

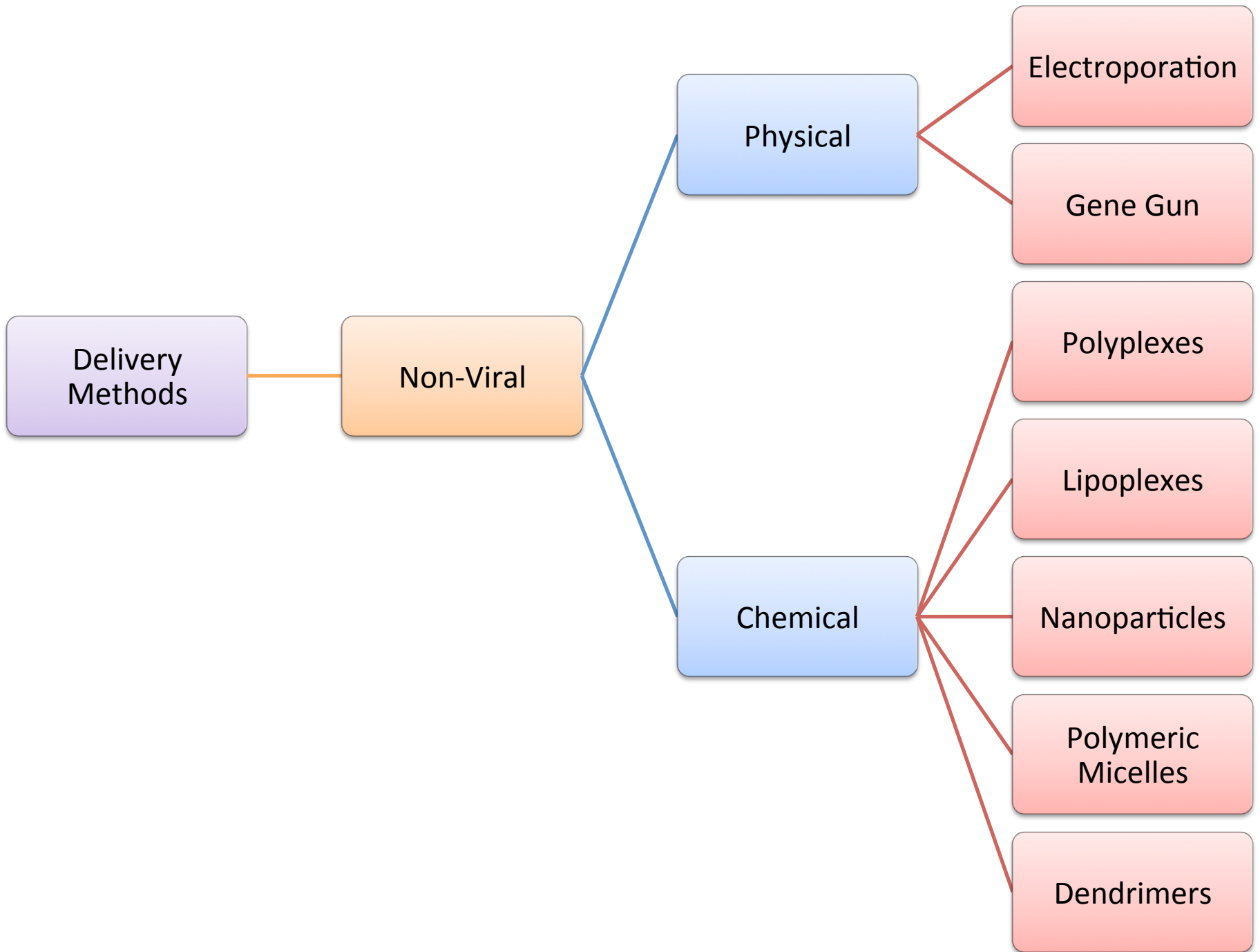
Methods of Gene Transfer (II)

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Objectives of this lecture

By the end of this lecture you will be able to:

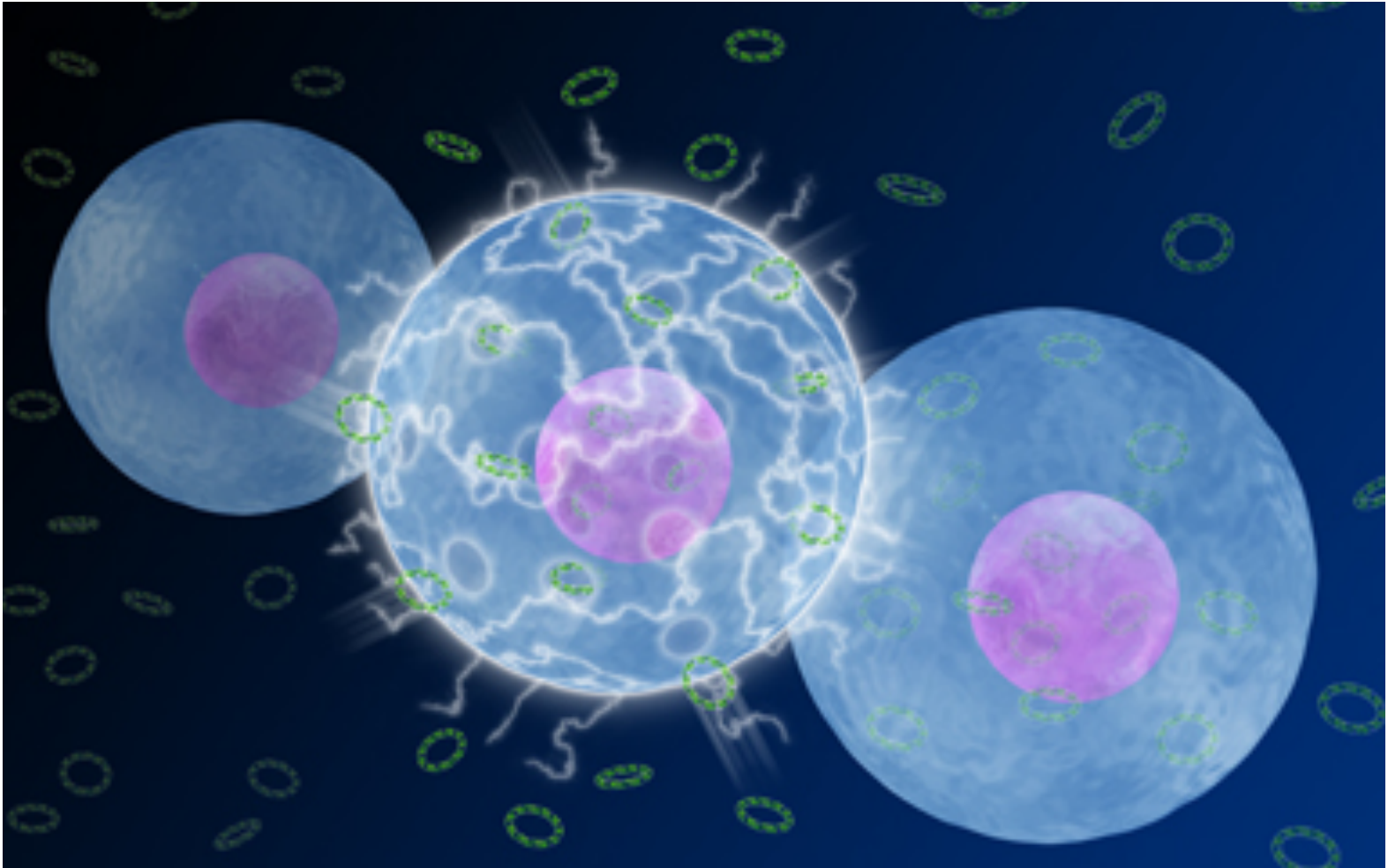
1. Differentiate between physical and chemical methods for gene transfer
2. Compare between several nanocarriers used for gene delivery
3. Appreciate the potential of non-viral method as a therapeutic strategy for gene therapy



