40th EUROPEAN CYSTIC FIBROSIS CONFERENCE
7 – 10 JUNE 2017 I SEVILLE, SPAIN

NEXT GENERATION SUSCEPTIBILITY TESTING: WHAT WORKS?

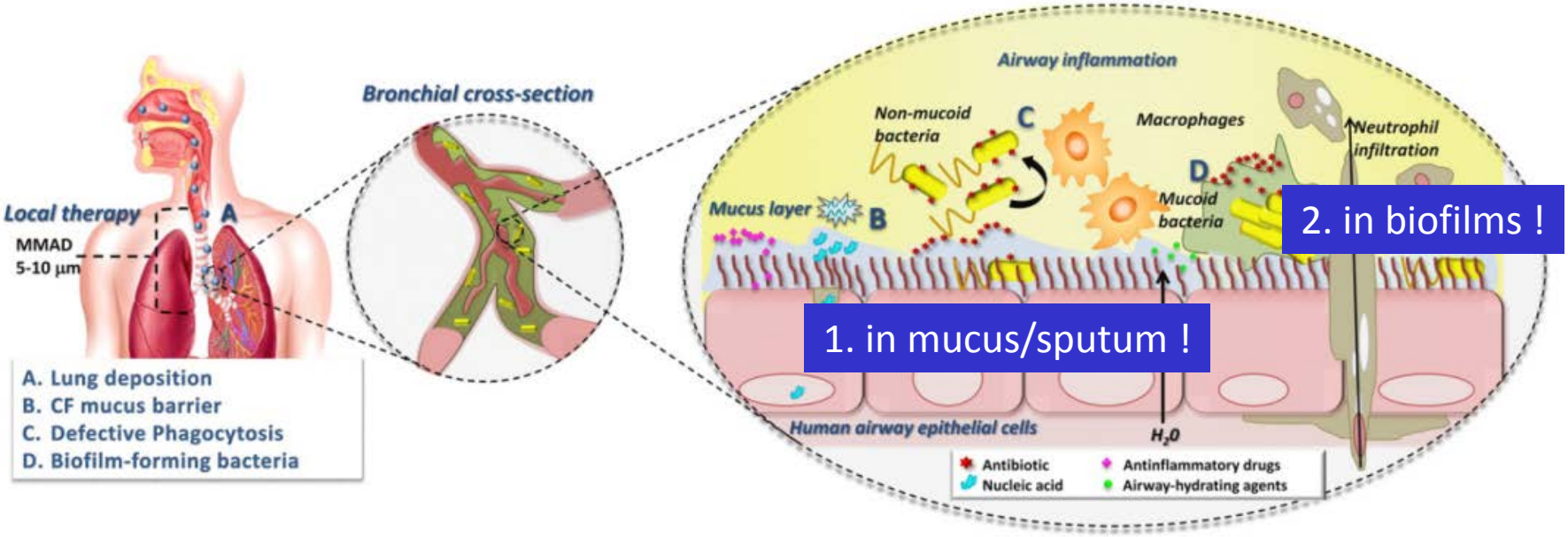
Improving antibiotic targeting *in vivo*

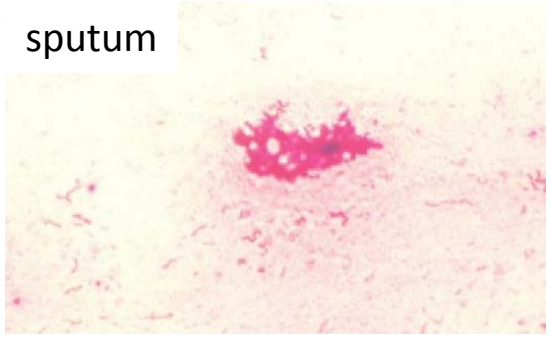
Françoise Van Bambeke, PharmD, PhD

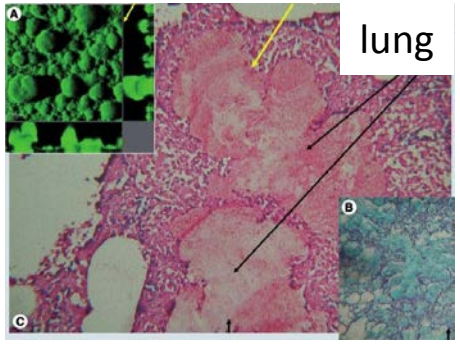
Pharmacologie cellulaire et moléculaire
Louvain Drug Research Institute
Université catholique de Louvain,
Brussels, Belgium

<www.facm.ucl.ac.be>

Where are bacteria (in CF) ? (1/2)



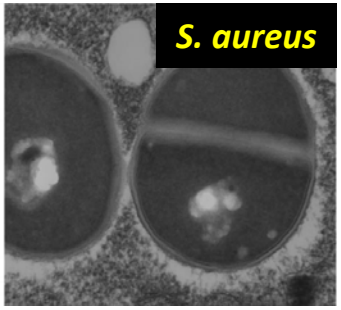


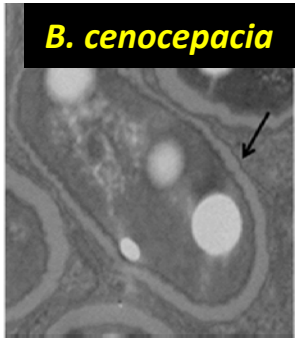


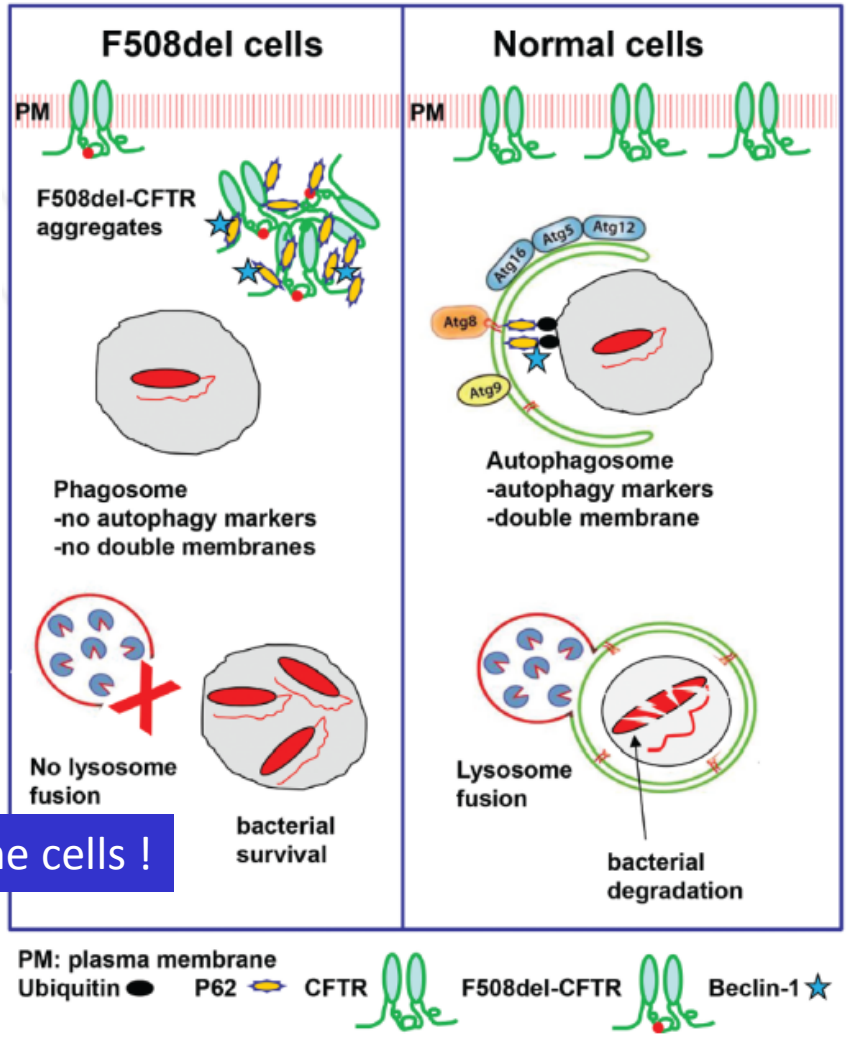
Hoiby et al, Future Microbiol. 2010; 5:1663-74

D'Angelo et al, Advanced Drug Delivery Reviews 2014; 75:92–111

Where are bacteria (in CF) ? (2/2)





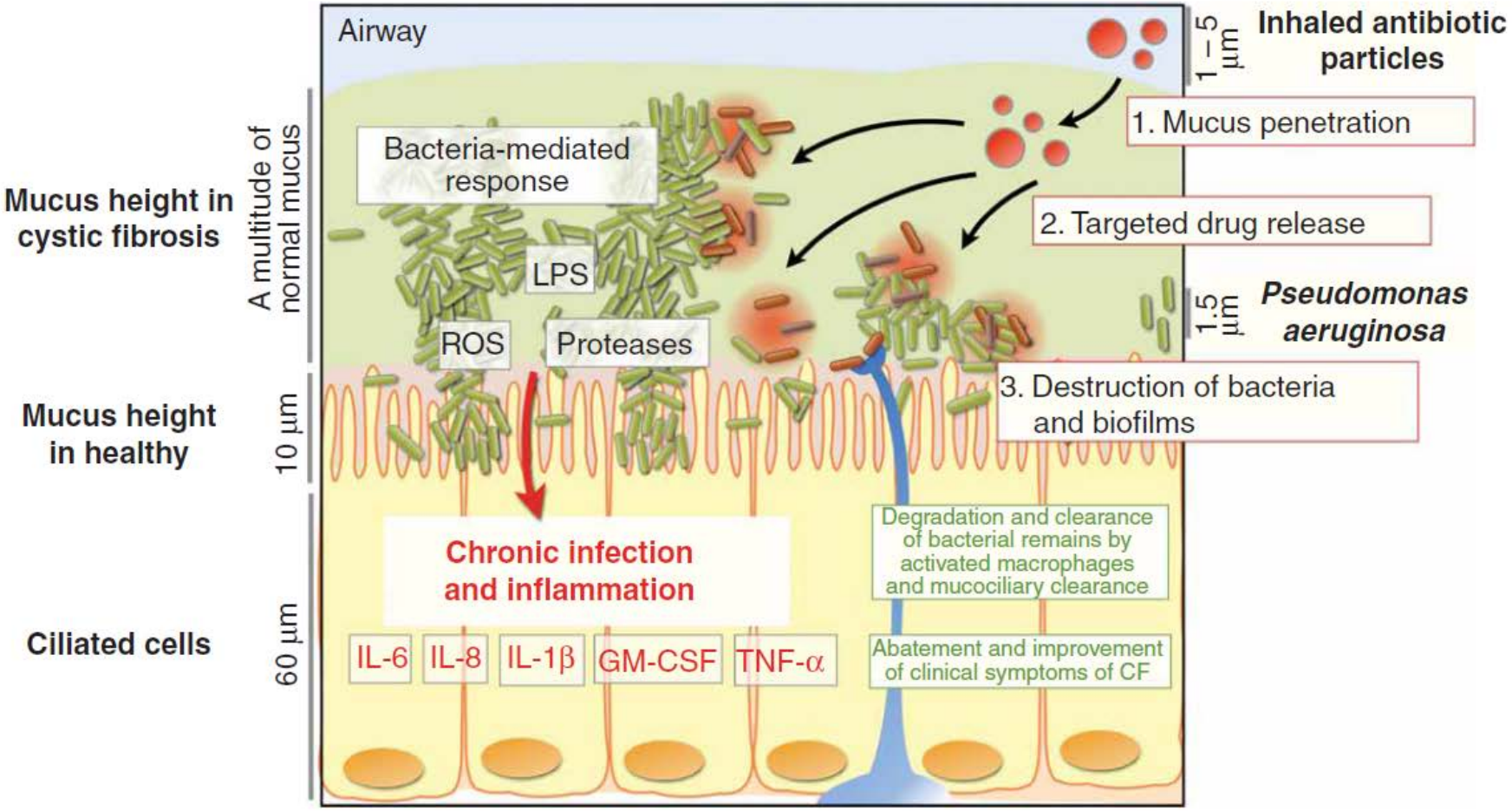


3. inside the cells !

Goldberg & Pier, Trends Microbiol. 2000; 8:514-20
Jarry & Cheung, Infect Immun. 2006;74:2568-77
Assani et al, PLoSone 2014; 9: e96681.

Cormet Boyaka et al, 2016; http://dx.doi.org/10.5772/64686

How to improve targeting of these bacteria ?

