

Activity of antibiotics against intracellular *S. aureus*

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Disclosures

- Grants-in-aid from Targanta Therapeutics and Theravance Inc.
- Member of the European Advisory board of Targanta Therapeutics

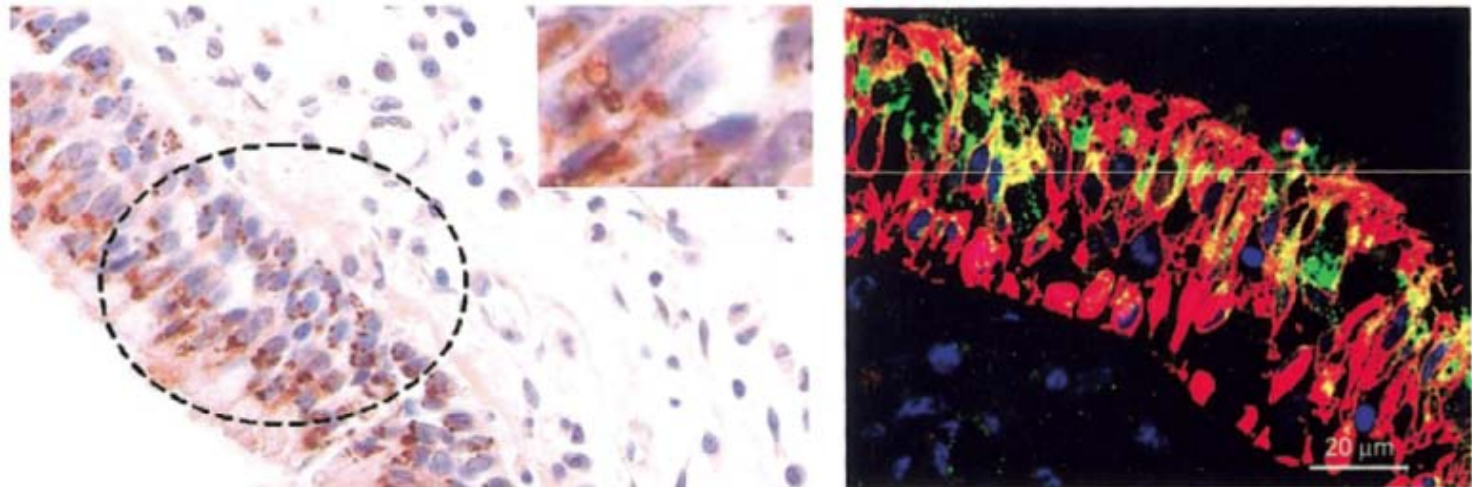
[< www.facm.ucl.ac.be >](http://www.facm.ucl.ac.be)

Intracellular *S. aureus* : is it important ?



Intracellular reservoir evidenced *in vivo*

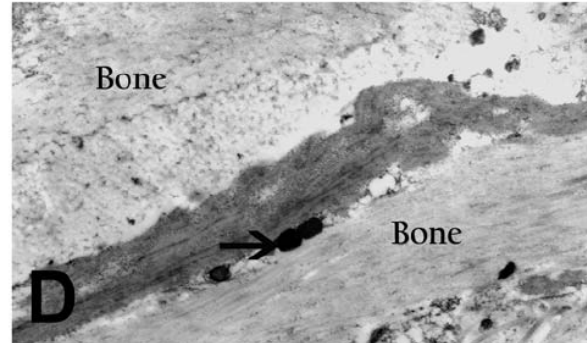
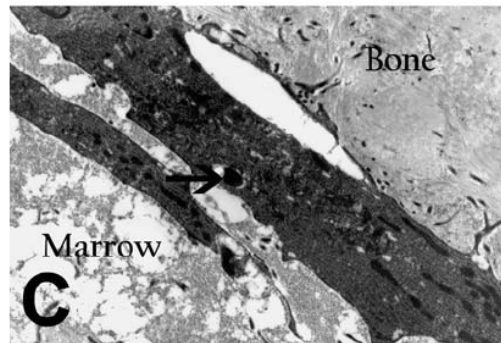
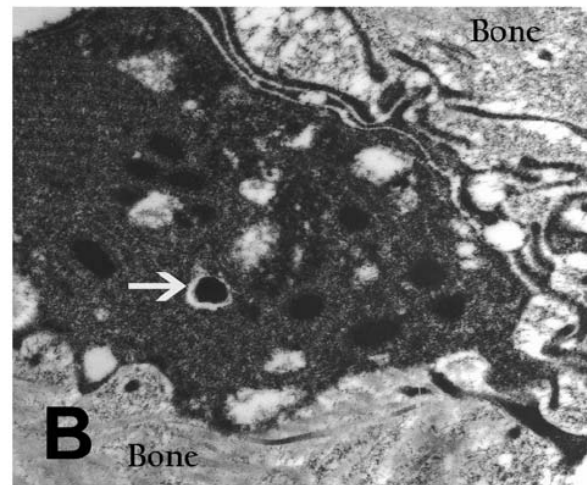
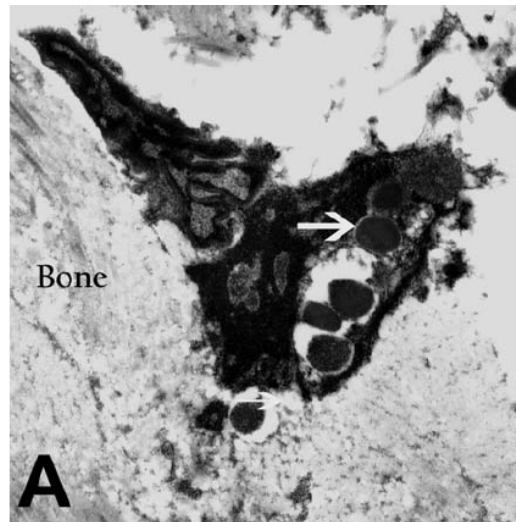
Evidence of an intracellular reservoir in the nasal mucosa of patients with recurrent *Staphylococcus aureus* rhinosinusitis



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

Intracellular reservoir evidenced *in vivo*

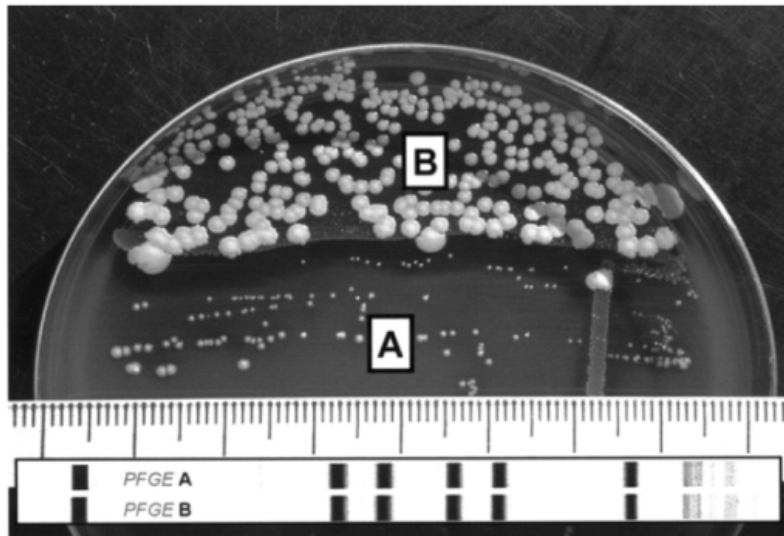
Evidence of an intracellular reservoir
in osteocytes (A,B), osteoblasts (C) and bone matrix
of a patient with recurrent osteomyelitis



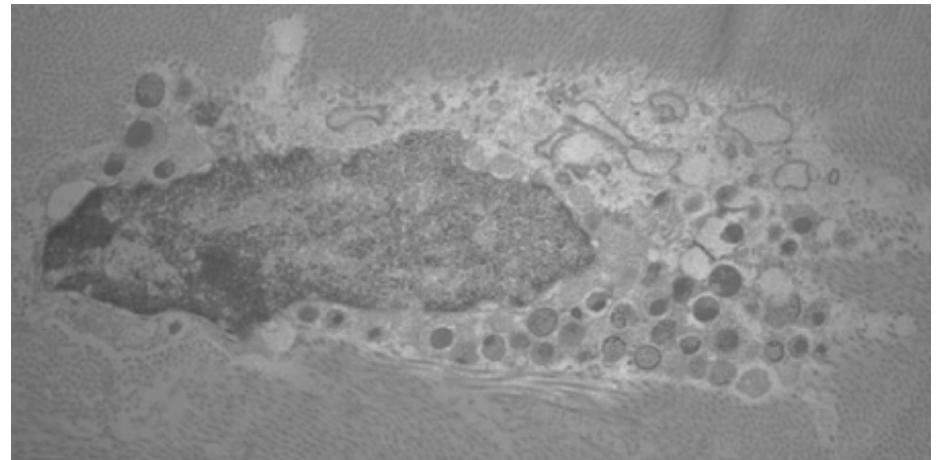
Bosse et al., J Bone Joint Surg Am. (2005) 87:1343-7

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.



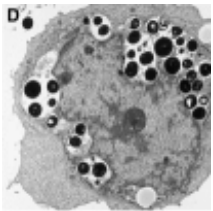
* Flucloxacillin, Ciprofloxacin + Rifampin, Vancomycin + Fosfomycin

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

S. aureus can survive and multiply in several cell types



Mechanisms of *Staphylococcus aureus* invasion of cultured osteoblasts.
Ellington et al. Microb Pathog. (1999) 26:317-23.



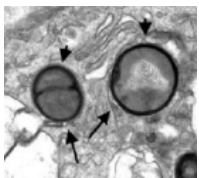
Invasion of human keratinocytes by *Staphylococcus aureus* and intracellular bacterial persistence represent haemolysin-independent virulence mechanisms that are followed by features of necrotic and apoptotic keratinocyte cell death.

Mempel et al. Br J Dermatol. (2002) 146:943-51.



In vitro ability of *Staphylococcus aureus* isolates from bacteraemic patients with and without metastatic complications to invade vascular endothelial cells.

Park et al. J Med Microbiol. (2007) 56:1290-5.



Staphylococcus aureus invasion of bovine mammary epithelial cells.

Almeida et al. J Dairy Sci. (1996) 79:1021-6.

Brouillette et al. Microb Pathog. (2003) 35:159-68.

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.

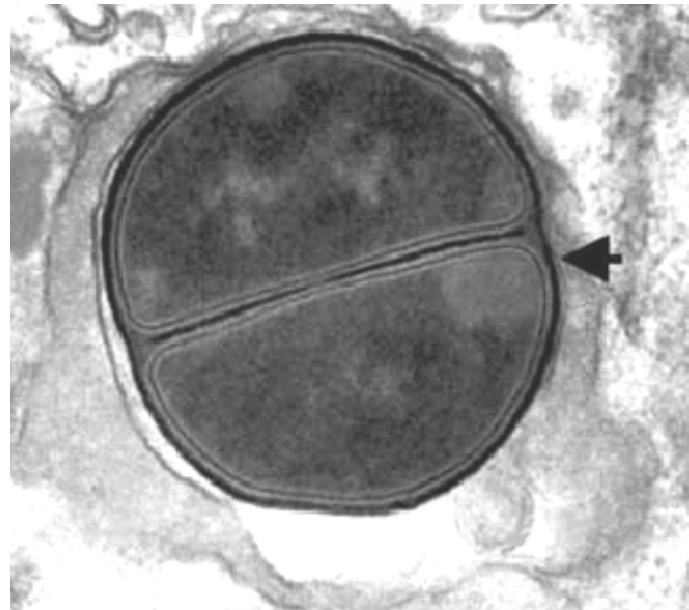
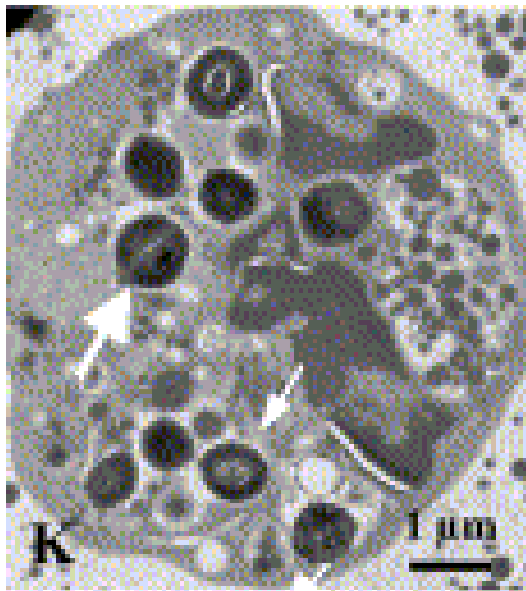


Demonstration of intracellular *Staphylococcus aureus* in bovine **mastitis** alveolar cells and macrophages isolated from naturally infected cow milk.

