

**Virulence and Resistance in *Staphylococcus aureus*:
2016 State of the Art, ESCMID Postgraduate
Education Course**

28 June - 1 July 2016, Lyon, France



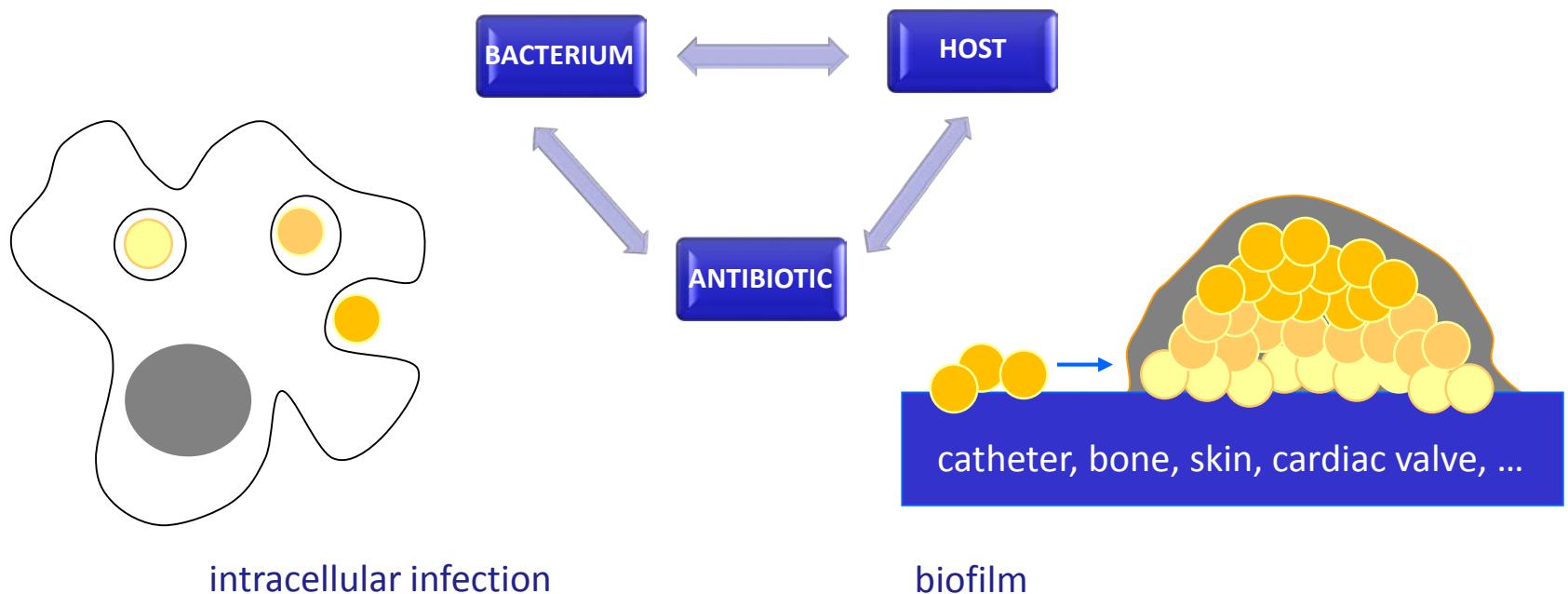
Anti-staphylococcal activity of antibiotics in biofilm and host cell

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Brussels, Belgium

<www.facm.ucl.ac.be>

Persistent forms of infection by *S. aureus*



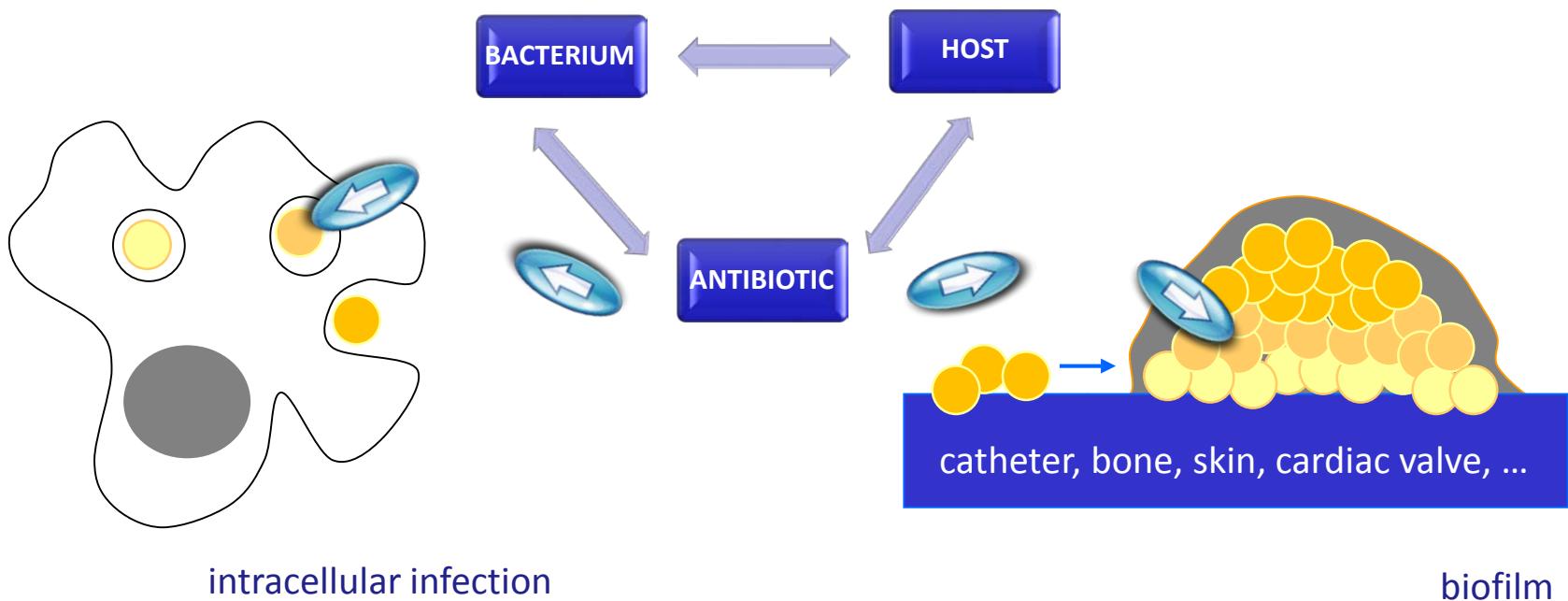
Persistent forms of infection by *S. aureus* and antibiotics

PK parameters:

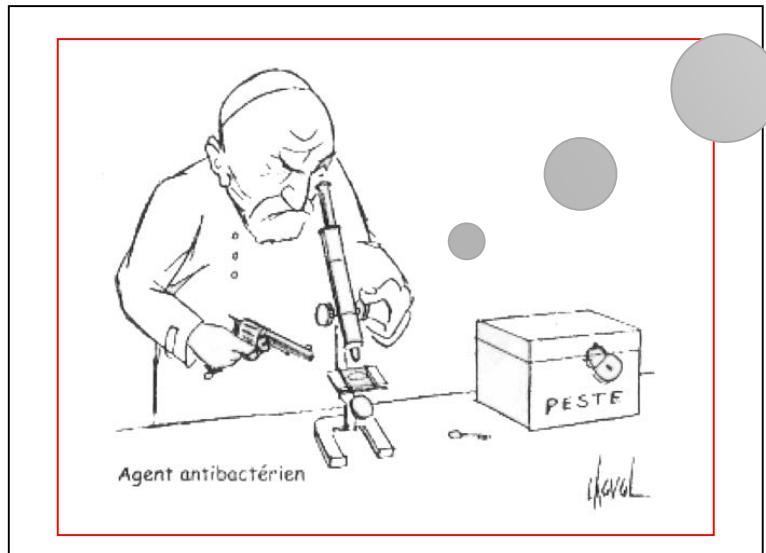
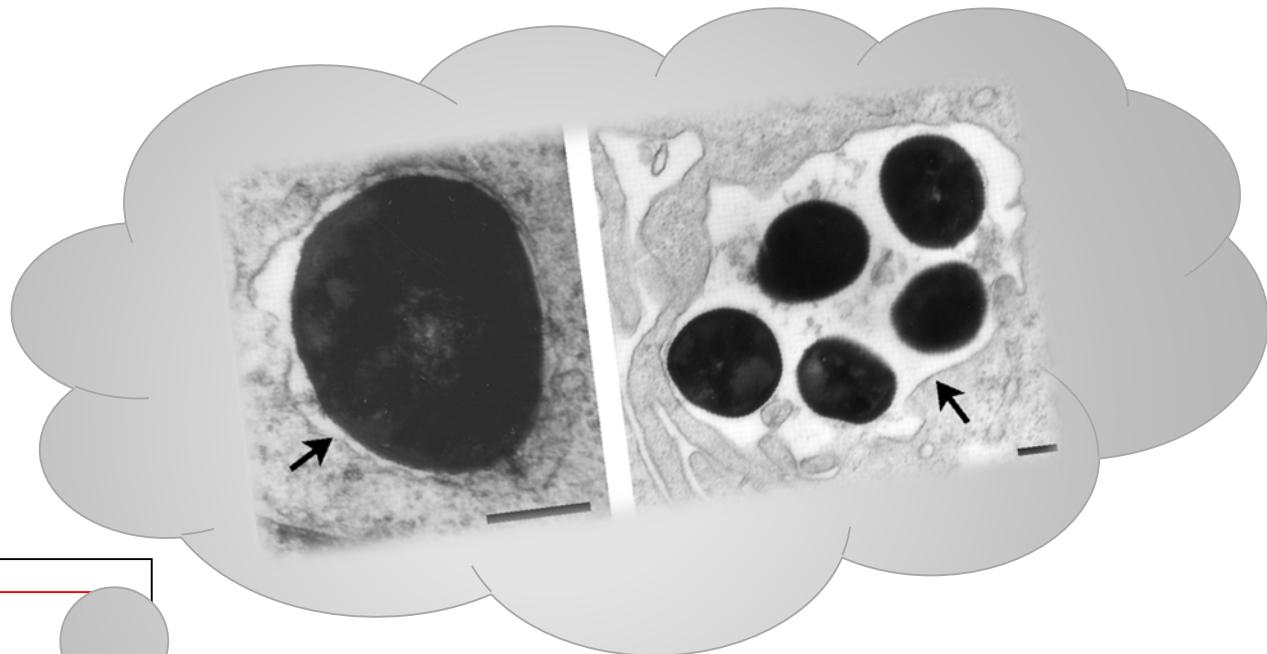
Access and accumulation
at the infection site

PD parameters:

- Expression of antibiotic activity
- Bacterial responsiveness
- Cooperation with the host

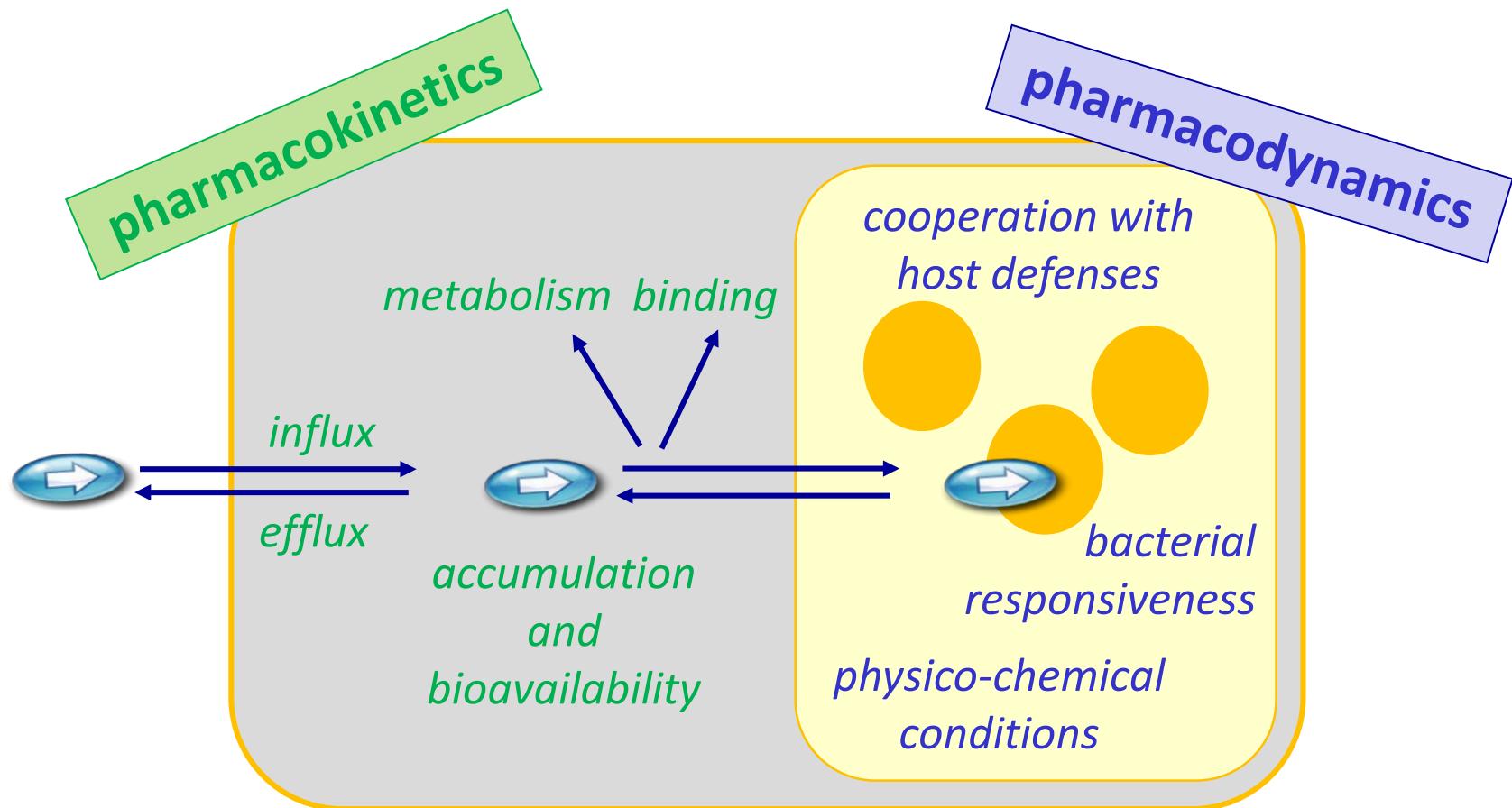


Intracellular infection



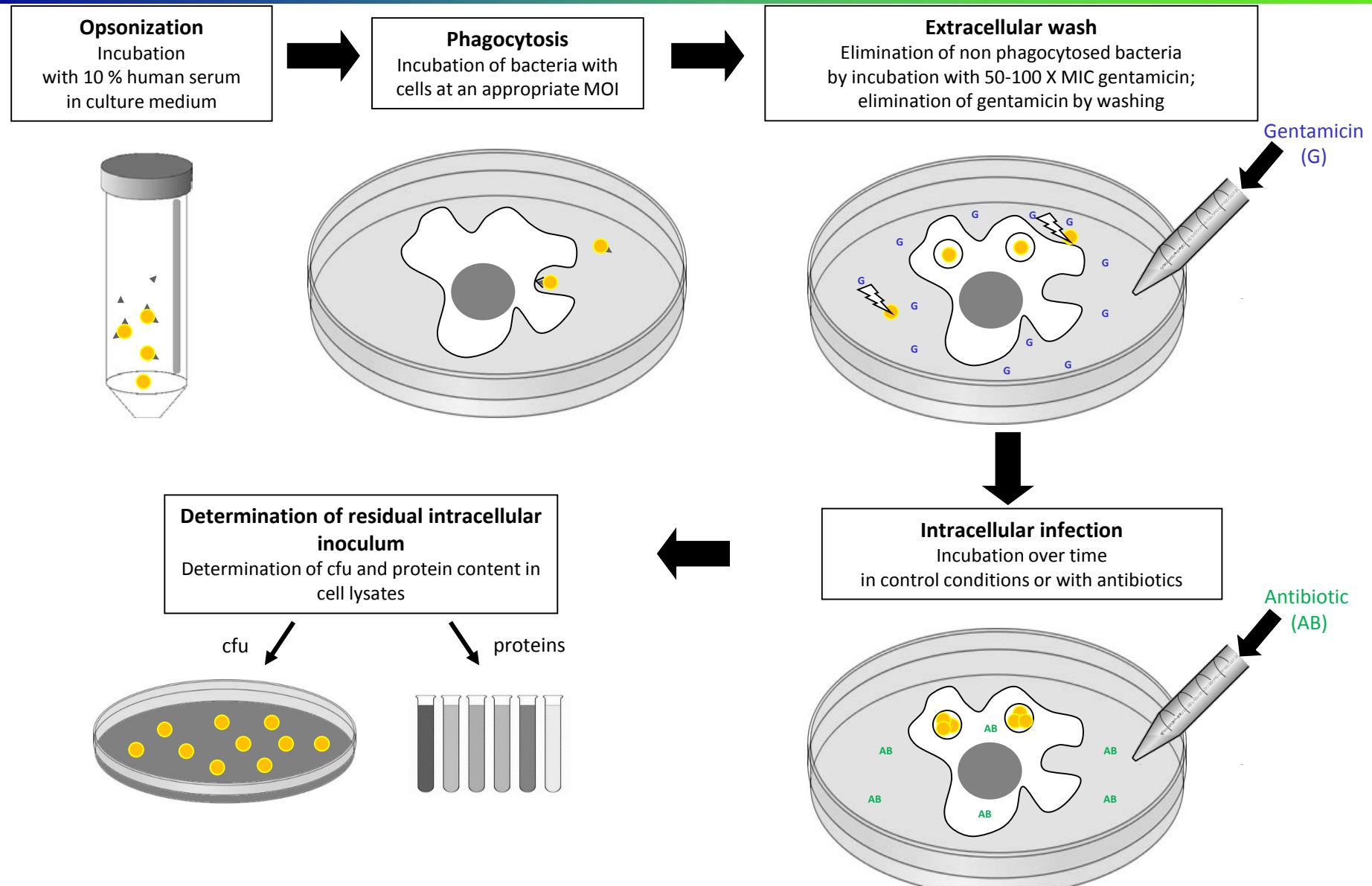
Nguyen et al, AAC (2009) 53: 4 1434-42

PK/PD parameters and intracellular activity

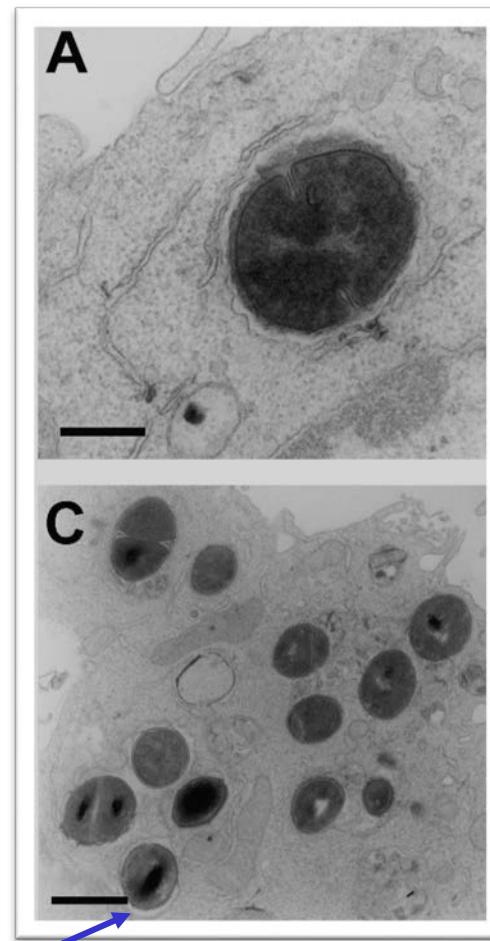
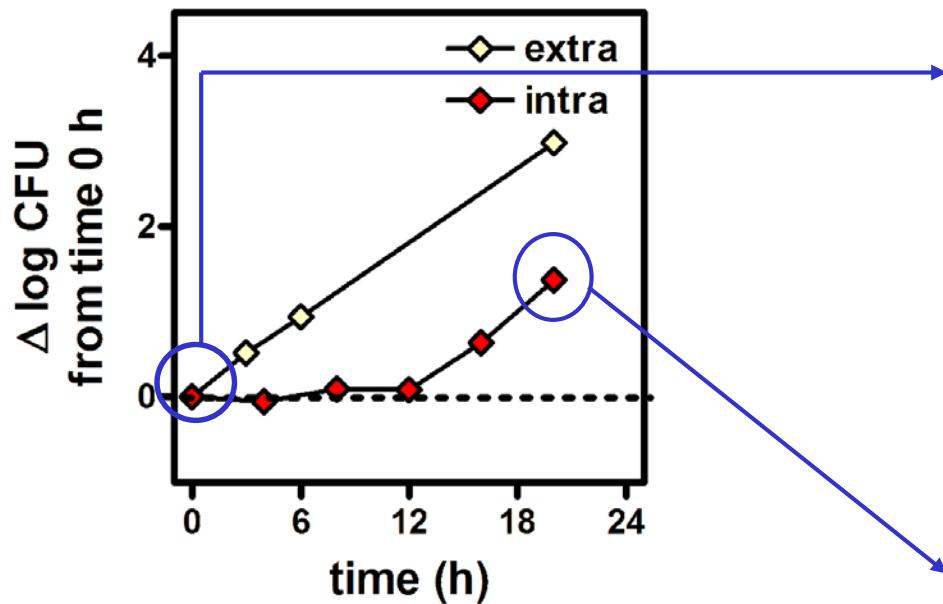


