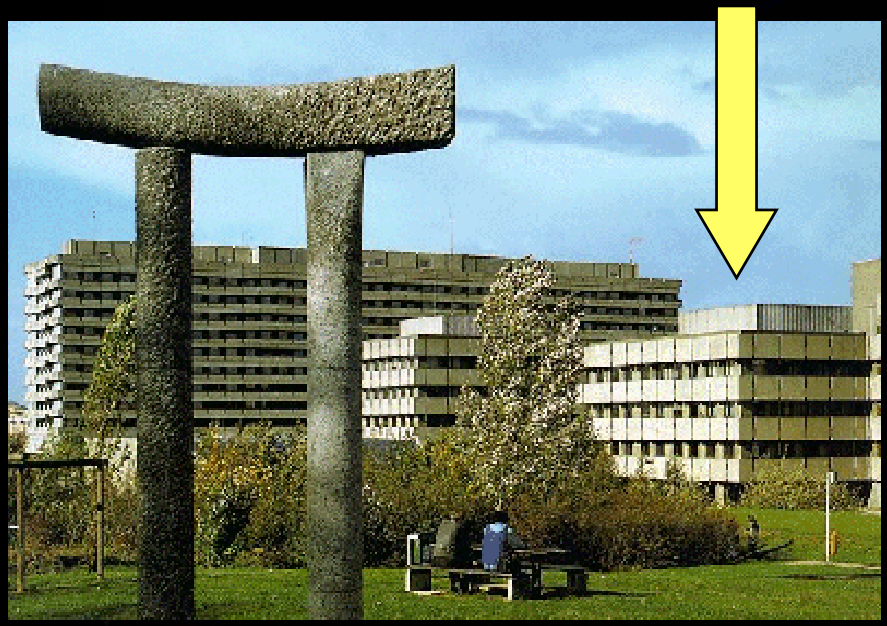
Antibiotic efflux pumps in eucaryotic cells: consequences for activity against intracellular bacteria

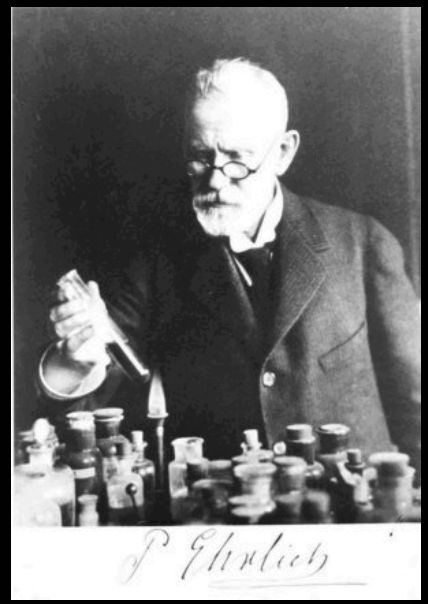
Unité de Pharmacologie
cellulaire et moléculaire

F. Van Bambeke

Magic bullets need to reach their target

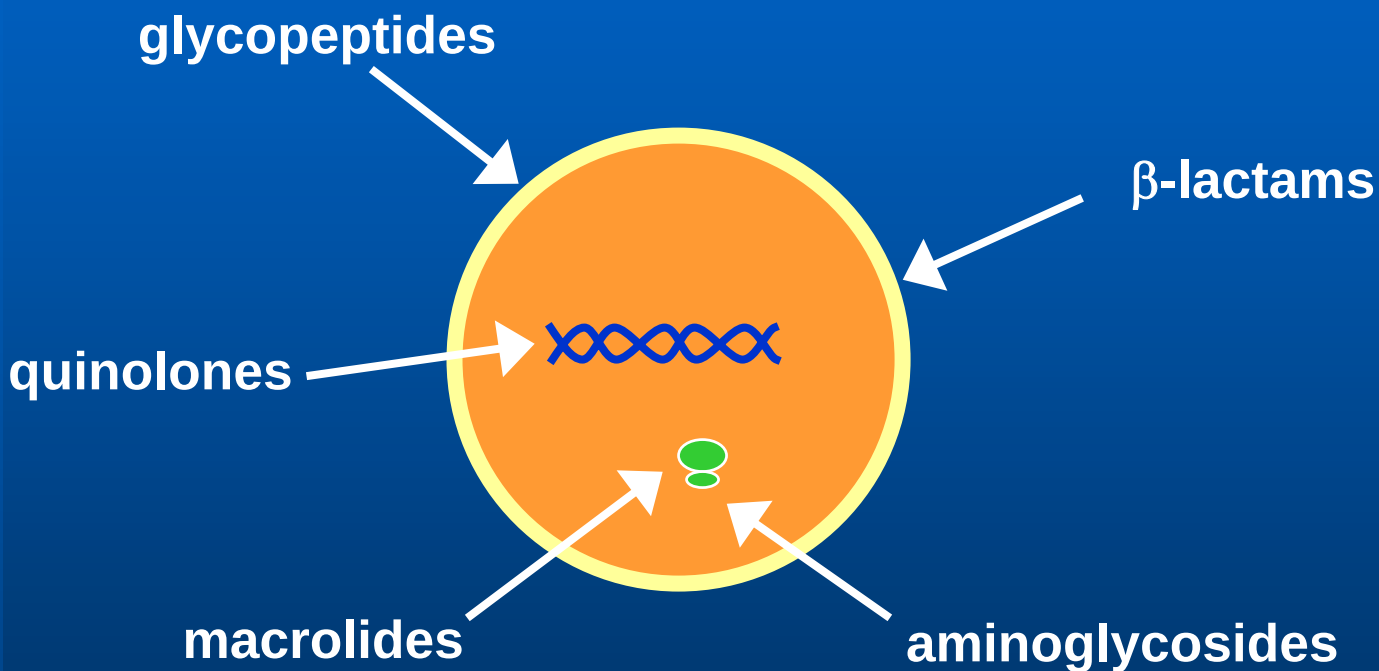
Paul Ehrlich (1854–1915)



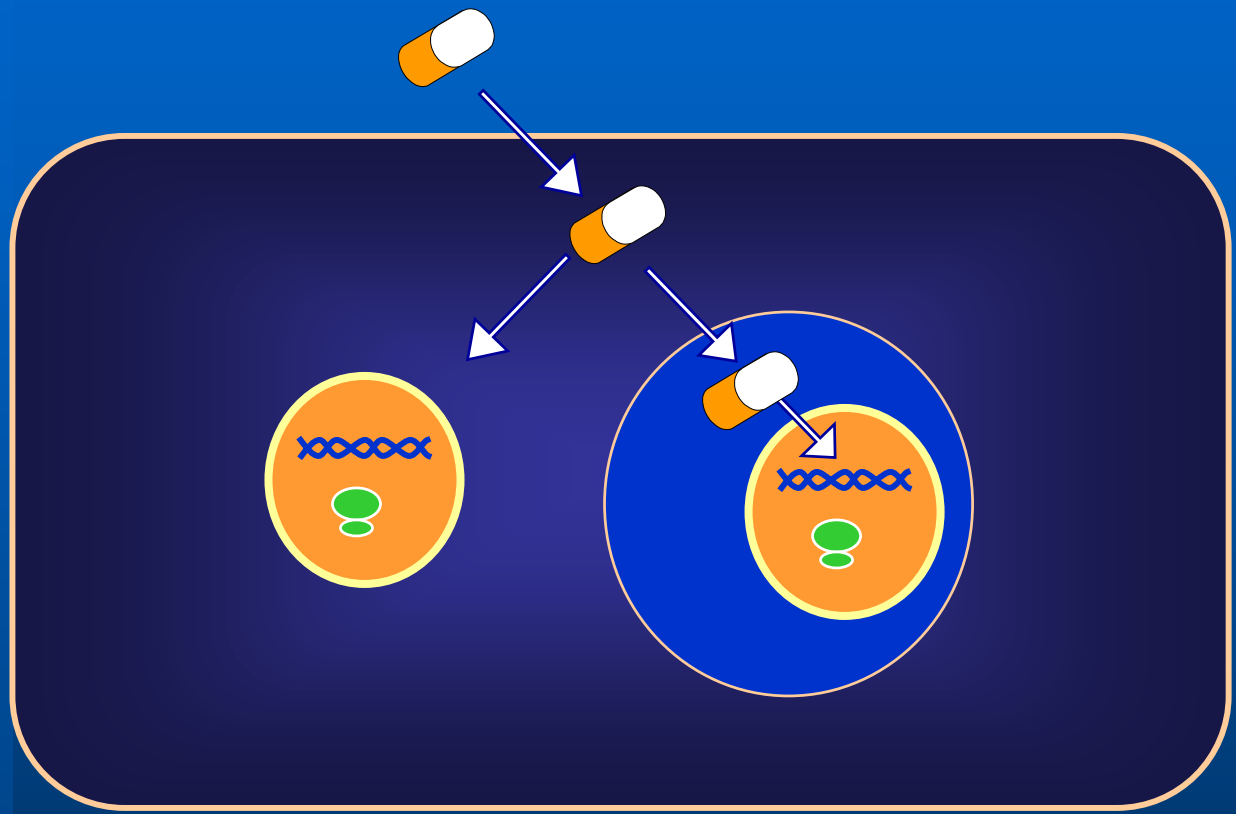


"…the goal is…to find chemical substances that have special affinities for pathogenic organisms and that, like *magic bullets*, go straight to their targets..."

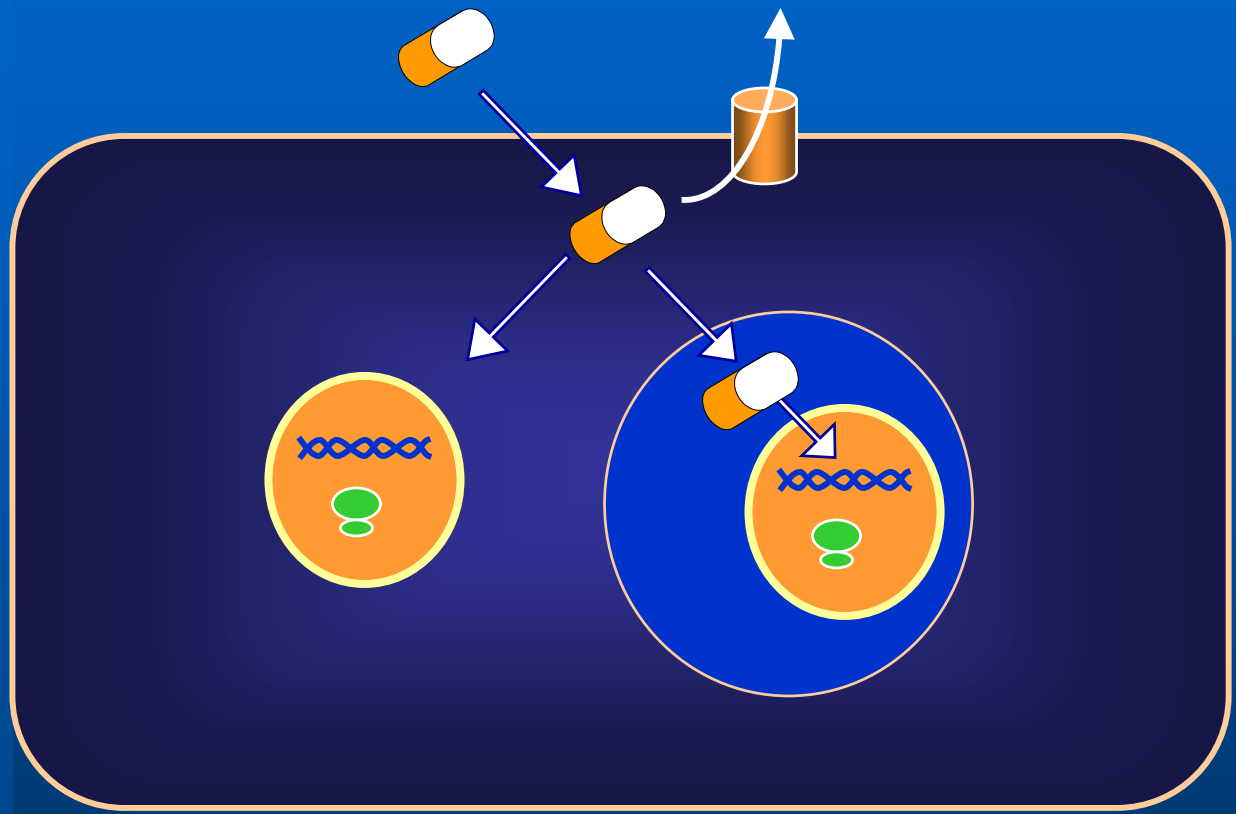
Magic bullets need to reach their target



Target accessibility is critical for intracellular activity

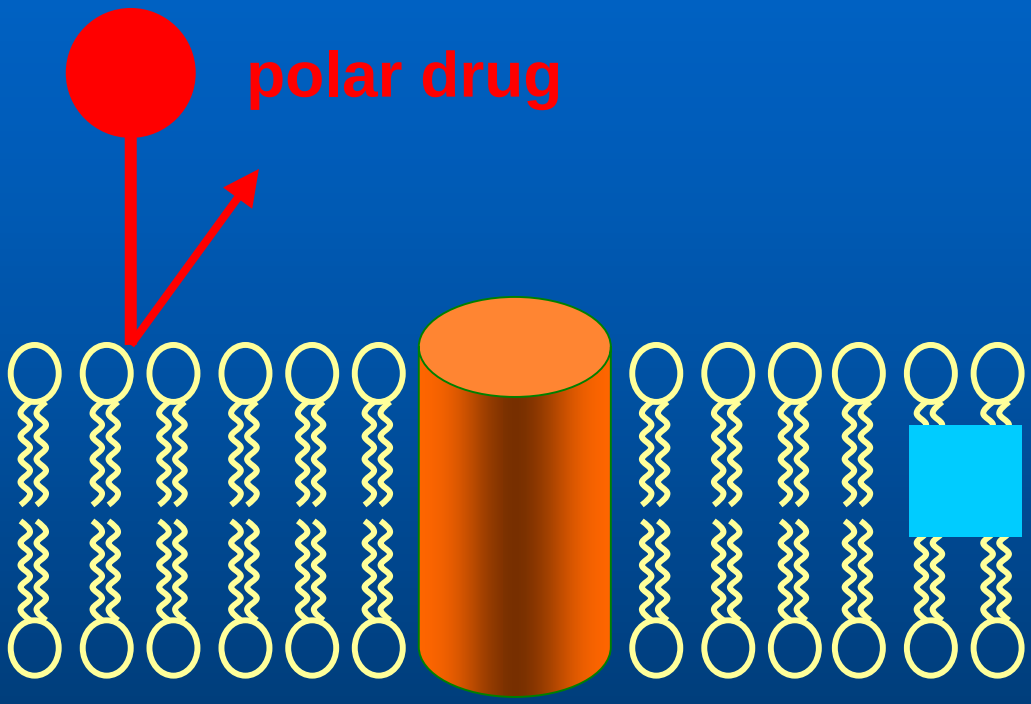


Active efflux reduces antibiotic cellular concentration



Why efflux transporters ?

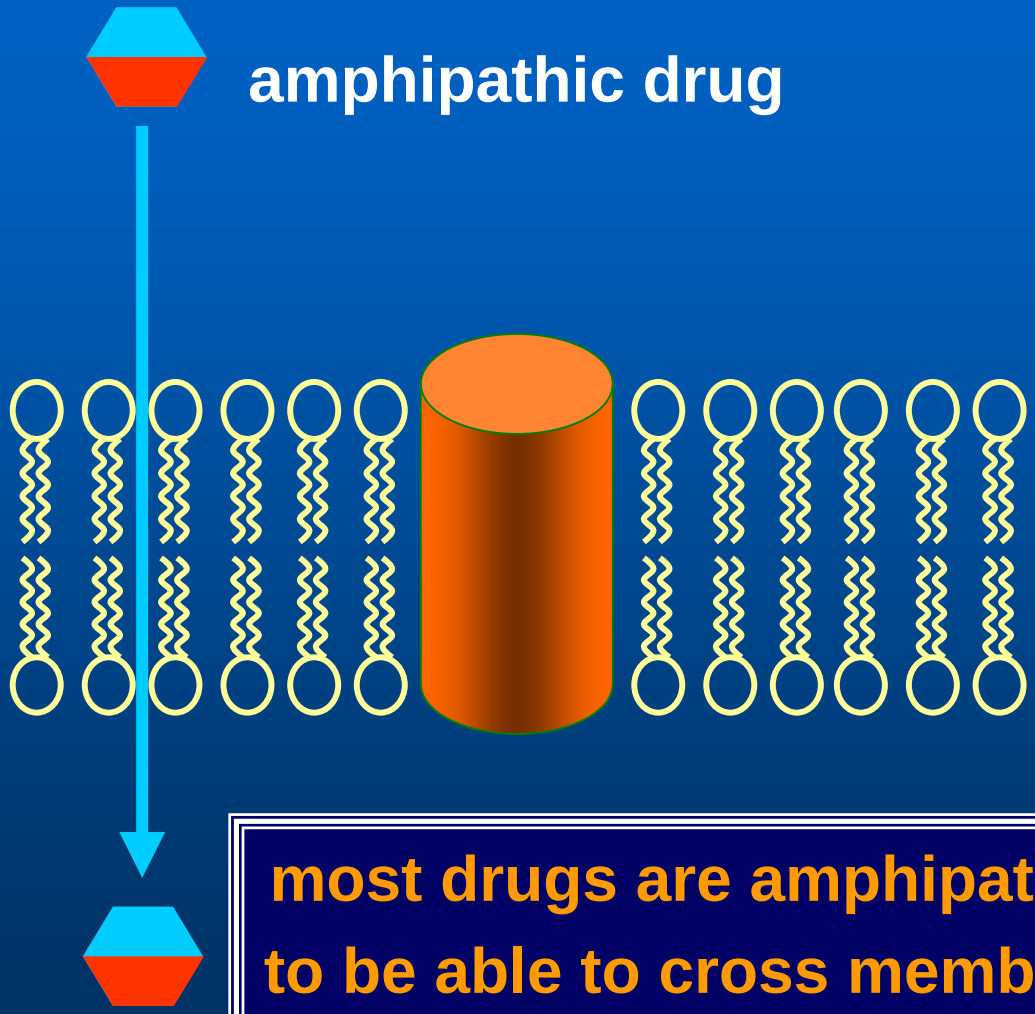
Why efflux transporters ?



physico-chemical properties are inadequate for reaching an intracellular target !

Van Bambeke *et al*. (2000) Biochem. Pharmacol. 60:457-70

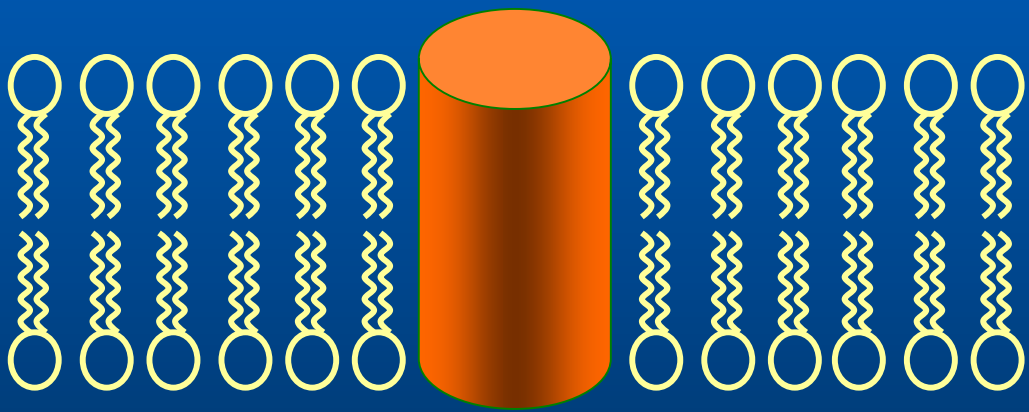
Why efflux transporters ?



most drugs are amphipathic by design, to be able to cross membrane barriers !

Van Bambeke *et al*. (2000) Biochem. Pharmacol. 60:457-70

Why efflux transporters ?



But a diffusible compound

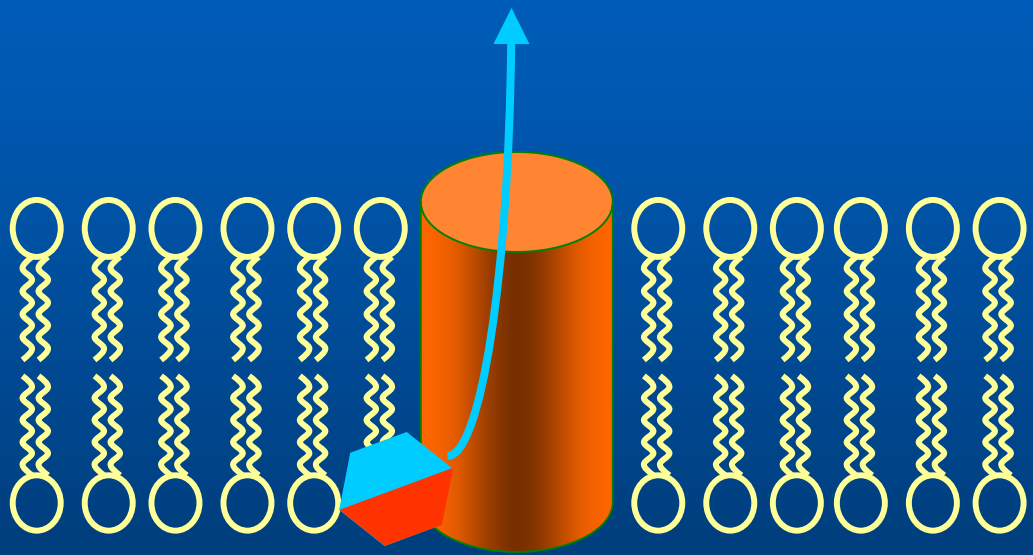
may have

potentially harmful effects !

Van Bambeke *et al*. (2000) Biochem. Pharmacol. 60:457-70

Why efflux transporters ?

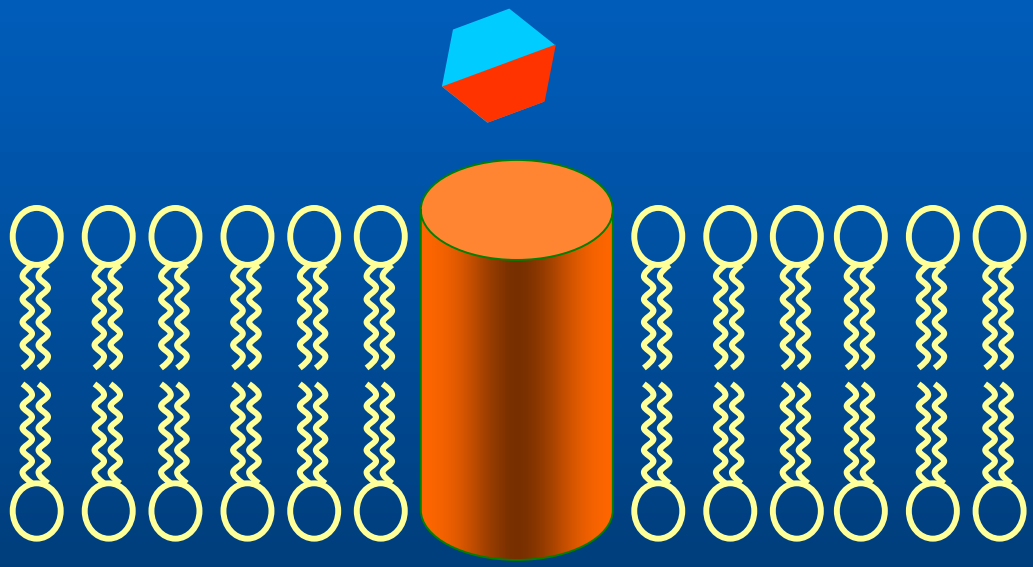
Extrusion by efflux pumps



Van Bambeke *et al*. (2000) Biochem. Pharmacol. 60:457-70

Why efflux transporters ?

