



*COLLOQUE ABC FRANCO - BELGE*  
/  
*FRENCH - BELGIAN ABC MEETING*

**Antibiotic efflux in eucaryotic cells:  
implications of ABC transporters  
and pharmacokinetic  
and pharmacodynamic consequences**

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# Antibiotics as substrates of MDR transporters

broad spectrum, several classes of drugs



## P-glycoprotein

- macrolides, rifampicin
- anticancer agents
- antidepressants, antiepileptics
- digoxin
- ...

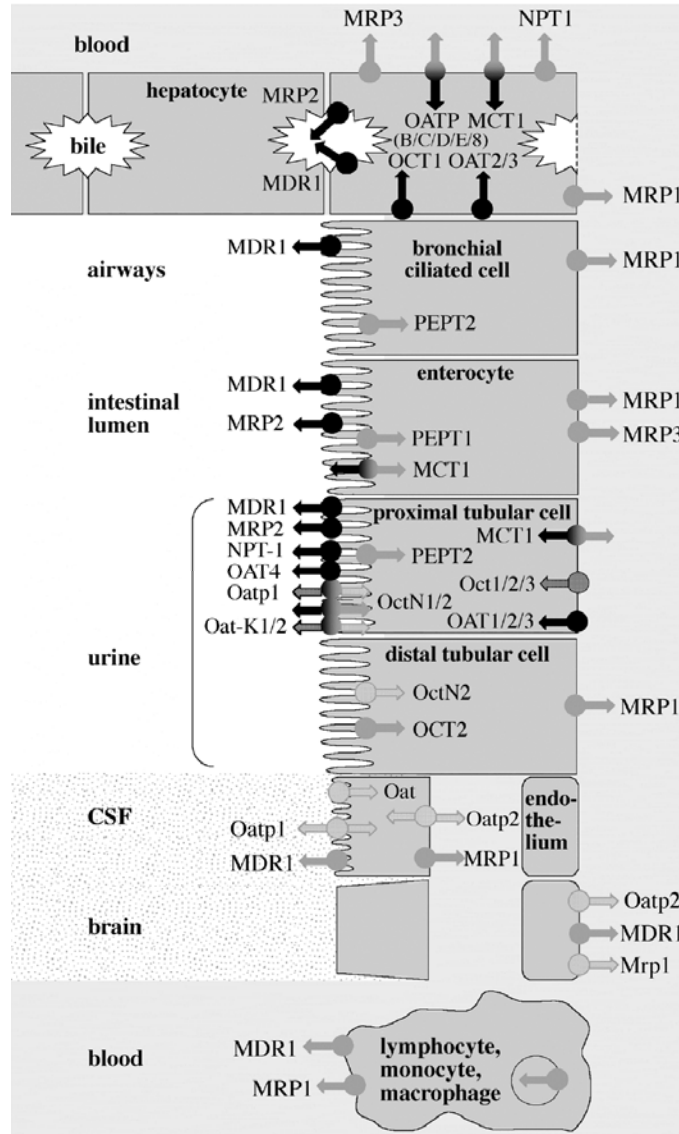
## MRP

- fluoroquinolones,  $\beta$ -lactams
- antiviral agents
- anticancer agents
- drug conjugates
- ...