Electroporation

- Momentary exposure of cells suspended in DNA solution to a high electrical field
- **Advantages:**
 - High transfection efficiency
- **Disadvantages:**
 - Damage of a significant number of cells

Electroporation

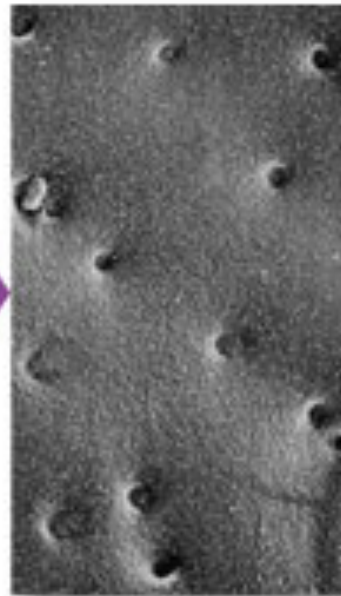


Electroporation

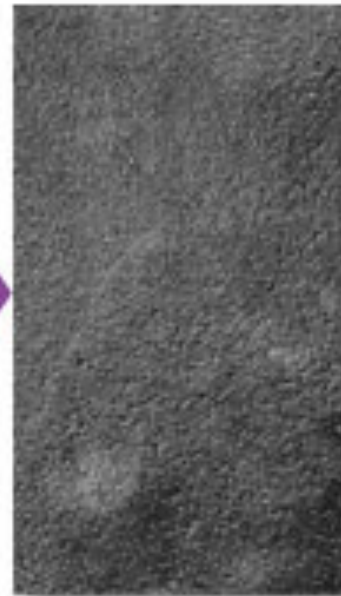
The phenomenon of electroporation



Cell membrane
before pulsing

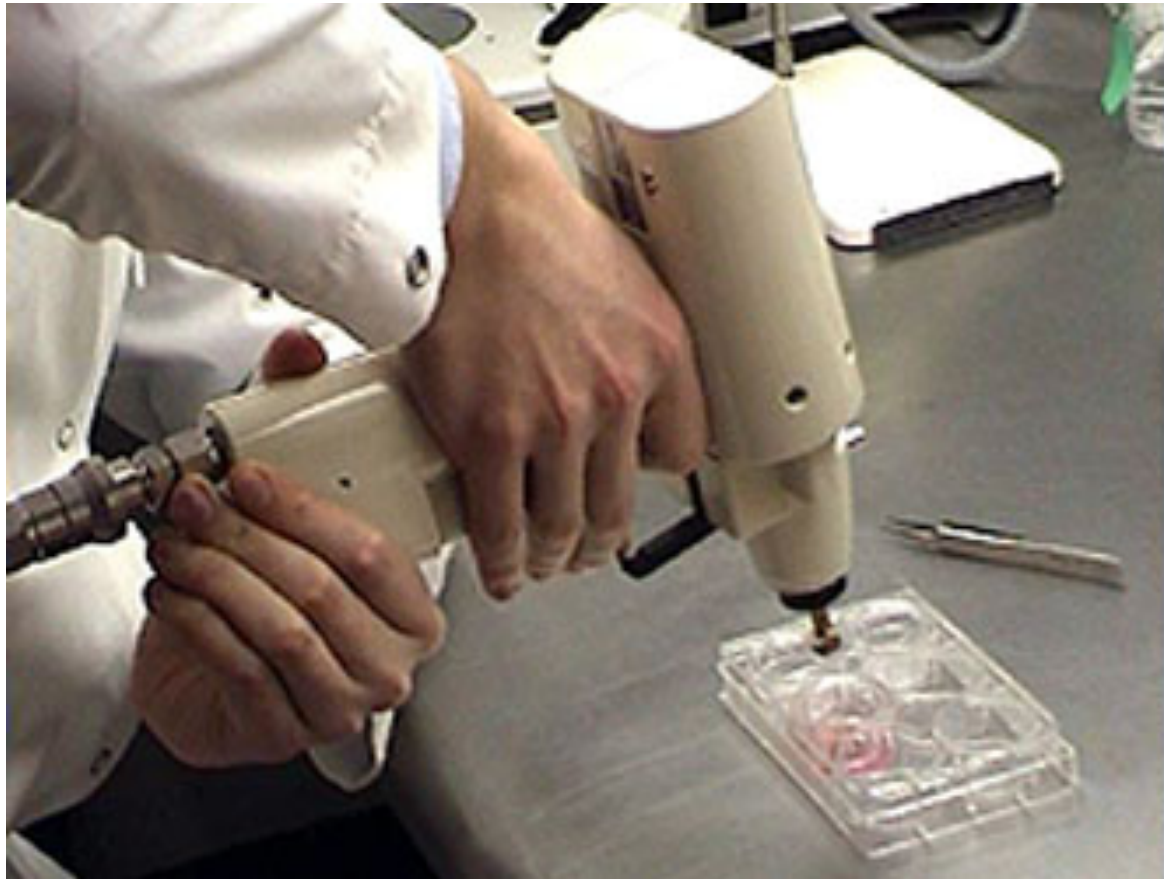


Cell membrane
during pulsing

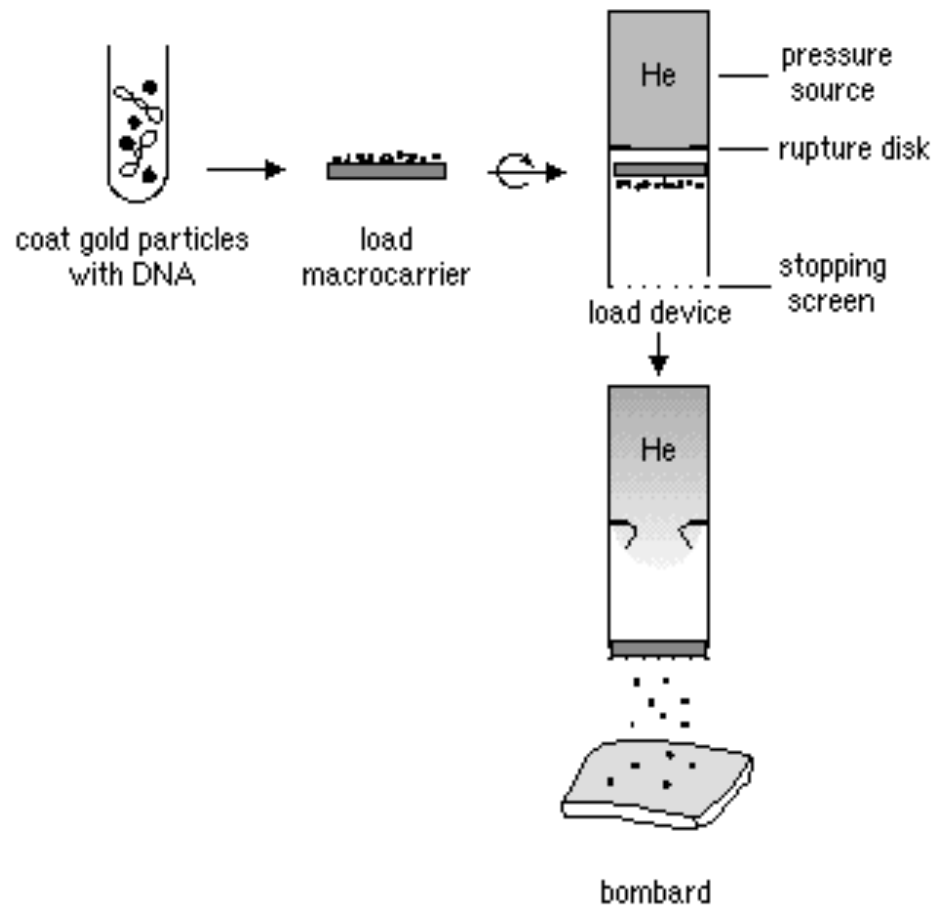


Cell membrane
after pulsing
(cell returns to
original state)

Gene Gun



Gene Gun



Gene Gun

- Also called Biolistic transformation
- **Advantages:**
 - Can be used in any cell type
- **Disadvantages:**
 - Damage of a significant number of cells
 - Low transfection efficiency
 - Reproducibility problems

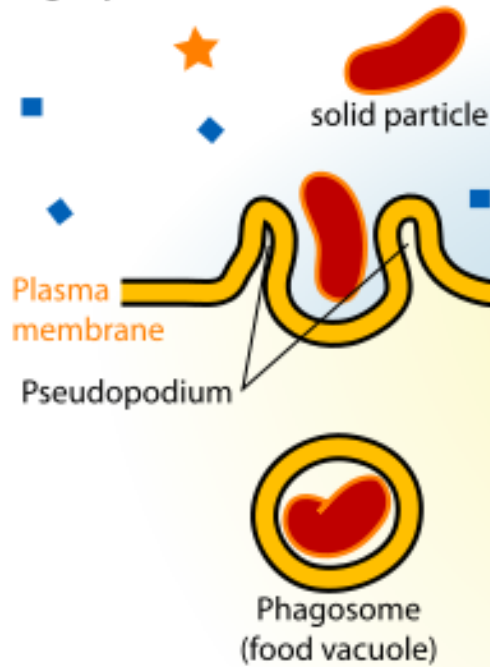
Co-precipitation

