Inhaled nanoparticles and microparticles to treat pulmonary biofilms

7th NanoFar School

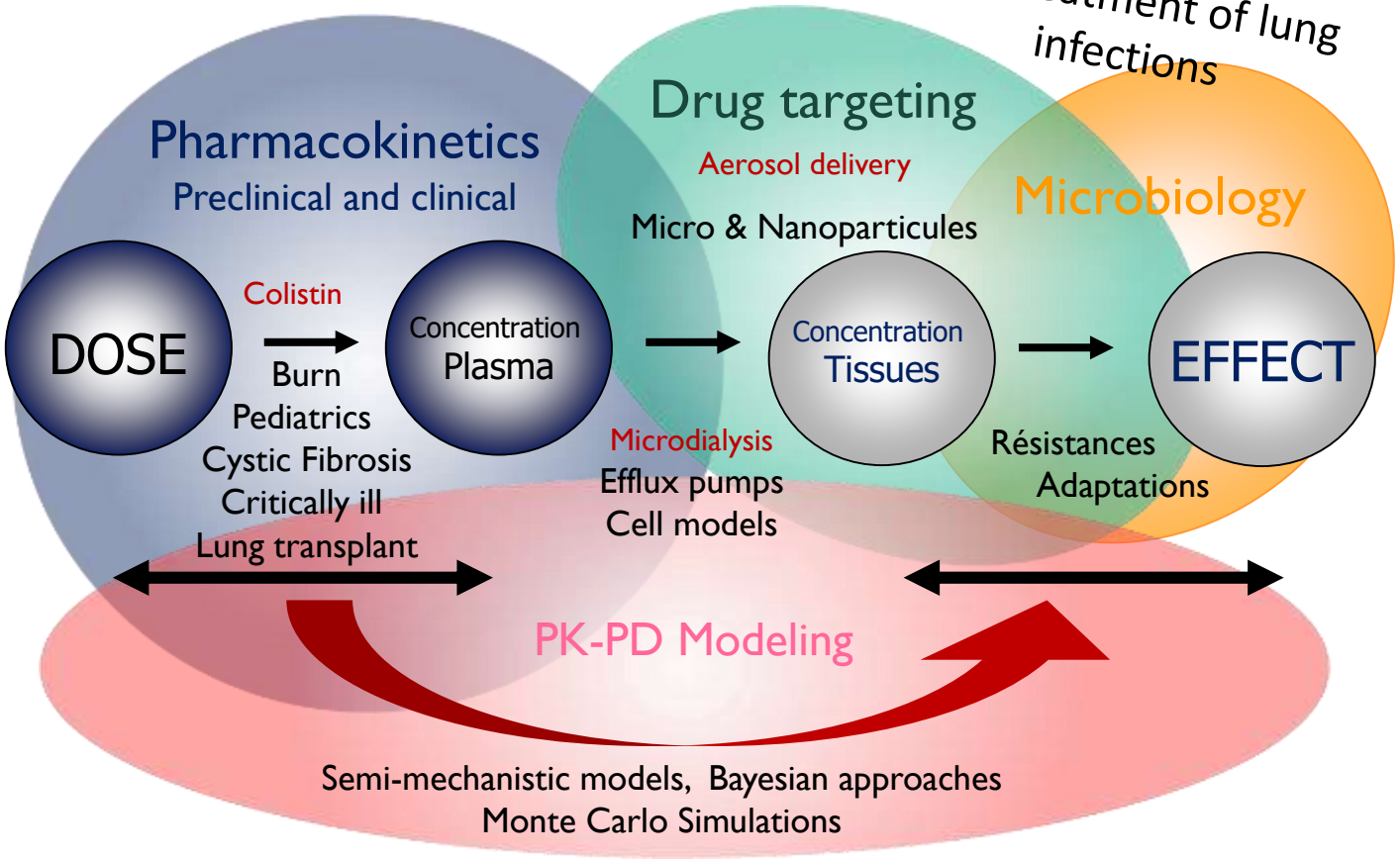
FREDERIC TEWES

Angers, June 2019

Inserm U1070

Pharmacology of antimicrobial agents

Antibiotics aerosol for the treatment of lung infections



World Health Organization

May 2017

WHO PRIORITY PATHOGENS LIST FOR R&D OF NEW ANTIBIOTICS

Priority 1: CRITICAL#

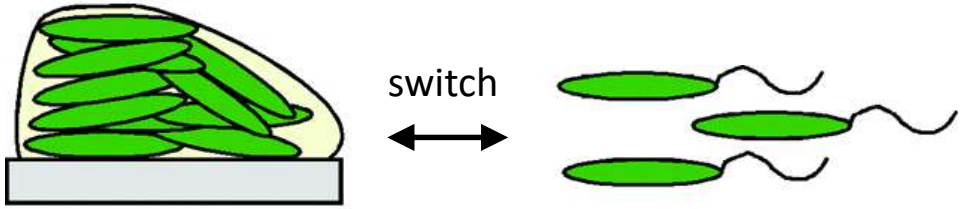
Acinetobacter baumannii, carbapenem-resistant

Pseudomonas aeruginosa, carbapenem-resistant

Enterobacteriaceae*, carbapenem-resistant, 3rd generation cephalosporin-resistant

Chronic versus acute infections to *Pseudomonas Aeruginosa* (PA)

PA is an opportunistic pathogen that can cause invasive infections, such as acute pneumonia, in immune compromised hosts. It also causes chronic infections that are impossible to eradicate, such as the chronic lung infection in cystic fibrosis (CF) patients



PA adapts to the host environment and undergoes changes which promote bacterial survival and evasion of host defenses.

chronic infections

- Persistent inflammation
- Low metabolic activity
- ATB resistance
- Low mobility (Flagellin ↓)
- ↓ host recognition
- bacterial biofilm

acute infections

- Planktonic bacteria (free swimming bacteria)
- High metabolic activity
- High virulence (T3SS, secreted proteases ↑)
- Can be eliminated by ATB and detected by host

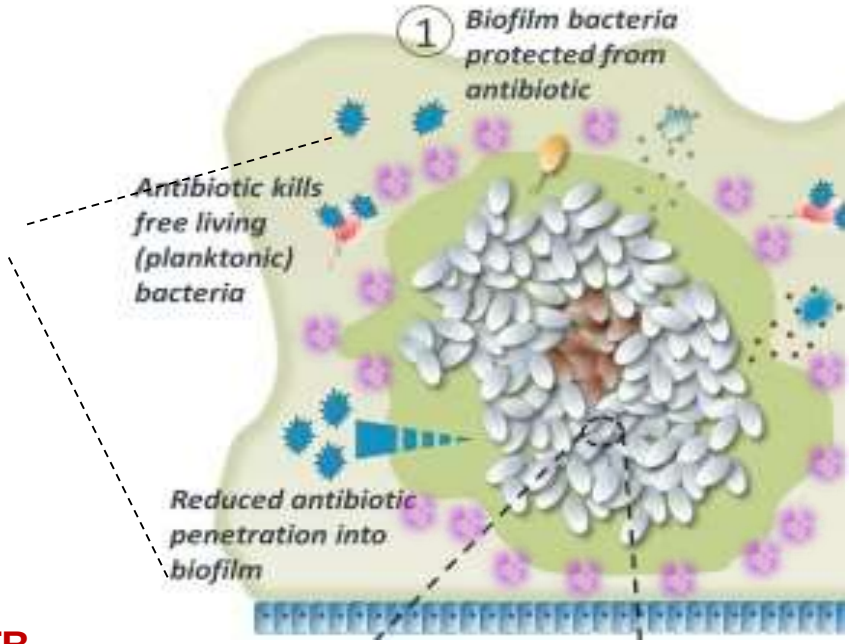
Pulmonary biofilms

Biofilm: Bacterial aggregate in a matrix of exopolymeric substance (anionic polysaccharides (alginate), proteins & DNA)

Clusters of *P. aeruginosa* biofilms (<50-100 µm wide) are found embedded in the **conducting airways mucus**, often surrounded by immune cells such as polymorphonuclear Neutrophils (PMN)

Mucus of CF patient (anionic polymer)

Biofilms contain regions with low metabolic activity, and this contributes to reduced susceptibility towards antibiotics



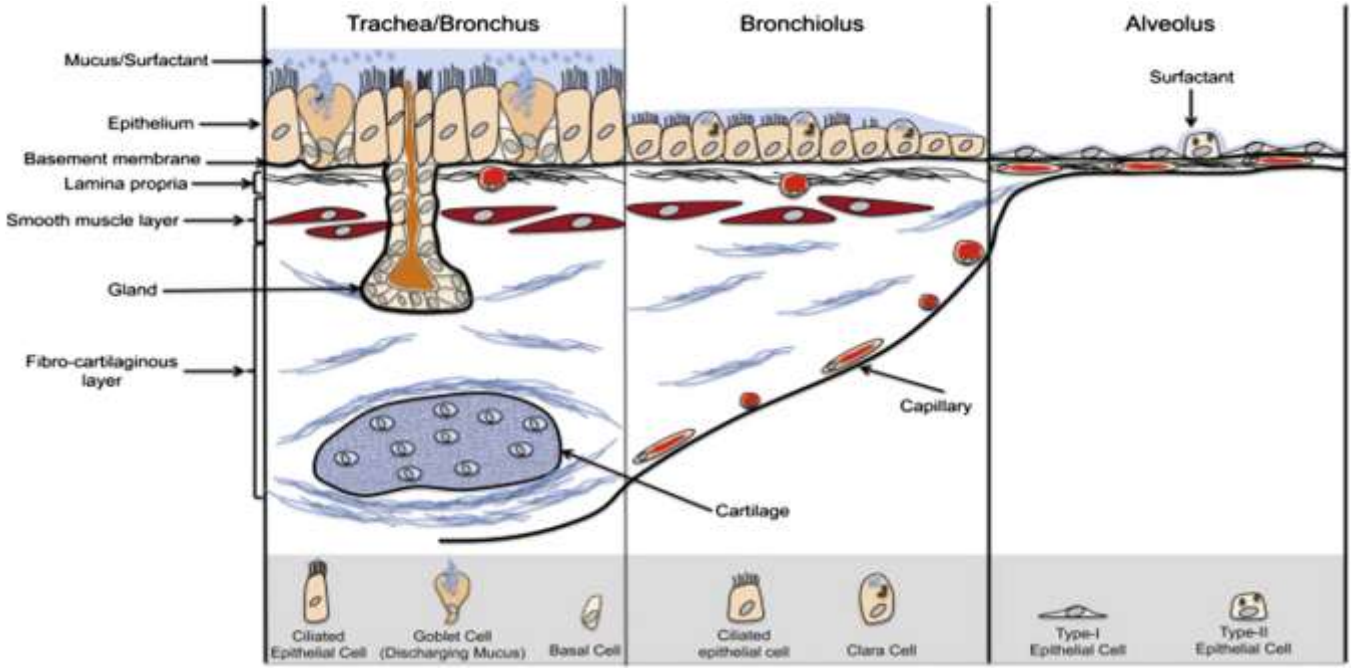
❖ **Three barriers prevent the penetration of ATB**

Bjarnsholt, Thomas, et al. *Pseudomonas aeruginosa* biofilms in the respiratory tract of cystic fibrosis patients. Pediatric pulmonology 44.6 (2009): 547-558.