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

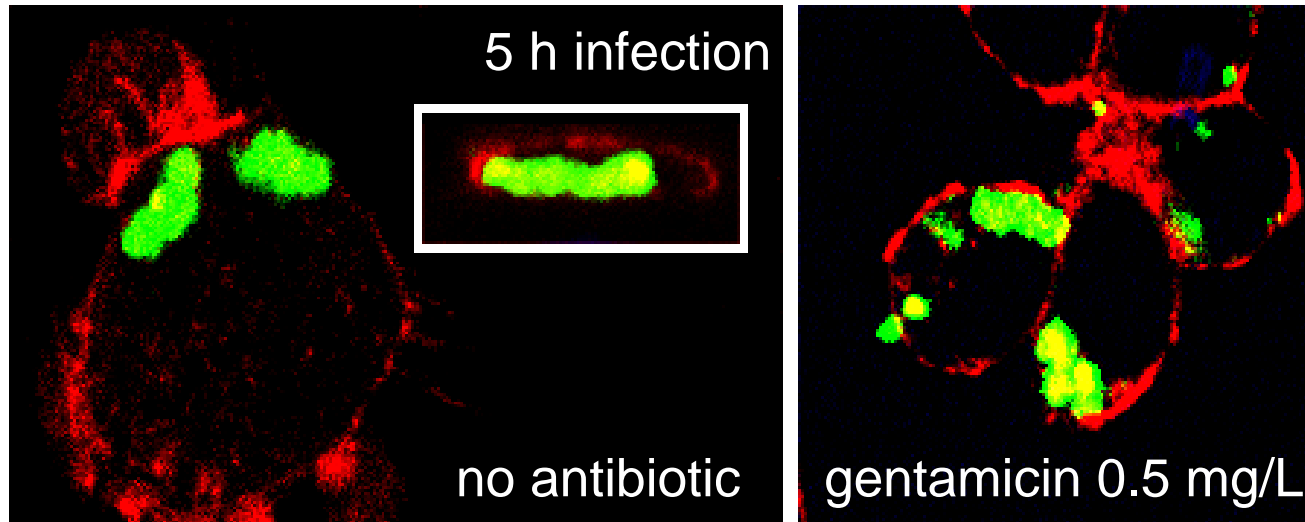


Brouillette et al., Vet Microbiol (2004) 101:253-262; Microb Pathog. (2003) 35:159-68

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



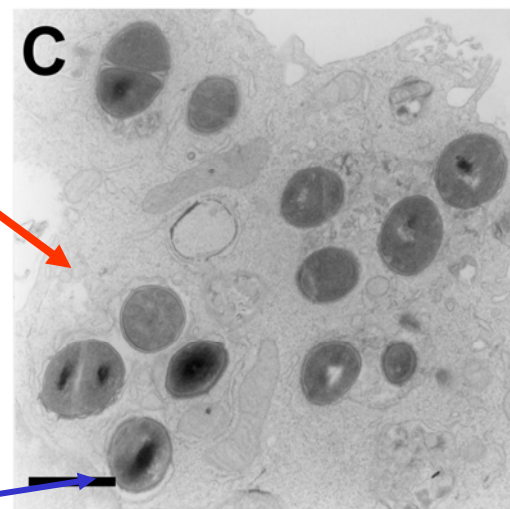
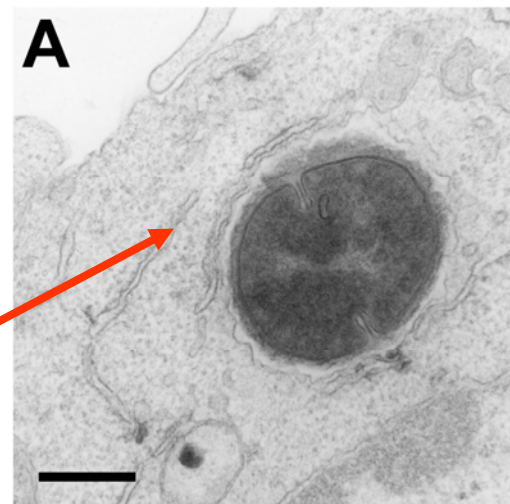
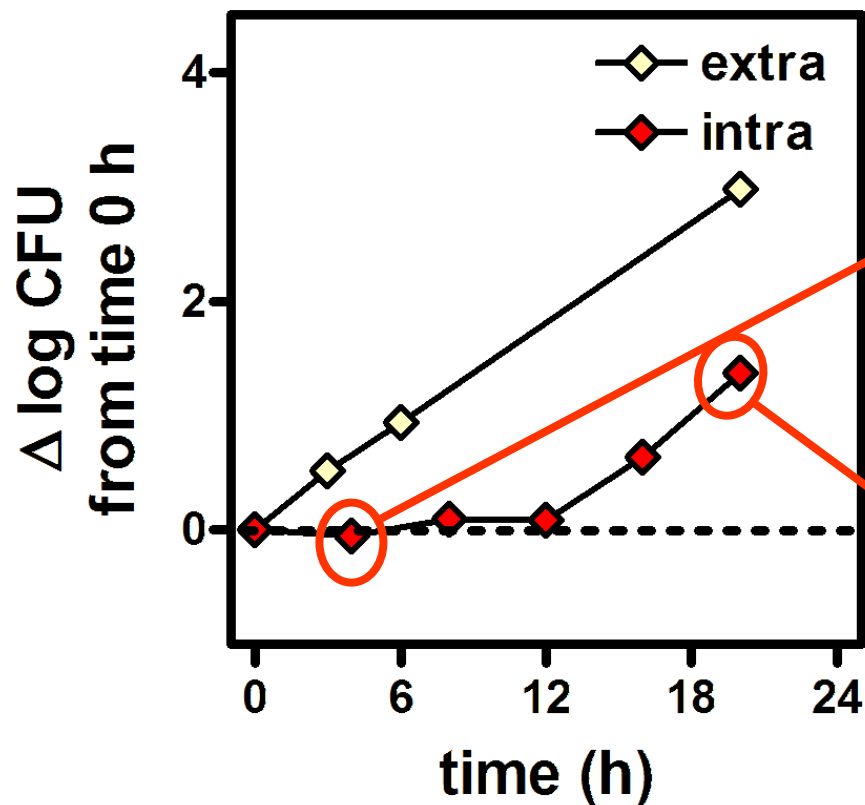
Setting up a model of intracellular infection over a 24 h period of time



- 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 µg/ml (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 . . .

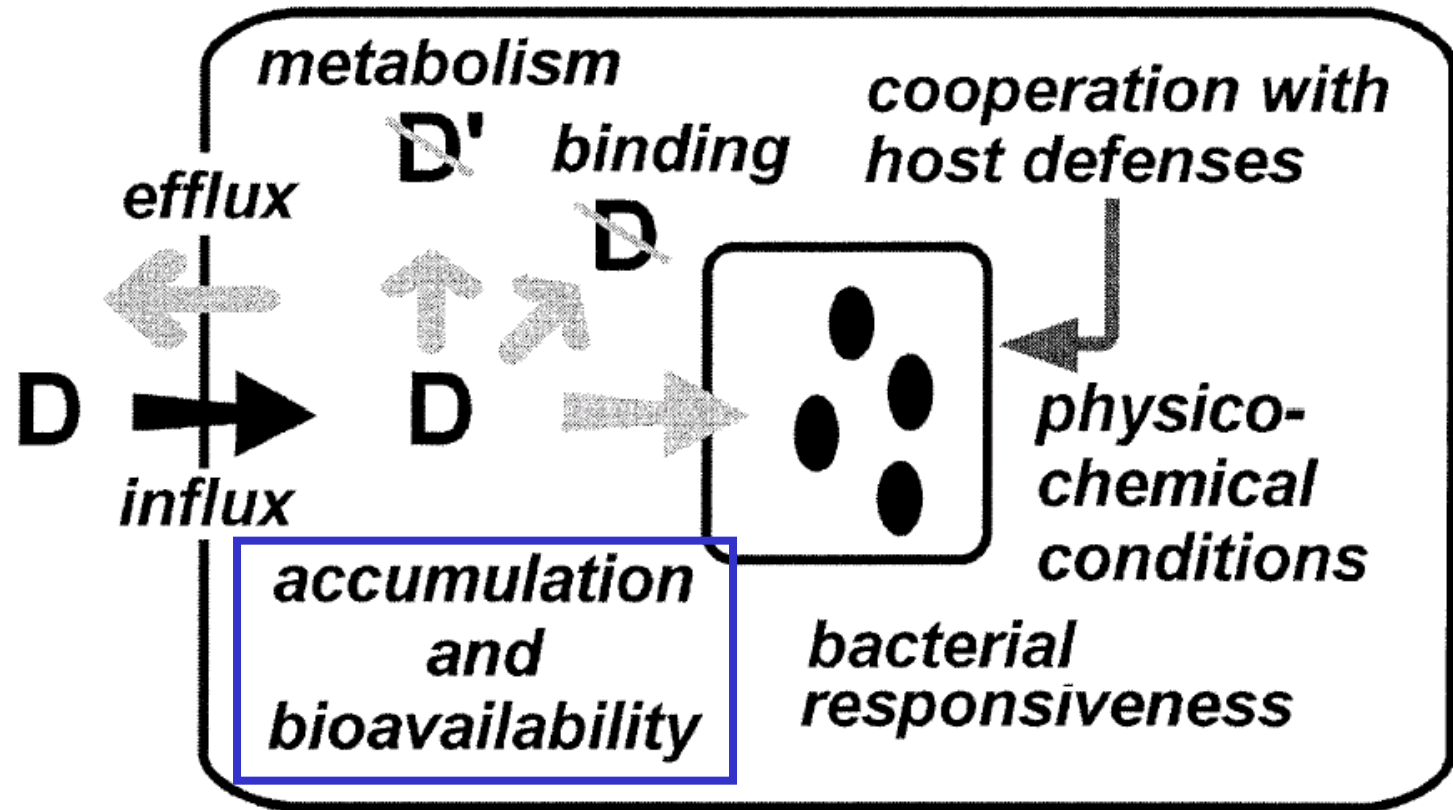
Very complicated ?



Very simple ?



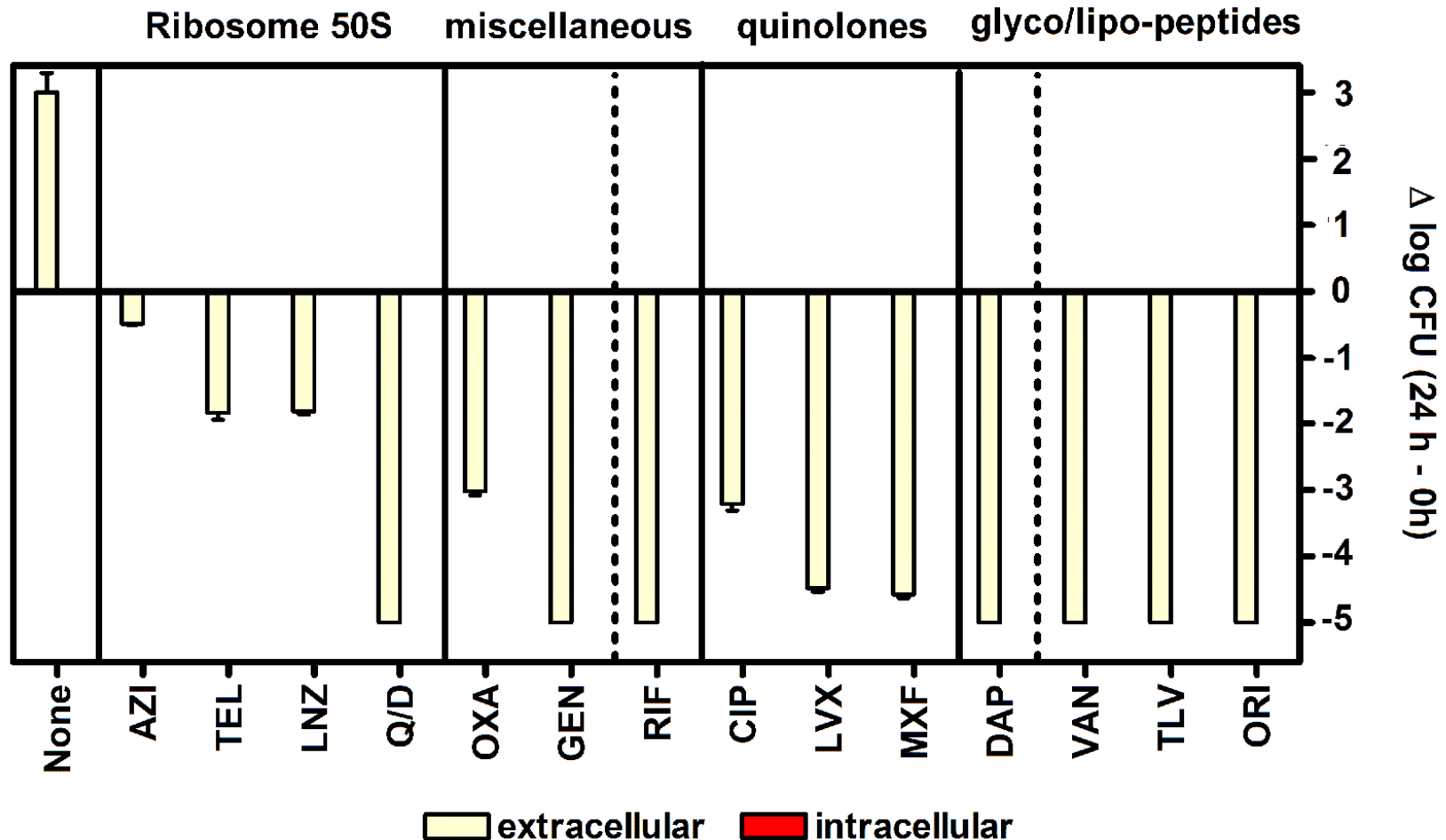
Intracellular vs extracellular activity of antibiotics : PK – PD in action



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

Extracellular vs intracellular activity at Cmax

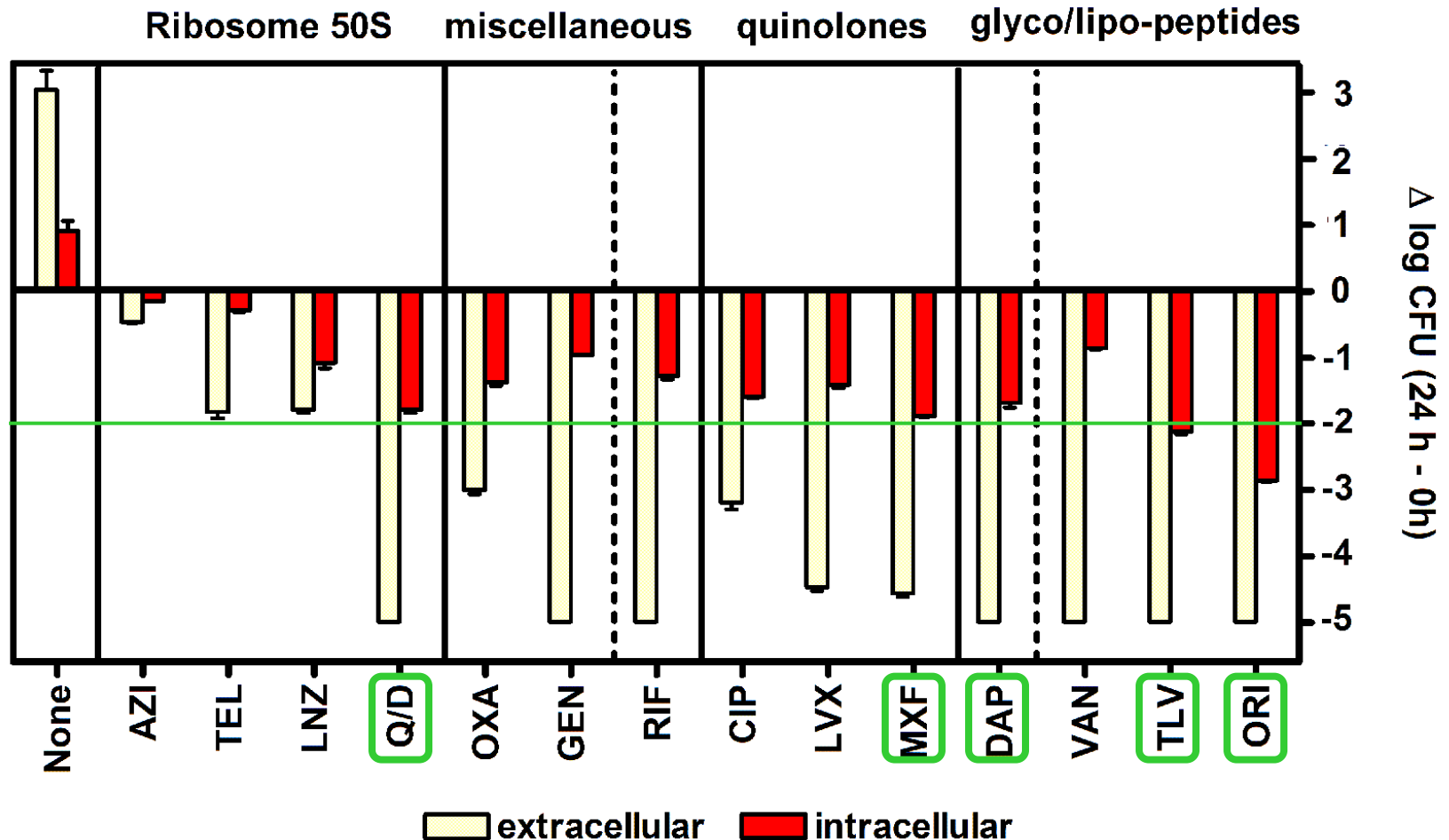
THP-1; 24 h, ATCC25923, antibiotics at Cmax