Carryn et al, Infect Dis Clin North Am (2003) 17:615-34

In vitro model of intracellular infection



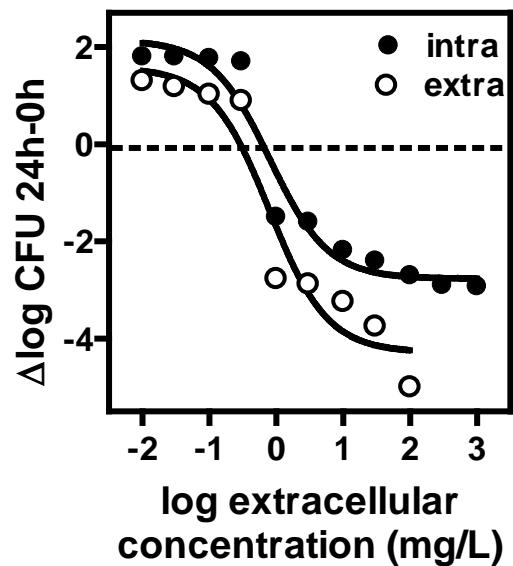
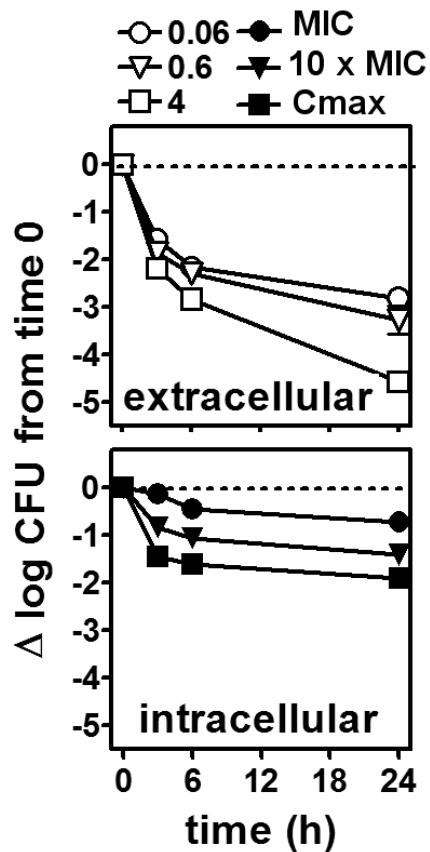
In vitro model of intracellular infection



remains in
vacuoles

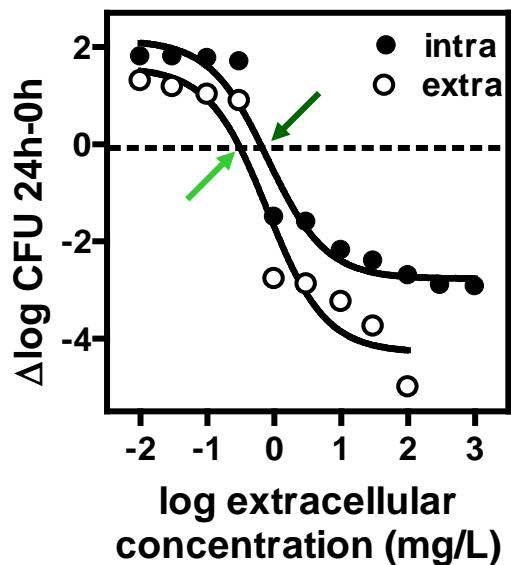
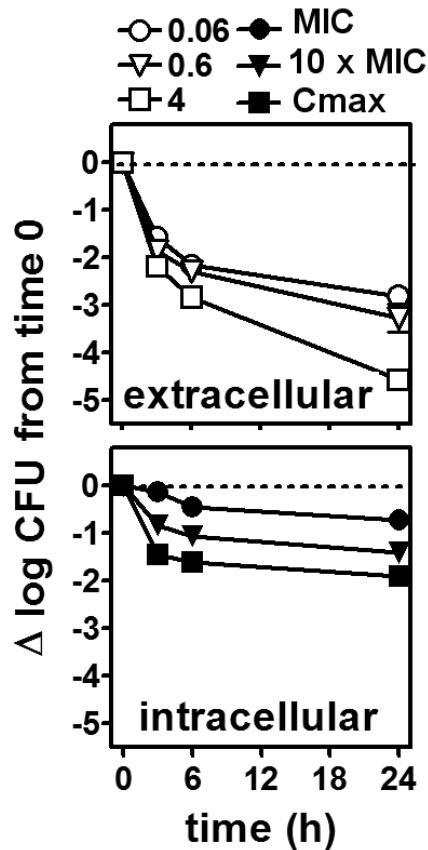
Setting-up appropriate models for the study of cellular activity of antibiotics

moxifloxacin & *S. aureus*



Setting-up appropriate models for the study of cellular activity of antibiotics

moxifloxacin & *S. aureus*

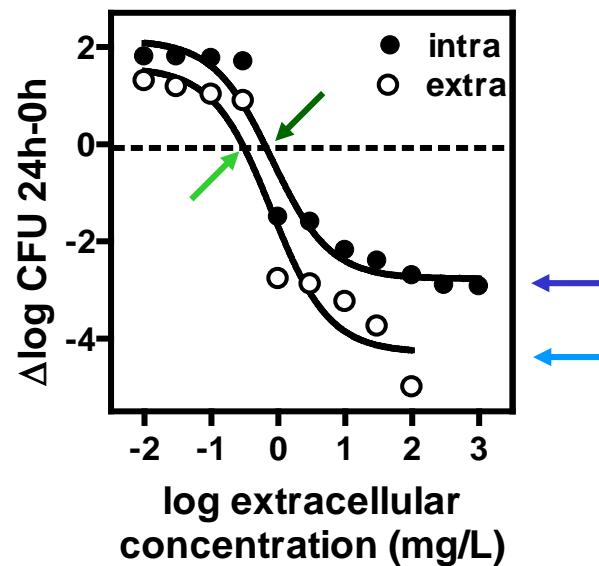
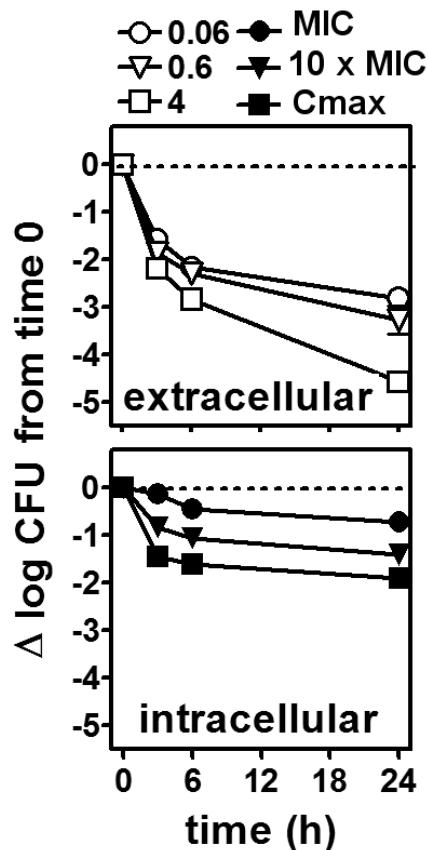


model	$C_{stat} (x \text{ MIC})$
extra	0.27
intra	0.63

relative
potency

Setting-up appropriate models for the study of cellular activity of antibiotics

moxifloxacin & *S. aureus*

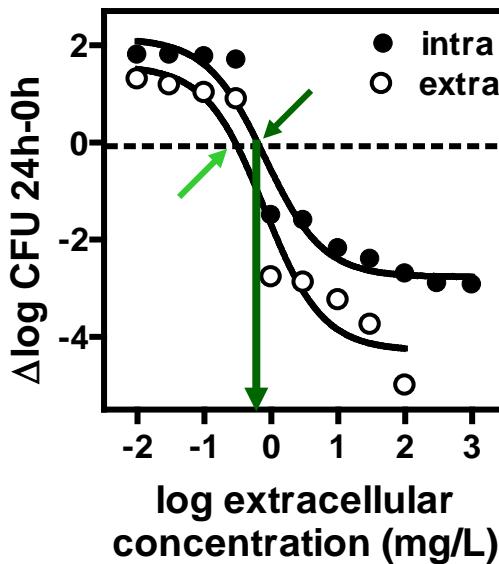


model	$C_{\text{stat}} (\times \text{MIC})$	E_{max}
extra	0.27	-3.86 (5.22 to 2.51)
intra	0.63	-2.77 (3.31 to 2.22)

relative
potency

maximal
efficacy

What do these parameters tell you ?



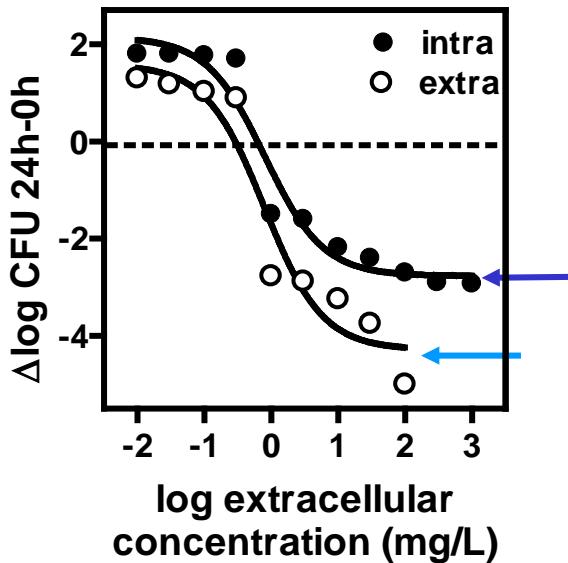
relative potency



- Estimation of the concentration needed to reach a specified effect
- Measure of the « intracellular MIC »
 - ⇒ « PK-related » parameter:
 - accumulation in the infected compartment
 - intracellular bioavailability
 - ⇒ influence of local environment on intrinsic activity
 - pH
 - oxidant species

In most cases
 $C_s \text{ intra} \geq C_s \text{ extra}$

What do these parameters tell you ?



maximal efficacy



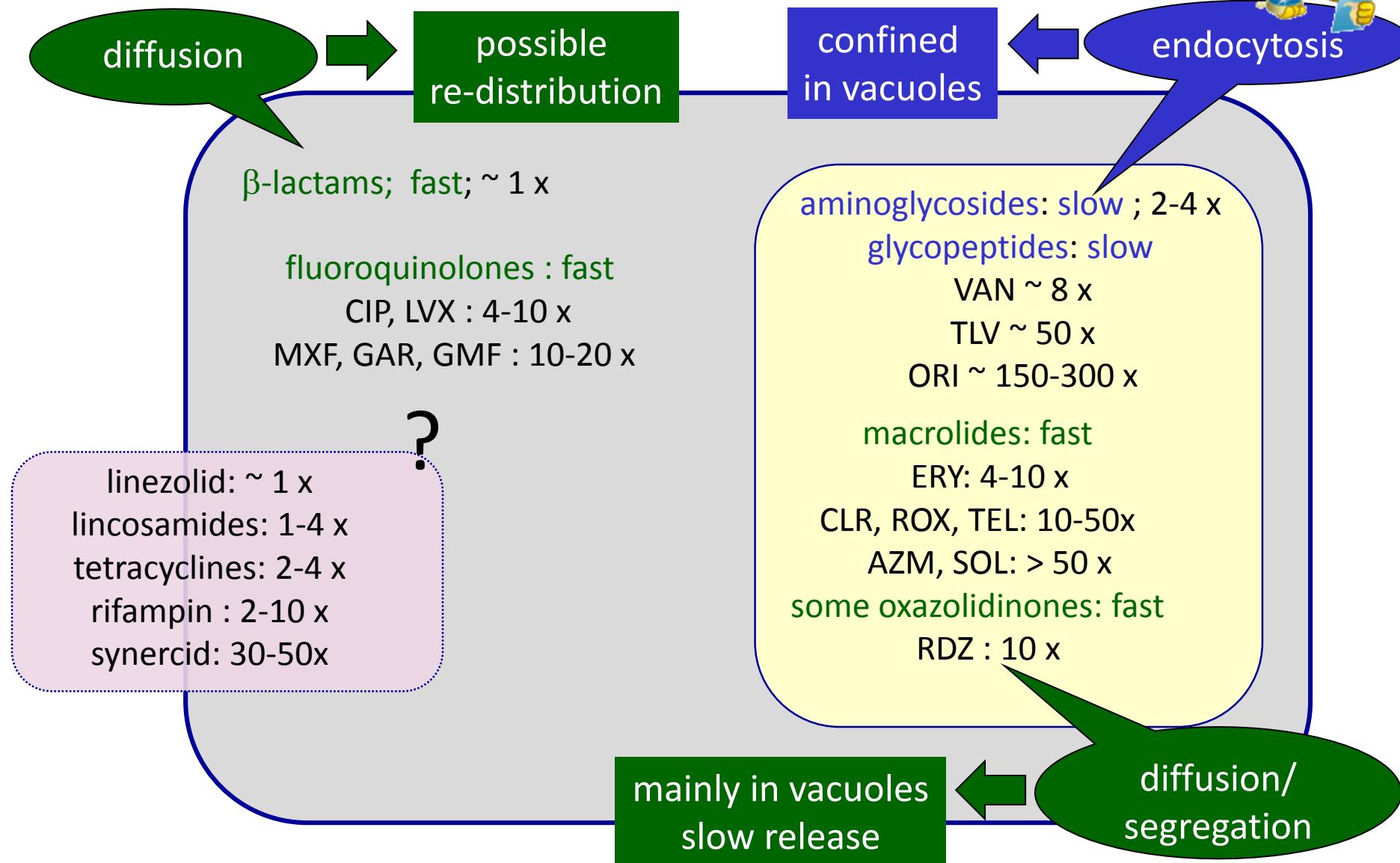
- Estimation of the maximal reduction in inoculum for an infinitely large concentration
- Measure of the killing capacity

⇒ « PD-related » parameter

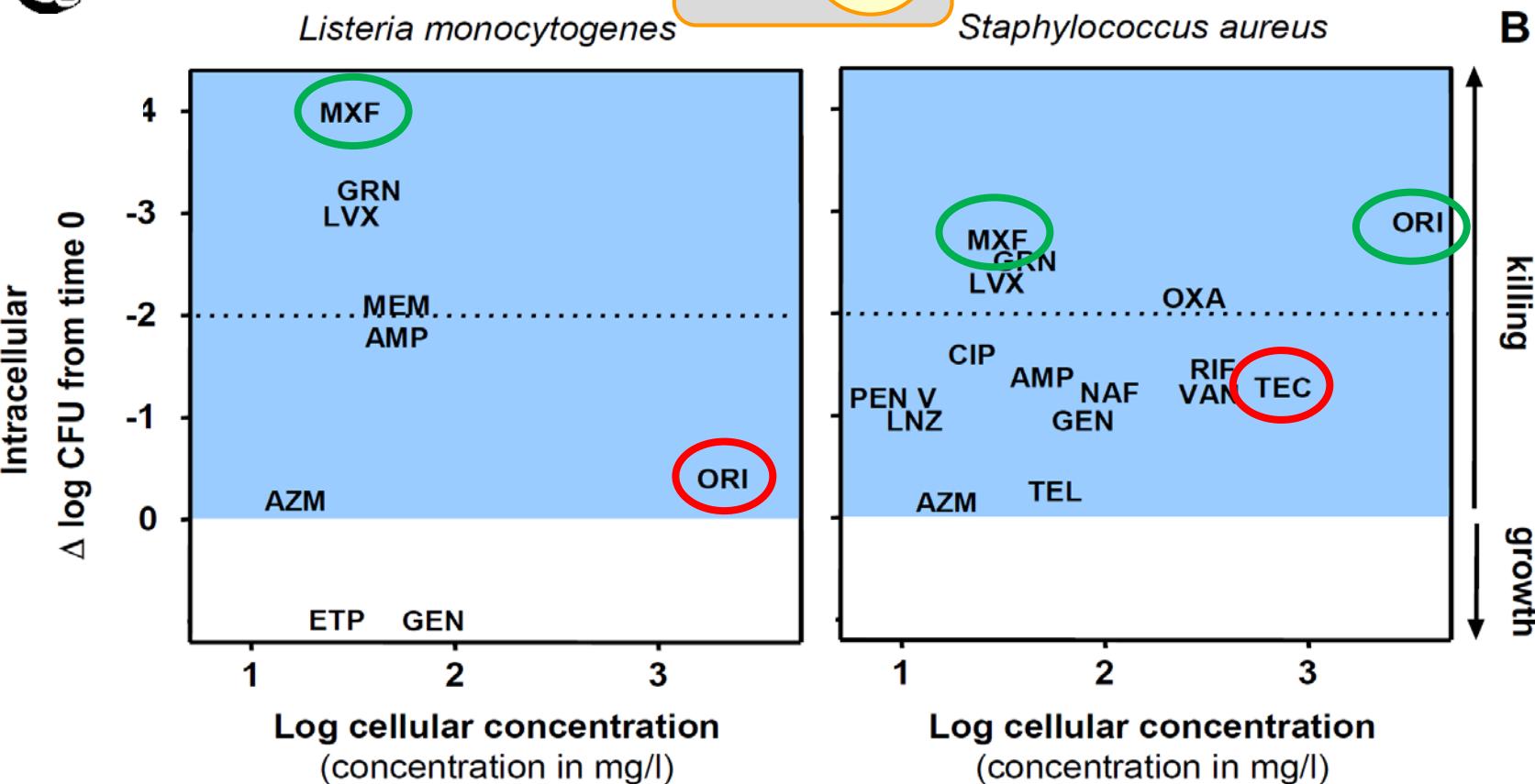
- mode of action of the drug
- bacterial responsiveness
- cooperation with host defenses

In most cases
 $E_{\text{max intra}} \lll E_{\text{max extra}}$

Antibiotic accumulation and subcellular distribution



Can we predict the intracellular activity based on intracellular concentrations?



