How to Target Intracellular Pathogens ?

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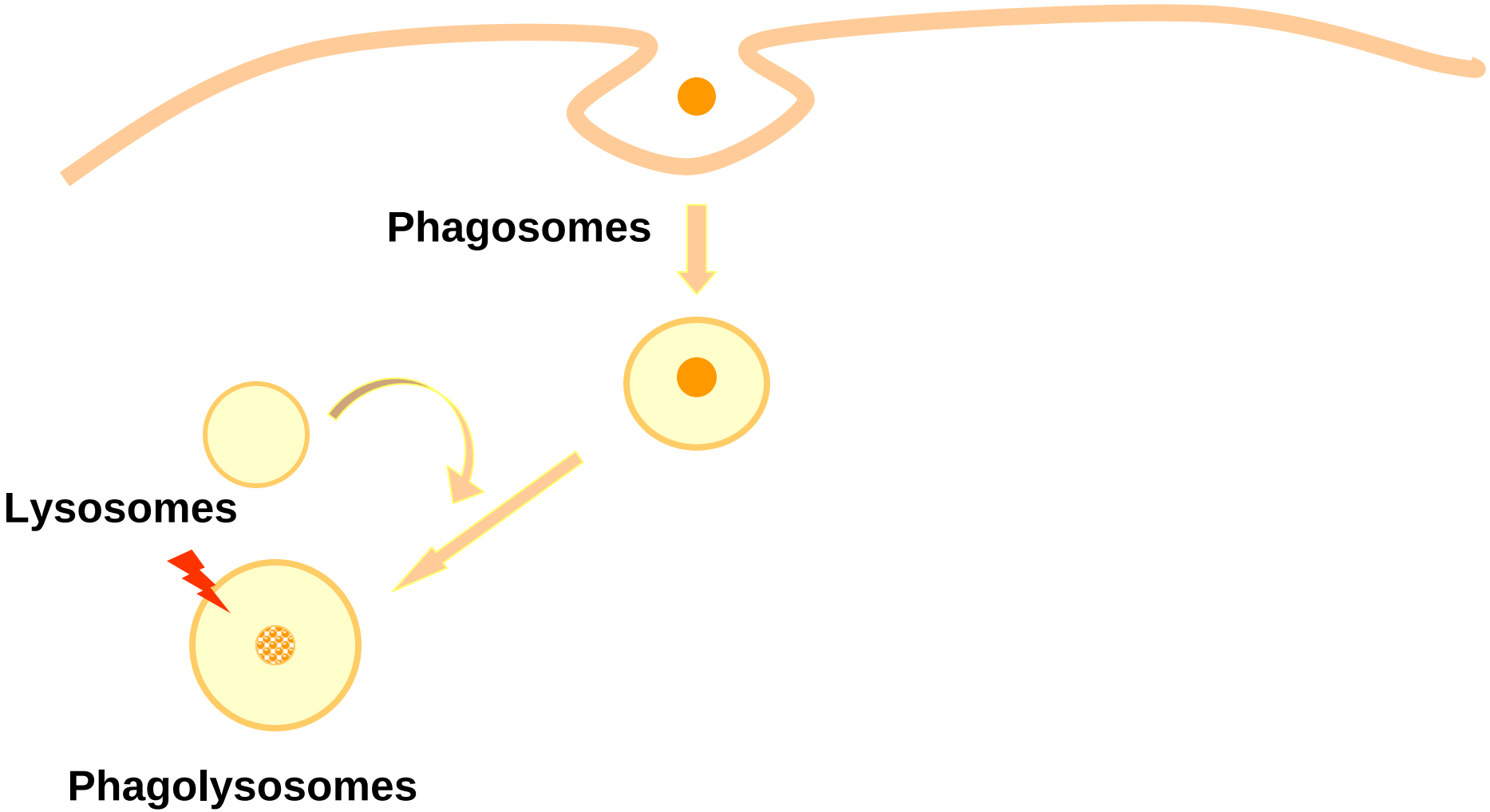
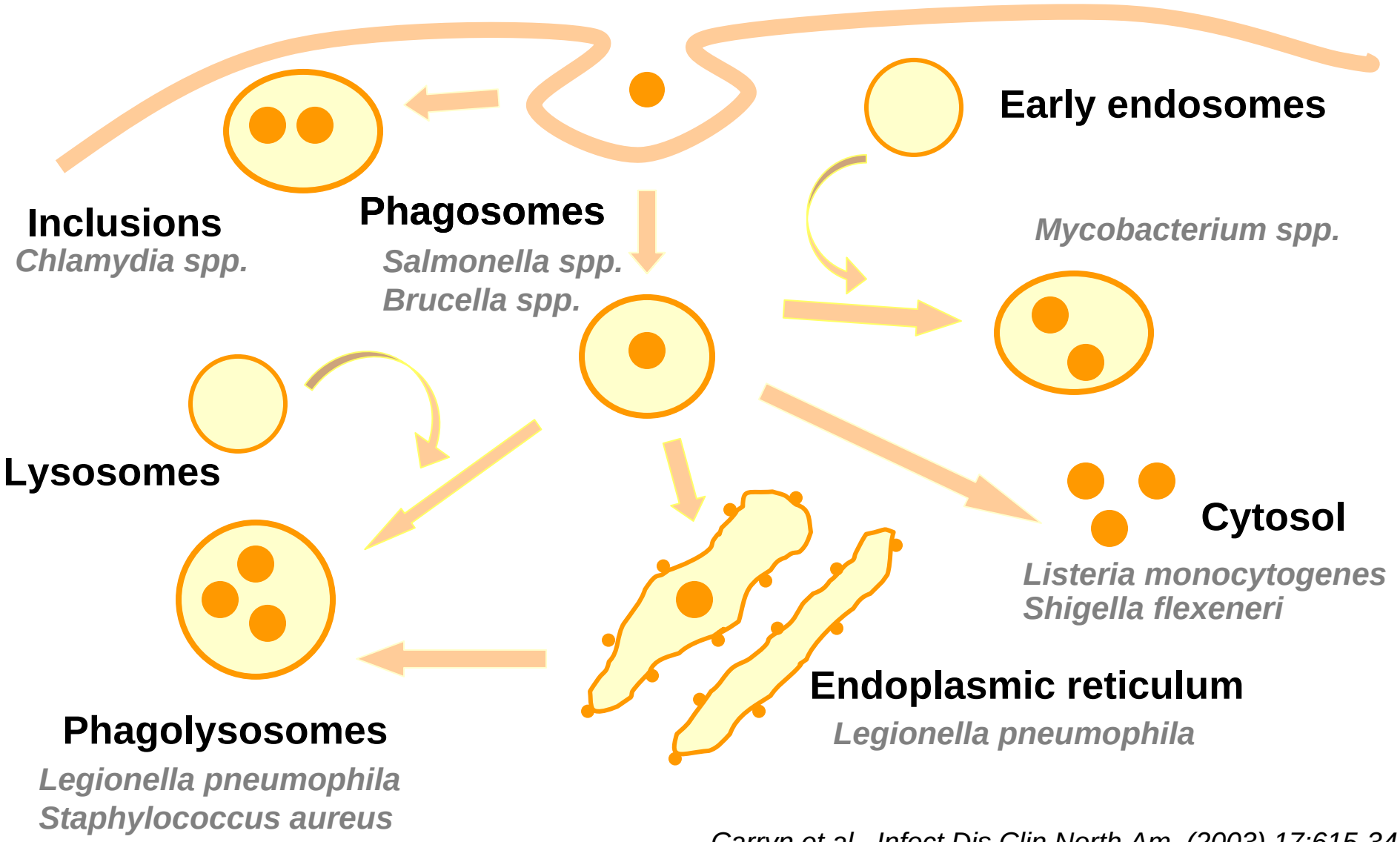
Disclosures:
Grant investigator from Theravance Inc. and Targanta Corp.
Member of the European Advisory Board of Targanta Corp.

Intracellular killing of bacteria by host cell defence mechanisms



Some bacteria can escape host cell defence mechanisms …



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

… that are difficult to treat !

Journal of Antimicrobial Chemotherapy (2004) **53**, 167–173

Antibiotic-induced persistence of cytotoxic *Staphylococcus aureus* in non-phagocytic cells

Oleg Krut, Herdis Sommer and Martin Krönke*

Institute for Medical Microbiology, Immunology and Hygiene, Medical Center University of Cologne, Goldenfelsstr. 19–21, 50935 Cologne, C…

BRIEF REPORTS • **CID 2001:32 (1 June)** • 1643

Intracellular Persistence of *Staphylococcus aureus* Small-Colony Variants within Keratinocytes: A Cause for Antibiotic Treatment Failure in a Patient with Darier's Disease

Christof von Eiff,[1] Karsten Becker,[1] Dieter Metze,[2] Gabriele Lubritz,[1] Johannes Hockmann,[2] Thomas Schwarz,[2] and Georg Peters[1]

[1]Institute of Medical Microbiology and [2]Department of Dermatology, Westfälische Wilhelms-Universität Münster, Münster, Germany

1: Postgrad Med J. 2000 Aug;76(898)

Pathophysiology of chronic bacterial osteomyelitis. Why do antibiotics fail so often?

Ciampolini J, Harding KG.

Wound Healing Research Unit, University of Wales College of Medicine, Cardiff, UK.

Journal of Antimicrobial Chemotherapy (2005) **55**, 383–386

Intracellular persistence of *Escherichia coli* in urinary bladders from mecillinam-treated mice

M. B. Kerrn[1,2]*, C. Struve[1], J. Blom[3], N. Frimodt-Møller[2] and K. A…

[1]Department of Bacteriology, Mycology and Parasitology, [2]National Center of Antimi… Control and [3]Department of Virology, Statens Serum Institut, Copenhagen,

Infection. 1992 Mar-Apr;20(2):99-100.

Fatal Legionella pneumophila pneumonia: treatment failure despite early sequential oral-parenteral amoxicillin- clavulanic acid therapy.

Hohl P, Buser U, Frei R.

Dept. of … dicine. University Hospital, Basel, Switzerland.

Int J Tuberc Lung Dis. 2004 Jan;8(1):31-8.

Development of acquired drug resistance in recurrent tuberculosis patients with various previous treatment outcomes.

Yoshiyama T, Yanai H, Rhiengtong D, Palittapongarnpim P, Nampaisan O, Supawitkul S, Uthaivorawit W, Mori T.

Epidemiology Division, Research Institute of Tuber… Kiyose, Tokyo, Japan.

JEADV (2001) 15, 405–409

Electron microscopic evidence of persistent chlamydial infection following treatment

EY Bragina,† MA Gomberg,‡* GA Dmitriev†

†Department of Microbiology, Central Institute of Skin and Venereal Diseases, ‡Laboratory of Viral Urogenital Infections, Central Institute of Skin and Venereal Diseases, Korolenko Str., 3, Moscow, 107076, Russia.

Clinical Infectious Diseases

Development of Listerial Meningitis during Ciprofloxacin Treatment

Nicholas M. Grumbach, Eleftherios Mylonakis, and Edward J. Wing

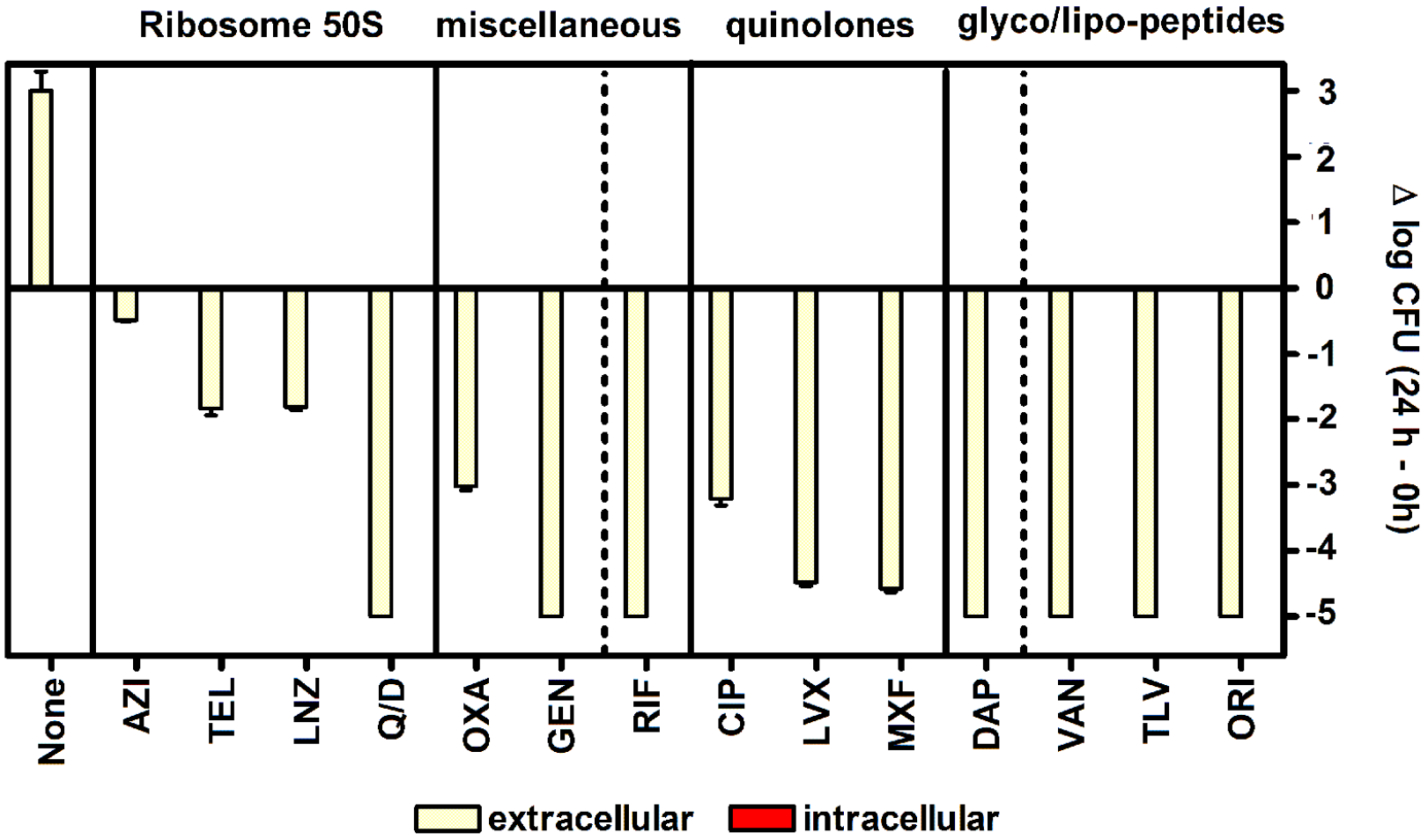
Dilemna to select the appropriate antibiotic …

highest cellular accumulation ?
best diffusibility ?
prolonged cellular retention ?
no cell toxicity ?
lowest MIC ?
cooperation with oxydative burst ?
cooperation with cytokines ?
other ?

Let’s start by a global view of the situation ….

