On the Biology (and Related Subjects) From the Point of View of a Magnetic Nanoparticle

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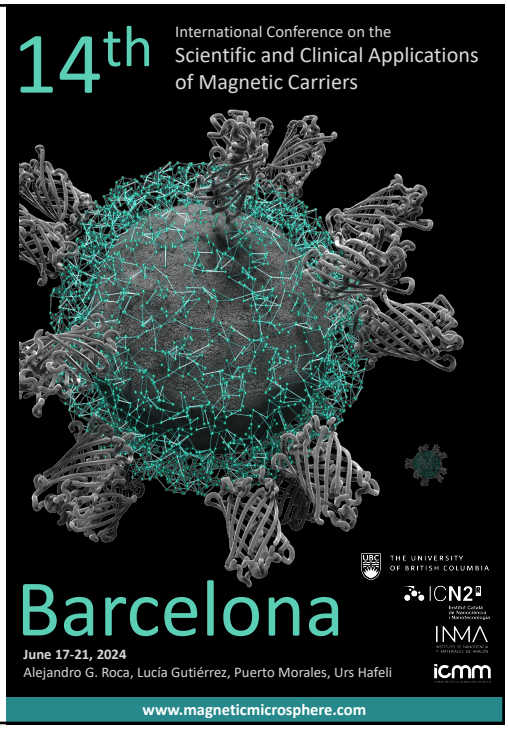

The Nano Odyssey: Understanding Nanoparticles in Biological Systems

-- Biology for non-biologist (taught by non-biologist) –

PART 1
19/6/24

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Is this tutorial for you?

Are you comfortable with this?

$$\vec{H}_i^{\text{dip}} = \sum_{i \neq j} V_j M_s r_{ij}^{-3} (-\hat{m}_j + 3\hat{r}_{ij}(\hat{m}_j \cdot \hat{r}_{ij}))$$

Yes

TEAM: Physics

No

Have you ever worked with cells?

No

TEAM: Chemistry

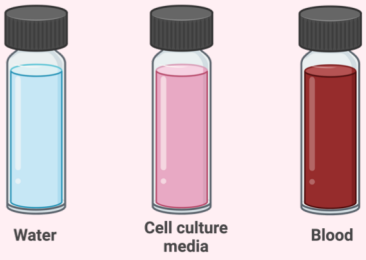
Yes

TEAM: Bio

Congratulations!
You can sleep longer!

Interactions of NPs with biological systems

Part 1: Fluids



Part 2: Cells

Part 3: Body

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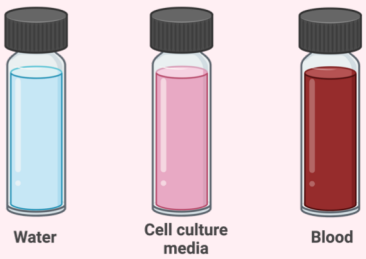
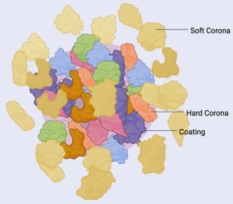
Interactions of NPs with biological systems

Part 1: Fluids



Protein corona formation



NPs sterility

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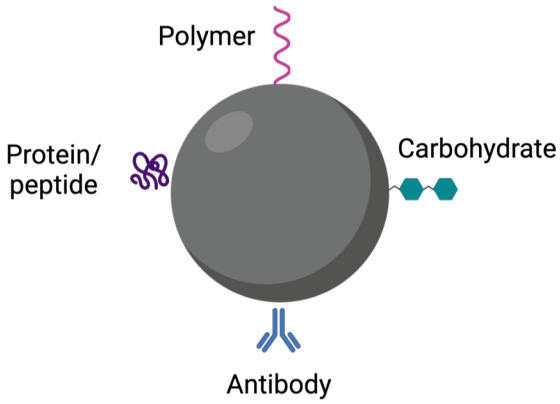
Introduction to the main character: The Magnetic Nanoparticle



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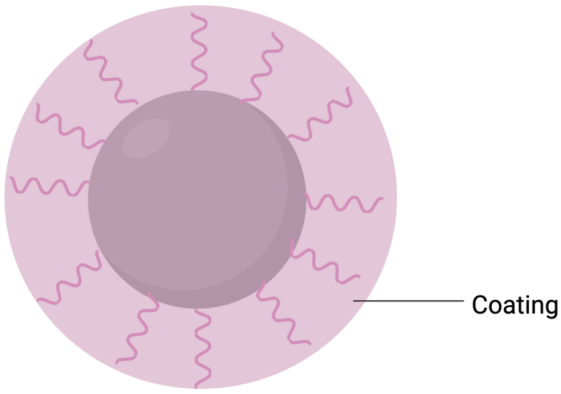
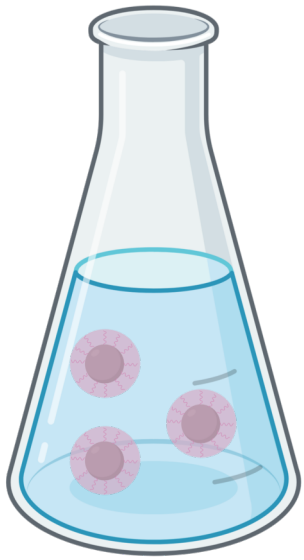
NP + coating



Synthetic identity



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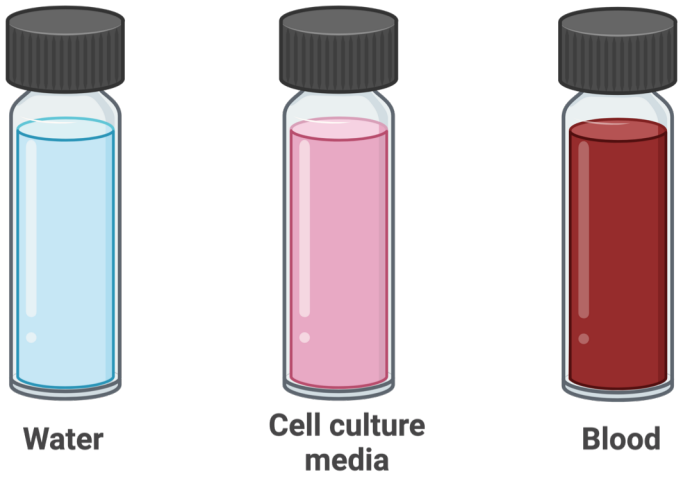
Fluids



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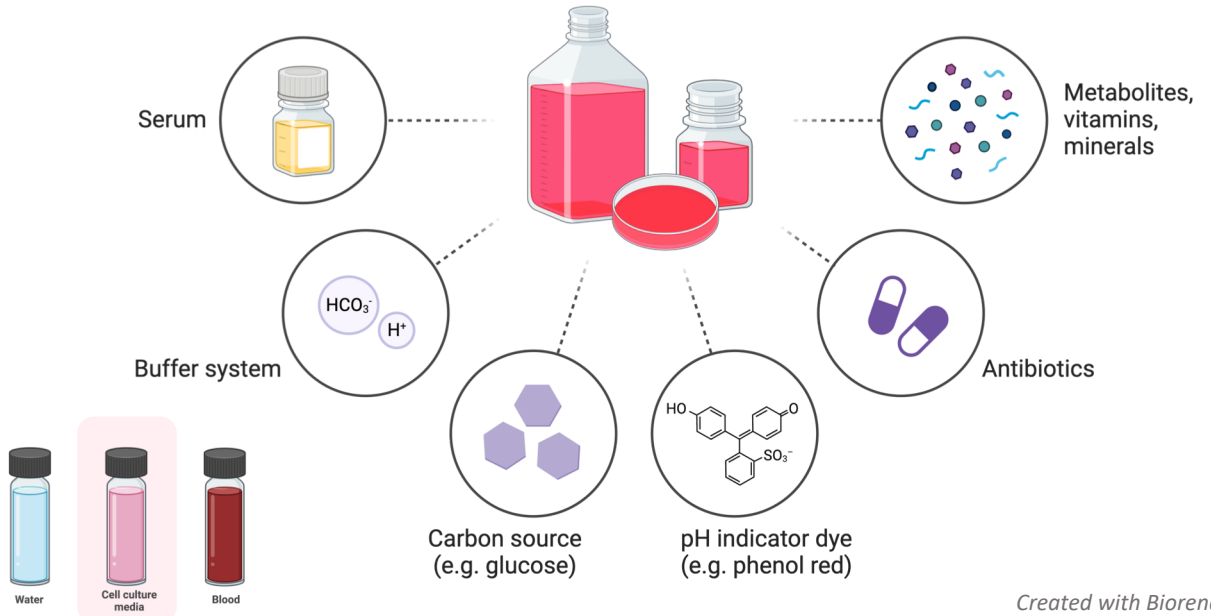
Cell culture media composition



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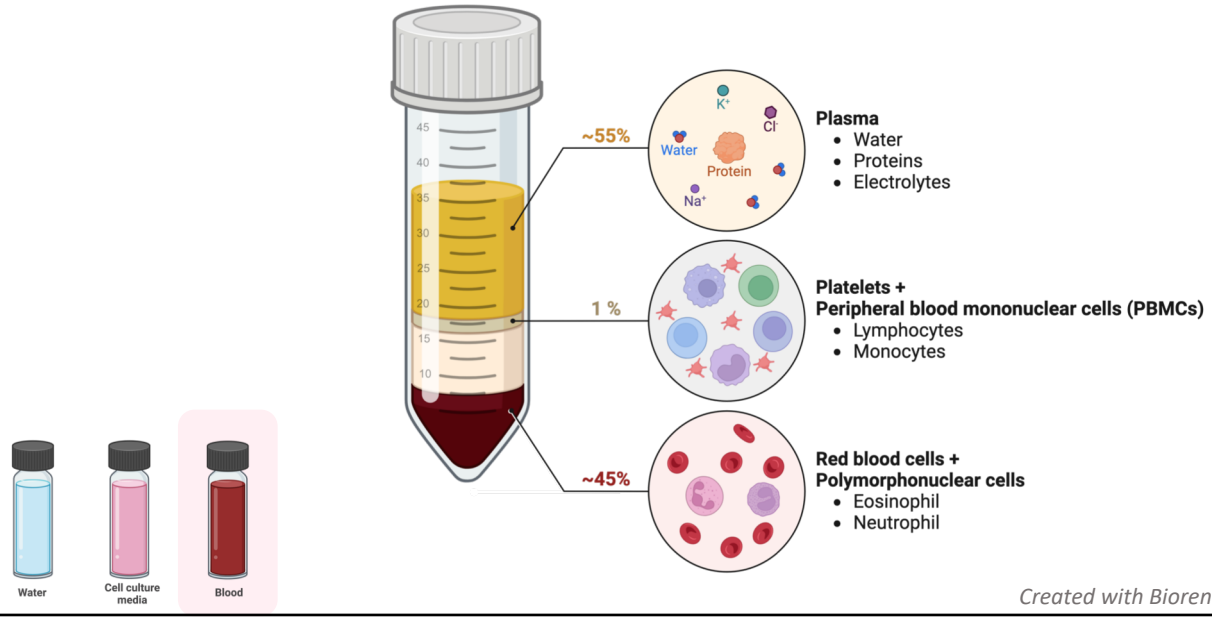
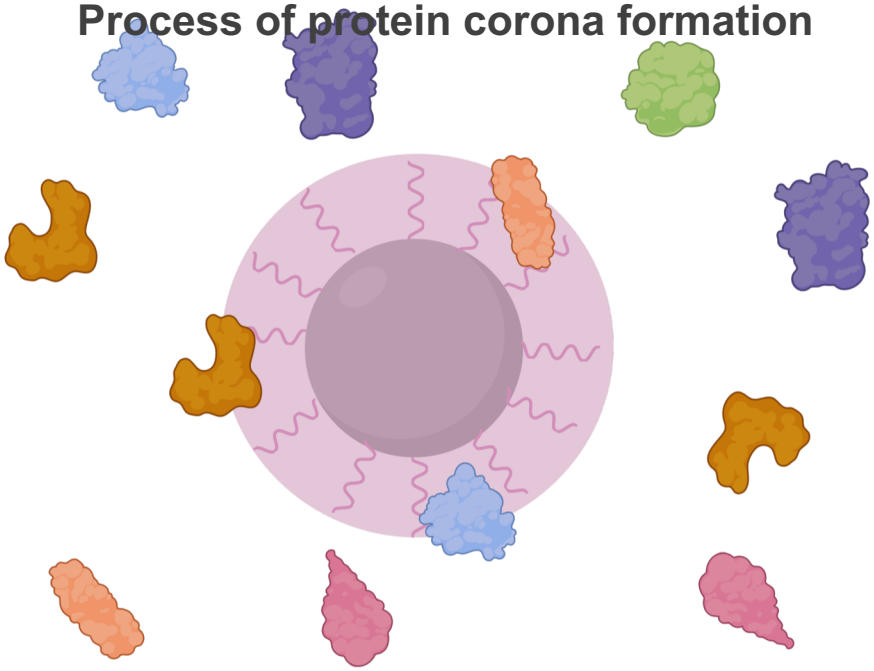
Blood composition



What will happen to the nanoparticle in this new world?

Protein corona formation

Process of protein corona formation



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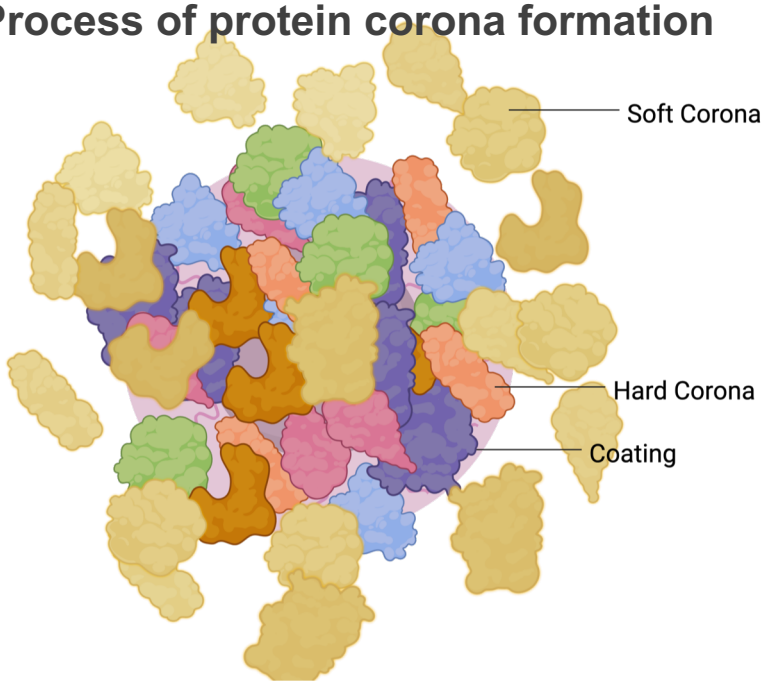
Process of protein corona formation



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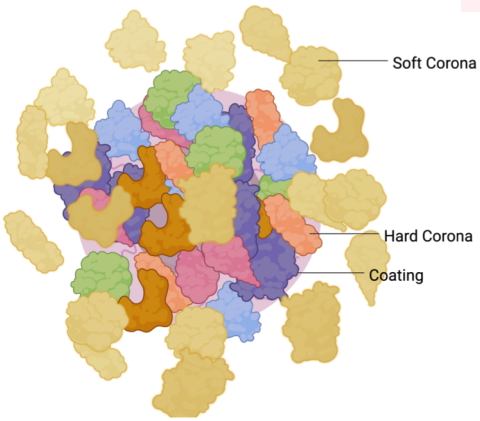
Properties of the protein corona

Dynamic



FIRST

- Proteins attach very fast to the surface
- Most abundant proteins (albumin, fibrinogen)

THEN

- Less abundant proteins will be bound
- Higher affinity proteins will remain

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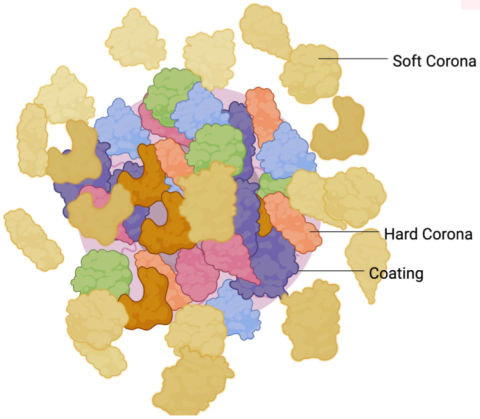
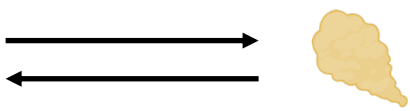
Properties of the protein corona

Dynamic



Over time they are replaced by proteins with lower concentration but higher affinity.



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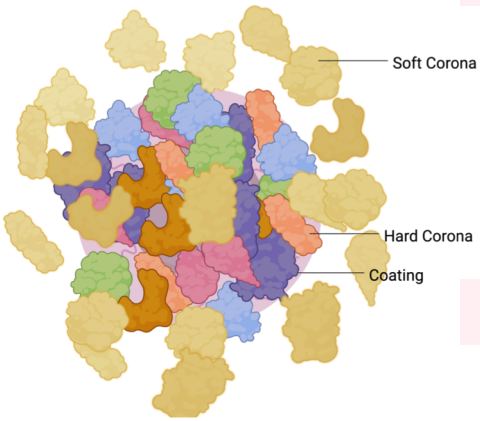
Parts of the protein corona



Hard corona

- Irreversibly bonded proteins

Soft corona

- Reversible bound

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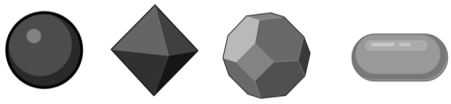
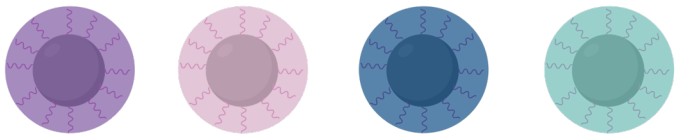
Parameters that affect the protein corona formation

