



THINKING OUT OF THE BOX TO BEAT BIOFILMS

Activity of drug combinations against staphylococcal biofilms

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Disclosures

- Research grants for studies on biofilms (last 5 years) : Cempra, Melinta therapeutics
- Common research programs with OneLife, subsidized by the *Region Wallonne*, Belgium
- Research grants for studies on other topics (last 5 years): Debiopharm, GSK, Merlion pharmaceuticals, SMB
- Advisory board: Bayer

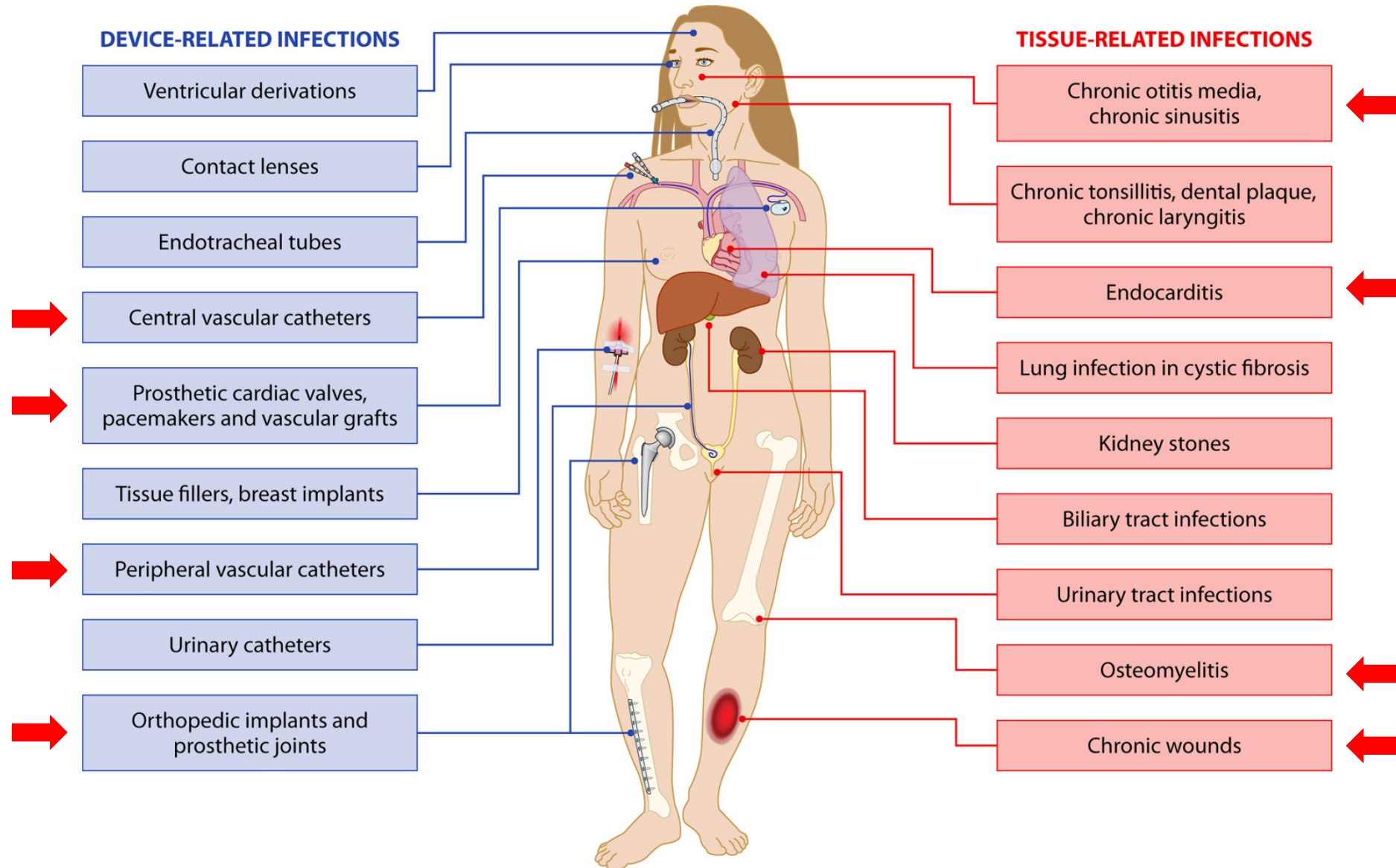
Staphylococcal biofilms



Staphylococcal biofilms ...a new point of view on how to cure them

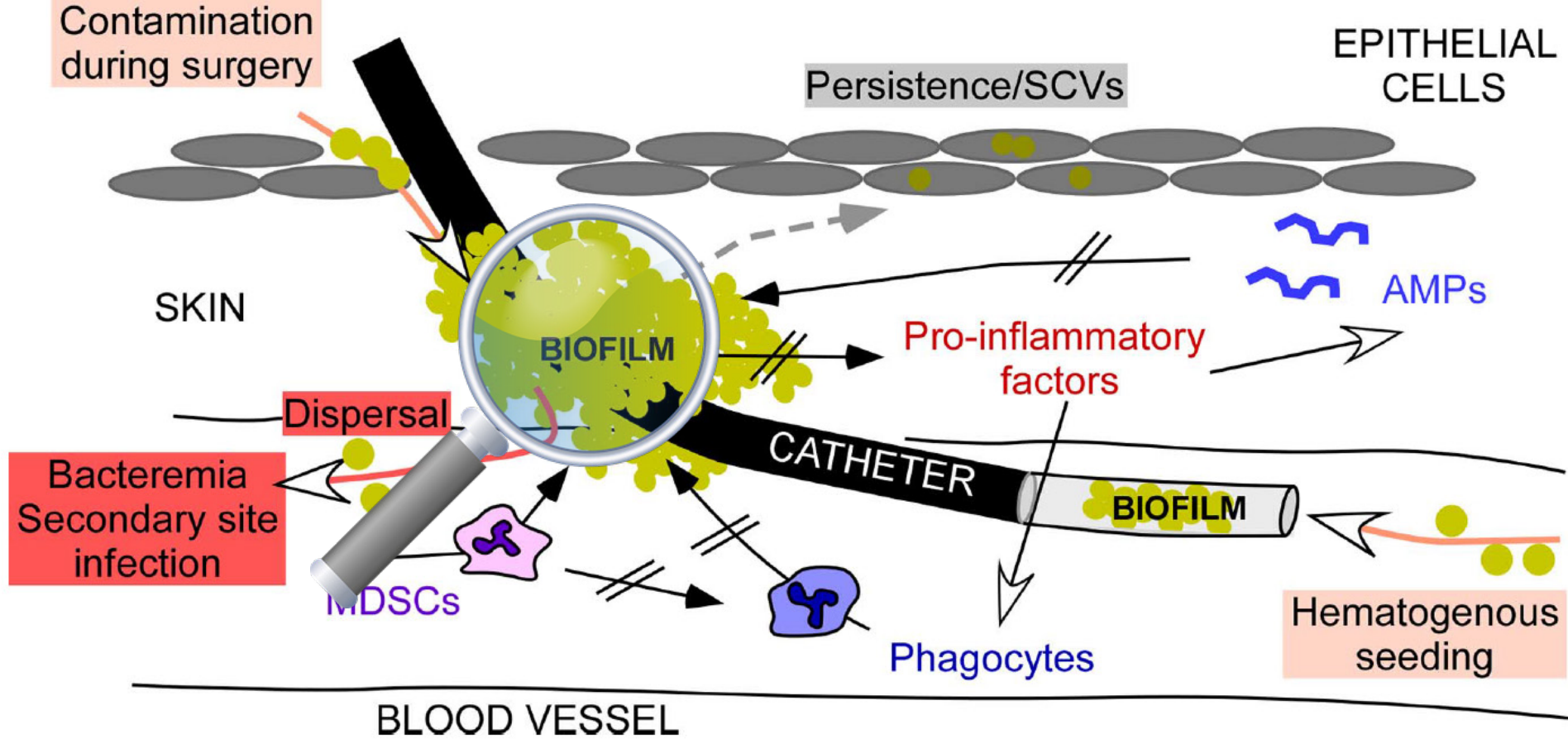


Staphylococcal biofilms and human infections



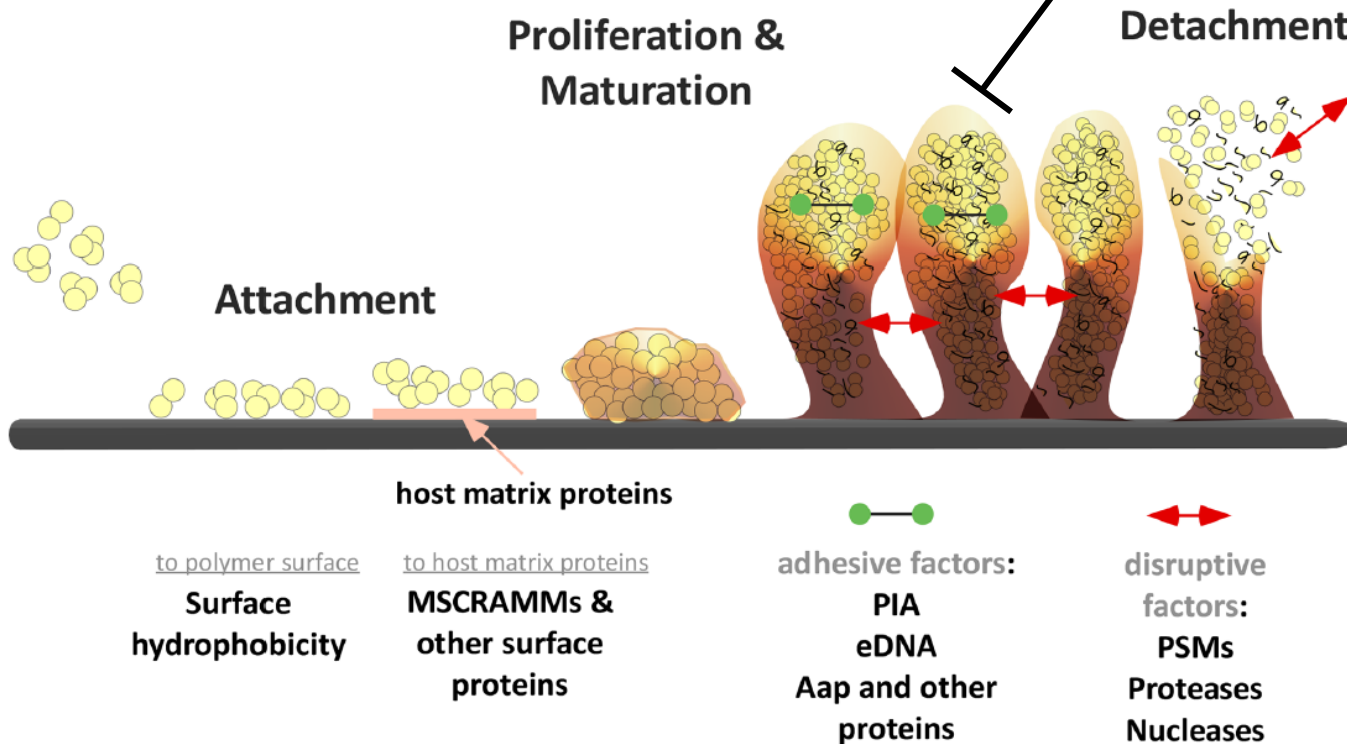
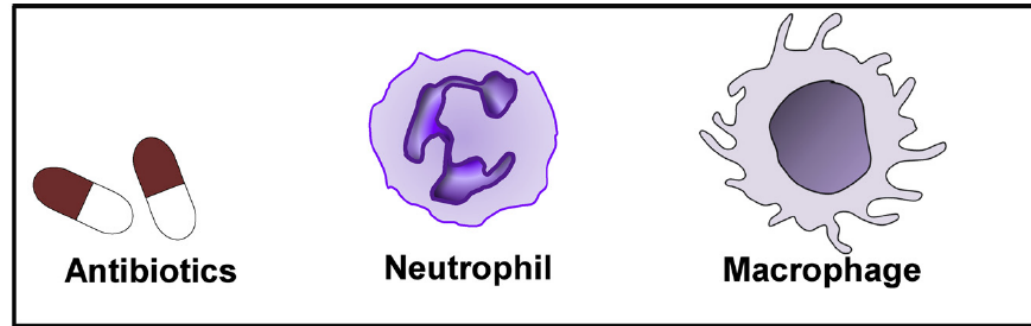
Lebeaux et al, *Microbiol Mol Biol Rev.* 2014;78:510-43

Staphylococcal biofilms: why do they cause persistent infections ? (1/2)



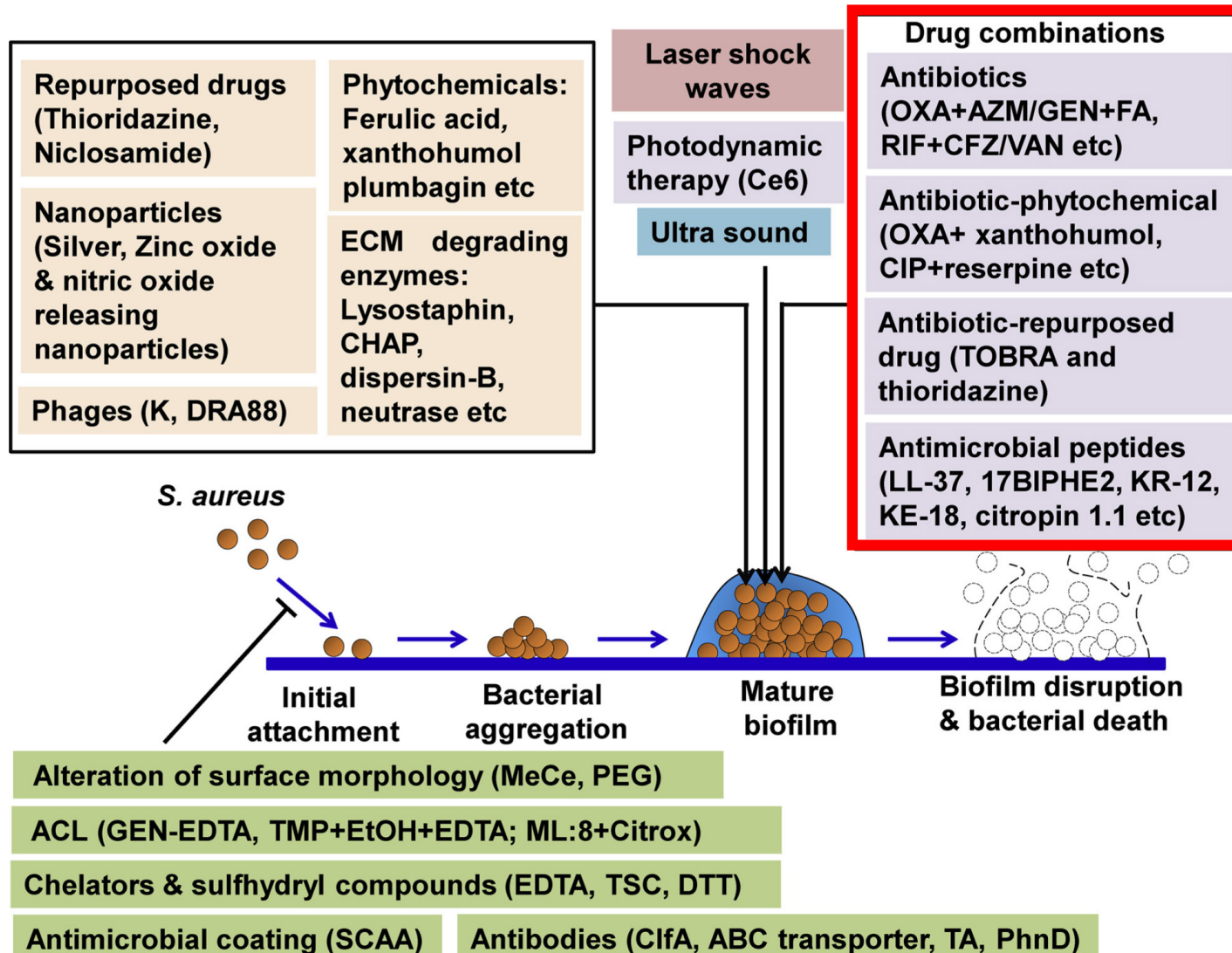
Otto, *Microbiol Spectrum* 2018; 6:GPP3-0023-2018

Staphylococcal biofilms: why do they cause persistent infections ? (2/2)

