PHARMACODYNAMICS IN THE INTRACELLULAR SPACE

Modulating Intracellular Antimicrobial Activity

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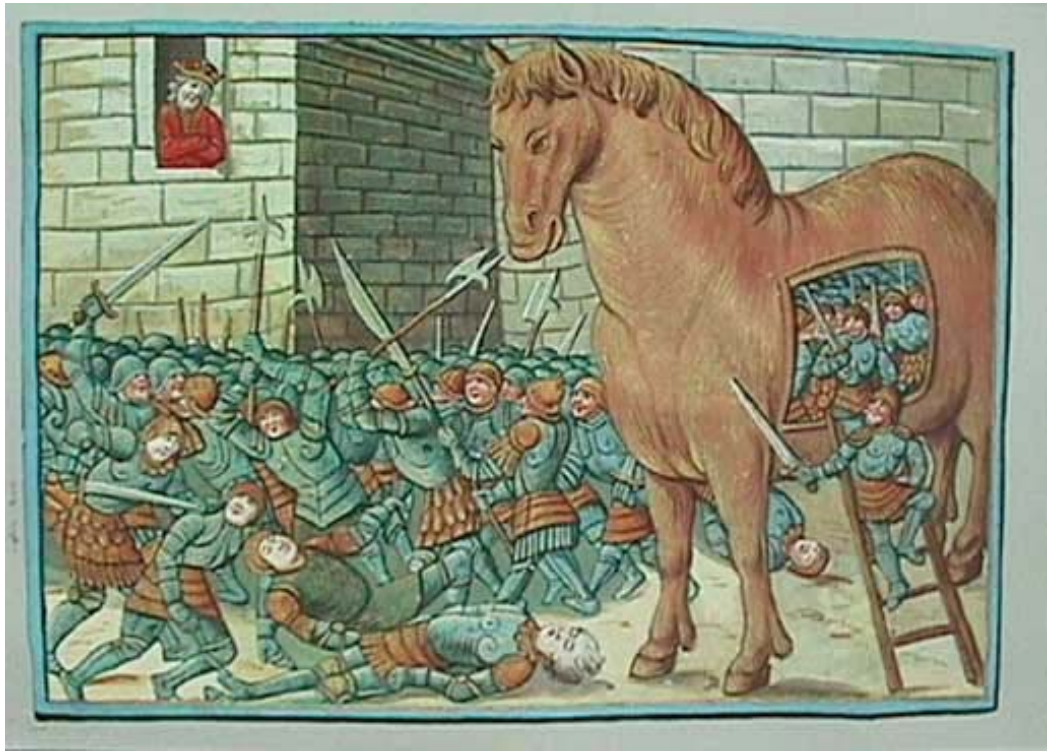
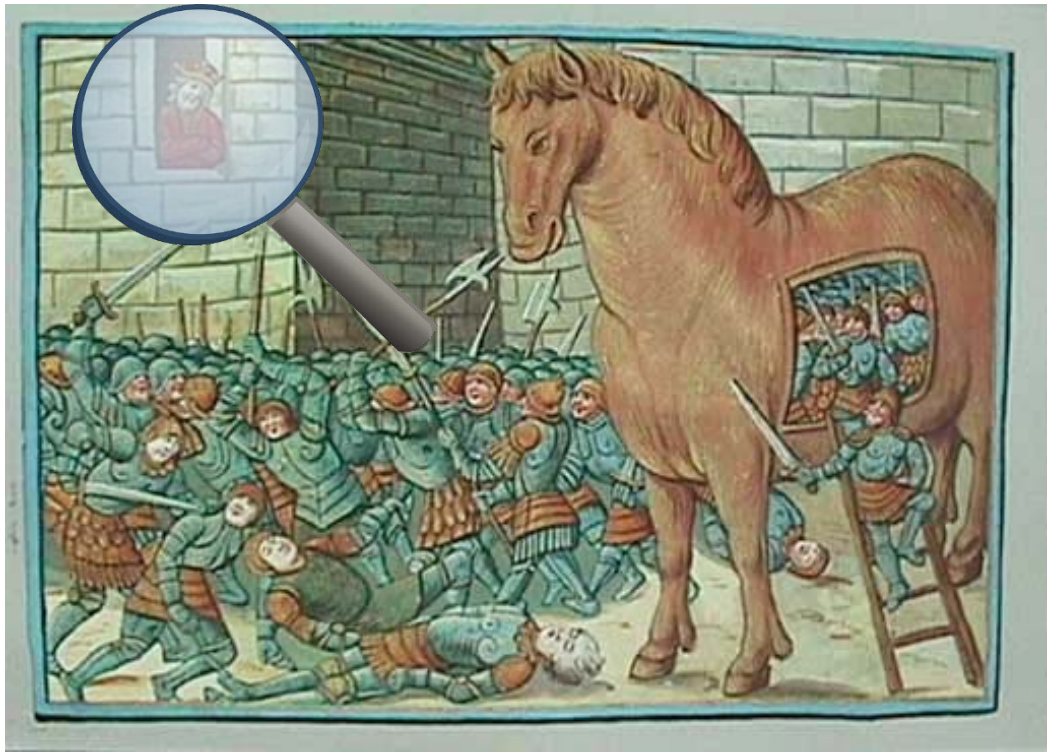
Intracellular life : a Trojan Horse strategy….



Intracellular life : a Trojan Horse strategy….

Protection
~ humoral host defenses
~antibiotics



Intracellular life : a Trojan Horse strategy….

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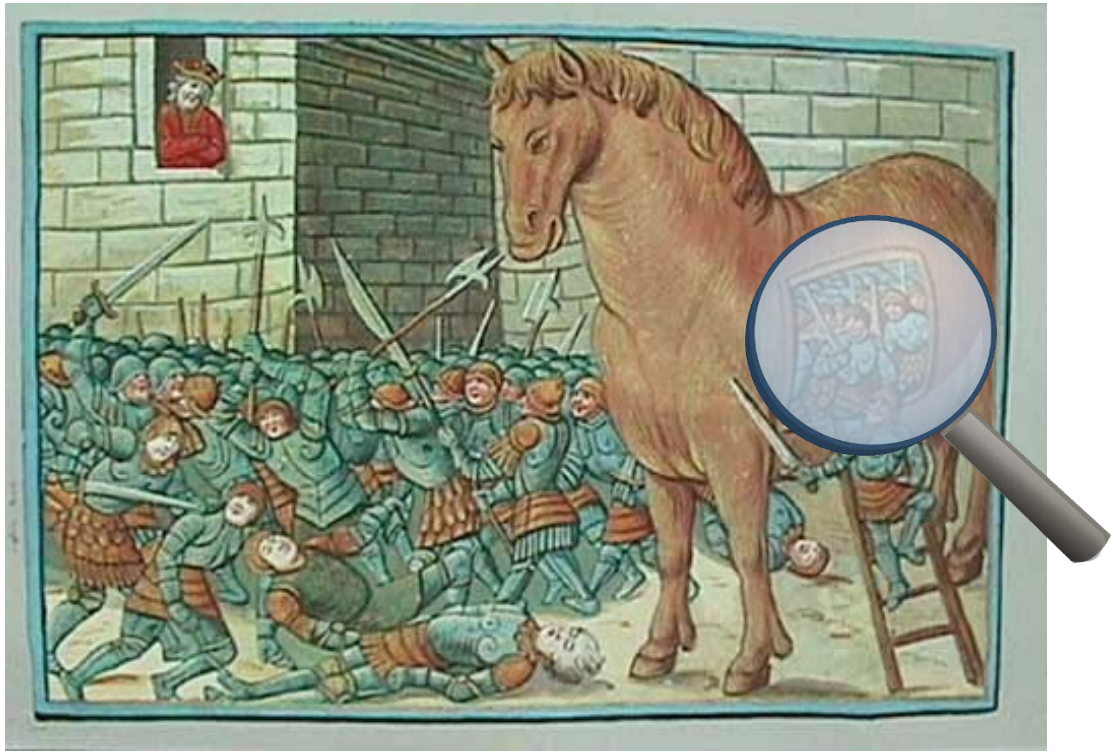
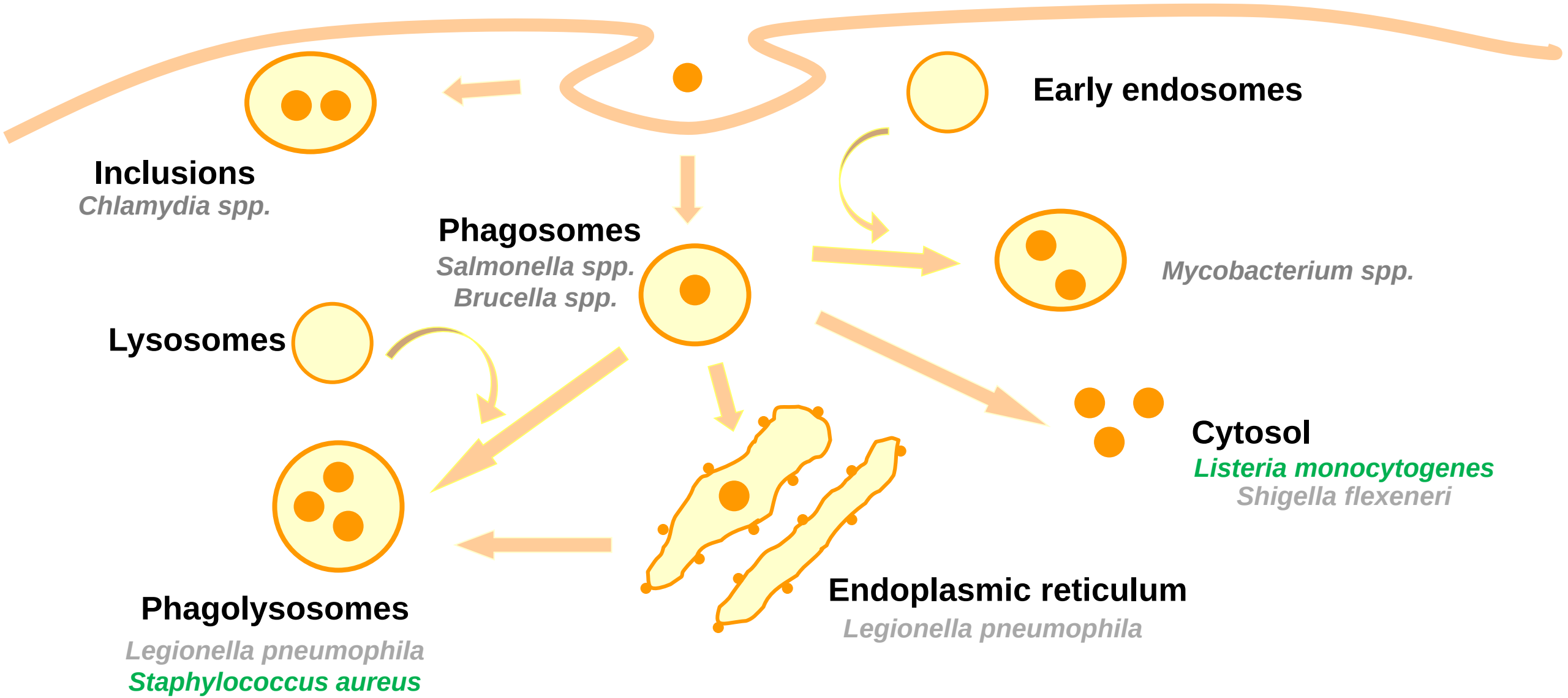


Persistence
(chronic infection)

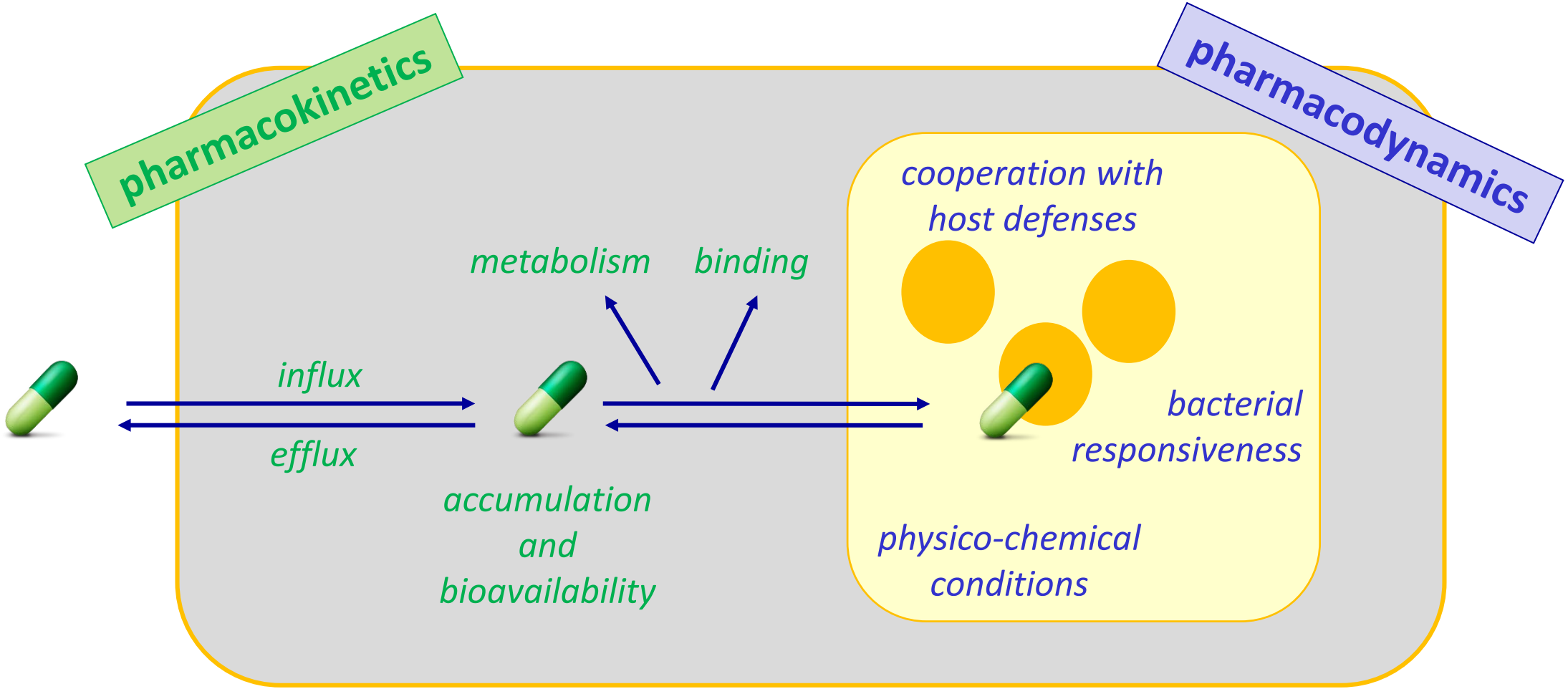
Invasion
(crossing barriers)
Recurrence

Intracellular life : where to go ?



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

PK/PD parameters against intracellular bacteria



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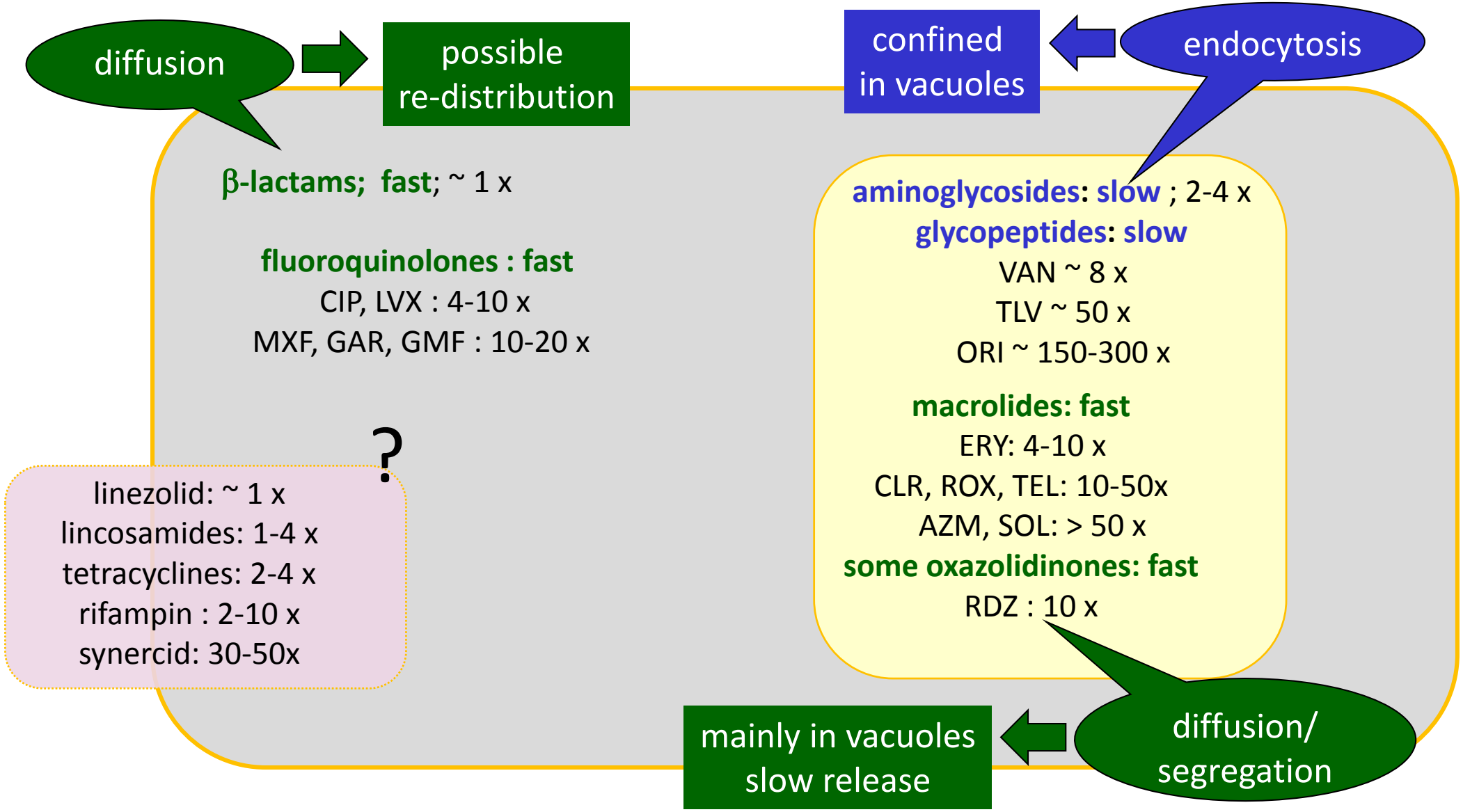
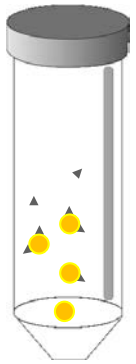
Antibiotic accumulation and subcellular distribution