Size

Shape



Coating



Charge | **Hydrophobicity** | **Rugosity**

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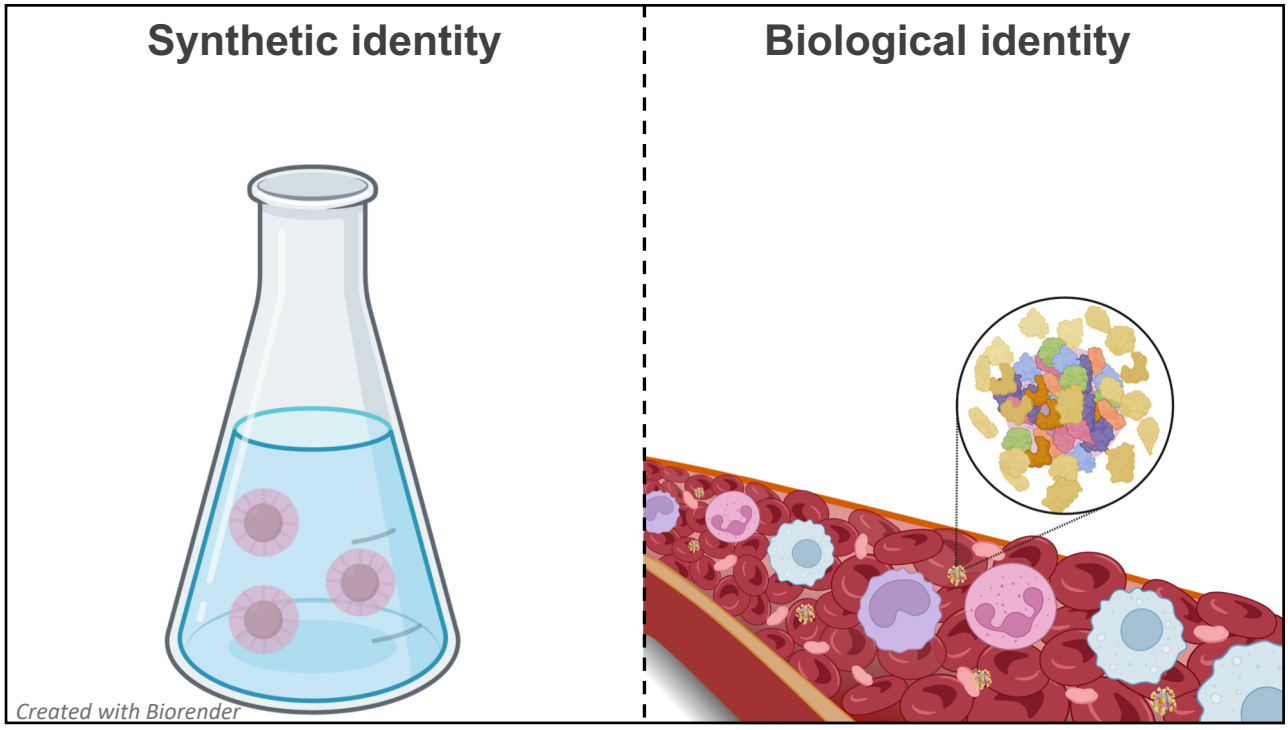
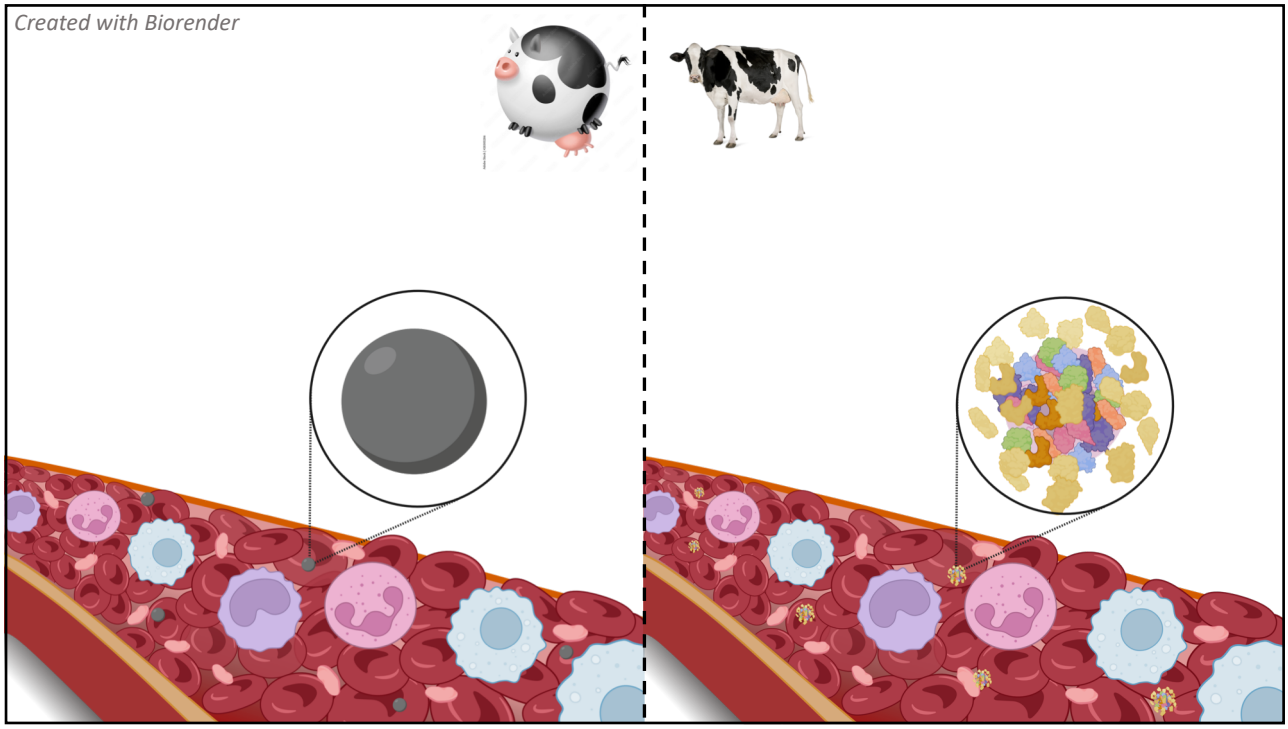
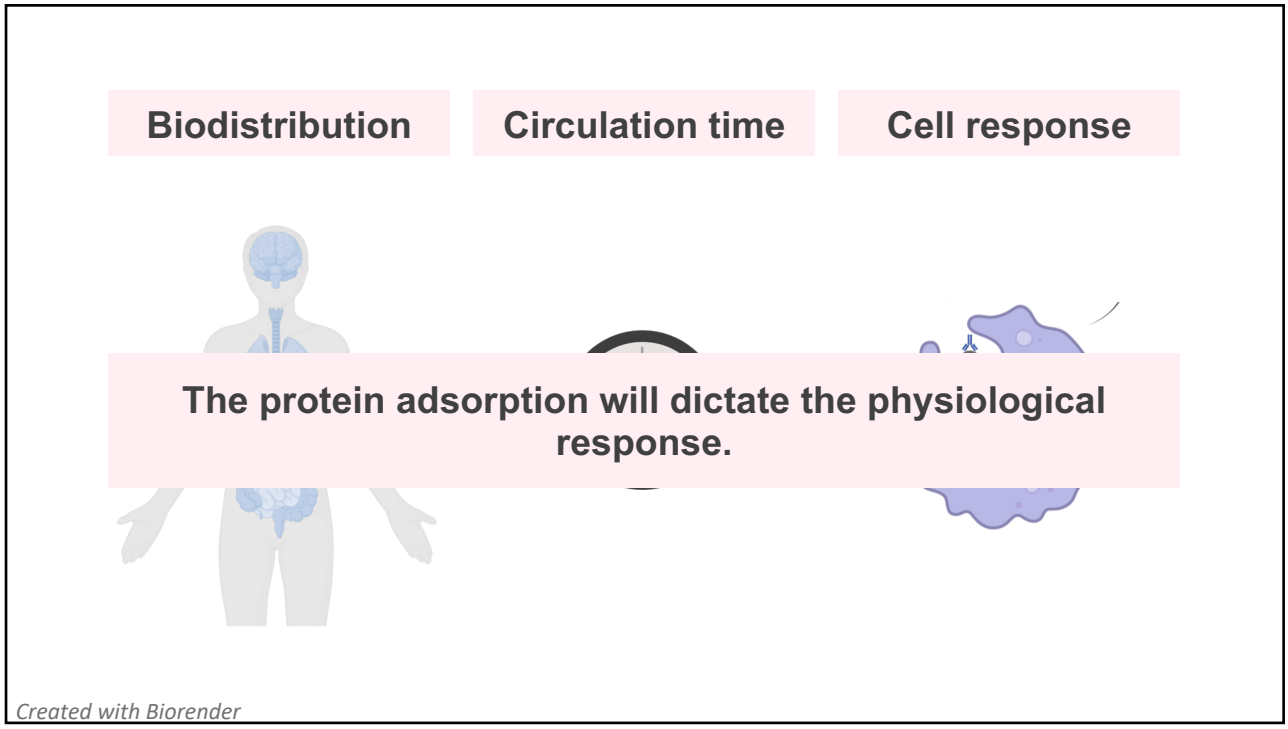
Why is this important?

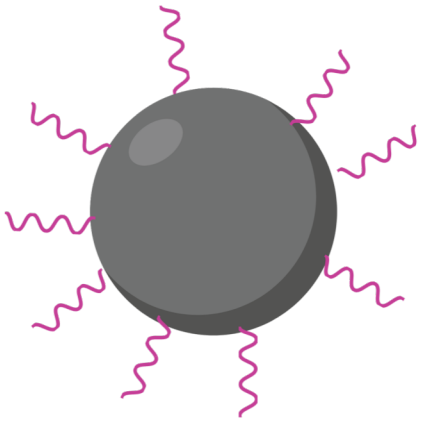



Created with Biorender

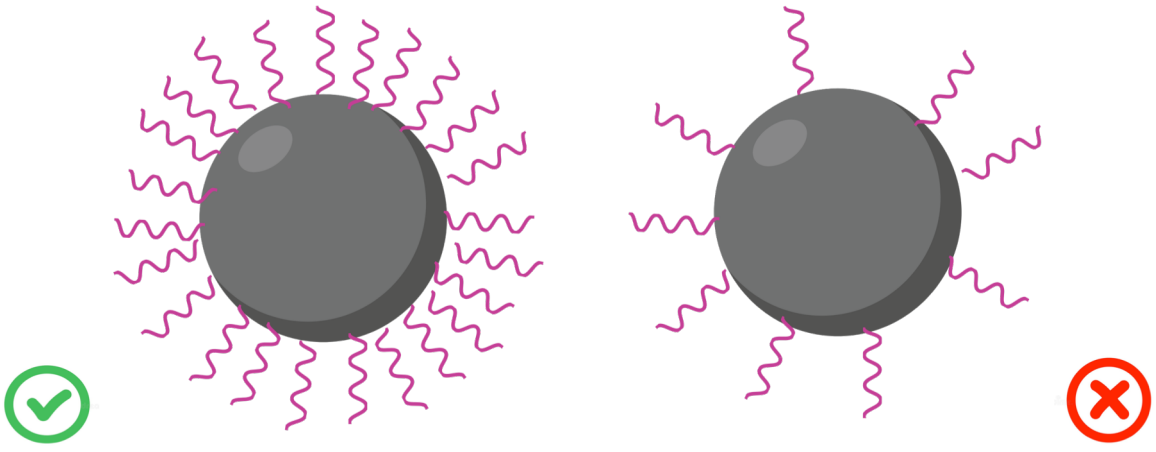

Biodistribution
Circulation time
Cell response
The protein adsorption will dictate the physiological response.
Created with Biorender

How to avoid protein binding?

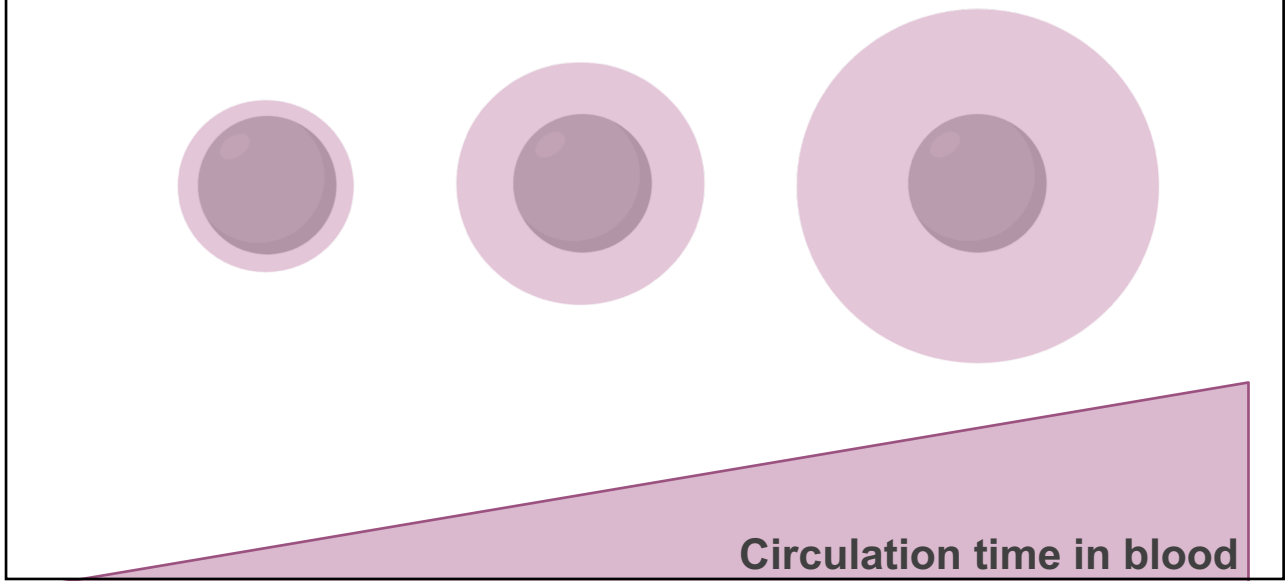
PEG: Polyethylene glycol



PEG Coating: DENSITY

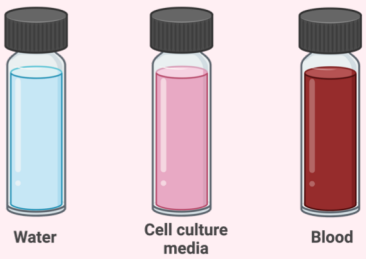


PEG Coating: MOLECULAR WEIGHT

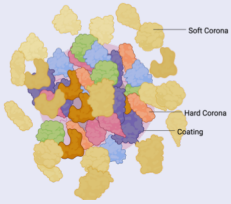


SUMMARY

Part 1: Fluids



Protein corona formation



1. Hard/Soft Corona
2. Dynamic
3. Biological identity
4. Coatings

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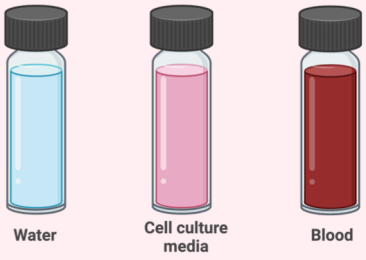
Interactions of NPs with biological systems

Part 1: Fluids



Protein corona formation

NPs sterility

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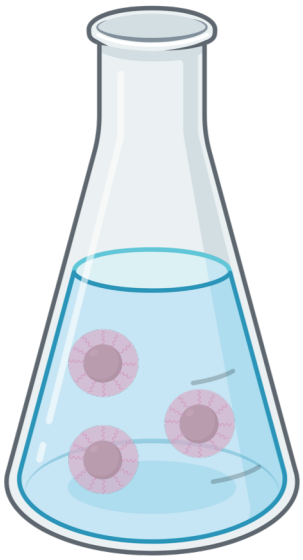
Sterility



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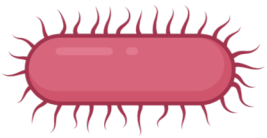
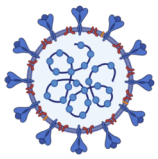
Sterilization

What to we want to remove?

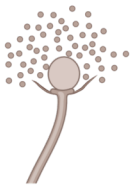


Bacteria



Virus

Fungi



Spores

Prions

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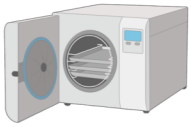
Sterilization

How do we remove it?

Heat

Chemicals

Irradiation



High pressure

Filtration

But…..

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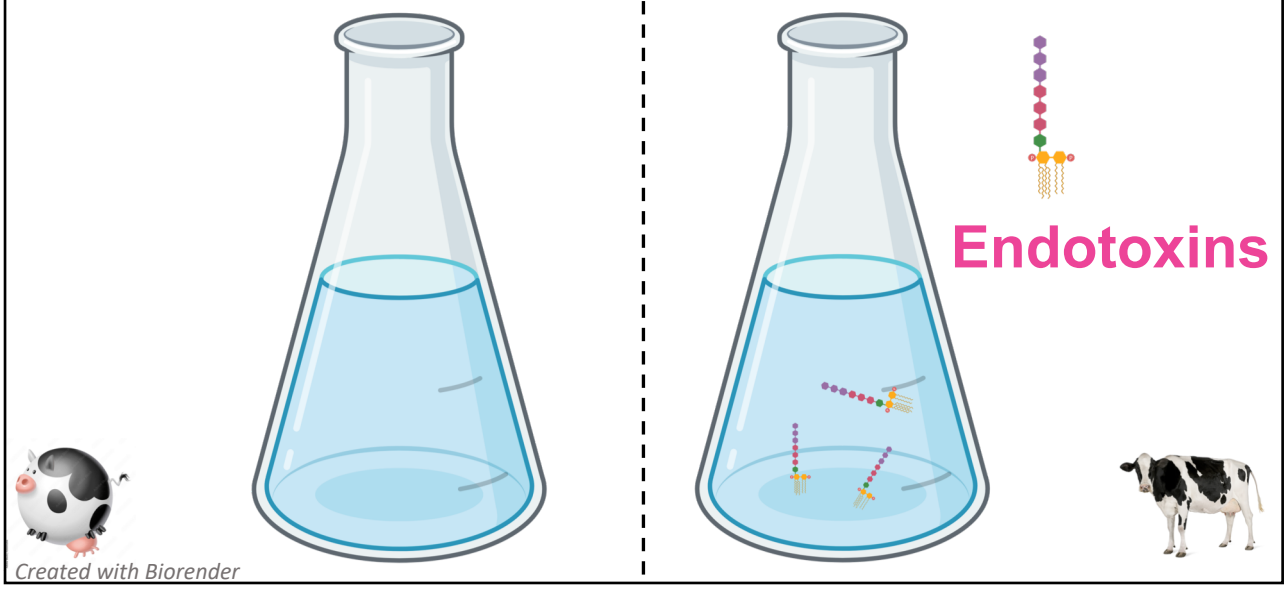
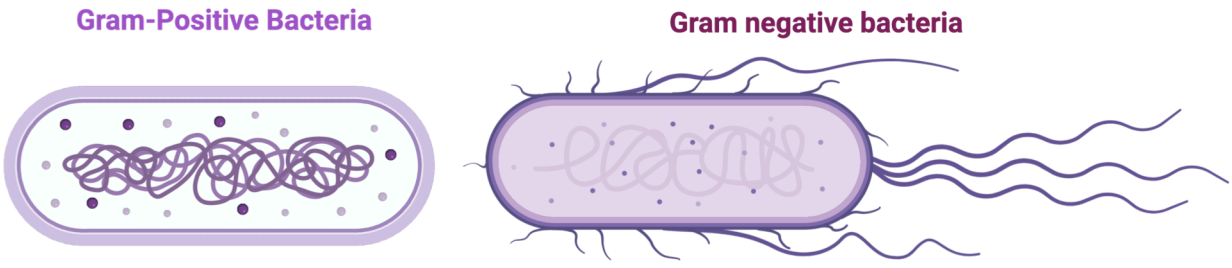
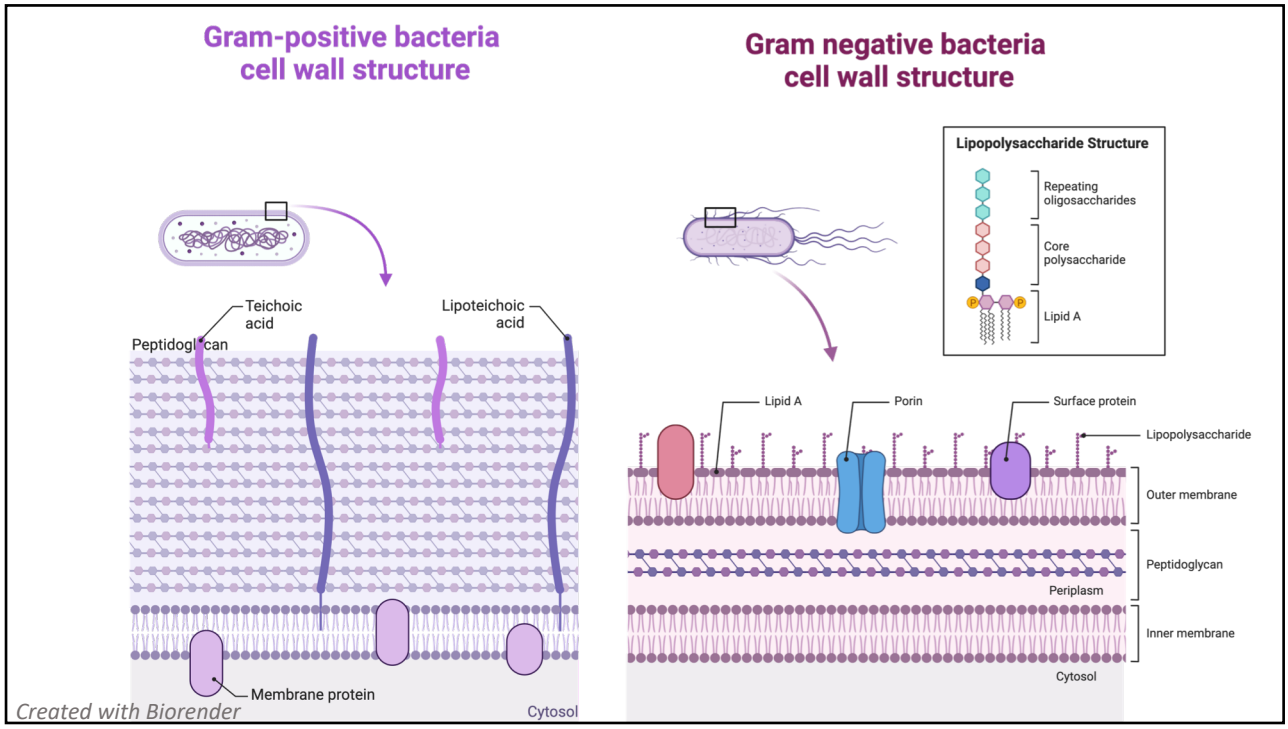
What you think you have vs what you really have

