Toxicology of Nanoparticles - Nanotoxicology

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CENATOX
Centre of Excelence for Nanotoxicology

„V neustálém cyklu proměn malé se stává velkým a velké nepatrným“

Lao‘c

Formation Aggregation Growth Birth

Termination Dissociation Decay Death

“Nothing is more damaging to a new truth than an old error.”
Johann Wolfgang von Goethe

Growth of Nanotechnology Sector

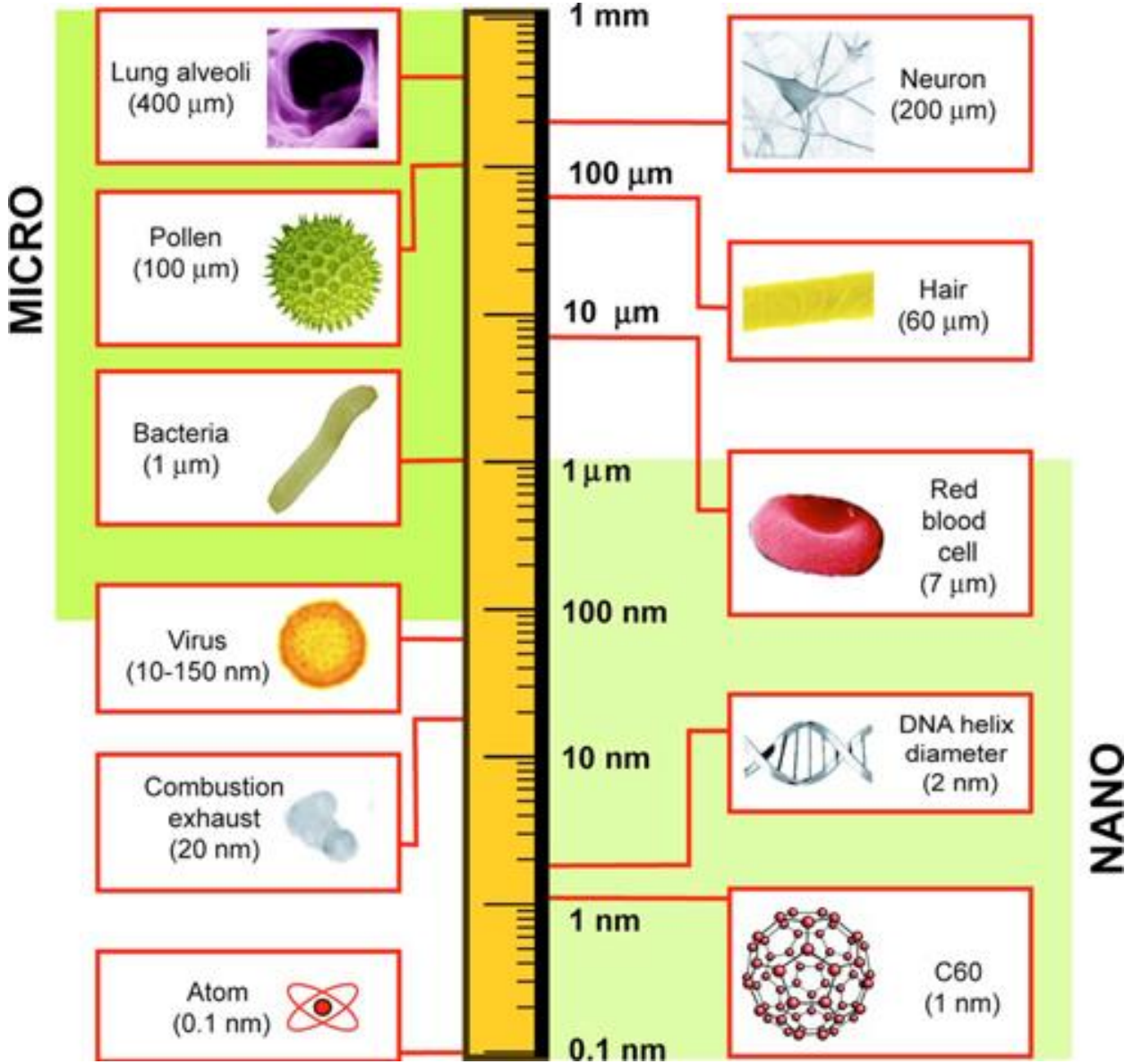
- 2001, National Nanotechnology Initiative (USA)
- 2002, Nanotechnology Network (Australia)
- 2005, $32 billion globally
- 2007, $147 billion
- 2010, ~ 1,015 products (24 nations)
- 2015: $2.6 trillion (projected)

Image source: www.eagleone.com

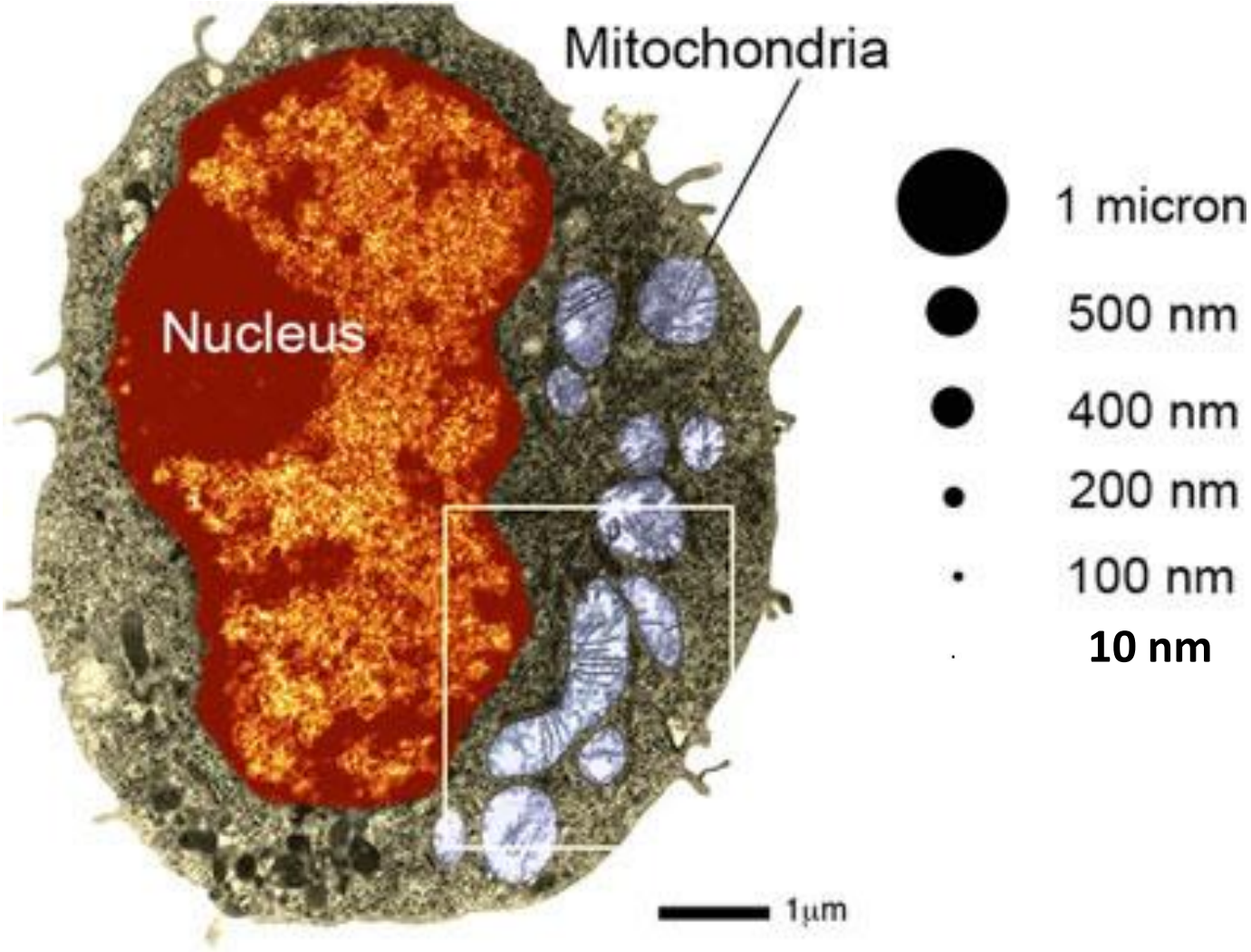
Project on Emerging Technologies

www.nanotechproject.org

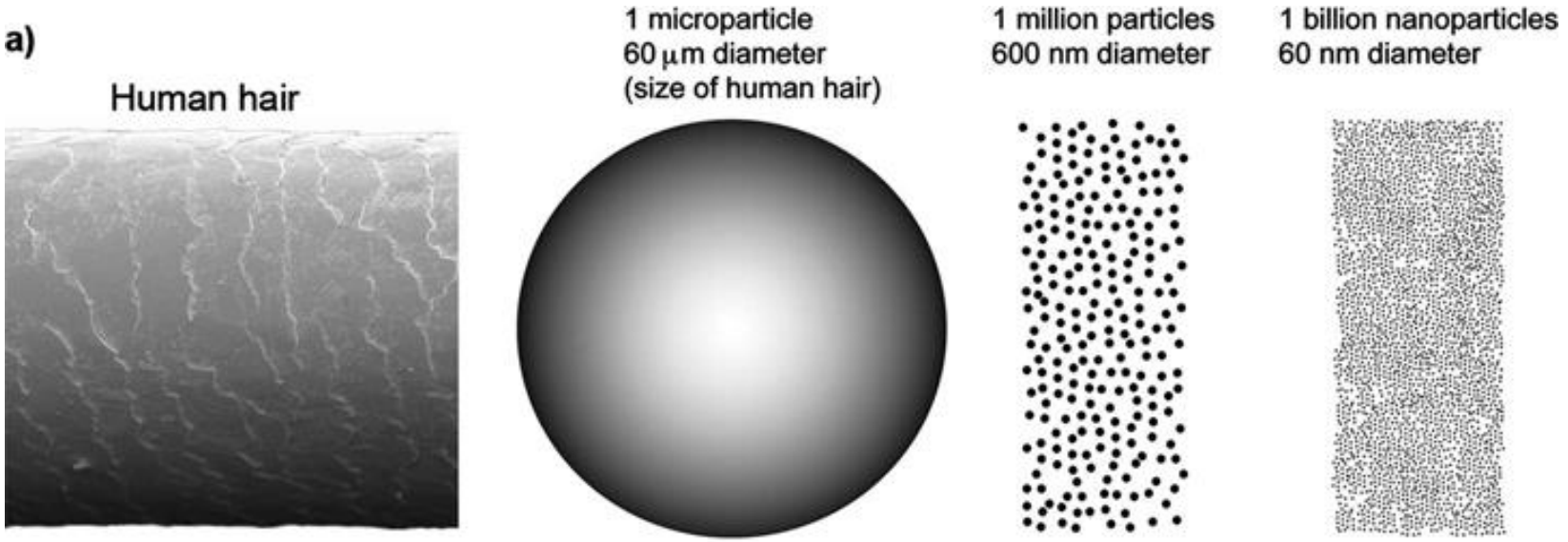
Log length scale showing size of nanomaterials compared to biological components and definition of “nano” and “micro” sizes

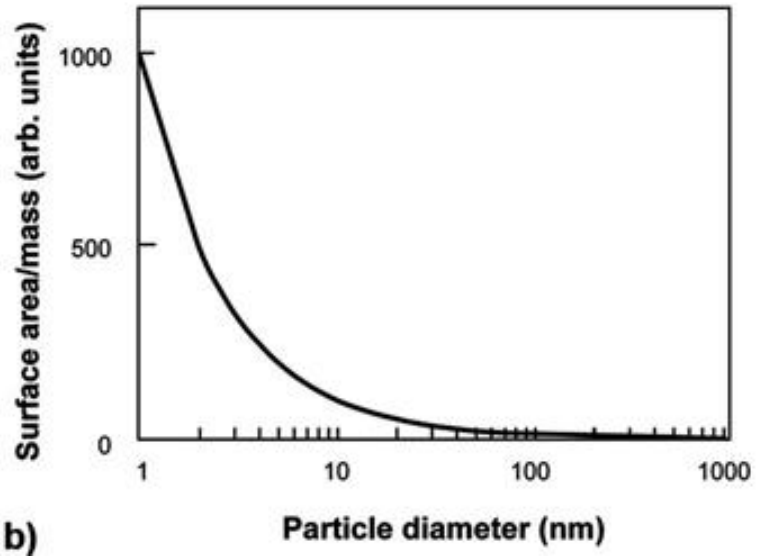


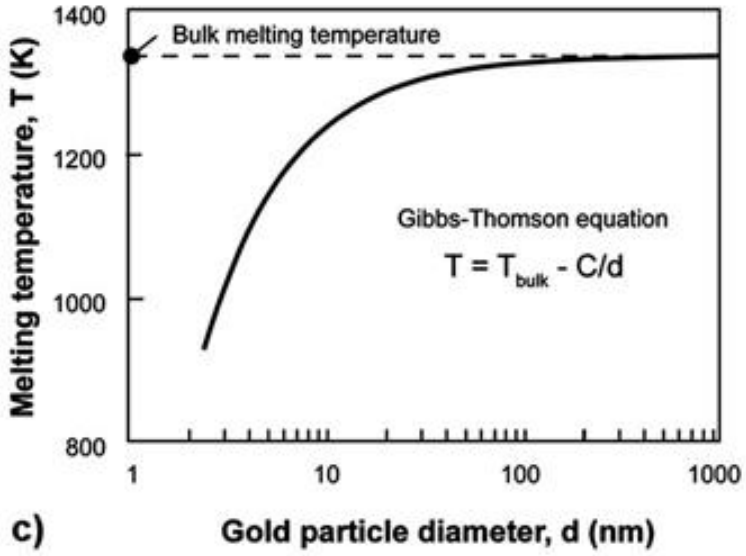
Comparison of rat macrophage cells size to nanoparticles size



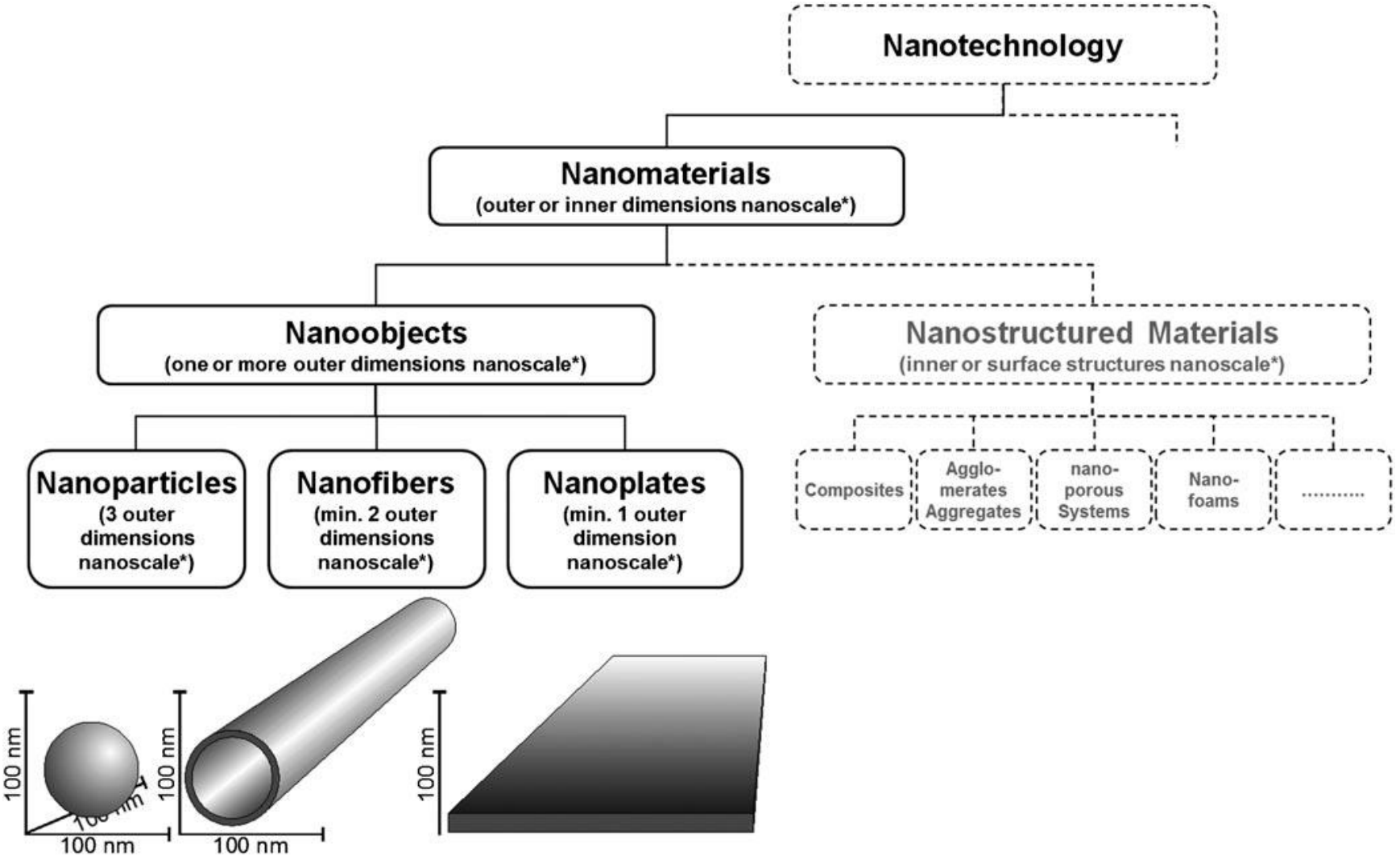
Microparticles versus nanoparticles







Nanoobjects



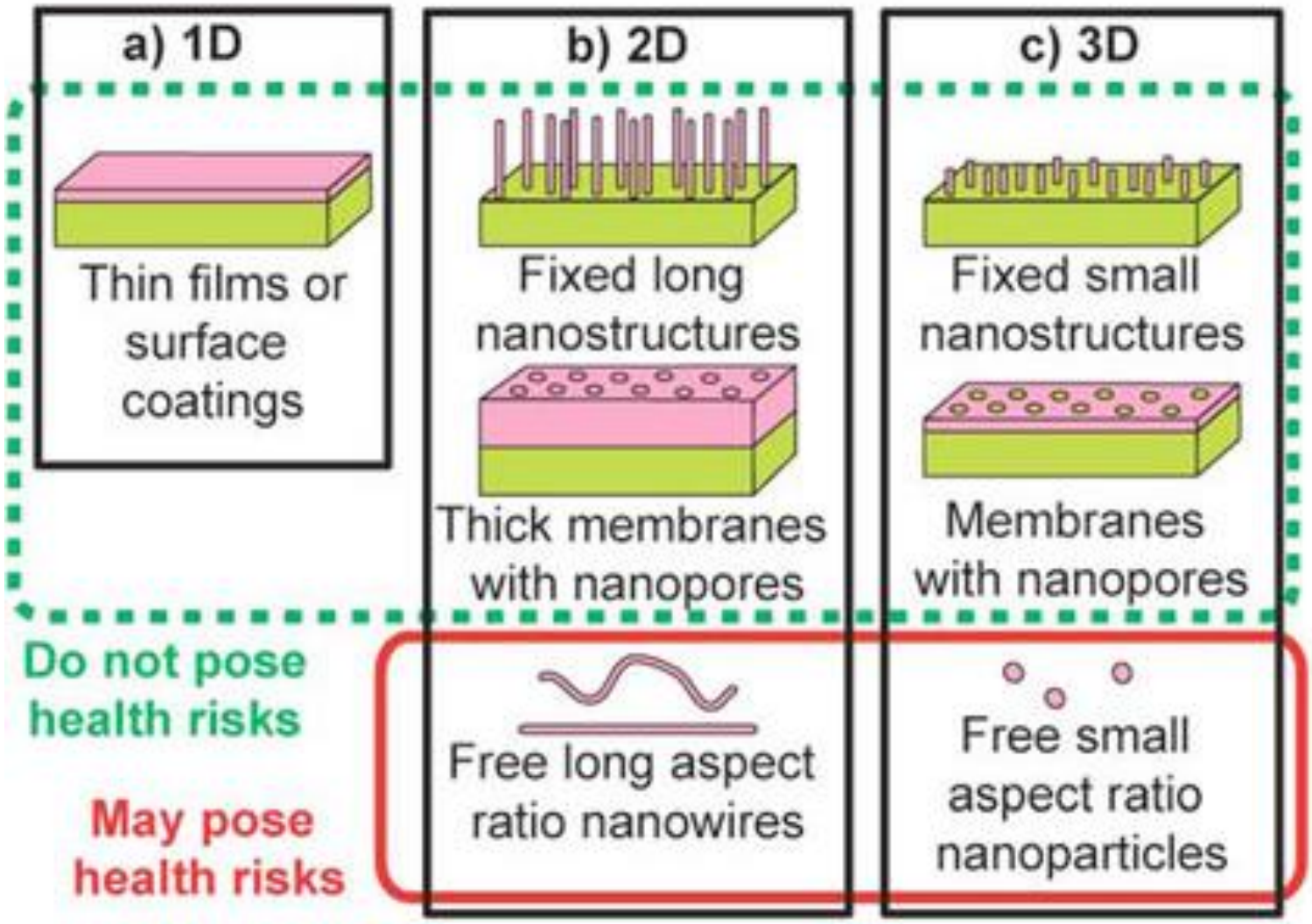
The ISO definition of nanoobjects. Included as nanoobjects are nanoparticles (nanoscale in all three dimensions), nanofibers (nanoscale in two dimensions), and nanoplates or nanolayers (nanoscale only in one dimension). * Nanoscale: a size of between 1 and 100 nm.

Main characteristics of Nanoparticles

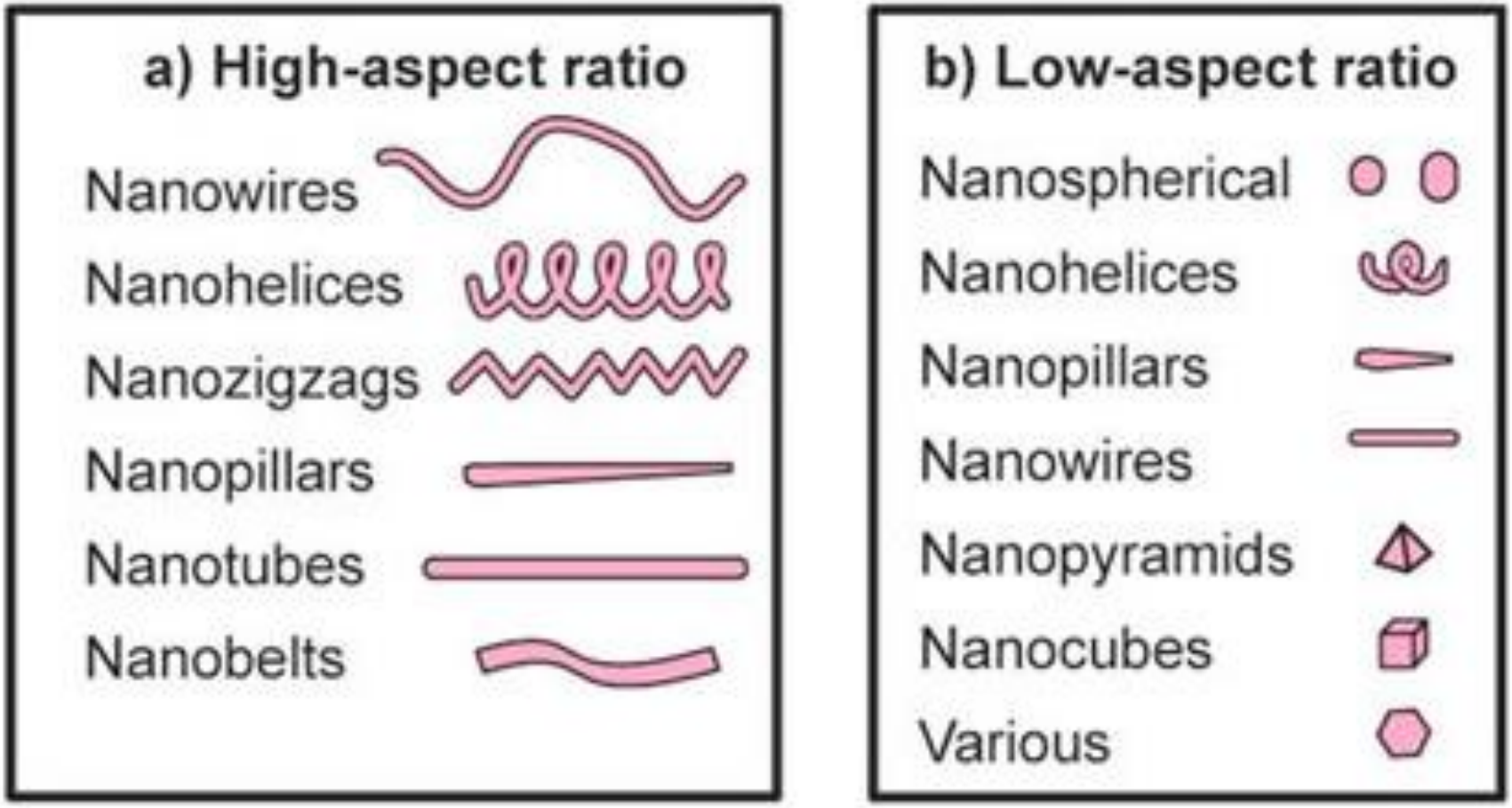
* Chemical composition, purity, impurities
* Particle size and size distribution
* Specific surface
* Morphology (crystalline/amorphous, shape)
* Surface chemistry, coating, functionalization
* Degree of agglomeration/aggregation and particle size distribution under experimental conditions (for example, media with/without proteins)
* Water solubility (differentiation between soluble, metastable, and biopersistent nanomaterials)
* Surface reactivity and/or surface load (zeta potential).

NANOPARTICLE CLASSIFICATION

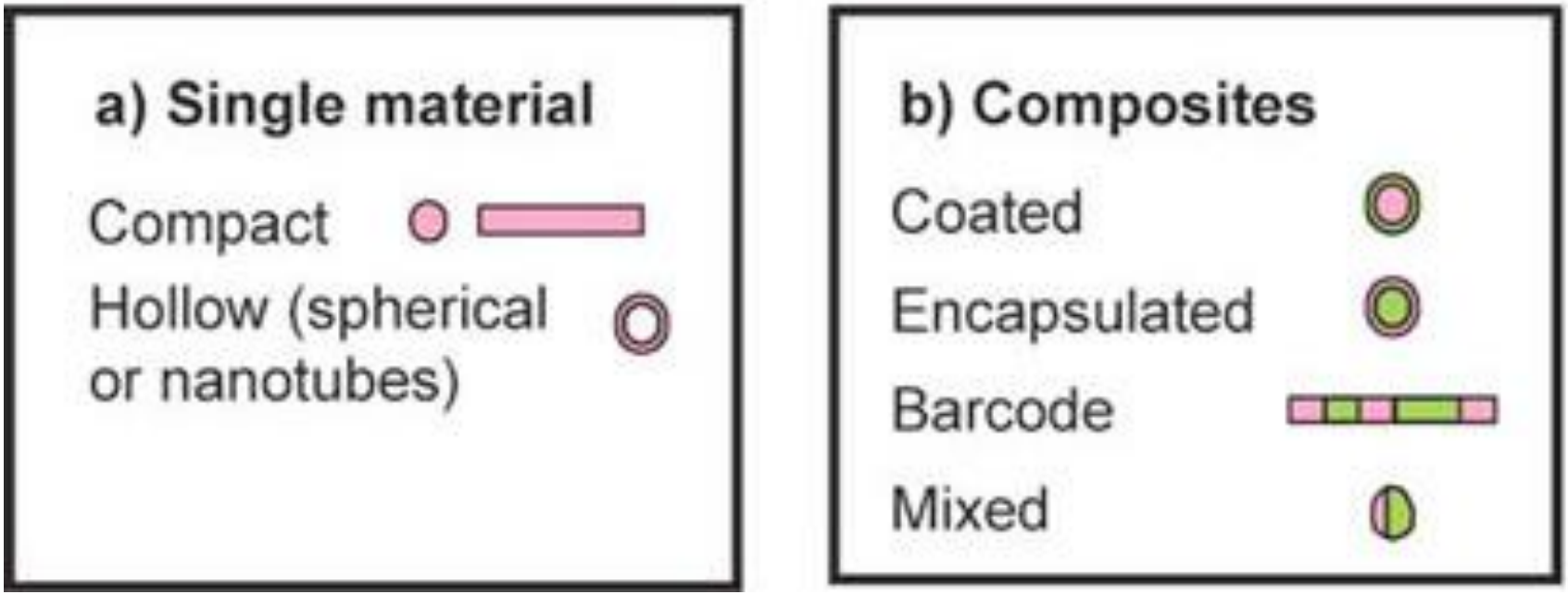
1. Dimensionality



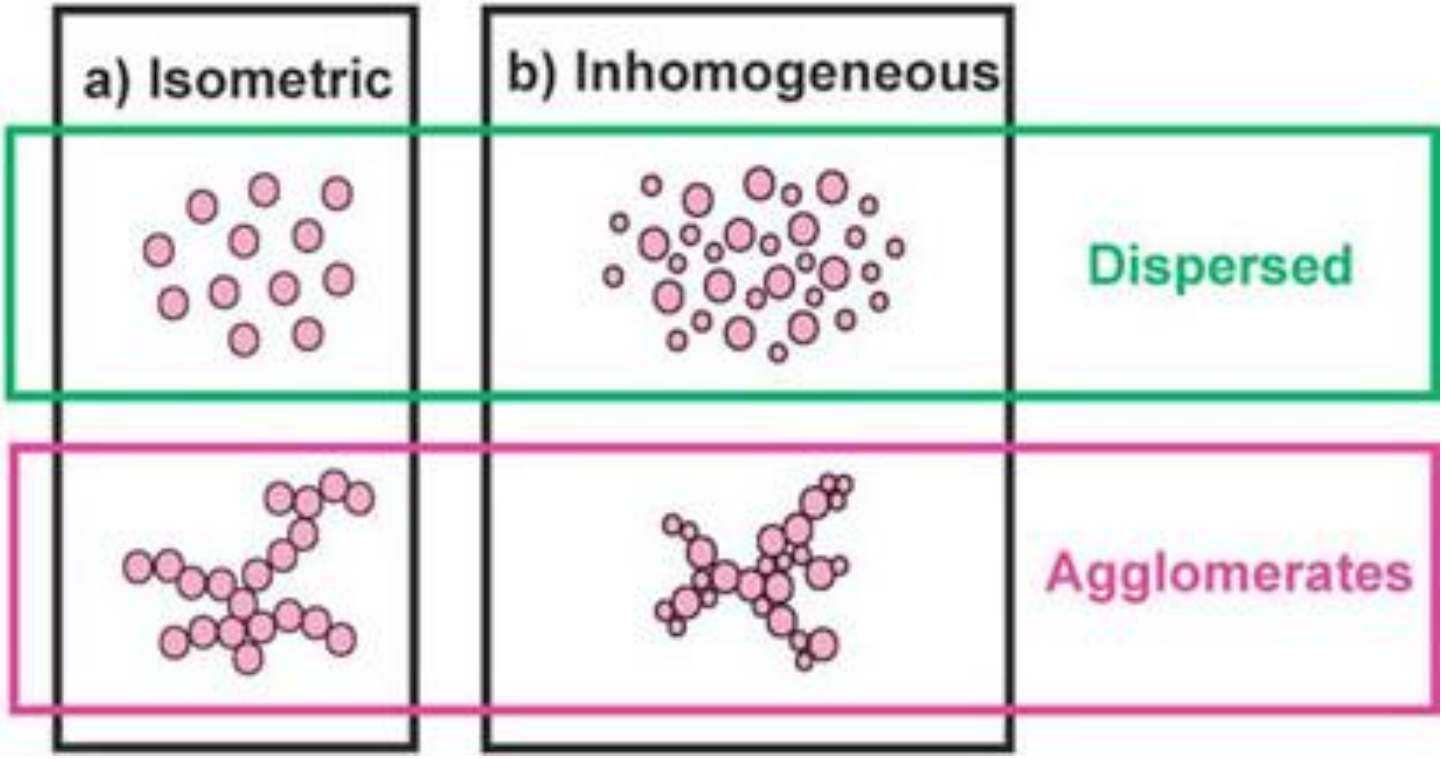
2. Morphology



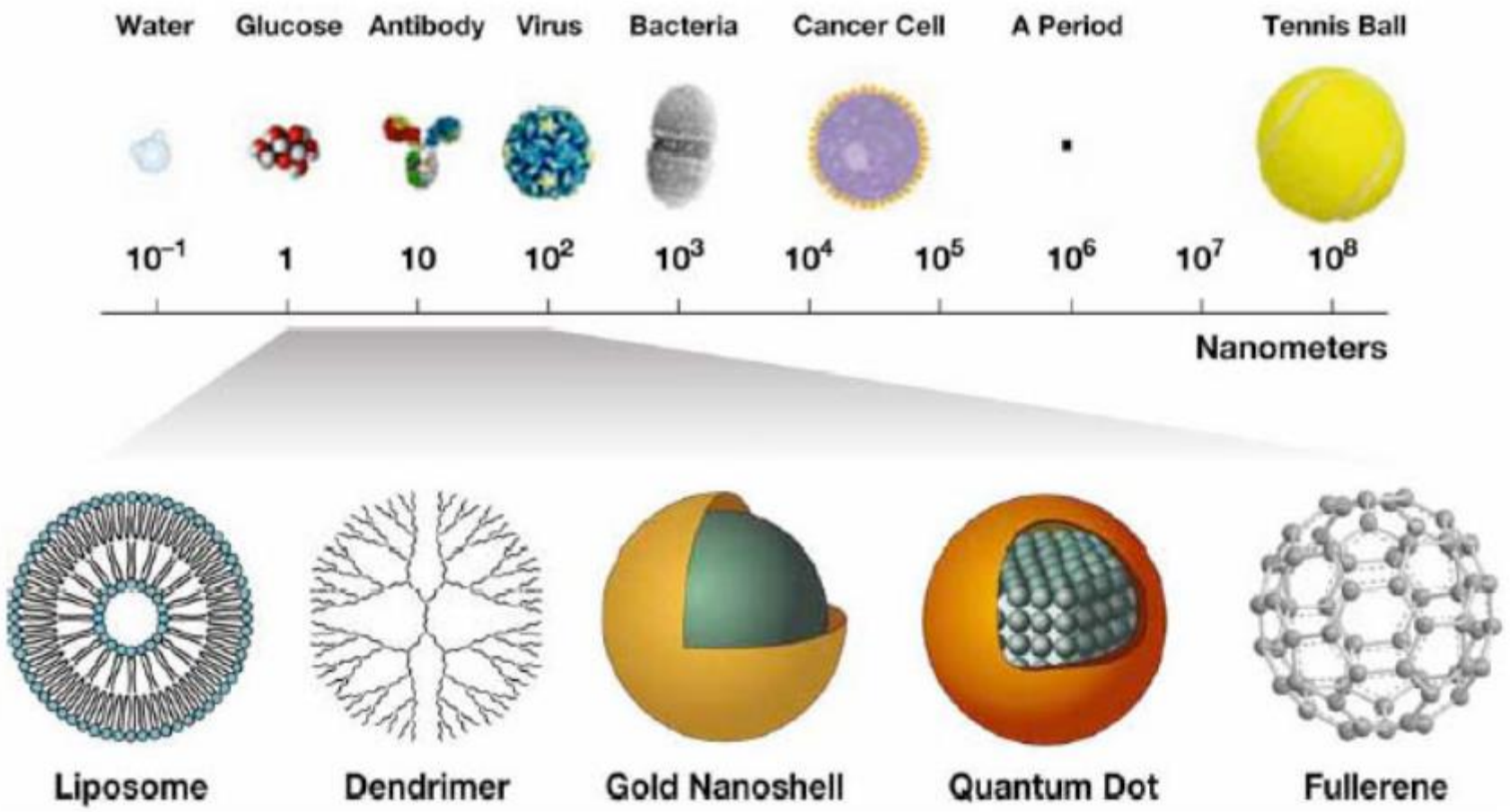
3. Structure and composition



Uniformity and agglomeration state



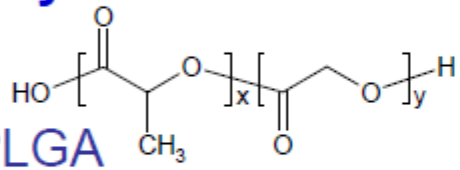
Future question: will the physico-chemically related effect be a common phenomenum between different nanoparticles?



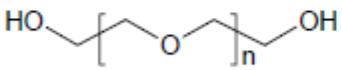
Types of Nanoparticles (NPs)

(after AJ Gormley & H. Ghandehari [2009])

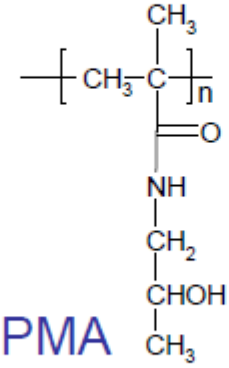
Polymeric



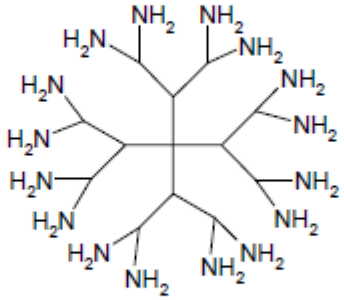
PLGA



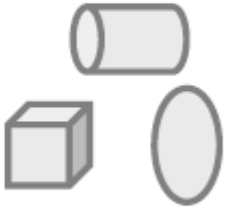
PEG



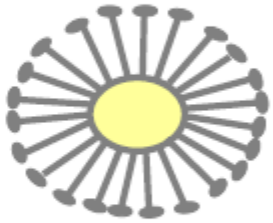
HPMA



Dendrimer

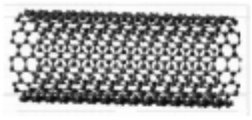


Particulate



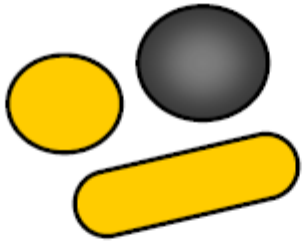
Liposome

Inorganic

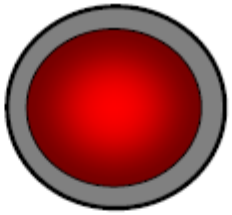


Carbon nanotubes

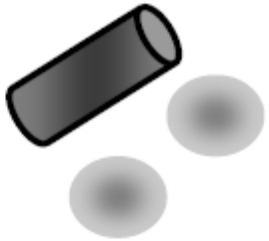
Fullerene



Gold and silver



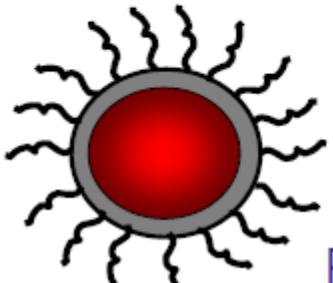
Quantum dots

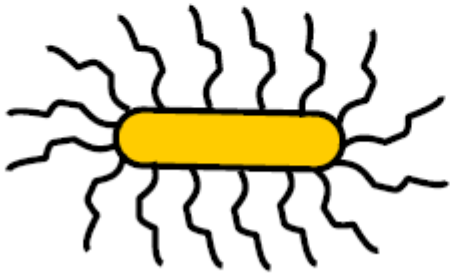


Silica

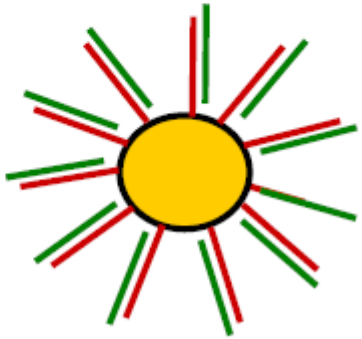
Metal oxides

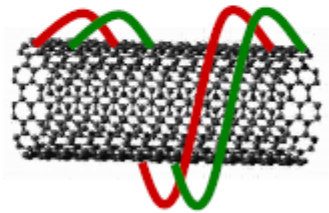
Hybrid





PEGylated NPs

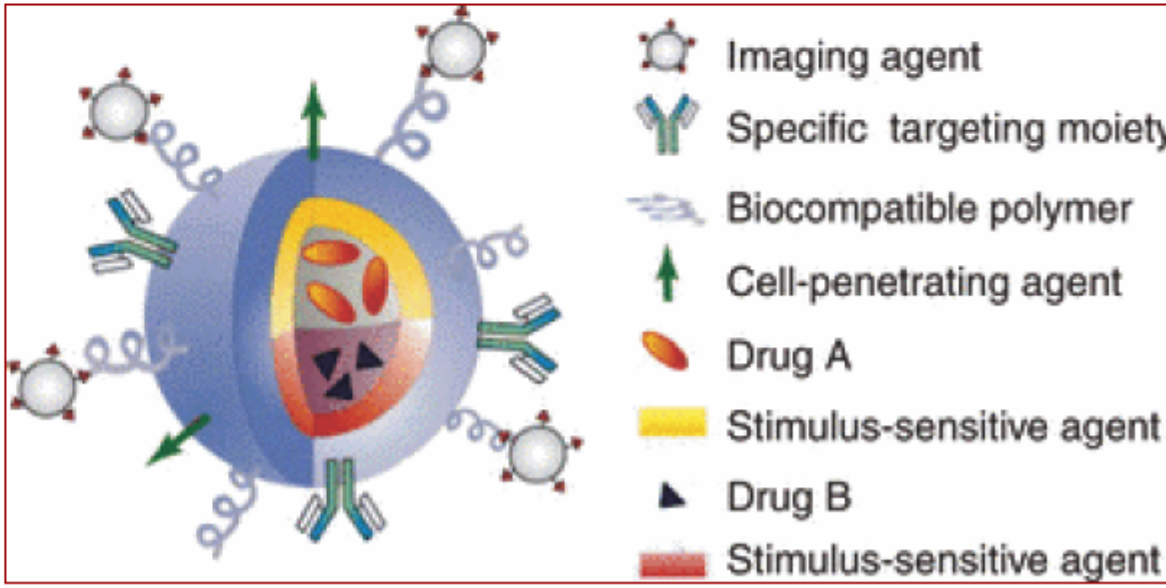




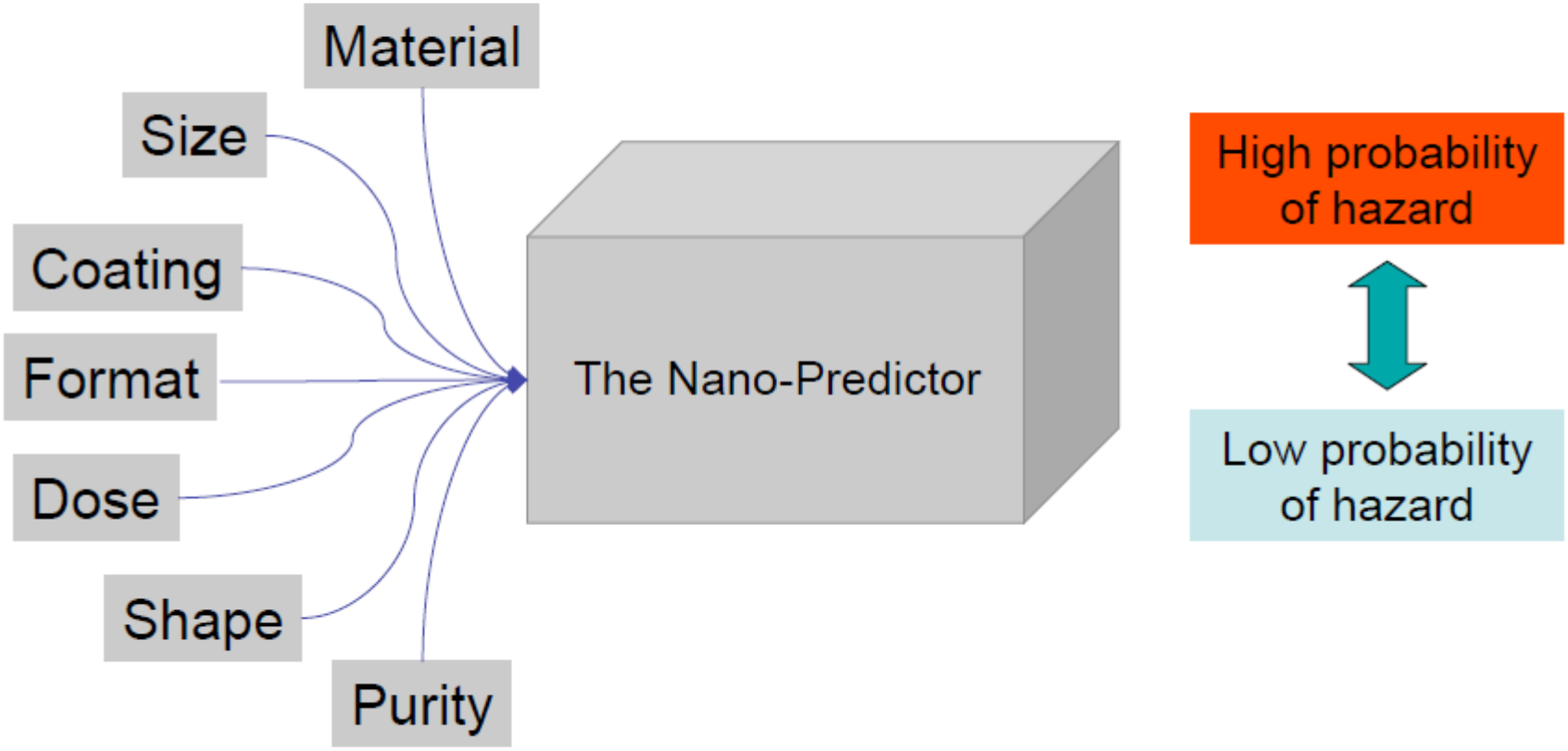
DNA NPs

Nanotechnology Innovation in Human and Veterinary Medicine

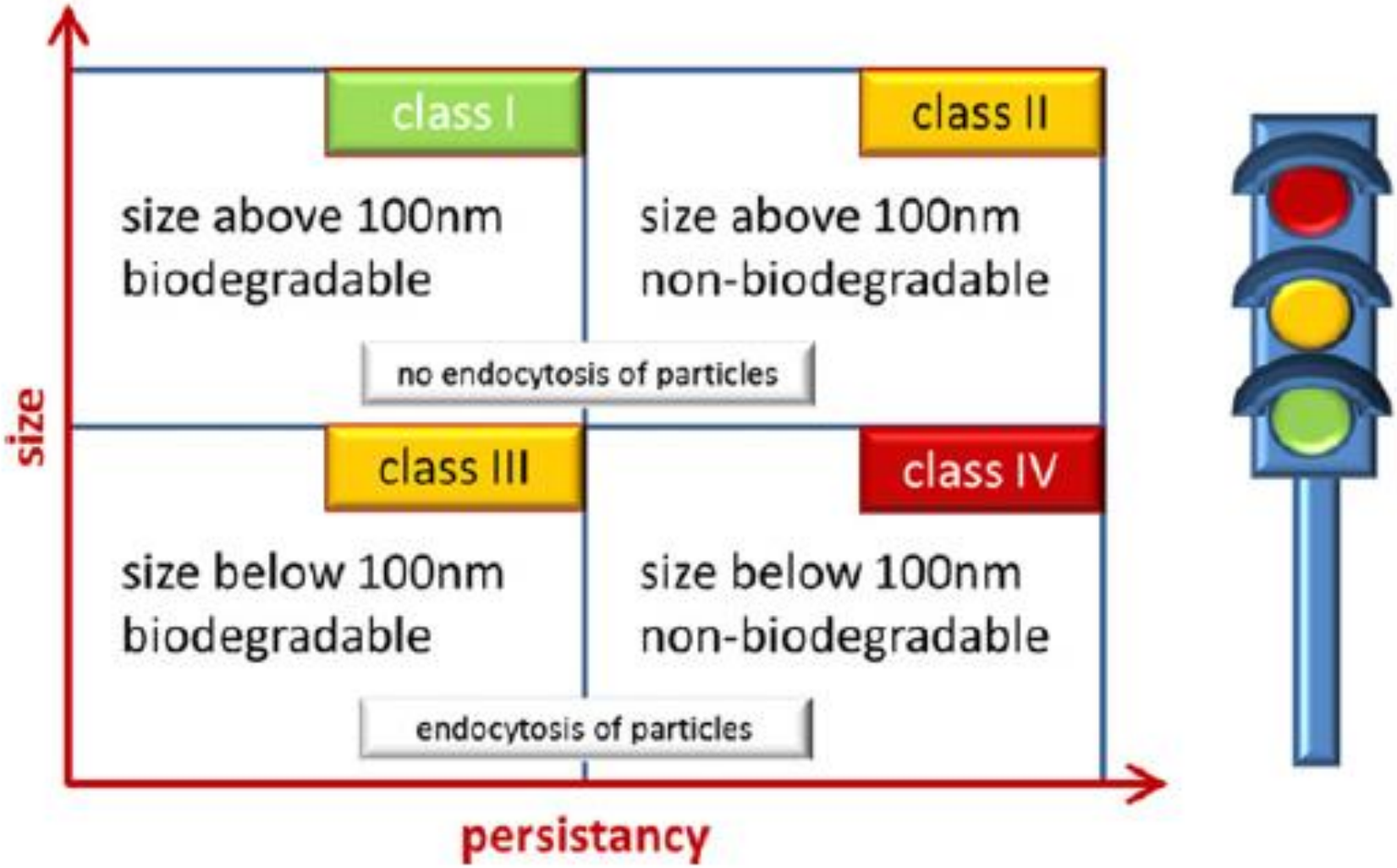
- Regenerative medicine/tissue engineering
  - tissue repair (e.g. cell sheets, nanofibres)
- Imaging and “point of care” diagnostics
- Biosensors (toxins, microbes, pollutants)
- Drug delivery
- Gene delivery
- Vaccine delivery
  - (nanoneedles)



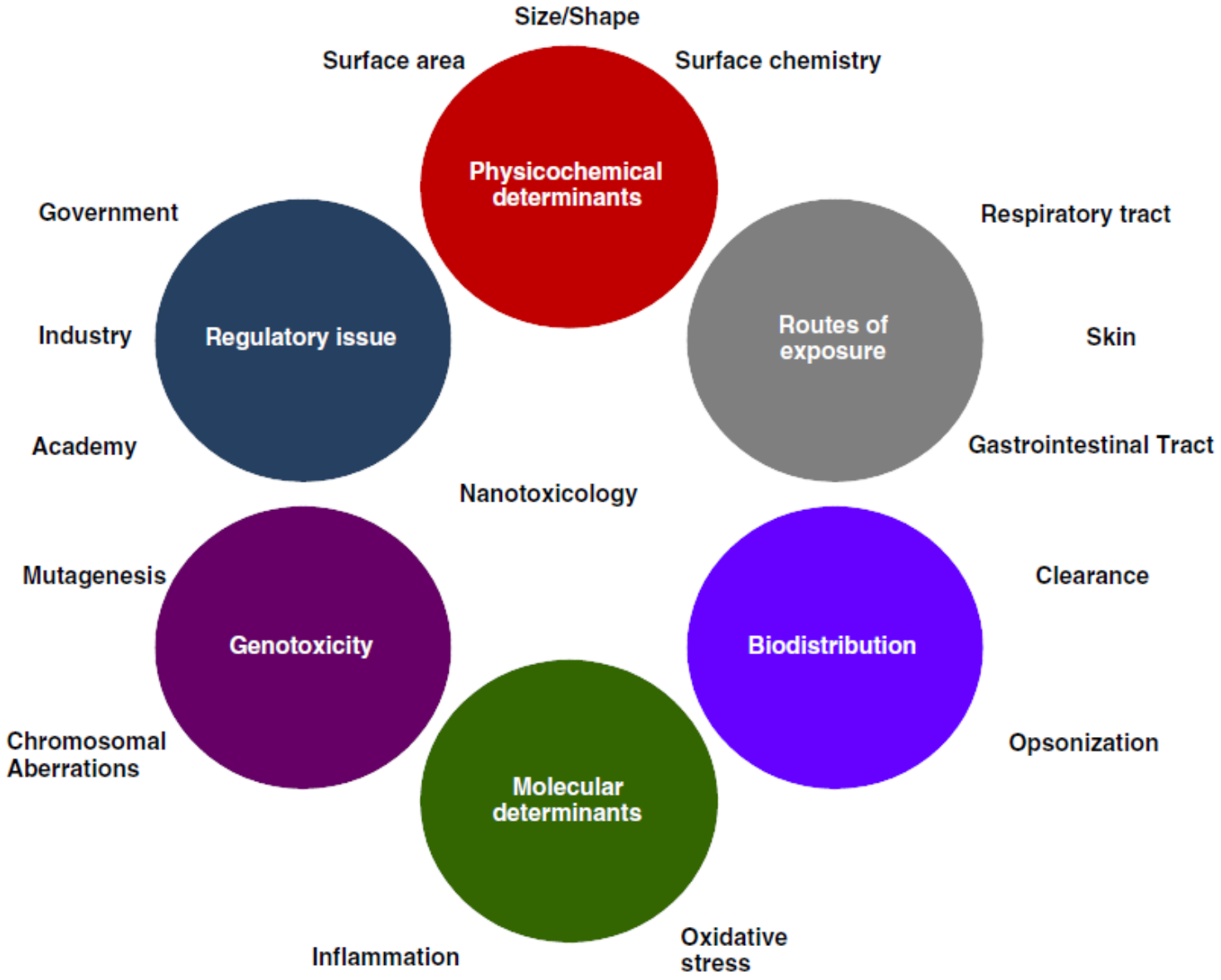
Nanosafety management



Nanotoxicological Classification System (NCS)



Complexity array of issues sururrounding toxicity of nanoparticles

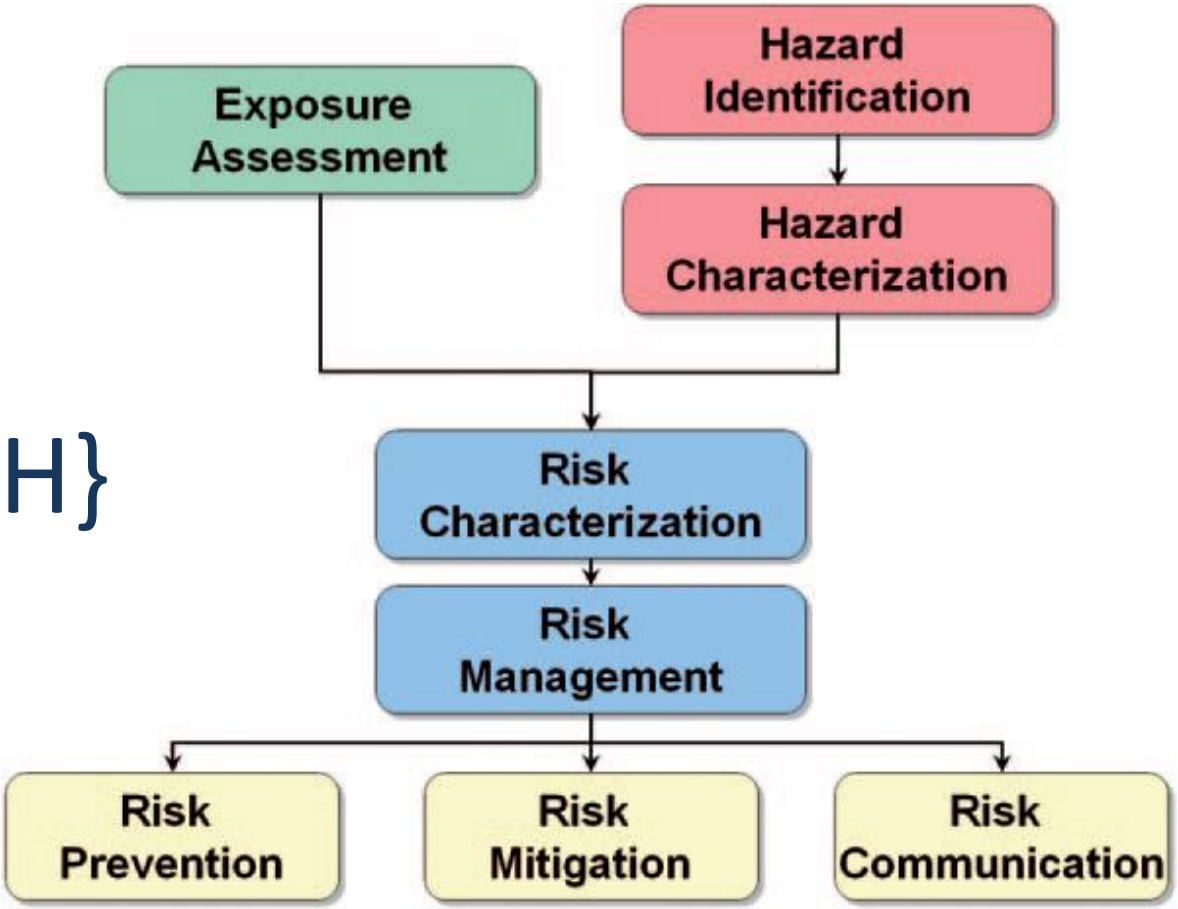


Unique features of nanomaterials

Size: 20–50nm enters CNS
<70 nm, able to escape defense system in vivo
High surface to mass ratio
High sharpness, surface charge, conductivity, solubility, durability and reactivity
Catalytic promotion of reactions
Ability to adsorb and carry other compounds
Ability to escape defense system in vivo
Ability to cross cellular and sub-cellular membranes
Surface coating (e.g., lecithin, albumin)
Enhance uptake by Type I/II pneumocytes
Transcytosis across capillary
Charged particle (higher inhaled deposition)

Assesment of the risk of nanoparticle´s adverese effects

$$R = F_p\{E,H\}$$



New in our present time is the **variety of additional materials and compounds** as well as the **wide range of possible applications**, which are expected to soon increase the loads on man, plants, animals, and environmental compartments and to raise issues of a **risk R** arising from **exposure E** to the new materials and of the biological effect or **hazards H** that may cause **biological effects**. **The probability P** of processes must also be considered, because a risk only occurs when there is a certain probability of the development of biological effects.

