Medical Biotechnology 2023'

Biological therapies

Lecture 9-10th

Nanoparticles for biotherapy

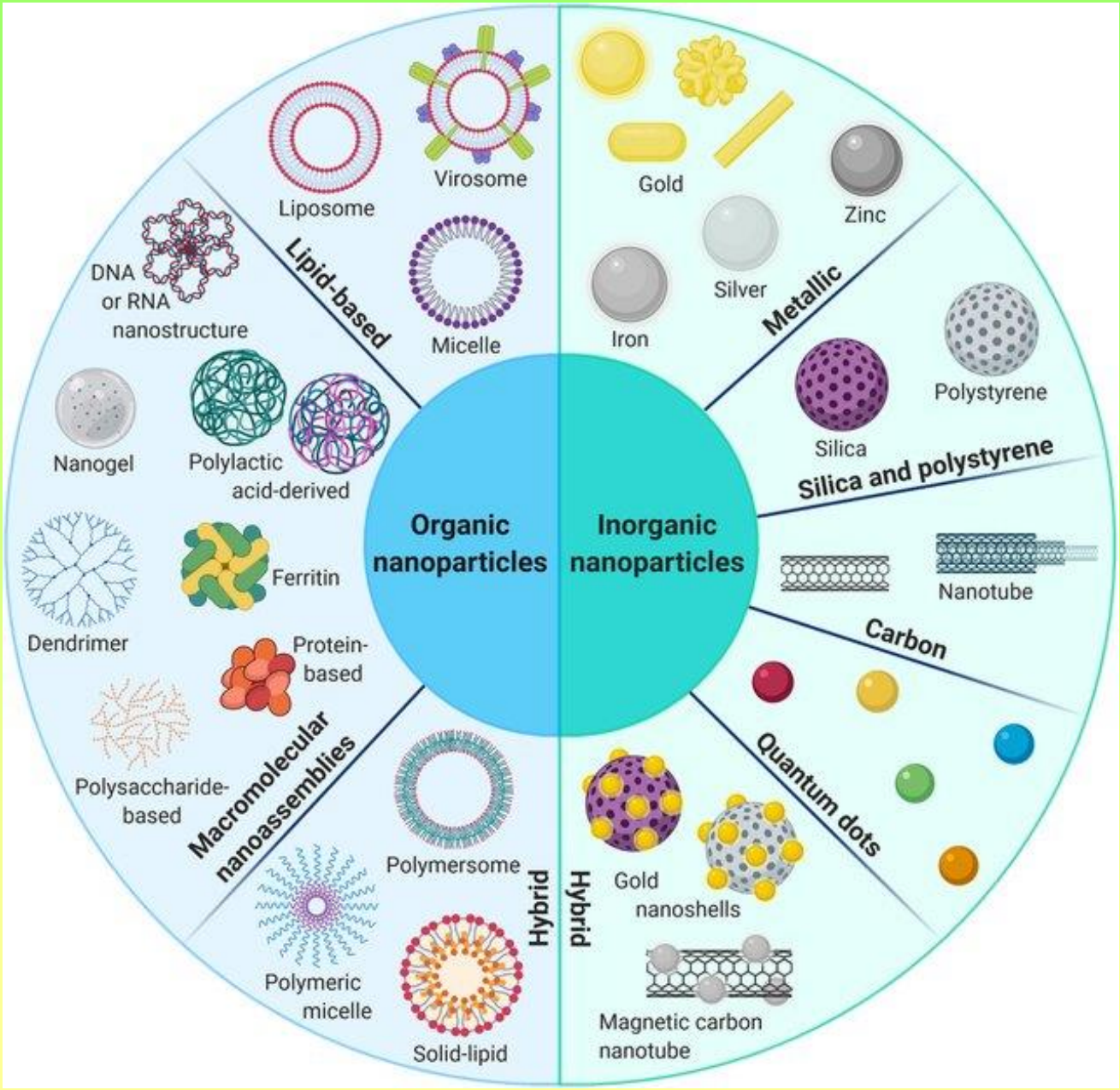
Definitions

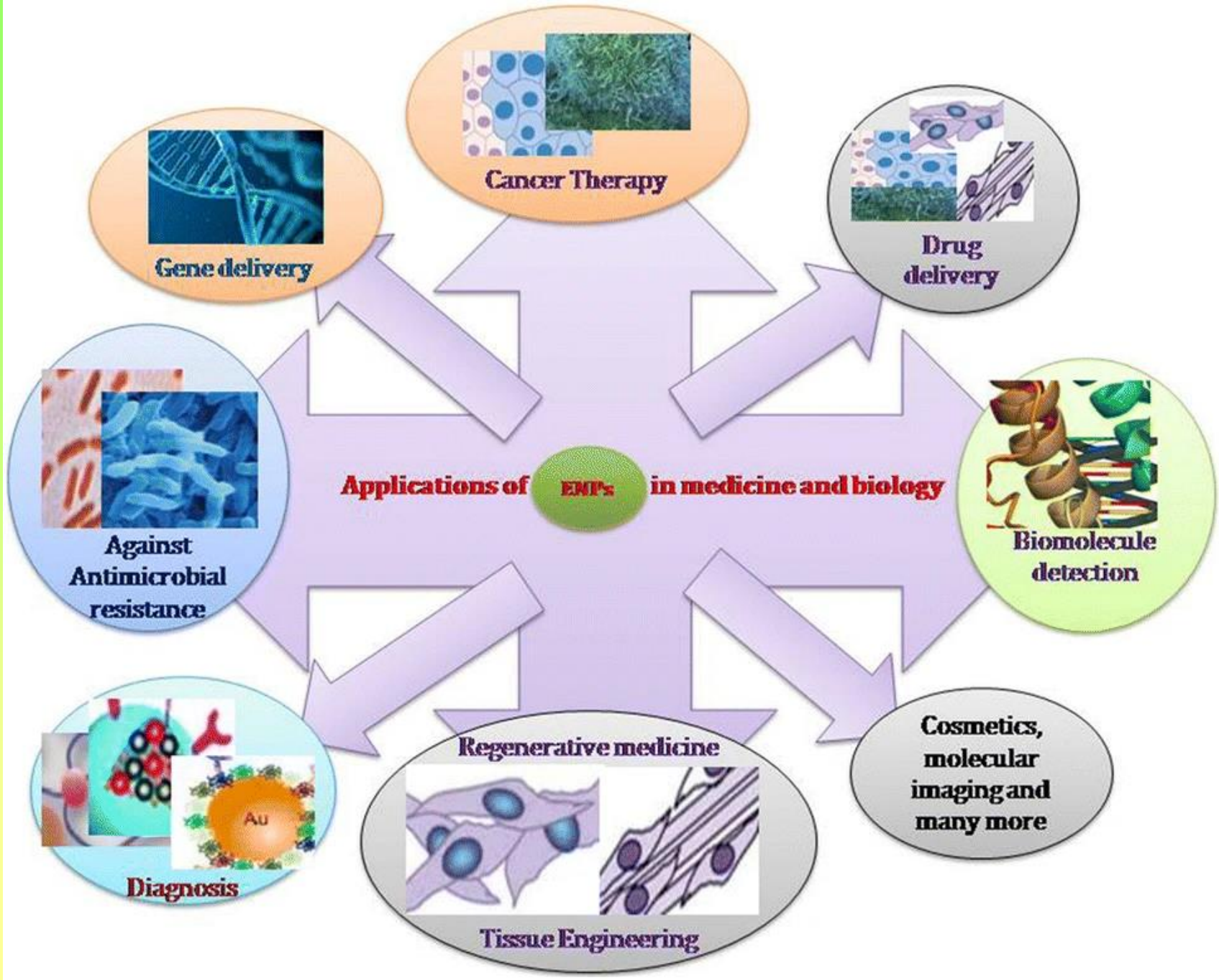
- Ultrafine particles, or **nanoparticles** are sized between **1 and 100 nanometers**.
- Nanoparticle research is currently an area of intense scientific interest due to a wide variety of potential applications in biomedical, optical and electronic fields. The strongly size-related properties of nanoparticles offer uncountable opportunities for surprising discoveries. The often unexpected and unprecedented behavior of nanoparticles bears great potential for innovative technological applications, but also poses great challenges to the scientists.

Nanoparticles in biological research

- **Living organisms** are built of cells that are typically **10-50 μm across**. However, the cell parts are much smaller and are in the sub-micron size domain. The **proteins** with a typical size of just **5 nm**, which is comparable with the dimensions of smallest manmade nanoparticles.
- Simple size comparison gives an idea of using nanoparticles as **very small probes** that would allow us to investigate cellular machinery **without** introducing too much **interference**. Understanding of biological processes on the nanoscale level is a strong driving force behind development of nanotechnology.

Main types of engineered nanoparticles



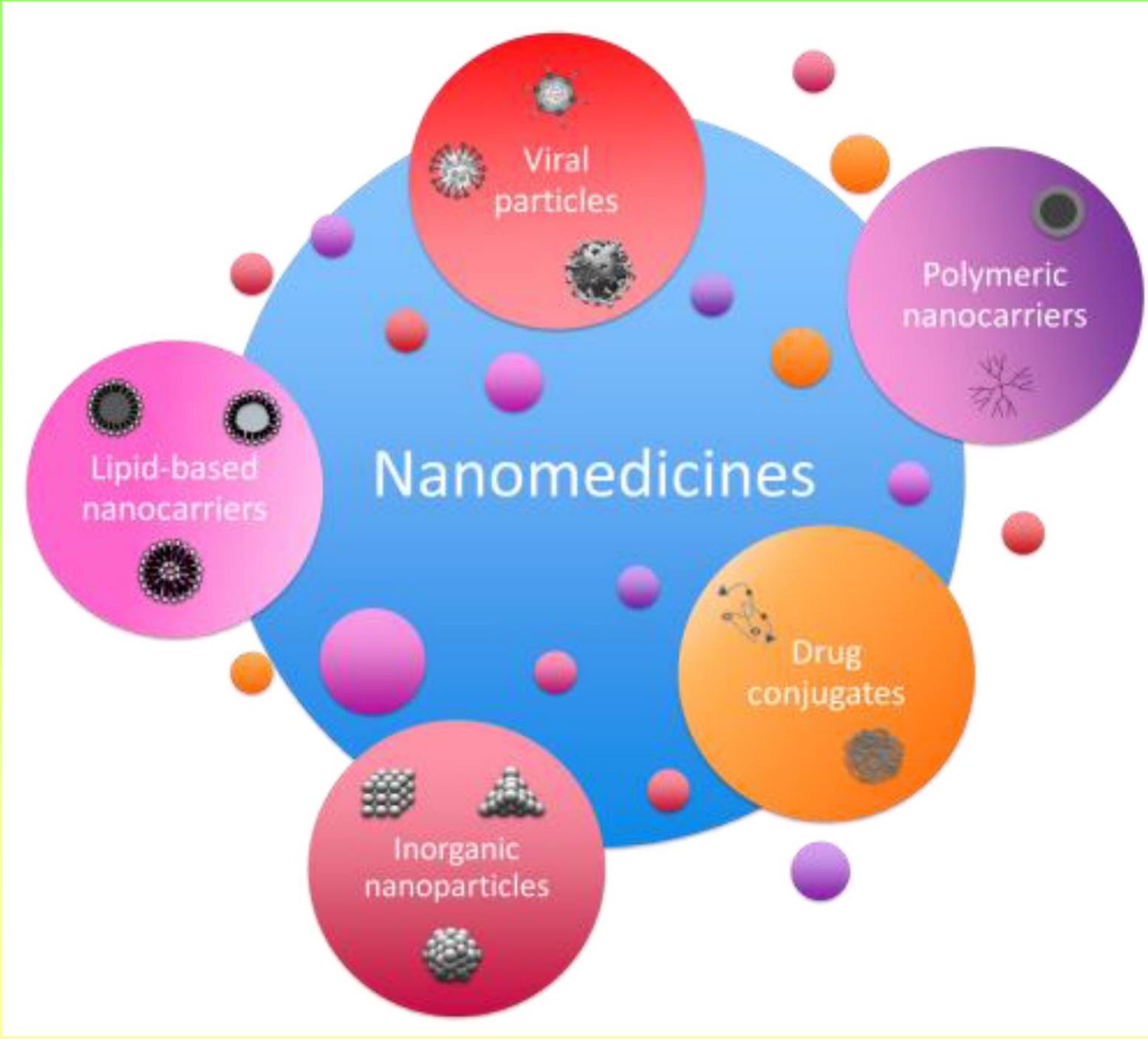

Cancer Therapy
Gene delivery
Drug delivery
Against Antimicrobial resistance
Applications of ENPs in medicine and biology
Biomolecule detection
Au
Diagnosis
Regenerative medicine
Tissue Engineering
Cosmetics, molecular imaging and many more

Nanoparticles in biology and medicine

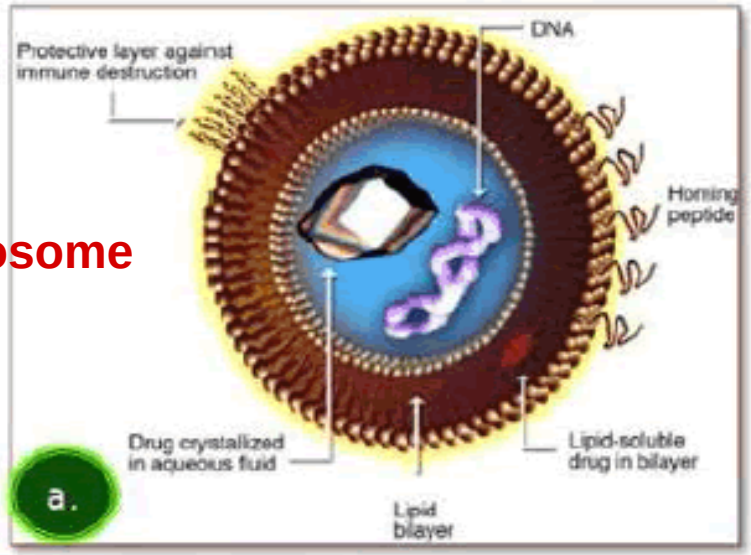
- Nanoparticles provide a useful platform, establishing **unique properties** with potentially wide-ranging **research and therapeutic** applications.
- For biological applications, the **surface coating should be polar** to give high aqueous solubility and prevent nanoparticle aggregation. In serum or on the cell surface, **highly charged** coatings promote non-specific binding, while polyethylen glycol (**PEG**) linked to terminal hydroxyl or methoxy groups prevent non-specific interactions.

