

A relevant *in vitro* model of the alveolus enables reliable studies of nanomaterial-cell interactions

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European
Commission

Horizon 2020
European Union funding
for Research & Innovation



SmartNanoTox
Smart Tools for Gauging Nano Hazards



Nanoparticles & us

we are constantly exposed to nanoparticles (NPs):

- natural NPs
- incidentally produced NPs
- deliberately produced and used NPs
- food, air, water, cosmetics, medicine, ...



NPs enter our bodies → and bloodstream → accumulate in tissues

Nanoparticles & us

we are constantly exposed to nanoparticles (NPs):

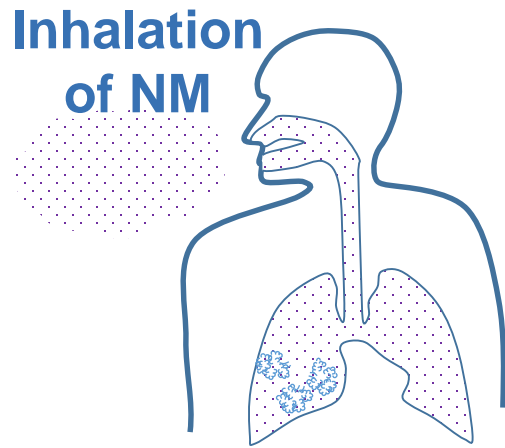
- natural NPs
- incidentally produced NPs
- deliberately produced and used NPs
- food, air, water, cosmetics, medicine, ...



NPs enter our bodies → and bloodstream → accumulate in tissues

consequences???

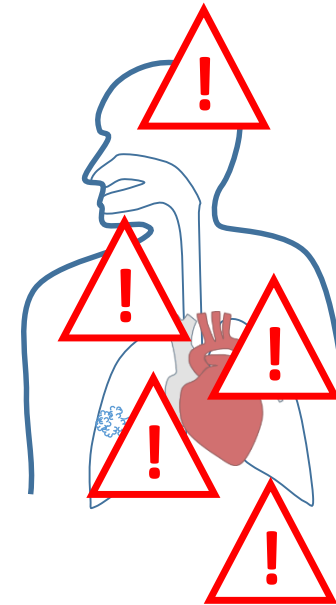
Nanosafety



correlation



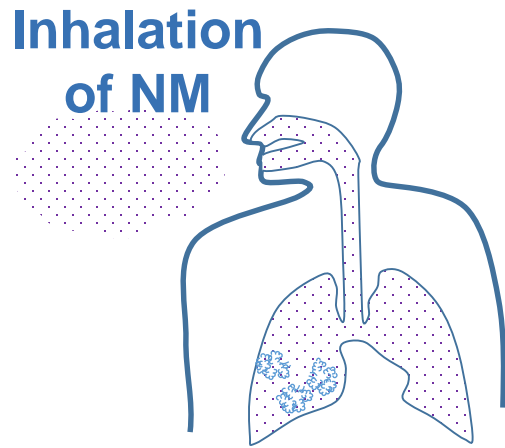
dose
exposure route
nanomaterial properties



neurodegenerative
disease
lung cancer
cardiovascular
disease
lung fibrosis
systemic
inflammation

our goal: testing and predicting nanomaterial health risks

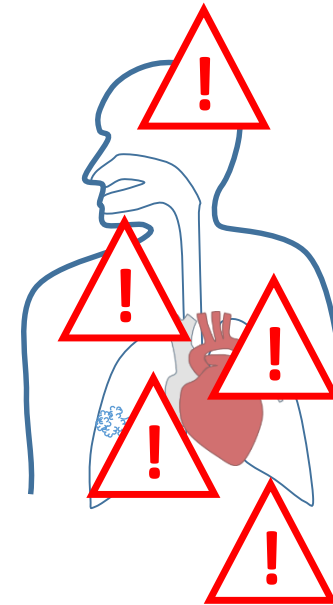
Nanosafety



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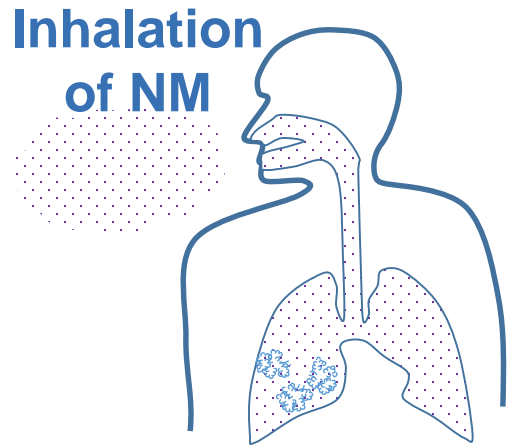
neurodegenerative
disease
lung cancer
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our goal: testing and predicting nanomaterial health risks

X
expensive,
unreliable

X
not possible

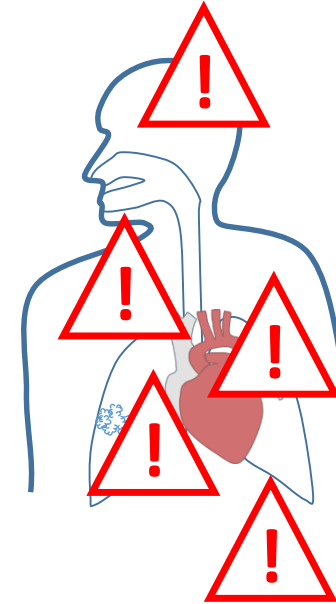
Nanosafety



correlation



dose
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neurodegenerative
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our goal: ~~testing~~ and ~~predicting~~ nanomaterial health risks

expensive,
unreliable

not possible

**we do not understand
the interactions!**

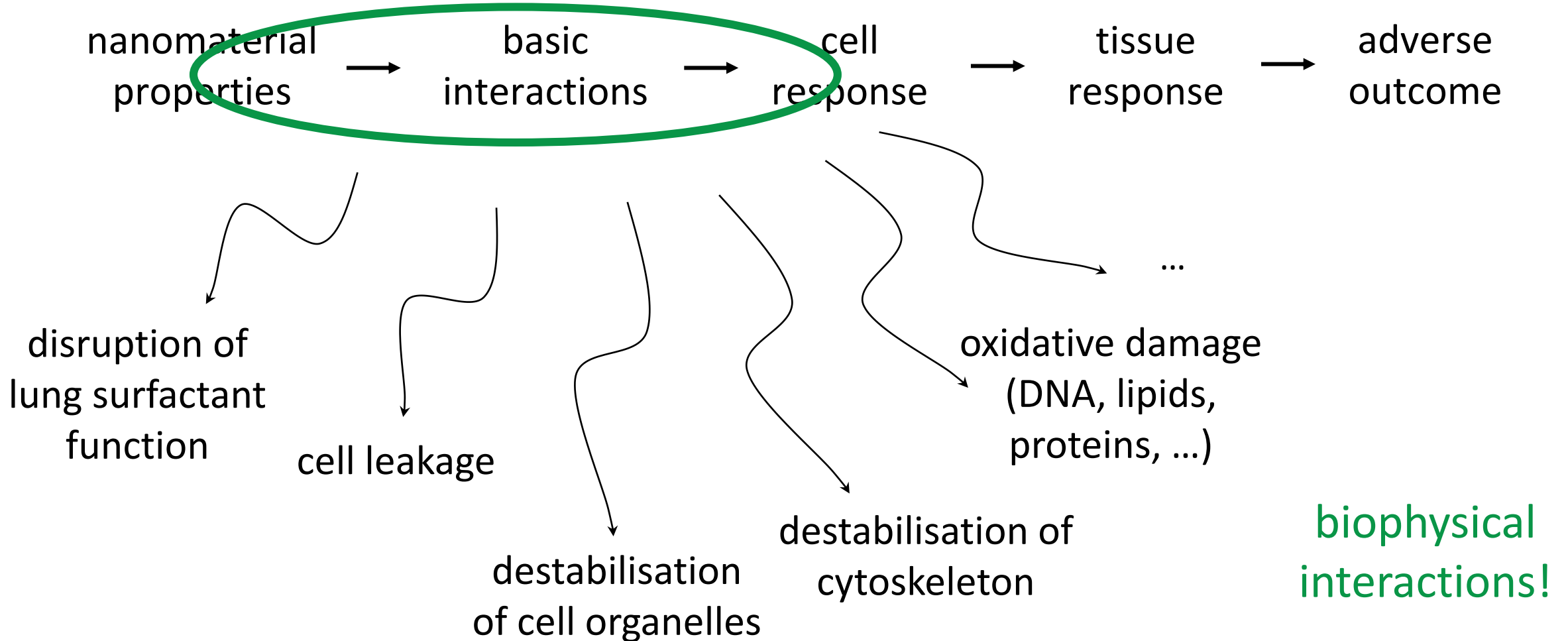
The search for molecular initiating events



