sensing (QS)

Examples of autoinducers:

**N-acyl
homoserine
lactone
autoinducers**
produced by
different
Gram-
negative
bacteria



R group



ADPITRQWGD

B. subtilis (ComX)

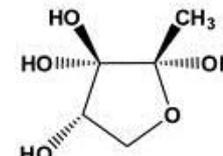
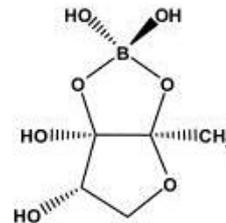
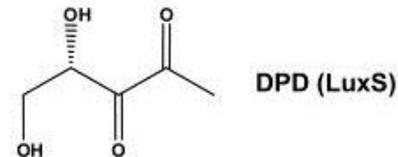
**Three peptide
autoinducers,**
produced by
Gram-positive
bacteria

ERGMT

B. subtilis (CSF)

EMRLSKFFRDFILQRKK

S. pneumoniae (CSP)



**DPD, the
precursor to AI-2.**
In the presence
of boron, AI-2
exists as S-THMF-
borate. In the
absence of
boron, AI-2 exists
as R-THMF.

INNOVATIVE STRATEGIES (II)

Polymers that interfere with quorum sensing (QS)

Bacterial function under QS control include:

Surface attachment

Extracellular polymer production

Biosurfactant synthesis

Sporulation

Competence

Bioluminescence

Secretion of nutrient sequestering compounds

Secretion of virulence factors

INNOVATIVE STRATEGIES (II)

Polymers that interfere with quorum sensing (QS)

Main strategies to affect QS:

- Use of enzymes with quorum-quenching activity
- Addition of a competitive molecule able to interfere with autoinducer receptors, including:
 - 1) N-Acyl homoserine lactone analogues
 - 2) Furanones

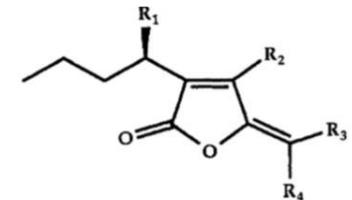
For instance, copolymers based on styrene and furanone have been synthesised or furanone was covalently grafted on the surface [5].

In Nature, *Delisa pulchra*, an endemic species of red algae from southeastern Australia, secretes anti-quorum substances to inhibit bacterial colonization on its surface.

(b) chemical secretions



red seaweed (*Delisea pulchra*)



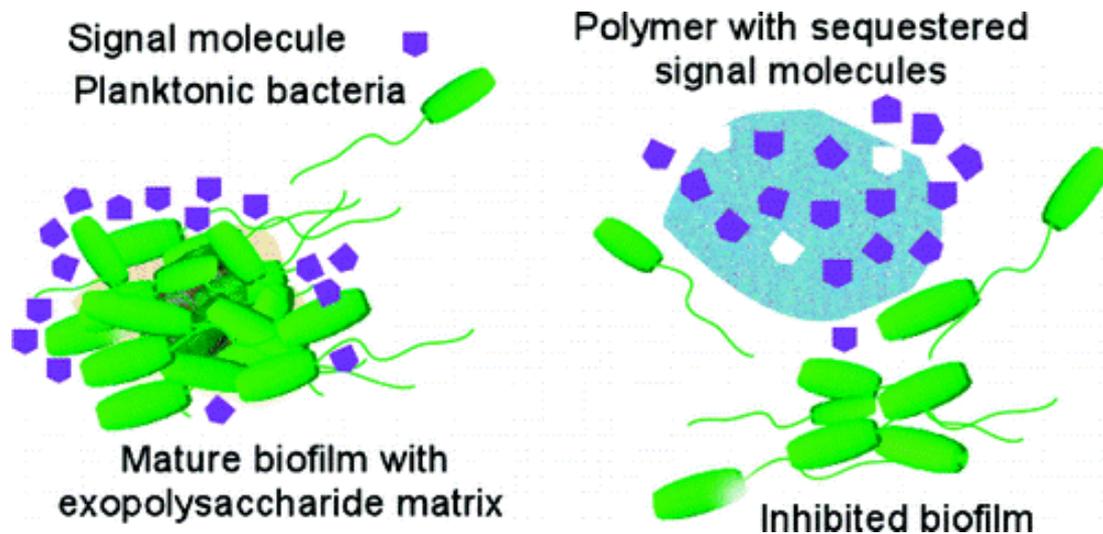
	R1	R2	R3	R4
compound 1	H	Br	Br	Br
compound 2	H	Br	H	Br
compound 3	OAc	Br	H	Br
compound 4	OH	Br	H	Br
compound 5	OAc	Br	H	I
compound 8	H	H	Br	Br

INNOVATIVE STRATEGIES (II)

Polymers that interfere with quorum sensing (QS)

Main strategies to affect QS:

- Molecular imprinting strategy: engineering of crosslinked polymers with cavities for capturing specific autoinducers.

