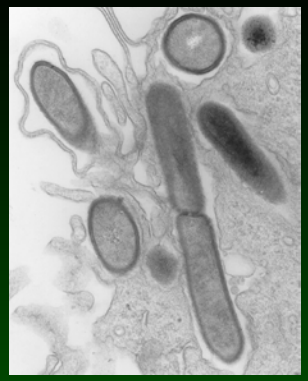
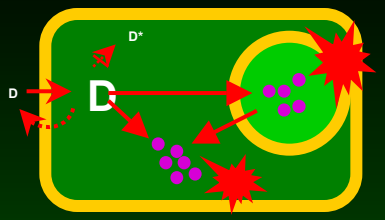


# Intracellular antibiotics

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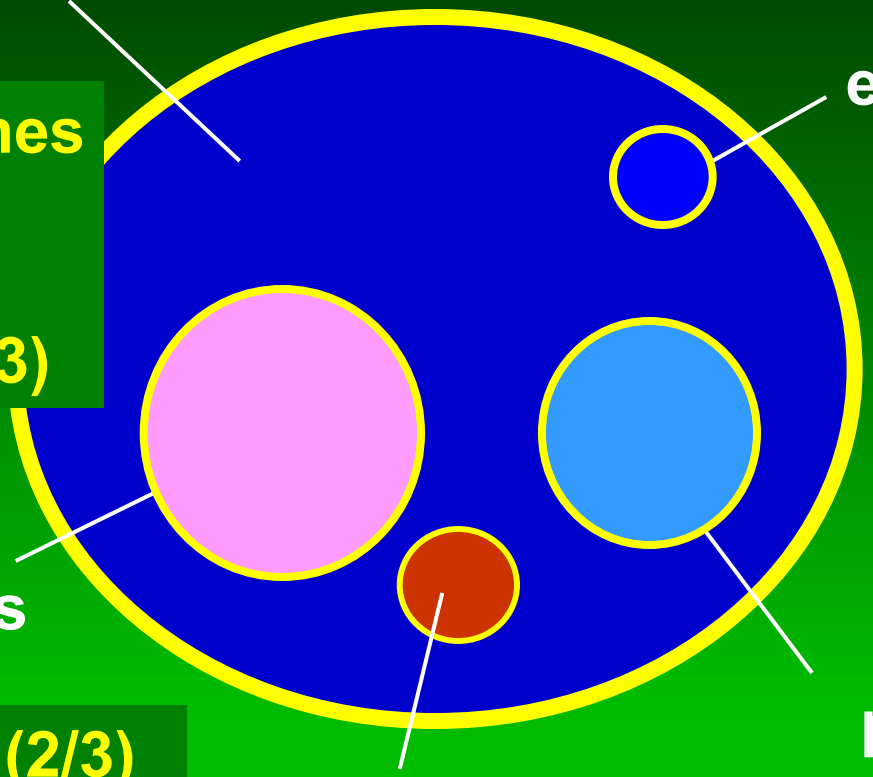
# Why intracellular antibiotics ?

# Which bacteria … and which diseases ...

- **Obligatory or mainly intracellular:**
  - **respiratory infections (pneumopathies):**
    - ***Chlamydia pneumoniae:*** **10% in children**
    - ***Legionella pneumophila:*** **frequent if immunosuppression**
    - ***Mycobacterium spp.:*** **frequent if immunosuppression**
  - **sexually transmitted diseases**
    - ***Chlamydia trachomatis:*** **most common pathogen**
  - **CNS infections + other sites:**
    - ***Listeria monocytogenes:*** **pregnant women; immunosuppression**

- **Facultative or mainly extracellular:**
  - **digestive tract infections**
    - ***Salmonella spp., Shigella spp.***
  - **respiratory, cutaneous, etc…tract infections**
    - ***Streptococcus spp., Staphylococcus spp.*** **etc...**

# Subcellular localization of antibiotics ?



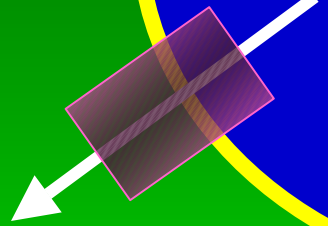

- fluoroquinolones
- beta-lactams
- ansamycins
- macrolides (1/3)

- macrolides (2/3)
- aminoglycosides

# Mechanisms of localisation and accumulation ...

cytosol

- fluoroquinolones



- Mechanism unknown (loose binding to lipids / lipoproteins ? …)
- Efflux demonstrated (MRP ?)

# Mechanisms of localisation and accumulation ...

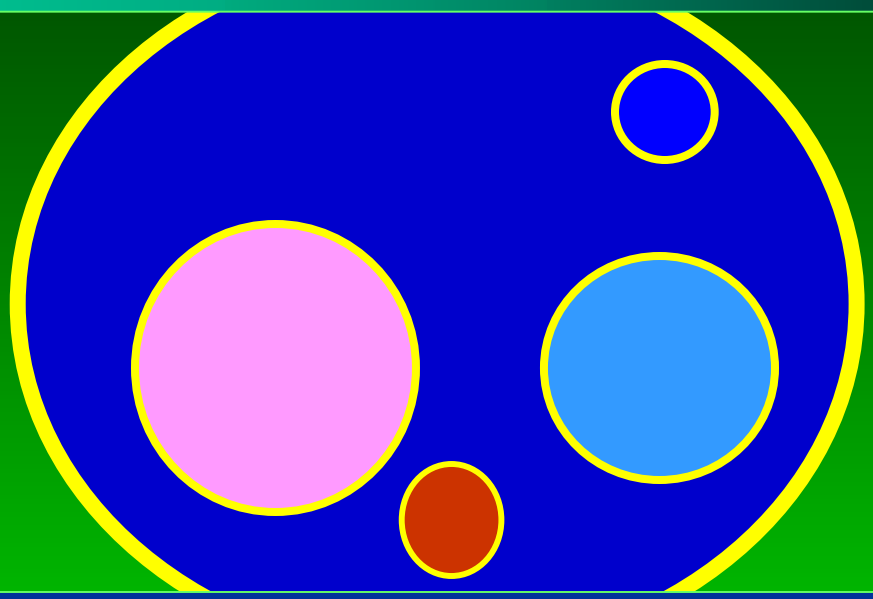
- proton trapping
- binding to phospholipids
- *for aminoglycosides: inability to cross membranes*

