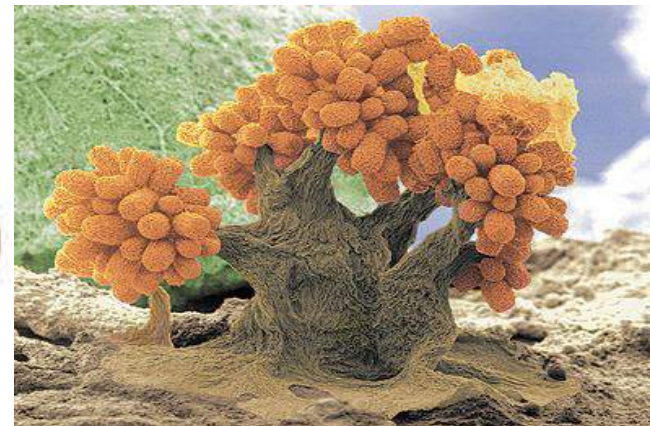
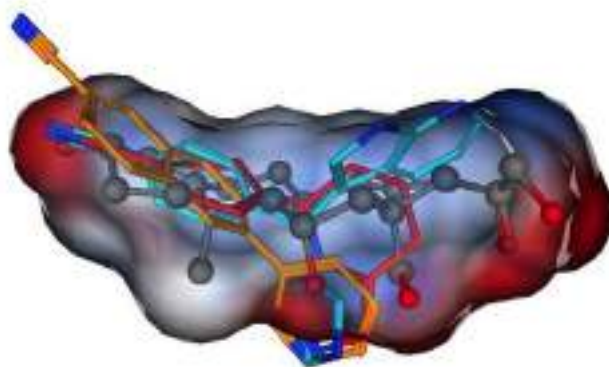
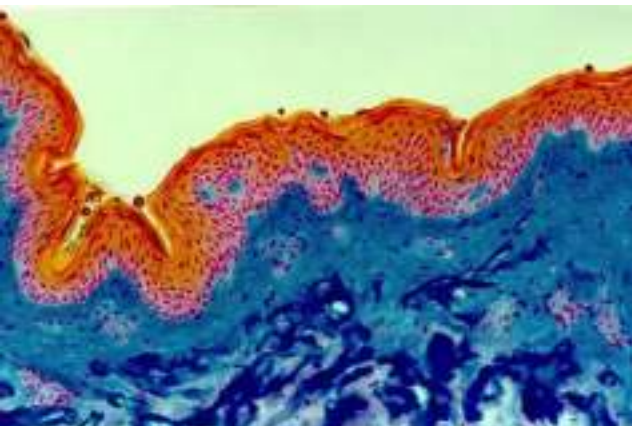


# Non-Invasive Drug Delivery across Epithelial Barriers for Combatting and Preventing Infectious Diseases

DDF Summit, Berlin, March 2017



Prof. Dr Claus-Michael Lehr

Helmholtz Institute for Pharmaceutical Research Saarland (HIPS)

Department of Drug Delivery (DDEL)

[claus-michael.lehr@helmholtz-hzi.de](mailto:claus-michael.lehr@helmholtz-hzi.de)

[www.helmholtz-hzi.de](http://www.helmholtz-hzi.de)



HELMHOLTZ  
ZENTRUM FÜR  
INFEKTIONSFORSCHUNG



UNIVERSITÄT  
DES  
SAARLANDES

# Non-University Research Organizations in Germany

(Funded from federal resources by the country, besides **108 universities** funded by the „Länder“ )

## Basic research



MAX-PLANCK-GESELLSCHAFT

**Max Planck Society**  
[www.mpg.de](http://www.mpg.de)

**83 institutes**  
**17,000 employees**



**Helmholtz Association**  
[www.helmholtz.de](http://www.helmholtz.de)

**18 centres**  
**37,000 employees**  
Total budget 4 bn. EUR/year



**Leibniz Association**  
[www.wgl.de](http://www.wgl.de)

**86 institutes**  
**17,500 employees**

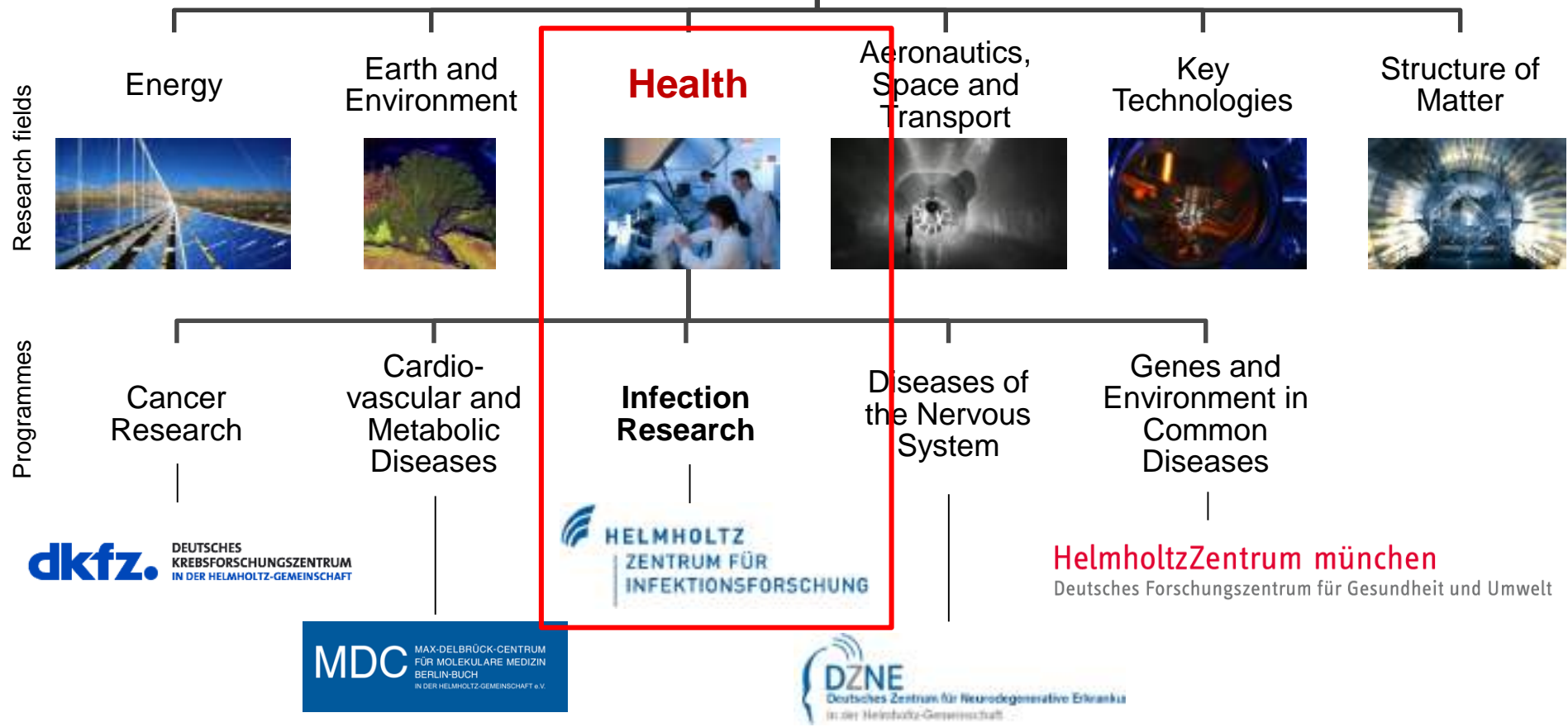


**Fraunhofer Society**  
[www.fraunhofer.de](http://www.fraunhofer.de)

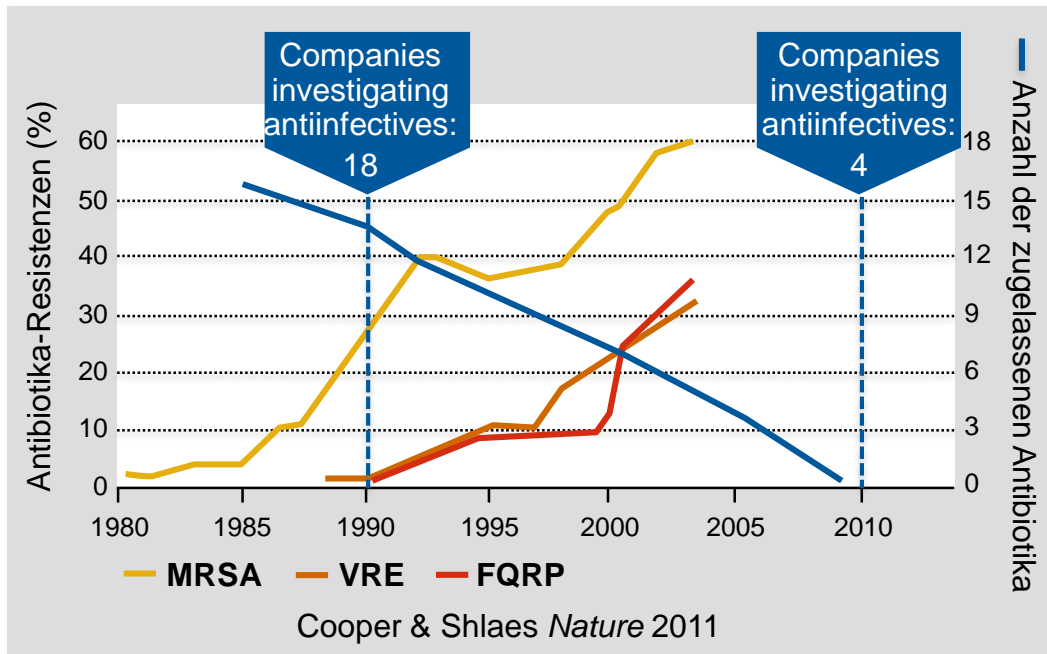
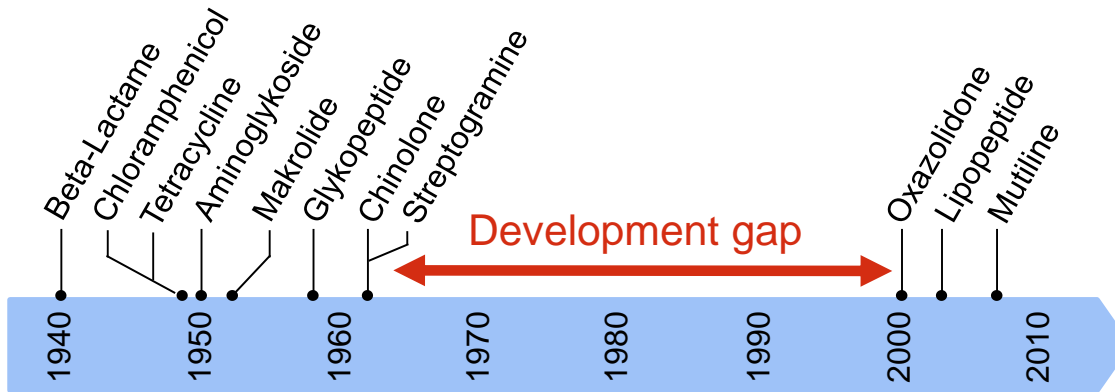
**66 institutes**  
**24,000 employees**

## Application

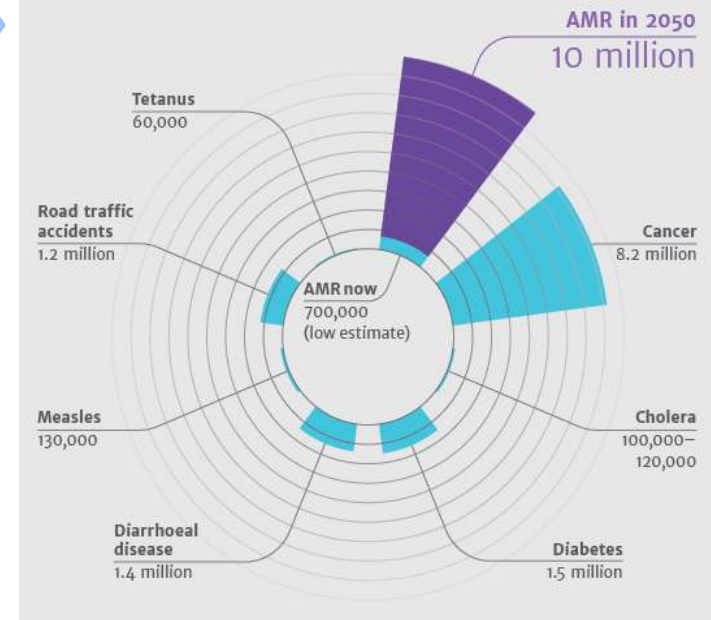
# Research Areas of the Helmholtz Association



# Why is there a research need for anti-infectives?



Deaths attributable to AMR every year compared to other major causes of death

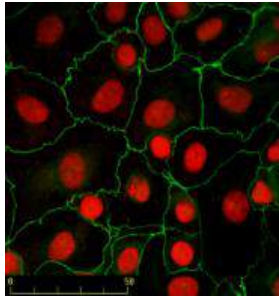


UK Review on Antimicrobial Resistance, Chaired by Jim O'Neill, Dec 2014

# Drug Delivery

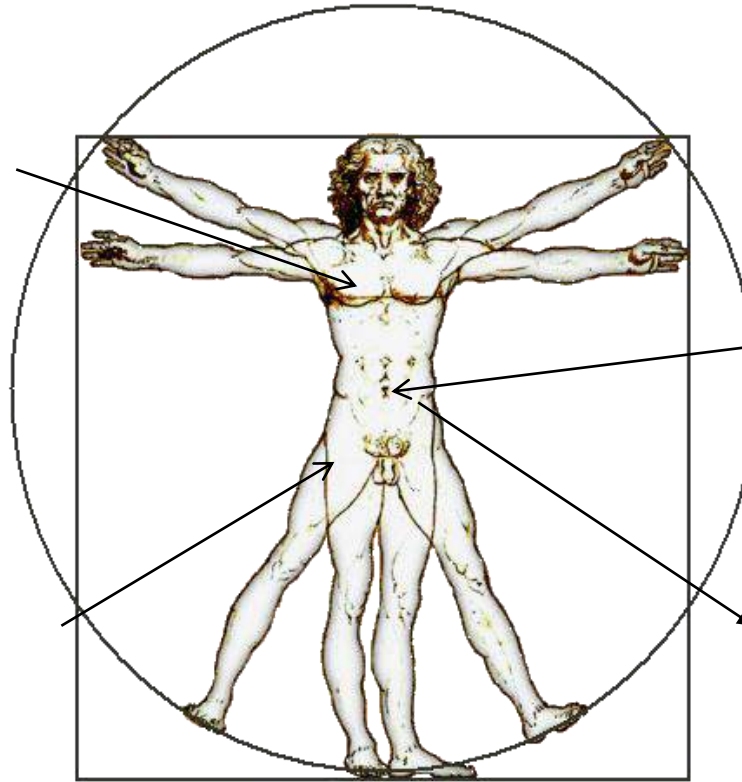
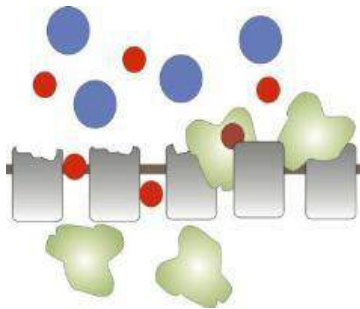
= getting molecules to their site of action;  
must be safe and efficient, preferentially non invasive

Lungs

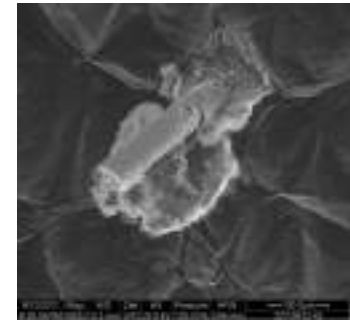


Monolayers of alveolar epithelial cells

Intestinal mucosa



Skin

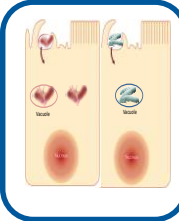
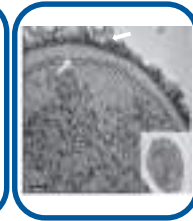


Bacterial Barriers

Biofilms

Bacterial envelop

Host cell



Barriers and Models for Drug Delivery in Health and Disease

Carriers and Technologies for Delivering Antiinfectives and Vaccines

Helmholtz-Institut für Pharmazeutische Forschung Saarland

# Overcoming Epithelial and Microbial Barriers:

## ■ THE GUT:

- Targeting inflamed mucosal areas in IBD
- Pseudobacterial nanocarriers to combat intracellular infections

## ■ THE SKIN: „Needle-free“ transfollicular vaccination

## ■ THE LUNGS:

- Modelling the air-blood barrier in vitro
- Mucus and surfactant as non-cellular barriers
- Towards some novel drug pulmonary nanocarriers

# Inflammatory bowel disease



Ulcerative colitis: restricted to colon and rectum, only mucosa effect

Crohn's disease: transmural inflammation, any part of the GI from mouth to anus, preferably terminal ileum and right colon

Symptoms:

Abdominal pain

Increased mucus production

Frequent defecation

Bloody diarrhea

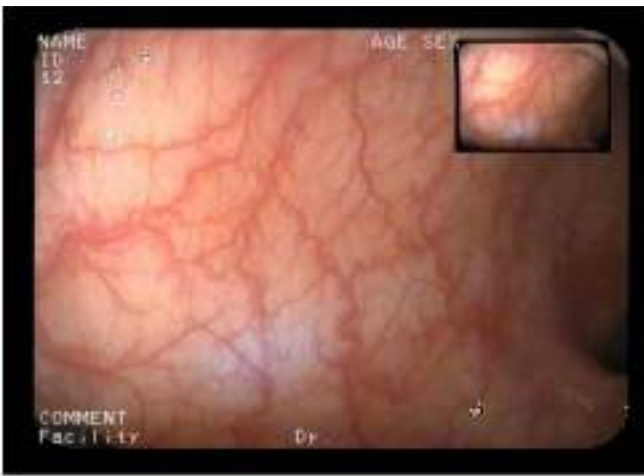
=> difficult to treat!

- short action after topical administration

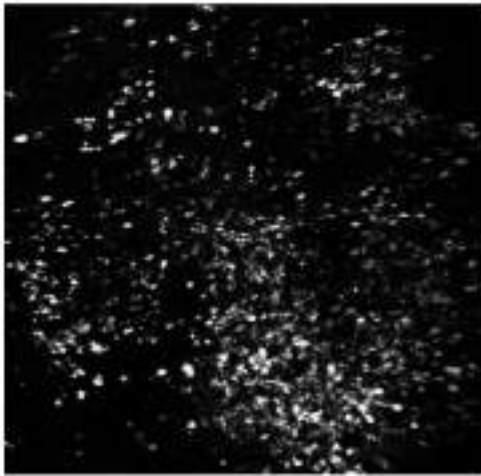
- oral/systemic therapy requires high doses; adverse effects

# Targeting the inflamed mucosa in patients with inflammatory bowel disease (IBD)

Colitis patient with moderately inflamed mucosa



Severely Inflamed Mucosa with flat ulcerations



Schmidt et al., J. Control. Rel. 165 (2013) 139–145

VOLUME 161, ISSUE 2, 26 JANUARY 2013 ISSN: 0169-8299

**journal of controlled release**

OFFICIAL JOURNAL OF THE CONTROLLED RELEASE SOCIETY AND THE JAPANESE SOCIETY OF DRUG DELIVERY SYSTEM

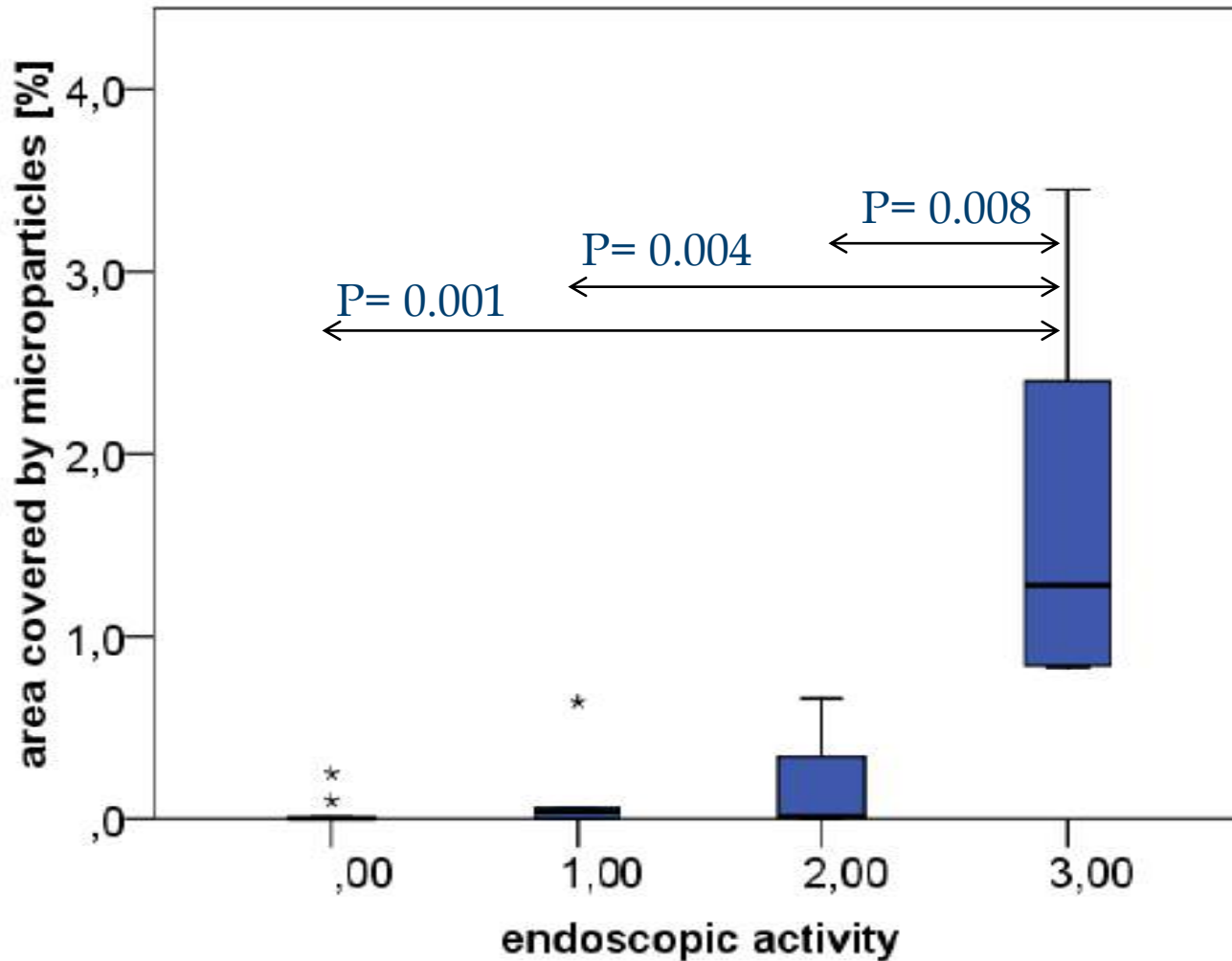
**COVER STORY**  
Targeted delivery nanosensors particles to inflamed intestinal mucosa in human