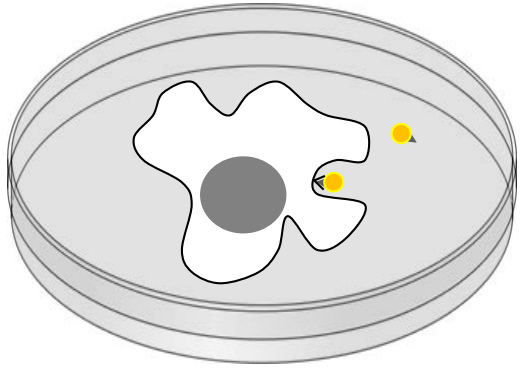


In vitro model of intracellular infection

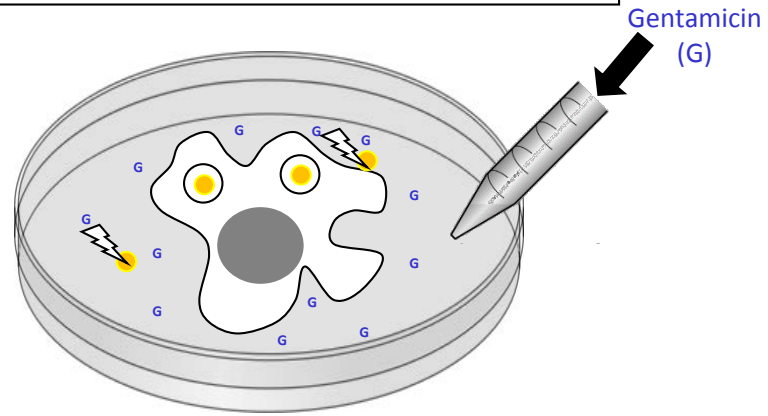
Opsonization
Incubation
with 10 % human serum
in culture medium



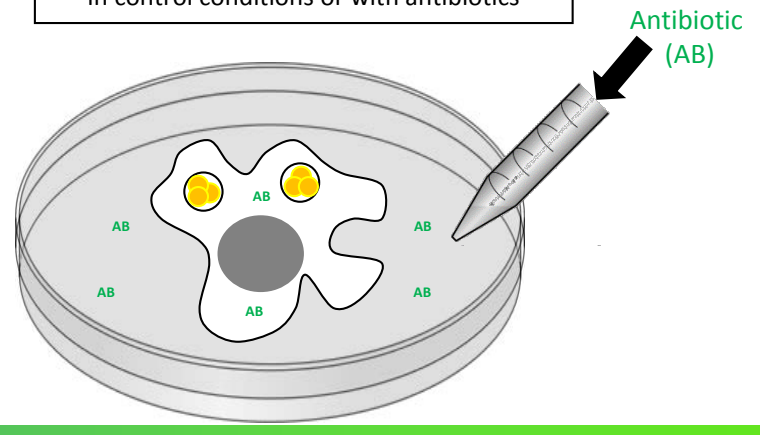
Phagocytosis
Incubation of bacteria with
cells at an appropriate MOI



Extracellular wash
Elimination of non phagocytosed bacteria
by incubation with 50-100 X MIC gentamicin;
elimination of gentamicin by washing

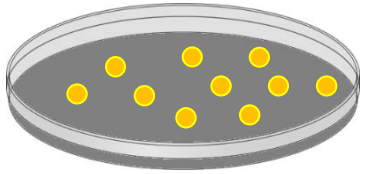


Intracellular infection
Incubation over time
in control conditions or with antibiotics

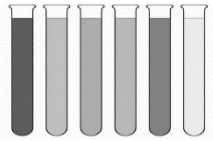


Determination of residual intracellular inoculum
Determination of cfu and protein content in cell lysates

cfu

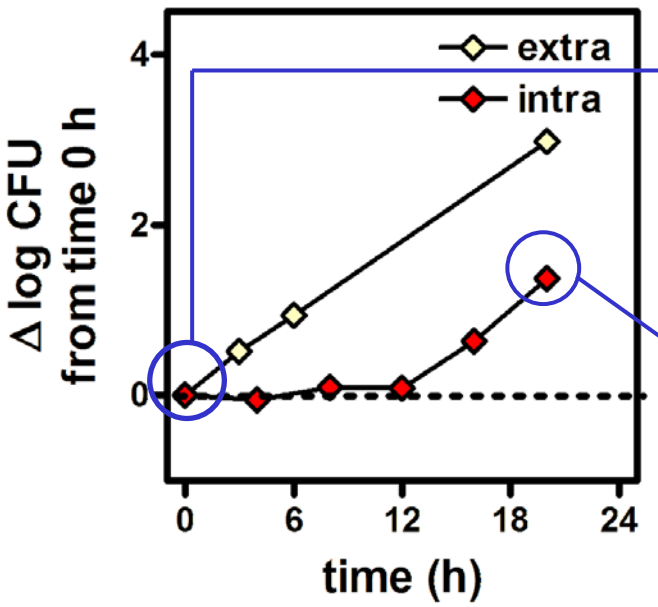


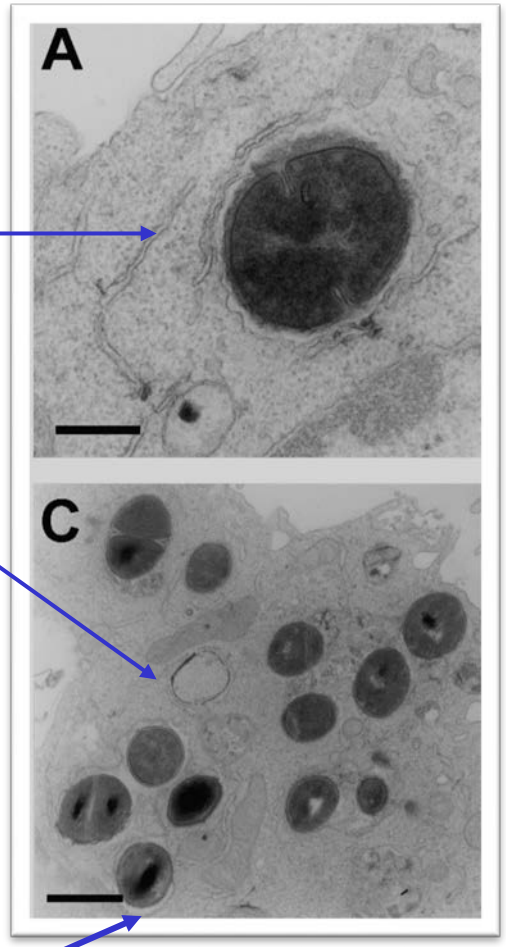
proteins



Buyck et al (2016) ISBN 978-1-4939-2854-5

In vitro model of intracellular infection

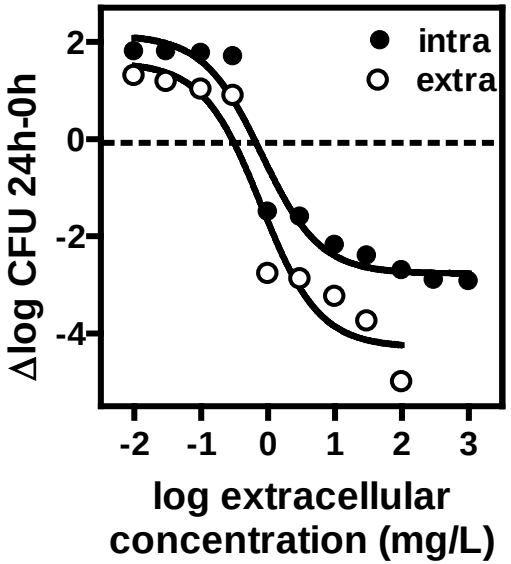




remains in vacuoles

+ AB

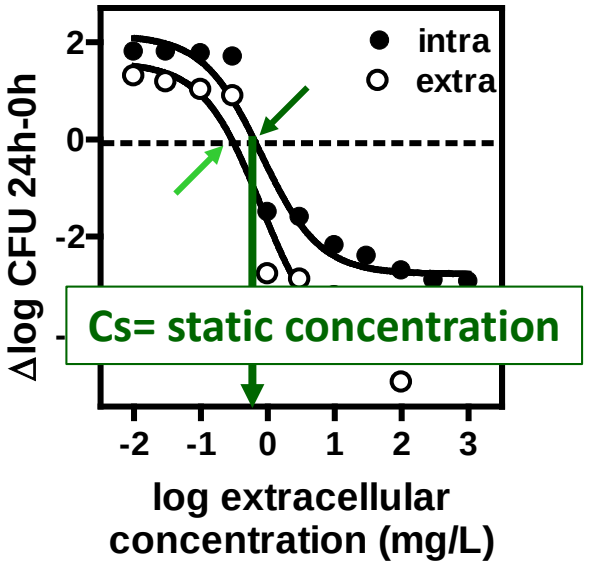
0.001 x MIC – 1,000 x MIC



Seral et al, AAC (2003) 47:2283-92

Barcia-Macay et al, AAC (2006) 50:841-51

PD parameters: what do they tell you ?



relative potency

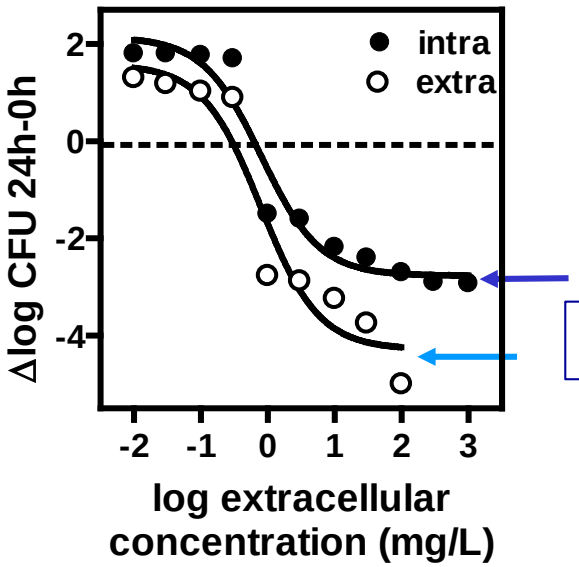
- Estimation of the concentration needed to reach a specified effect
- Measure of the « intracellular MIC »

⇨ « PK-related » parameter:
- accumulation in the infected compartment
- intracellular bioavailability

⇨ influence of local environment on intrinsic activity
- pH
- oxidant species

In most cases
Cs intra ≥ Cs extra

PD parameters: what do they tell you ?



Emax = max. efficacy

maximal efficacy

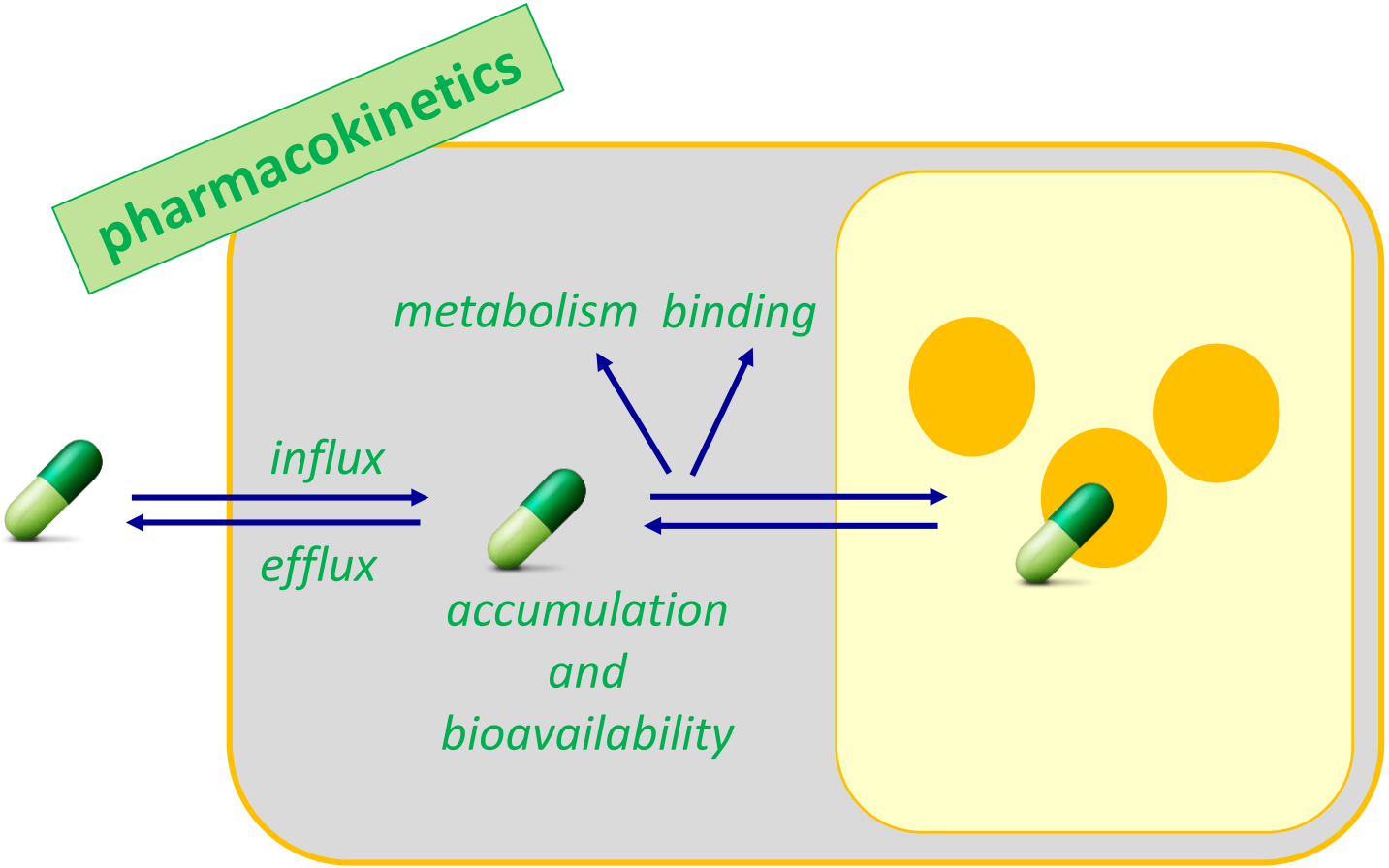
- Estimation of the maximal reduction in inoculum for an infinitely large concentration
- Measure of the killing capacity

⇨ « PD-related » parameter

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

In most cases
Emax intra <<< Emax extra

Modulating intracellular potency by modulating PK



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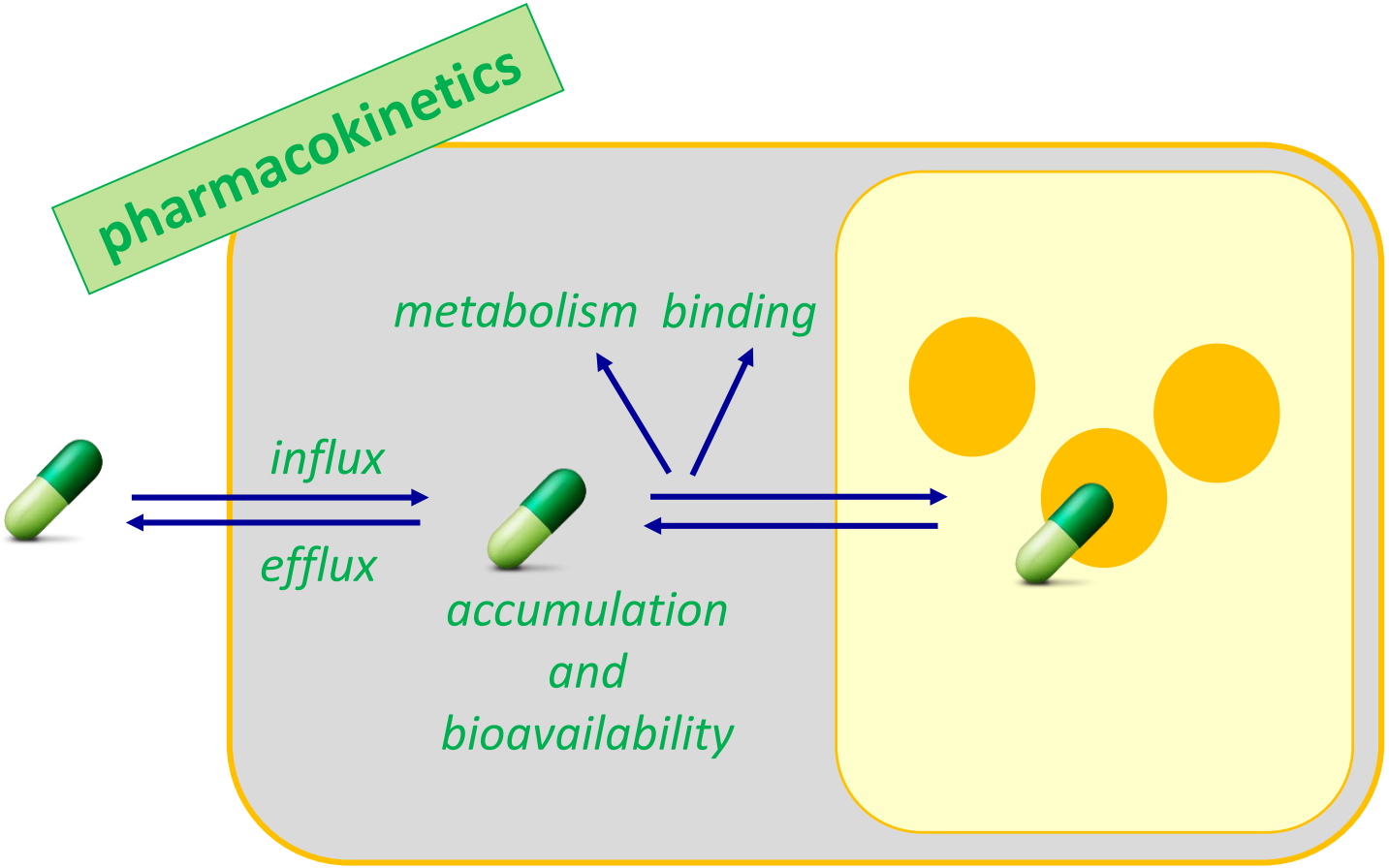
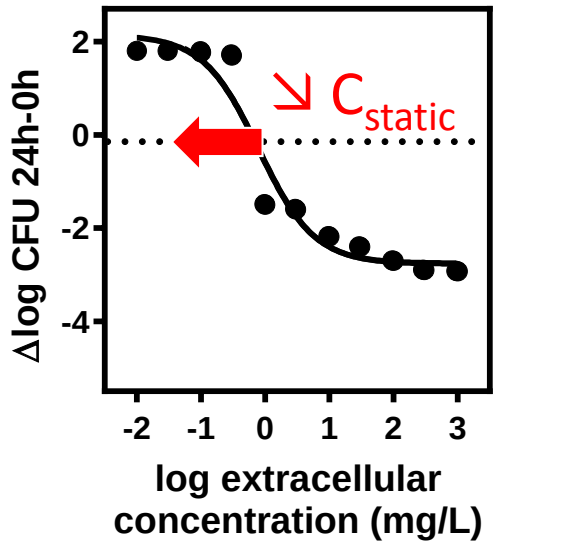
Modulating intracellular potency by modulating PK



