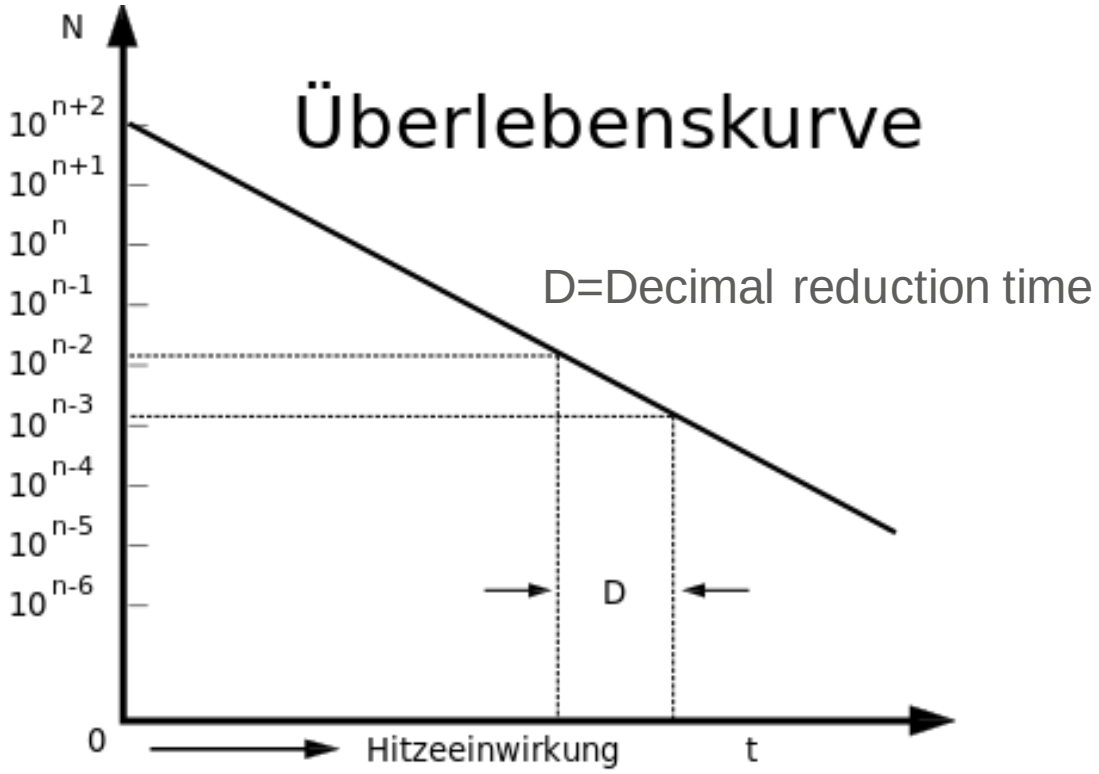
Nachtrag: Sterility and Sterilization of Materials

Sterile: free of living microorganisms, including spores and other active biological agents (prions, viruses)

Manufacturing: sterile process or terminal sterilization

Sterilization (10^{-6})
Disinfection (10^{-5})

- Heat
- Filtration
- Radiation
- Chemicals



[Bin im Garten]

Heat

moist heat sterilization (autoclaving)
121°C, 20 min or 134°C, 18 min

dry heat sterilization
160-180°C, 30-120 min

!

Potential effects:
Degradation of heat sensitive components
Disruption of physical structure
Agglomeration

[Systec]

Sterile Filtration

passage through sterile filters (0.2 µm) into a sterile container
for heat labile substances and suspensions

Compatibility of filter materials with solvents
and contained substances

Potential effects:
Stripping off adsorbed substances
Loss of materials/substances by retention/adsorption

Not suitable for highly viscous solutions

[Millipore]

Gamma Irradiation

Ionizing radiation emitted by radioisotopes, e.g. Cobalt-60

Electromagnetic radiation

Potential effects:
Formation of reactive free radicals/ cascades of free radical reactions
Formation of peroxides
Crosslinking or chain scission

Chemical Sterilization

Ethylene oxide (C_2H_4O), epoxide

toxic compound

(mutagen: changing the genetic material, DNA

clastogen: induces breakage of chromosomes)

Potential effects:
Alkylation of compounds
toxic residues

Classification of Medical Products by Type of Contact)

In contact with body surfaces
- Intact skin (e.g. electrodes, external prostheses, bandages)
- Mucous membranes (e.g. contact lenses, dental prostheses)
- Harmed or damaged skin (e.g. wound dressing)

From the outside in contact with the inner body
- vasculature, indirect (e.g. transfusion devices, tubing)
- Tissue, bones, dentin (e.g. dental cement, fillings)
- Circulating blood (e.g. intravascular catheter, dialysers)

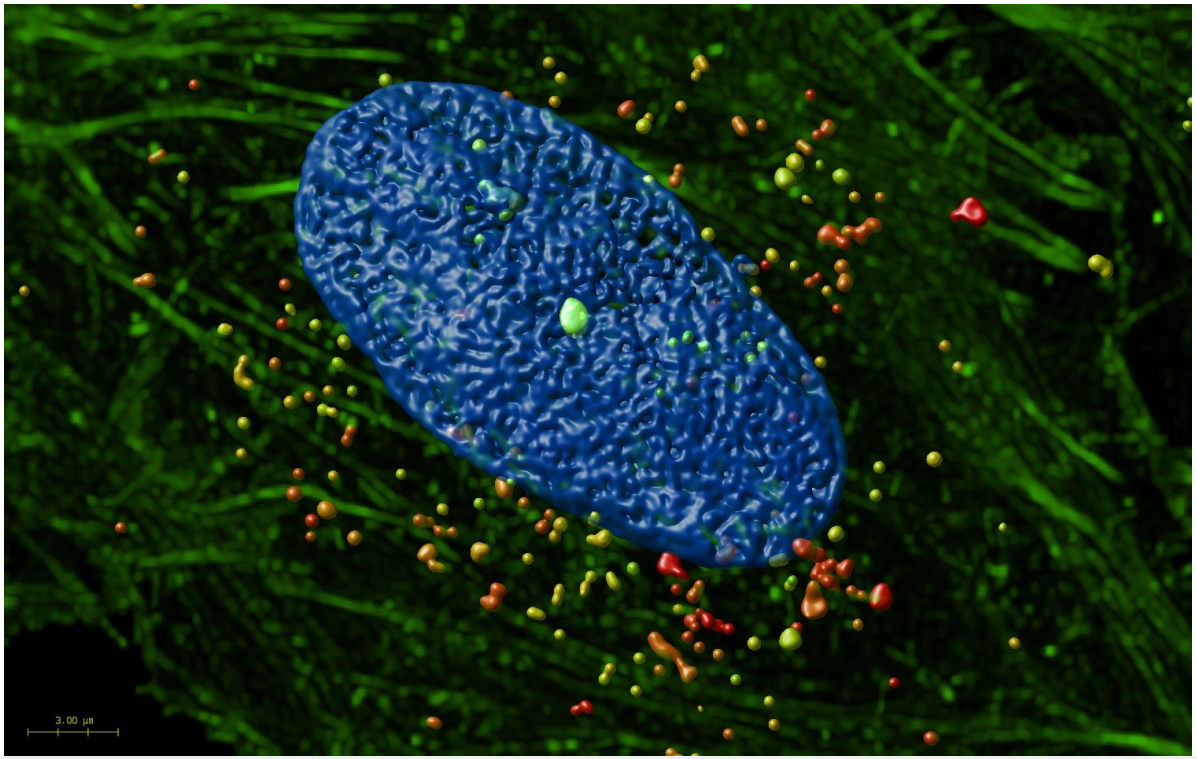
Implantable
- tissue, bone (e.g. nails, plates, prostheses, pacemakers)
- Blood (e.g. heart valves, stents)

Types of Contact Duration and Biompatibility

- Short-term (up to 24 h)
- Prolonged (24 h – 30 days)
- Permanent (> 30 days)

- Systemic Toxicity (by release of constituents into the body)
  acute (within 24 h), subacute (up to 28 days)
  subchronic (10 % of the life span), chronic effects (> 10% of the life span)

- Skin, eye or mucous membrane irritation (e.g. inflammation)
- Hemolysis (lysis of red blood cells)
- Thrombogenicity (potential for clot formation)
- Sensitization (including an immune response)
- Genotoxicity (DNA destruction, mutation, chromosomal aberration)
- Cancerogenity (tumorigenic potential)
- Reproductive toxicity (e.g. embryonic development)



Nanosafety
July 12, 2019

Dr. Annette Kraegeloh
INM - Leibniz Institute for New Materials

Nanomaterials Definition

"Nanomaterial" means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm.

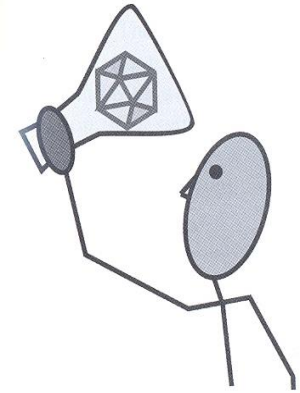
In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %.

Fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials.

[COMMISSION RECOMMENDATION on the definition of nanomaterial, 2011]

a specific surface per unit volume of greater than 60 m^2/cm^3

Nanomaterial Types

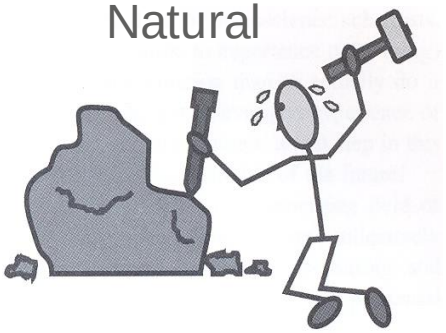


hydrothermal reactor [INM]

Manufactured

Incidental

Natural



Nanomaterials Characterization

measurement methods:

Electron Microscopy,

Dynamic Light Scattering,

Centrifugal Liquid Sedimentation,

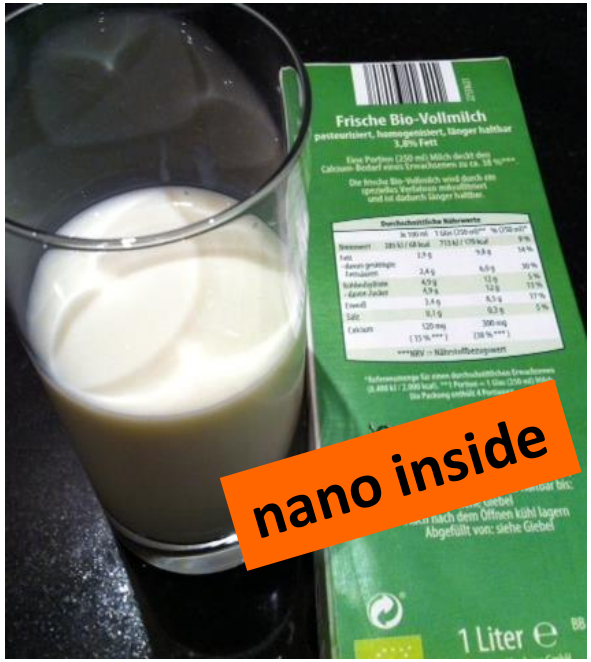
Field Flow Fractionation,

Particle Tracking Analysis,

Atomic Force Microscopy,

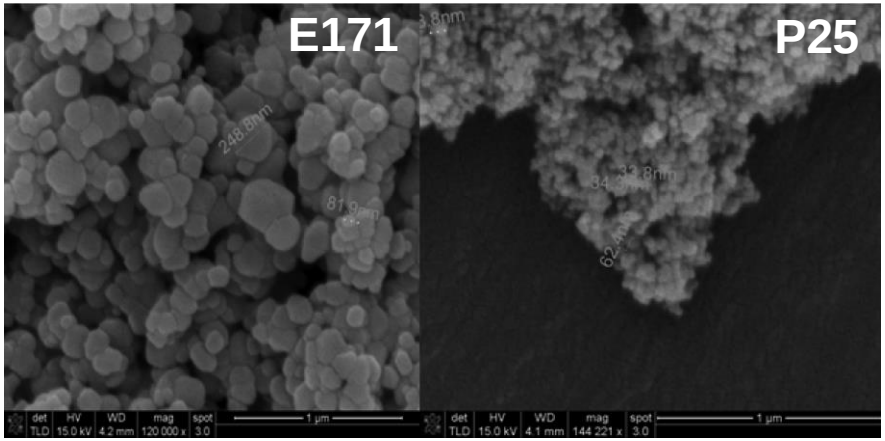
BET (N_2 adsorption by Brunauer, Emmet, Teller)

[JRC reference report 2012]



Nanomaterial or Not?