Helmholtz-Institute for Pharmaceutical Research Saarland





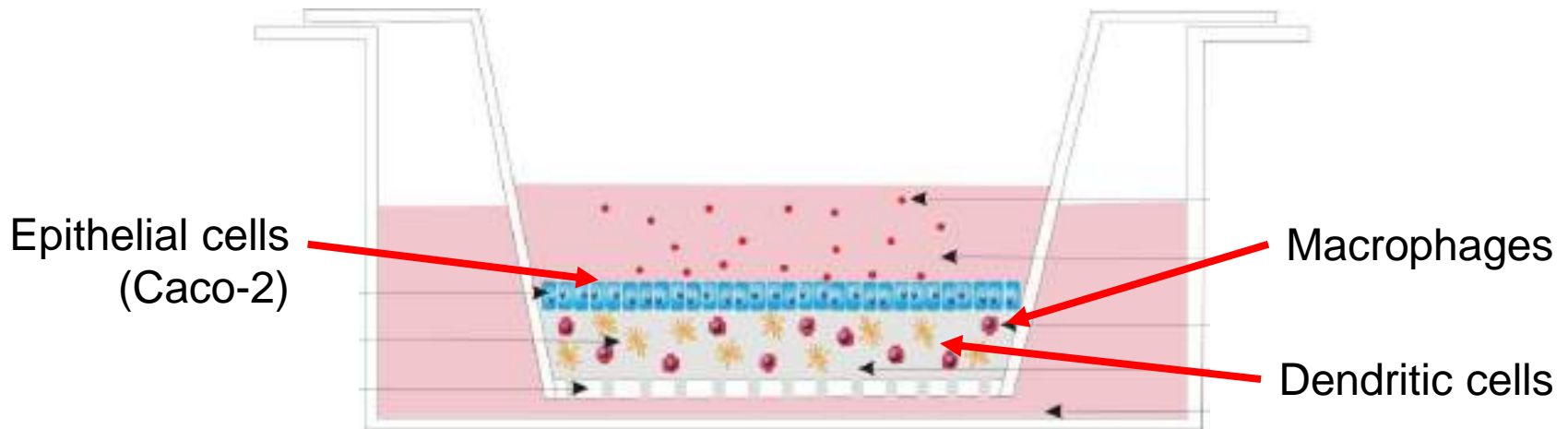
# Accumulation of microparticles in rectal mucosa of IBD patients



Schmidt et al., J. Control. Rel. 165 (2013) 139-145

r Pharmaceutical Research Saarland

# 3D Triple Co-culture Model of the *inflamed* intestinal mucosa



Rhineland-Platinate Research Award 2010

German Federal Research Award 2011 for  
Alternative Methods to Animal testing

CAAT Award for Best paper in ALTEX 2012

Leonhard et al., Mol. Pharm., 2010

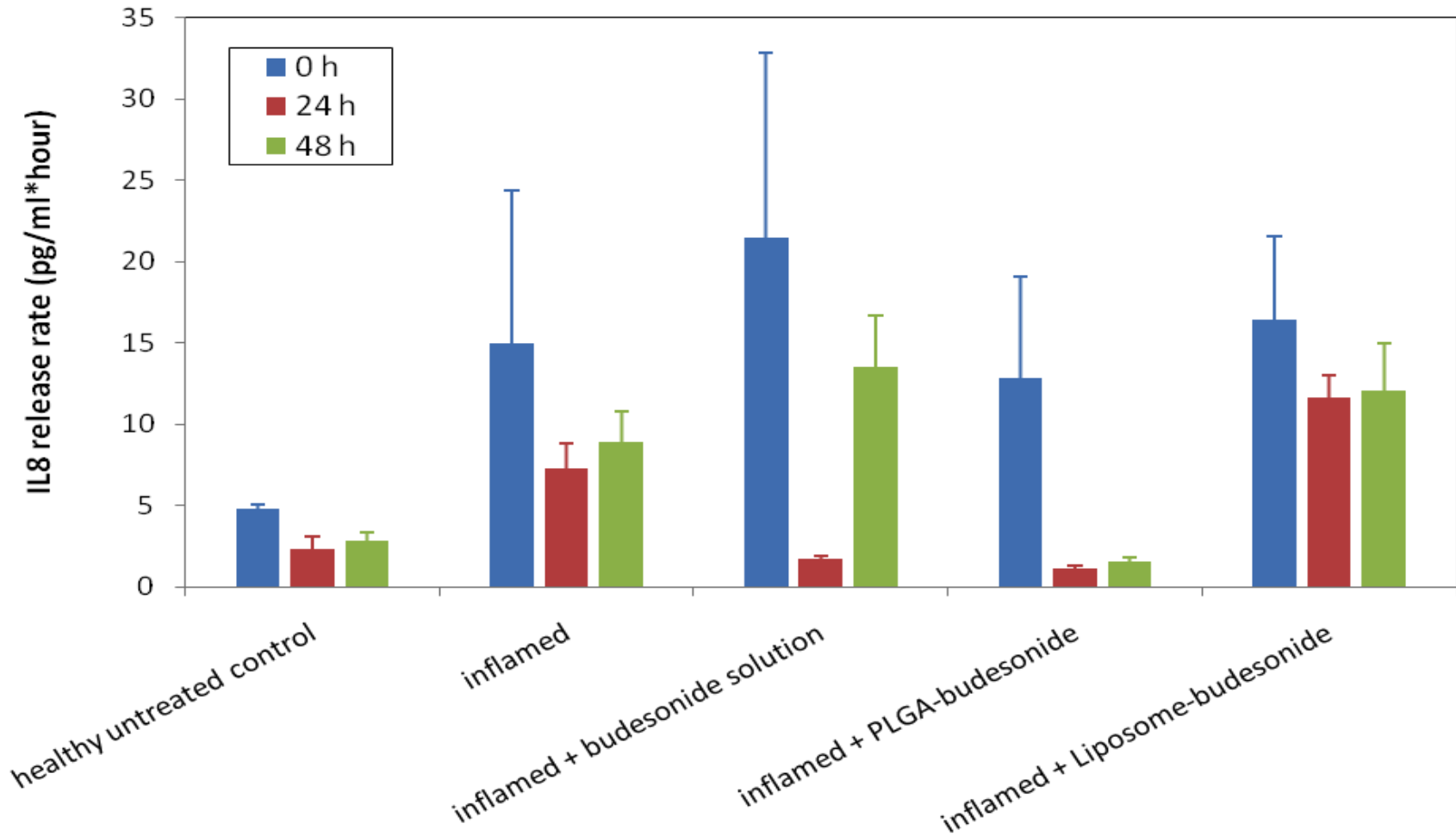


Helmholtz-Institute for Pharmaceutical Research Saarland

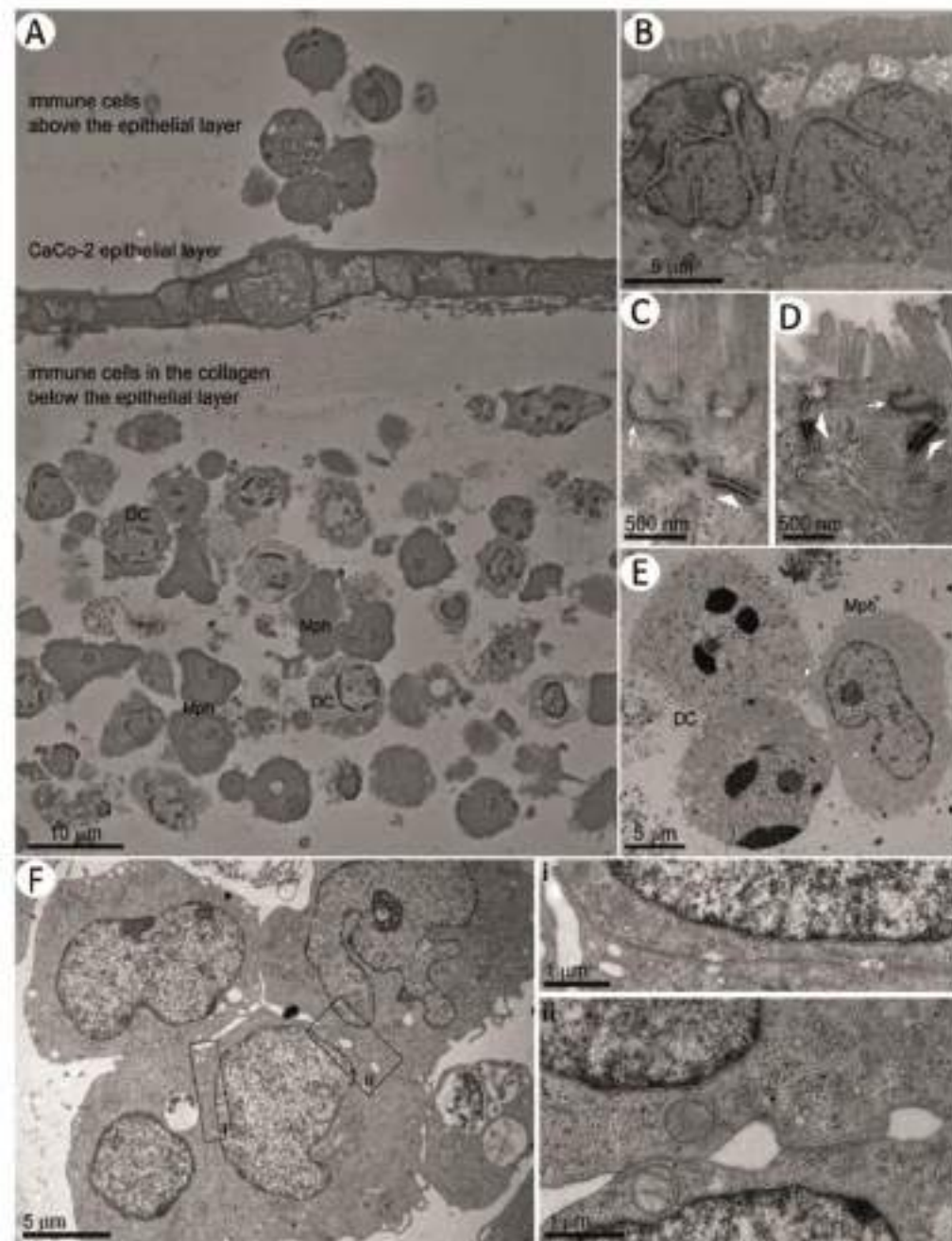


# Effect of budesonide treatment on inflammation:

## Solution <-> Nanoparticles <-> Liposomes



Leonard et al., ALTEX 29 (2012) 275-85



## Next step: Replacing primary cells by human cell lines

(A,B): Caco-2 cells representing the epithelial layer, with immune cells on top and within the collagen matrix.

(C,D): Cell junctions of Caco-2 layer:  
- tight junctions (thin arrows)  
- Desmosomes (fat arrows)

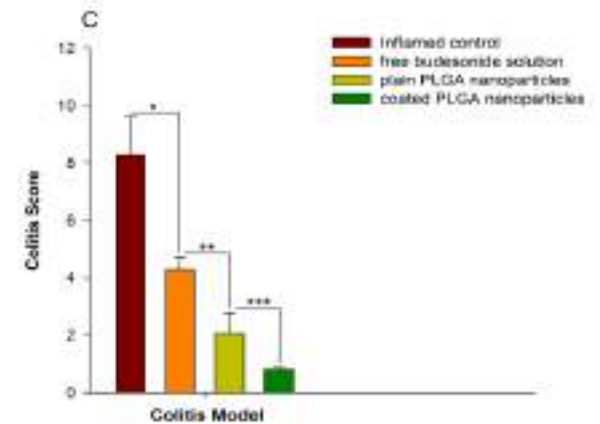
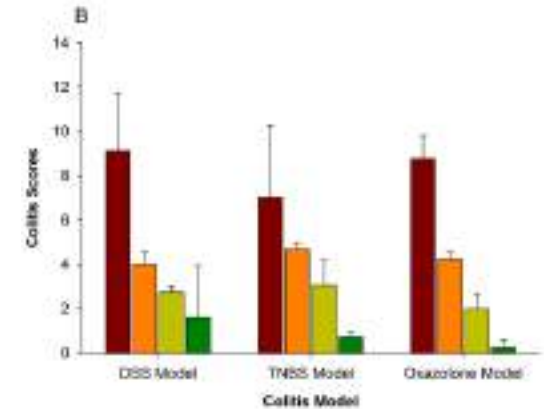
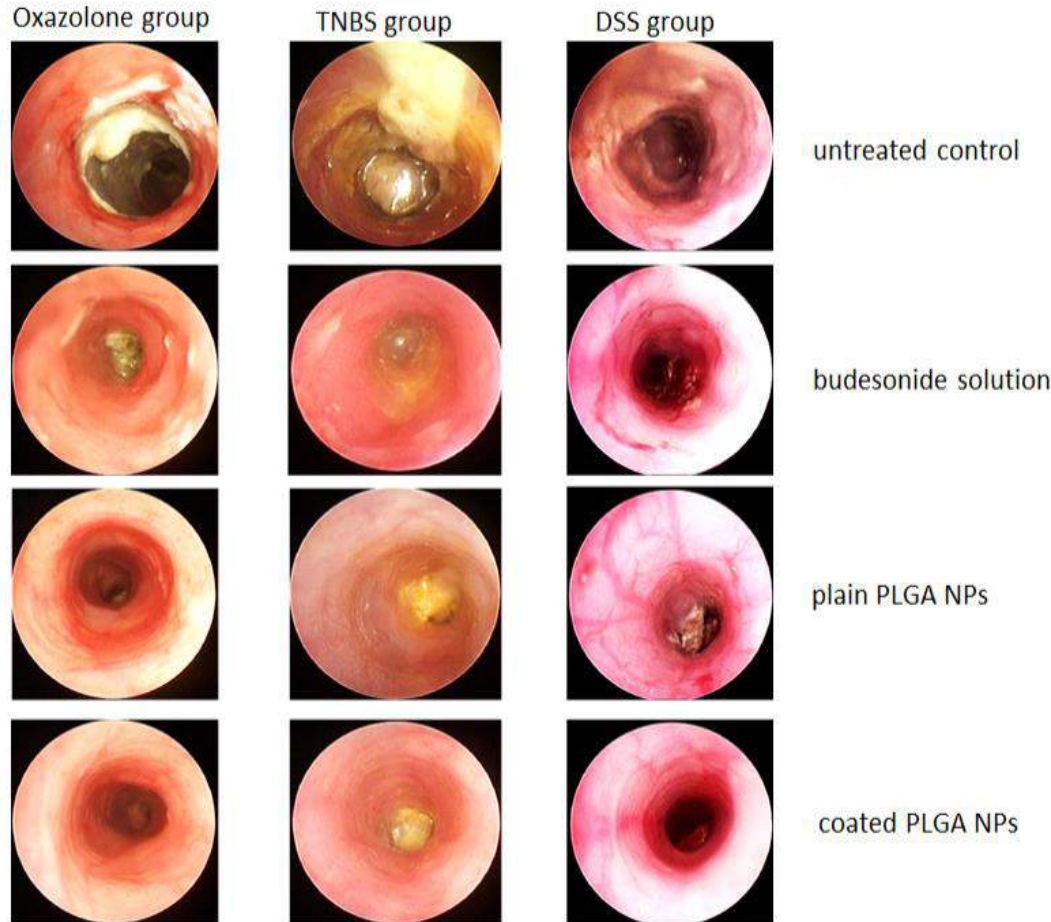
(E,F): Macrophages (Mph) and dendritic cells (DC) show can be distinguished by their structure

Inserts (i,ii): I interaction between immune cells and collagen matrix

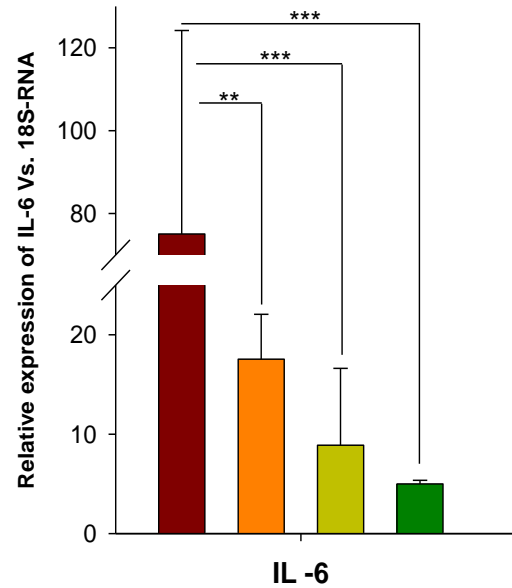
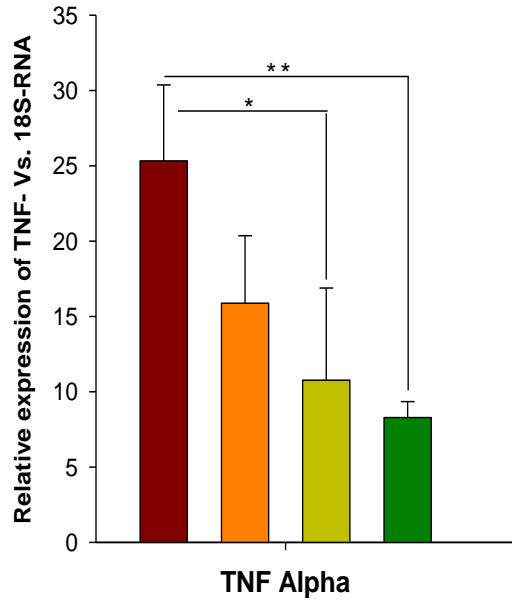
*Susewind et al.,  
Nanotoxicology 2015*

Helmholtz-Institute for Pharmaceutical Research Saarland

But for the time being, animal experiments are still needed:  
 Budesonide loaded nanoparticles with pH-sensitive coating for improved mucosal targeting in mouse models of inflammatory bowel diseases

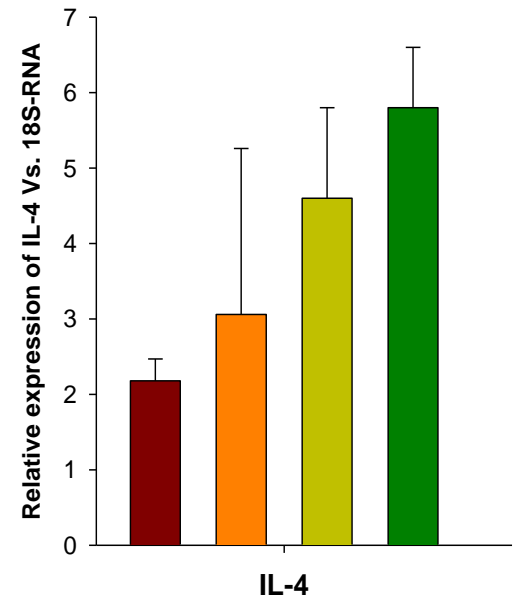
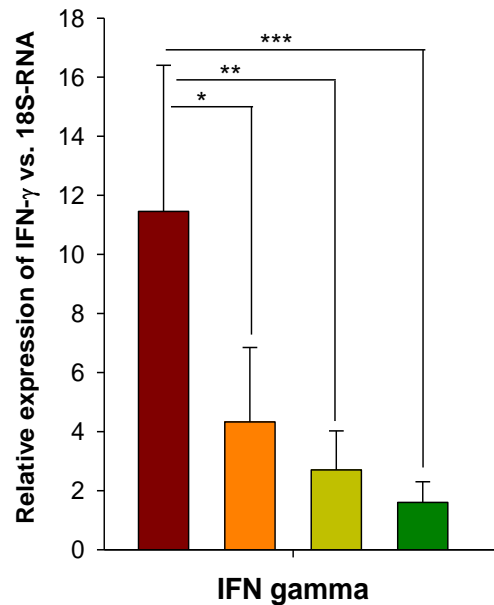
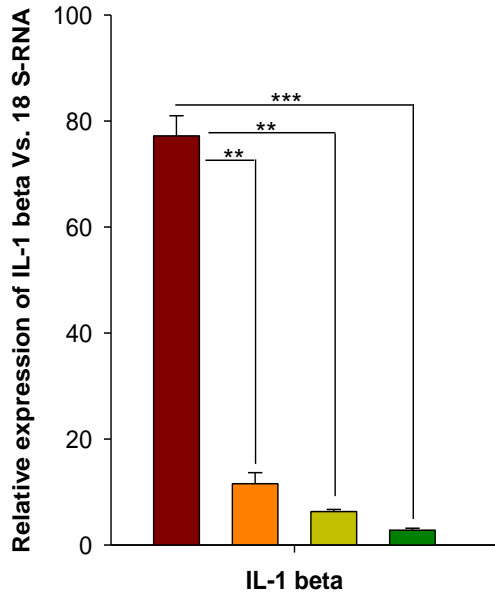


# Expression of Cytokines in Inflamed and Treated Groups



- Inflamed control
- free budesonide solution
- uncoated PLGA nanoparticles
- coated PLGA nanoparticles

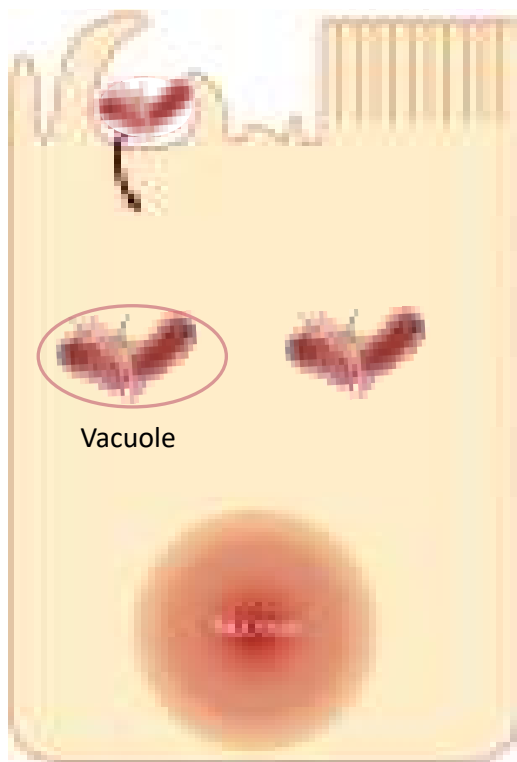
( $p < 0.05 = *$ ,  $p < 0.01 = **$ ,  $p < 0.001 = ***$ )



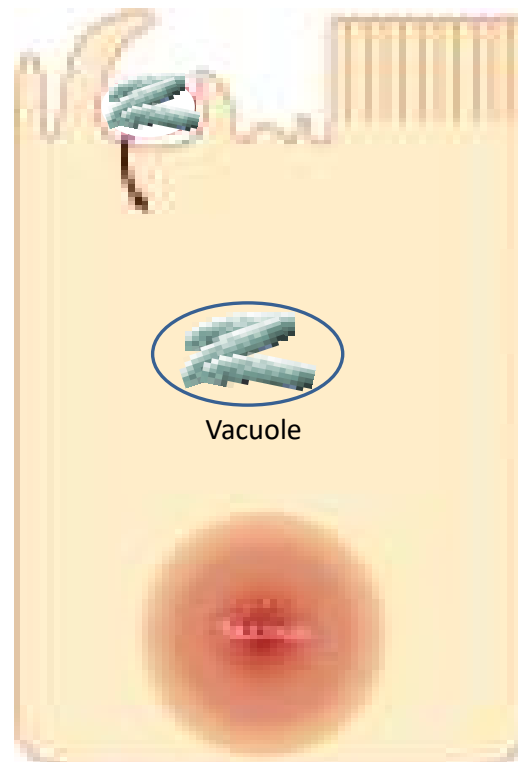
# SWITCHING GEARS: Intracellular infections

**PROBLEM: Bacteria search protection behind membrane structures of the host cell**

*Salmonella*



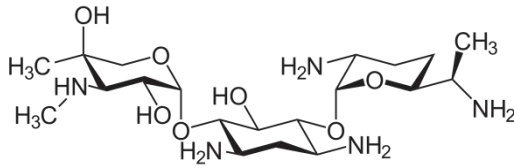
*Yersinia*



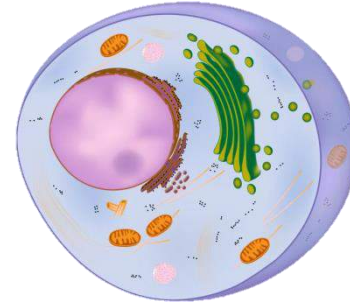
Helmholtz-Institute for Pharmaceutical Research Saarland

Modified from: Donnenberg, M.S. (1999). Nature 401, 218-219.