Extrusion by efflux pumps

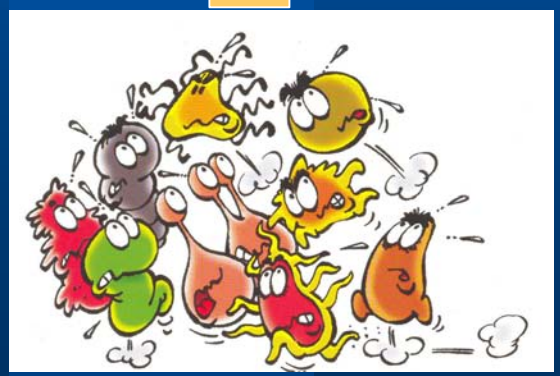


general mean of protection
against cell invasion by diffusible molecules

Van Bambeke *et al*. (2000) Biochem. Pharmacol. 60:457-70

Typical ‘toxic’ diffusible substances as substrates for efflux pumps

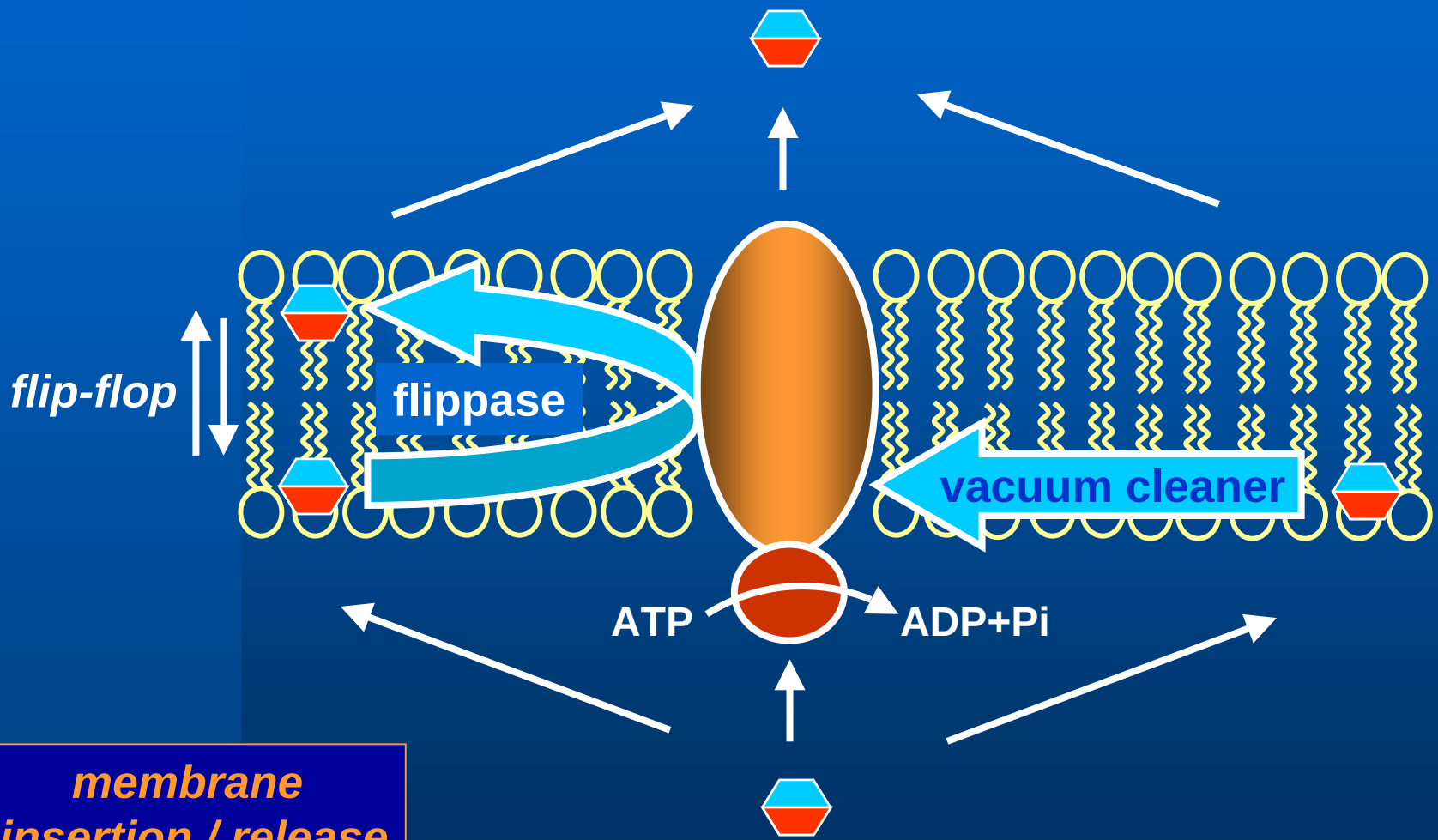
antibiotics



antifungals

anticancer agents

Mechanisms of active efflux

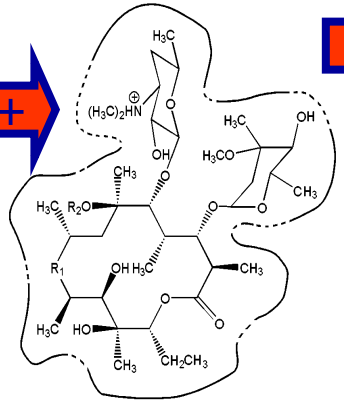


Van Bambeke *et al*. (2000) Biochem. Pharmacol. 60:457-70

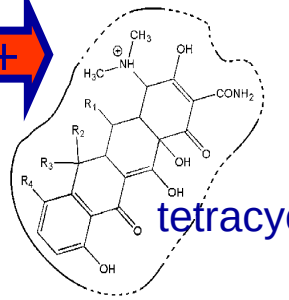
Most antibiotics are amphiphilic !

cationic amphiphiles

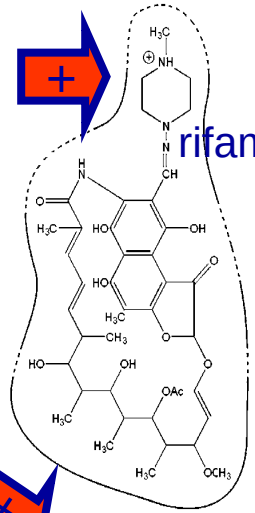
macrolide



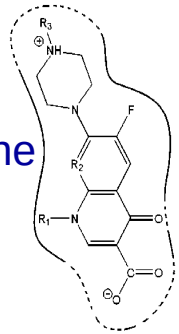
tetracycline



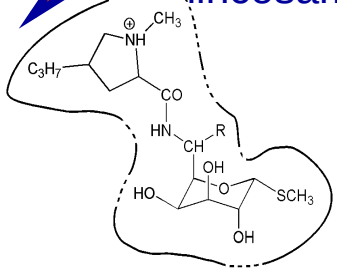
rifampicin



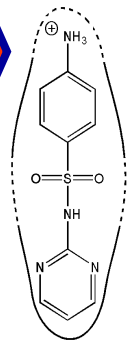
fluoroquinolone



lincosamide

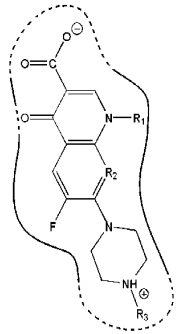


sulfamide

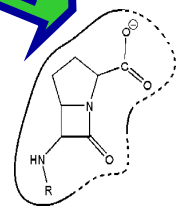


anionic amphiphiles

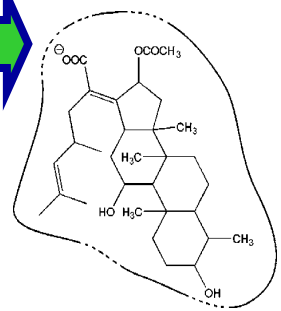
fluoroquinolone



β-lactam



fusidic acid



Van Bambeke *et al*. Biochem. Pharmacol. (2000) 60: 457-470

Antibiotics as substrates of efflux pumps

Antibiotic class	bacteria Gram (+)	bacteria Gram(-)	fungi	superior eucaryotes
β-lactams	●	●	●	●
fusidic acid		●		
macrolides	●	●	●	●
streptogramins	●			●
tetracyclines	●	●	●	●
aminoglycosides		●	●	
chloramphenicol	●	●	●	
rifamycins				●
sulfamides			●	
trimethoprim		●		
fluoroquinolones	●	●		●

Van Bambeke *et al*. (2000) Biochem. Pharmacol. 60:457-70

Consequences of antibiotic efflux from eucaryotic cells

- **alteration of pharmacokinetics**
  - single cell: accumulation, localization
  - whole organism: absorption, distribution, elimination
- **alteration of pharmacodynamics**
  - cellular level: activity against intracellular bacteria
  - body level: drug concentration in the infected compartment

Van Bambeke *et al*, JAC (2003) 51:1067-1077

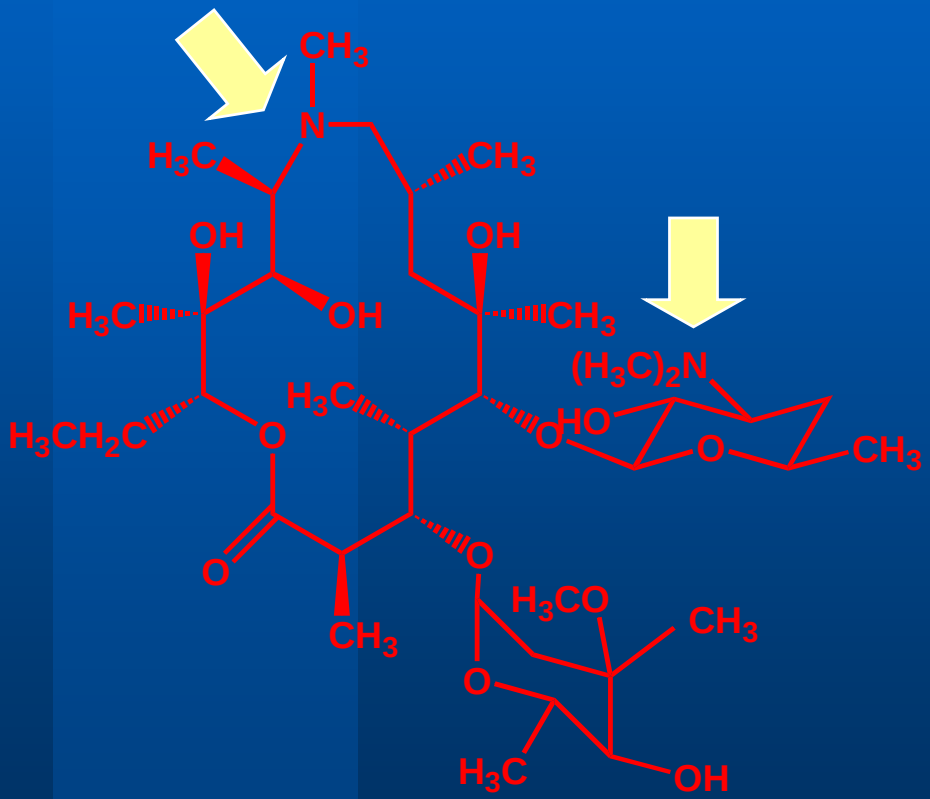
Antibiotics as substrates of efflux pumps

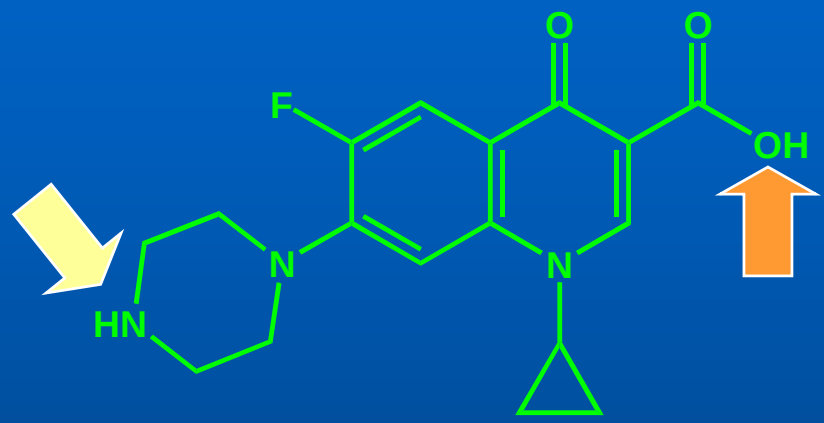
Antibiotic class	bacteria Gram (+)	bacteria Gram(-)	fungi	superior eucaryotes
β-lactams	●	●	●	●
fusidic acid		●		
macrolides	●	●	●	●
streptogramins	●			●
tetracyclines	●	●	●	●
aminoglycosides		●	●	
chloramphenicol	●	●	●	
rifamycins				●
sulfamides			●	
trimethoprim		●		
fluoroquinolones	●	●		●

Van Bambeke *et al*. (2000) Biochem. Pharmacol. 60:457-70

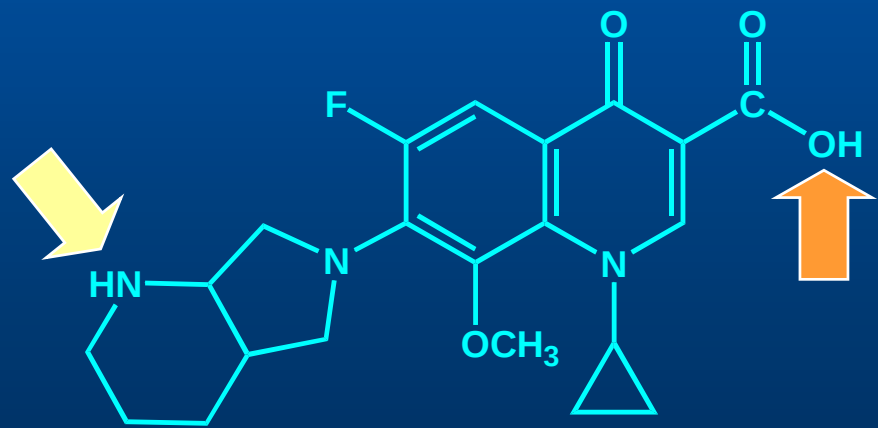
Antibiotics as substrates of efflux pumps

azithromycin





ciprofloxacin



moxifloxacin

Macrolides and quinolones as cell-associated antibiotics

Infection. 1995;23 Suppl 1:S10-4.

Clinical relevance of intracellular and extracellular concentrations of macrolides.

Carbon C.

C.H.U. Bichat-Claude Bernard, Paris, France.

The serum levels of the three macrolides--roxithromycin, clarithromycin and azithromycin--vary considerably. The prediction of the antibacterial effect against extracellular pathogens is based on circulating concentrations of free drug, peak and trough levels, the rate of killing, and the presence of a post-antibiotic effect. Intracellular activity depends on the distribution of the antibiotic and the localization of the bacteria, and is variable. Roxithromycin uptake is greater than that of erythromycin. The intracellular half-life may be long for some compounds (azithromycin > roxithromycin). The intracellular distribution is bimodal, both in the lysosomes and the cytoplasm, but the mechanisms of uptake have not yet been established. At low pH, accumulation is low and macrolides are less active in an acidic medium. Intracellular concentrations cannot readily be predicted on the basis of extracellular levels. Different models have shown that the greater the intracellular concentration, the better the clinical effect. In addition, the transport of macrolides by cells into the infected focus may play an important role in the therapeutic outcome. These factors influence the clinical indications for macrolides, their dosing regimens and breakpoints. In future, macrolides will be developed that are more selective for intracellular infections, while others, which will achieve significant serum levels, will be useful for a broader range of diseases. However, new compounds should be evaluated in different models of infection before clinical studies are instituted. The analysis of failures remains the most important approach in defining concentration/effect relationships.

Infection. 1991;19 Suppl 7:S365-71.

Quinolones in the treatment of lower respiratory tract infections caused by intracellular pathogens.

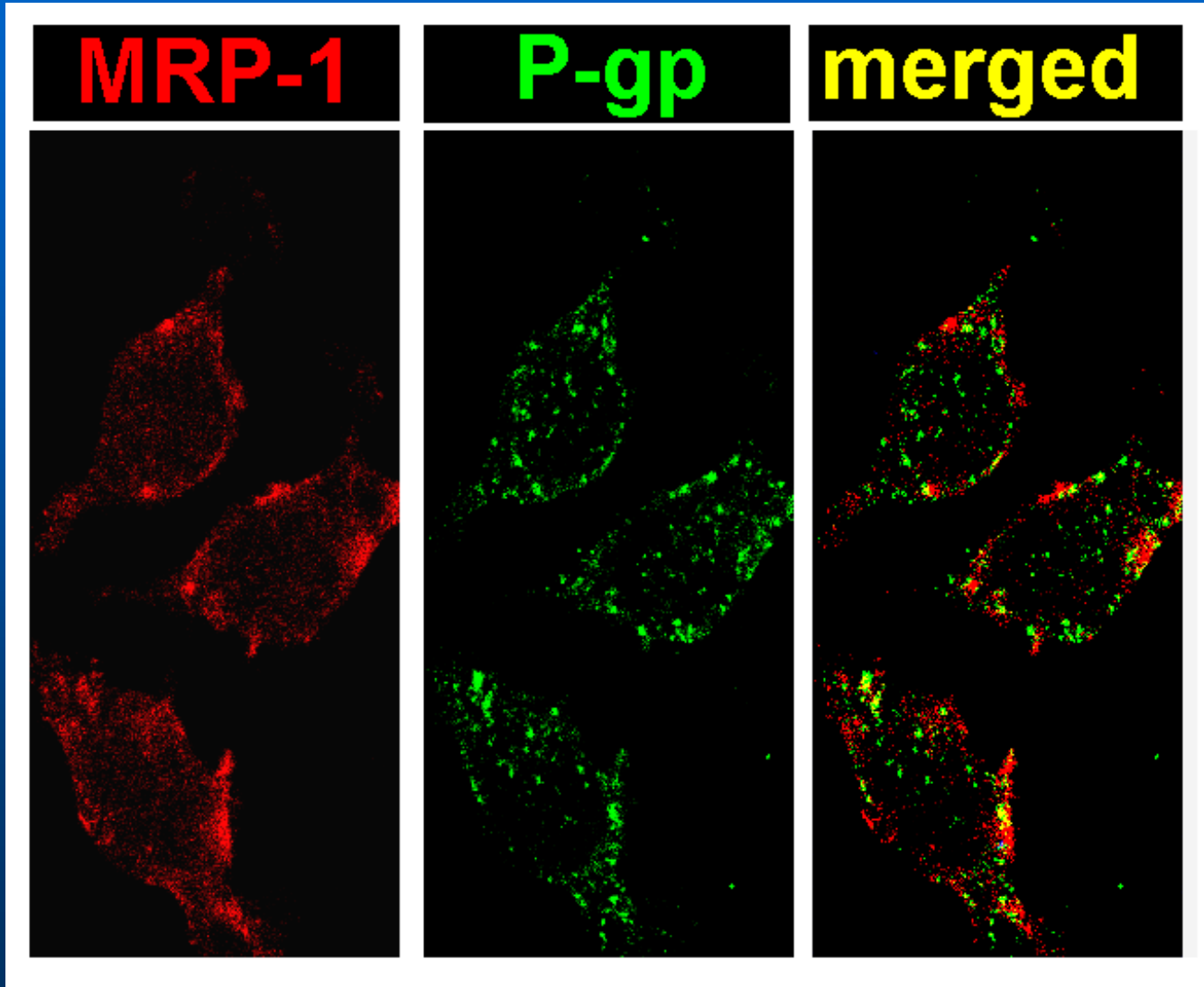
Chidiac C, Mouton Y.

Department of Infectious Diseases, University of Lille II, Central Hospital, Tourcoing, France.

Intracellular pathogens are inhibited to varying degrees, depending upon the strain of the organism and the quinolone tested. Quinolones achieve levels in the lower respiratory tract that equal or exceed serum concentrations, and they also achieve good intracellular concentrations. Experimental models of intracellular infection have demonstrated the efficacy of ciprofloxacin, difloxacin, fleroxacin, ofloxacin and pefloxacin. Animal models of experimental legionellosis have confirmed in vivo their efficacy in this field. Thus, quinolones appear to be a safe and efficacious alternative treatment in lower respiratory tract infection (LRTI) due to intracellular pathogens. Considering the in vitro and experimental studies, quinolones should play an important role in the treatment of LRTI caused by intracellular pathogens, and prospective controlled studies are strongly recommended.

Characterization of antibiotic efflux pumps in macrophages

Efflux pumps expressed in J774 macrophages



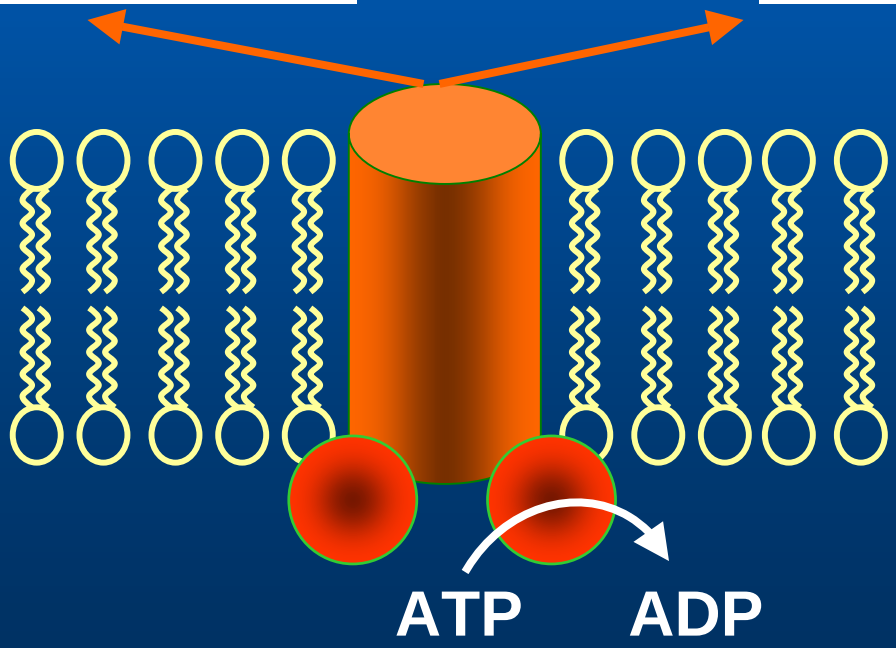
ABC multidrug transporters

cationic amphiphiles

anionic amphiphiles

MDR-1 (P-glycoprotein)

MRP1-10



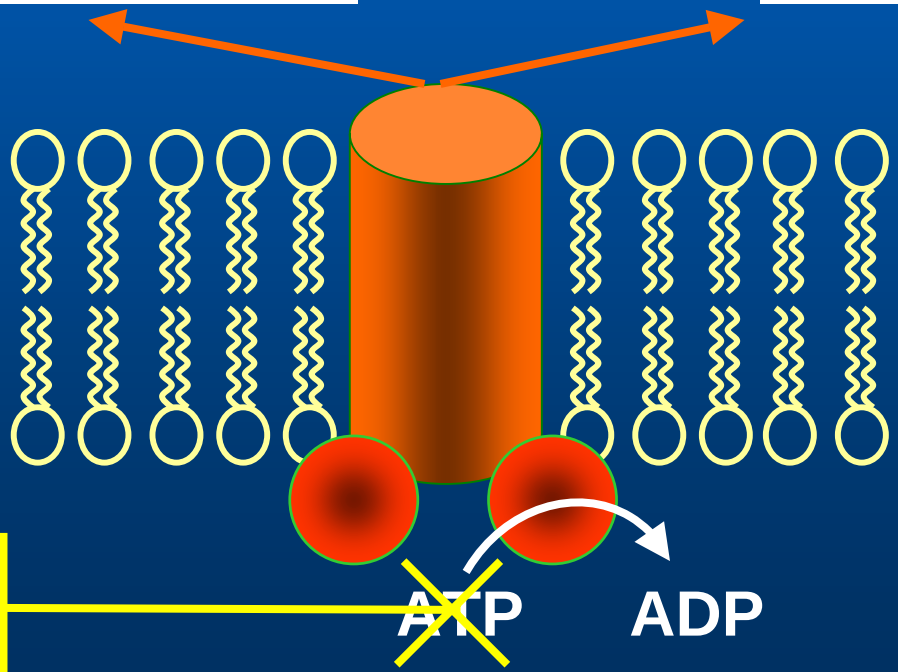
How to inhibit ABC transporters ?

cationic amphiphiles

anionic amphiphiles

MDR-1 (P-glycoprotein)

MRP1-10



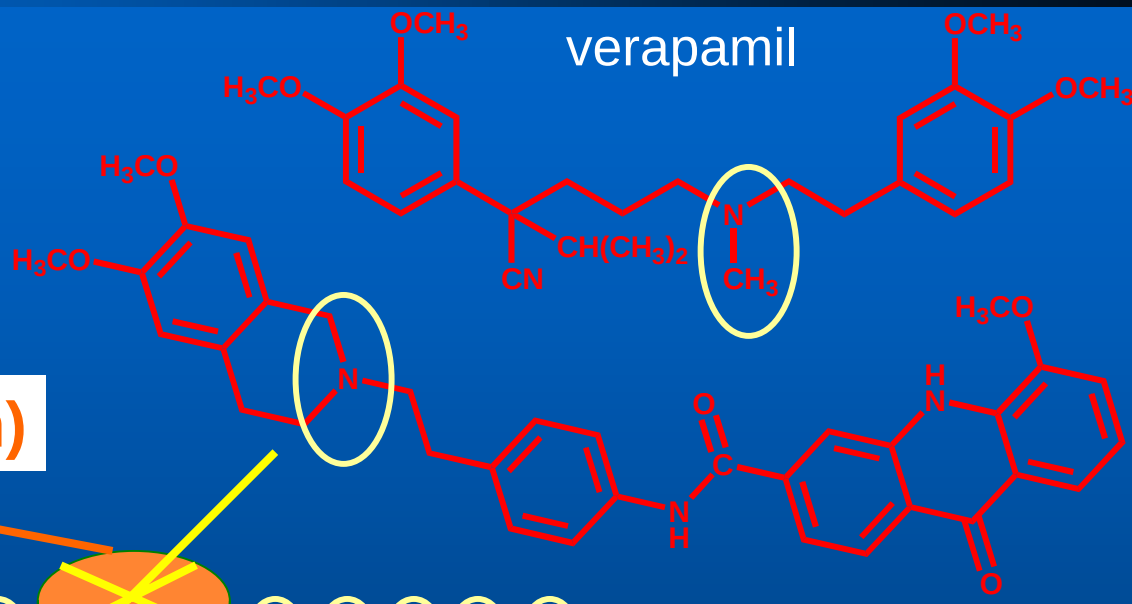
deoxyglucose
NaN_3

How to inhibit ABC transporters ?

