Deciphering the activity of antibiotics against intracellular *Staphylococcus aureus* with the help of PK/PD (pharmacokinetics/pharmacodynamics).

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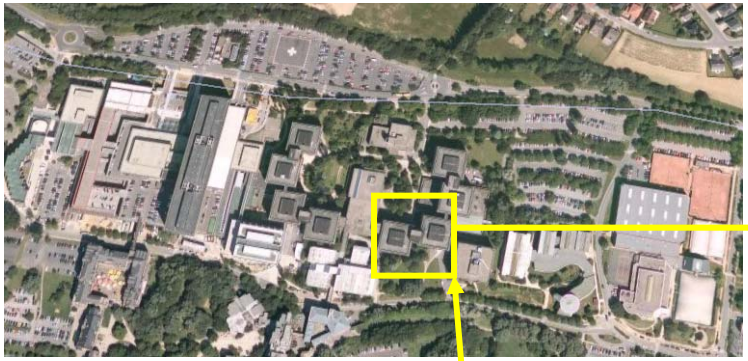
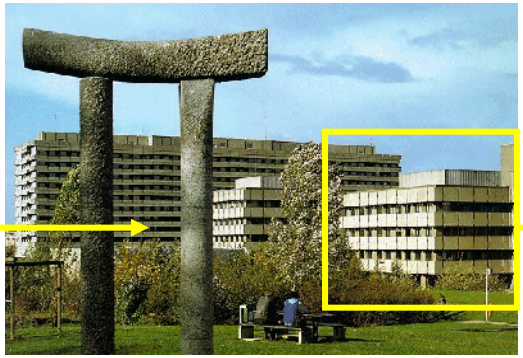
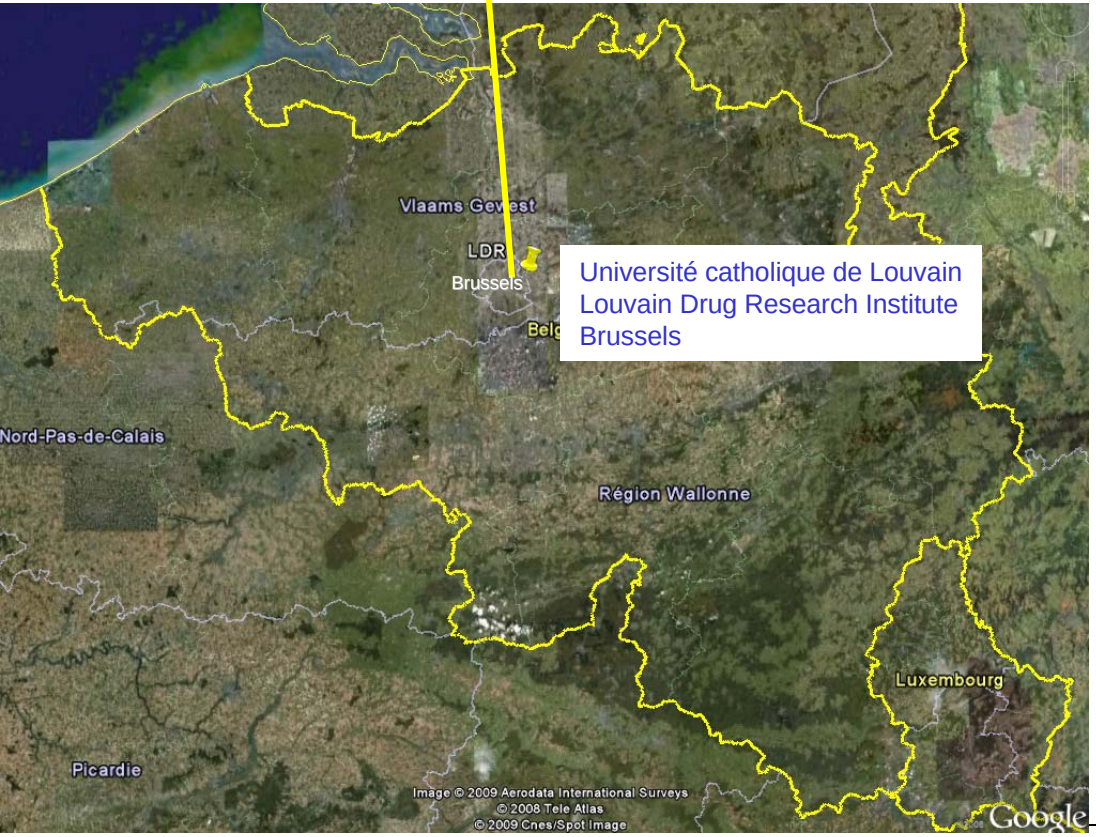
From where do I come from ?







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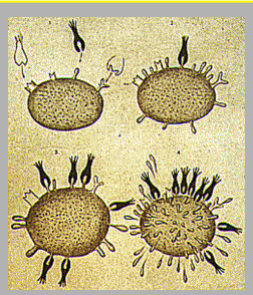


"corpora non agunt nisi fixata"

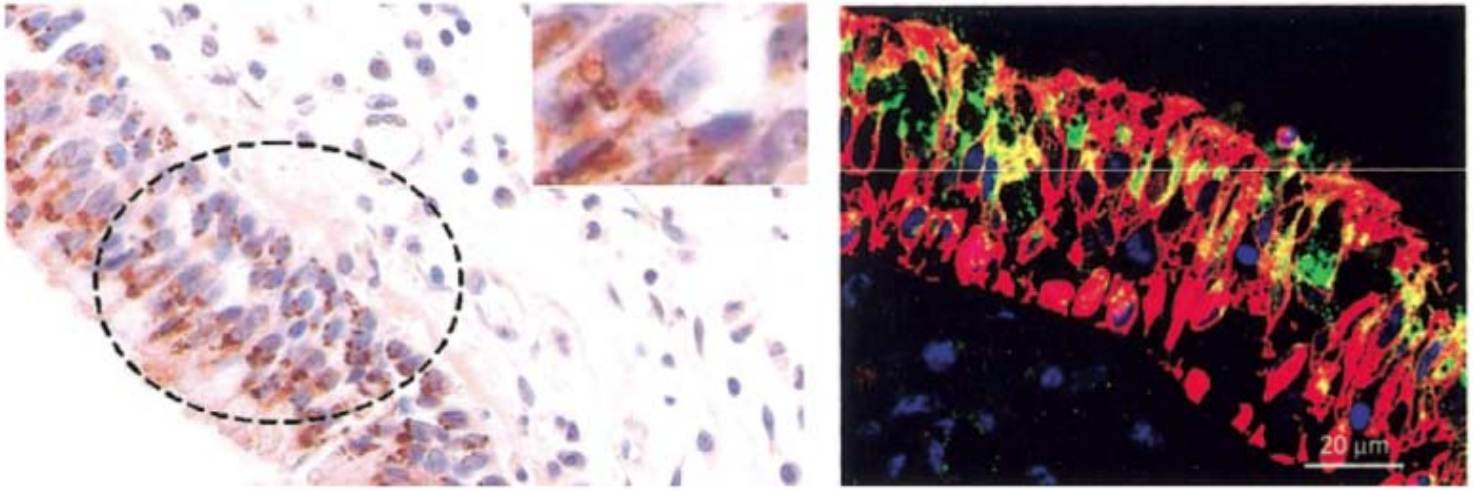
"The goal is … to find
chemical substances
that have special affinities for
pathogenic organisms and that,
like magic bullets,
go straight to their targets"

Intracellular *S. aureus* : is it important ?

Brussels: atomium built for the universal exposition in 1958
(crystal structure of iron)

Intracellular reservoir evidenced *in vivo*

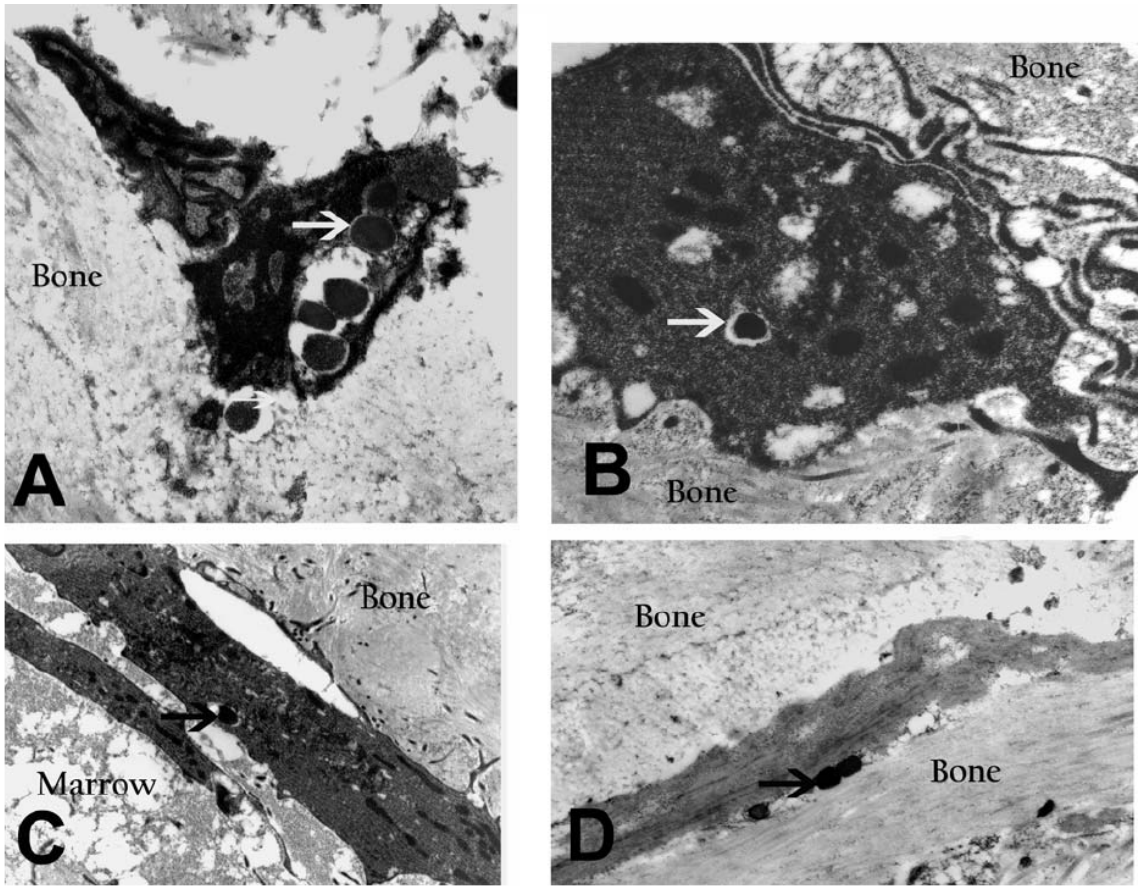
Evidence of an intracellular reservoir in the nasal mucosa of patients with recurrent *Staphylococcus aureus* rhinosinusitis



Clement et al., J Infect Dis. (2005) 192:1023-8

Intracellular reservoir evidenced *in vivo*

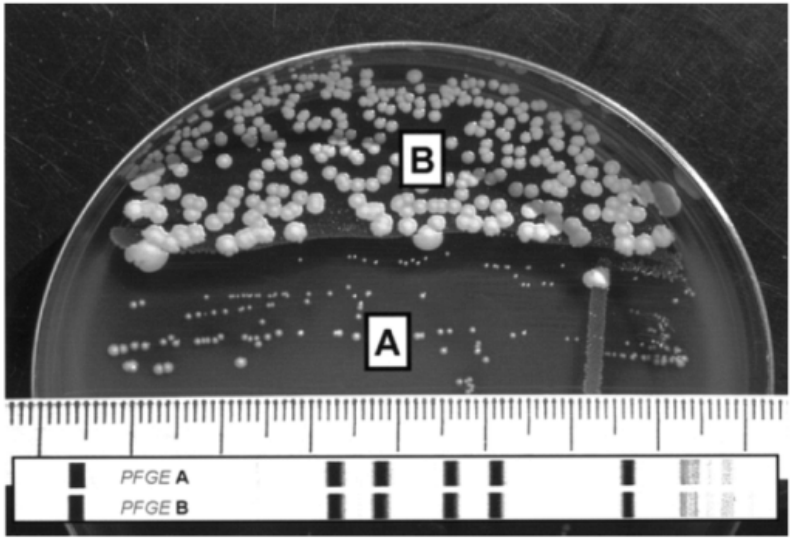
Evidence of an intracellular reservoir in osteocytes (A,B), osteoblasts (C) and bone matrix of a patient with recurrent osteomyelitis



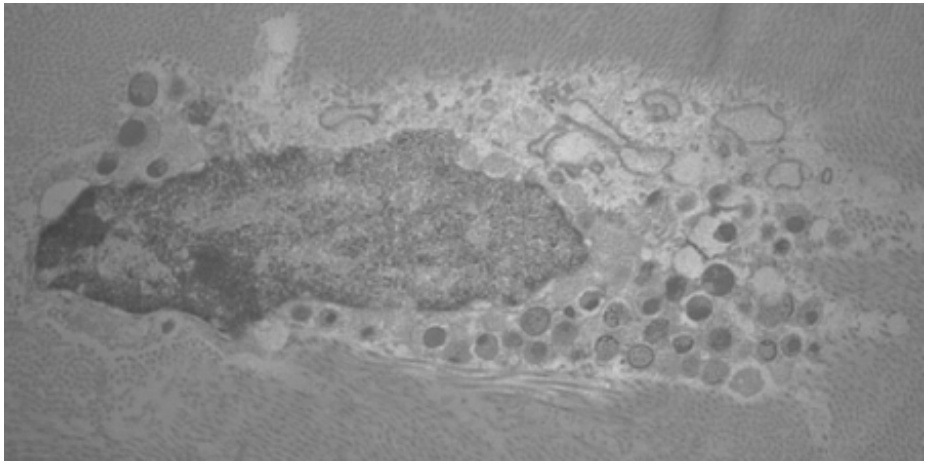
Bosse et al., J Bone Joint Surg Am. (2005) 87:1343-7

Intracellular reservoir evidenced *in vivo*

Evidence of Small Colony Variants and of intracellular *S. aureus* after treatment failure * in patients with prosthetic joint infections



Small colony variant *(A)* and normal-phenotype *Staphylococcus aureus (B)* isolated from patient 1 on Columbia blood agar.



* Fluclox, CIP+ RIF, VAN + FEP

Sendi et al., Clin Infect Dis. (2006) 43:961-7

S. aureus can survive and multiply in several cell types

Intracellular *Staphylococcus aureus*. A mechanism for the indolence of osteomyelitis.
Ellington et al. J. Bone Joint Surg Br. (2003) 85:918-21

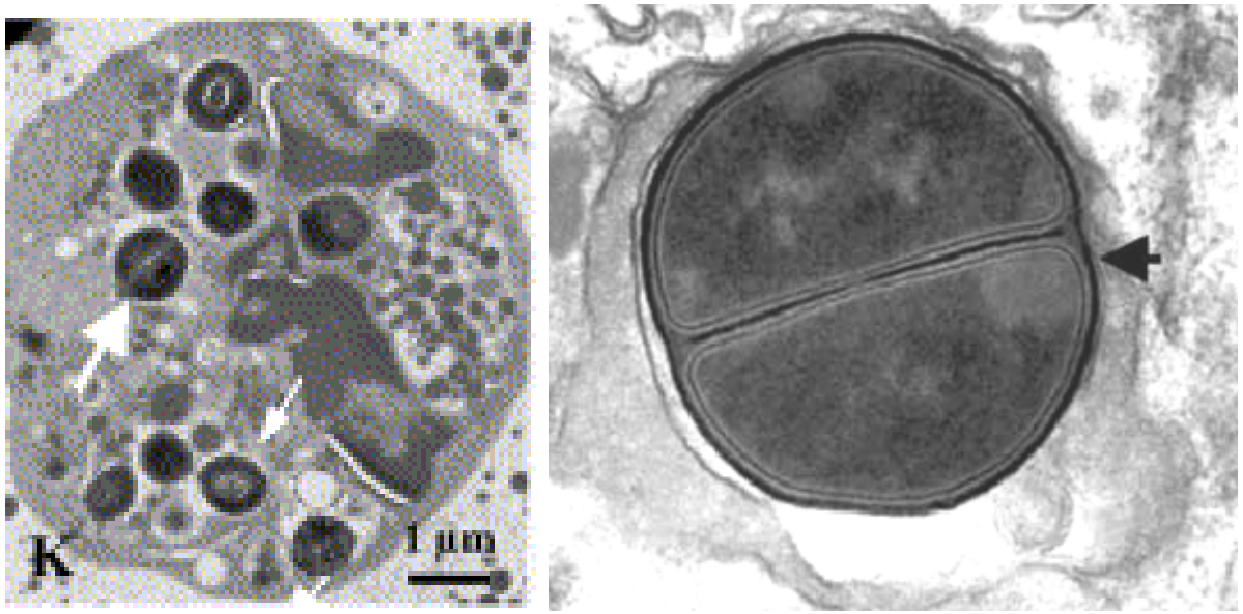
Intracellular persistence of *Staphylococcus aureus* small-colony variants within keratinocytes: a cause for antibiotic treatment failure in a patient with darier's disease.
Von Eiff et al. Clin Infect Dis. (2001) 32:1643-7

Phagocytosis of *Staphylococcus aureus* by cultured bovine aortic endothelial cells: model for postadherence events in endovascular infections.
Hamill et al. Infect Immun. (1986) 54:833-6.

Demonstration of intracellular *Staphylococcus aureus* in bovine mastitis alveolar cells and macrophages isolated from naturally infected cow milk.
Hebert et al. FEMS Microbiol. Lett. (2000) 193:57-72.

S. aureus can survive and multiply in several cell types including phagocytic cells

PMN and macrophages

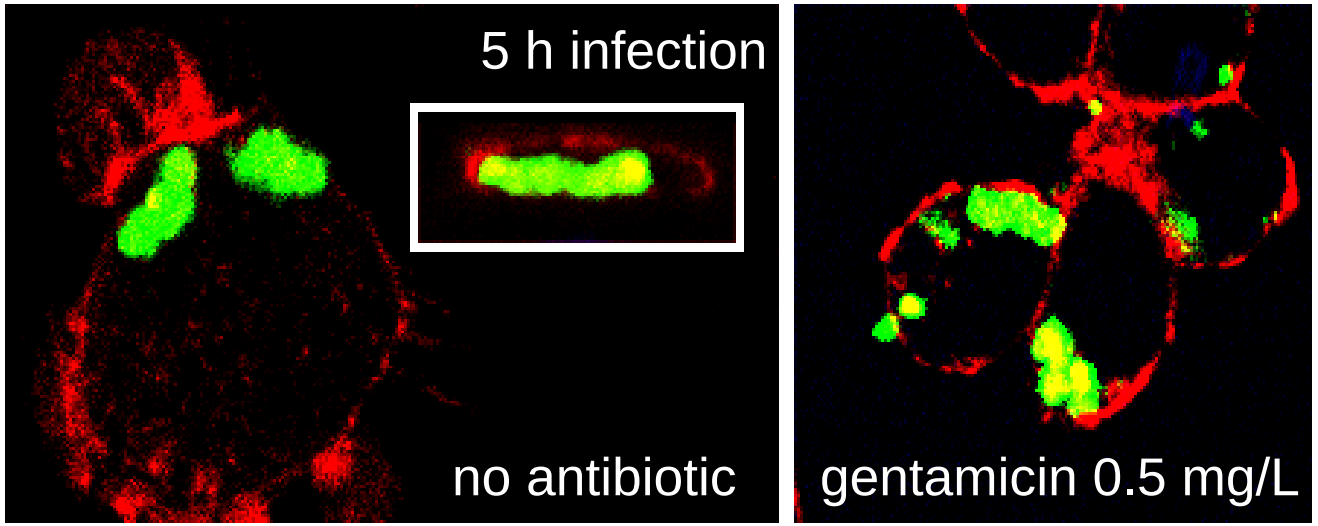


Brouillette et al., Vet Microbiol (2004) 101:253-262; Microb Pathog. (2003) 35:159-68

In vitro models : a possible way for studying antibiotic activity towards intracellular *S. aureus*

Brussels last week

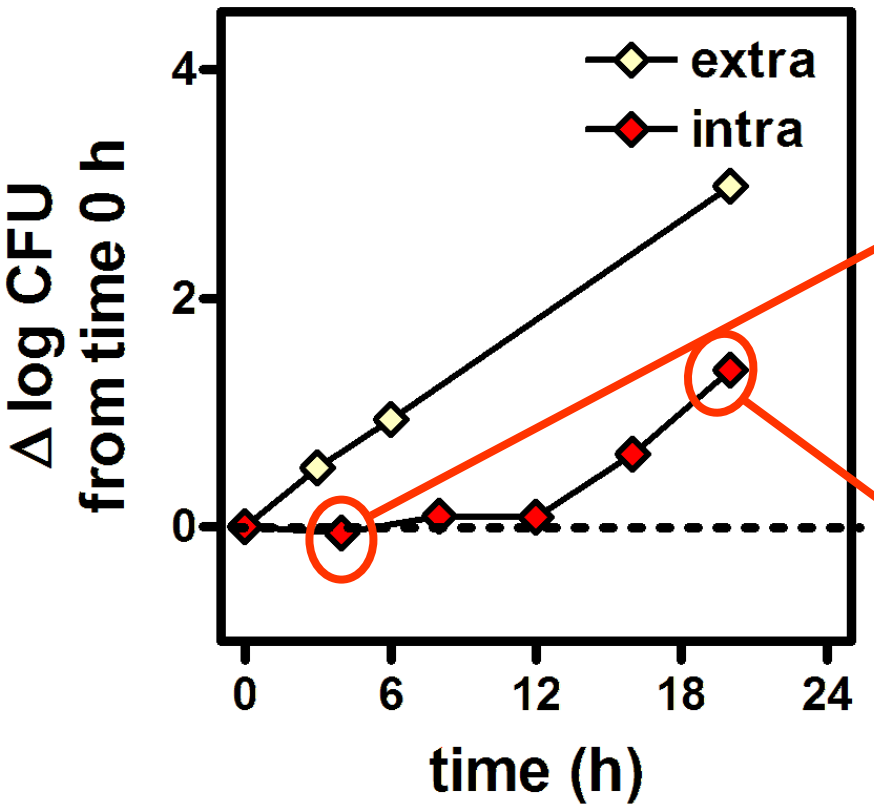
Setting up a model of intracellular infection over a 24 h period of time

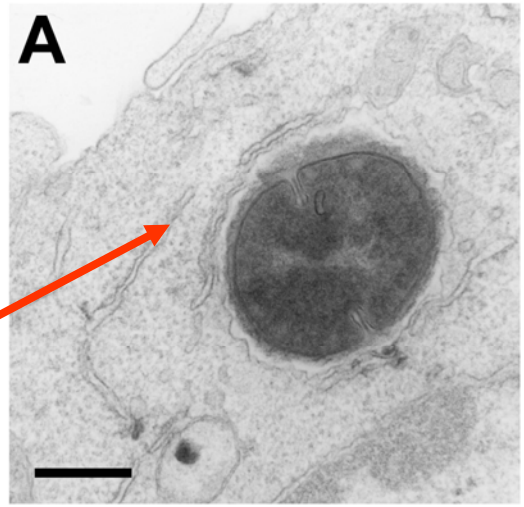


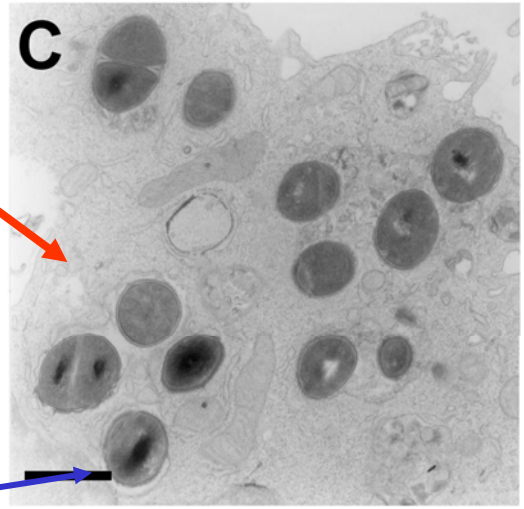
- infection of macrophages (with opsonized bacteria)
  - Mouse (J774; 5 bact/cell)
  - Human (THP-1; 4 bact/cell)
- washing with GEN 50 µg/ml to eliminate extracellular bacteria
- incubation for up to 24 h with
  - GEN (0.5-1 x MIC)
  - antibiotic under study

Seral et al., Antimicrob. Agents Chemother. (2003) 47:2283-2292

Description of the model : how does *S. aureus* grow intracellularly ?







remains in vacuoles

Seral et al., Antimicrob. Agents Chemother. (2003) 47:2283-2292

Measuring the intracellular activity of antibiotics . . .

