



Antibiotic transporters: From Discovery to Clinical Implications

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Influenced largely by

- Françoise Van Bambeke, Béatrice Marquez, Youri Glupczynski (UCL) and Martine Prevost (ULB)
- my participation to the *European Committee for Antibiotic Susceptibility Testing (EUCAST)*

The slides are available from <http://www.facm.ucl.ac.be> – follow "Lectures"

Steps and Challenges of efflux in antibacterial chemotherapy

A microscopic image showing several clusters of spherical bacteria, likely Gram-negative cocci, arranged in pairs and small groups. The bacteria are stained, showing some internal structure and possibly flagella or pili.

- **recognizing its existence:**
is it a significant mechanism of resistance ?
- **which pumps ...**
is antibiotic efflux different from other drug efflux ?
- **defining its role:**
does it need to change our vision on (and decisions about) existing antibiotics ?
- **setting up diagnostic techniques:**
how can we detect it today (and do we need to do this ?)
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 - can we make non-effluxed drugs ?
 - and what about efflux inhibitors ?
 - is efflux important in pharmacokinetics/drug interactions ?

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Historical observations on tetracyclines ...

□ 1: [Nature](#), 1963 Oct 26;200:384-5.

DISAPPEARANCE OF OXYTETRACYCLINE
ACCUMULATION IN THE CELLS OF MULTIPLE
DRUG-RESISTANT ESCHERICHIA COLI.

[IZAKI K](#), [ARIMA K](#).

PMID: 14087909 [PubMed - indexed for MEDLINE]



Who remembers that car ?



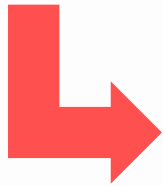
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Who remembers that graph ?

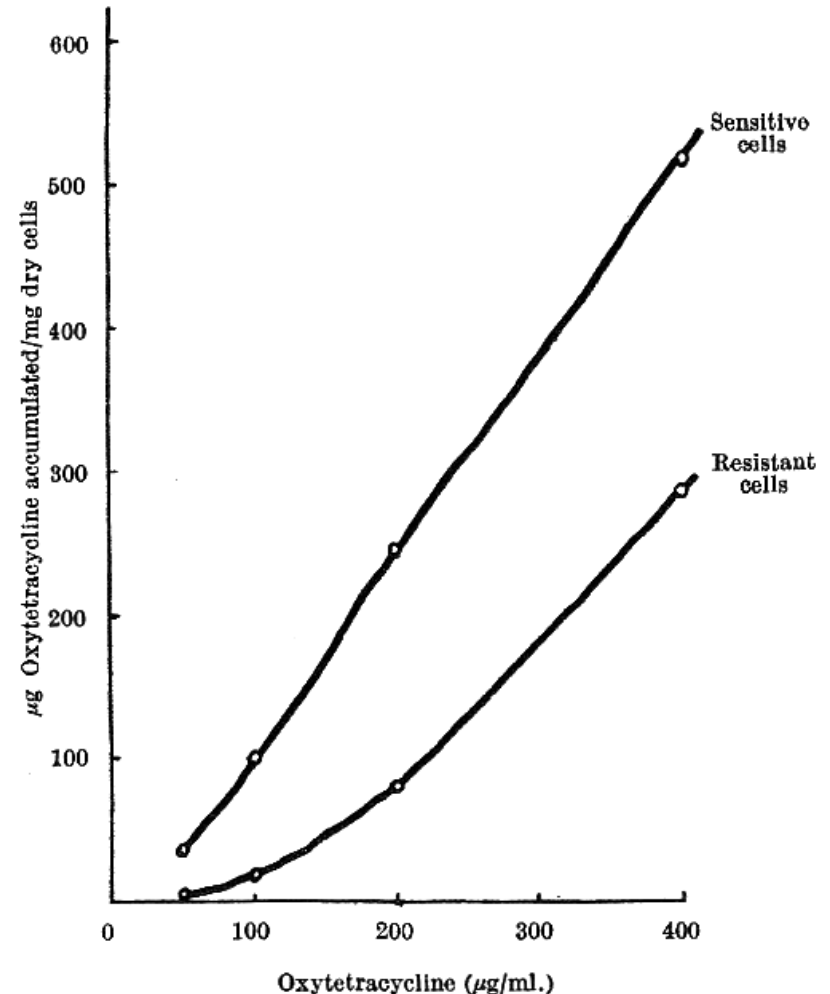


Fig. 1. Accumulation of oxytetracycline in *E. coli* K-12 at various concentrations of oxytetracycline added. The reaction mixture contains 1 ml. suspension (0.7 mg dry weight) oxytetracycline hydrochloride, 1 ml. (0.5-4.0 mg/ml.) and 1 ml. of 10 per cent (w/v) glucose, 2 per cent K_2HPO_4 , and 0.1 per cent $MgSO_4 \cdot 7H_2O$ respectively in a total volume of 10 ml. Incubation was carried out aerobically at 30° C for 90 min

Historical observations on tetracyclines ...

54

Biochem. J. (1965) **94**, 54

Resistance of *Escherichia coli* to Tetracyclines

BY T. J. FRANKLIN AND A. GODFREY
*Imperial Chemical Industries Ltd. (Pharmaceuticals Division),
Alderley Park, Macclesfield, Cheshire*

(Received 23 March 1964)

1. A strain of *Escherichia coli* highly resistant to chlortetracycline and partially cross-resistant to tetracycline has been isolated. 2. The nitro-reductase system of the resistant cells was inhibited to a smaller extent by chlortetracycline than was the corresponding enzyme of sensitive cells. 3. The incorporation of leucine *in vitro* into the ribosomal protein of cell-free preparations from sensitive and resistant cells was equally inhibited by chlortetracycline. 4. Resistant cells accumulated much less chlortetracycline and tetracycline than did sensitive cells when both were cultured in the presence of these drugs. 5. The uptake of tetracycline by both sensitive and resistant *E. coli* was dependent on the presence of glucose in the medium. 6. Fractionation of cells cultured in medium containing [¹⁴C]chlortetracycline indicated that the largest proportion of radioactivity in sensitive cells was in the fraction consisting mainly of cell-wall material. There was no concentration of radioactivity in any one fraction of the resistant cells. 7. No evidence could be obtained for a specific tetracycline-excretion system in the resistant cells. 8. The significance of these results in relation to current theories of the antibiotic action of and resistance to the tetracycline drugs is discussed.



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Historical observations on tetracyclines ...

15 years later...

Proc. Natl. Acad. Sci. USA
Vol. 77, No. 7, pp. 3974–3977 July 1980
Biochemistry

Active efflux of tetracycline encoded by four genetically different tetracycline resistance determinants in *Escherichia coli*

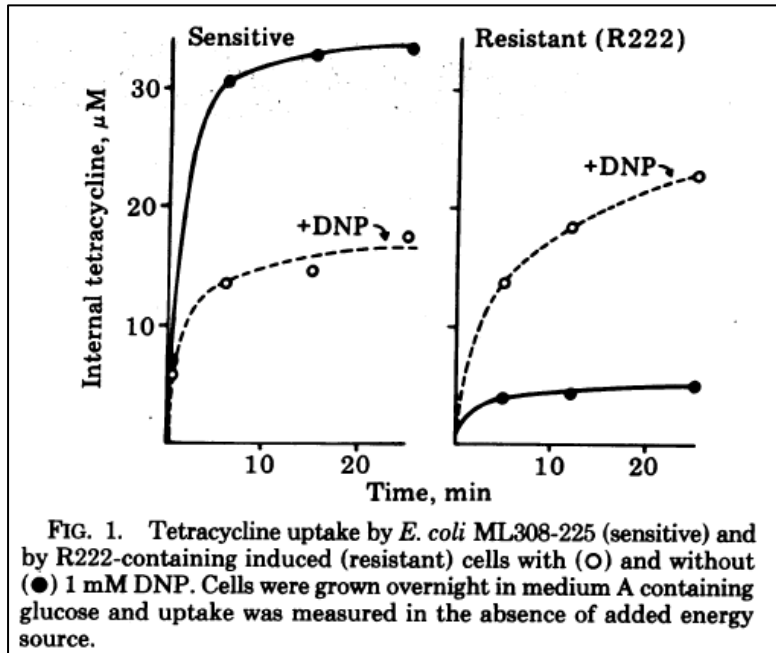
(everted membrane vesicles/tetracycline transport/transposon Tn10/plasmids)

Laura McMurry, Richard E. Petrucci, Jr., and Stuart B. Levy*

Department of Molecular Biology and Microbiology and Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts 02111

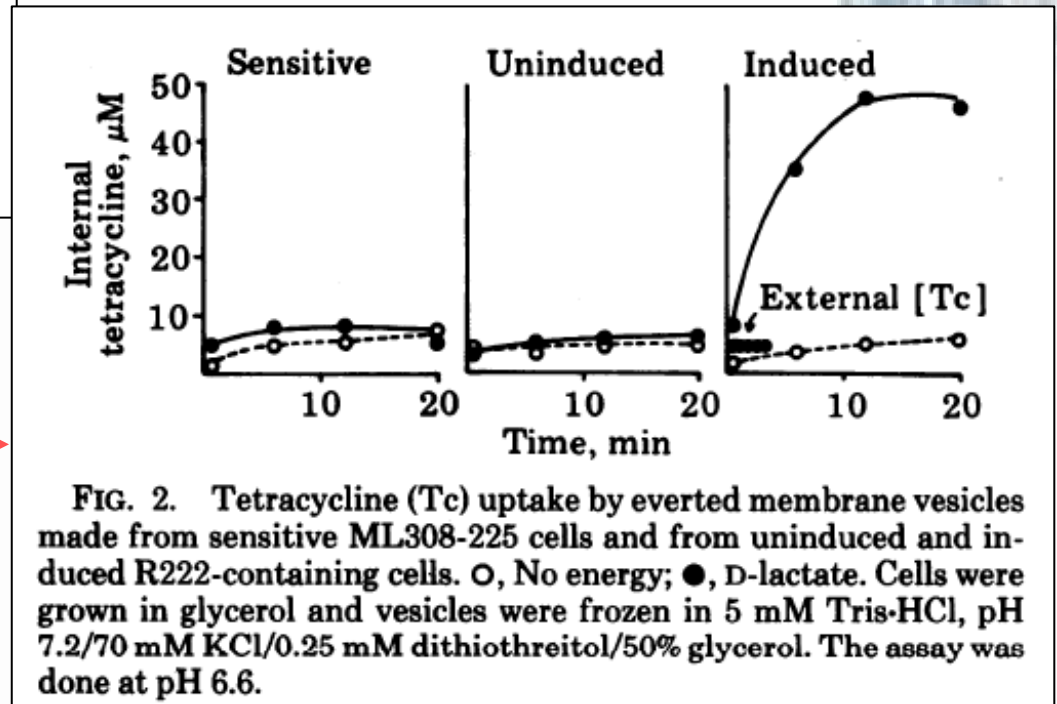
Communicated by Boris Magasanik, April 21, 1980

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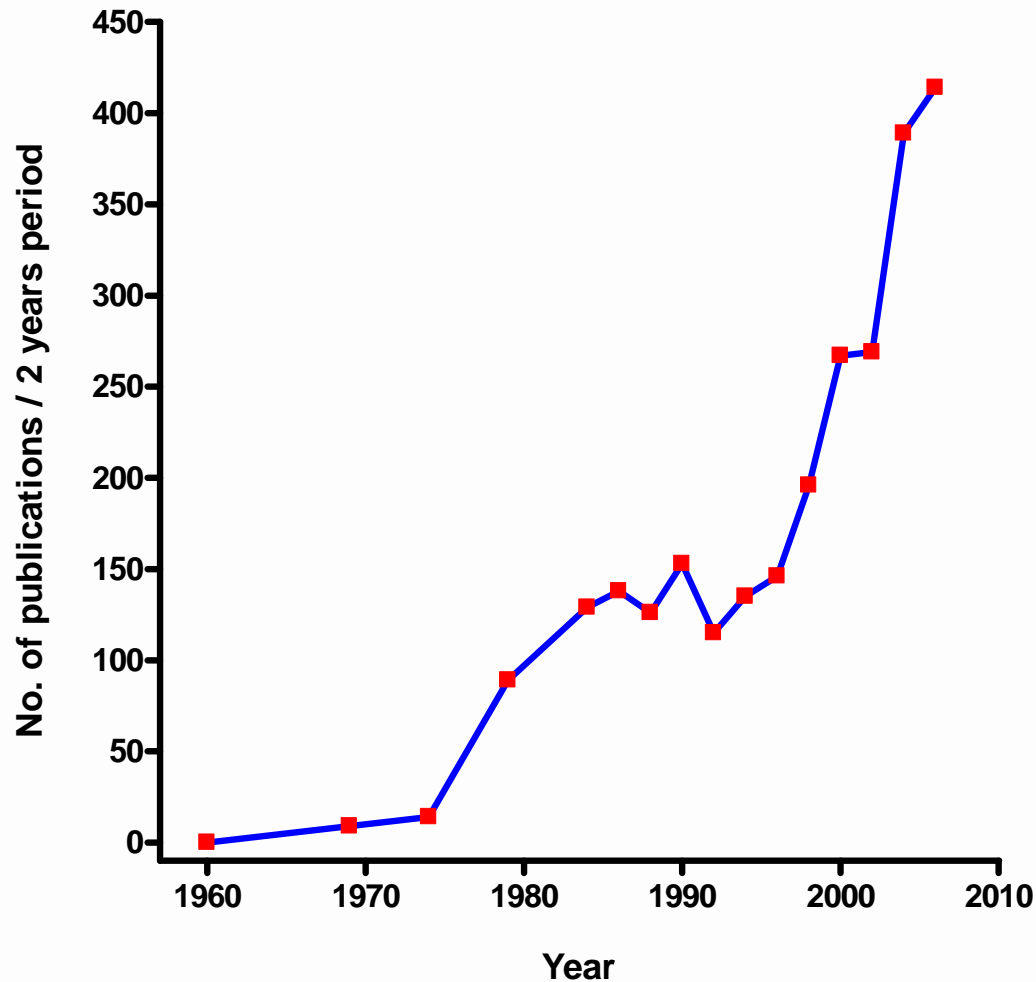
Whole bacteria

Everted membranes



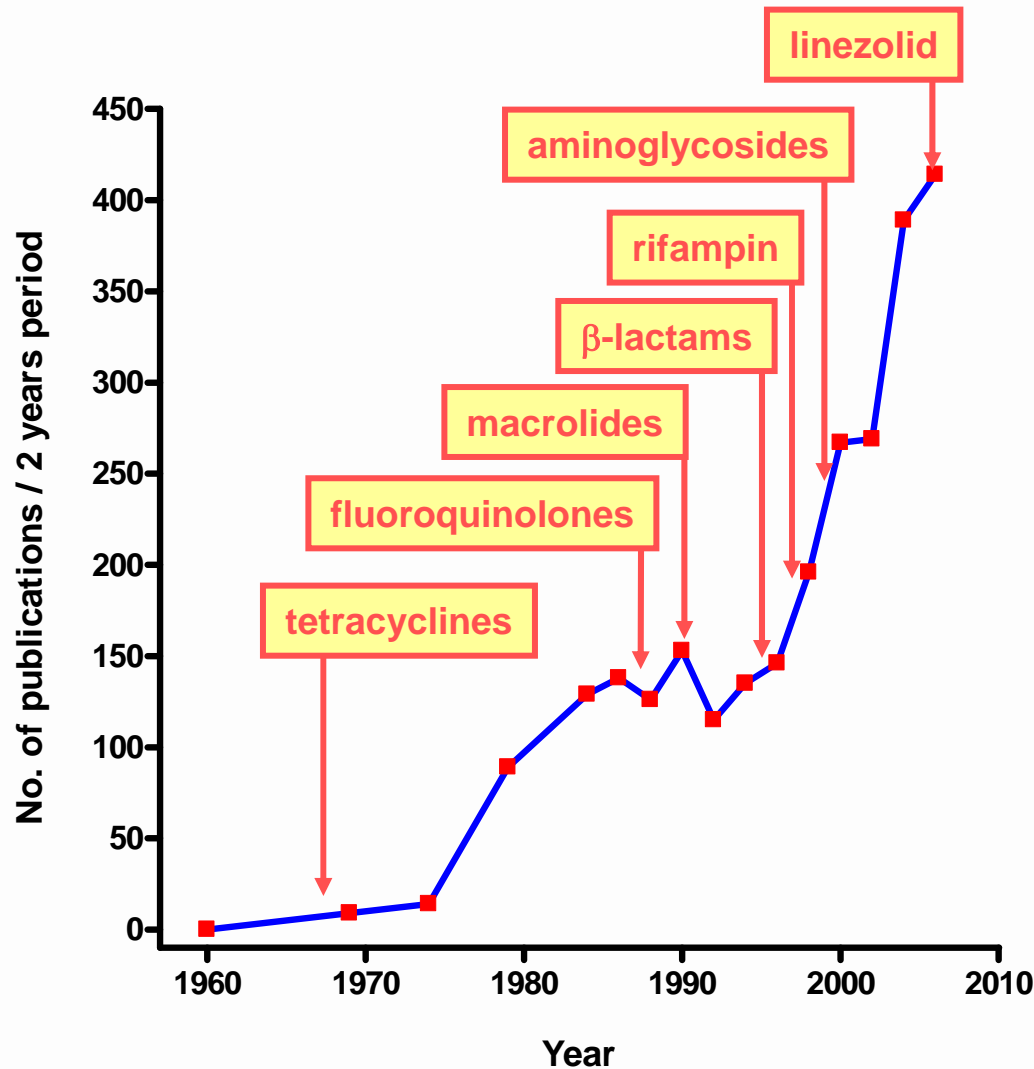
McMurry et al., PNAS 1980; 77:3974-3977

Historical trends ...