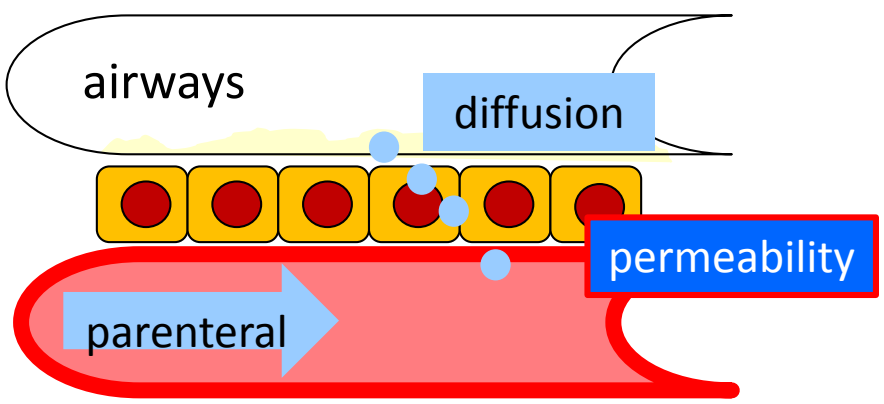


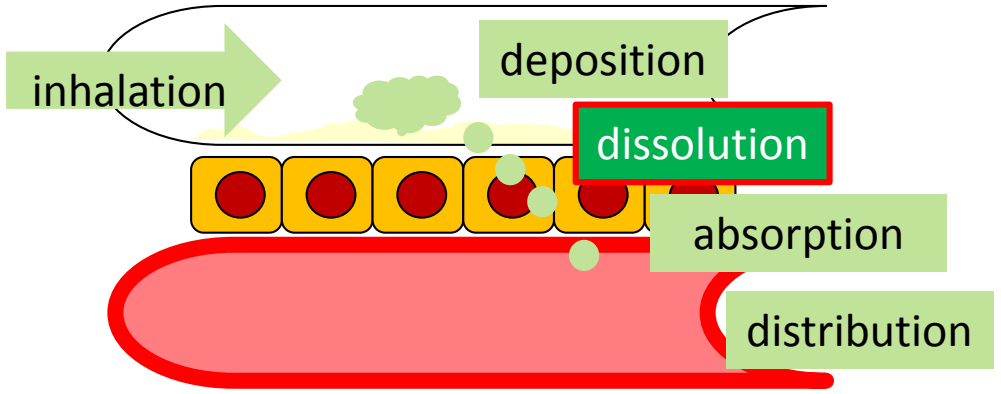
Klinger-Strobel et al, Expert Opin Drug Deliv. 2015; 12:1351-74

How to improve targeting of these bacteria ?

1. Mucus penetration

Administration by inhalation: for which drugs ?





Biopharmaceutics Classification System

fluoroquinolones

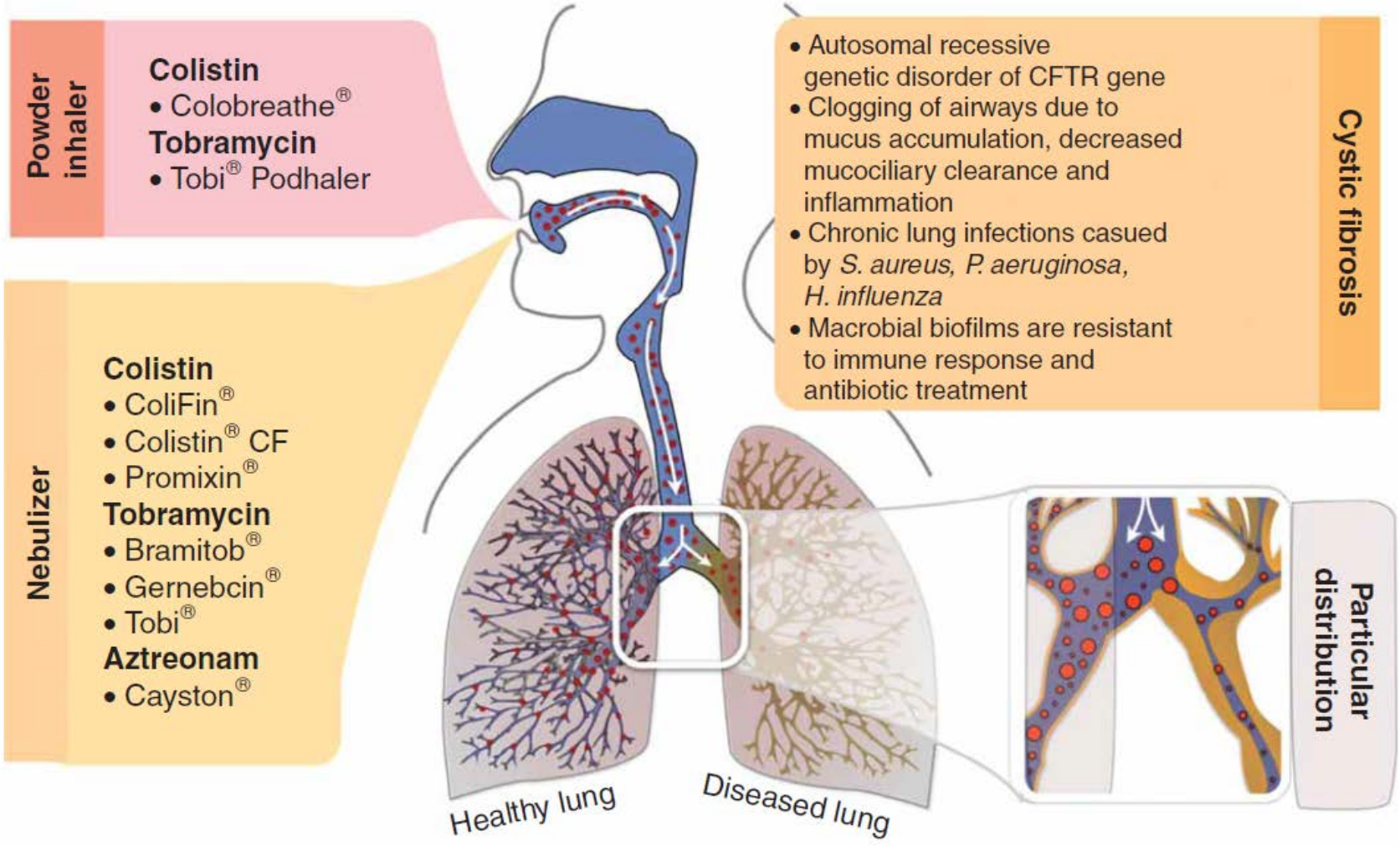
Class I	Class II
high solubility high permeability	low solubility high permeability

pulmonary conc.
inhalation >> IV/PO

aminoglycosides
polymyxins
β-lactams

Class III	Class IV
high solubility low permeability	low solubility low permeability

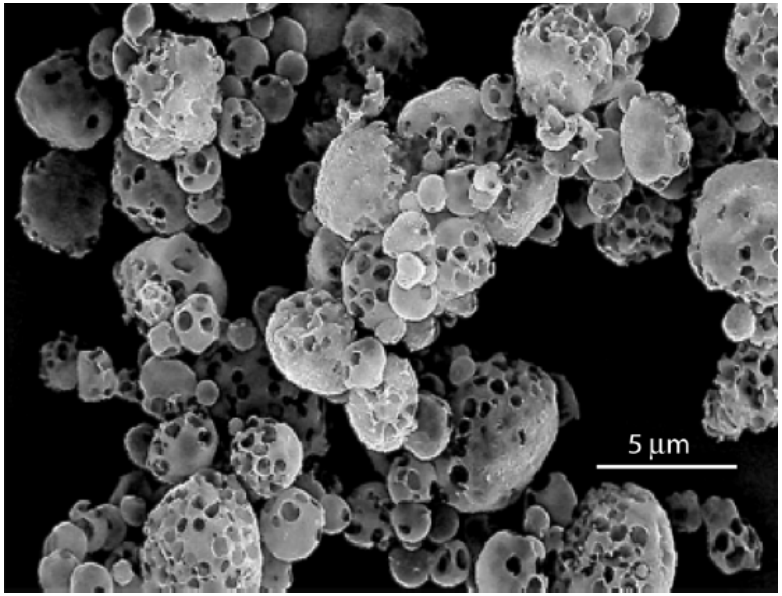
Antibiotics by inhalation available today



Klinger-Strobel et al, Expert Opin Drug Deliv. 2015; 12:1351-74

Antibiotics by inhalation: do they reach their target ?

Tobramycin powder for inhalation



Parameter	Sputum values		Serum values	
	TIP 112 mg	TIS 300 mg	TIP 112 mg	TIS 300 mg
C_{max} (µg/mL)	1,048	737	1.02	1.04
AUC_{∞} (µg · h/mL)	1,740	1,302	5.1	5.3
AUC_{12} (µg · h/mL)	1,307	974	4.6	4.8
T_{max} (h)	0.5	0.5	1.0	1.0
$t_{½}$ (h)	2.2	1.7	3.1	3.0

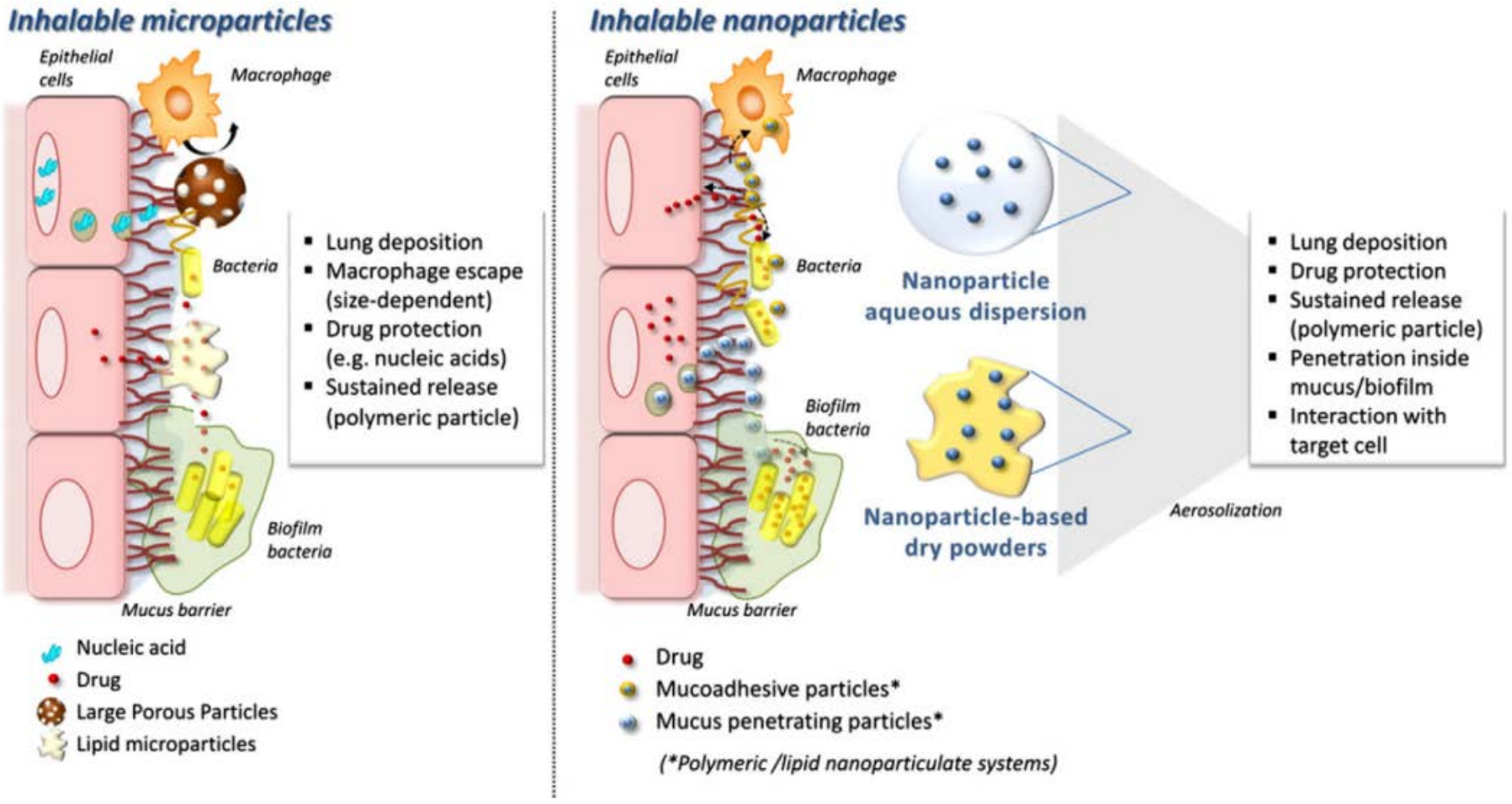
TIP was administered via the T-326 Inhaler and TIS was administered via PARI LC® PLUS nebulizer

Globally similar PK but easier use for TIP (powder) than for TIS (solution)

Miller et al, Mol. Pharmaceutics 2015; 12:2582–93

Geller et al, Pediatr Pulmonol. 2007;42:307–13.