Extracellular vs intracellular activity against *S.aureus* at Cmax

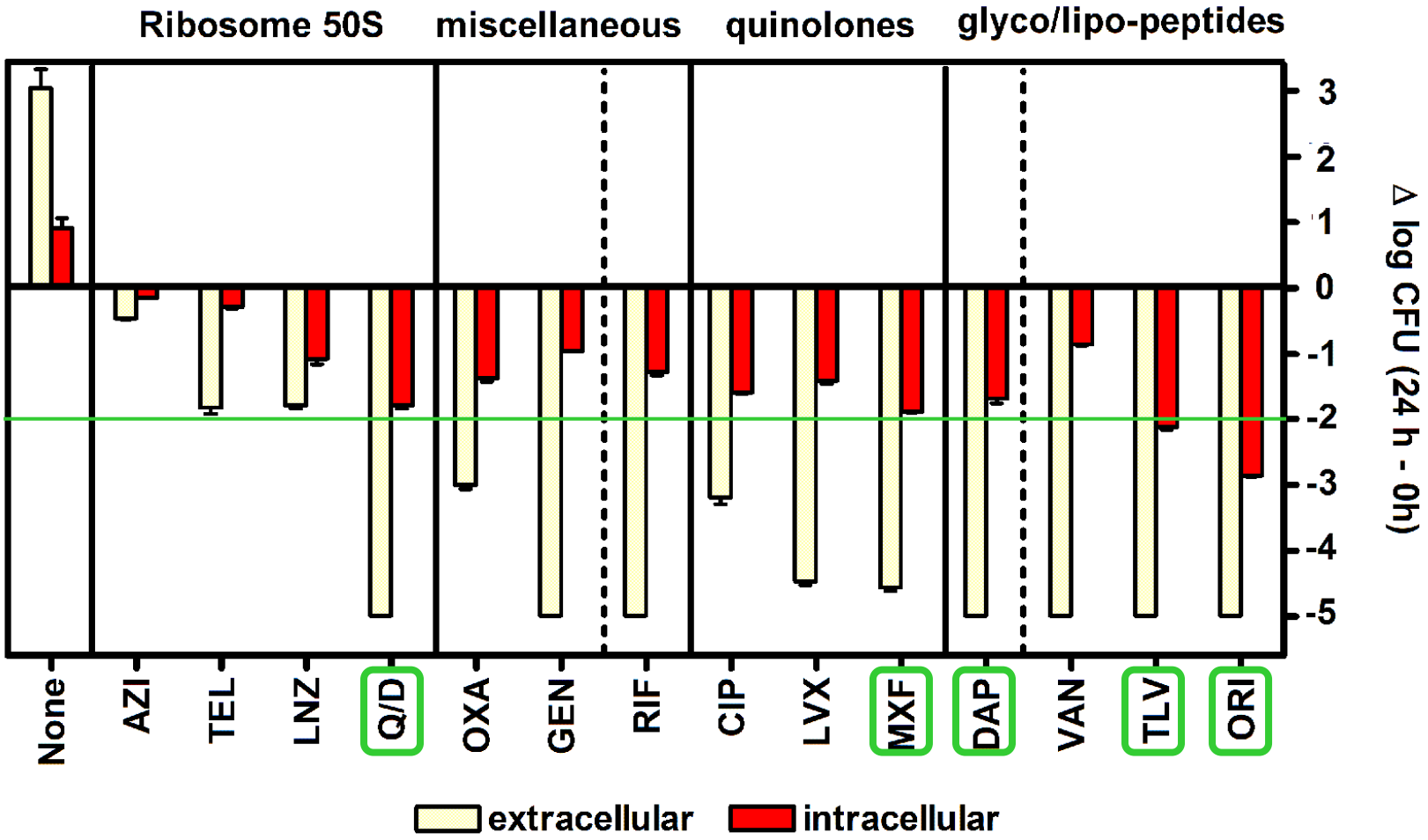
THP-1; 24 h, ATCC25923, antibiotics at Cmax



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

Extracellular vs intracellular activity against *S.aureus* at Cmax

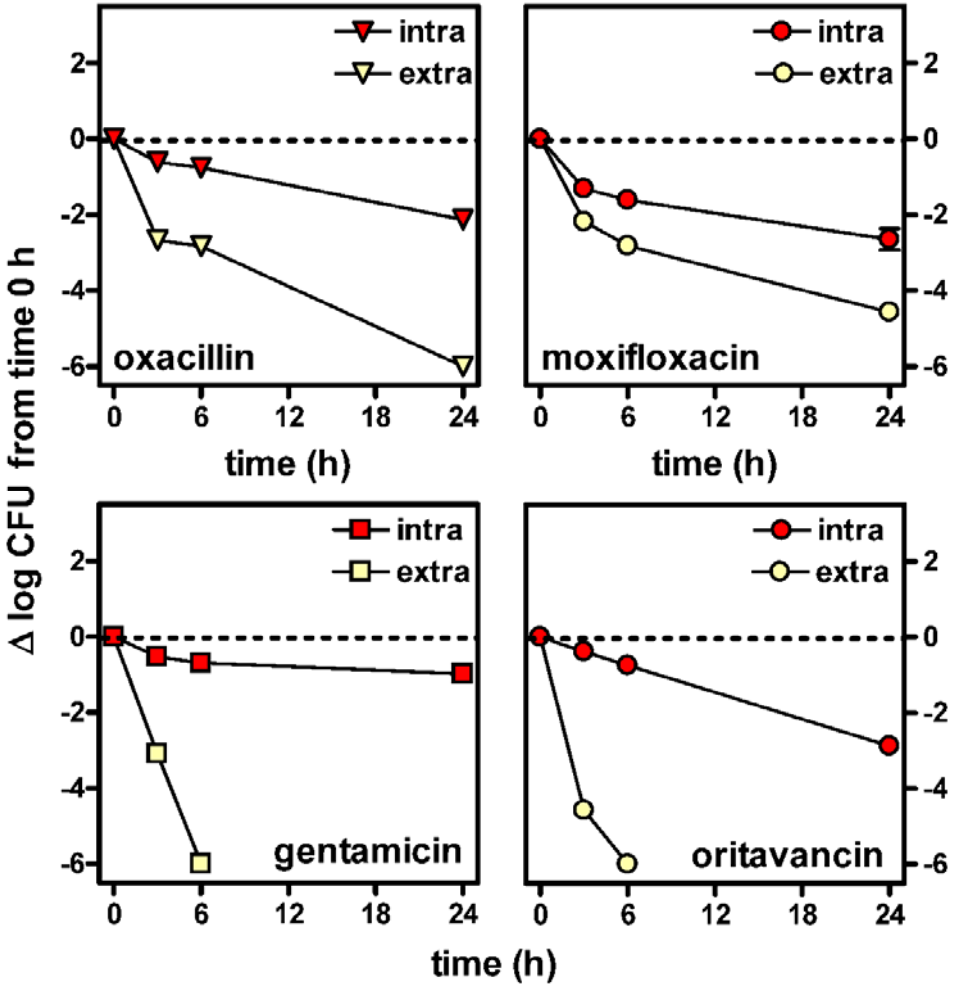
THP-1; 24 h, ATCC25923, antibiotics at Cmax



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

Pharmacodynamic relationships: time-effects against *S.aureus* at Cmax

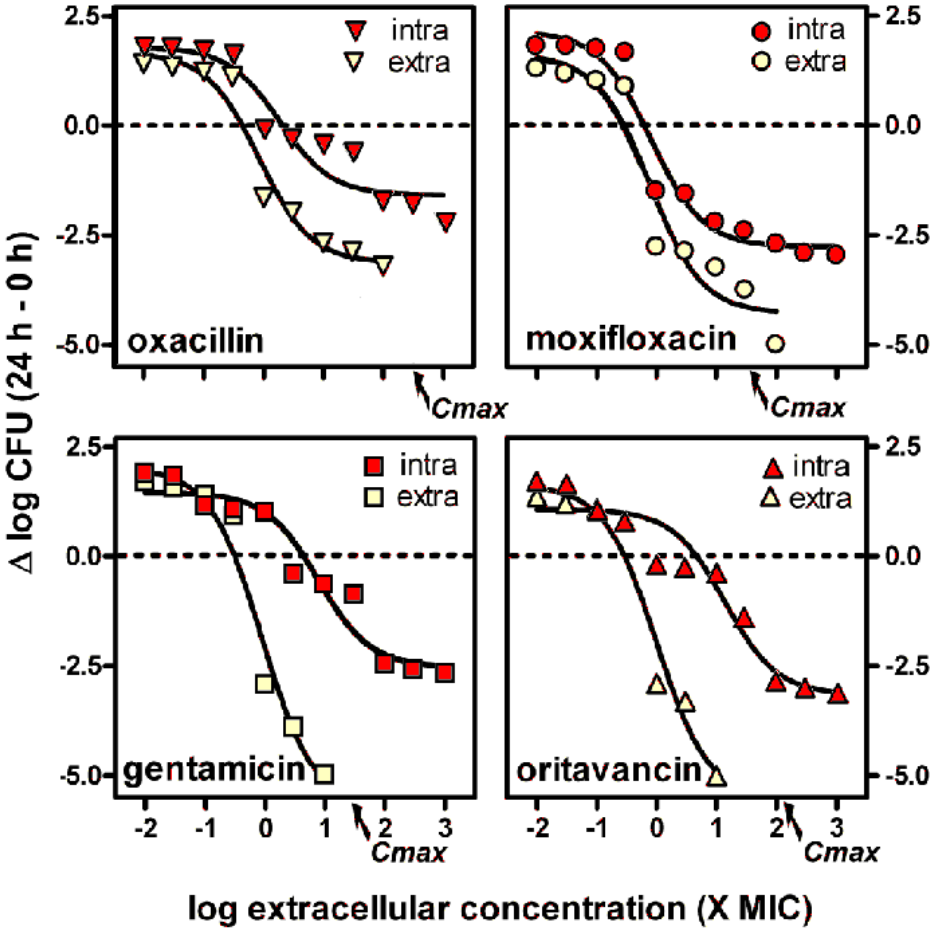
Slower killing rate intracellularly



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

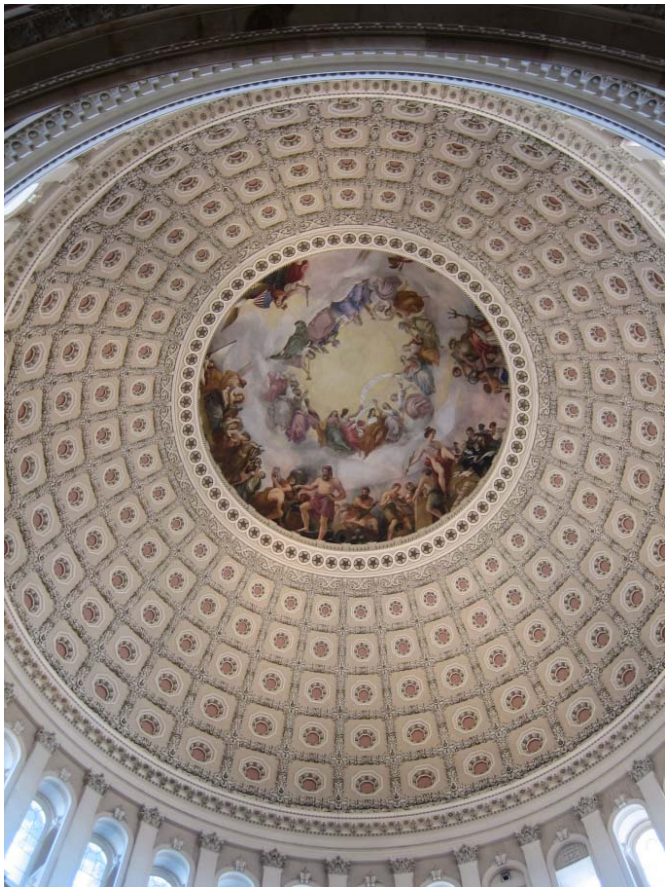
Pharmacodynamic relationships: concentration-effects against *S.aureus* at 24 h

Concentration-dependent killing; lower Emax intracellularly



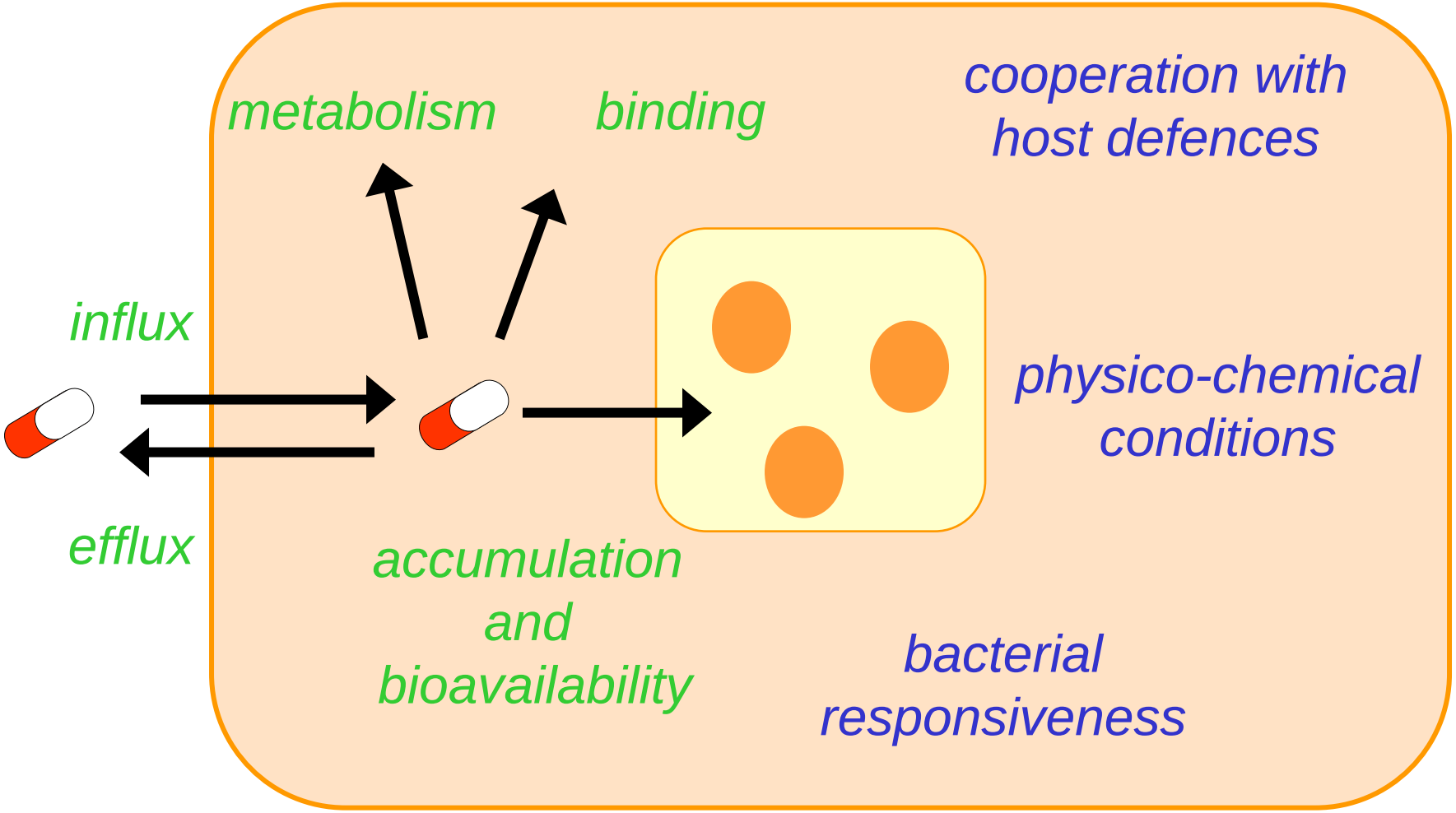
Barcia-Macay et al., AAC (2006) 50:841-51

From extracellular to intracellular :



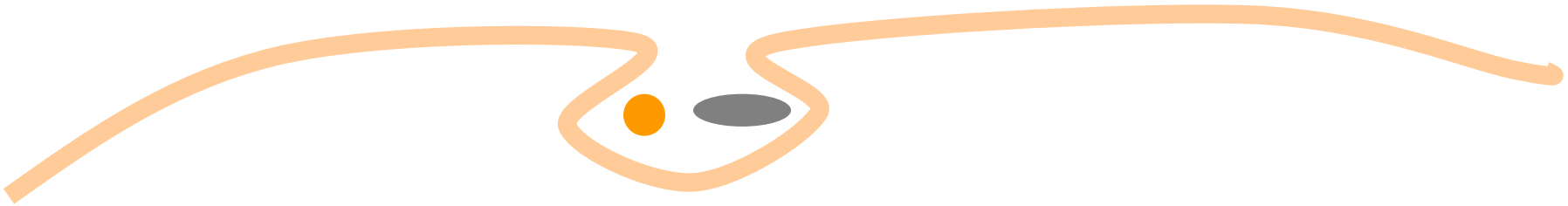
can we apply the same PK/PD concepts ?