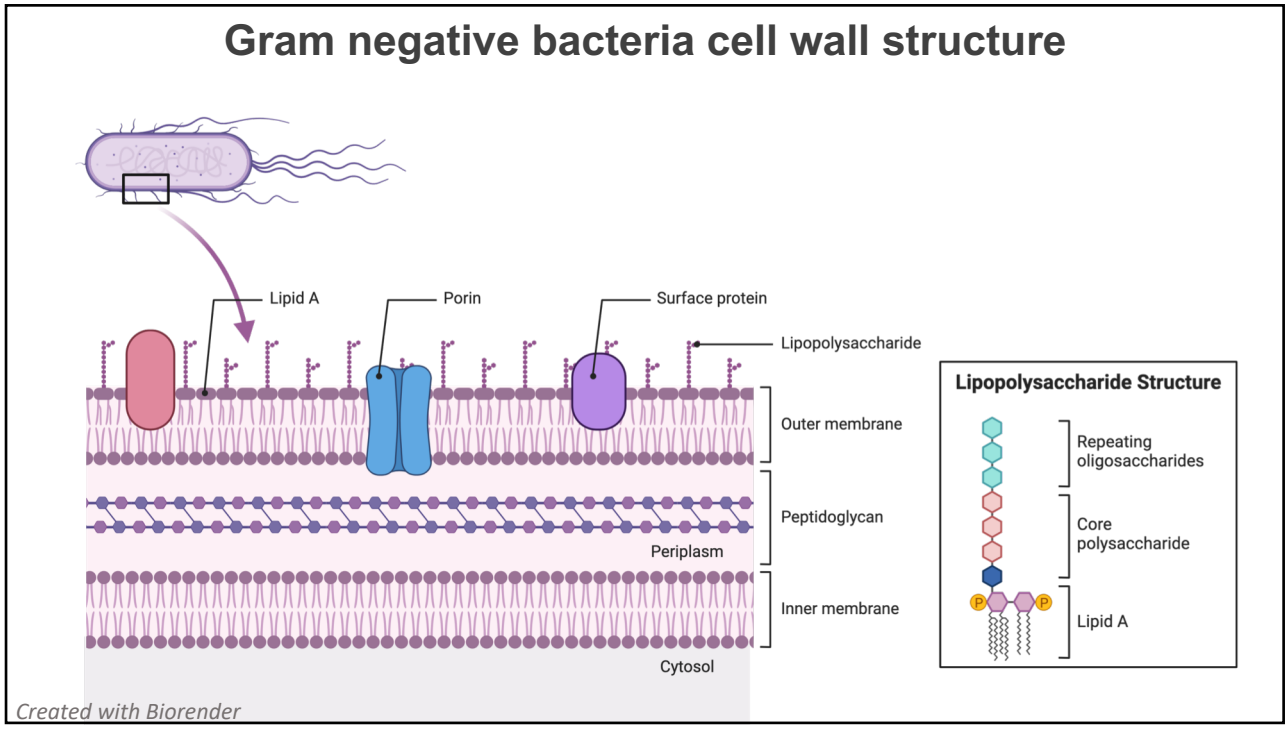


Where do endotoxins come from?

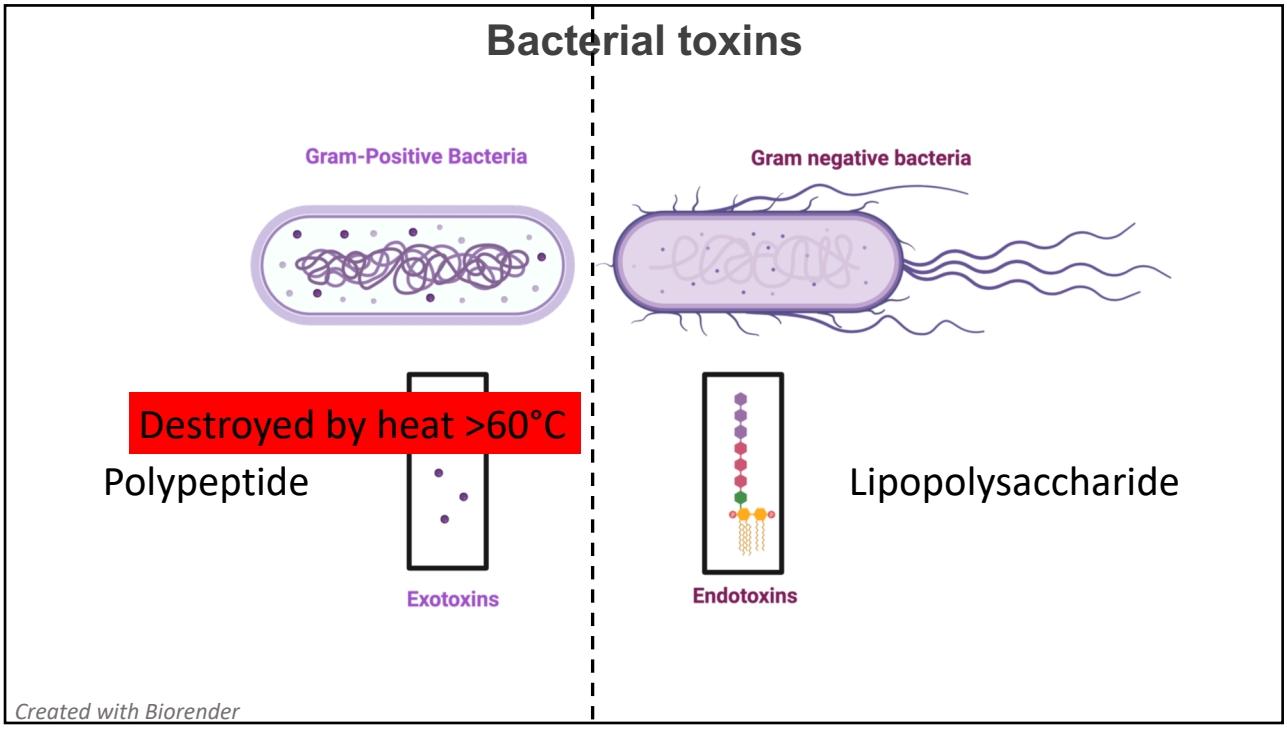
Bacteria




Gram-positive bacteria
cell wall structure
Teichoic
acid
Lipoteichoic
acid
Peptidoglycan
Membrane protein
Cytosol
Gram negative bacteria
cell wall structure
Lipopolysaccharide Structure
Repeating
oligosaccharides
Core
polysaccharide
Lipid A
Lipid A
Porin
Surface protein
Lipopolysaccharide
Outer membrane
Peptidoglycan
Periplasm
Inner membrane
Cytosol
Created with Biorender


Gram negative bacteria cell wall structure
Lipid A
Porin
Surface protein
Lipopolysaccharide
Outer membrane
Peptidoglycan
Periplasm
Inner membrane
Cytosol
Lipopolysaccharide Structure
Repeating
oligosaccharides
Core
polysaccharide
Lipid A
Created with Biorender

Bacterial toxins



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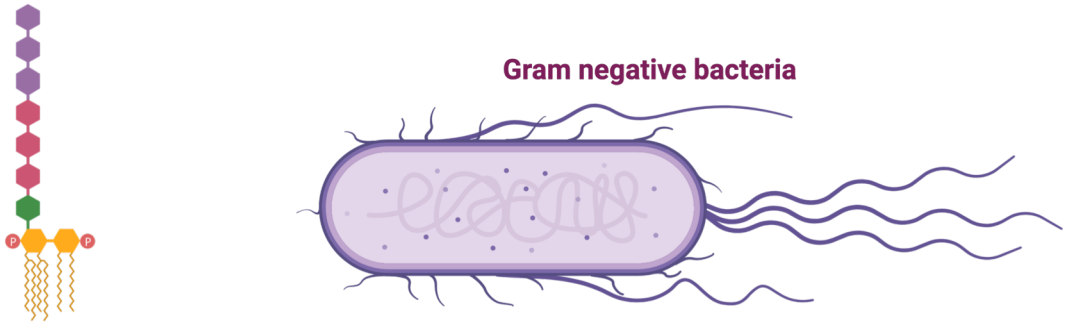
Endotoxin release



When the bacteria is alive	When the bacteria dead
• Slow release • Low concentration	• Fast release • High concentration

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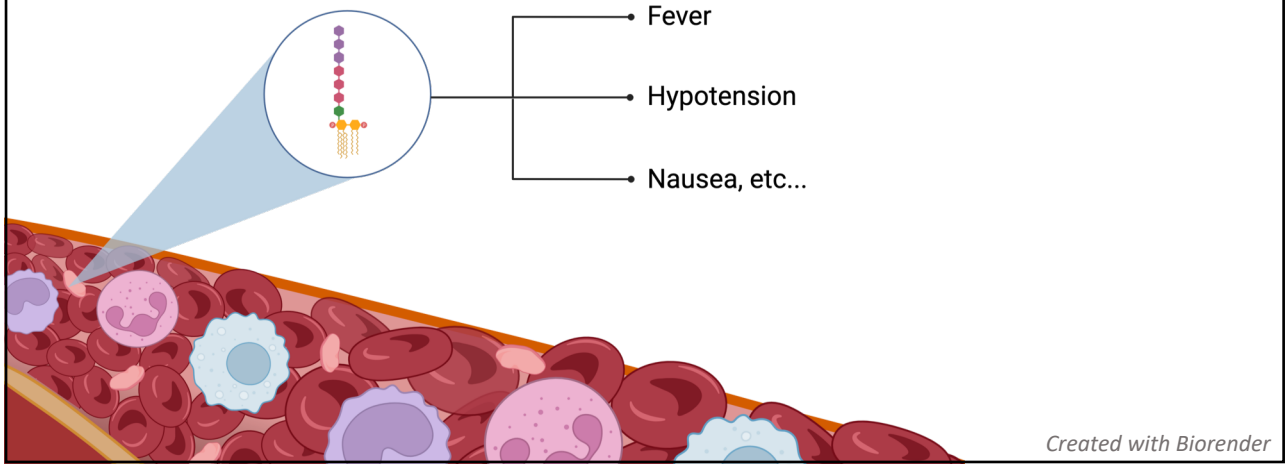
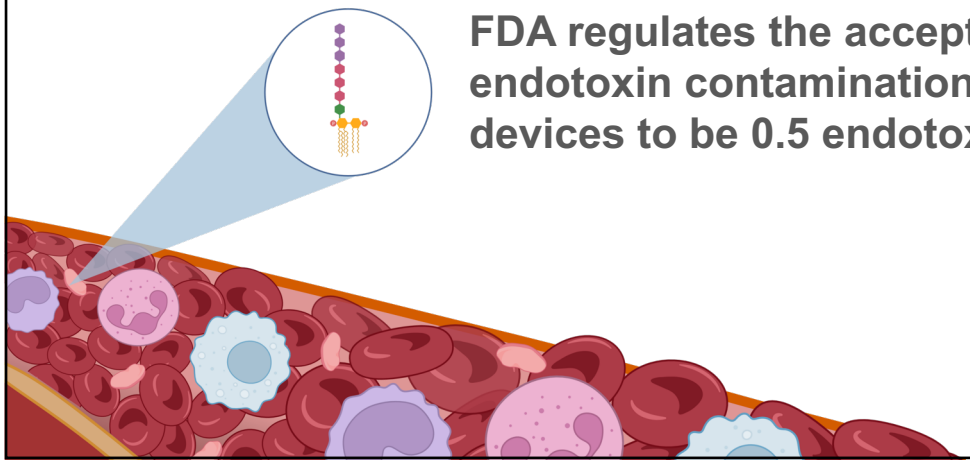
Main problems of endotoxins



Main problems of endotoxins

FDA

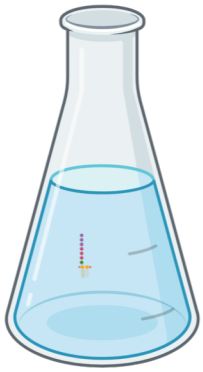
FDA regulates the acceptable level of endotoxin contamination with medical devices to be 0.5 endotoxin units/ml



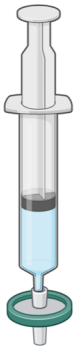
Main problem of endotoxins

FDA

"It is difficult to remove endotoxins from products once present."



Water soluble



Pass through filters

Heat stable

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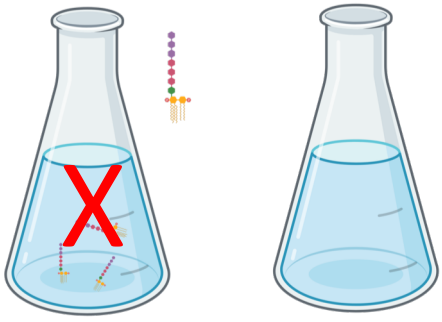
Reccomendations

FDA

"It is difficult to remove endotoxins from products once present. It is far better to keep finished products and components relatively endotoxin-free rather than have to remove it once present"



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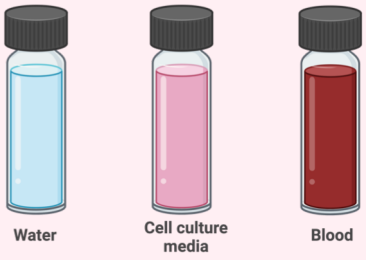
SUMMARY

Part 1: Fluids



1. Sterilization process
2. What are endotoxins?
3. FDA limitations
4. Recommendations

NPs sterility

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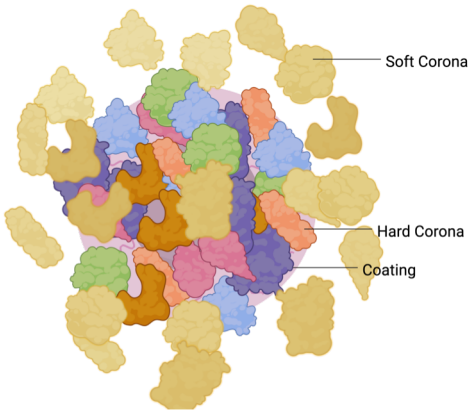
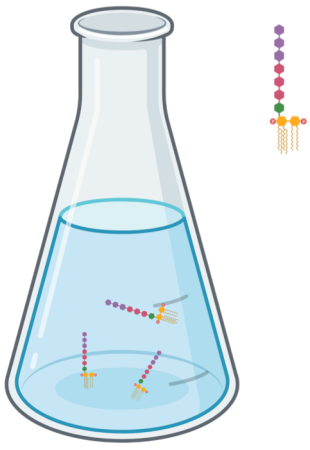
Summary of Part 1





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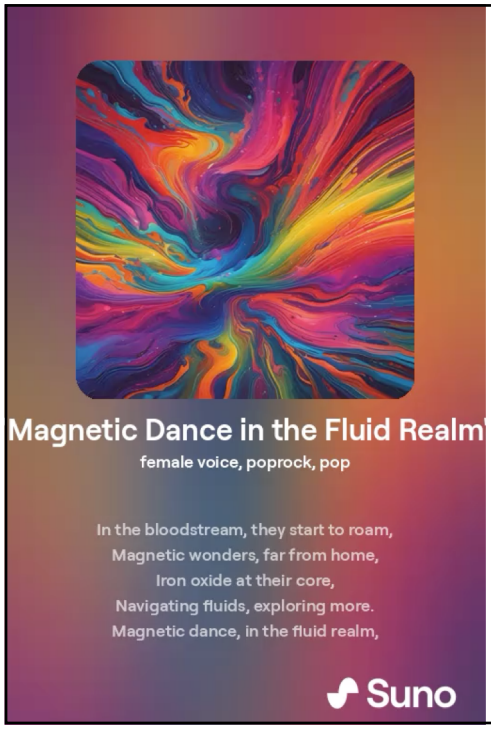

TUTORIAL PLAYLIST
Session 1: Introduction and Interactions at the Fluid Level

The Nano Odyssey: Understanding Nanoparticles in Biological Systems

-- Biology for non-biologist (taught by non-biologist) –

PART 2

Lucía Gutiérrez

INMA INSTITUTO DE NANOCIENCIA Y MATERIALES DE ARAGÓN CSIC Universidad Zaragoza

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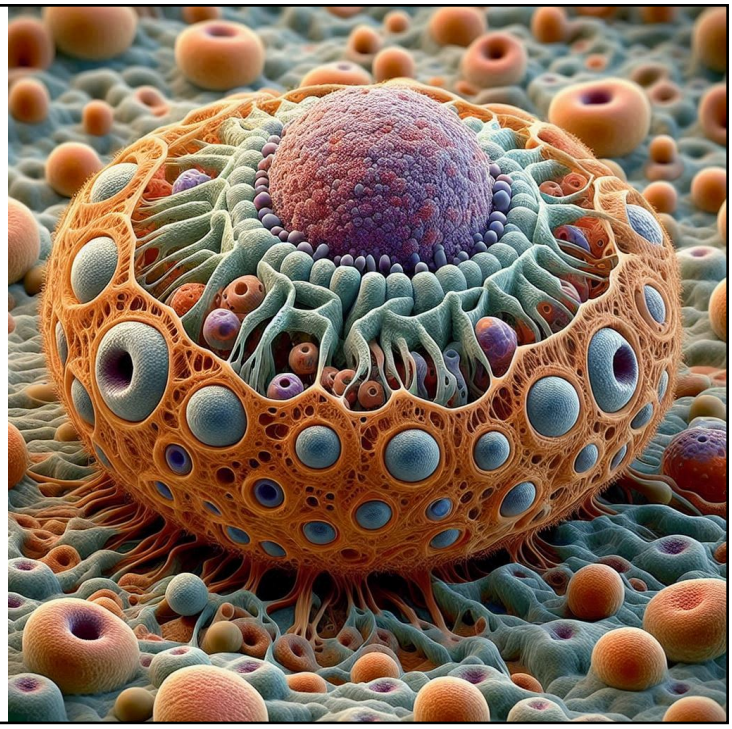
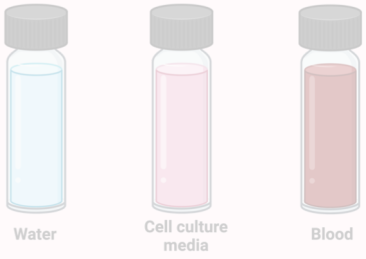
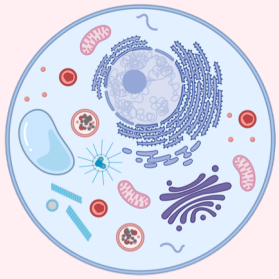


Interactions of NPs with biological systems

Part 1: Fluids



Part 2: Cells



Part 3: Body

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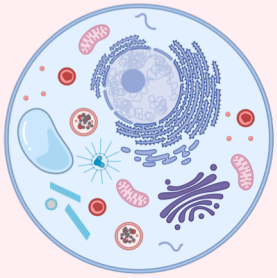
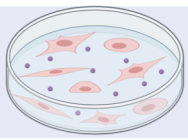
Interactions of NPs with biological systems

Part 2: Cells



Cell culture



NP uptake

Intracellular NP fate

Toxicity

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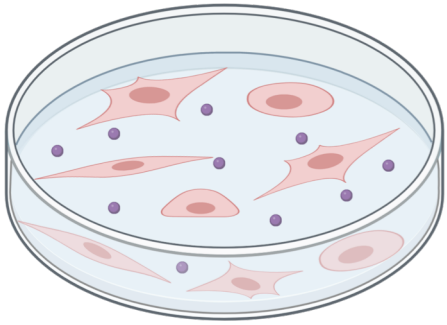
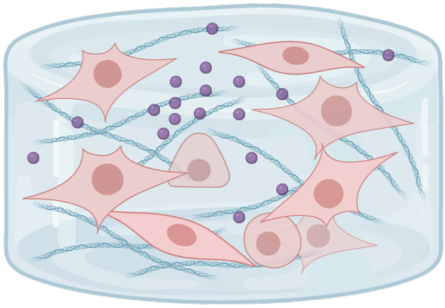
Cell culture types

2D vs 3D





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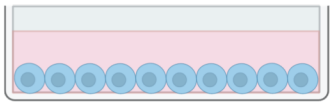
Cell culture types

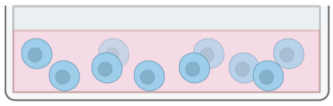
2D cell cultures

Adherent

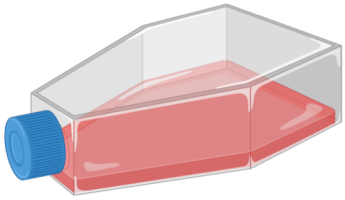


- Cells are attached to solid surface
- Grown as monolayers

Suspension



- Cells are free-floating in liquid medium



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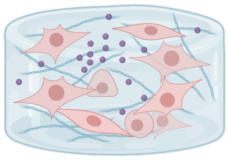
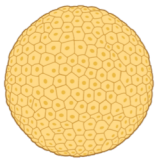
Cell culture types

3D cell cultures

3D matrix

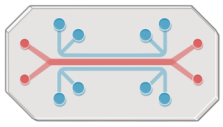


Spheroid



Organoid

Organ-on-chip



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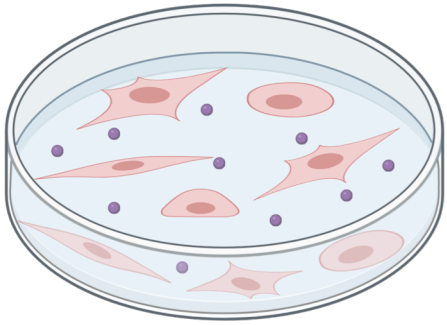
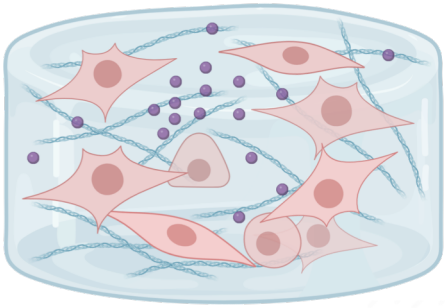
Why is this important?