# Some antibiotics, such as e.g. Gentamicin, are too hydrophilic to permeate across cellular membranes



**Gentamicin**

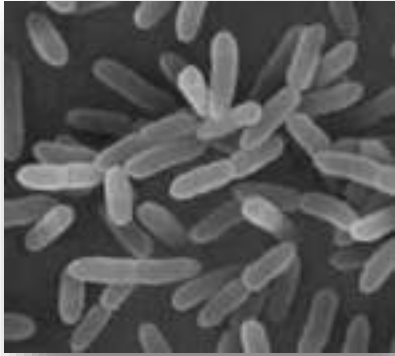


**Cell**



# Yersinia Invasin – a bacterial protein mediating intracellular uptake

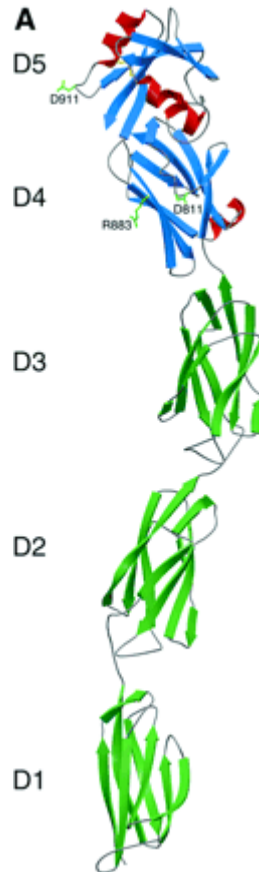
## *Yersinia pseudotuberculosis*



Gram negative

Enteropathogenic bacteria

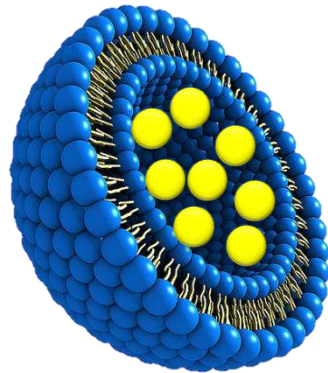
Gastroenteritis



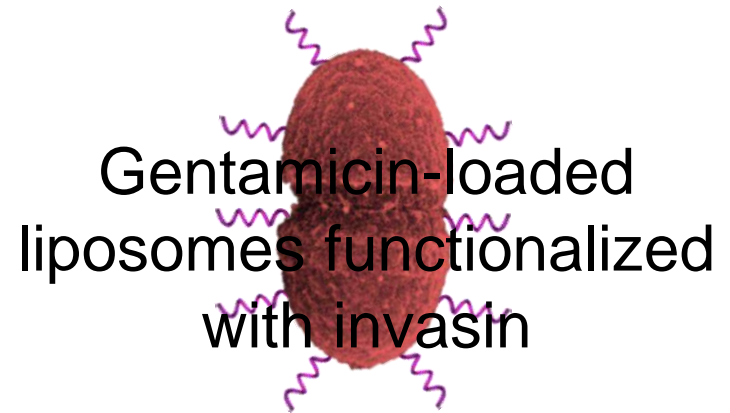
Outer membrane protein

Expressed on the surface of  
*Yersinia pseudotuberculosis*

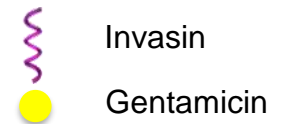
# Apply Invasin from *Y. pseudotuberculosis* to enable uptake of nanocarrier!



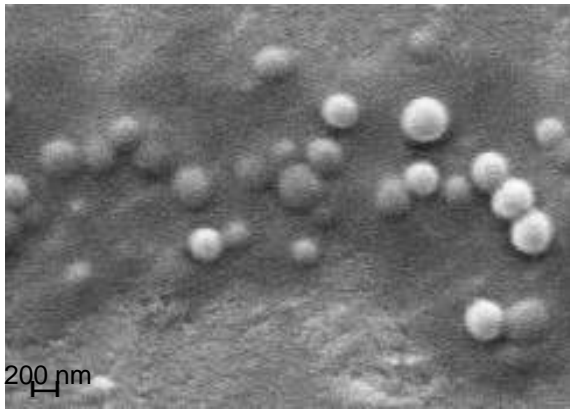
Gentamicin-loaded liposome



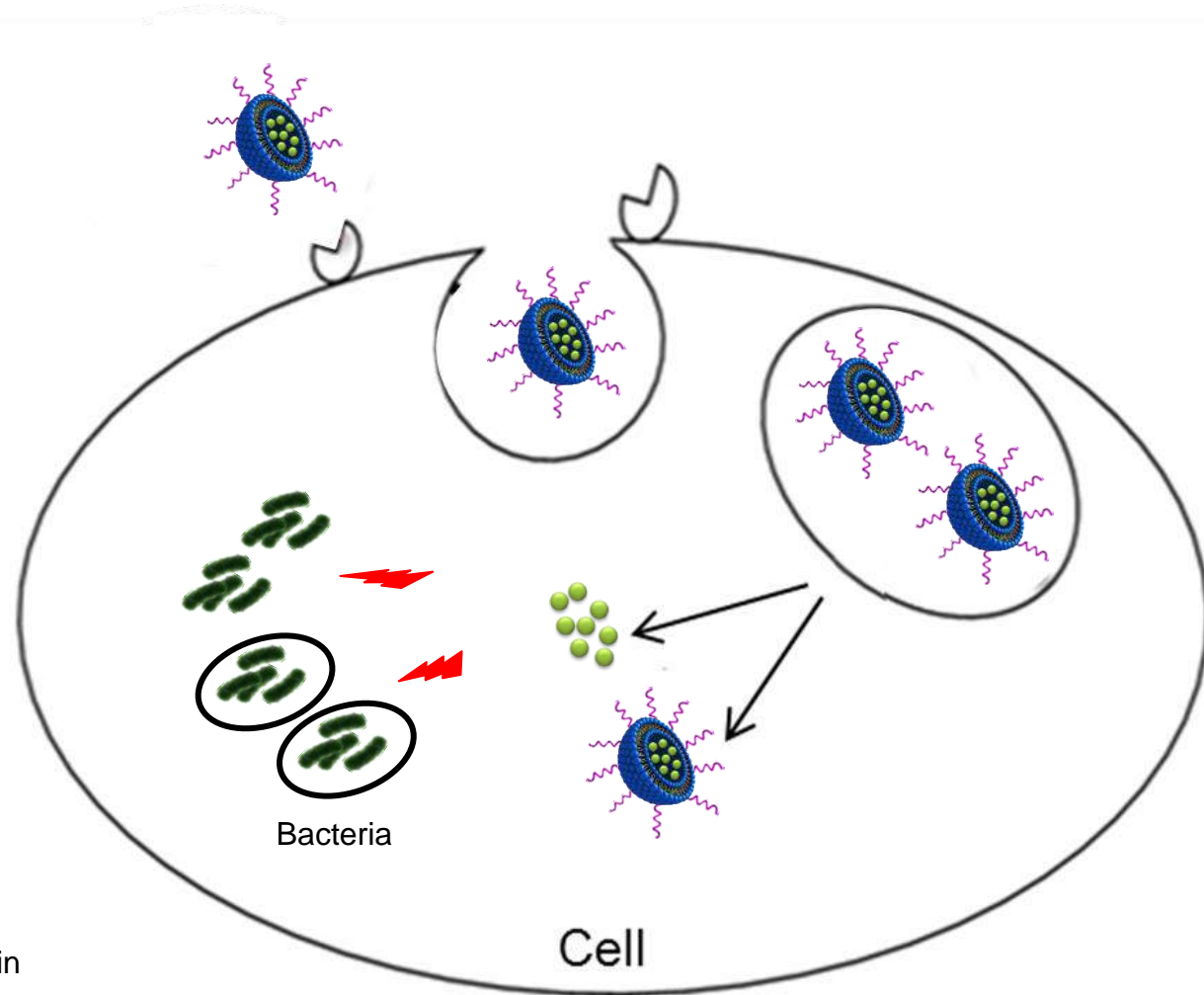
*Y. pseudotuberculosis*







# Improving intracellular delivery of anti-infectives by invasin-decorated nanocarriers



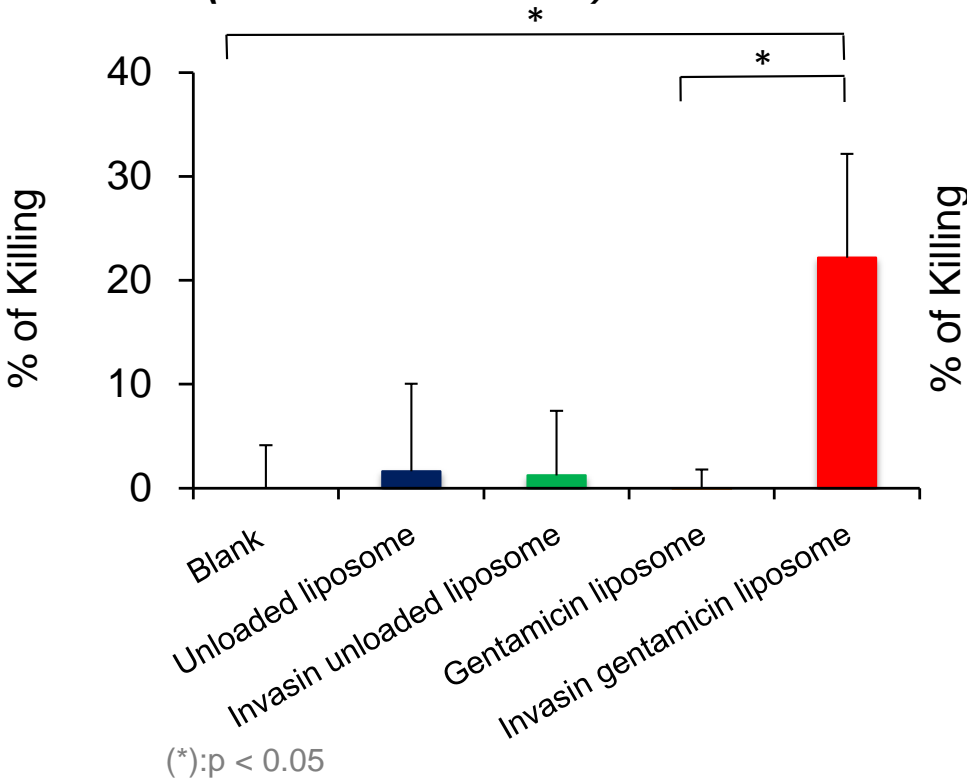
SEM image of gentamicin-loaded liposomes



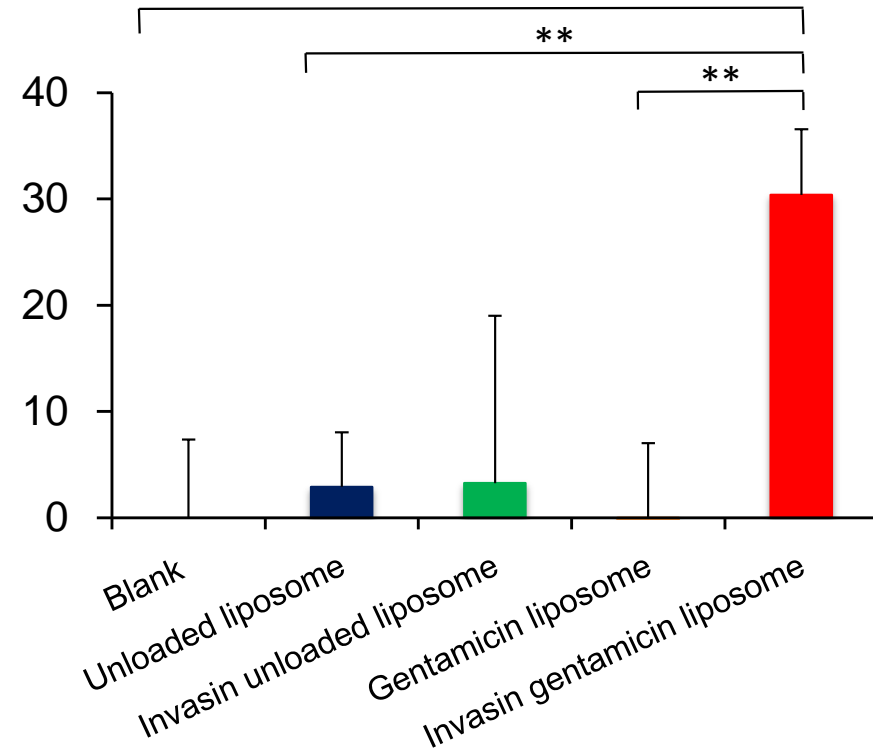
-  Invasin
-   $\beta$ 1-Integrin
-  Gentamicin
-  Bacteria

# Impact of invasin-decorated nanocarriers for gentamicin on intracellular infections

**Salmonella enterica**  
(after 1 h treatment)



**Yersinia pseudotuberculosis**  
(after 2 h treatment)



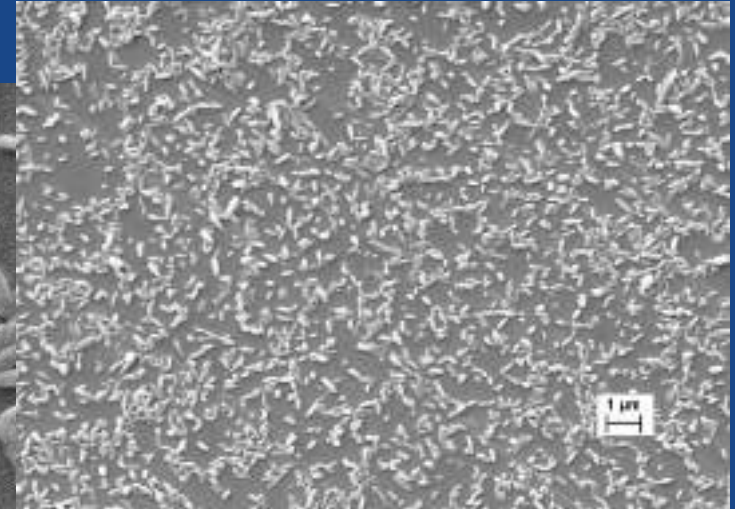
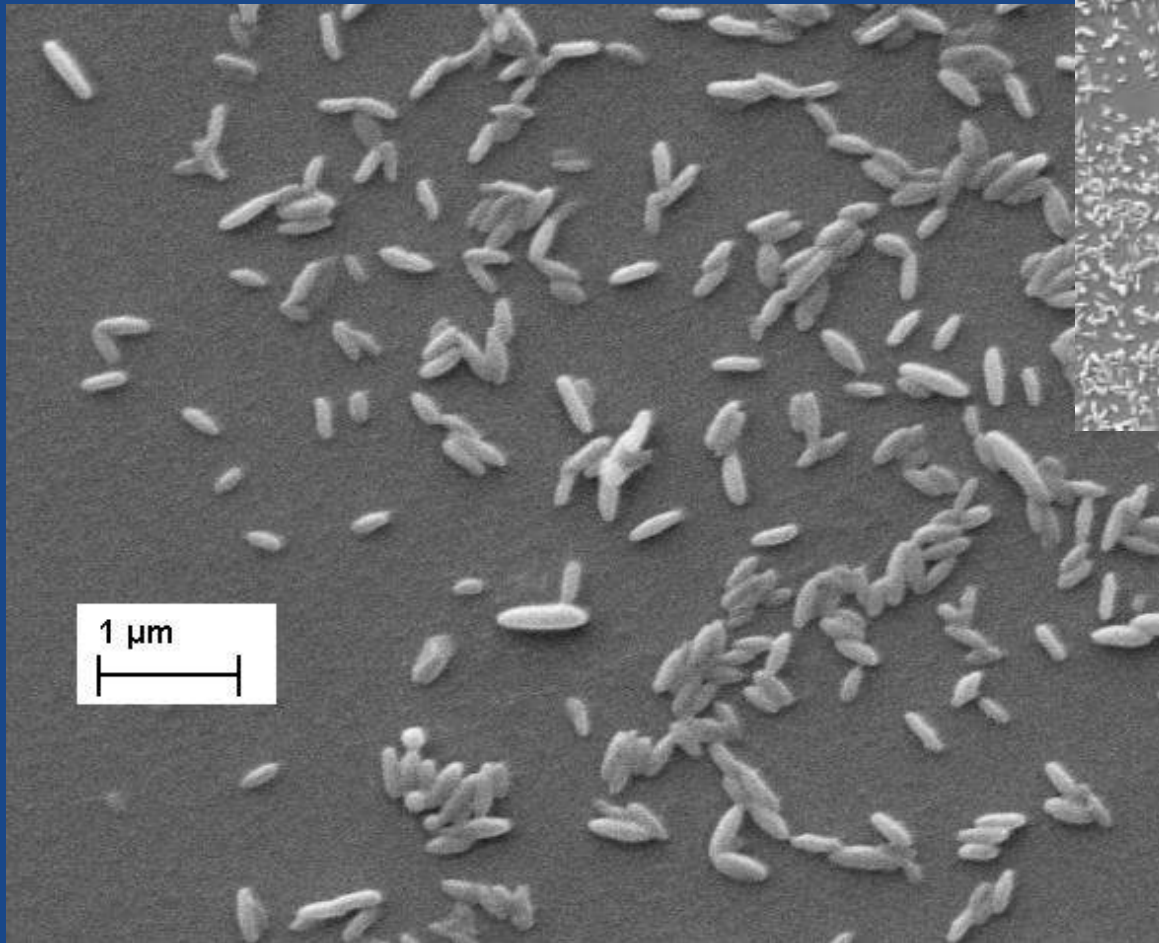
Lehr, C.-M. et al. WO2016/024008.

Menina, S. et al. RSC Advances (2016) 6: 41622-41629.

Labouta, H. et al. Journal of Controlled Release (2015) 220: 414-424.

Helmholtz-Institute for Pharmaceutical Research Saarland

# Outlook: Aspherical “rice-like” nanocarriers: Will they mimick bacteria even better?



A. Castoldi, PhD Project

Helmholtz-Institute for Pharmaceutical Research Saarland

# Overcoming Epithelial and Microbial Barriers:

## ■ THE GUT:

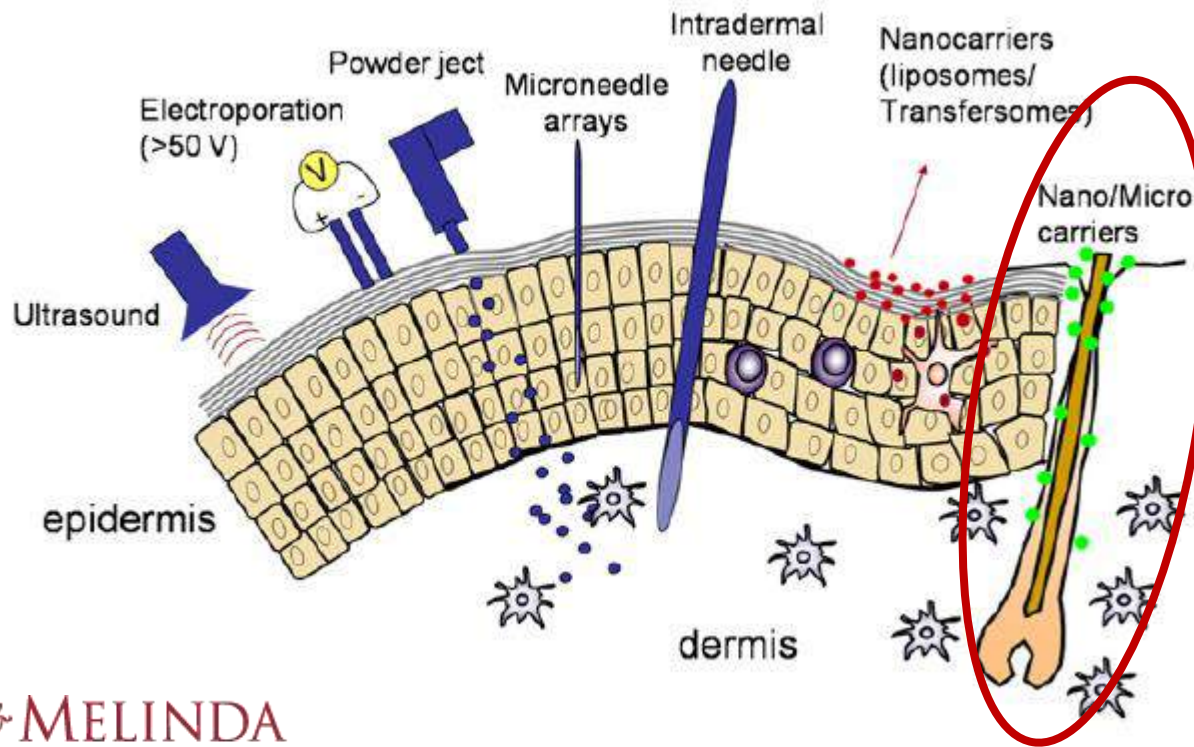
- Targeting inflamed mucosal areas
- Pseudobacterial nanocarriers to combat intracellular infections

## ■ THE SKIN: „Needle-free“ transfollicular vaccination

## ■ THE LUNGS:

- Modelling the air-blood barrier in vitro
- Mucus and surfactant as non-cellular barriers
- Towards some novel pulmonary nanocarriers

# Various approaches for Transdermal vaccination



BILL & MELINDA  
GATES foundation

Bal et al., J. Control. Release. 2010,266-82

Bal et al., J. Control. Release. 2010,266-82

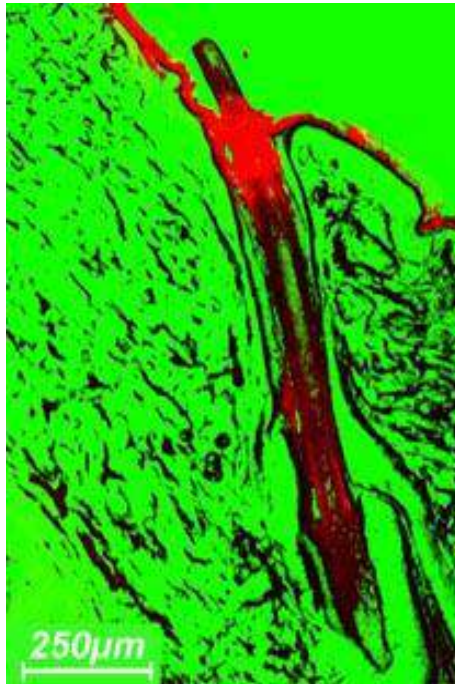
- ✎ Either invasive methods or reducing skin barrier properties
- ✎ Suboptimal for certain applications such as mass vaccination campaigns
- ✎ Special device required

Ankit Mittal, PhD Thesis, 2014

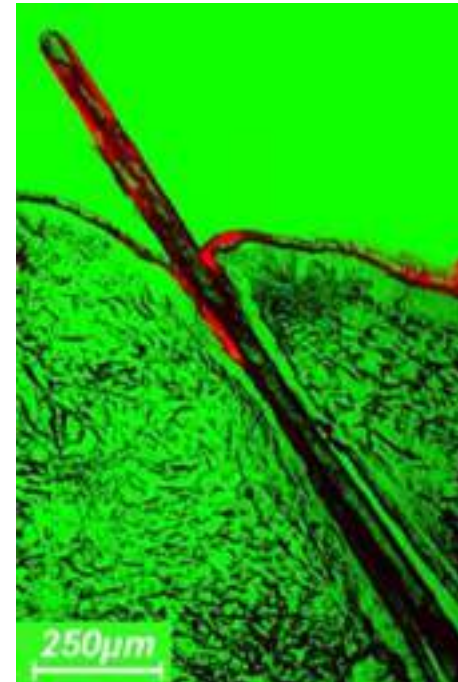
Helmholtz-Institute for Pharmaceutical Research Saarland

# Nanoparticles do not permeate the stratum corneum, but they do penetrate into hair follicles!

pig ear skin (in vitro), after massage  
PLGA NP's ( 320 nm )



