

Are intracellular drug concentrations relevant for efficacy ?

A discussion about accumulation, efflux and activity ...



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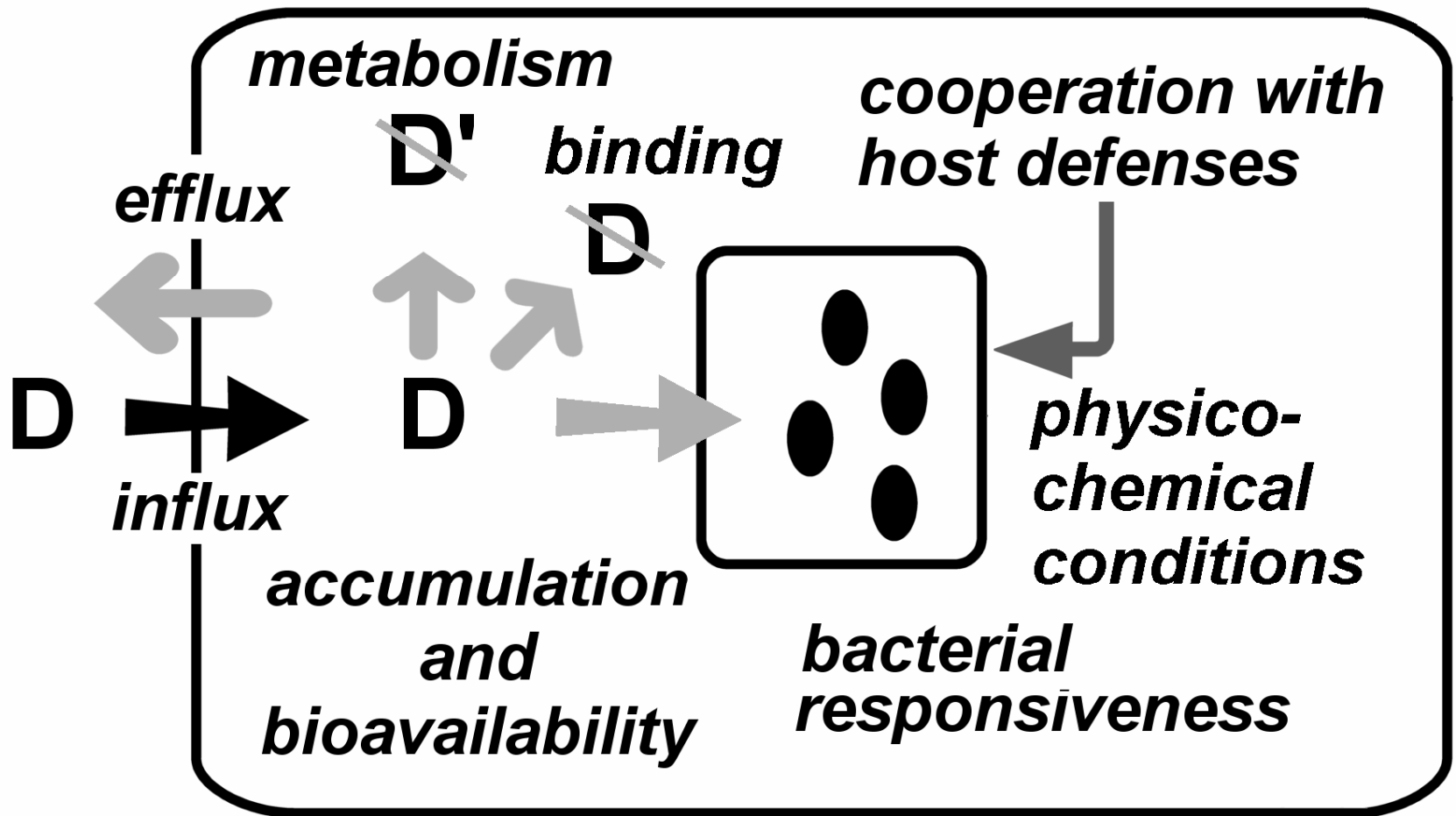
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Najaarsvergadering – Amsterdam – 16 november 2006

A simple figure ...



Carryn *et al.* Infect Dis Clin North Am. 2003 Sep;17(3):615-34.

First statements ...

- If a drug does not accumulate, it cannot be active ...

Quick answer:

this is correct if you mean "*it does not get in cells at all...*"

More elaborate answer:

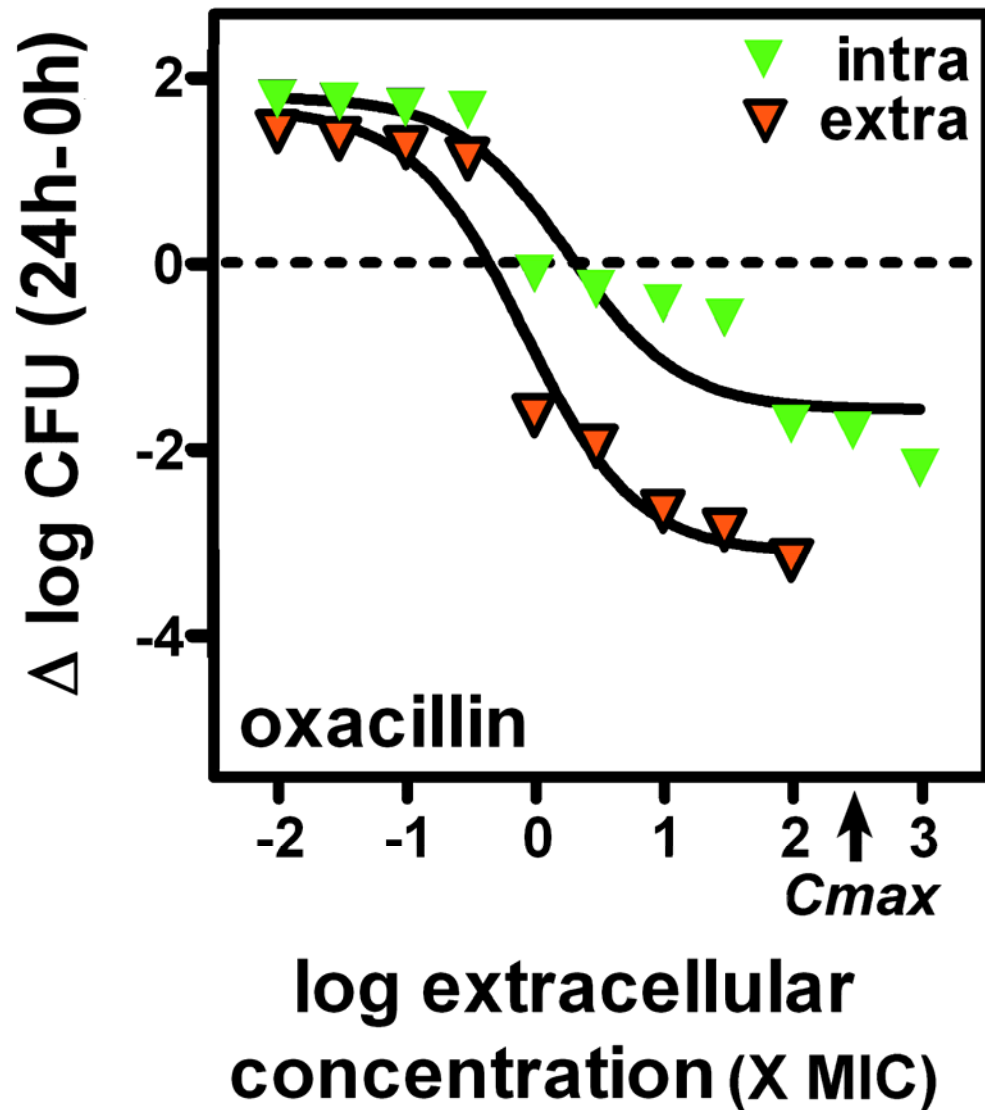
no "accumulation" does not mean that the drug is not present, and if present, it may be active if above the critical concentration for sufficient time

...

Experimental evidence:

β -lactams, known for "no accumulation" are active against intraphagocytic *L. monocytogenes* and *S. aureus* if their extracellular concentration is large enough... and if you let them enough time to act...

Intraphagocytic *S. aureus* and β -lactams

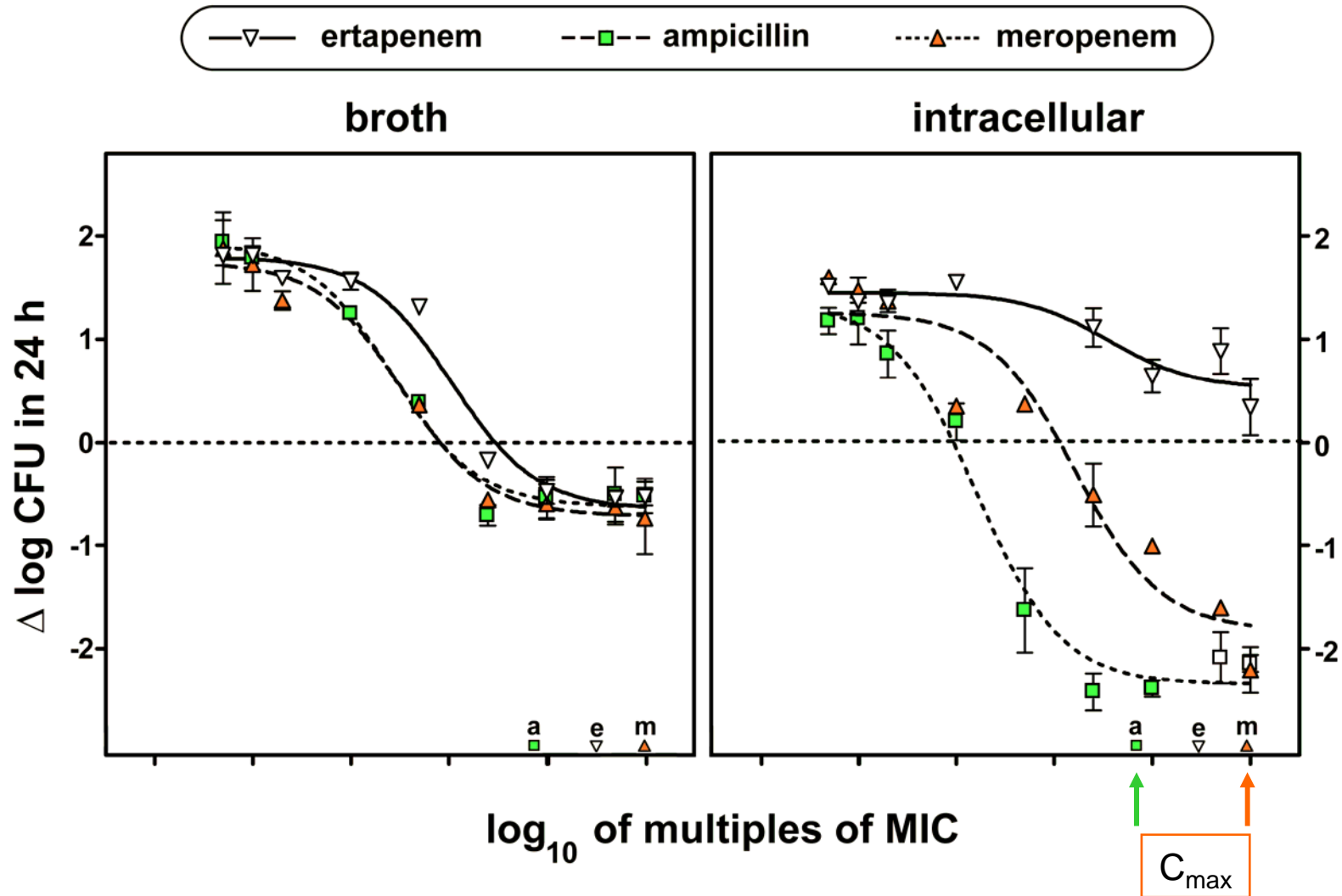


➤ 24 h model

➤ C_{\max} = 63 mg/L (total)

Barcia-Macay et al. M, Antimicrob Agents Chemother. 2006 Mar;50(3):841-51.

Intraphagocytic *L. monocytogenes* and β -lactams



Lemaire et al. J Antimicrob Chemother. 2005;55(6):897-904.

Observation ...

- The activity of β -lactams is larger than anticipated...

Quick answer:

Their concentration may simply be large enough

More elaborate answer:

The intracellular milieu may favor their activity ...