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



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

In vitro models for intracellular PK/PD studies



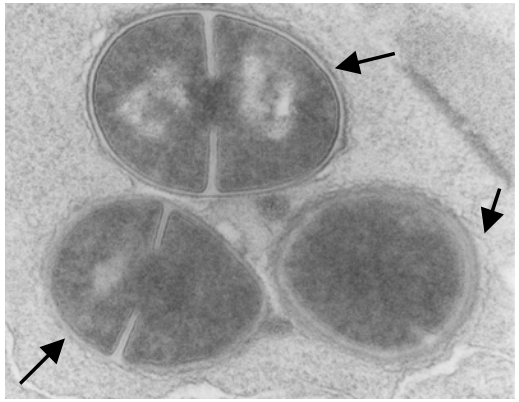
Lysosomes

Cytosol

Listeria monocytogenes

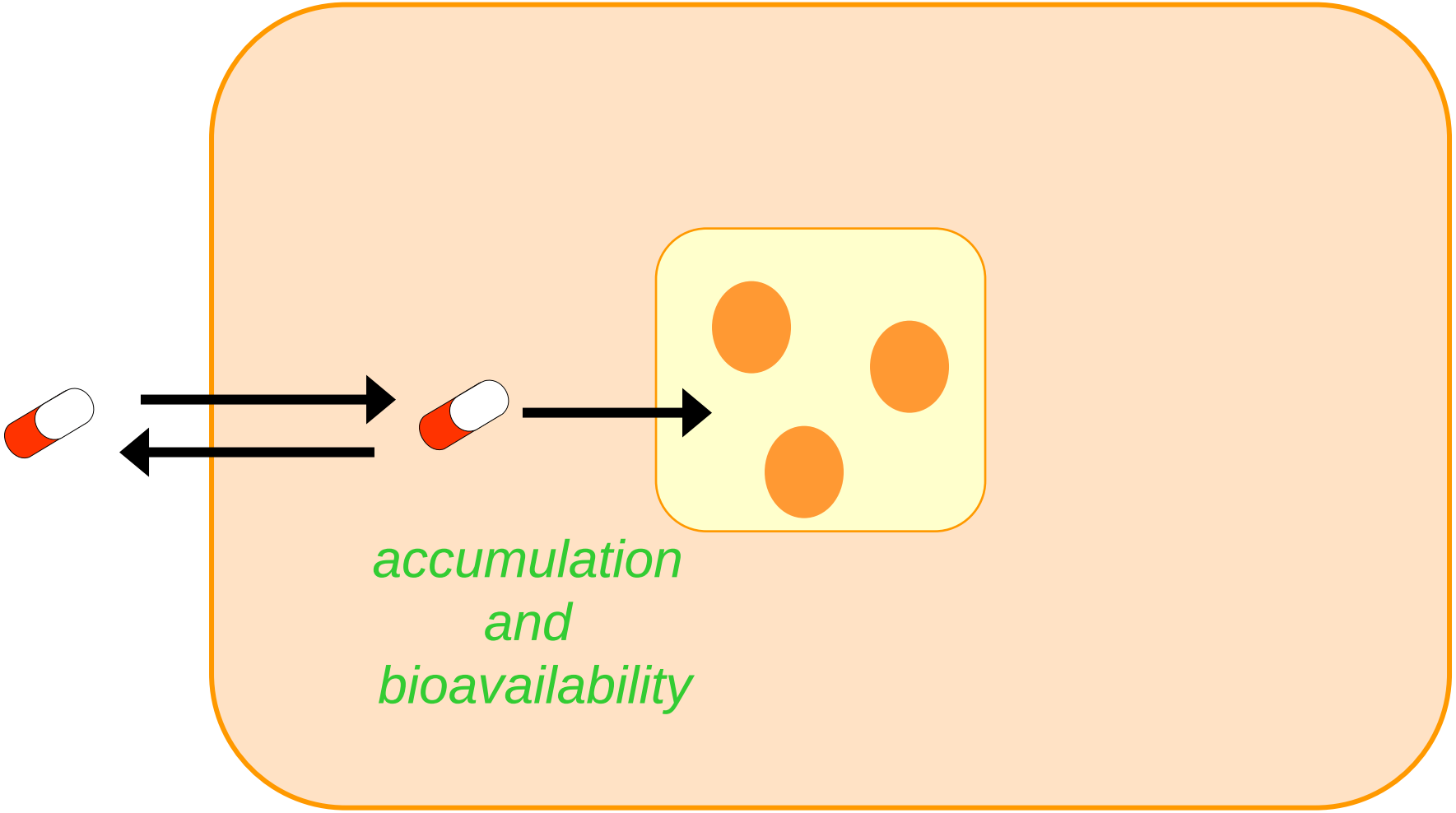
Phagolysosomes

Staphylococcus aureus



Do you like pharmacokinetics ?

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



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

Antibiotic accumulation and subcellular distribution

β-lactams; fast; ~ 1 x

fluoroquinolones : fast
CIP, LVX : 4-10 x
MXF, GAR, GMF : 10-20 x

aminoglycosides: slow ; 2-4 x

glycopeptides: slow
VAN ~ 8 x
TLV ~ 50 x
ORI ~ 150-300 x

macrolides: fast
ERY: 4-10 x
CLR, ROX, TEL: 10-50x
AZM: > 50 x

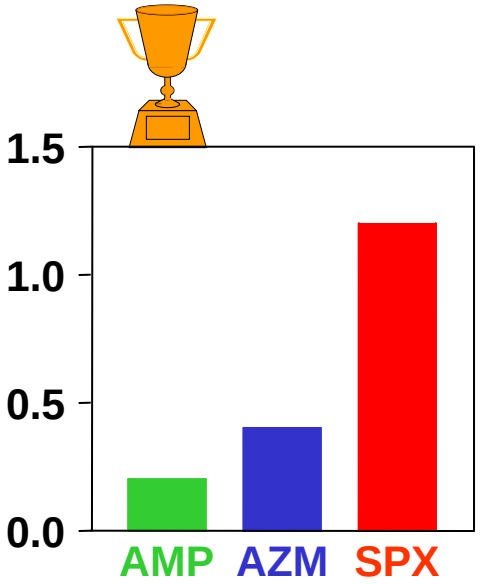
?

linezolid: ~ 1 x
Lincosamides: 1-4 x
Tetracyclines: 2-4 x
Rifampin : 2-10 x

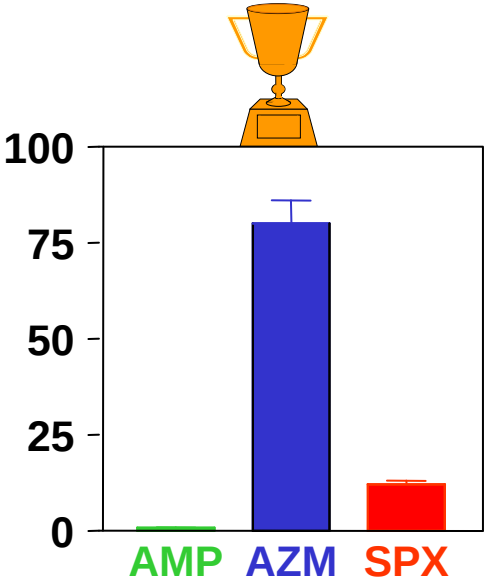
Van Bambeke et al., Curr Opin Drug Discov Devel. (2006) 9:218-30

Can we simply predict intracellular activity based on MIC and antibiotic accumulation?

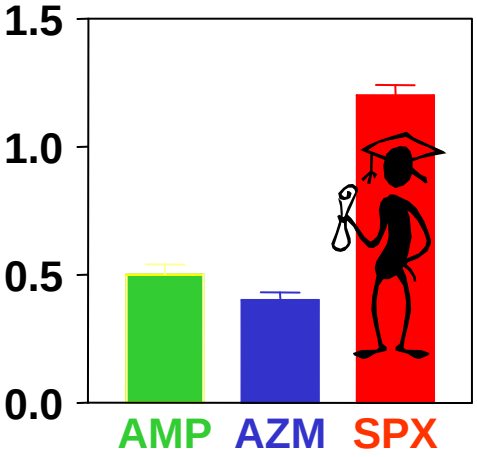
MIC
(L. monocytogenes)



antibiotic accumulation

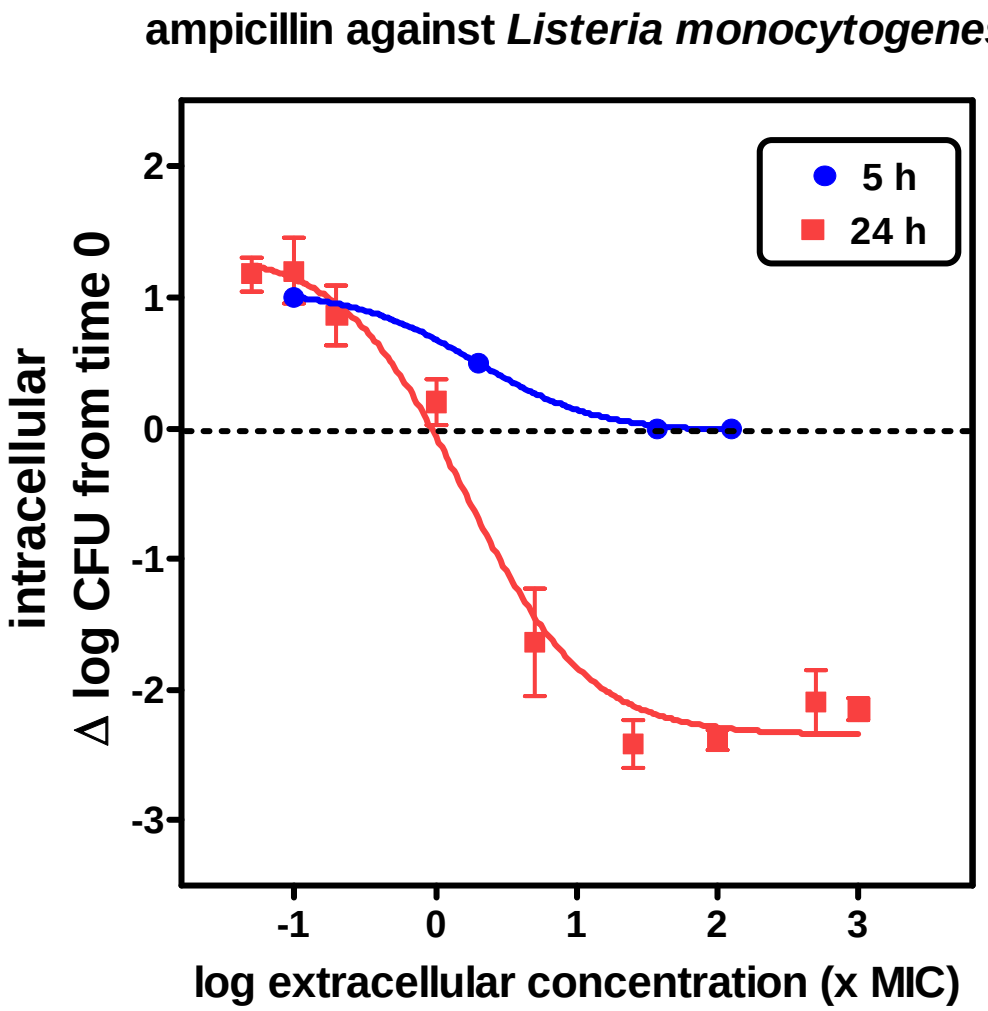


activity on intracellular *Listeria*
(5 h; 10 x MIC)



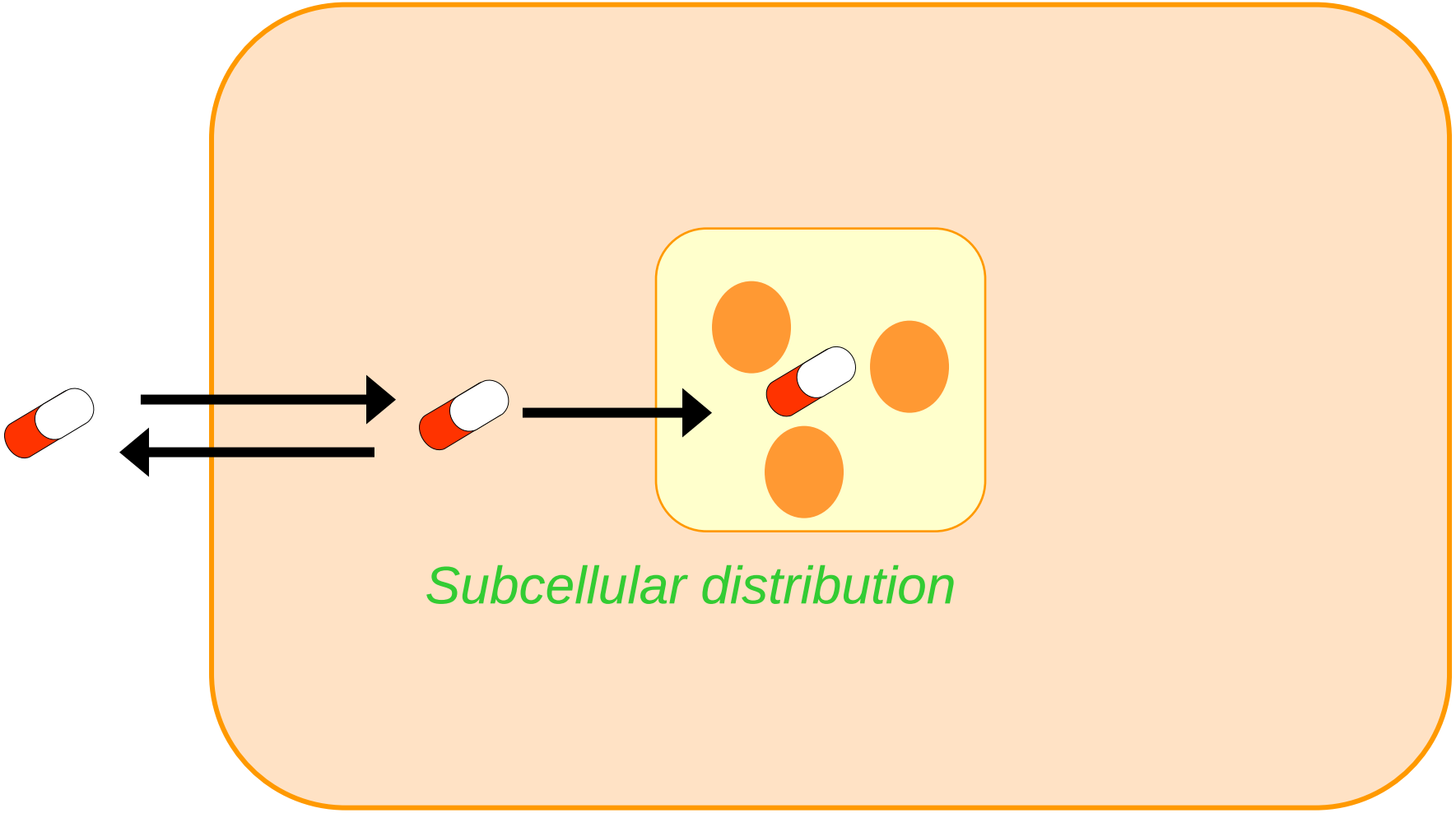
Ouadrhiri et al (1999) AAC 43:1242-51

Importance of optimizing time and concentration …



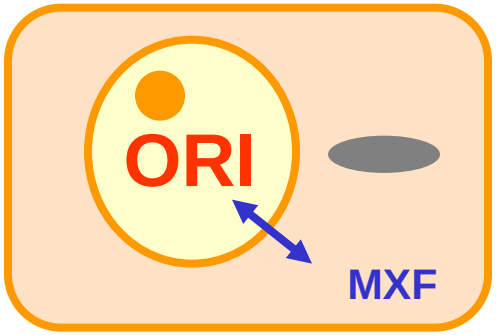
adapted from Lemaire et al., JAC (2005) 55:897-904

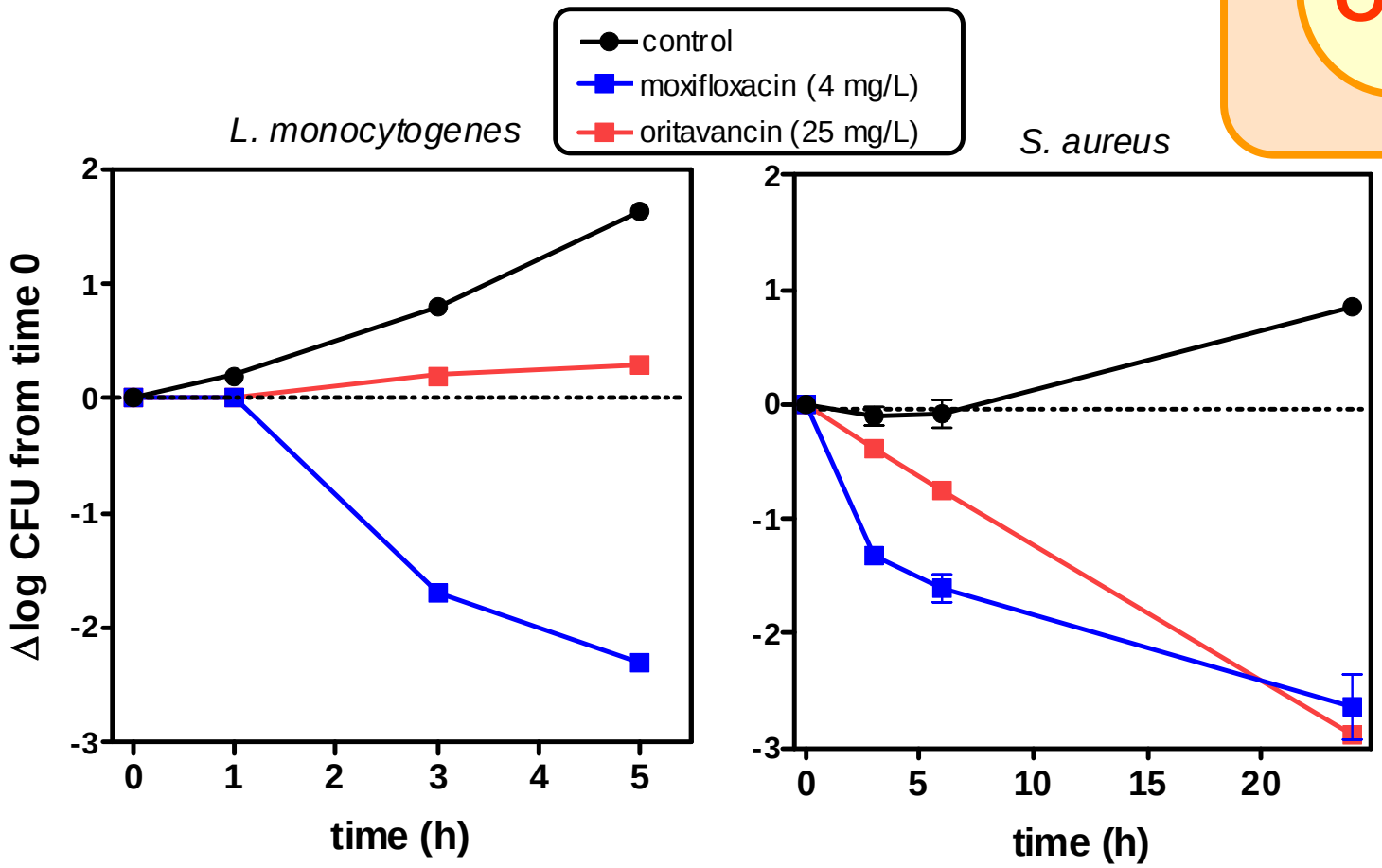
Intracellular vs extracellular activity of antibiotics : PK – PD in action