# Antibiotics as substrates of MDR transporters

➔ Pharmacokinetic implications

➔ Pharmacodynamic implications



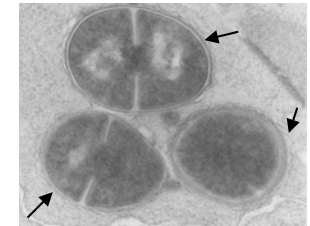
## intracellular infections

*L. monocytogenes*

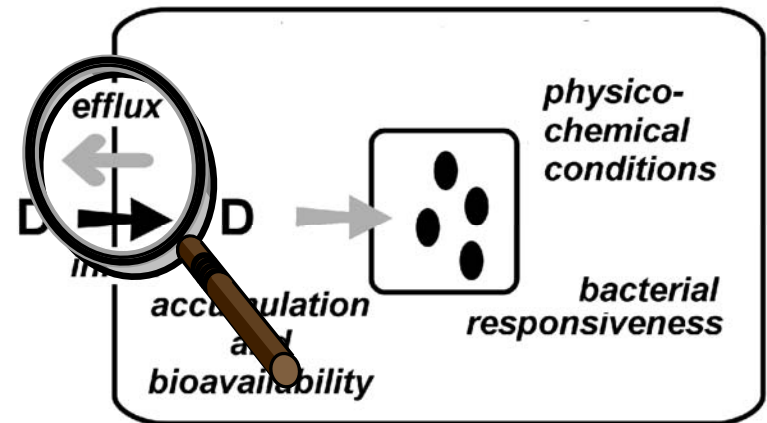


cytosol

*S. aureus*

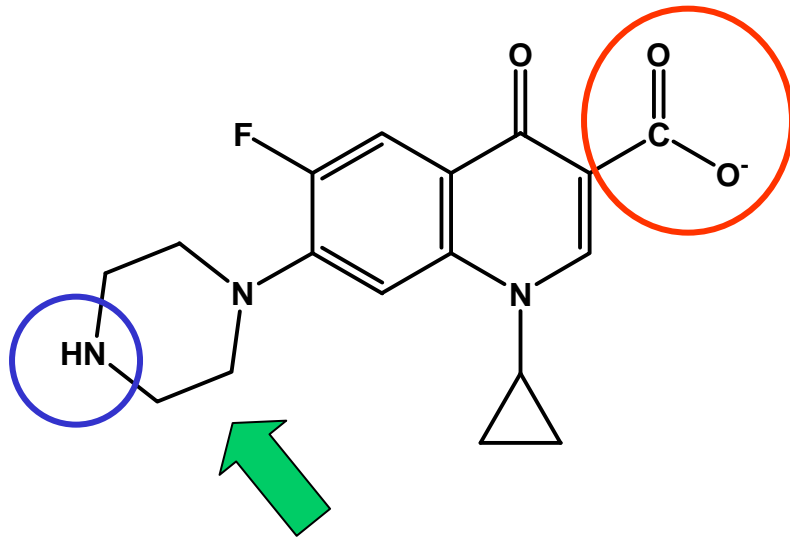


phagolysosomes

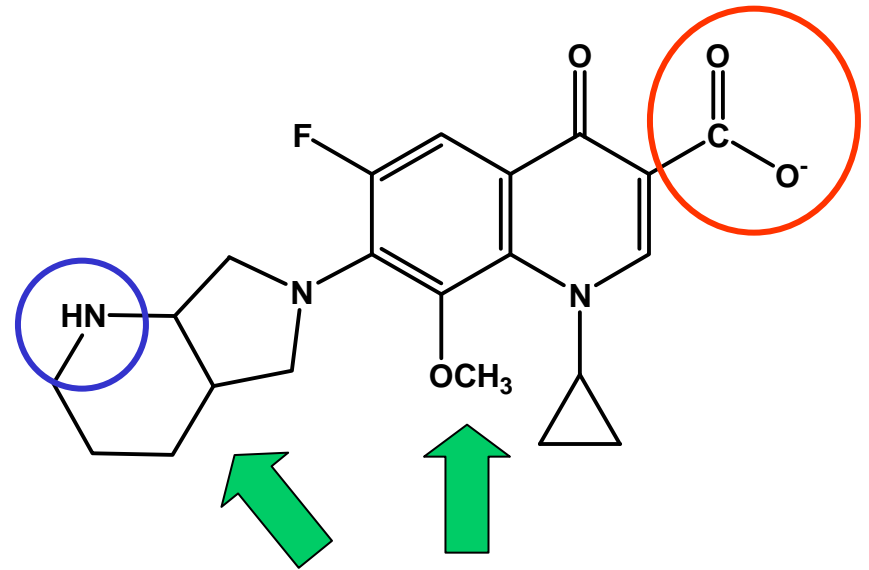


# Different behavior of closely structurally-related quinolones

## ciprofloxacin

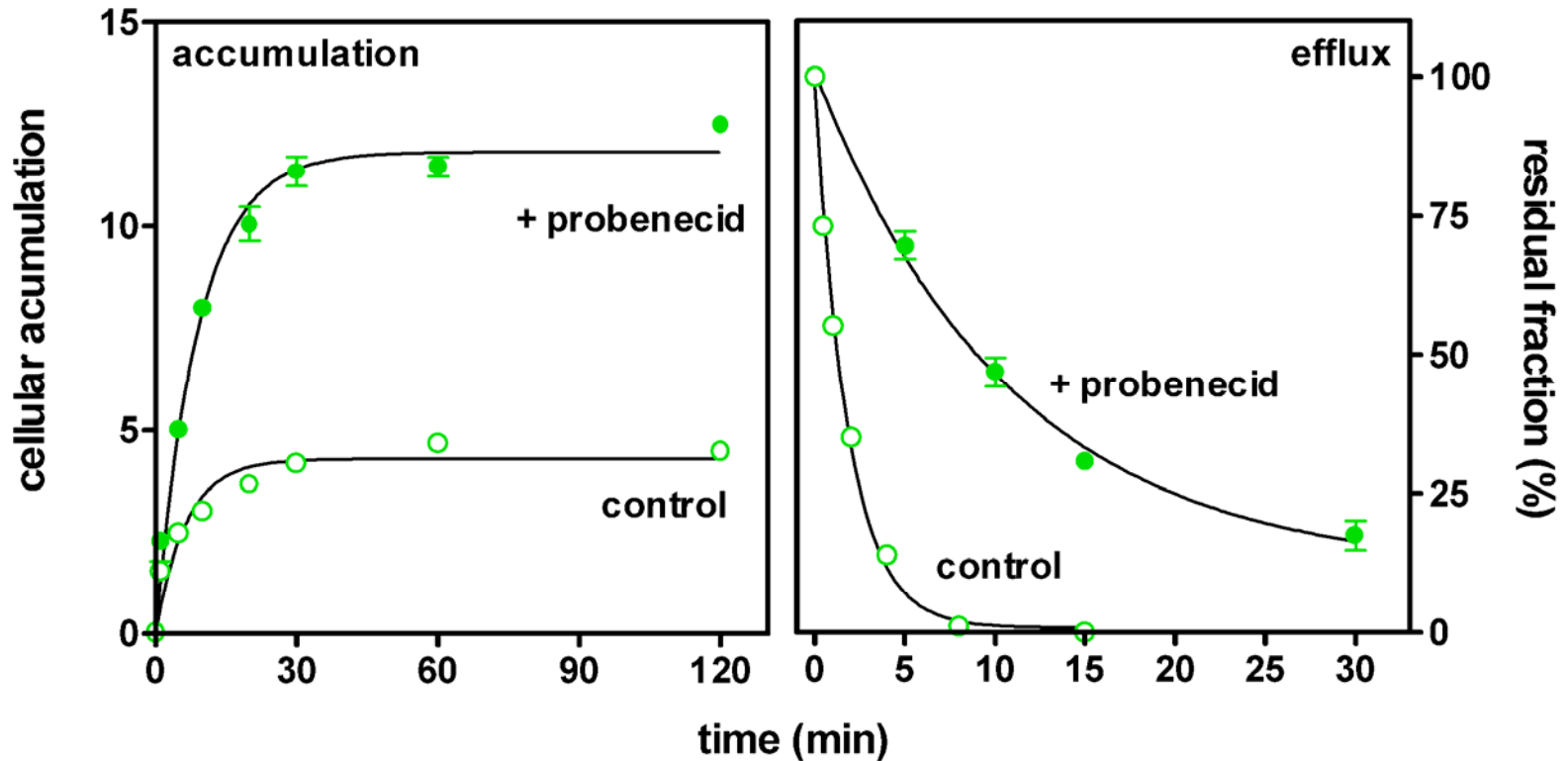


## moxifloxacin



# Kinetics of accumulation and efflux for ciprofloxacin

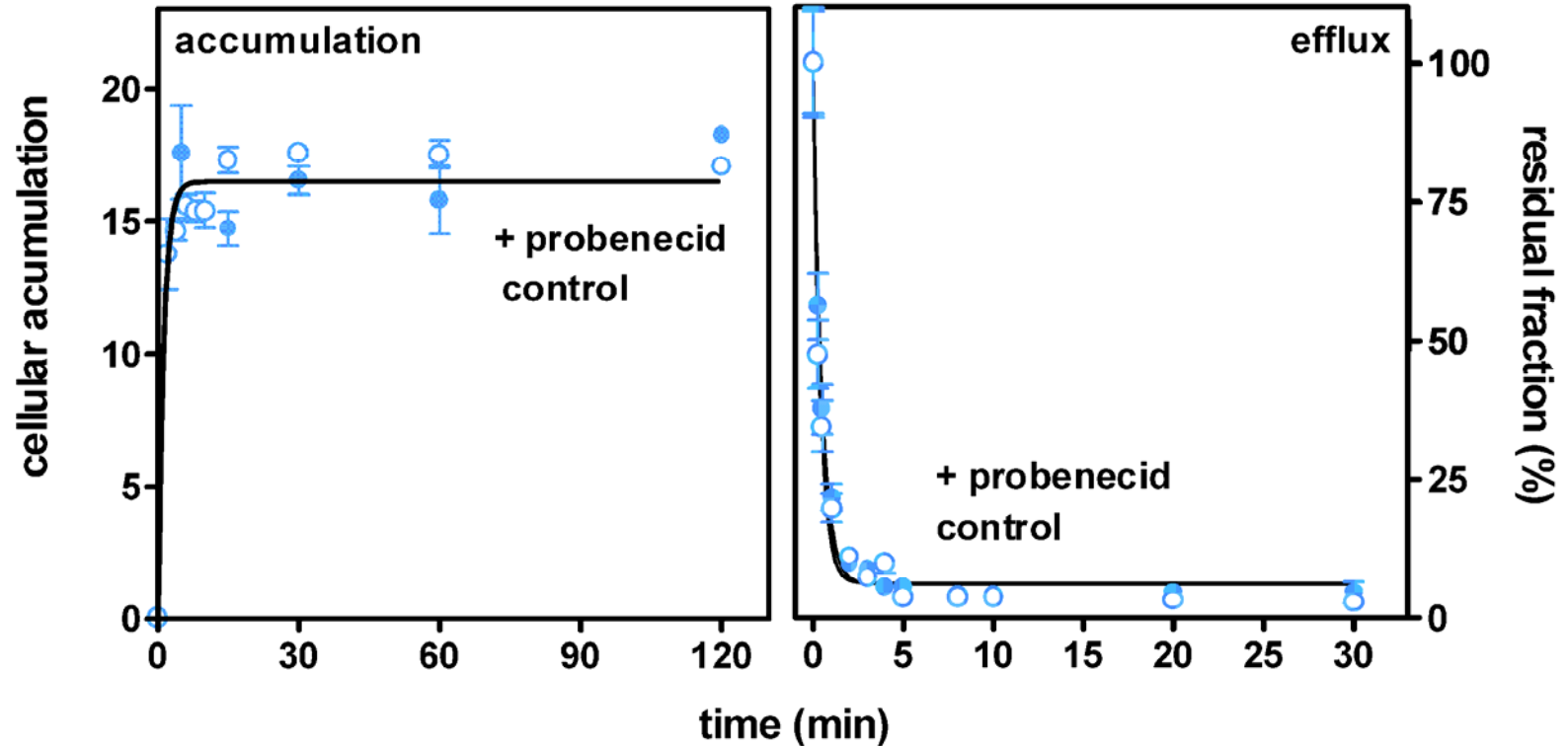
both accumulation and efflux markedly affected by MRP inhibitors