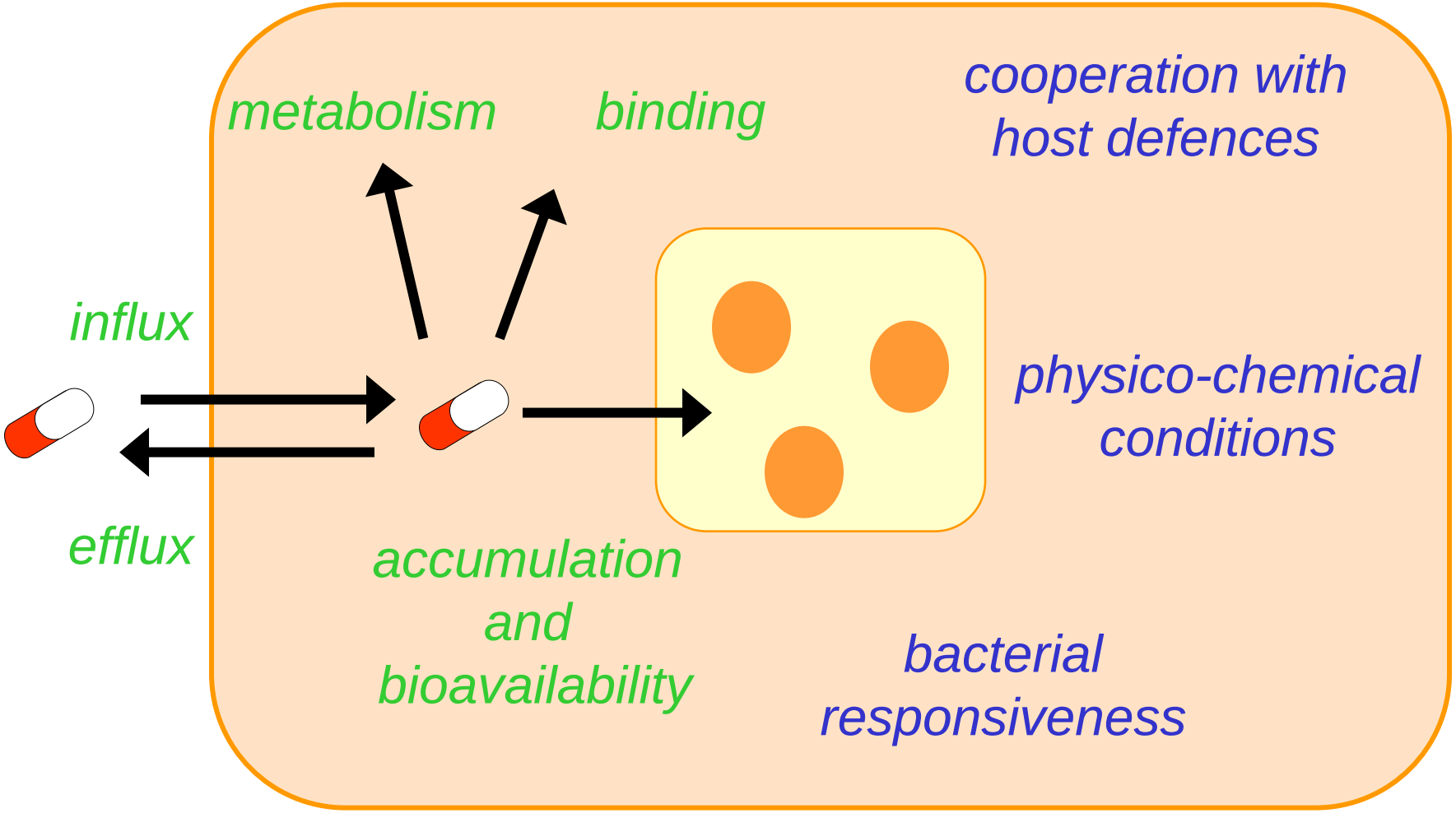
Very complicated ?

Rubens

Very simple ?

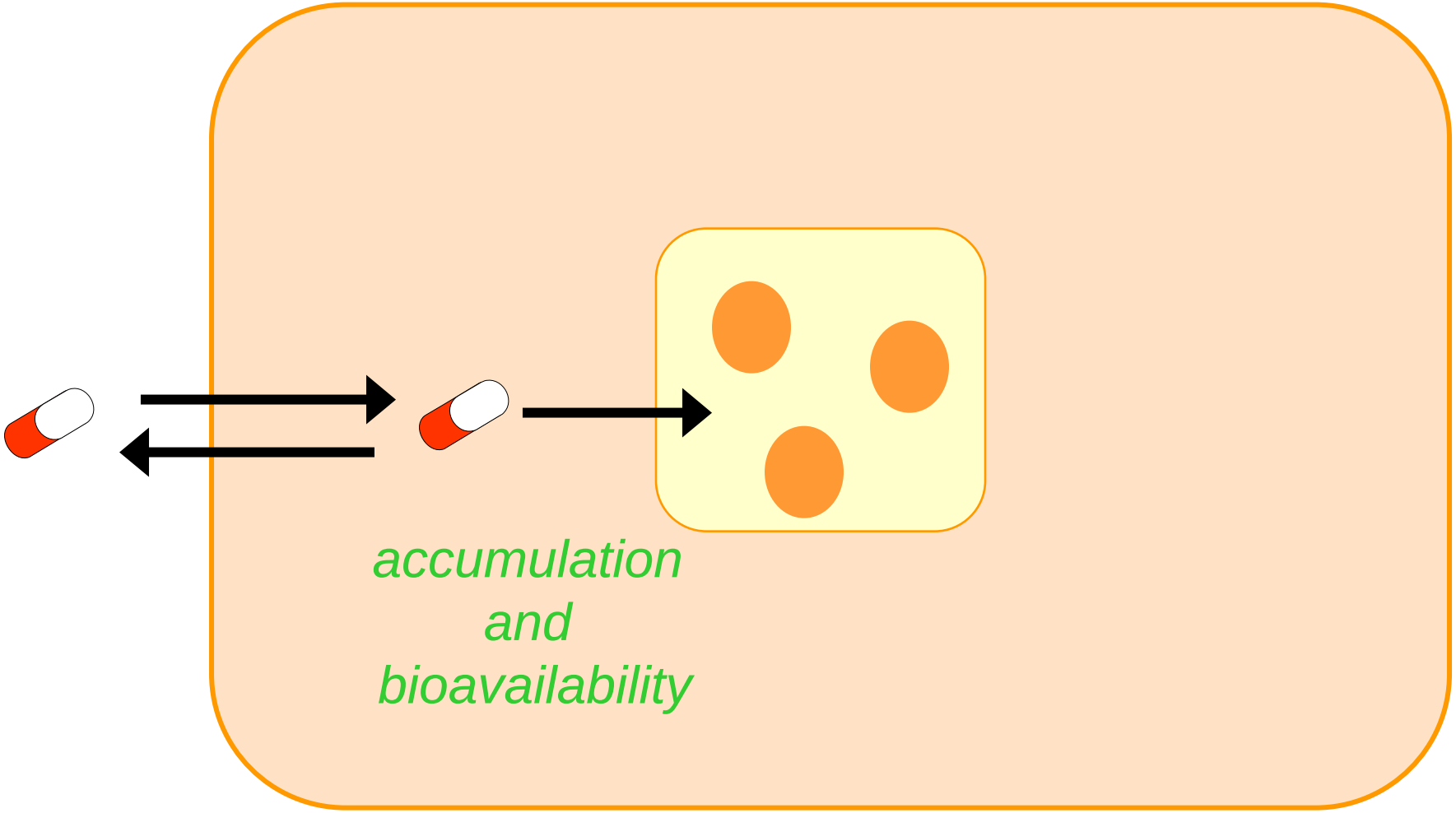
Folon

Intracellular vs extracellular activity of antibiotics : PK – PD in action



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

Intracellular vs extracellular activity of antibiotics : PK – PD in action



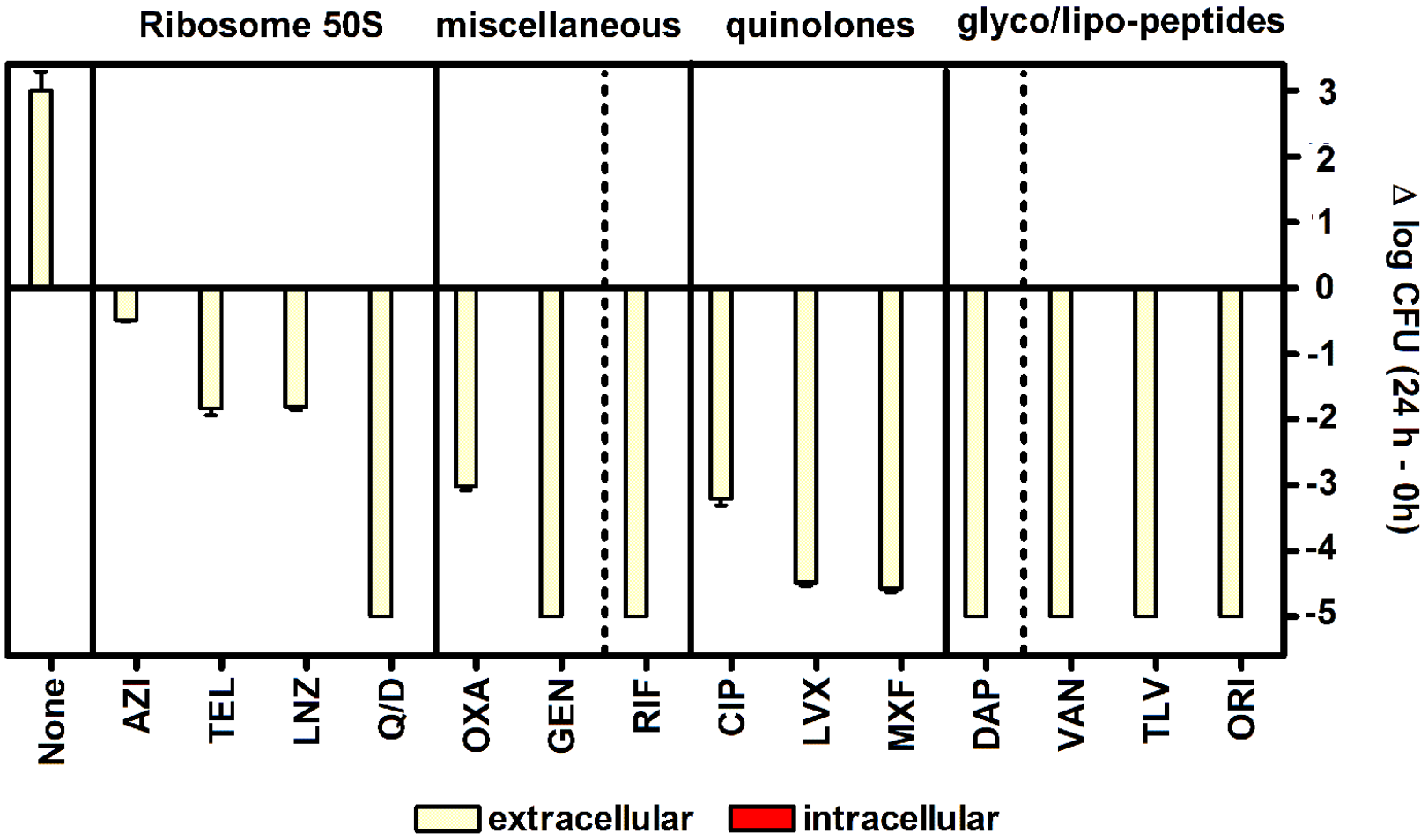
Carryn et al., Infect Dis Clin North Am. (2003) 17:615-34

Drug targeting is essential

Belgian classical comic

Extracellular vs intracellular activity at Cmax

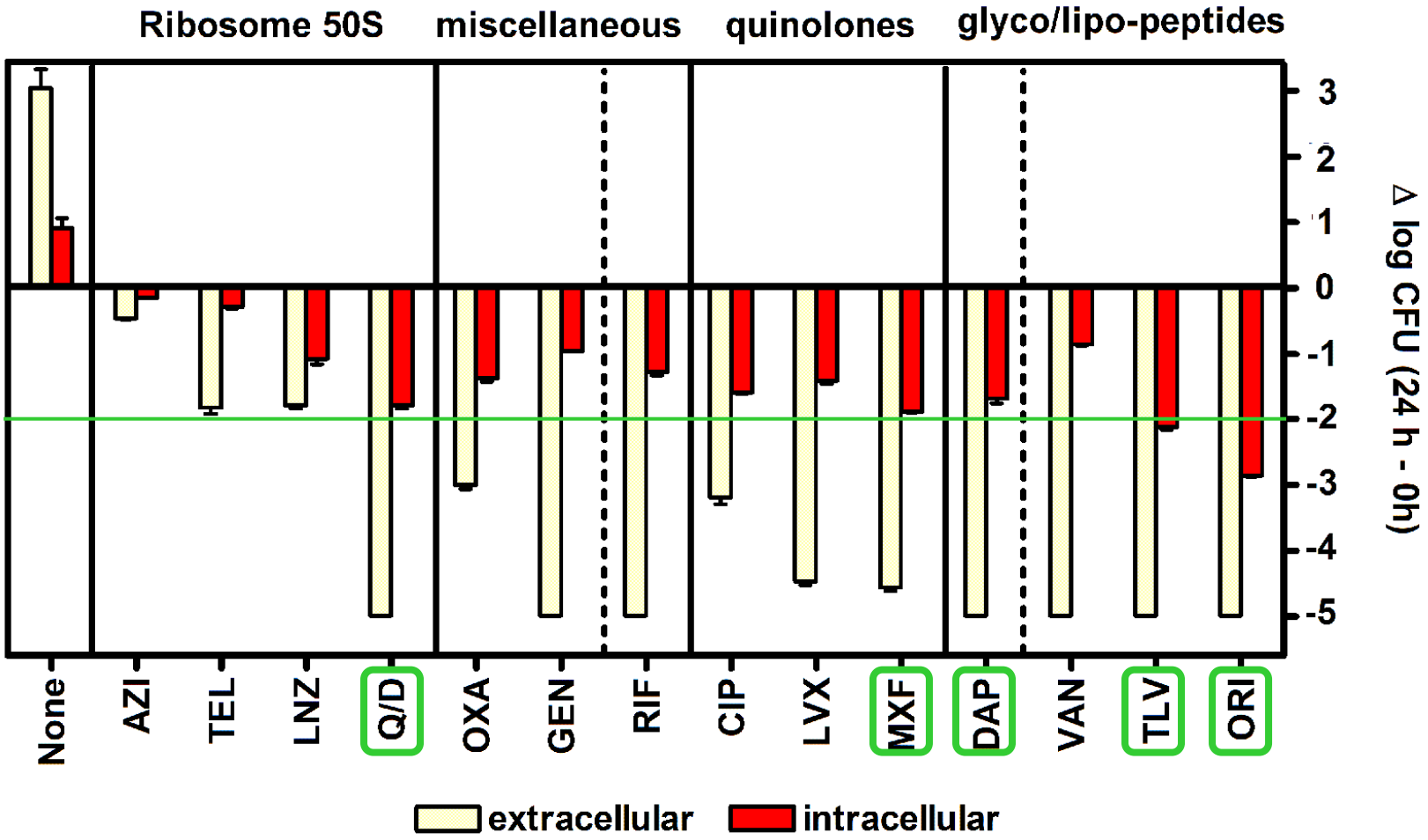
THP-1; 24 h, ATCC25923, antibiotics at Cmax



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

Extracellular vs intracellular activity at Cmax

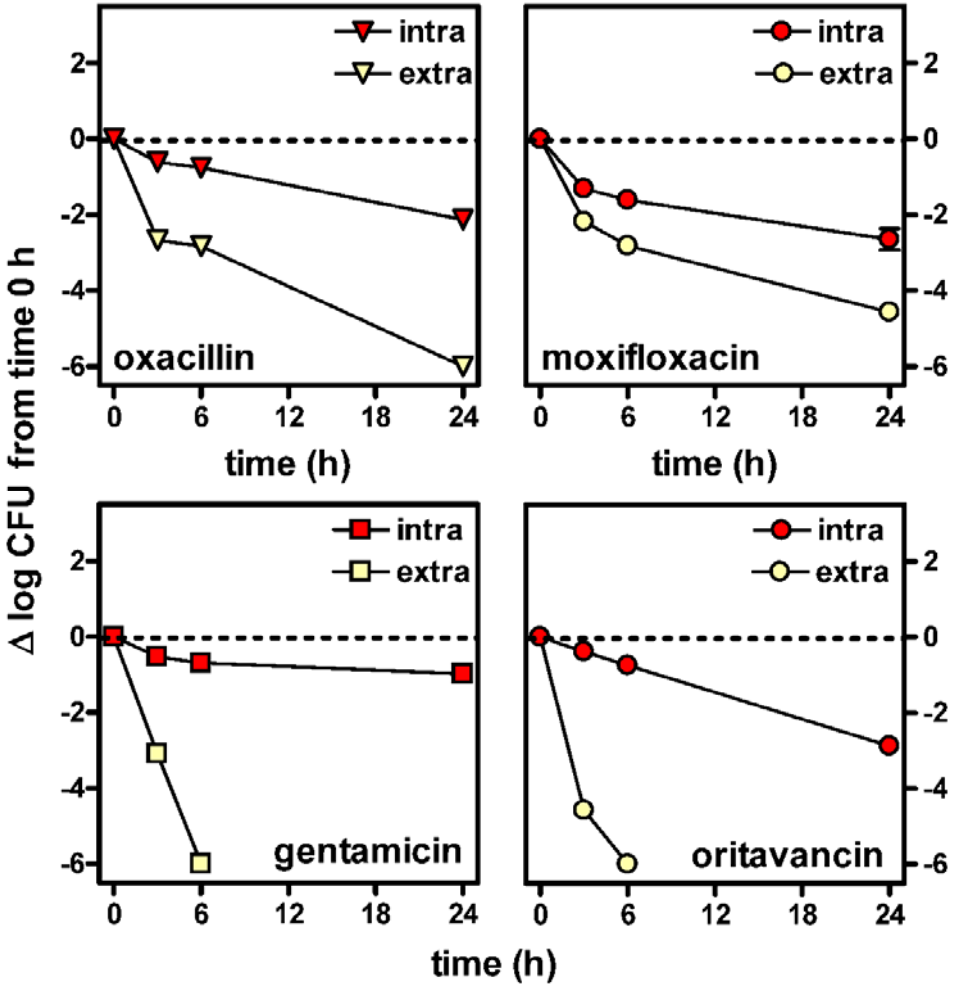
THP-1; 24 h, ATCC25923, antibiotics at Cmax



