TUTORIAL
Biology for the Physicist, Chemist and Engineer

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Schedule

- Part 1
  - Introduction
    - Nanoparticles in biomedicine
      - Magnetic Nanoparticles/Nanomaterials
      - Biokinetics
  - Interaction with Biological Fluids
    - General
      - Blood
- Part 2
  - Interaction with Biological Borders
    - Cells
    - Tissues/Organs
  - Cytotoxicity
- Part 3
  - Biodistribution
  - Degradation
  - Elimination

Schedule - Part I

- Part 1
  - Introduction
    - Nanoparticles in biomedicine
      - Magnetic Nanoparticles/Nanomaterials
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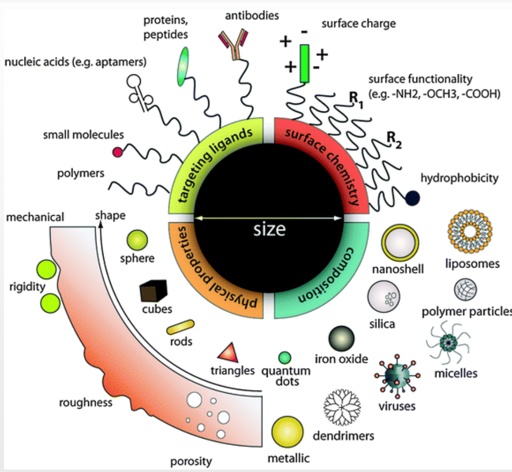
  - Interaction with Biological Fluids
    - General
      - Blood
        - » Compounds – molecules and cells
        - » Physico-chemical properties
    - Stability
- Part 2
- Part 3

Biomedical applications of nanoparticles

- Drug delivery
- Hyperthermia
- Targeting of cells
- Tracking of cells
- Monitoring of diagnostic parameters

World of nanoparticles



Composition
magnetic particle

Physical properties
spheres

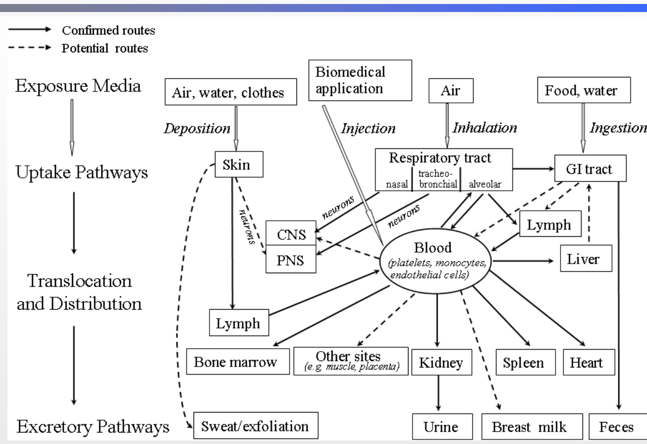
Targeting ligands
face biological systems

Surface chemistry
face biological systems

Chou LYT, Ming K, Chan WCW
Chem. Soc. Rev., 2011, 40, 233-245

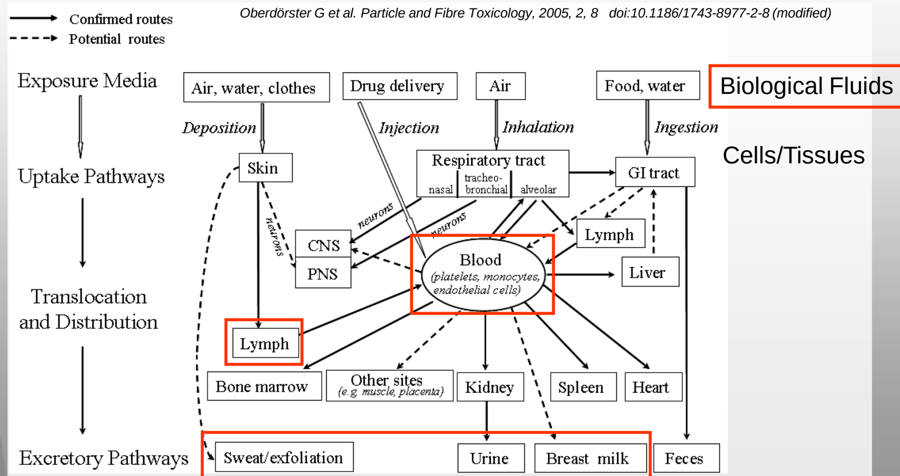
Natural nanoparticles

Biokinetics of Nanosized Particles



Oberdörster G et al. Particle and Fibre Toxicology, 2005, 2, 8 doi:10.1186/1743-8977-2-8 (modified)

Nanosized Particles in Biofluids

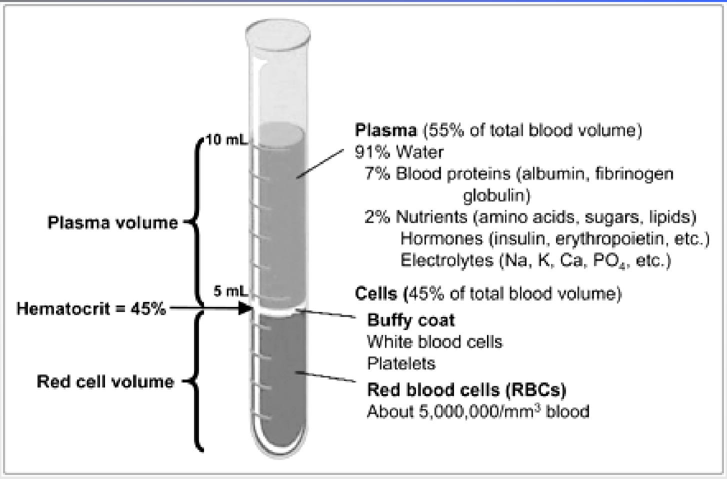


Oberdörster G et al. Particle and Fibre Toxicology, 2005, 2, 8 doi:10.1186/1743-8977-2-8 (modified)

Biological systems

- Biological Fluids
  - **Blood**
  - Lymphe
  - Liquor
  - Urine
  - Ascites
  - Sweet
  - (Breast milk)
- Cells
- Organisms/Organs