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

# Kinetics of accumulation and efflux for moxifloxacin

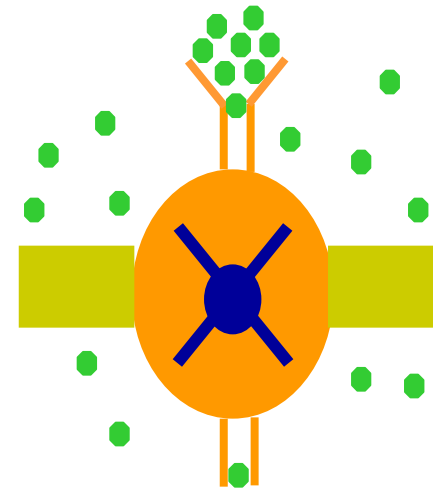
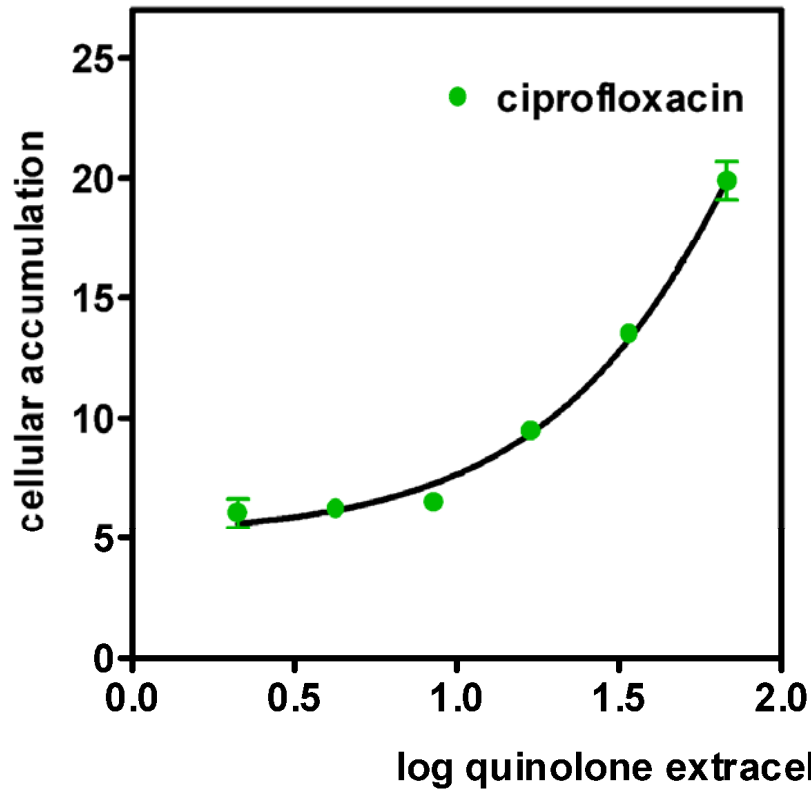
neither accumulation nor efflux affected by MRP inhibitors



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

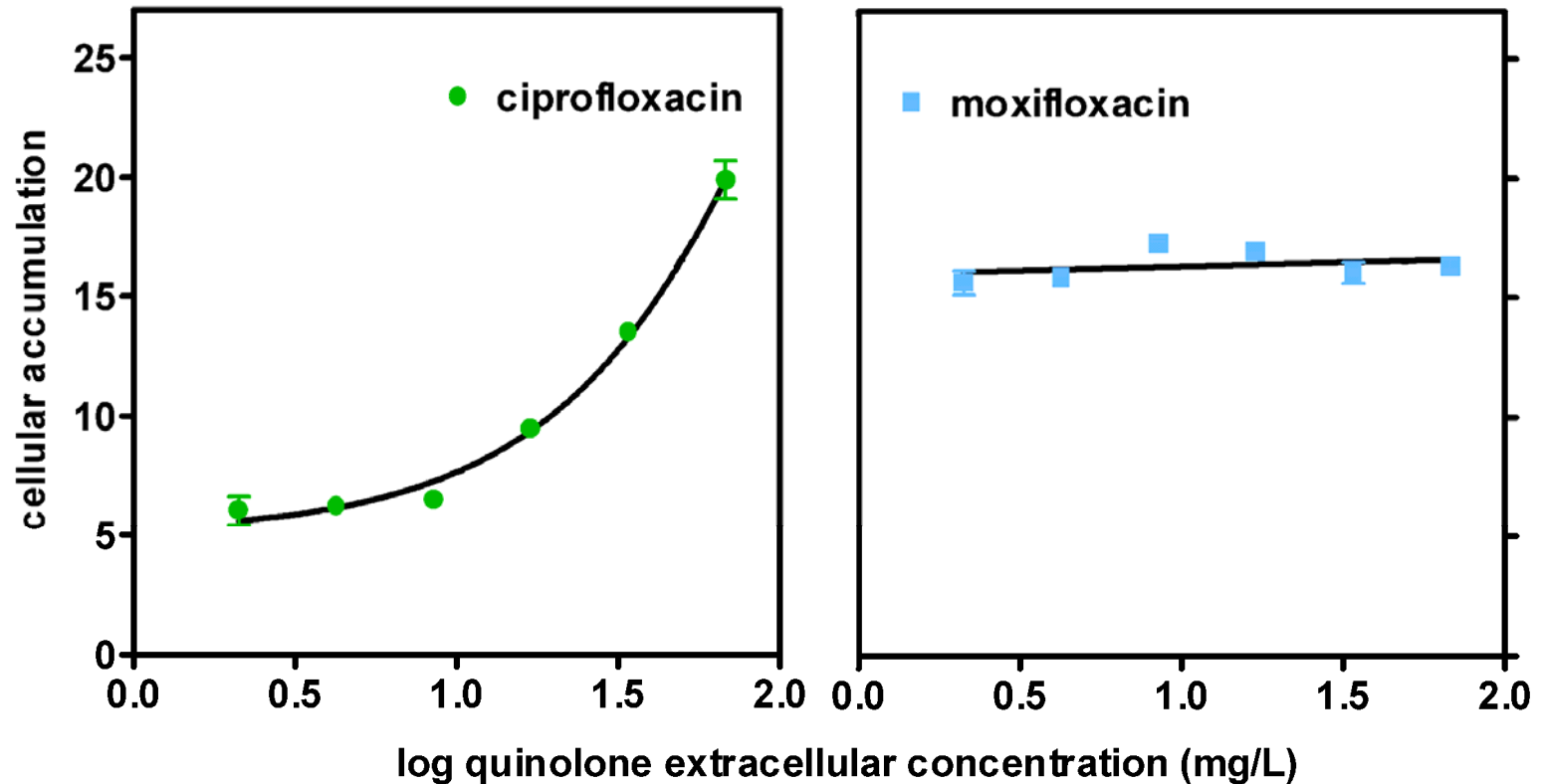
# Quinolones as inhibitors of ciprofloxacin efflux