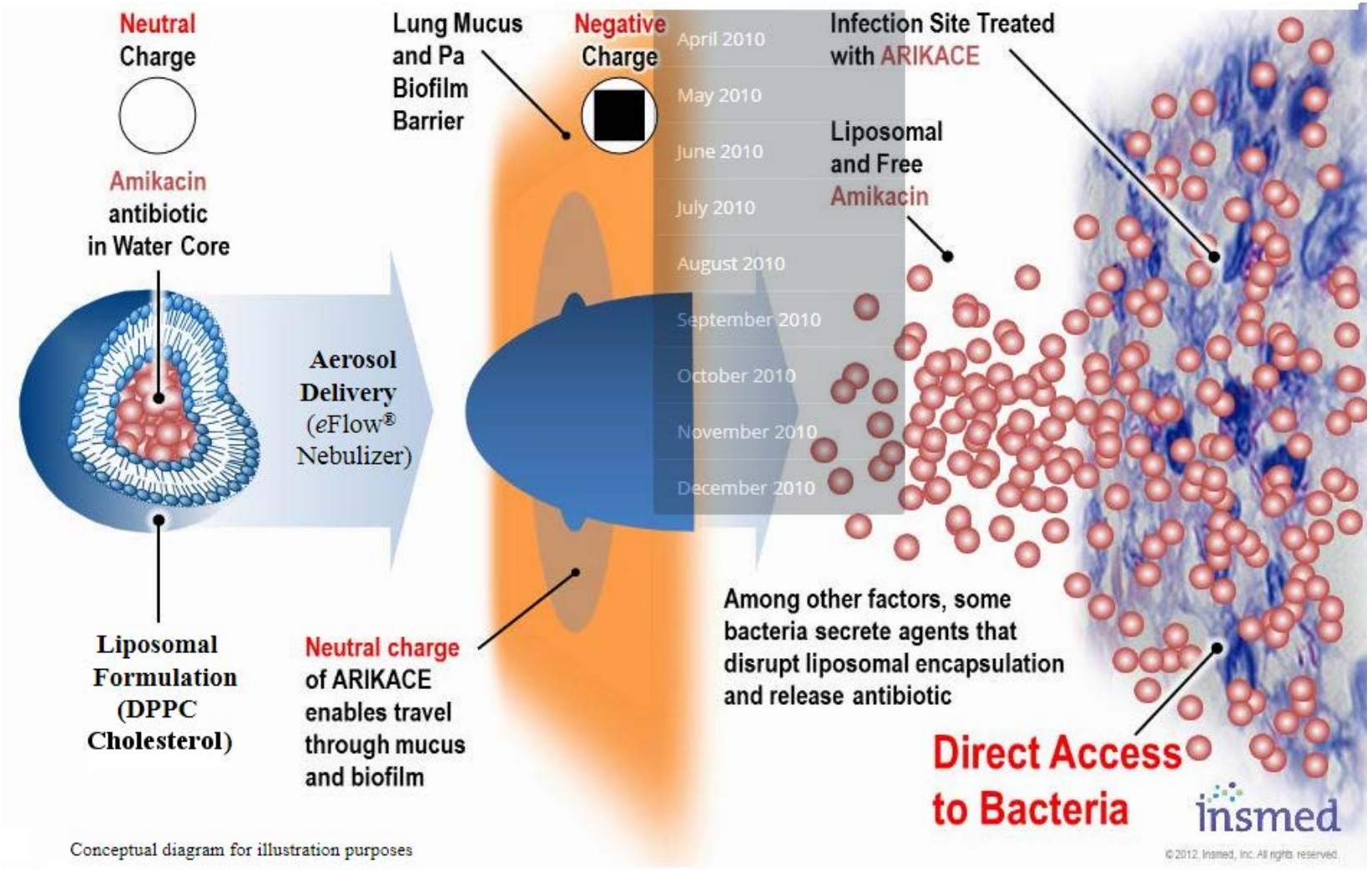
Delivery systems to increase drug concentrations



D'Angelo et al, Advanced Drug Delivery Reviews 2014; 75:92–111

Antibiotics by inhalation: can we improve local concentrations ?

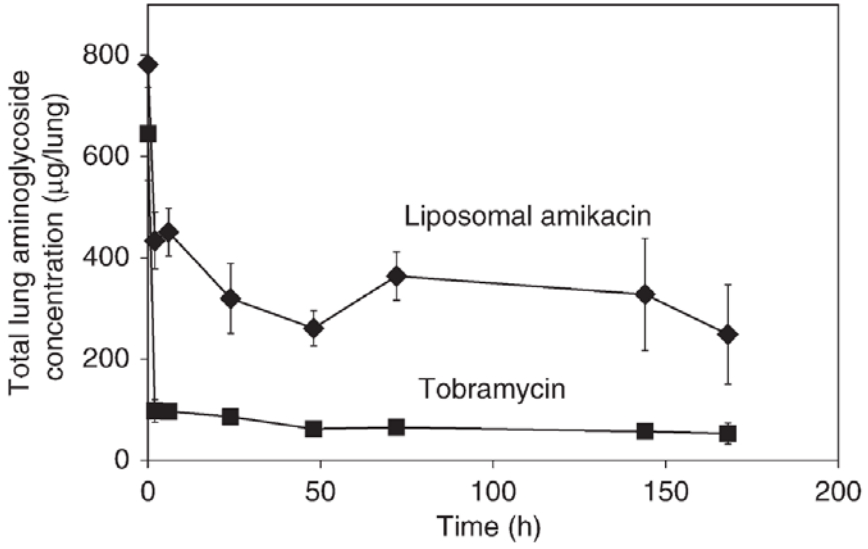
Liposomal formulations



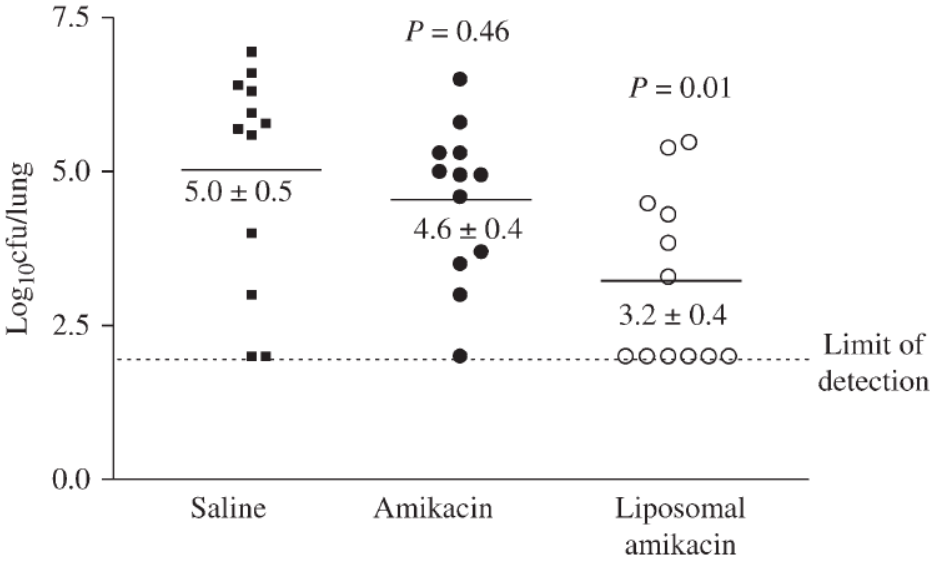
Antibiotics by inhalation: can we improve local concentrations ?

Liposomal formulations

PK in rats at equivalent doses



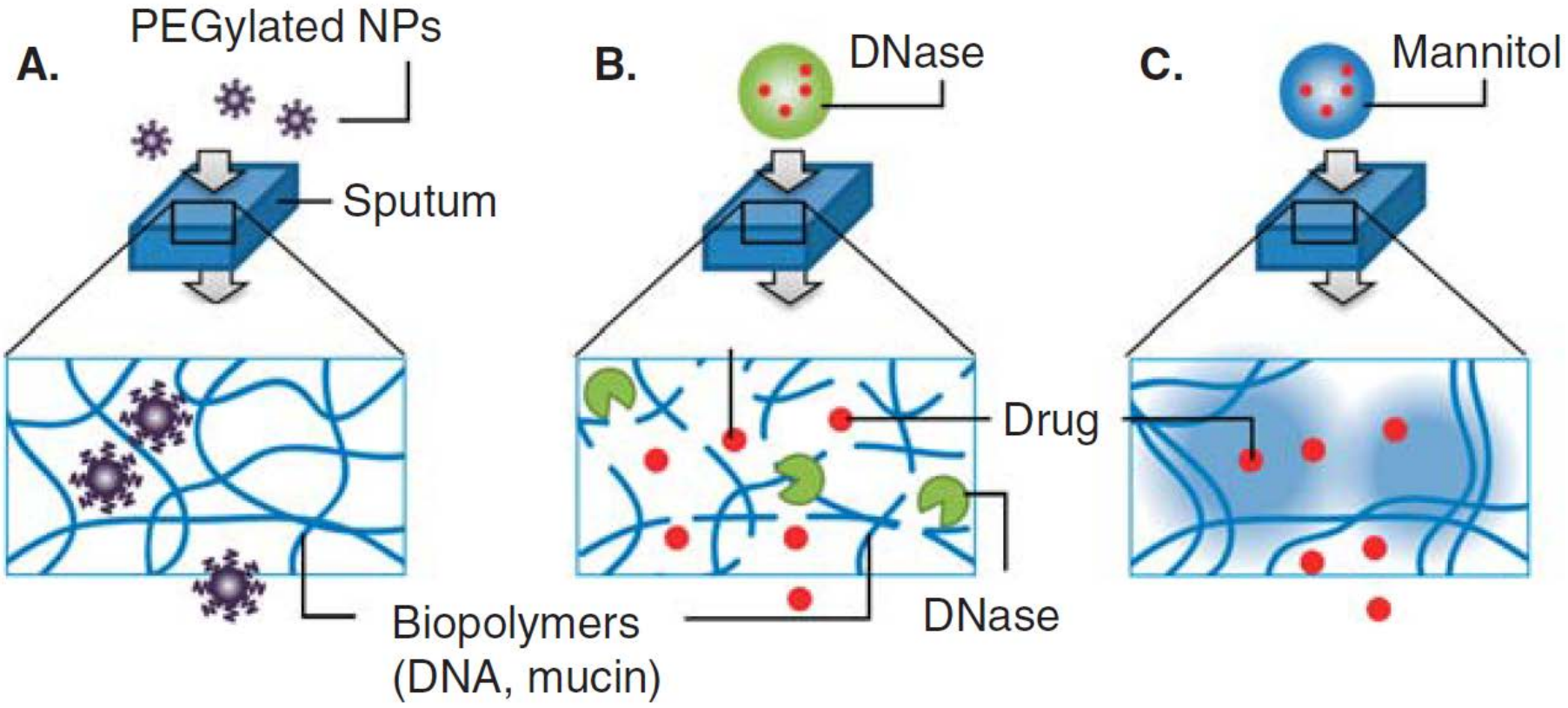
efficacy in rats at equivalent doses



Higher concentration in lung and better efficacy for liposomal formulation

Meers et al, JAC 2008; 61, 859–868

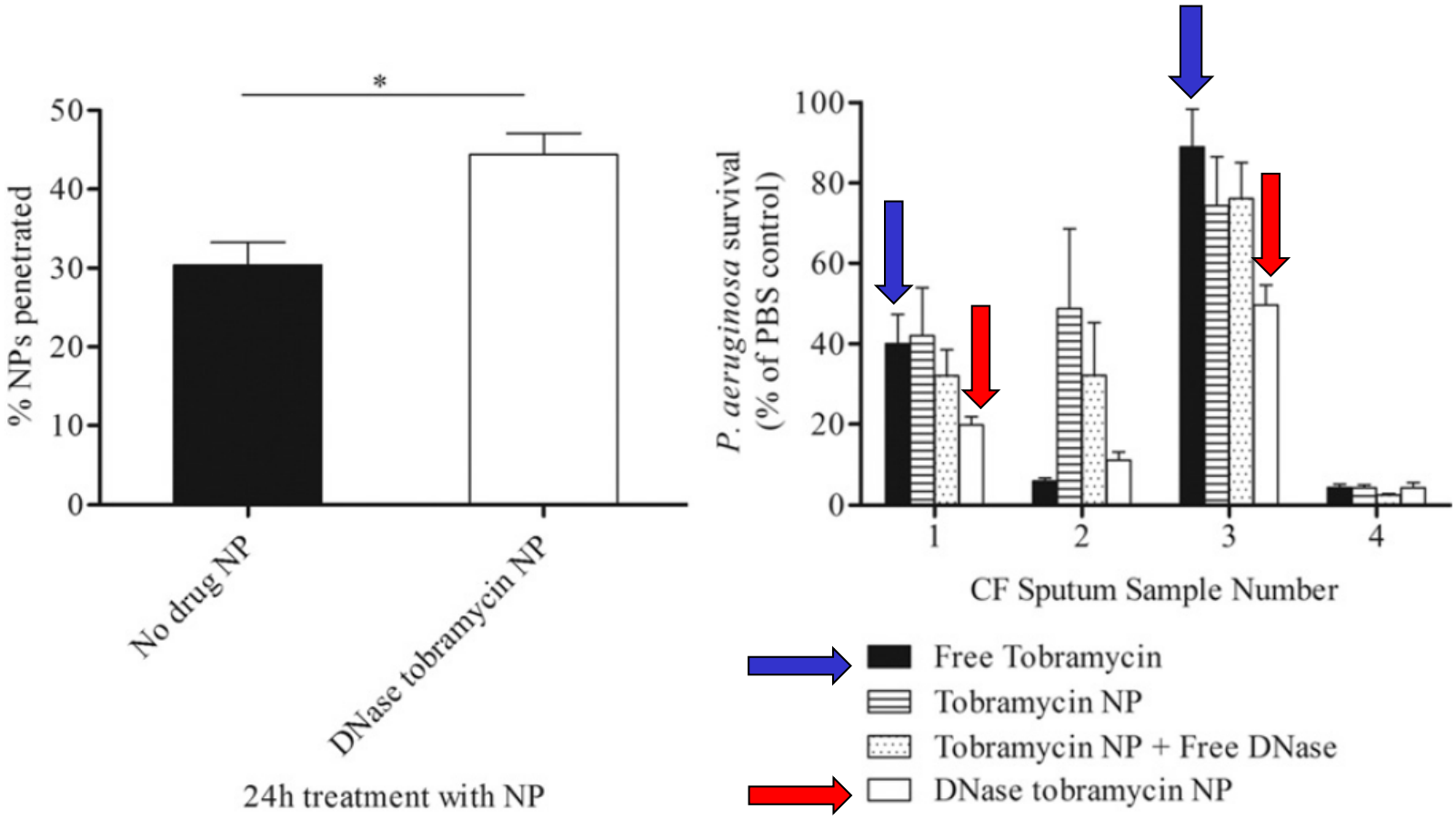
Approaches to enhance transport through CF sputum