No. of publications
in PubMed with
keywords:
antibiotic & efflux

Historical landmarks ...



Successive
description of efflux-
mediated resistance
for major antibiotics

The present situation ...

- Efflux has, slowly but surely, been shown to affect most if not all major antibiotic classes ...

? Are there classes that will never show efflux-mediated resistance ?

May be only those which must act on bacterial surface ?

- ? glycopeptides [vancomycin...],
- ? lipoglycopeptides [telavancin],
- ? lipopeptides [daptomycin], ...



1: [Antimicrob Agents Chemother.](#) 2007 Aug;51(8):2748-57. Epub 2007 Jun 4.

Modulation of the cellular accumulation and intracellular activity of daptomycin towards phagocytized *Staphylococcus aureus* by the P-glycoprotein (MDR1) efflux transporter in human THP-1 macrophages and madin-darby canine kidney cells.

[Lemaire S](#), [Van Bambeke F](#), [Mingeot-Leclercq MP](#), [Tulkens PM](#).

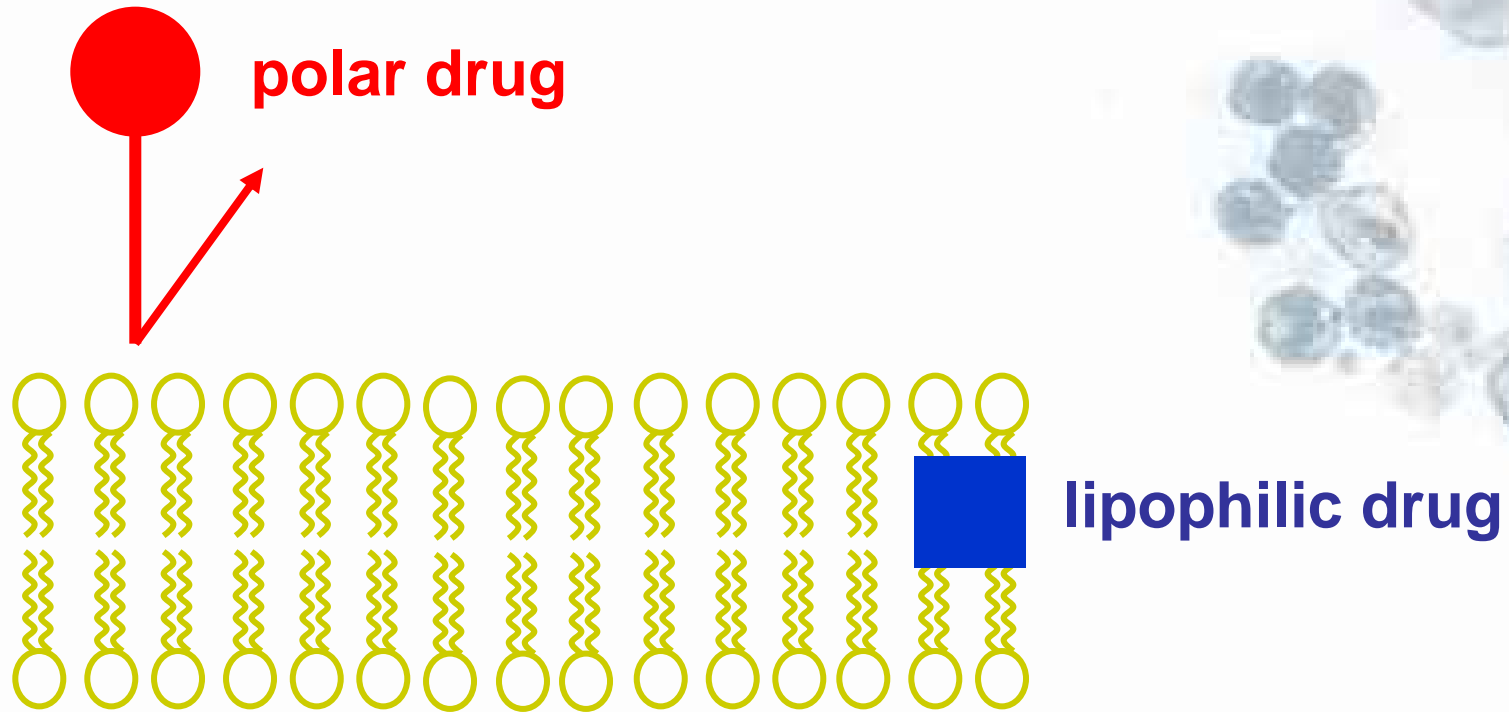
Unité de Pharmacologie Cellulaire et Moléculaire, Université Catholique de Louvain, Mounier 73, Brussels, Belgium.

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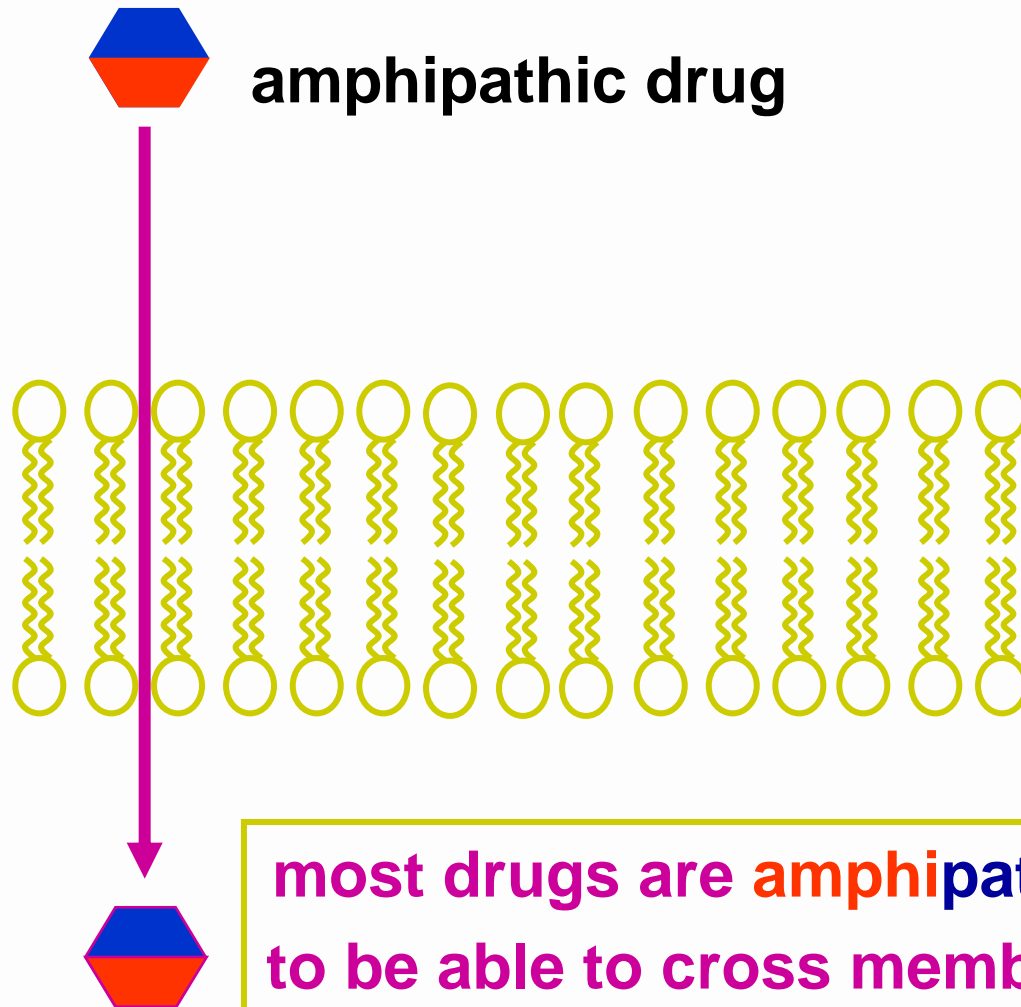
Why do we have efflux ?



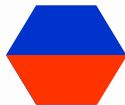
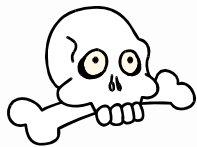
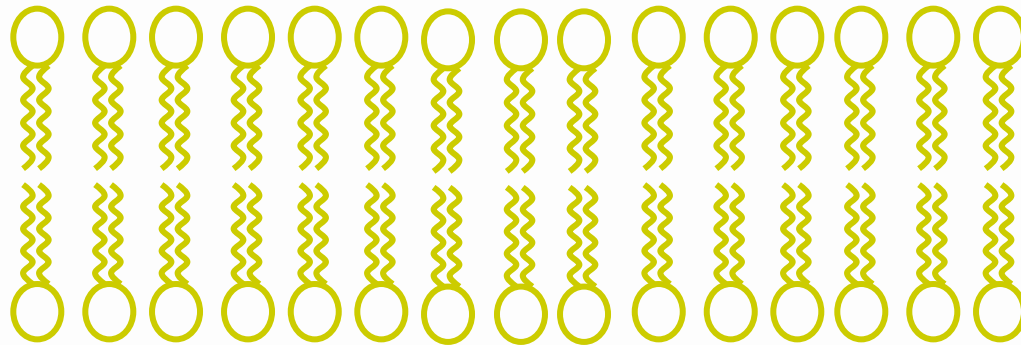
- **polar drugs do not reach intracellular targets !**
- **Lipophilic drugs get stucked in the bilayer !**



Reaching an intracellular target ...



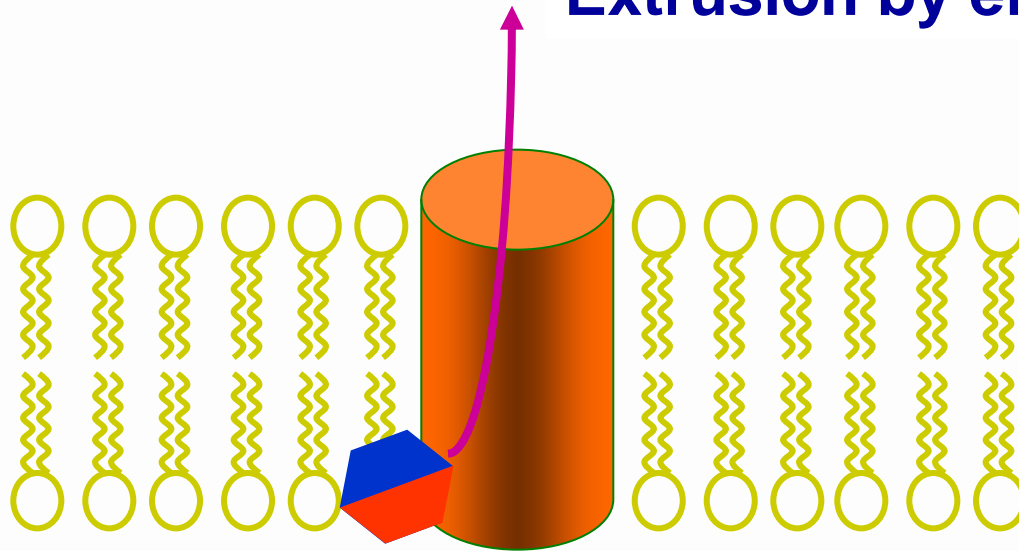
Intracellular chemotherapeutic agents



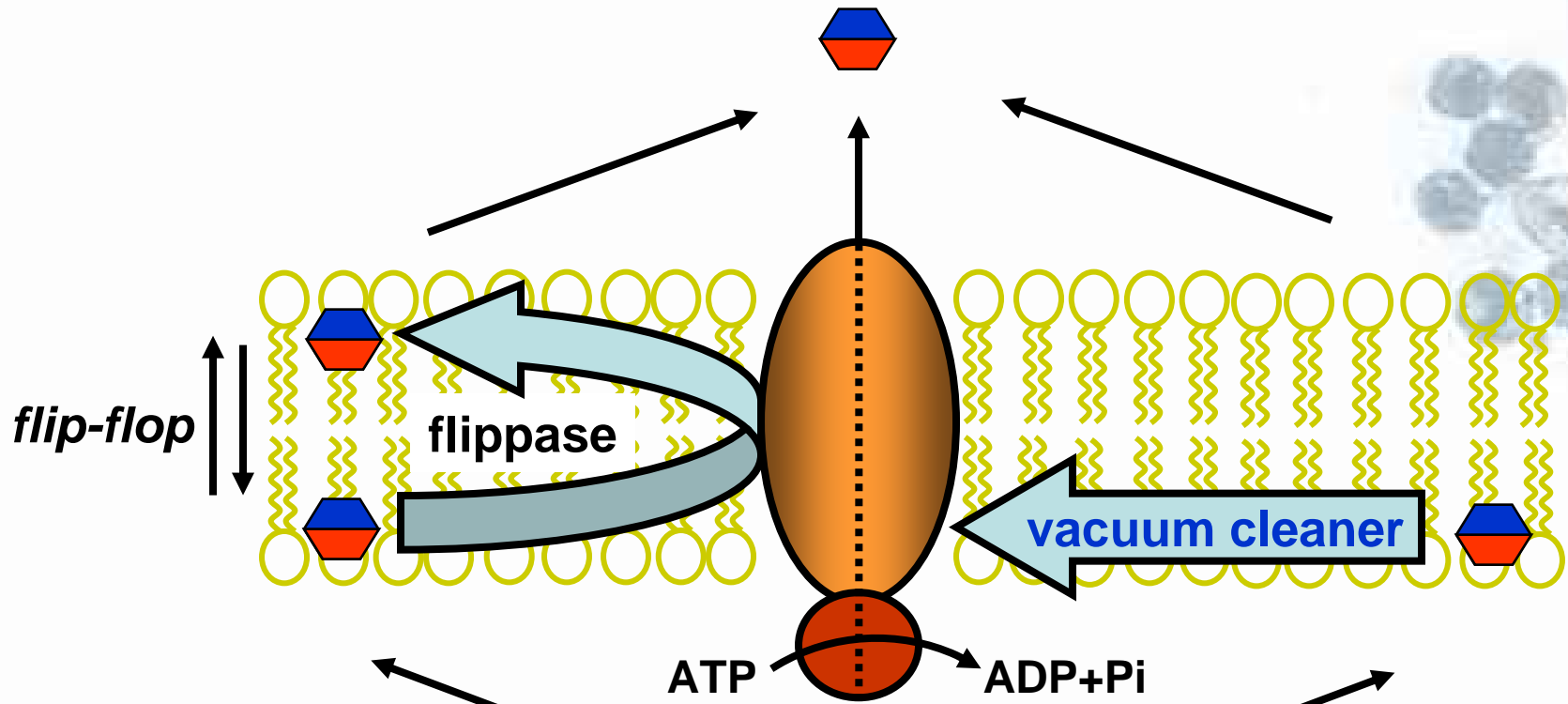
But once inside the cell, any compound may have potentially harmful effects

Proteins with efflux capabilities may have been selected out to help cells getting rid of diffusible, potentially toxic compounds

Extrusion by efflux pumps



General mechanisms of drug efflux

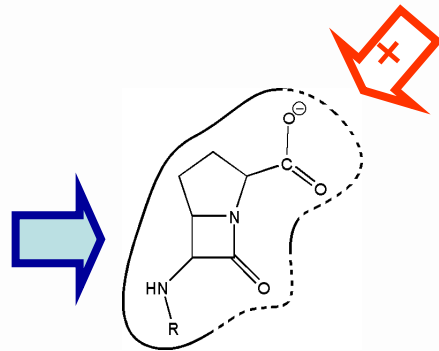


**membrane
insertion / release**

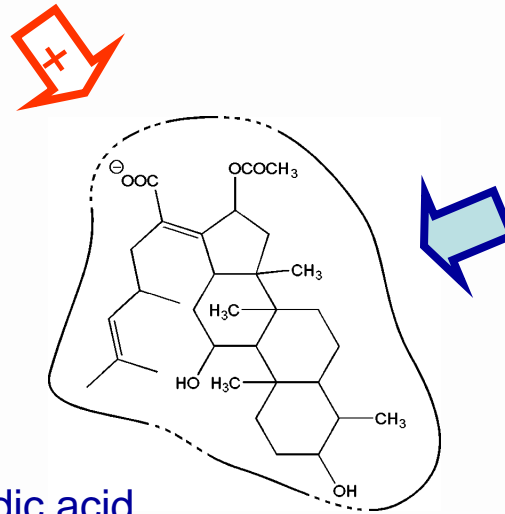
Van Bambeke *et al.* (2000) *Biochem. Pharmacol.* 60:457-70

Most antibiotics are amphiphilic !