Targeting area : the conducting zone

Conducting zone

Respiratory zone



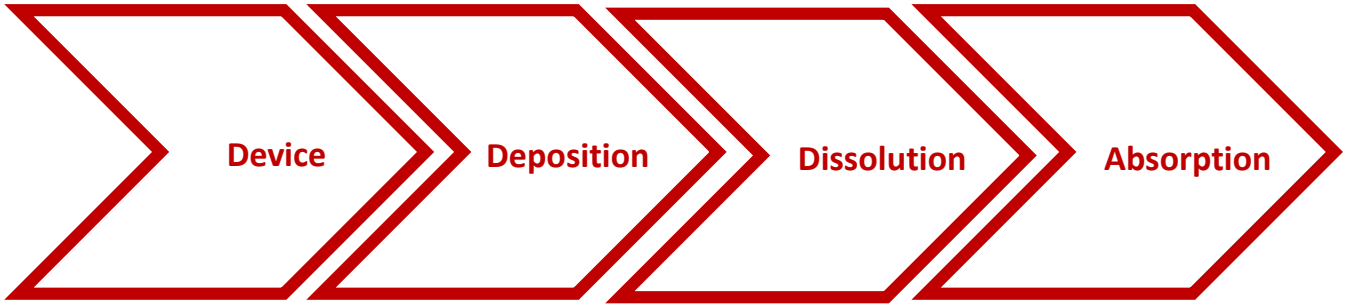
Bjarnsholt, Thomas, et al. *Pseudomonas aeruginosa* biofilms in the respiratory tract of cystic fibrosis patients. Pediatric pulmonology 44.6 (2009): 547-558.

- Aerosol delivery *vs* IV or oral administration?

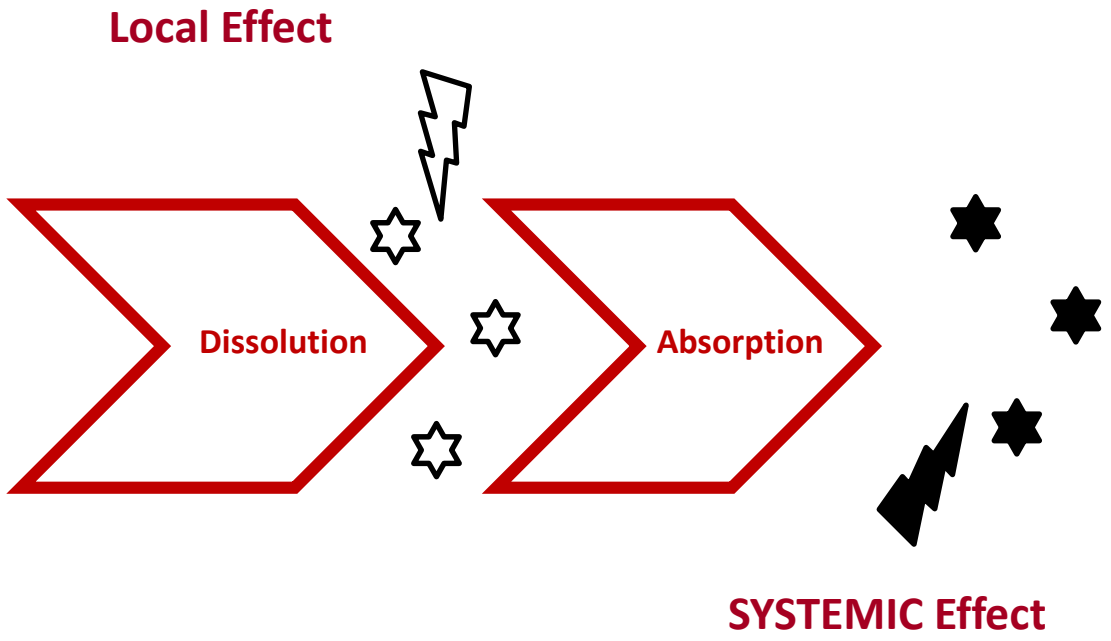
- Higher local conc. / Lower systemic conc.

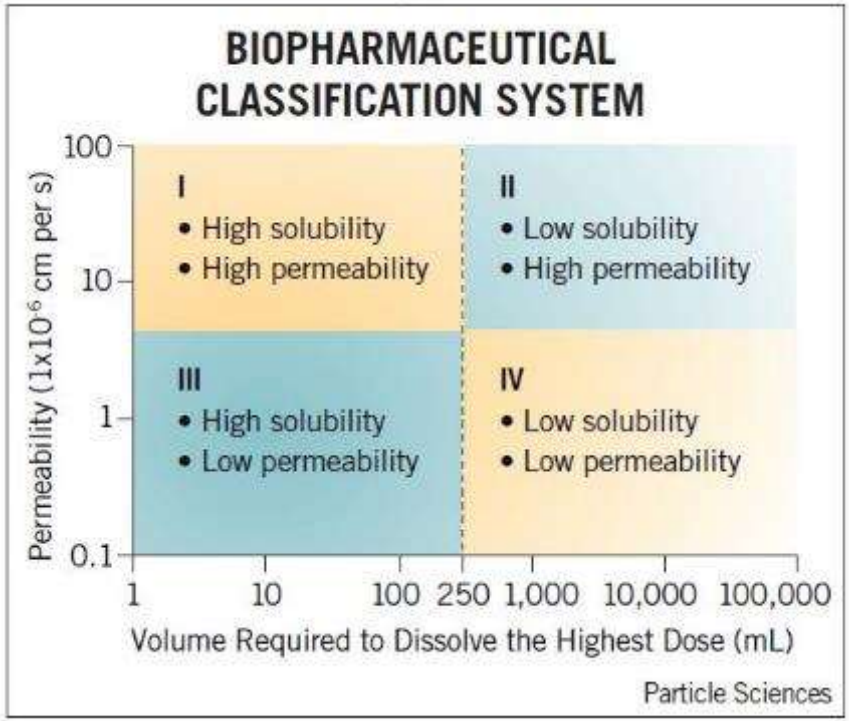
Is it always true ?

Main factors controlling antibiotics lung conc. ?

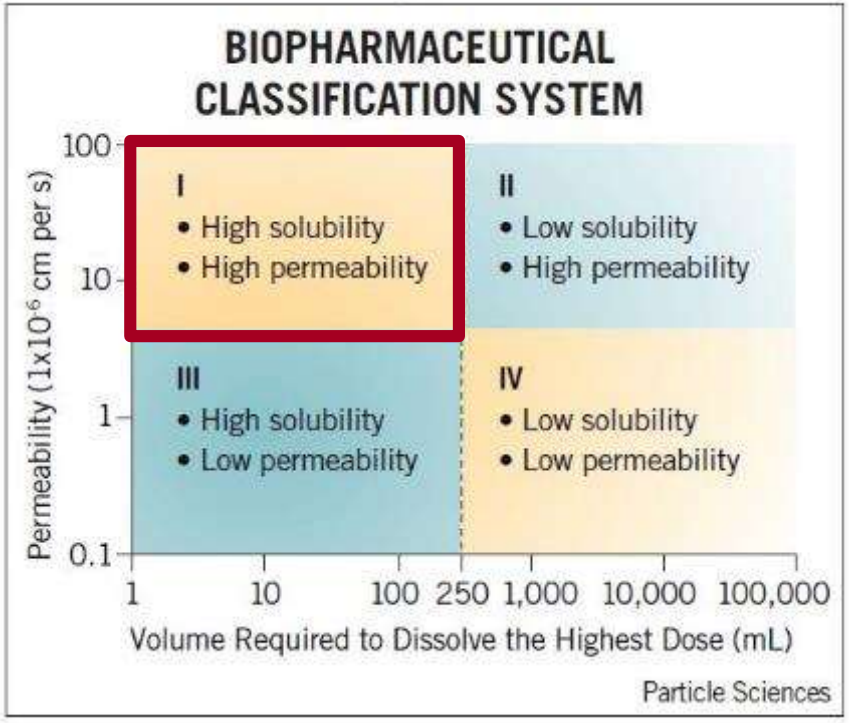


What is the objective ?




BIOPHARMACEUTICAL CLASSIFICATION SYSTEM
Permeability (1x10⁻⁶ cm per s)
100
10
1
0.1
I
• High solubility
• High permeability
II
• Low solubility
• High permeability
III
• High solubility
• Low permeability
IV
• Low solubility
• Low permeability
1
10
100
250
1,000
10,000
100,000
Volume Required to Dissolve the Highest Dose (mL)
Particle Sciences

Systemic


BIOPHARMACEUTICAL
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I
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III
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• Low permeability
IV
• Low solubility
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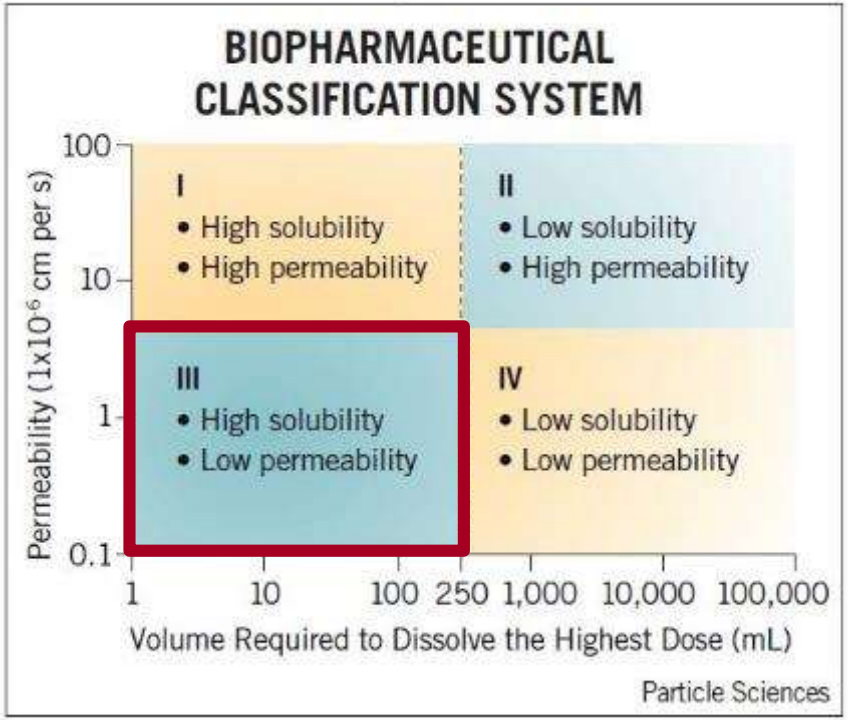
Permeability (1x10⁻⁶ cm per s)
100
10
1
0.1
1
10
100
250
1,000
10,000
100,000
Volume Required to Dissolve the Highest Dose (mL)
Particle Sciences