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

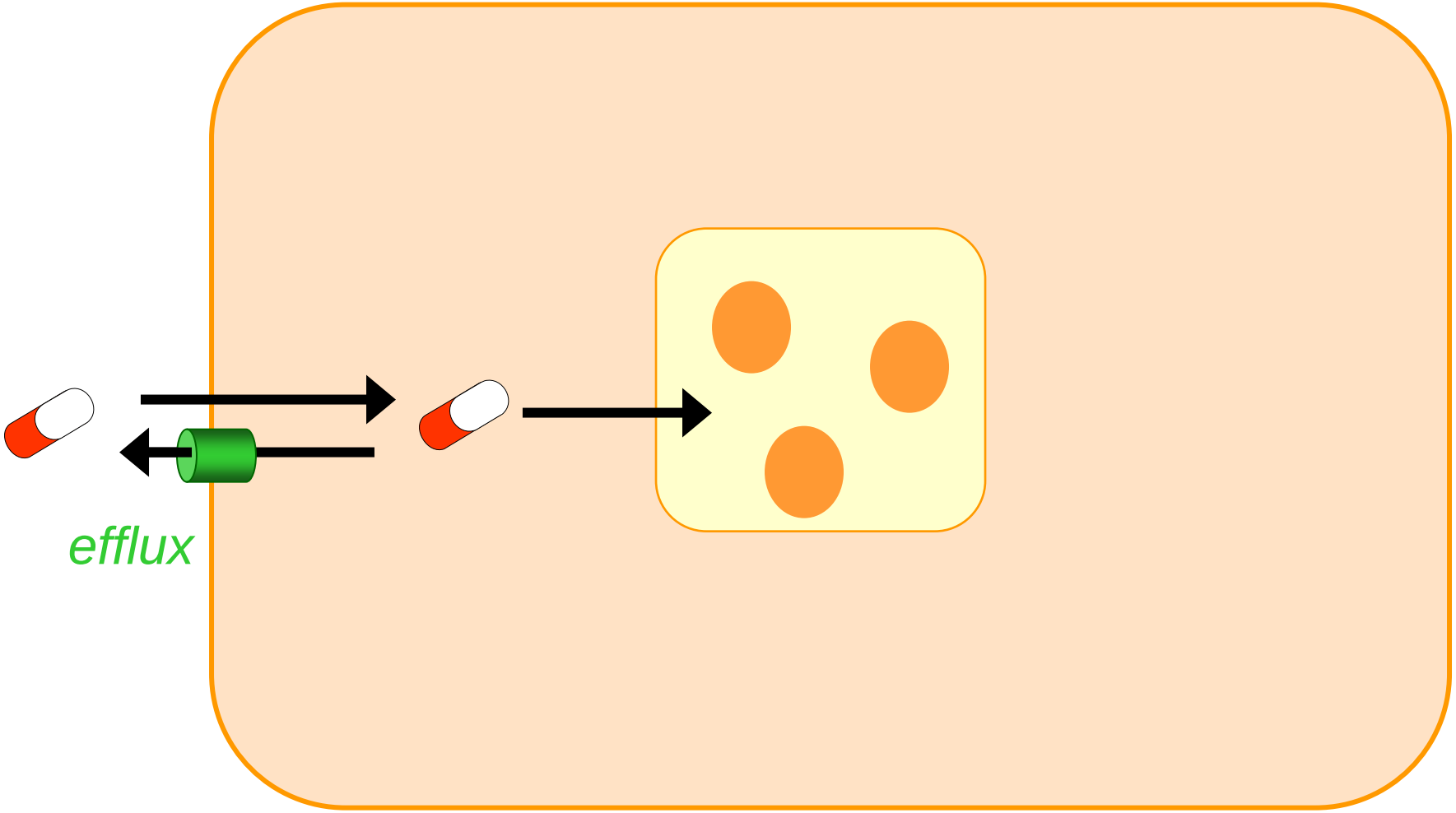
Importance of reaching intracellular bacteria …





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

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

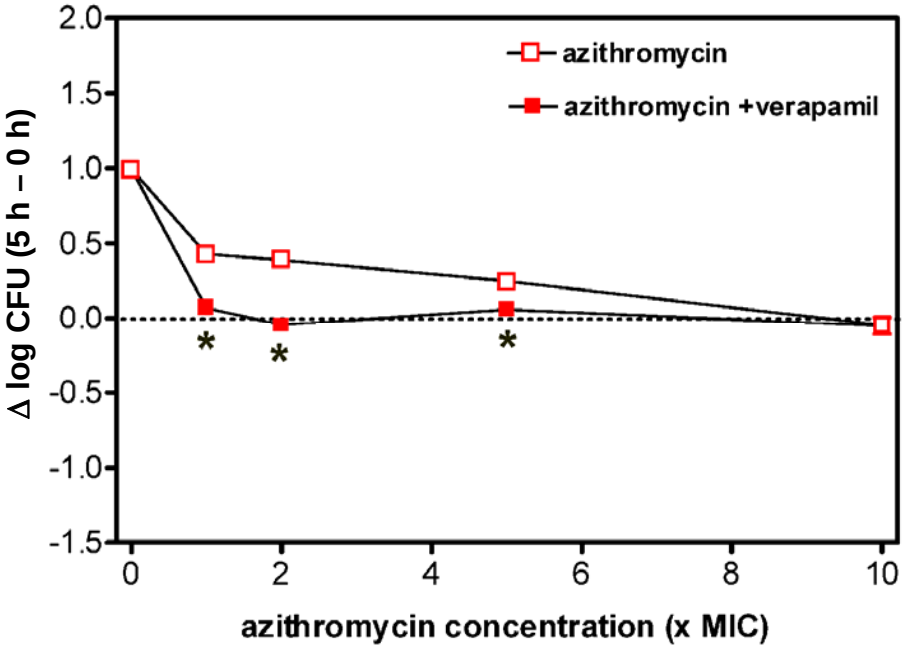


Carryn et al., Infect Dis Clin North Am. (2003) 17:615-34

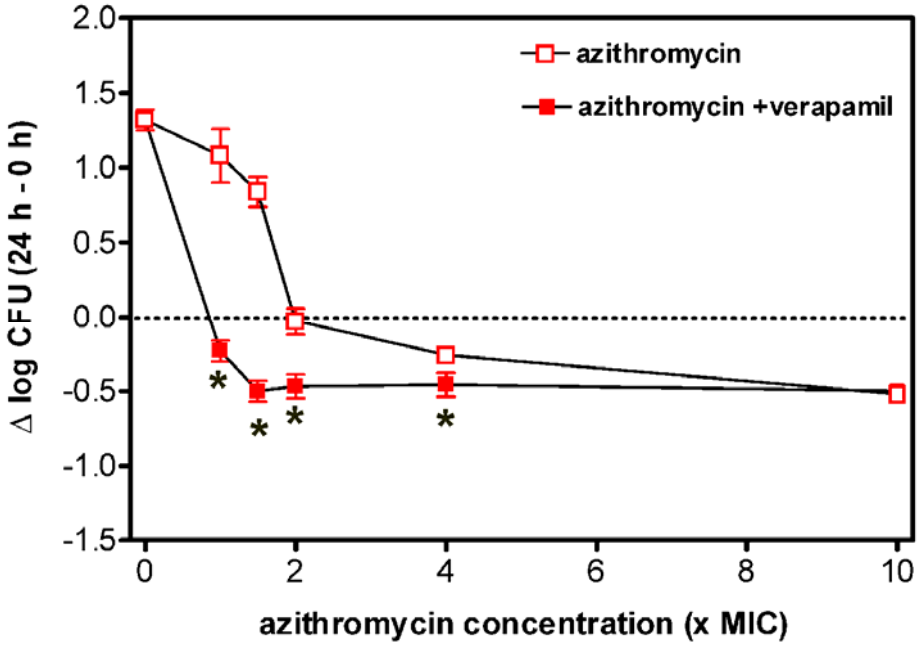
Influence of pump inhibitors on intracellular activity

Inhibition of P-gp by verapamil enhances azithromycin activity against *L. monocytogenes* and *S. aureus*

L. monocytogenes



S. aureus

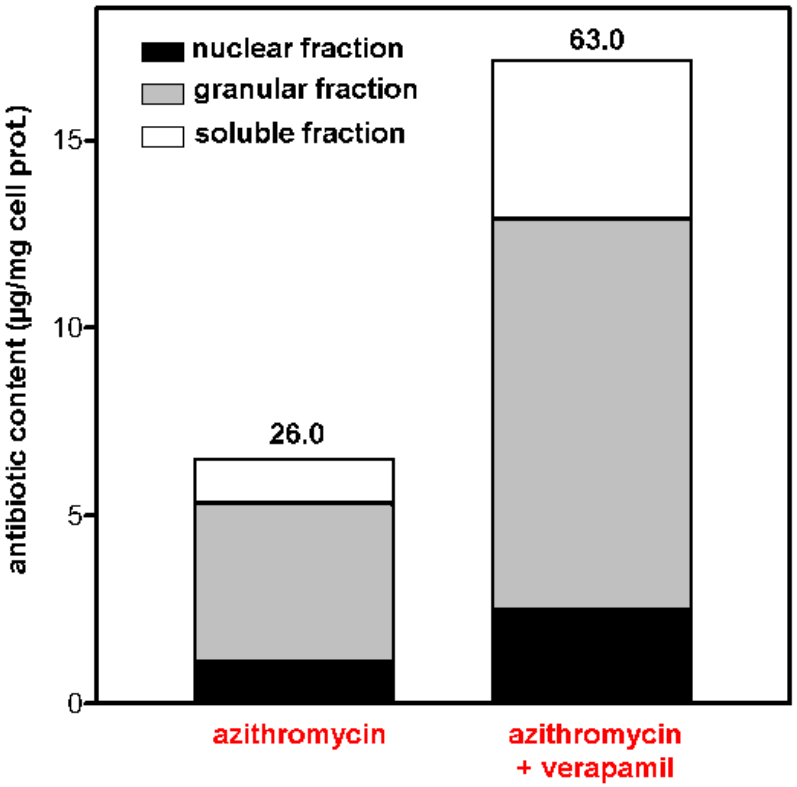


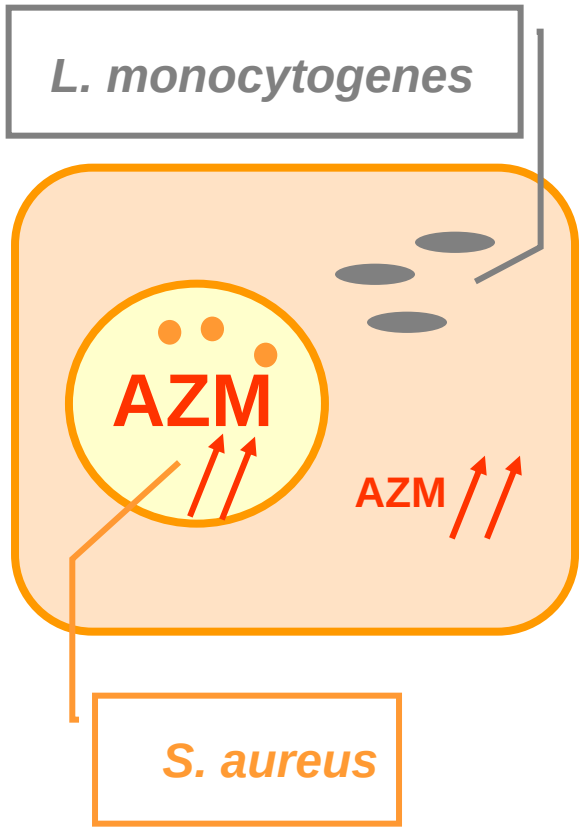
verapamil 20 µM; 24 h

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

Influence of pump inhibitors on antibiotic distribution

Inhibition of P-gp by verapamil enhances azithromycin concentration in cytosol and vacuoles



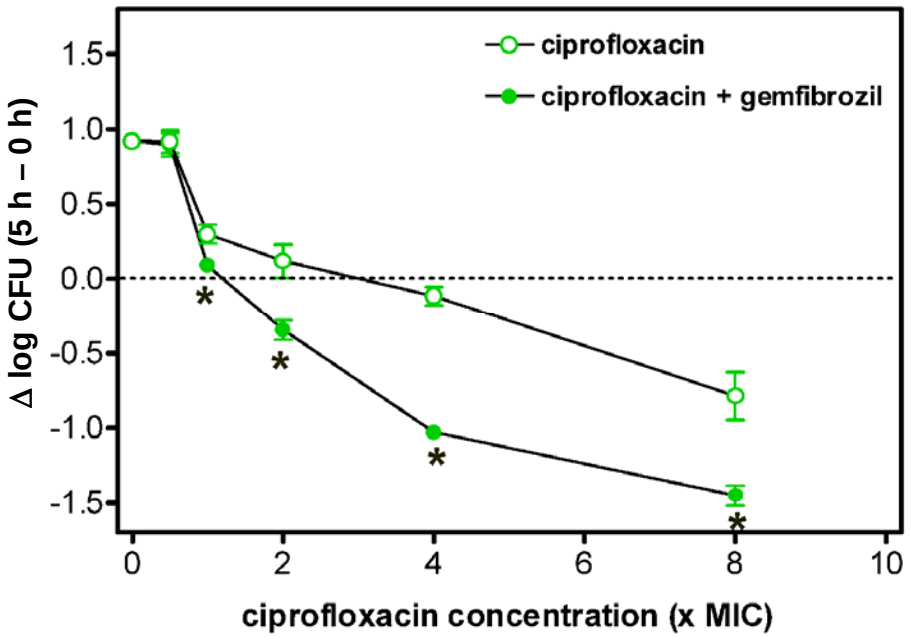


Seral et al., JAC (2003) 51:1167-73

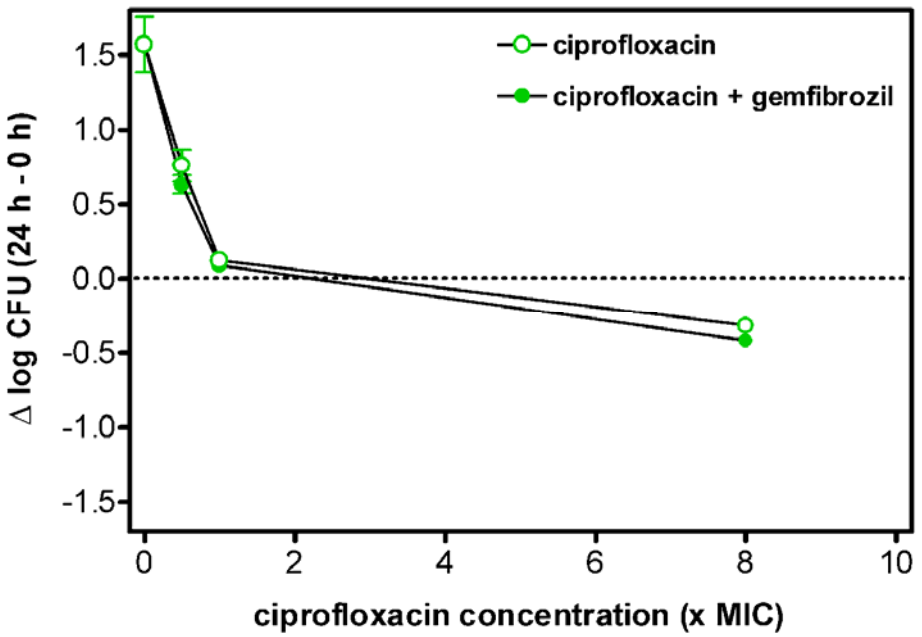
Influence of pump inhibitors on intracellular activity