lysososomes

- macrolides
- aminoglycosides

# Subcellular bioavailability of antibiotics ?

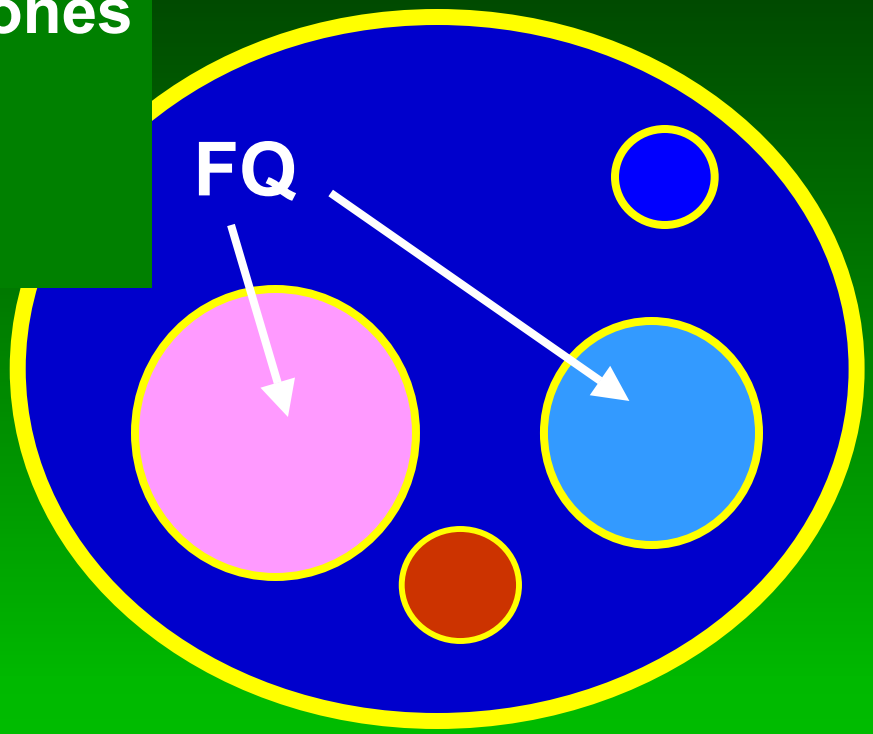
High Fair Nil



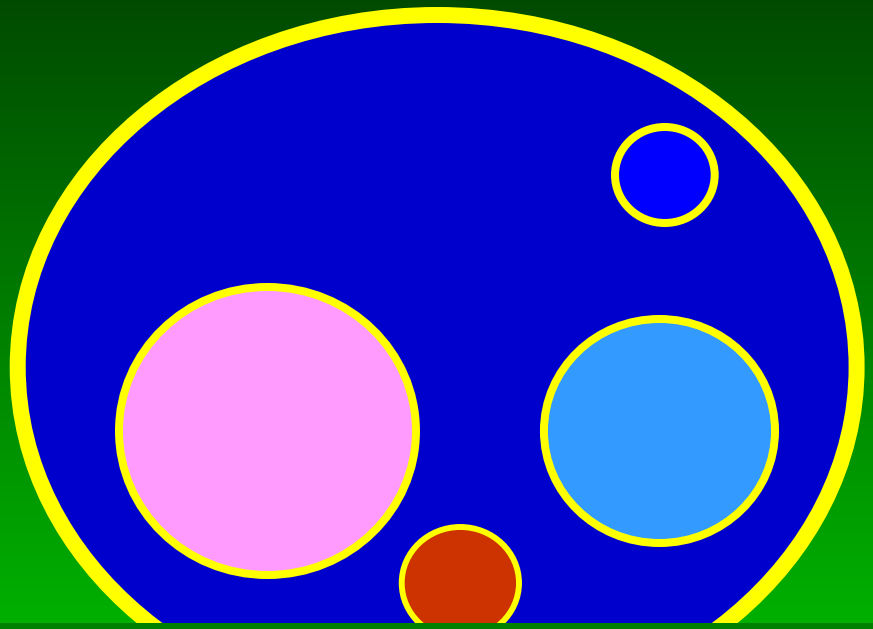
FQ / Ansamyc. / cytosol. ML lysosom. ML / AG

# Subcellular bioavailability of antibiotics ?

**Fluoroquinolones move easily across membranes**



# Subcellular bioavailability of antibiotics ?



**aminoglycosides**
**and lysosomal macrolides**
**reamain largely if not totally sequestered**
**in an acidic environment ...**

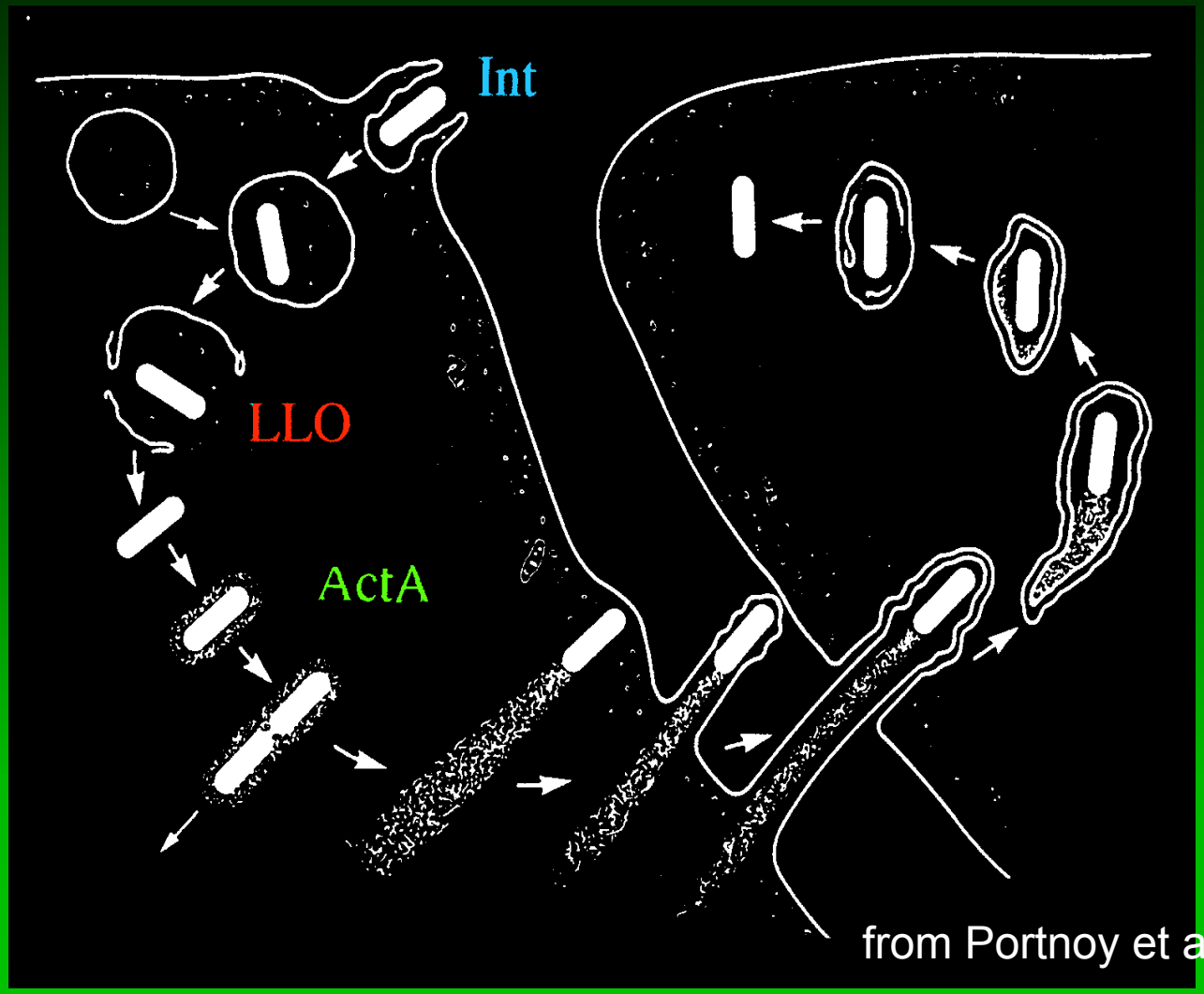
# Illustration: the Listeria story

**antibiotics:**

- **ampicillin/meropenem**
- **azithromycin**
- **sparfloxacin/moxifloxcin**
- **pivampicillin**