Ibrahim et al, Expert Opin. Drug Deliv. 2011; 8:451-66

Approaches to enhance transport through CF sputum

Nanoparticles (AB + DNase)



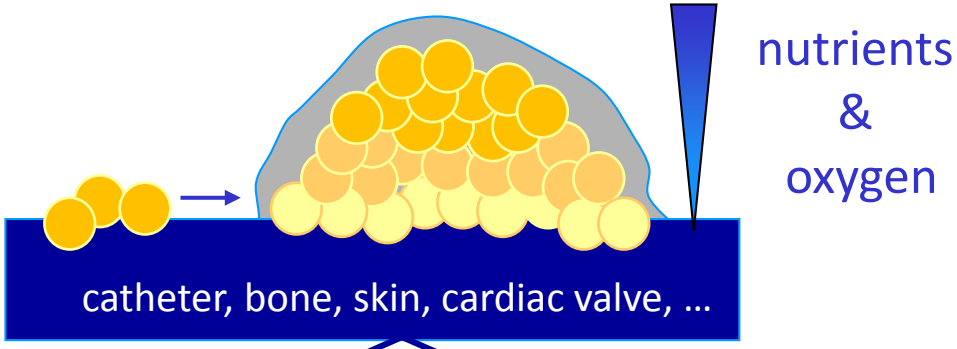
Higher penetration in mucus
and better efficacy against strains non responding to free TOB

Deacon et al, J Control Release 2015; 198 :55–61

How to improve targeting of these bacteria ?

2. Biofilm penetration

Antibiotic PK/PD parameters in biofilms



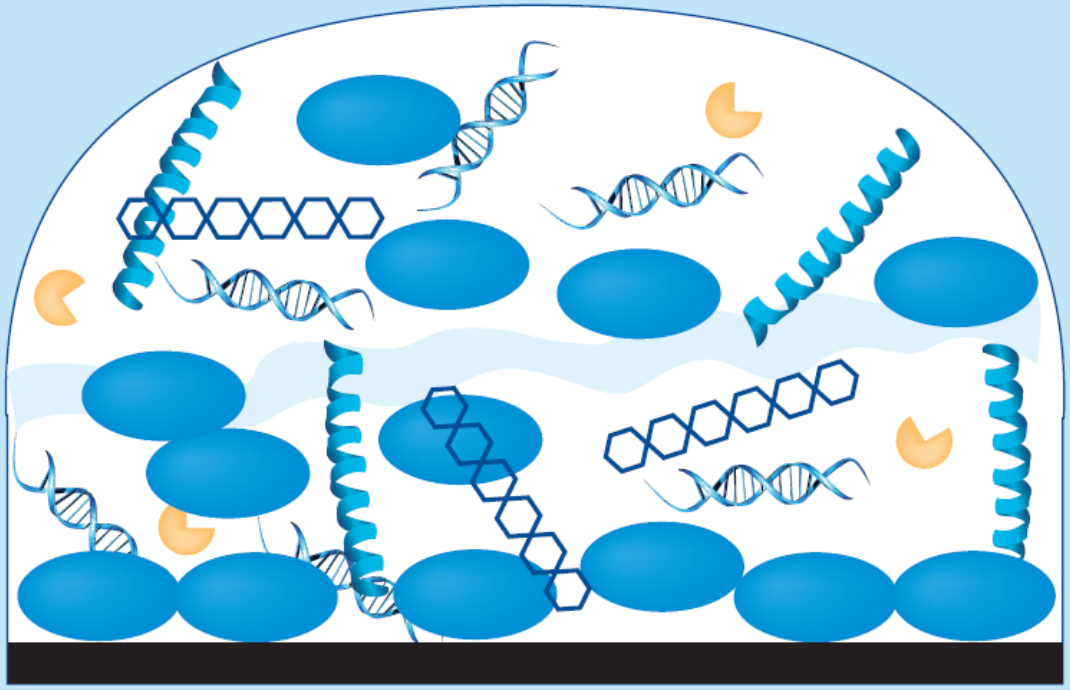
pharmacokinetics

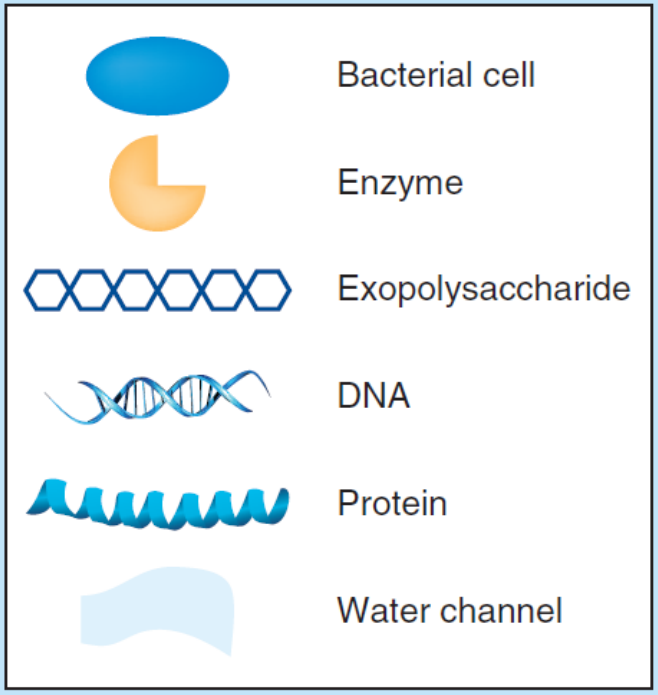
- diffusibility through the matrix
- bioavailability within the biofilm
- access to bacteria
- efflux out of bacteria

pharmacodynamics

- bacterial responsiveness (metabolic activity of bacteria)
- antibiotic expression of activity (local environment [O_2, pH, ..])

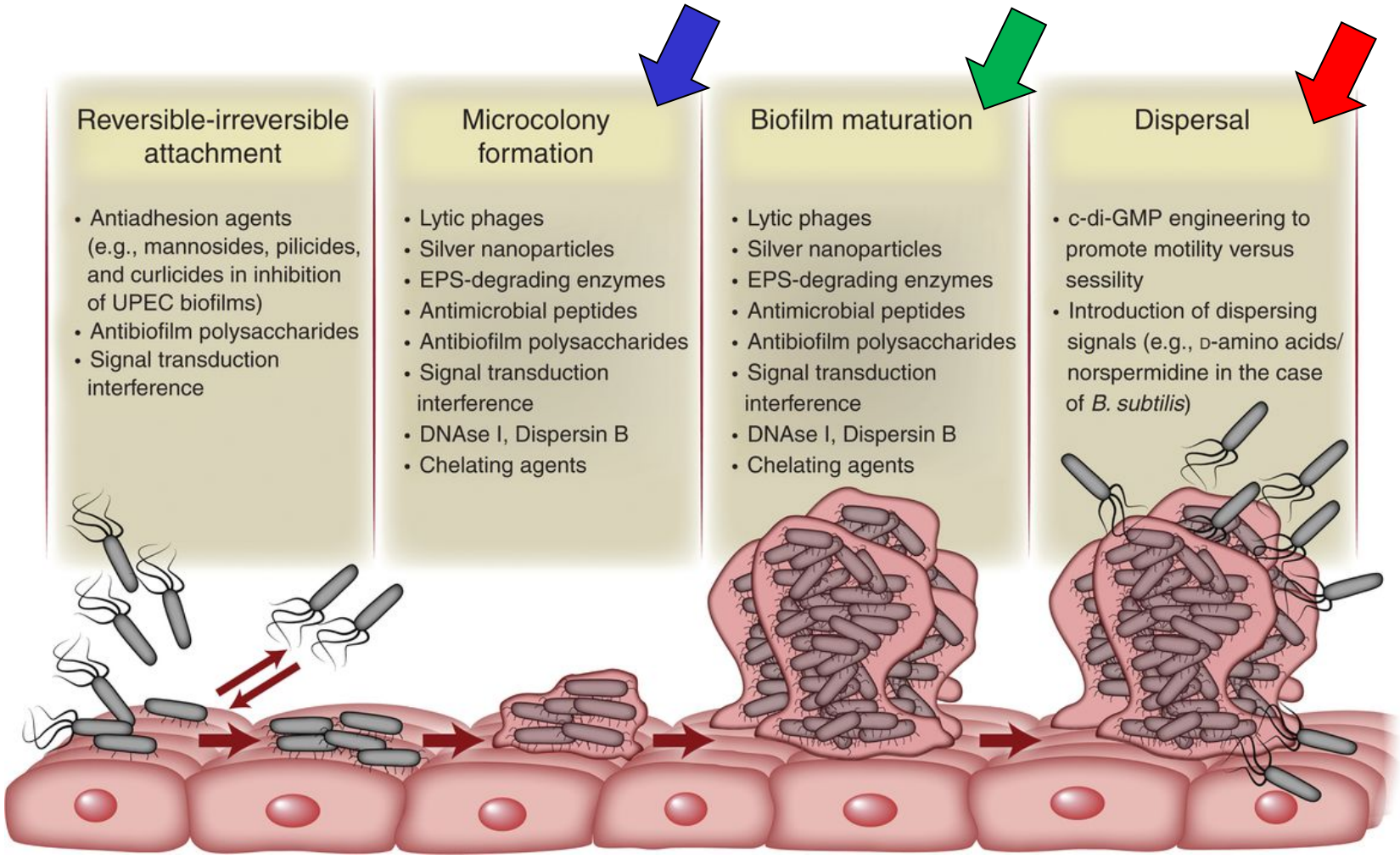
Biofilm matrix: what is it made of ?





Rabin et al., Future Med. Chem. 2015; 7:493–512

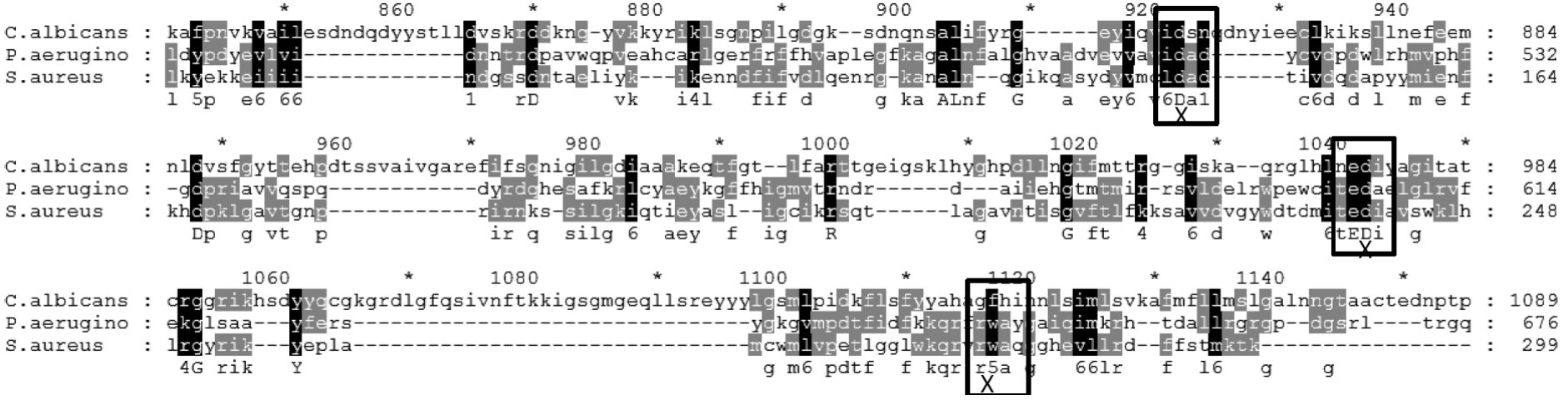
Antibiofilm strategies ~ antibiotic penetration



Kostakioti et al. Cold Spring Harb Perspect Med 2013;3:a010306

Strategies to increase antibiotic penetration in biofilms

Inhibiting matrix biosynthesis



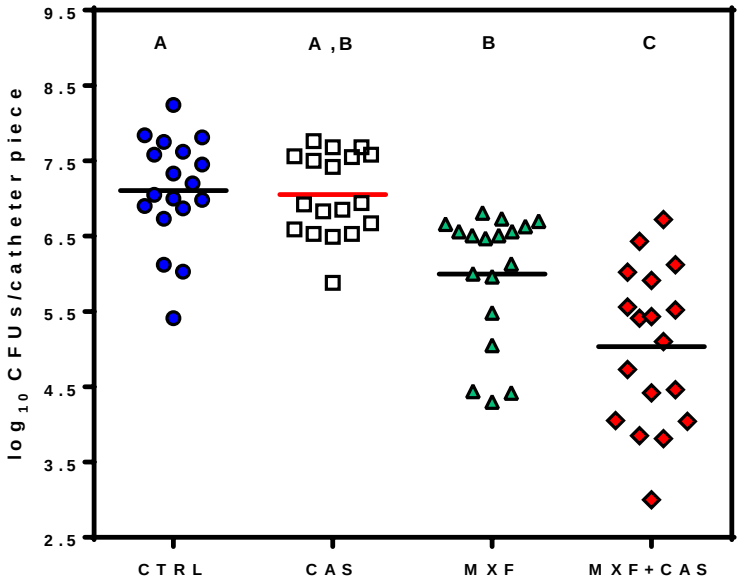
Enzymes involved in the synthesis of N-acetylglycosamine polymers of biofilm matrix in *S. aureus* and *P. aeruginosa* share homology with 1,3-β-D-glucan synthase [Target for echinocandins in fungi]

Siala et al., Nat. Commun. 2016; 7:13286; Kissoyan et al, Biofouling 2016; 32:779–86.