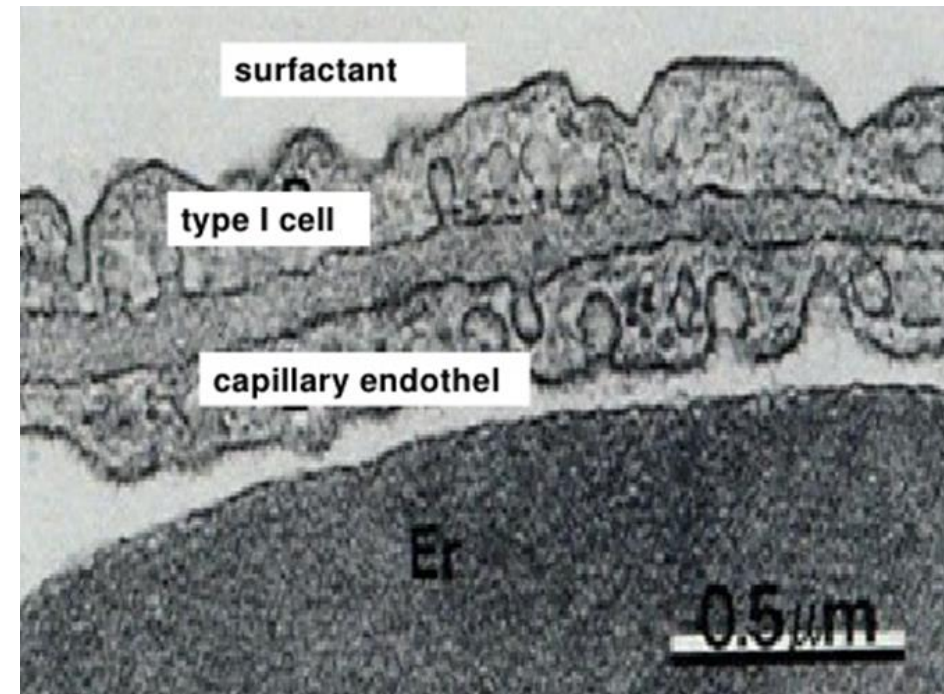
The search for molecular initiating events



The search for molecular initiating events



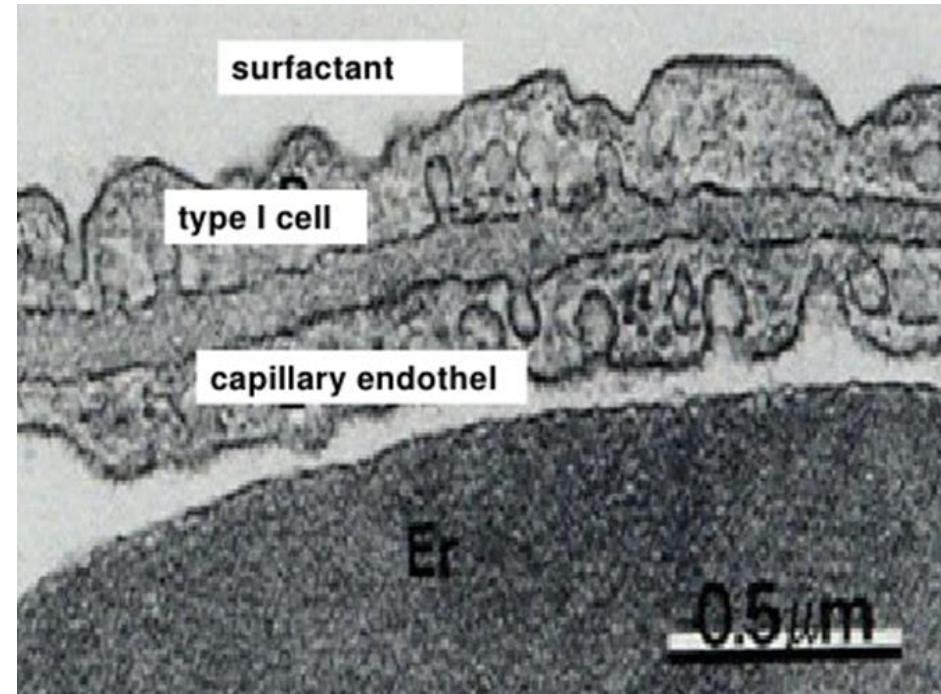
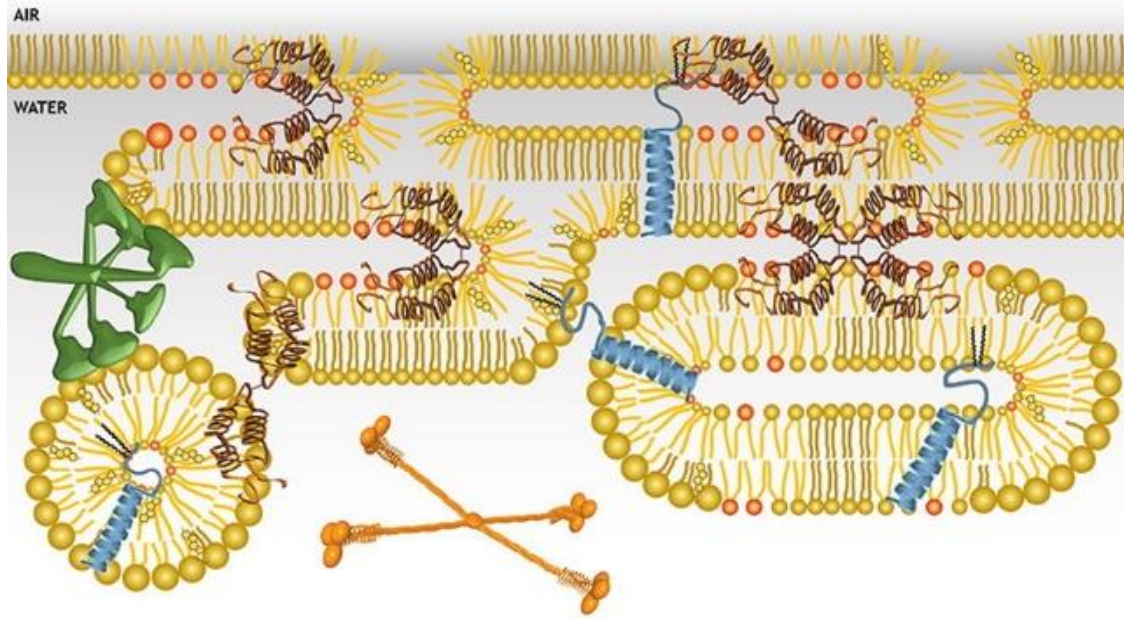
Relevant *in vitro* model?



air

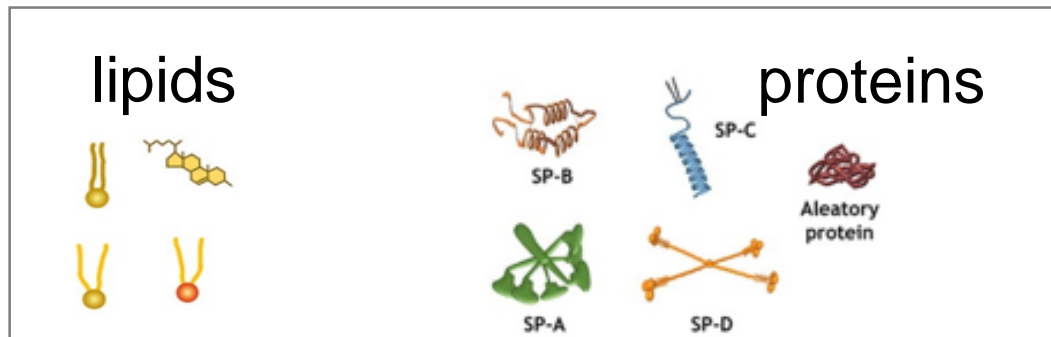
blood

Relevant *in vitro* model?