TiO_2:



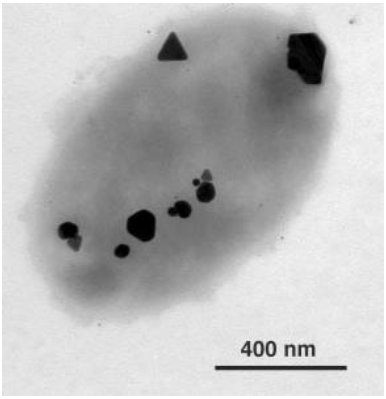
E171:
110 nm (30-400 nm) (SEM)
36% < 100 nm (TEM)
150 nm (0,75% BSA, DLS)

P25:
30-40 nm (TEM), 21 nm (SEM)

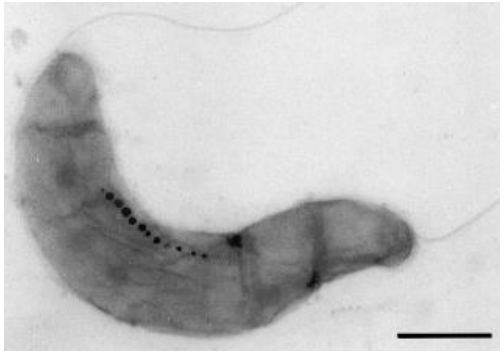
136 nm, 30% of the particles < 100 nm
BET surface: 50 m^2/g

[Weir 2012; Bundschuh 2012; NanoCare Final Report 2009]

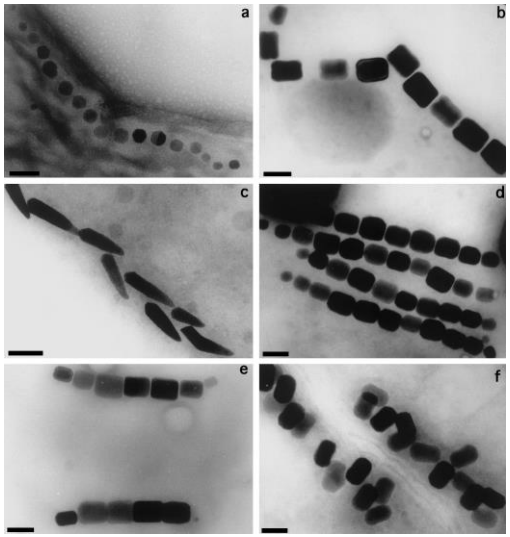
Biological Nanostructures (Inorganic)

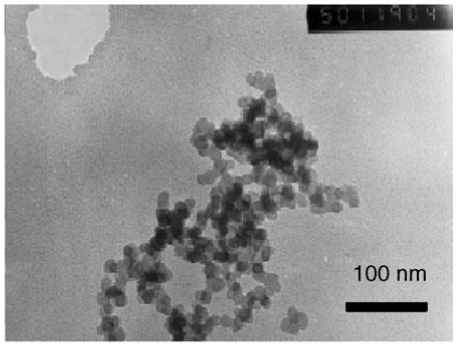


[Klaus et al., 1999]

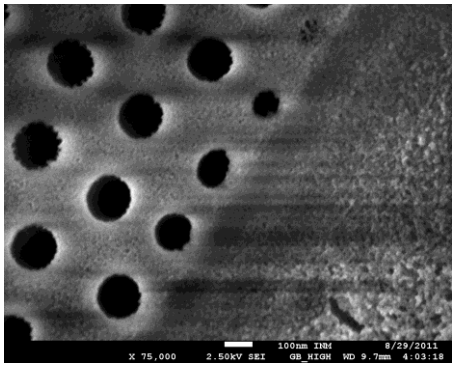


[Schüler and Frankel, 1999]



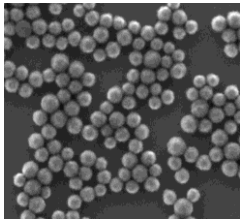
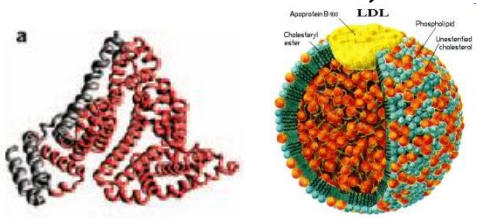
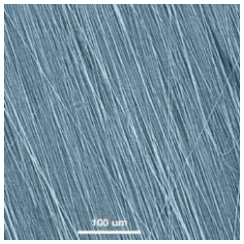
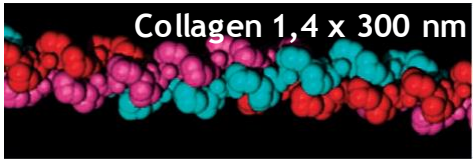
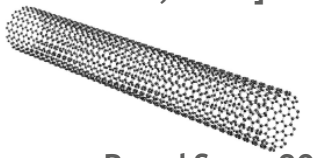
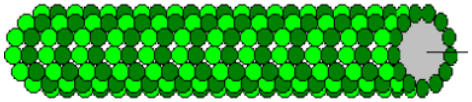
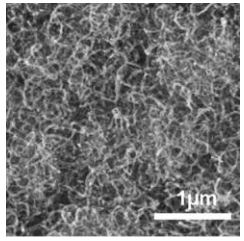
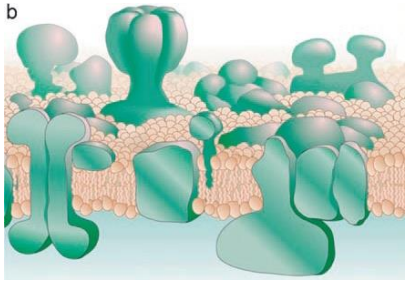


horse tail: SiO_2 structures
[Curry und Perry, 2007]



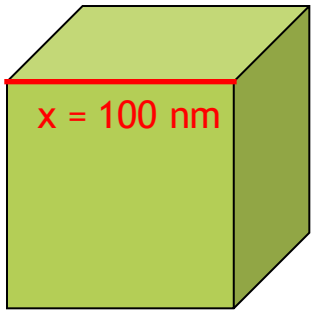
diatoms: SiO_2 structures
[INM]

Biological Nanostructures (Organic)

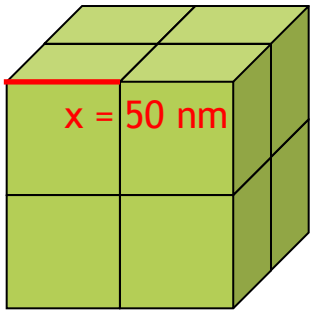
Nanostructure	Engineered	Biological
❑ Nanoparticles		Serumalbumin 8 x 3 nm, LDL 20 nm  [Ferrer et al., 2001]
❑ Nanofibres	 [Dersch et al., 2005]	 [Mann, 2008; Röcker et al., 2009]
❑ Nanotubes	 [Nanorep. Royal Soc., 2004]	Microtubules 25 nm 
❑ Nanostructured Surfaces	 [Veith et al., 2008]	 Cytoplasmic membrane 5 nm [Mann, 2008]