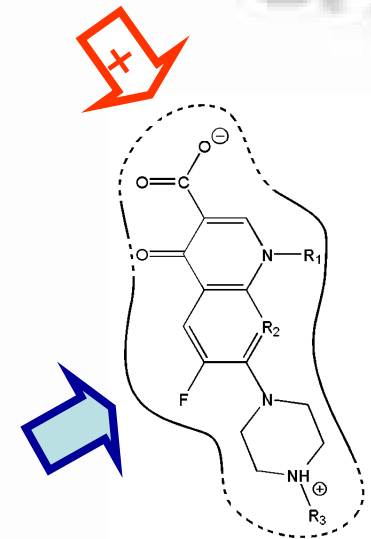
anionic amphiphiles



β -lactam



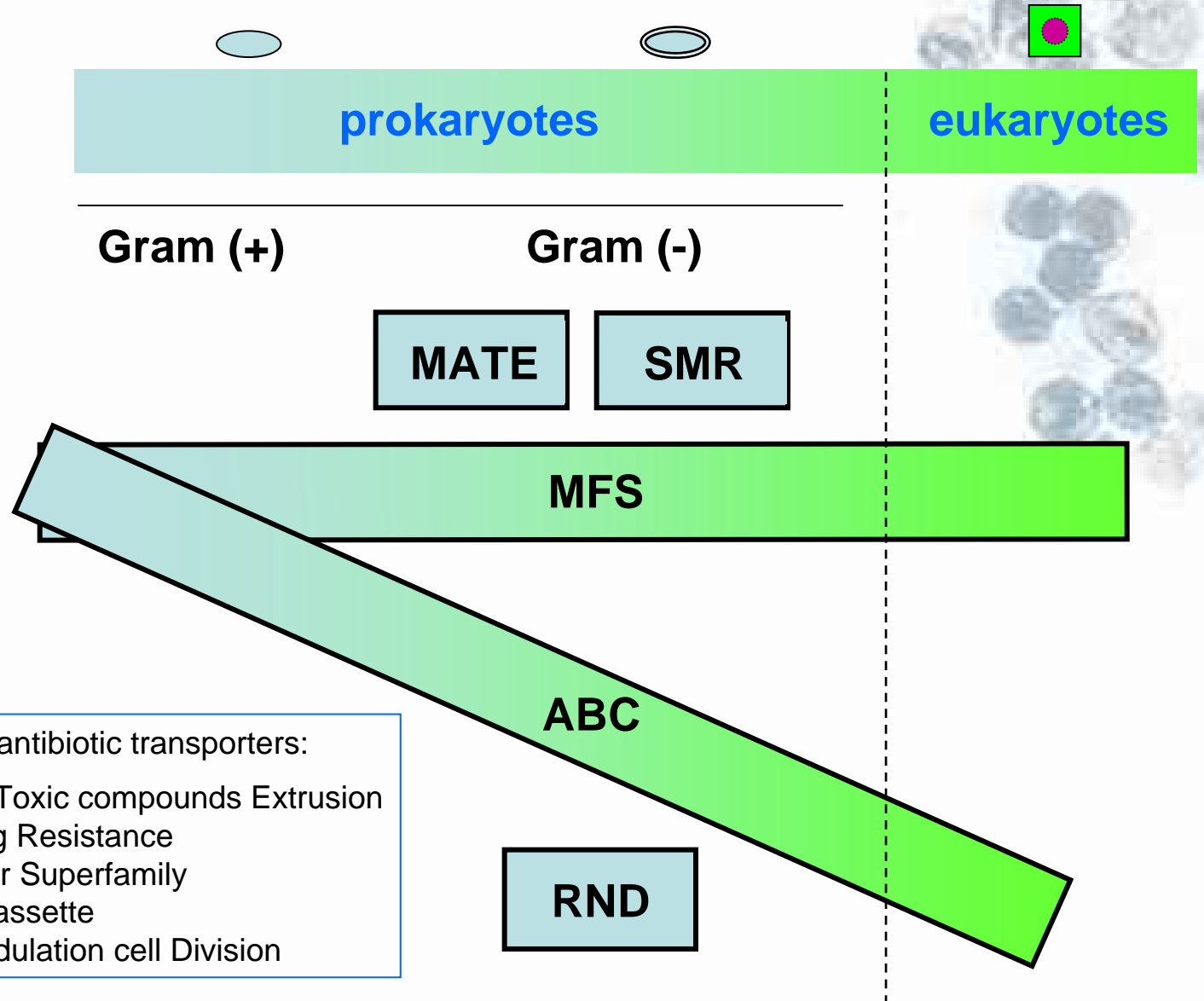
fusidic acid



fluoroquinolone



But antibiotic efflux transporters are everywhere



Main (super)families of antibiotic transporters:

- MATE: Multidrug And Toxic compounds Extrusion
- SMR: Small Multidrug Resistance
- MFS: Major Facilitator Superfamily
- ABC: ATP Binding Cassette
- RND: Resistance Nodulation cell Division

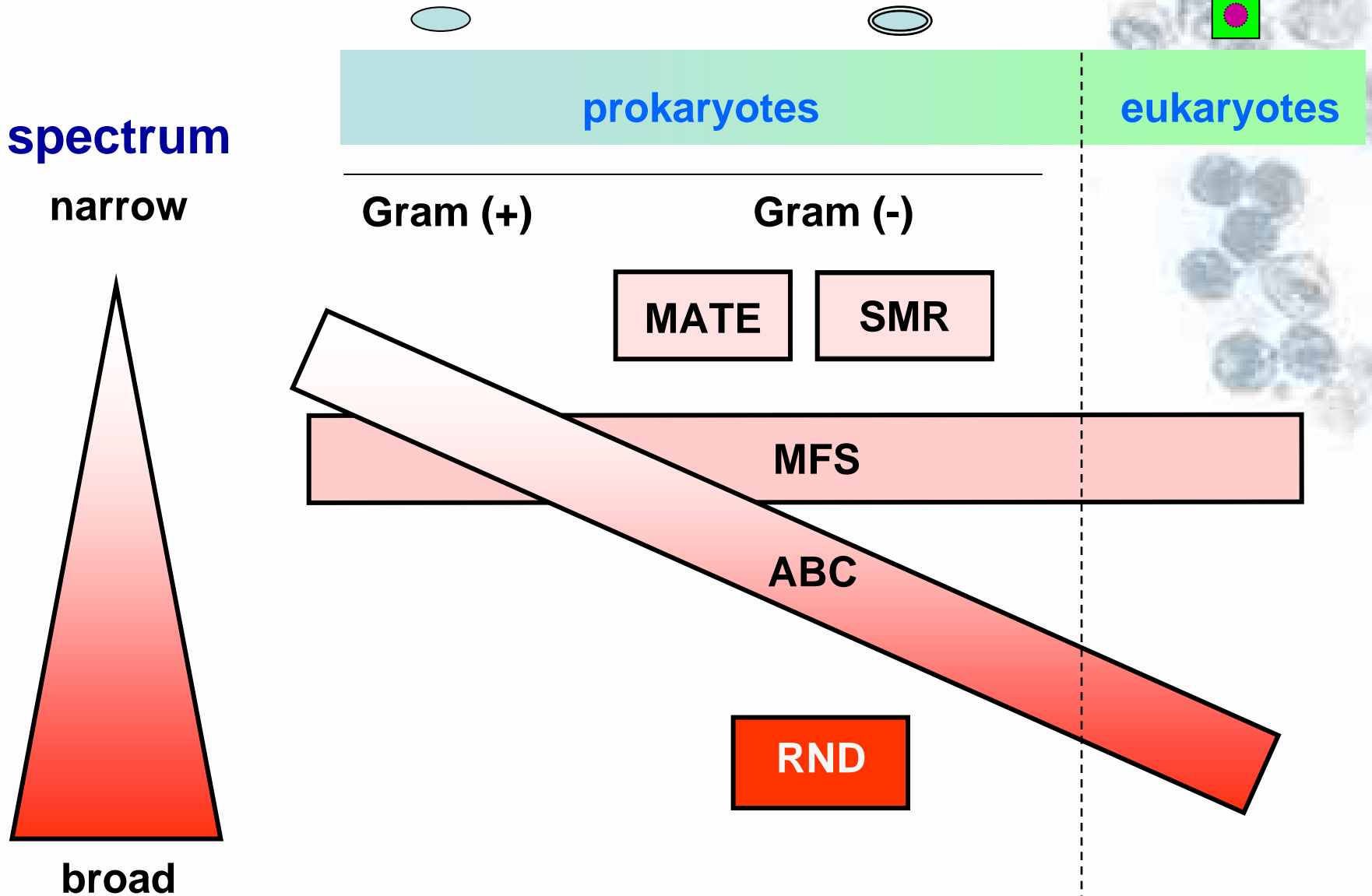
Mesaros *et al.* (2005) *Louvain médical*. 124:308-20

Antibiotics as substrates of efflux pumps across living organisms

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

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

Antibiotic transporter spectra ...



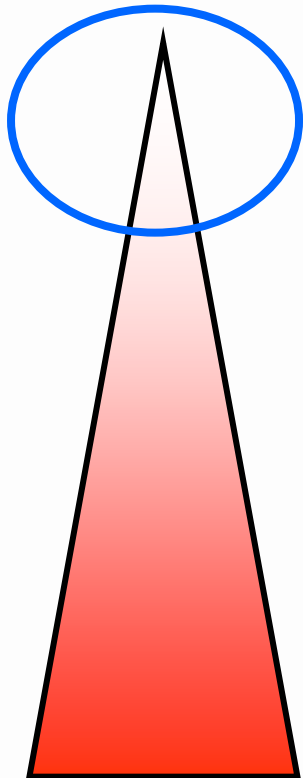
Mesaros *et al.* (2005) *Louvain médical*. 124:308-20

Efflux as a mechanism of export in antibiotic producers



spectrum

narrow



specific for the produced antibiotic

ABC

OleB or C of *S. antibioticus*
→ oleandomycin
SmB of *S. ambofaciens*
→ macrolides
Tlrc of *S. fradiae*
→ tylosin

MFS

Ptr of *S. pristinaespiralis*
→ pristinamycin
LmrA of *S. lincolnensis*
→ lincomycin
RifP of *A. mediterranei*
→ rifampicin

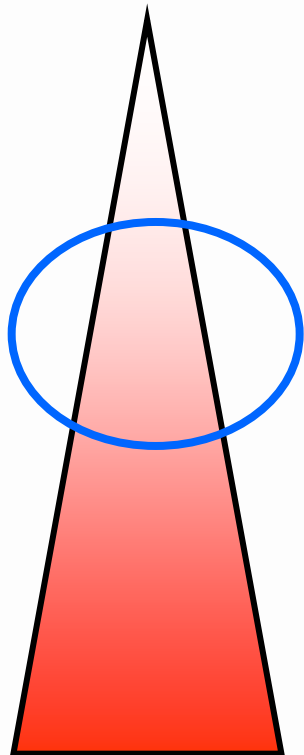
broad

Efflux as a mechanism of resistance in Gram-positive bacteria



spectrum

narrow



broad

specific for one (or a few) families of drugs

ABC

PatA/PatB of *S. pneumoniae*

→ FQ, chl

MsrA of *S. epidermidis*

→ erythromycin

MFS

NorA of *S. aureus*

→ FQ, Tet, chl

MefE of *S. pneumoniae*

→ ML

PmrA of *S. pneumoniae*

→ FQ

MefA of *S. pyogenes*

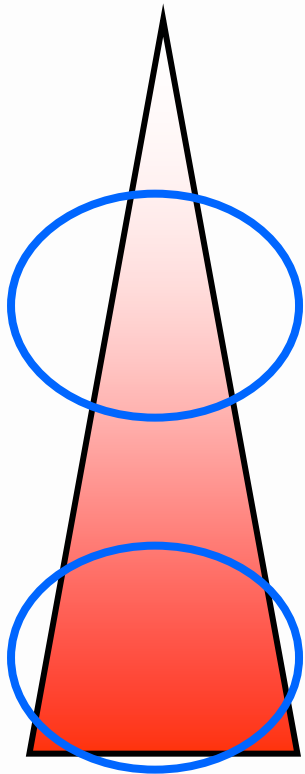
→ ML

Efflux as a mechanism of resistance in Gram-negative bacteria



spectrum

narrow



specific for one (or a few) families of drugs

MFS

TetA of *E. coli*

→ Tet

MsrA of *S. epidermidis*

→ erythromycin

broad spectrum, conferring cross-resistance

RND

MexAB-OprM of *P. aeruginosa*

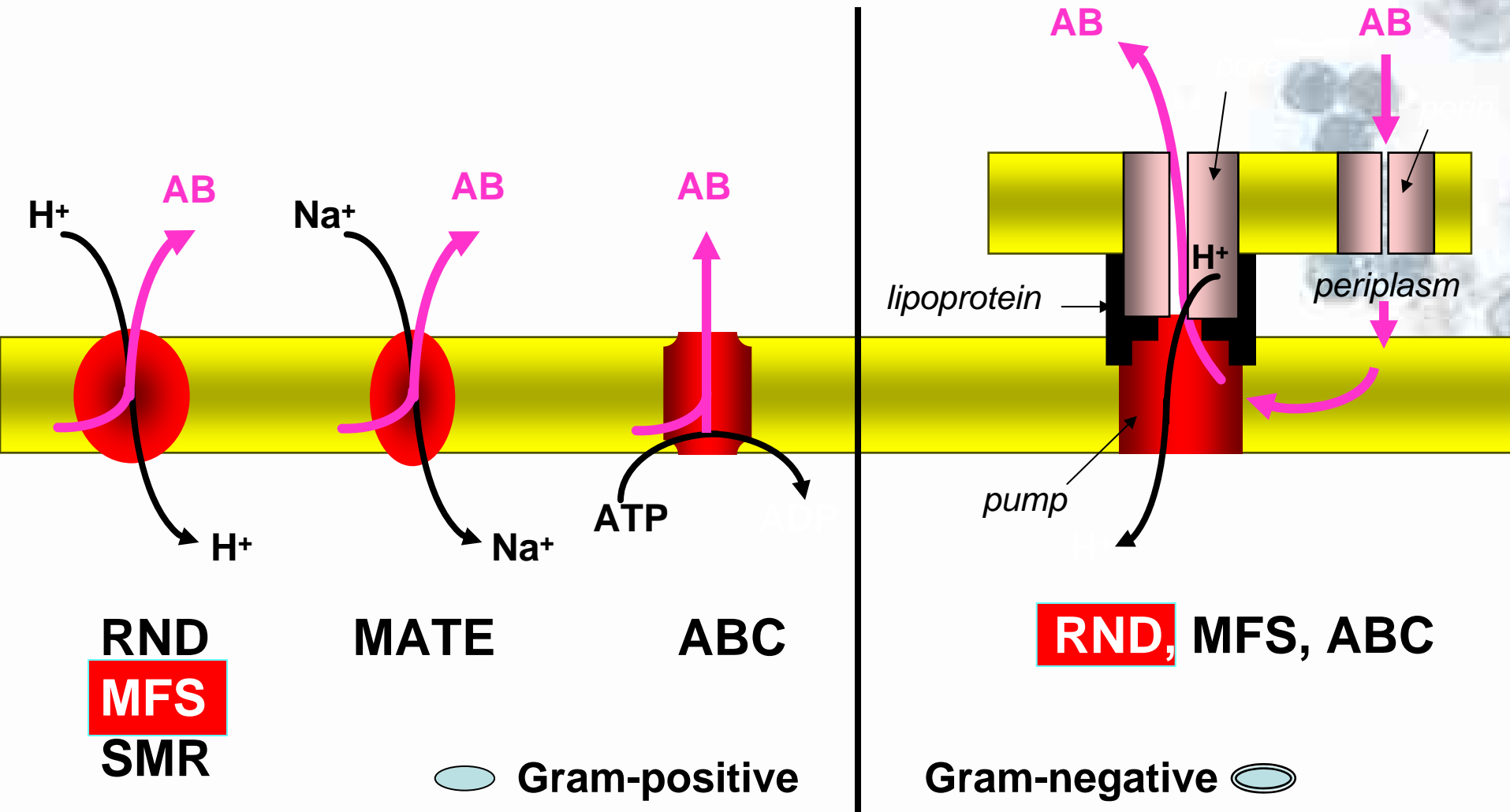
→ β -lac, FQ, Tet, ML, chl, rif, sulf

AcrAB-TolC of *E. coli*

→ β -lac, FQ, Tet, ML, chl, rif, sulf

broad

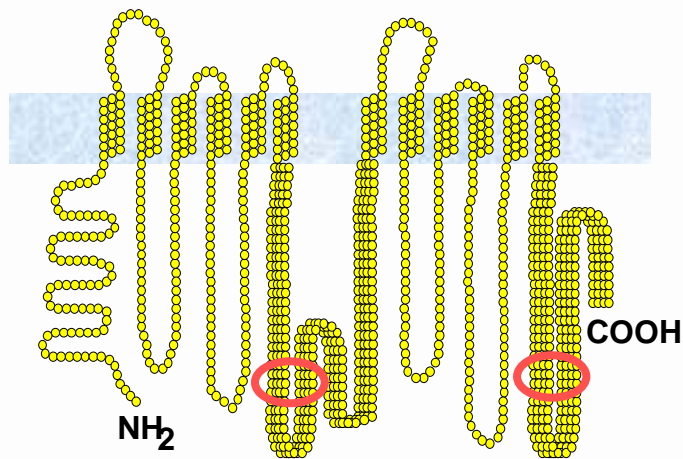
Topological organization of efflux transporters in procaryotes



