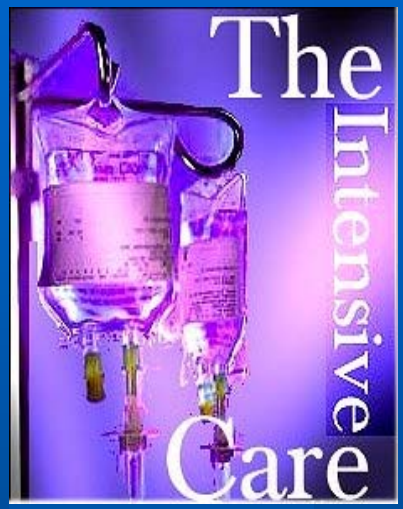
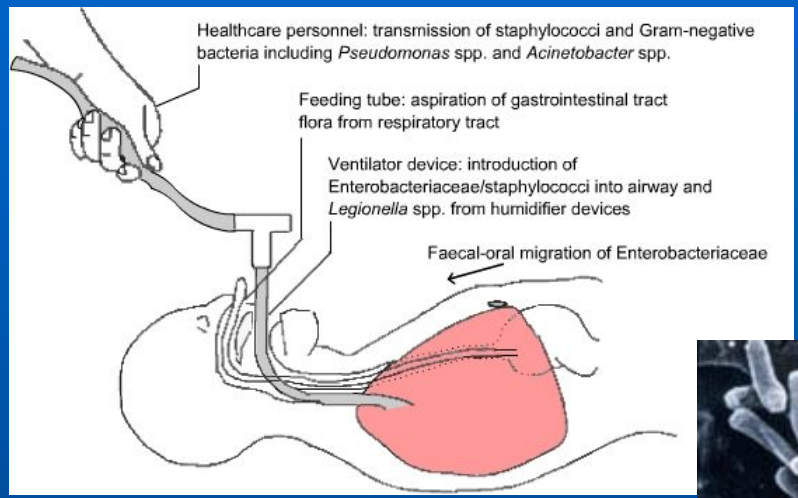
Inhibiting efflux pumps to restore antibiotic activity against *Pseudomonas aeruginosa*

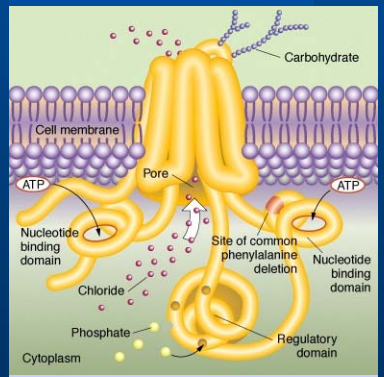
Unité de Pharmacologie
cellulaire et moléculaire

F. Van Bambeke

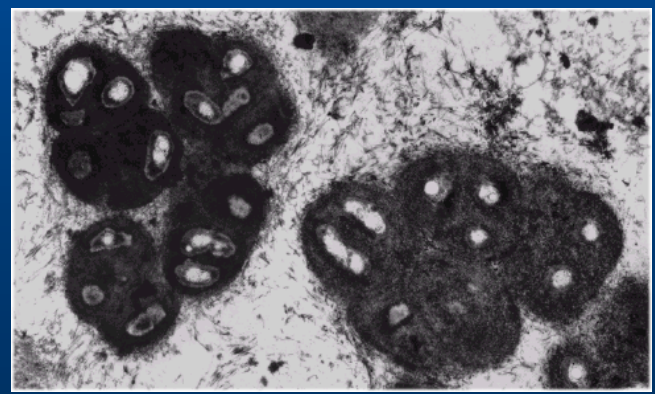
P. aeruginosa, a pathogen for vulnerable patients





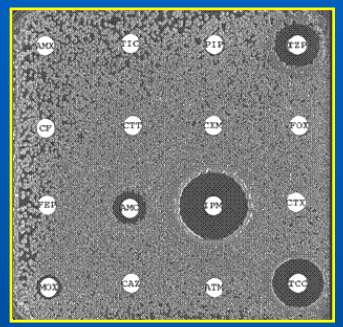


cystic fibrosis



Sadikot *et al,* Am J Respir Crit Care Med. 2005

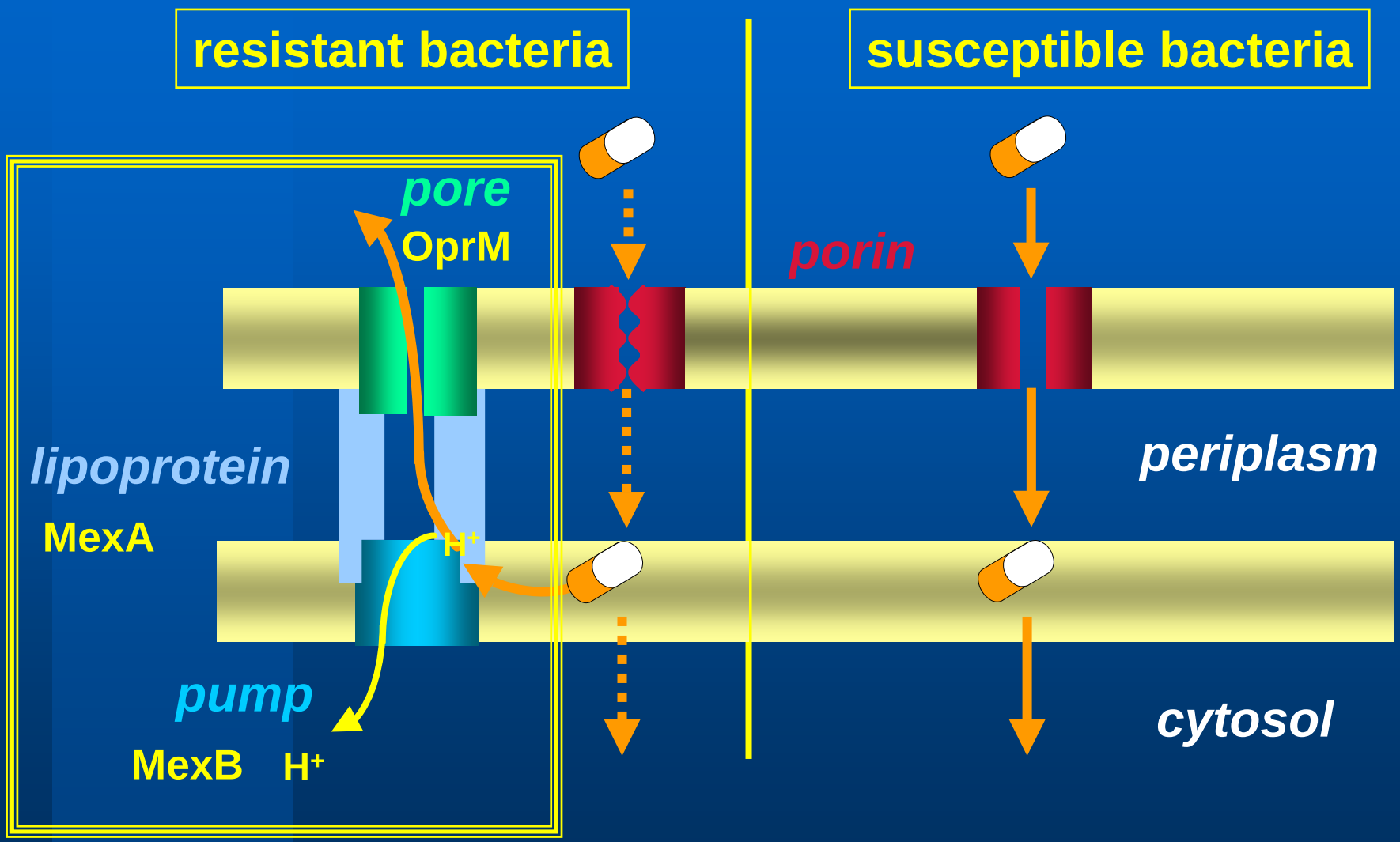
P. aeruginosa, a multiresistant pathogen



