

Intracellular Antibiotic Kinetics and Dynamics : A Reflexion of Reality

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Why intracellular antibiotics ?

antibiotic

The Cell

bacteria

Black Box...

Intracellular antibiotics: the issues

1. which bacteria and where ?



2. which antibiotics accumulate ?

3. influx vs efflux ?

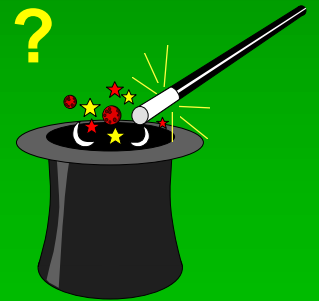
3. where are antibiotics in cells ?



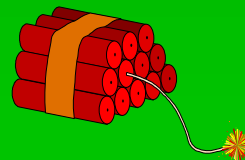
4. intracellular expression of activity ?

5. bacterial responsiveness ?

6. cooperation with host defenses ?



7. any toxicity ?



Which antibiotics accumulate in cells ?

- beta-lactams: $\leq 1x$
- aminoglycosides: <1 to $2x$
- ansamycins: $2-3x$
- tetracyclines: $2-4x$
- fluoroquinolones: $5 - 20x$
- macrolides: 4 to $> 100x$ *
- glycopeptides: 1 to $400x$!! **

* azithromycin, ketolides

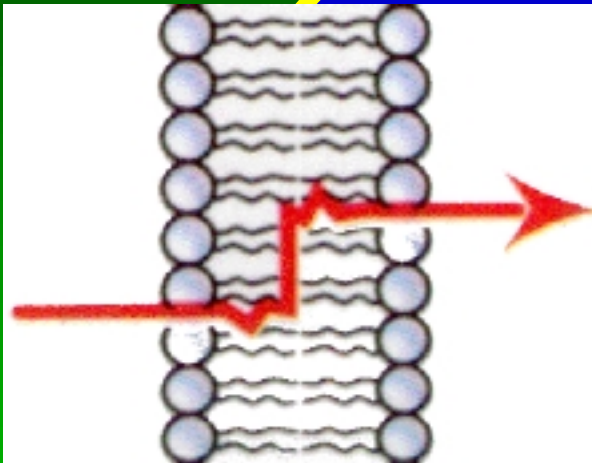
** LY 333328

How do antibiotics penetrate in cells ?

- **trans-membrane influx**
 - ▶ **diffusion**
 - ▶ **carrier mediated**
- **endocytosis**

How do antibiotics penetrate in cells ?

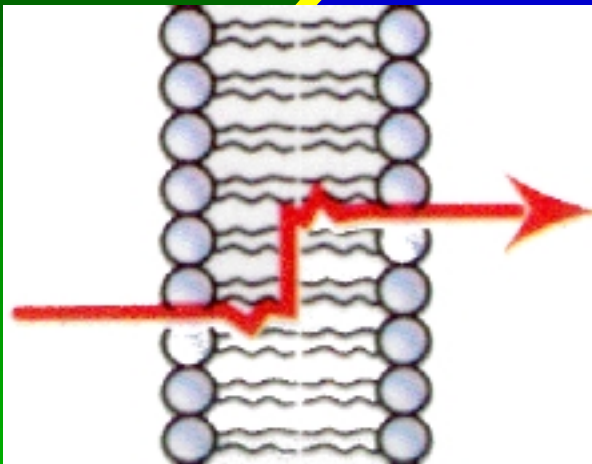
diffusion



- **amphiphilic compounds**
- **fast**
- **non-saturable**
- **no competition by analogues**

How do antibiotics penetrate in cells ?

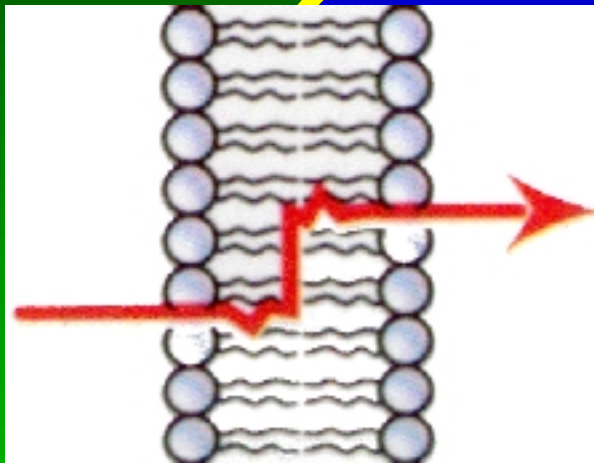
diffusion



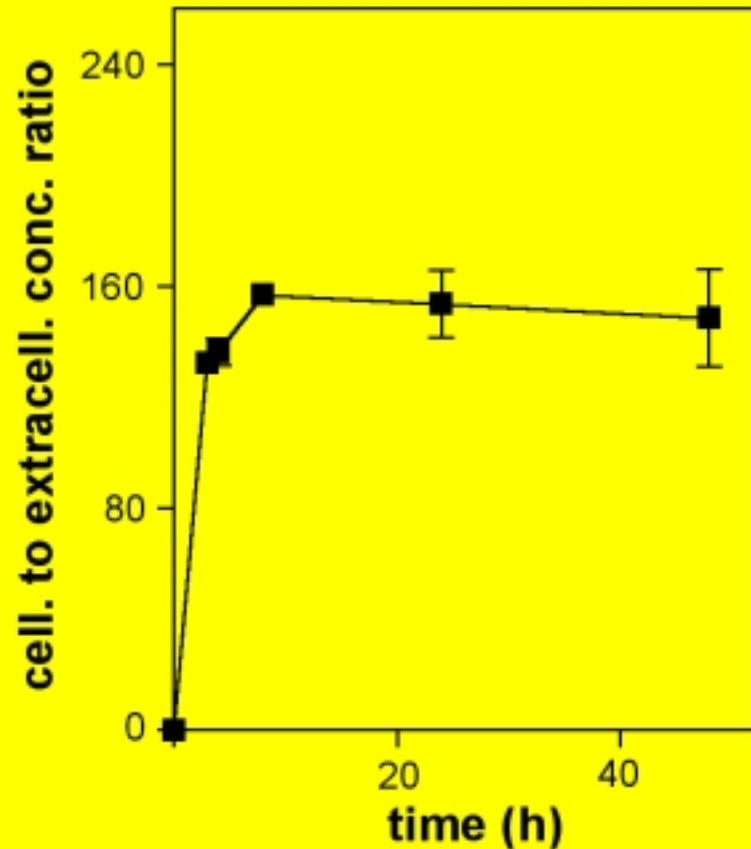
- ▶ macrolides
- ▶ fluoroquinolones
- ▶ tetracyclines
- ▶ ansamycines
- ▶ β -lactams,
- ▶ ...

How do antibiotics penetrate in cells ?

diffusion



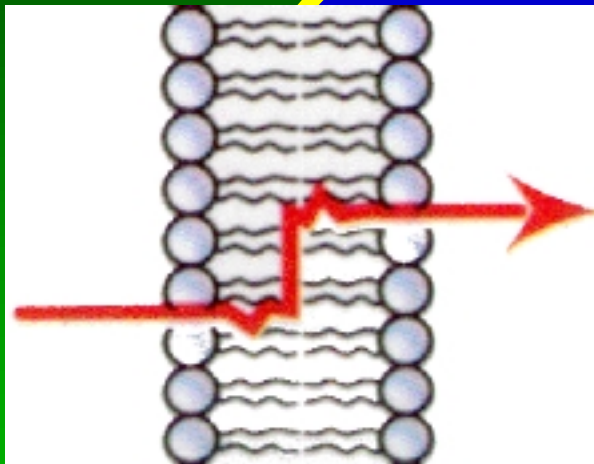
Azithromycin accumulation
in rat embryo fibroblats



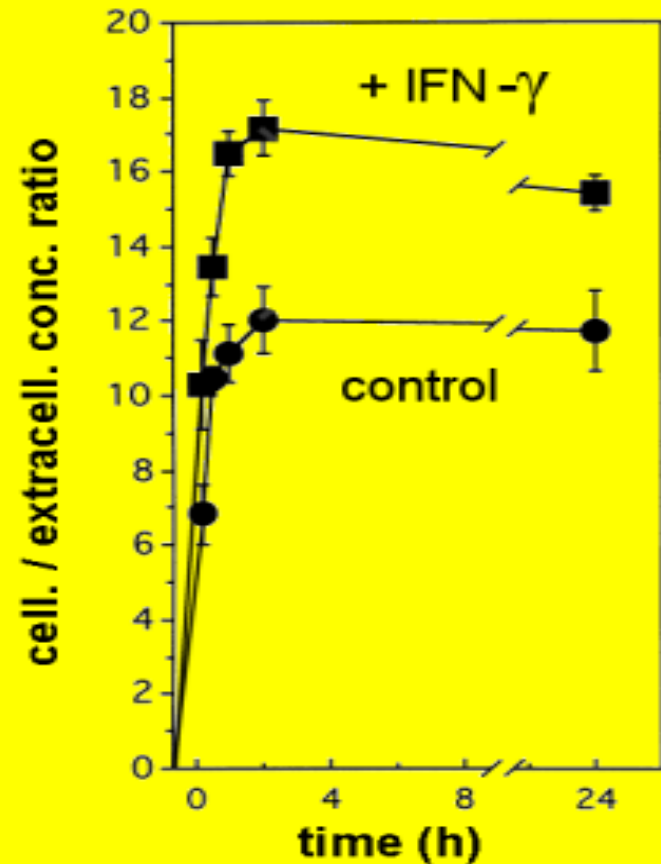
Tyteca et al., EJCB, 2001, in press

How do antibiotics penetrate in cells ?

diffusion



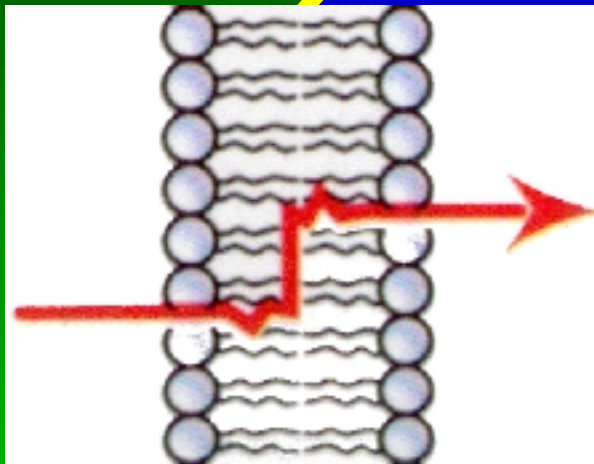
Sparfloxacin accumulation in THP-1 macrophages



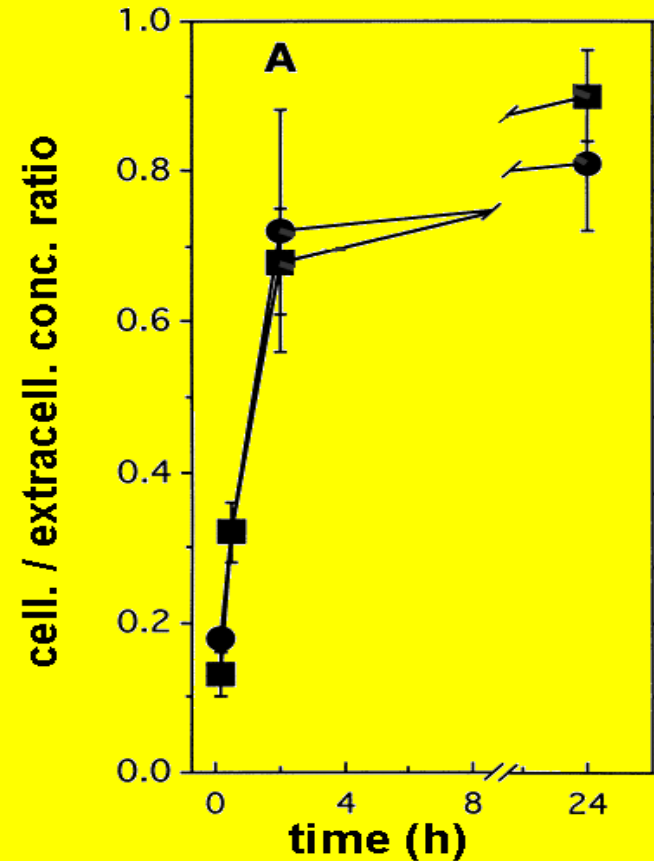
Quadrhiri et al., AAC, 1999

How do antibiotics penetrate in cells ?

diffusion



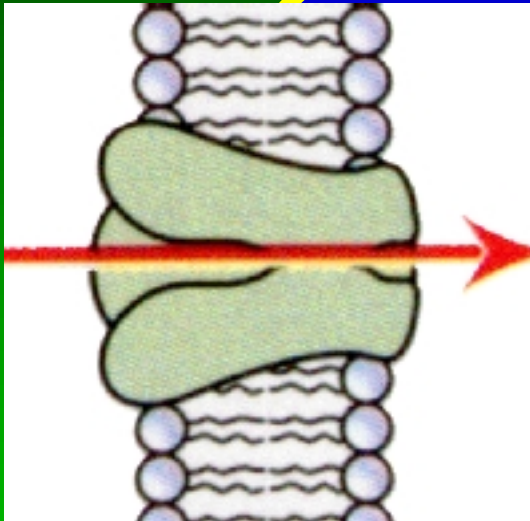
Ampicillin accumulation in THP-1 macrophages



Quadrhiri et al., AAC, 1999

How do antibiotics penetrate in cells ?

