Intracellular *Staphylococcus aureus*, an emerging link to persistent and relapsing infections:

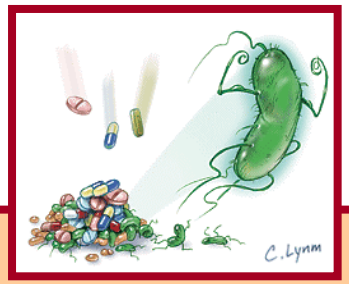
Factors influencing the activity of antimicrobials against intracellular *S. aureus*

Sandrine Lemaire

Promotor : Paul M. Tulkens
Co-promotor : Françoise Van Bambeke

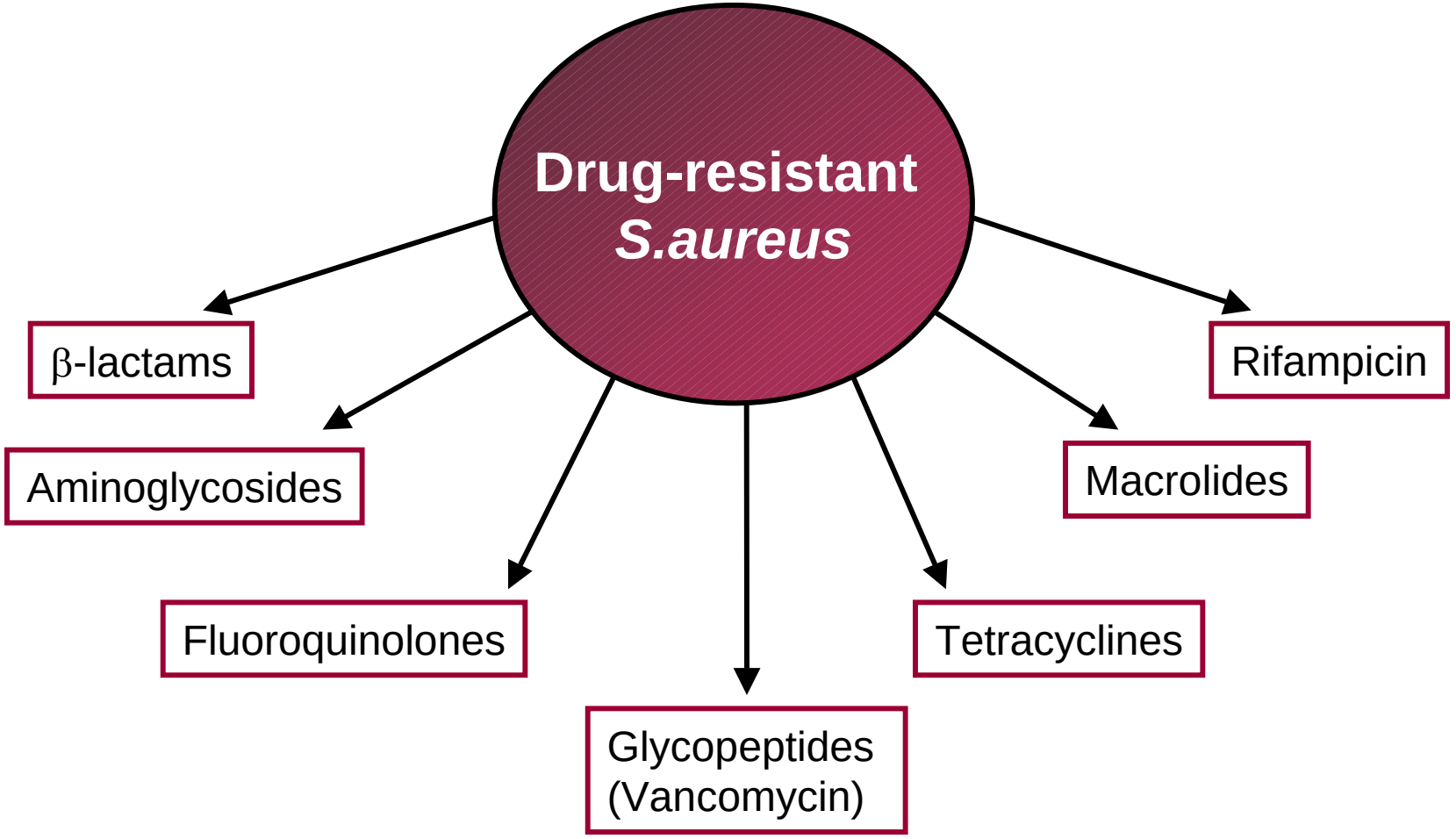
Unité de Pharmacologie cellulaire et moléculaire

What makes *Staphylococcus aureus* so difficult-to-treat ?



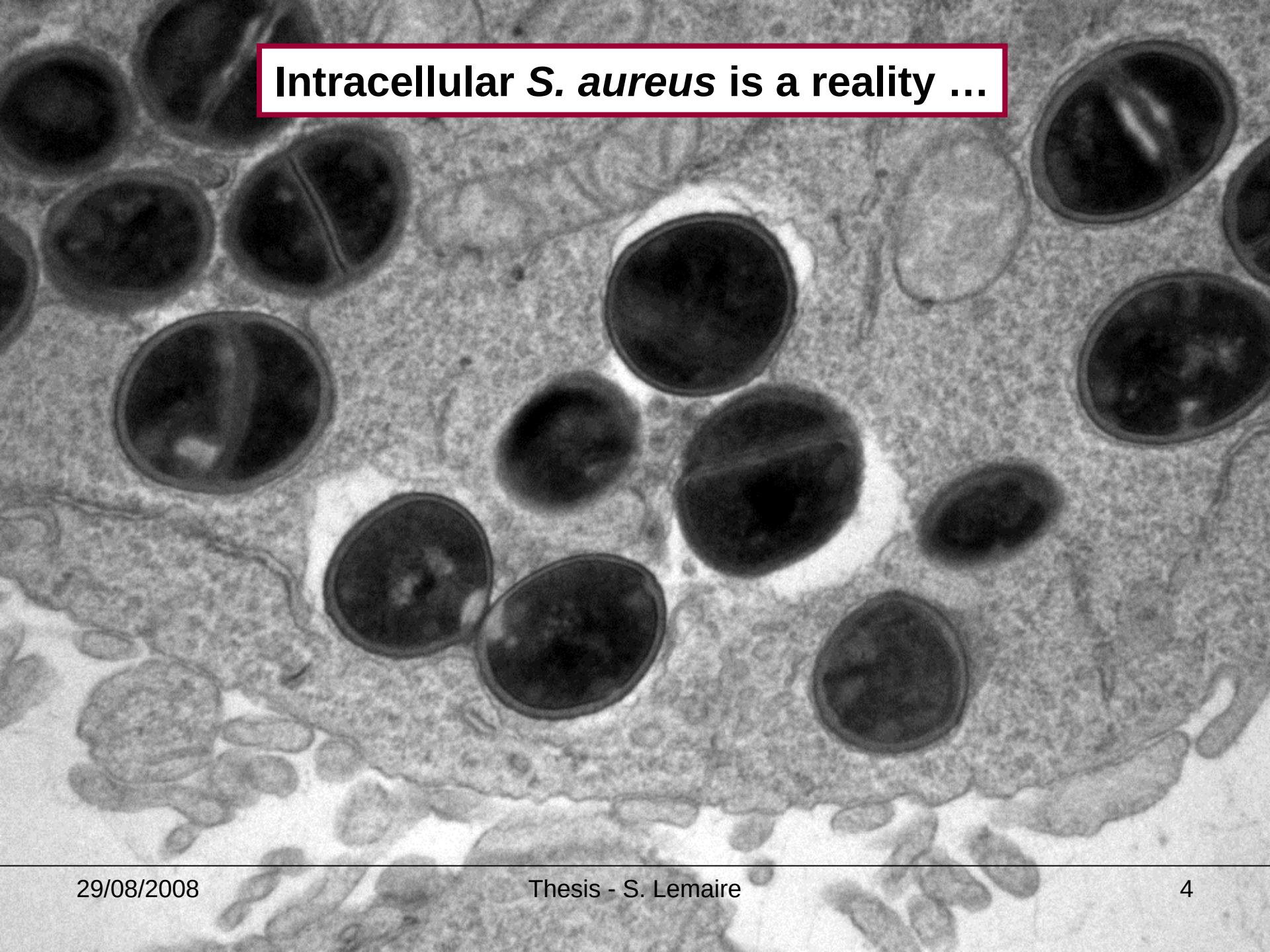
- **Rising resistance to antimicrobials … reaching the limit of clinical application …**
- **Production of biofilms**
- **Difficulty in eradicating intracellular *S. aureus*, which probably results in recurrences, relapses and selection of drug-resistant organisms.**

The era of antibiotic resistance …

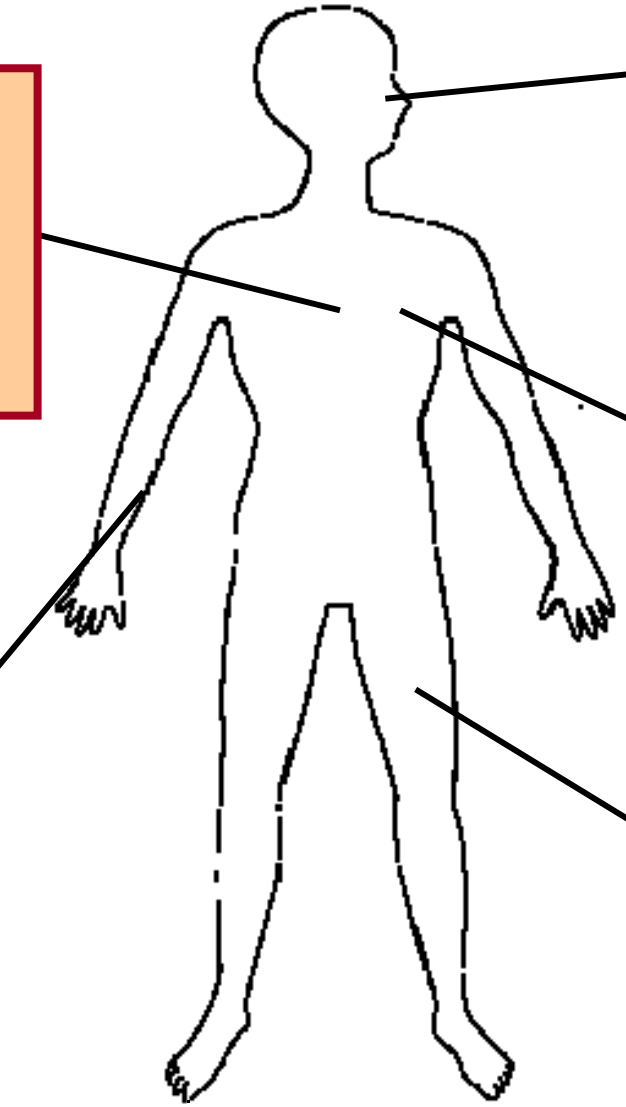


+ Selection of isolates less susceptibles to novel antimicrobials (linezolid, daptomycin, …)

Intracellular *S. aureus* is a reality …



Intracellular *S. aureus*



PULMONARY INFECTIONS ASSOCIATED WITH CYSTIC FIBROSIS
Jarry and Cheung, Infect Immun, 2006

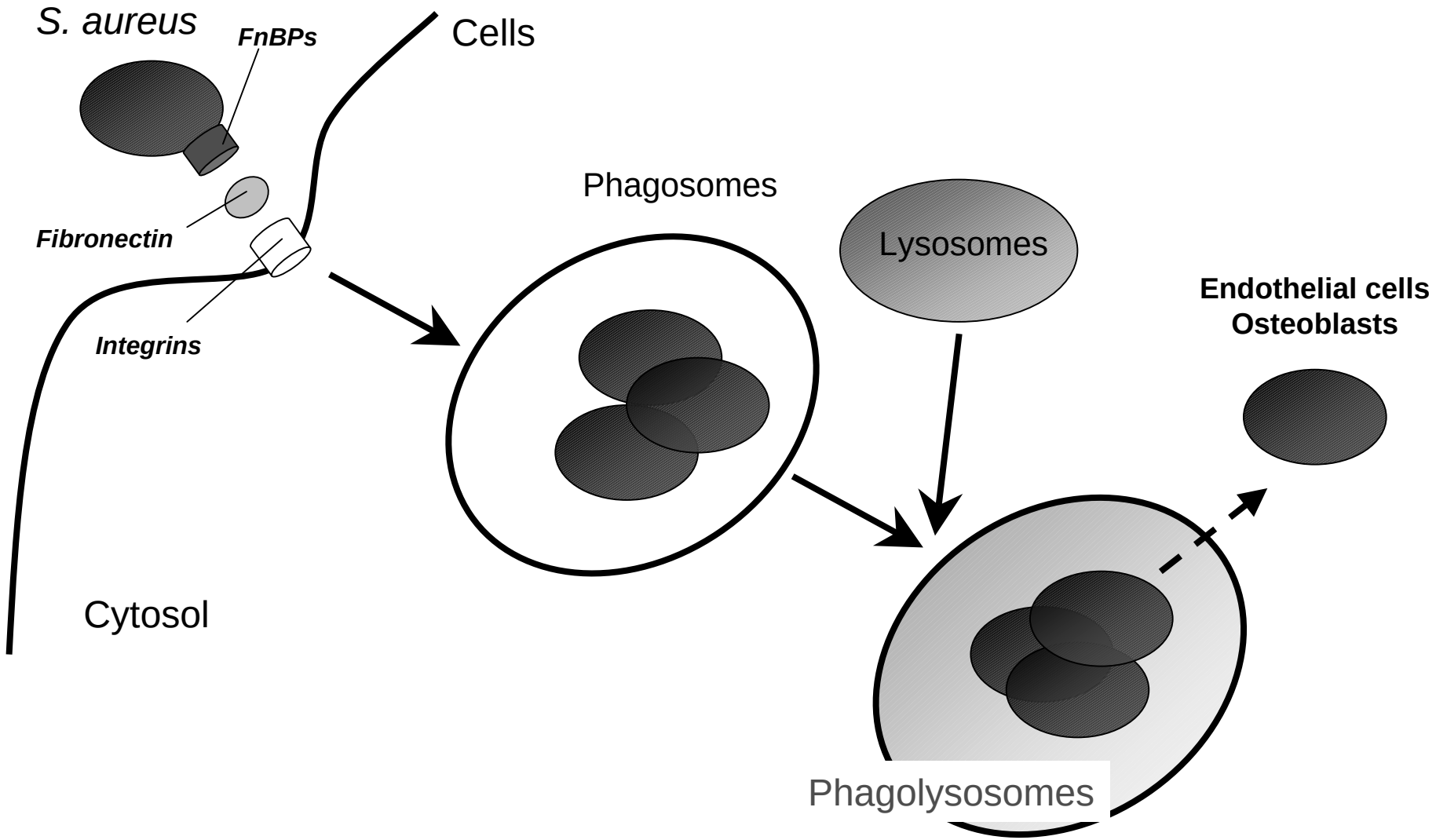
RECURRENT RHINOSINUSITIS
Clement et al, J Infect Dis, 2005