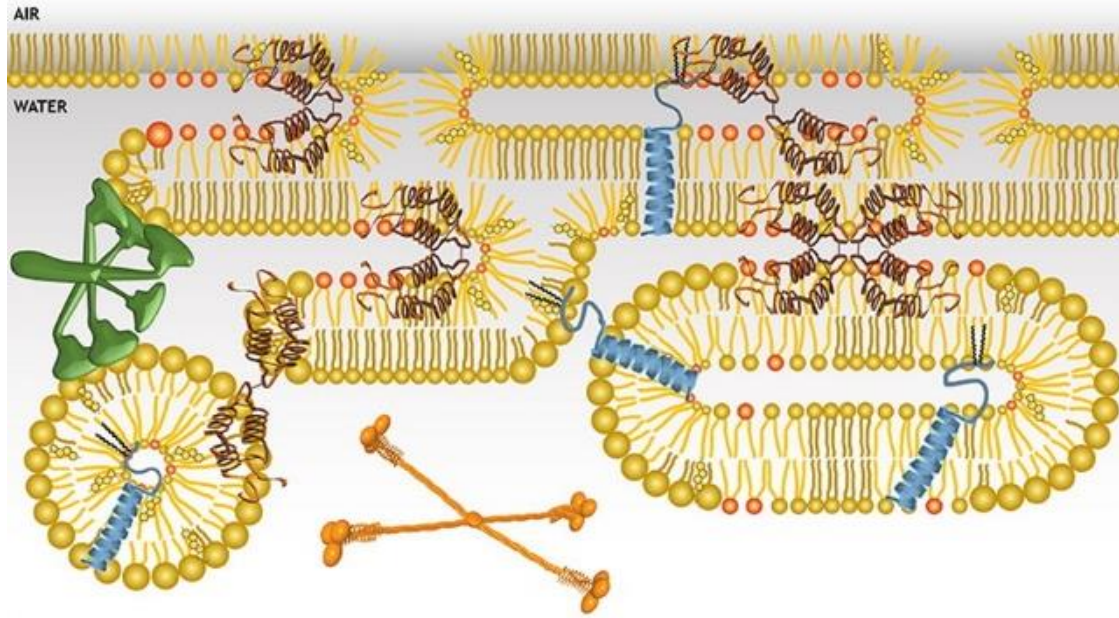


air

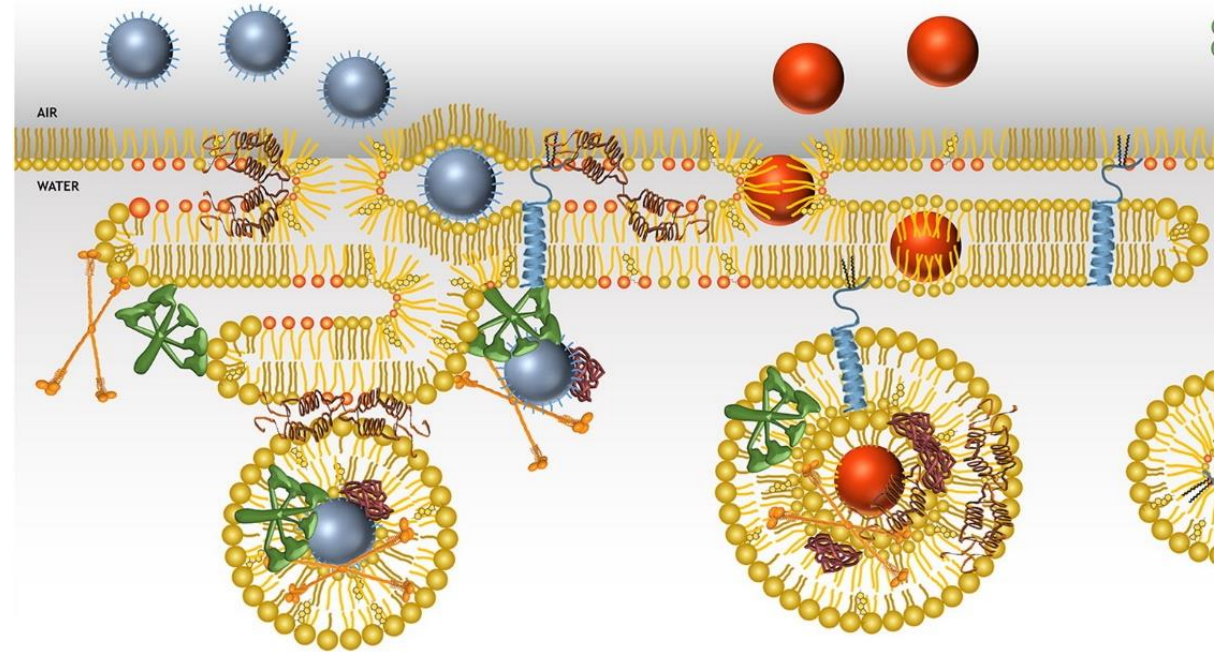
blood



Lung surfactant



Biomolecular corona

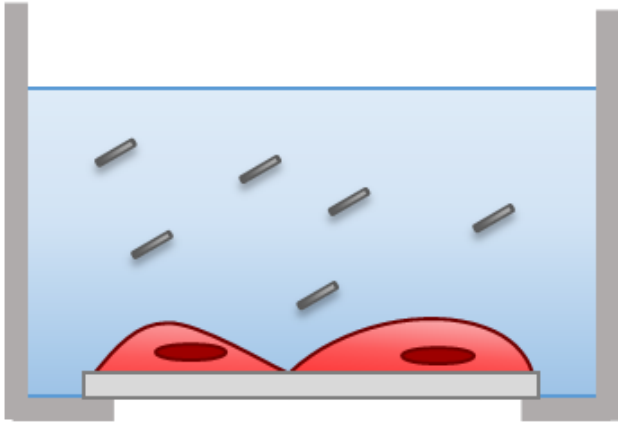


<p>lipids</p>	<p>proteins</p>	<p>nanoparticles</p>
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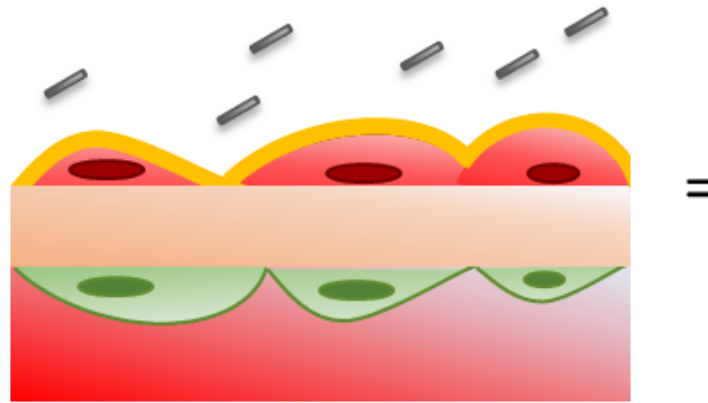
corona depends on NP properties

defines the biological fate of the NM!