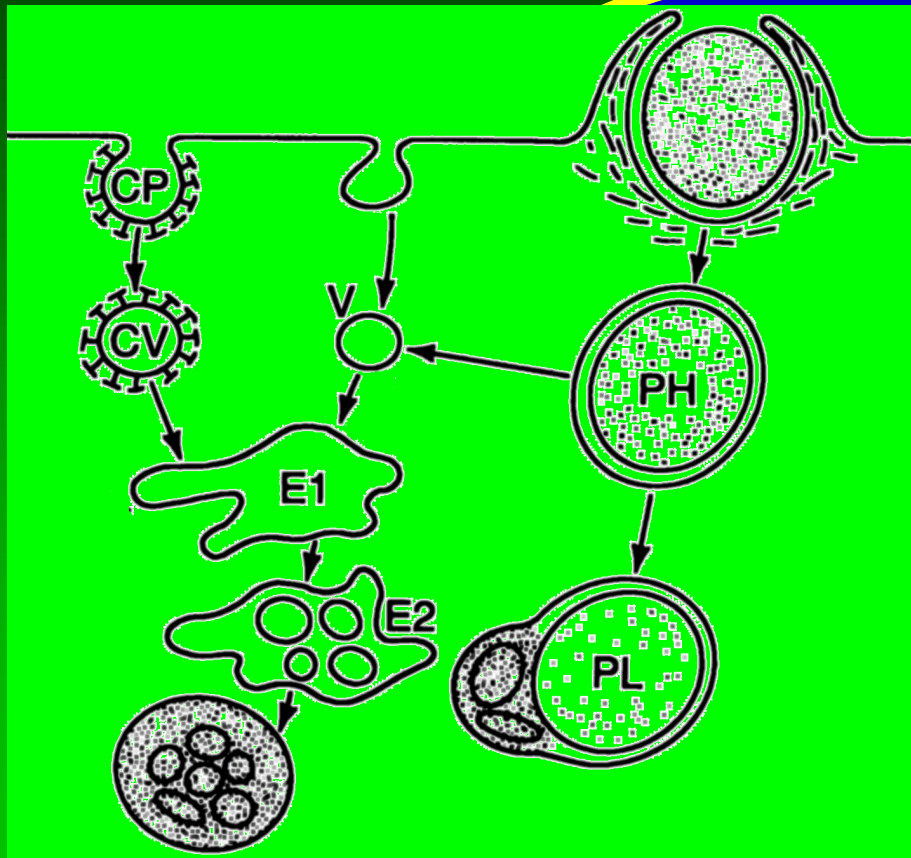
carrier-mediated influx



- specific structure
- (energy-dependent)
- saturable
- competition by analogues

How do antibiotics penetrate in cells ?

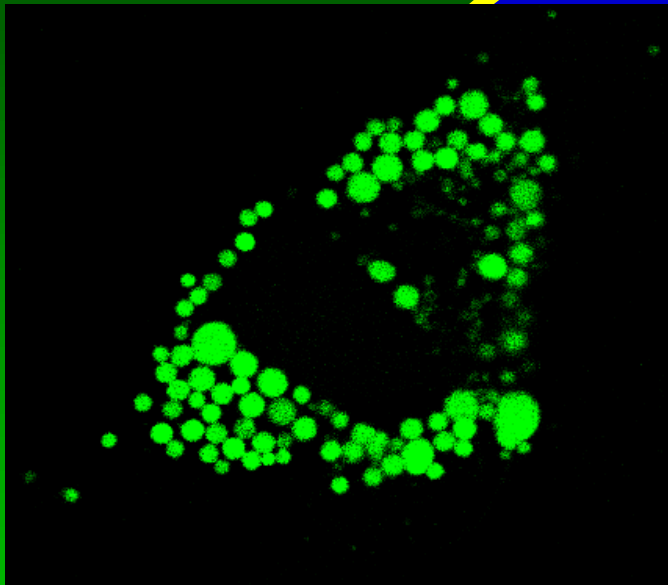
endocytosis



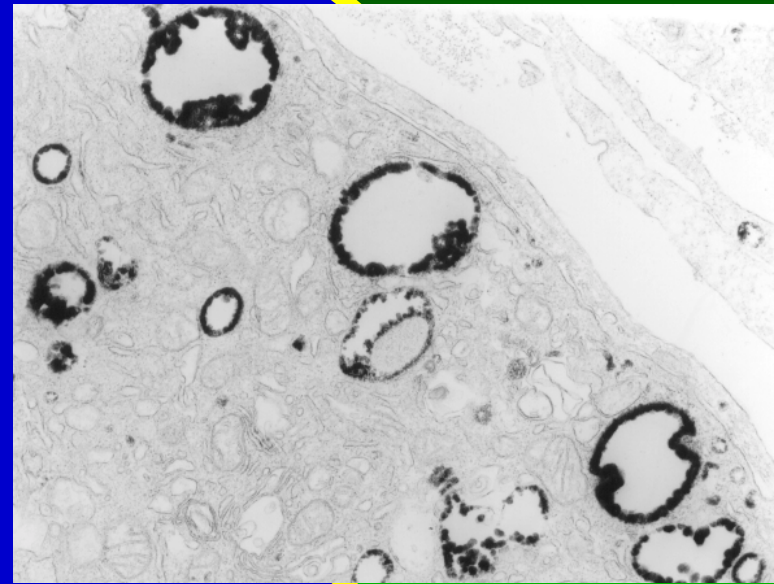
- non-permeant drugs
- slow unless membrane-bound, or receptor-mediated
- confined to vacuolar system

How do antibiotics penetrate in cells ?

endocytosis in fibroblats ...



Uptake of LY

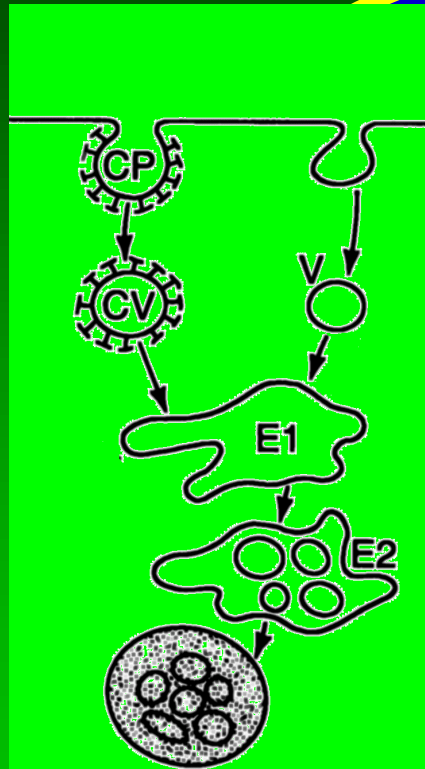


Uptake of HRP

Tyteca et al., EJCB, 2001, in press

How do antibiotics penetrate in cells ?

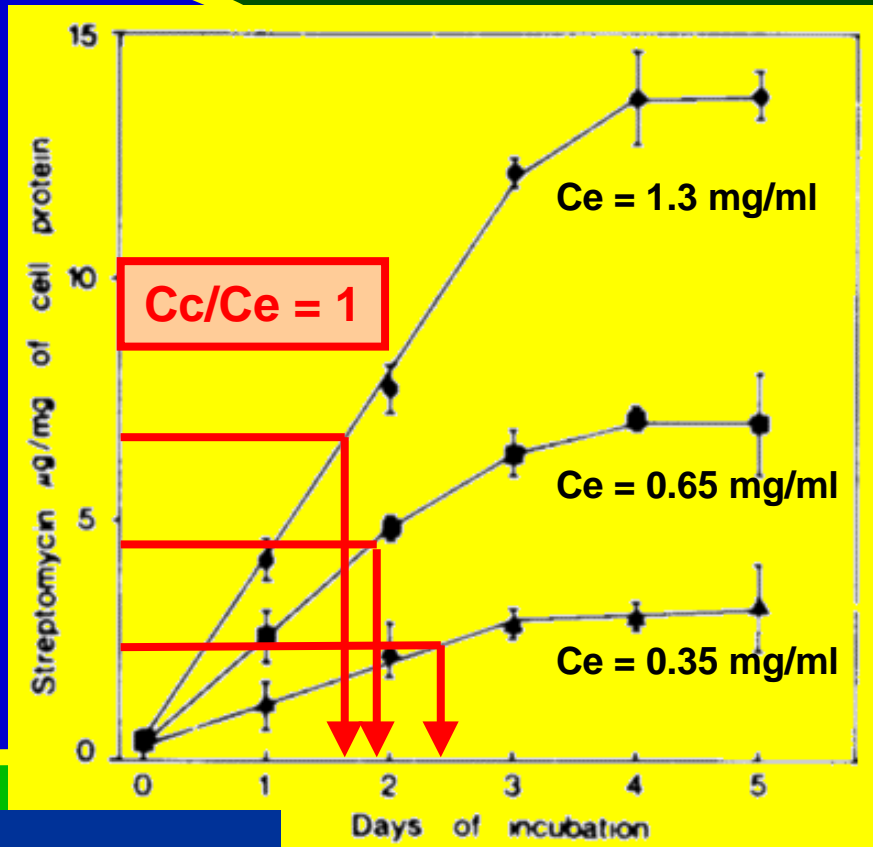
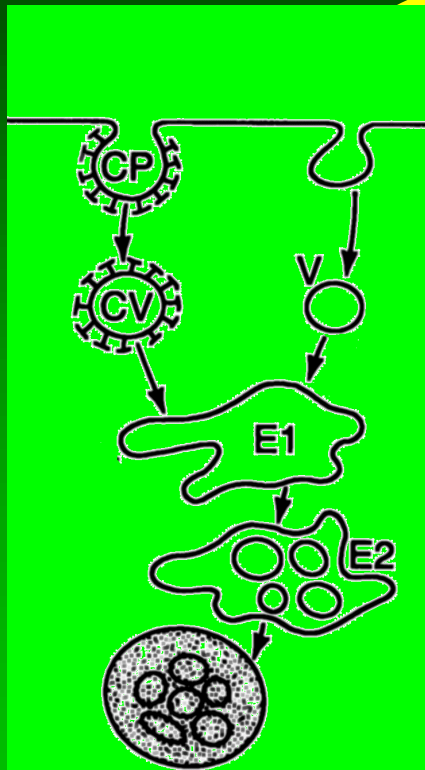
pinocytosis



- ▶ aminoglycosides
- ▶ glycopeptides (?)