Nanomedicine

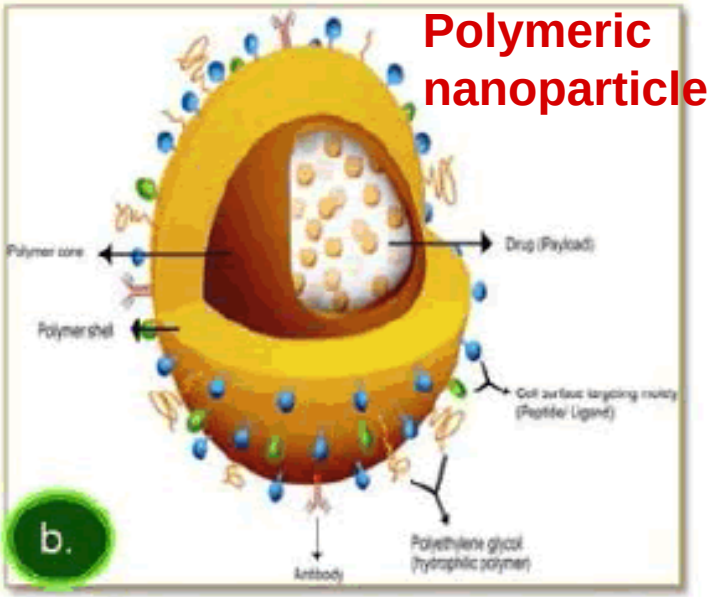
- Nanomedicine is the **medical application** of nanotechnology: it ranges from therapeutic applications of **nanomaterials**, to **nano-electronic biosensors**, and even possible future applications of molecular nano-technology **in diagnostics and therapy**.
- **Current problems** for nanomedicine involve understanding the issues related to **toxicity and environmental impact** of nanoscale non-degradable materials.


Viral particles
Polymeric nanocarriers
Lipid-based nanocarriers
Nanomedicines
Drug conjugates
Inorganic nanoparticles

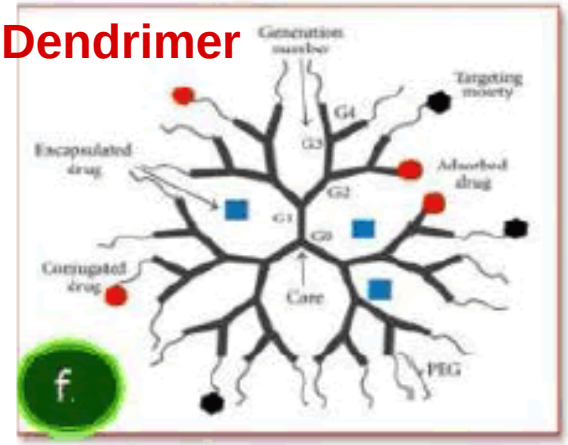
Liposome



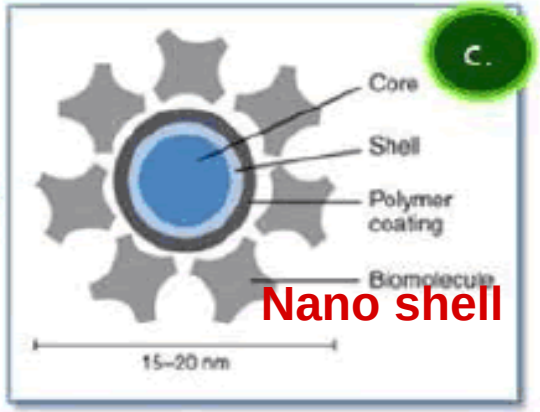
Polymeric nanoparticle



Dendrimer

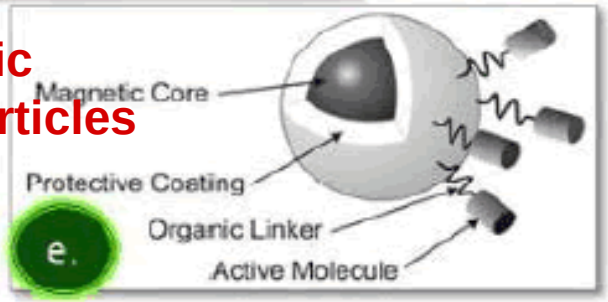


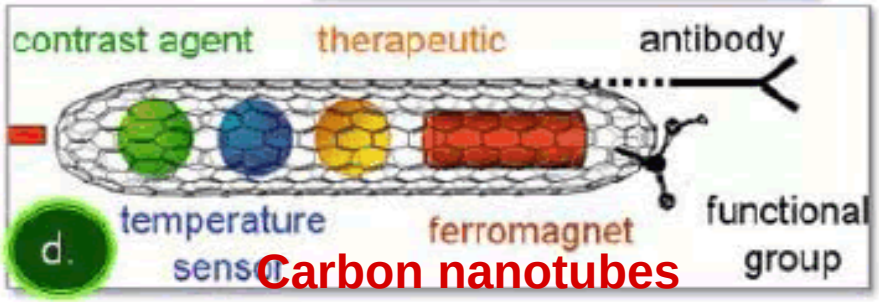
Therapeutic platforms



Nano shell

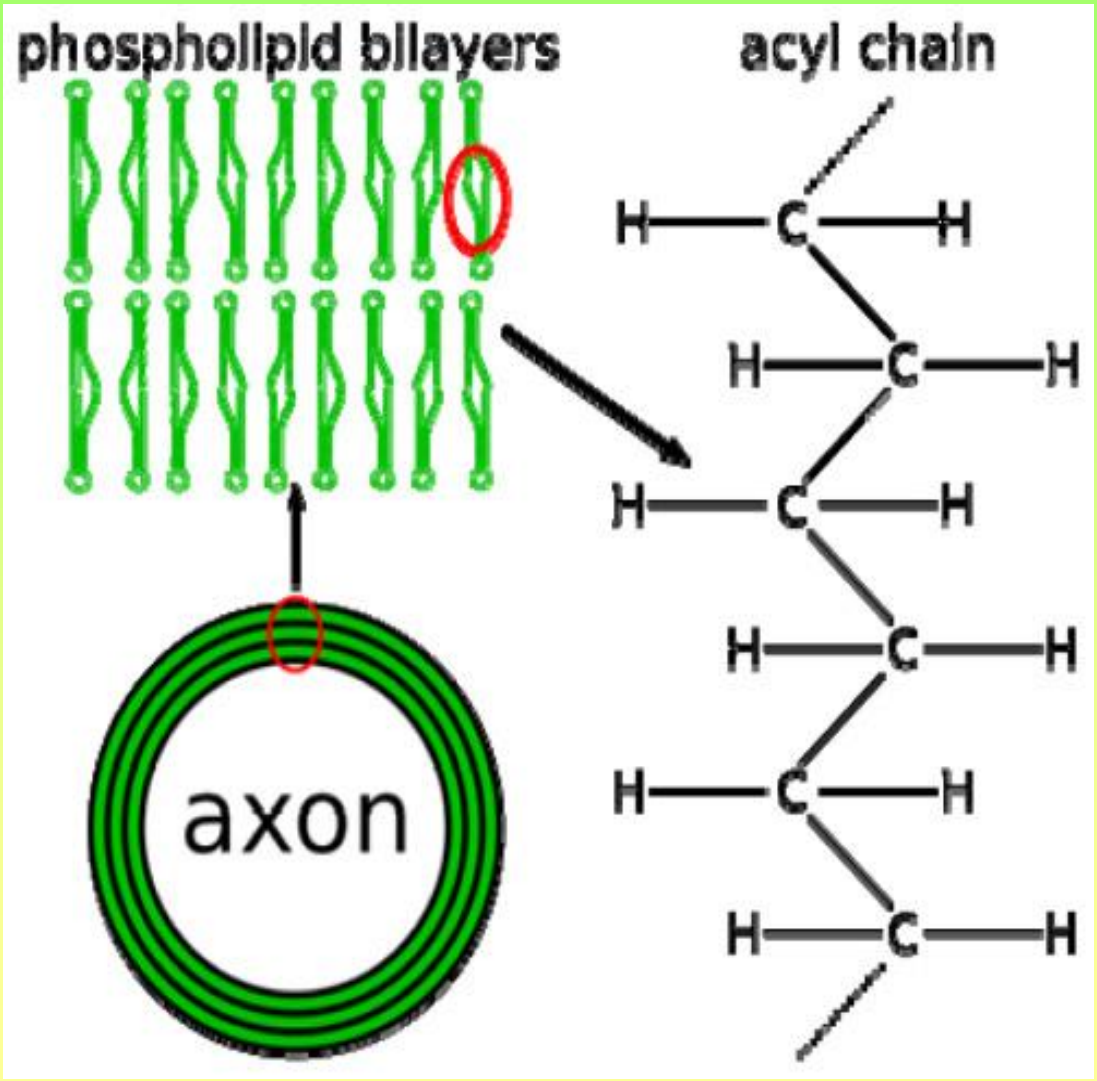
Magnetic nanoparticles

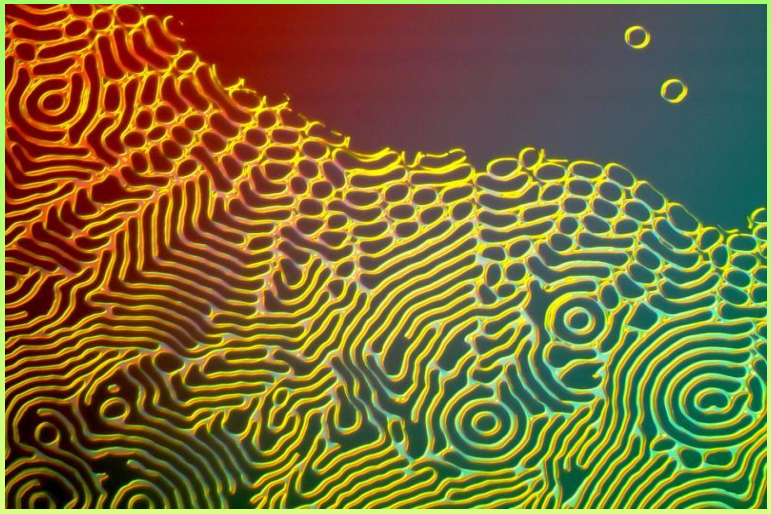


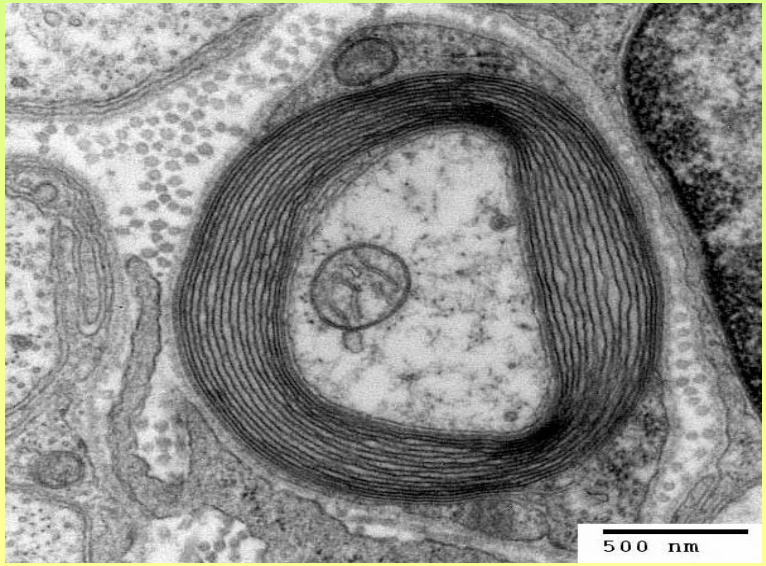


Carbon nanotubes

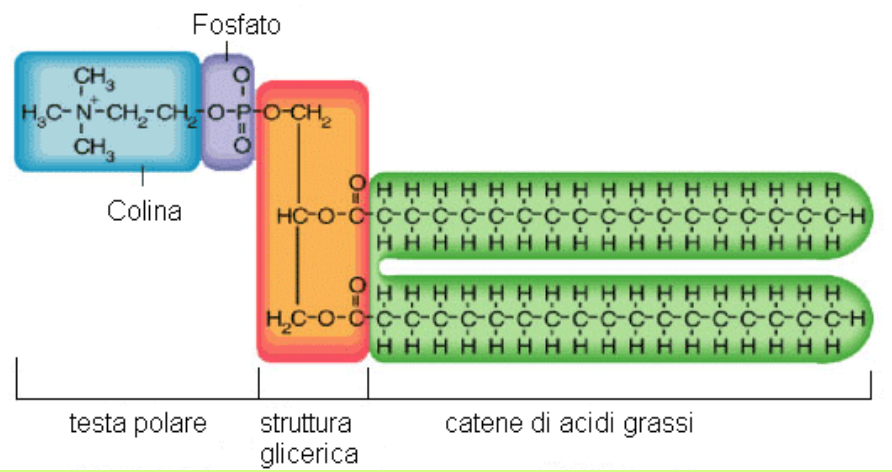
„Myelin figures”

