UNIVERSITÀ DEGLI STUDI DI TERAMO

Nanotecnologies:

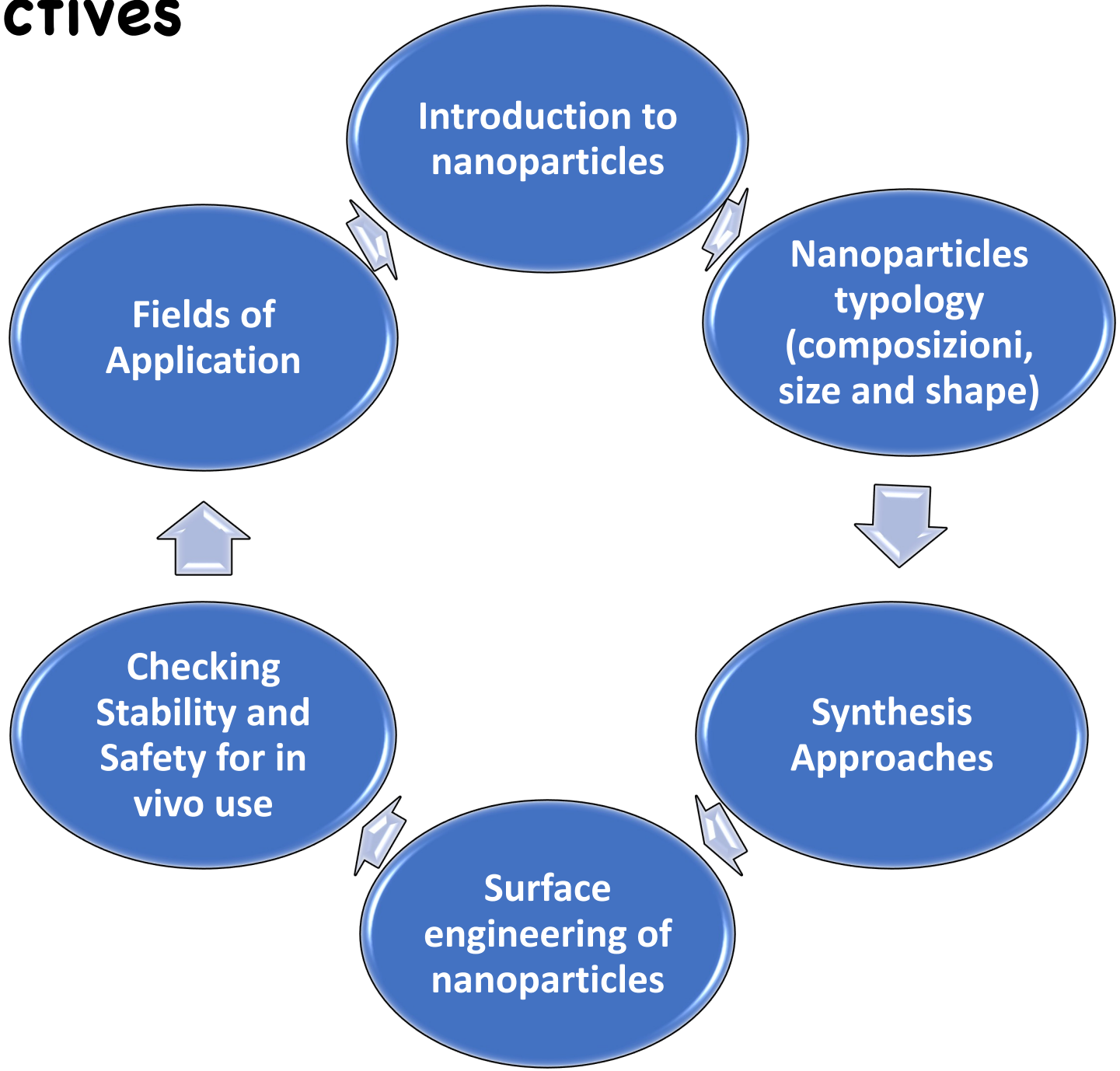
Applications in drug delivery ed imaging

Corso di Laurea Magistrale in Biotecnologie Avanzate
AA 2021-2022

Corso: Tecnologie per la produzione di dispositivi biomimetici (3CFU)

LECTURE 3
LECTURE 4

Learning objectives



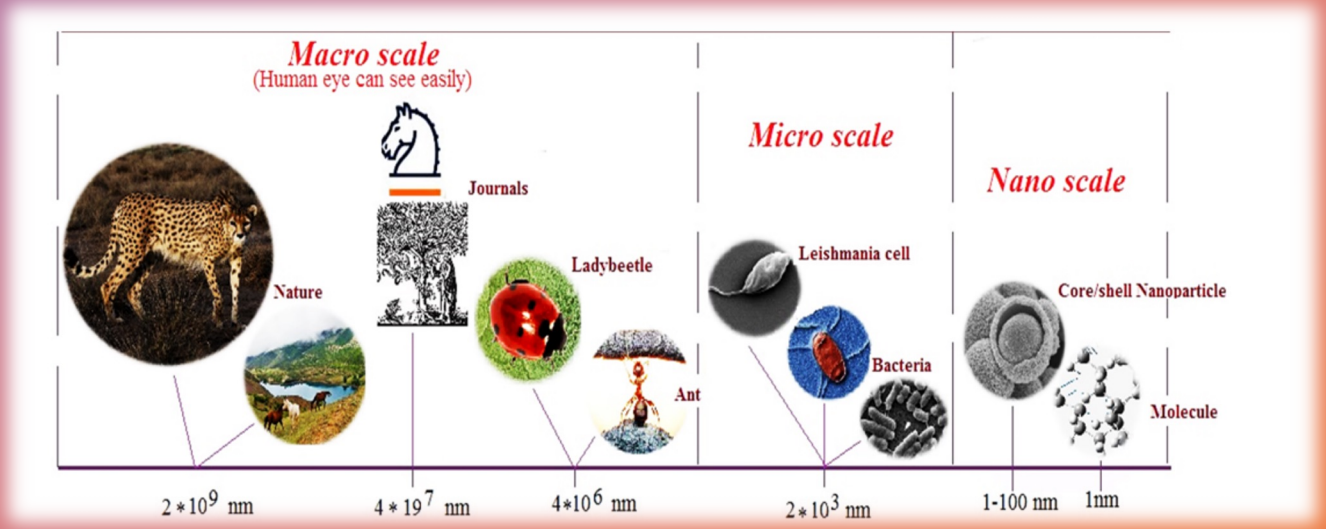
Etymology

Nanos (greek word):

dwarf or extremely small

Nanoparticles (NP)

NP are solid colloidal particles ranging from 1 to 1000nm in size, they consist of macromolecular materials in which the active compounds (drugs or biologically active material) is dissolved, entrapped or encapsulated, or absorbed.

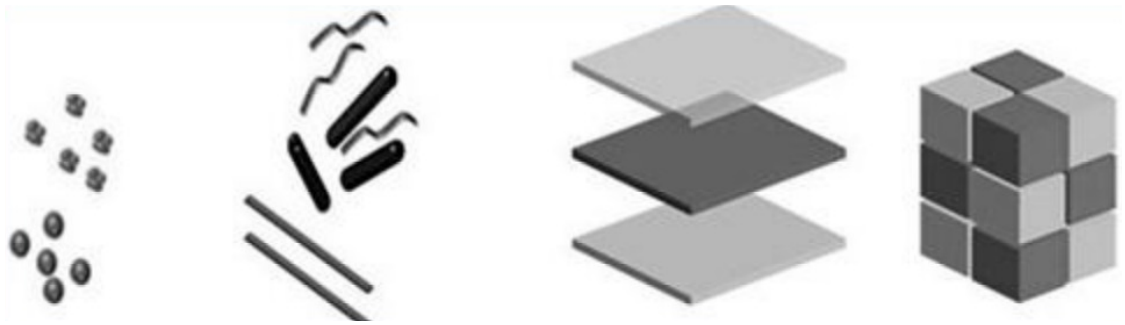


Khatami M e al., 2018

Classification of NP based on their dimension

Siegel classification

Zero-dimensional nanomaterials	One-dimentional nanomaterials	Two-dimensional nanomaterials	Three-dimensional nanomaterials
Here, all dimensions (x, y, z) are at nanoscale, i.e., no dimensions are greater than 100 nm. It includes nanospheres and nanoclusters.	Here, two dimensions (x, y) are at nanoscale and the other is outside the nanoscale. This leads to needle shaped nanomaterials. It includes nanofibres, nanotubes, nanorods, and nanowires.	Here, one dimension (x) is at nanoscale and the other two are outside the nanoscale. The 2D nanomaterials exhibit platelike shapes. It includes nanofilms, nanolayers and nanocoatings with nanometre thickness.	Not confined to the nanoscale in any dimension. These materials have three arbitrary dimensions above 100 nm. The bulk (3D) nanomaterials are composed of a multiple arrangement of nanosize crystals in different orientations. It includes dispersions of nanoparticles, bundles of nanowires and nanotubes as well as multinanolayers (polycrystals) in which the 0D, 1D and 2D structural elements are in close contact with each other and form interfaces.

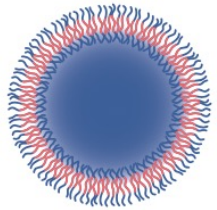


0D ***1D*** ***2D*** ***3D***

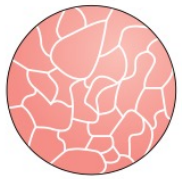
K. Mohapatra, 2020 "Chemistry_Nanomaterials" http://www.gcekjr.ac.in/pdf/lectures/2020/7166--_2nd%20Semester_ALL.pdf

Classification of NP based on their material composition

Polymeric

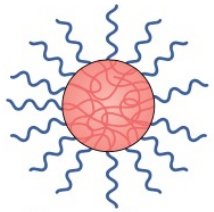


Polymersome



Dendrimer

Polymer micelle



Nanosphere

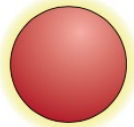
1-1000nm

Inorganic

Silica NP

Quantum dot

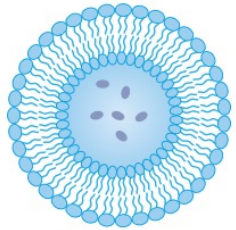
Iron oxide NP



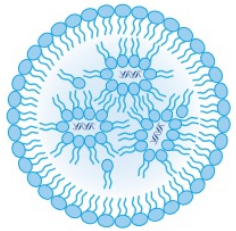
Gold NP

20-150nm

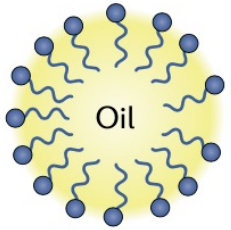
Lipid-based



Liposome



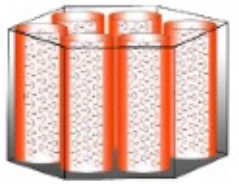
Lipid NP

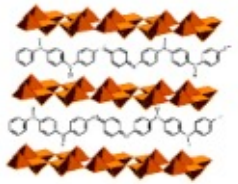


Emulsion

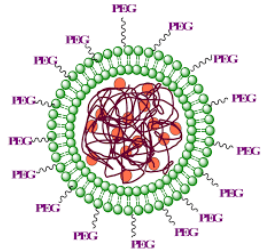
10-1000nm

Hybrid *





Inorganic-Organic



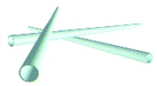
Lipidic-organic

Adapted from Michell MJ et al., 2021

*** Examples hybrids:**
Inorganic-Organic: Polyaniline-Vanadium Oxide
Organic-Inorganic: Polypyrrole- Silicium
Lipidic-organic: Liposome- PEG

Inorganic NP

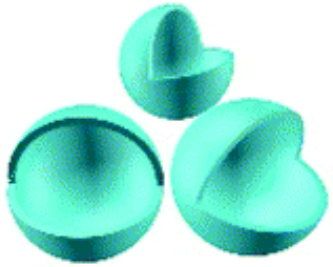
shapes



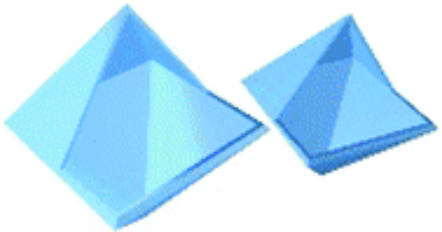
rods or tubes

stars

wires

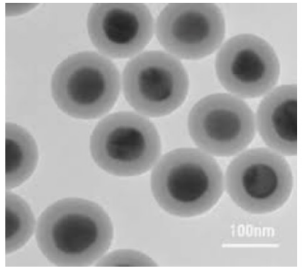


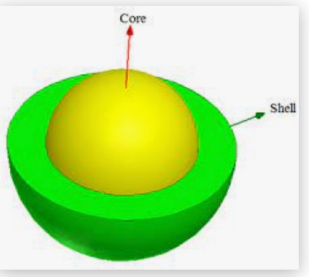
spherical



pyramids

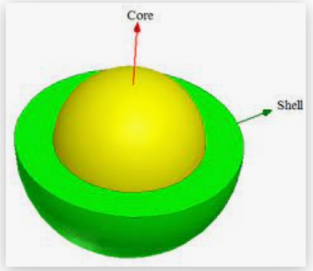
Adapted from Wu Z et al., 2016





Core-shell structure

Adapted form Khatami M e al., 2018



Inorganic NP core composition:

Metals or other chemical elements

1. Metal-based NP:
silica, manganese, gold, silver, lanthanide, molybdenum, ruthenium, rubidium, gadolinium, and zinc elements

2. Metal oxide-based NP:
iron oxide, superparamagnetic iron oxide (SPIO), ultrasmall superparamagnetic iron oxide (USPIO), titanium oxide and cobalt iron oxide elements

3. Metal sulfide or phosphide-based NP:
quantum dots

4. Mineral-based NP:
hidroxyapatite and selenium elements

Inorganic NP shell composition

Metals or **organic polymers** that:

1. protect the core from chemical interactions with the external environment
2. serves as a substrate for conjugation with biomolecules such as antibodies, peptides or oligonucleotides (**Functionalization**)
3. preserves NP stability avoiding aggregation

Polymeric NP

- Constituted by a polymeric matrix core

- The polymeric matrix can be loaded with bioactive molecules (**functionalization**)

- They are categorized into two forms:

1. **spheres** (the bioactive molecule is dispersed within a polymer matrix)

2. **capsules** (the bioactive compound is placed in the core of the particle covered by a layer of polymer)

PEG: poly(ethylene glycol)
PLGA: poly(lactide-co- glycolide)
PS: polystyrene
PCL: poly(epsilon-caprolactone)
PLA: poly(lactide)
PMPC: poly(2- methacryloyloxy) ethyl phosphorylcholine (PMPC) PGS: poly(glycerol-co-sebacate)
PDPA-PEO: poly (diphenylamine)-poly(ethylene oxide) (PDPA-PEO)
MEH-PP: poly[2-methoxy-5-(2-ethylhexyloxy)- 1,4-phenylenevinylene]

Lipid-based NP

SYNTHETIC FORMULATION:

Liposomes: with size < 200 nm, spherical vesicles with an aqueous core and bilayer lipid membrane. They have the capacity to encapsulate diverse bioactive compounds, which can be included into the aqueous core or at the bilayer interface

Solid lipid NP (SLN, solid lipids): spherical in shape and consist of a solid lipid core stabilized by a surfactant. This construct can be used to deliver both hydrophilic and hydrophobic bioactive molecules (functionalization)

Nanostructured lipid carriers (NLC): with sizes ranging from 10–1000 nm, are a combination of liquid and solid lipids.

NATURAL FORMULATION:

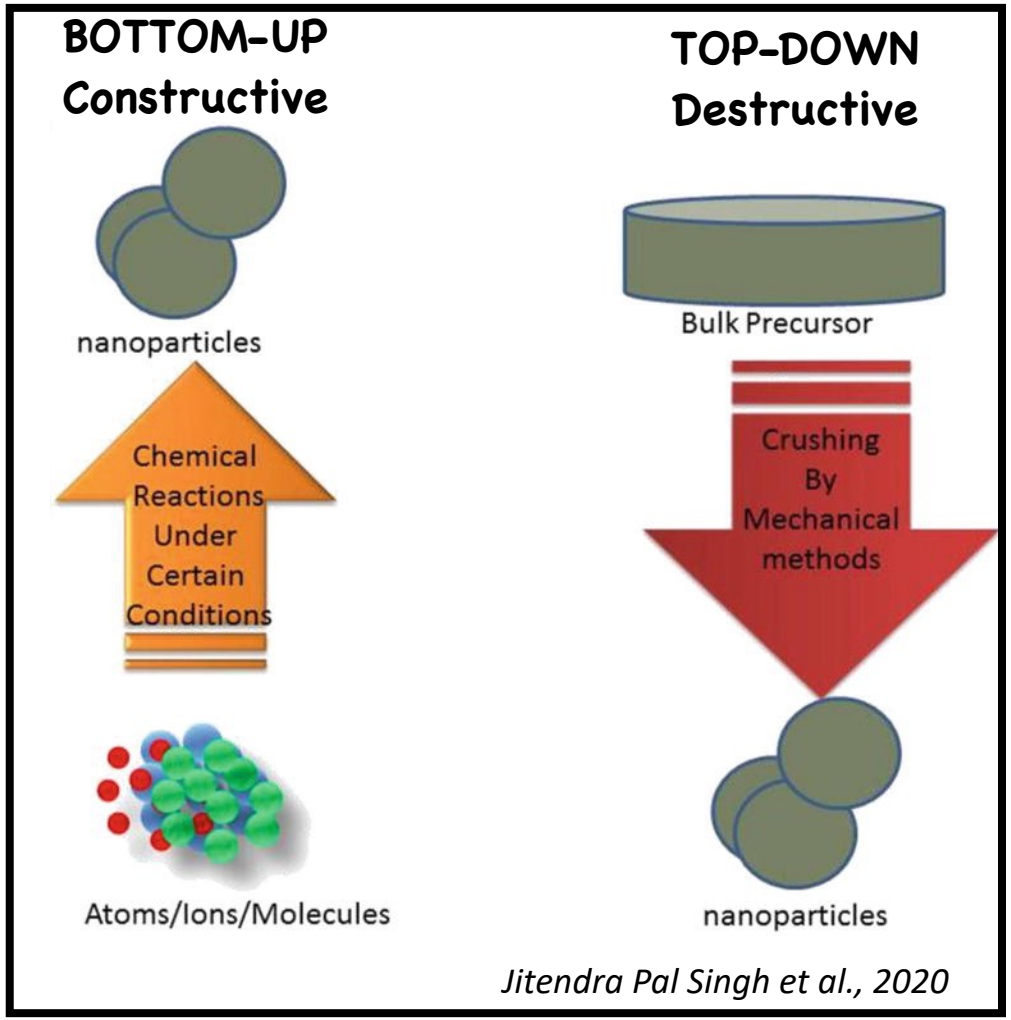
Cell- derived membrane lipidic vesicles, with a small size range (40–100 nm) are naturally derived lipid NP versus the synthetic lipidic formulation

Hybrid NP

Hybrid NPs are constructed from at least two different kind of NP, to overcome the limits of single-component nanoparticles, to improve properties, to achieve new properties not possible for single nanoparticles, and/or to achieve multiple functionalities for single nanoparticles