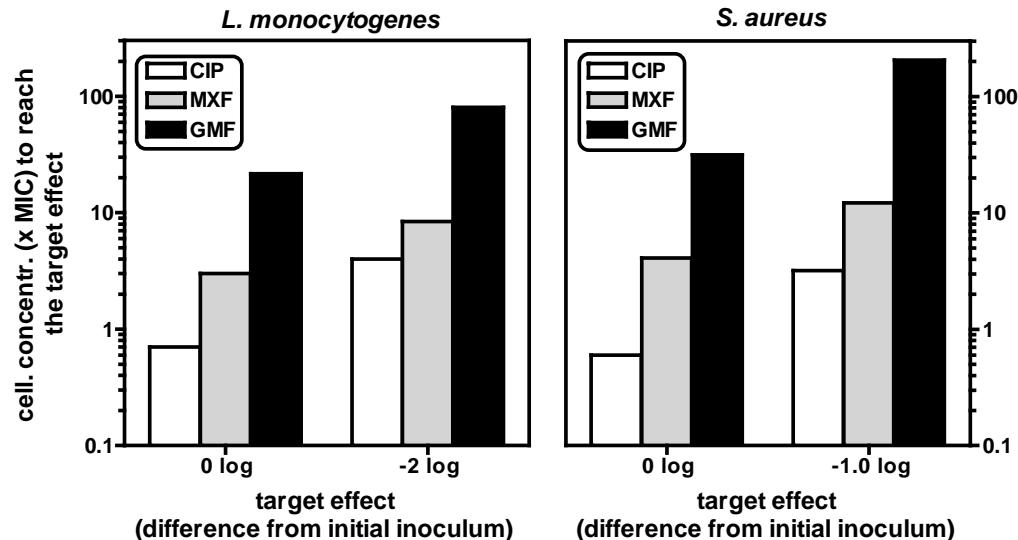
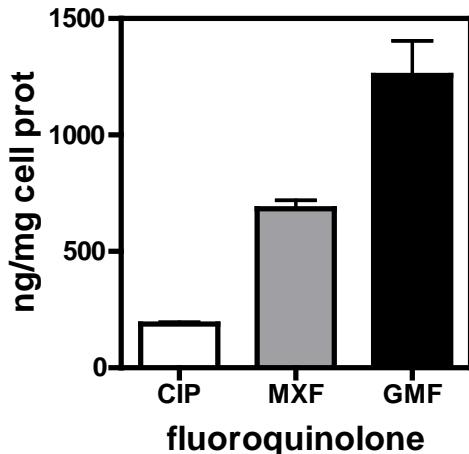
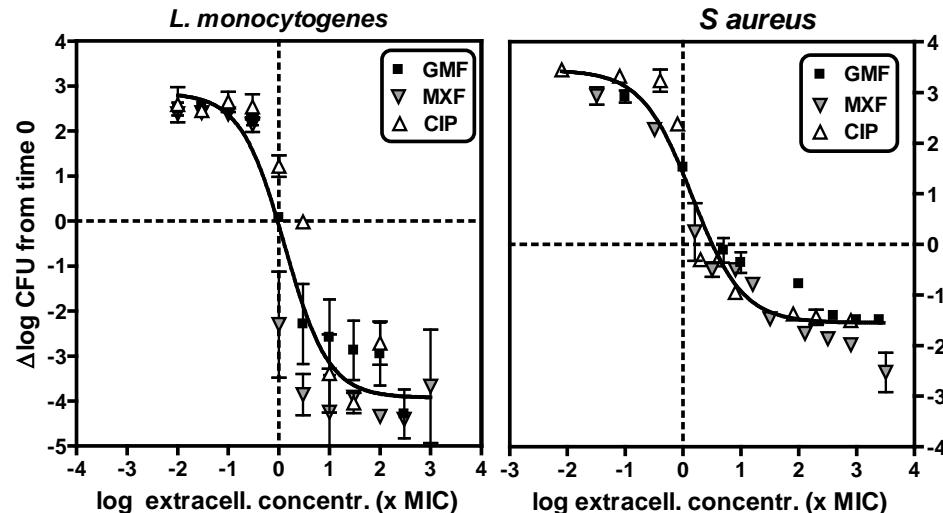
No correlation between intracellular concentration and intracellular activity

Importance of accumulation/bioavailability

Fluoroquinolones against *L. monocytogenes* vs. *S. aureus*



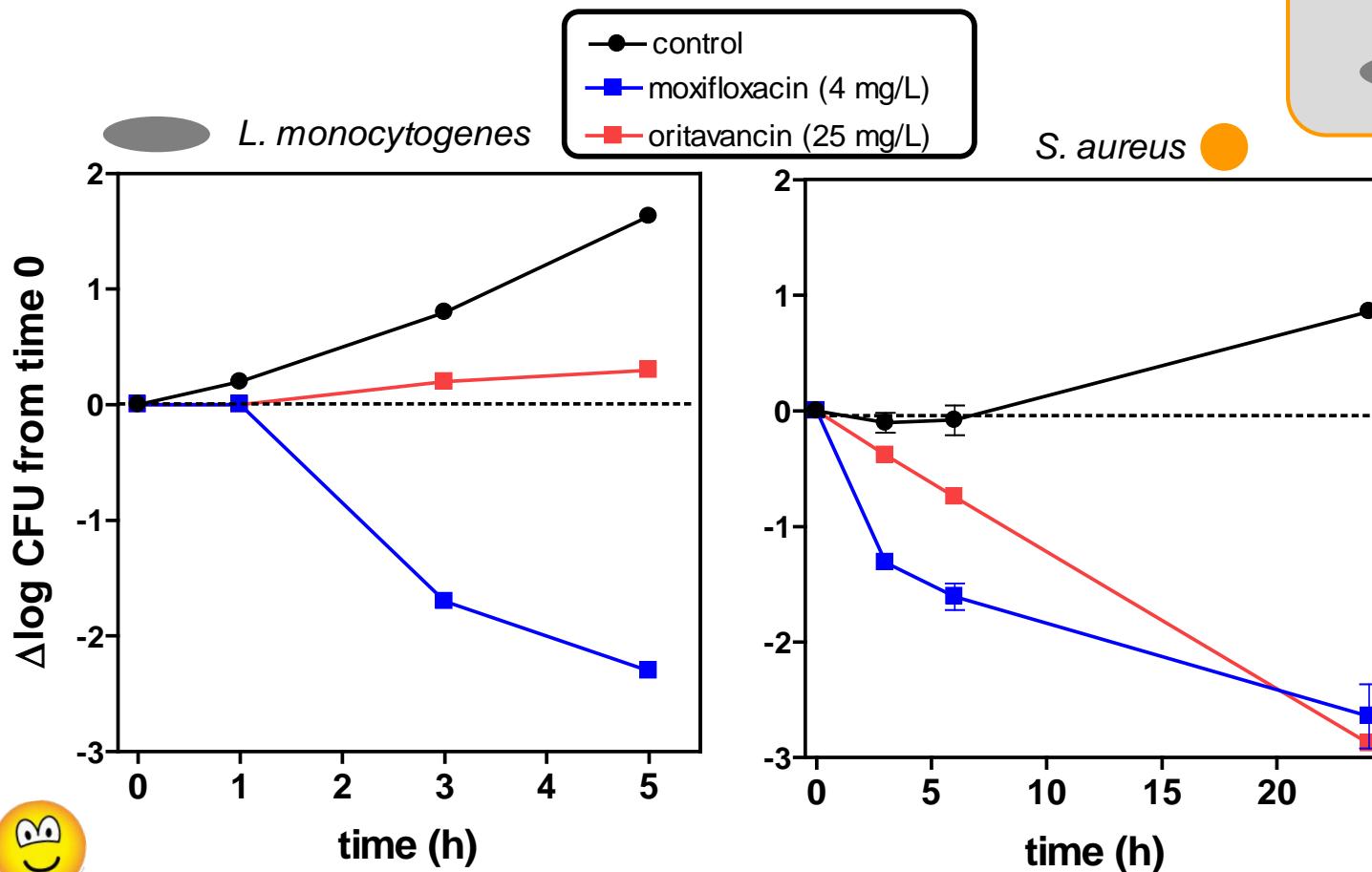
intracellular
bioavailability ?



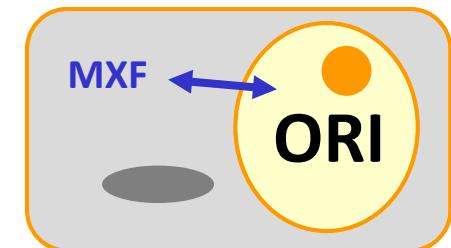
Vallet et al, IJAA (2011) 38:249-256.

Importance of subcellular distribution

Moxifloxacin & oritavancin against *L. monocytogenes* vs. *S. aureus*



AB needs to have access
to the infected compartment

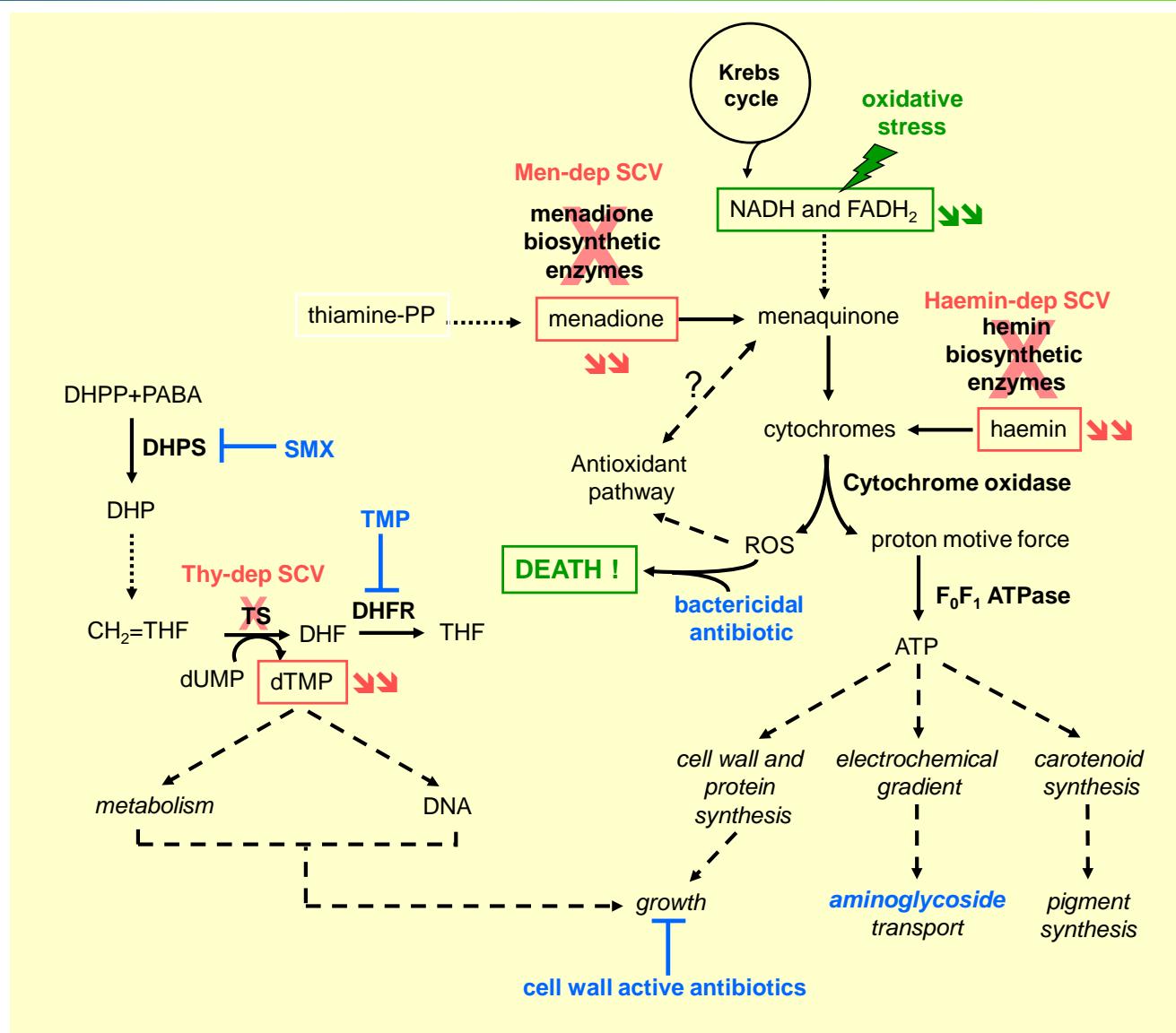
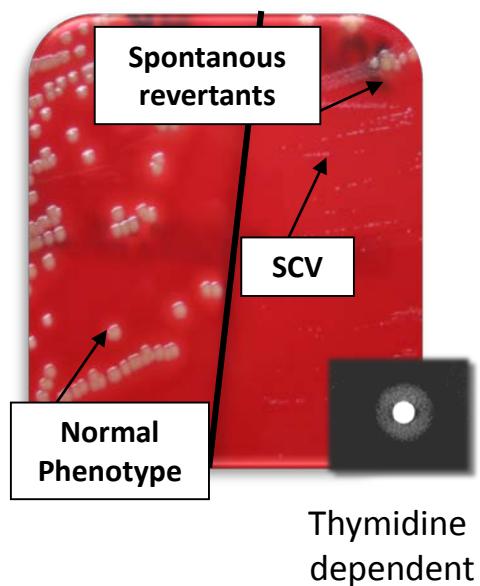


adapted from Carryn et al, AAC (2002) 46:2095-2103
Van Bambeke et al, AAC (2004) 48:2853-60
Barcia-Macay et al, AAC (2006) 50:841-51

Importance of bacterial phenotype

comparison : isogenic strains
with different phenotypes

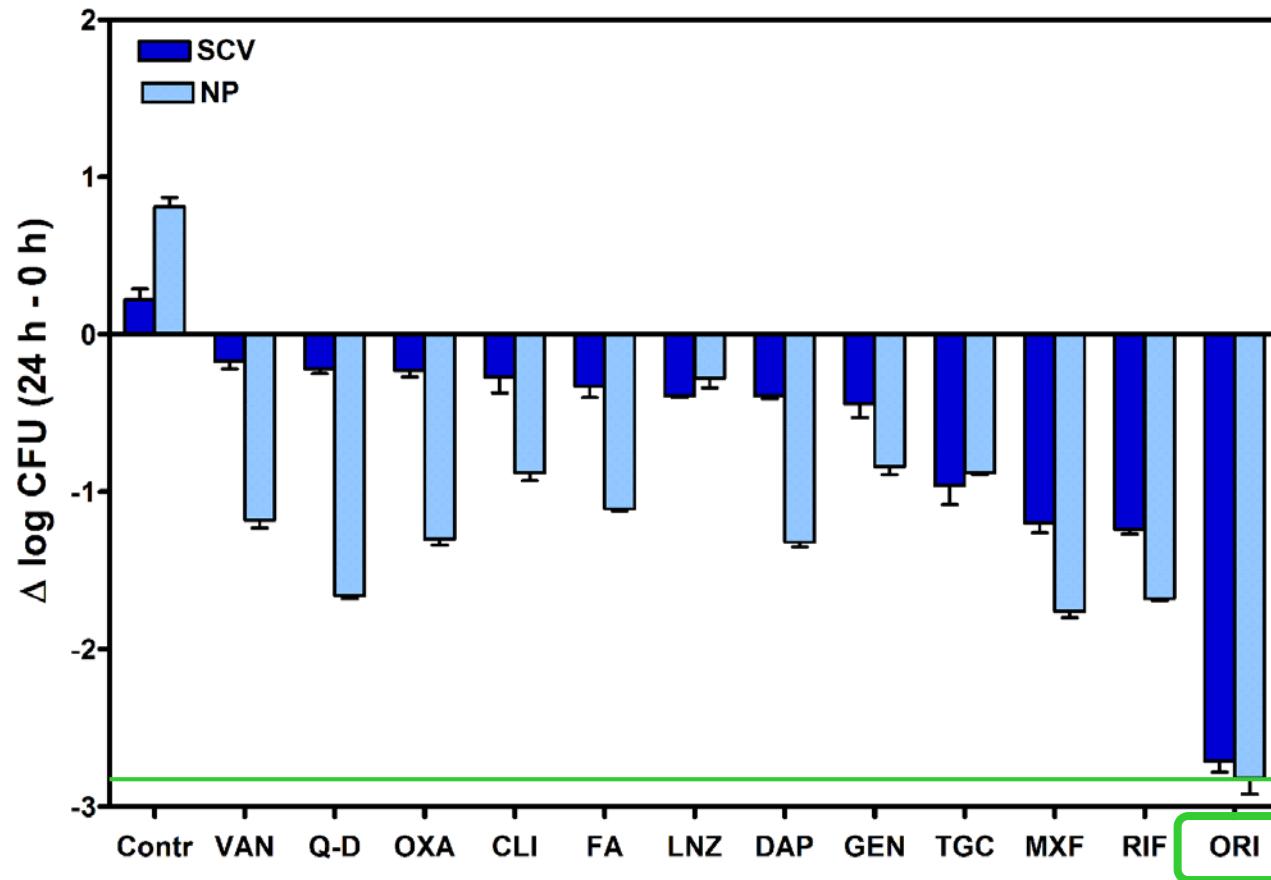
SCV vs normal phenotype



Importance of bacterial phenotype

PD

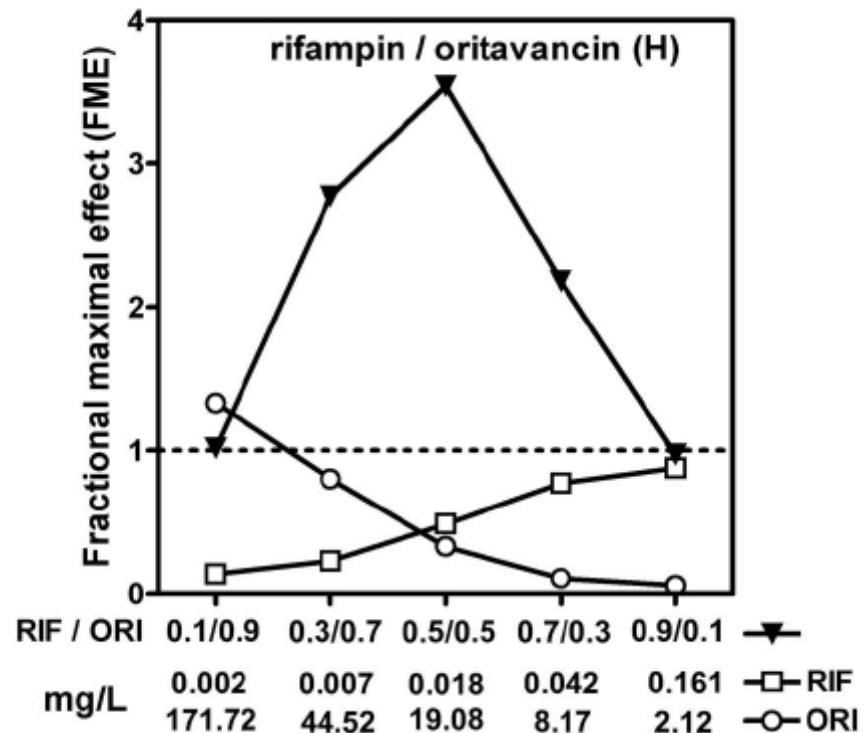
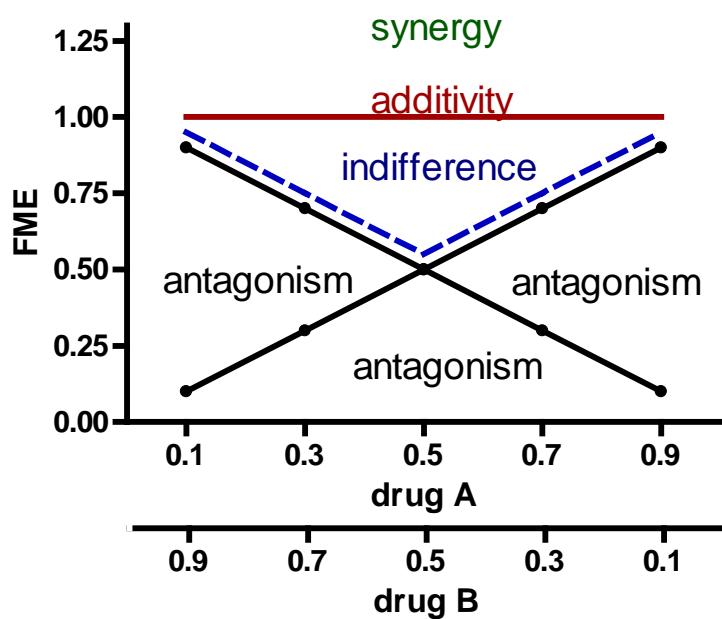
comparison : isogenic strains with different phenotypes



Efficacy lower against SCV, possibly related to slower metabolism ?