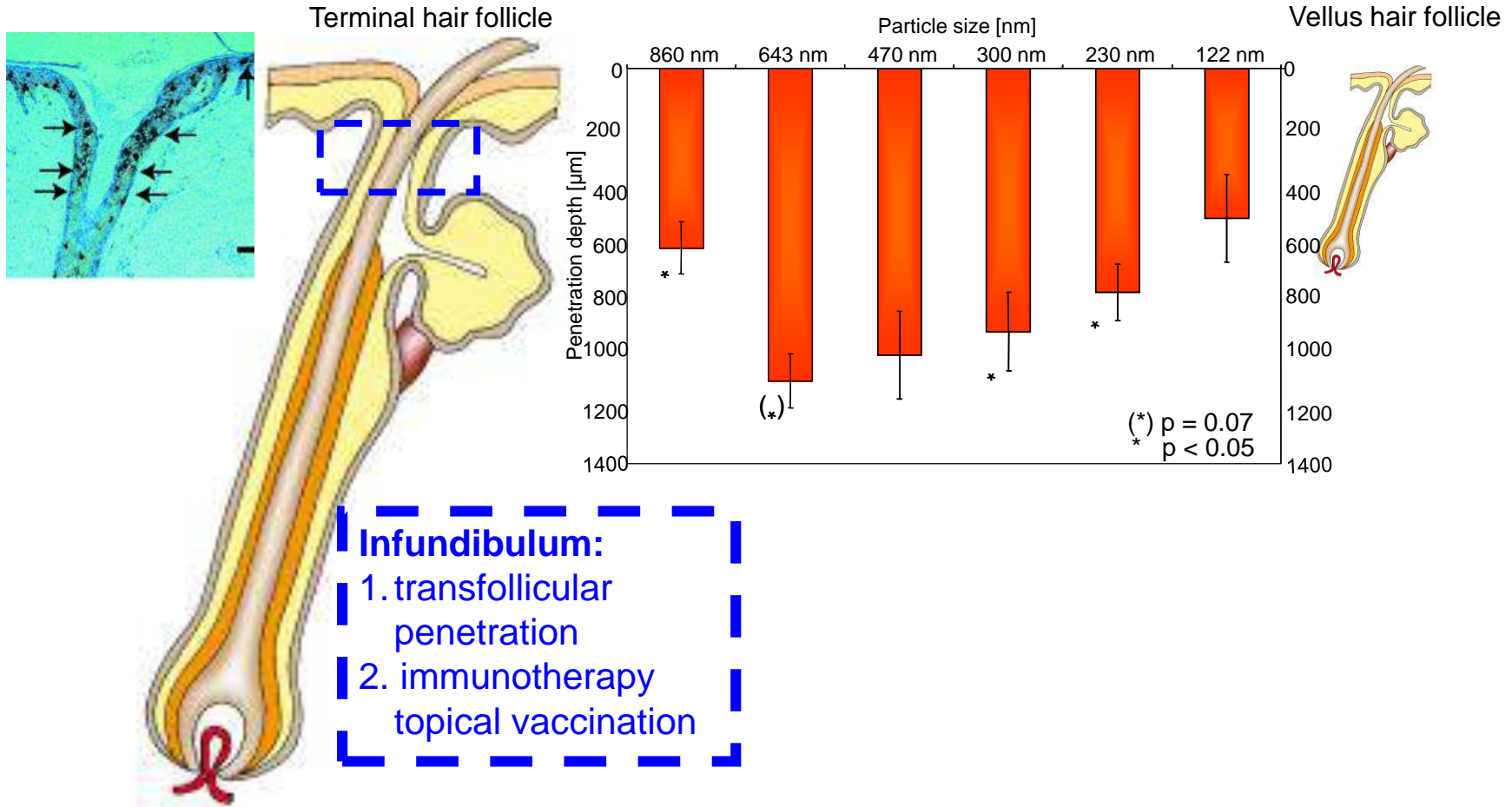
Fluorescent particles



Fluorescent dye

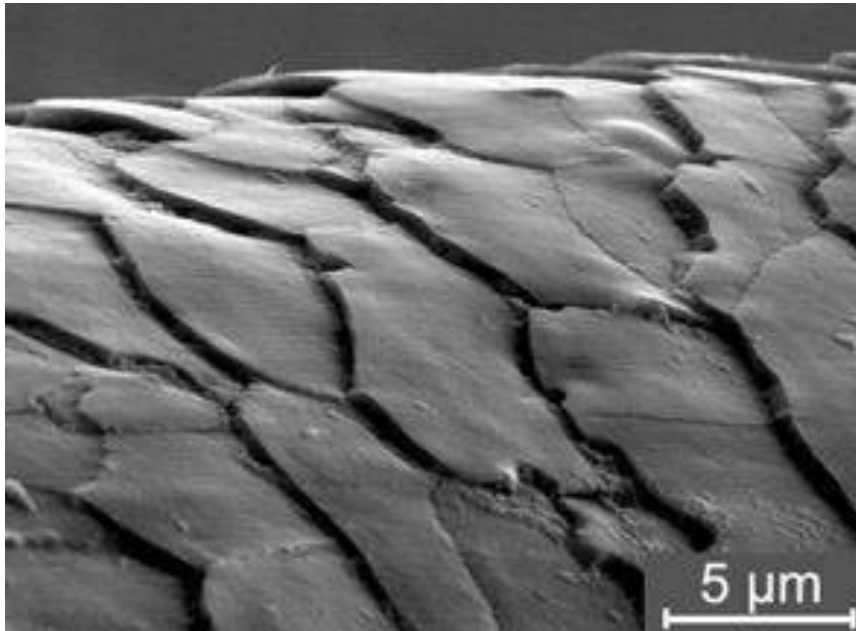


# BUT SIZE DOES MATTER: Effect on follicular penetration (pig ear)



Slide courtesy of J. Lademann, Charité Berlin

# Hair follicle pump



Courtesy Prof J . Lademann, Berlin

# First vaccination experiment:

## Formulations tested:

- 1. Intact Skin**
- Blank Chit-PLGA NPs + c-di AMP
  - OVA in solution
  - OVA Chit-PLGA NPs
  - OVA Chit-PLGA NPs + c-di AMP

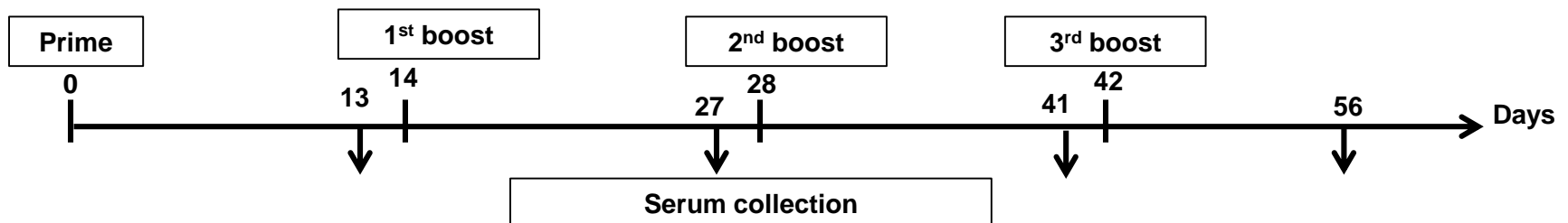


C57BL/6 mice

**Dose:** 200 µg OVA (in 60µl), **N** = 5

**Analysed:** - OVA Specific IgG titres  
- Cellular response

- 2. Tape stripped skin**
- OVA in solution
  - OVA Chit-PLGA NPs

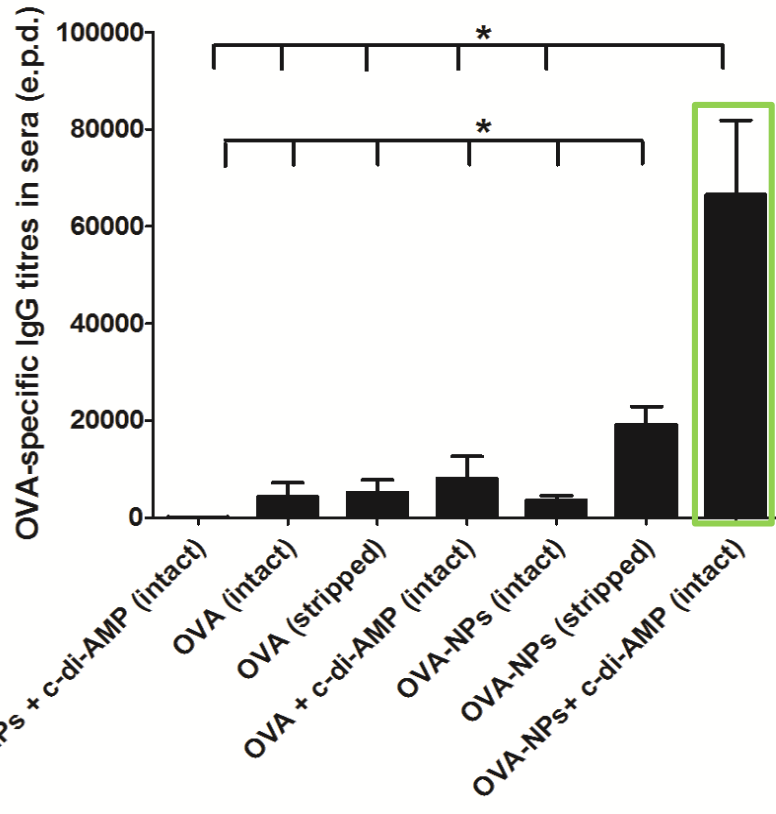


# Transfollicular Vaccination: Humoral Immune Response

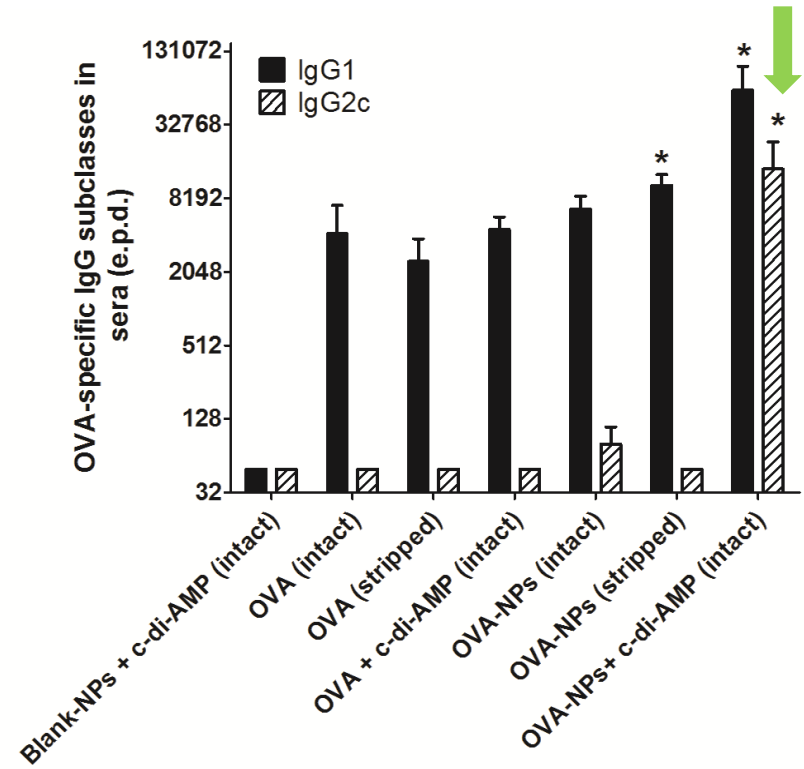
(After 3<sup>rd</sup> Boost)



## IgG Response



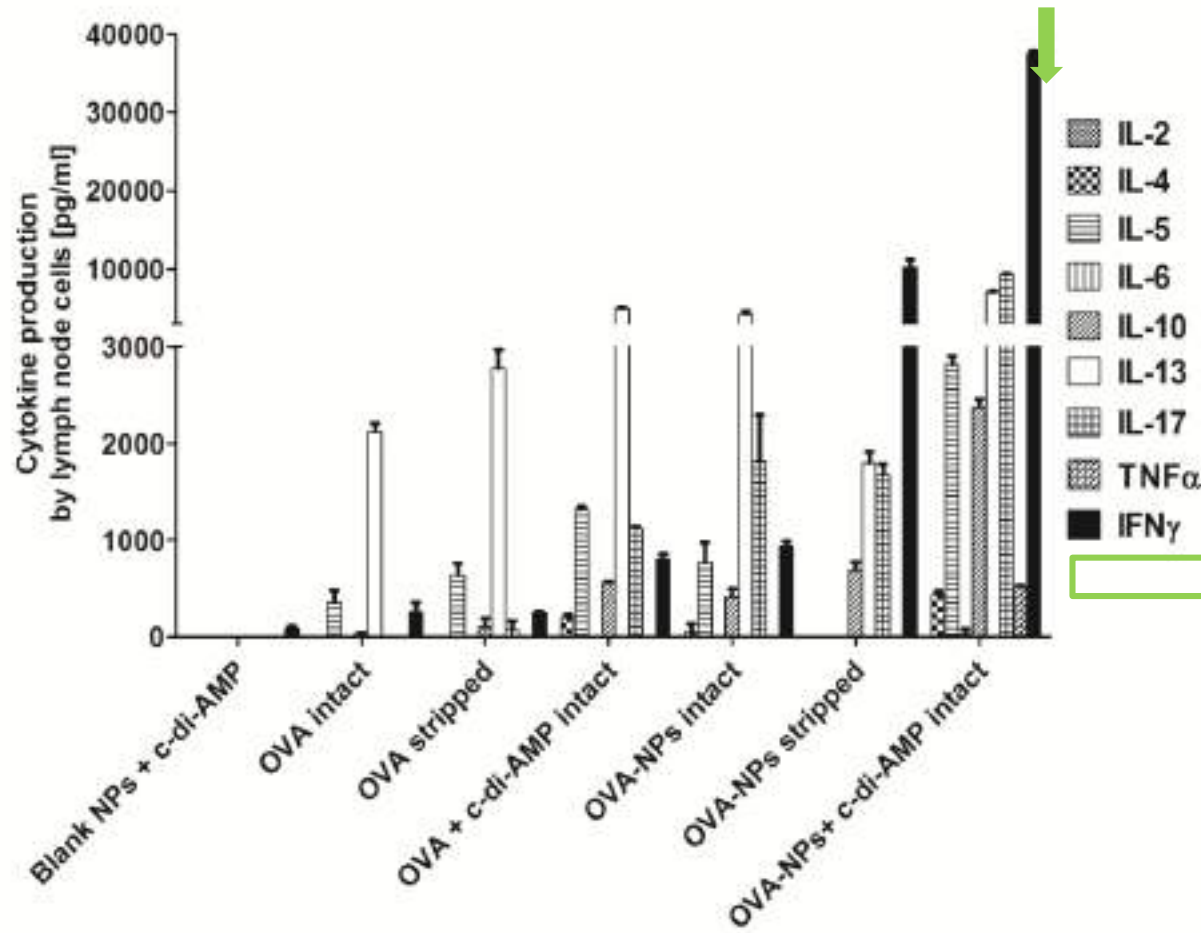
## IgG Subclasses



# Transfollicular Vaccination: Cellular Immune Response

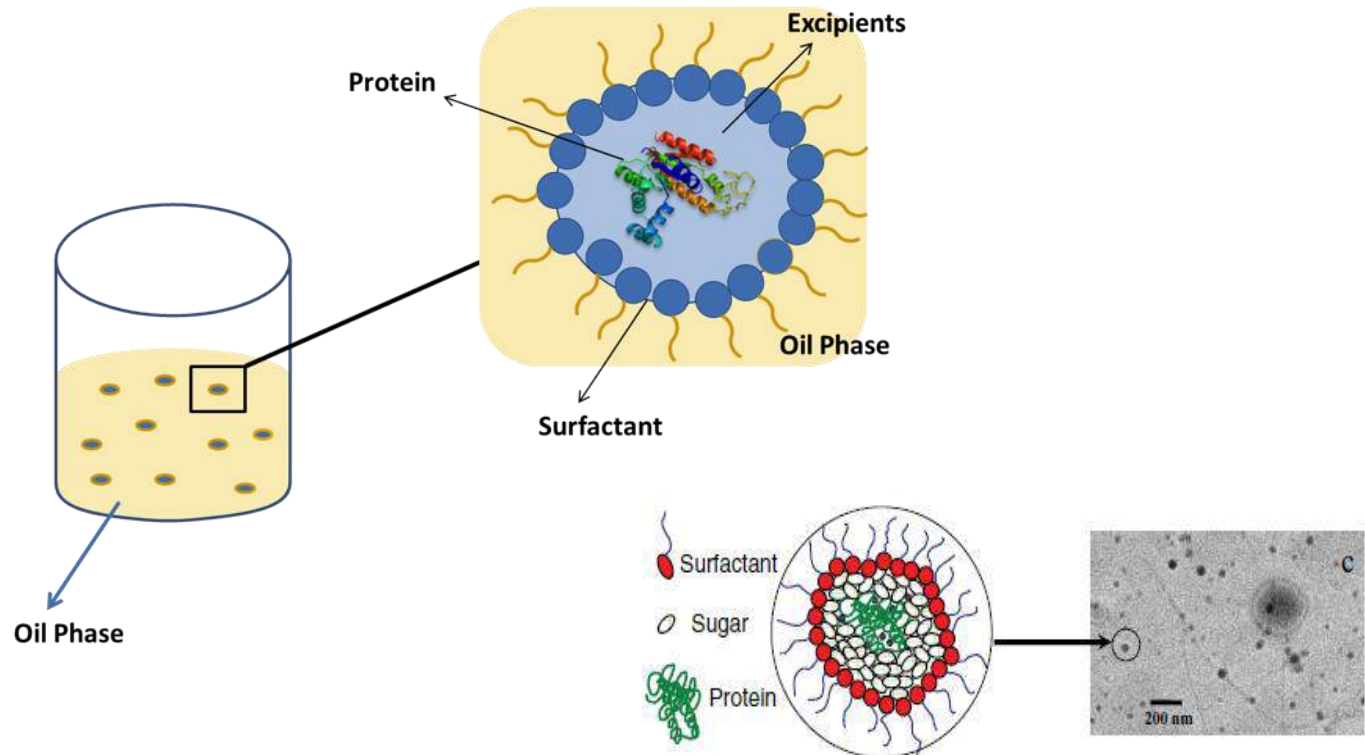
(After 3<sup>rd</sup> Boost)

## Cytokine Response



# Can better particles do better?

e.g. Inverse Micellar Sugar Glass (IMSG) nanoparticles

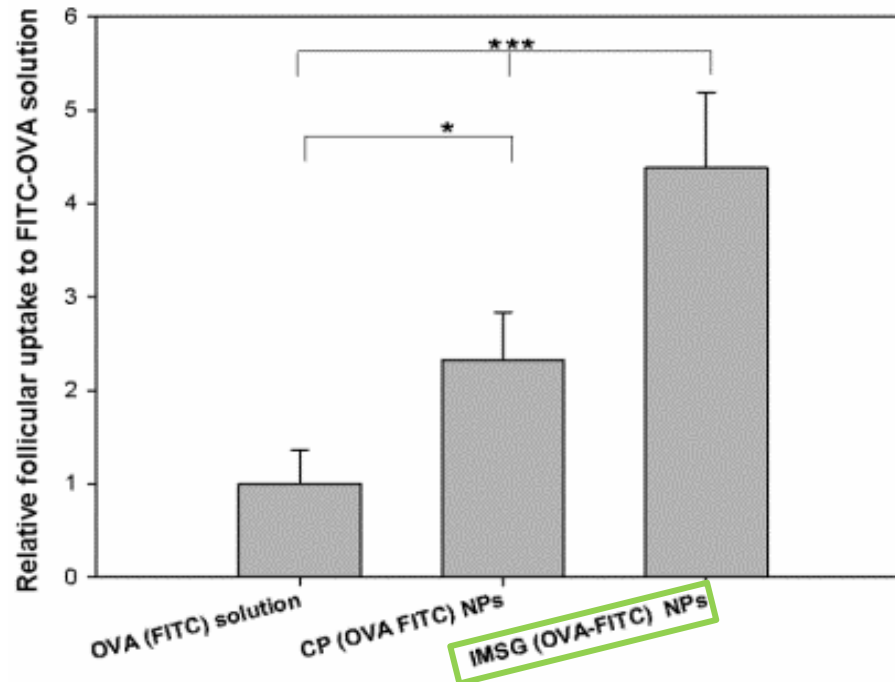
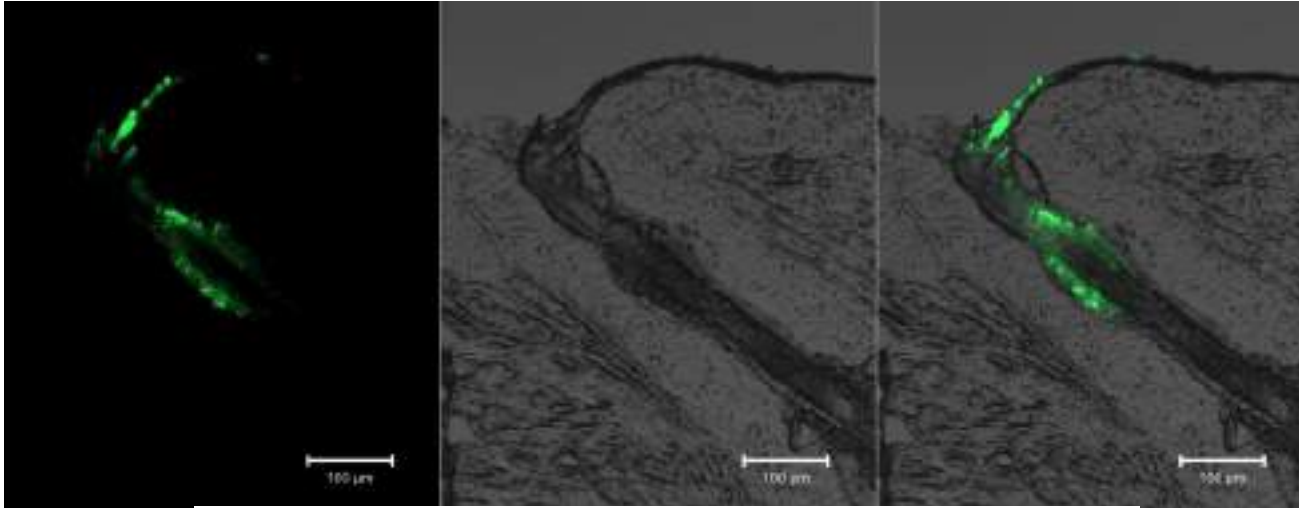


## Advantages....

- Efficient incorporation and stabilization of proteins
- Compatible with the sebum of the follicles
- Incorporation of both adjuvant and protein inside the particles

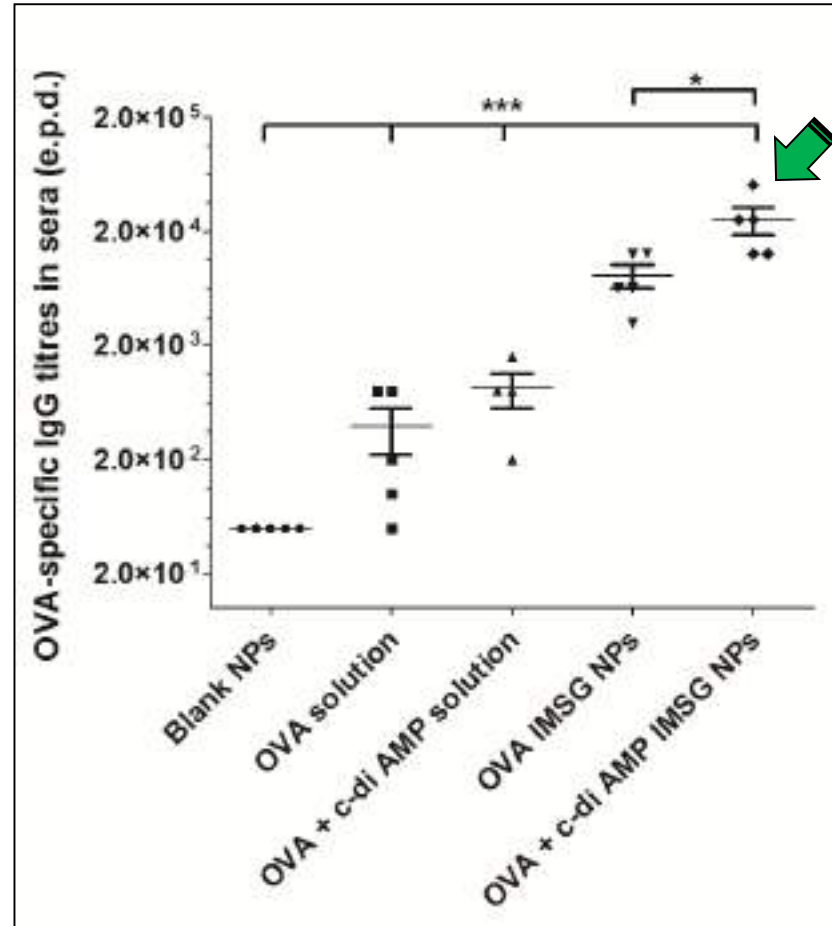
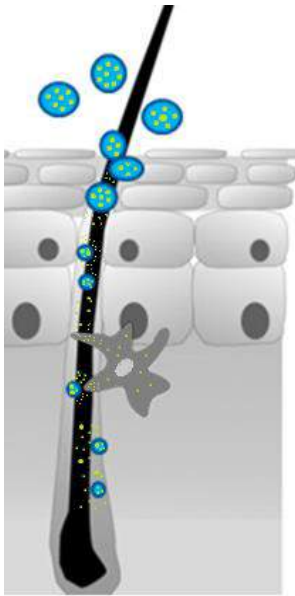
*Giri et al. Adv. Mater. 2011: 4861-7*