Cell culture types

2D vs 3D





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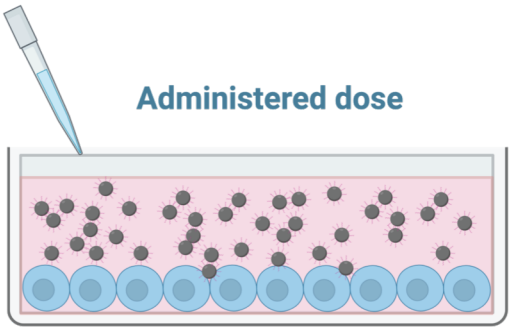
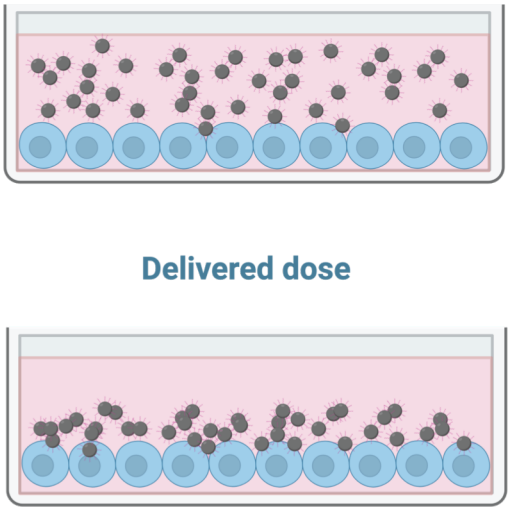
Cell culture

What do cells feel?





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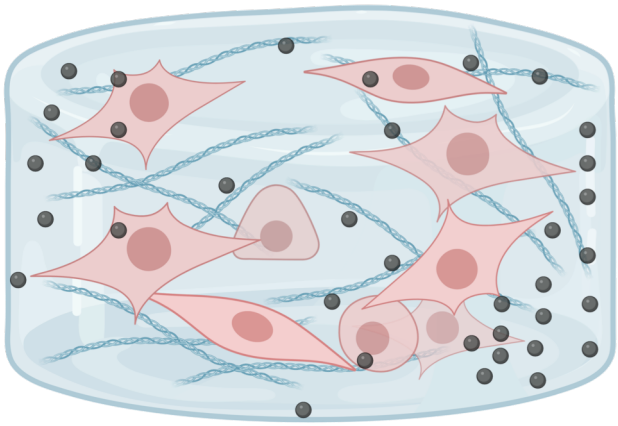
Cell culture

What do cells feel?

Delivered dose



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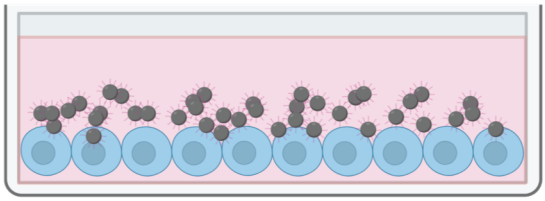
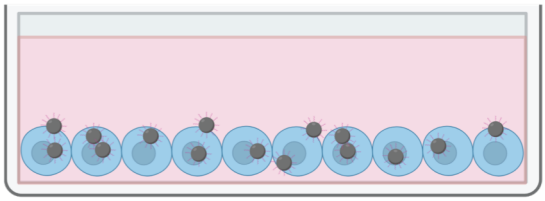
Cell culture

What do cells feel?

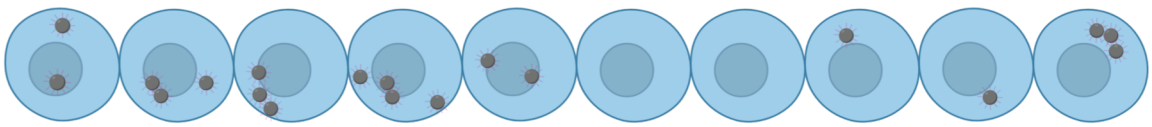
Delivered dose



NPs uptaken



Average NP uptake (?)



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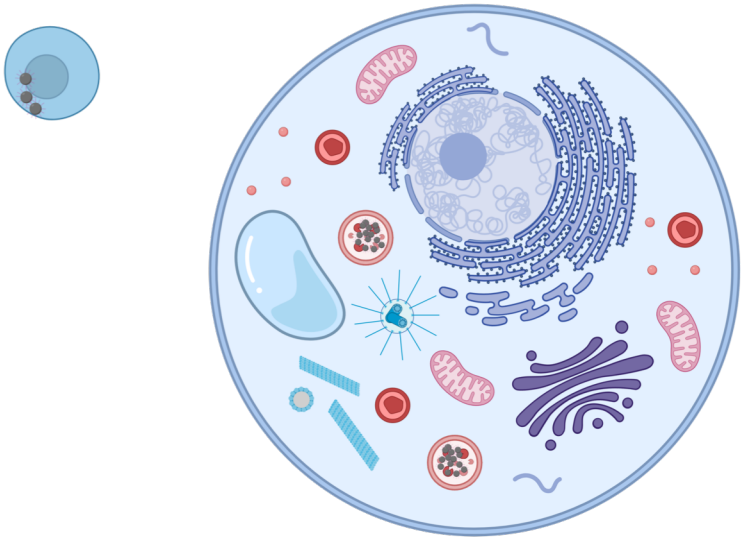
Things we often forget

Cell culture

Average concentration vs Local concentration



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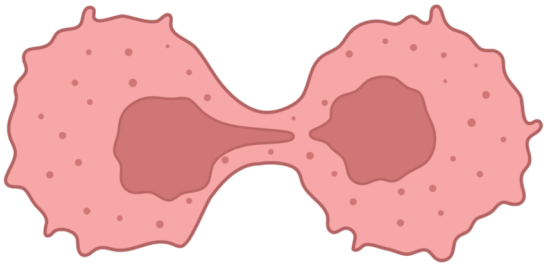
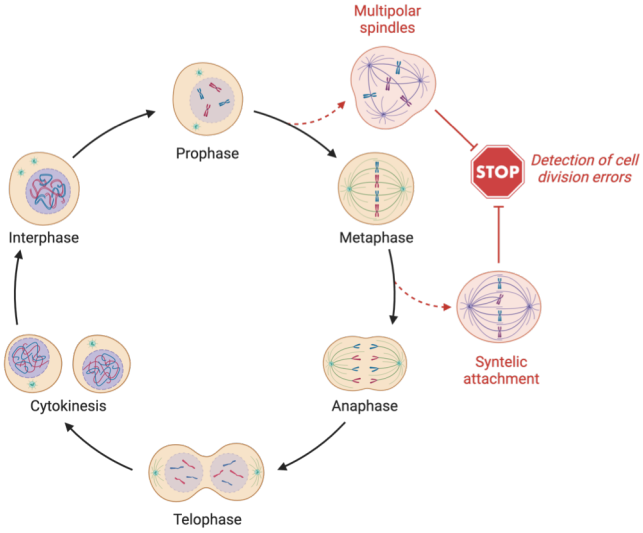
Things we often forget

Dynamics





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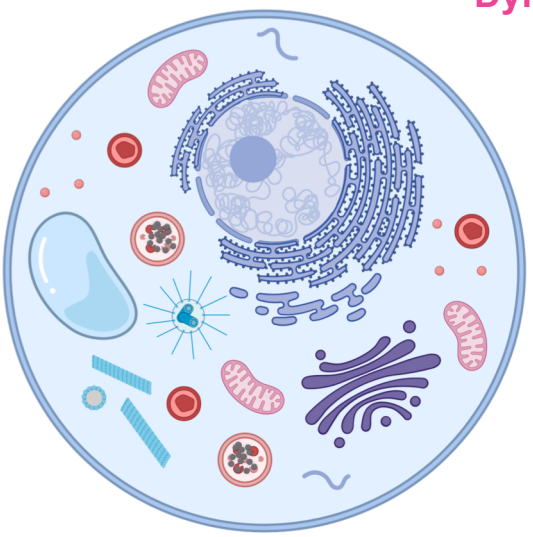
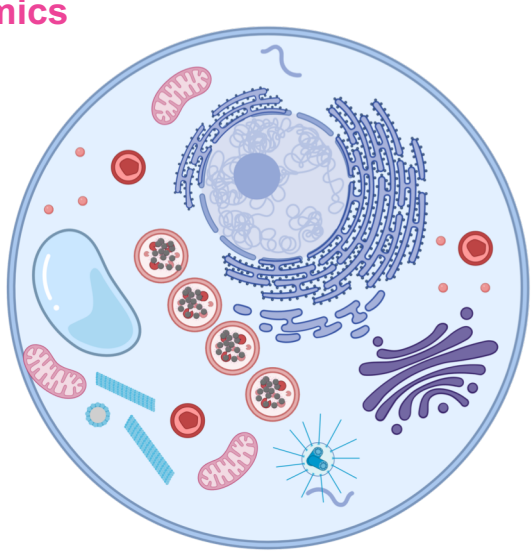
Things we often forget

Dynamics





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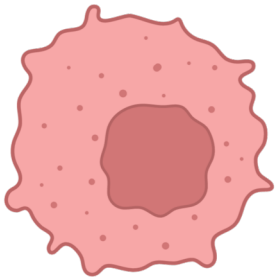
Talk by Yilian Fernández Afonso: Session 11

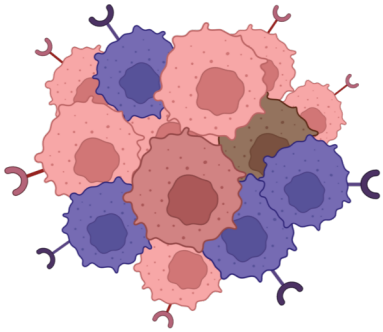
Things we often forget

Healthy cell

Tumor cell



Tumor heterogeneity



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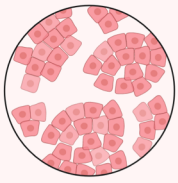
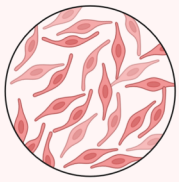
Things we often forget

Epithelial



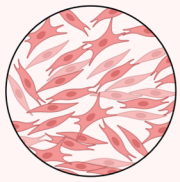
- Squamous, columnar or cuboidal-shaped

Endothelial



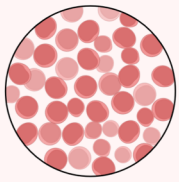
- Rounded outline
- Elongated

Fibroblast-like



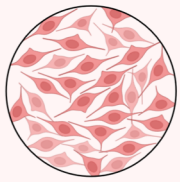
- Spindle-shaped
- Elongated

Lymphoblast-like



- Spherical-shaped
- Cells range in size

Neuronal



- Round, pyramidal, or spindle-shaped

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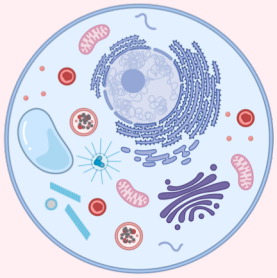
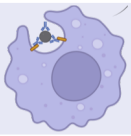
Interactions of NPs with biological systems

Part 2: Cells



Cell culture

NP uptake



Intracellular NP fate

Toxicity

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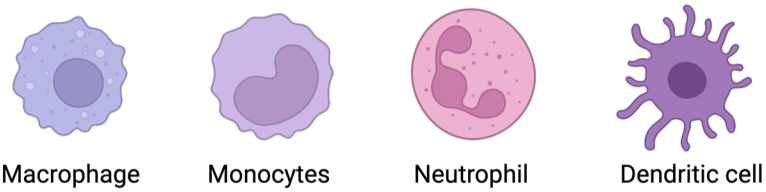
Nanoparticle entry into cells

Mechanisms of NP uptake

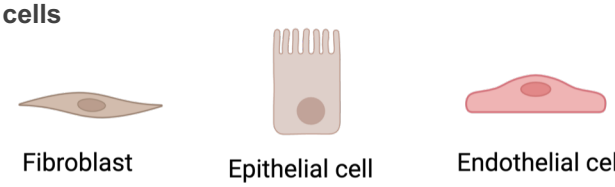
- **Phagocytosis** — Professional phagocytes
- **Pinocytosis** — Many cell types
  - Macropinocytosis
  - Clathrin-mediated endocytosis
  - Caveolin-mediated endocytosis
  - Others…

Phagocytosis

Professional phagocytes



Other types of cells



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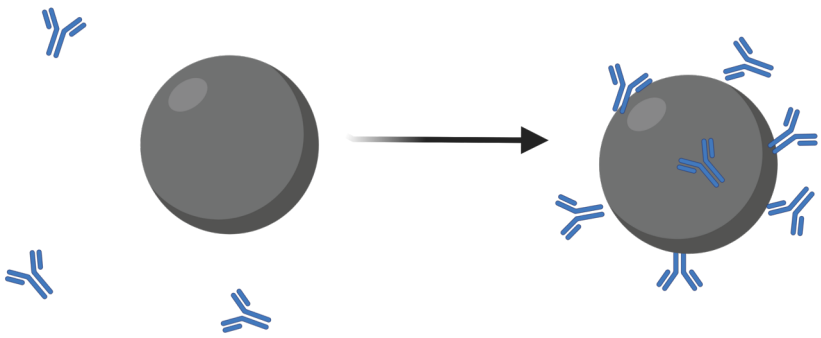
Phagocytosis

Opsonization



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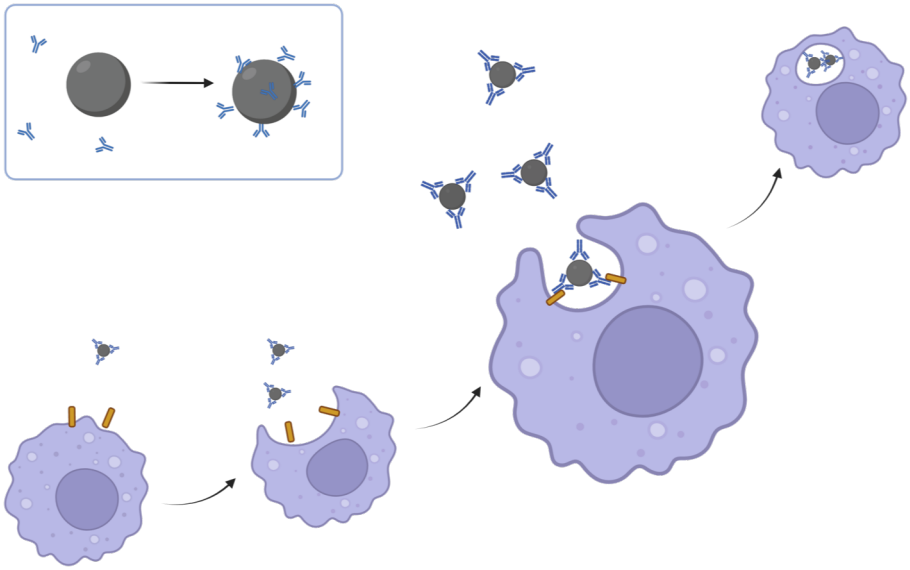
Phagocytosis

Opsonization



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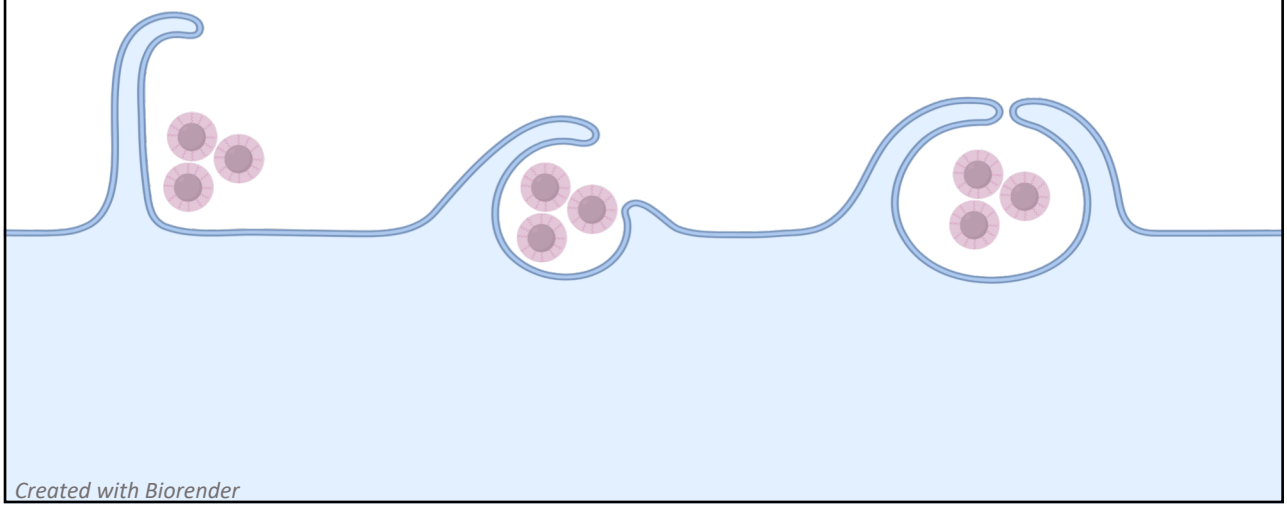
Nanoparticle entry into cells

Mechanisms of NP uptake

- **Phagocytosis**
  - Professional phagocytes
- **Pinocytosis**
  - Many cell types
    - **Macropinocytosis**
    - **Clathrin-mediated endocytosis**
    - **Caveolin-mediated endocytosis**
    - **Others…**

Macropinocytosis



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Nanoparticle entry into cells

