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

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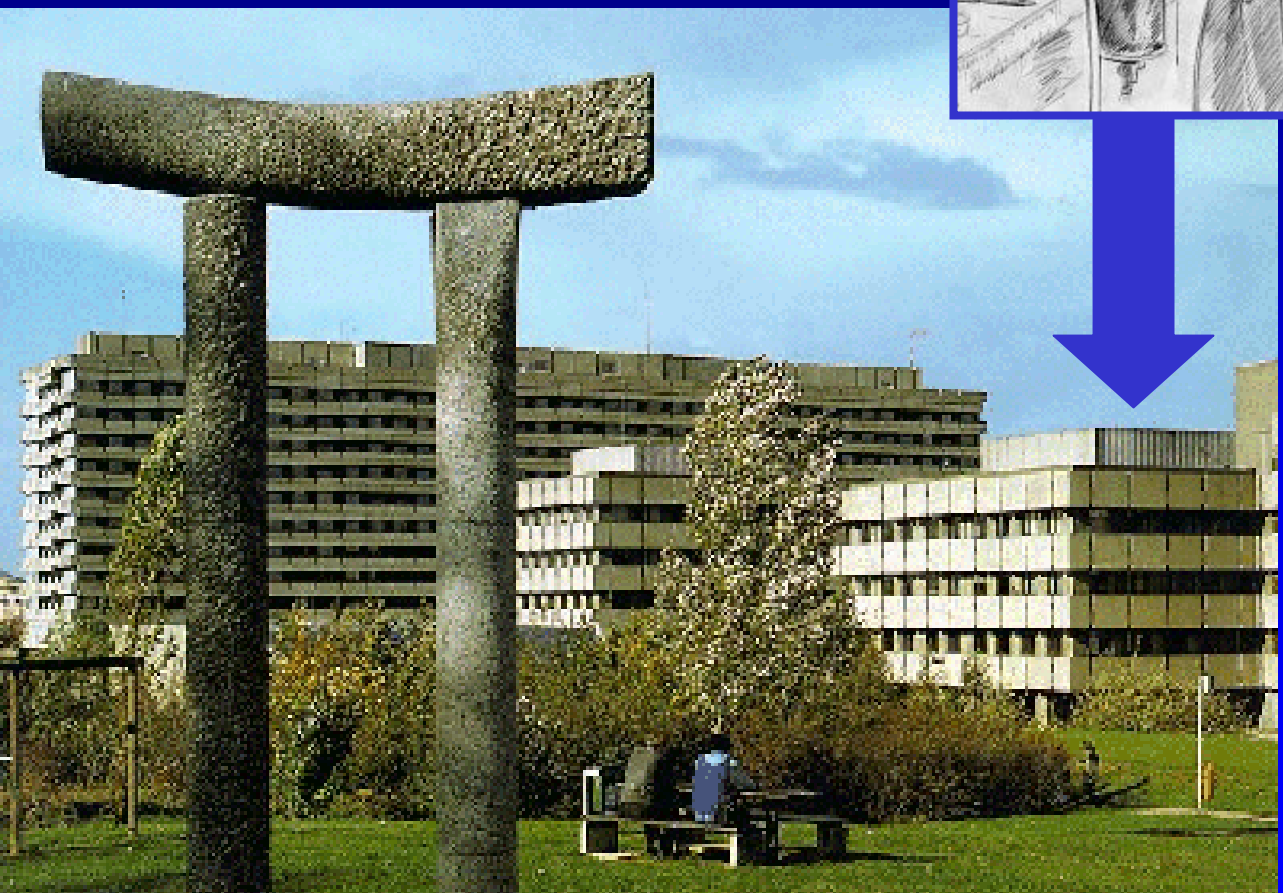
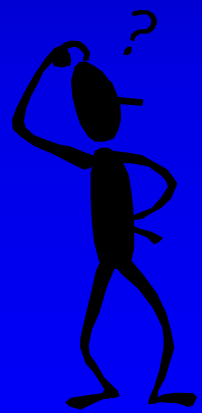
Bienvenue à
L'UCL-BRUXELLES
pharmacy

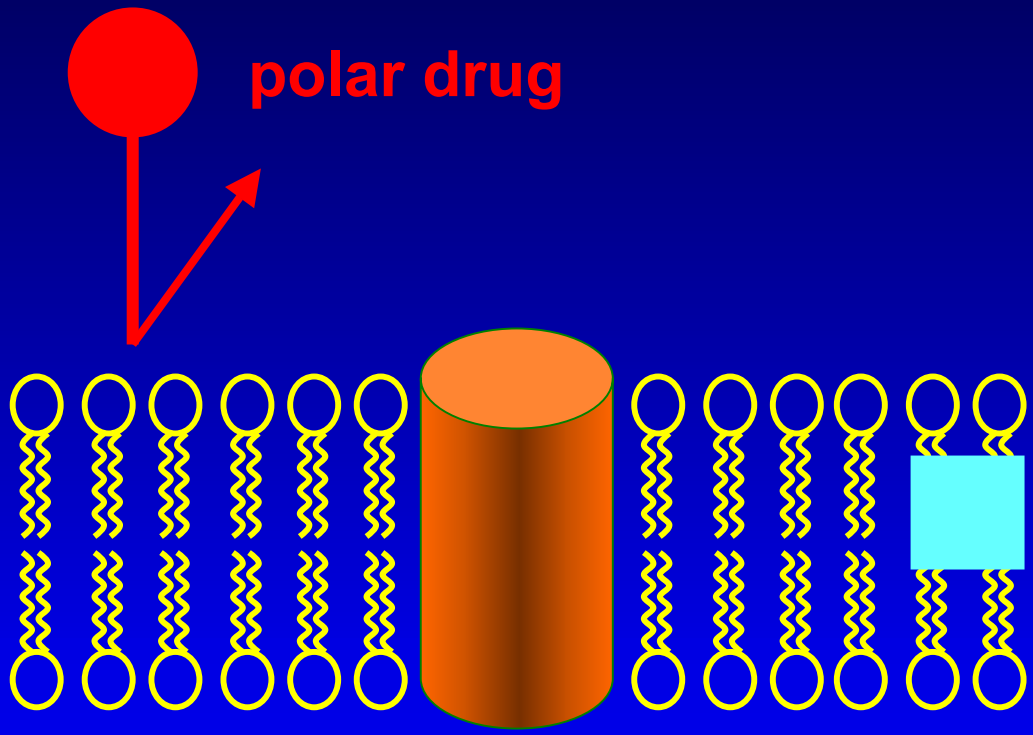
antibiotics as magic bullets



already concerned in efflux ?

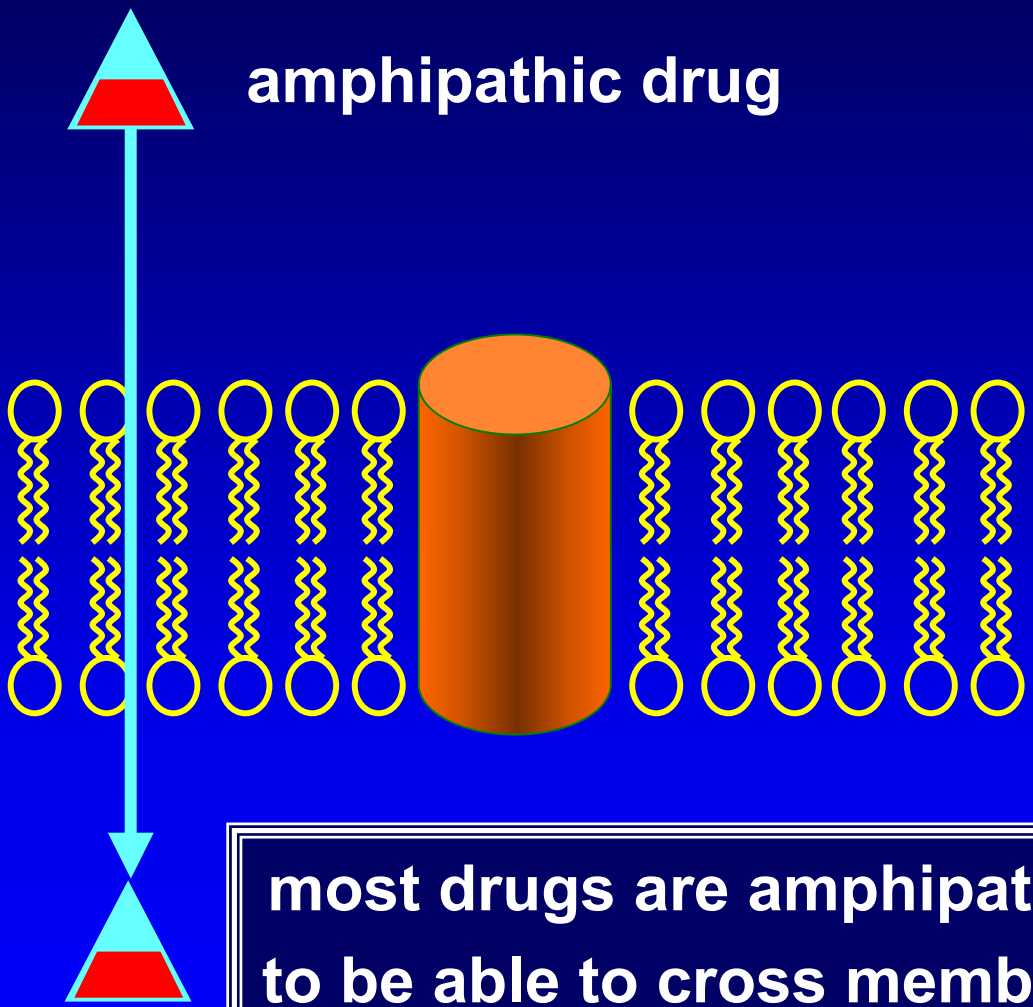


Why drug efflux transporters ?



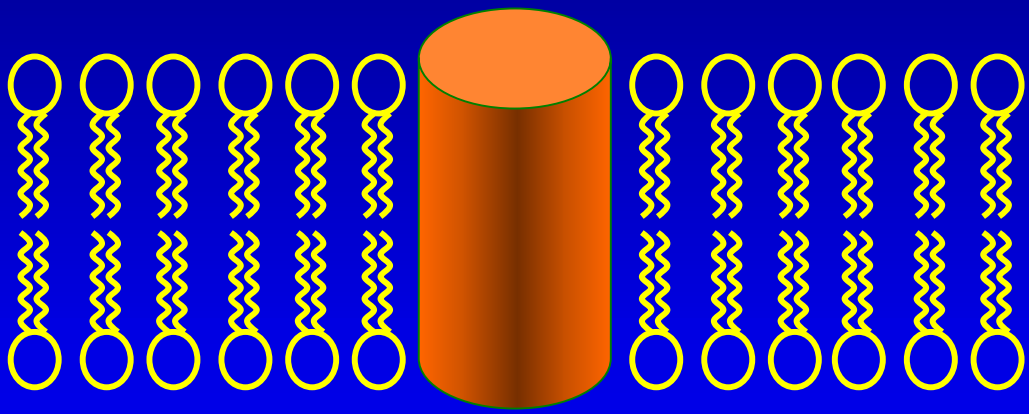
physico-chemical properties are inadequate for reaching an intracellular target !

Why drug efflux transporters ?



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

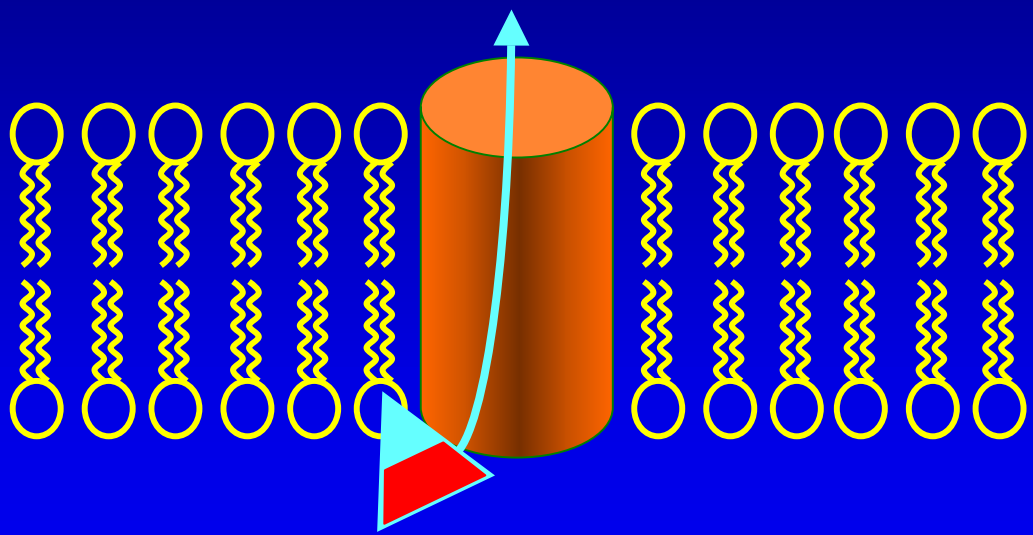
Why drug efflux transporters ?



But a diffusible compound may have **potentially harmful effects !**

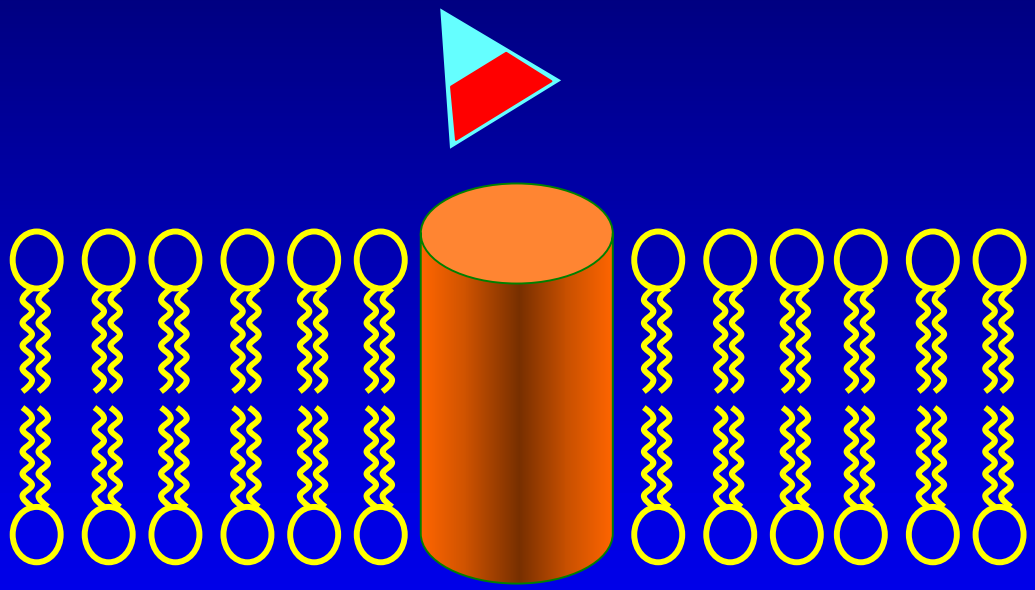
Why drug efflux transporters ?

Extrusion by efflux pumps



Why drug efflux transporters ?

Extrusion by efflux pumps



general mean of protection
against cell invasion by diffusible molecules

Typical ‘toxic’ diffusible substances well known as substrates for efflux pumps

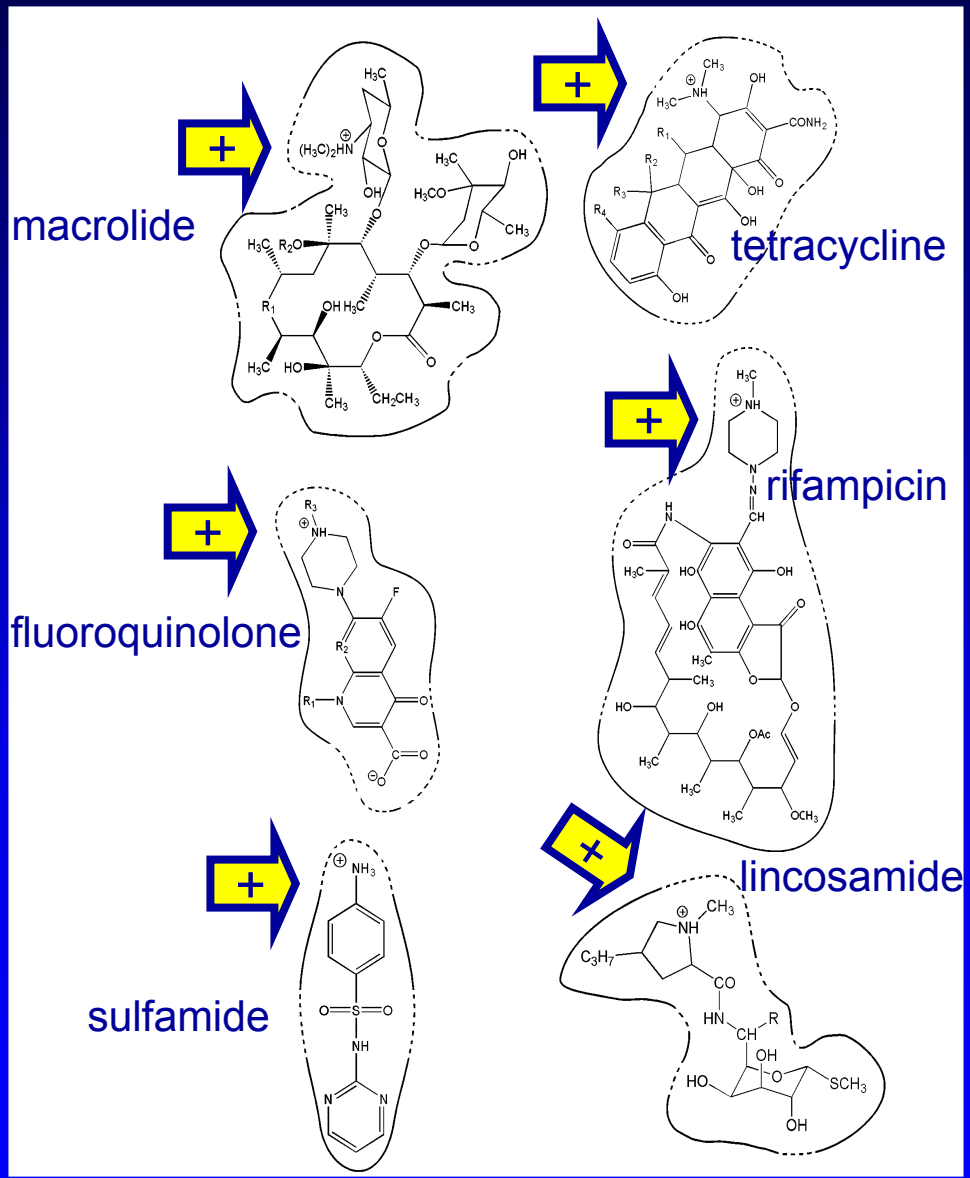
antibiotics

antifungals

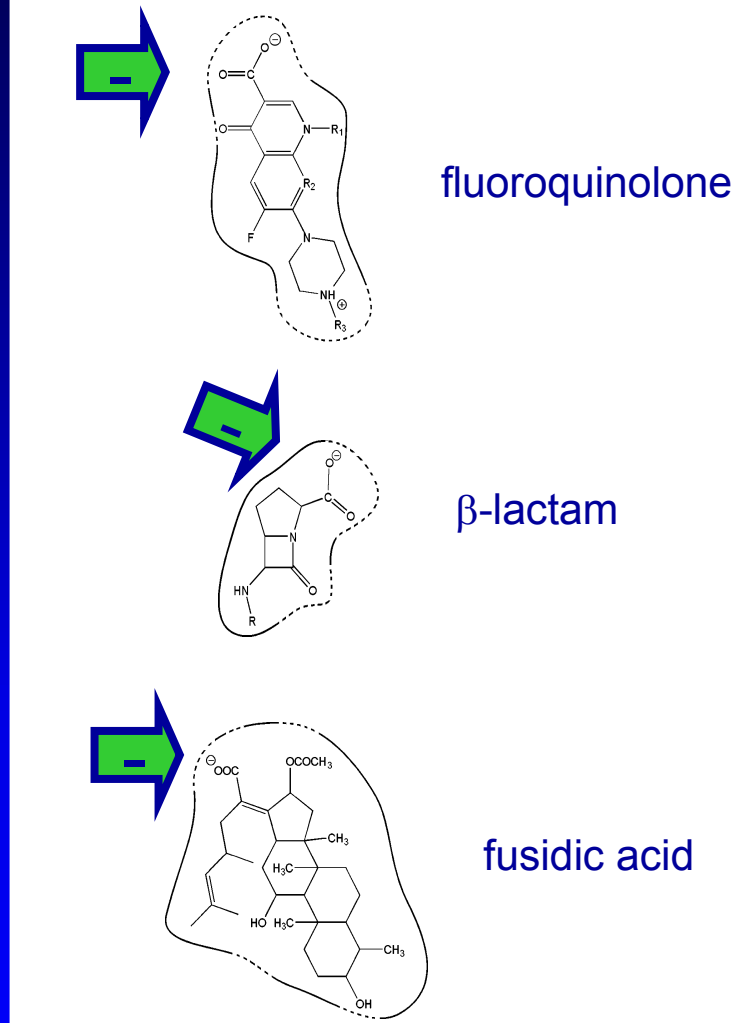
anticancer agents

Most antibiotics are amphiphilic !