- **Acquired resistance**
  - **β-lactams:** production of β-lactamases
  - **Aminoglycosides:** production of modifying enzymes
  - **Fluoroquinolones**: target mutations
- **Constitutive resistance**
  - **Low permeability**
    - Porins
    - Efflux pumps

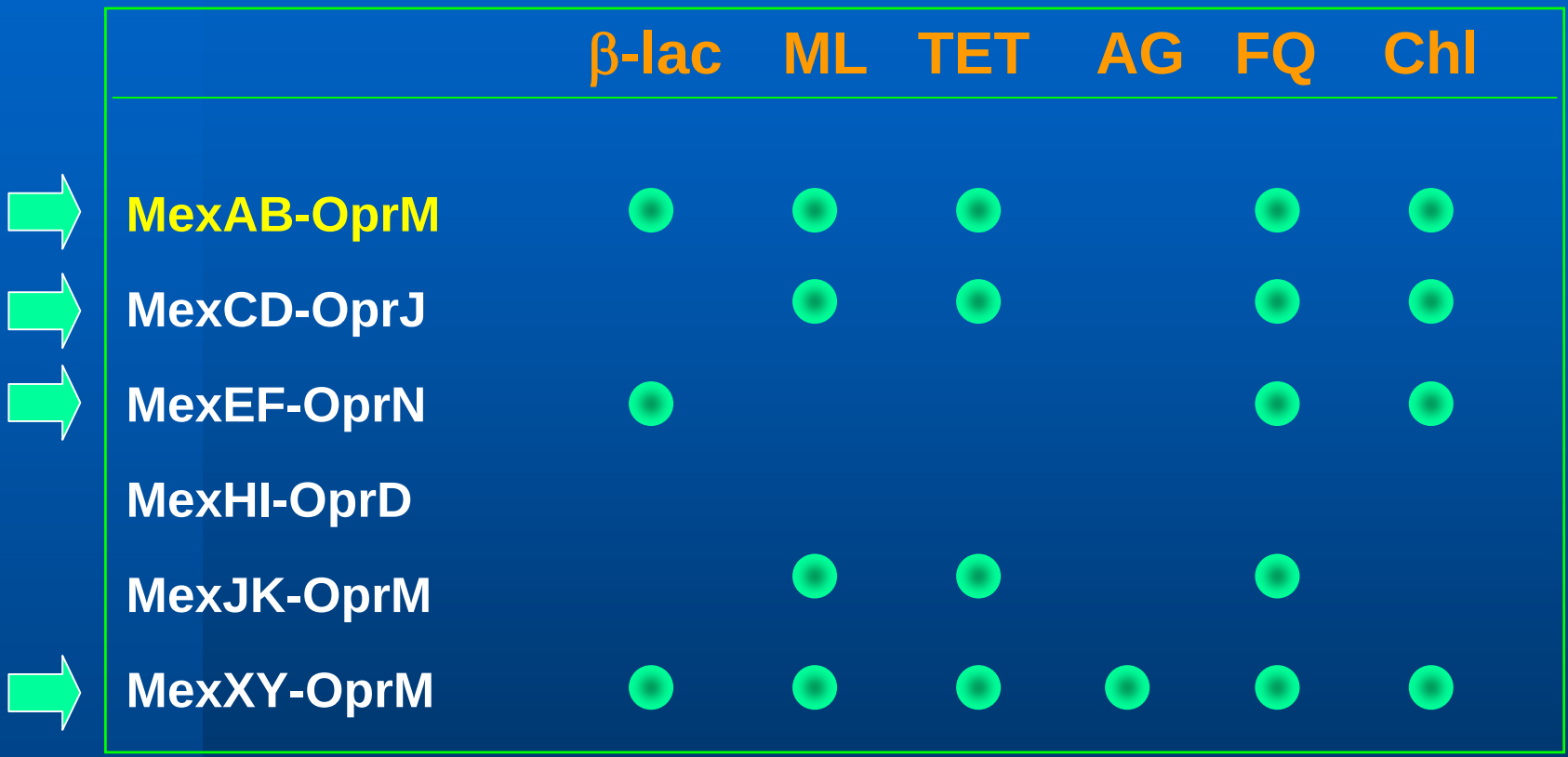
Pier & Ramphal, PPID (2005), chap 216

Low permeability and constitutive resistance



expressed in wild-type strains!

Main efflux pumps in *P. aeruginosa*



constitutive expression; inducible expression

Van Bambeke et *al*. JAC (2003) 51:1055-65; Aeschlimann, Pharmacotherapy (2003) 23:916-24

Disruption of efflux pumps increases susceptibility to antibiotics

	MIC	
antibiotic	**WT strain**	**disruptant**
carbenicillin	32	**< 0.25**
cefepime	2	1
norfloxacin	> 8	1
ciprofloxacin	2	**0.1**
chloramphenicol	16	4

Li *et al*. AAC (1995) 39:1948-53

Disruption of efflux pumps decreases selection of target mutations

Frequency of levofloxacin-resistant mutants in *P. aeruginosa* with deletions of the efflux pump operons

Pump status	Frequency of LVX-resistant mutants
WT	2×10^7 - 4×10^7
Δ MexAB-OprM	2×10^7 - 4×10^7
Δ MexCD-OprJ	2×10^7 - 4×10^7
Δ MexEF-OprN ;	2×10^7 - 4×10^7
Δ MexAB-OprM; Δ MexEF-OprN	2×10^7 - 10^7
Δ MexCD-OprJ; Δ MexEF-OprN	2×10^6
Δ MexAB-OprM; Δ MexCD-OprJ	1×10^9
Δ MexAB-OprM; Δ MexCD-OprJ; Δ MexEF-OprN	**$<1 \times 10^{11}$**

Selection of mutants in FQ target undetectable if ALL pumps are disrupted

Lomovskaya *et al,* AAC (1999) 43:1340-46

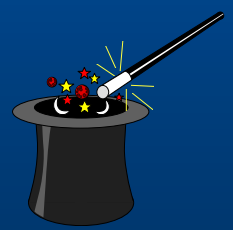
Inhibiting efflux as a strategy to improve antibiotic efficacy

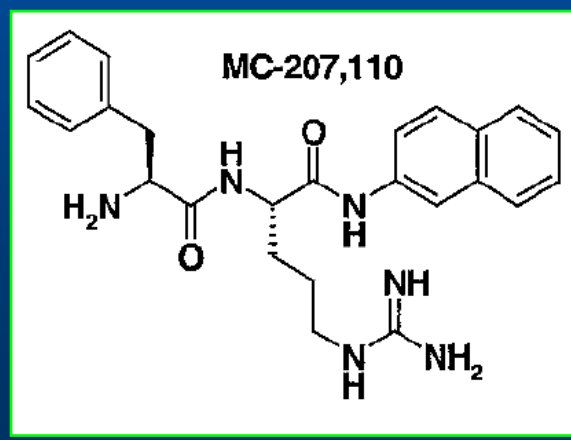
- Discovery of inhibitors of efflux pumps (EPI)
- In vitro activity
- Mode of action
- Structure-activity relationships
- Potential uses