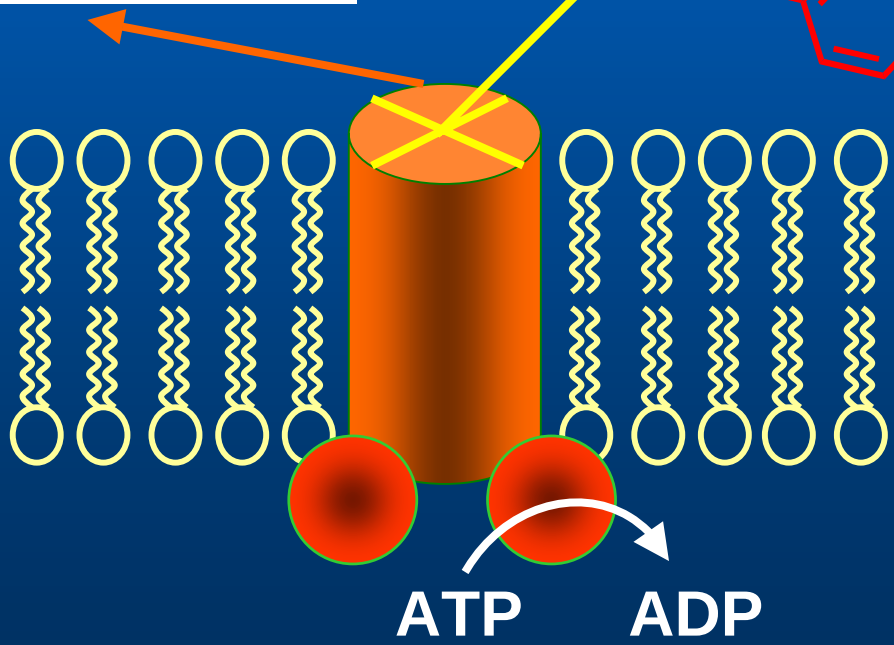
cationic amphiphiles

MDR-1 (P-glycoprotein)

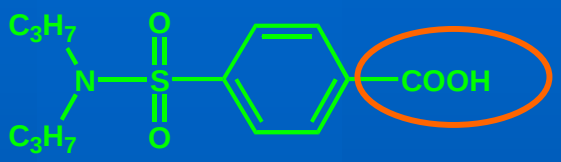
verapamil



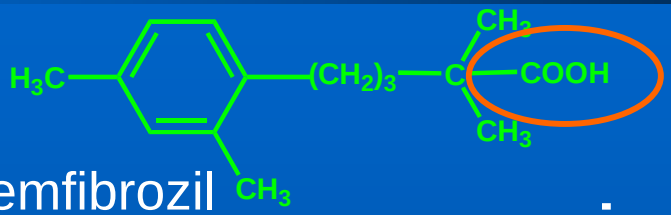
GF120918



How to inhibit ABC transporters ?

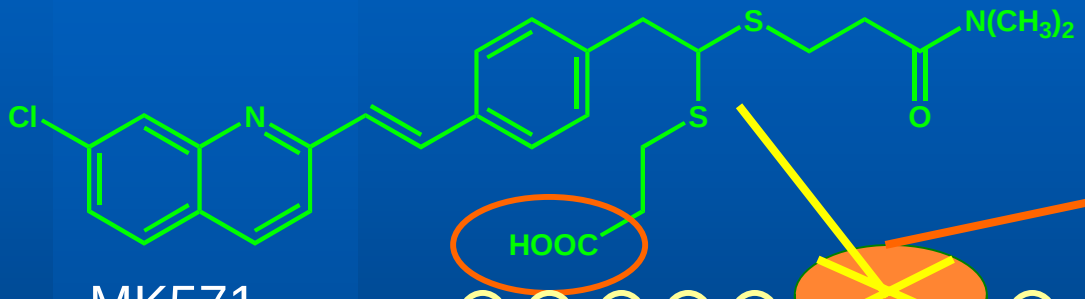


probenecid



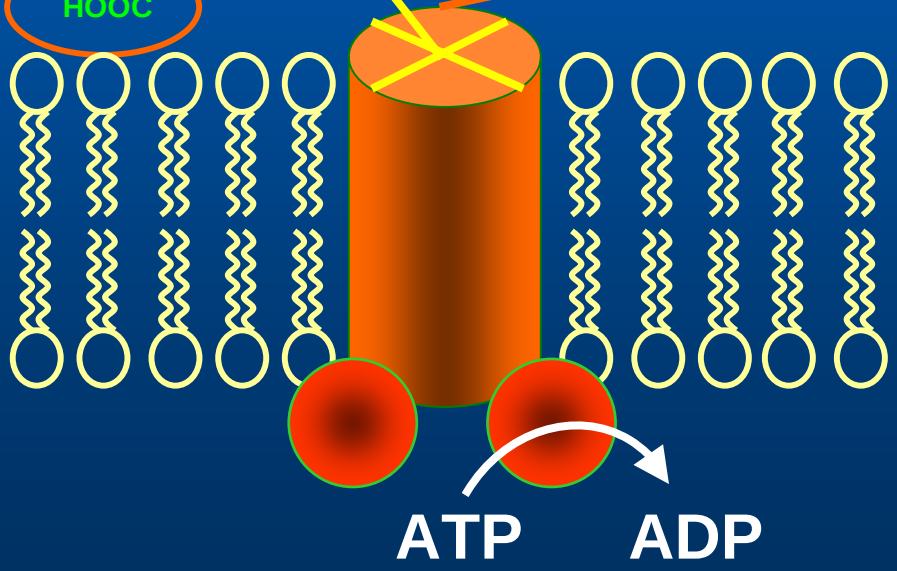
gemfibrozil

anionic amphiphiles



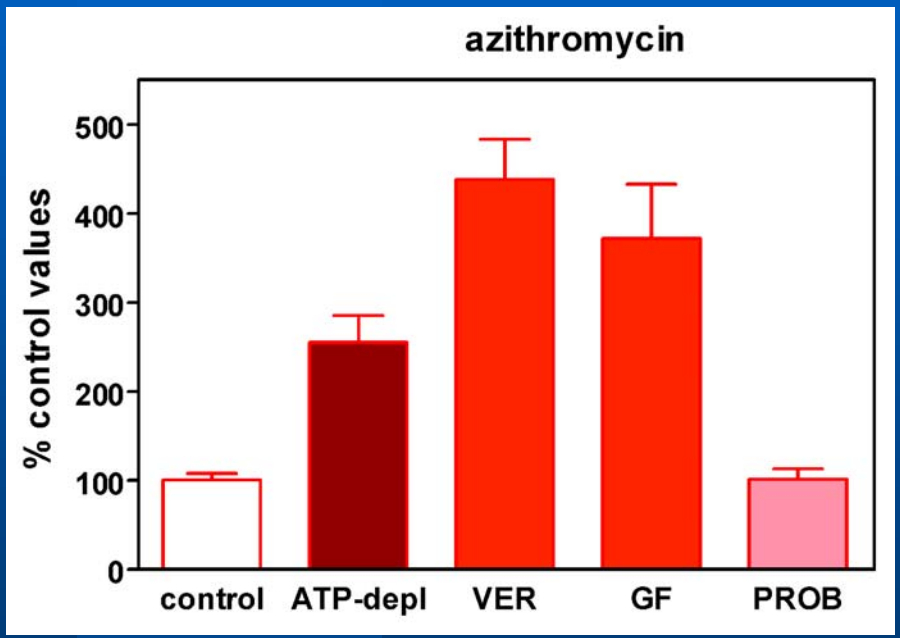
MK571

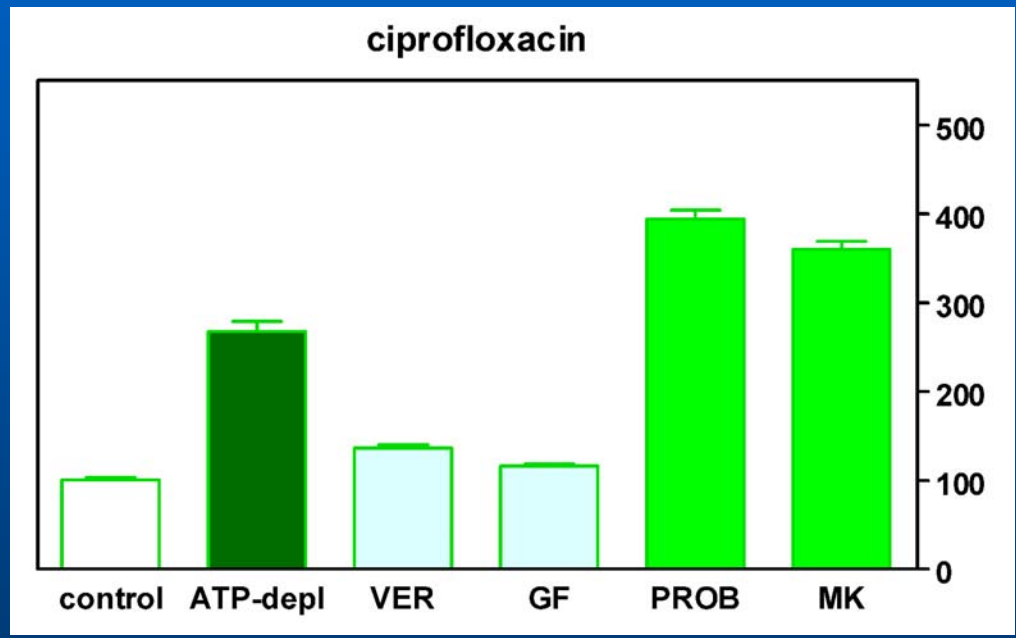
MRP1-10



Differential recognition by MDR pumps

Influence of ATP-depletion and pump inhibitors on accumulation at equilibrium





azithromycin & P-glycoprotein

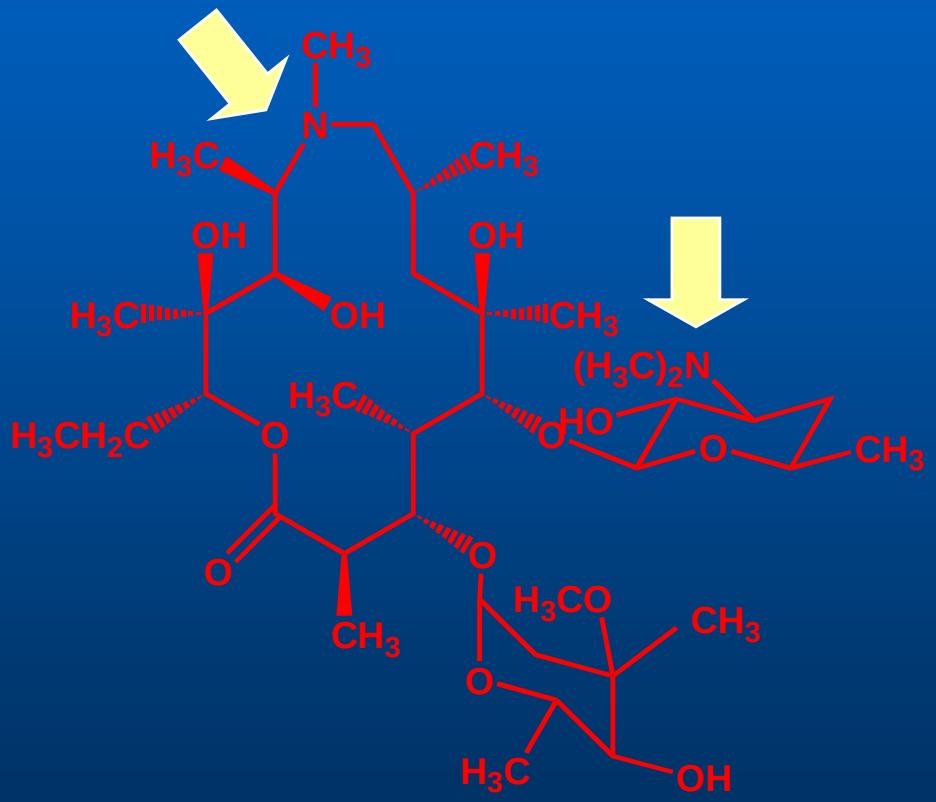
ciprofloxacin & MRP

extracell. conc. 5 mg/L; AZM 3 h; CIP 2 h

Michot *et al*. AAC (2004) 48:2673-82

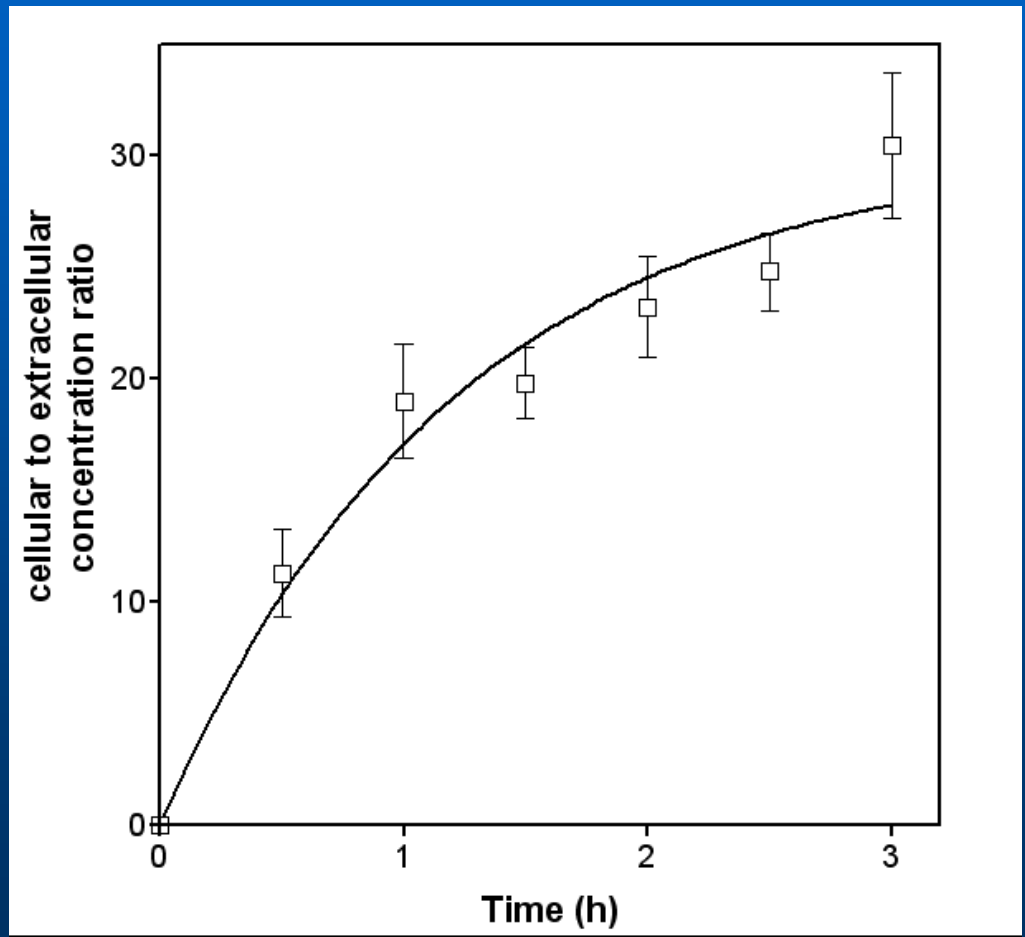
Efflux of macrolides: azithromycin

azithromycin



Kinetics of accumulation

Azithromycin accumulates to high levels in eucaryotic cells

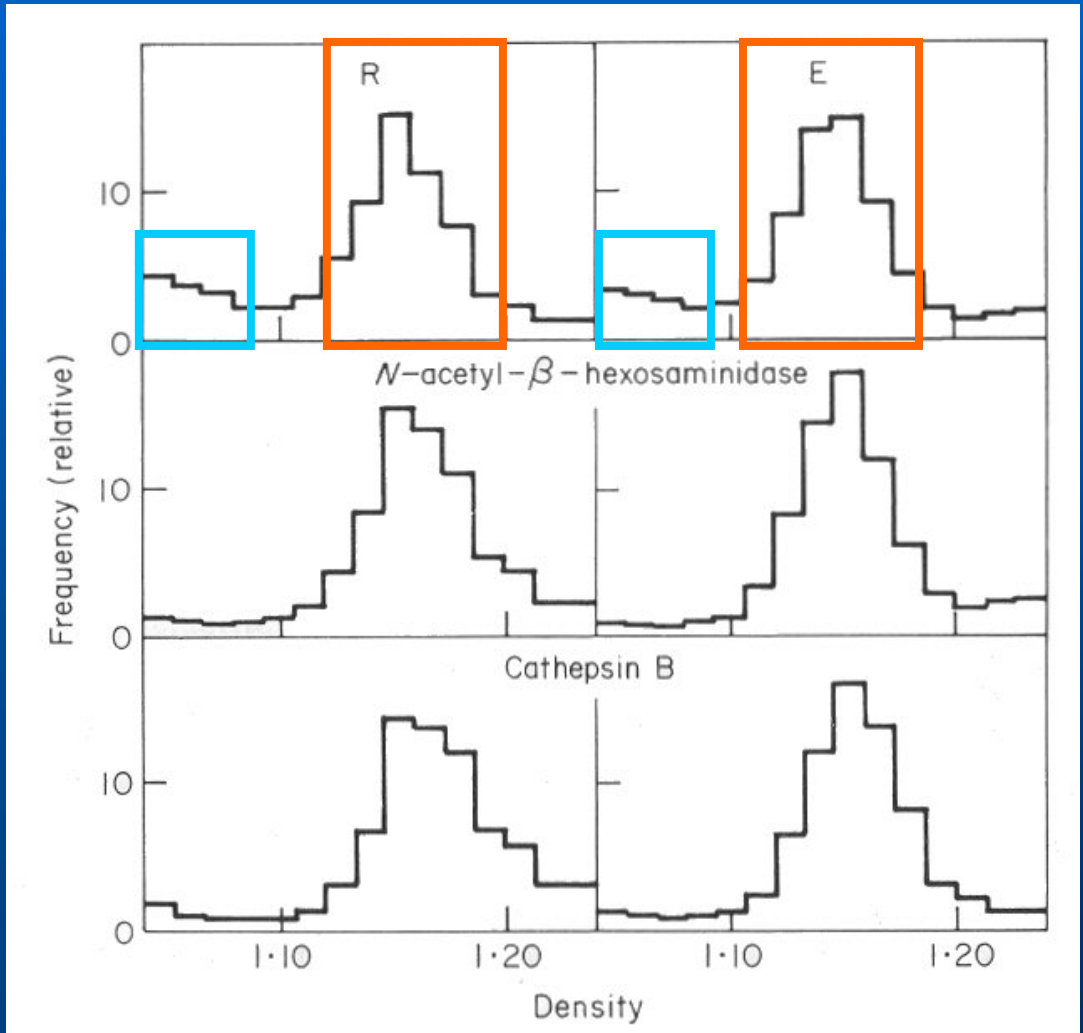


extracell. conc. 5 mg/L

Seral *et al* (2003) AAC 47:1047-51

Macrolide subcellular distribution

Macrolides accumulate in the lysosomal compartment

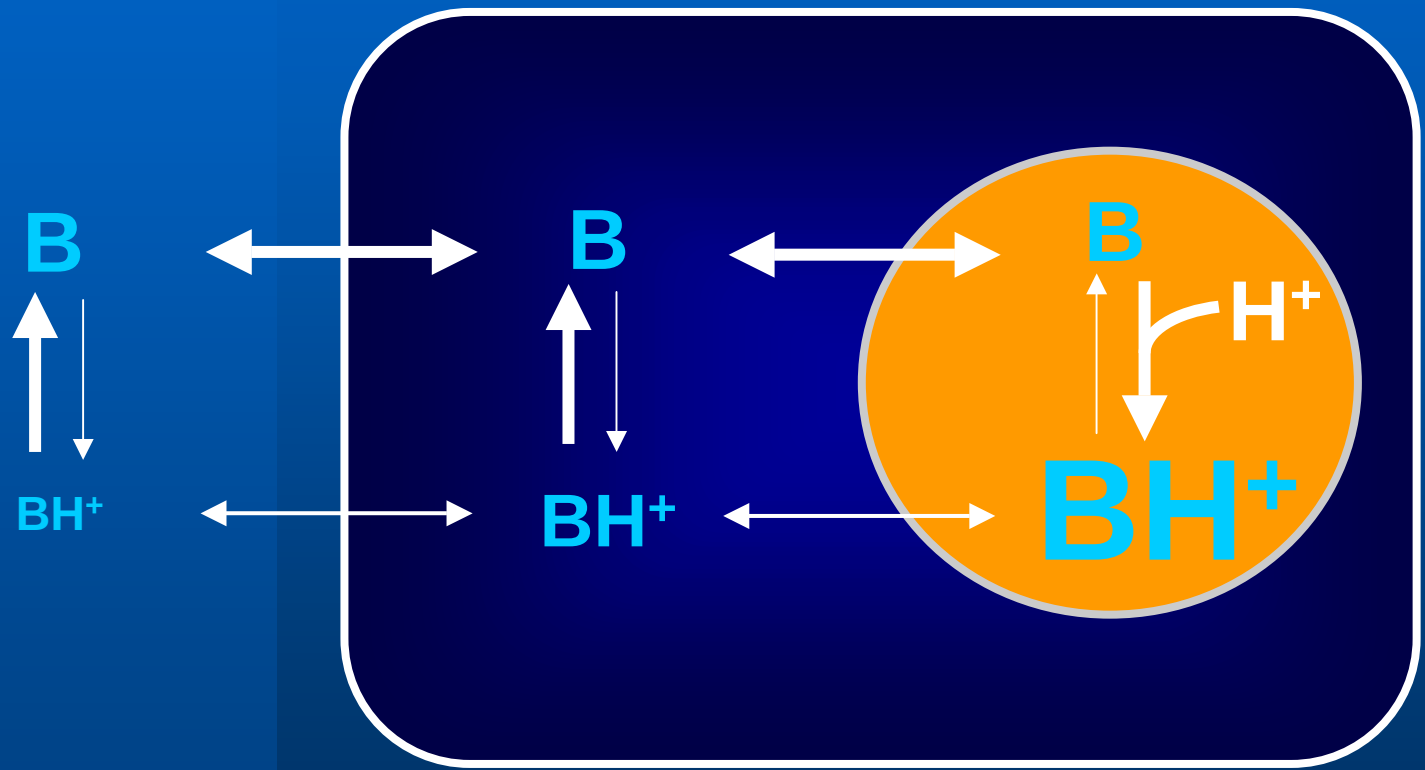


lysosomal enzymes

Carlier *et al*, JAC (1987) 20 Suppl B:47-56

Mechanism of accumulation for macrolides

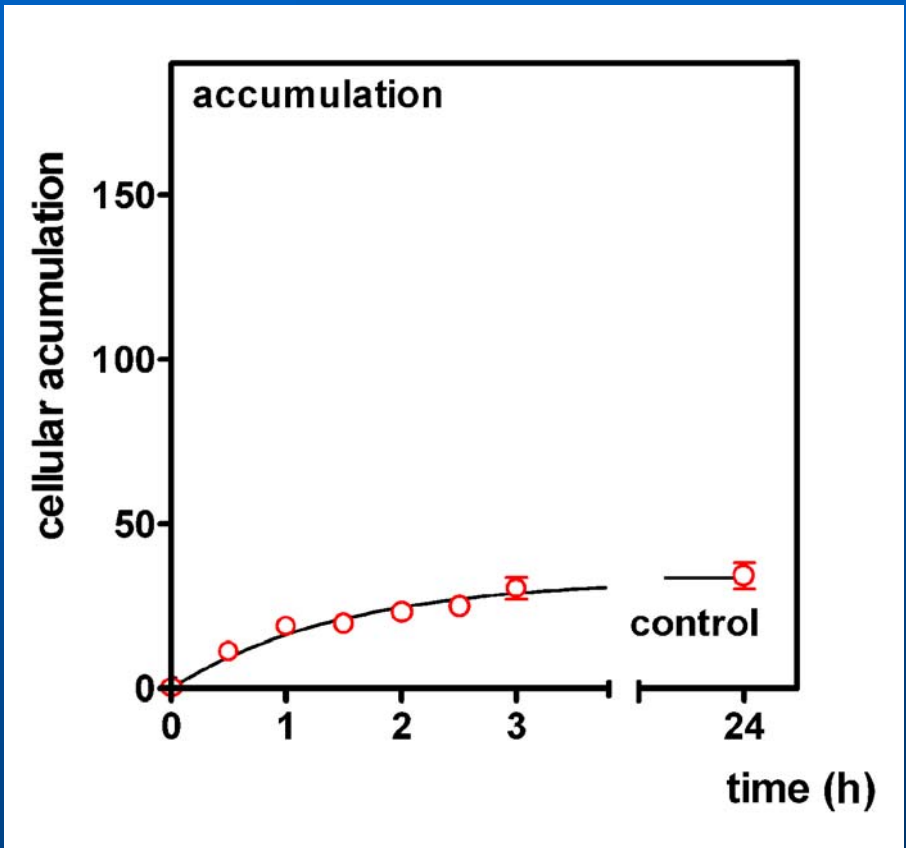
Macrolide accumulation proceeds by diffusion / segregation