# Localization and follicular uptake of nanoparticles



OVA-FITC IMSG NPs (green)

# Transfollicular antigen delivery by IMSG-NP's: Humoral immune response (after 2° boost)



Mittal et al. *Vaccine* (2013)  
Mittal, Schulze et al. *Nanomedicine* (2014)  
Mittal, Schulze et al. *Journal Controlled Release* (2015)



# Overcoming Epithelial and Microbial Barriers:

## ■ THE GUT:

- Targeting inflamed mucosal areas
- Pseudobacterial nanocarriers to combat intracellular infections

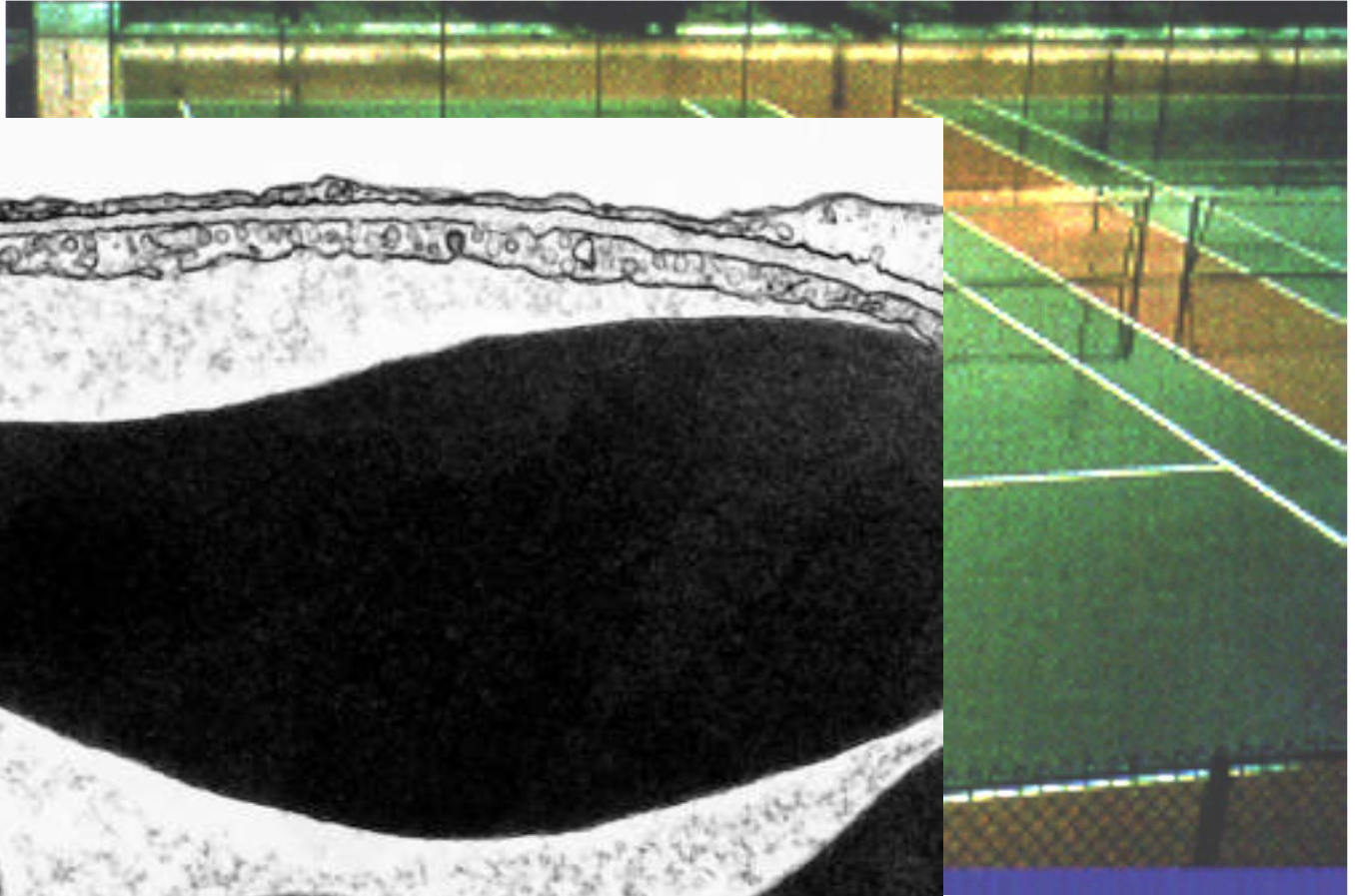
## ■ THE SKIN: „Needle-free“ transfollicular vaccination

## ■ THE LUNGS:

- **Modelling the air-blood barrier in vitro**
- **Mucus and surfactant as non-cellular barriers**
- **Towards some novel pulmonary nanocarriers**

# Pulmonary Drug Delivery

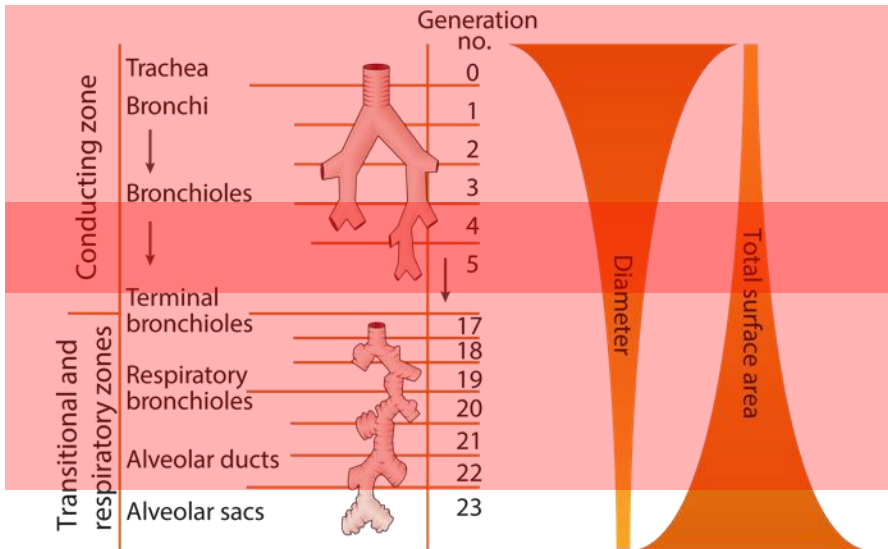
## A promising new area for Nanomedicine



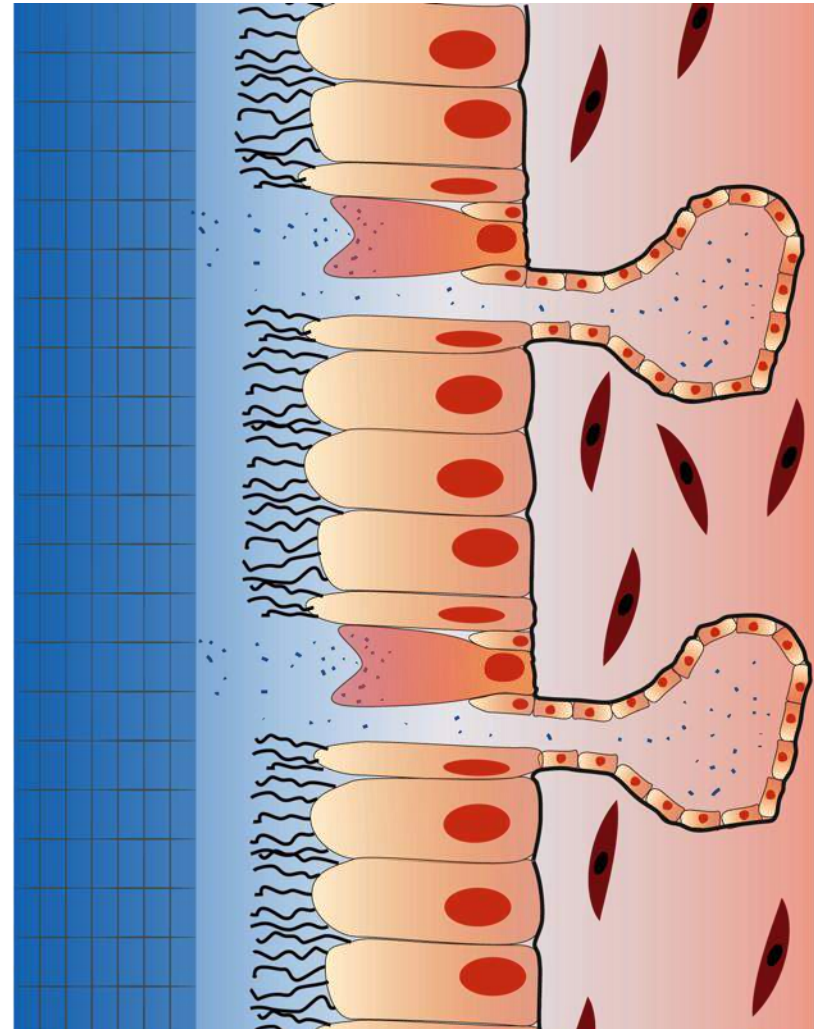
courtesy P. Gehr, Bern

courtesy J. Patton, USA

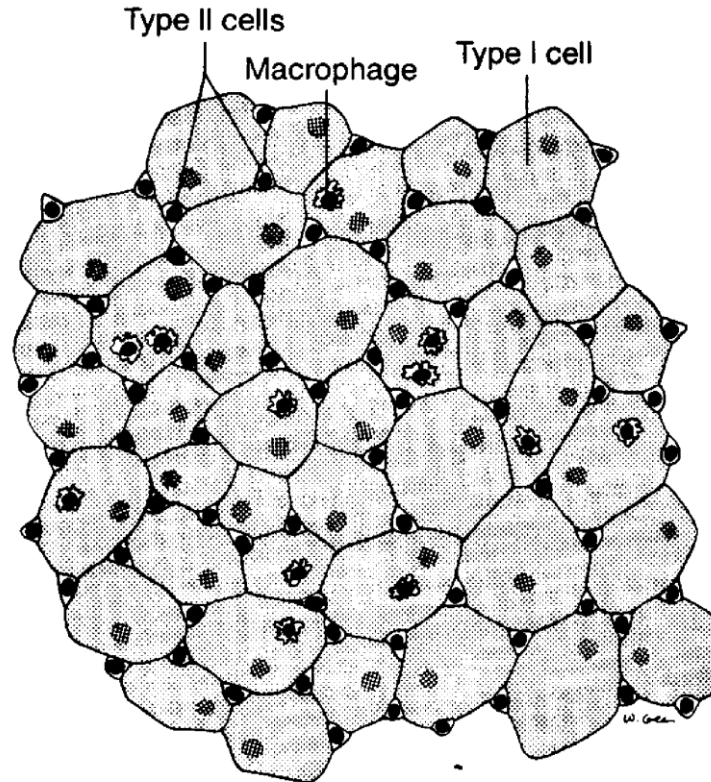
# Along the respiratory tract the epithelium changes!



- Mucus layer
- Ciliated cells
- Goblet cells

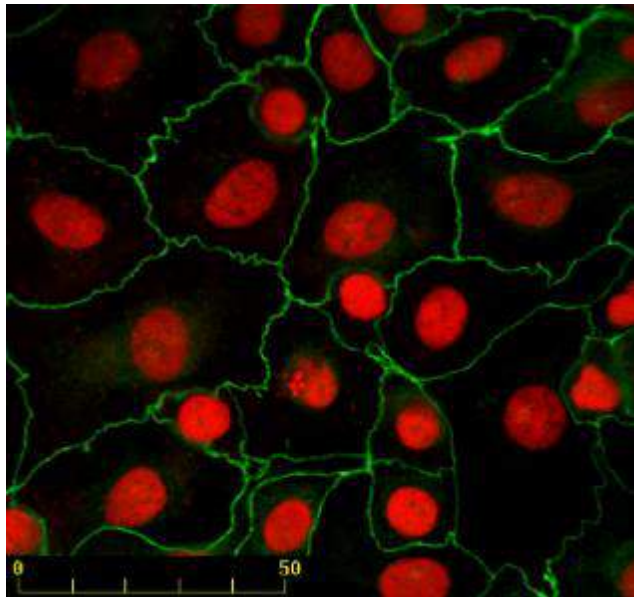


# The alveolar epithelium has mainly three cell types



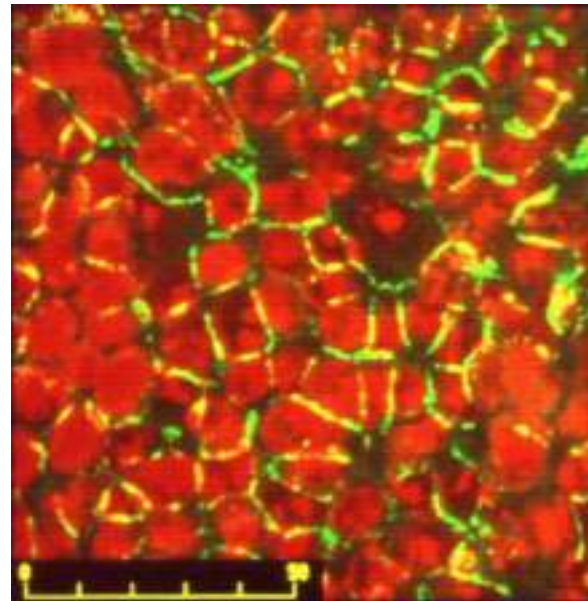
# Human alveolar epithelial cells culture models: Need to express tight junctions!

human alveolar epithelial cells  
in primary culture, day 8



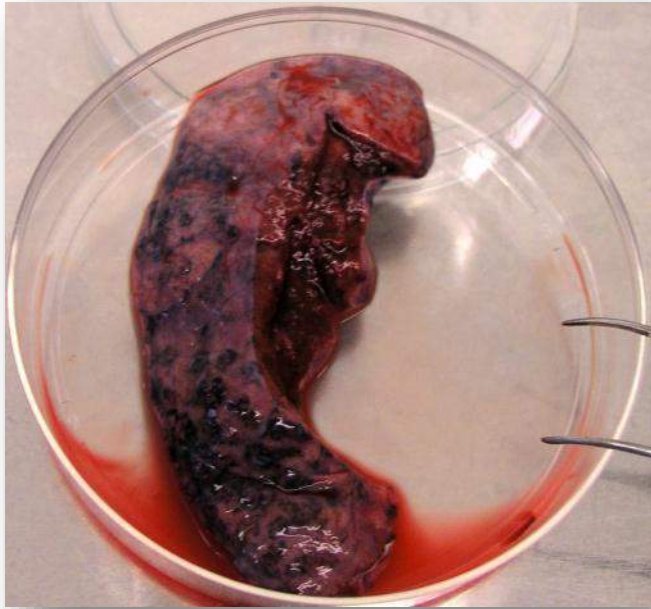
TEER > 1500  $\Omega$  cm<sup>2</sup>

A549 cell line, day 8



TEER < 100  $\Omega$  cm<sup>2</sup>

# Isolating human alveolar epithelial cells: a cumbersome procedure!



Lung tissue samples from patients  
undergoing lung resection surgery



Chopping, trypsination



Removal of undesired cell types with  
„physical“ methods  
(e.g. erythrocytes, macrophages)



Positive selection of epithelial cells (mainly  
alveolar type II cells) with anti-EpCAM AB

Elbert et al. 1999 Pharm Res  
Fuchs et al. 2003 Cell Tissue Res  
Daum et al. 2012 Meth Mol Bio

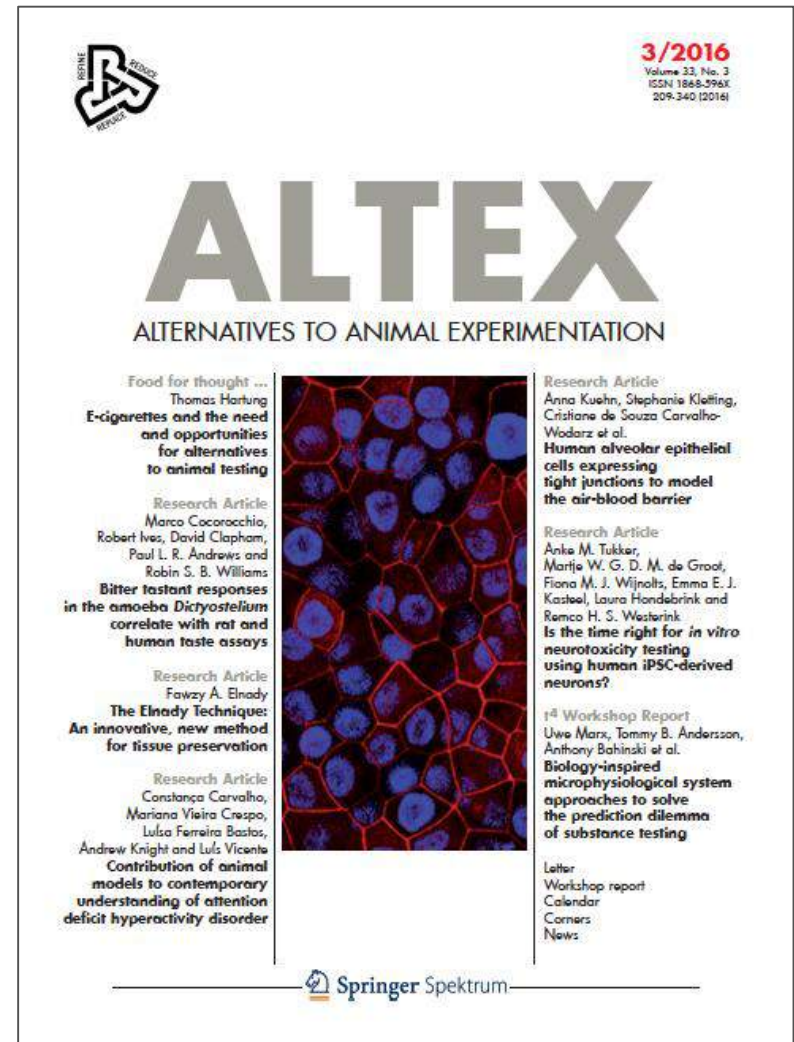
# Generating an AT I-like cell line with prolonged lifespan and significant barrier properties

## our approach

”mild proliferators”  
i.e. 33 different genes  
in different combinations  
under **constitutive** promoter

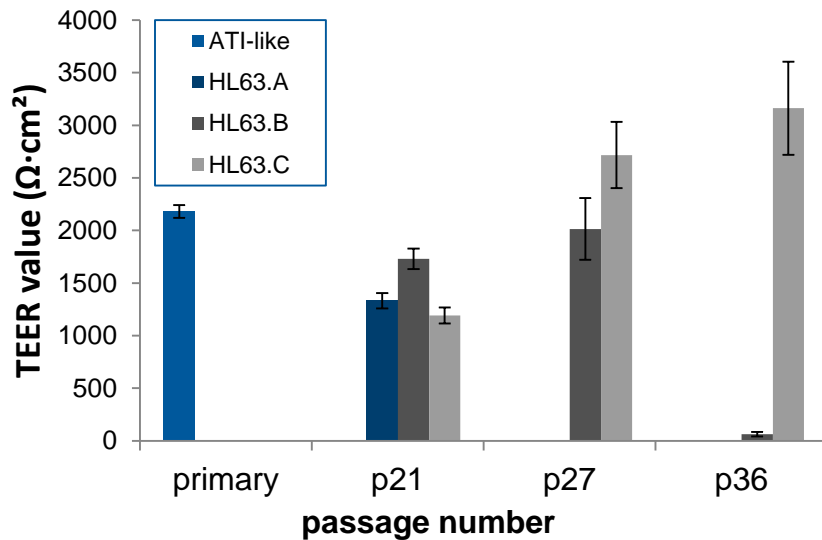
- more than 50 transfections
- splitting/passaging/selection
- characterization

**7 cell lines with prolonged lifespan,  
3 of them exhibiting TEER >1000  $\Omega\text{cm}^2$   
1 remaining stable over >30 passages**

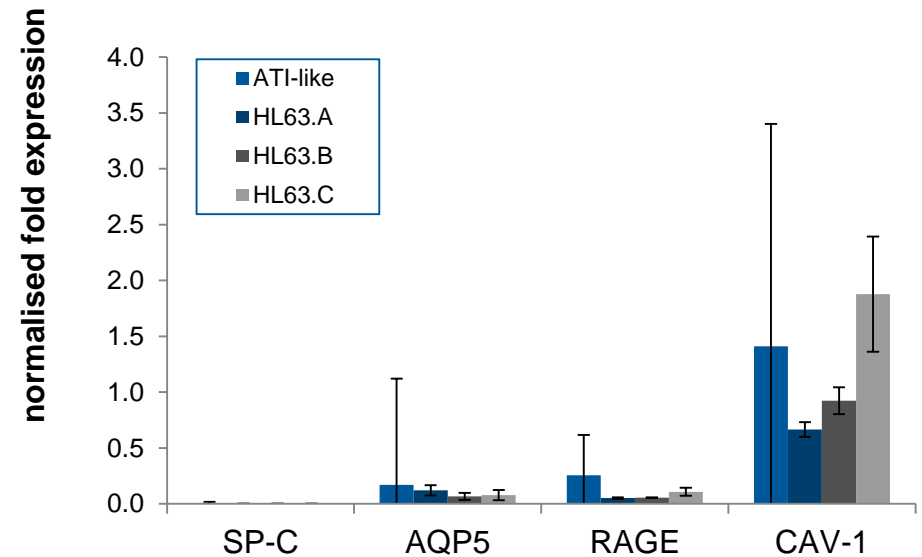


# hAELVI – the first human alveolar epithelial Lenti virus immortalized cells line with tight junctions and AT1-characteristics over several passages

maximum TEER

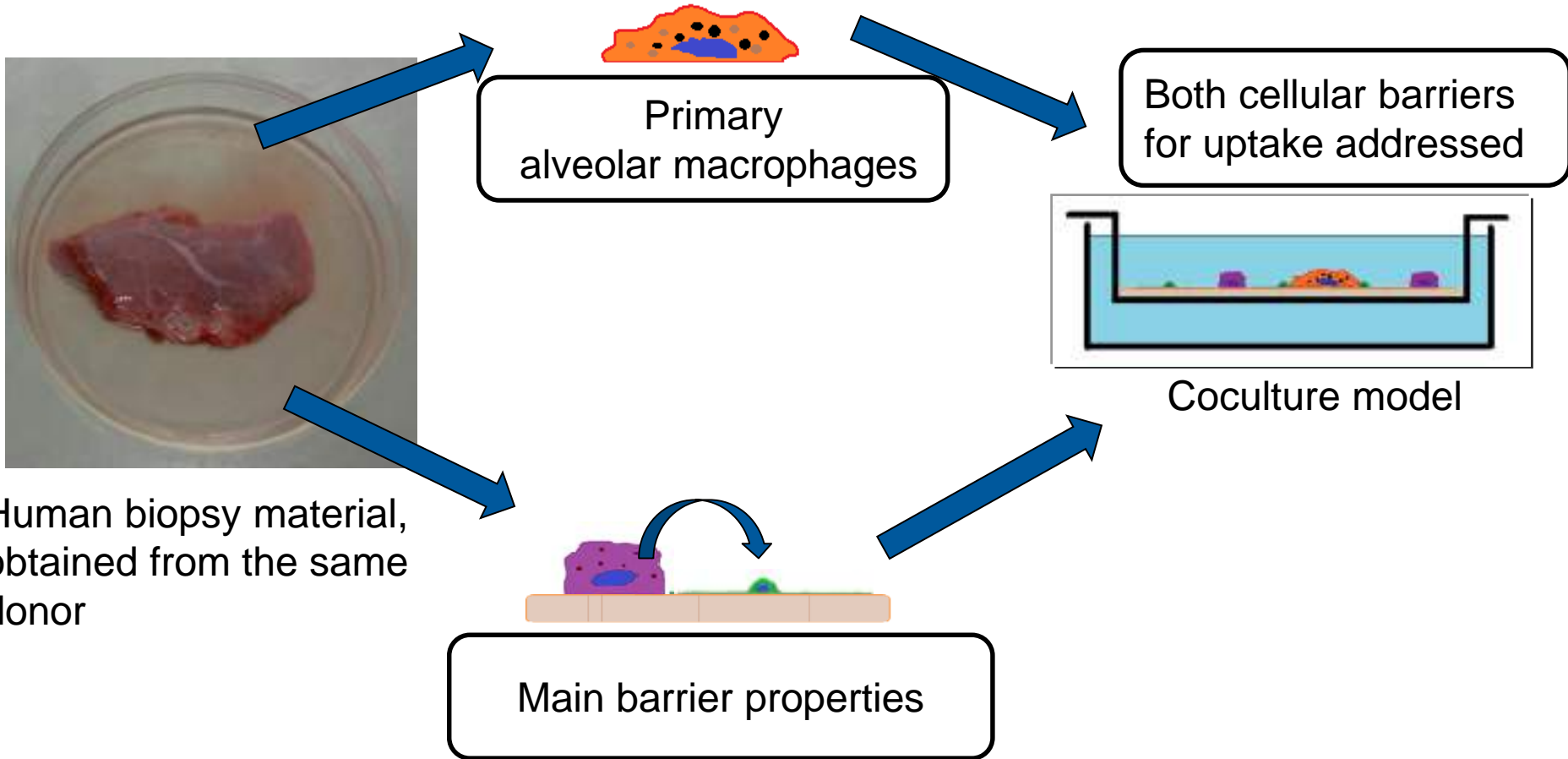


lung-specific marker expression





# Human Alveolar Epithelial Cells (hAEpC) and Macrophages in autologous primary coculture

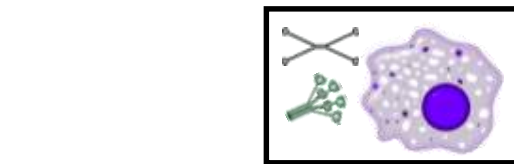


Hittinger M, et al., ATLA - *Altern Lab Anim*. 2016;44(4):337-347.  
Hittinger M, et al., ATLA - *Altern Lab Anim*. 2016;44(4):349-360.