NP Synthesis Approaches

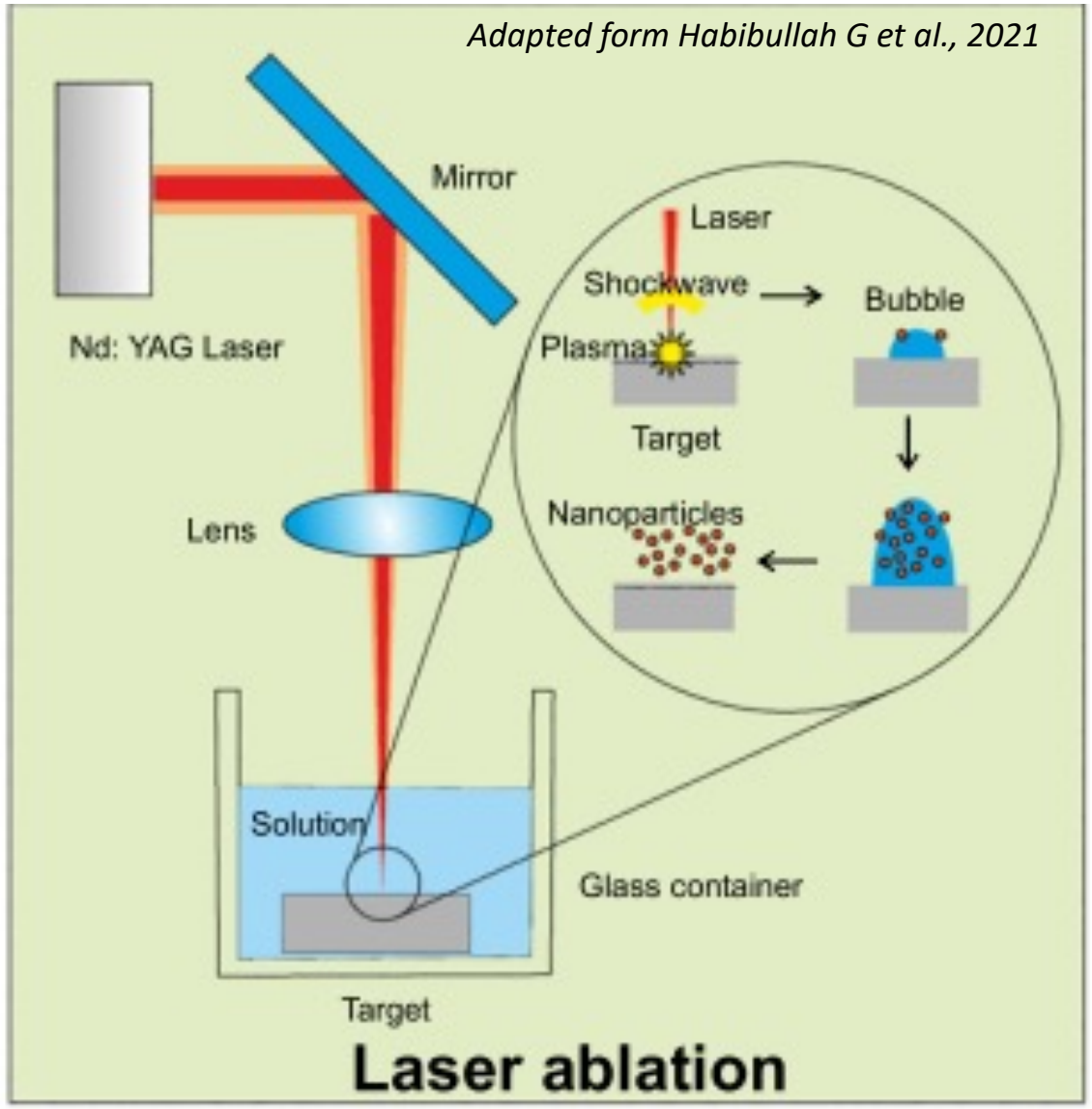
BOTTOM-UP
NP are produced by the self-assembly of the atoms, the molecules or the clusters



TOP-DOWN
It involves breaking bulk materials into smaller particles of nano-dimensions using various physical and chemical methods

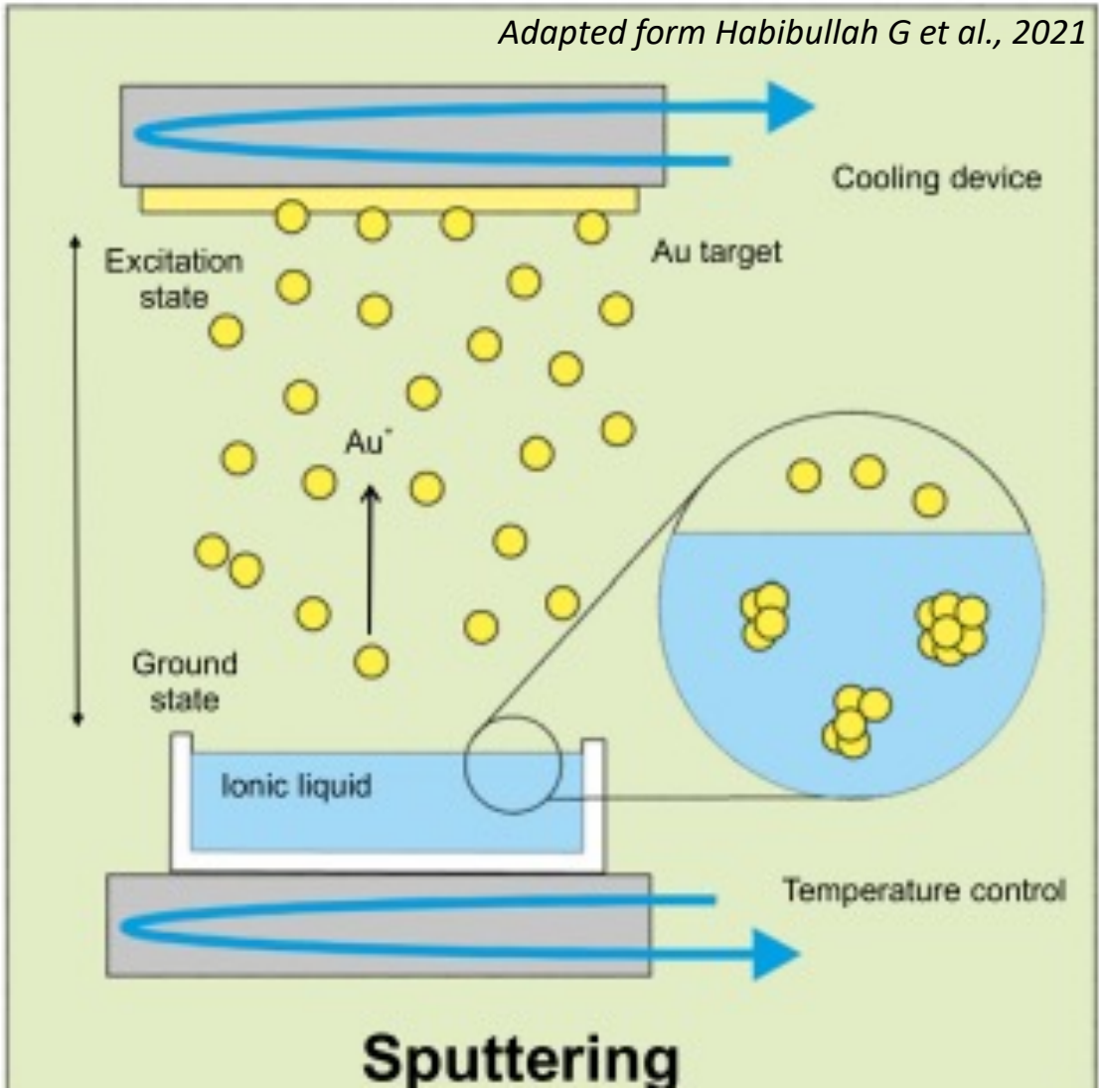
Inorganic NP Synthesis Approaches

TOP-DOWN



Nucleation:
Formazione, a partire da una fase solida, liquida o gassosa, di aggregati di atomi o molecole (detti nuclei) di un'altra fase, in grado di accrescersi fino a produrre particelle di dimensione microscopica o superior

In a laser ablation process, a solid surface (generally a plate of pure metal) is **irradiated** with a laser beam. Nanoparticles are generated by **nucleation and formation of laser-vaporized species** in a background gas.



Deposition of NPs as a thin layer generated by the **collision of ions over the substrate** and followed by their **aggregation**. It is defined as a physical vapor deposition technique

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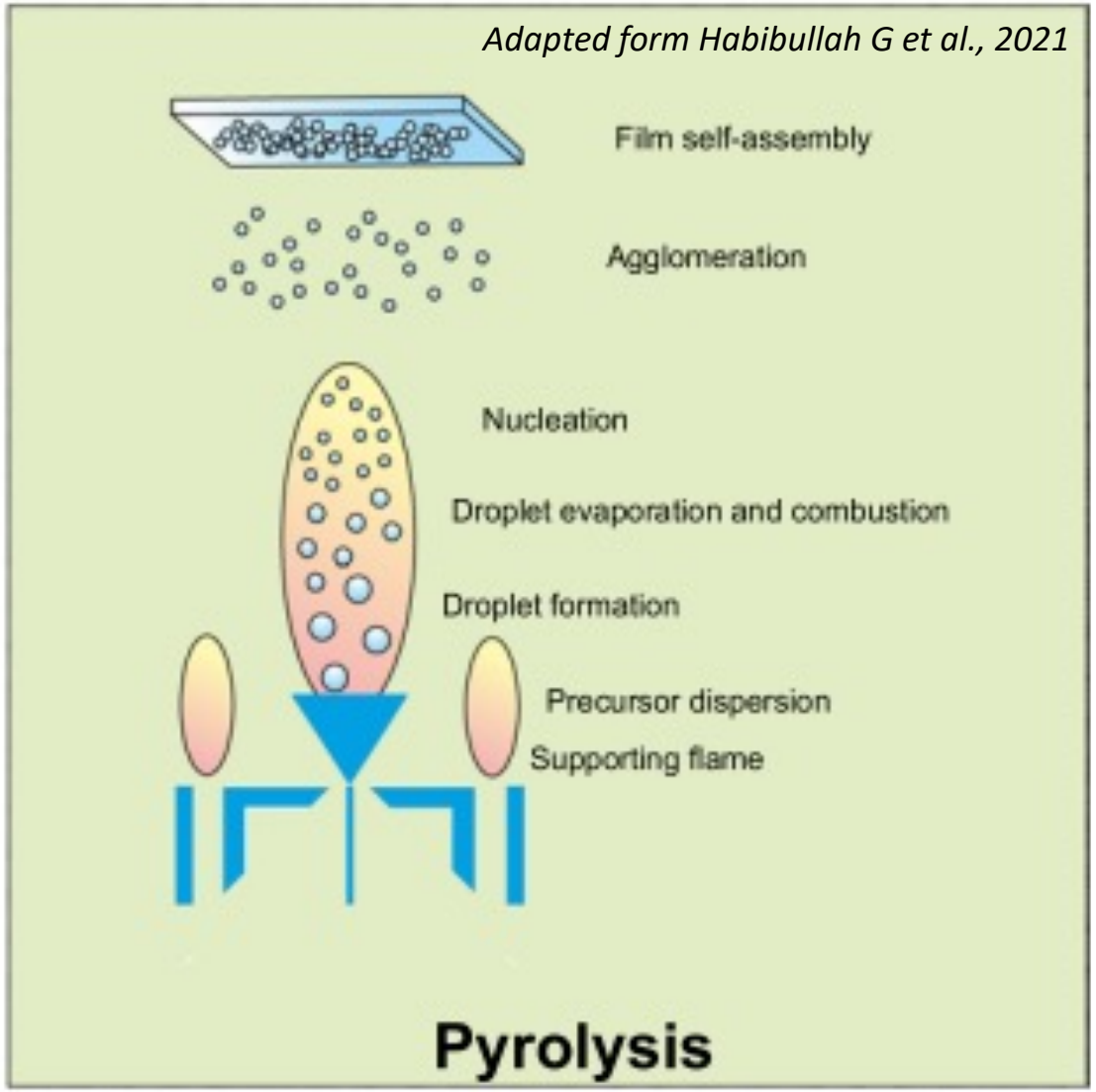
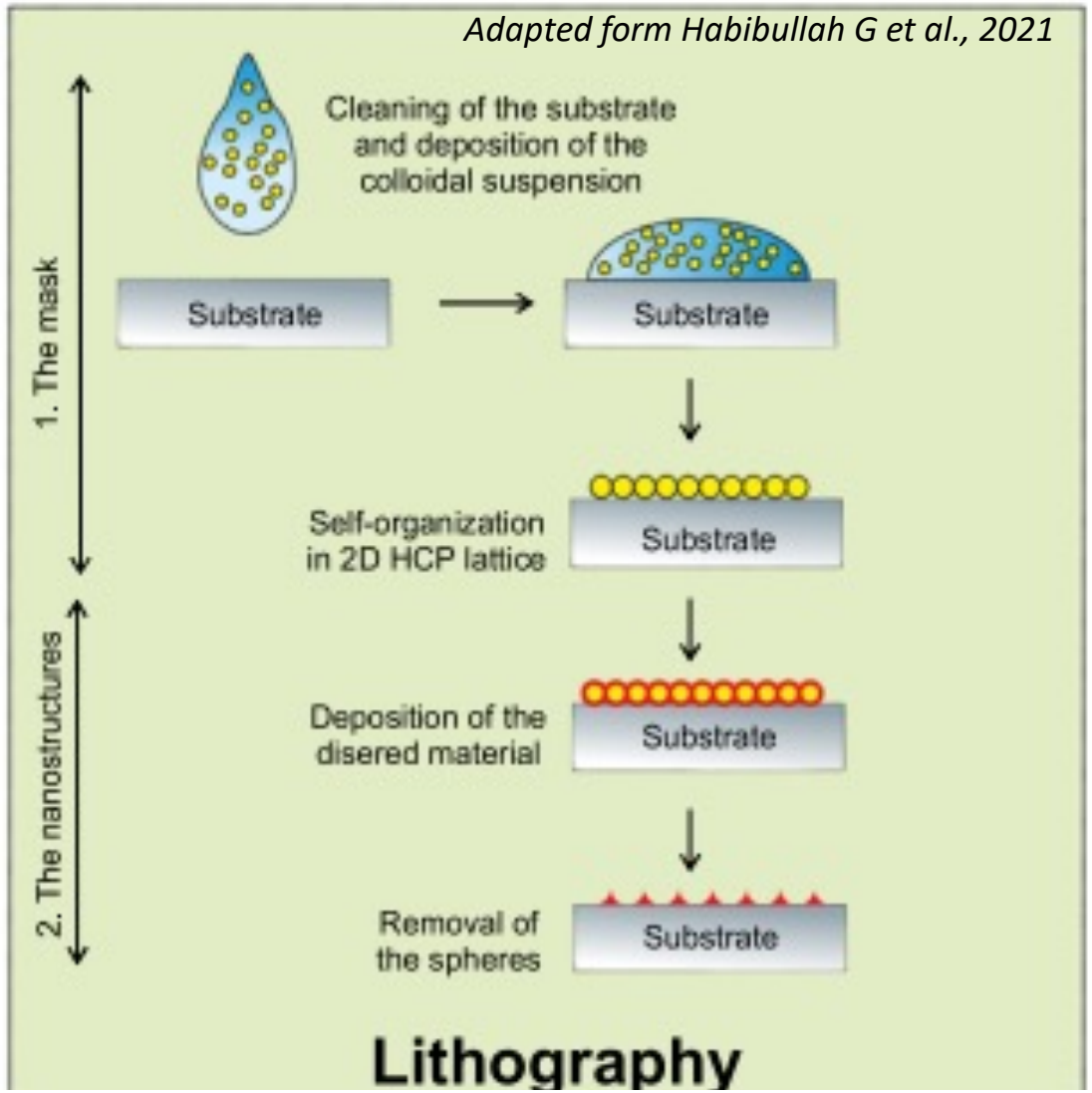
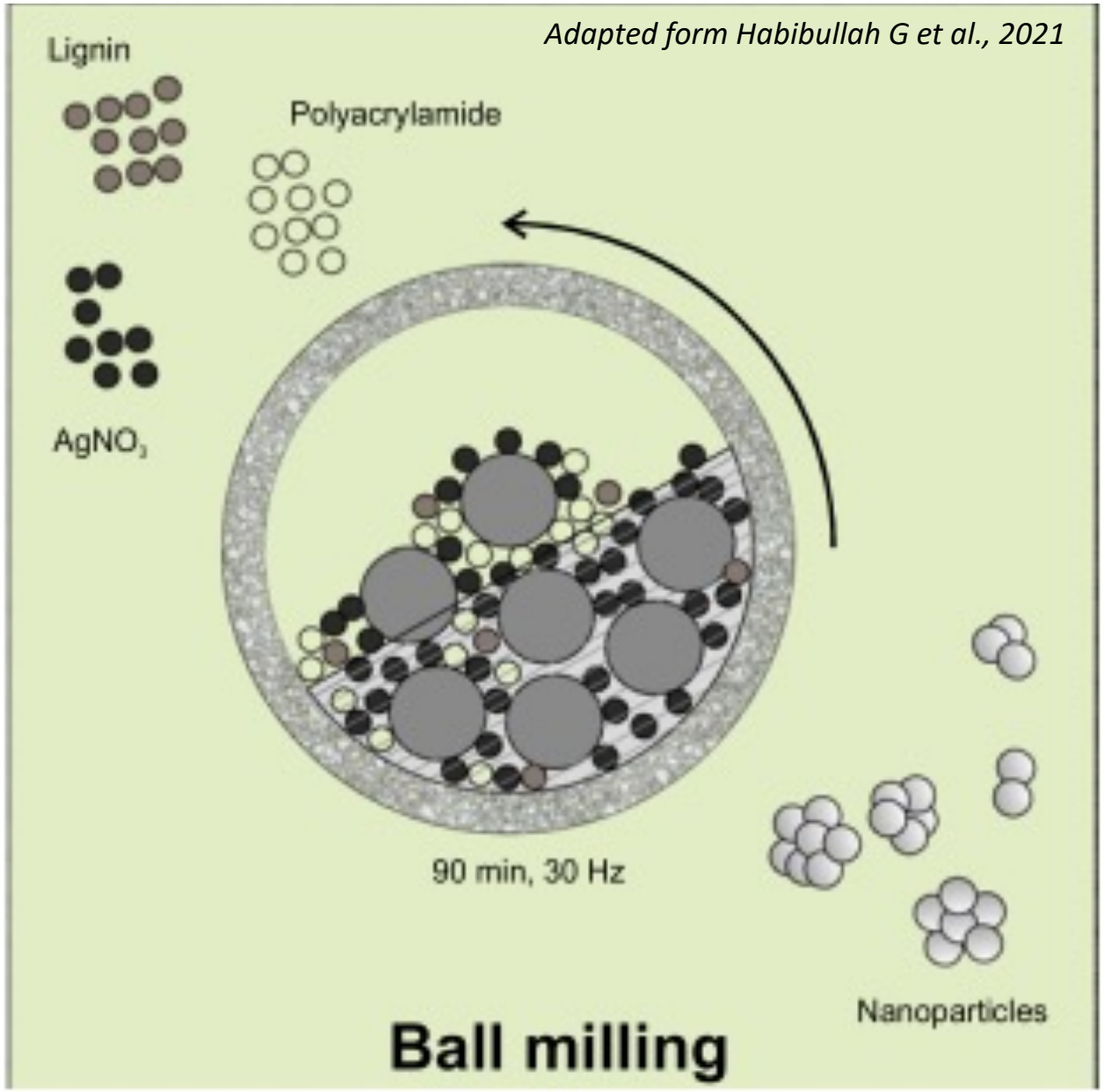


Thermal decomposition. It is an endothermic chemical decomposition process that **uses heat to break the compound's chemical bonds,** resulting in decomposition of the precursor forcing it into a chemical reaction producing NPs along with other by-products in the form of ash.