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

Extracellular vs intracellular activity at Cmax

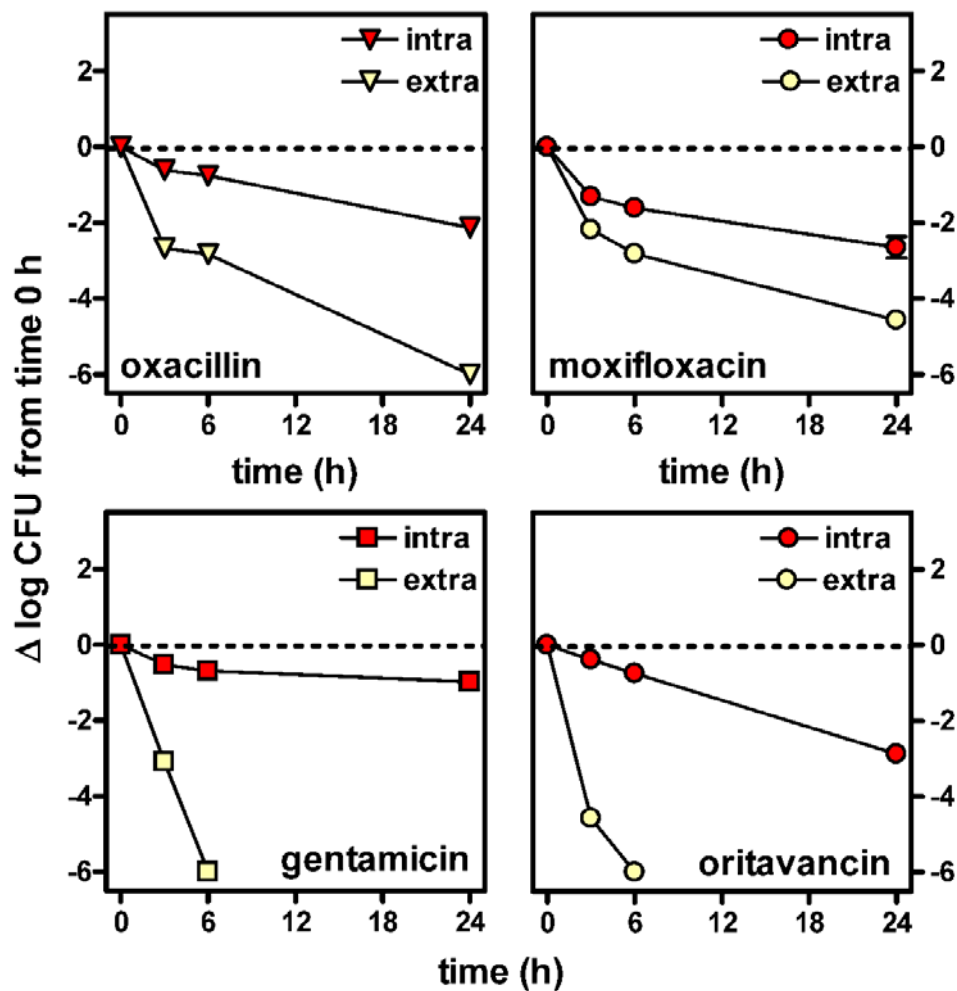
THP-1; 24 h, ATCC25923, antibiotics at Cmax



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

Pharmacodynamic relationships: time-effects at Cmax

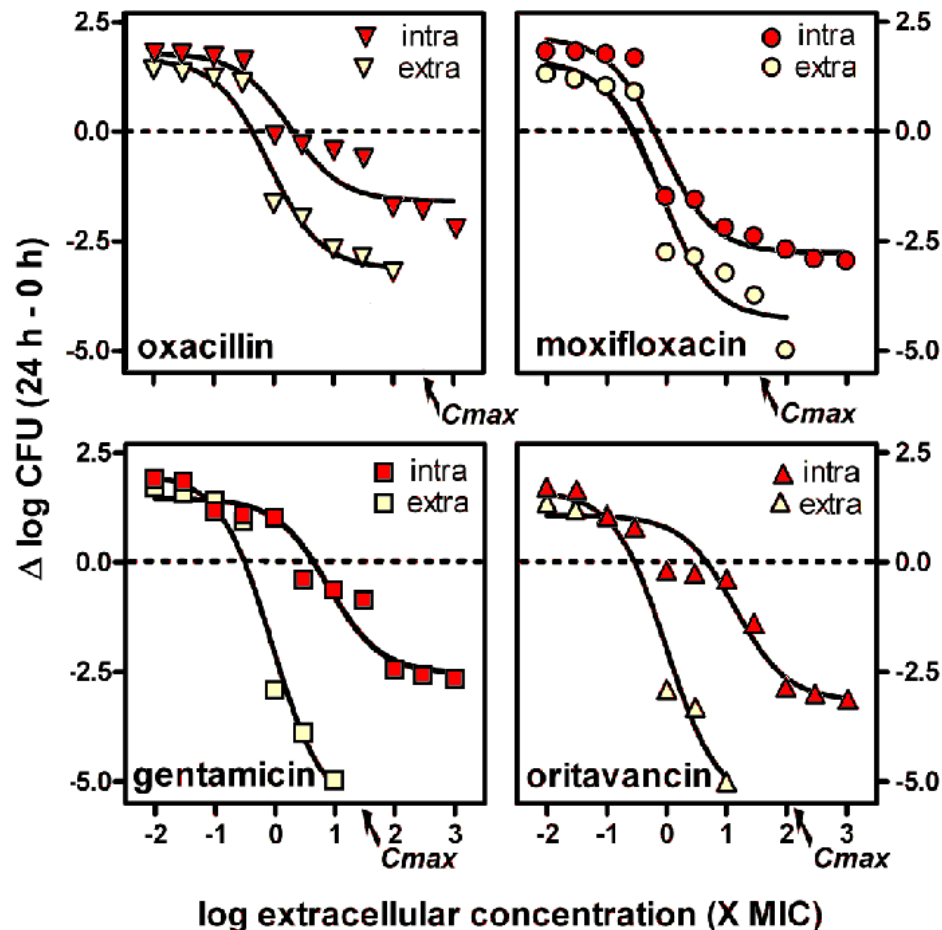
Slower killing rate intracellularly



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

Pharmacodynamic relationships: concentration-effects at 24 h

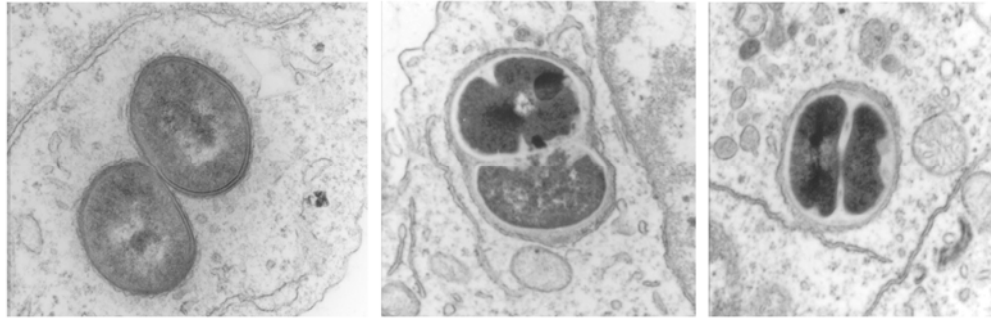
Concentration-dependent killing; lower Emax intracellularly



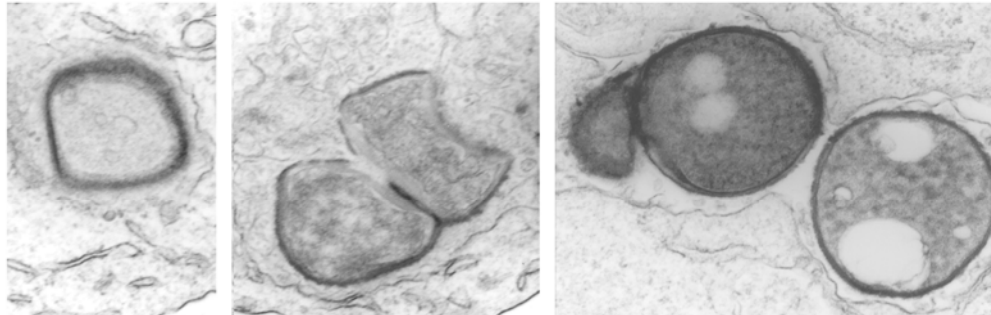
Barcia-Macay et al., *Antimicrob Agents Chemother.* (2006) 50:841-51

Intracellular killing is visible for antibiotics working on cell wall

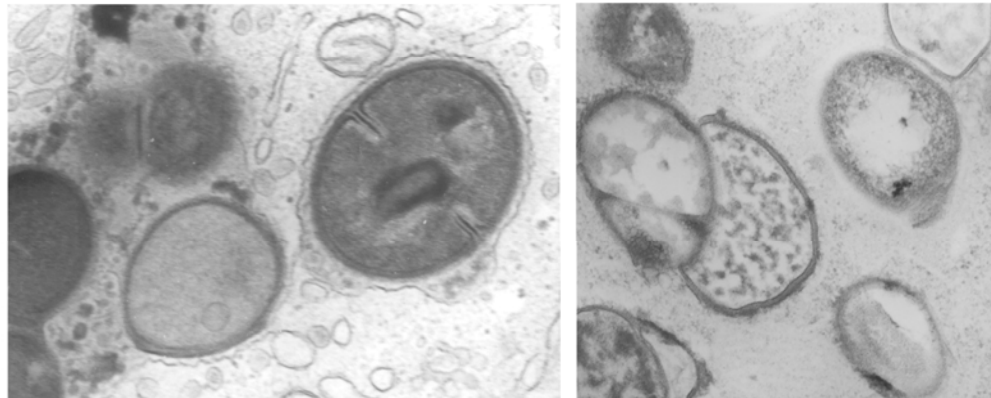
control



oxacillin



oritavancin

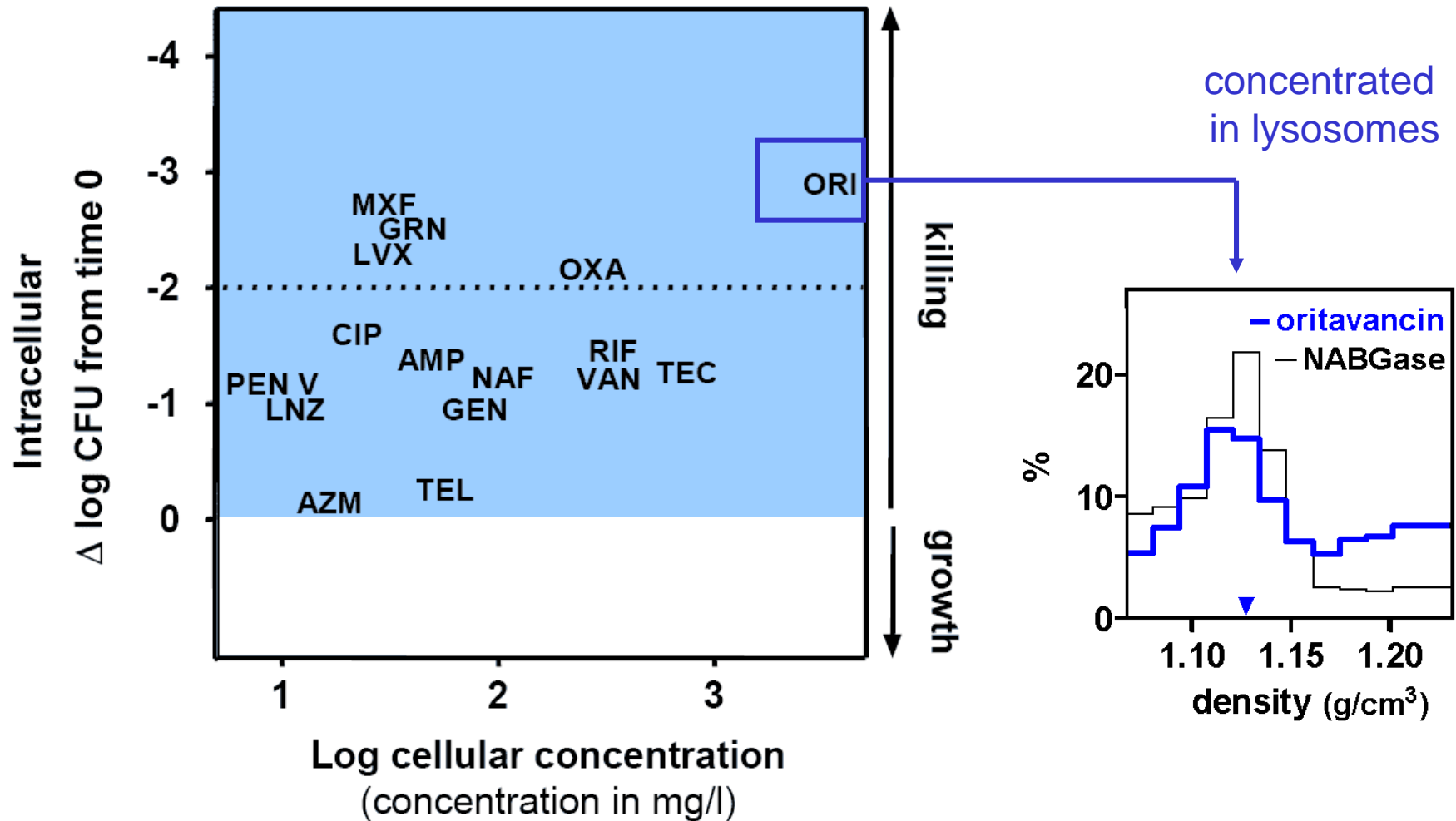


Barcia-Macay et al., Antimicrob Agents Chemother. (2006) 50:841-51

Any relationship between activity and accumulation ?