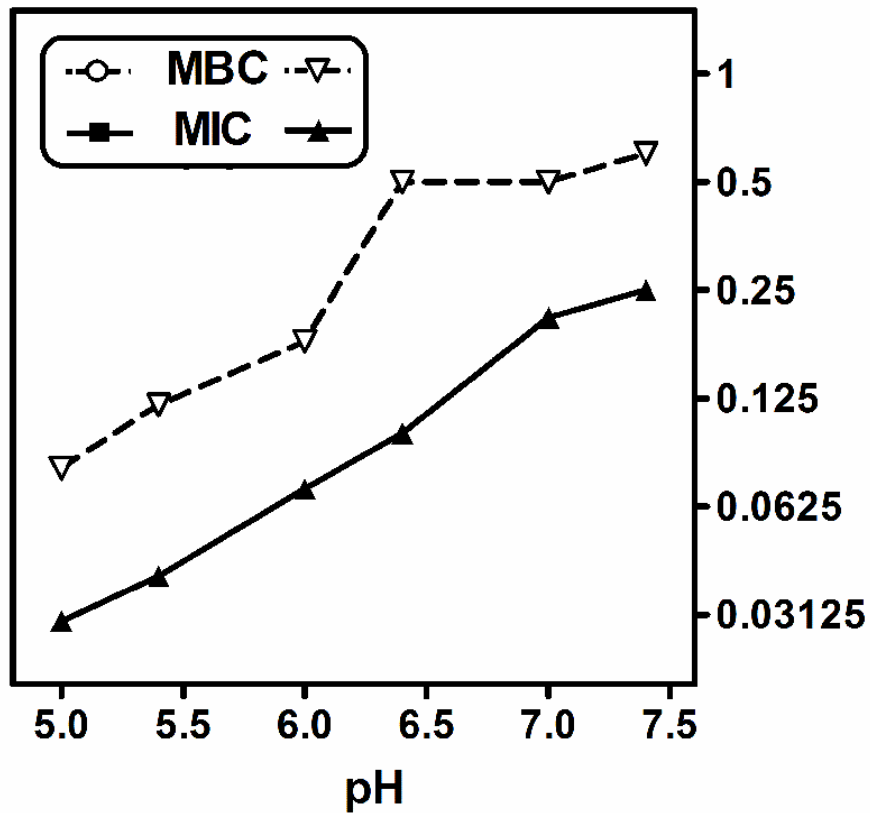
Experimental evidence for a potential explanation:

- Acid pH increases the activity of β -lactams against intraphagocytic *S. aureus*...
- We do not have it (yet) for *L.monocytogenes* ...

Acid pH favors the activity of β -lactams ...

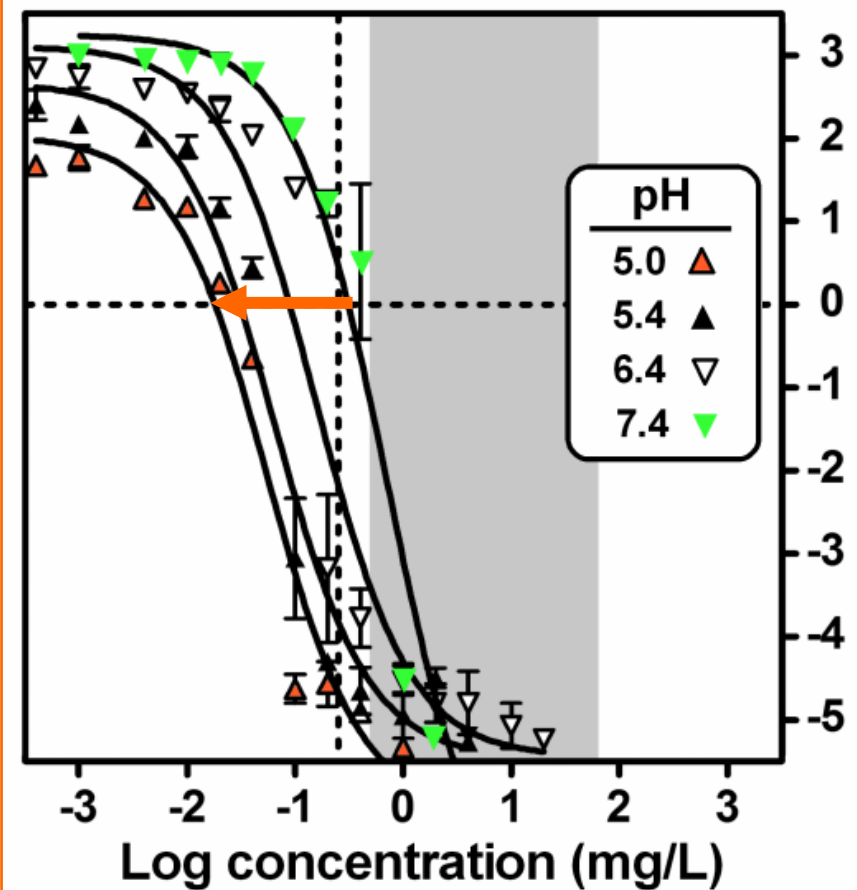
extracellular activity

oxacillin

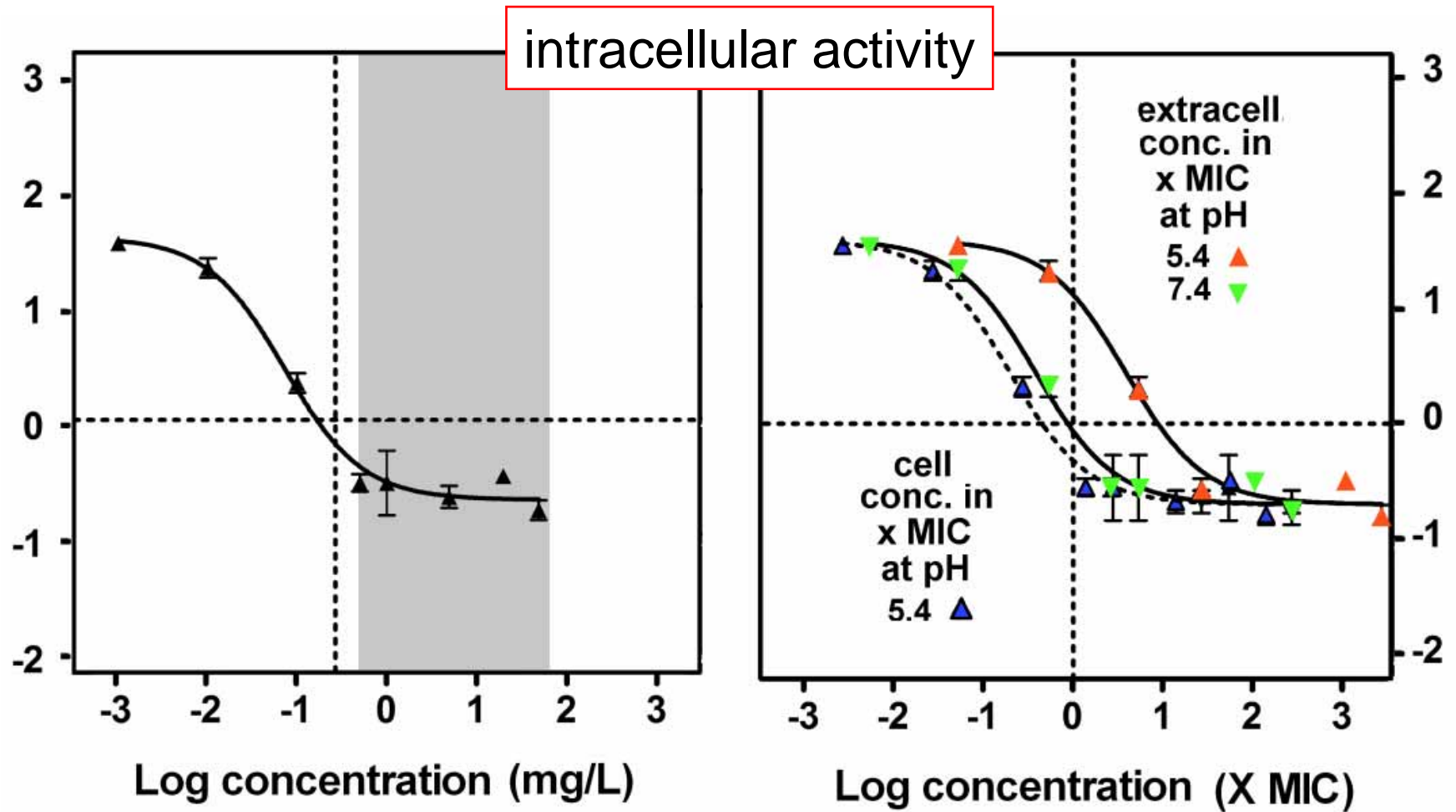


Baudoux et al., J. Antimicrob. Chemother., in press.

oxacillin



And acidity compensates for poor intracellular accumulation ...



Baudoux *et al.*, J. Antimicrob. Chemother., in press.

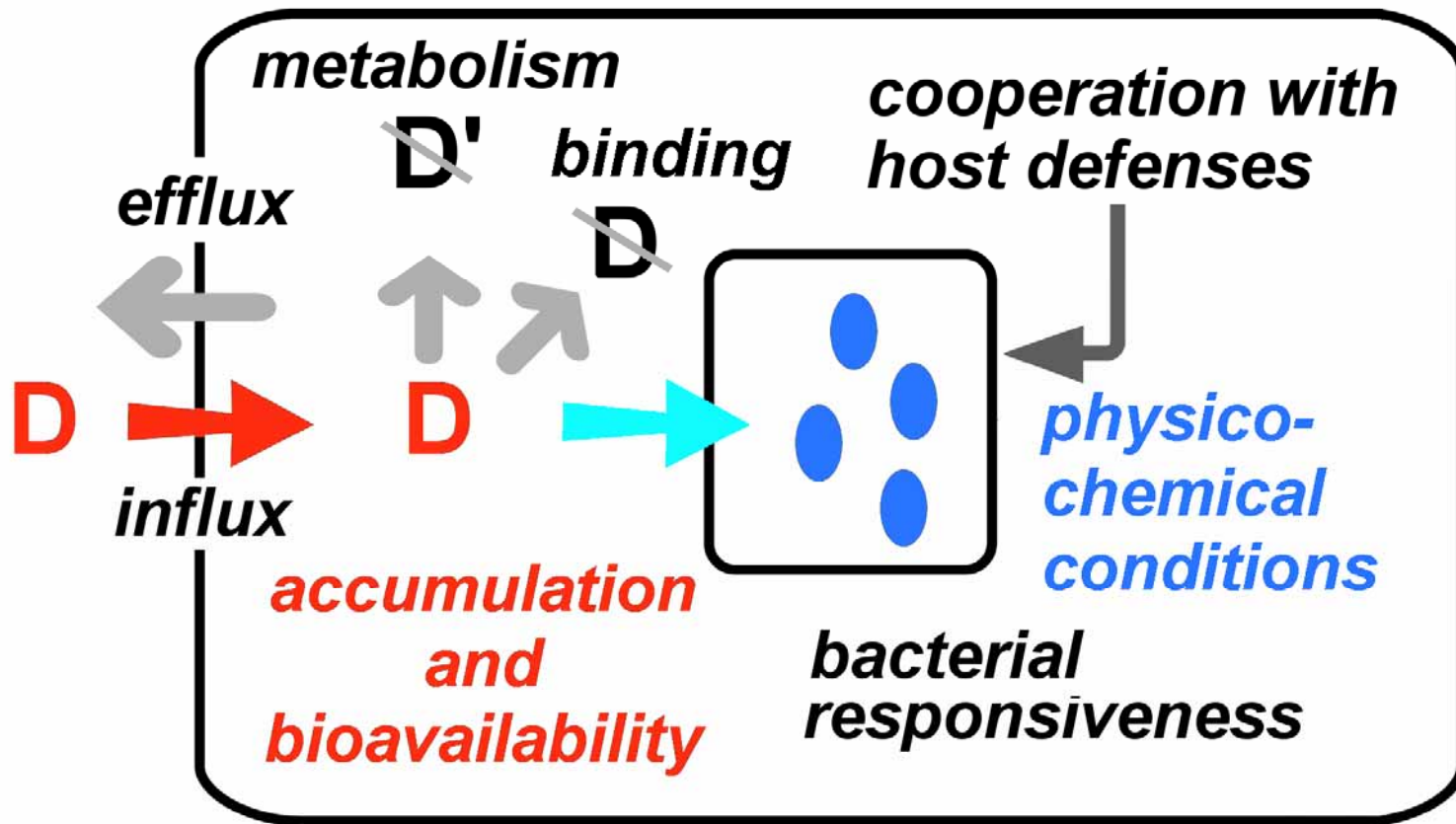
What about other antibiotics ...

- **Aminoglycosides** accumulate (slowly) in phagolysosomes but their activity is defeated by the acid pH for phagolysosomal organisms (*S. aureus...*) >< β -lactams... !

They are inactive against *L. monocytogenes* (not present in the cytosol ...)

- **Macrolides** accumulate ... but their activity is severely defeated by the acid pH ... and they are only bacteriostatic...
- **Quinolones** accumulate modestly, but their activity is maintained at acid pH ... and they have access to most intracellular compartments... Yet, their intracellular activity is at most similar to their extracellular activity ...