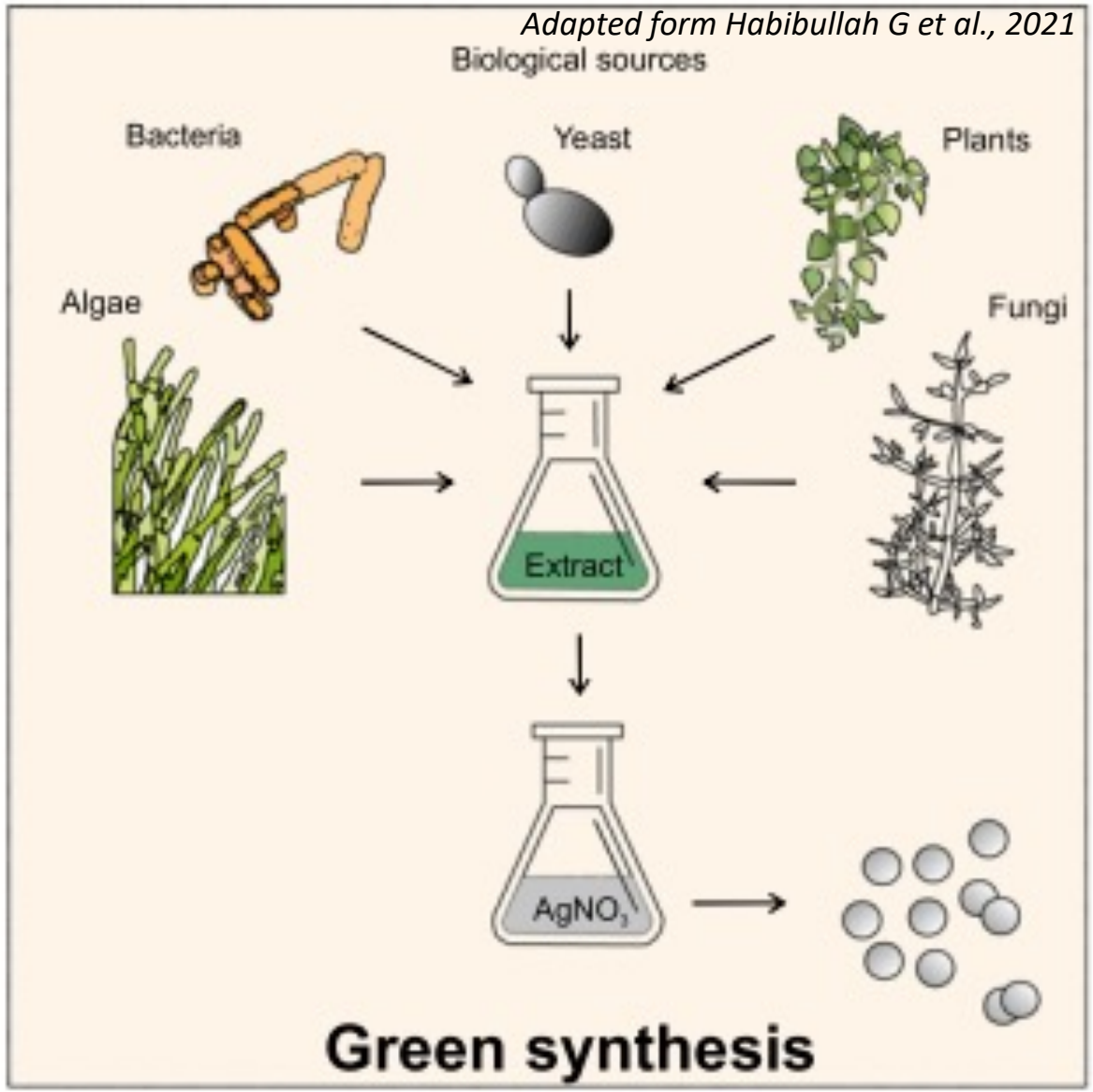
This method is based on the deposition of the **desidered material** on a substrate (e.g.silicon) to produce **regular and homogenous arrays of nanoparticles** with different sizes and with precisely controlled spacings.



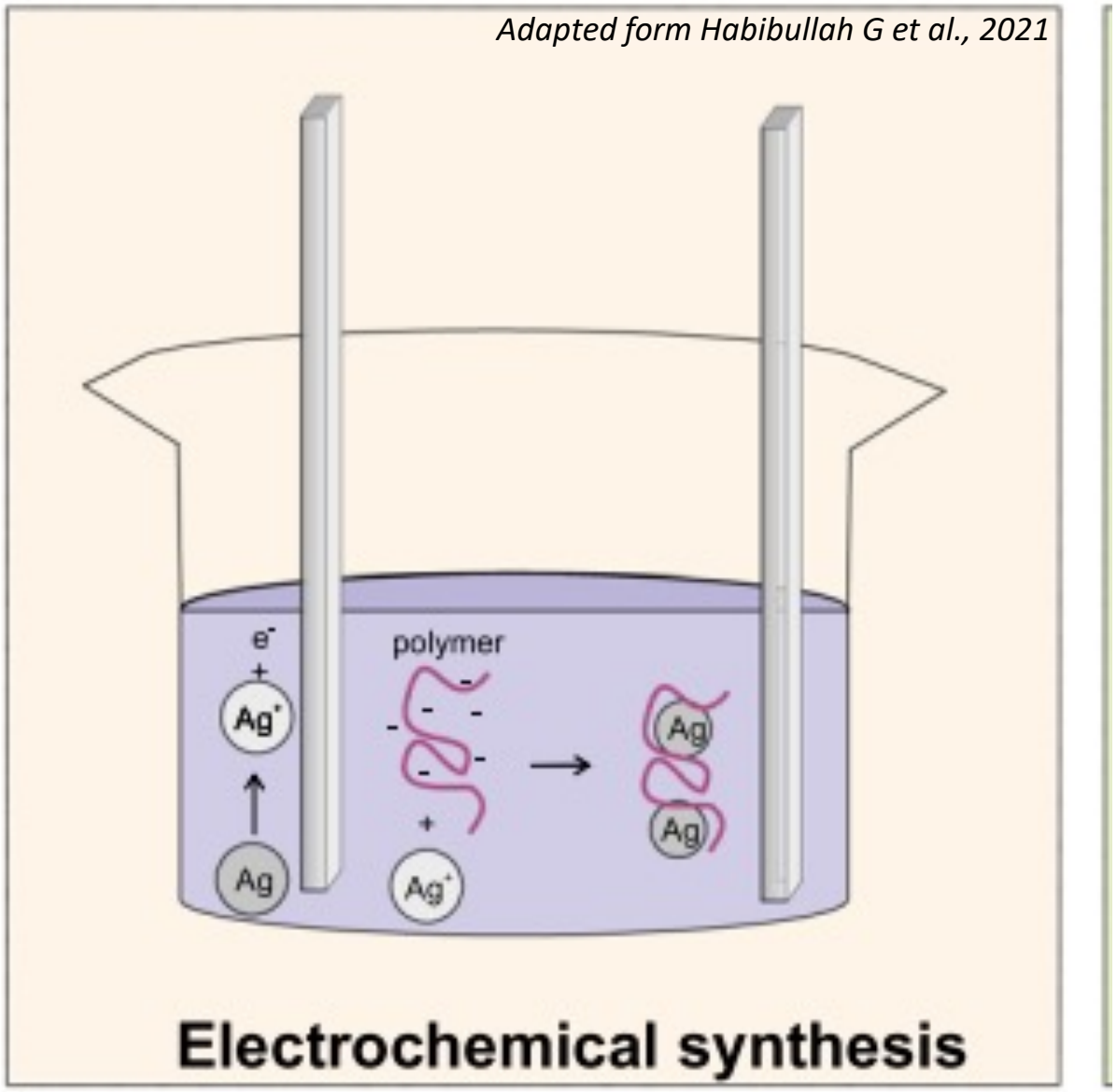
The kinetic energy of the **rollers/balls** (AgNO3, polyacrylammide, lignin) is transferred to the bulk material, which results in the **reduction in grain size**

BOTTOM-UP

Adapted form Habibullah G et al., 2021

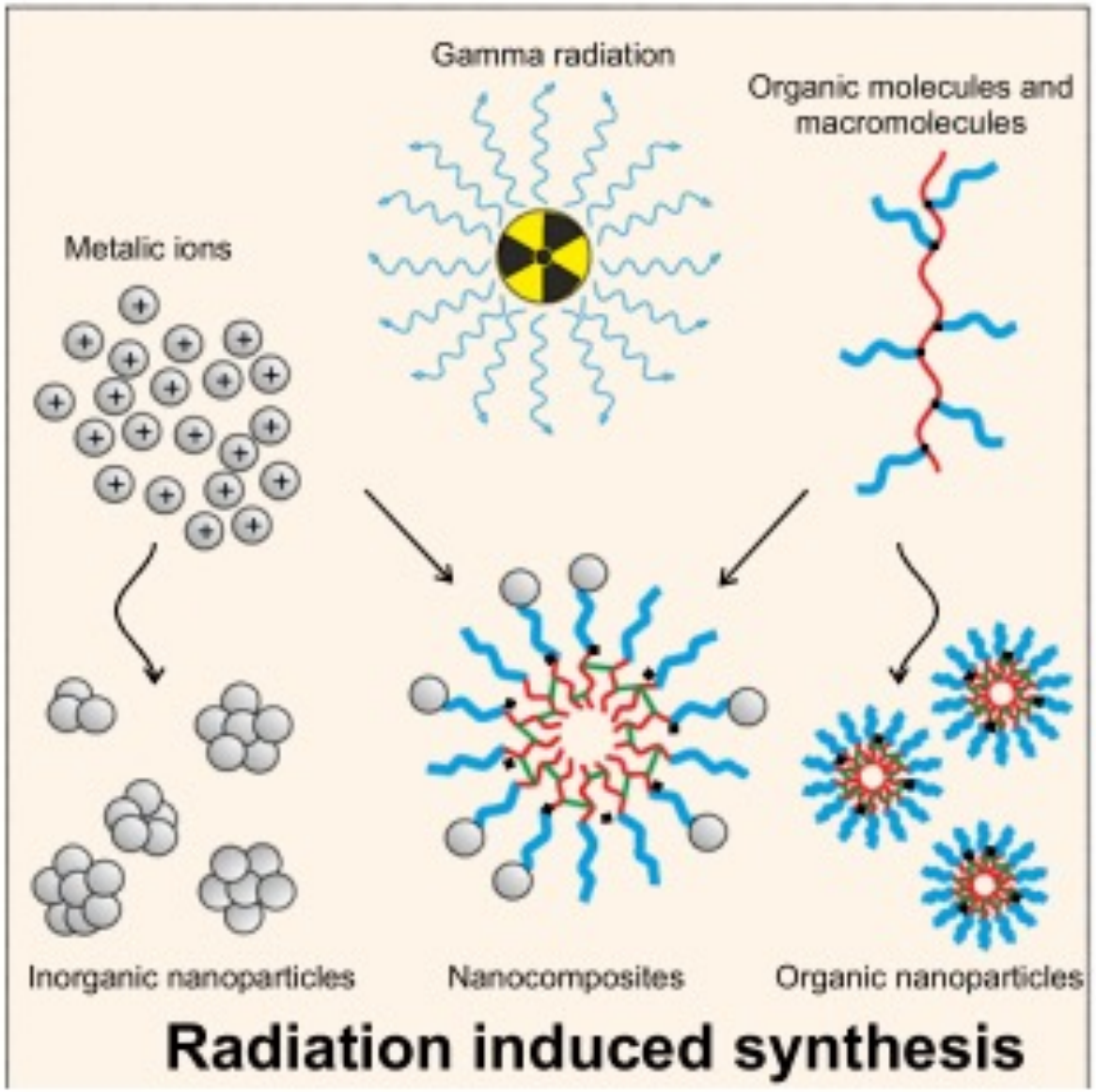


Green synthetic methods employing plant extracts, microorganisms and biopolymers have proven to be potent candidates for replacing chemical methods of NP synthesis (reaction catalisis by enzymes or specific chemical elements)

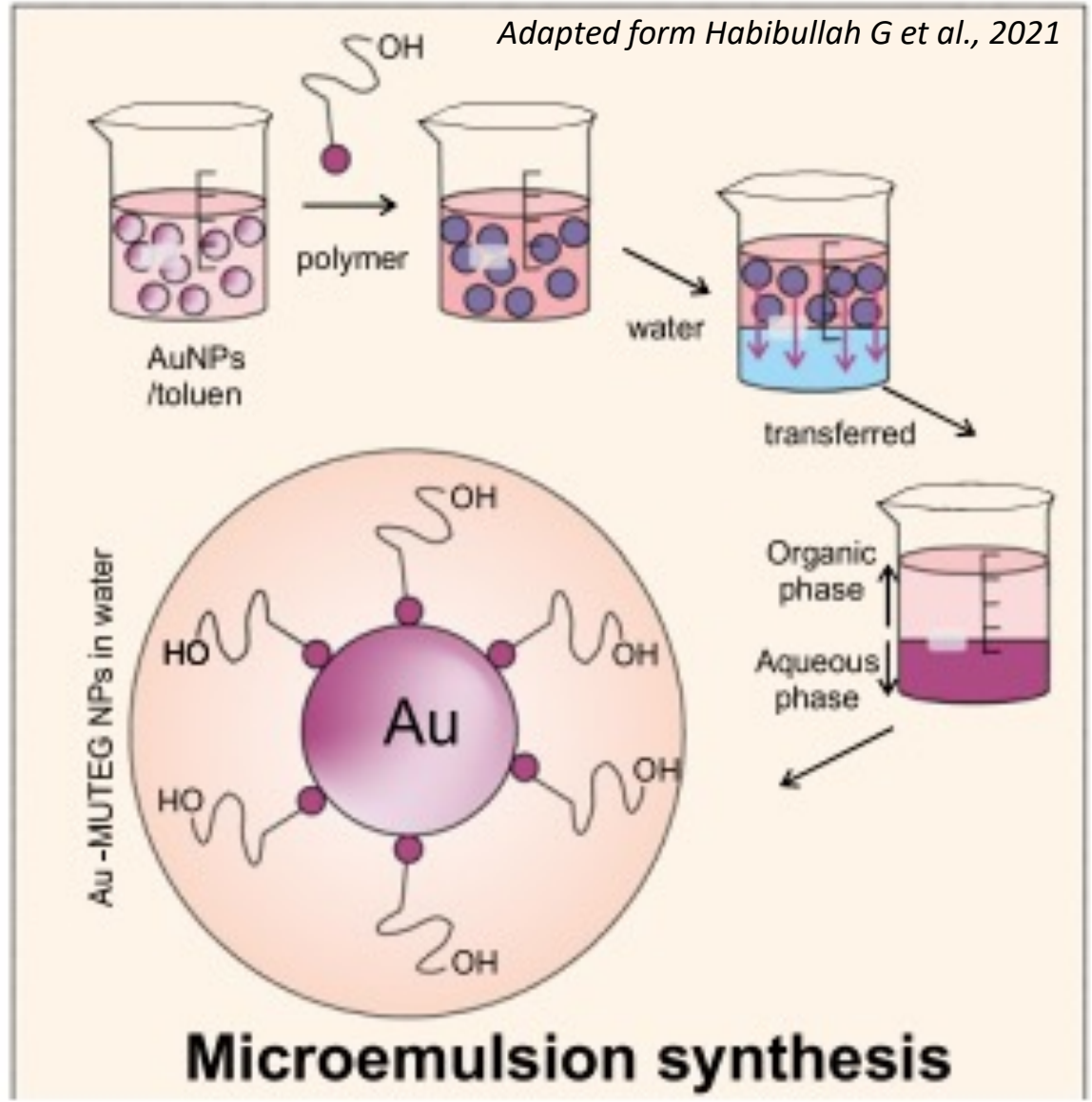


Dissolution of a metal sheet from the anode to achieve the **deposition of metal salt on the cathode** of an electrochemical cell in the presence of an electrolyte to produce nanoparticles

Adapted form Habibullah G et al., 2021



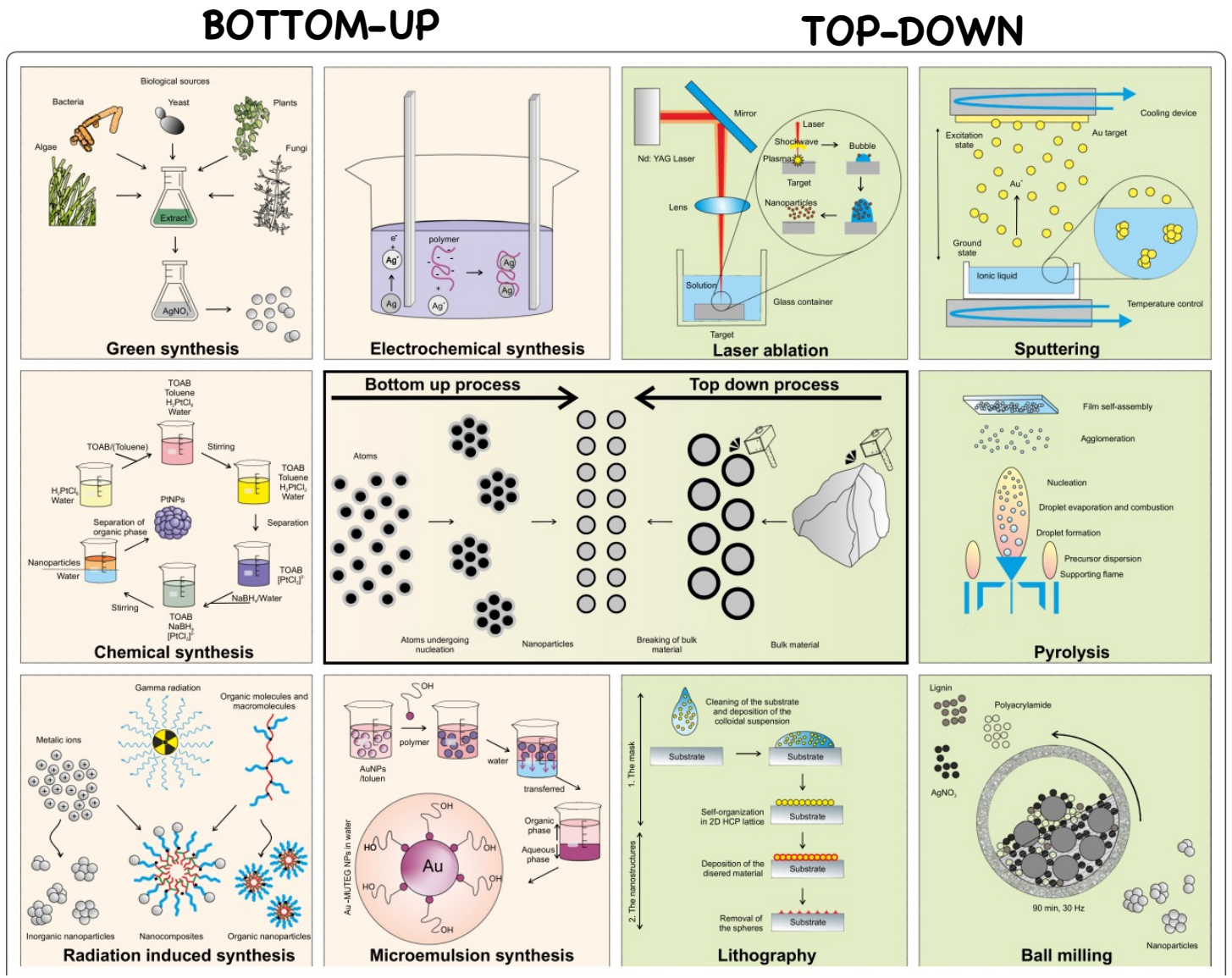
For metal NP. This method employs **ionizing radiation** (gamma and X-rays and UV-light) for the synthesis of **metal nanoparticles**. Reaction occours in aqueous solutions.



It consists in a mixtures of two immiscible liquids in the presence of a surfactant.
Two separate microemulsions are prepared, one containing the **ionic salt** and another containing the **reducing agent** produced in an amphiphilic environment.