Physiologically relevant *in vitro* model

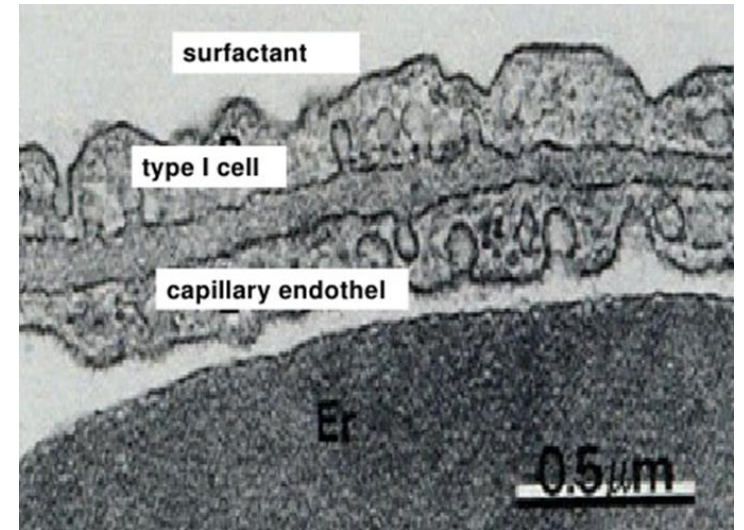


submerged *in vitro* model



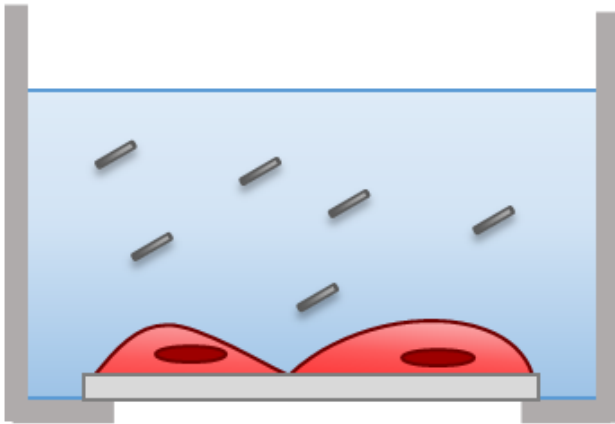
alveolus

=

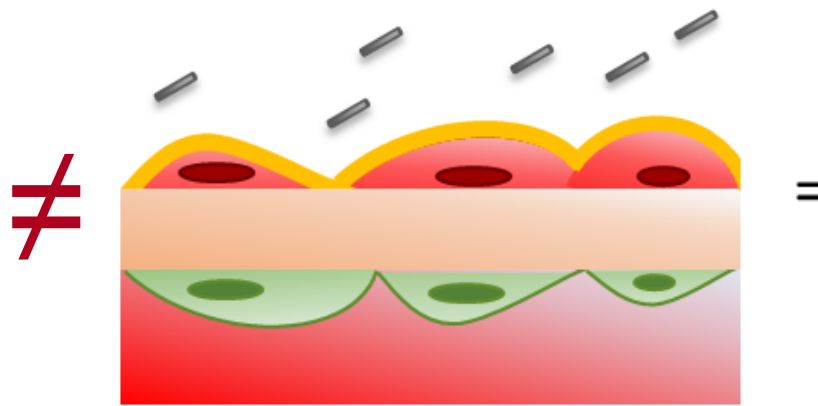


Physiologically relevant *in vitro* model

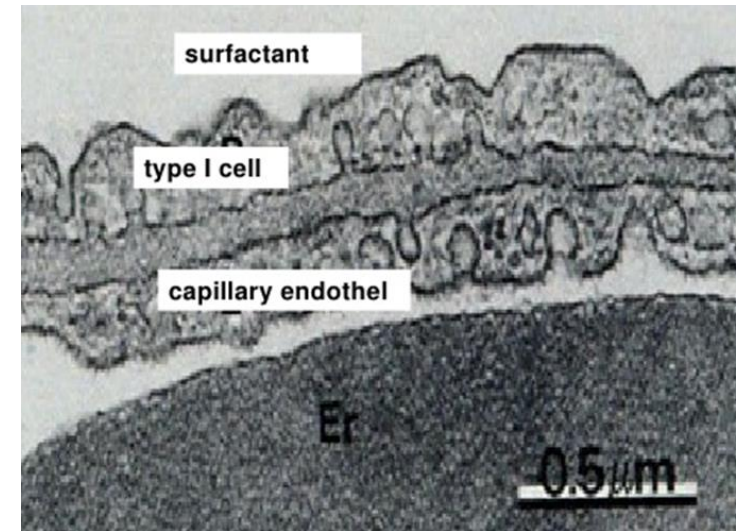
different NP corona \rightarrow different interactions \rightarrow different conclusions!



submerged *in vitro* model

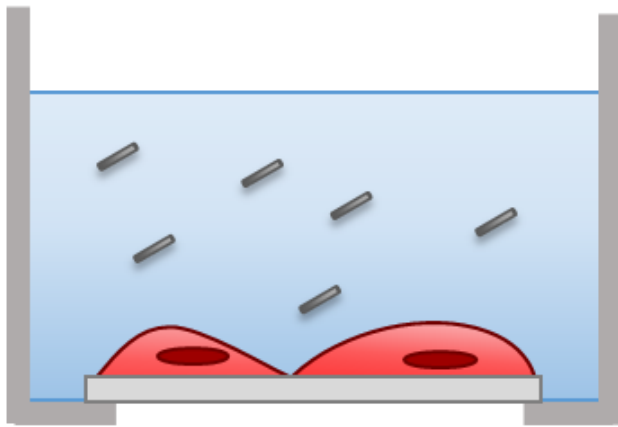


alveolus



Physiologically relevant *in vitro* model

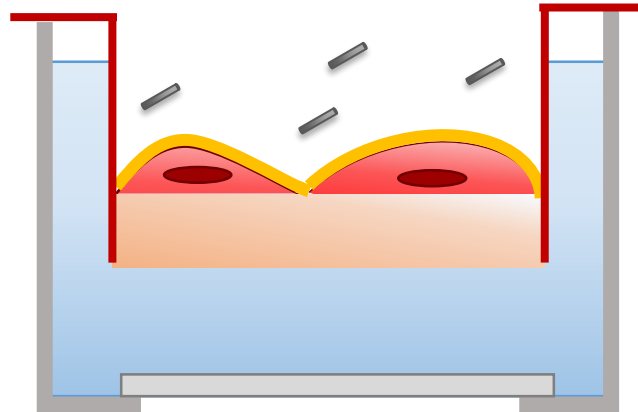
different NP corona \rightarrow different interactions \rightarrow different conclusions!



submerged *in vitro* model

NPs in cell medium

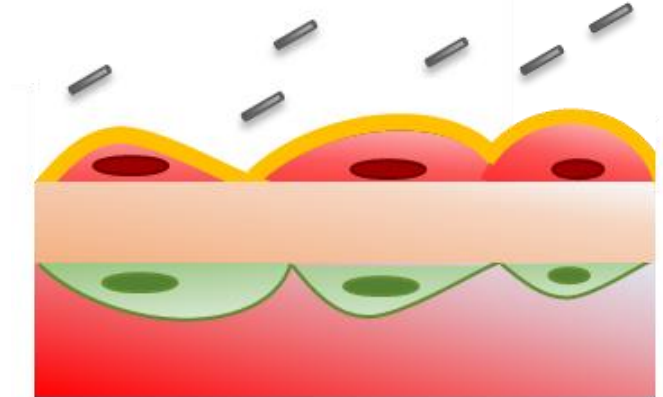
\neq



our proposed *in vitro* model

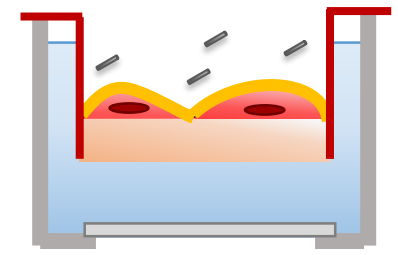
surfactant
airborne NPs

\approx



alveolus
surfactant
airborne NPs

Surfactant layer deposition

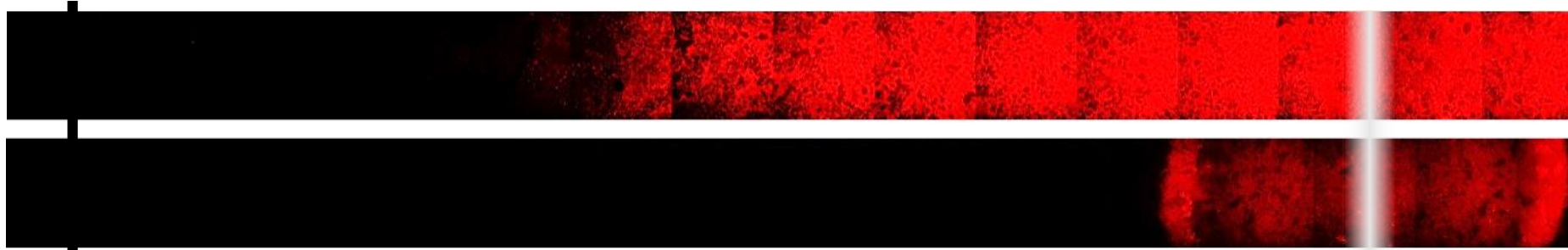


~ μm of medium

- application of 10 monolayers of **lung surfactant** (with all lipids and proteins)
- applied and filmed at 37 °C

Surfactant layer deposition

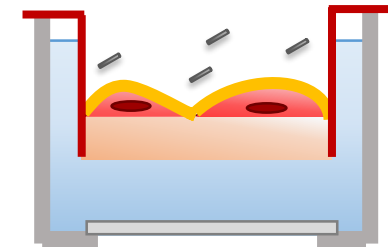
edge of dish



pipette spot



1 mm



~ μm of medium

~ 10 nm of medium

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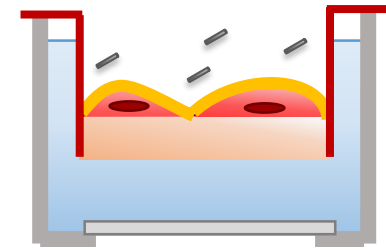
Surfactant layer deposition