Size Dependent Properties of Nanoobjects 1

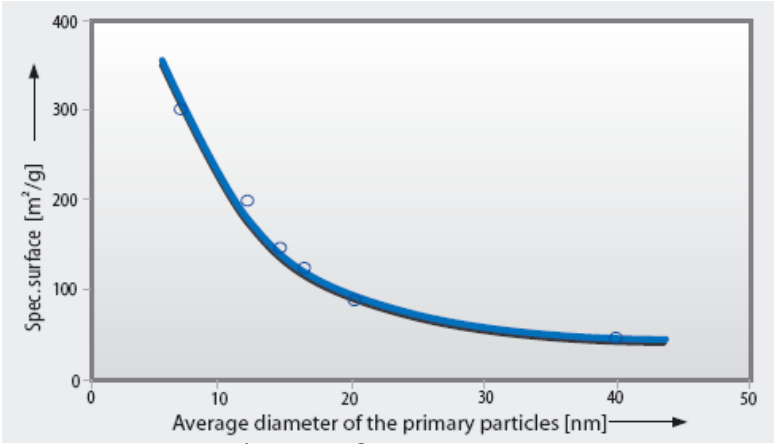
High Surface to Volume Ratio



O = 6 10^4 nm^2
V = 1 10^6 nm^3

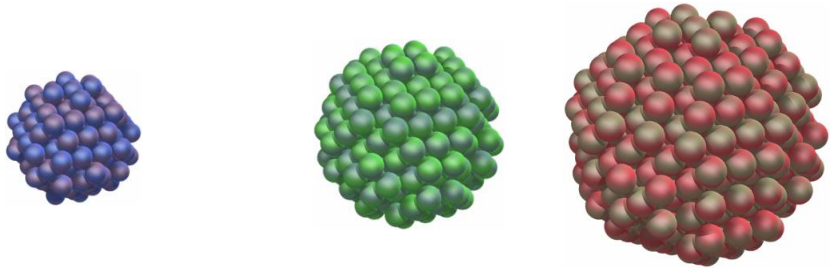


O = 1,5 10^4 nm^2
V = 1,3 10^5 nm^3



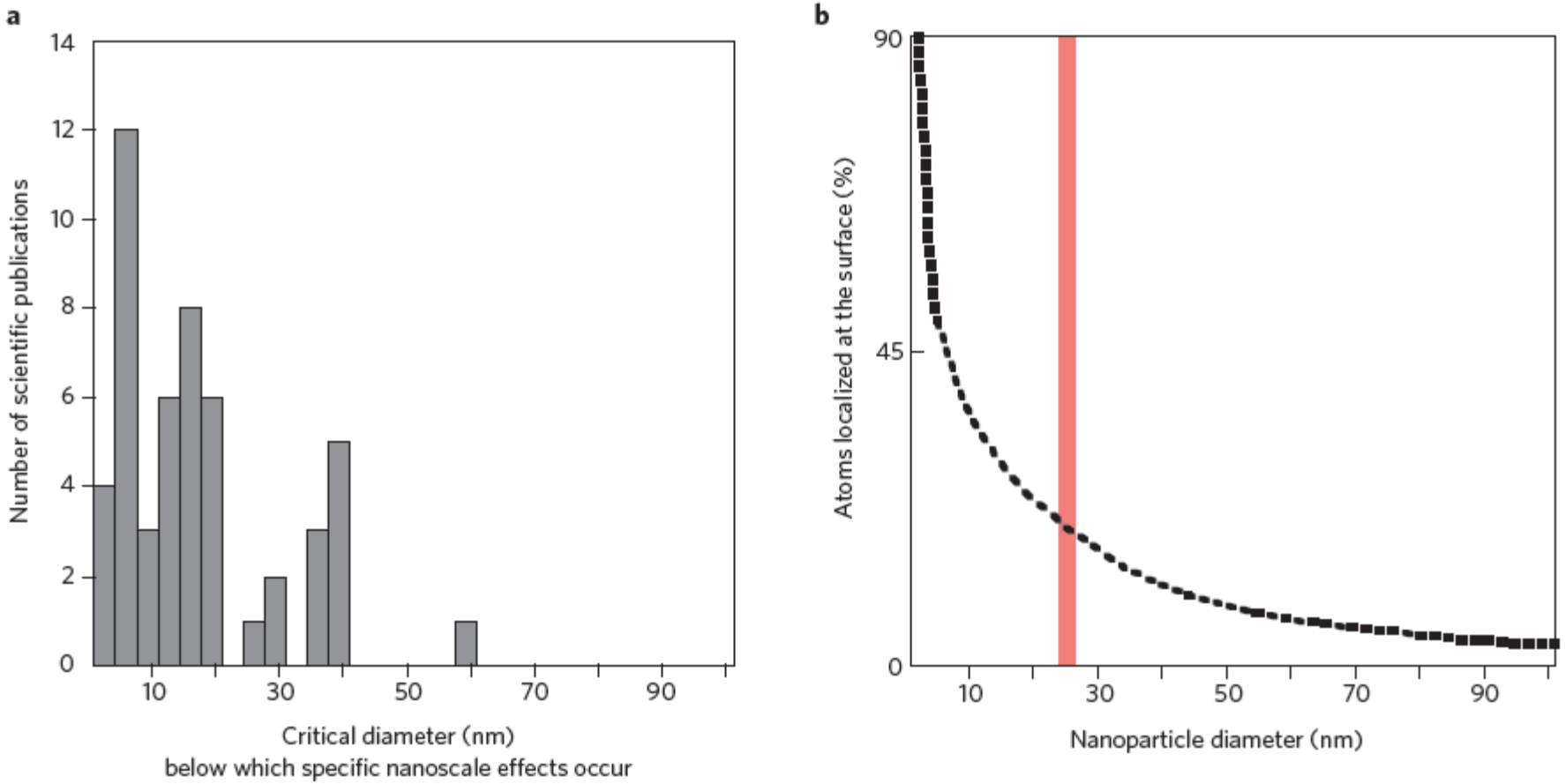
[Techn. Bull. Fine Part.]

Luminescence



CdSe Quantum Dots [Niemeyer, 2005]

Size Dependent Properties of Nanoobjects 2

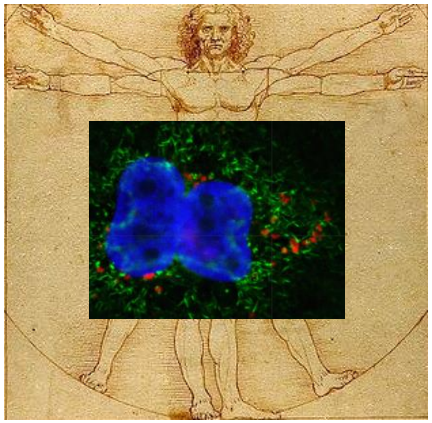


[Auffan et al., 2009]

Safety Aspects and Nanotoxicology

Production and Manufacturing

Usage by Consumers



Biomedical Applications

Hazard?

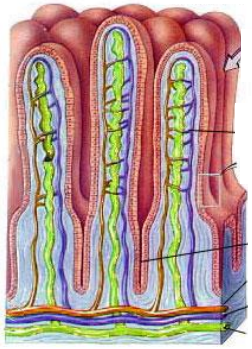
Exposure?

Risk?

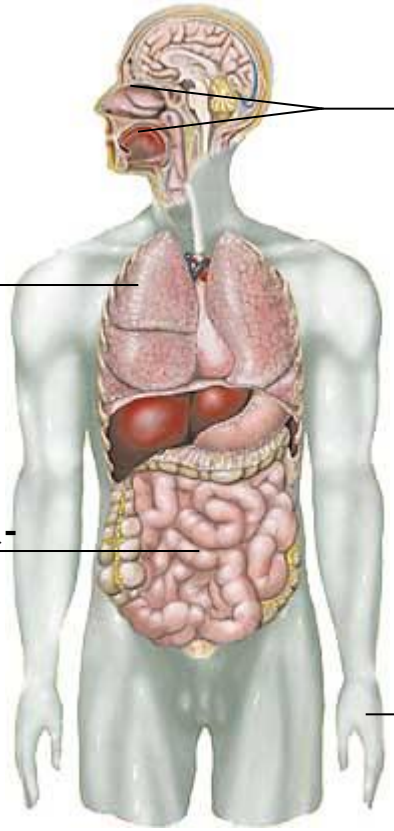
Environmental Issues

Potential Routes of Entry into the Human Body

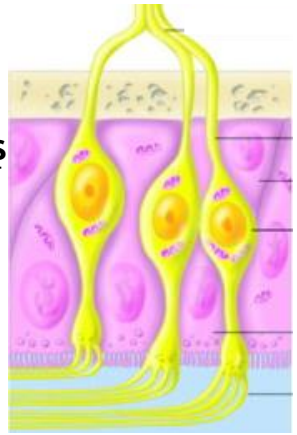
lung (~100 m^2)



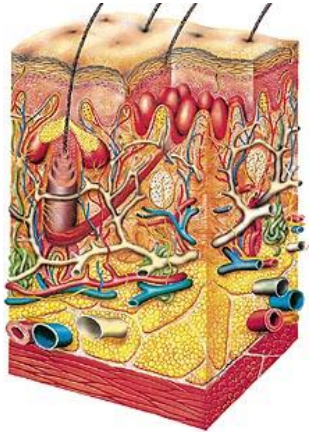
gastro-intestinal-tract (~200 m^2)



mucous membranes

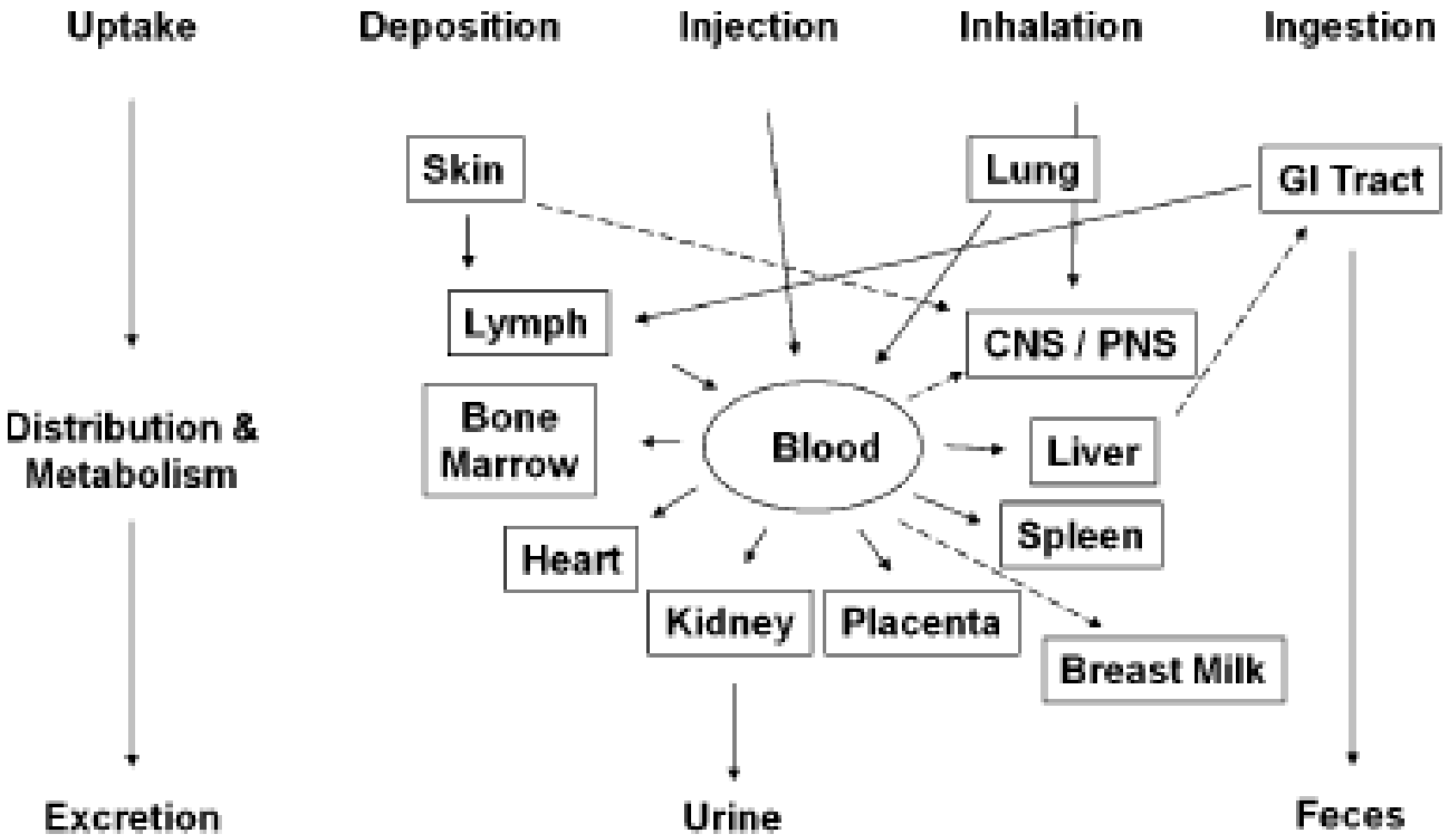


skin (~2 m^2)



[Oberdörster *et al.*, 2005]

Translocation Routes of Nanoparticles within the Body !