Advantages:
1. provides control over the fnal product formation with more **homogeneous size, shape** (physical parameters) and **chemical composition**

2. Less expensive



Habibullah G et al., 2021

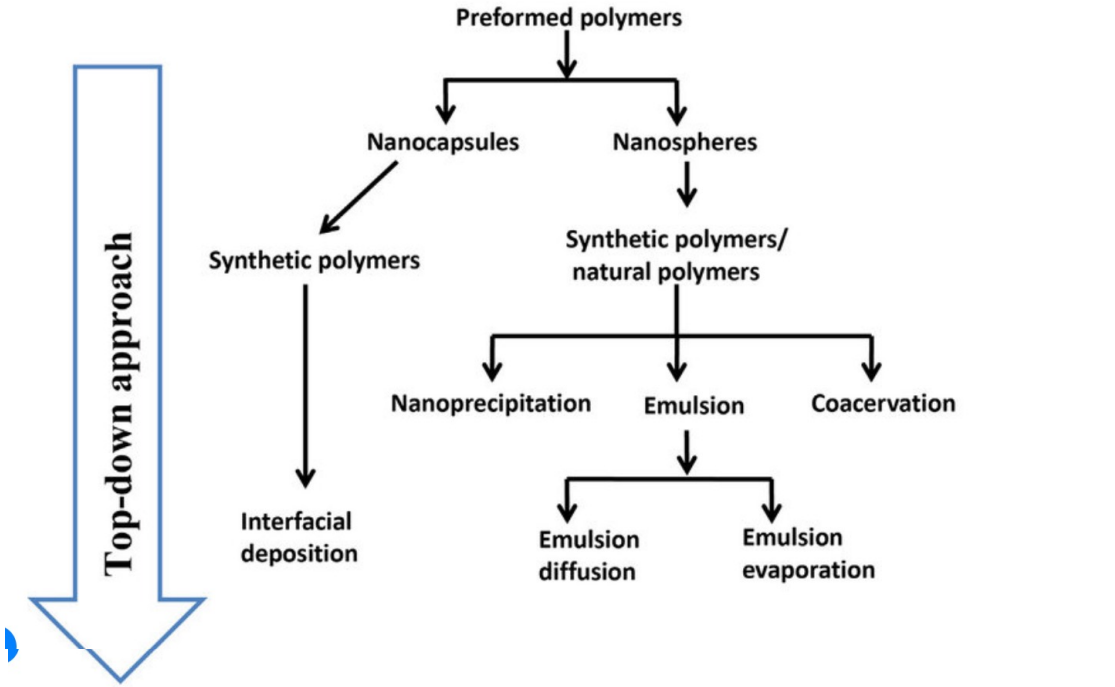
Involve externally controlled processes of **cutting, milling and shaping** the materials into the desired order and shape

Major imitations:
1. the imperfect surface structure of the resulting NP, which substantially affects their physical and chemical properties

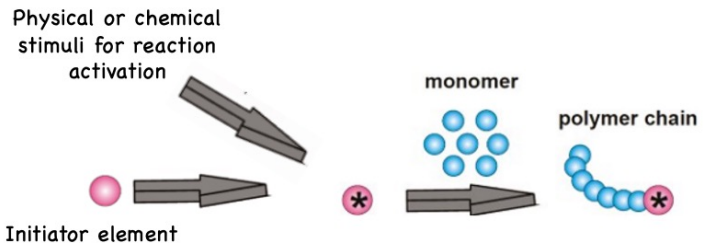
2. this method requires an enormous amount of energy to maintain the high-pressure and high-temperature conditions during the synthetic procedure, making the process expensive

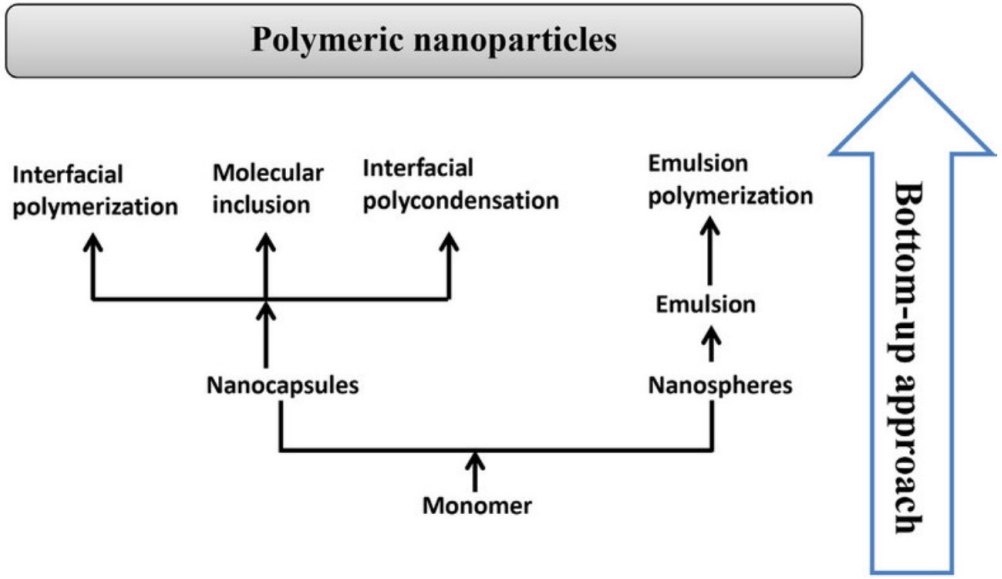
Polymeric NP Synthesis Approaches

Dispersion approaches of preformed polymers



Polymerization of monomers

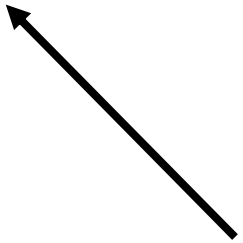




Reaction ambient: Non-solvent phase or solvent phase

Stabilizators: tensioactive or surfactants

Synthesis approaches and material composition should guarantee NP … overtime



STABILITY PRESERVATION

FACTORS INFLUENCING NP STABILITY:

Environmental stresses
such as extended storage, pH and mineral composition, thermal processing, freeze–thaw cycling, dehydration, mechanical stress and light exposure

Table 5. Key parameters defining NP stability and strategies to determine stability preservation.

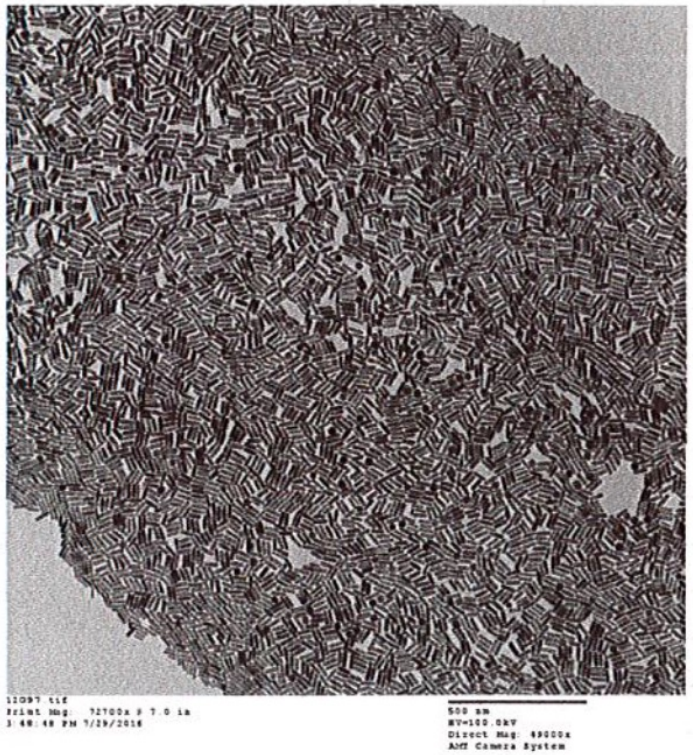
	NP Stability	Definition	Approaches Used for Characterization of NP Stability	
			Physical	Chemical
1	Aggregation	Preservation of NPs upon collisions	Dynamic light scattering	Single particle inductively coupled plasma mass spectrometry UV–visible spectroscopy
2	Core Composition	Unchanged chemistry of the core during the use	X-ray diffraction	Single particle inductively coupled plasma mass spectrometry UV–visible spectroscopy Surface-enhanced Raman scattering X-ray photoelectron spectroscopy Energy dispersive X-ray
3	Shape	Preservation of NP architecture during the use	Transmission electron microscopy Scanning electron microscopy X-ray diffraction Atomic force microscopy	Single particle inductively coupled plasma-mass spectrometry UV–visible spectroscopy
4	Size	Preservation of NP dimension during use or storage	Dynamic light scattering Scanning electron microscopy Transmission electron microscopy Small-angle X-ray scattering Atomic force microscopy	Single particle inductively coupled plasma-mass spectrometry UV–visible spectroscopy
5	Surface chemistry	Preservation of the native surface functionality	Low energy ion scattering X-ray photoelectron spectroscopy	Single particle inductively coupled plasma-mass spectrometry UV–visible spectroscopy Surface-enhanced Raman scattering X-ray photoelectron spectroscopy Energy dispersive X-ray

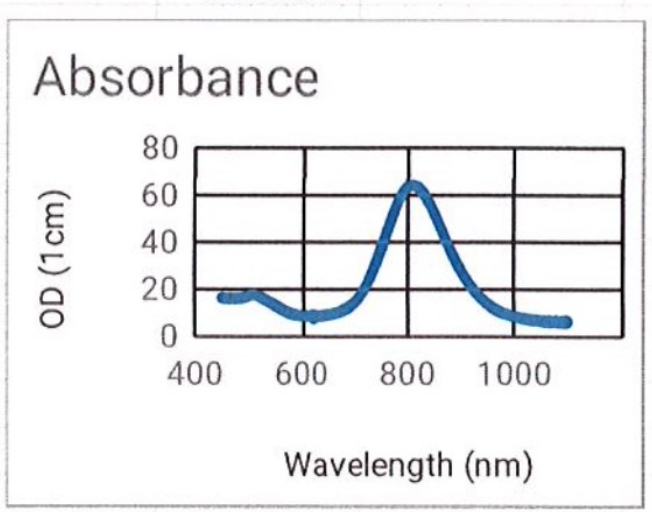
Peserico A et al., 2022

Case study (1)
Indirect check of NP stability

Gold nanorods (colloidal)

Abs pick: 809nm





How can I indirectly check stability during their use?

NP functionalization

NP conjugation with bioactive molecules (MOIETIES)

- **TARGETING/UPTAKE**

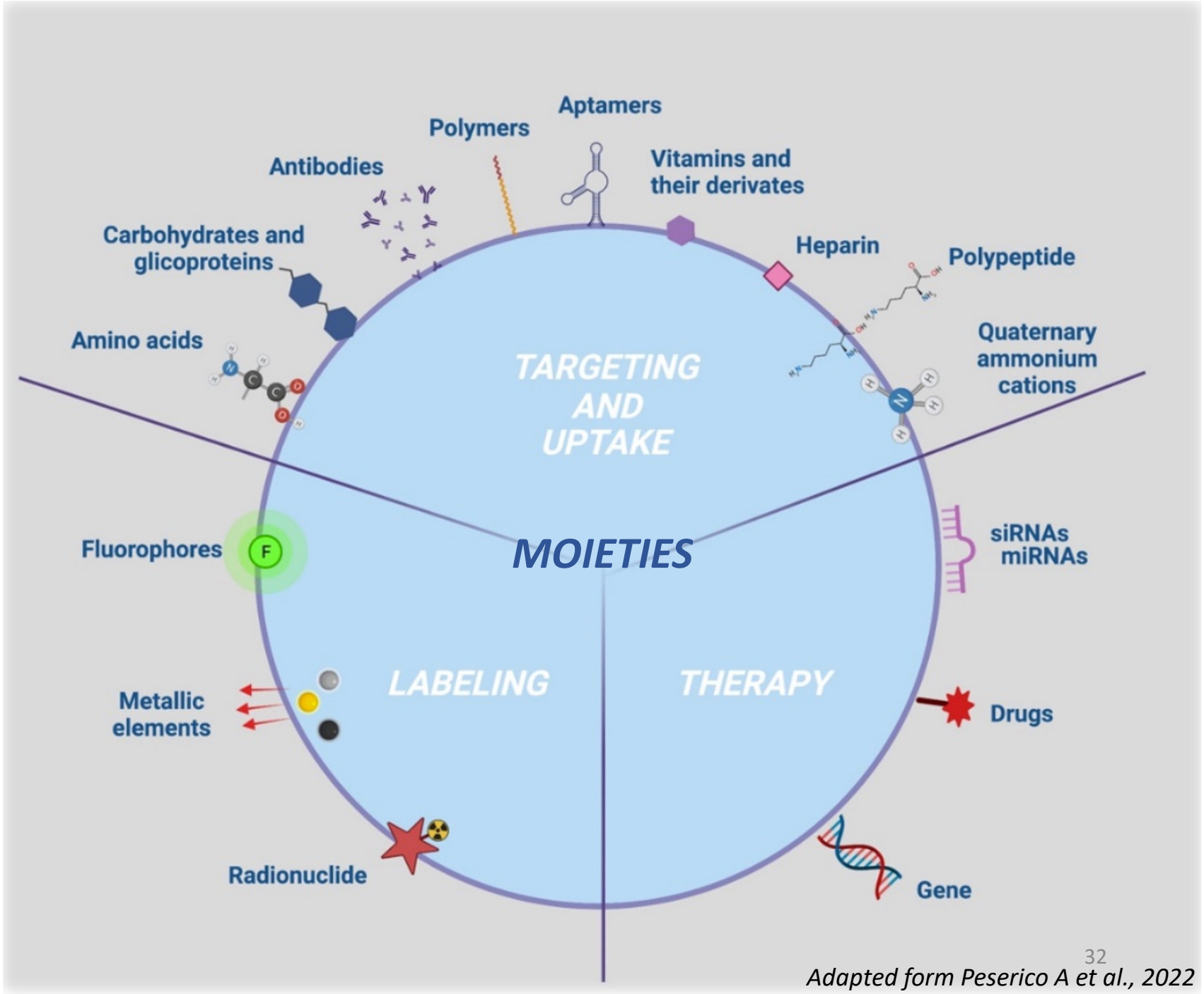
Selection of cells to be targeted and facilitation of NP internalization

- **THERAPY**

Affect positively or negatively target cell functions

- **LABELING**

Tracking of NP delivery



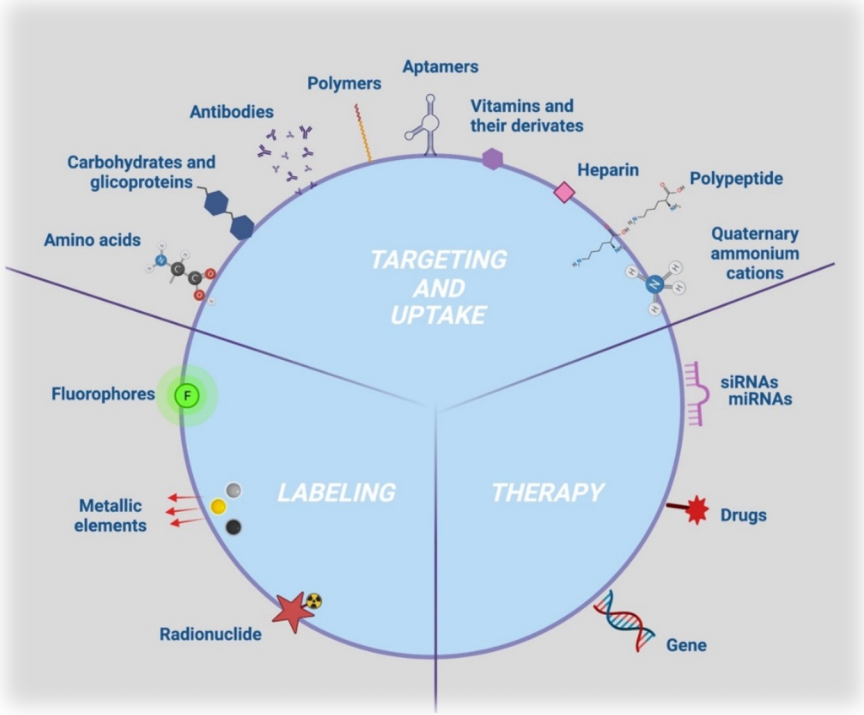
Adapted form Peserico A et al., 2022

Targeting and uptake moieties

MOIETIES WITH ACTIVE ACTION

Elicit a targeted NP uptake (internalization) by capturing specific cell biomarkers such as antigens/receptors

- **Antibodies** for specific cancer cell antigens
- **Folic acid** and **riboflavin vitamins** due to overexpression of their receptors on cancer cells
- **Aptamers** which recognize specific receptors on the cell surface
- **Carbohydrates** (dextran, carbodextran, chitosan, glucose, beta cyclodextrin, and transferrin) to avoid immune response



Peserico A et al., 2022

MOIETIES WITH PASSIVE ACTION

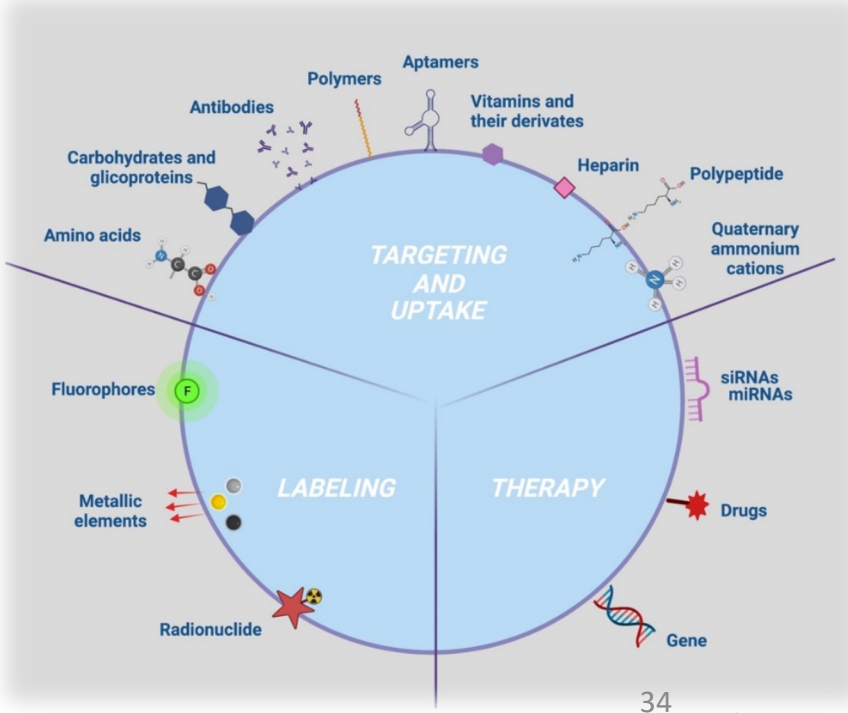
Enhance NP permeation and retention based on their biocompatibility

- **Polymers**
- **Heparin**
- **Quaternary ammonium cations**
- Polypeptide **polylysine**
- **Histidine** amino acid, which, thanks to their positive charge, stabilize NP and mediate the electrostatic interaction with the cell membrane, improving the endocytosis

Terapheutic moieties

Several therapeutic NP have been developed for both self-reporting disease and/or tissue damage and delivering therapy.
Therapy followed by imaging might be useful to test reactions in order to treat and identify patients in which therapy has an effect with the goal of providing personalized therapy for individual patients.

miRNA
siRNA
genes
drugs or **compounds**
with key roles in the modulation of cell proliferation and differentiation



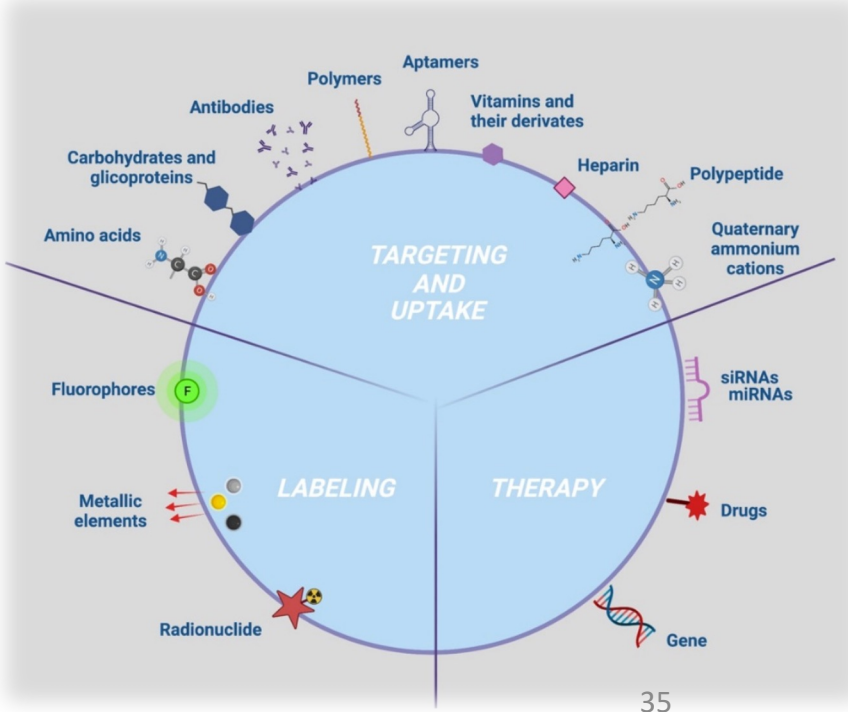
Peserico A et al., 2022

Labeling moieties

Molecules with optical properties working as contrast agent:

- **Fluorophore**
- **Bioluminescent dyes**
- **Isotopes** or **chemical elements with high molecular weight or magnetic properties**

For inorganic NP, a combinatorial usage of contrast agents represents an effective strategy for multimodal in vitro and/or in vivo tracking, as it could allow the limitations found with the use of a single-tracking approach to be overcome.