High throughput screening for the discovery of efflux pumps inhibitors

librairy of 200,000 synthetic and natural compounds

in vitro screening in combination with levofloxacin against *P. aeruginosa* overexpressing Mex pumps





Renau *et al*. J. Med. Chem. (1999) 42: 4928-31; Lomovskaya *et al*. JMMB (2001) 3: 225-36

Inhibiting efflux as a strategy to improve antibiotic efficacy

- Discovery of inhibitors of efflux pumps (EPI)
- In vitro activity
- Mode of action
- Structure-activity relationships
- Potential uses

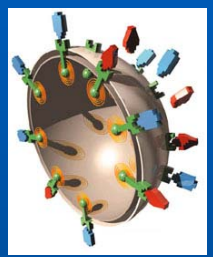
In vitro activity of EPI

EPI are as effective as disruption of pump genes to restore antibiotic efficacy !

	MIC ratio	
antibiotic	**WT strain / Δ MexAB-OprM**	**AB / AB + MC-207,110**
levofloxacin	64	32
sparfloxacin	32	128
erythromycin	32	32
chloramphenicol	512	128

Lomovskaya *et al,* AAC (2001) 45:105-116

Inhibiting efflux as a strategy to improve antibiotic efficacy



- Discovery of inhibitors of efflux pumps (EPI)
- In vitro activity
- Mode of action
- Structure-activity relationships
- Potential uses

Characteristics of the ideal EPI

- ✓ Enhance activity of AB in efflux pumps overproducers by inhibiting efflux

- ✓ Not affect AB activity in strains lacking efflux pumps
- ✓ Not potentiate activity of AB that are not effluxed
- ✓ Not affect proton gradients across the inner membrane

- ✓ Not affect eucaryotic efflux pumps

Lomovskaya *et al.* AAC (2001) 45:105-116

Characteristics of the ideal EPI

- ✓ **Enhance activity of AB in efflux pumps overproducers by inhibiting efflux**

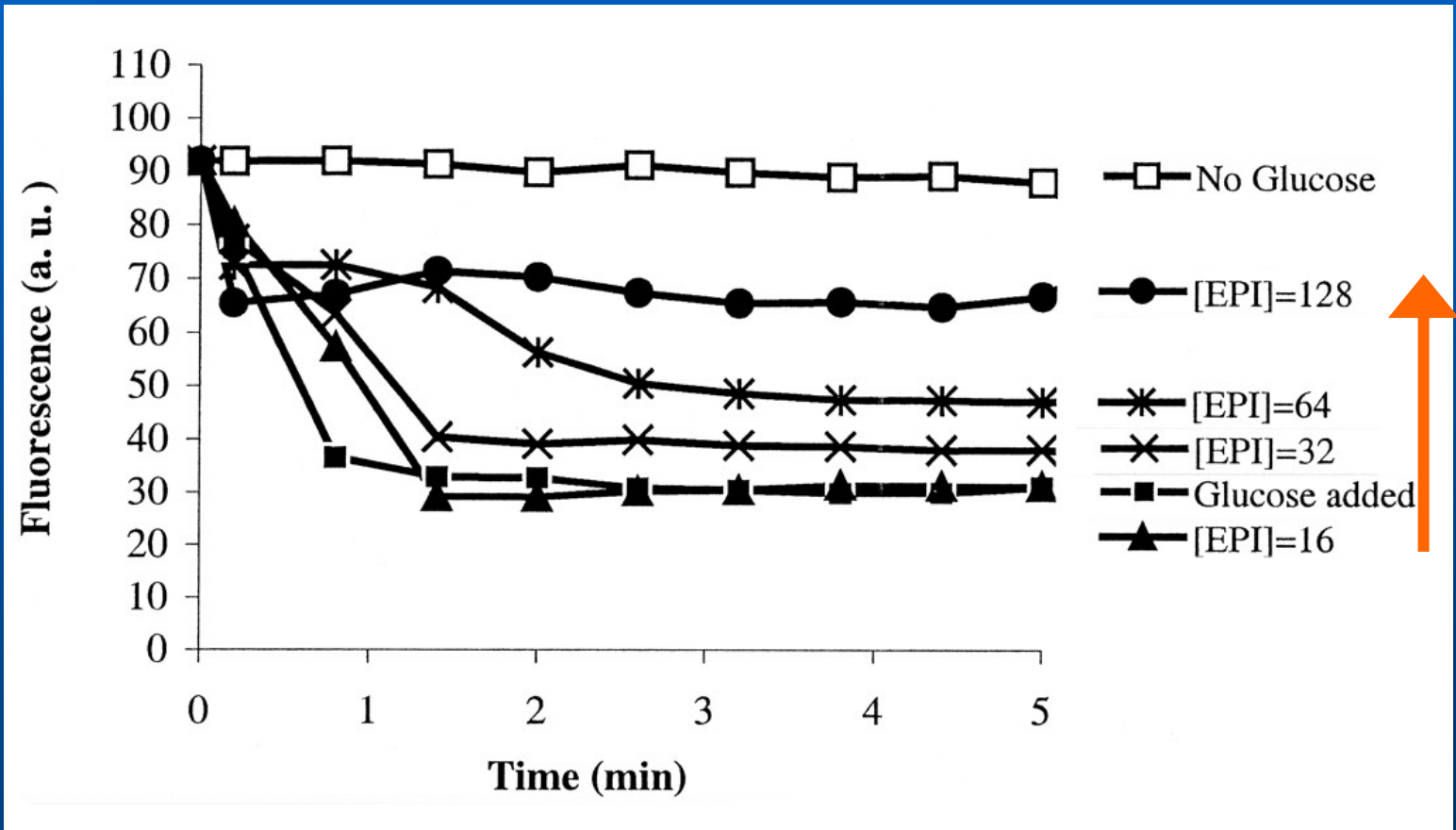
- ✓ **Not affect AB activity in strains lacking efflux pumps**
- ✓ **Not potentiate activity of AB that are not effluxed**
- ✓ **Not affect proton gradients across the inner membrane**

- ✓ **Not affect eucaryotic efflux pumps**

Lomovskaya *et al.* AAC (2001) 45:105-116

EPI as inhibitors of efflux pumps

Concentration-dependent inhibition of *N*-phenyl-1-naphtylamine efflux



Ocaktan *et al*. JBC (1997) 272: 21964-69; Lomovskaya *et al.* AAC (2001) 45:105-116

Characteristics of the ideal EPI

- ✓ Enhance activity of AB in efflux pumps overproducers by inhibiting efflux

- ✓ Not affect AB activity in strains lacking efflux pumps
- ✓ Not potentiate activity of AB that are not effluxed
- ✓ Not affect proton gradients across the inner membrane

- ✓ Not affect eucaryotic efflux pumps

Lomovskaya *et al.* AAC (2001) 45:105-116

EPI are active only on efflux pumps producers

	AB / AB + MC-207,110 MIC ratio	
antibiotic	**MexAB-OprM (+) strain**	**Δ MexAB-OprM**
levofloxacin	32	2
sparfloxacin	128	4
carbenicillin	512	4
erythromycin	32	4
chloramphenicol	128	2