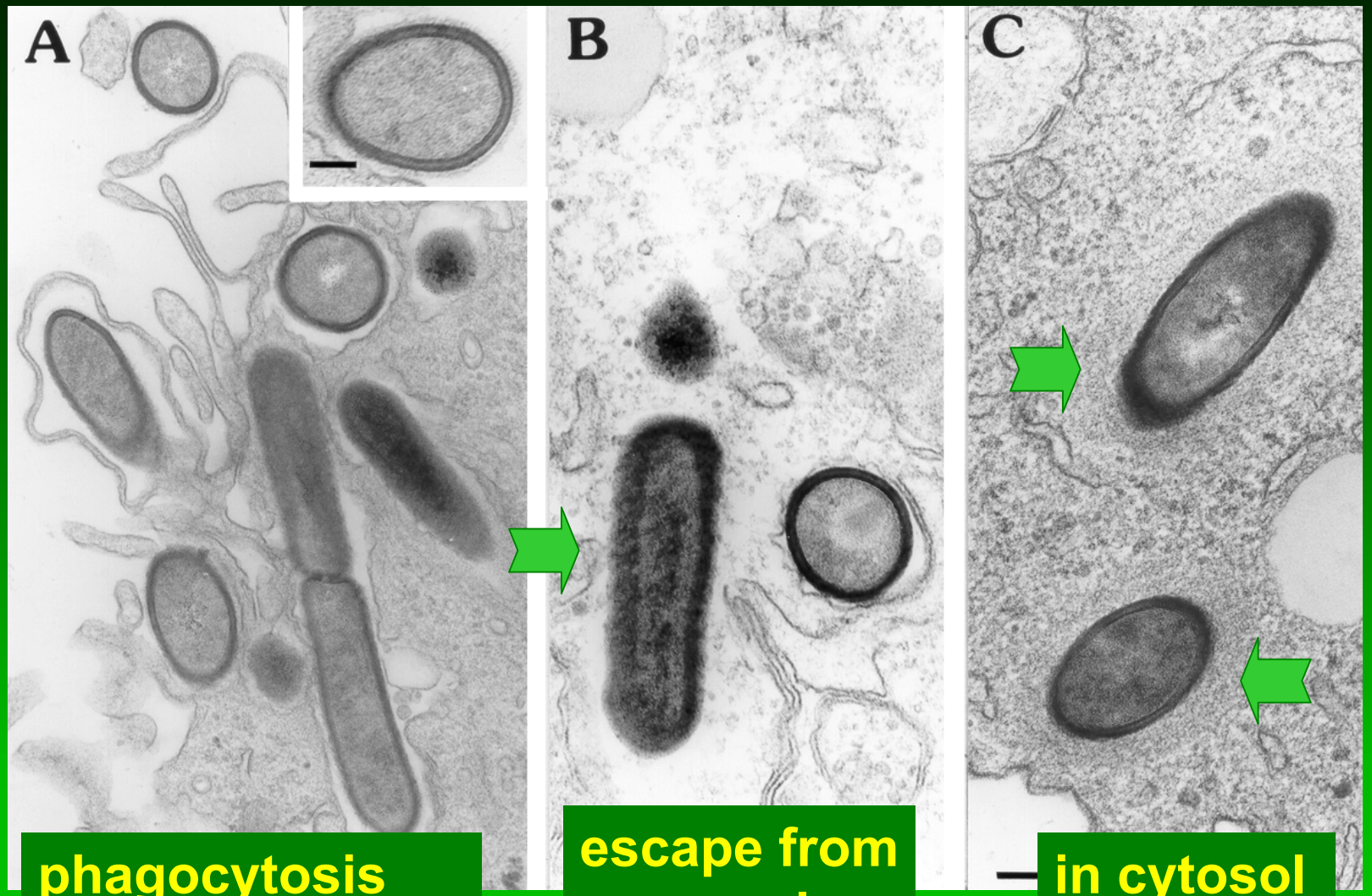
***Listeria monocytogenes***
**hly+**

# Intracellular infection cycle of *Listeria monocytogenes* hly$^{+}$

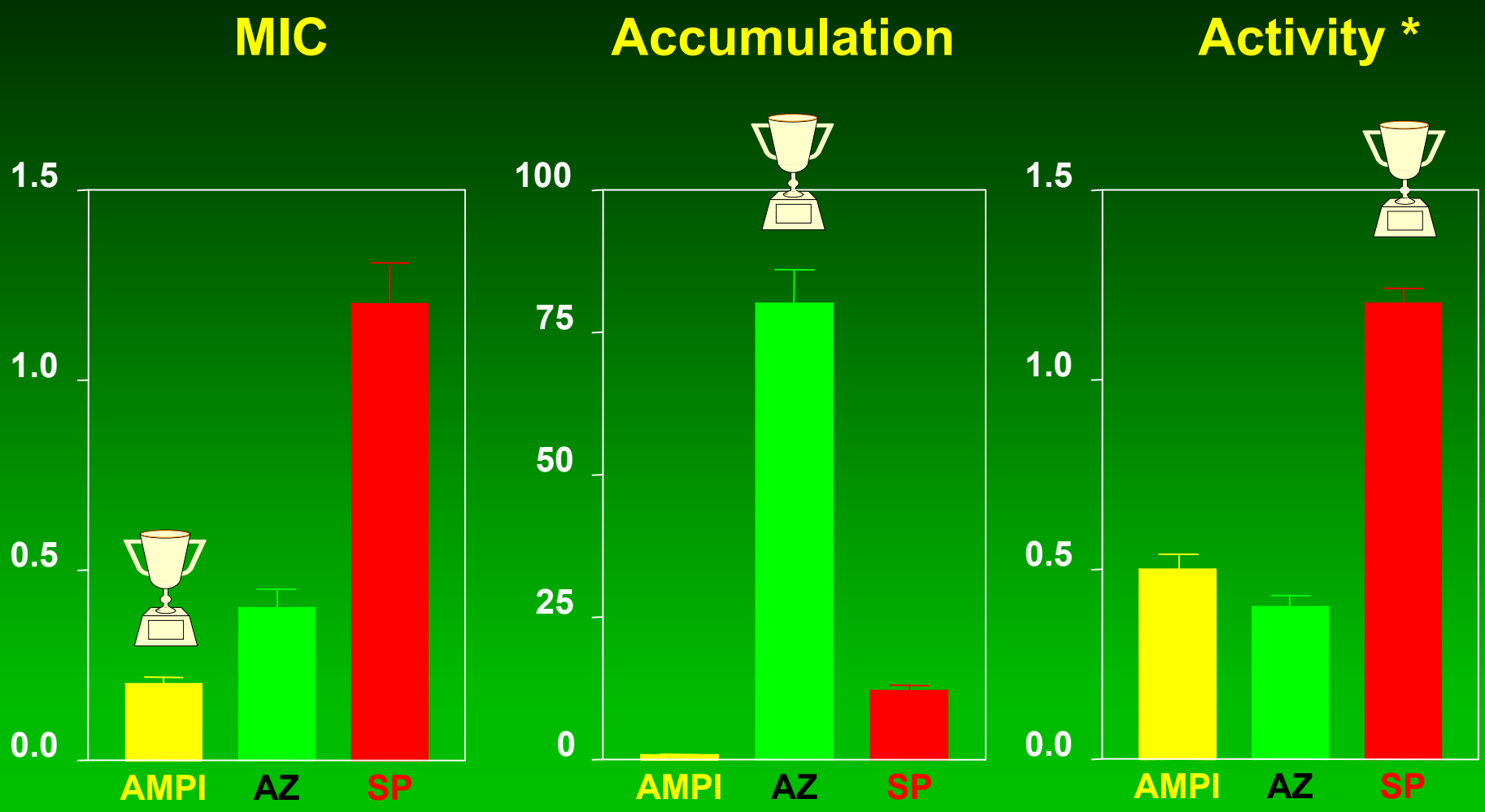



from Portnoy et al.

# Following the intracellular fate of *Listeria m.* by EM



# 1st question: is there a simple relation between MIC, accumulation and intracellular activity (5 h model)




Ouadhriri et al., AAC,1999

* log CFU 5h
Ce = 10 x MIC

# *Listeria m. and* ampicillin

Ampicillin is poorly active against intracellular *Listeria m.* in spite of its favourable MIC;

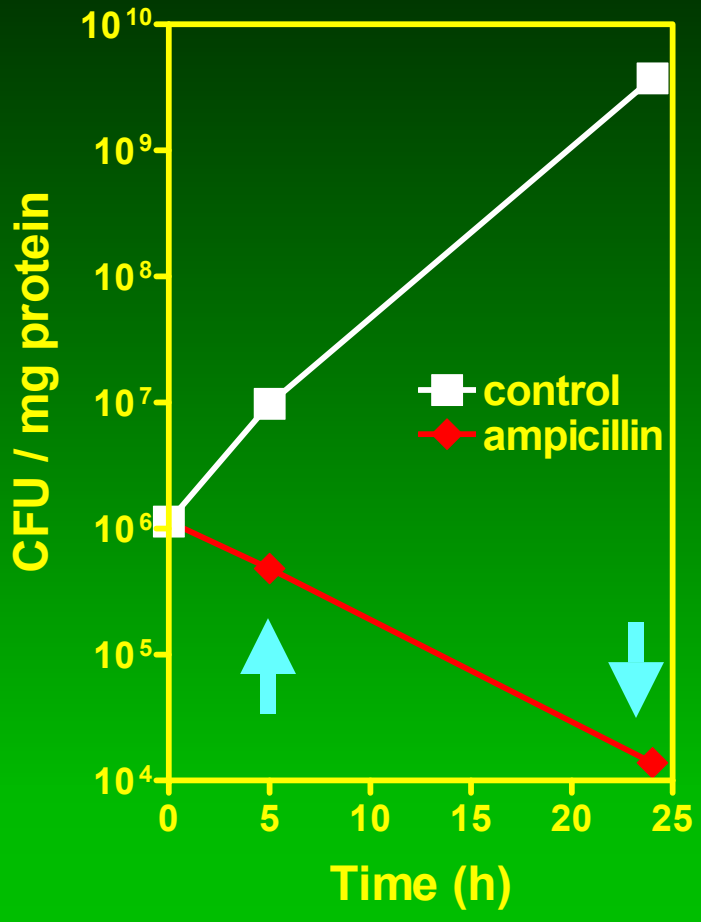
➡ lack of accumulation ...

Why do you keep ampicillin ?