Peserico A et al., 2022

NP Sterilization prior in vivo delivery

- Nanoparticles intended for parenteral use should be sterilized to be pyrogen free before using on animals or humans.
- Sterilization is achieved by using aseptic technique throughout preparation, processing and formulation or by autoclaving or using γ- irradiation.
- Autoclaving and γ- irradiation show impact on the physicochemical properties of the particles with modification of particle size stability and drug release characteristics.
- Sterilization is a critical step and should be systematically investigated during formulation development stage.

NP applications in biomedicine

CANCER MEDICINE

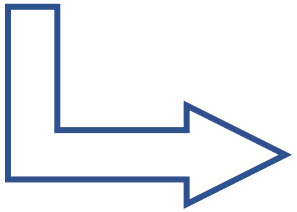
Aim: diagnosis and/or treatment of cancer

- Tracking of tumor foci
- Drug delivery (CHX or bioactive compound)

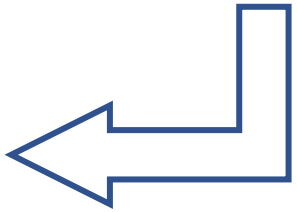
REGENERATIVE MEDICINE

Aim: monitoring cell trasplantation procedures and/or enhancing tissue regeneration

- Tracking of trasplanted cells
- Immunomodulatory factors delivery



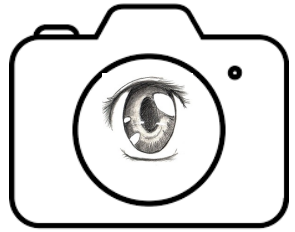
HOW?



HOW

DIAGNOSIS?

NP works as a contrast agent to be followed by imaging techniques



HOW

THERAPY?

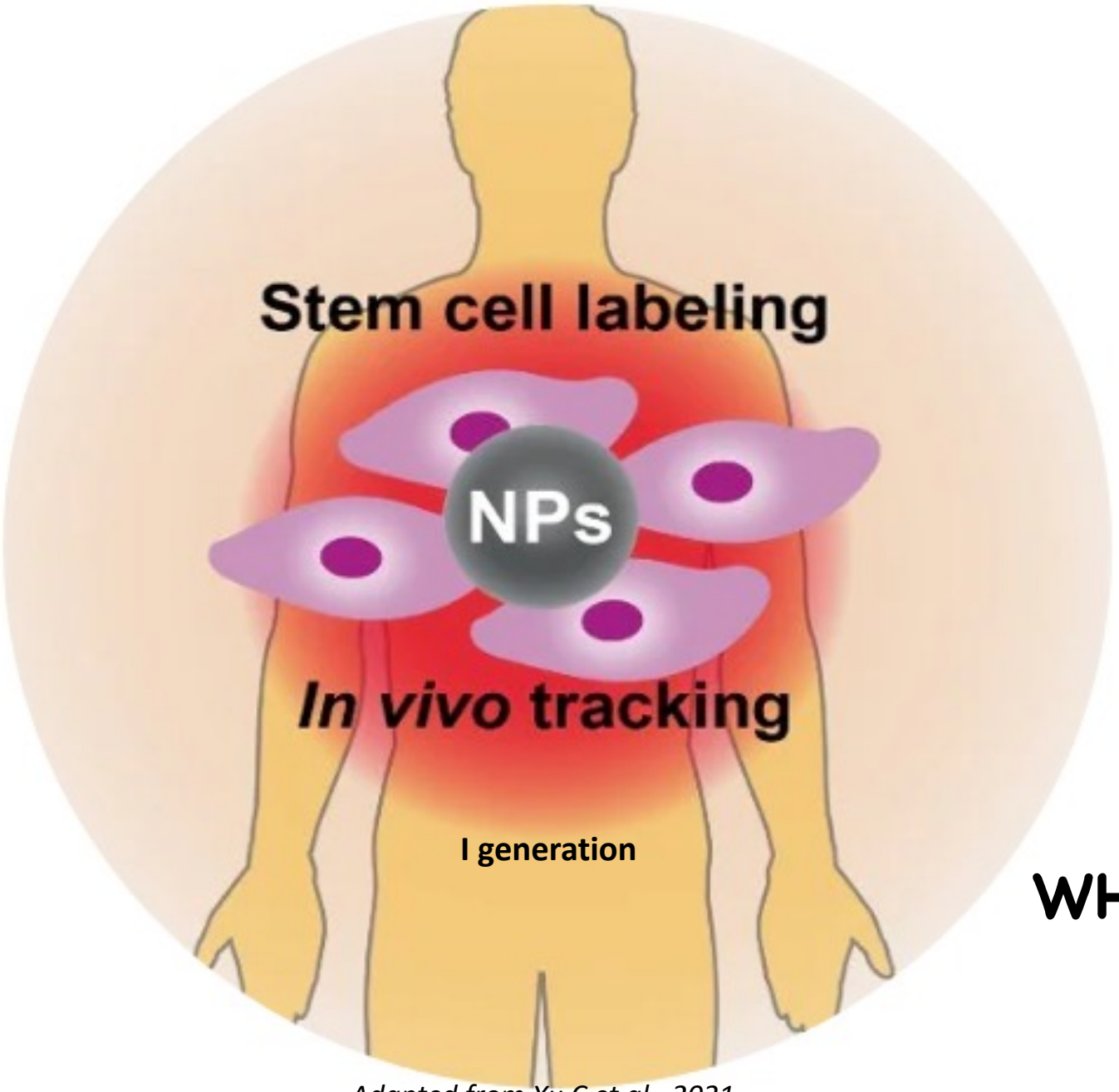
NP carries a drug

For in vivo application the most suitable solution is systemic NP administration

HOW

NP finds its way to tumor or damaged tissue?

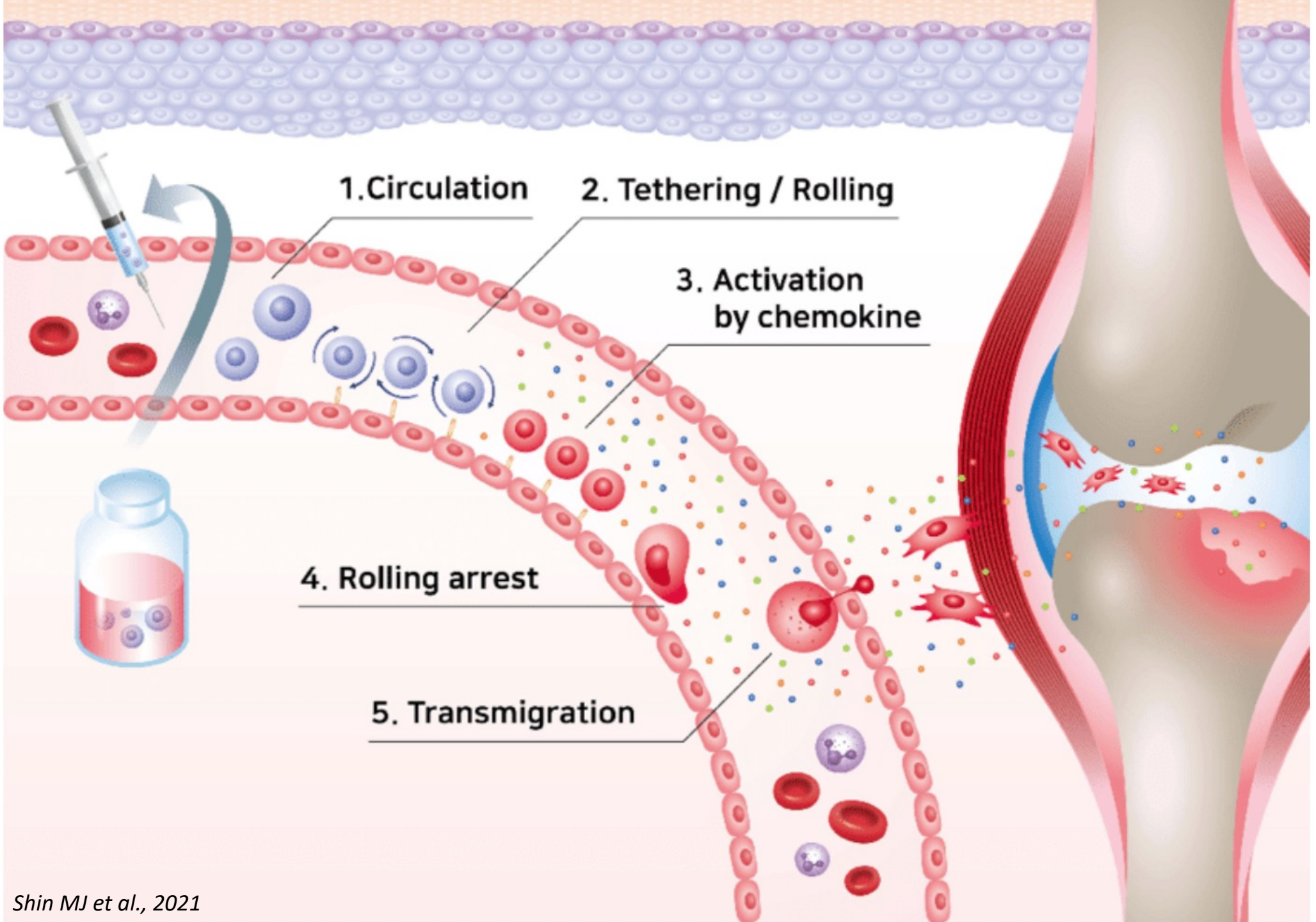
when incorporated by a cell able to home



WHY STEM CELLS?

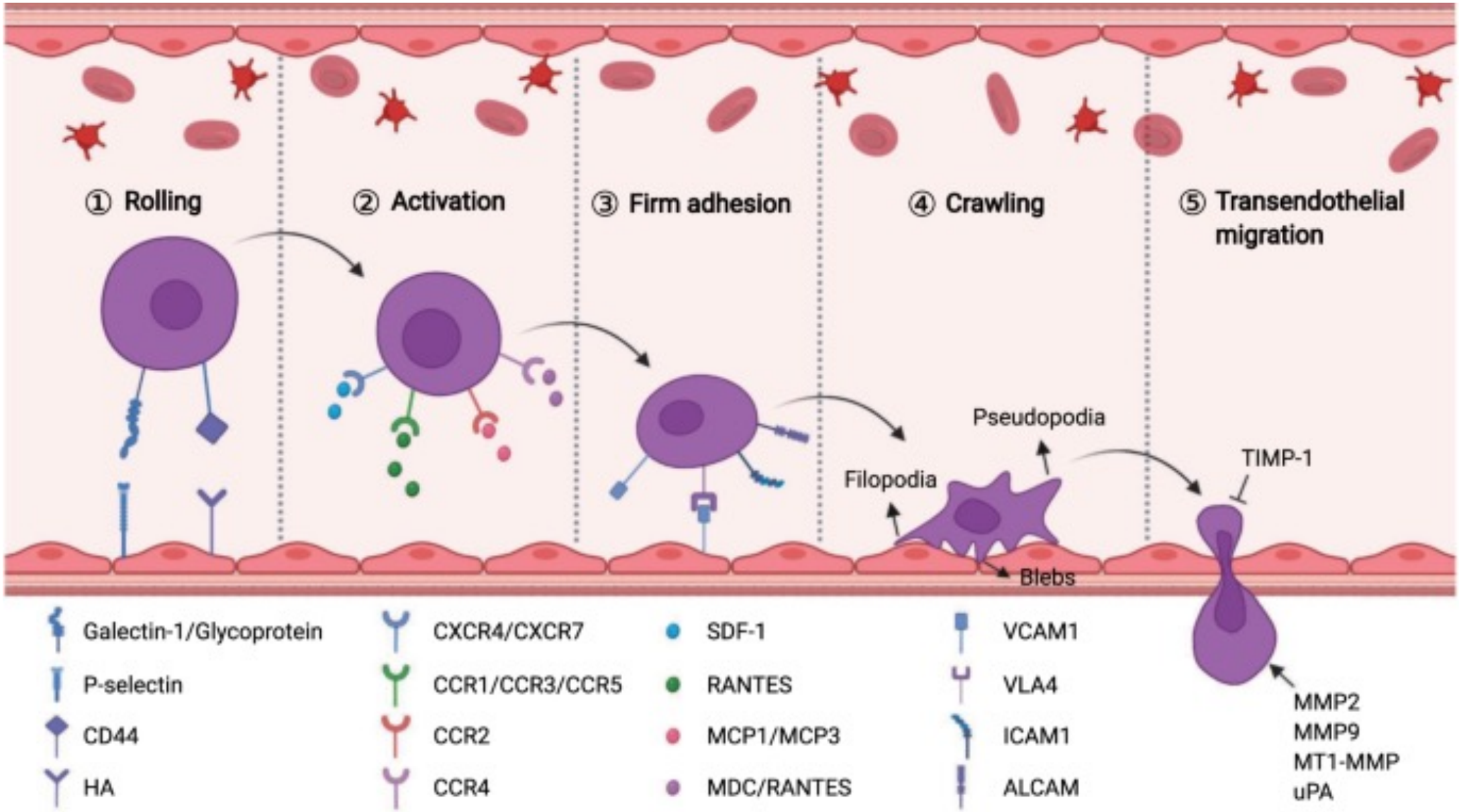
Adapted from Xu C et al., 2021

STEM CELL HOMING



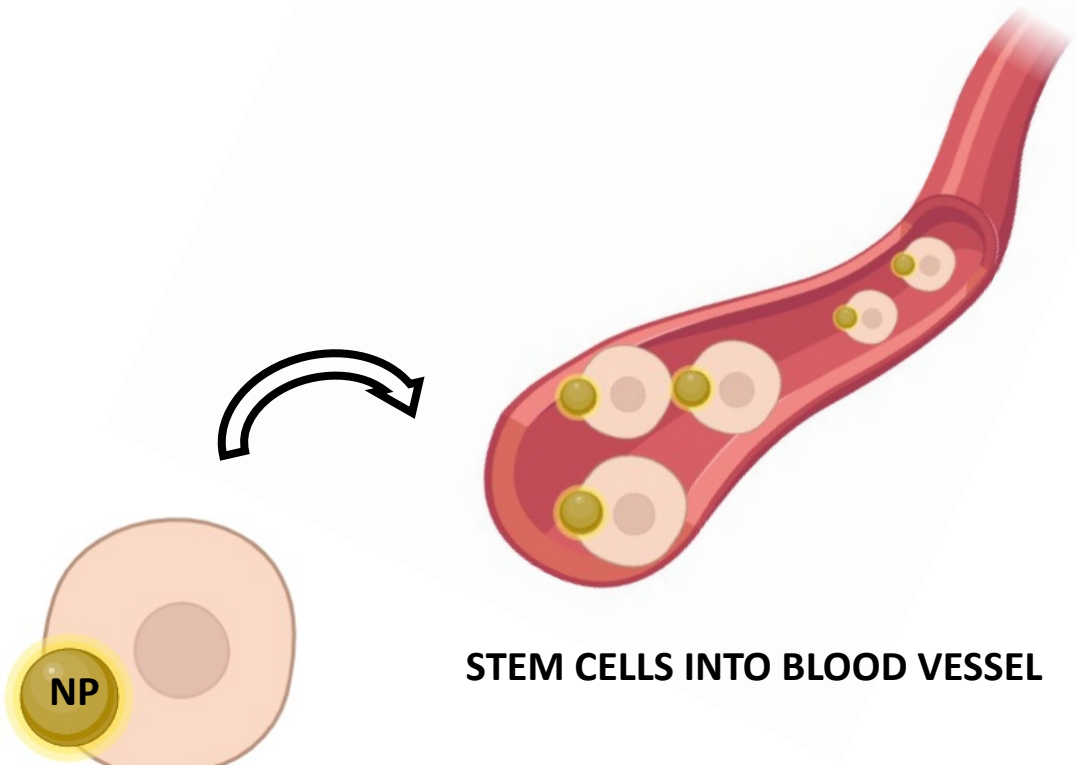
Shin MJ et al., 2021

HOW DO STEM CELLS FIND THEIR WAY HOME?