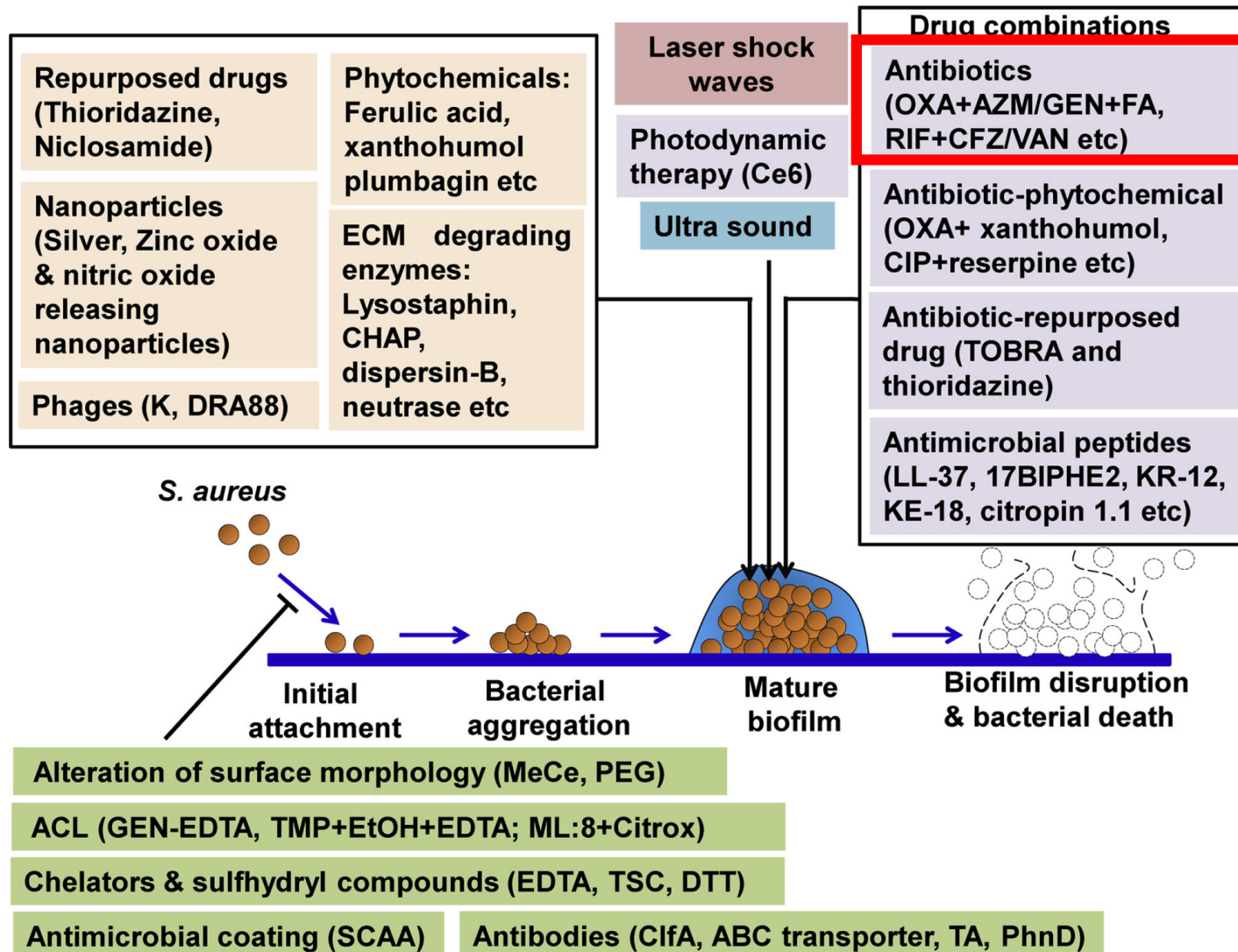


Suresh et al, *Int J Med Microbiol.* 2019;309:1-12
 Otto, *Microbiol Spectrum* 2018; 6:GPP3-0023-2018

Staphylococcal biofilms: strategies currently under investigation

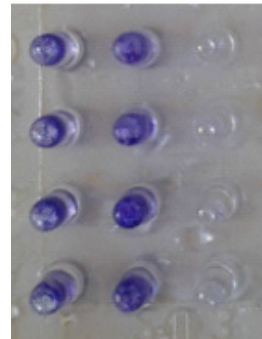
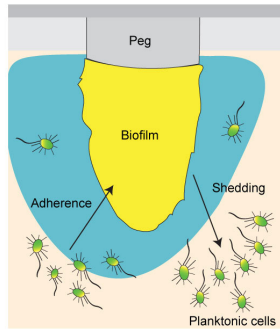
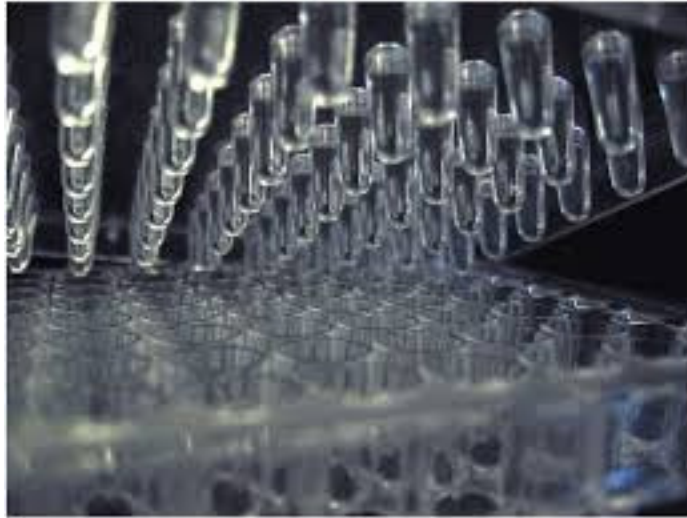


Staphylococcal biofilms: strategies currently under investigation



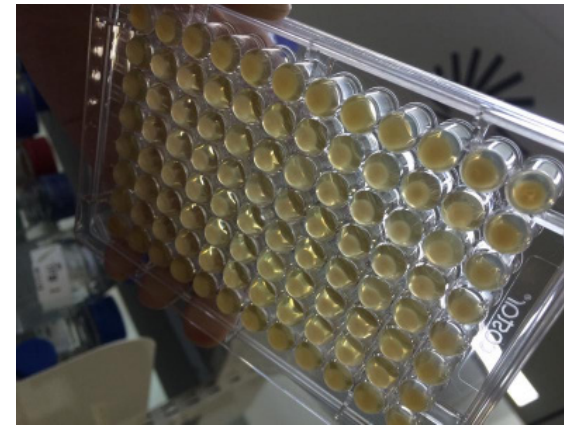
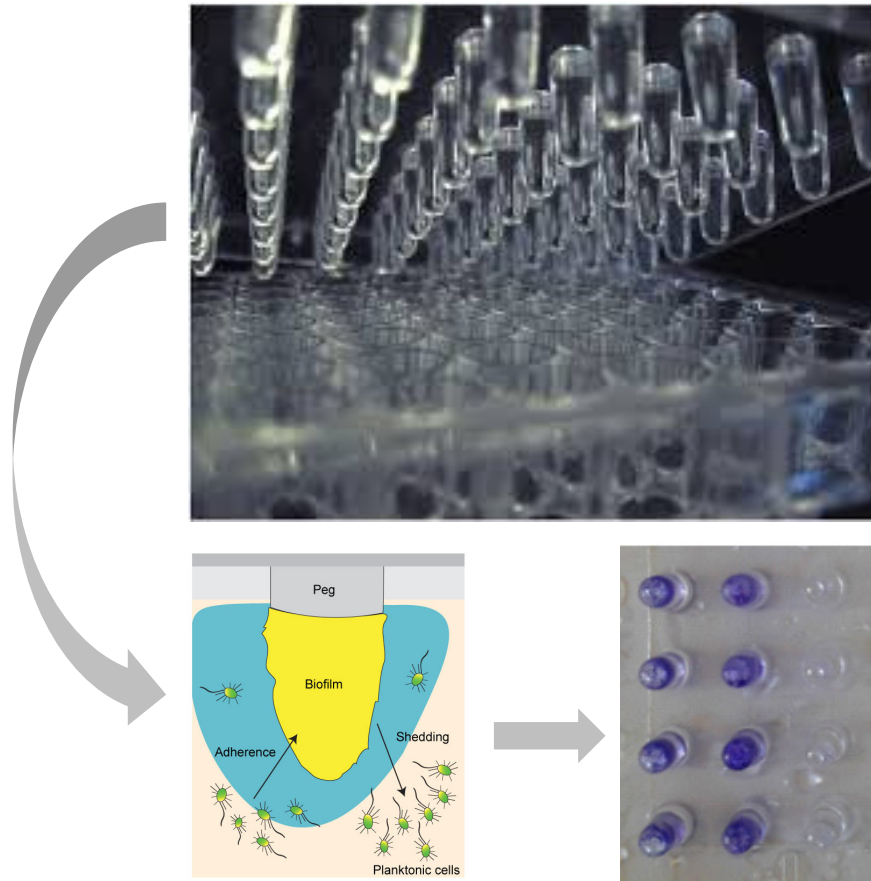
Antibiotic combinations: in vitro models

MBEC (Calgary device)

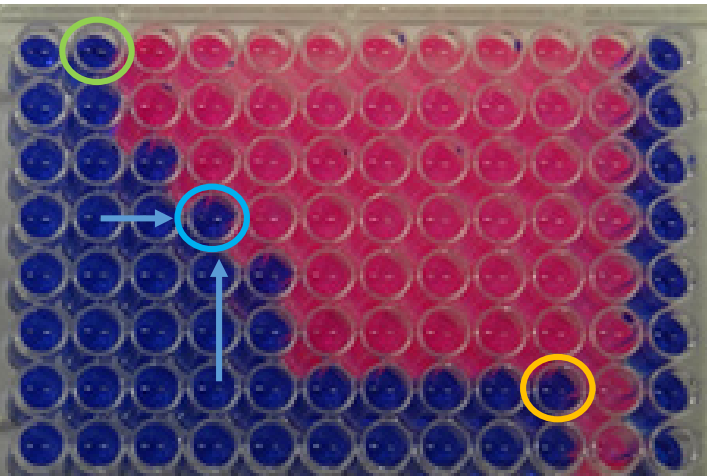


Antibiotic combinations: in vitro models

MBEC - checkerboard



drug A

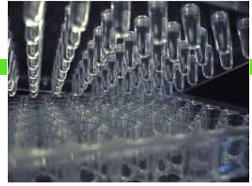


Metabolically

- inactive
- active

drug B

Antibiotic combinations: in vitro models



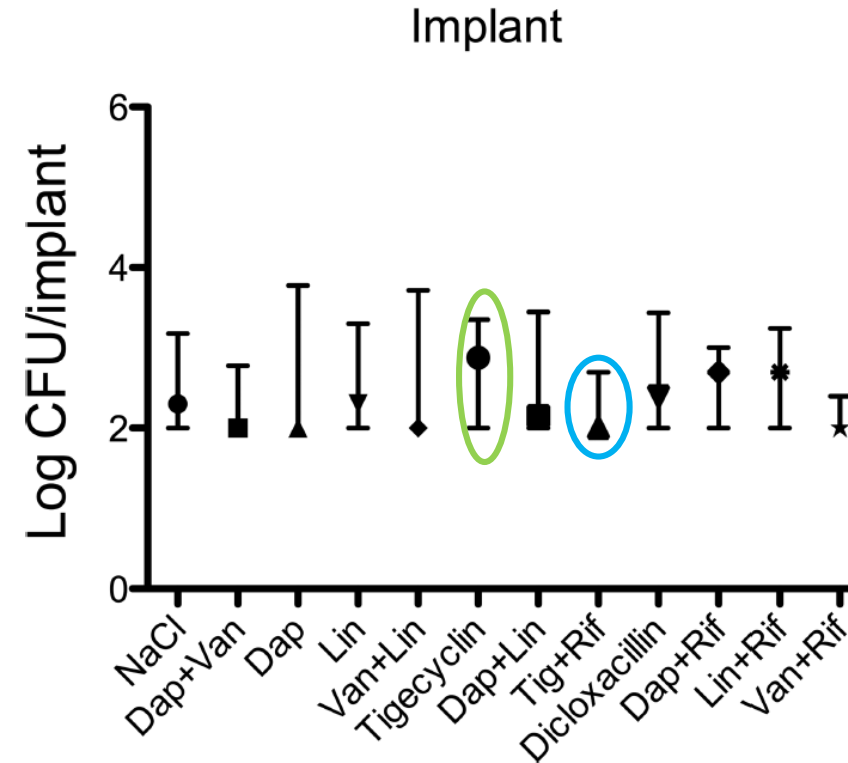
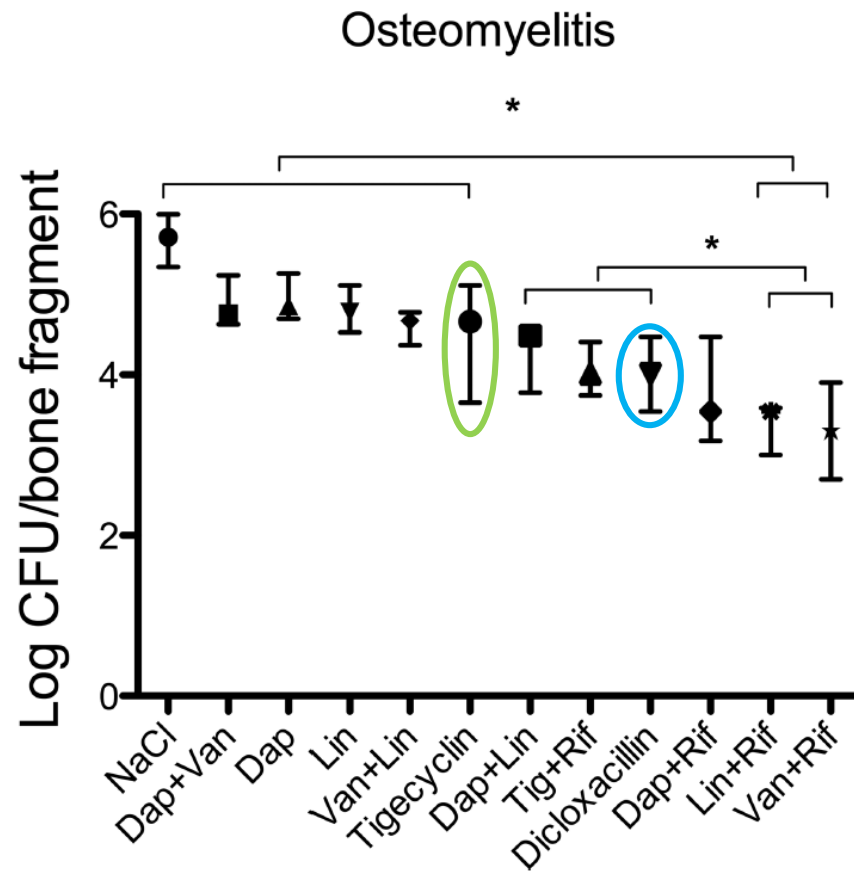
MBEC - checkerboard

	MIC (mg L ⁻¹)	MBEC (mg L ⁻¹)
Vancomycin	2	>2048
Daptomycin	0.38	1024
Linezolid	1	>1024
Tigecycline	0.25	1024
Rifampicin	0.008	N/A
Dicloxacillin	0.125 ^a	512

	Rifampicin (10 mg L ⁻¹)	Vancomycin (25 mg L ⁻¹)	Daptomycin (130 mg L ⁻¹)	Linezolid (10 mg L ⁻¹)
Vancomycin (mg L ⁻¹)	256	*	256	512
Daptomycin (mg L ⁻¹)	1024	>2048	*	>2048
Linezolid (mg L ⁻¹)	>1024	>1024	>1024	*
Tigecycline (mg L ⁻¹)	8	N/A	N/A	N/A

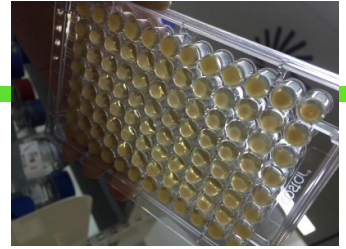
But still >> clinically achievable concentrations !

