

Chapter 3

Lipid-Based Theranostics

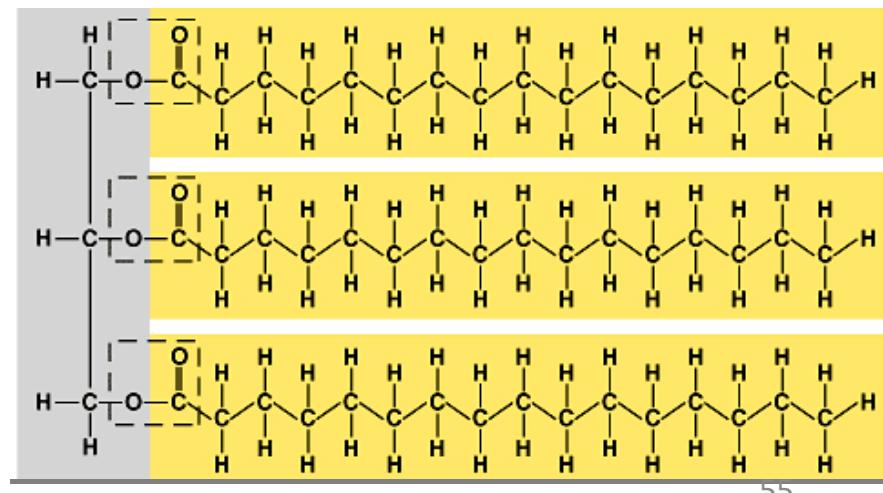
What is a lipid?

Lipids - one class of large biological molecules that do not form polymers

- composed of C, H, O
 - long hydrocarbon chain
- 4 types of lipids
 - fats
 - phospholipids
 - steroids
 - waxes

Properties

- The unifying feature of lipids is having little or no affinity for water (water fearing)
- Lipids are hydrophobic because they consist mostly of hydrocarbons, which form nonpolar covalent bonds
- The most biologically important lipids are fats, phospholipids, and steroids



Phospholipids

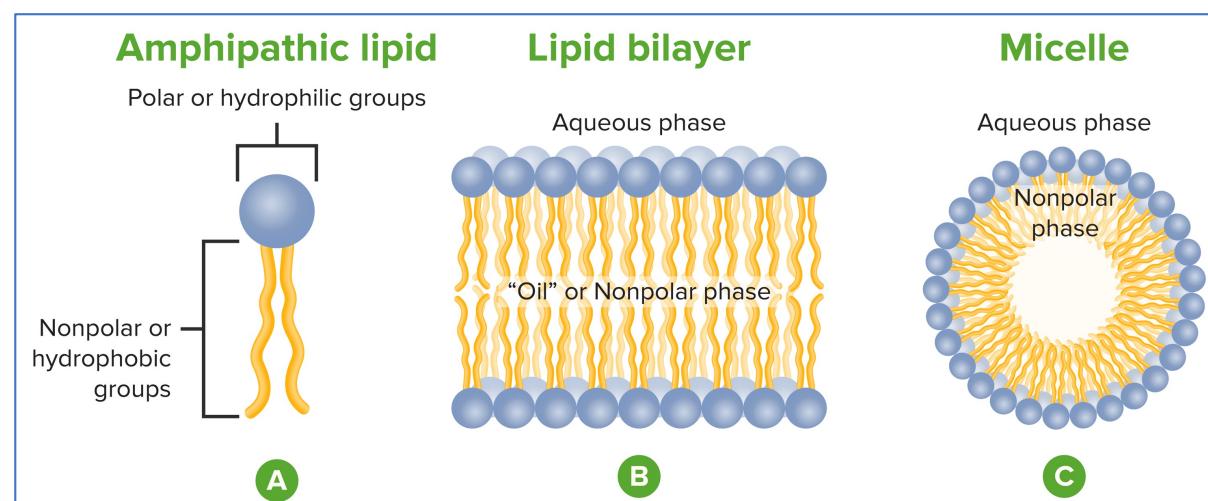
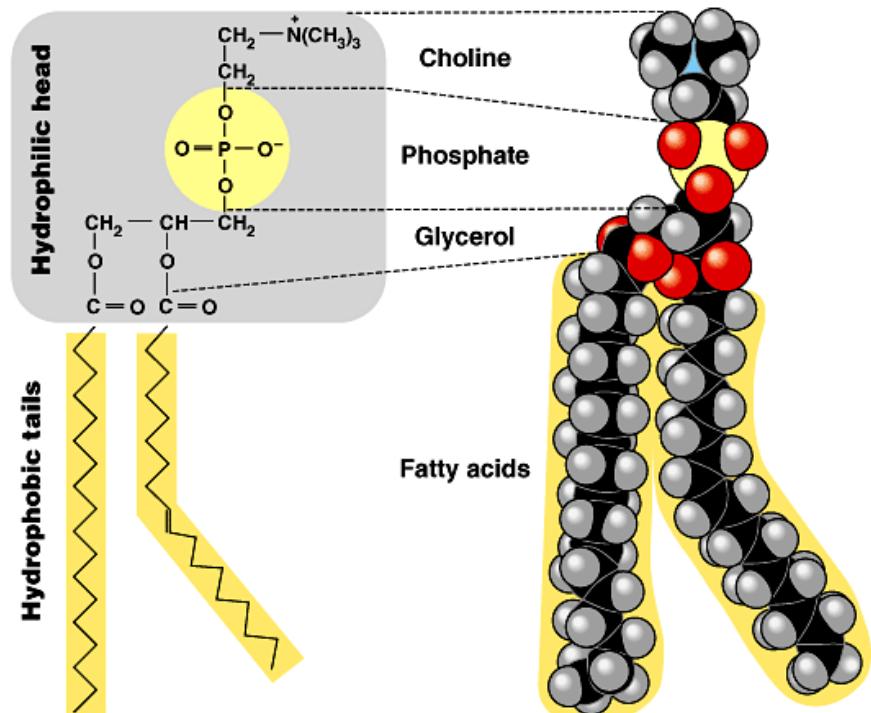
➤ Phospholipids (amphipathic)

- Hydrophobic or hydrophilic?
 - fatty acid tails = hydrophobic
 - PO_4 = hydrophilic head
 - dual “personality”

It likes water
& also pushes
it away!