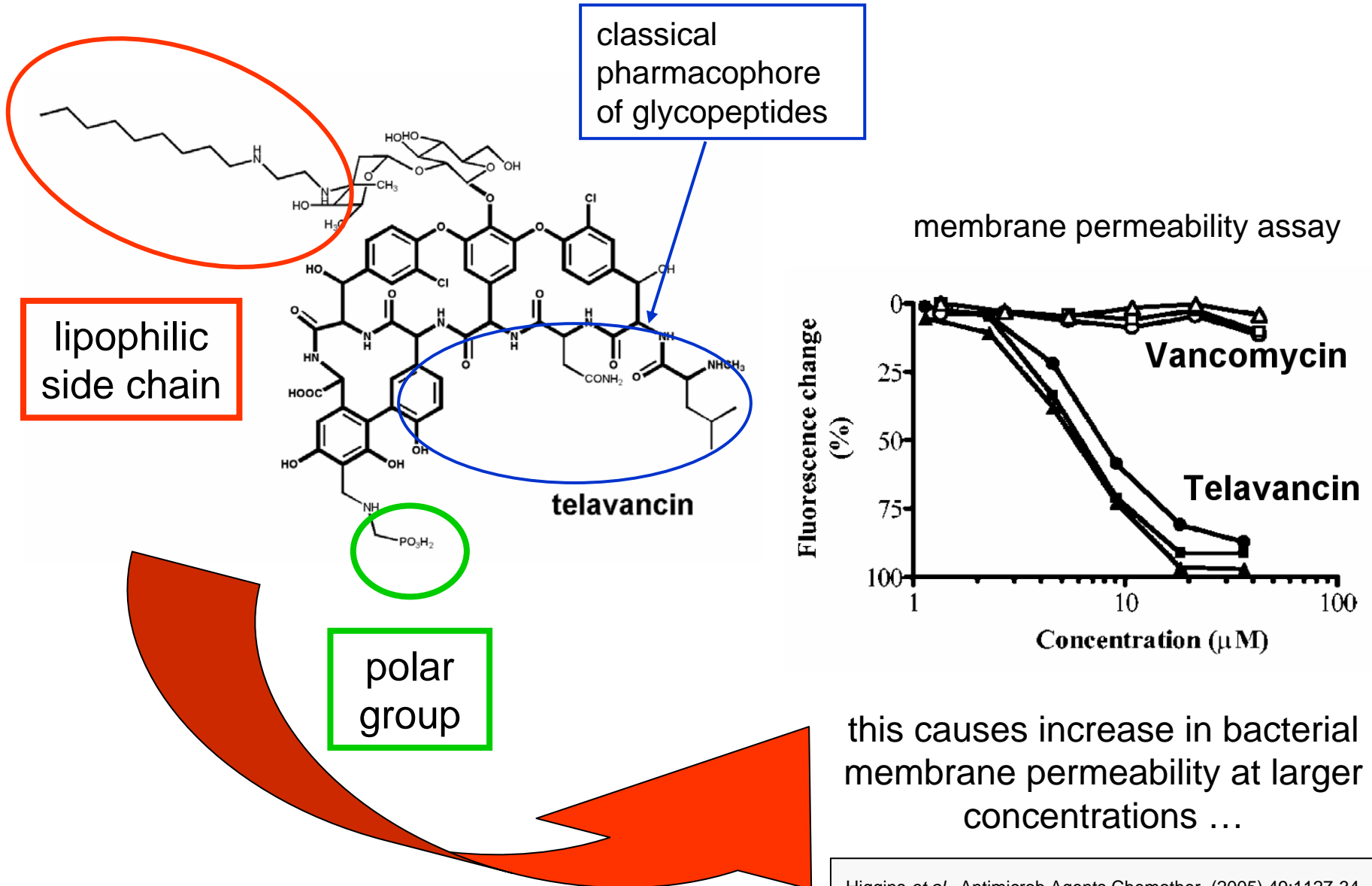
A not so simple figure ...



Activity = accumulation x bioavailability x favorable conditions

Carryn *et al.* Infect Dis Clin North Am. 2003 Sep;17(3):615-34.

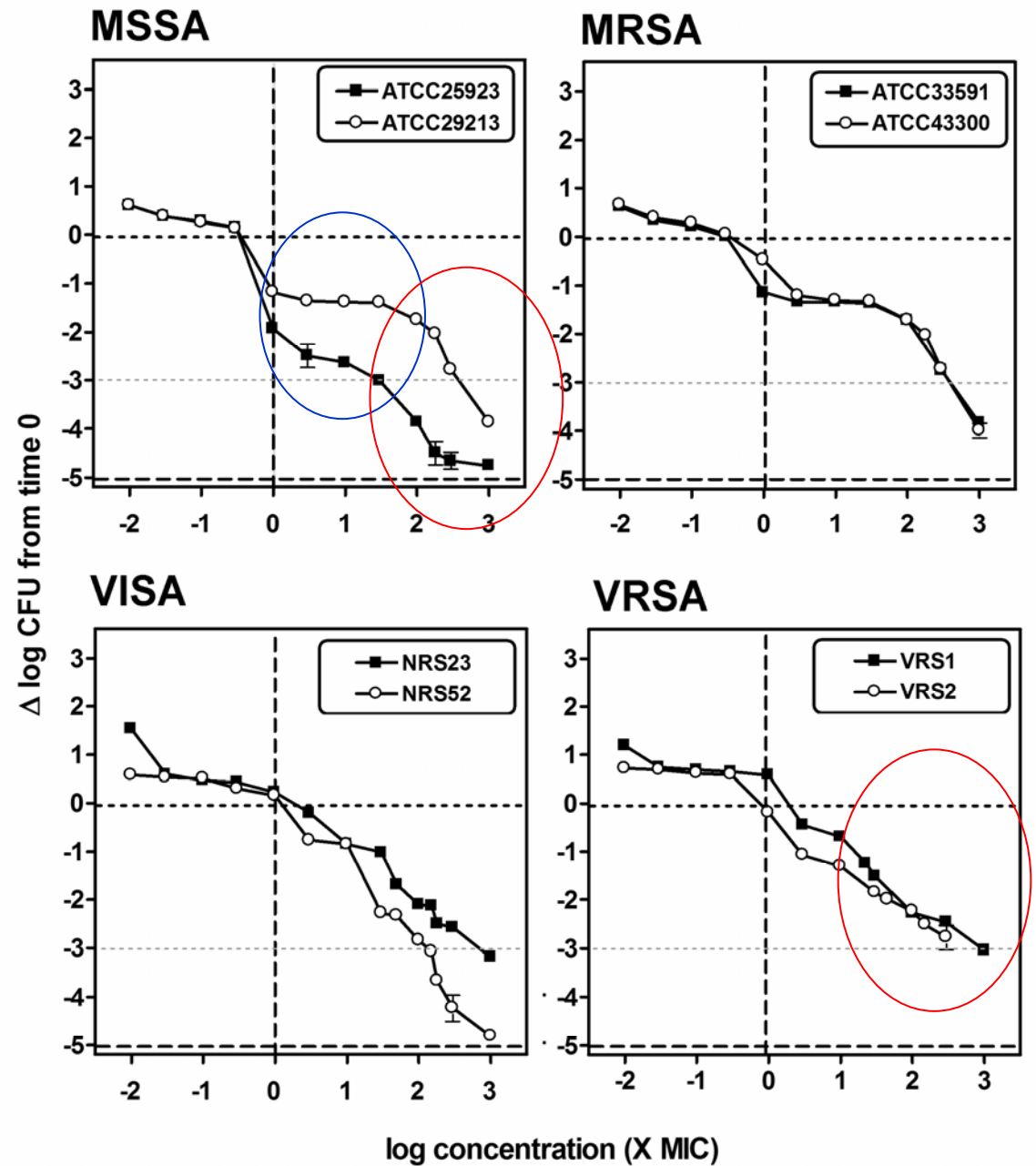
A few words about bacterial response: the case of telavancin and VRSA...



Higgins *et al.*, Antimicrob Agents Chemother. (2005) 49:1127-34

Televancin dual
mode of action ?

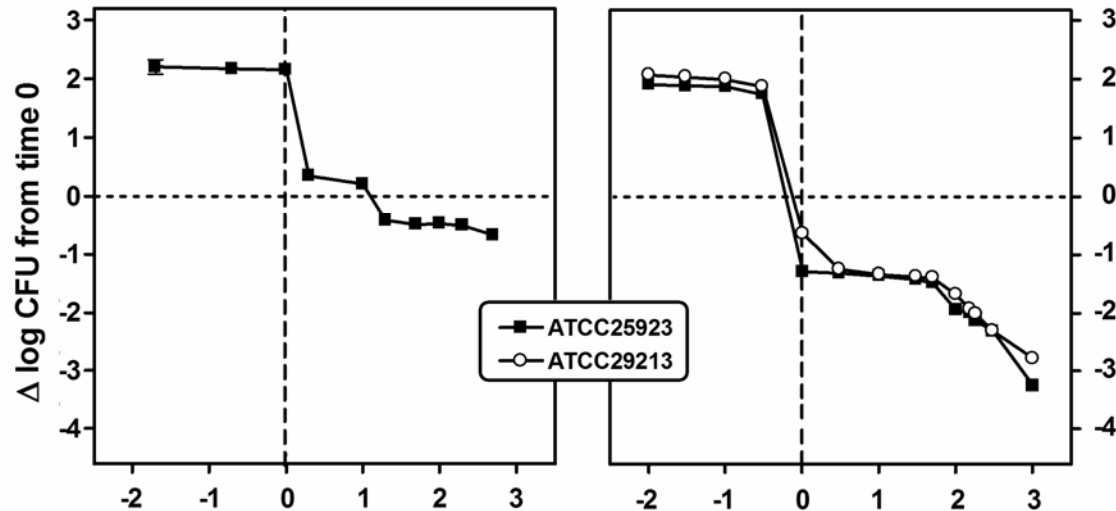
3 h kill curves
extracellular
bacteria



vancomycin

telavancin

MSSA



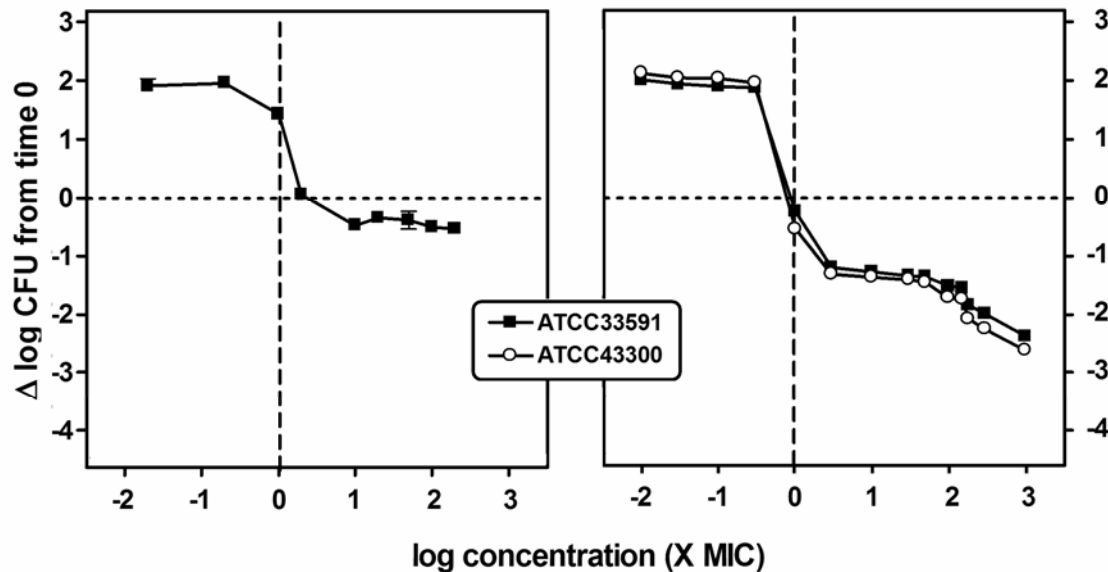
Intracellular activity
of telavancin
vs.

vancomycin:

➔ MSSA

➔ MRSA

MRSA



24h CFU ↘ at C_{max} :

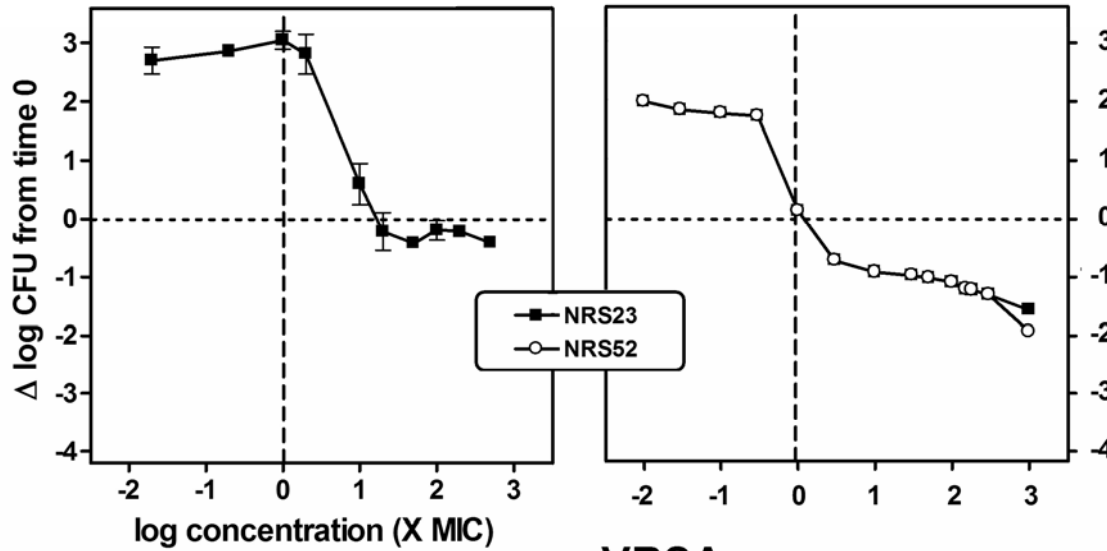
• vanco: ~ 0.5 log

• TLV: ~ 2 log

vancomycin

telavancin

VISA



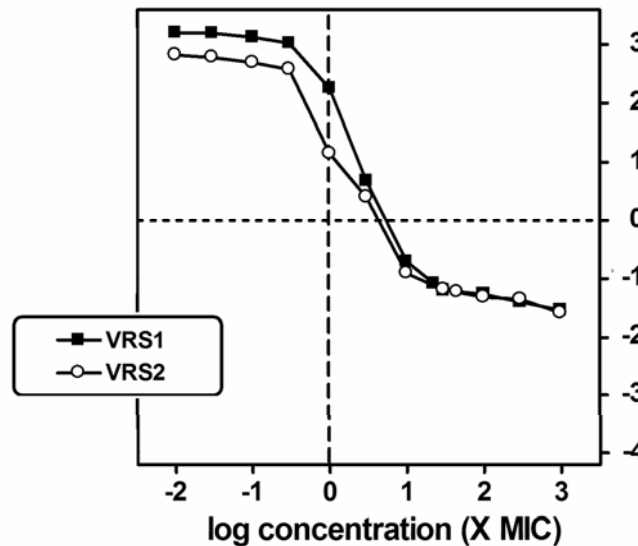
Intracellular activity
of telavancin
vs.