Antibiotic combinations: from in vitro to in vivo models

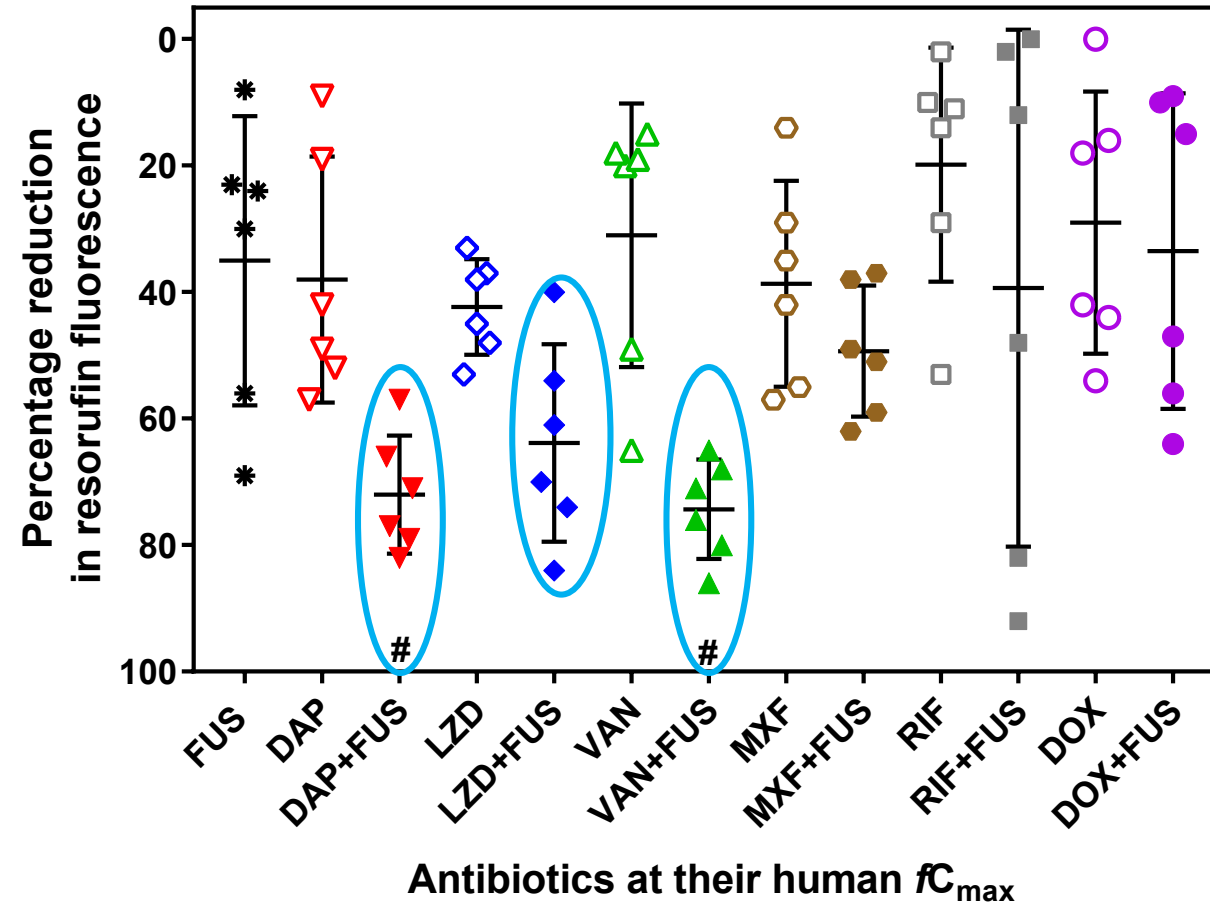


Combinations do not seem more efficacious in vivo than drugs alone ...

Antibiotic combinations: in vitro models

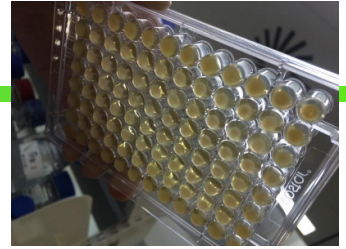


Combinations in microplates at fixed concentrations (free human C_{max})

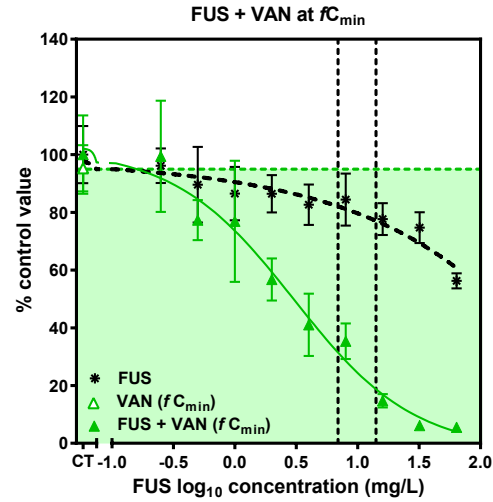
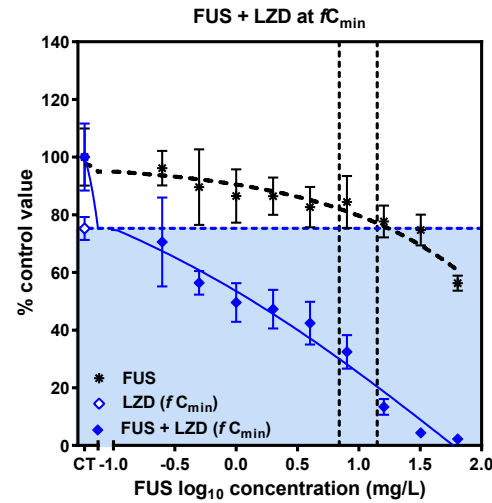
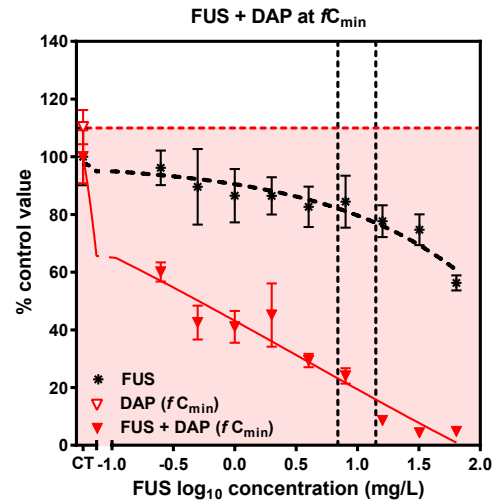


Combinations more effective at clinically achievable concentrations

Antibiotic combinations: in vitro models



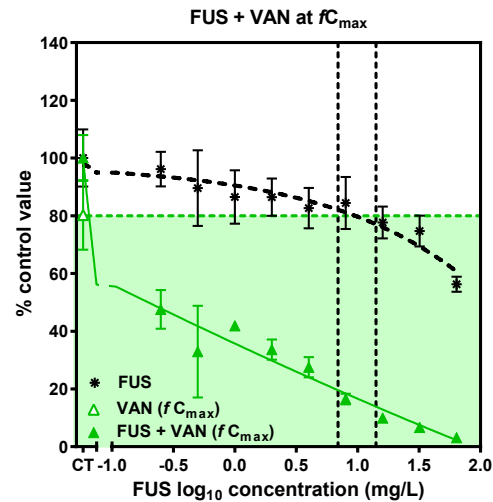
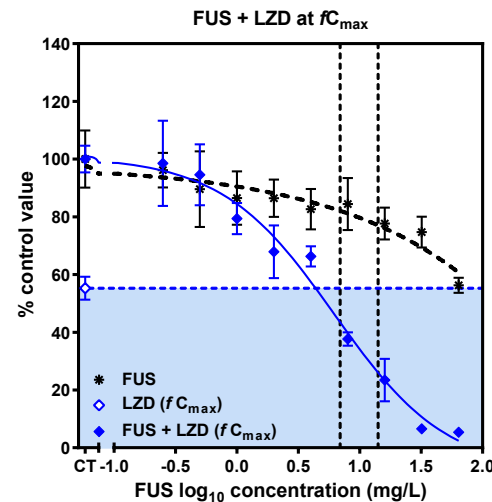
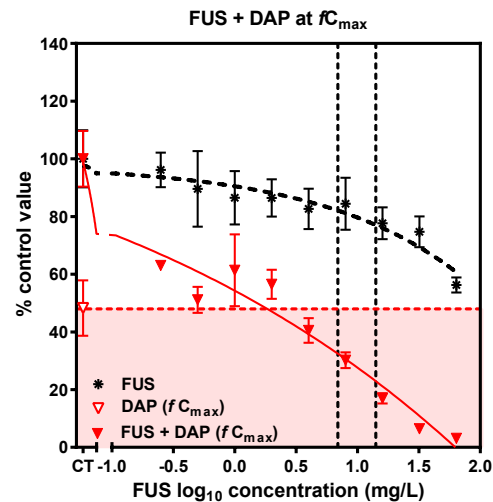
Combinations in microplates at variable concentrations



Combined drug at fC_{min} alone

FUS alone [variable conc.]

Combined drug at fC_{min}
+ FUS [variable conc.]



Combined drug at fC_{max} alone

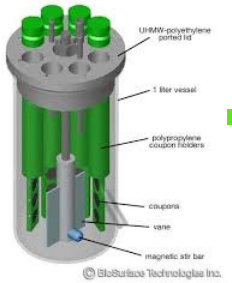
FUS alone [variable conc.]

Combined drug at fC_{max}
+ FUS [variable conc.]

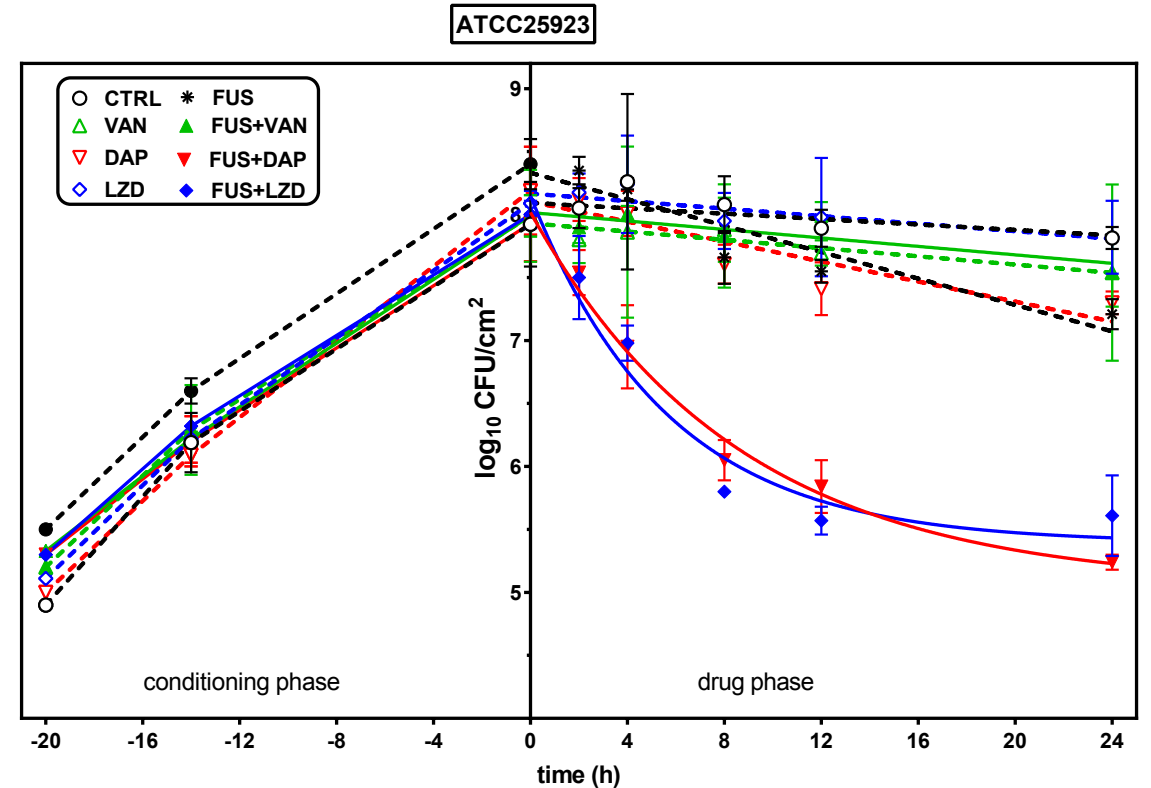
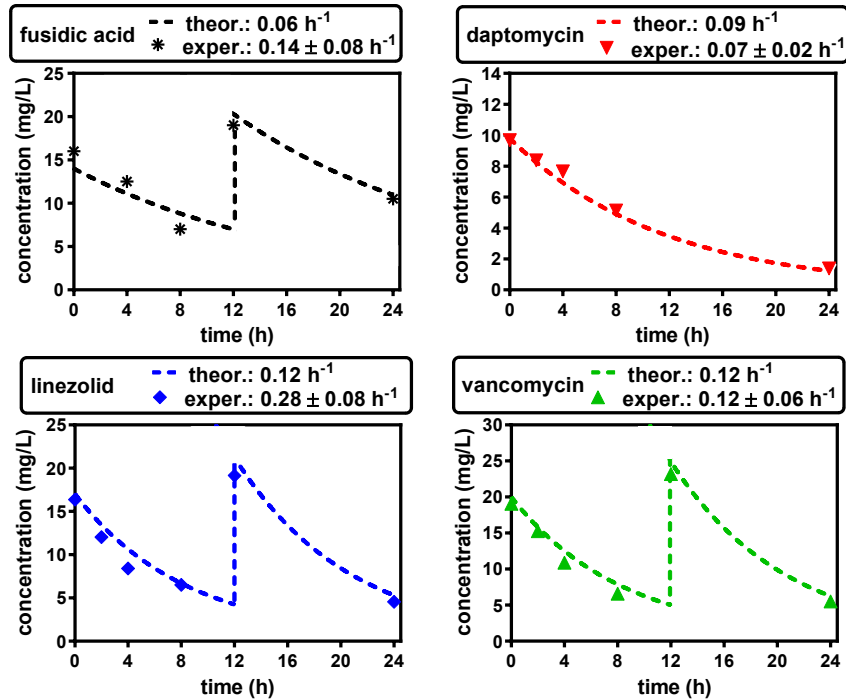
Combinations more effective

Siala et al, Antimicrob Agents Chemother. 2018;62:pii: e00598-18

Antibiotic combinations: in vitro models

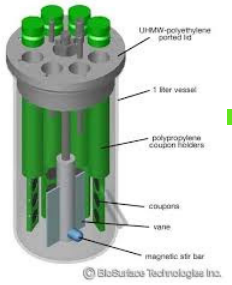


Combinations in CDC bioreactor (mimicking human PK)

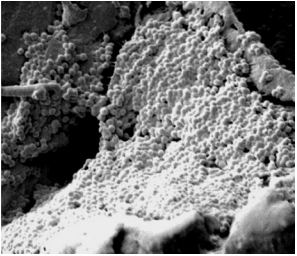


Combinations of FUS + LZD and DAP still more effective
...but not combinations with VAN ?

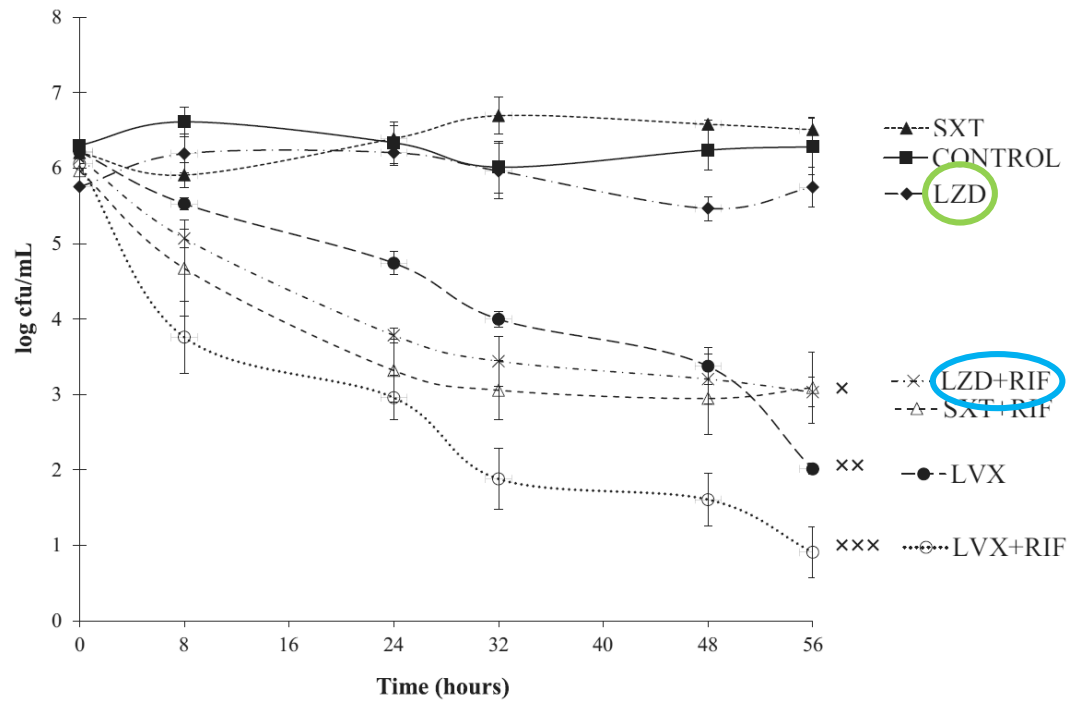
Antibiotic combinations: in vitro models