Lomovskaya *et al.* AAC (2001) 45:105-116

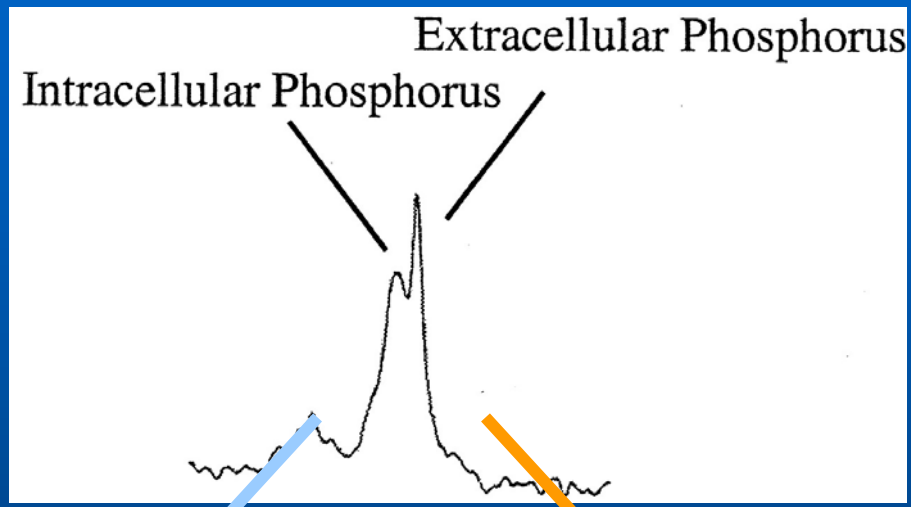
EPI enhance activity of efflux pump substrates only

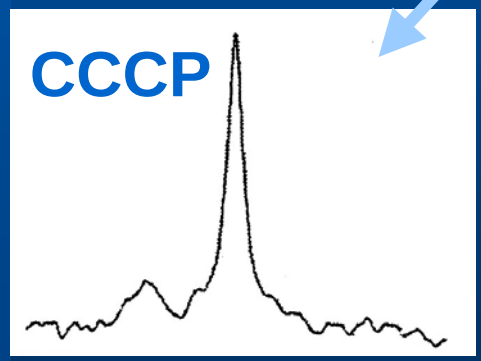
	AB / AB + MC-207,110 MIC ratio	
antibiotic	**MexAB-OprM (+) strain**	**Δ MexAB-OprM**
levofloxacin	32	2
sparfloxacin	128	4
carbenicillin	512	4
erythromycin	32	4
chloramphenicol	128	2
imipenem	1	1
gentamicin	1	1

Lomovskaya *et al.* AAC (2001) 45:105-116

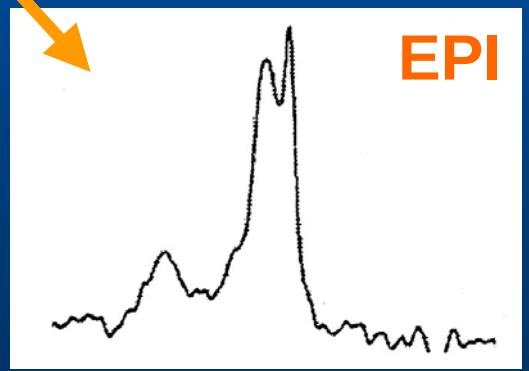
EPI does not affect proton gradients across the IM

NMR spectra of ^{31}P to detect pH gradients





carbonyl cyanide m-chlorophenylhydrazone

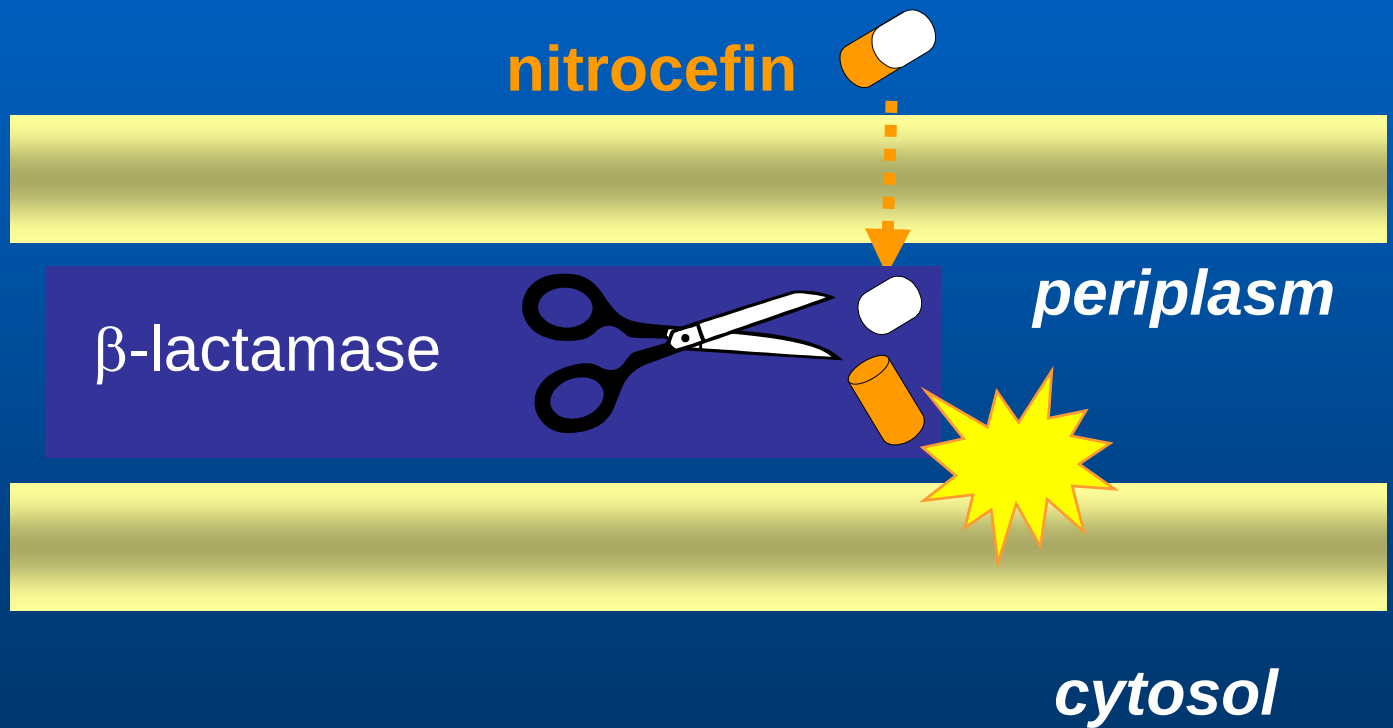


other mode of action ?