Local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100
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1
0.1
1
10
100
250
1,000
10,000
100,000
Volume Required to Dissolve the Highest Dose (mL)
Particle Sciences

Antimicrobial Agents and Chemotherapy

2014

Biopharmaceutical Characterization of Nebulized Antimicrobial Agents in Rats: 1. Ciprofloxacin, Moxifloxacin, and Grepafloxacin

Aline Vidal Lacerda Gontijo,[a,c,d] Julien Brillault,[a,c] Nicolas Grégoire,[a,c] Isabelle Lamarche,[a,c] Patrice Gobin,[a,b] William Couet,[a,b,c] Sandrine Marchand[a,b,c]

Inserm U1070, Pôle Biologie Santé, Poitiers, France[a]; Service de Toxicologie-Pharmacocinétique, CHU de Poitiers, Poitiers, France[b]; Université de Poitiers, UFR Médecine-

Biopharmaceutical Characterization of Nebulized Antimicrobial Agents in Rats: 2. Colistin

Aline Vidal Lacerda Gontijo,[a,b,d] Nicolas Grégoire,[a,b] Isabelle Lan
Sandrine Marchand[a,b,c]

Inserm U1070, Pôle Biologie Santé, Poitiers, France[a]; Université de Poitiers, UFR Mé
Pharmacocinétique, Poitiers, France[c]; CAPES Foundation, Ministry of Education of

2015

Biopharmaceutical Characterization of Nebulized Antimicrobial Agents in Rats: 3. Tobramycin

Sandrine Marchand,[a,b,c] Nicolas Grégoire,[a,b] Julien Brillault,[a,b] Isabelle Lamarche,[a,b] Patrice Gobin,[a,c] William Couet[a,b,c]

Inserm U1070, Pôle Biologie Santé, Poitiers, France[a]; Université de Poitiers, UFR Médecine-Pharmacie, Poitiers, France[b]; CHU Poitiers, Service de Toxicologie-Pharmacocinétique, Poitiers, France[c]

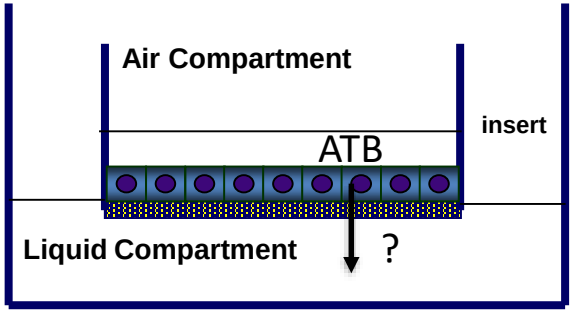
1) In-vitro experiments transepithelial transport across Calu-3 cell monolayers

apparent permeability → Papp

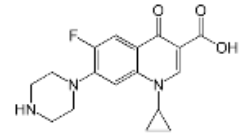
Human lung epithelial cell line

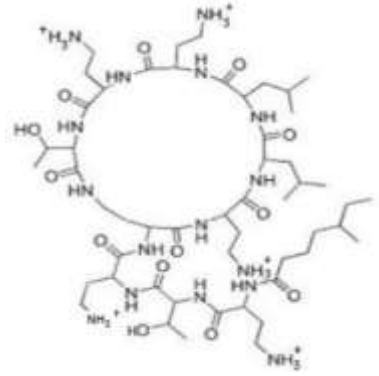
Epithelial cells : thigh junctions

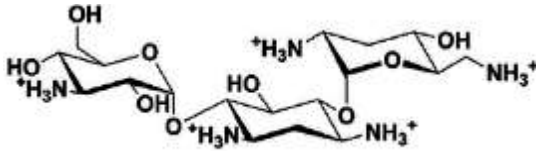
Efflux transport systems: **P-gp**



	Solubility (water, mg.mL^{-1})		Log P	Perm Papp (
CIP	1,35	0.28	-0,81	0.1
MOX	0,168	2.9	-0,5	5
COL	0,238	-2.9	-8,1	0.0
AZT	0,0429		-3,1	0.0
TOB	53,7		-6,3	





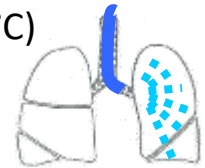


2) Standardized in-vivo protocol in rats

- **Routes of administration:**
  - Nebulization
  - IV infusion

MicroSprayer IA-1B

- **Simultaneous sampling (5x6)**
  - Blood (intracardiac puncture) ⇨ **Plasma drug concentrations**
  - BAL (1 mL NaCL 0.9% - 37°C)

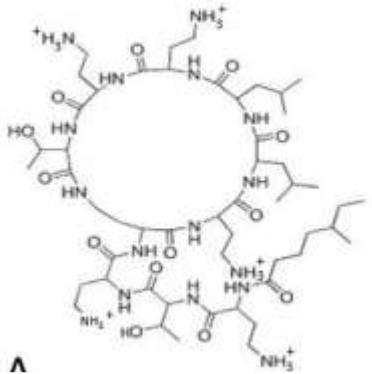


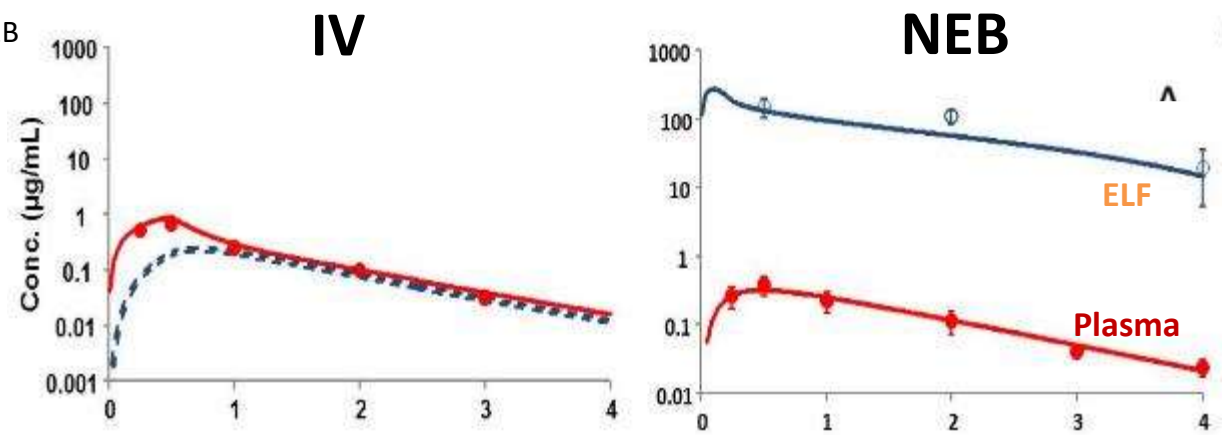
⇨ **Epithelial lining fluid (ELF) concentrations**

- **LC-MS/MS assay**

Penn Century
MicroSprayer IA-1B

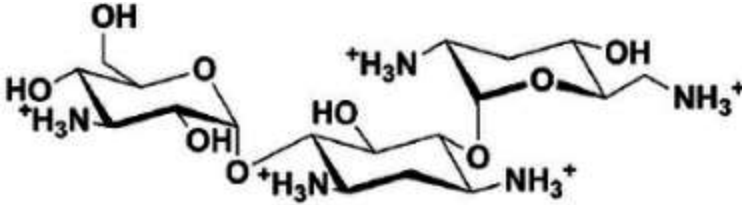
Class III: a) Colistin

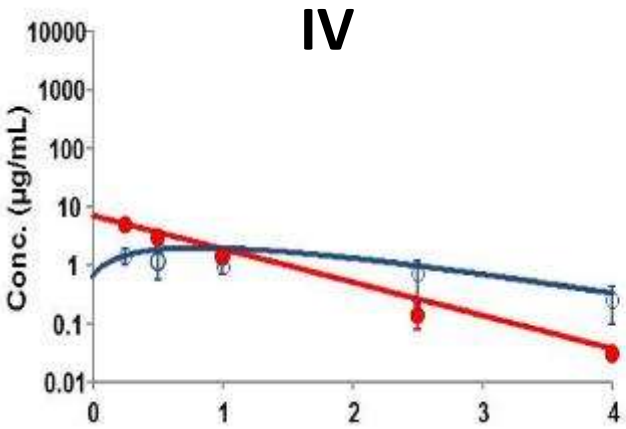


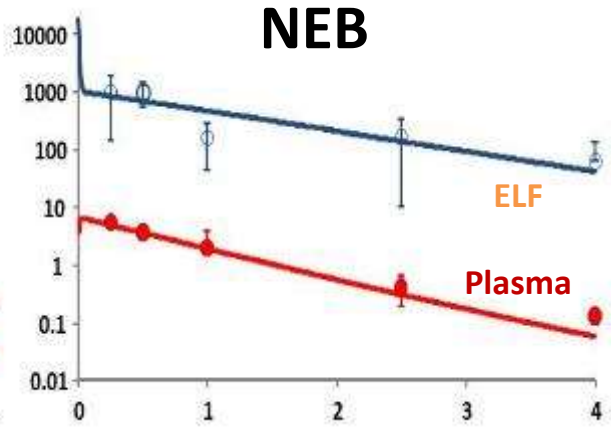


(Gontijo et al., AAC 2014)

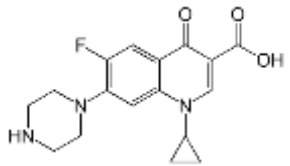
Class III: b) Tobramycin

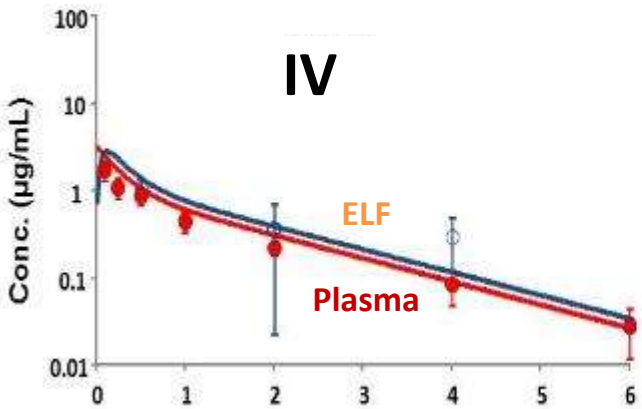


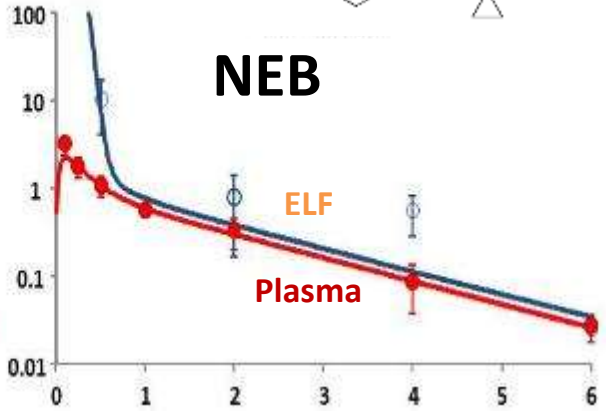




Class II: Ciprofloxacin







Gontijo, A. V. L., Brillault, J., Grégoire, N., Lamarche, I., Gobin, P., Couet, W., & Marchand, S. (2014). Biopharmaceutical characterization of nebulized antimicrobial agents in rats: 1. Ciprofloxacin, moxifloxacin, and grepafloxacin. Antimicrobial agents and chemotherapy, 58(7), 3942-3949.