Mechanisms of NP uptake

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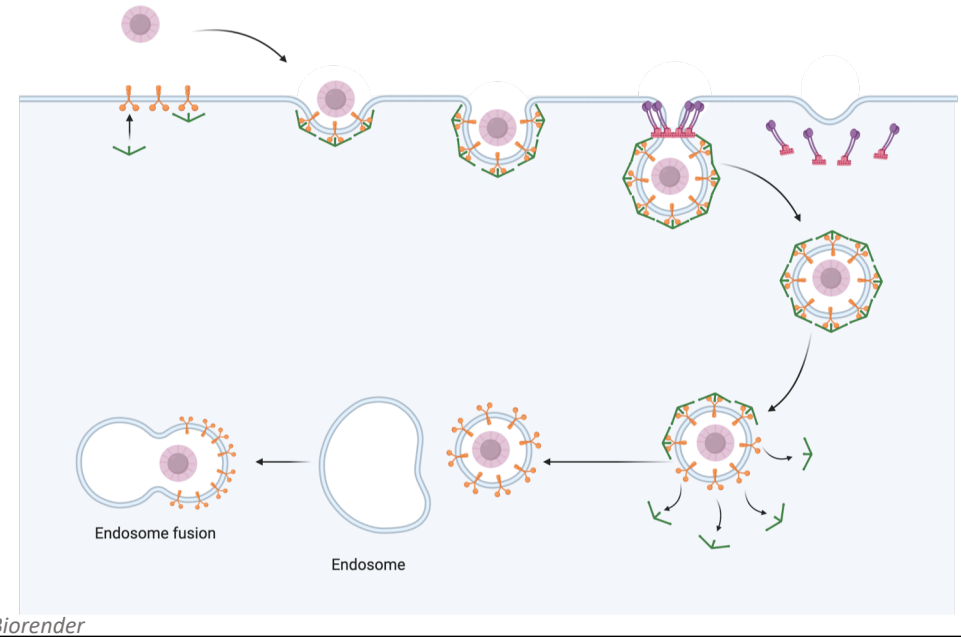
  - Macropinocytosis
  - Clathrin-mediated endocytosis
  - Caveolin-mediated endocytosis
  - Others…

Clathrin-meadiated endocytosis



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Nanoparticle entry into cells

Mechanisms of NP uptake

- **Phagocytosis**
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- **Pinocytosis**
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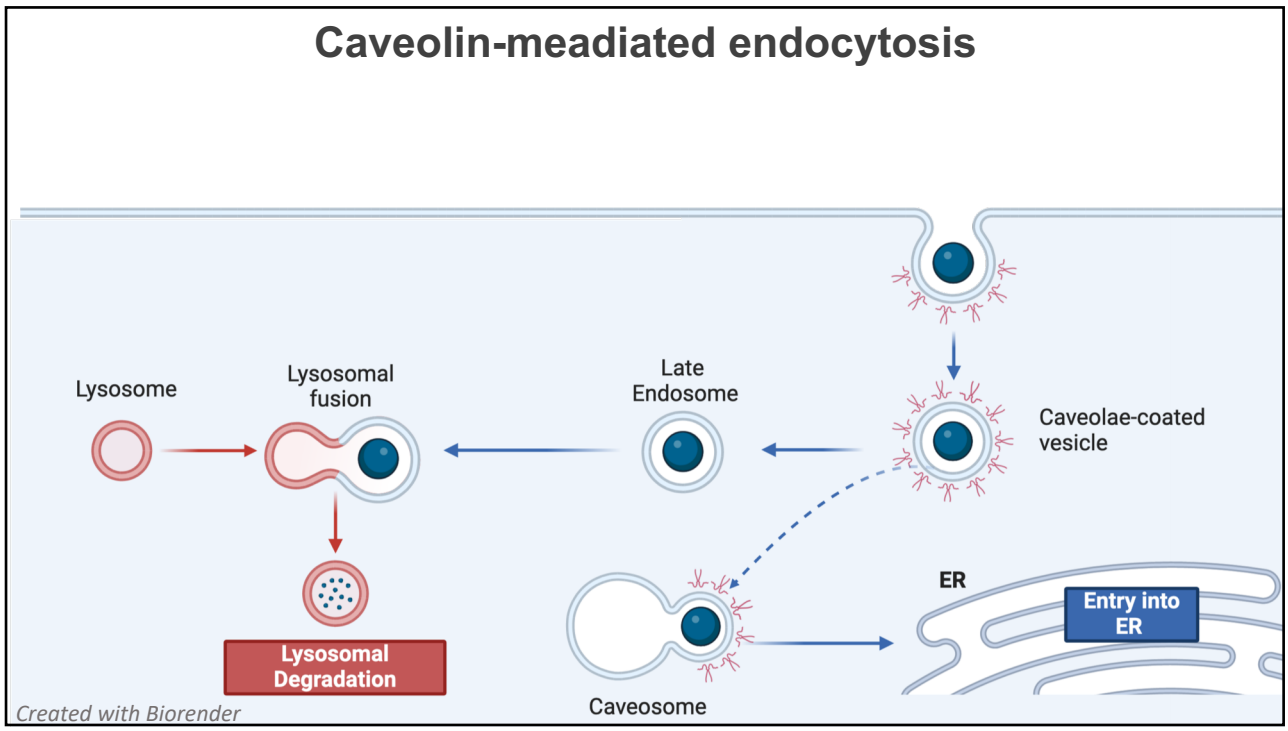
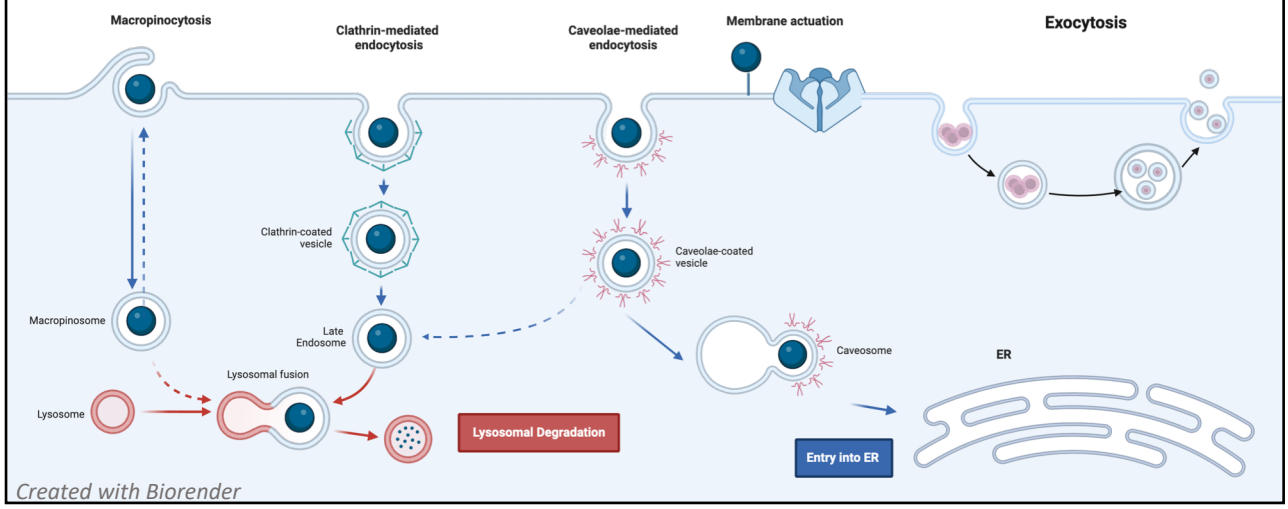
    - Macropinocytosis
    - Clathrin-mediated endocytosis
    - **Caveolin-mediated endocytosis**
    - Others…

Caveolin-meadiated endocytosis



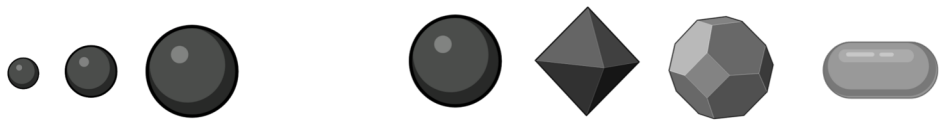
Summary of nanoparticles uptake



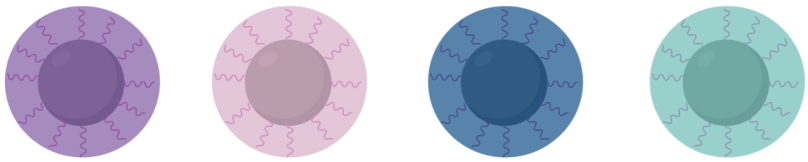
Parameters that affect NP uptake

Size

Shape



Coating

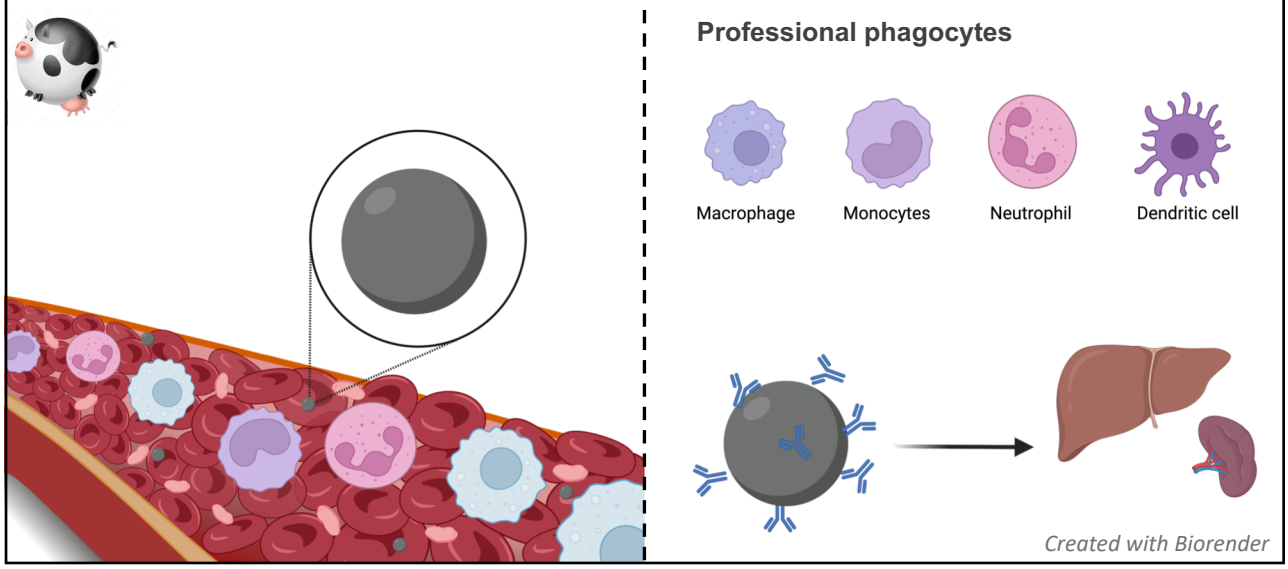


Charge	Hydrophobicity	Rugosity

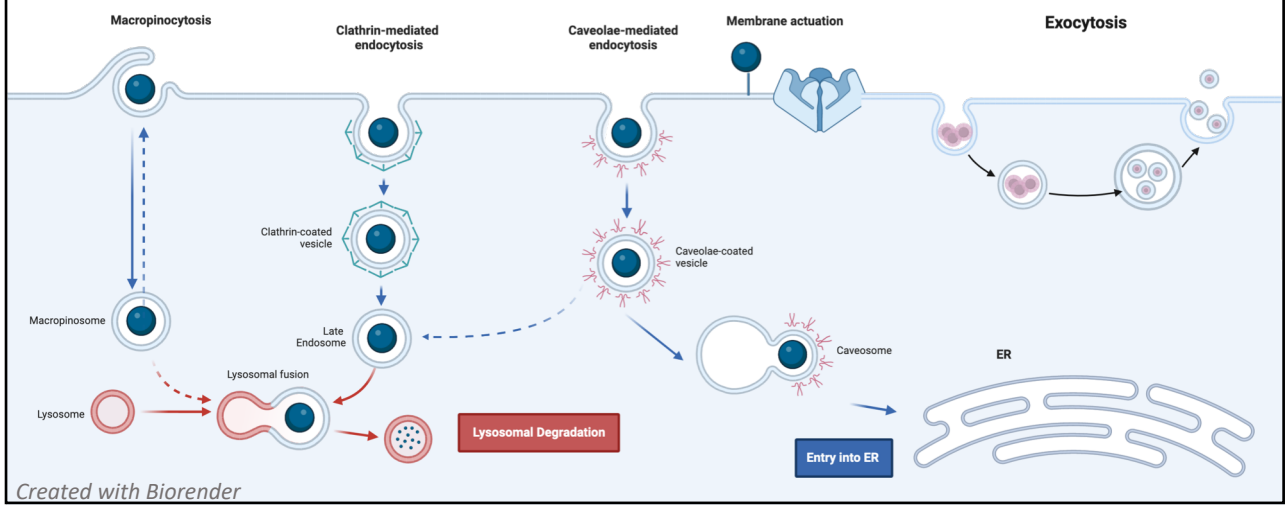
Why is this important?

Why is this important?

Fate of the particle (in the body)

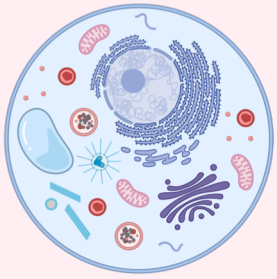


What happens to the particles when they are in?



Interactions of NPs with biological systems

Part 2: Cells



Cell culture

NP uptake

Intracellular NP fate

Toxicity

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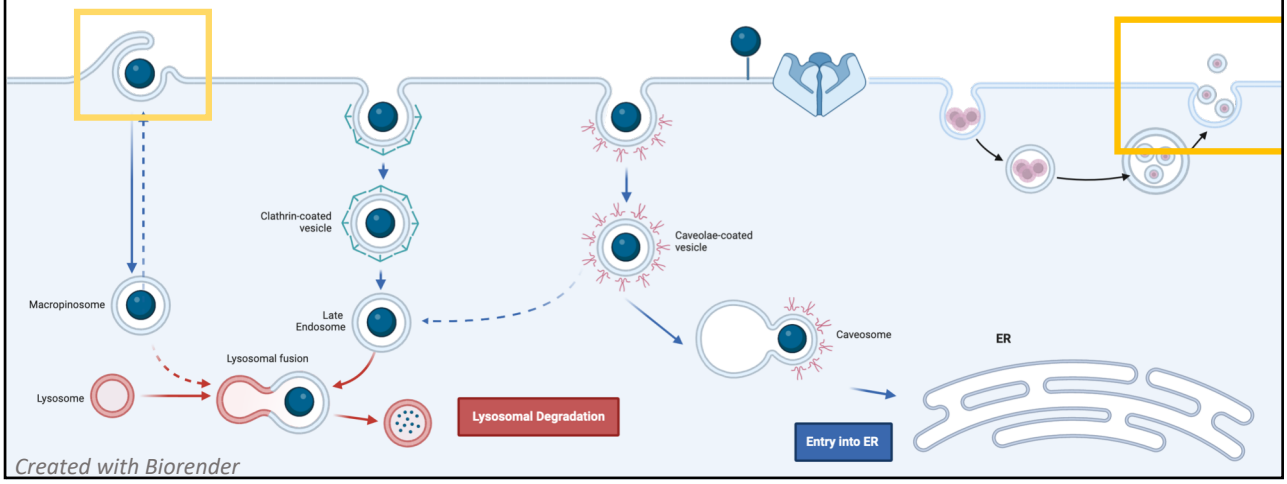
What happens to the particles when they are in?

Exocytosis → **Time frame for treatment**



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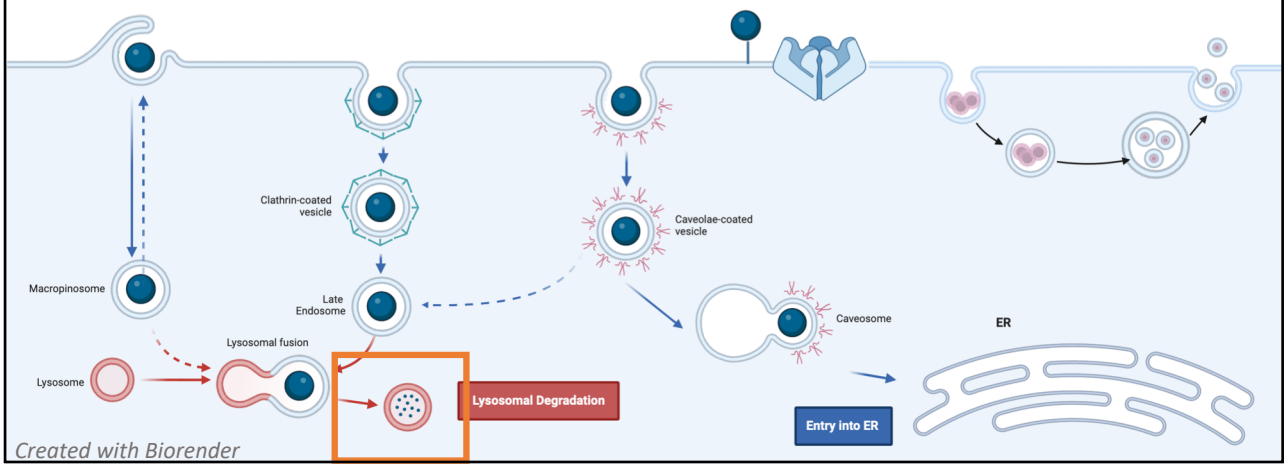
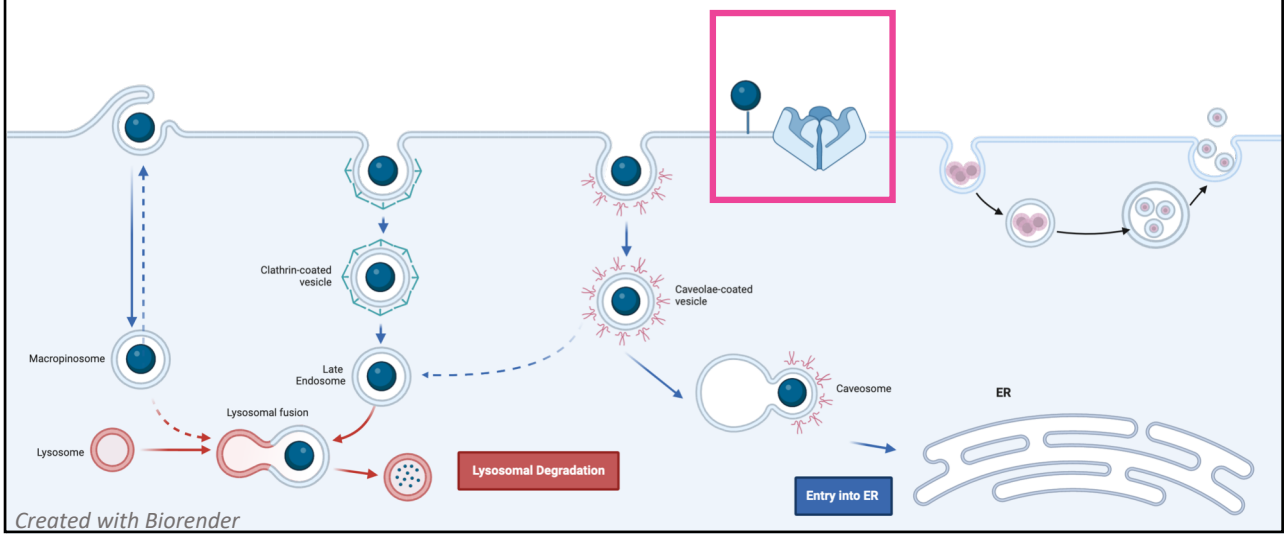
What happens to the particles when they are in?

Degradation → **Time frame for treatment**



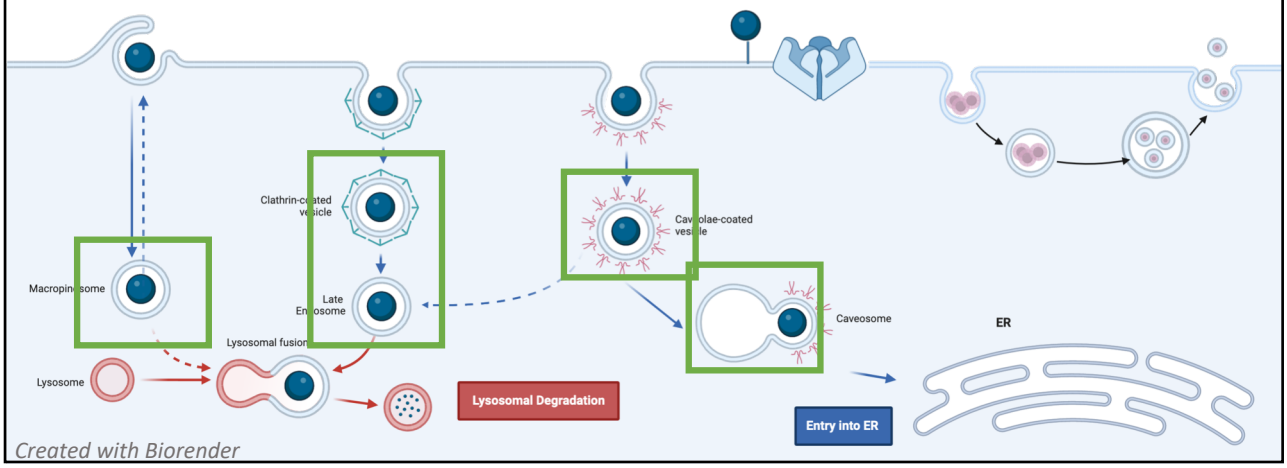
Do we always want them in?