Blood content



Nanoparticles enter a new world

organic solvents ▶

Ions

aqueous solution ▶

Ions
Amino acids
Proteins
Lipids
DNA
RNA
Carbohydrates
Hormones
Cytokines
Small molecules
Erythrocytes
Macrophages
Lymphozytes
Granulozytes
Platelets

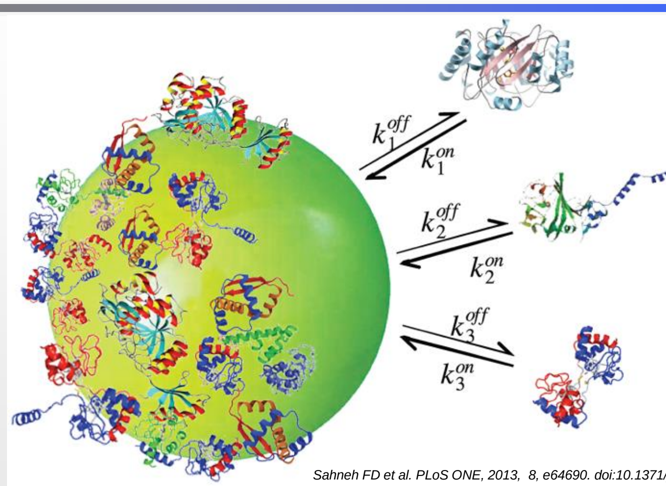
aqueous solution

patients' characterisitcs

Blood interferes with Nanoparticles

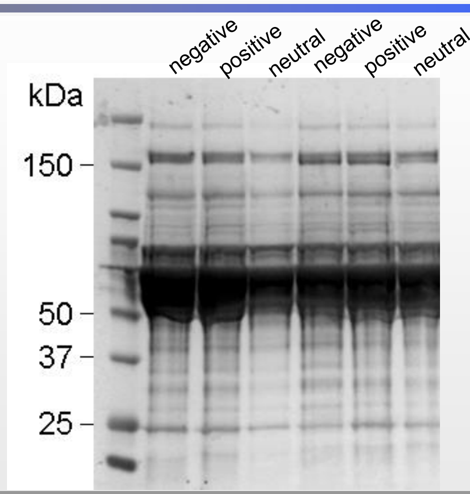
- protein corona formation
  - immediately
  - depend on the physico-chemical properties of the nanoparticles
    - shell composition
    - surface charge
  - changes surface charge
- affecting colloidal stability
  - agglomeration

Protein Corona – formation protein binding kinetics

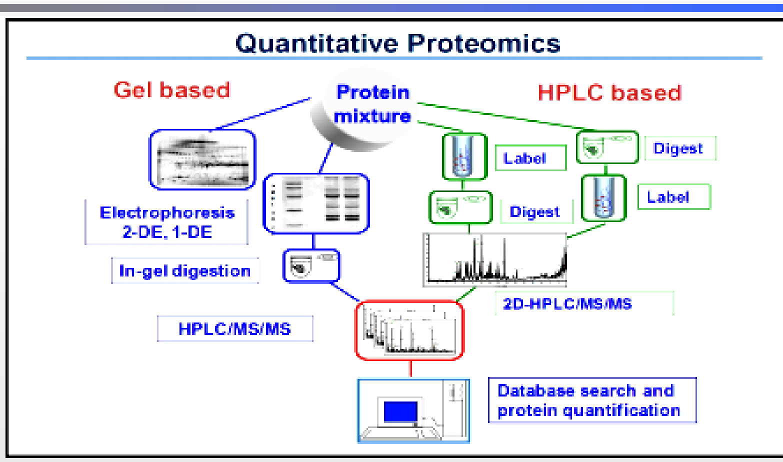


Sahneh FD et al. PLoS ONE, 2013, 8, e64690. doi:10.1371/journal.pone.0064690

Protein distribution on nanoparticles – a simple approach

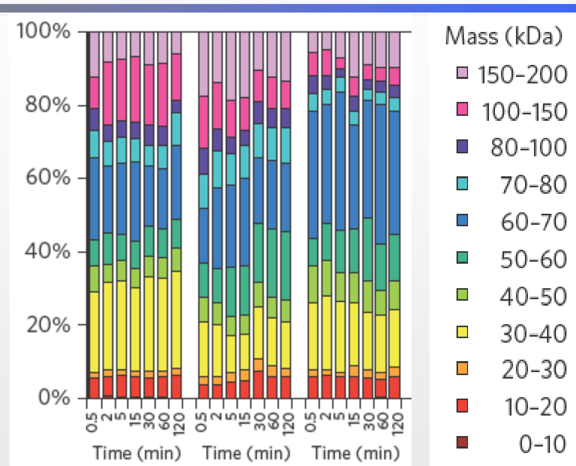


Protein distribution on nanoparticles – a quantitative approach



http://www.biotech.uconn.edu/msf/

Protein distribution on nanoparticles – complex protein patterns

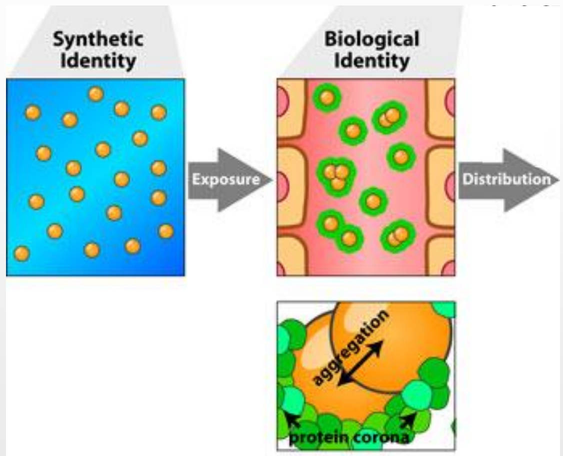


Tenzer S et al., Nature Nanotechnology, 2013, 8, 772-781

Protein corona formation

- is a dynamic process
- and dependent on
  - the protein composition
  - the time of contact
  - the temperature
- proteins are eliminated from medium
- affecting cell interaction