De Duve *et al,* Biochem Pharmacol. (1974) 23: 2495-531

Kinetics of accumulation and efflux for azithromycin

Azithromycin concentration is high but still suboptimal …

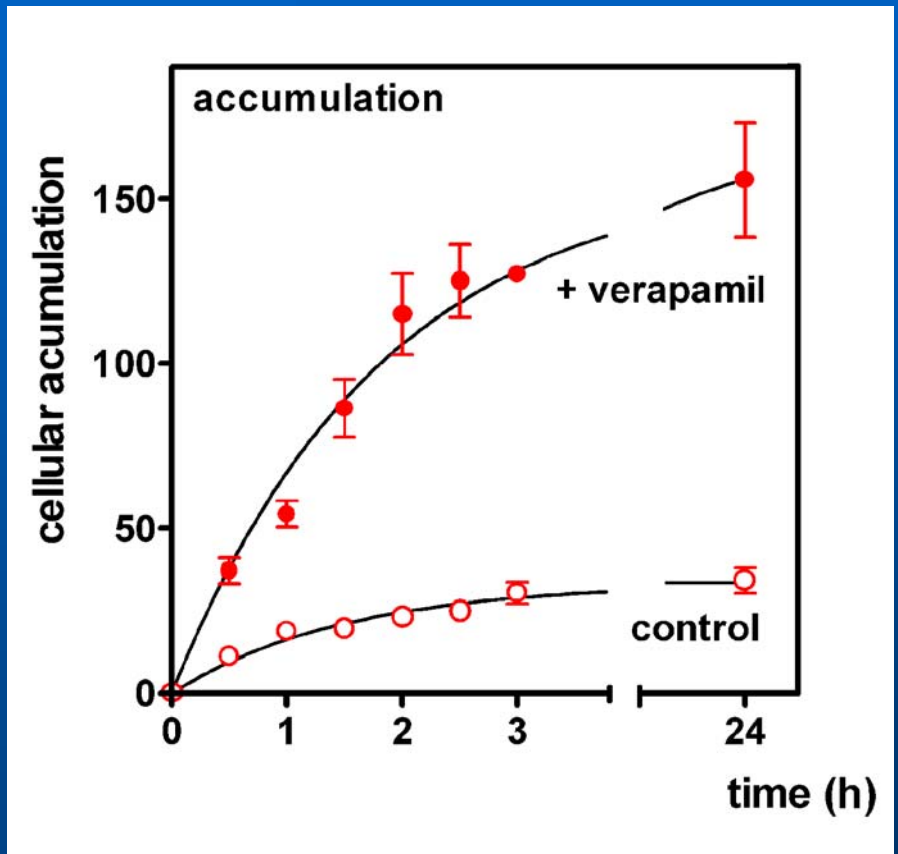


extracell. conc. 5 mg/L

Seral *et al* (2003) AAC 47:1047-51

Kinetics of accumulation and efflux for azithromycin

Inhibition of P-gp by verapamil increases accumulation

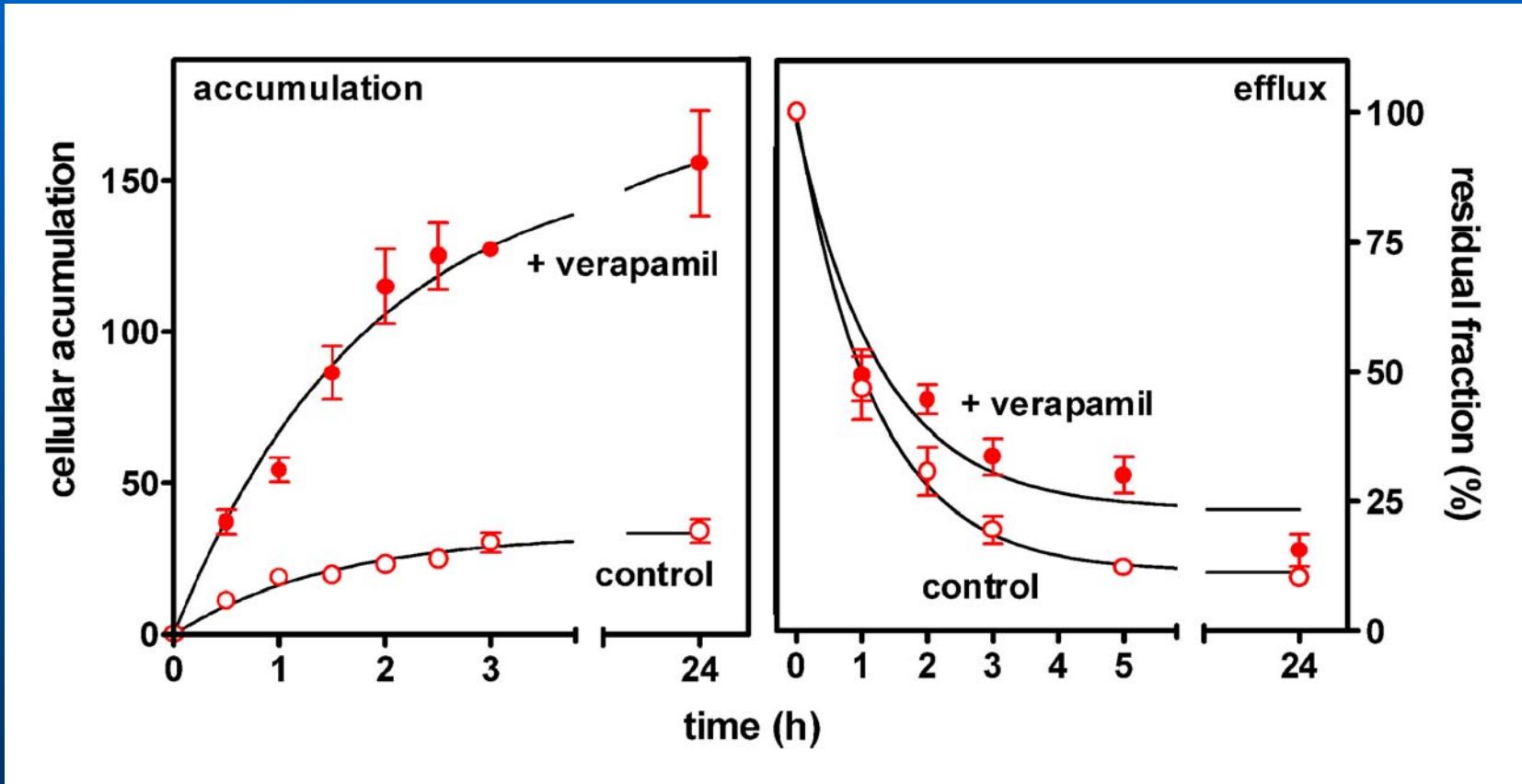


extracell. conc. 5 mg/L; verapamil 20 µM

Seral *et al* (2003) AAC 47:1047-51

Kinetics of accumulation and efflux for azithromycin

Accumulation markedly increased; efflux marginally affected

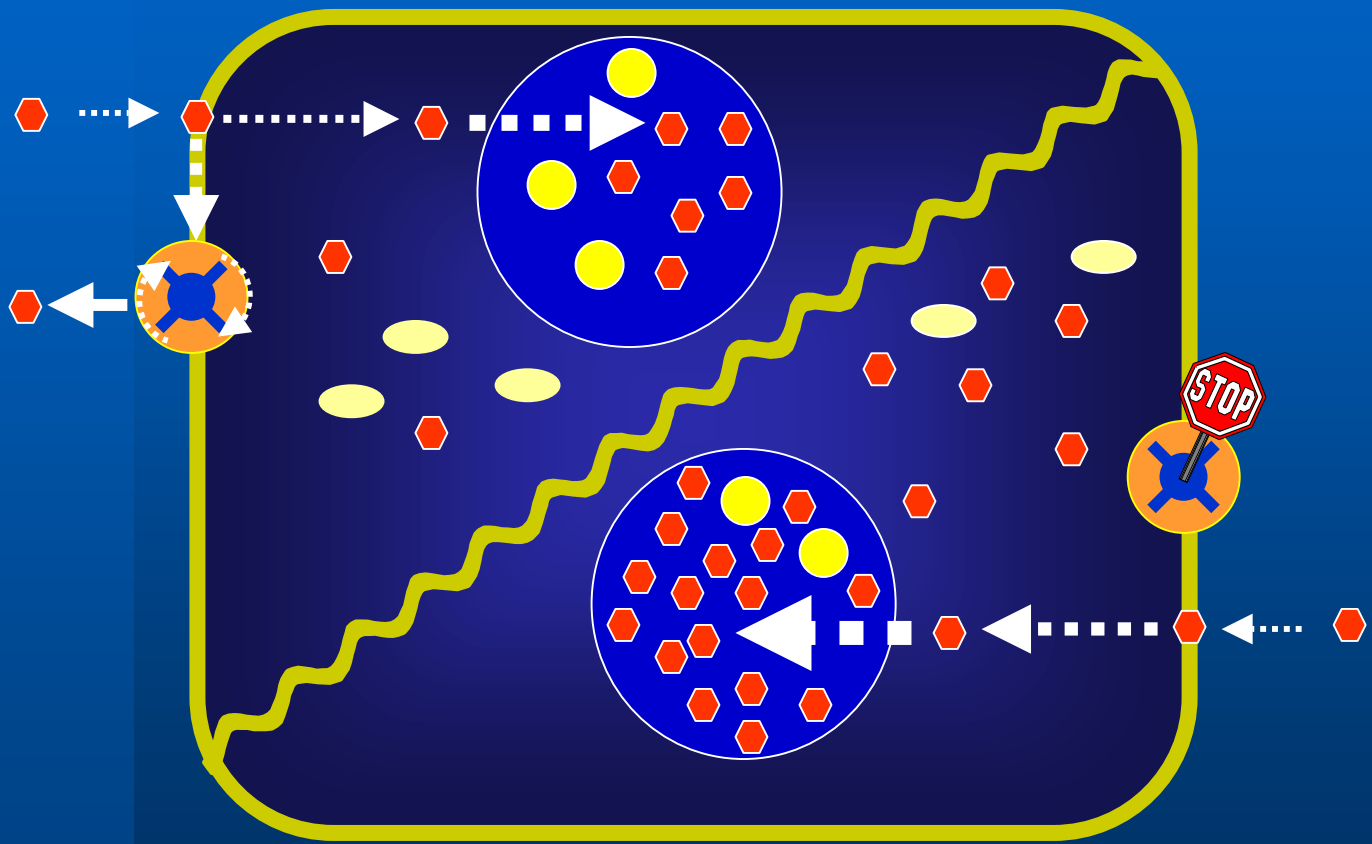


extracell. conc. 5 mg/L; verapamil 20 µM

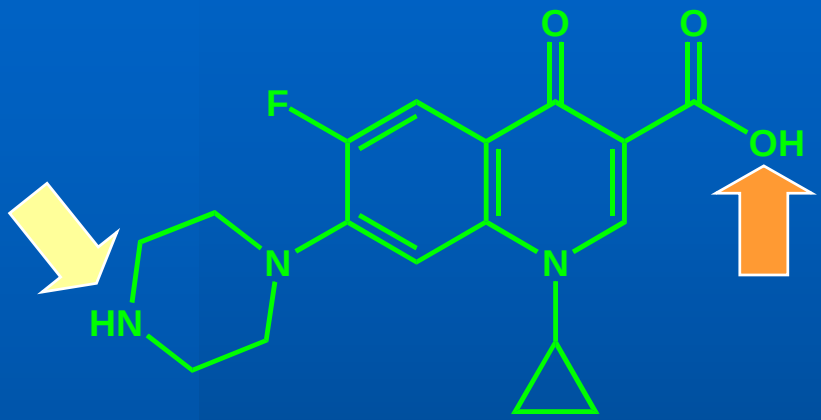
Seral *et al* (2003) AAC 47:1047-51

Azithromycin, ‘kick-back’ model

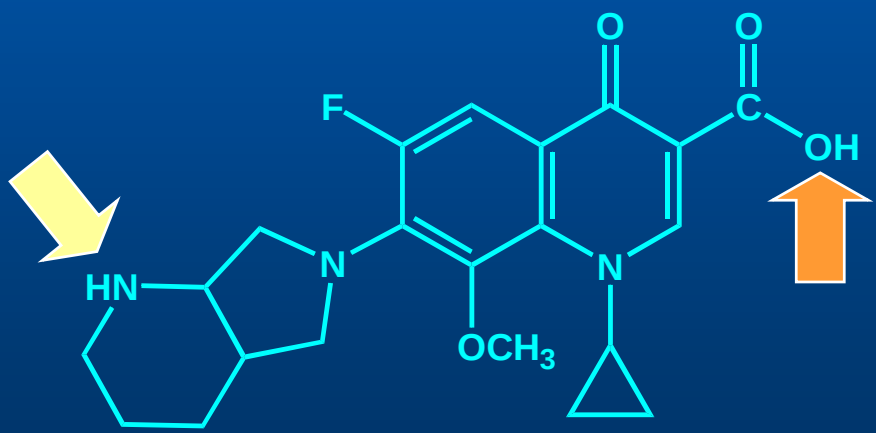
Gaj *et al*. (1998) Biochem. Pharmacol. 55:1199-211



Efflux of quinones (ciprofloxacin, moxifloxacin)



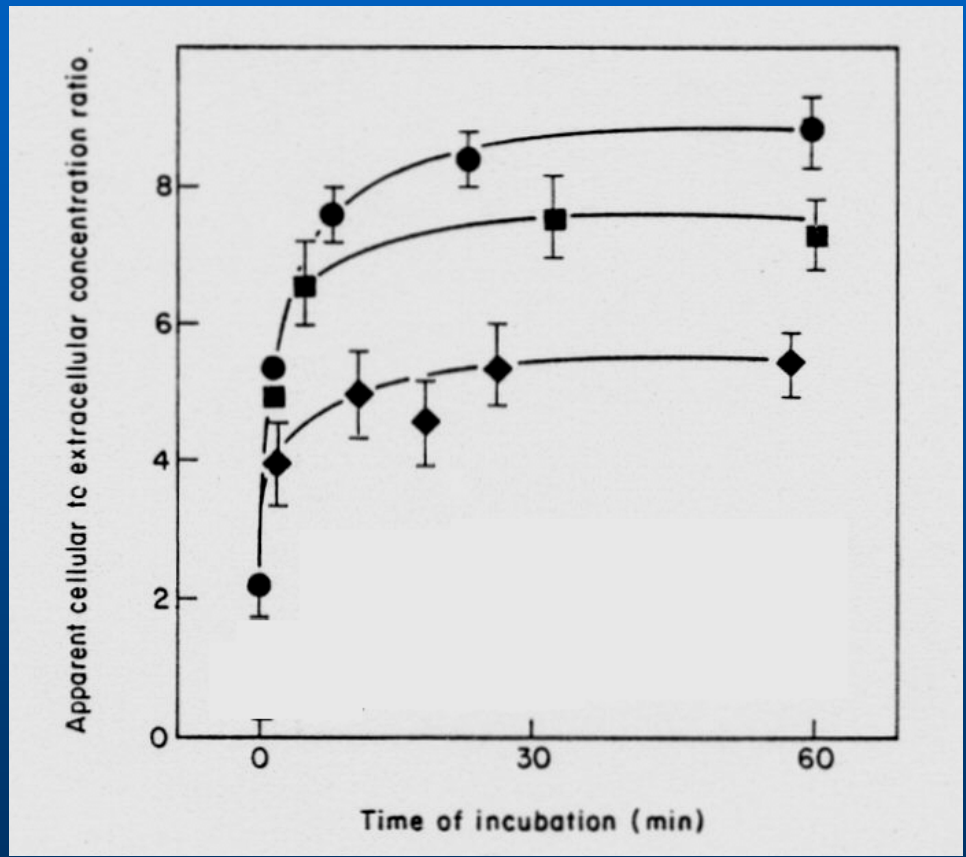
ciprofloxacin



moxifloxacin

Kinetics of accumulation

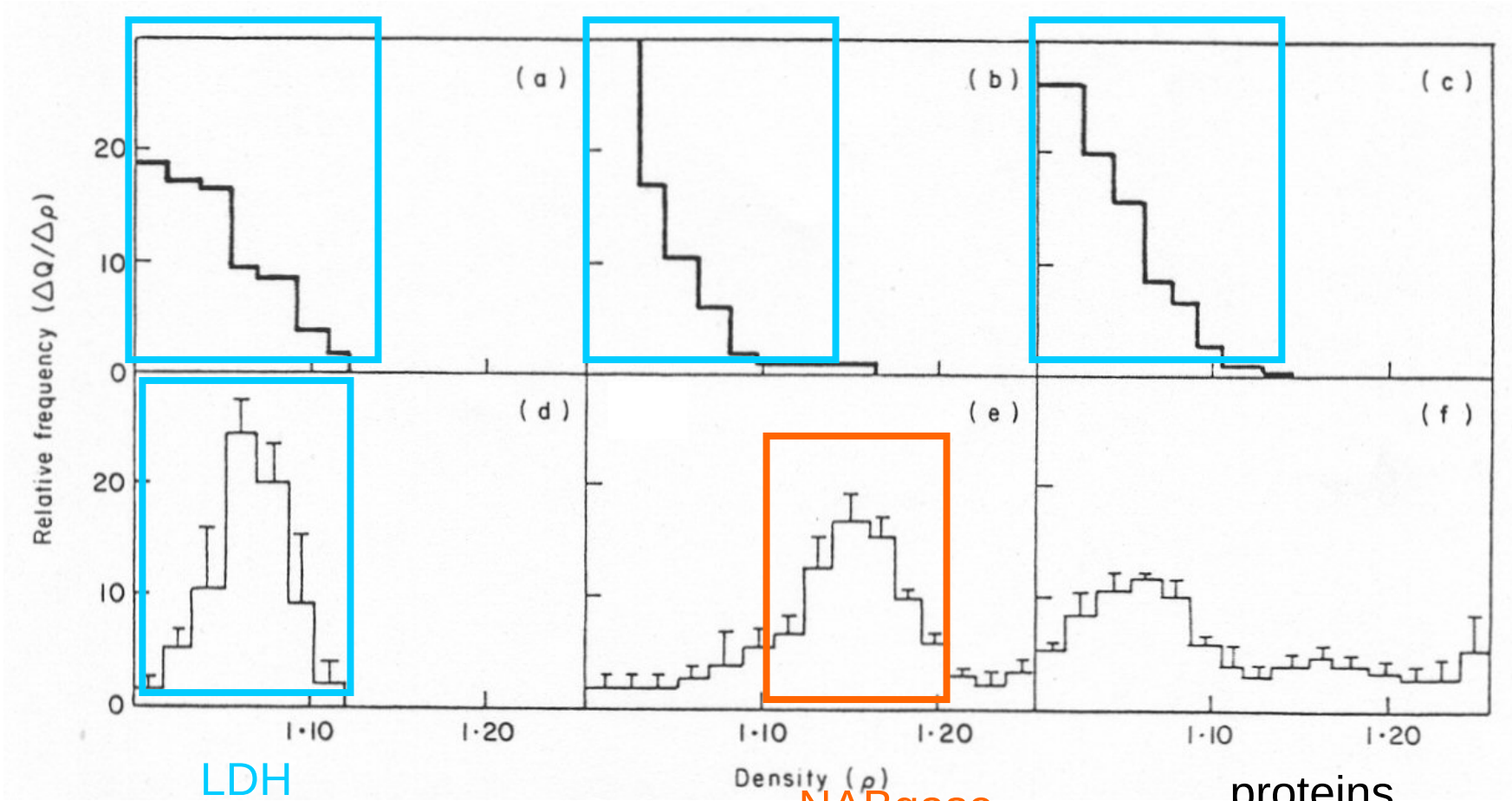
Quinolones accumulate to moderate levels in eucaryotic cells



Carlier *et al* JAC (1990) 26 Suppl B:27-39

Quinolone subcellular distribution

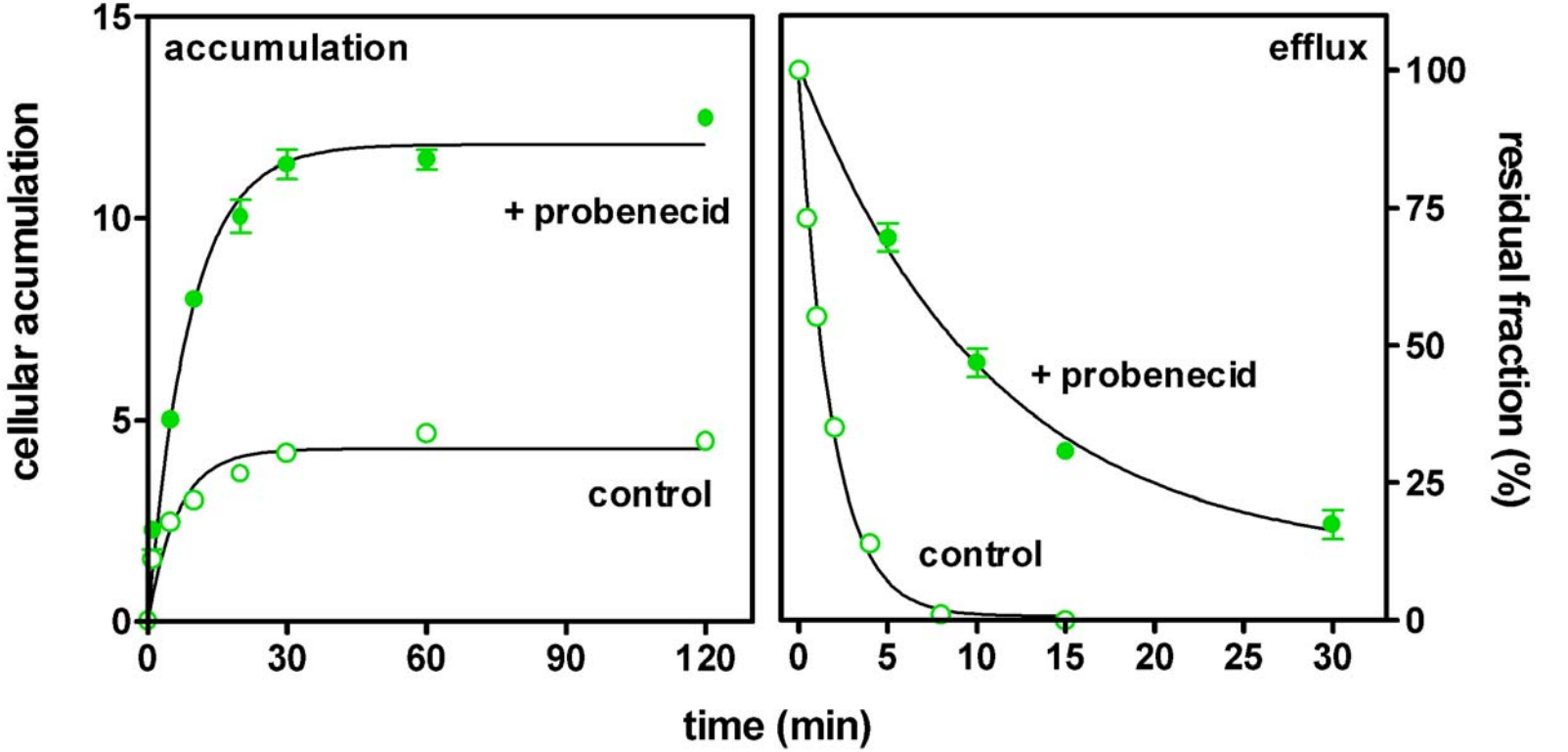
Quinolones are found in the soluble fraction