Timeframe for biomedical application



What happens to the particles when they are in?

Accumulation → **Toxicity**



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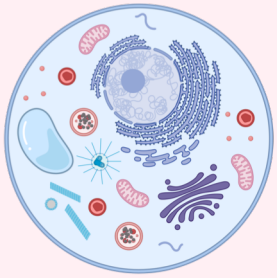
Interactions of NPs with biological systems

Part 2: Cells



Cell culture

NP uptake

Intracellular NP fate

Toxicity

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Toxicity: how do NPs damage cells?

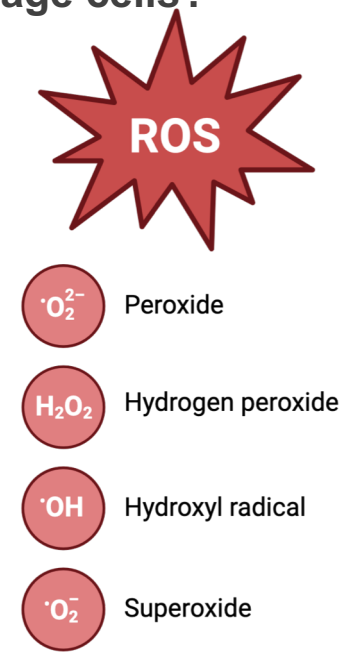
- **Reactive Oxygen Species**
- Cytoskeleton Damage
- Proinflamatory Markers
- Cell Organelle Damage
- Cell membrane disruption
- DNA Damage

Toxicity: how do NPs damage cells?

- **Reactive Oxygen Species**

Fenton and Haber-Weiss reactions

ROS can cause severe damage to the DNA, protein, and cells



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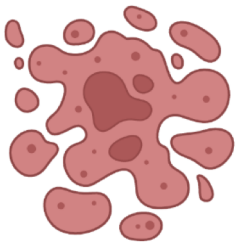
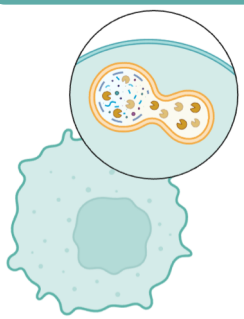
Types of cell death

Apoptosis



Necrosis

Autophagy



** There are many other types, but these three are the main ones…

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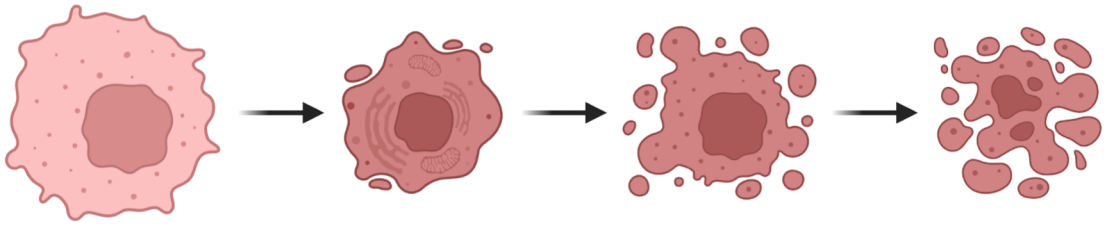
Stages of Apoptosis



Stage 1

Non-apoptotic cancer cell

Stage 2

Cell shrinkage and chromatin condensation

Stage 3

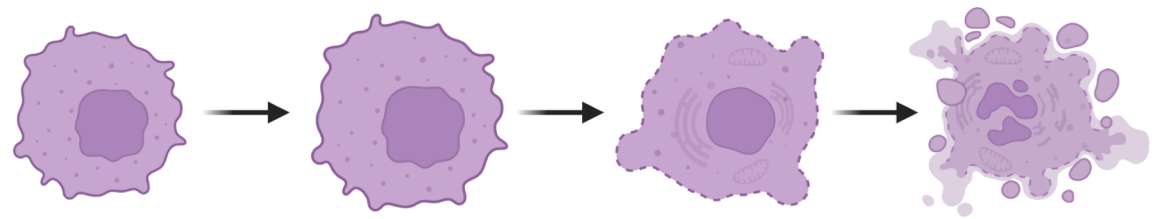
Nuclear fragmentation and membrane blebbing

Stage 4

Apoptotic body formation

Created with Biorender

Stages of Necrosis

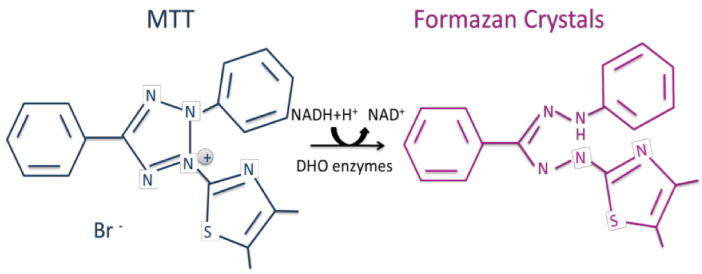


Stage 1	Stage 2	Stage 3	Stage 4
Non-necrotic cancer cell	Swelling of the cell and organelles	Rupture of cell membrane	Cell content release

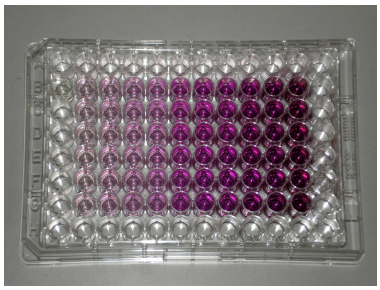
Created with Biorender

Toxicity: how do we measure cell death?

MTT: Absorbance



Absorbance measurement at 570nm

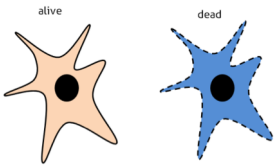


MTT- (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)

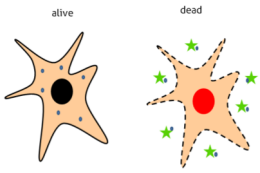
Toxicity: how do we measure cell death?

Membrane permeability: Microscopy

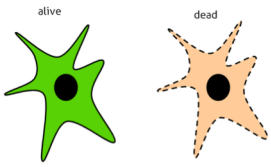
Trypan Blue



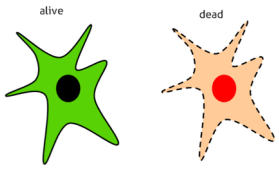
Lactate deshidrogenase (LDH)



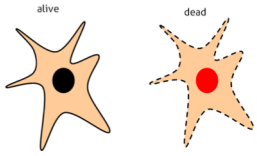
Fluorescein diacetate



Calcein-AM

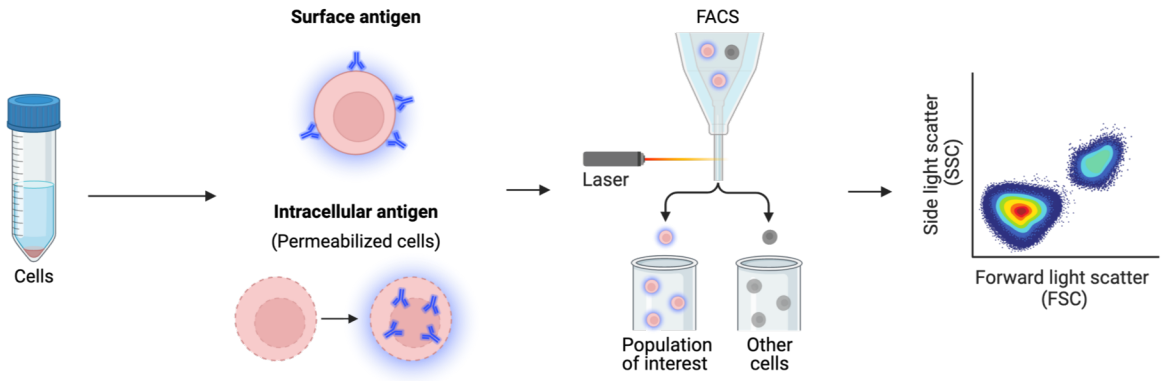


Fluoresecent DNA intercalants



Toxicity: how do we measure cell death?

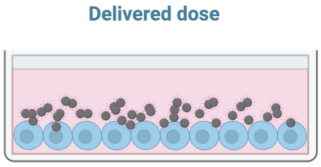
Flow citometry

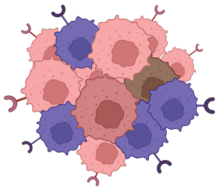


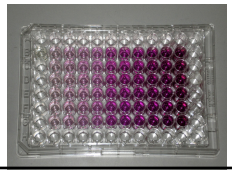
Created with Biorender

Challenges

- What is the particle concentration?
- How many cell types do we test?
- Timeframe for toxicity studies
- Interferences of the NPs with some of the toxicity tests

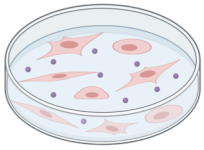


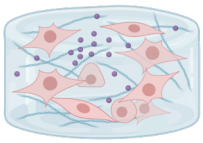




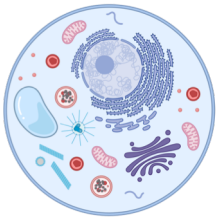
Summary

Cell culture types

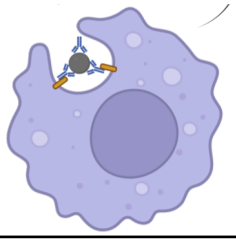




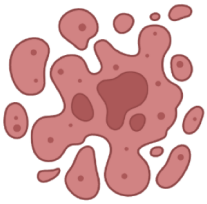
Intracellular fate of NPs

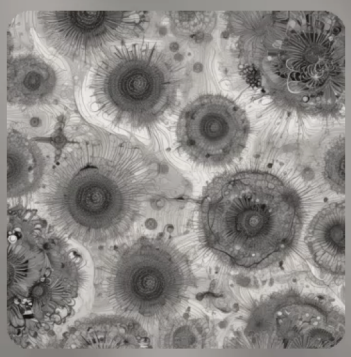


Mechanisms of cell uptake



NPs toxicity





Magnetic Magic Inside the Cell

rock, male voice

Through the membrane, they do pass,
Endocytosis, happens fast,
Iron oxide, at the core,
Entering cells, explore some more.
Magnetic magic, inside the cell,

Suno

TUTORIAL PLAYLIST
Session 2: Interactions at the Cellular Level

The Nano Odyssey: Understanding Nanoparticles in Biological Systems

-- Biology for non-biologist (taught by non-biologist) –

PART 3

Lucía Gutiérrez

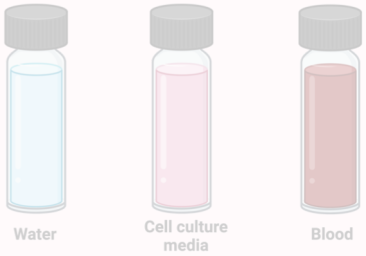
INMA INSTITUTO DE NANOCIENCIA Y MATERIALES DE ARAGÓN CSIC Universidad Zaragoza

Bionanosurf

MAGMEET 2024 BARCELONA

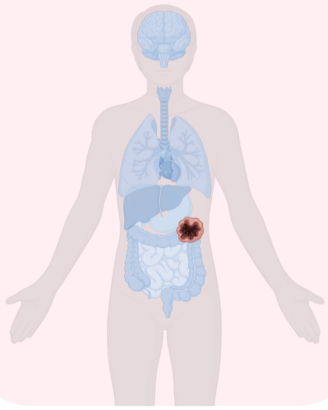
Interactions of NPs with biological systems

Part 1: Fluids



Part 2: Cells

Part 3: Body



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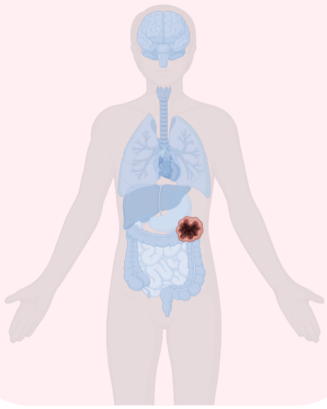
Interactions of NPs with biological systems

Part 3: Body



- **Administration routes**
- Animal models
- Tracking NPs in the body

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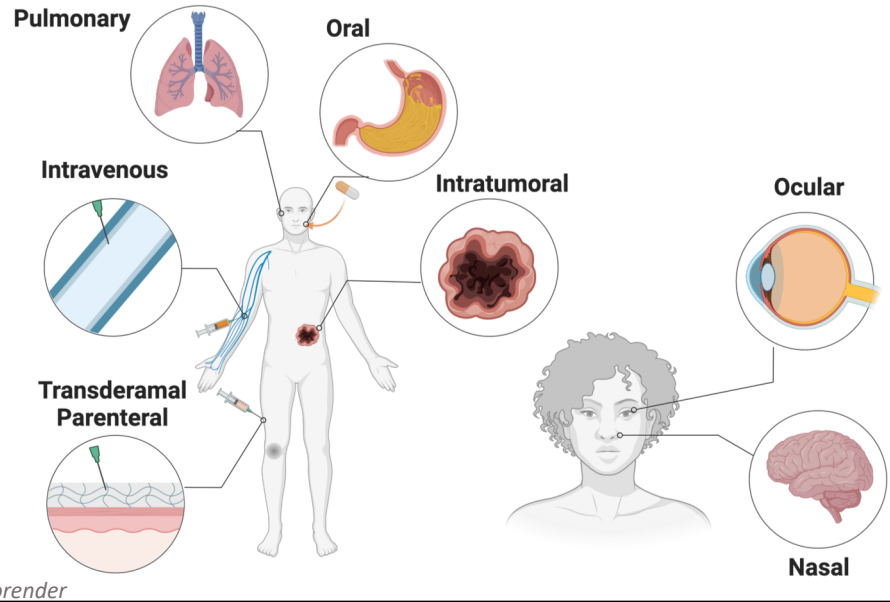
Interactions at the Organ and Body Level

Administration routes



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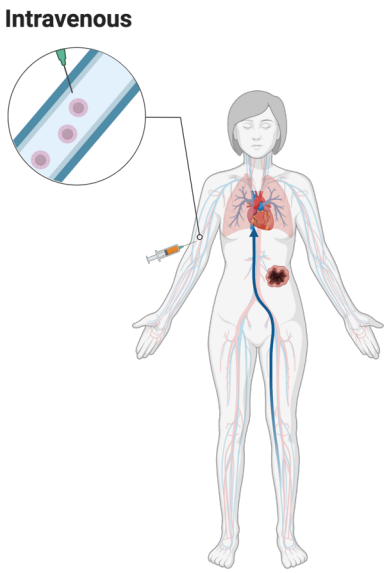
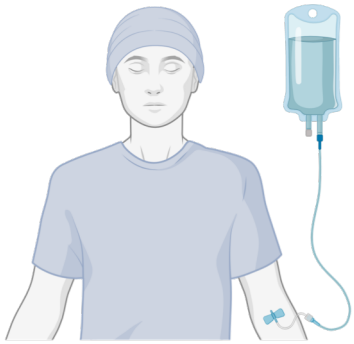
Intravenous administration

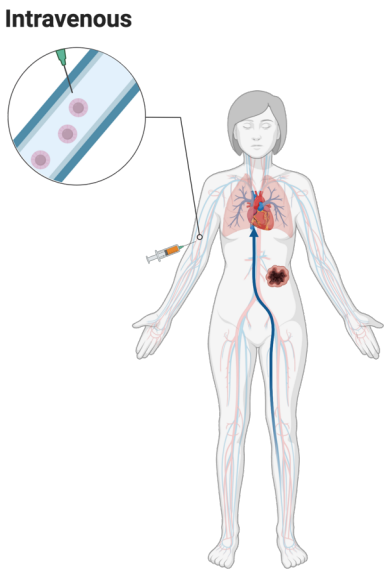


Advantage: easy

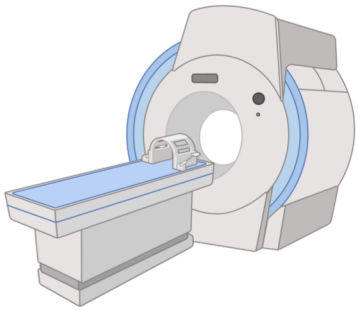


Created with Biorender

Intravenous administration



Circulation time & Clearance from the blood



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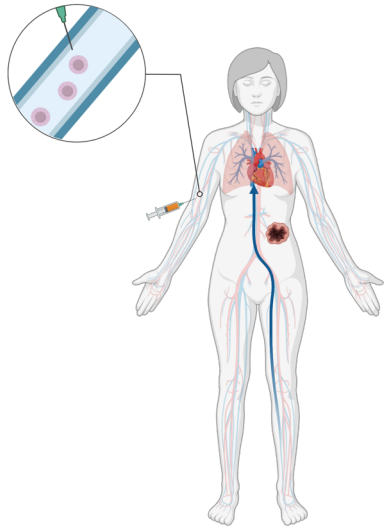
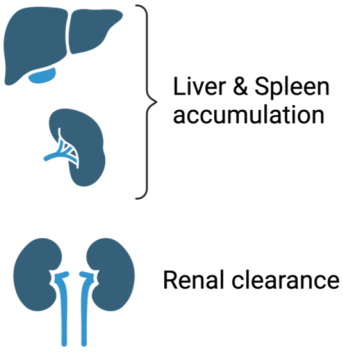
Intravenous administration



Biodistribution and clearance



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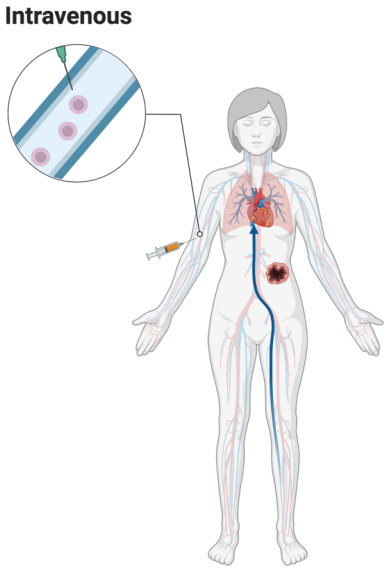
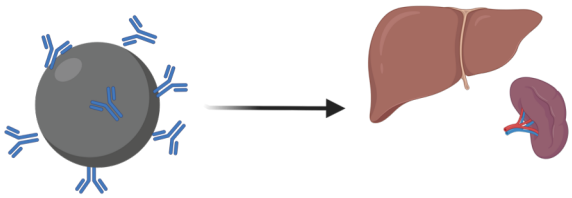
Intravenous administration



PROBLEM!

Particles will mainly be accumulated in the liver and the spleen



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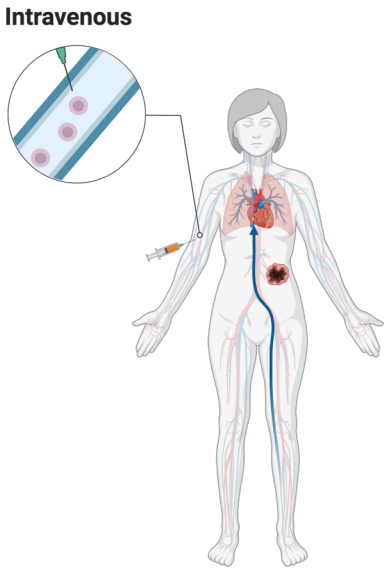
Intravenous administration



PROBLEM!