Protein Corona leads to a biological identity

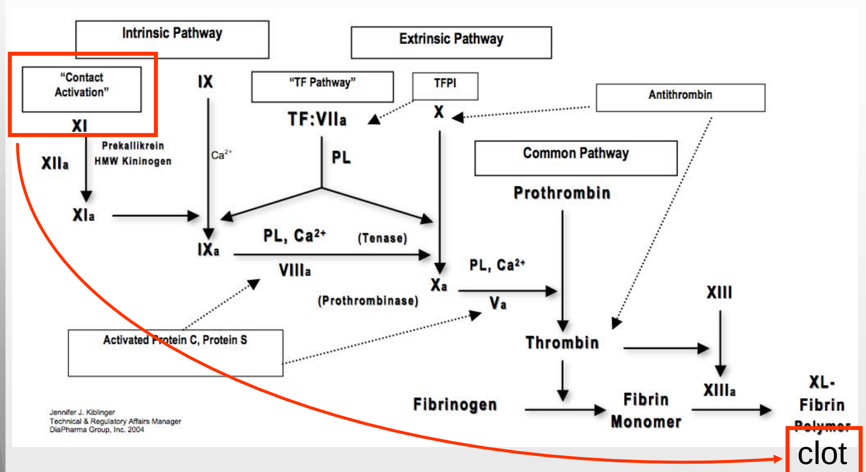


nanowerk.com

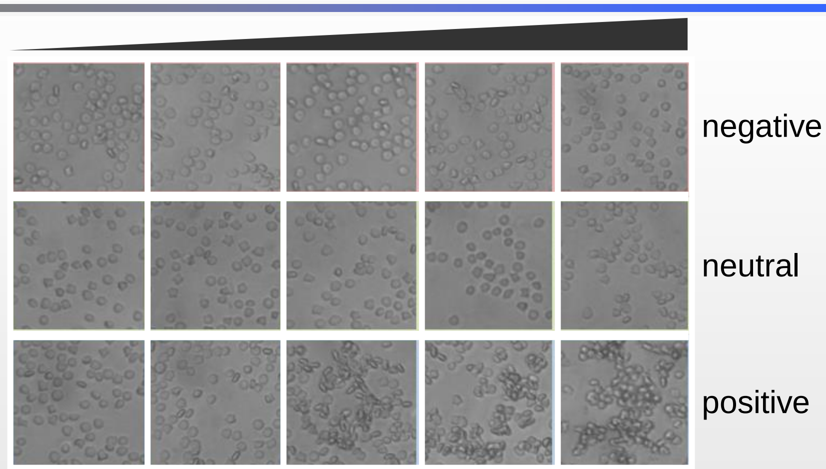
Blood interferes with Nanoparticles

- Coaggulation
  - prothrombin from tissue
  - activated clotting tissue
  - activation of coaggulation factor XI
- Clot formation
- Platelet aggregation
- Activation of complement system
- Aggregation of erythrocytes
- Hemolysis (of erythrocytes)

The Coagulation Cascade



Charge dependent erythrocyte aggregation



Florian Schlenk, Inst. of Pharmacy, Friedrich-Schiller-University Jena

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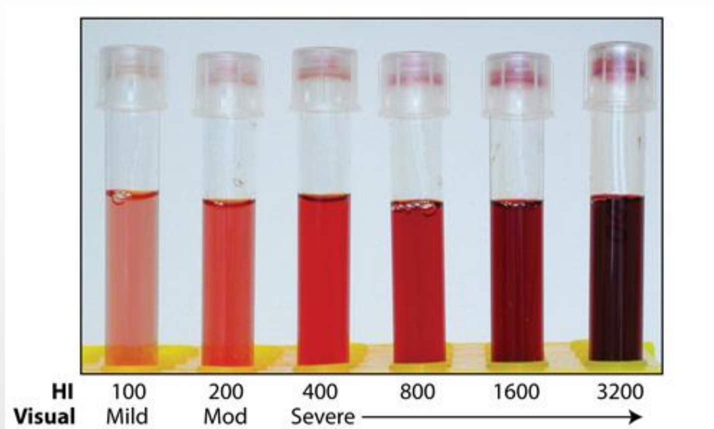
Nanoparticles can cause hemolysis



https://ahdc.vet.cornell.edu/sects/ClinPath/

Nanoparticles interact with blood cells

- Erythrocytes
- Macrophages
- Platelets
- Granulocytes
- Immune cells

Summary – Part I

- Complex reactions of nanoparticles with blood
- Depending on physico-chemical properties of nanoparticles
- Depending on the biological properties of the blood
- Protein corona formation

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Schedule – Part II

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    - Cells
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  - Cytotoxicity
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  - Degradation
  - Elimination

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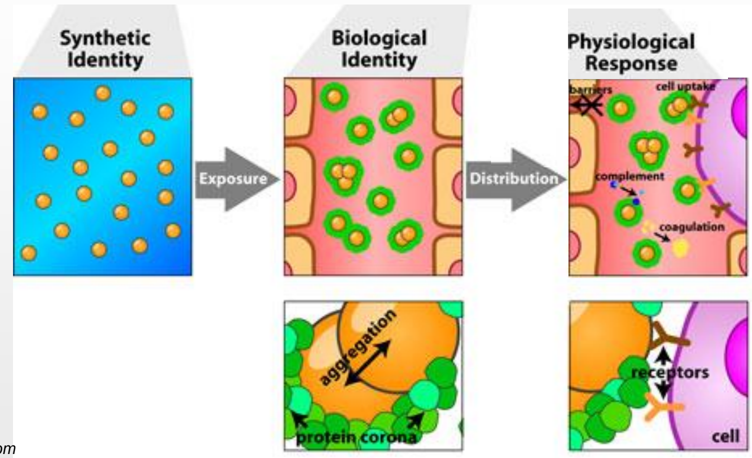
Protein Corona leads to a biological identity



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Biological systems

- Biological Fluids
- Cells
  - Cell surface
  - Membrane
  - Intracellular “space”
    - Cytoplasm
    - Organells
      - Endosome/lysosome
      - Mitochondria
      - Nucleus
      - Golgi apparatus
- Organisms/Organs