vancomycin:

➔ VISA

➔ VRSA

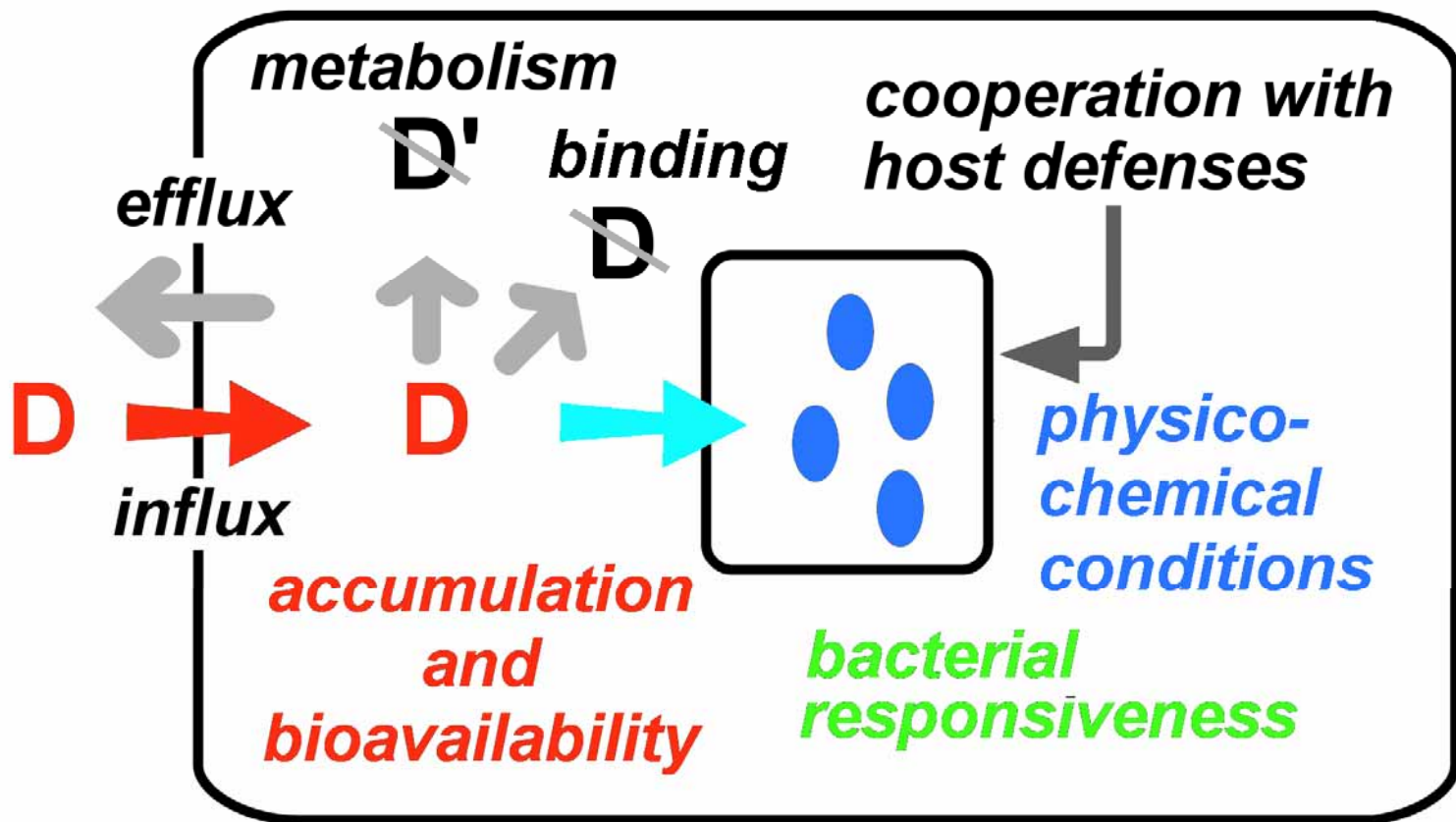
VRSA



24h CFU \searrow at C_{max} :

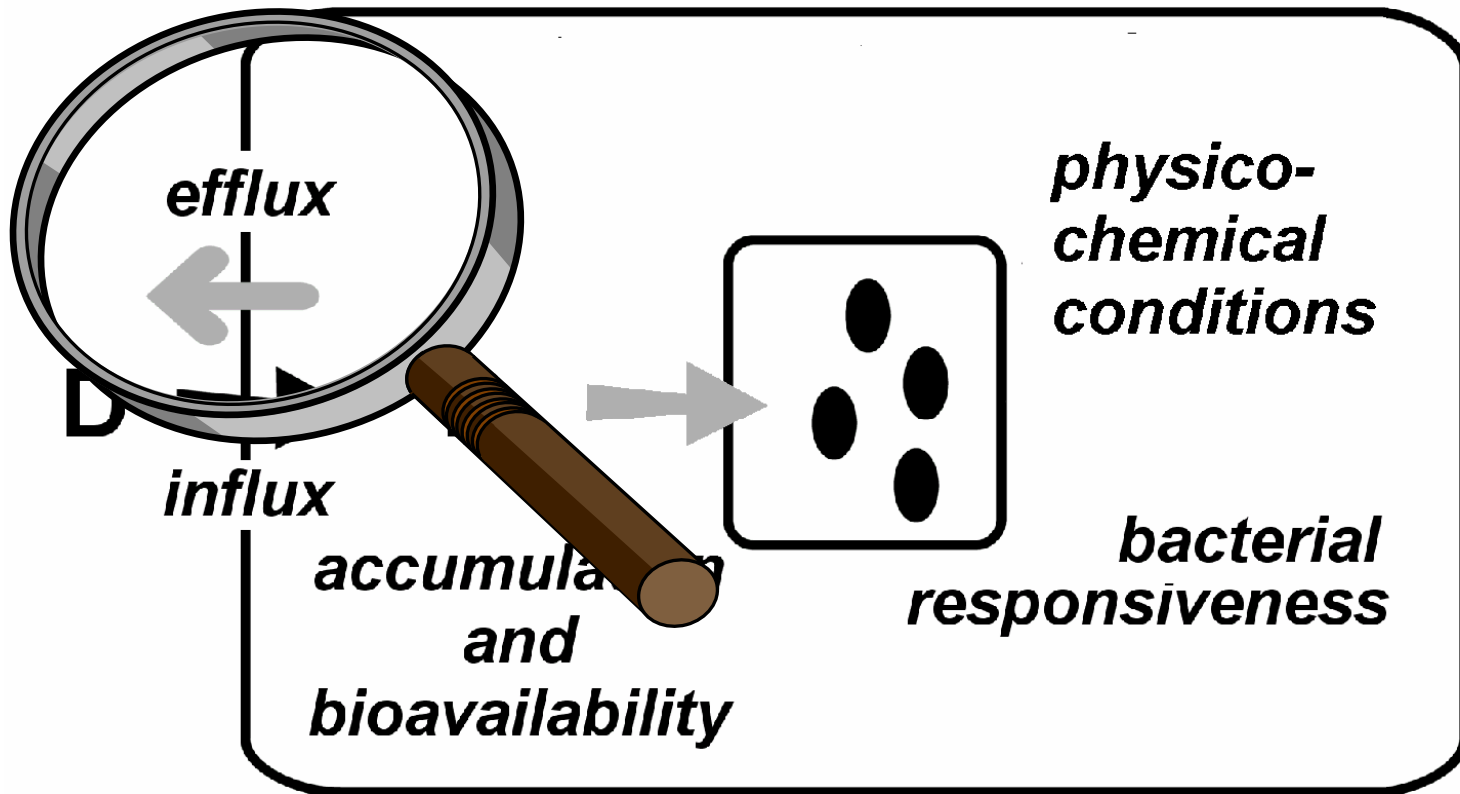
- vanco: static
- TLV: $\sim 1.2 \log$

The picture gets a bit more complex ...



Activity = accumulation x bioavailability x favorable conditions x bacterial responsiveness

Efflux from eucaryotic cells and intracellular activity



The story of the eucaryotic ABC transporters

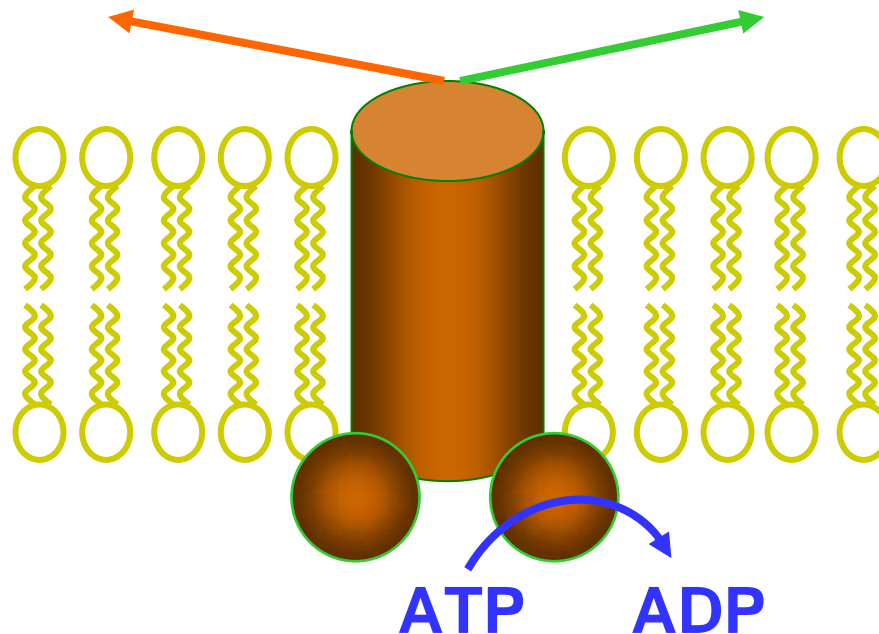


**cationic
amphiphiles**

**anionic
amphiphiles**

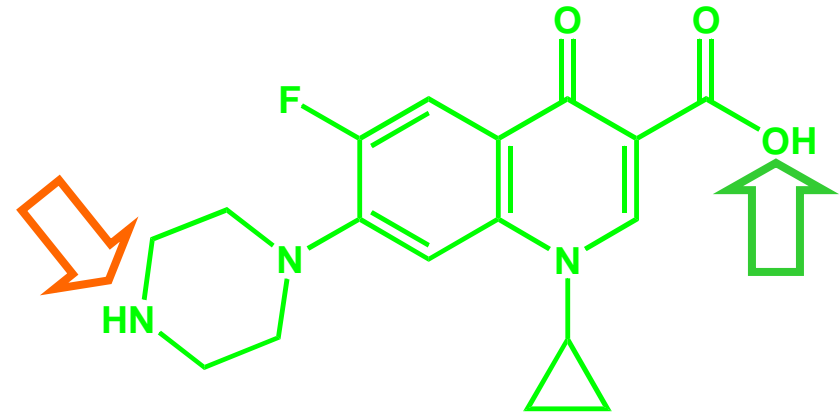
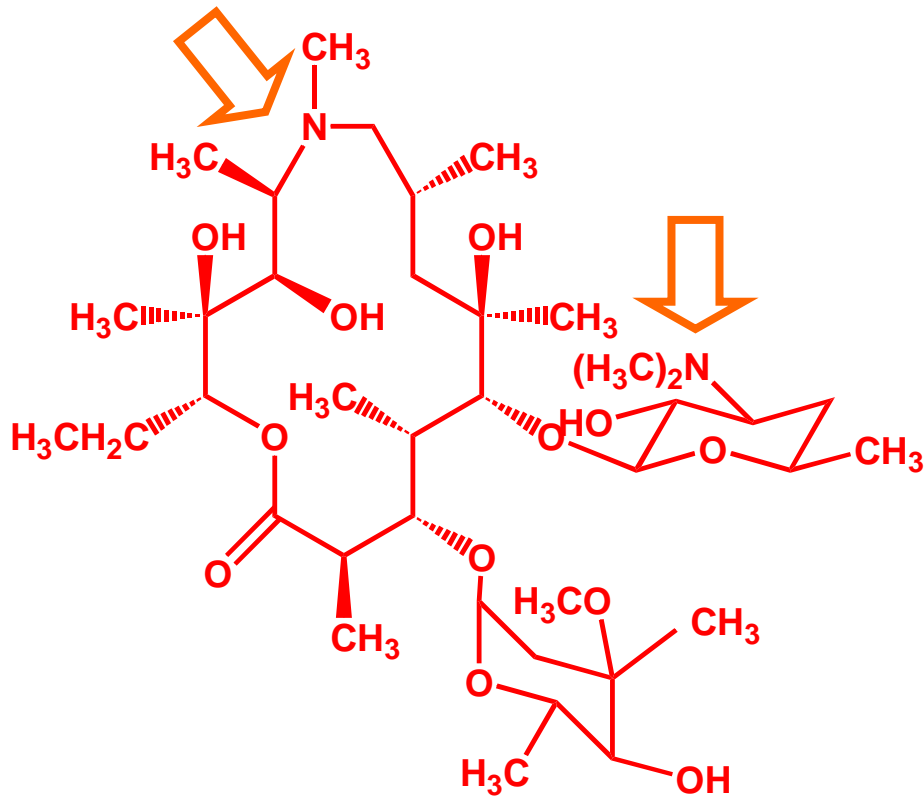
MDR-1 (P-glycoprotein)

MRP1-10



Antibiotics as substrates of efflux pumps

Azithromycin is cationic



Ciprofloxacin is zwitterionic

How to inhibit ABC transporters ?