ENDOCARDITIS
Sinha and Herrmann, Thromb Haemost, 2005

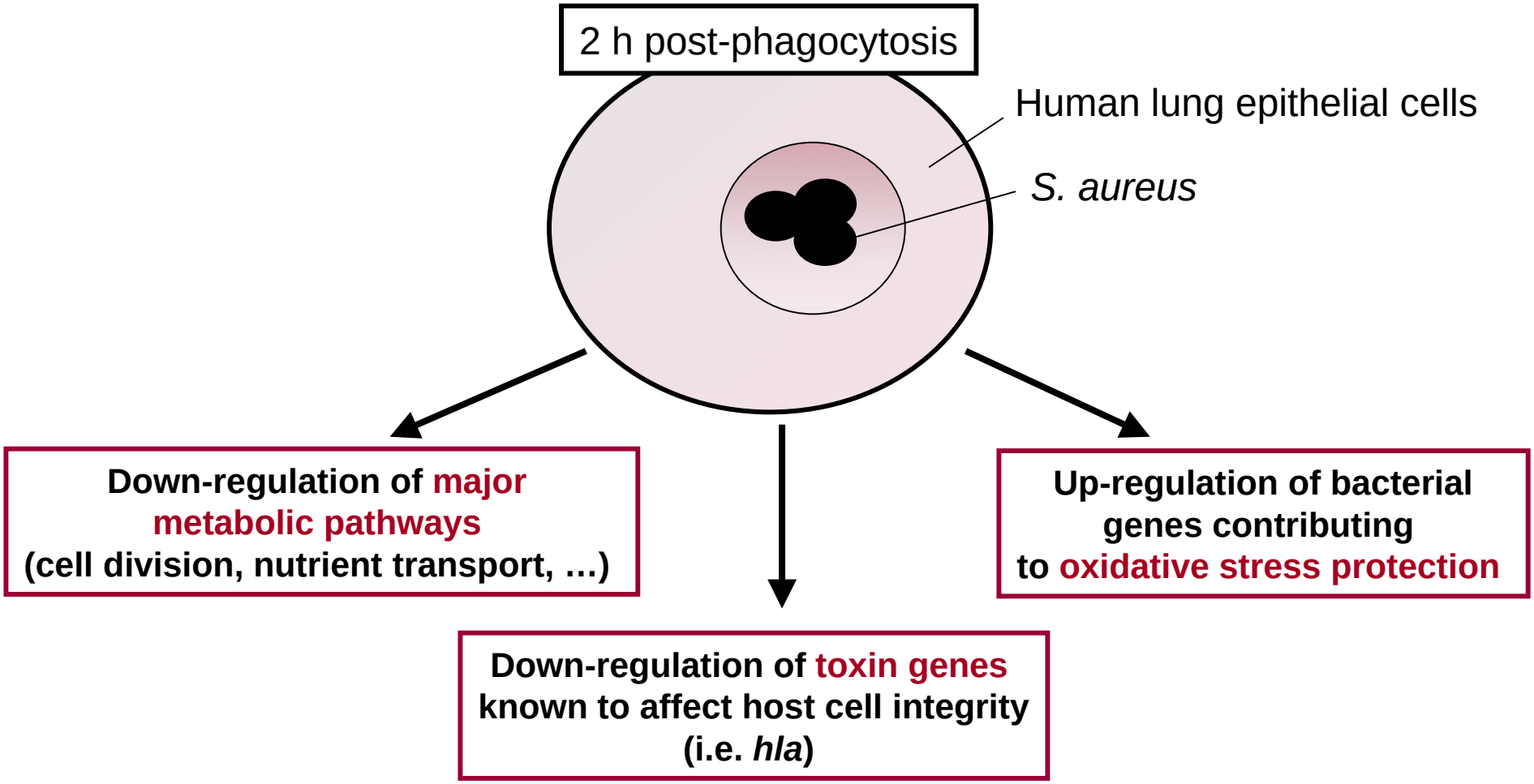
COMPLICATED SKIN INFECTIONS
Mempel et al, Br J Dermatol., 2002

OSTEOMYELITIS
Ellington et al, J Bone Joint Surg Br., 2003

Intracellular lifestyle of *S. aureus*

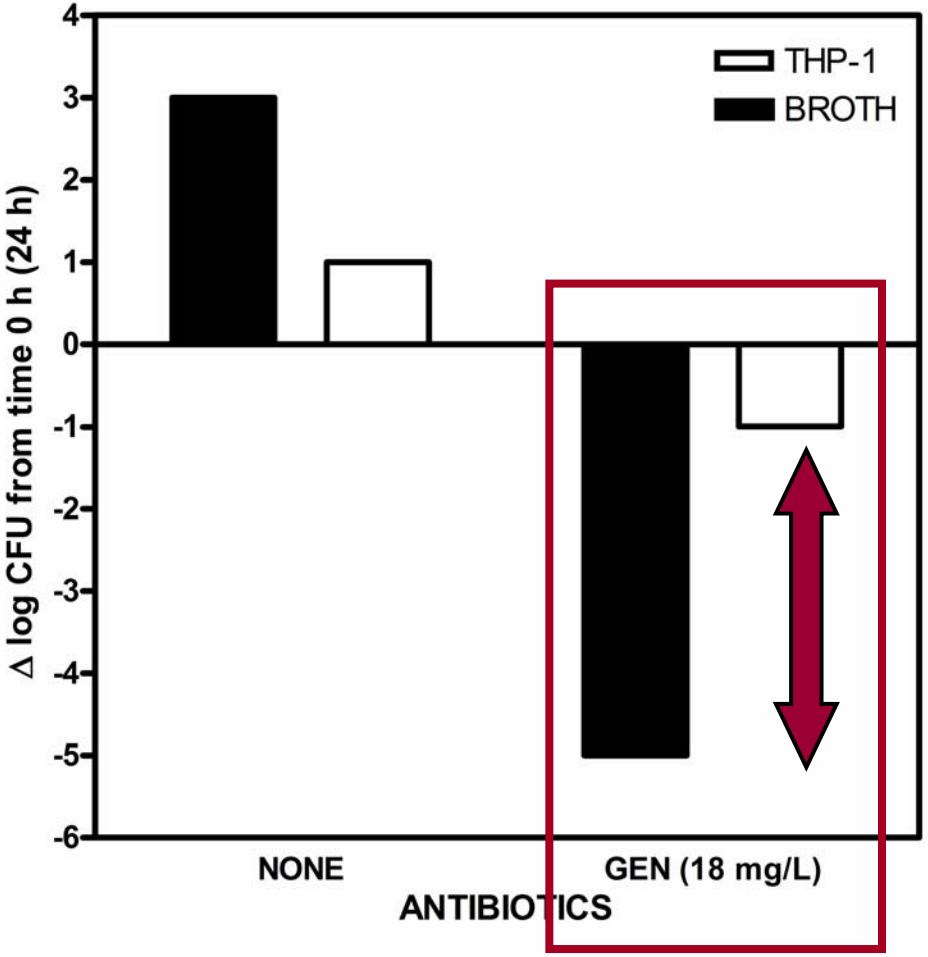


S. aureus reprograms its transcriptome once it reaches the cellular environment …



Garzoni et al, BMC Genomics, 2007

Intracellular *S. aureus (i)*

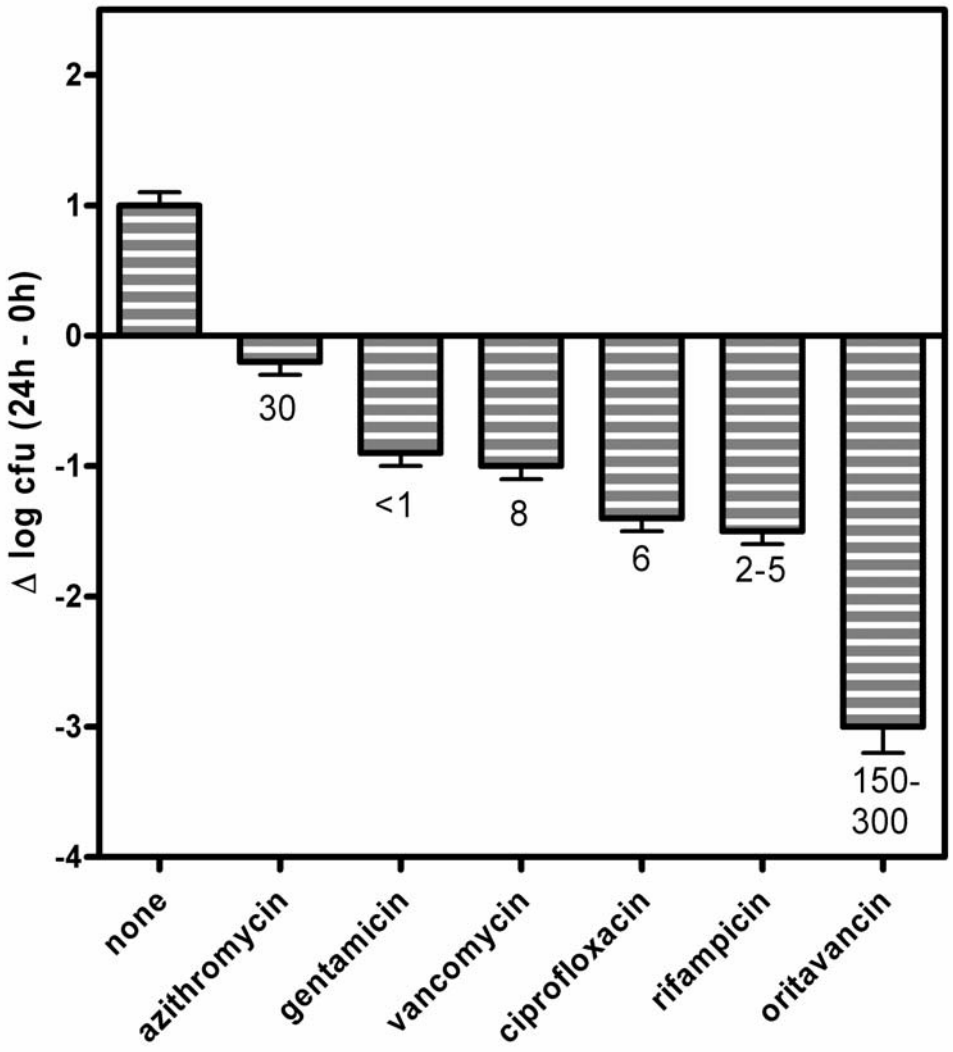


The intracellular environment:

- **delays the growth rate of this organism**
- **confines mostly living bacteria to membrane-bounded vesicles (phagolysosomes)**
- **protects *S. aureus* from the lethal action of most antimicrobials**

Barcia-Macay et al., Antimicrob. Agents and Chemother, 2006

Intracellular *S. aureus (ii)*



- **Macrolides** are only static against intracellular forms (role of acidic pH)
- **Gentamicin, vancomycin, ciprofloxacin, and rifampicin** never yield a truly bactericidal effect against intracellular *S. aureus*
- **Oritavancin** proves active against intracellular *S. aureus*

Barcia-Macay et al, AAC, 2006
Baudoux et al, JAC, 2007

Aim of the thesis

- **Optimization of cellular models (phagocytes vs. non-professional phagocytes)**
- **Pharmacological evaluation of antibiotic activity (time- and dose-response studies)**
- **Determination of bacterial/cellular factors affecting the intracellular activity of antimicrobials**

PART I:

Evaluation of the activity of antibiotics against intracellular forms of *S. aureus*

1. Are β-lactams active against intracellular *S. aureus* ?

- « **Intracellular protection** of *S. aureus* from the lethal action of penicillins (cloxacillin) »

  (Craven and Anderson, J Dairy Res, 1984)