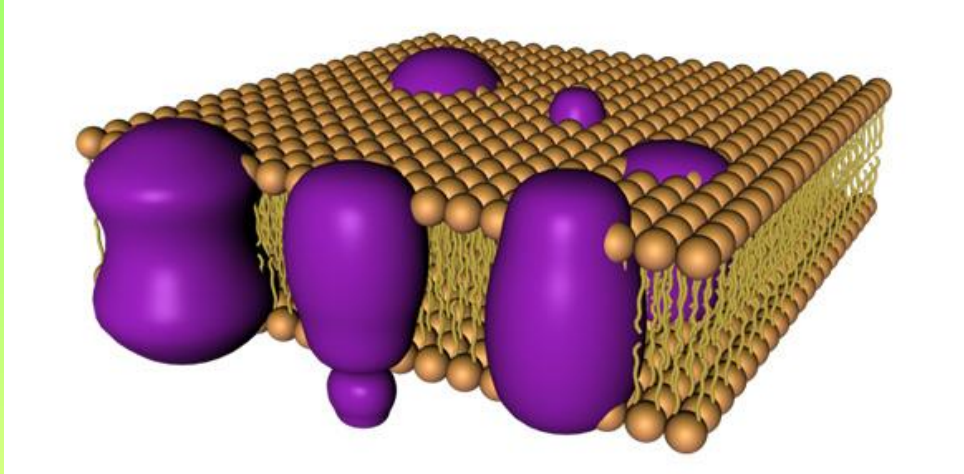


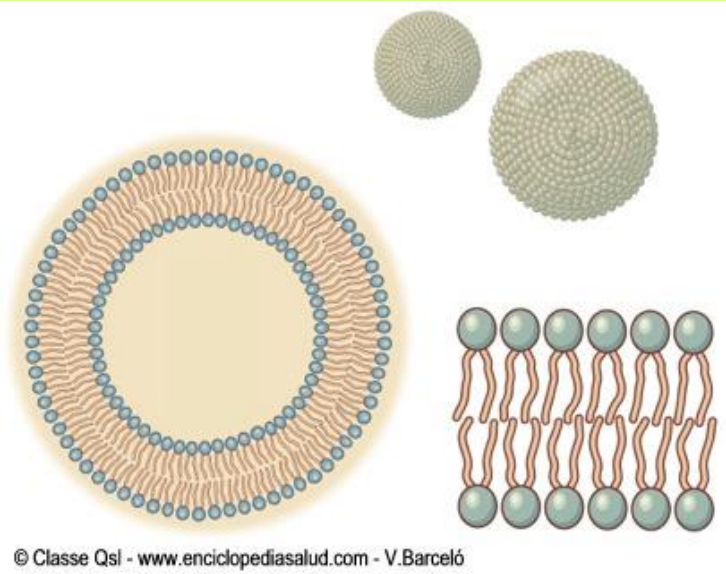


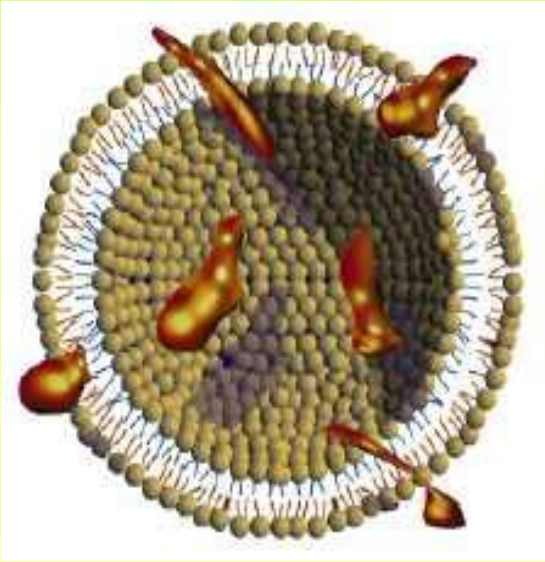


Phospholipid liposome





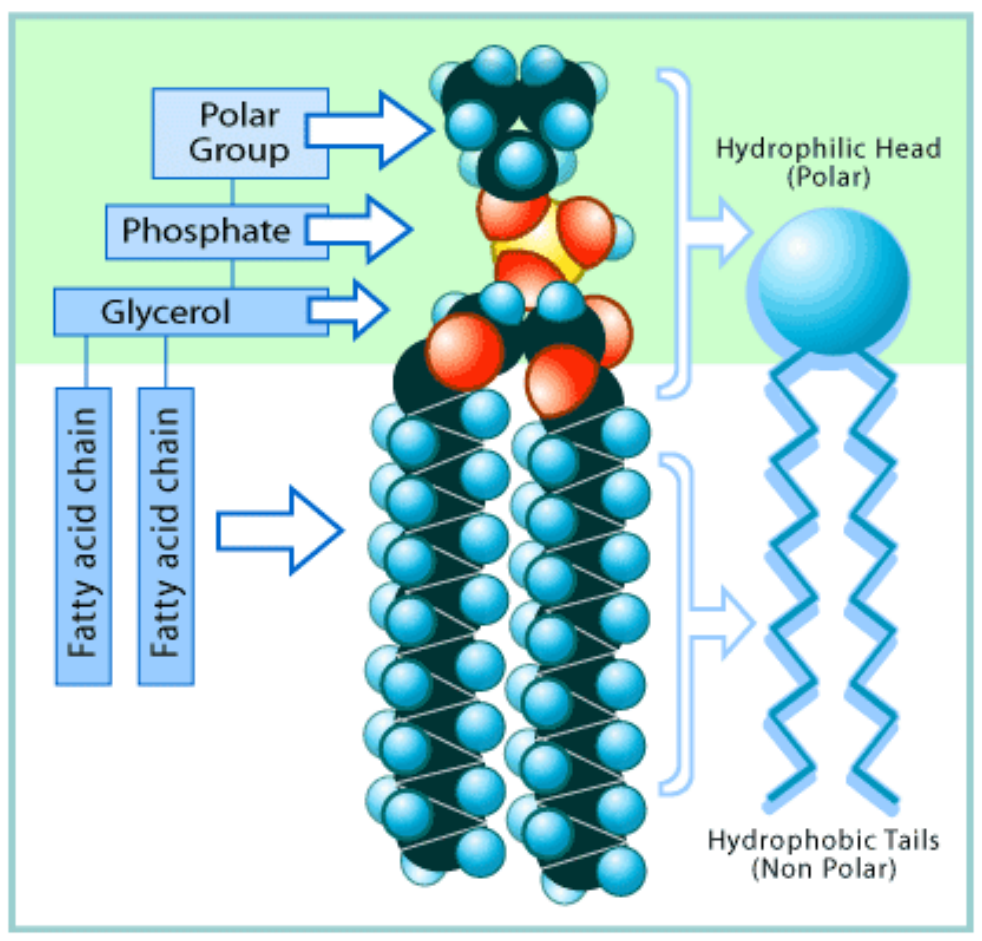


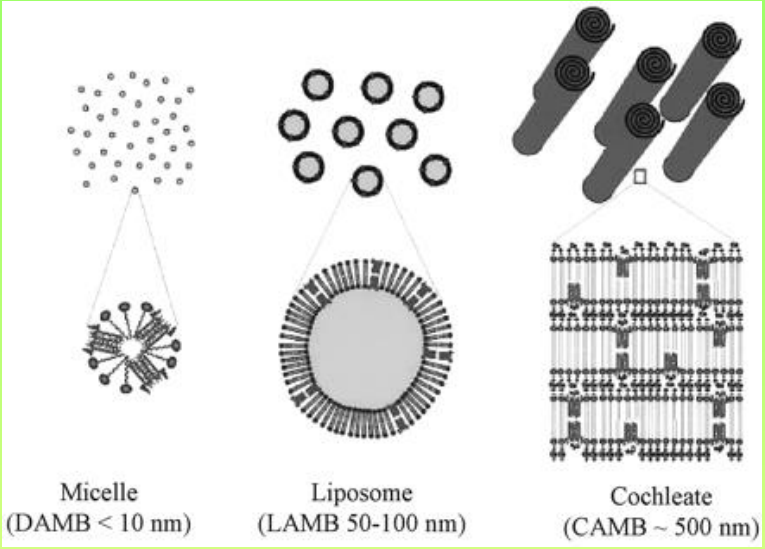


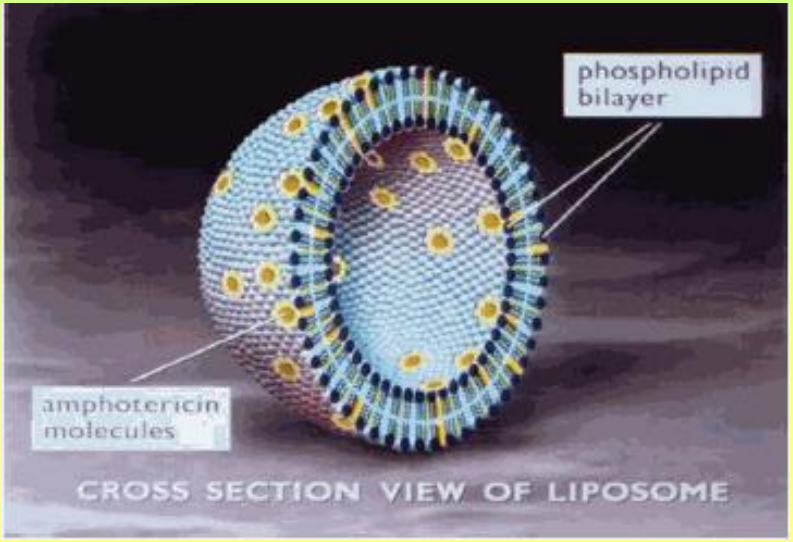
Application fields

- Drug delivery
- Cancer targeting biotherapies
- Immunotherapies targeting cell surface antigens for modulation of immune response
- Immunomodulatory complexes for vaccine development
- Influencing metabolic and endocrine regulations with artificial nano-transporters

Pharmaceutical application of liposome

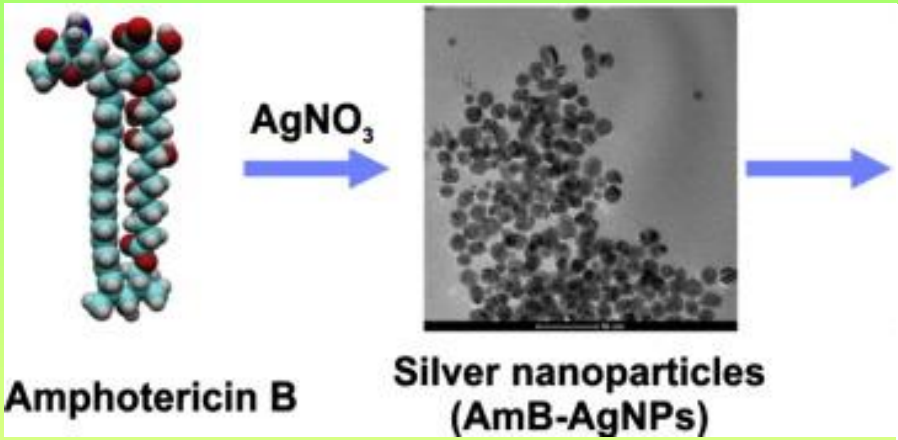




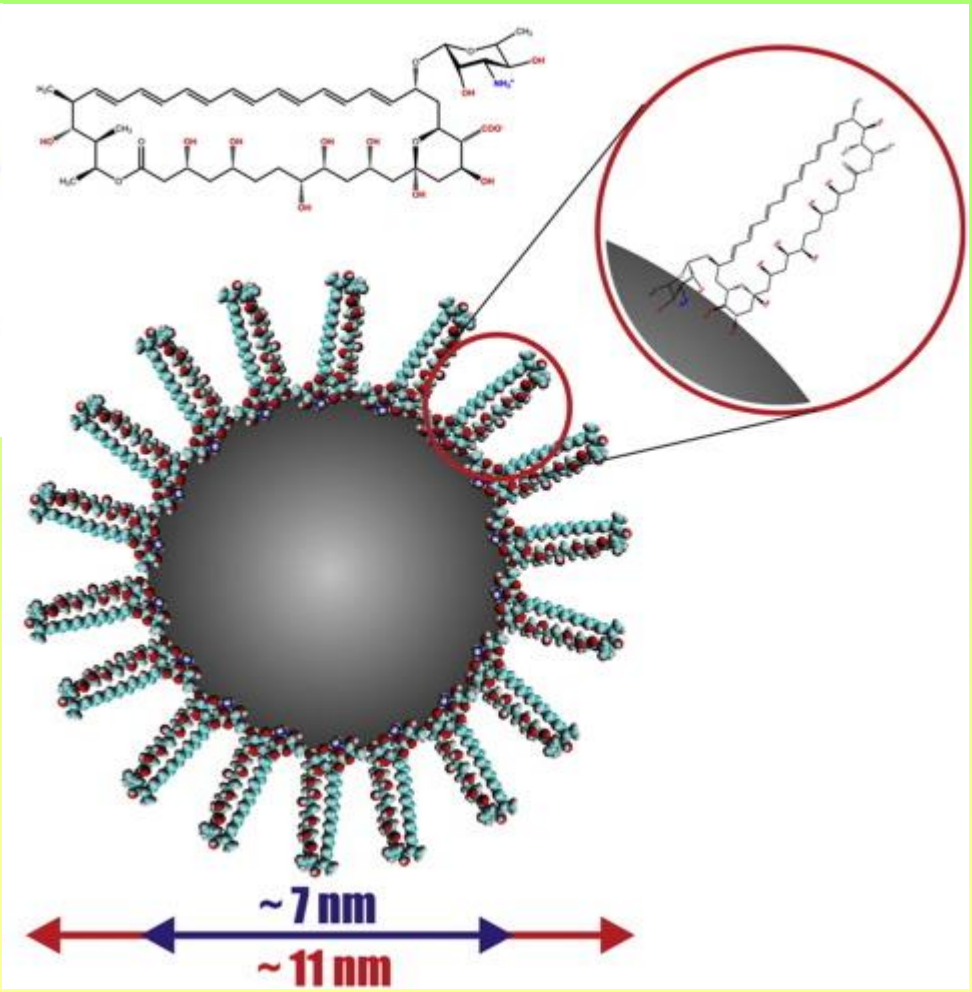


Amphotericin B containing liposome

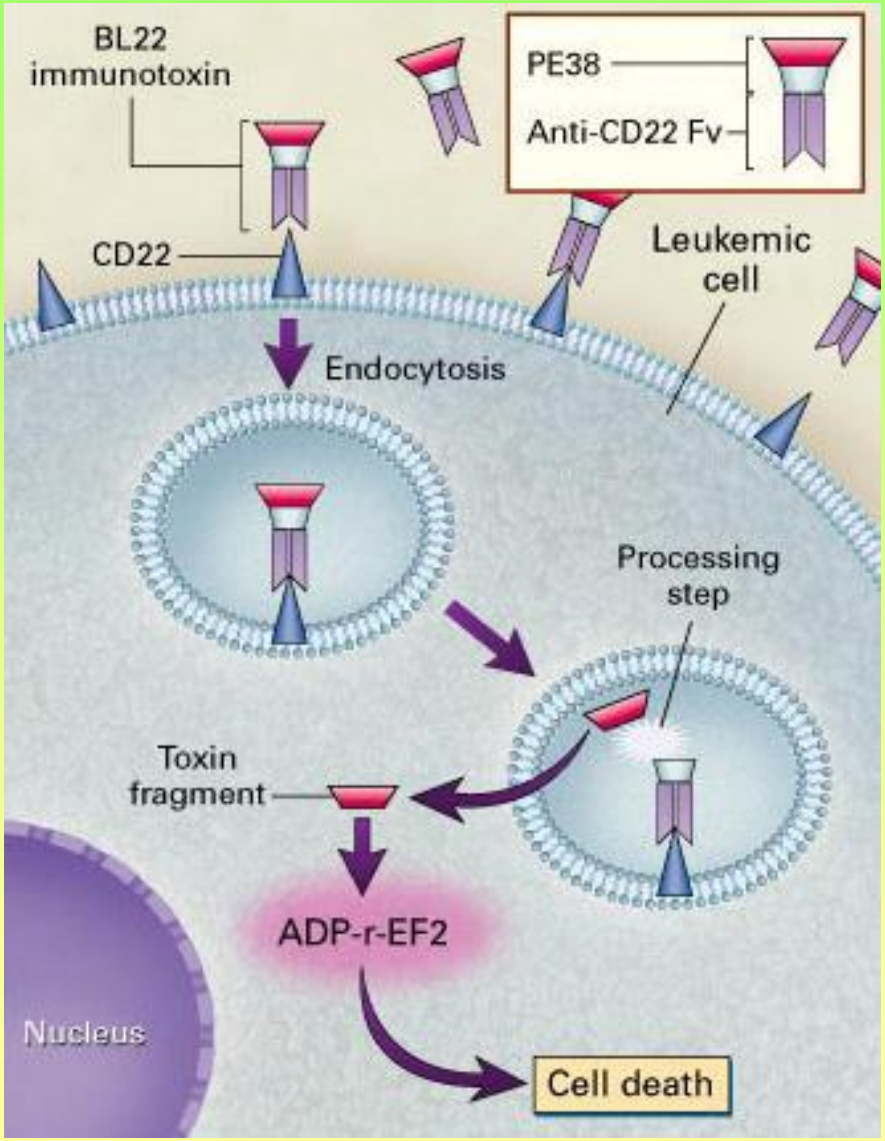
Drug delivery with reduced toxicity and synergism