- Binding of DNA (-ve) into macromolecular complexes e.g. Calcium Phosphate crystals (+ve). Uptake by **endocytosis**.
- **Advantages:**
 - Inexpensive and simple to perform
 - High transfection efficiency
- **Disadvantages:**
 - Cytotoxicity

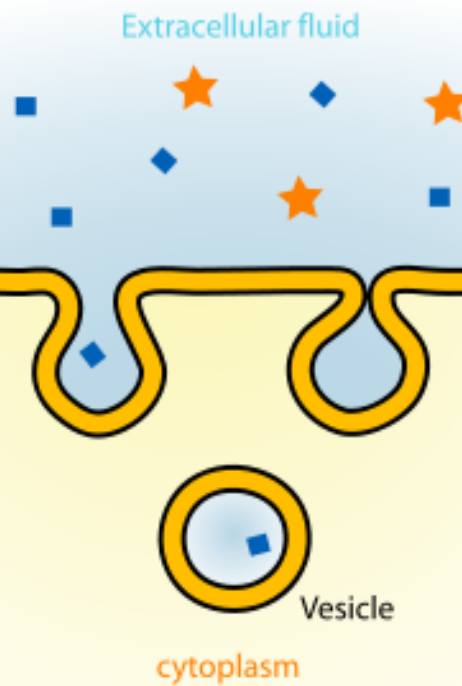
Endocytosis

Endocytosis

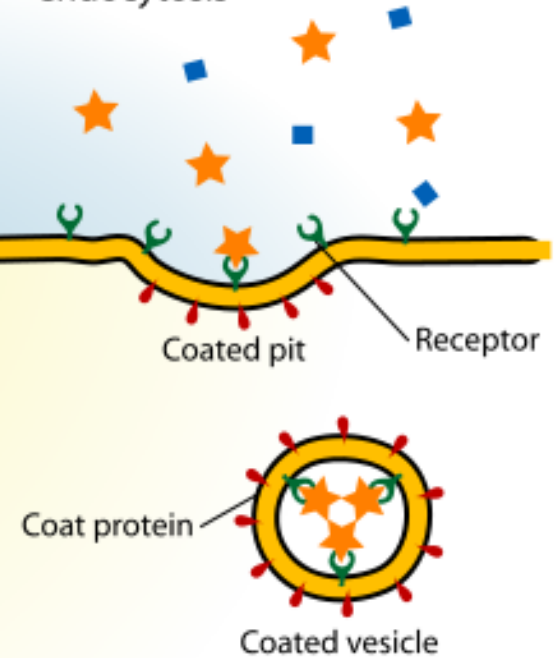
Phagocytosis



Pinocytosis



Receptor-mediated endocytosis



Nanoparticles

- Polyplexes
- Lipoplexes
- Polymeric NPs
- Polymeric micelles
- Dendrimers

Nanobiotechnology

- **Nanotechnology “nanotech”:**
 - The field of manipulating matter on nanometer range (1 to 100 nm)
- **Nanomedicine “nanomed”:**
 - The application of nanotechnology in diagnosis and therapy
- **Nanopharmaceuticals:**
 - Therapeutics consisting of at least two components (drug/carrier) in size range of (1 to 1000 nm)
- **Nanobiotechnology:**
 - The use of nanomaterials in biotechnology products