cationic amphiphiles



anionic amphiphiles



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

Antibiotic classes recognized by efflux pumps in different types of organisms

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

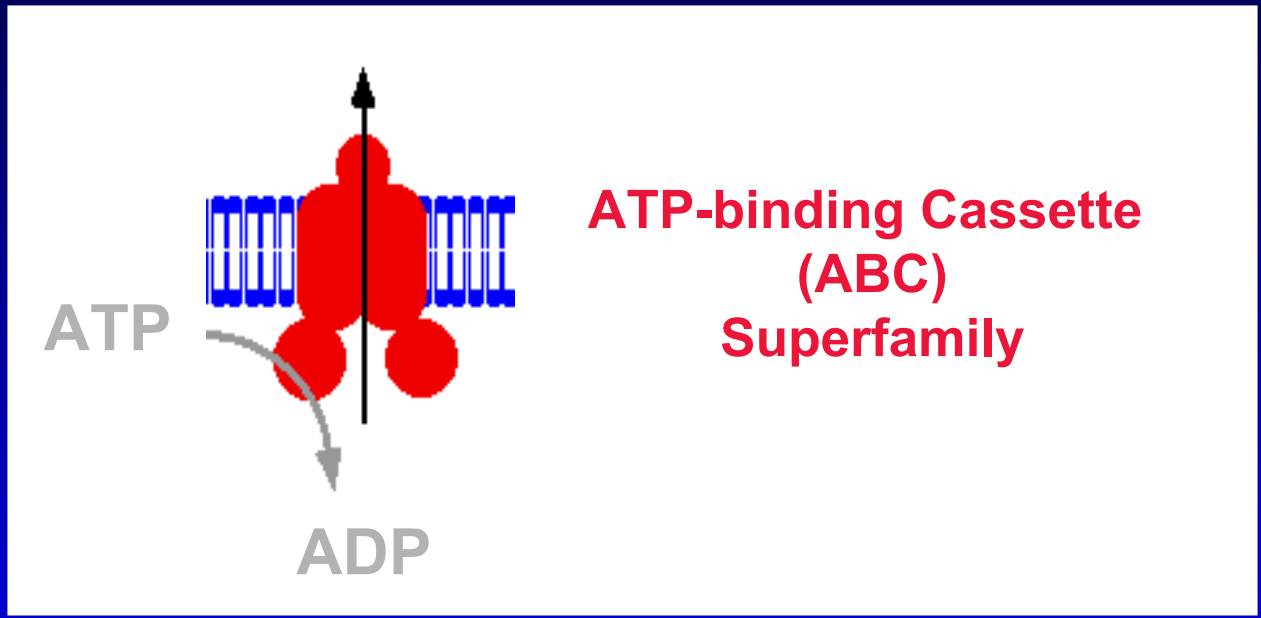
Consequences of antibiotic efflux from eucaryotic cells

- **alteration of pharmacokinetics**
  - whole organism: absorption, distribution, elimination
  - single cell: accumulation, localization

- **alteration of pharmacodynamics**
  - body level: drug concentration in the infected compartment
  - cellular level: activity against intracellular bacteria

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

Main multidrug resistance efflux pumps in eucaryotic cells



MDR-1 (P-glycoprotein)

cationic amphiphiles

MRP1-9

anionic amphiphiles

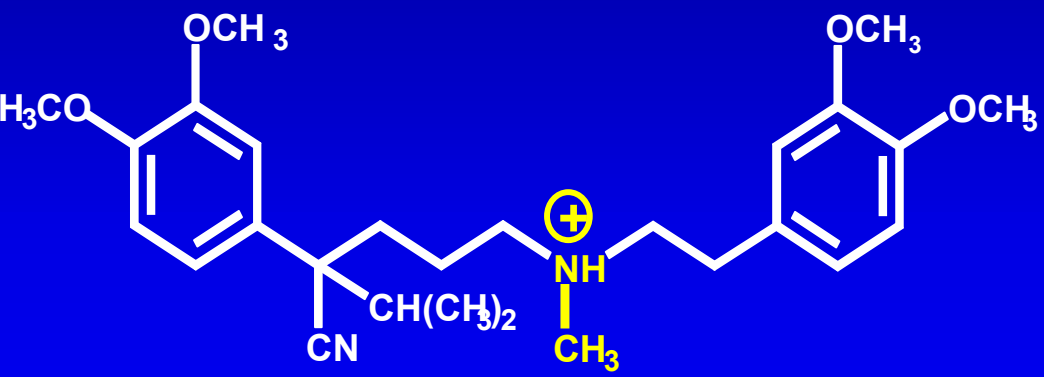
Inhibitors and substrates share the same physicochemical properties

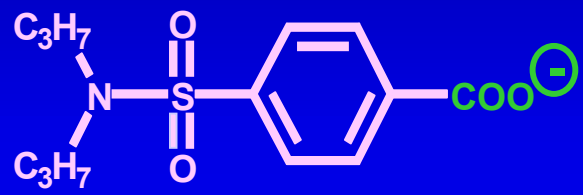
MDR-1 (P-glycoprotein)

MRP1-9

cationic amphiphiles

anionic amphiphiles

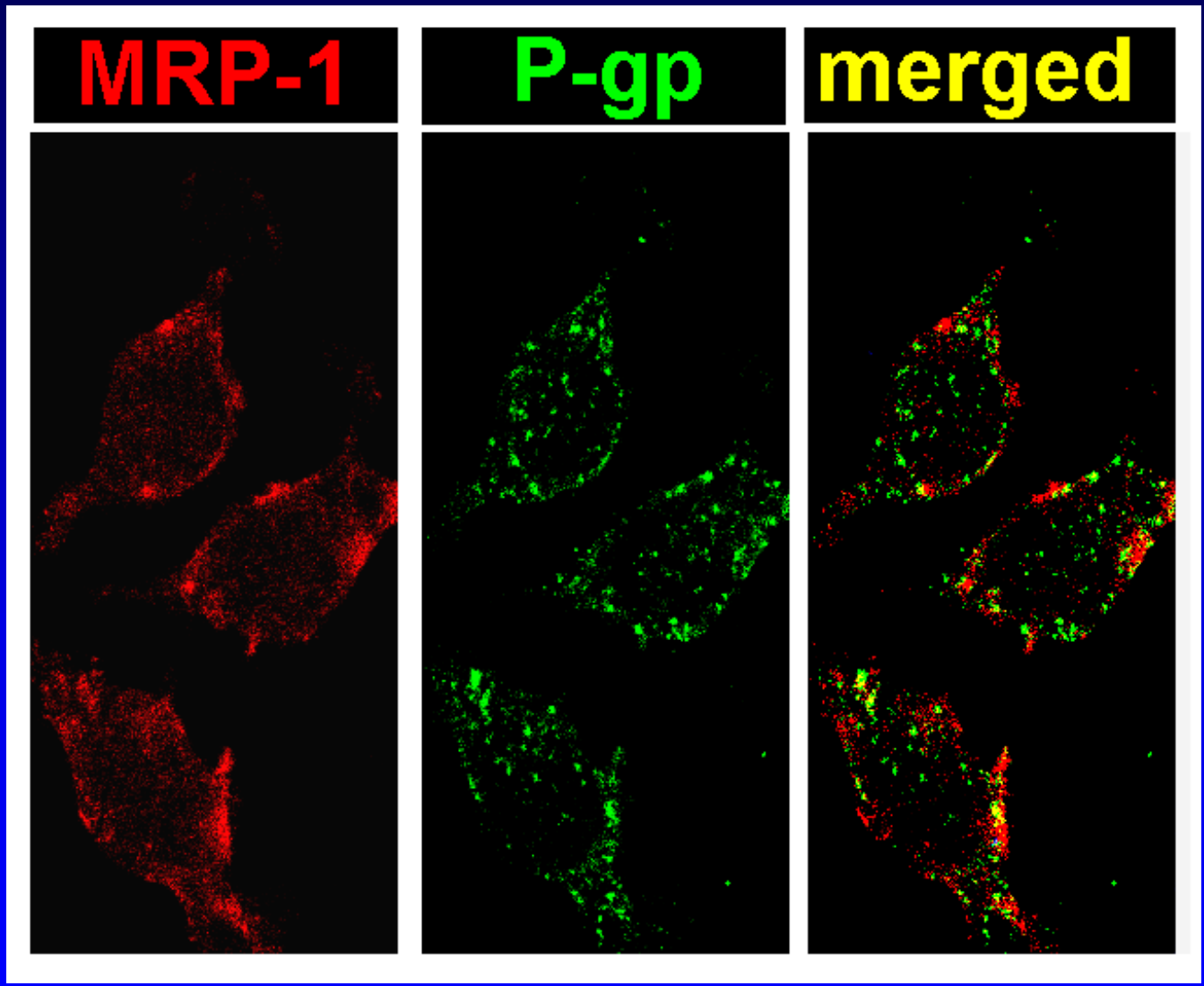




verapamil

probenecid

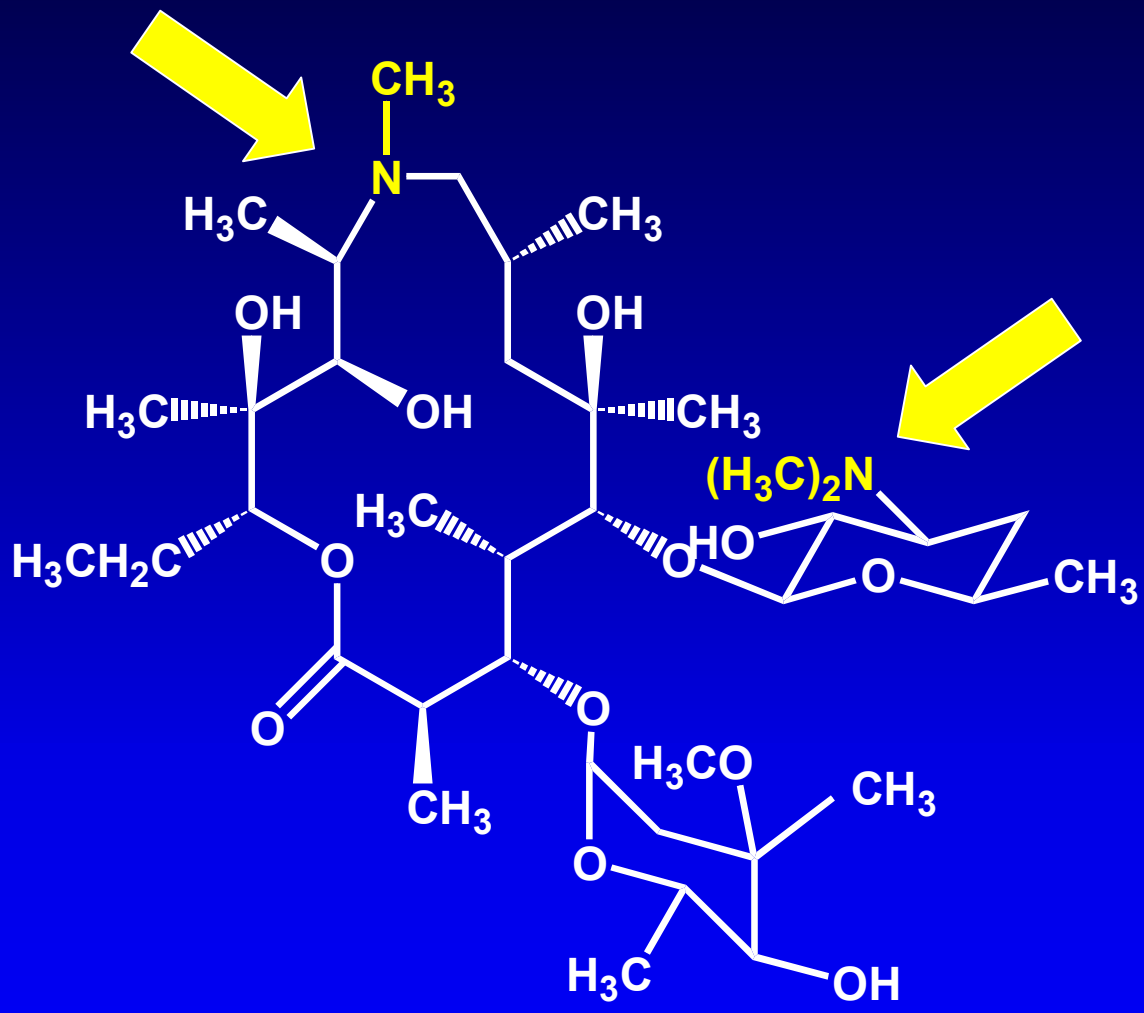
Macrophages express at least MRP-1 and P-gp



1. Influence of efflux pumps on antibiotic cellular pharmacokinetics

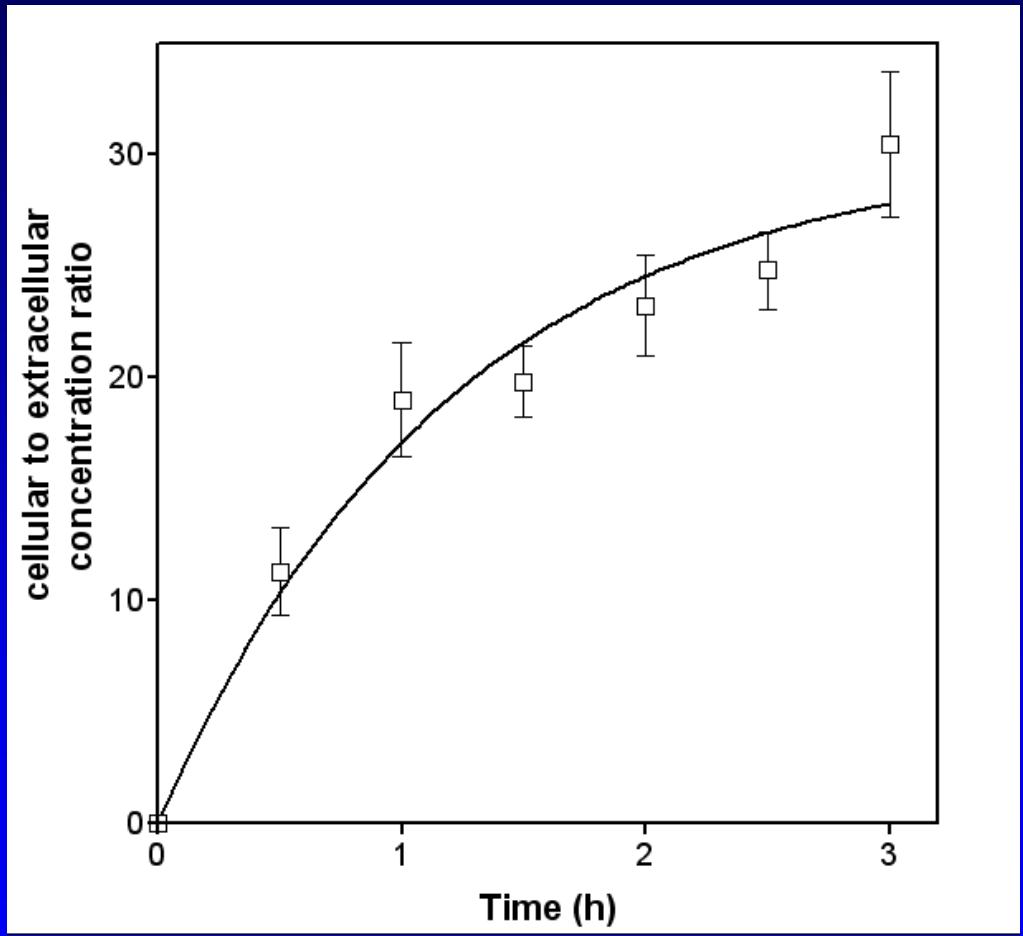
macrolides

Macrolide story



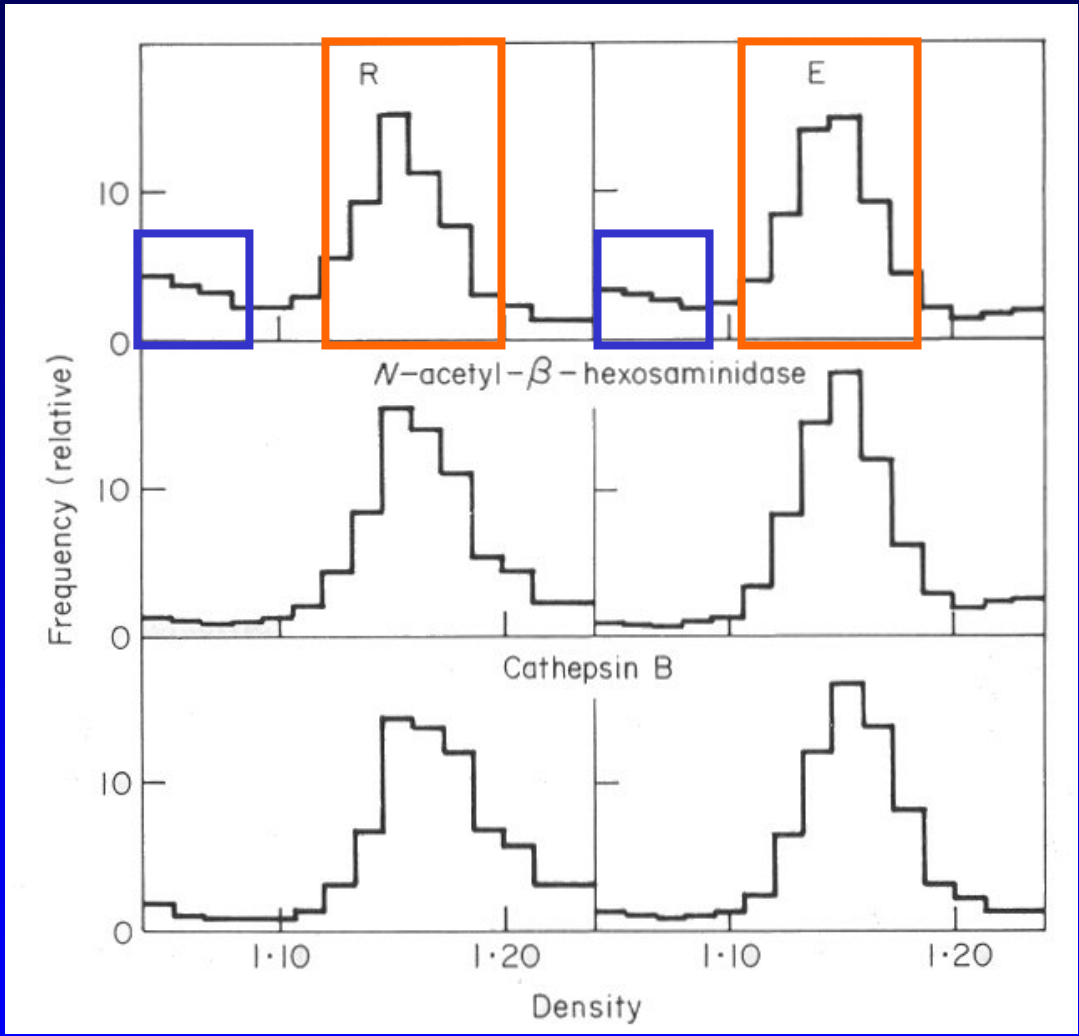
Azithromycin is a dicationic amphiphilic molecule

Azithromycin accumulates to high levels in eucaryotic cells



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

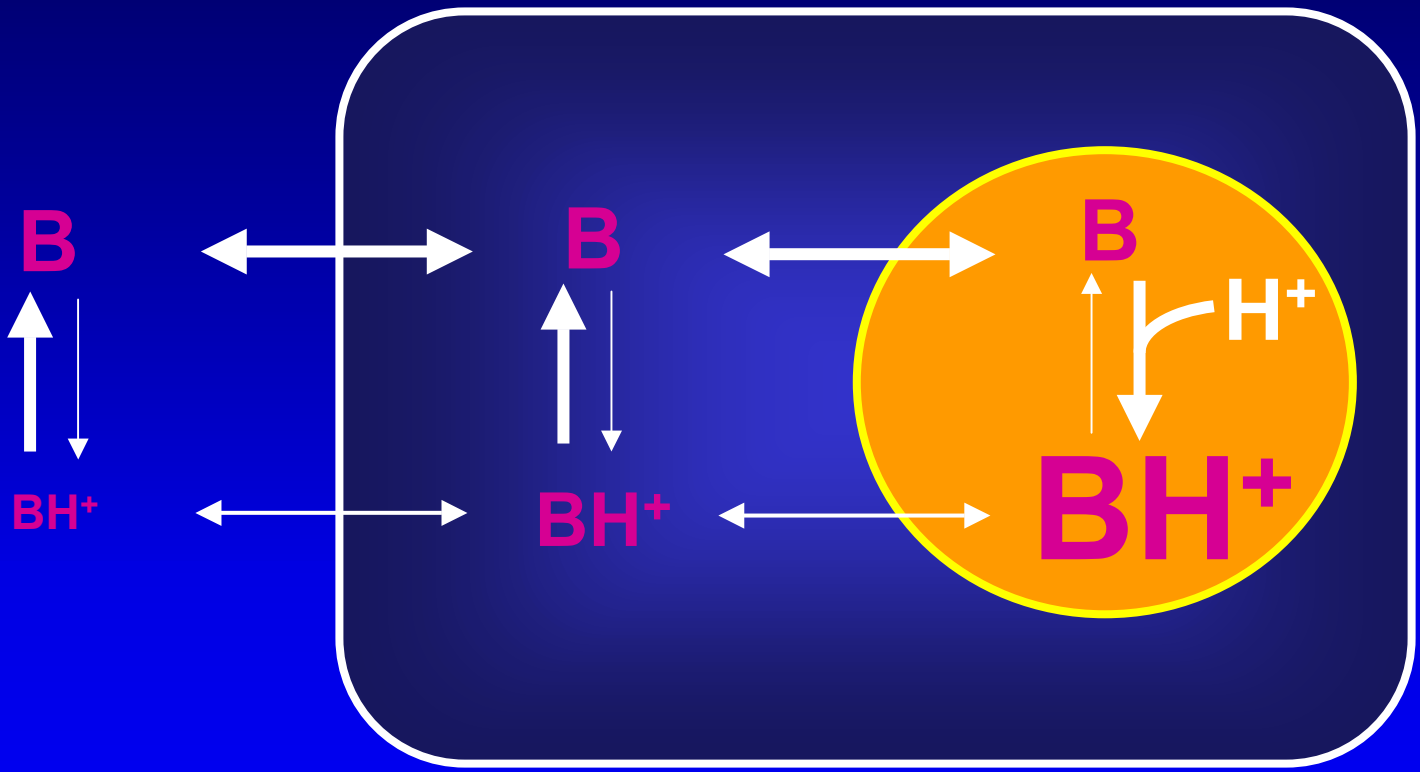
macrolides accumulate mainly in the lysosomal compartment