Carlier *et al* JAC (1990) 26 Suppl B:27-39

Kinetics of accumulation and efflux for ciprofloxacin

both accumulation and efflux markedly affected

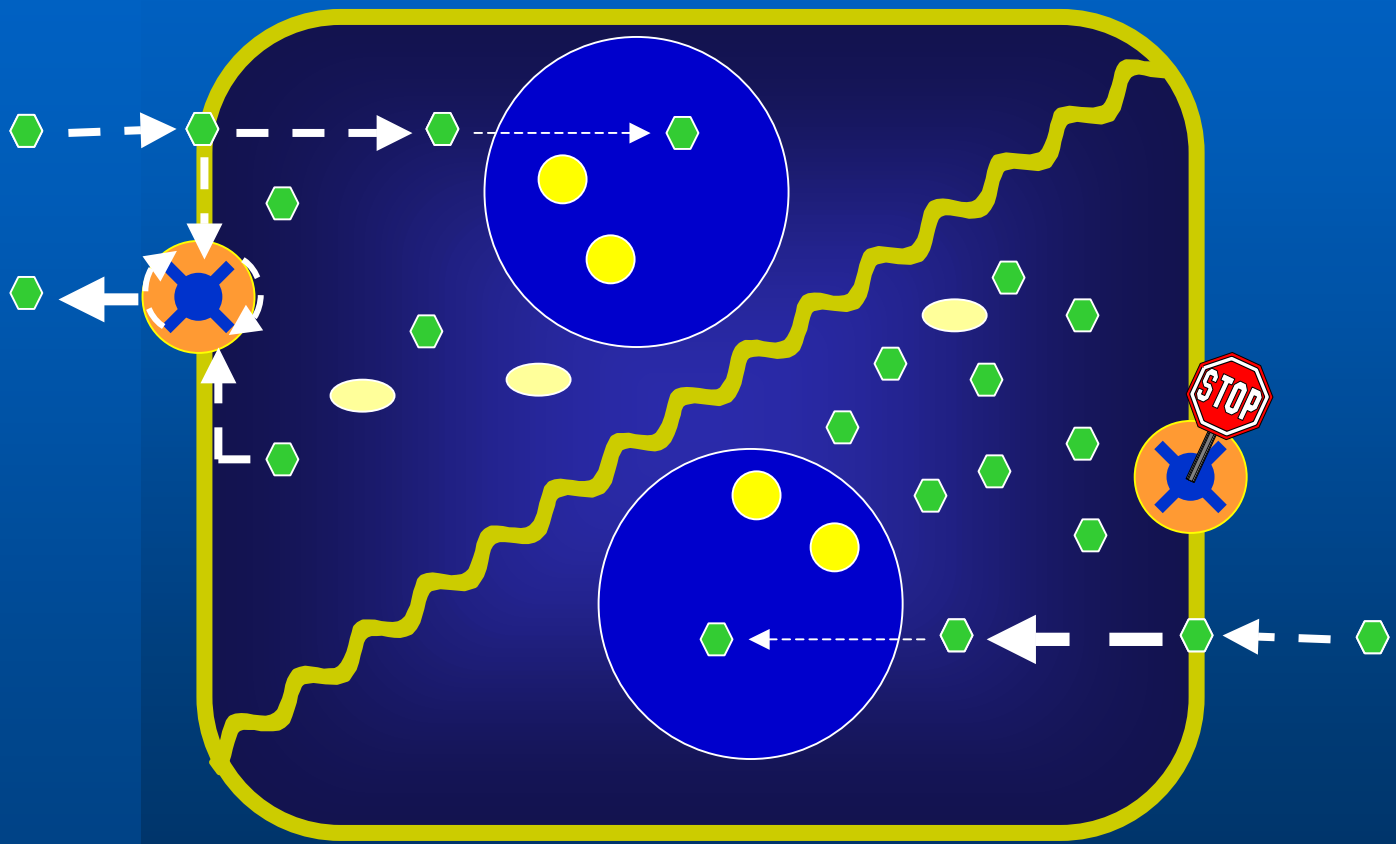


extracell. conc. 17 mg/L; probenecid 5 mM

Michot *et al*. AAC (2004) 48:2673-82

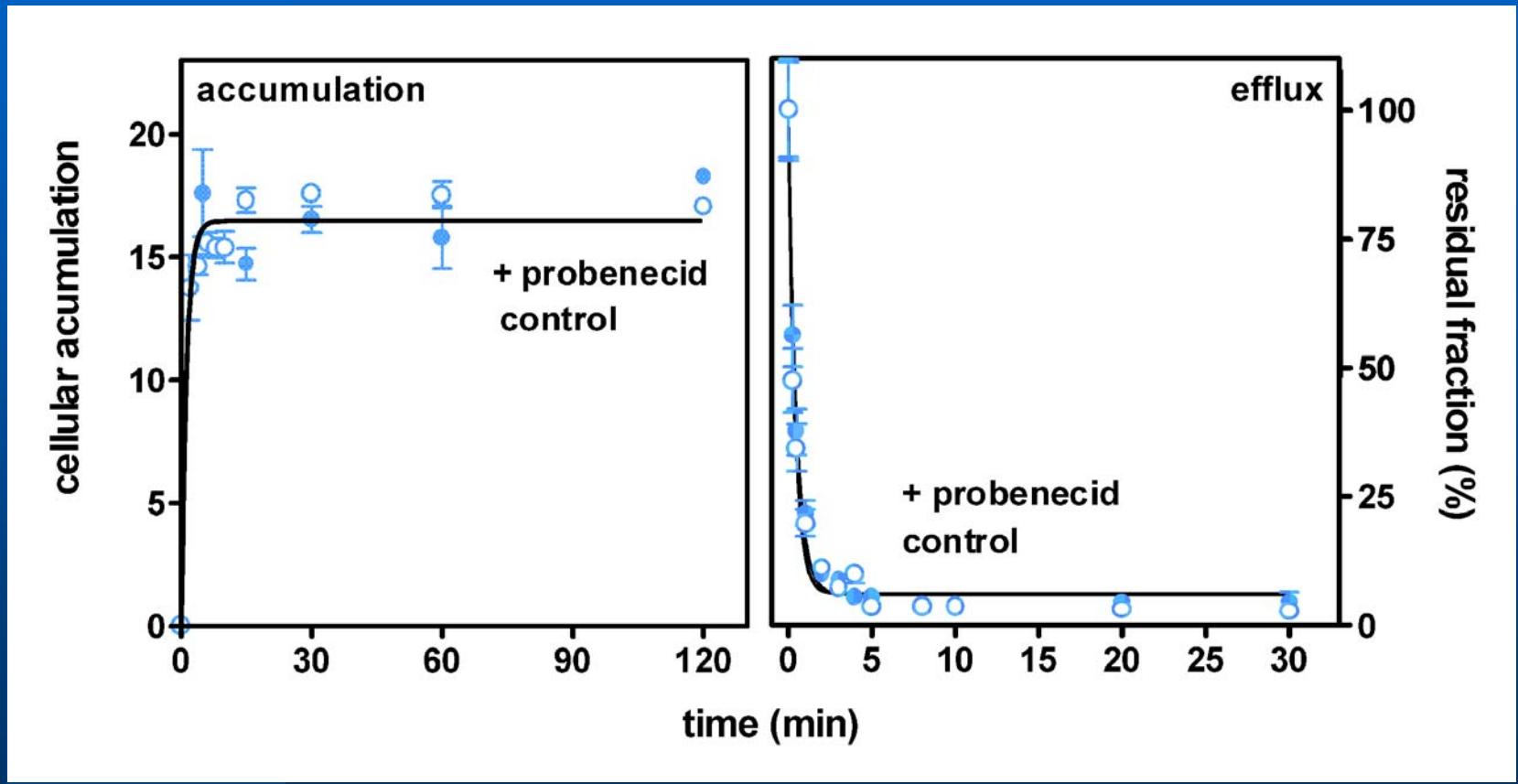
Ciprofloxacin, classical model

Kolaczkowski & Goffeau (1997) Pharmacol. Ther. 76:219-42



Kinetics of accumulation and efflux for moxifloxacin

neither accumulation nor efflux affected

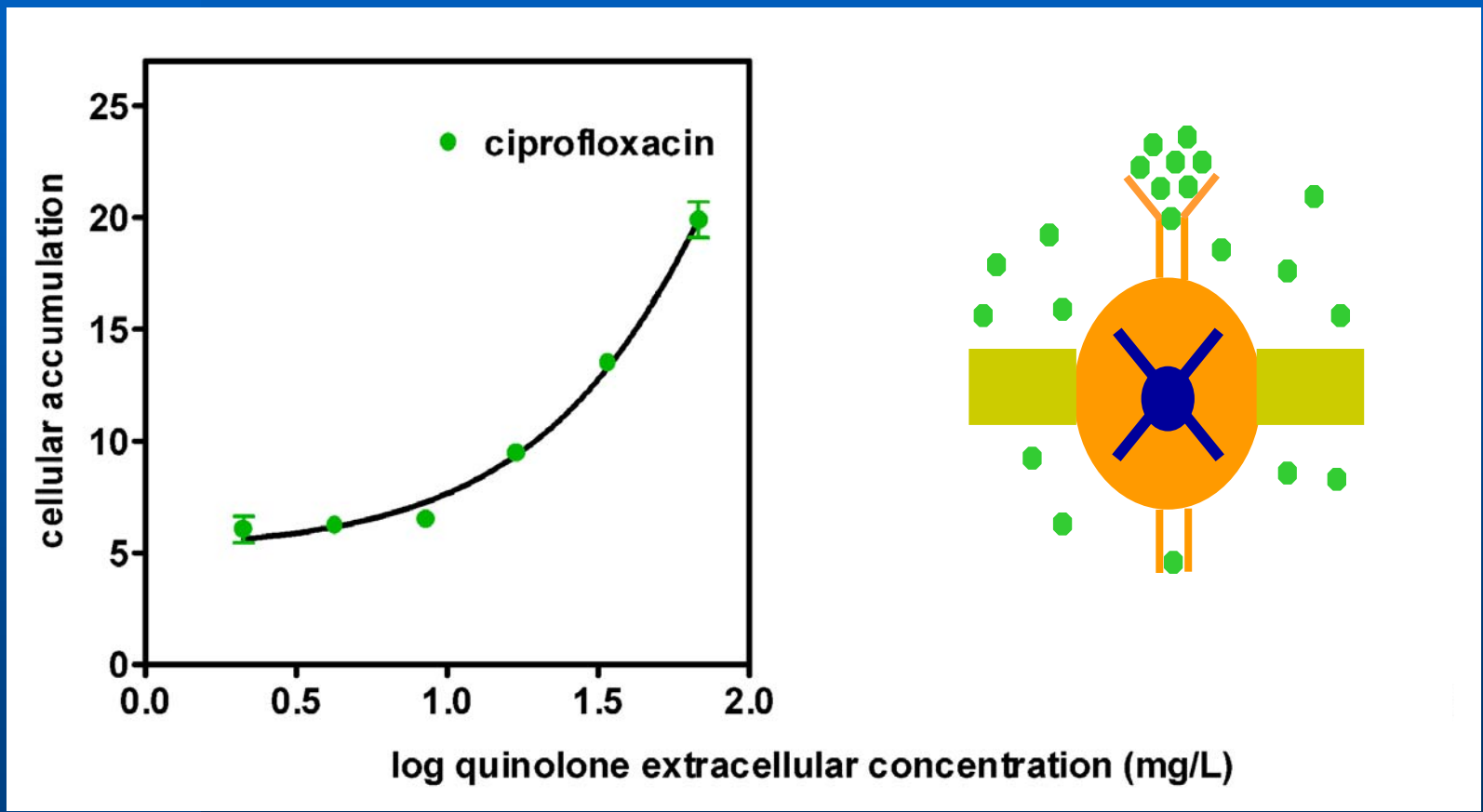


extracell. conc. 17 mg/L; probenecid 5 mM

Michot *et al*. AAC (2005) 49:2429-37

Quinolones as inhibitors of ciprofloxacin efflux

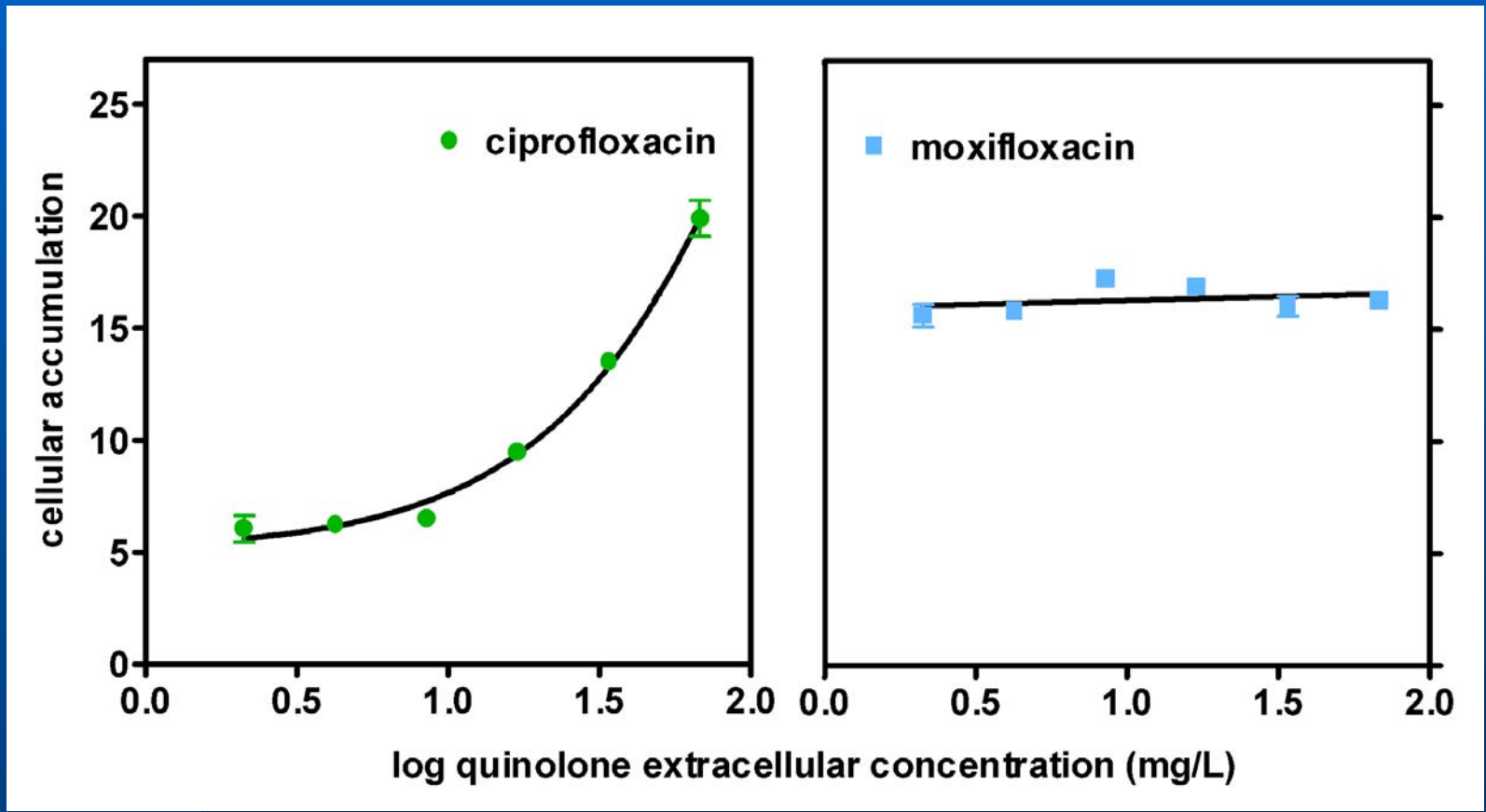
- ciprofloxacin efflux inhibited by ciprofloxacin



Michot *et al*. AAC (2005) 49:2429-37

Quinolones as inhibitors of ciprofloxacin efflux

- ciprofloxacin efflux inhibited by ciprofloxacin
- moxifloxacin not affected



Michot *et al*. AAC (2005) 49:2429-37

Quinolones as inhibitors of ciprofloxacin efflux

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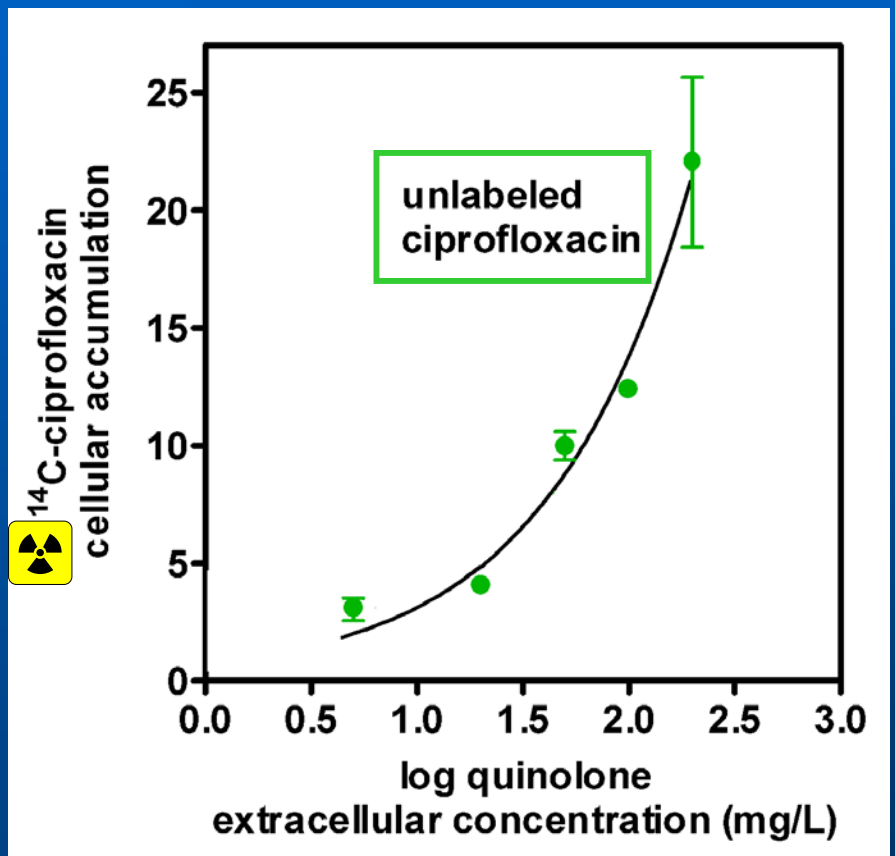
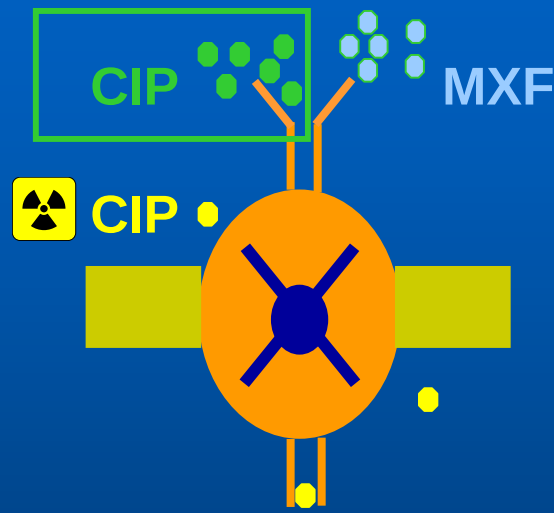
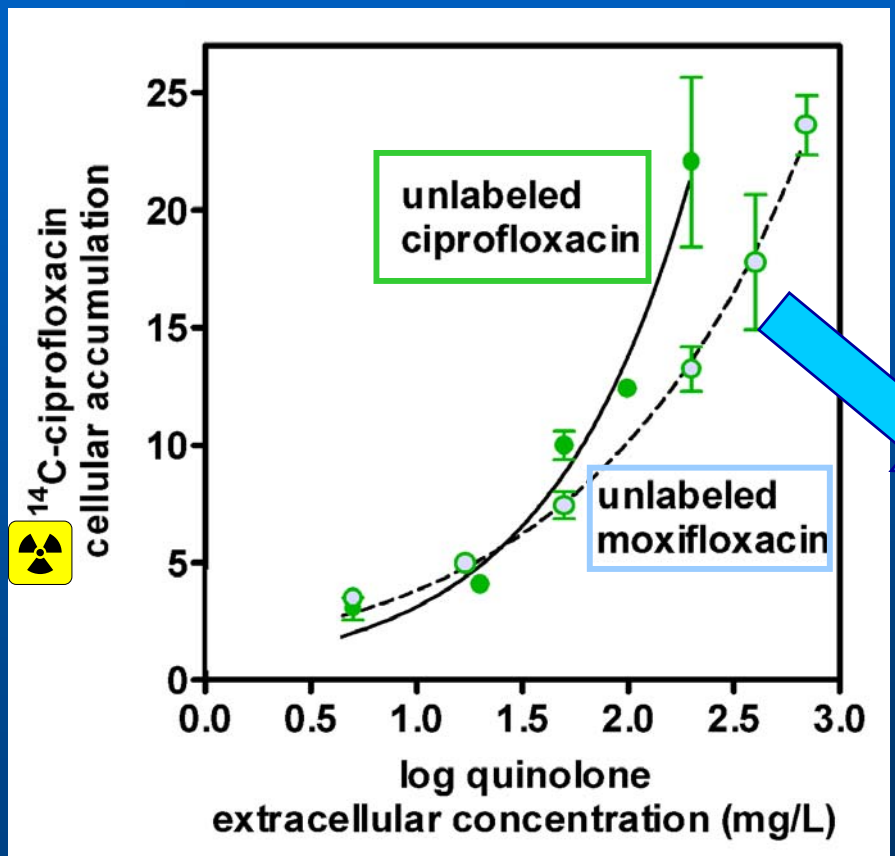


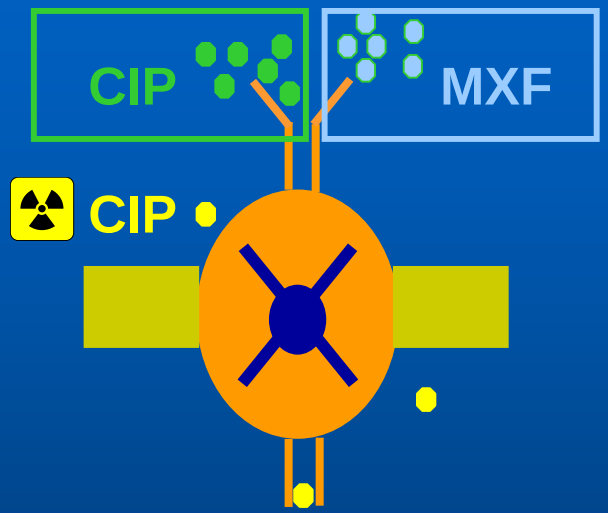


Michot *et al*. AAC (2005) 49:2429-37

Quinolones as inhibitors of ciprofloxacin efflux

- ciprofloxacin efflux inhibited by ciprofloxacin
  moxifloxacin



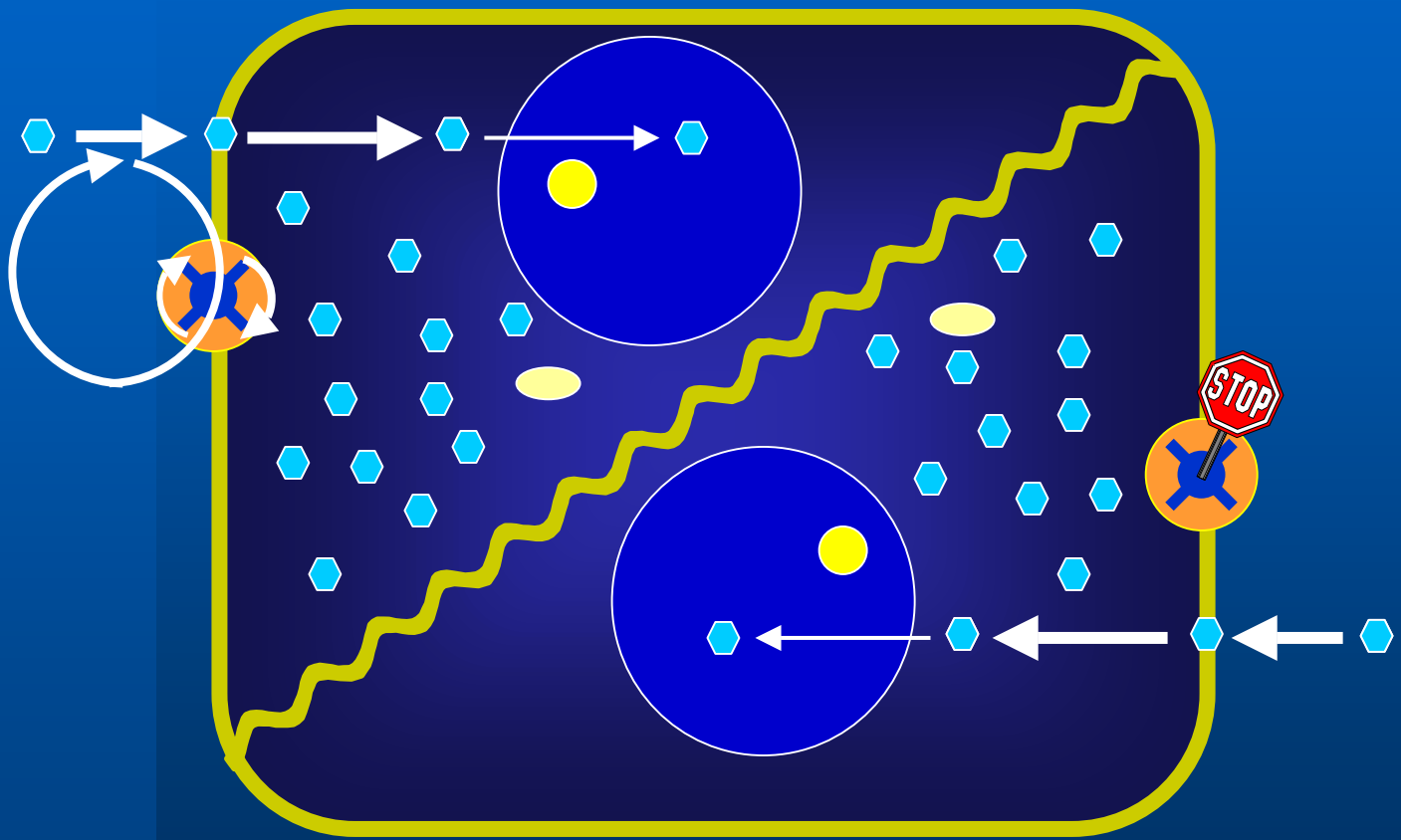


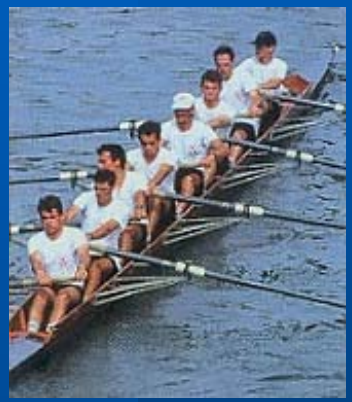
moxifloxacin also able to interact with the transporter !