Nanoscale

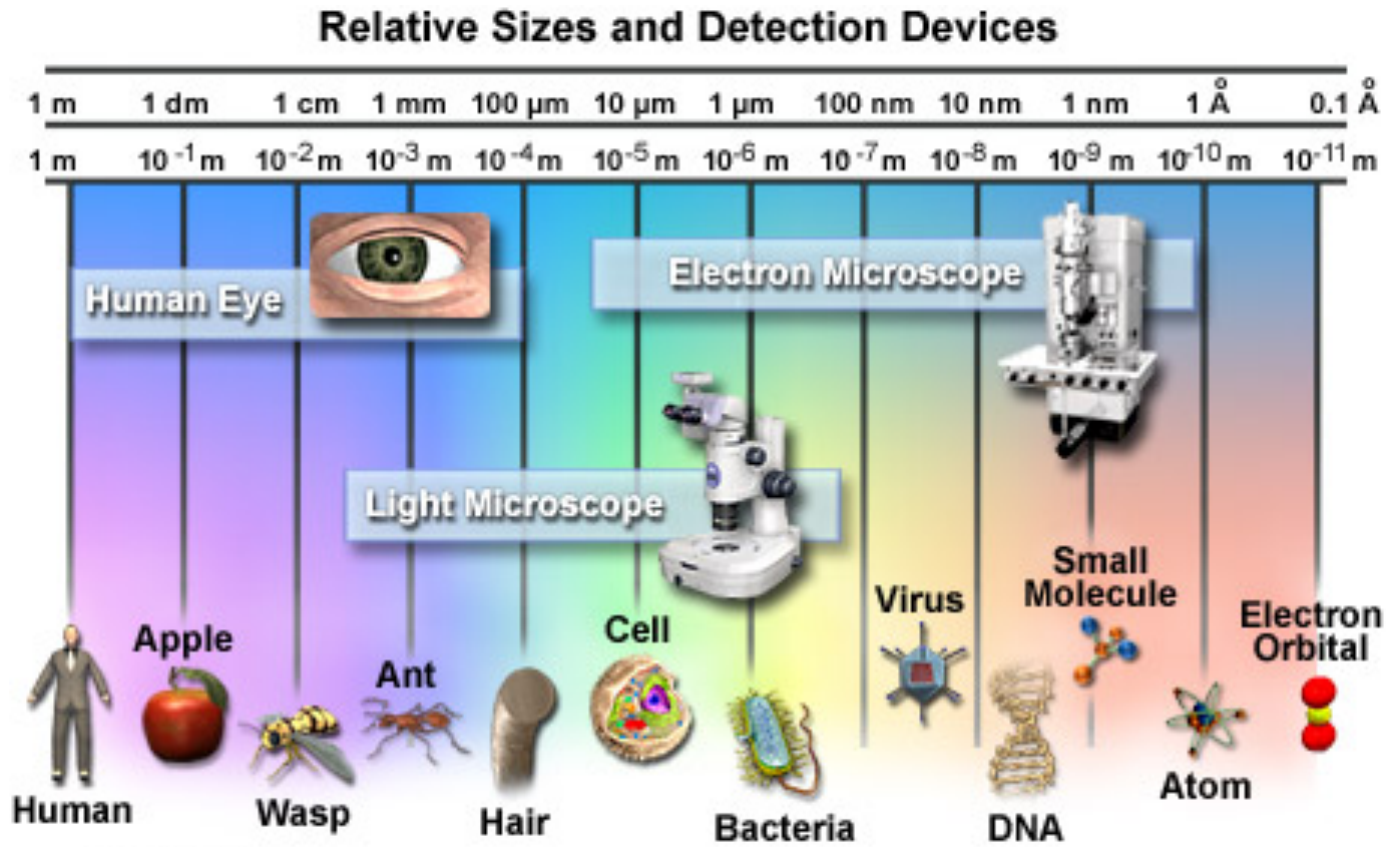
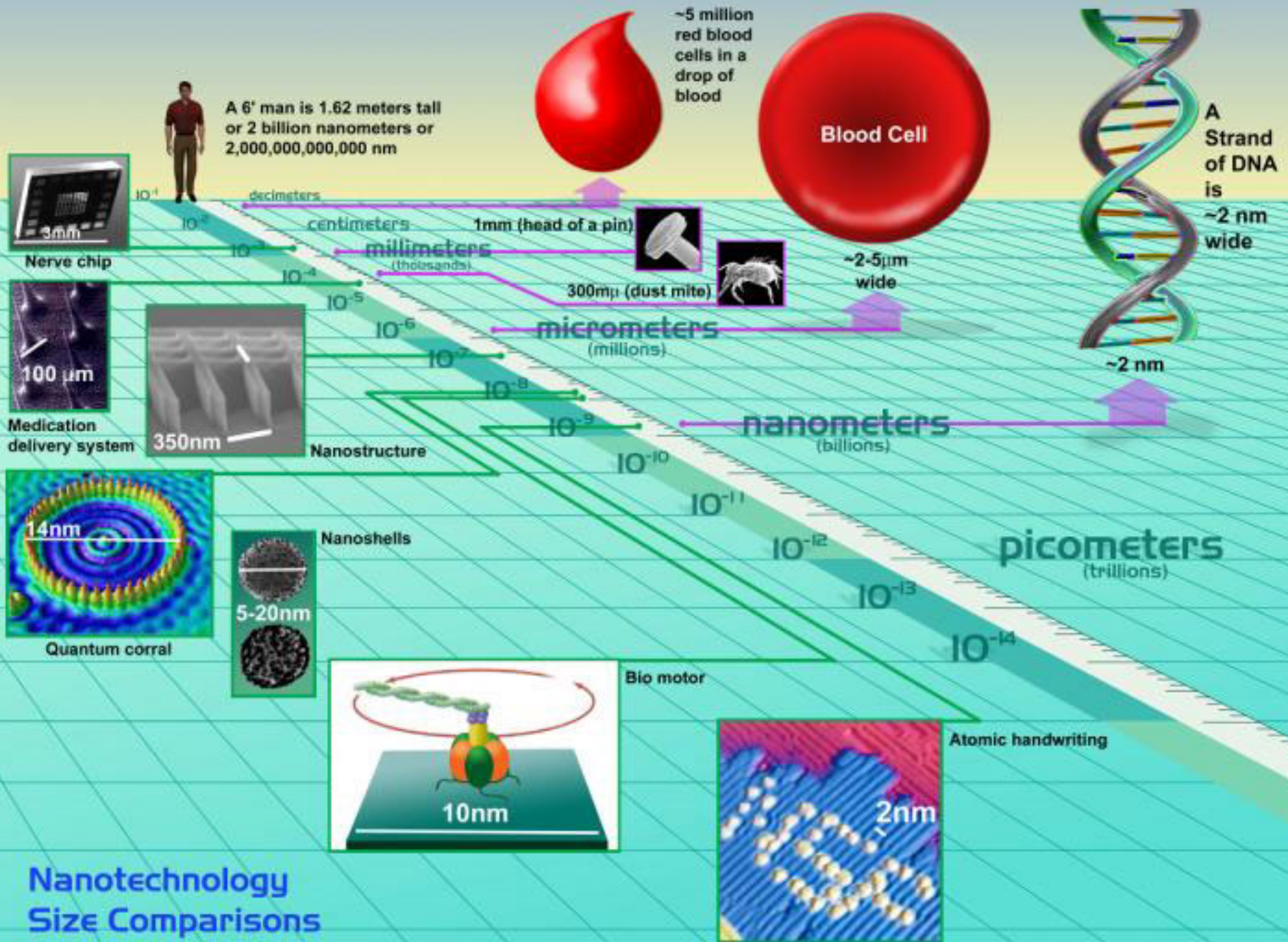
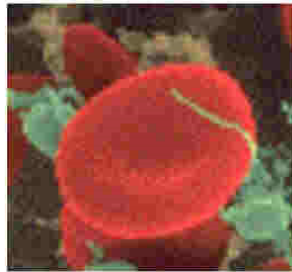