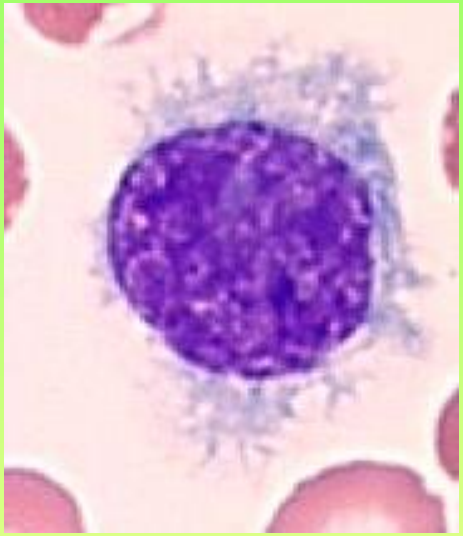


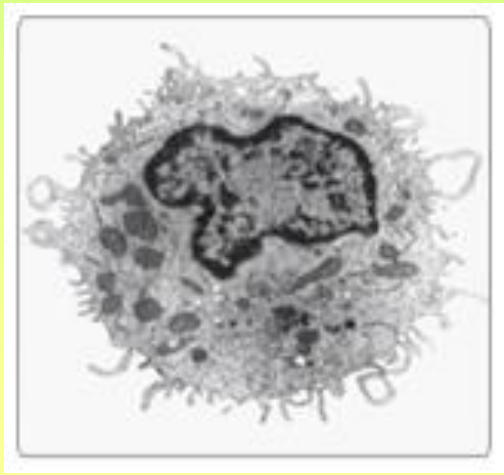
Amphotericin B is a commonly used for the treatment of severe fungal infections. Toxic side effects of the Amphotericin B is high, but a **new approach** in using a combination with **silver nanoparticles** reduces the toxicity and synergistic antifungal effects are between Amphotericin B and silver nanoparticles.



Immunotoxin therapy of „Hairy Cell” leukaemia by BL22

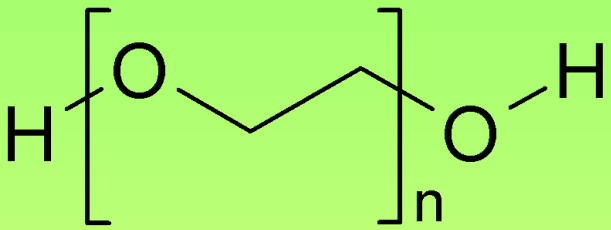




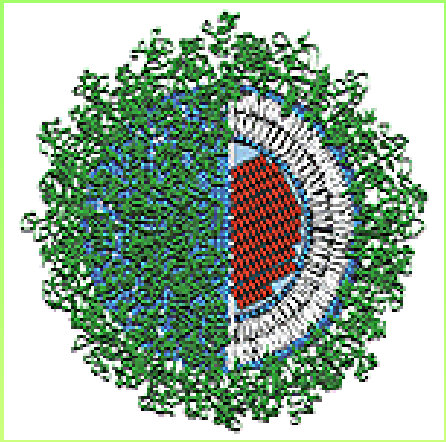


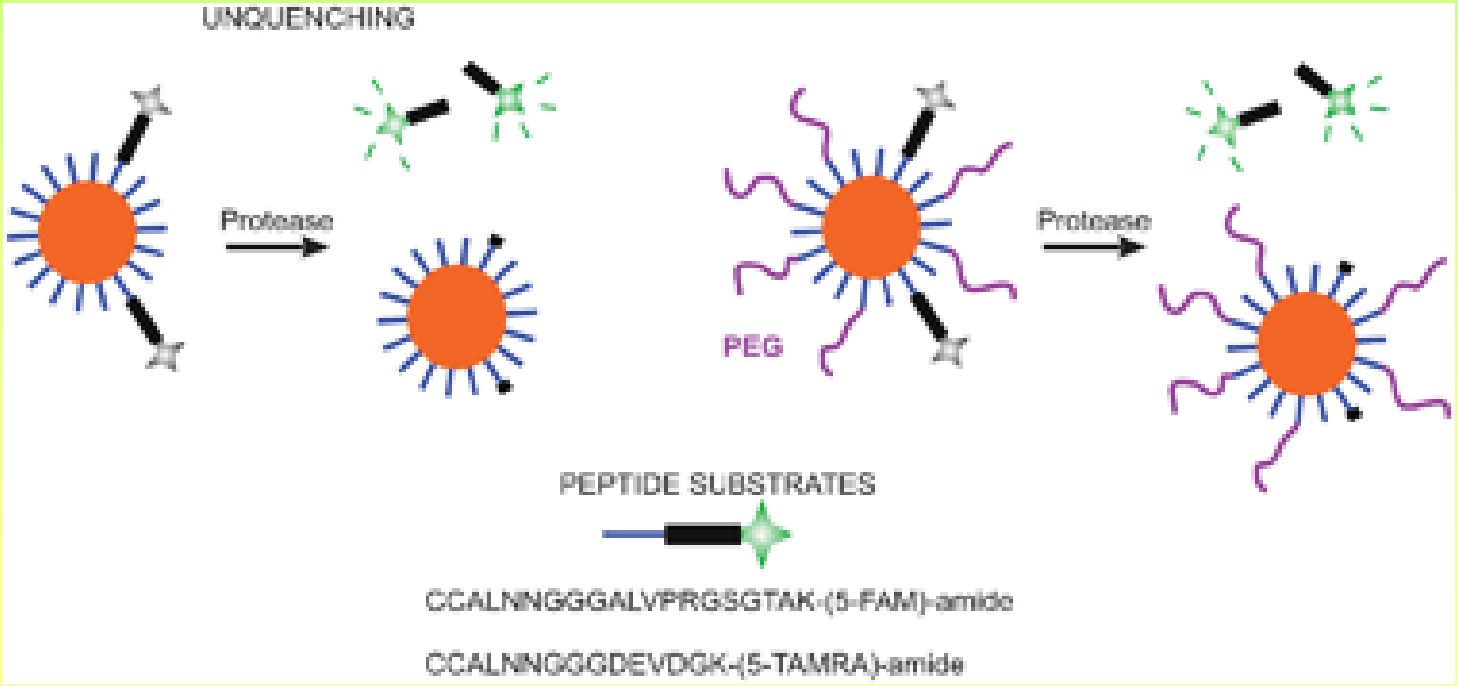
PE38 = Pseudomonas exotoxin

PEGylated liposomes

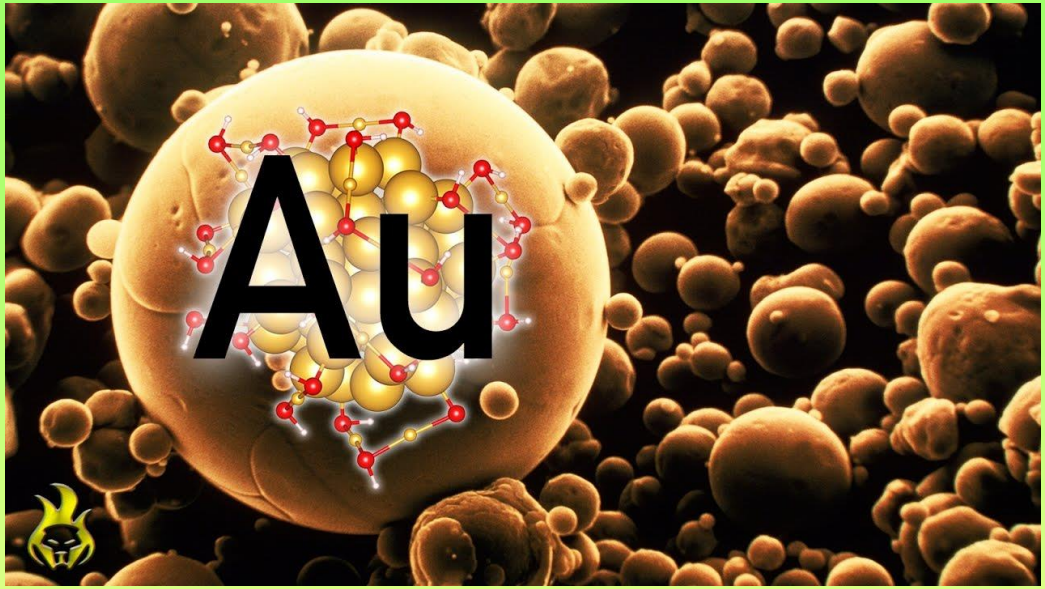


Polyetilenglycol



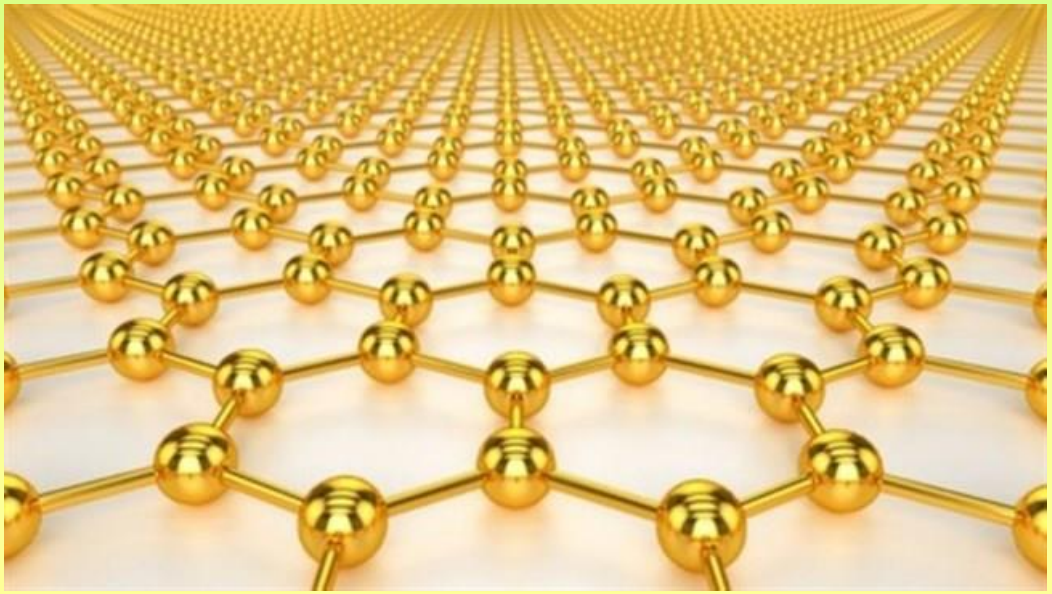


Gold nanoparticles



Colloidal gold is a sol or colloidal suspension in a fluid, usually water. The particles less than 100 nm red or blue/purple color.