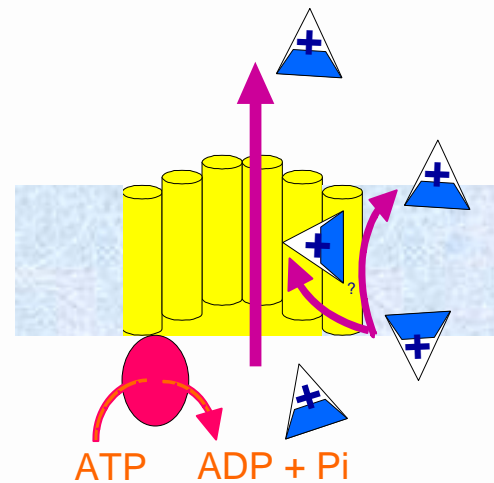
Common antibiotic-efflux pumps in eucaryotes (1/2)

- **Multiple Drug Resistance (PgP)** 









TOPOLOGY



MECHANISM



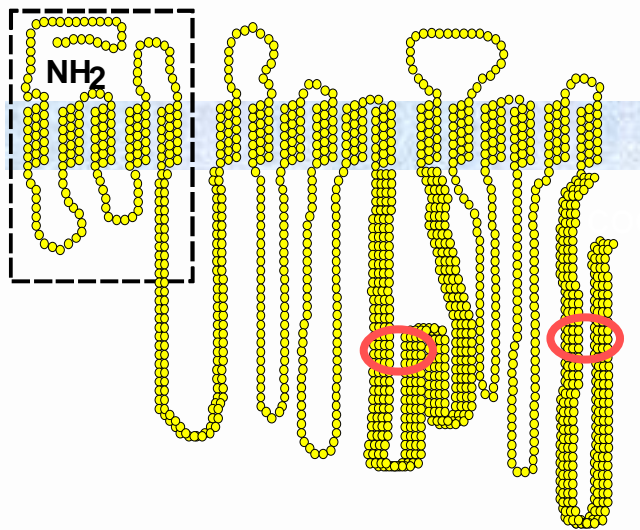
ANTIBIOTICS

-  tetracyclines
-  fluoroquinolones
-  macrolides
-  lincosamides
-  rifampicin
-  daptomycin
-  chloramphenicol
-  aminoglycosides

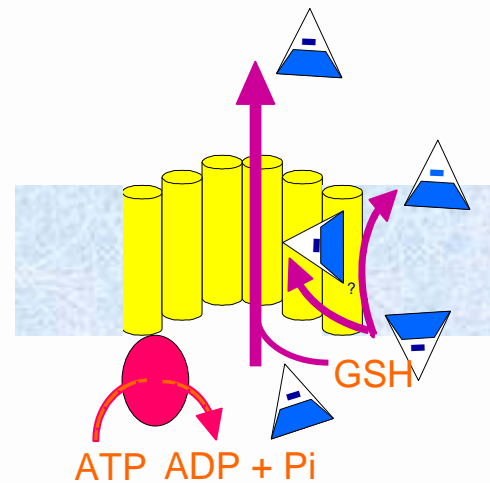
Common antibiotic-efflux pumps in eucaryotes (2/2)

- **Multidrug Resistance Proteins (MRP)** 

TOPOLOGY



MECHANISM



ANTIBIOTICS



fluoroquinolones



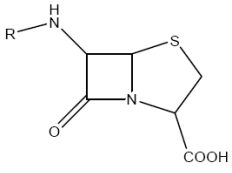
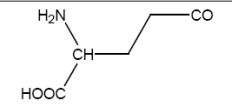
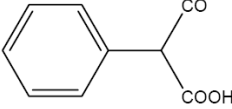
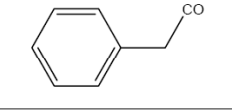
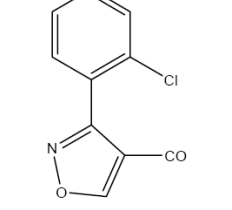
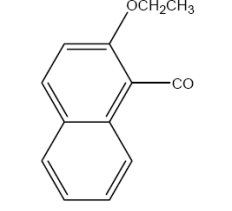
tetracyclines
macrolides

Molecular determinants of antibiotic efflux

- lipophilicity
- modulation by (small) structural changes
- anionic / cationic character
- capacity to bind to phospholipids
- and ... multi-ligand binding sites ...



Differential efflux of β -lactams by AcrB of *S. typhimurium*

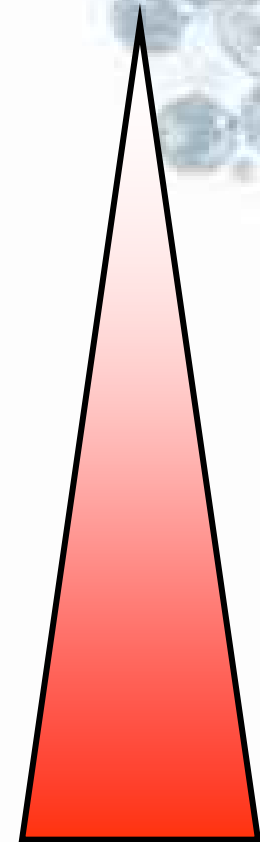
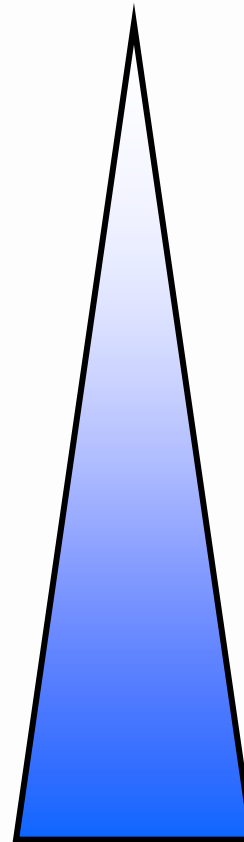
| R (Side chain) | molecule | side-chain partition coefficient | MIC ratio ^a |
|--|---------------|----------------------------------|------------------------|
|  | | | |
|  | penicillin N | 0 | 1 |
|  | carbenicillin | 80 | 4 |
|  | penicillin G | 270 | 32 |
|  | cloxacillin | 890 | 256 |
|  | nafcillin | 4200 | 128 |

^a ratio between the MIC of a *S. typhimurium* resistant mutant (AcrAB-ToIC overproducer) and of its susceptible parental strain

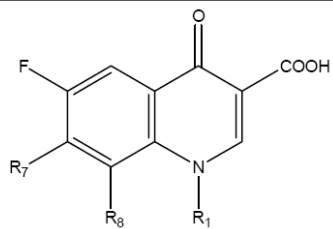
β -lactams & broad spectrum RND

Lipophilicity of the side chain


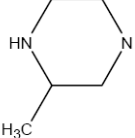

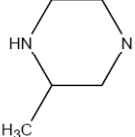

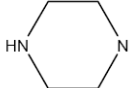
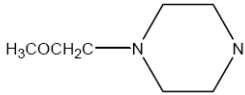
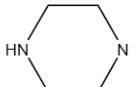
Resistance



Differential efflux of fluoroquinolones by NorA of *S. aureus*



Relationship between structure, bulkiness, and hydrophobicity of selected quinolone substituents and MIC of bacteria resistant by efflux.

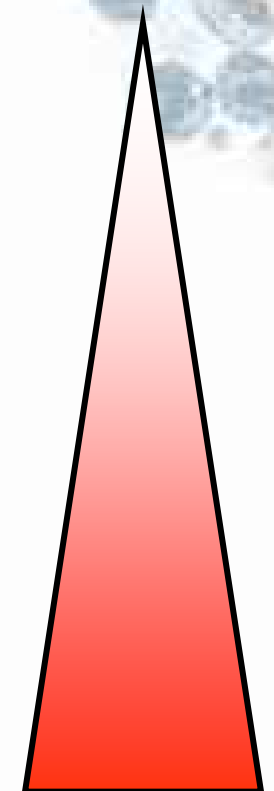
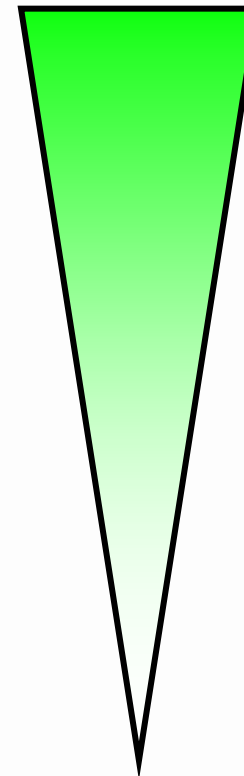
| molecule | R ₁ | R ₇ | R ₈ | MIC ratio ^a |
|---------------|---|---|------------------|------------------------|
| (a) |  |  | OCF ₃ | 2 |
| (b) |  |  | OCH ₃ | 4 |
| ciprofloxacin |  |  | H | 16 |
| (c) | C ₂ H ₅ |  | H | 16 |
| norfloxacin | C ₂ H ₅ |  | H | 64 |

^a ratio between the MIC of a *S. aureus* resistant mutant (NorA overproducer) and of its susceptible parental strain.

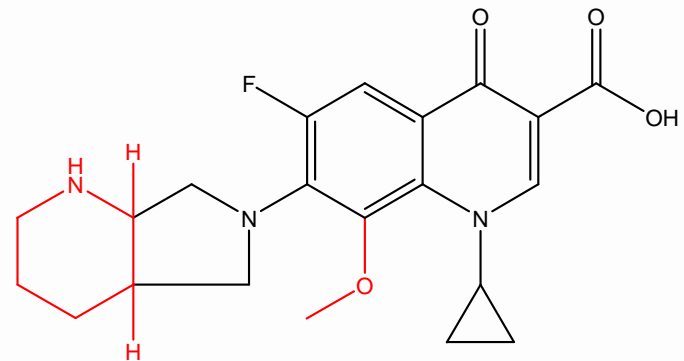
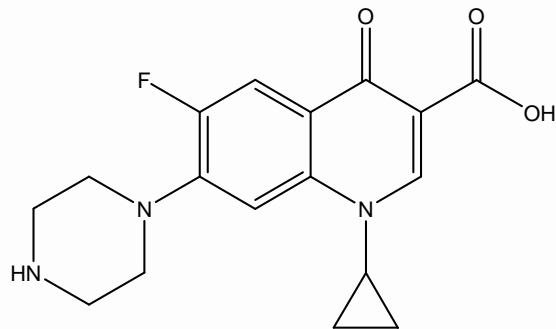
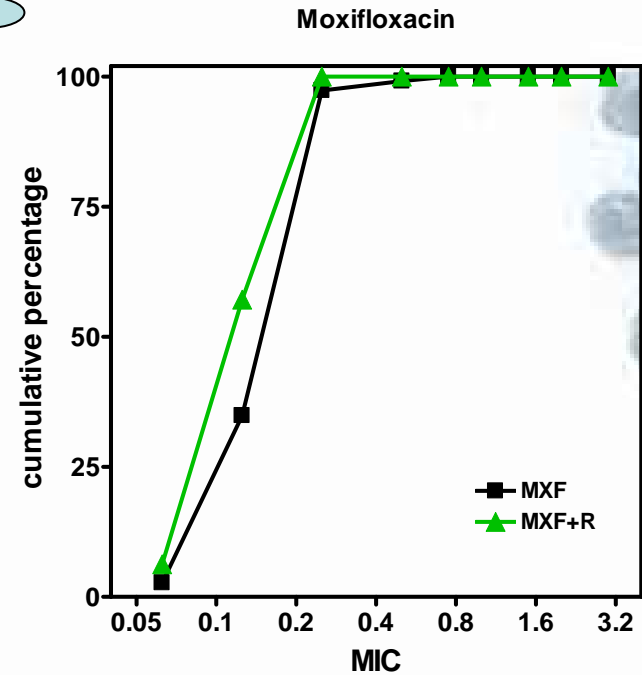
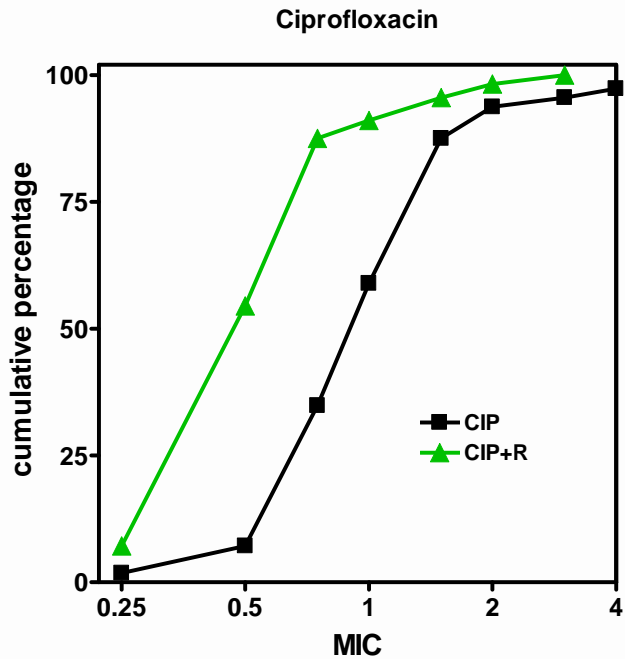
Quinolones & narrow spectrum MFS

Bulkiness in 7 and 8

Resistance

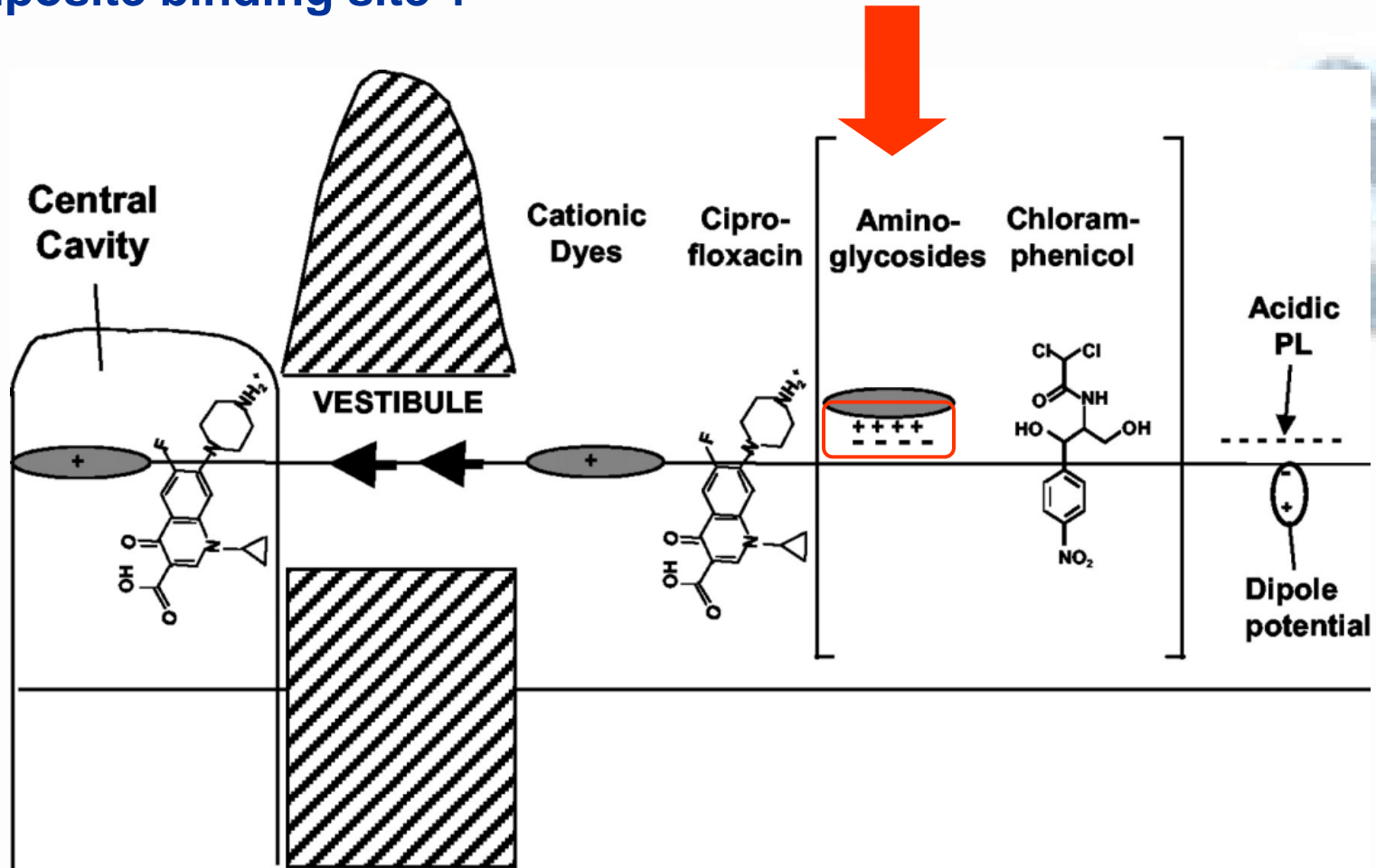


Moxifloxacin vs. ciprofloxacin for *S. pneumoniae* in Brussels : influence of reserpine ... (MFS [PmrA ?])