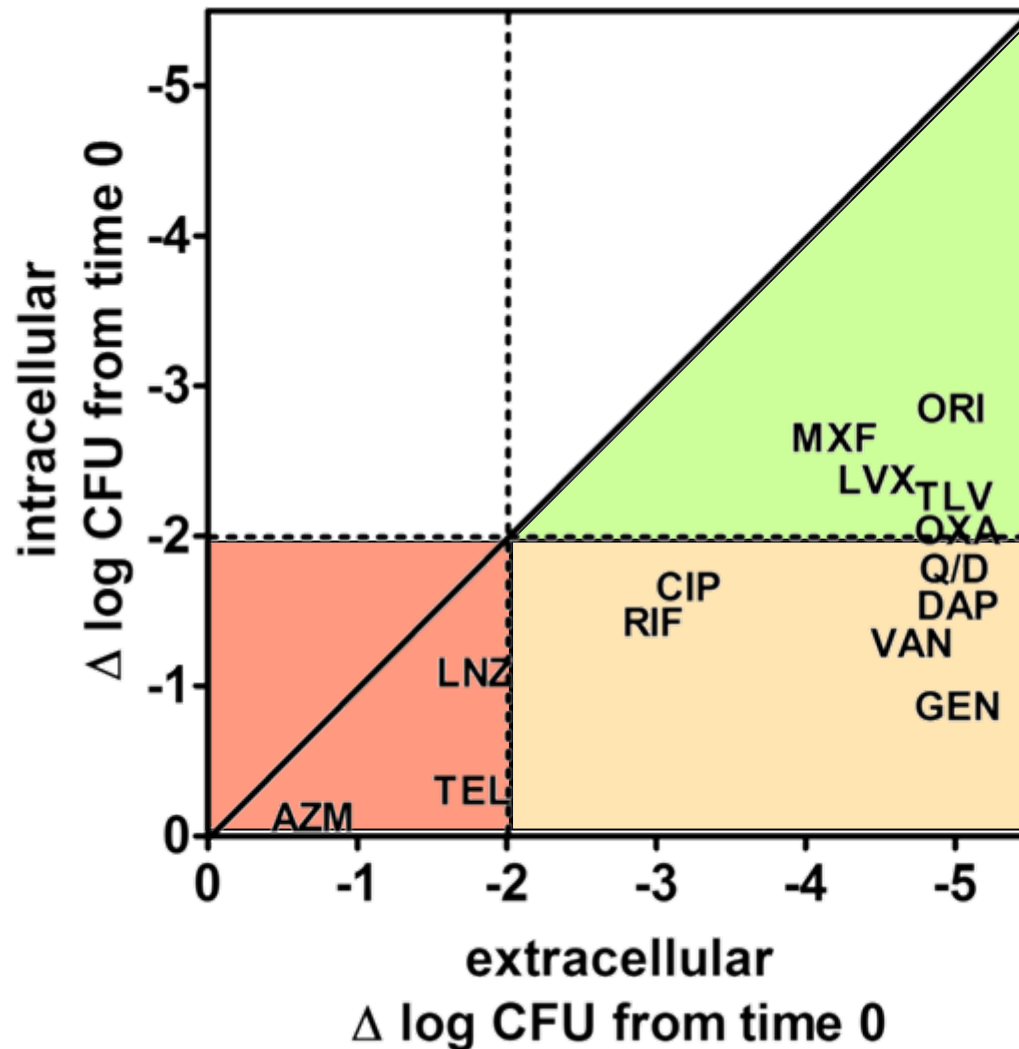
THP-1; 24 h, ATCC25923, antibiotics at Cmax

Staphylococcus aureus



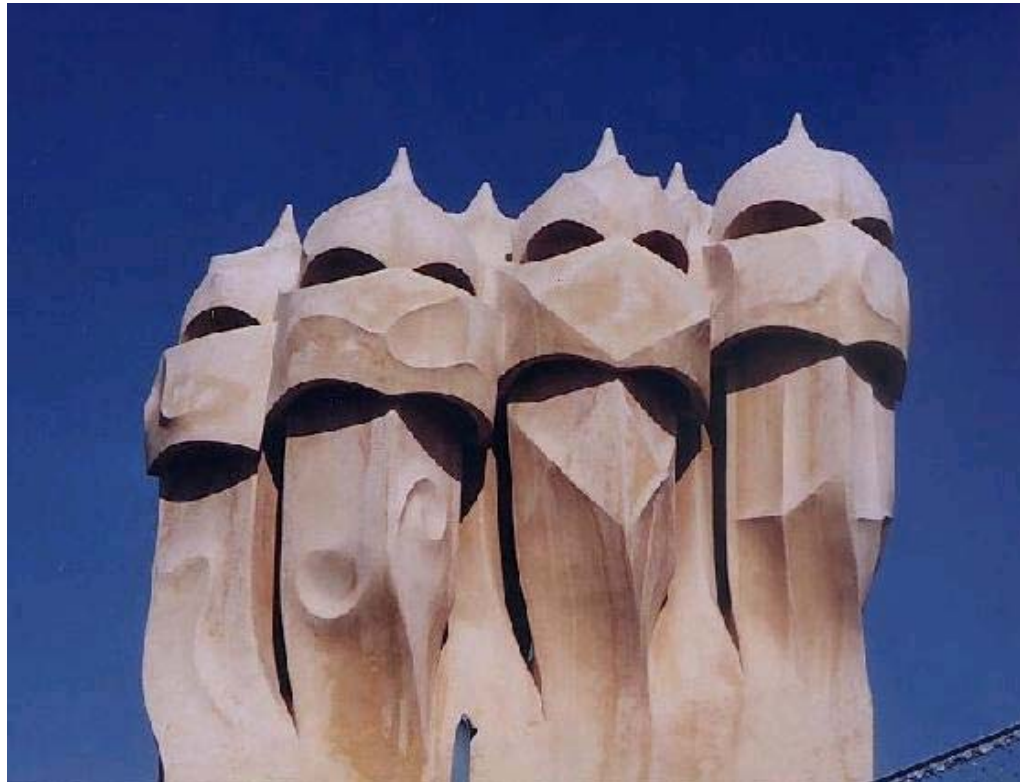
Van Bambeke et al., *Curr Opin Drug Discov Devel.* (2006) 9:218-30
Van Bambeke et al., *Antimicrob Agents Chemother.* (2004) 48:2853-60

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

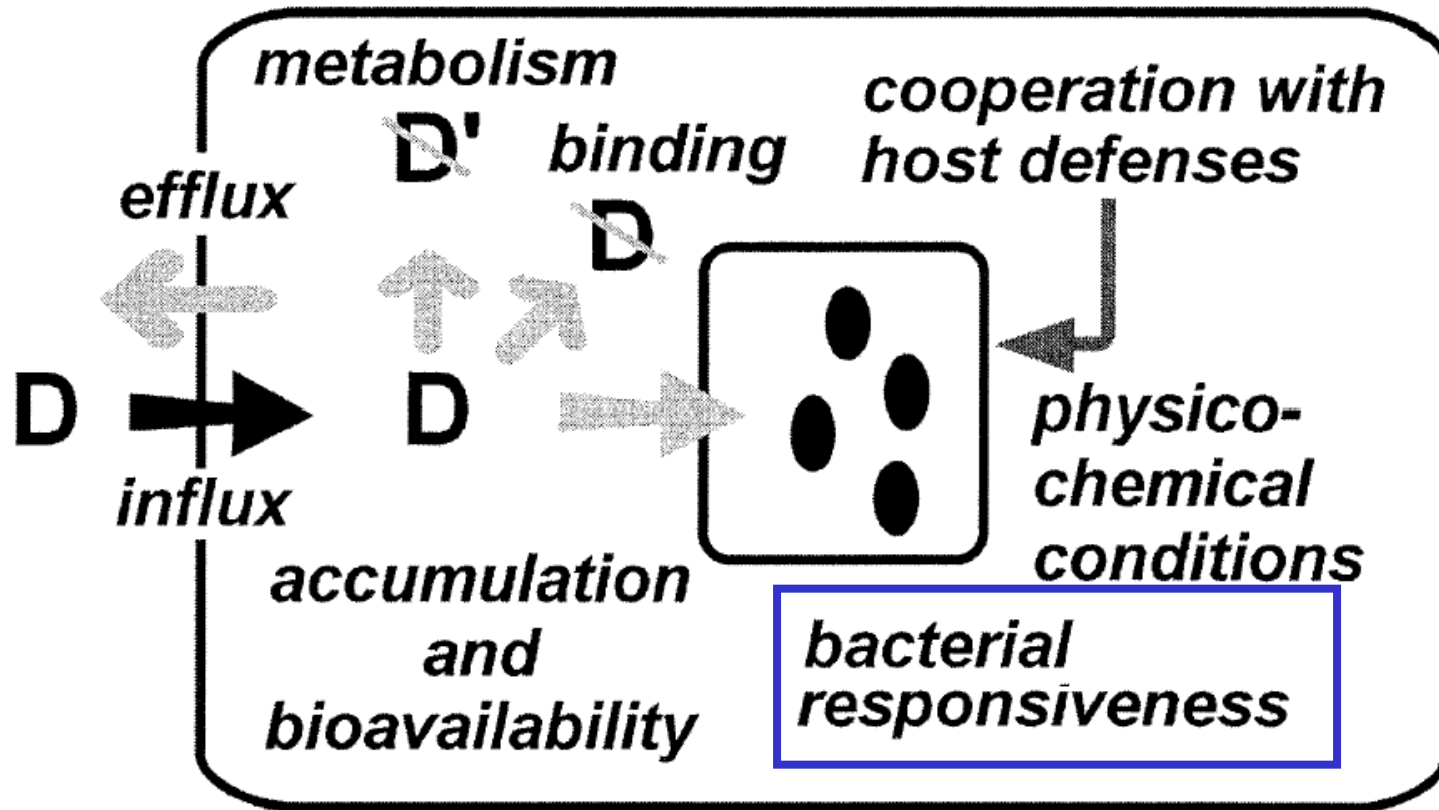


Adapted from Van Bambeke et al., *Curr Opin Drug Discov Devel.* (2006) 9:218-30

What about resistant strains ?

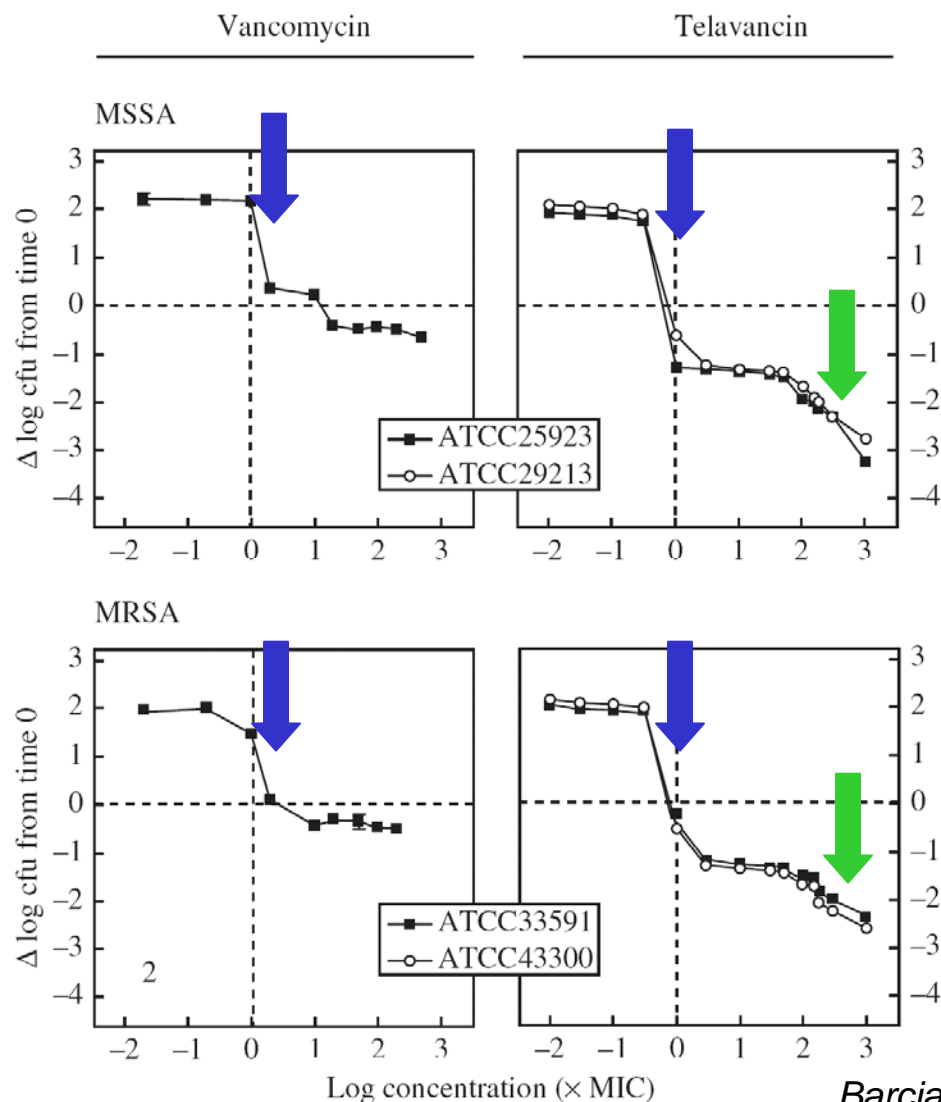


intracellular vs extracellular activity of antibiotics : PK – PD in action

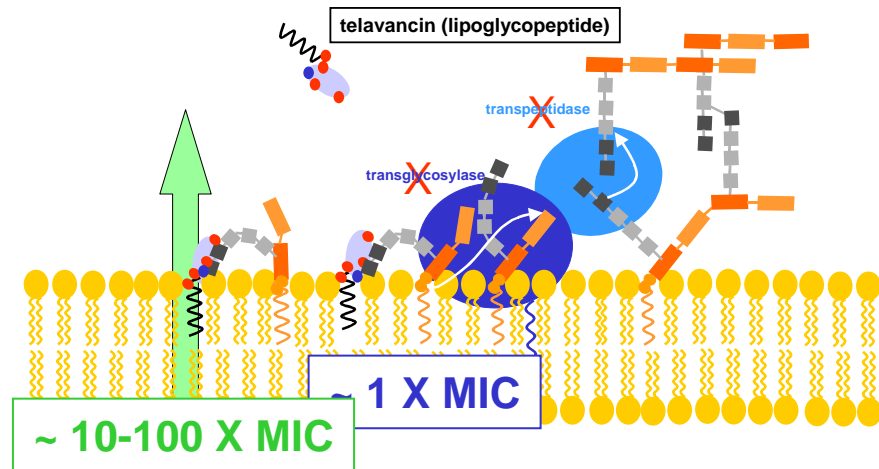


MSSA, MRSA, (VISA, VRSA)

a lipoglycopeptide shows bimodal effects towards Vanco-S strains...