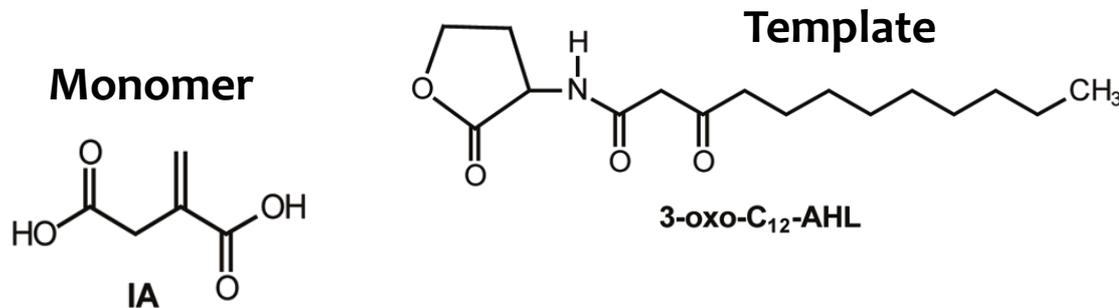


INNOVATIVE STRATEGIES (II)

Polymers that interfere with quorum sensing (QS)

Main strategies to affect QS:

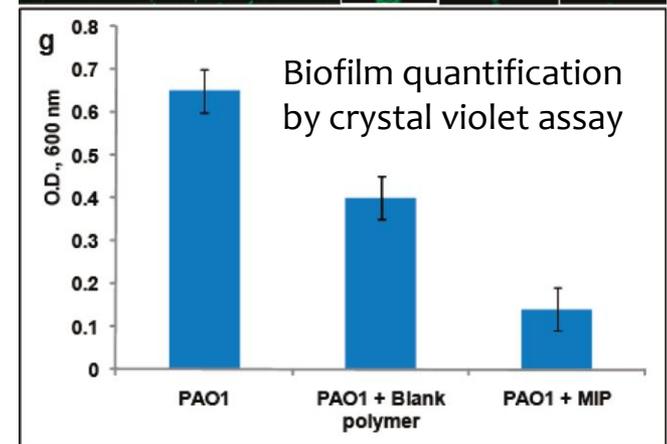
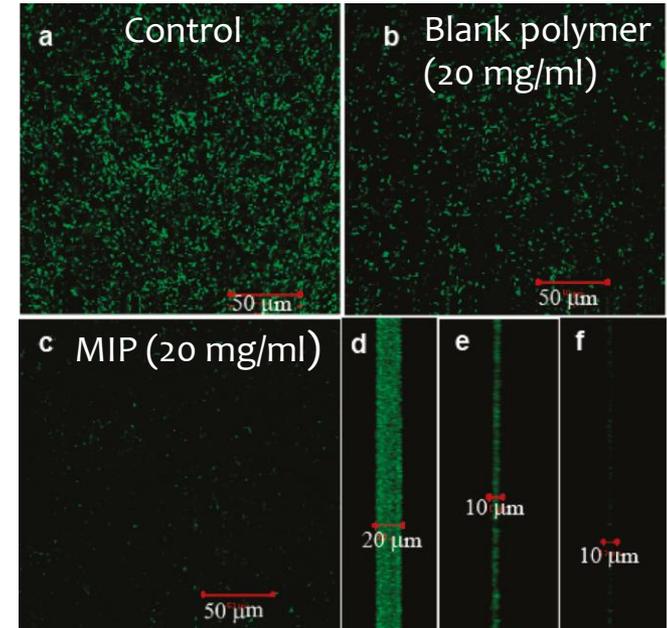
- Molecular imprinting strategy: engineering of crosslinked polymers with cavities for capturing specific autoinducers.



P. Aeruginosa biofilm

Biofilms were visualized by staining with fluorescent wheat germ agglutinin-Alexa Fluor 488 conjugate (WGA) that binds specifically to N-acetylglucosaminyl and sialic acid residues and quantified by crystal violet assay.

Biofilm on glass slide



CONCLUSIONS

- For **polymers mimicking AMPs**, it is important to tailor the hydrophilicity to hydrophobicity ratio (biocompatibility vs. antimicrobial activity)
- **Polymers mimicking AMPs in the form of armed nanoparticles** are particularly effective, due to their high charge density.
- **MIP can be exploited to interfere with QS** avoiding the use of potentially toxic autoinducer inhibitors such as furanones

Such alternative strategies to antibiotics have the advantage not to induce resistance mechanisms.

Thank you for the attention