# Mucus and Surfactant as non-cellular Barriers of the Respiratory Tract

## Peripheral Lung

=> **Surfactant**, Alveolar macrophages



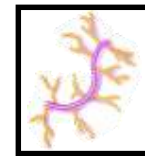
0.09-0.89  $\mu\text{m}^a$

$\sim 0.2 - 10 \mu\text{m}^c$



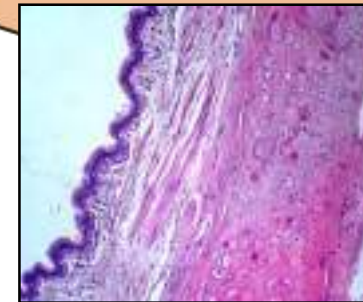
## Central Lung

=> **Mucus** (Glycoproteins), Cilia, Mucociliary Clearance



2-70  $\mu\text{m}^b$

$\sim 20-60 \mu\text{m}^c$



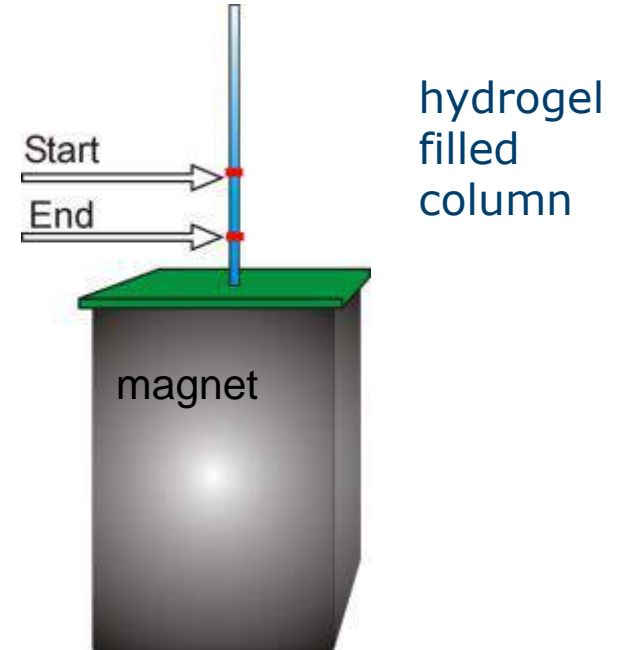
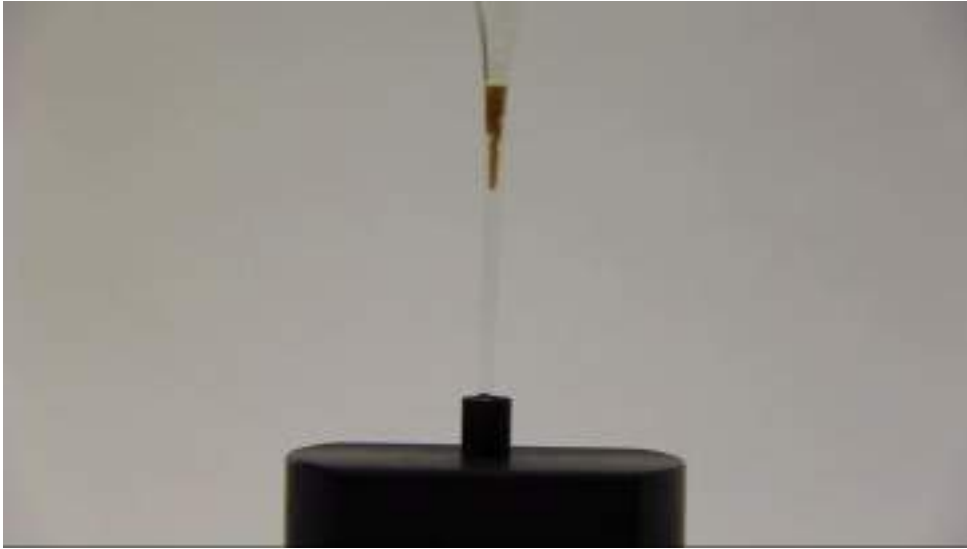
**Lung liquid surface layer**

**Cell layer**

Deutsche  
Forschungsgemeinschaft

**DFG**

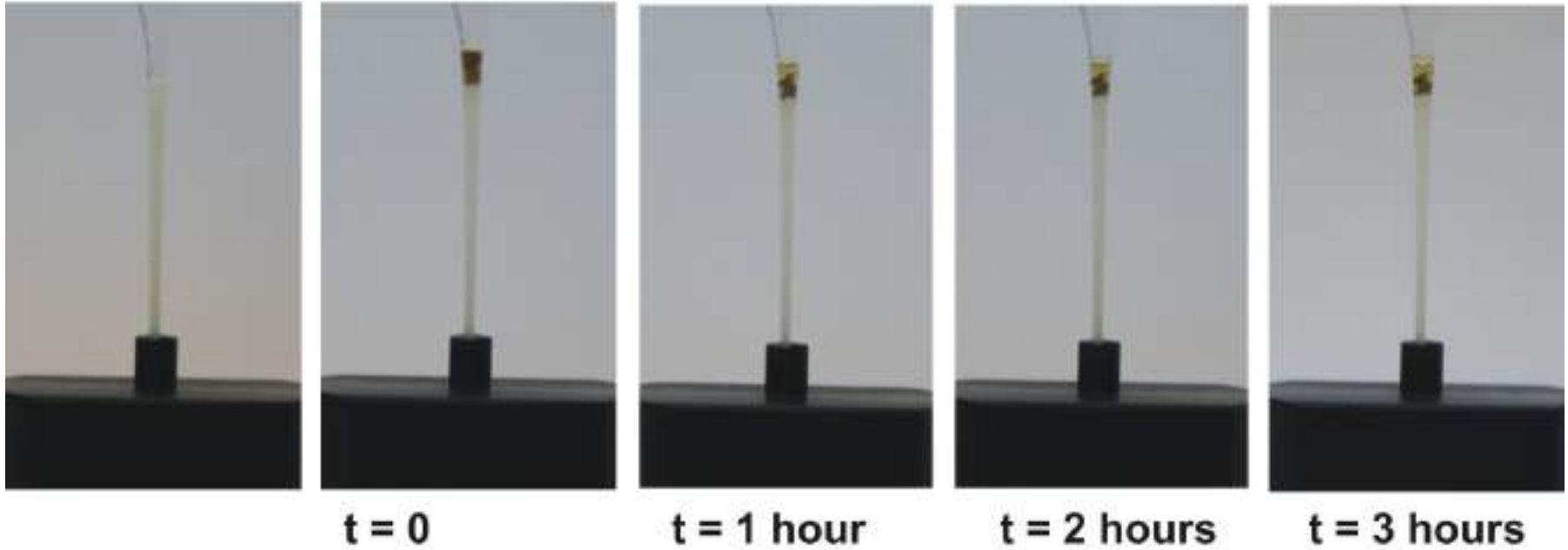
# Penetration of a model hydrogel



→ **Particles (250 nm diameter) penetrate rather easily through a model gel made of 1% hydroxyethyl-cellulose (HEC)**

## But what about mucus?

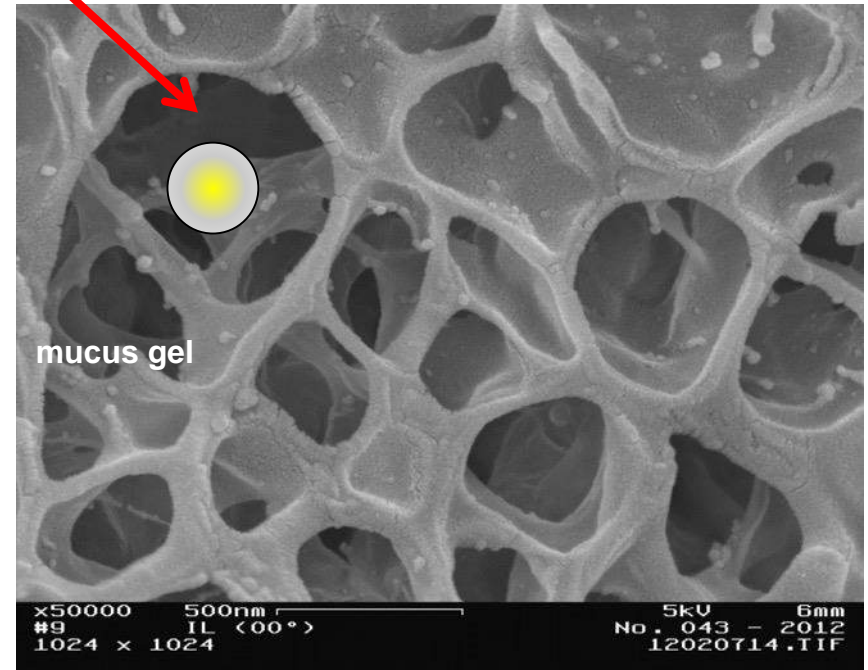
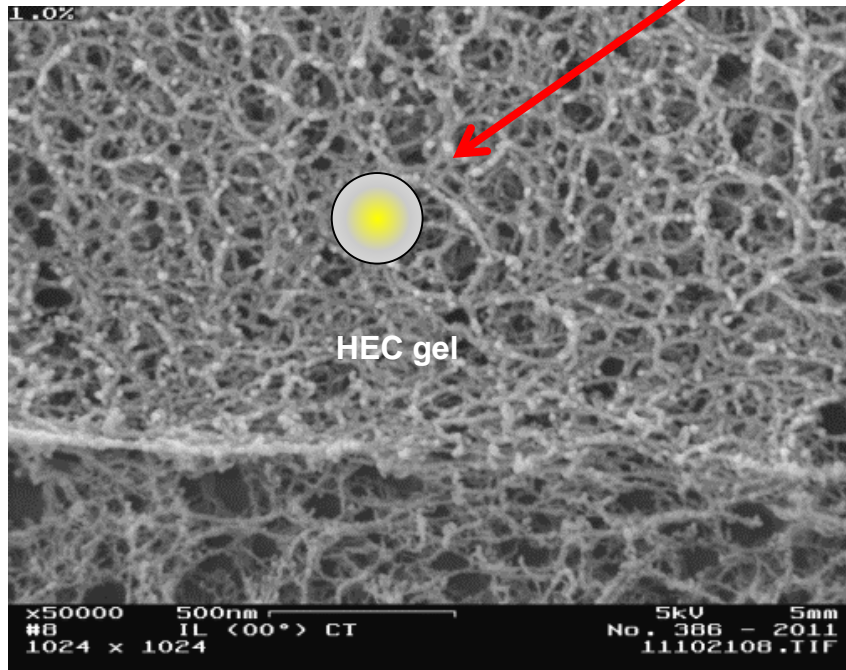
### mucus filled capillary



**In spite of strong magnetic field,  
virtually no penetration of mucus by  
magnetite NP's could be observed!**

# Cryo-SEM and optical tweezers reveal relationship between microstructure and nanoparticle penetration of pulmonary mucus

250nm particle



## HEC gel:

- **SMALL** pores/meshes
- **THIN** (flexible?) scaffold

## Mucus gel:

- **LARGE** pores,
- **THICK** (stiff?) scaffold

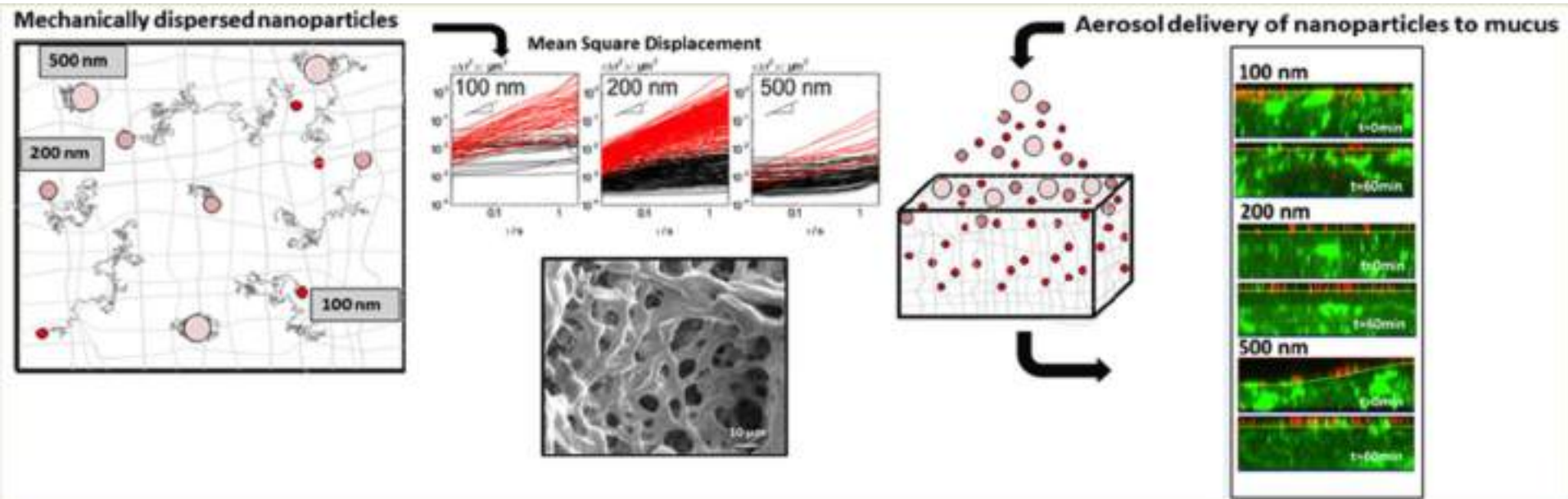
# Size-Limited Penetration of Nanoparticles into Porcine Respiratory Mucus after Aerosol Deposition

Xabier Murgia,<sup>†,‡</sup> Paul Pawelzyk,<sup>†,§</sup> Ulrich F. Schaefer,<sup>||</sup> Christian Wagner,<sup>⊥</sup> Norbert Willenbacher,<sup>§</sup> and Claus-Michael Lehr<sup>\*,‡,||</sup>

<sup>‡</sup>Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research (HZI),

<sup>||</sup>Biopharmaceutics and Pharmaceutical Technology, Department of Pharmacy, and <sup>⊥</sup>Experimental Physics, Saarland University, 66123 Saarbruecken, Germany

<sup>§</sup>Institute for Mechanical Process Engineering and Mechanics, Karlsruhe Institute of Technology (KIT), 76131 Karlsruhe, Germany

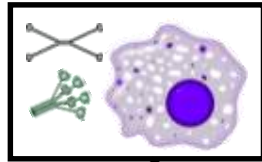


# Clearance of particles from different regions of the respiratory tract

## Macrophage Clearance

### Peripheral Lung

=> **Surfactant**, Alveolar macrophages

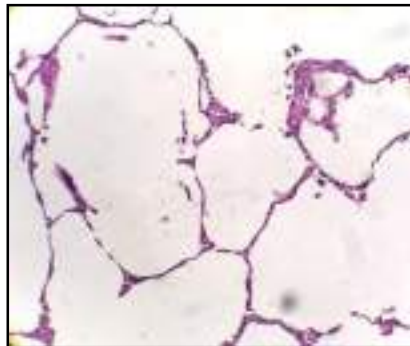


**Lung liquid surface layer**

0.09-0.89  $\mu\text{m}^{\text{a}}$

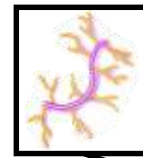
**Cell layer**

$\sim 0.2 - 10 \mu\text{m}^{\text{c}}$



### Central Lung

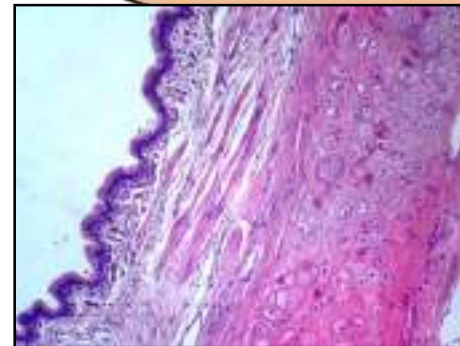
=> **Mucus** (Glycoproteins), Cilia, Mucociliary Clearance



2-70  $\mu\text{m}^{\text{b}}$



$\sim 20-60 \mu\text{m}^{\text{c}}$



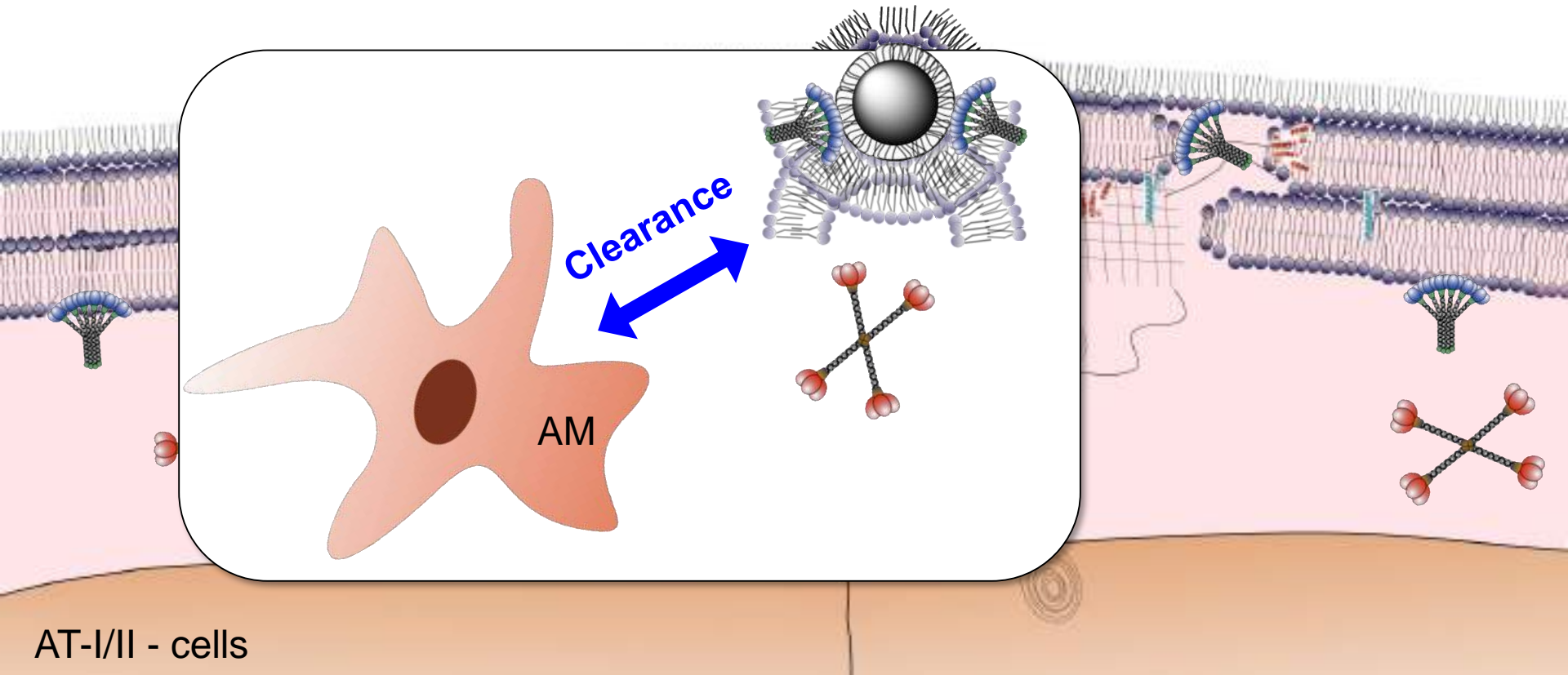
<sup>a</sup> Bastacky et al., J Appl Physiol 1995, 79 (5), 1615-28.

<sup>b</sup> Sims et al., The American journal of physiology 1997, 273 (5 Pt 1), L1036-41.