Figure 1

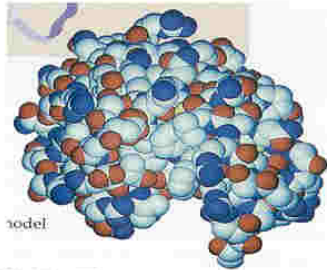
Nanoparticles



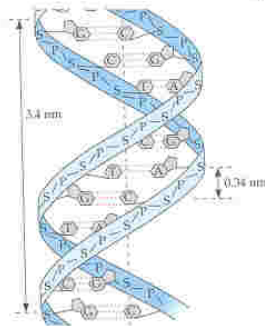
Microns to Nanometers -- Biological/Chemical/Atomic



Plant,
Animal Cell



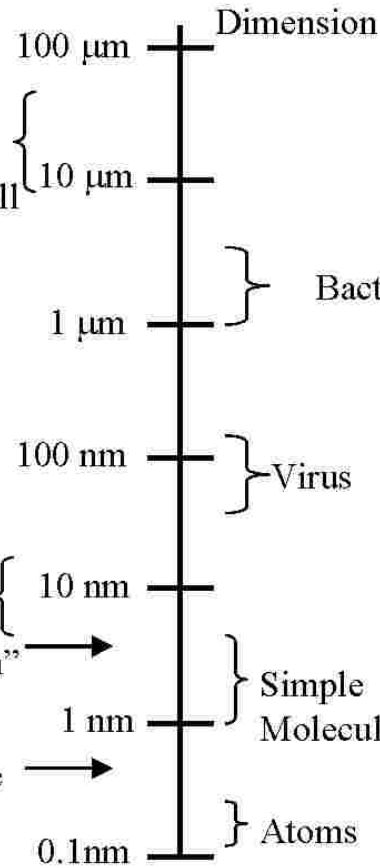
protein



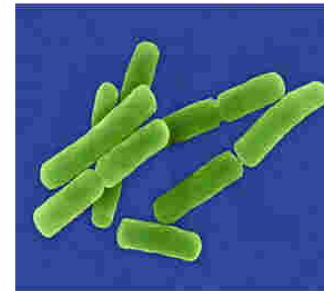
Protein

DNA "turn"

DNA base



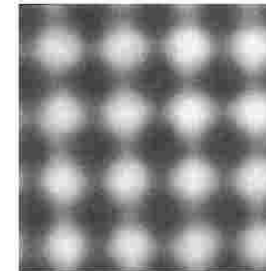
Bacteria



Virus



Simple
Molecules



Atoms

Nanoparticles

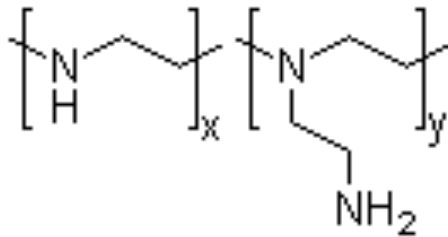
- Polyplexes
- Lipoplexes
- Polymeric NPs
- Polymeric micelles
- Dendrimers

Polyplexes

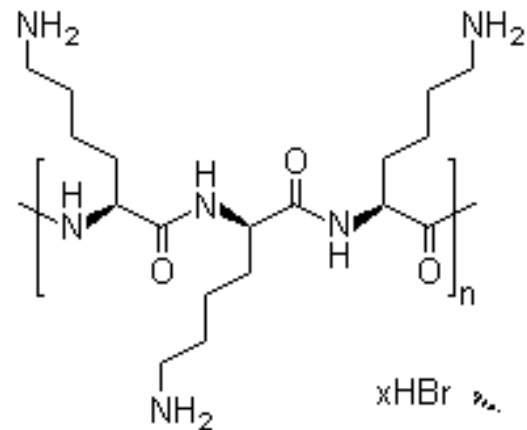
- They are complexes of cationic polymers with nucleic acids
- Polyplex formation is regulated by electrostatic interaction, which is affected by:
 - pH of the media
 - Ionic strength
 - Cationic density

Polyplexes

- Examples:
- Polyethylenimine (PEI)
- Poly-L-Lysine (PLL)