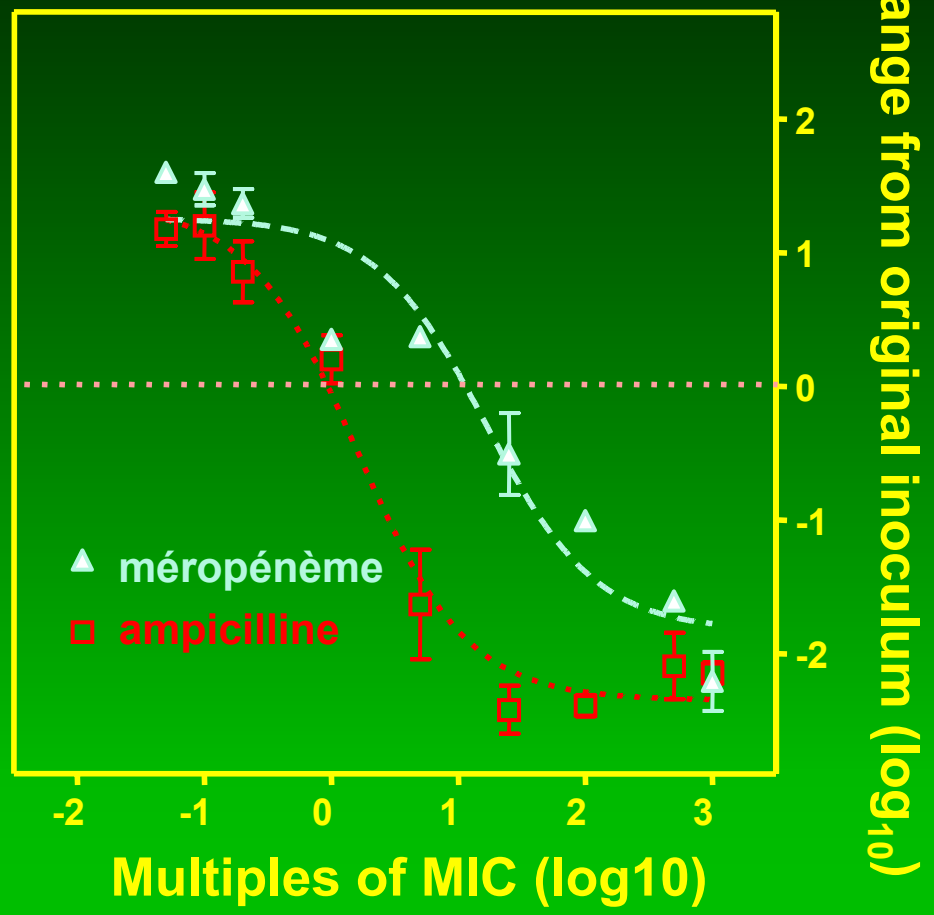
- ➔ extracellular bacteria
- ➔ get intracellular activity with very large doses ??

  **(but β-lactams are NOT dose-dependent…)**
- ➔ **but may be you just have to wait ...**

# β-lactams become bactericidal intracellularly after 24h and if you give a dose high enough




(Carryn et al., 2003, *JAC* 51:1051-52




(Lemaire et al, 2004, unpublished

# *Listeria m.* and azithromycin

Azitromycin is poorly active against intracellular *Listeria m.* in spite of its exceptionally large intracellular concentration

→ most azithromycin is trapped in lysosomes

→ azithromycin is poorly bactericidal

**Is there a future for macrolides ?**

# *Listeria m. and* fluoroquinolones

In a pharmacological model*, fluoroquinolones appear most active in spite of relatively unfavourable MICs and modest cellular accumulation compared to macrolides …