Lomovskaya *et al.* AAC (2001) 45:105-116

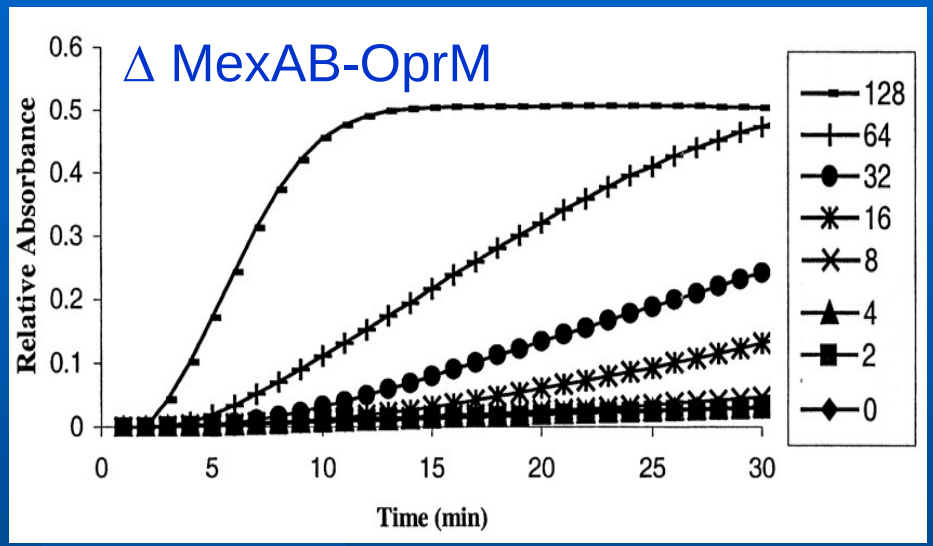
EPI as permeabilizing agents in strains lacking efflux pumps ?

Testing the hydrolysis rate of a non permeant β-lactam

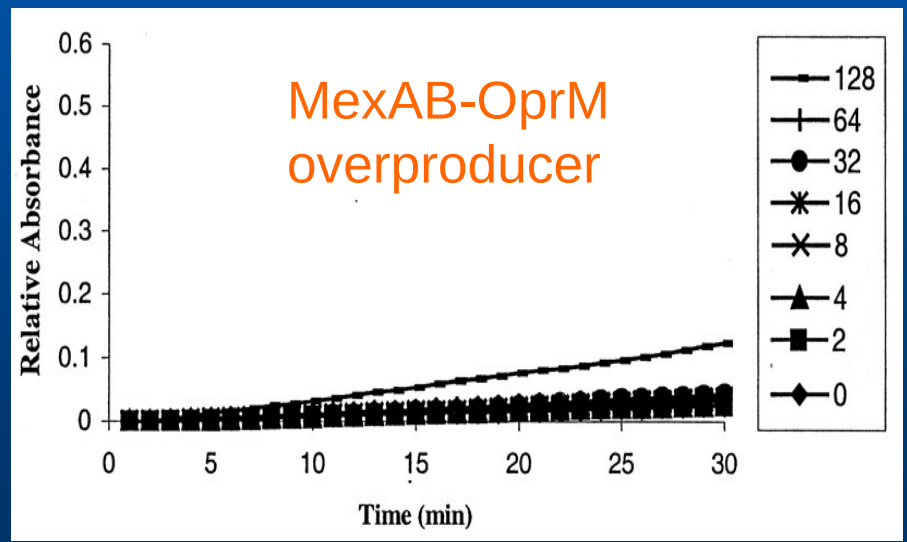


Lomovskaya *et al.* AAC (2001) 45:105-116

EPI as permeabilizing agents in strains lacking efflux pumps ?



EPI increase OM permeability when MexAB-OprM not functional



EPI as substrates of efflux pumps ?

Lomovskaya *et al.* AAC (2001) 45:105-116

EPI as substrates of efflux pumps ?

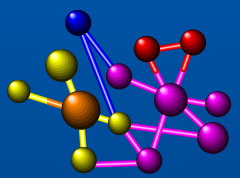
EPI intrinsic antibacterial activity appears only in strains lacking efflux pumps

strain	MIC of MC-207,110 (mg/L)
MexAB-OprM overexpressing strain	>512
Δ MexAB-OprM, Δ MexCD-OprJ, Δ MexEF-OprN	64

Lomovskaya *et al,* AAC (2001) 45:105-116

Inhibiting efflux as a strategy to improve antibiotic efficacy

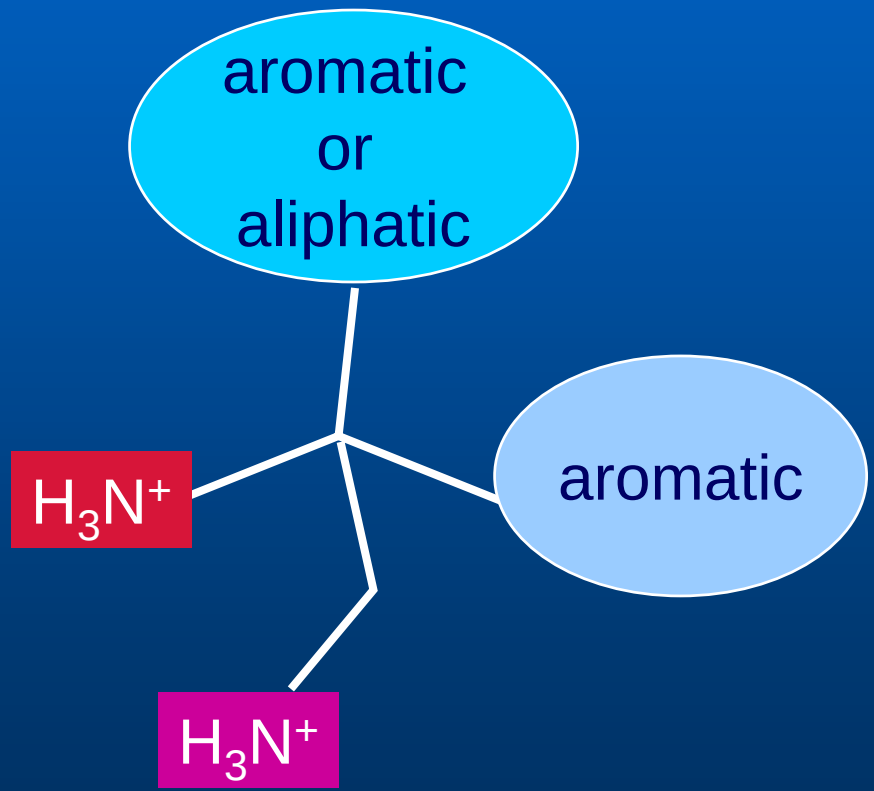
- Discovery of inhibitors of efflux pumps (EPI)
- In vitro activity
- Mode of action