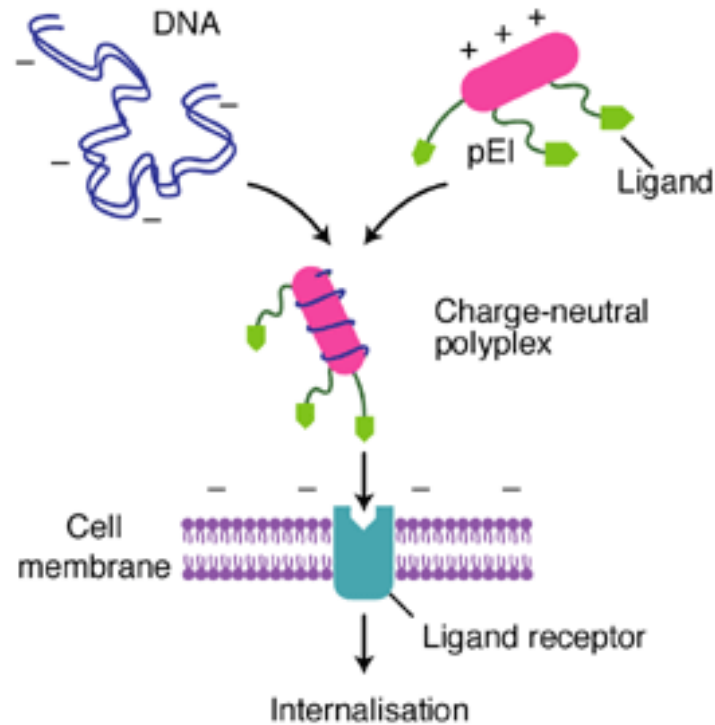


PEI



PLL

Polyplexes



Polyplexes

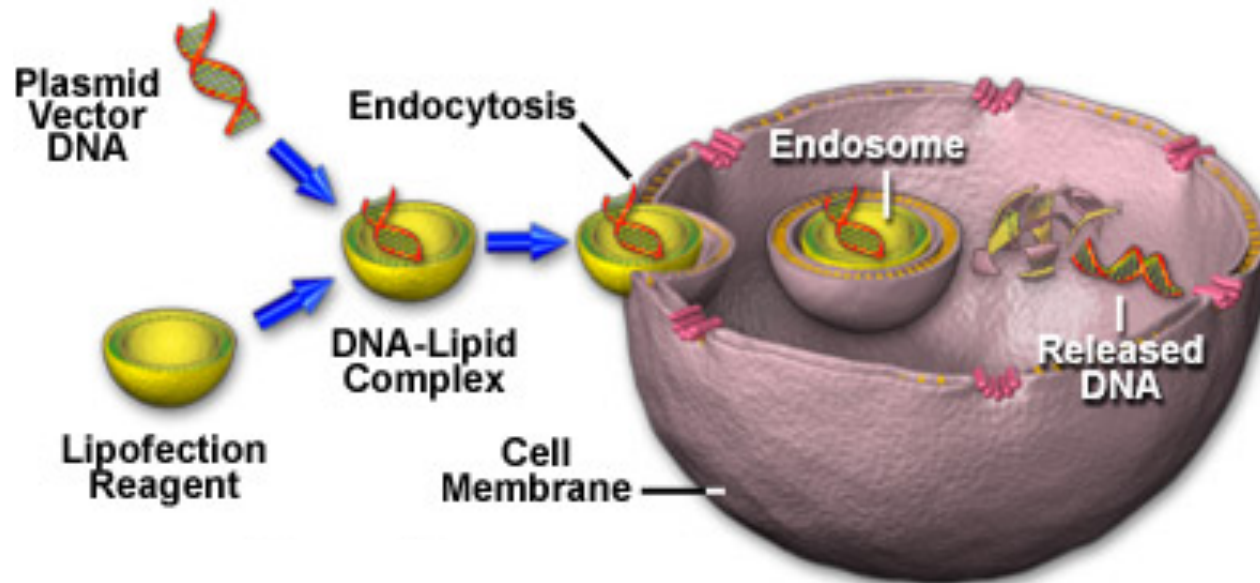
- **Advantages:**
 - Easy to formulate
 - Simple chemical modification
- **Disadvantages:**
 - Cytotoxicity
 - Stability problems

Lipoplexes

- They are complexes of cationic lipids or liposomes with nucleic acids
- Lipoplex formation is regulated by electrostatic interaction and lipid constituents of the carrier
- Transfection using lipoplexes is known as *Lipofection*

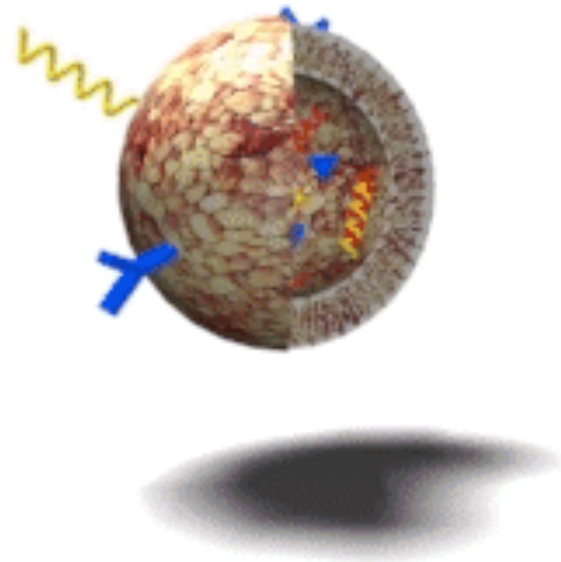
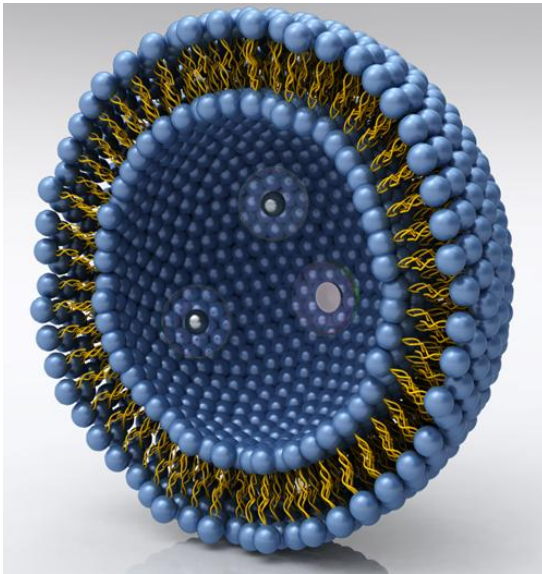
Lipofection