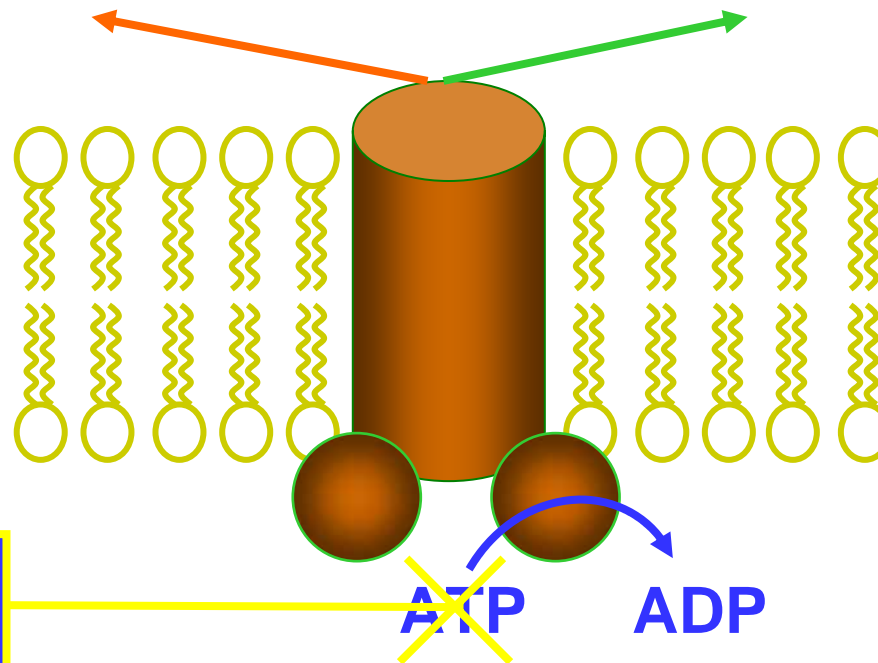


**cationic
amphiphiles**

**anionic
amphiphiles**

MDR-1 (P-glycoprotein)

MRP1-10

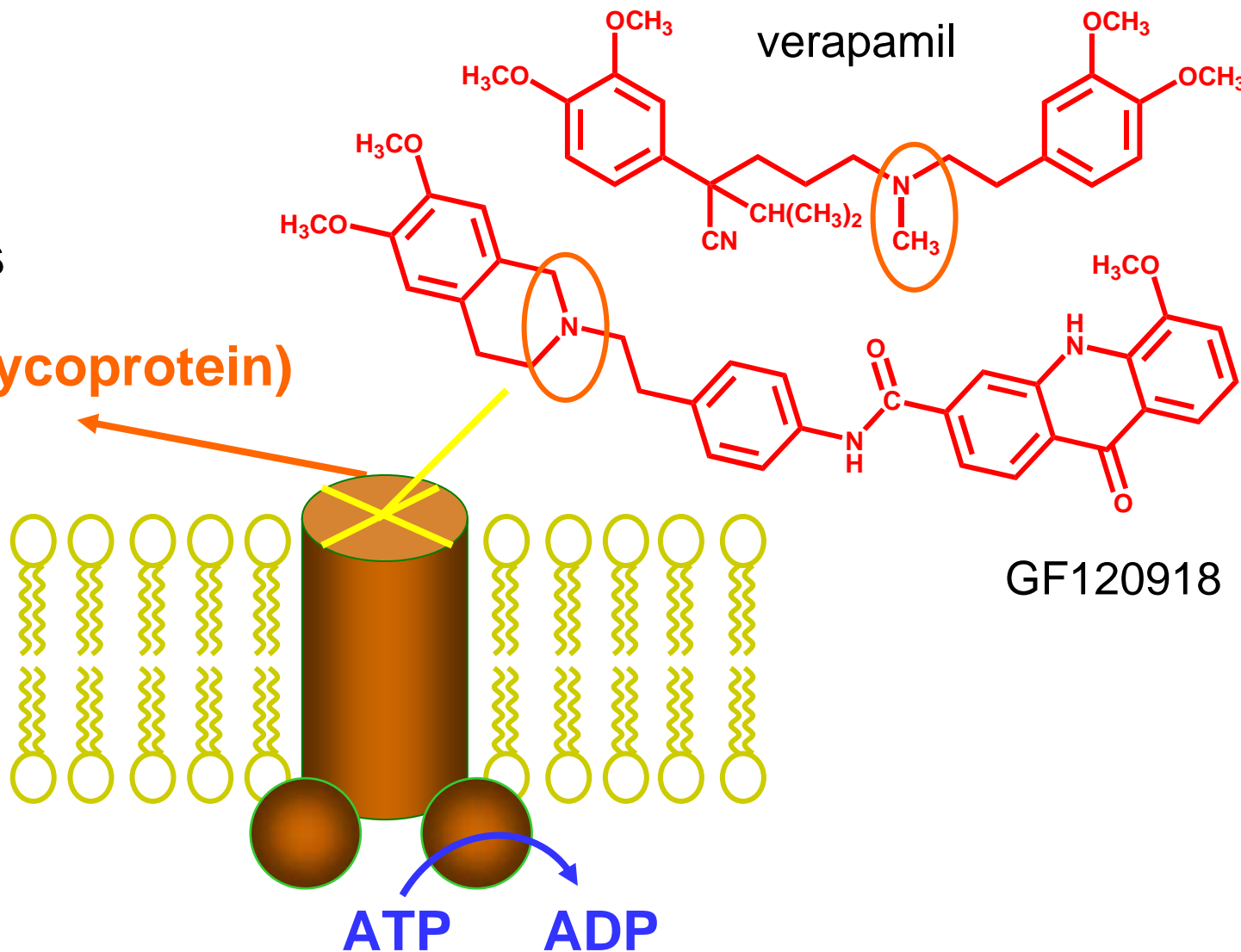


deoxyglucose
 NaN_3

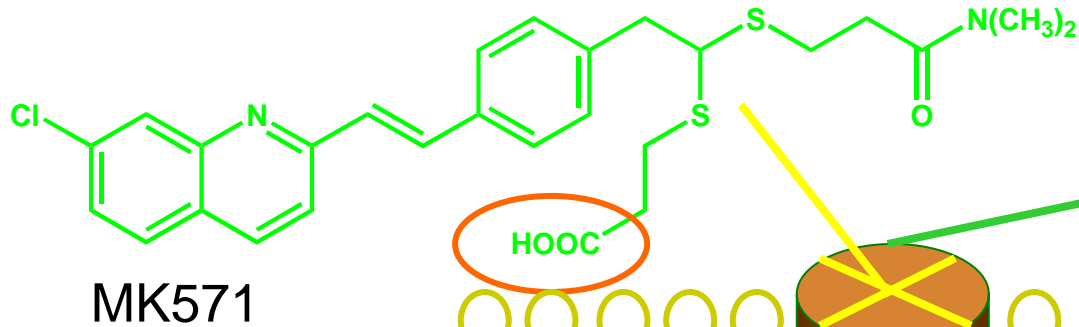
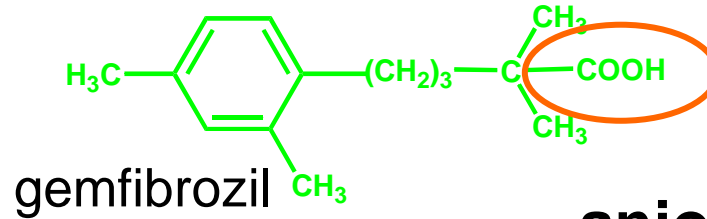
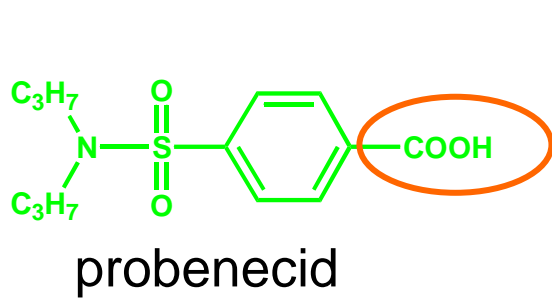
How to inhibit ABC transporters ?

**cationic
amphiphiles**

MDR-1 (P-glycoprotein)

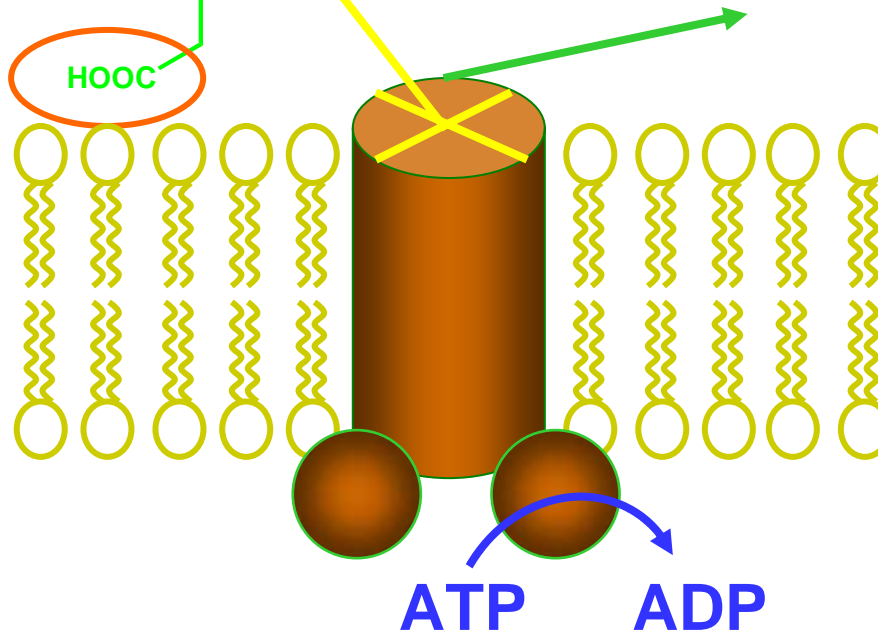


How to inhibit ABC transporters ?



**anionic
amphiphiles**

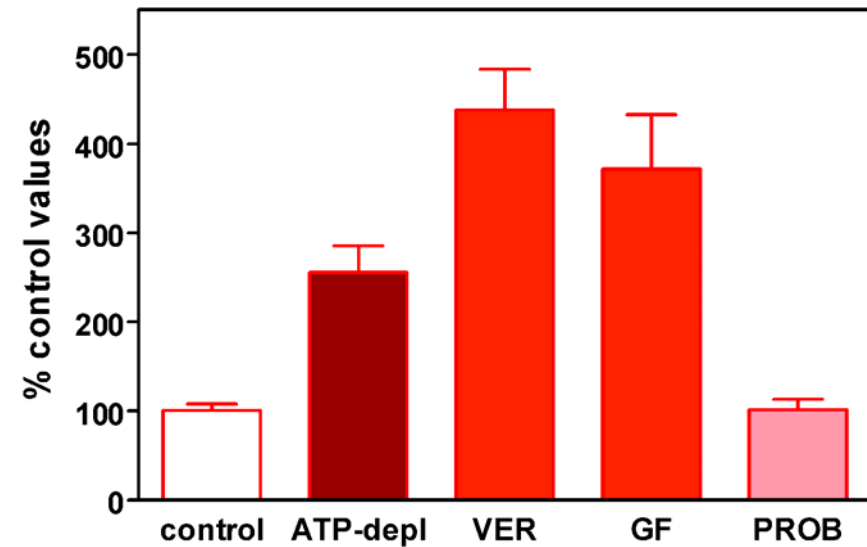
MRP1-10



Differential recognition by MDR pumps

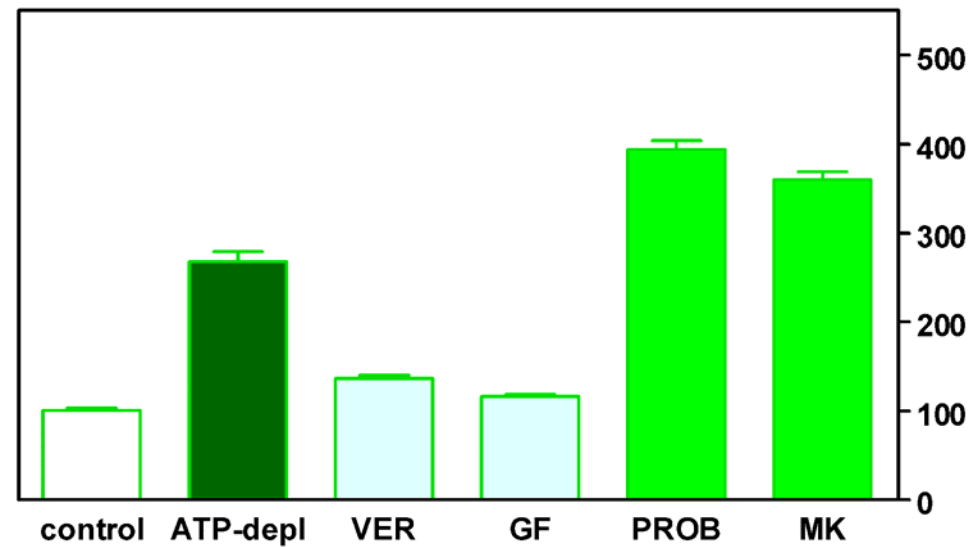
Influence of ATP-depletion and pump inhibitors on accumulation at equilibrium