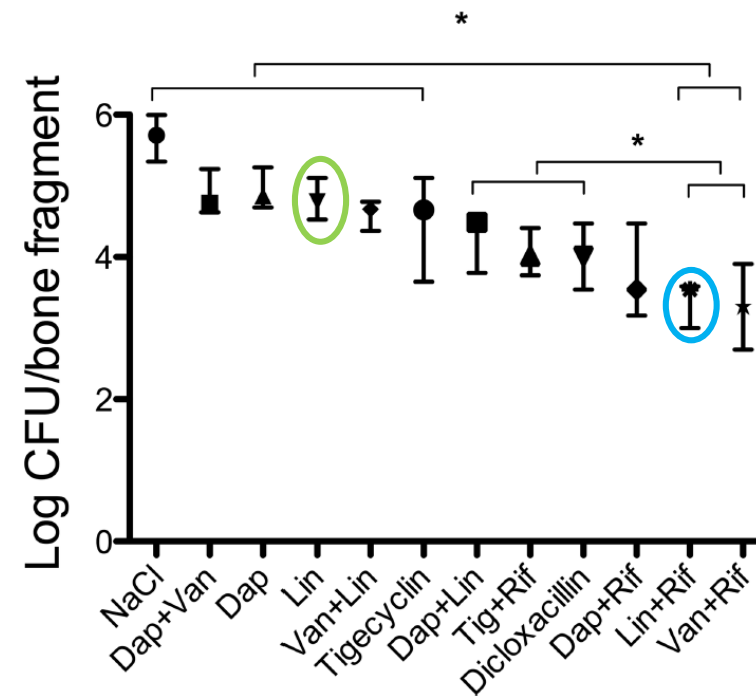
Combinations in CDC bioreactor (mimicking human PK)



Biofilm grown in CDC reactor



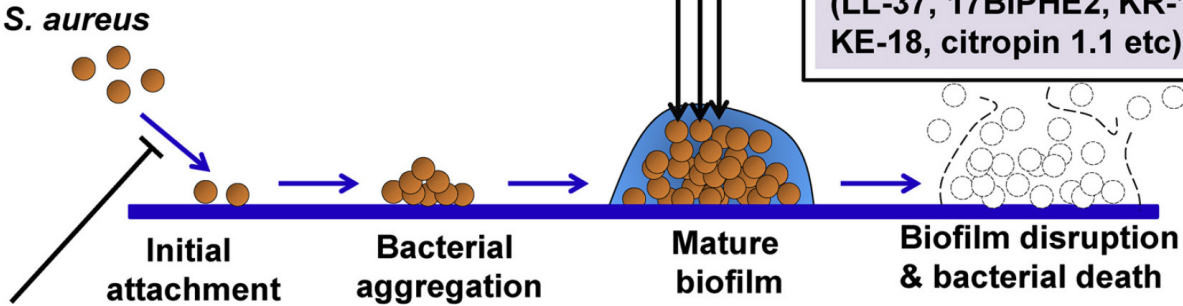
Osteomyelitis



Staphylococcal biofilms: strategies currently under investigation

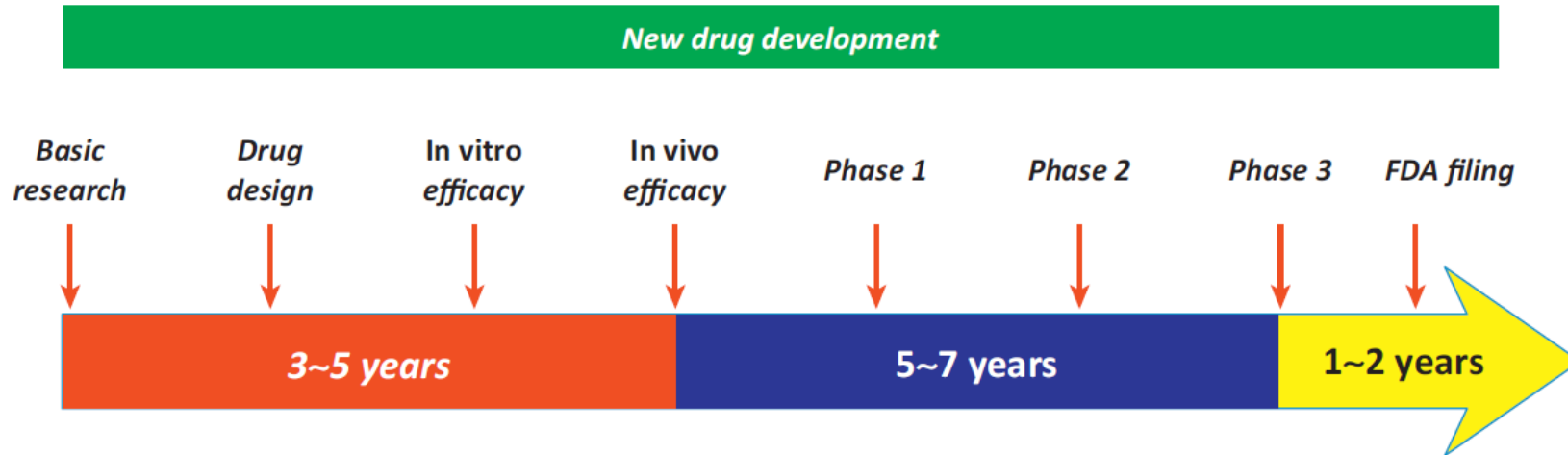


<p>Repurposed drugs (Thioridazine, Niclosamide)</p>	<p>Phytochemicals: Ferulic acid, xanthohumol, plumbagin etc</p>	<p>Laser shock waves</p> <p>Photodynamic therapy (Ce6)</p> <p>Ultra sound</p>	<p>Drug combinations</p> <p>Antibiotics (OXA+AZM/GEN+FA, RIF+CFZ/VAN etc)</p>
<p>Nanoparticles (Silver, Zinc oxide & nitric oxide releasing nanoparticles)</p>	<p>ECM degrading enzymes: Lysostaphin, CHAP, dispersin-B, neutrase etc</p>		<p>Antibiotic-phytochemical (OXA+ xanthohumol, CIP+reserpine etc)</p>
<p>Phages (K, DRA88)</p>			<p>Antibiotic-repurposed drug (TOBRA and thioridazine)</p>
			<p>Antimicrobial peptides (LL-37, 17BIPHE2, KR-12, KE-18, citropin 1.1 etc)</p>



- Alteration of surface morphology (MeCe, PEG)
- ACL (GEN-EDTA, TMP+EtOH+EDTA; ML:8+Citrox)
- Chelators & sulfhydryl compounds (EDTA, TSC, DTT)
- Antimicrobial coating (SCAA)
- Antibodies (ClfA, ABC transporter, TA, PhnD)

Drug repurposing as a source of potentiators



**Rediscovery of old drugs
for new uses**

International Journal of Antimicrobial Agents 49 (2017) 315–320

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journal homepage: www.elsevier.com/locate/ijantimicag

ELSEVIER

International Society of Chemotherapy for Infection and Cancer

Screening a repurposing library for potentiators of antibiotics against *Staphylococcus aureus* biofilms

Freija Van den Driessche, Gilles Brackman, Rosalie Swimberghe, Petra Rigole, Tom Coenye *

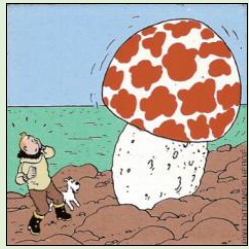
Laboratory of Pharmaceutical Microbiology, Ghent University, Ottergemsesteenweg 460, 9000 Gent, Belgium

CrossMark

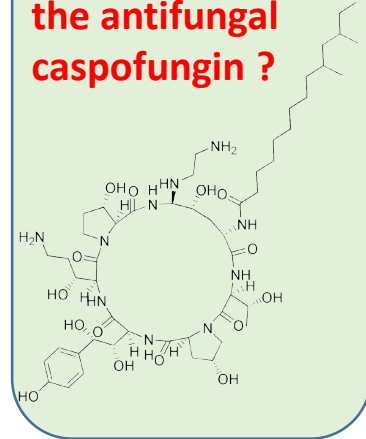
... but often active at suprathreshold concentrations and/or only in vitro

Gupta et al, Trends Pharmacol Sci. 2013;34:508-17

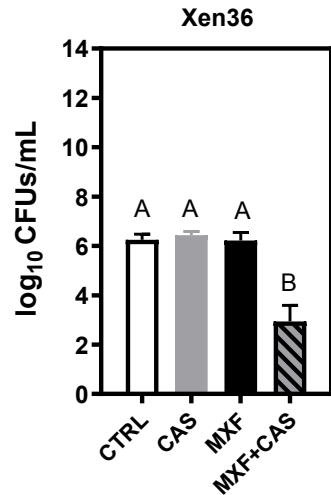
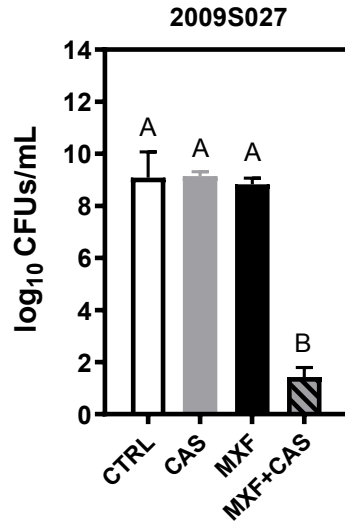
Drug repurposing as a source of potentiators: a possibly successful story ?



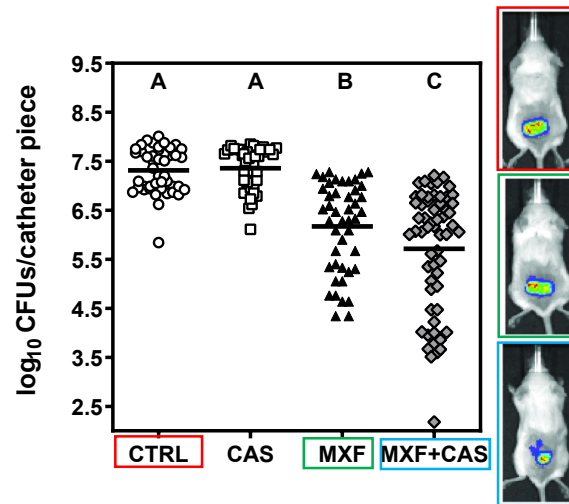
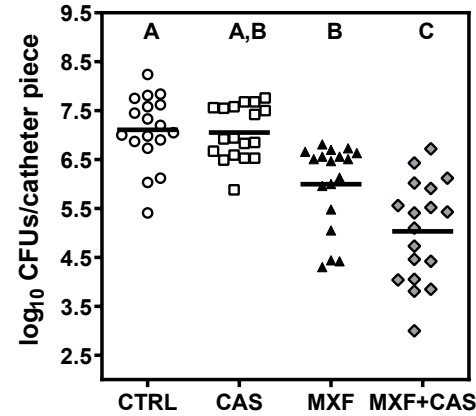
What about the antifungal caspofungin ?



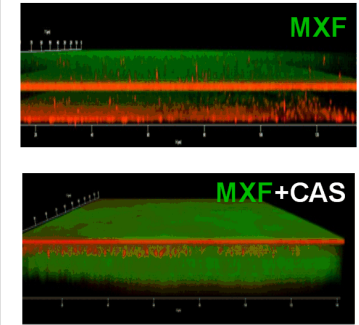
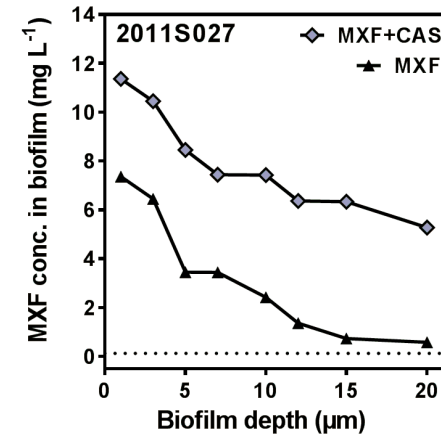
catheters in vitro



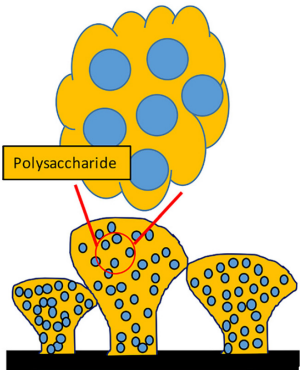
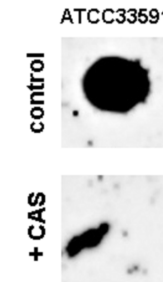
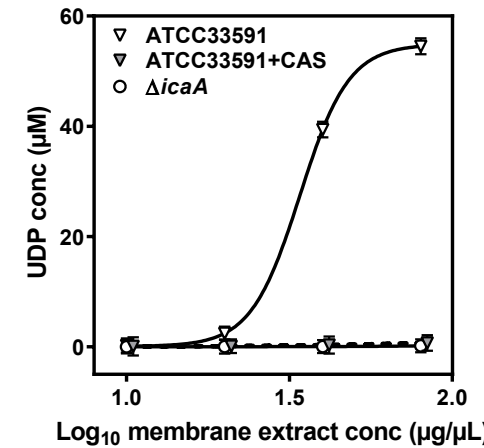
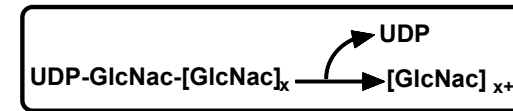
catheters in vivo



↗ penetration of MXF in biofilms



↘ Activity of IcaA

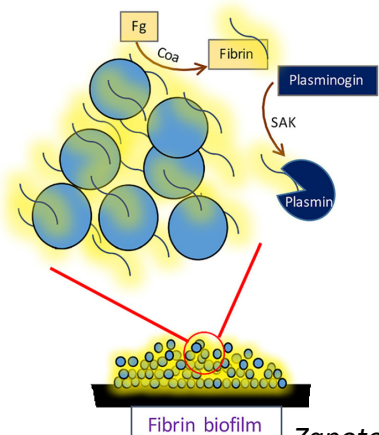


Zapotoczna et al., PLoS Pathog. 2016;12:e1005671

Siala et al, Nature Commun. 2016; 7:13286

Combining antibiotics with drugs having a complementary action

Fibrinolytic agents

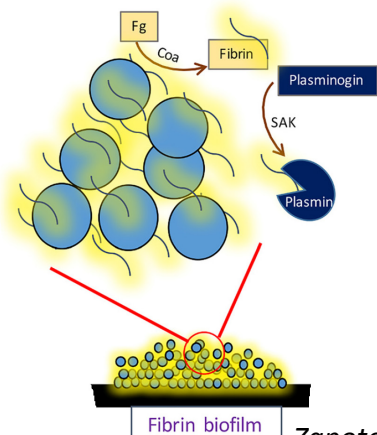
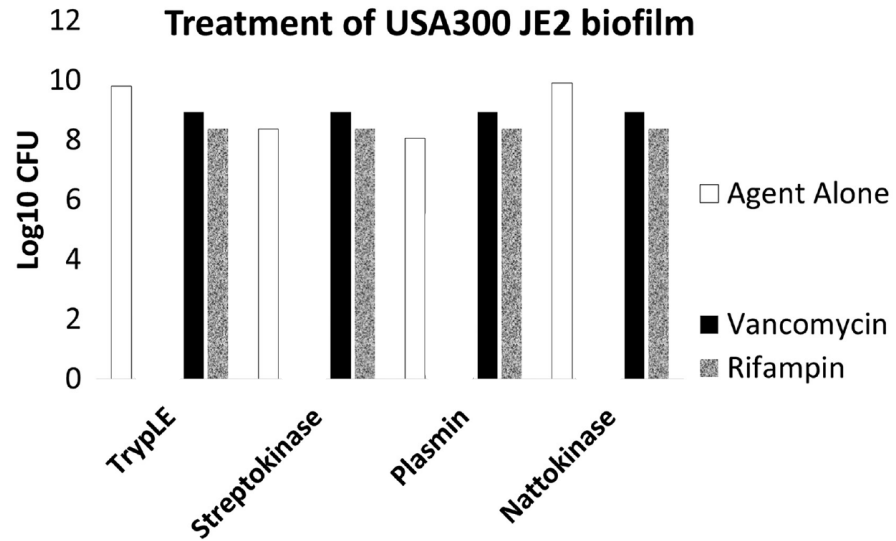