Nguyen et al, AAC (2009) 53:1434–42

Antibiotic combinations

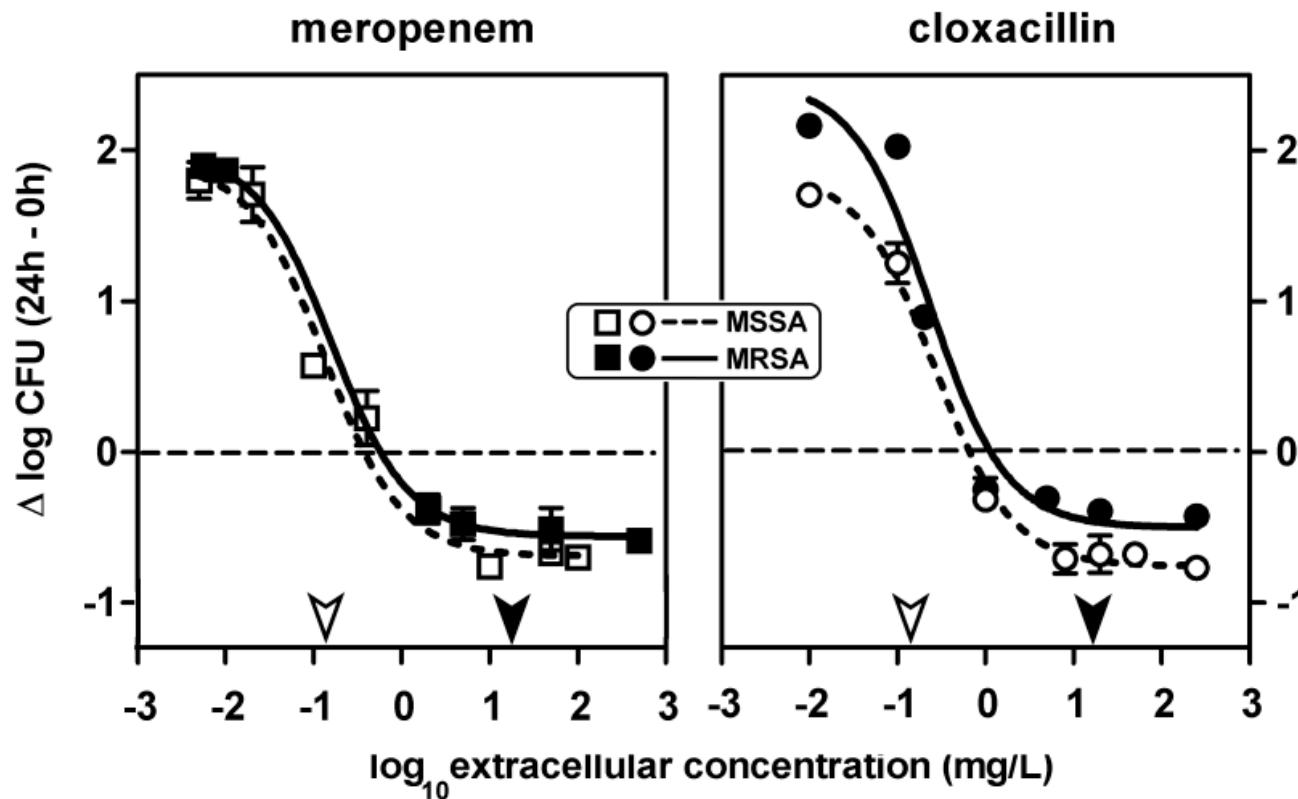


Combinations – synergism !

Influence of intracellular pH

PD

MRSA are as susceptible as MSSA to β -lactams when intracellular !

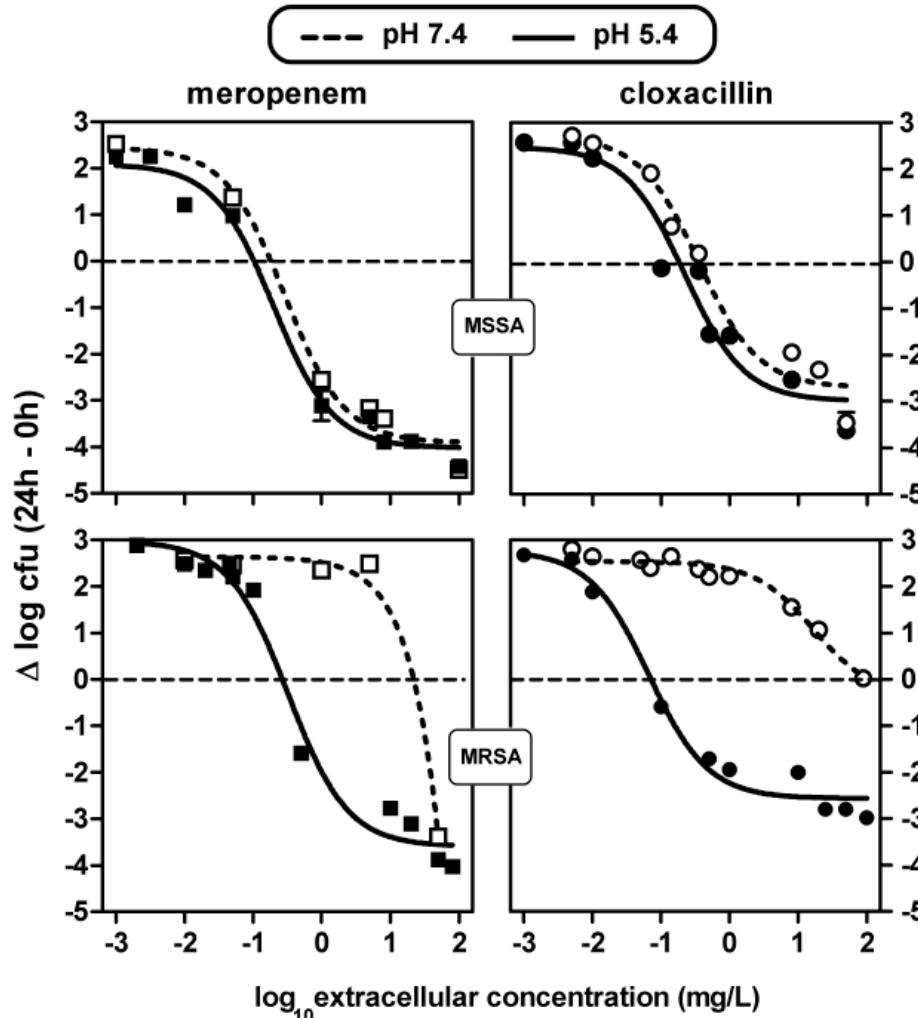


Lemaire et al., AAC (2007) 51:1627-32

Influence of intracellular pH



MRSA are as susceptible as MSSA in broth at acidic pH



Lemaire et al., AAC (2007) 51:1627-32

PBP2a conformation is modified by acidic pH

PD

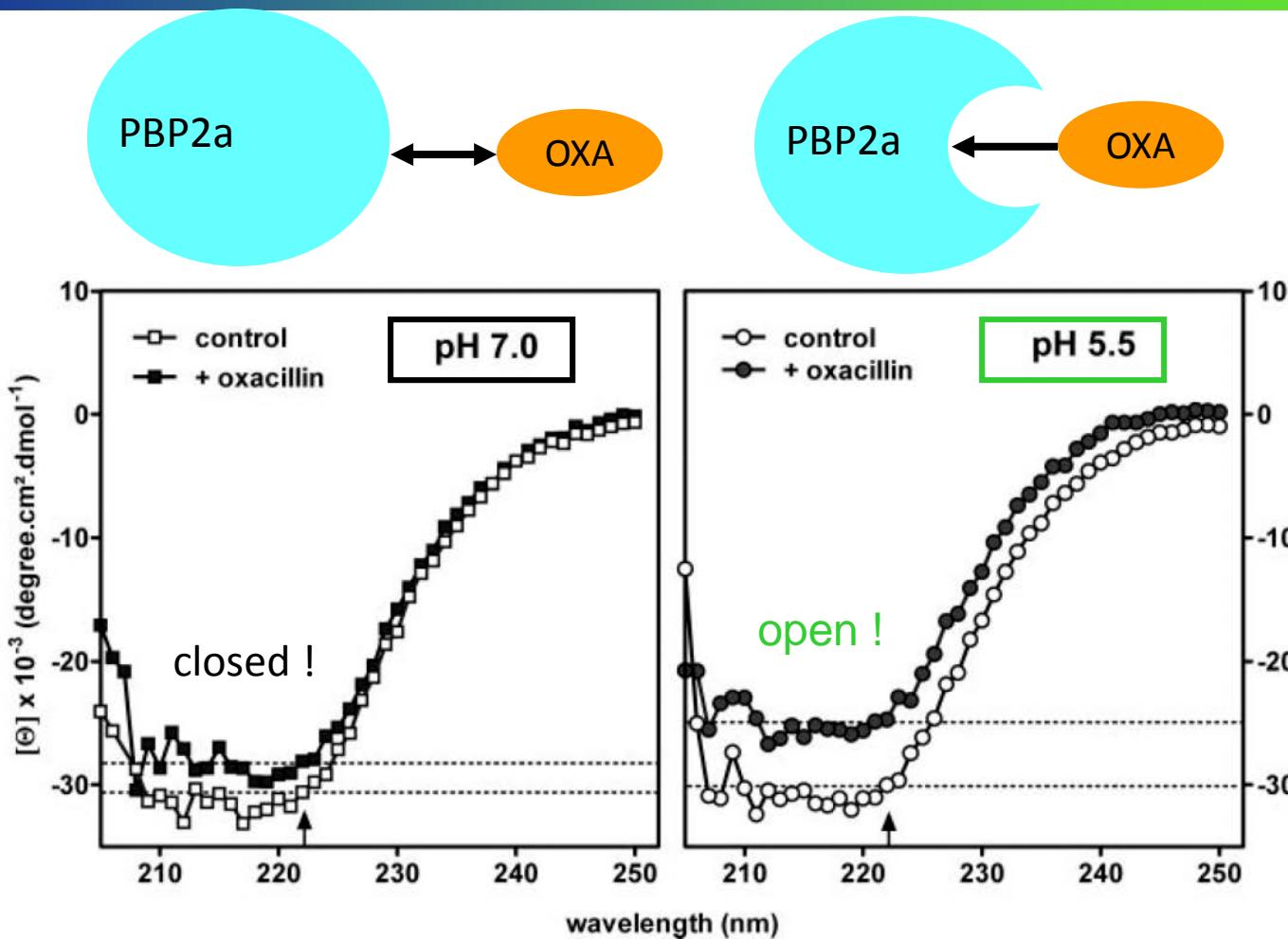
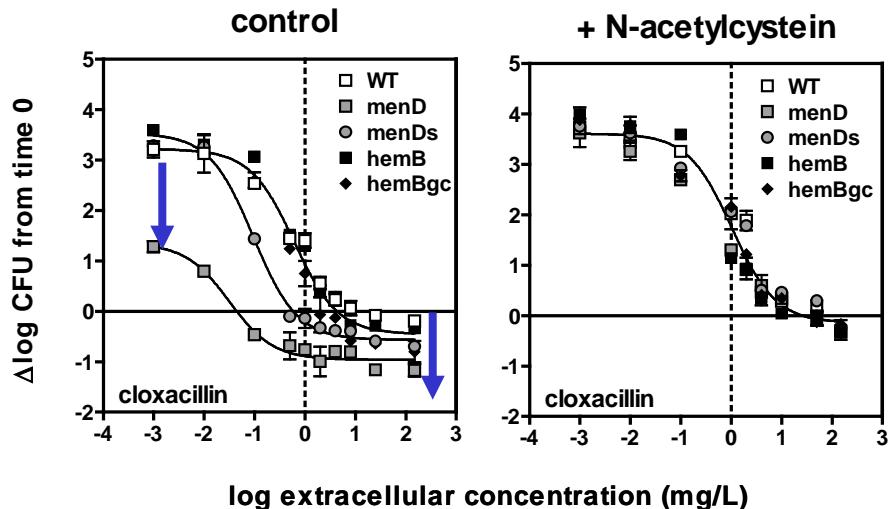


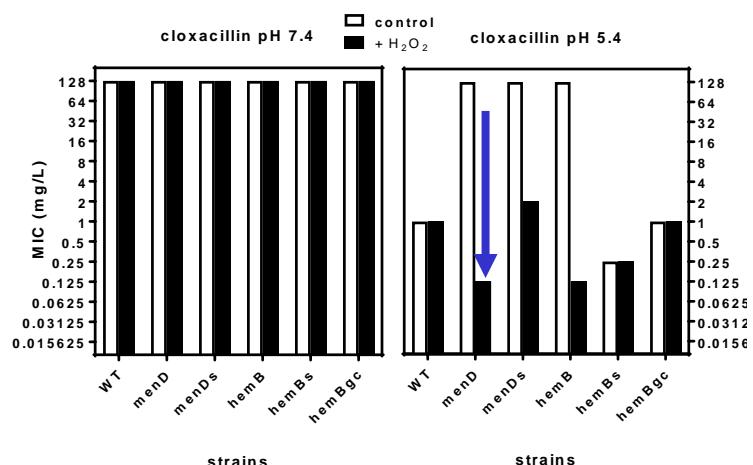
FIGURE 4. Circular dichroic spectra of PBP 2a at pH 7.0 (left panel) and pH 5.5 (right panel) in the absence (open symbols) and in the presence (closed symbols) of oxacillin (30 μ M) for 30 min at 25 °C. The thin dotted lines in each graph represent minima of PBP 2a molar ellipticity at 222 nm (vertical arrow on the abscissa) for each condition. The spectrum of oxacillin has been subtracted from all data points.

Importance of cell defense mechanisms

comparison : isogenic strains with different phenotypes



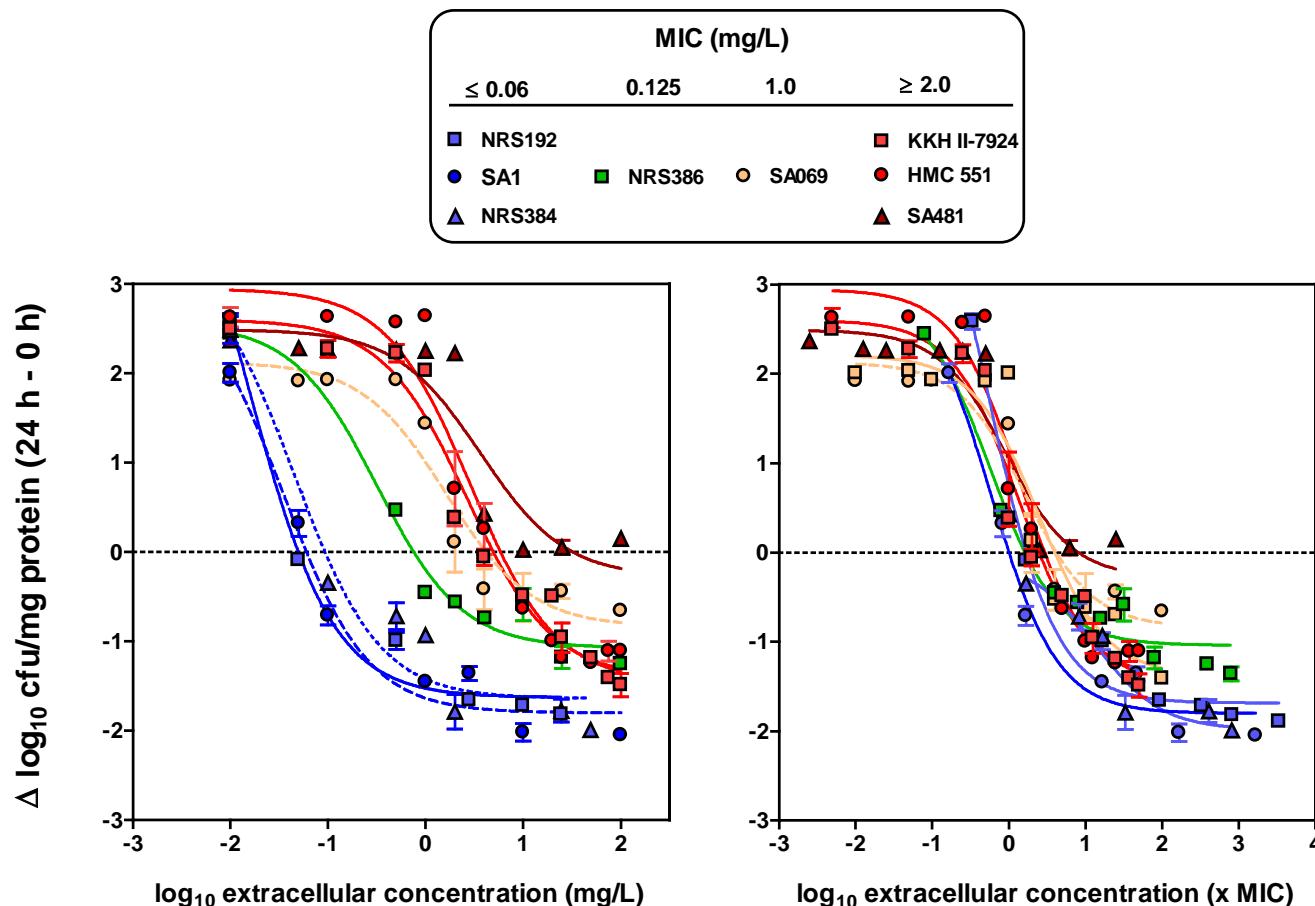
The menadione SCV
is hypersusceptible
intracellularly because of
the combined effect
of acidic pH and oxidant species

Comparison of a single antibiotic towards several strains



comparison : moxifloxacin against increasingly resistant MRSA

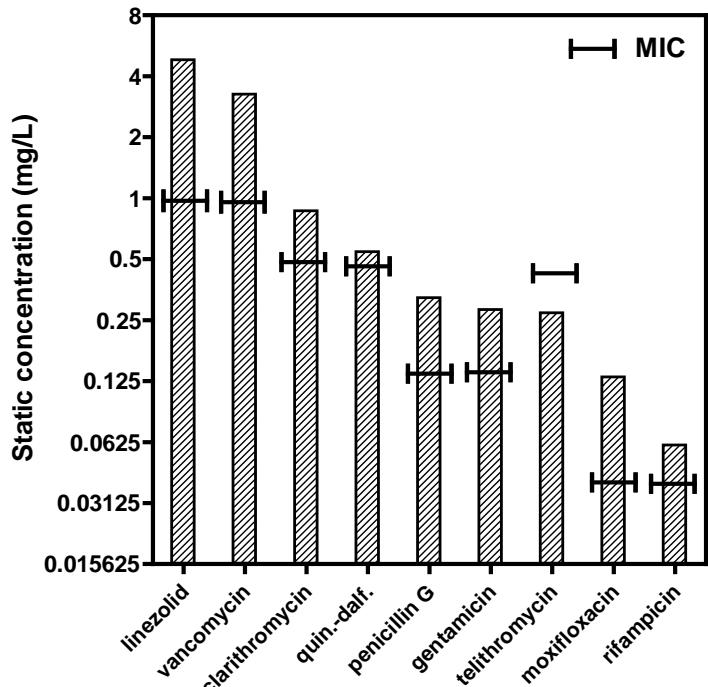
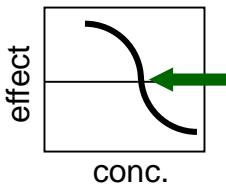


$C_s \sim \text{MIC}$ 😎

Comparison of several antibiotics towards a single strain

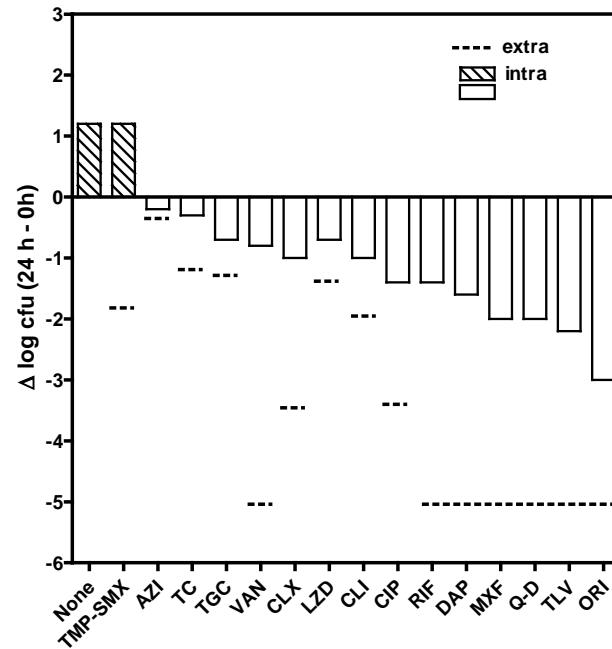
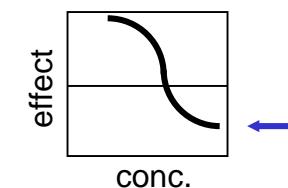


Relative potency ?