azithromycin



**azithromycin
&
P-glycoprotein**

ciprofloxacin



**ciprofloxacin
&
MRP**

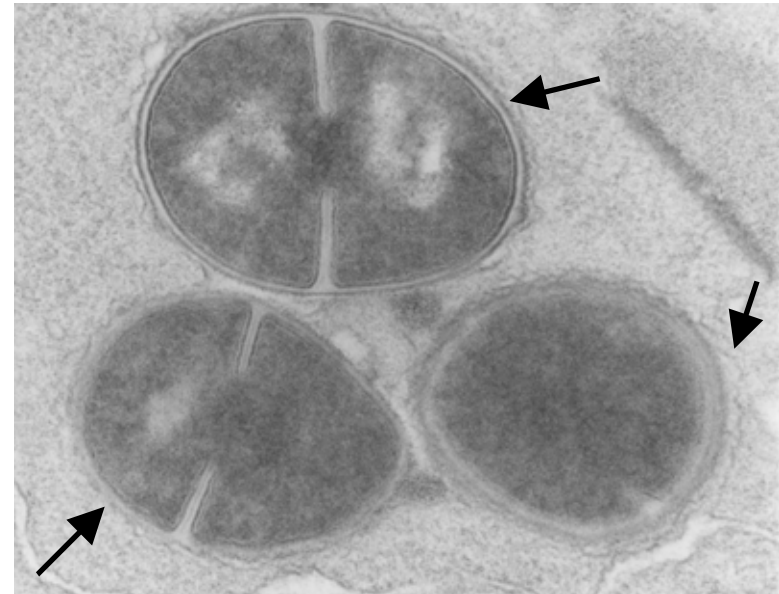
Models of intracellular infection

L. monocytogenes



cytosol

S. aureus

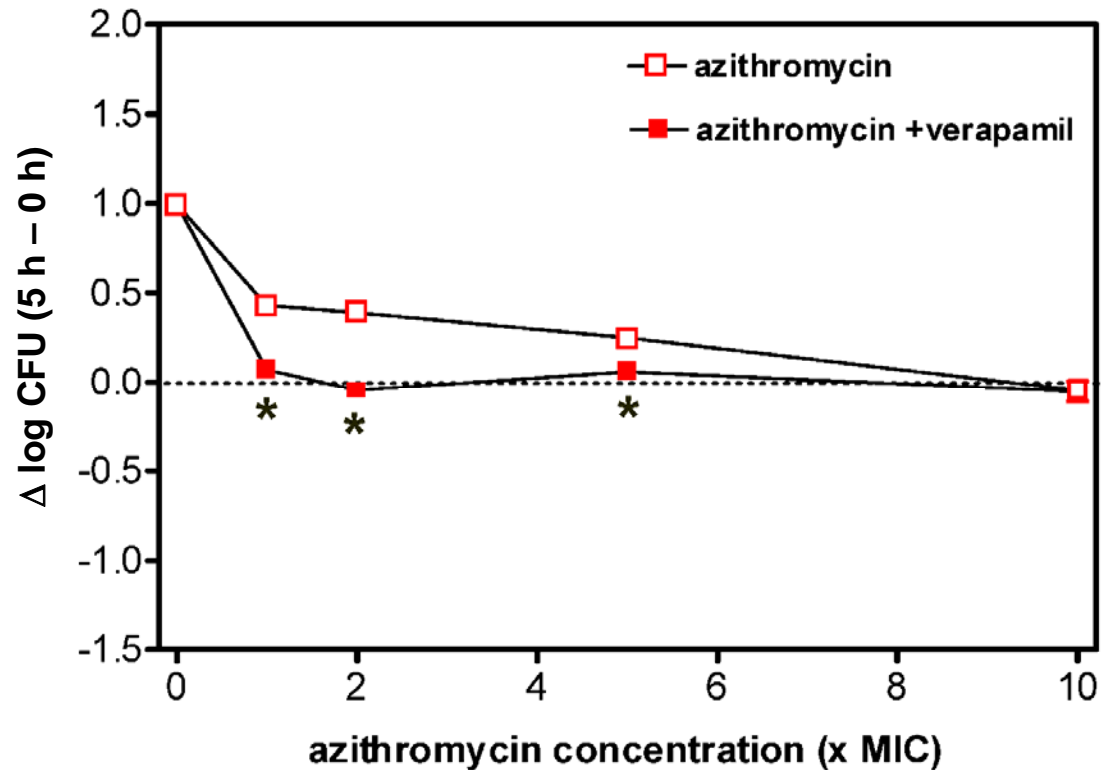
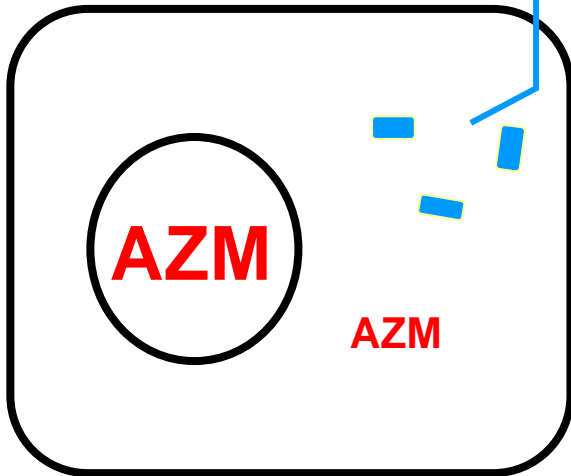


phagolysosomes

Influence of pump inhibitors on intracellular activity

azithromycin and *L. monocytogenes*

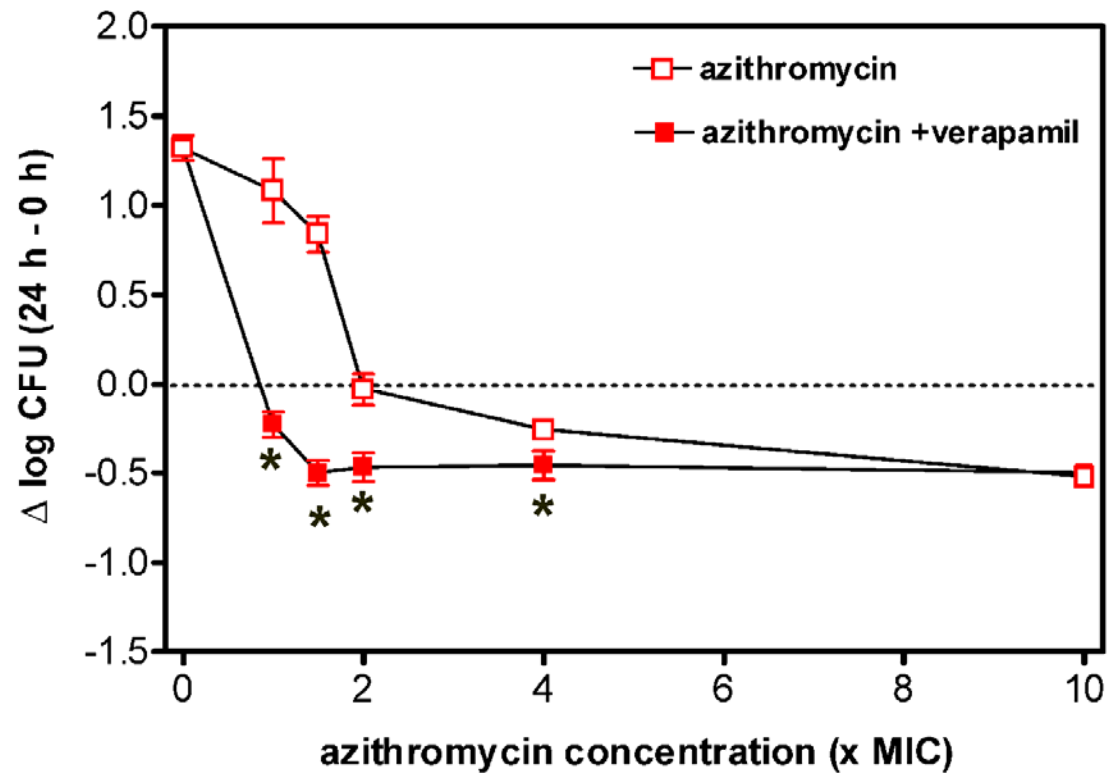
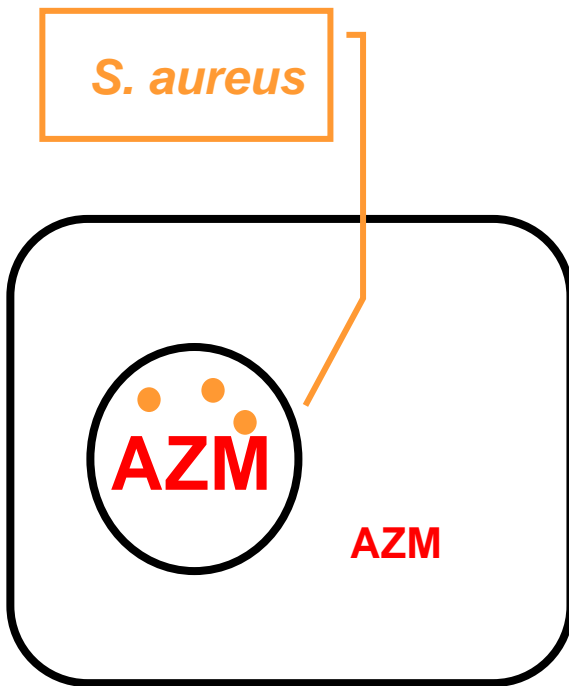
L. monocytogenes