edge of dish

pipette spot



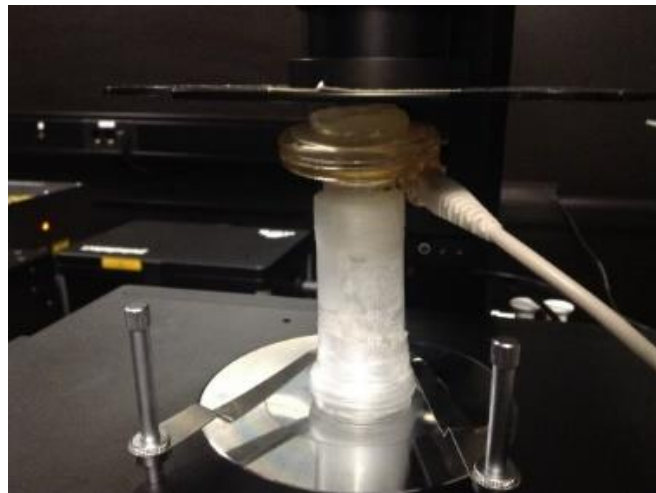
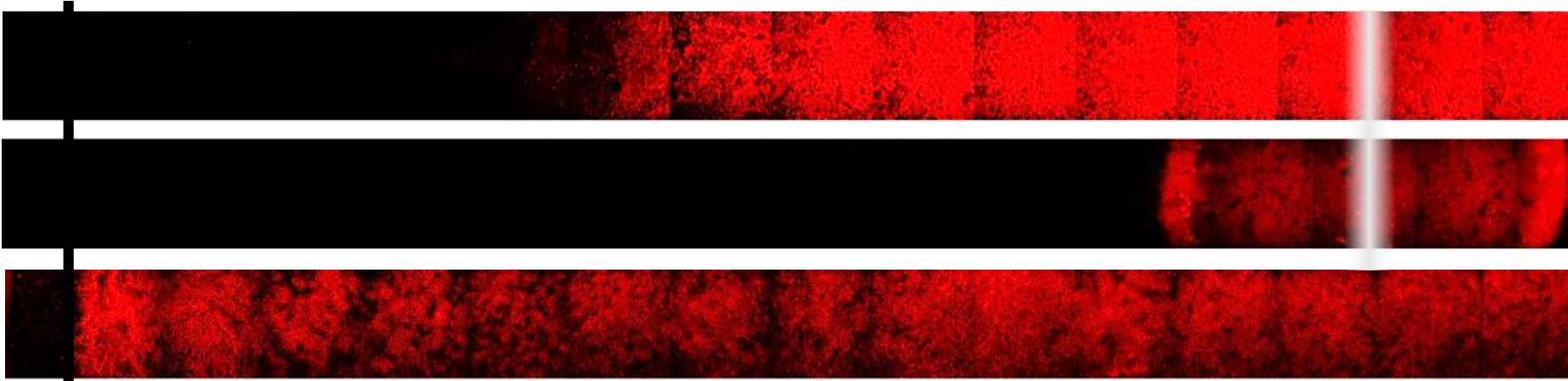
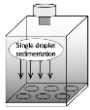
1 mm



~ μm of medium

~ 10 nm of medium

~ 10 nm of medium



- application of 10 monolayers of **lung surfactant** (with all lipids and proteins)
- applied and filmed at 37 °C

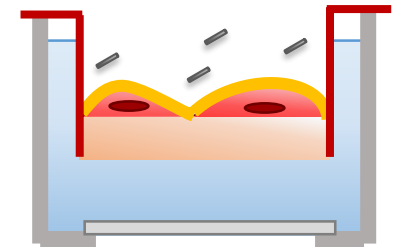
Surfactant layer deposition

edge of dish

pipette spot



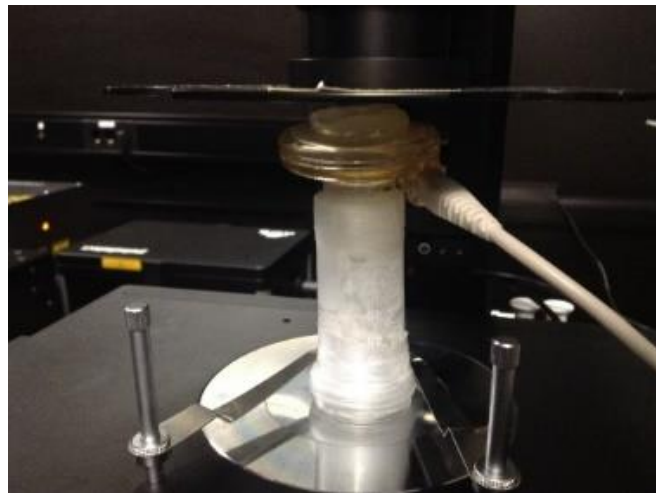
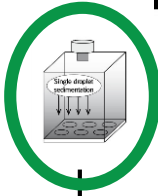
1 mm



~ μm of medium

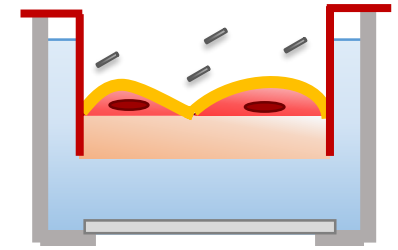
~ 10 nm of medium

~ 10 nm of medium



- application of 10 monolayers of **lung surfactant** (with all lipids and proteins)
- applied and filmed at 37 °C

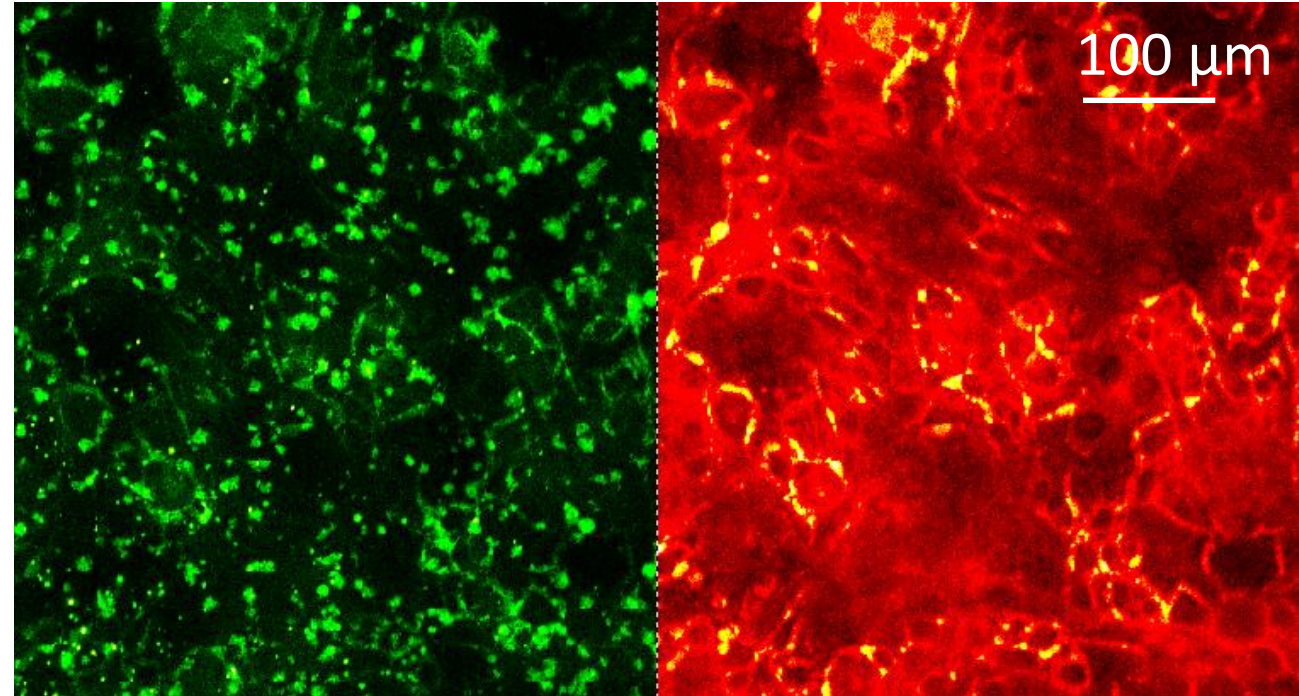
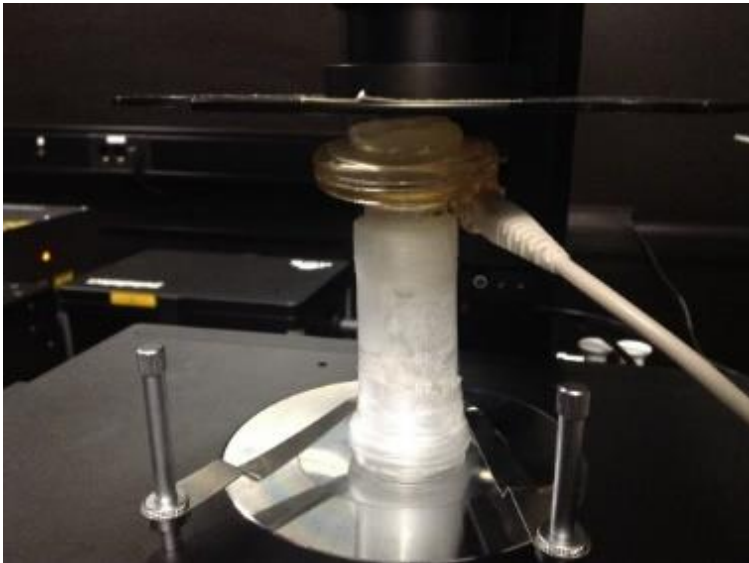
Nanoparticle deposition



Non-labeled LA4 cells (shadows)

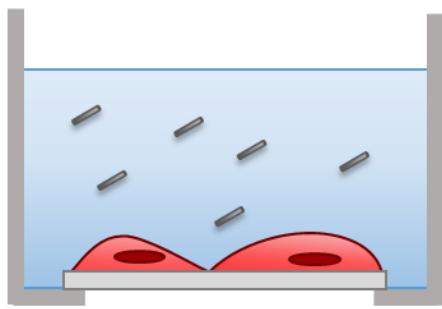
ATTO 594 on TiO₂ nanotubes

STARRED DPPE (1:1000 to surf.)