Zapotoczna et al., *PLoS Pathog.* 2016;12:e1005671

Combining antibiotics with drugs having a complementary action

Fibrinolytic agents

catheters in vitro



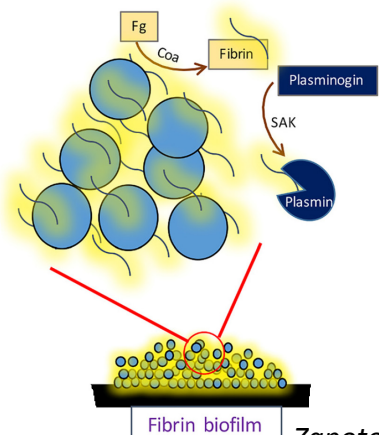
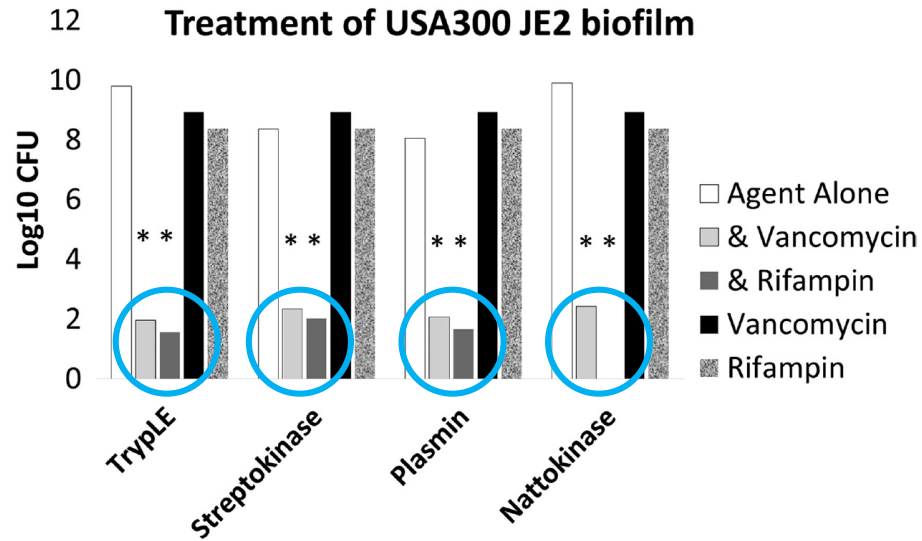
Zapotoczna et al., *PLoS Pathog.* 2016;12:e1005671

Hogan et al, *Antimicrob Agents Chemother.* 2018;62. pii: e02008-17

Combining antibiotics with drugs having a complementary action

Fibrinolytic agents

catheters in vitro



Zapotoczna et al., *PLoS Pathog.* 2016;12:e1005671

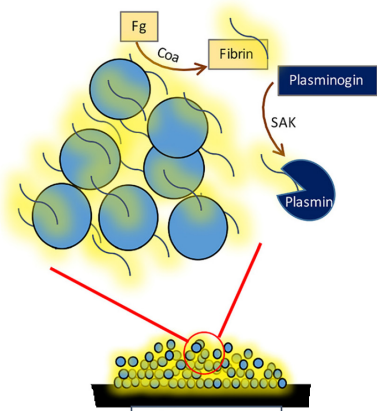
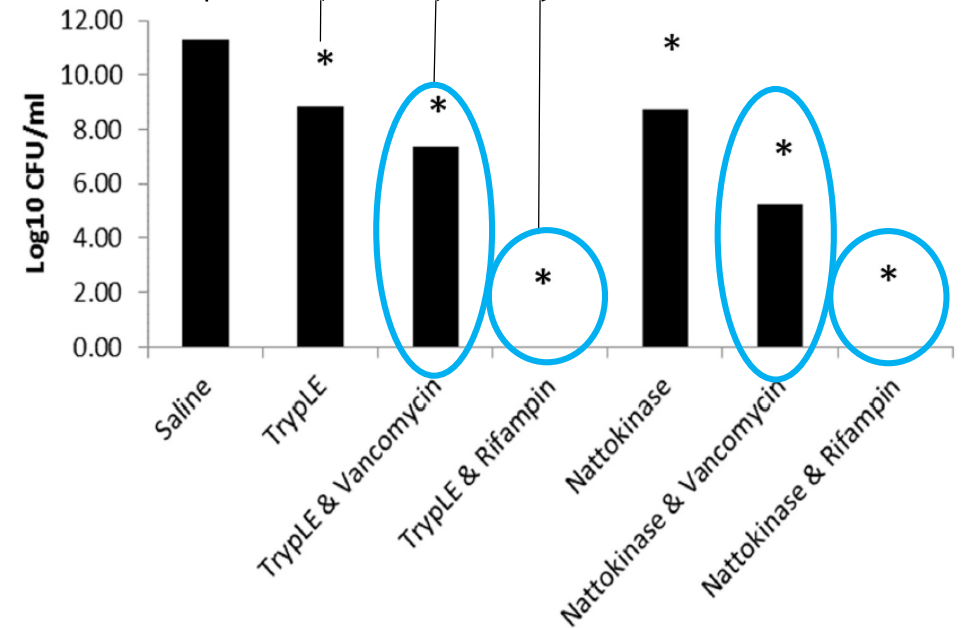
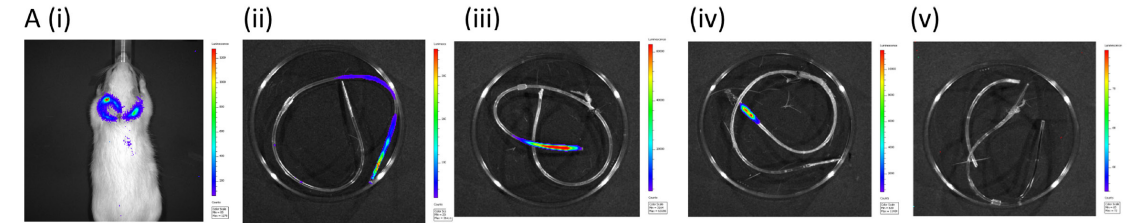
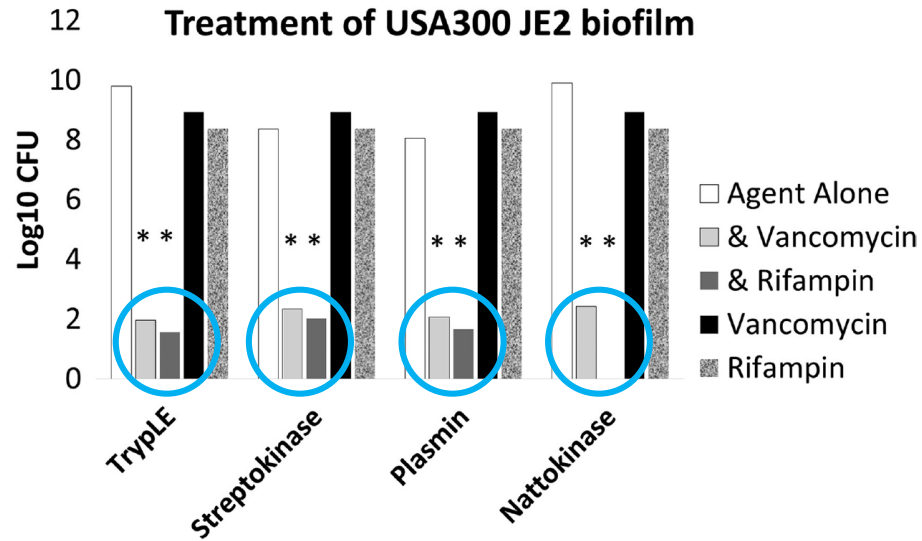
Hogan et al, *Antimicrob Agents Chemother.* 2018;62. pii: e02008-17

Combining antibiotics with drugs having a complementary action

Fibrinolytic agents

catheters in vitro

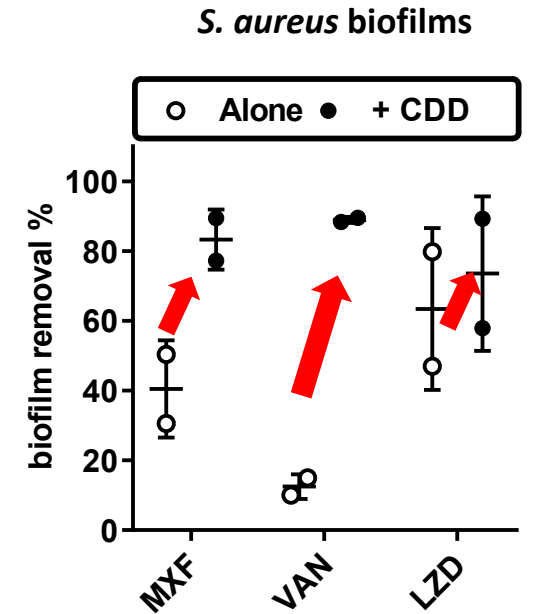
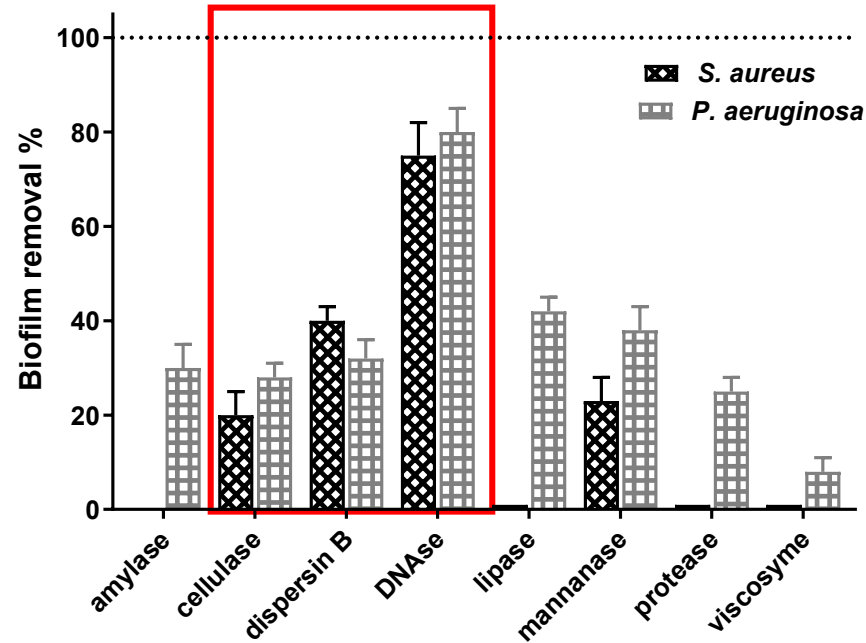
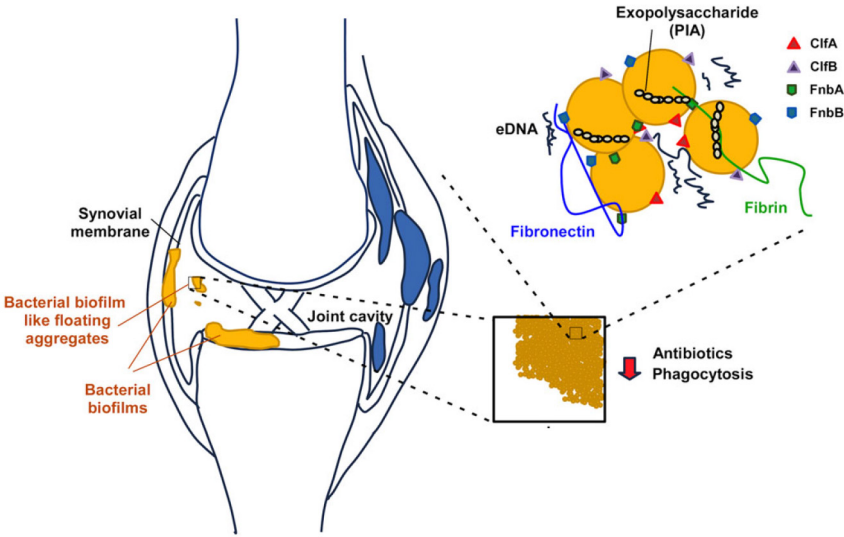
catheters in vivo



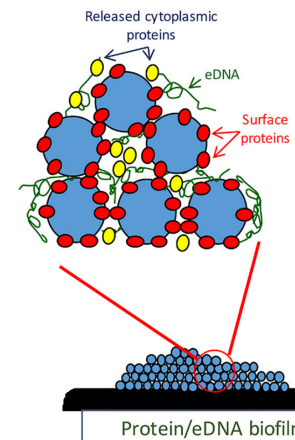
Fibrin biofilm | Zapotoczna et al., PLoS Pathog. 2016;12:e1005671