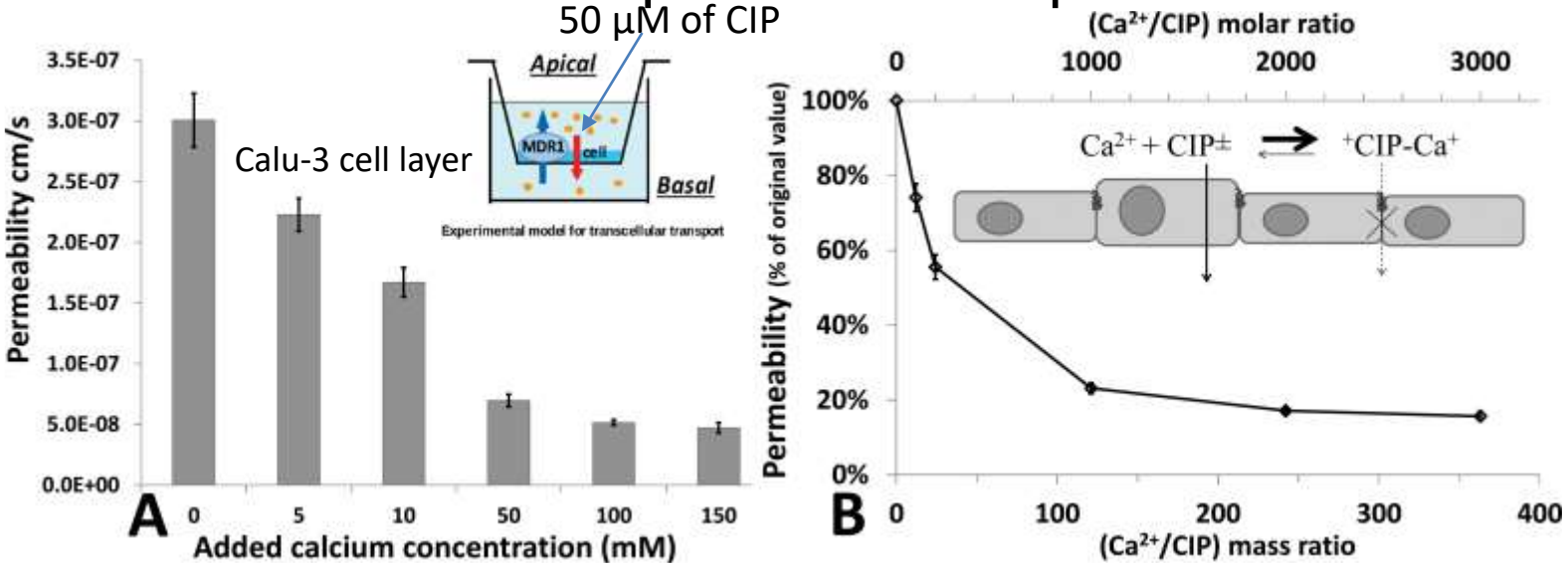
Ciprofloxacin pulmonary delivery/ *P. aeruginosa*

- CIP has a good activity against *P. aeruginosa*
- → high permeability
- → rapid equilibrium between ELF and plasma

How can we maintain CIP in the lung after pulmonary administration?

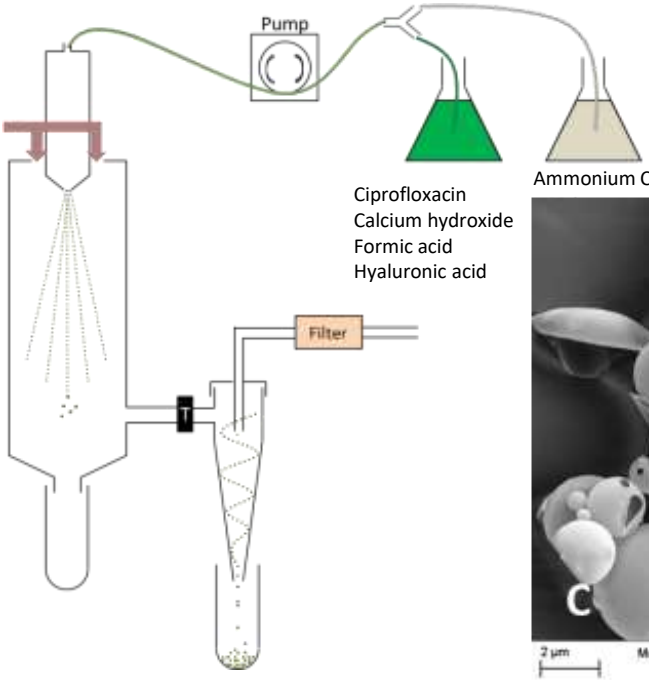
CIP Pulmonary Permability Control

Oral absorption of fluoroquinolones is



Tewes, F., Brillault, J., Lamy, B., O'Connell, P., Olivier, J. C., Couet, W., & Healy, A. M. (2015). Ciprofloxacin-Loaded Inorganic–Organic Composite Microparticles To Treat Bacterial Lung Infection. *Molecular pharmaceutics*, *13*(1), 100-112.

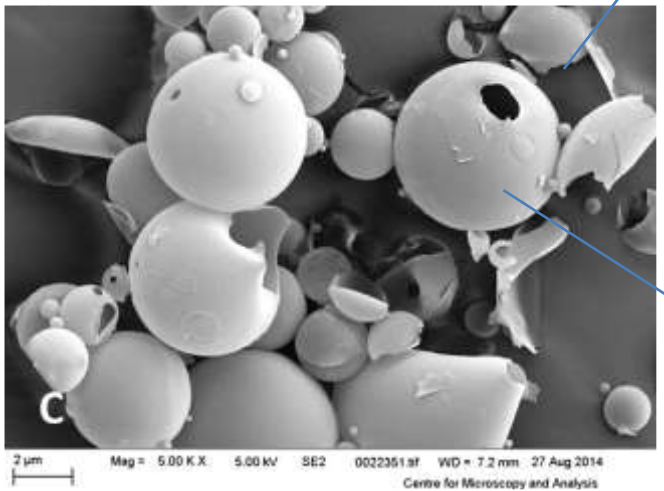
CIP Pulmonary delivery



Ciprofloxacin
Calcium hydroxide
Formic acid
Hyaluronic acid

Ammonium Carbonate

Büchi B-290 Mini Spray Dryer



Amorphous Calcium carbonate

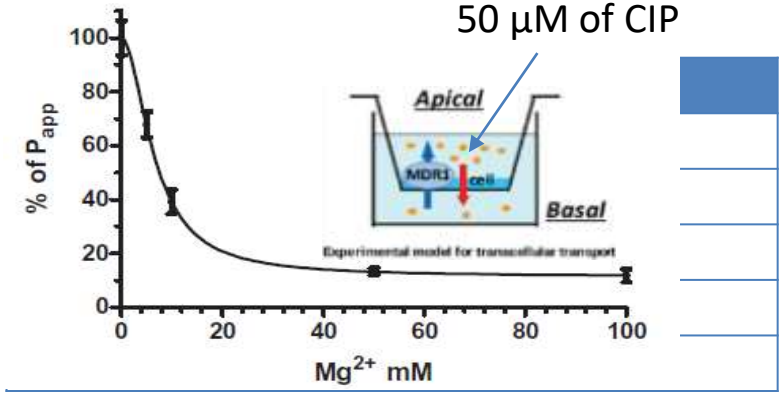
Good Aerodynamic properties

Tewes, F., Brillault, J., Lamy, B., O'Connell, P., Olivier, J. C., Couet, W., & Healy, A. M. (2015). Ciprofloxacin-Loaded Inorganic–Organic Composite Microparticles To Treat Bacterial Lung Infection. *Molecular pharmaceutics*, *13*(1), 100-112.

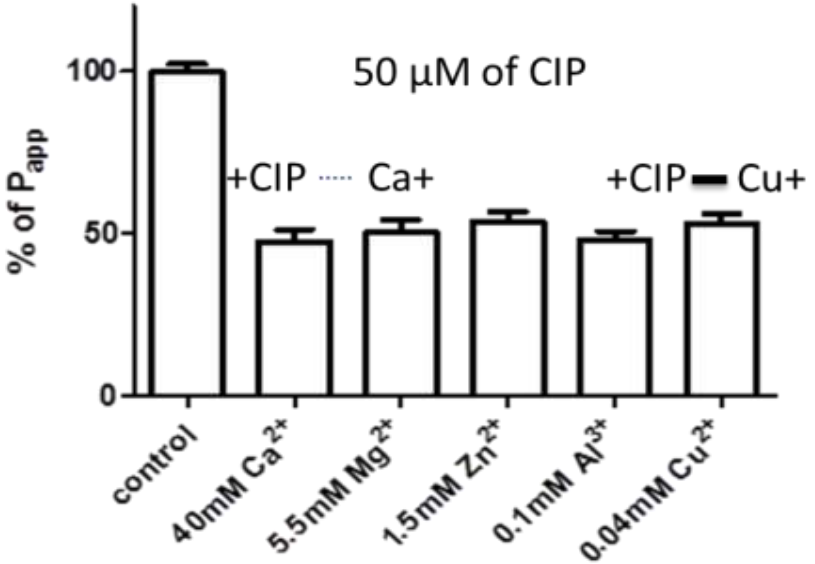
CIP Pulmonary Permability Control

CIP-metal complex formation decrease its apparent permeability across pulmonary epithelium model

The more stable the metal-CIP complex, the higher the CIP apparent permeability decrease.