<sup>c</sup> Steimer et al., J Aerosol Med Pulm D (2005) vol. 18 (2) pp. 137-82

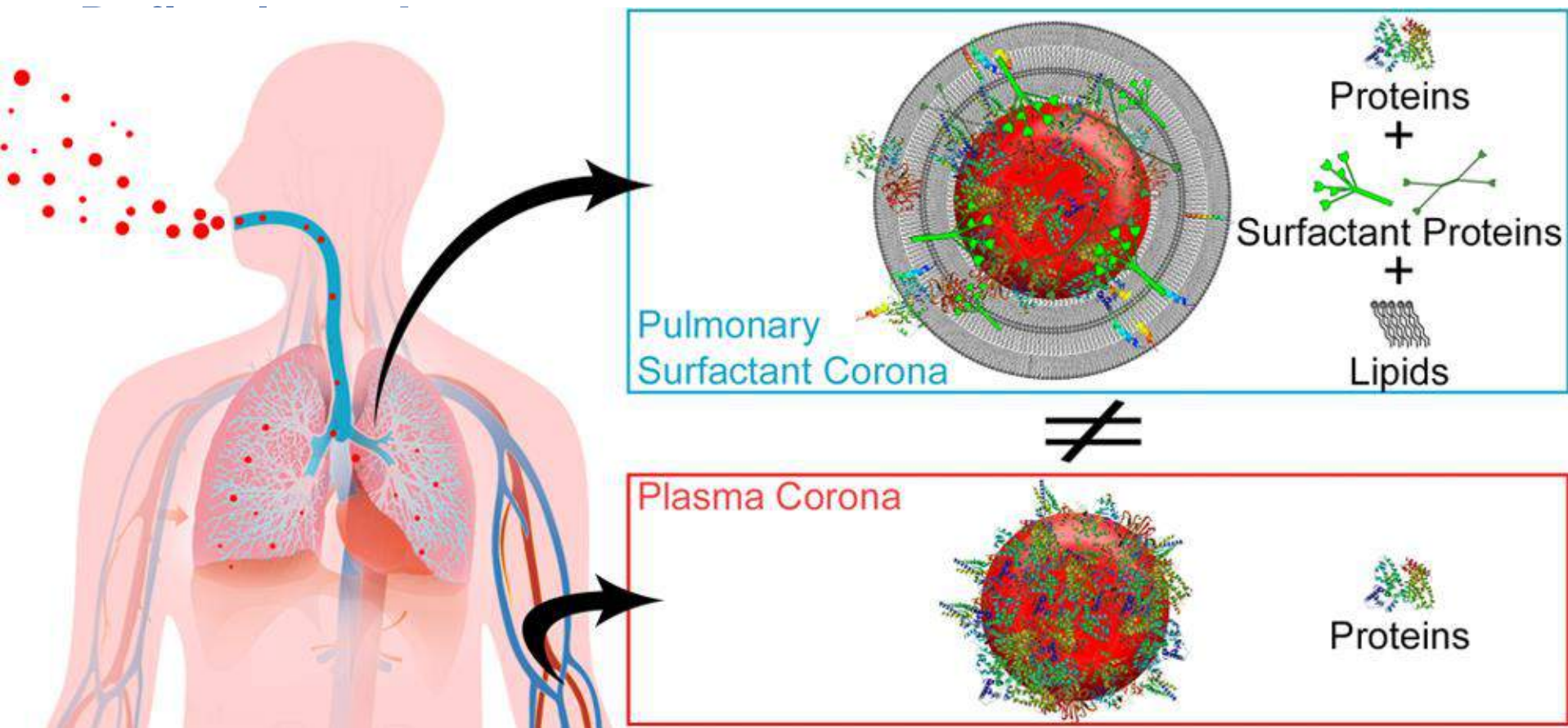
# Nanoparticles in the Deep Lungs

- i) Deposition
- ii) Immersion
- iii) Interaction with molecules („Corona-Formation“)
- iv) Cellular Response



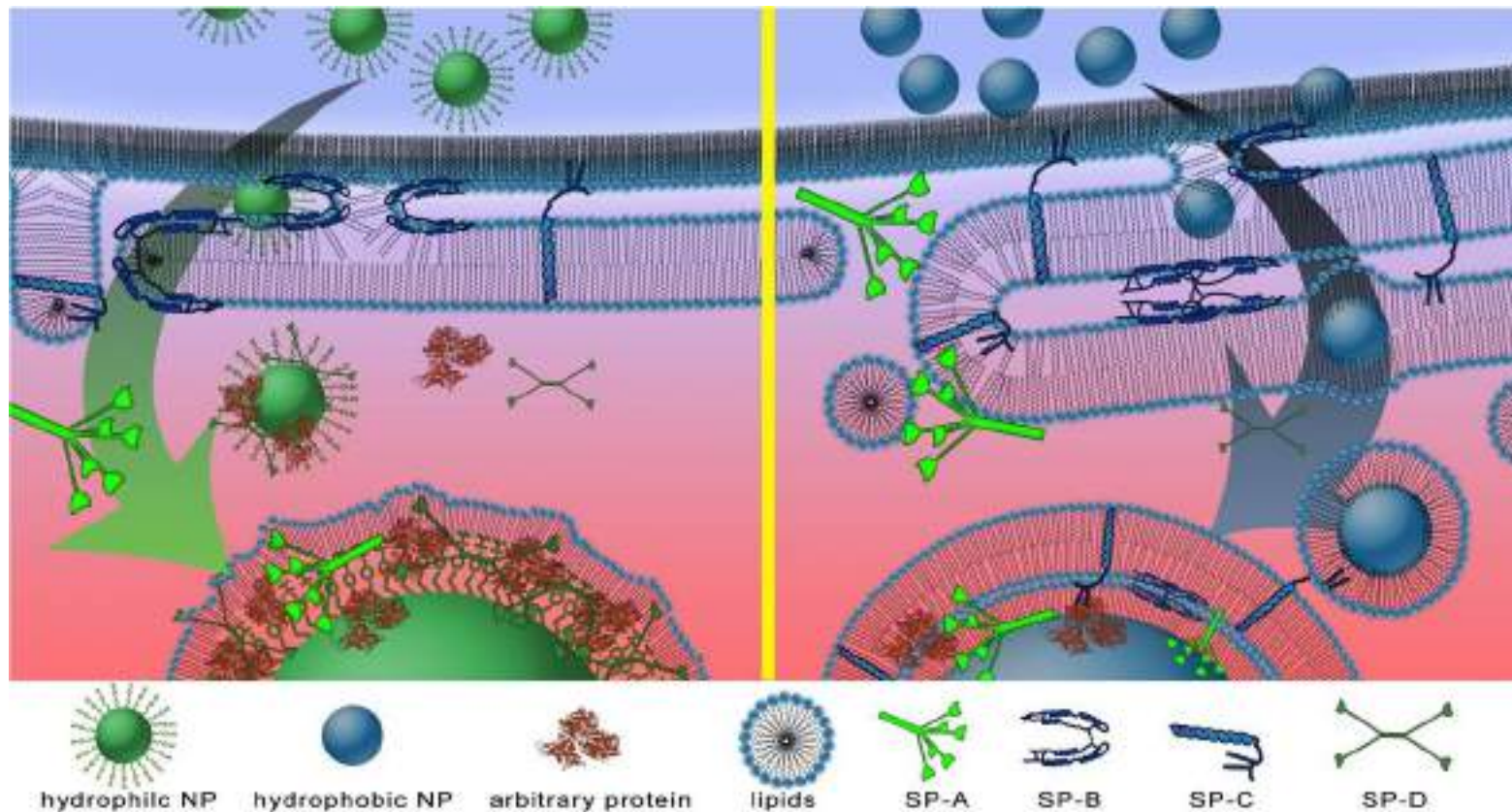


# The corona formed around inhaled NP's is different to the one formed in plasma!



## Surprising result:

The different Surfactant Proteins appear to act as „primer“ for the same phospholipids!



- Individual protein corona allows even hydrophilic NPs to adsorb lipids onto their surface
- Highly efficient mechanism to mask every surface by lipid adsorption

## Consequence:

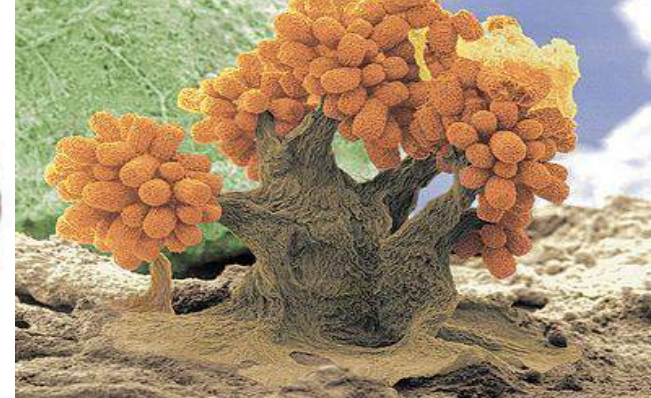
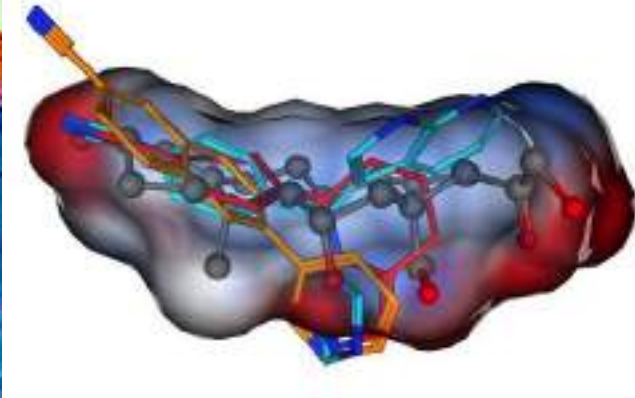
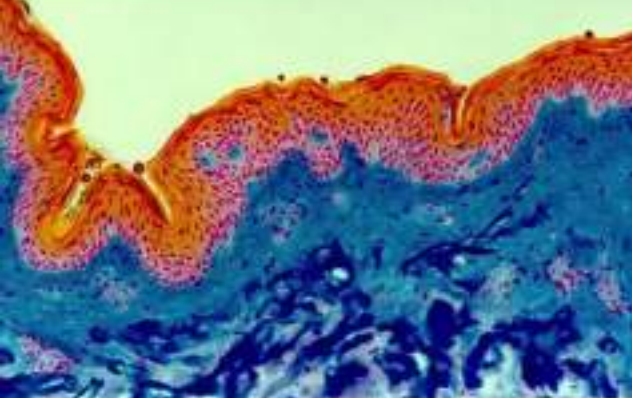
# The Interplay of Lung Surfactant Proteins and Lipids Assimilates the Macrophage Clearance of Nanoparticles



... This could be good regarding the safety of (technical) nanomaterials,

but at the same time a challenge for targeting nanomedicines...

Ruge, C.A., et al. PLoS ONE. 7, e40775 (2012).

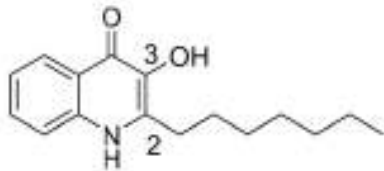
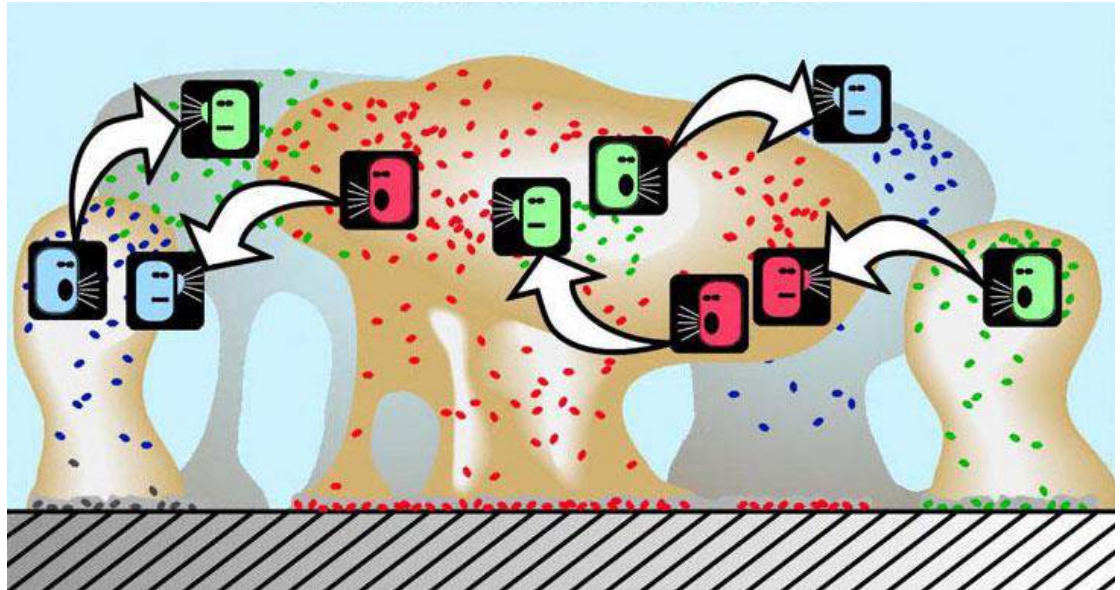


# Ultra-small, mucus-penetrating solid lipid nanoparticles (SLN) for improved pulmonary delivery of novel anti-infectives

Noha Nafee, Ayman Husari, Christine K. Maurer, Cenbin Lu, Chiara de Rossi, Anke Steinbach, Rolf W. Hartmann, Claus-Michael Lehr and Marc Schneider

# Bacterial biofilm - Quorum Sensing

- **Quorum sensing (QS)**, intracellular signal, produced by *P. aeruginosa*, responsible of cell-to-cell communication

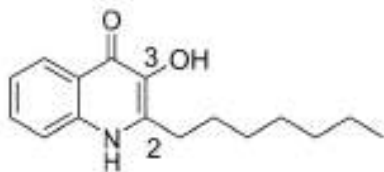
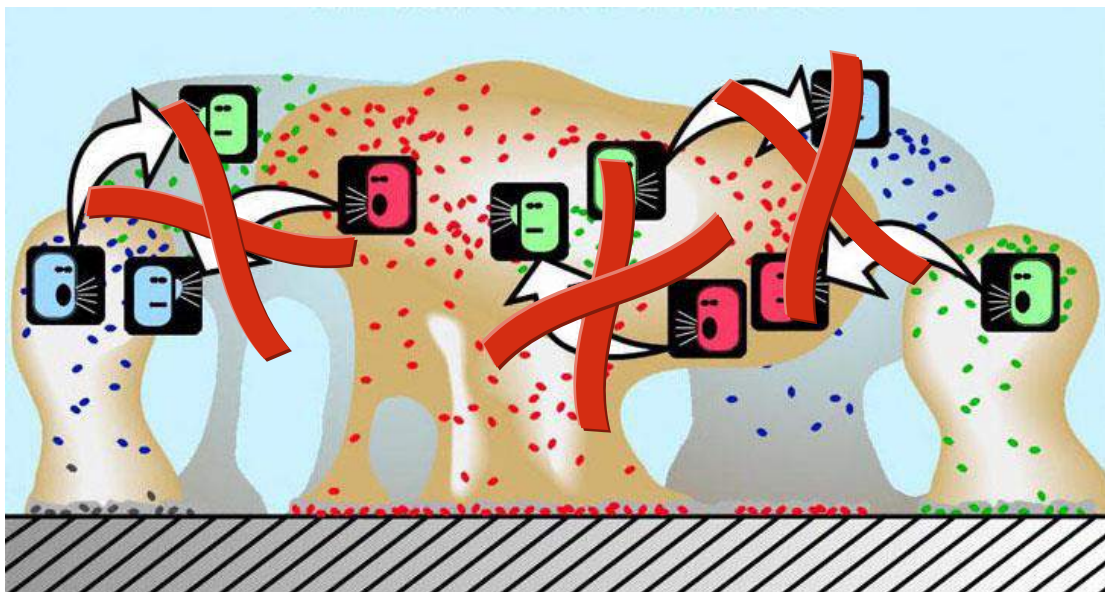


PQS

Pseudomonas Quorum Sensing (PQS)

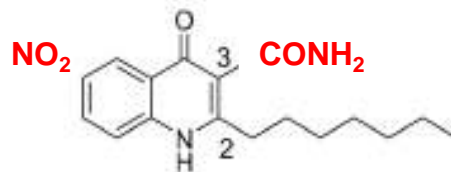
# Quorum Sensing Inhibitors

- Cell-Cell communication



PQS

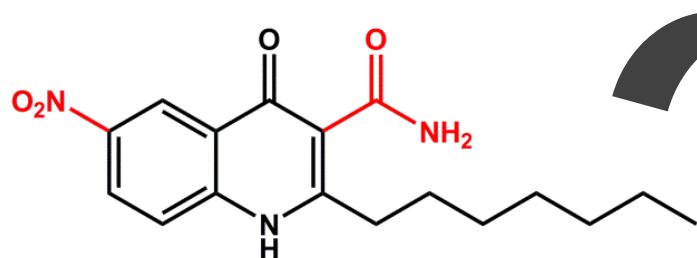
Pseudomonas Quorum Sensing (PQS)



HIPS 378

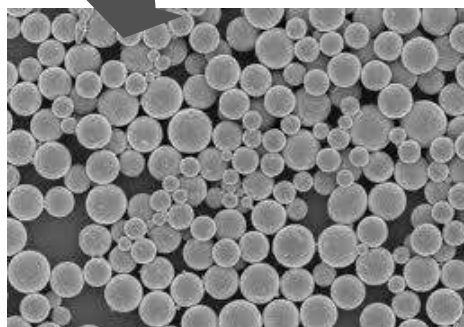
PQS inhibitor

# Nanocarriers for QSI



QSI

Lipophilic!!!



Solid Lipid Nanoparticles (SLNs)

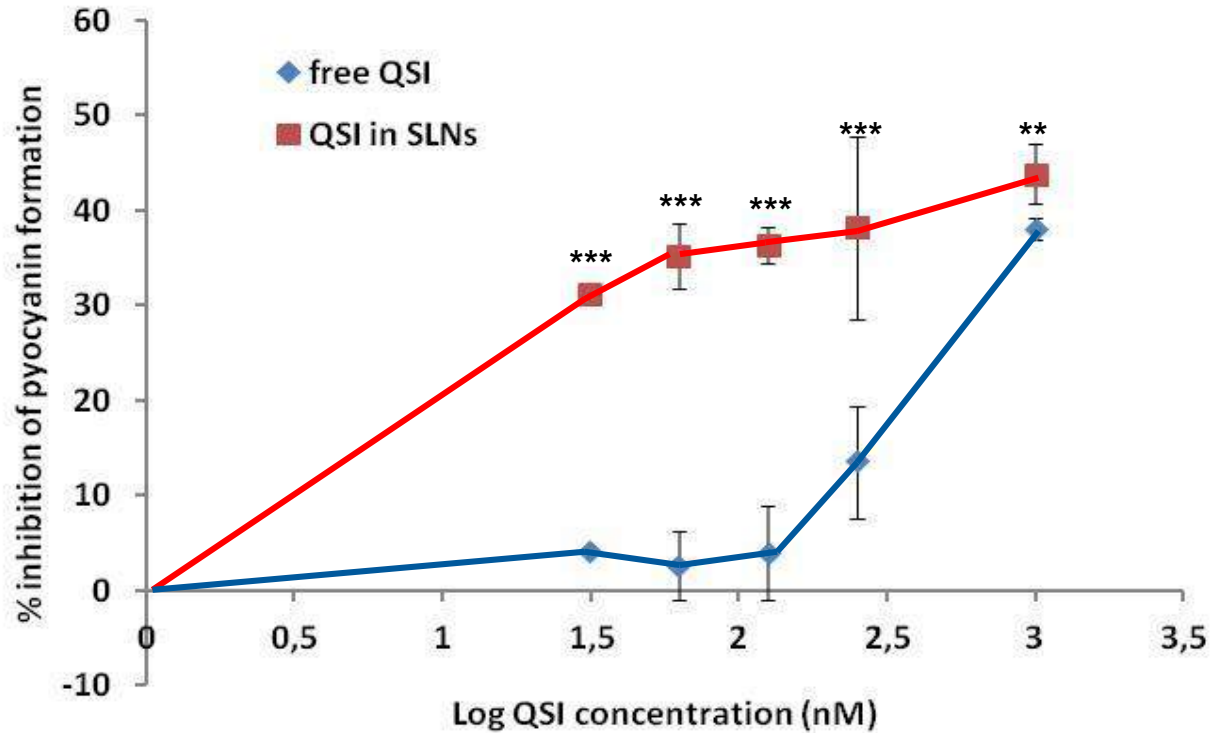
- Deposit in the bronchial region
- Penetrate mucus and biofilms
- Deliver the payload in a controlled manner