How do antibiotics penetrate in cells ?

aminoglycosides in fibroblasts

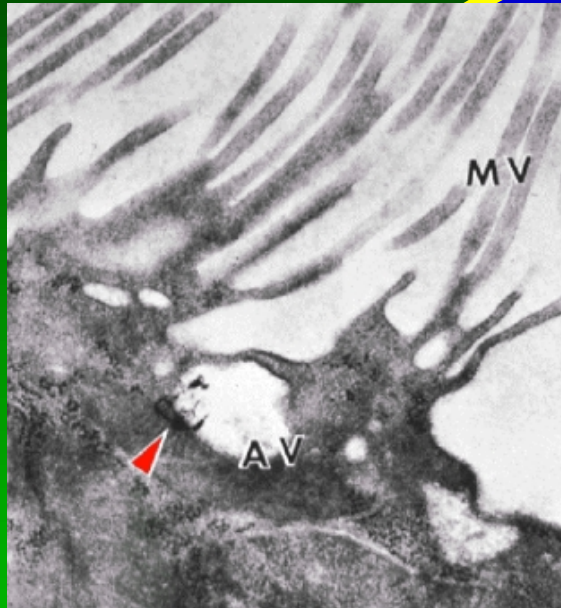


- ▶ Slow (days...)
- ▶ ill-effective (2-4 fold)

Tulkens & Trouet, 1978

How do antibiotics penetrate in cells ?

receptor-mediated pinocytosis in kidney cortex



binding to

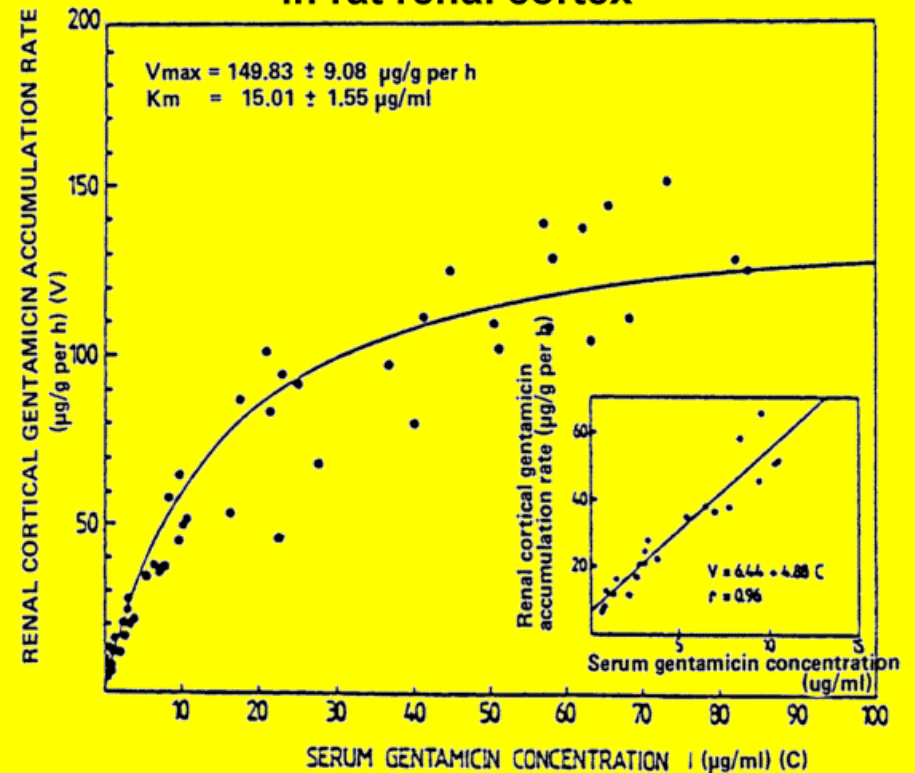
- megalin

(Moeströp et al., 1995)

- acidic phospholipids

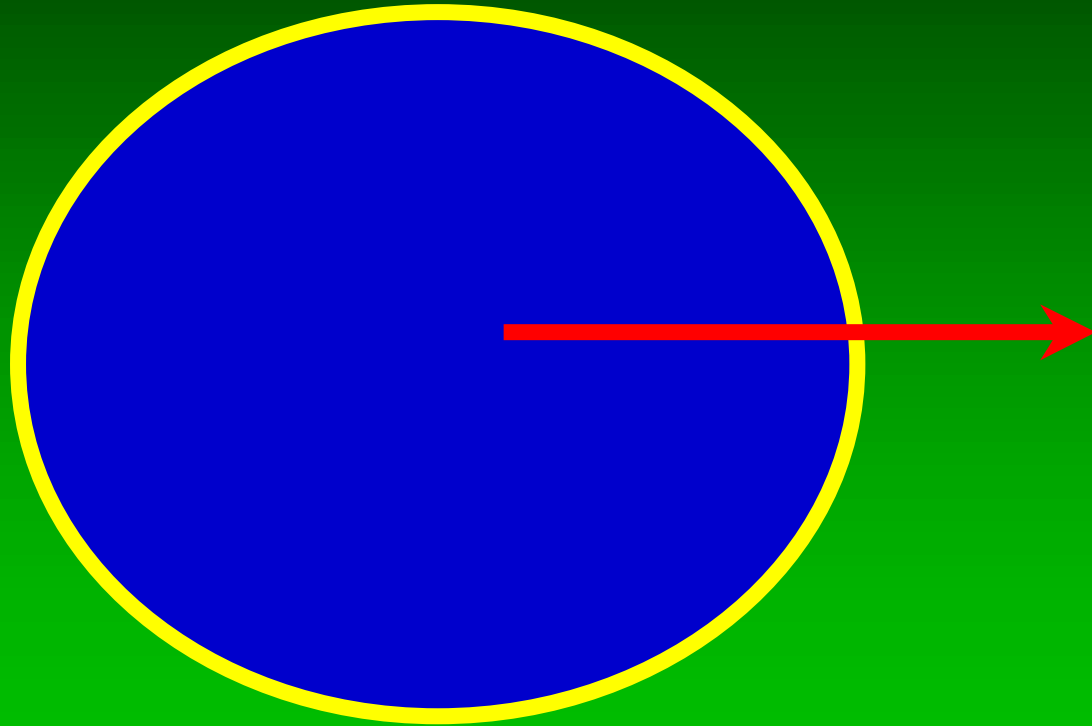
(Humes et al, 1983)

In vivo accumulation of gentamicin in rat renal cortex



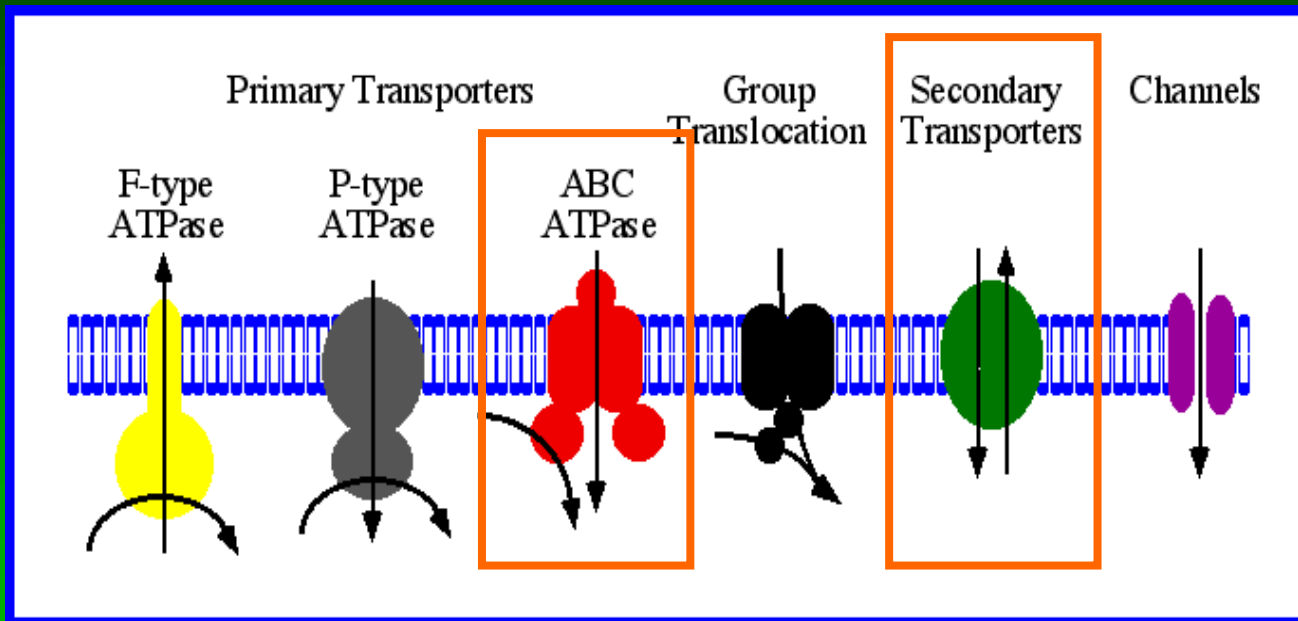
Giuliano *et al.*, J. Pharm. Exp. Ther., 1986

Antibiotics efflux ?



Transporters - data bases

<http://www-biology.ucsd.edu/~msaier/transport/titlepage.html>




**main
 drug
 transporters**

- Classification page** → combination of phylogenetic and functional information
- Transport analysis page** → comparison of transporters in complete genomes
- Phylogenetic page** → phylogenetic trees of transporters families

Saier, 2000

Transporters involved in the efflux of antibiotics from eucaryotic cells

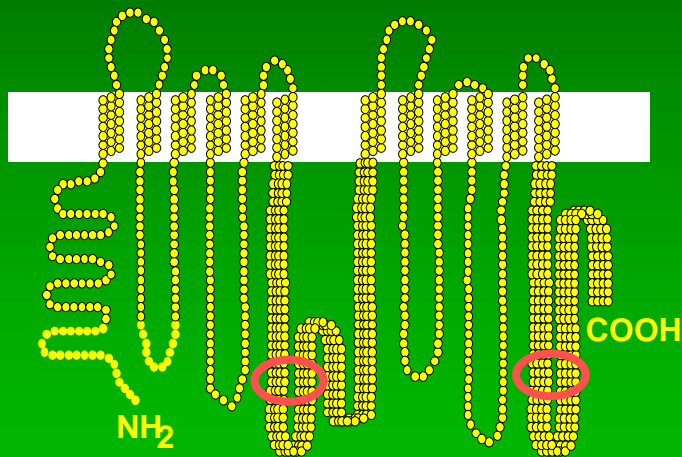


superfamily	transporter	physiol. substrates	antibiotics
ABC	MDR1	phospholipids	fluoroquinolones macrolides β-lactams tetracyclines streptogramins
	MRP1	phospholipids leukotrienes conjugates	fluroquinolones macrolides rifamycins
	MRP2	conjugates	fluoroquinolones β-lactams
MFS	NPT1	phosphates	β-lactams
OAT	OATP1	bile salts steroids	β-lactams

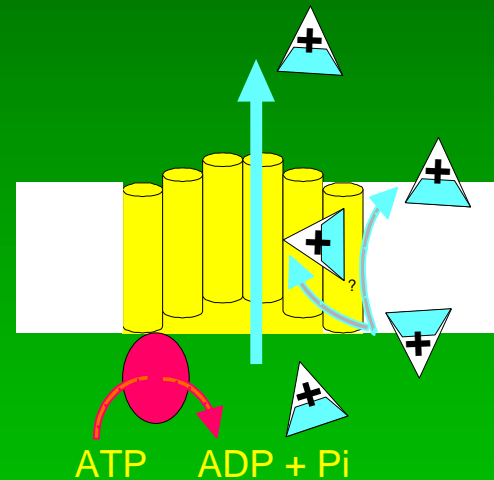
Most frequent antibiotic-pumps in eucaryotes (1/2)

Multiple Drug Resistance (MDR also known as PgP)

TOPOLOGY



MECHANISM



ANTIBIOTICS

-  tetracyclines
-  fluoroquinolones
-  erythromycin
-  lincosamides
-  rifampicin

-  chloramphenicol

-  aminoglycosides

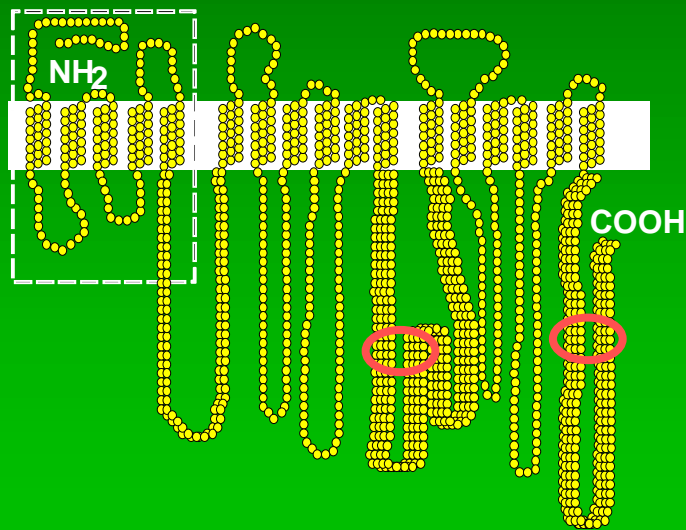
Van Bambeke et al., Biochem. Pharmacol. 2000

Most frequent antibiotic-pumps in eucaryotes (2/2)

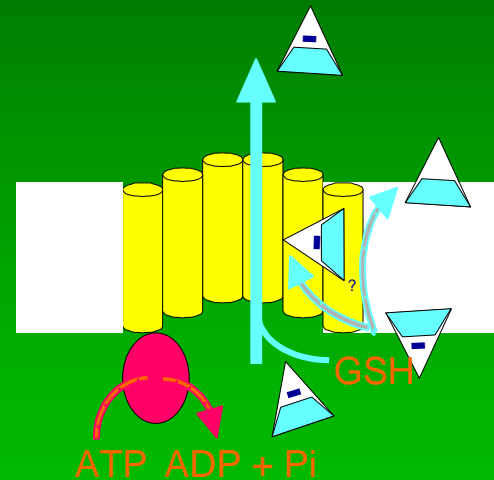


Multidrug Resistance Proteins (MRP)