Inhibition of MRP by gemfibrozil enhances ciprofloxacin activity against *L. monocytogenes* only

L. monocytogenes



S. aureus

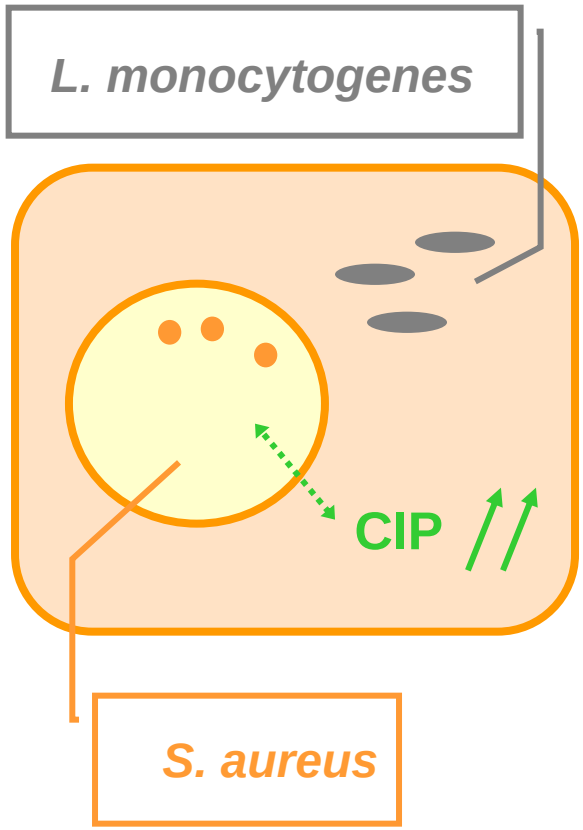


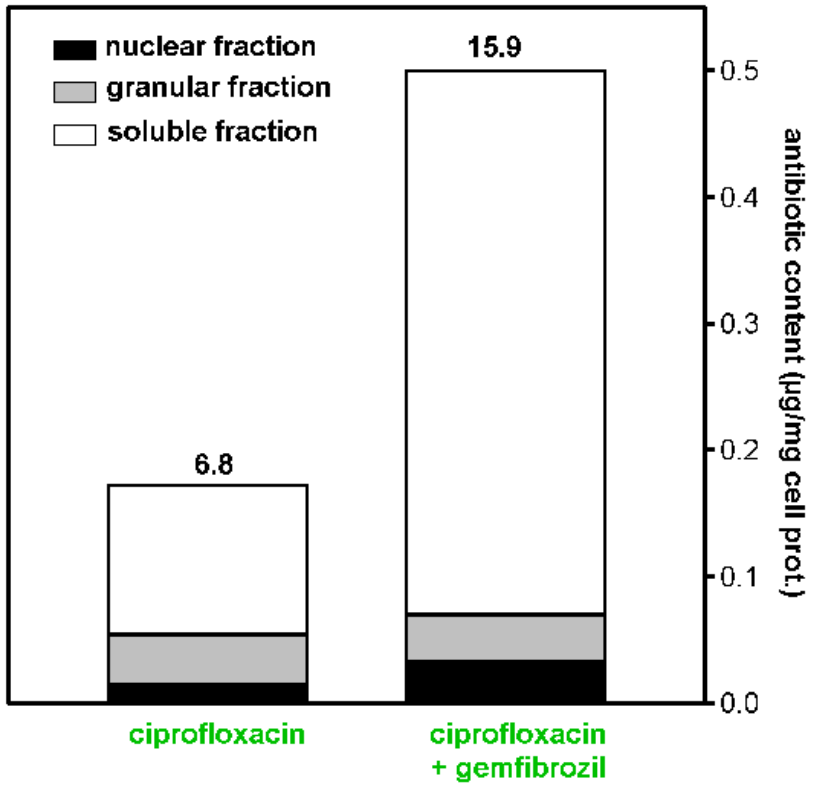
Seral et al., JAC (2003) 51:1167-73

verapamil 20 µM; 24 h

Influence of pump inhibitors on antibiotic distribution

Inhibition of MRP by gemfibrozil enhances ciprofloxacin concentration in the cytosol

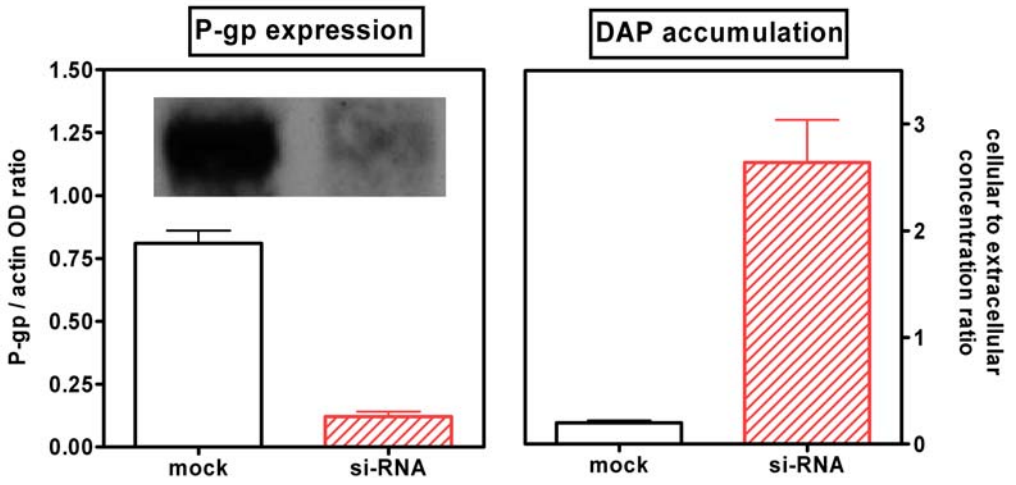


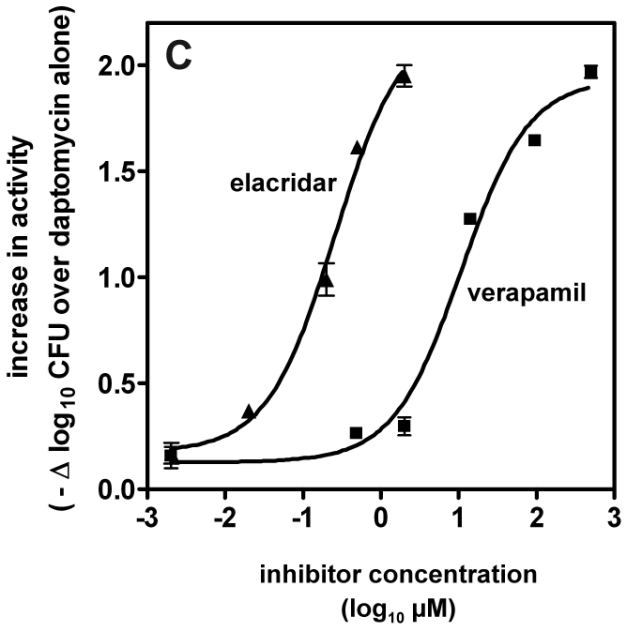


Seral et al., JAC (2003) 51:1167-73

Daptomycin, an unexepected substrate for P-gp

The cellular accumulation and intracellular activity of daptomycin are increased upon inhibition or under-expression of P-gp



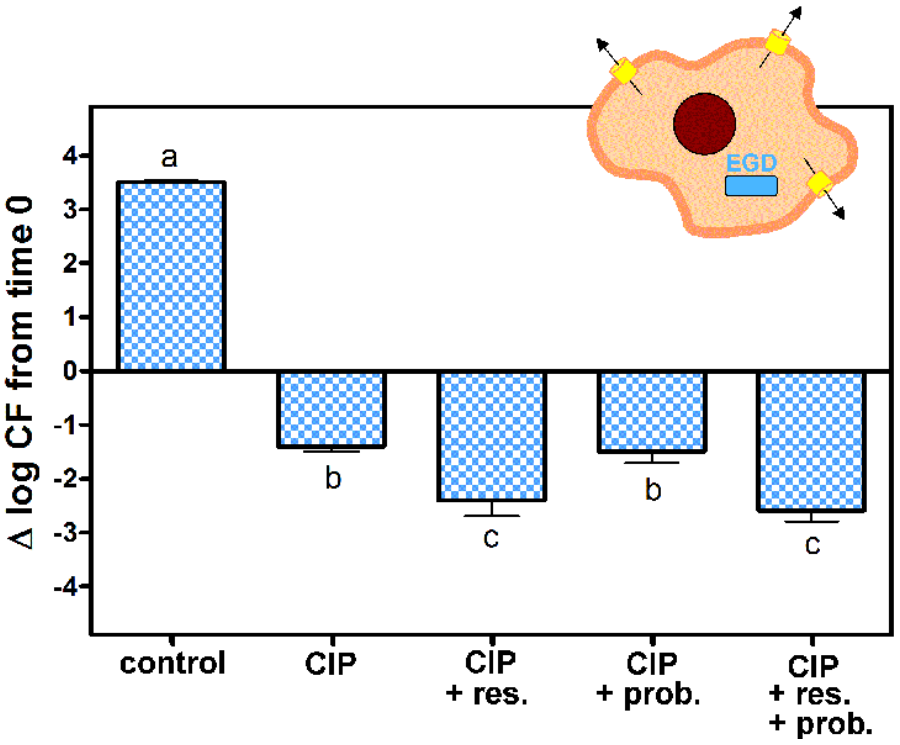


Lemaire et al., AAC (2007) 51:2748-57

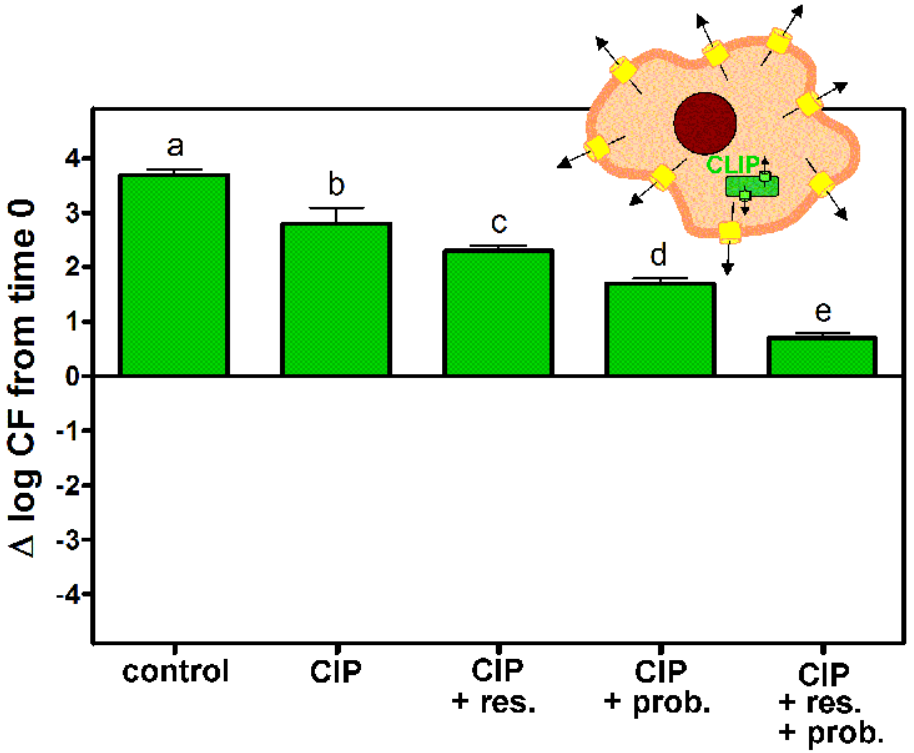
Cooperation between efflux pumps of bacteria and of macrophages