SOURCES OF NANOPARTICLES AND THEIR HEALTH EFFECTS

Natural sources of nanoparticles (90%)
Nanoparticles are abundant in nature, as they are produced in many natural processes, including photochemical reactions, volcanic eruptions, forest fires, and simple erosion, and by plants and animals, e.g., shedding of skin and hair.

Human activities (10%) —cars, industry and charcoal burning

The most significant components of total global atmospheric aerosols are:
Mineral aerosols primarily from soil deflation wind erosion with a minor component 1% from volcanoes 16.8 Mt
Sea salt 3.6 Mt
Natural and anthropogenic sulfates 3.3 Mt
Products of biomass burning excluding soot 1.8 Mt, and of industrial sources including soot 1.4 Mt

Natural and anthropogenic non-methane hydrocarbons 1.3 Mt
Natural and anthropogenic nitrates 0.6 Mt,
Biological debris 0.5 Mt
Extraterrestrial dust

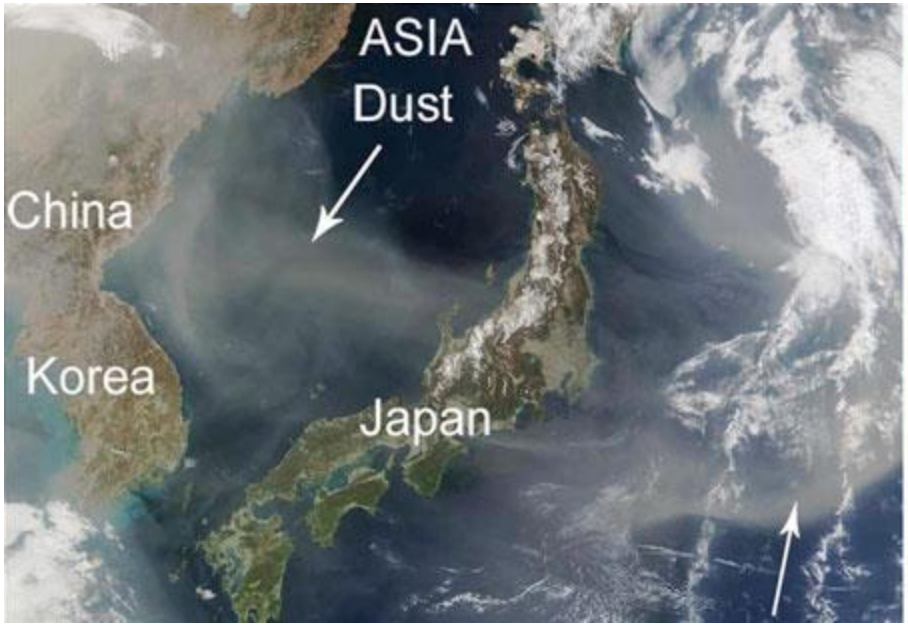
Ten major sources of dust in the world

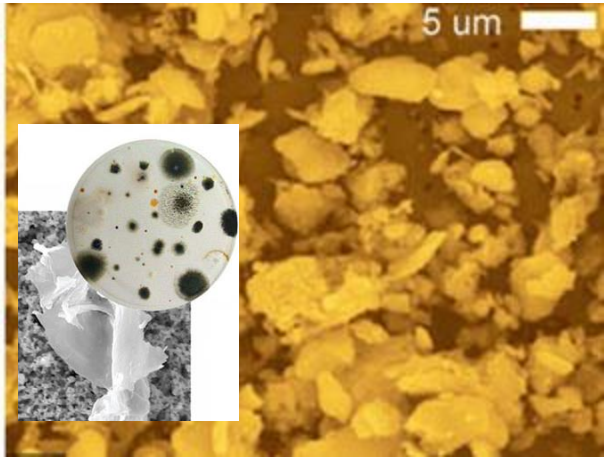
Kdo umí kráčet, nezanechává stop

Lao´c

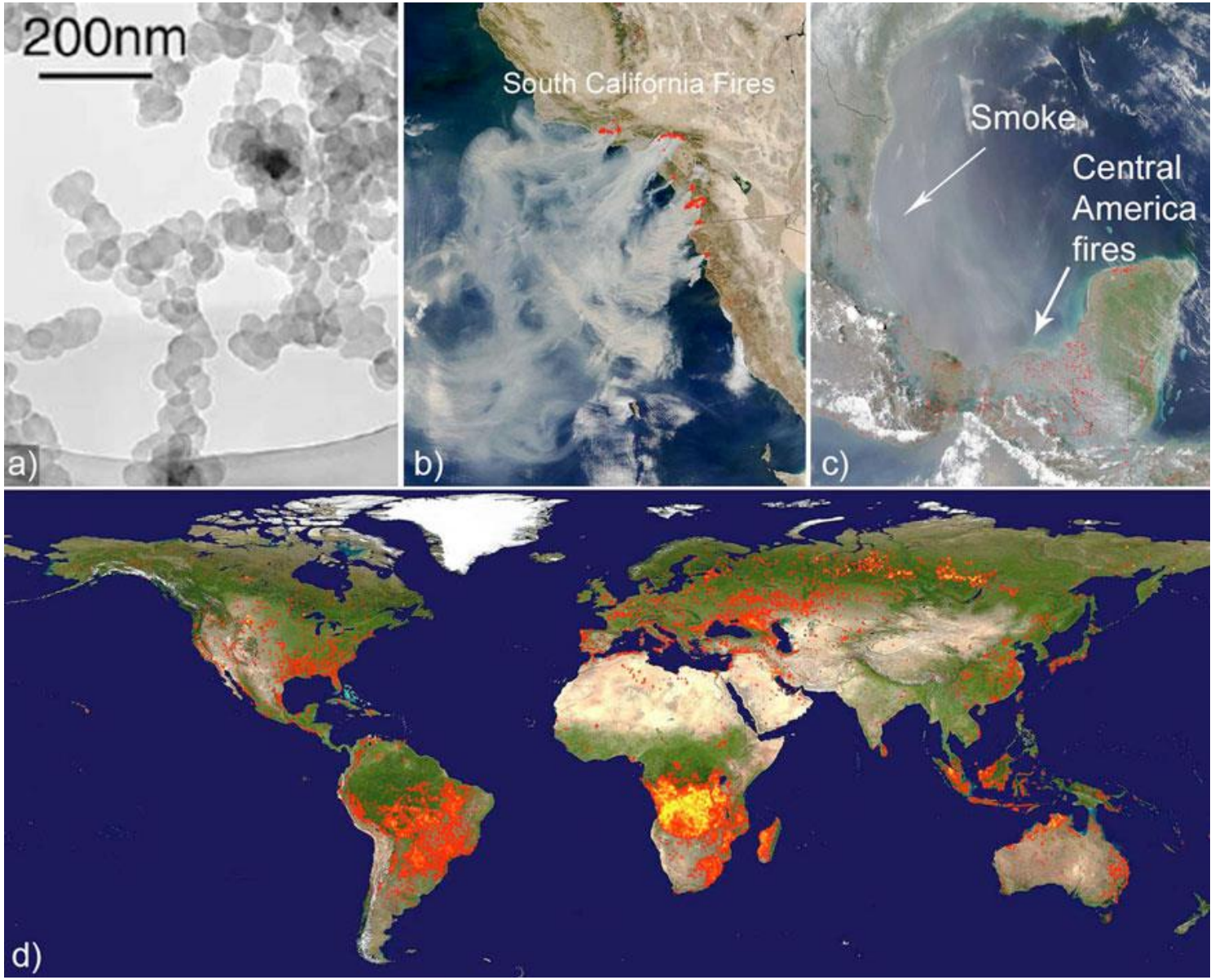
Sand storms visualized at macro and microscale

Satellite image showing dust blowing off mainland China over the Sea of Japan and Pacific Ocean





Forest fires and grass fires

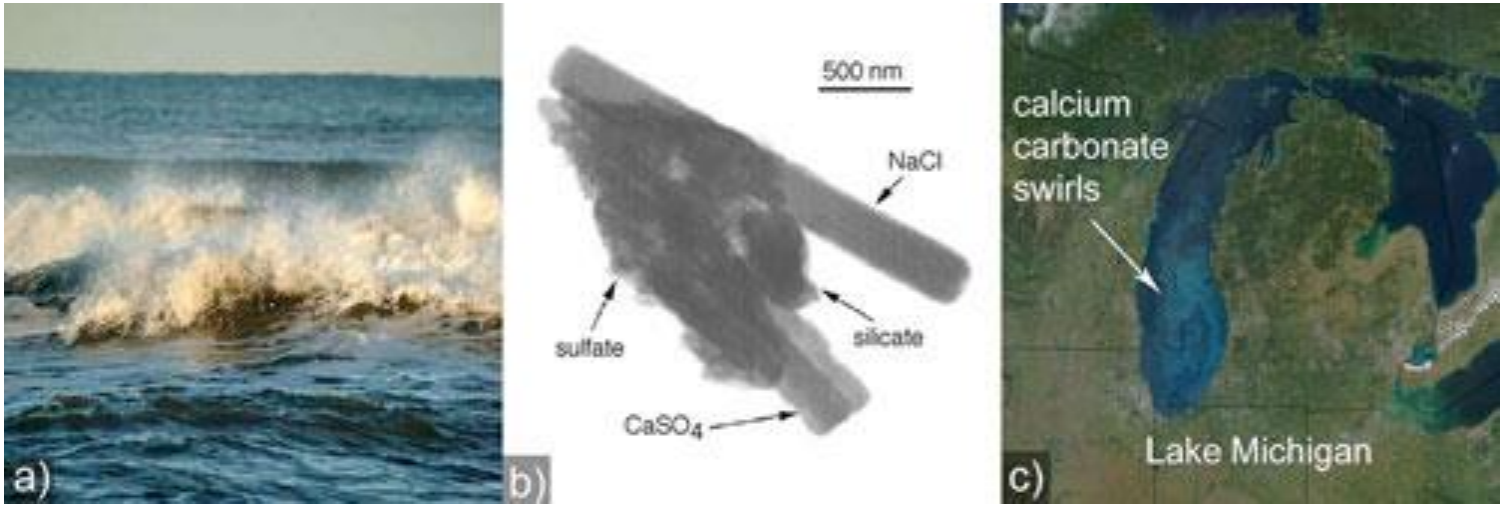


Volcanoes

The quantity of particles released into the atmosphere is enormous; a single volcanic eruption can eject up to 30×10^6 tons of ash. Volcanic ash that reaches the upper troposphere and the stratosphere the two lowest layers of the atmosphere can spread worldwide and affect all areas of the Earth for years.

Ocean and water evaporation

Sea spray from ocean waves

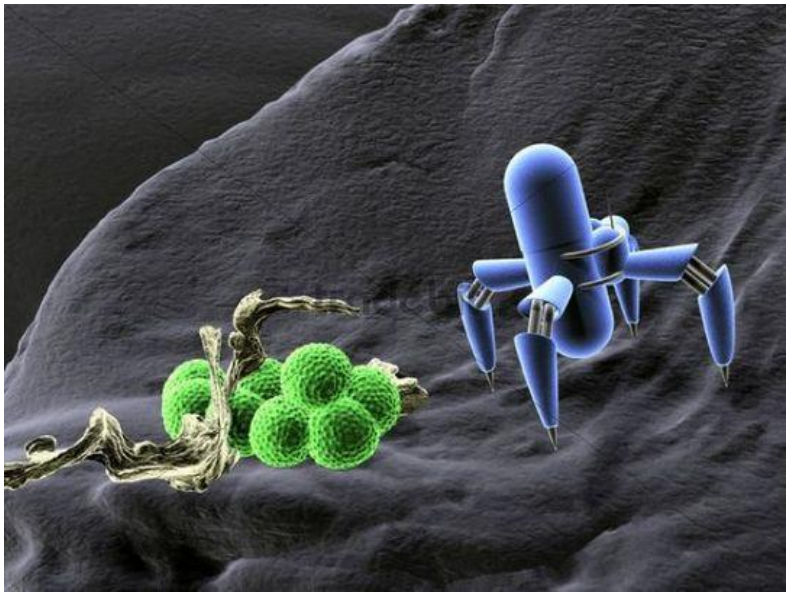


Nano-Organisms

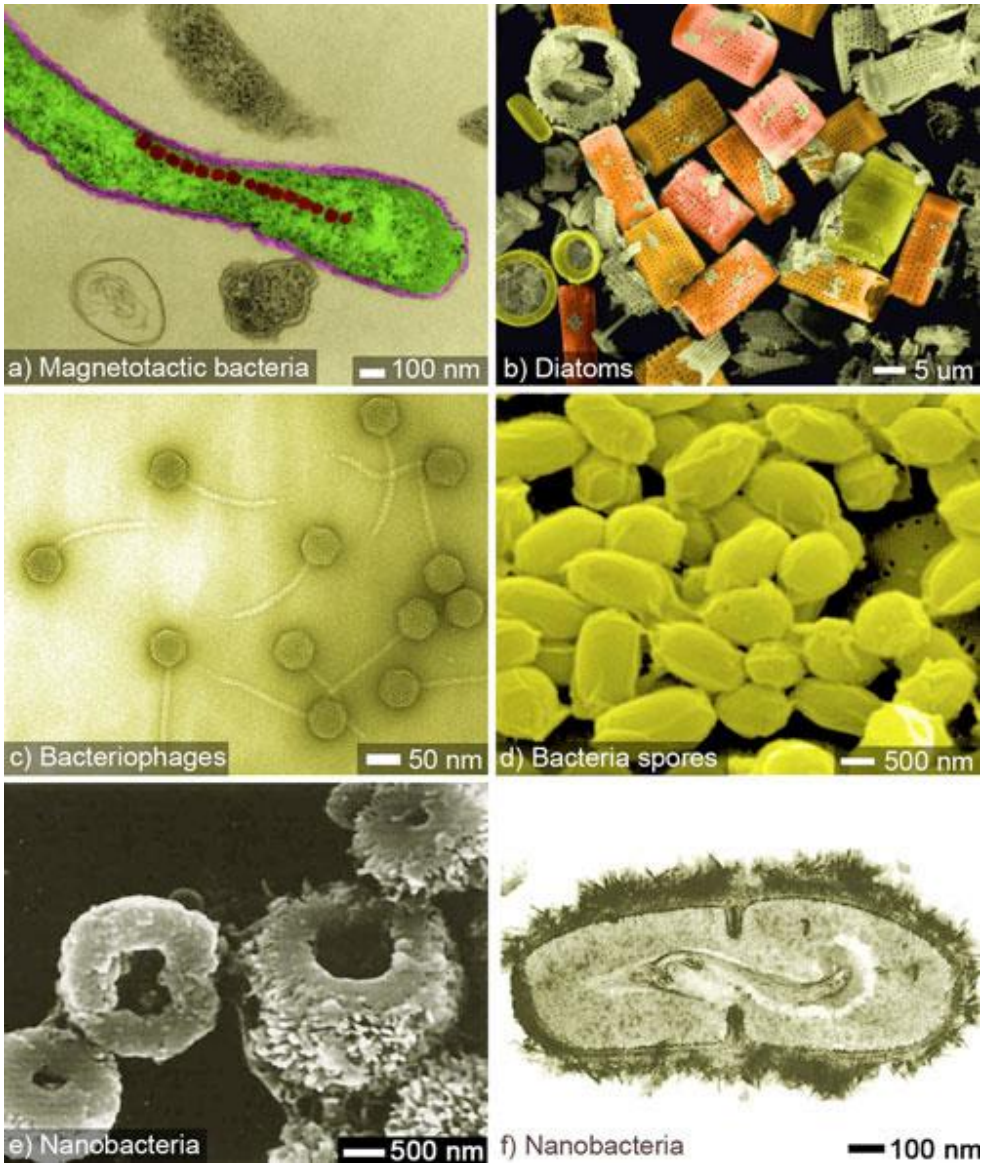
Many organisms are smaller than a few microns including viruses 10–400 nm and some bacteria 30 nm–700 m.

Nanoorganisms or their components - bacteria, viruses, cells, and their organelles. Cells, bacteria, and viruses are self-organizing, selfreplicating, dissipative structures with a shorter-lived structure than inorganic solids. Nanoorganisms generally dissipate when their supply of energy is exhausted. In contrast, nanoparticles are typically inorganic solids that require no supply of energy to remain in a stable form.

Nanorobots



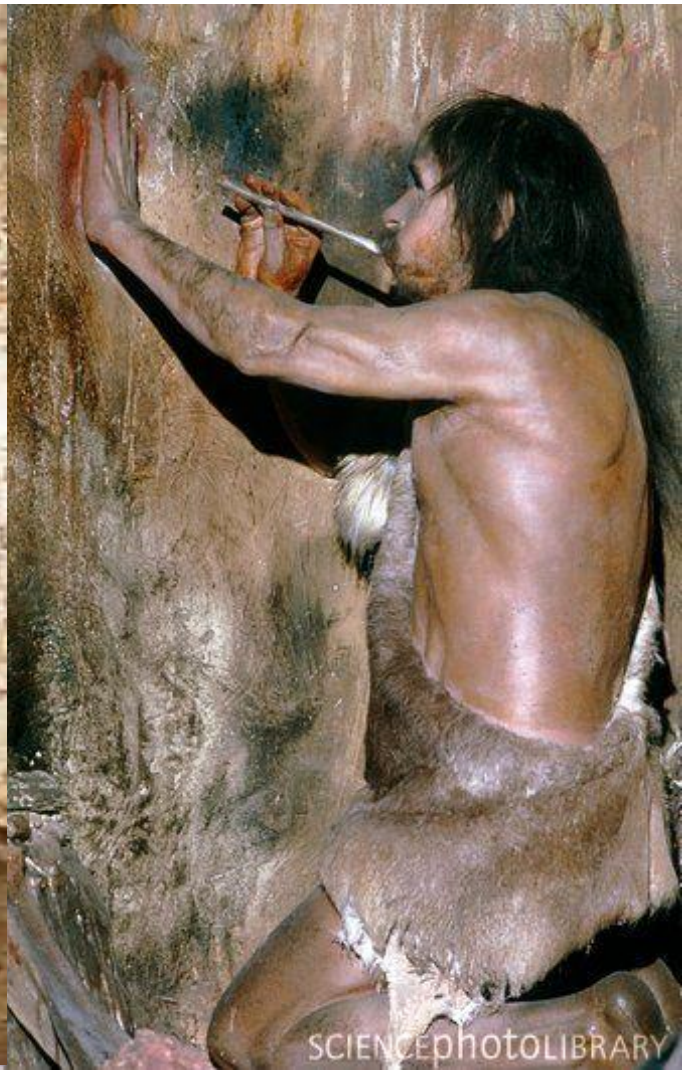
Organisms in the nanoscale range or producing solid-state nanoscale debris



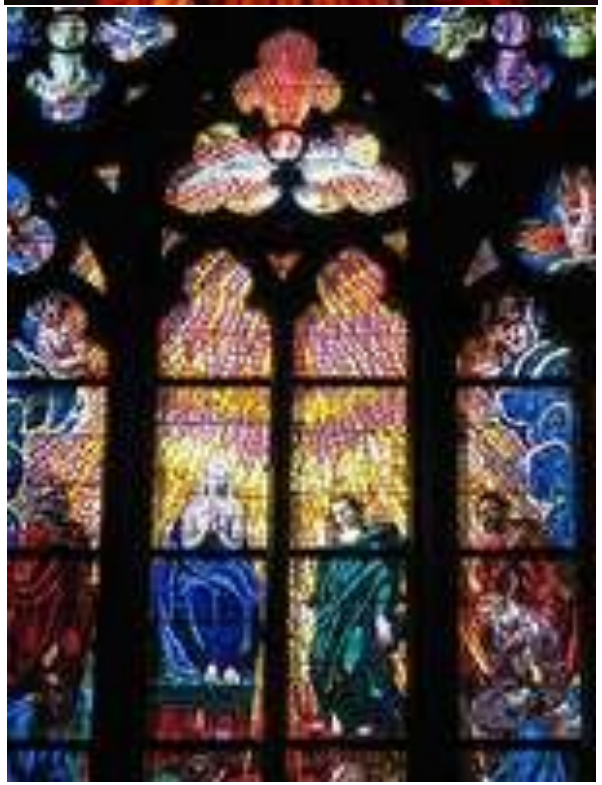
Anthropogenic nanomaterials

Humans have created nanomaterials for millennia, as they are by-products of simple combustion with sizes down to several nanometers and food cooking, and more recently, chemical manufacturing, welding, ore refining and smelting, combustion in vehicle and airplane engines, combustion of treated pulverized sewage sludge, and combustion of coal and fuel oil for power generation.

History of nanomaterials application



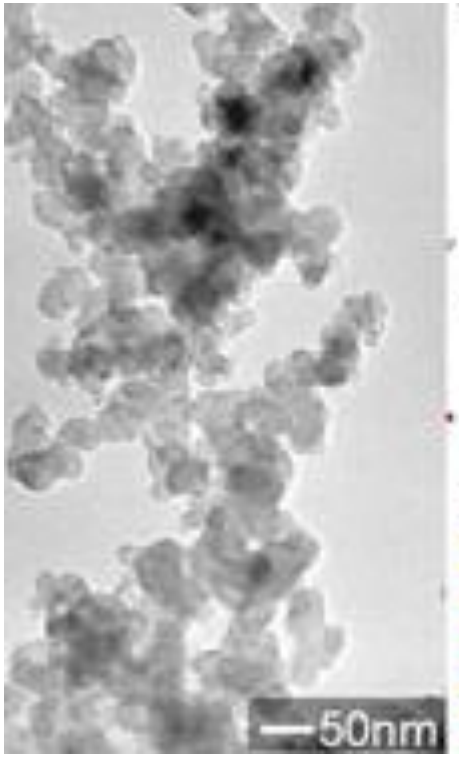
History of nanomaterials application

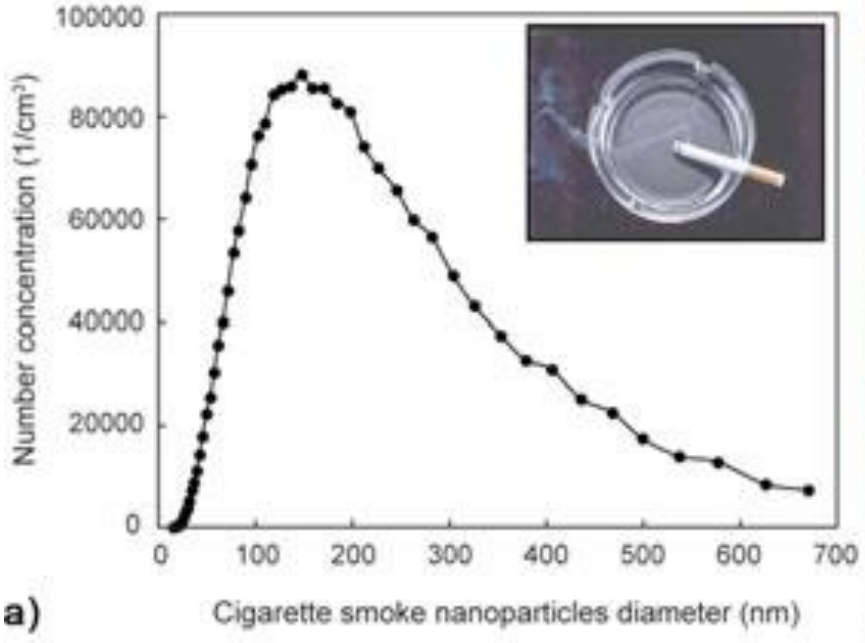


Indoor pollution

Humans and their activities generate considerable amountsof particulate matter indoors. Nanoparticles are generated through common indoor activities, such as cooking, smoking, cleaning, and combustion (e.g. candles and fireplaces). Examples of indoor nanoparticles are textile fibers, skin particles, spores, dust mite droppings, chemicals, and smoke from candles, cooking, and cigarettes. Poorly ventilated stoves using biomass fuels wood, crop residue, dung, and coal are mainly responsible for the death of an estimated $1.6x10^6$ people annually, from which more than a half are children under the age of 5.

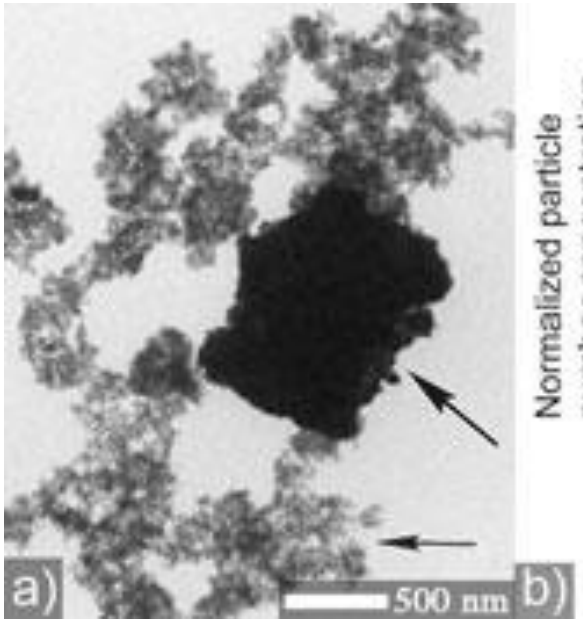
Indoor air can be ten times more polluted than outdoor air!!!

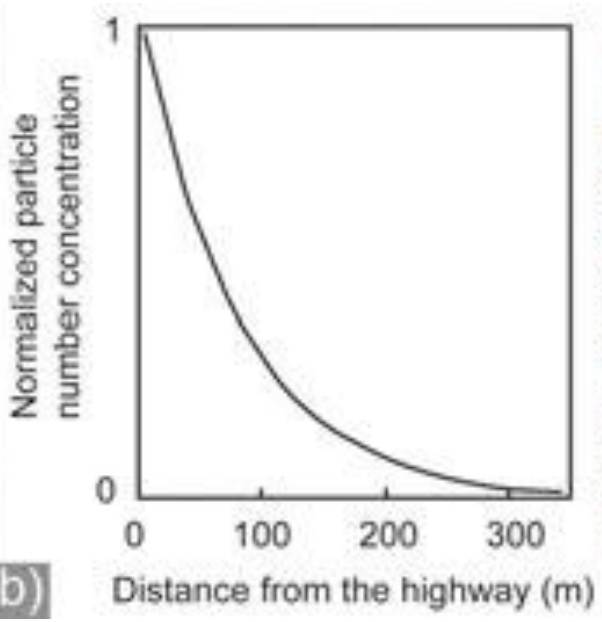




100 000 chemical components and compounds

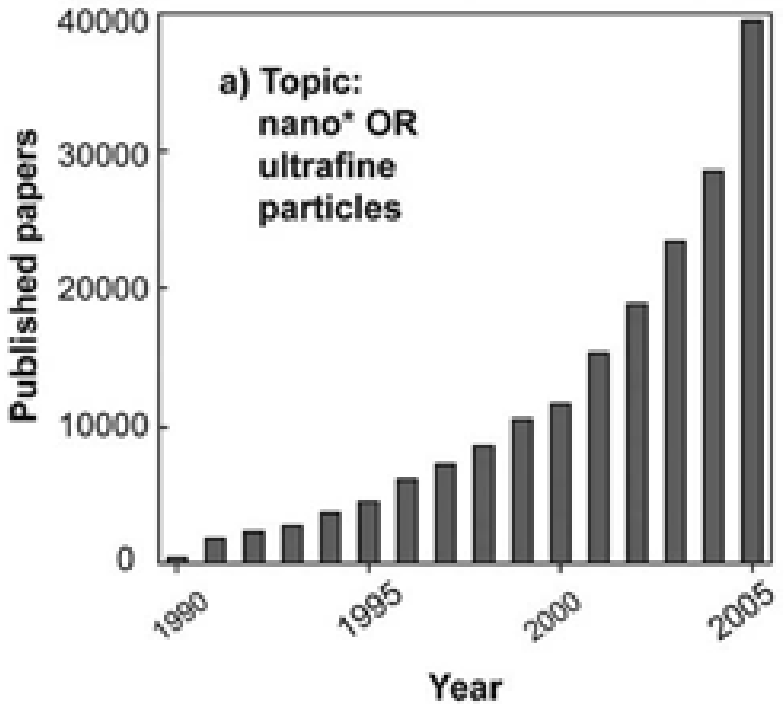
Tipical engine exhaust particles consisting of carbon aggregates around a larger mineral particle



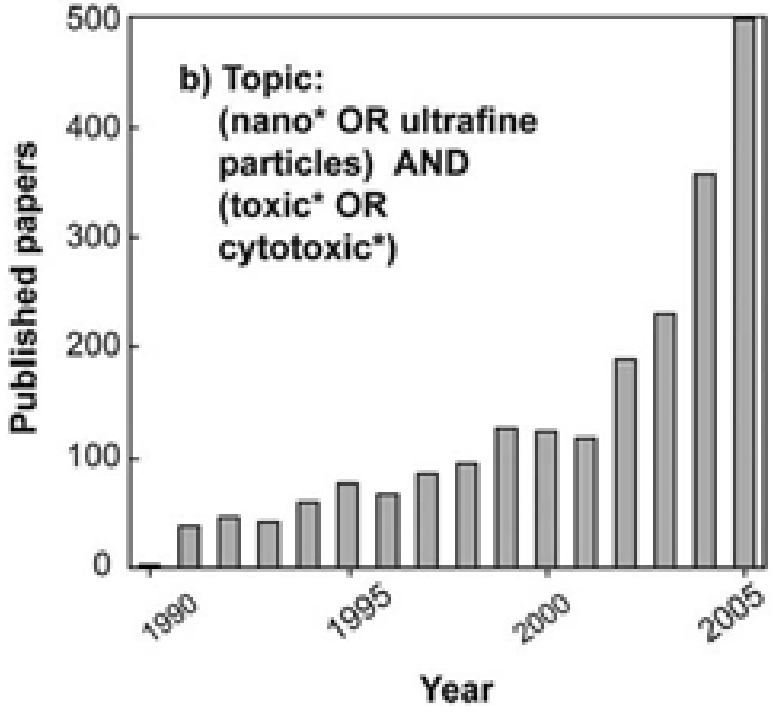


Nanotoxikology

Statistics on scientific articles published on a) nanomaterials and b) their toxicity



2005-2012 502 301 publications in WOS



2005-2012 14 949 publications in WOS

Nanotoxicology

"All things are poison and nothing is without poison; only the dose makes a thing not a poison."

Almost all substances are toxic under the right conditions.

The dose makes the poison.

Paracelsus (1493–1541)

Nanotoxicology in the 21st Century Will Rely Heavily on In Vitro Studies

In Vitro Studies are Currently Useless for Quantitative Assessment



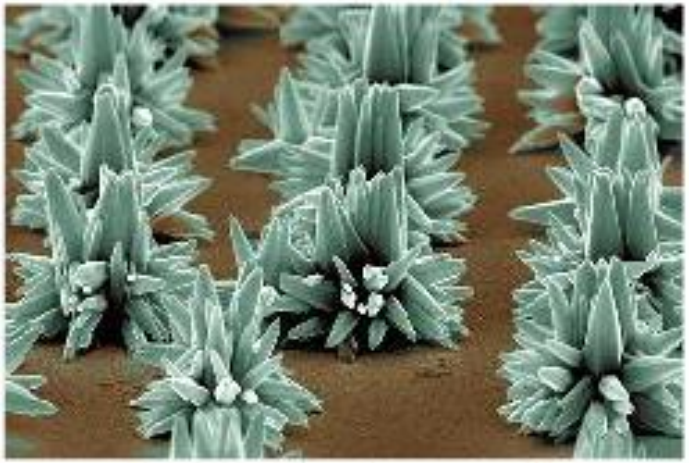
Toxicity of nanoparticles

Nanoparticles arise from a wide variety of natural and man-made sources and have a diverse array of biological, chemical, and physical properties.

The toxicity of these particles can be roughly divided into two categories:

1) the enhanced delivery of toxic agents

2) toxicity induced the properties of the particle itself (e.g. size, shape, surface sharpness, solubility)

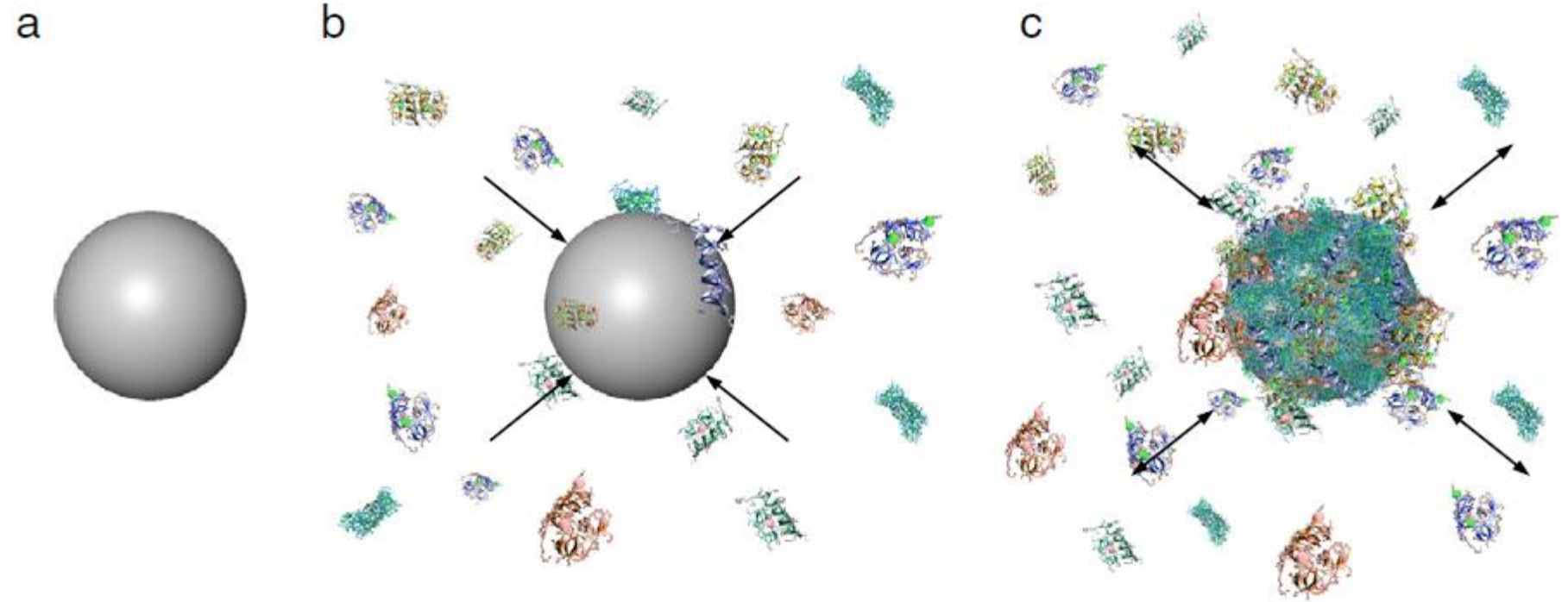


Zinc Oxide Nanoparticles

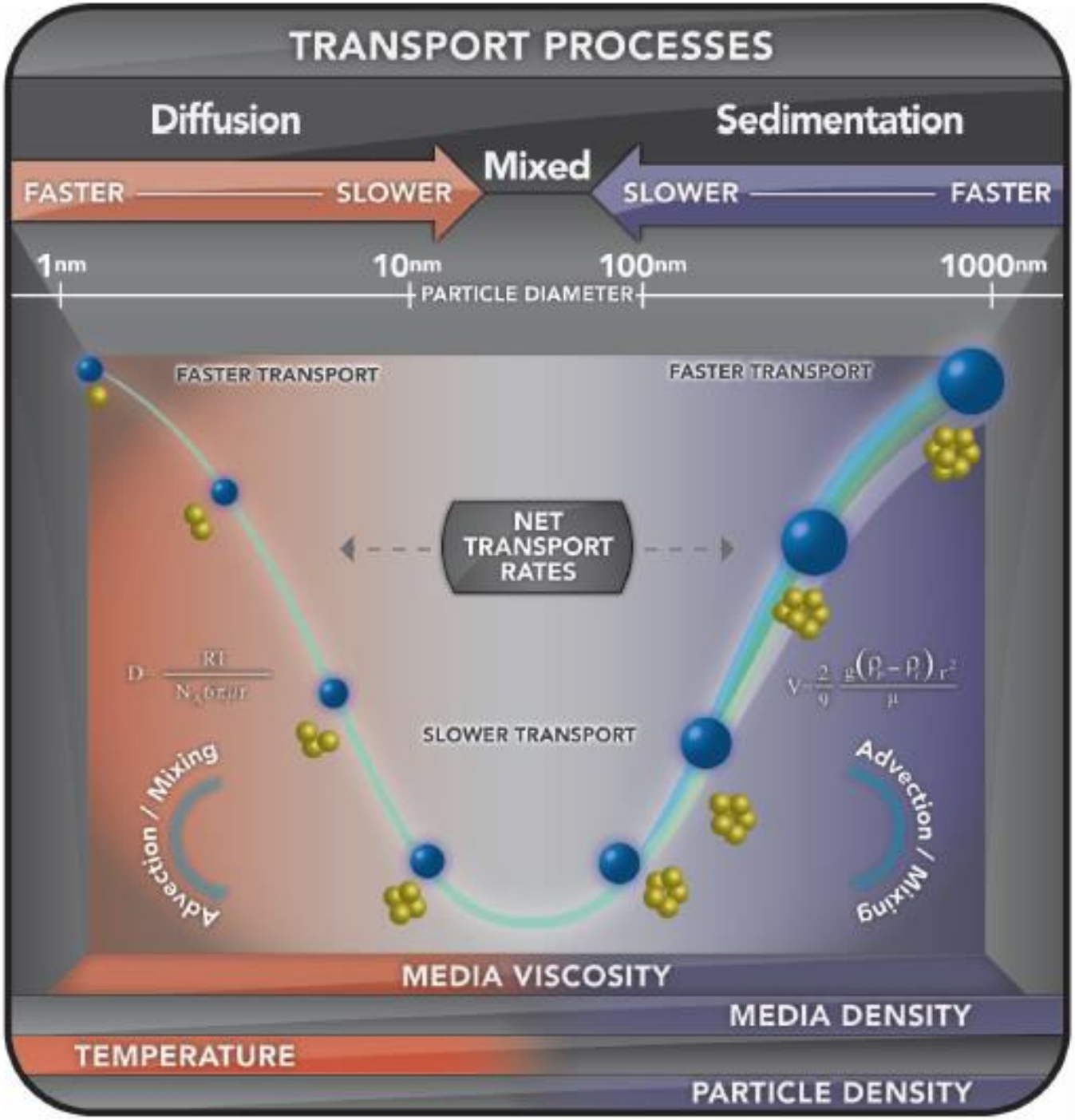
In vitro tests

Nanoparticle surface—increased reactivity

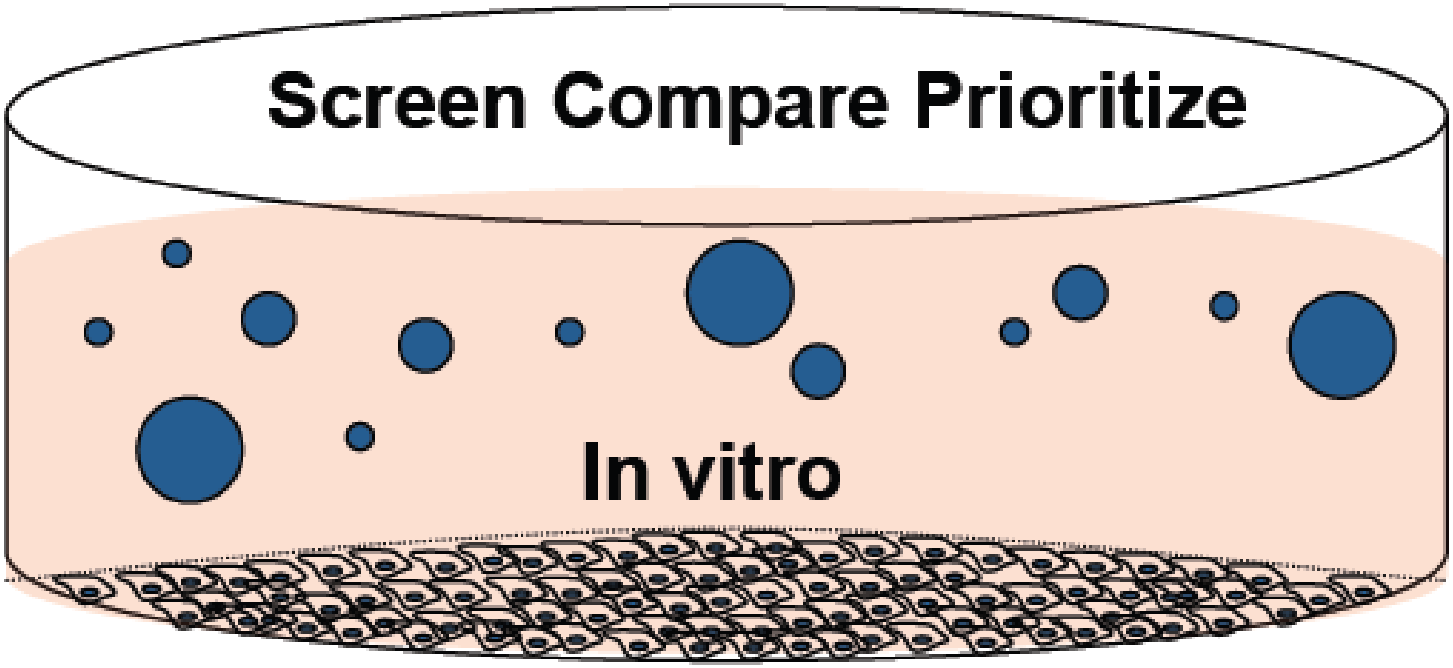
Research has shown that it is not the nanomaterial itself but the “corona” that mainly defines the properties of the “particle-plus-corona” compound. This makes it necessary to understand not only the nanomaterial but also the nanoparticle environment when testing for nanotoxicity



Formation of nanoparticle corona: a) “bare” particle, b) nanoparticle in contact with proteins, c) corona formation. The corona can consist of a “hard corona”, proteins firmly attached to the surface, or a “soft corona” of proteins which are only weakly bound to the nanoparticles and form an equilibrium layer with the surrounding matrix.


TRANSPORT PROCESSES
Diffusion
Sedimentation
Mixed
FASTER
SLOWER
SLOWER
FASTER
1nm
10nm
100nm
1000nm
PARTICLE DIAMETER
FASTER TRANSPORT
FASTER TRANSPORT
NET TRANSPORT RATES
SLOWER TRANSPORT
Advection / Mixing
Advection / Mixing
MEDIA VISCOSITY
MEDIA DENSITY
TEMPERATURE
PARTICLE DENSITY

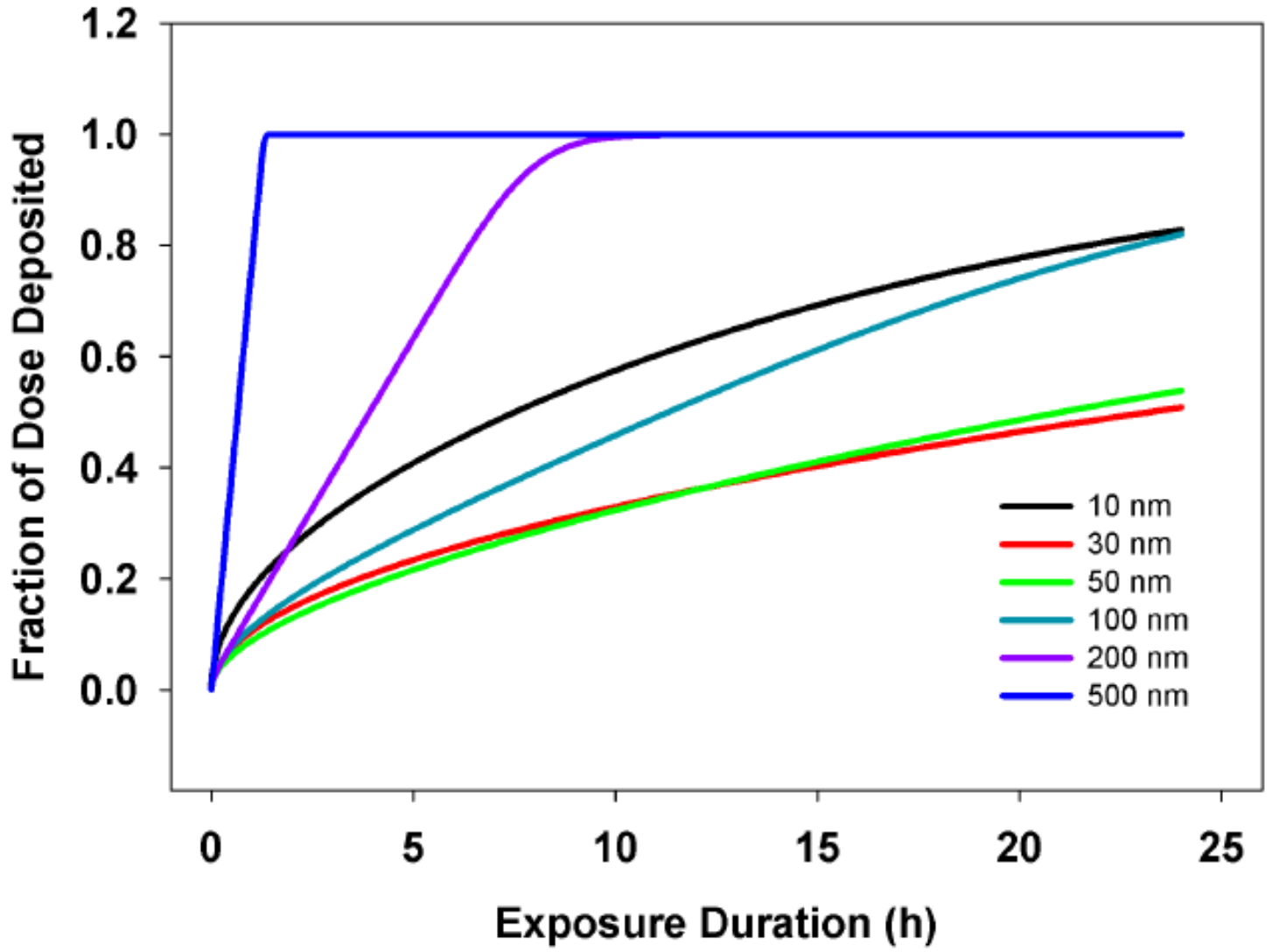
In vitro testing



A dosimetry paradigm for chemicals focused on target tissue dosimetry has existed for decades

Transport Rates for TiO2.

ISDD calculated fraction of nano and micron scale TiO2 particles delivered to cells over the duration of a 24 hour in vitro study with a media height 3.1 mm. Different rates of particle transport result in different time-courses for delivery to cells, which is only complete for large particles by 24 hours.



Artificial nanoparticles

- **Engineered nanoparticles represent a novel toxicological challenge**. They are completely novel in evolutionary terms, the evidence shows that they gain can access to the body, particularly through inhalation, and then translocate within the body to distant sites at low doses.

- **A critical step in nanotoxicology** is to **characterise the nanomaterial** under examination and this is much more difficult than is the case in classical toxicology because of the multitude of variables in the parameter space. These include: particle size, roughness, shape, charge, composition and surface coating. The latter can change depending upon the matrix into which it is introduced.

- **Estimation of nanoparticle "dose" is complex**, requiring a number of direct and/or indirect technologies to determine how many particles are reaching defined targets. Testing nanoparticle toxicity based on estimates of **realistic human exposure** is required instead of testing unrealistically high nanoparticle doses with no relevance to real-world exposures.

Dose and in vitro toxicology

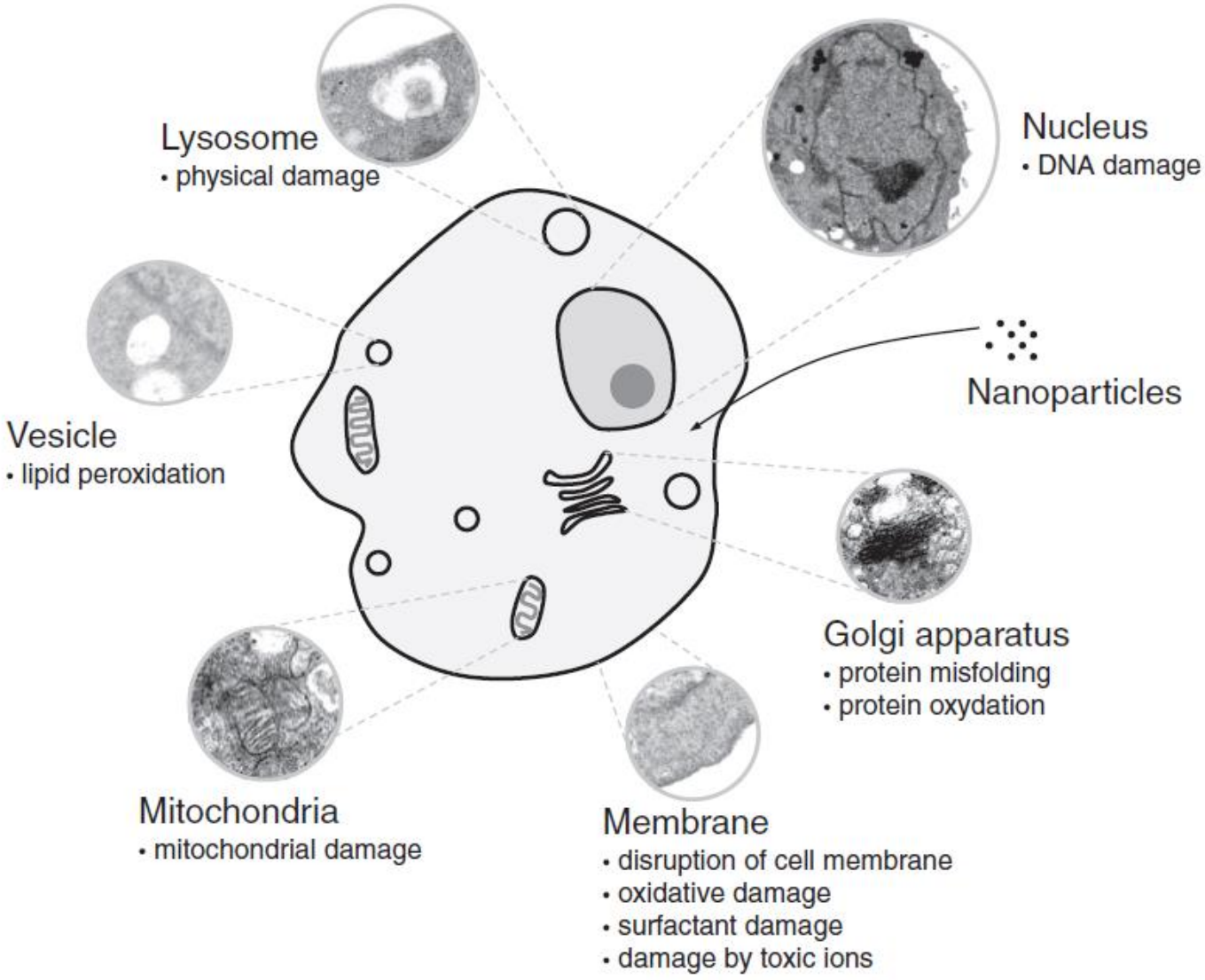
- Dose In Vitro is Misunderstood, Unmeasured or Misrepresented

- Particles are not chemicals! They settle and diffuse at very different rates depending on the density, size and agglomeration status

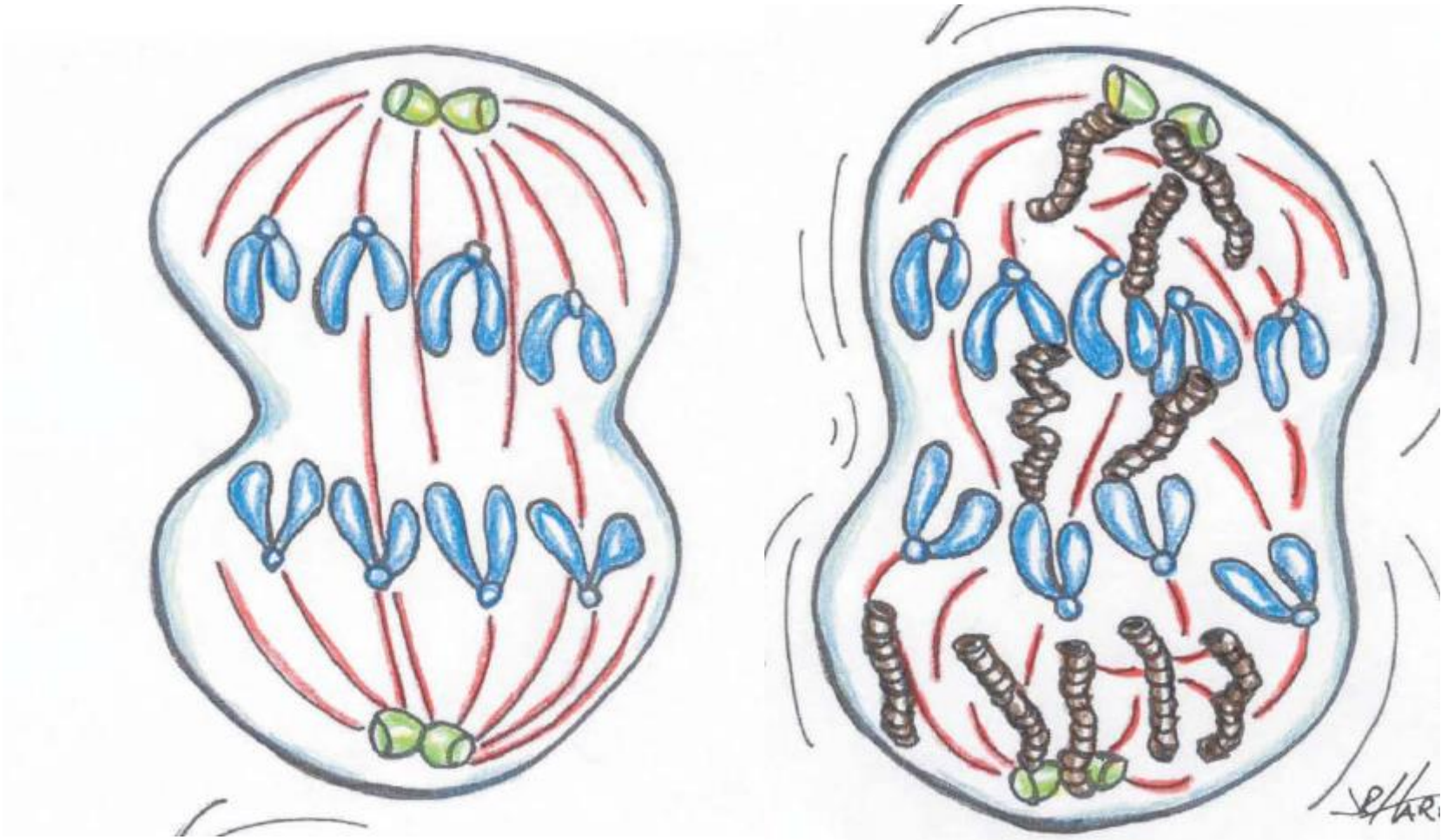
- Nominal media doses, the standard, (µg/ml, µg/plate surface) **misrepresent dose by 1 to 6 orders of magnitude**
- Nominal media doses cannot be related to target tissue (e.g. lung, liver, gut) doses from in vitro studies.