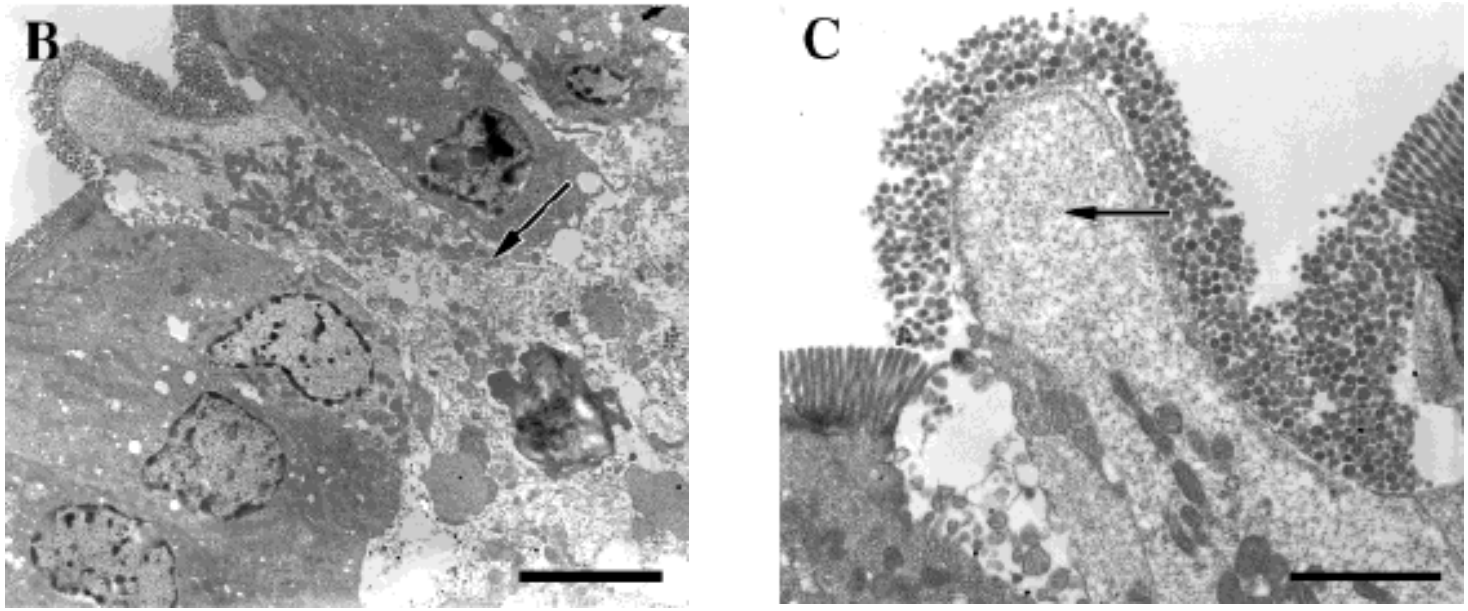


(Nel et al., 2006)

Tissue Distribution of Gold Nanoparticles after Oral Uptake

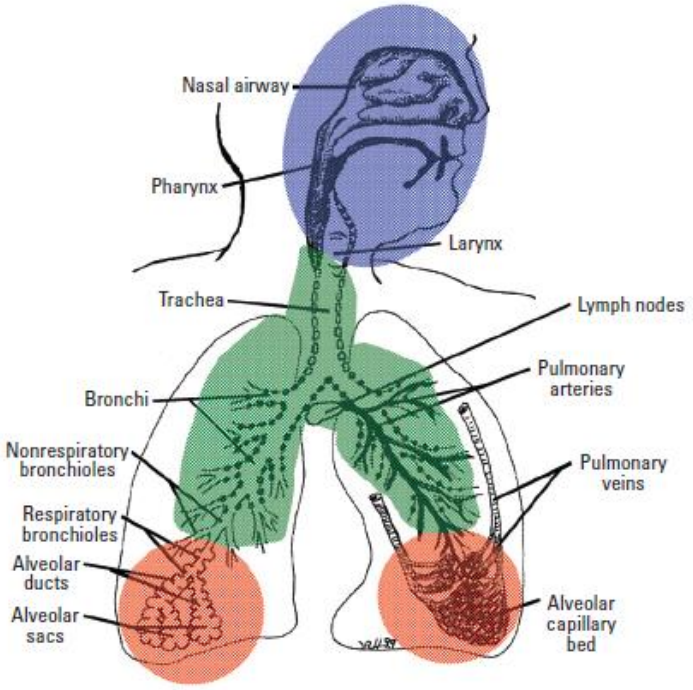
Table 1. Colloidal Gold Distribution (PPB, $ng_{Au}/g_{tissue} \pm$ S.E.) at the Organ/Tissue Level of BALB/c Mice[a]

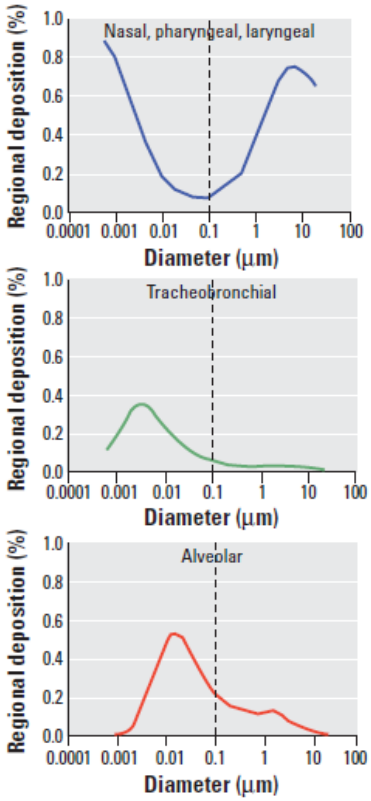
	4 nm	10 nm	28 nm	58 nm
Blood	6.83 ± 0.69	0.77 ± 0.07	0.47 ± 0.20	-0.09 ± 0.10
Brain	4.67 ± 0.26	2.06 ± 0.78	0.70 ± 0.20	2.40 ± 1.30
Lung	32.42 ± 9.37	8.55 ± 4.90	0.80 ± 0.35	-1.43 ± 0.37
Heart	15.05 ± 2.05	7.27 ± 3.25	1.51 ± 0.78	-2.37 ± 0.86
Kidney	75.40 ± 10.65	17.36 ± 1.51	6.19 ± 1.51	1.46 ± 0.33
Spleen	20.25 ± 2.27	7.01 ± 4.65	13.74 ± 13.48	-0.65 ± 1.20
Liver	21.27 ± 3.62	2.78 ± 0.30	1.16 ± 0.03	0.37 ± 0.16
S. intestine	38.83 ± 9.16	13.89 ± 3.11	214.95 ± 150.49	17.59 ± 2.53
Stomach	22.44 ± 4.66	19.99 ± 7.72	37.92 ± 30.42	19.56 ± 9.87

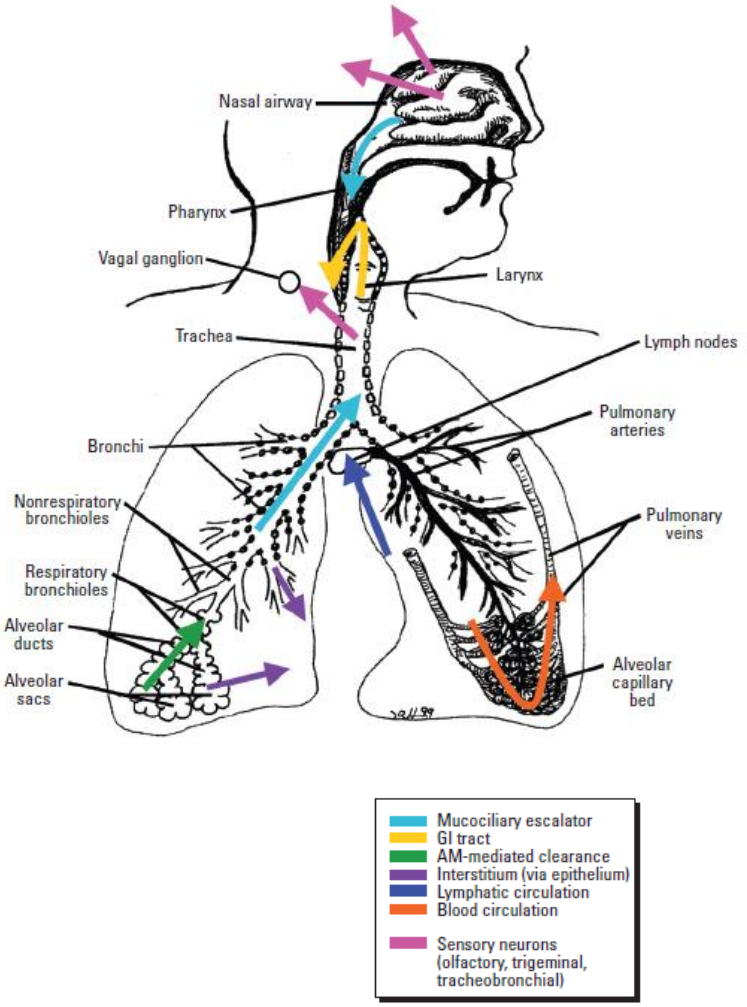


(Hillyer et al., 2001)