Ciprofloxacin and *Listeria*

Wild-type cells and bacteria



cells and bacteria overproducing efflux pumps for ciprofloxacin

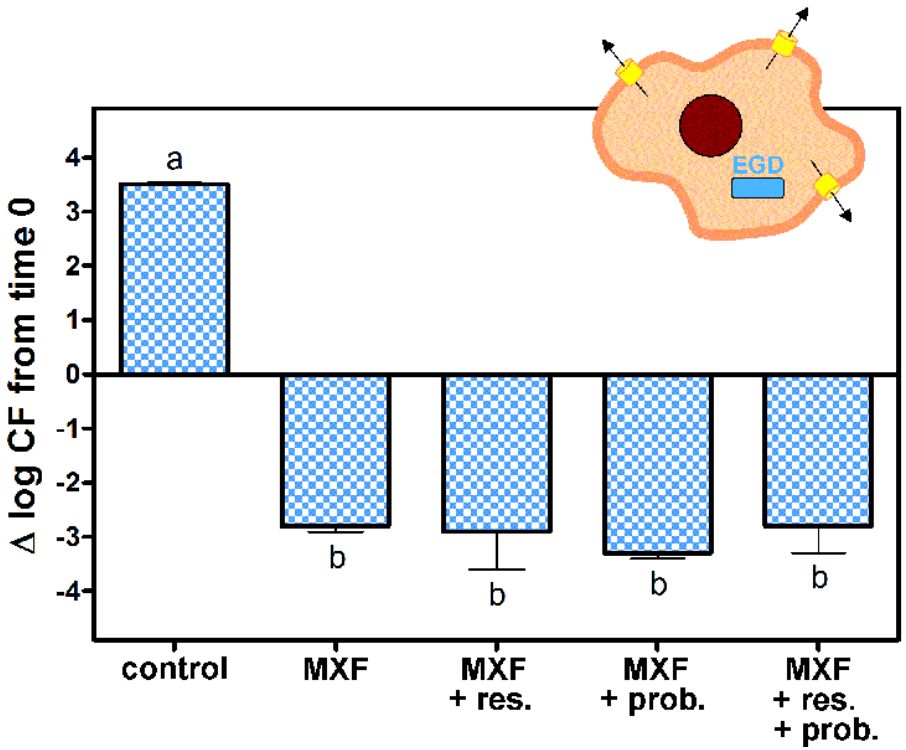


Lismond et al., AAC (2008) 52:3040-46

Cooperation between efflux pumps of bacteria and of macrophages

Moxifloxacin and *Listeria*

Wild-type cells and bacteria



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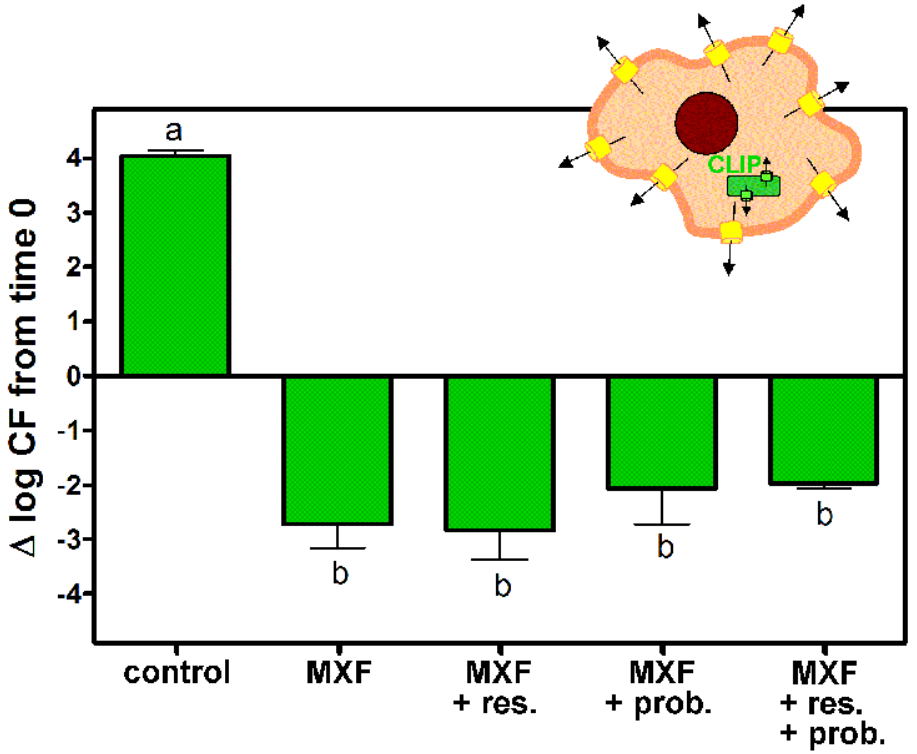
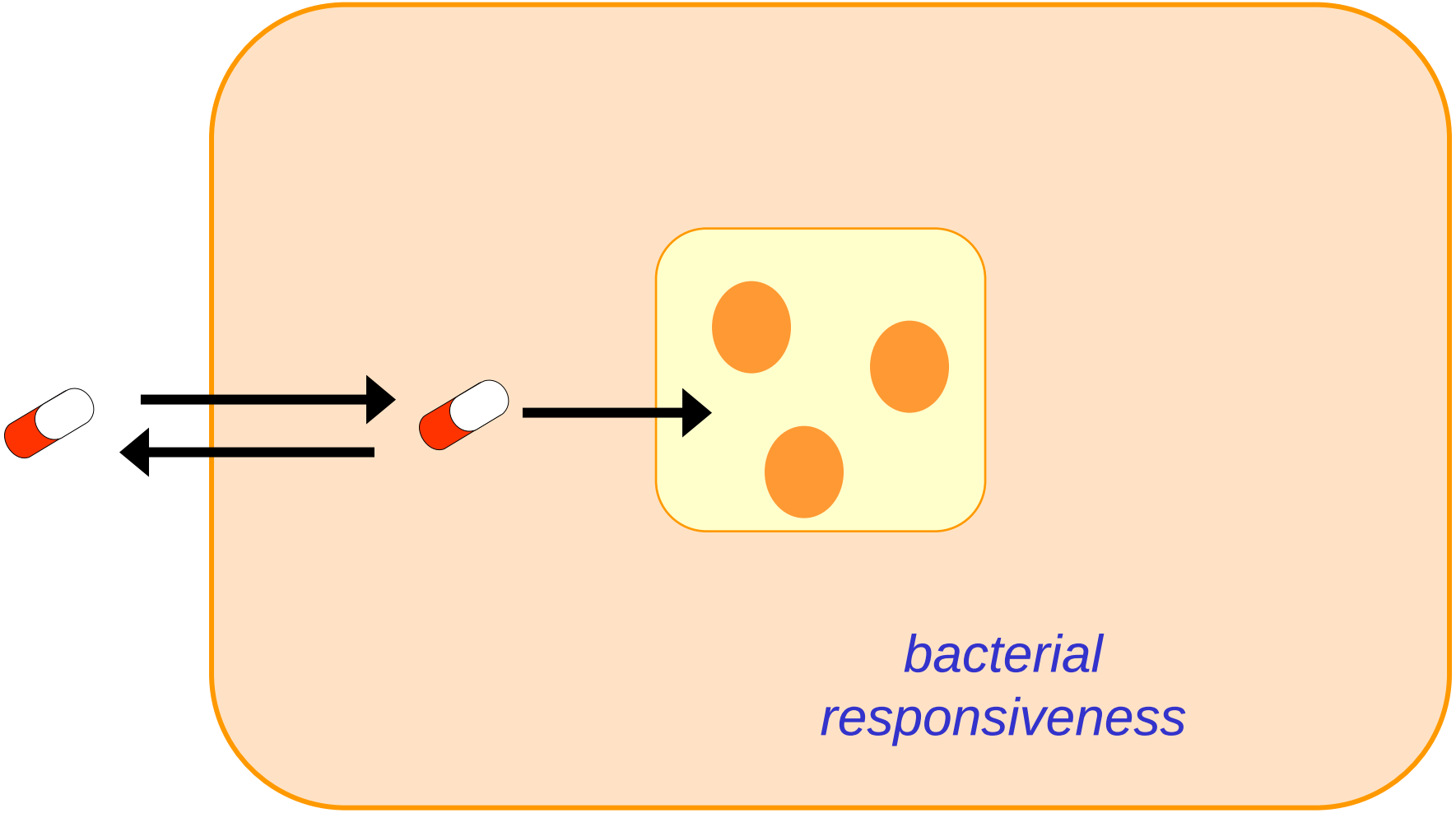


Lismond et al., AAC (2008) 52:3040-46

Do you prefer pharmacodynamics ?

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



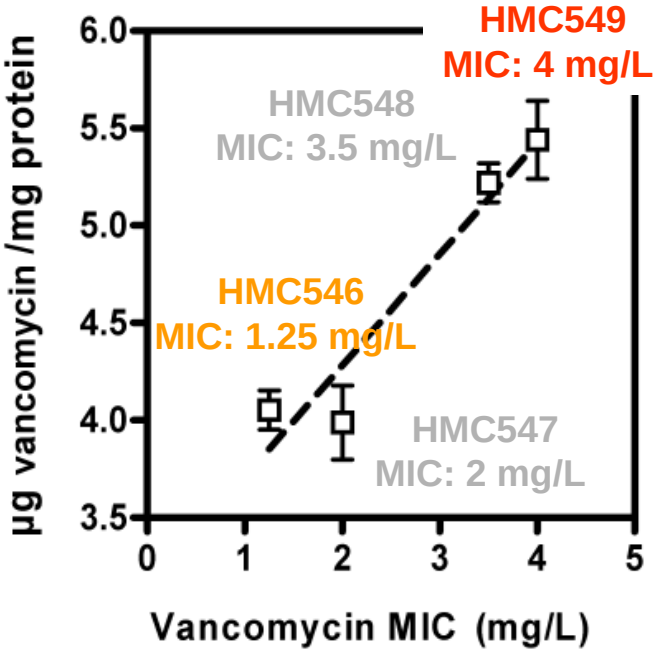
Carryn et al., Infect Dis Clin North Am. (2003) 17:615-34

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

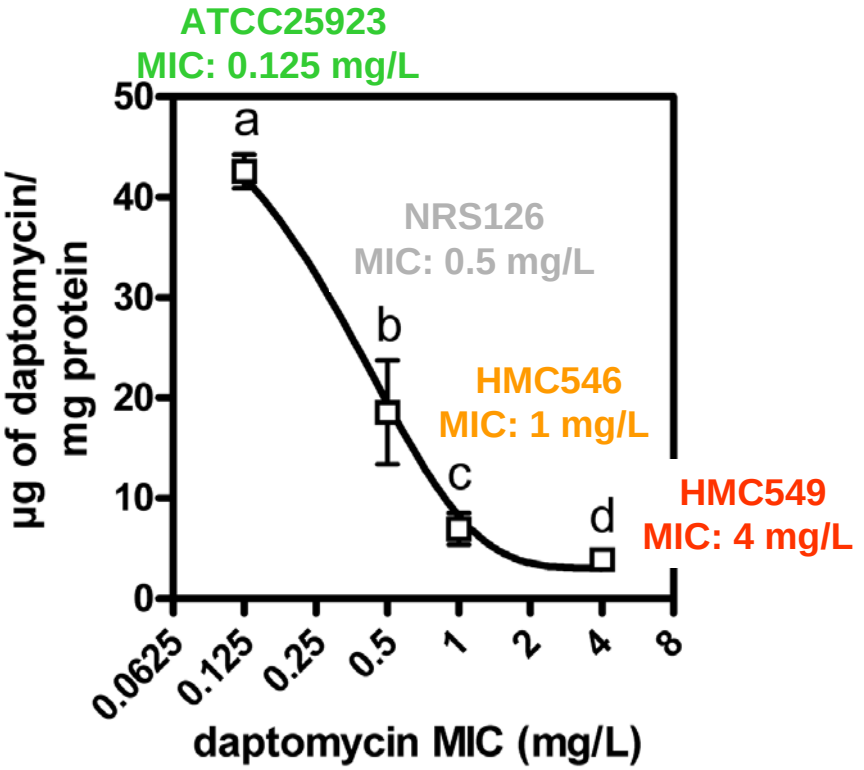
Julian et al. AAC (2007) 51:3445-8.