TOPOLOGY



MECHANISM



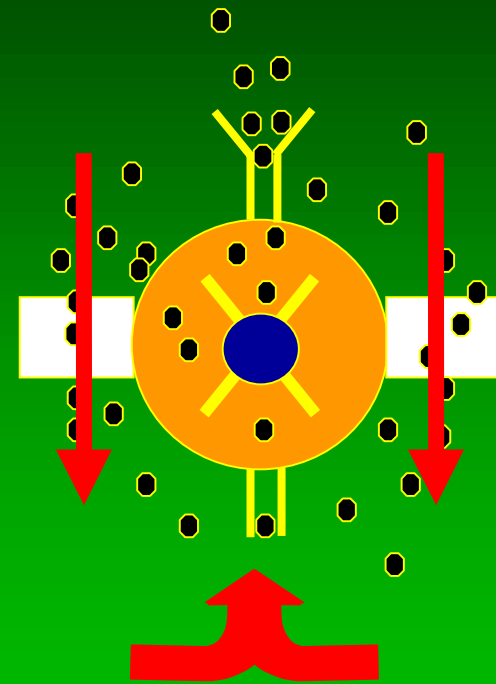
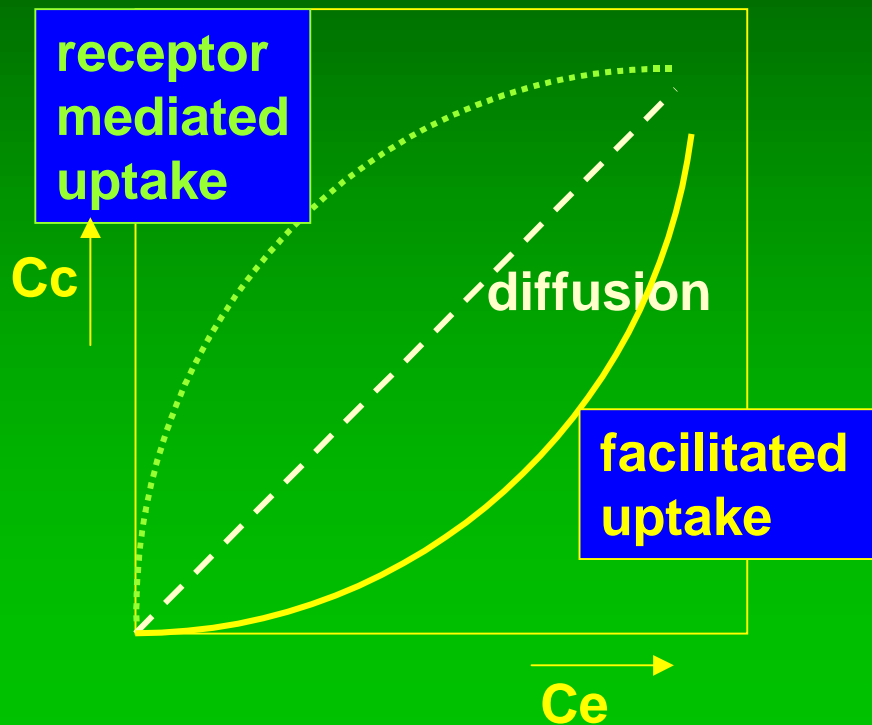
ANTIBIOTICS

-  fluoroquinolones
-  tetracyclines
macrolides

Van Bambeke et al., Biochem. Pharmacol. 2000

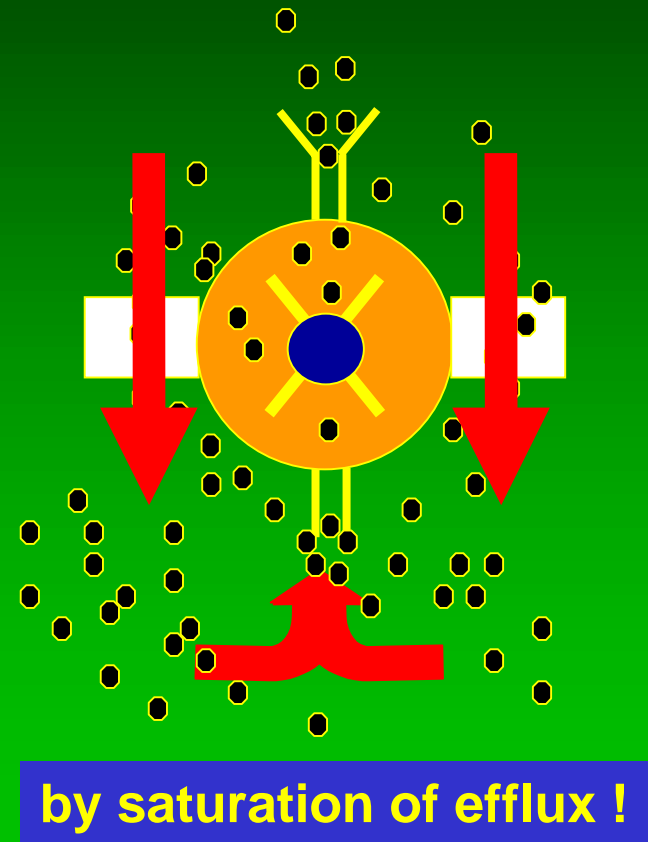
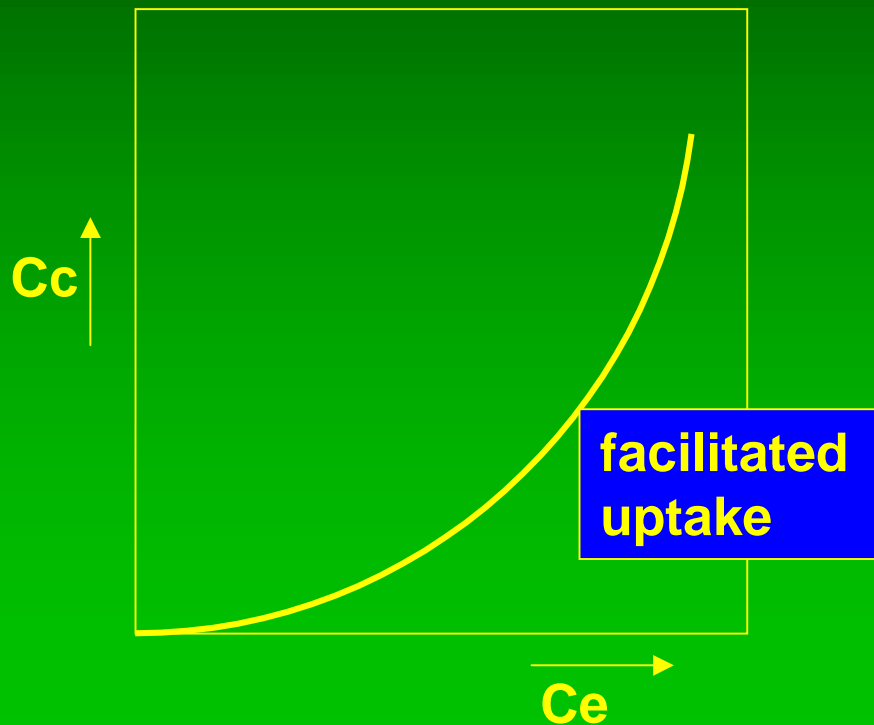
Evidencing active efflux ...

non linear accumulation kinetics ...



Evidencing active efflux ...

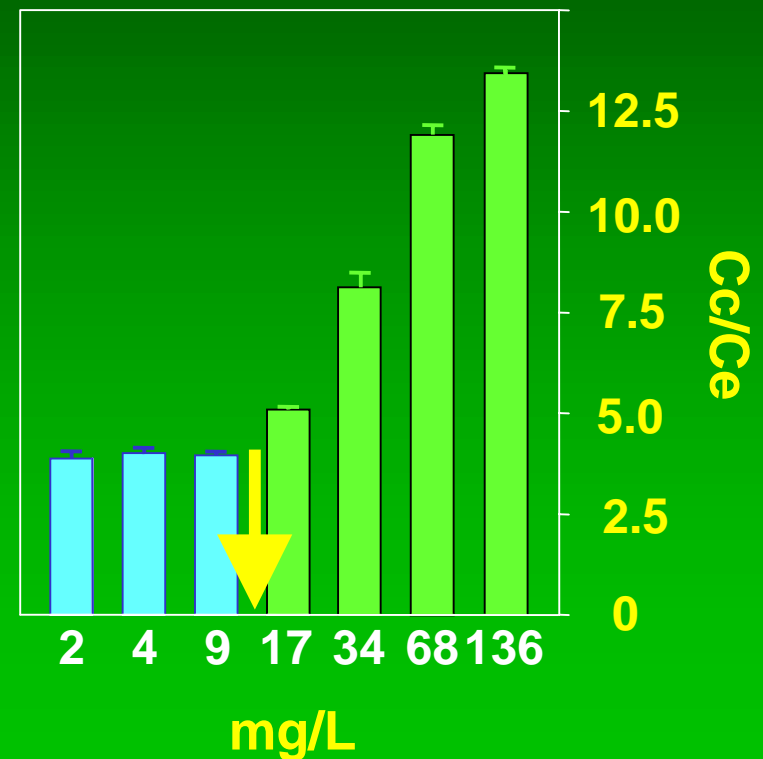
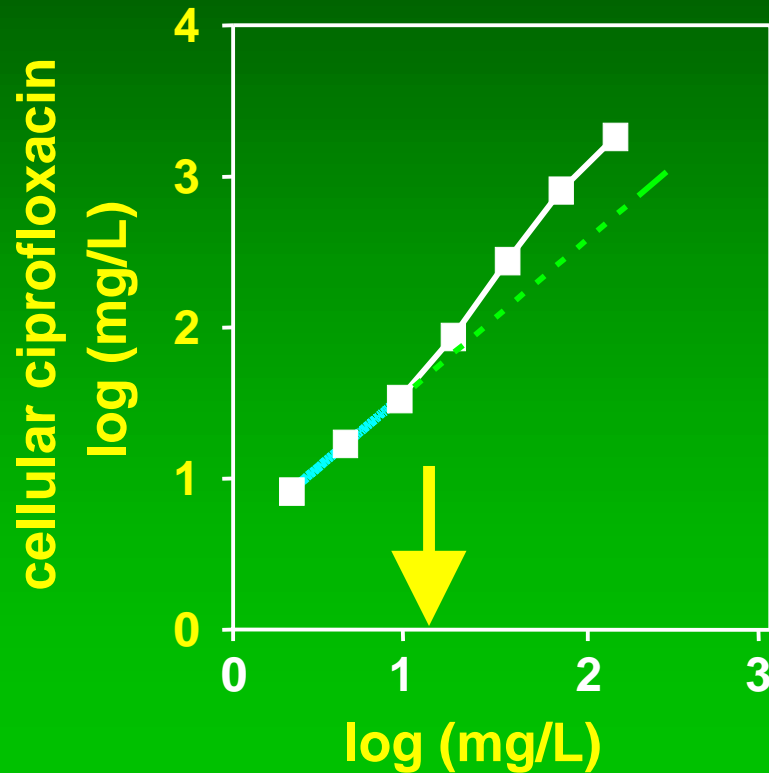
non linear accumulation kinetics ...



Active efflux of ciprofloxacin

Ciprofloxacin accumulation is facilitated upon increase of its extracellular concentration

Michot et al., ICAAC 2000



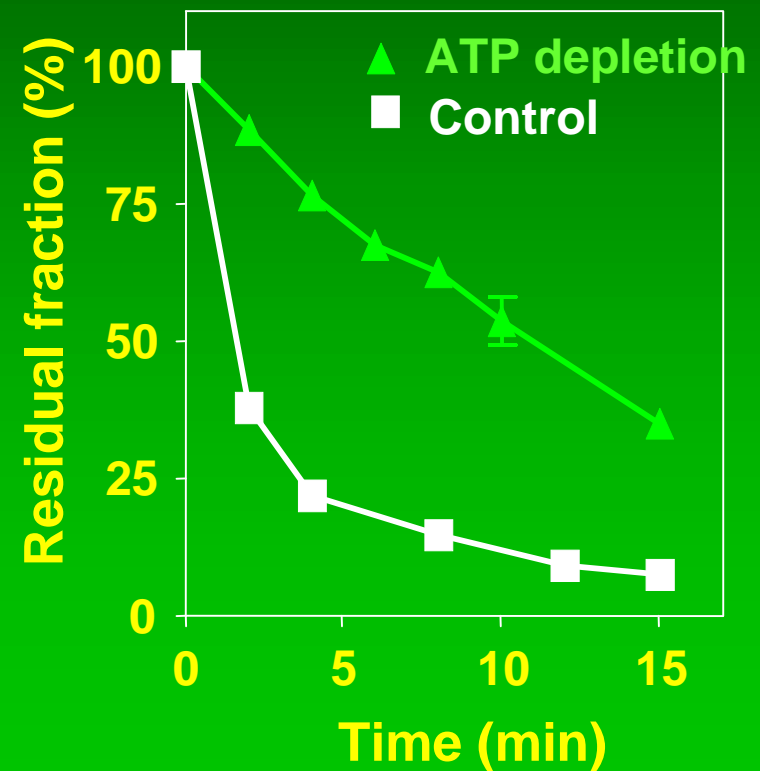
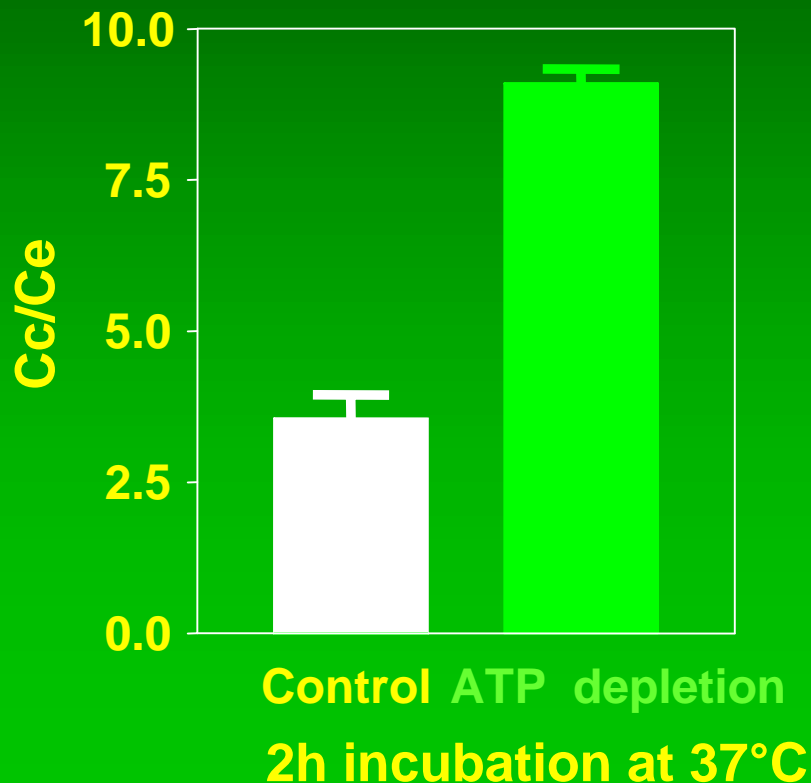
extracellular [ciprofloxacin] - 2h incubation at 37°C