Membranes—interface between nanoparticles and cells

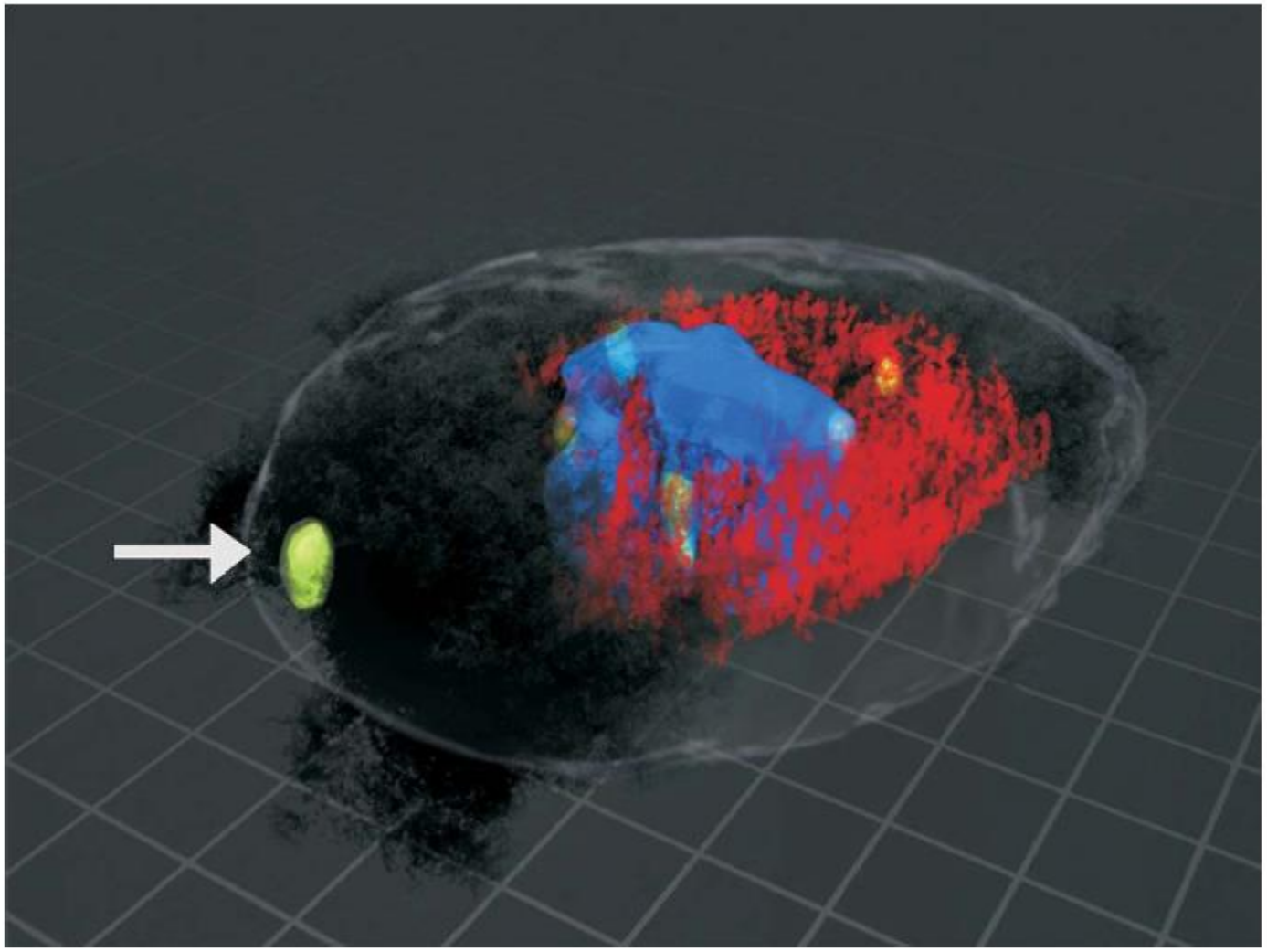
- In a cellular context, membranes, which are phospho-lipid bilayers, partition different intracellular compartments which each have specific functions.



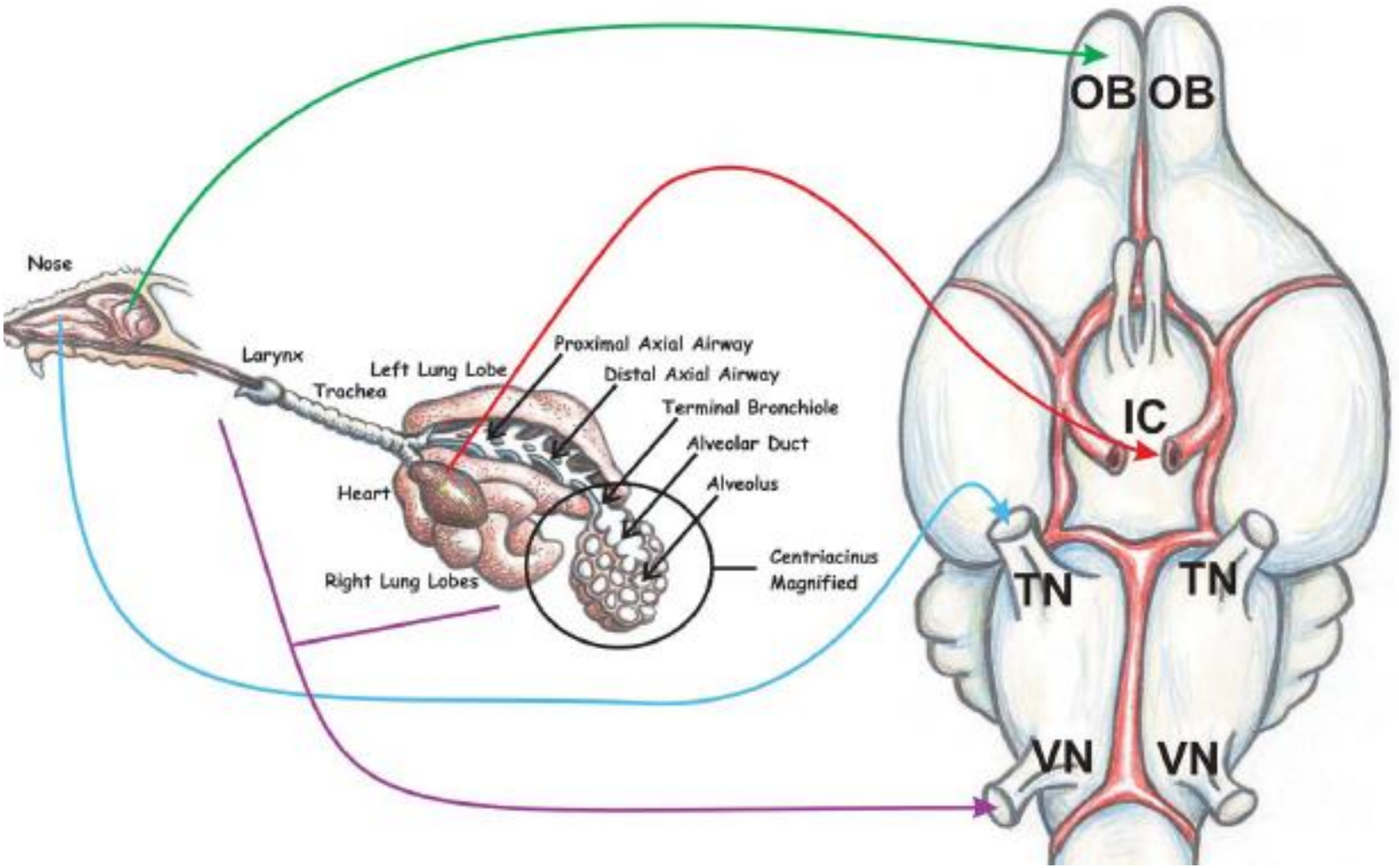
Potential interactions between NPs and the dividing cel



3D reconstruction showing the interaction of the centrosome, the DNA, and the microtubules with SWCNTs

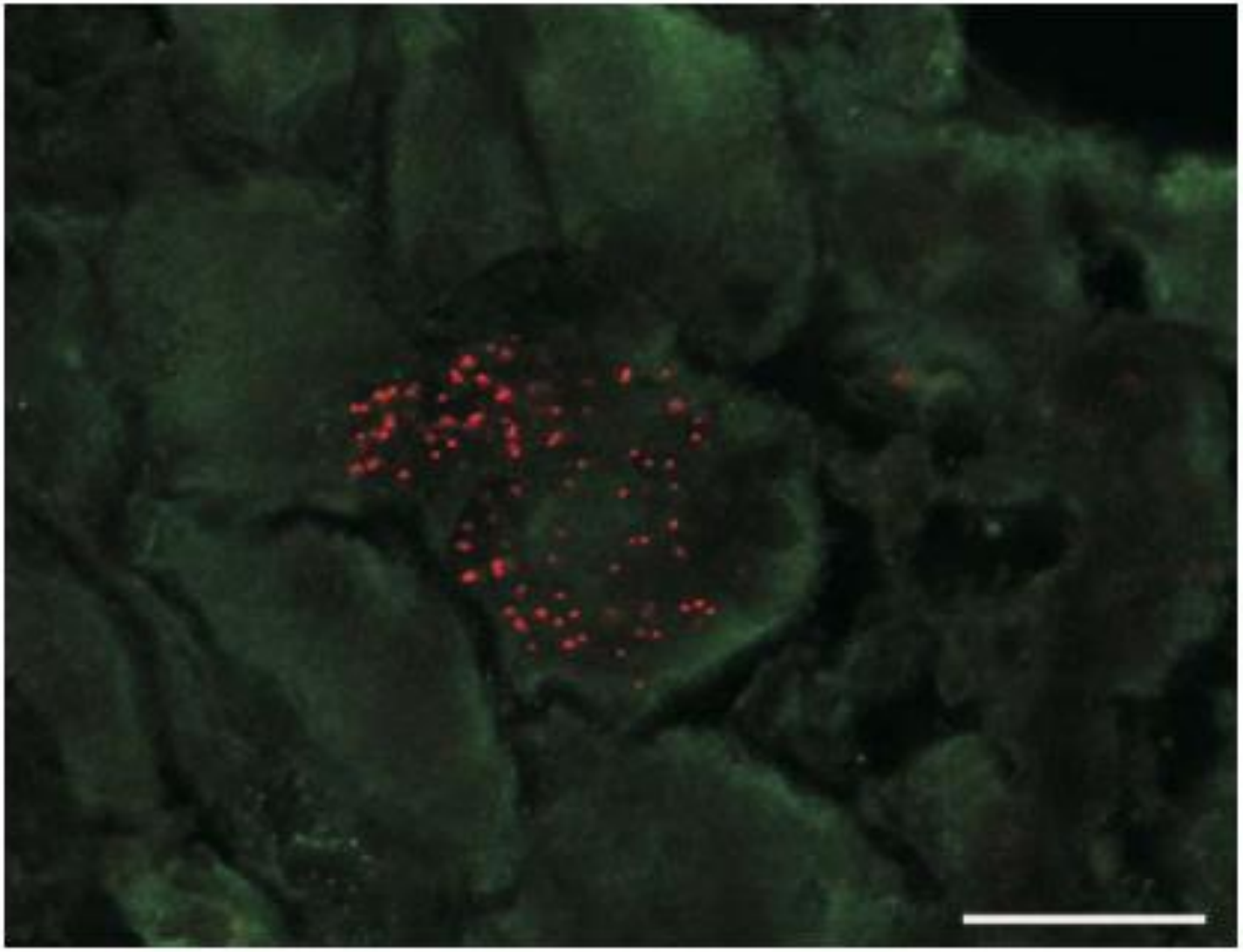


The major potential pathways for inhaled NP transport into the brain



NPs can be carried to the brain via the vasculature, neuronal transport from the olfactory neurons in the olfactory epithelium of the nose and then through the cribiform plate to the olfactory bulb, neuronal transport from sensory nerves of the nasal passageways to the trigeminal nerve, or neuronal transport from sensory nerves of the trachea and intrapulmonary airway to the vagus nerve

Red rhodamine beads in a neuron cell body of the jugular ganglion demonstrate neuronal transport of particles from the trachea of a rat after intratracheal instillation of fluorescent beads



Toxic Warnings

1997 - *Titanium dioxide/zinc oxide* nanoparticles from sunscreen are found to cause free radicals in skin cells, damaging DNA. (Oxford University and Montreal University) Dunford, Salinaro et al.

March 2002 – … *engineered nanoparticles* accumulate in the organs of lab animals and are taken up by cells…“ Dr. Mark Wiesner

March 2003 - ... studies on effects of *nanotubes* on the lungs of rats produced more toxic response than quartz dust.“ „Scientists from DuPont Haskell laboratory present varying but still worrying findings on nanotube toxicity.

Nanotubes can be highly toxic." - Dr. Robert Hunter (NASA researcher)

March 2003 - Dr. Howard: the smaller the particle, the higher its likely toxicity and that *nanoparticles* have various routes into the body and across membranes such as the blood brain barrier. ETC Group

July 2003 - Nature reports on work by CBEN scientist Mason Tomson that shows *buckyballs* can travel unhindered through the soil. "Unpublished studies by the team show that the nanoparticles could easily be absorbed by earthworms, possibly allowing them to move up the food-chain and reach humans" - Dr. Vicki Colvin, the Center's director.

Nanoparticles and the environment

- Nanoparticles released into the environment **interact with air, water and soil.** This often **changes the surface properties** of the particles which can result in **particle aggregation or changes in particle charge** and other surface properties. These effects have been studied in **water ecosystems and soil** and show the importance of understanding nanoparticles and their environmental setting as a "complex" that needs to be looked at in its entirety in order to understand particle behaviour in the environment. A current debate addresses whether nanoparticles can cause toxicity as a contaminant in, for example, soil or water, via a "piggyback" mechanism on natural organic matter.

Nanoparticles and cells—in-vitro nanotoxicology

- Unicellular organisms, which ruled the Earth for approximately the first 3 billion years of life, exist by engulfing matter, usually particulate, from their immediate environment.
- There are the **two basic mechanisms, phagocytosis and endocytosis**. The former is generally for large particles and requires a “recognition” step while the latter is for the trans-membrane transport of liquids and molecules.
- Only by **understanding particle uptake dynamics** and pathways in a quantitative way, can nanotoxicological conclusions be drawn.

Mechanisms on the nano-bio interface

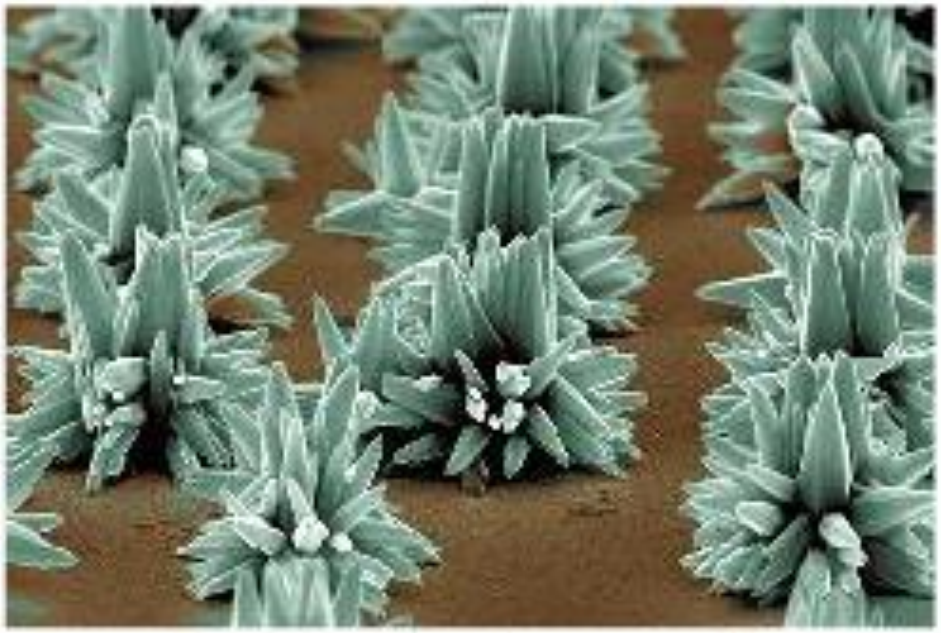
- Mechanisms on the nano-bio interface can be either **chemical or physical**.

- Chemical mechanisms include the **production of reactive oxygen species (ROS)**, dissolution and release of toxic ions, disturbance of the electron/ion cell membrane transport activity, oxidative damage through catalysis, lipid peroxidation or surfactant properties. **ROS** is considered as being **the main underlying chemical process in nanotoxicology**, leading to secondary processes that can ultimately cause cell damage and even cell death.

- Moreover, ROS is one of the main factors involved in **inflammatory processes**

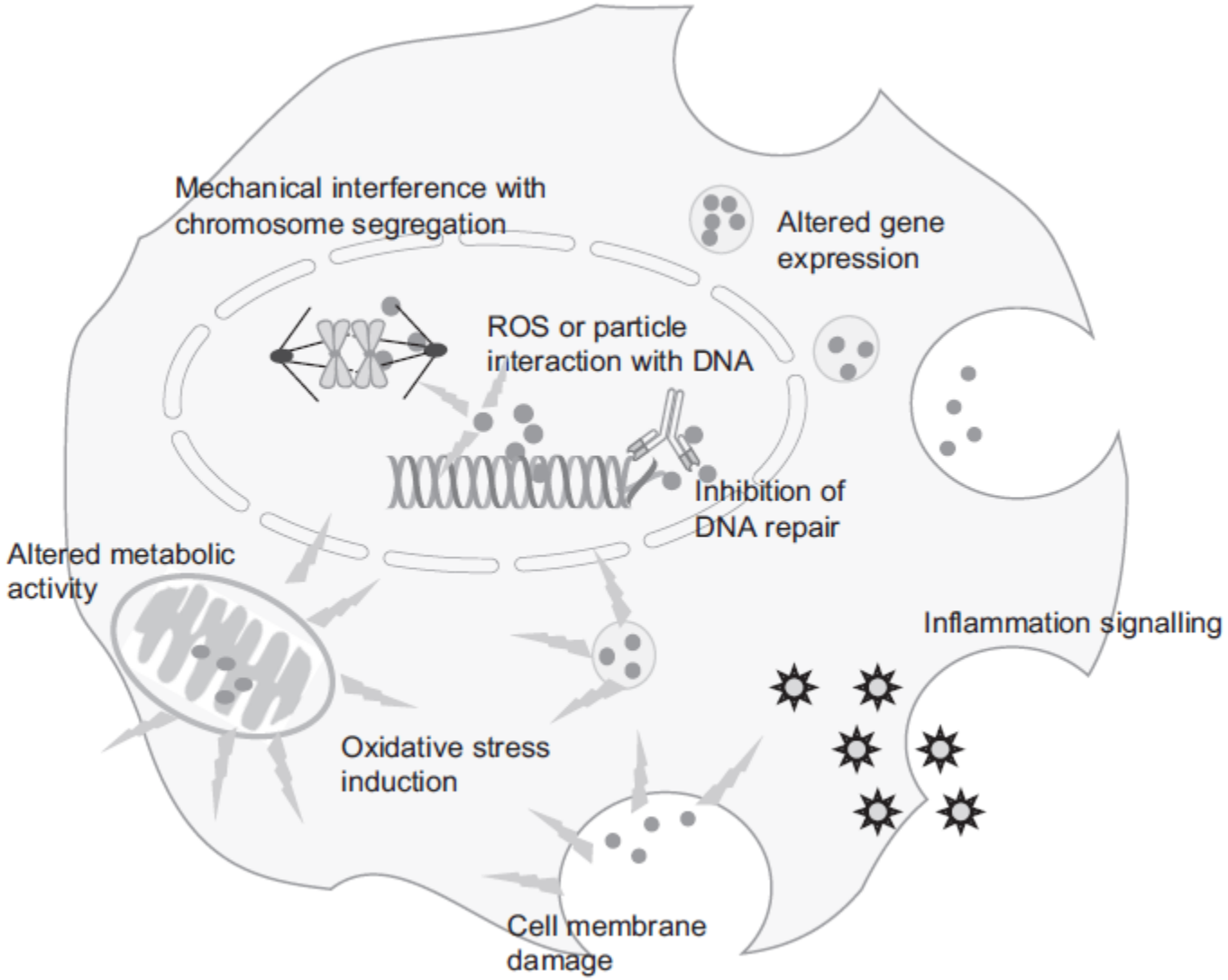
Physical mechanisms at the nano-bio interface

- Physical mechanisms at the nano-bio interface are mainly a result of particle size and surface properties. This includes disruption of: membranes, membrane activity, transport processes, protein conformation/folding and protein aggregation /fibrillation.

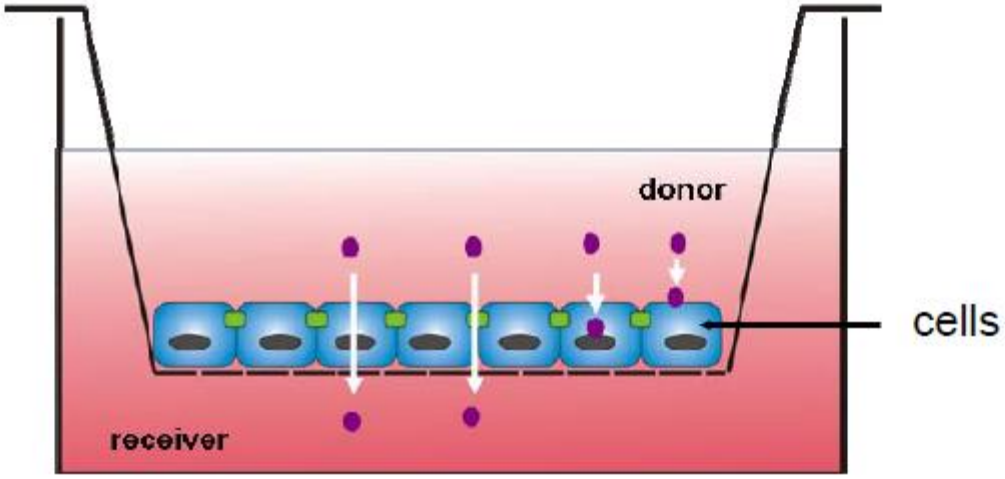


Zinc Oxide Nanoparticles

Possible interactions of ENMs with the cell and



Transport of metal oxide nanomaterials through cell monolayer- a model



The Transwell system:

epithelial cells (blue) separate the apical (donor) from the basolateral (receiver) compartment.

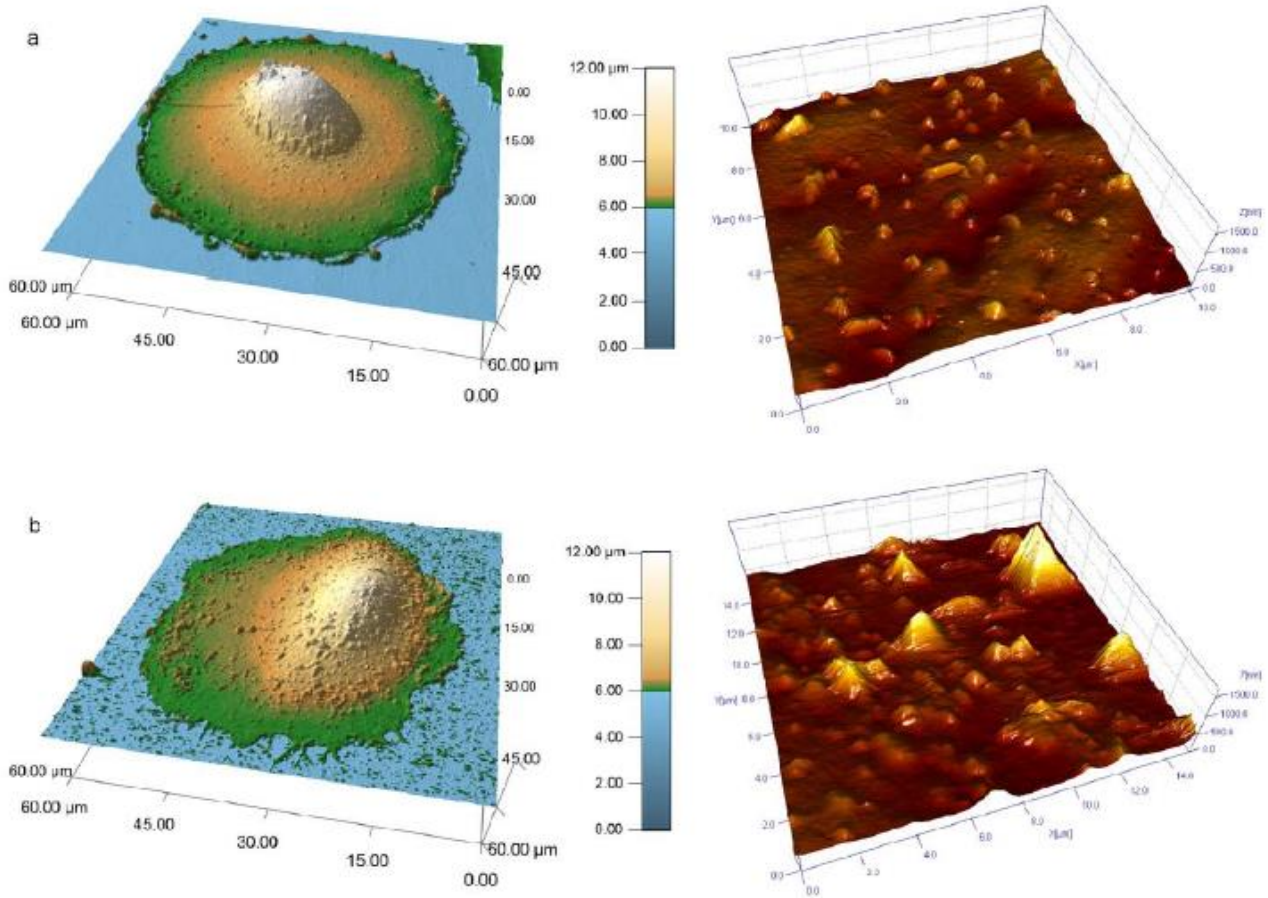
Intercellular and intracellular transport of particles can be investigated as well as uptake into or adhesion of particles onto cell.

AFM analysis of nanoparticle-cell interactions

Topographic AFM-images of a whole RLE-6TN-cell (left) and magnified parts of the cell surface (right). Untreated cells (a) and incubated with CeO_2-particles (b)

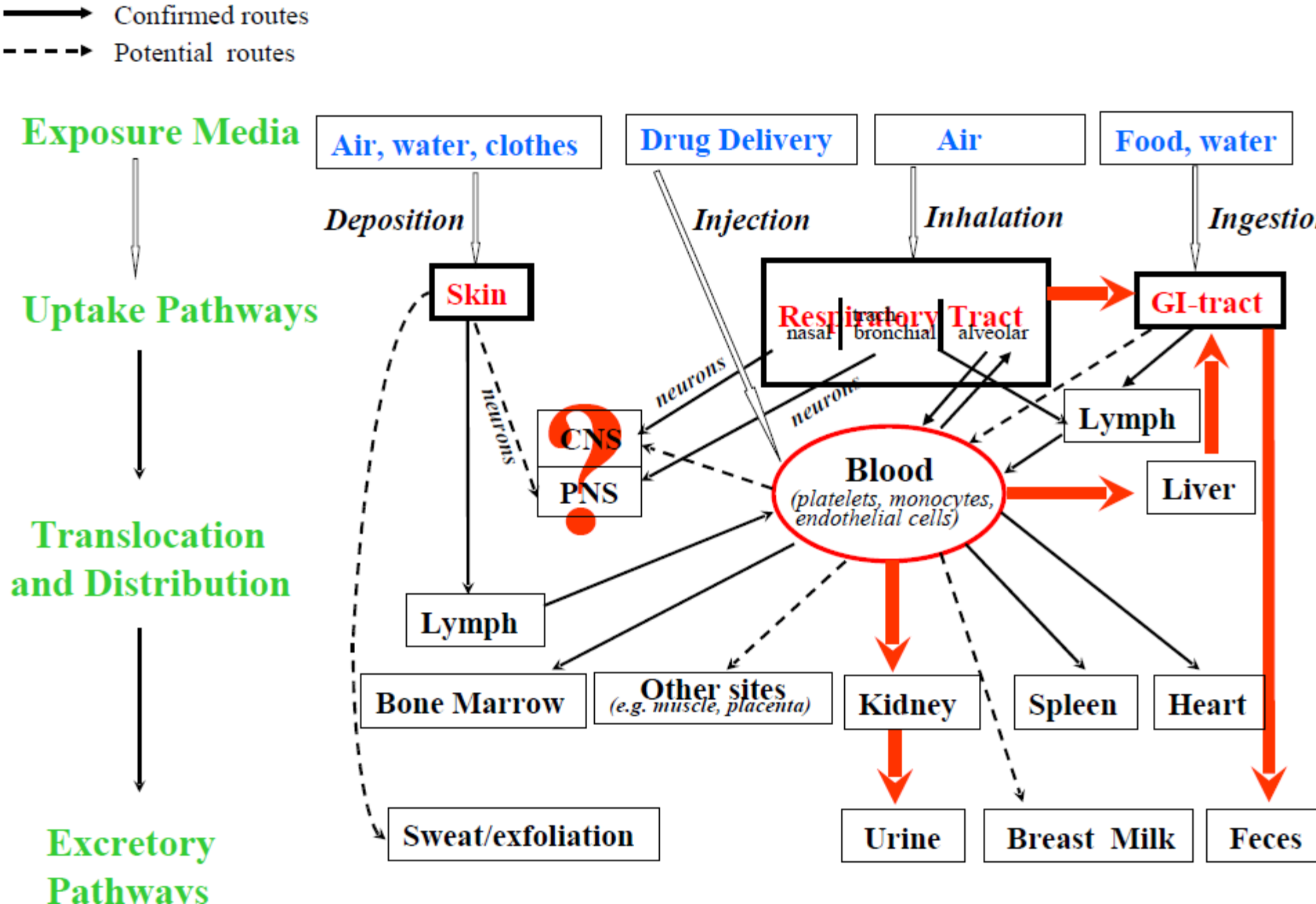
Cells are fixed and imaged in buffer solution.

Magnified images a: 10 µm, b: 15 µm scan size.

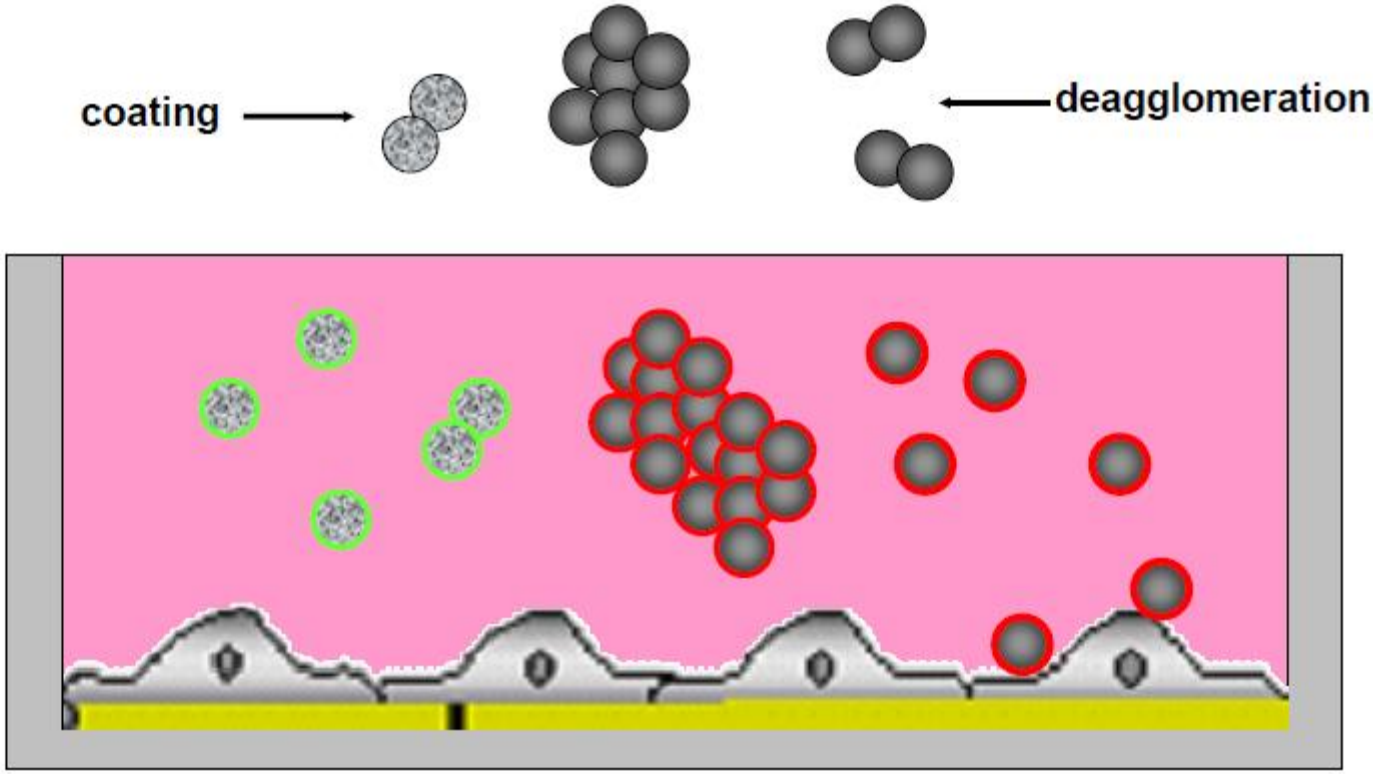


Nanomaterials are bound on the cellular surface.

Elimination Pathways of Nanosized Particles

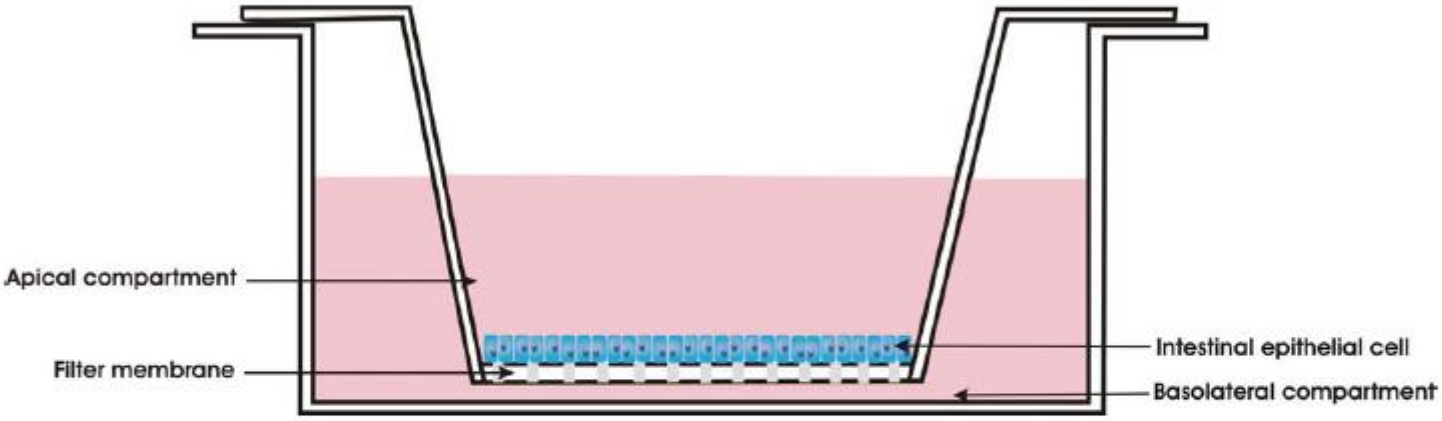


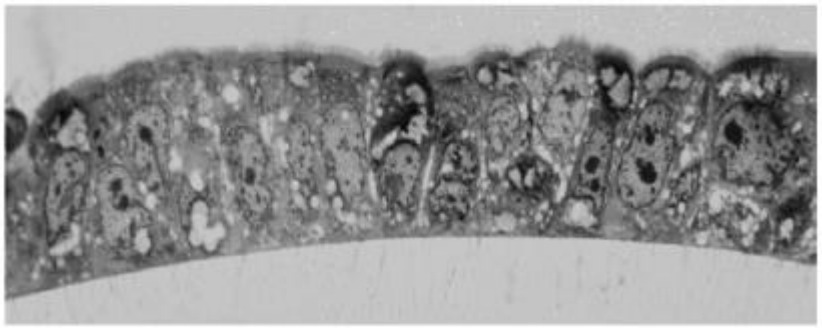
Nanoparticles in media



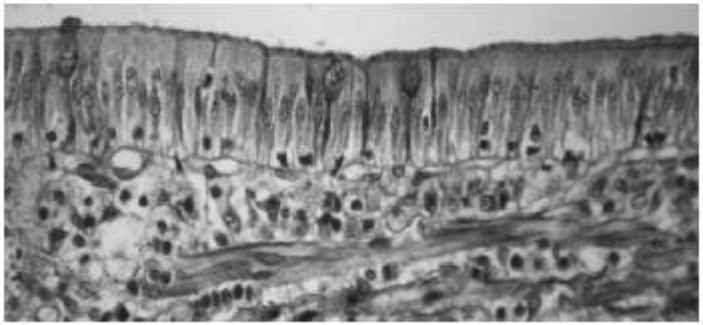
- Nanoparticles can interact and can be coated with proteins
- Nanoparticles can deagglomerate

In vitro test sytems for oral bioavailability



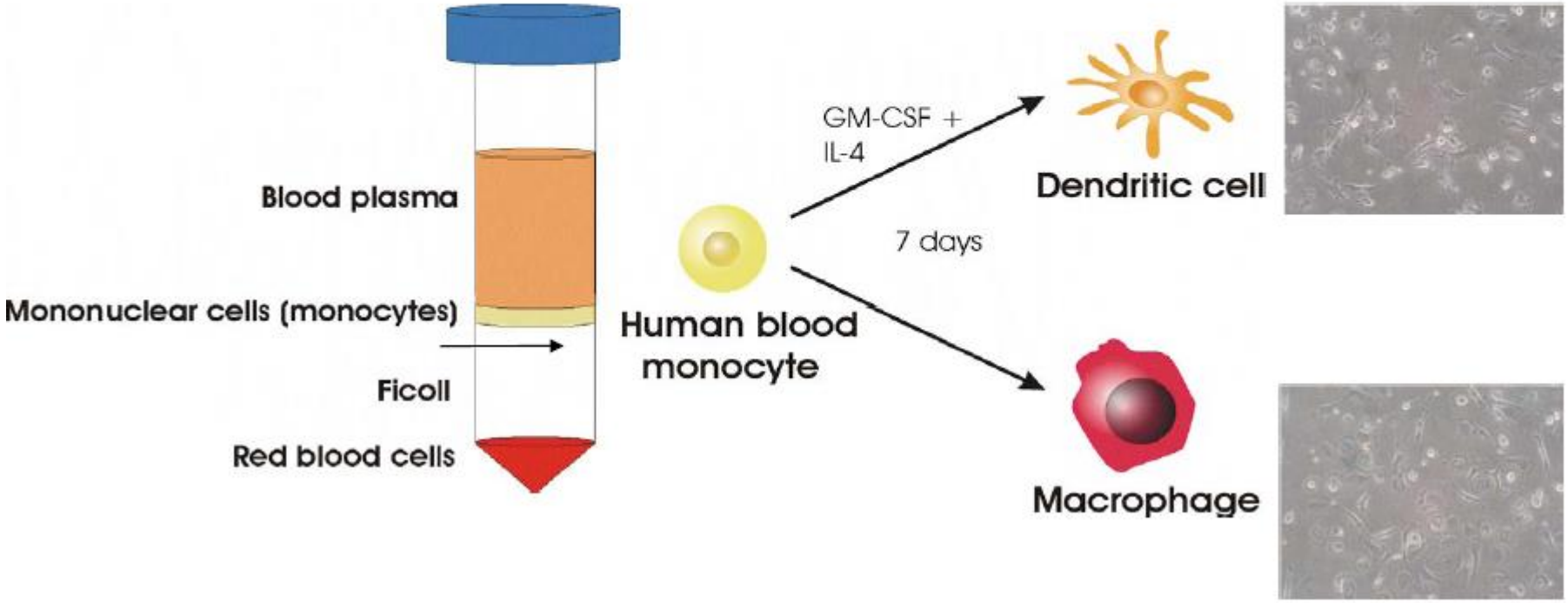


Caco-2 monolayer

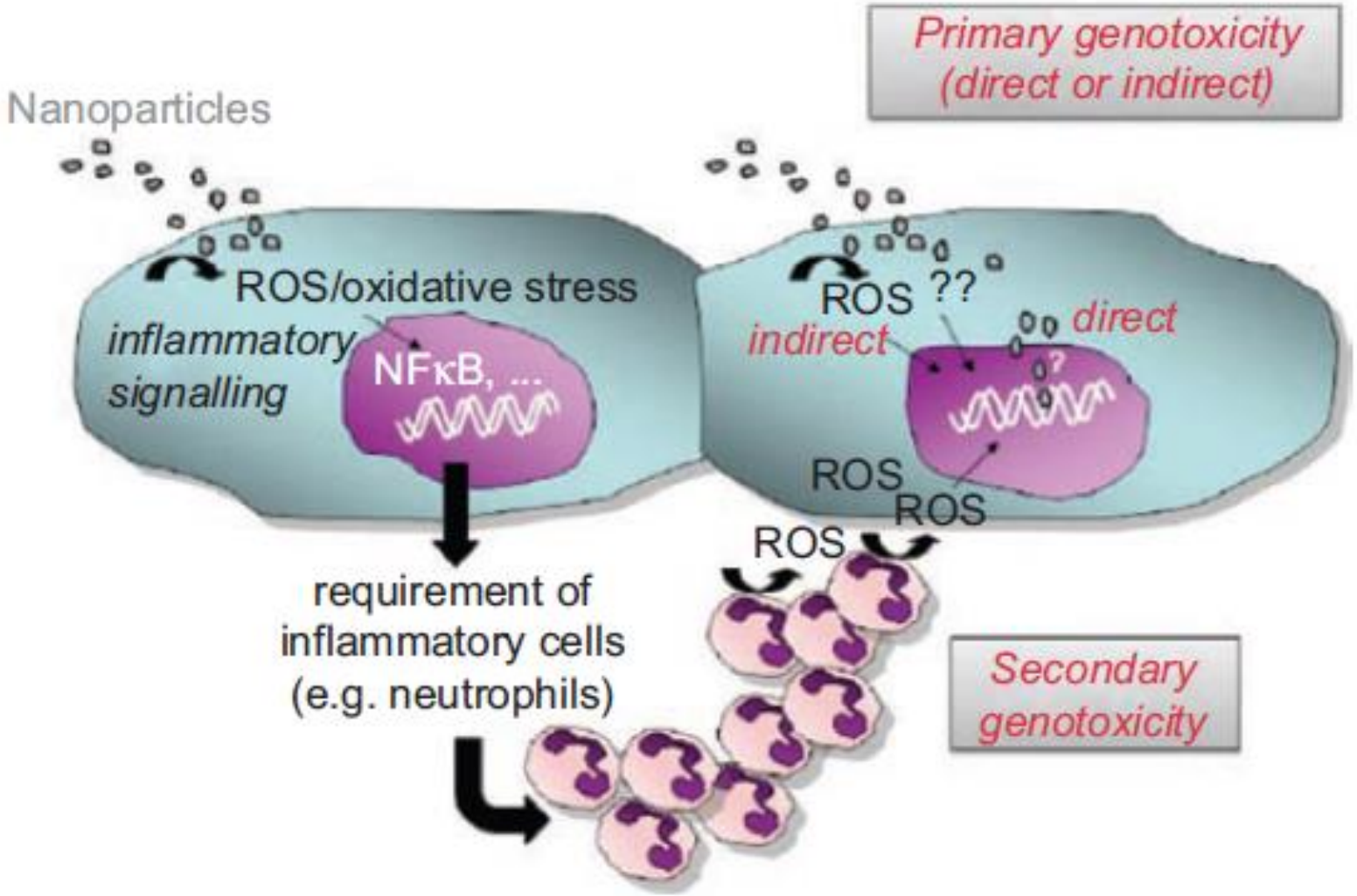


Intestinal mucosa

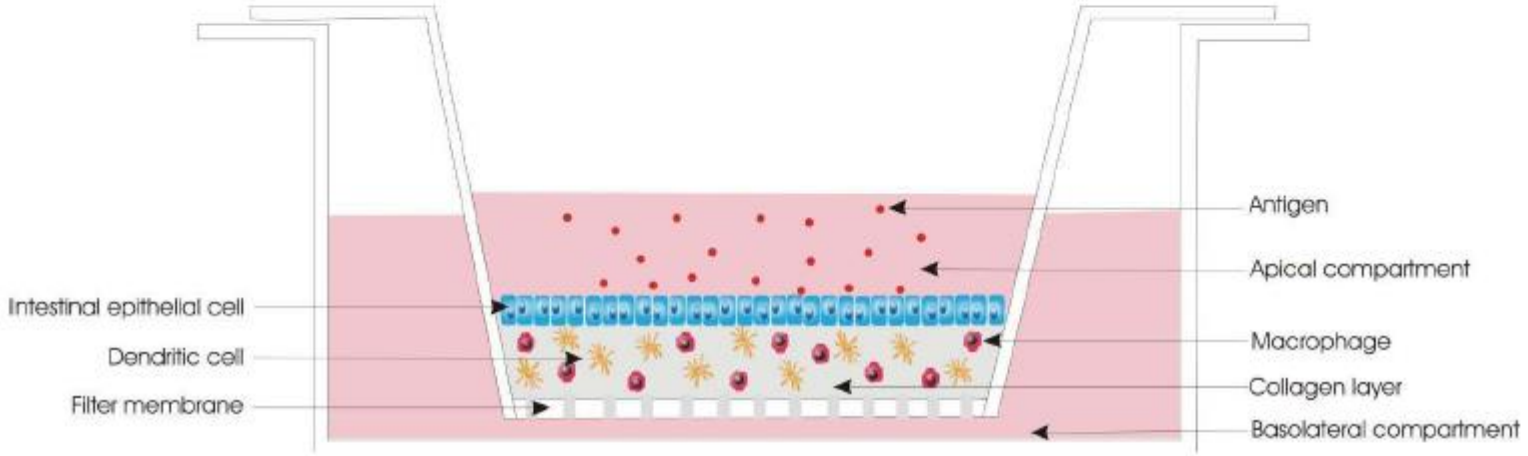
Adding complexity: immune cells



Schematic diagram of the mechanisms of “primary” and “secondary” genotoxicity in nanoparticle exposed cells



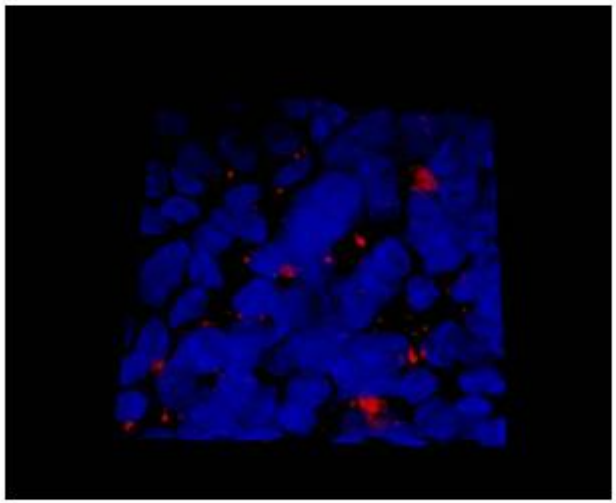
3D in vitro model of the inflamed intestinal mucosa

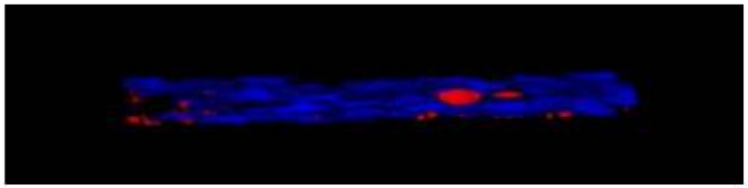


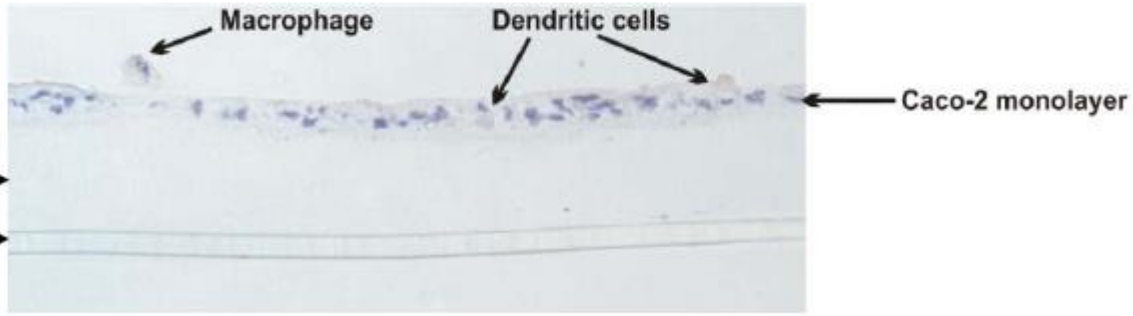
- Co-culture of Caco-2 intestinal epithelial cells with blood derived dendritric cells and macrophages
- Stimulation of inflammation by addition of lipopolysaccharides or pro-inflammatory cytokines (interleukin-1β) to the cell culture medium
- Should reflect the relevant pathophysiological changes occuring in vivo: release of pro-inflammatory markers (IL-8, TNF-α), re-organisation of thight junctional proteins, reduced barrier function, increased mucus production

Pathophysiological changes in the 3D model

Infiltration of immunocompetent cells (macrophages + dendritic cells)



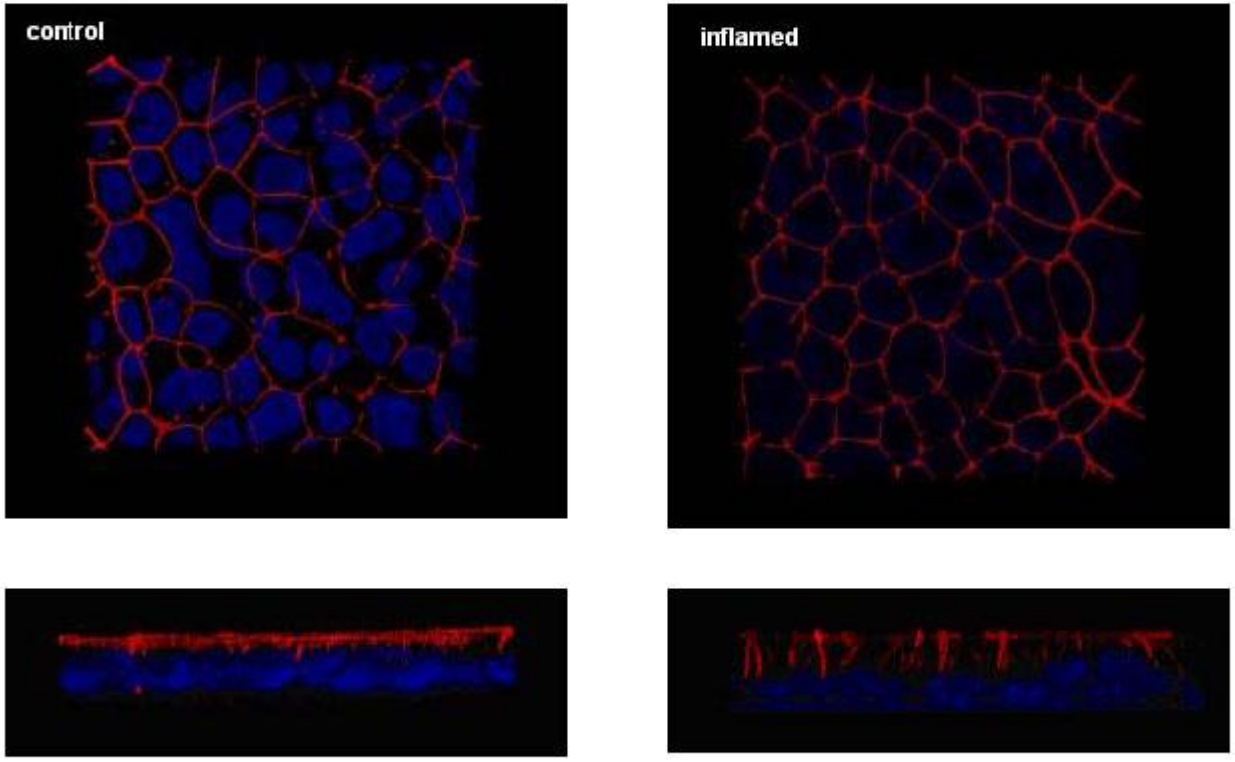




Leonard et al, Mol Pharm: 7(6), 2103-19 *(2010)*

Pathophysiological changes in the 3D model

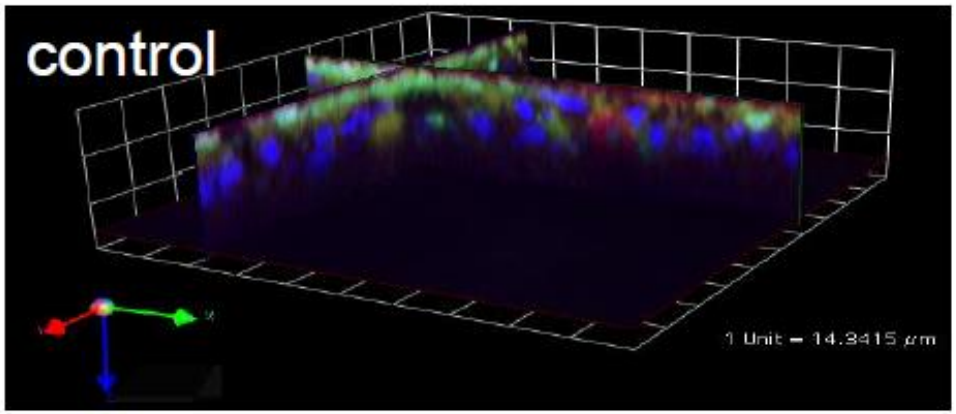
Changes in tight junctional organization (ZO-1) …