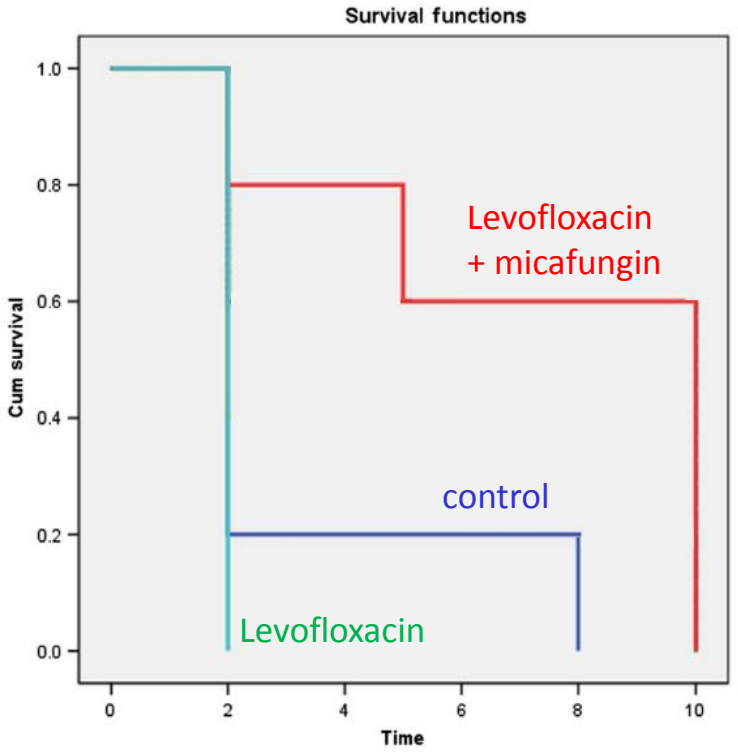
Strategies to increase antibiotic penetration in biofilms

Inhibiting matrix biosynthesis

S. aureus
implanted catheter infection



P. aeruginosa
intraperitoneal infection



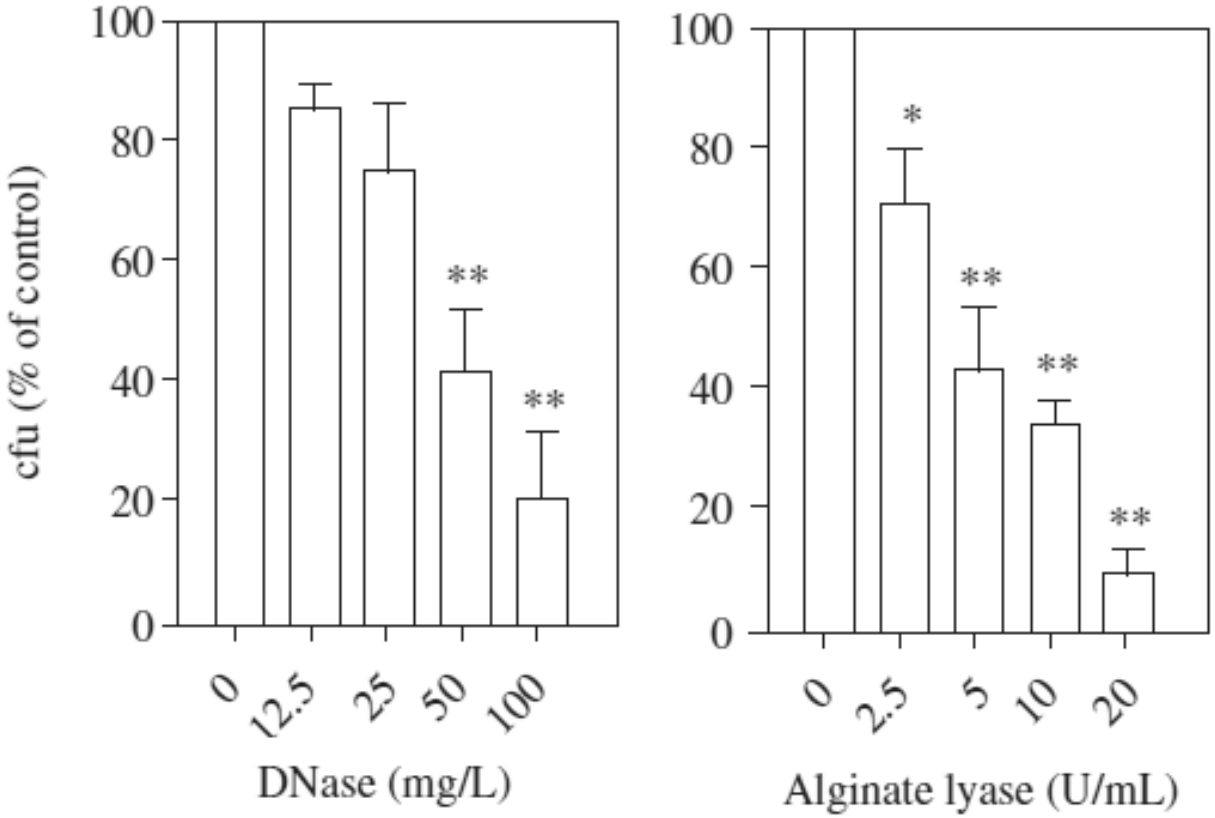
Echinocandins are synergistic with fluoroquinolones in vivo

Siala et al., Nat. Commun. 2016; 7:13286; Kissoyan et al, Biofouling 2016; 32:779–86.

Strategies to increase antibiotic penetration in biofilms

Degrading preformed matrix by enzymes

P. aeruginosa and tobramycin (512 mg/L)



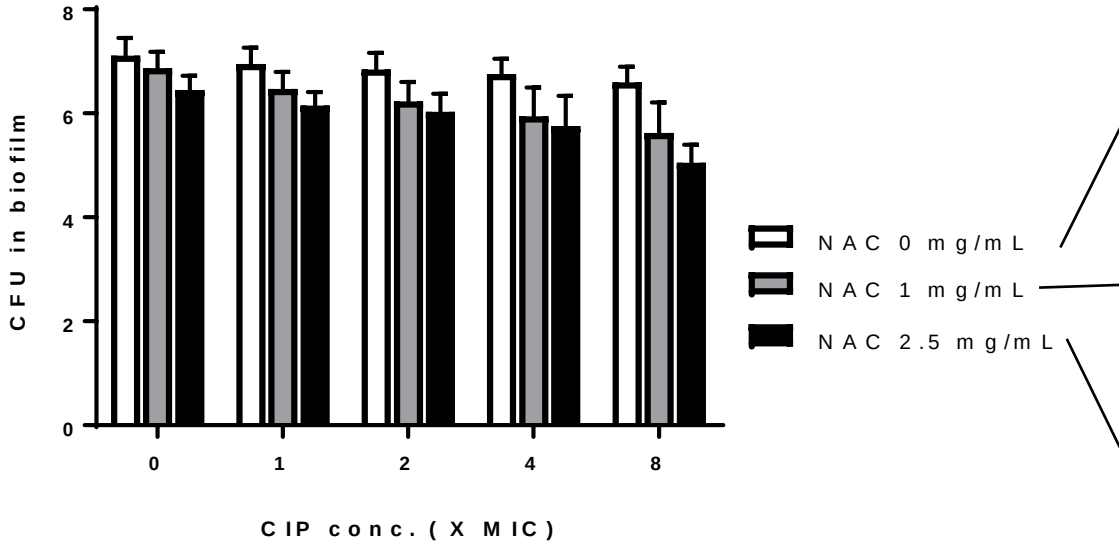
Enzymes degrading matrix constituents ↗ antibiotic activity in biofilms

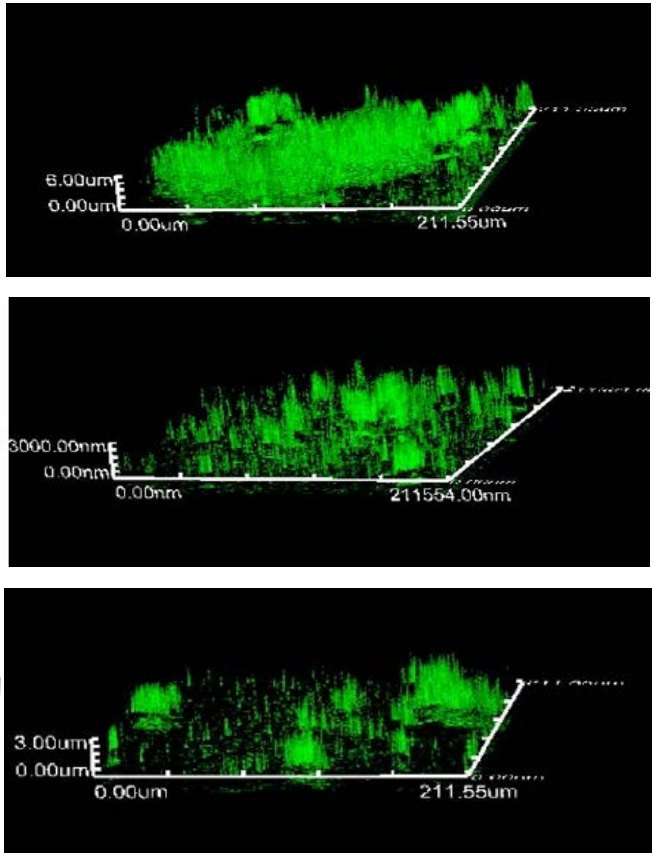
Alipour et al., JAC 2009; 64:317-25.

Antibiotic PK/PD parameters in biofilms

N-acetyl-cysteine (anti-oxidant + reducing disulfure bridges)

Ciprofloxacin and *Pseudomonas* biofilms





NAC increases CIP activity against biofilms in vitro…

Zhao & Liu BMC Microbiology 2010; 10:140

Antibiotic PK/PD parameters in biofilms

N-acetyl-cysteine (anti-oxidant + reducing disulfure bridges)

… But does it work in patients ? 900 mg 3 x/day for 24 weeks

Summary data for changes in primary and selected secondary endpoints from week 0 to week 24.