Size Dependent Distribution of Particles in the Respiratory Tract

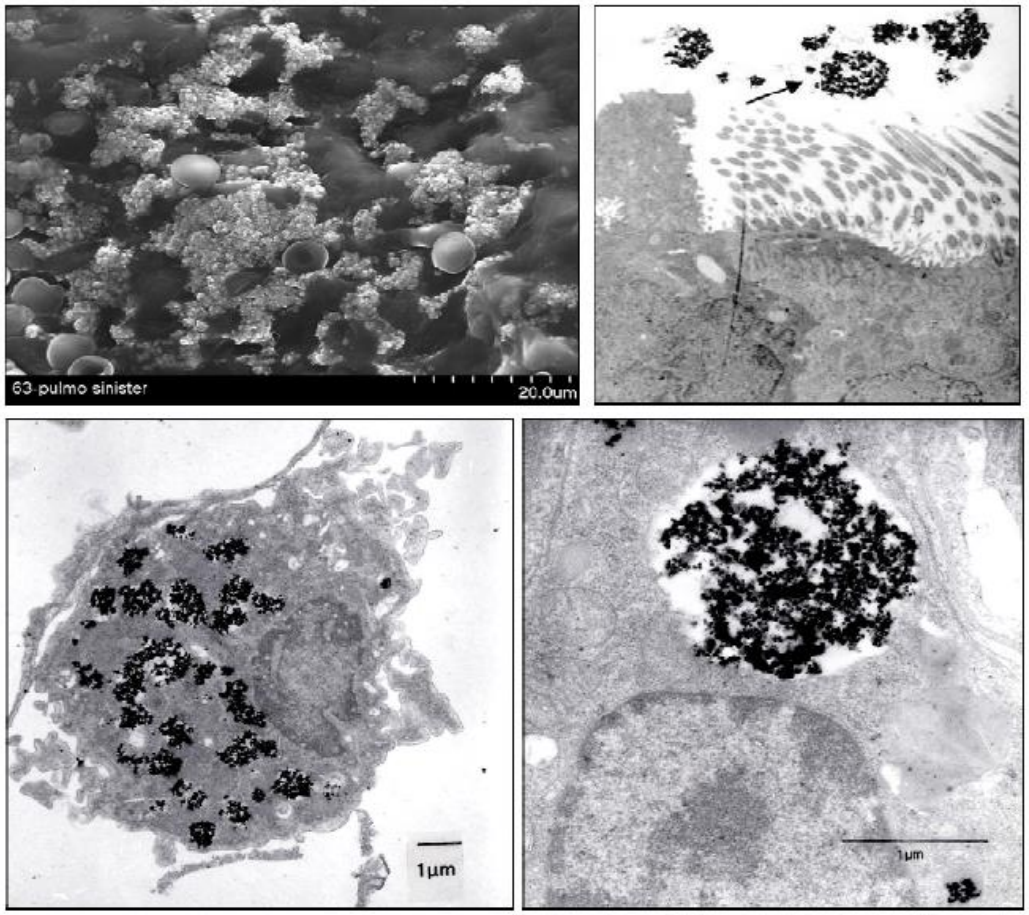






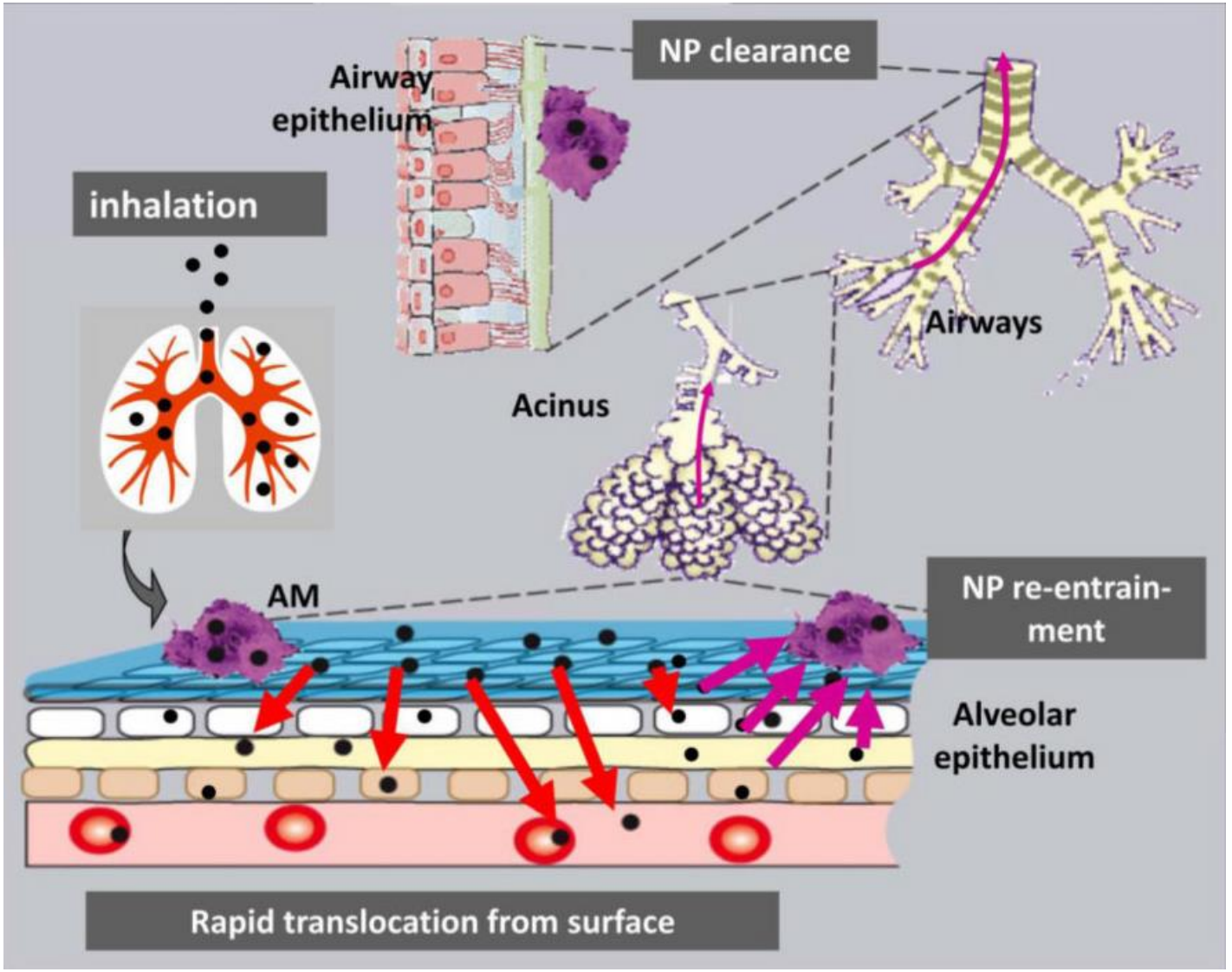
[Oberdörster *et al.*, 2005]

Deposition of Nanoparticles after Inhalation



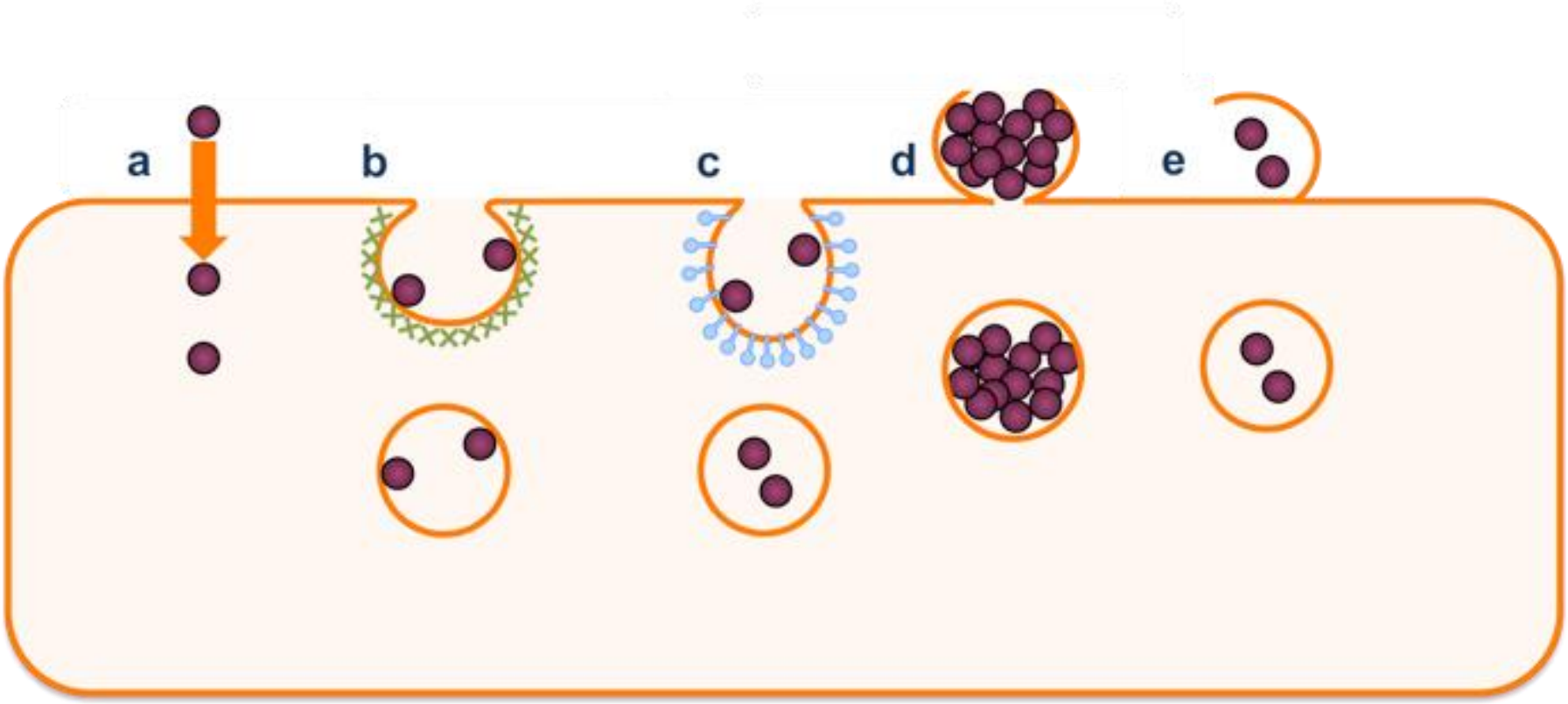
[NanoCare Report, 2009]

Deposition and Relocation of Nanoobjects in the Lung !



(Kreyling et al., 2013)

Endocytosis of Nanoparticles



penetration

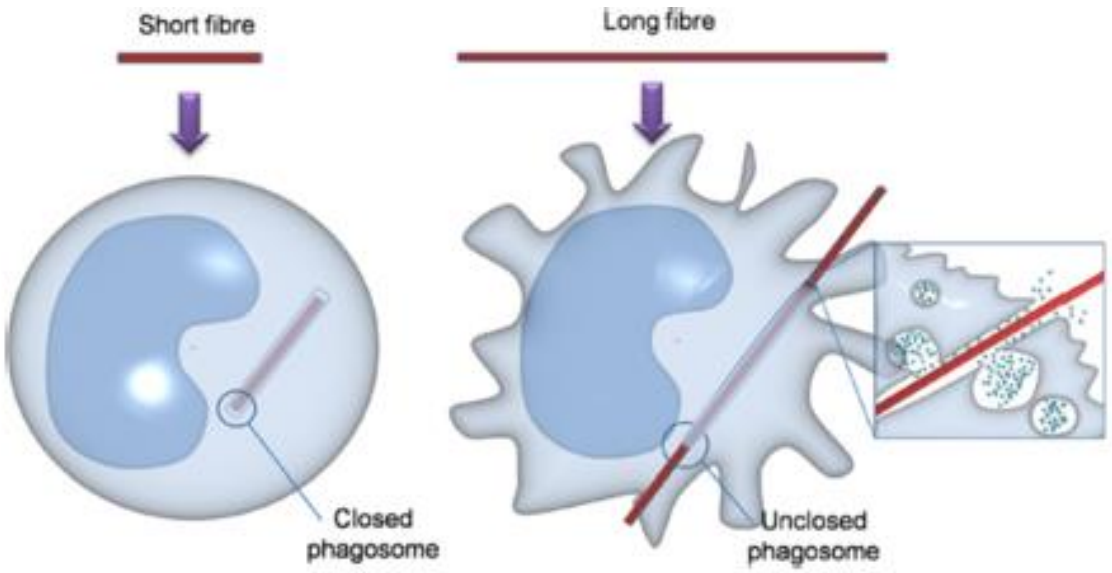
clathrin-mediated

caveolin-mediated

phagocytosis

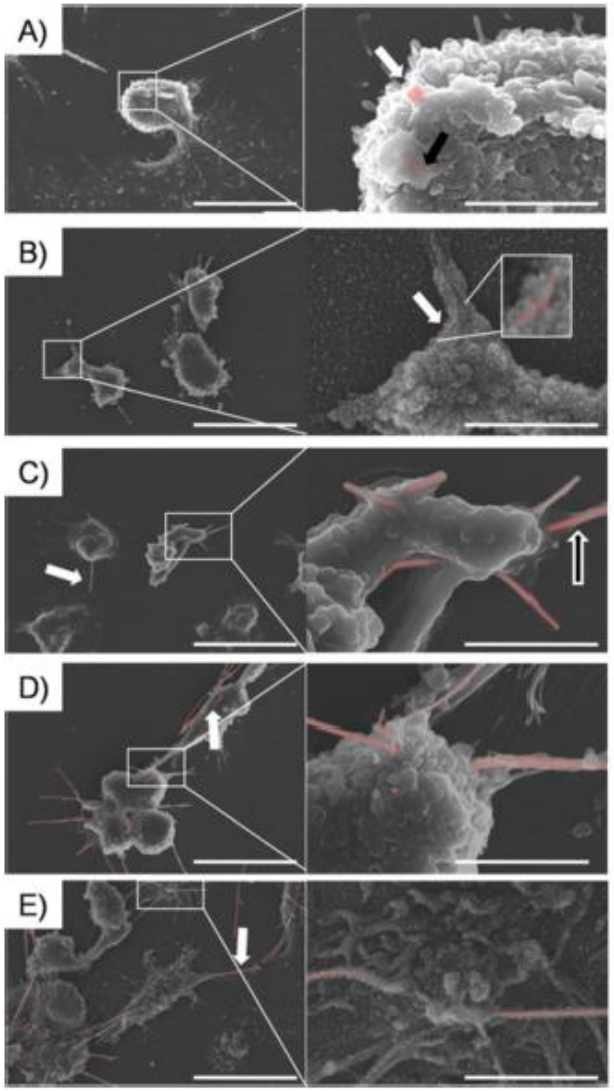
macropinocytosis

Frustrated Phagocytosis of Fibres Contributes to Toxicity !