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

Pharmacodynamic relationships: time-effects at Cmax

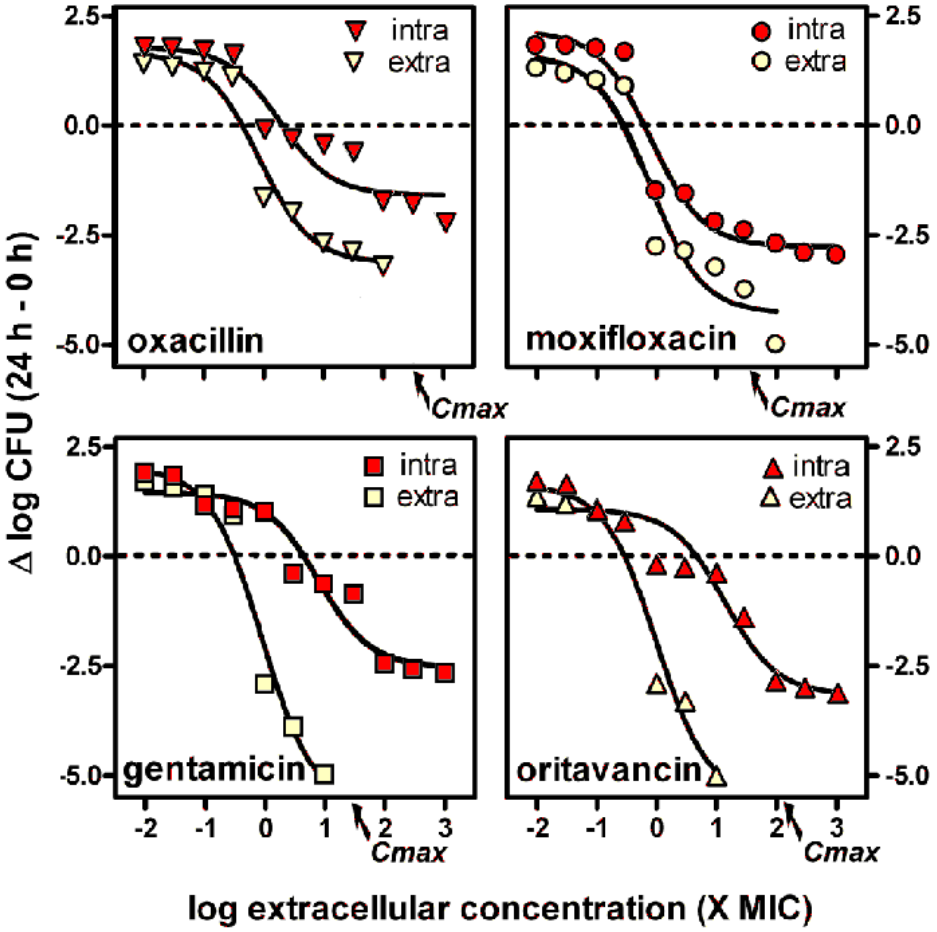
Slower killing rate intracellularly



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

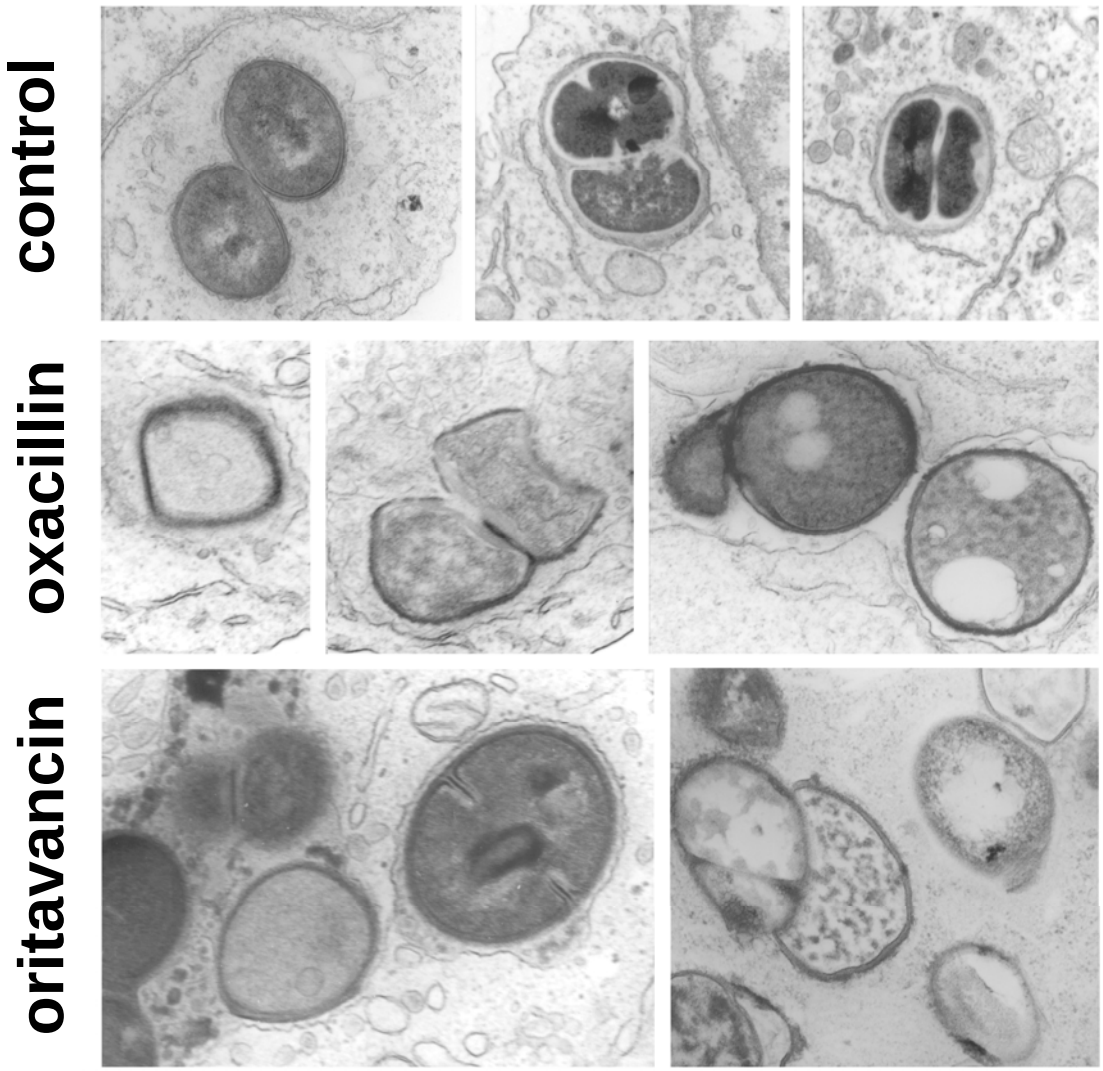
Pharmacodynamic relationships: concentration-effects at 24 h

Concentration-dependent killing; lower Emax intracellularly



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

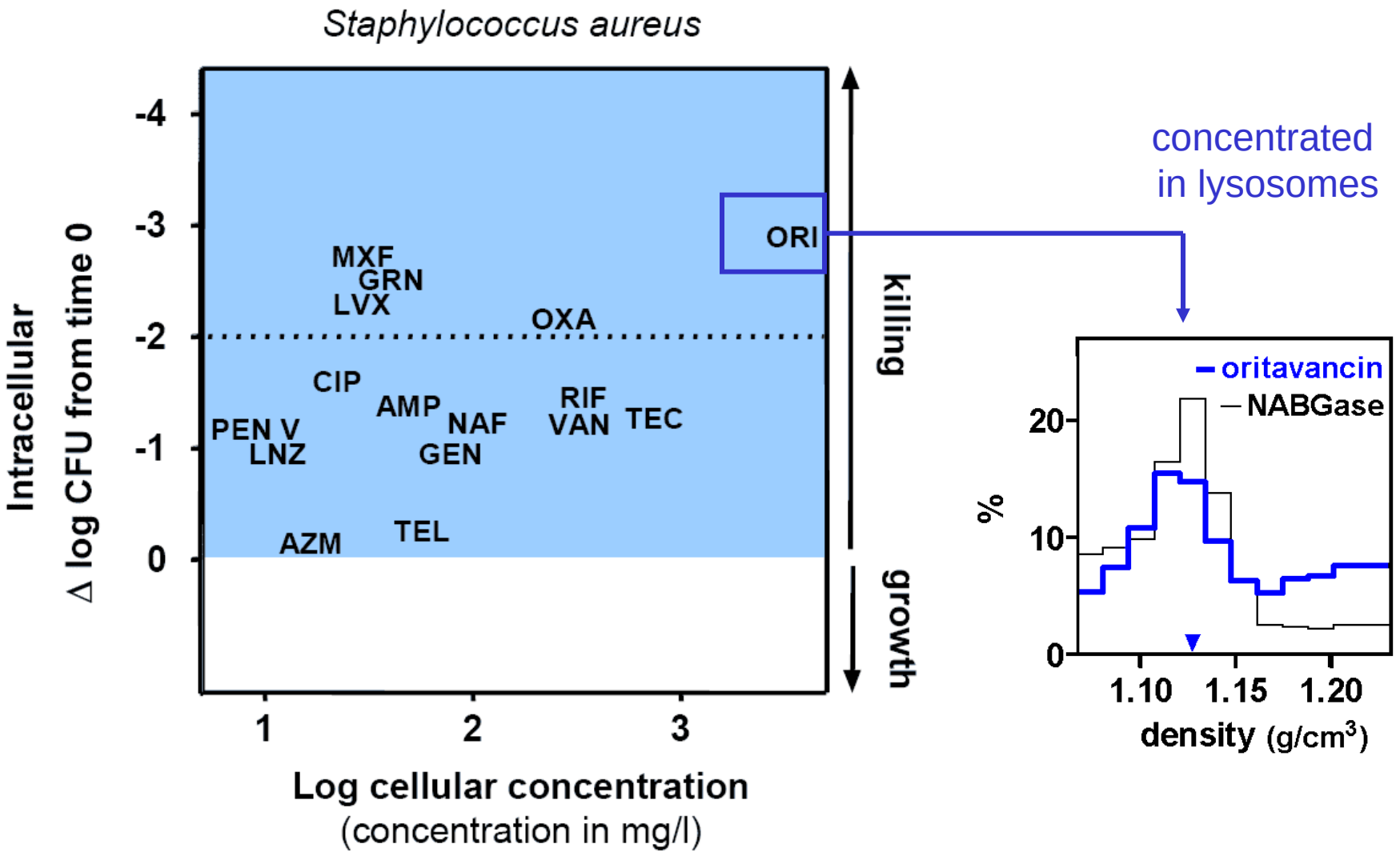
Intracellular killing is visible for antibiotics working on cell wall



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

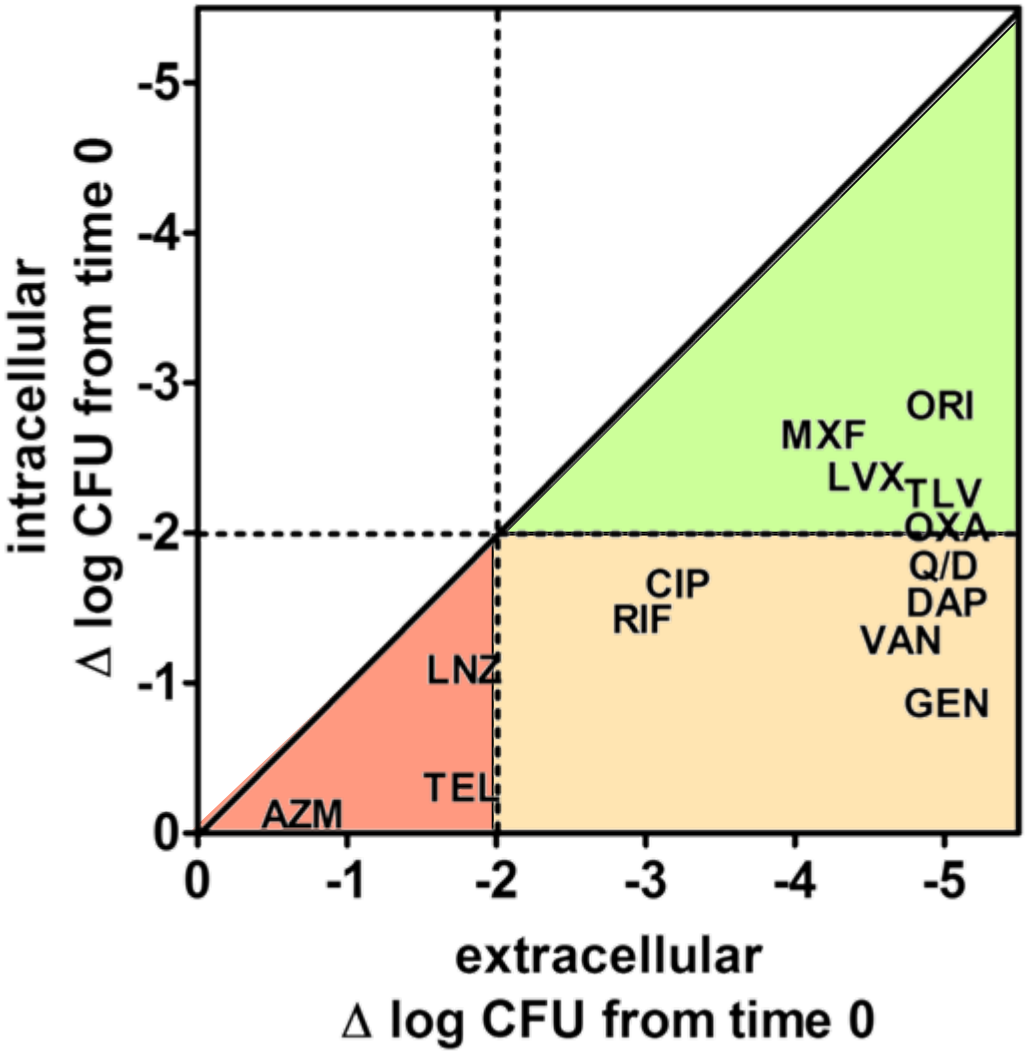
Any relationship between activity and accumulation ?

THP-1; 24 h, ATCC25923, antibiotics at Cmax



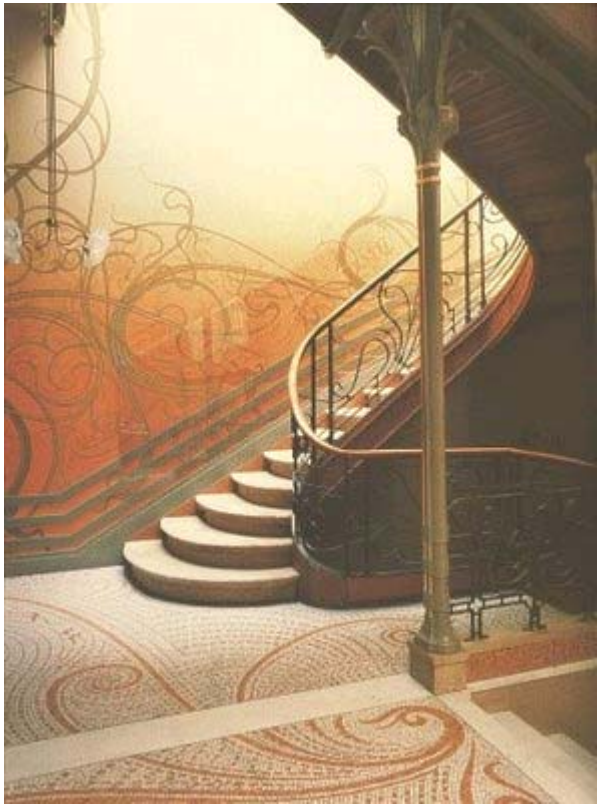
Van Bambeke et al., Curr Opin Drug Discov Devel. (2006) 9:218-30
Van Bambeke et al., Antimicrob Agents Chemother. (2004) 48:2853-60

Smart choice of antibiotics based on balanced extra- / intra- activity



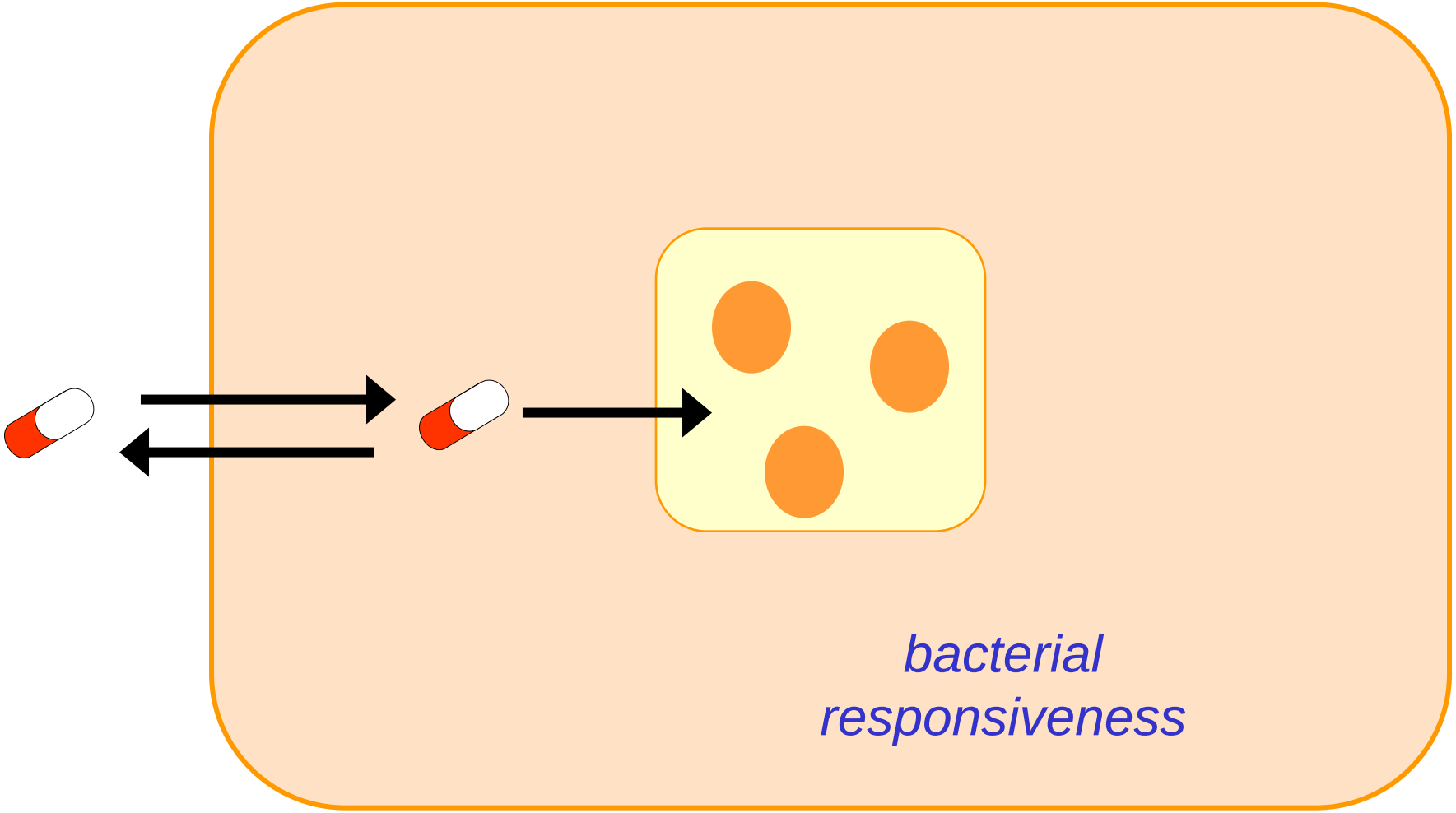
Adapted from Van Bambeke et al., Curr Opin Drug Discov Devel. (2006) 9:218-30

Do resistant strains escape antibiotics intracellularly ?