... because of dual mode of action ?

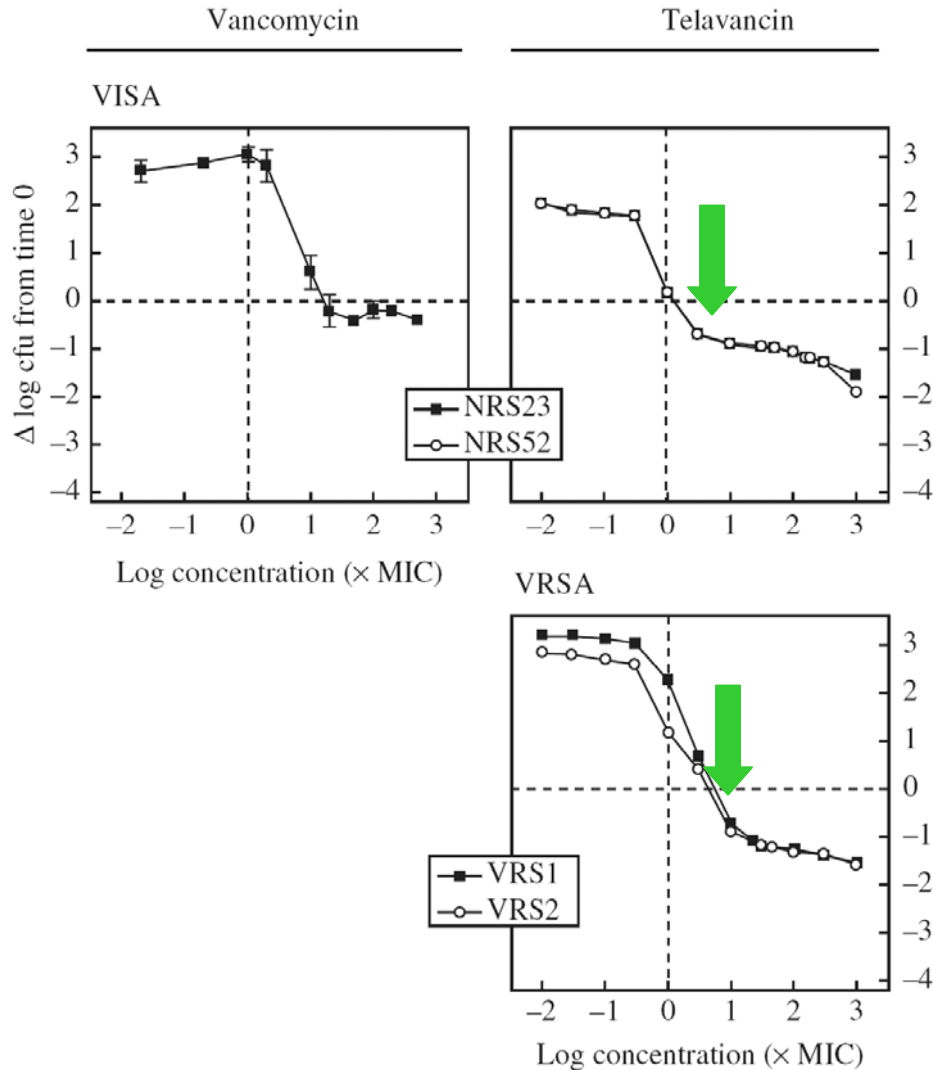


based on Higgins et al AAC (2005) 49: 1127-34

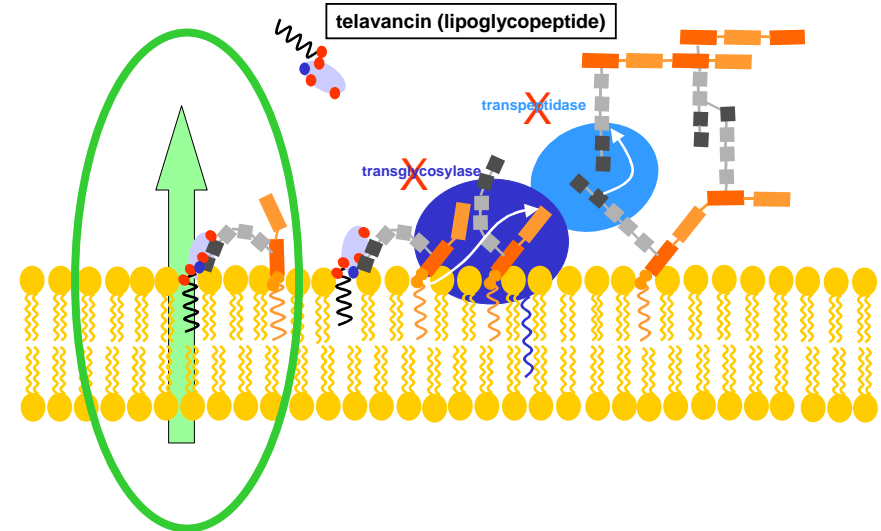
Barcia-Macay et al., J Antimicrob Chemother. (2006) 58:1177-84

(MSSA, MRSA), VISA, VRSA

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



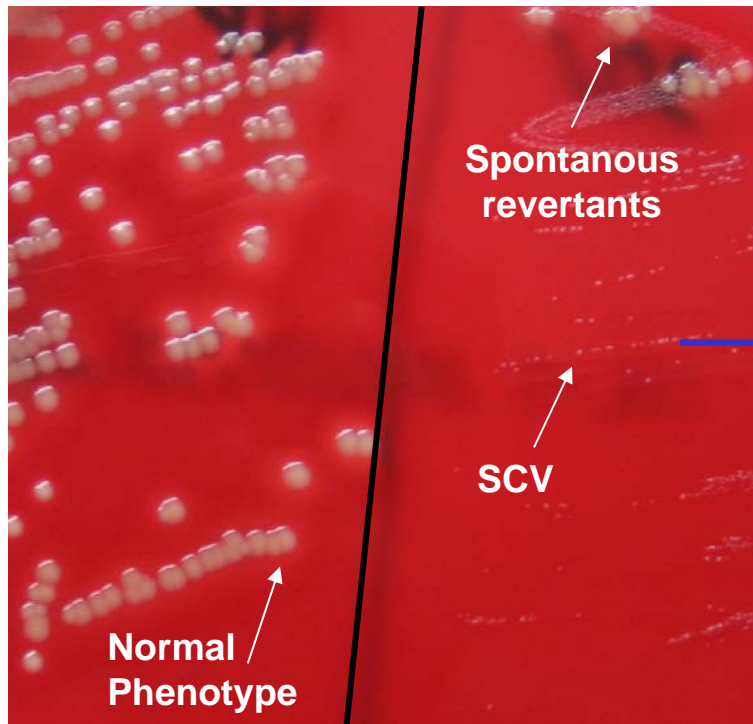
... because only one mode of action left ?



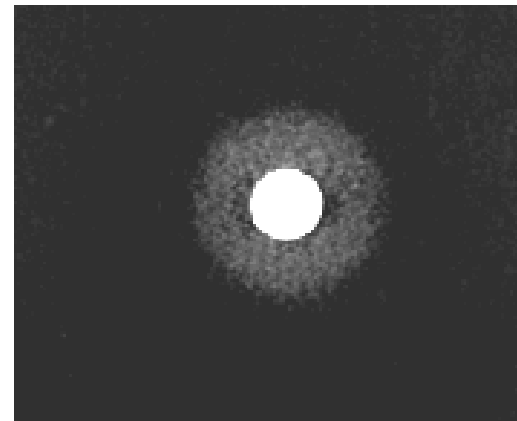
Barcia-Macay et al., *J Antimicrob Chemother.* (2006) 58:1177-84