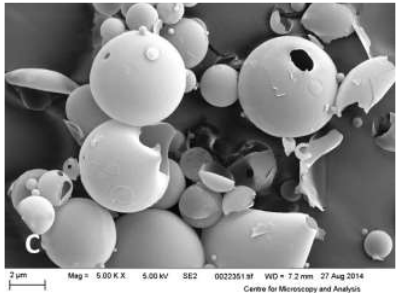
Influence of added Mg^{2+} concentration on the apparent permeability for CIP



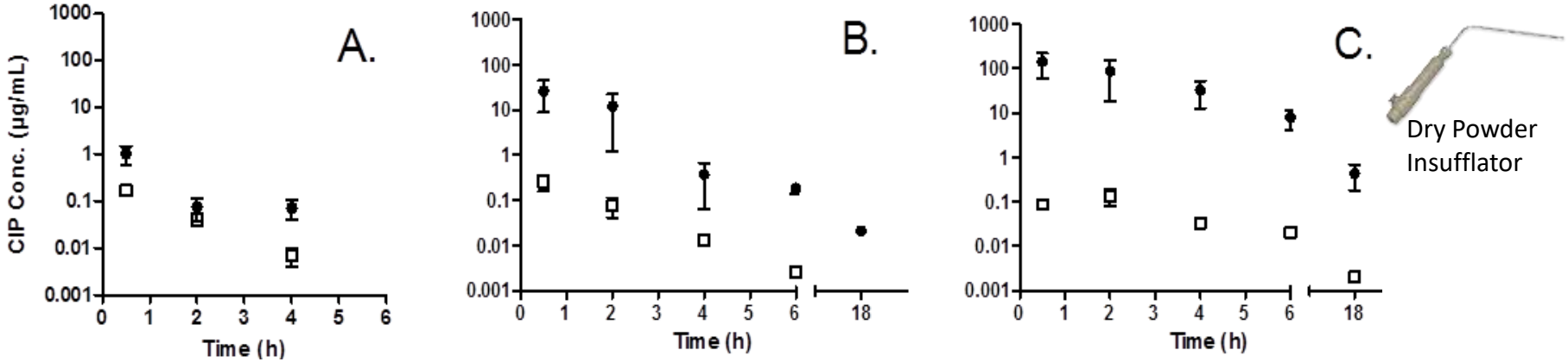
Papp of CIP in conditions of 80% complexation. Cations concentrations were choose to complex 80% of CIP. Data are expressed as the percentage of the control Papp for CIP (means ± S.E.M., n = 8 to 12)

Brillault, J., Tewes, F., et al. (**2017**). In vitro biopharmaceutical evaluation of ciprofloxacin/metal cation complexes for pulmonary administration. *European Journal of Pharmaceutical Sciences*, 97, 92-98.

CIP Pulmonary Concentration Control



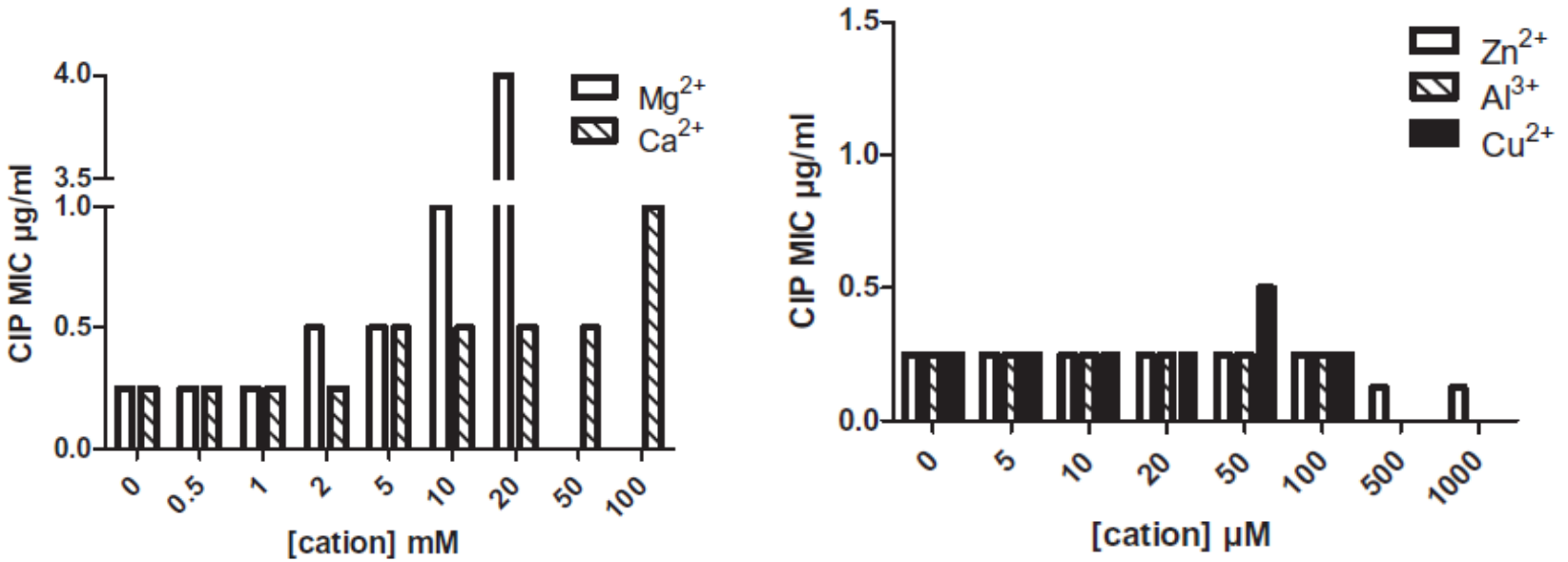
In vivo, the more stable the metal-CIP complex, the higher the extracellular concentration of CIP in the lung.



Mean unbound CIP normalized concentrations in ELF (black circle) and in plasma (open square) versus time after (A) IT nebulization of a CIP solution, (B) IT administration of CIP-Ca microparticles (F2a), or (C) IT administration of CIP-Cu microparticles (F2b). **Dose = 0.35 mg/kg**

Lamy, B., Tewes, F., et al. (**2018**). New aerosol formulation to control ciprofloxacin pulmonary concentration. *Journal of Controlled Release*, 271, 118-126.

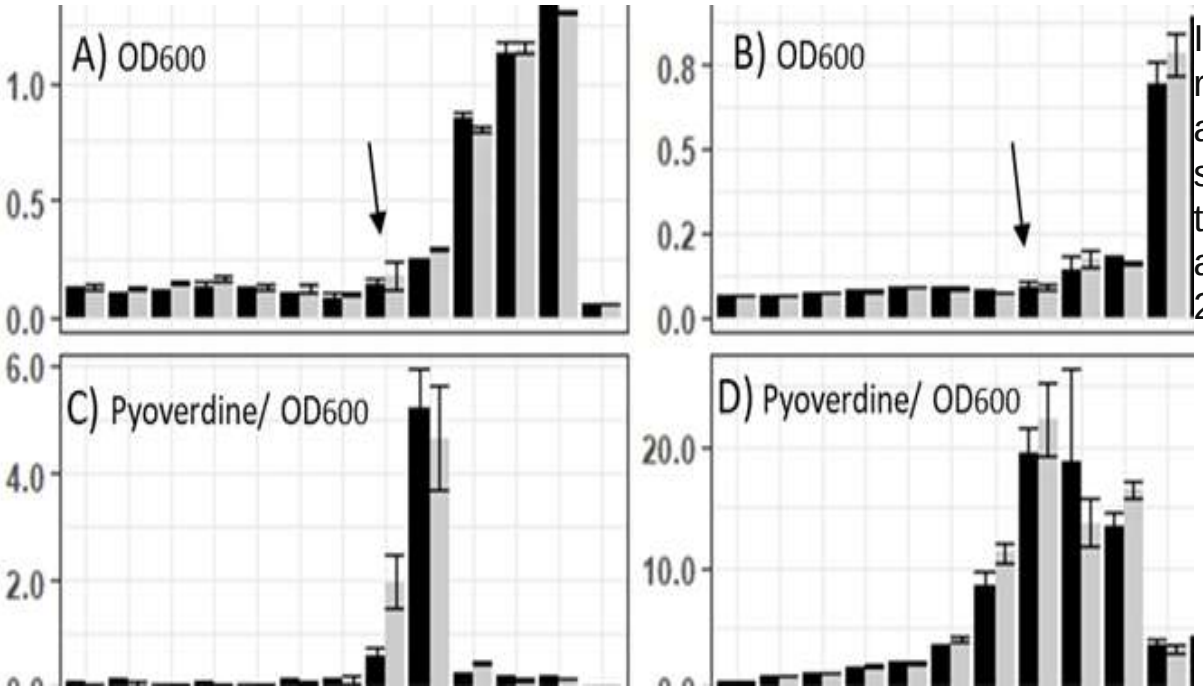
In vitro antibacterial efficacy in vitro against Planktonic *P. aeruginosa*



CIP apparent MIC against *P. aeruginosa* in the presence of increasing cation concentrations

Brillault, J., Tewes, F., et al. (**2017**). In vitro biopharmaceutical evaluation of ciprofloxacin/metal cation complexes for pulmonary administration. European Journal of Pharmaceutical Sciences, 97, 92-98.