Art Nouveau in Brussels

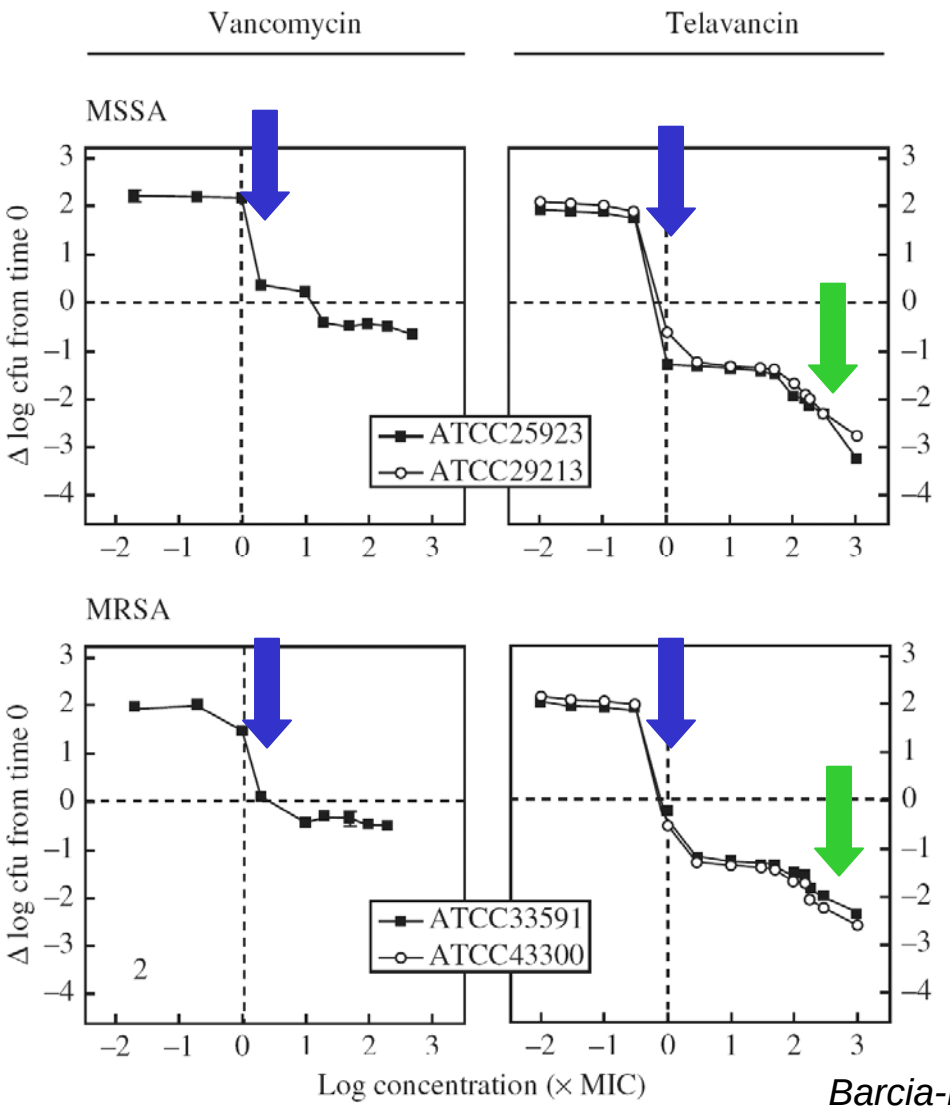
Intracellular vs extracellular activity of antibiotics : PK – PD in action



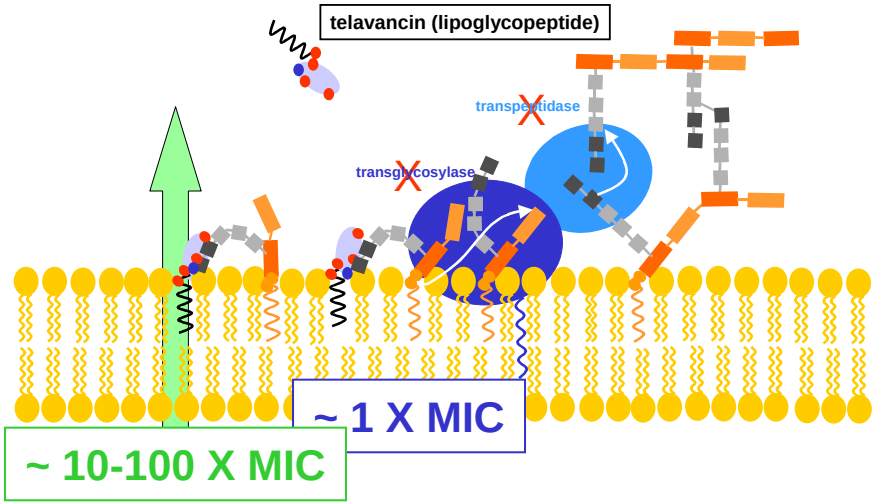
Carryn et al., Infect Dis Clin North Am. (2003) 17:615-34

MSSA, MRSA, (VISA, VRSA)

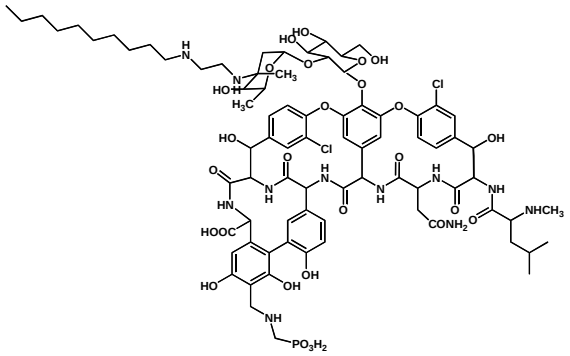
a lipoglycopeptide shows bimodal effects towards Vanco-S strains…



… because of dual mode of action ?



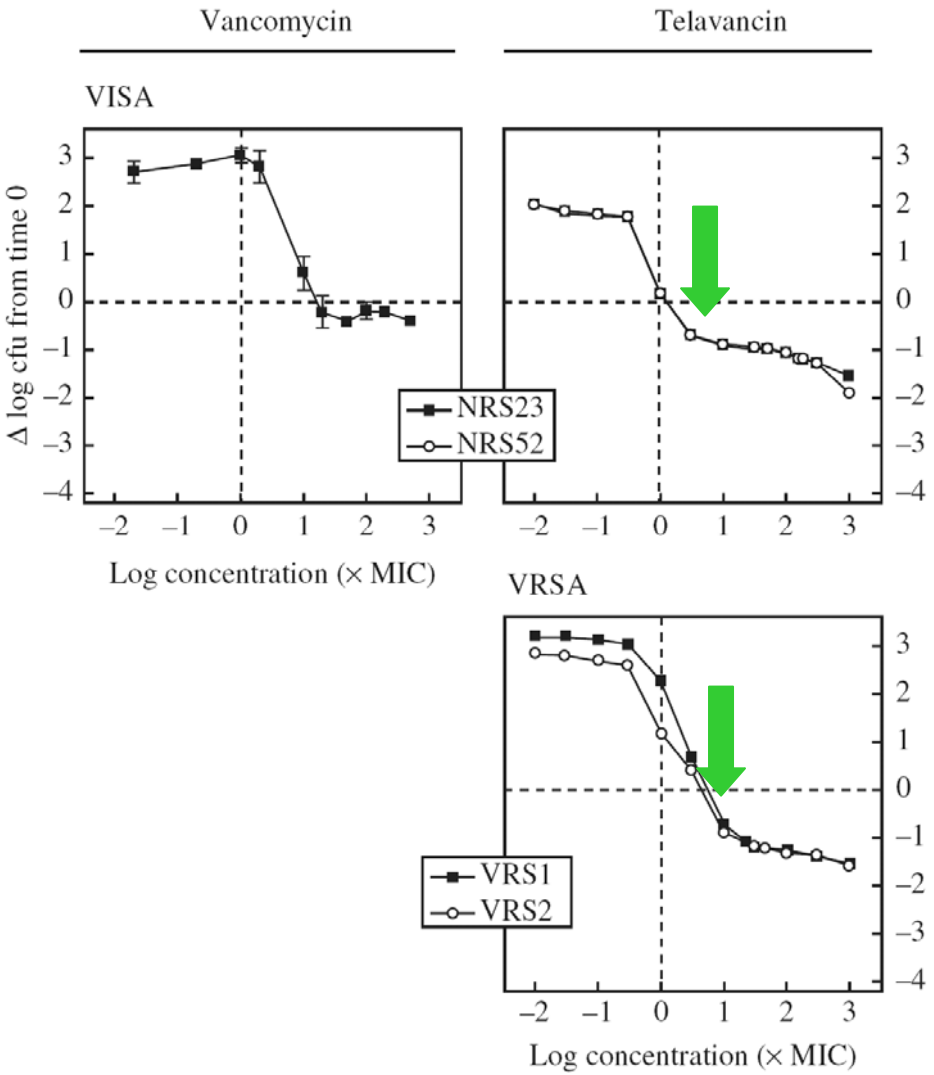
based on Higgins et al AAC (2005) 49: 1127-34



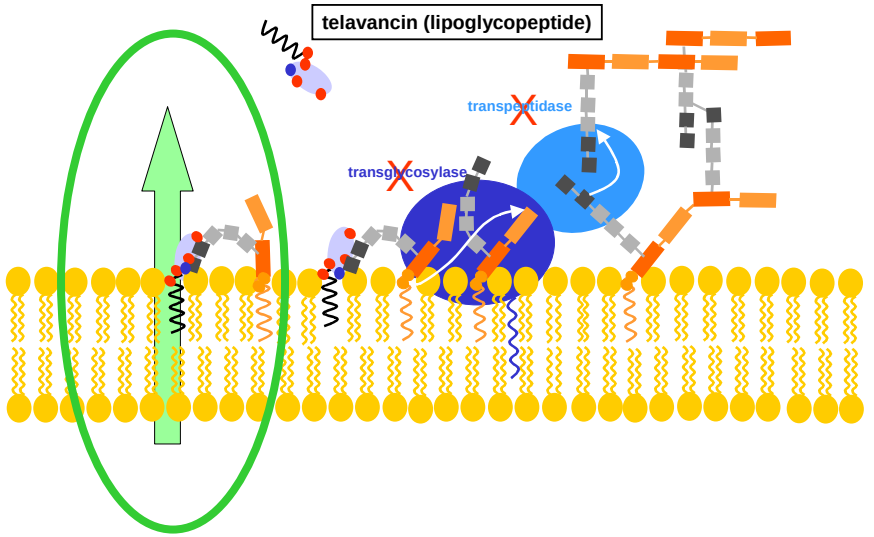
Barcia-Macay et al., J Antimicrob Chemother. (2006) 58:1177-84

(MSSA, MRSA), VISA, VRSA

a lipoglycopeptide shows unimodal effects towards Vanco-I/R strains…



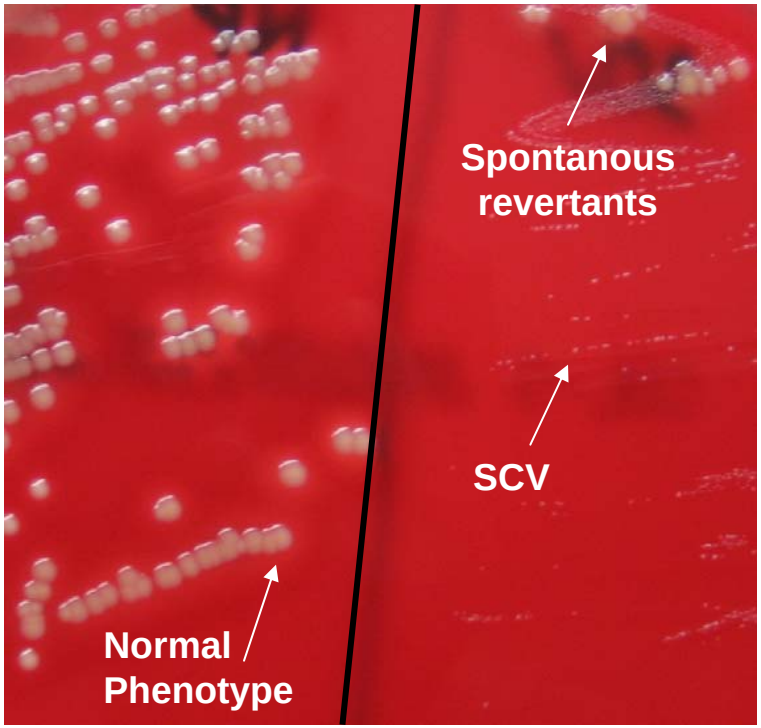
… because only one mode of action left ?



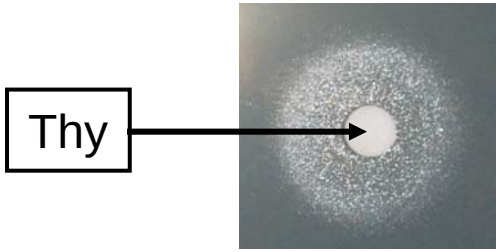
Barcia-Macay et al., J Antimicrob Chemother. (2006) 58:1177-84

SCV isolated from a cystic fibrosis patient

Vergison et al. J Antimicrob Chemother. 2007 59:893-9.

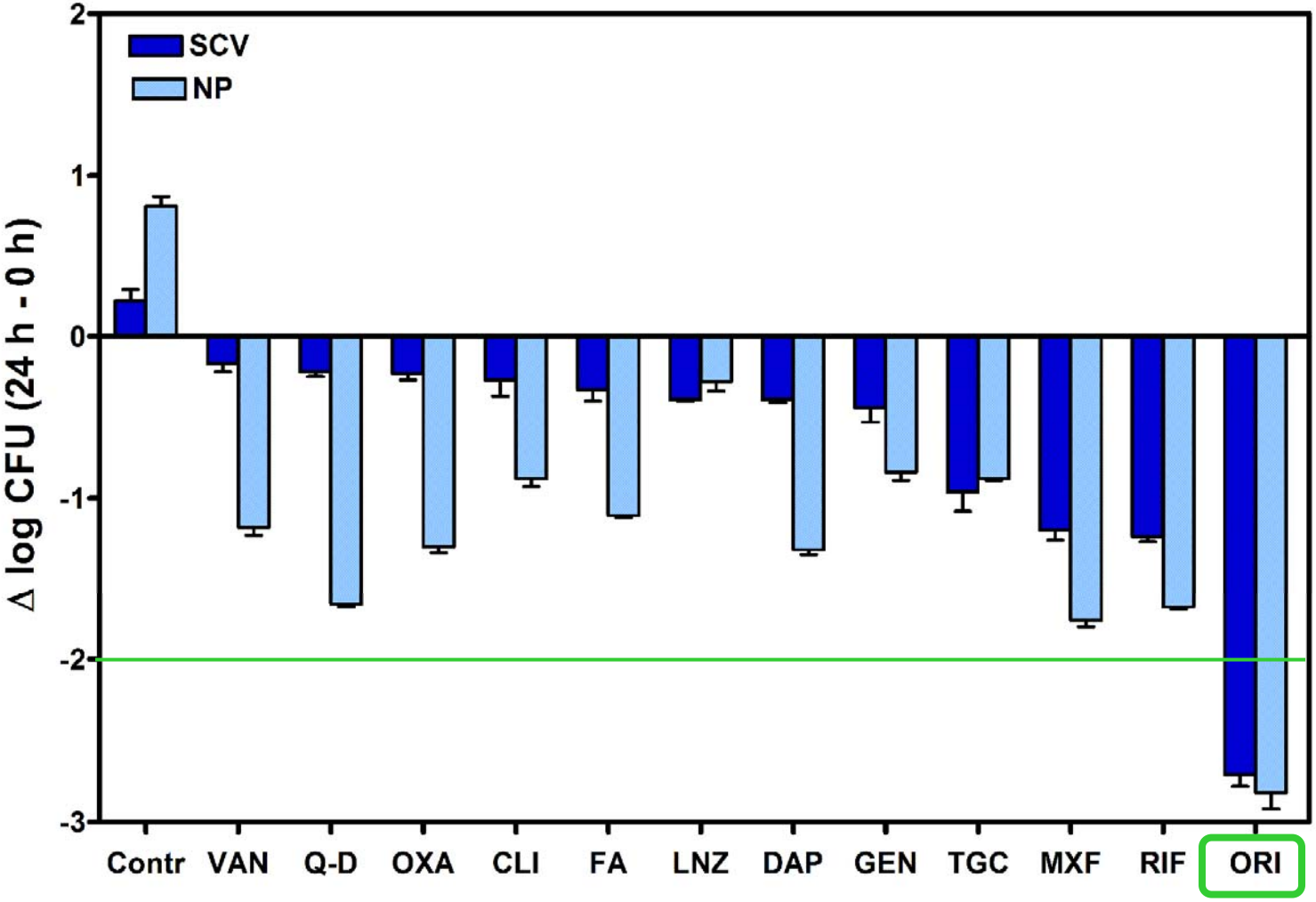


Thymidine dependent



Intracellular activity, SCV vs normal phenotype

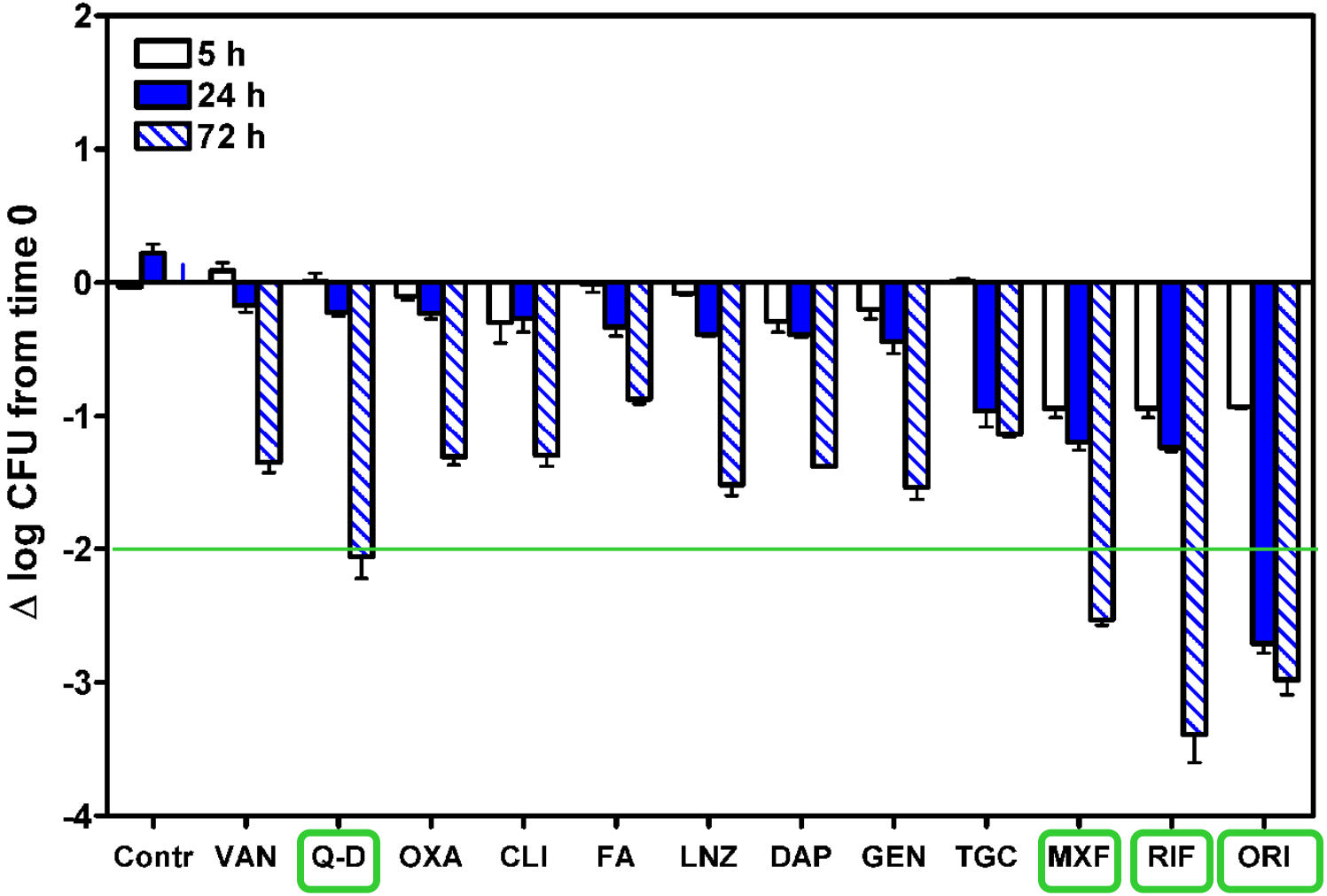
THP-1; 24 h, antibiotics at Cmax



Nguyen et al, RICAI 2007, poster 325

Intracellular activity, SCV over time

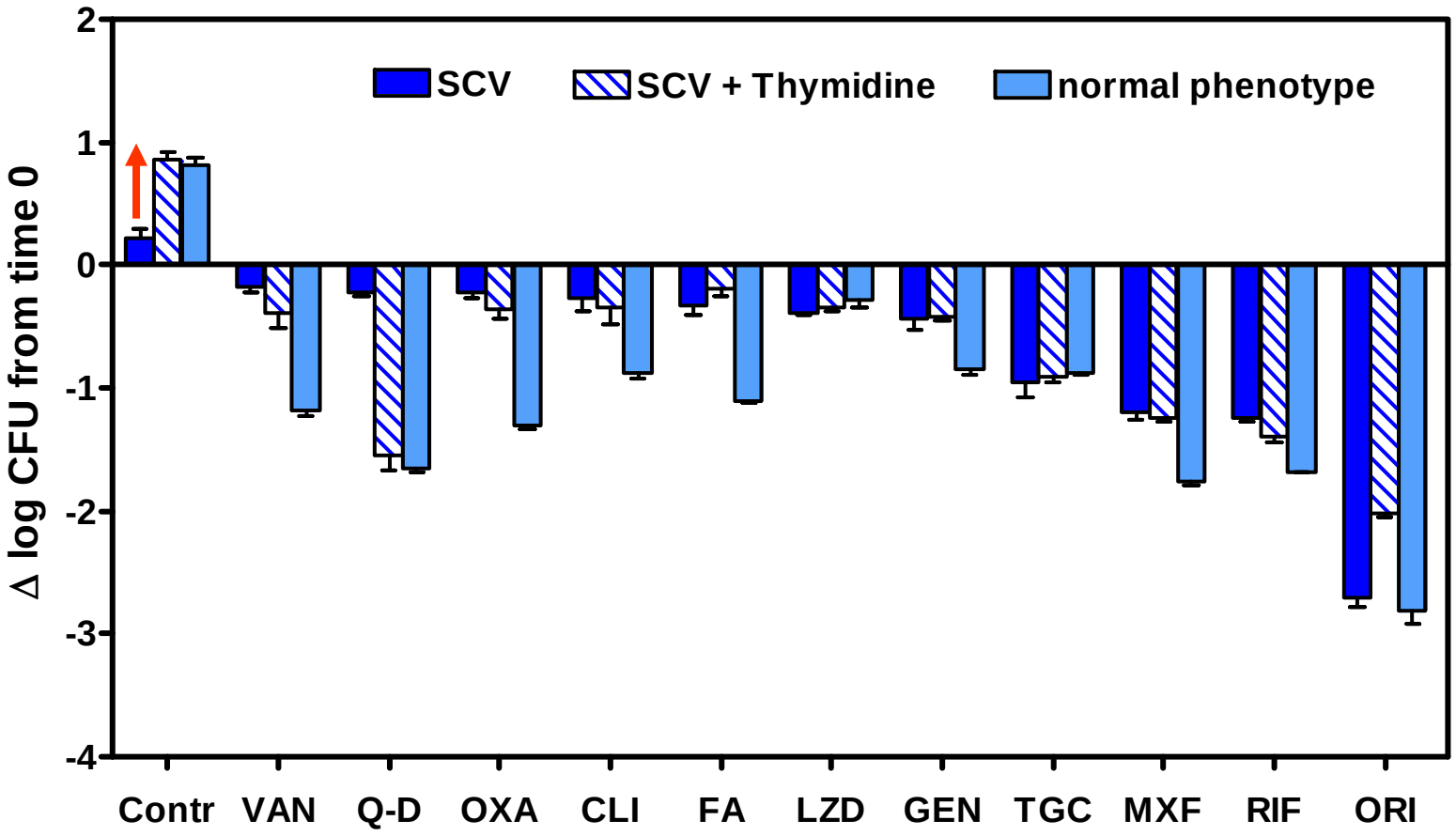
THP-1; SCV, antibiotics at Cmax for up to 3 days