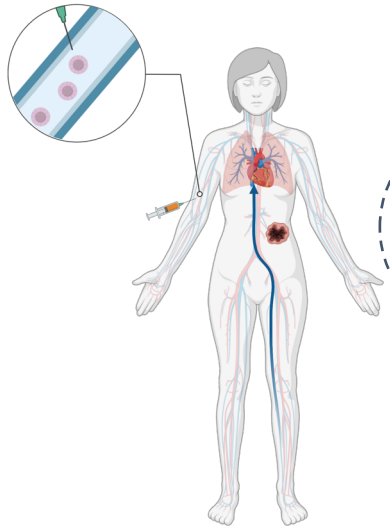
Small particles (< 5nm) will be eliminated through the kidneys

Renal clearance

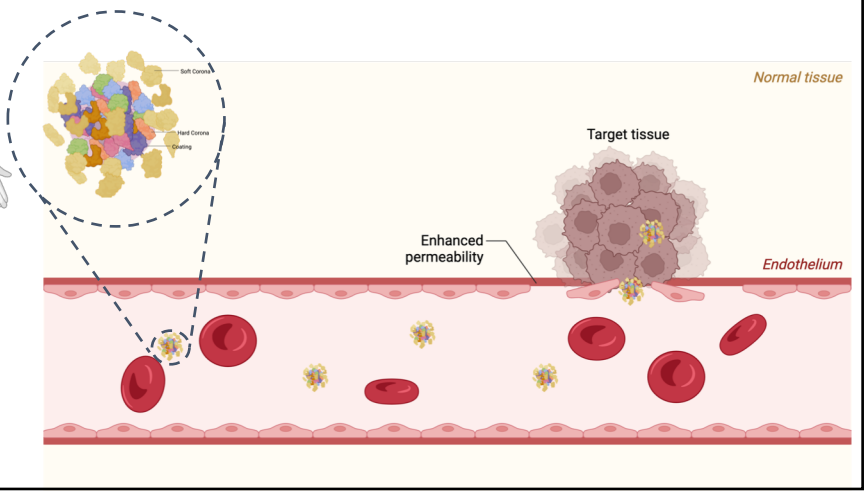
Created with Biorender

Intravenous administration

Intravenous



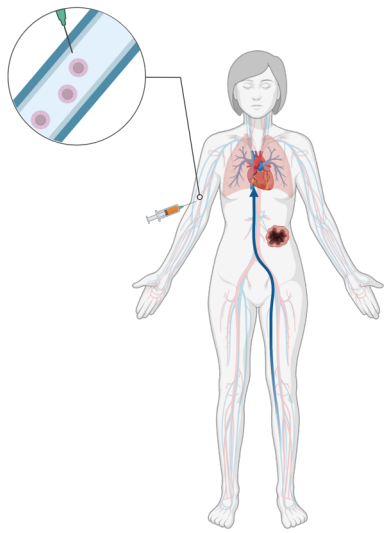
Mechanism of accumulation
EPR effect
(Enhanced Permeability and Retention)



Created with Biorender

Intravenous administration

Intravenous



PROBLEM!

1 % of particles arrive to the tumor

Analysis of nanoparticle delivery to tumours

Stefan Wilhelm, Anthony J. Tavares, Qin Dai, Seiichi Ohta, Julie Audet, Harold F. Dvorak & Warren C. W. Chan

Nature Reviews Materials **1**, Article number: 16014 (2016) | Cite this article

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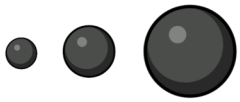
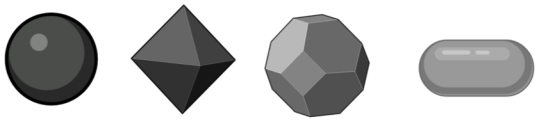
Parameters that affect the biodistribution

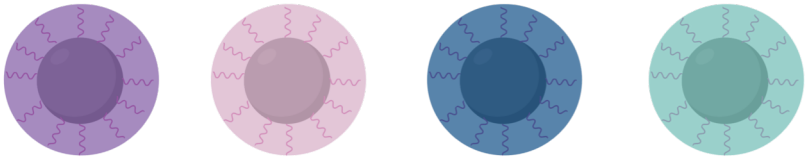
Size



Shape



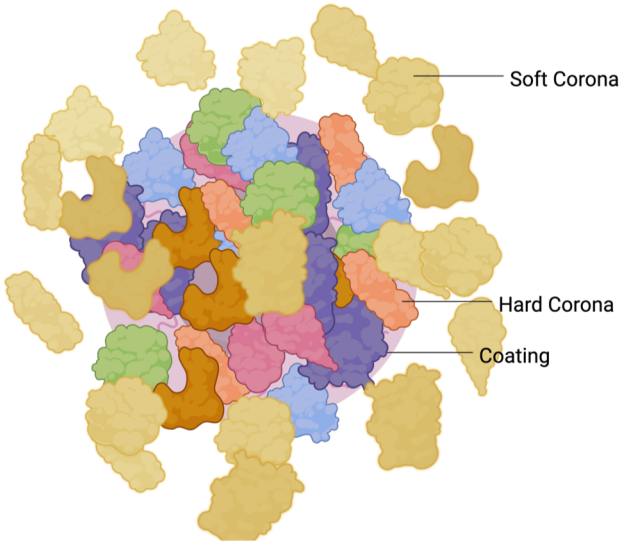
Coating



Charge	Hydrophobicity	Rugosity

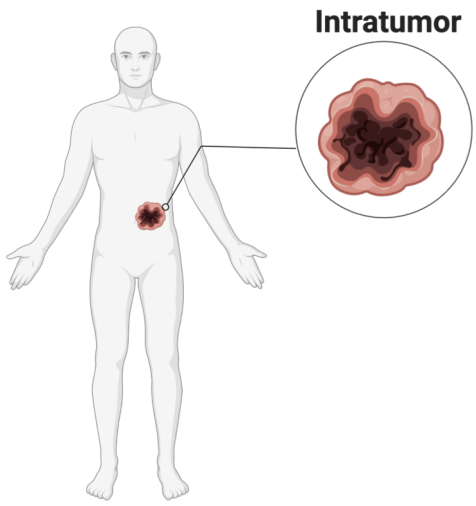
Parameters that affect the biodistribution

Protein Corona



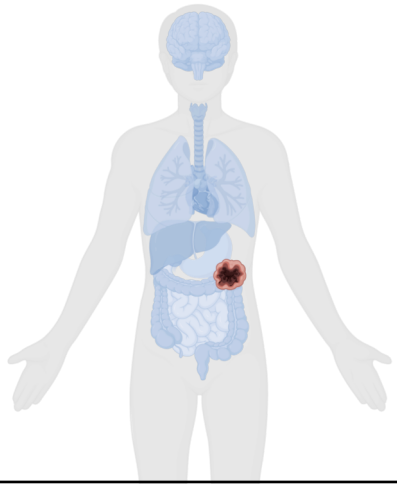
Created with Biorender

Intratumor administration



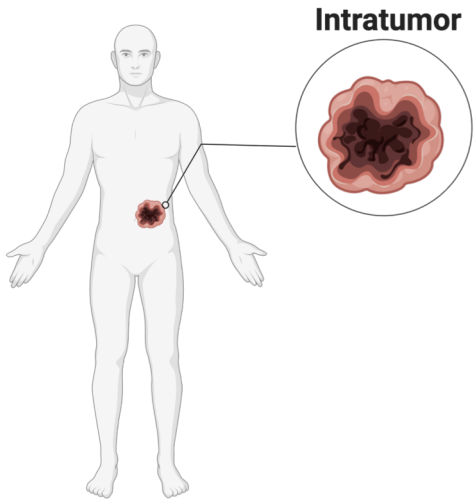
Advantage

No other organs are affected



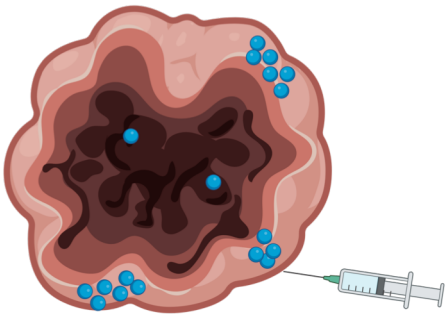
Created with Biorender

Intratumor administration



Advantage

Larger dose delivered to the target area



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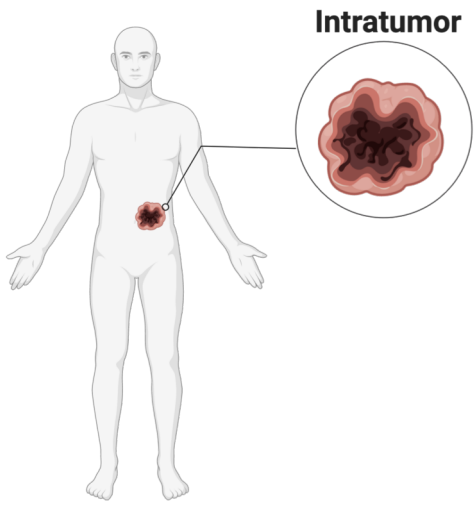
Intratumor administration



PROBLEM!

Need of surgery (?)

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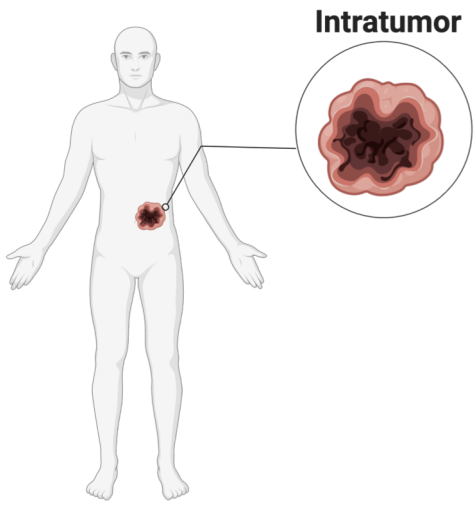
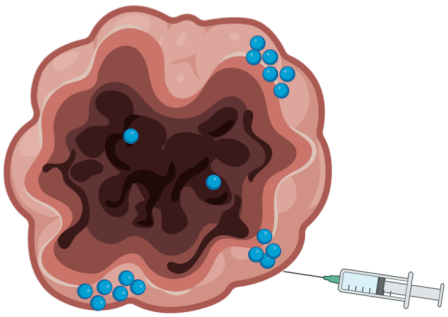
Intratumor administration



PROBLEM!

Heterogeneous distribution

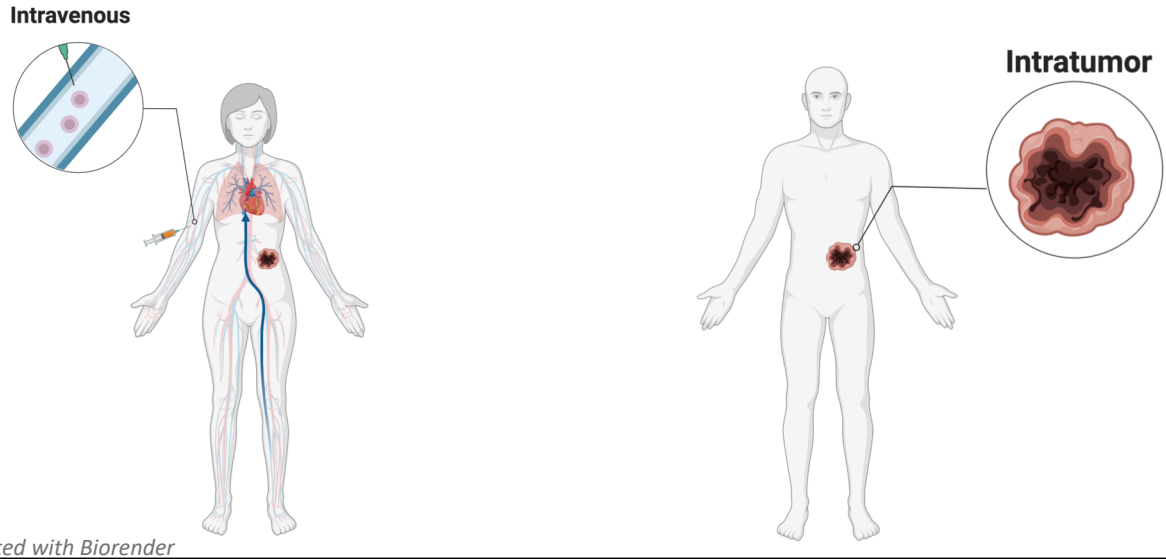


Created with Biorender

Why is this important?

Why is this important?

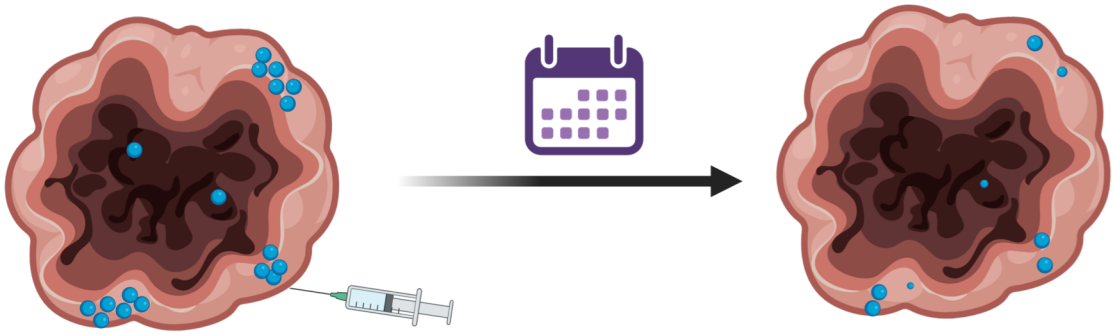
Route selection to achieve the desired amount of NPs at the target area



Created with Biorender

Why is this important?

The amount of particles that reach the tissue determines the degradation speed

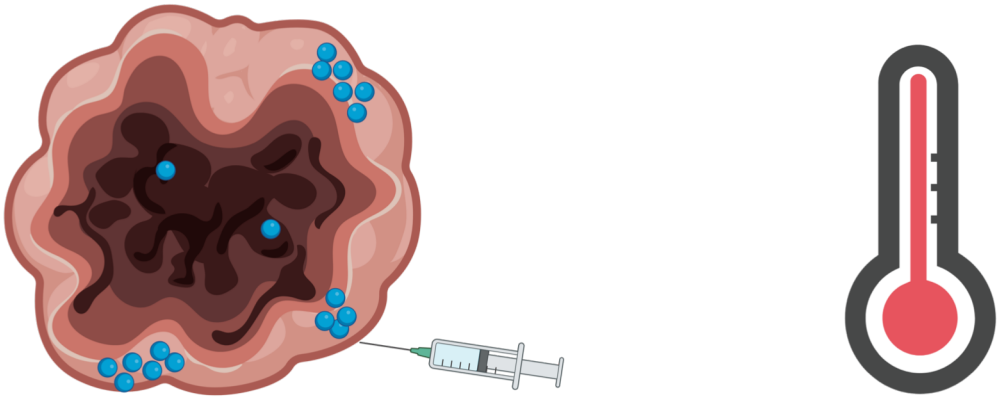


Need of repeated treatments?

Created with Biorender

Why is this important?

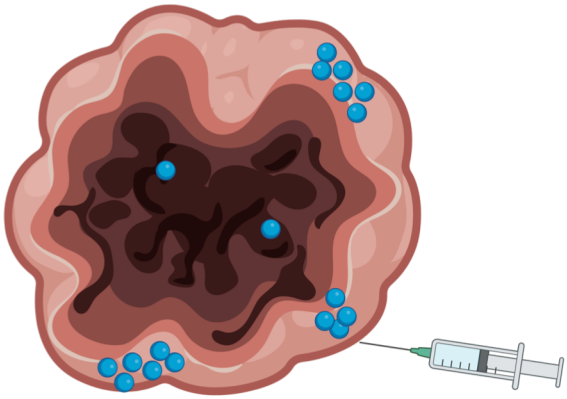
Heterogeneous distribution: heat gradients or treatment gradients

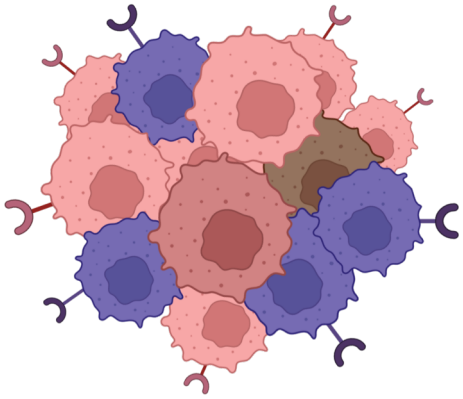


Created with Biorender

Why is this important?

Heterogeneous distribution: Untreated cells





Resistance?

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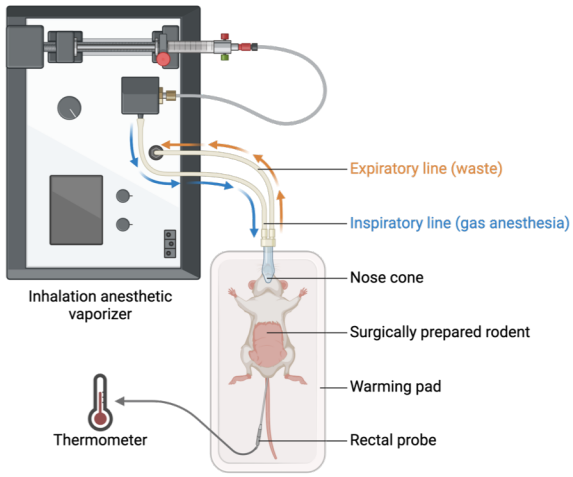
Things we often forget

Parameters that affect the biodistribution

Anesthesia



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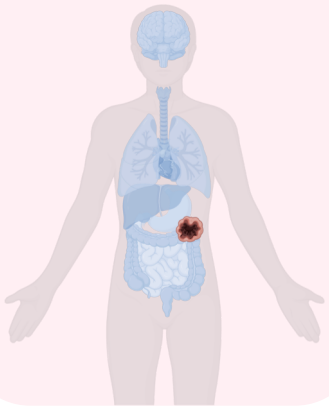
Interactions of NPs with biological systems

Part 3: Body



Administration routes

Animal models

Tracking NPs in the body

Created with Biorender

Animal models

Immunodeficient /immunocompetent

Immunocompetent mouse

Immunodeficient mouse

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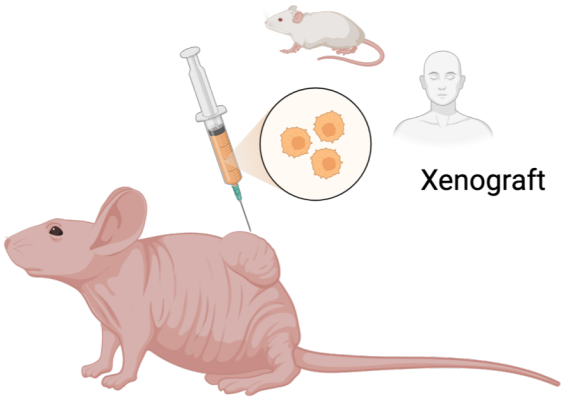
Animal models

Xenograft /allograft

Allograft

Immunocompetent mouse



Immunodeficient mouse

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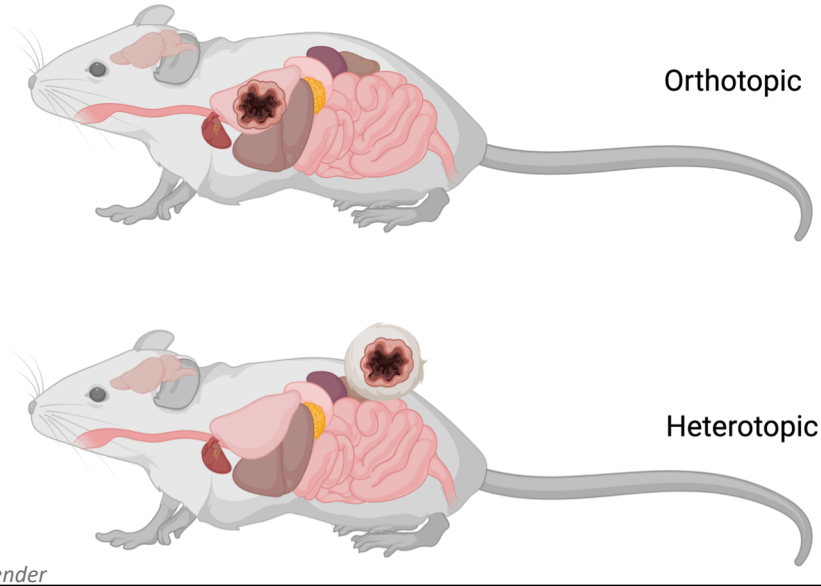
Animal models