Variable	Treatment effect (95% CI)	p-Value
FEV_1 (% pred)	4.4 (0.83, 7.9)	0.02
FEV_1 (L)	0.15 (0.03, 0.28)	0.02
Sputum neutr. elastase activity (log_{10})	0.21 (−0.07, 0.48)	0.14
Sputum neutrophil count (log_{10})	2.6 (−12.1, 17.3)	0.73
Sputum IL-8 (log_{10})	0.19 (−0.03, 0.42)	0.09
Plasma IL-8 (log10)	−0.1 (−0.33, 0.14)	0.42
GSH in whole blood	64.2 (−177.6, 305.9)	0.60
Incidence of pulmonary exacerbation	−0.08 (−0.30, 0.14)	0.48
New use of antibiotics	0.08 (−0.14, 0.29)	0.50
CFQ-R respiratory domain	−0.34 (−6.3, 5.67)	0.91
CFRSD number of resp sx	−0.15 (−1.1, 0.8)	0.75

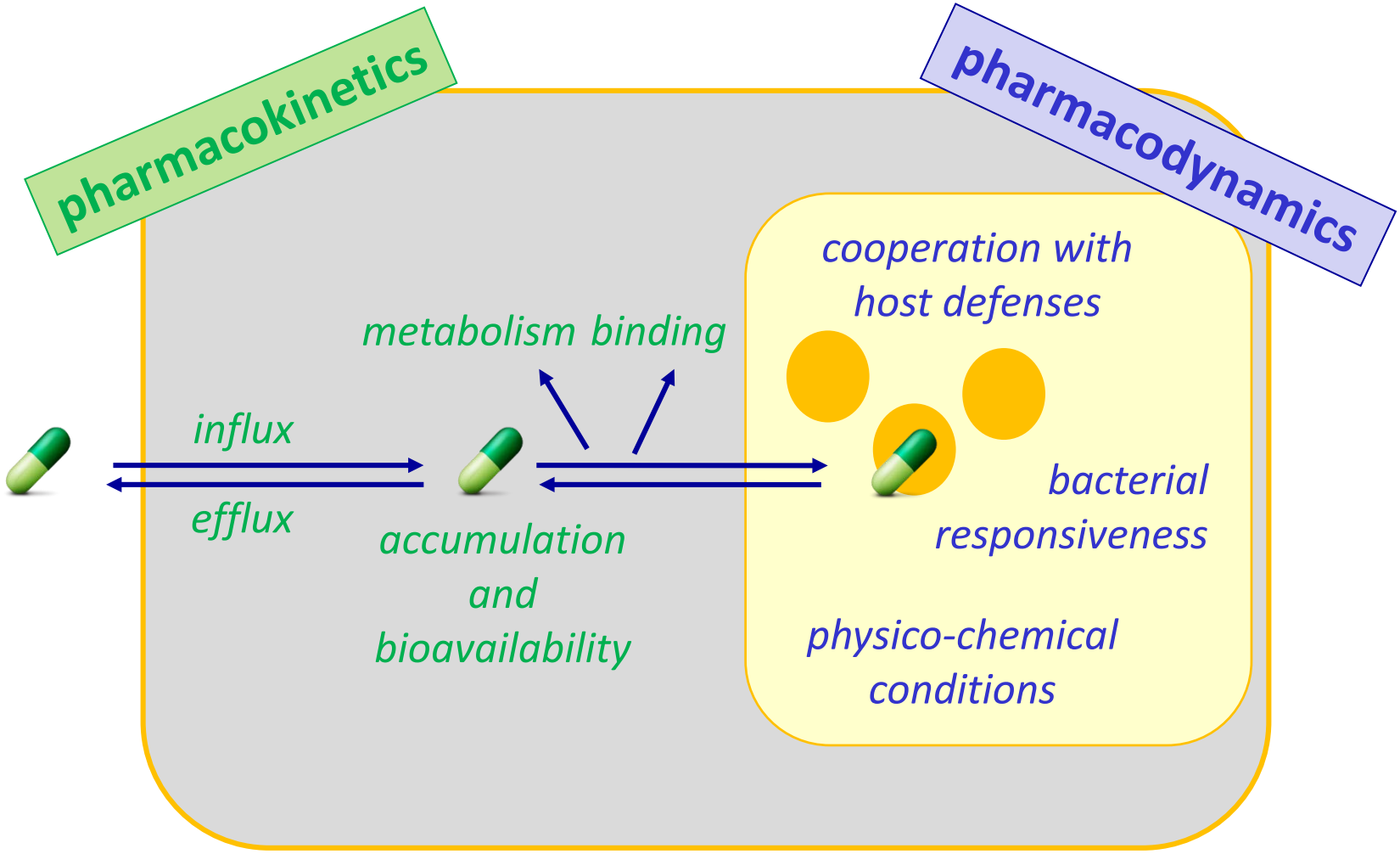
95% point wise confidence intervals (using t-distribution approximation) are included at each time point. Similar changes were measured in FEF 25–75% (see online supplement).

Conrad et al. , J. Cystic Fibrosis 2015; 14:219–227

How to improve targeting of these bacteria ?

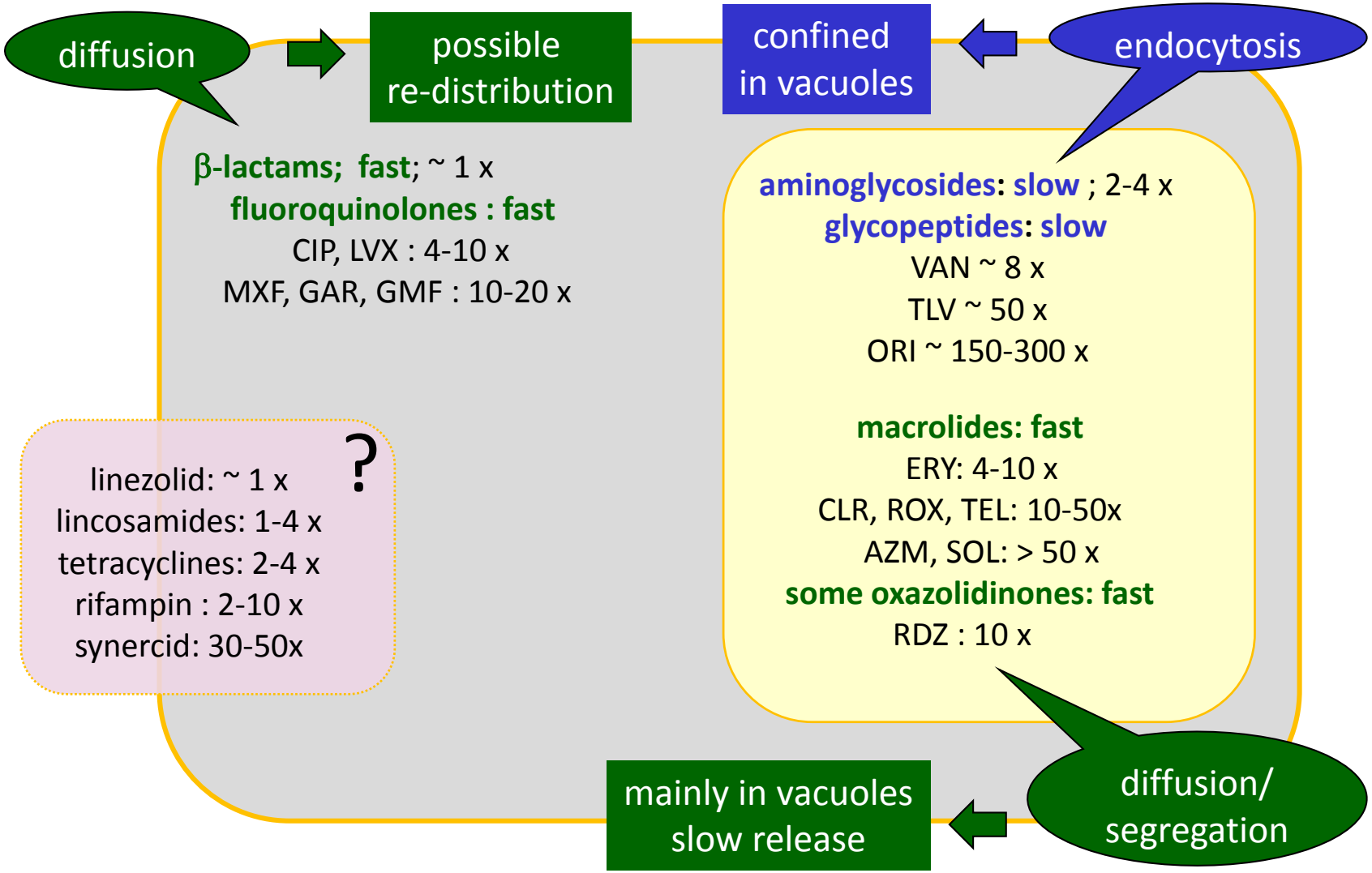
3. Intracellular targeting

PK/PD parameters against intracellular bacteria

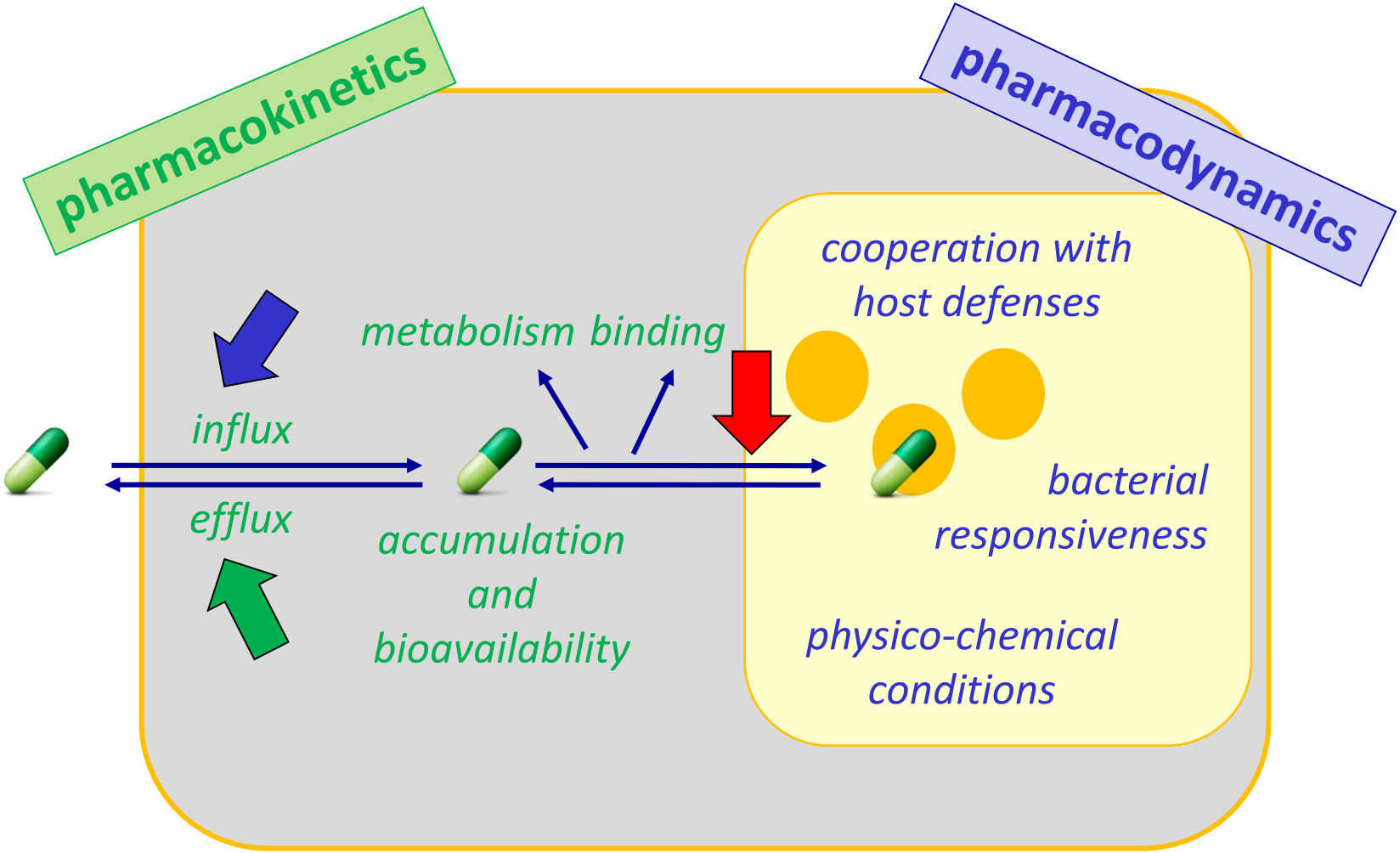


Carryn et al, Infect Dis Clin North Am 2003; 17:615-34

Antibiotic accumulation and subcellular distribution

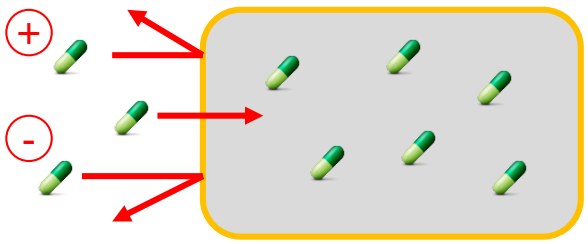


Strategies to increase antibiotic cellular concentrations



Strategies to increase antibiotic cellular concentrations

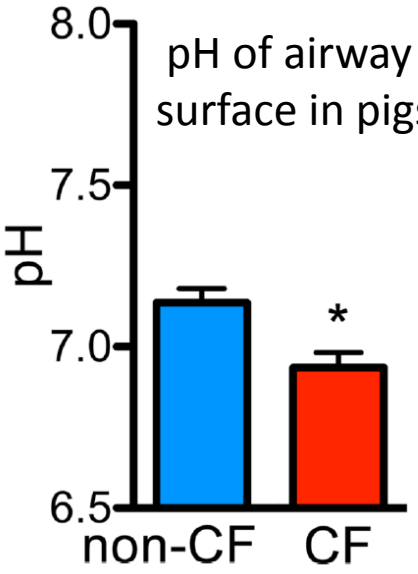
Influx and pH gradient

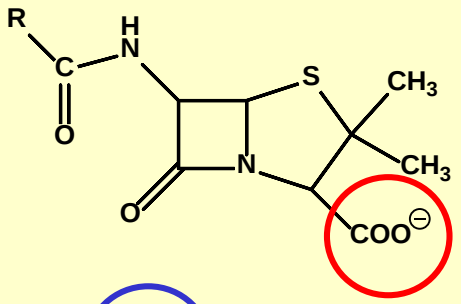


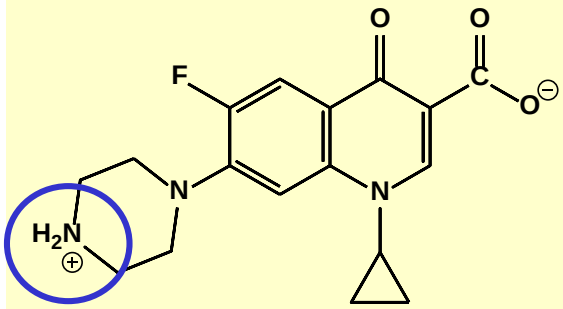
Neutral/zwitterionic molecules are more diffusible