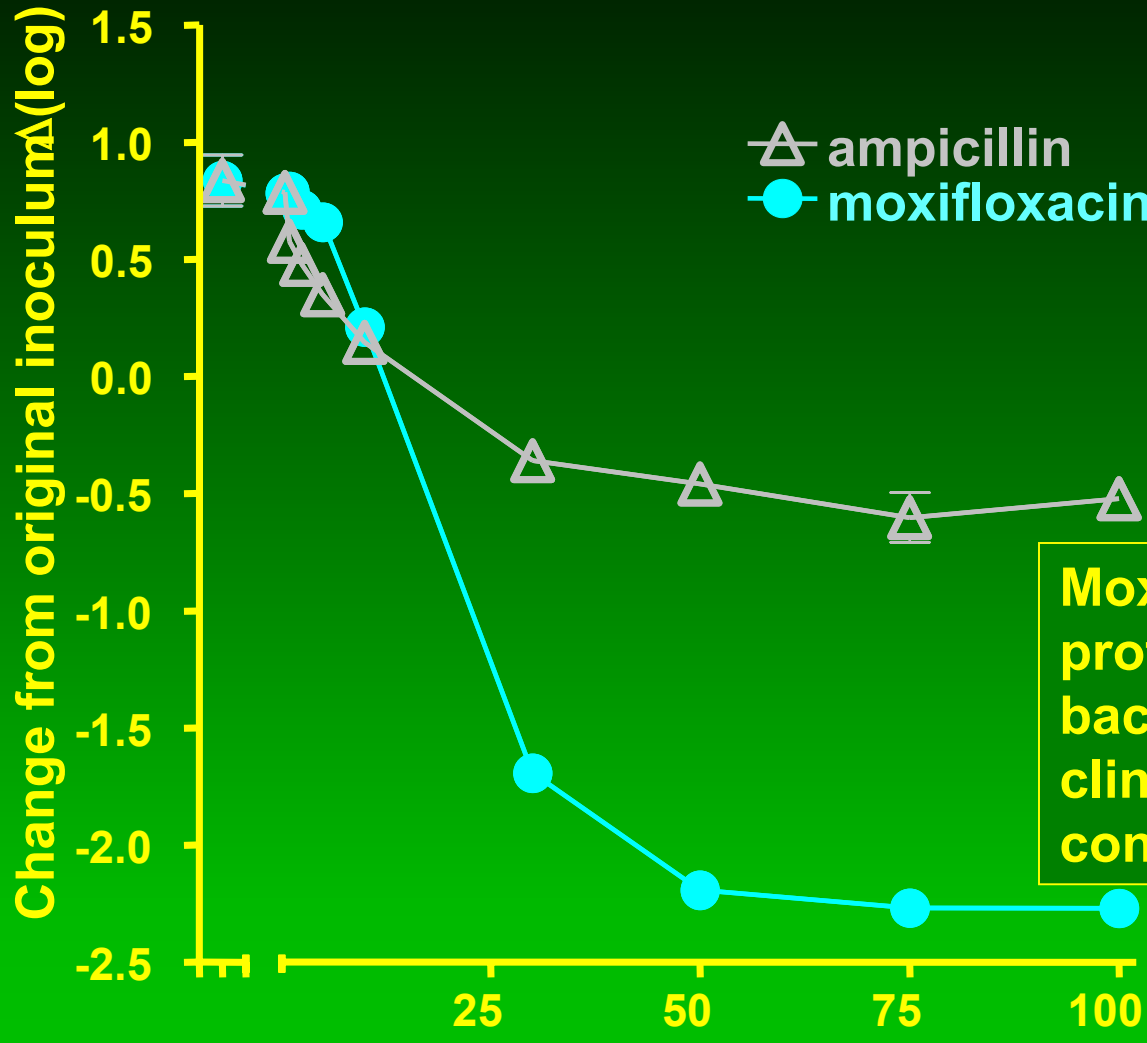
Fluoroquinolones

⇒ have a large subcellular bioavailability

⇒ are highly bactericidal

* all Ce = 10 X the MIC

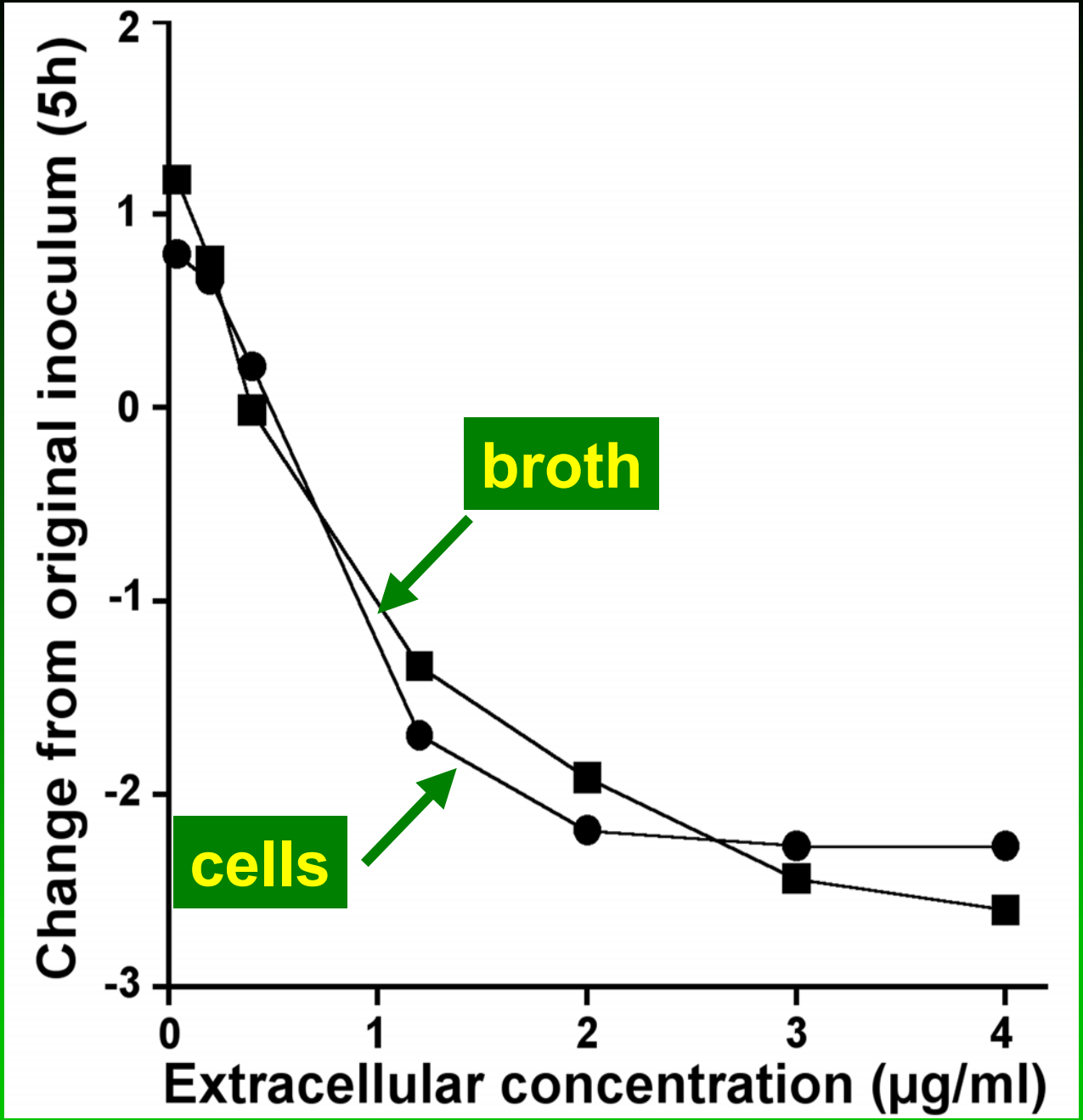
# Comparative intracellular activities at multiples of $C_{max}$




**Moxifloxacin is profoundly bactericidal at clinically achievable concentrations**

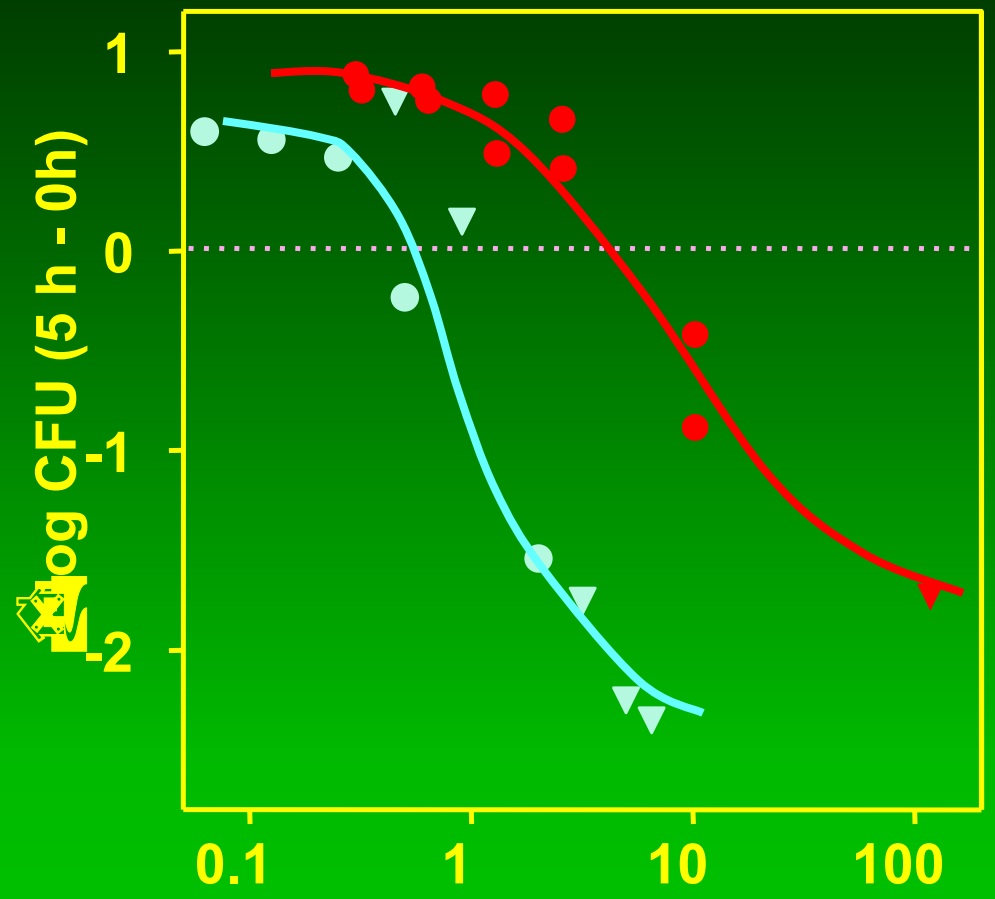
(Carryn et al., 2002, *AAC* 43:2095-103)

**However, intracellular moxifloxacin is NOT more active intracellularly than extracellularly when using the extracellular concentration as comparison basis ...**




Carryn et al., AAC, 2002

# And this is obvious if you compare intracellular and extracellular activities <u>at the same</u> apparent concentration * / MIC ratio ….