- ciprofloxacin efflux inhibited by ciprofloxacin



# Quinolones as inhibitors of ciprofloxacin efflux

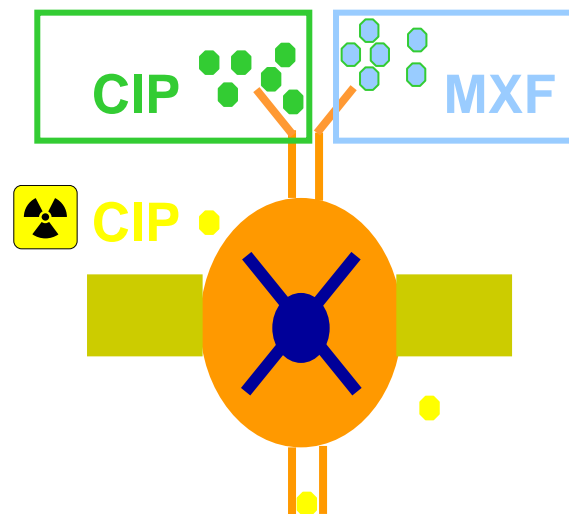
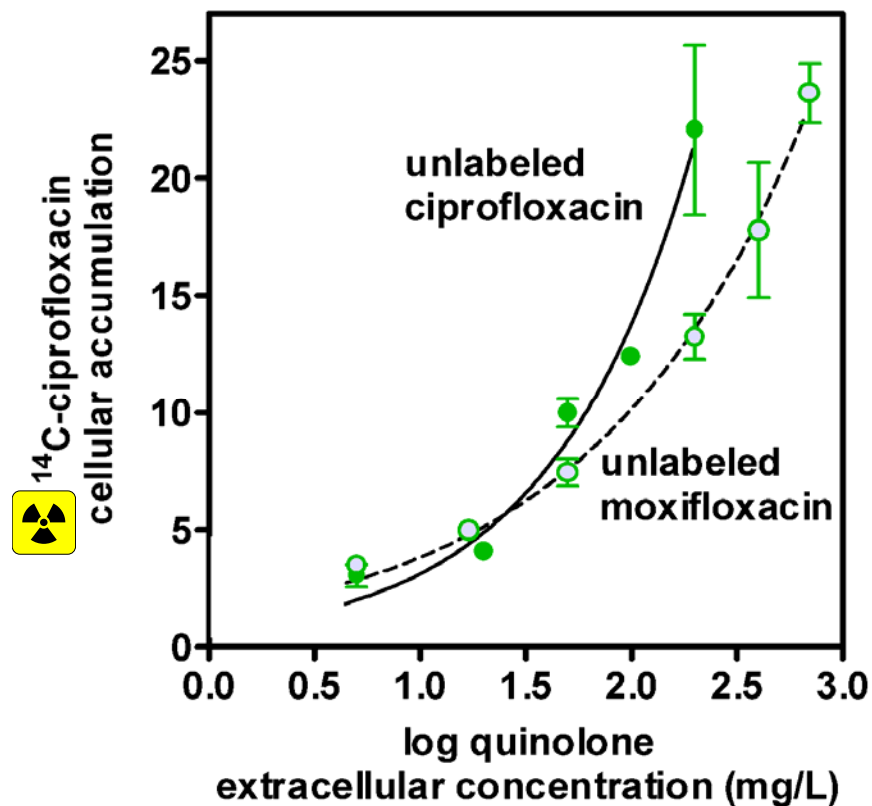
- ciprofloxacin efflux inhibited by ciprofloxacin
- moxifloxacin not affected





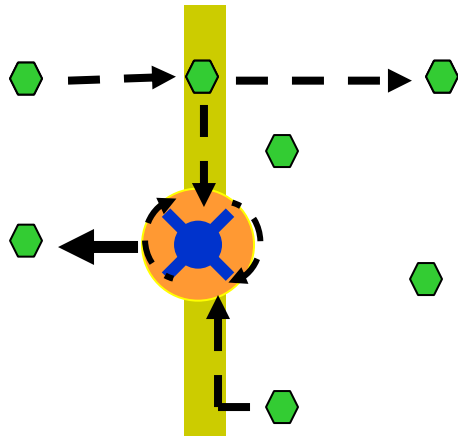
# Quinolones as inhibitors of ciprofloxacin efflux

- ciprofloxacin efflux inhibited by ciprofloxacin  
moxifloxacin



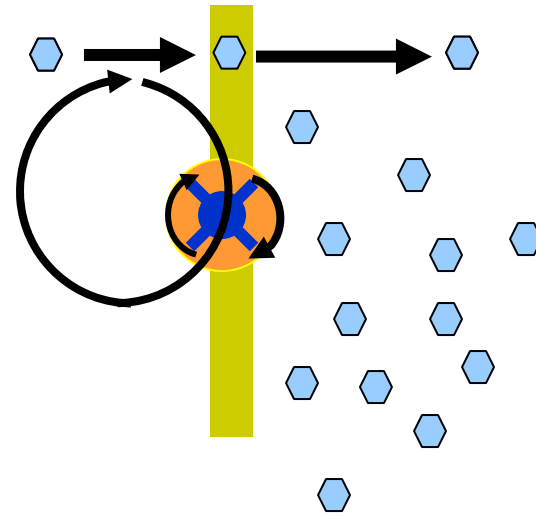
# Putative mechanism of transport of quinolones by MRP

**ciprofloxacin**



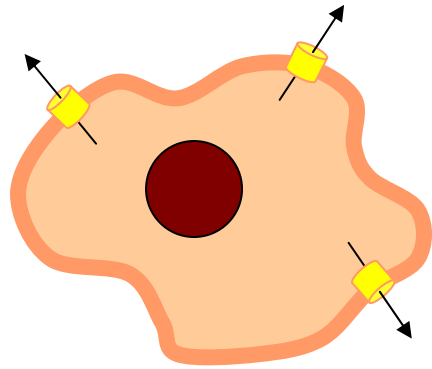
" classical " model

**moxifloxacin**

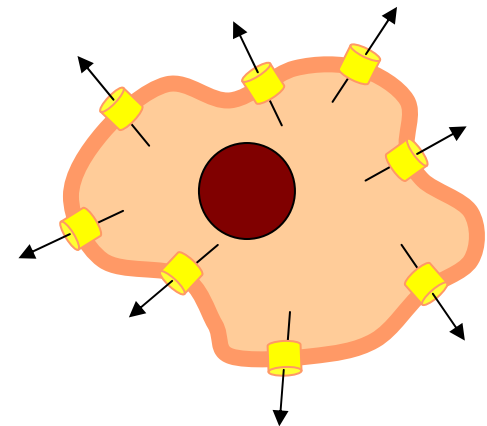


" futile cycle "

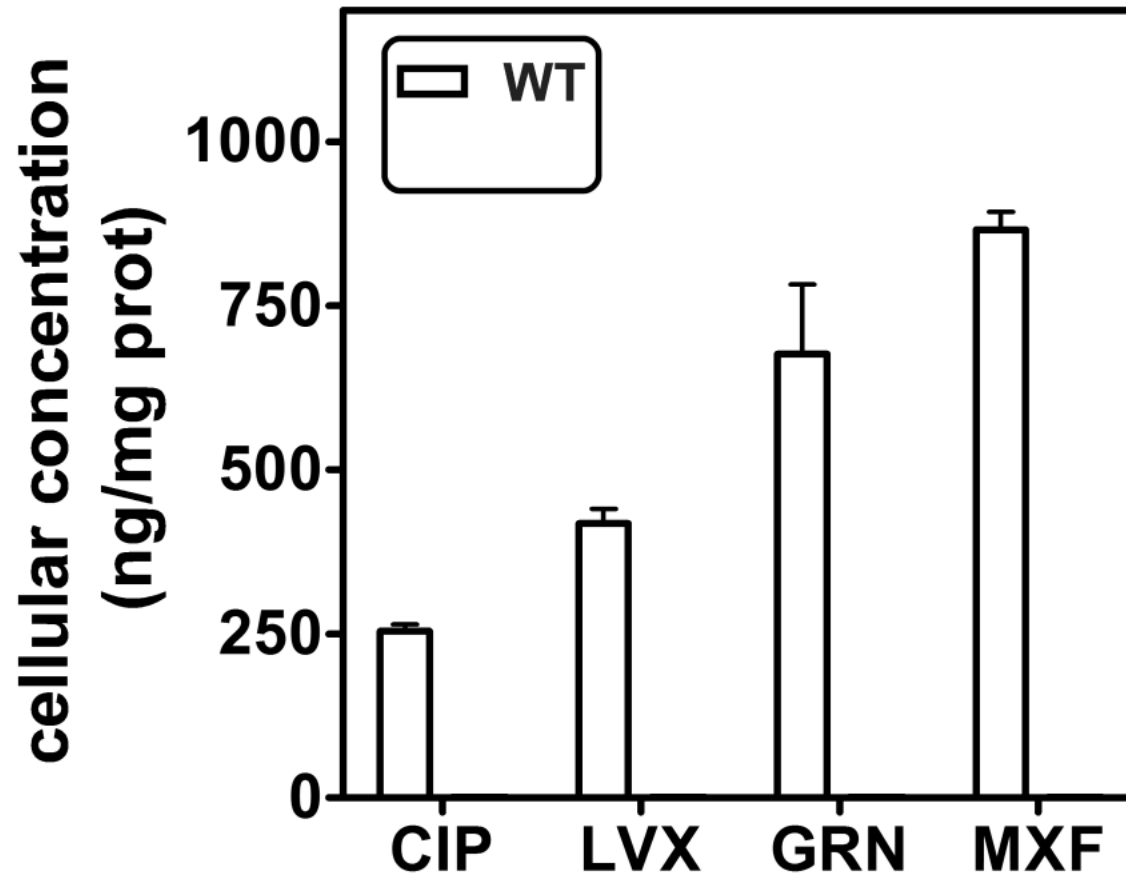
# Can we make eukaryotic cells resistant to antibiotics ?