Yuan M et al., 2022

REGENERATIVE MEDICINE

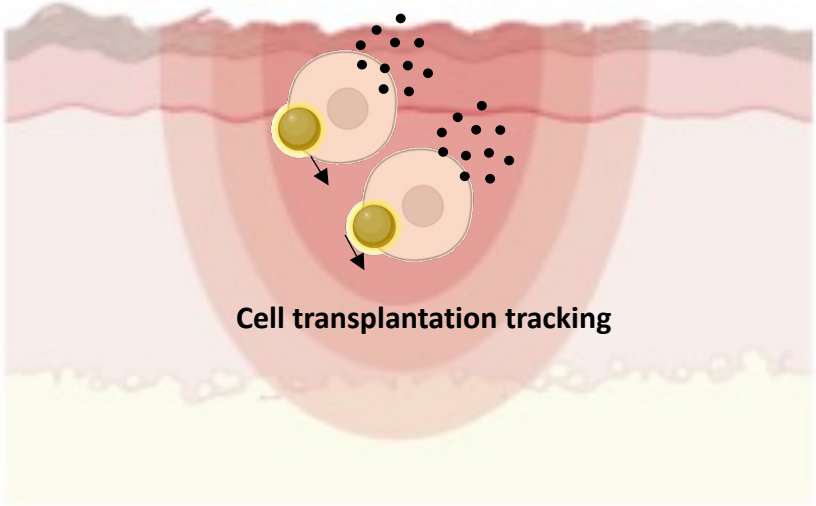


STEM CELL

STEM CELLS INTO BLOOD VESSEL

homing

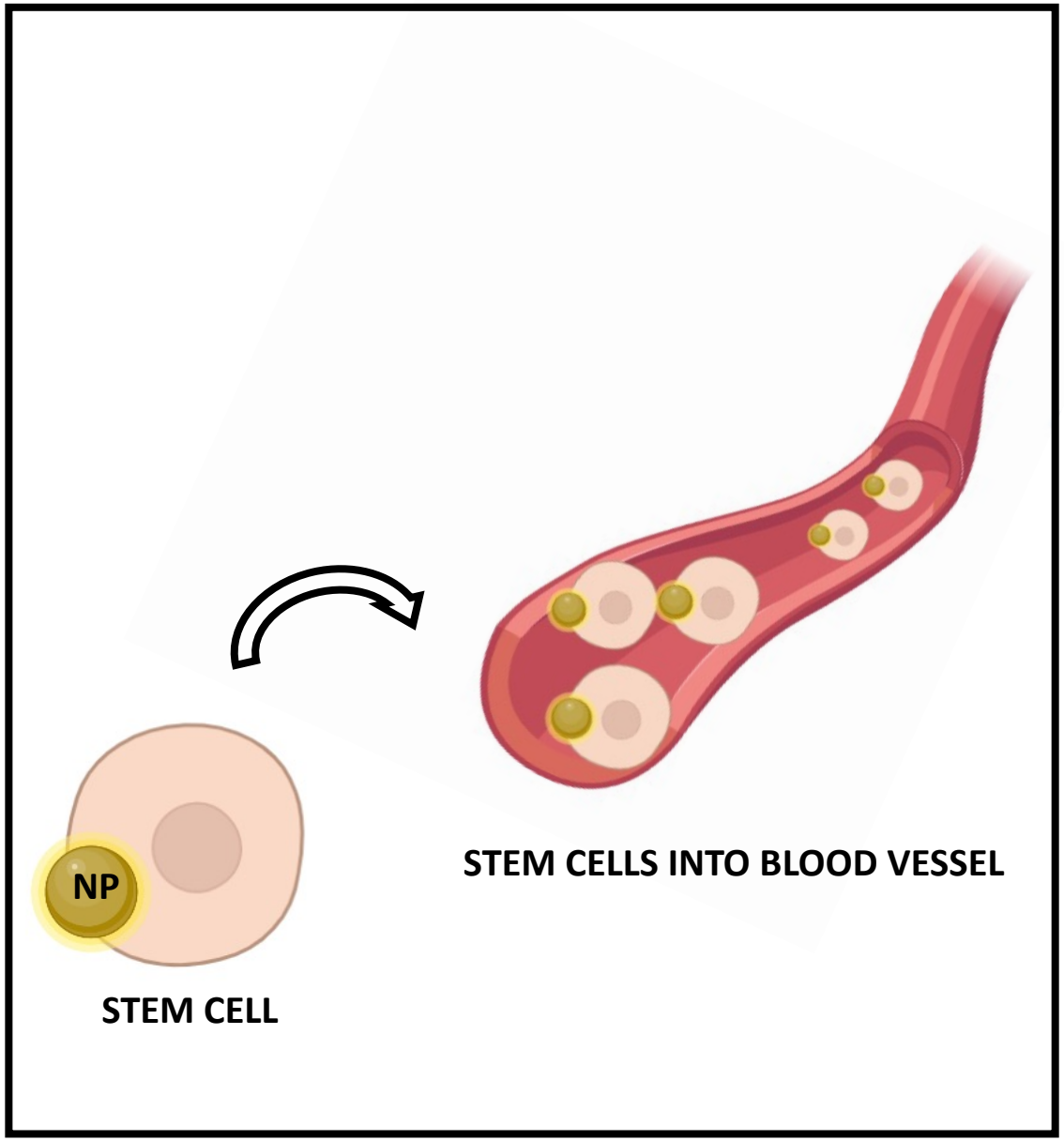
DAMAGED TISSUE



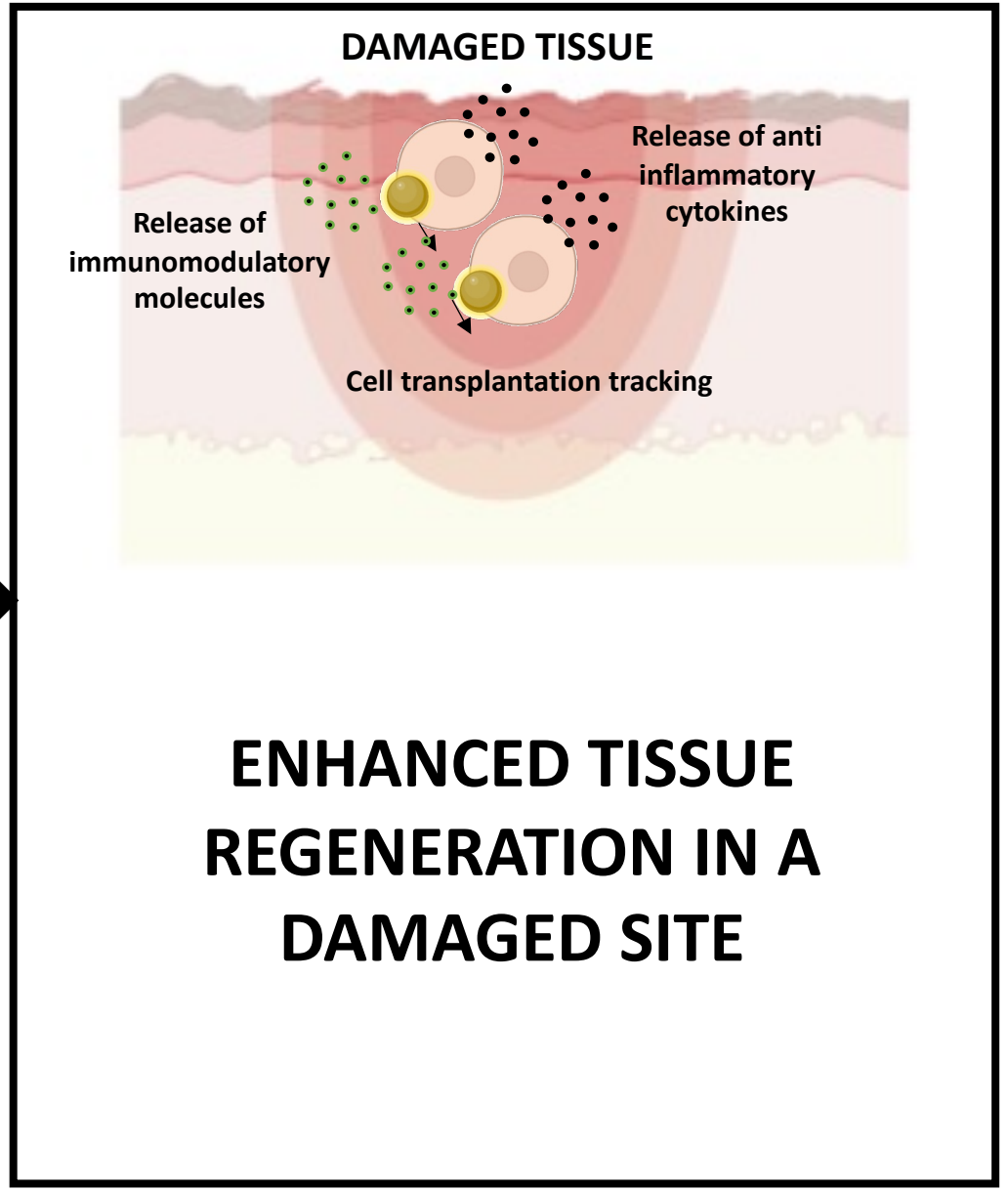
EXPLOITATION OF STEM CELL PROPERTIES

- Self renewing
- Undifferentiated. Can differentiate into any organ-specific cell, depending on their origin
- **Immunomodulation**