Nguyen et al., ICAAC 2007, poster A1437

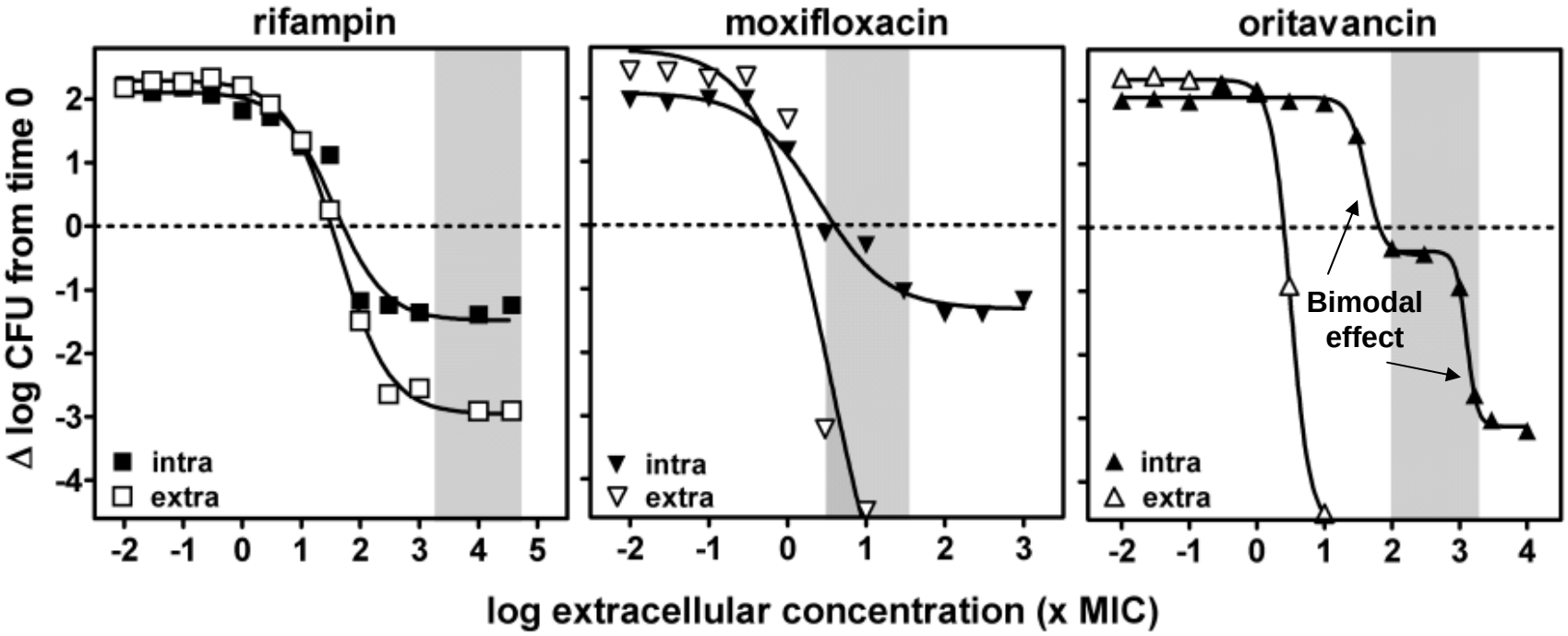
Intracellular activity, thymidine supplementation

THP-1; SCV, antibiotics at Cmax for up to 3 days



Thymidine supplementation restores intracellular growth
but does not affect the activity of most antibiotics

Dose-response curves of the 3 most active antibiotics against extra- and intra-cellular SCV (24 h of exposure)



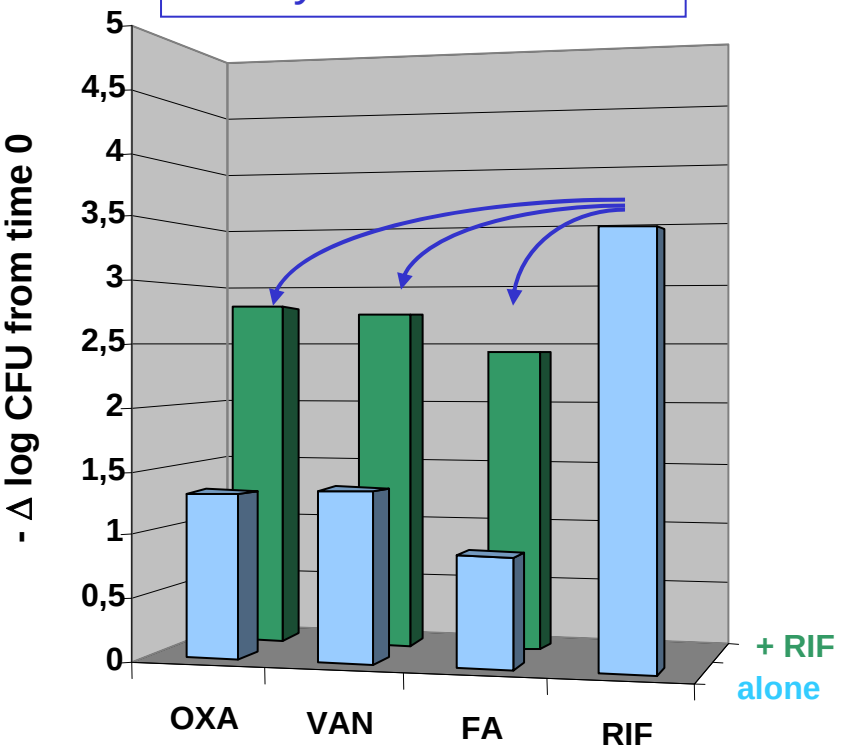
Gray zones: clinically-relevant range of concentrations

- Extracellular activity:
  - all drugs show concentration-dependent bacteridal effects
- Intracellular activity:
  - RIF and MXF show markedly reduced activity
  - ORI shows a bimodal effect with maximal activity ≈ 3 log

Intracellular activity of combinations against SCV

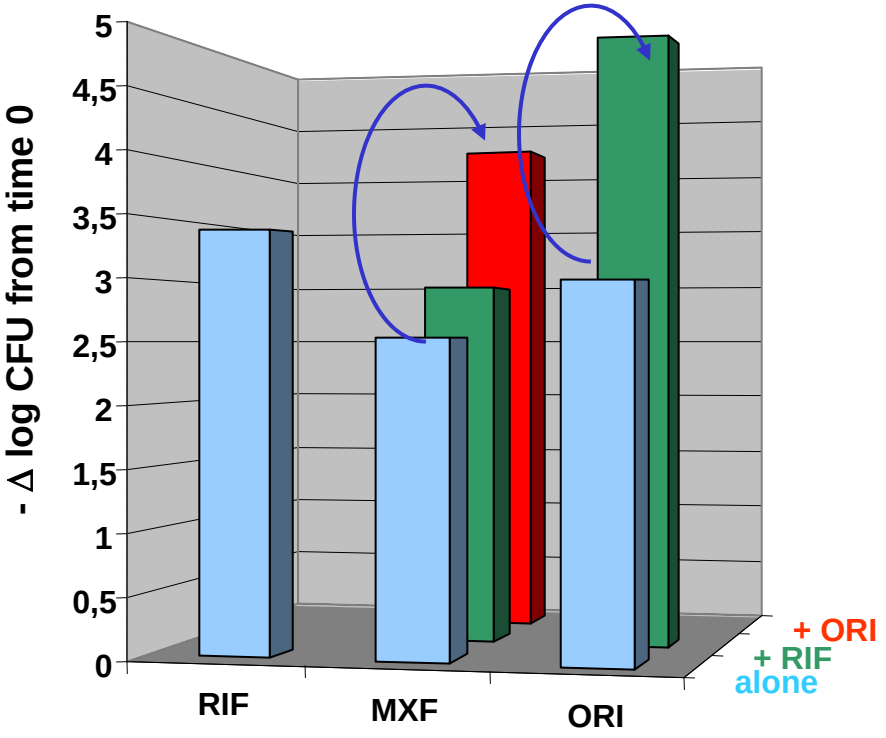
THP-1; SCV, antibiotics at Cmax for 3 days

Poorly active AB + RIF



Slightly less active than RIF alone

Combinations between the most active AB



Combinations with ORI are synergistic

Nguyen et al., ECCMID 2008, poster 1059

Activity of combinations with ORI against intracellular SCV

Fractional maximal effect (FME) approach

- Handle the nonlinear pharmacodynamics exhibited by antibiotics
- Analyse the combinations with calculated and not arbitrarily chosen concentrations

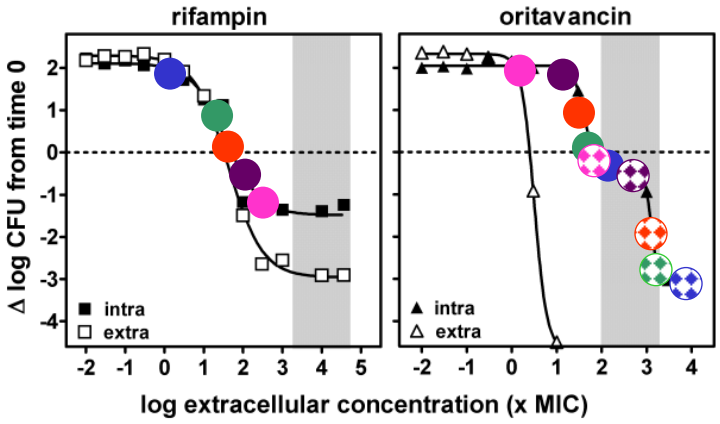
Effect (E): decrease of inoculum after 24 h. Sigmoid E_{max} model $\Rightarrow$ E_{max}, EC_{50}

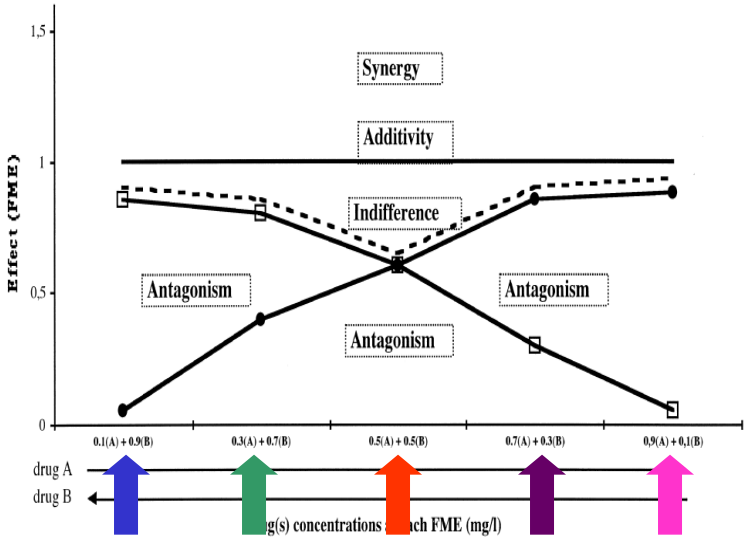
$$E = \frac{E_{max} \cdot C^n}{EC_{50}^n + C^n}$$

ATBs (A et B) are combined to a FME =1.

5 pairs: 0.1 FME_A + 0.9 FME_B, 0.3 FME_A + 0.7 FME_B, 0.5 FME_A + 0.5 FME_B, 0.7 FME_A + 0.3 FME_B, 0.9 FME_A + 0.1 FME_B

Correspoding concentration to be tested alone and in combination:

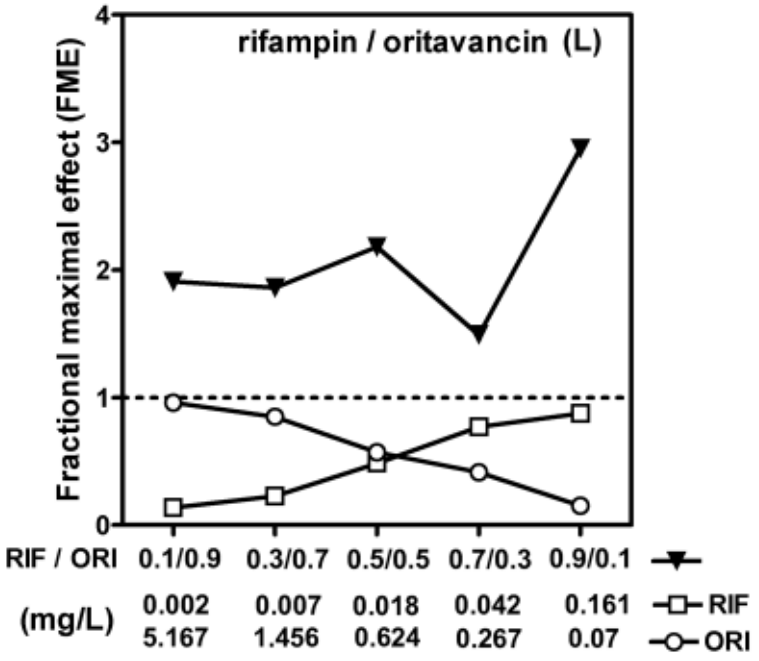


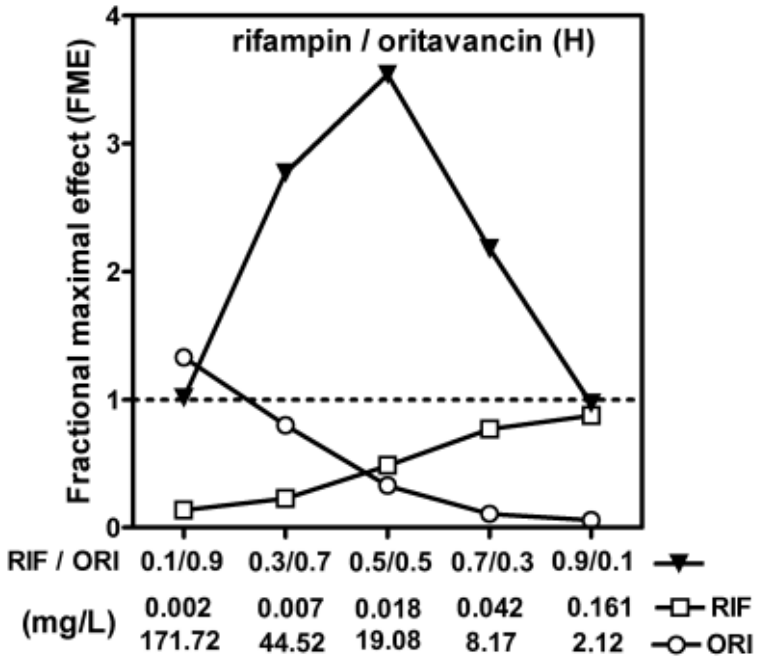


Desbiolles et al, Antimicrob. Agents Chemother. (2001) 45: 3328-33

Activity of RIF-ORI combination against intracellular SCV

Fractional maximal effect (FME) approach





FME > 1 : synergistic; = 1: additive

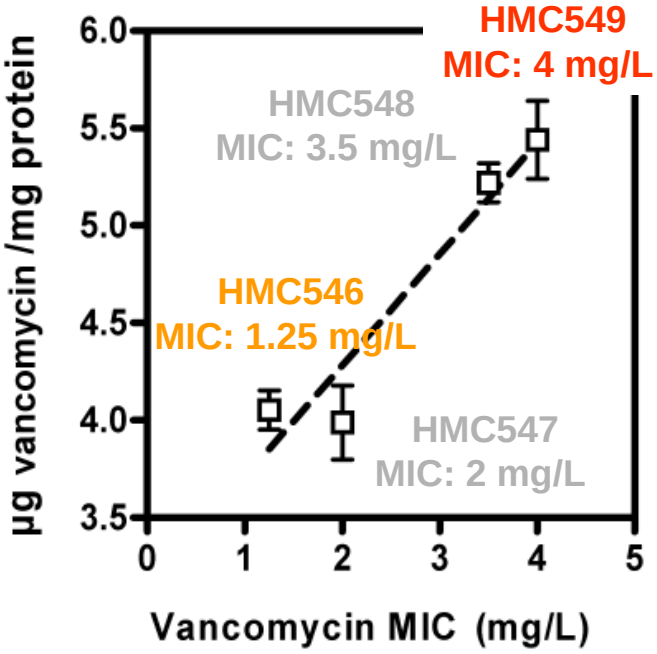
RIF-ORI combination is highly synergistic over a wide range of concentration ratios

VISA and DAP-resistant strains isolated from a patient with endocarditis

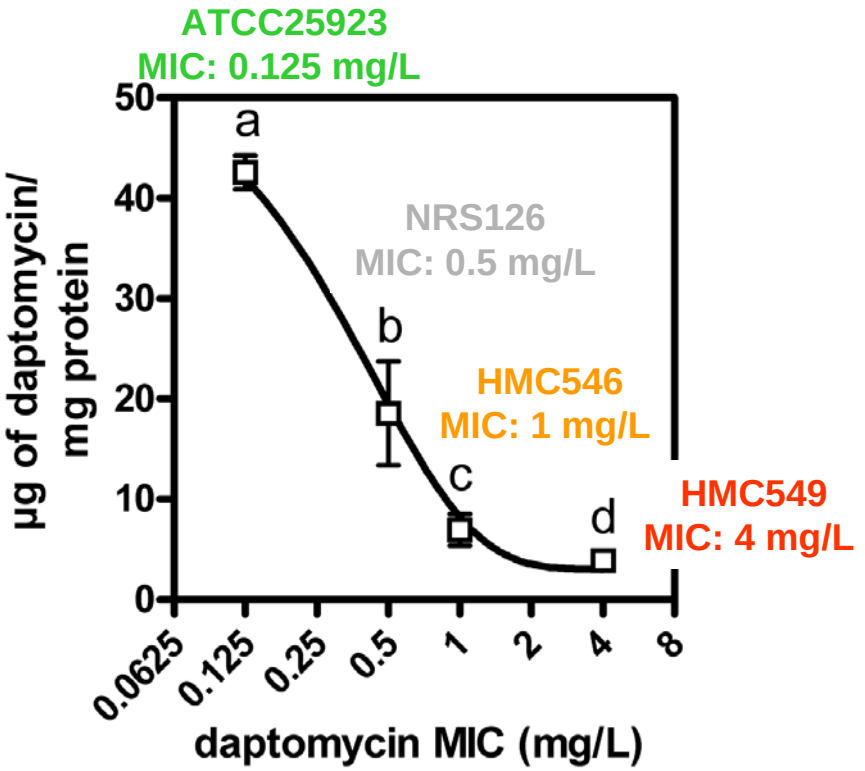
Julian et al. Antimicrob Agents Chemother. 2007 51:3445-8.