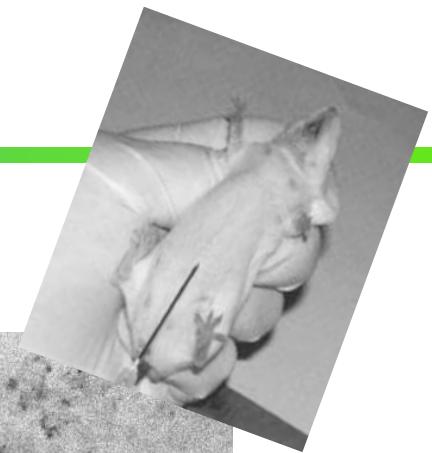
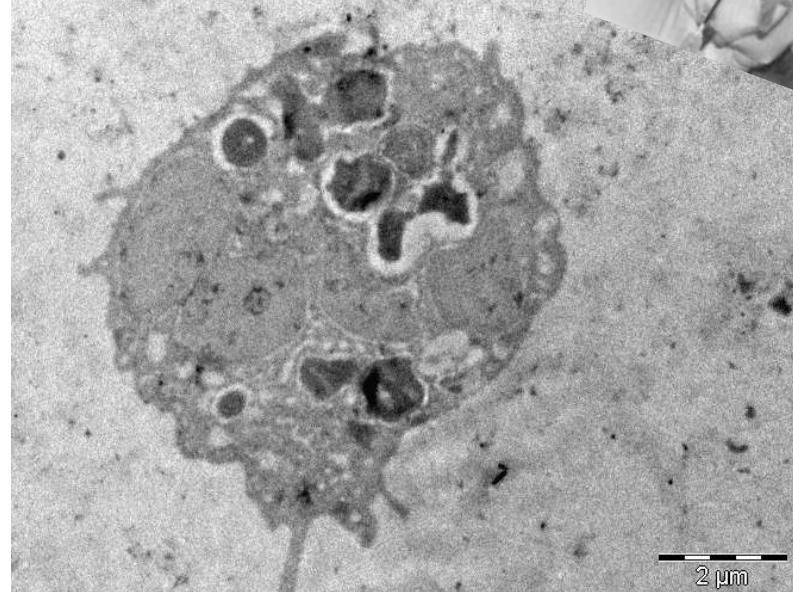
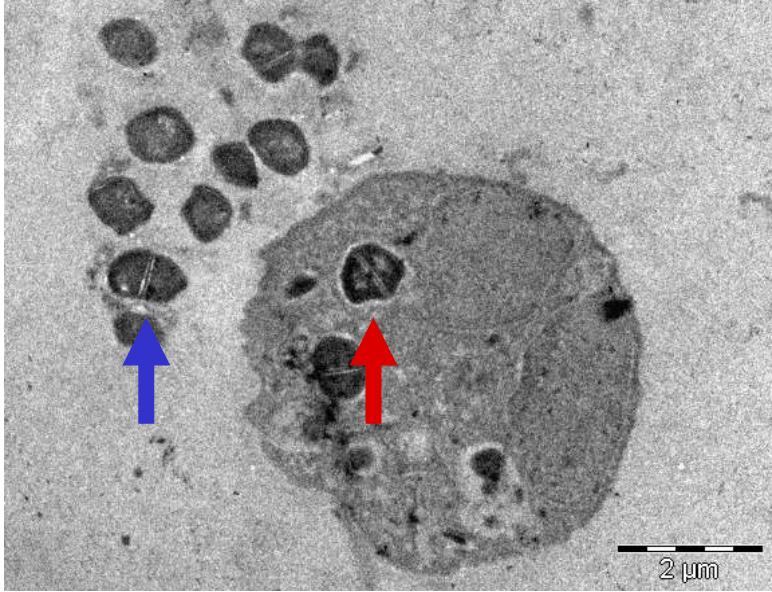
$C_s \sim MIC$

Maximal efficacy?



$Emax_{intra} \ll Emax_{extra}$

From in vitro to in vivo : The mouse peritonitis model



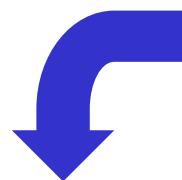
Electron microscopy of peritoneal fluid post infection with *S. aureus*

- **Extracellular *S. aureus***
- **Intracellular *S. aureus***

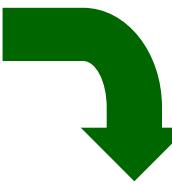
in vitro vs in vivo



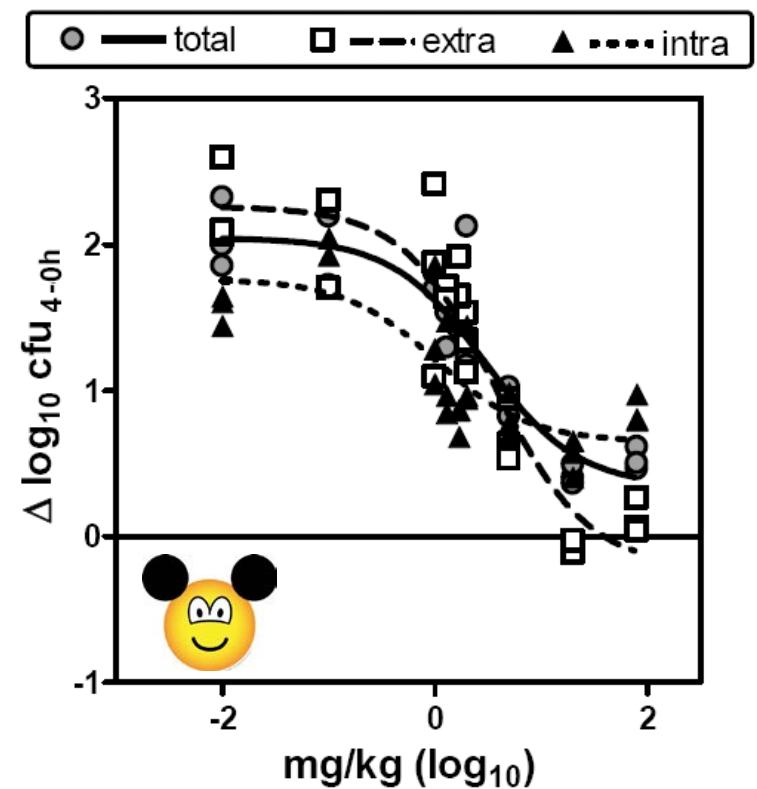
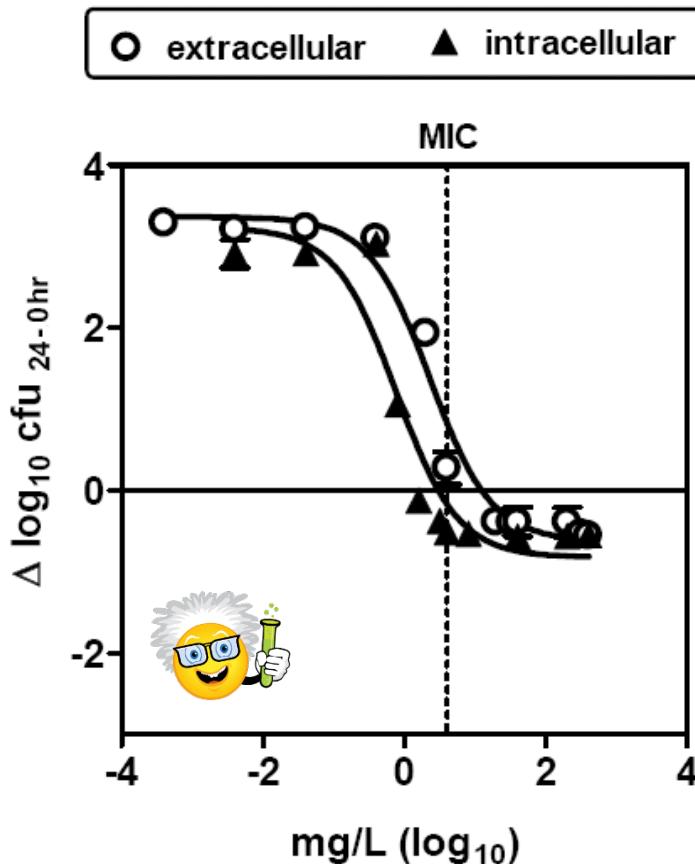
in vitro
(macrophages)



Linezolid & *S. aureus*

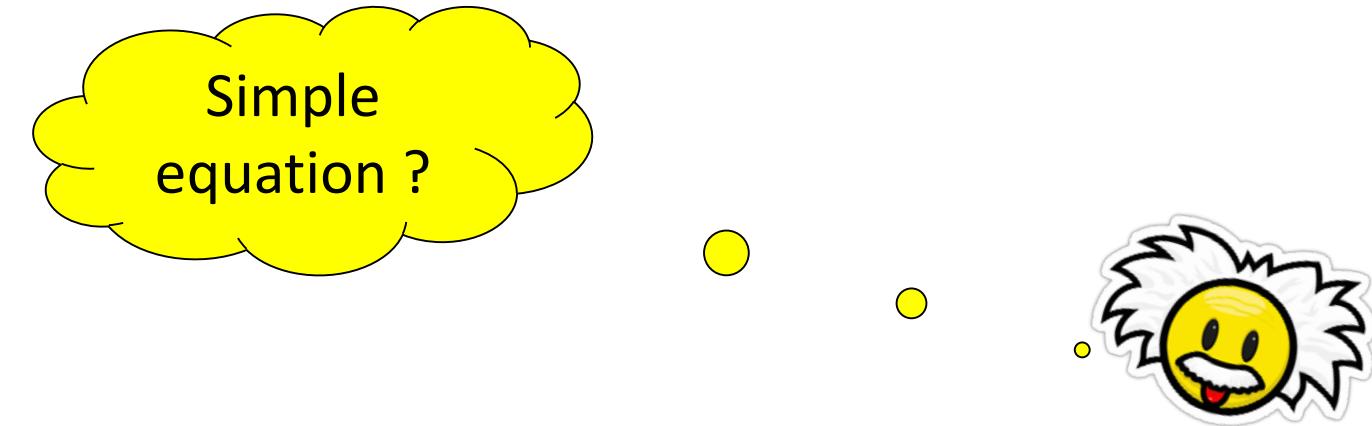


in vivo
(peritonitis)



Sandberg et al, JAC (2010) 65:962-973

Antibiotics and intracellular *S. aureus*: take home message



Intracellular activity X MIC x accumulation



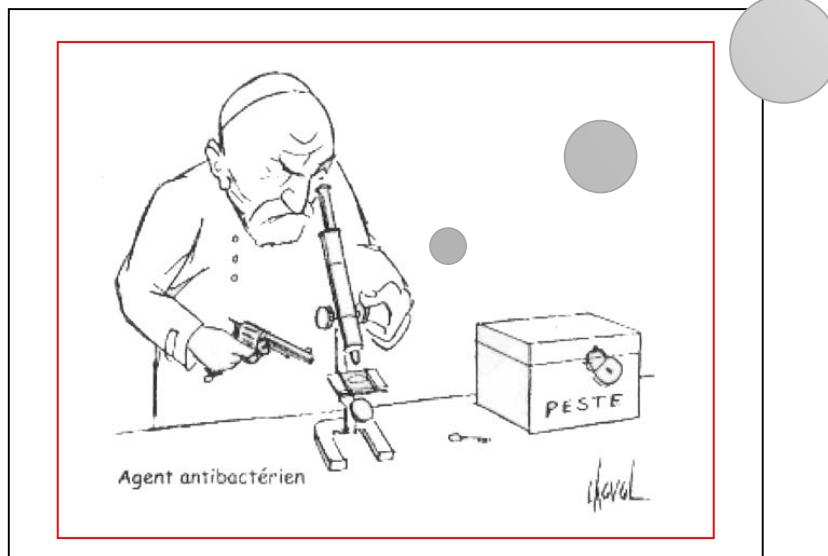
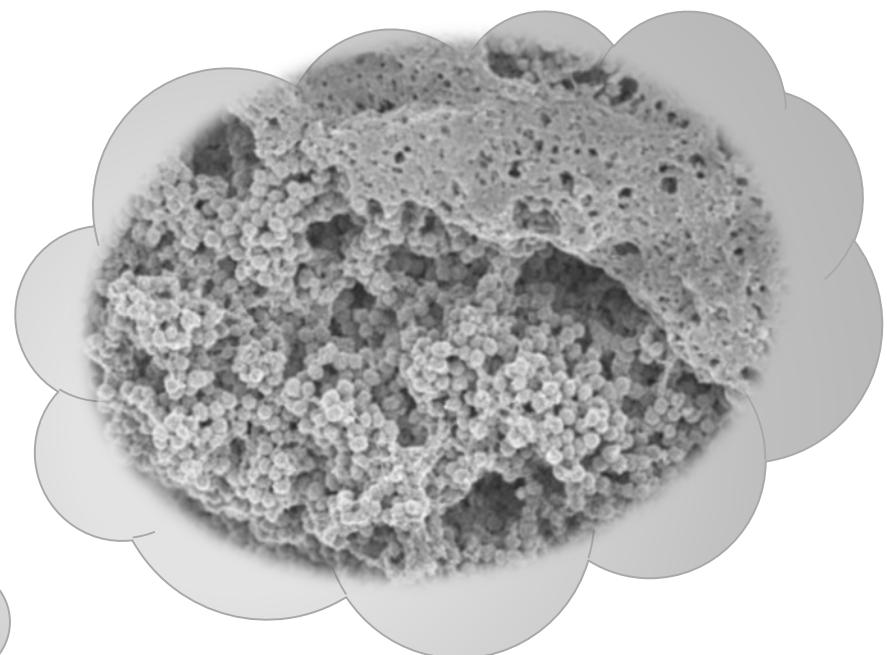
Antibiotics and intracellular *S. aureus*: take home message

“shopping list”

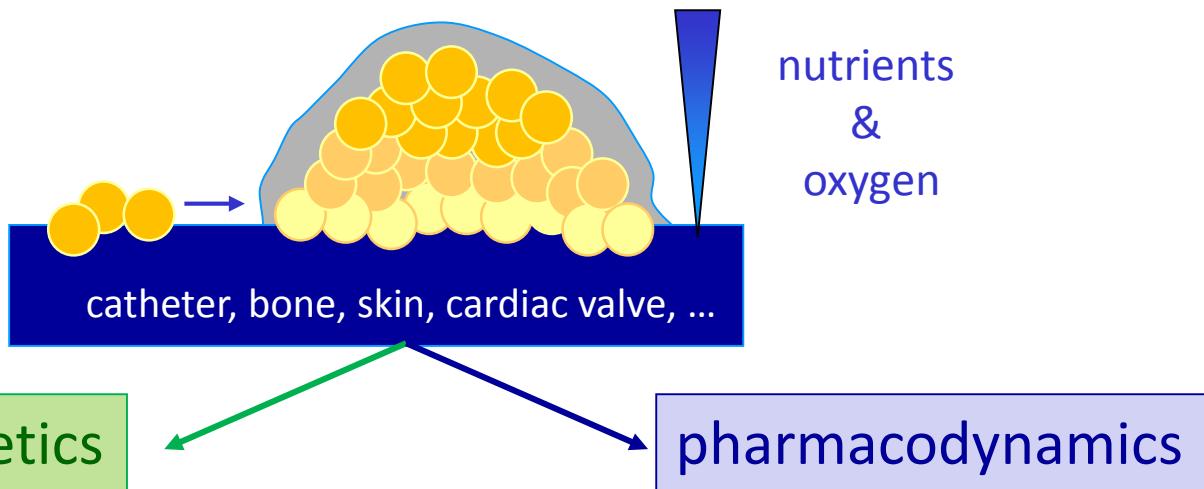
- ✓ high intracellular bioavailability
- ✓ capacity to rejoin the infected compartment
- ✓ not substrate for efflux pumps
- ✓ low MIC at both neutral and acidic pH
- ✓ highly bactericidal, including against slow growing bacteria
- ✓ no cell toxicity
- ✓ cooperation with cell defense mechanisms



Biofilms



PK/PD parameters and activity in biofilms



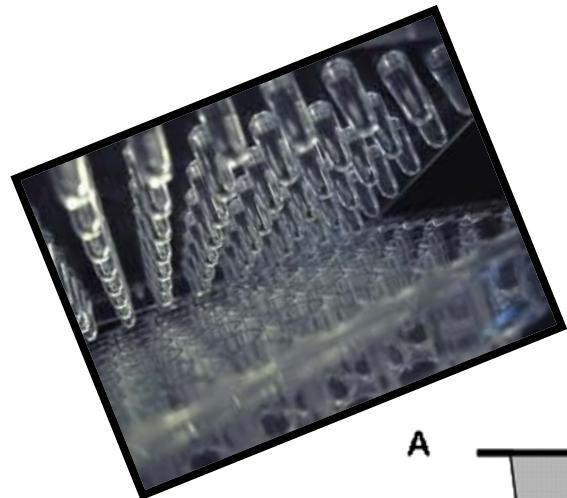
- diffusibility through the matrix
- bioavailability within the biofilm
- access to bacteria
- efflux out of bacteria

- bacterial responsiveness (metabolic activity of bacteria)
- antibiotic expression of activity (local environment [O₂, pH, ..])

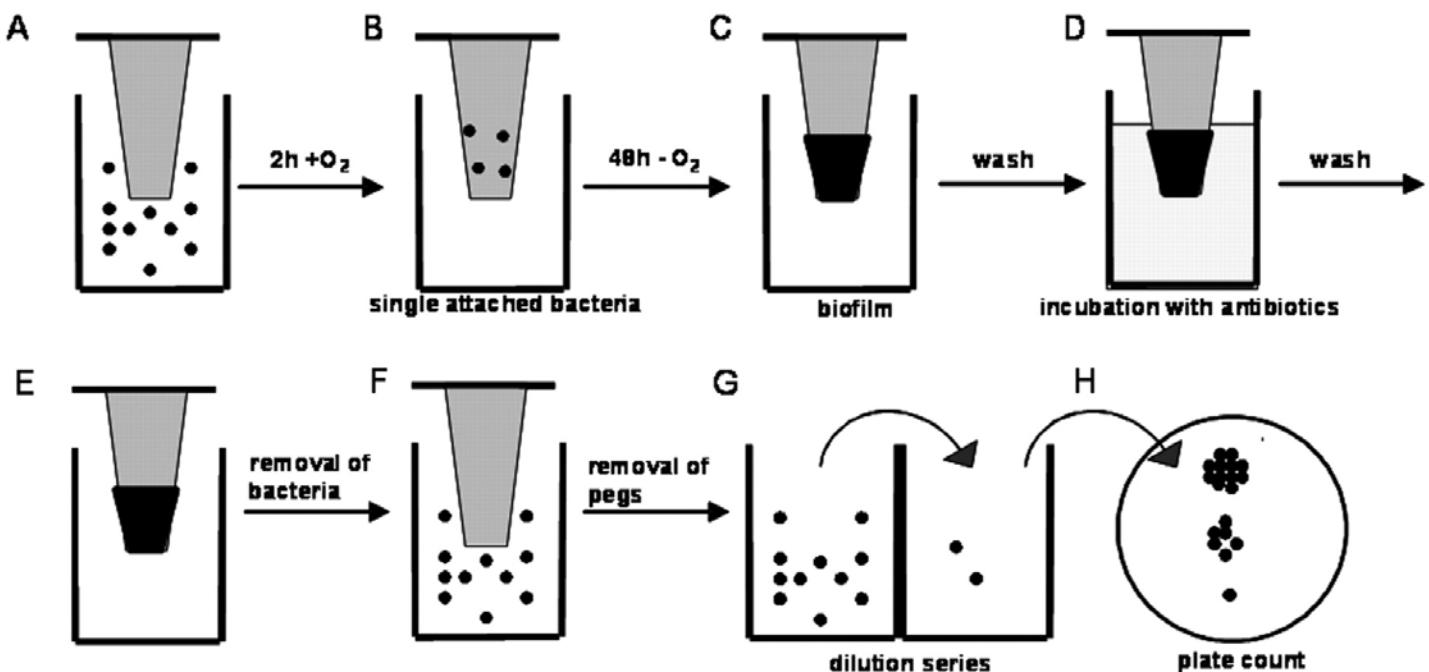
PD parameters: planktonic vs. biofilm cultures

Parameter	Abbreviation	Definition
Minimal inhibitory concentration	MIC	The lowest concentration of an antibiotic that inhibits the visible growth of a planktonic culture after overnight incubation
Minimal biofilm inhibitory concentration	MBIC	The lowest concentrations of an antibiotic that resulted in an OD650 difference at or below 10% (1 Log difference in growth after 6 h of incubation) of the mean of two positive control well readings.
Minimal bactericidal concentration	MBC	The lowest concentration of an antibiotic producing a 99.9% CFUs reduction of the initial inoculum of a planktonic culture.
Biofilm bactericidal concentration	BBC	The lowest concentration of an antibiotic producing a 99.9% reduction of the CFUs recovered from a biofilm culture compared to growth control.
Minimal biofilm eradication concentration	MBEC	The lowest concentration of an antibiotic that prevents visible growth in the recovery medium used to collect biofilm cells.
Biofilm prevention concentration	BPC	Same as MBIC but bacterial inoculation and antibiotic exposure occur simultaneously.