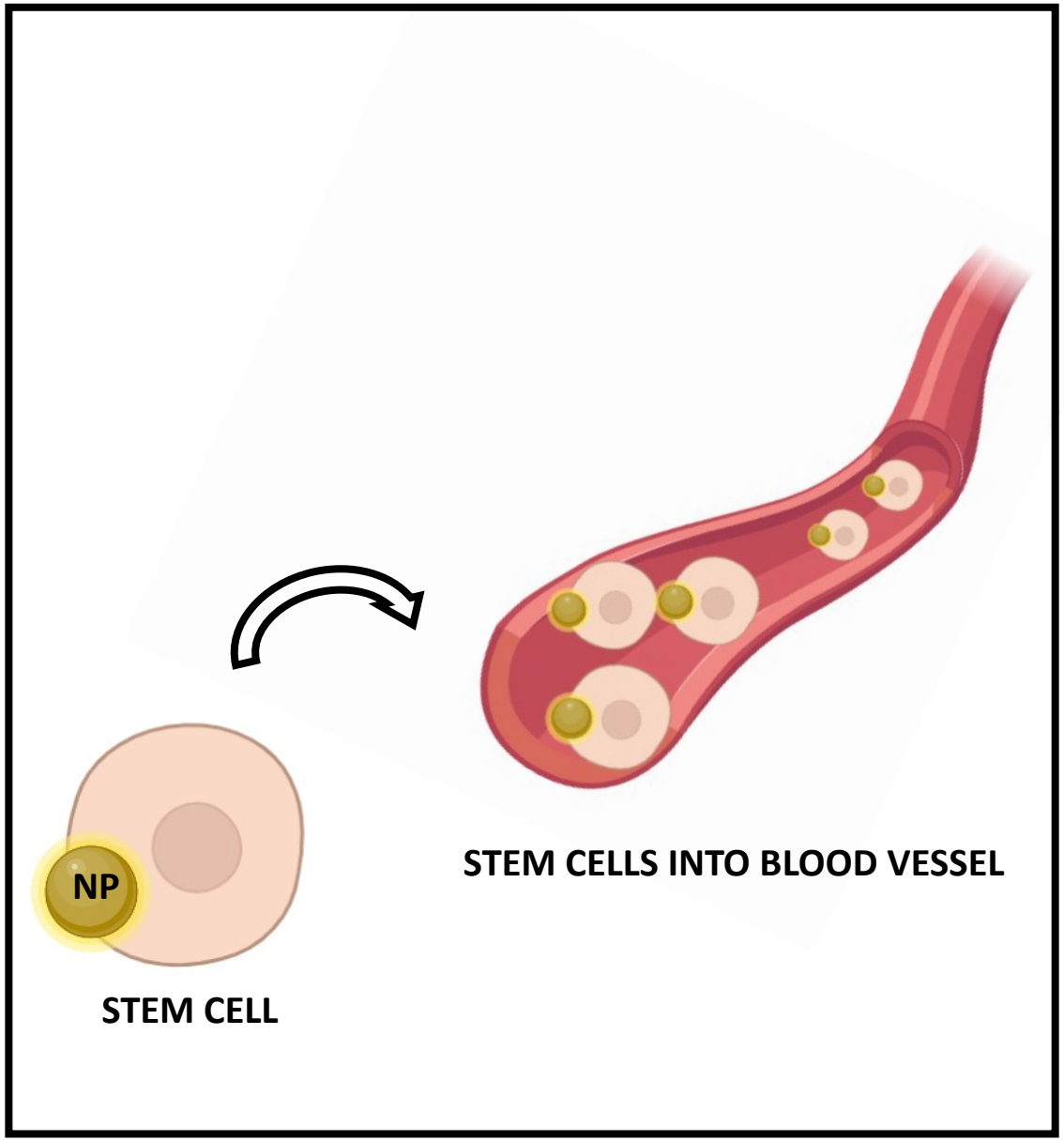
REGENERATIVE MEDICINE



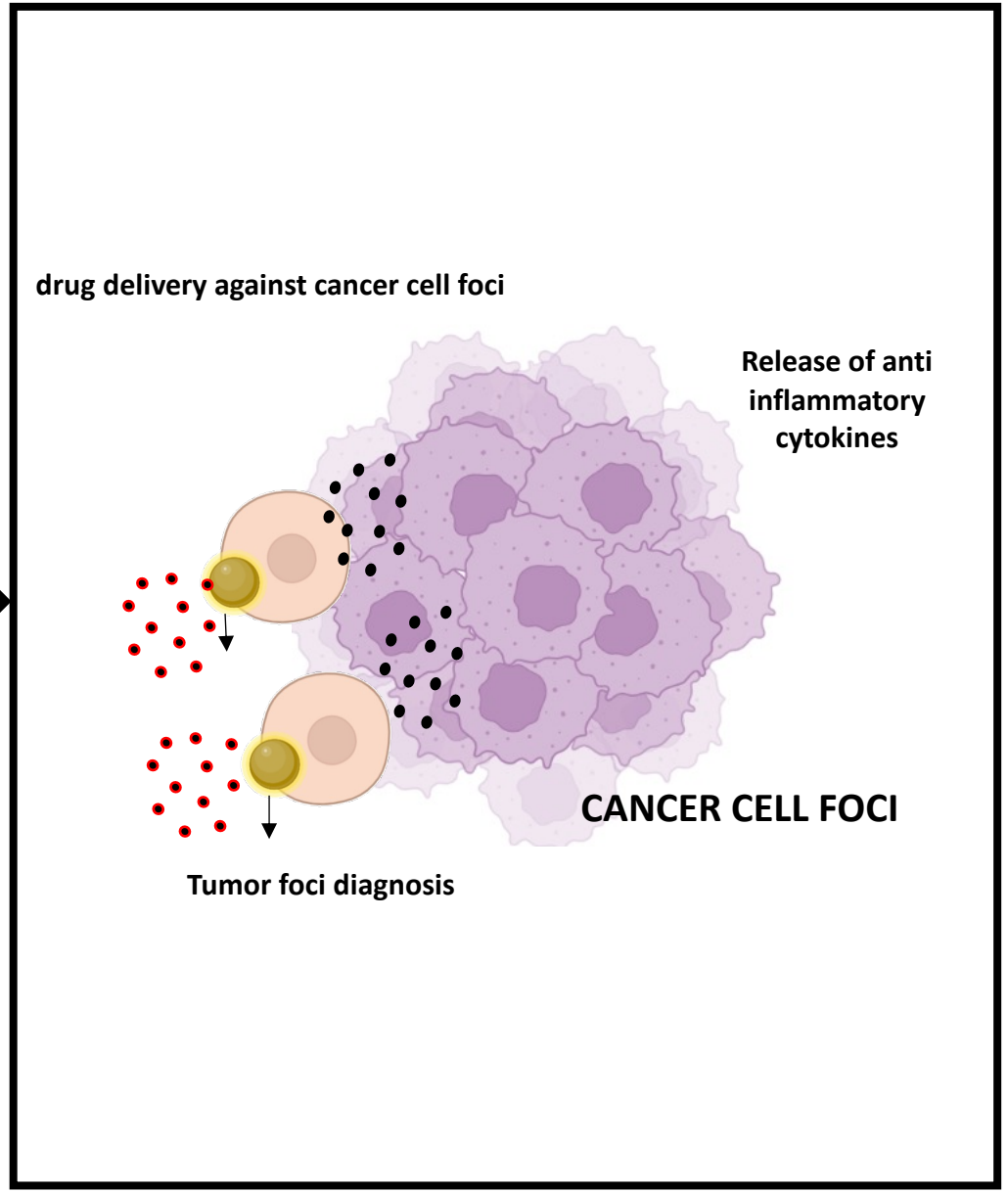
homing



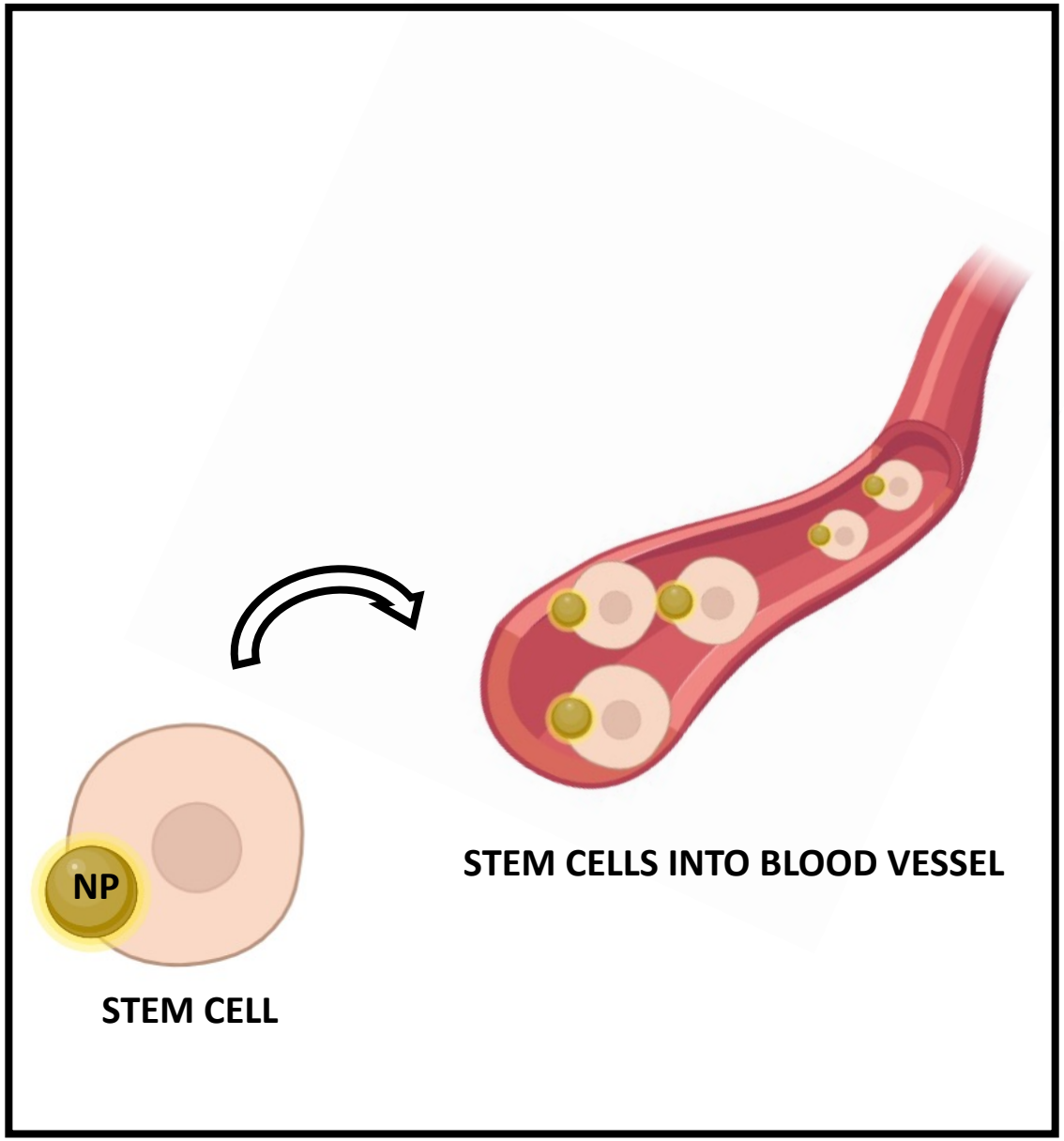
CANCER MEDICINE



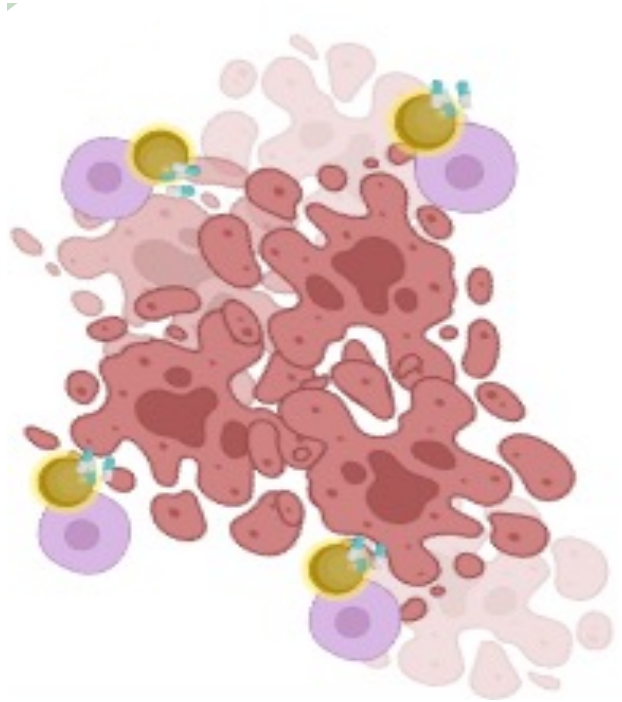
homing



CANCER MEDICINE



homing

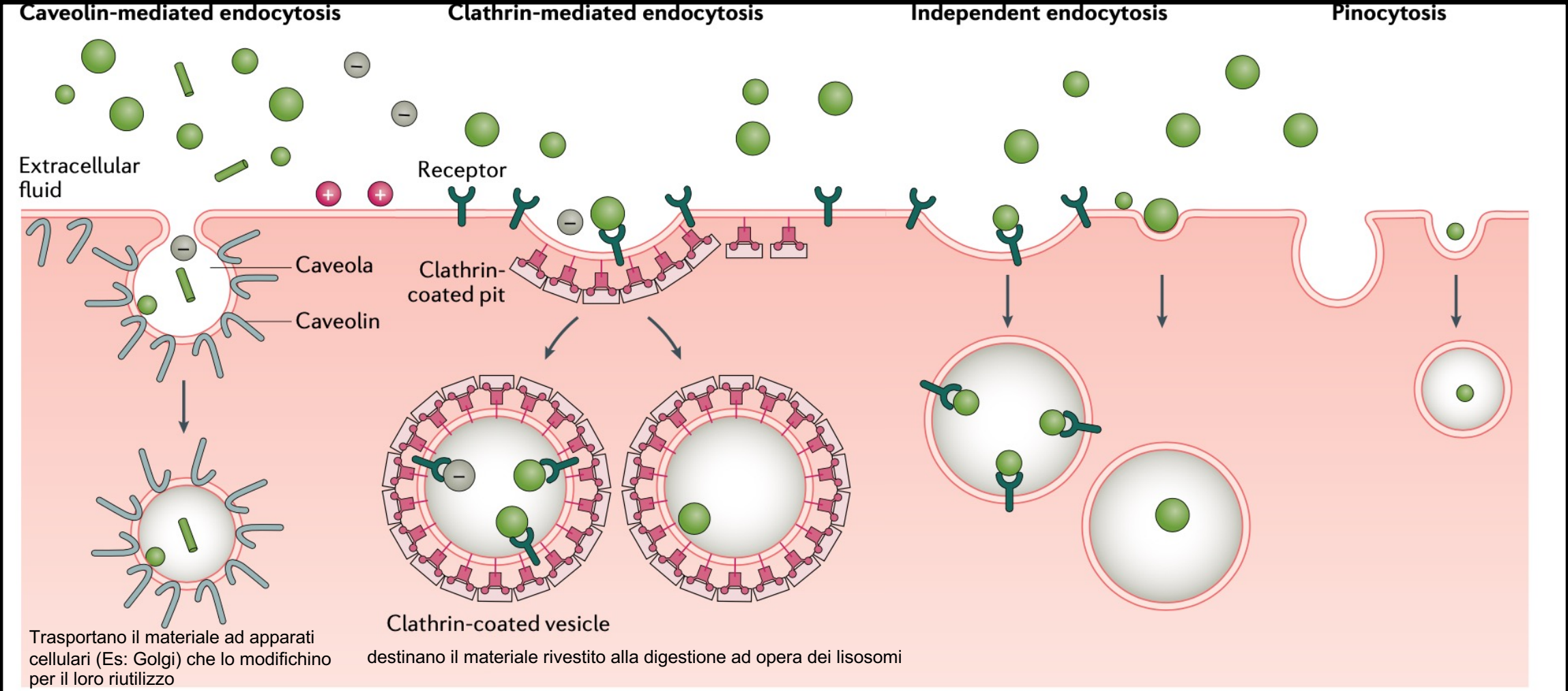


CANCER CELL DEATH

Key features defyning a cell as good carrier of NP

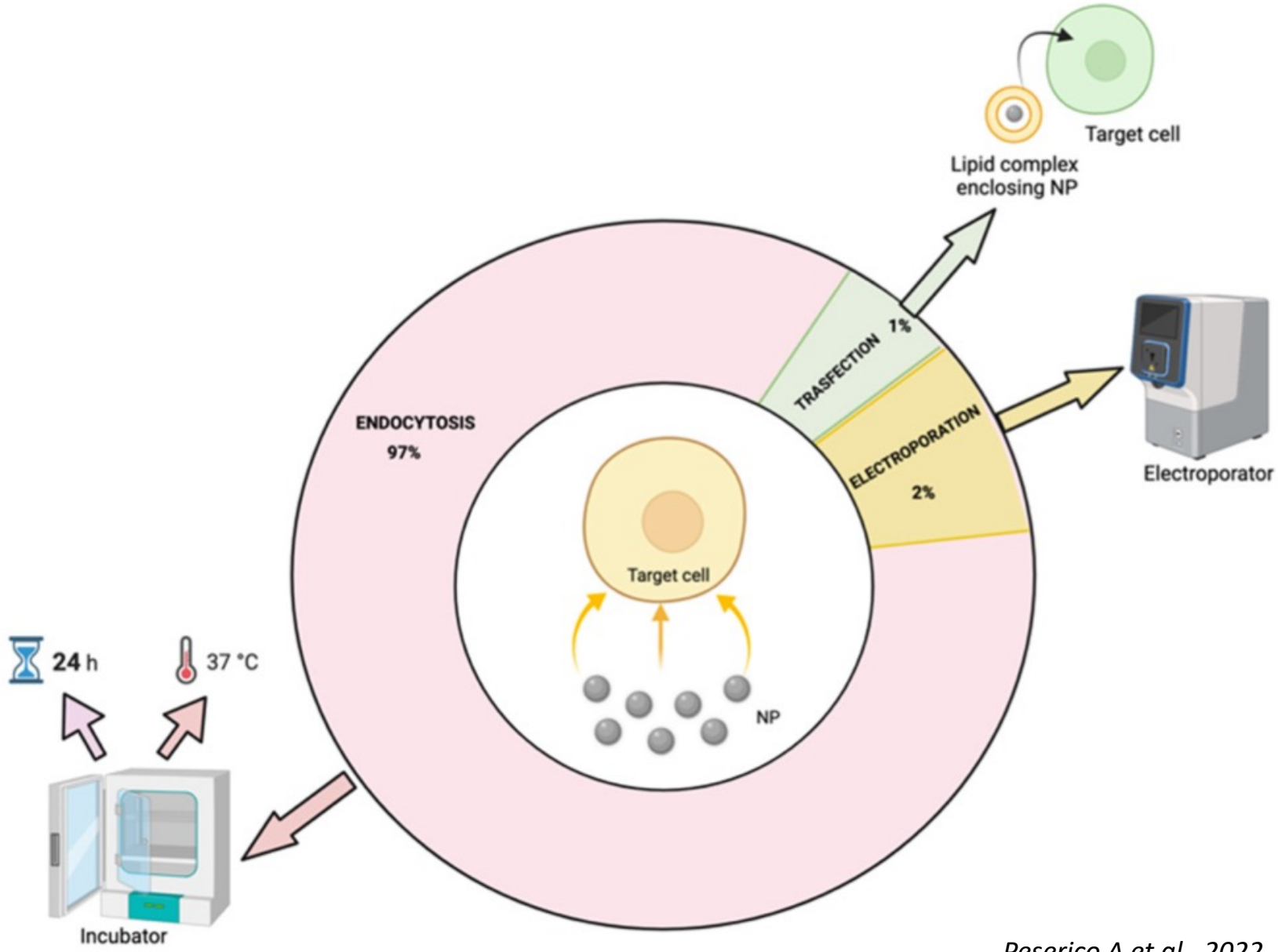
- The cells must be able to incorporate exogenous material not requiring particular in vitro manipulation
- Migratory and homing ability
- Immunomodulatory capacity
- Medium doubling time to avoid as much as the dilution effect of the NP incorporated to each cell division

FIND THE RIGHT RATIO!



Michell MJ et al., 2021

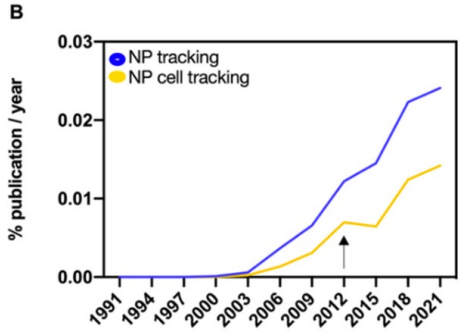
NP CELL INTERNALIZATION MECHANISMS



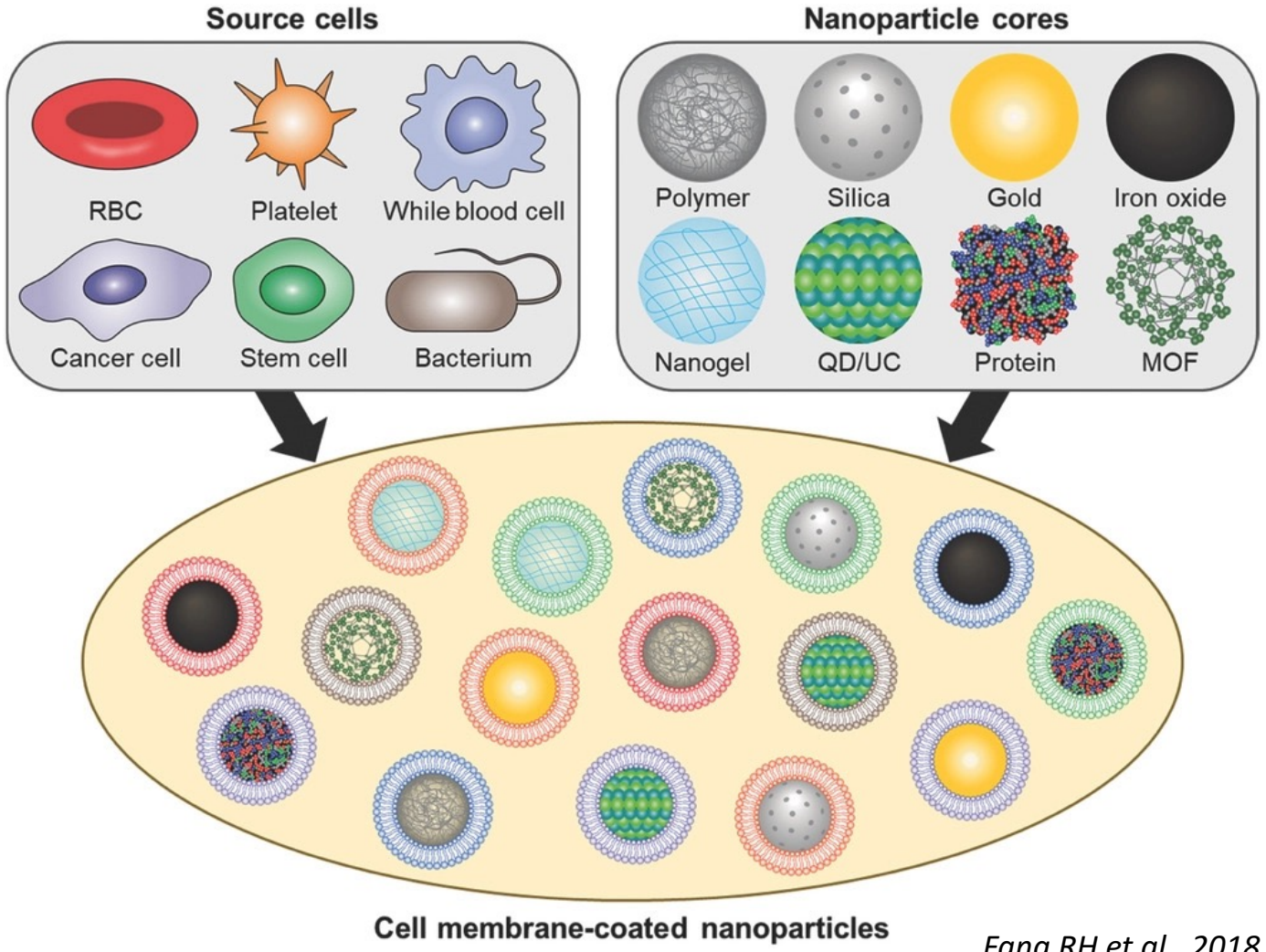
Peserico A et al., 2022

Cell Membrane-coated NP

II generation of NP for cell tracking (diagnosis) and therapy

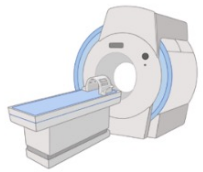
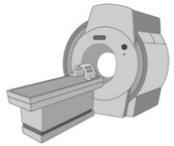
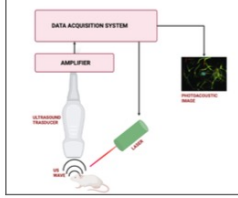


Peserico A et al., 2022



Fang RH et al., 2018

Imaging devices in vivo

IMAGING DEVICE	OPERATIONAL PRINCIPLE	TARGET TISSUE	ADVANTAGES AND DISADVANTAGES
MAGNETIC RESONANCE IMAGING * 	Magnetic resonance imaging (MRI) uses powerful magnets to create a strong magnetic field that compels protons in the body to align with it. The MRI sensors can detect the energy produced as the protons realign with the magnetic field when the radiofrequency field is switched off and build a picture of these signals.	Soft tissue	Advantages: High spatial resolution; Detailed anatomical information of specific organs; Non-ionizing radiation. Disadvantages: Slow imaging speed; Long scanning time.
COMPUTED TOMOGRAPHY 	CT employs a narrow beam of X-rays that is targeted at a patient and swiftly rotated around the body, creating signals that are analyzed by the machine's computer to create cross-sectional pictures of the body.	Hard tissues	Advantages: High temporal resolution; No depth penetration limit; Inexpensive; Offers quantitative information on contrast agents in vivo. Commonly available in hospitals and research facilities.
* **PHOTOACOUSTIC IMAGING** 	PAI irradiates tissues using pulsed laser light, which causes pressure waves because to the elevated warmth and volume. These pressure waves are monitored using a high-frequency ultrasound transducer, and a 3D reconstruction is done.	It adapts very well to structures that contain blood.	Advantages: Excellent contrast; High spatial resolution; High sensitivity. Disadvantages: Shallow detection depth; Lack of stability.
OPTICAL IMAGING 	In vivo optical imaging is involved in the collection of a photographic picture of the body under white light, which allows for the quantification of a bioluminescent (BLI) or fluorescent (FI) signal overlaid on the image. The bioluminescent or fluorescent signal is represented as an intensity map and expressed in photons per second.	Different biological samples: in vitro cells, ex vivo tissue, in vivo imaging of living organism.	Advantages: Semi-quantitative planar image; Signal intensity proportional to the number of viable or actively expressing cells; Disadvantages: Without background anatomical information.

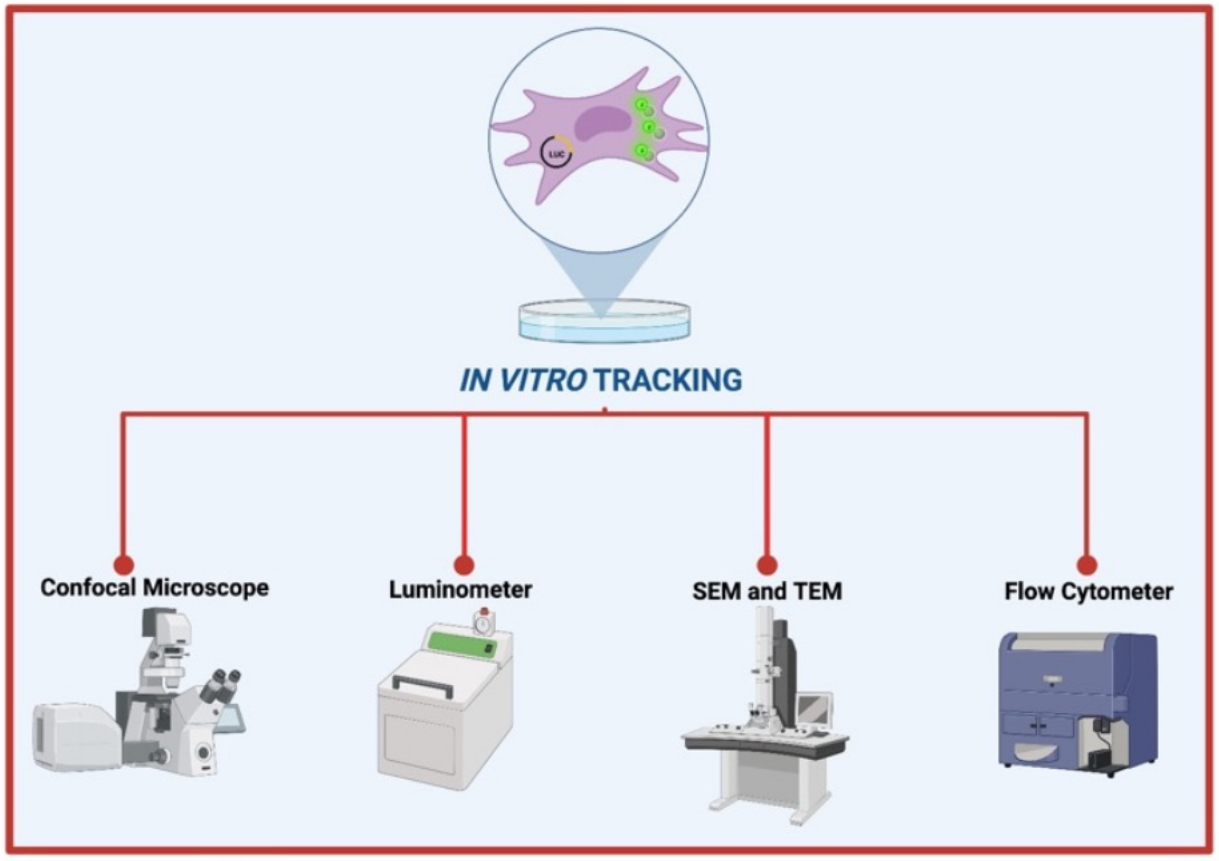
Chemical elements with magnetic properties