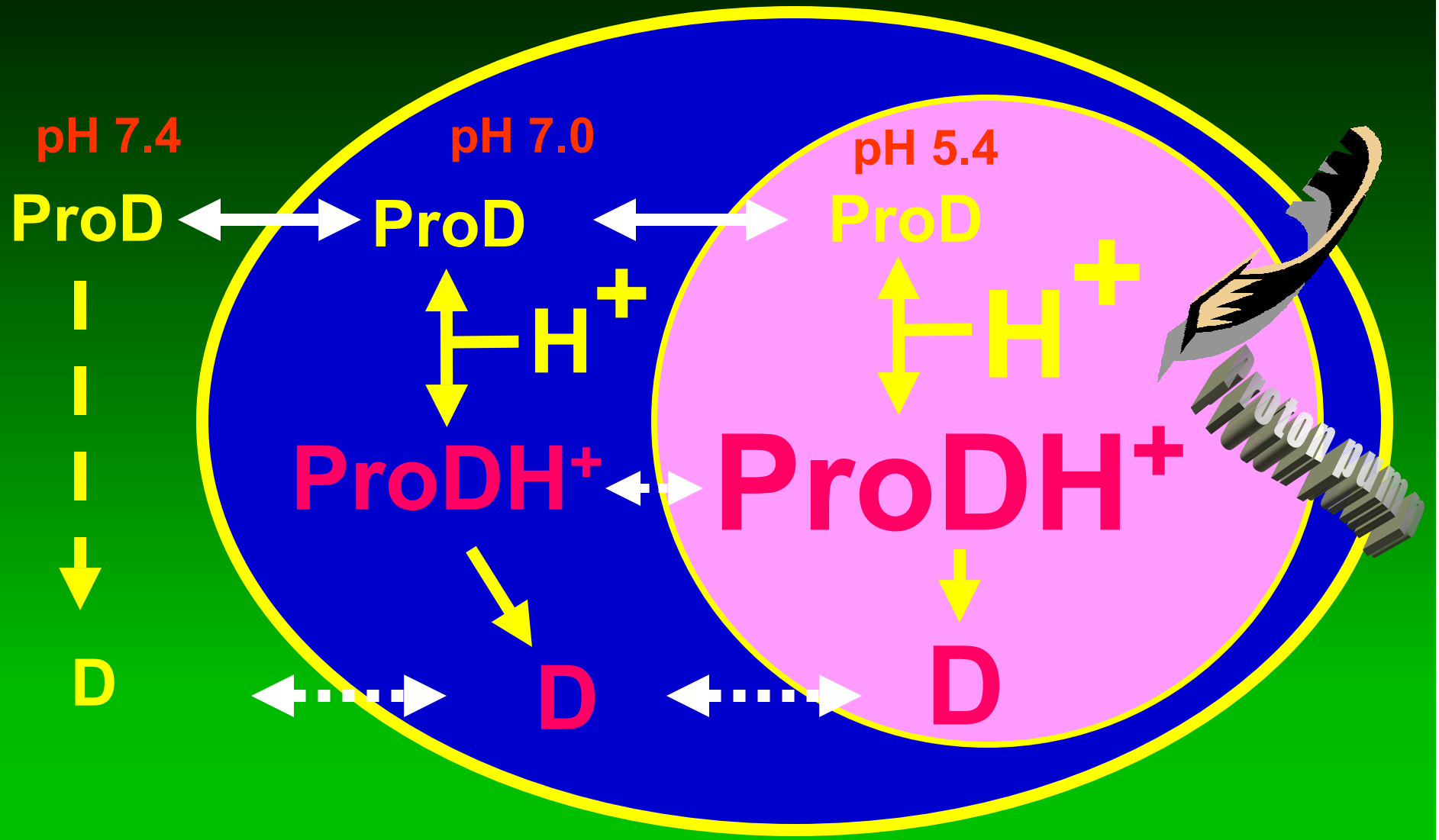

extracellular
- levofloxacine
- moxifloxacine

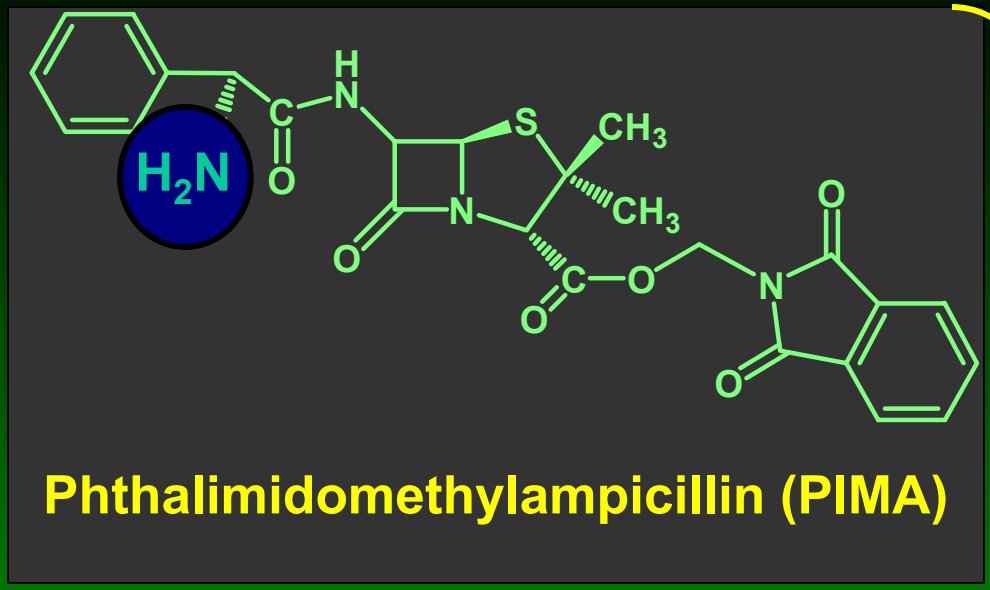
intracellular
- levofloxacine
- moxifloxacine

Seral et al., unpublished

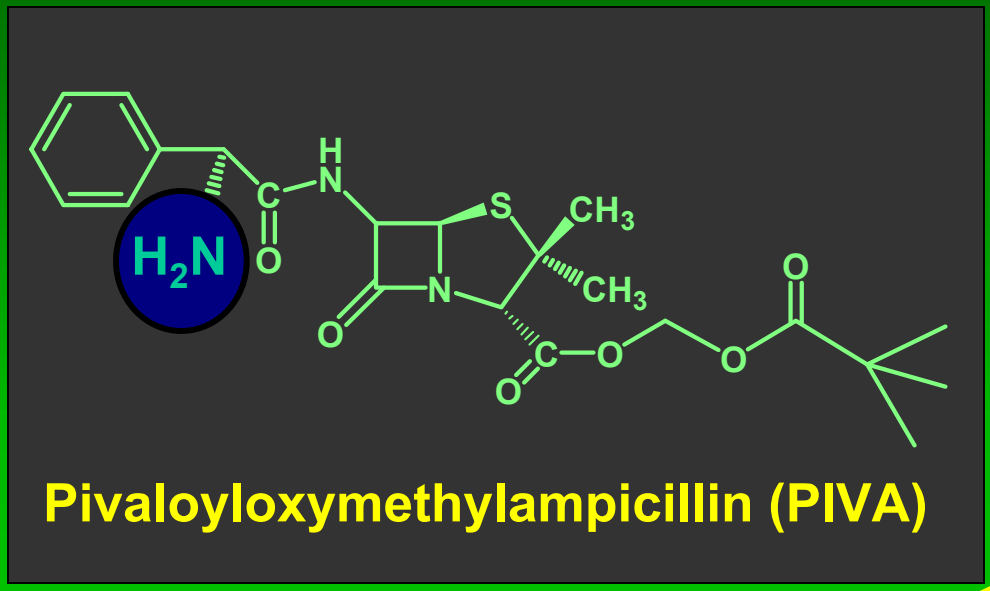
extracellular = actual
intracellular = calculated

# A basic prodrug of a β-lactam ?






Phthalimidomethylampicillin (PIMA)




Pivaloyloxymethylampicillin (PIVA)