↗ relative potency



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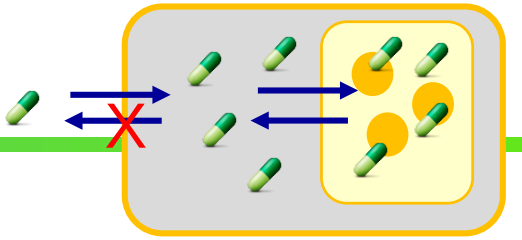
Increasing accumulation by inhibiting efflux



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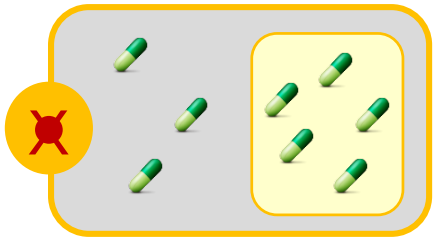
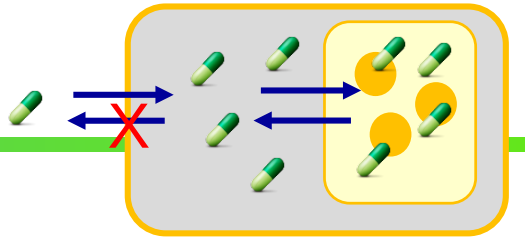
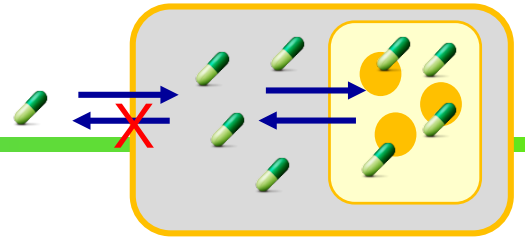


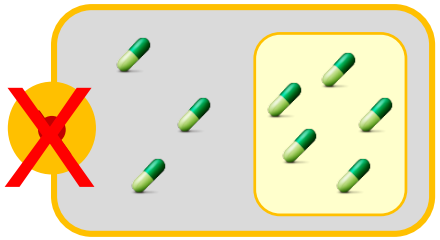
Azithromycin is substrate for P-glycoprotein



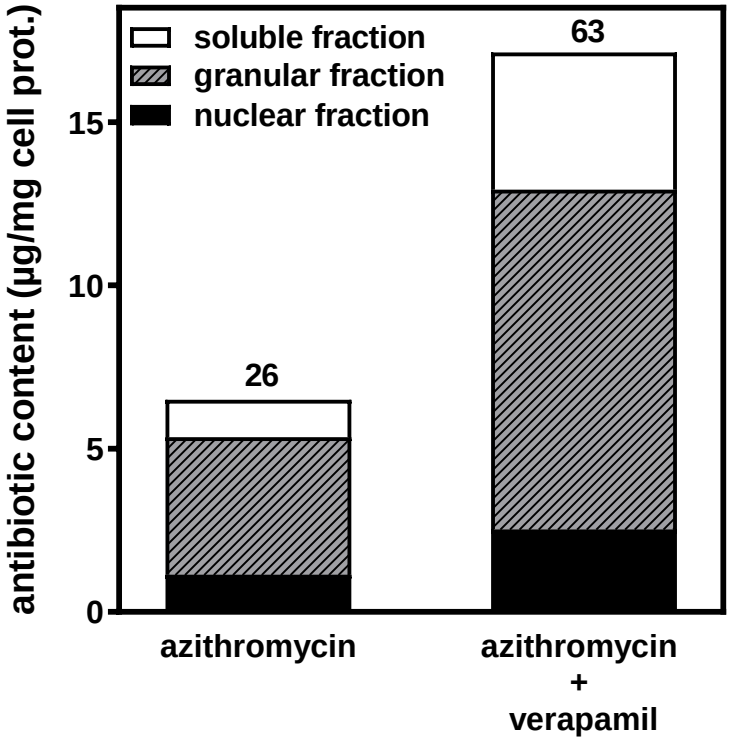
Seral et al. JAC (2003) 51:1167-73

Increasing accumulation by inhibiting efflux





Verapamil is an inhibitor of P-glycoprotein
→ increase in azithromycin accumulation (cytosol/organelles)



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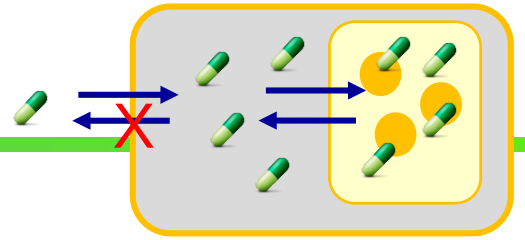
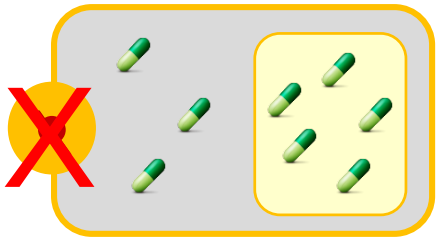
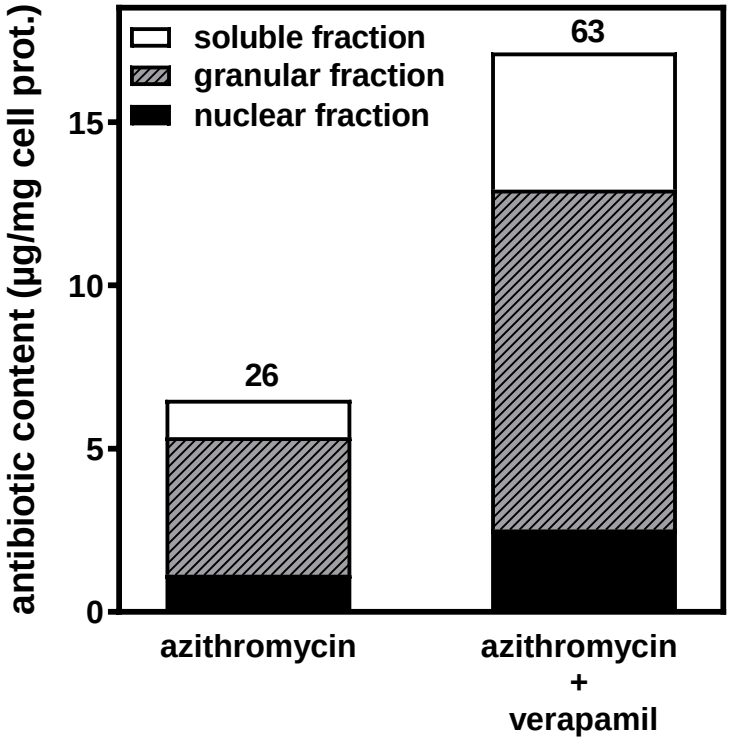
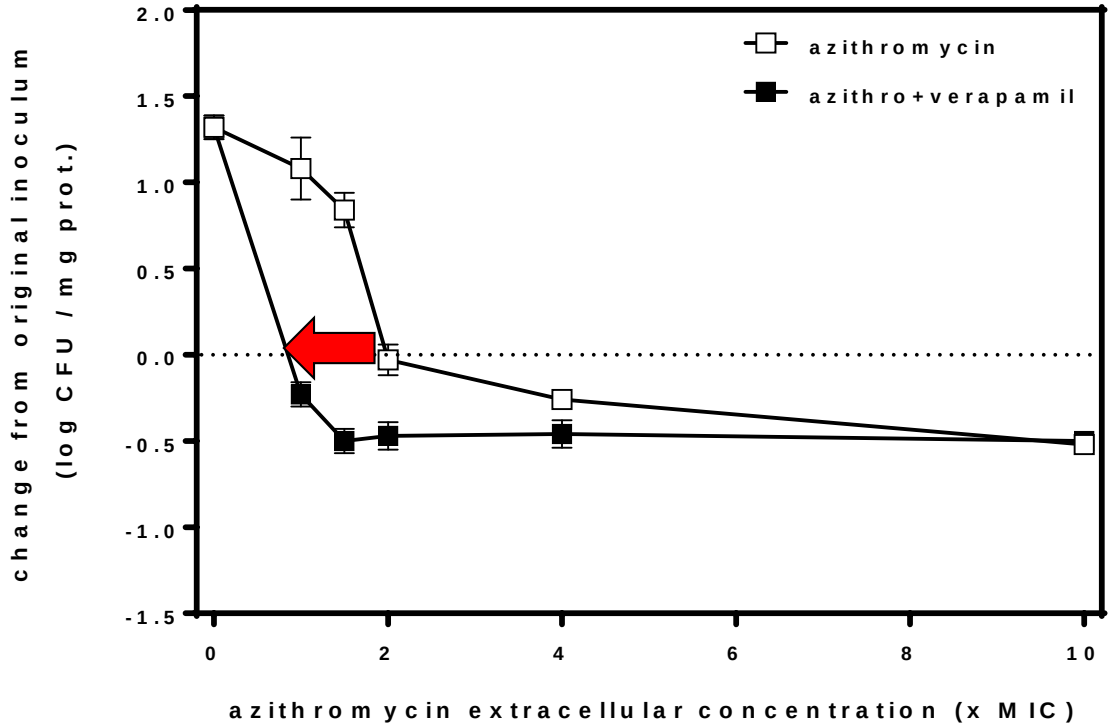




Verapamil is an inhibitor of P-glycoprotein
→ increase in azithromycin accumulation (cytosol/organelles)
→ Increase in relative potency against intracellular *S. aureus*





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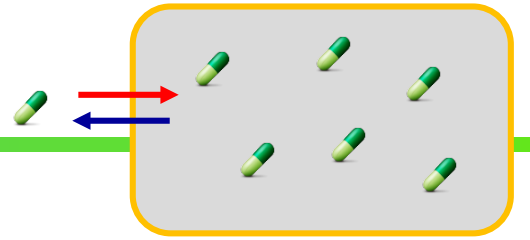
Increasing accumulation by improving diffusibility



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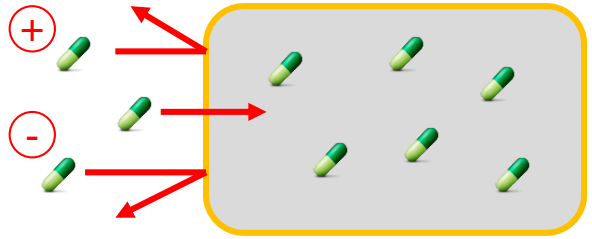
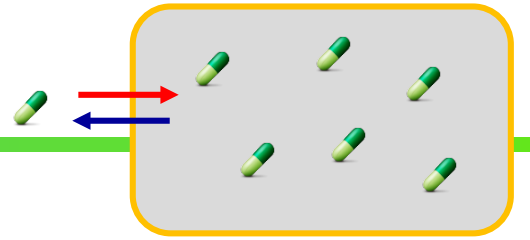
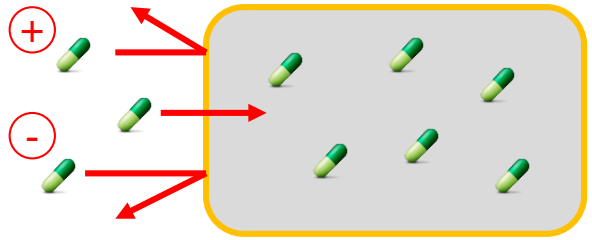
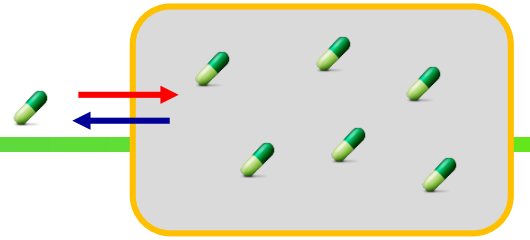




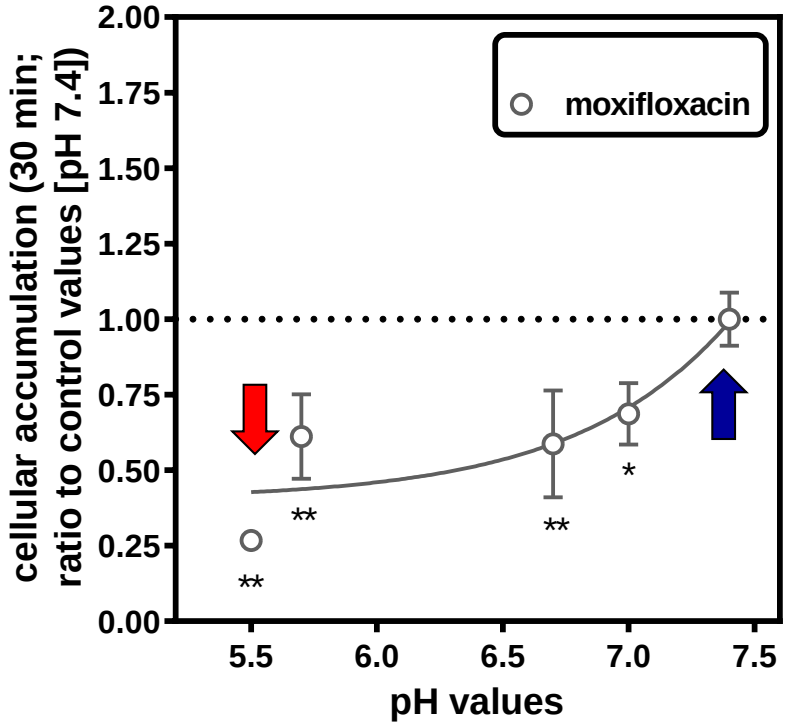
Neutral/zwitterionic molecules are more diffusible

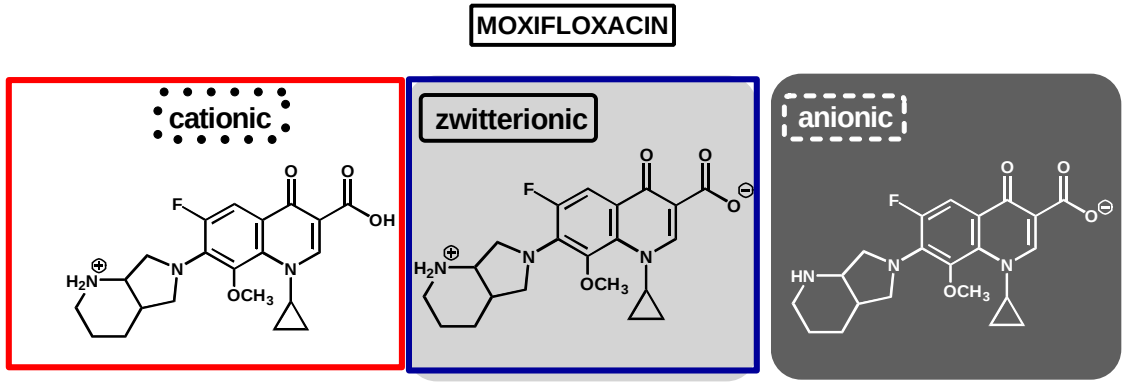
Increasing accumulation by improving diffusibility

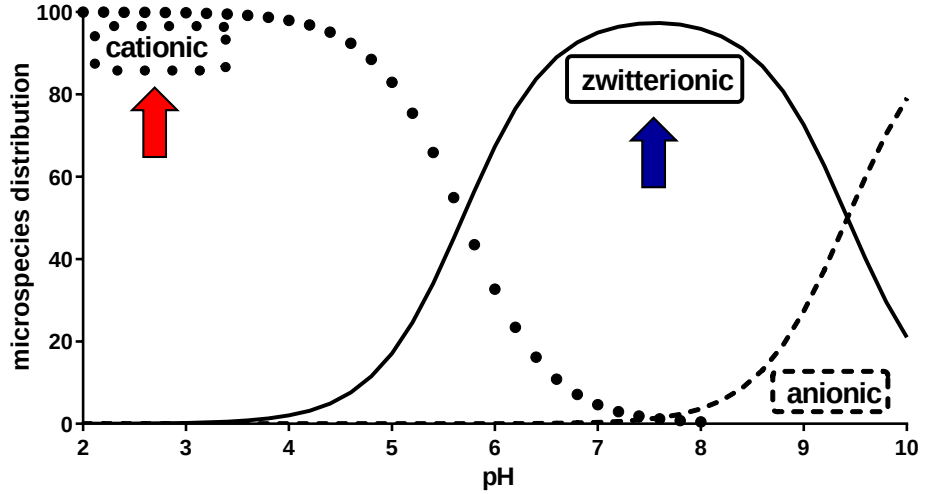




Neutral/zwitterionic molecules are more diffusible
→ accumulation lower for most fluoroquinolones at acidic pH

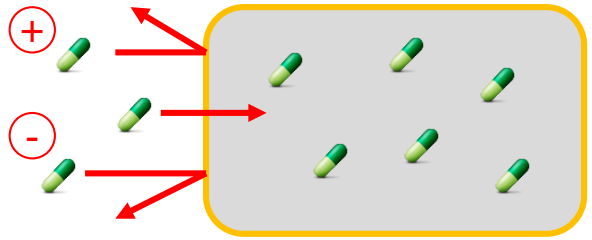


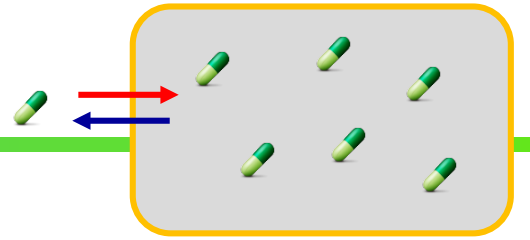




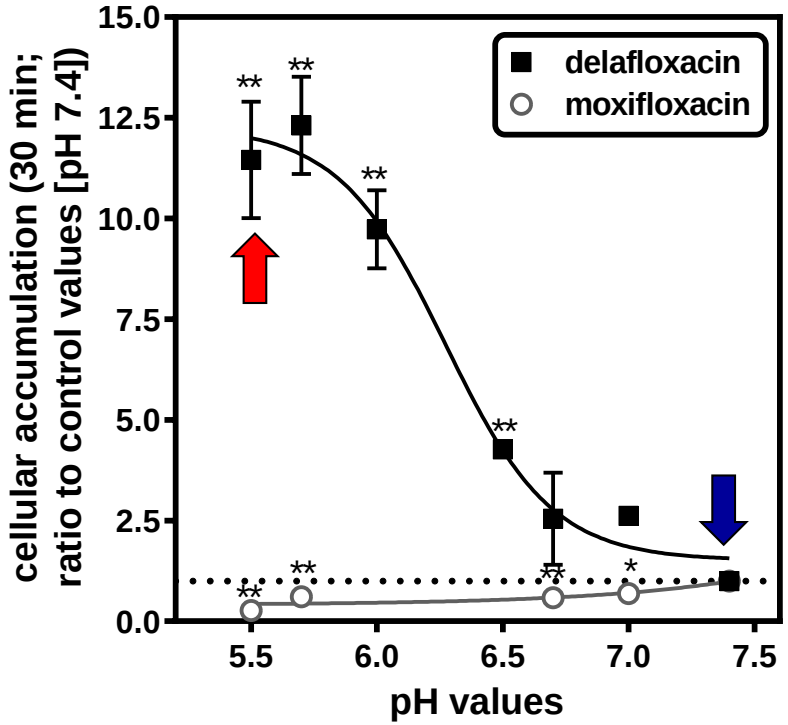
Lemaire et al. AAC (2011) 55:649-58; Van Bambeke, Future Microbiology (2015) 10:1111–23