Characterization of the transporter(s)

ATP depletion increases ciprofloxacin accumulation and decreases ciprofloxacin efflux

Michot et al., ICAAC 2000

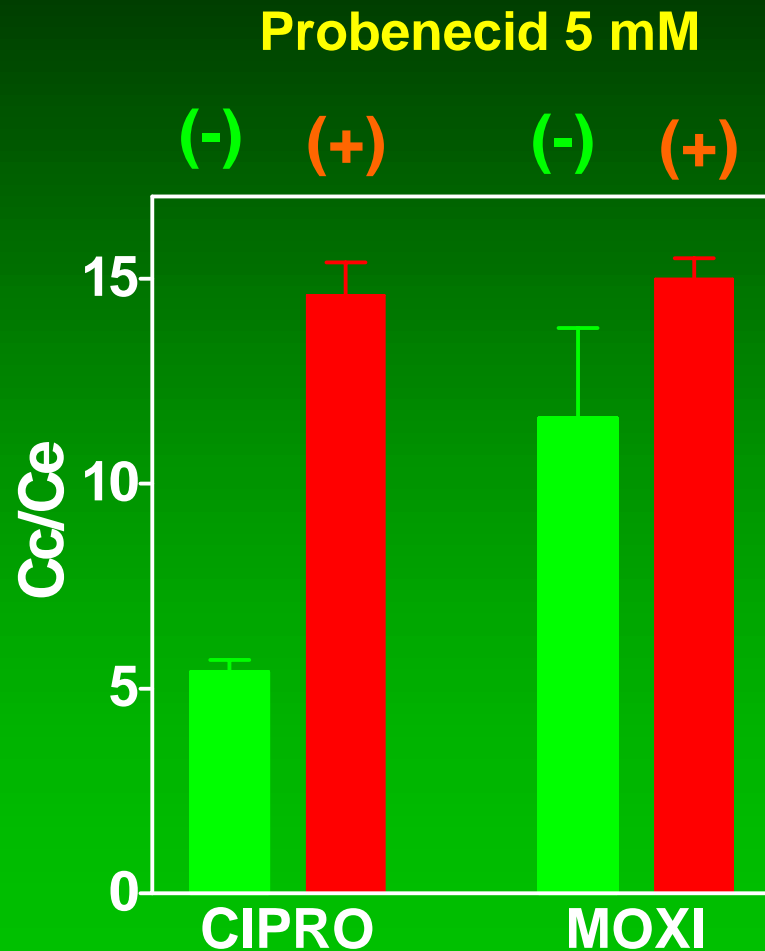
$C_e = 50 \mu\text{M}$ (17 $\mu\text{g/ml}$)



Not all fluoroquinolones are subject to active efflux ...

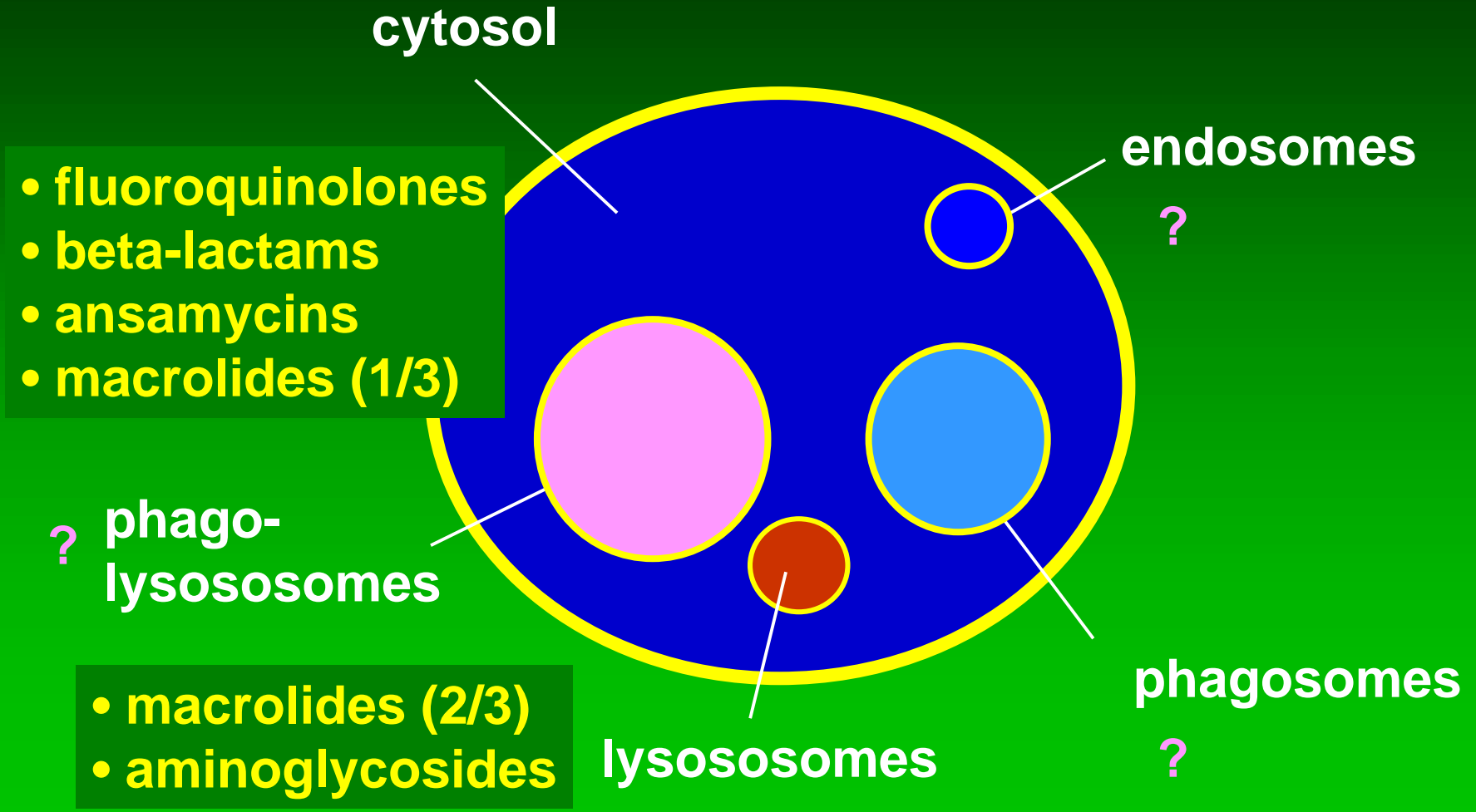
Influence of probenecid * on ciprofloxacin and moxifloxacin accumulation in J774 macrophages

* inhibitor of FQ efflux
Cao et al., 1992



Michot et al., ICAAC 2000

Subcellular localization ?



Mechanisms of localisation and accumulation ...

cytosol

- **fluoroquinolones**

**Mechanism unknown
(loose binding to
lipid-containing proteins ? ...)**

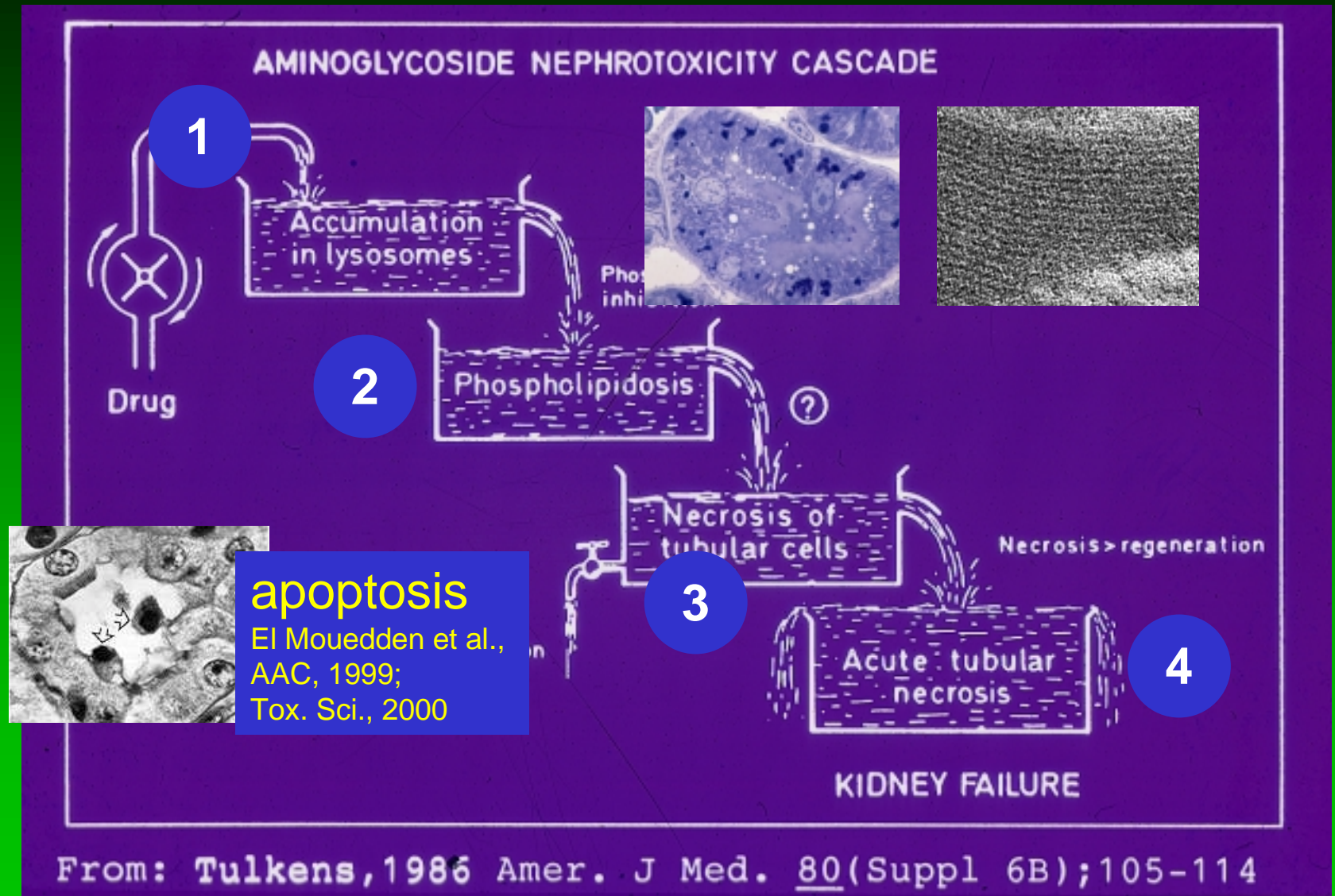
Mechanisms of localisation and accumulation ...