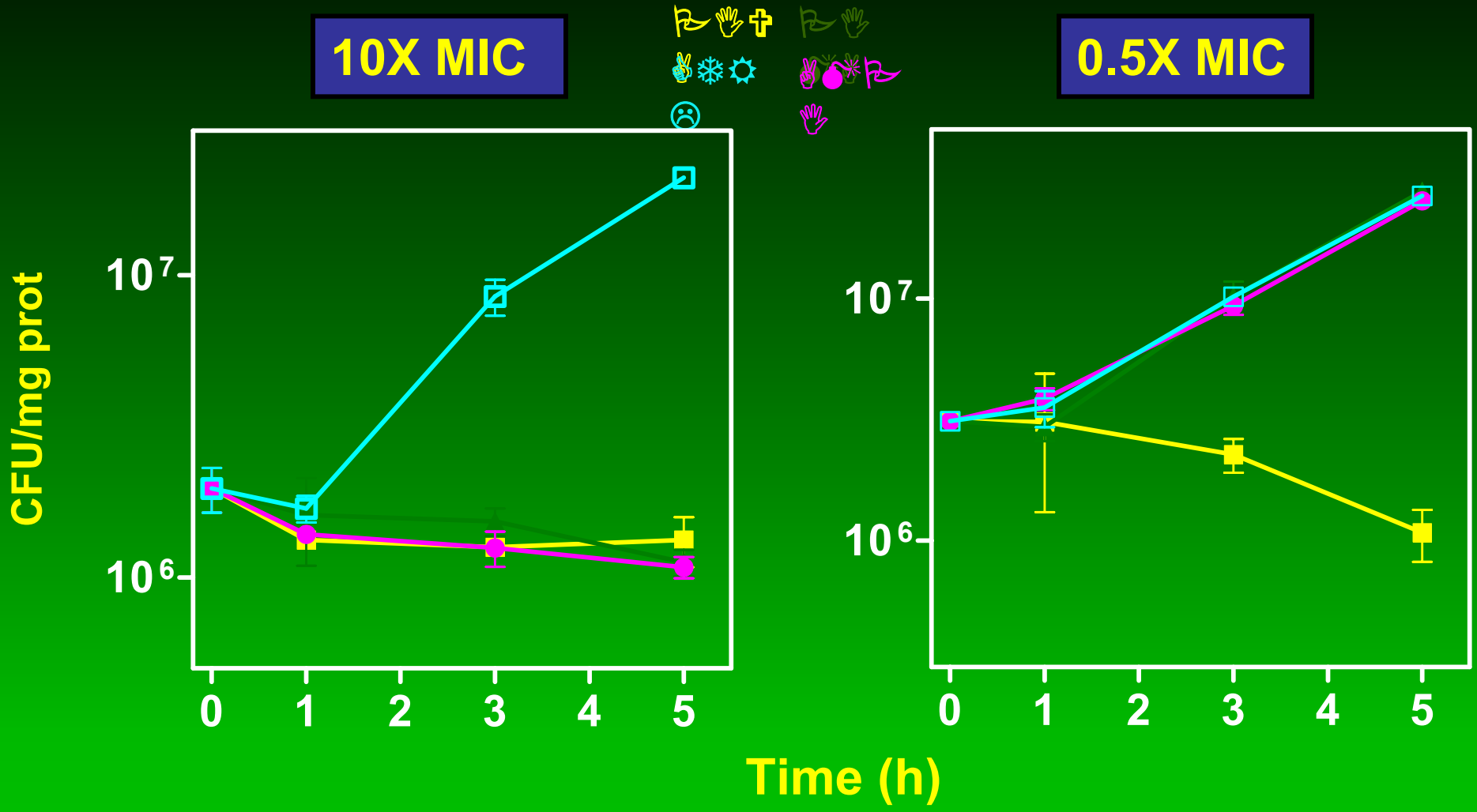
**Basic compounds that**

- **regenerate ampicillin**
- **accumulate in J774 macrophages**

Fan et al, Bioorg. Med. Chem. Let.1997

Paternotte et al, Biorg. Med. Chem. 2001

# Intracellular activity for extracellular concentrations of ...

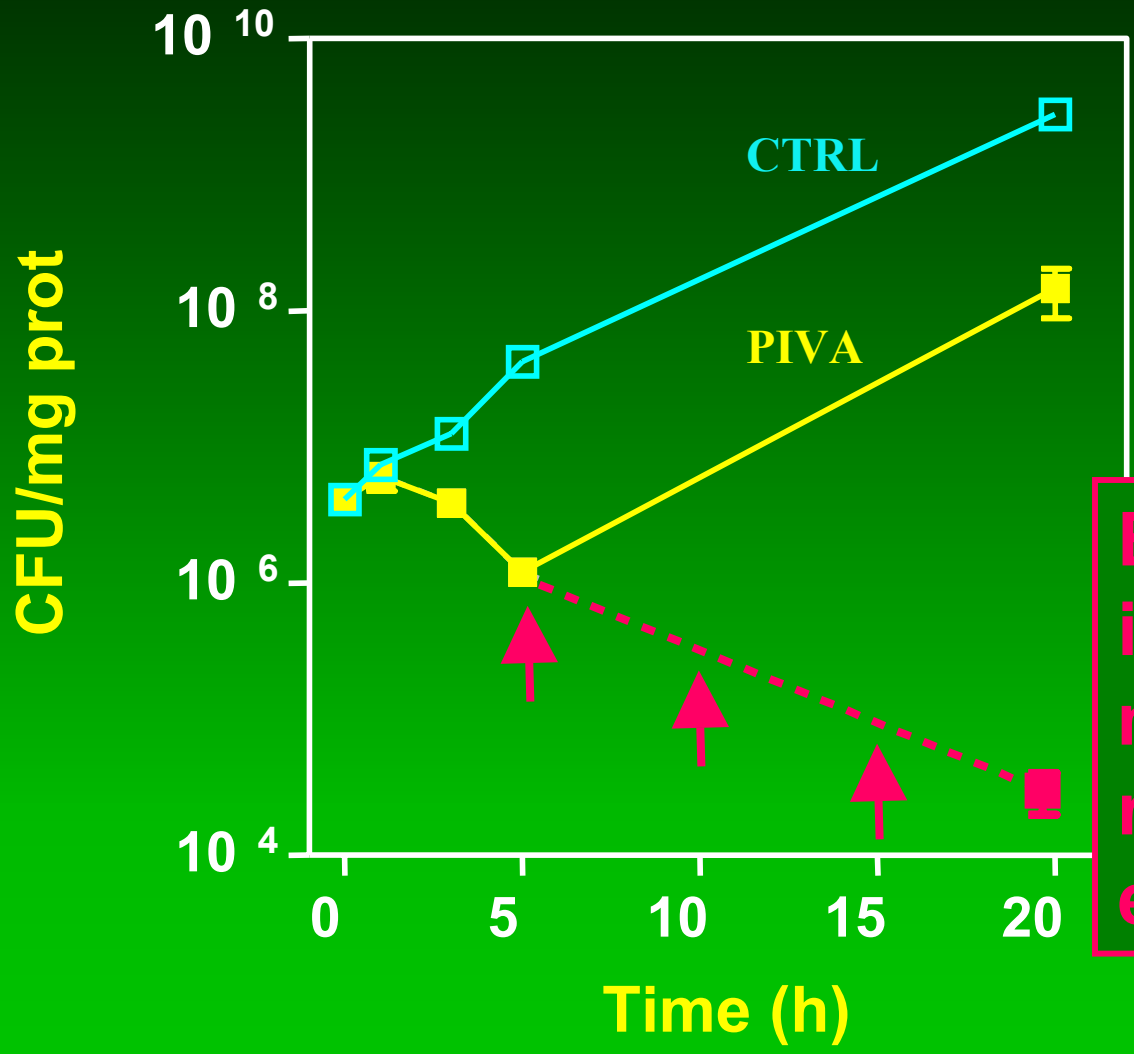



Same activity for PIVA, PIMA and AMPI

Only PIVA is active

Chanteux et al, JAC, 2003

# At low extracellular concentration, PIVA loses its activity after 5 h if the medium is not renewed



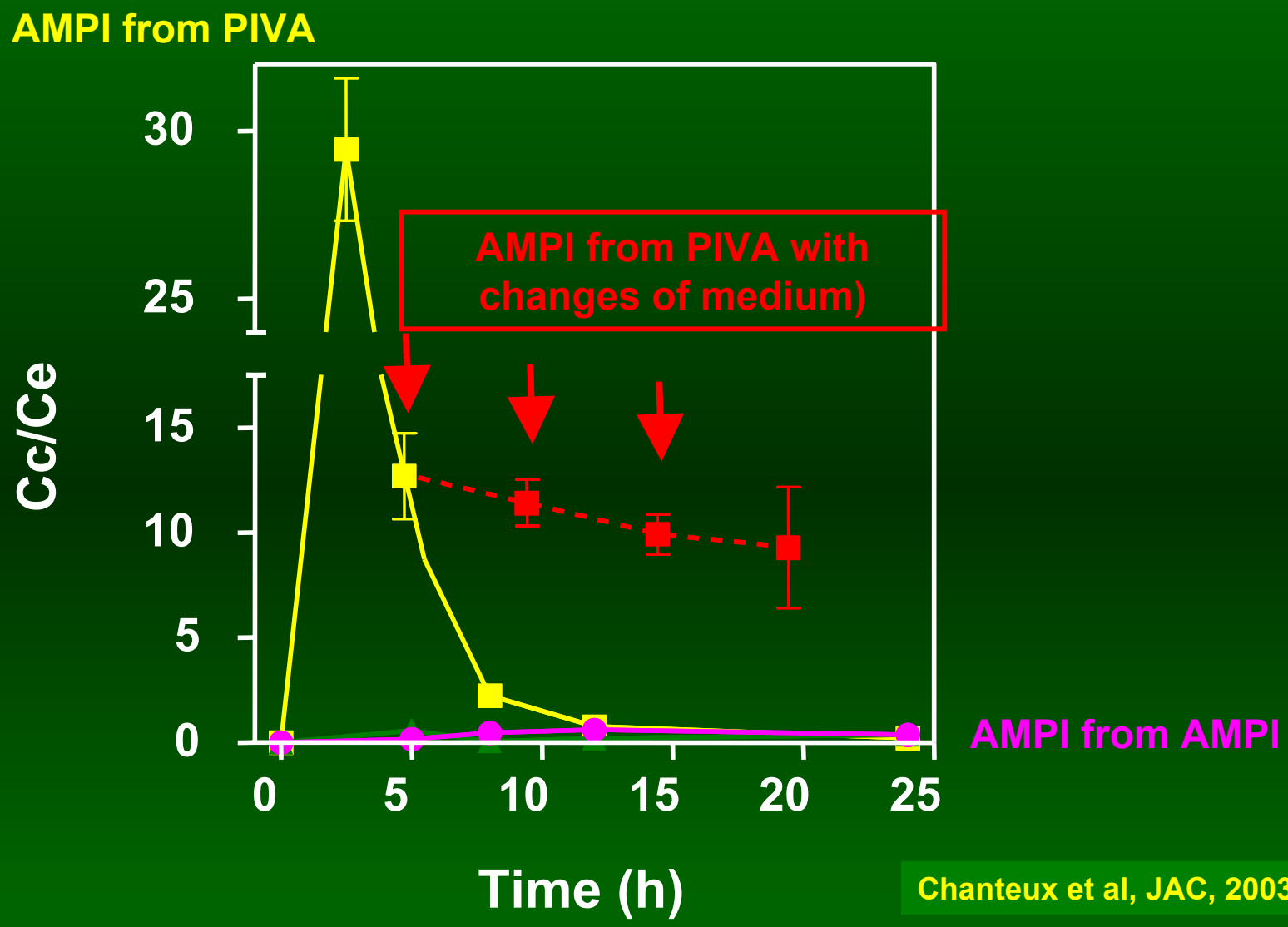

Extracellular concentration

**0.5X MIC**

**But maintains it if the medium is renewed every 5 h**

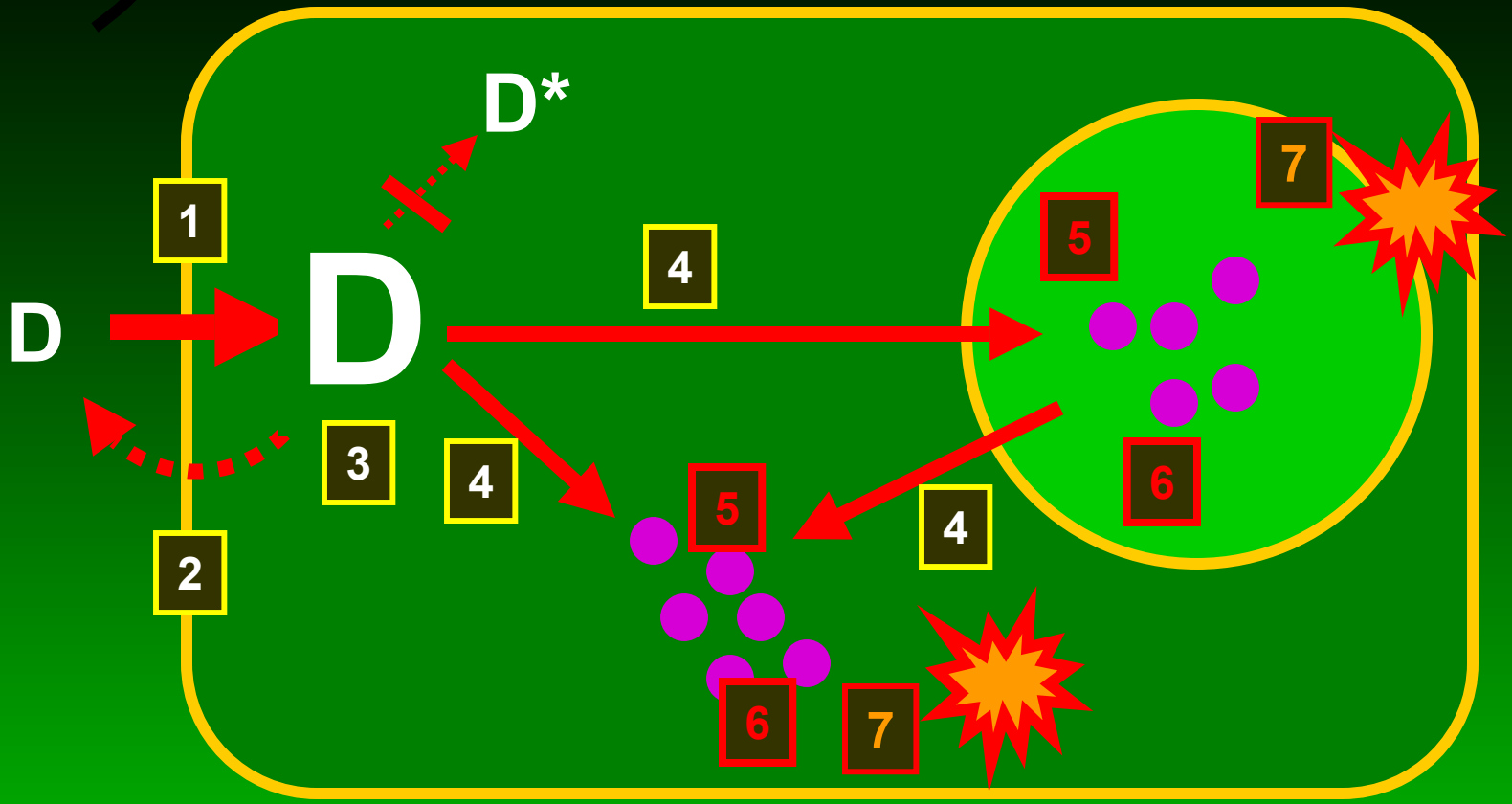