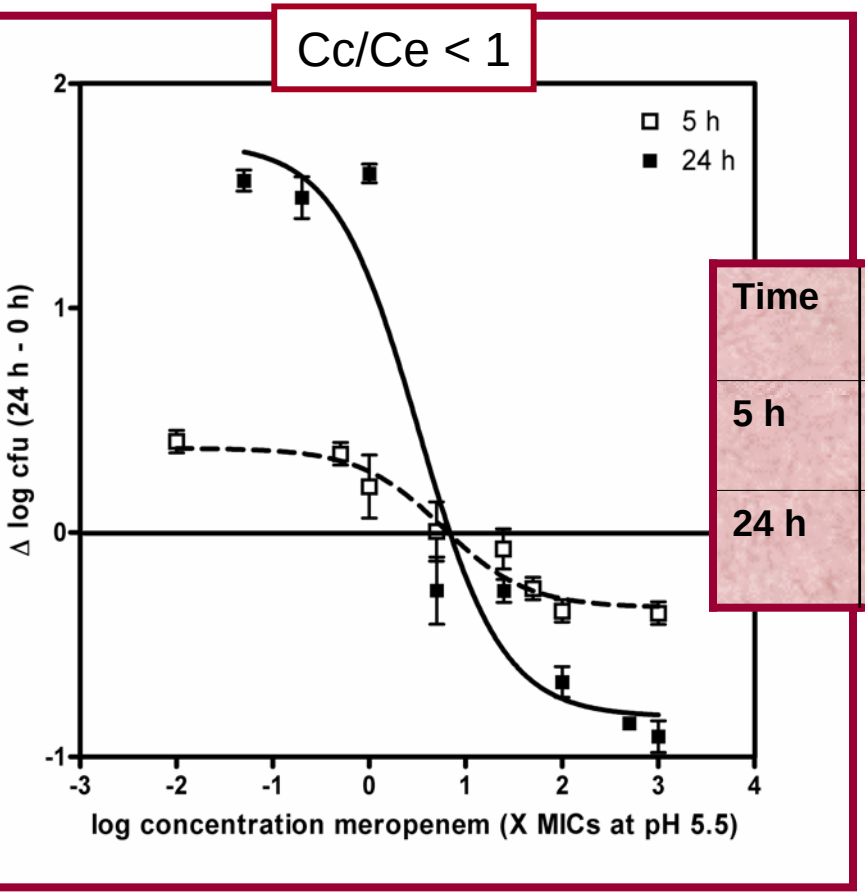
- « Cloxacillin **failed to kill** intracellular *Staphylococci* »

  (Craven and Anderson, Am J Vet Res, 1983)

- « Penicillin has **no intracellular effect** against intracellular *S. aureus* during a 3 h incubation period »

  (Hand and King-Thompson, Antimicrob Agents and Chemother, 1986)

But … β-lactams ARE active intracellularly …



β-lactams exert concentration- and time-dependent effects against intracellular *S. aureus*

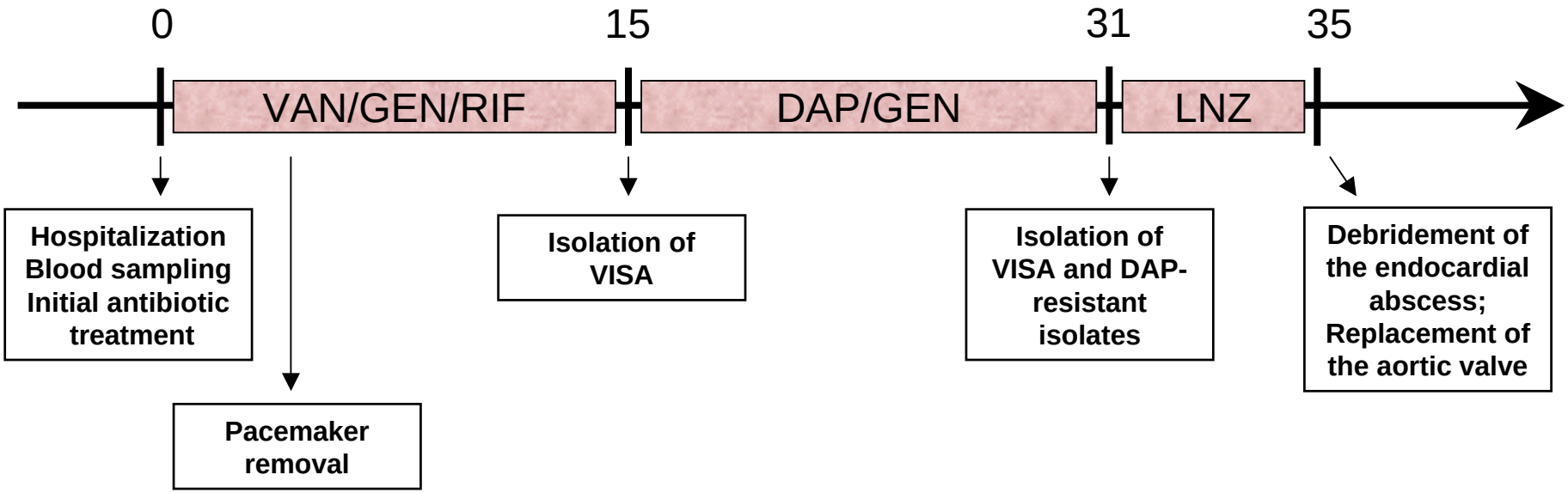
Time	EC_{50} (with CI)	Static dose	E_{max}	R^2
5 h	5.85 (0.77 to 13.64)	~ 6.46	-0.34 ± 0.05	0.958
24 h	3.30 (1.68 to 20.33)	~ 6.92	- 0.83 ± 0.02	0.950

Lemaire et al, JAC, 2005

2. Is there a correlation between treatment failure and insufficient activity of most antimicrobial agents ?

Case report:

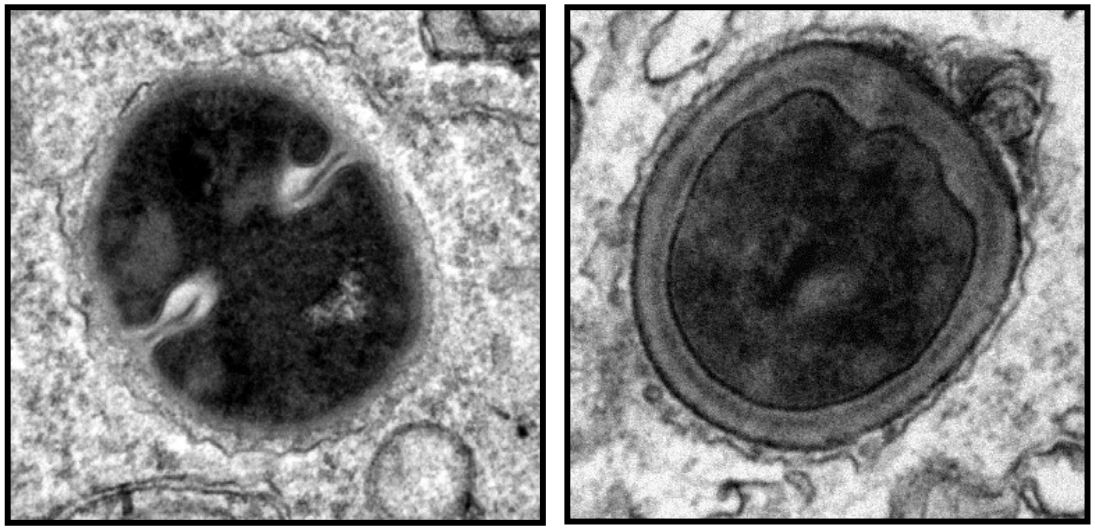
- A 65-year patient, with aortic valve replacement in 1991
- Coronary artery disease, sick sinus syndrome, and diabetes mellitus
- Hospitalization for fevers that developped two weeks after pacemaker placement
- MRSA bacteriemia and endocarditis



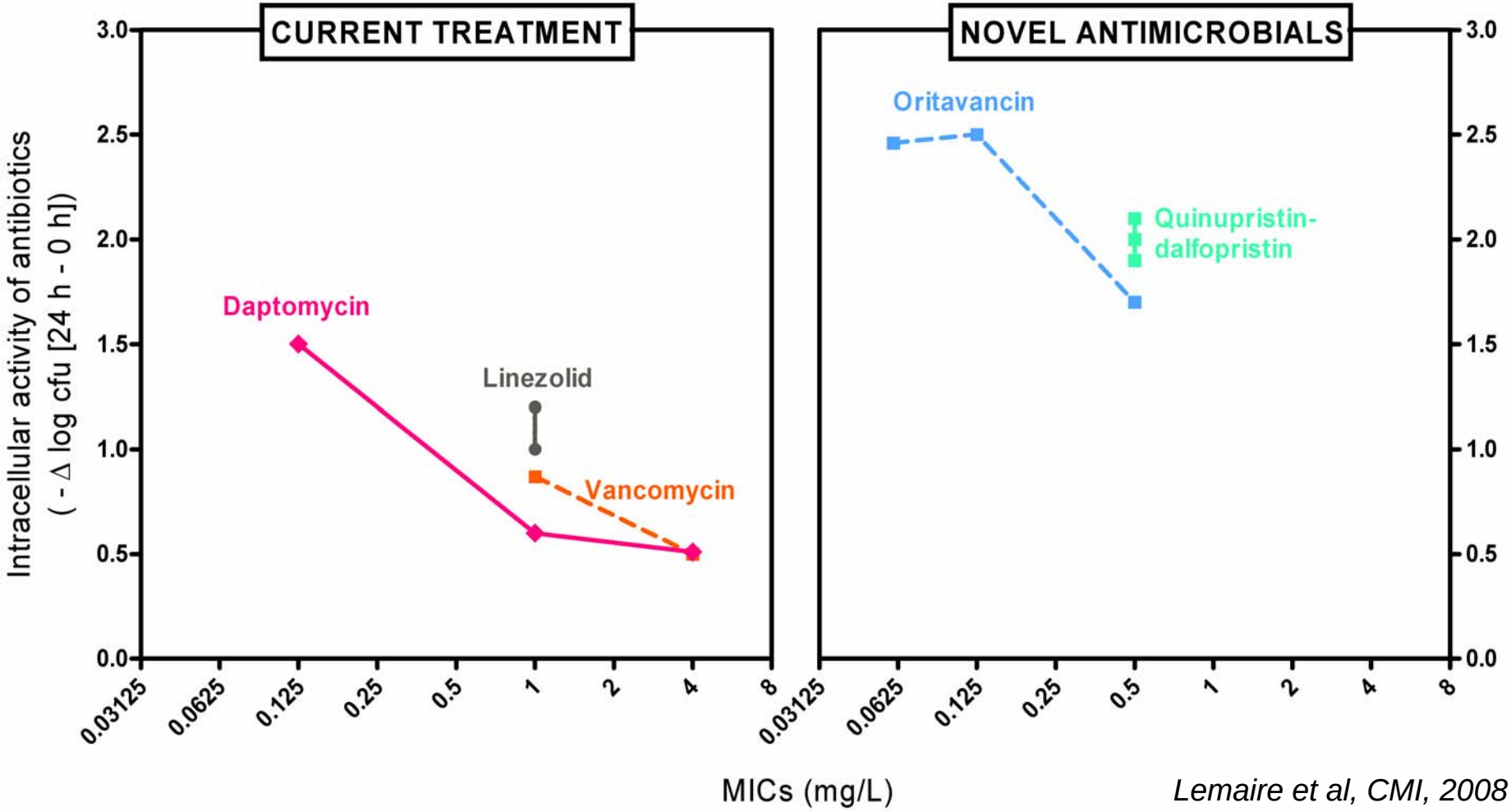
Julian et al, AAC, 2007

Vancomycin Intermediate *S. aureus*

- Selection of VISA since 1996 in patients receiving prolonged courses of vancomycin therapy
- Global metabolic defects affecting cell-wall synthesis, overall structure, and autolysis
- Reduced susceptibility to vancomycin and daptomycin