- Structure-activity relationships
- Potential uses

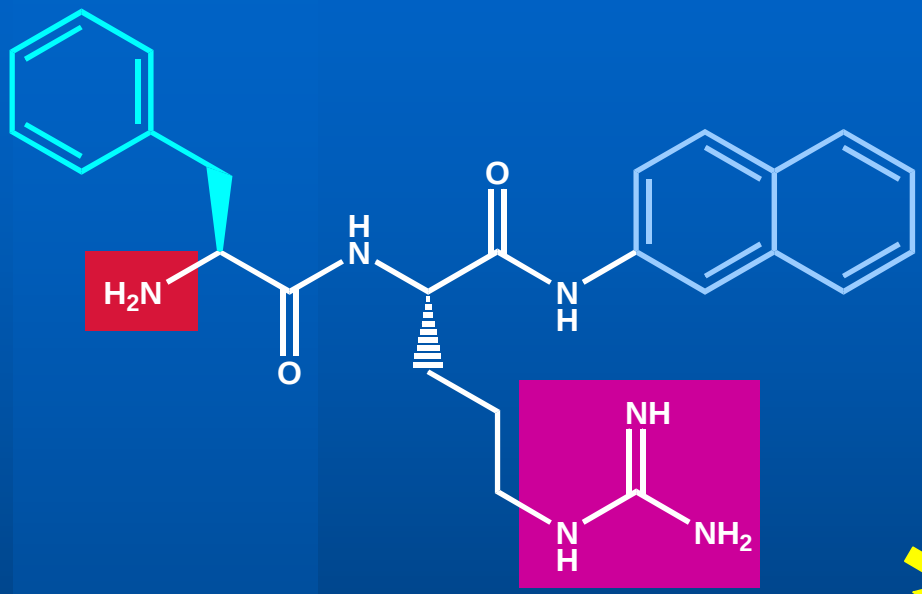
EPI pharmacophore

EPI are derivatives of dipeptides



Watkins *et al,* ICAAC (2001) 339

Improving stability in biological media: MC-207,110 ➔ MC-02,595



L-Phe-L-Arg-β-naphthylamide

D-Orn-D-hPhe-aminoquinoline

Renau *et al.* Bioorg. Med. Chem Lett. (2001) 11:663-7

Improving stability in biological media: MC-207,110 ➔ MC-02,595

- **switching amino-acids position keeps activity**
- **using D-series amino-acids confers stability**

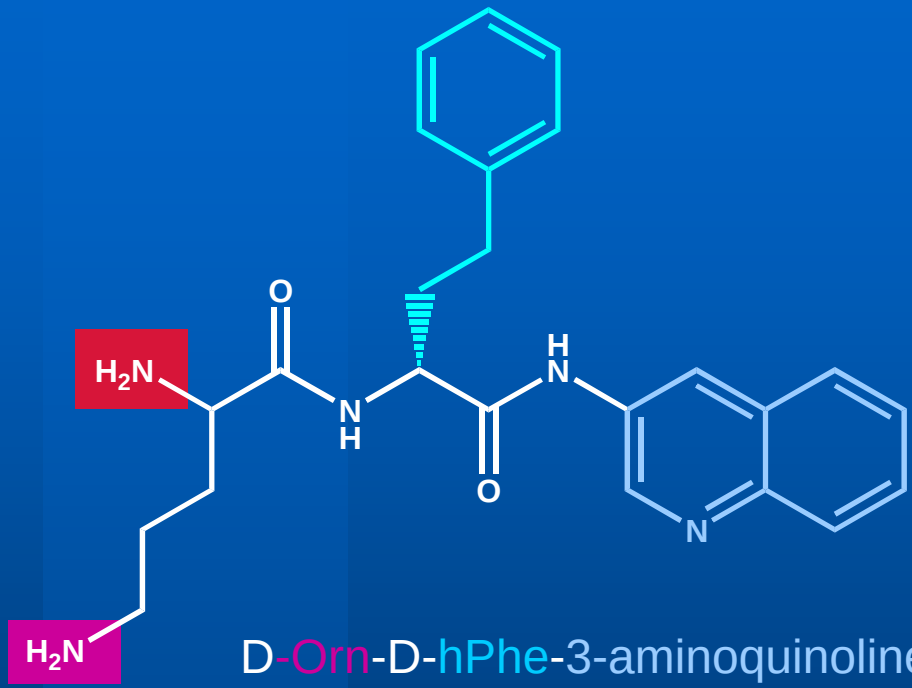
compound		MPC_8 * (mg/L)	$t_{1/2}$ in rat serum
L-Phe-**L**-Arg-β-naphthylamide	**MC-207,110**	10	5 min
L-hPhe-**L**-Orn- β-naphthylamide		5	< 10 min
L-Orn-**L**-hPhe-aminoquinoline		20	ND
D-Orn-**D**-hPhe-aminoquinoline	**MC-02,595**	10	**> 24 H**

* EPI conc. reducing LVX MIC 8-fold

Renau *et al.* Bioorg. Med. Chem Lett. (2001) 11:663-7

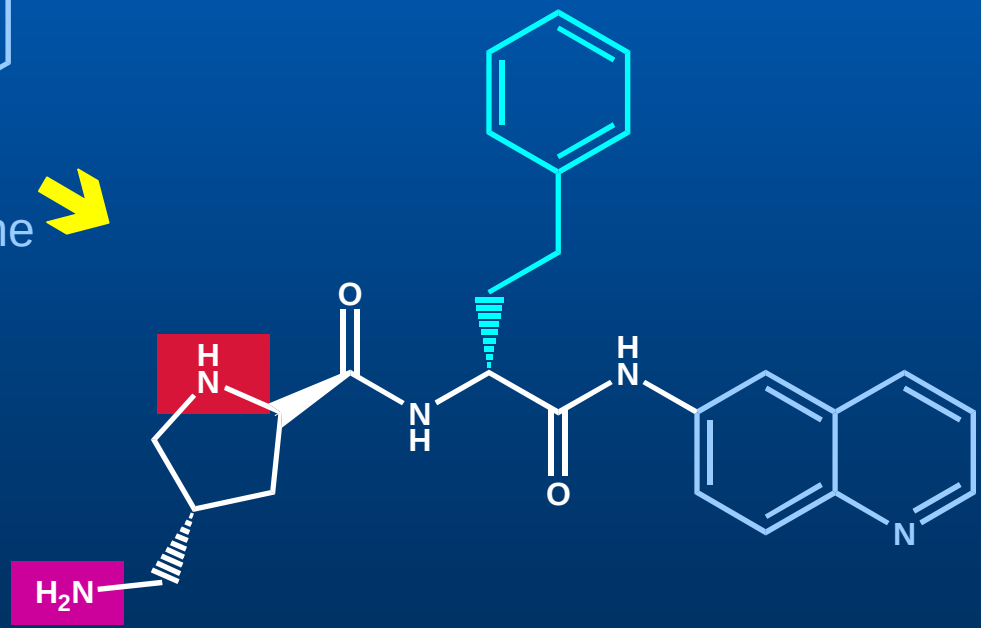
Improving safety profile:

MC- 02,595 ➔ MC-04,124



D-Orn-D-hPhe-3-aminoquinoline

4-aminomethyl-
2-pyrrolidinecarboxamide (Pro)
-D-hPhe-6-aminoquinoline



Renau *et al.* Bioorg. Med. Chem Lett. (2003) 13:2755-58

Improving safety profile:

MC- 02,259 ➔ MC-04,124

- **aminated side chain causes toxicity**
- **conformationally restricted analogues keep activity**

compound		MPC_8 * (mg/L)	MLD # in rat serum
L-Phe-**L**-Arg-β-naphthylamide	**MC-207,110**	10	< 25
D-Orn-**D**-hPhe-aminoquinoline	**MC-02,595**	5	< 25
D-Ala-**D**-hPhe-aminoquinoline		20	125
MC-04,124		10	**> 100**