lysosomal enzymes

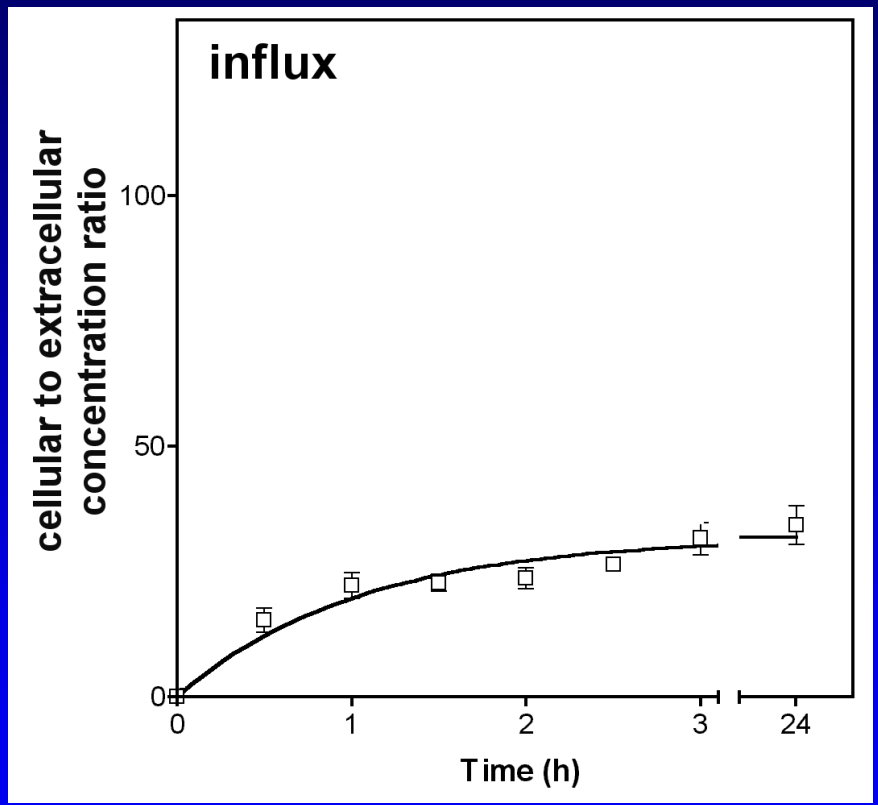
Carlier *et al,* JAC (1987) 20 Suppl B:47-56

macrolide accumulation proceeds by diffusion / segregation



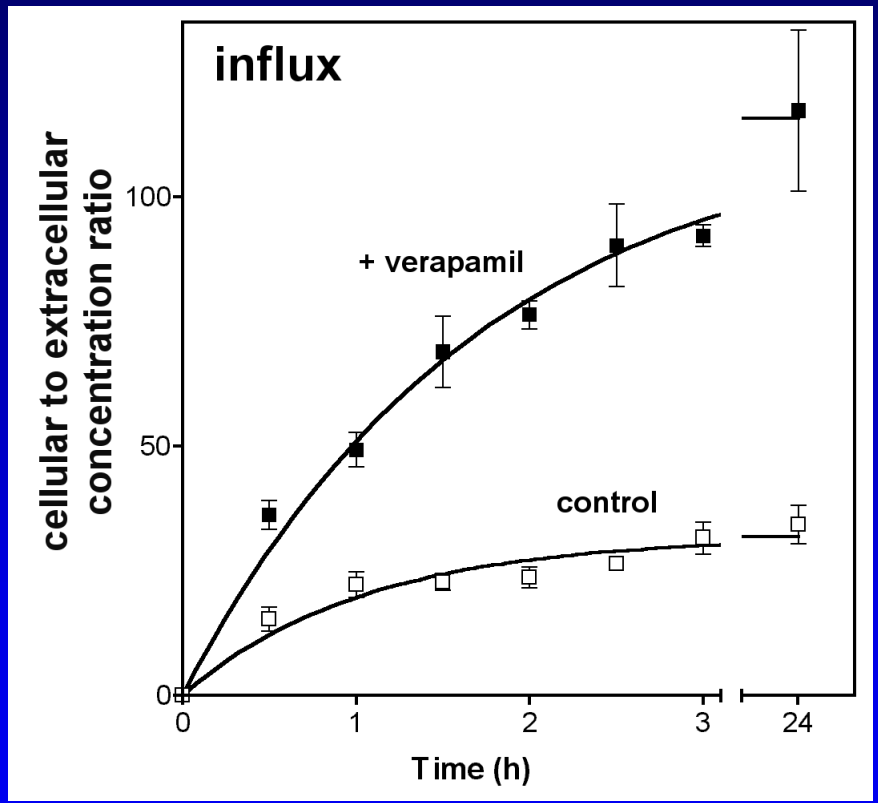
De Duve *et al,* Biochem Pharmacol. (1974) 23: 2495-531.

Azithromycin concentration is high but still suboptimal …



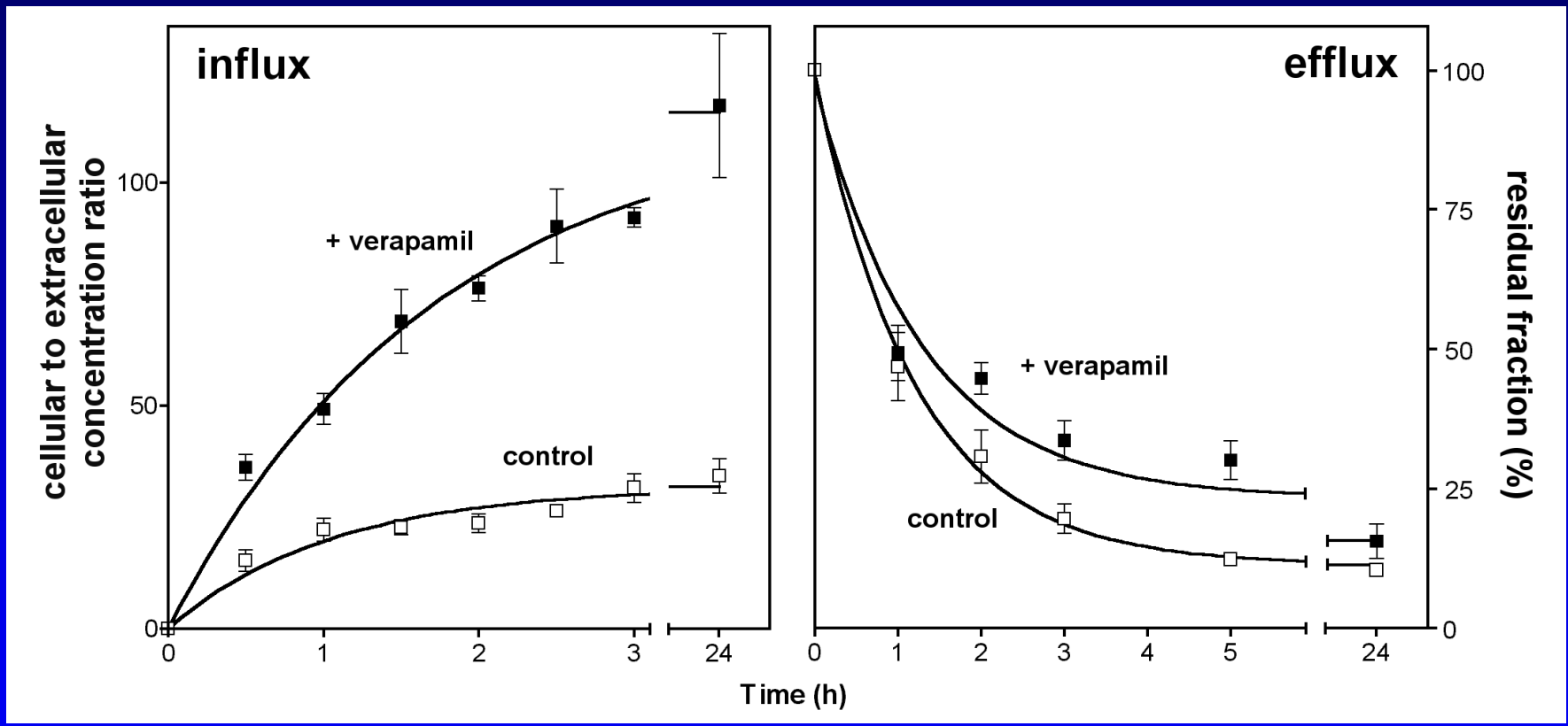
Seral *et al*, AAC (2003) 47:1047-51.

Inhibition of P-gp by verapamil increases accumulation



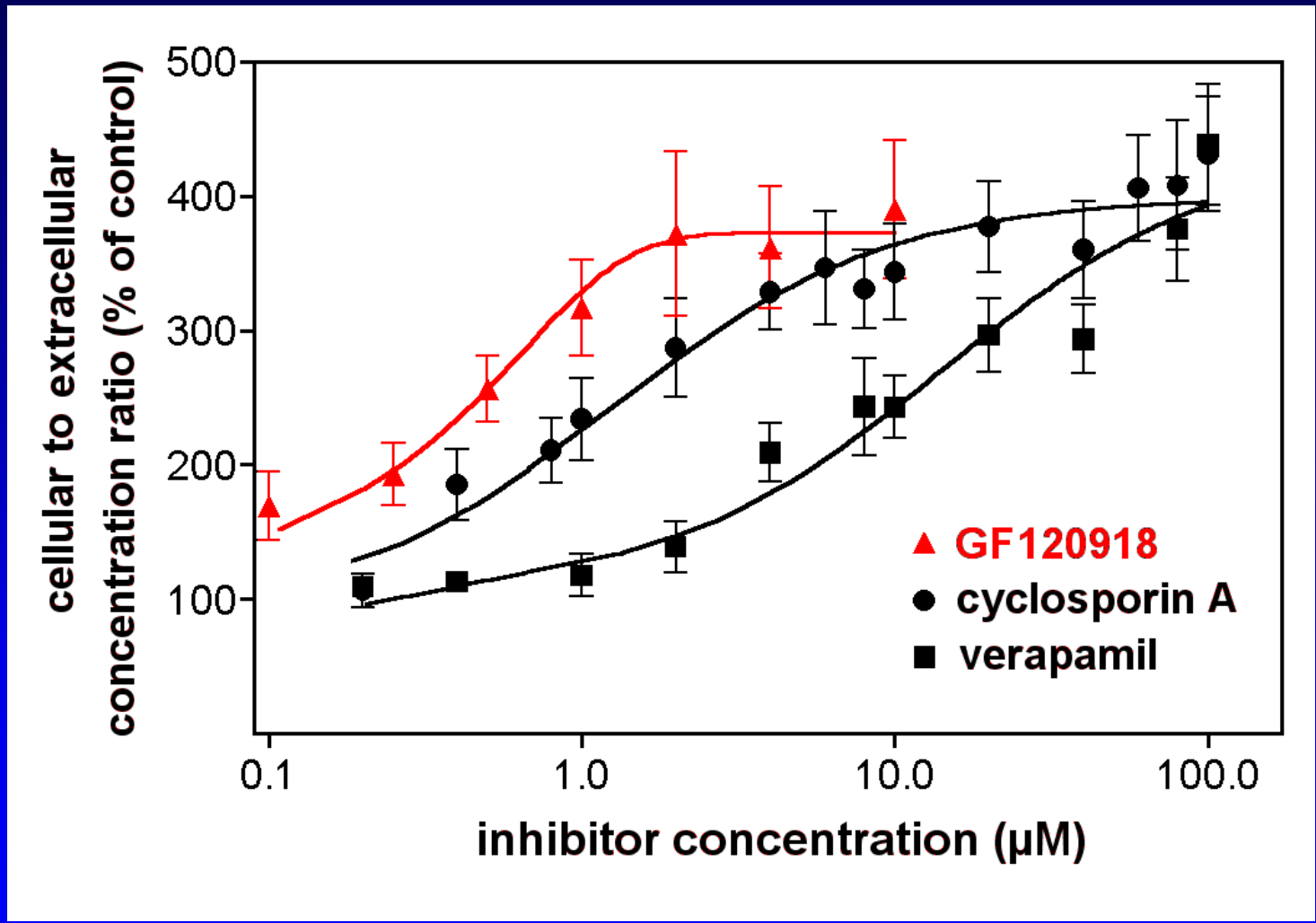
Seral *et al*, AAC (2003) 47:1047-51.

Inhibition of P-gp by verapamil increases accumulation and slightly slows down efflux



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

Similar effects are obtained with more specific inhibitors of P-gp



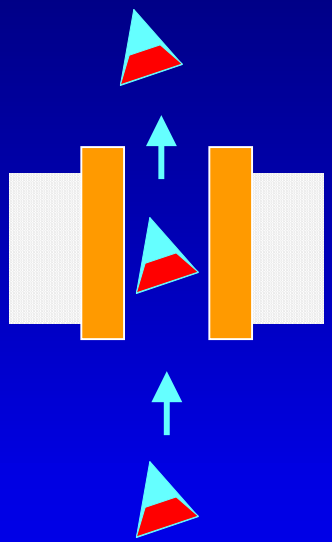
Seral *et al*, AAC (2003) 47:1047-51.

How can efflux pumps increase azithromycin accumulation without markedly affecting its efflux ?

➡ mechanism of action of efflux pumps

Efflux pumps as pores ?

Probably not ...



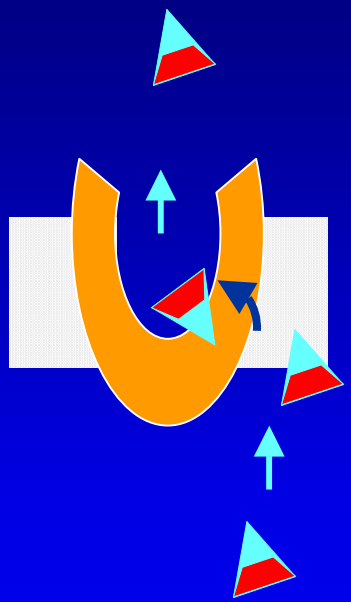
Experimental evidences

- substrates and inhibitors are amphiphilic
- rates and kinetics of efflux are not directly related to the cytosolic drug content

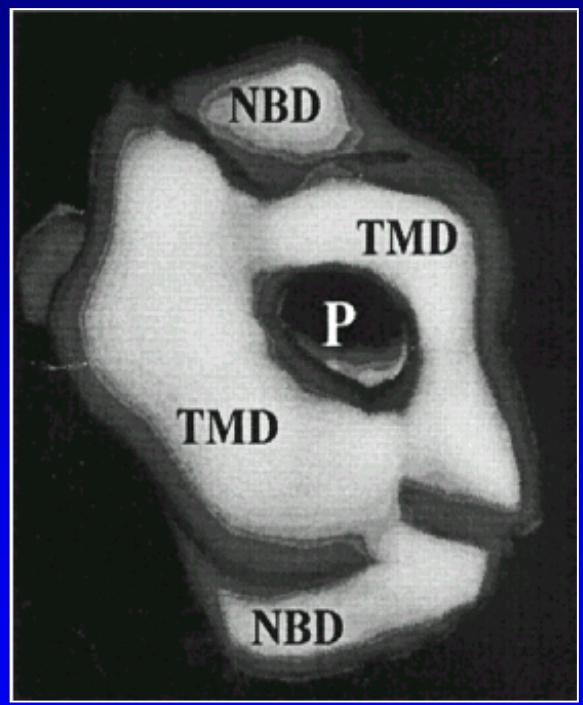
Higgins *et al*, Trends Biochem Sci (1992) 17:18-21
Wadkins *et al*, Biochem Biophys Acta (1993) 1153:225-236
Bolhuis *et al*, EMBO J (1996) 15:4239-4245
Ashida *et al*, J Theor Biol (1998) 195:219-232

Efflux pumps as vacuum cleaners ?

Possibly ...



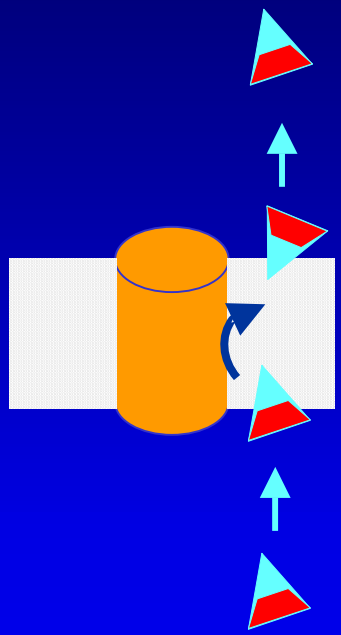
Structural evidence



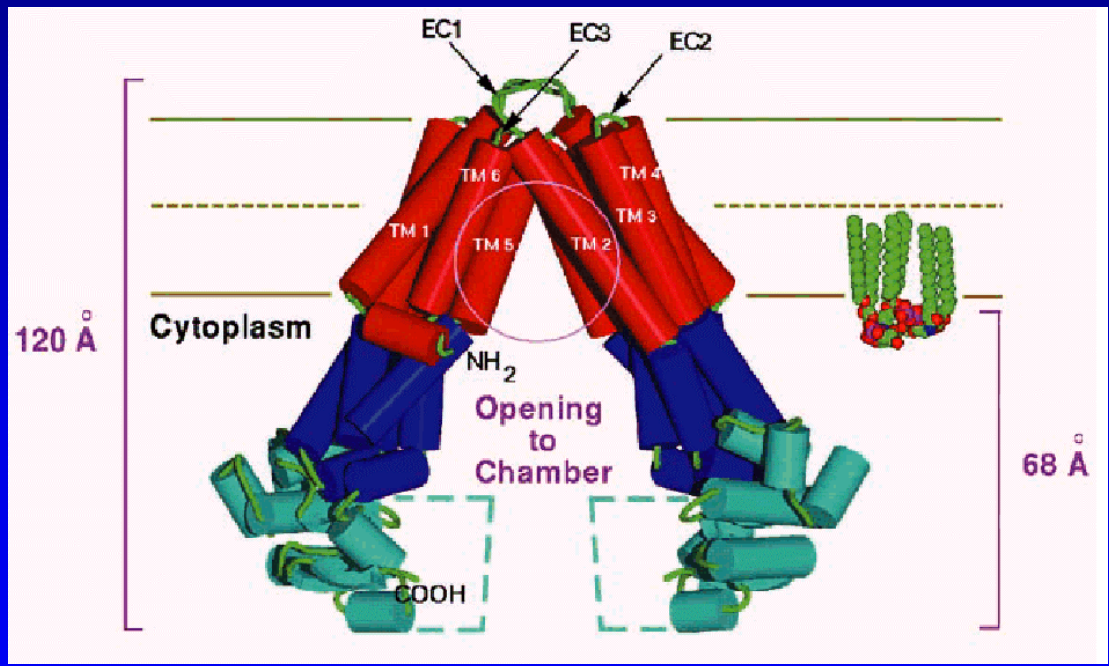
Rosenberg *et al*, JBC (1997) 272:10685-94

Efflux pumps as flippases ?

Possibly ...

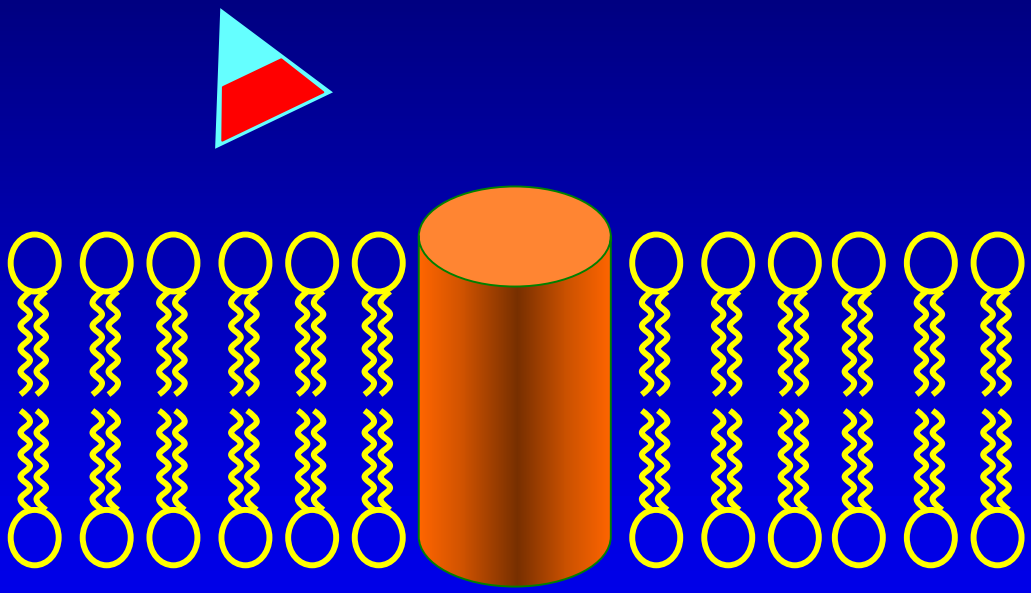


X-Ray structure of the lipid A transporter from *E. coli*, an homolog of P-glycoprotein

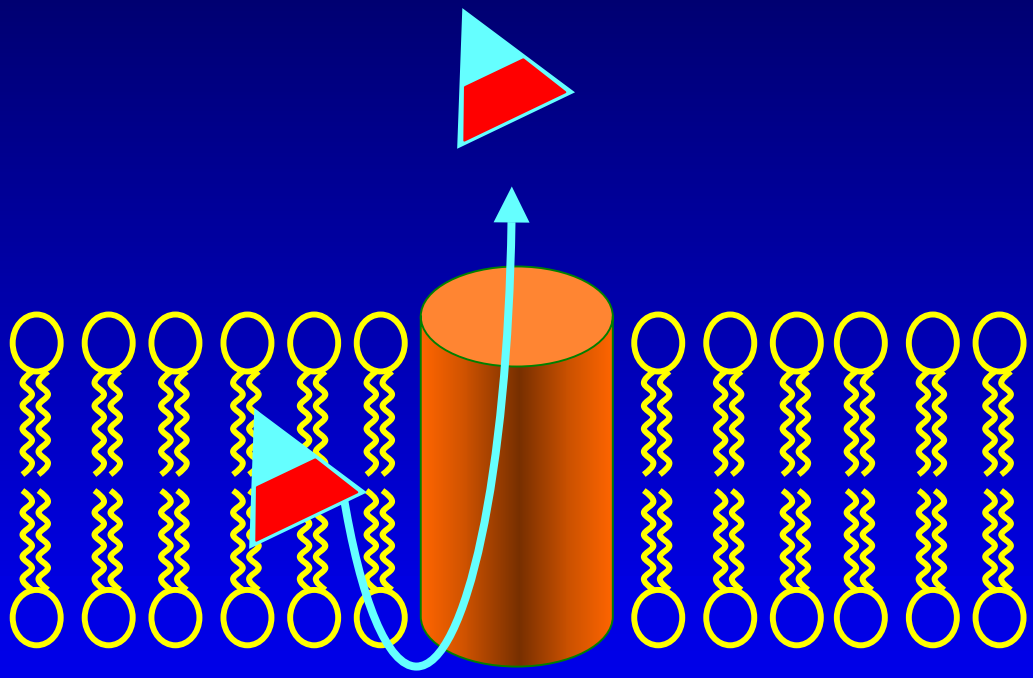


Chang *et al*, Science (2001) 293: 1793-1800

substrates potentially extruded from the membrane !



substrates potentially extruded from the membrane without having seen the cytosol !