In vitro static models: Calgary Biofilm Device



Determination of
Minimal Biofilm Eradication Concentration
(MBEC)

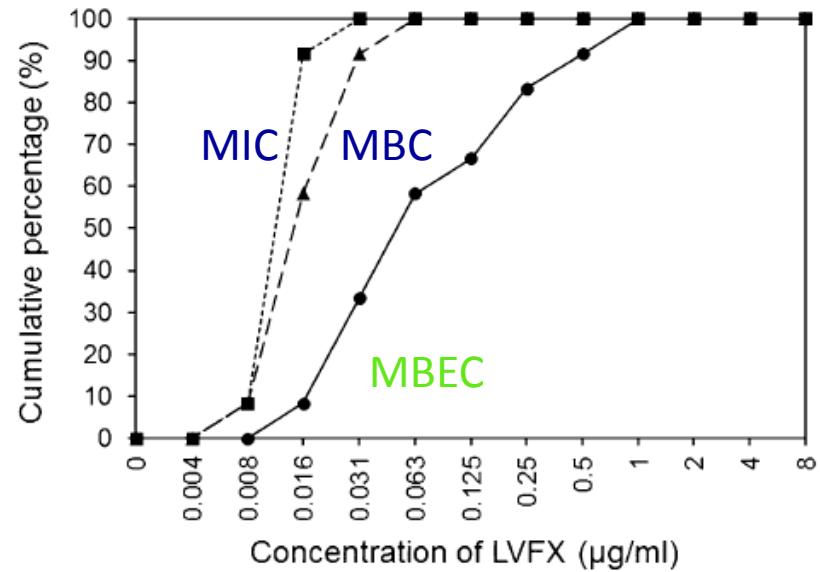
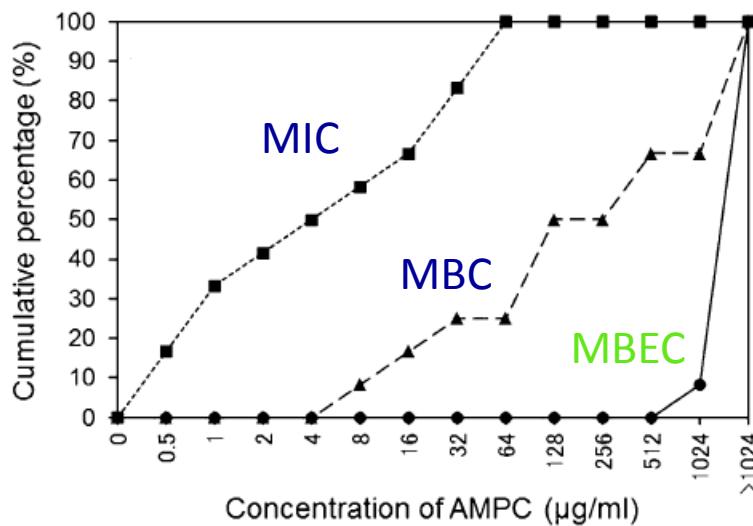




PD parameters: planktonic vs. biofilm cultures



Ampicillin and levofloxacin vs. *H. influenzae* from middle ear fluid



slowly bactericidal antibiotic:
MBEC >> MBC >>MIC

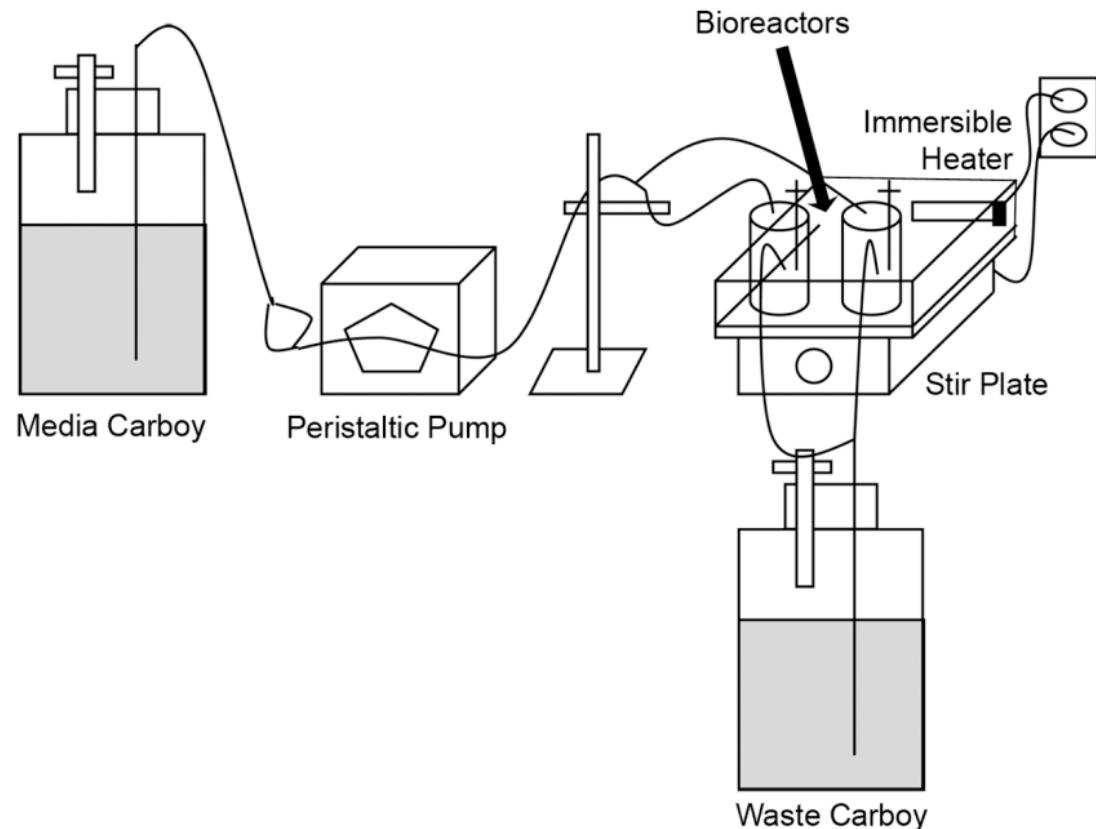
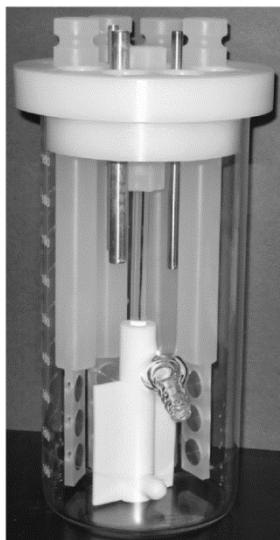
rapidly bactericidal antibiotic:
MBEC > MBC ~ MIC

Dynamic models: bioreactors



CDC reactor:

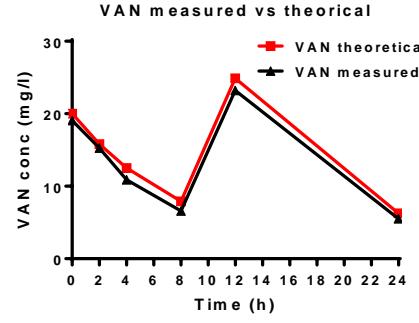
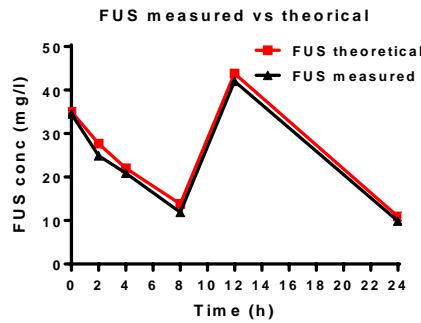
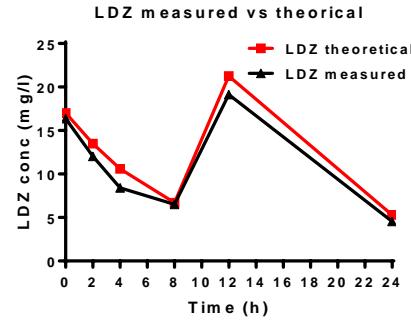
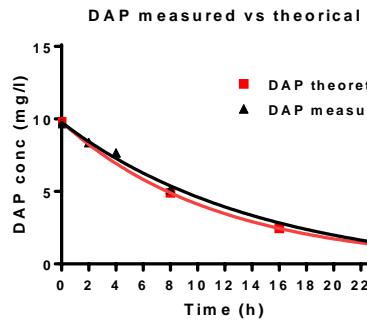
- constant mixing by stirring
→ kinetic experiments with change in medium composition over time
- high shear stress



Stewart et al, PLoS One (2012) 7:e50560

Antibiotics and *S. aureus* - biofilms and intracellular forms

Antibiotic activity - mimicking human exposure

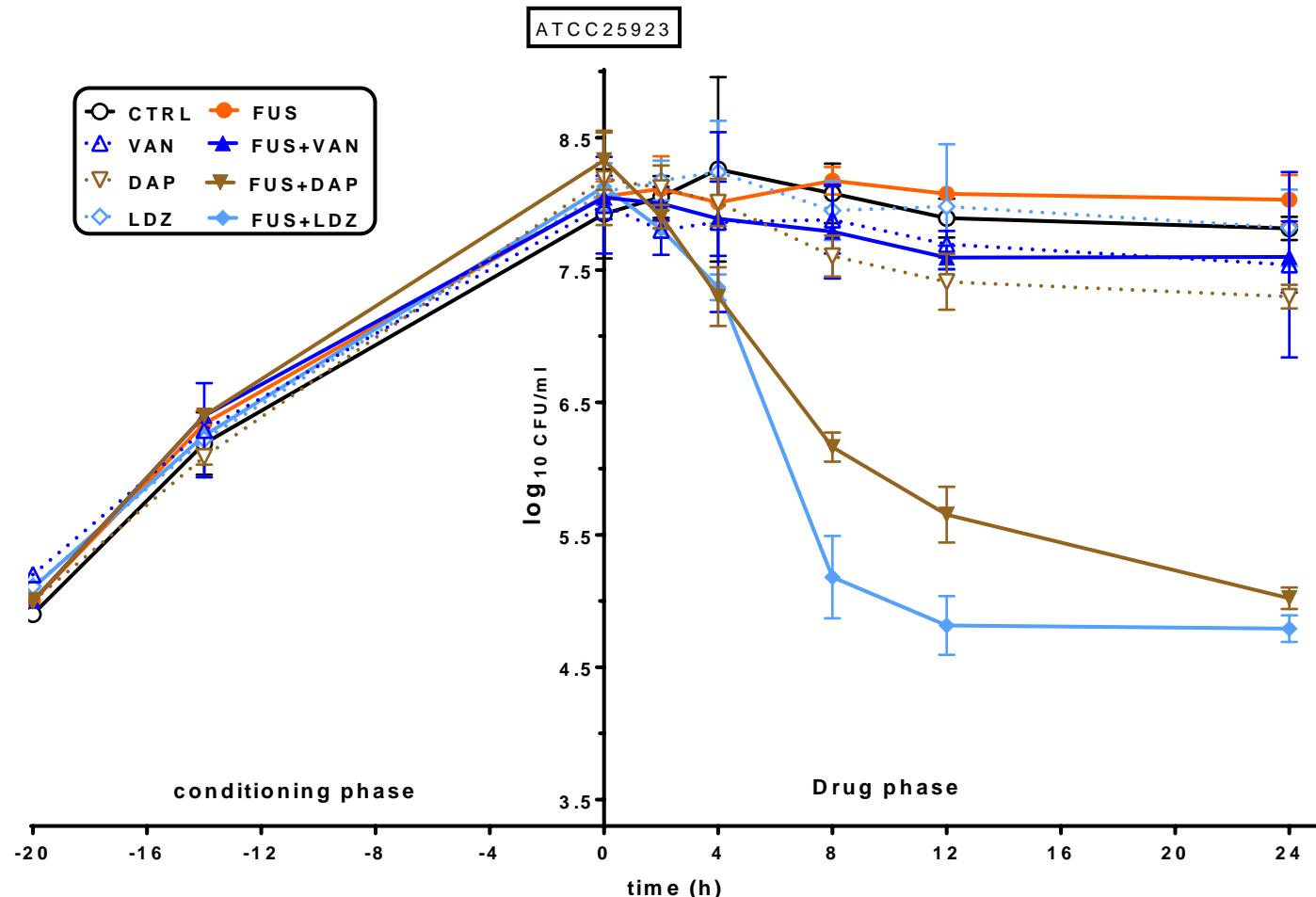


SHERV.NET



antibiotic	fCmax (mg/L)	fCmin (mg/L)	k _{calc} / t _{1/2} (h ⁻¹) / (h)	k measured (h ⁻¹)
DAP	9.4	0.7	0.09 (8h)	0.07 ± 0.02
VAN	20	2.5	0.12 (6h)	0.12 ± 0.06
LZD	17	9	0.12 (6h)	0.28 ± 0.08
FUS	35	11	0.12 (6h)	0.15 ± 0.08

Antibiotic activity - mimicking human exposure



Combination much more active

In vitro models for PK/PD studies: 96-well polystyrene plates

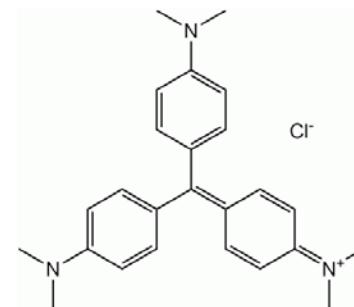
appropriate
dyes
to evaluate biomass or
bacterial load



Quantifying biomass and metabolic activity in biofilms

biofilm mass

crystal violet



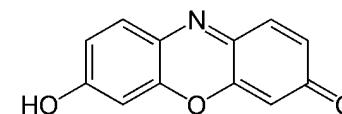
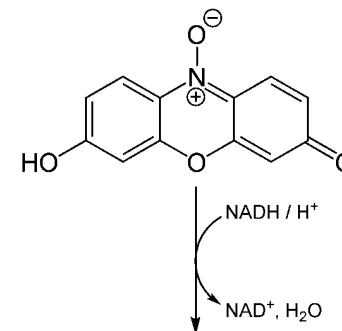
Christensen et al, *Infect. Immun.* (1982) 37:318–26

metabolic activity

resazurin



resorufin



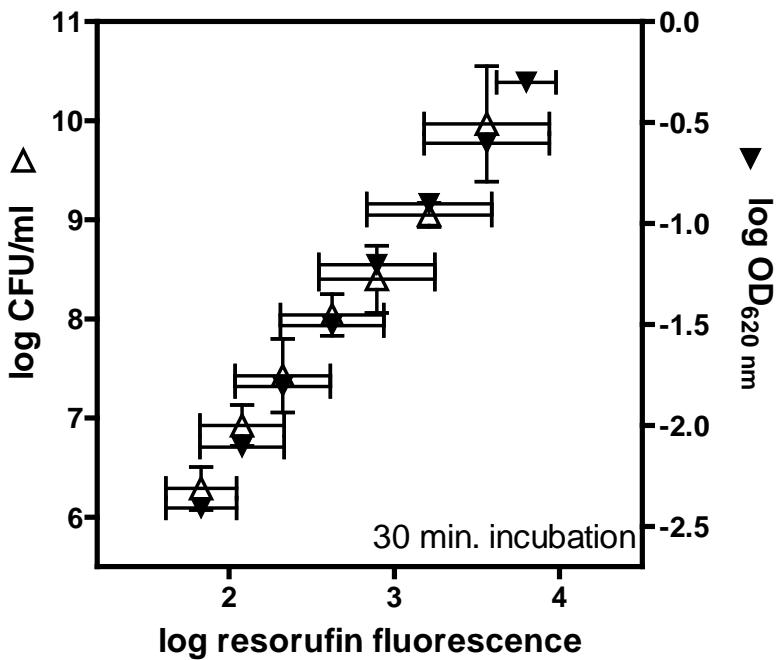
Tote et al, *Lett. Appl. Microbiol.* (2008) 46:249–254