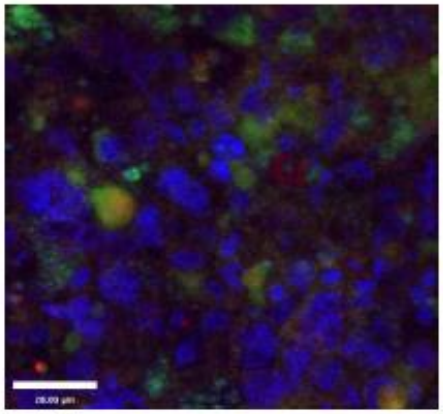


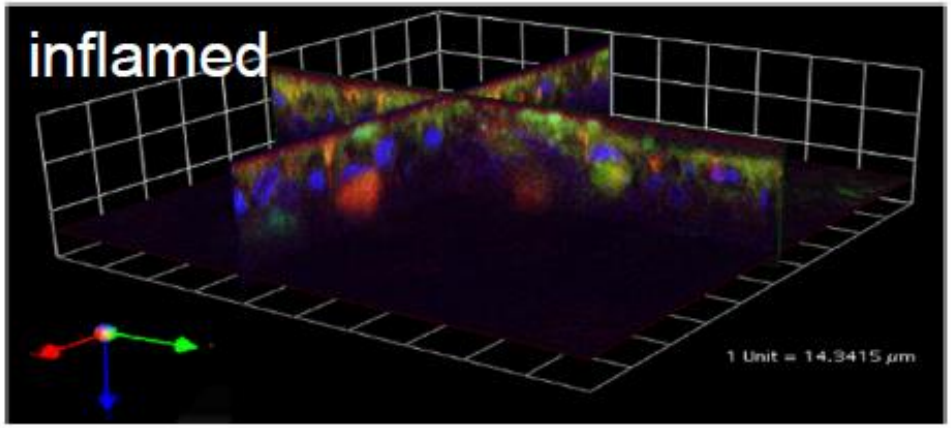
Leonard et al, Mol Pharm: 7(6), 2103-19 *(2010)*

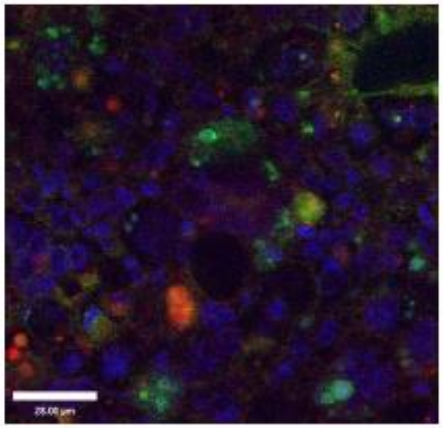
Pathophysiological changes in the 3D model

Increased activity of immune cells



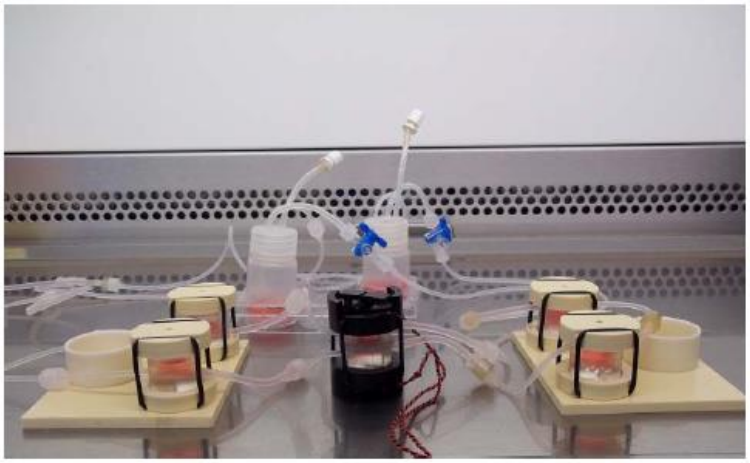


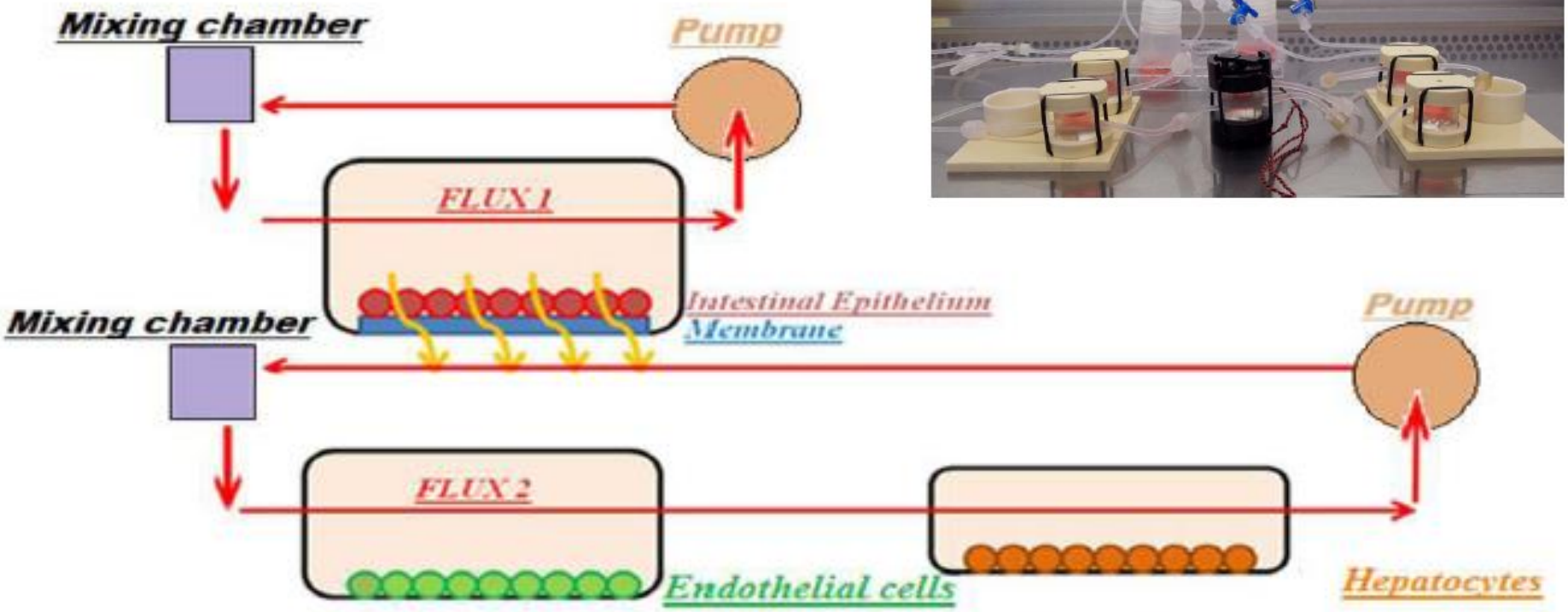




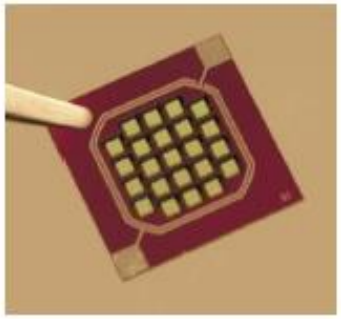
Leonard et al, Mol Pharm: 7(6), 2103-19 *(2010)*

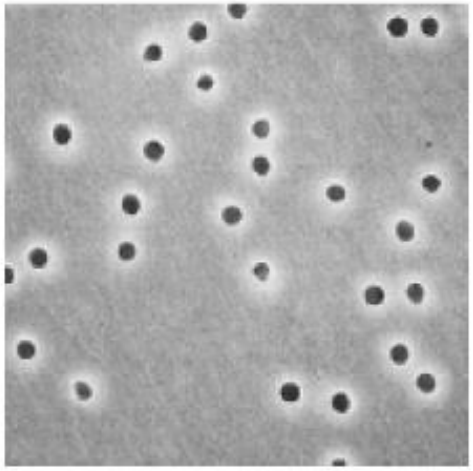
Other applications of the 3D model of the inflamed intestinal mucosa: nanotoxicology

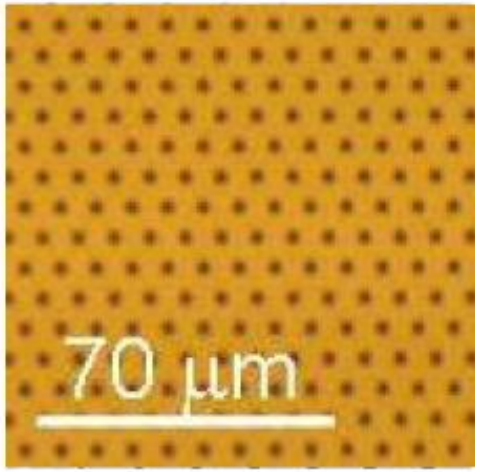


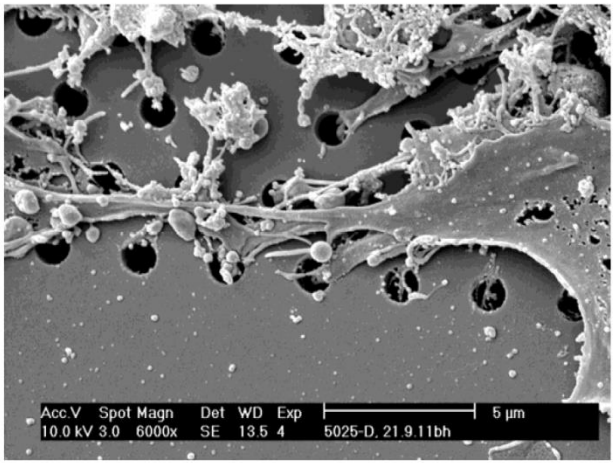


It‘s only a matter of support: new directions for advanced intestinal cell models





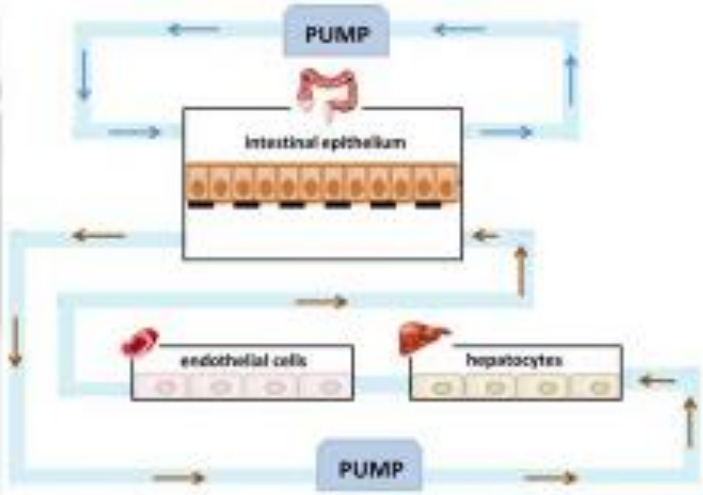




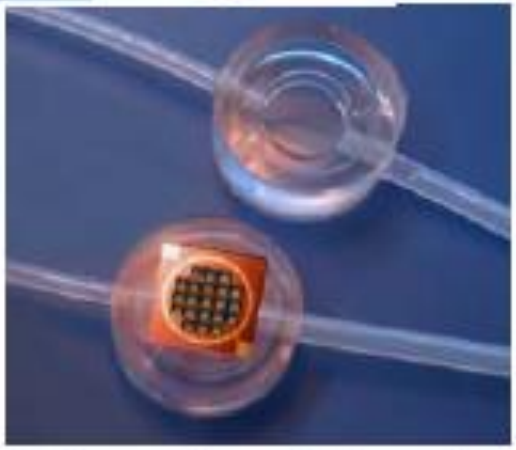
HELMHOLTZ

Nanotox by ingestion: InLiveTox

- Goals:
  - move towards replacing in vivo experiments in nanotox
  - combine models of intestine, blood vessels, liver in one system
  - Compare results in fluidics system and in rats



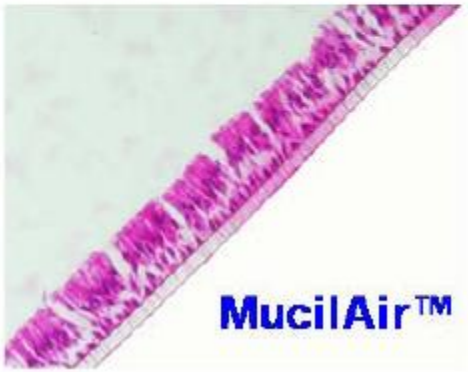
- CSEM's rôle
  - Coordinator
  - Integration of ultrathin membranes and electrical measurements

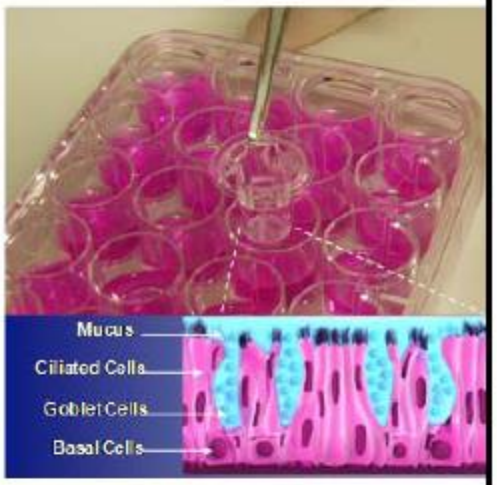


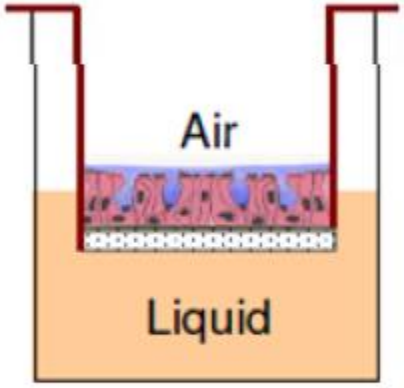
Better electrical measurements for lung models:

- Model airway epithelia can be cultured at the air/liquid interface.
- These models are especially relevant for airborne substances e.g nanoparticles
- Electrical measurements require them to be submerged in liquid

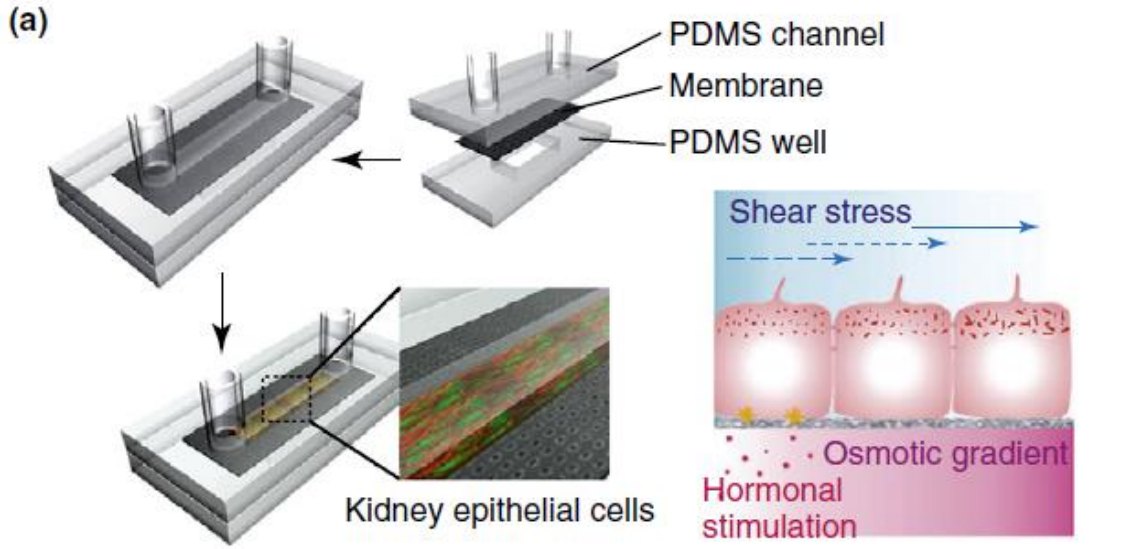
- CSEM has developed a new electrode system allowing electrical measurements at the air/liquid interface

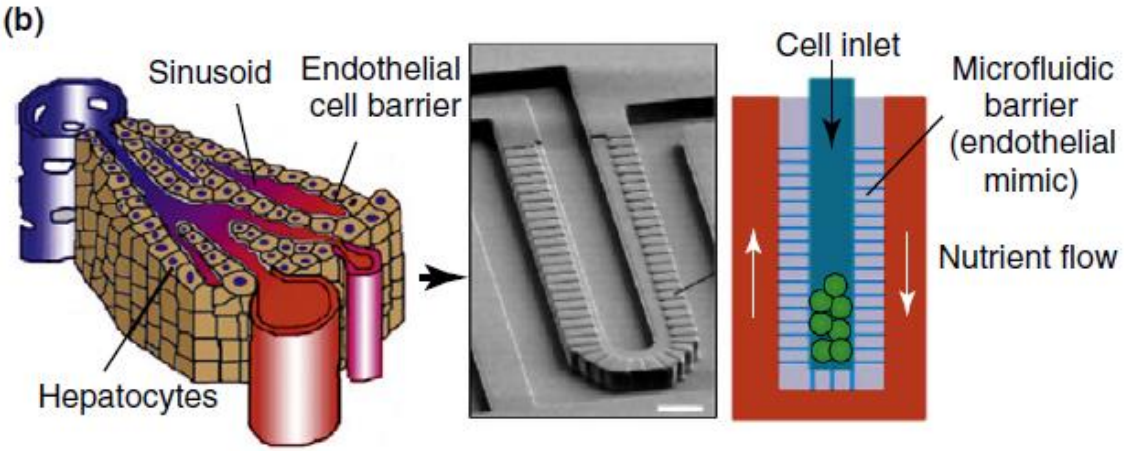


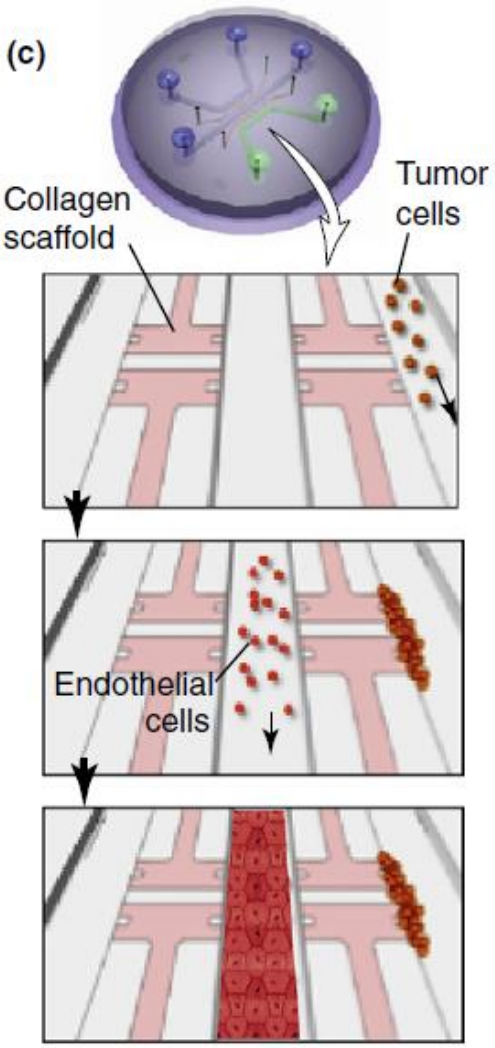




Microengineered organs-on-chips







TRENDS in Cell Biology

Effect of size

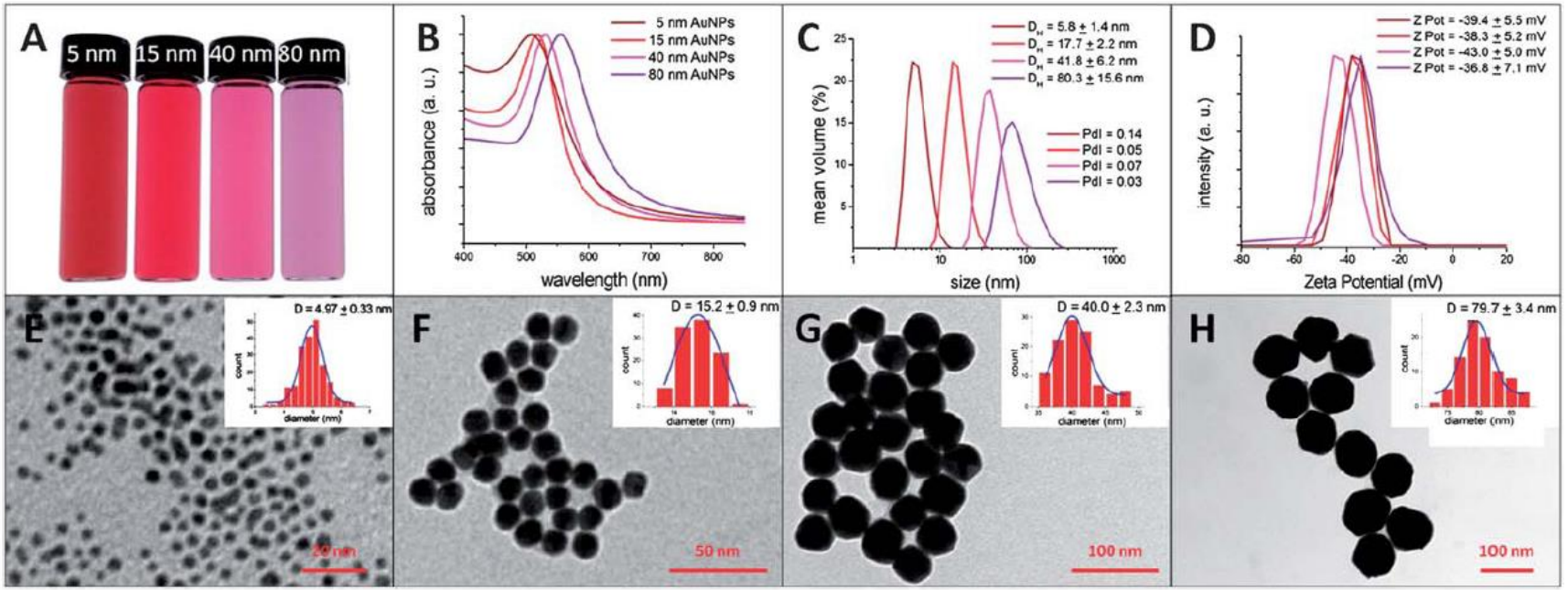
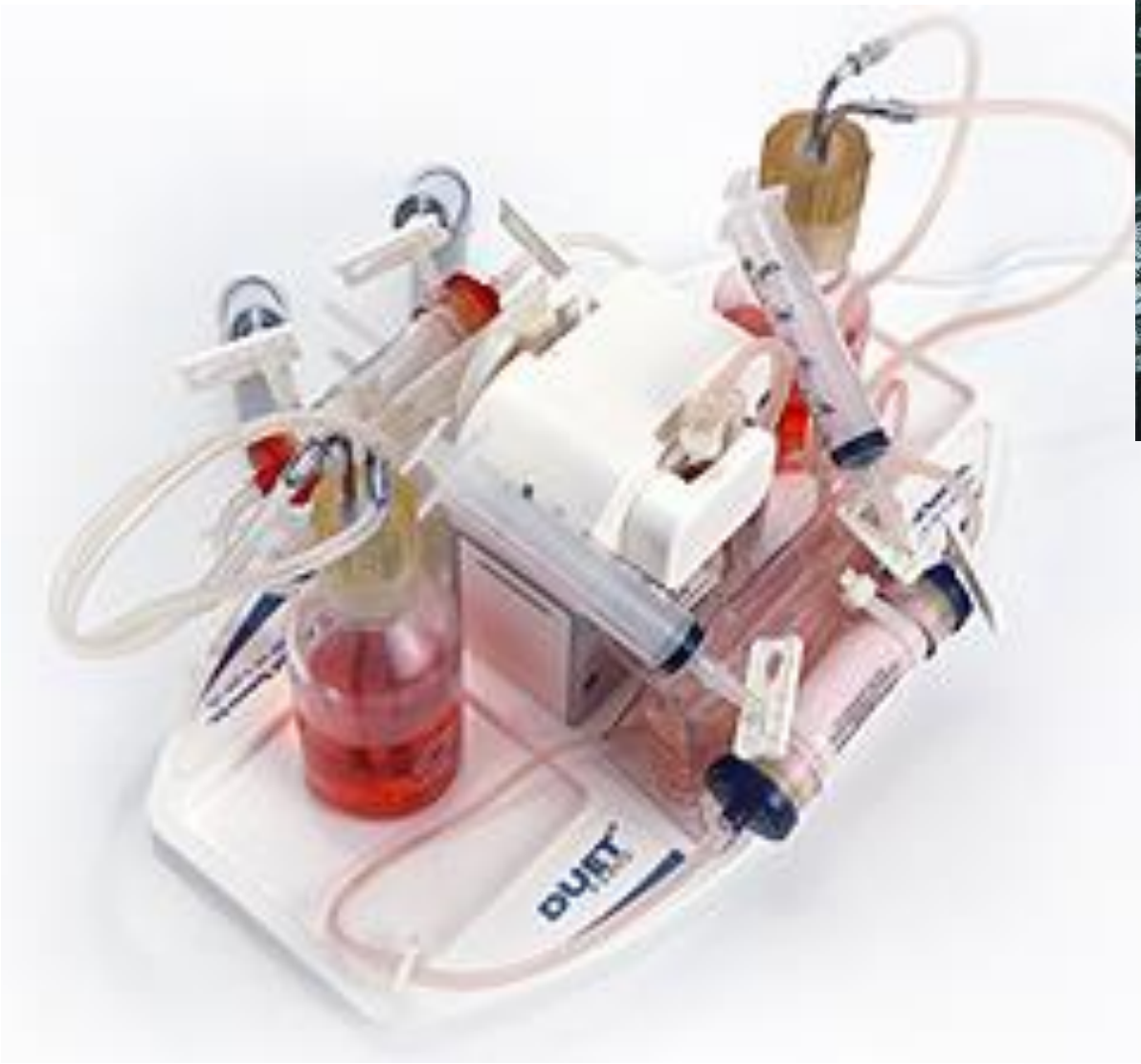
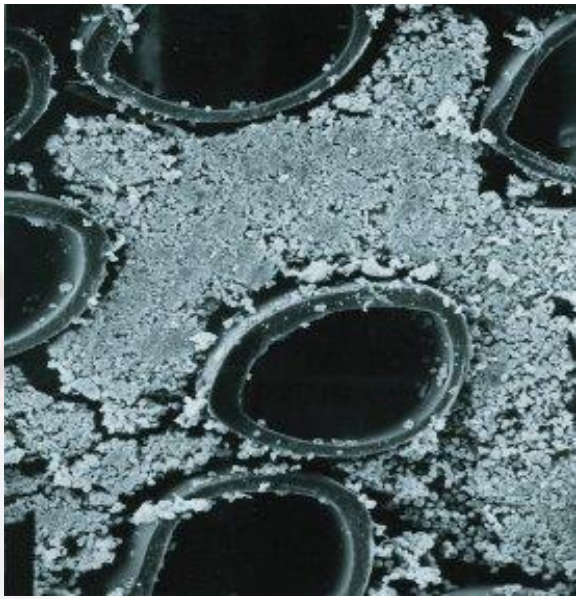
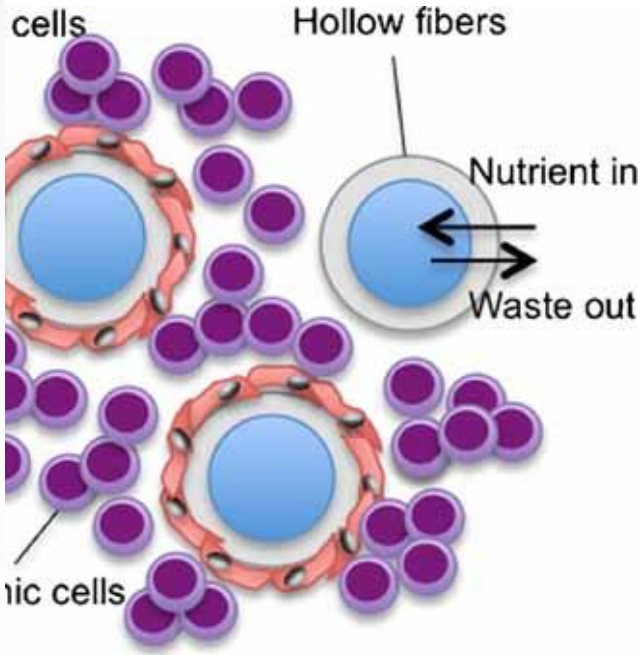


Fig. 1 (A) Photograph of a vial of 5, 15, 40, and 80 nm citrate-capped AuNPs freshly prepared; (B) SPR absorption spectra; (C) DLS analyses and (D) *Z*-potential analyses; (E–H) representative TEM image and size distribution analyses obtained from more than 100 NPs in random fields.

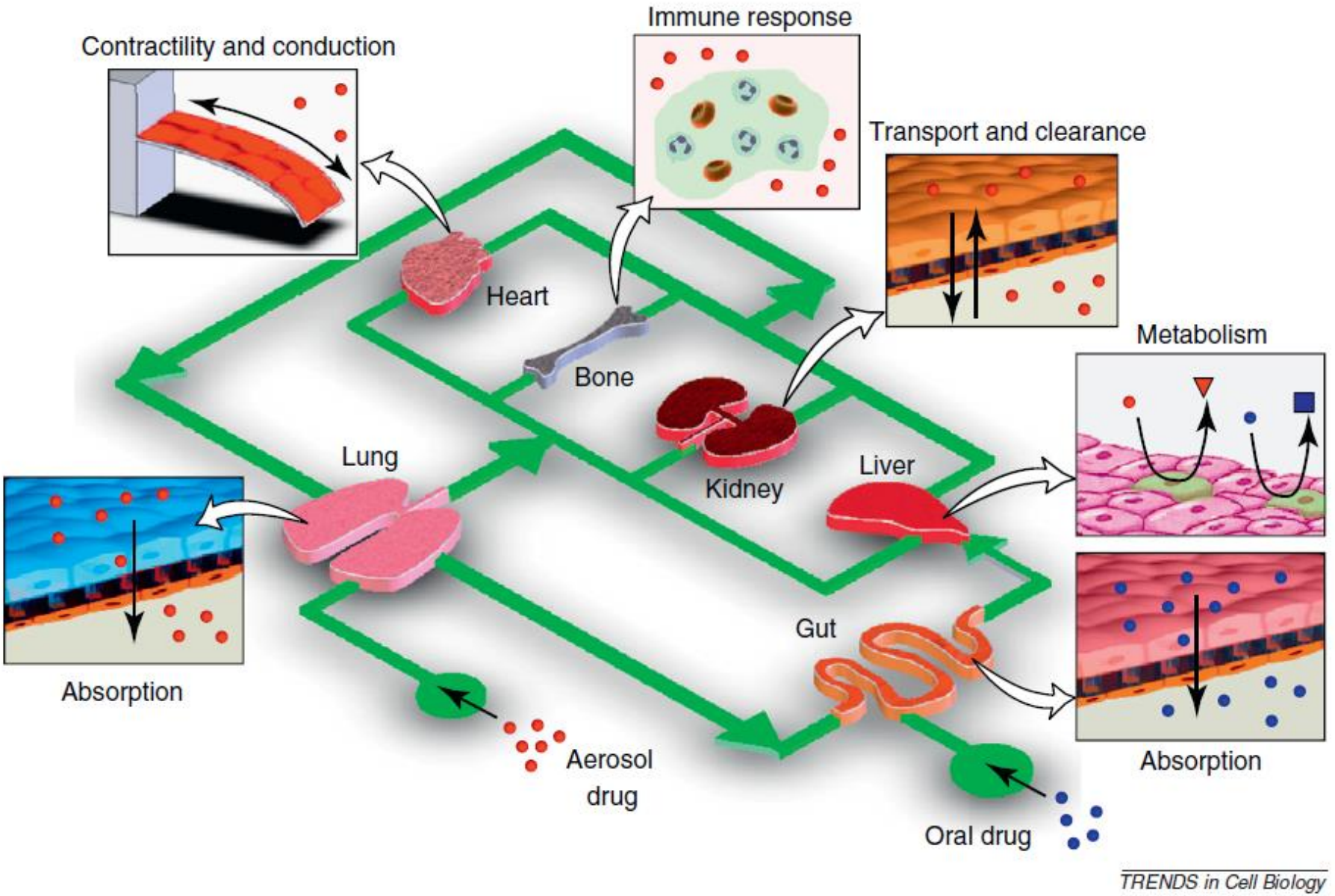
Hollow fibre model



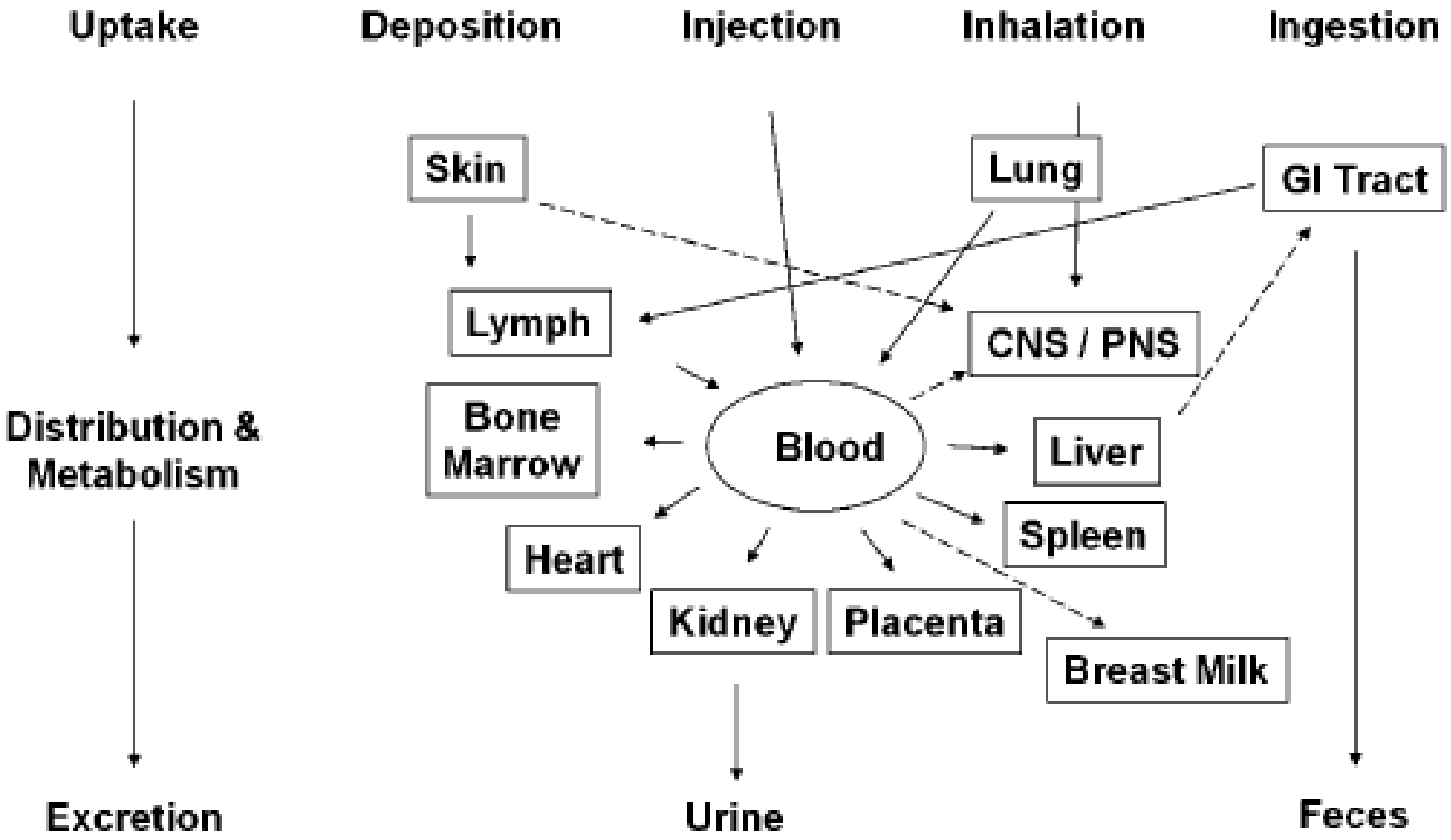




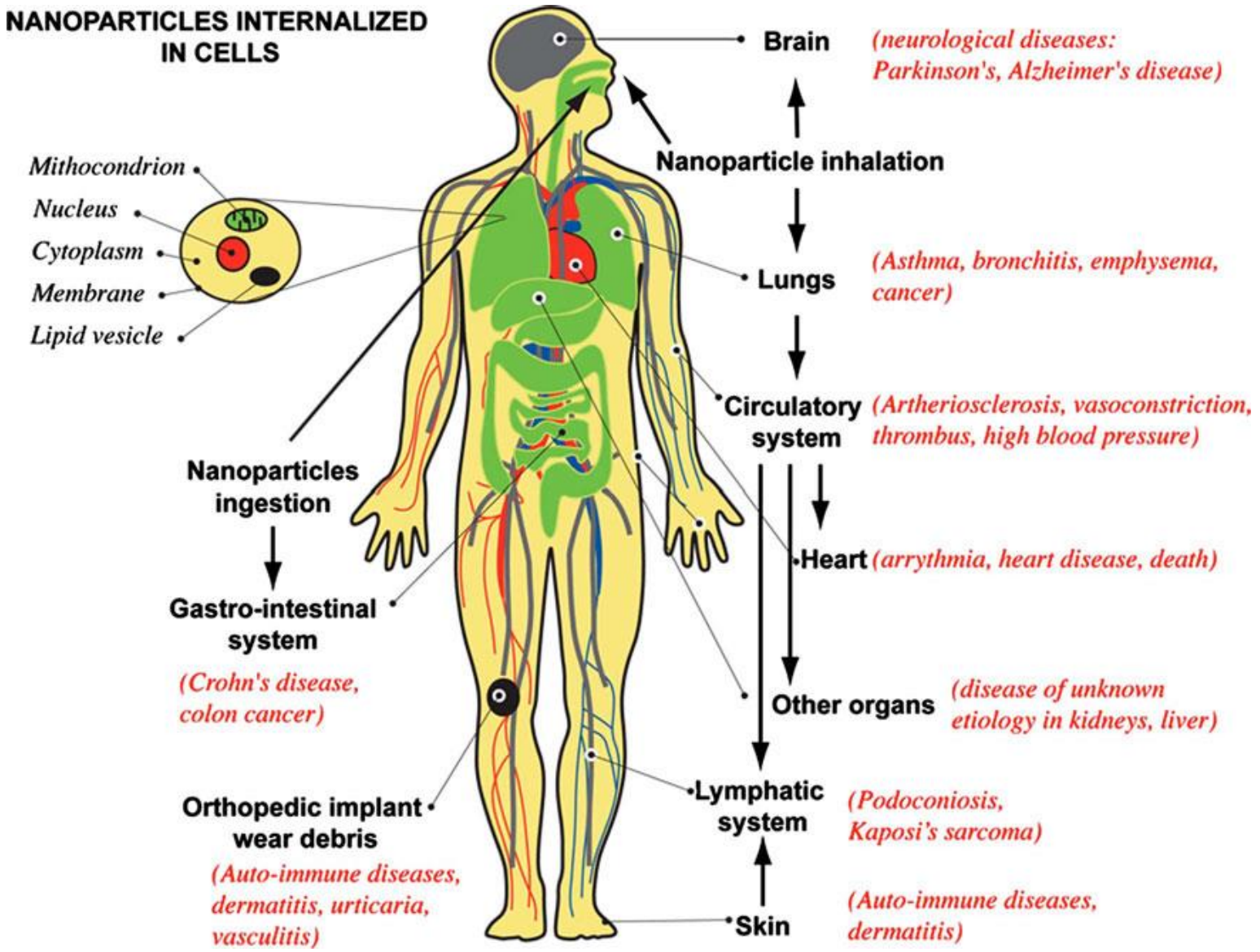
The human-on-a-chip concept



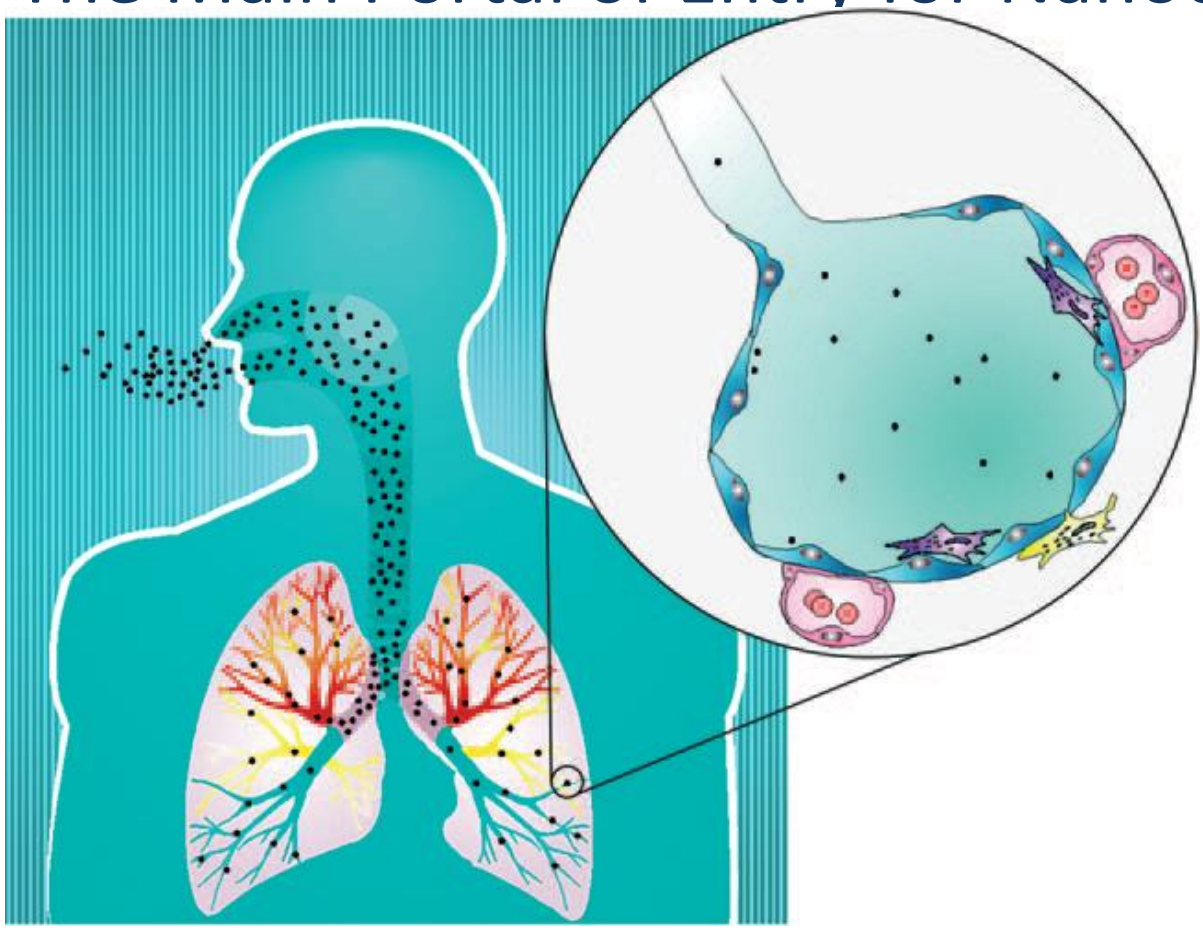
Nanoparticles pahtways within the body



Schematics of human body with pathways of exposure to nanoparticles



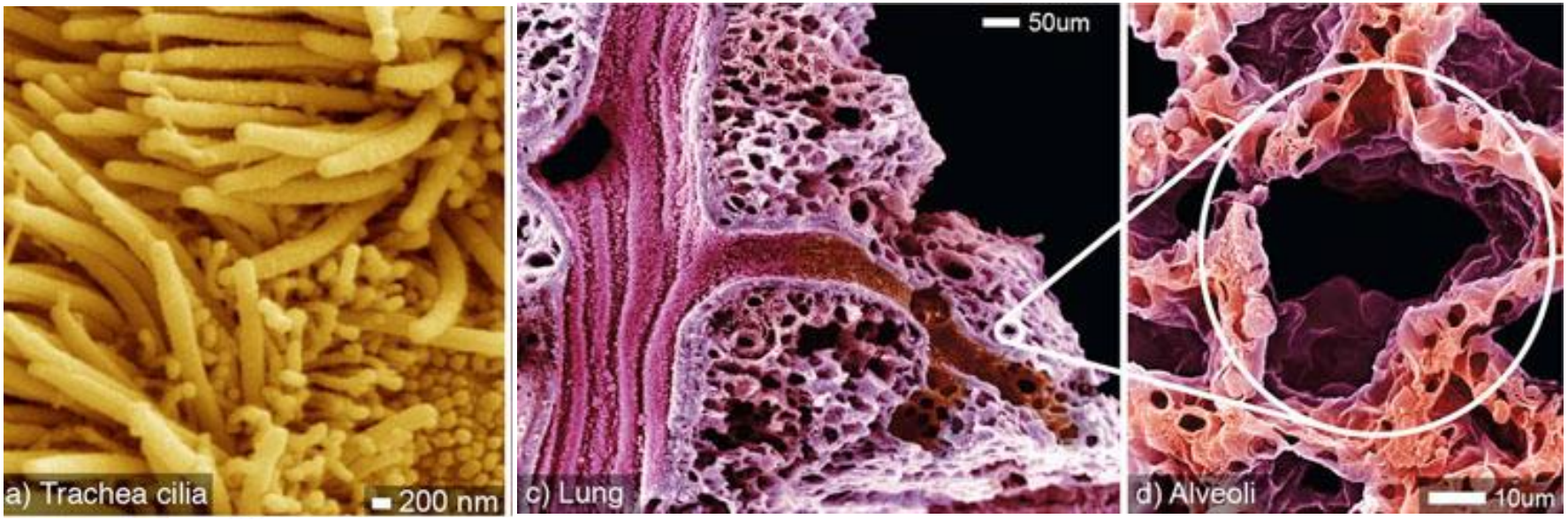
The Lung: The Main Portal of Entry for Nanoobjects

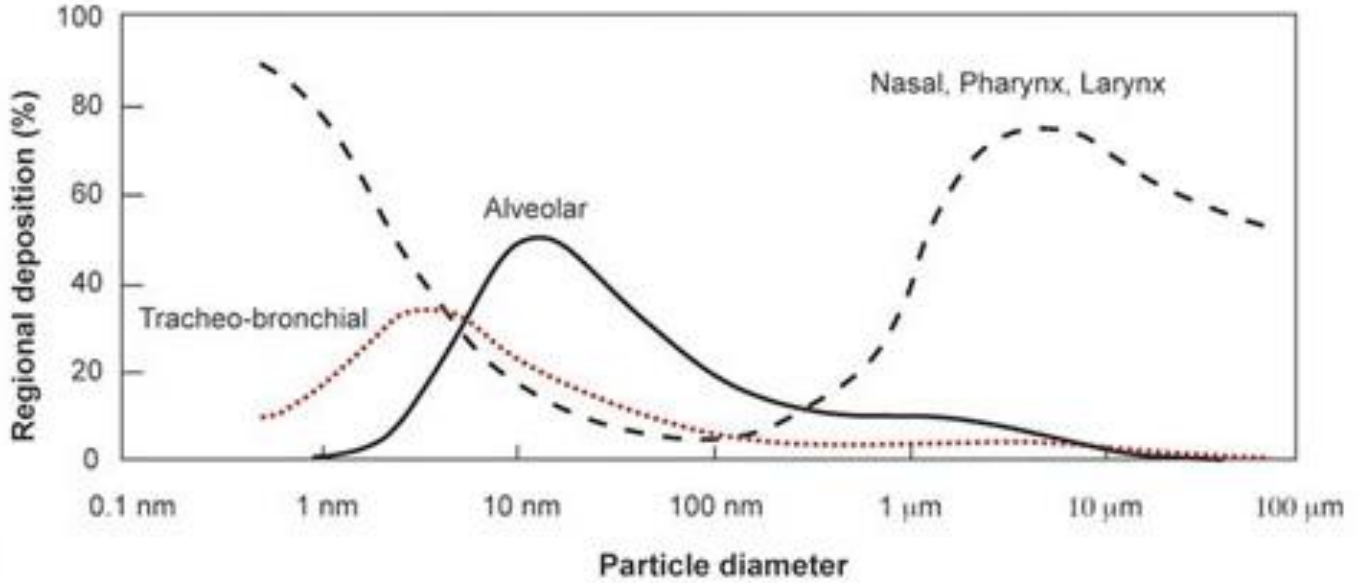


There are 300 million alveoli to facilitate this gas exchange via diffusion, encompassing a surface area of approximately 140 m^2.

Inhaled particles that are smaller than 2.5 mm (PM2.5) have access to the alveolar structures of the deep lung and may, in high doses, induce inflammation. A very small portion of the nanoparticles can cross the air–blood barrier and will be distributed via the bloodstream (red). Within the alveoli, most of the particles will be phagocytized by macrophages (purple) or dendritic cells (yellow) or may also be taken up by epithelial cells (blue).