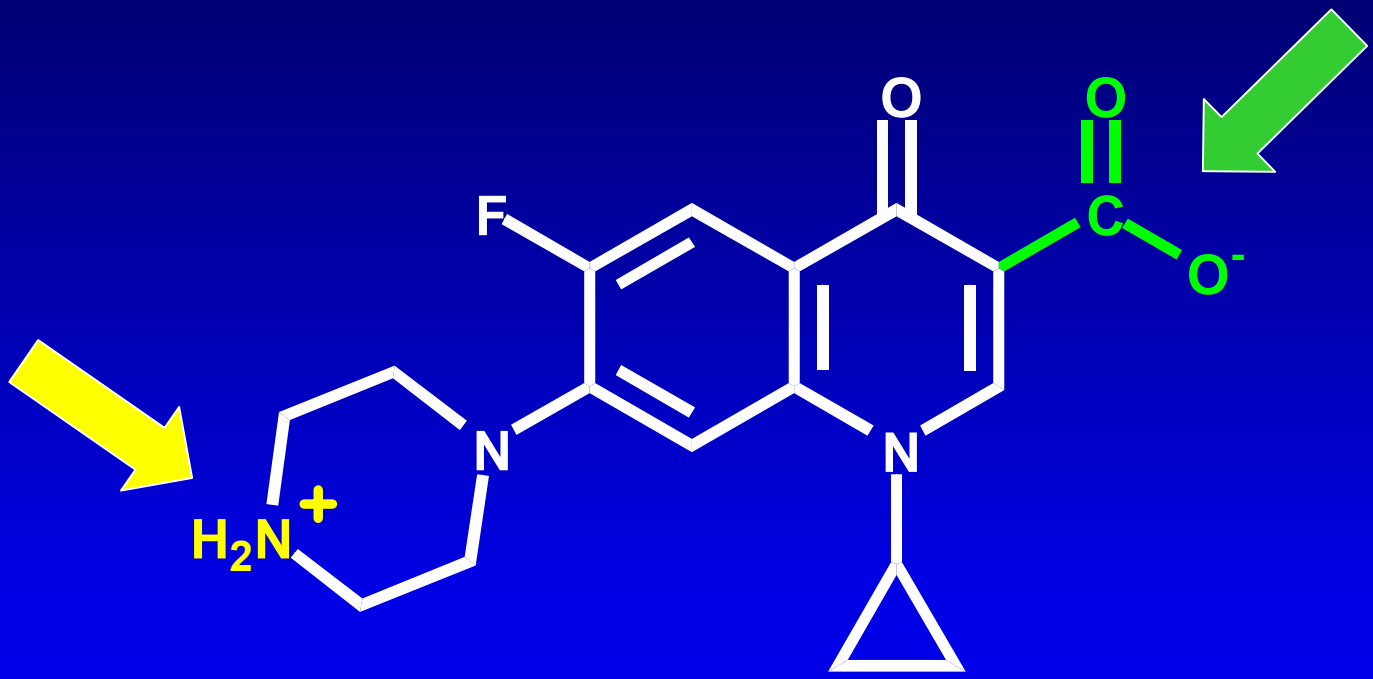


Marbeuf-Gueye *et al* (1999) BBA 1450:374-384

2. Influence of efflux pumps on antibiotic cellular pharmacokinetics

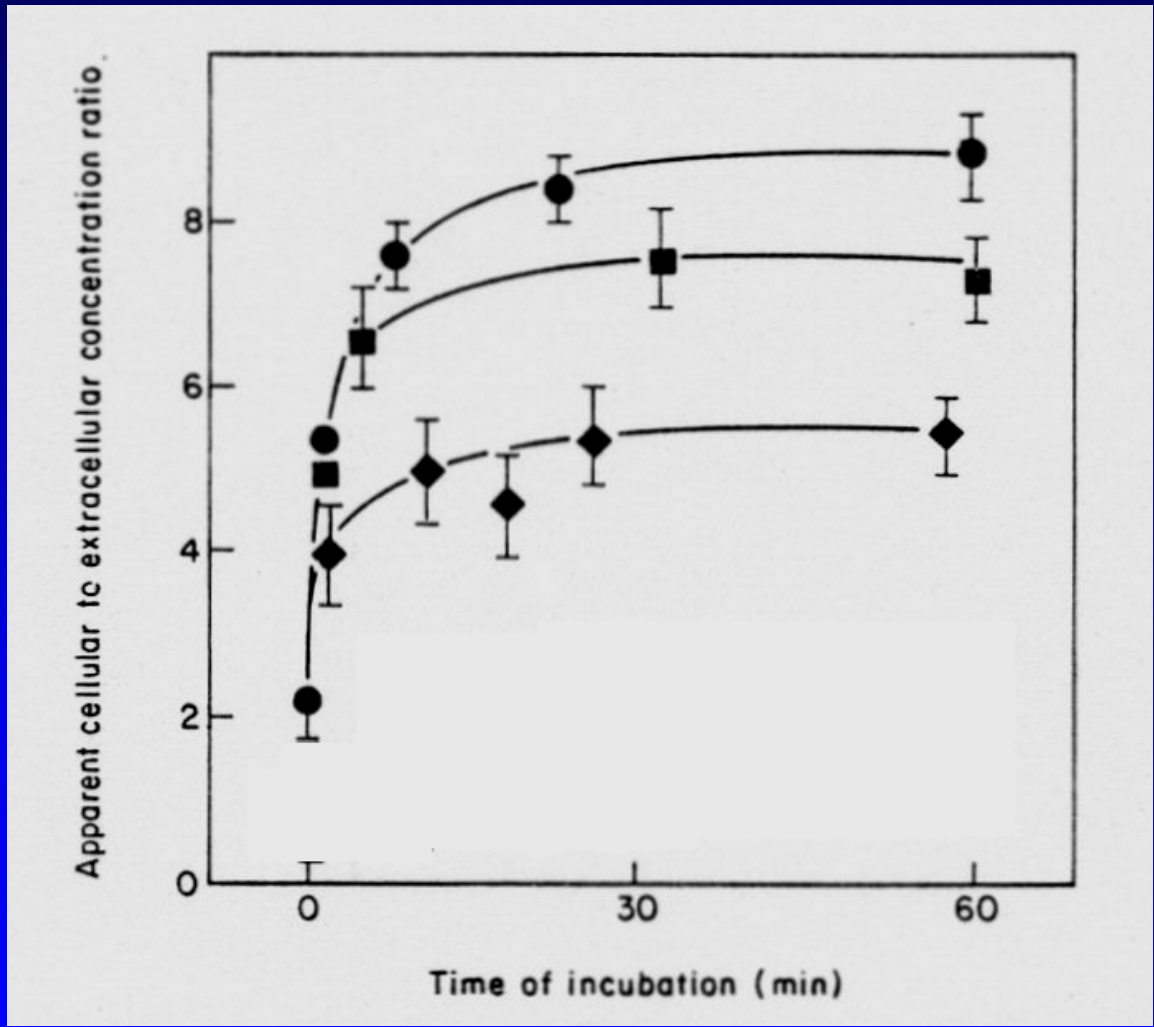
quinolones

Quinolone story



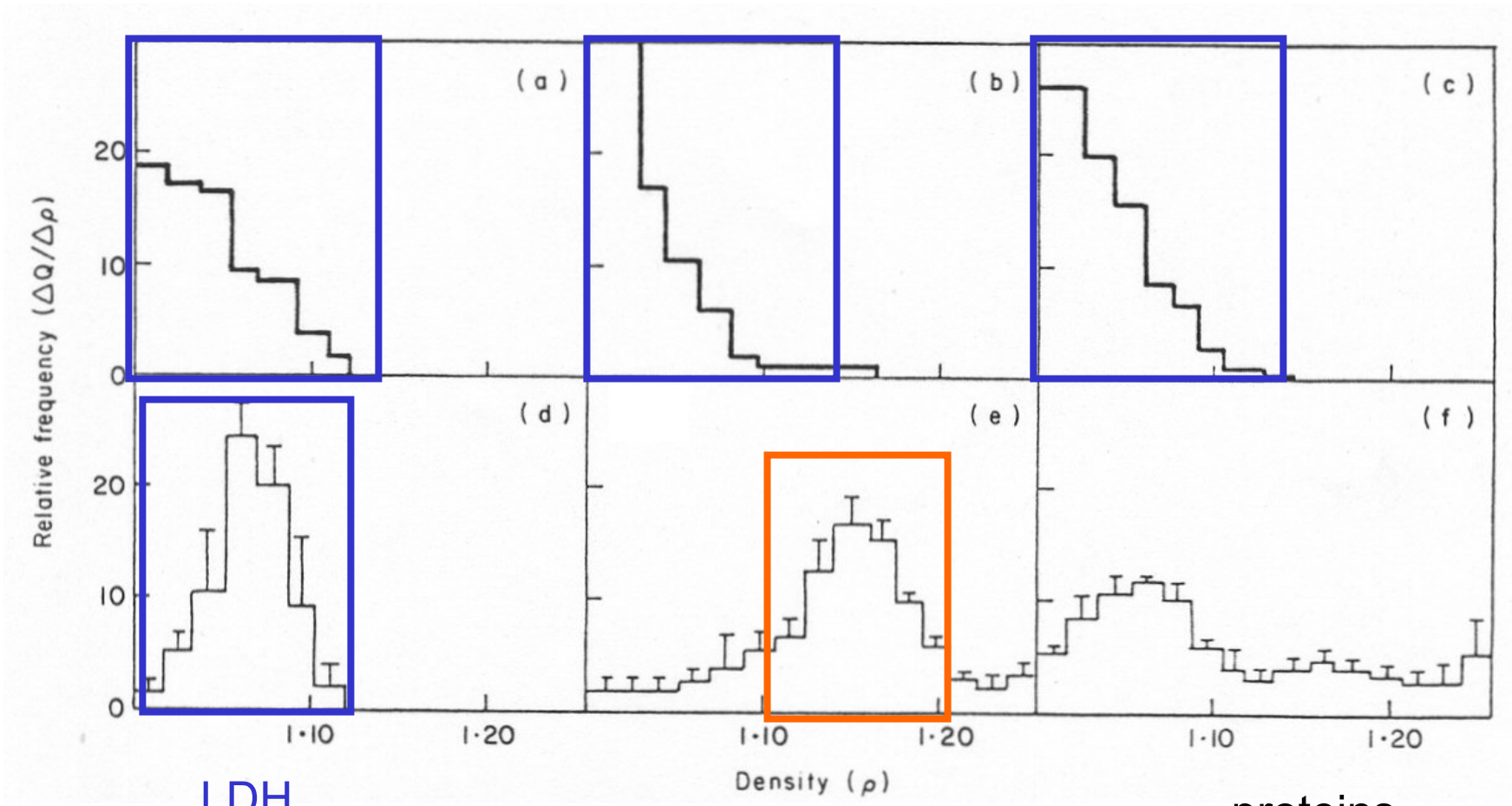
Ciprofloxacin is a zwitterionic amphiphilic molecule

Quinolones accumulate to moderate levels in eucaryotic cells



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

Quinolones are found in the soluble fraction of cell homogenates



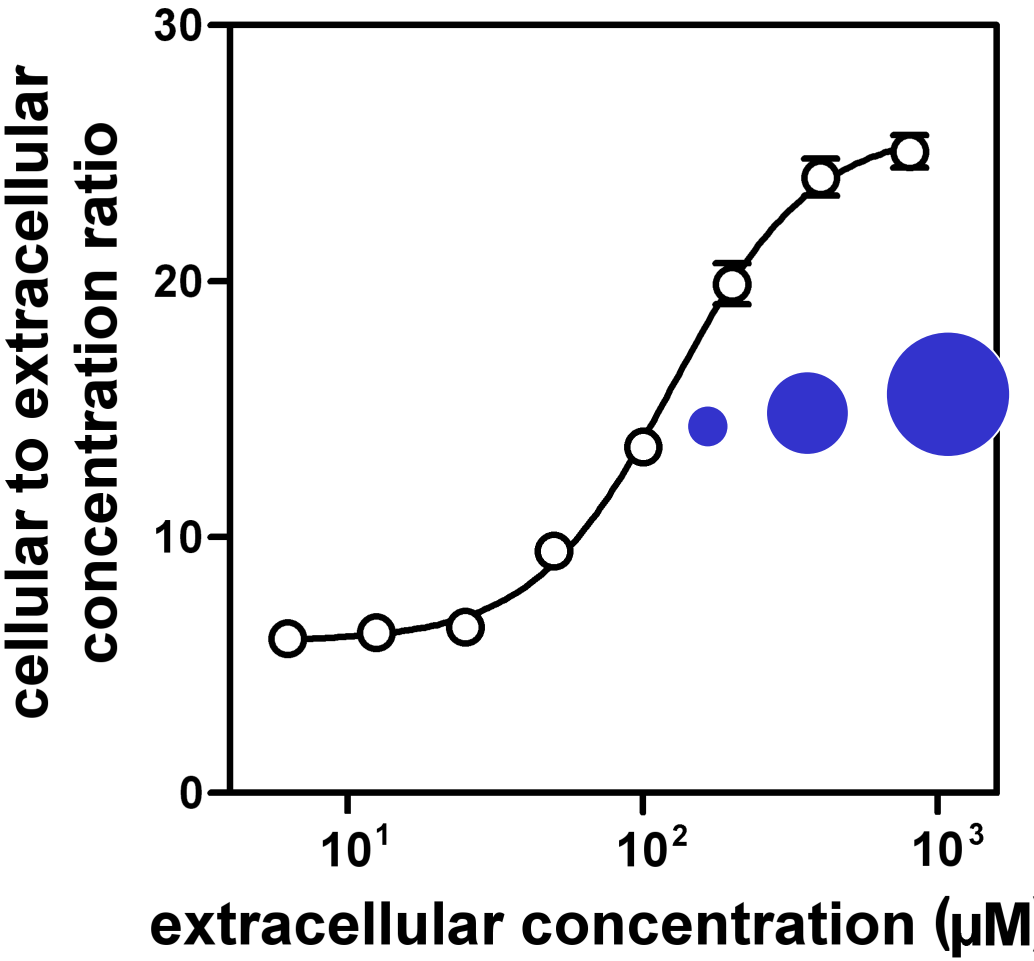
LDH (cytosol)

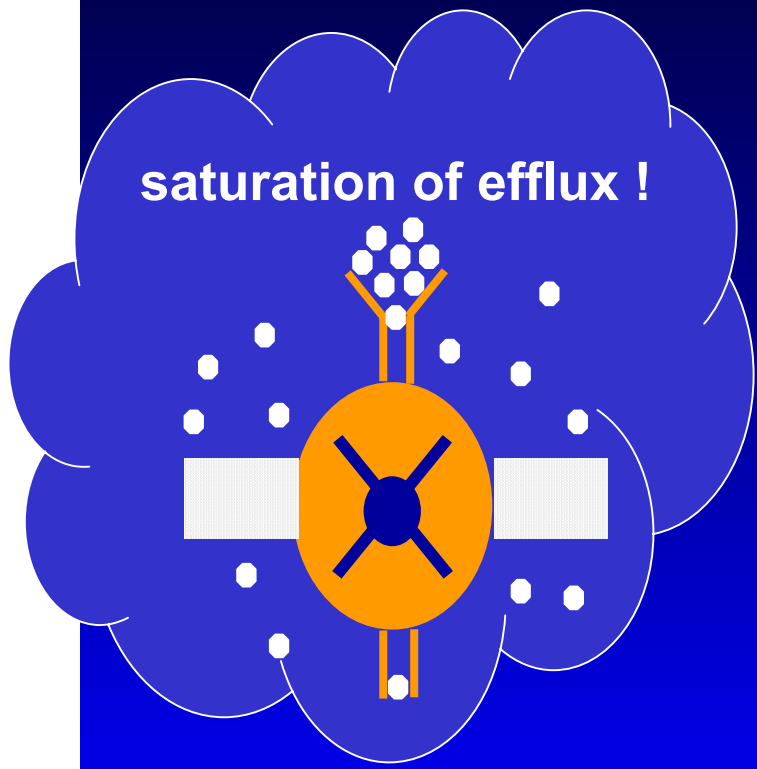
NABgase (lysosomes)

proteins

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

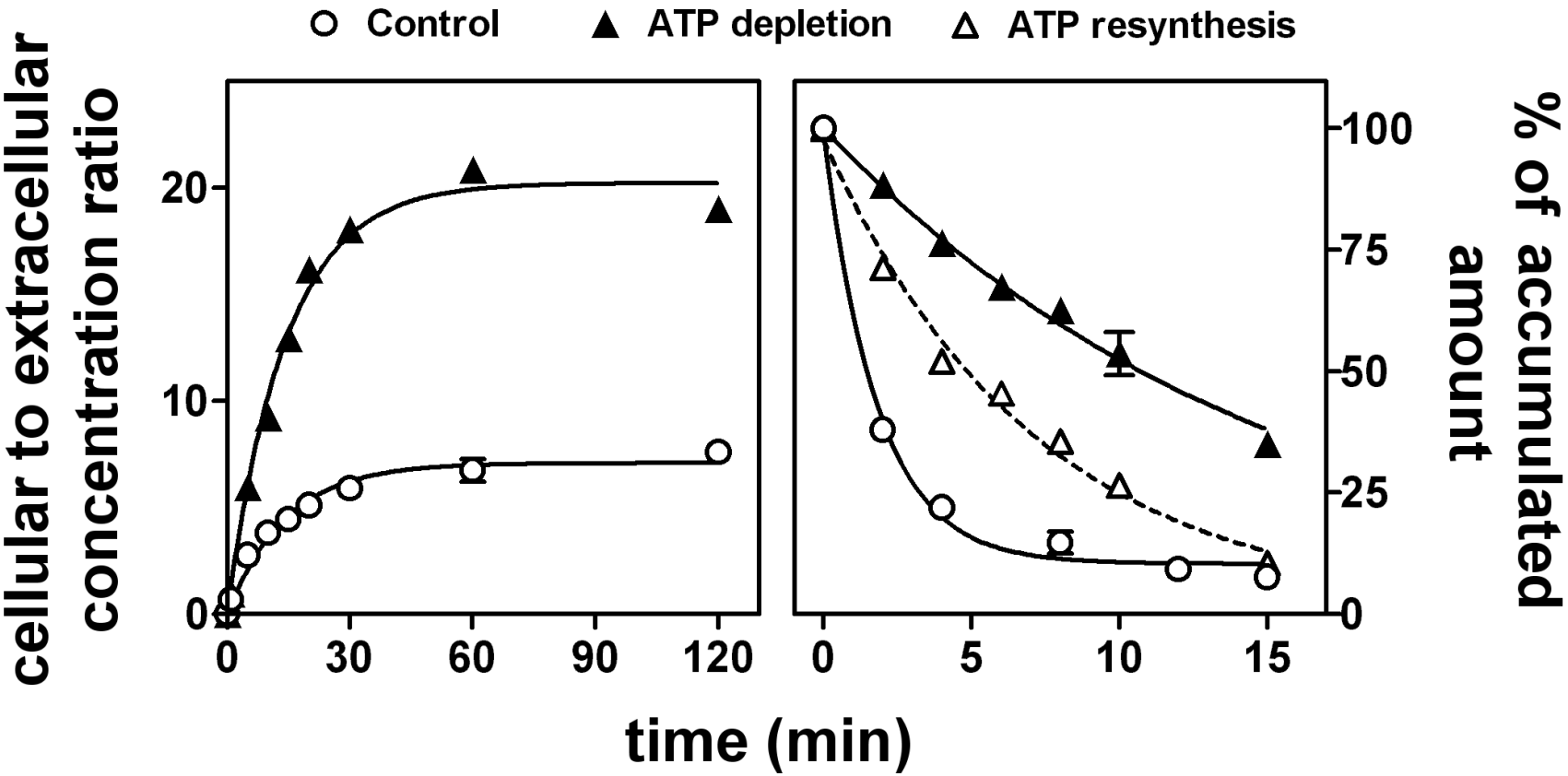
Ciprofloxacin facilitates its own uptake