* EPI conc. reducing LVX MIC 8-fold

\# dose causing > 66% letality

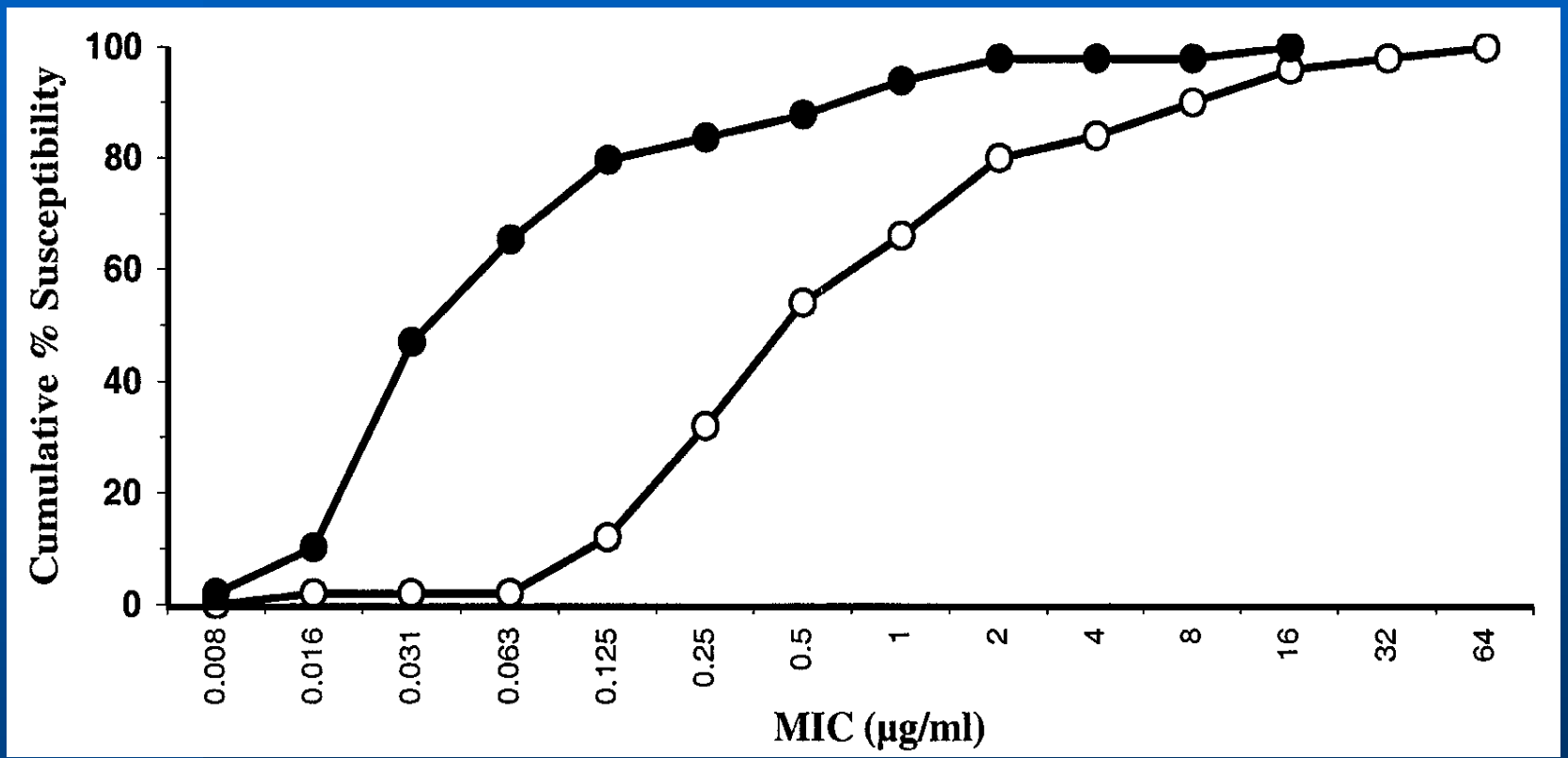
Renau *et al.* Bioorg. Med. Chem Lett. (2003) 13:2755-58

Inhibiting efflux as a strategy to improve antibiotic efficacy

- Discovery of inhibitors of efflux pumps (EPI)
- In vitro activity
- Mode of action
- Structure-activity relationships
- Potential uses

EPI increases susceptibility of clinical isolates

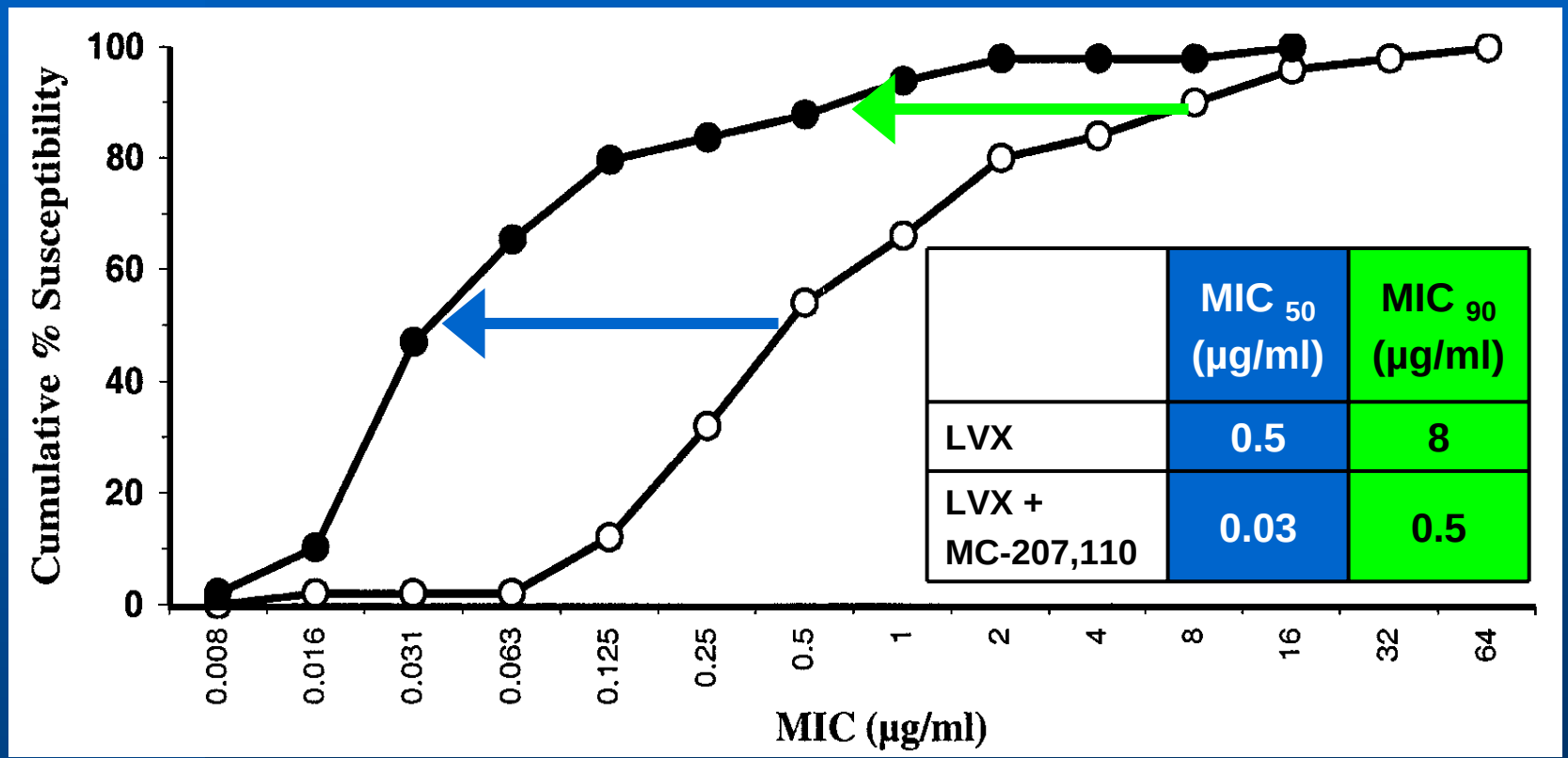
MIC distribution for levofloxacin in clinical isolates of *P. aeruginosa*