Lipid-Mediated Transfection in Mammalian Cells



Lipoplexes

- Examples:
- Lipofectin
- Lipofectamine



Lipoplexes

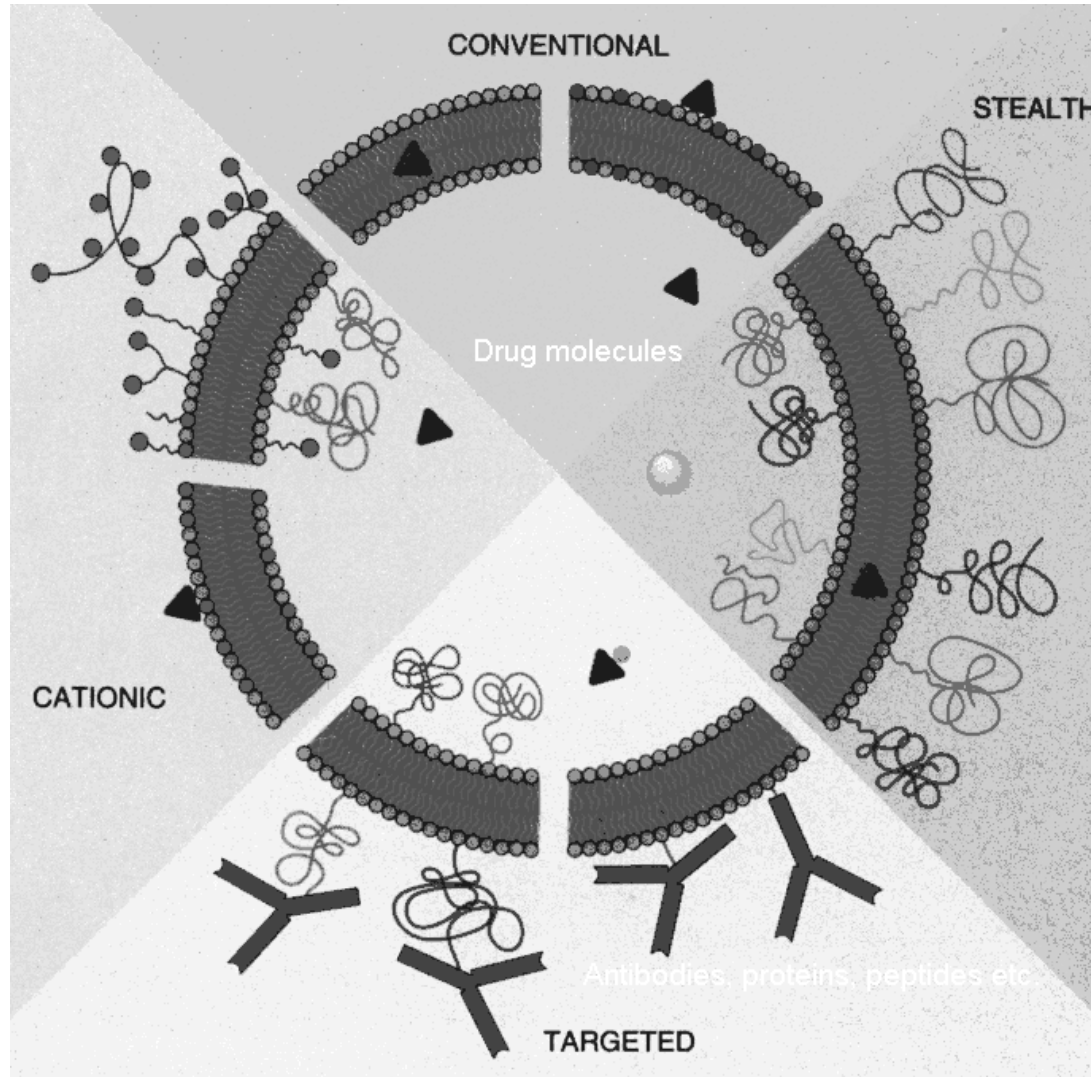
- **Advantages:**

- Versatility
- Protection of DNA
- Fusion with cell membrane
- Biocompatibility

- **Disadvantages:**

- Labor intensive
- Expensive
- Stability
- Clearance by RES (***bonus question***)

Liposome versatility

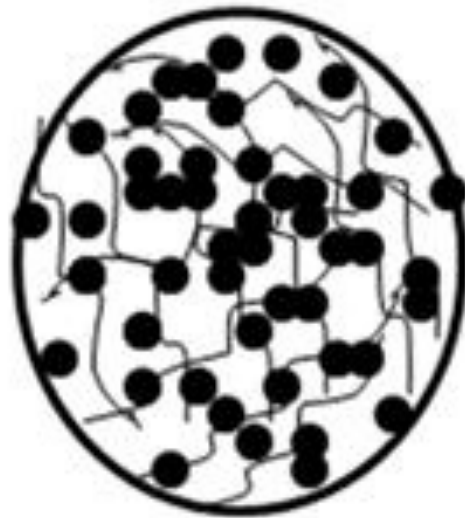


Polymeric Nanoparticles

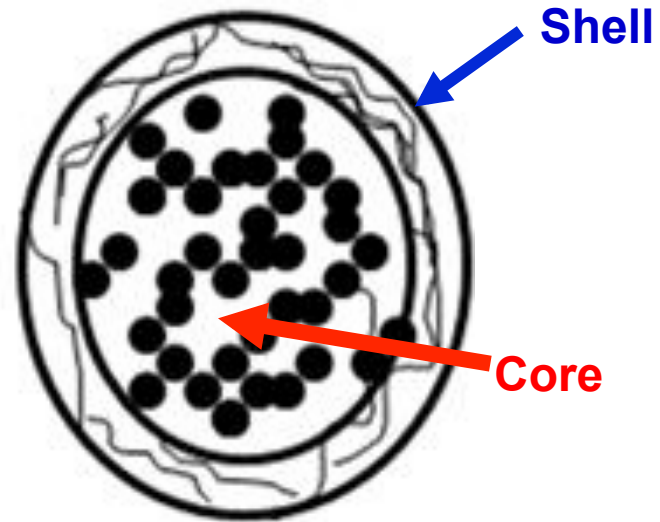
- Nanoparticles made out of polymers
- Natural polymers:
 - Chitosan
 - Albumin
 - Gelatin
- Synthetic Polymers:
 - Polylactic acid (PLA)
 - Polyglycolic acid (PGA)
 - Poly(lactic-co-glycolic acid) (PLGA)

Polymeric Nanoparticles

- Polymeric NPs can be prepared to become *Nanospheres* or *Nanocapsules*



Nanosphere



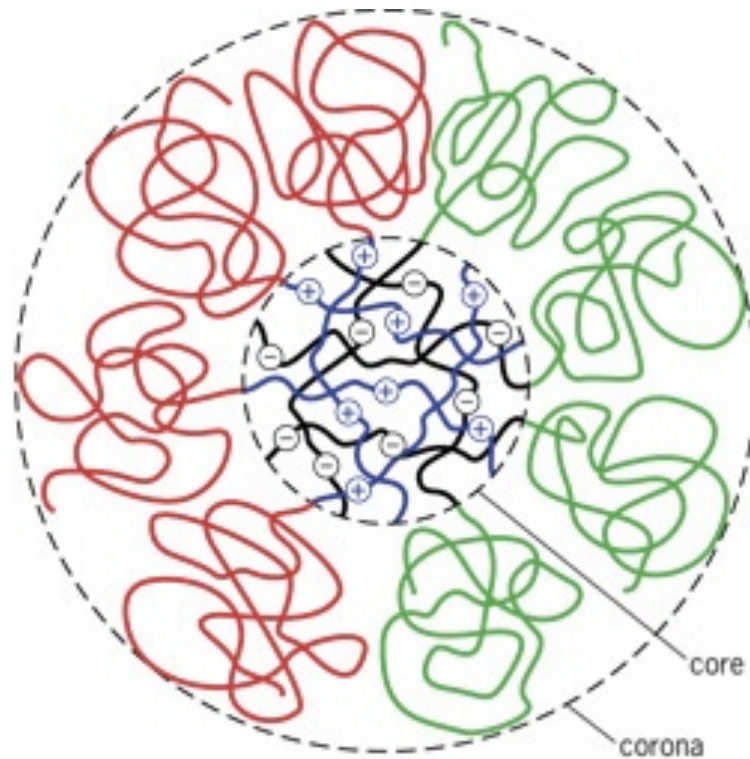
Nanocapsule

Polymeric Nanoparticles

- **Advantages:**
 - Biocompatibility
 - Biodegradability
 - Loading of more than one gene or drug
- **Disadvantages:**
 - Tedious preparation
 - Degradation of DNA during preparation
 - Can't be easily modified

Polymeric Micelles

- Amphiphilic polymer chains that self-assemble into nano-sized spherical structures in aqueous medium



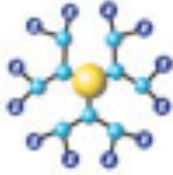
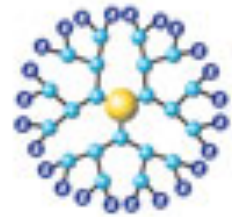
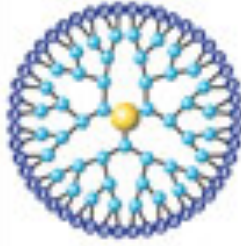