Reduced susceptibility associated with

increased amount of bound vancomycin



decreased amount of bound daptomycin

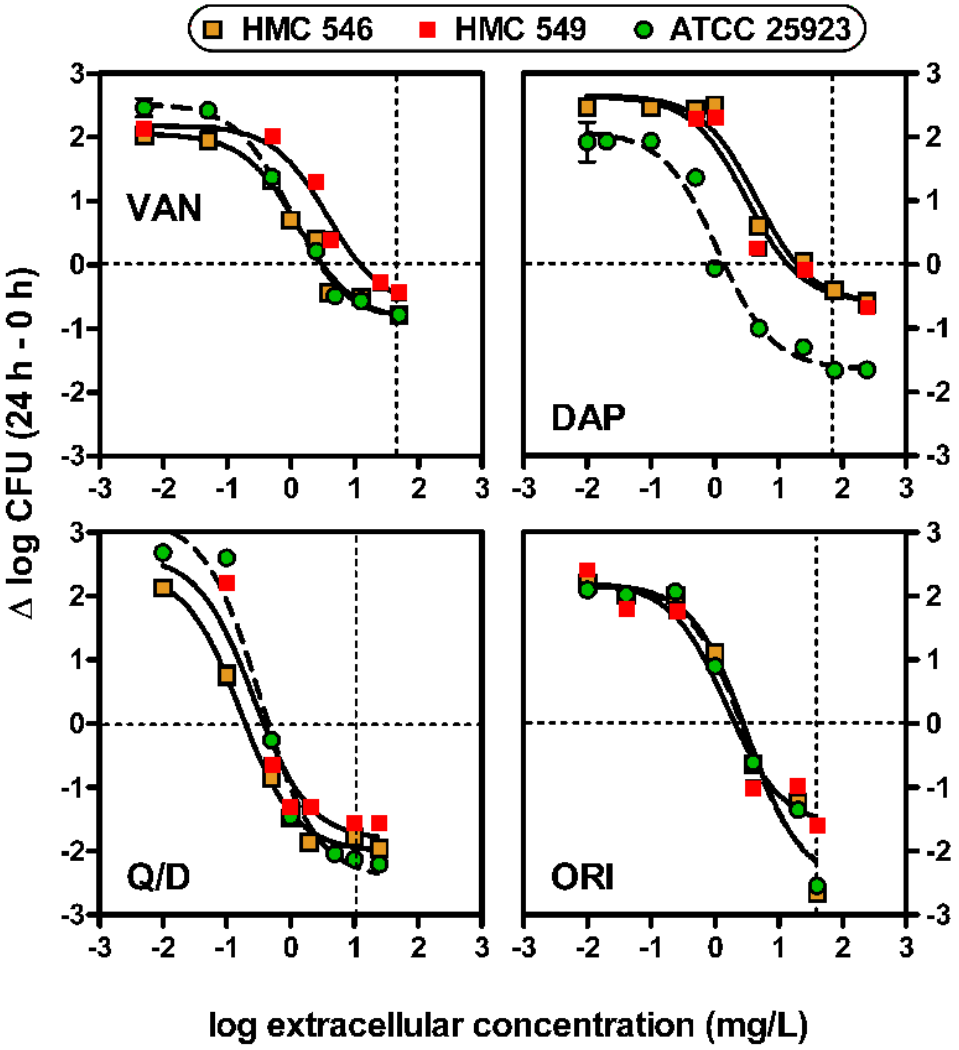


Lemaire et al., Clin. Microbiol. Infect. (2008) 14:766-77

Intracellular activity against VISA and DAP-resistant strains isolated from a patient with endocarditis

higher intracellular EC_{50}

no effect of resistance phenotype



higher intracellular EC_{50}

lower intracellular Emax

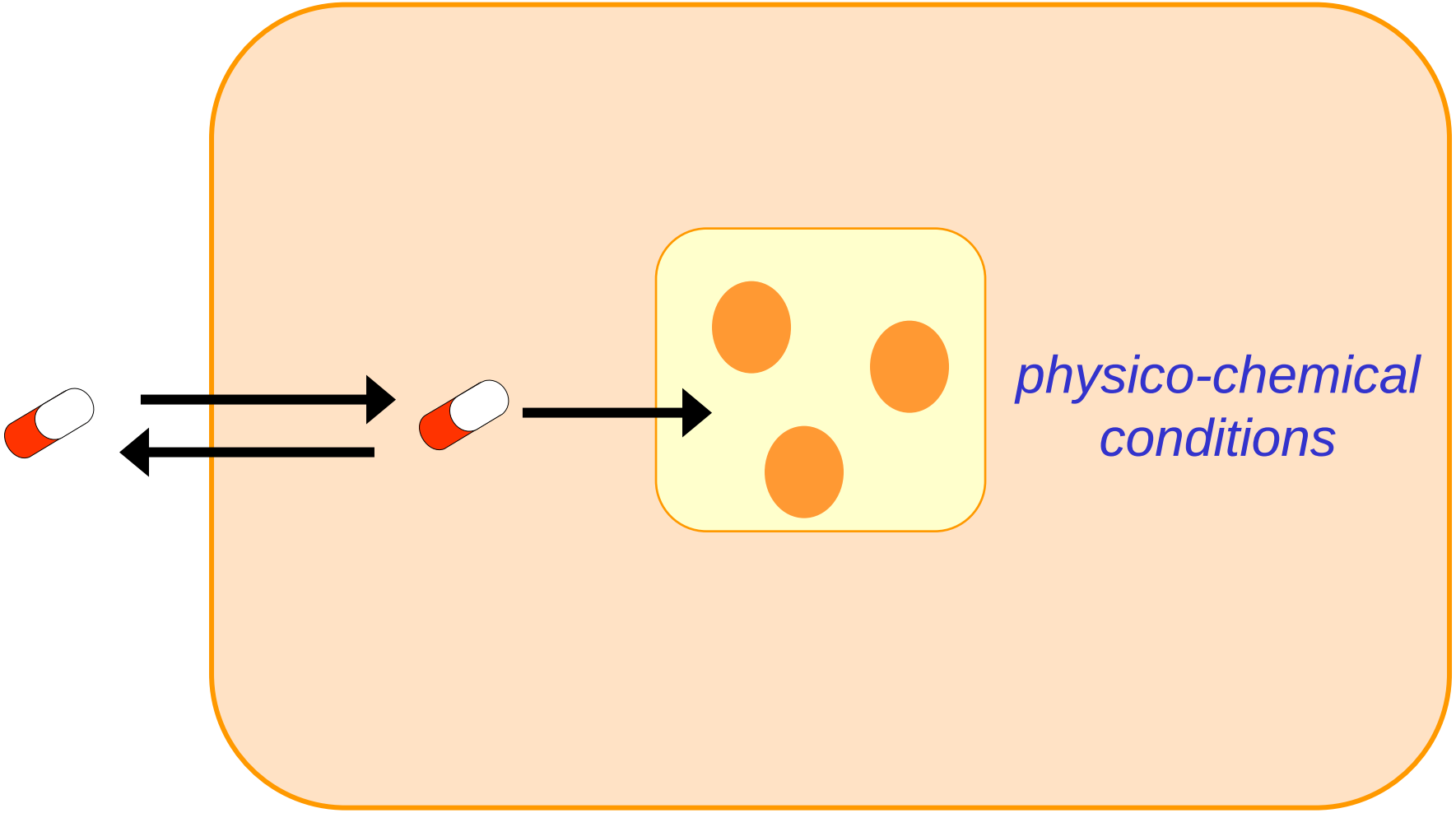
no effect of resistance phenotype

Lemaire et al., Clin. Microbiol. Infect. (2008) 14:766-77

Cellular factors affecting antibiotic intracellular activity

Brussels Grand-Place
Flower carpet

Intracellular vs extracellular activity of antibiotics : PK – PD in action



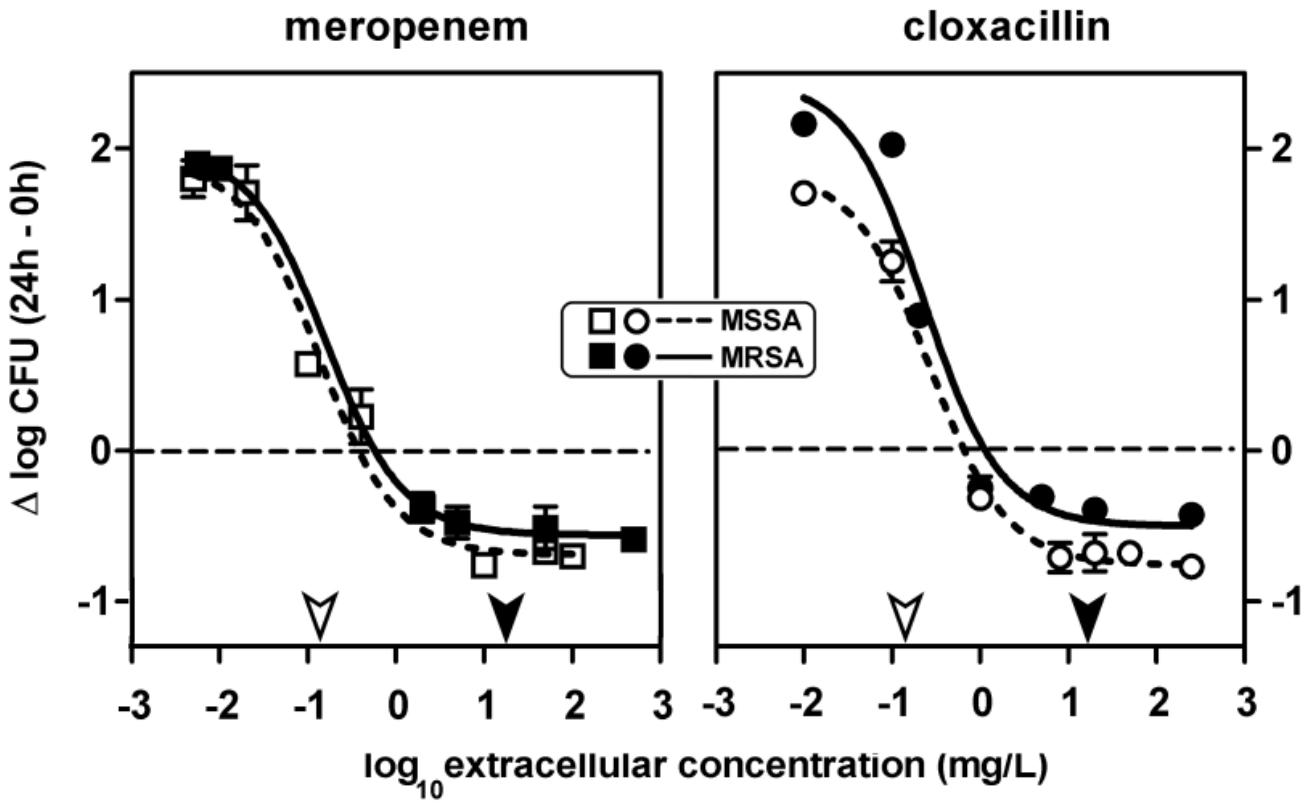
Carryn et al., Infect Dis Clin North Am. (2003) 17:615-34

acid pH of lysosomes

Famous Belgian bier

MRSA vs MSSA: intracellular activity of β-lactams

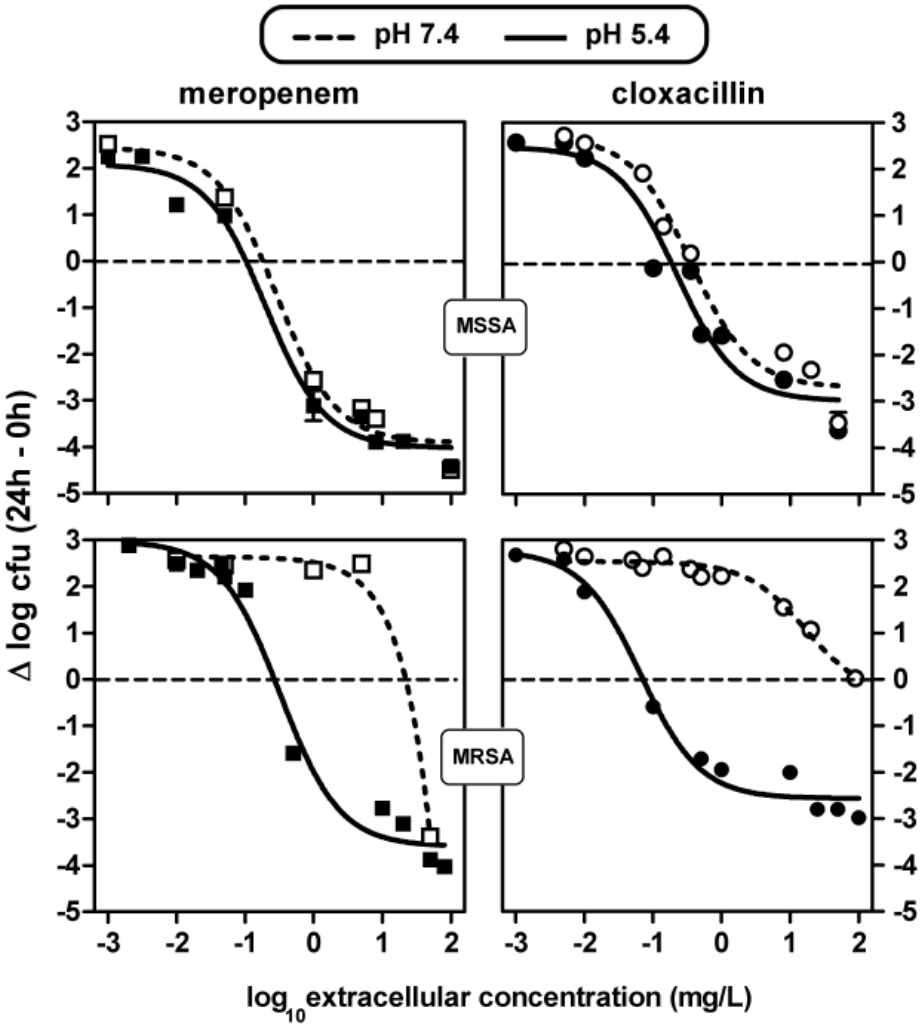
MRSA are as susceptible as MSSA to β-lactams when intracellular !



Lemaire et al., Antimicrob. Agents Chemother. (2007) 51:1627-1632

MRSA vs MSSA: extracellular activity of β-lactams

MRSA are as susceptible as MSSA in broth at acidic pH

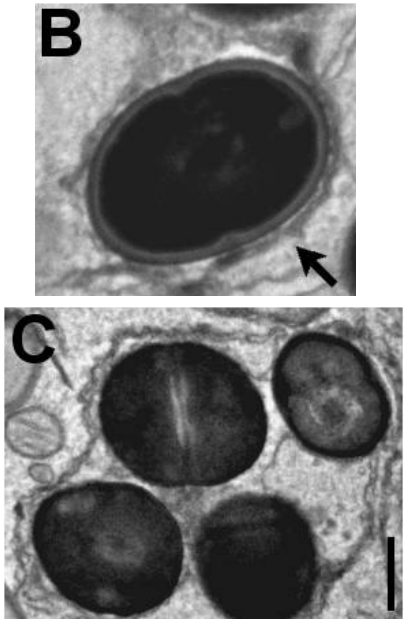


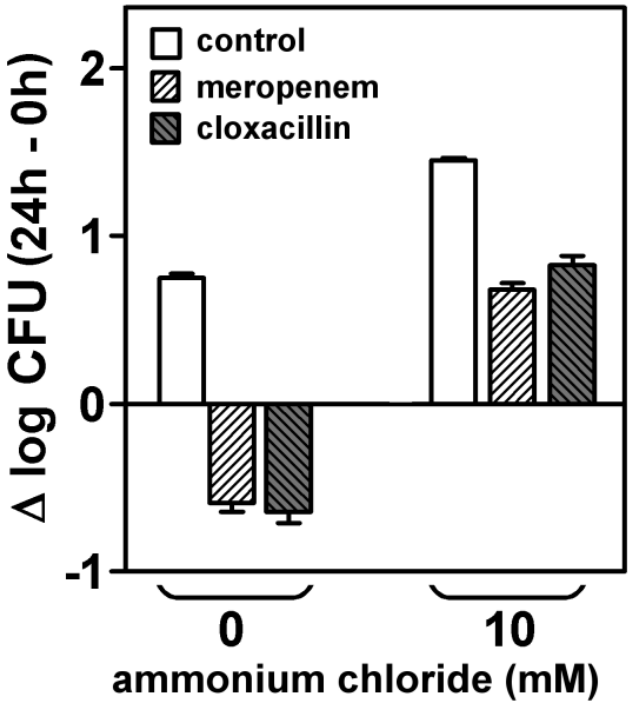
Lemaire et al., Antimicrob. Agents Chemother. (2007) 51:1627-1632

MRSA vs MSSA: extracellular activity of β-lactams

Neutralization of lysosomes makes intracellular MRSA resistant to β-lactams !

MRSA are inside [acidic] vacuoles





Lemaire et al., Antimicrob. Agents Chemother. (2007) 51:1627-1632

PBP2a conformation is modified by acidic pH

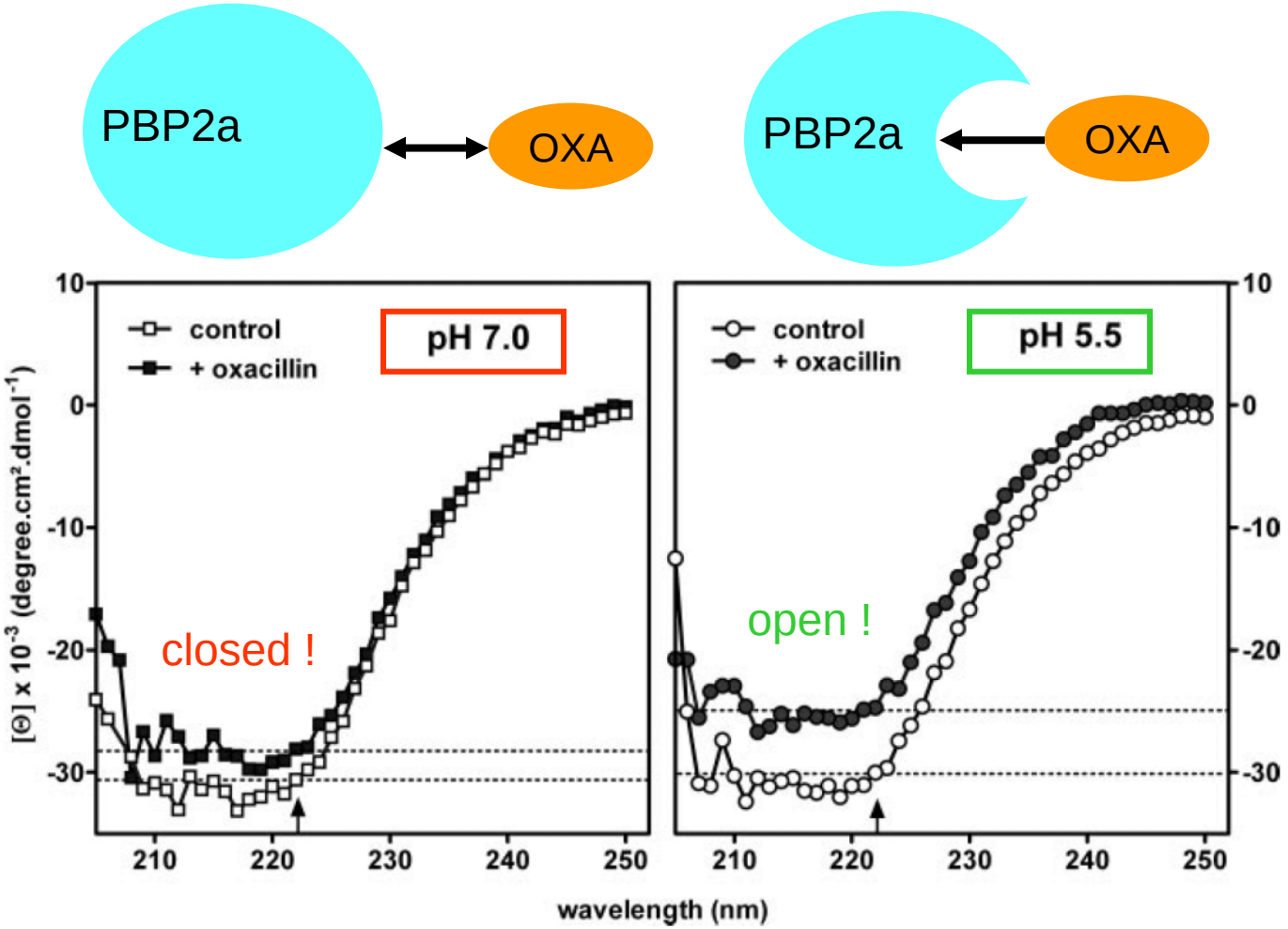


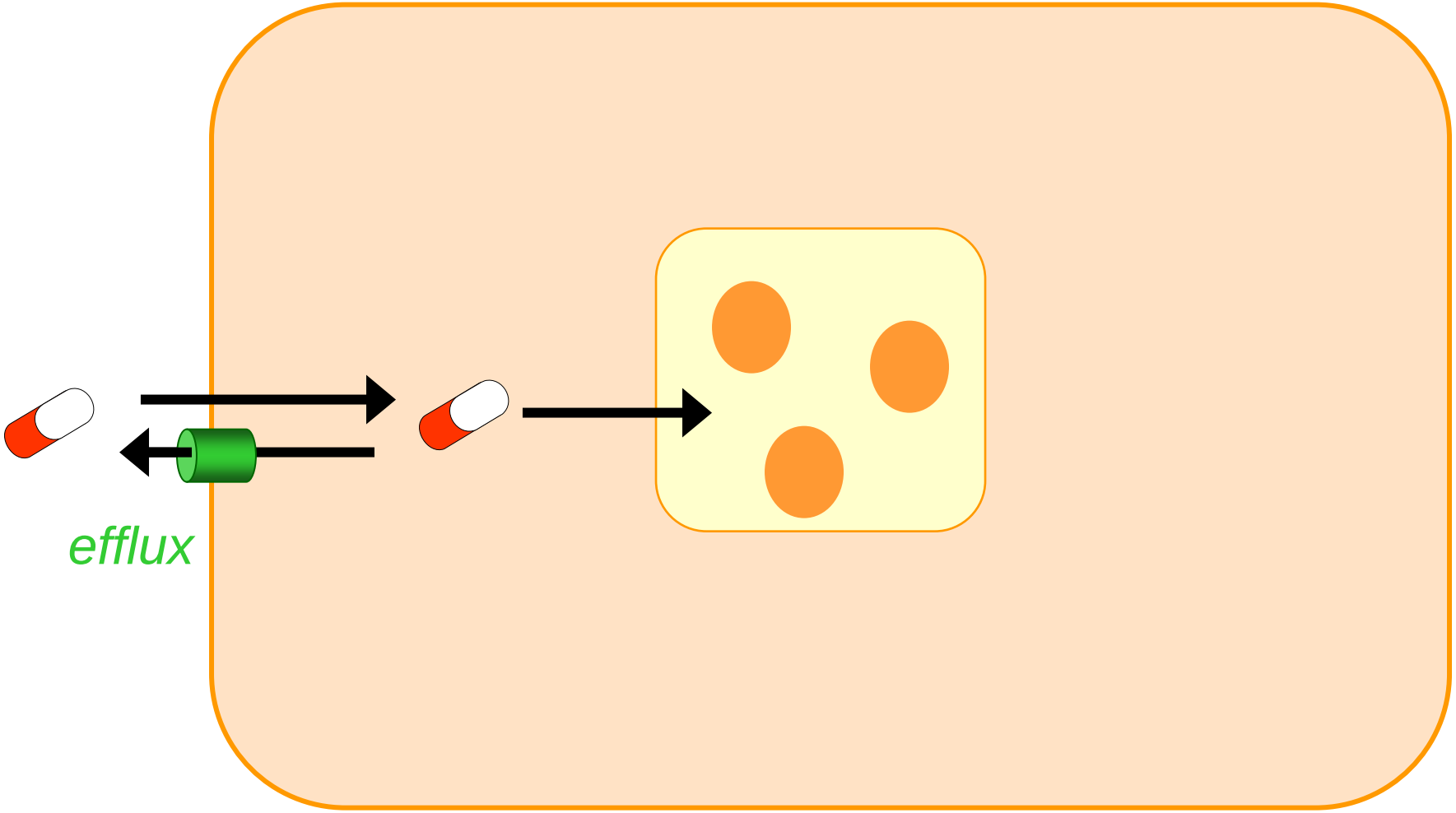
FIGURE 4. **Circular dichroic spectra of PBP 2a at pH 7. 0 (*left panel*) and pH 5.5 (*right panel*) in the absence (*open symbols*) and in the presence (*closed symbols*) of oxacillin (30 μM) for 30 min at 25 °C.** The *thin dotted lines* in each graph represent minima of PBP 2a molar ellipticity at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