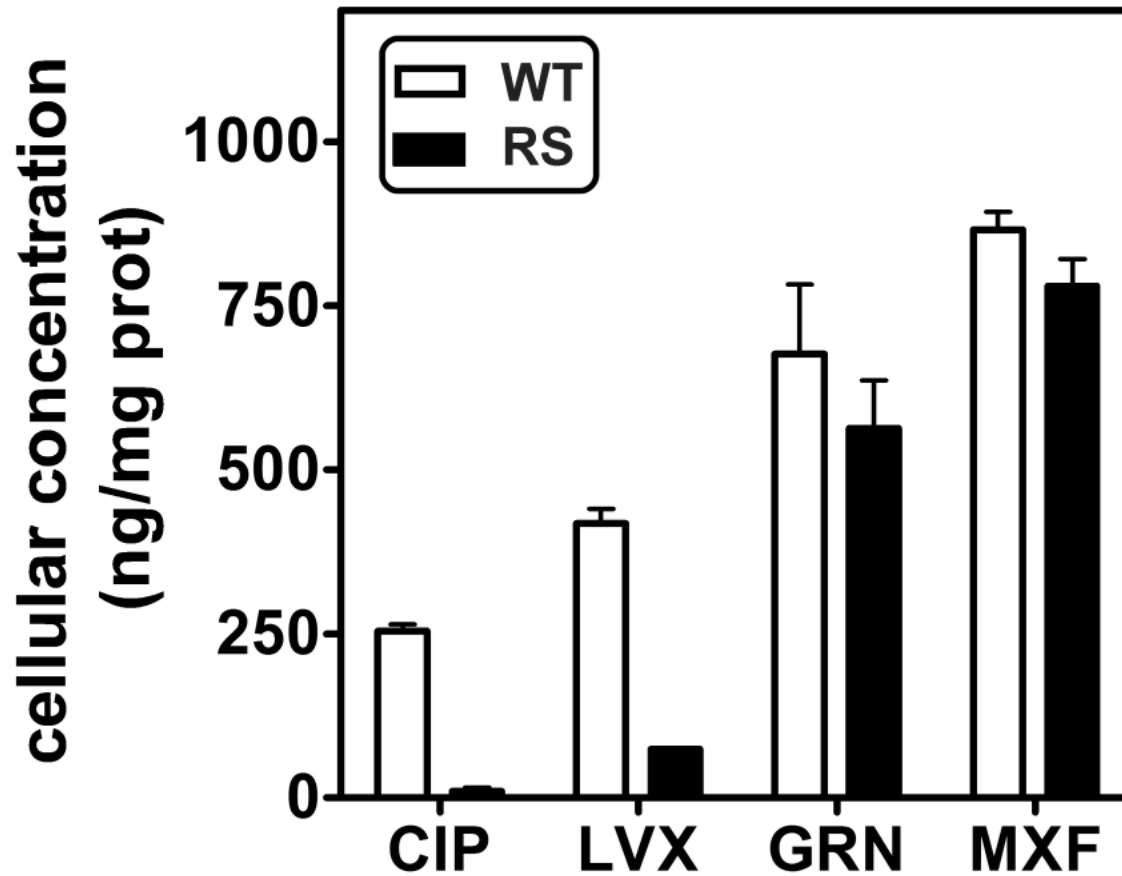
Chronical exposure of J774 macrophages to increasing concentrations of ciprofloxacin



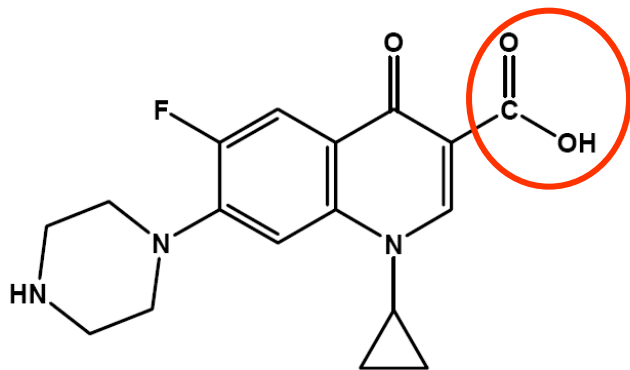
# Reduced drug accumulation in resistant macrophages



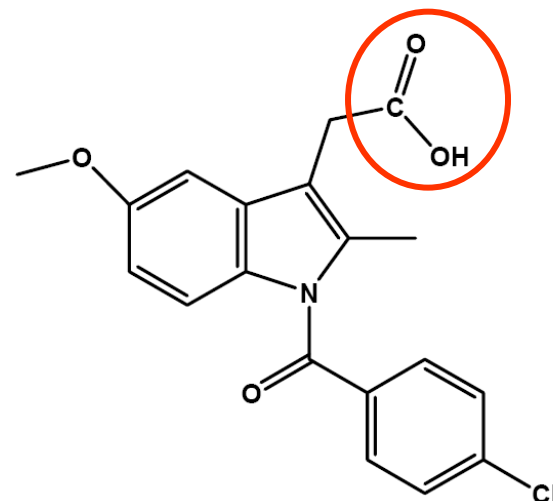
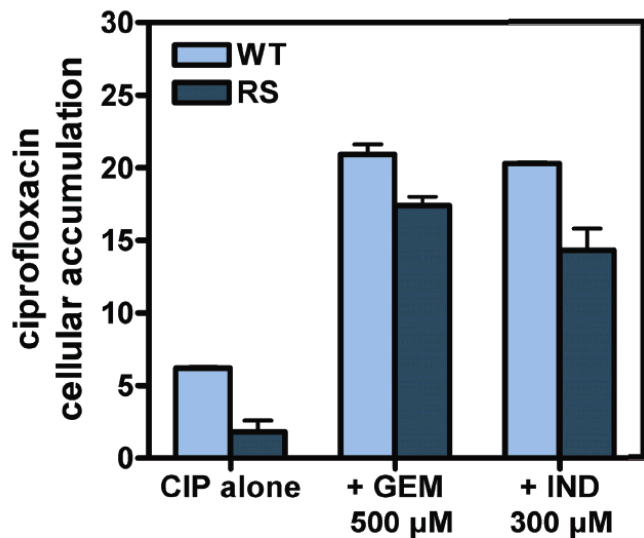
# Reduced drug accumulation in resistant macrophages



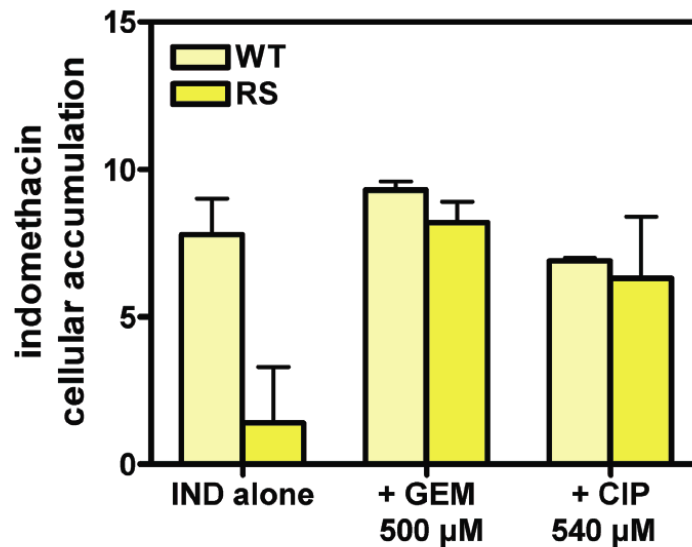
# Competition for efflux as a mechanism for drug interaction