Due to their optical, electronic, and molecular-recognition properties, gold nanoparticles are generally used both in research and industrial applications.
The properties of colloidal gold nanoparticles, and thus their applications, depend strongly upon their size and shape



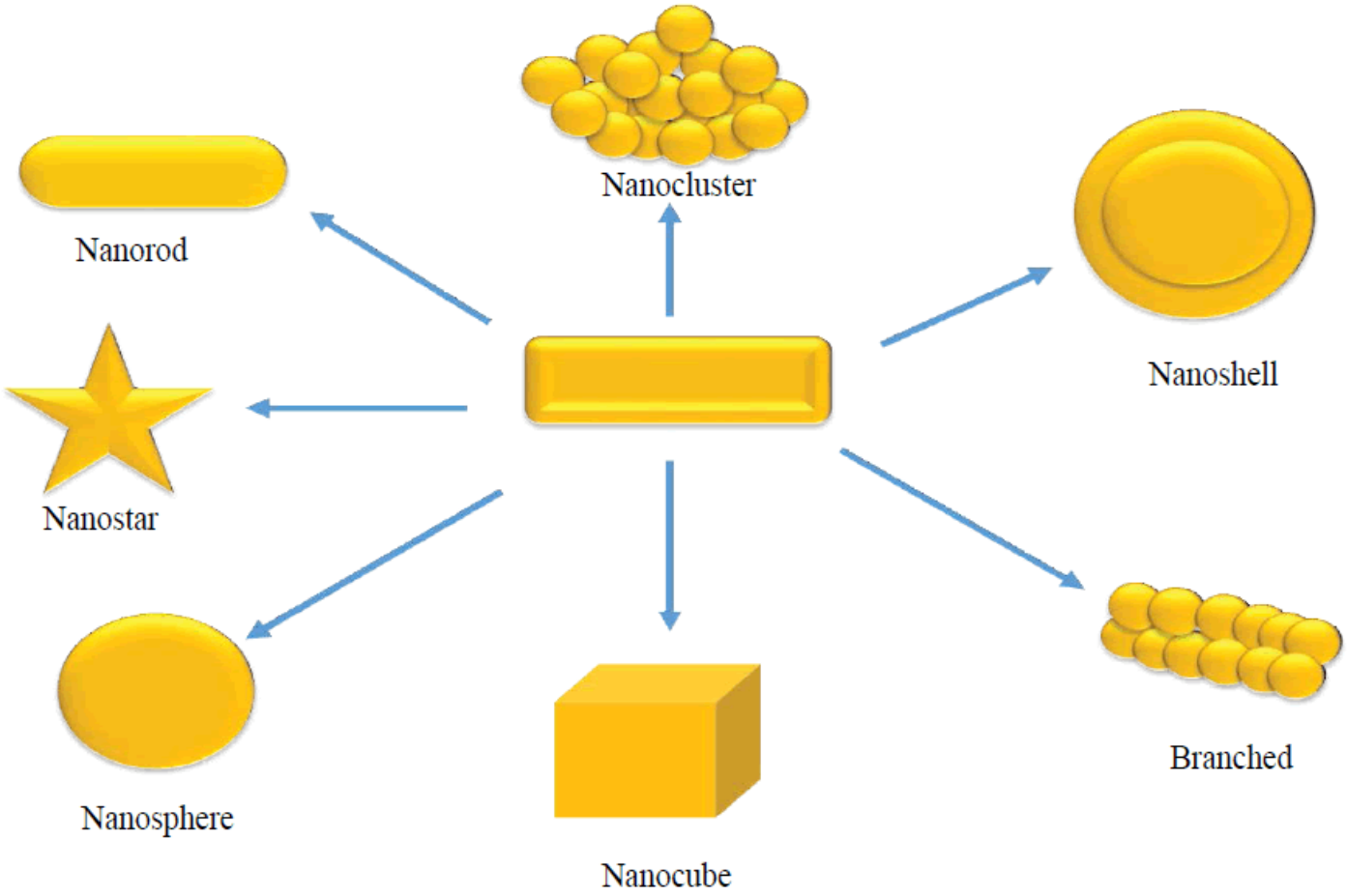
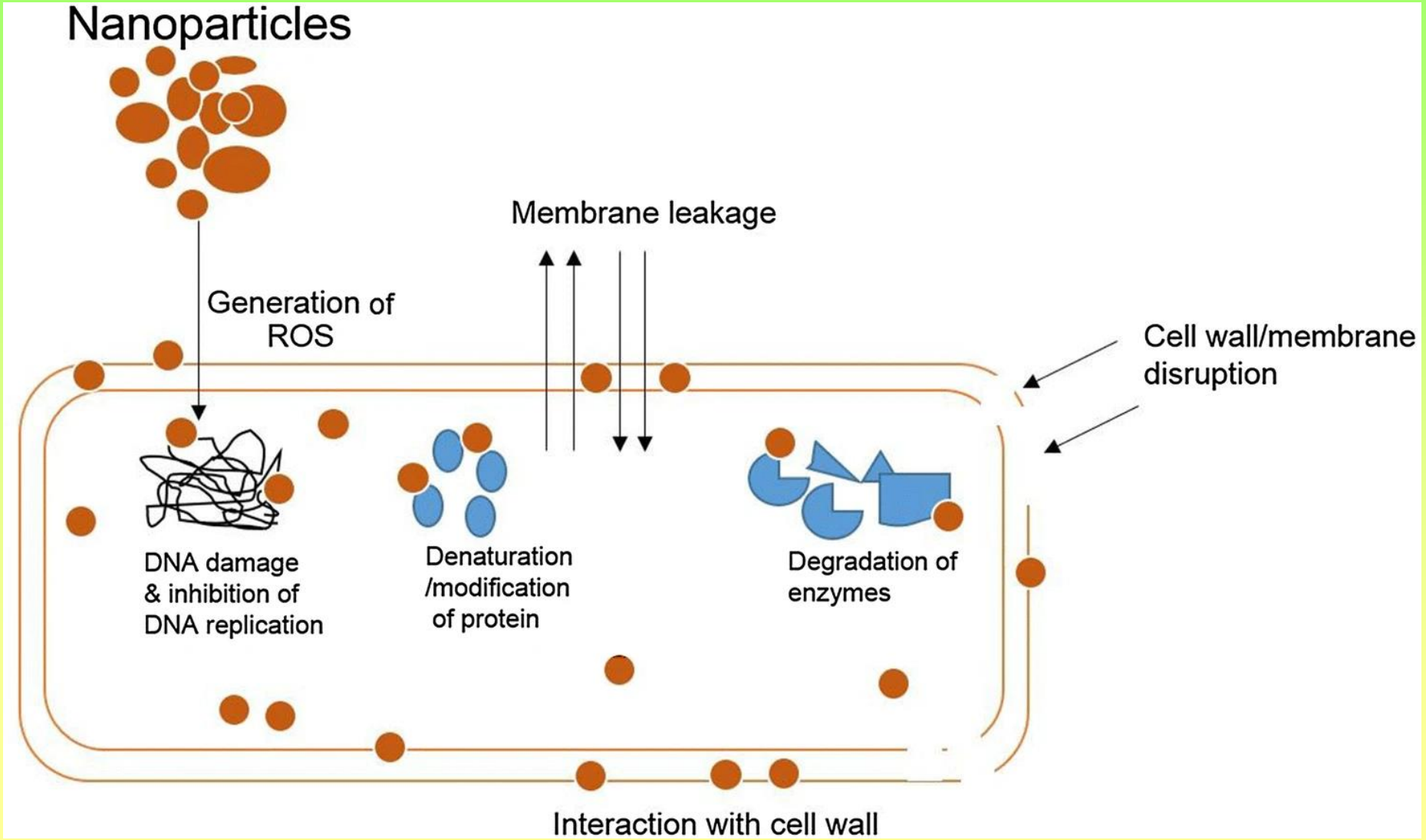
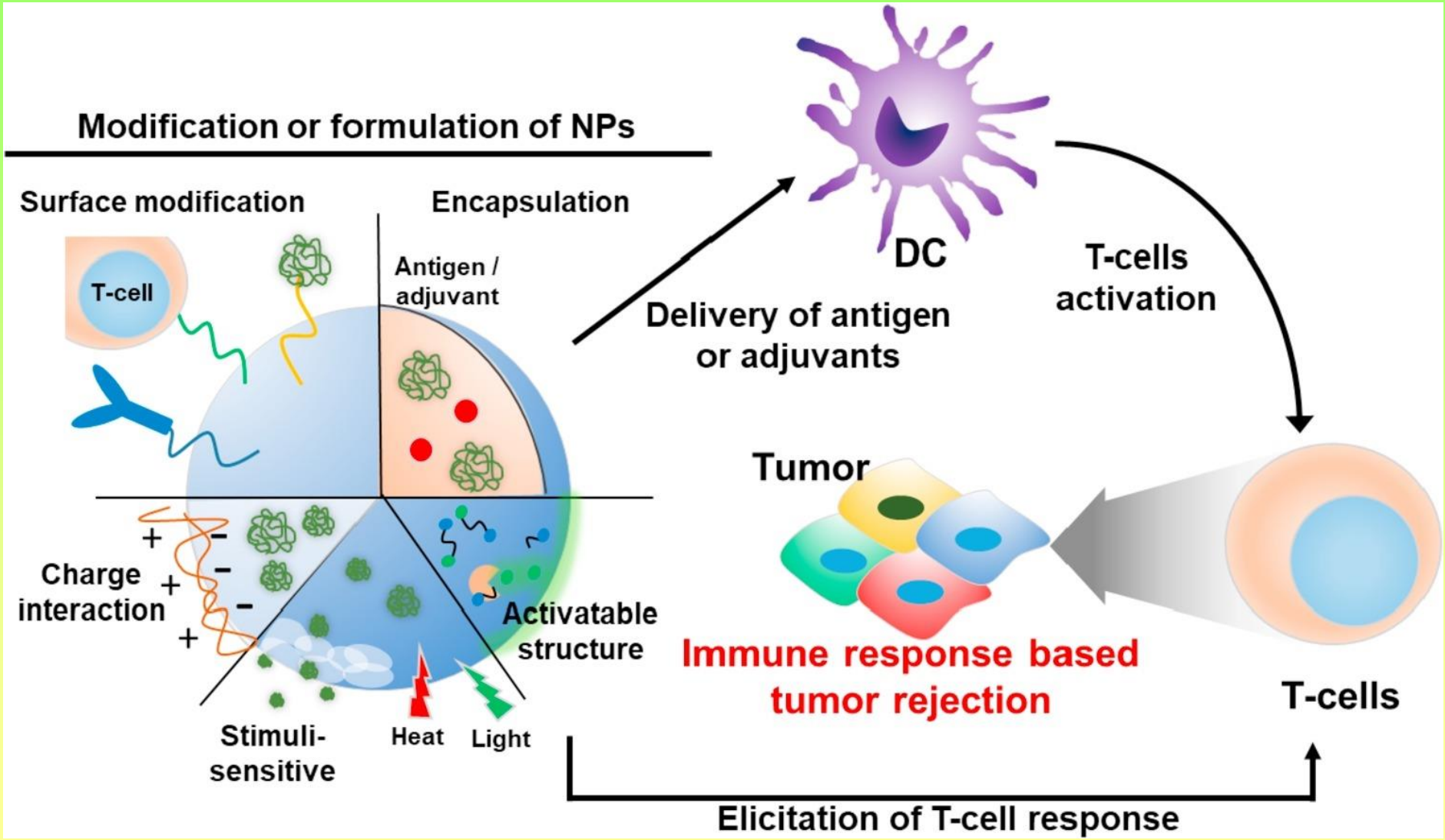


Figure 1: Various shapes of gold nanoparticles.

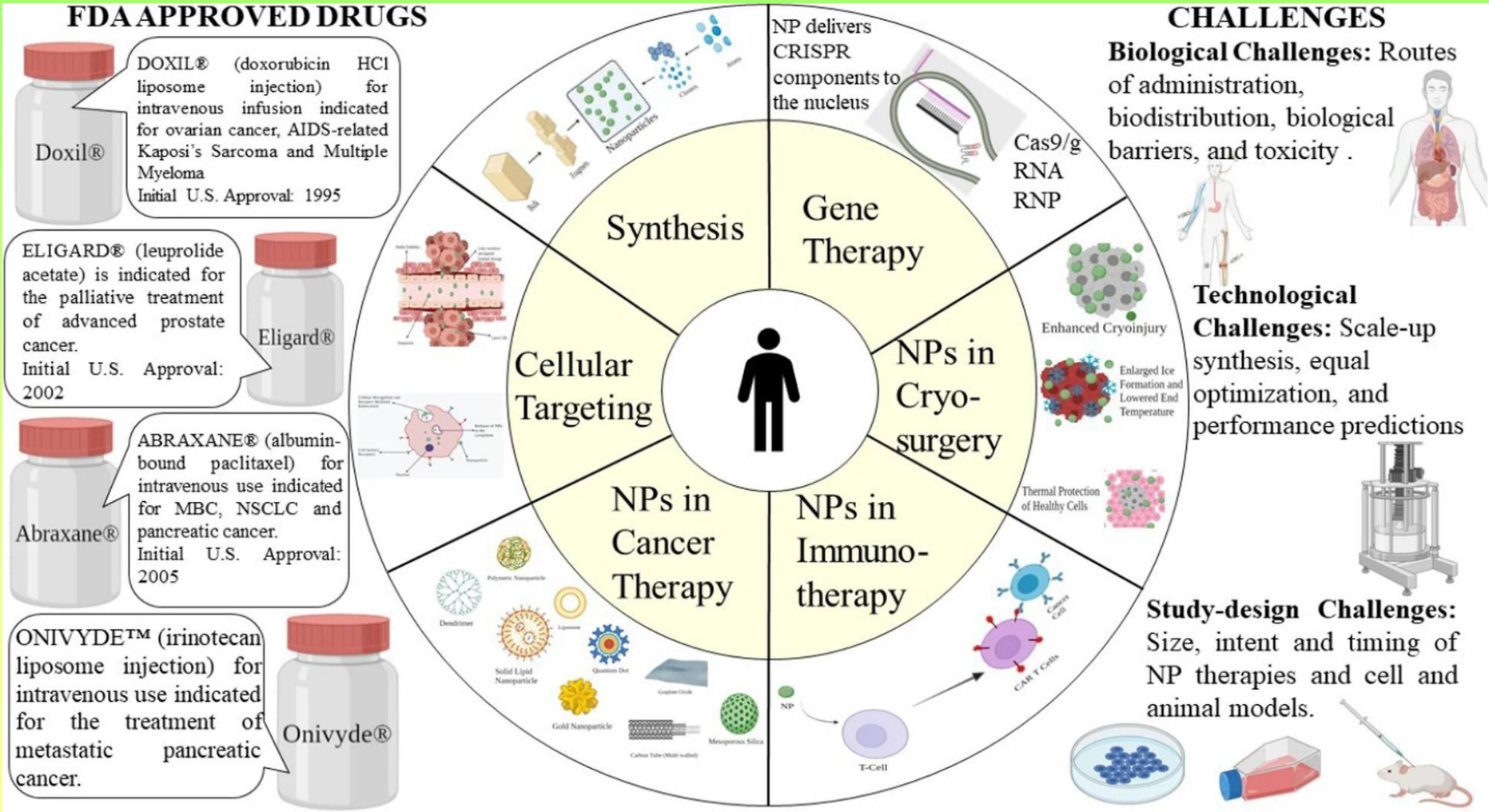
Mode of action of engineered nanoparticles against microorganisms