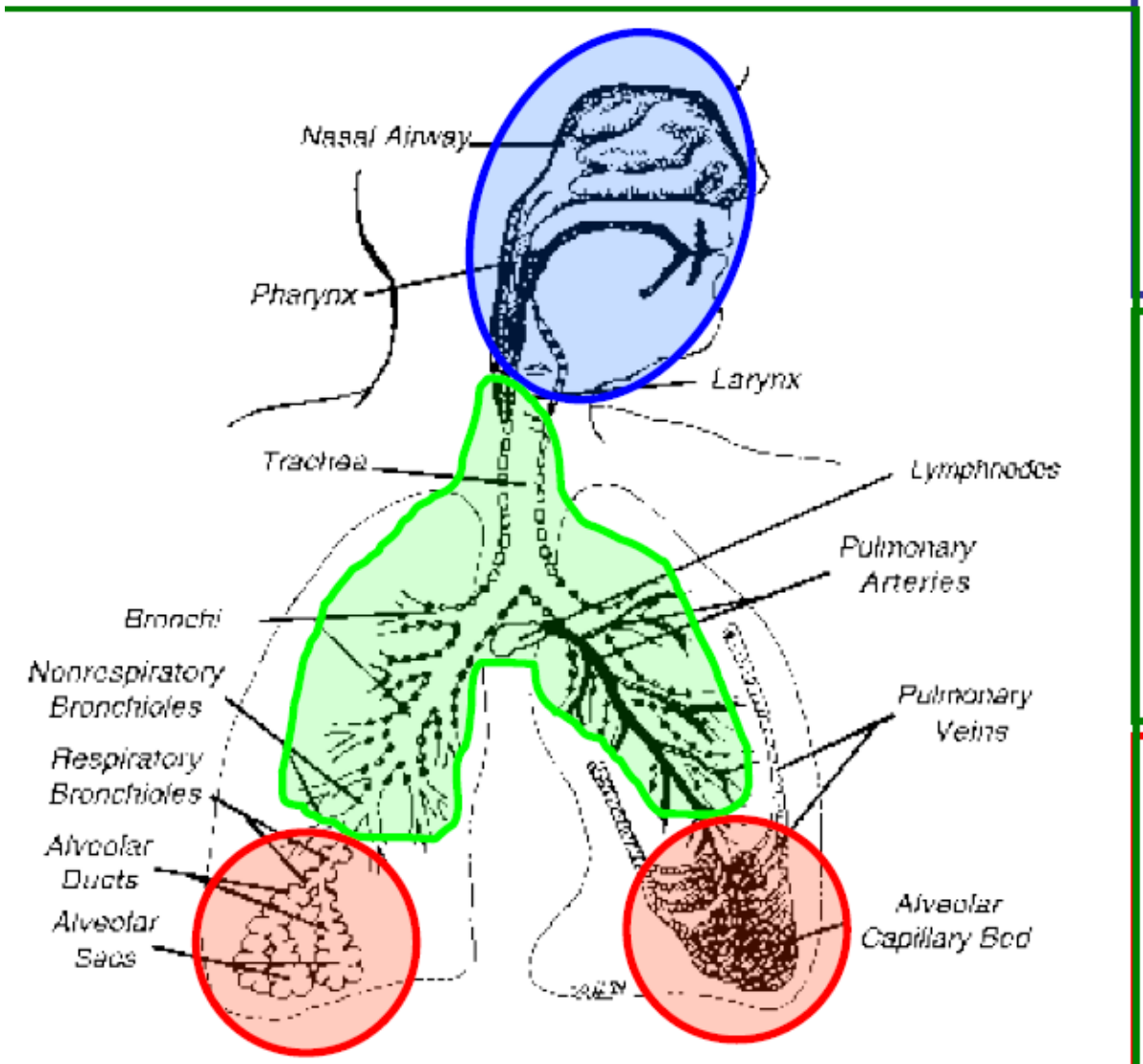
Effect of the particle size on deposition in respiratory tract

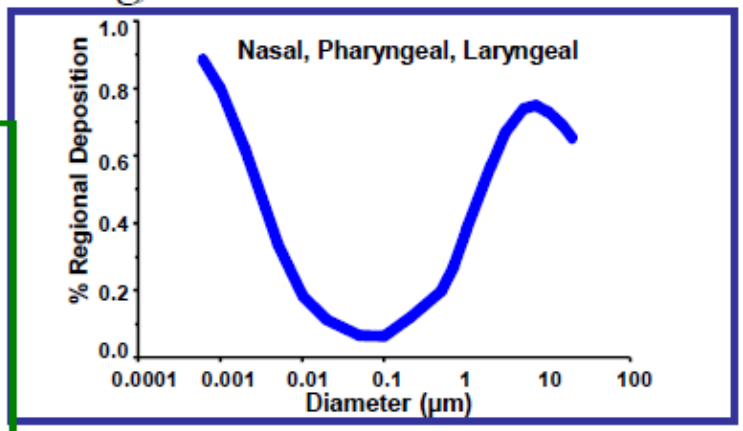


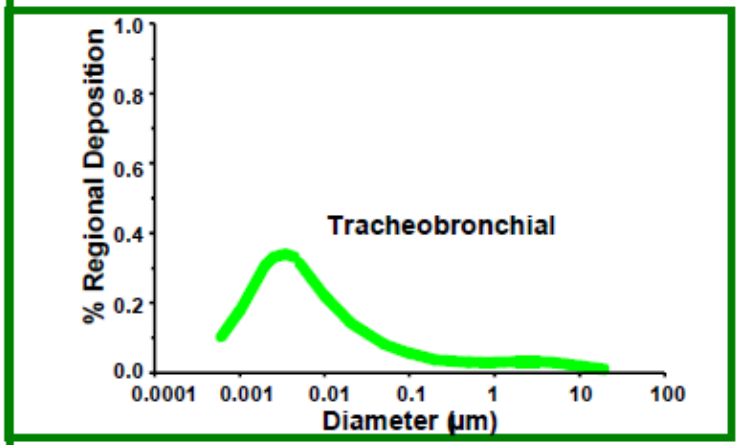


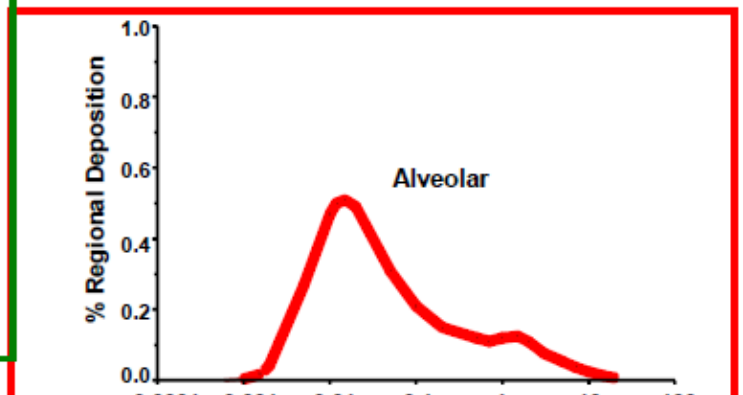
Fractional Deposition of Inhaled Particles in the Human Respiratory Tract

(ICRP Model, 1994; Nose-breathing)

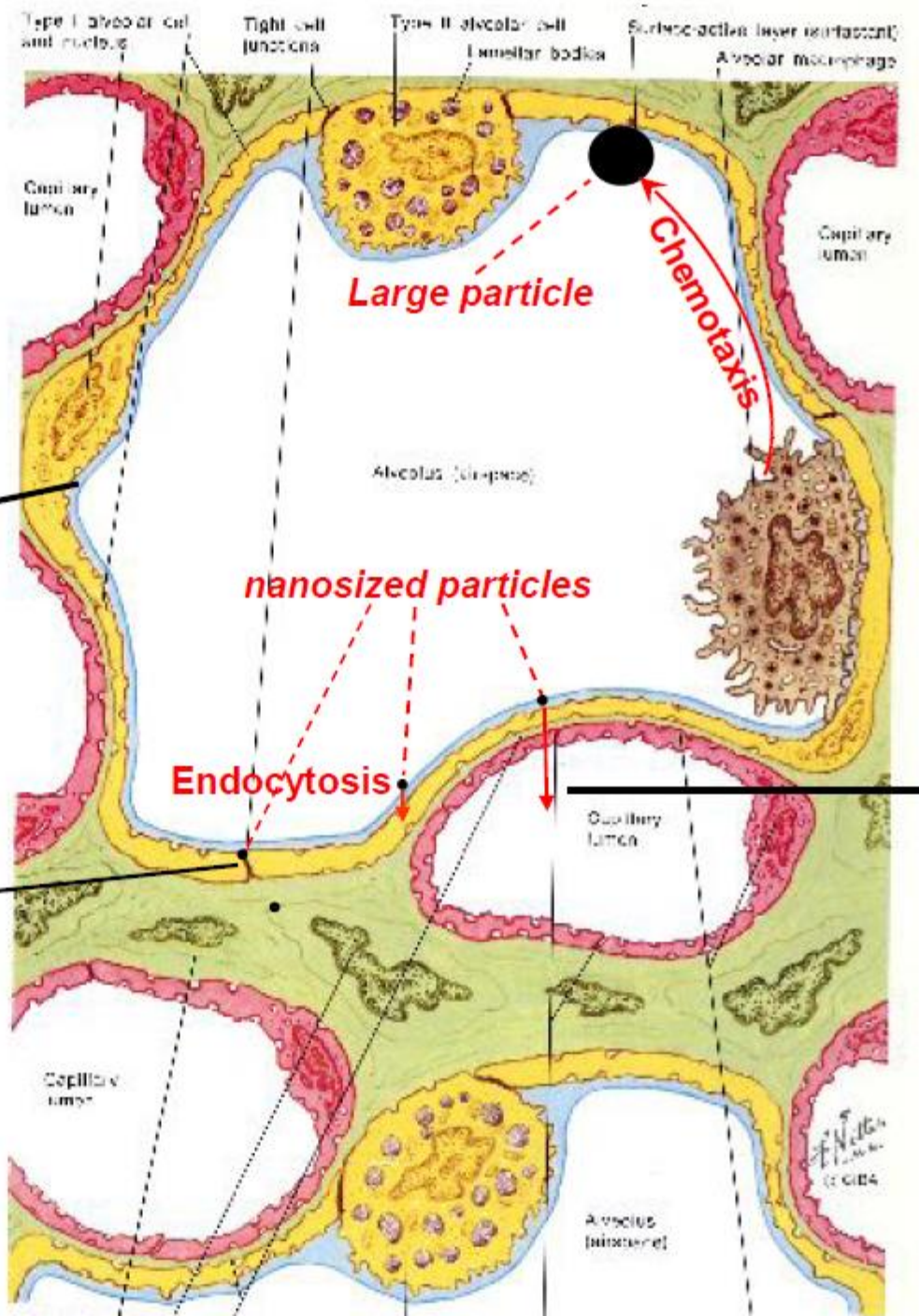








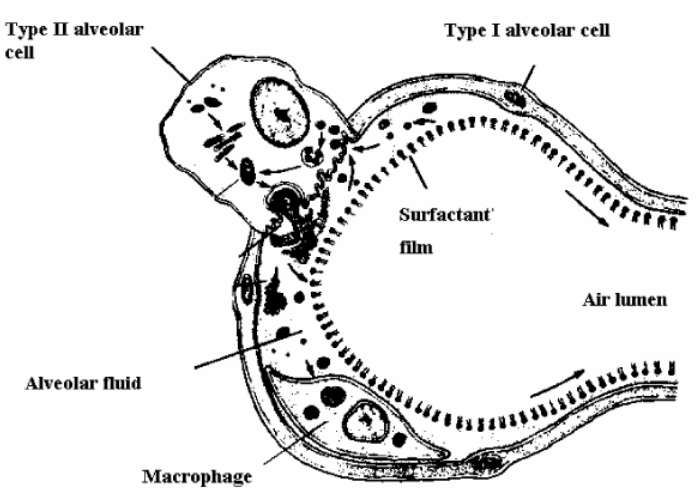
Ultrastructure of Pulmonary Alveoli and Capillaries

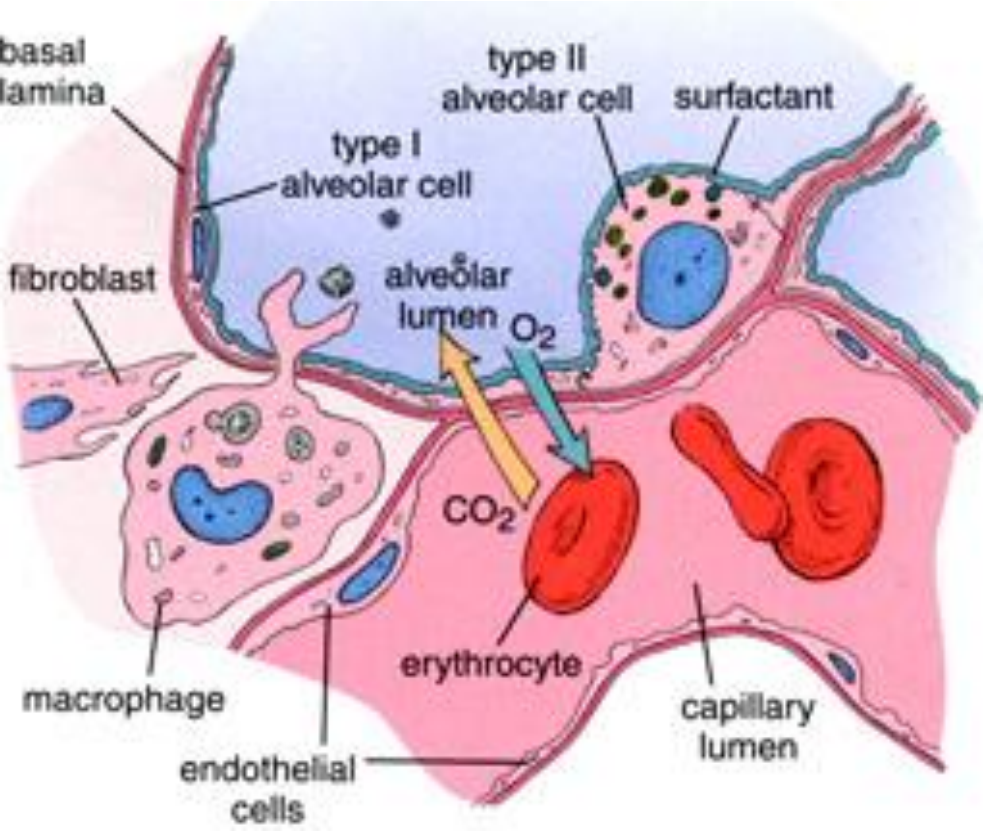


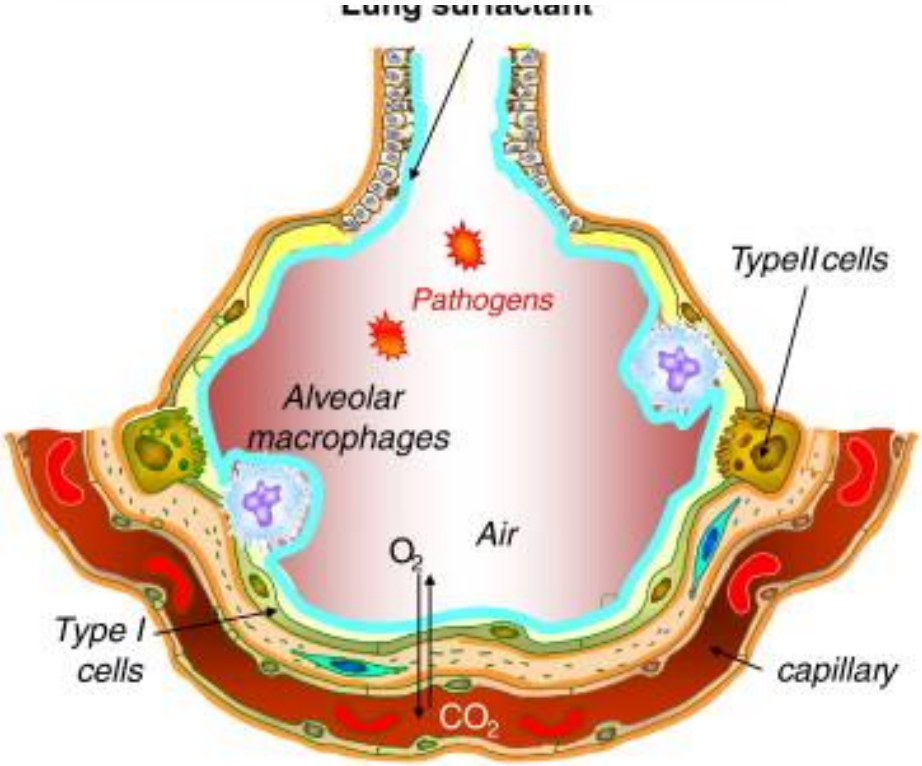
Surfactant layer:
Phospholipids, proteins

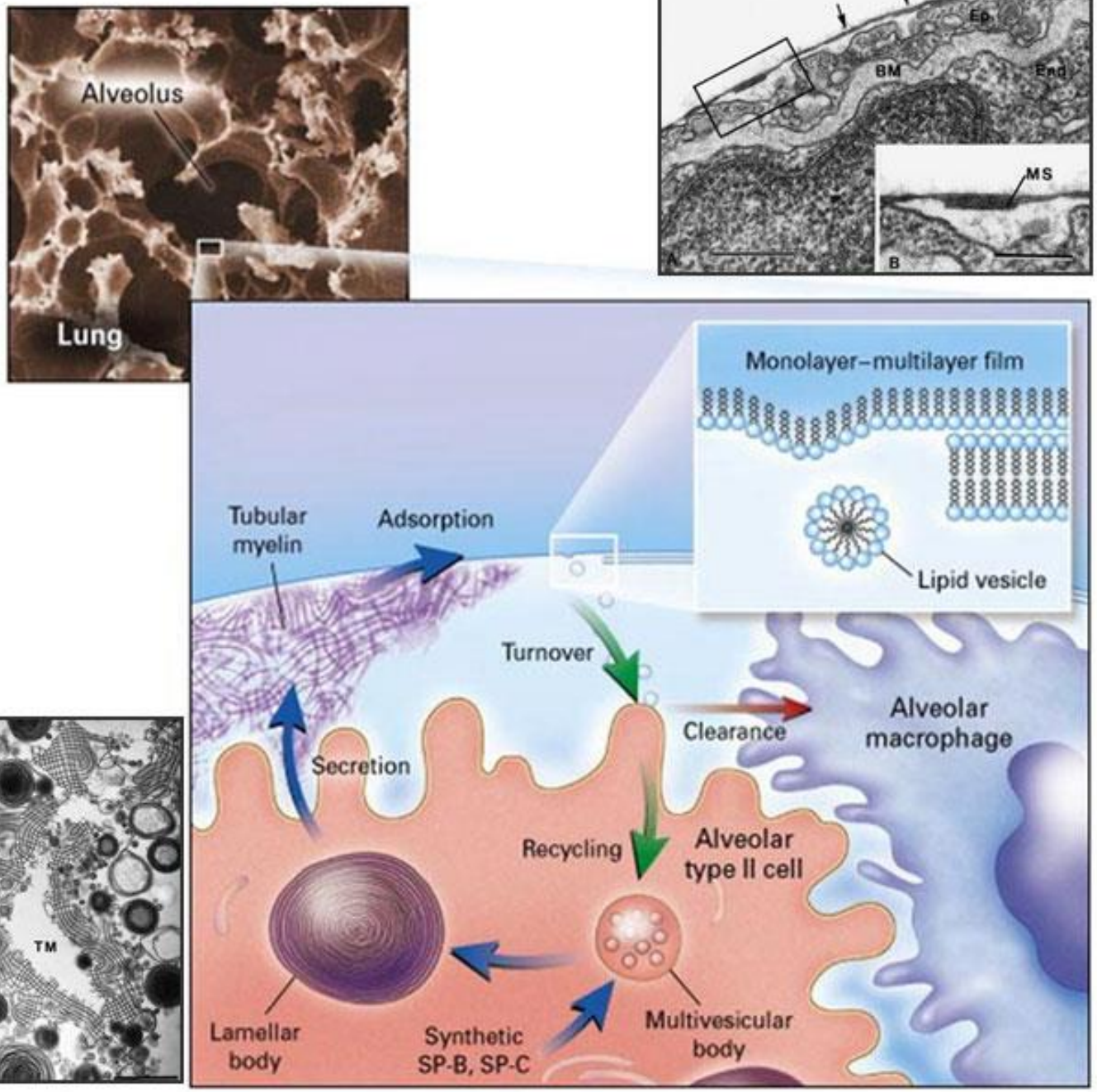
Tight junction
(paracellular)
Translocation

Transcell
Transloca

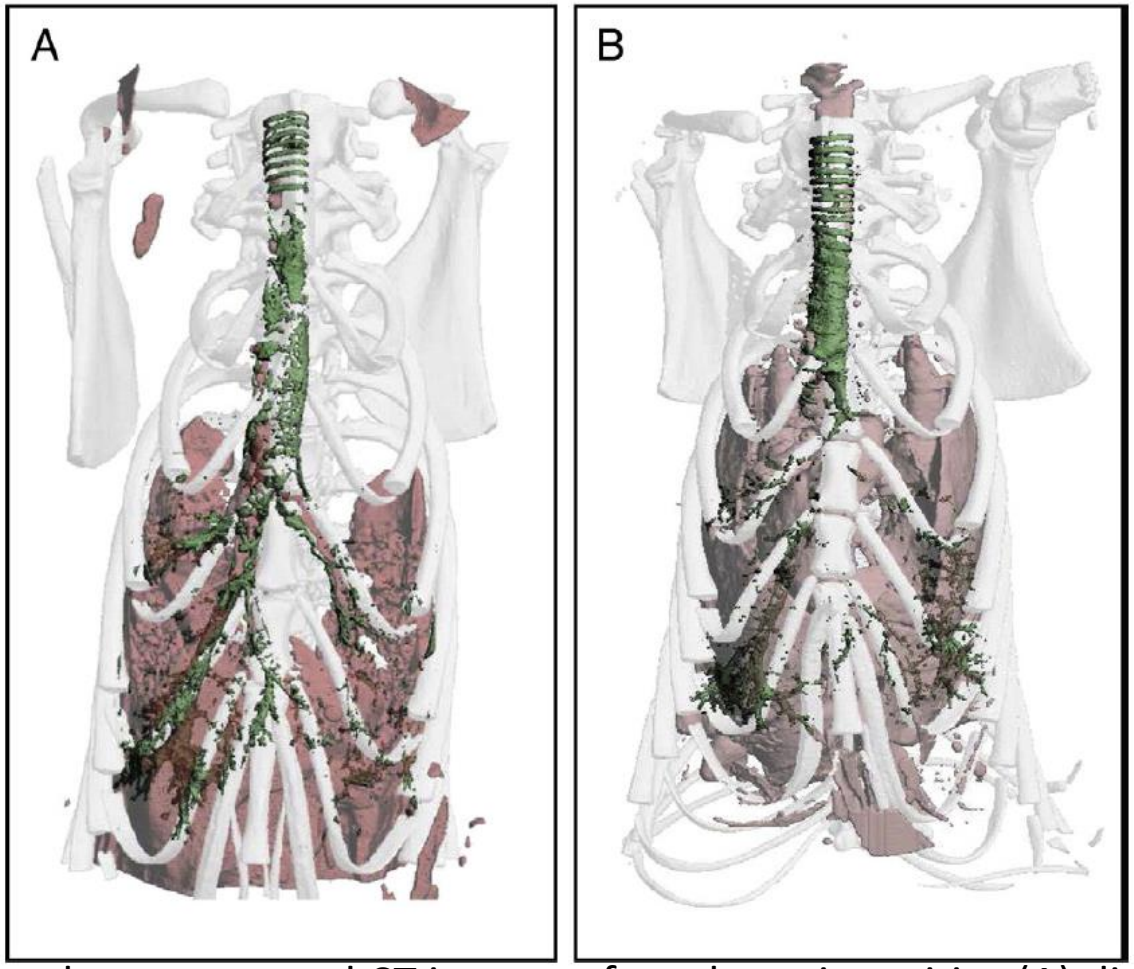

Type II alveolar cell
Type I alveolar cell
Surfactant film
Air lumen
Alveolar fluid
Macrophage


basal lamina
type II alveolar cell
surfactant
type I alveolar cell
fibroblast
alveolar lumen
O_2
CO_2
erythrocyte
macrophage
endothelial cells
capillary lumen


Pathogens
TypeII cells
Alveolar macrophages
Air
O_2
Type I cells
capillary
CO_2

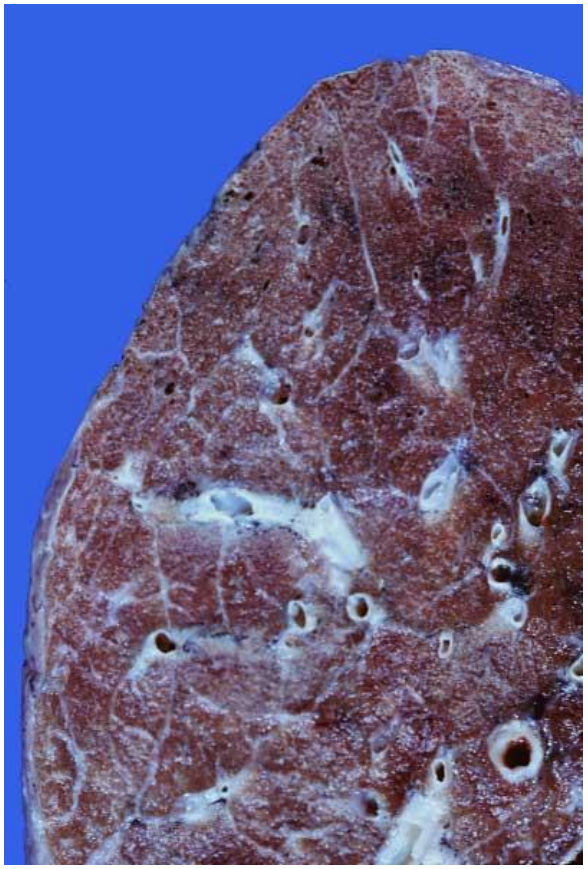

Alveolus
Lung
Ep
BM
End
MS
A
B
Monolayer–multilayer film
Lipid vesicle
Tubular myelin
Adsorption
Turnover
Clearance
Alveolar macrophage
Secretion
Recycling
Alveolar type II cell
Lamellar body
Synthetic SP-B, SP-C
Multivesicular body
LB
TM

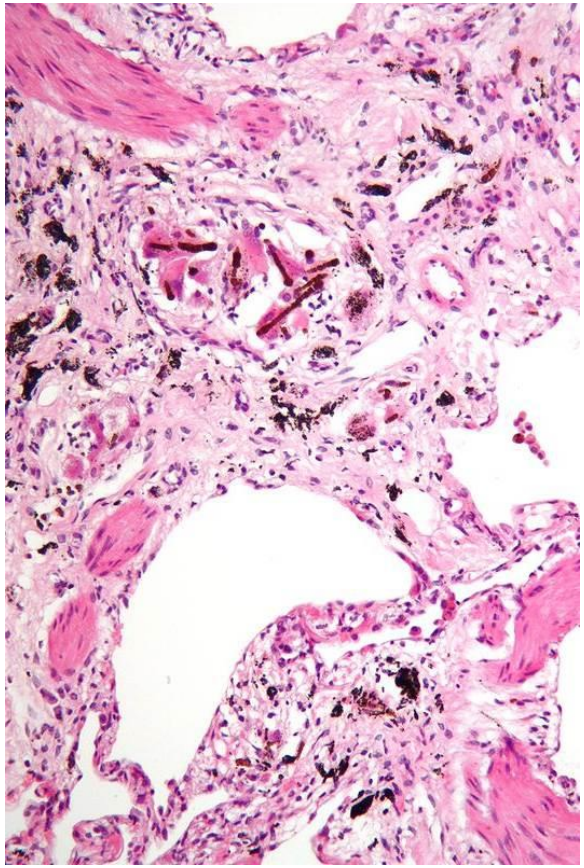
Deposition of NP in respiratory tract

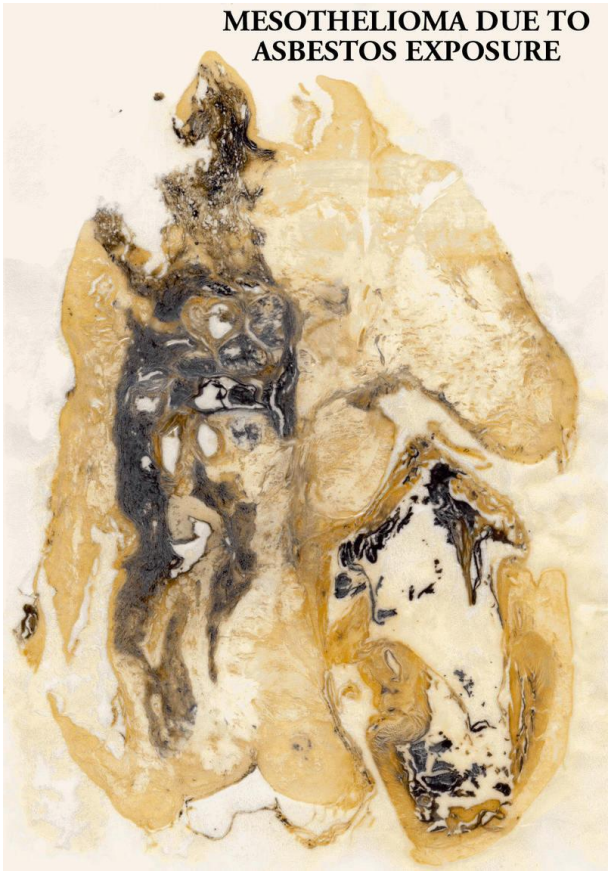


Three-dimensional reconstructed CT images of rat thoracic cavities (A) directly and (B) 2 hours after insufflation of nanocluster dry powder. Note that directly after insufflation, visualization of the respiratory bronchioles and some alveolar structures in the lung periphery is evident (A). After 2 hours the nanoclusters are cleared from the central airways, with contrast more localized to the trachea or lung periphery. Nanoclusters (green); soft tissue (pink).

Toxic effect of asbestos







Carbon nanotubes

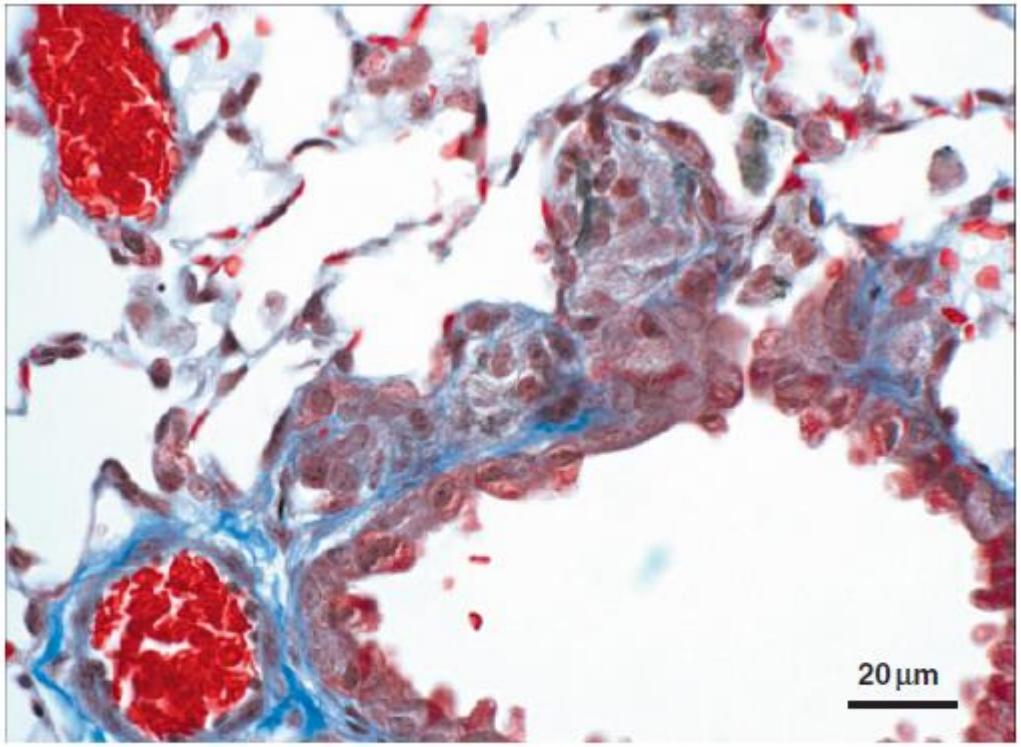


Fig. 2. Representative image of lung section from the SWCNT inhalation study (5 mg/m^3 5 h/day, 4 days) depicting granuloma formation on day 28 post treatment. Fibrosis i indicated by blue staining in this Masson's Trichrome stained section of the lung.

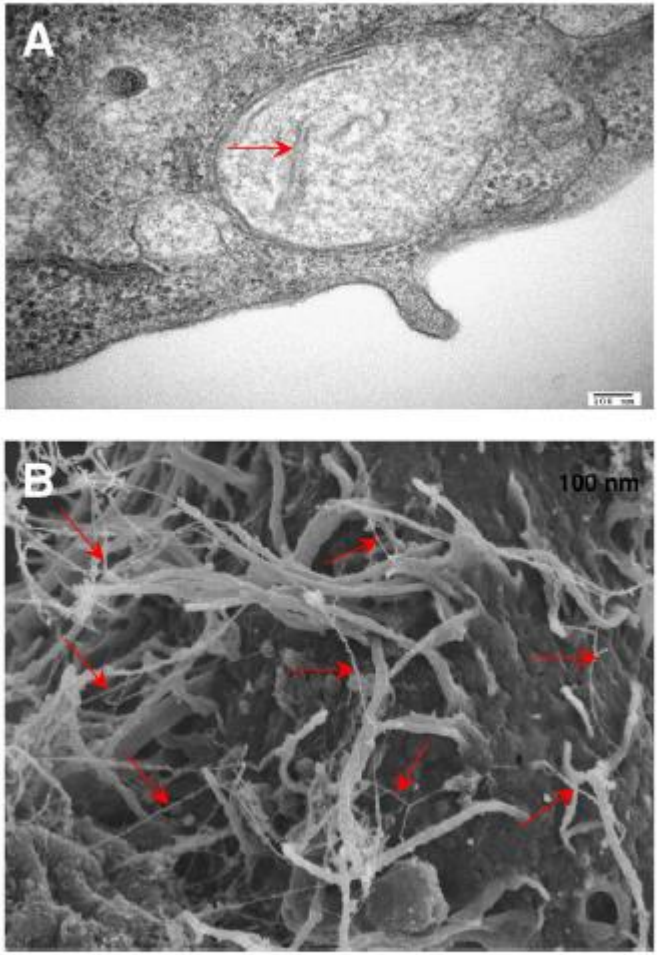
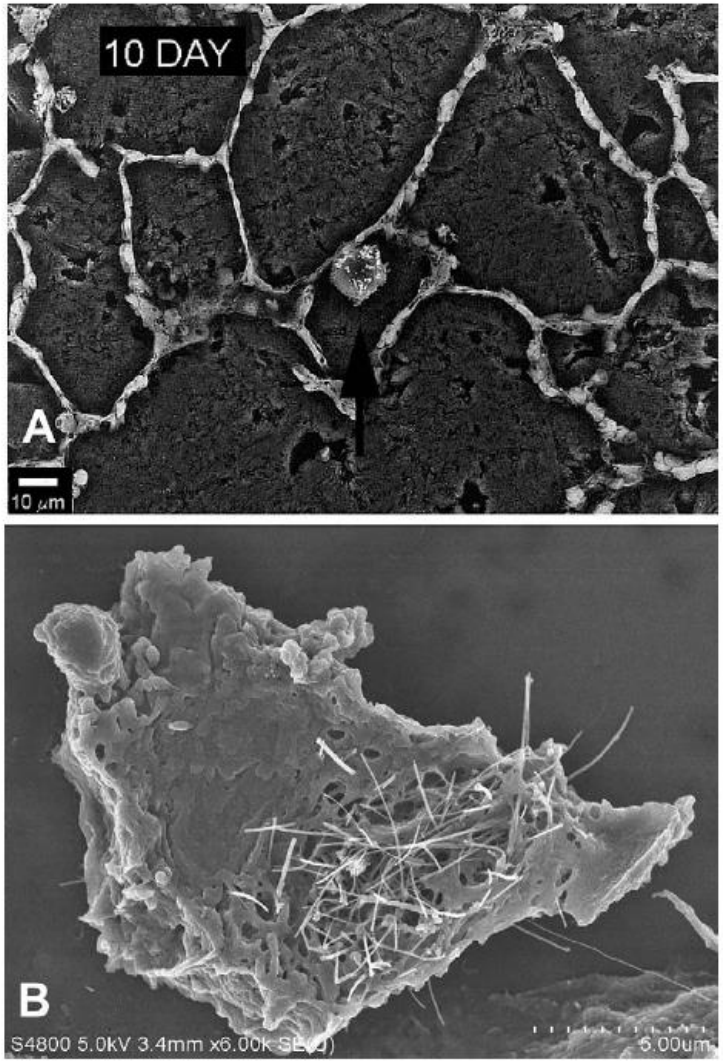
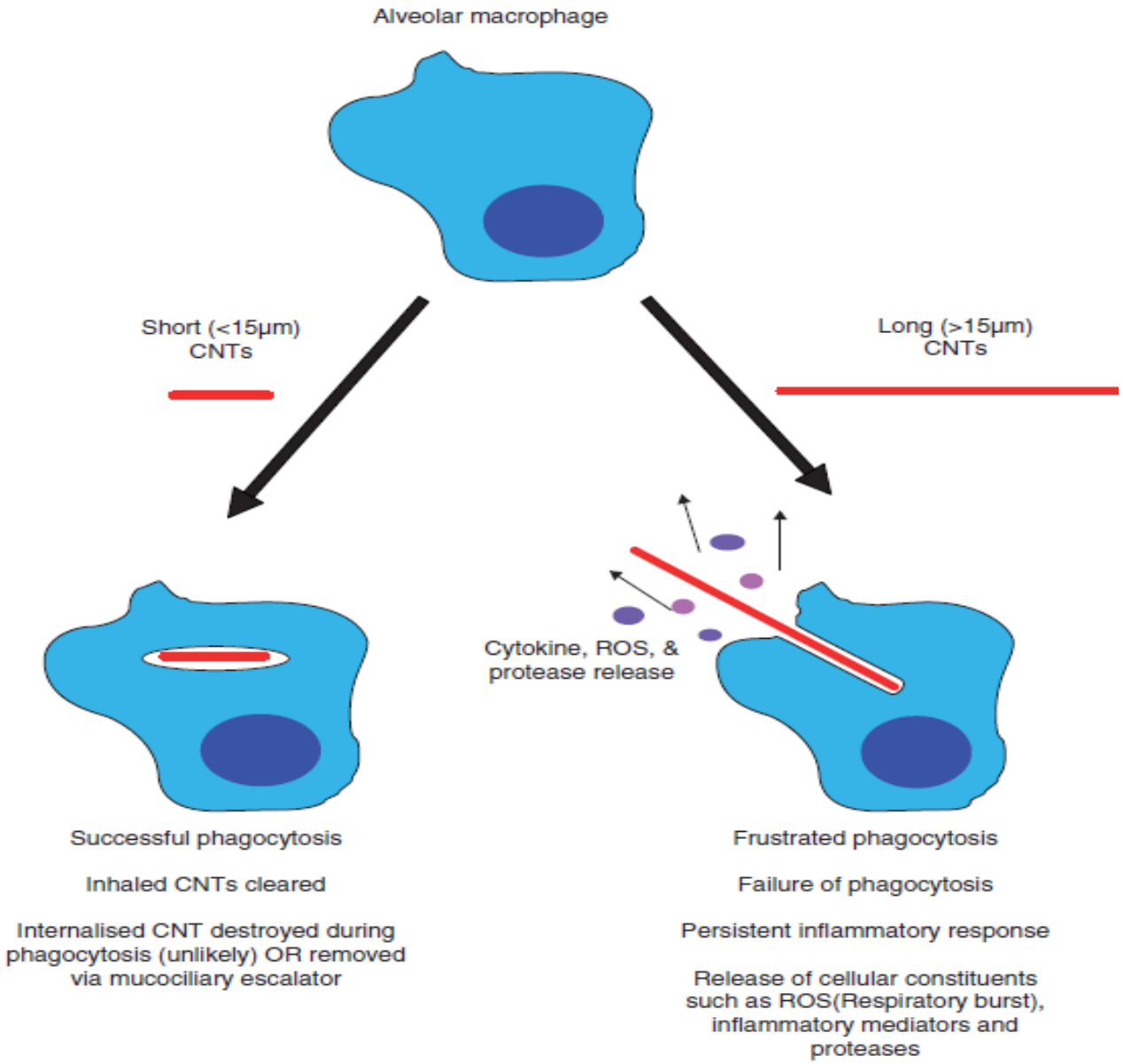


Fig. 1. Representative transmission electron (**A**) and scanning electron (**B**) micrographs of RAW264.7 macrophages with engulfed PS-coated SWCNT. RAW264.7 macrophages (0.3×10^6 cells/ml) were incubated for 2 h with PS-coated SWCNT. Arrows indicate SWCNT.

Scanning electron micrographs of particulates in tissue section. (A) Backscatter SEM image of lung section 10 days after silica inhalation. An alveolar macrophage loaded with fine-sized silica particles is identified by the arrow.



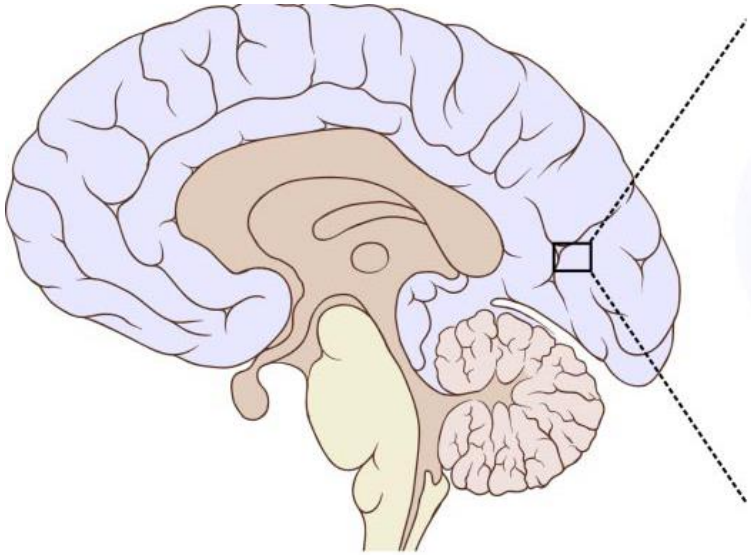

Alveolar macrophage
Short (<15μm) CNTs
Long (>15μm) CNTs
Cytokine, ROS, & protease release
Successful phagocytosis
Inhaled CNTs cleared
Internalised CNT destroyed during phagocytosis (unlikely) OR removed via mucociliary escalator
Frustrated phagocytosis
Failure of phagocytosis
Persistent inflammatory response
Release of cellular constituents such as ROS(Respiratory burst), inflammatory mediators and proteases

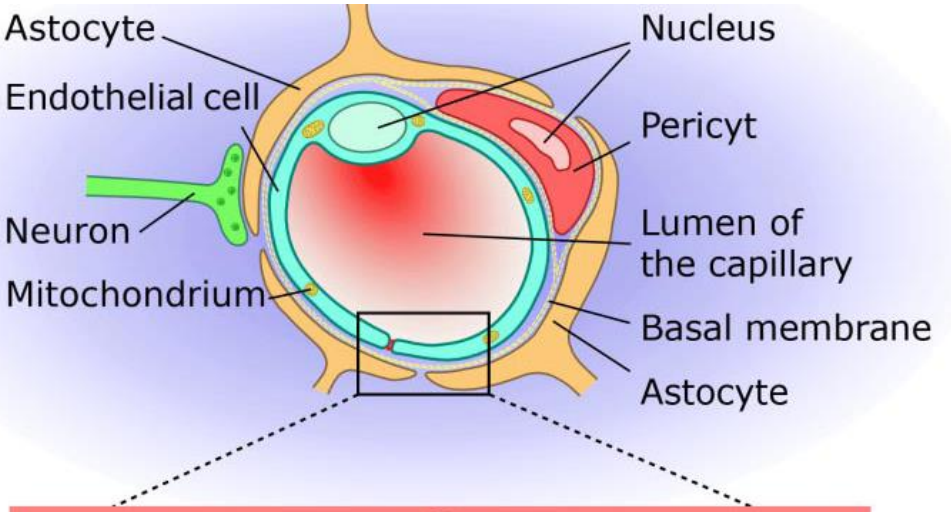
Uptake of Nanoparticles via the Olfactory Nerve: Bypassing the Blood–Brain Barrier

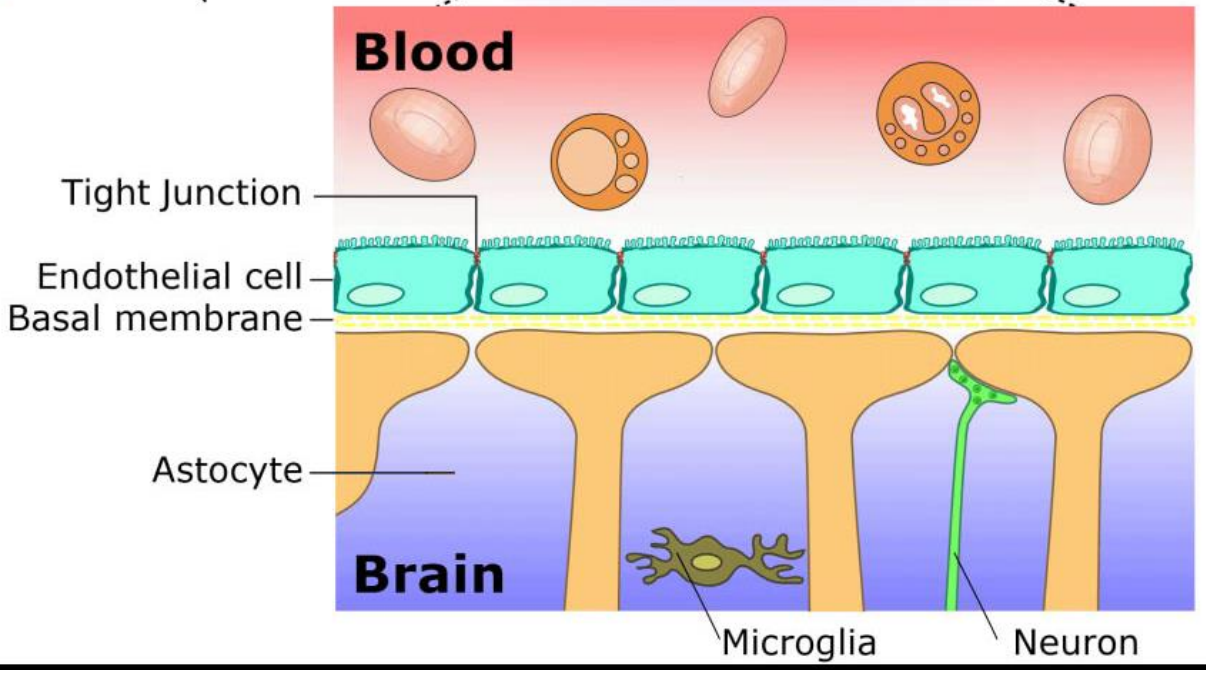
Another quite significant uptake pathway is available to nanoobjects owing to their small sizes. The particles can be incorporated via the nerve fibers in the area of the olfactory epithelium. Instillation/inhalation tests on rodents using different particles have demonstrated that nanoscale carbon particles, gold particles, manganese oxide particles, and others are conveyed by transsynaptic transport.

Nanoparticles can reach the brain directly by passing the olfactory epithelium and the nervus olfactorius located in the roof of the nose. It is also conceivable that systemic uptakes take place via the *nervus trigeminus* und the sensoric nerve fibers in the tracheobronchial tract. The quantities reaching the brain via the olfactory nerve are very small; however, they bypass the blood–brain barrier.

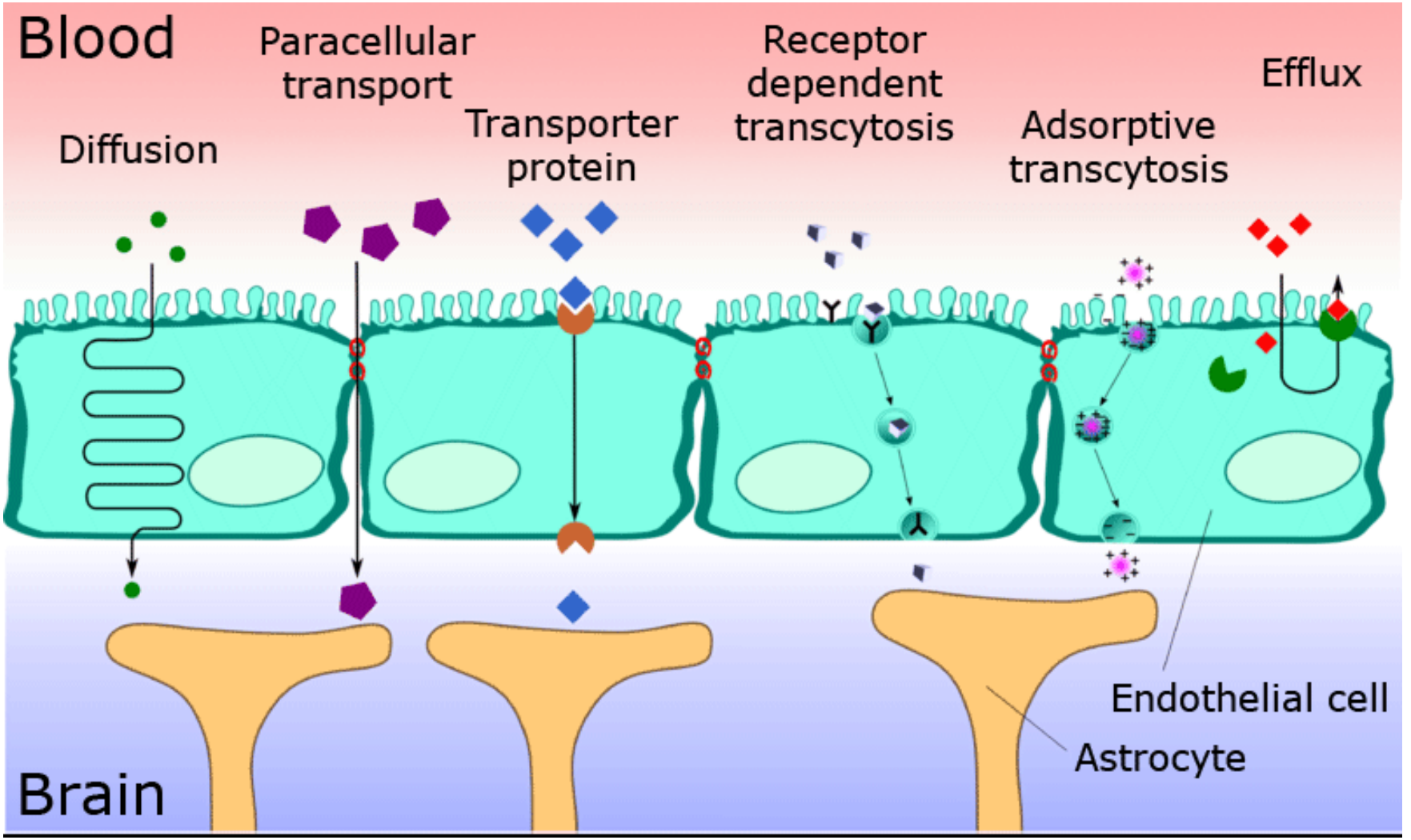
Blood-Brain Barrier



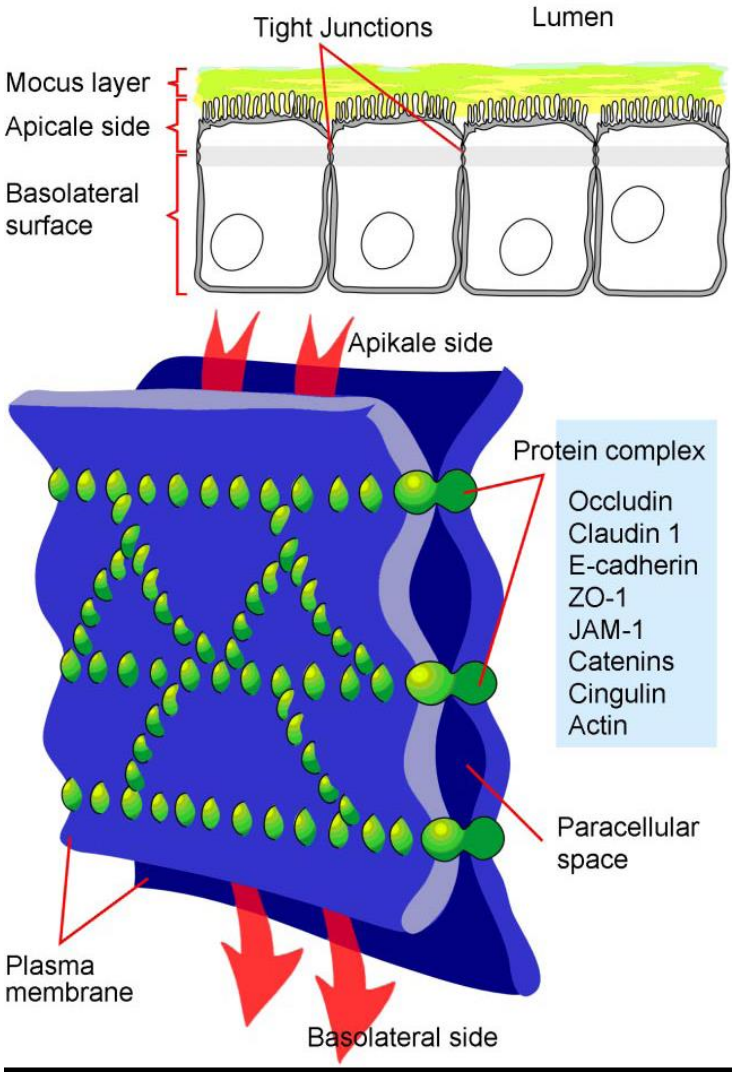


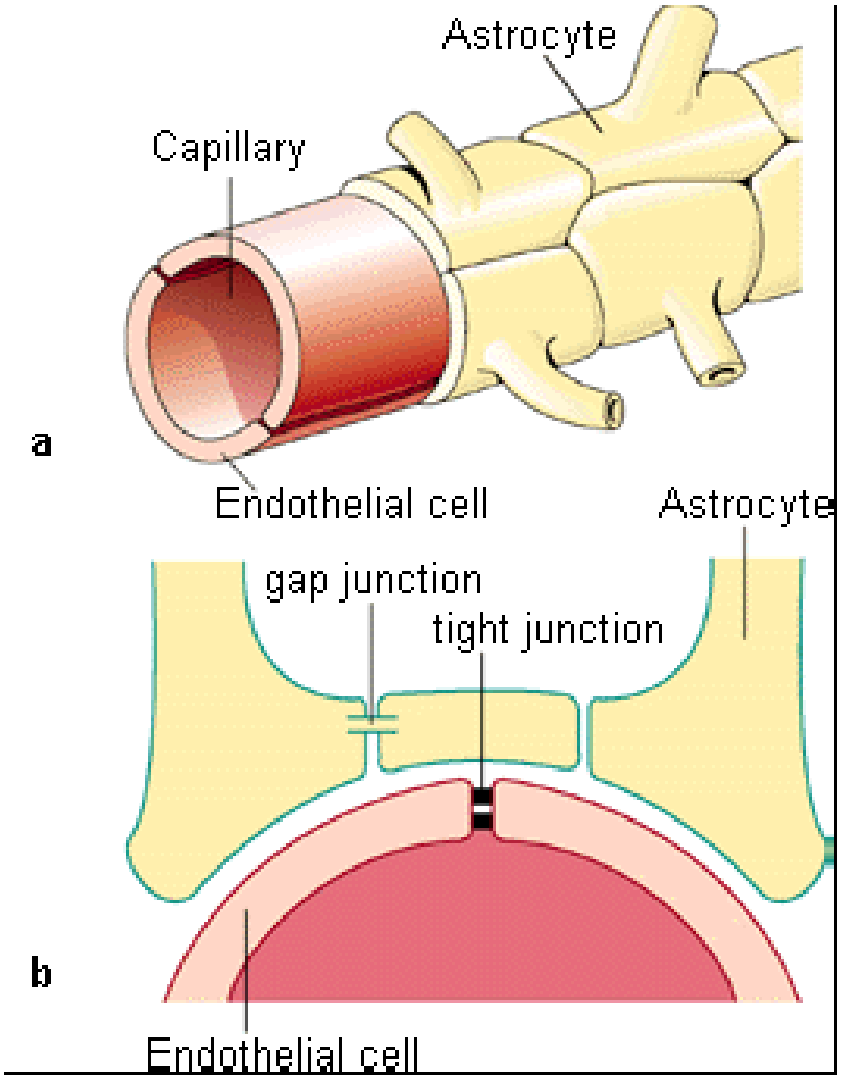


Transport through Blood-Brain Barrier

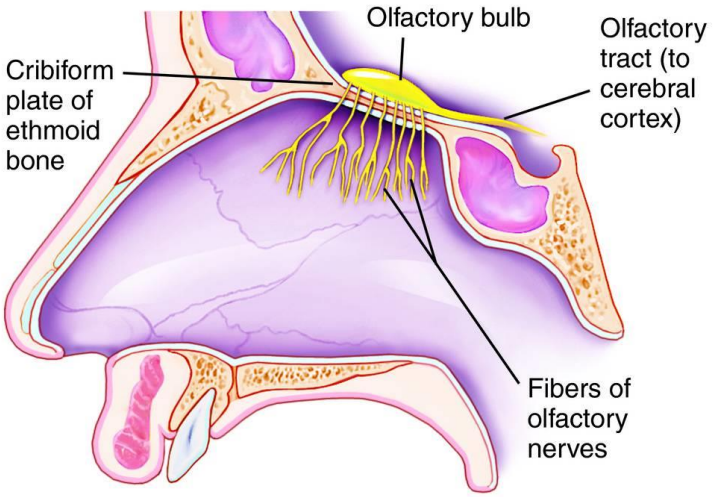


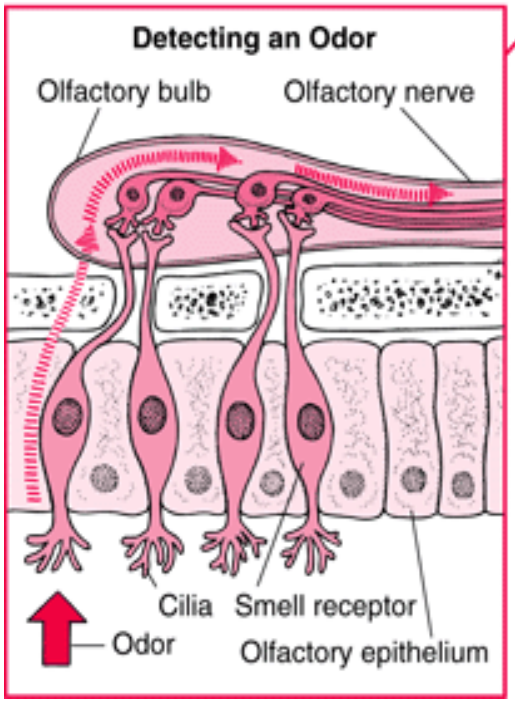
Tight junction

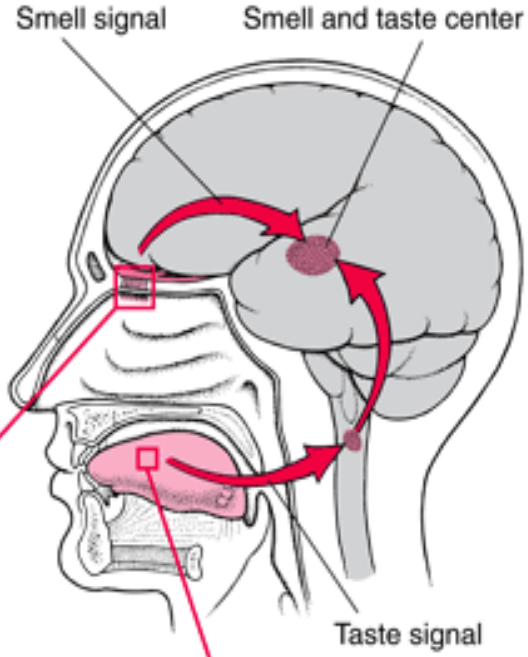


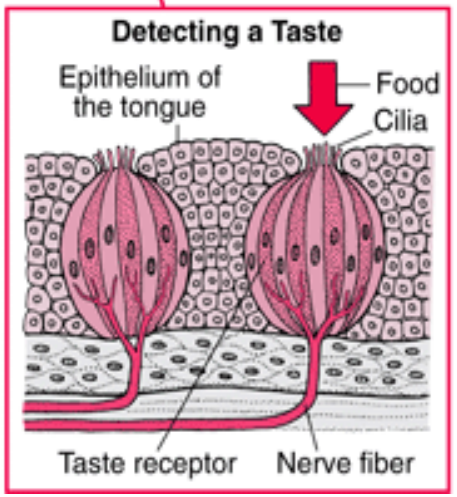


Anatomy of Olfactory bulb



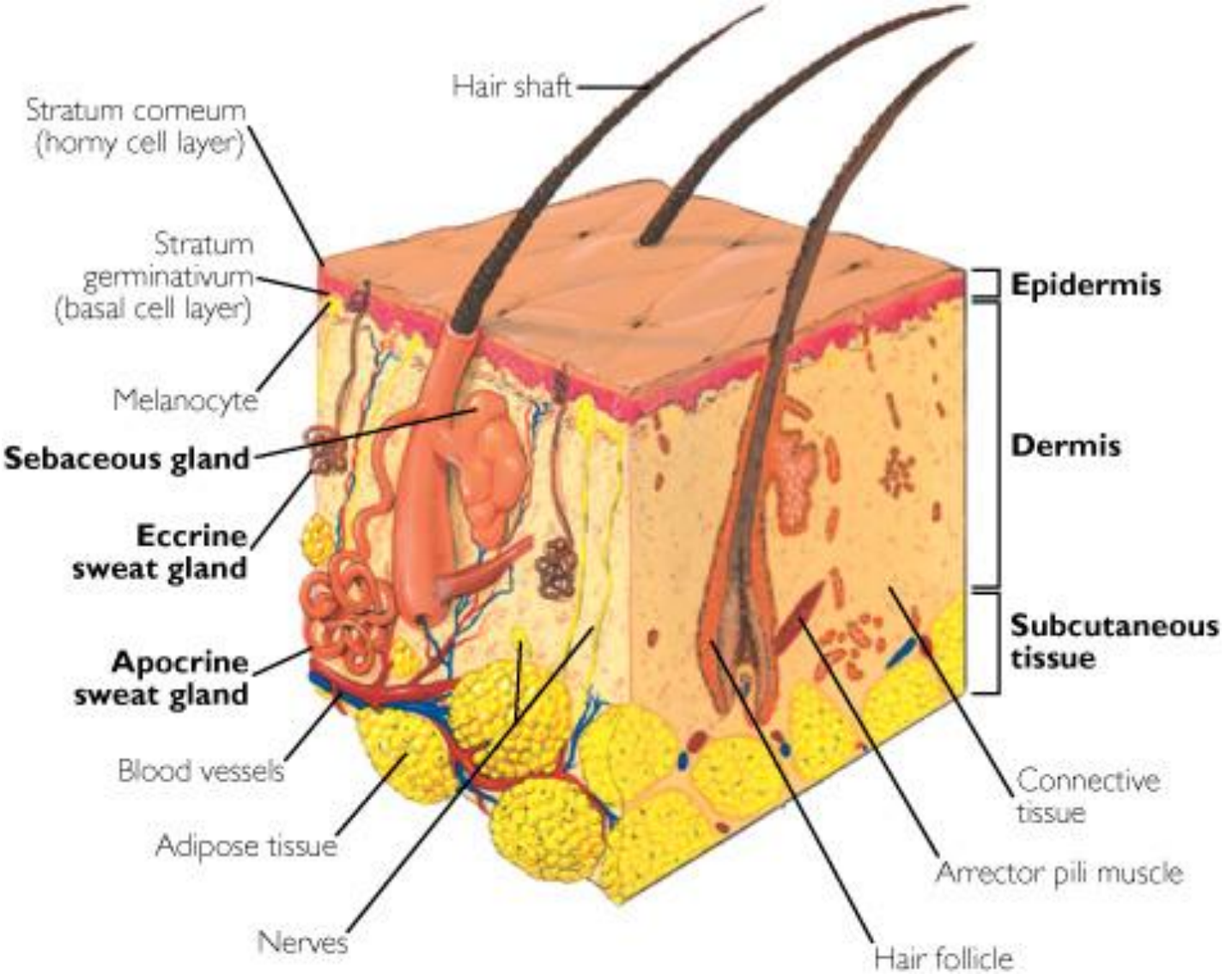




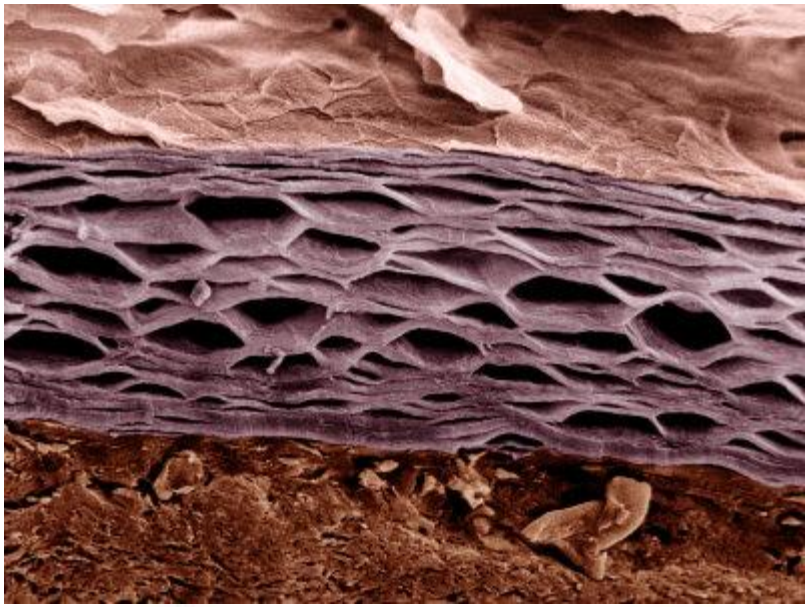


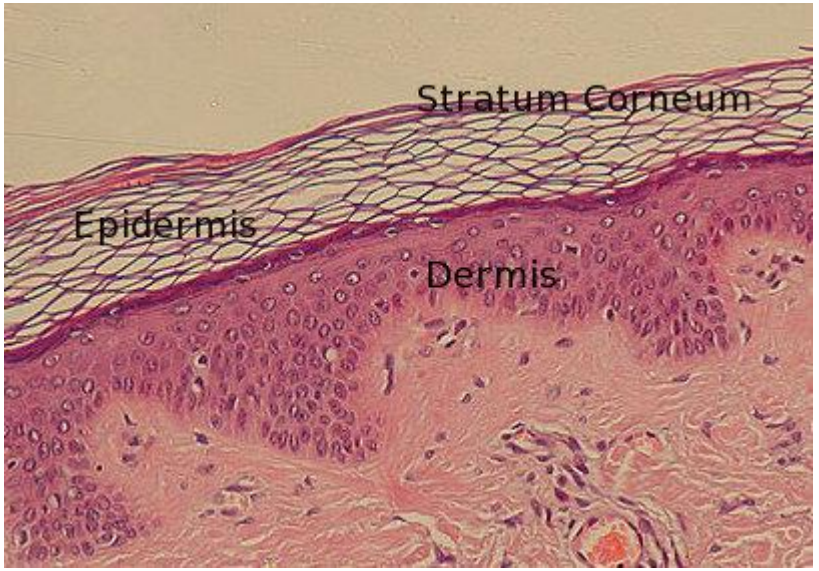
Healthy Skin: An Effective Barrier Against Many Nanomaterials