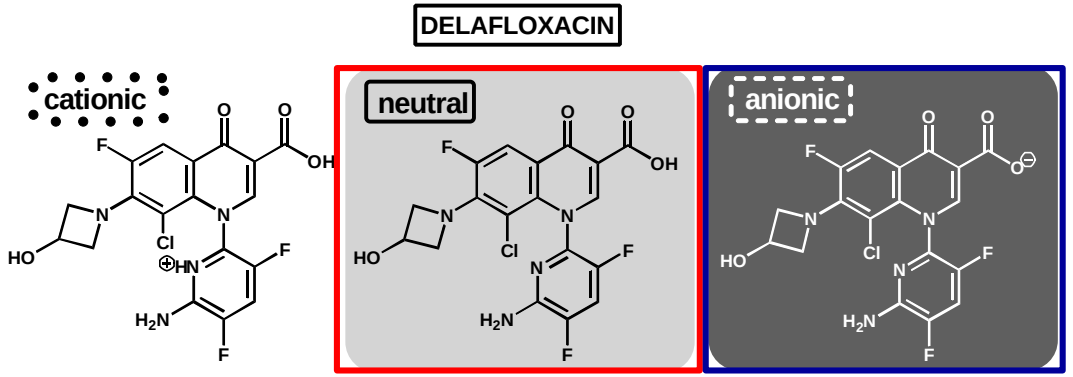
Increasing accumulation by improving diffusibility

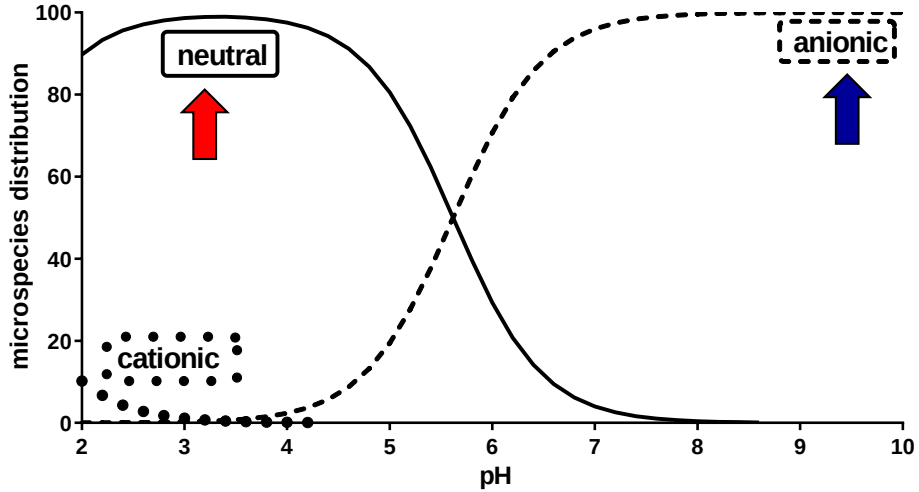




Neutral/zwitterionic molecules are more diffusible
→ accumulation higher for acidic fluoroquinolones (delafloxacin/finafloxacin)







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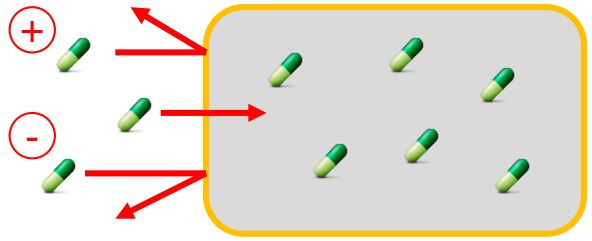
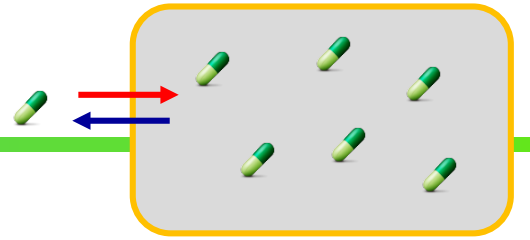
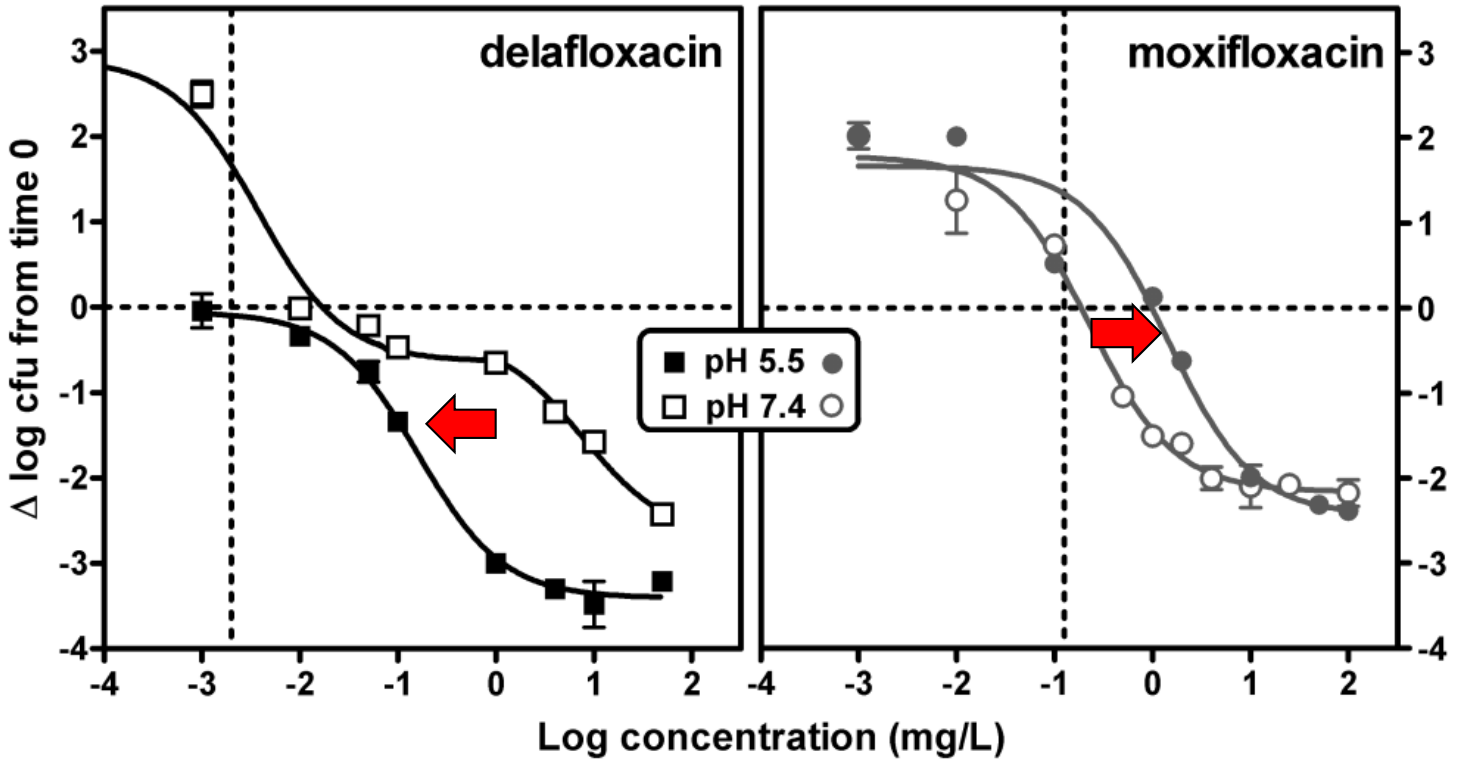




Neutral/zwitterionic molecules are more diffusible

→ Increase in relative potency for acidic molecules in acidic environments



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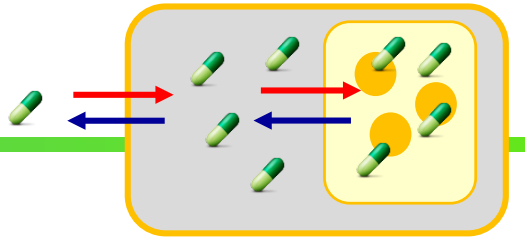
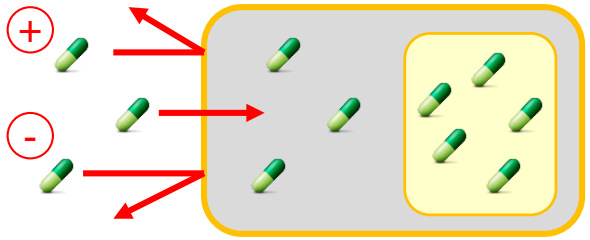




Neutral/zwitterionic molecules are more diffusible

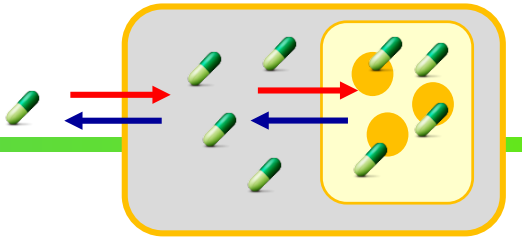
Is this relevant ?

pH is acidic in many environments :

- skin surfaces (pH 4.2–5.9)
- mouth (pH 5–7)
- vagina (pH 4.2–6.6)
- urinary tract (pH 4.6–7)

- pus, infected peritoneal fluid, and drainage fluid (6.6-6.8)

Delivery systems to modulate accumulation/distribution



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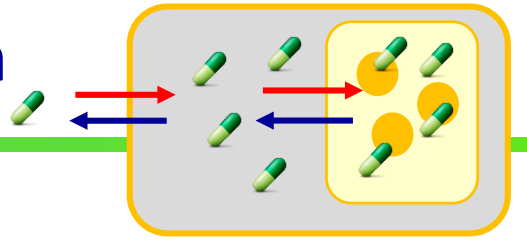
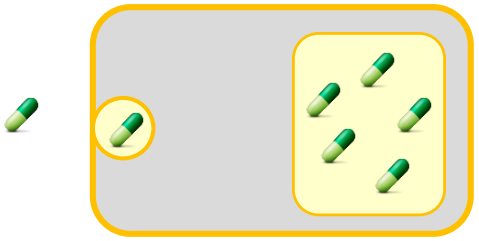
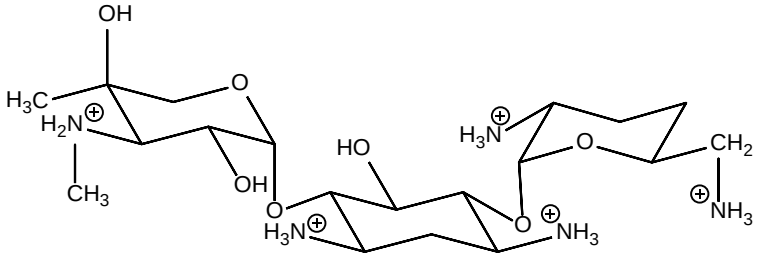
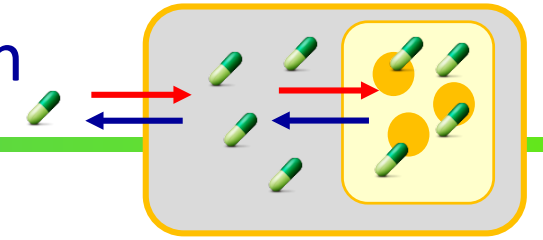


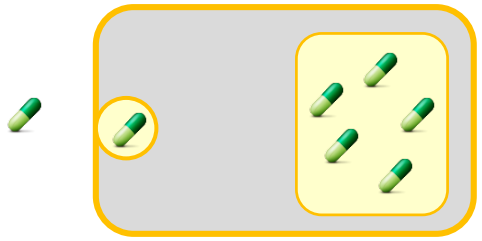


Aminoglycosides are not diffusible (too polar ...)
and accumulate in lysosomes by endocytosis

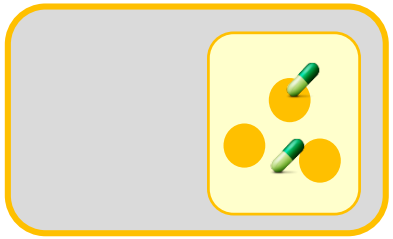


Delivery systems to modulate accumulation/distribution



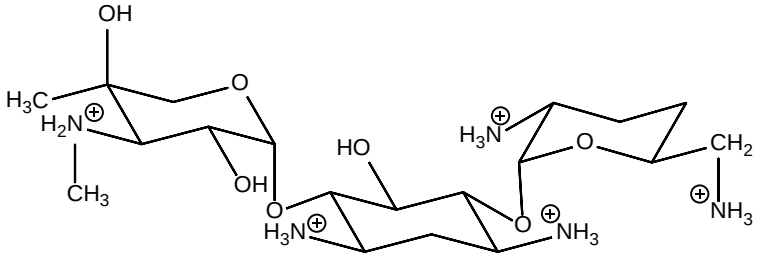


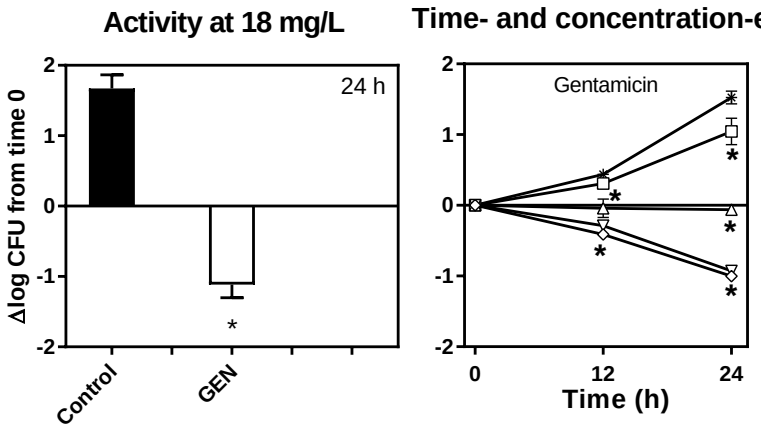
S. aureus



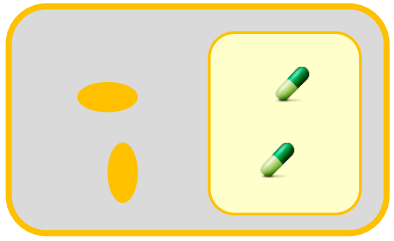
Aminoglycosides are not diffusible and accumulate in lysosomes by endocytosis

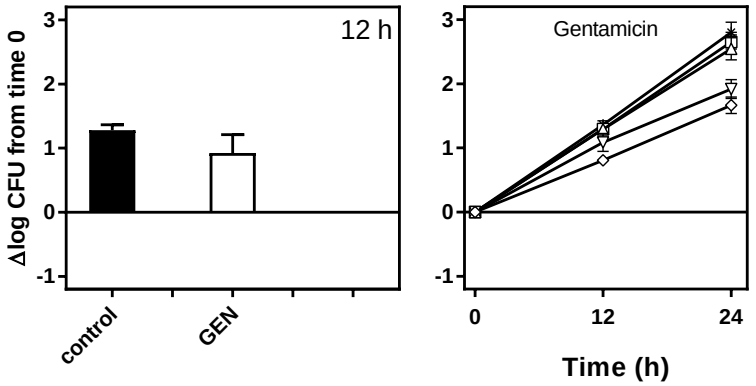
→ No activity against a cytosolic bacterium





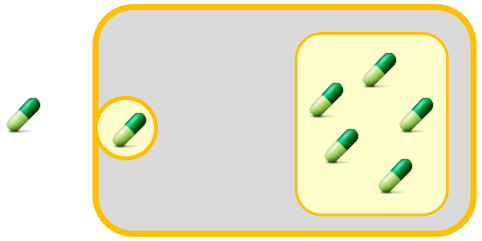
L. monocytogenes





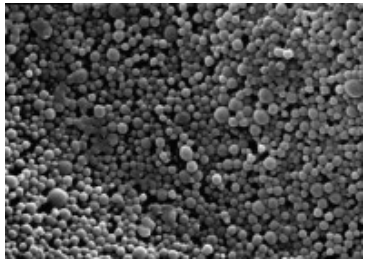
Imbuluzqueta et al. JAC (2012) 67:2158-64

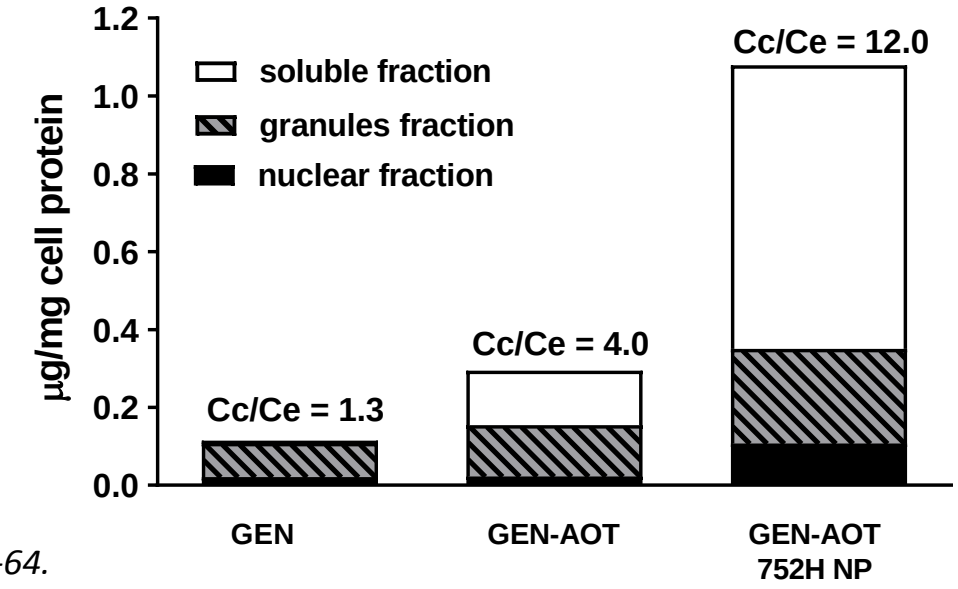
Vectors to modulate accumulation/distribution



Aminoglycosides are not diffusible and accumulate in lysosomes by endocytosis
→ No activity against a cytosolic bacterium
→ Nanoparticle formulation for cytosolic release

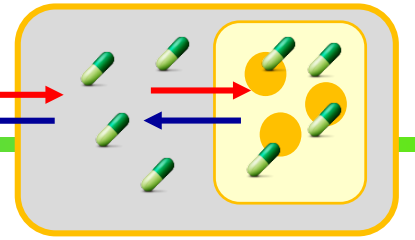
gentamicin (GEN) + surfactant (AOT [bis(2-ethylhexyl) sulfosuccinate sodium salt])
+ poly(D,L-lactide-co-glycolide) (PLGA)

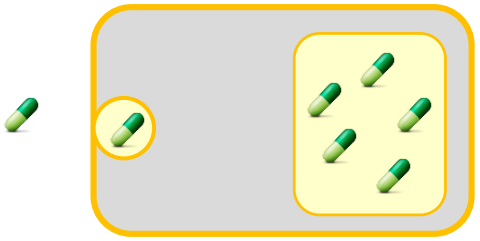




Imbuluzqueta et al. Acta Biomater. (2011) 7:1599-608; JAC (2012) 67:2158-64.