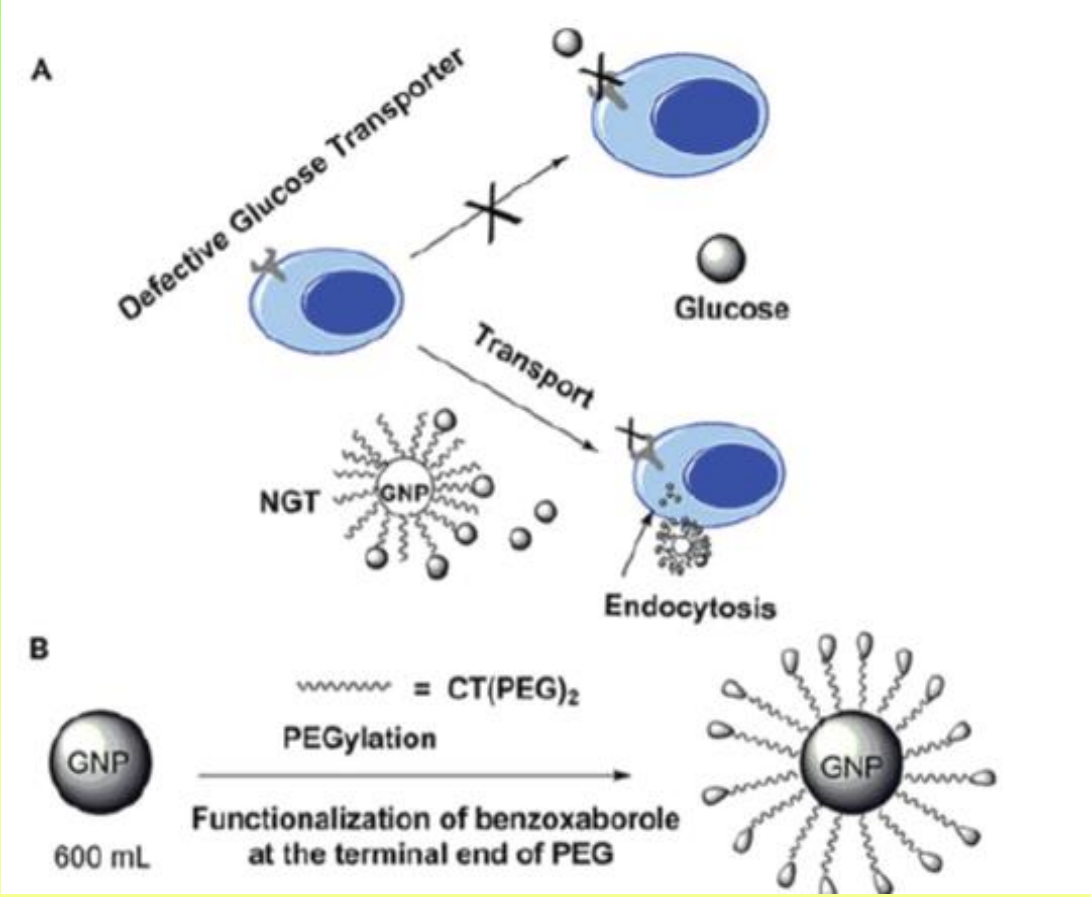
Engineered nanoparticles for cancer therapy



Nanoparticles for cancer therapy

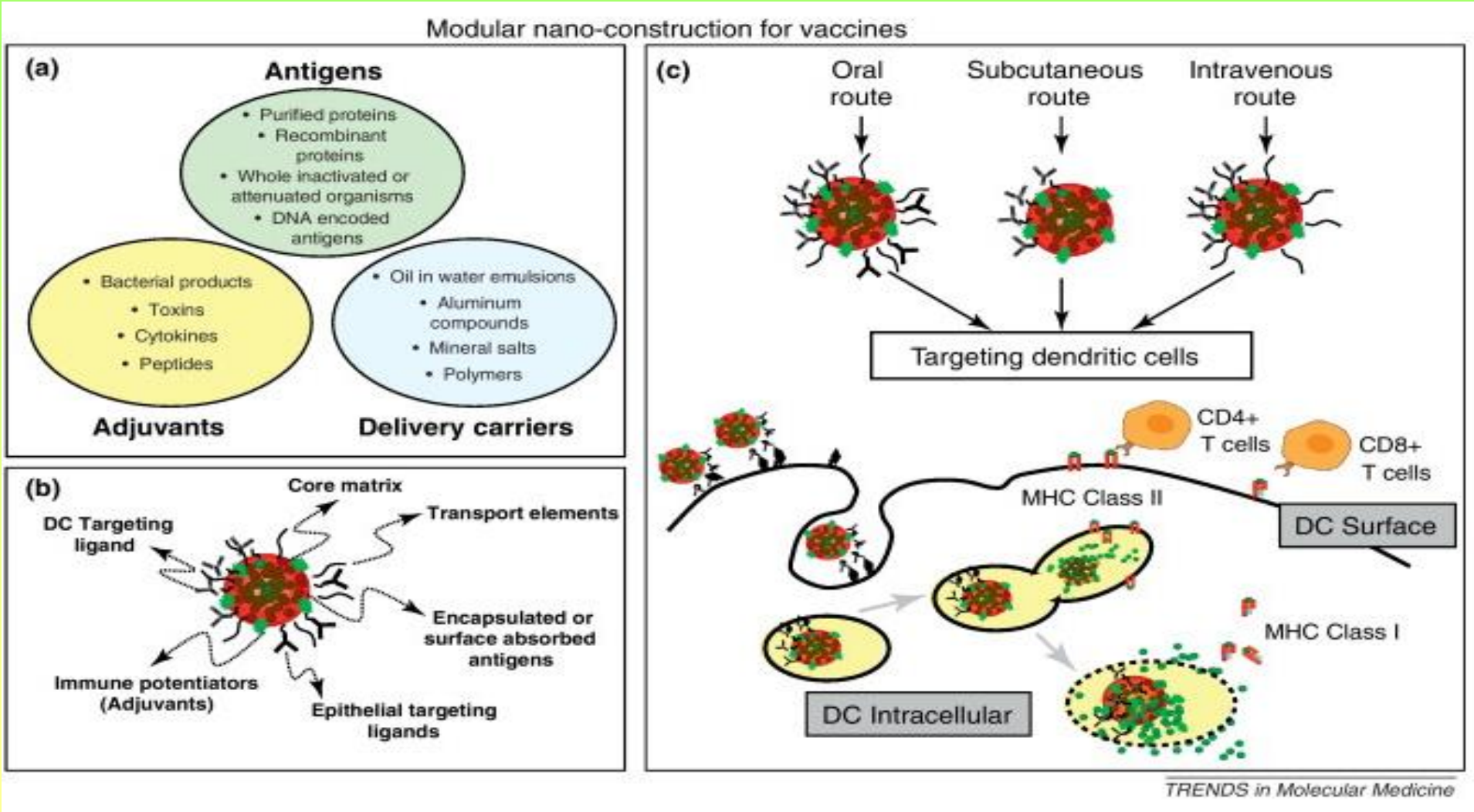


Modulation of glucose transport by engineered bio-mimetic gold nanoparticles



In heredited metabolic or endocrine diseases synthetic bio-mimetic transporters can optimize the transport deficit E.g. in glucose transport. Using GNP for transport management of various metabolites is emerging. This strategy could be extended for future application to the transport problems where extracellular biologic and metabolite burdens could be potential threat to the physiology.

Vaccine development using nanoparticles



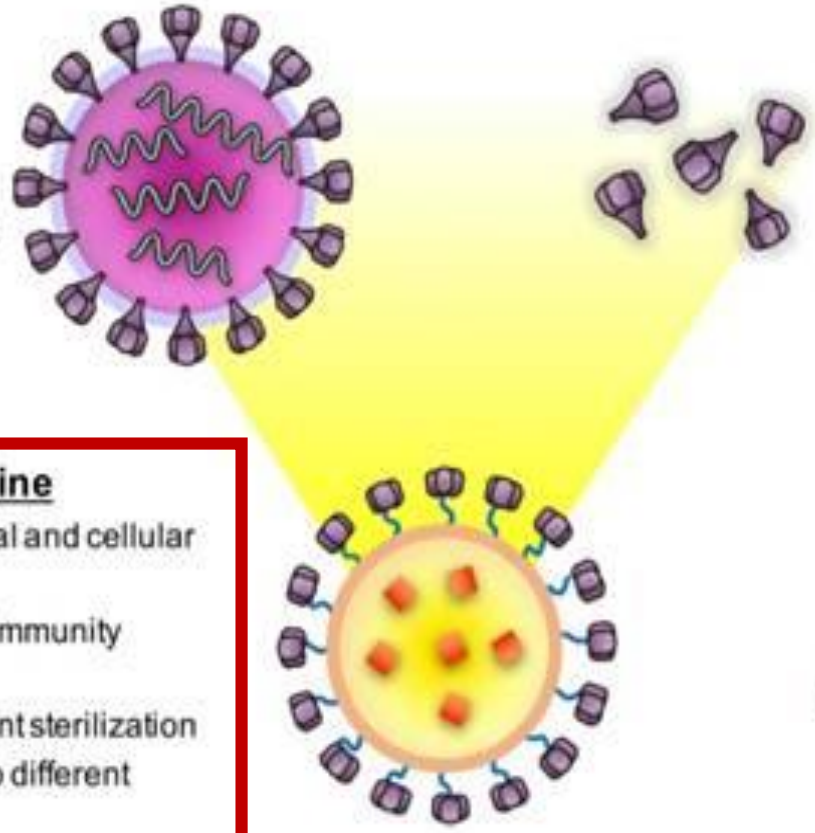
Vaccine development

Viral vaccine

- Elicits strong immune response
- Provides long-lasting immunity
- Susceptible to denaturation
- Susceptible to contamination
- Risk of reversion to pathogenic form

Subunit vaccine

- No risk of infection
- Safe to administer in immunocompromised , pregnant women, and the elderly
- Poor cellular immune responses
- Poor induction of immunological memory



Nanoparticle vaccine

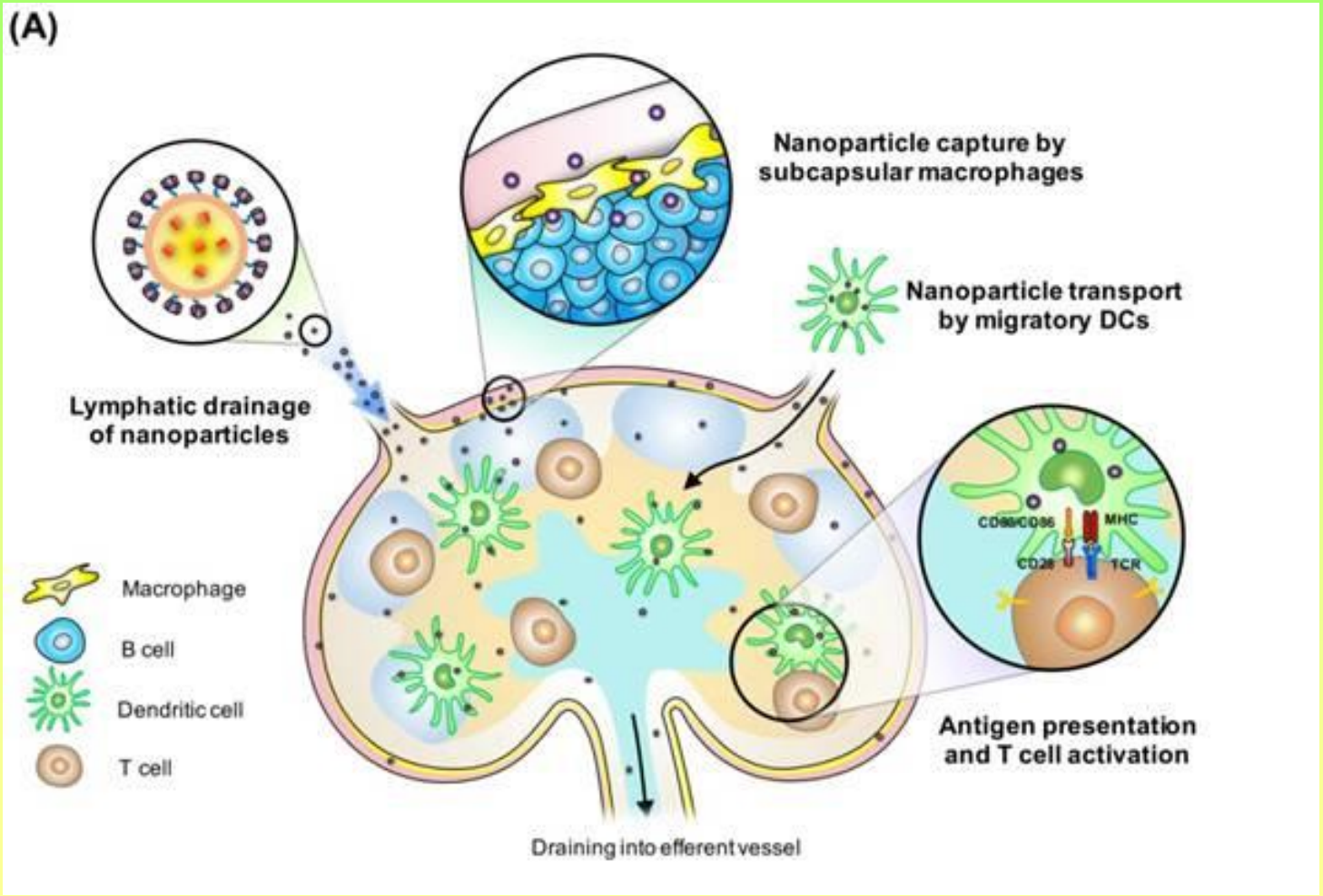
- Elicits strong humoral and cellular immune responses
- Induce long-lasting immunity
- No risk of infection
- Amenable to stringent sterilization
- Readily adaptable to different pathogenic threats