Chanteux et al, JAC, 2003

# PIVA releases large amount of intracellular ampicillin




Chanteux et al, JAC, 2003

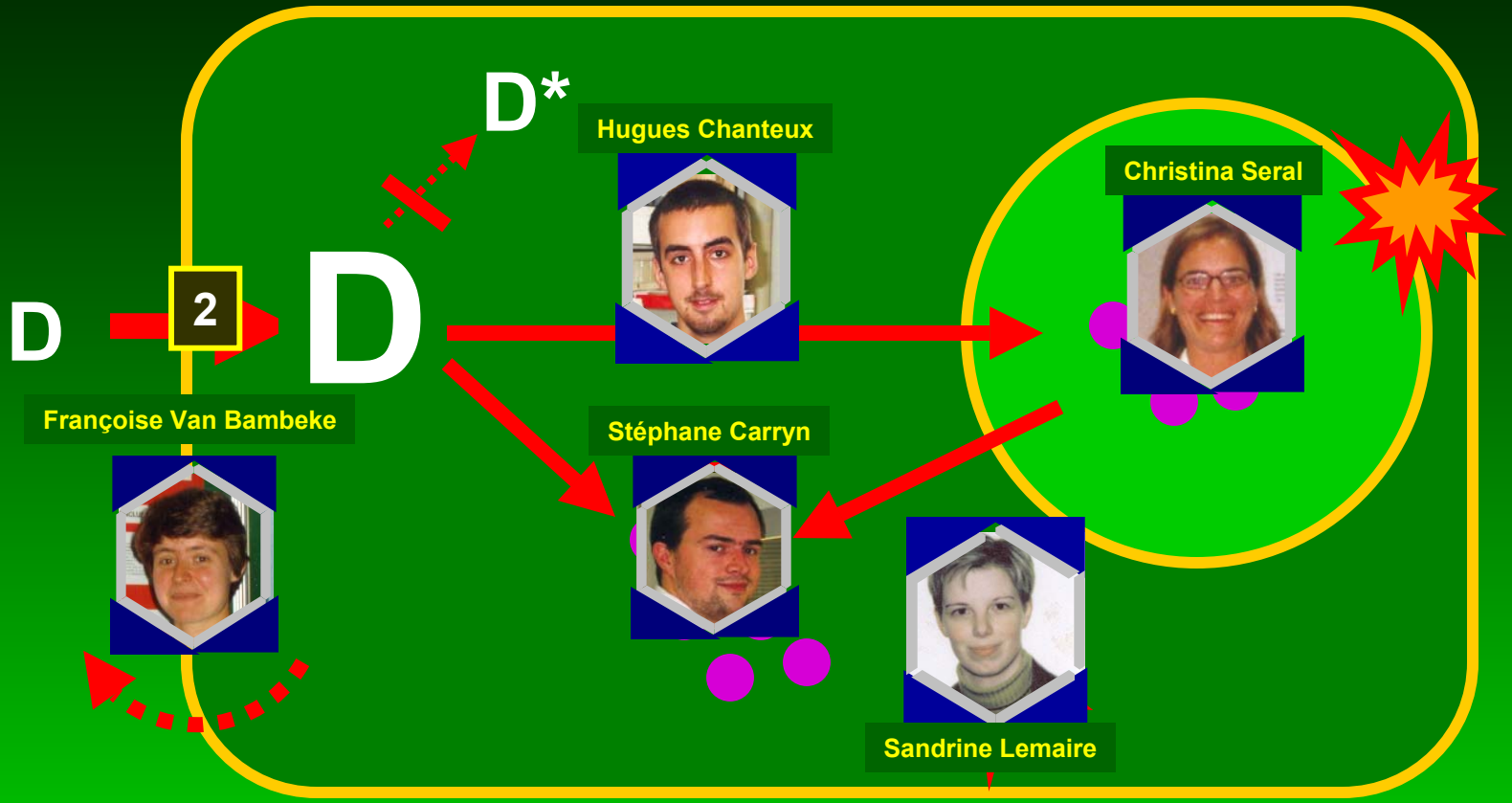
# The seven pillars of intracellular / intratissular activity ?




1. Penetration
2. No efflux
3. Accumulation
4. Subcell. bioavailability

5. Expression of activity
6. Bacterial responsiveness and pharmacodynamics
7. Cooper. with host def.

# The 6 pillars of intracellular / intratissular accumulation and activity of antibiotics...




Françoise Van Bambeke, Stéphane Carryn, Cristina Seral, Hugues Chanteux, Sandrine Lemaire ...