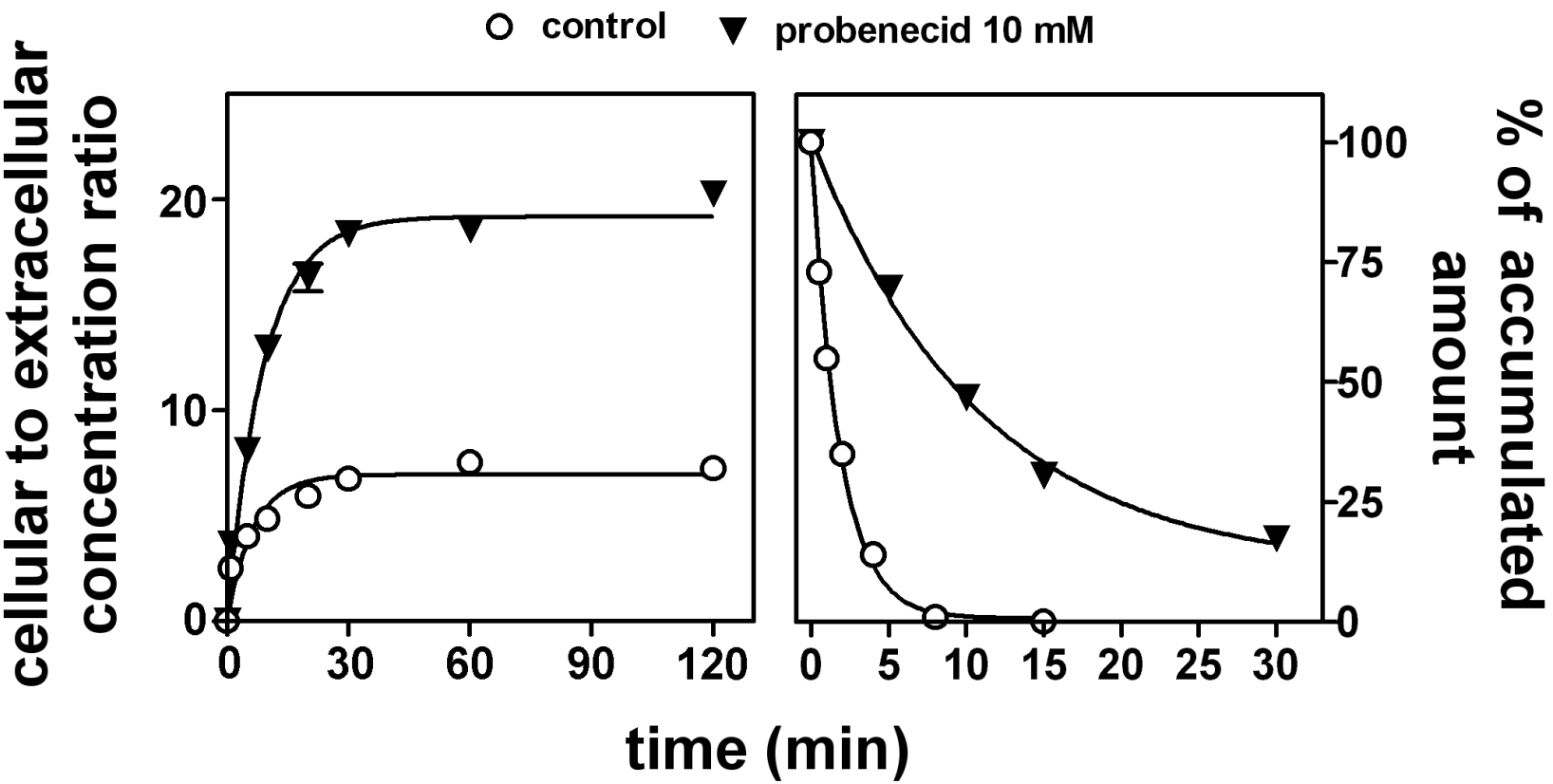
Michot *et al*, AAC (2004) 7: in press

Ciprofloxacin accumulation and efflux in ATP-depleted cells



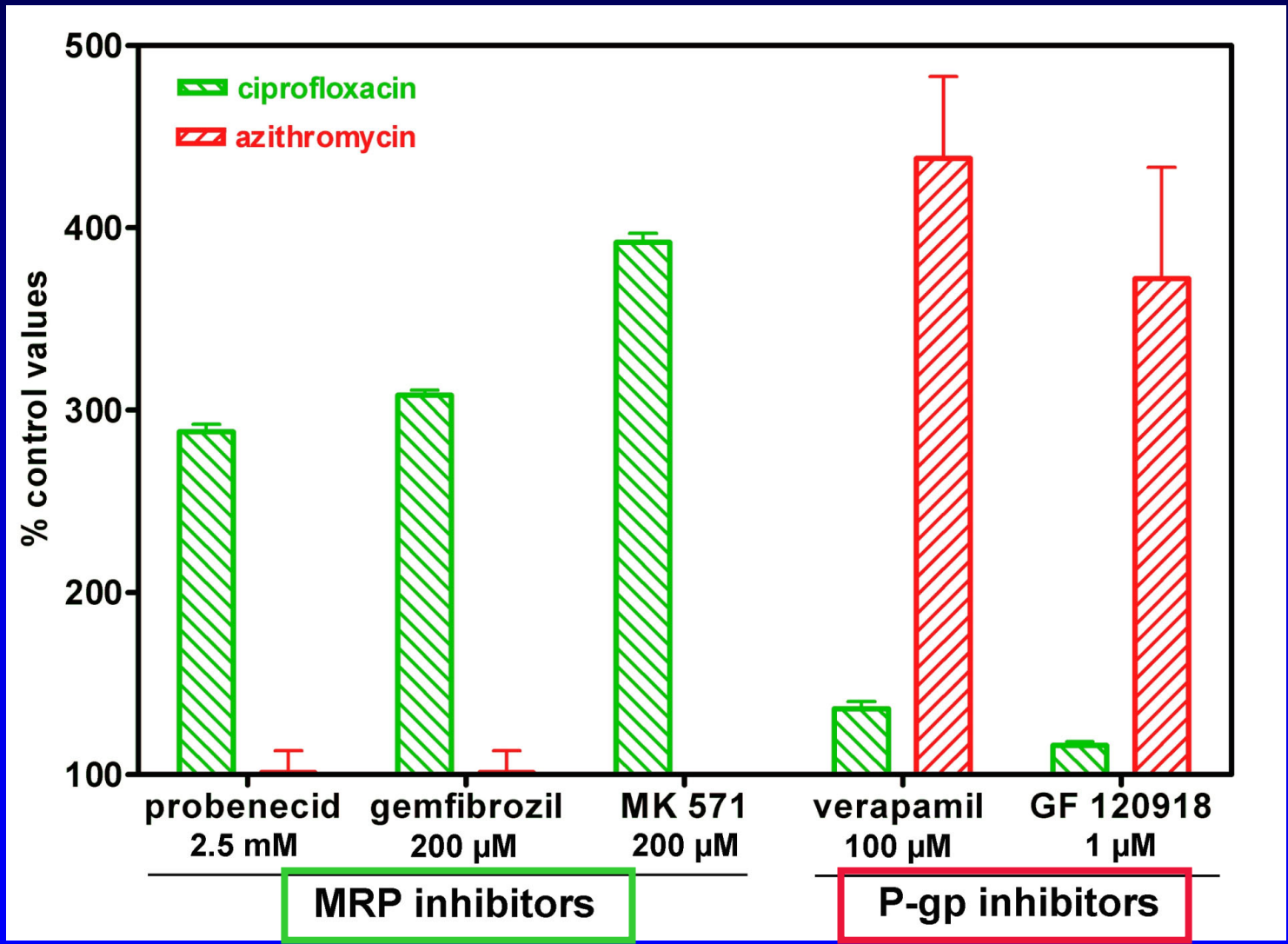
Michot *et al*, AAC (2004) 7: in press

Ciprofloxacin accumulation and efflux in probenecid-treated cells



Michot *et al*, AAC (2004) 7: in press

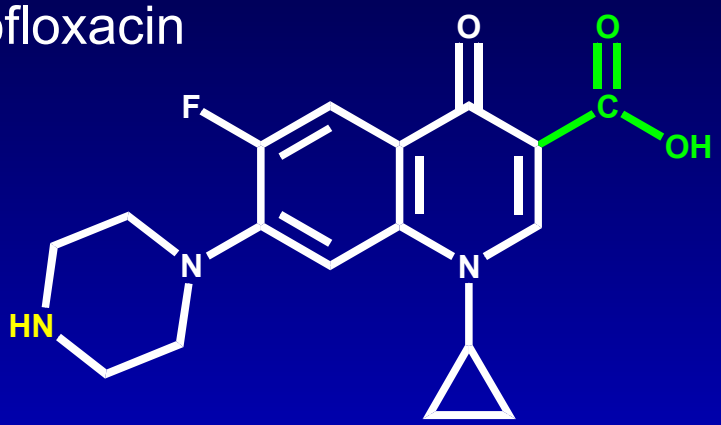
Differential effects of pump inhibitors on ciprofloxacin and azithromycin accumulation



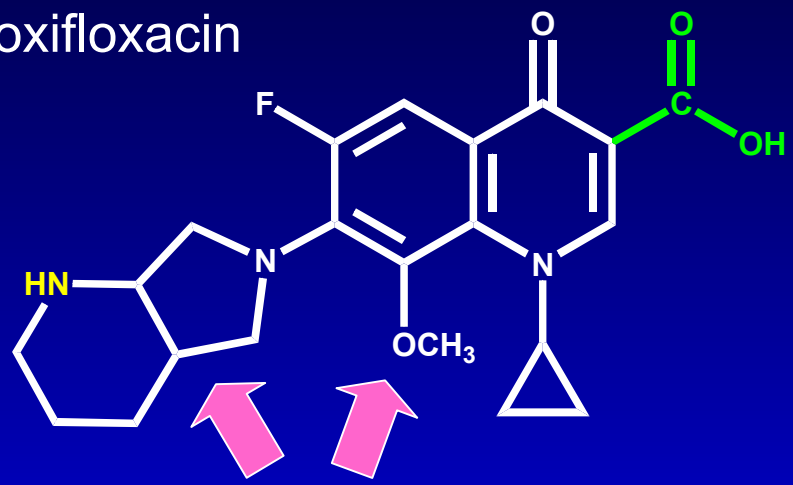
data from Michot et al, AAC, 2004

Have all quinolones been made equal ?

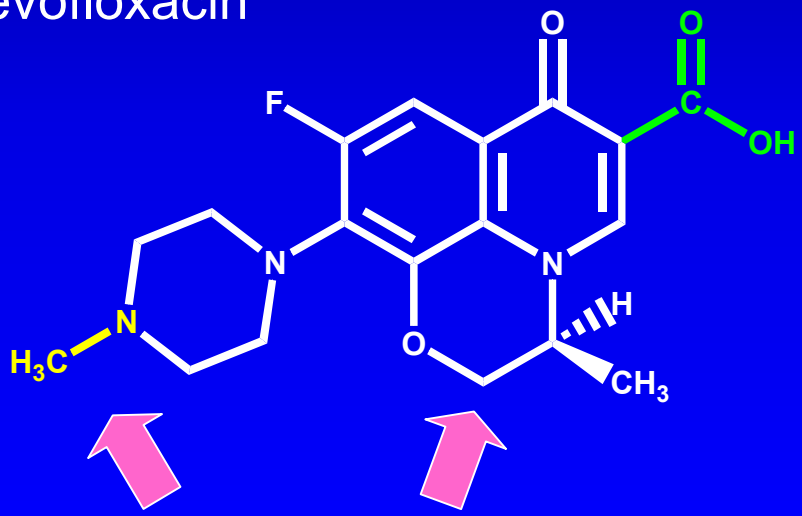
ciprofloxacin



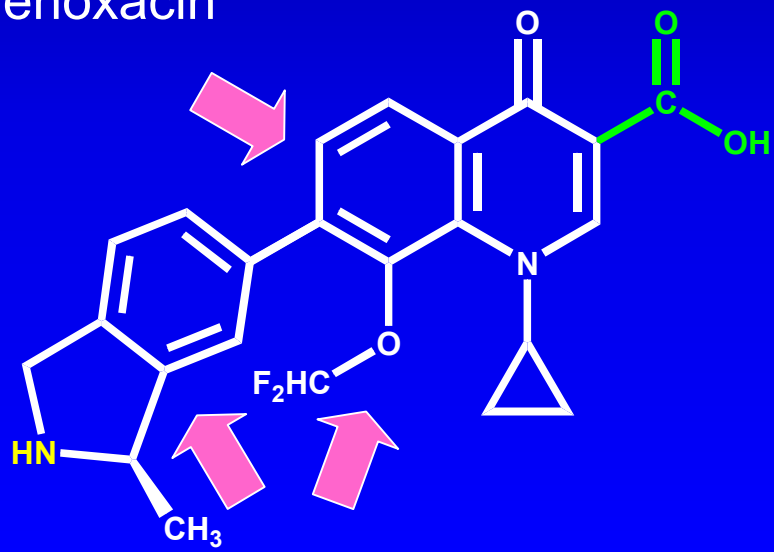
moxifloxacin



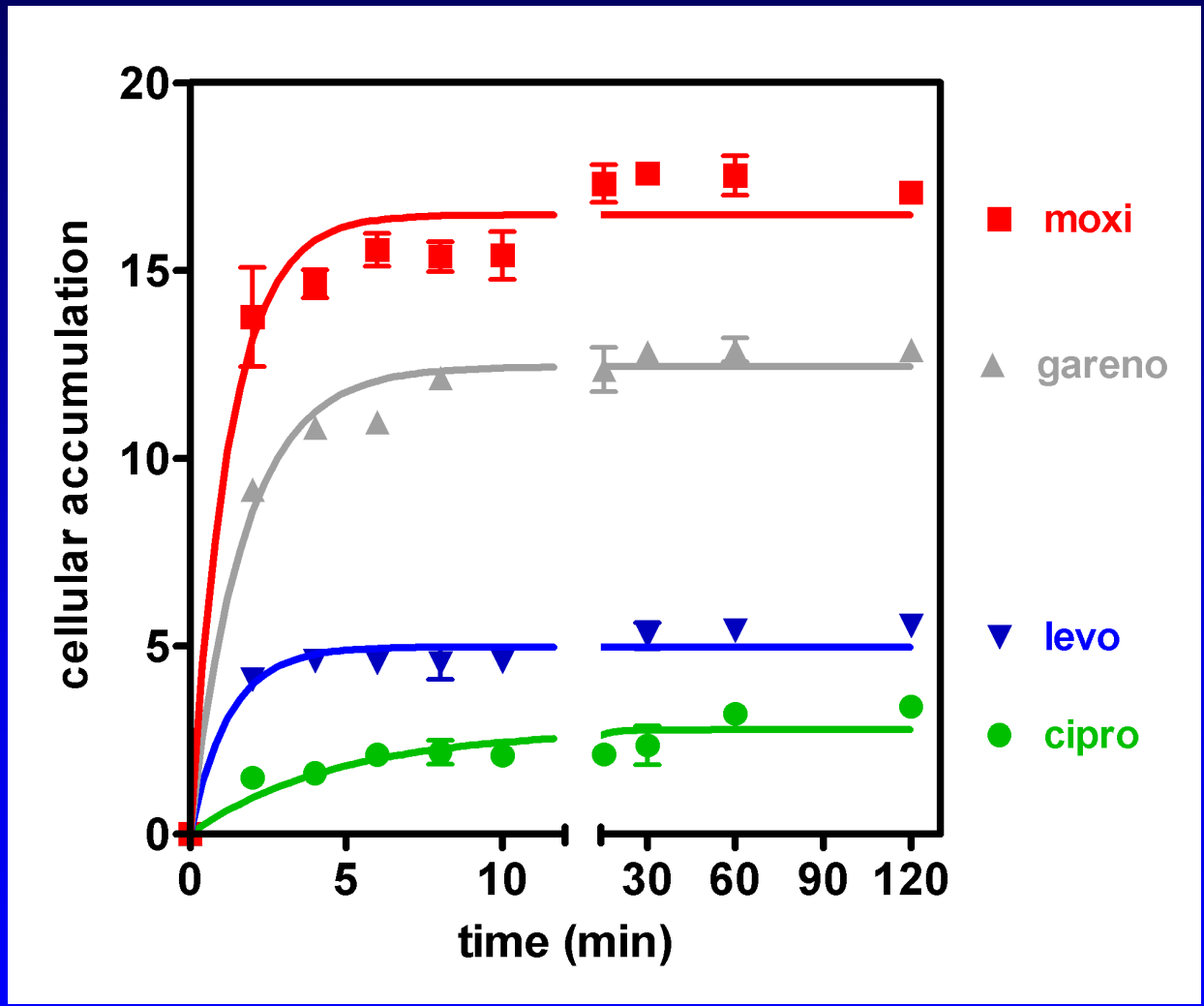
levofloxacin



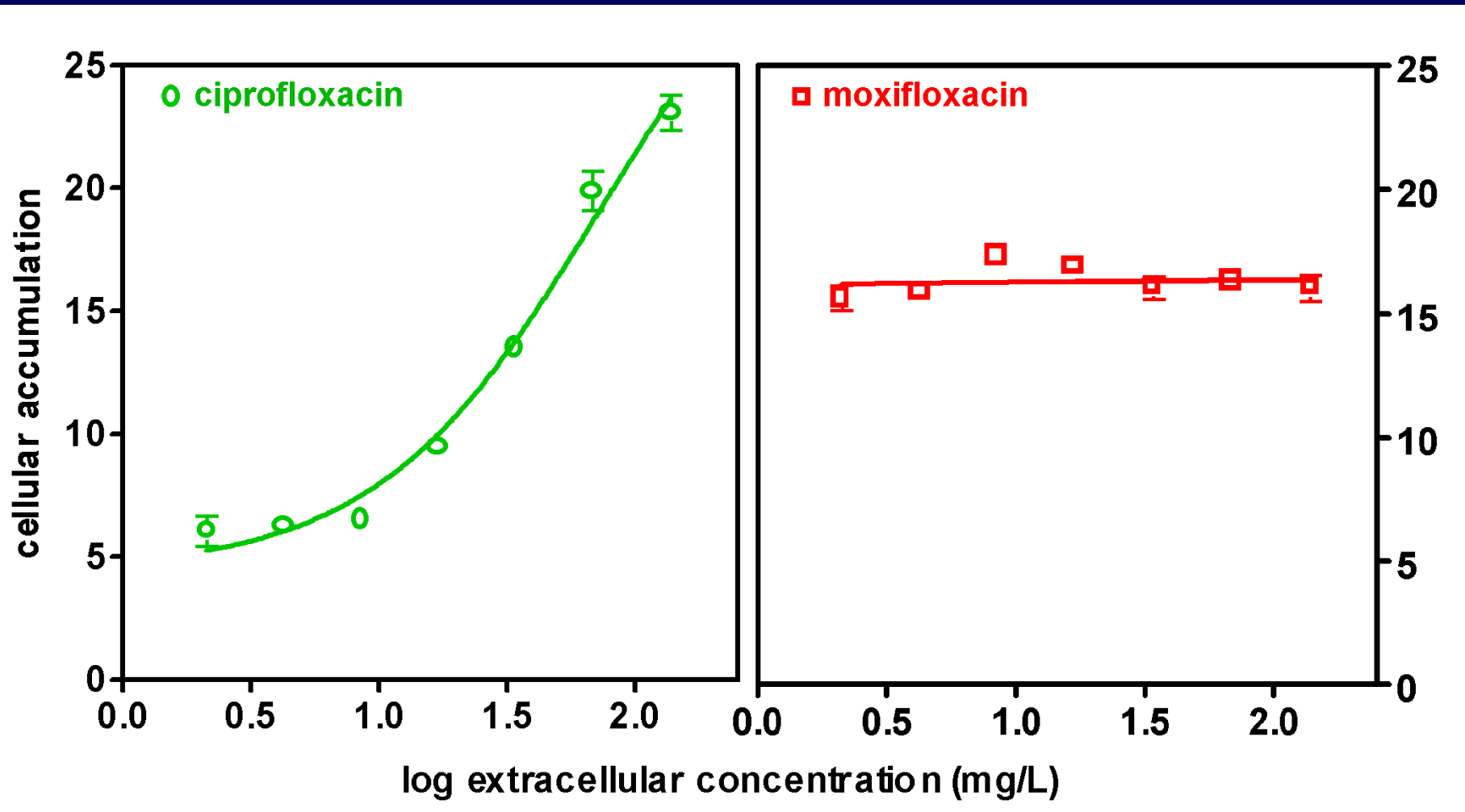
garenoxacin



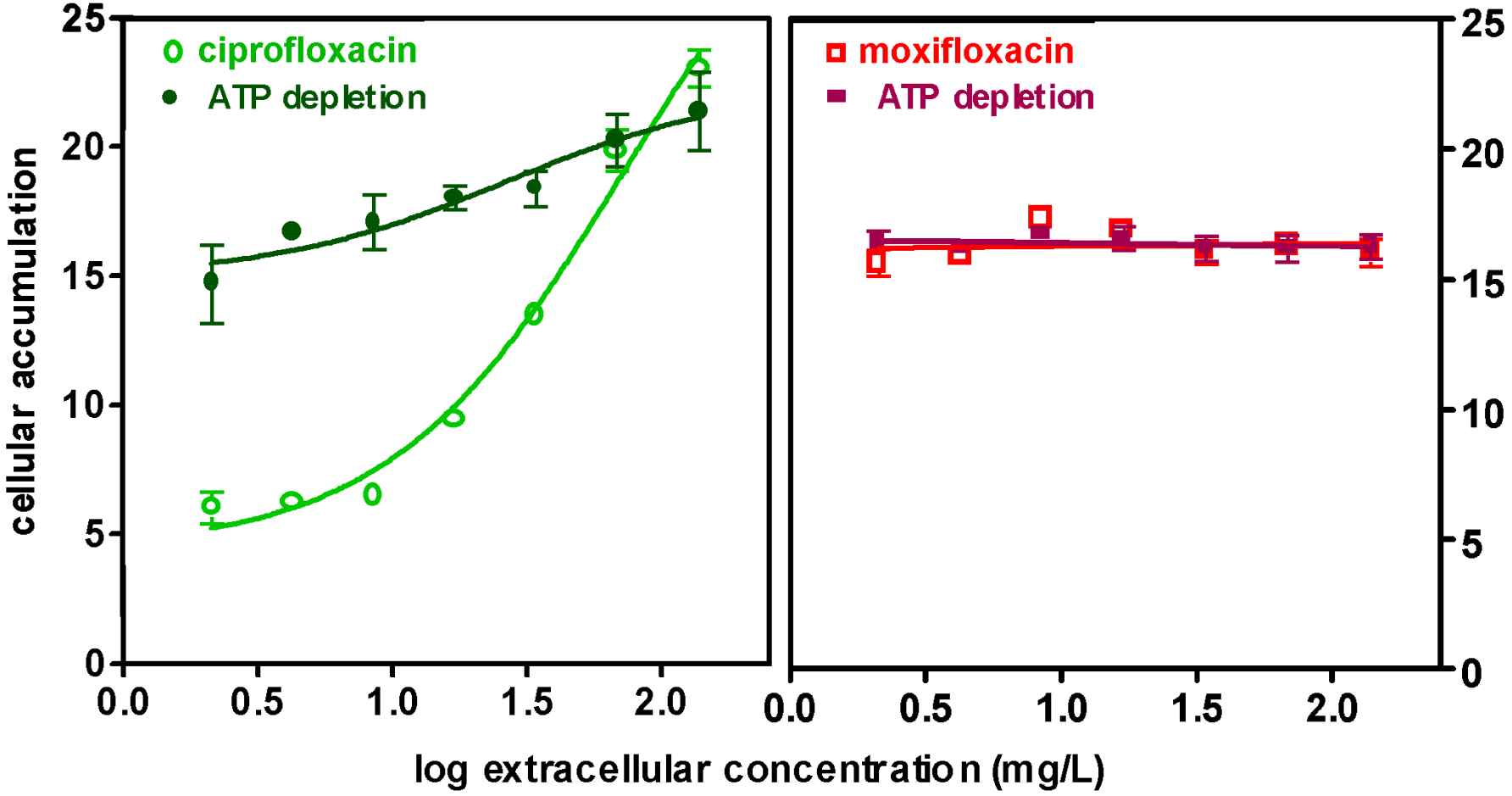
Quinolones markedly differ by their accumulation level