Reduced susceptibility associated with

increased amount of bound vancomycin



decreased amount of bound daptomycin

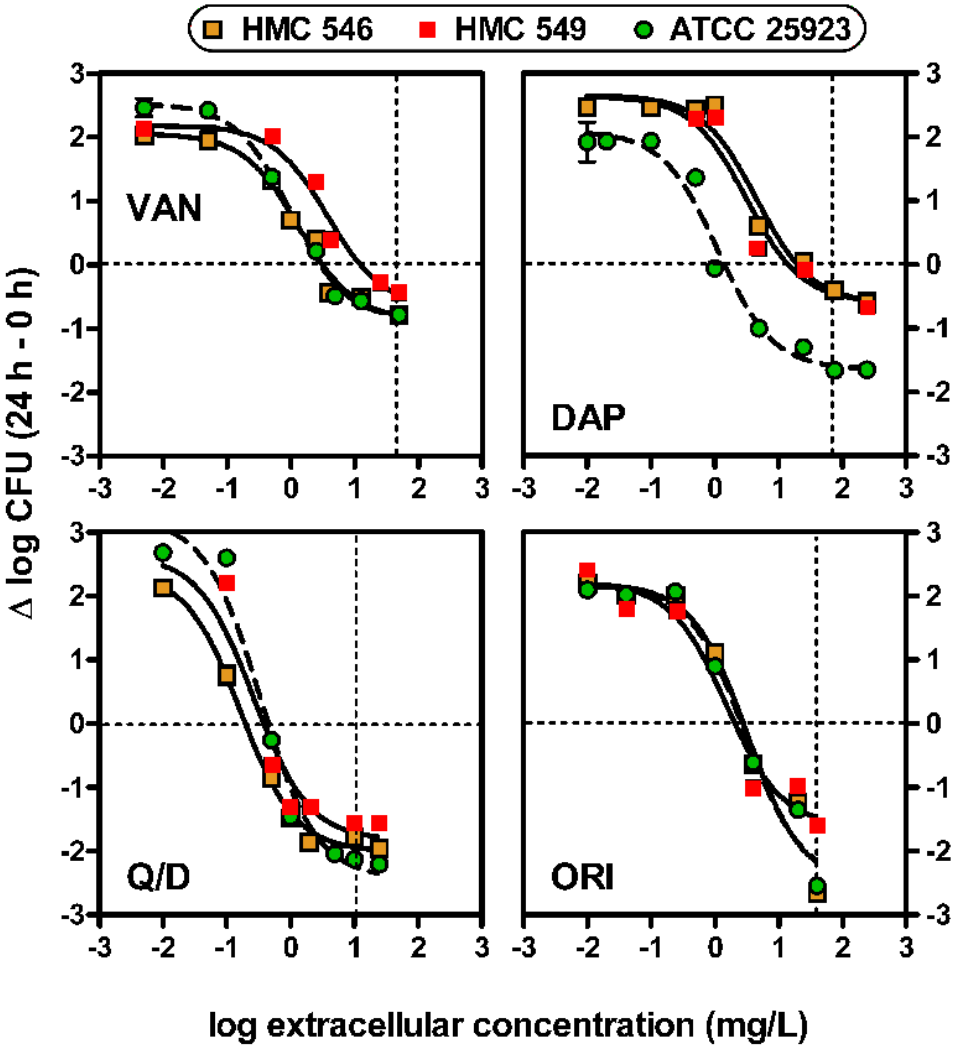


Lemaire et al., CMI (2008) 14:766-77

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}

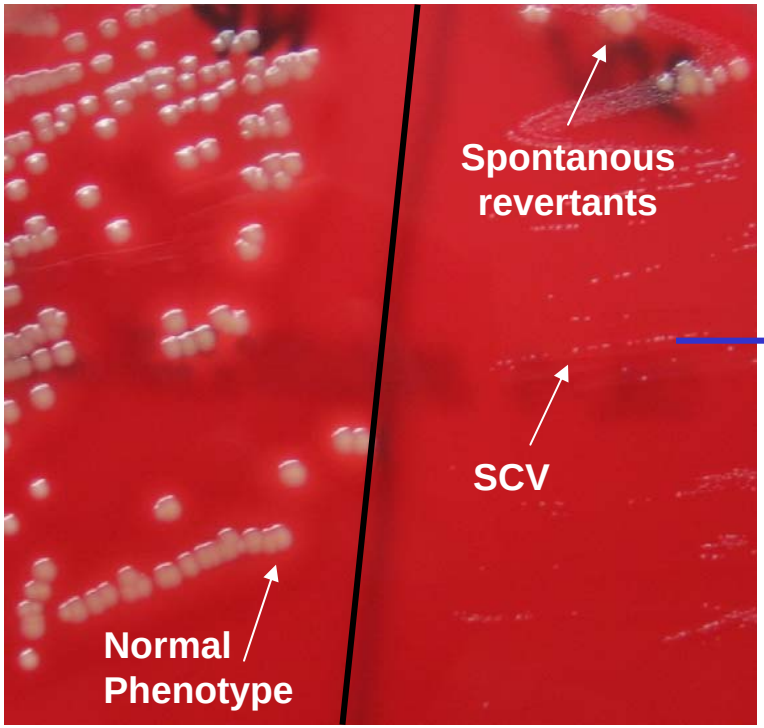
lower intracellular Emax

no effect of resistance phenotype

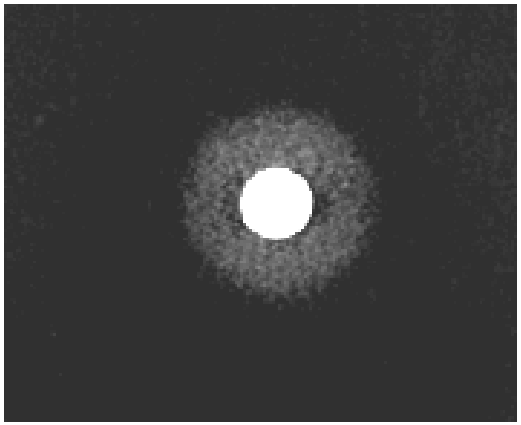
Lemaire et al., CMI (2008) 14:766-77

SCV isolated from a cystic fibrosis patient

Vergison et al. JAC (2007) 59:893-9

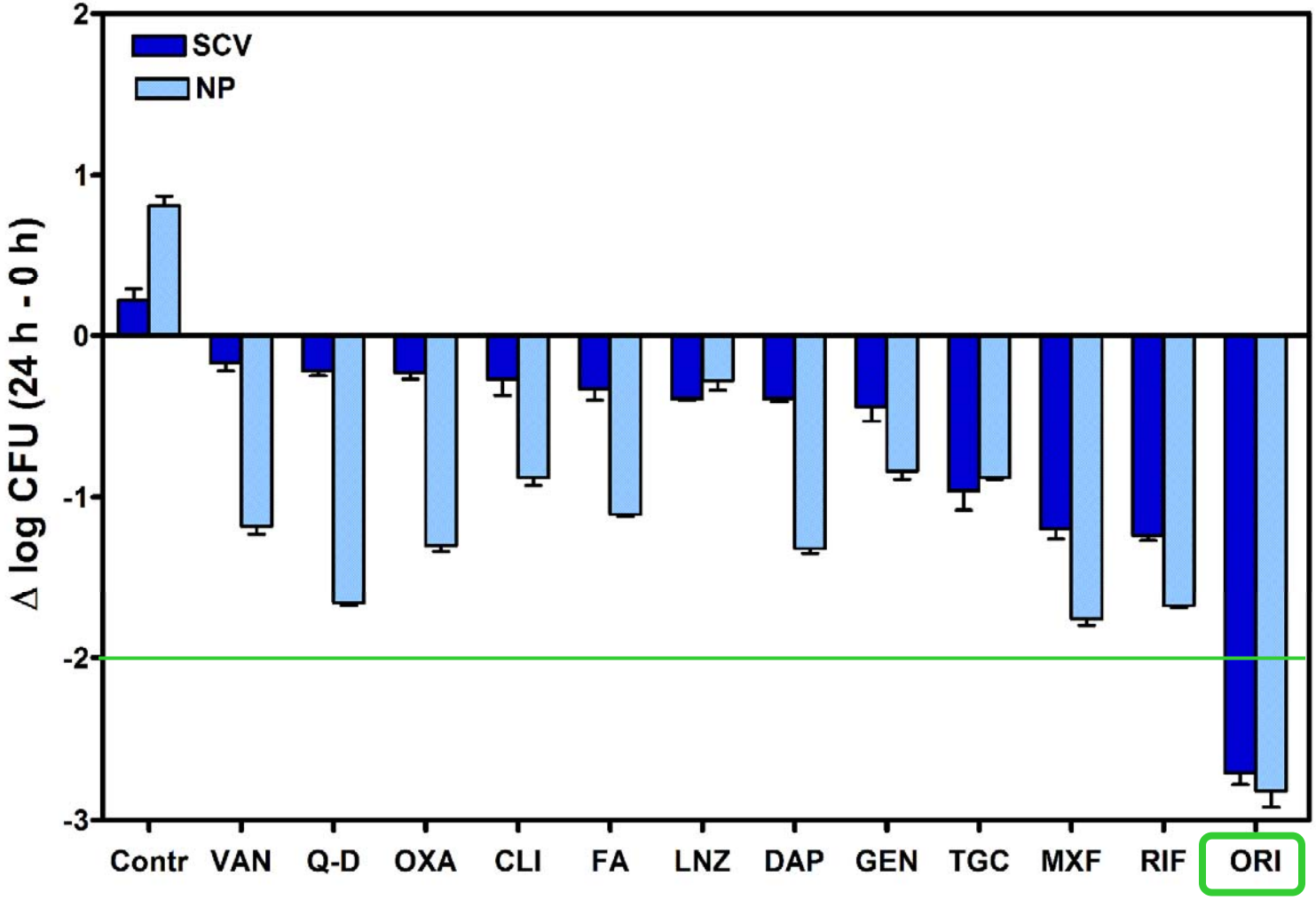


Thymidine dependent



Intracellular activity, SCV *vs*. normal phenotype

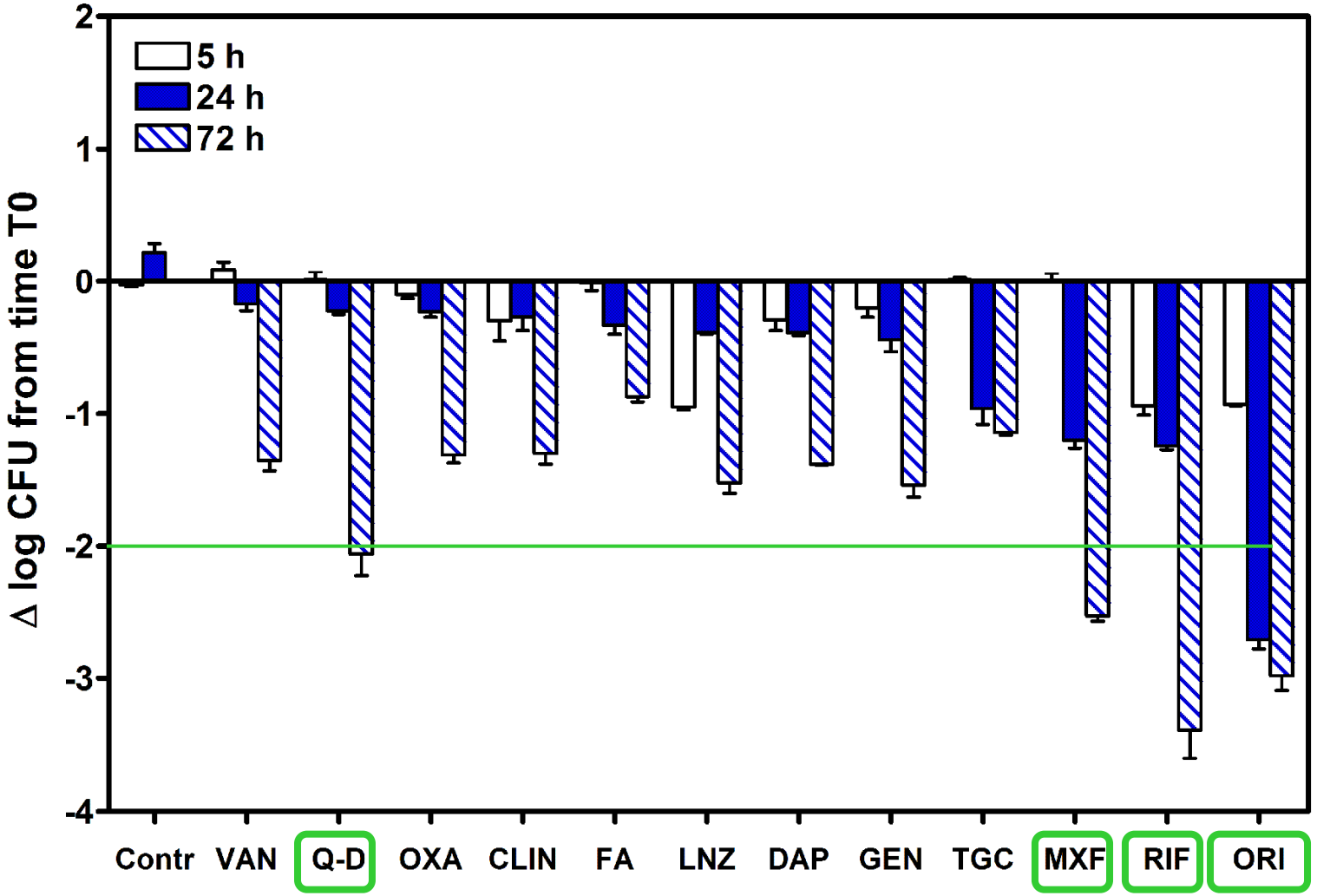
THP-1; 24 h, antibiotics at Cmax



Nguyen et al, RICAI 2007, poster 325

Intracellular activity, SCV over time

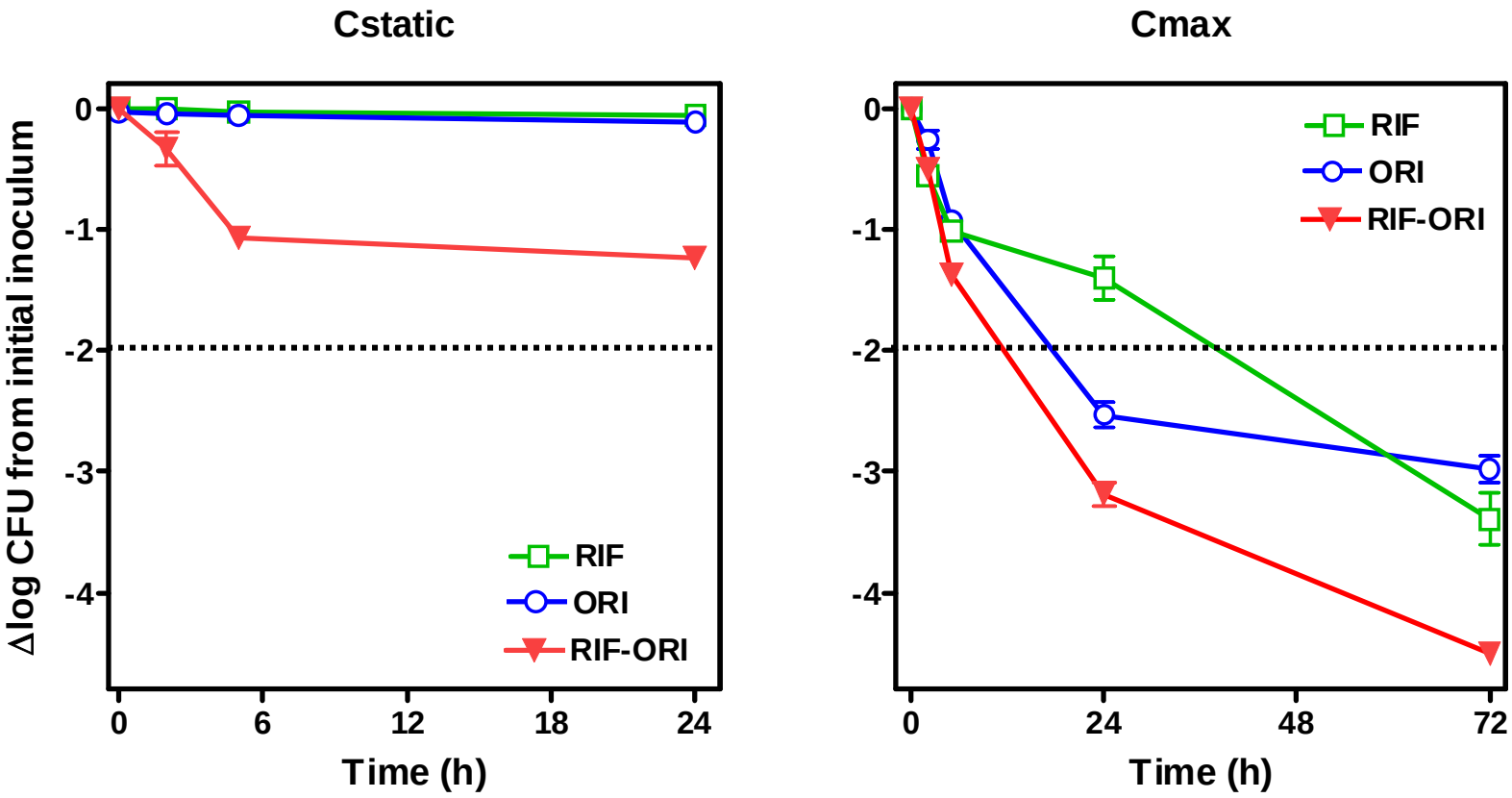
THP-1; SCV, antibiotics at Cmax for up to 3 days



Nguyen et al., ICAAC 2007, poster A1437

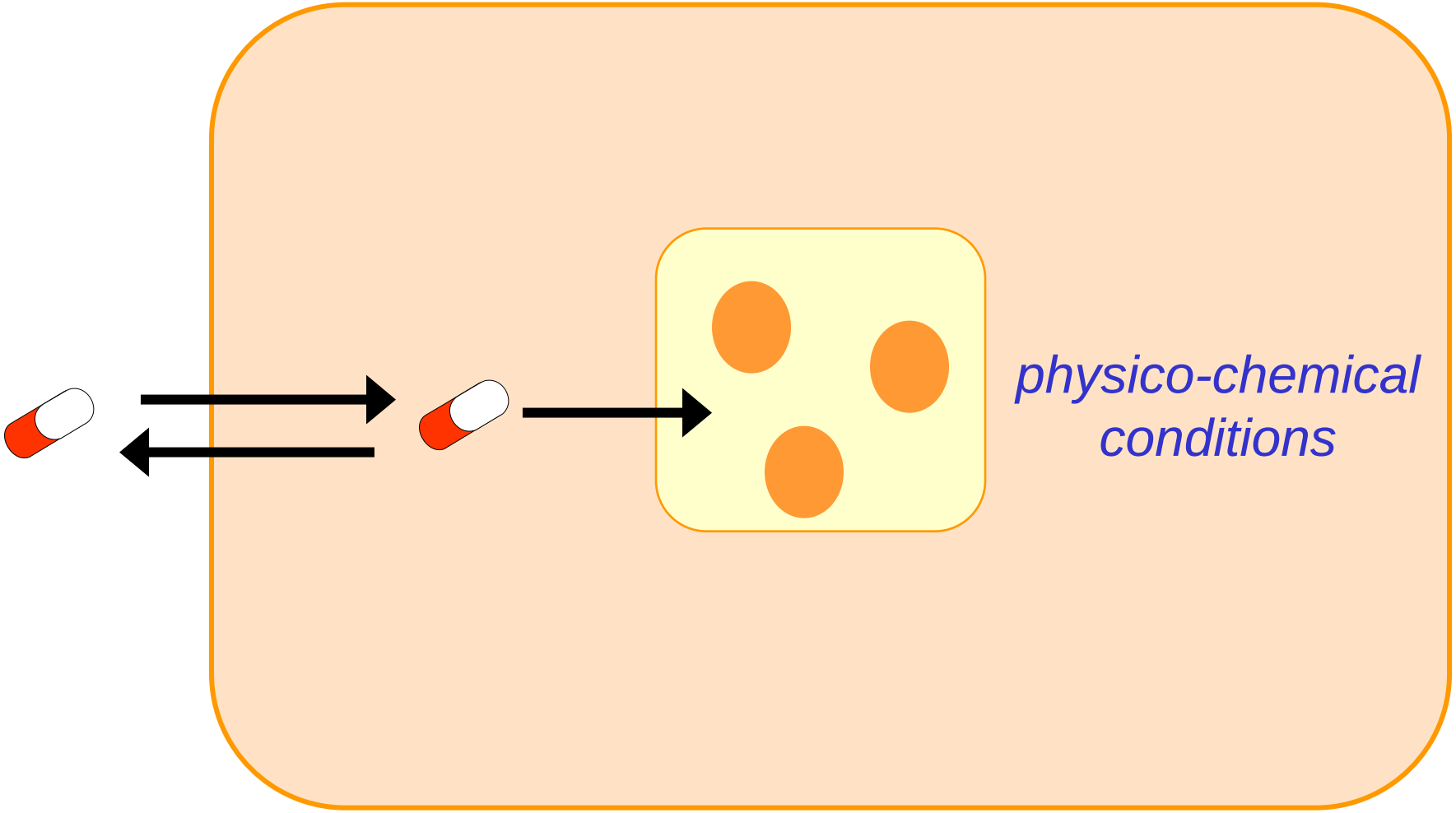
Can we do better with combinations ?

THP-1; SCV, antibiotics at Cstatic for up to 24 h or at Cmax for up to 3 days



Nguyen et al., ECCMID 2008, poster 1059

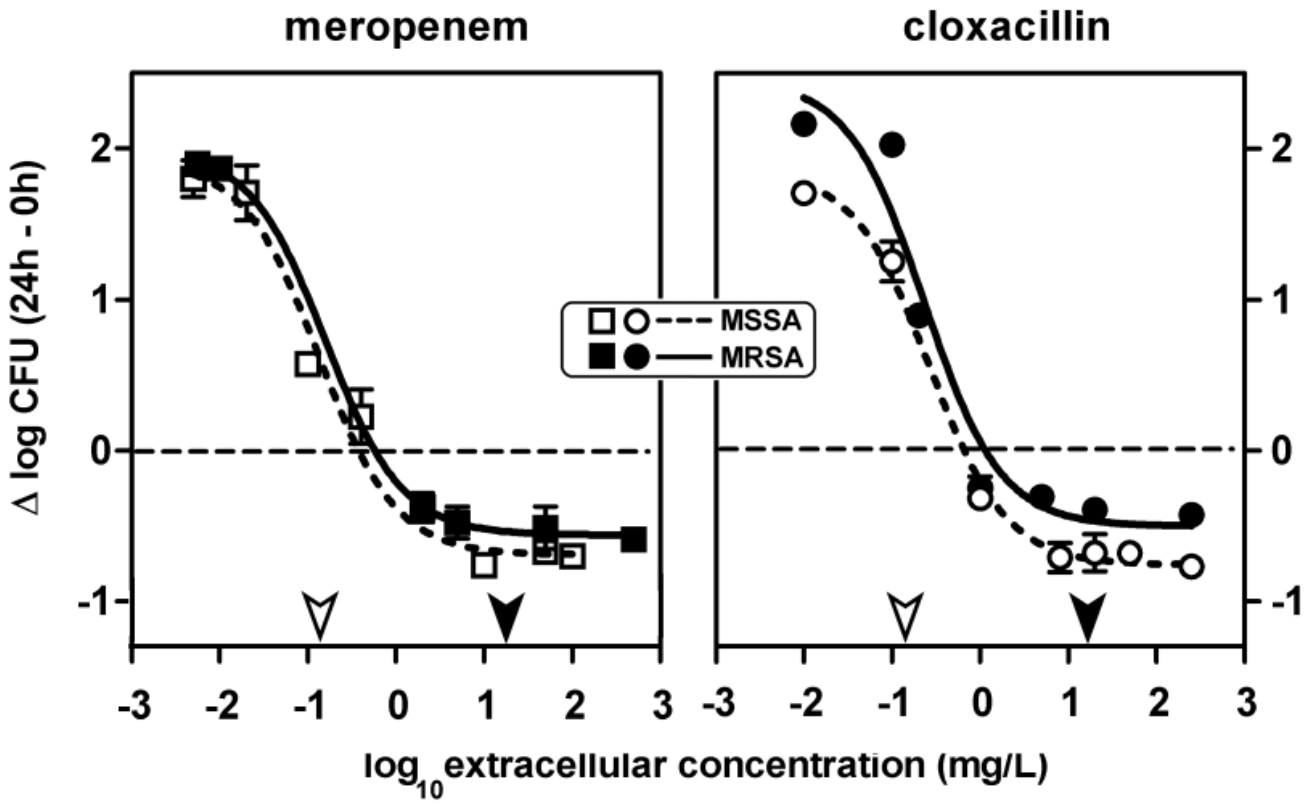
Intracellular vs extracellular activity of antibiotics : PK – PD in action



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

MRSA *vs.* MSSA: intracellular activity of β-lactams

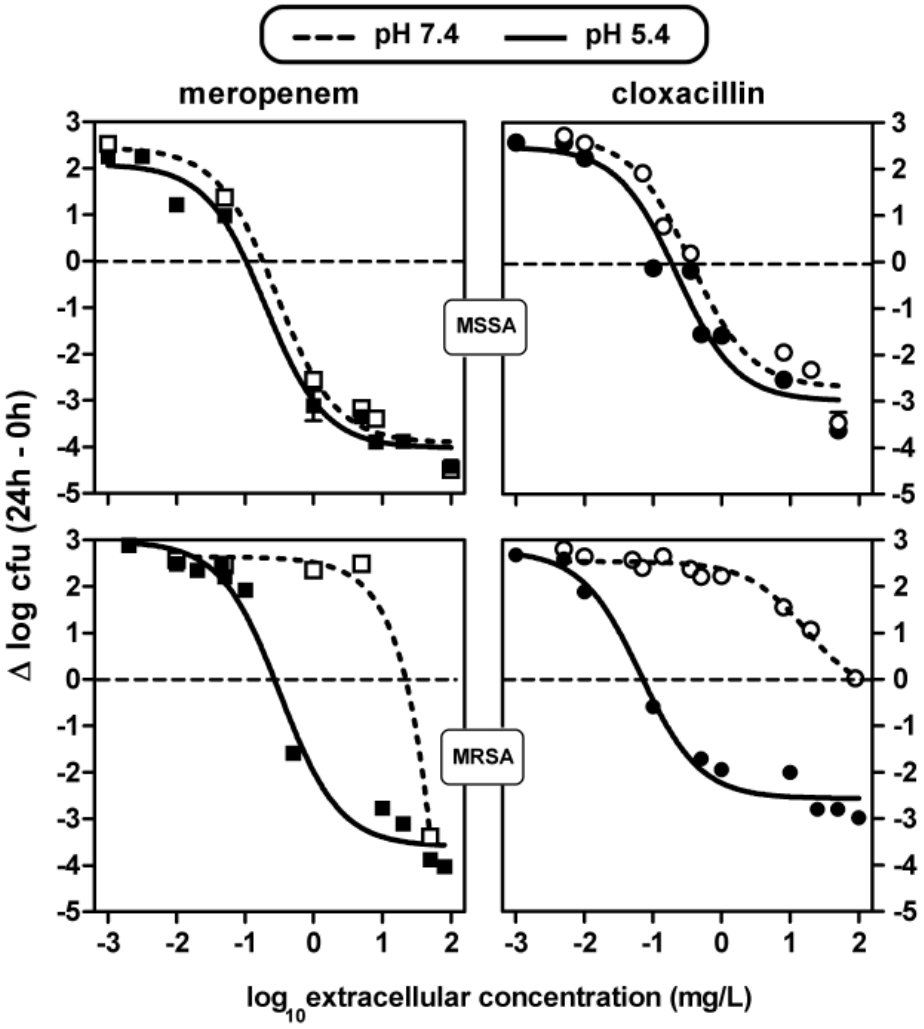
MRSA are as susceptible as MSSA to β-lactams when intracellular !