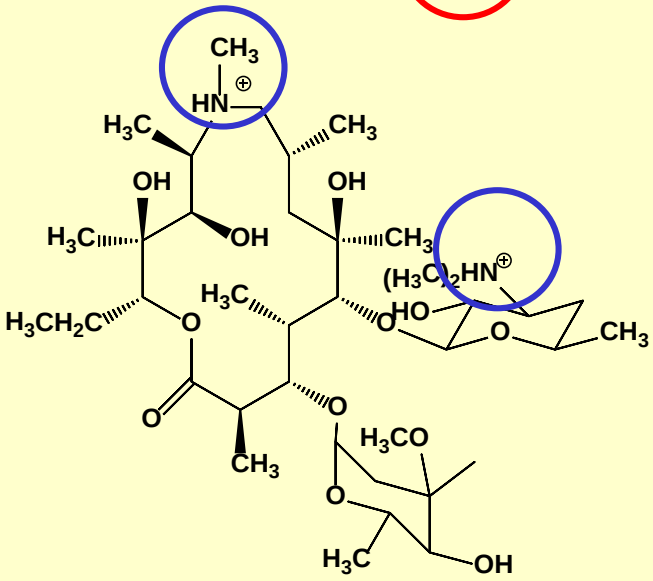
If extracellular pH ↘:

- ↗ uptake of acidic molecules
- ↘ uptake of basic molecules





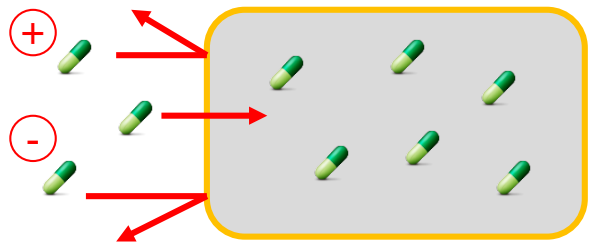




Pezzulo et al, Nature 2013; 487: 109–113

Strategies to increase antibiotic cellular concentrations

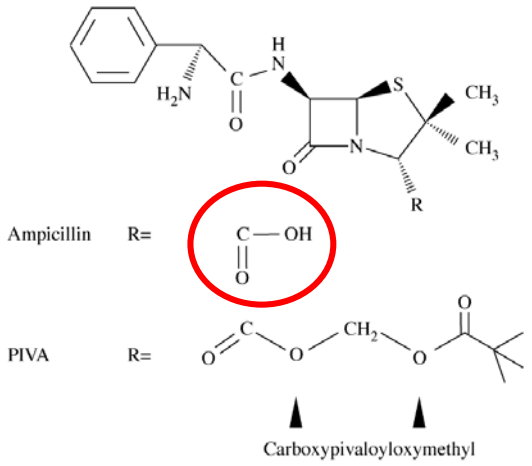
β-lactams

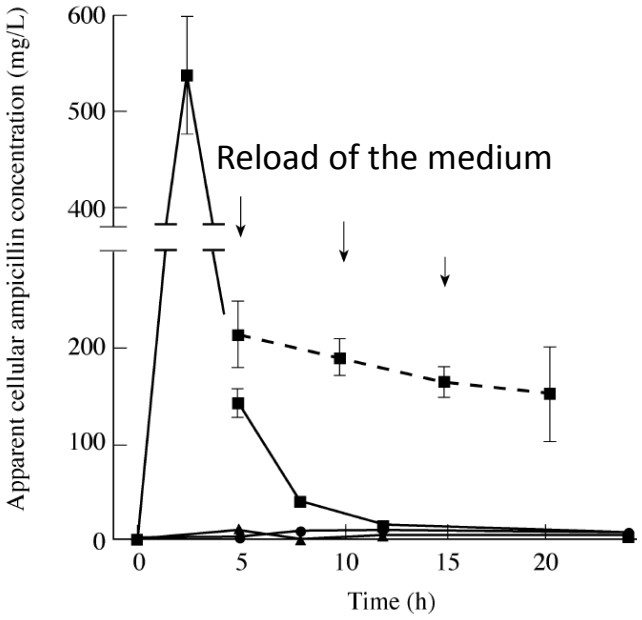


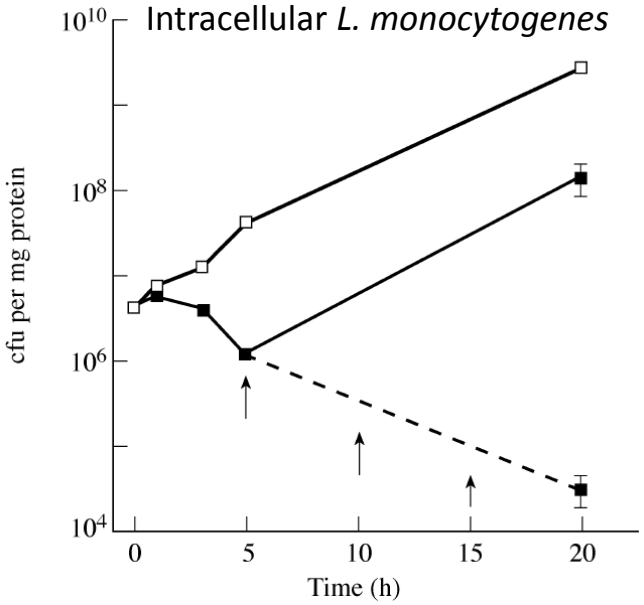
Neutral/zwitterionic molecules are more diffusible

→ Masking the charges to change diffusibility

Ampicillin prodrug: accumulation and activity



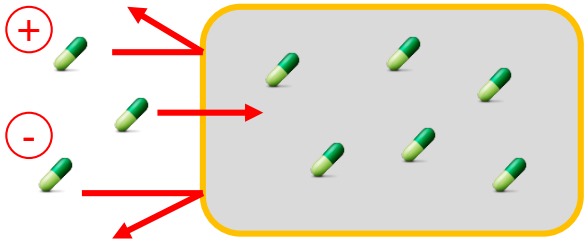




Chanteux et al., JAC 2003; 52:610-15

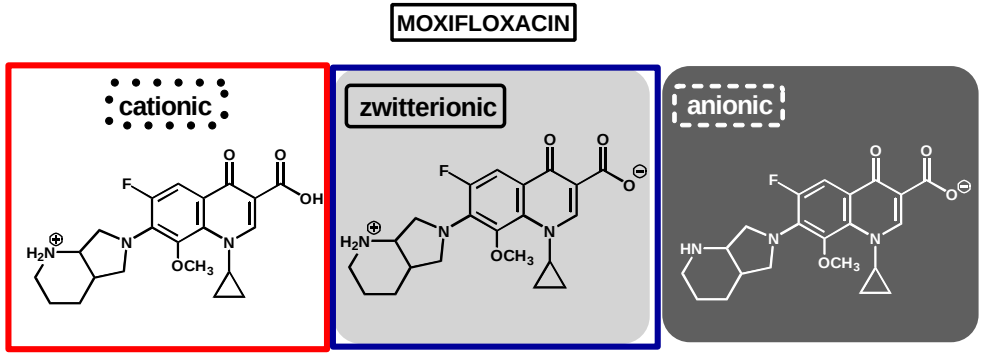
Increasing accumulation by improving diffusibility

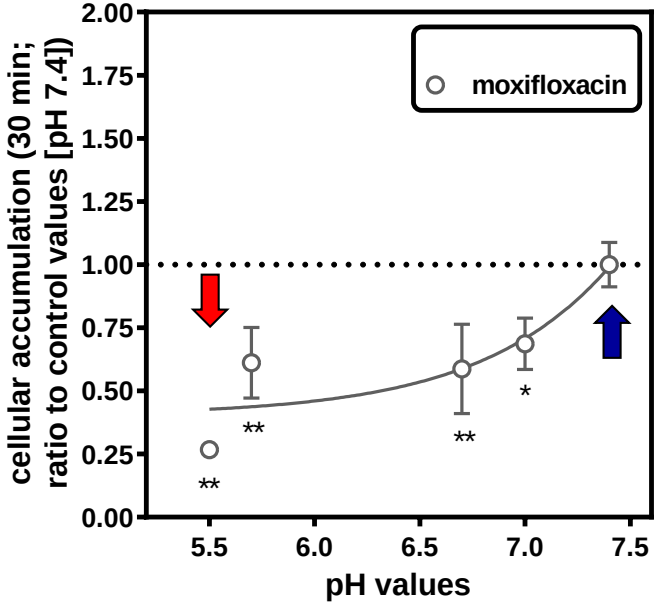
fluoroquinolones

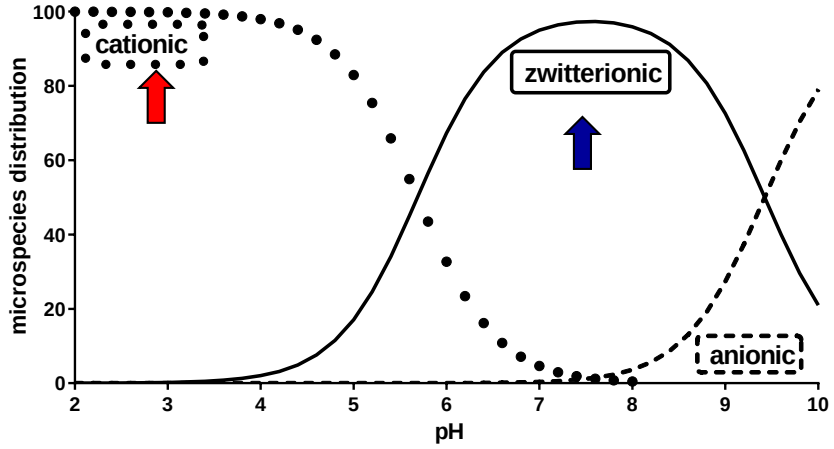


Neutral/zwitterionic molecules are more diffusible

→ accumulation lower for most fluoroquinolones at acidic pH



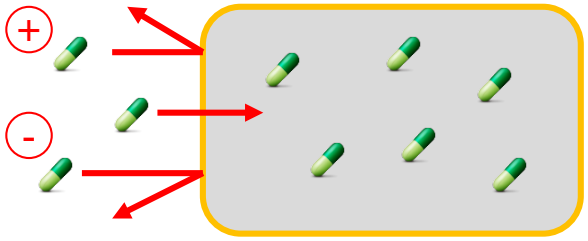




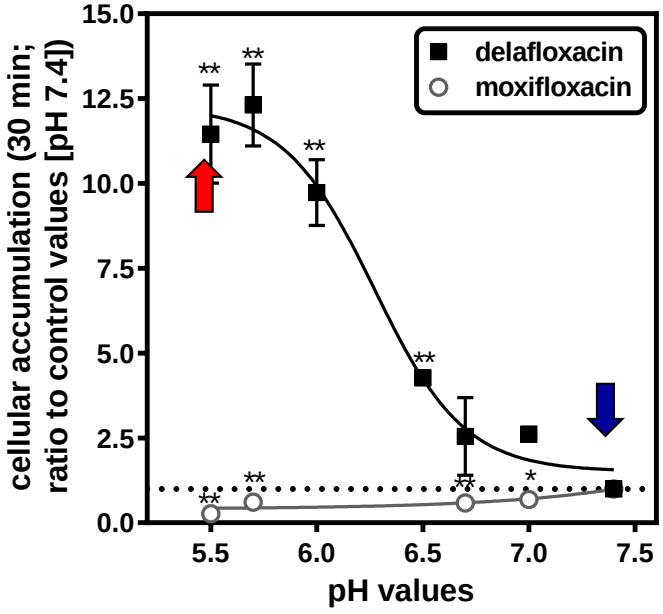
Lemaire et al. AAC 2011; 55:649-58; Van Bambeke, Future Microbiology 2015; 10:1111–23