Aminoglycosides as unexpected substrates for efflux pumps (RND)

Composite binding site ?



Yu *et al.* (2003) *J. Bacteriol.* 185:5657-64

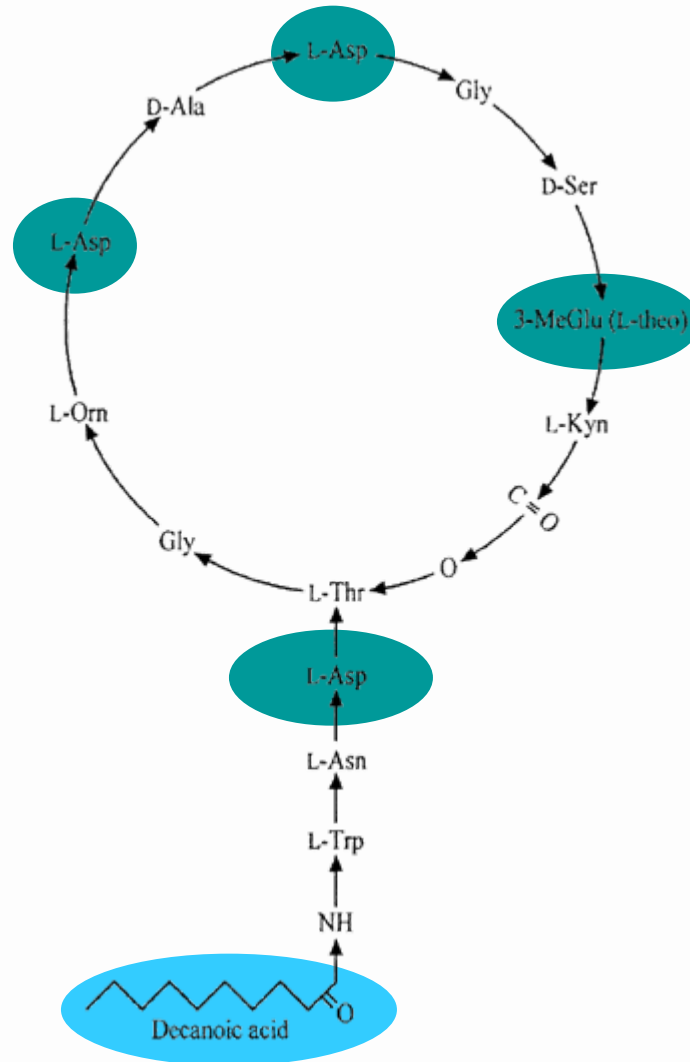
Another unexpected substrate

daptomycin

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

Highly polar molecule !

lipophilic chain

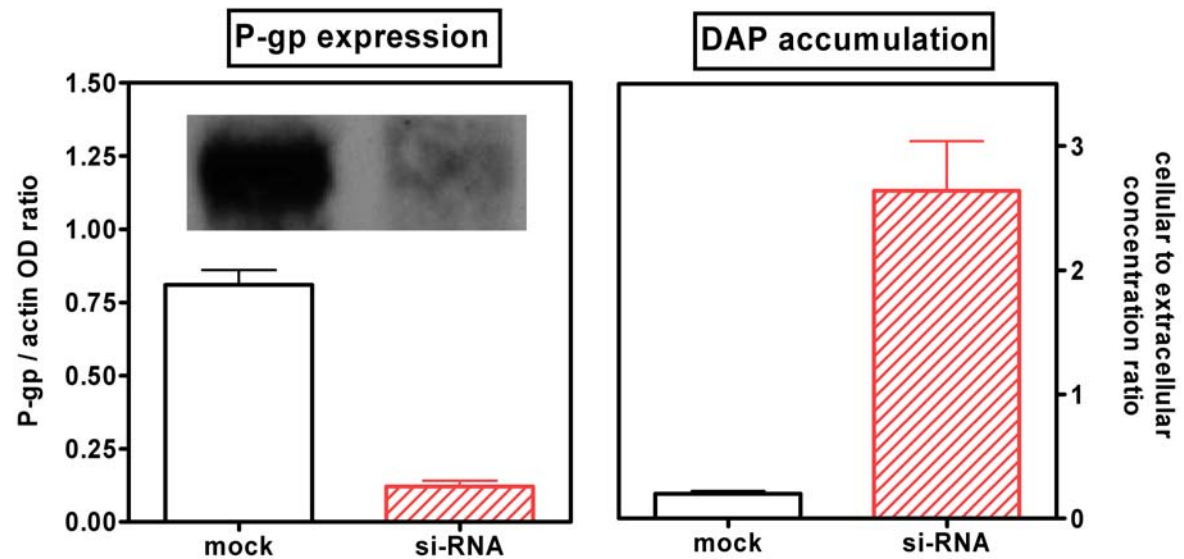
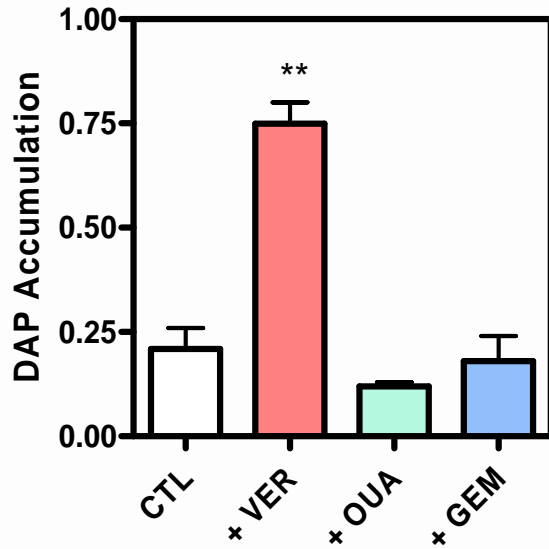


polyanionic
cyclic peptide



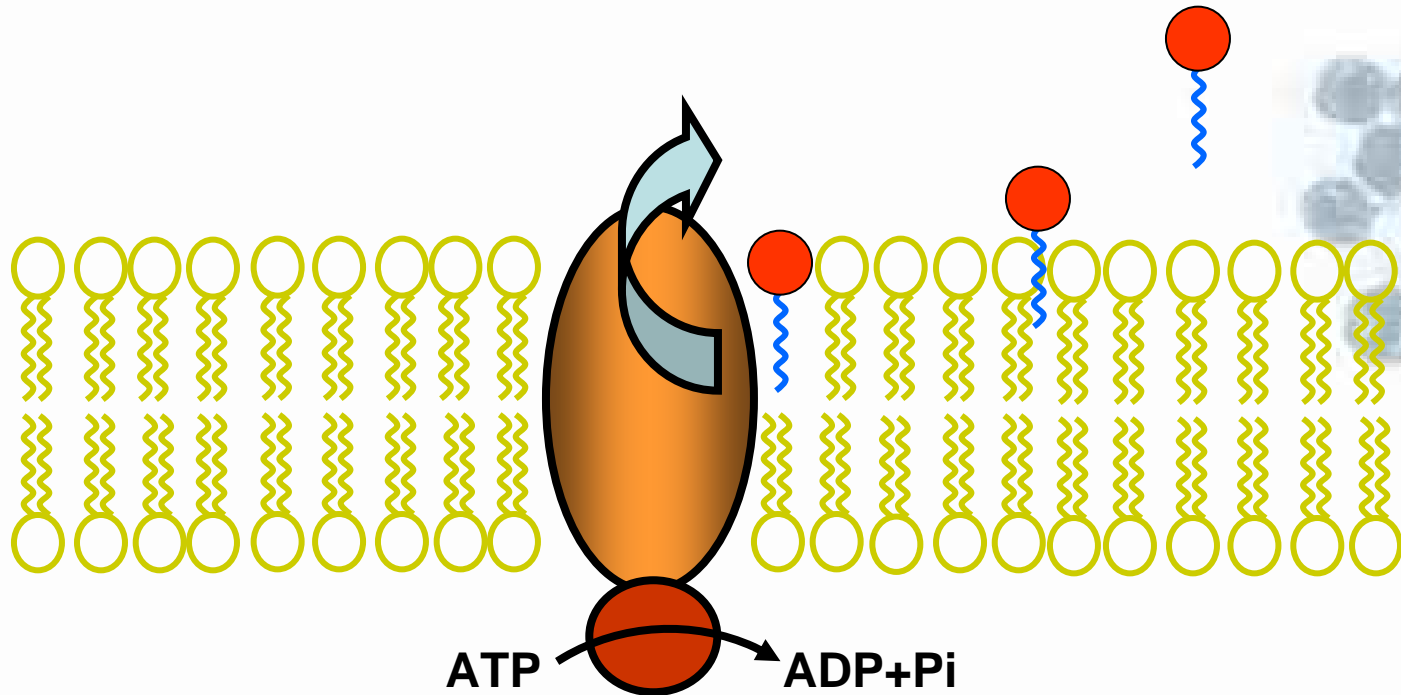
Daptomycin is substrate of P-gp

Daptomycin accumulation in macrophages is inversely proportional to P-gp activity and expression level



Lemaire *et al.* AAC 2007; 51(8):2748-57

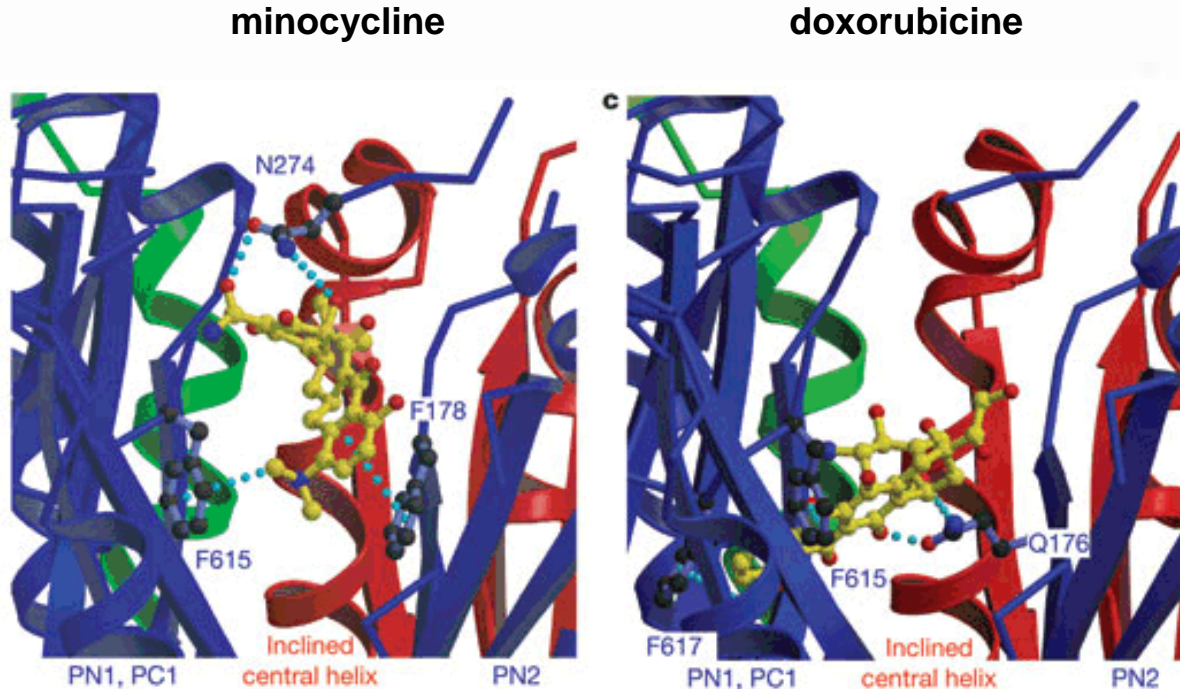
Putative mechanism of daptomycin transport by P-gp



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

Lemaire *et al.* AAC 2007; 51(8):2748-57

Multi-ligand binding sites: the binding domain of AcrB (RND) in *E. coli*



- The methyl moiety of the 7-dimethylamino group and the C-ring of minocycline interact with Phe 178 and Phe 615, respectively. Two oxygen atoms of the 1-oxo and 2-amido groups interact with Asn 274
- Doxorubicin interacts with Phe 615, similar to minocycline, but interacts with Gln 176 and Phe 617 instead of Asn 274 and Phe 178, respectively.

Murakami et al. Nature 2006;443:173-9

Steps and Challenges of efflux in antibacterial chemotherapy

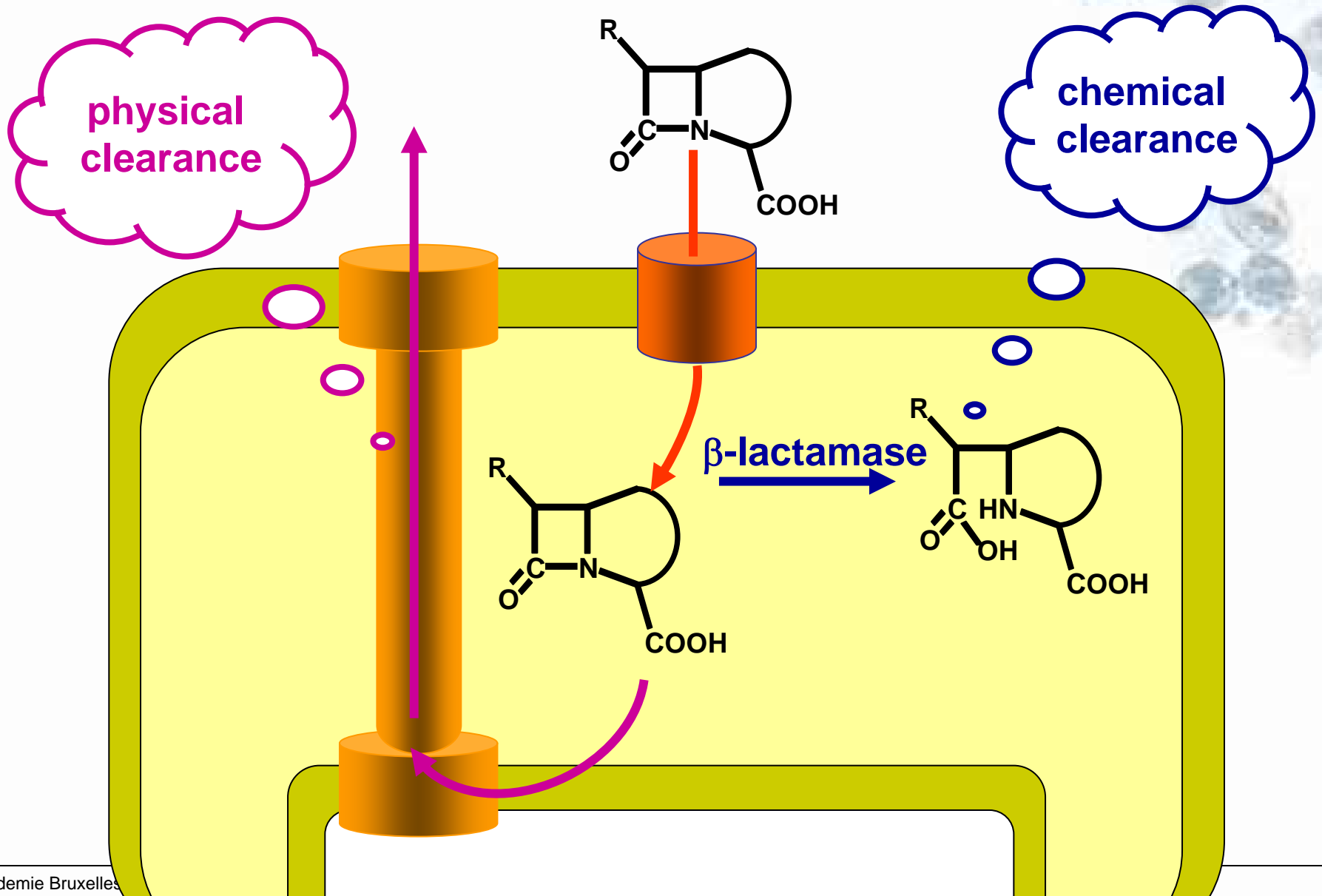
- **recognizing its existence:**
is it a significant mechanism of resistance ?
- **which pumps ...**
is antibiotic efflux different from other drug efflux ?
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does it need to change our vision on (and decisions about) existing antibiotics ?
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 - is efflux important in pharmacokinetics/drug interactions ?