contrasting effect of quinolone concentration on their accumulation

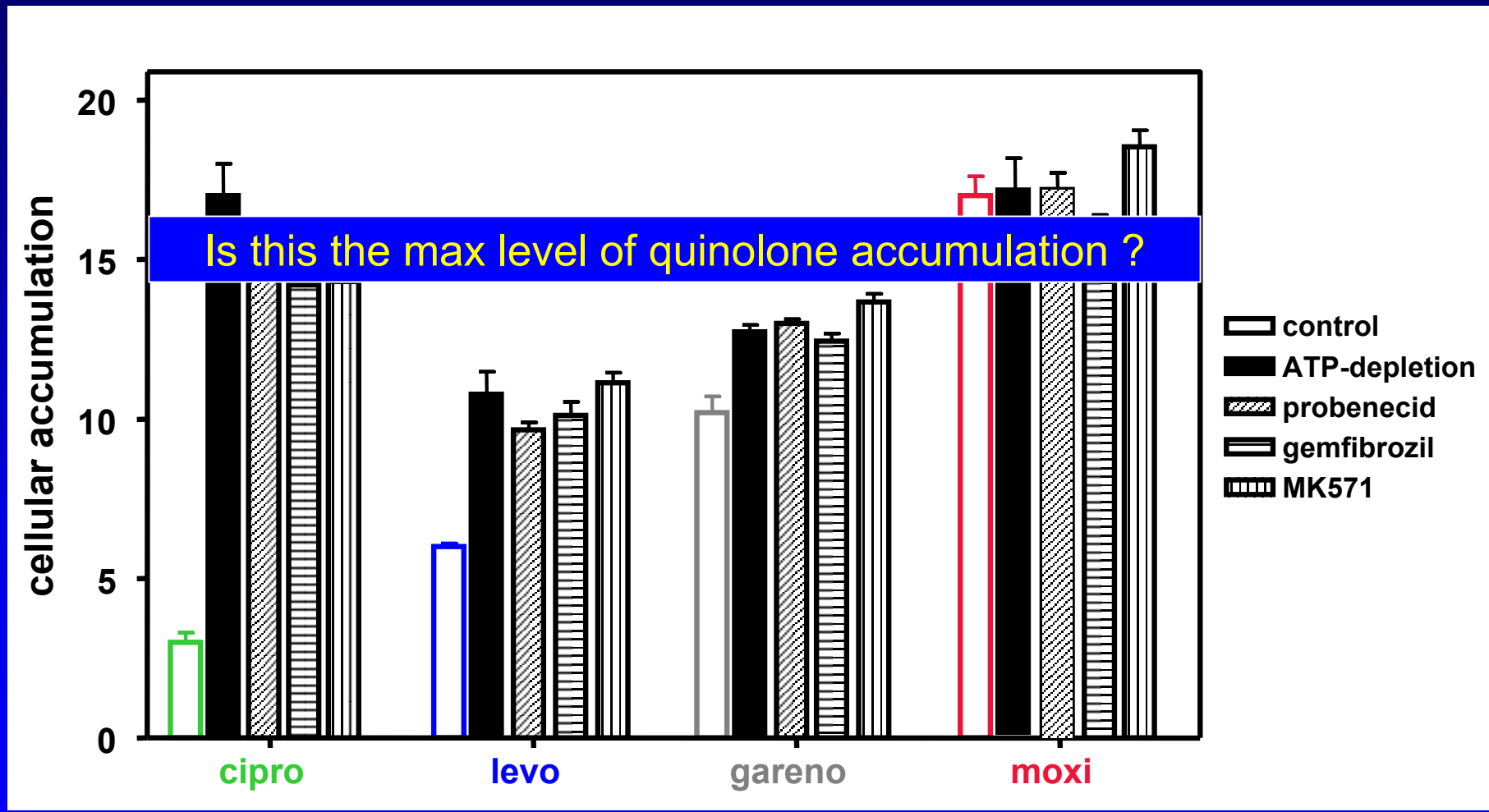


contrasting effect of ATP-depletion on quinolone accumulation



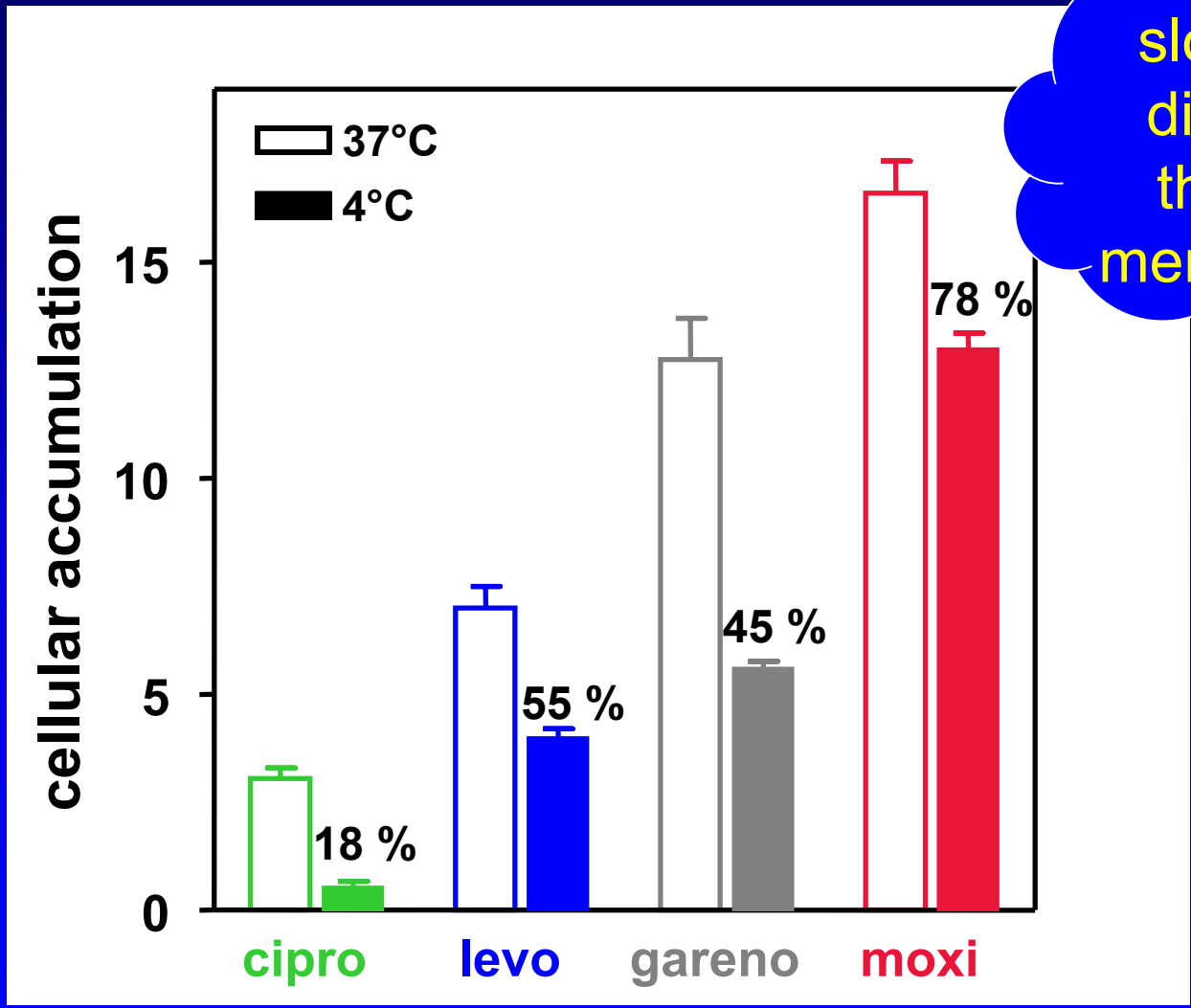
comparative effect of pump inhibition on quinolone accumulation

2 h incubation, 5 mg/L, inhibitors :



contrasting effect of temperature on quinolone accumulation – cooling

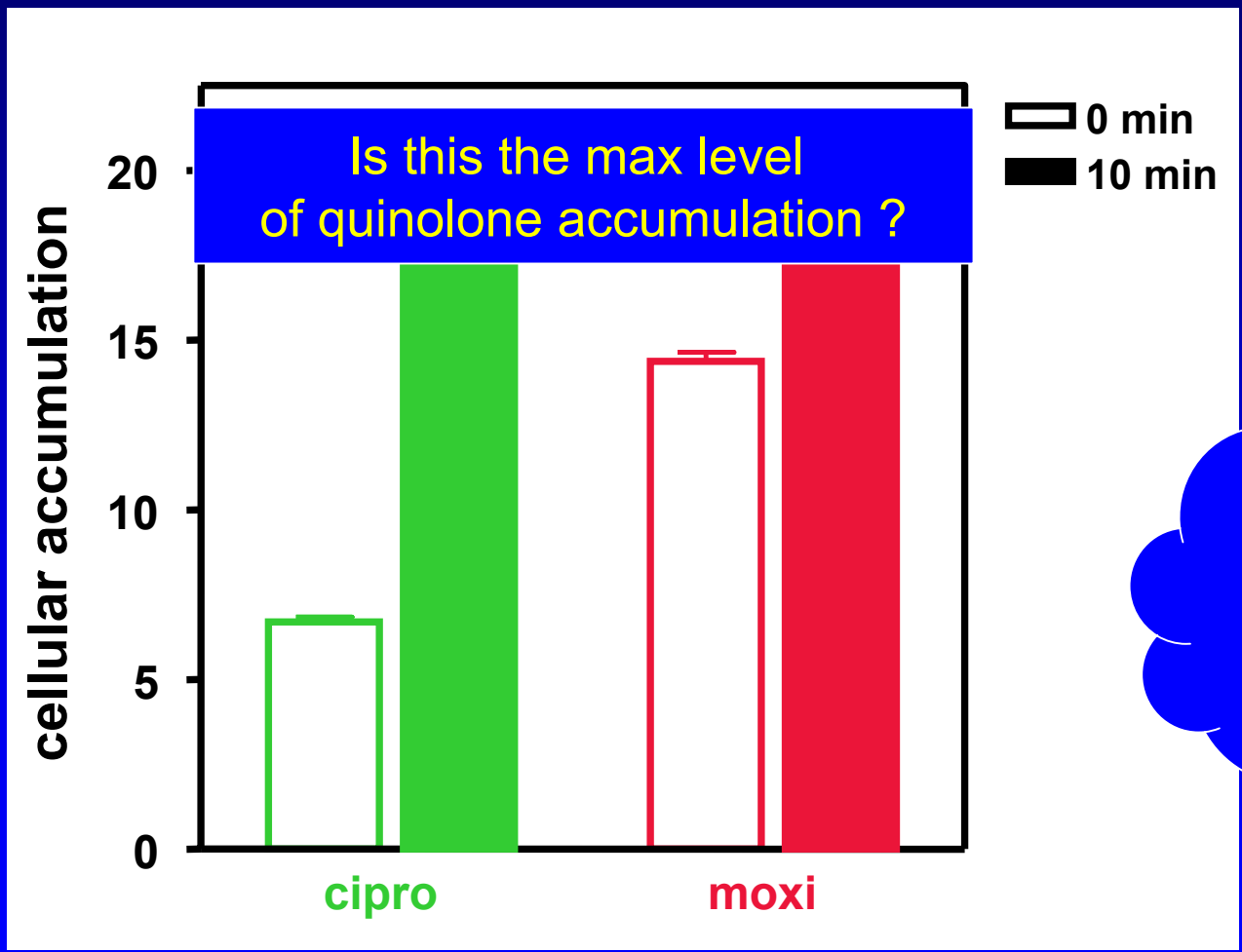
2 h incubation, 5 mg/L, temperature :

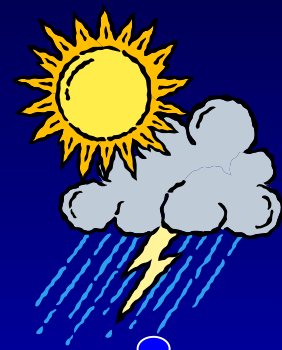


slowered diffusion through membranes

contrasting effect of temperature on quinolone accumulation – heating

2 h incubation at 37°C, 17 mg/L, preexposure to 56°C:



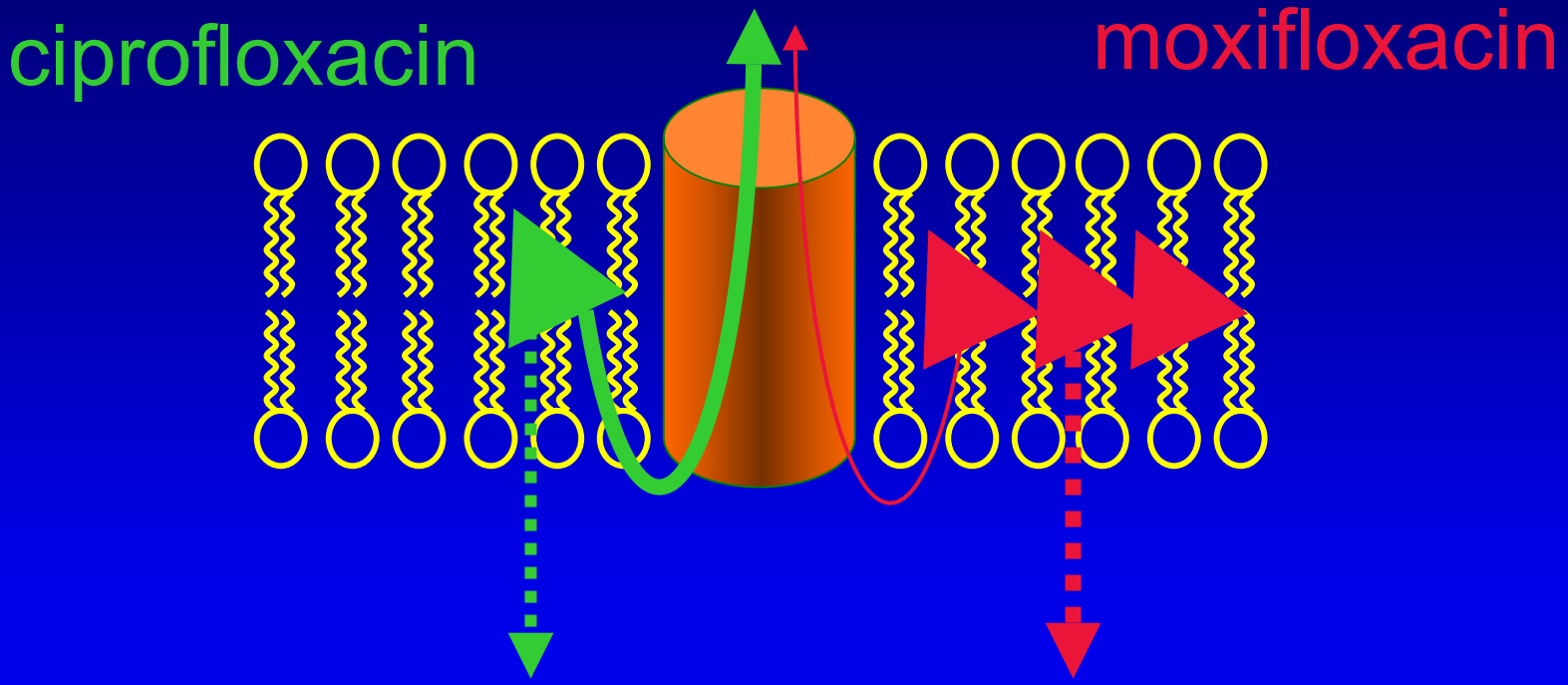


active processes impaired in death cells

quinolone accumulation is passive but efflux is active

➡ actual sorting site may be at the cell surface

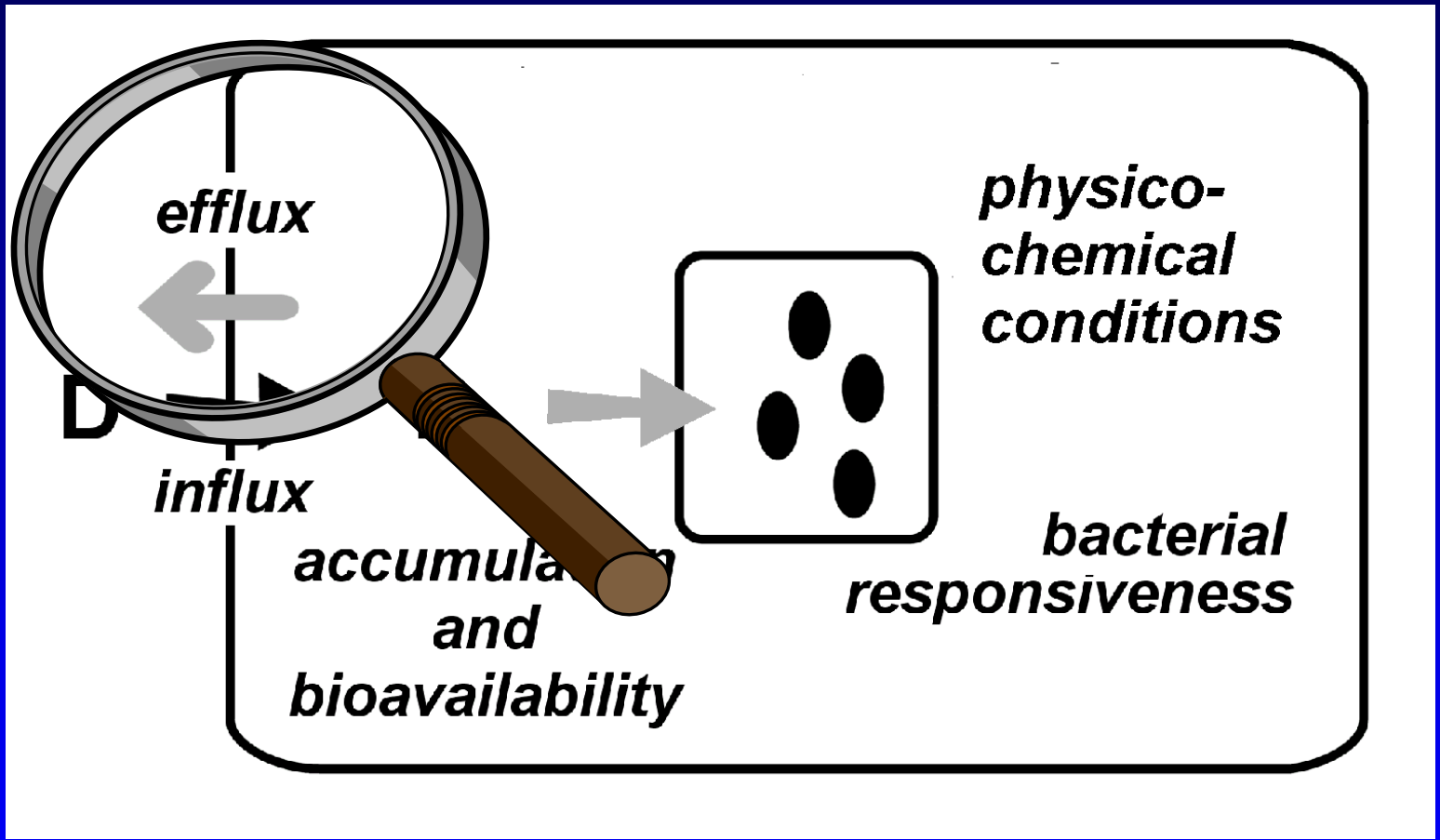
accumulation is inversely related to recognition by efflux transporters



Is the amount of drug reaching the cellular medium high enough to kill intracellular bacteria ?

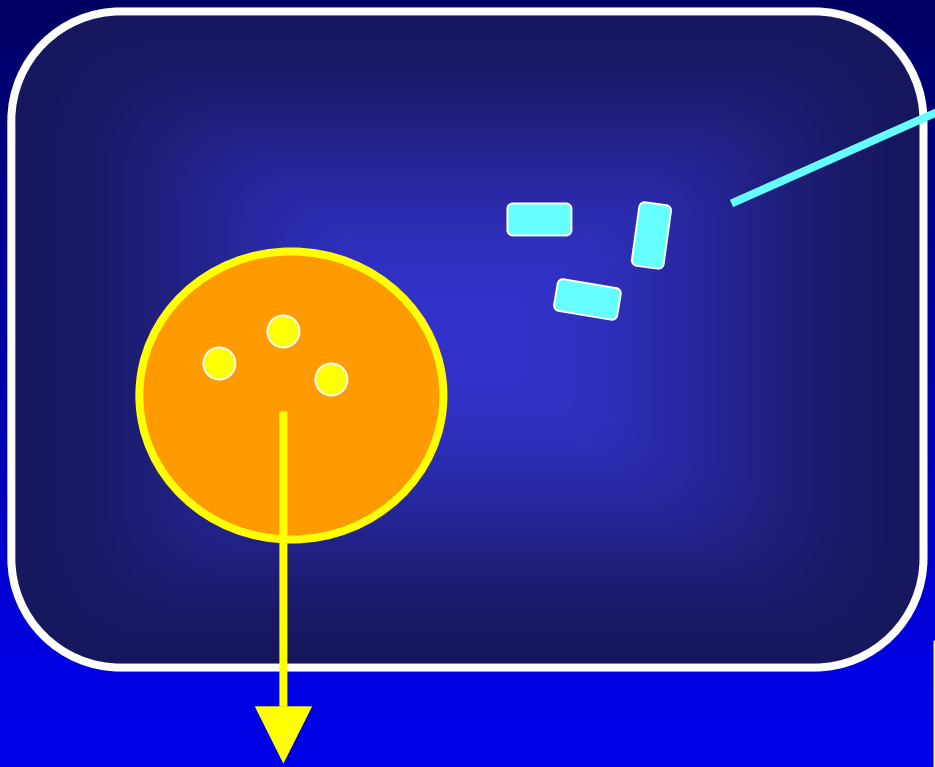
3. Influence of efflux pumps on antibiotic cellular pharmacodynamics

Does efflux from macrophages confer ‘resistance’ against intracellular infections ?