2. Is there a correlation between treatment failure and insufficient activity of most antimicrobial agents ?

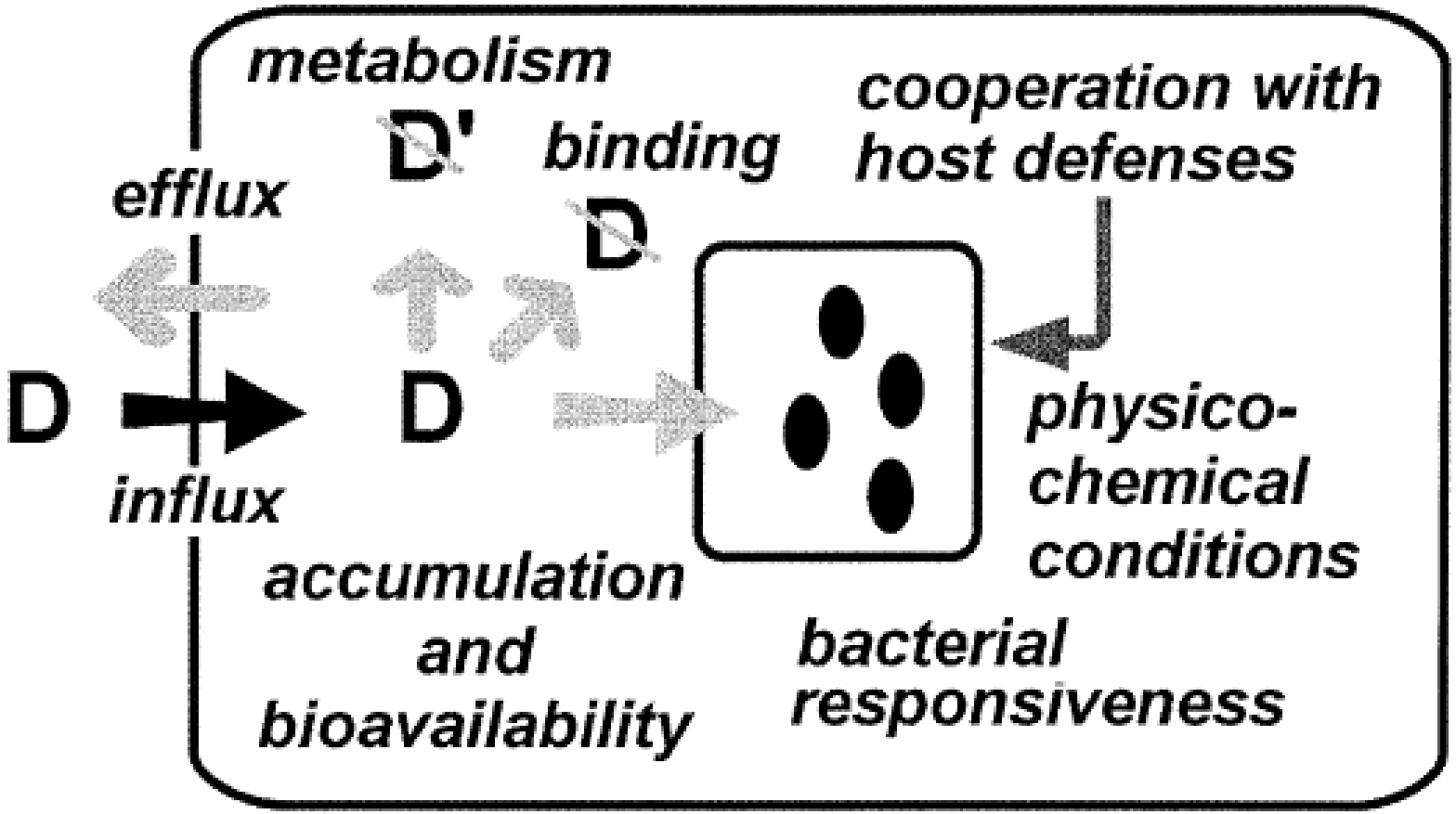


Lemaire et al, CMI, 2008

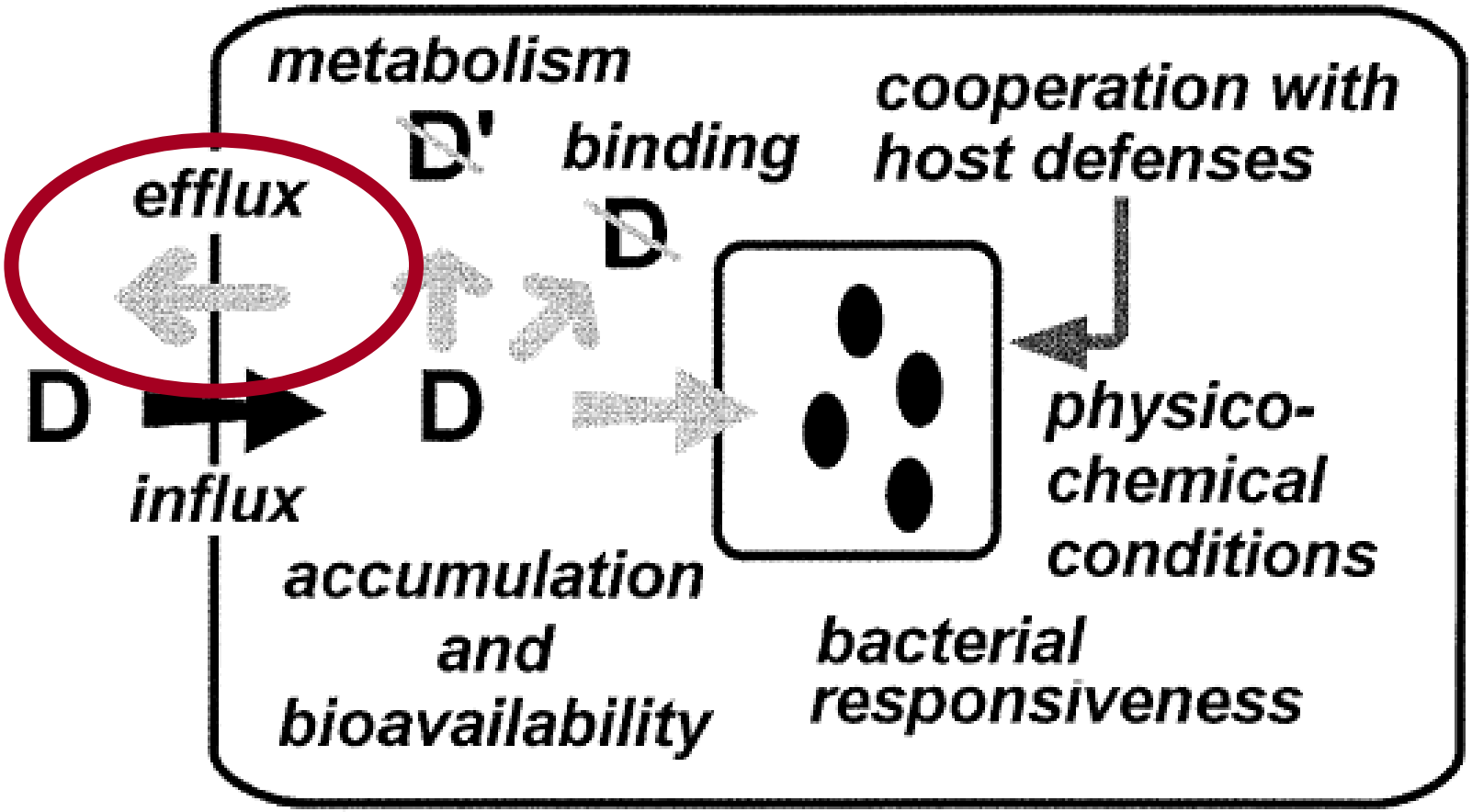
PART II:

Modulation of the activity of antibiotics by the cellular milieu

Factors modifying the activity of antibiotics against intracellular pathogens



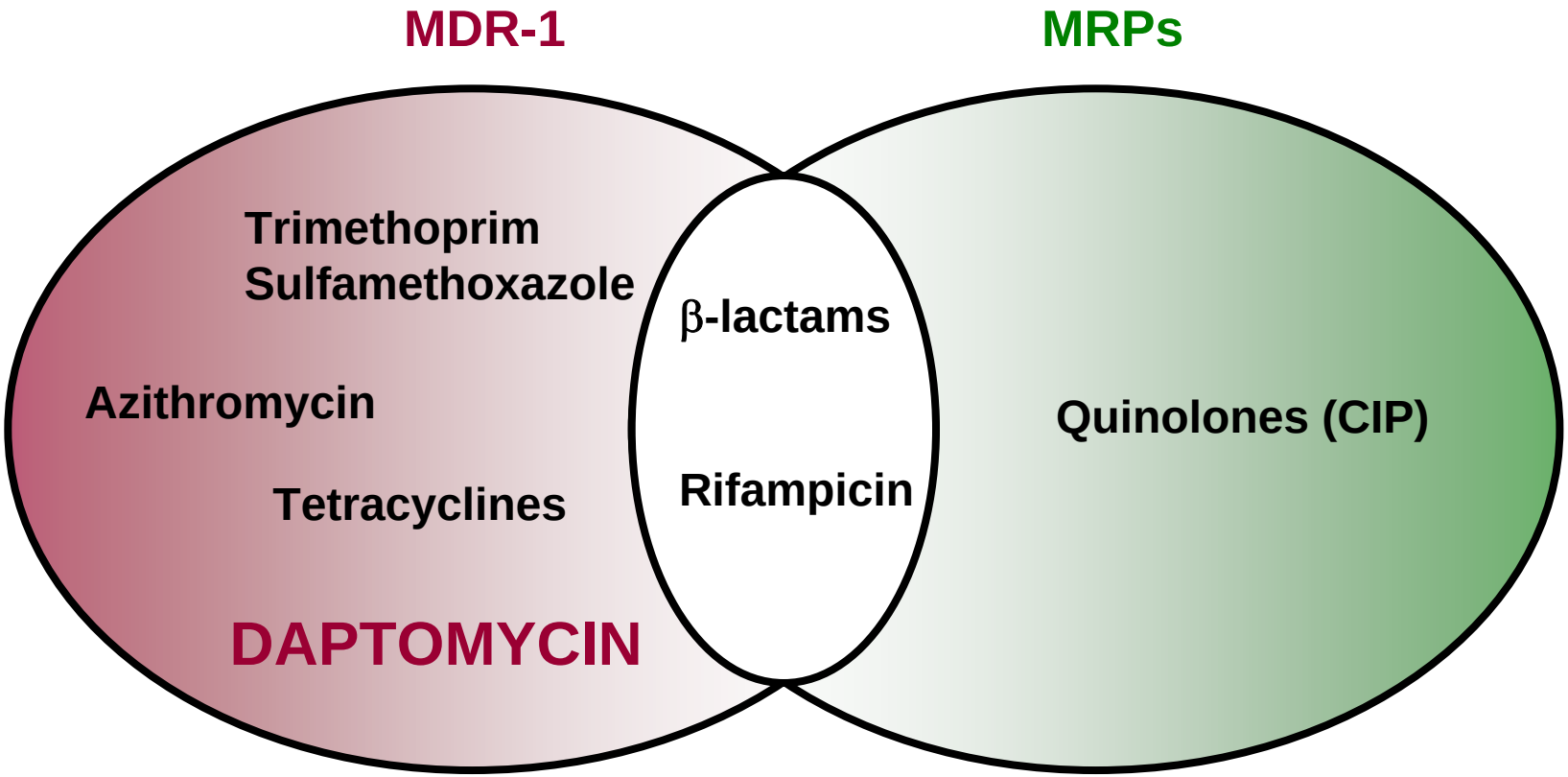
Carryn et al., Infect Dis Clin N Am, 2003



Eukaryotic efflux transporters can modulate the cellular concentration and the intracellular activity of antibiotics

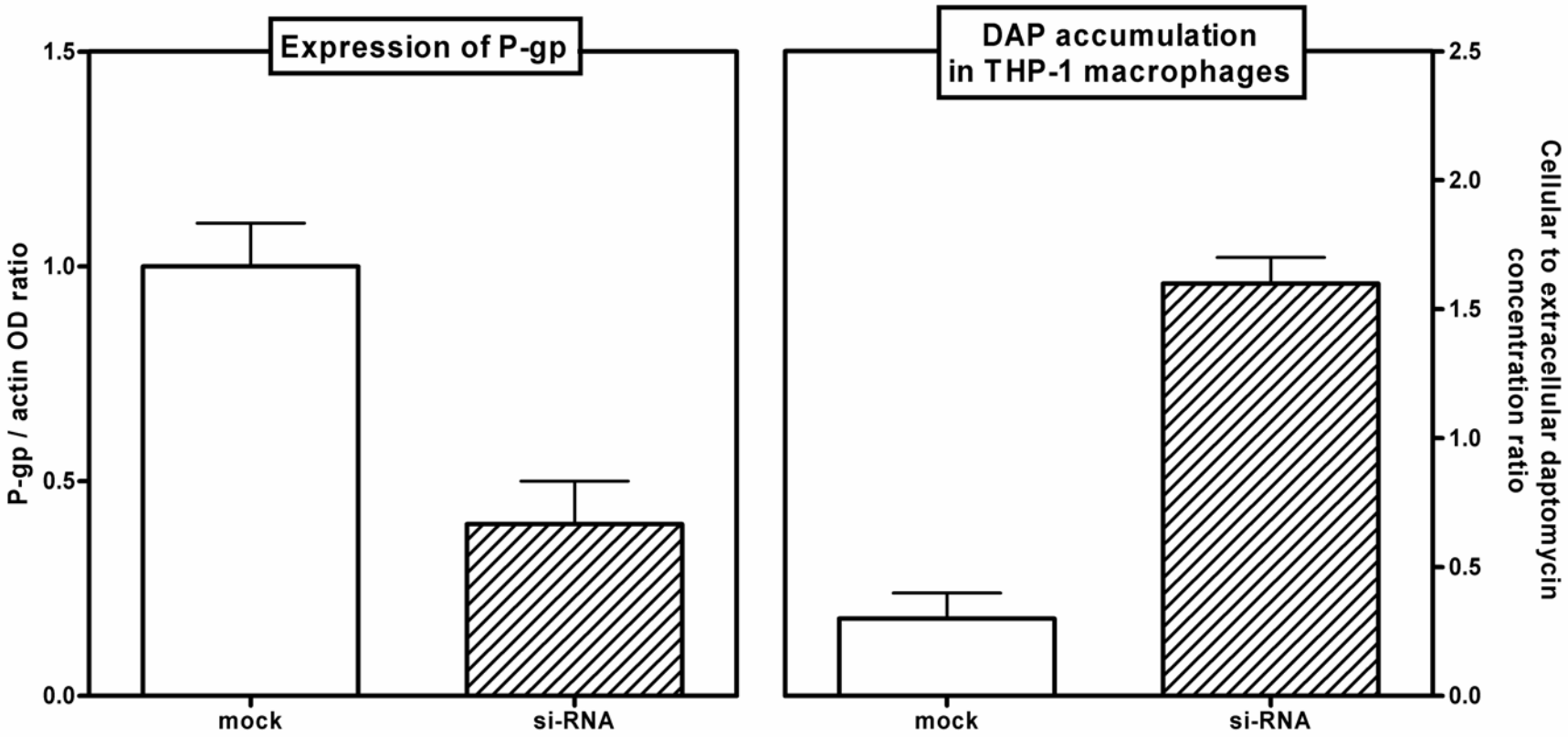
Carryn et al., Infect Dis Clin N Am, 2003