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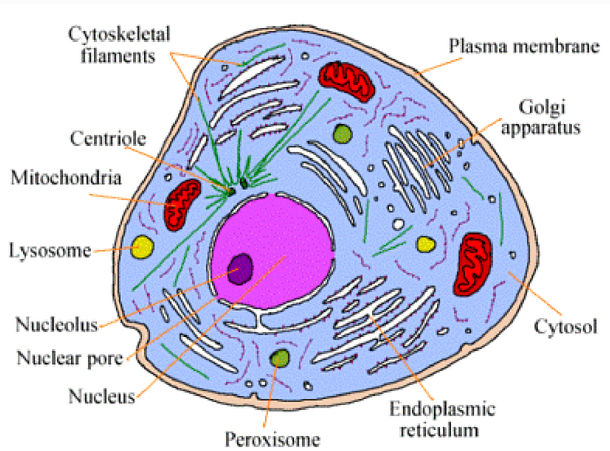
Cells and their abilities to interact with nanoparticles

- Professional phagocytes (Macrophages)
- Proliferating cells
- Cells with transport functions (endothelial cells)
- Docking sites (e.g. growth factor receptors, channels and pores)

A human cell



Compartmentalization
- pH
- enzyme content
- dynamic

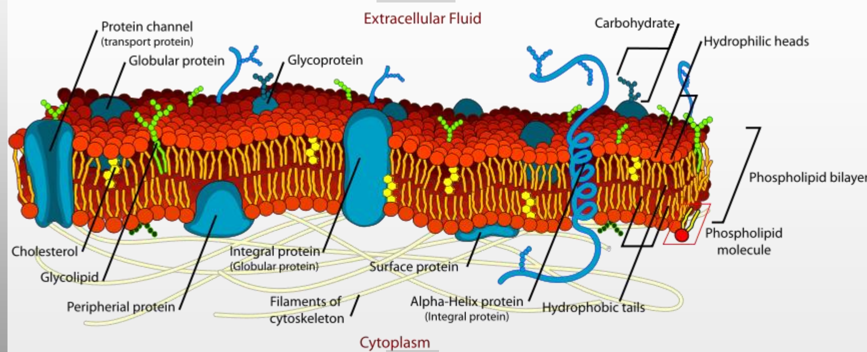
→ functional areas

Most critical
- mitochondria
- nucleus

TutorVista.com

Cell membrane

out Extracellular matrix

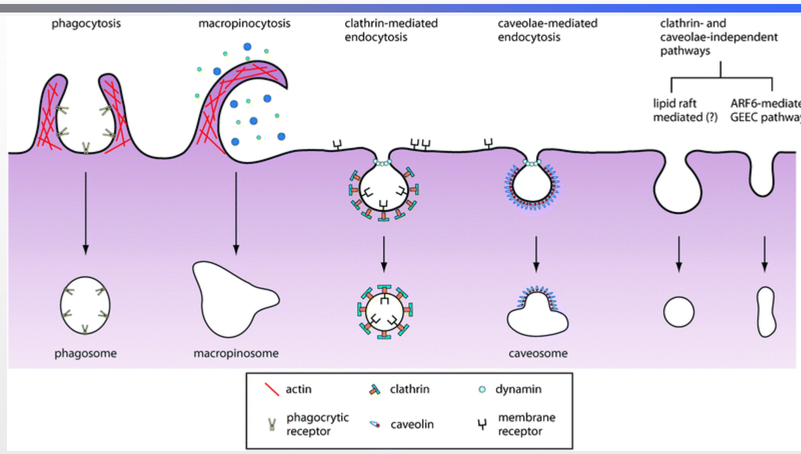


in

Entry into the cell

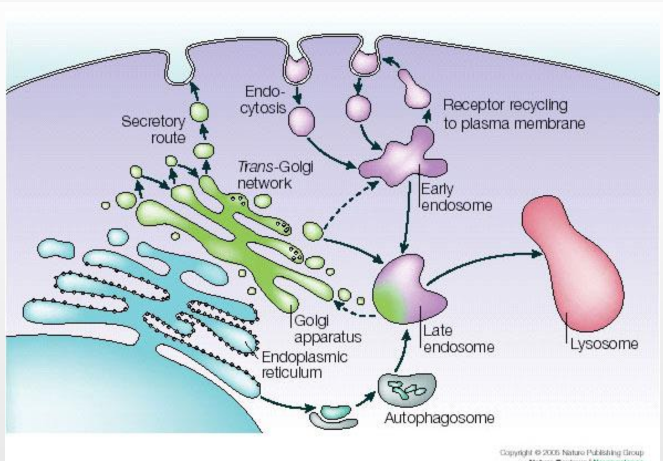
- Cell membrane of a vital cell is a very restrictive border
- Ion gradients
- Passage through the cell membrane
  - Membrane channel
  - Receptors/binding site induces change in membrane
  - Membrane turn over
  - Penetrating peptides
  - Forced entry (Transfection, e.g. Magnetofection)

Endocytosis

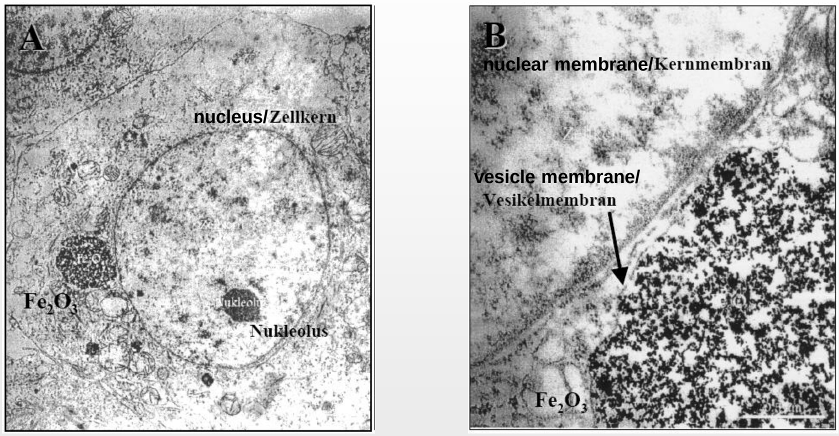


Chou LYT, Ming K, Chan WCW Chem. Soc. Rev., 2011, 40, 233-245

Intracellular fate of the nanoparticles



Nanoparticles are located in lysosomes



Wagner K et al., Appl. Organomet. Chem., 2004, 18, 514

Intracellular fate of the nanoparticles

- Nanoparticle shells are degraded in the lysosome
- Liberation of nanoparticles
  - Proton sponge effect
- Interaction with components of the cytoplasm
  - Nucleic acids (messenger RNA, transfer-RNA)
  - Ribosomes
  - protein folding machinery