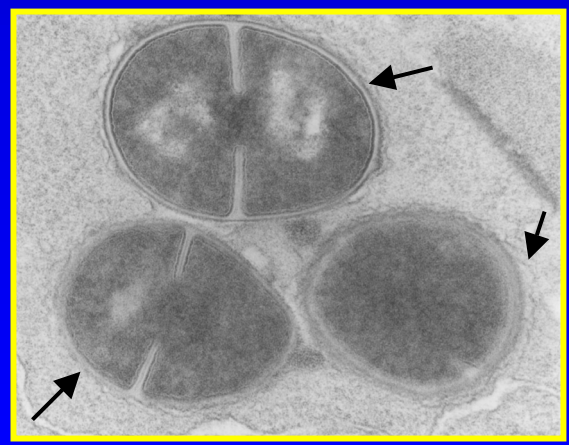
Carryn *et al*, Infect Dis Clin North Am. (2003) 17:615-34

Does efflux affect the intracellular activity of these antibiotics ?

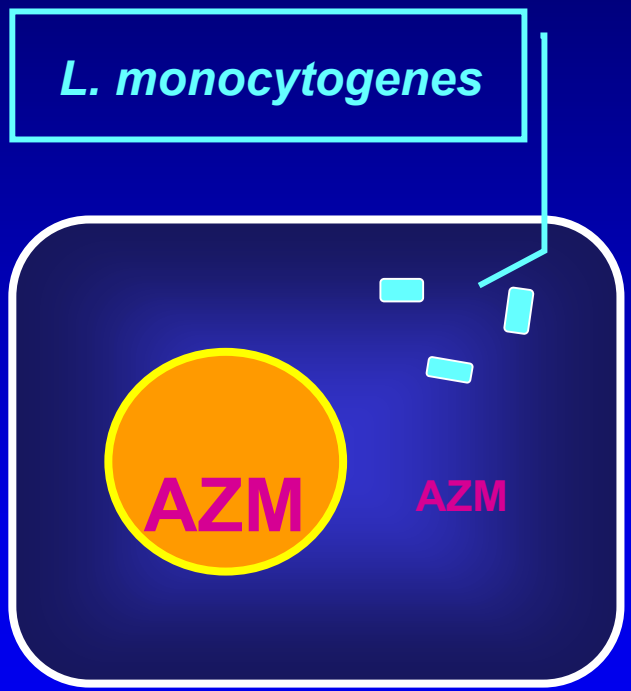


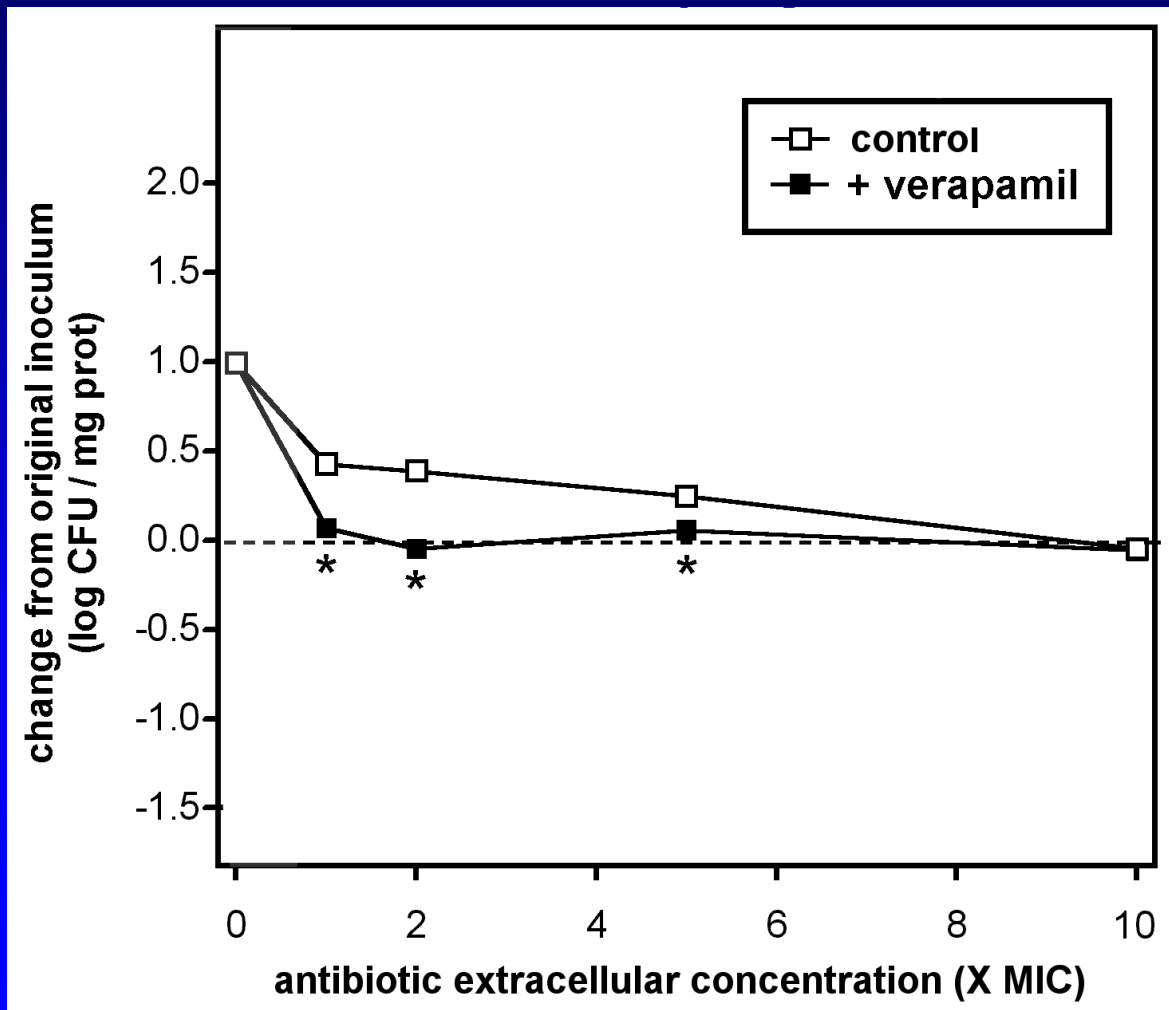
Listeria monocytogenes

Staphylococcus aureus



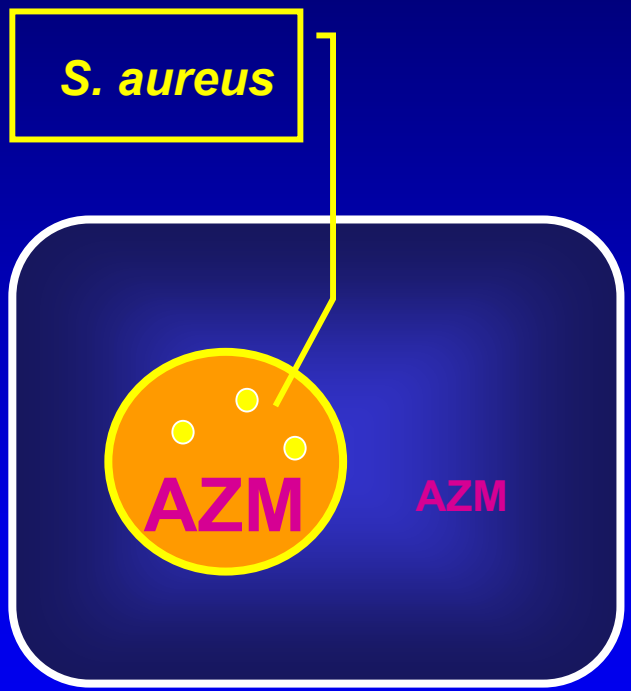
Verapamil increases azithromycin activity against *L. monocytogenes*

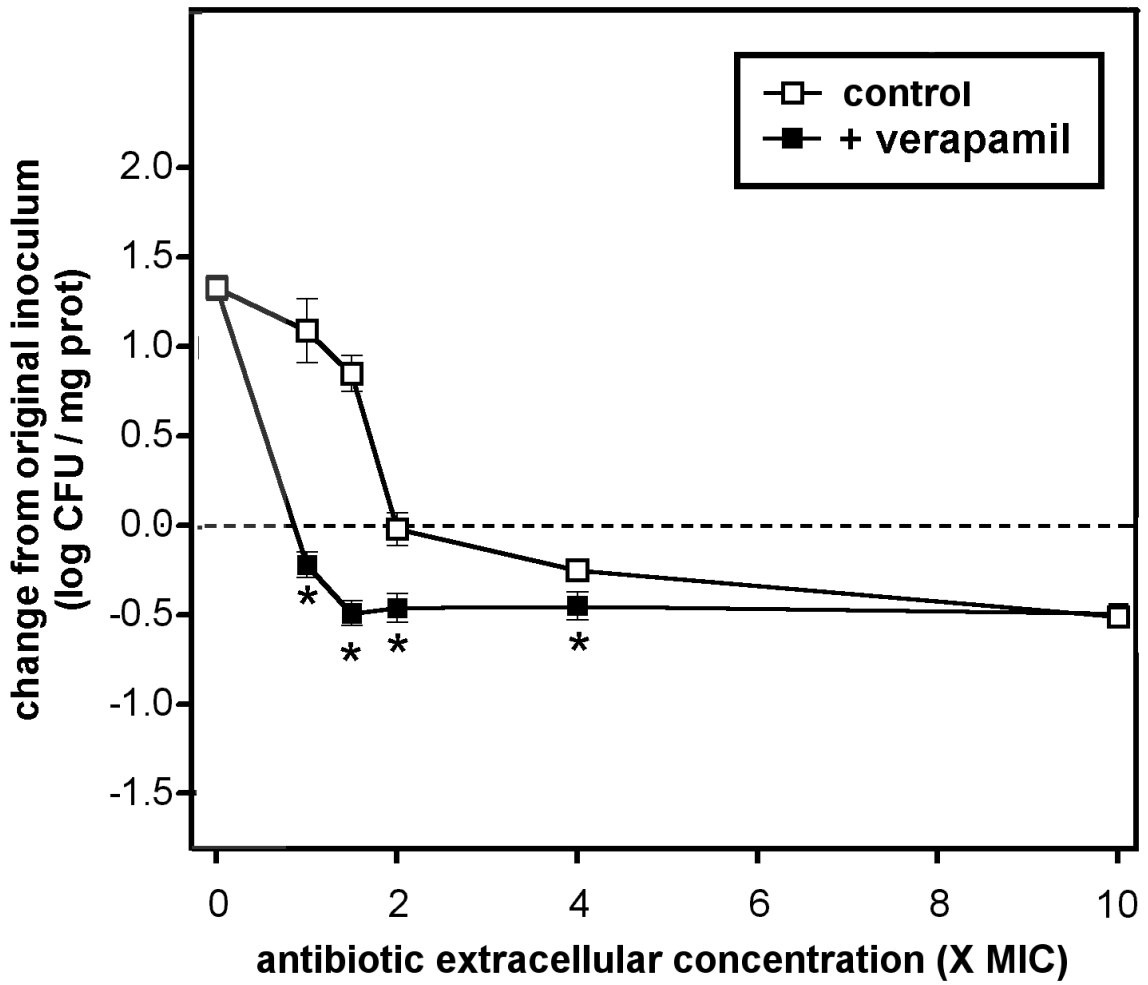




Seral *et al*, JAC (2003) 51:1167-73

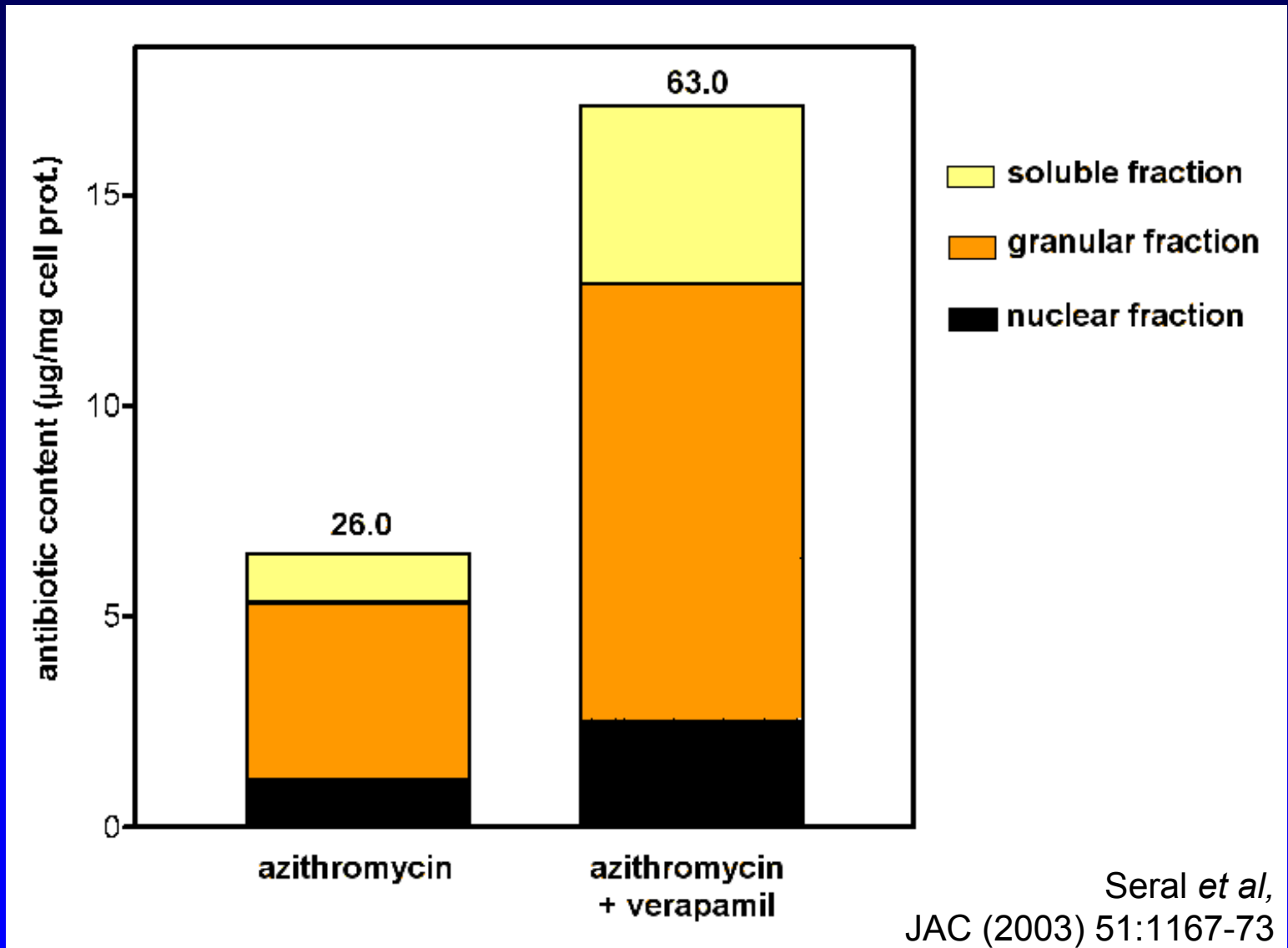
Verapamil increases azithromycin activity against *S. aureus*





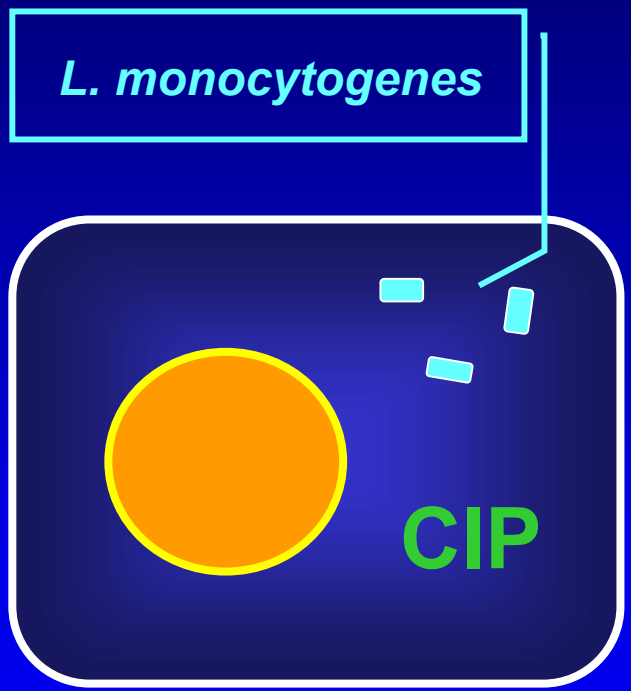
Seral *et al*, JAC (2003) 51:1167-73

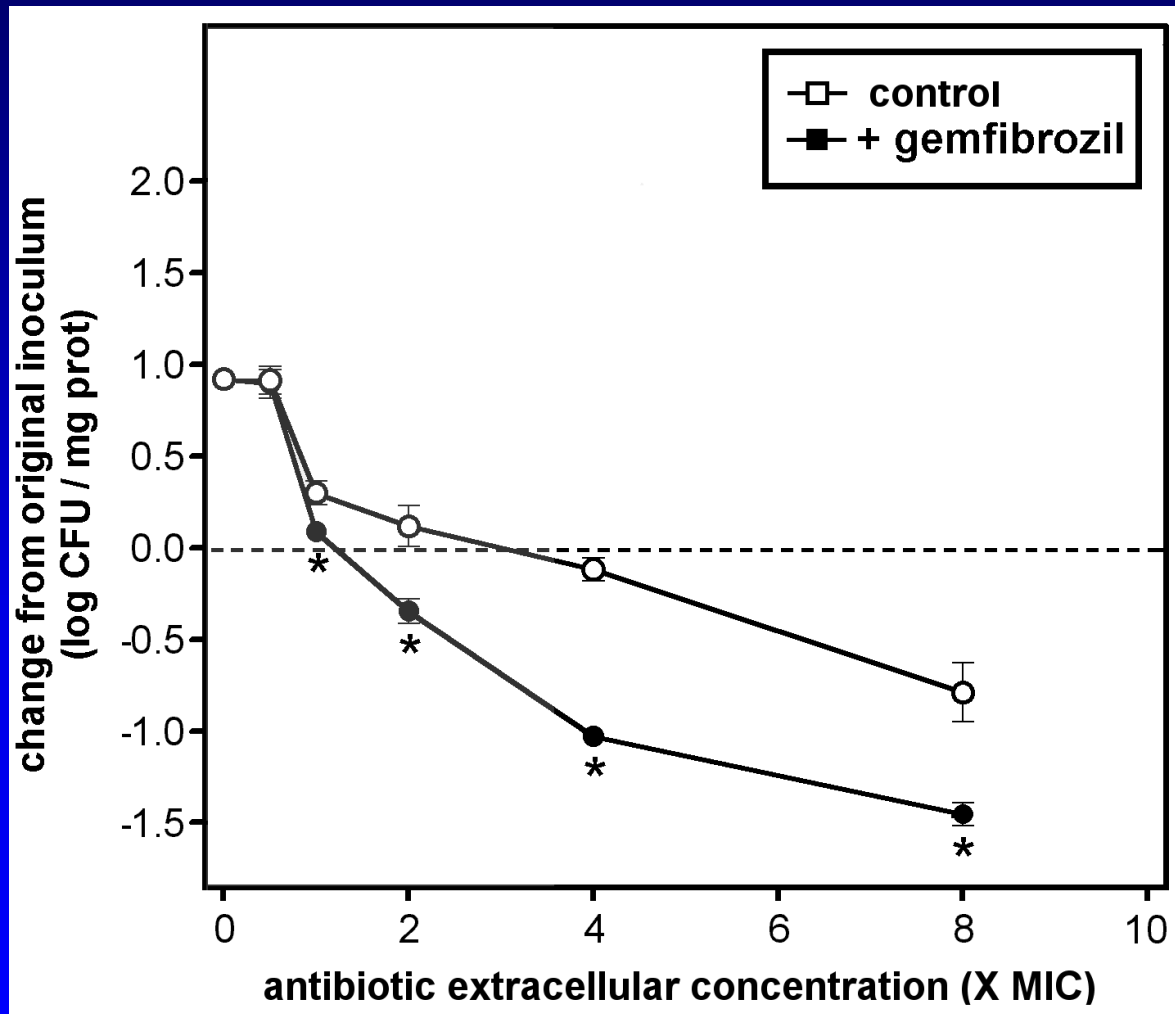
Verapamil increases azithromycin conc. both in the soluble and granular fractions



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

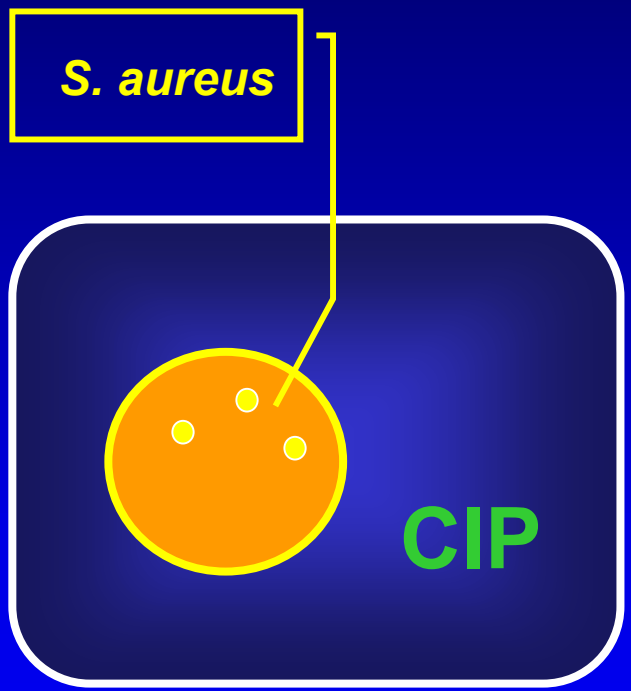
Gemfibrozil increases ciprofloxacin activity against *L. monocytogenes*