Delivery systems to modulate accumulation/distribution

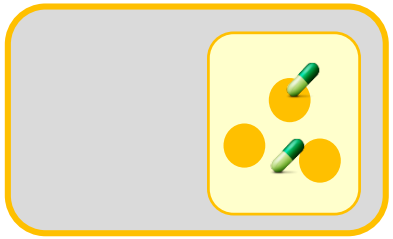


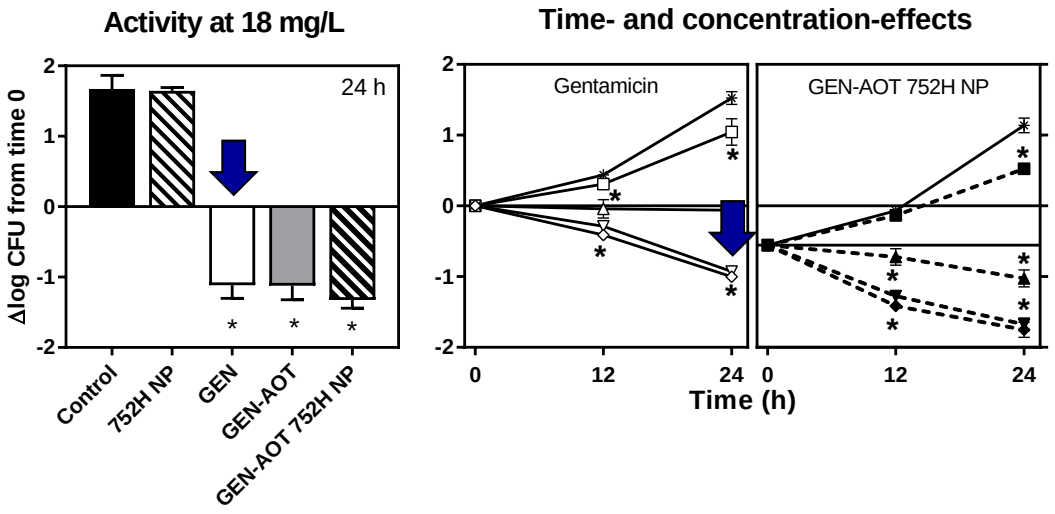


Aminoglycosides are not diffusible and accumulate in lysosomes by endocytosis

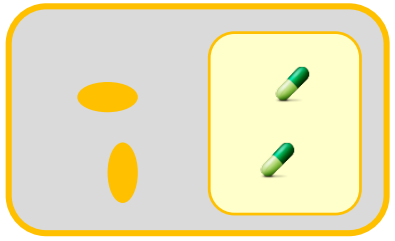
→ Delivering them in the cytosol make them active on cytosolic bacteria

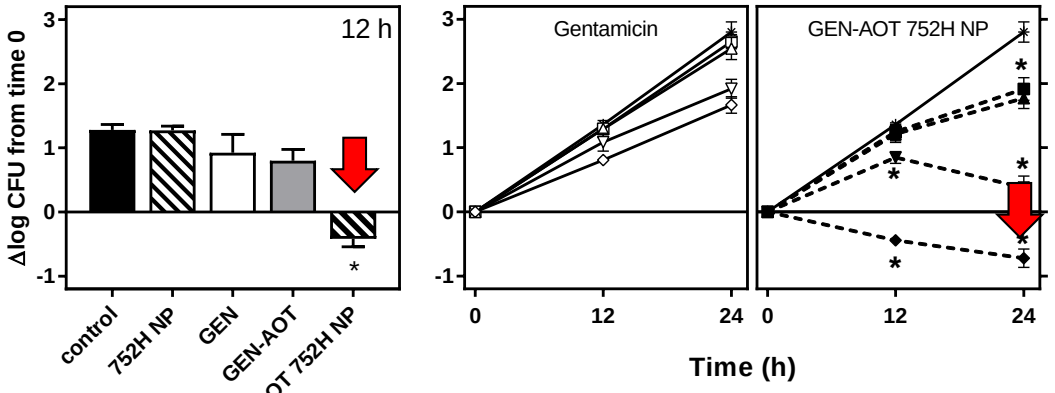
S. aureus





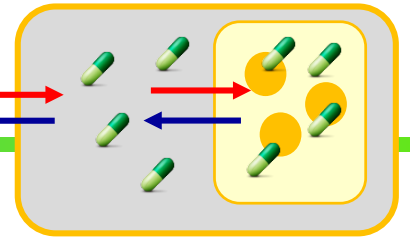
L. monocytogenes

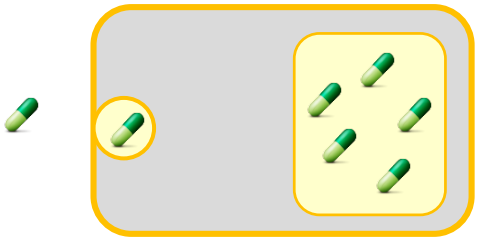




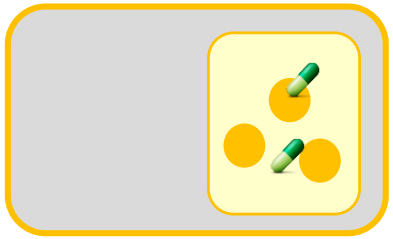
Imbuluzqueta et al. JAC (2012) 67:2158-64

Delivery systems to modulate accumulation/distribution

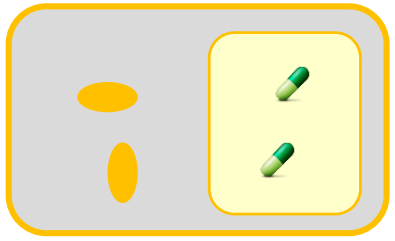




S. aureus

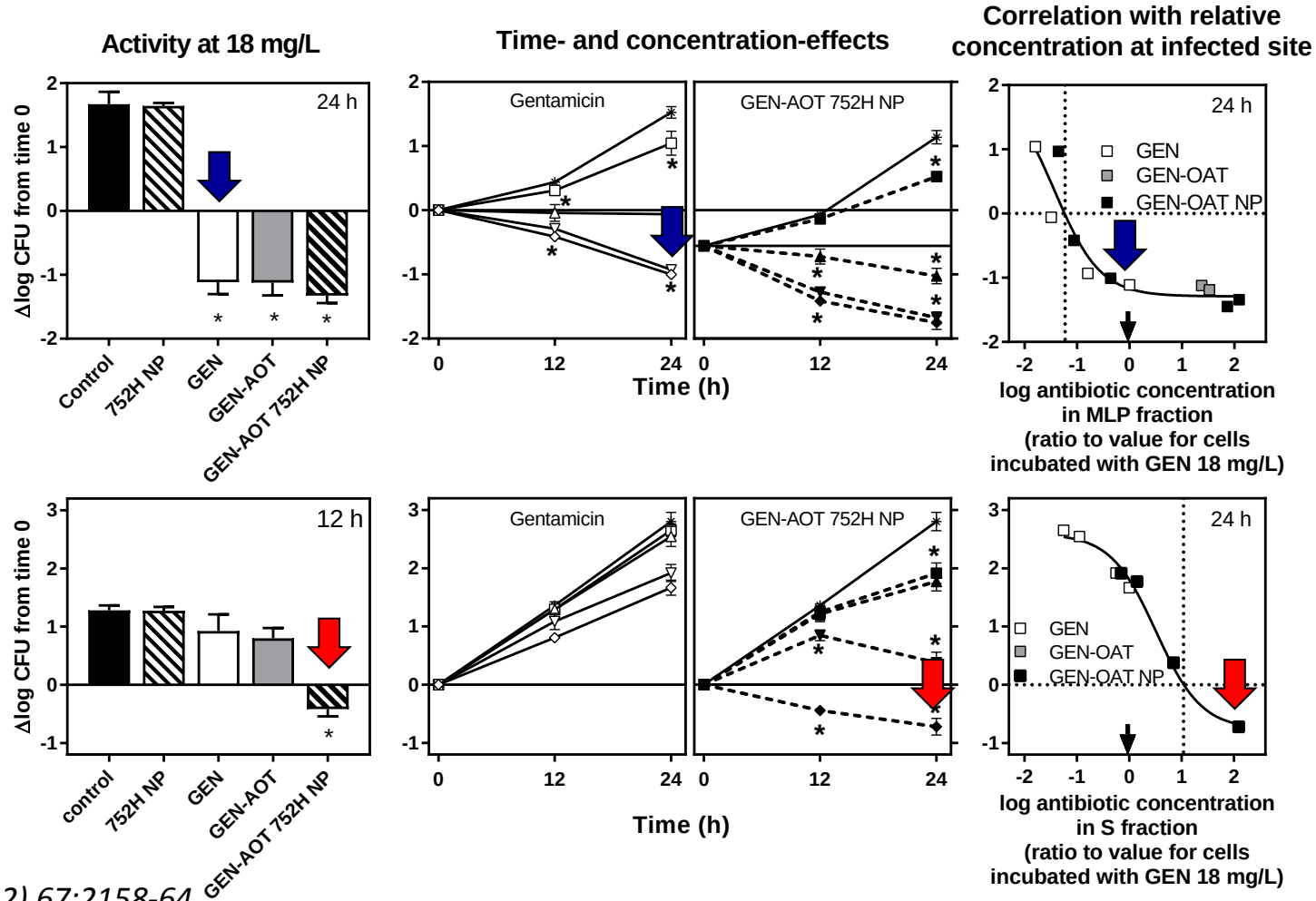


L. monocytogenes



Aminoglycosides are not diffusible and accumulate in lysosomes by endocytosis

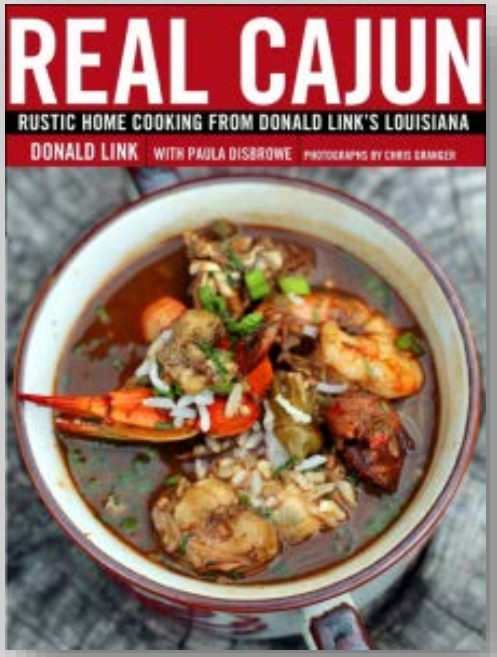
→ Delivering them in the cytosol make them active on cytosolic bacteria



Imbuluzqueta et al. JAC (2012) 67:2158-64

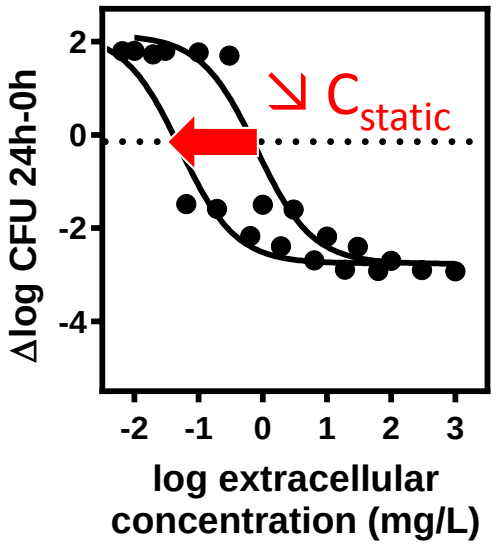
Influence of the intracellular environment on potency

Local environment can also modulate the intrinsic activity of antibiotics (“intracellular MIC”)



physico-chemical conditions

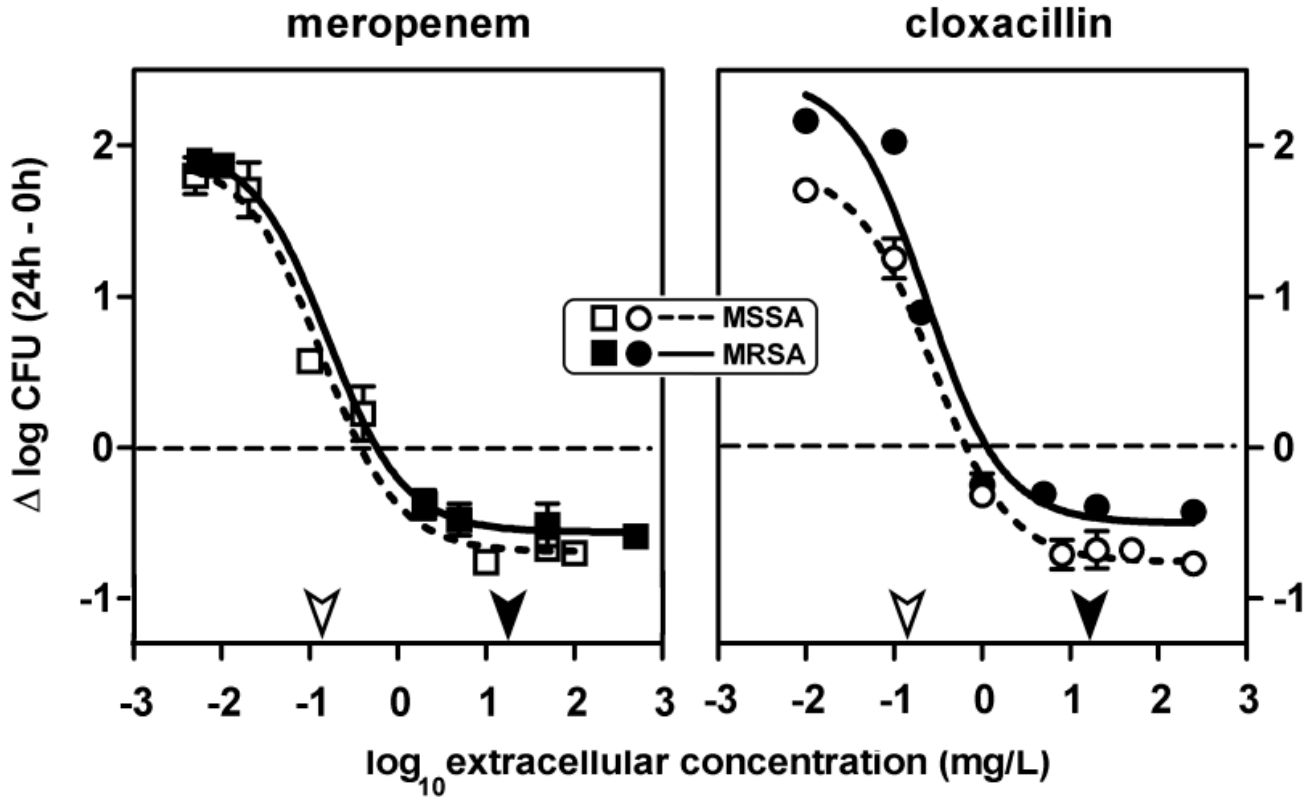
↗ relative potency



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

Impact of intracellular pH on activity

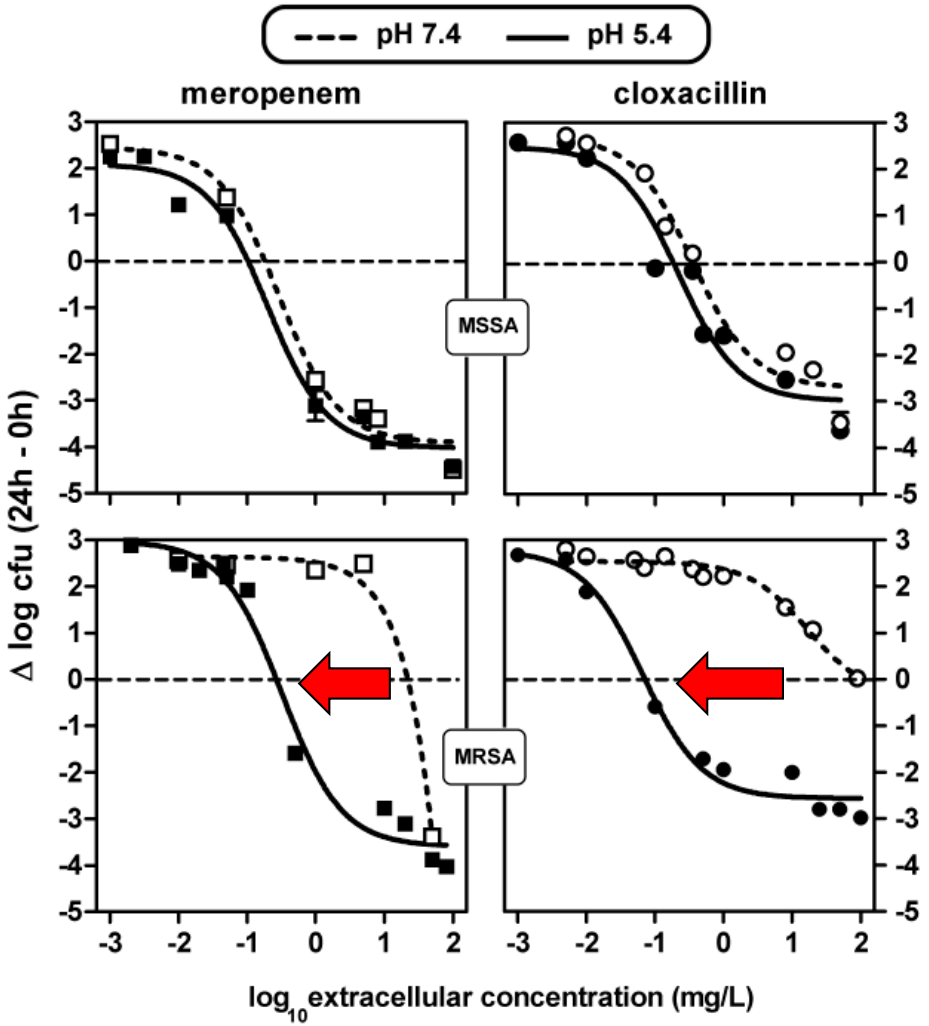
MRSA are as susceptible as MSSA to β-lactams when intracellular !