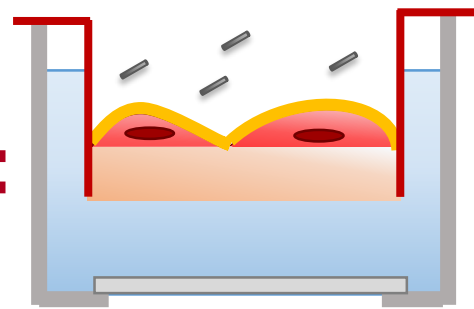
- 1st application of 10 monolayers of **lung surfactant** (with all lipids and proteins) via nebulization
- 2nd application of airborne **nanomaterial** via nebulization

Future upgrade: *in vitro* model of air-blood barrier for high-resolution live imaging



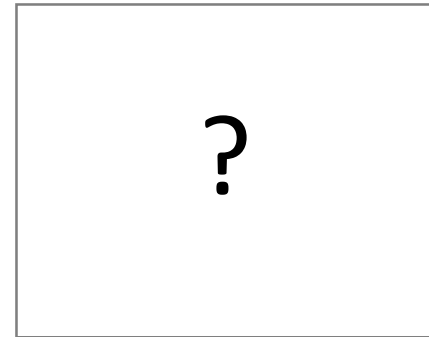
submerged *in vitro* model

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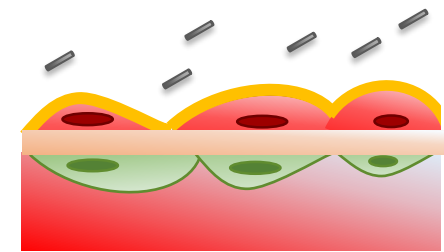


our current *in vitro* model

≈



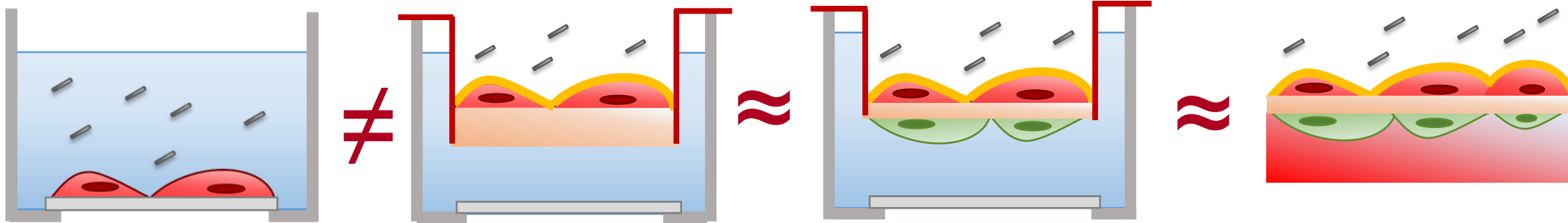
≈



alveolus

coculture (macrophages and capillary cells)
thin membrane

Future upgrade: *in vitro* model of air-blood barrier for high-resolution live imaging