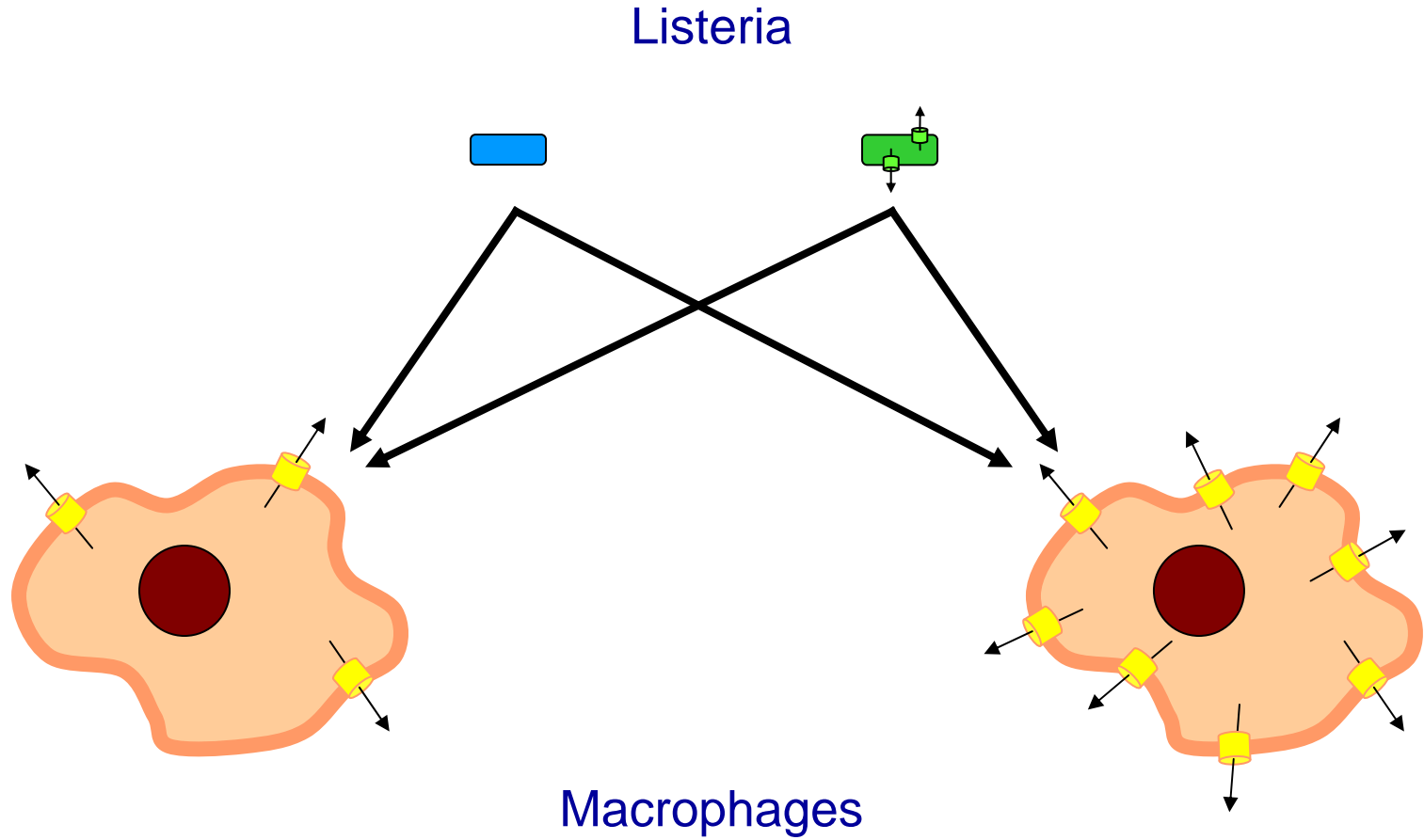
ciprofloxacin



indomethacin



# Coworking between bacteria and macrophage pumps to reduce ciprofloxacin activity

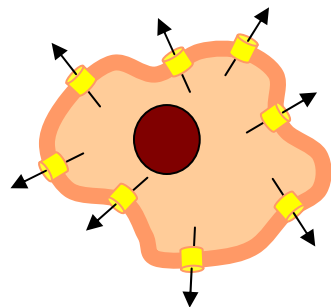
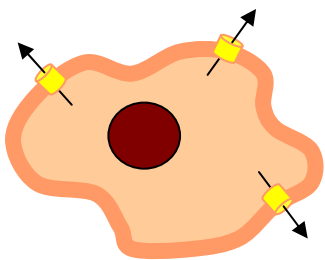


# Coworking between bacteria and macrophage pumps to reduce ciprofloxacin activity

Listeria



Macrophages



FQ	MIC (mg/L)			
	EGD		CLIP	
	Res. (-)	Res. (+)	Res. (-)	Res. (+)
CIP	1.2	1.0	5.0	1.0
MXF	0.6	0.6	0.5	0.25

CIP R; MXF S

FQ	Cellular concentration (ng/mg prot)			
	WT		RS	
	Prob. (-)	Prob. (+)	Prob. (-)	Prob. (+)
CIP	72	263	23	159
MXF	262	208	241	257

CIP R; MXF S



# Coworking between bacteria and macrophage pumps to reduce ciprofloxacin activity

Same substrate specificity of the MFS procaryotic pump and of the ABC eucaryotic pump !

FQ	MIC (mg/L)			
	EGD		CLIP	
	Res. (-)	Res. (+)	Res. (-)	Res. (+)
CIP	1.2	1.0	5.0	1.0
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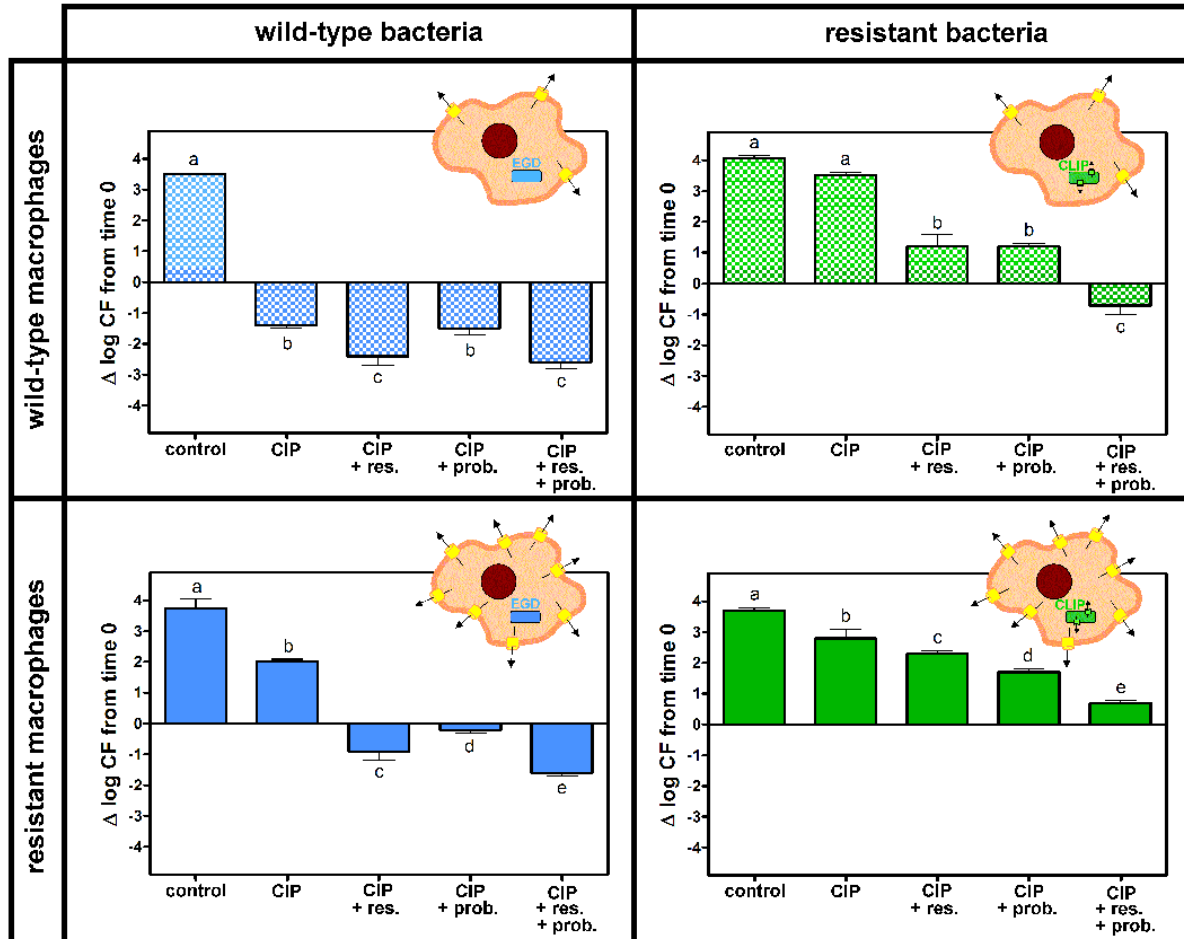
CIP R; MXF S