Orthotopic /heterotopic



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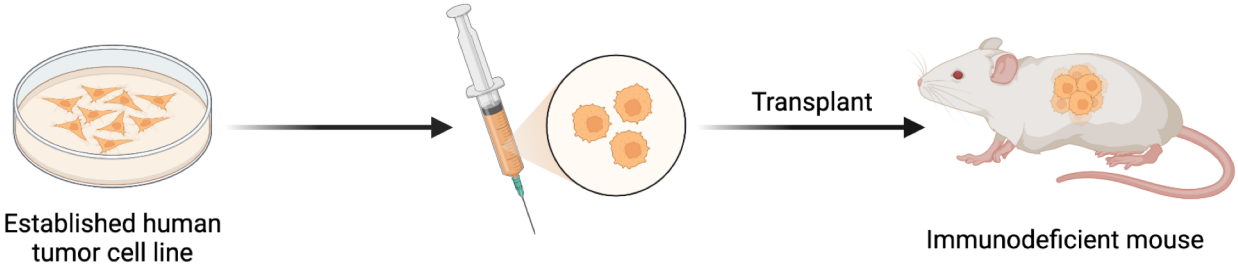
Cell Line-Derived Xenograft Models



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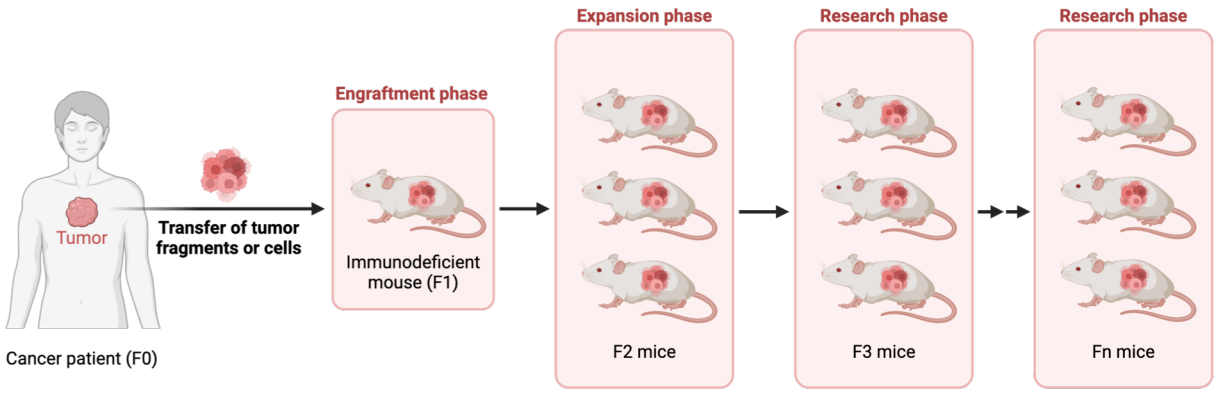
Patient-Derived Xenograft (PDX) Models



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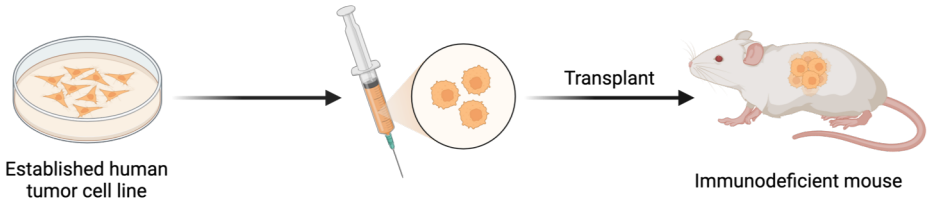
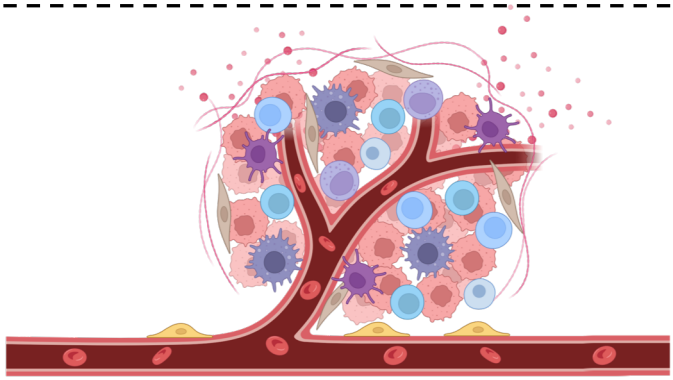
Why is this important?

Theory vs reality





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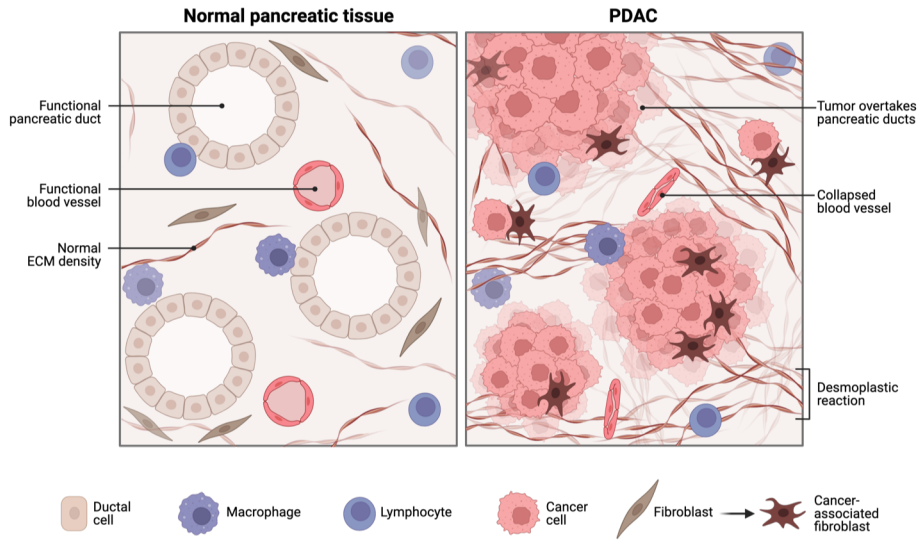
Pancreatic Ductal Adenocarcinoma (PDAC)



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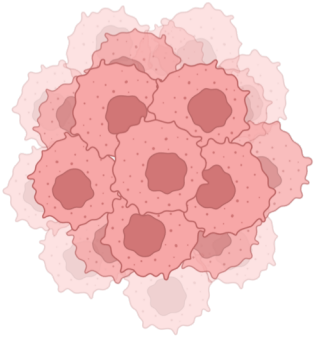
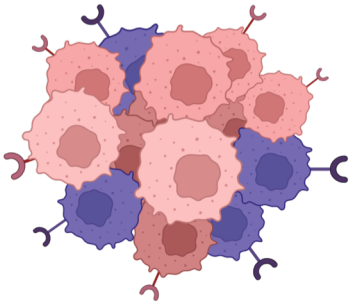
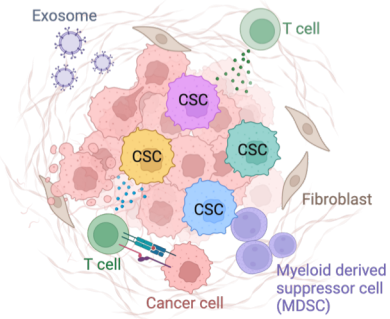
Theory vs reality: IMMUNE RESPONSE







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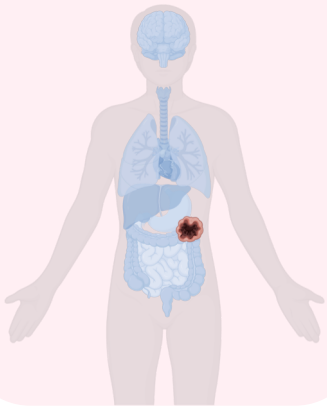
Interactions of NPs with biological systems

Part 3: Body



Administration routes

Animal models

Tracking NPs in the body

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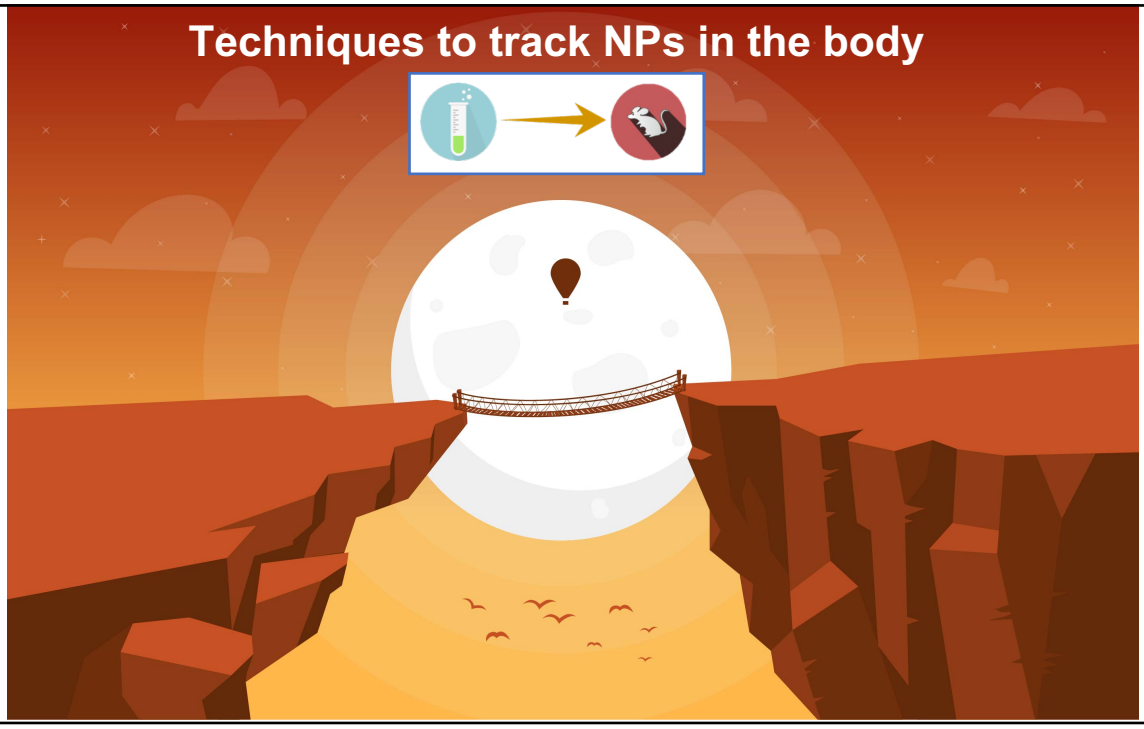
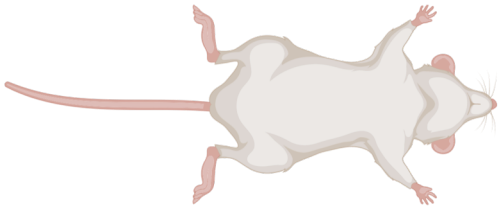
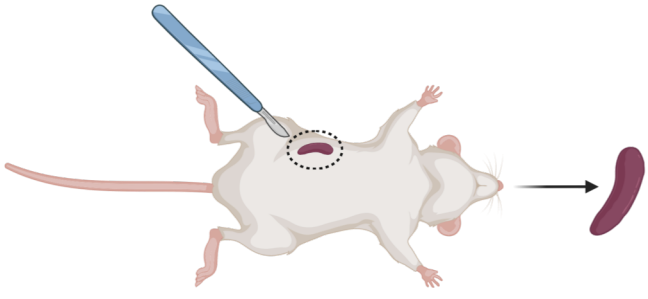
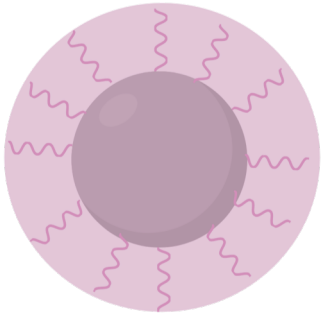


Techniques to track NPs in the body

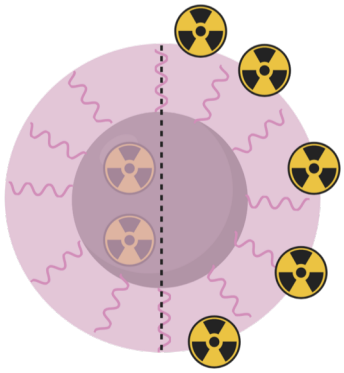
In vivo	Ex vivo
	
• More expensive • Hospital type facilities	• Sacrifice of animals

Techniques to track NPs in the body

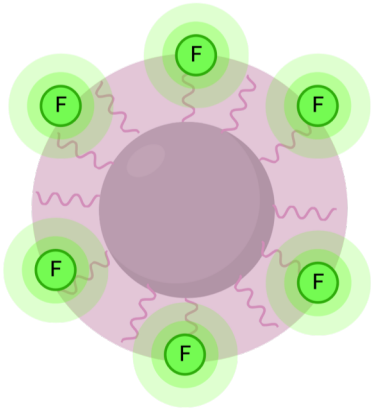
NPs



Modified NPs



Radionuclide



Fluorophore

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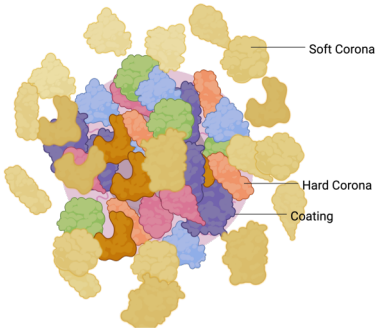
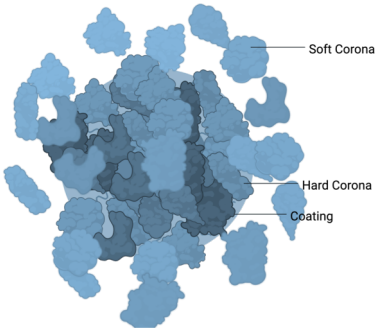
Techniques to track NPs in the body

NPs



Modified NPs



?

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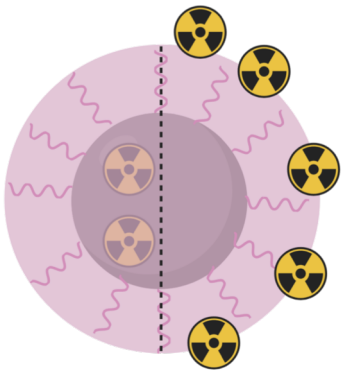
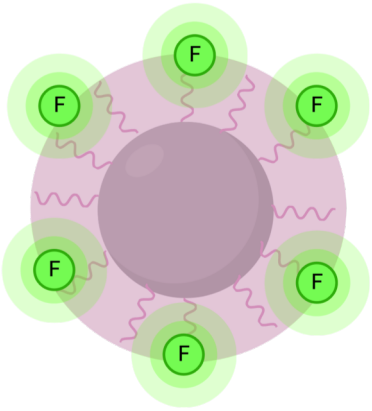
Techniques to track NPs in the body

What are we really measuring?

Leakage?

Modified NPs





Radionuclide

Fluorophore

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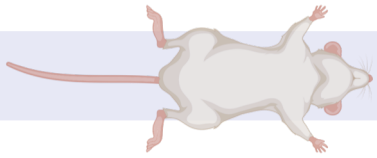
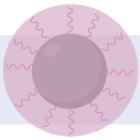
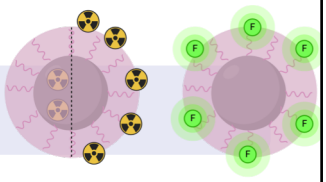
Tracking NPs In vivo



NPs



Modified NPs



MRI

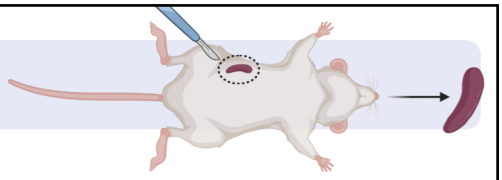
MPI

PET

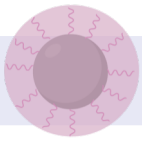
SPECT

Fluorescence

Tracking NPs Ex vivo

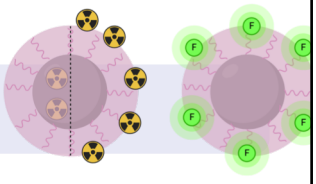


NPs



- Optical Microscopy
- Electron Microscopy
- Elemental Analysis *
- Magnetic Measurements
- Others…

Modified NPs

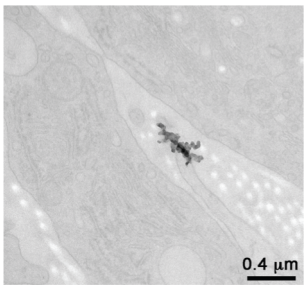


- Autoradiography
- Fluorescence

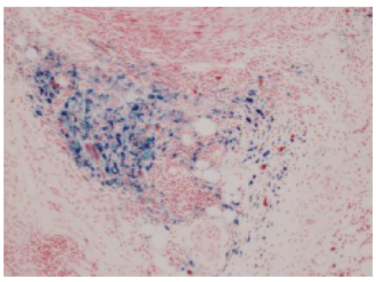
Techniques to track NPs in the body

Ideal option: Combine several techniques

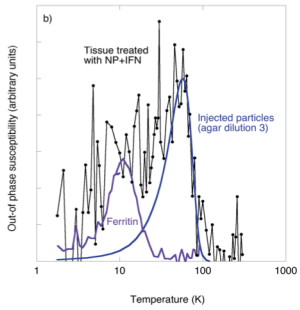
TEM



Optical Microscopy



Magnetometry



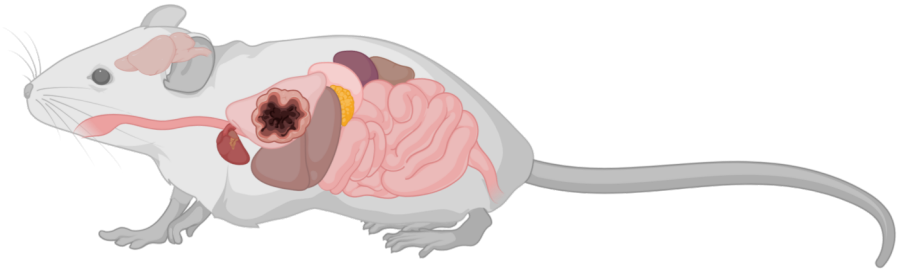
Local information (at different legth scales)

Global information

Why is this important?

Why is this important?

Biodistribution



Degradation

- **Are the particles where I want them to be?**
- **How long do they remain the same?**

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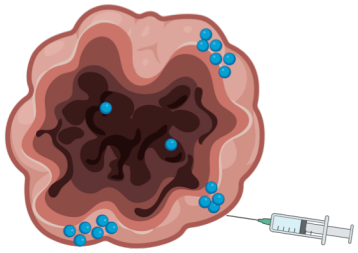
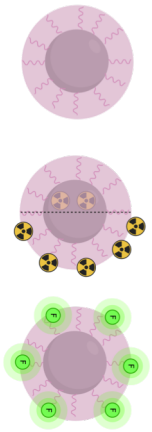
Summary

Administration routes



Animal models

Tracking particles



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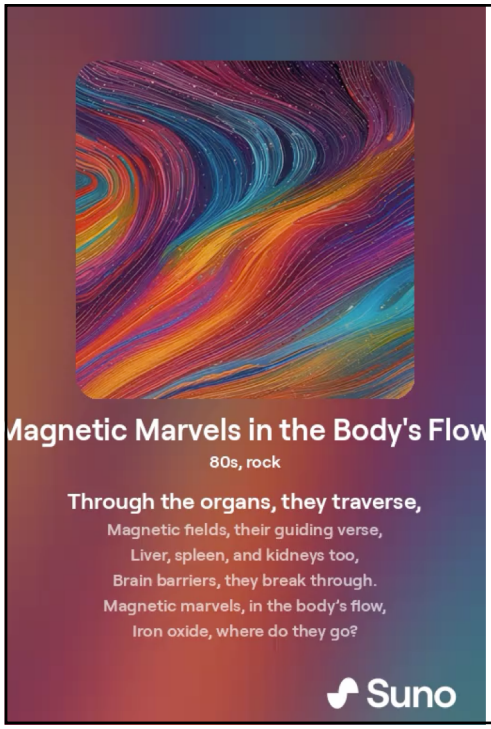

TUTORIAL PLAYLIST
Session3: Interactions at the Organ and Body Level

Thank you !

You can contact me at lu@unizar.es