Pulmonary DDS

# Inhibition of Pyocyanin<sup>\*)</sup> production

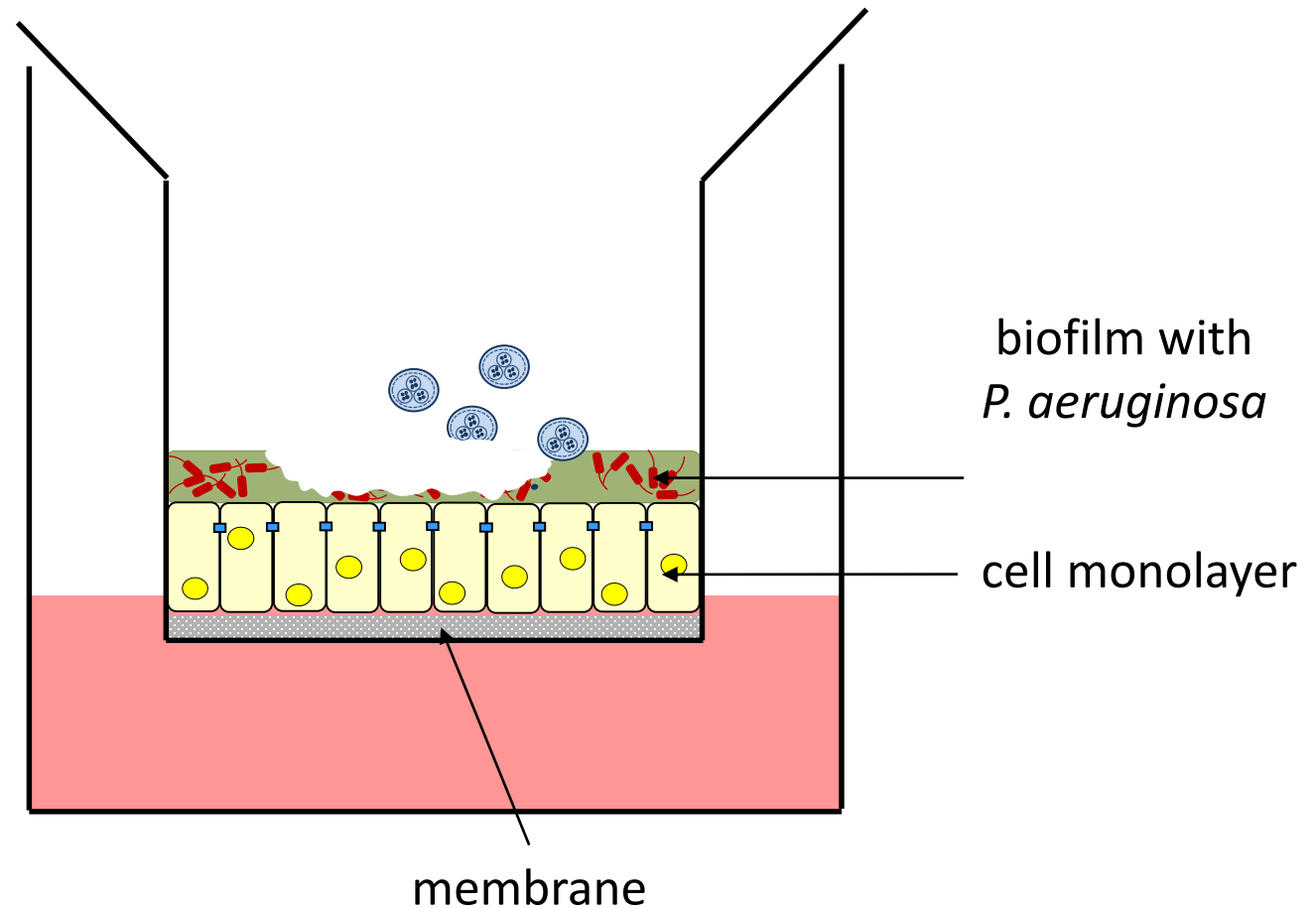
\*) virulence factor of *P. aeruginosa*



**Sevenfold increase in anti-virulence activity by QSI-SLNs**

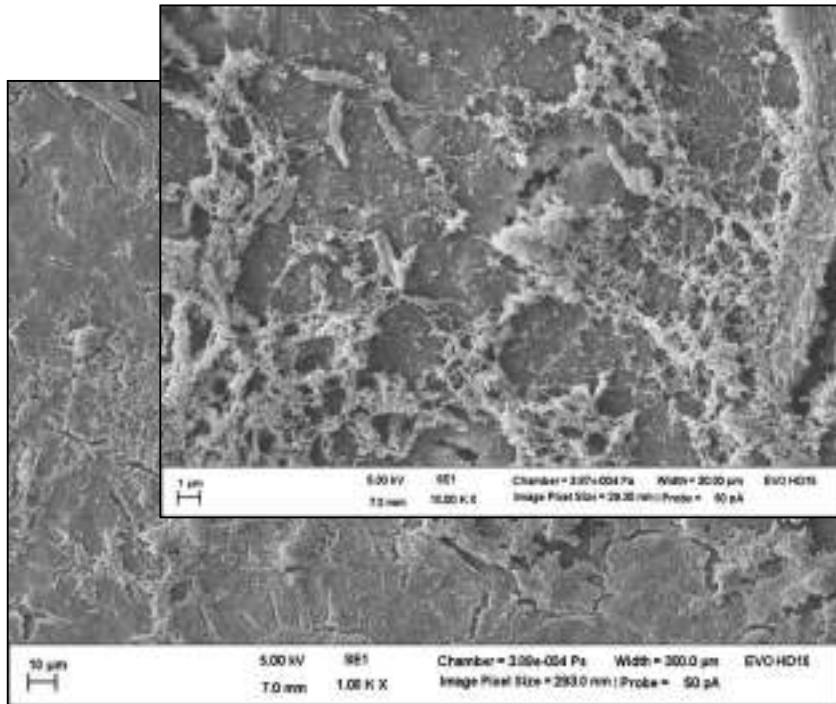


# Ongoing: Bacterial-epithelial co-cultures for testing of aerosolized antibiotic nanocarriers

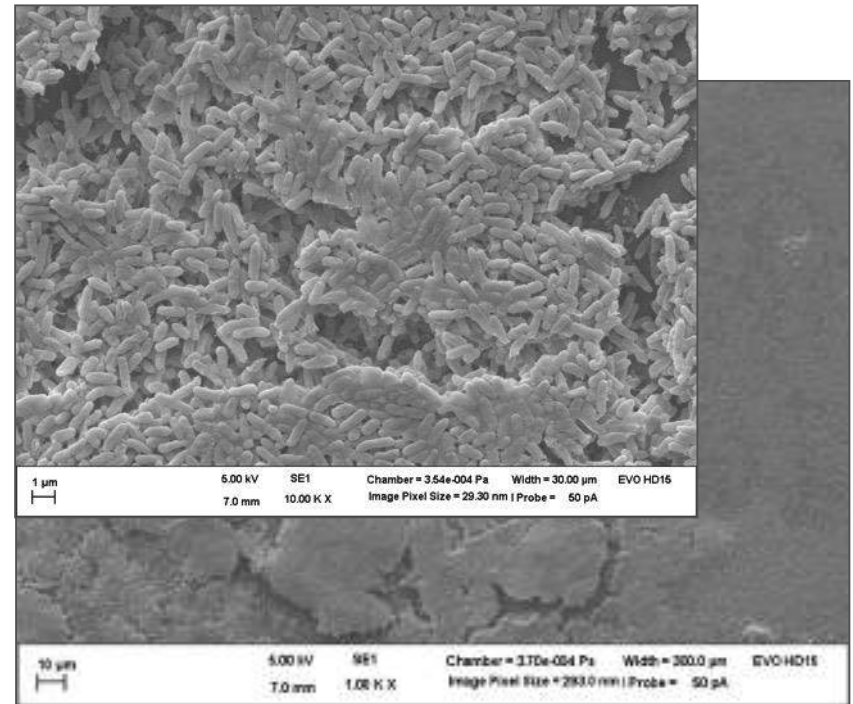


# First results:

## SEM images after 24 h of infection and treatment with NPs



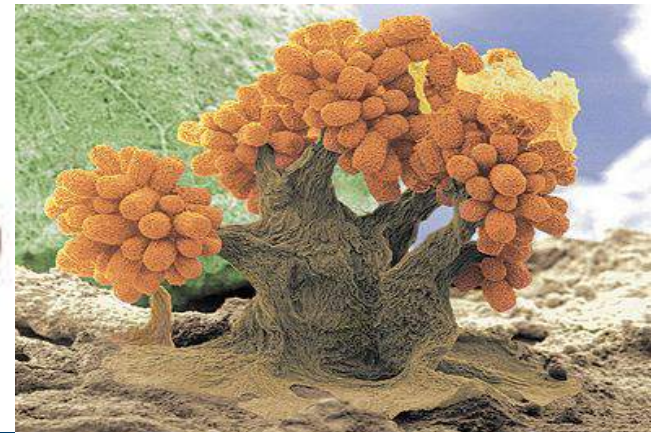
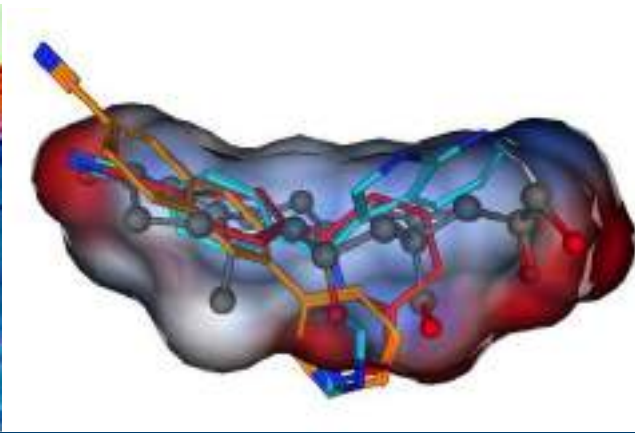
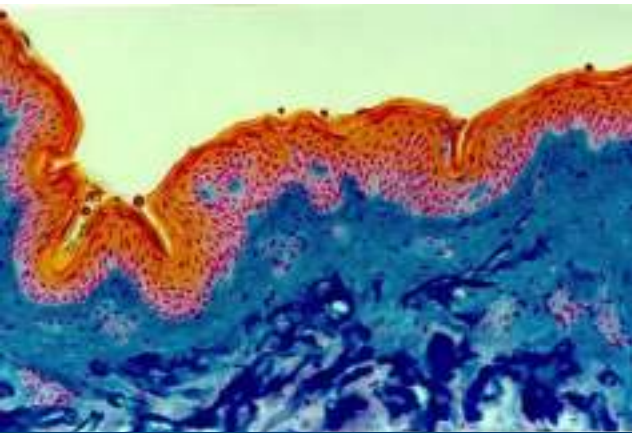
Infected and treated with **drug-loaded** NPs



Infected and treated with **drug-free** NPs

**Drug-loaded particles appear to kill the bacteria,  
but to make the epithelial cells survive the infection!**

J. Juntke et al, in preparation



# Biodegradable nanocarriers for delivering Biopharmaceuticals

[brigitta.loretz@helmholtz-hzi.de](mailto:brigitta.loretz@helmholtz-hzi.de)

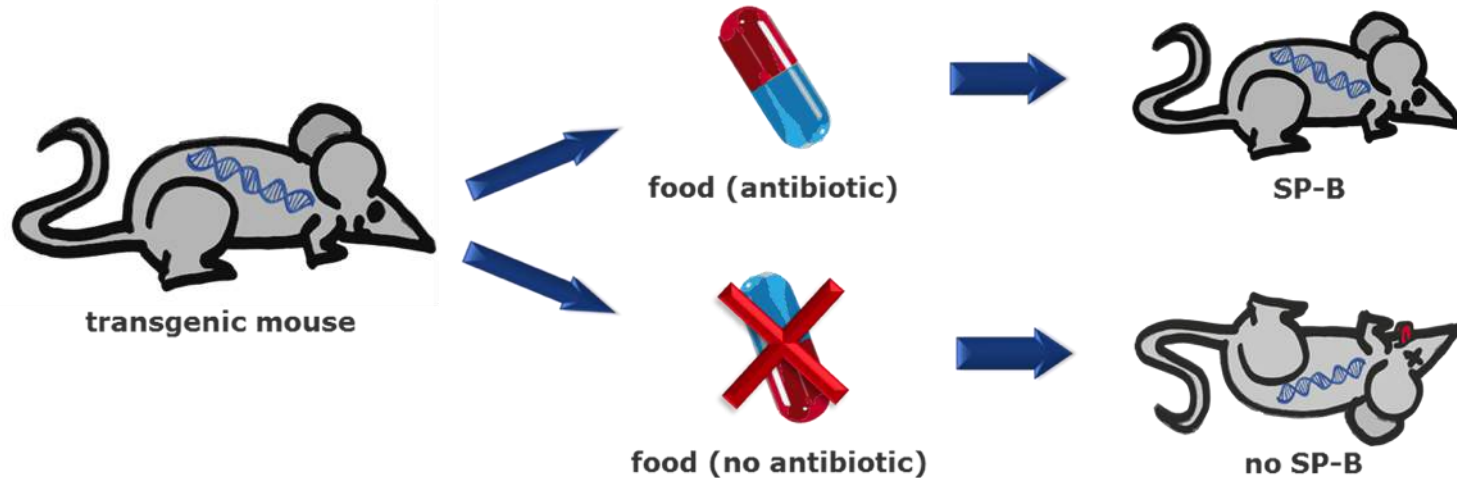
Helmholtz-Institute for Pharmaceutical Research Saarland  
Department Drug Delivery

Helmholtz-Institut für Pharmazeutische Forschung Saarland

# Genome editing using Chitosan PLGA Nanoparticles for treatment of lethal SP-B deficiency

cooperation: Dr. Michael Kormann, Tübingen

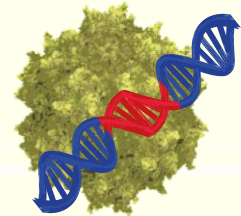
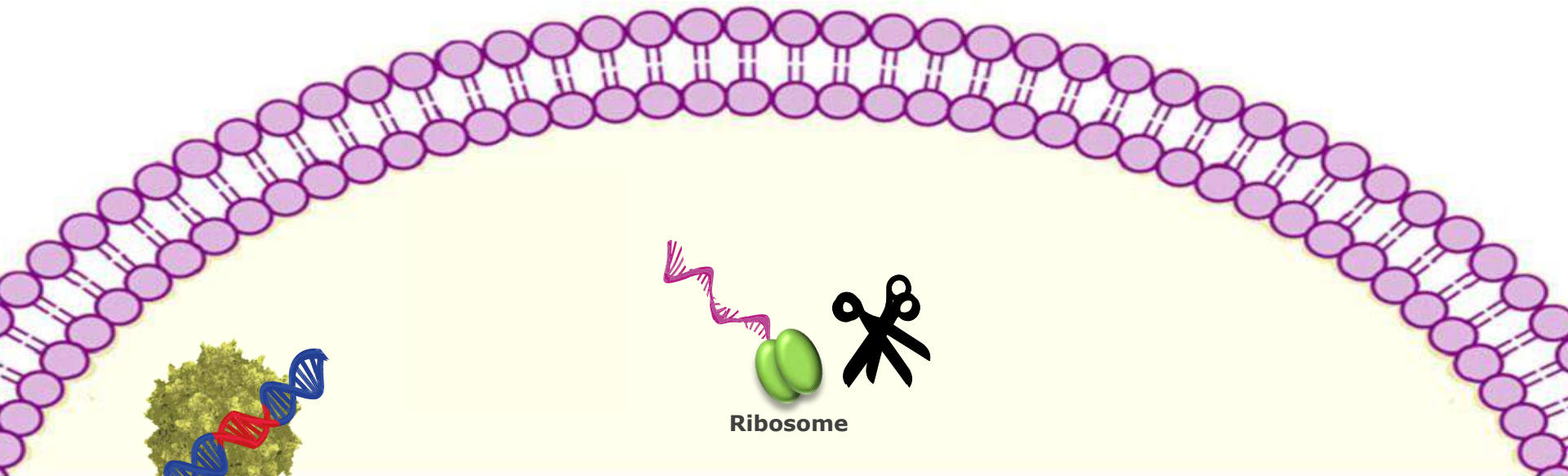
**Transgenic mouse model:** SP-B gene under control of a Doxycycline-dependent promoter; mice only survive as long as they get DOX



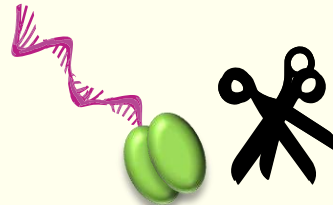
„Therapy“ = Insertion of a constitutive (DOX-independent) promoter  
=> If successful, mice can survive w/o DOX.

# “Genome Editing” = Double Transfection of

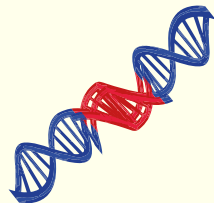
- 1.) DNA encoding the correct Template, and
- 2.) mRNA encoding a sequence-specific Endonuclease



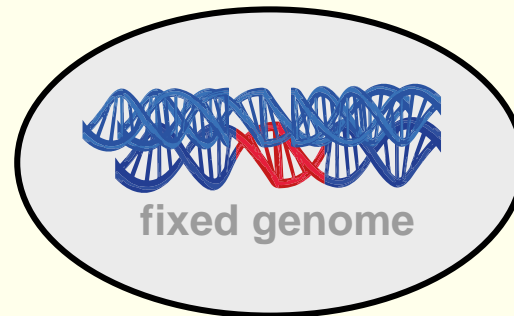
AAV



Ribosome

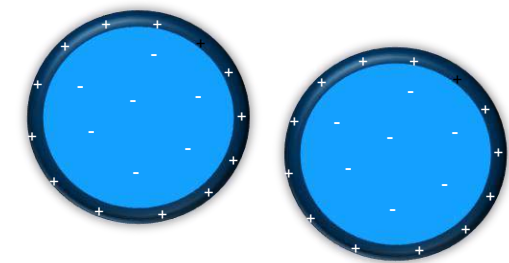


**Expression of the missing protein !**

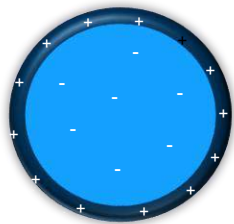


fixed genome

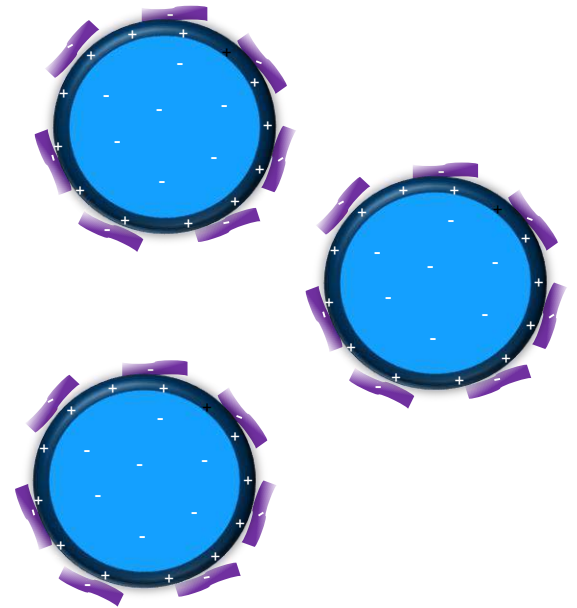
# Chitosan-PLGA NPs as Carrier for Nucleotide Delivery



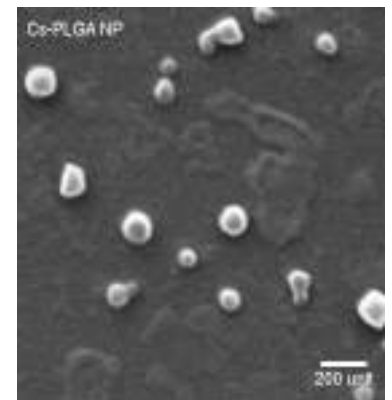
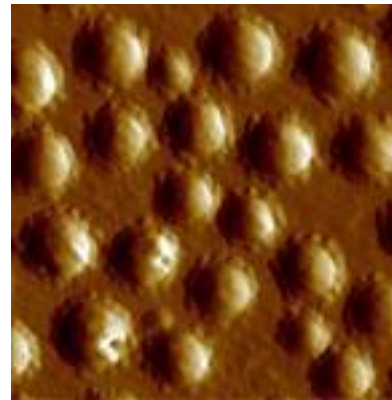
blank PLGA-Chitosan-NP  
~160 nm in size, ~ + 30 mV



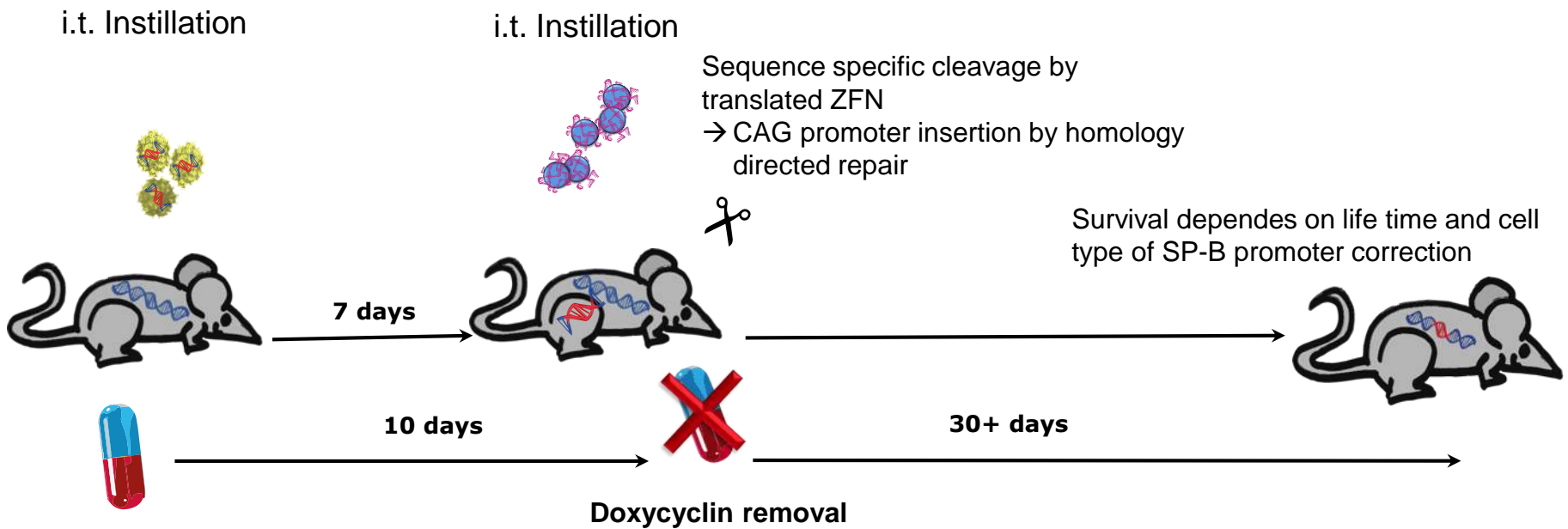
+  
Nucleotides



Beisner J., et al., *Lung Cancer*, 2010  
Taetz S. et al., *Eur.J.Pharm.Biopharm.* 2009  
Nafee N. et al, *Nanomedicine NBT*, 2007  
Kumar M.R.V. et al, *Biomaterials*, 2004

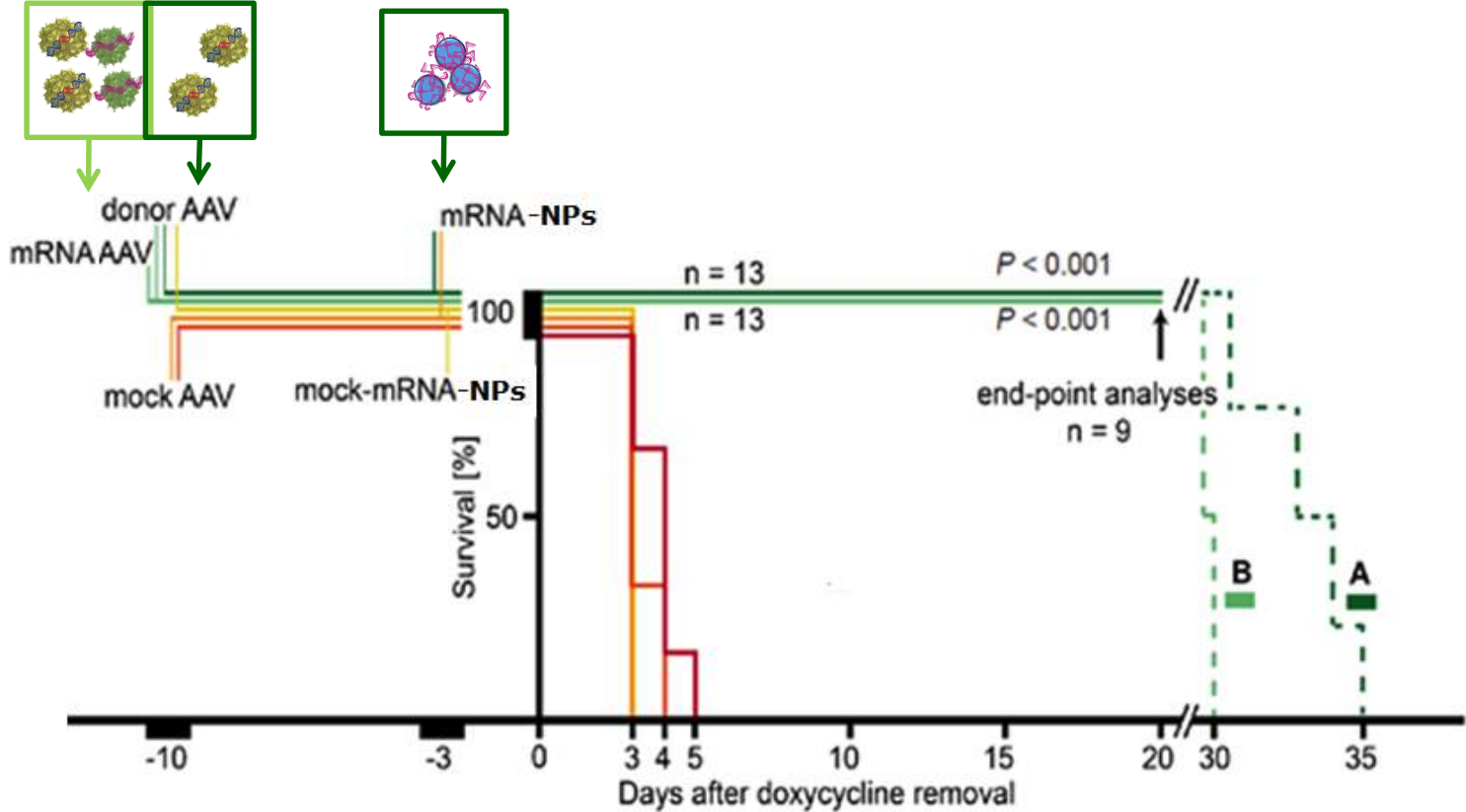


# Treatment Scheme of the Double Transfection



# In vivo genome editing using nuclease-encoding mRNA corrects SP-B deficiency

© 2015 Nature America, Inc. All rights reserved.



controls, n = 6

**C** donor AAV6 + mockRNA-NP

**D** mock AAV6 + mRNA-NP

**E** mock AAV6

**F** no treatment

main groups, n = 13

**A** donor AAV6 + mRNA-NP

**B** donor AAV6 + mRNA AAV6

Mahini et al., **NATURE BIOTECHNOLOGY** 2015 Jun;33(6):584–6.




# Acknowledgements





Research & Development

**PharmBioTec**  
GmbH

 Founded in 2010 by Prof.'s Hartmann, Lehr and Müller

 100% subsidiary of Saarland University

 What we offer:

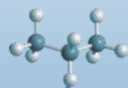
- *In vitro* models of biological barriers (skin, GI, lung)
- Formulations and drug delivery systems; nanomedicine
- Biotech Processes & Analytics of Proteins & Peptides
- Generation of compounds and libraries



Target Discovery



**Hit  
Identification**



**Hit to Lead  
Optimization**



**Preclinical  
Development**



Clinical  
Development



**Regulatory  
Approval  
Manufacturing**



**Europäische Union**

Europäischer Fonds für regionale Entwicklung  
Investition in Ihre Zukunft



Helmholtz-Institut für Pharmazeutische Forschung Saarland