Antibacterial efficacy in vitro against Biofilm of *P. aeruginosa*

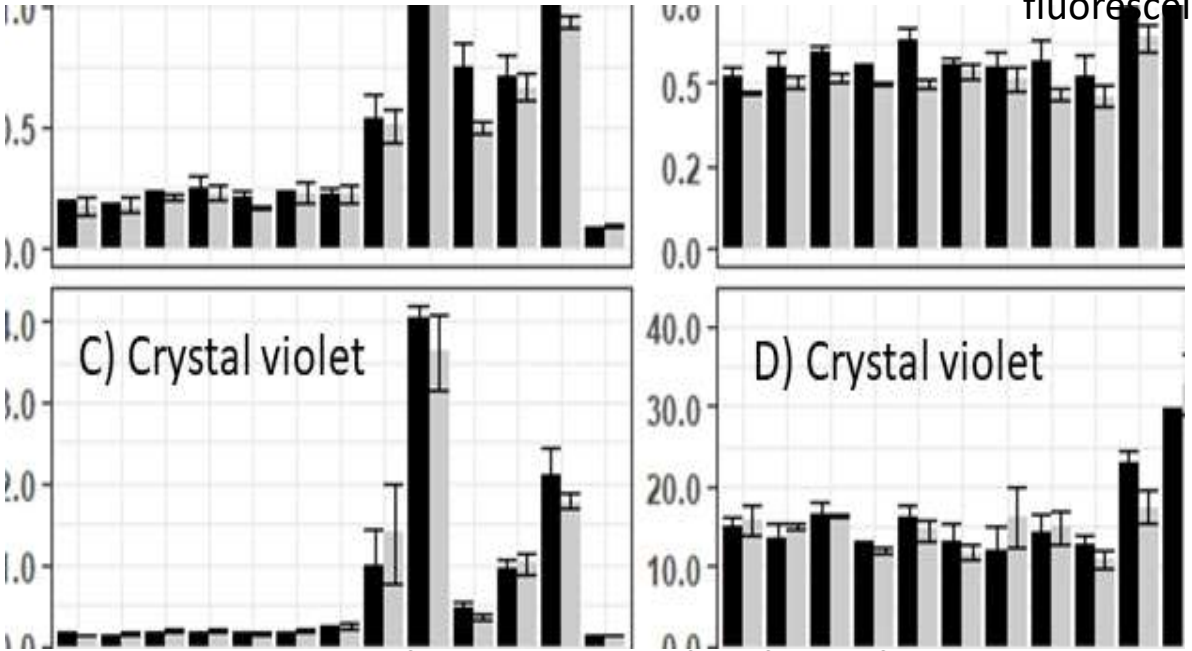


In vitro evaluation of the response of planktonic P. aeruginosa PAO1 strain (left side) and PA14 strain (right side) to various concentrations of CIP and CIP-Cu complex after 20 to 24 h.

High CIP Concentration Was Required to Avoid an Increase in P. aeruginosa Virulence

Tewes, F., Bahamondez-Canas, T. F., & Smyth, H. D. (**2019**). Efficacy of Ciprofloxacin and Its Copper Complex against Pseudomonas aeruginosa Biofilms. AAPS PharmSciTech, 20(5), 205.

In vitro antibacterial efficacy in vitro against Biofilm of *P. aeruginosa*



fluorescein diacetate (FDA) hydrolysis

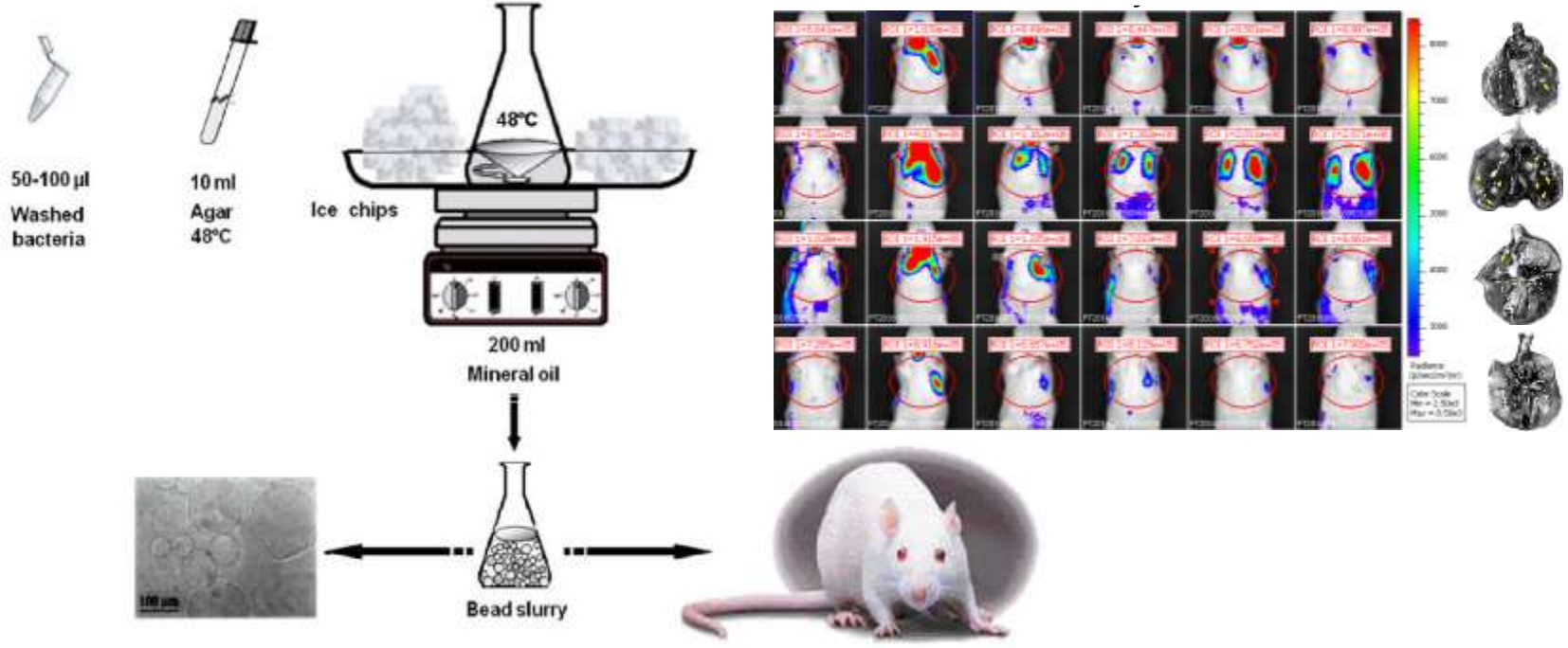
In vitro evaluation of the response of biofilms of P. aeruginosa

When Treating CIP Susceptible P. aeruginosa Biofilms, High CIP Concentration Should Be Achieved in the Vicinity of the Biofilm as Low CIP Concentration Stimulates Biofilm Production

Tewes, F., Bahamondez-Canas, T. F., & Smyth, H. D. (**2019**). Efficacy of Ciprofloxacin and Its Copper Complex against Pseudomonas aeruginosa Biofilms. AAPS PharmSciTech, 20(5), 205.

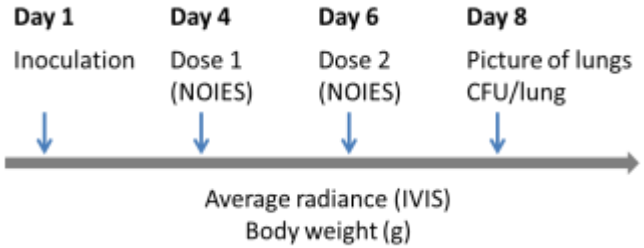
Antibacterial efficacy in vivo in a lung chronic infection model

Bioluminescent PAO1::p16Slux tagged by chromosomal integration of p16S*lux was* enmeshed in agar beads and instilled to rats to reproduce the lung pathology of cystic fibrosis patients with advanced chronic pulmonary disease. Infection was monitored by measuring the bioluminescence.

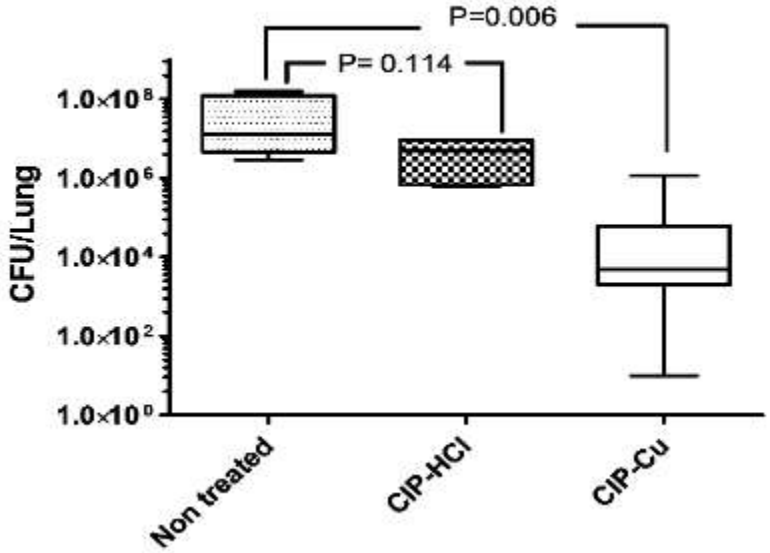


Antibacterial efficacy in vivo in rats

nose only exposure inhalation system (NOEIS)

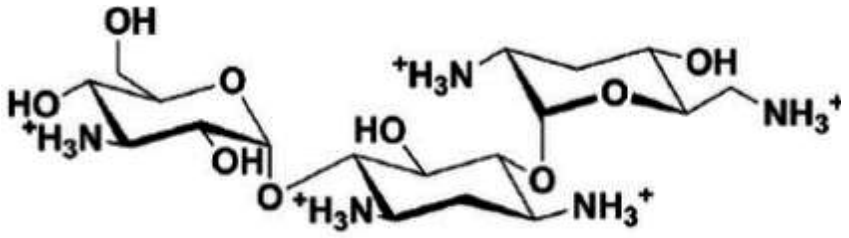


CIP dose 0.35 mg/kg



In vivo efficacy of CIP-Cu in a *P. aeruginosa* chronic lung infection model. Rats were treated on days 4 and 6 with CIP-Cu or CIP·HCl, and the lungs were harvested on day 8. Box and whisker plot of *P. aeruginosa* surviving colony forming units (CFU) per lung on day 8. Whiskers represent the minimal and maximal values (n = 4-6)

TOBRAMYCIN against biofilms?