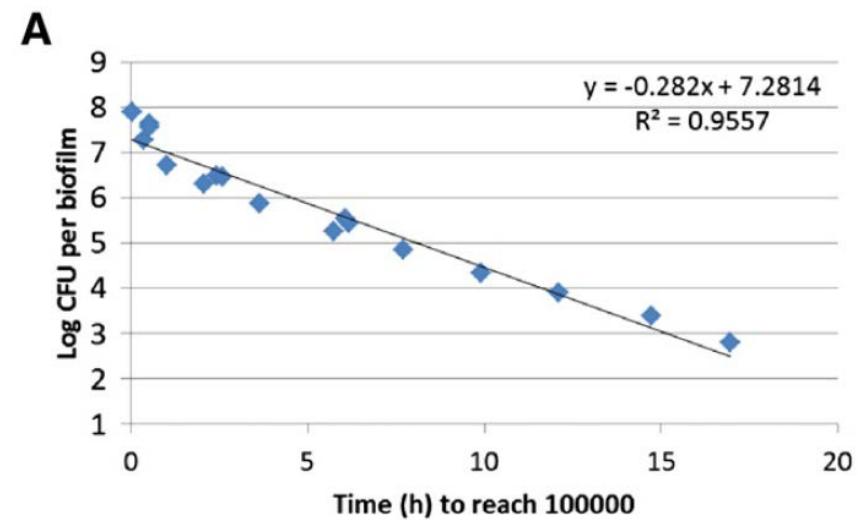
CFU counting vs. RF fluorescence



relation between fluorescence
and bacterial inoculum for *S. aureus*

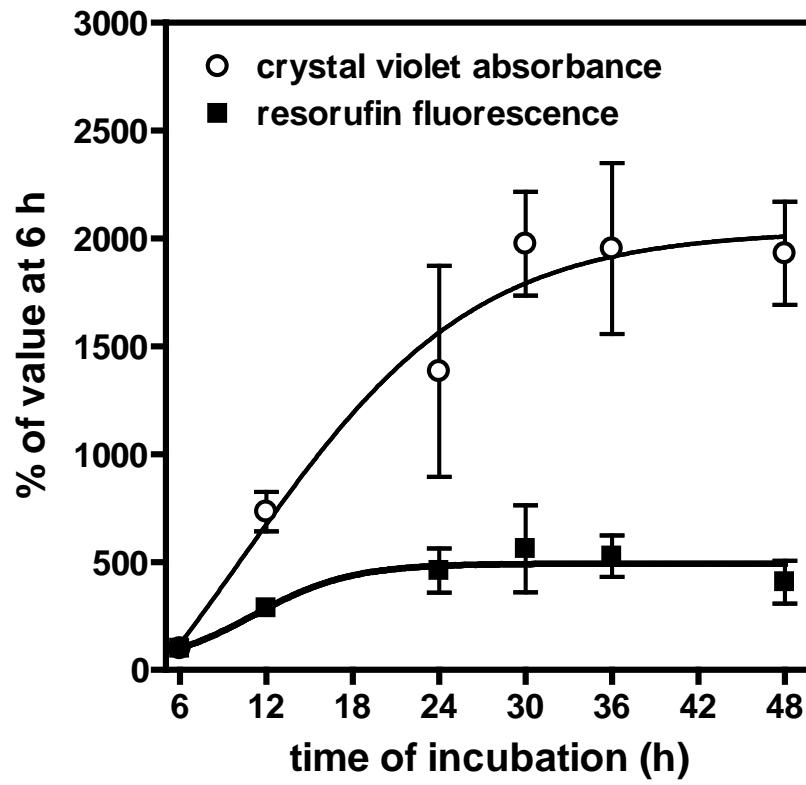


CFU & RF signal proportional



sensitivity depending on incubation time

S. aureus model: growth kinetics

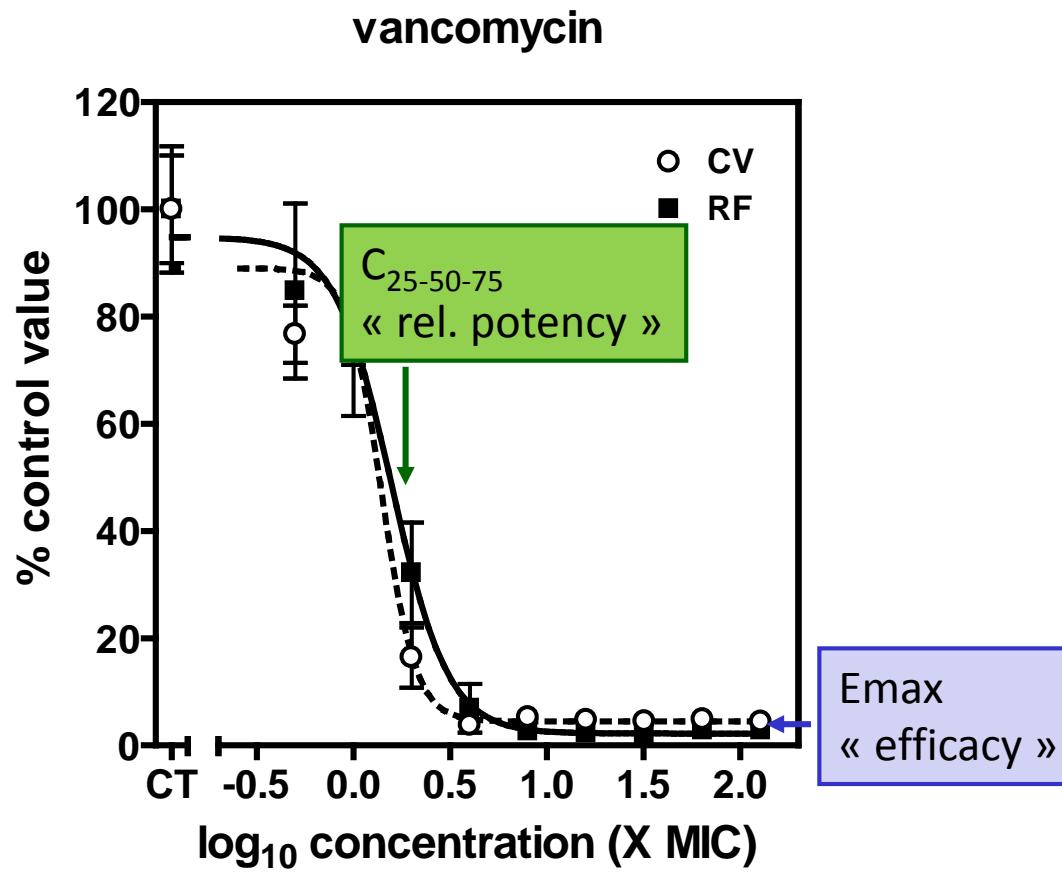


↑
young biofilm ↑
mature biofilm

Pharmacodynamic model for antibiotic activity

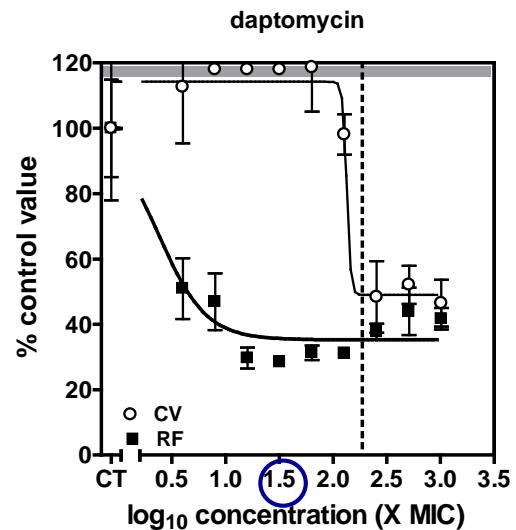
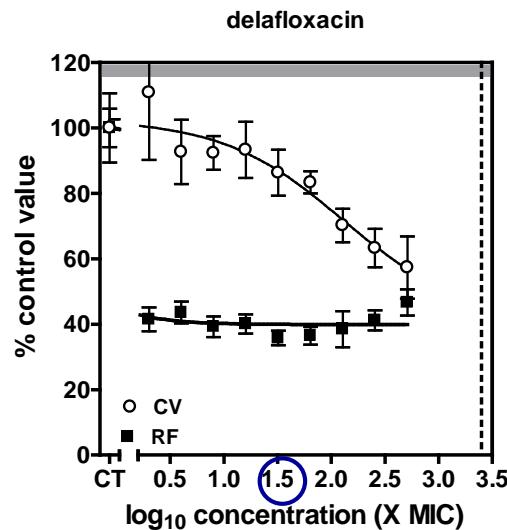
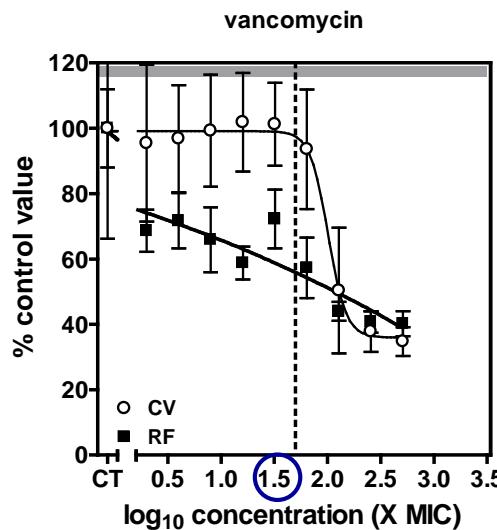


An example with young biofilm of *S. aureus*

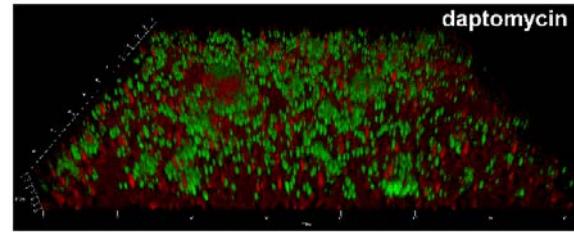
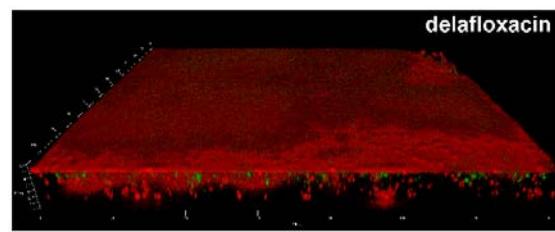
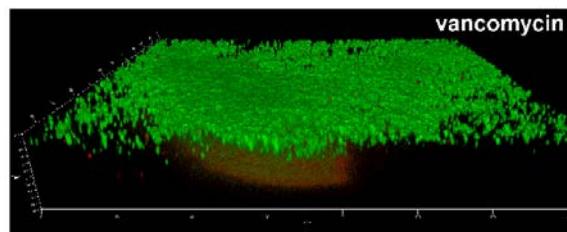


S. aureus mature biofilms: comparison of drugs

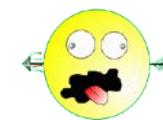
ATCC33591 (MRSA)



life
green
dead
red

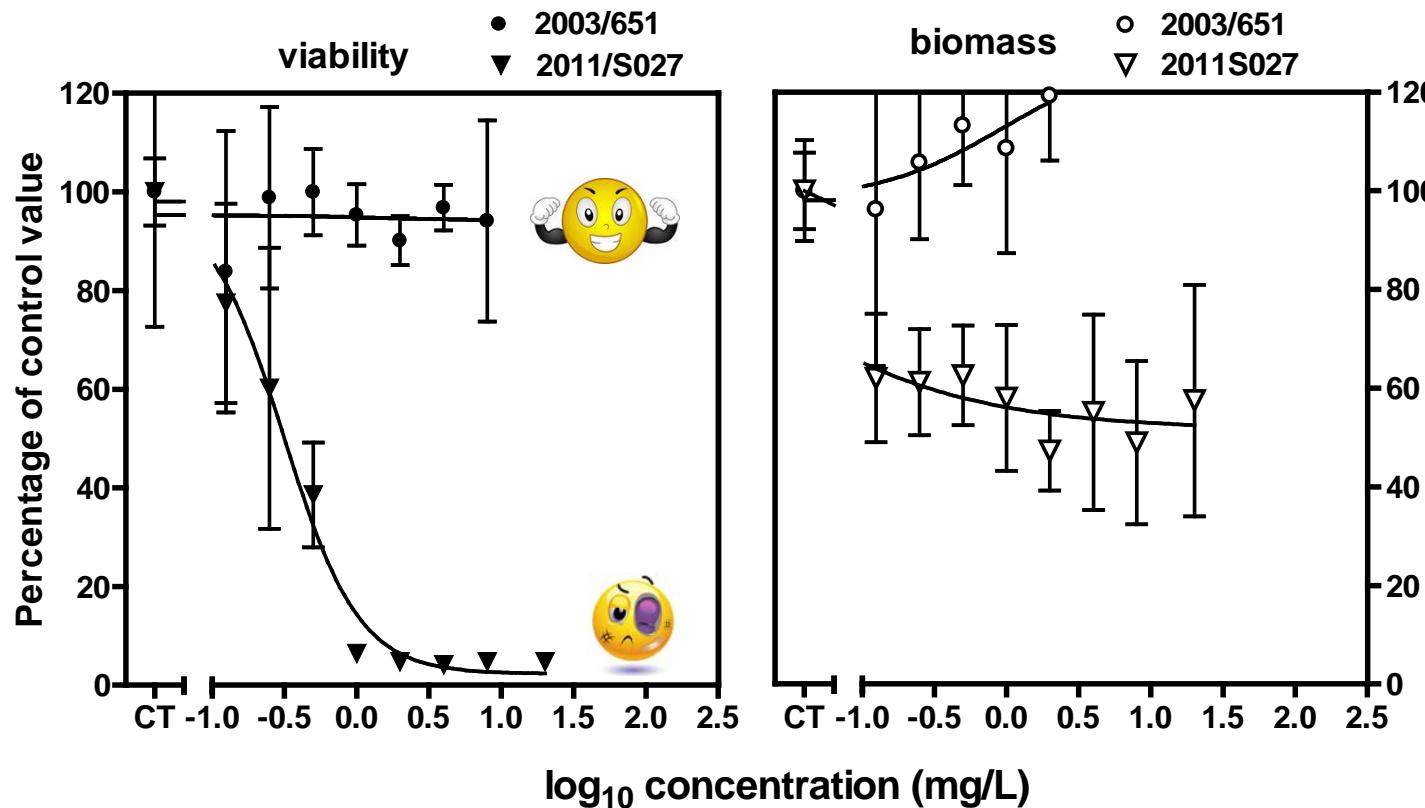


- more active on viability than on matrix
- huge difference among drugs



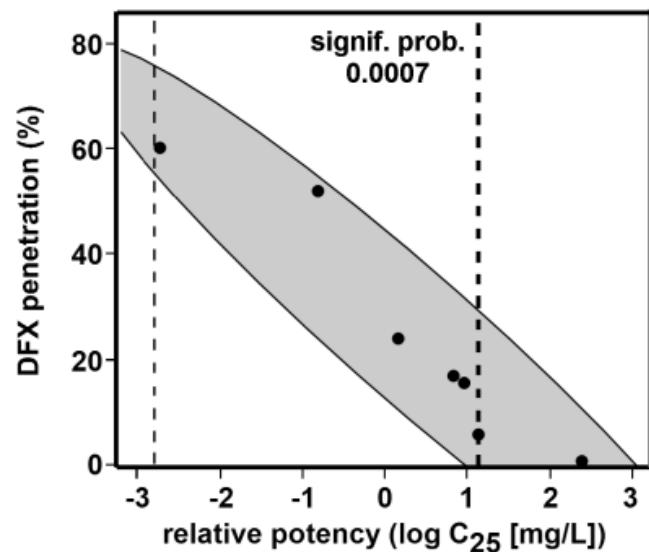
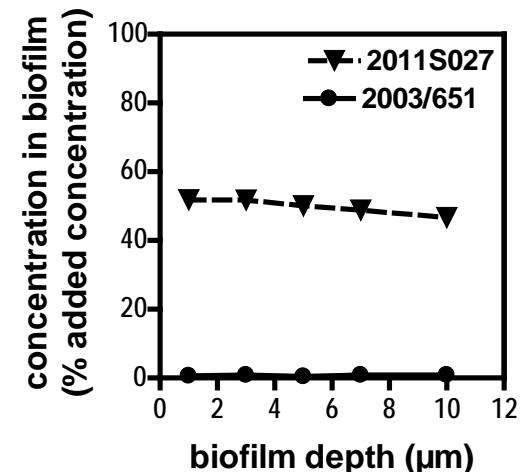
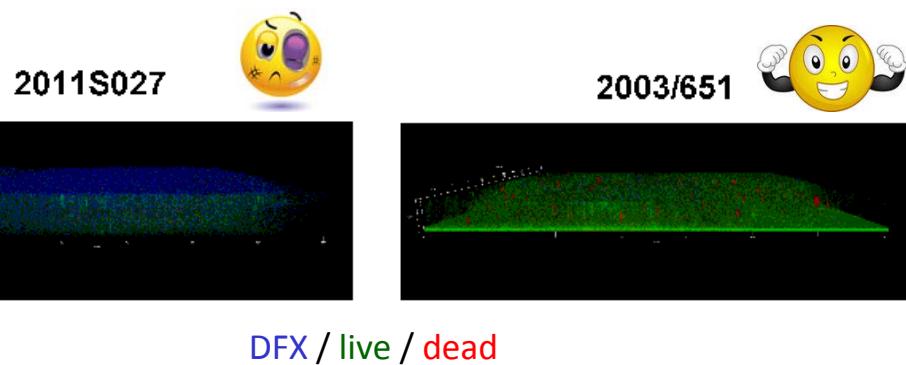
Parameters affecting antibiotic activity in biofilms

2 clinical isolates of *S. aureus*- *delafloxacin*



What makes the difference ?

PK parameter: antibiotic penetration

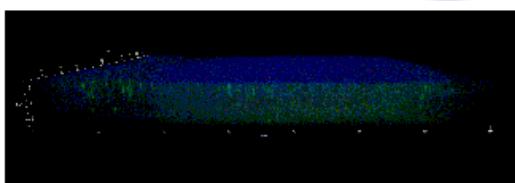


Correlation
antibiotic penetration – potency
in biofilms

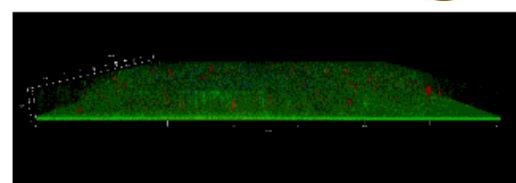


PK parameter: antibiotic penetration

2011S027

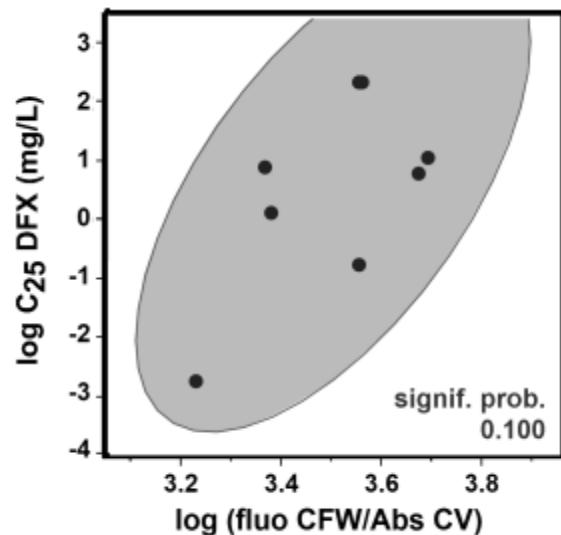
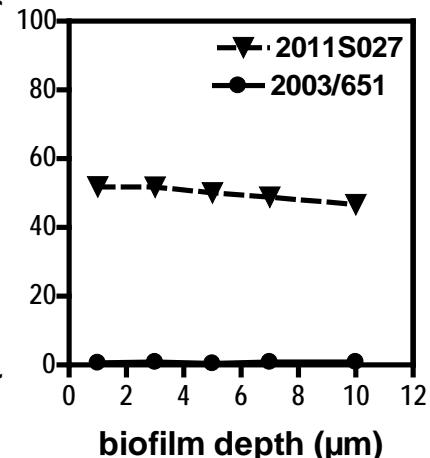


2003/651



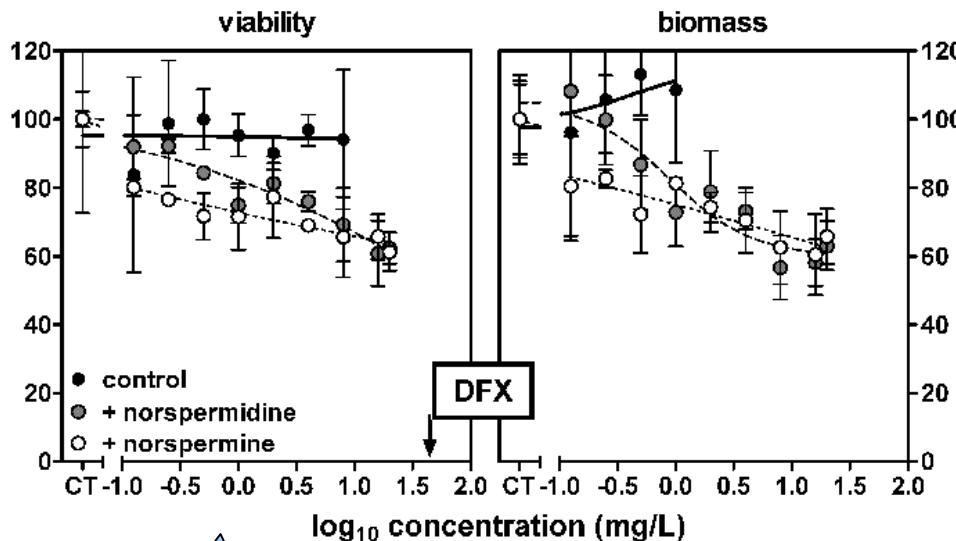
DFX / live / dead

concentration in biofilm
(% added concentration)

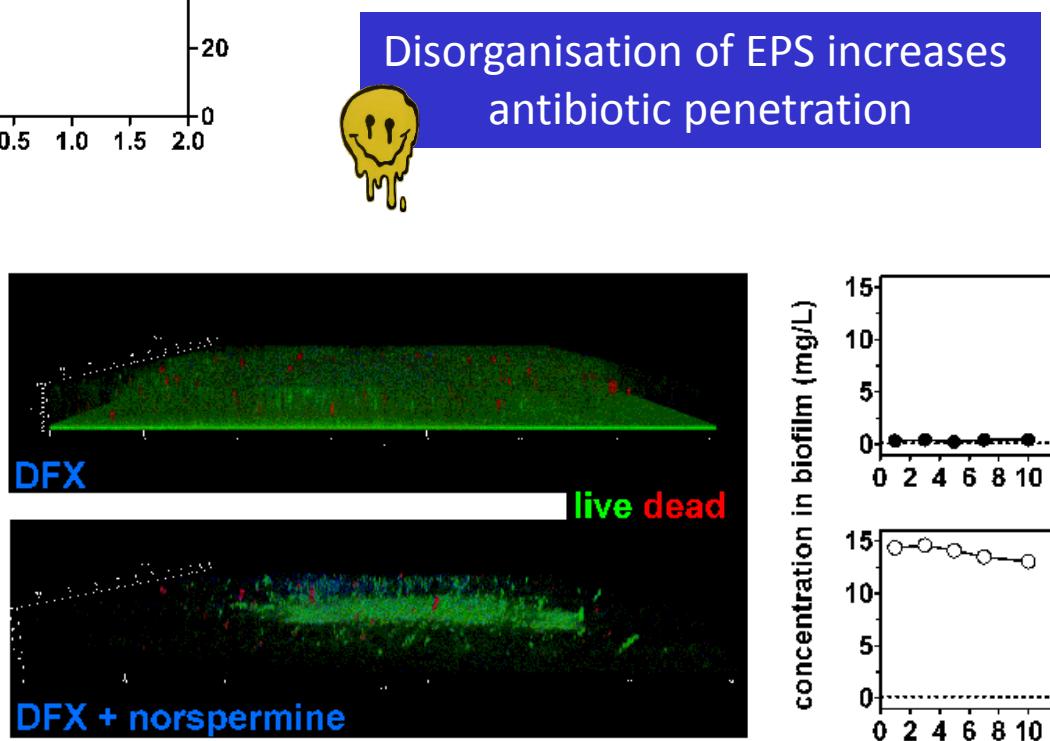


antibiotic penetration depends
on matrix polysaccharide content

Matrix disorganisation



Norspermidine reduced the concentrations (30 x) and the diameters (2 x) of exopolysaccharide supramolecular particles.