SCV isolated from a cystic fibrosis patient

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

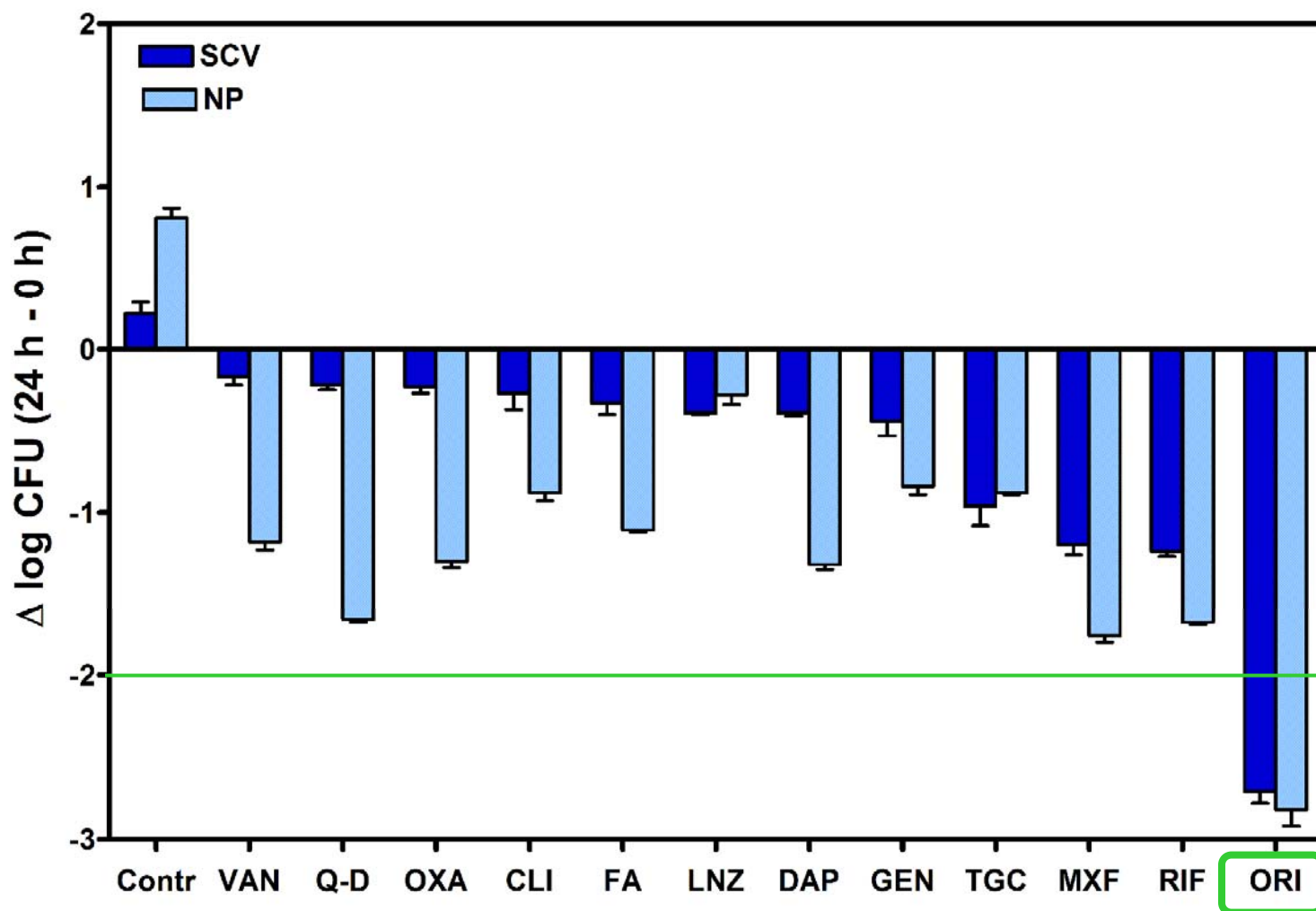


Thymidine dependent



Intracellular activity, SCV vs normal phenotype

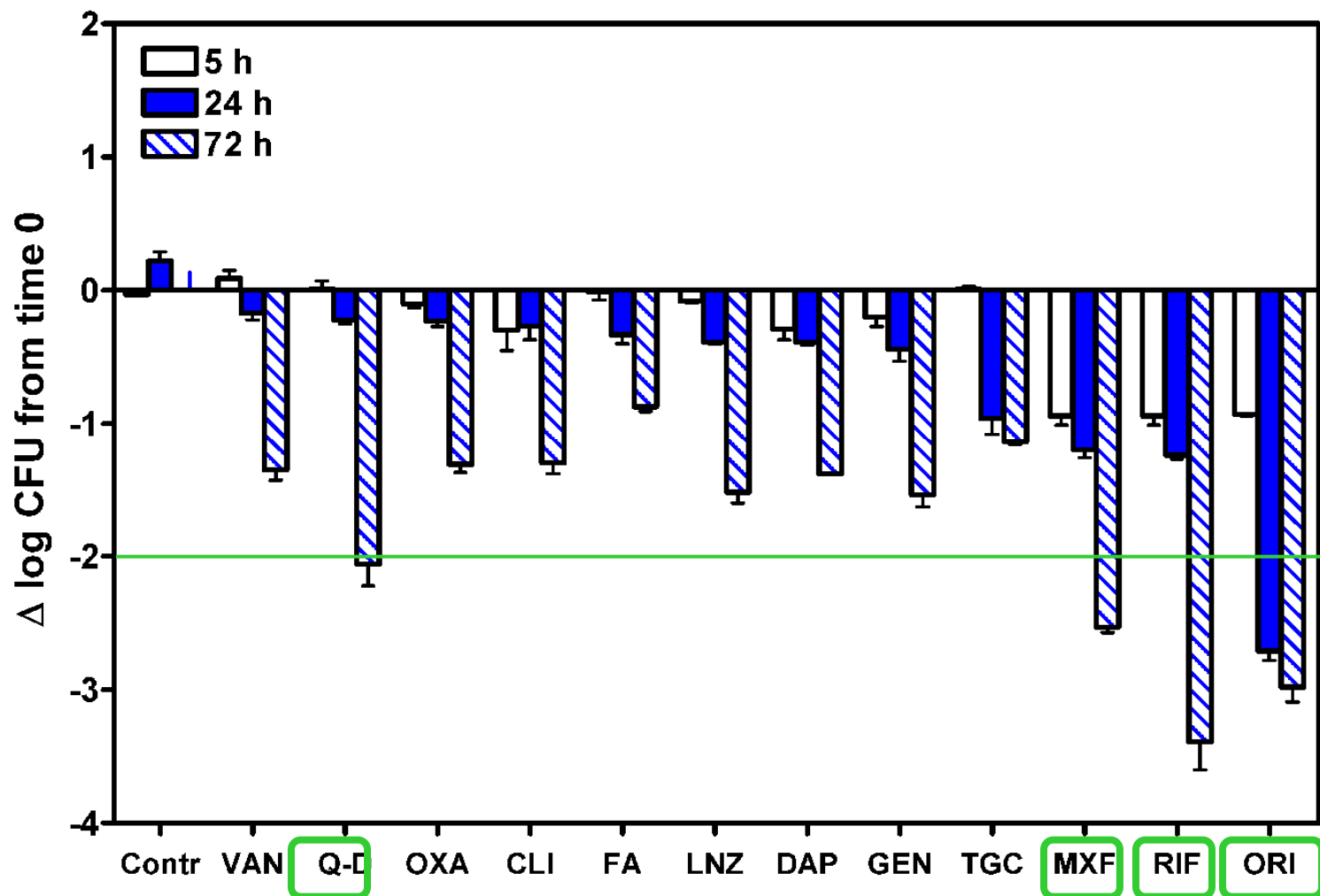
THP-1; 24 h, antibiotics at C_{max}



Nguyen et al, RICA1 2007, poster 325

Intracellular activity, SCV over time

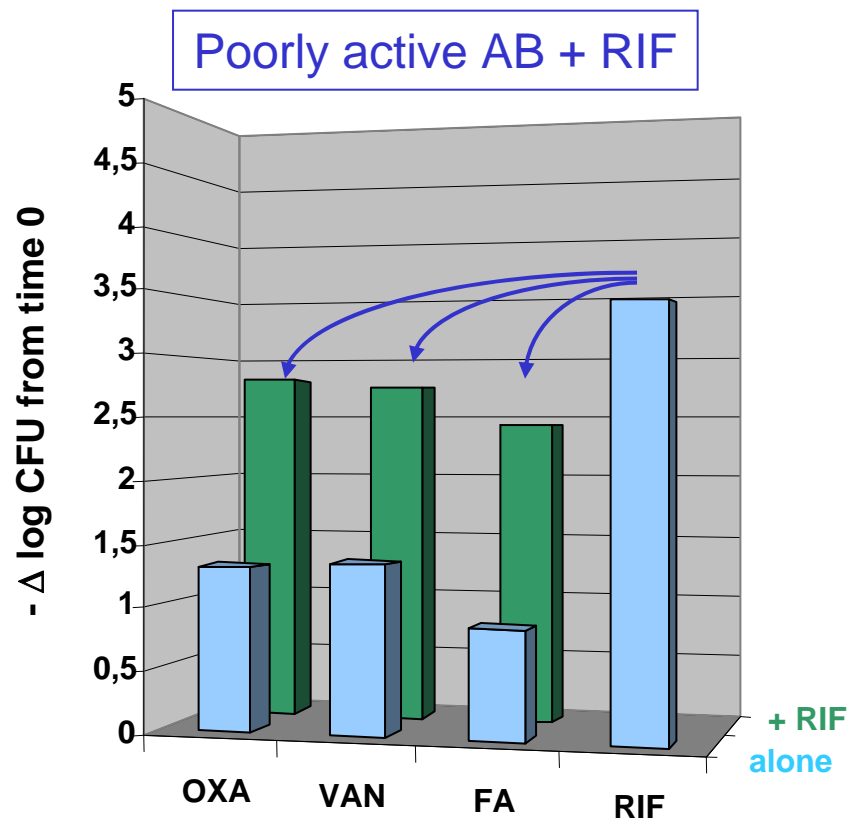
THP-1; SCV, antibiotics at Cmax for up to 3 days



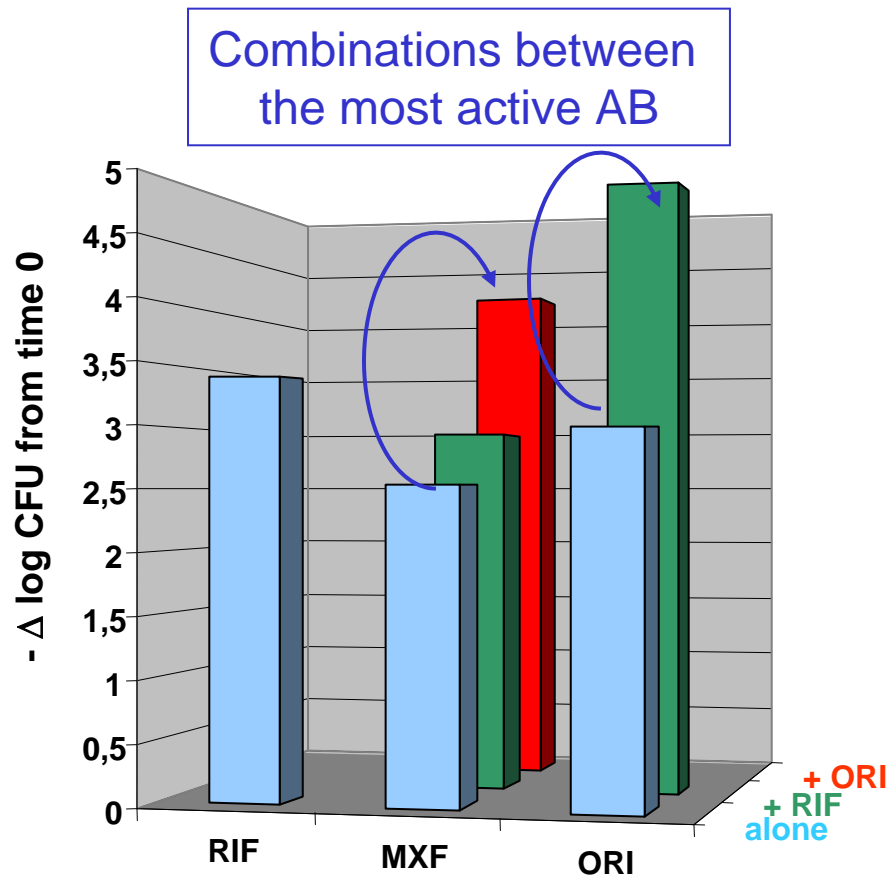
Nguyen et al., ICAAC 2007, poster A1437

Intracellular activity of combinations against SCV

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



Slightly less active than RIF alone



Combinations with ORI are synergistic

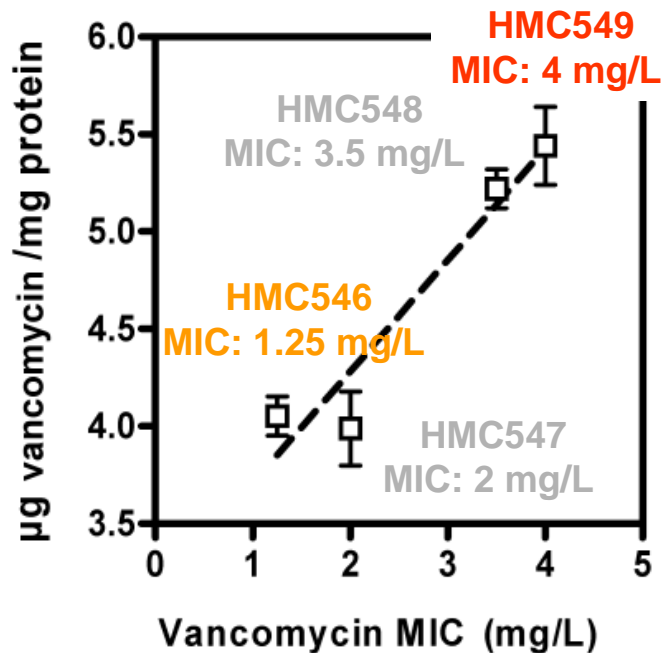
Nguyen et al., ECCMID 2008, poster 1059

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

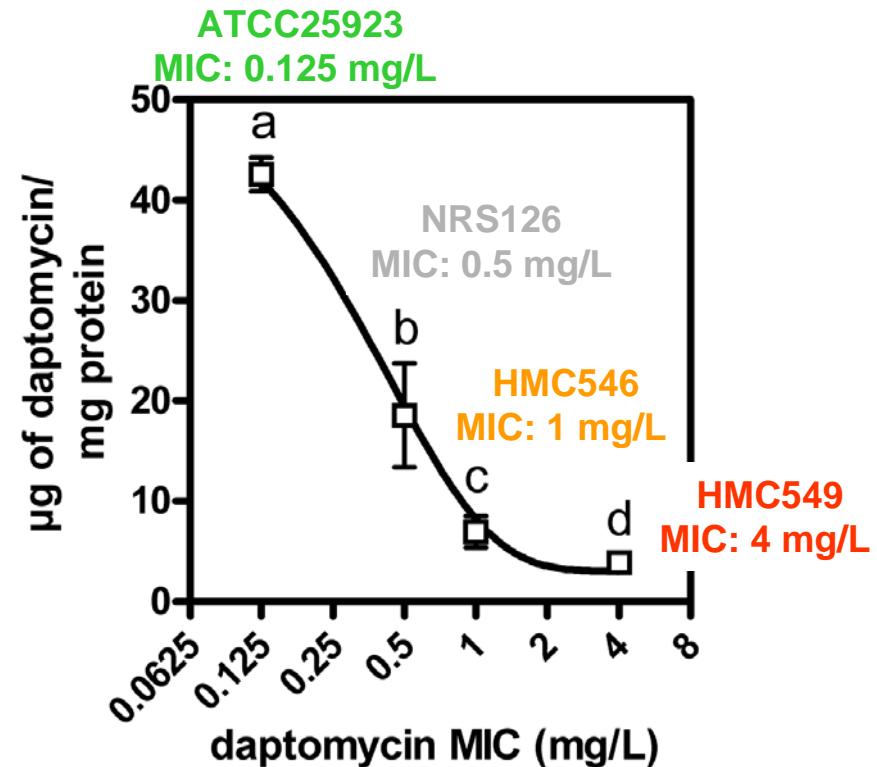
Julian et al. *Antimicrob Agents Chemother.* 2007 51:3445-8.