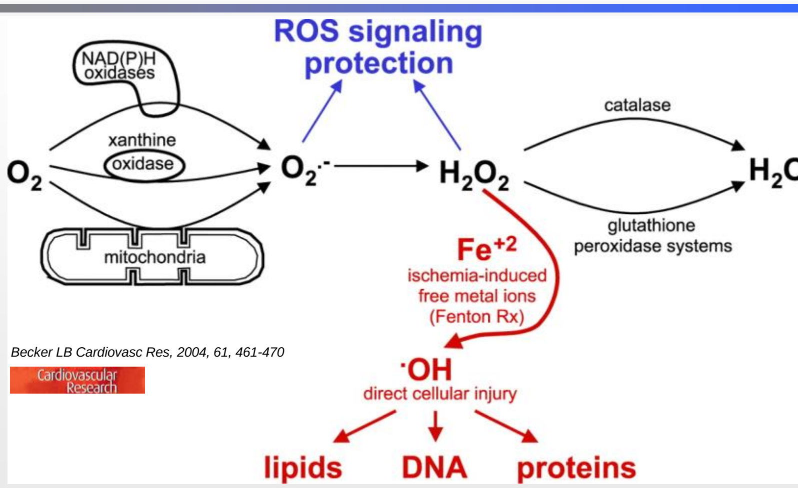
Intracellular fate of the nanoparticles

- Penetration into mitochondra or the nucleus
- Impairing essential cellular functions
  - Energy production
  - Genomic DNA
- Cytotoxicity

Mechanisms of cytotoxicity

- concentration of nanoparticles
  - causes oxidative stress by production of reactive oxygen species (ROS)
  - activation of transcription factors for proinflammatory mediators
- High surface/size ratio of nanoparticles facilitates generation of free radicals by redox cycling

Reactive oxygen metabolism



Mechanisms of cytotoxicity

- Liberation of iron ions
- Overload of iron ions
  - Disturbance of iron homeostasis (balance) causing aberrant cellular processes: cytotoxicity, enzymatic reactions, inflammation, DNA damage → carcinogenesis
- Oxidation status of iron is critical (Fe_{2+}/Fe_{3+} ratio)
  - Fe_{3+} is more potent in causing DNA damage than Fe_{2+}
  - Magnetite (Fe_3O_4) causes more oxidative DNA lesions than Maghemite (Fe_2O_3)

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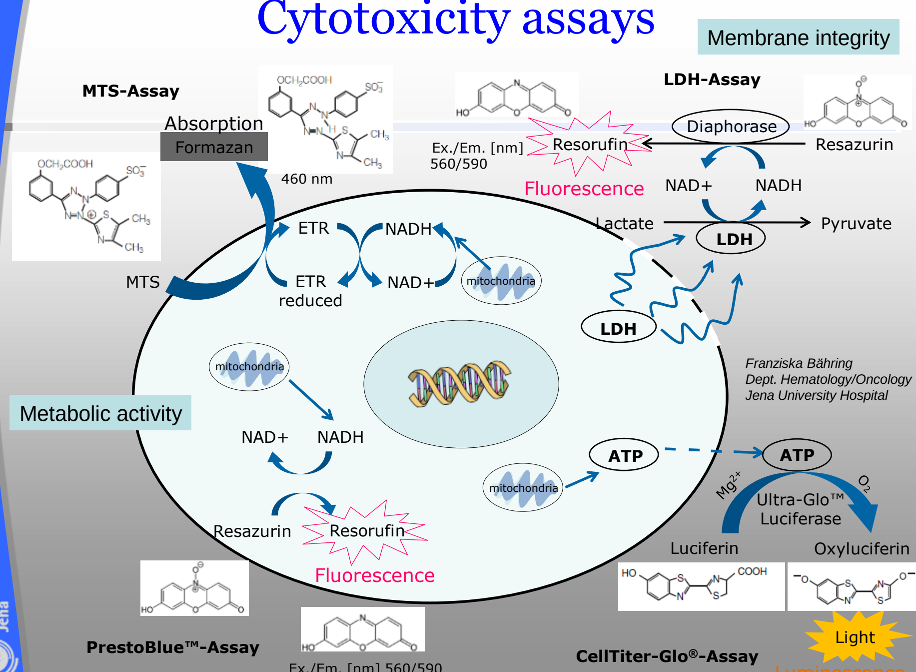
Evaluation of Cytotoxicity

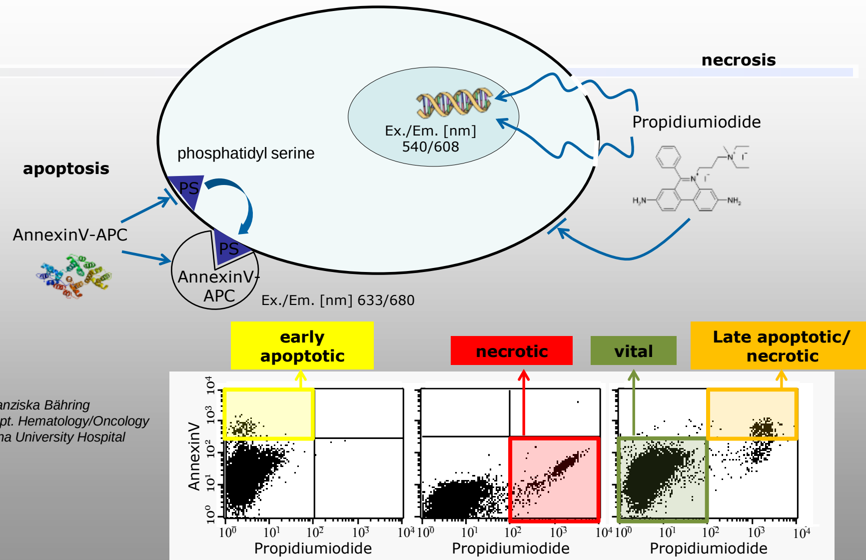
- Using *in vitro* models:
  - fast
  - simple
  - low costs
  - high-throughput
  - no ethics

- Methods:
  - Metabolic activity
  - Membrane integrity
  - Proliferation
  - Apoptosis