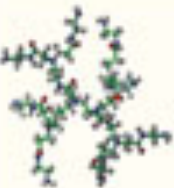
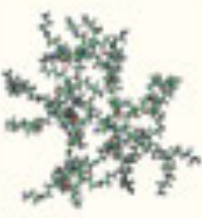
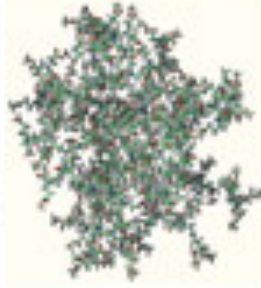
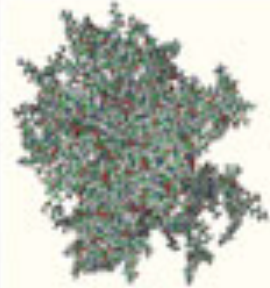


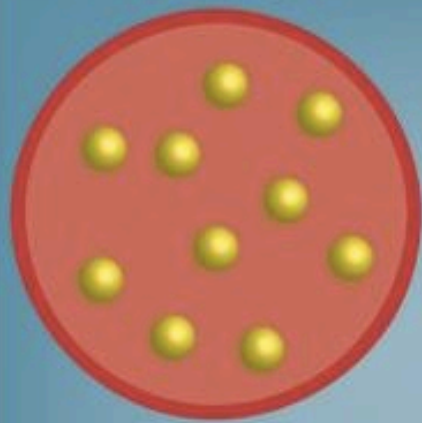
Polymeric Micelles

- **Advantages:**
 - Biocompatibility
 - Biodegradability
 - Easy to prepare
 - Chemical modification is easy
- **Disadvantages:**
 - Highly unstable
 - Must be made fresh

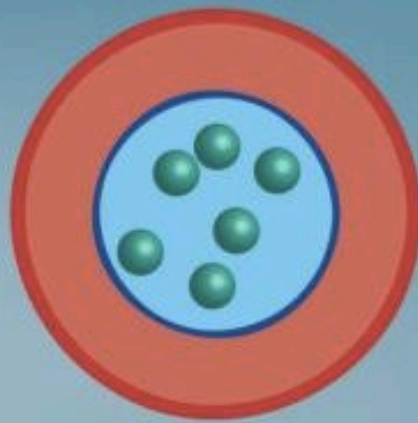
Dendrimers

- Highly-branched polymeric macromolecules

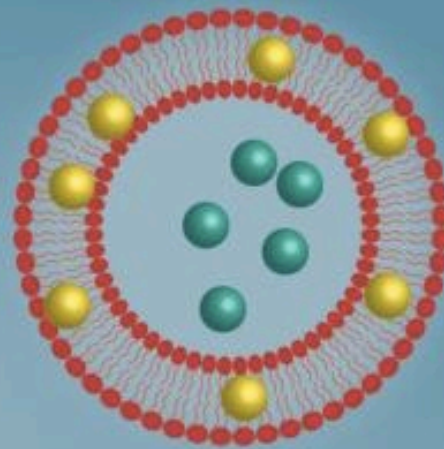
Generation	G0	G1	G2	G3	G4
# of Surface Groups	3	6	12	24	48
Diameter (nm)	1.4	1.9	2.6	3.6	4.4
2D Graphical Representation					
3D Chemical Structure View					



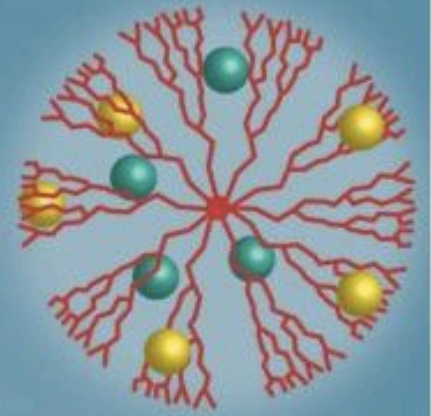
Nanospheres



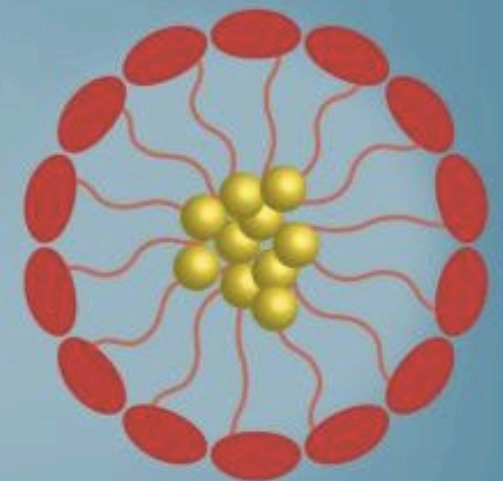
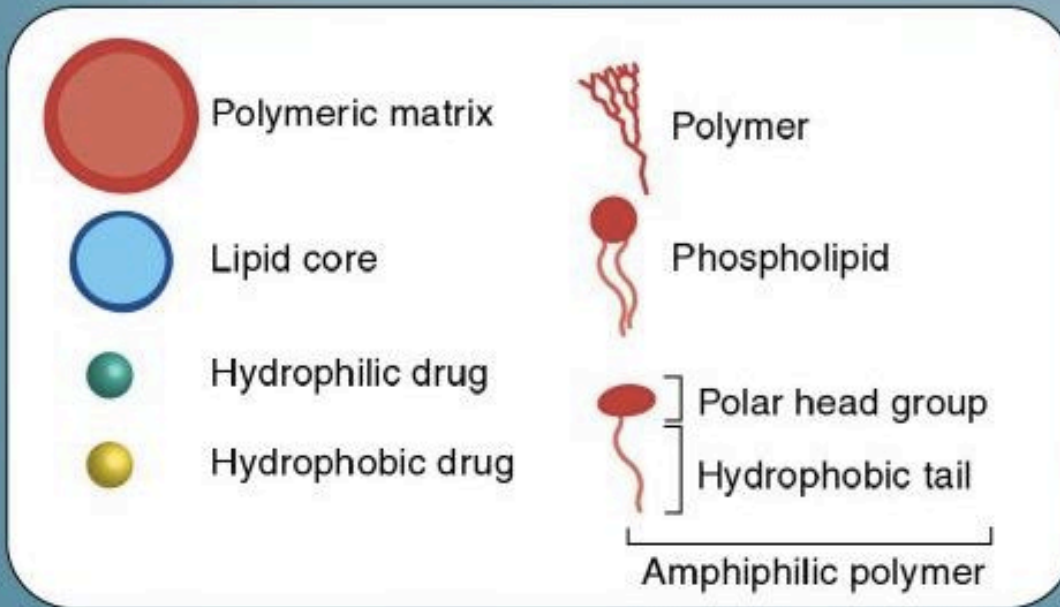
Nanocapsules



Liposomes



Dendrimers



Polymeric micelles

You are now able to:

- ✓ Differentiate between physical and chemical methods for gene transfer
- ✓ Compare between several nanocarriers used for gene delivery
- ✓ Appreciate the potential of non-viral method as a therapeutic strategy for gene therapy