Michot *et al*. AAC (2005) 49:2429-37

Moxifloxacin, ‘futile-cycle’ model

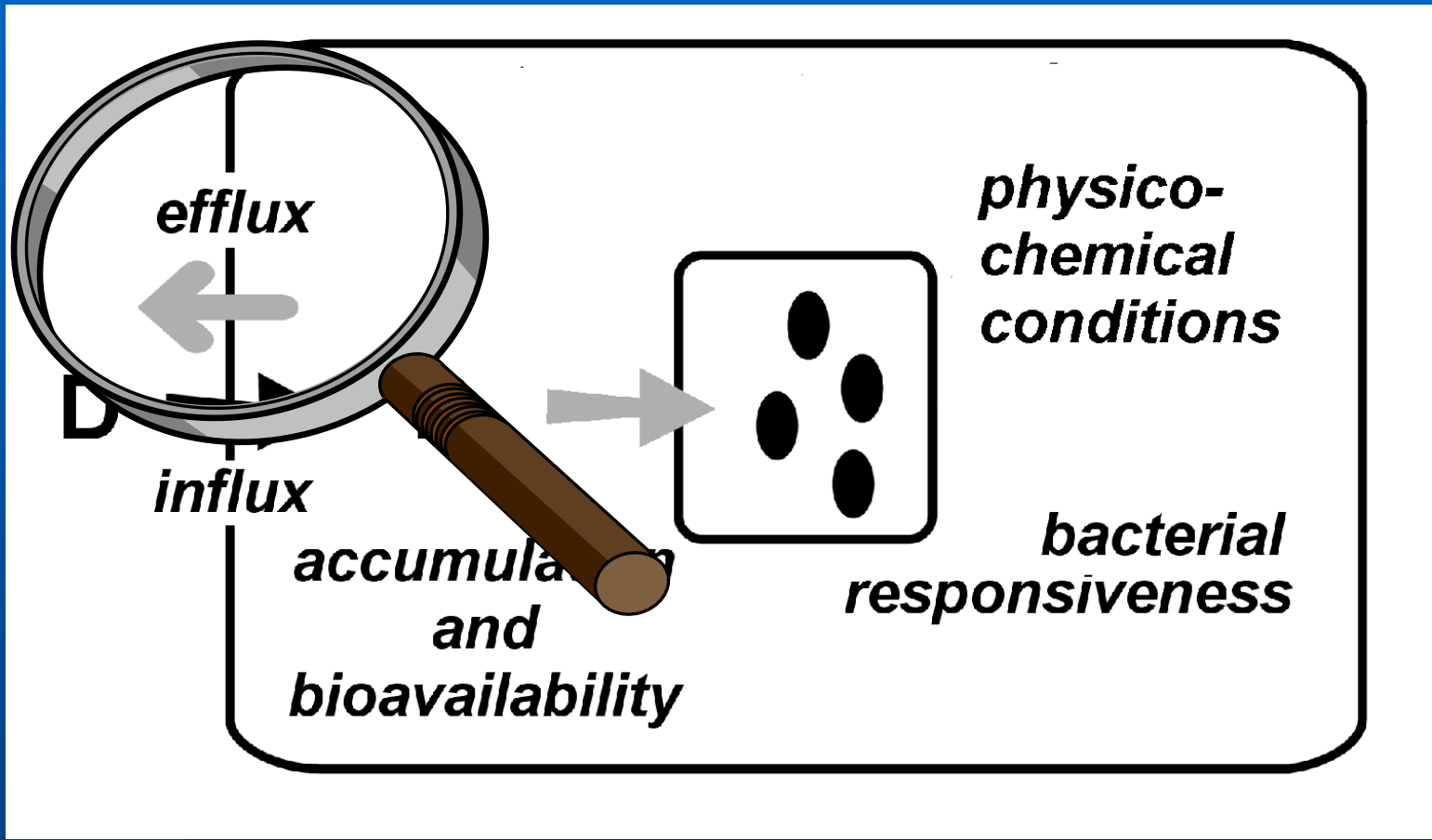
Eytan *et al*. (1996) JBC 271:12897-902





Influence of efflux pumps on antibiotic activity against intracellular infections

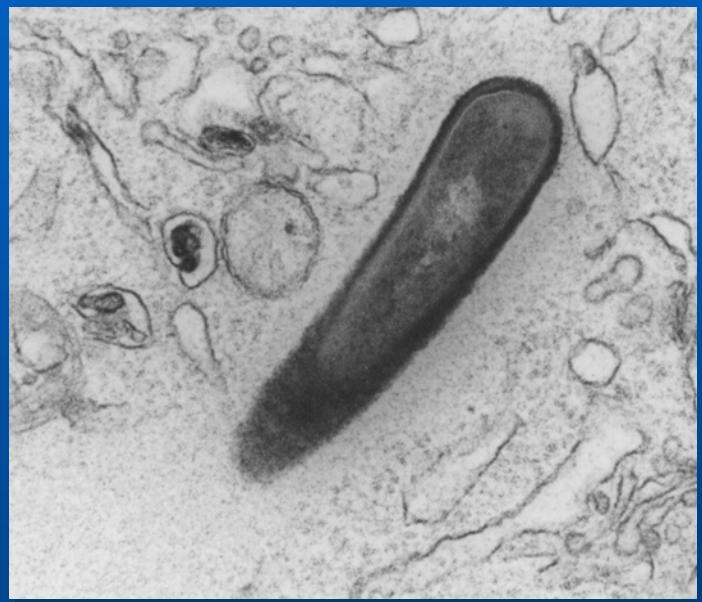
Efflux from eucaryotic cells and intracellular activity