Siala et al, AAC (2014) 58:6385-98

PD parameter: environmental pH

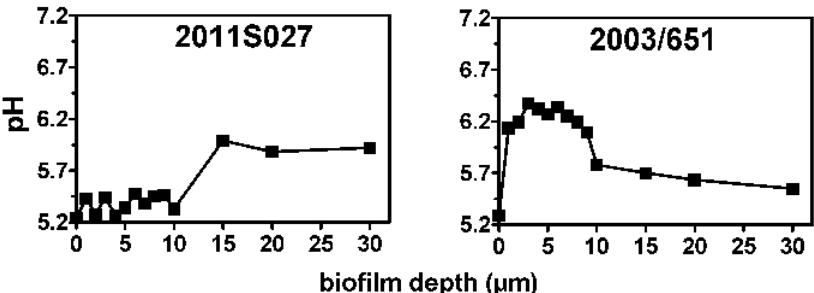
PD



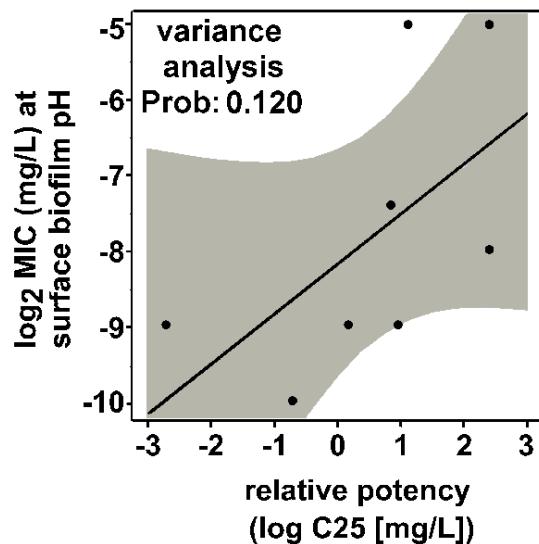
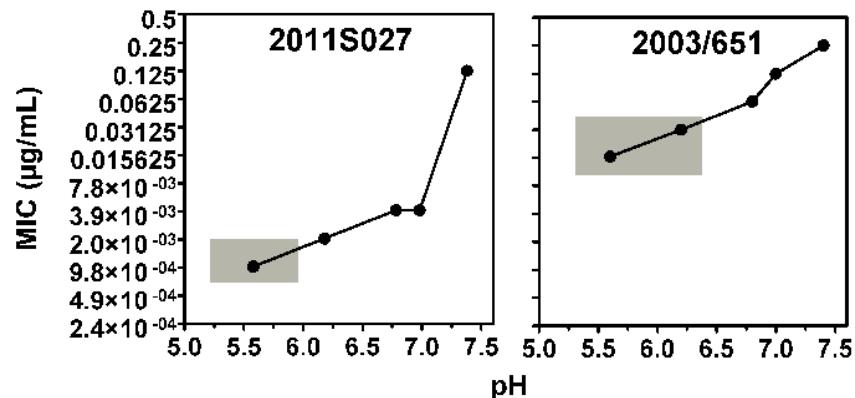
Biofilm pH



basic



Influence of pH on delafloxacin MIC



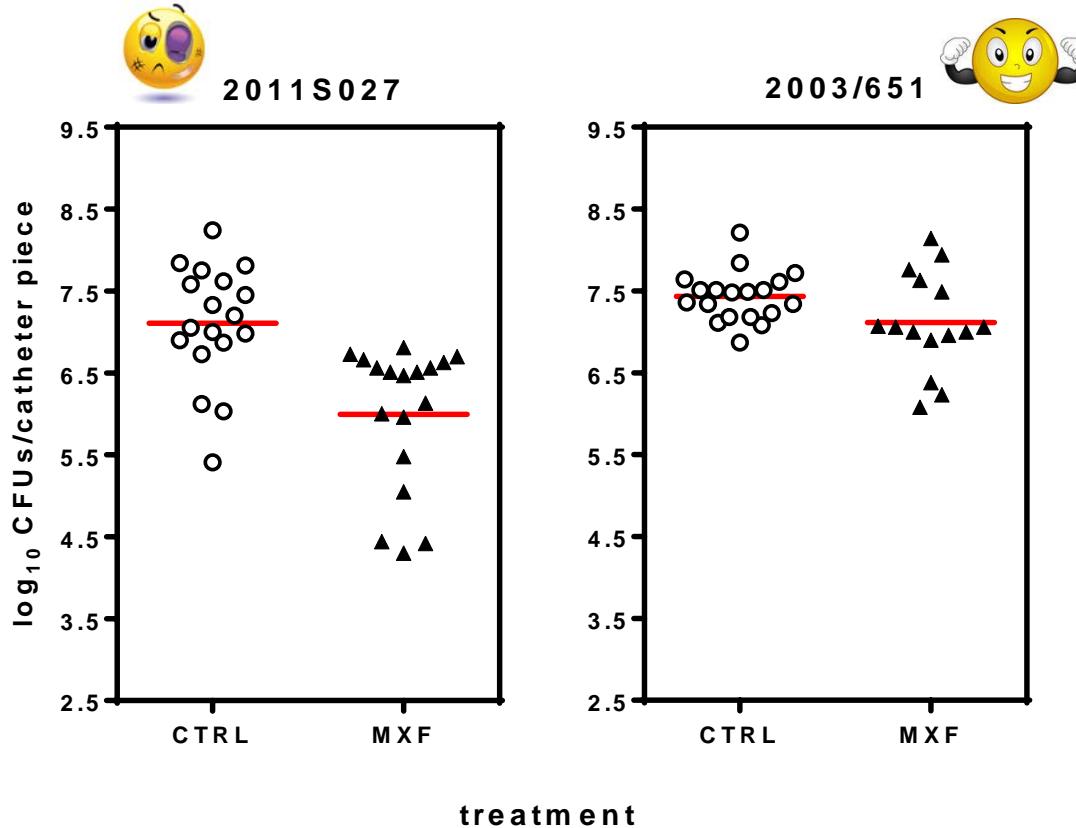
Correlation
MIC at biofilm pH – potency
in biofilms



Siala et al, AAC (2014) 58:6385-98



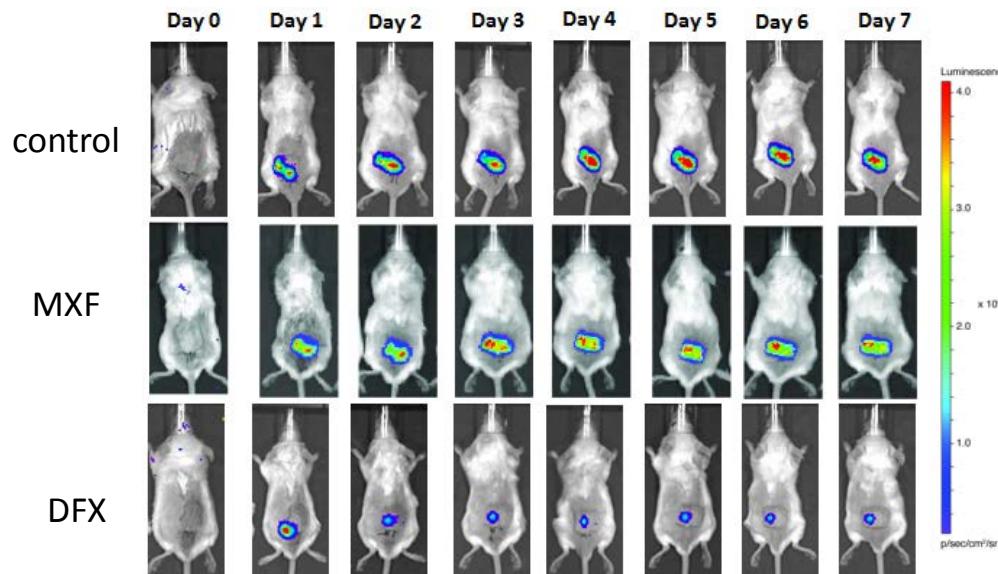
in vitro vs in vivo



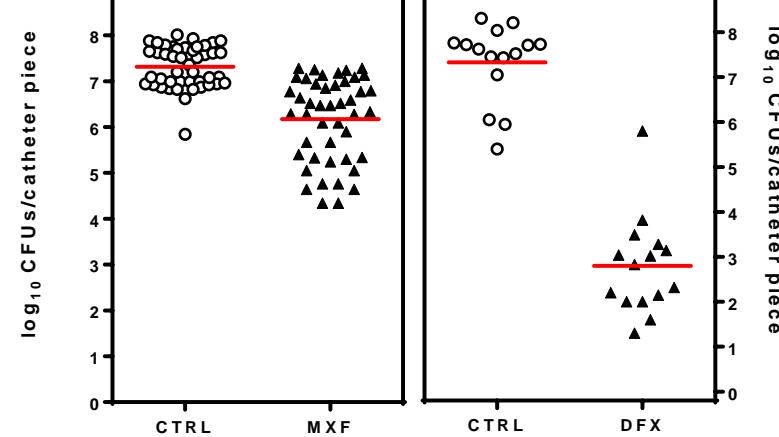
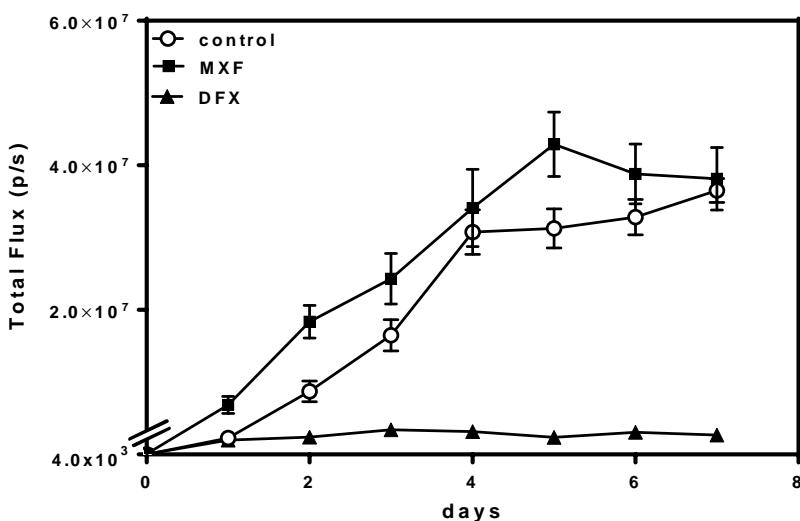
Siala et al, submitted for publication



in vitro vs in vivo



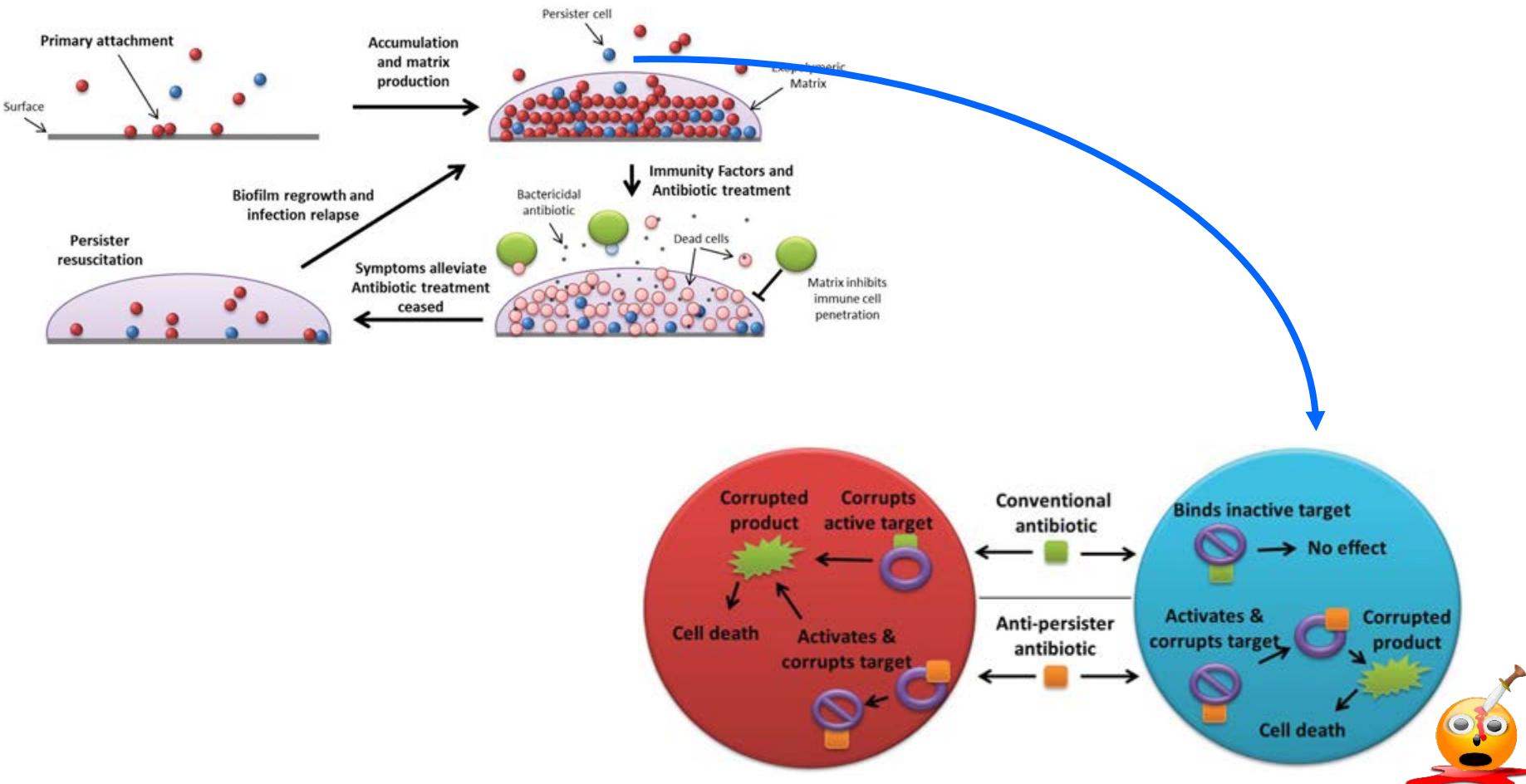
Xen 36



Siala et al, unpublished

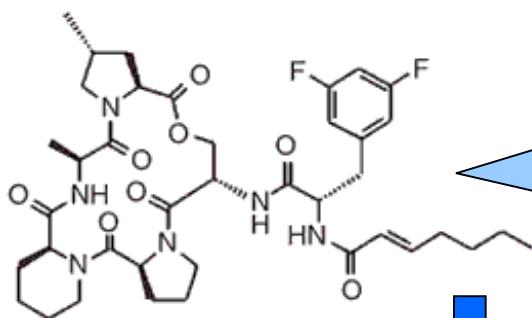
PD parameter: importance of metabolic state

PD



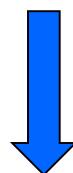
Coulon, Bioassays (2014) 36: 991–6

Anti-“persister” and biofilms

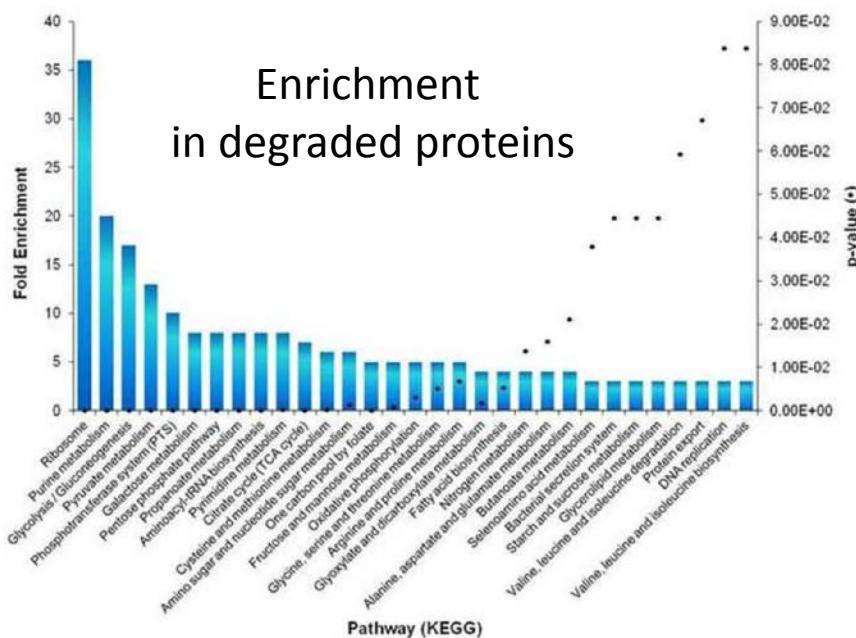


ADEP 4

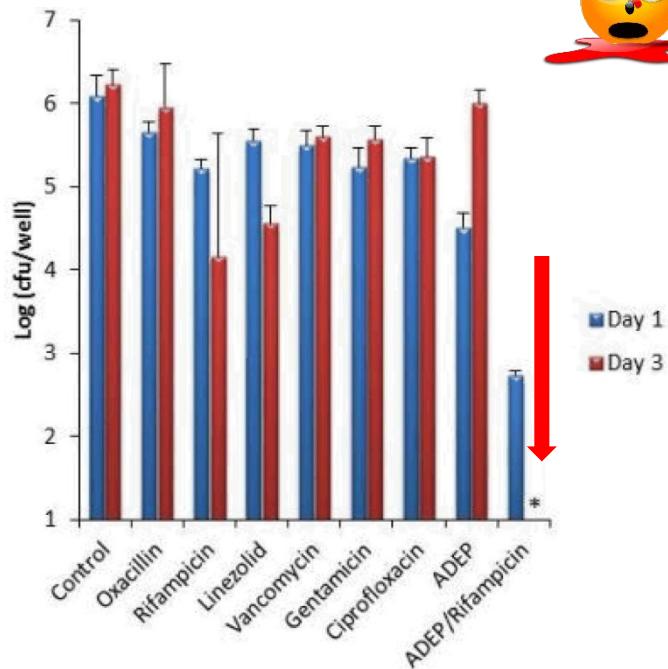
targets ClpP, core unit of a major bacterial protease complex.



Enrichment
in degraded proteins



Synergistic with antibiotics in biofilms



Coulon et al, Nature (2013) 503: 365–70

Antibiotics and *S. aureus* biofilm: take home message

How do biofilms counter-act antibiotic activity ?



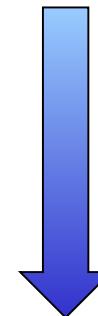
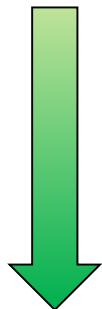
MATRIX

- ✓ Composition
- ✓ Biophysical properties

BACTERIA

- ✓ Metabolic state

Useful strategies ?



Destructing the matrix

Waking-up dormant cells

Acknowledgments – intracellular infection



Paul
Tulkens



Cristina
Seral



Maritza
Barcia



Sandrine
Lemaire



Frédéric
Peyrusson



Huang Anh
Nguyen



Pierre
Baudoux



Laetitia
Garcia



Anne
Sandberg



Niels
Frimodt-Möller



LA LIBERTÉ DE CHERCHER



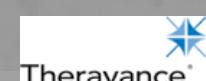
Transparency declaration



cempra



The
Medicines
Company



Acknowledgments - biofilms



Julia Bauer



Wafi Siala



KU LEUVEN



Sona Kucharíková



Patrick Van Dijck



LA LIBERTÉ DE CHERCHER



Wallonie



EMPOWERING RESEARCH

Transparency declaration



The
Antibiotics
Company

Acknowledgments – other collaborations

- In Belgium ...

O. Denis, Université libre de Bruxelles

H. Rodriguez-Villalobos, Université catholique de Louvain

- Outside Belgium

B. Kahl, University of Muenster, Germany

P. Appelbaum, Hershey Medical Center, PA

S. Mobashery, University of Notre-Dame, IL

Acknowledgments



Thank you
for your attention !