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

Impact of intracellular pH on activity

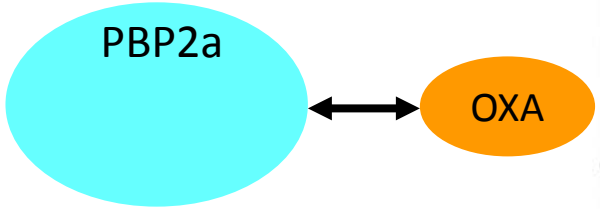
In broth, at acidic pH, MRSA are as susceptible as MSSA to β-lactams !

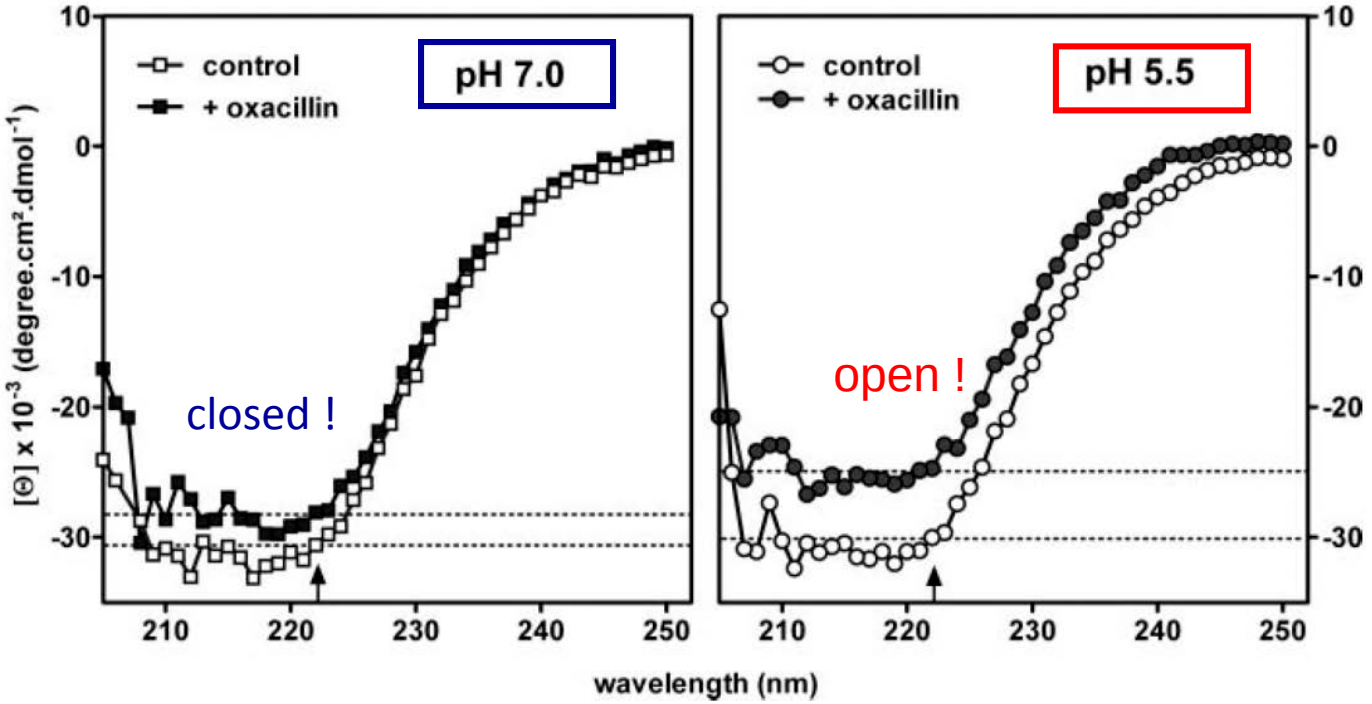


Lemaire et al., AAC (2007) 51:1627-32

Impact of intracellular pH on activity

At acidic pH, the conformation of PBP2a is modified, allowing for the access of β-lactams !





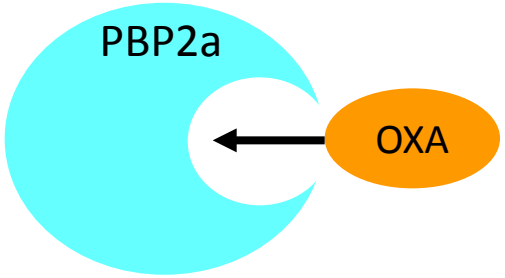
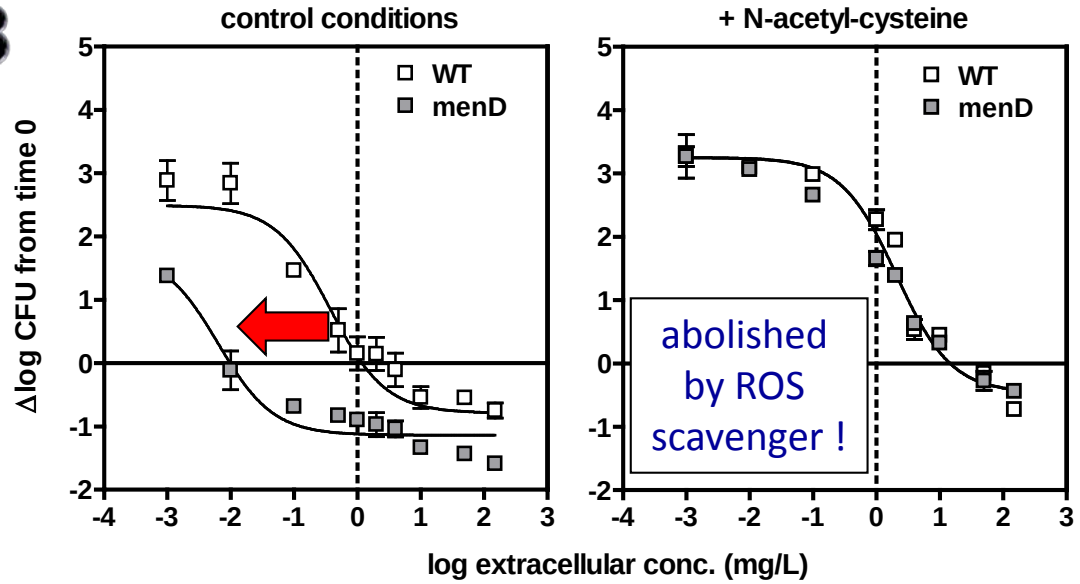


FIGURE 4. **Circular dichroic spectra of PBP 2a at pH 7. 0 (*left panel*) and pH 5.5 (*right panel*) in the absence (*open symbols*) and in the presence (*closed symbols*) of oxacillin (30 μM) for 30 min at 25 °C.** The *thin dotted lines* in each graph represent minima of PBP 2a molar ellipticity at 222 nm (*vertical arrow* on the *abscissa*) for each condition. The spectrum of oxacillin has been subtracted from all data points.

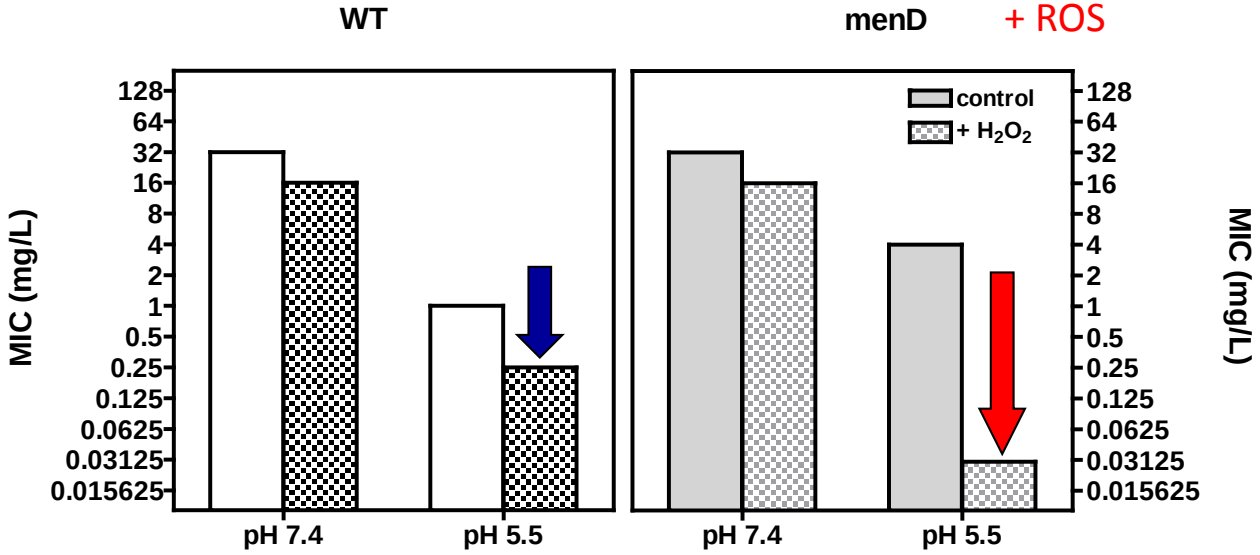
Lemaire et al., J Biol Chem (2008) 283:12769-76

Impact of oxidant species on activity

Menadione-dependent SCV of MRSA are hypersusceptible to β-lactams intracellularly ….

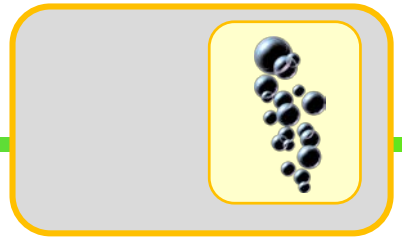


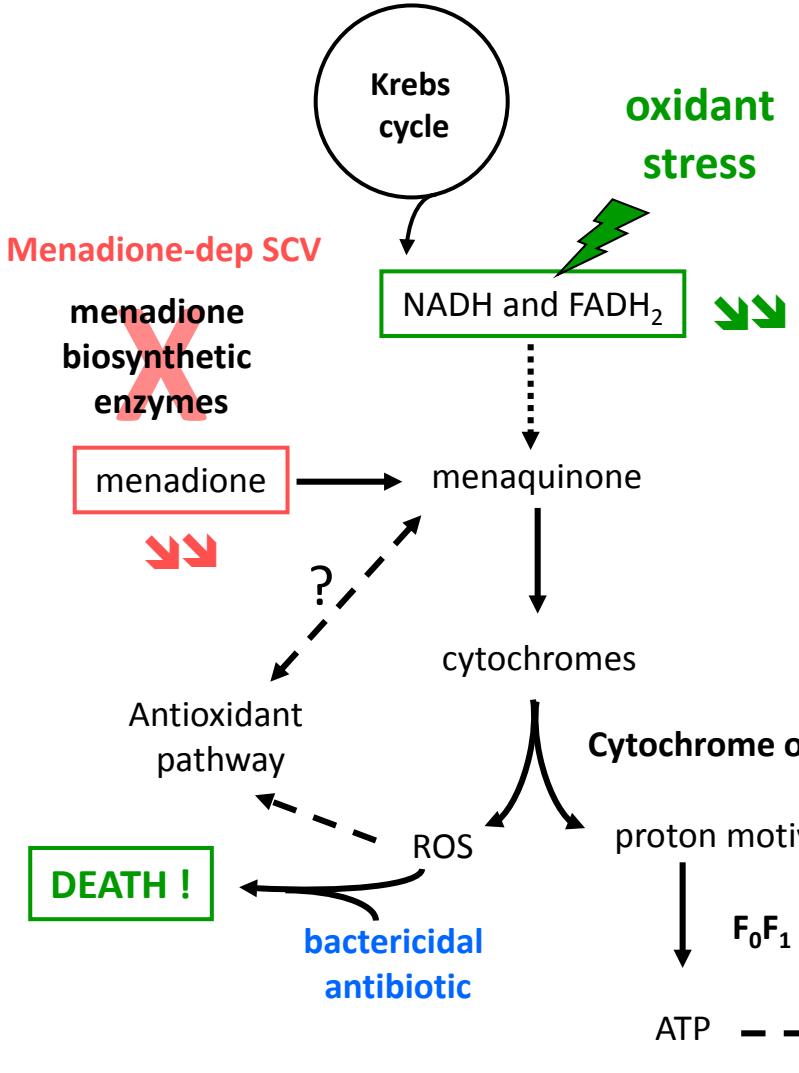
MIC ↘↘ if acid pH + ROS



Garcia et al., JAC (2012) 67:2873-81

Impact of oxidant species on activity





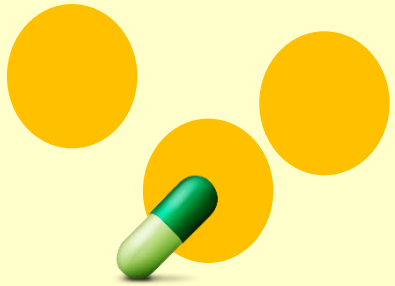
Exogenous oxidant species
may compensate
for reduced ROS production
in menadione-dependent SCVs

Garcia et al., JAC (2013) 68:1455-64

Modulating intracellular activity by modifying PD

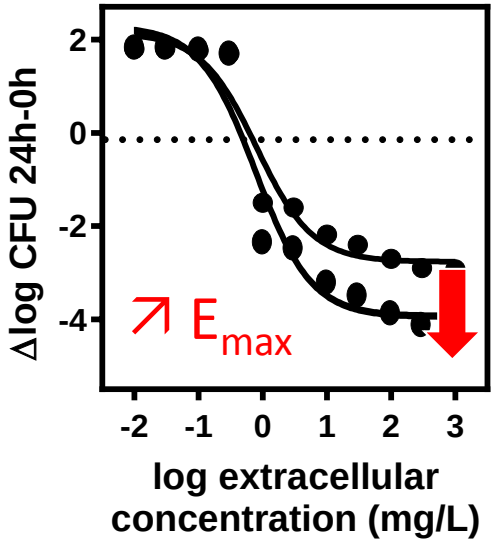
pharmacodynamics

cooperation with host defenses



bacterial responsiveness

↗ Maximal efficacy



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

Bacterial responsiveness and PD

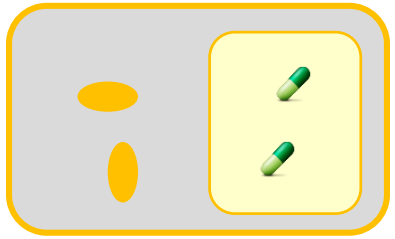
bacterial responsiveness

Bacterial responsiveness and PD

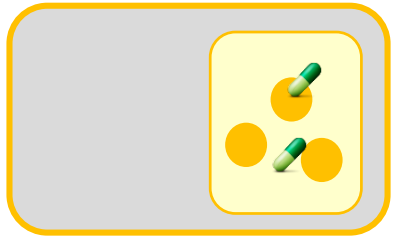
bacterial responsiveness

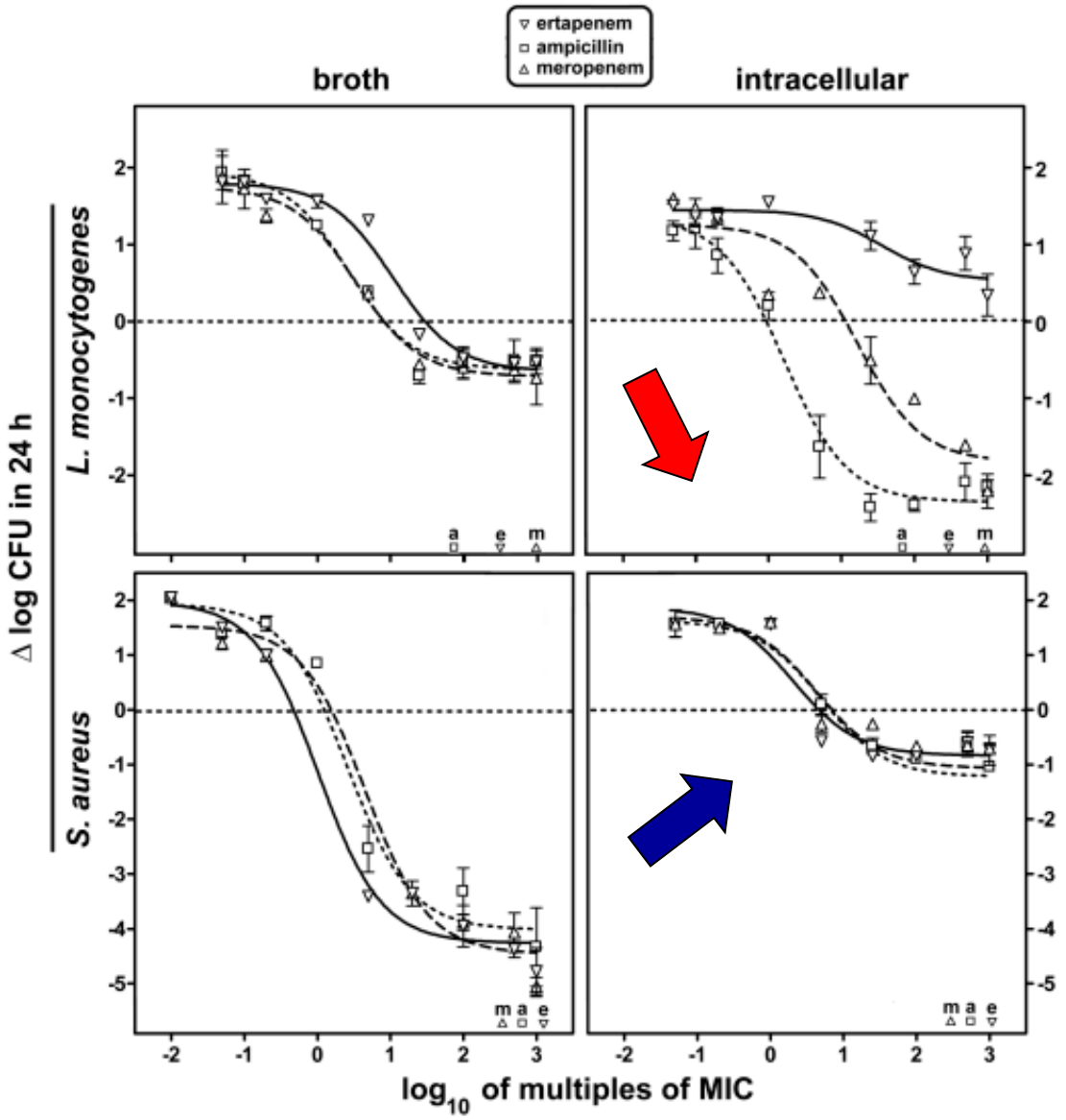
Differences among species

L. monocytogenes



S. aureus





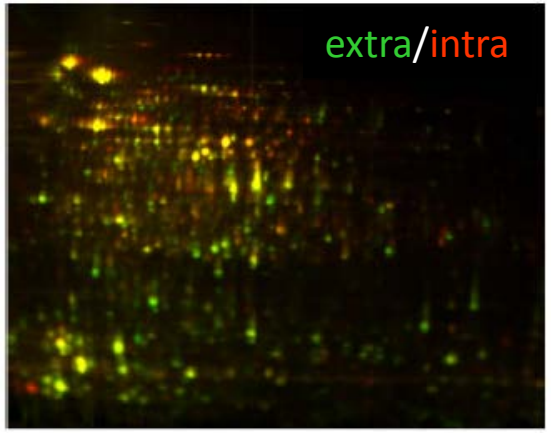
Why are β-lactams more active against intracellular *Listeria* ?