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

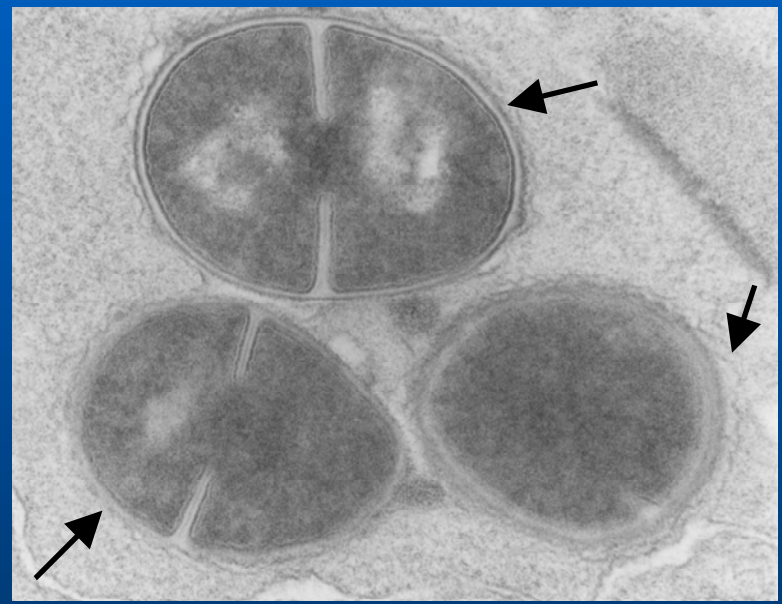
Models of intracellular infection

L. monocytogenes



cytosol

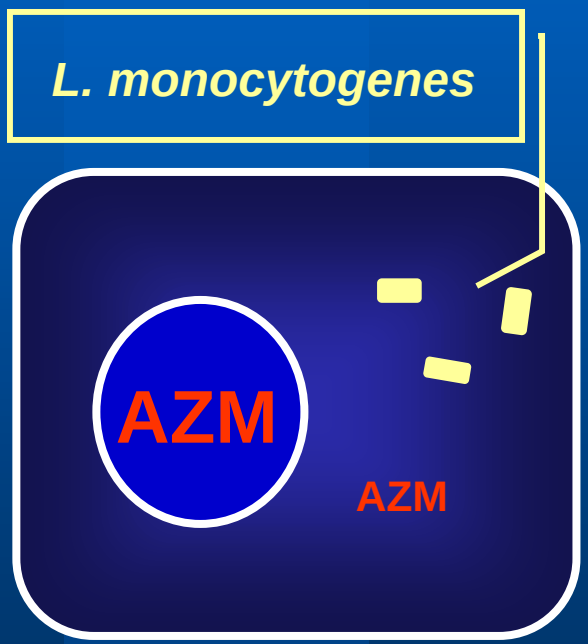
S. aureus

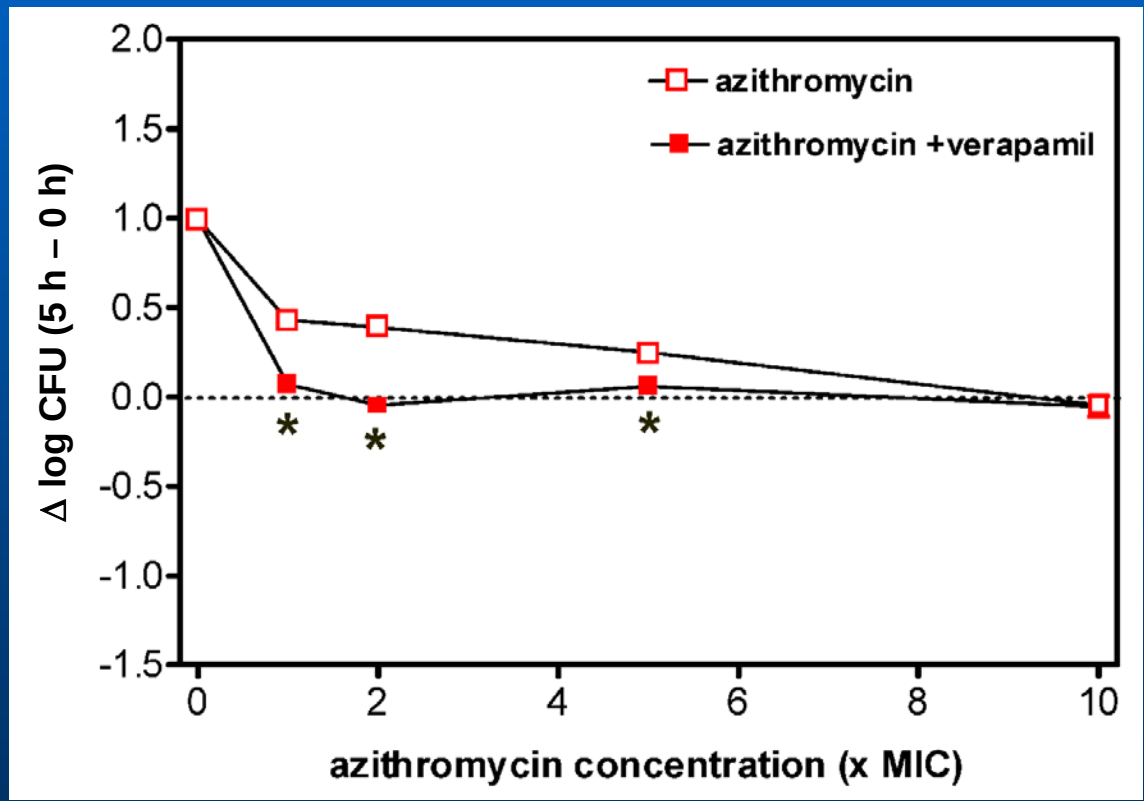


phagolysosomes

Influence of pump inhibitors on intracellular activity

azithromycin and *L. monocytogenes*



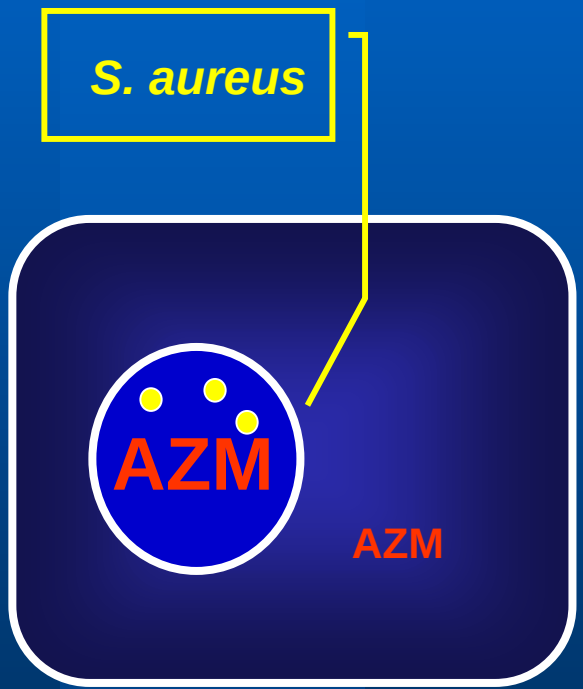


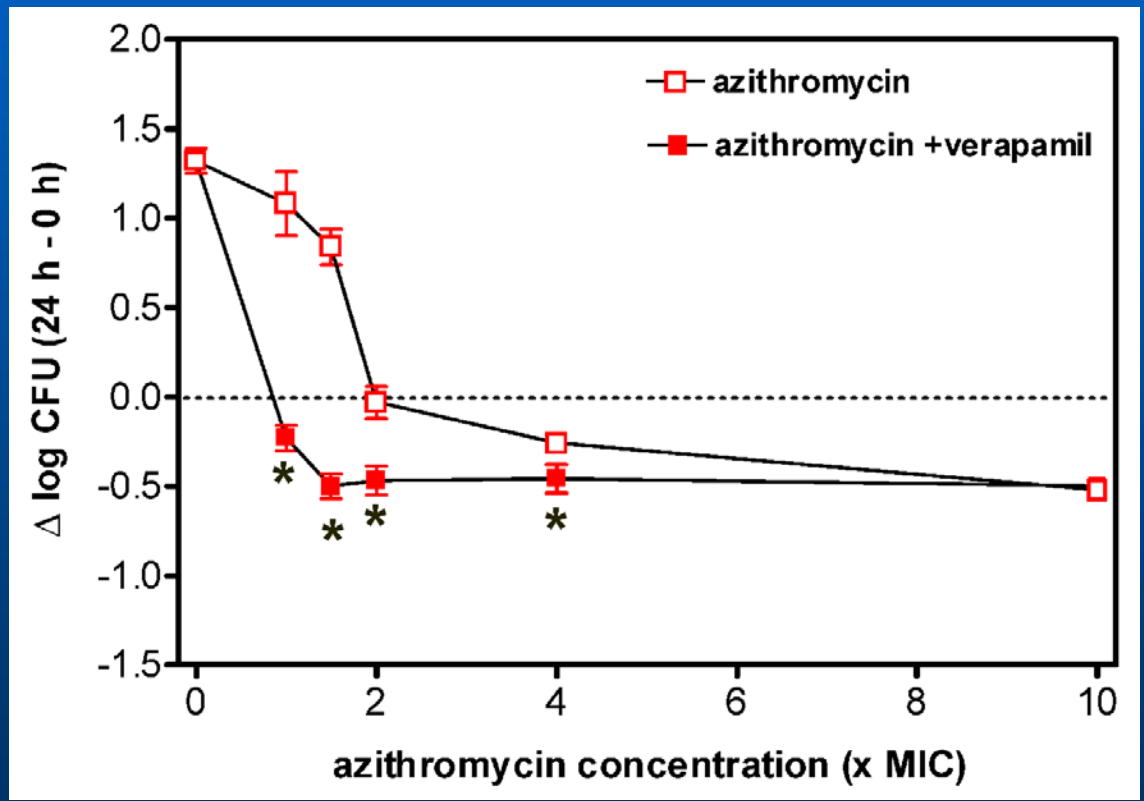
verapamil 20 µM; 24 h

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

Influence of pump inhibitors on intracellular activity

azithromycin and *S. aureus*



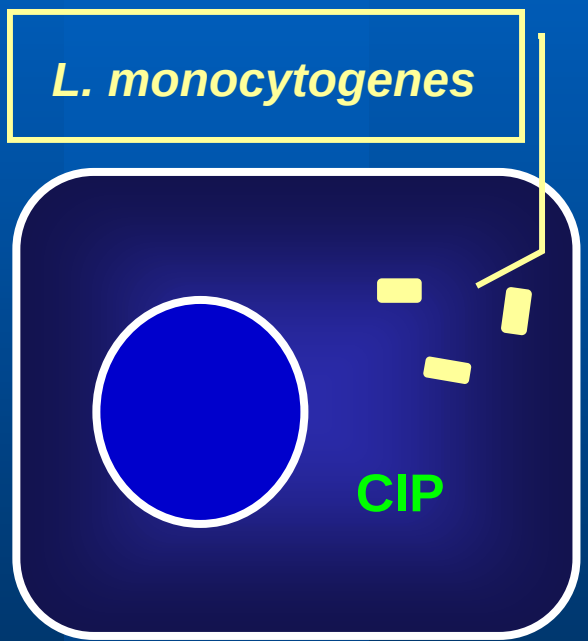


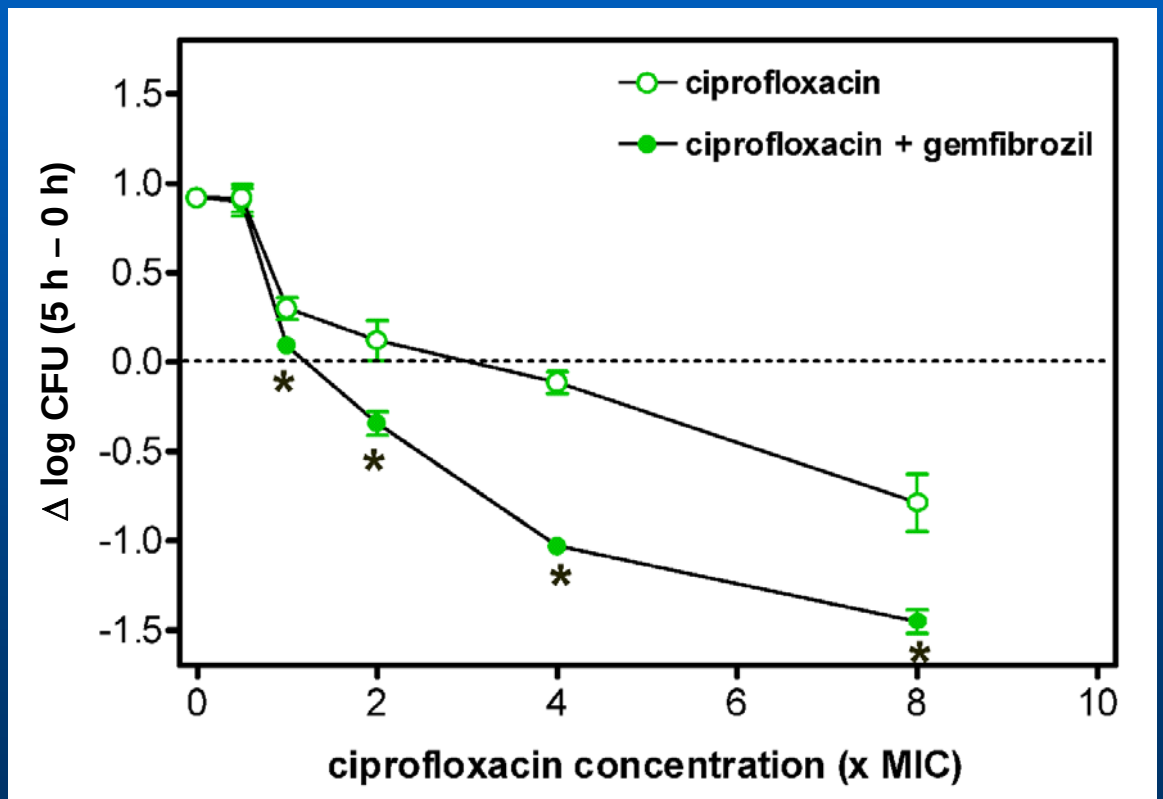
verapamil 20 µM; 24 h

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

Influence of pump inhibitors on intracellular activity

ciprofloxacin and *L. monocytogenes*



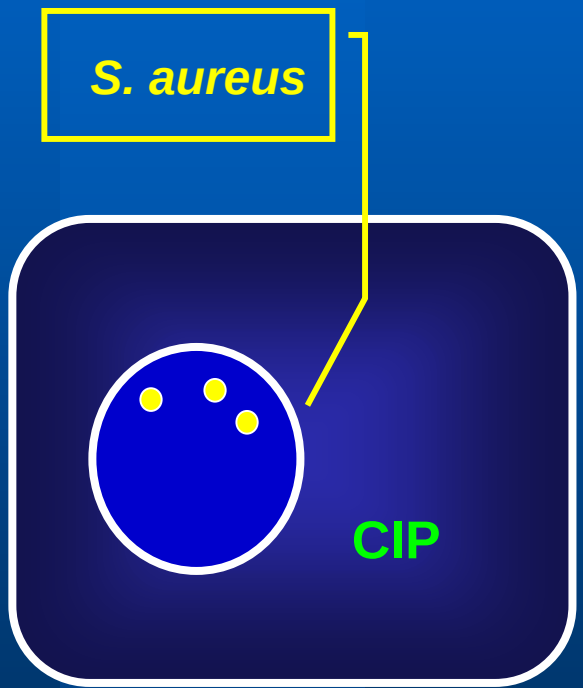


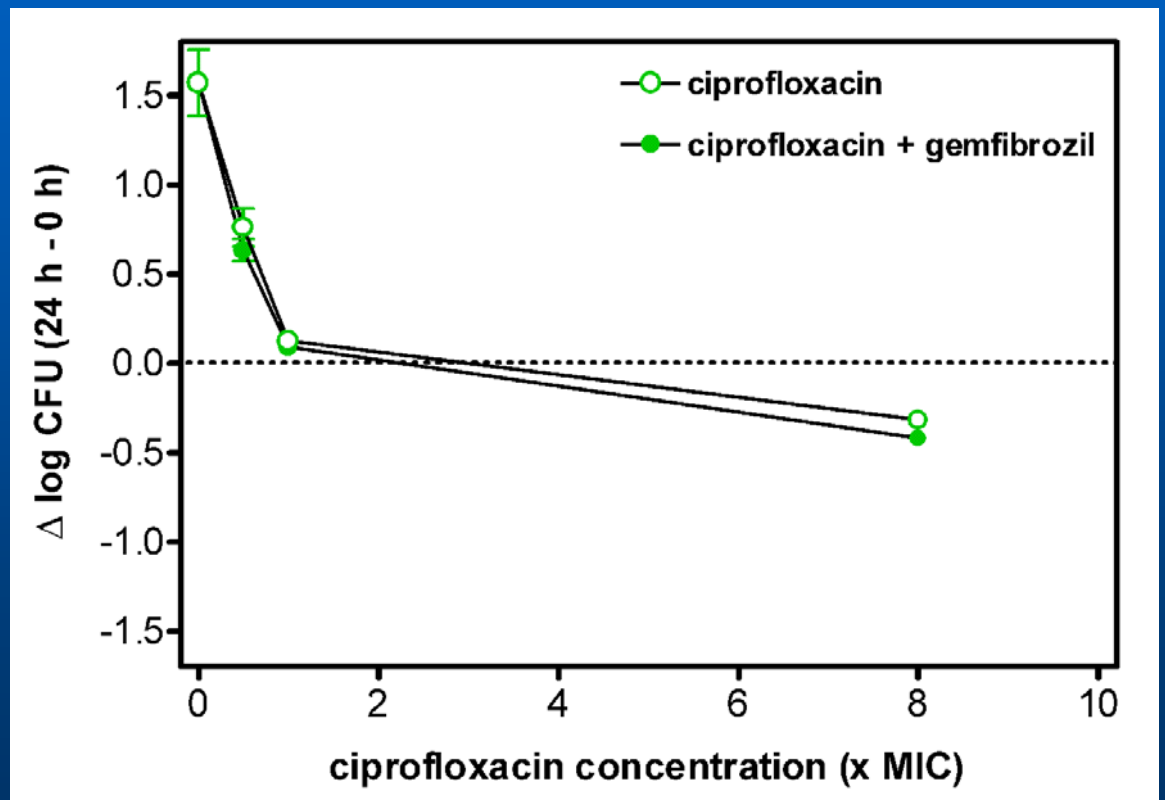
gemfibrozil 250 µM; 24 h

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

Influence of pump inhibitors on intracellular activity

ciprofloxacin and *S. aureus*



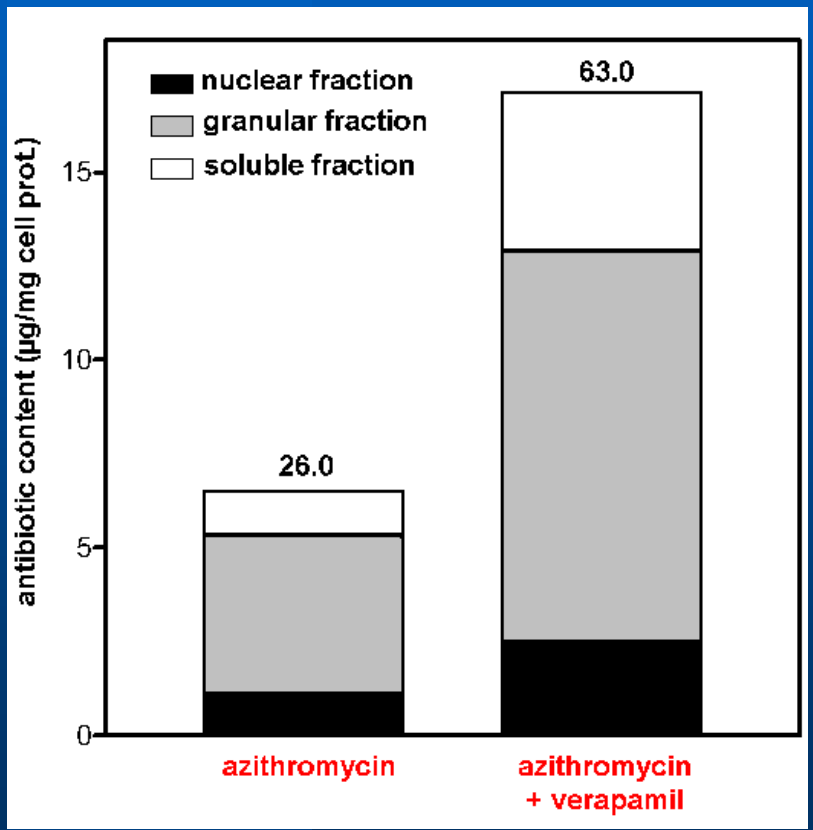


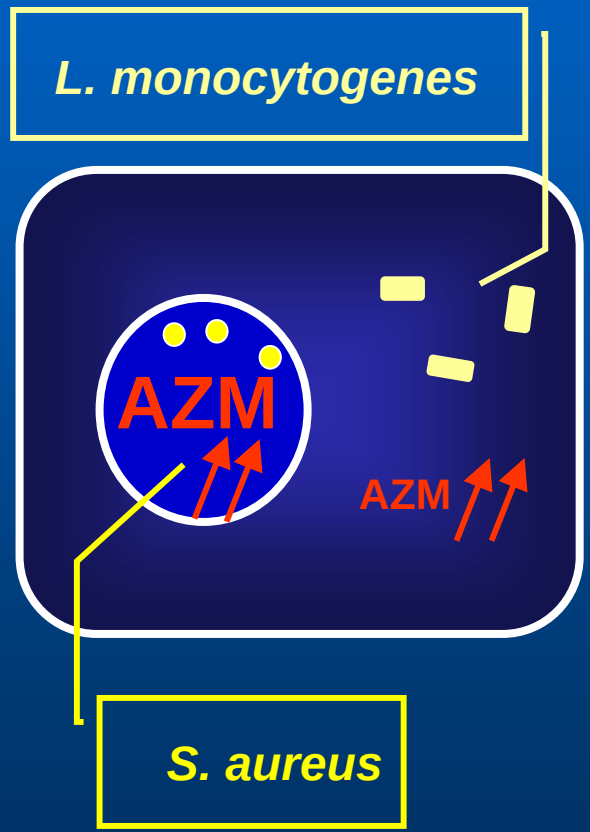
gemfibrozil 250 µM; 24 h

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

Influence of pump inhibitors on antibiotic distribution

verapamil enhances azithromycin concentration in cytosol and vacuoles

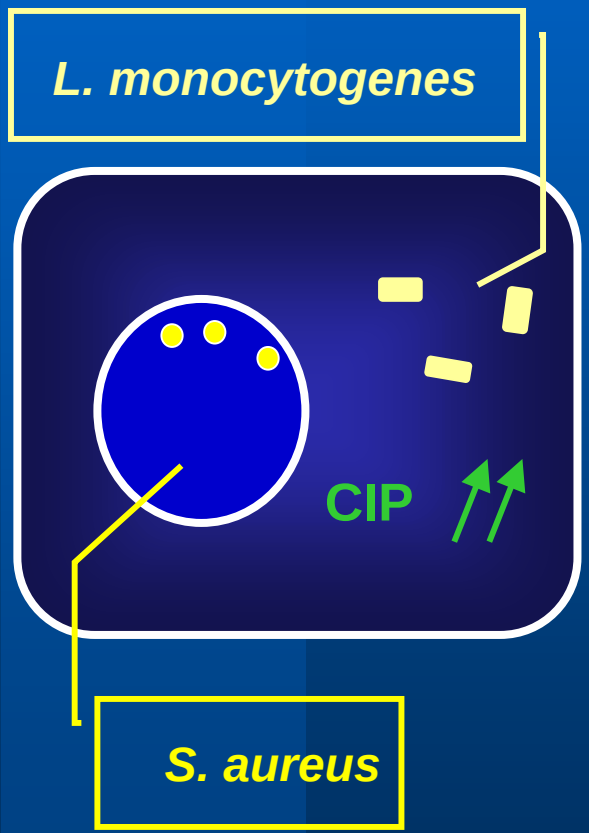


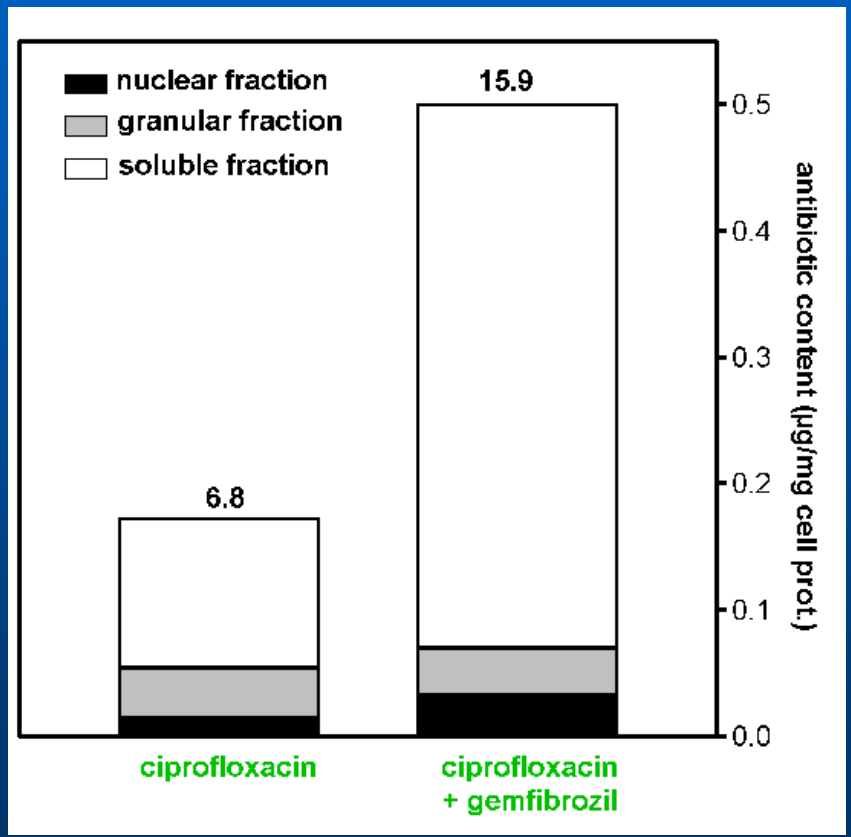


Seral *et al* (2003) JAC 51:1167-73

Influence of pump inhibitors on antibiotic distribution

gemfibrozil enhances ciprofloxacin cytosolic content





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

Conclusion

constitutive efflux of antibiotics in macrophages

pharmacokinetics:
suboptimal cellular accumulation

pharmacodynamics:
suboptimal intracell. activity

pharmacology:
differences in affinity within a AB class

pharmacokinetics:
wide spectrum transporters

resistance ?

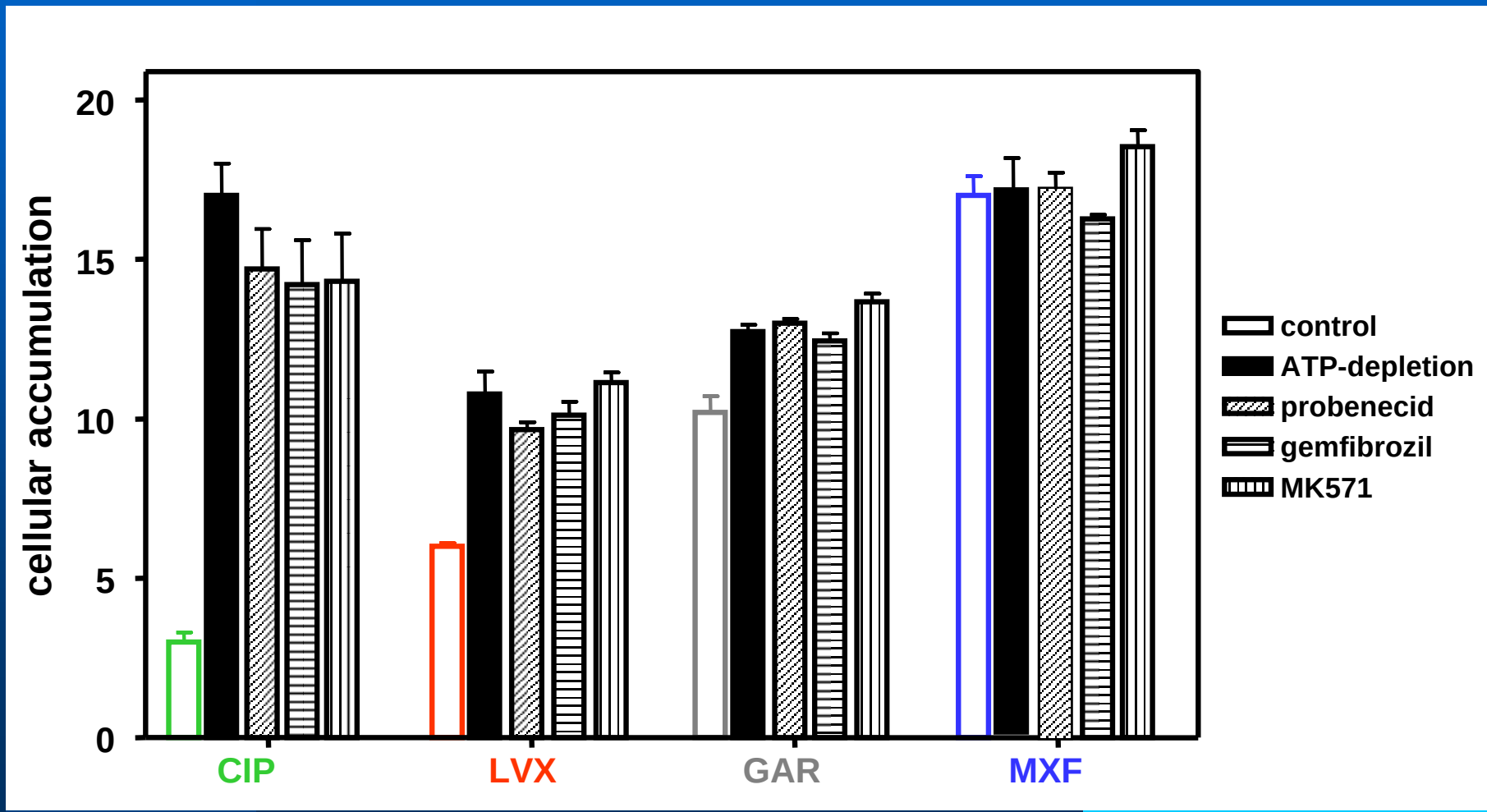
drug interactions ?

Perspectives for the future of chemotherapy

Perspectives for the future of chemotherapy

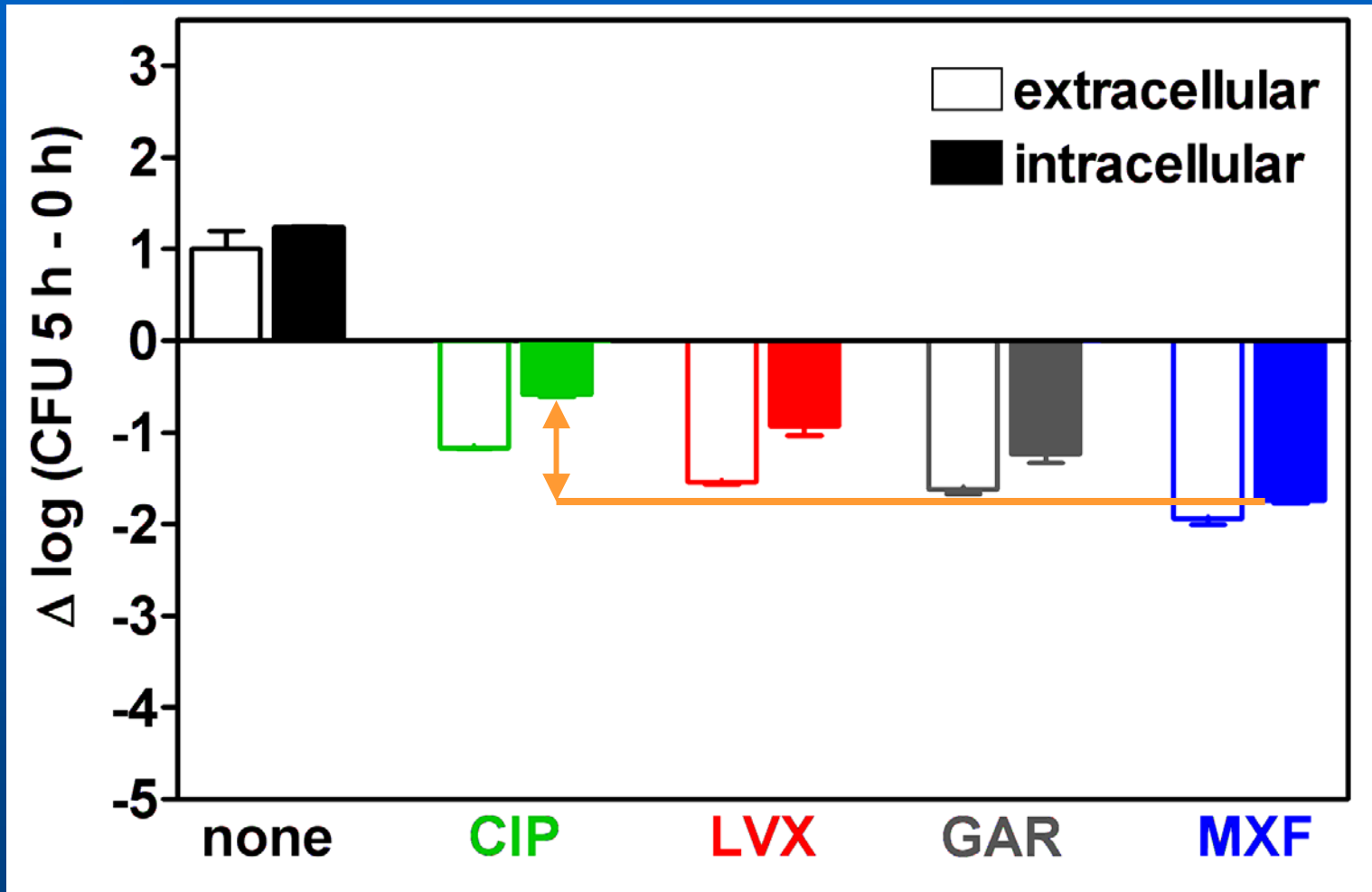
- use of poor substrates of efflux pumps (moxi vs cipro)
- development of specific inhibitors of efflux pumps
- caution for « cross – resistance » with other substrates (over – expression of efflux pumps)

Quinolones differ by the susceptibility to efflux



Michot *et al*. AAC (2005) 49:2429-37

Quinolones differ by their activity against intracellular Listeria



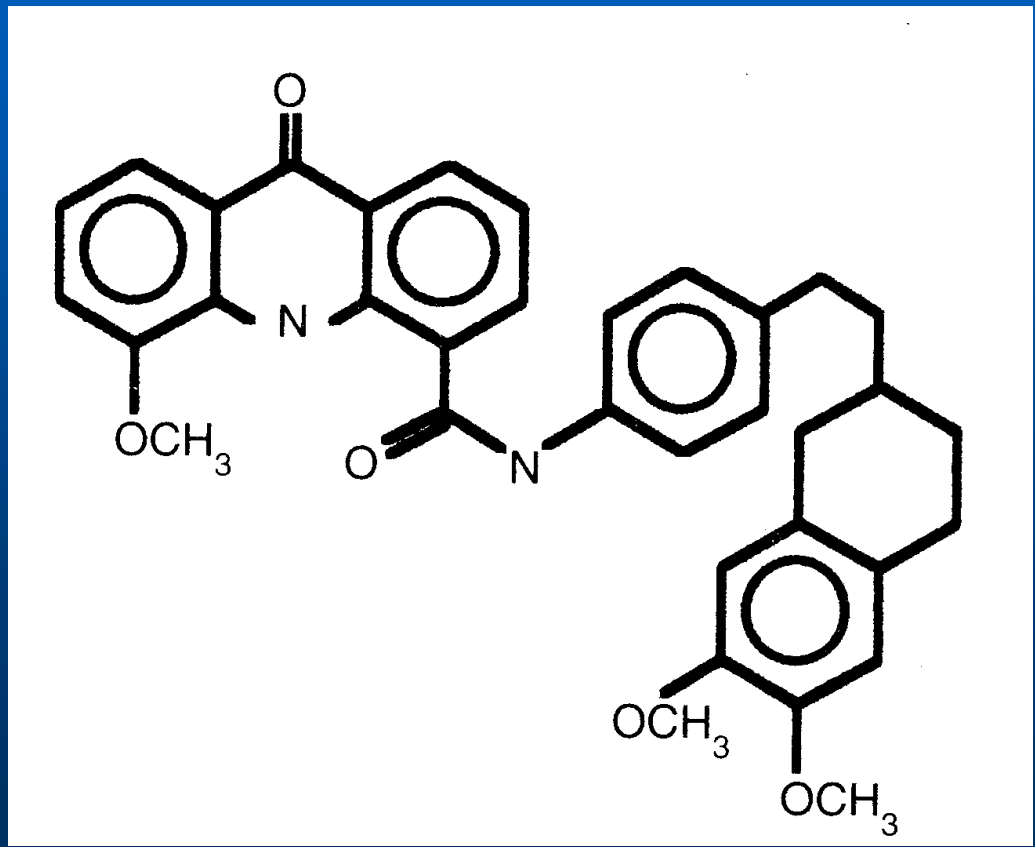
Seral *et al*. JAC (2005) 5:511-7

Perspectives for the future of chemotherapy

- use of poor substrates of efflux pumps (moxi vs cipro)
- development of specific inhibitors of efflux pumps
- caution for « cross – resistance » with other substrates (over – expression of efflux pumps)

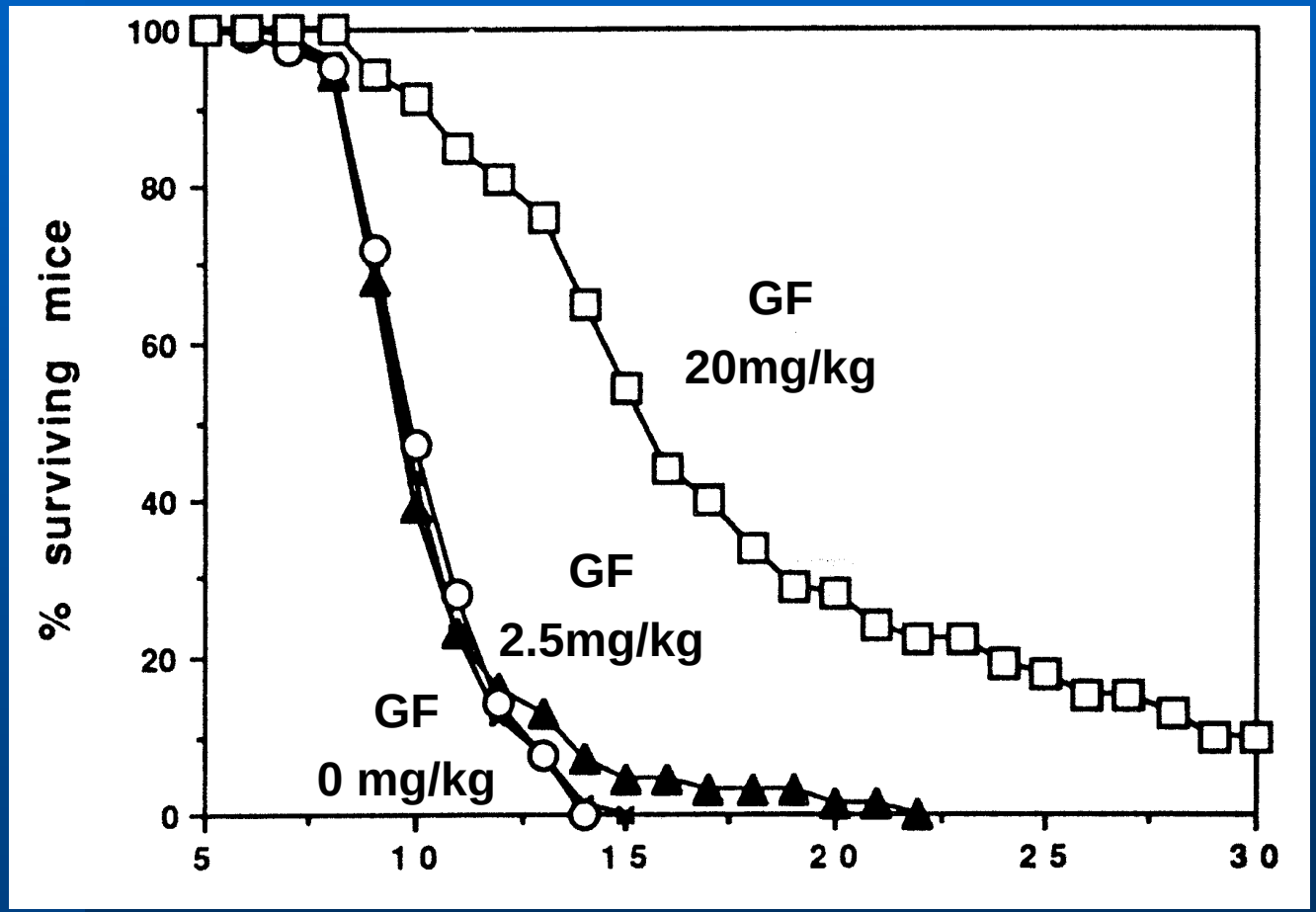
Specific inhibitors

GF120918 : a specific MDR inhibitor currently in clinical evaluation in cancer chemotherapy



Specific inhibitors

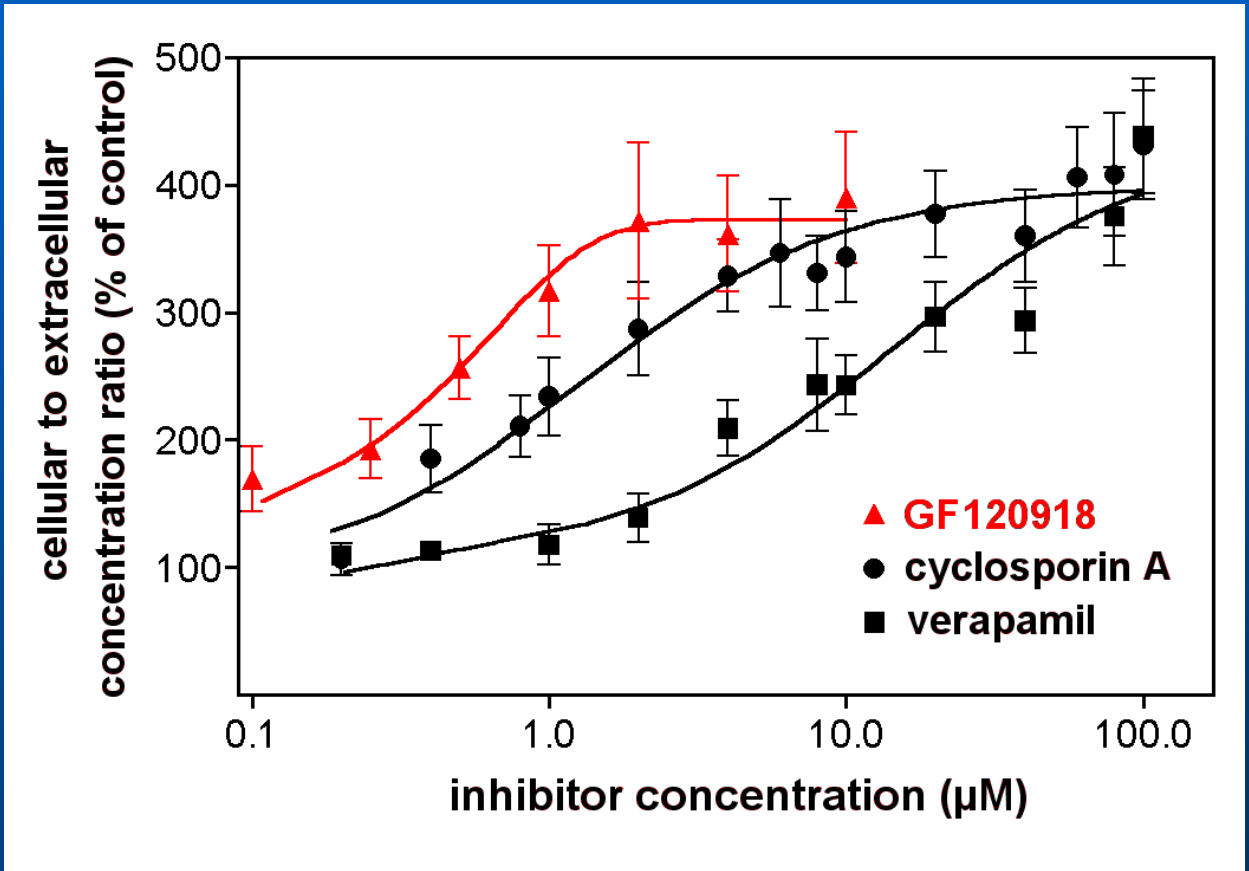
GF120918 increases efficacy of doxorubicin in mice with resistant tumors



Hyafil *et al*, Cancer Res. (1993) 53: 4595-4602

Specific inhibitors

GF120918 is more potent than verapamil to increase azithromycin cellular accumulation



Seral *et al*, AAC (2003) 47:1047-1051

Perspectives for the future of chemotherapy

- use of poor substrates of efflux pumps (moxi vs cipro)
- development of specific inhibitors of efflux pumps
- caution for « cross – resistance » with other substrates (over – expression of efflux pumps)

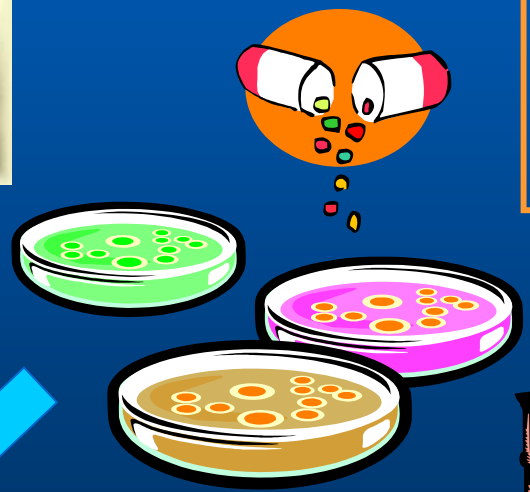
Over-expression of efflux pumps as mechanism of resistance

anticancer agent

antibiotic ?????

Over-expression of efflux pumps as mechanism of resistance

How to get resistant cells ?