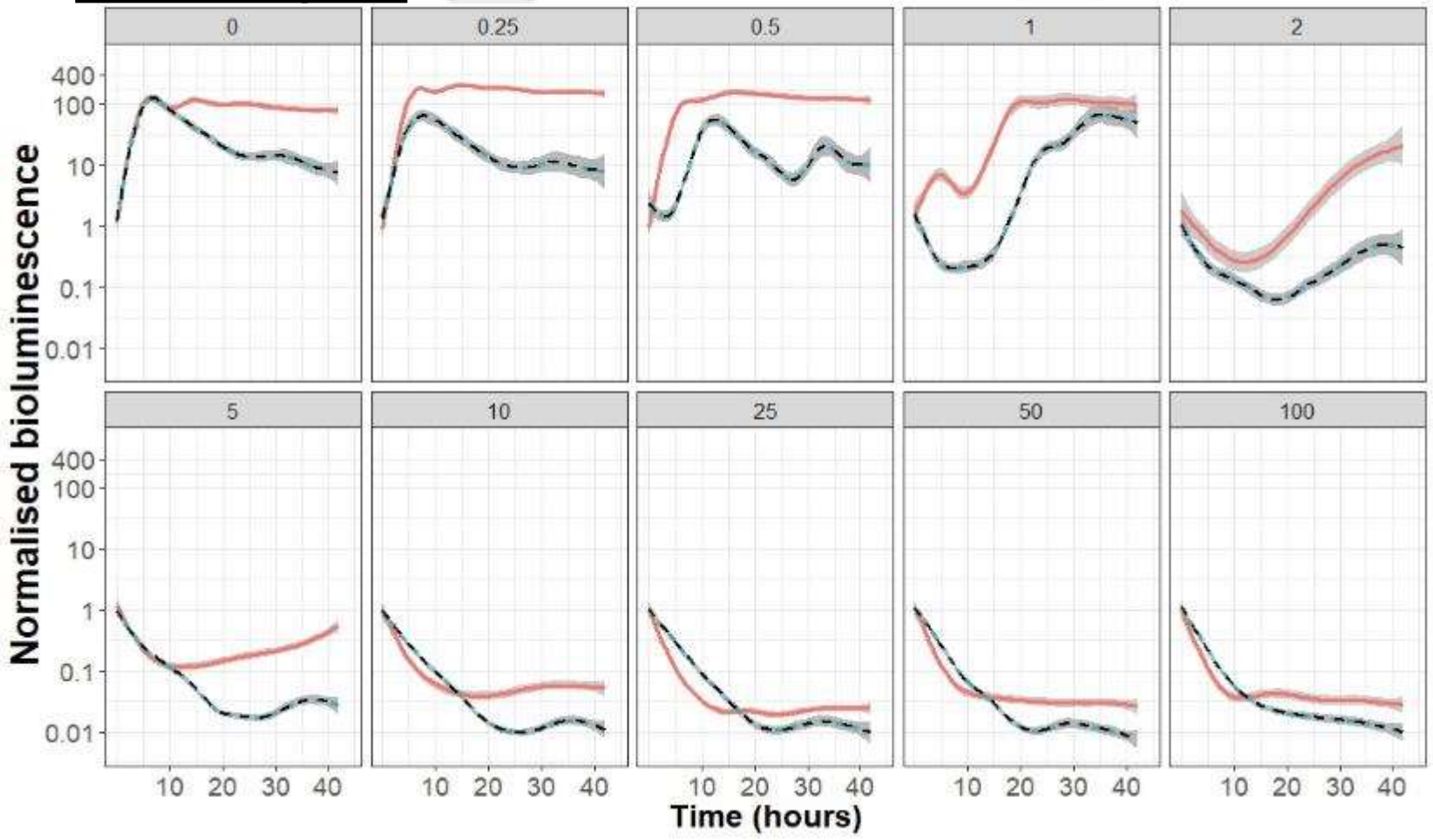


Tobramycin (cation pH7.4)

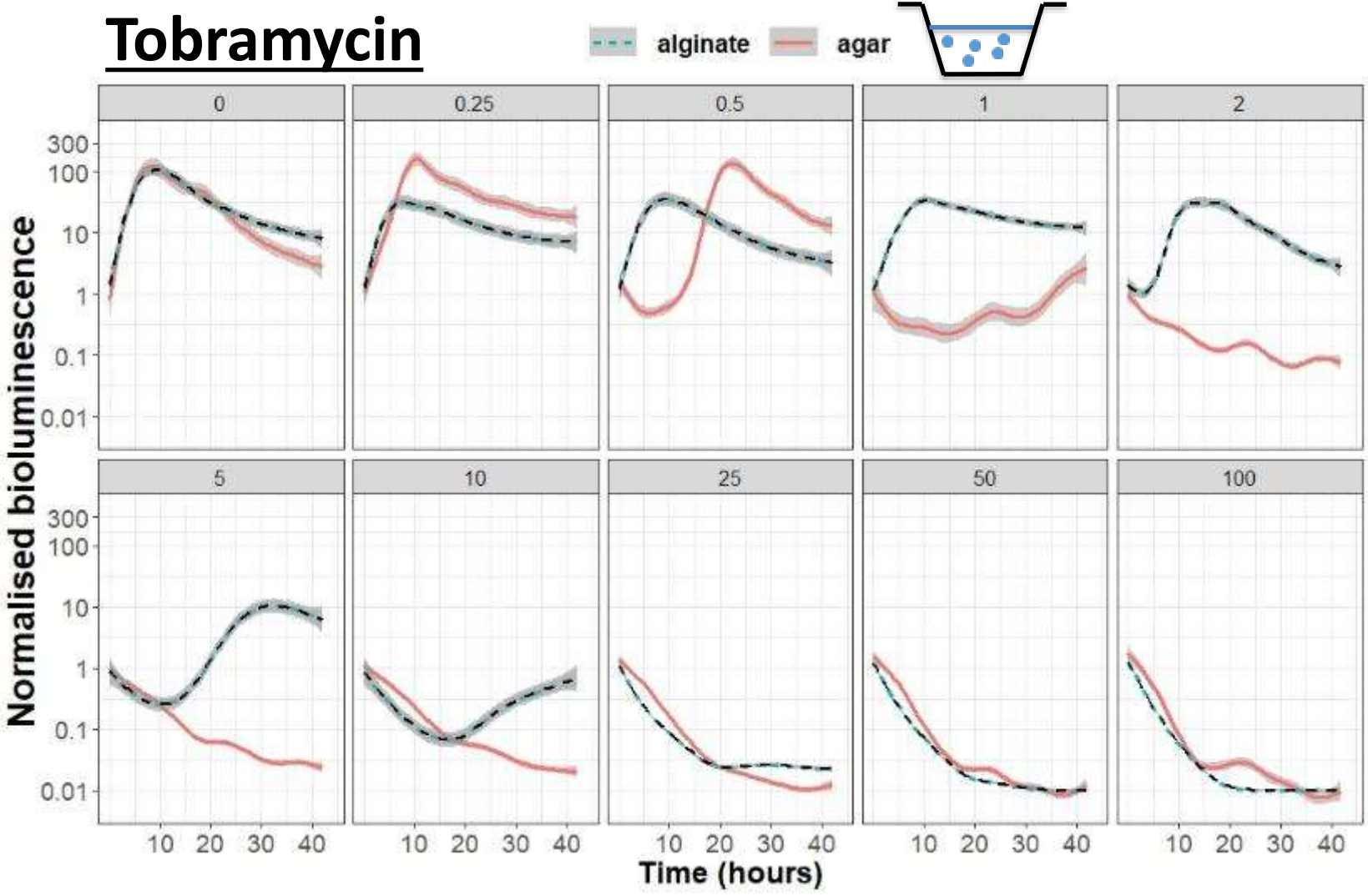
Interaction of the positive charges with the biofilm?

Tobramycin

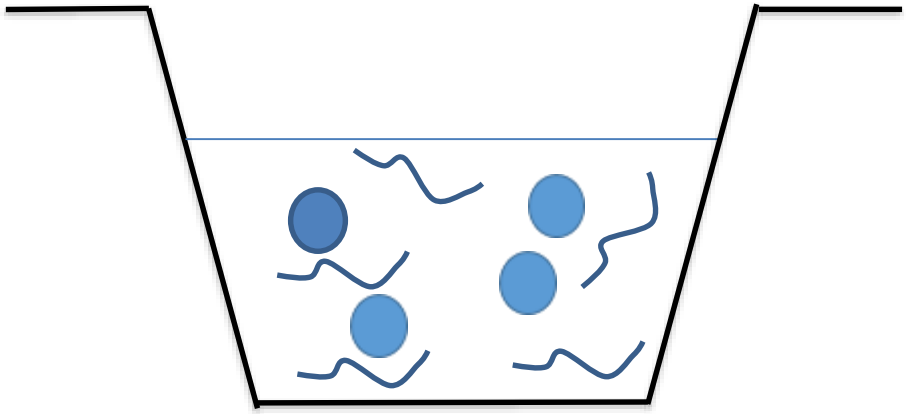

planktonic
biofilm


0
0.25
0.5
1
2
5
10
25
50
100
Normalised bioluminescence
400
100
10
1
0.1
0.01
10
20
30
40
Time (hours)

Tobramycin


alginate
agar
0
0.25
0.5
1
2
5
10
25
50
100
300
100
10
1
0.1
0.01
Normalised bioluminescence
10
20
30
40
Time (hours)

Effect of mucus on time-kill curves



Alginate beads dispersed in ASM to mimic in vivo chronic infections

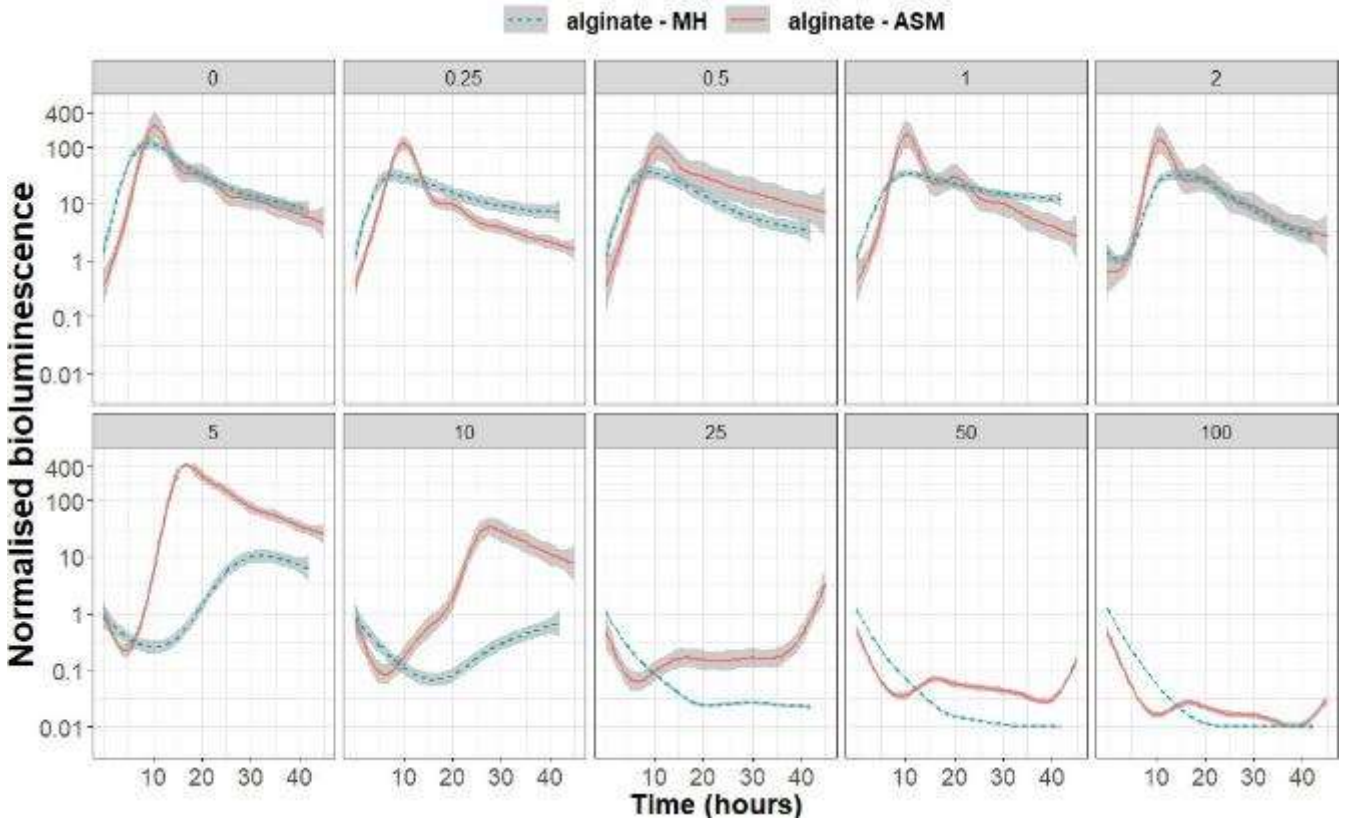
Alginate beads
Bioluminescent PA

Artificial sputum medium (ASM) mimic the sputum of cystic fibrosis

- 5 g/L mucin from pig stomach
- 4 g/L salmon sperm DNA
- 0.5% (v/v) egg yolk emulsion
- 5.9 mg/L of DTPA
- Minerals and amino acids
- pH = 7

Kirchner, Sebastian, et al. Journal of visualized experiments: JoVE 64 (2012).
Sriramulu, Dinesh D., et al. Journal of medical microbiology 54.7 (2005): 667-676.