Active efflux of antibiotics (i)



TIGECYCLINE

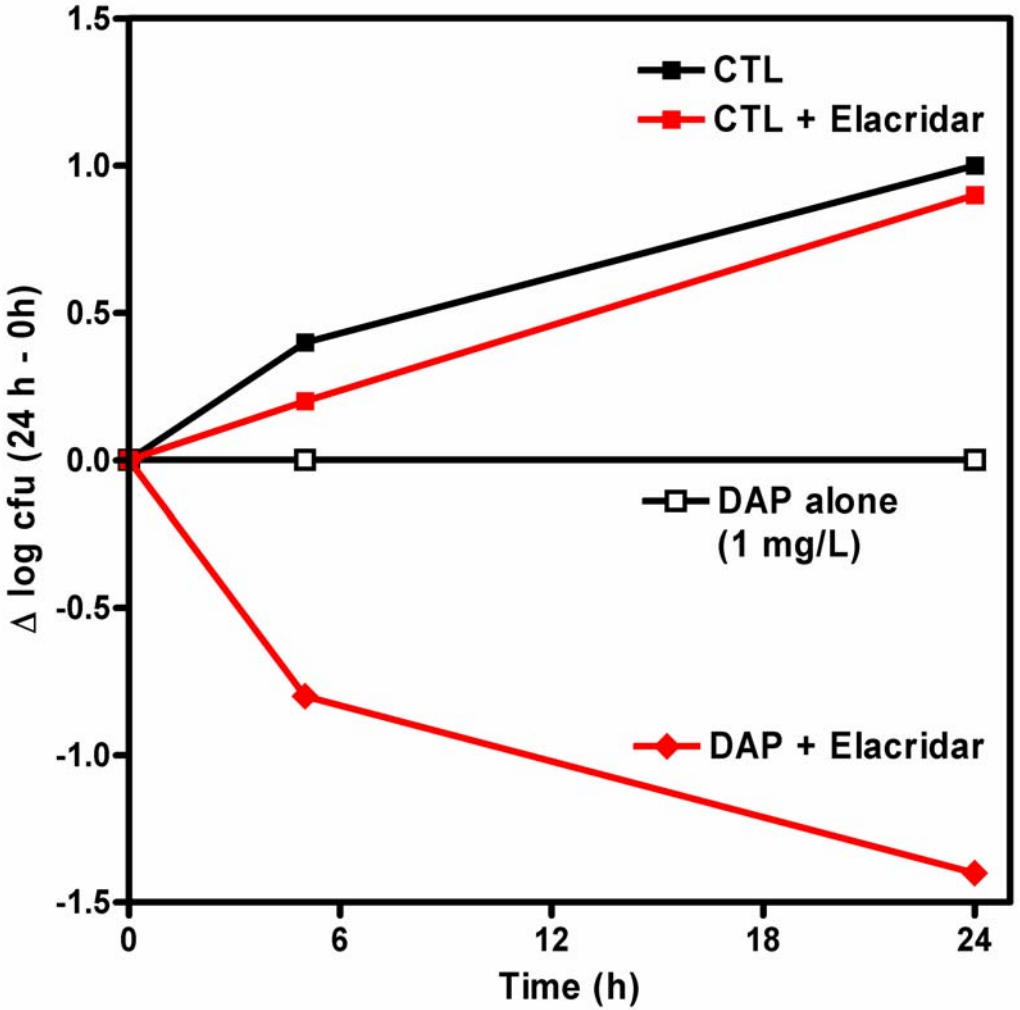
Active efflux of antibiotics (ii)



Decreasing P-gp expression is associated with an increased cellular accumulation of DAP

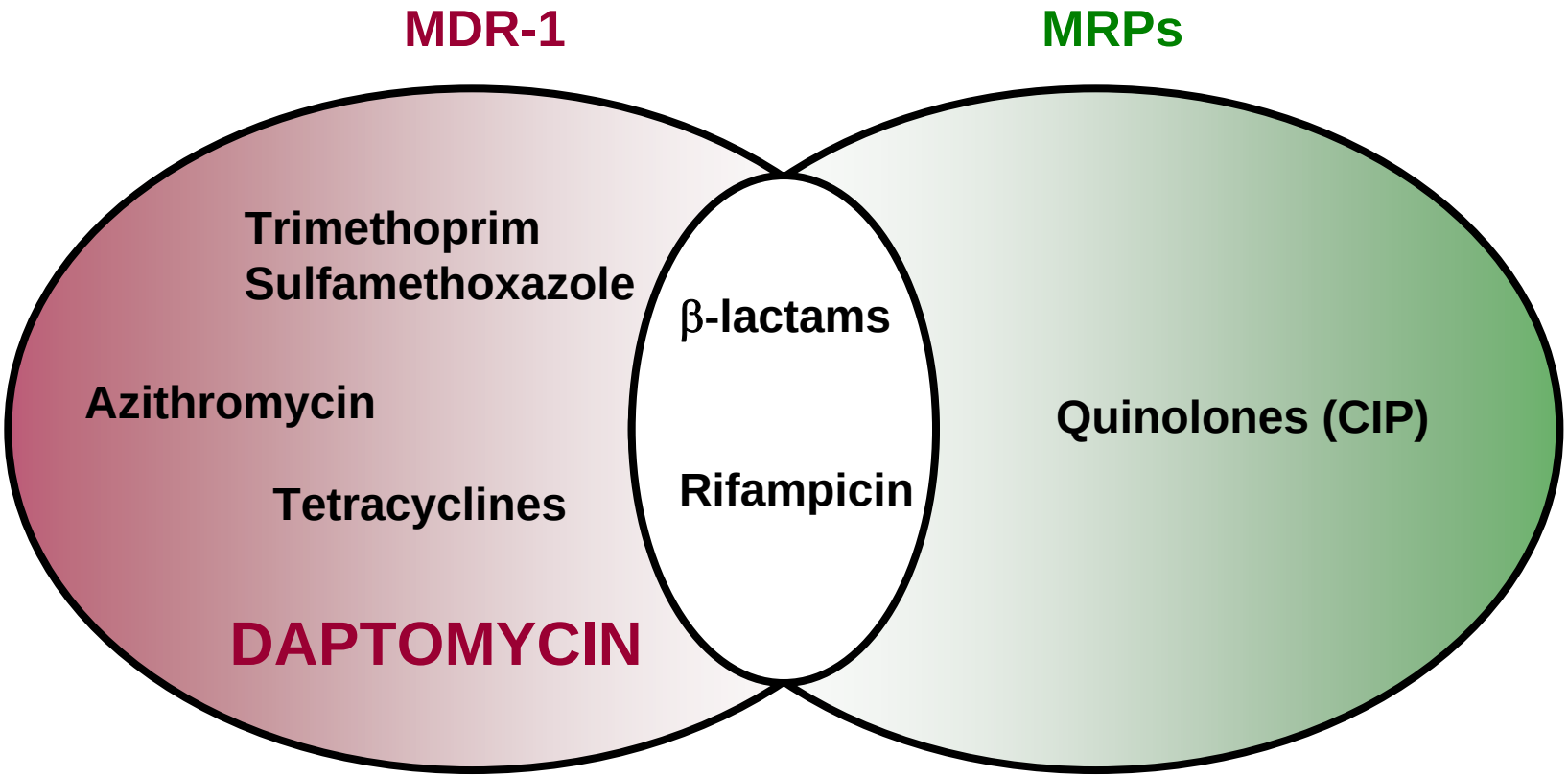
Lemaire et al, AAC, 2007

Active efflux of antibiotics (iii)

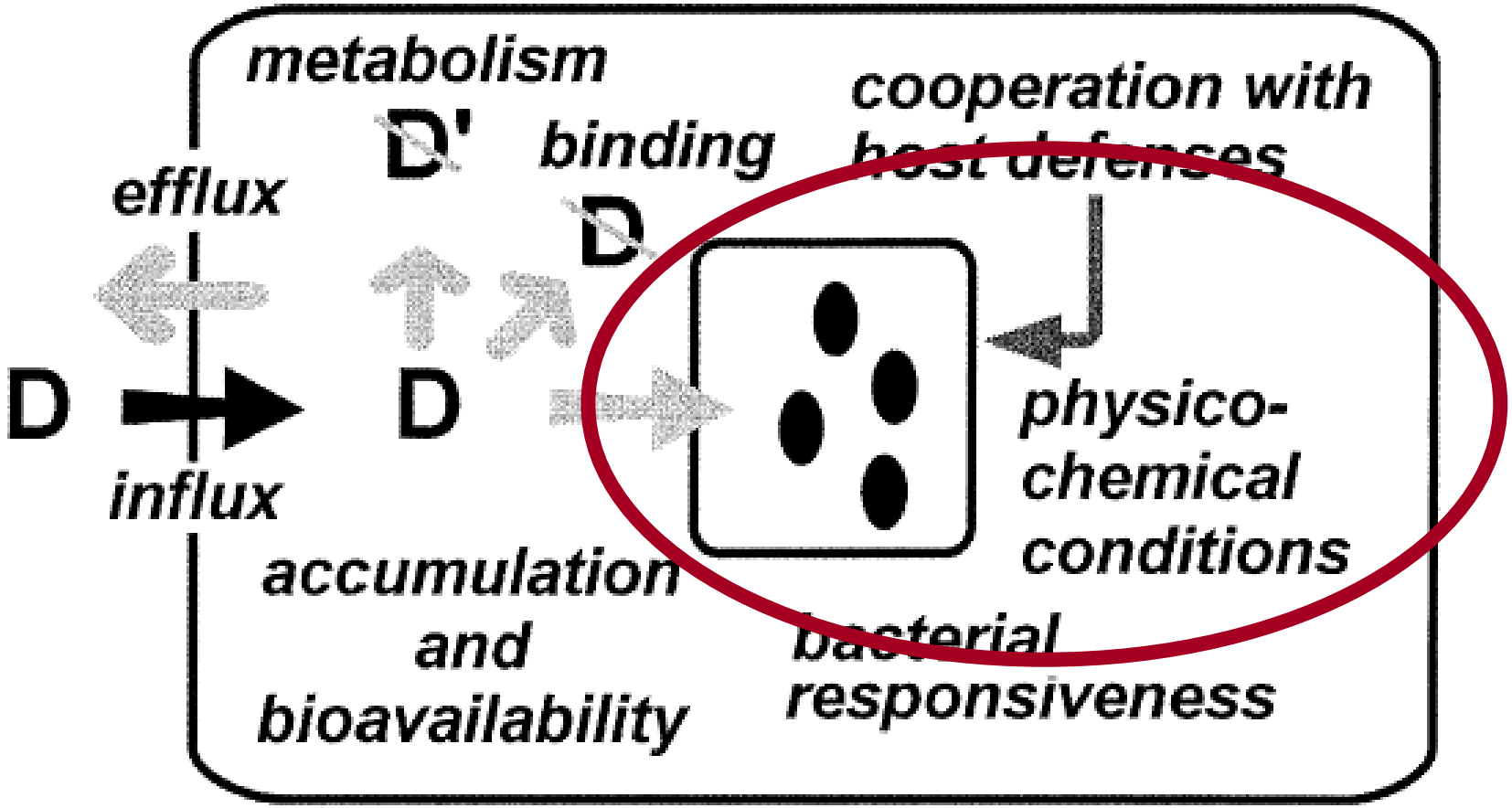


Lemaire et al, AAC, 2007

Active efflux of antibiotics (i)