Viral antigen

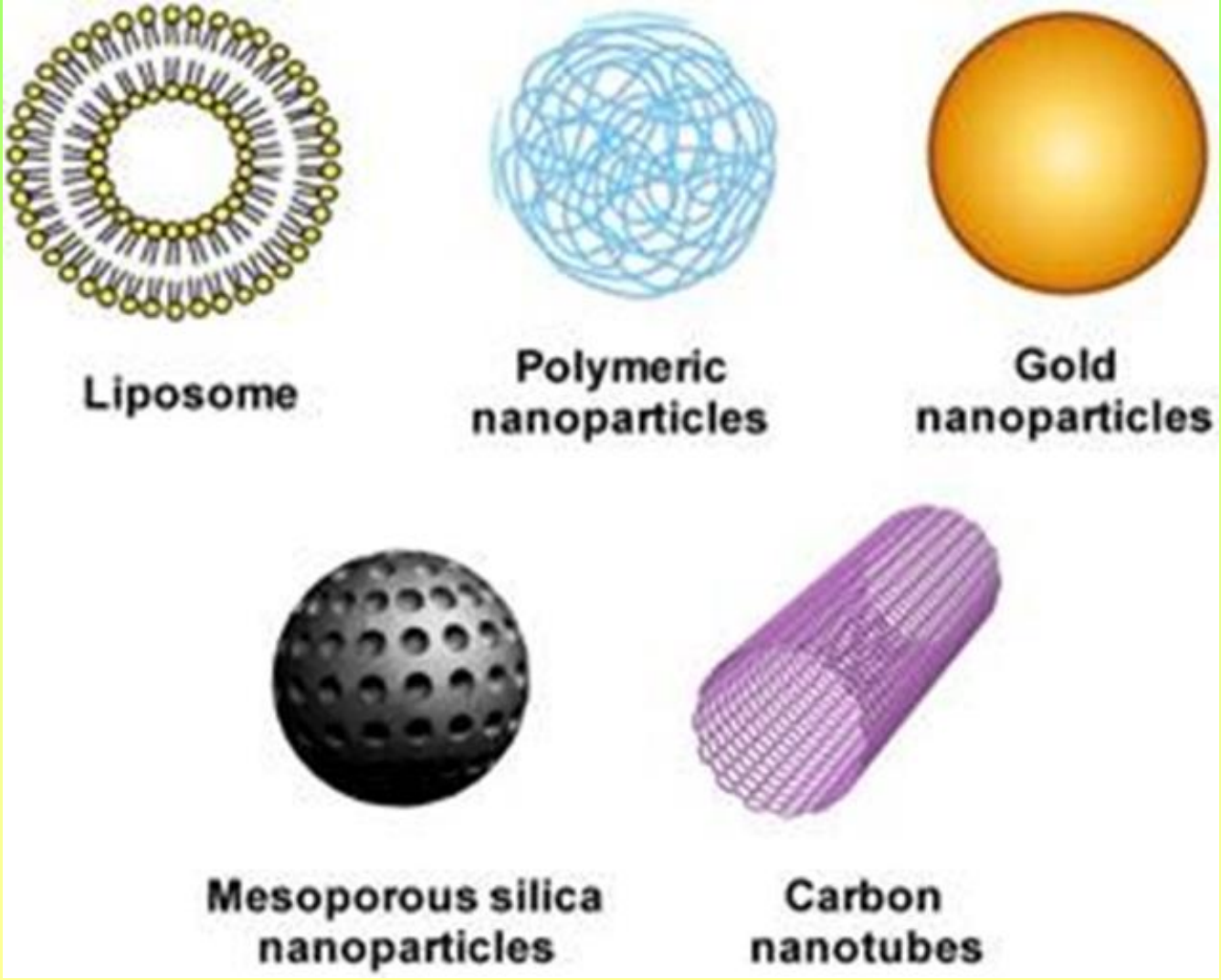
DNA/RNA

Adjuvant

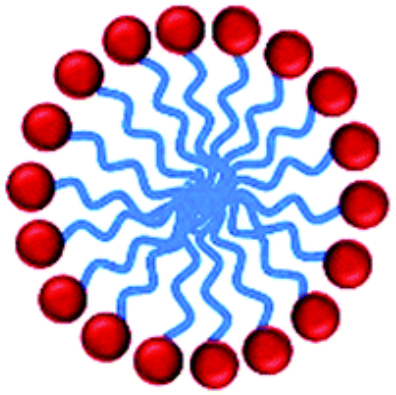
Lymph node delivery by nanoparticle vaccines



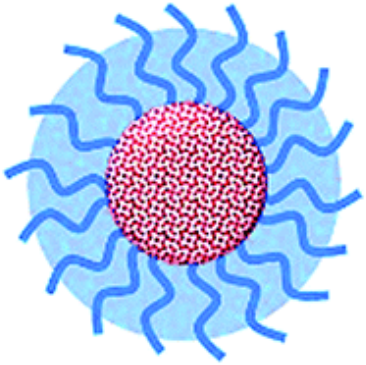
Synthetic nanoparticles for vaccine delivery



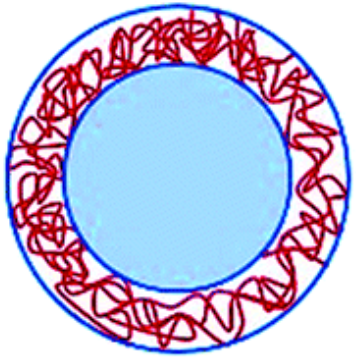
POLYMERIC NANOCARRIERS (PNCs)



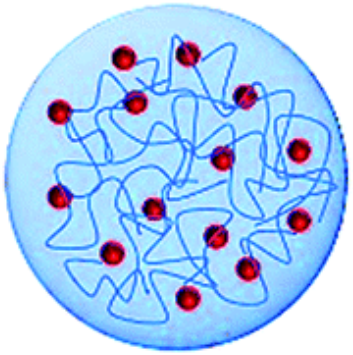
Polymeric Micelles (PMs)



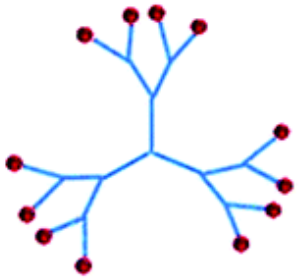
Polymeric Hybrid Nanoparticles (PHNPs)



Polymeric Nanocapsules (PNCPs)



Polymeric Nanogels (PNGs)

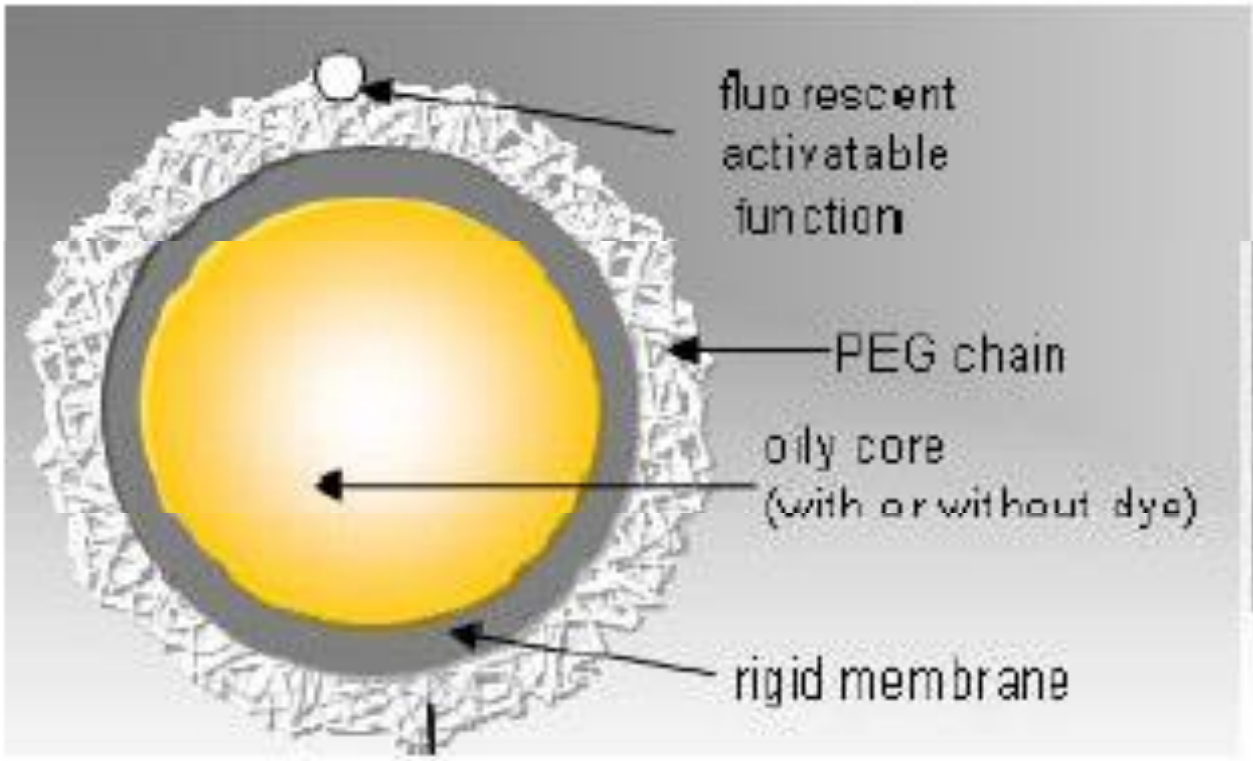


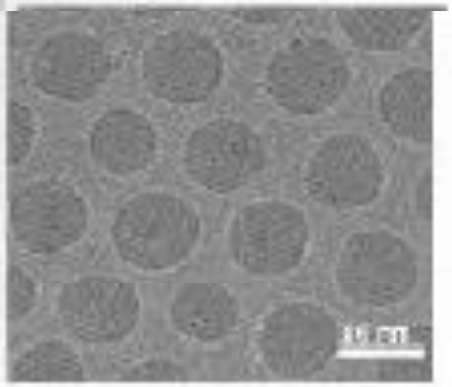
Polymeric Dendrimers (PDDs)

Lymphocarriers

- Antigen specific **immunostimulating constructs** capable of inducing effective antitumoral cellular responses. These nanocarriers are called **IMMUNO-LYMPHOCARRIERS**

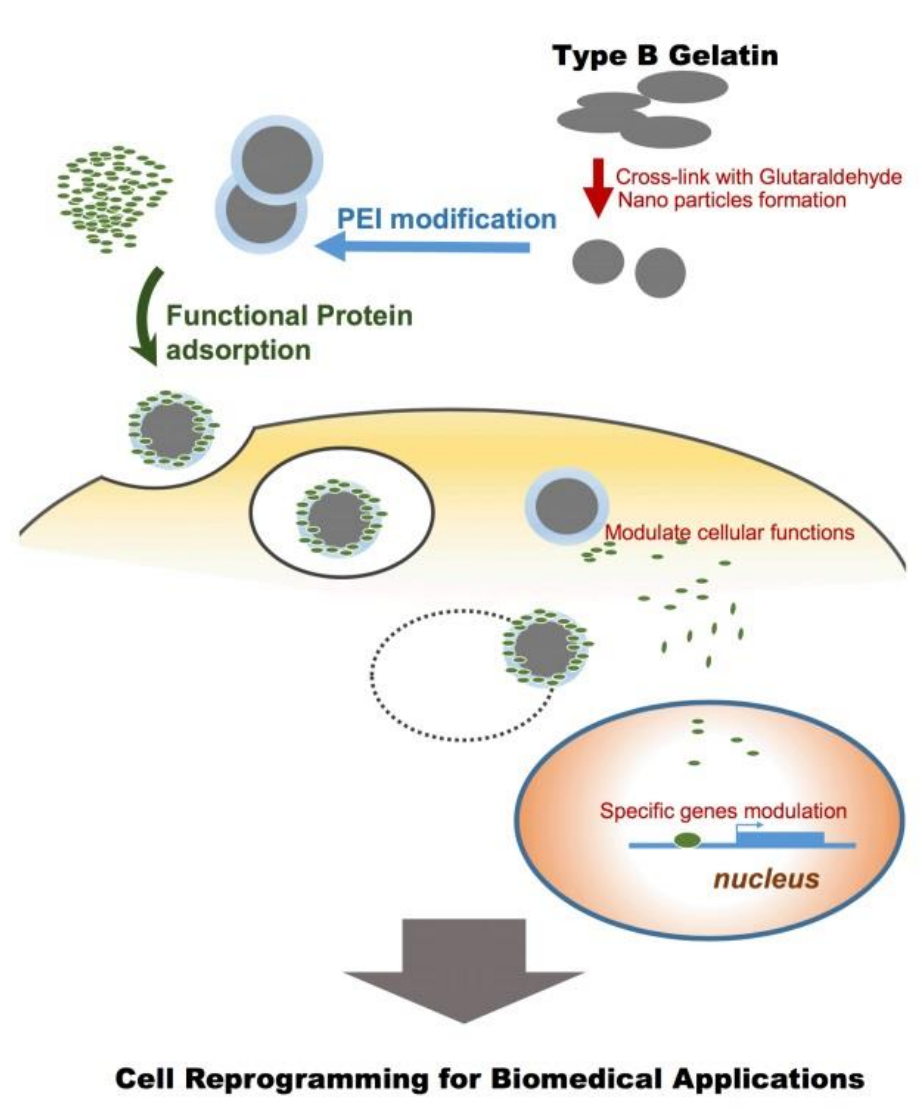
- They are decorated with antibodies against metastatic cell markers (e.g. CXCR4). These cell - targeted nanocarriers are called **TARGETED LYMPHOCARRIERS**





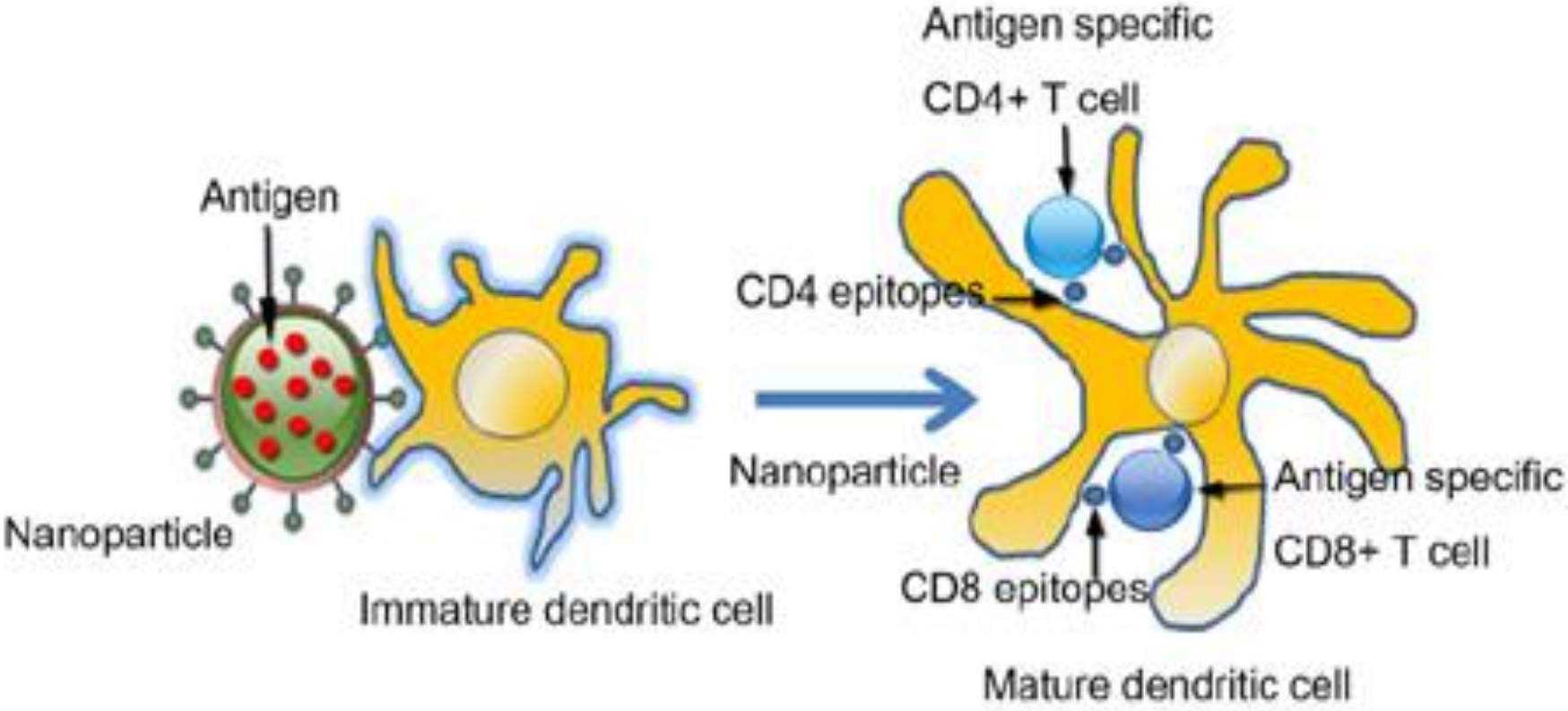
Schematic illustration of a PEG-coated lipid nanocapsule LYMPHOCARRIERS (left).
Transmission electron microscopy image of the lipid nanocapsules after cryo-fracture (right).