Reduced susceptibility associated with

increased amount
of bound vancomycin

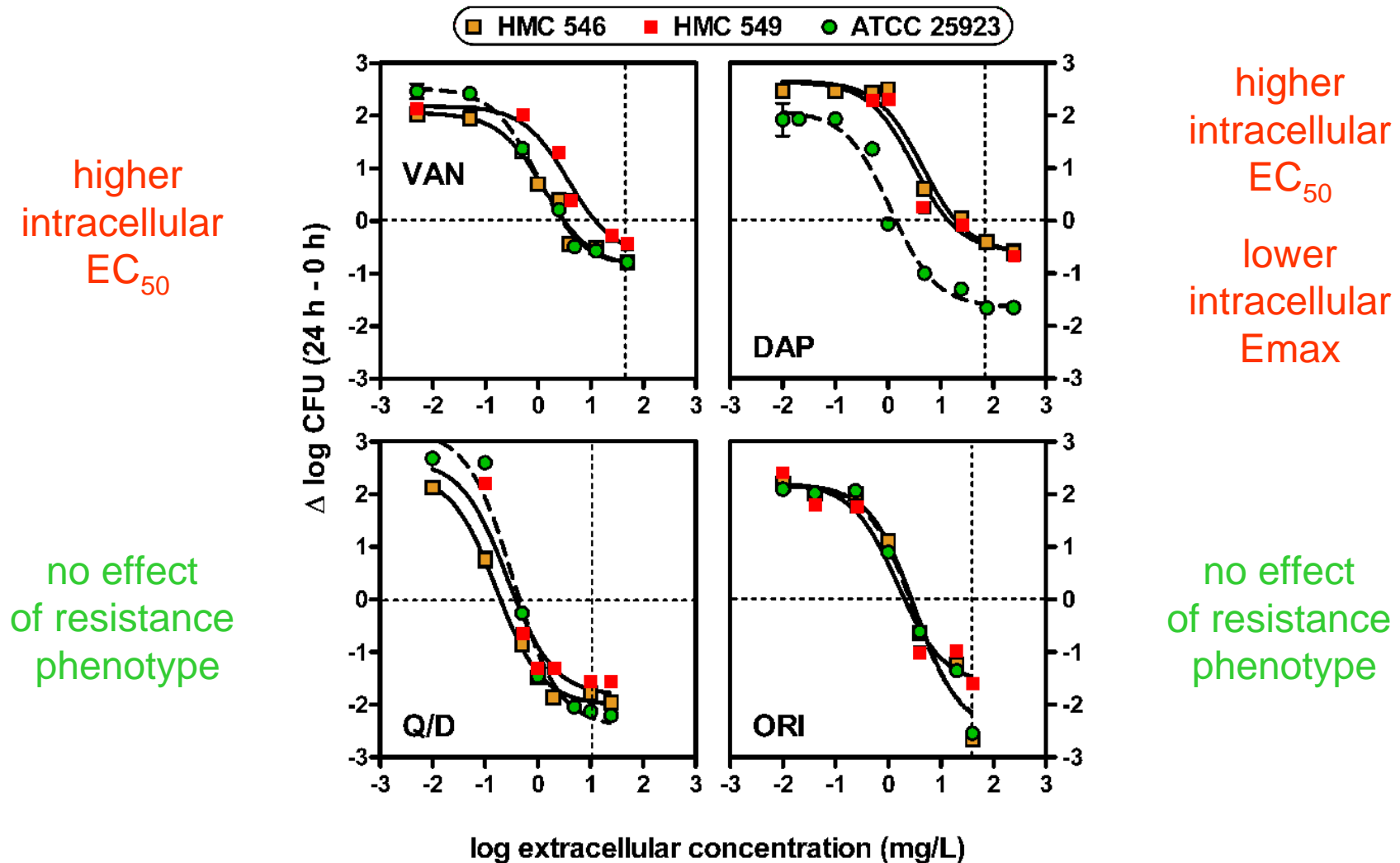


decreased amount
of bound daptomycin



Lemaire et al., *Clin. Microbiol. Infect.* (2008) in the press

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

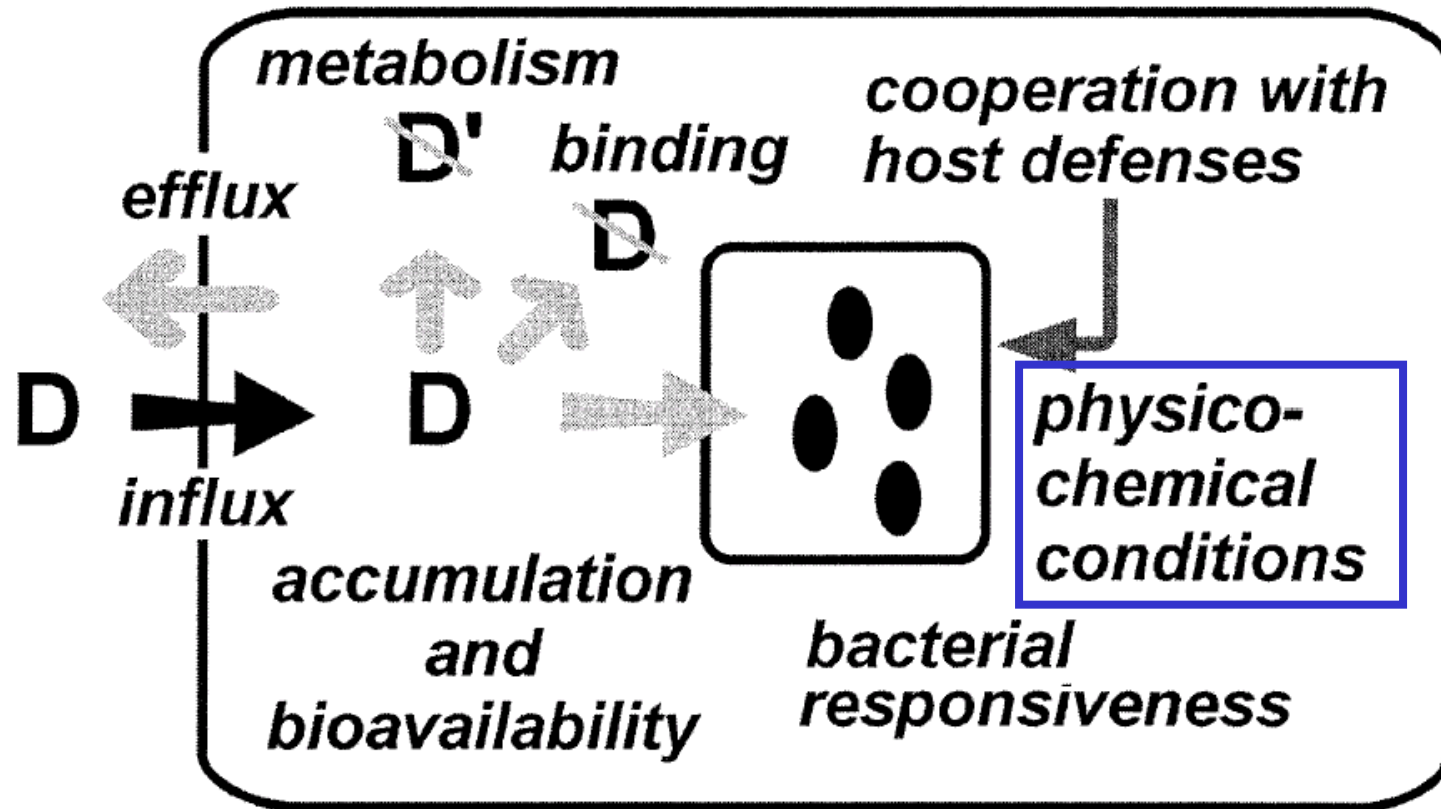


Lemaire et al., Clin. Microbiol. Infect. (2008) in the press

Cellular factors affecting antibiotic intracellular activity



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



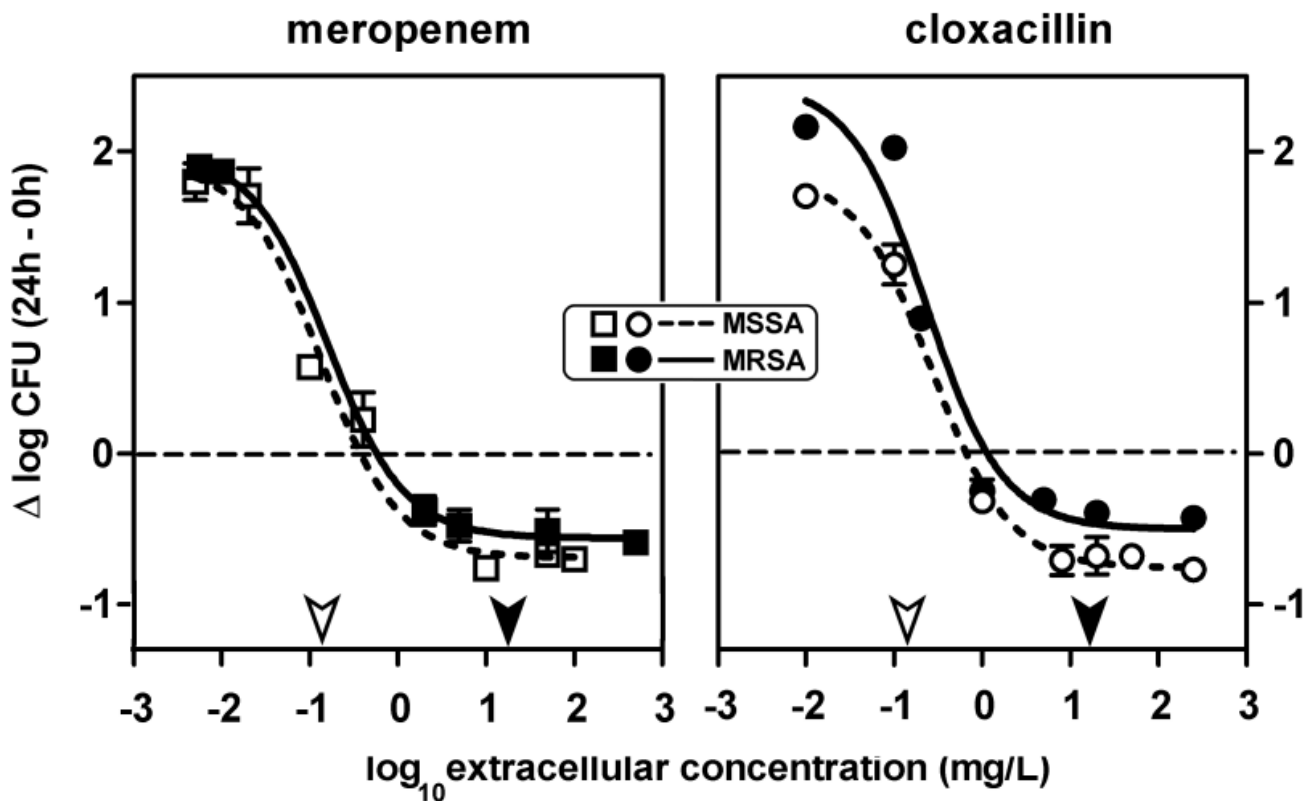
Carryn et al., *Infect Dis Clin North Am.* (2003) 17:615-34

acid pH of lysosomes



MRSA vs MSSA: intracellular activity of β -lactams

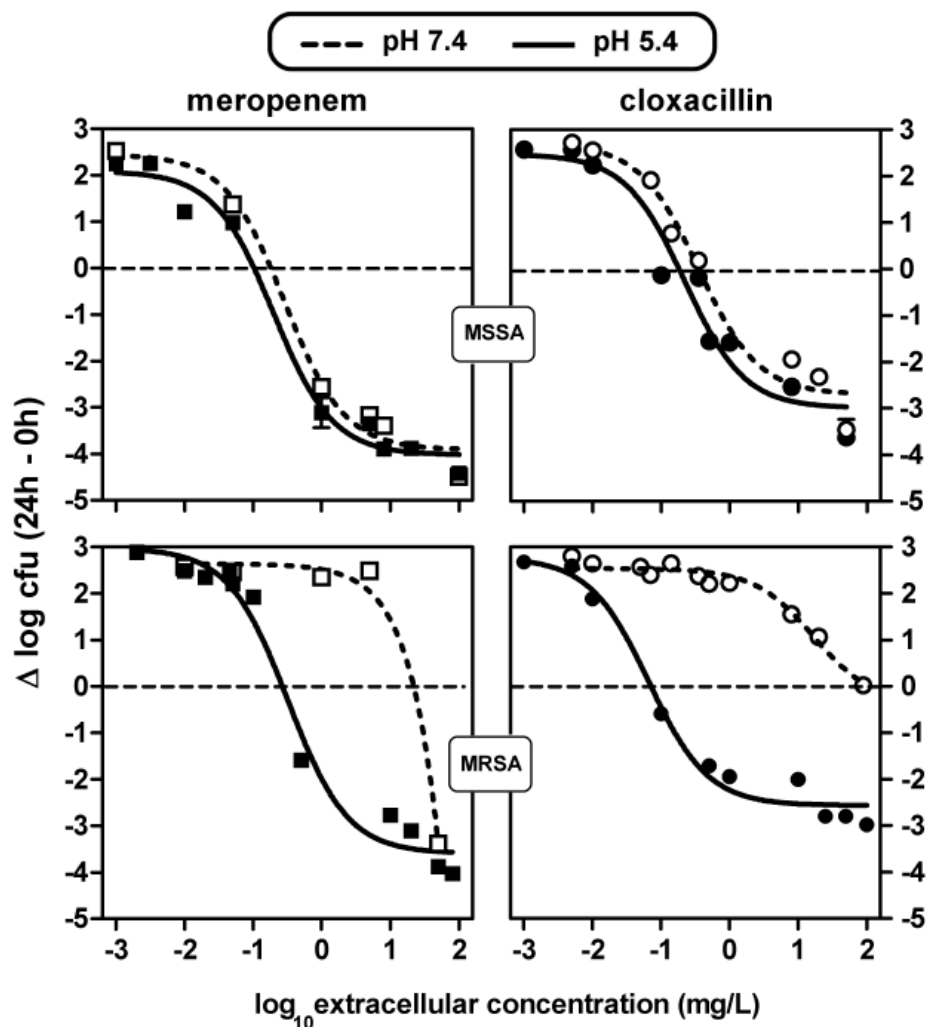
MRSA are as susceptible as MSSA to β -lactams when intracellular !



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

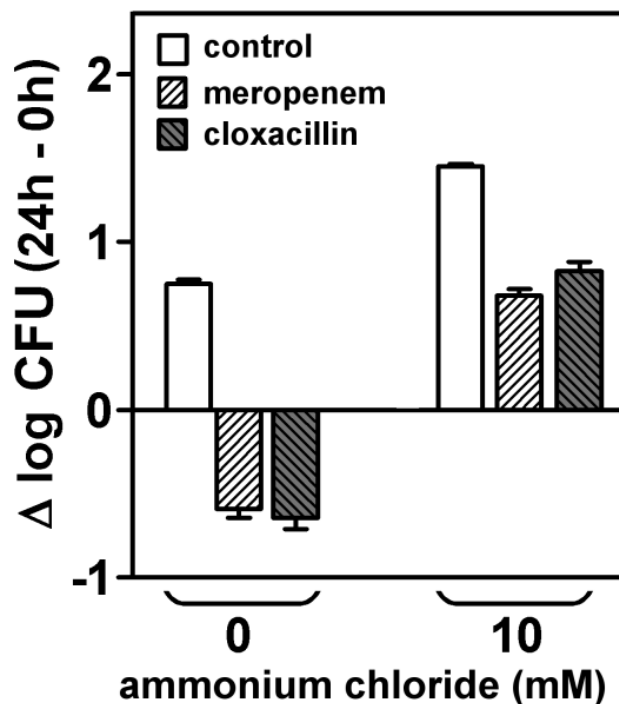
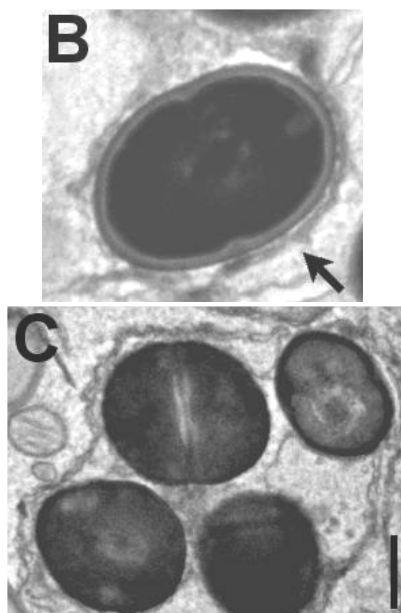