Lemaire et al. JAC (2005) 55:897–904

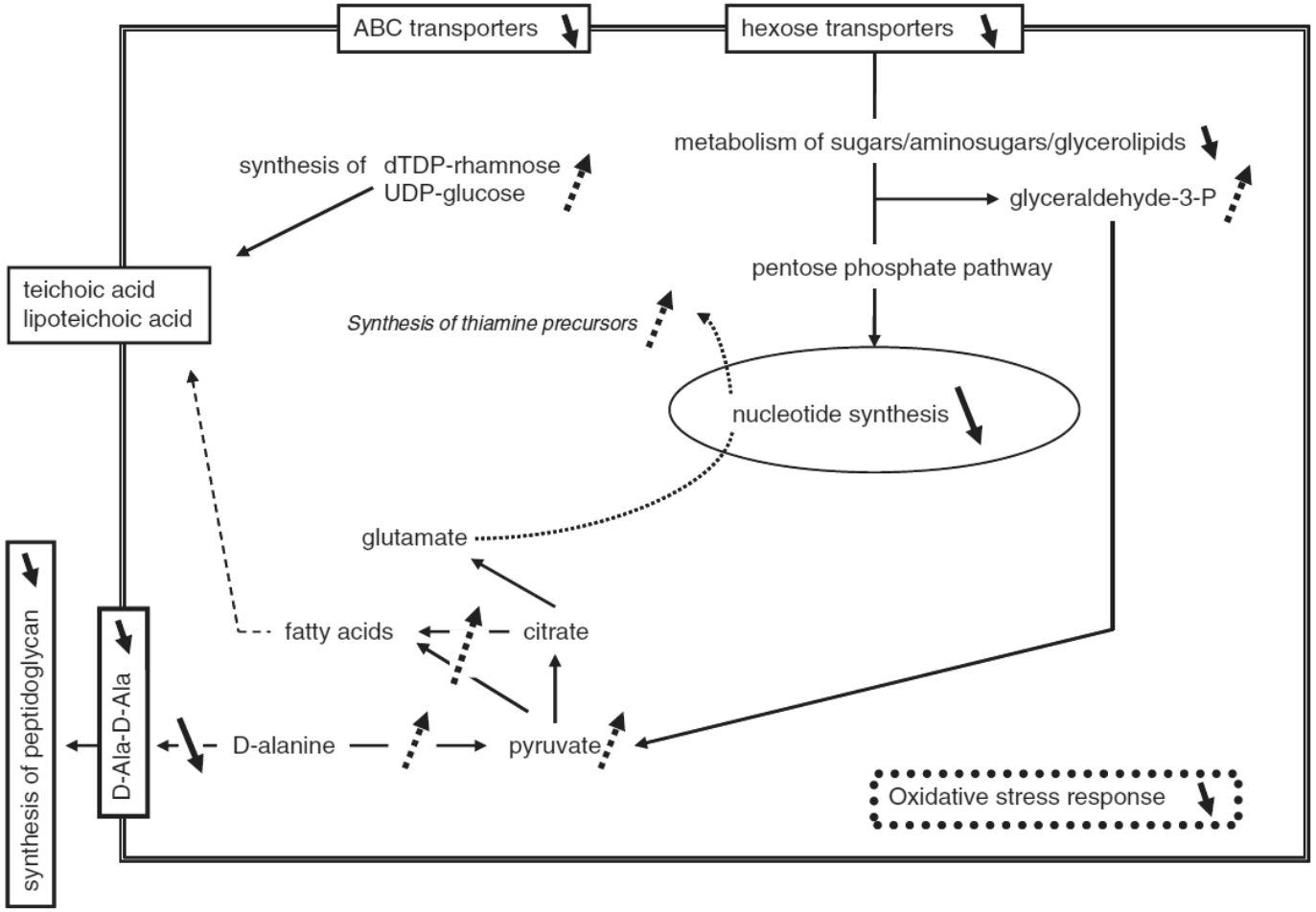
Bacterial responsiveness and PD

bacterial responsiveness

Proteomic analysis of extra- vs intra-cellular *Listeria*



Reduced cell wall synthesis

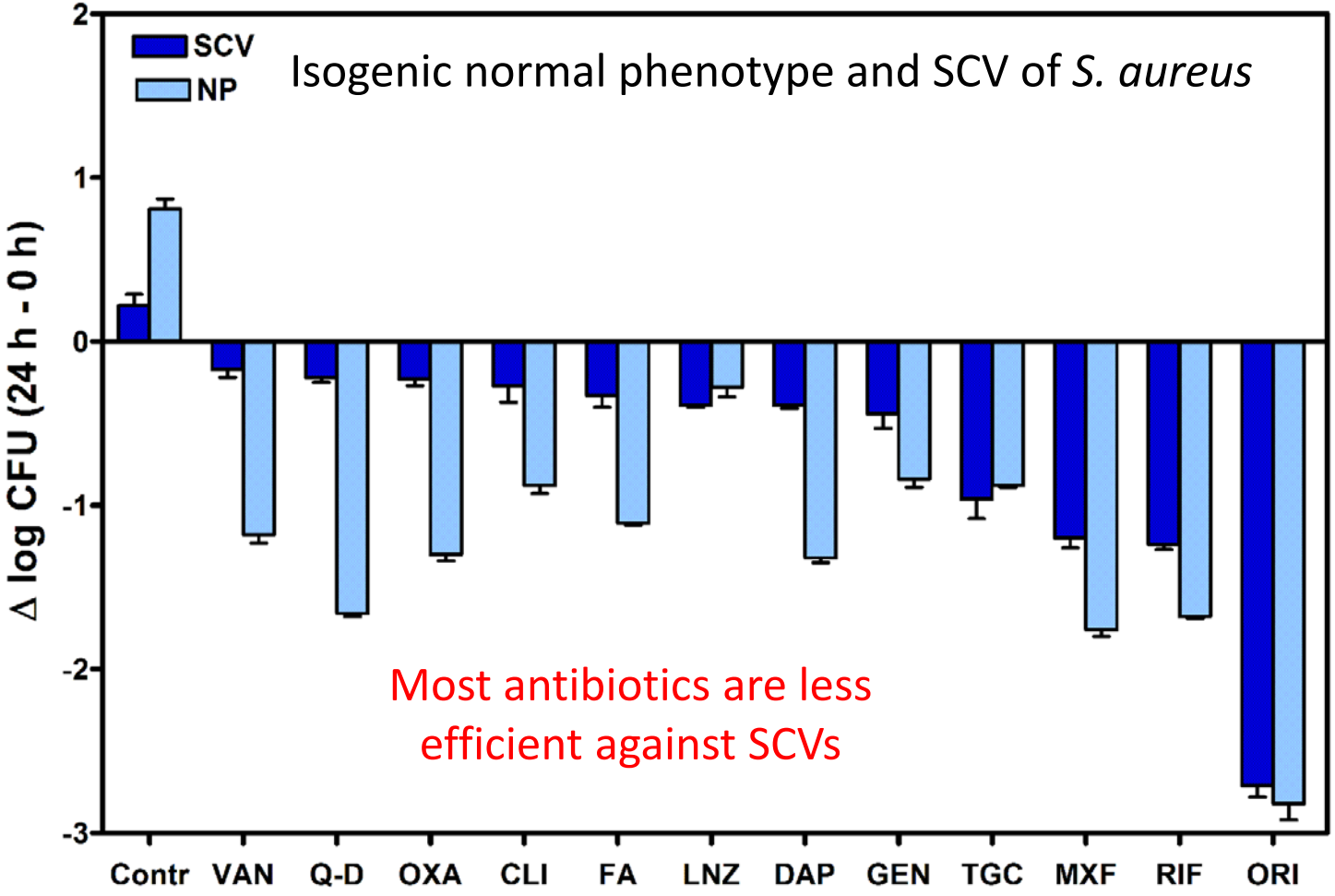


Van de Velde et al. Proteomics (2009) 9:5484-96

Bacterial responsiveness and PD

bacterial responsiveness

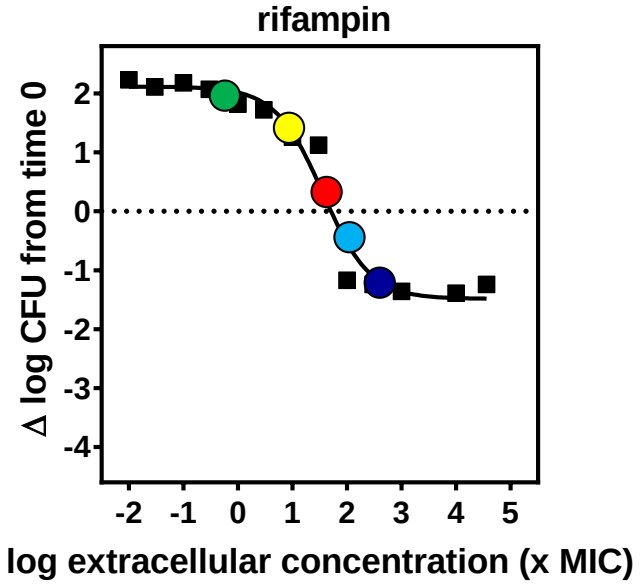
Differences among phenotypes



Nguyen et al, AAC (2009) 53:1434–42

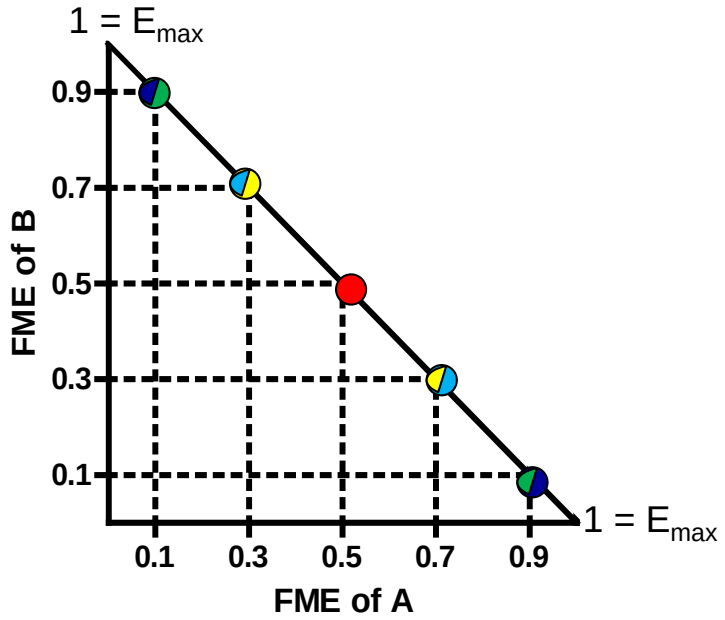
Bacterial responsiveness and PD

Combining drugs as a way to improve efficacy ?

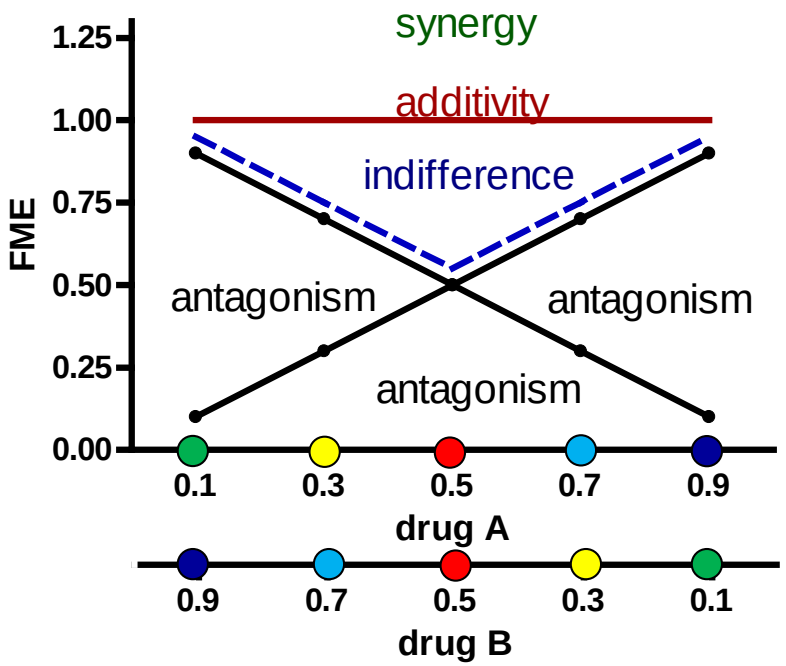


$$C_A = \frac{FME_A.EC_{50A}}{1-FME_A}$$

$$C_B = \frac{FME_B.EC_{50B}}{1-FME_B}$$



$$FME_{comb.} = FME_A + FME_B = 1$$

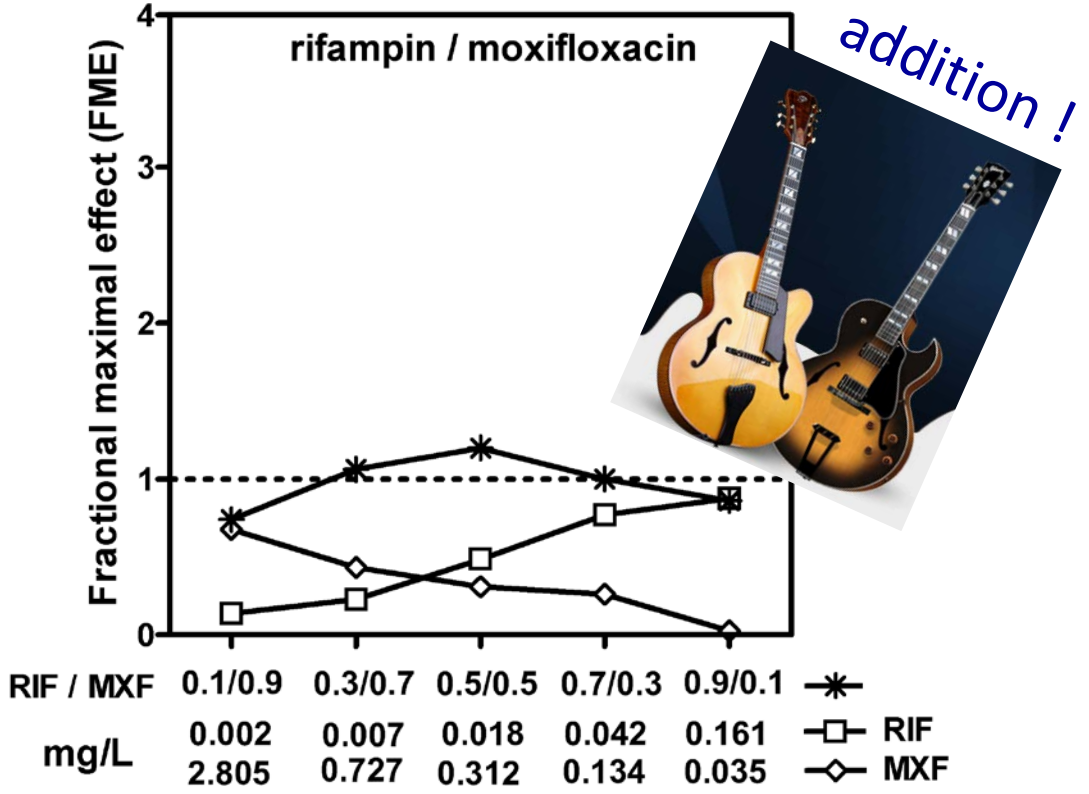


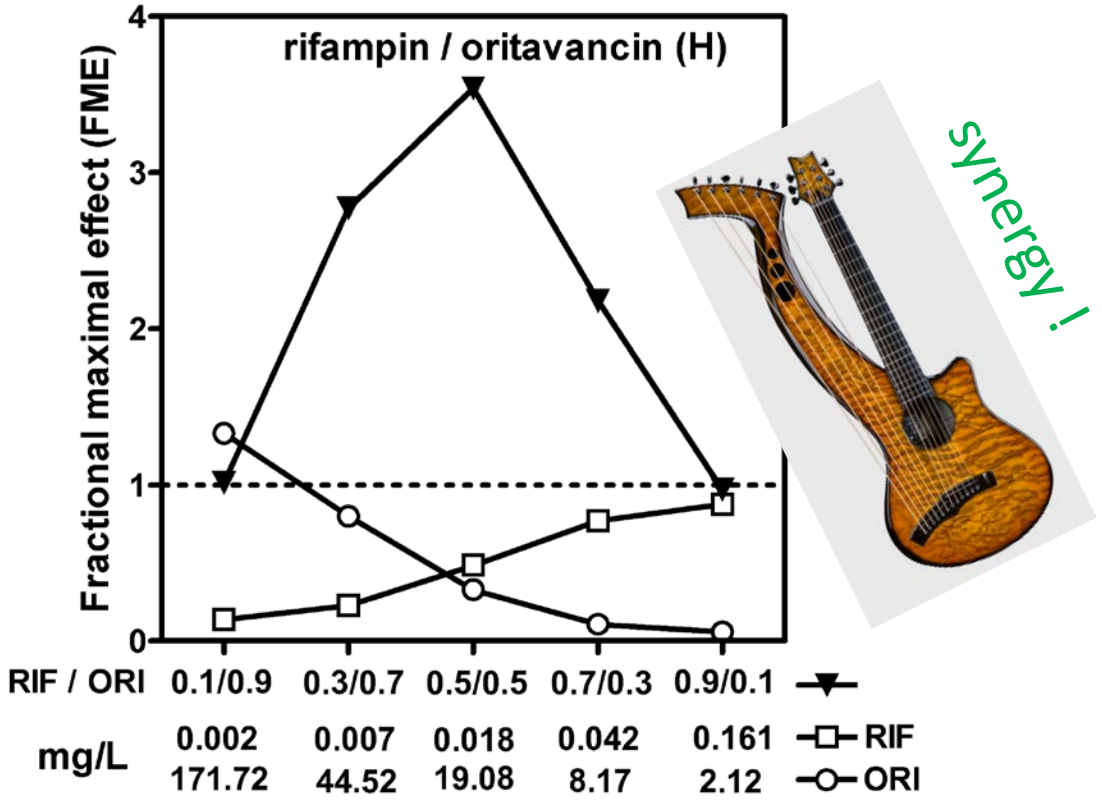
Nguyen et al, AAC (2009) 53:1443–49

Bacterial responsiveness and PD

bacterial responsiveness

Combining drugs as a way to improve efficacy ?