Cytotoxicity assays



Cytotoxicity assays



Franziska Bähring
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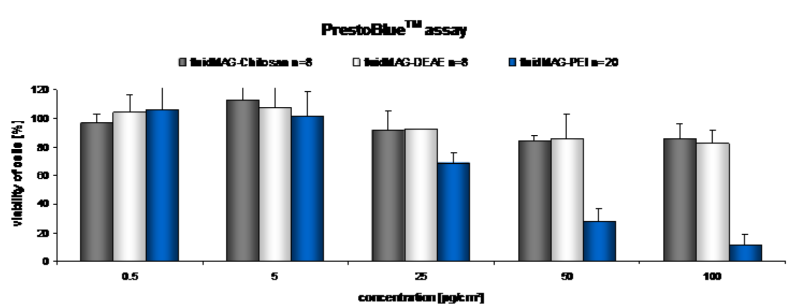
Jena University Hospital

Cytotoxicity assays - example



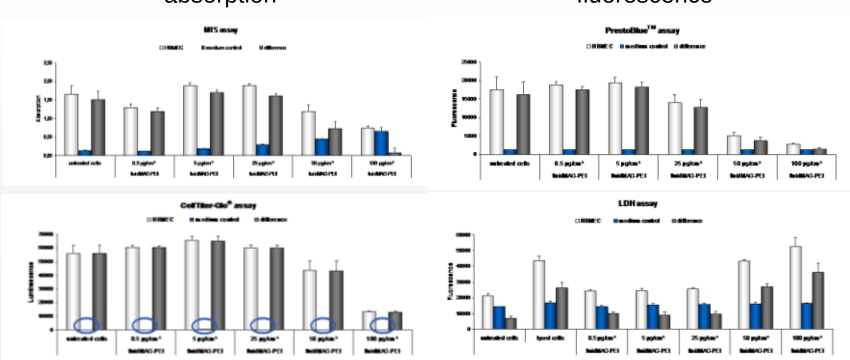
Bähring F et al., IEEE Transactions on Magnetics, 2013, 49, 383-388

Evaluation of Cytotoxicity

- Using *in vitro* models
- Caution!
  - Interference with assay components
  - Little/no correlation to *in vivo* situation
    - because of
      - no perfect cell-cell contact
      - no perfect cell-matrix contact
      - mostly 2-dimensional cell culture models instead of 3-dimensional cell culture models

Cytotoxicity assays – assay components affect measurements

absorption fluorescence



luminescence fluorescence

Bähring F et al., IEEE Transactions on Magnetics, 2013, 49, 383-388

Nanoparticle derived cytotoxicity

- Cytotoxicity correlates with nanoparticle
  - Size
  - Shape
  - Surface conditions
    - Charge

~~e.g. rods show lower endocytosis rates than spheres~~

Summary – Part II

- Nanoparticles can enter cells via different routes
- The endosomal/lysosomal pathway is the most common
- Nanoparticles can affect mitochondrial and nuclear processes and can cause cytotoxicity
- Comprehensive analysis of potential cytotoxicity of nanoparticles is crucial for their future biomedical application

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Schedule – Part III

- Part 1
- Part 2
- Part 3
  - Biodistribution
  - Degradation
    - Shell components
    - Core materials
  - Elimination

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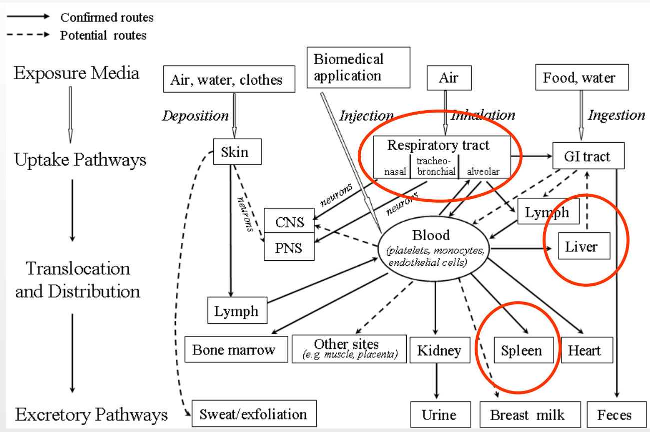
Biokinetics of Nanosized Particles



Oberdörster G et al. Particle and Fibre Toxicology,2005, 2, 8 doi:10.1186/1743-8977-2-8 (modified)

Fate of nanoparticles

- depends on physico-chemical characteristics
  - Size
  - Size distribution
  - Surface structure
  - Charge
  - Shell material
- depends on composition of the protein corona
  - Biological identity
- depends on interaction tissue/cells

Applying nanoparticles into organisms

- Open questions
  - Pharmacokinetics
    - Organ specific distribution
    - unspecific/artificial interactions with tissues and cells
    - Degradation, elimination, clearance
    - Effect of increased iron content
    - repeated application (MRT, drug delivery)

Biodistribution

Applying nanoparticles into organisms:

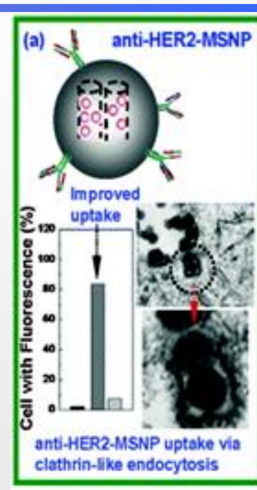
- Rapid clearance from circulation
- Distribution
  - >80% liver, >5% spleen, 1-2% bone marrow
- Nanoparticles affect homeostasis of organs, especially liver and kidney
- Liver
  - Metabolic capacity is very high; 10% of the liver is able to fulfil all essential functions
  - high regenerative potential
- But: sensitive to inflammation

Biodistribution

- **Directed** distribution (to cells; to overcome barriers)
  - Binding proteins on cell surface
    - Receptors, e.g. HER2 receptor
      - Ligand
      - Antibody
    - Glycoproteins, e.g. Mucin1
  - Tight junctions
  - Transcellular transport (Transcytosis)
    - Endothelia
    - Airway-epithelia
    - Blood-brain-barrier
    - Gastrointestinal duct
  - … using a magnet
  - Blocking cellular interaction/uptake