Lemaire et al., *Antimicrob. Agents Chemother.* (2007) 51:1627-1632

MRSA vs MSSA: extracellular activity of β -lactams

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

MRSA are inside
[acidic] vacuoles

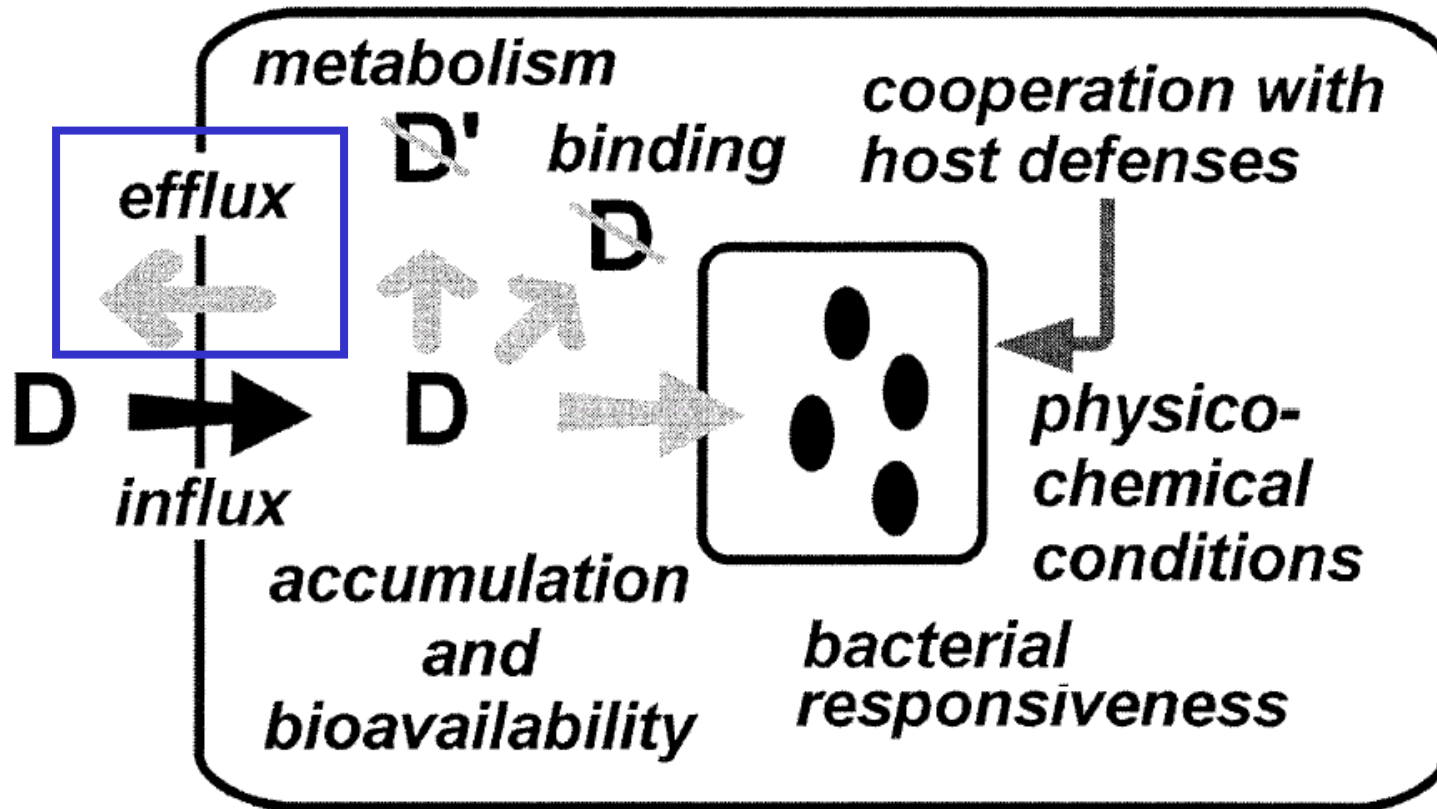


Lemaire et al., *Antimicrob. Agents Chemother.* (2007) 51:1627-1632

Efflux pumps



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

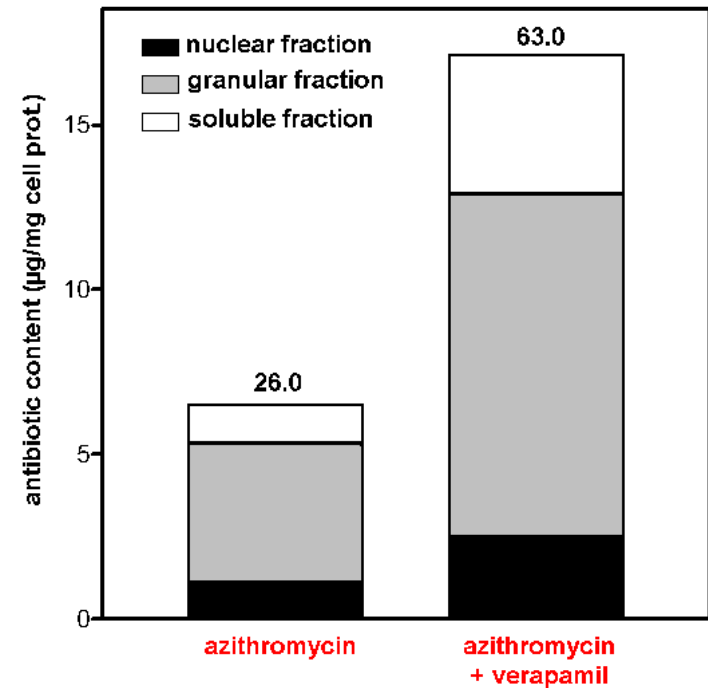
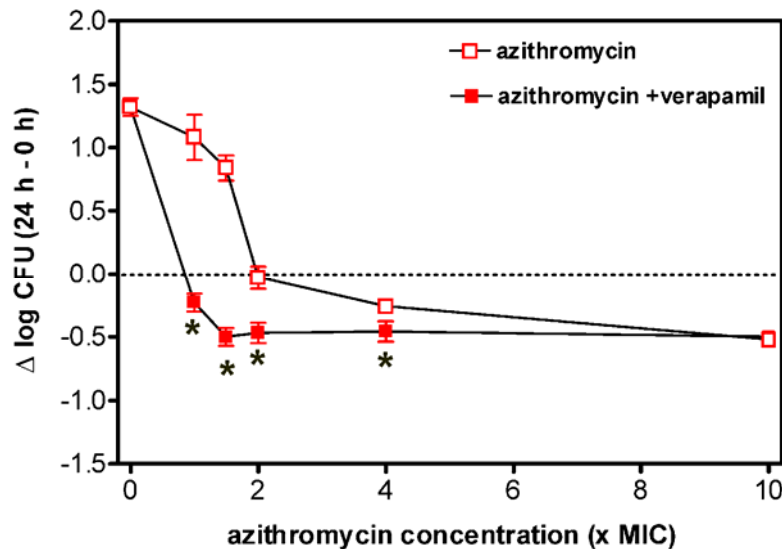


Carryn et al., *Infect Dis Clin North Am.* (2003) 17:615-34

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

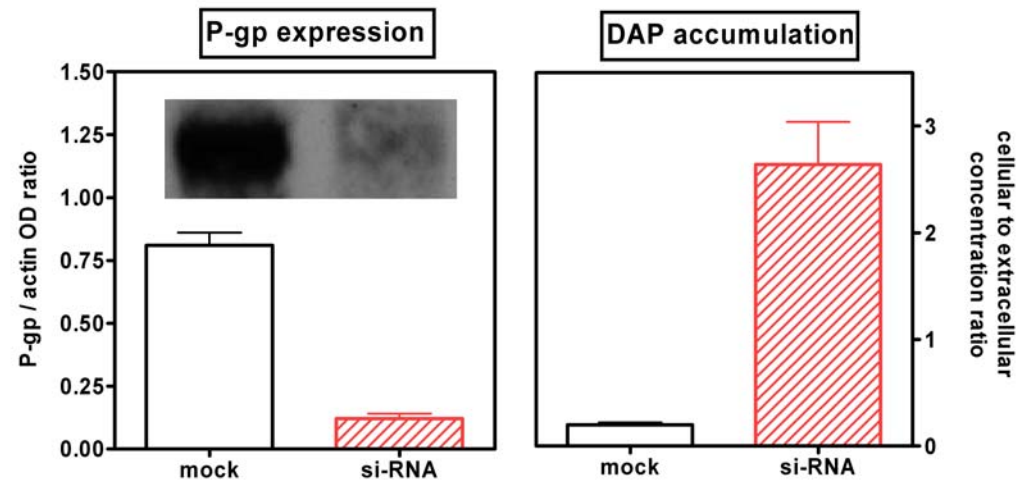
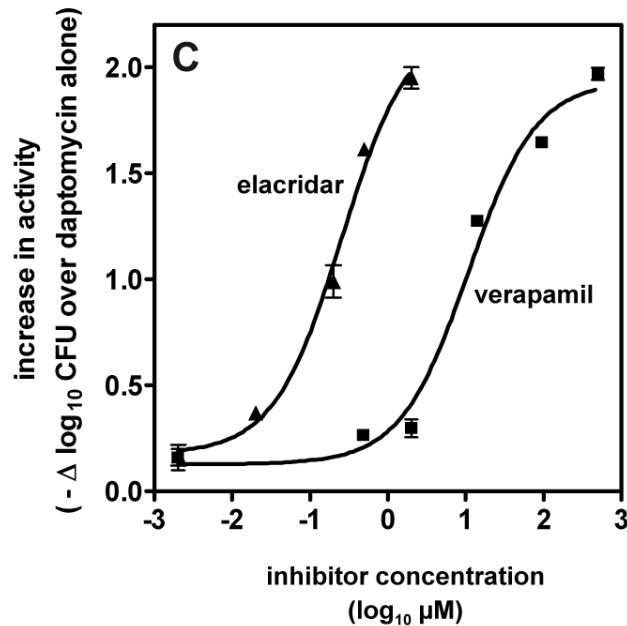


Seral et al., J. Antimicrob. Chemother. (2003) 51:1167-73

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

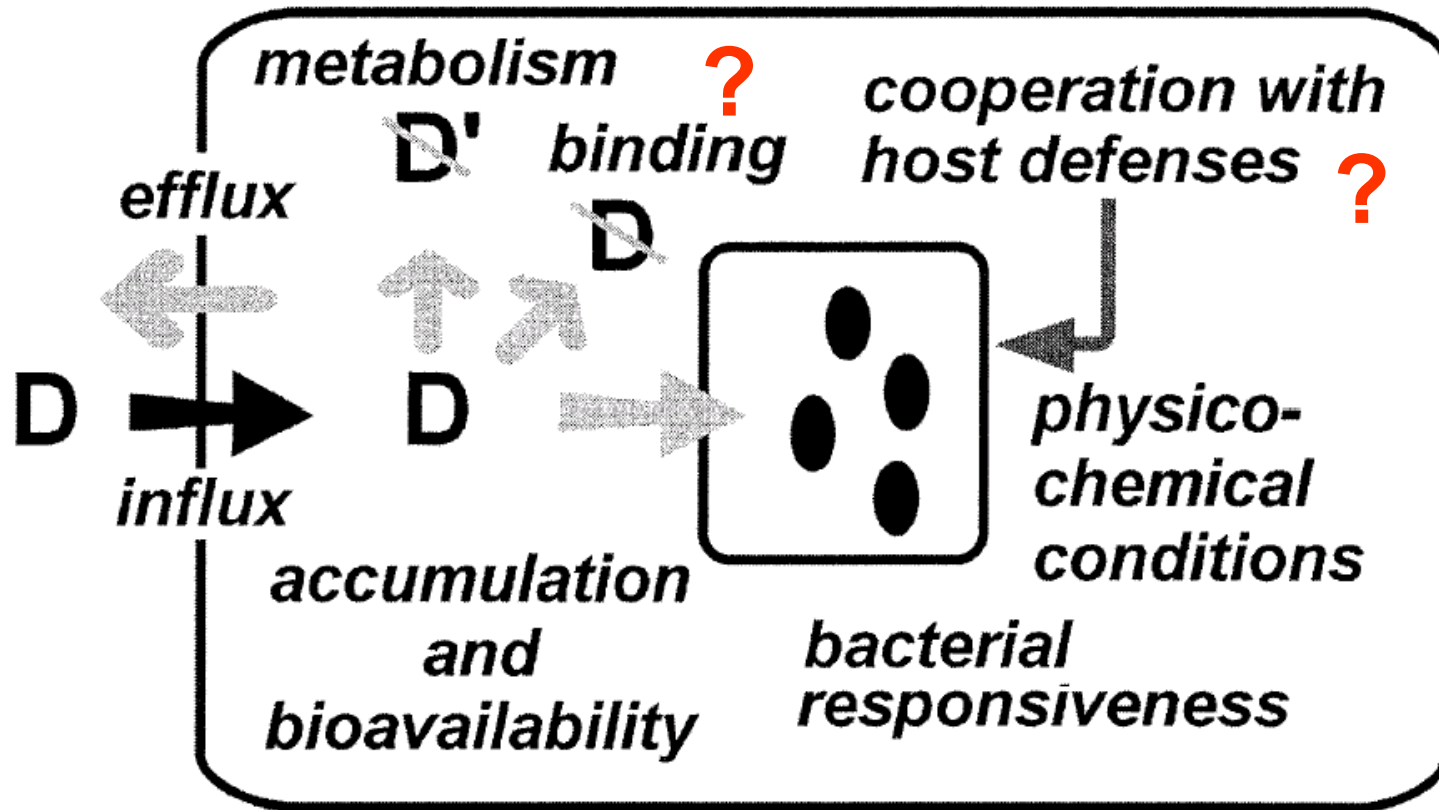
- intracellular activity
- accumulation in lysosomes

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



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

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

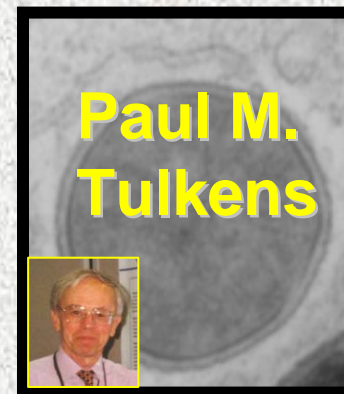
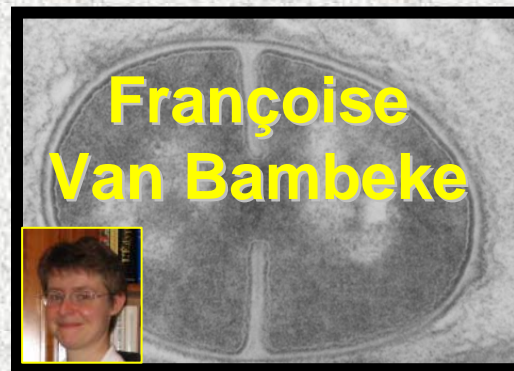
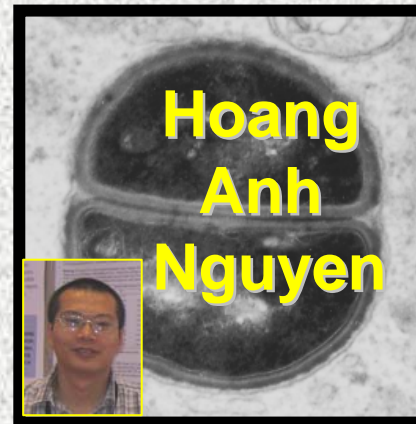
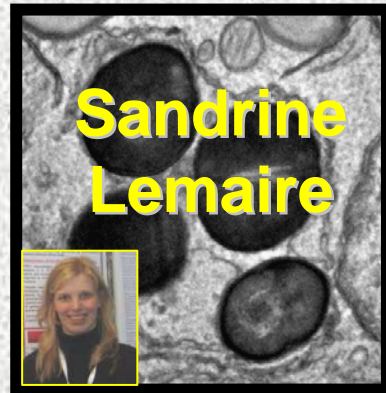


How are all these parameters inter-connected ?

Still a lot of work ahead ...



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