submerged *in vitro* model

our current *in vitro* model

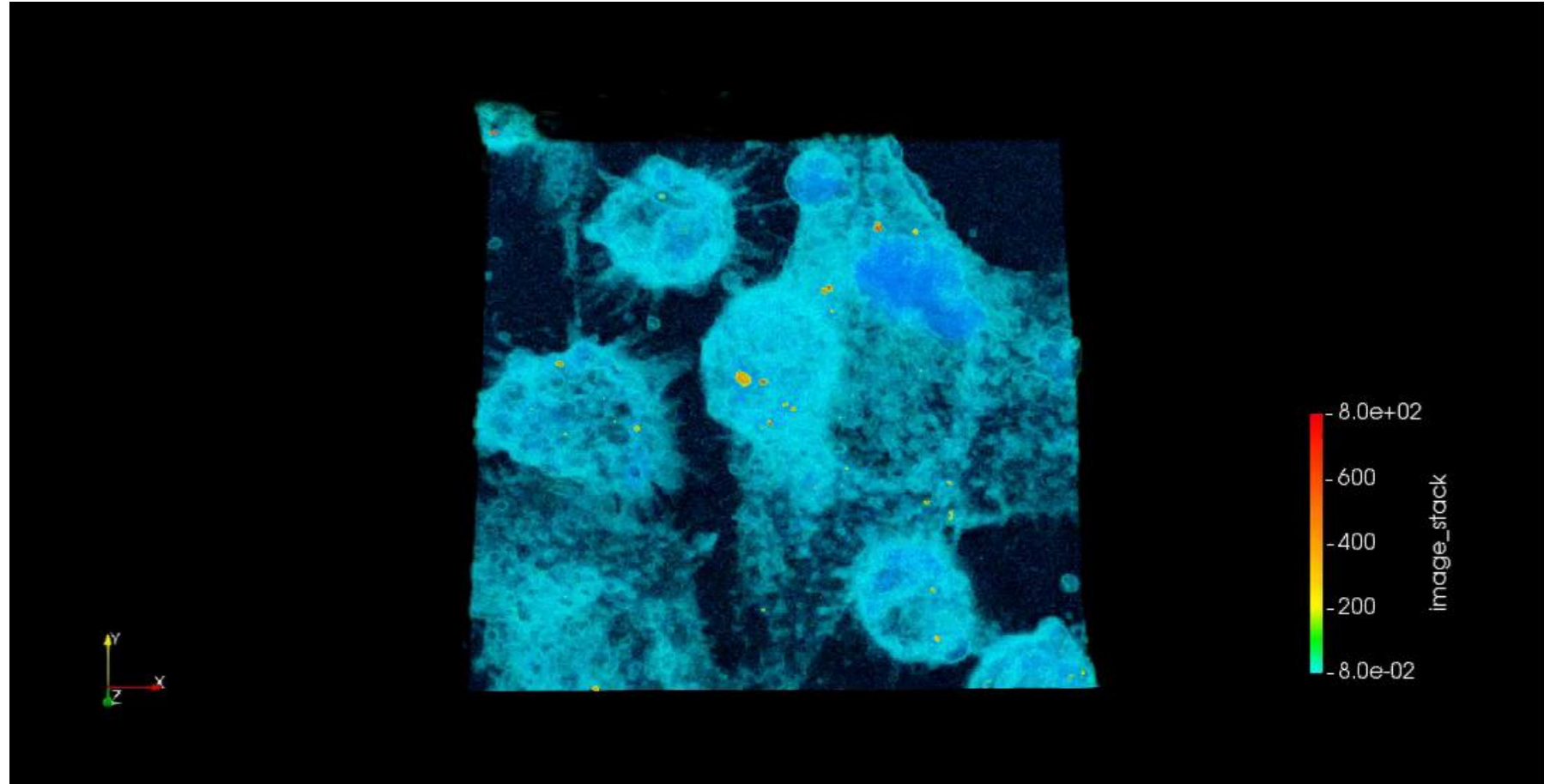
proposed *in vitro* model

alveolus

coculture (macrophages and capillary cells)
thin membrane

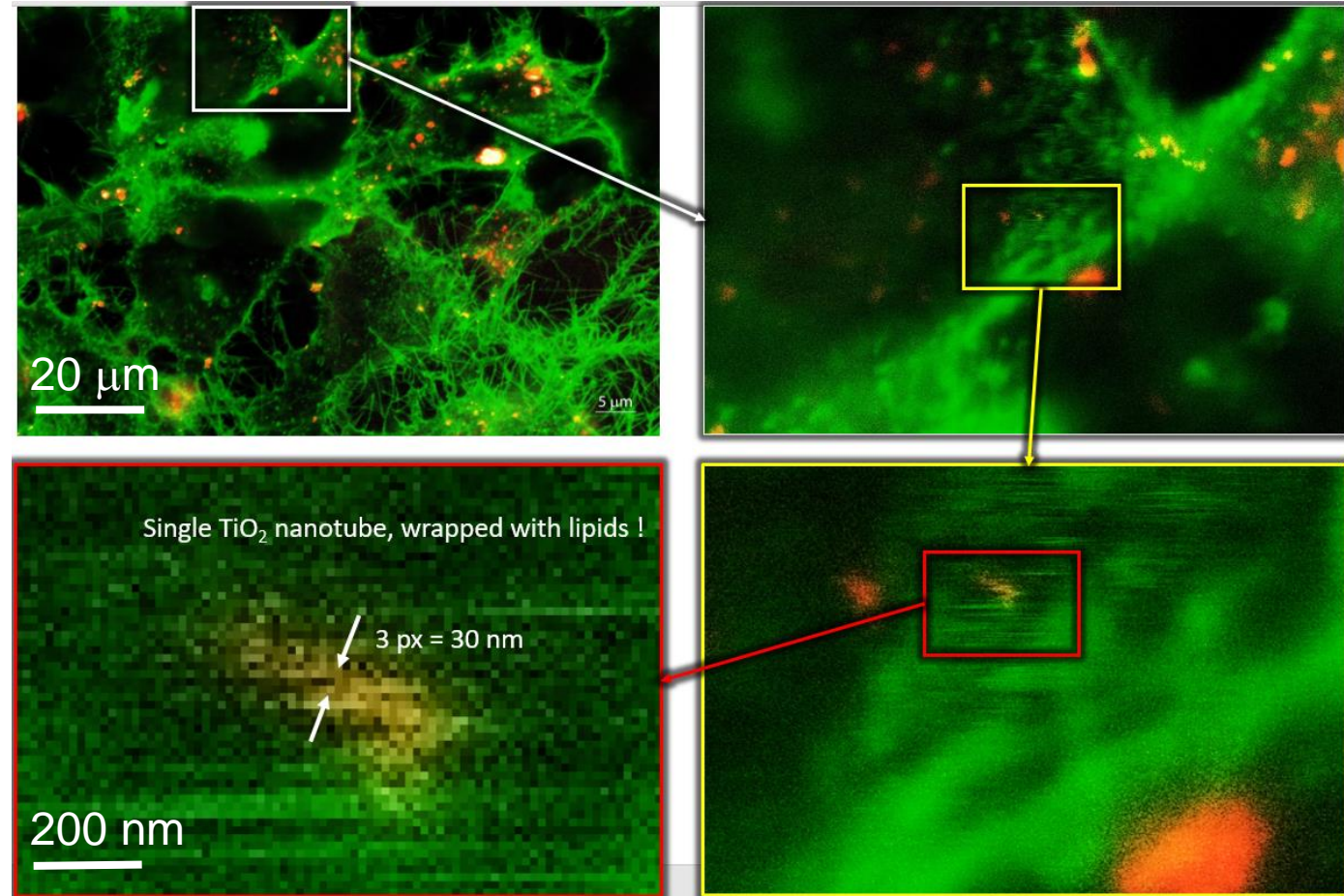
Future upgrade: *in vitro* model of air-blood barrier for high-resolution live imaging

- coculture of epithelial lung cells and macrophages, incubated with TiO_2 nanotubes
- confocal 3D image from a series of z-stacks, filmed on living cells

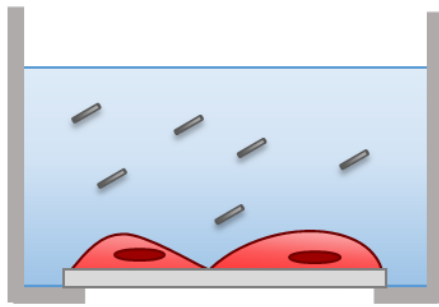


Future upgrade: *in vitro* model of air-blood barrier for high-resolution live imaging

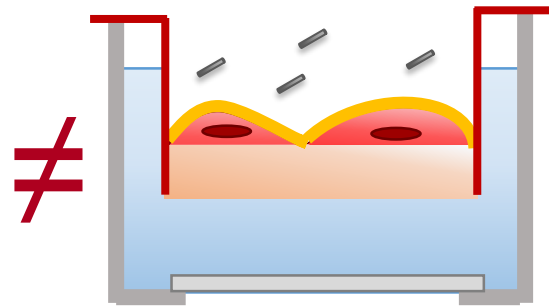
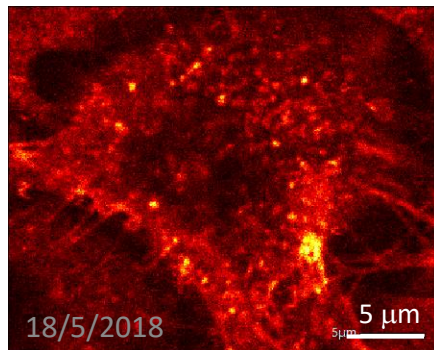
- epithelial lung cells, incubated with TiO_2 nanotubes
- STED panorama of living cells



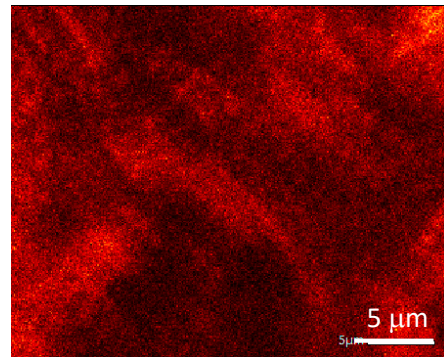
Future upgrade: *in vitro* model of air-blood barrier for high-resolution live imaging



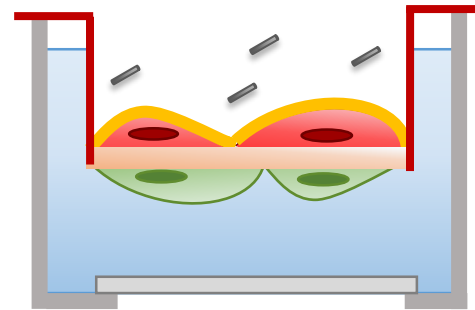
submerged *in vitro* model



our current *in vitro* model



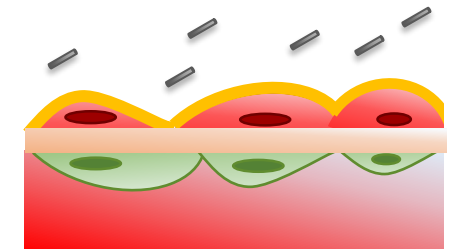
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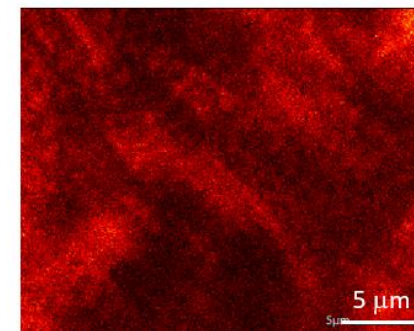
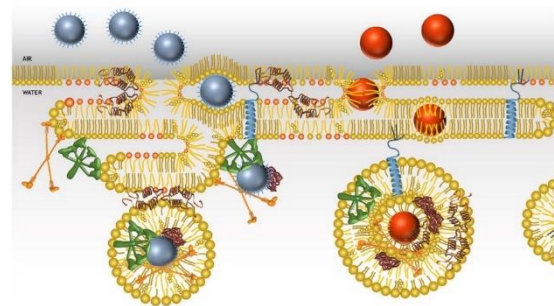
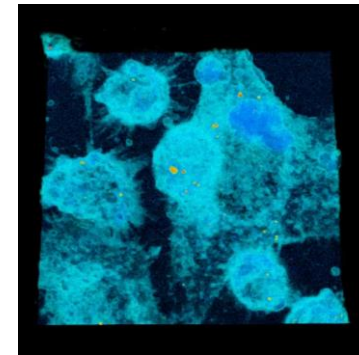
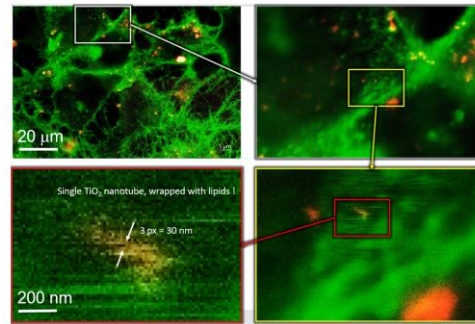
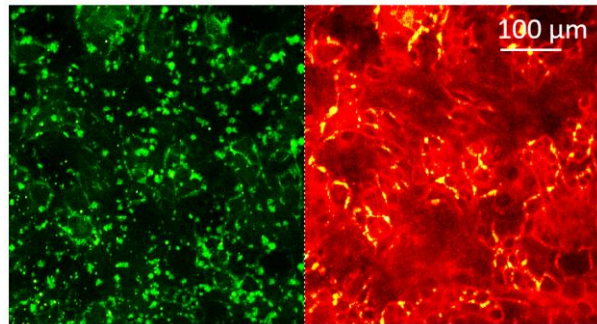
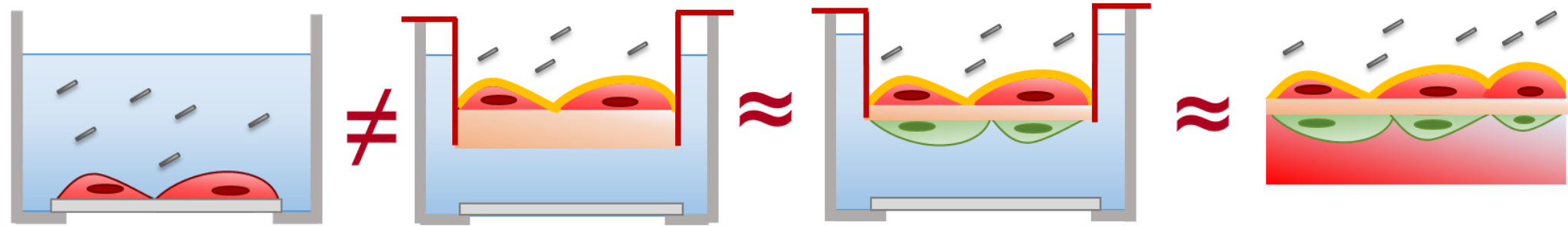
proposed *in vitro* model