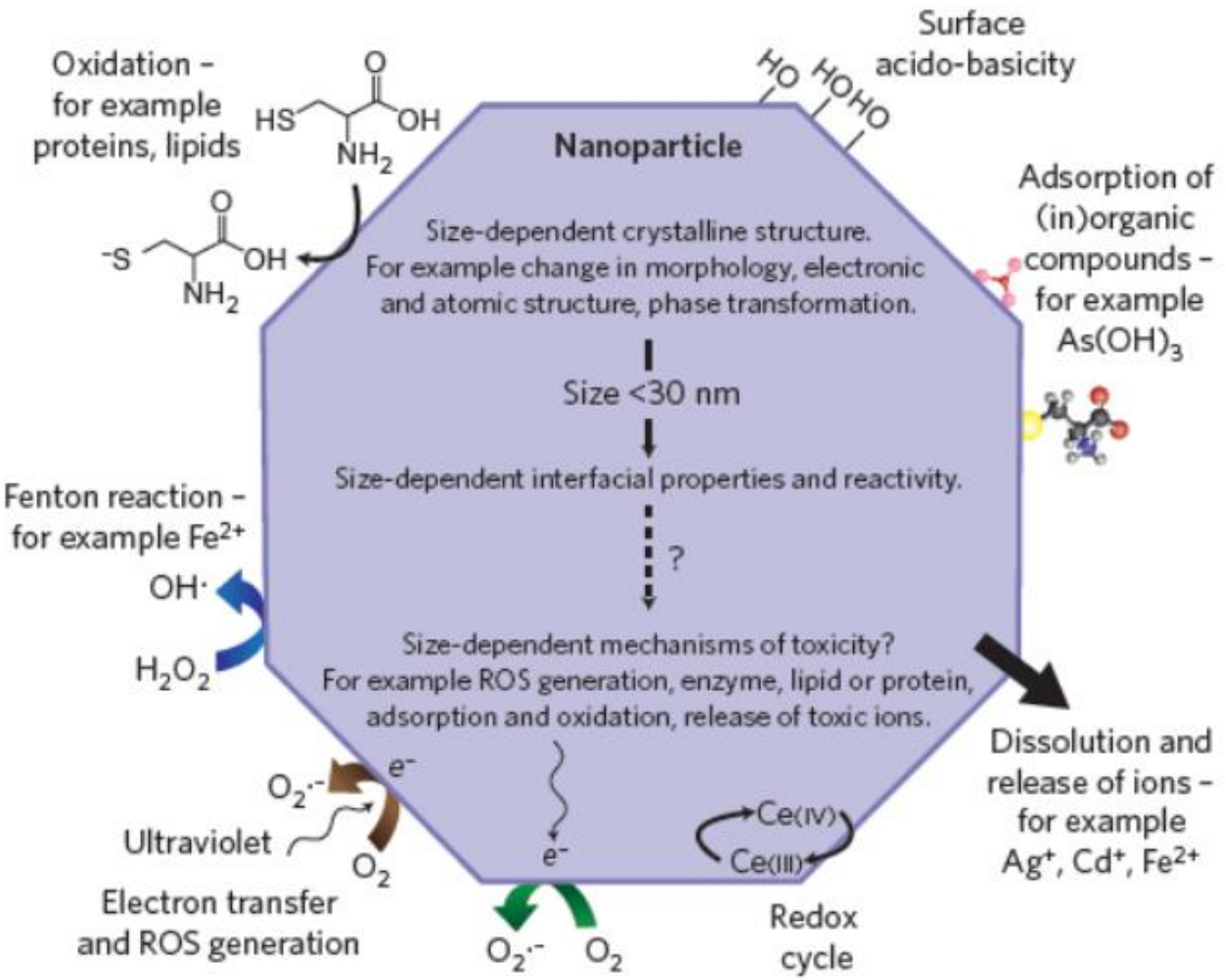
phagocytosis

frustrated phagocytosis inflammation



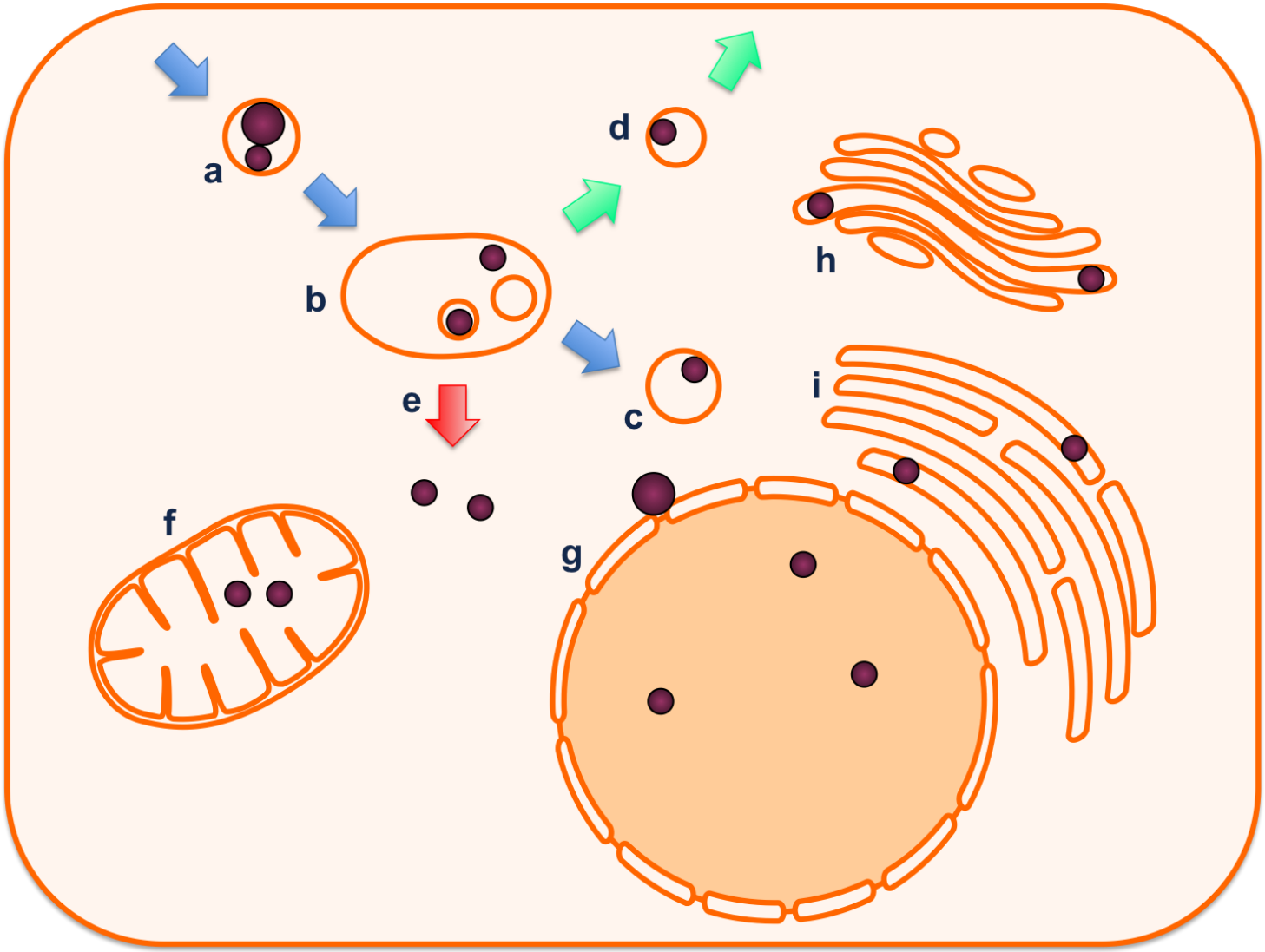
(Schinwald and Donaldson, 2012)

Biologically Relevant Properties of Nanoobjects



[Auffan 2009]