Hogan et al, Antimicrob Agents Chemother. 2018;62. pii: e02008-17

Combining antibiotics with enzymes destroying the matrix



Dastgheyb et al, *J Infect Dis.* 2015;211:641–50

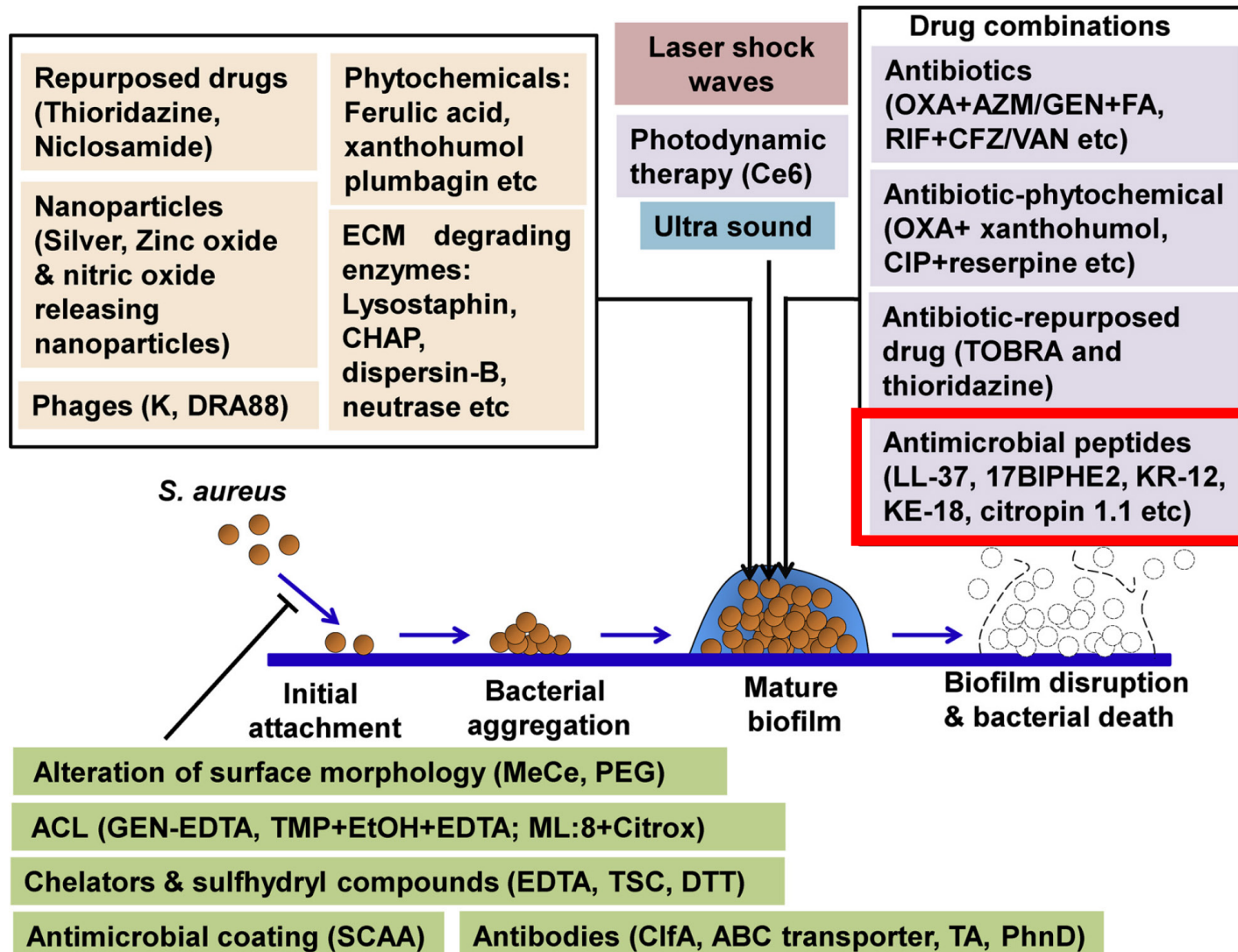


Zapotoczna et al., *PLoS Pathog.* 2016;12:e1005671

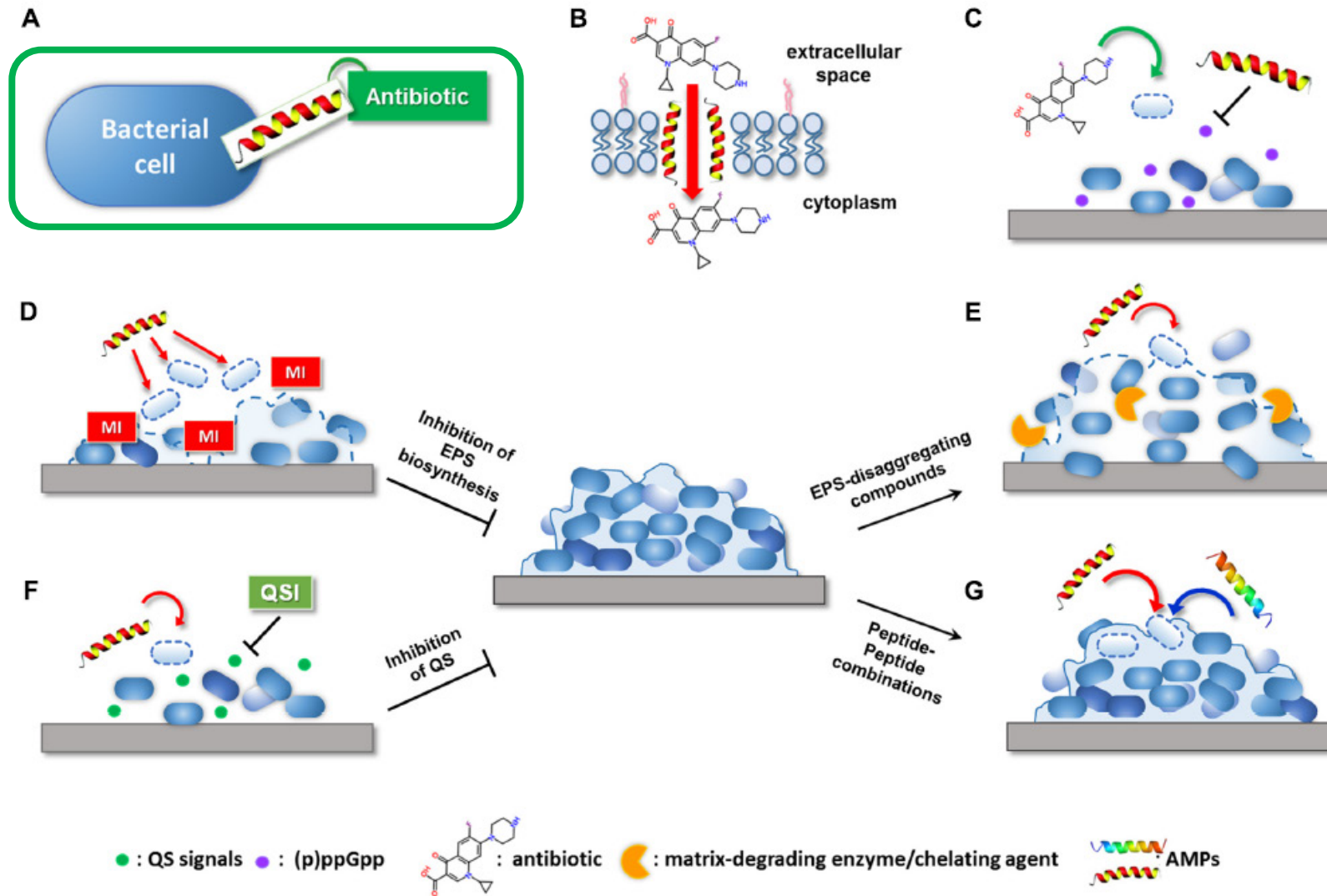


Siala et al., *ECCMID 2018; O0081*

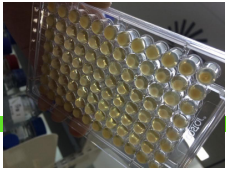
Staphylococcal biofilms: strategies currently under investigation



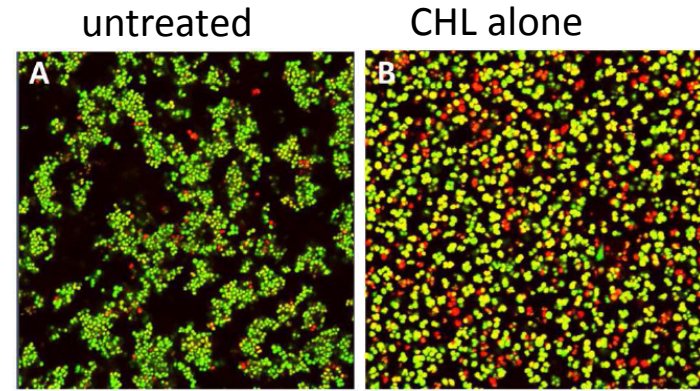
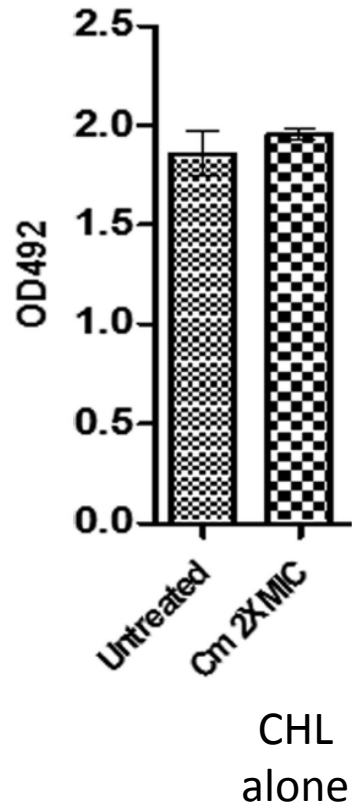
Mode of action of antimicrobial peptides against biofilms



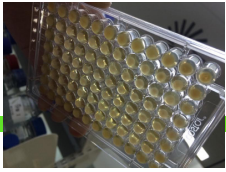
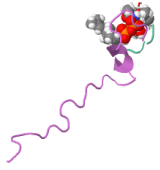
An example with nisin ...



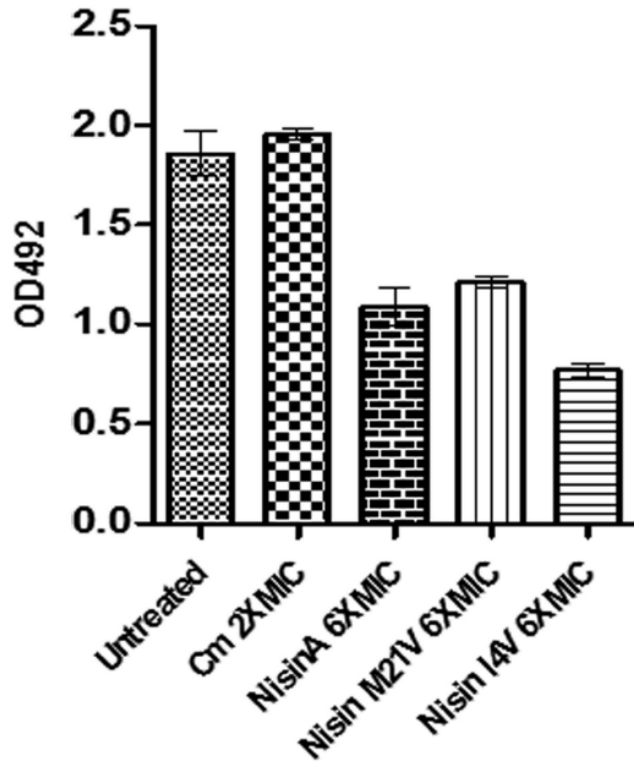
Nisin and derivatives thereof



An example with nisin ...



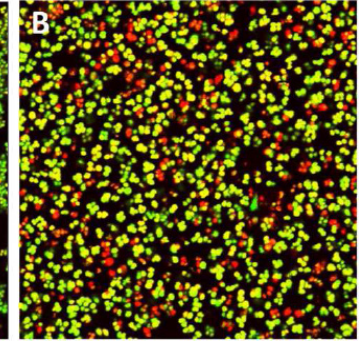
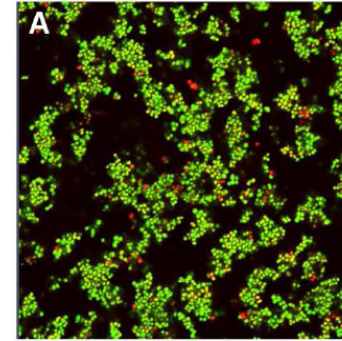
Nisin and derivatives thereof



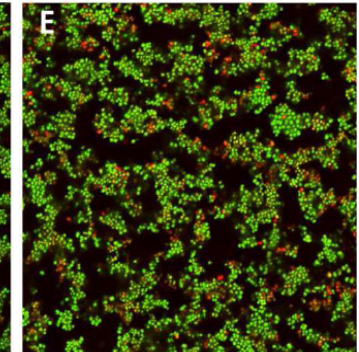
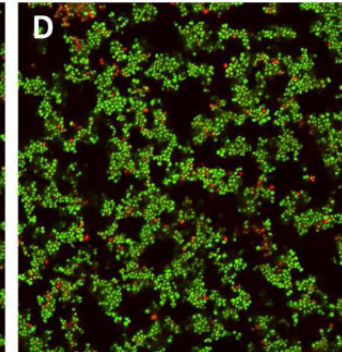
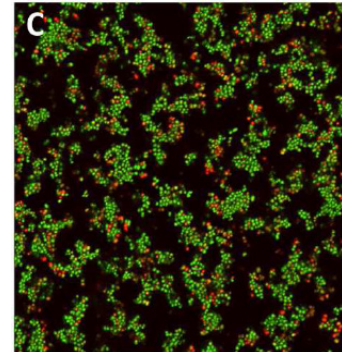
Nisin & derivatives
alone

untreated

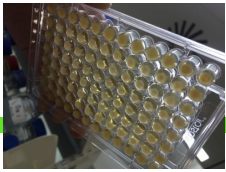
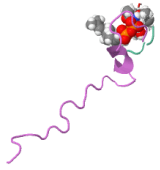
CHL alone



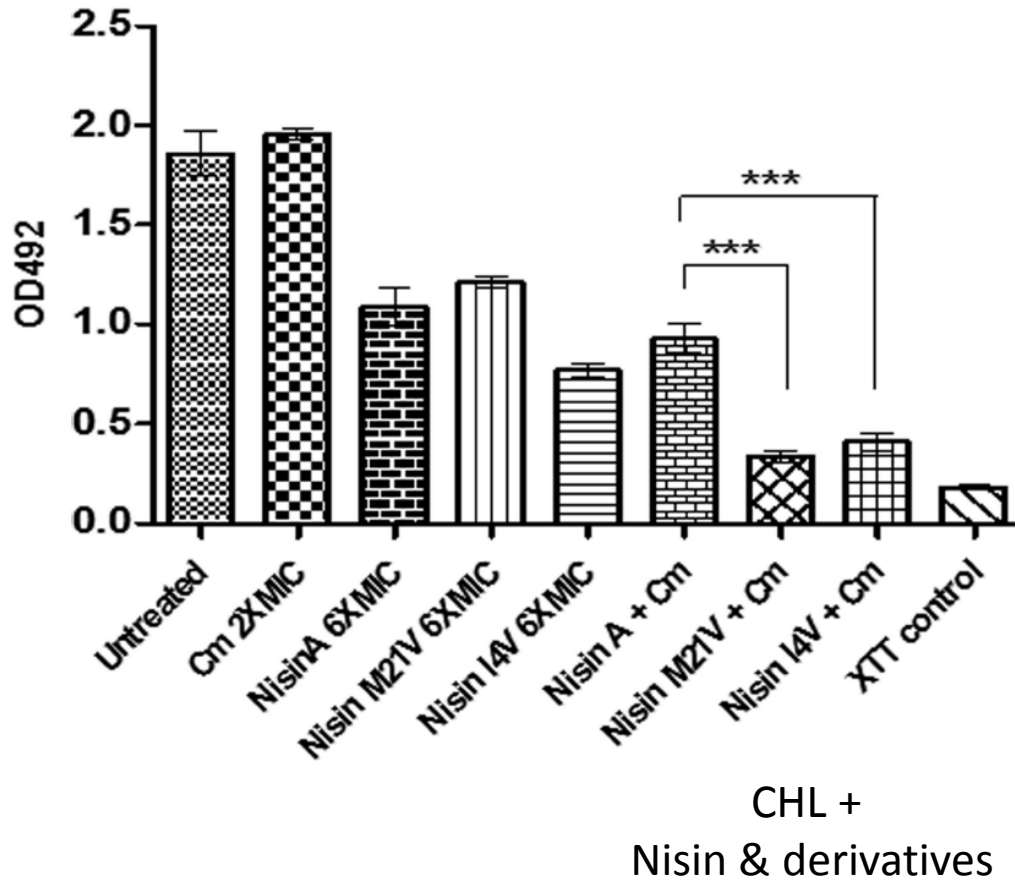
Nisin & derivatives alone



An example with nisin ...

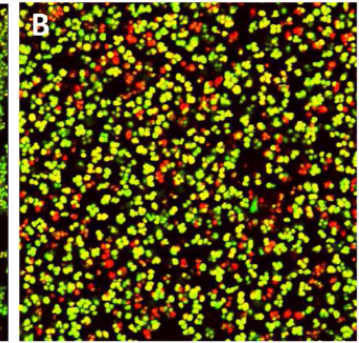
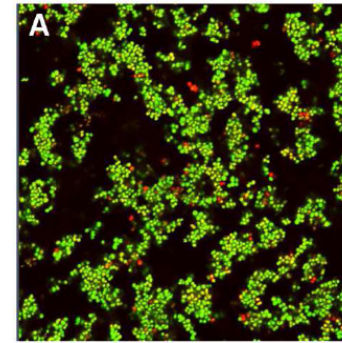


Nisin and derivatives thereof

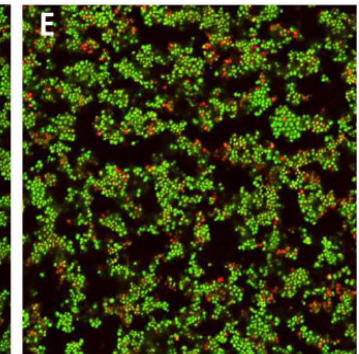
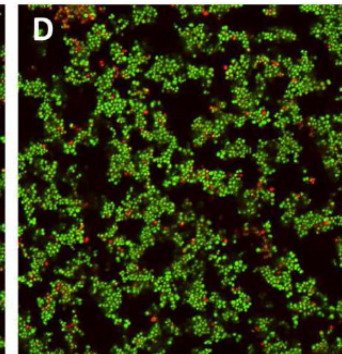
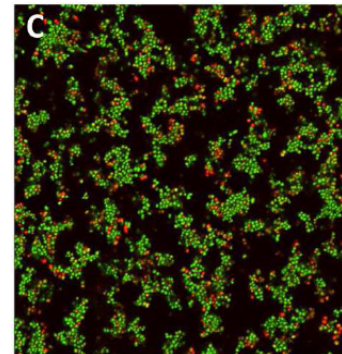


untreated

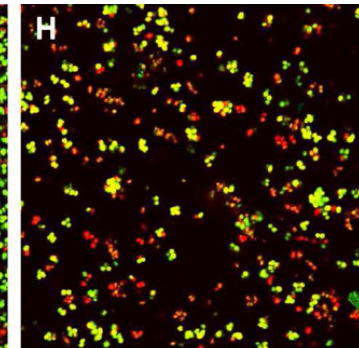
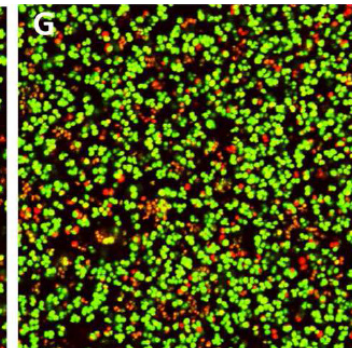
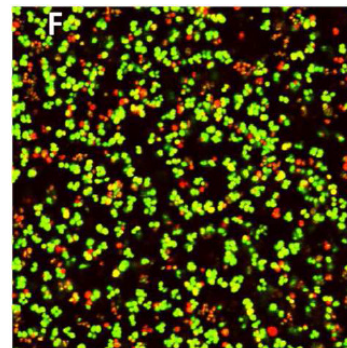
CHL alone