Lemaire et al., JBC (2008) 283:12769-76

Efflux pumps

Manneken Pis, who saved Brussels from fire by urinating on a burning fuse

Intracellular vs extracellular activity of antibiotics : PK – PD in action

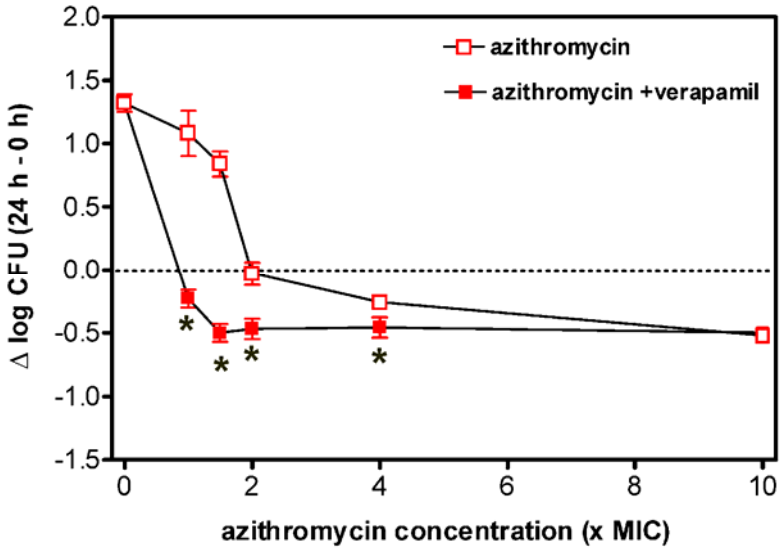


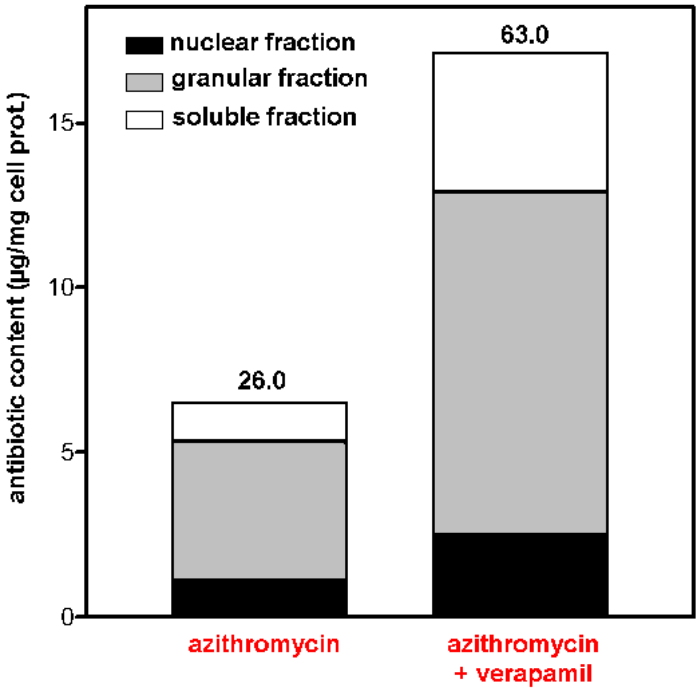
Carryn et al., Infect Dis Clin North Am. (2003) 17:615-34

P-gp as a cellular mechanism of resistance to intracellular efficacy of antibiotics

- intracellular activity
- accumulation in lysosomes

of **azithromycin** are increased by P-glycoprotein inhibitors



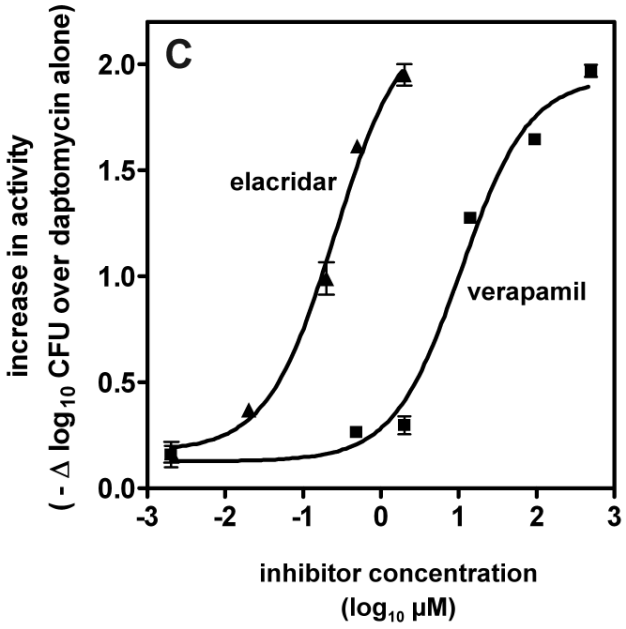


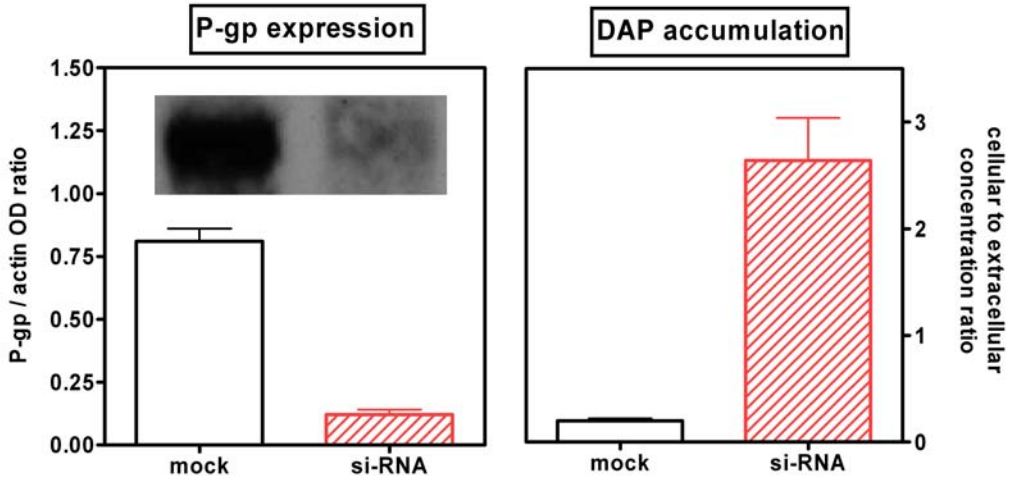
Seral et al., J. Antimicrob. Chemother. (2003) 51:1167-73

P-gp as a cellular mechanism of resistance to intracellular efficacy of antibiotics

- intracellular activity
- accumulation in lysosomes

of **daptomycin** are increased upon P-glycoprotein inhibition or under-expression



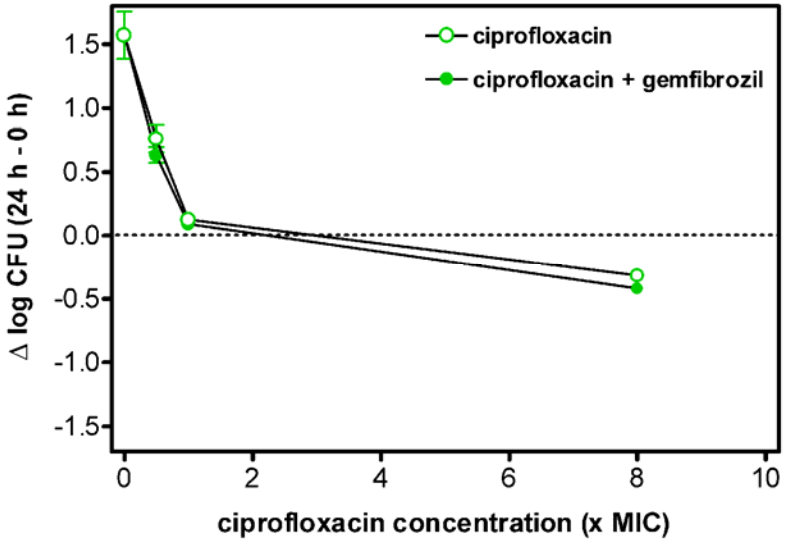


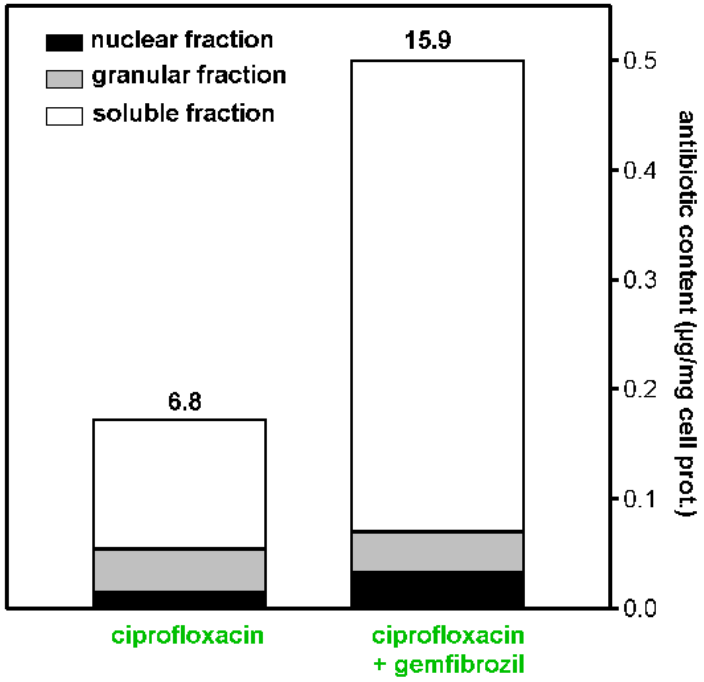
Lemaire et al., Antimicrob. Agents Chemother. (2007) 51:2748-2757

But again targeting the infected compartment is important ….

- intracellular activity
- accumulation in lysosomes

} of **ciprofloxacin** are NOT increased by MRP inhibitors



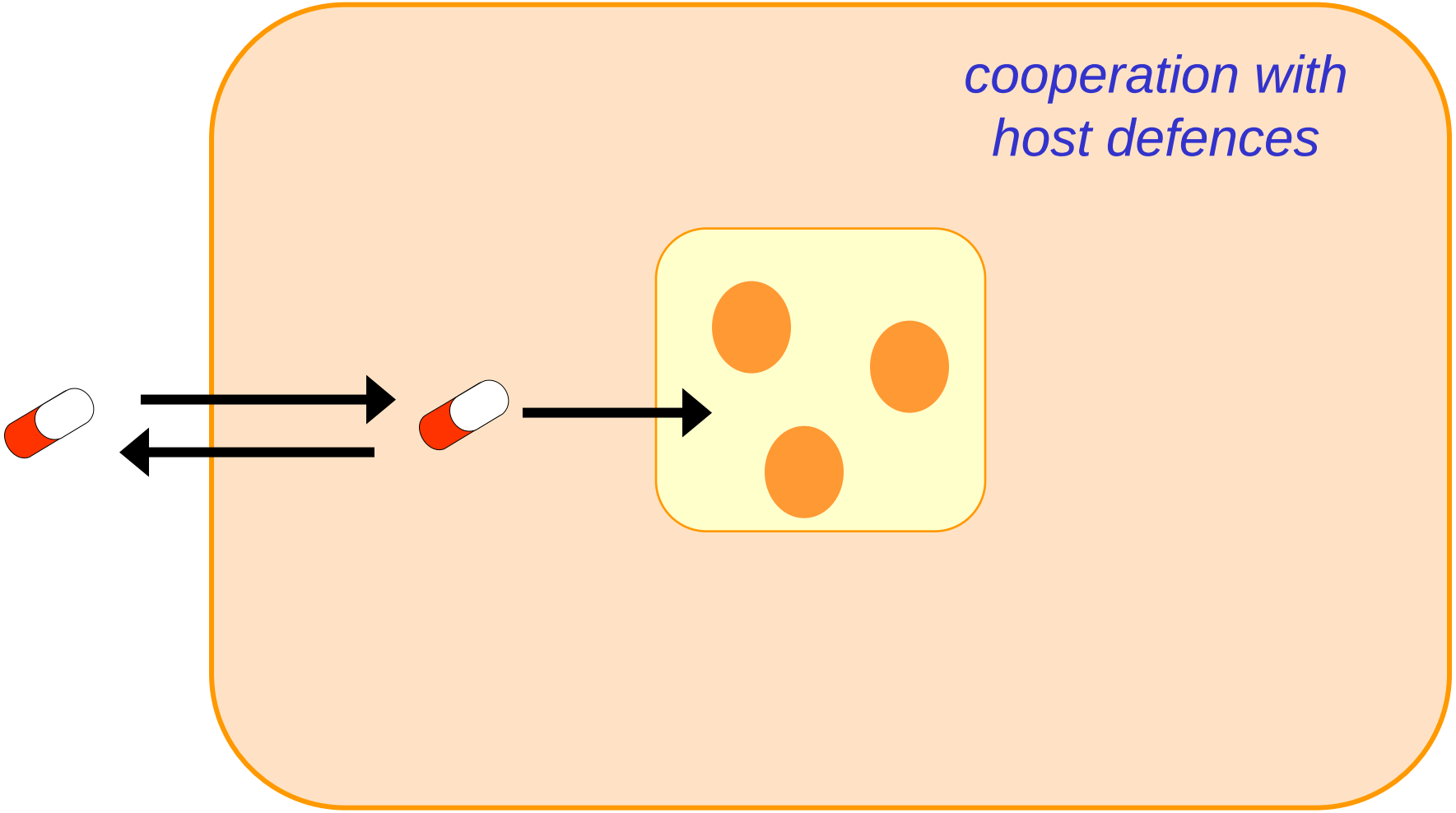


Seral et al., J. Antimicrob. Chemother. (2003) 51:1167-73

Cell metabolic state

Belgian gastronomy

Intracellular vs extracellular activity of antibiotics : PK – PD in action

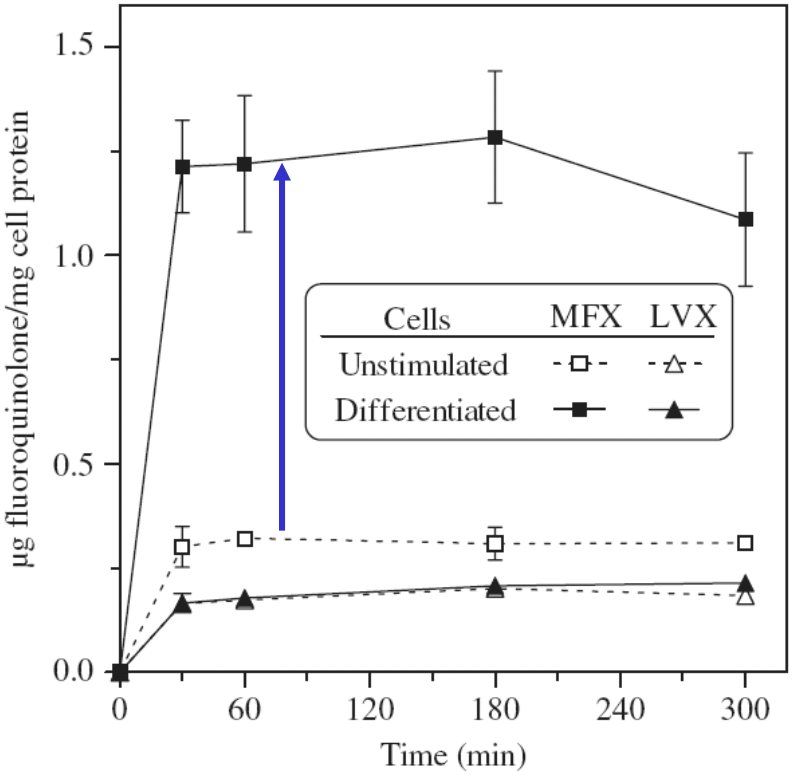


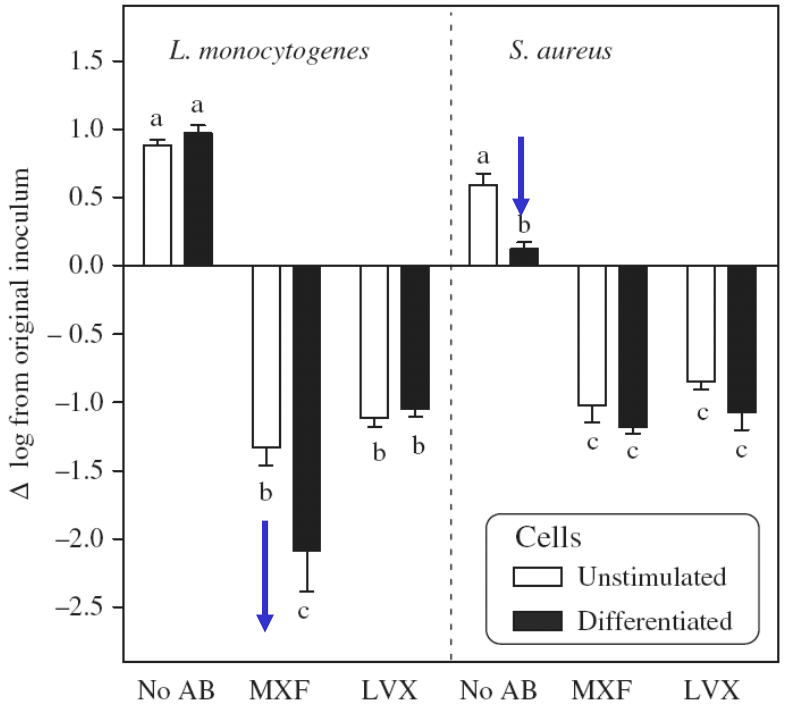
Carryn et al., Infect Dis Clin North Am. (2003) 17:615-34

Cooperation between fluoroquinolones and PMA against *Listeria monocytogenes*

PMA increases
the cellular concentration
of MXF but not of LVX

PMA
- reduces the cellular growth of *S. aureus*
- increases the intracellular activity of MXF against *Listeria* only





Van de Velde et al., JAC (2008) 62:518-21

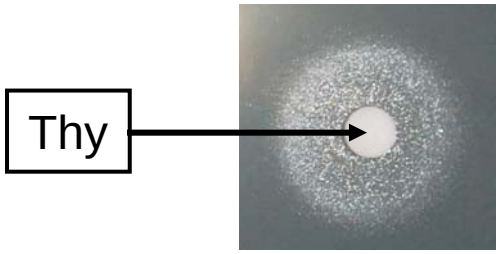
How can these models help the clinican ?

Hôpital Notre Dame à la Rose, Lessines

In vitro models to predict failure/success ?

- SCV isolated from a patient
  - with complicated prosthetic vascular graft infection and bacteraemia,
  - unsuccessfully treated successively with
    - cotrimoxazole (SMX/TMP),
    - minocycline (MIN),
    - a combination of vancomycin and rifampin (VAN-RIF)
    - a combination of linezolid and rifampin (LNZ-RIF)

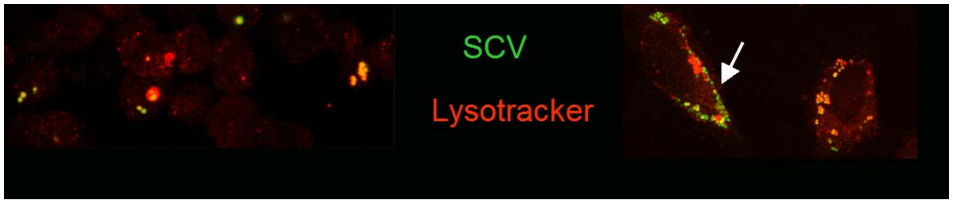
- thymidine-auxotrophic MRSA, growing as tiny, non-pigmented and non-hemolytic colonies on Columbia blood agar.



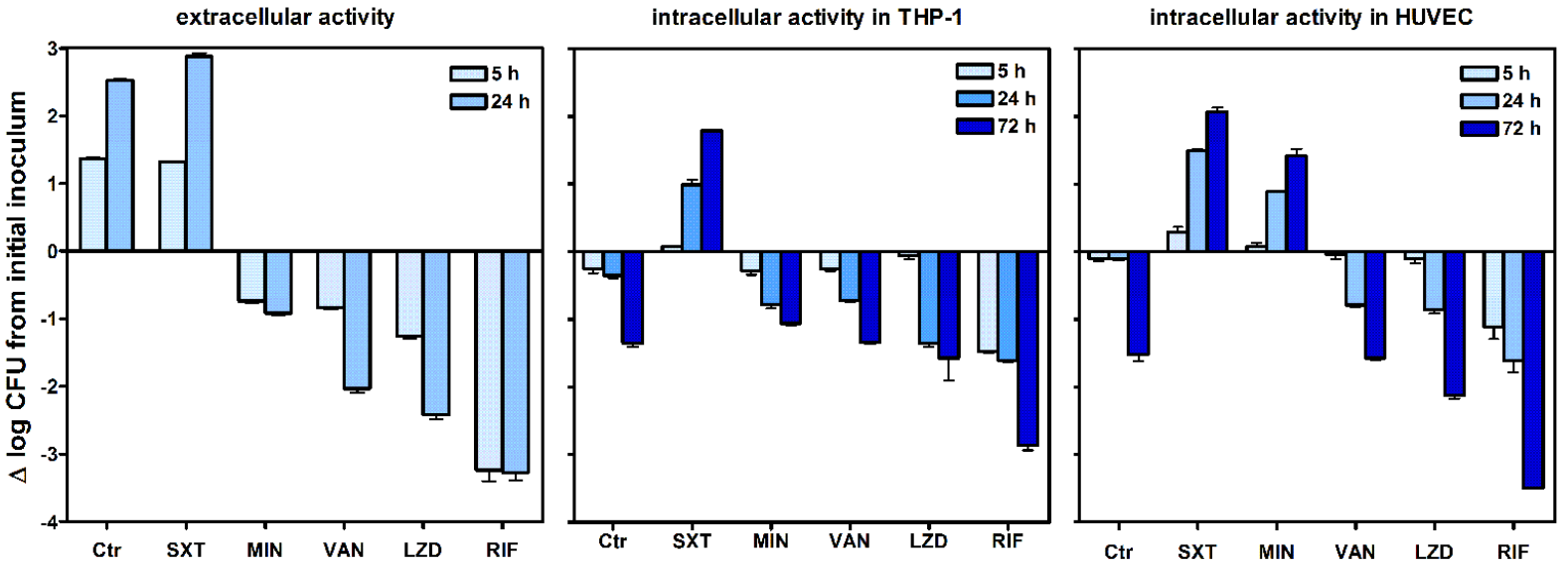
- resistant to OXA, SXT, CLI, LIN, ERY, quinupristin and TET.