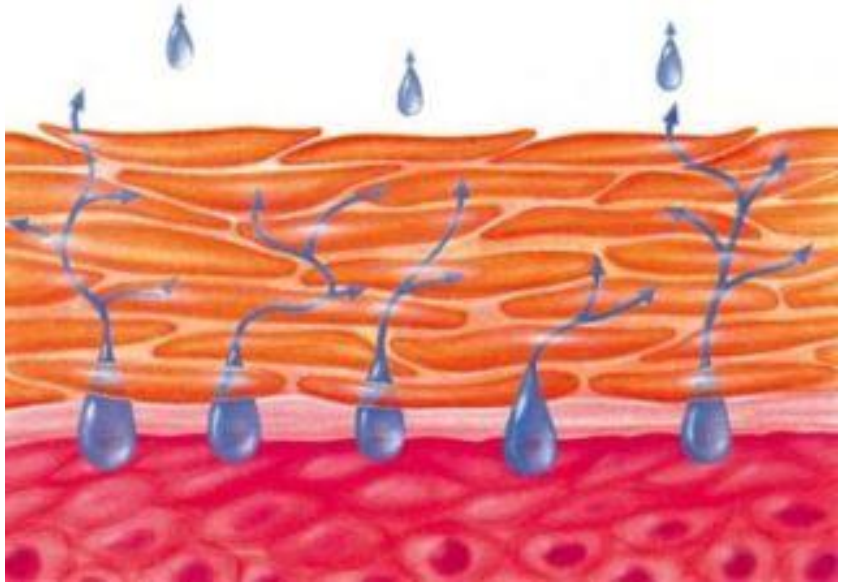
The corneal layer of stressed or diseased skin is not intact



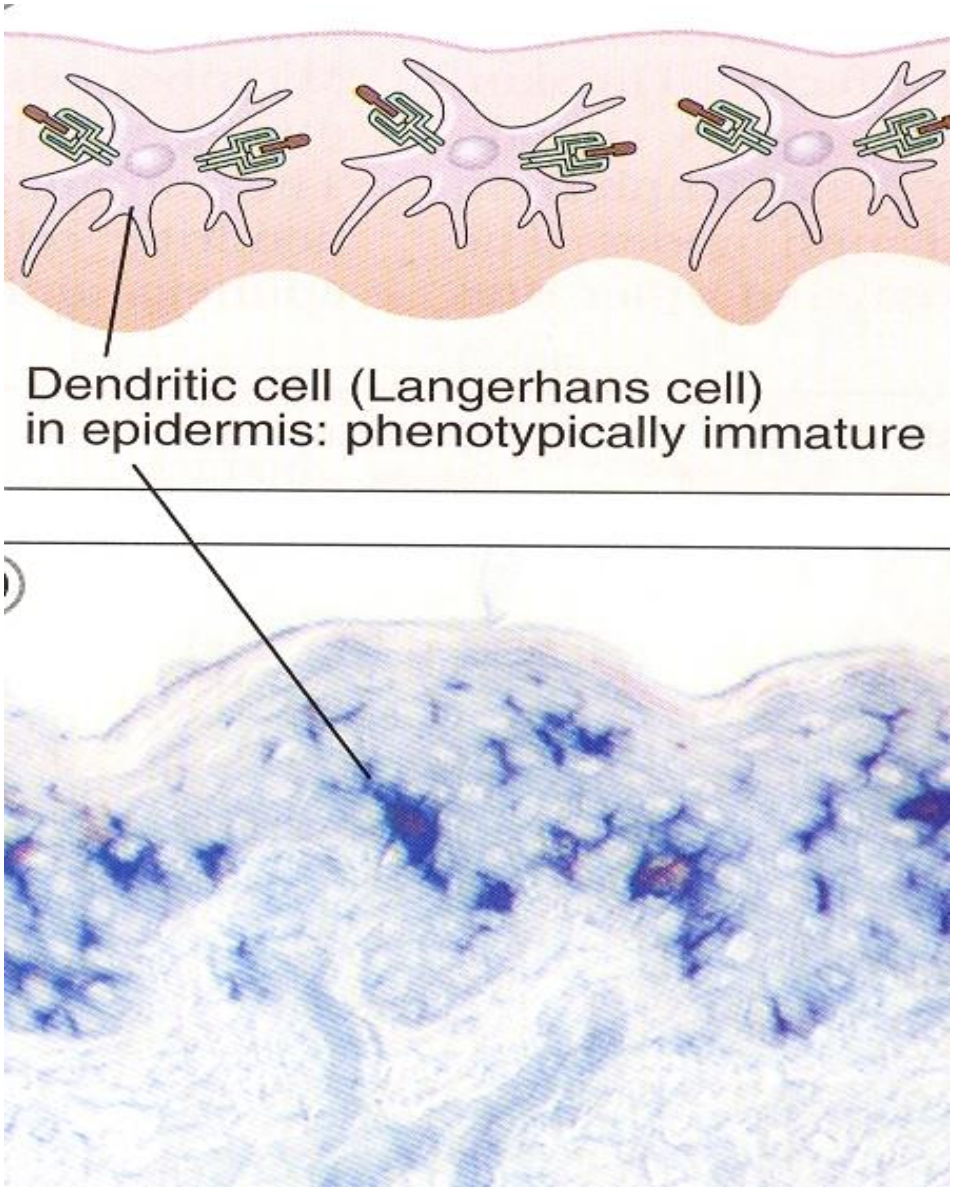
Stratum corneum





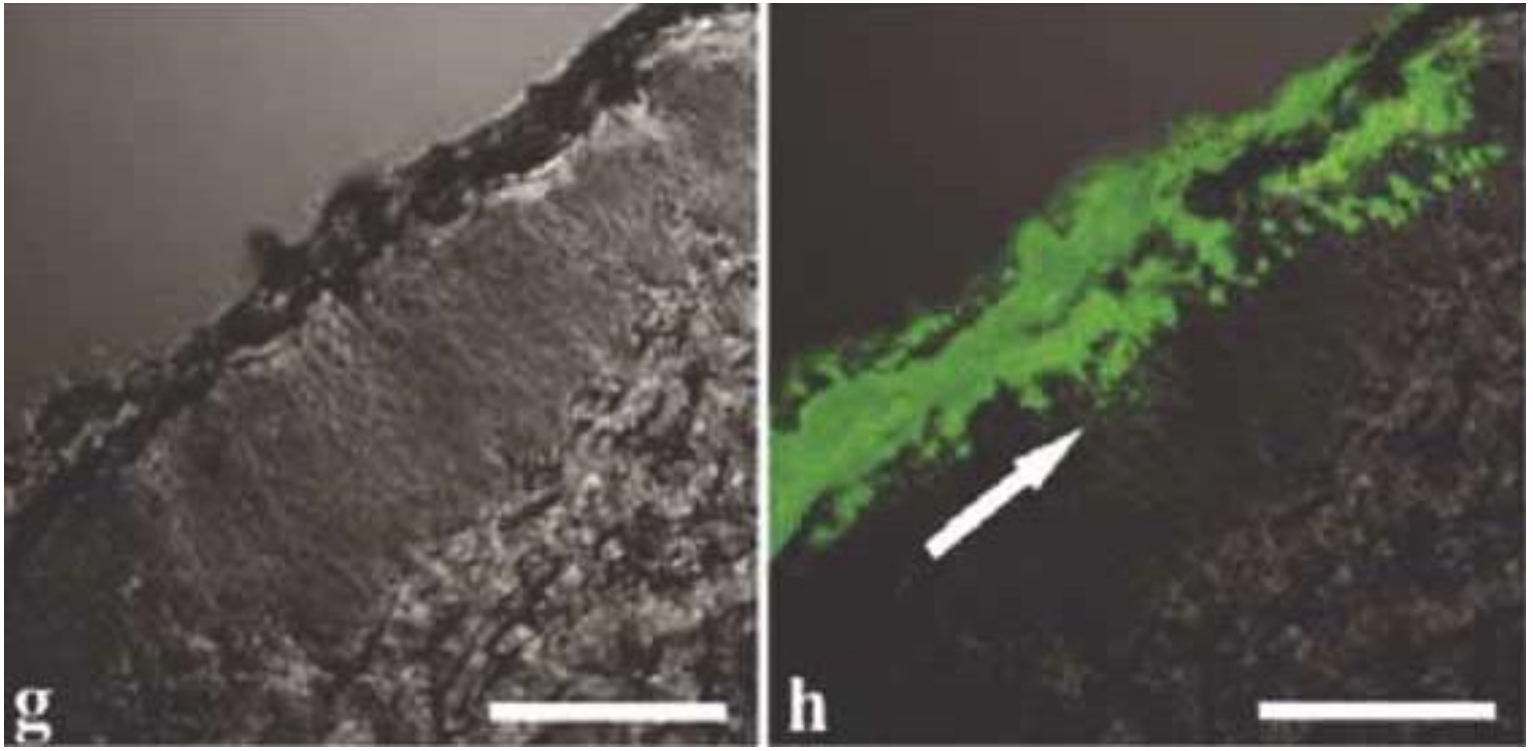


Induction of autoimmune diseases and hypersensitivity



Penetration of QDots through skin

Very small particles (<10 nm) are capable of penetrating through to the epidermis or dermis. Particle surface coatings or functionalization, which are often used to prevent agglomeration, may strongly influence the penetration.



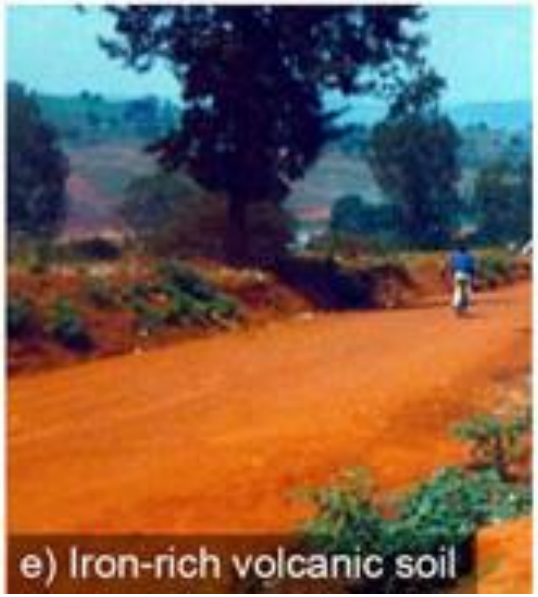
Confocal scanning microscopy of porcine skin treated with QD 565 for 8 h. (g-i) QD-COOH. Confocal-DIC overlay with the green QD fluorescence channel shows QD localization within the epidermis or dermis (arrows). Scale bars equal 50 µm.

Volcanoes and health effects

Short-term effects of ash on health include respiratory effects nose and throat irritation, and bronchitic symptoms and eye and skin irritation

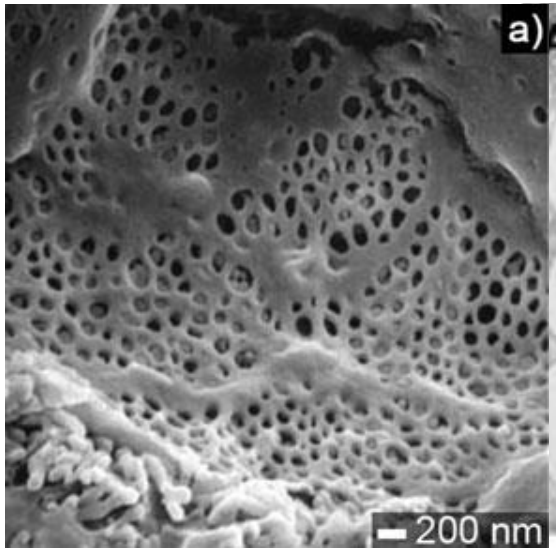
Long-term exposure to volcanic particulate pollution - the barefoot agricultural populations living in parts of the world containing volcanic soils, such as Africa, Mediterranean, and Central America. A large percentage of this population is affected by diseases of lympho-endothelial origin (Podocoinosis, Kaposi´s sarkoma) (cross-complication with AIDS)

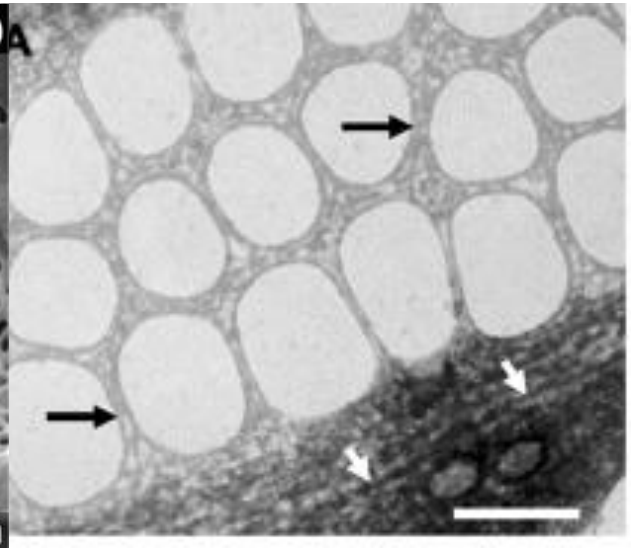

d) Podoconiosis

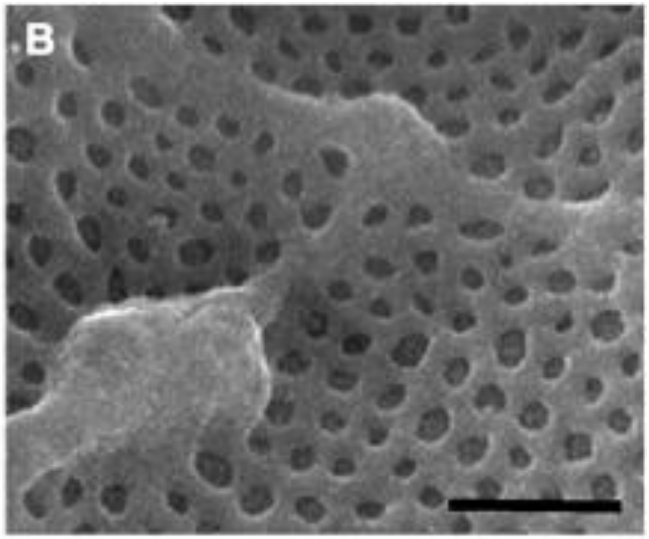

e) Iron-rich volcanic soil

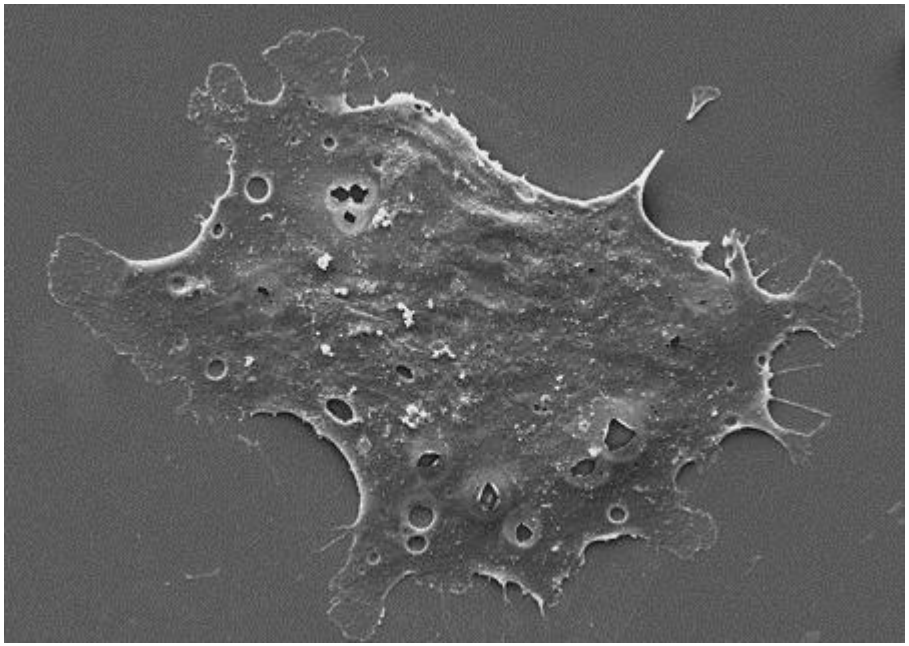

f) Kaposi's sarcoma

Liver and kidney fenestrations









Application of nanoparticles in medicine

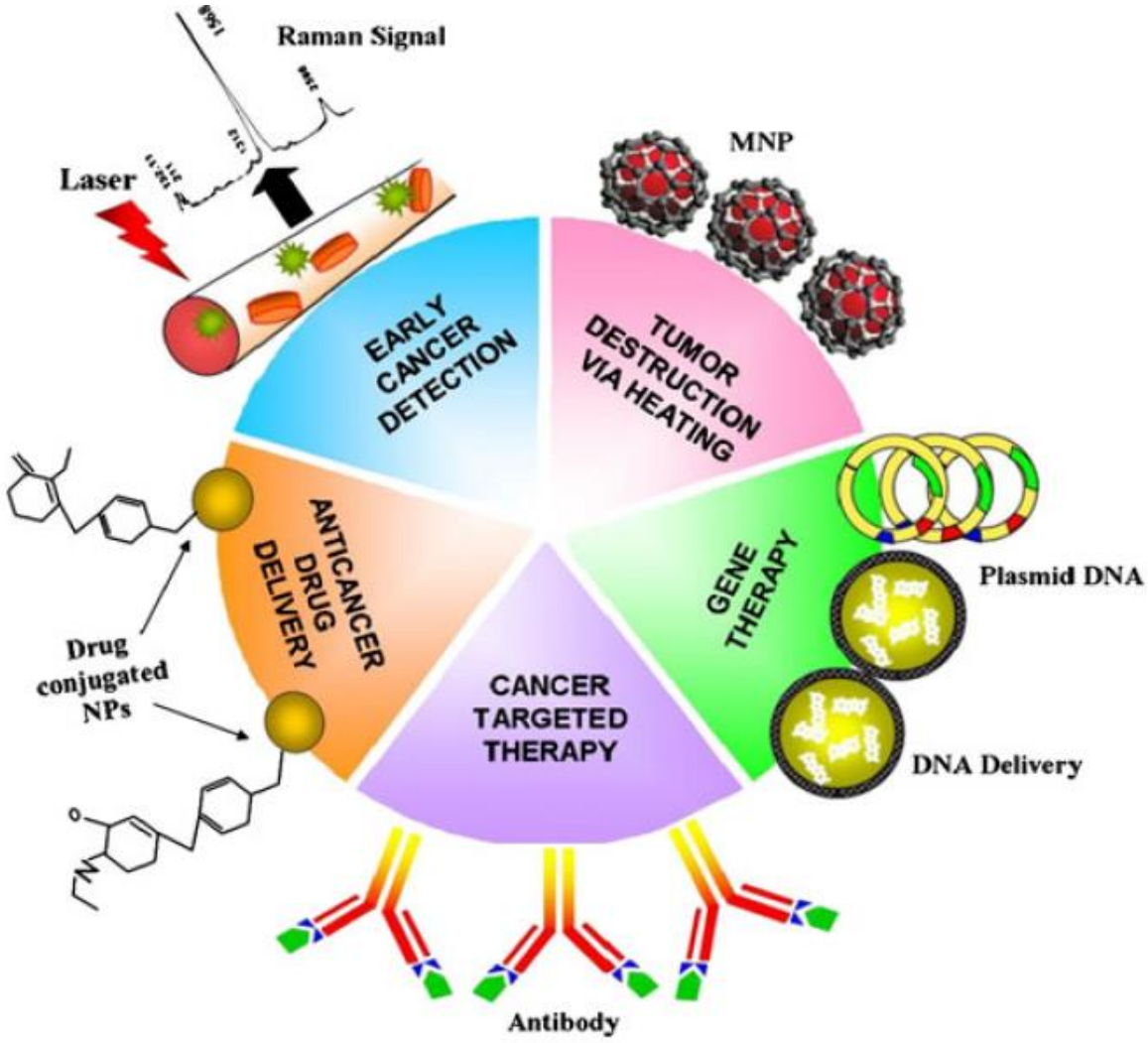
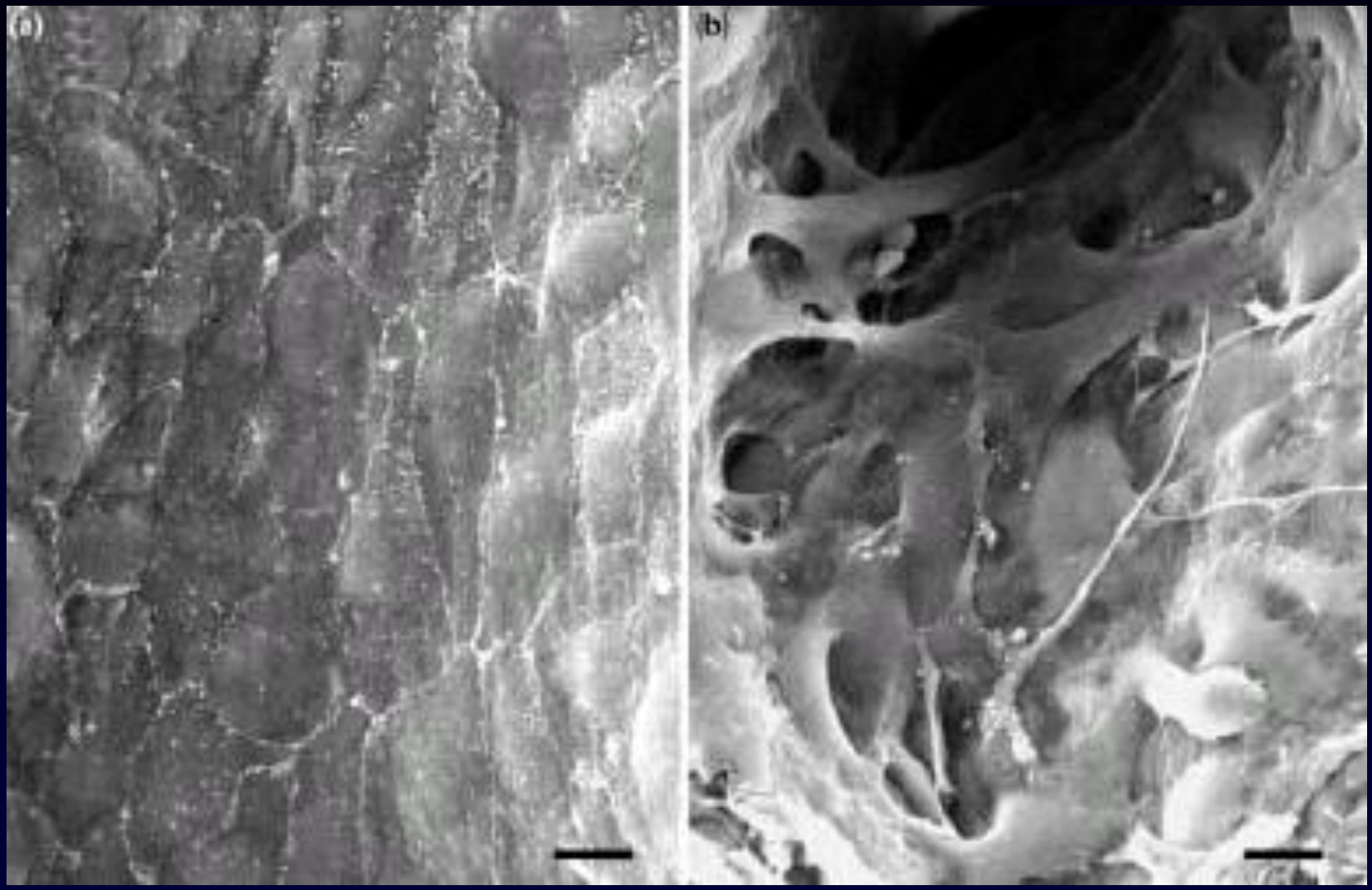
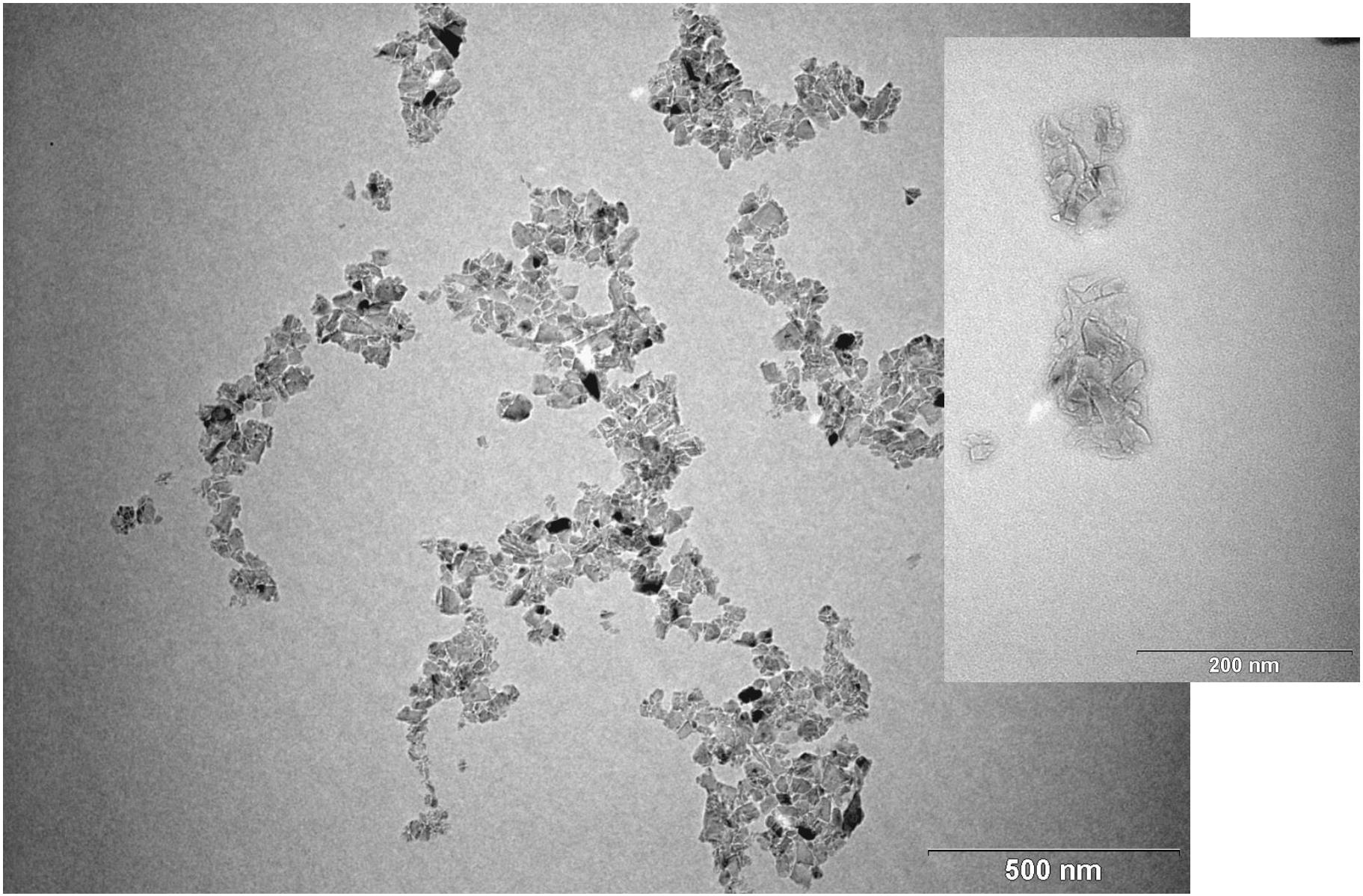


Figure 1. Schematic diagram showing nanotechnology-based approaches for cancer research related to early cancer detection, anti-cancer drug delivery, cancer-targeted therapy, gene therapy and tumor destruction via hyperthermia.

Normal vs tumour blood vessels

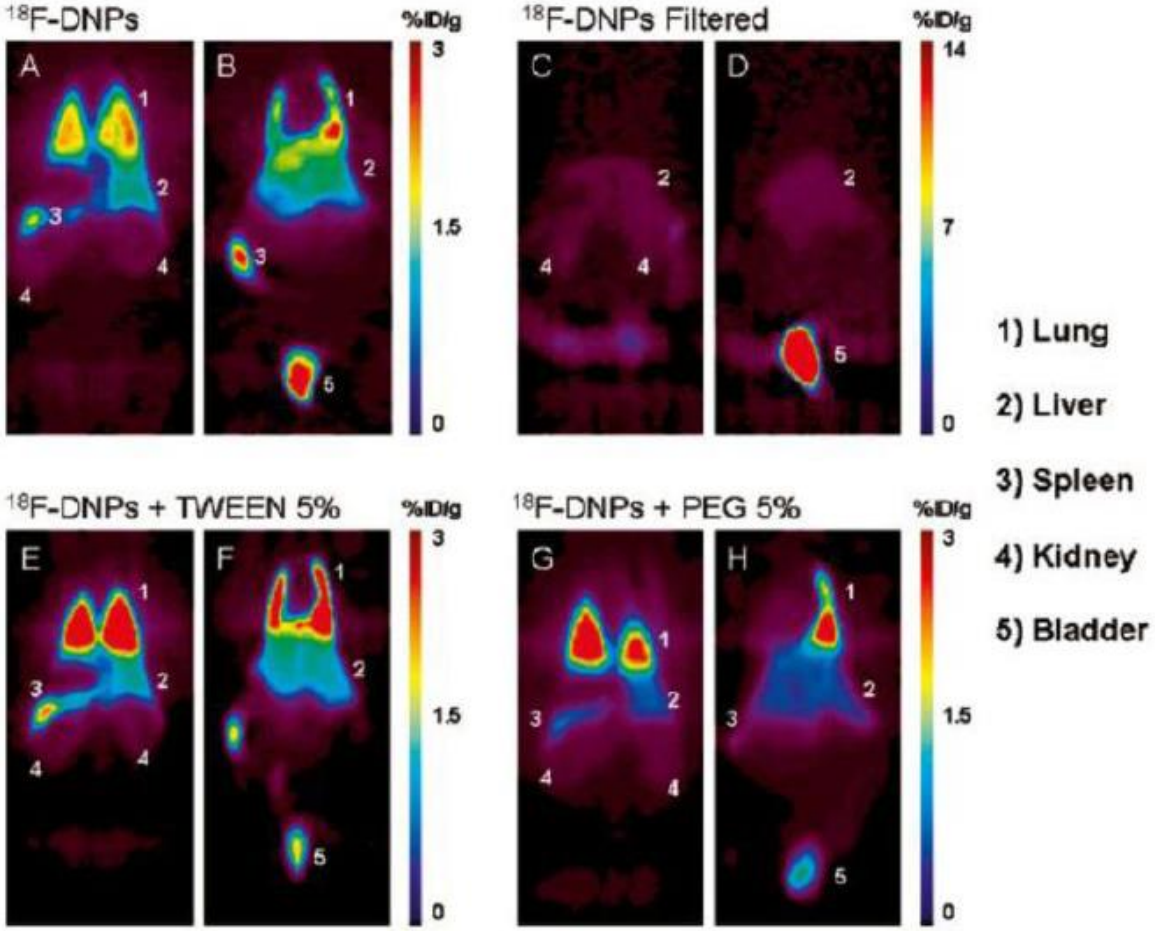


Nanodiamonds

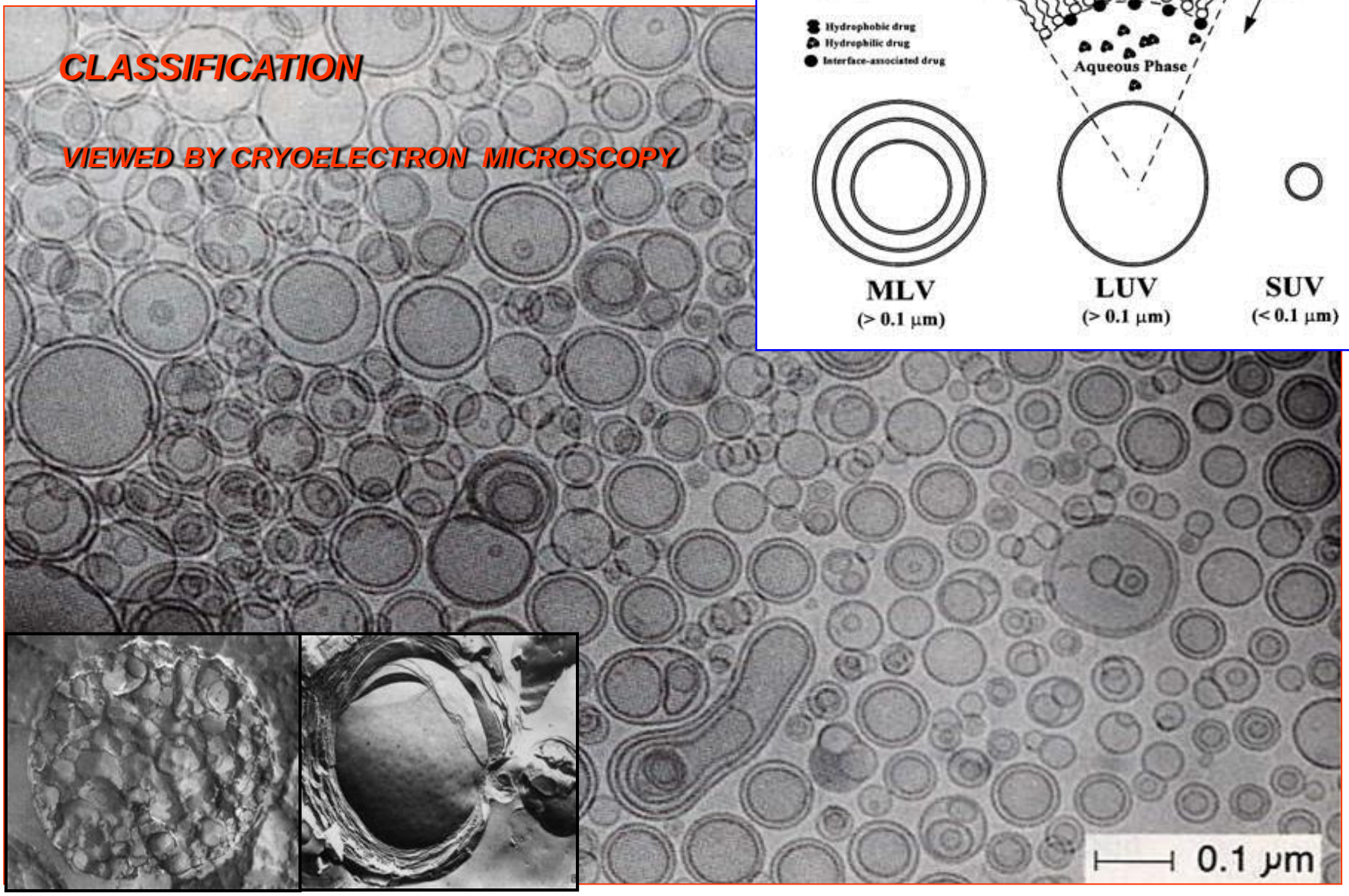


Coronal sections of PET images acquired 120 min after injection of four different ND preparations

Images show animals injected with 18F-NDs dorsal (A) to ventral (B), with filtered 18F-NDs dorsal (C) to ventral (D), with 18F-NDs + Tween 80 dorsal (E) to ventral (F), and finally with 18F-NDs + PEG8000 dorsal (G) to ventral (H). Organs that exhibited an elevated uptake of the radiolabeled compound are labeled with numbers for easy identification.

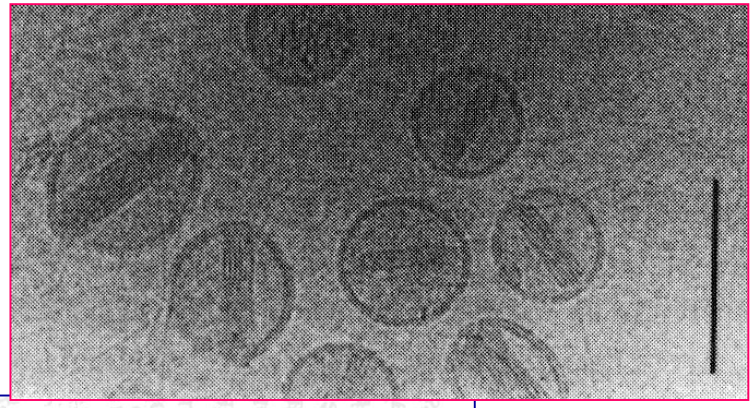


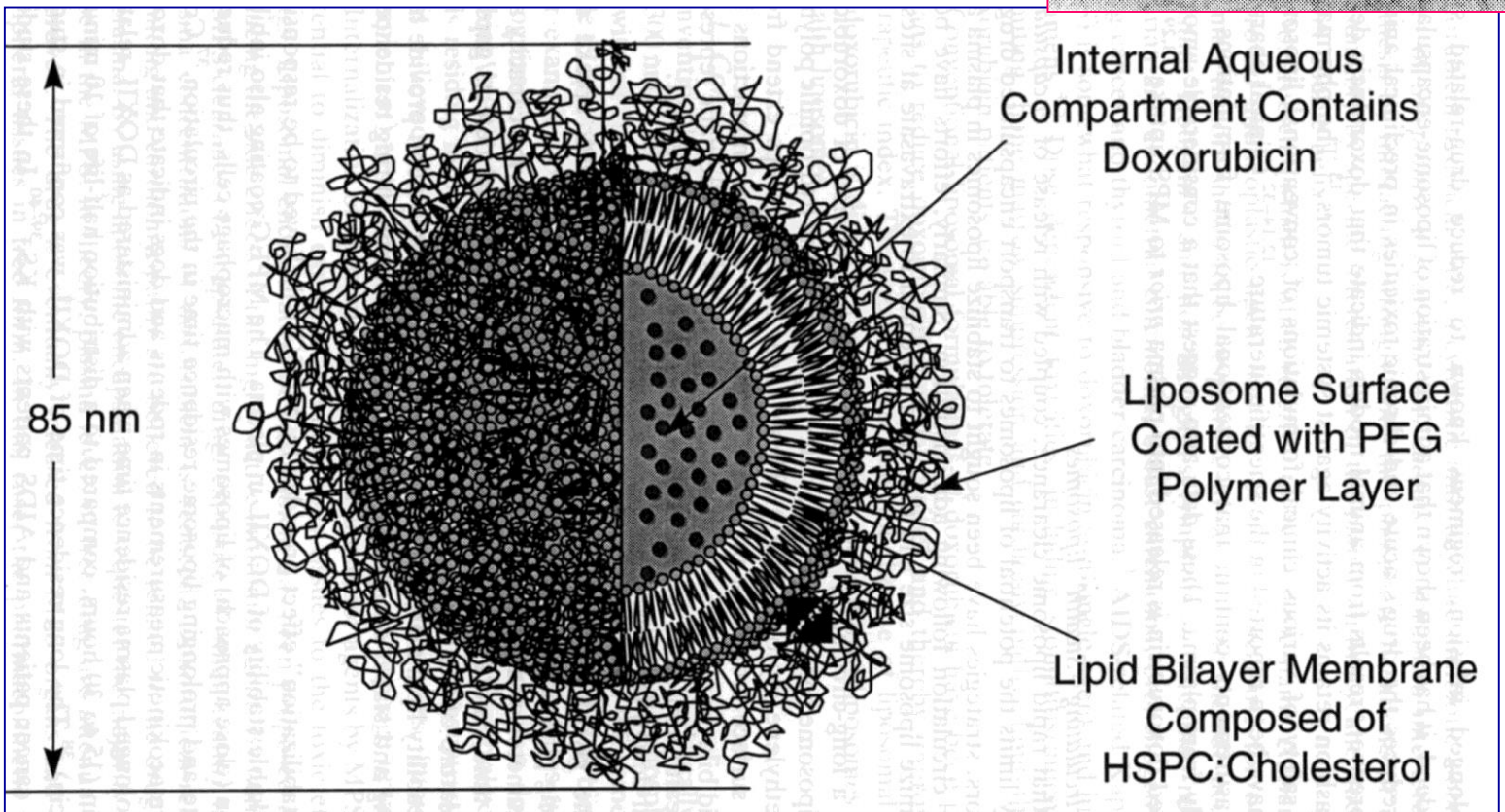
LIPOSOMES


CLASSIFICATION
VIEWED BY CRYOELECTRON MICROSCOPY
Phospholipid bilayer
40-50 A°
Hydrophobic drug
Hydrophilic drug
Interface-associated drug
Aqueous Phase
MLV
(> 0.1 μm)
LUV
(> 0.1 μm)
SUV
(< 0.1 μm)
0.1 μm

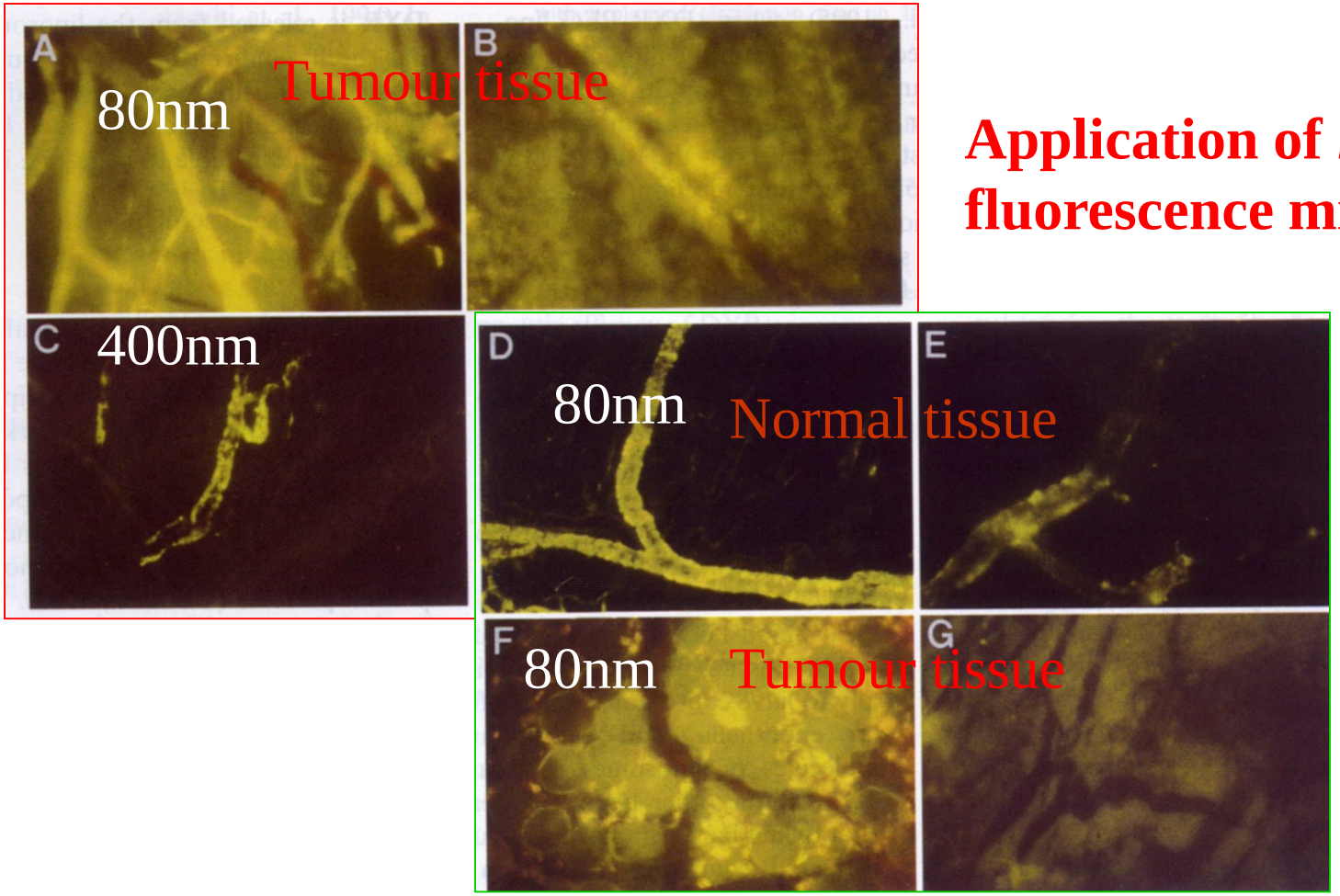
Stealth liposomes

Doxorubicin





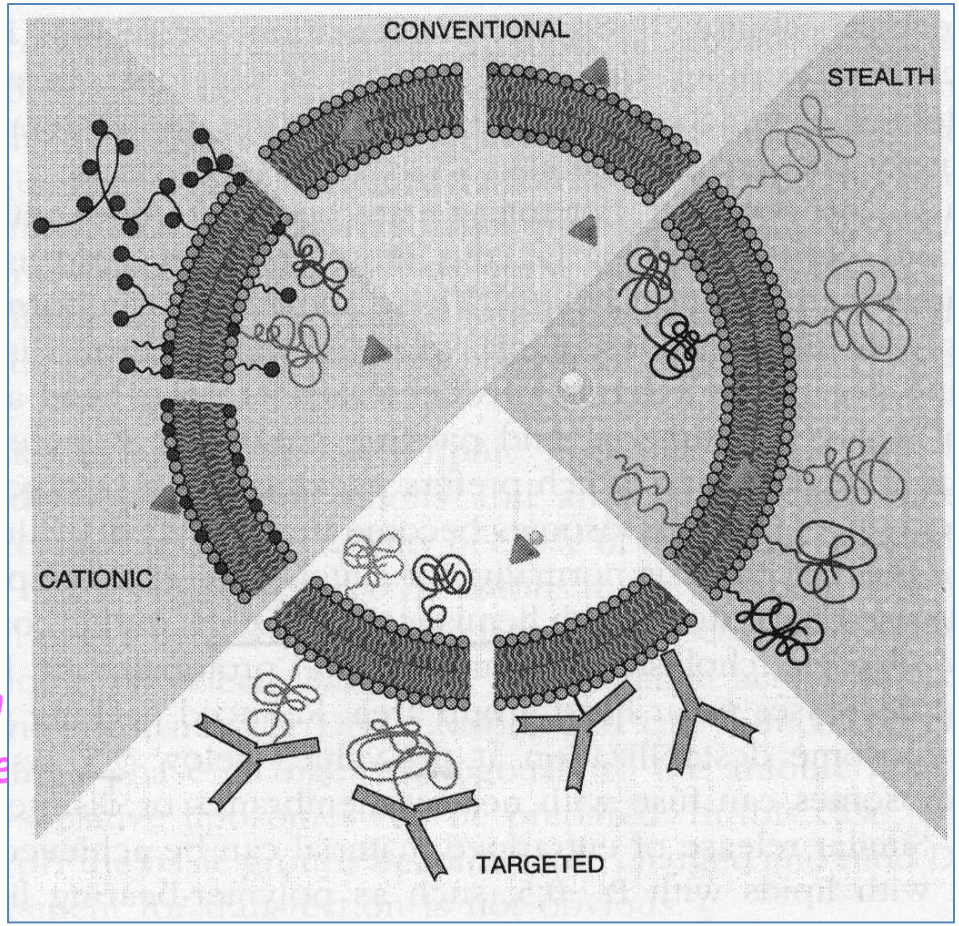
Penetration of Stealth liposomes into normal and tumour tissues



Application of *in vivo* fluorescence microscopy

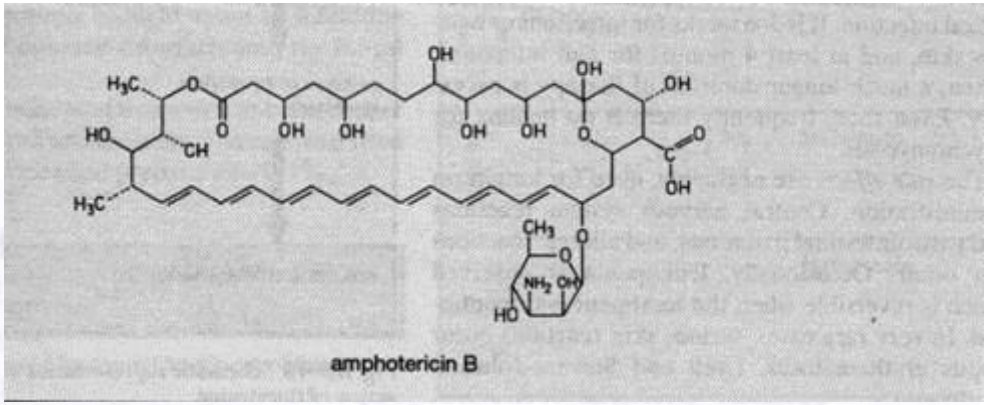
Classes of liposomes based on their functionality