TIGECYCLINE

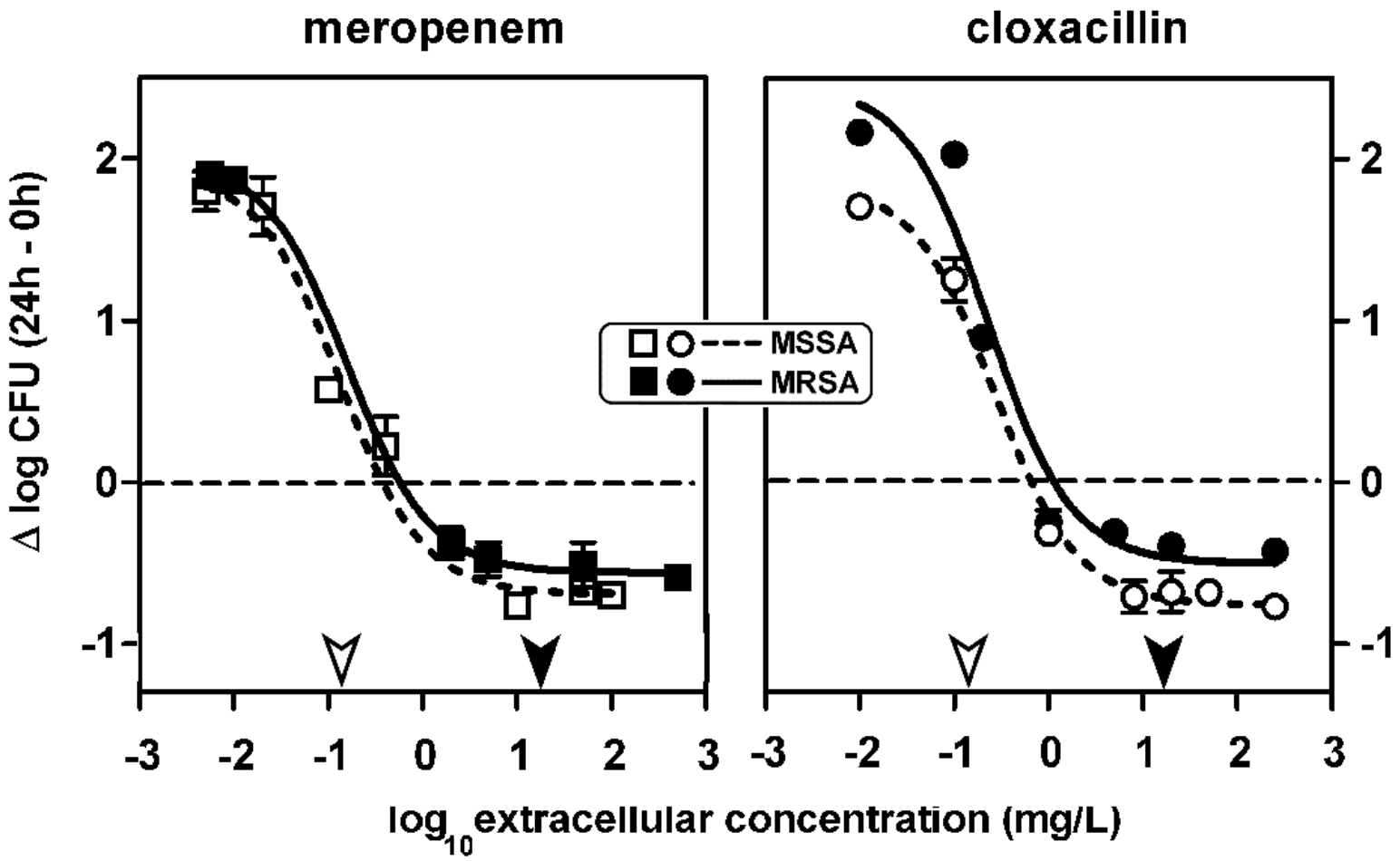


The conditions prevailing in the infected site are a major determinant that may modulate the pharmacodynamic properties of antibiotics

Carryn et al., Infect Dis Clin N Am, 2003

Role of acidic pH (i)

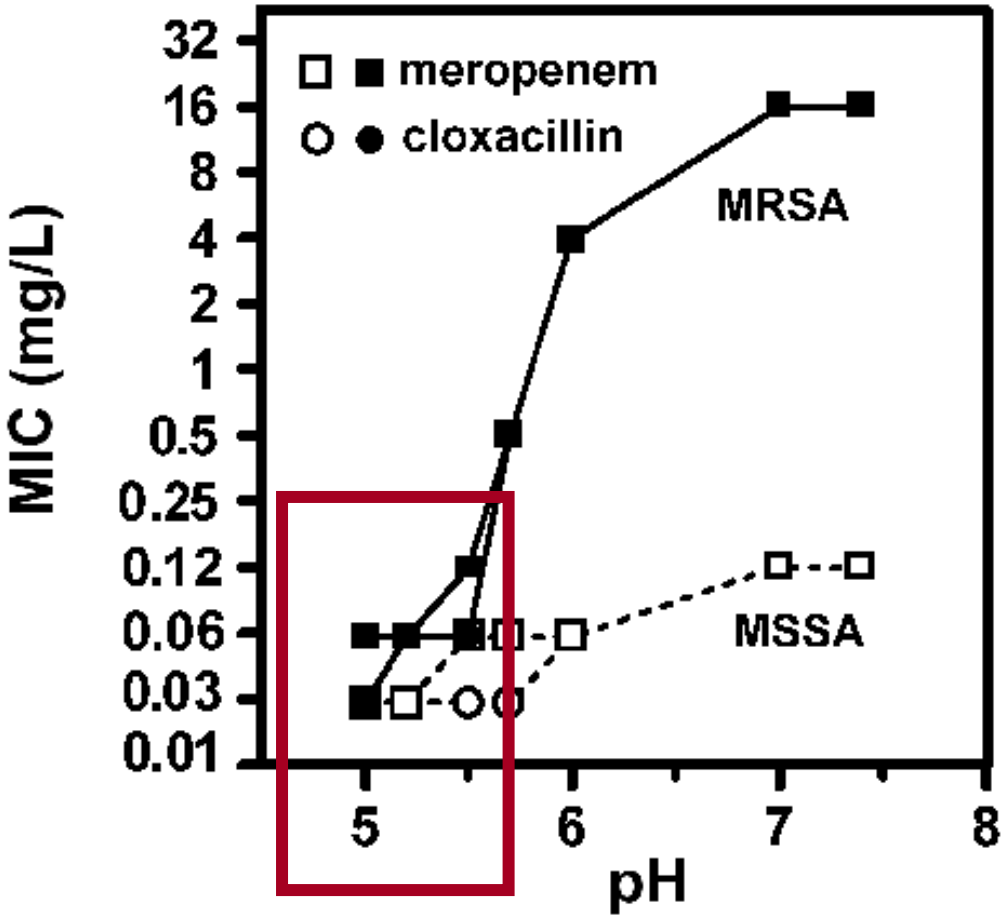
Activity in THP-1 macrophages



Lemaire et al, AAC, 2007

Role of acidic pH (ii)

Activity in pH-adjusted broth

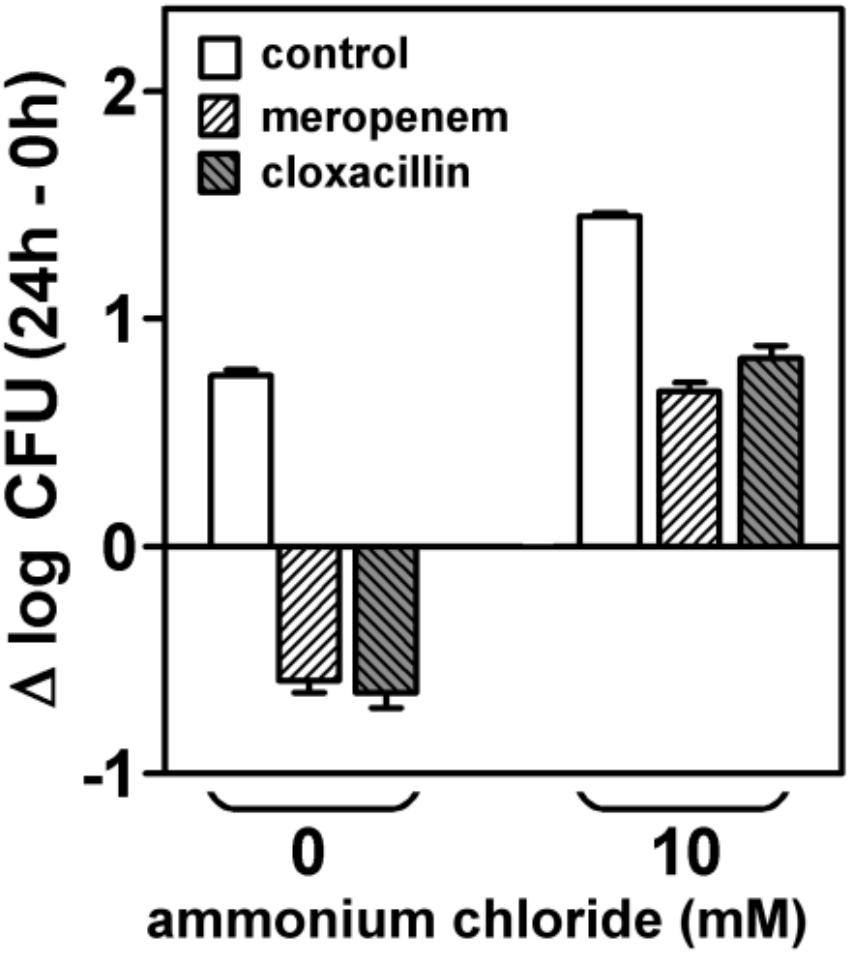


Acidic pH restores the susceptibility of MRSA to β-lactams

Lemaire et al, AAC, 2007

Role of acidic pH (iii)

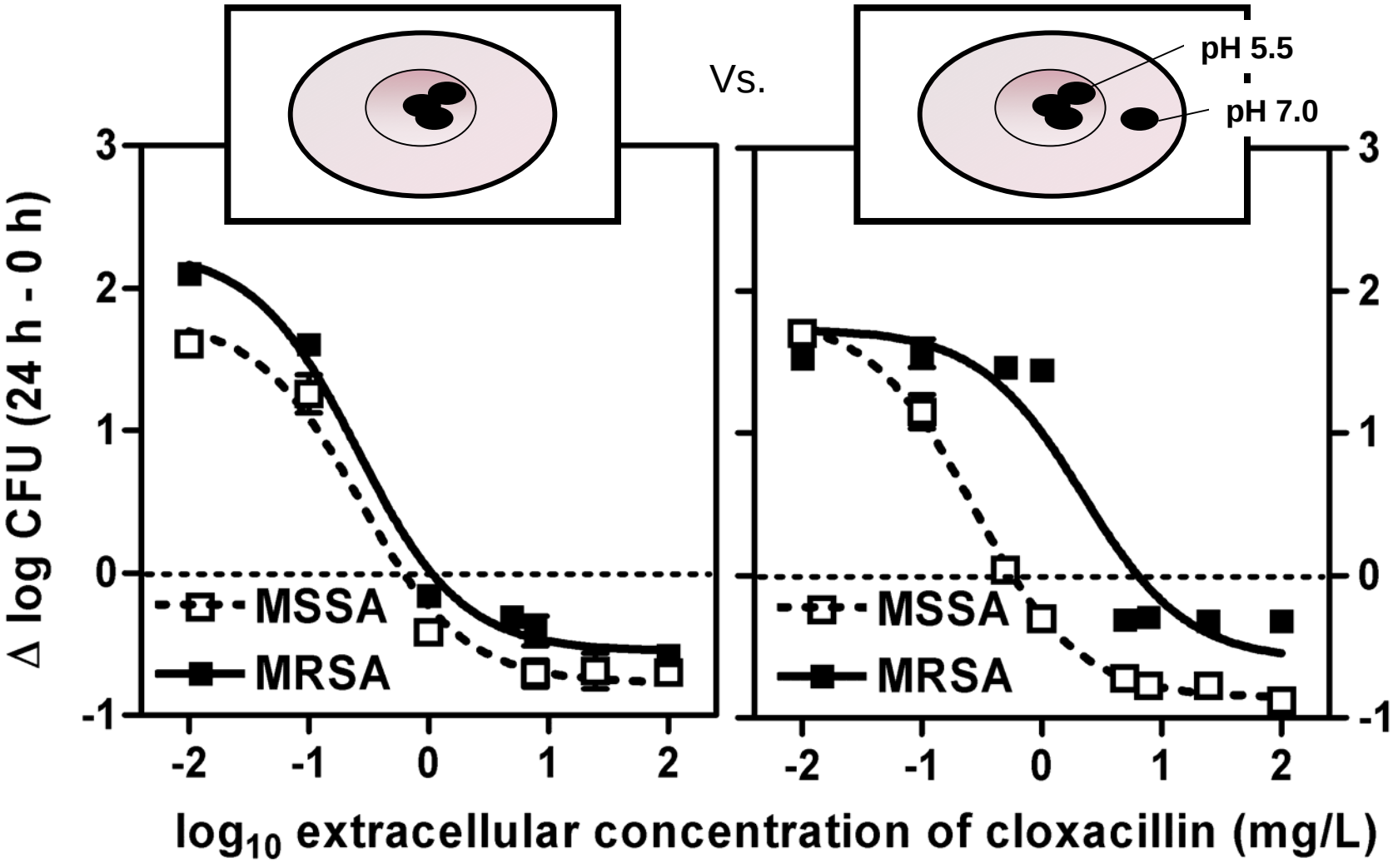
Activity in THP-1 macrophages



Neutralization of the lysosomal pH makes MRSA insensitive to β-lactams

Lemaire et al, AAC, 2007

Role of acidic pH (iv)



PART III:

Why MRSA becomes susceptibles to beta-lactams in acidic environments ?