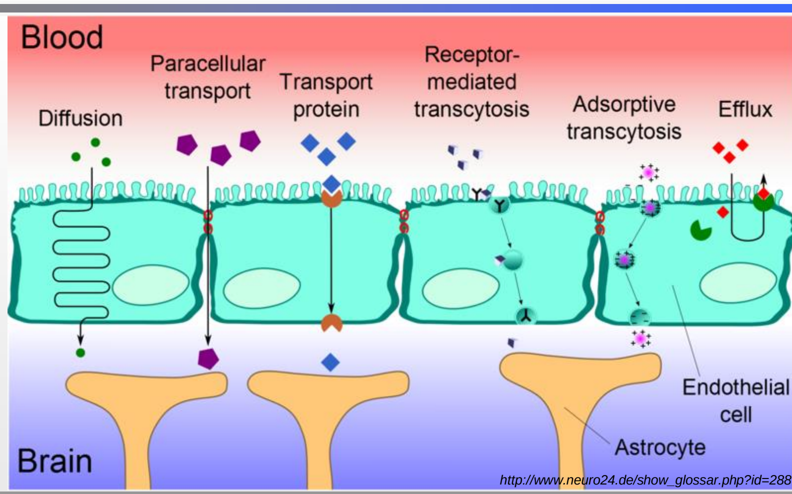
Biodistribution

- Receptor-mediated distribution



Mai WX and Meng H Integr. Biol., 2013, 5, 19

Biodistribution - barriers



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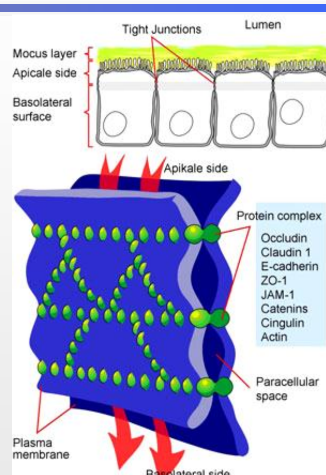
Biodistribution - barriers

- Tight junctions



http://php.med.unsw.edu.au/cellbiology/images/c/c3/Tight_junction3.jpg

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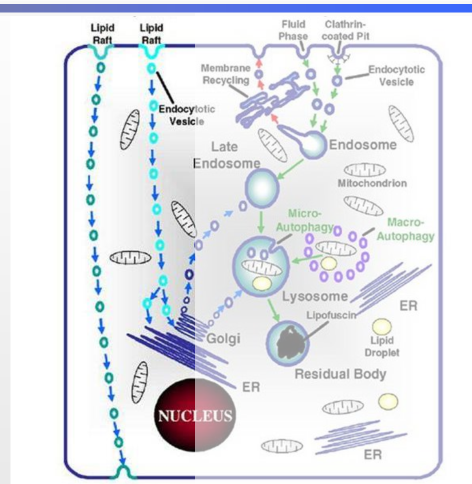
Biodistribution - barriers

- Transcytosis

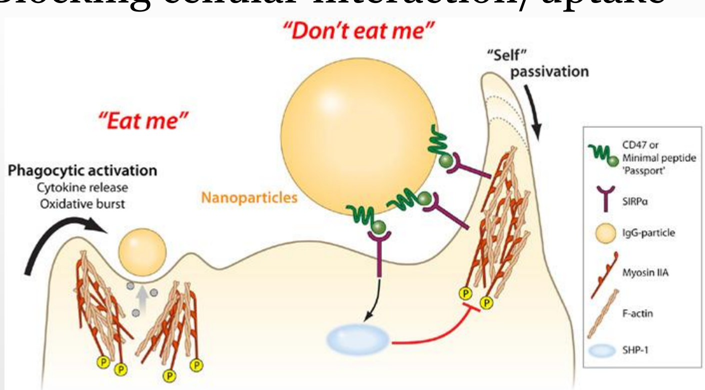
Caveolae-mediated
Endocytosis



http://www.iitk.ac.in/infocell/

Biodistribution

- Blocking cellular interaction/uptake



Biodistribution

- Methods to study distribution
  - Tissue sections/Histology (e.g. Prussian blue)
  - Magnetic resonance imaging (MRI)
  - Magnetic particle spectroscopy (MPS)
  - Magnetic Particle Imaging (MPI)
  - Magnetrelaxometry
  - Fluorescence based methods
  - Multi-spectral Optoacustic Imaging
  - …

Degradation

Degradation

- Polysaccharide shells are rapidly degraded
- Magnetite/Maghemite particles
  - Degradation needs several weeks
- PEG and other compounds can hinder degradation and clearance of iron oxide based cores (stealth particles)

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Degradation

- Magnetite/Maghemite particles
  - Degradation needs several weeks
  - Change of iron homeostasis
  - Oxidative stress
  - ROS production
  - High intracellular iron content leads to increased risk of cancer (e.g. liver cancer: spindle cell carcinoma, pleomorphic carcinoma)

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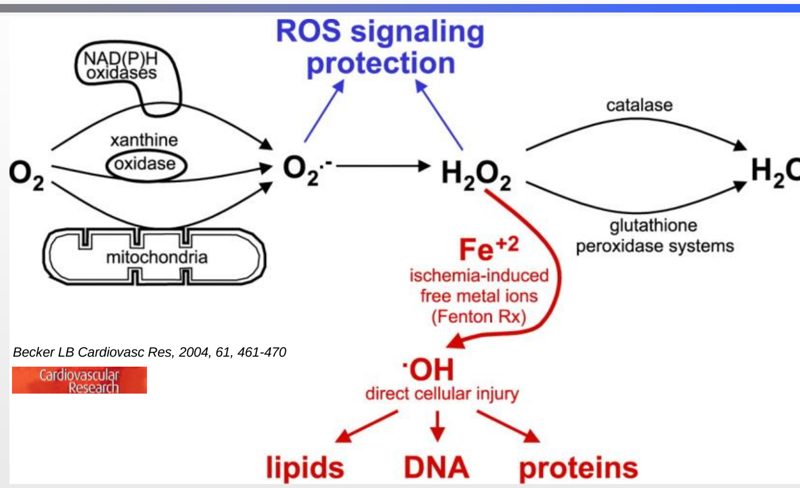
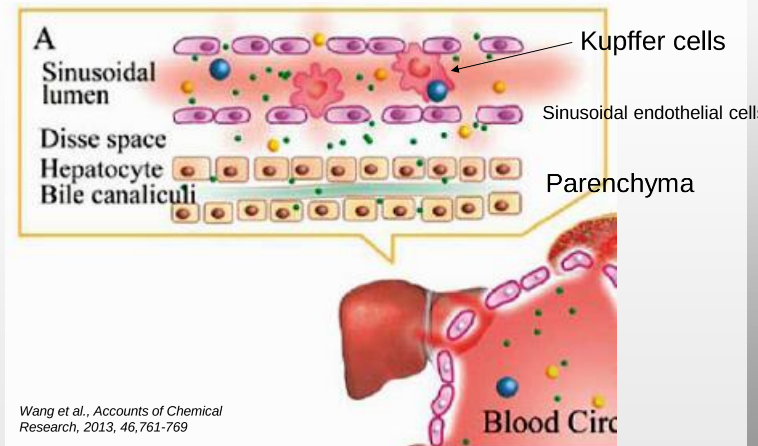
Reactive oxygen metabolism