coculture
thin membrane

≈



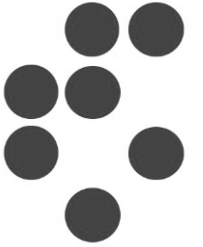
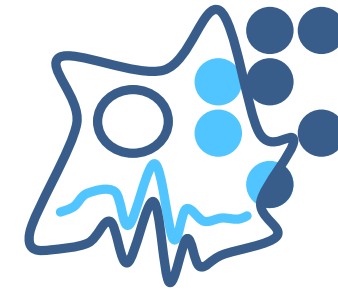
alveolus



Interested in *in vitro* models? We have a **PhD position** open!

Interested in **super-resolution imaging** (2PE-STED-FLIM)? We have a **postdoc position** open!

<http://lbf.ijs.si/>
janez.strancar@ijs.si



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SmartNanoTox
Smart Tools for Gauging Nano Hazards

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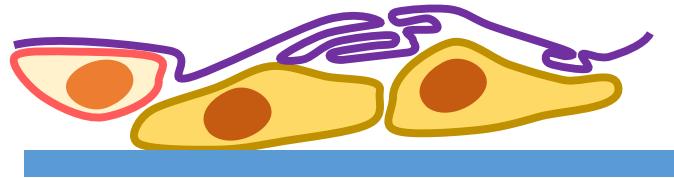
<http://lbf.ijs.si/>
janez.strancar@ijs.si



physiologically relevant in vitro models



contaminated air



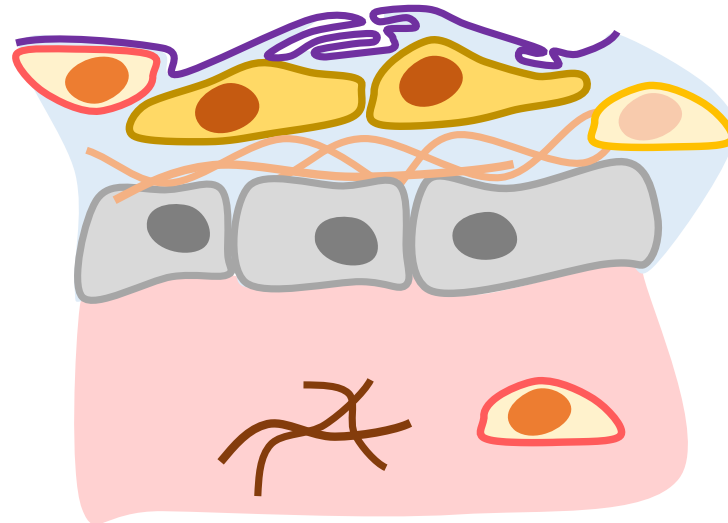
tissue barrier model

Current Lung barrier model: surfactant,
epithelium, immune cells

physiologically relevant in vitro models



contaminated air

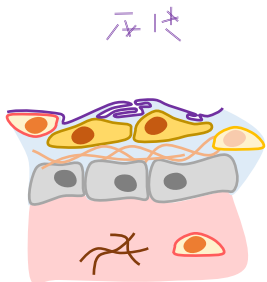


tissue barrier model

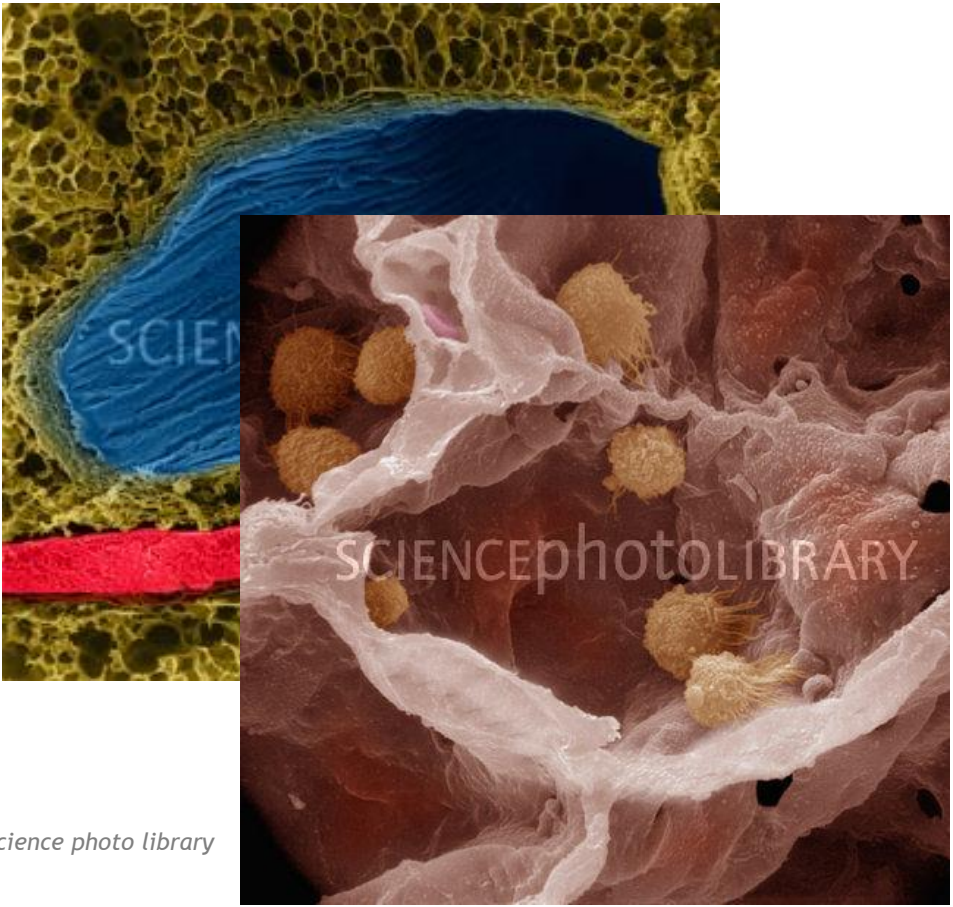
circulation model

Lung barrier model: surfactant, epithelium,
fibroblasts & matrix, endothelium

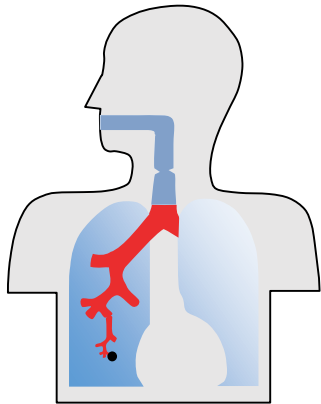
Circulation model: immune cells &
coagulation factors



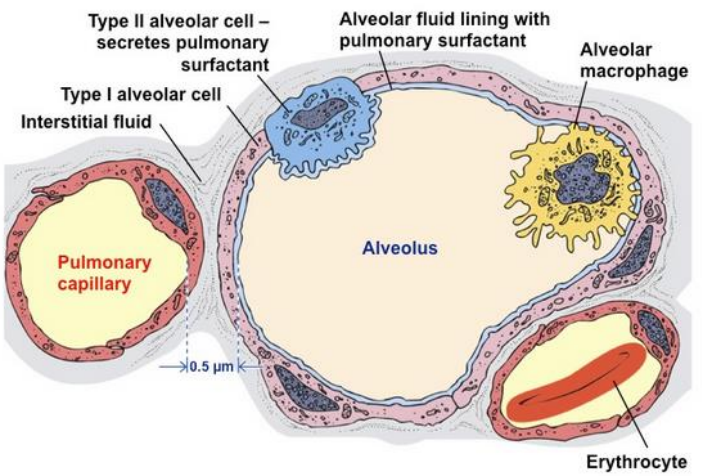
The alveolus



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