- step-wise increase in CIP concentration
- several passages at each step

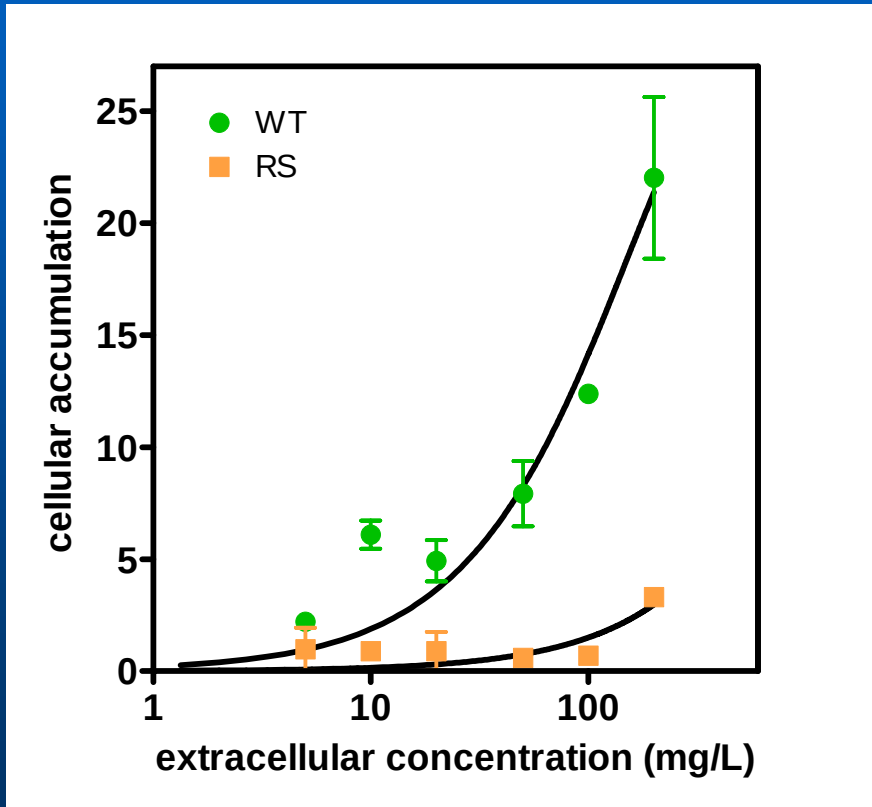
time and drug consumming!

multifactorial multidrug resistance

Gottesman *et al*, Methods Enzymol. (1998) 292: 248-58

Over-expression of efflux pumps as mechanism of resistance

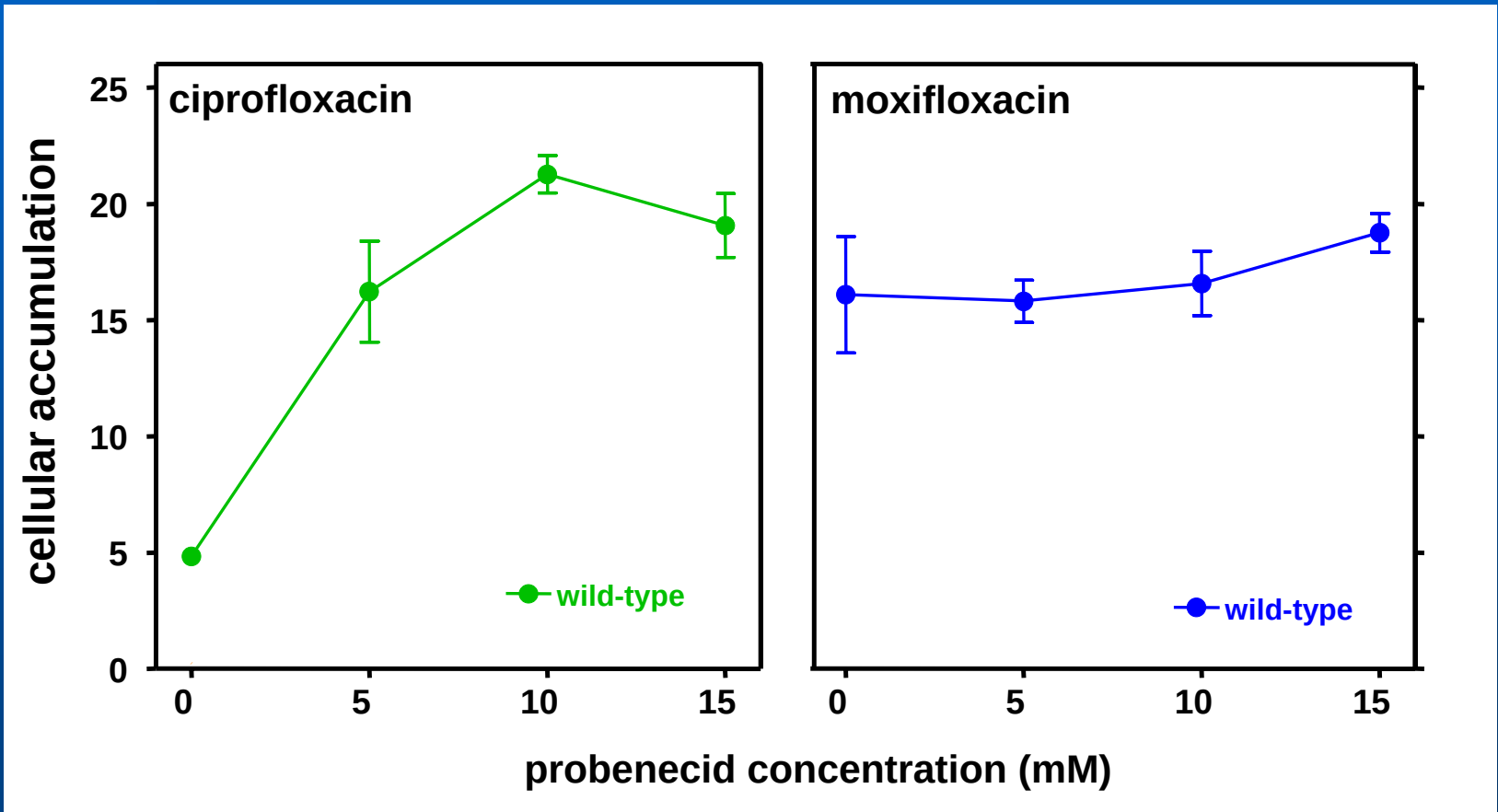
Ciprofloxacin accumulation is reduced in resistant cells



Heremans *et al*. ICAAC 2004

Over-expression of efflux pumps as mechanism of resistance

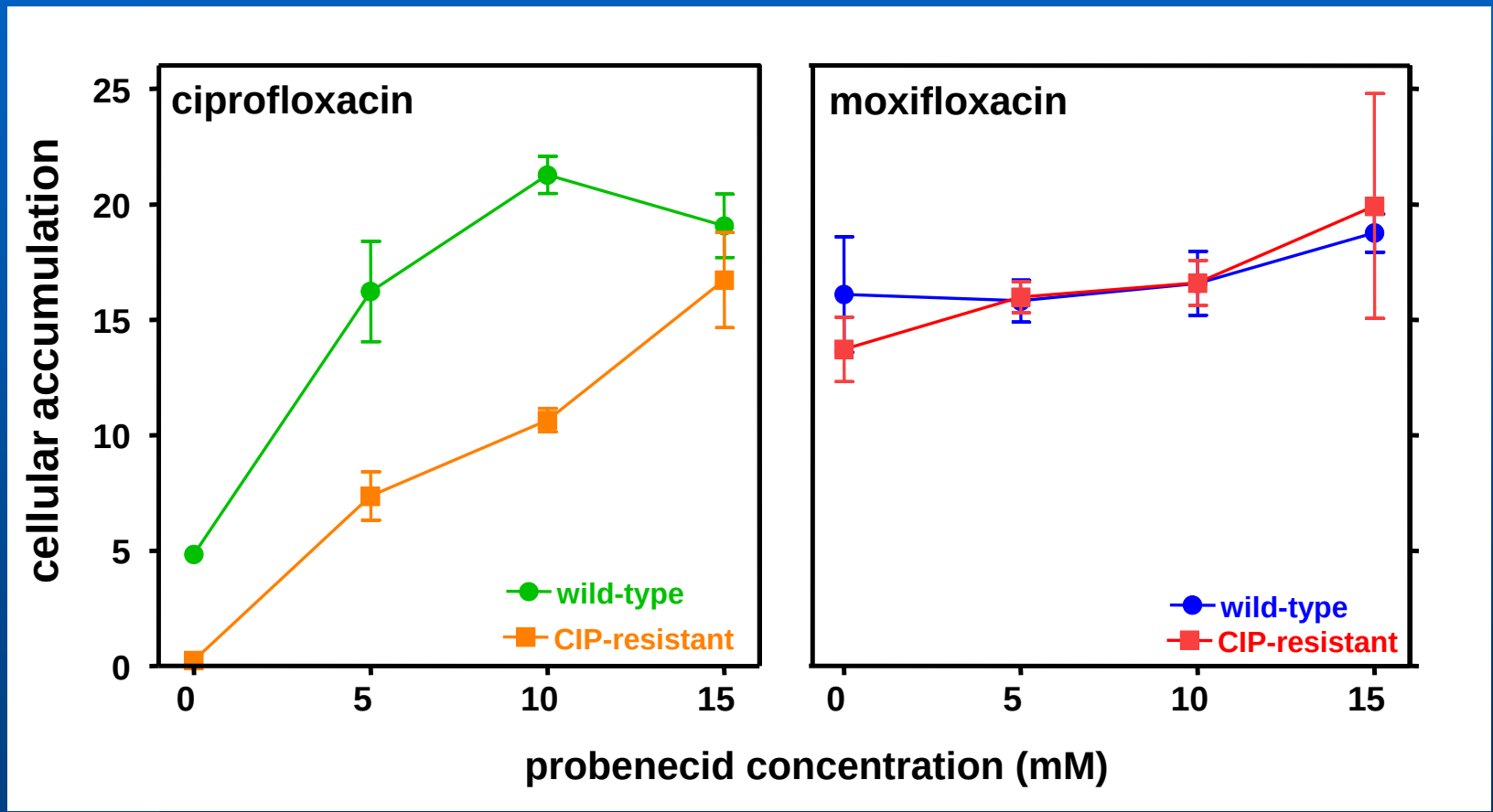
influence of probenecid on quinolone accumulation in wild-type cells



Heremans *et al*. ICAAC 2004

Over-expression of efflux pumps as mechanism of resistance

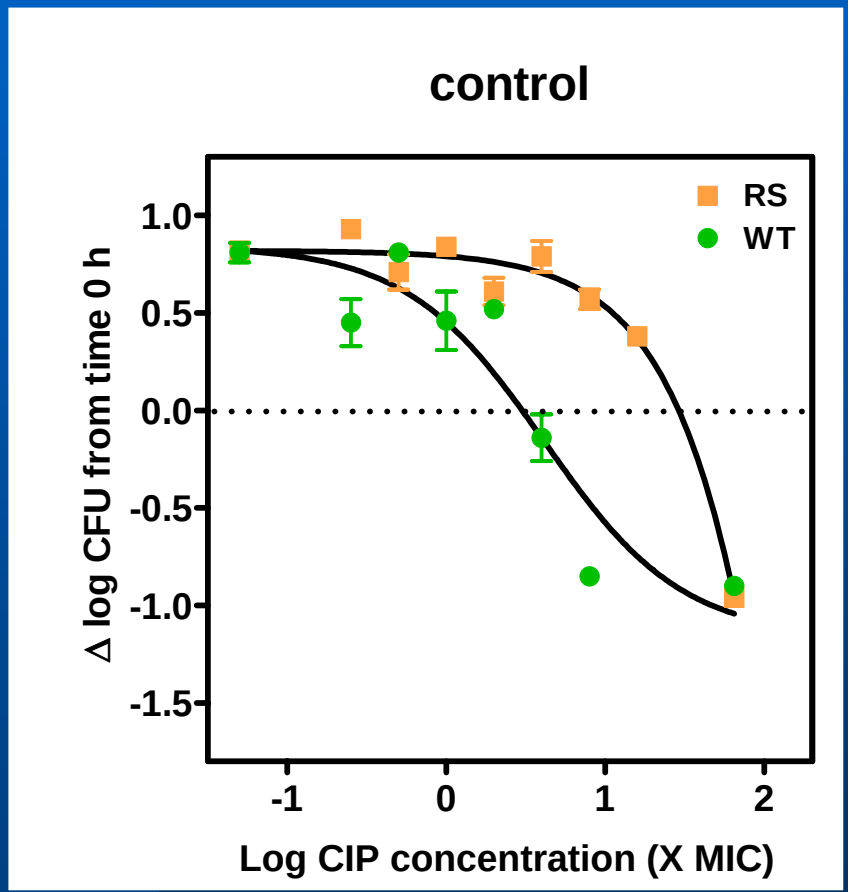
influence of probenecid on quinolone accumulation in wild-type and CIP-resistant cells



Heremans *et al*. ICAAC 2004

Over-expression of efflux pumps as mechanism of resistance

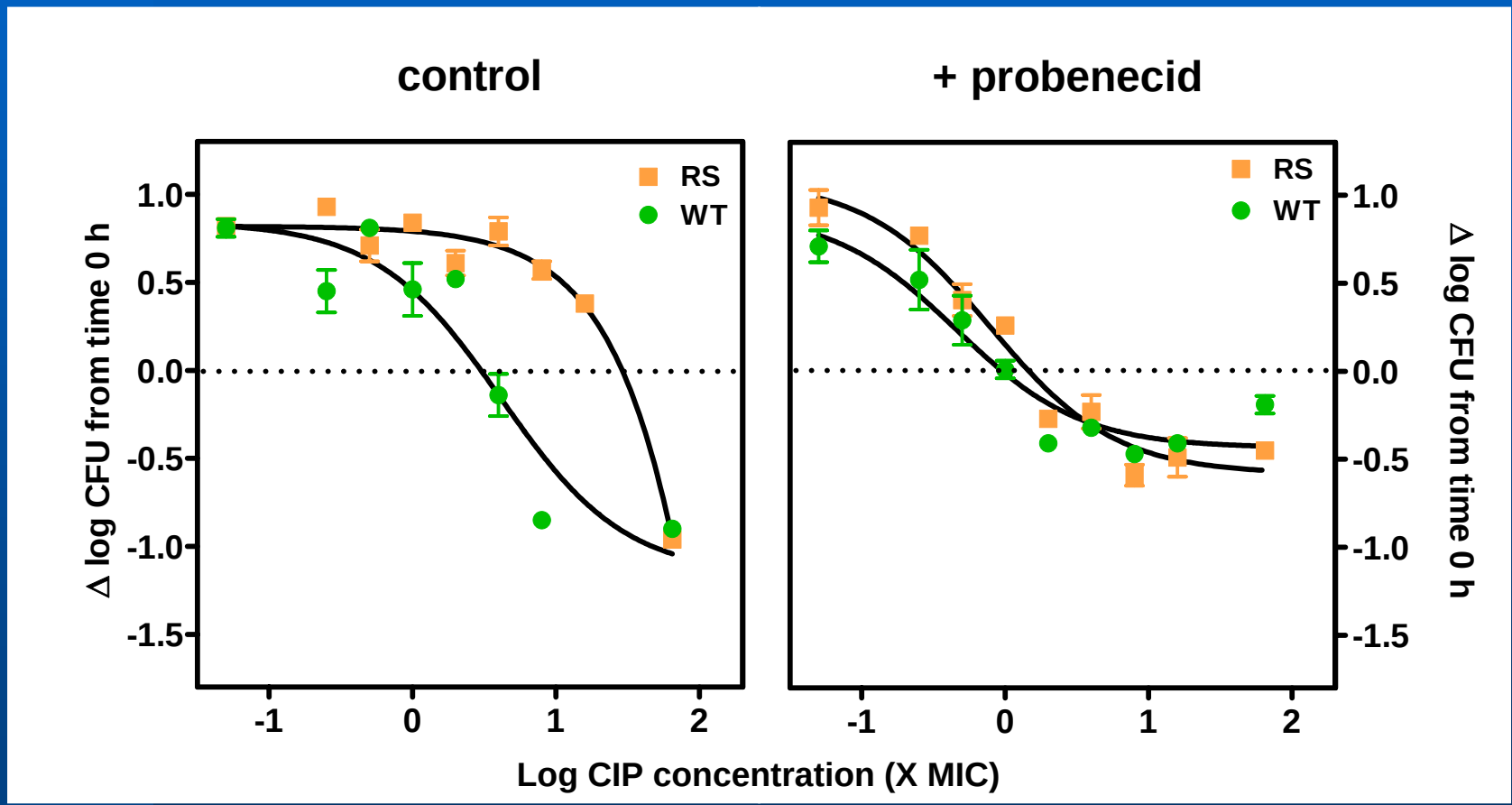
CIP is ineffective in CIP-resistant cells infected by *L. monocytogenes*



Heremans *et al*. ECCMID 2005

Over-expression of efflux pumps as mechanism of resistance

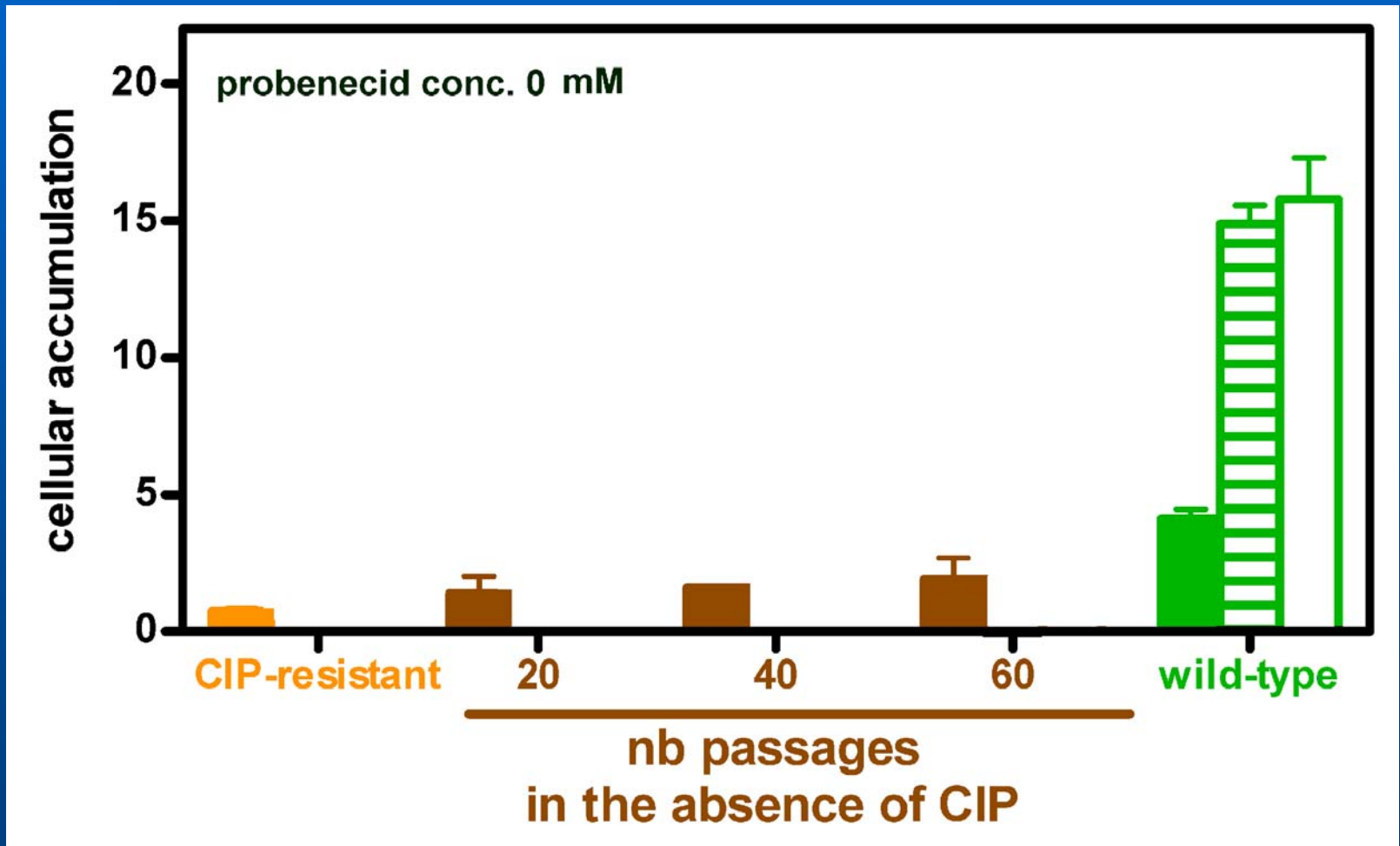
Probenecid restores CIP activity in CIP-resistant cells infected by *L. monocytogenes*



Heremans *et al*. ECCMID 2005

Over-expression of efflux pumps as mechanism of resistance

the CIP-resistant phenotype is not easily reversible



Heremans *et al*. ICAAC 2004

Over-expression of efflux pumps as mechanism of resistance

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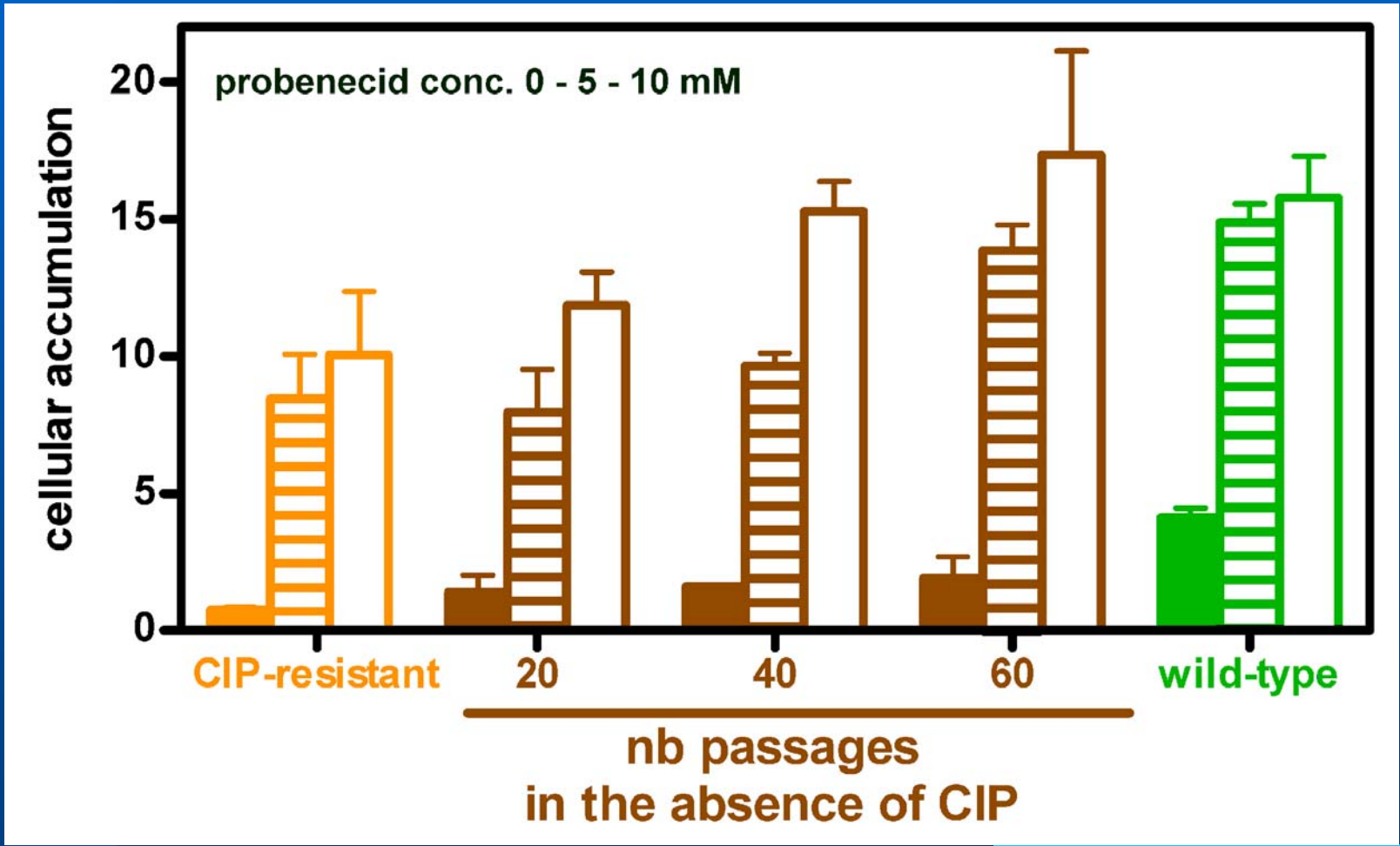


Heremans *et al*. ICAAC 2004

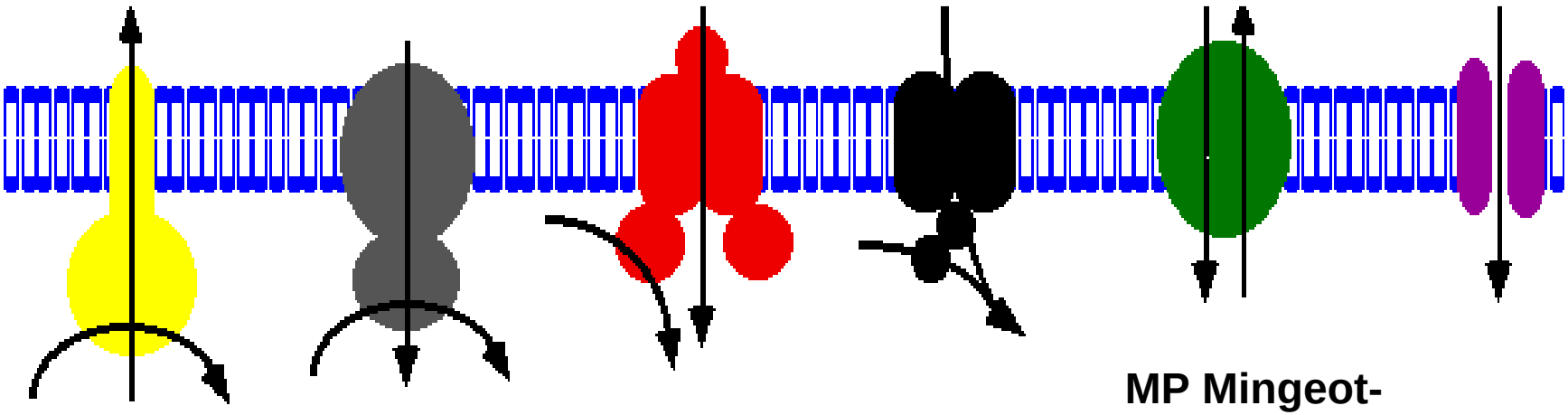
Take home message

constitutive efflux is part of the game

→Take it into account

- **in the choice of your « magic bullets » …**
- **for their optimal targeting**

Thanks to …



F Van Bambeke JM Michot C Seral M Heremans MP Mingeot-Leclercq PM Tulkens

come and see us at <www.md.ucl.ac.be/facm>