Clearance

Clearance - liver



Wang et al., Accounts of Chemical Research, 2013, 46,761-769

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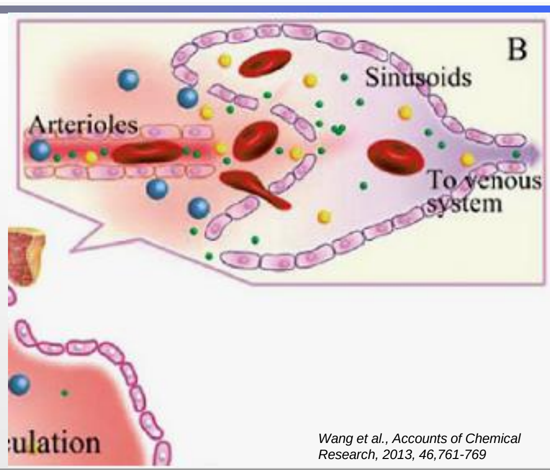
Clearance - spleen



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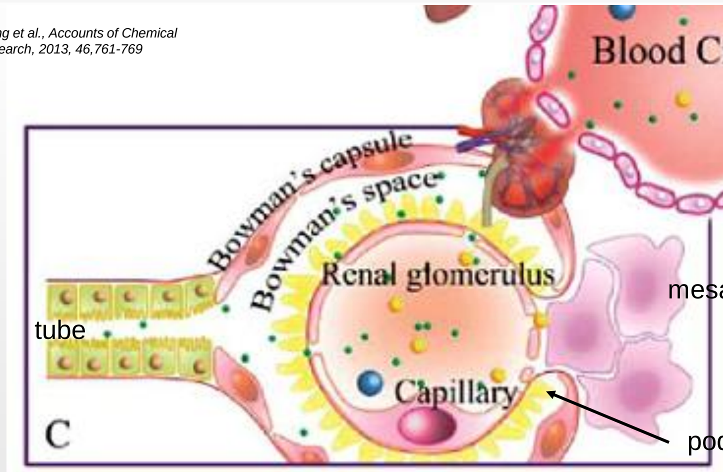
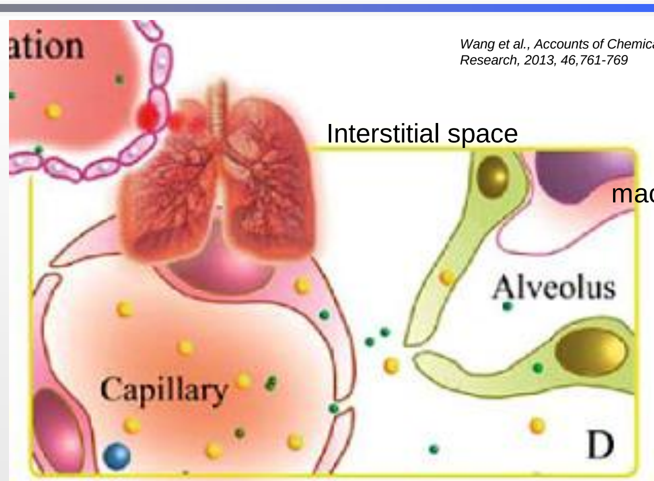
Clearance - kidney

Wang et al., Accounts of Chemical Research, 2013, 46,761-769

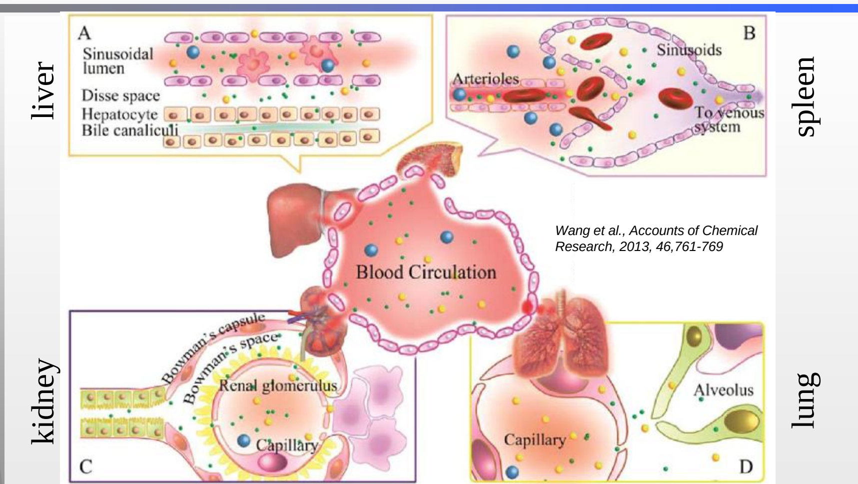


Clearance - lung

Wang et al., Accounts of Chemical Research, 2013, 46,761-769



Clearance



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Summary - Part III

- Vertebrates are complex
- Little is known about long term effects of nanomaterials in organisms
- *In vivo* application of nanomaterials need global and in-depth understanding of biological consequences
- A Gold standard for cytotoxicity determination is needed

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Perspectives

- High content approaches
- Systems biology
- Collecting data – extend the nanoparticle databases
- Bridge the gap between *in vitro* and *in vivo*

Take home message

- Nanomaterials are amazing tools -
- Biology offers great opportunites -
Lets bring them together!

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