Chemical elements with high molecular weight

Chemical elements with high molecular weight; Carbon elements

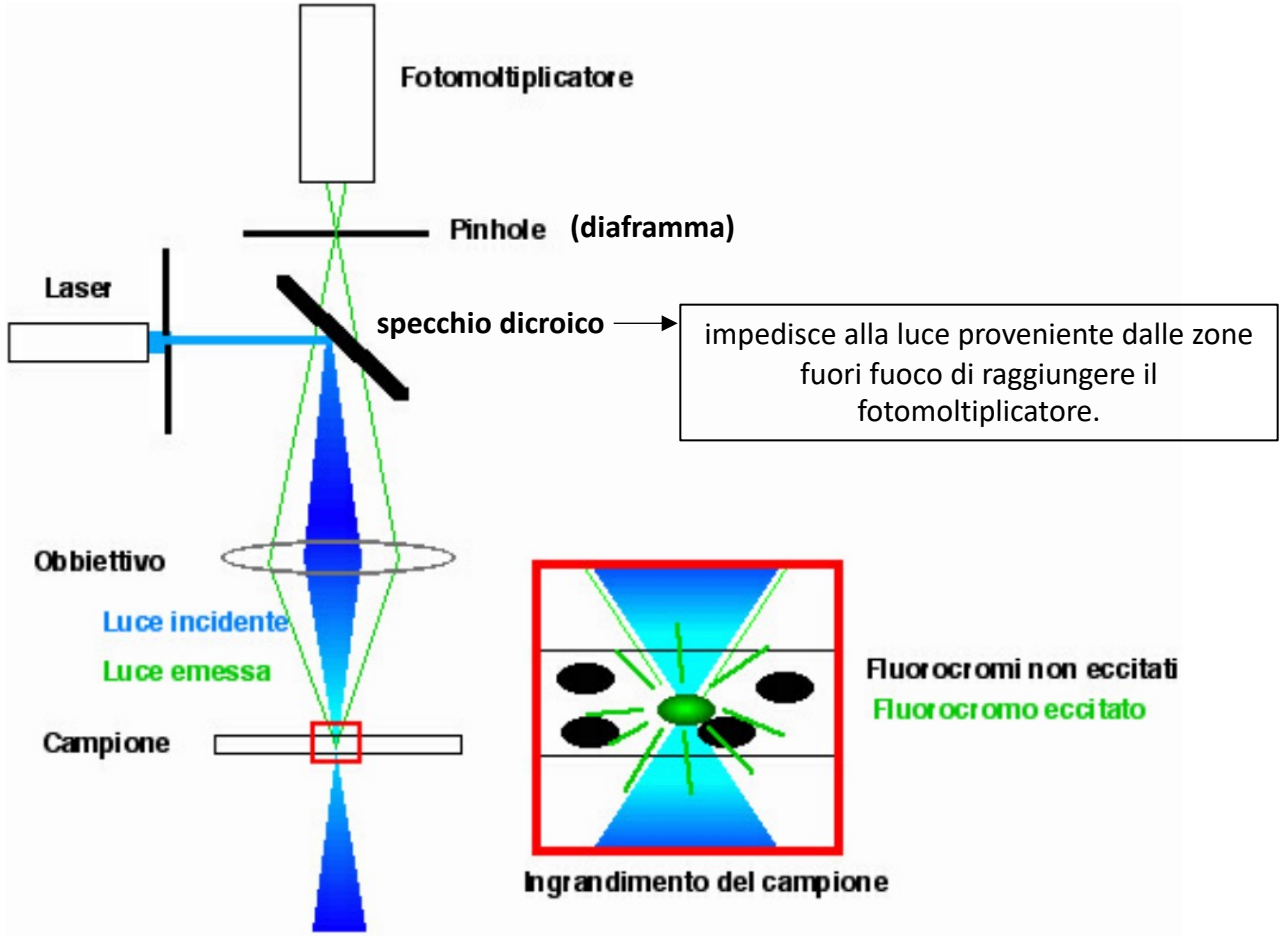
Fluorophores and Bioluminescents

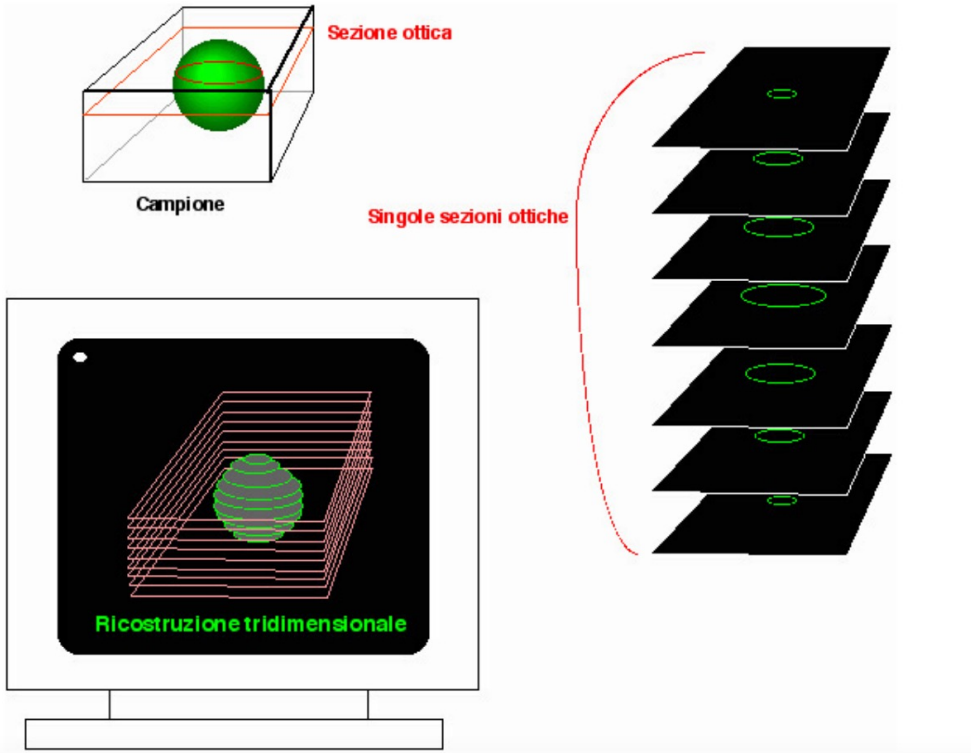
Imaging devices in vitro



Confocal Microscope

La luce emessa dai fluorocromi presenti nel campione viene catturata dalle lenti dell'obbiettivo e deviata da uno specchio dicroico su un fotomoltiplicatore, che trasforma l'intensità luminosa rilevata in un segnale elettrico di intensità proporzionale, segnale digitalizzato per la costruzione dell'immagine.





Ogni punto del campione verrà a corrispondere ad un pixel dello schermo. L'accostamento di tutti i singoli pixel corrispondenti ai punti scanditi dal fascio laser nel campione darà così l'immagina finale.

Spostando lungo l'asse verticale il campione dopo ogni scansione, è possibile eseguire serie di scansioni successive corrispondenti a piani focali via via più profondi all'interno del campione. Queste scansioni prendono il nome di **sezioni ottiche** e la loro sovrapposizione ordinata consente di ricostruire un'immagine complessiva dell'intero volume scandito, in cui tutti i piani sono contemporaneamente a fuoco.

Luminometer

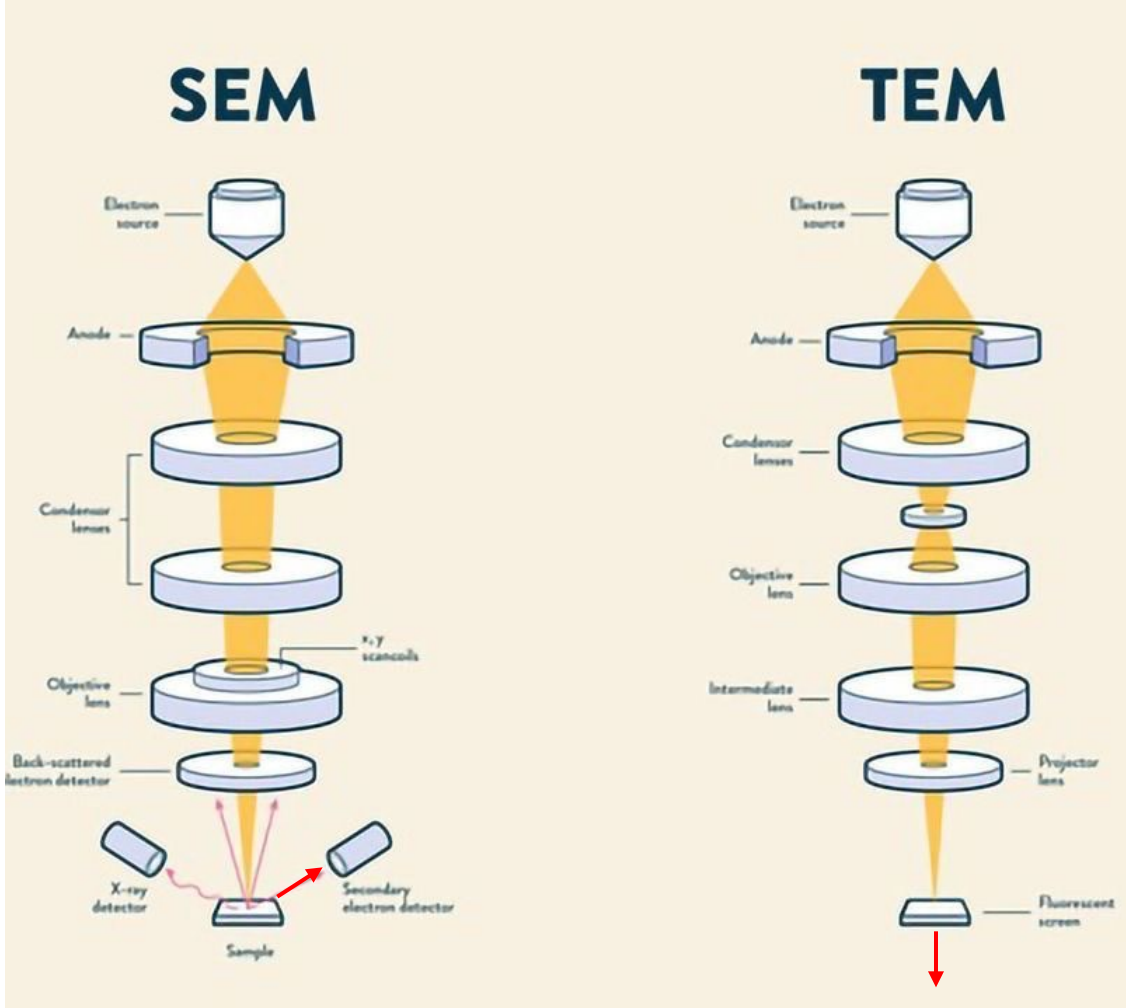
Lettura dell'emissione di fotoni nello spettro visibile.

Sfrutta il fenomeno della bioluminescenza in cui giocano un ruolo chiave 2 elementi:

- substrato organico che emette la luce (luciferina)
- enzima catalizzatore (luciferasi)

Nella maggior parte dei casi il fenomeno è appunto dovuto alla luciferina, che in presenza di ATP (adenosintrifosfato), magnesio e dell'enzima luciferasi, cede elettroni, i quali, passando ad un livello minore di energia, liberano energia sotto forma di luce.

Scanning Electron Microscopy (SEM) and Transmission electron Microscopy (TEM)



SEM creates an image by detecting reflected electrons

TEM uses transmitted electrons (electrons that are passing through the sample) to create an image.

As a result, TEM offers valuable information on the inner structure of the sample, such as crystal structure, morphology and stress state information, while SEM provides information on the sample's surface and its composition.

Signal source: electrons beam
How the signal is transmitted: electron beam pass trought electromagnetic and electrostatic lenses in a high vacuum chamber.

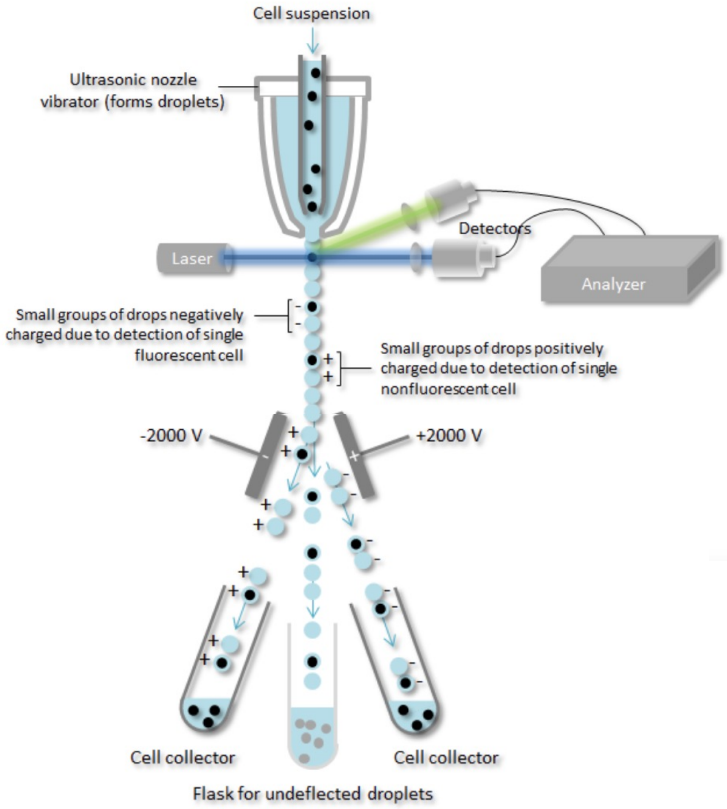
Flow Cytometer

- Caratterizzazione sia a livello qualitativo sia quantitativo di una sospensione cellulare o di particelle.

- Analisi contemporanea di molteplici parametri sia fisici (dimensione e complessità cellulare) sia biochimici/molecolari (es. presenza di specifici antigeni cellulari).

Operational principle:
Il principio si basa sull'impiego di una sorgente luminosa che emette a lunghezza d'onda variabile intercettando perpendicolarmente le singole cellule che fluiscono in un flusso costante e lineare:

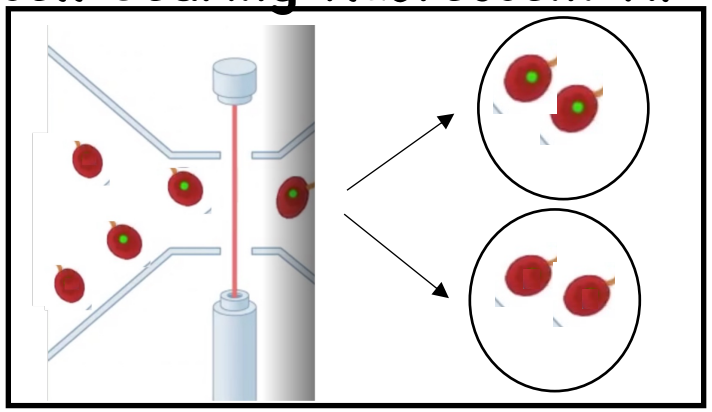
- i raggi direttamente deviati dalla cellula "scatter" forniscono le informazioni fisiche.
- le fluorescenze di emissione forniscono le informazioni legate al target cellulare che si è deciso di studiare (es. particelle incorporate, sottopopolazioni cell).
- 2 detector, uno che misura la dimensione (forward scatter; FSC) ed uno che misura la complessita o granulosità cellulare (side scatter; SSC)



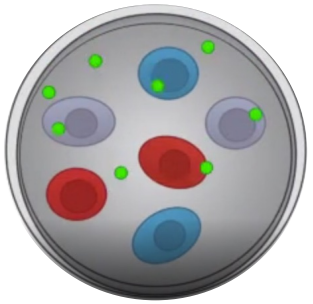
N.B. Esistono due tipi di citometria a flusso: una di tipo "non-sorting" che raccoglie i dati di "scatter" ed emissioni di fluorescenza, ed una di tipo "sorting" che sfrutta tali informazioni per poter separare singole sottopopolazioni cellulari con determinati parametri, partendo dalla popolazione eterogenea iniziale.

Flow cytometry Application examples:

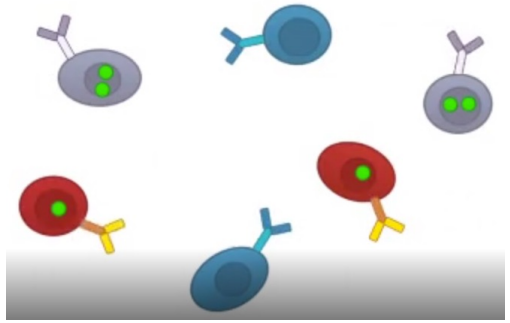
1. Sorting and quantification of cell bearing fluorescent NP prior in vivo administration



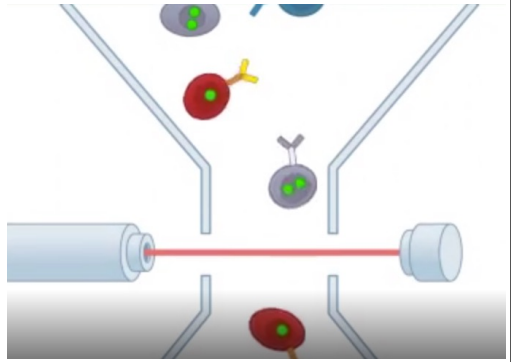
2. Identification of fluorescent NP interaction with different cell populations



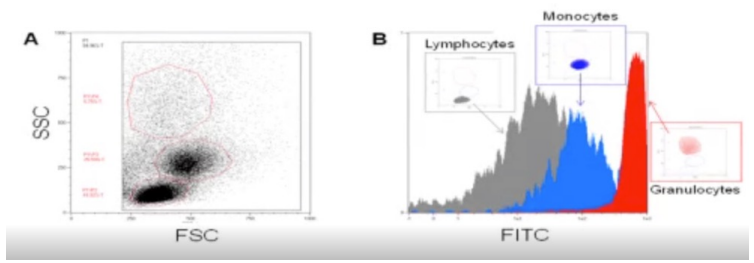
NP cell uptake



Cell labeling with ab against specific cell type markers



Flow cytometry analysis



Interaction of each cell type with NP can be measured

STUDY QUESTIONS

- Ho un paziente con tumore alla mammella in stadio avanzato, occorre valutare la presenza di eventuali metastasi. Primo tessuto target di metastatizzazione del tumore alla mammella è l'osso.

1. Quale approccio diagnostico supportato da NP?
2. Quale NP?

Se volessi far terapia?

- Ho un paziente con danno tissutale al fegato che necessita di trapianto per risoluzione.

1. Come posso monitorare il trapianto cellulare?
2. Posso fare terapia anti-infiammatoria?

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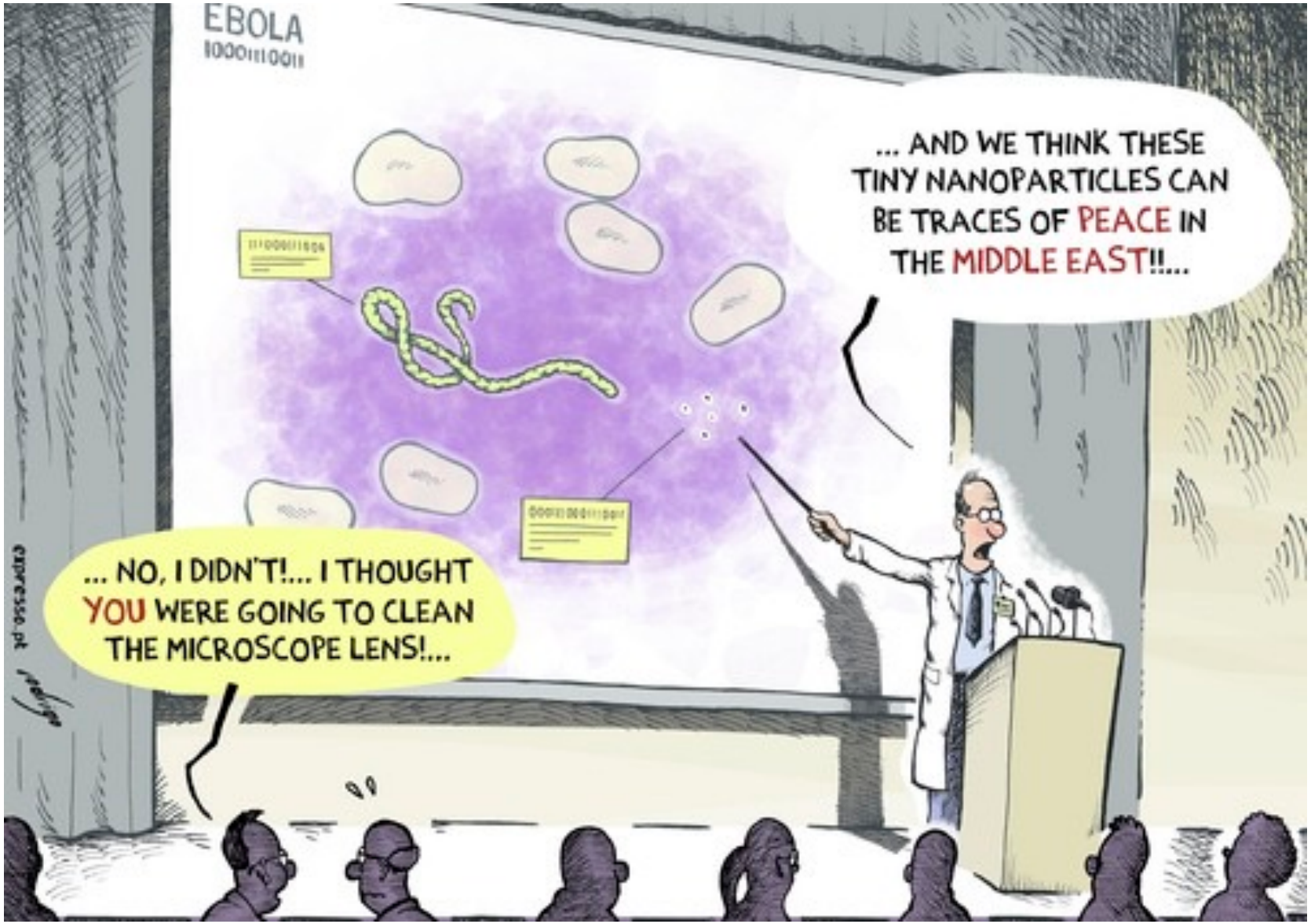
- Link to TC operational principles: https://www.youtube.com/watch?v=IH49HLC-wYg
- Link to PA imaging operational principles: https://www.youtube.com/watch?v=sJ9ZWnvZkRI


EBOLA
... AND WE THINK THESE TINY NANOPARTICLES CAN BE TRACES OF PEACE IN THE MIDDLE EAST!!...
... NO, I DIDN'T!... I THOUGHT YOU WERE GOING TO CLEAN THE MICROSCOPE LENS!...