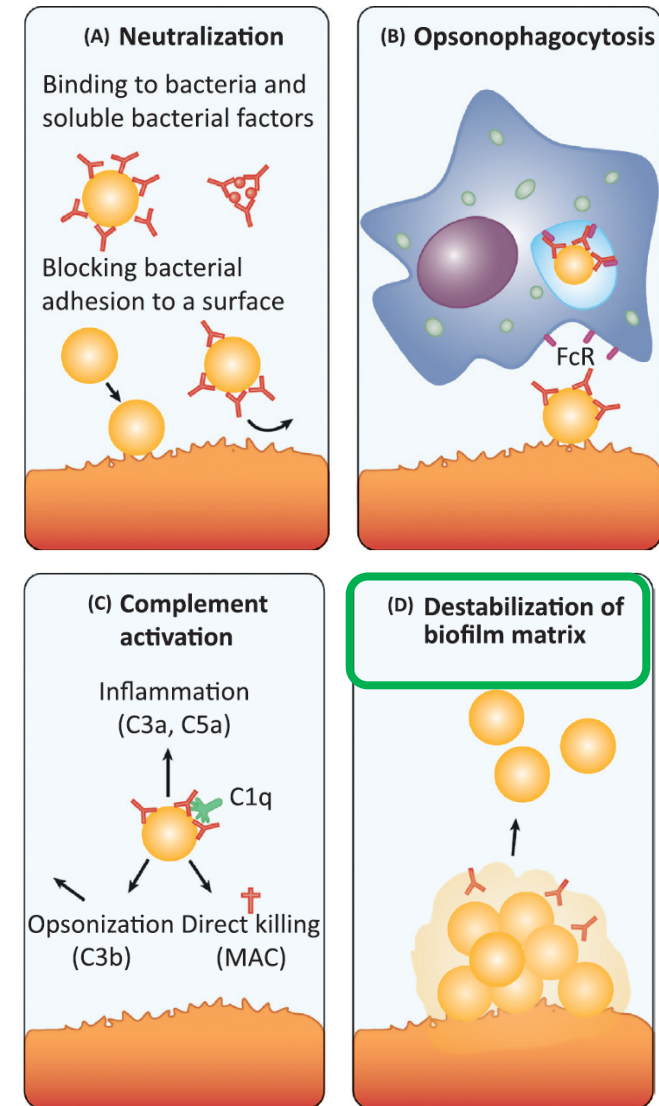
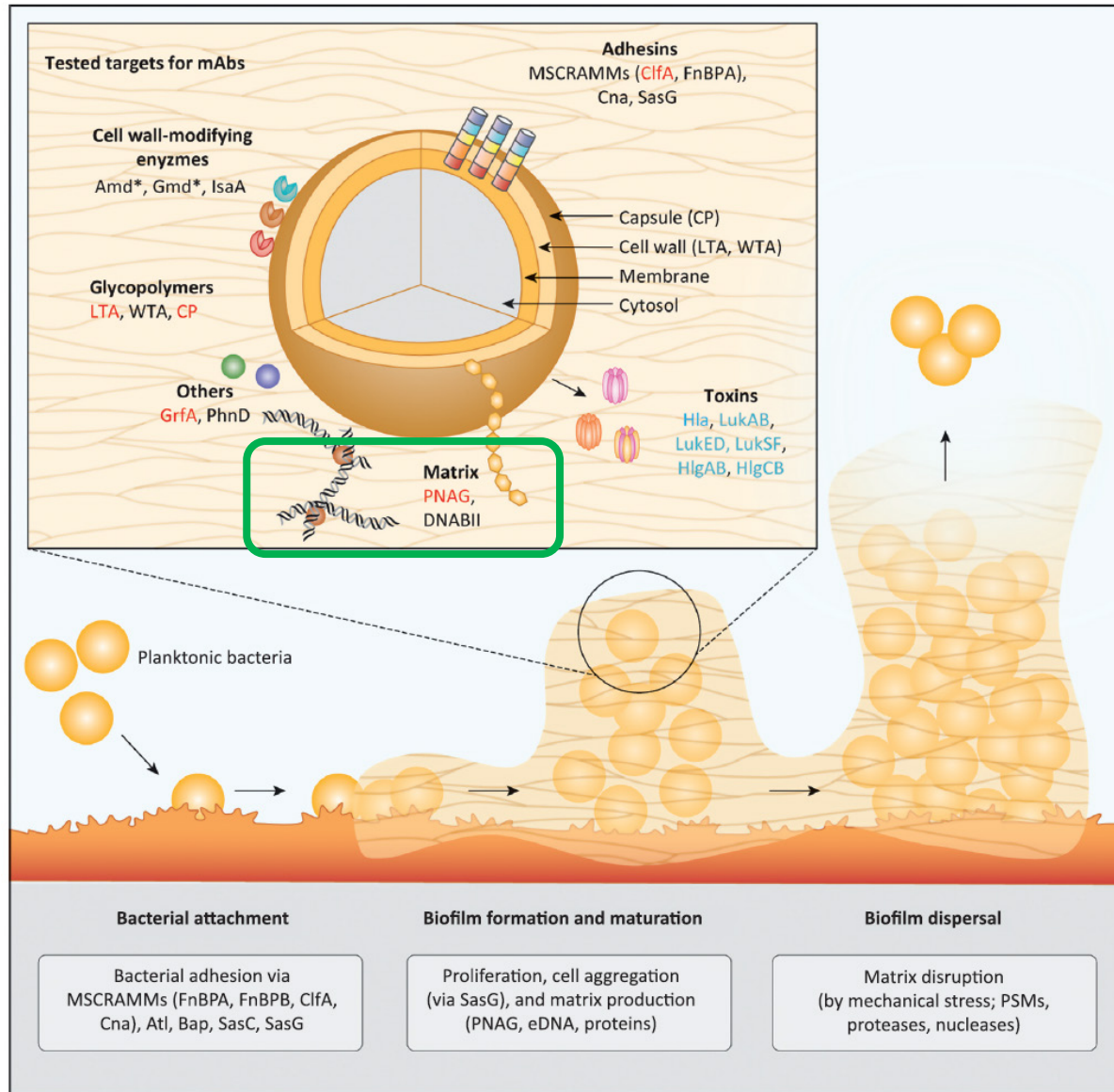
Nisin & derivatives alone



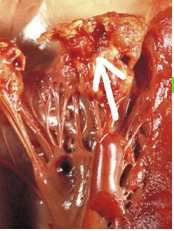
CHL + Nisin & derivatives



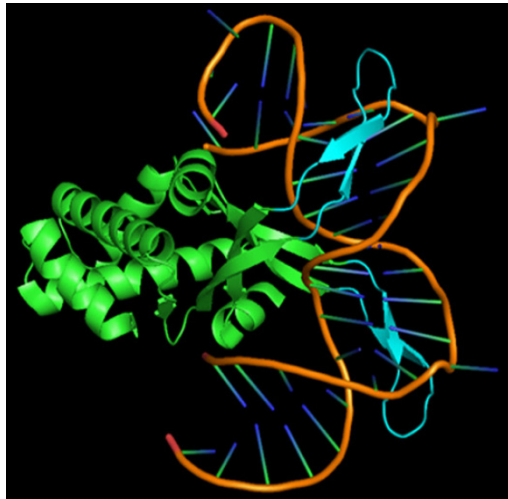
Mode of action of antibodies against biofilms



Antibodies against biofilms to destroy the matrix



Dimer of histone-like prot. stabilizing eDNA

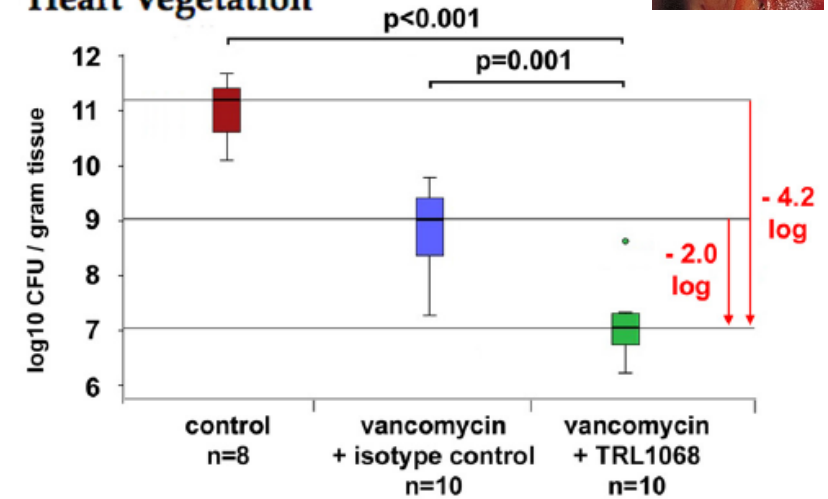


Epitope targeted by Ab TRL1068

eDNA

In vivo (endocarditis)

Heart Vegetation



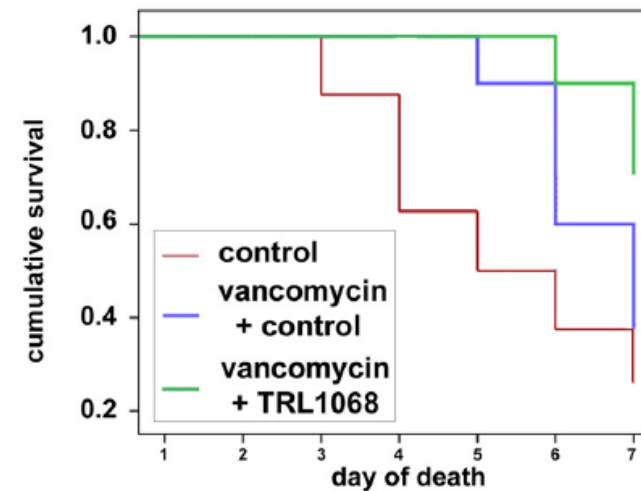
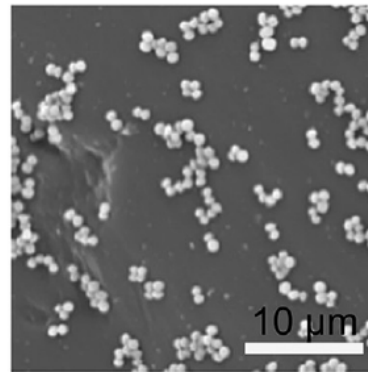
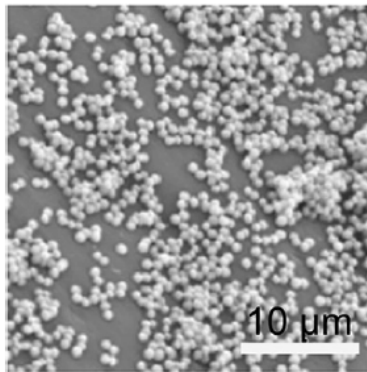
In vitro (PEGs)



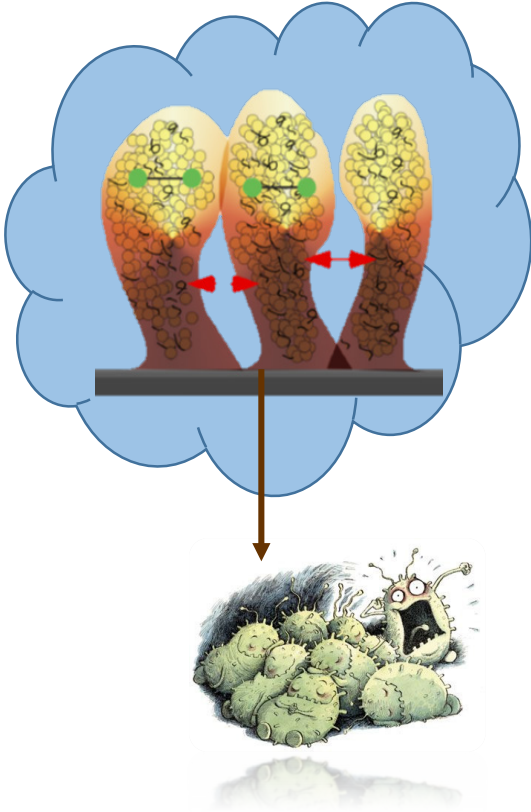
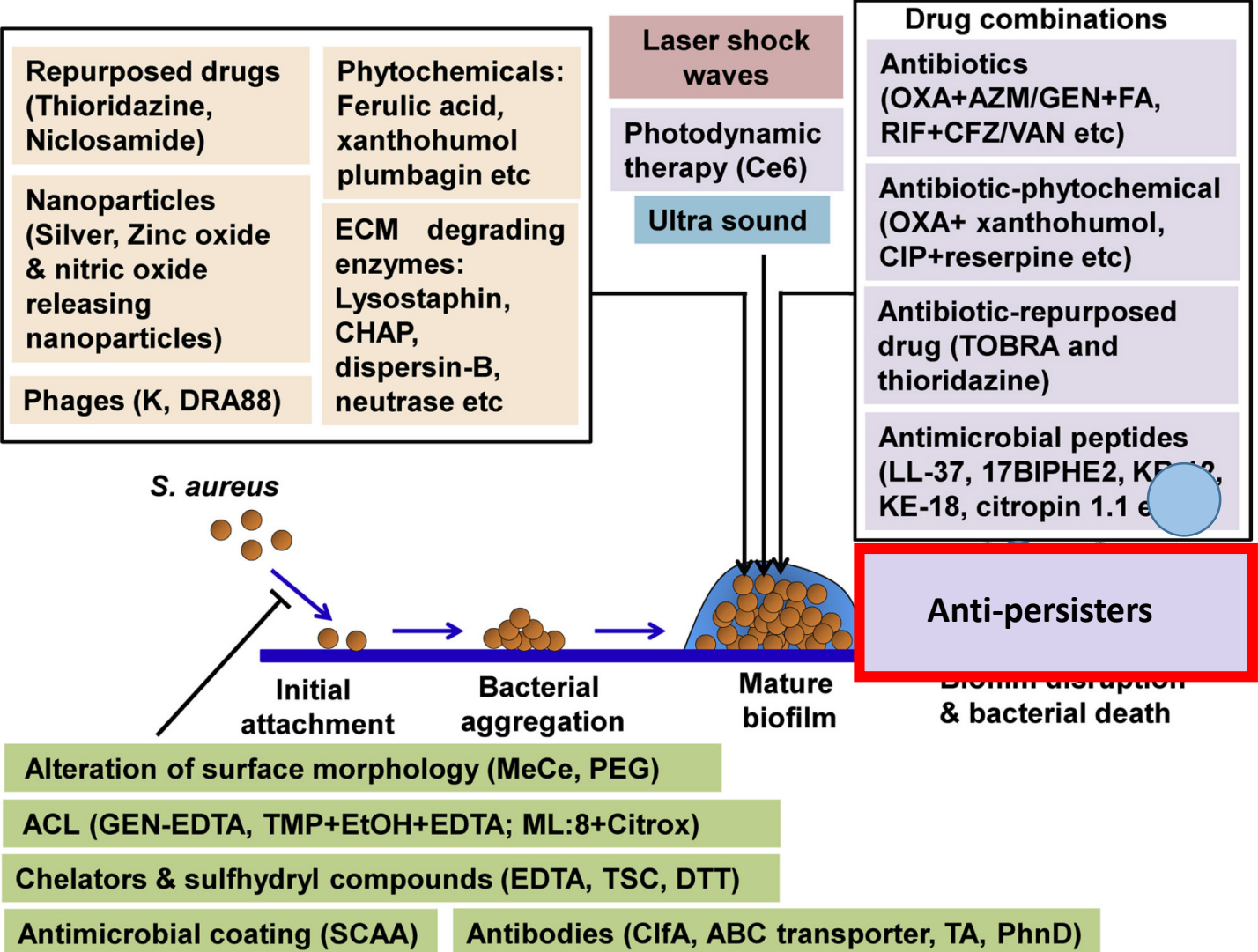
Staphylococcus aureus

Growth Control

TRL1068: 1.2 µg/mL

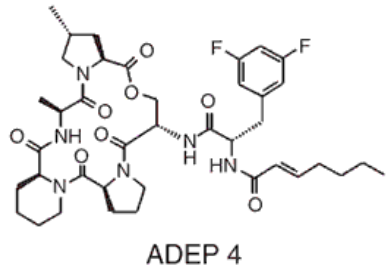
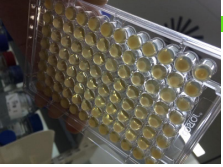


Staphylococcal biofilms: strategies currently under investigation



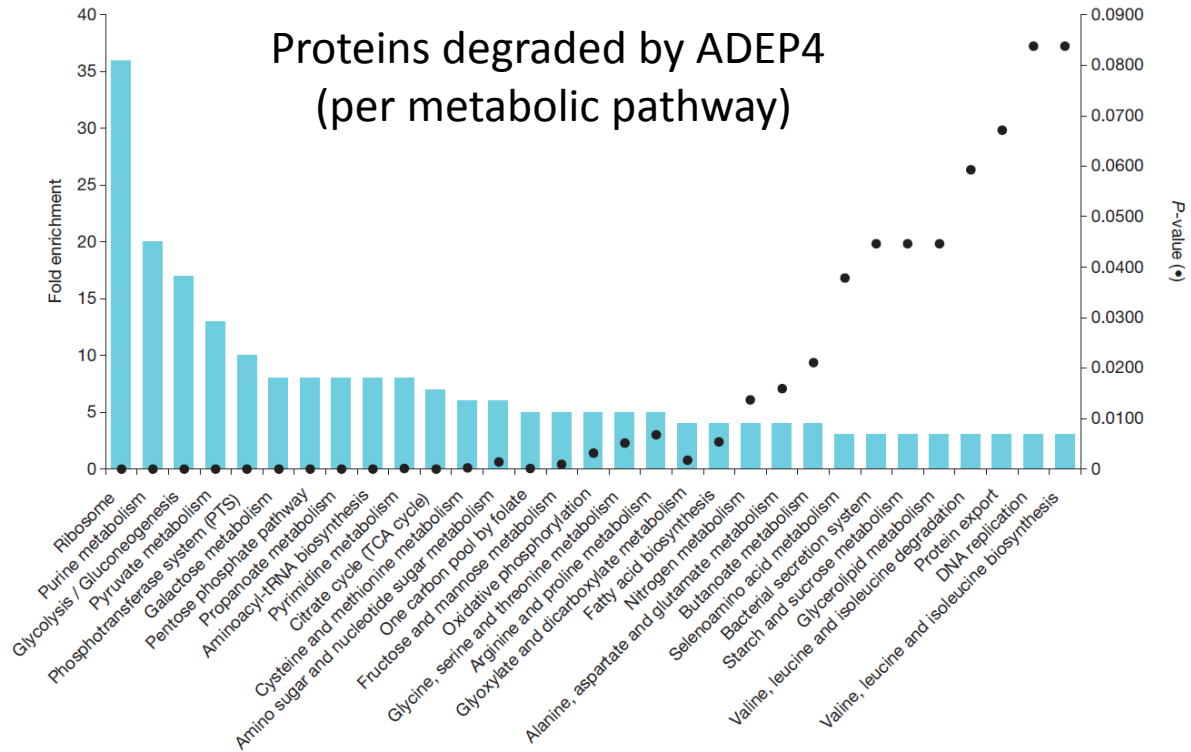
Suresh et al, Int J Med Microbiol. 2019;309:1-12