Potential Cellular Targets of Nanoobjects



Cellular Effects Induced by Nanoobjects

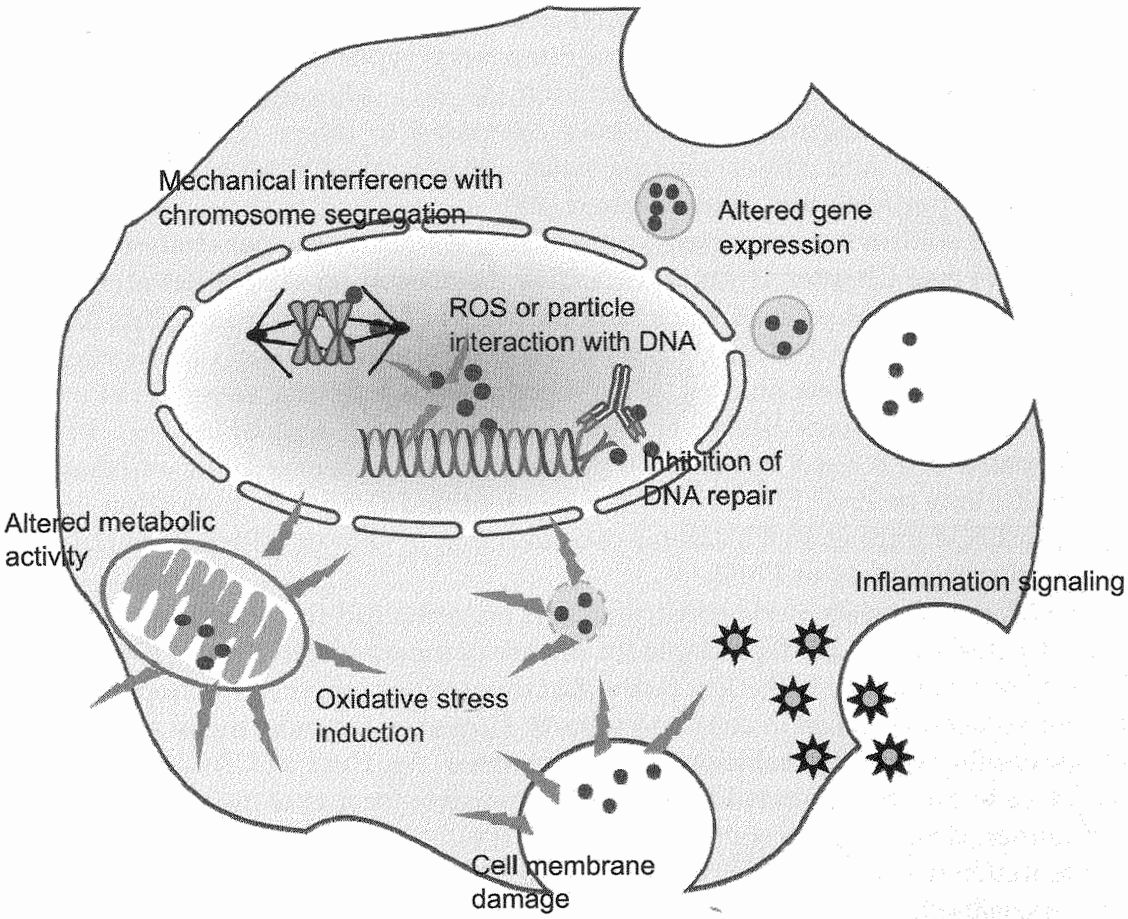
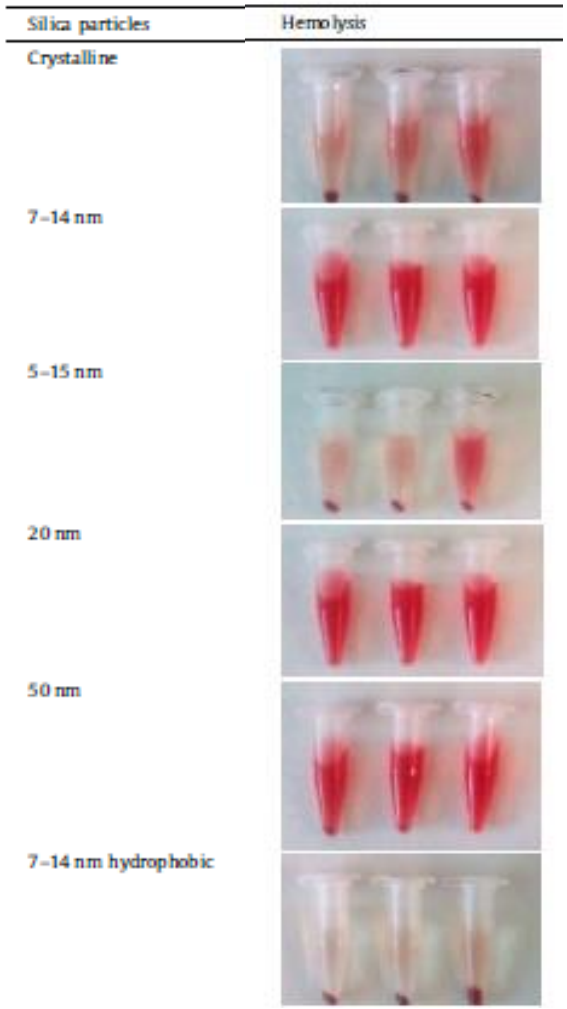
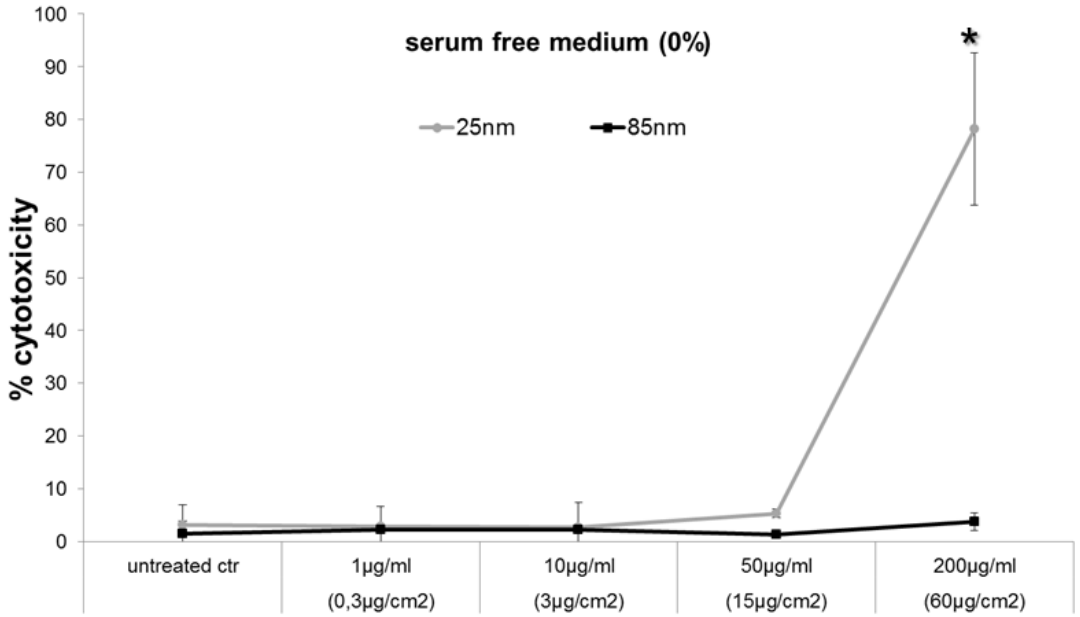


FIGURE 1.4 Possible interactions of ENM with the cell and subcellular structures. Suggested mechanisms underlying nanoparticle-induced responses at the cellular level which, at sufficiently high or persistent levels, potentially can lead to altered tissue function and damage.

(Fadeel et al., 2012)

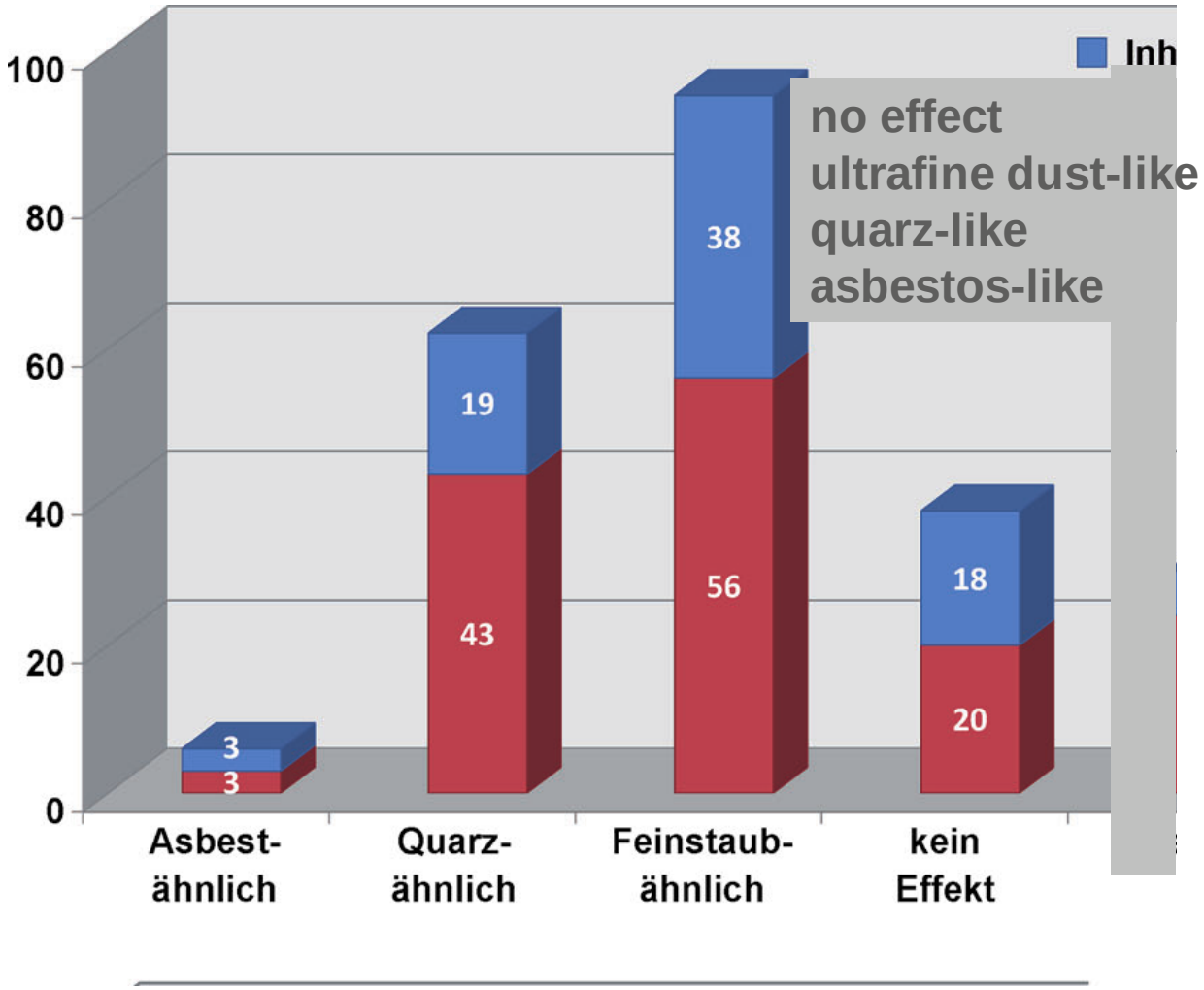
Effects of Nanoparticles on the Integrity of Whole Cells





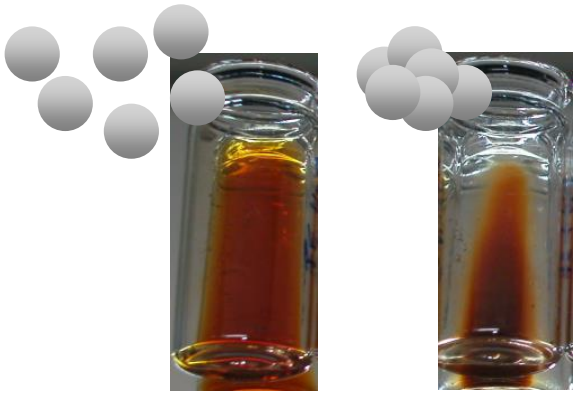
(Shi et al., 2012; Peuschel and Ruckelshausen 2015)