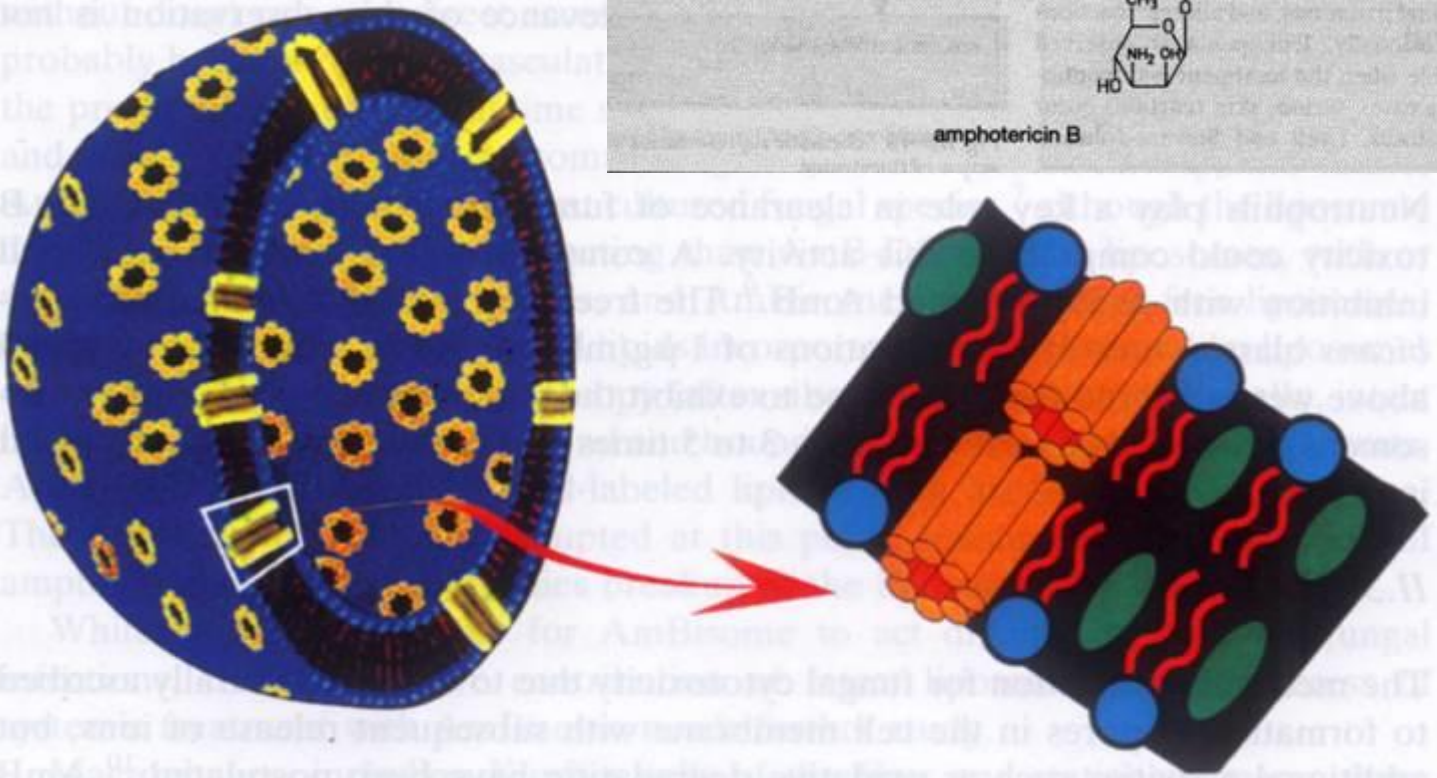
- **Conventional liposomes** *nonspecific interaction with melieu*
- **Stealth liposomes** *sterically shielded , low non-specific interactions, long circulating*
- **Targeted liposomes** *specific interaction via coupled ligand*
- **Polymorphic-*Cationic*** *change their phase upon inter-action with specific agents, pH or temperature sensitive*



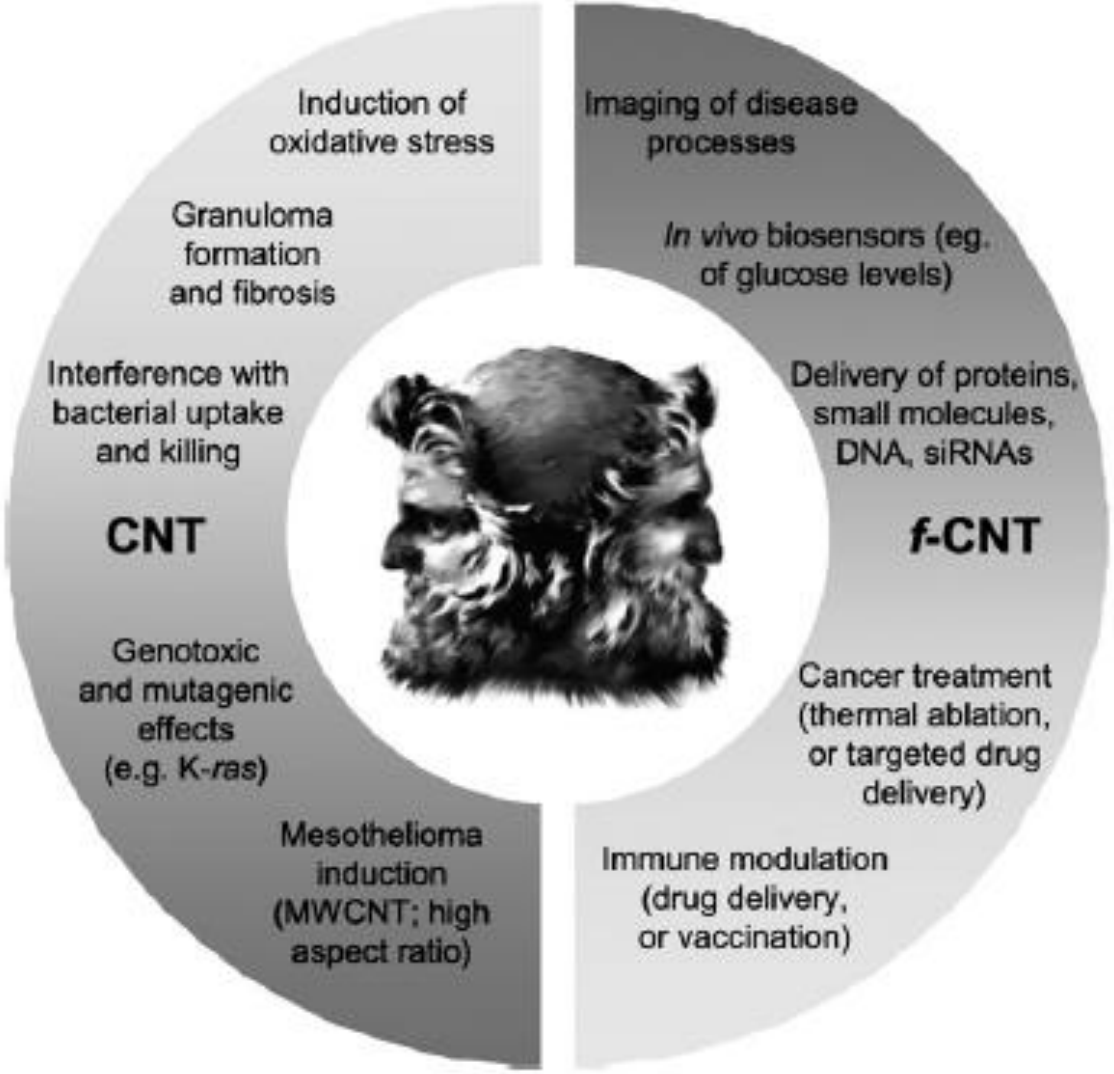
Liposomal antimicrobial drugs

Amphotericin B

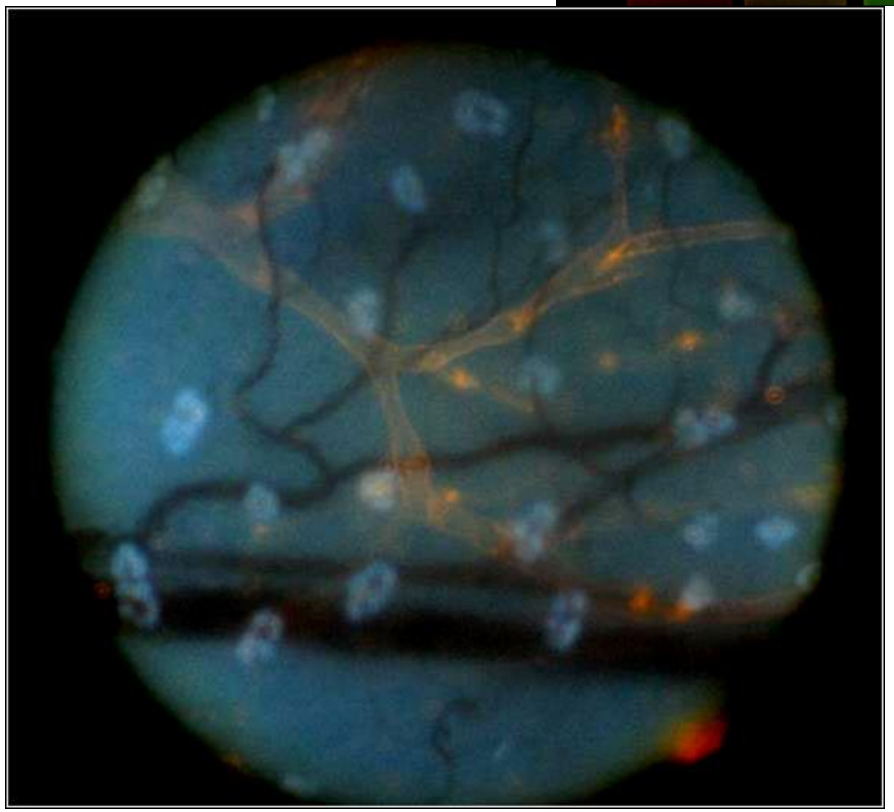


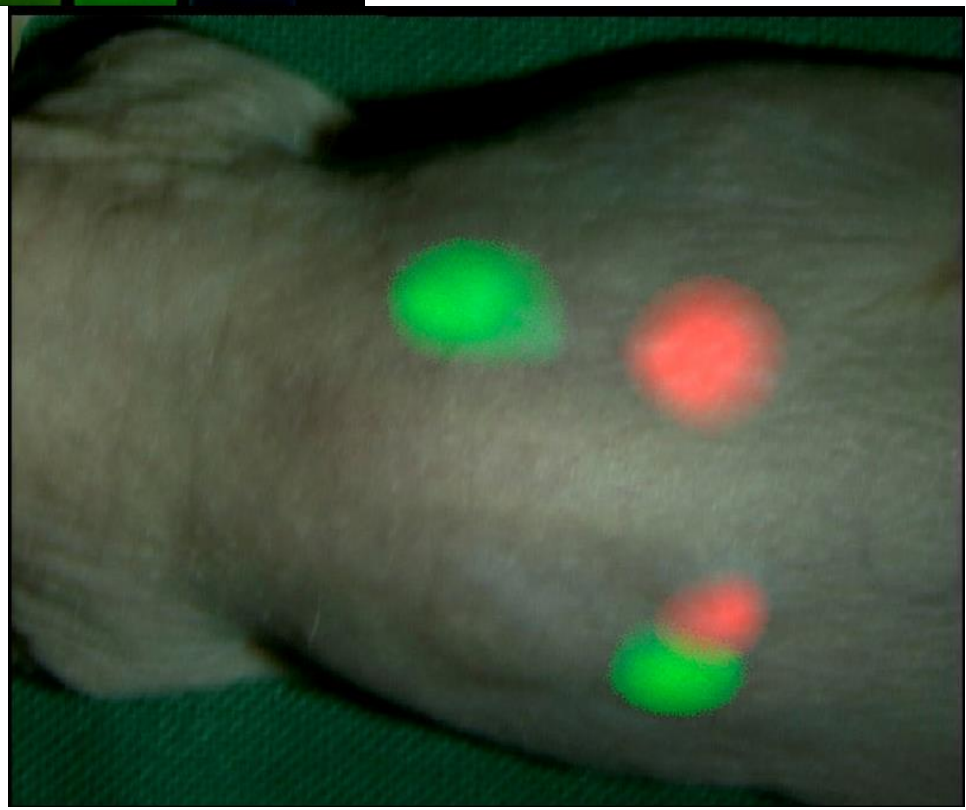


Application of carbon nanotubes

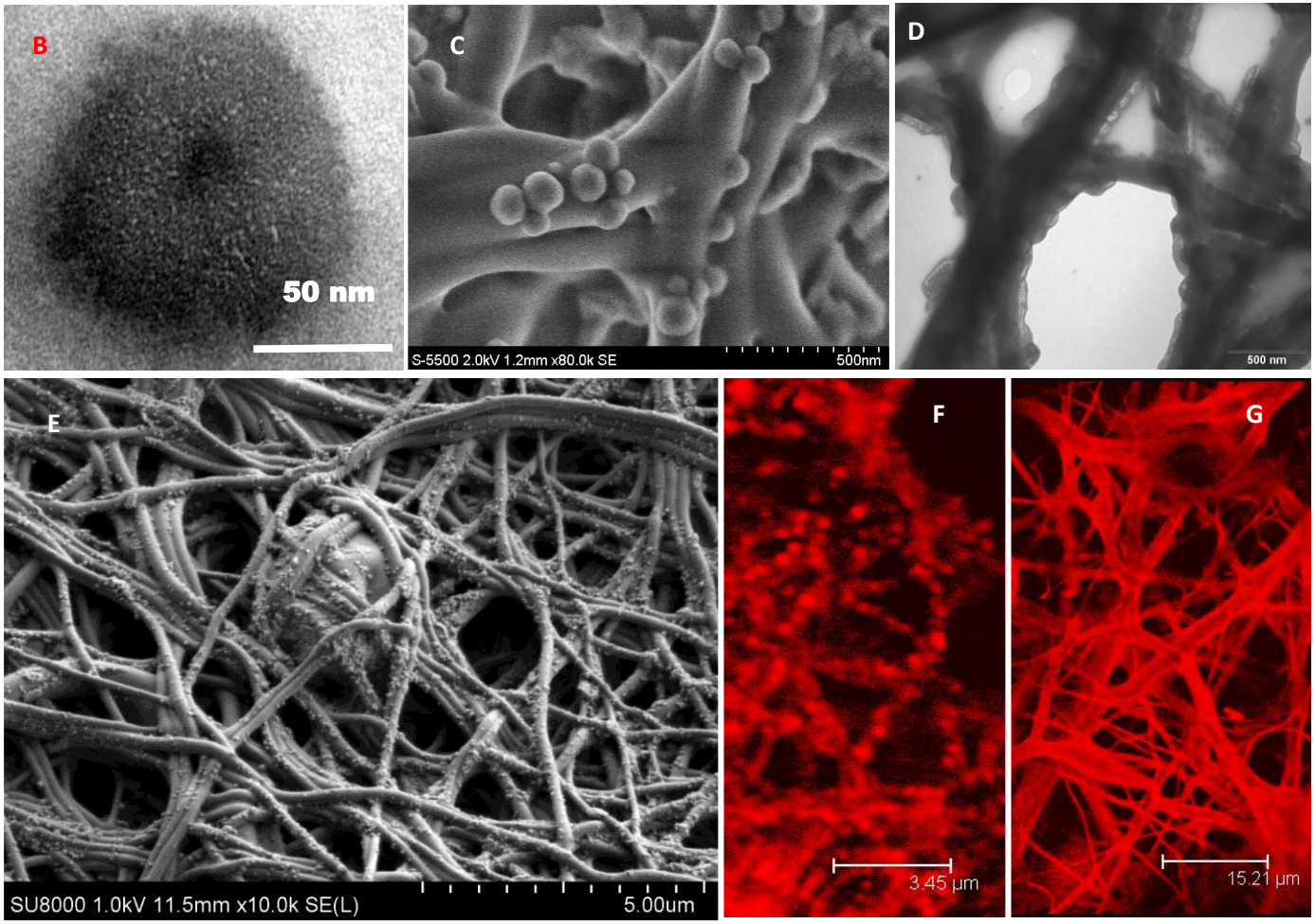


Quatnum Dots

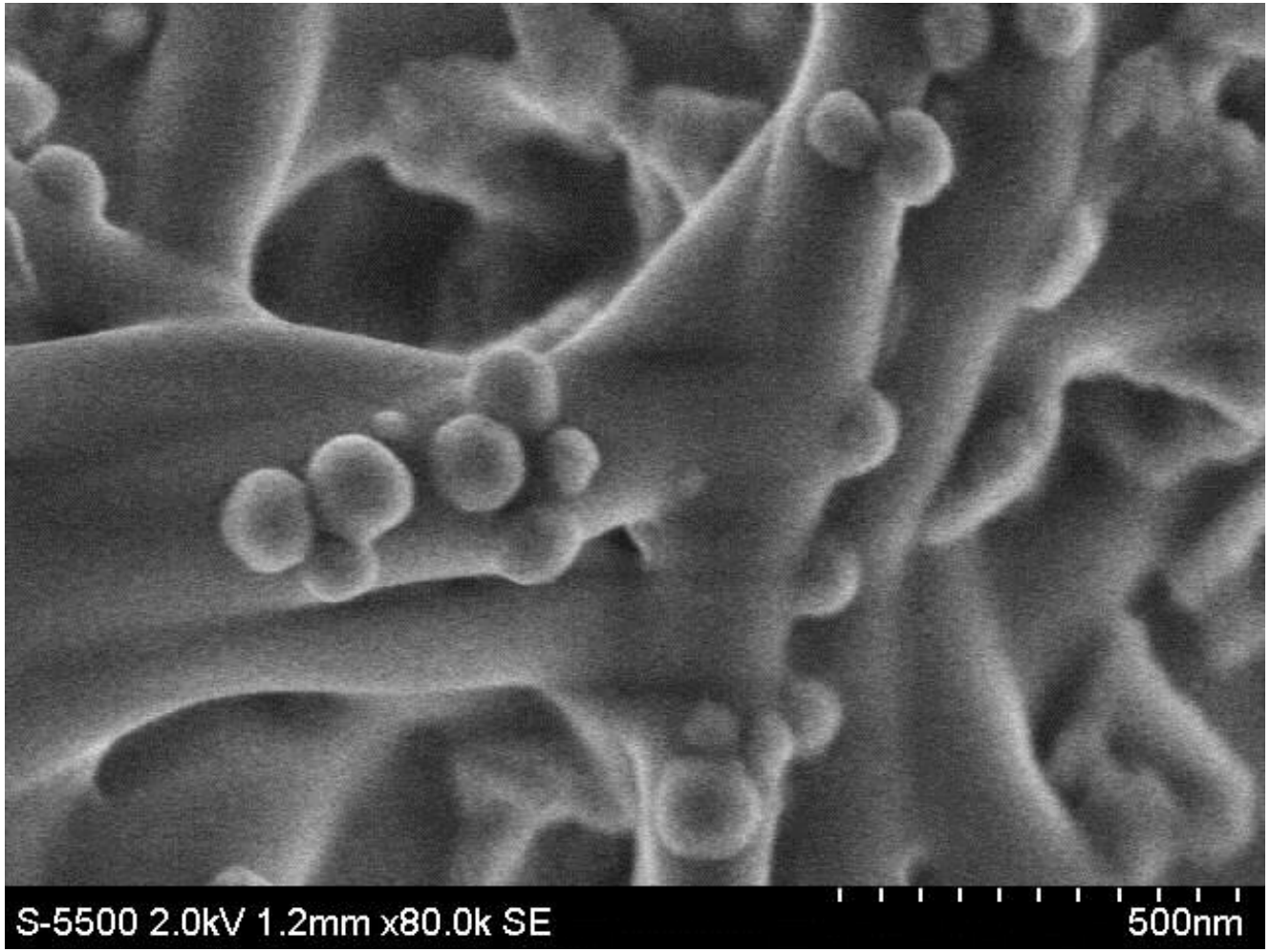




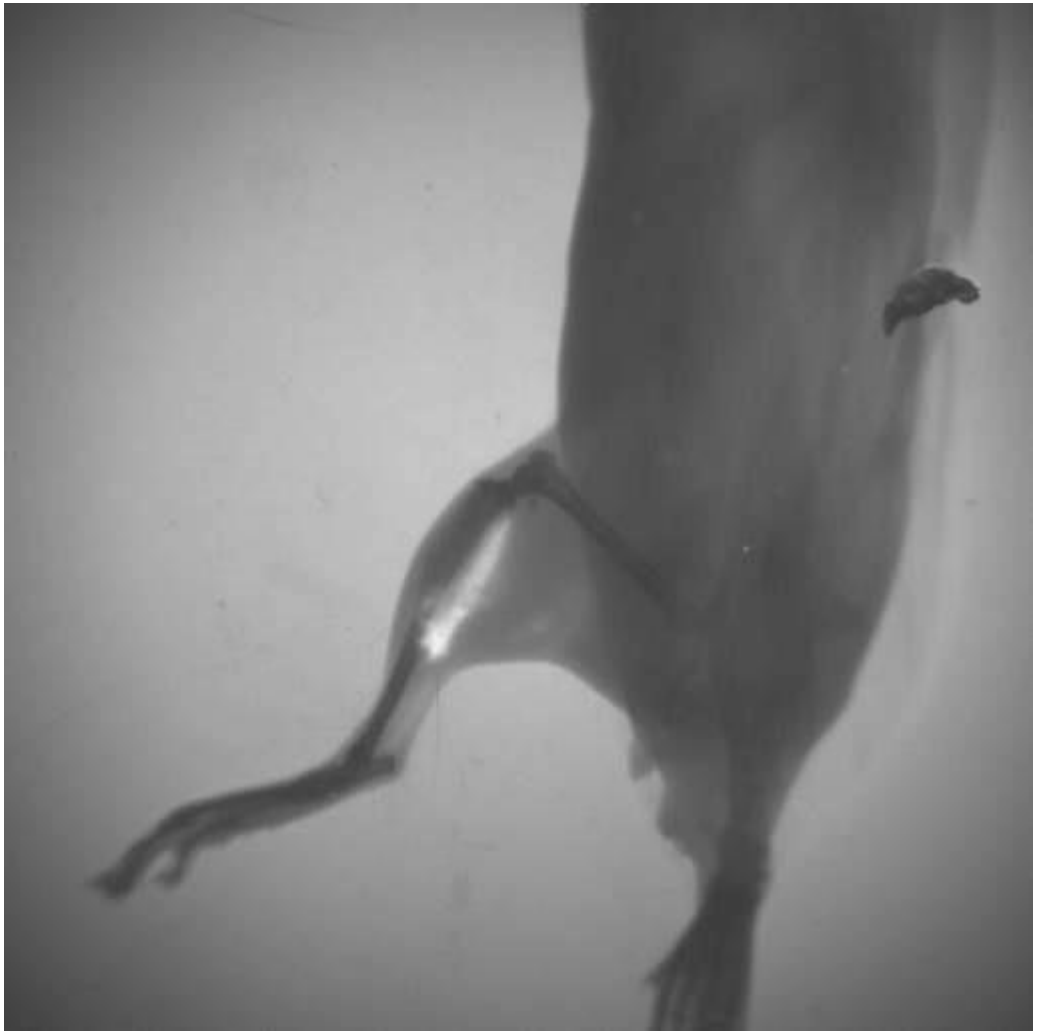
Nanofibres and nano textiles

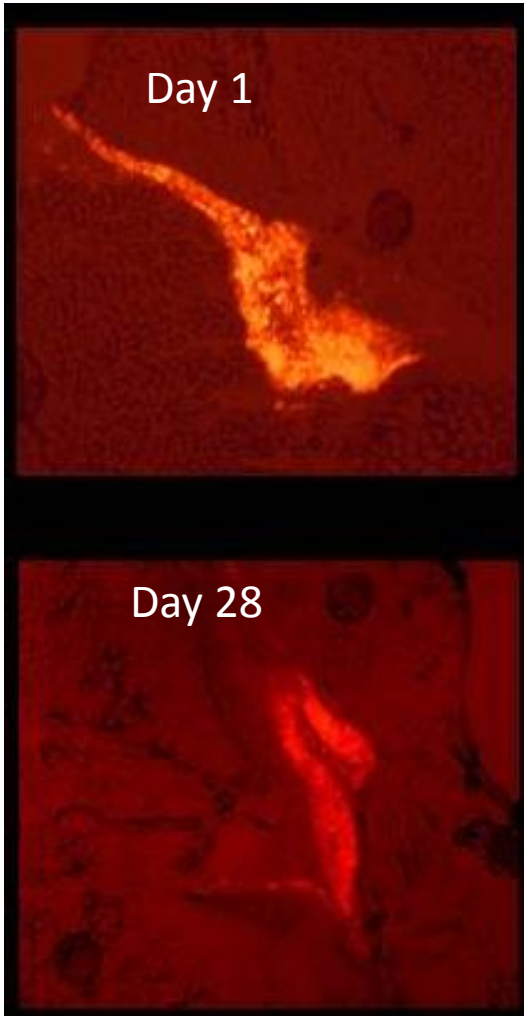


Functionalised liposomes and nanofibre textile



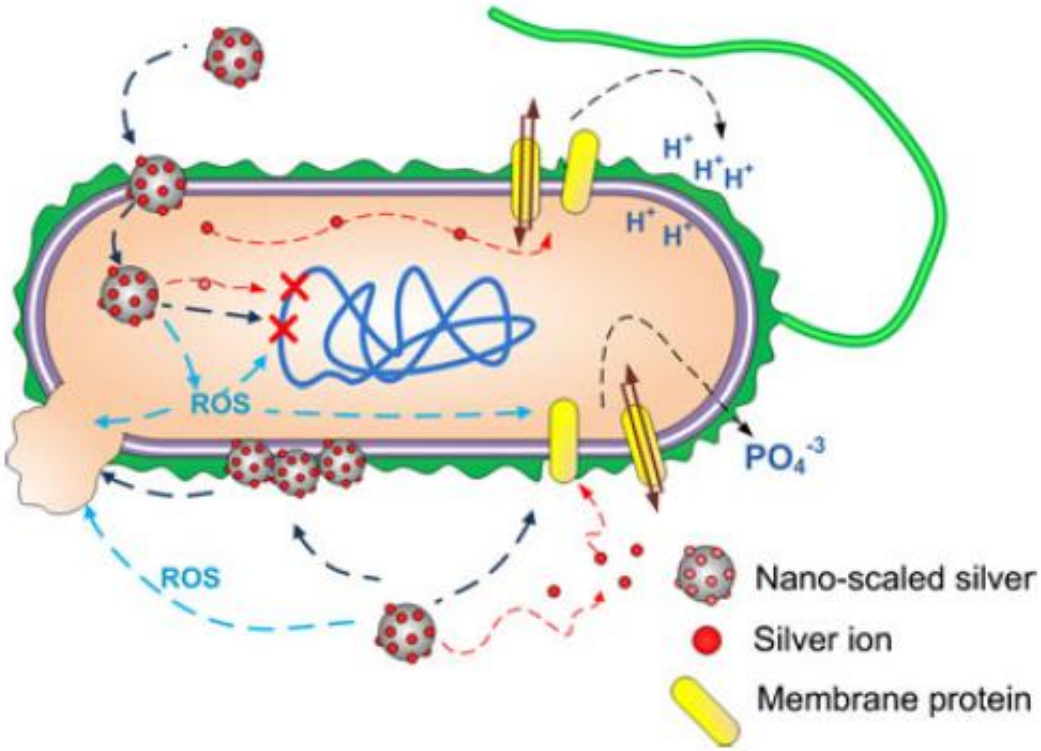
Visualizace liposom-DNA depotu fluorescenčním celotělním zobrazením

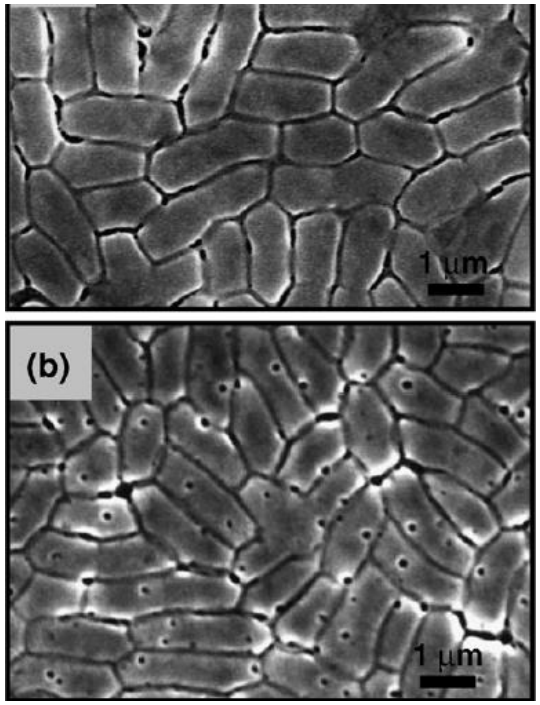




Application of nanosilver

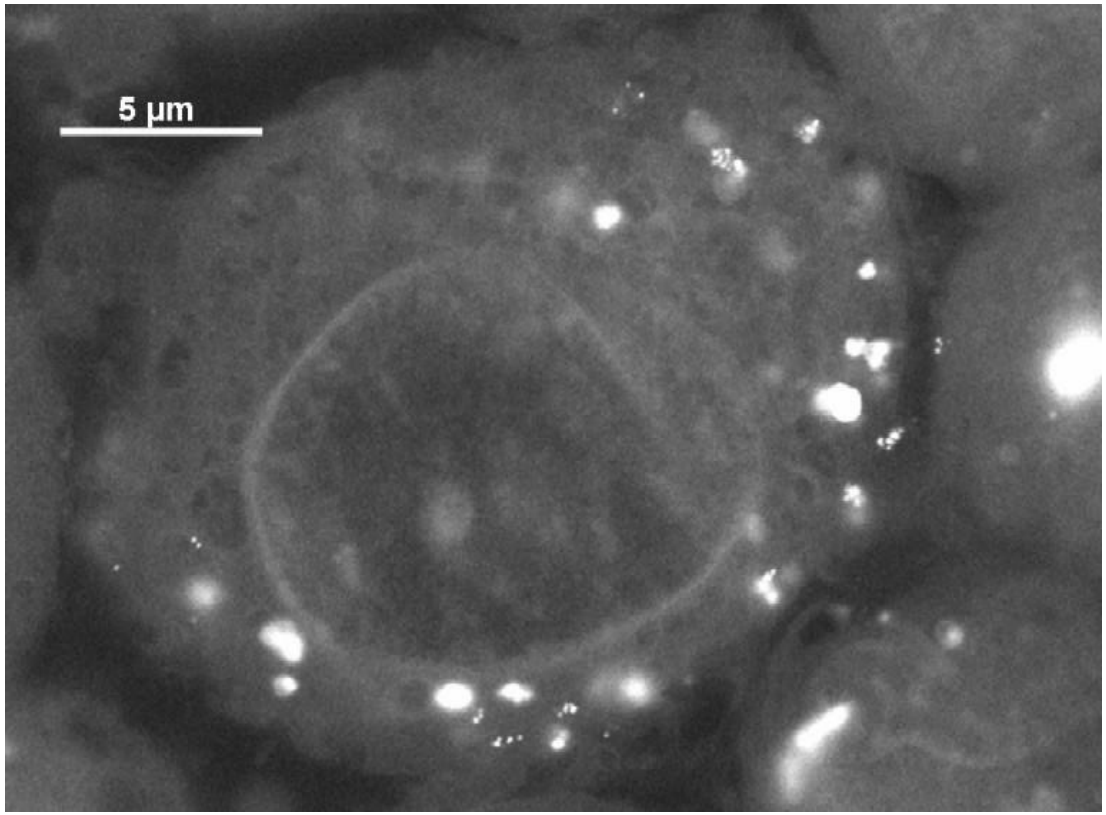
Nano-scaled silver may:
(1) release silver ions and generate ROS
2) interact with membrane proteins affecting their correct function
3) accumulate in the cell membrane affecting membrane permeability
4) enter into the cell where it can generate ROS, release silver ions, and affect DNA. Generated ROS may also affect DNA, cell membrane, and membrane proteins, and silver ion release will likely affect DNA and membrane proteins



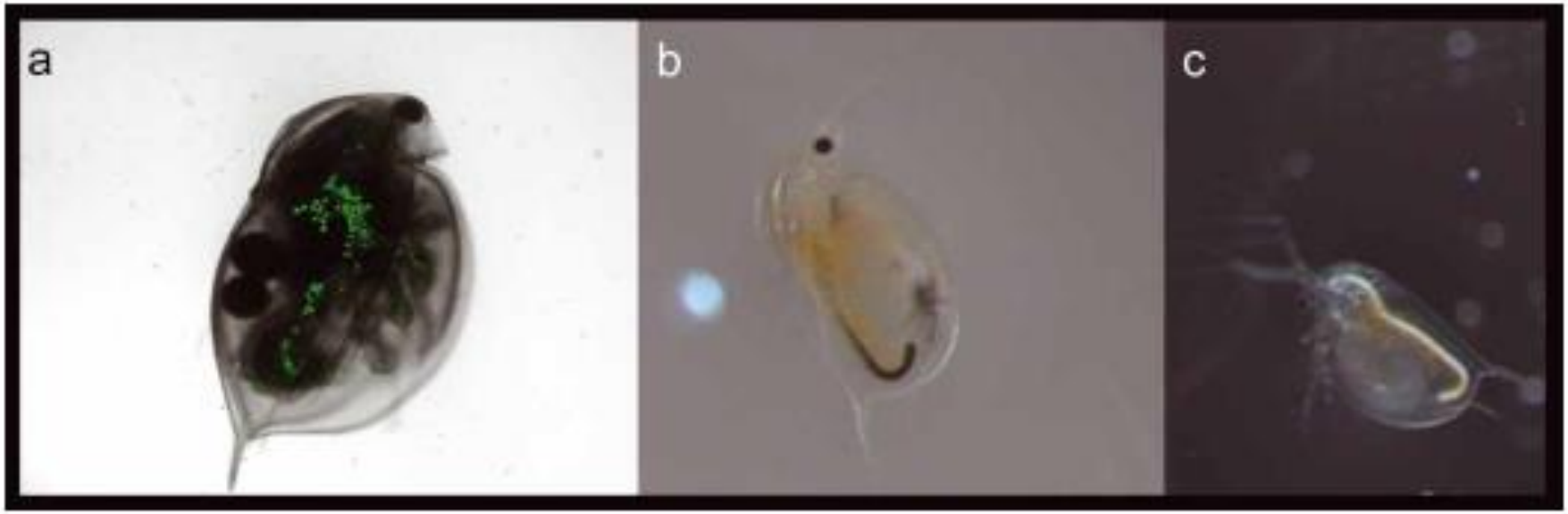


Ecology

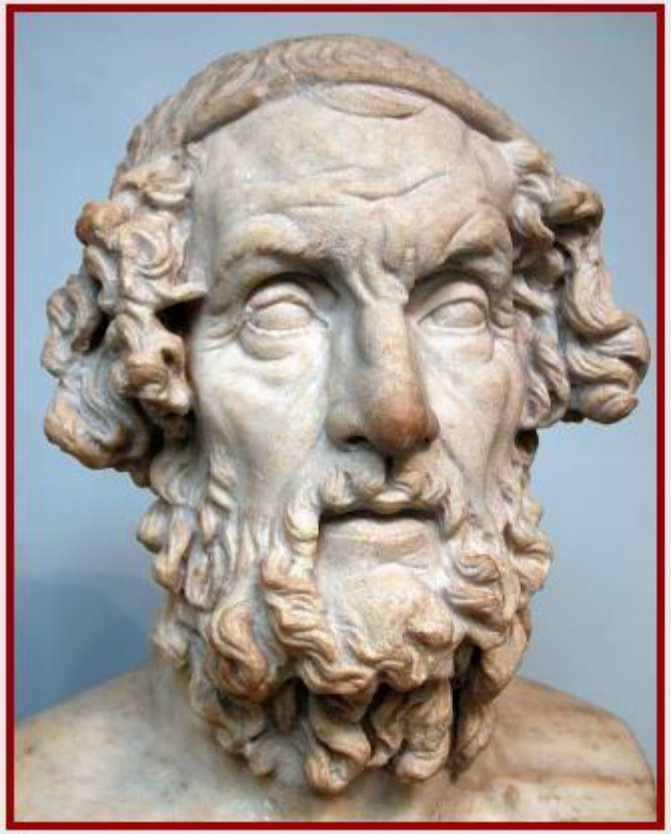
- In the development and commercialization of nanoscale particles also potential hazards for humans and the environment occurring during application and production have to be taken into account.
- rainbow trout gill cells with internalized tungsten carbide



Accummulation of NP in aquatic organisms

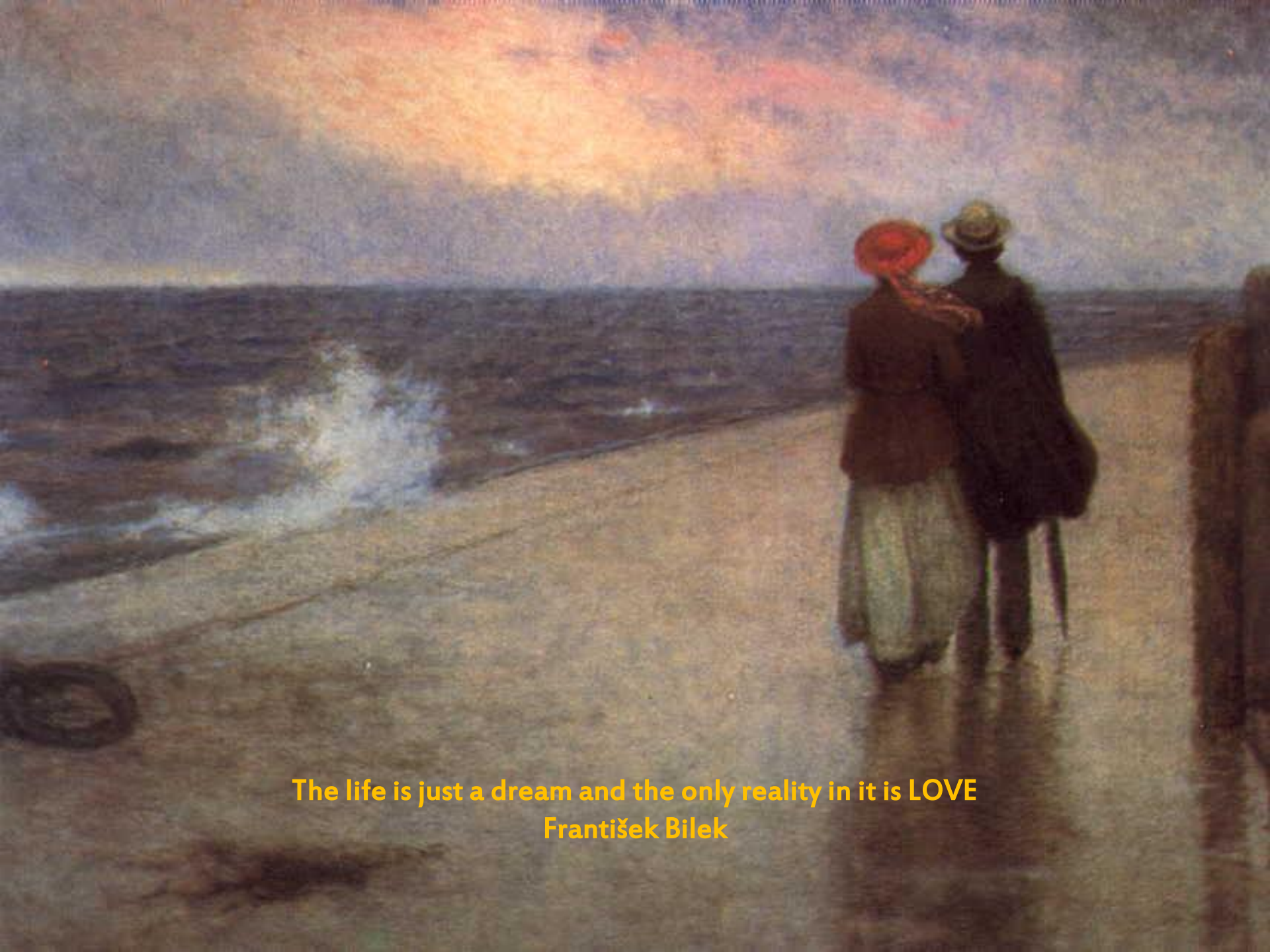


Daphnia magna exposed to (a) 20 nm fluorescent carboxylated polystyrene beads (2.64 mg/l for 1 h), (b) 14 nm carbon black (1 mg/l for 48 h) or (c) 25 nm TiO2 particles (1 mg/l for 48 h).



"Once harm has been done, even a fool understands it."

Homer
(The Illiad, ca. 8th century BC)


The life is just a dream and the only reality in it is LOVE
František Bilek


Thank you for your attention

Methods for characterisation of nanoparticles

Separation Techniques: GPC, FFF, Flow Cytometry

Light scattering: SLS/MALS (R_g), DLS (R_h)

Microscopic methods: TEM, SEM, AFM, Confocal microscopy

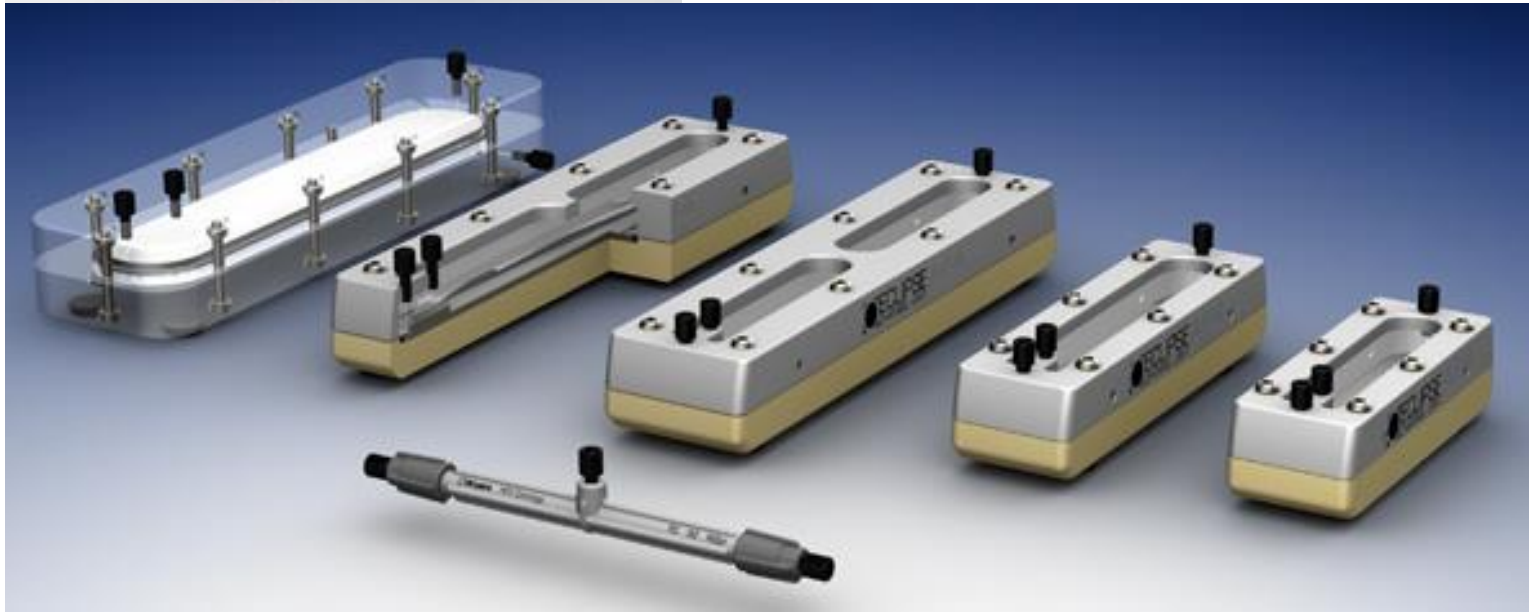
Single particle tracikng analysis: NanoSight

Conductivity: particle counters (iZON)

FFF – velikost analytu

Separační rozsah

Fiel Flow Fractionation



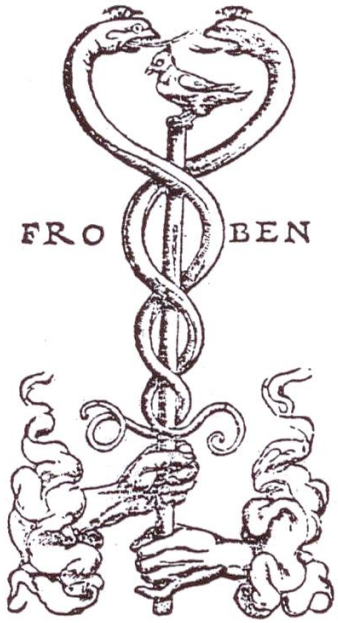
Georgius Agricola Doctor

GEORGII AGRICOLAE

DE RE METALLICA LIBRI XII▸ QVIbus Officia, Inſtrumenta, Machinæ, ac omnia deniq; ad Metallicam ſpectantia, non modo luculentiſſimè deſcribuntur, ſed & per effigies, ſuis locis inſertas, adiunctis Latinis, Germanicisq; appellationibus ita ob oculos ponuntur, ut clarius tradi non poſſint.

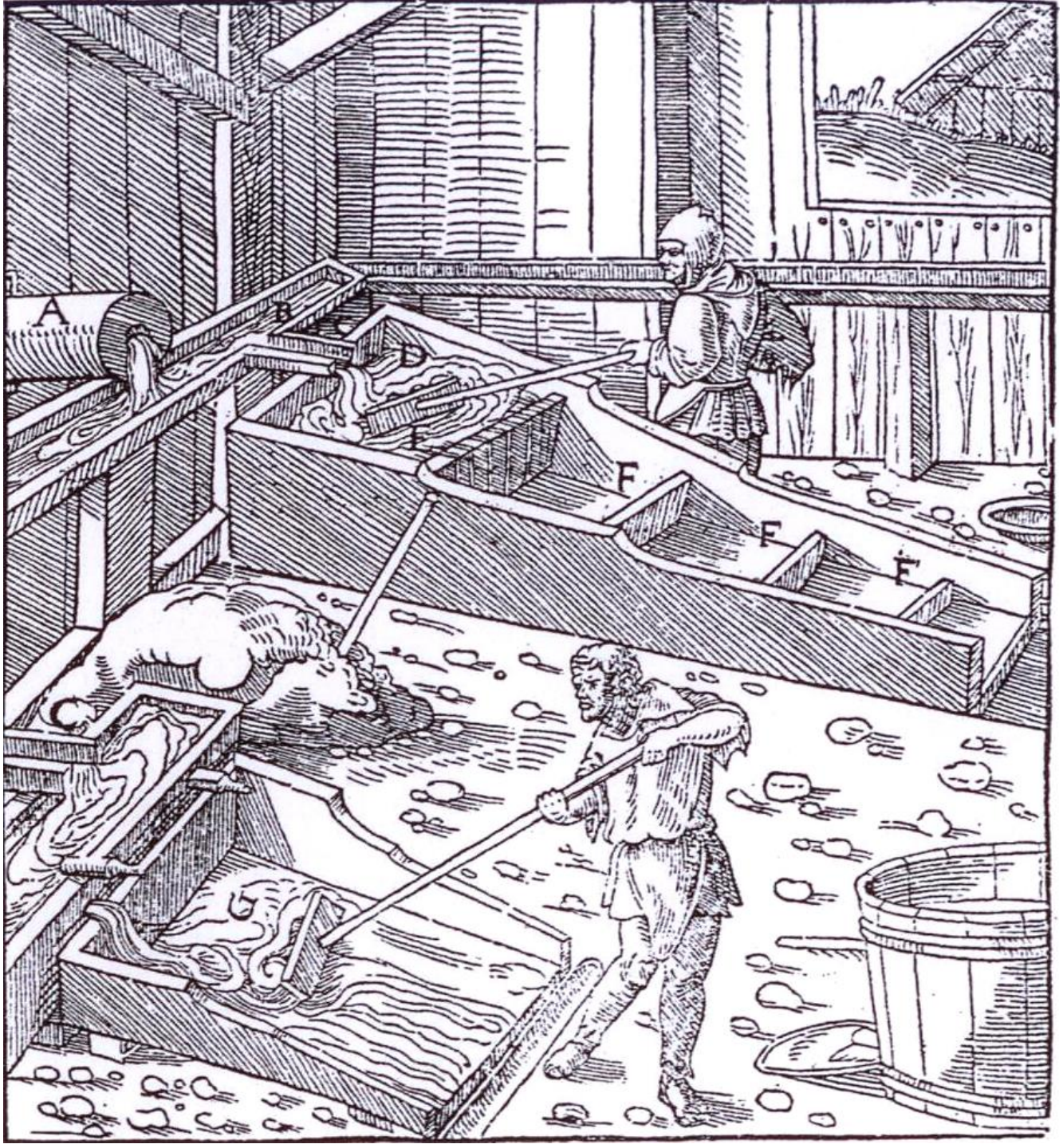
EIVSDEM

DE ANIMANTIBVS SVBTERRANEIS Liber, ab Autore recognitus: cum Indicibus diuerſis, quicquid in opere tractatum eſt, pulchrè demonſtrantibus.

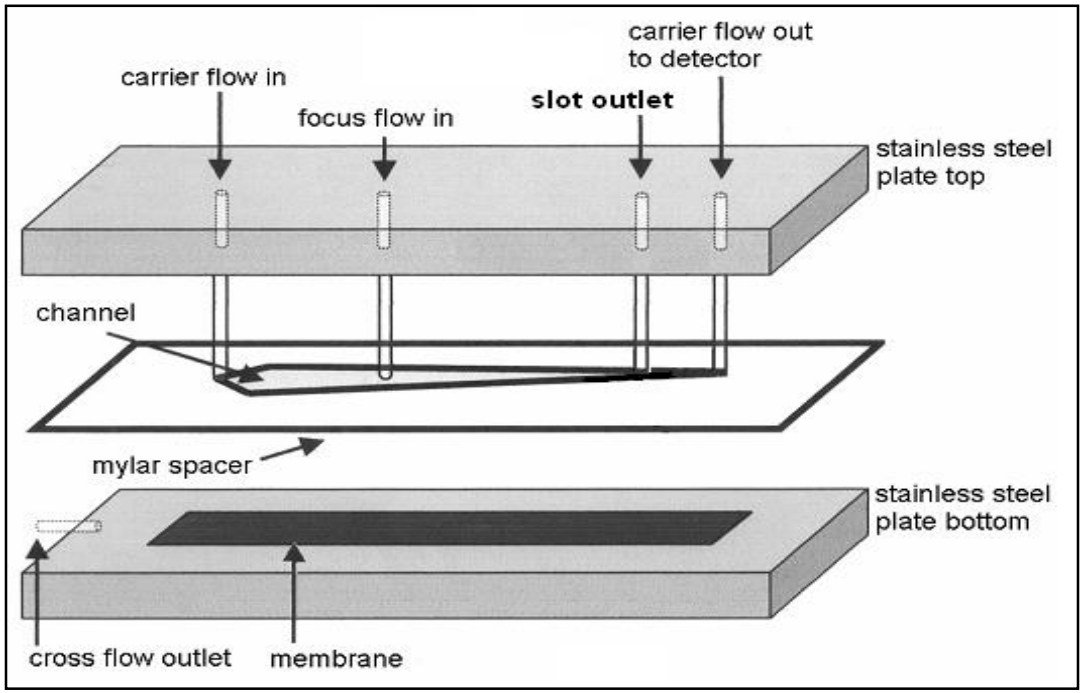


BASILEAE M▸ D▸ LVI▸

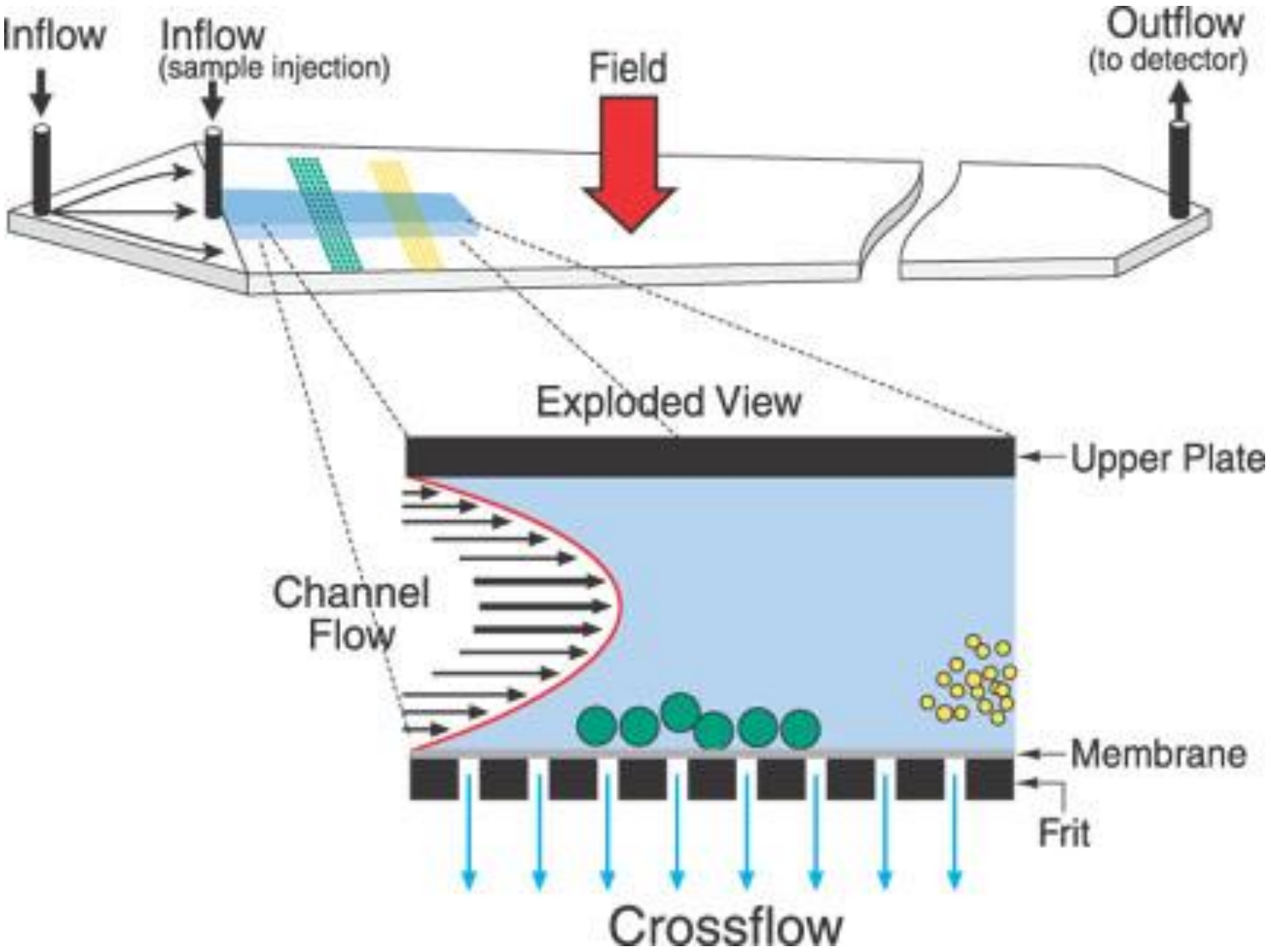
Cum Priuilegio Imperatoris in annos v.
& Galliarum Regis ad Sexennium.