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

MRSA *vs.* MSSA: extracellular activity of β-lactams

MRSA are as susceptible as MSSA in broth at acidic pH

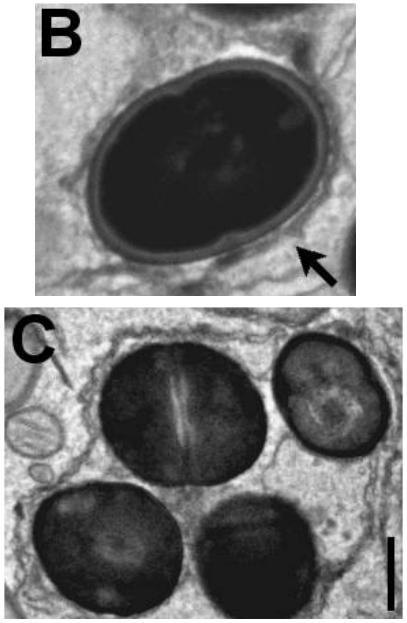


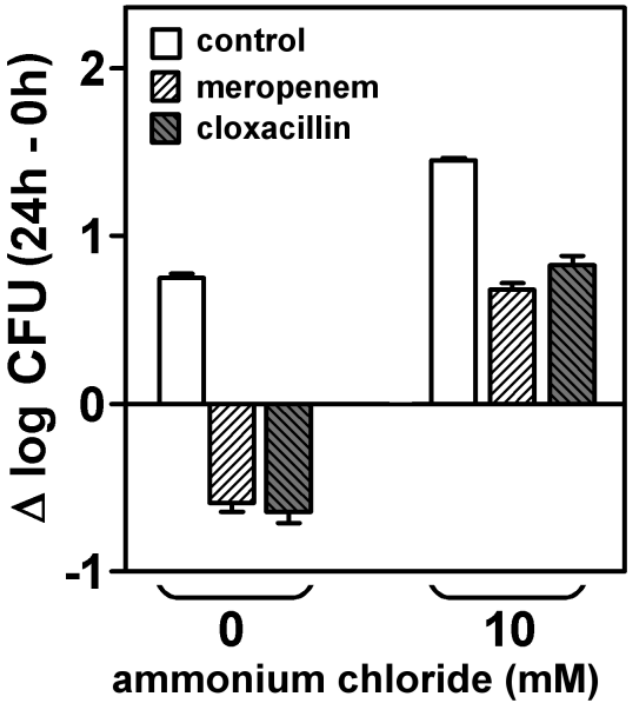
Lemaire et al., AAC (2007) 51:1627-32

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., AAC (2007) 51:1627-32

PBP2a conformation is modified by acidic pH

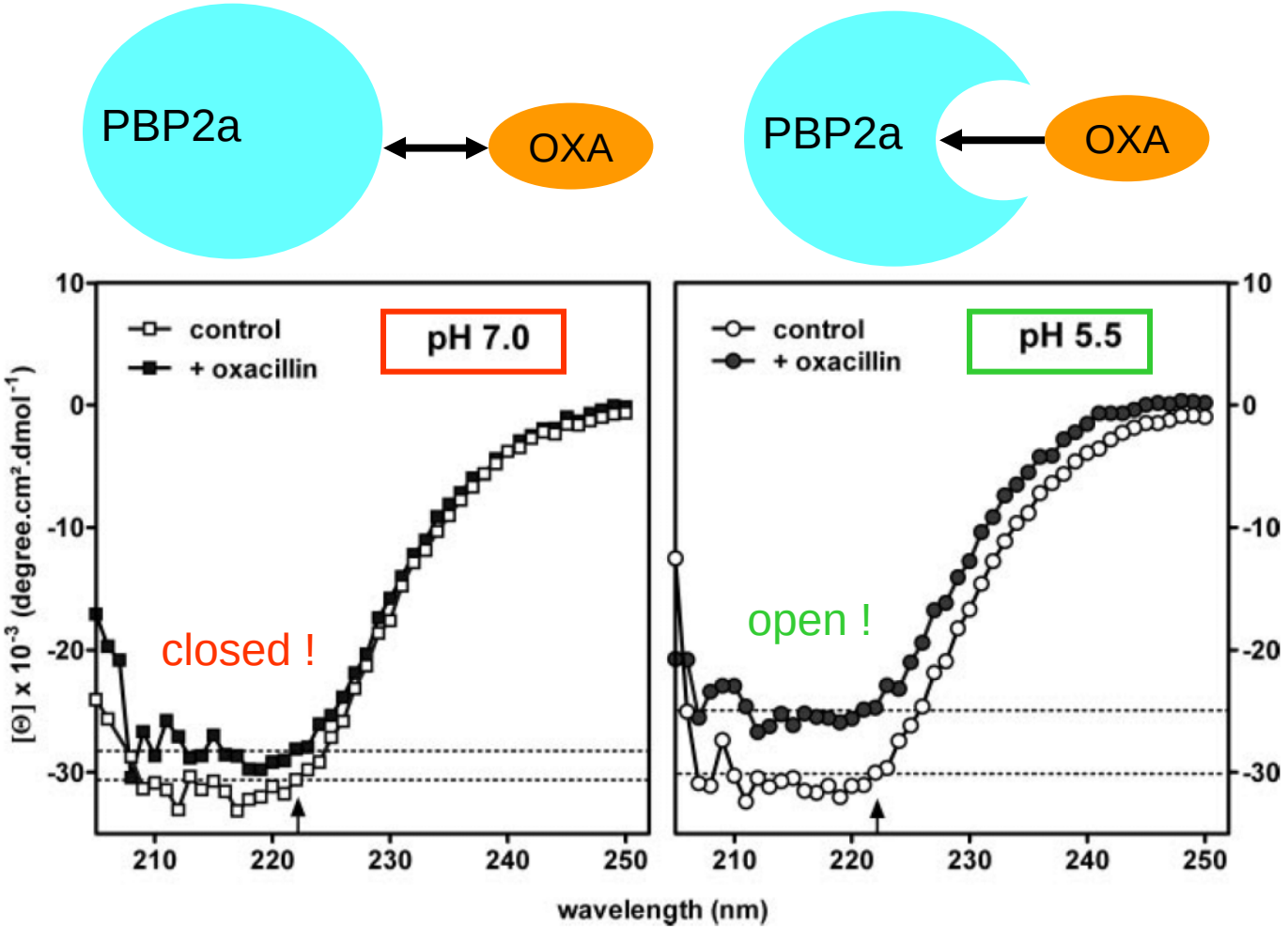
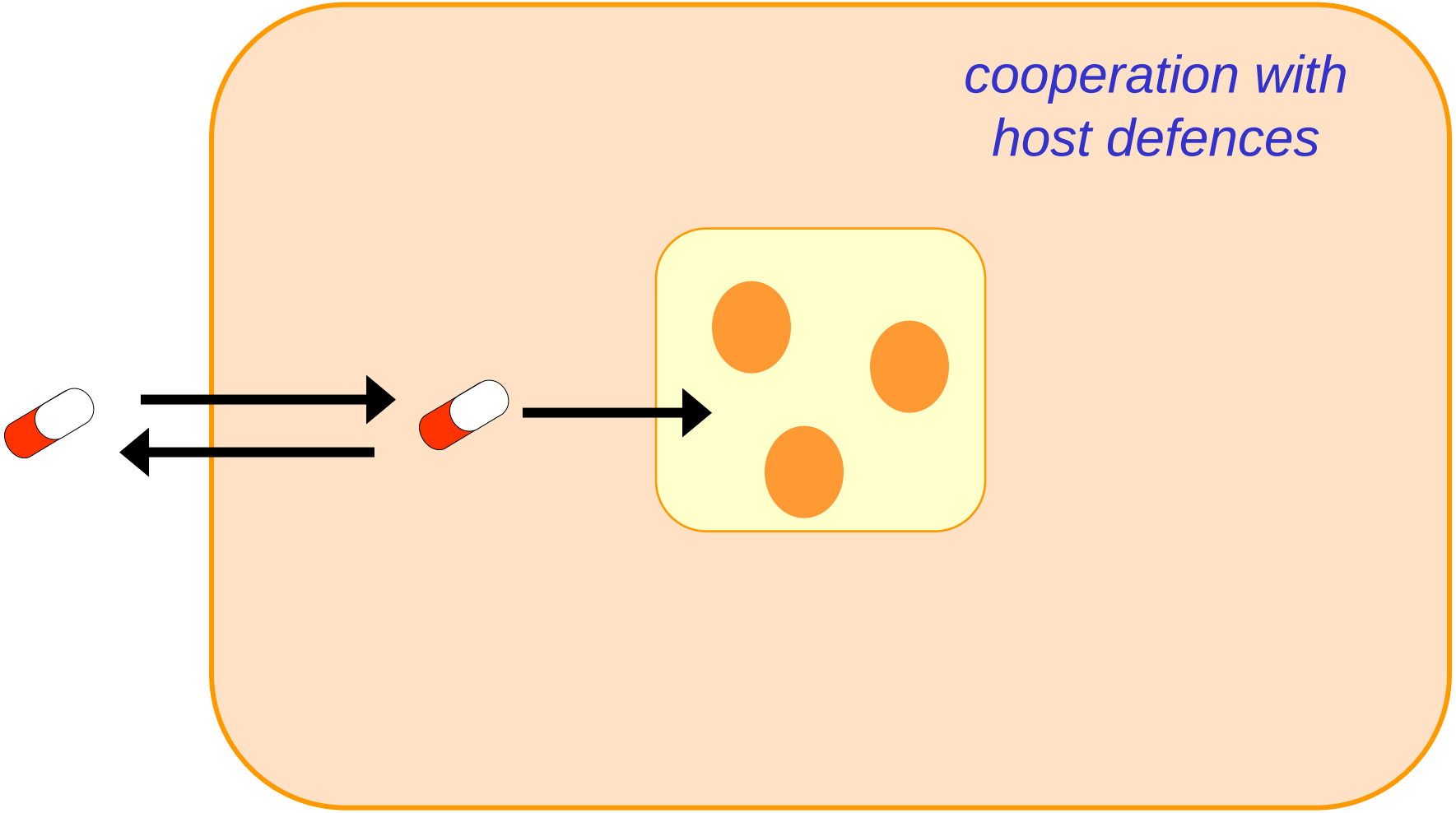


FIGURE 4. **Circular dichroic spectra of PBP 2a at pH 7. 0 (*left panel*) and pH 5.5 (*right panel*) in the absence (*open symbols*) and in the presence (*closed symbols*) of oxacillin (30 μM) for 30 min at 25 °C.** The *thin dotted lines* in each graph represent minima of PBP 2a molar ellipticity at 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

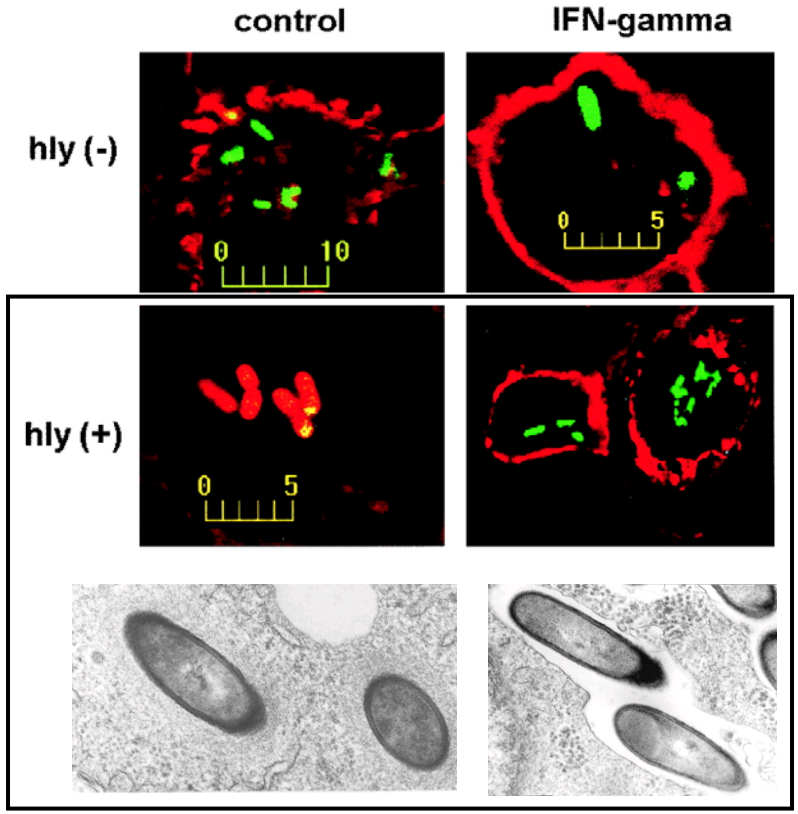
Intracellular vs extracellular activity of antibiotics : PK – PD in action



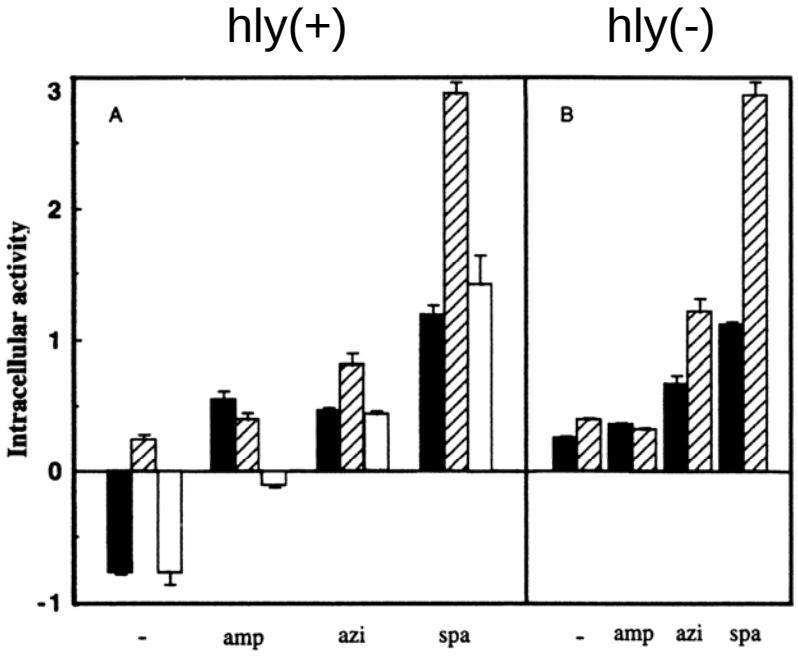
Carryn et al., Infect Dis Clin North Am. (2003) 17:615-34

Cooperation between fluoroquinolones and INF-γ against *Listeria monocytogenes*

IFN- γ prevents *Listeria* escape from phagosome



fluoroquinolones cooperate with oxydative burst



- control
- + IFN- γ
- + IFN- γ , LMMA , catalase

Ouadrhiri et al., AAC (1999) 43:1242-51

Have you made your choice ?

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

Take home message

1. identify your target

2. choose the right weapon

you may avoid serious problems ...

Our « intracellular » team

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