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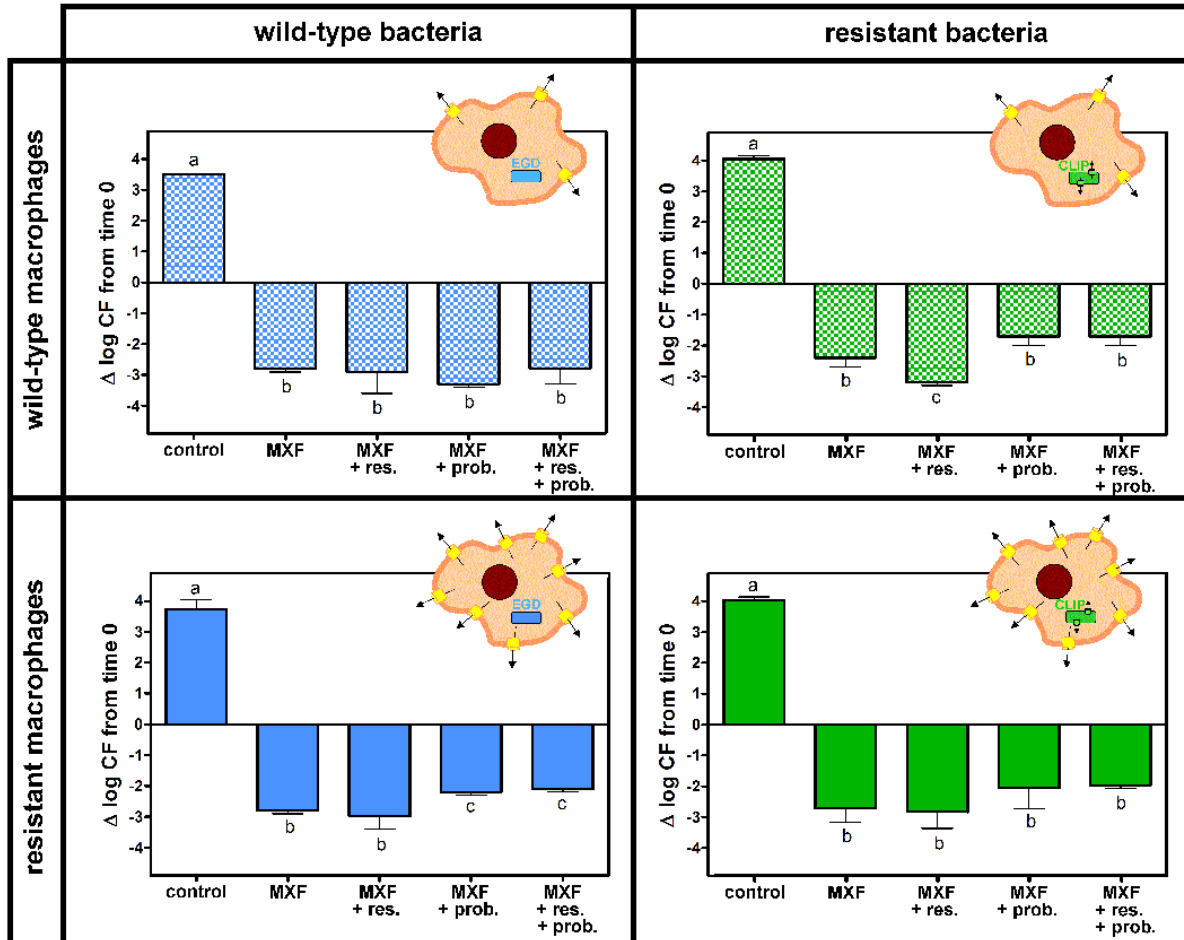
# Coworking between bacteria and macrophage pumps to reduce ciprofloxacin activity

## ciprofloxacin



# No effect of bacteria and macrophage pumps on moxifloxacin activity

## moxifloxacin



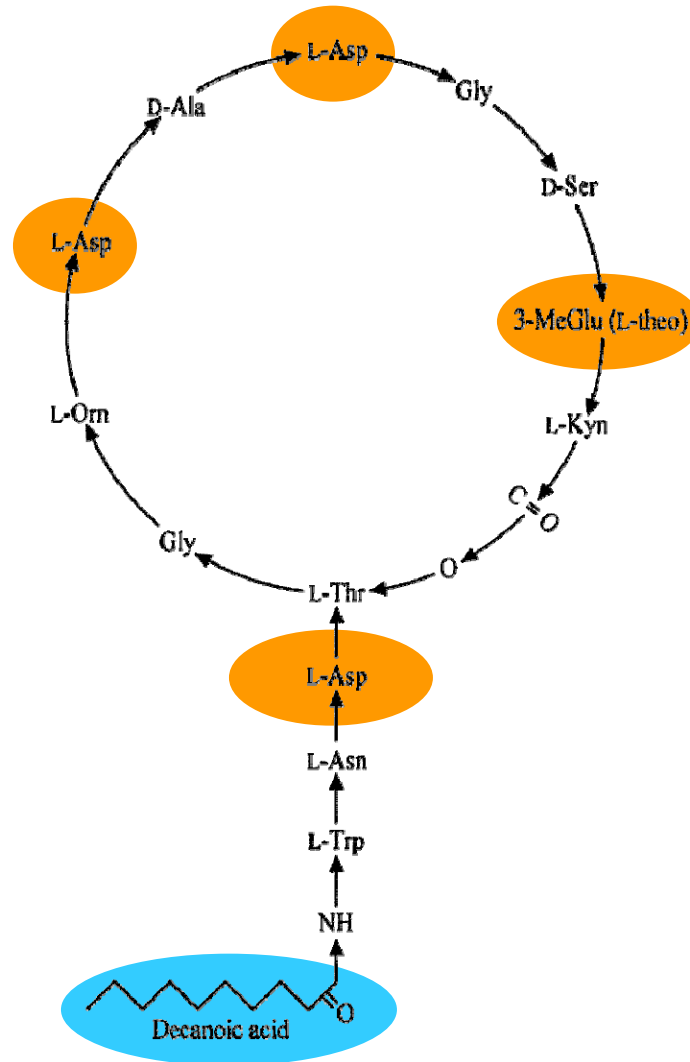
# Daptomycin, an unexpected substrate for efflux pumps

daptomycin

Log P = - 4  
Log D = -9.5 at pH 7

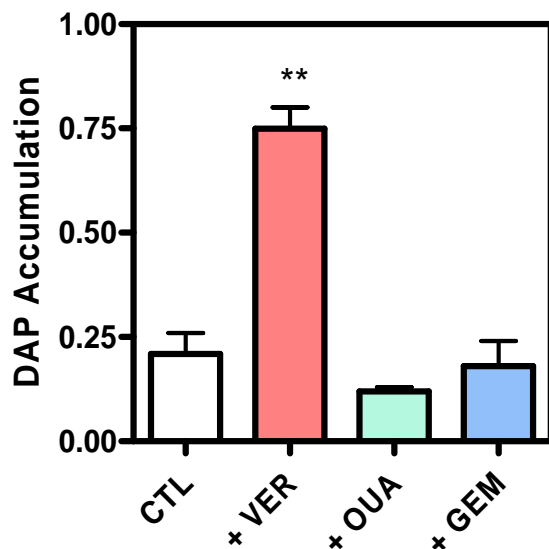
Highly polar molecule !