A
B
C
D
F
F
F
G

- **Příčný tok pro separaci**
- **Separace založena na velikosti/MW**
- **Separační rozsah 10^3 – 10^{12} Da, 1 nm – 10 µm**
- **Temperace kanálu**

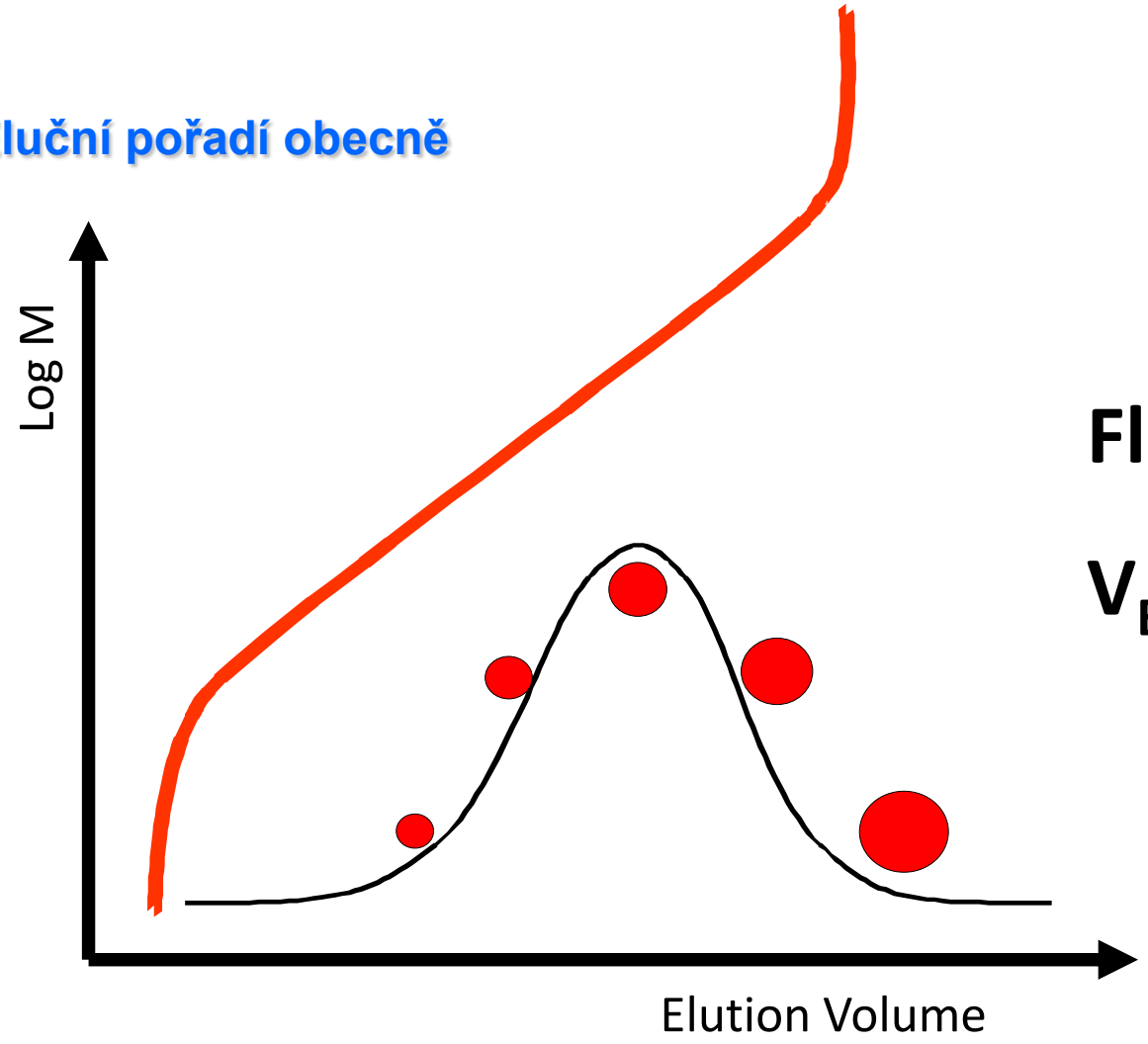


Princip AF4


Inflow
Inflow
(sample injection)
Field
Outflow
(to detector)
Exploded View
Upper Plate
Channel
Flow
Membrane
Frit
Crossflow

Eluční pořadí ve FFF

Eluční pořadí obecně

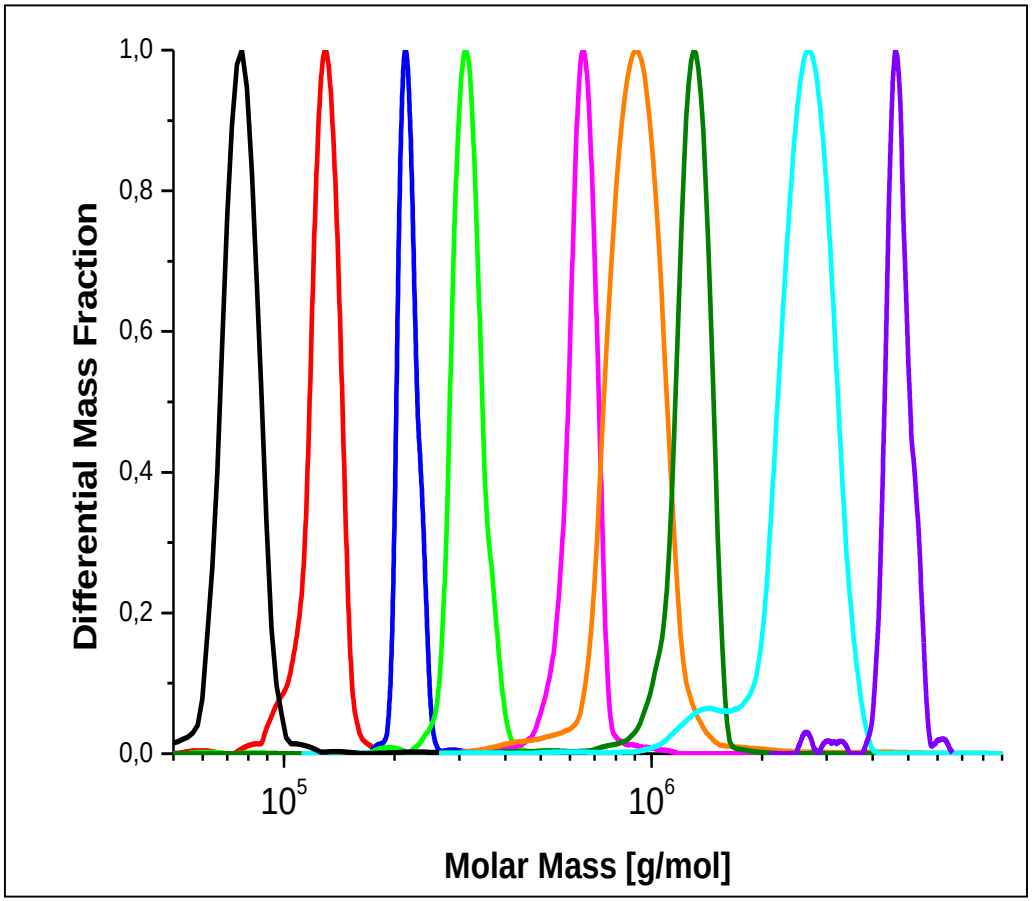


Flow FFF:

$V_E \sim 1/D \sim R_h$

FFF Aplikace

Distribuce molekulové hmotnosti (AF4-MALS)



PS-standardy v THF

M_p [kg/mol] Výrobce	M_p [kg/mol] AF4
68	77
127	129
246	213
310	313
659	651
1040	907
1260	1321
2750	2691
5000	4623

qNano

qNano uses unique nanopore-based detection to enable the physical properties of a wide range of particle types to be accurately measured.

Detailed Multi-Parameter Analysis.

Particle-by-particle measurement through qNano enables detailed determination of:

- Particle Size
- Concentration
- Particle Interactions
- Particle Zeta-potential

Nanopore-based detection allows thousands of particles to be measured individually, providing far greater detail and accuracy than light-based techniques. Learn More.

Click Here to Request Further Information / Quote

Applications & Particle Types

A wide range of biological and synthetic particle types, spanning 50 nm - 10 µm, can be measured, across a broad range of research fields. Examples are listed below. Learn More.

qNano

Drug Delivery Research
Liposomes
Nanobubbles
Polymeric Drug Delivery
"Smart" Particles

Virus Quantitation
Viral Vaccines
Adenovirus
Lentivirus

Biomedical Diagnostics Research
Functionalized Particles for Immunodetection
Dye-doped Particles

Microvesicle Research & Haematology
Microparticles & Exosomes
Whole Blood Cells
Platelets

Industrial Research
Paint Pigments
Food & Beverage (Milk)

Microbiology
Viruses
Bacteria
Yeast

Other
Silica, Polystyrene, Polymers

The qNano is a complete

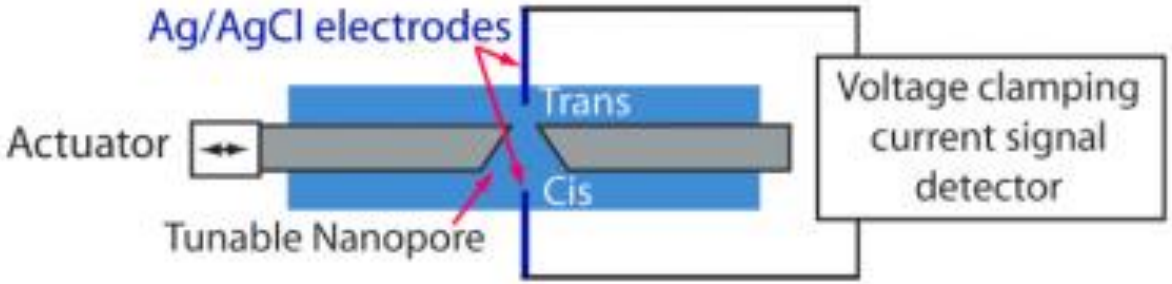


Figure 1. A sensitive signal detector monitors current flow across the nanopore.

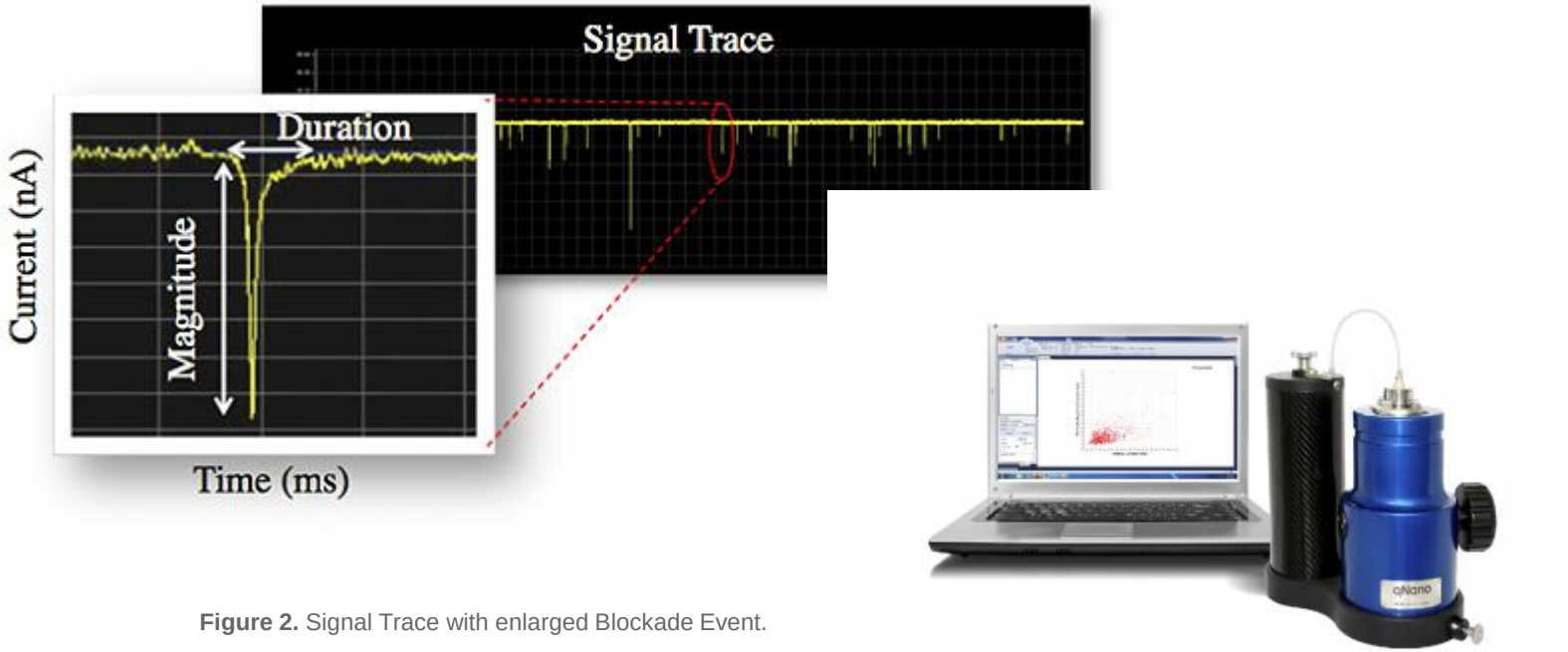


Figure 2. Signal Trace with enlarged Blockade Event.

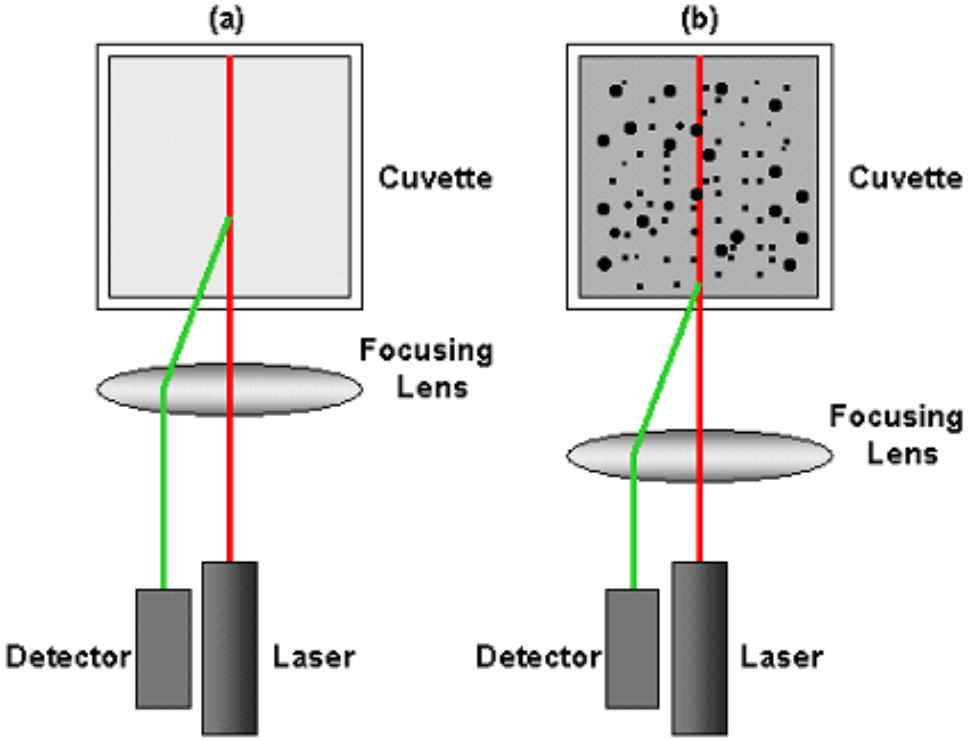
- Laboratory of Bionanotechnology

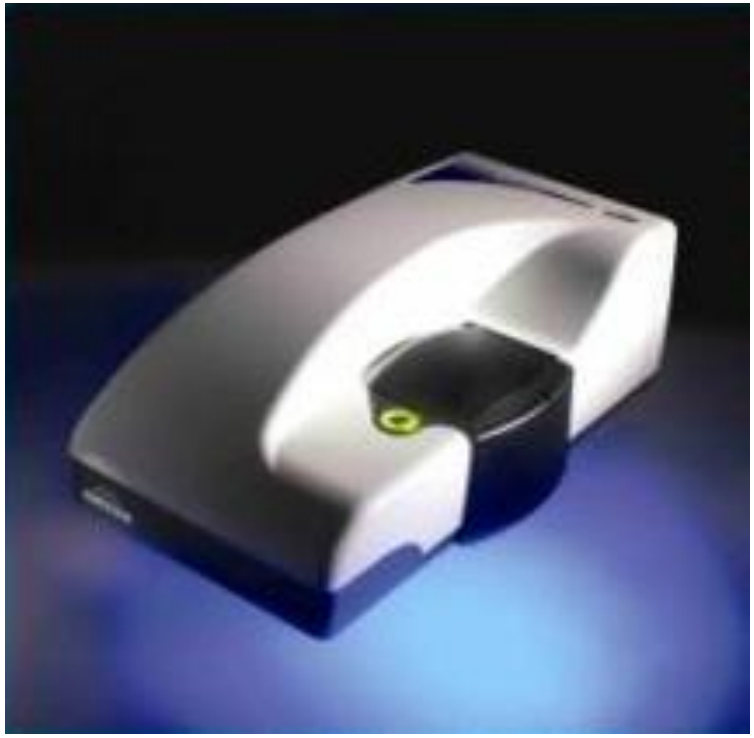
Laboratory of Bionanotechnology

Dynamic light scattering
Benefits of sizing by DLS

- Non-invasive
- High sensitivity (< 0.1 mg/mL for typical proteins)
- Low volume (12 µL)
- Scattering intensity is proportional to the square of the protein molecular weight, making the technique ideal for identifying the presence of trace amounts of aggregate.

New dynamic light scattering technology for high sensitivity and measurement at high concentration (NIBS) (Non-Invasive Back-scatter) technology extends the range of sizes and concentrations of samples that can be measured.
The sizing capability in the Zetasizer Nano S and Nano ZS instruments detect the scattering information at 173°. This is known as backscatter detection. In addition, the optics are not in contact with the sample and hence the detection optics are said to be non-invasive.





Pohyblivá zaostřovací čočka umožňuje snímání signálů z různé hloubky kyvety a tak eliminuje u koncentrovaných vzorků vícenásobný rozptyl (viz obr b)

Zetasizer Nano ZS, Malvern

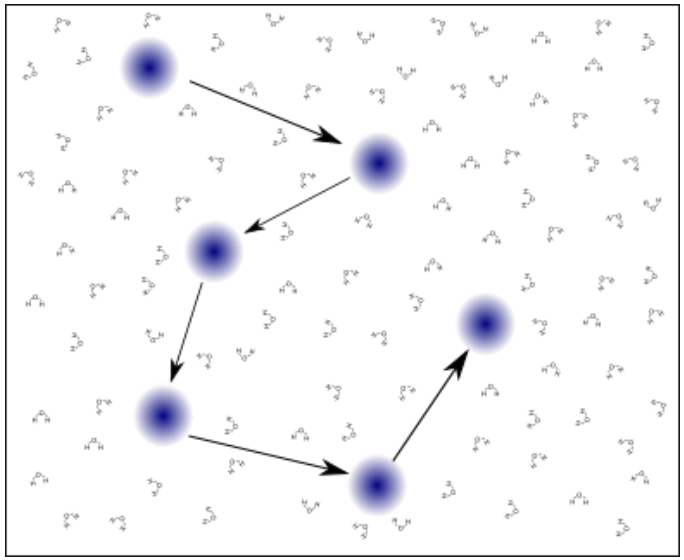
Parameter	Value
Sizing range	0.6 nm to 6 um Diam
Concentration range	0.1 mg/mL Lys to 30w%
Min sizing sample volume	12 uL
Min zeta sample volume	0.75 mL
Temperature control	2 to 90 °C
Conductivity range	0 to 200 mS/cm
Laser	3 mW 633 nm HeNe
Detector	APD

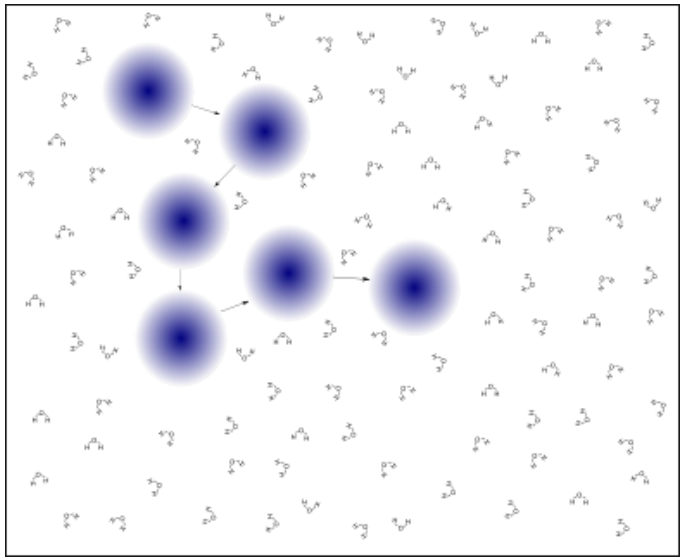
- Crystal screening
- Protein & polymer characterization
- CMC measurements
- Drug delivery systems
- Formulation stability
- Biological assemblies
- Virus & vaccine characterization
- Macromolecular critical points

Principle Behind Dynamic Light Scattering

Particles, emulsions and molecules in suspension undergo Brownian motion. This is the motion induced by the bombardment by solvent molecules that themselves are moving due to their thermal energy. If the particles or molecules are illuminated with a laser, the intensity of the scattered light fluctuates at a rate that is dependent upon the size of the particles as smaller particles are “kicked” further by the solvent molecules and move more rapidly. Analysis of these intensity fluctuations yields the velocity of the Brownian motion and hence the particle size using the Stokes-Einstein relationship.

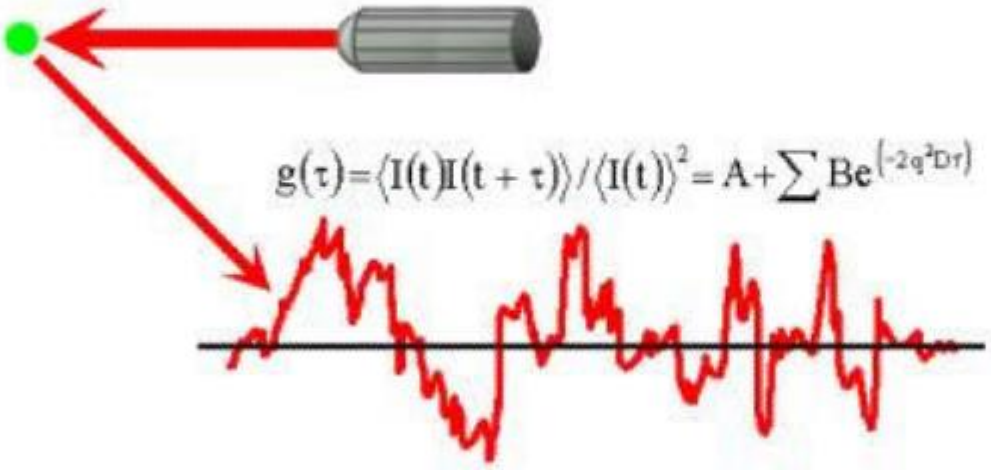
$$R_h = \frac{kT}{6\pi\eta D}$$





Dynamic Light Scattering

Fluctuations are a result of Brownian motion and can be correlated with the particle diffusion coefficient and size.

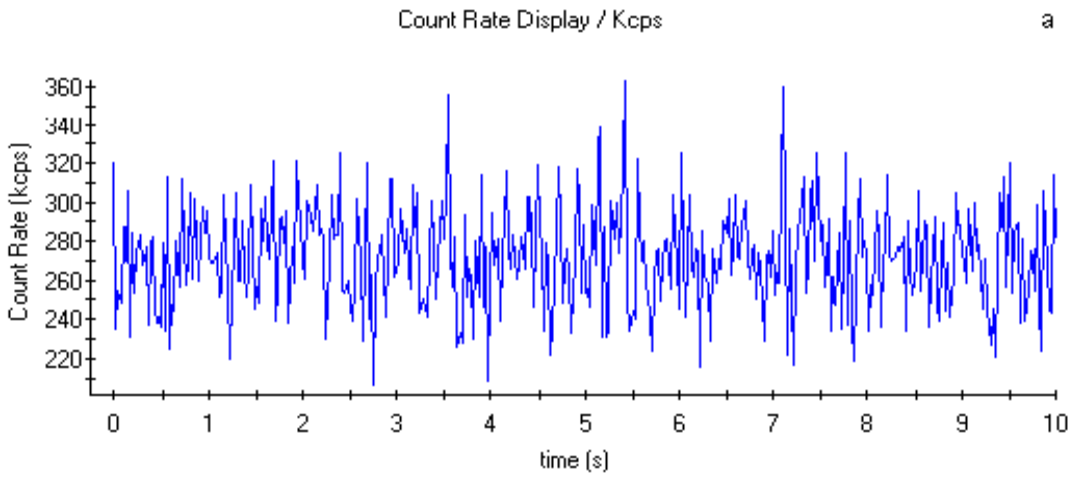


Stokes-Einstein

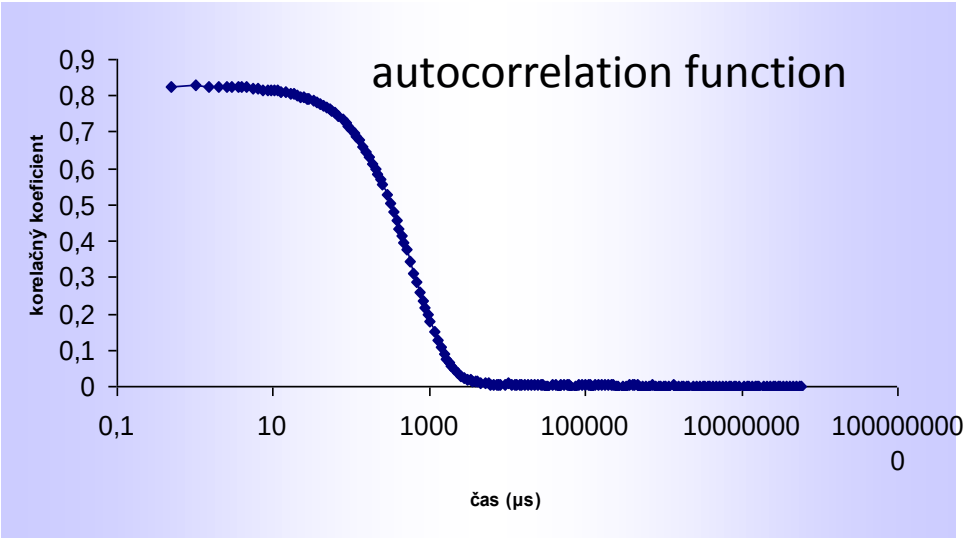
$$R_H = \frac{kT}{6\pi\eta D}$$

q = Scattering vector
R_H = Radius
T = Temperature

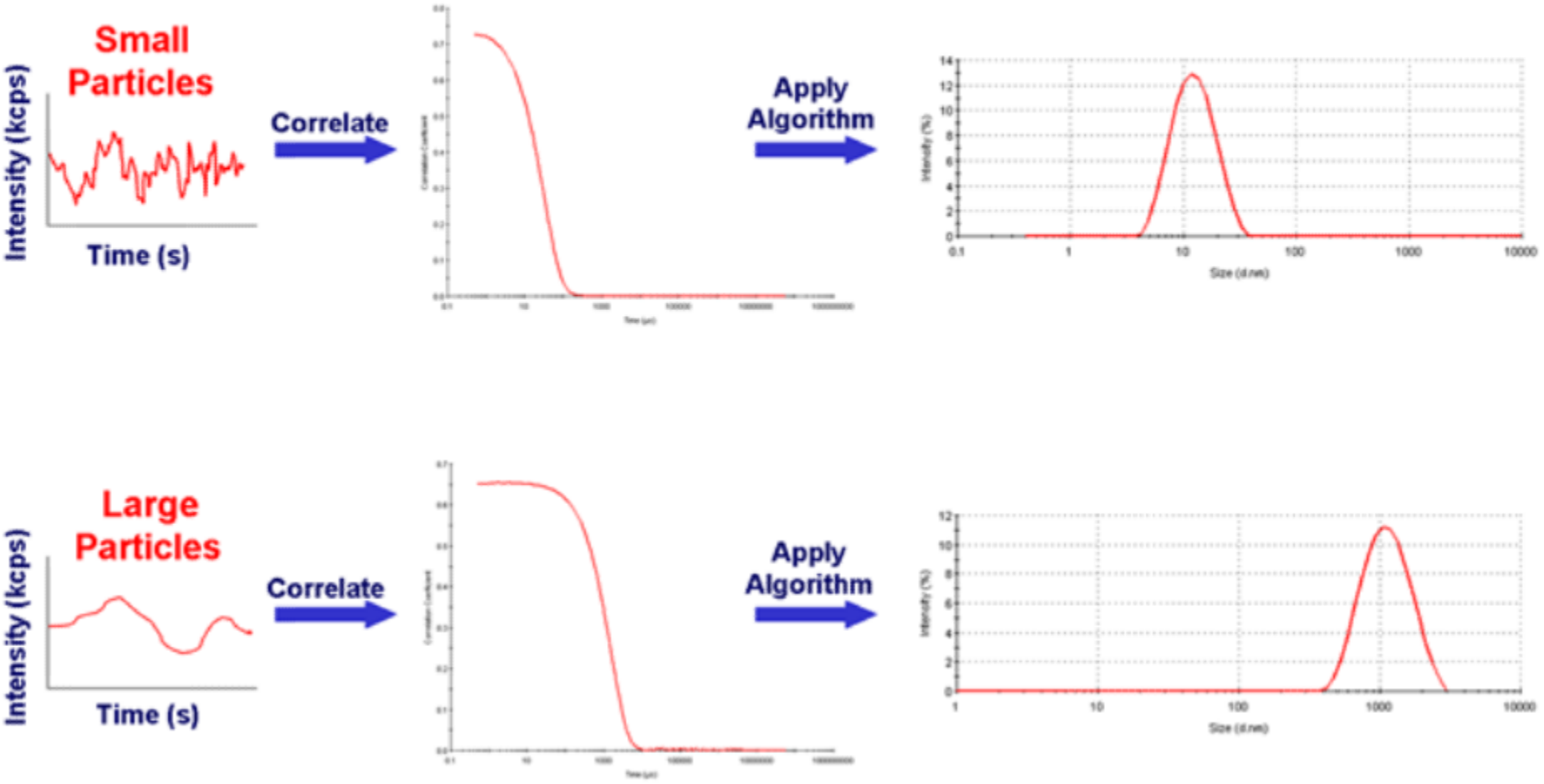
D = Diffusion coefficient
k = Boltzmann constant
η = Solvent viscosity



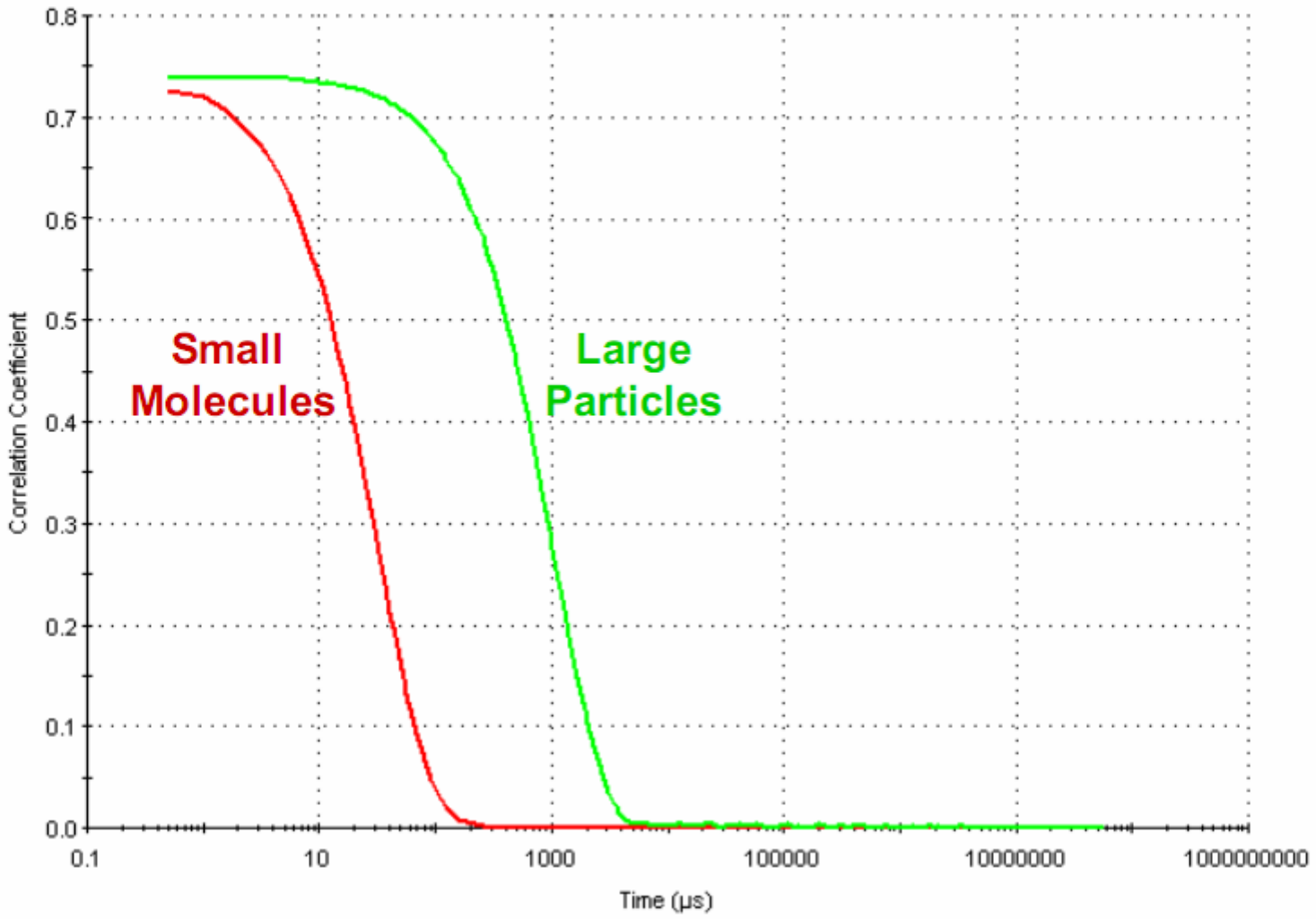
$$g(\tau)=\frac{\langle I(t)I(t+\tau)\rangle}{\langle I(t)\rangle^2}=A+\sum Be^{-2q^2D\tau}$$



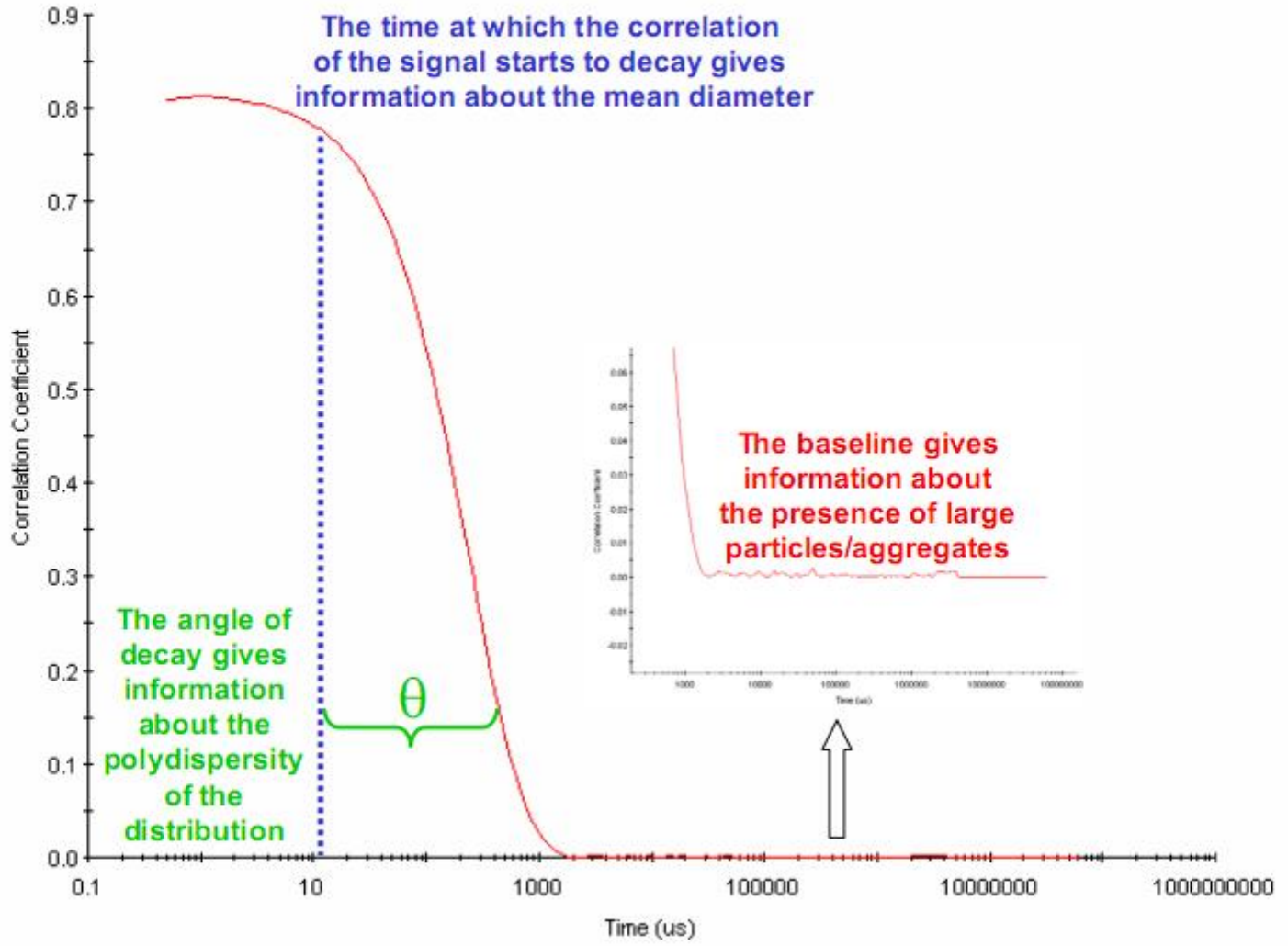
Intensity Fluctuations, Correlation and Size Distributions



Correlogram Interpretation

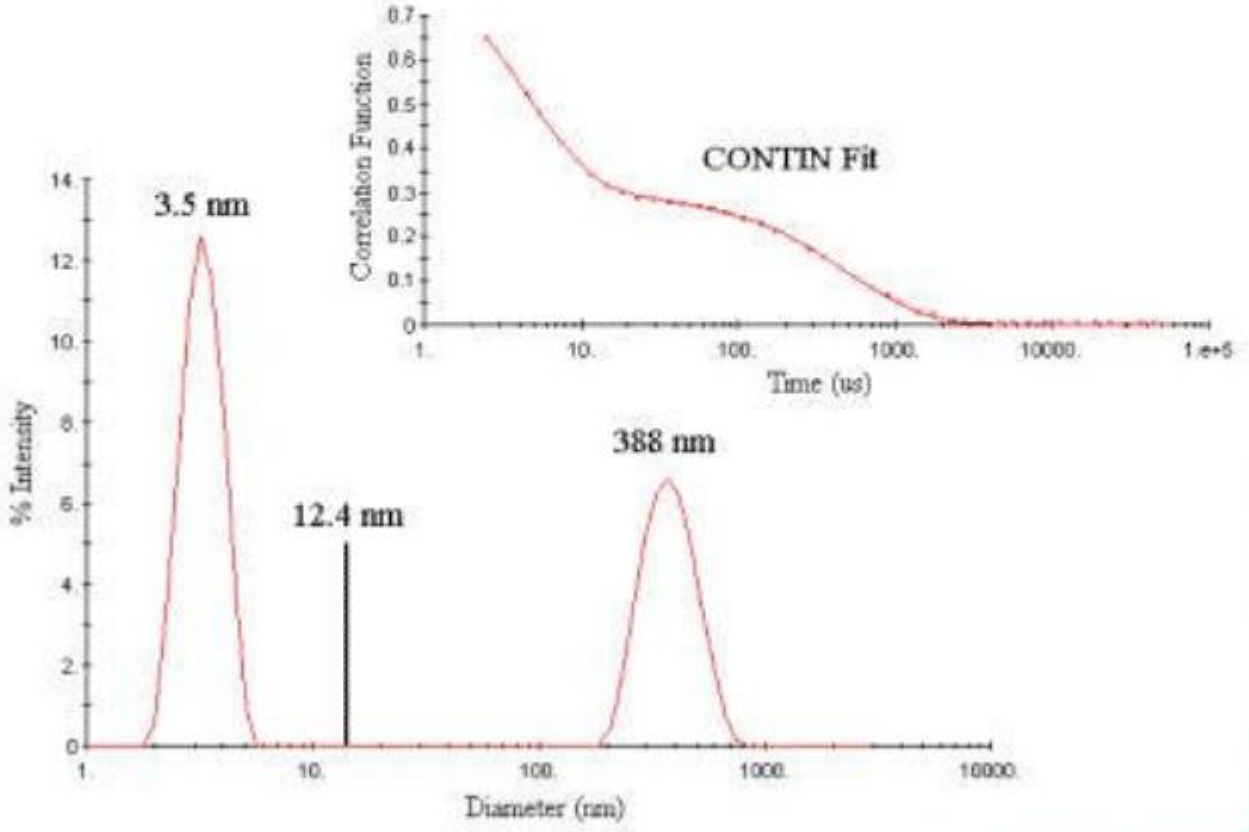


Correlogram Interpretation



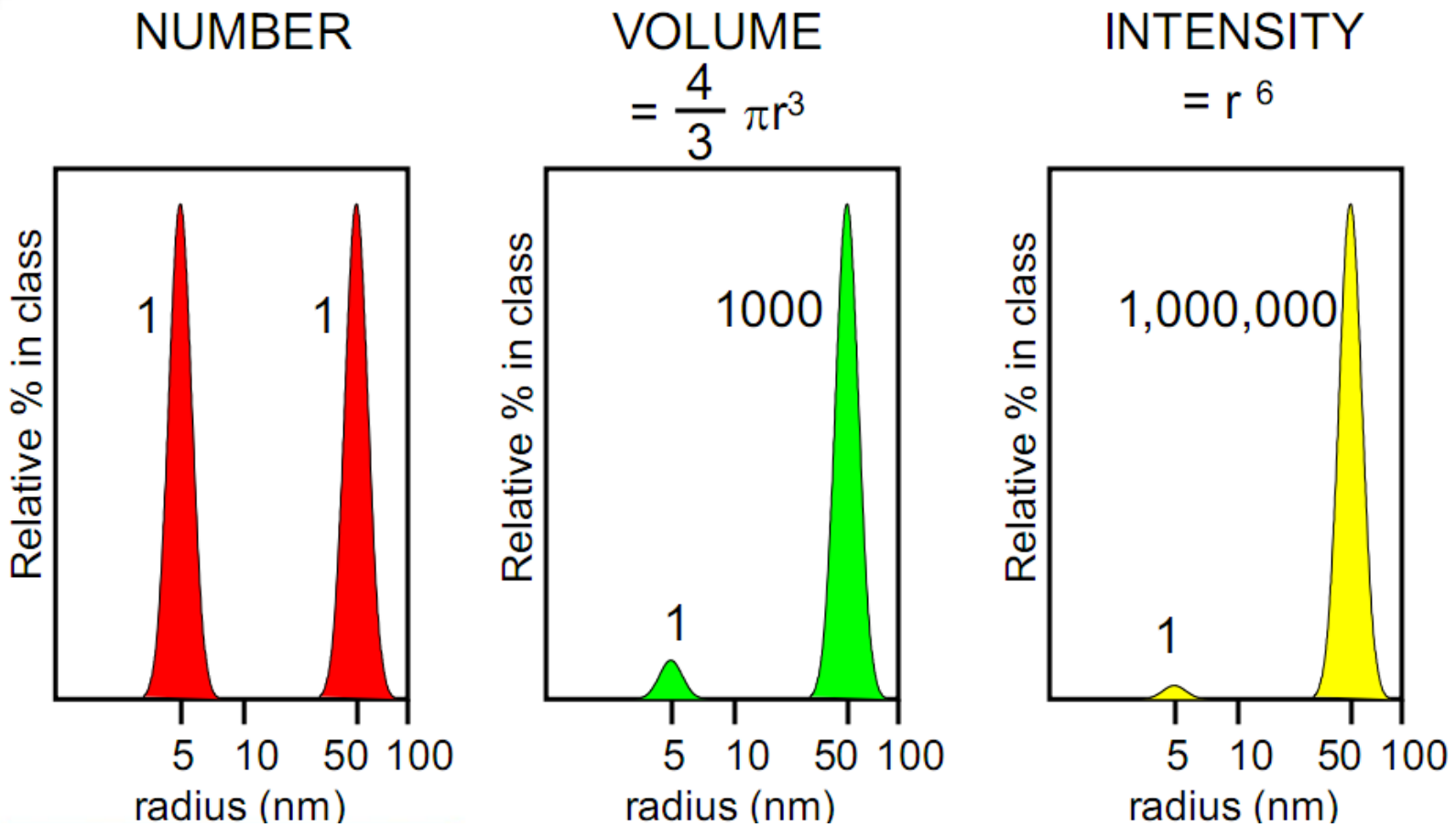
Distribution by DLS

Comparison of Z average (Cumulants) size to multi-modal (CONTIN) distribution results.



Intensity, Volume And Number Distributions

Mixture containing equal numbers of 5 and 50nm spherical particles

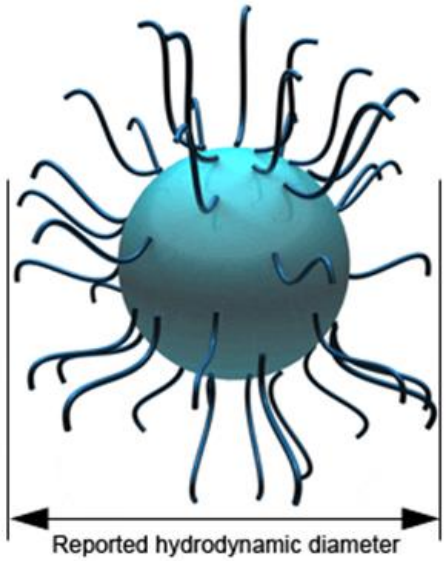


Advantages Dynamic Light Scattering technology

Accurate, reliable and repeatable particle size analysis in one or two minutes
Measurement in the native environment of the material
Mean size only requires knowledge of the viscosity of the liquid
Simple or no sample preparation, high concentration, turbid samples can be measured directly
Simple set up and fully automated measurement
Size measurement of sizes < 1nm
Size measurement of molecules with MW < 1000Da
Low volume requirement (as little as 12µL)

What does Dynamic Light Scattering actually measure?

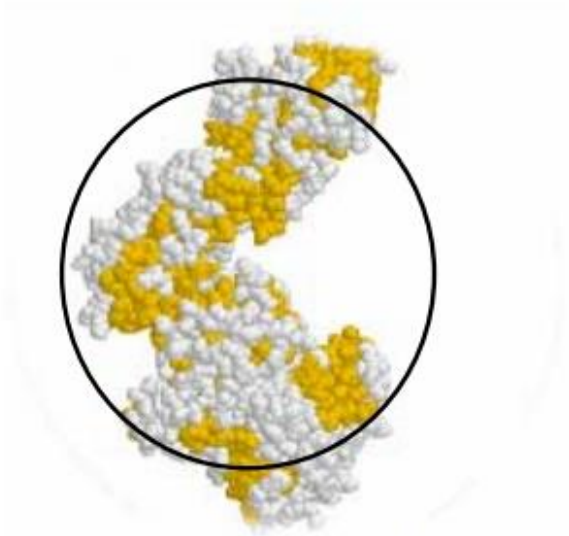
The diameter that is measured in Dynamic Light Scattering is called the hydrodynamic diameter and refers to how a particle diffuses within a fluid. The diameter obtained by this technique is that of a sphere that has the same translational diffusion coefficient as the particle being measured.
The translational diffusion coefficient will depend not only on the size of the particle "core", but also on any surface structure, as well as the concentration and type of ions in the medium. This means that the size can be larger than measured by electron microscopy, for example, where the particle is removed from its native environment.



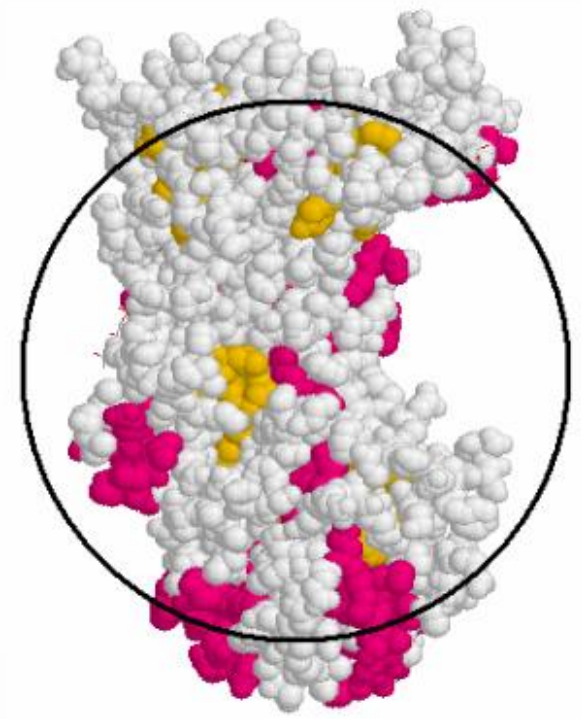
Comparative protein R_h values

Lysozyme
M_W=14.5 kDa
R_H=1.9 nm

Insulin - pH 7
M_W=34.2 kDa
R_H=2.7 nm



Immunoglobulin G
M_W=160 kDa
R_H=7.1 nm

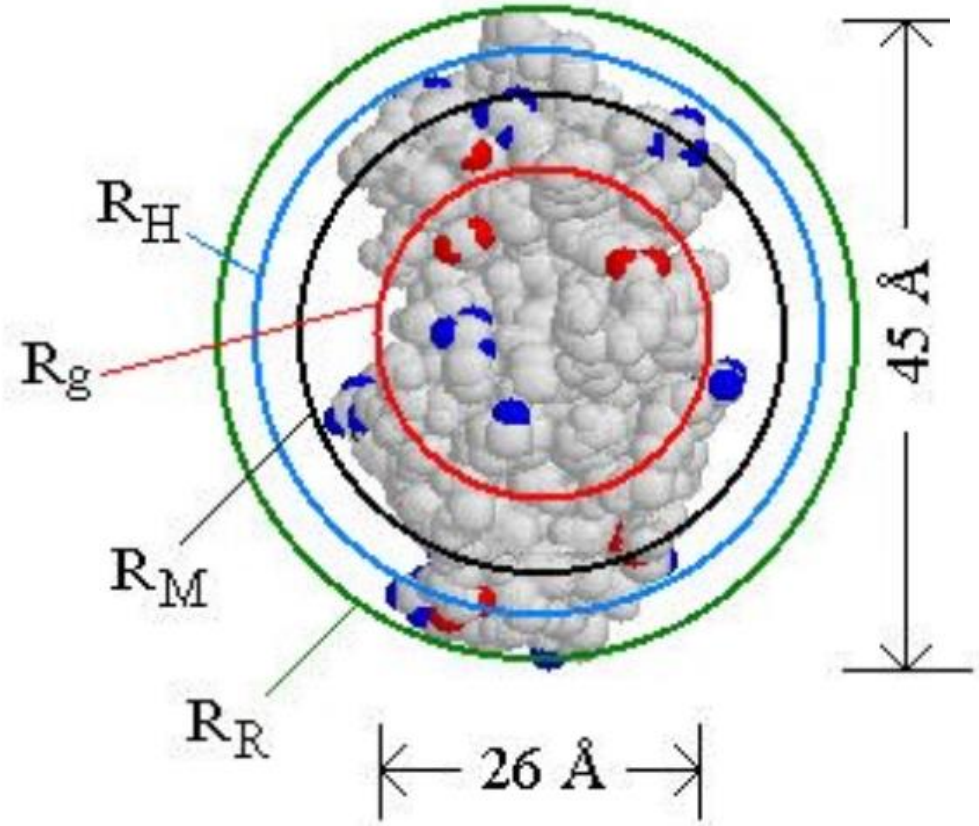


Thyroglobulin
M_W=650 kDa
R_H=10.1 nm

5 nm

Various expressinos and aproximation of protein shape

$$R_g^2 = \frac{\sum m_i r_i^2}{\sum m_i}$$



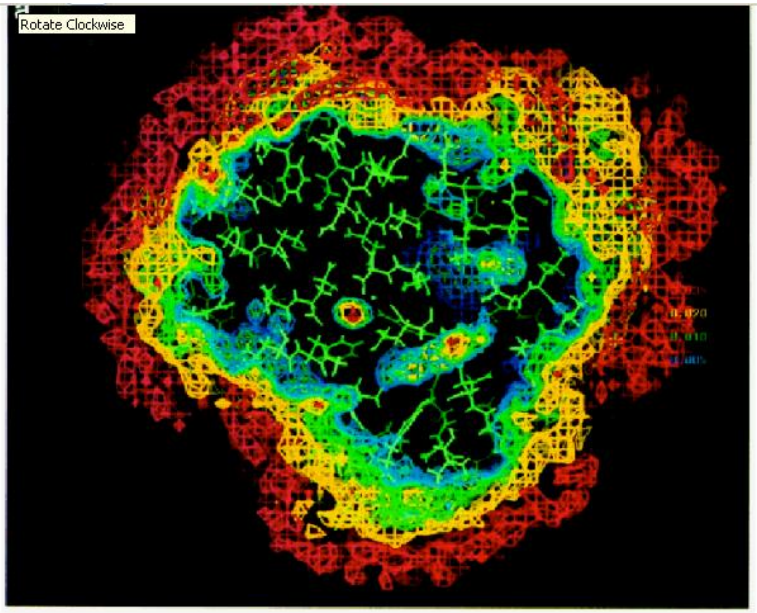
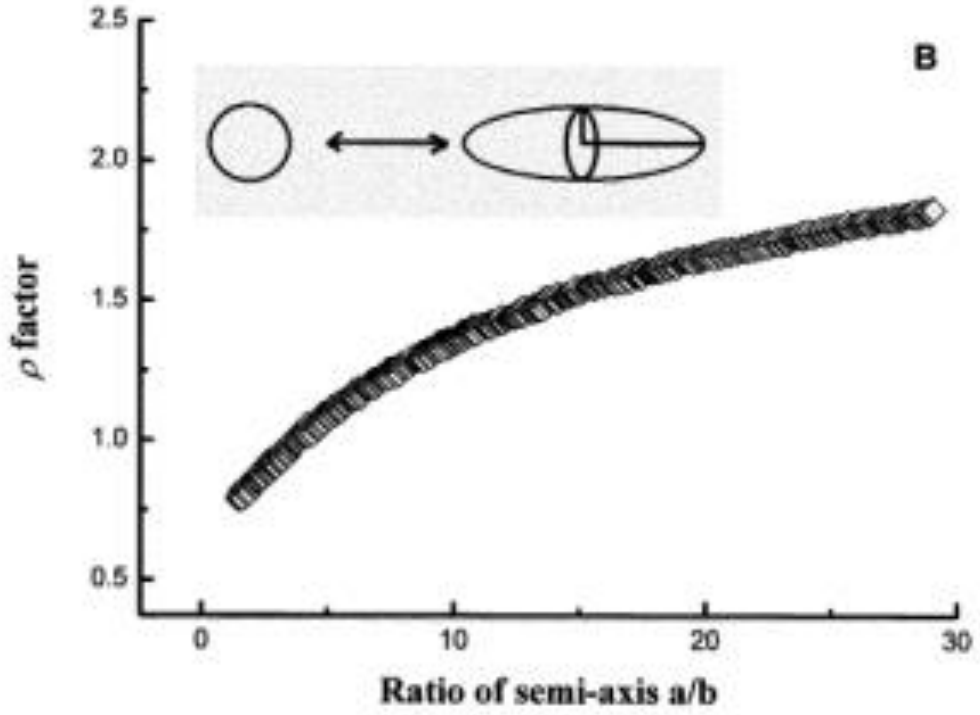


FIGURE 1. Three-dimensional density. Three-dimensional solvent number density distribution around myoglobin is shown as a slice computed from a molecular dynamics trajectory. The solvent density is overlaid with an average structure of myoglobin and contoured at 0.005 $Å^{-3}$ (blue), 0.01 $Å^{-3}$ (green), 0.02 $Å^{-3}$ (yellow), and 0.035 $Å^{-3}$ (red). The bulk solvent density is 0.033 $Å^{-3}$. (a) Density from simulation, (b) density from prediction. Reproduced with permission of the authors from ref 24.

Makarov et al (1998) *Biopolymers* **45**, 469.

Shape analysis: shape factor p = Rg/Rh



For	$\rho = R_g/R_h$
Sphere	0.774
Coil	0.816
Rod	1.732

Light scattering data

- Hydrodynamic Radius
- Distribution & Polydispersity
- Solution Composition
- Molecular Weight
- 2nd Virial Coefficient
- Conformation
- Shape Estimates
- Zeta Potential
- pI & Charge Estimates
- Formulation Stability