Lomovskaya *et al*. JMMB (2001) 3: 225-36

EPI increases susceptibility of clinical isolates

MIC distribution for levofloxacin in clinical isolates of *P. aeruginosa*



	MIC $_{50}$ (µg/ml)	MIC $_{90}$ (µg/ml)
LVX	0.5	8
LVX + MC-207,110	0.03	0.5

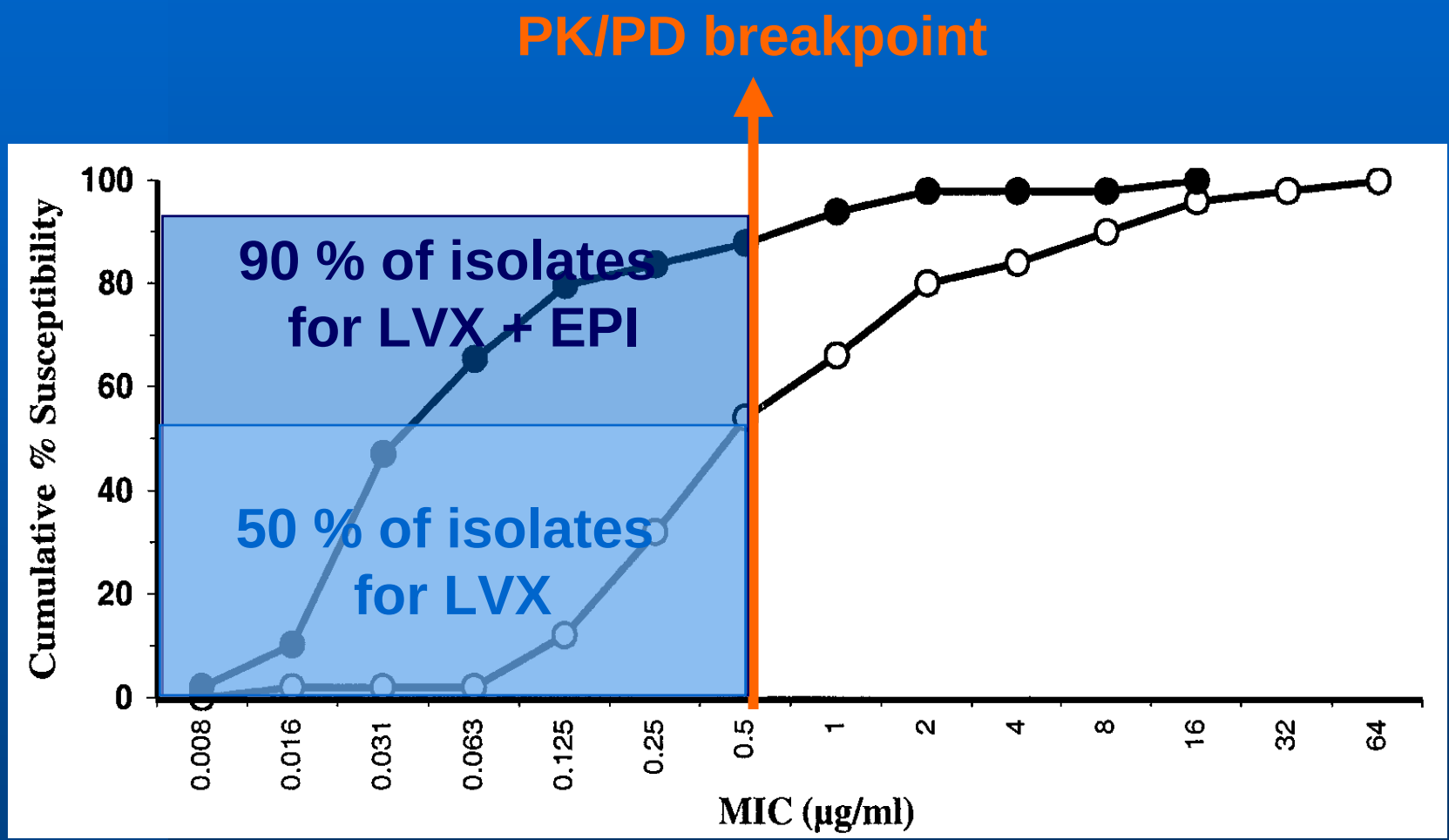
Lomovskaya *et al*. JMMB (2001) 3: 225-36

PK-PD breakpoints for levofloxacin

dose/24 h (mg)	AUC * (mg/L x h)	PK/PD bkpt (AUC/MIC= 125)
500	47	0.4
750	71	0.6
1000	94	0.8

* US prescrib. inf. (adult of 60 kg) of LEVAQUIN®

EPI helps reaching PK/PD criteria of effectiveness



EPI as adjuvant therapy

EPI (MC-04,124) potentiates levofloxacin activity in *P. aeruginosa* mouse thigh model

regimen	LVX MIC (mg/L)	effective regrowth time (h)	max Δ log CFU
LVX (30 mg/kg)	2	3	0.1
LVX (30 mg/kg) + MC-04,124 (25 mg/kg)	0.125	13	3.6

Griffith *et al*. ICAAC (2001) F-340

EPI as screening tool for resistance by efflux

efflux pump	β-lac	ML	TET	AG	FQ	Chl
MexAB-OprM	carbenicillin					
MexCD-OprJ		erythromycin				
MexEF-OprN	imipenem					
MexXY-OprM				gentamicin		

constitutive expression; inducible expression

Mesaros *et al*, ECCMID (2005) and ongoing work

EPI as screening tool for resistance by efflux

genotype	MIC (mg/L)			
	carbenicillin EPI(-)/EPI(+)	erythromycin EPI(-)/EPI(+)	imipenem EPI(-)/EPI(+)	gentamicin EPI(-)/EPI(+)
wild-type	1	16	1	1
MexAB-OprM	4	16	1	1
MexCD-OprJ	1	64	1	1
MexEF-OprN	1	8	4	1
MexXY-OprM	2	16	1	8

Strains received from P. Plésiat, Besançon

Mesaros *et al,* ECCMID (2005) and ongoing work

Perspectives for future research

- **Demonstration of the mode of action**
  - Efflux of EPI
  - Competition for transport with known substrates
- **Study of the interaction between EPI and efflux pumps**
  - 3D-models and docking
  - Comparison of binding site of substrates and inhibitors
- **Development of specific inhibitors as diagnostic tools**
  - MexAB-OprM
    - Nakayama *et al* (2003) Biorg. Med. Chem. Lett 13: 4205-08

Perspectives for future research

- **Definition of potential clinical interest**
  - Exploration of activity spectrum
  - Animal models of infections by resistant pathogens
  - Further study of pharmacokinetic and pharmacodynamic properties
  - Toxicological evaluation