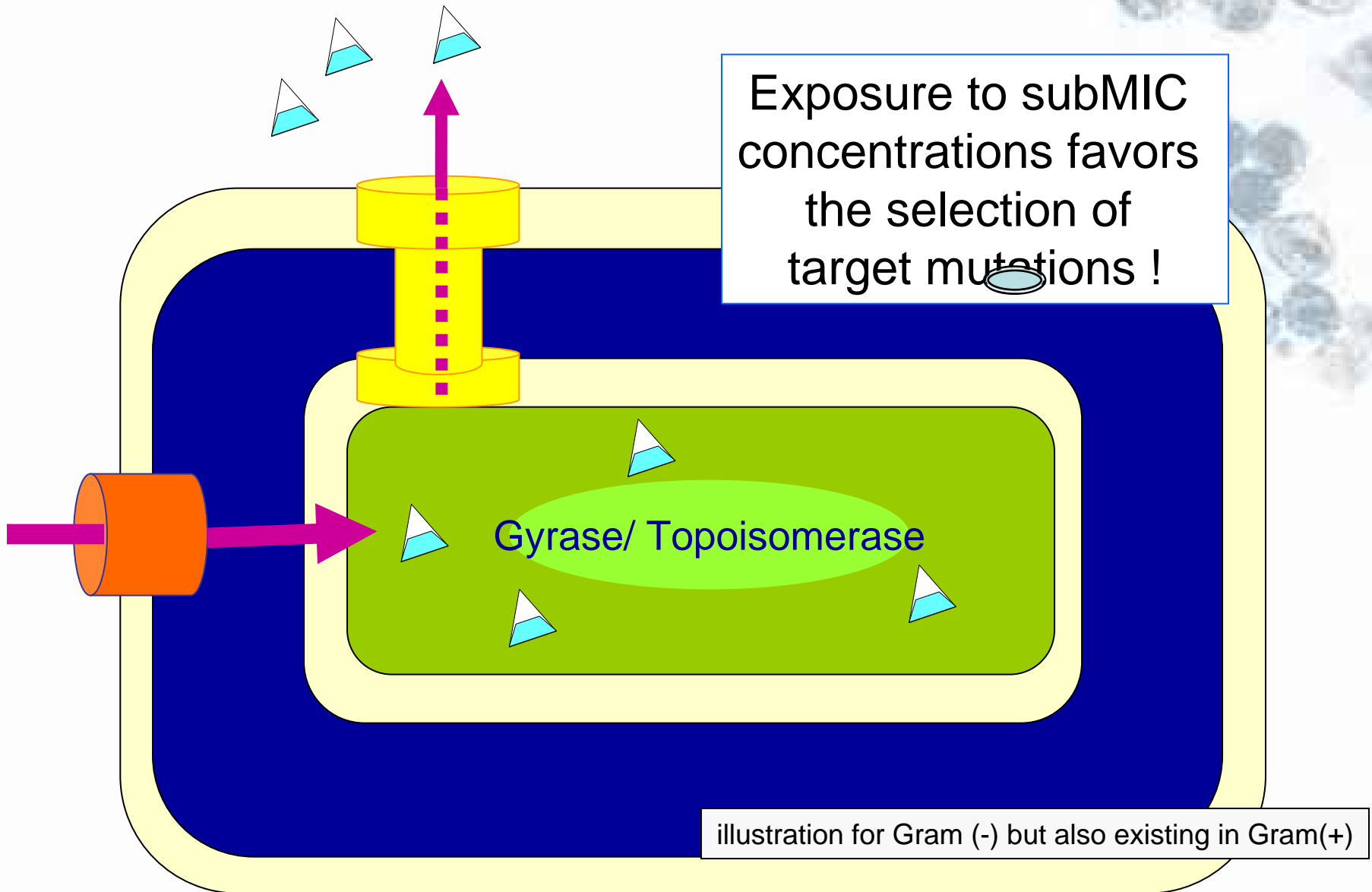
Impact of efflux on bacterial susceptibility

- Bacterial efflux
 - cooperation with other mechanisms of resistance, leading to high level resistance
 - suboptimal exposure of the antibiotic target, leading to selection of less susceptible mutants
 - low level resistance if alone, but nearing the limit of the PK/PD breakpoint (and new EUCAST breakpoints)
- Efflux from eucaryotic cells
 - decrease of intracellular activity

β -lactams: cooperation between efflux and β -lactamase(s) in Gram-negative bacteria



Efflux and selection of resistance to Fluoroquinolones



Efflux and resistance in pathogenic bacteria

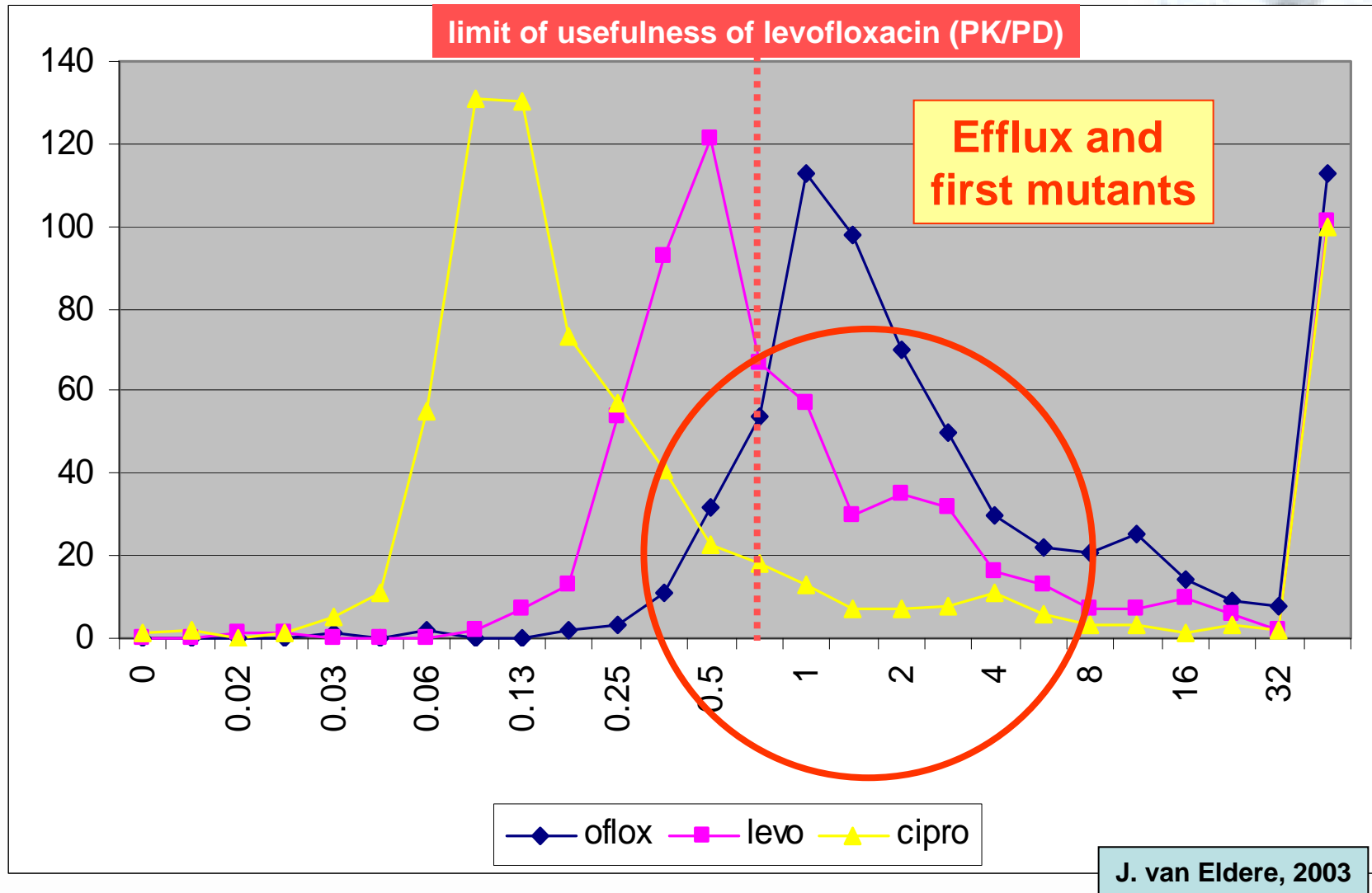


1 bacteria → **several pumps** → **multiresistance**

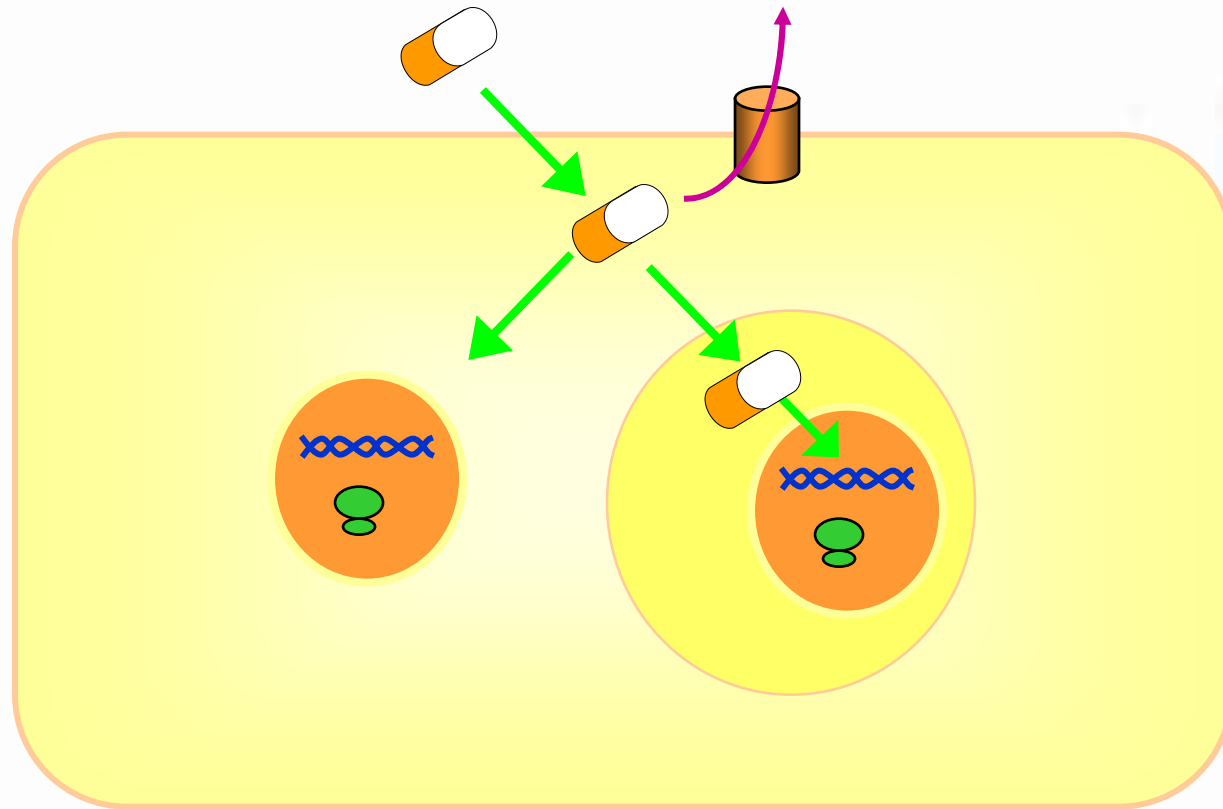
1 pump → **several classes of antibiotics** → **crossresistance**

1 class of antibiotics → **several pumps** → **efficacy of inhibitors ?**

Fluoroquinolones and *P. aeruginosa* at the Gaslhuisberg Academic Hospital (Belgium)



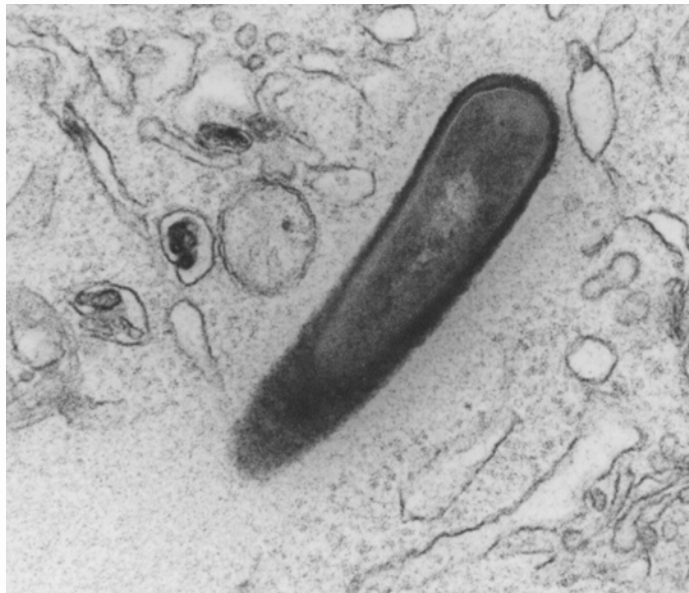
Antibiotic efflux from eucaryotic cells and "intracellular resistance"



Reduction in intracellular drug concentration
can result in inefficacy against intracellular bacteria

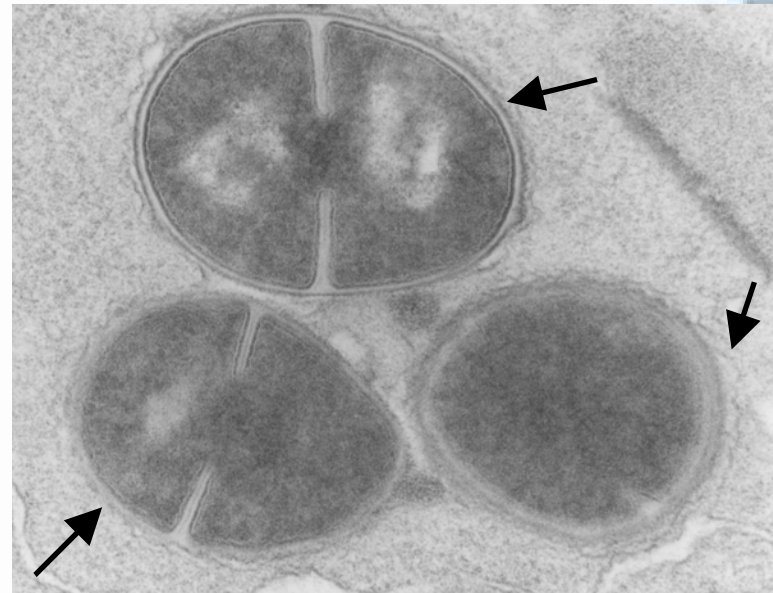
Models of intracellular infection

L. monocytogenes

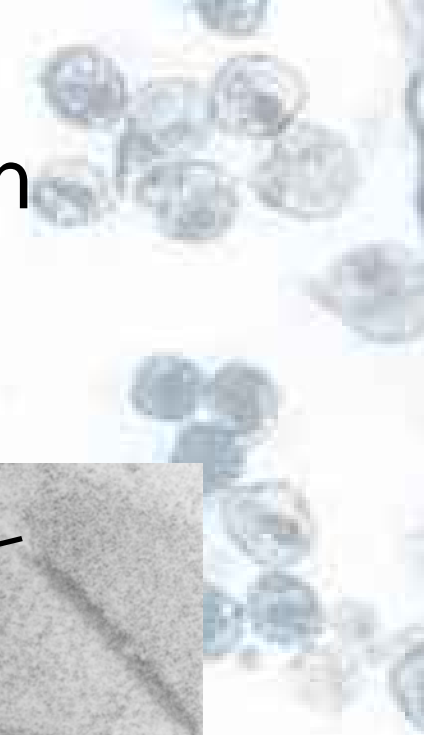


cytosol

S. aureus



phagolysosomes

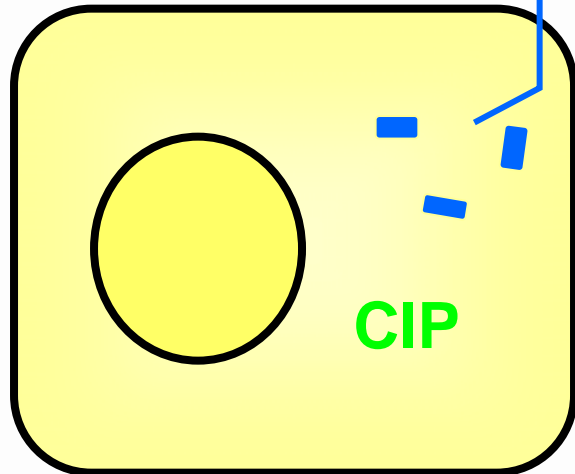


Influence of an inhibitor * of an eucaryotic antibiotic transporter (MRP) on the intracellular activity of an antibiotic