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

Influence of pump inhibitors on intracellular activity

azithromycin and *S. aureus*



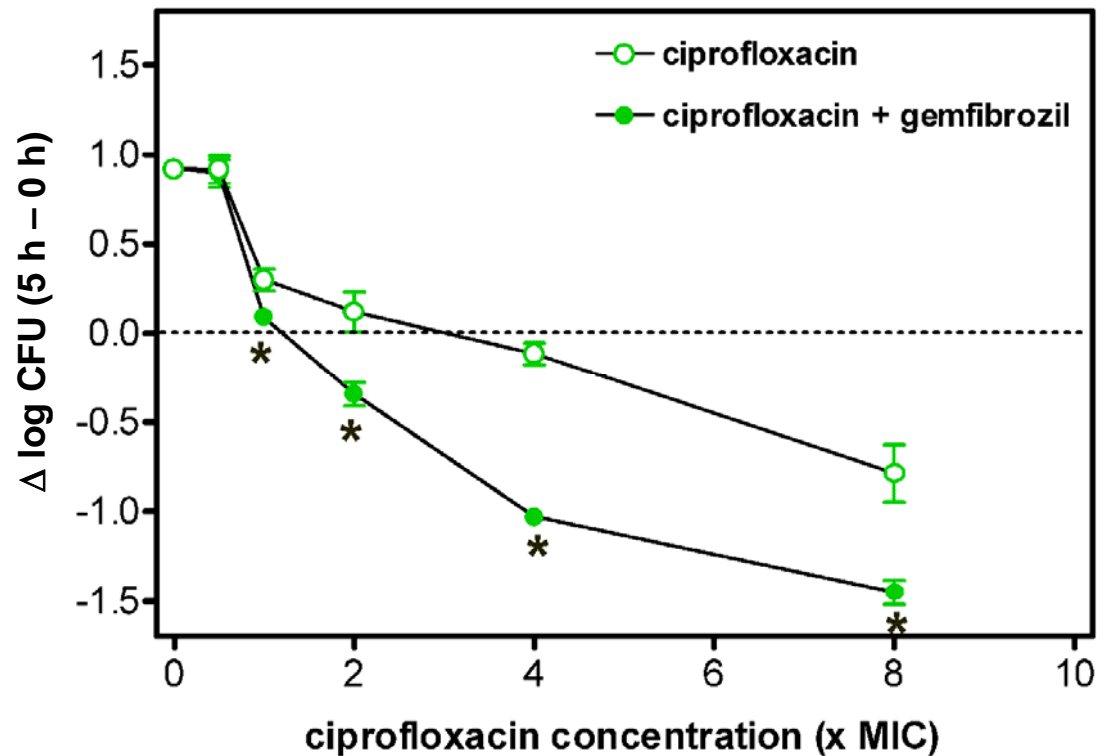
Seral *et al* (2003) JAC 51:1167-73

Influence of pump inhibitors on intracellular activity

ciprofloxacin and *L. monocytogenes*

L. monocytogenes

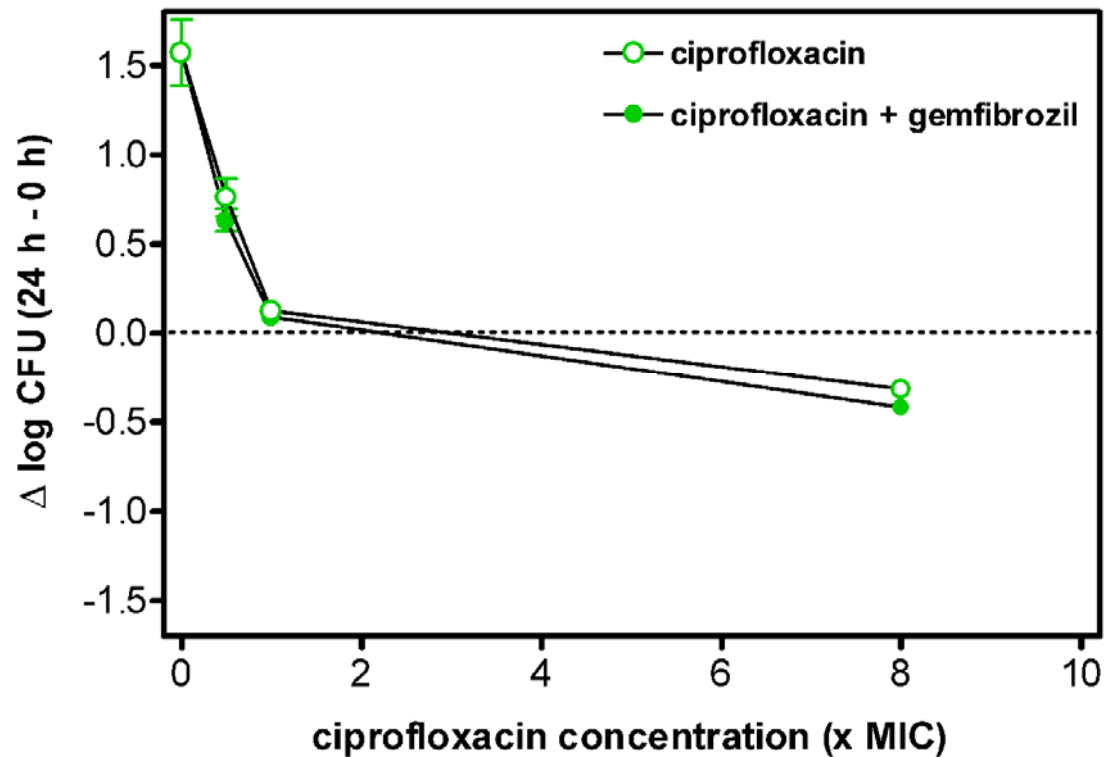
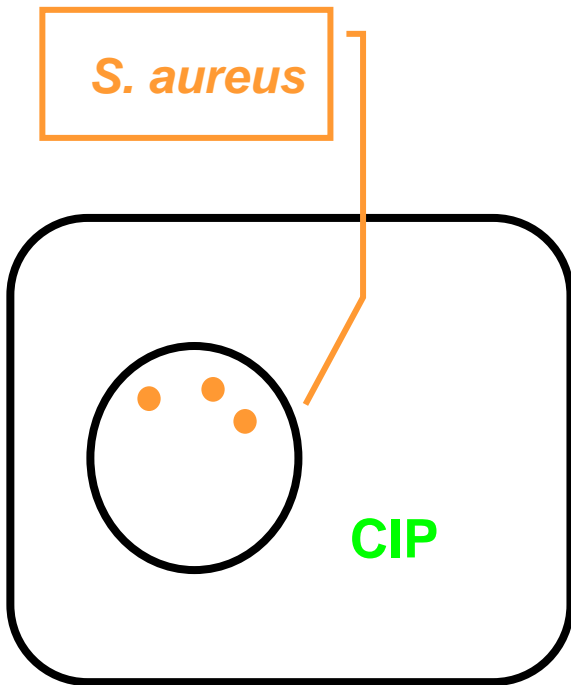
CIP



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

Influence of pump inhibitors on intracellular activity

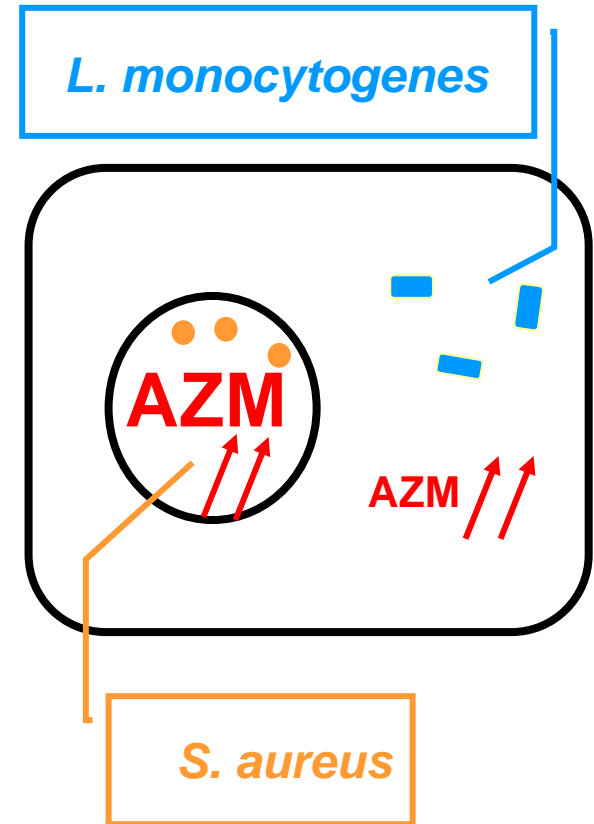
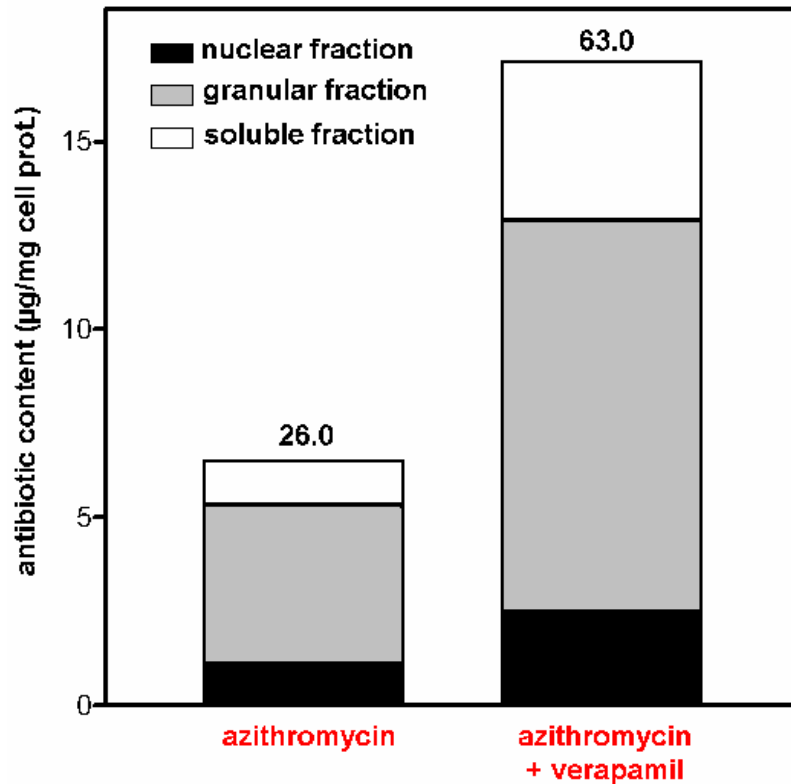
ciprofloxacin and *S. aureus*



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

Influence of pump inhibitors on antibiotic distribution

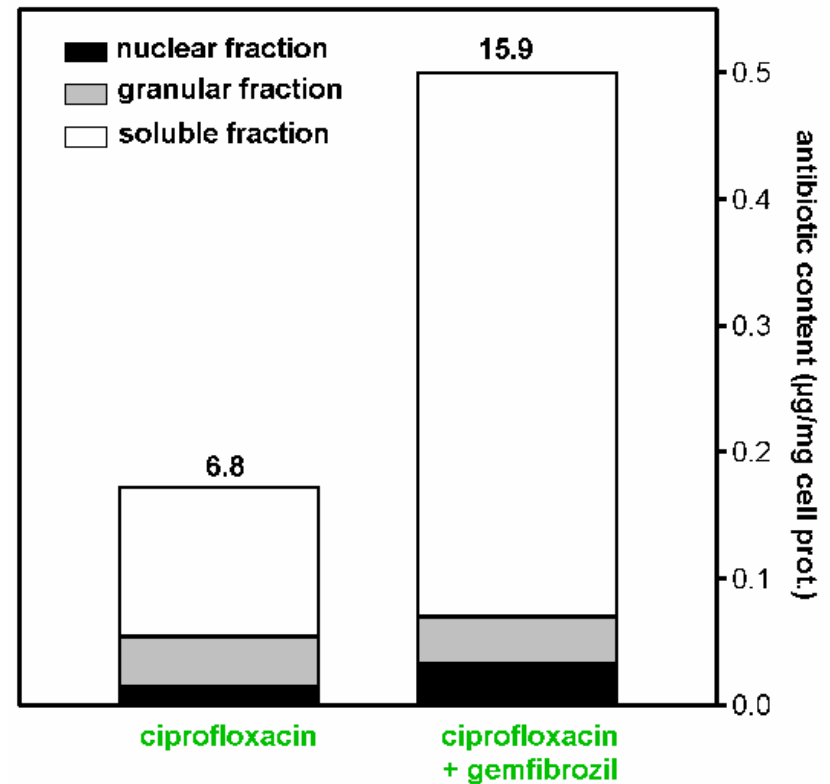
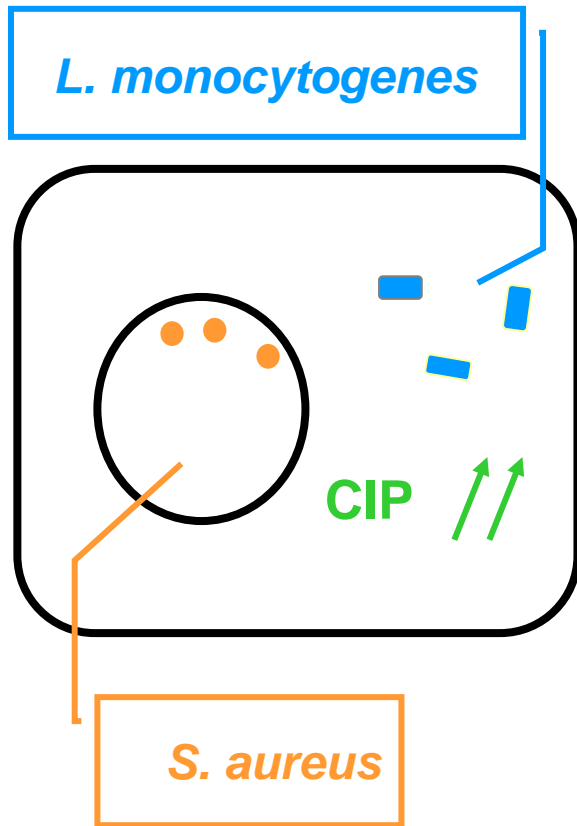
verapamil enhances azithromycin concentration in cytosol and vacuoles



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

Influence of pump inhibitors on antibiotic distribution

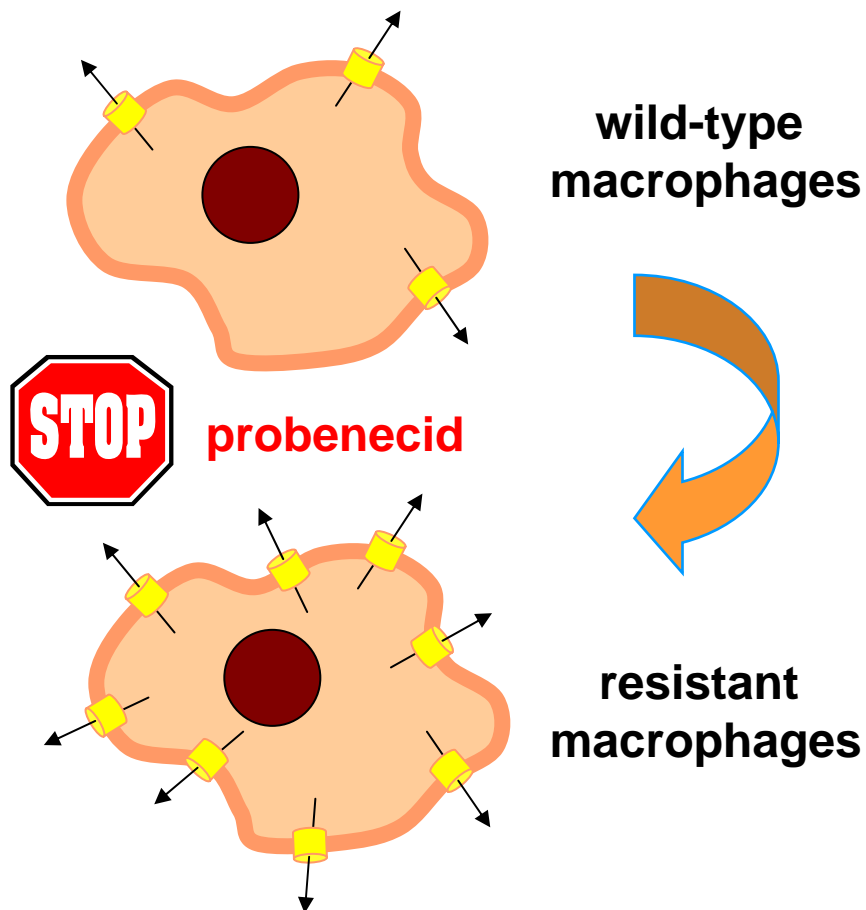
gemfibrozil increases ciprofloxacin cytosolic content **ONLY**



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

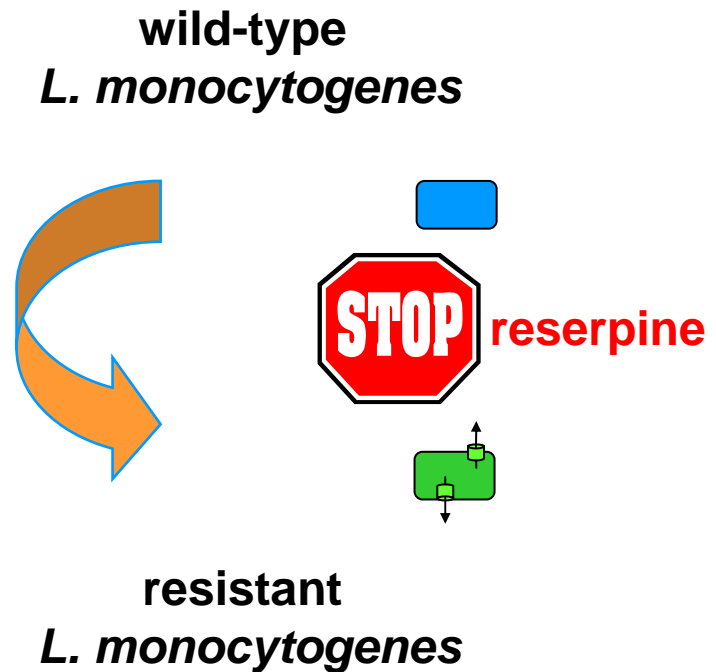
Over-expression of efflux pumps as mechanism of « resistance »

in eucaryotic cells ...