Intracellular protein delivery by cationic Polyethyleneimine-Modified Gelatin Nanoparticles



Intracellular protein delivery may provide a safe and non-genome integrated strategy for targeting abnormal or specific cells for applications in **cell reprogramming therapy**. The PEI-modified gelatin particle may provide a **biodegradable** and highly efficient protein delivery system for use in **regenerative medicine and cancer therapy**.

Nanoparticle based dendritic cell maturation as a vaccine carrier for cancer immunotherapy



Examples of PEG-Modified Polypeptides and Their Clinical Applications

Name	Chemistry	Disease Targeted	Clinical Stage	Pharmacokinetics/ Pharmacodynamics		
Pegademase (Adagen)	1st generation adenosine deaminase (AD)	Severe combined immuno-deficiency (SCID)	Approved (Enzon)	1,800X more AD activity than red blood cells		
Pegaspargase (Oncaspar)	1st generation L-asparaginase	Acute lymphocytic leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia	Approved (Enzon)	Half life: unPEG, 20 hours; pegylated, 357 hours		
PegIntron	1st generation IFN-α2b	Hepatitis C	Approved (Schering-Plough)		Intron-A	PegIntron
				Clearance rate	1	1/7
				Half life (h)	1	5
				Dosage/wk	3	1
Pegasys	2nd generation PEG 40KD IFN-α2b	Hepatitis C	Approved (Hoffmann-La Roche)		Intron-A	Pegasys
				Clearance rate	1	1/100
				Half life (h)	9	77
				Dosage/wk	3	1

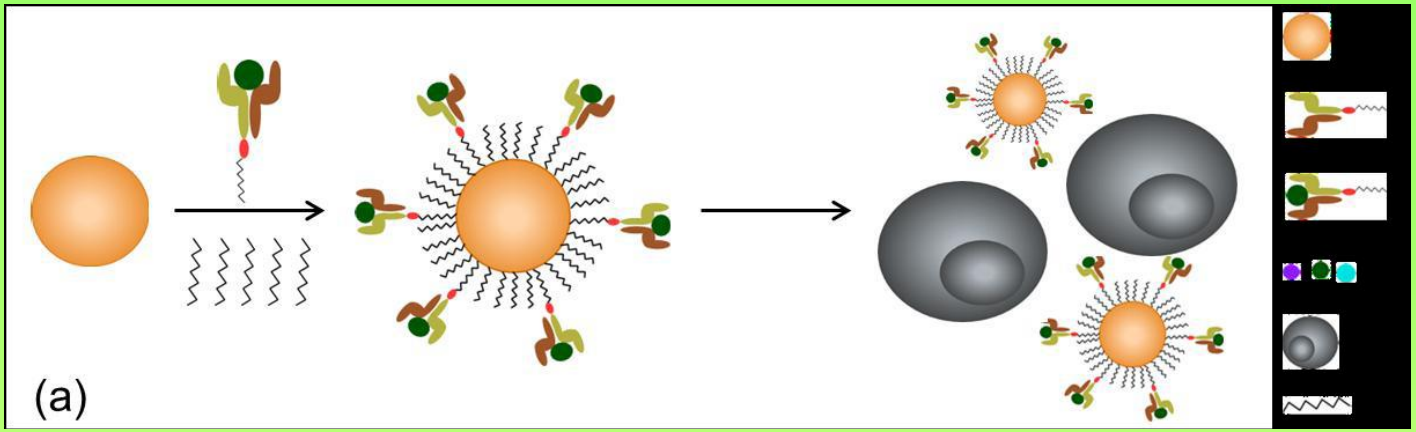
Possible application fields of the nano-immuno-therapy

- Nanoparticles for “combinatorial auto antigen therapy”
- Lymphocarriers
- Lymphotropic nanocarriers for antigen presentation “IMMUNO LYMPHOCARRIERS”

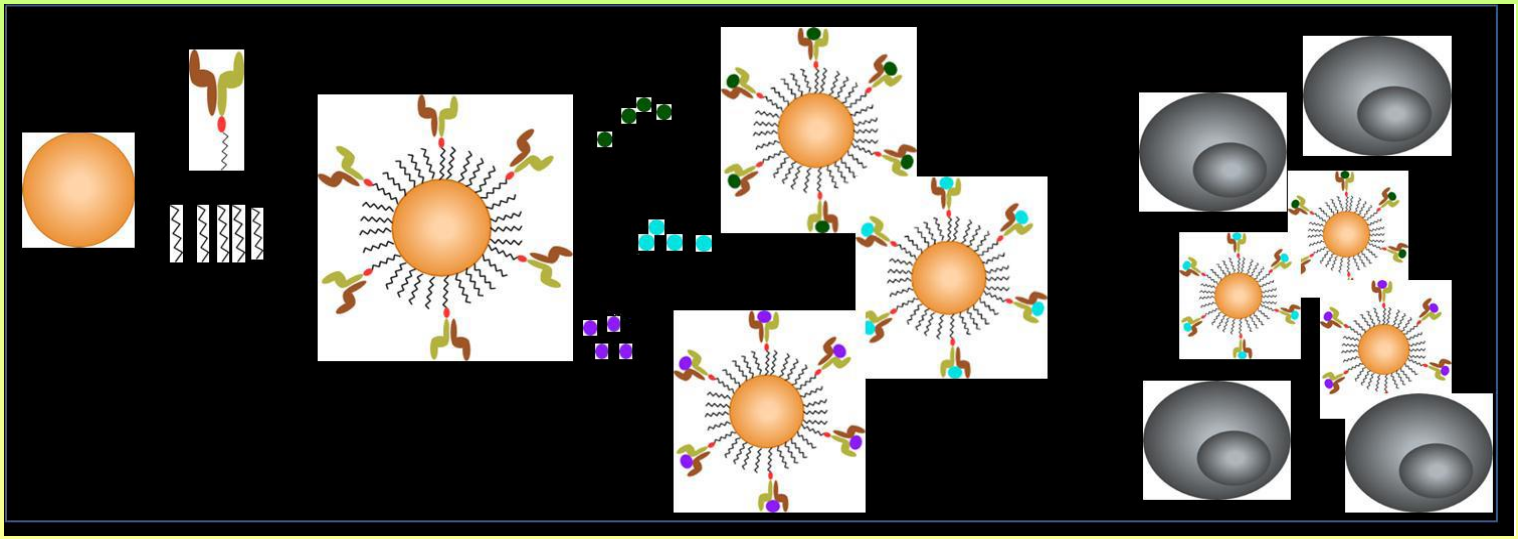
Nanoparticles for “combinatorial auto antigen therapy”

Aim and theoretical background:

- Complex molecular system that will induce **antigen-specific tolerance** to well established T-cell epitopes
- It is enable the **selective suppression** of certain parts of the immune system that are specifically associated with the targeted antigen
- The goal is be achieved through the **development of a library of Self MHC class II/peptide presenting biocompatible nanoparticles**, and their use for the **manipulation of CD4+ autoreactive T-cells** to prevent or suppress the tissue destruction.



(a) Gold Nanoparticles are functionalized with PEG + MHC-peptide complexes. The functional NPs are designed to interact with T-Cells.



(b) NPs are functionalized with PEG+MHC complex, free of peptides. Various peptides are then loaded on the MHC NPs. The functional NPs are designed to interact with T-Cells

Method

- Clone and express MHC class II covalently linked to the peptide associated with the autoimmune disease.
- The antigenic peptide is cloned into the MHC class II molecule, and the antigenic peptide is introduced to the N-terminus of one of the extracellular domains of the recombinant MHC class II through a flexible linker.
- A thiolated polyethylene glycol (**PEG**) monolayer is introduced to the C-terminus.

Examples for practical application of combinatorial auto-antigen therapy

- Biotherapies developed for autoimmune diseases (such as T1D) has been to target autoreactive T-cells and to **generate specific immune tolerance** while keeping the ability to respond to exogenous antigens.

- Strategies such as the injection of high doses of soluble peptides, NPs for the **manipulation of CD8+ T-cells**, and soluble MHC tetramers, have already been shown to be effective in animal models. Clinical trials are established.

Gold nanoparticle as a suitable technical opportunity for lyphocarriers

- **Gold NPs** are a common tool in biology, chemistry, engineering and medicine.
- Gold NPs can be synthesized reproducibly and chemically modified with a variety of functional groups.
- Their physical properties, including unique optical properties, robustness, and high surface area, make them a highly attractive platform for numerous biological applications.
- In analogy to proteins, NPs can be used as a multivalent receptor to enhance low-affinity interactions. Their biocompatibility is well demonstrated in several experiments.

LYMPHOCARRIERS

- Prototypes of lymphotropic nanocarriers consisting of **lipid nanocapsules** prepared according to mild and easily scalable techniques. (For example, the **Phase-Inversion temperature emulsification technique** produce **20 nm size**, and the self-emulsification of lipid mixtures for the formation of nanocarriers with a suitable size for lymphatic targeting - i.e. particle sizes **below 100 nm** and very narrow particle size distributions – can be produce).
- These two novel lipid nanocarriers that mimic lipoproteins are useful for preparation of **nanocapsules** selected from non-toxic and biocompatible lipids, polymers (e.g. polyaminoacids and polyesters), polymer-lipid conjugates (e.g. **PEGylated lipids**).
- **Polymer** (chitosan-PEG or PEG) **coated lipid carriers** made by self-emulsification or made by the phase-inversion temperature technique.
- Lymphocarriers are loaded with docetaxel as a model of **anticancer drugs**.

Lymphotropic nanocarriers for antigen presentation "IMMUNO-LYMPHOCARRIERS"

- LYMPHOCARRIERS coated with **MHC-Ig fusion proteins and loaded with relevant antigenic peptides and with a costimulatory signal B7.1-Ig**, ultimately leading to the formation of IMMUNO LYMPHOCARRIERS.
- The bioconjugation of the antigens/danger signals onto the particle surface can test by ELISA and immunofluorescence
- Immunization in **artificial antigen presenting carriers** have already shown **antitumoral activity**.

High effectiveness = high advantage and high risk

None-degradable materials: risk for the environment

Green Chemistry

- Green chemistry is an area of chemistry and chemical engineering focused on the designing of products and processes that **minimize the use and generation of hazardous substances** (E.g. nanoparticles).
- The overarching goals of green chemistry — namely, **more resource-efficient and inherently safer design** of molecules, materials, products, and processes — can be pursued in a wide range of contexts.
- **Introduction of Green Chemistry Regulations is essential to the nanotechnology!**

Green chemistry principles

Green Chemistry Prinicples	Designing Greener Nanomaterial and Nanomaterial production methods	Practicing Green Nanoscience
• Prevent waste (P1) • Tom economy (P2) • Less hazardous chemical synthesis (P3) • Desinging safer chemicals (P4) • Safer solvents or reaction media (P5) • Design for energy efficiency (P6) • Renewable feedstocks (P7) • Reduce derivatives (P8) • Catalysis (P9) • Design for degradation (P10) • Real time monitoring and proces control (P11) • Inherently safer chemistry (P12)	Design of safer nanomaterials (P4, P12) Design for reduced environmental impact (P7, P10) Design for waste reduction (P1, P5, P8) Design fir process safety (P3, P5, P7, P12) Design for nanomaterials efficiency (P2, P5, P9, P11) Design for energy efficiency (P6, P9, P11)	Determine the biological impact of nanoparticle size, surface area, surface functionality; utilize this knowlledge to design effective safer materials that possess desired physical properties; avoid incorporation of toxic elements in nano[article composition. Study nanomaterilas degradation and fate in the environment; design material to degrade to harmless subunits or products. An approach involves avoiding the use of hazardous elements in nanoparticle formulation; the use of hazardless, bio-based, nanoparticle feed tocks may be a key. Elimate solvent-intensive purification by utilizing selective nano syntheses-resulting in grater purity and monodispersity; develop new purification methods, e.g. Nanofiltration, that minimize solvent use; tilize bottom up approaches to enhance materials efficiency and elements steps. Design and develop advance synthesis that utlize more benign reagents and solvents tha used in discovery preparations; utlize mor benign feed-stocks, derived from renewable sources, if possible, identify repalcements for highly toxic and pyrophoric reagents. Design new, compact synthetic strategies; optimize incorporation raw material in products through bottom up approaches, use alternative reaction media and catalysis to enhance reaction selectivity; develop real time monitoring to guide process control in complex nanoparticle syntheses. Pressure efficient synthetic pathways that can be carried out at ambient temperature rather than elevated tremperature; use of non-covalent ad bottom up assembly method near ambient temperature, utilize real time monitoring to optimize reaction chemistry and minimize energy costs.

Nano hazard symbols