Antipersister compounds to help antibiotics eradicating bacteria in biofilms

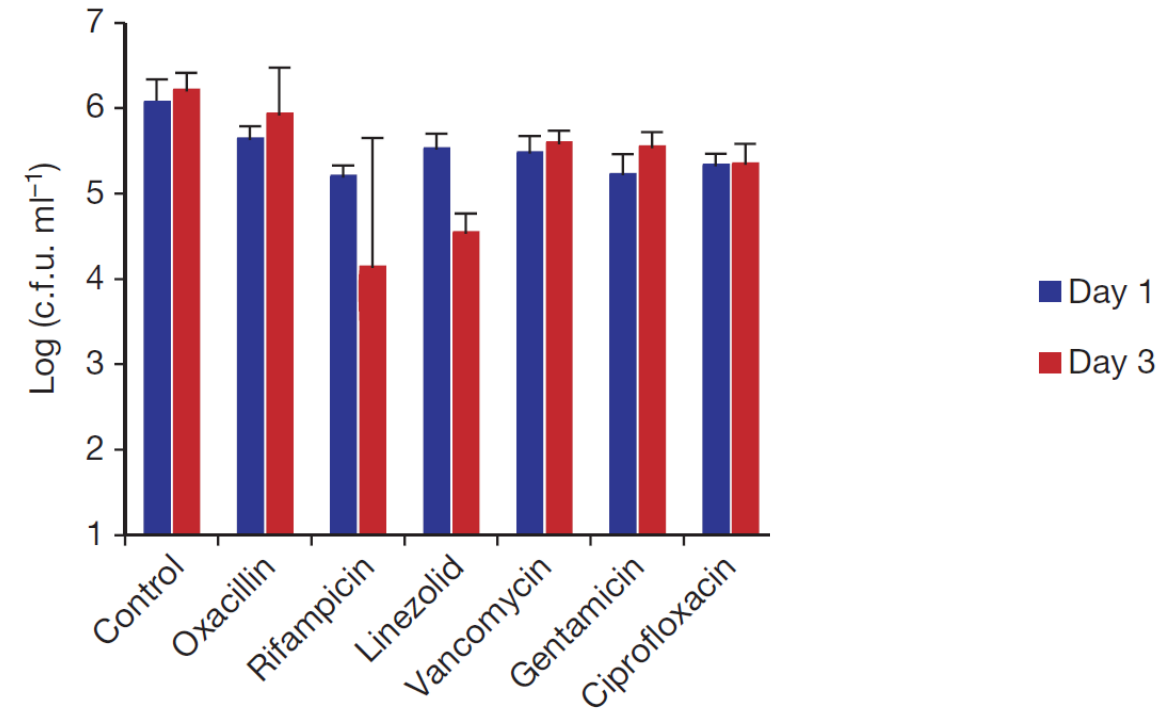


targets ClpP, core unit of a major bacterial protease complex.

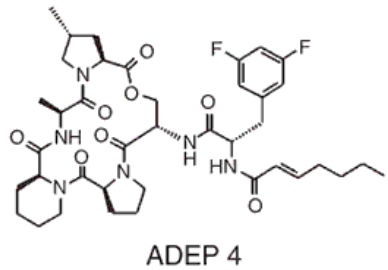
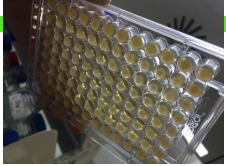
Proteins degraded by ADEP4 (per metabolic pathway)



Biofilm in vitro

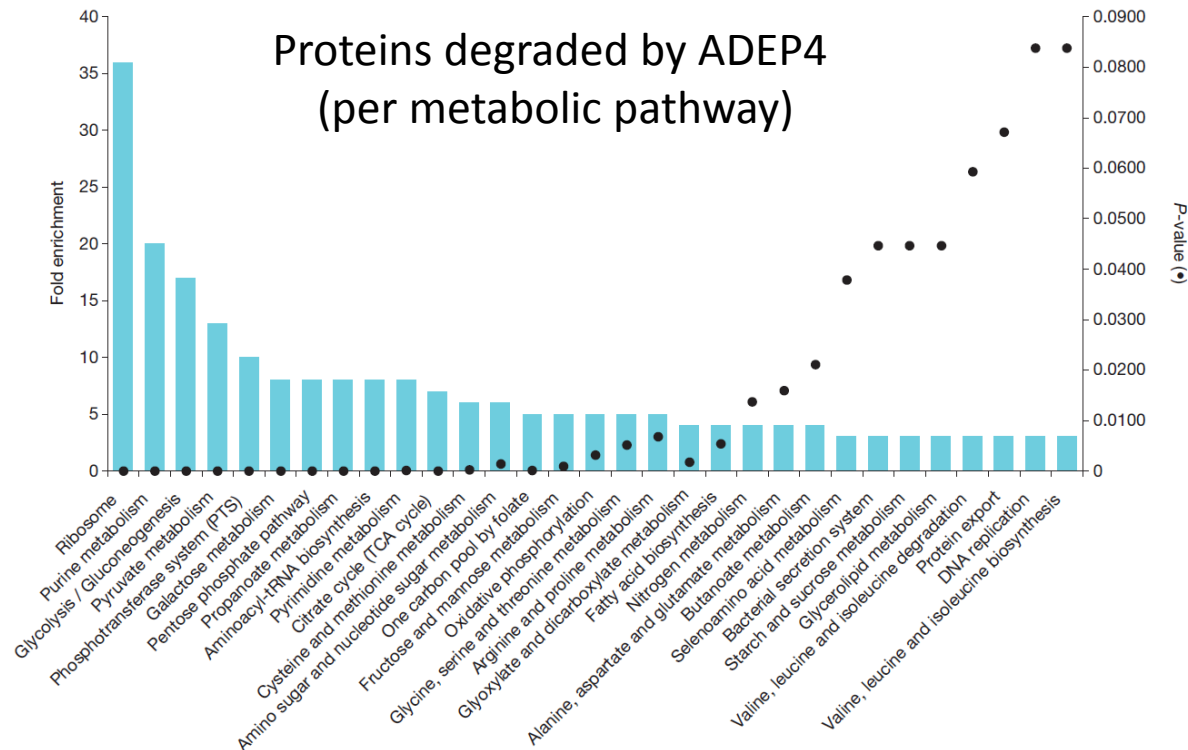


Antipersister compounds to help antibiotics eradicating bacteria in biofilms

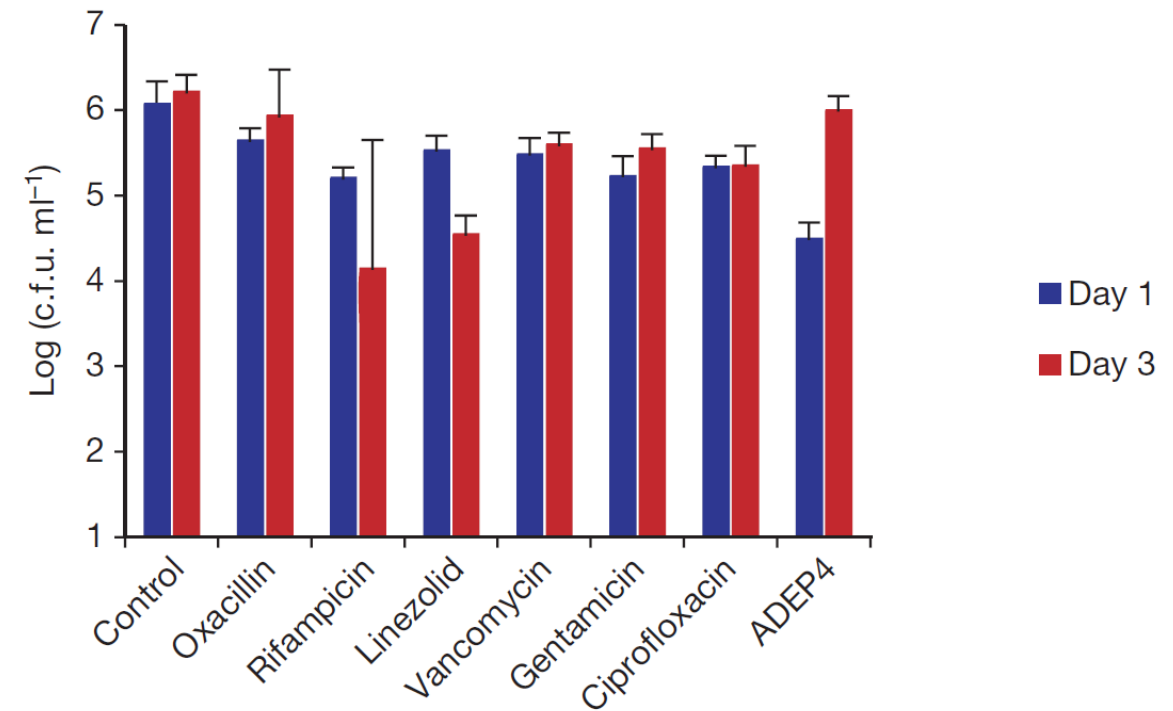


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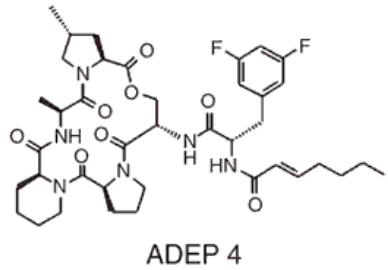
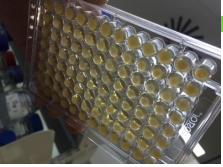
Proteins degraded by ADEP4 (per metabolic pathway)



Biofilm in vitro



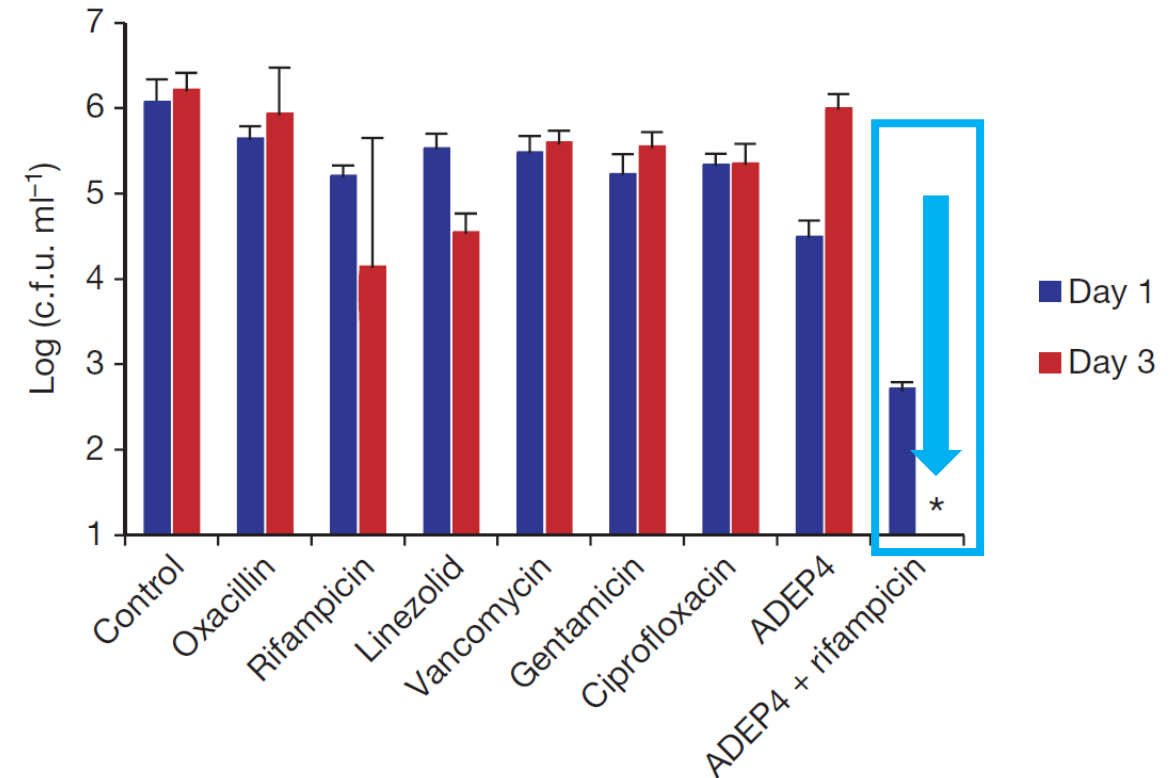
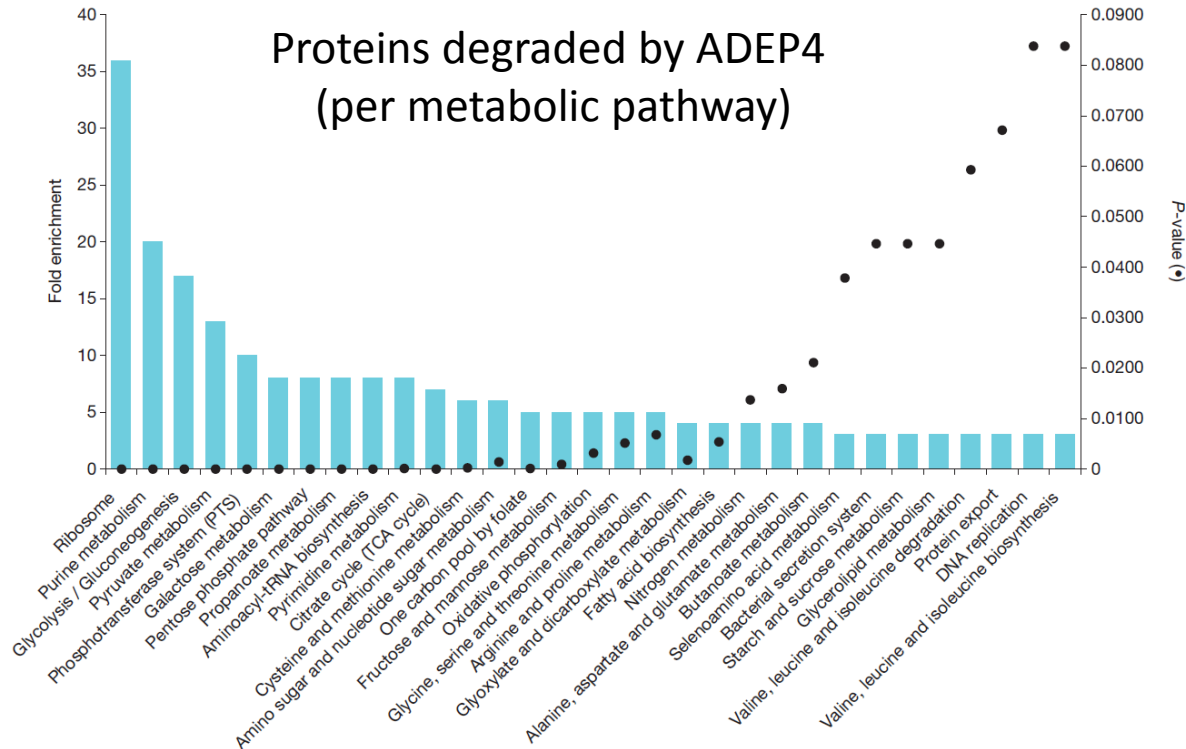
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targets ClpP, core unit of a major bacterial protease complex.

Biofilm in vitro

Proteins degraded by ADEP4 (per metabolic pathway)



Conlon et al, Nature 2013;503: 365–70

Staphylococcal biofilms: which are the best weapons to combine ?



Staphylococcal biofilms: which are the best weapons to combine?



- **Antibiotic combinations:**

- Useful to prevent resistance
 - Frequent synergy in vitro
 - Sometimes synergy in vivo
- } rifampicin / other bactericidal drugs ?

- **Repurposed drugs as potentiators:**

- May accelerate development
- But consider active concentrations vs. therapeutic concentrations

- **Peptides and antibodies:**

- Promising, some are in clinical development
- But ADME issues

- **Antipersisters:**

- Highly effective
- Currently only preclinical preliminary data available



Acknowledgments

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Bauer



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Yvan
Diaz Iglesias

KU LEUVEN



Sona
Kucharíková



Patrick
Van Dijck