In vivo Effects of Nanoobjects

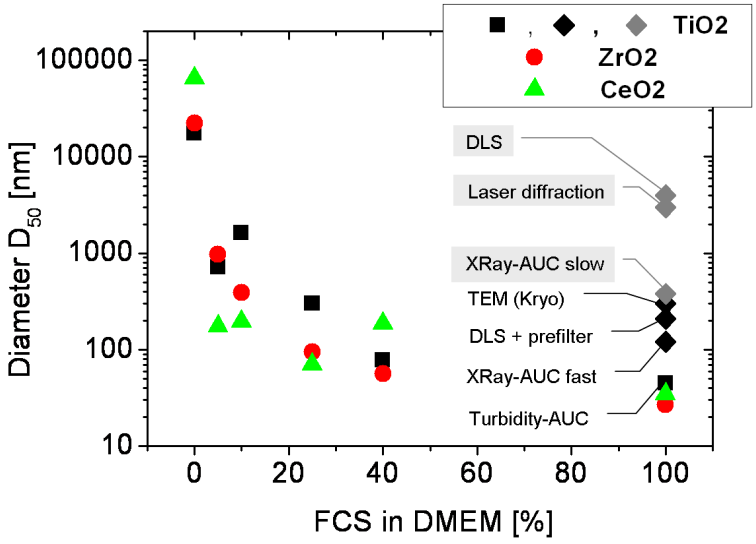


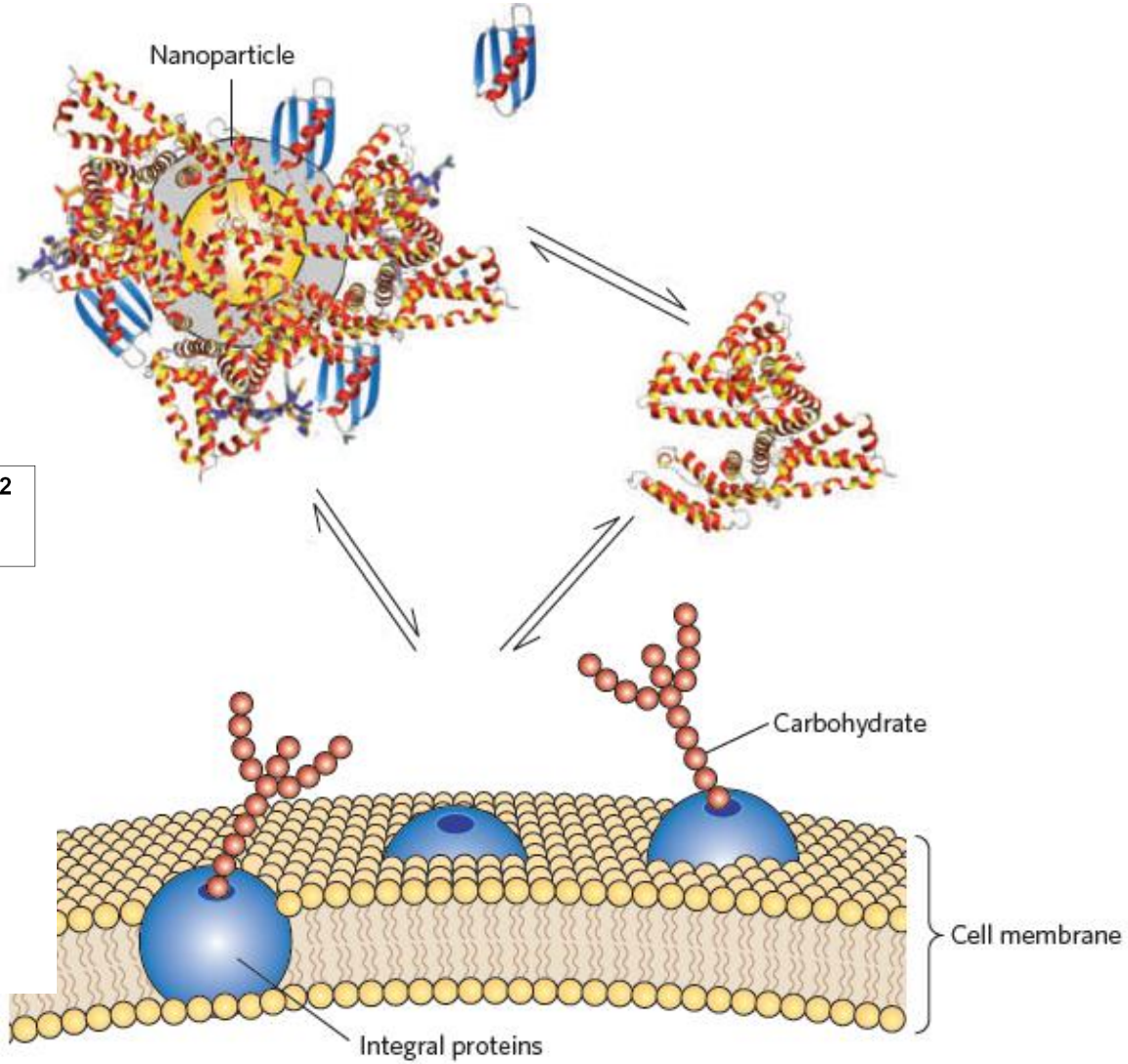
[Krug 2014]

Influence of Proteins on Nanoobjects



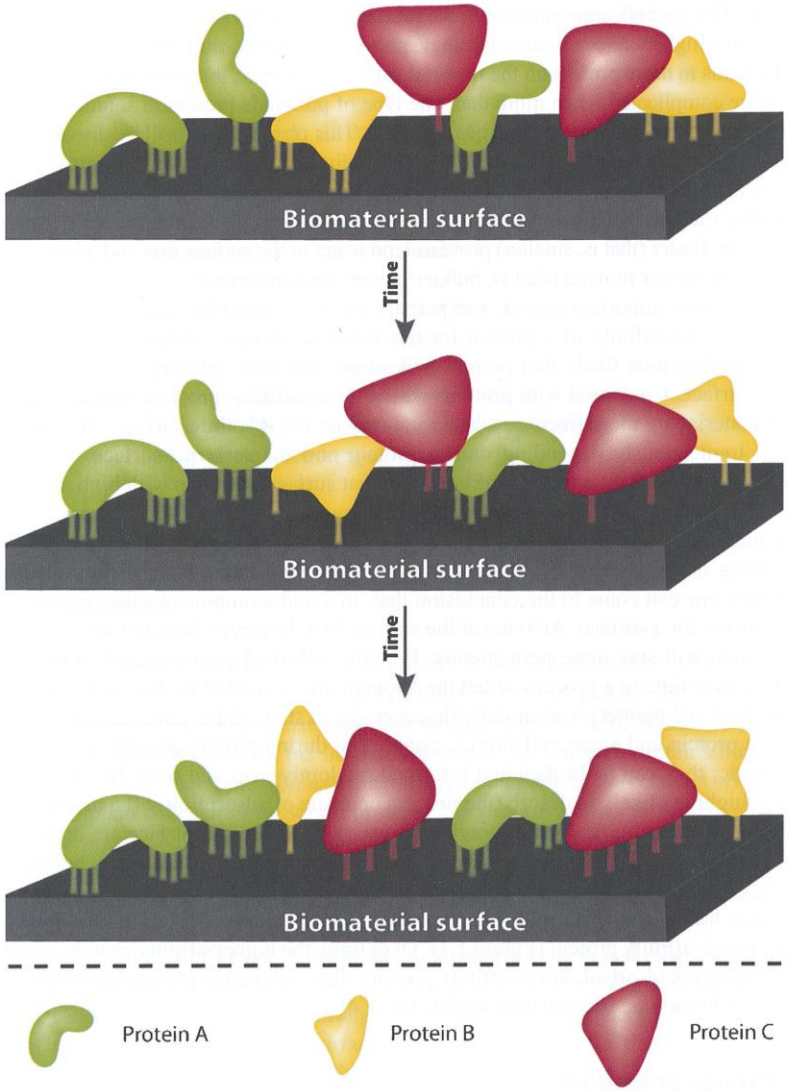
Agglomeration





[Röcker et al., 2009; Lynch et al., 2009; NanoCare 2009

Protein Adsorption to Biomaterials



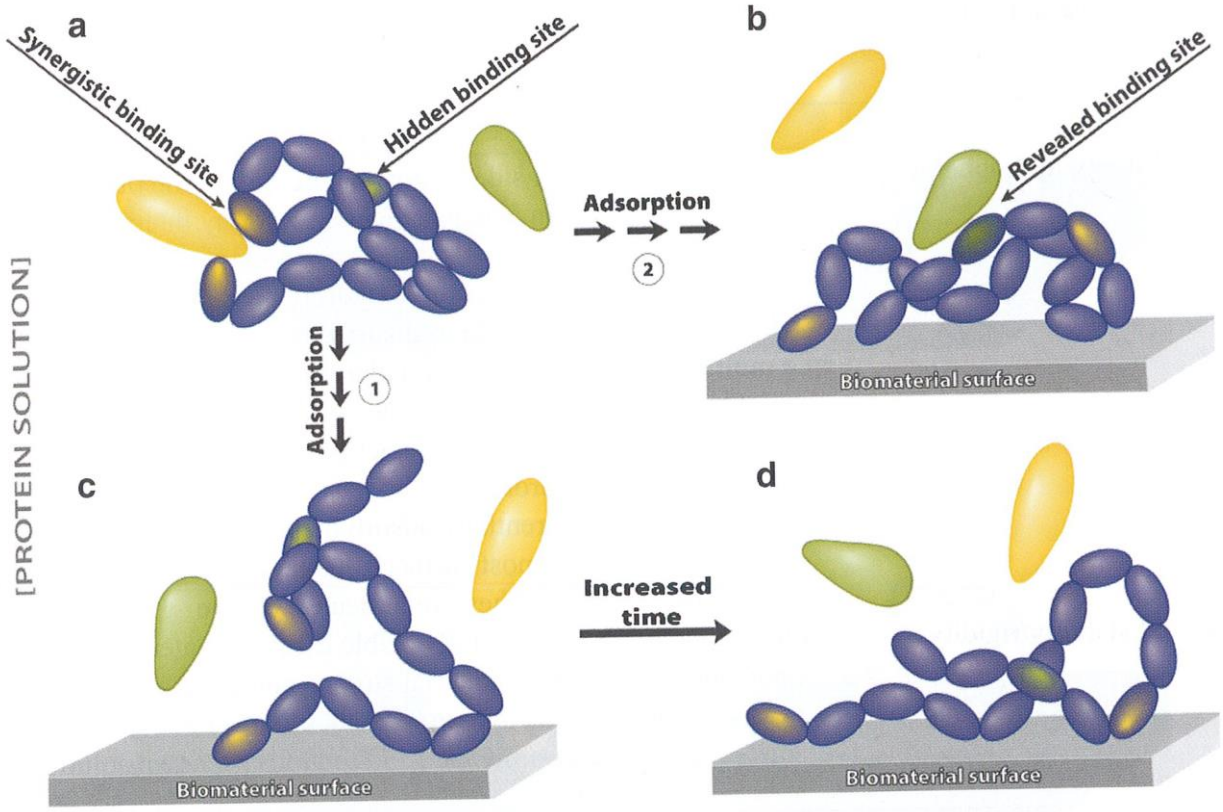
Protein adsorption is dependent on:

- **protein concentration**
- **rate of diffusion**
- **affinity**

Vroman Effect

(Figure 1.1, Puleo and Bizios, 2009)

Protein Conformational Changes upon Adsorption on Surfaces



Protein properties affecting adsorption:

- **hydrophilicity/ hydrophobicity**
- **size**
- **charge**
- **structural stability**

(Figure 1.2, Puleo and Bizios, 2009)

Material Surface Properties Affecting Protein Adsorption

Surface Property	Description
hydrophilicity/ hydrophobicity	hydrophobic surfaces tend to adsorb more proteins
charge	opposite charges between surface and protein promote protein adsorption
topography	increased roughness provide increased surface area
chemistry	chemical composition dictates the types of bonds between protein and material surface

(Puleo and Bizios, 2009)

Questions

- ❑ What are nanomaterials, how are they defined? What are natural, incidental, manufactured nanomaterials?
- ❑ How are size, surface and volume of nanoobjects interrelated?
- ❑ What are important biologically relevant properties of nanoobjects, apart from size?
- ❑ what are important entry routes of nanoobjects into the body?
- ❑ Describe the inhalative pathway of nanoobjects!
- ❑ How do nanoobjects enter cells? What is frustrated phagocytosis?
- ❑ What effects do nanoobjects have on a cellular level?
- ❑How do proteins interact with material surfaces?
- ❑ Which techniques are used to sterilise biomaterials?

Literature

Books:

- Fadeel et al. (2012) Adverse Effects of Engineered Nanomaterials

Articles:

- Krug and Wick (2011) Nanotoxicology an interdisciplinary challenge, Angewandte Chemie Int. 50: 1260
- Krug (2014) Nanotoxikologie, Angew. Chemie 126: 12502

Written Exam: July 19, 2019

2nd try: September 20, 2019 (10-12) HS A, D2.2