Nguyen et al, AAC (2009) 53:1443-49

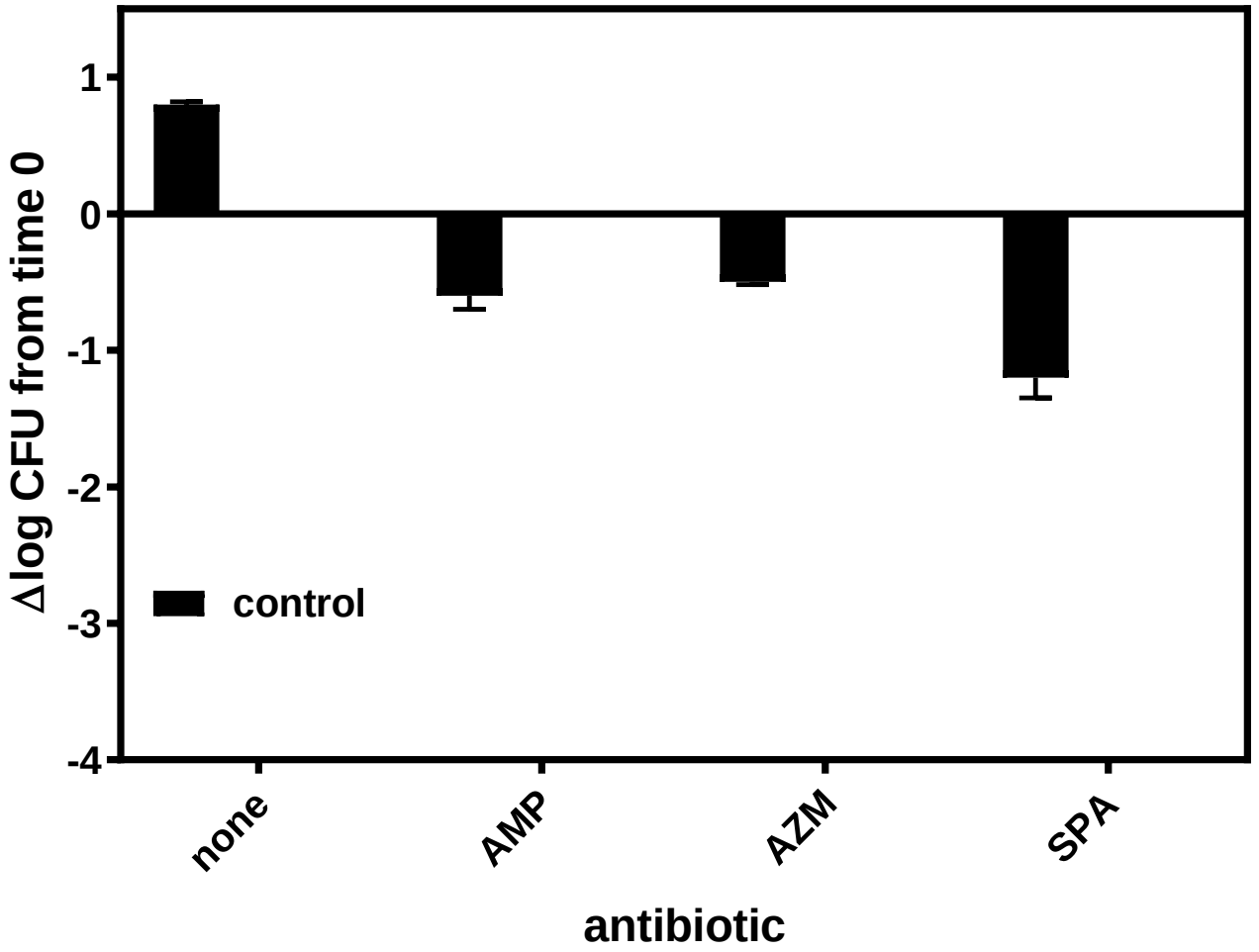
Cooperation with host defense and PD

host defenses

Cooperation with host defense and PD

host defenses

Influence of Interferon-γ on antibiotic activity towards intracellular *L. monocytogenes*



Ouadhriri et al, AAC (1999) 43:1241-51

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host defenses

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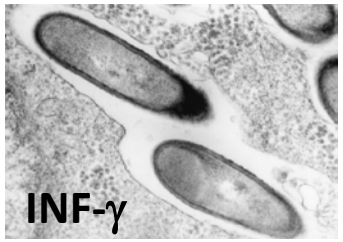
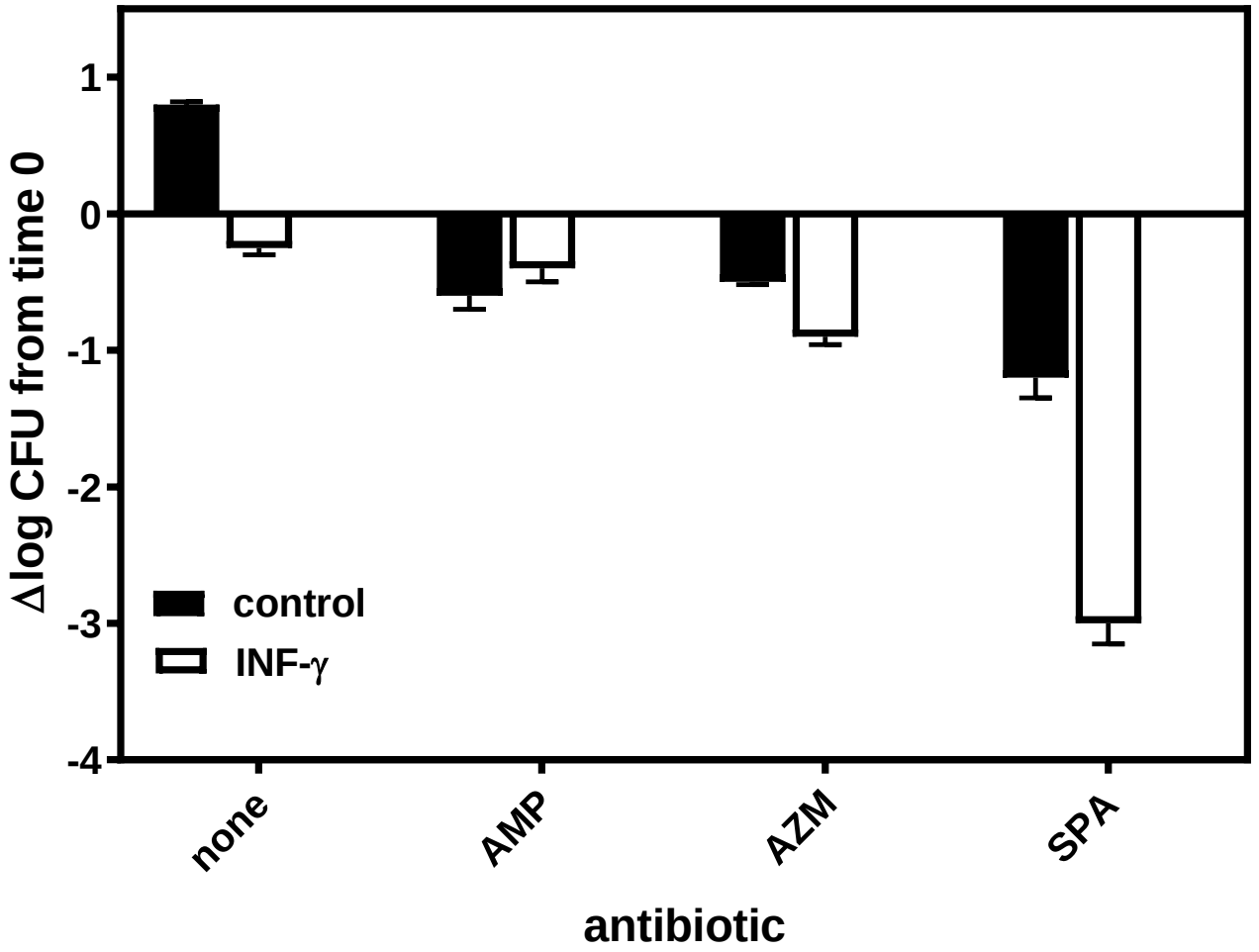




+ INF-γ:

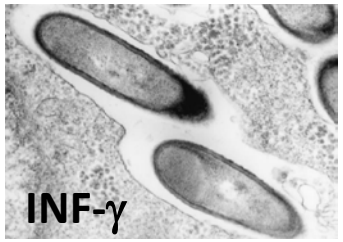
- Bacteria confined in vacuoles
- FQ (and ML) more active

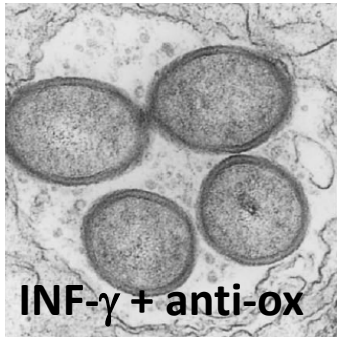
Ouadhriri et al, AAC (1999) 43:1241-51

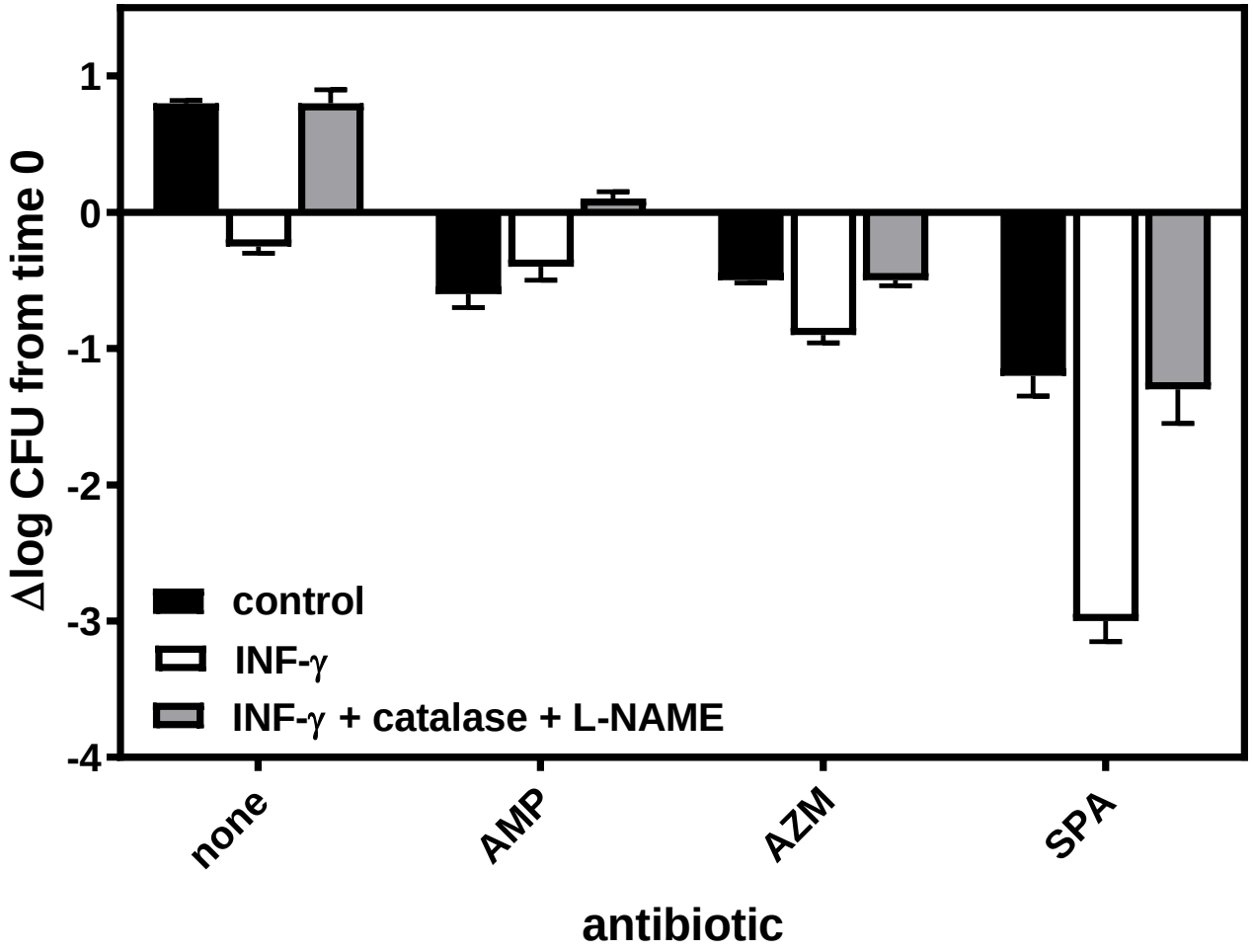
Cooperation with host defense and PD

host defenses

Influence of Interferon-γ on antibiotic activity towards intracellular *L. monocytogenes*







+ INF-γ and antioxidants:

- Bacteria confined in vacuoles
- AB activity ~ control conditions

Cooperation between AB and oxidant species

Ouadhriri et al, AAC (1999) 43:1241-51

Conclusions: Can we modulate intracellular antimicrobial activity ?

Improve potency ? **PROBABLY FEASIBLE !**

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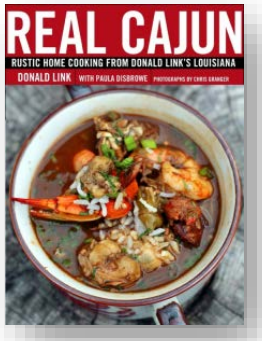
- 

reduce intracellular MIC (pH, oxidant species; difficult to change in practice…)

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  Use drug combinations
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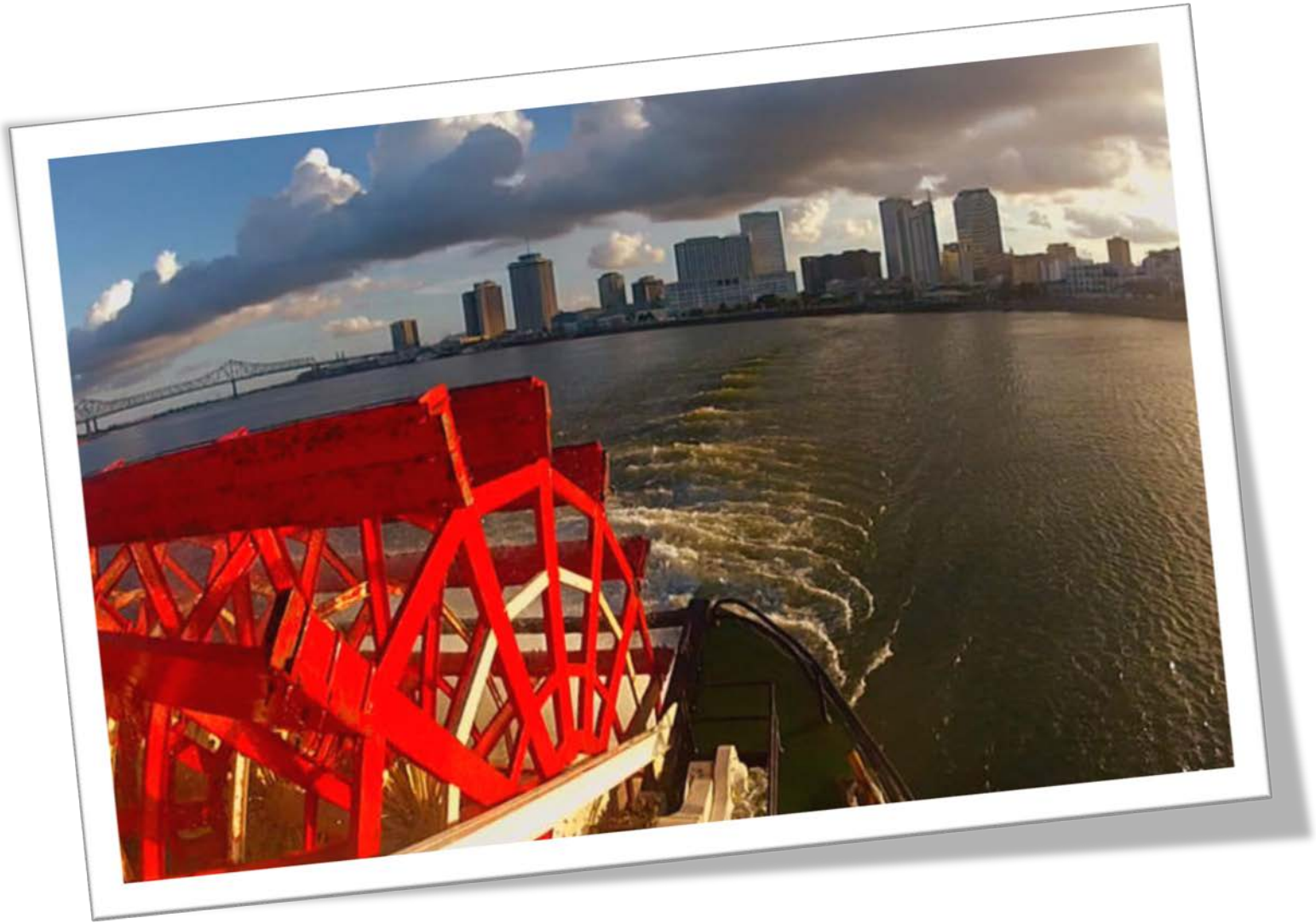
  make bacteria more responsive
  (do not neglect slow growing phenotypes [SCV]…)
- 
  boost cell defense mechanisms

Have a safe trip back home …



Acknowledgments

Paul Tulkens

Youssef Ouadhriri

Cristina Seral

Maritza Barcia

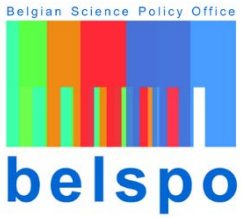
Huang Anh Nguyen

Sandrine Lemaire

Edurne Imbuluzqueta

Sébastien Van de Velde

Laetitia Garcia



Transparency declaration
Research grants from …