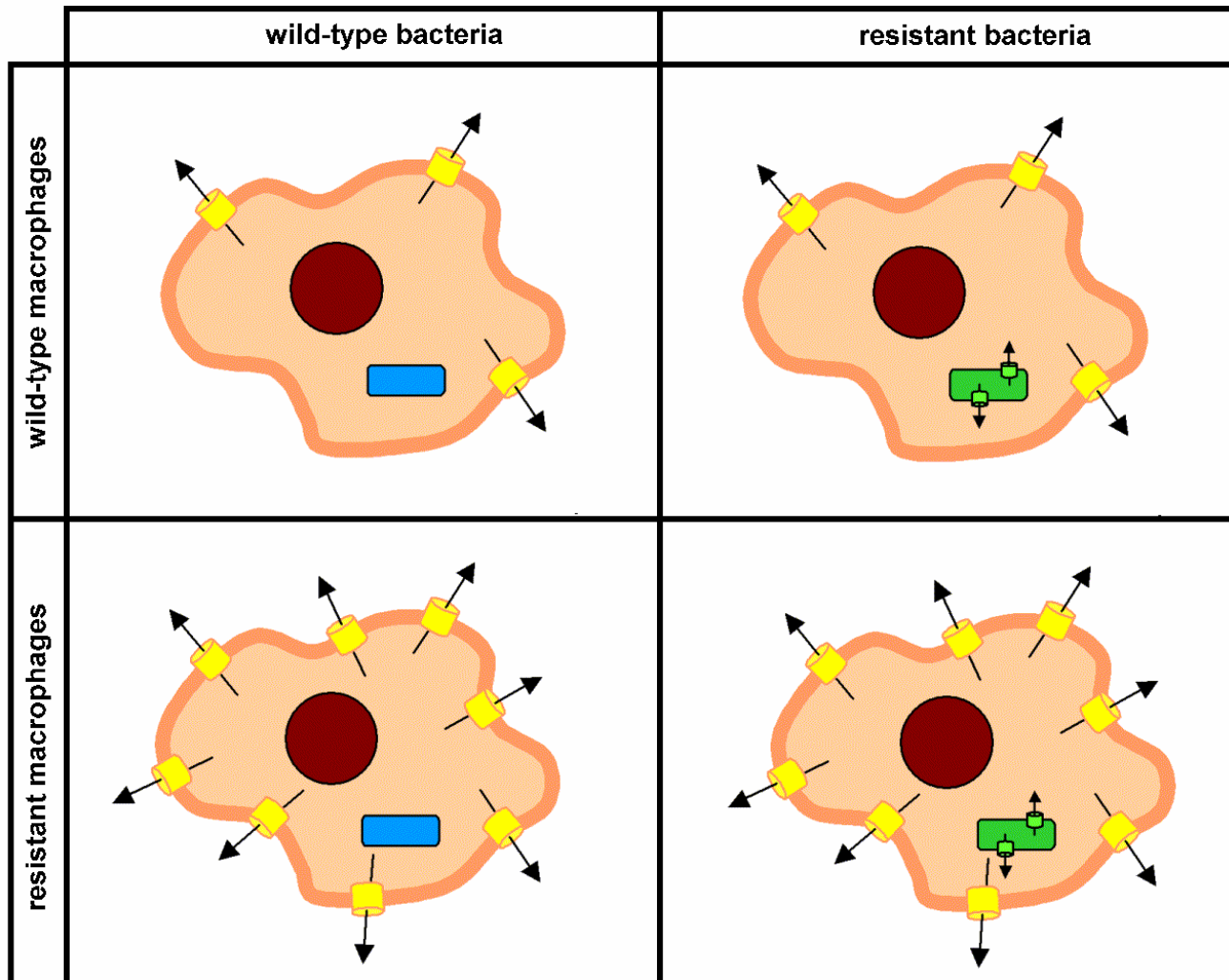
Michot *et al*, AAC (2006) 50:1689-95

in prokaryotic cells ...



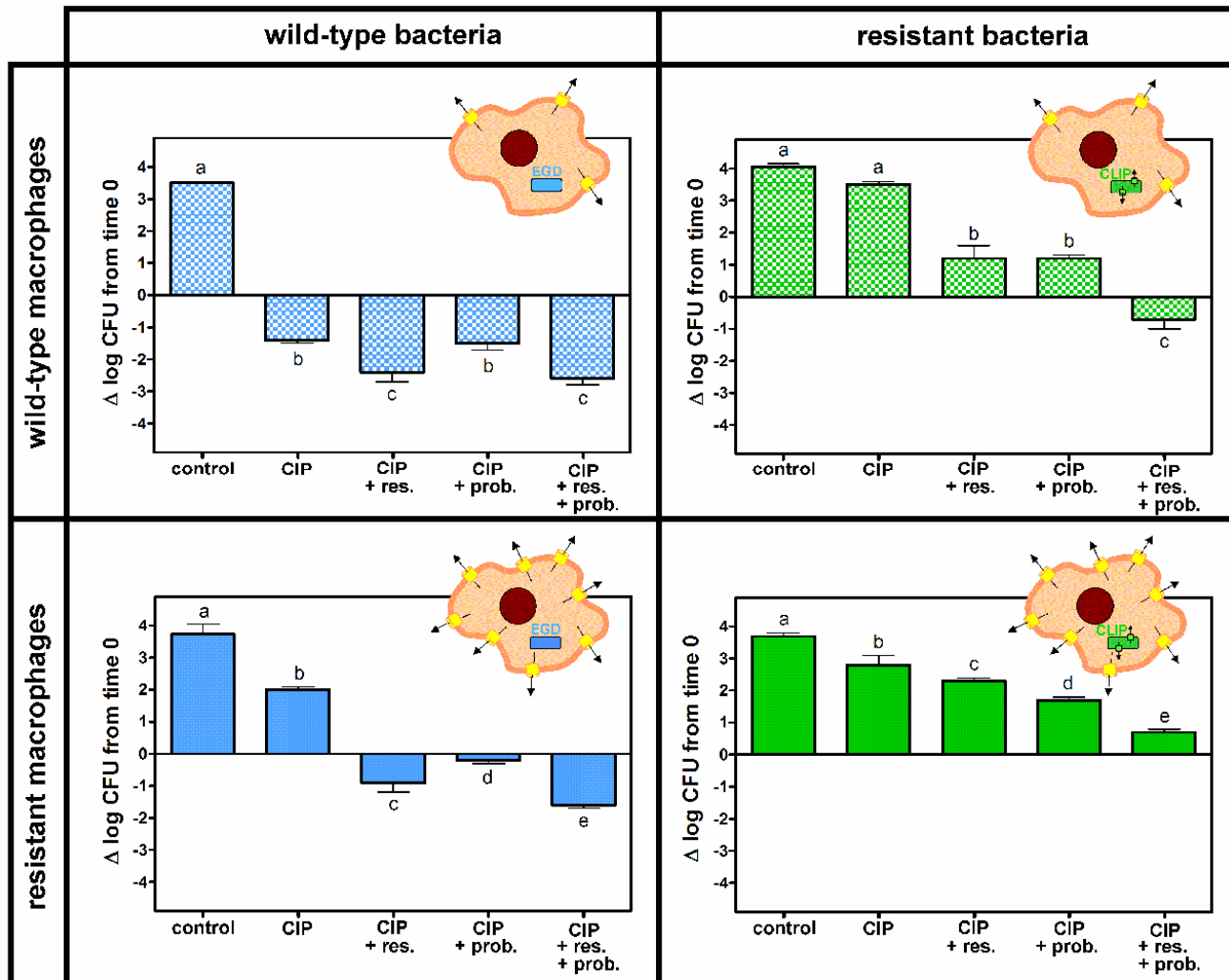
Godreuil *et al*, AAC (2003) 47:704-8

Cooperation between procaryotic and eucaryotic efflux pumps

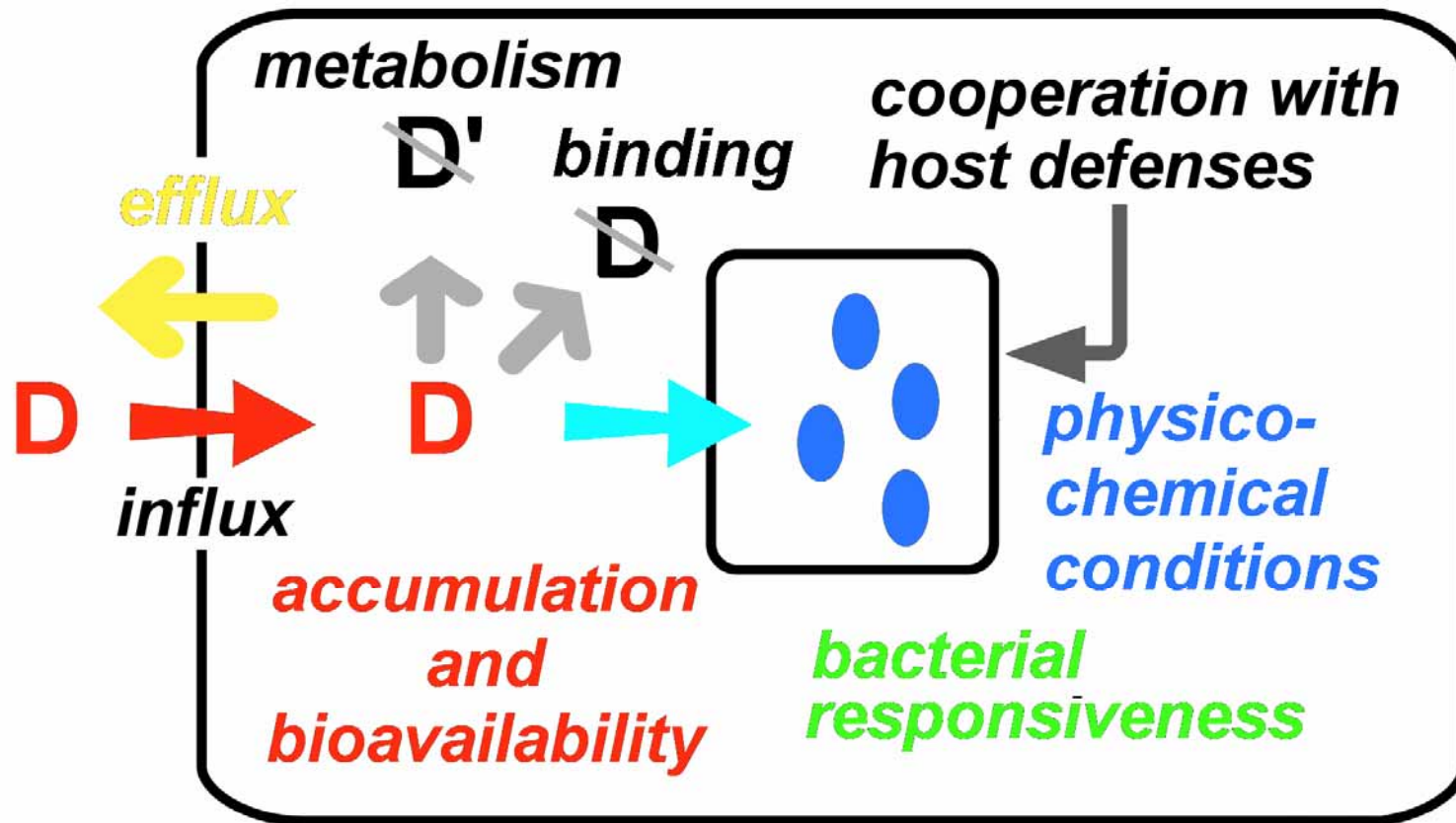


Cooperation between procaryotic and eucaryotic efflux pumps

ciprofloxacin



To conclude ...



$$\text{Activity} = \frac{\text{accumulation} \times \text{bioavailability} \times \text{favorable conditions} \times \text{bacterial responsiveness}}{\text{efflux}}$$