MRSA (Methicillin-Resistant *S. aureus*)

1950 1960 1970 1980 1990 2000 2010

Selection of MRSA

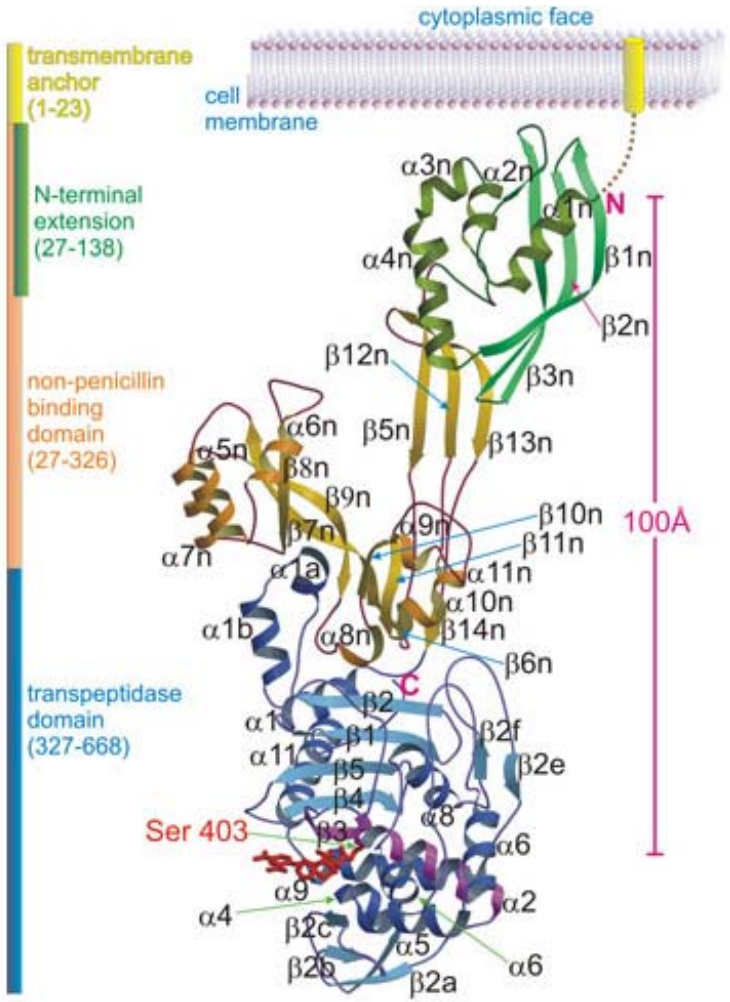
Since 1970's, MRSA were increasingly associated with health-care associated infections

Emergence of MRSA in the community:
- **Abscess (spider bites) and severe skin infections,**
- **Necrotizing pulmonary infections**

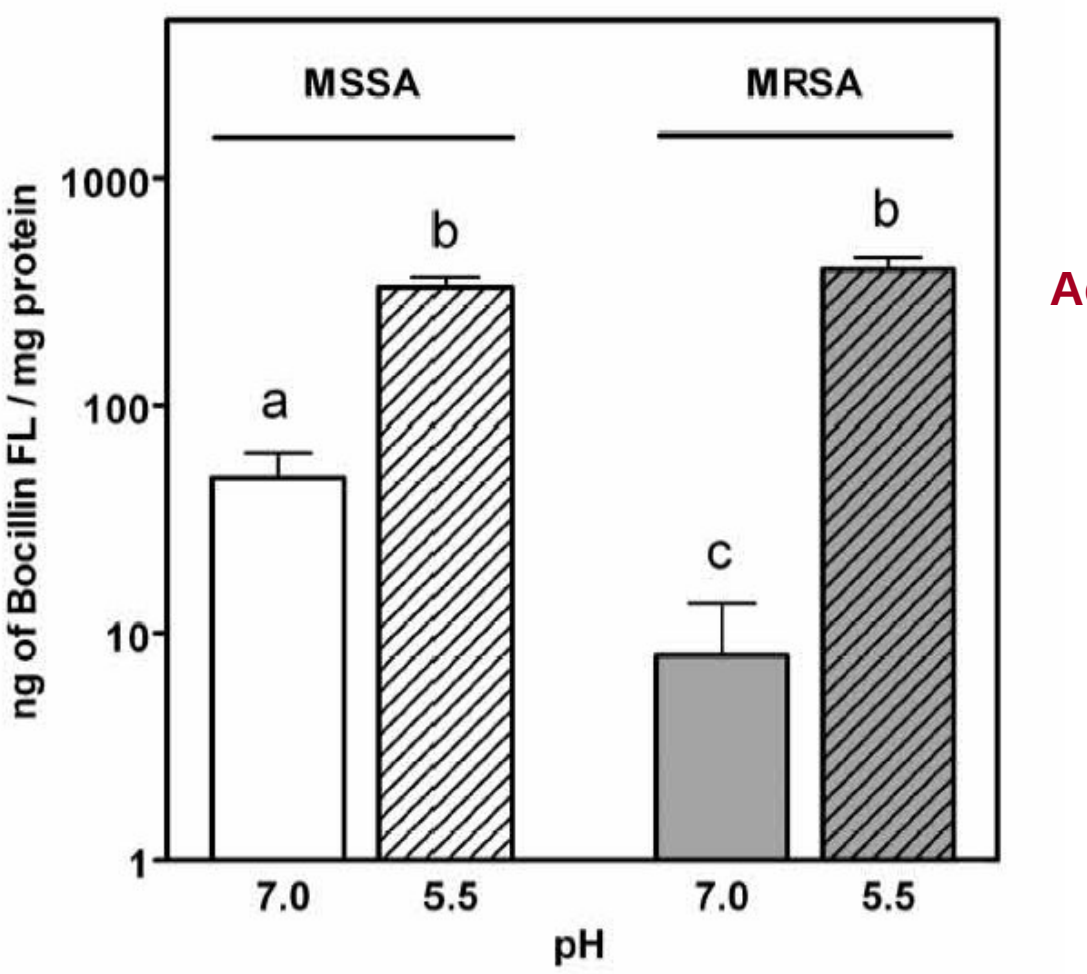
Emergence of MRSA in animals

Mechanism of resistance

- The ***mecA* gene** is the central genetic component of methicillin resistance
- Gene coding for an additional, 78 kDa- Penicillin Binding Protein (termed **PBP2a or PBP2'**)
- PBP2a has a general, **low-affinity for β-lactams (closed conformation)**
- PBP2a has **transpeptidase** activity (subtituting therefore to the function of native PBPs when these latter are inhibited by β-lactams)



Role of acidic pH (i)

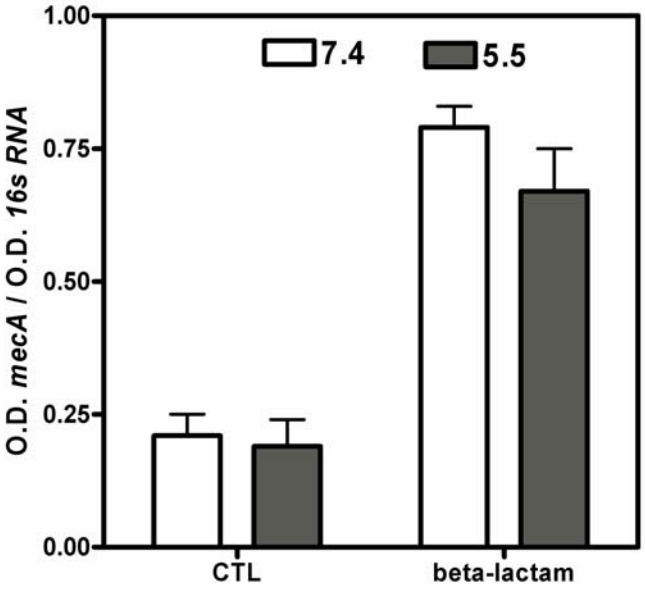


Acidic pH increases significantly the binding of a fluorescent Penicillin (Bocillin FL) to whole cell-wall bacteria

Lemaire et al, JBC, 2008

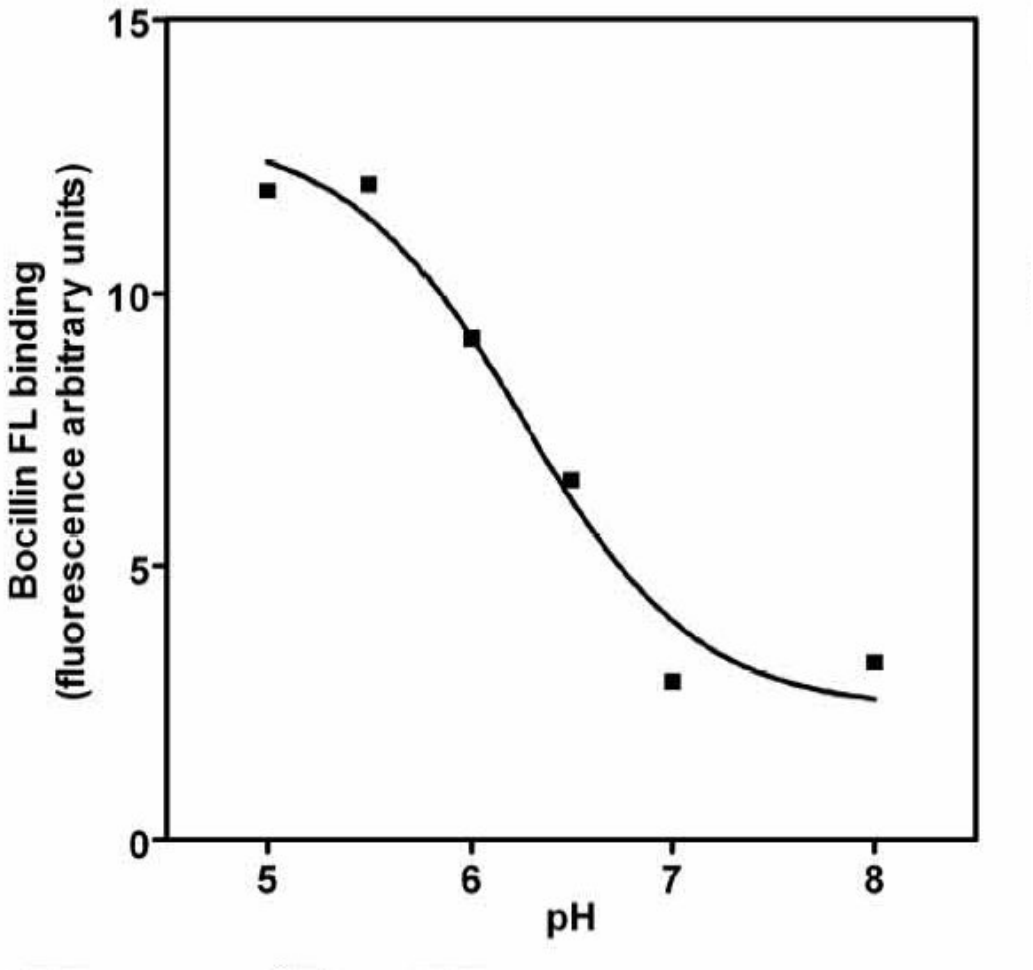
Role of acidic pH (ii)

- Hypothesis #1: Does acidic pH decrease the expression of *mecA* ?



- Hypothesis #2: Does acidic pH increase the binding of beta-lactam to PBP2a ?

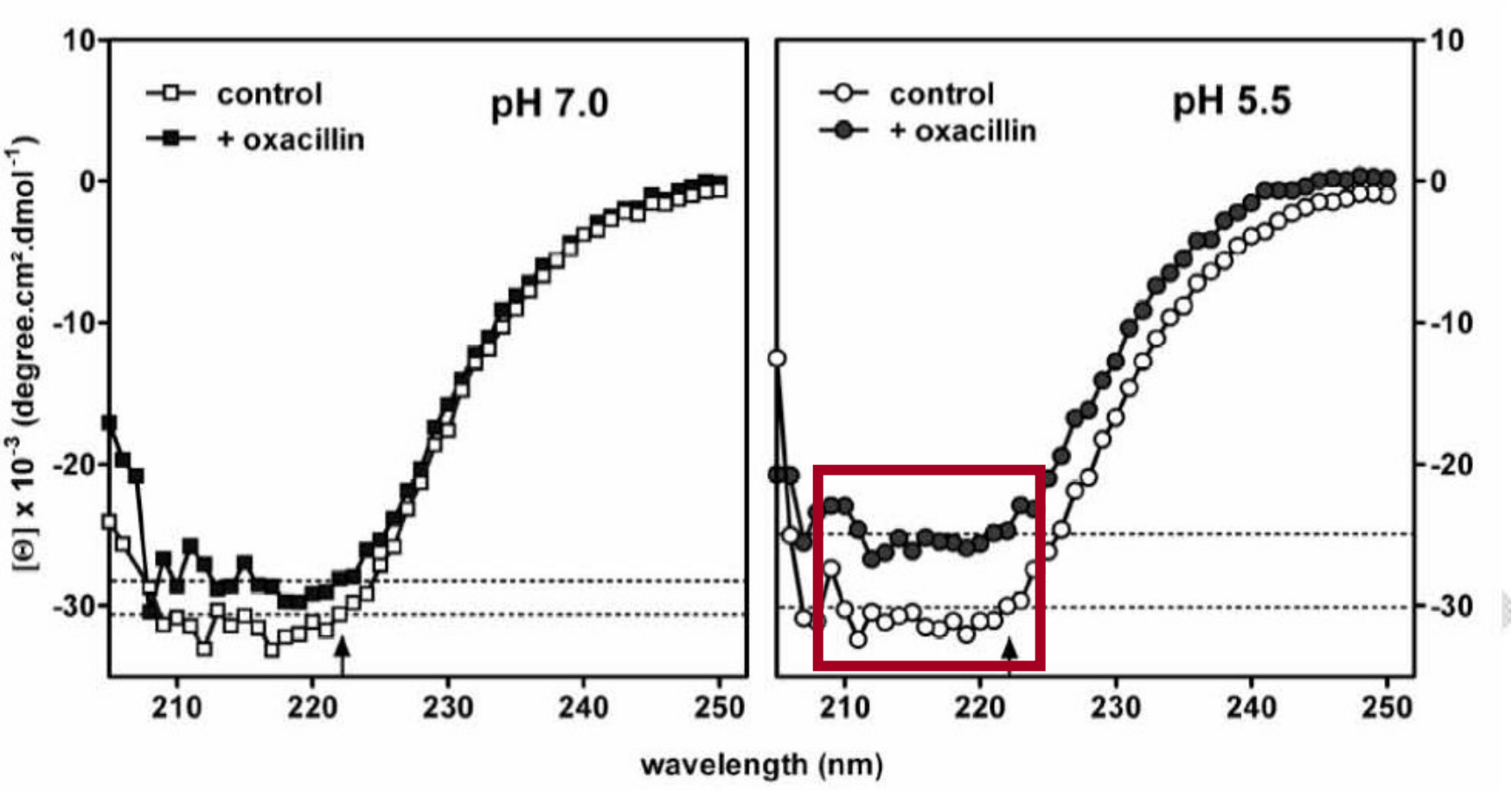
Role of acidic pH (iii)