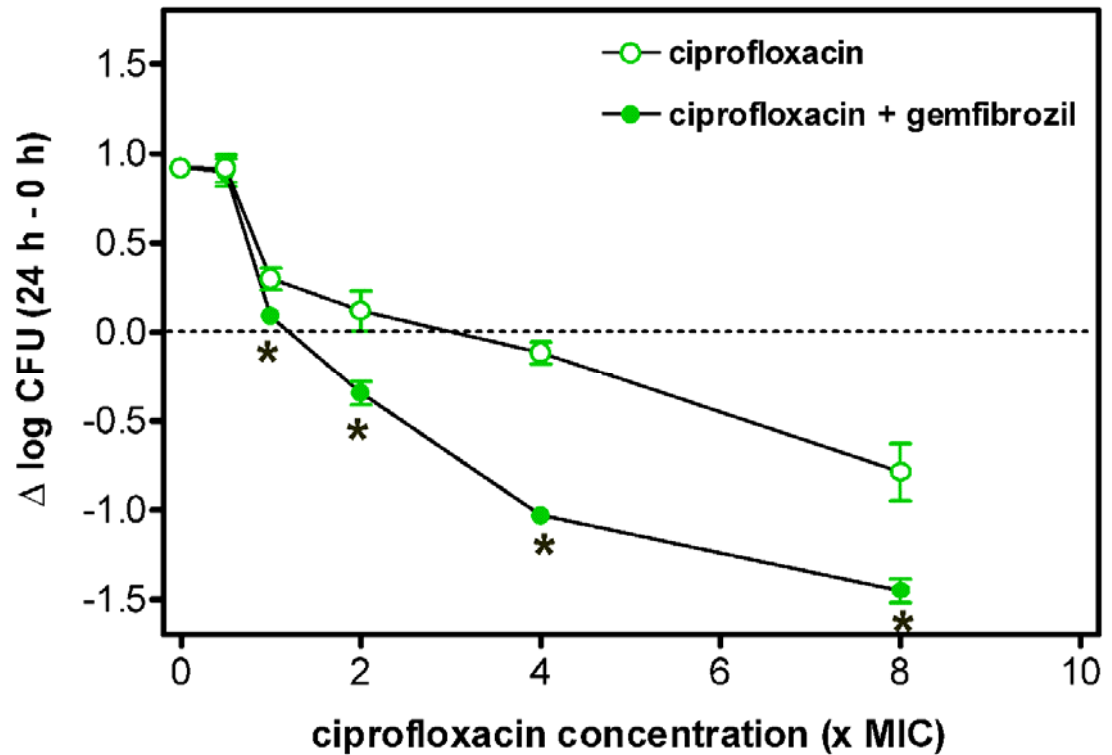
ciprofloxacin and *L. monocytogenes*



L. monocytogenes



* *gemfibrozil* 250 μ M; 24 h

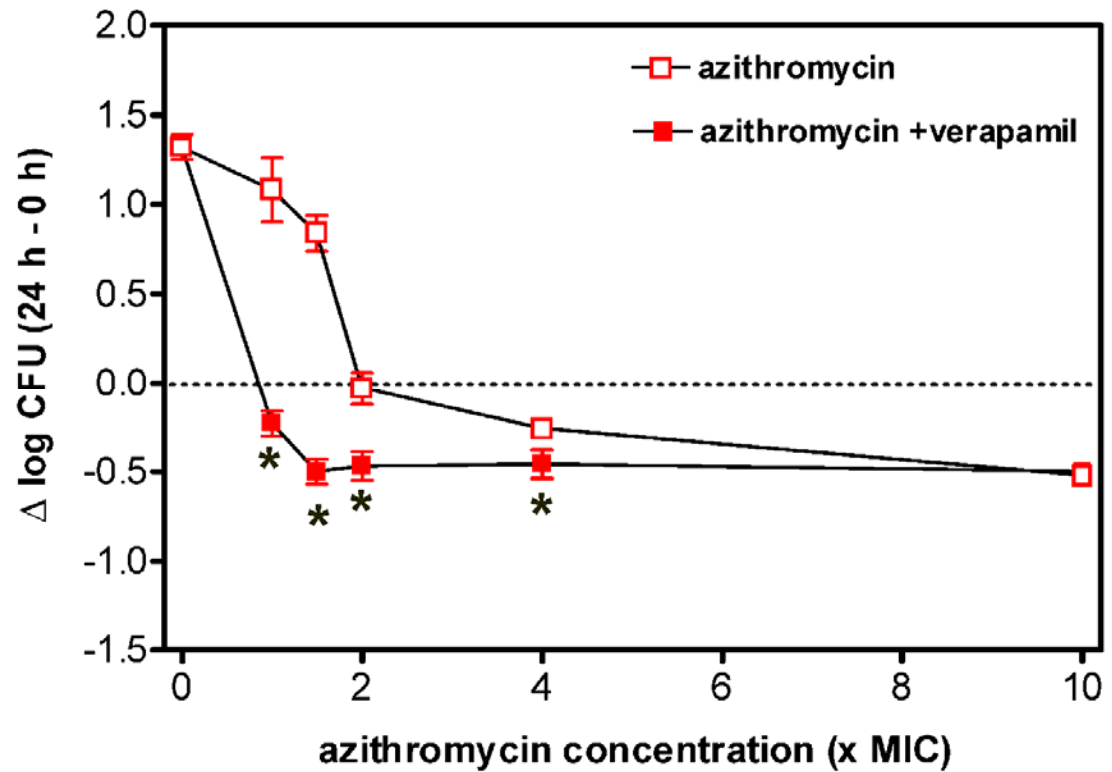
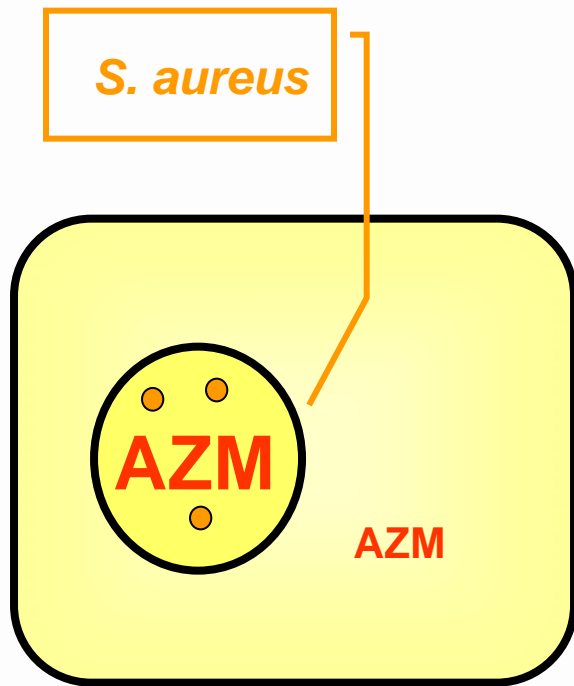


Seral *et al.* (2003) JAC 51:1167-73

Influence of an inhibitor * of an eucaryotic antibiotic transporter (P-gp) on the intracellular activity of an antibiotic

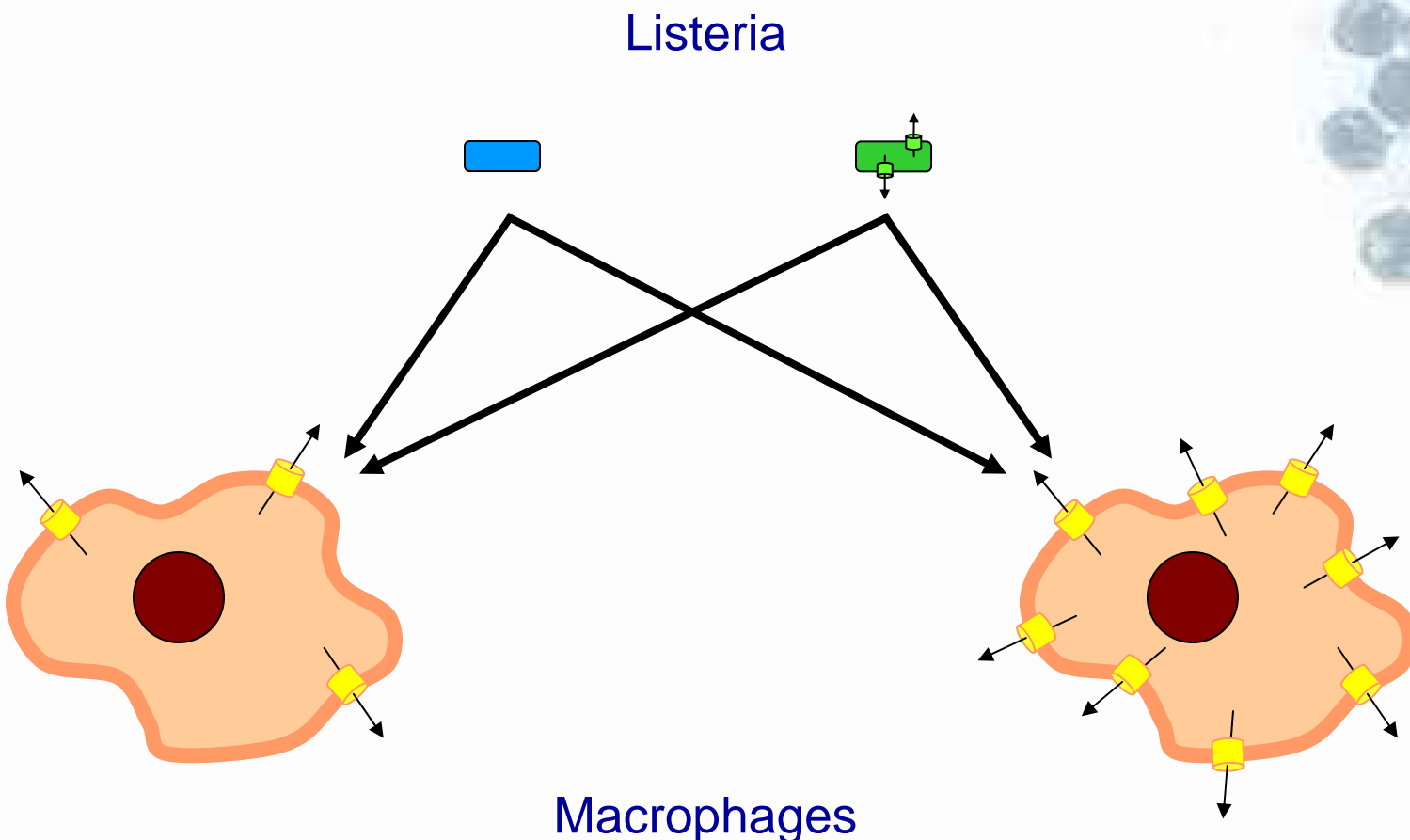
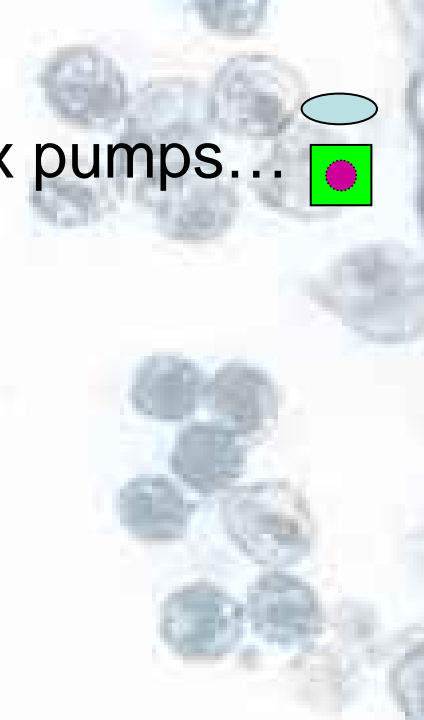


azithromycin and *S. aureus*



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

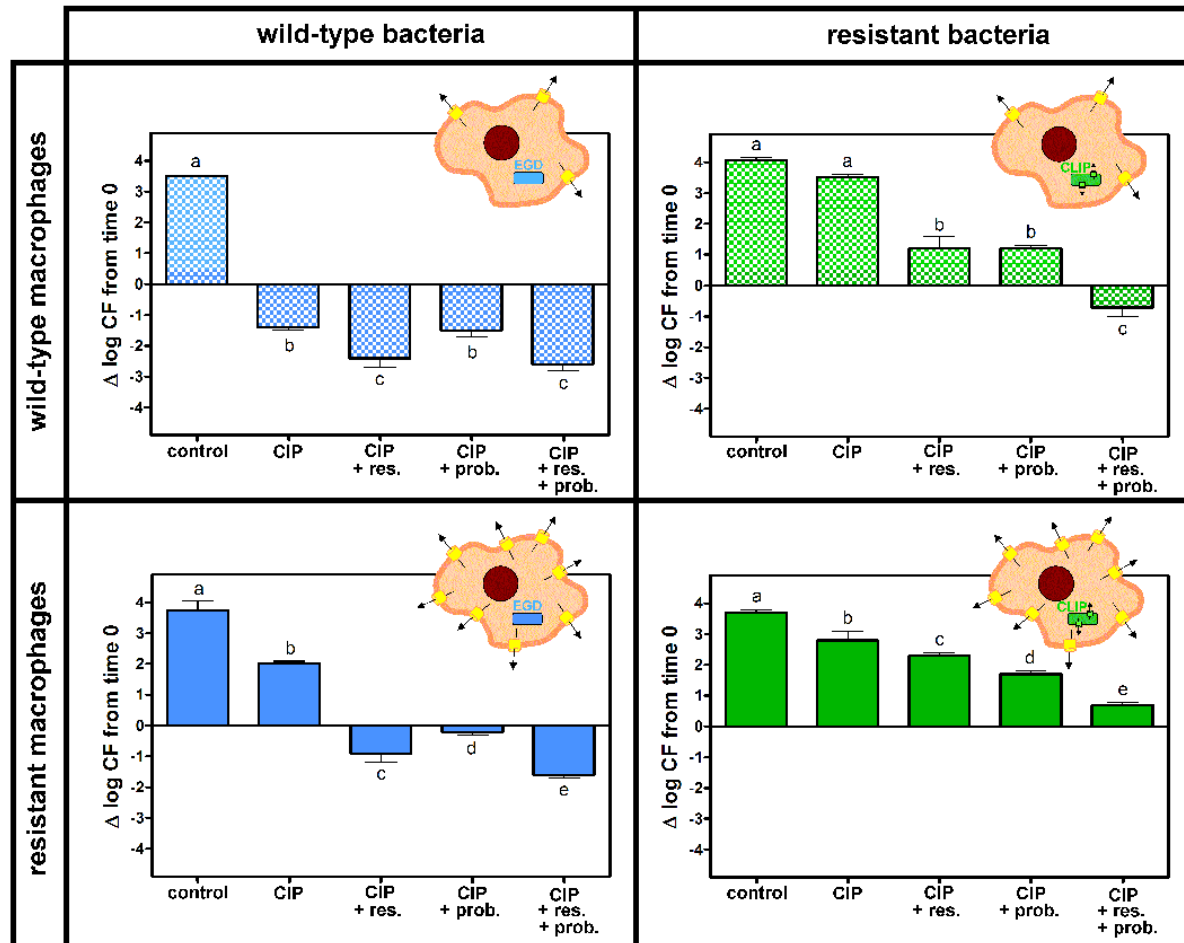
Cooperation between bacterial and eucaryotic efflux pumps...



Cooperation between bacterial and eucaryotic efflux



ciprofloxacin



Steps and Challenges of efflux in antibacterial chemotherapy

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Diagnostic approaches ...