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

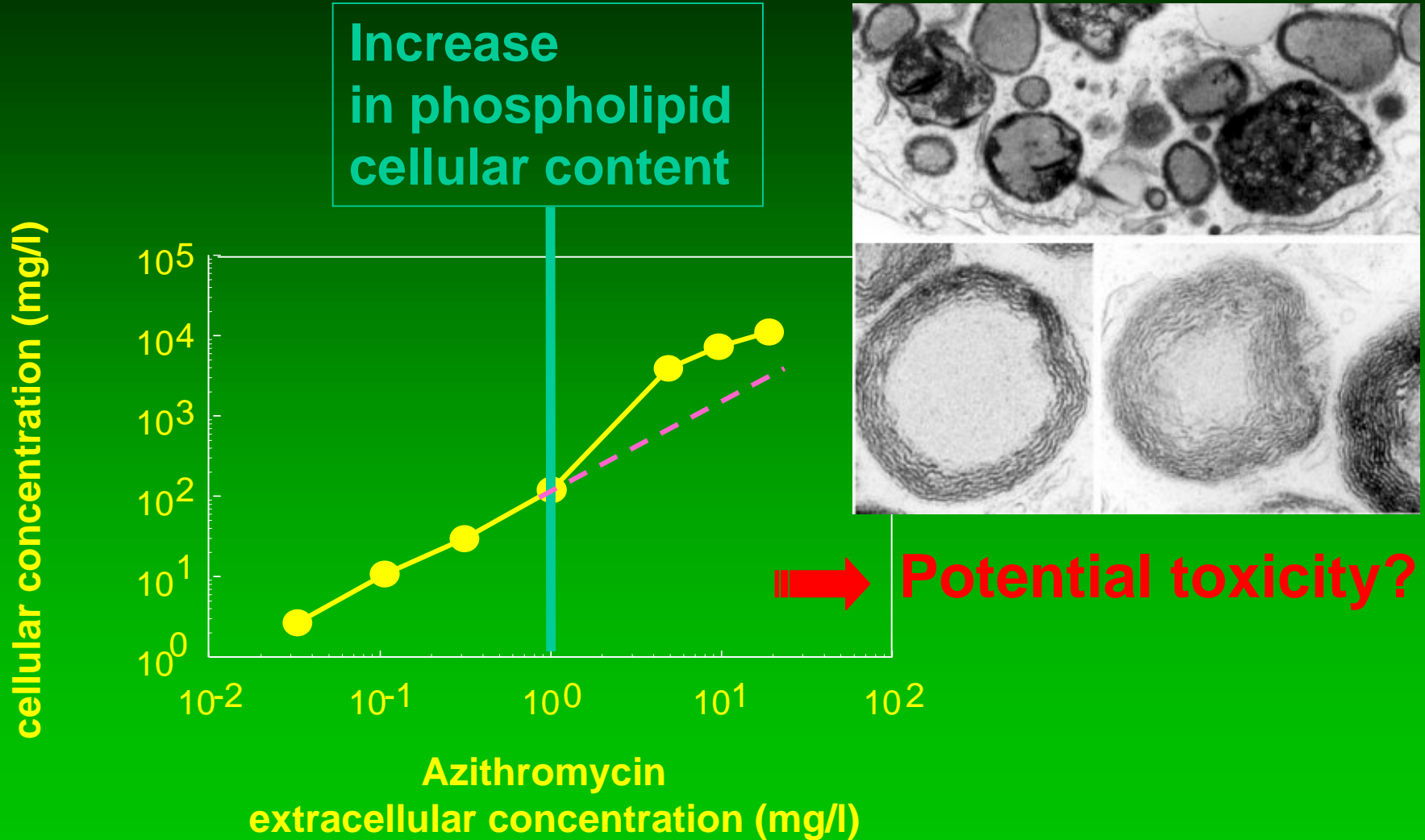
- macrolides
- aminoglycosides

lysosomes

Mechanisms of localisation and accumulation ...



Mechanisms of localisation and accumulation ...



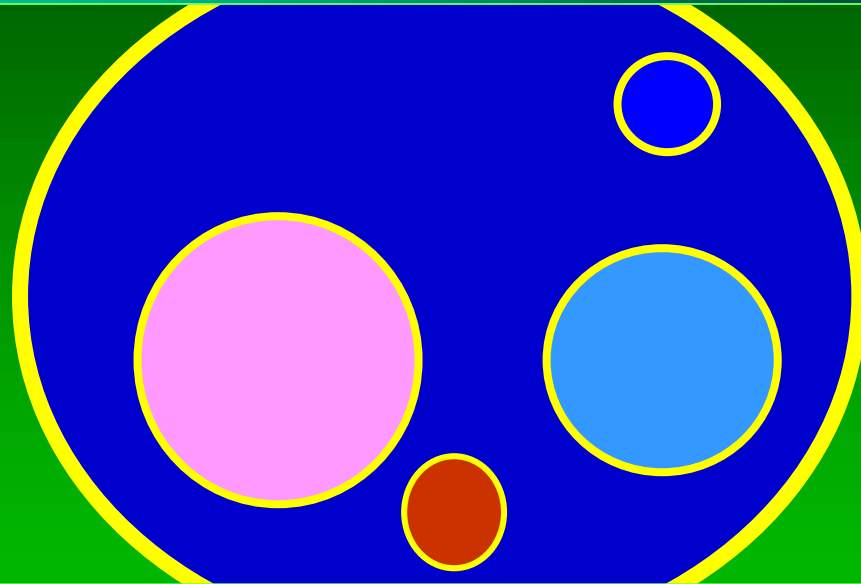
Van Bambeke et al., JAC, 1998

Subcellular bioavailability of antibiotics ?

High

Fair

Nil

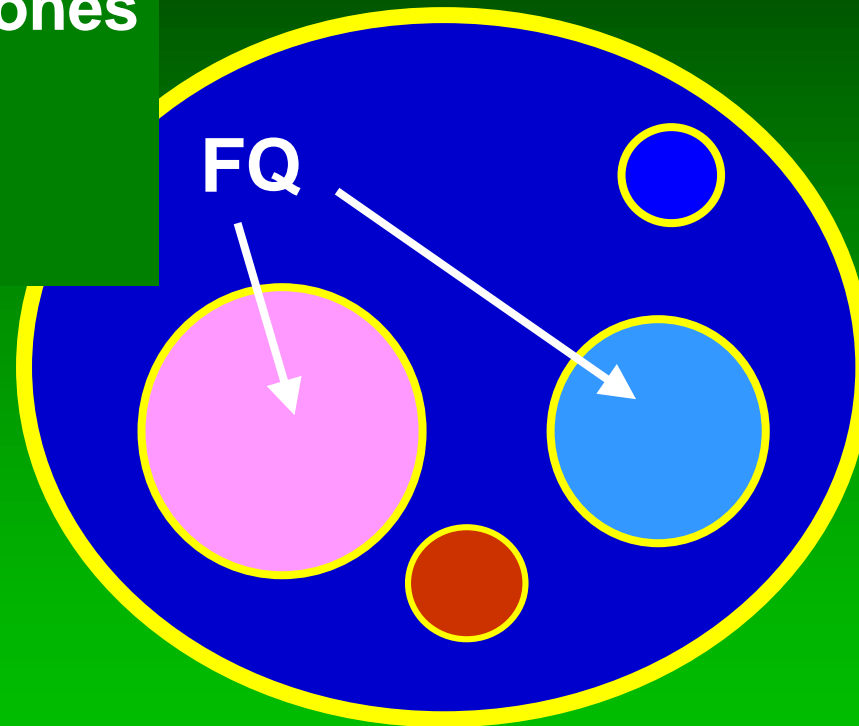


FQ / Ansamyc. / cytosol. ML

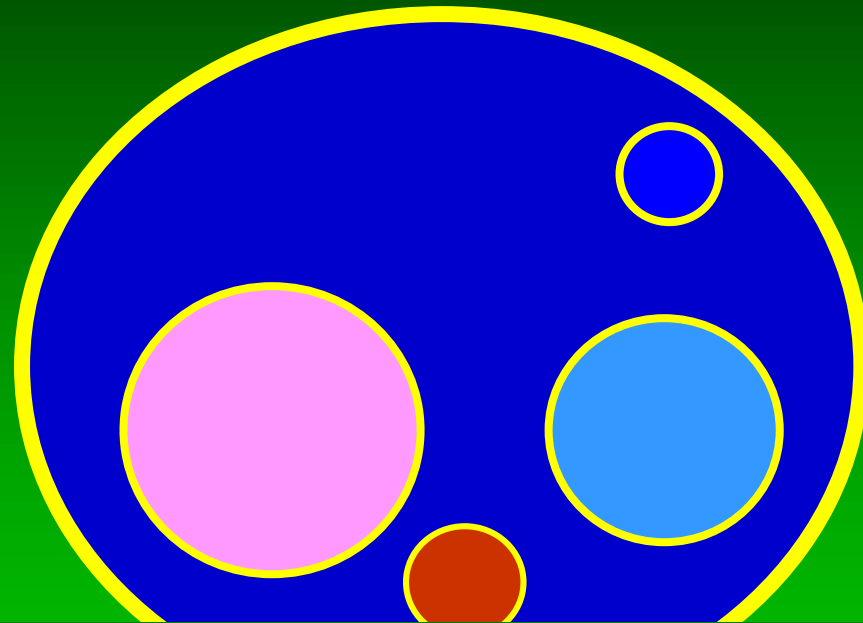
lysosom. ML / AG

Subcellular bioavailability of antibiotics ?

**Fluoroquinolones
move easily
across
membranes**



Subcellular bioavailability of antibiotics ?



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

Illustration: the Listeria story

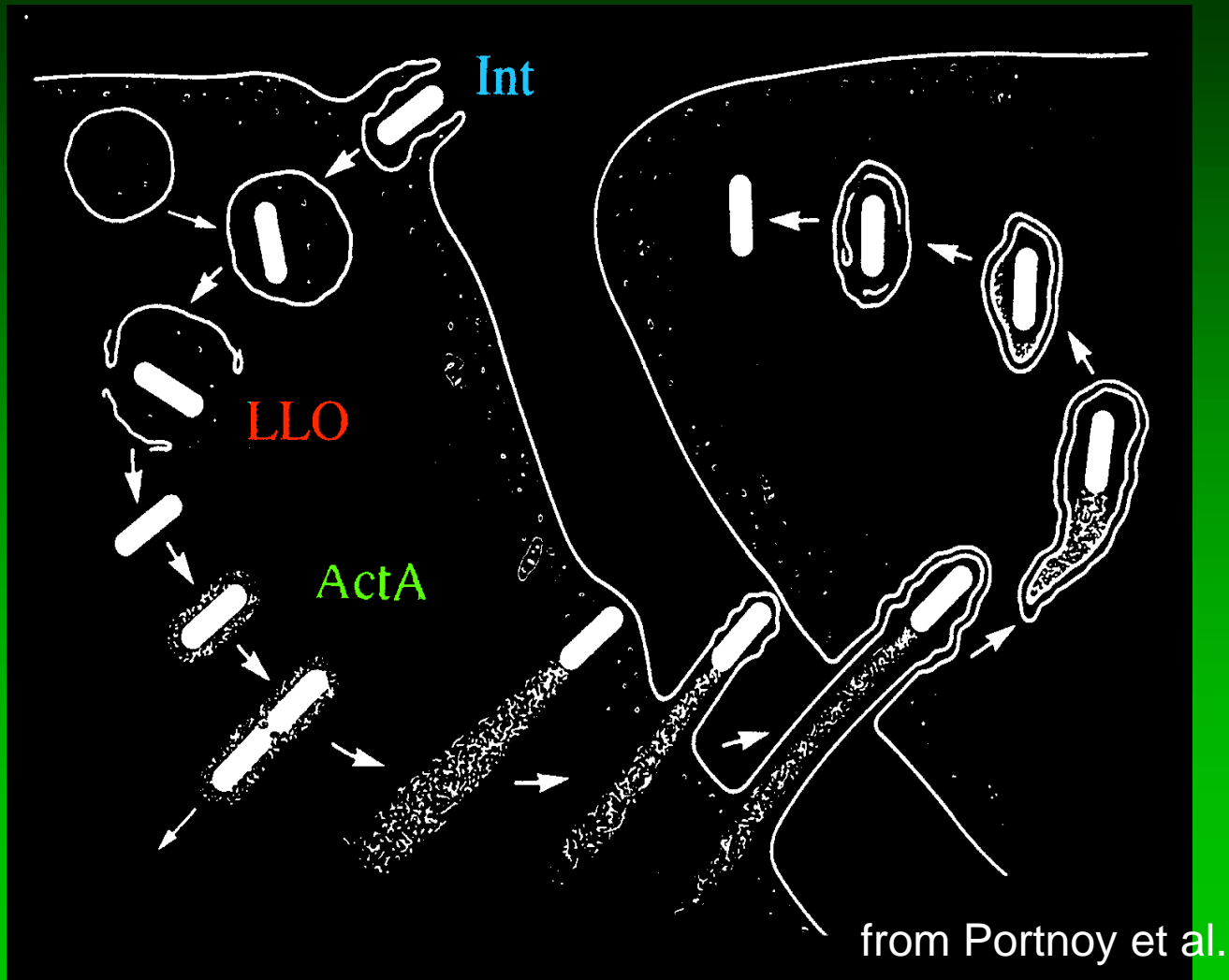
antibiotics:

- ampicillin
- azithromycin
- sparfloxacin

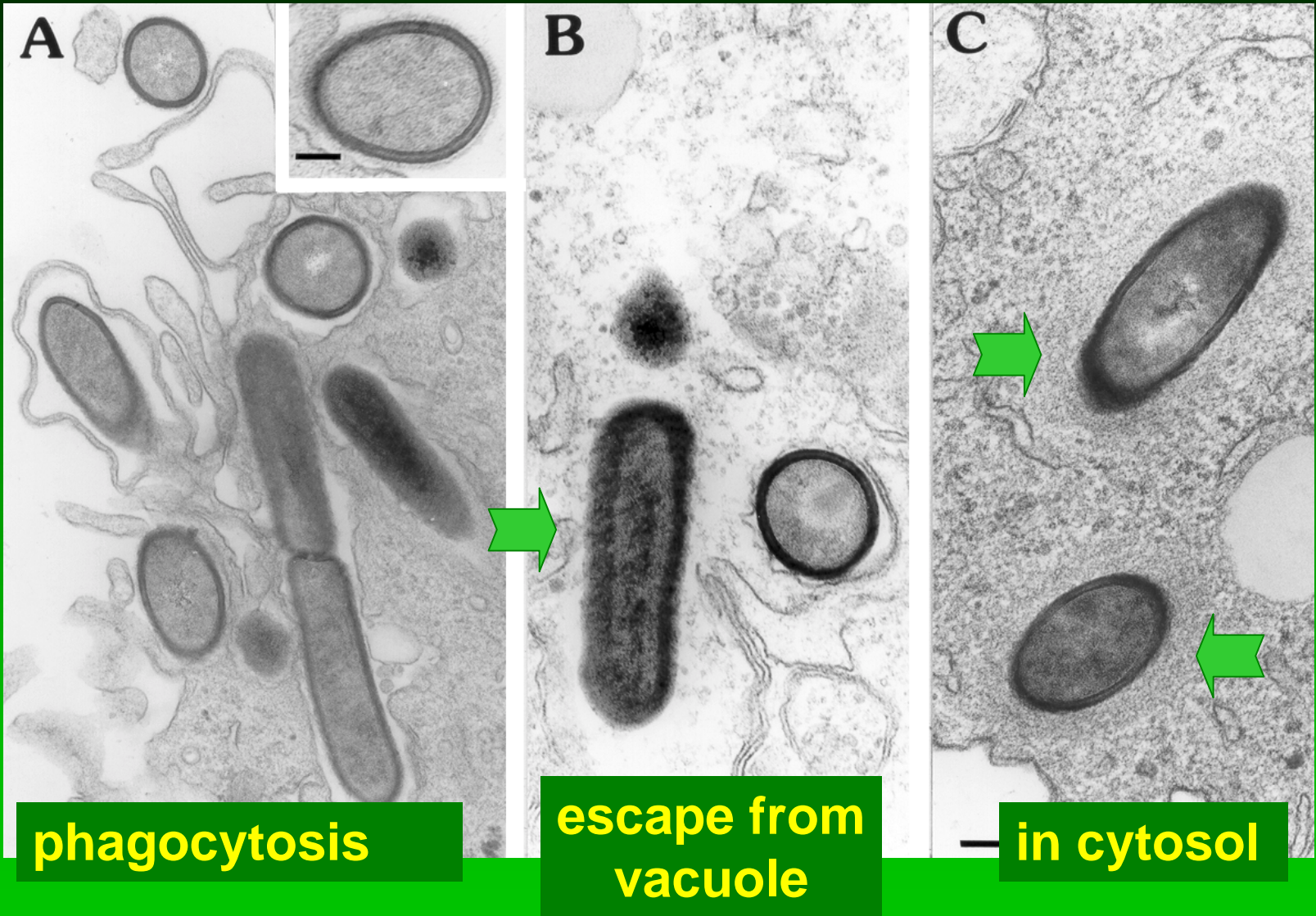
Listeria monocytogenes
hly+



Intracellular infection cycle of *Listeria monocytogenes* hly⁺

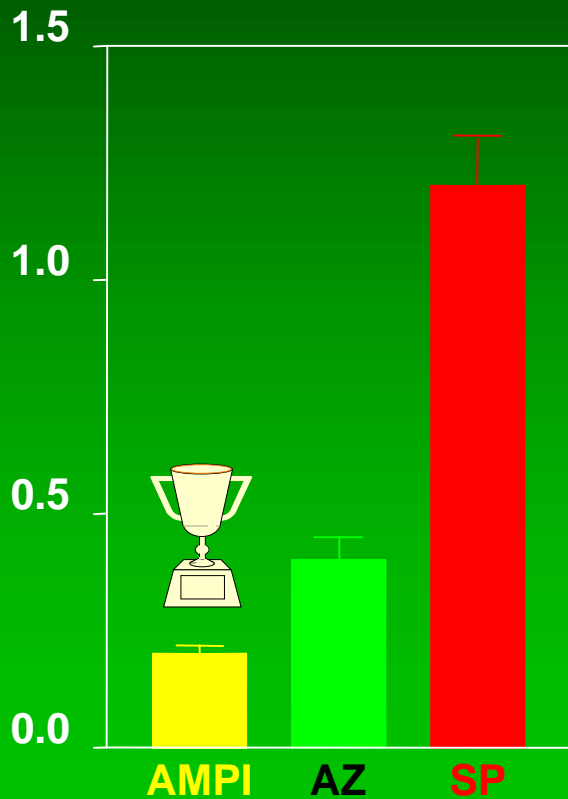


Following the intracellular fate of *Listeria m.* by EM

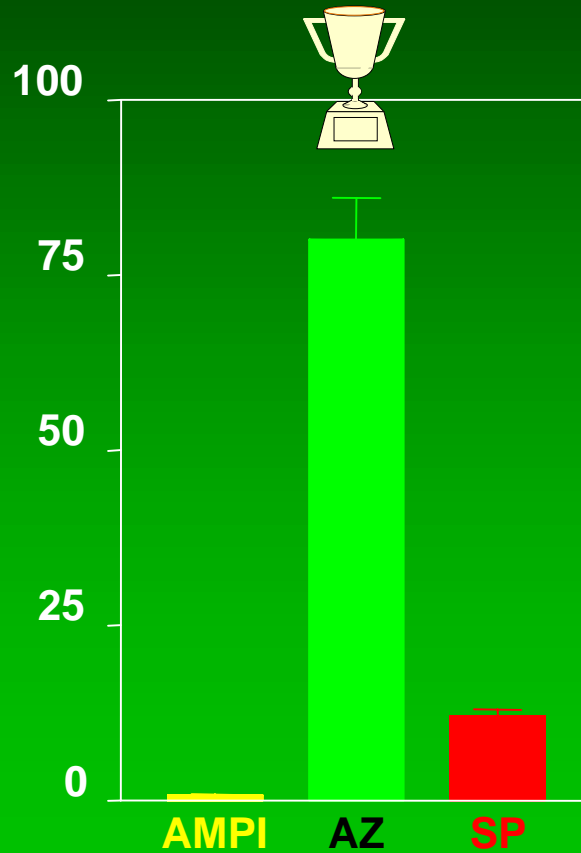


MIC, accumulation and activity against cytosolic *Listeria m.* ...

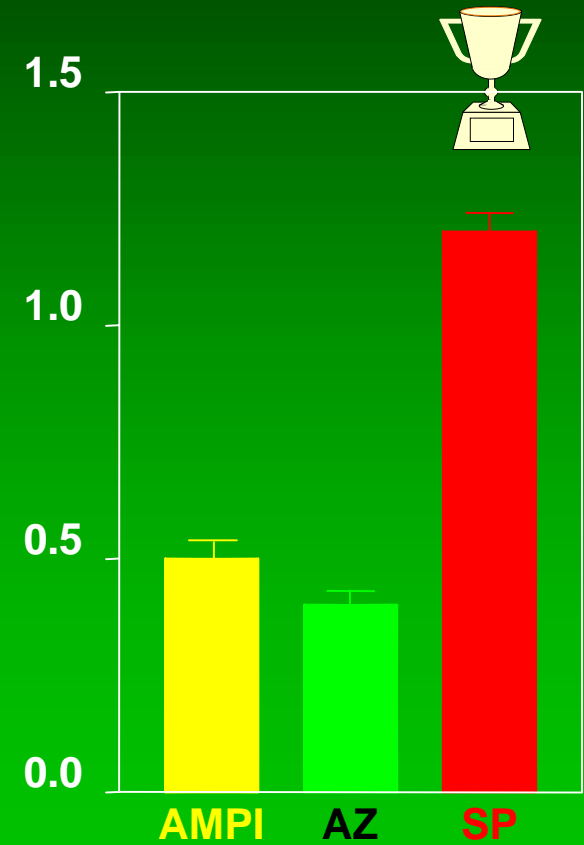
MIC



Accumulation



Activity *



Quadhriri et al., AAC, 1999

* $\Delta \log \text{CFU } 5\text{h}$
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...)

Listeria m. and azithromycin

Azithromycin is also 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 this pharmacological model *, sparfloxacin IS the most active in spite of a unfavourable MIC (1.4 µg/ml) and modest cellular accumulation (12 x)

Fluoroquinolones

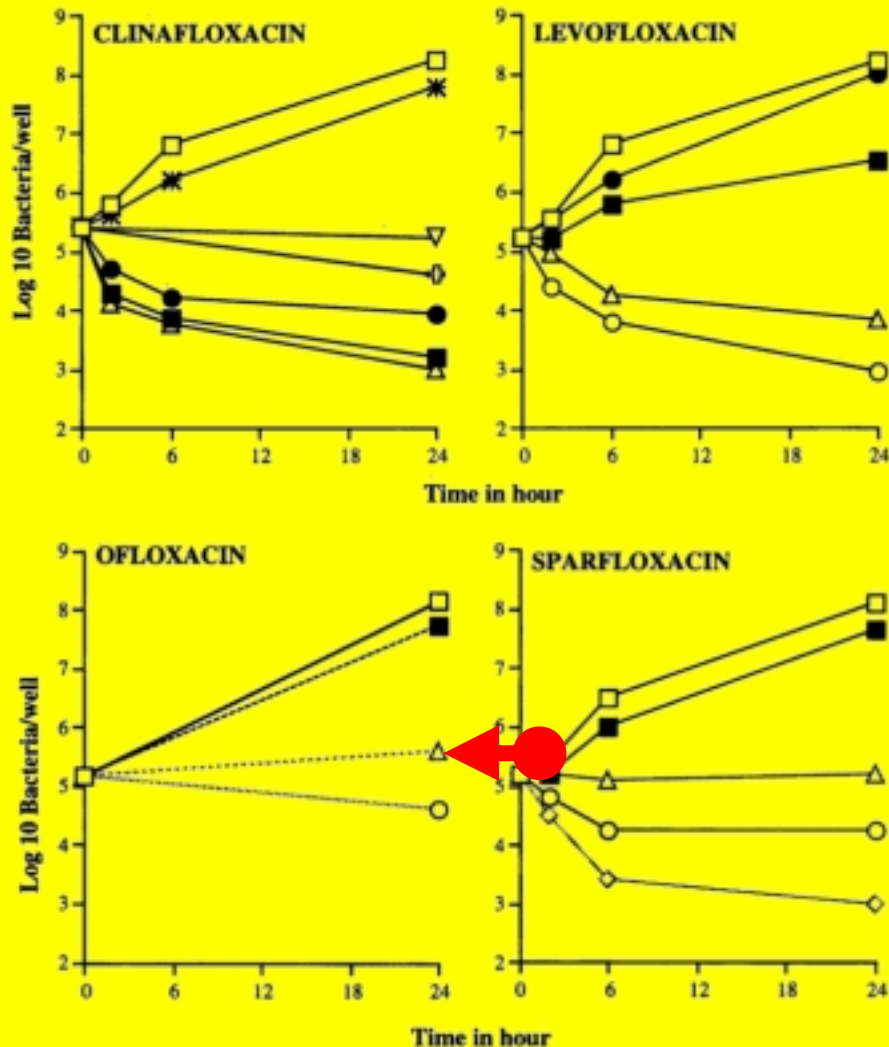
- ➔ have a large subcellular bioavailability
- ➔ are highly bactericidal

Why don't you use fluoroquinolones today ?

➔ too low intrinsic activity ** ...