PBP2a binds more avidly beta-lactams at acidic pH

Lemaire et al, JBC, 2008

Role of acidic pH (iv)



At acidic pH, PBP2a undergoes a conformational change in the presence of oxacillin, consistent with the opening of the active site

Lemaire et al, JBC, 2008

Discussion and perspectives

Interest of the model

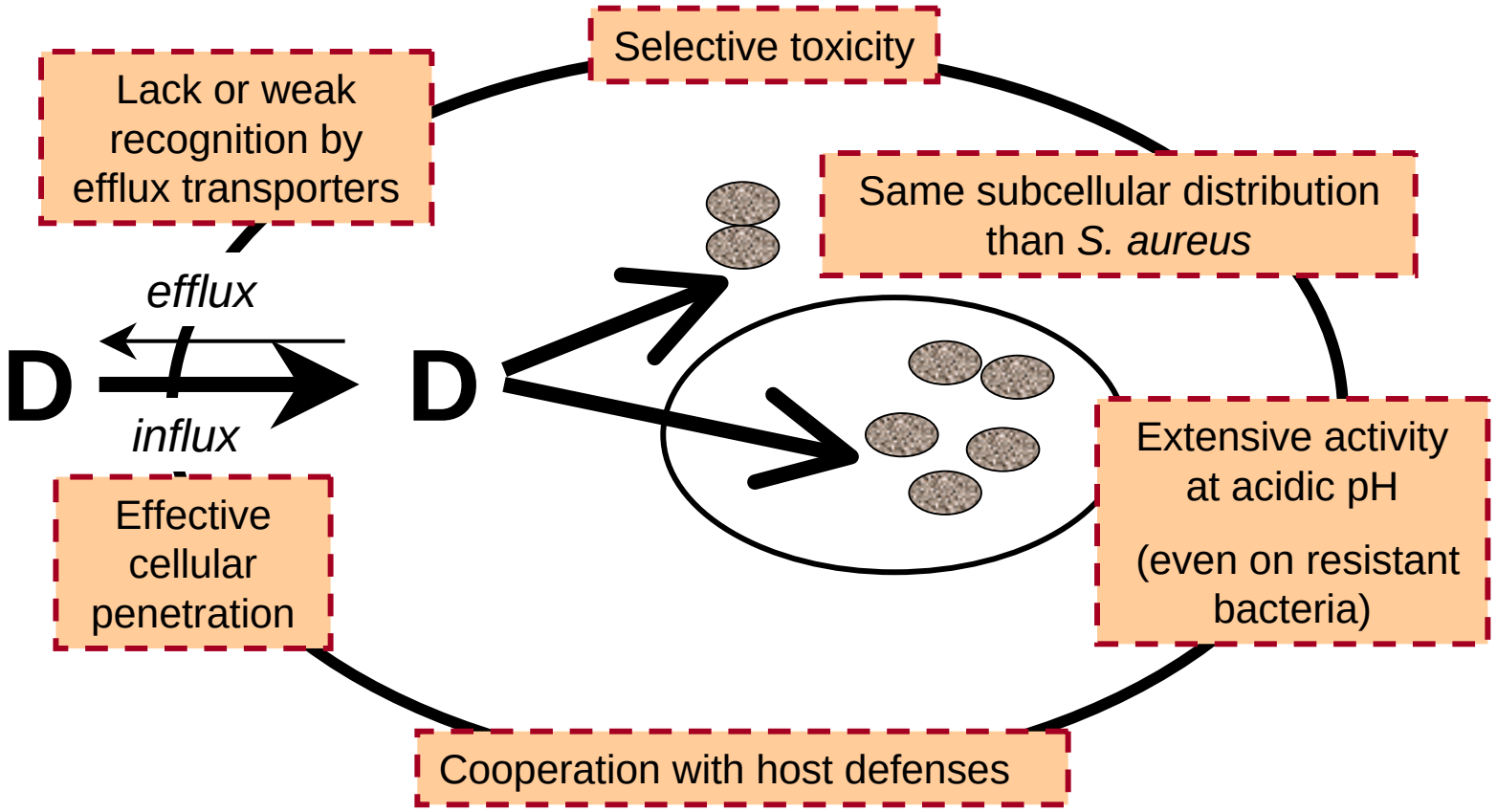
Usefulness of cellular models:

- To critically examine the pharmacodynamic properties of antibiotics (role in the early development of novel antibiotics)

- To systematically compare the activity of antibiotics against drug-resistant isolates

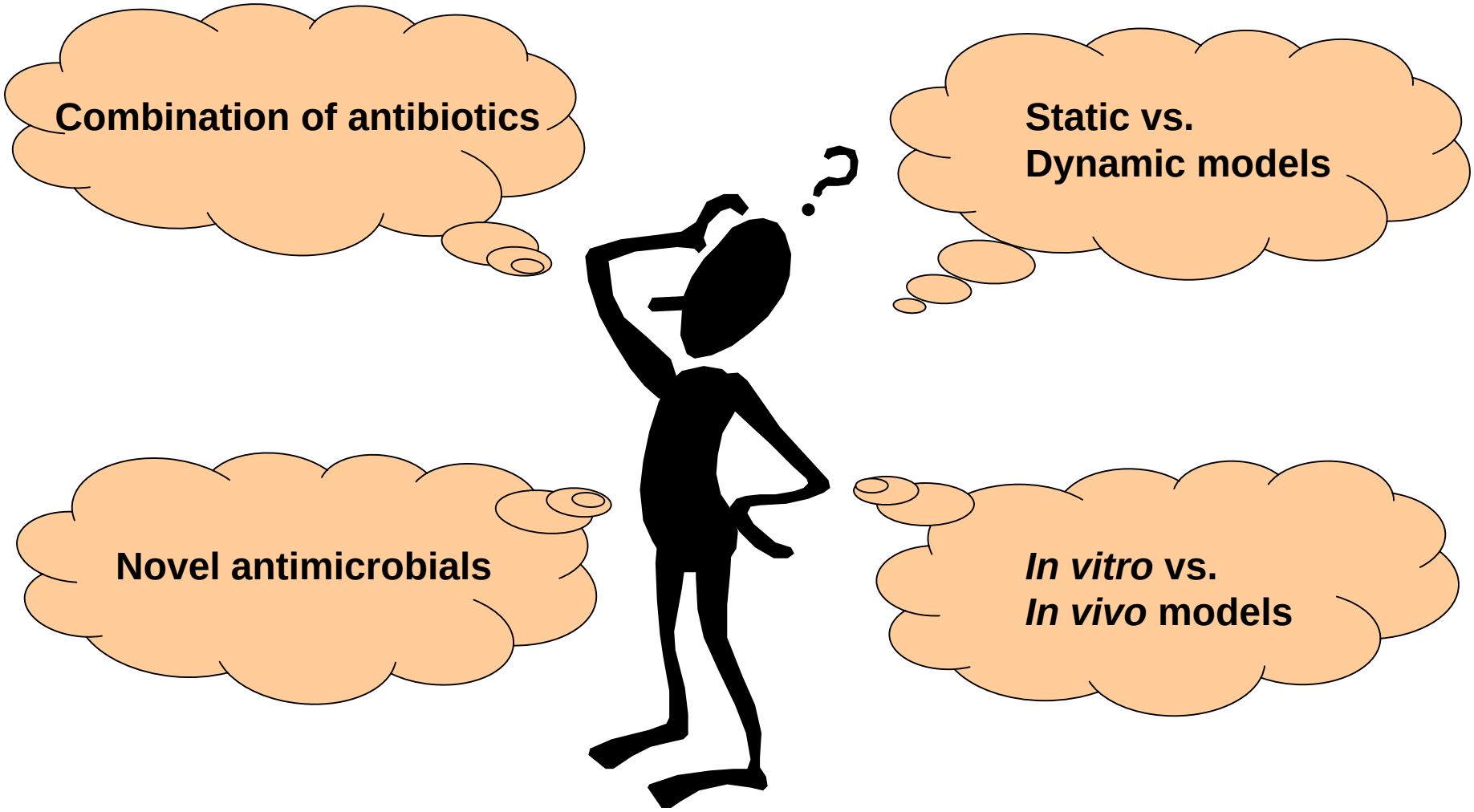
- To compare the activity of antibiotics in relevant cellular models of infections

Can we define the ideal antibiotic against intracellular *S. aureus* ?



Antibiotics should not only kill extracellular *S. aureus*, but also intracellular ones

Perspectives (i)



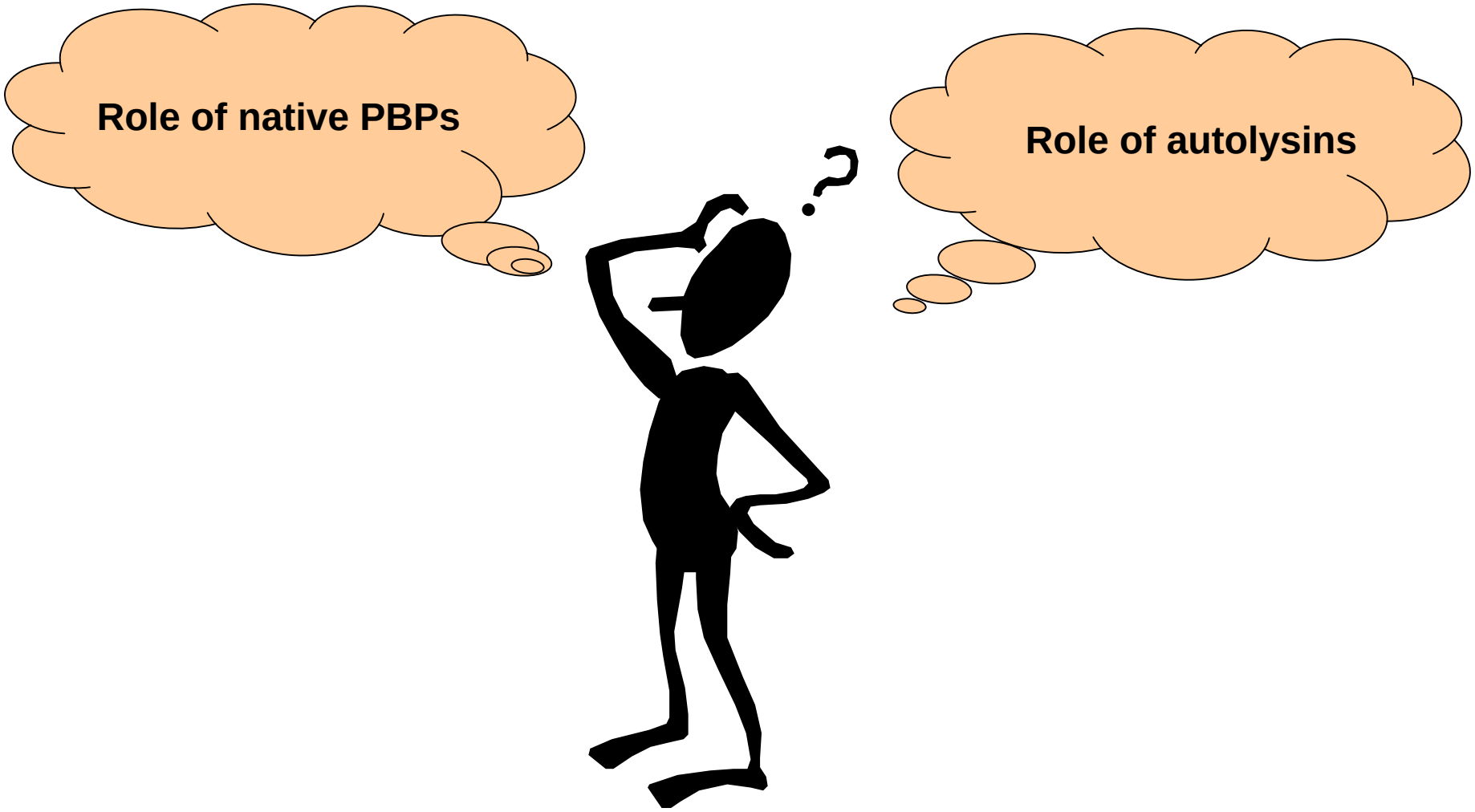
Discussion (ii)

B) PART II: Restoration of the susceptibility of MRSA to beta-lactams

The understanding of the molecular and biochemical mechanisms underlying this process is of profound significance because:

- it might reveal some potential targets
- it might be relevant of clinical situations where acidic conditions are present (skin surface, vagina, urinary tract)
- it might increase the efficacy of novel anti-MRSA cephalosporins (ceftobiprole)

Perspectives (ii)



Thanks to …

- **Professors P.M. Tulkens, F. Van Bambeke and M.-P. Mingeot-Leclercq**
- **Members of the jury**
- **Collaborators :**

  - Professors Y. Glupczynski and P.C. Appelbaum
  - Professors S. Mobashery, S. Vakulenko, and Dr Cosimo Fuda
  - Queen Astrid Military hospital (D. De Vos, J.-P. Pirnet)

- **F.R.I.A.**
- **All the old and new FACM-istes …**
- **My parents and friends**

Thanks for your attention