lipophilic chain

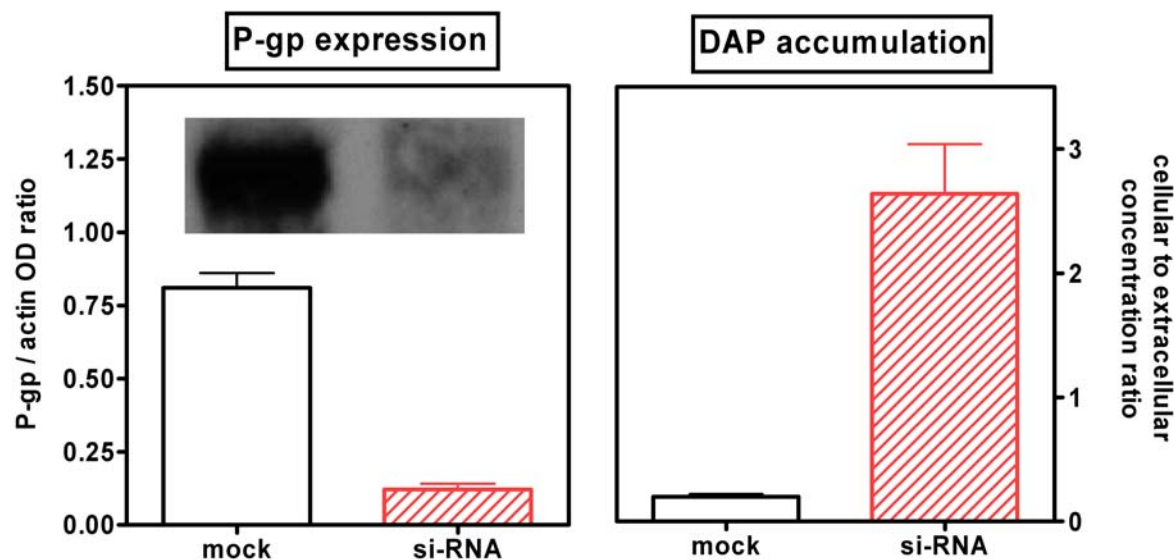


polyanionic  
cyclic peptide

# Daptomycin is substrate of P-gp

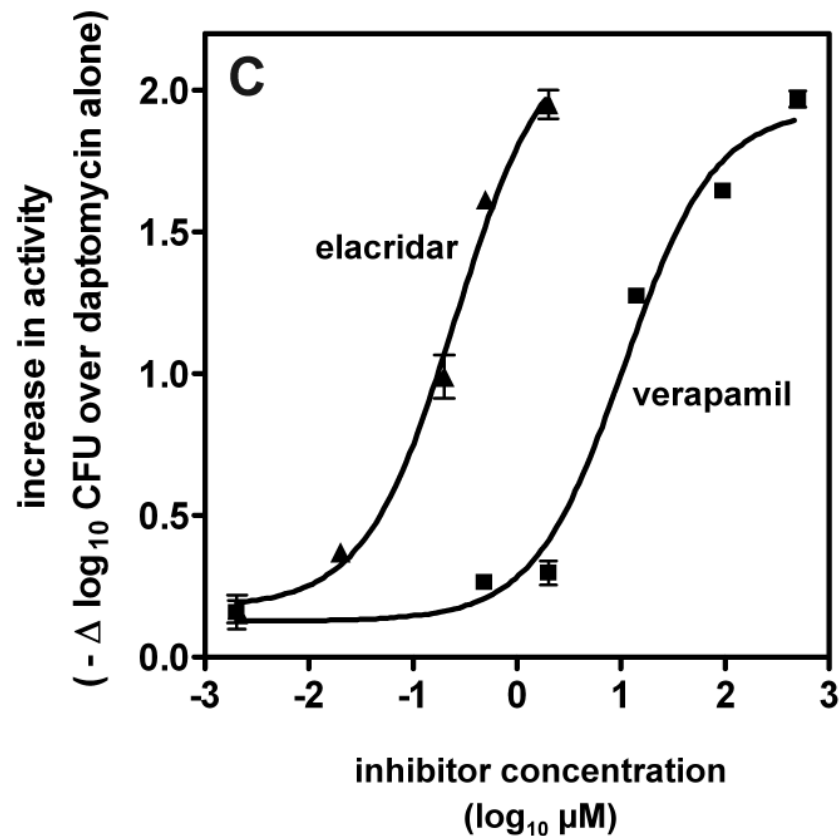


Daptomycin accumulation proportional to P-gp activity and expression level

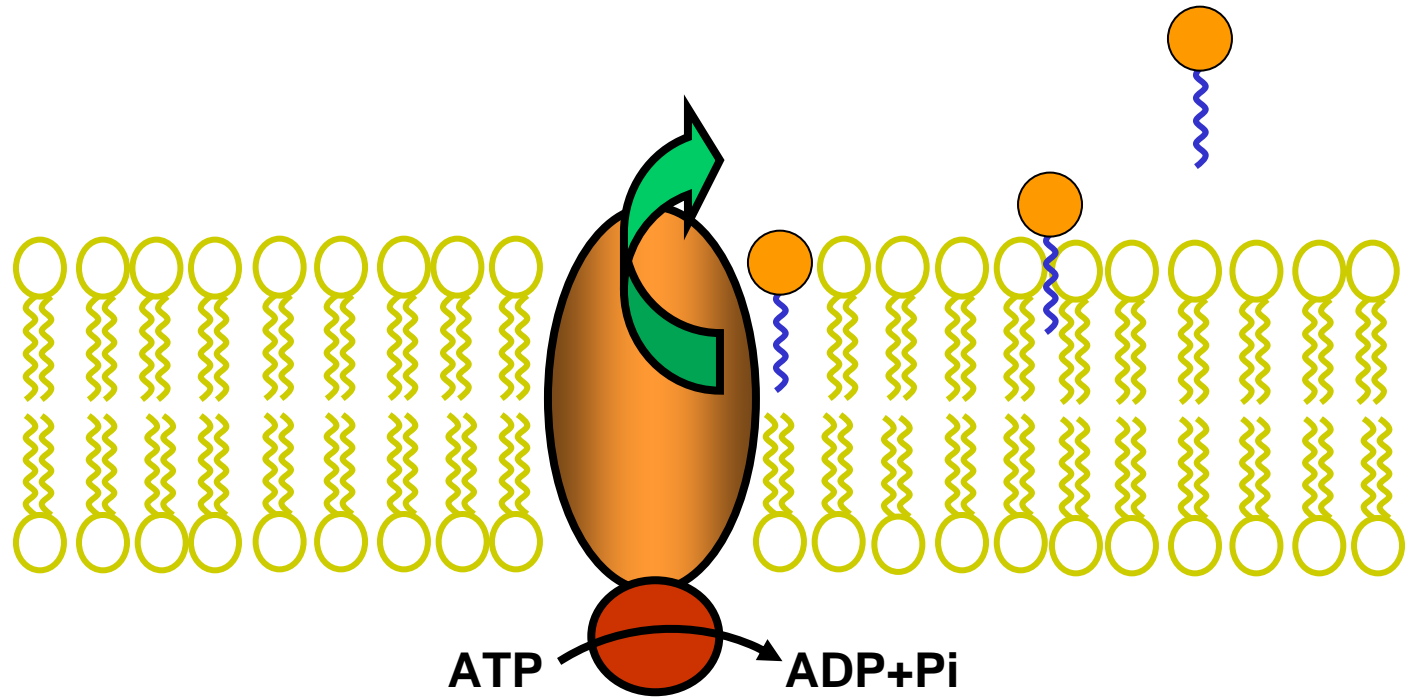


# Daptomycin is substrate of P-gp

Daptomycin intracellular activity is increased in the presence of P-gp inhibitors



# Putative mechanism of daptomycin transport by P-gp



anchoring in the membrane towards the hydrophobic chain  
and extrusion from the membrane

# The past and present efflux team in Brussels