Nguyen et al., ECCMID (2008) - A970

In vitro models to predict failure/success ?



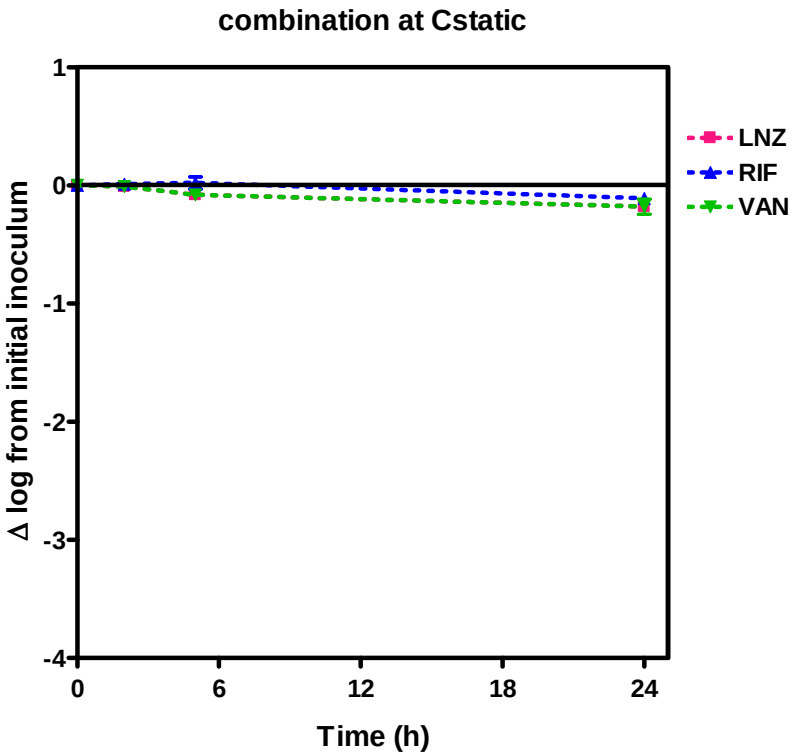
THP-1 macrophages HUVEC endothelial cells



Nguyen et al., ECCMID (2008) - A970

In vitro models to predict failure/success ?

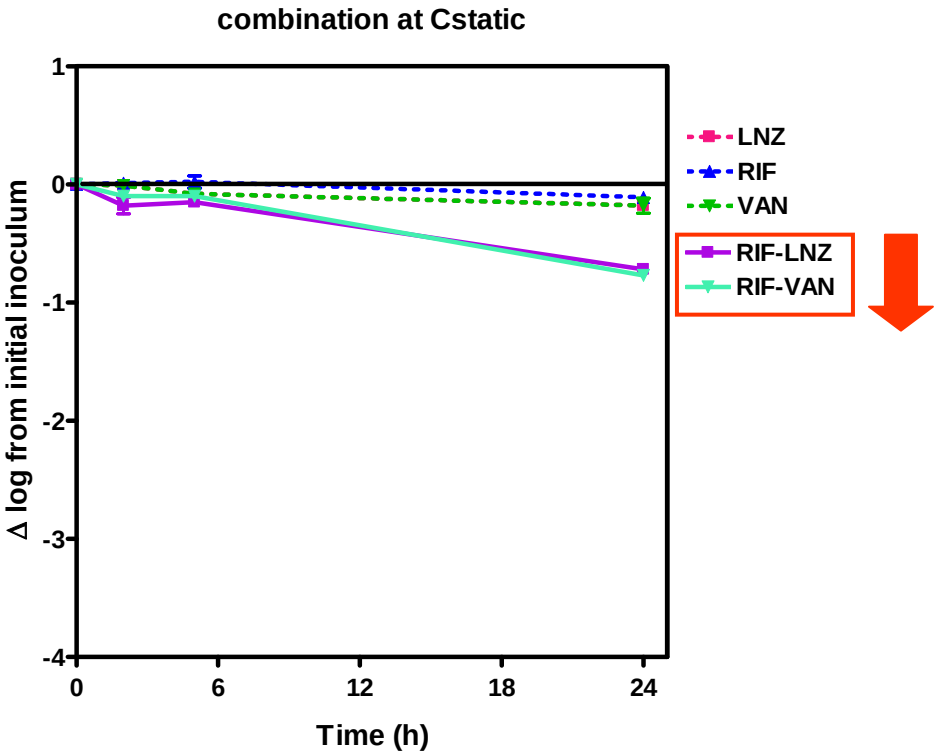
combinations received by the patient



Nguyen et al., ECCMID (2008) - A970

In vitro models to predict failure/success ?

combinations received by the patient



Nguyen et al., ECCMID (2008) - A970

In vitro models to predict failure/success ?

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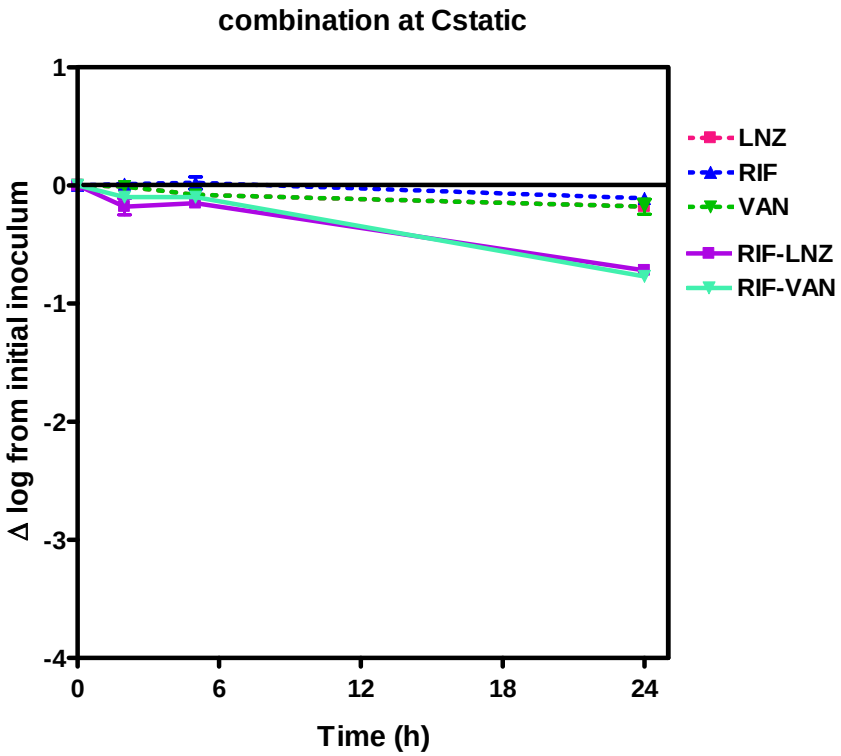

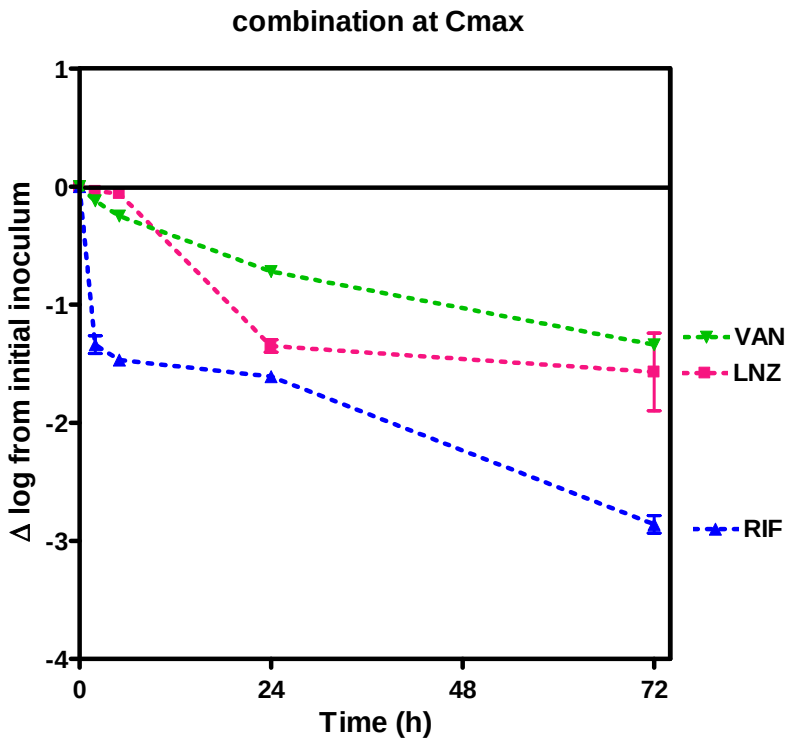

Nguyen et al., ECCMID (2008) - A970

In vitro models to predict failure/success ?

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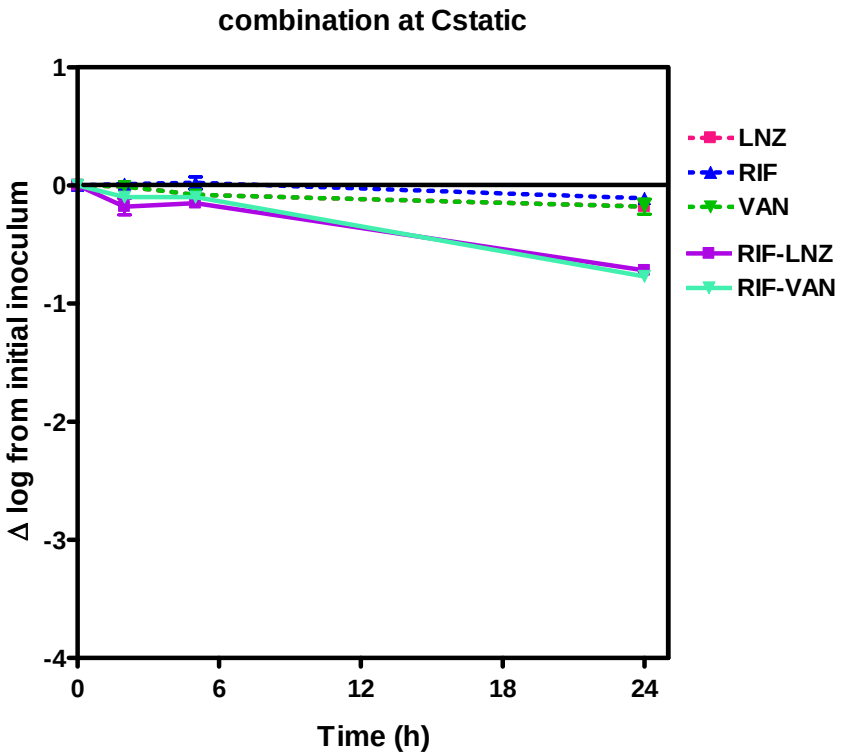
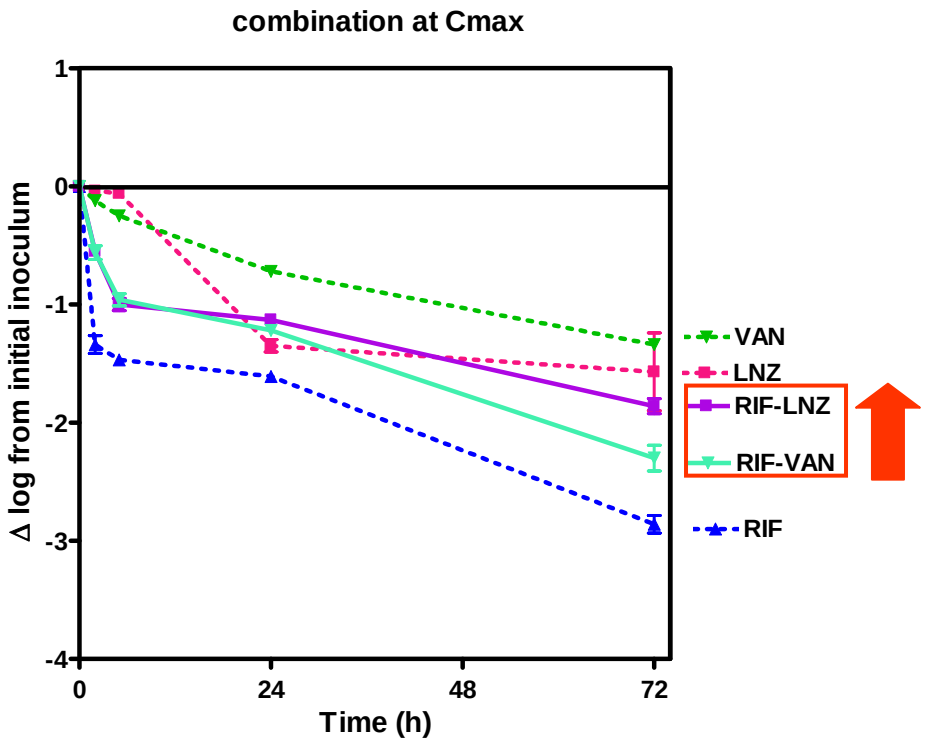
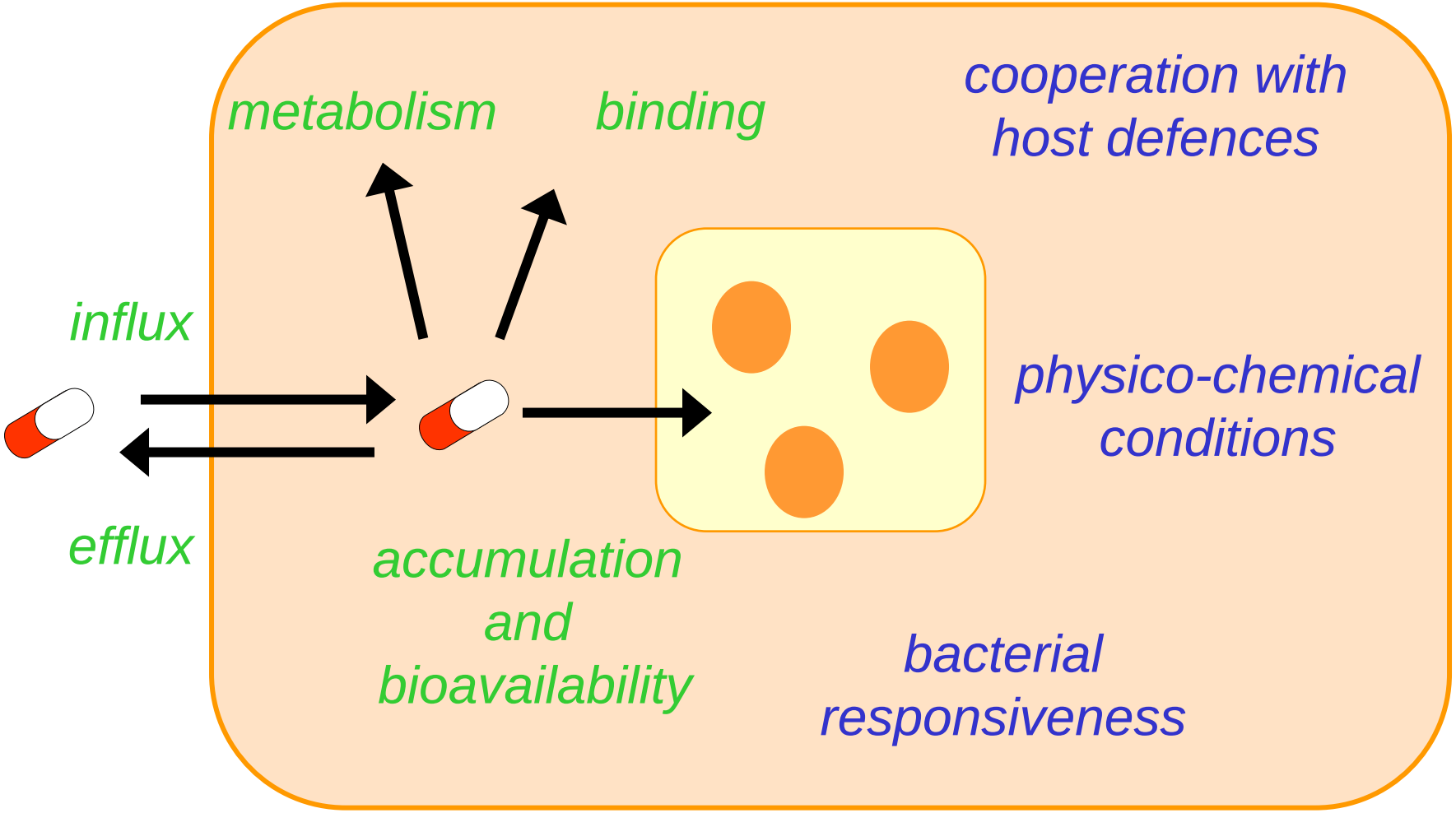




Nguyen et al., ECCMID (2008) - A970

Intracellular vs extracellular activity of antibiotics : PK – PD in action

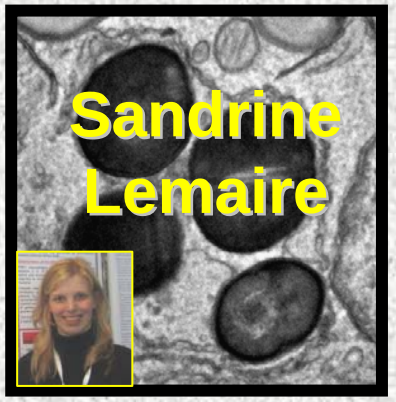


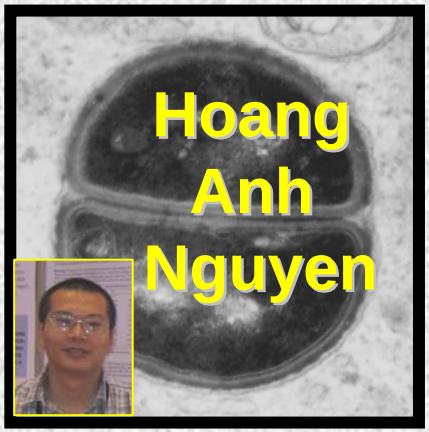
Carryn et al., Infect Dis Clin North Am. (2003) 17:615-34

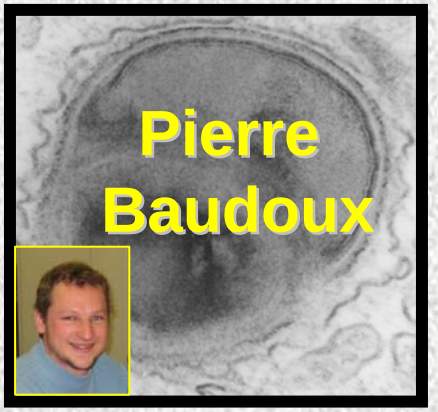
Still a lot of work ahead to fully understand …

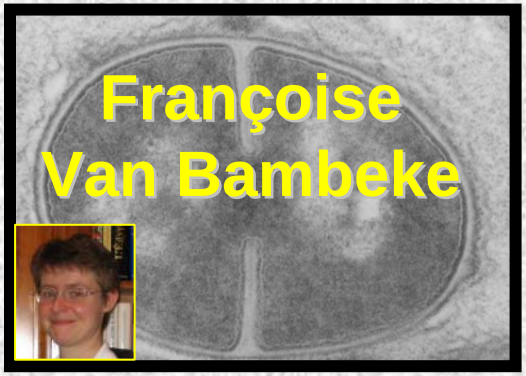
Magritte, Belgian surrealism

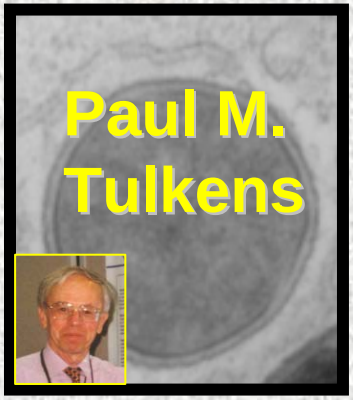
Our "Staph" team

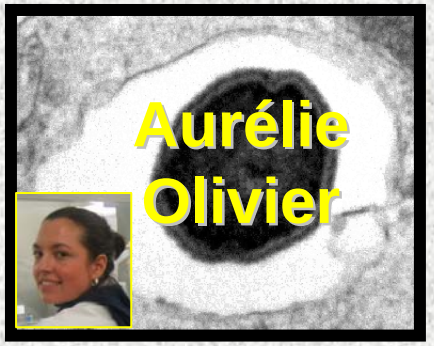












In collaboration with :

- Y. Glupczynski, cliniques universitaires de l'UCL à Mont-Godinne, Yvoir, Belgium
- A. Vergison, O. Denis, M. Struelens, Hôpital Erasme, ULB, Brussels, Belgium
- P. Appelbaum, Hershey Medical Center, Hershey, PA, USA

We wish you a Happy New Year !