* all $C_e = 10 \times$ the MIC

Activities of fluoroquinolones against intracellular *Listeria m.* (Michelet et al., AAC, 1997)

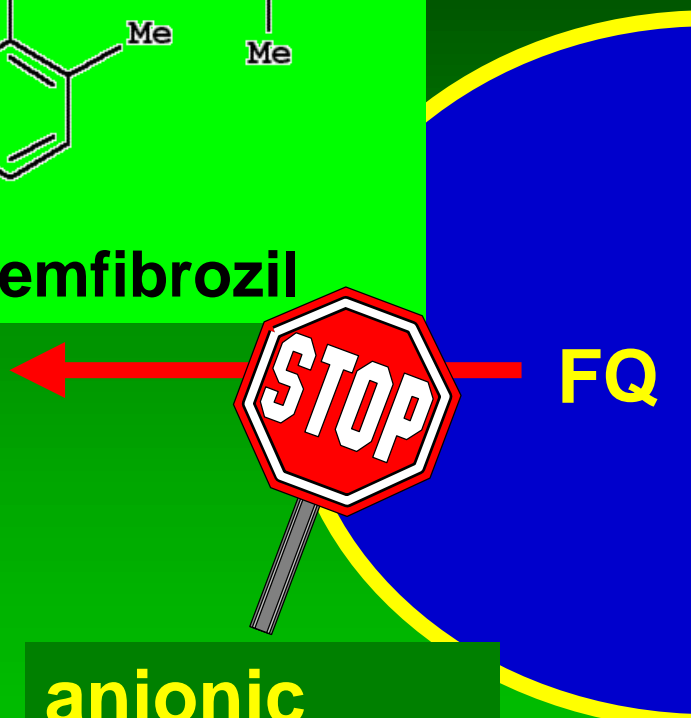
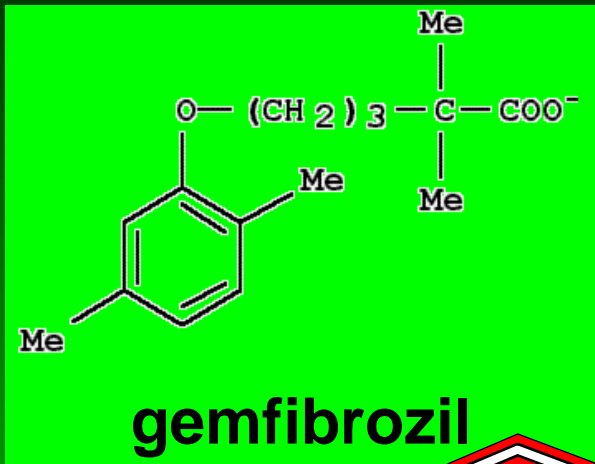


- Activity is concentration-dependent

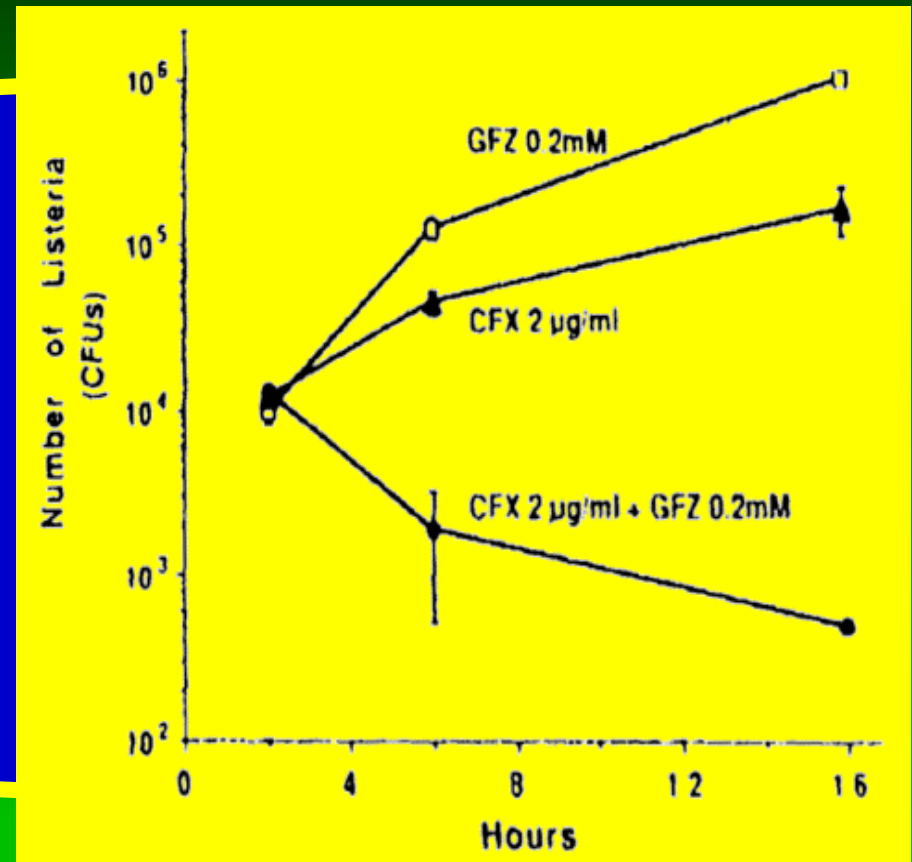
BUT...

- Activity at $C_e \approx C_{max}$ ($2\mu\text{g/ml}$) is probably insufficient (levofloxacin ?)

Improving the activity of fluoroquinolones by inhibiting efflux

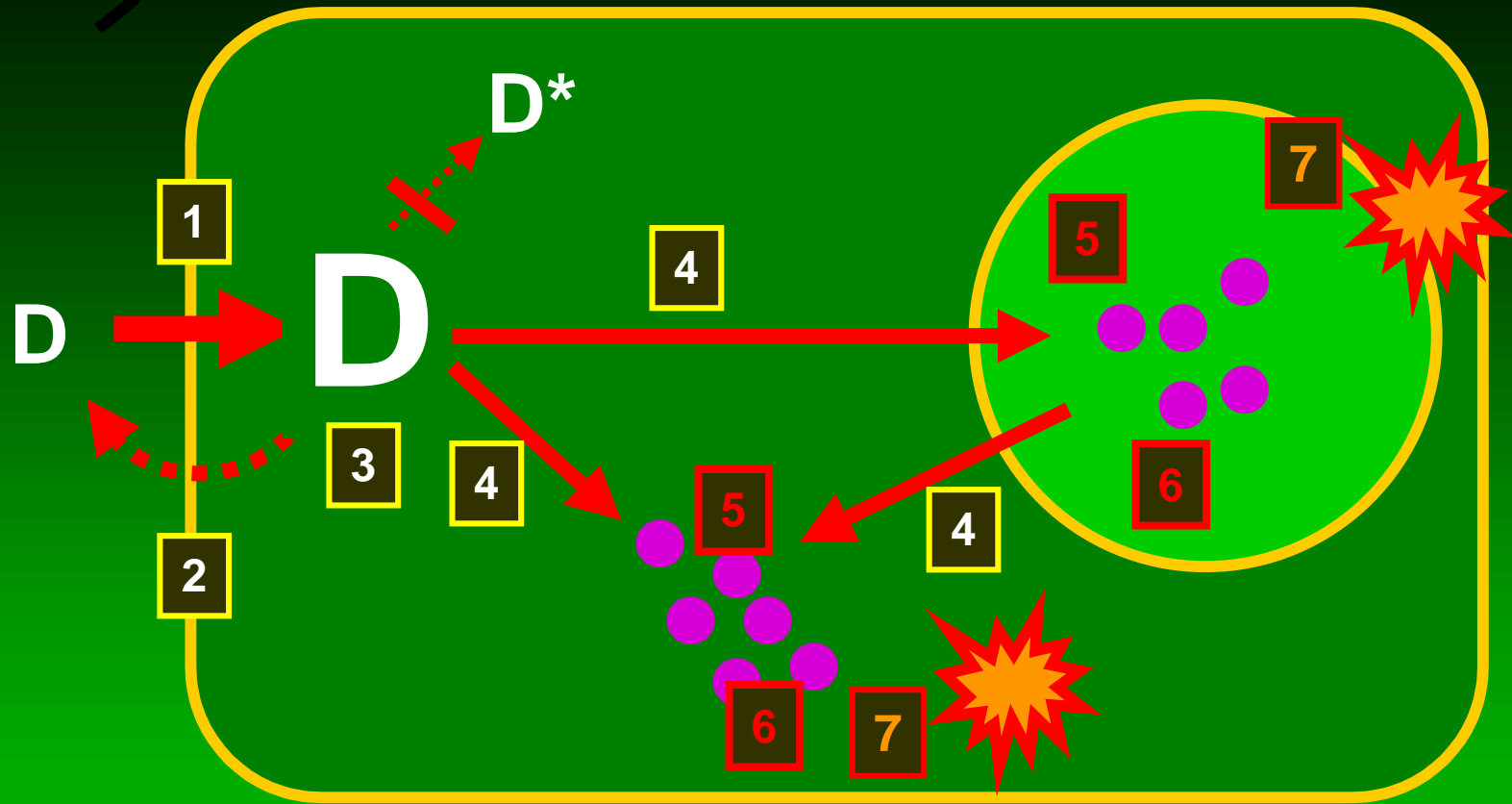


**anionic
amphiphiles**



Rudin et al, J. Exp. Med. 1992

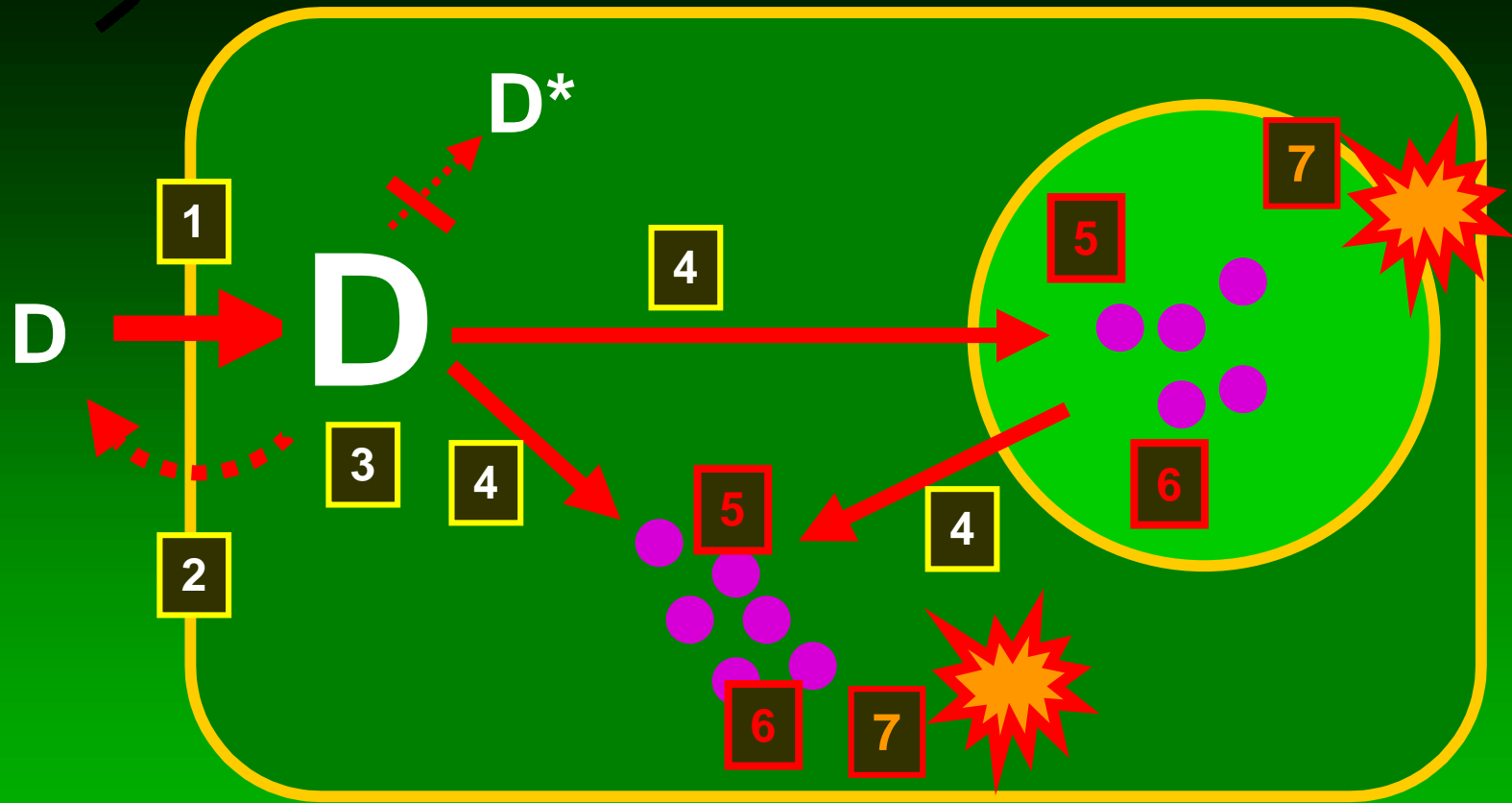
The seven pillars of intracellular activity ?



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

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

Co-workers on intracellular activity ...



M.P. Mingeot, D. Tyteca
J.M. Michot, C. Seral
M.B. Carlier, A. Zenebergh
Y.Chanteux, M. Bouvier d'Yvoire

C. Renard, H. Fan, E. Sonveaux
S. Carryn, F. Van Bambeke,
and ISAP...
B. Scorneaux, Y. Ouadrhiri,
I. Paternotte,