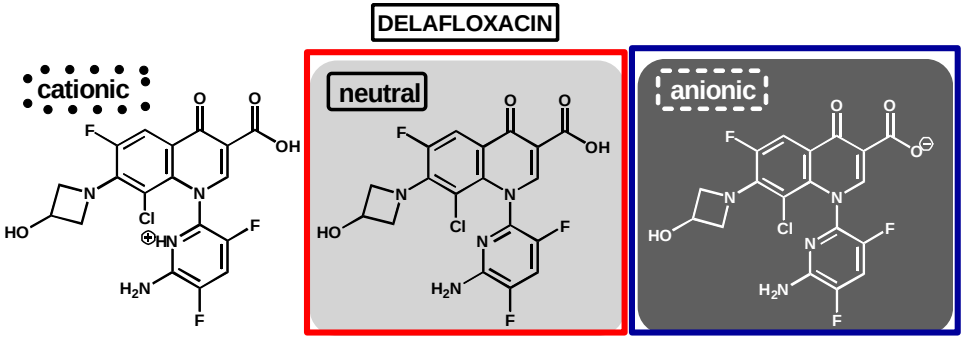
Increasing accumulation by improving diffusibility

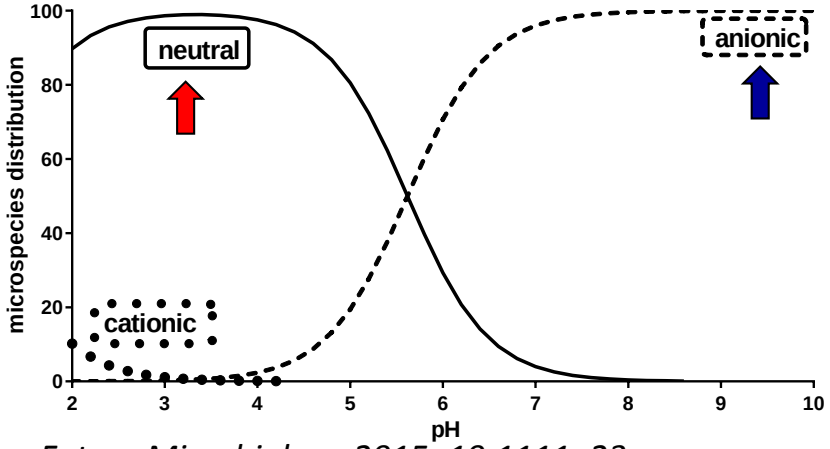
fluoroquinolones



Neutral/zwitterionic molecules are more diffusible
→ accumulation higher for acidic fluoroquinolones (delafloxacin/finafloxacin)



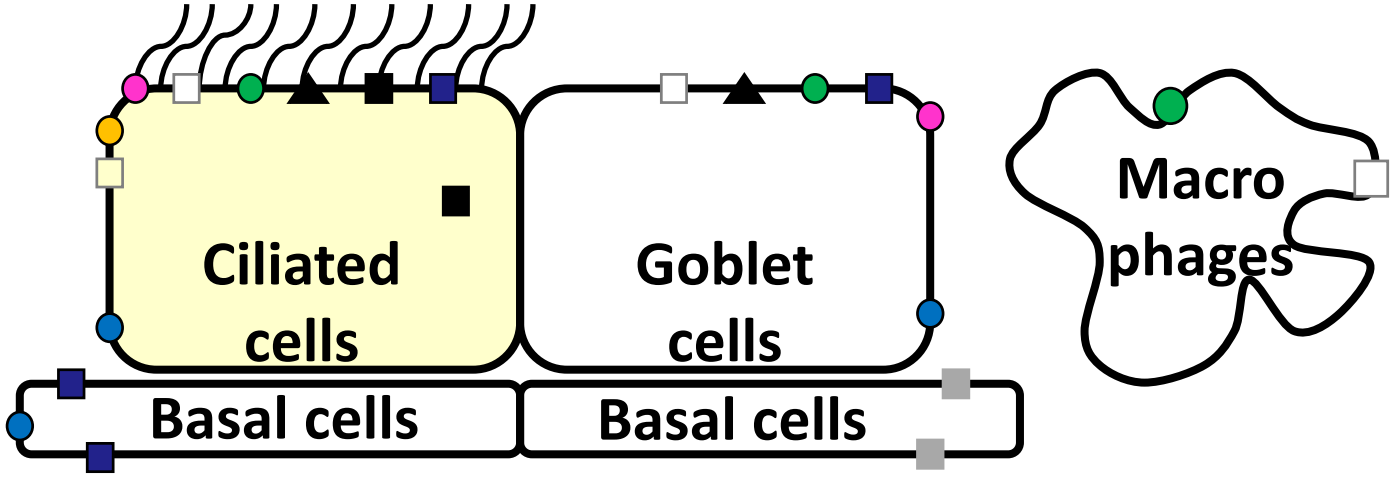




Lemaire et al. AAC 2011; 55:649-58; Van Bambeke, Future Microbiology 2015; 10:1111–23

Strategies to increase antibiotic cellular concentrations

Inhibition of efflux



- P-gp
- MRP1
- MRP2*
- BCRP*
- PEPT1-2
- OCT1
- OCT2
- OCT3
- OCTN1
- OCTN2

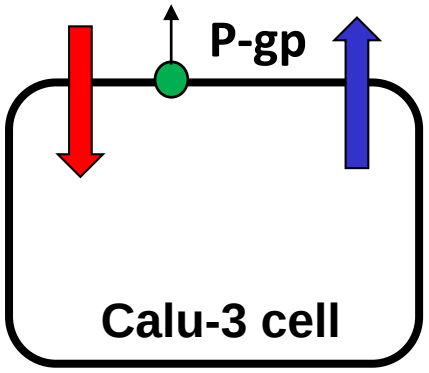
* Conflicting data

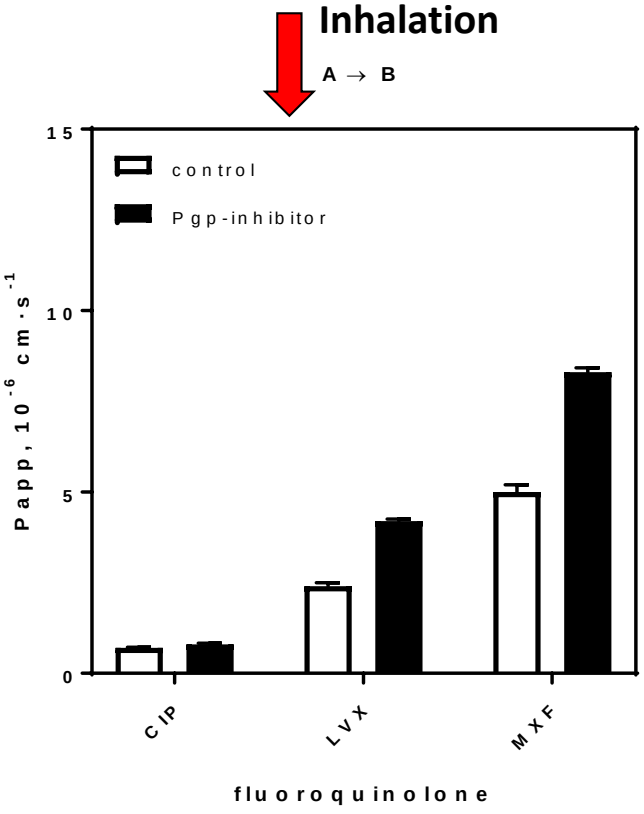
- Fluoroquinolones
- Macrolides
- β-lactams
- Rifampin

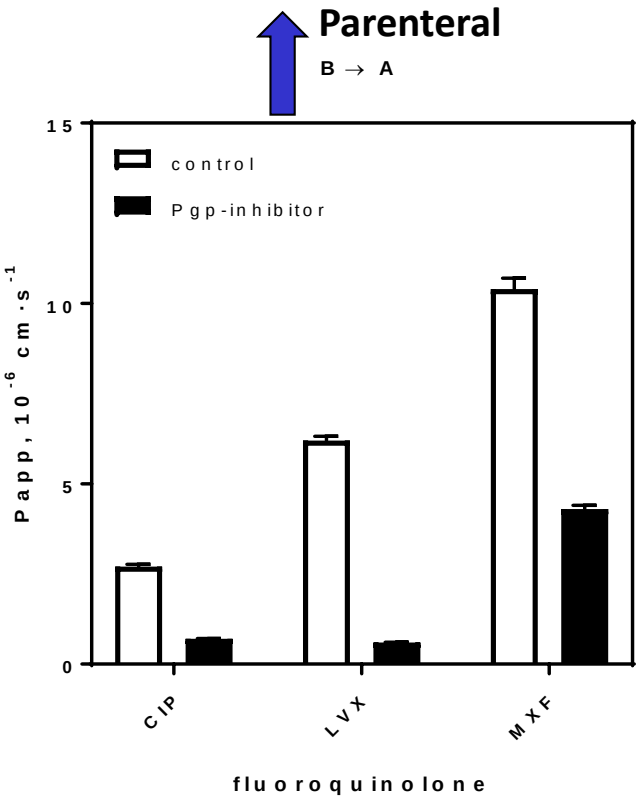
Adapted from
Bosquillon, J Pharm Sci, 2010; 2240-55;
Van Bambeke et al, JAC 2003; 51:1067-1077

Strategies to increase antibiotic cellular concentrations

Inhibition of efflux







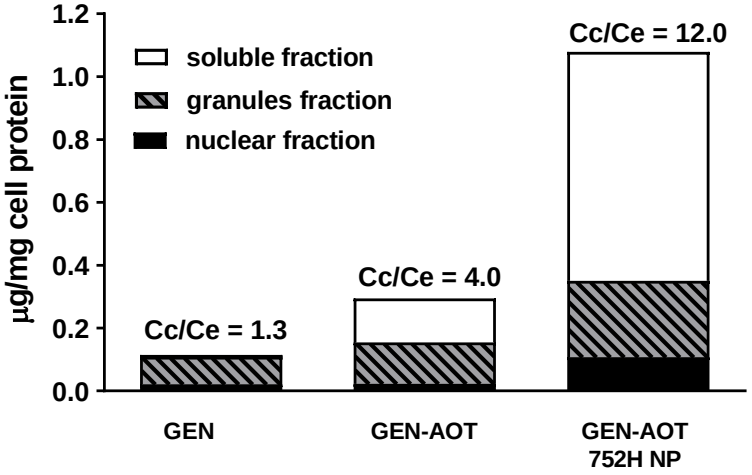
Pgp activity modulates fluoroquinonolone concentrations to different extents

Adapted from
Brillault et al, AAC 2010; 54: 543–5

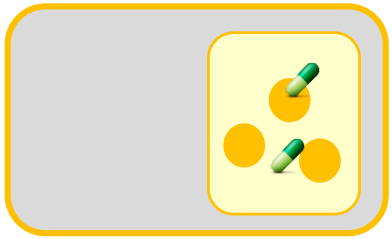
Strategies to increase antibiotic cellular concentrations

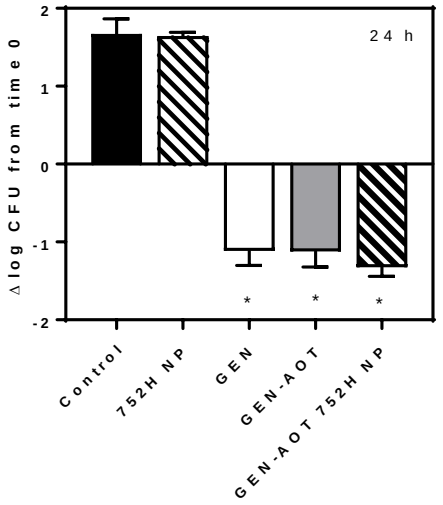
Modulation of distribution: use of delivery systems

gentamicin (GEN) + surfactant (AOT [bis(2-ethylhexyl) sulfosuccinate sodium salt]) + poly(D,L-lactide-co-glycolide) (PLGA)

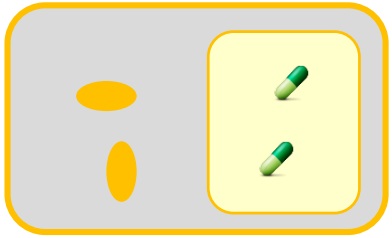


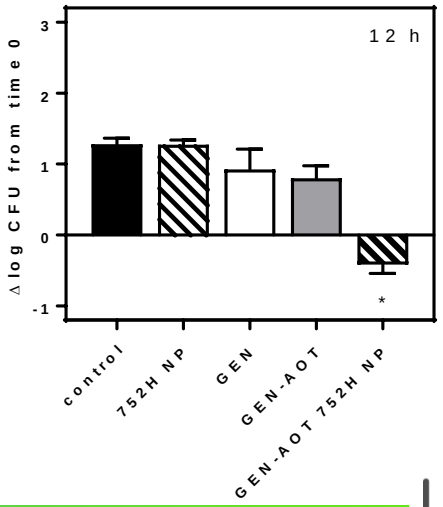
S. aureus





L. monocytogenes





Delivery systems can help delivering antibiotics to different subcellular compartments

Imbuluzqueta et al. Acta Biomater. 2011; 7:1599-608; JAC 2012; 67:2158-64.

Take home messages

- Antibiotic access to bacteria is made difficult in CF lung by mucus/specific modes of life
- Enzymes and NAC already used in the clinics but no direct demonstration of their adjuvant efficacy towards infections
- Many strategies evaluated *in vitro* still lacking *in vivo* and/or clinical evaluation
- A lot of work needed ….

https://loonylabs.org/2015/01/04/antibiotic-resistance-2/

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