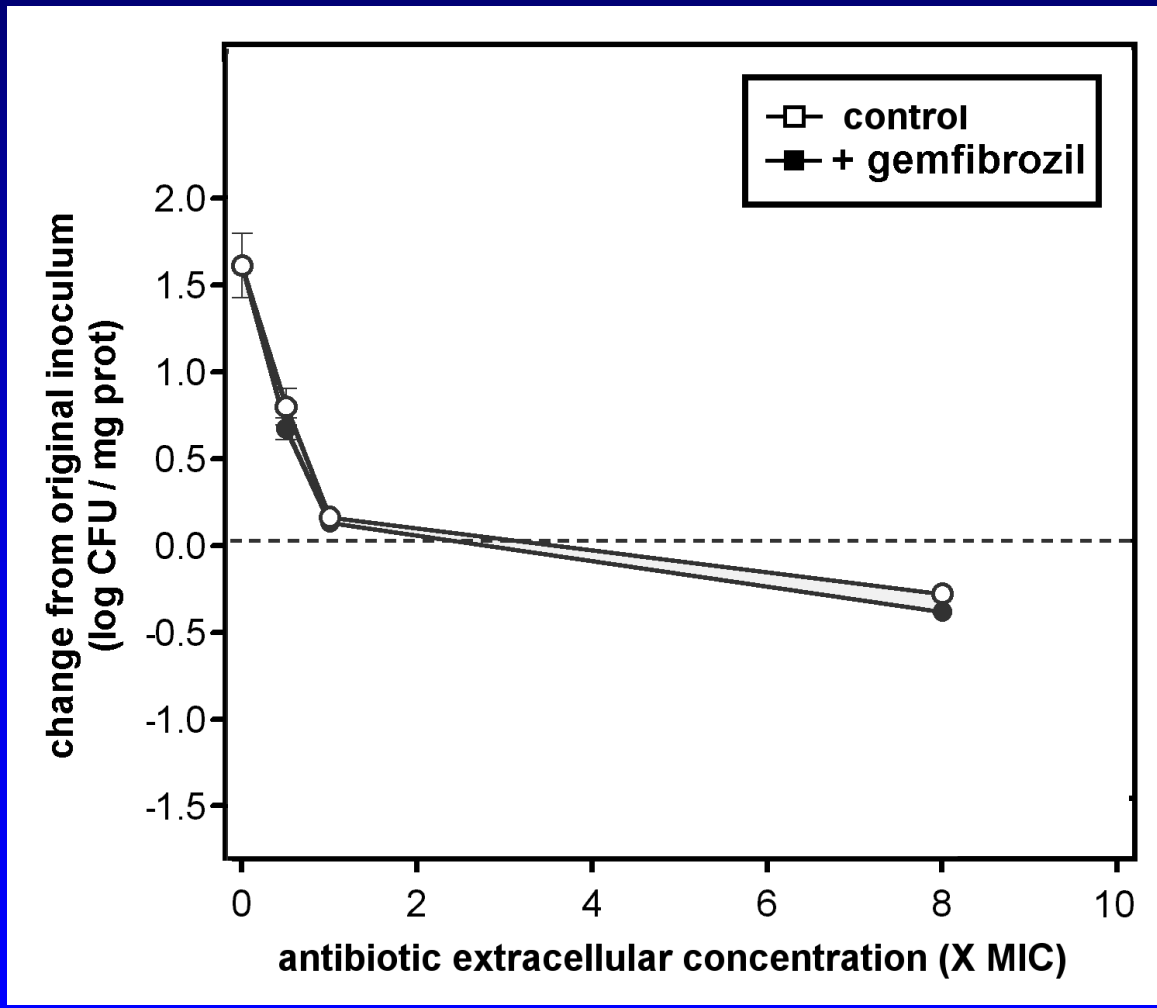




Seral *et al*, JAC (2003) 51:1167-73

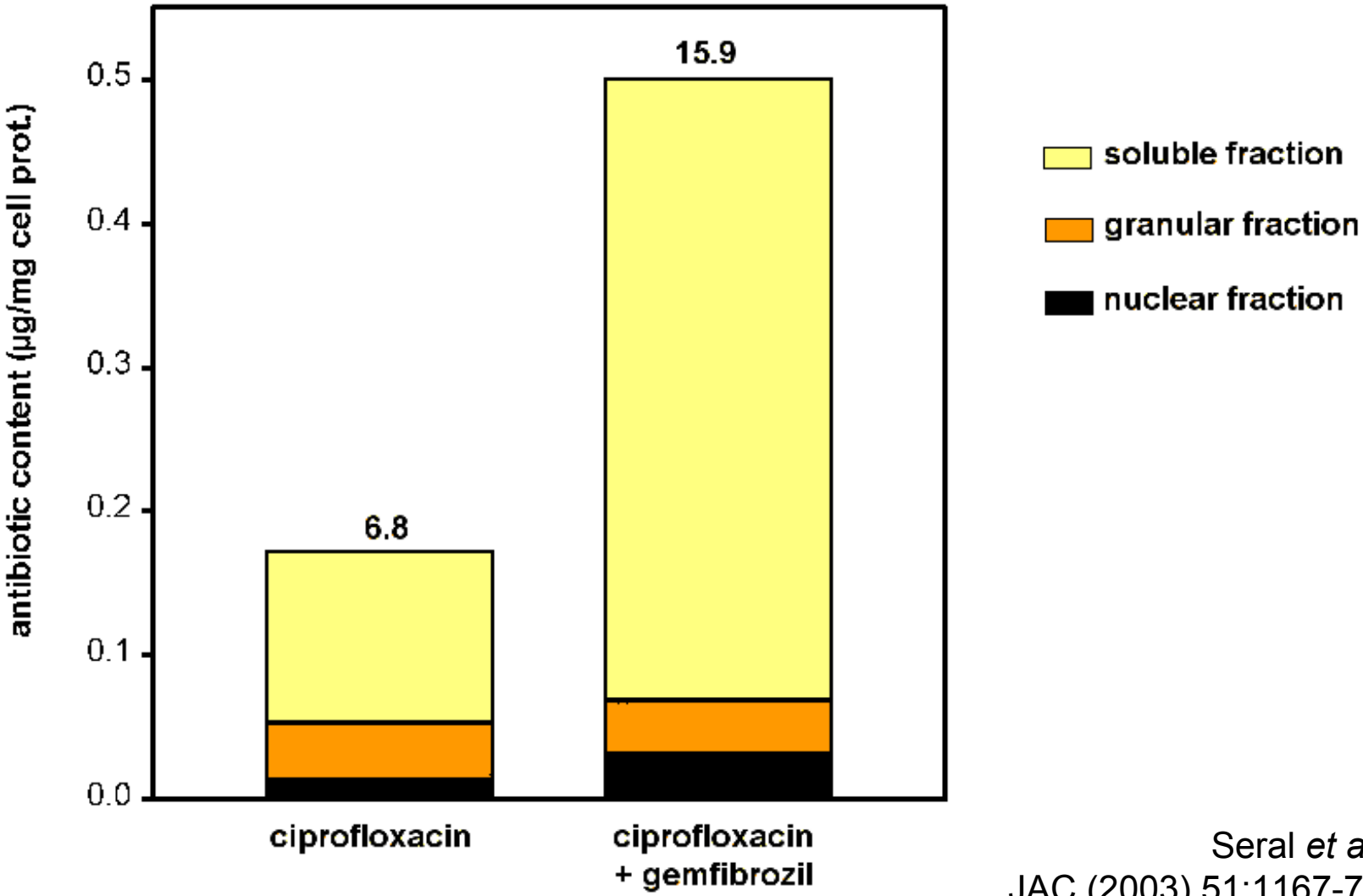
Gemfibrozil does not increases ciprofloxacin activity against *S. aureus*





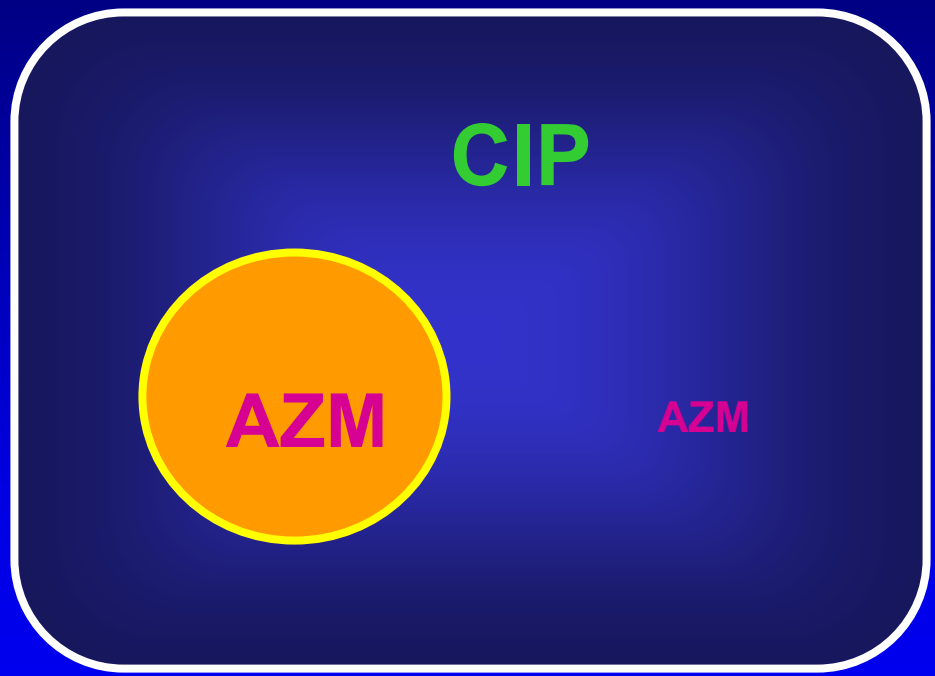
Seral *et al*, JAC (2003) 51:1167-73

Gemfibrozil increases ciprofloxacin conc. in the soluble fraction only



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

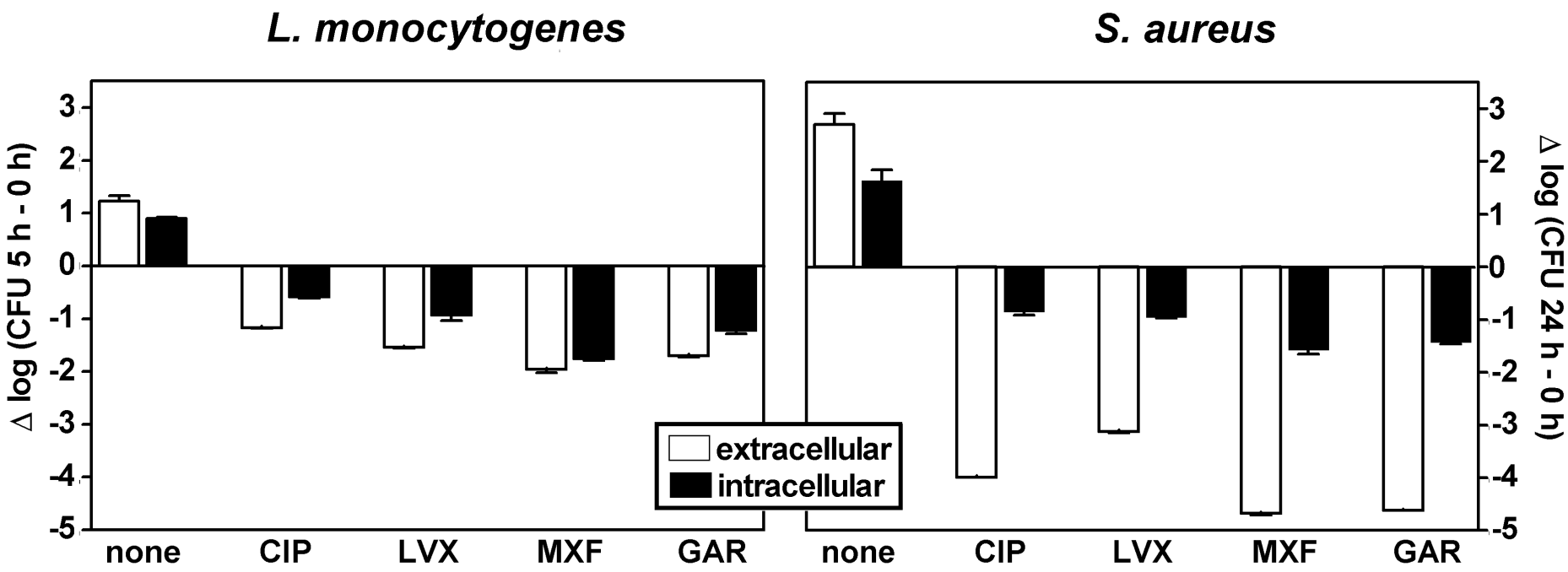
Inhibition of efflux pumps may increase antibiotic activity in the compartments where they accumulate



Strategies for the future of antibiotherapy of intracellular infections

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

moxi/gareno are more active than cipro/levo against *L. monocytogenes* et *S. aureus*



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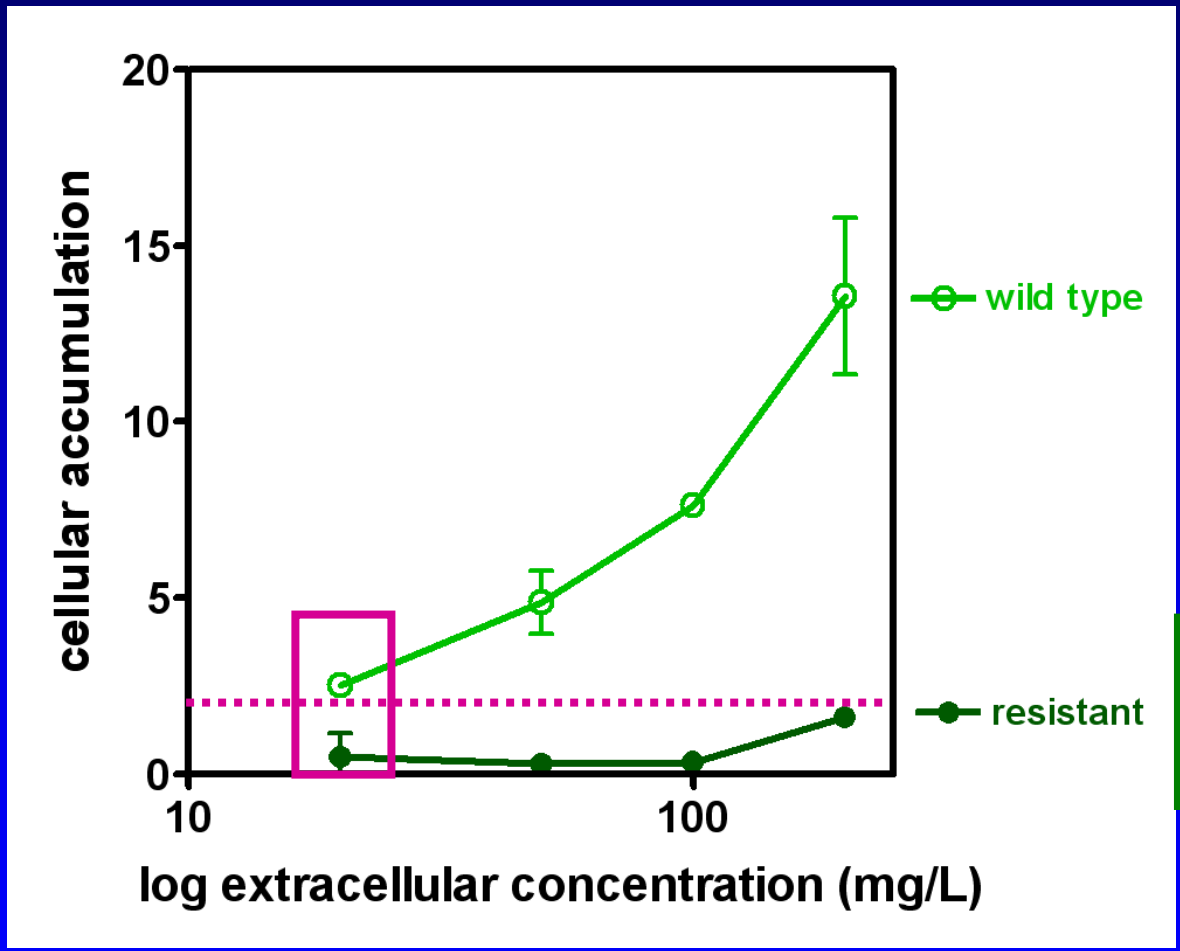
Over-expression of efflux pumps as mechanism of resistance

anticancer agent

antibiotic ?????

Ciprofloxacin selects over-expression of efflux pumps as mechanism of resistance

2 h incubation at 37°C



macrophages exposed to increasing conc. of cipro (up to 80 mg/L)

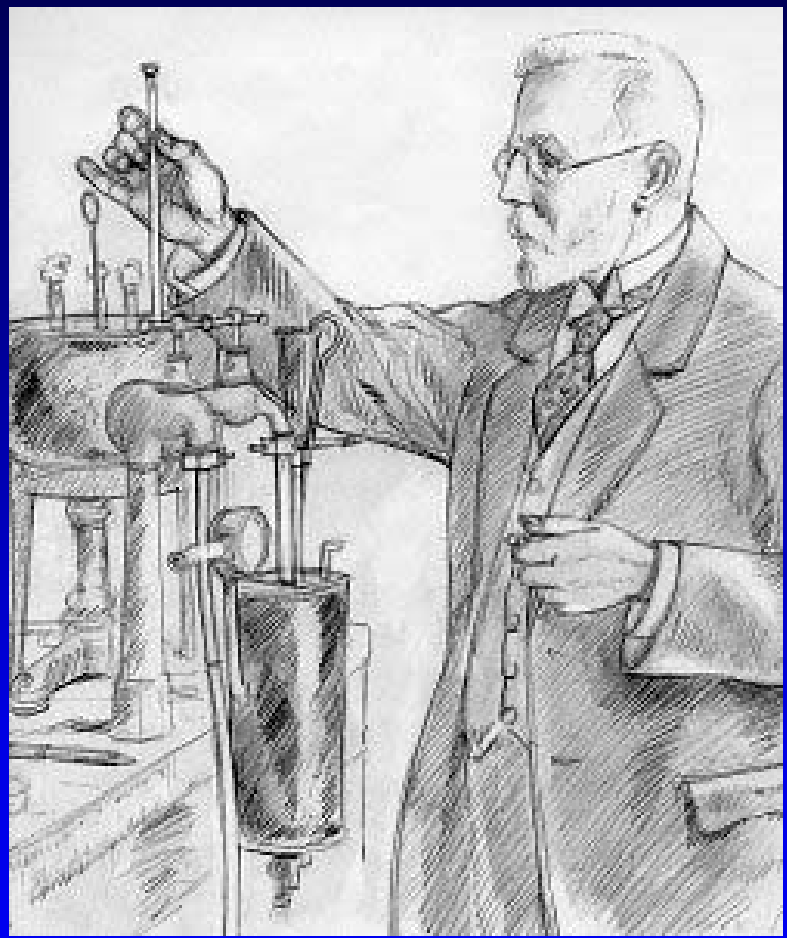
Strategies for the future of antibiotherapy of intracellular infections

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

Inhibitors of efflux transporters …

should help you to keep your stuff in ...

Inhibitors of efflux transporters …

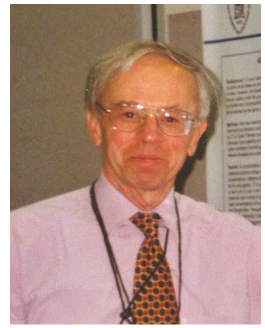


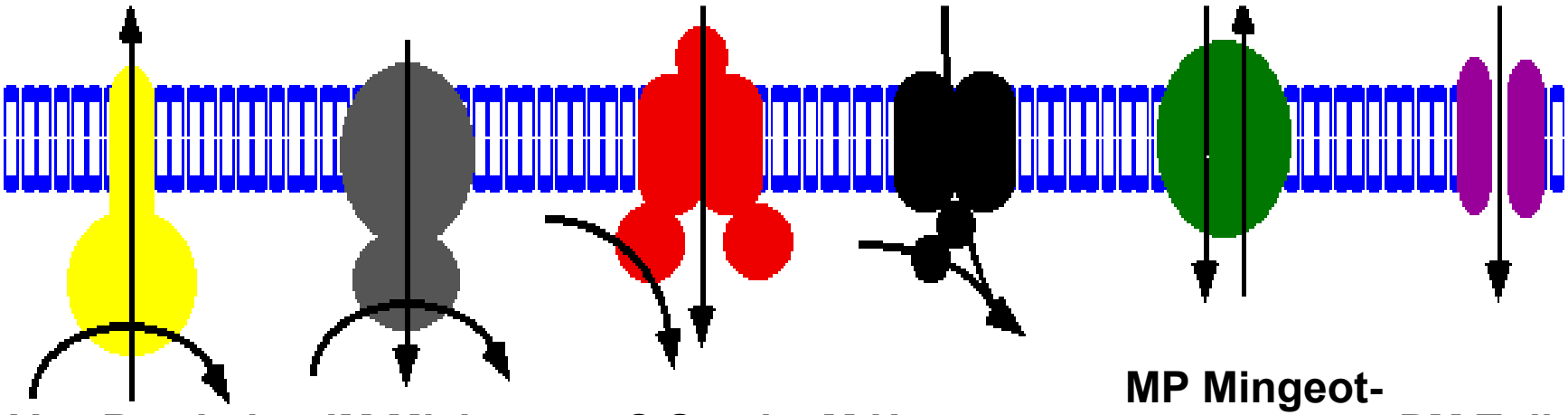
But be careful not to turn off a useful pump ...

Thanks to ...







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

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