JAC

Journal of Antimicrobial Chemotherapy (2007) **59**, 378–386

doi:10.1093/jac/dk1504

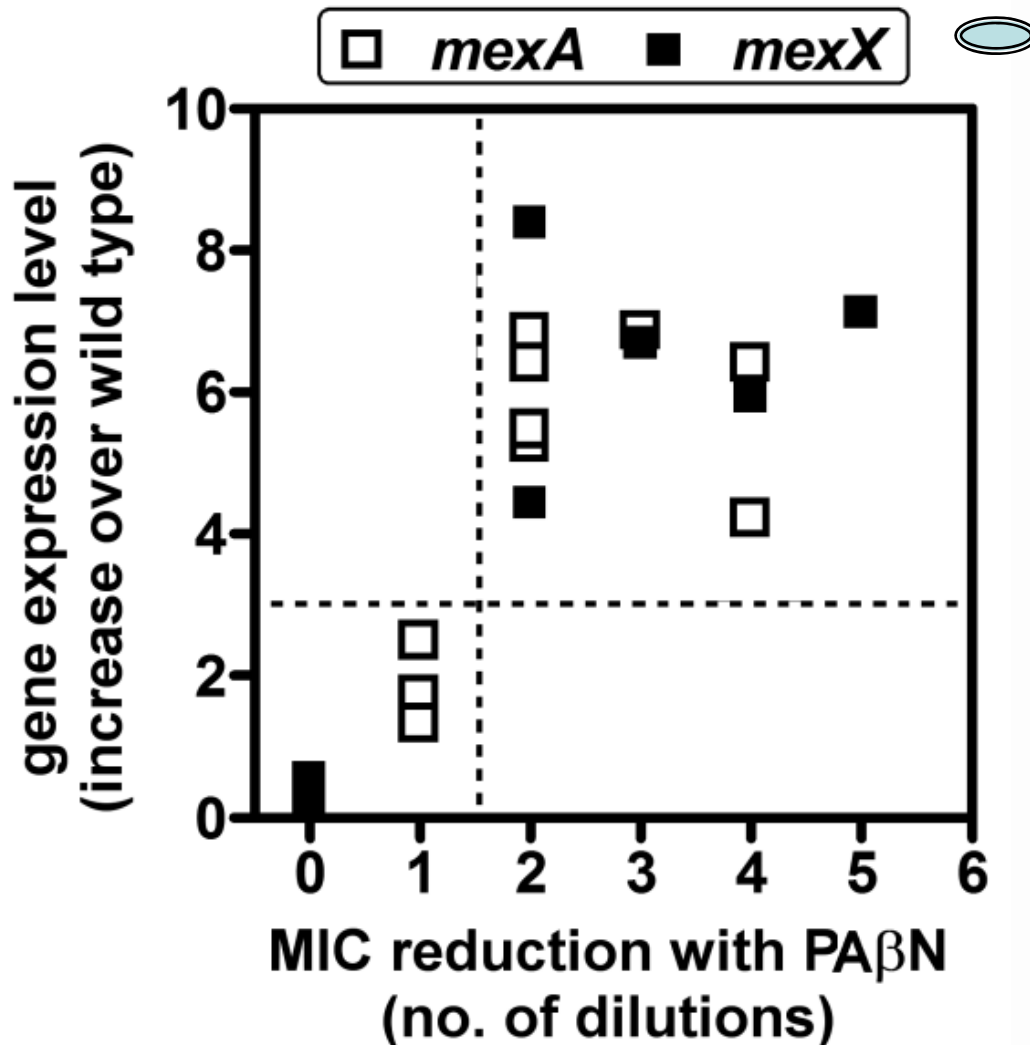
Advance Access publication 8 February 2007

A combined phenotypic and genotypic method for the detection of Mex efflux pumps in *Pseudomonas aeruginosa*

Narcisa Mesaros¹, Youri Glupczynski², Laëtitia Avrain¹, Nancy E. Caceres¹, Paul M. Tulkens^{1*} and Françoise Van Bambeke¹

¹Unité de Pharmacologie cellulaire et moléculaire, Brussels, Université catholique de Louvain, UCL 7370 avenue E. Mounier 73, B-1200 Bruxelles, Belgium; ²Laboratoire de Microbiologie, Cliniques universitaires UCL de Mont-Godinne, avenue G. Therasse 1, B-5530 Yvoir, Belgium

Diagnostic approaches ...



Correlation between the level of expression (PCR) of constitutive Mex pumps and the effect of PA β N on the MIC of **reporter antibiotics** (carbenicillin for *mexA* and gentamicin for *mexX*).

Data are grouped in two quadrants of potentially different diagnostic significance

- lower left, no or minimally meaningful efflux-mediated decrease of susceptibility
- upper right, efflux is likely to be the cause of the decreased susceptibility).

Mesaros et al., J Antimicrob Chemother. 2007; 59:378-86.



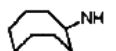


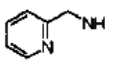
Steps and Challenges of efflux in antibacterial chemotherapy

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The final selection of tigecycline was the result of a systematic research to combine the **hydrophobic moiety** AND the **additional aminogroup** in a substituent attached to position 9

Table 2. In Vitro Antibacterial Activity of Compounds 13–25.

| | | Organism; minimum inhibitory concentration (MIC) µg/mg | | | | | | | | | | |
|----------------|--|--|---------------------------------|----------------------------------|----------------------------------|---|---|---|---|---|--|-------------------------------|
| R ₁ | | <i>E. coli</i> UBMS 88-1 Tet B | <i>E. coli</i> PRP1 Tet A | <i>E. coli</i> J3272 Tet C | <i>E. coli</i> J3272 Tet D | <i>E. coli</i> UBMS 90-4 Tet M | <i>E. coli</i> UBMS 90-5 sensitive | <i>S. aureus</i> UBMS 88-7 Tet K | <i>S. aureus</i> UBMS 90-1 Tet M | <i>S. aureus</i> UBMS 90-3 sensitive | <i>S. aureus</i> Smith sensitive | Enterococcus ATCC 29212 |
| 13 | MeNH | 1 | 16 | 8 | 0.5 | NT | 1 | 16 | 1 | 0.5 | 0.5 | 0.5 |
| 14 | <i>n</i> -PrNH | 0.5 | 2 | 0.5 | 0.12 | 0.25 | 0.5 | 2 | 0.5 | 0.25 | 0.25 | 0.25 |
| 15 | <i>n</i> -BuNH | 0.5 | 1 | 0.5 | 0.25 | 0.25 | 0.5 | 2 | 0.5 | 0.25 | 0.12 | 0.12 |
| 16 | <i>t</i> -BuNH | 0.5 | 0.25 | 0.25 | 0.12 | 0.12 | 0.25 | 0.5 | 0.12 | 0.25 | 0.25 | 0.12 |
| 17 | <i>n</i> -HexylNH | 0.5 | 0.5 | 0.5 | 0.12 | 0.25 | 0.25 | 2 | 0.25 | 0.06 | 0.12 | 0.12 |
| 18 | UndecylNH | 32 | 32 | 32 | 32 | 32 | 16 | 2 | 16 | 0.5 | 0.5 | 2 |
| 19 |  NH | 4 | 32 | 8 | 2 | 2 | 2 | 4 | 0.5 | 0.5 | 0.25 | 0.25 |
| 20 |  NH | 0.25 | 1 | 0.25 | 0.12 | 0.25 | 0.25 | 2 | 0.25 | 0.12 | 0.25 | 0.12 |
| 21 |  NH | 4 | 2 | 2 | 0.5 | 2 | 4 | 0.5 | 1 | 0.25 | 0.5 | 0.25 |
| 22 |  NH | 0.5 | 1 | 0.5 | 0.25 | 0.5 | 0.5 | 0.5 | 0.5 | 0.25 | 0.25 | 0.12 |
| 23 |  NH | 0.5 | 4 | 0.5 | 0.25 | 0.5 | 0.5 | 4 | 1 | 0.5 | 0.5 | 0.25 |
| 24 | PhCH ₂ NH | 2 | 4 | 2 | 0.5 | 0.5 | 0.5 | 2 | 0.5 | 0.25 | 0.25 | 0.25 |
| 25 |  NH | 16 | 32 | 16 | 8 | 8 | 16 | 32 | 8 | 4 | 4 | 2 |

tigecycline

Can we make clinically-useful inhibitors of bacterial efflux ?



Recent Patents on Anti-Infective Drug Discovery, 2006, 1, 157-175

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Inhibitors of Bacterial Efflux Pumps as Adjuvants in Antibiotic Treatments and Diagnostic Tools for Detection of Resistance by Efflux

Françoise Van Bambeke^{1,*}, Jean-Marie Pagès² and Ving J. Lee³

¹*Unité de Pharmacologie cellulaire et moléculaire, Université catholique de Louvain, Brussels, Belgium* ²*EA2197 Enveloppe Bactérienne, Perméabilité et Antibiotiques, Faculté de Médecine, Université de la Méditerranée, Marseille, France;* ³*CB Research and Development, Inc. (Adesis, Inc), New Castle, DE, USA*

BIOCHEMICAL PHARMACOLOGY 71 (2006) 910-918

Practical applications and feasibility of efflux pump inhibitors in the clinic—A vision for applied use

Olga Lomovskaya, Keith A. Bostian *

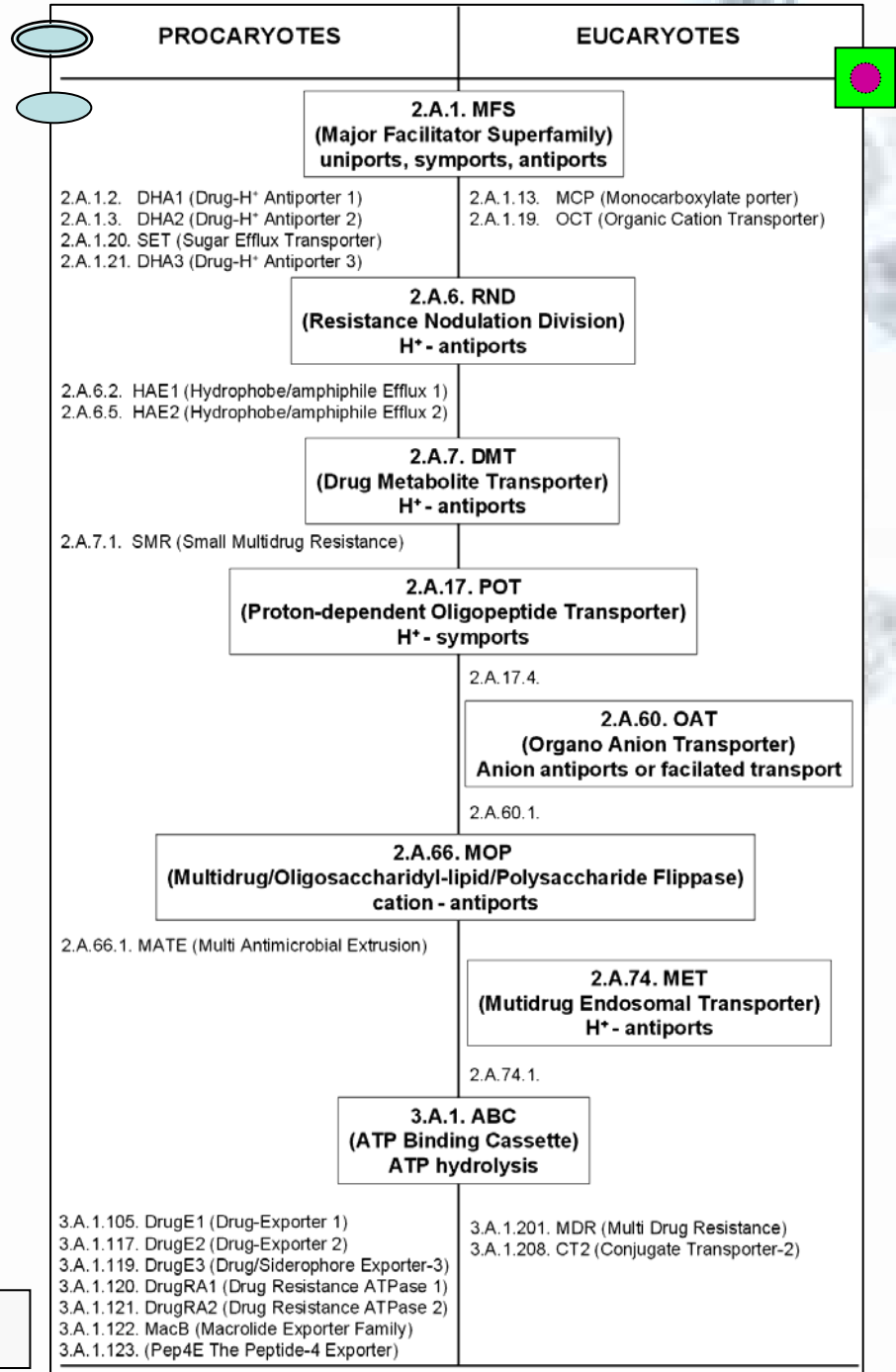
Mpex Pharmaceuticals Inc., 3030 Bunker Hill Street, San Diego, CA 92109, United States

Clinically useful inhibitors of antibiotic efflux pumps: the bottom line ...

It will be difficult because...

- Many procaryotic transporters have eucaryotic homologues;
- antibiotics are (for that reason or another ...) substrate to many of them... (sometimes in an unanticipated fashion)
- and so will probably be many inhibitors...

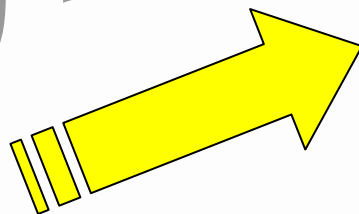
Van Bambeke et al. (2003)
J Antimicrob Chemother 51: 1055-1065.



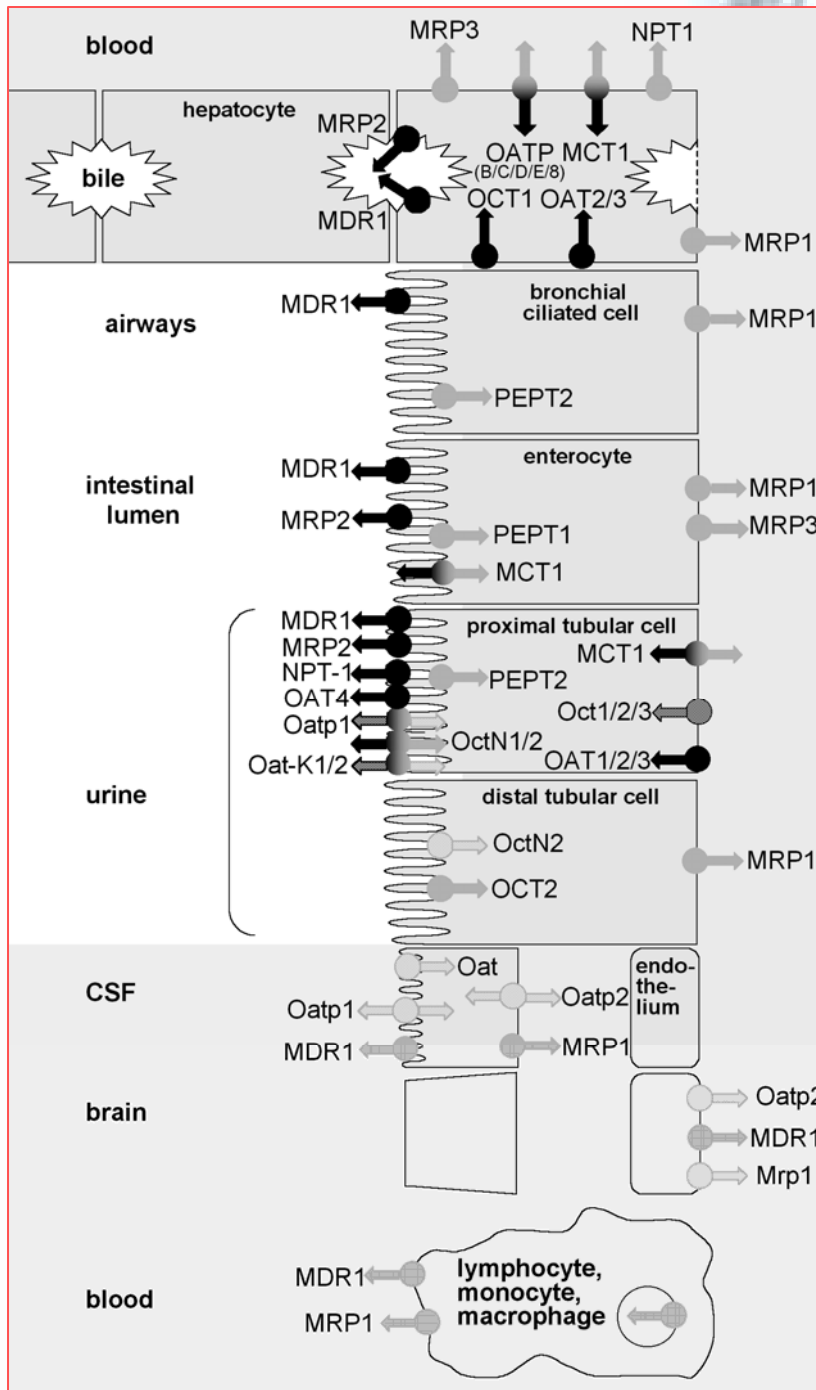
Is efflux important in pharmacokinetics ?



Yes, !!!



Van Bambeke et al. (2003)
J Antimicrob Chemother 51: 1055-1065.



antibiotic transporters in various human tissues

Let us believe in pumps... (each has its own set of challenges)



**the fire pump...
(save your life)**



**the gas pump...
(keeps you moving)**



**the pump for everything ...
(white product)**



**the little amateur one...
but with great expectations ...**



The past and present "pump team" at UCL