Effect of mucus on Tobramycin time-kill curves



Results in agreement with Müller *et al.*

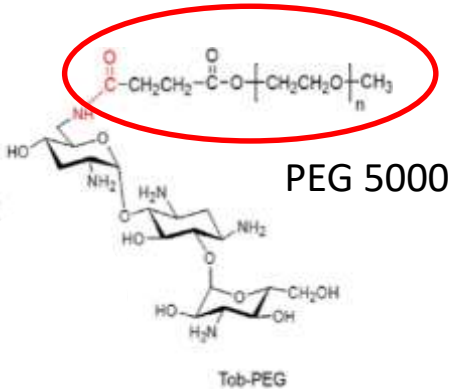
Human airway mucus alters susceptibility of P. aeruginosa biofilms to tobramycin, but not colistin.

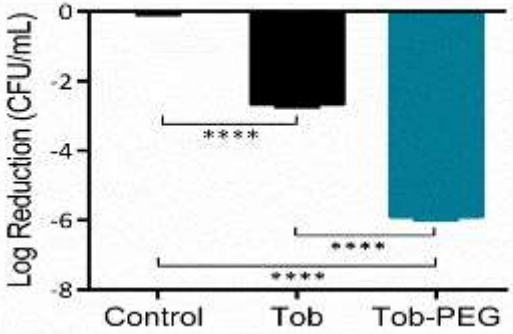
Journal of Antimicrobial Chemotherapy (2018).

A significant reduction of tobramycin efficacy when *P. aeruginosa* biofilms were grown in the presence of mucus-like medium

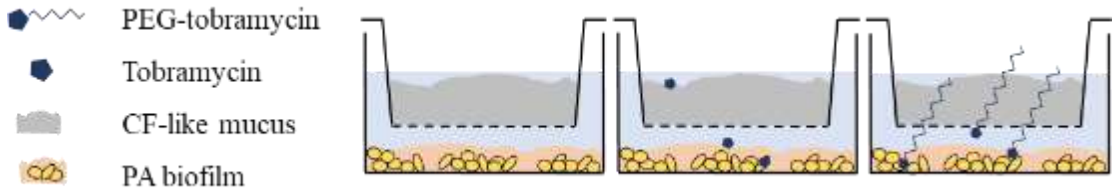
Biofilms in the presence of mucus-like medium respond differently to tobramycin

PEGylation of Tobramycin Improves biofilm antimicrobial activity





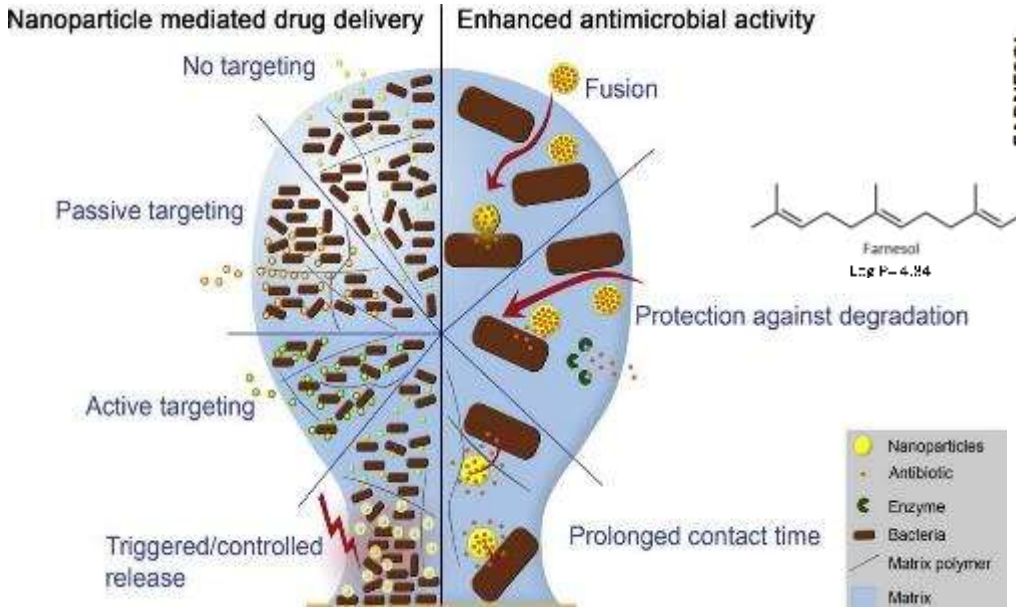
Log-reduction of PA biofilms after treatment with TOB-PEG



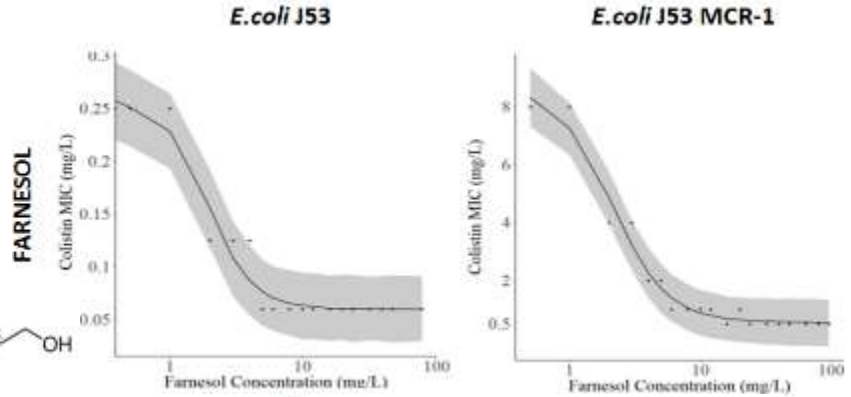
Bahamondez-Canas, T. F., Zhang, H., Tewes, F., Leal, J., Smyth, H. D. (2018) . PEGylation of tobramycin improves mucus penetration and antimicrobial activity against Pseudomonas aeruginosa biofilms in vitro. Molecular pharmaceutics, 15(4), (2018) p 1643-1652.

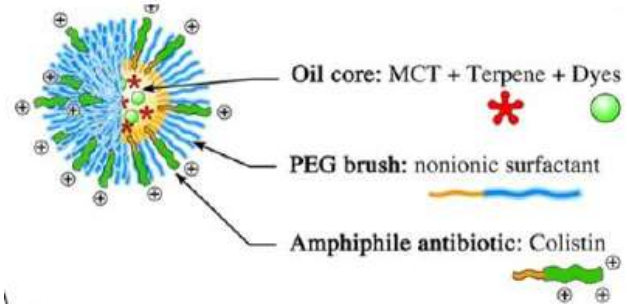
Why nanoparticles to treat pulmonary biofilms?

Improving diffusion
Improving interactions with bacteria and overcome drug efflux pump
Synergic effect with ATB (metal nanoparticles)



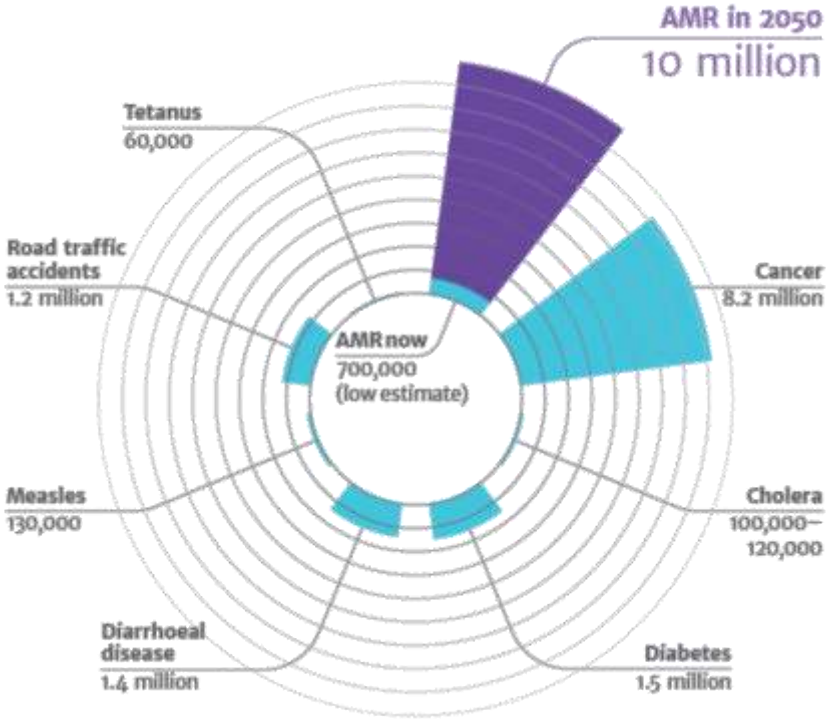
Journal of Controlled Release Volume 190, 2014, Pages 607-623





Lipid nanoparticles to deliver colistin and a terpenic adjuvant

The Review on Antimicrobial Resistance Chaired by Jim O'Neill December 2014



Antimicrobial-resistant (AMR) infections **currently** claim at least 700,000 lives each year

If the rise in resistance to ATB remains the same,
The impact of the AMR by 2050 would lead to 10 million people dying every year

Thank you!

- Julien Brillault
- Sandrine Marchand
- Barbara Lamy
- Isabelle Lamarche
- …

Inserm U1070

- Anne Marie Healy

Trinity College (TCD)

- Hugh Smyth
- Tania Bahamondez-Canas

The University of Texas at Austin