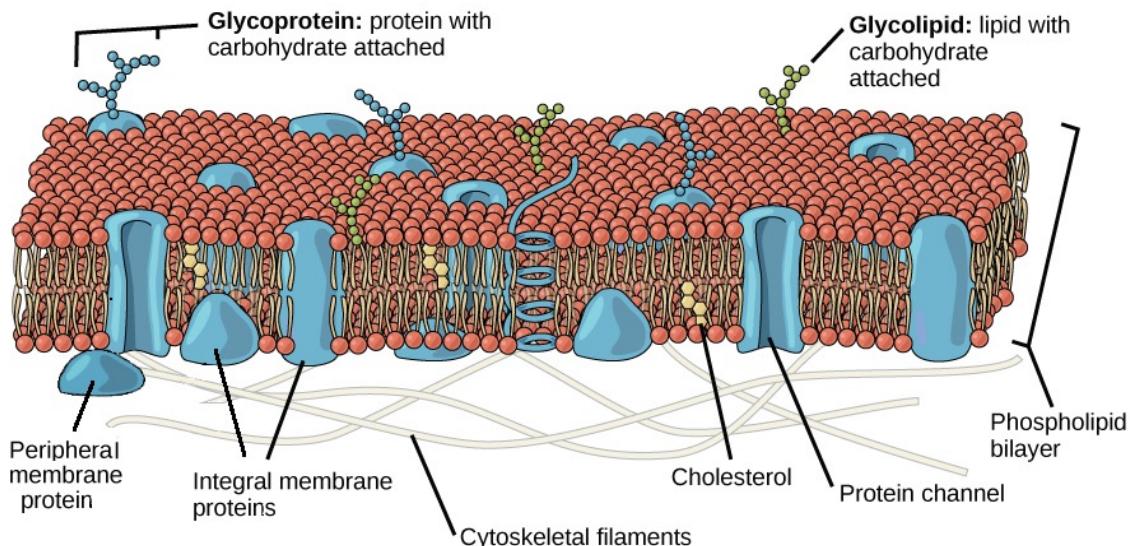


interaction with
 H_2O is complex
& very important!

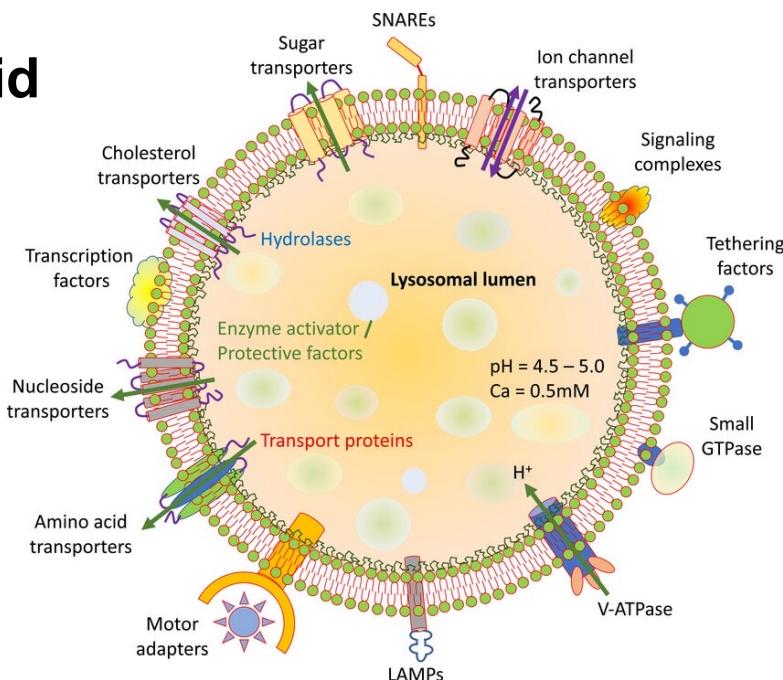


Phospholipid bilayer membrane

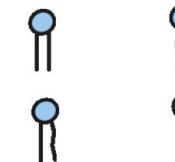
Cell membrane



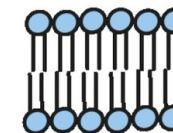
Lysosome lipid bilayer



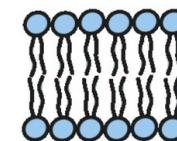
Different lipids form bilayers with different properties



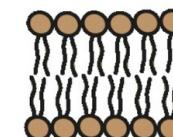
bilayer-forming lipids can have saturated or unsaturated chains, or be asymmetric
can also have charged headgroups



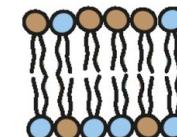
bilayer formed of saturated lipid chains e.g. DMPC, DMPG



bilayer formed of unsaturated lipid chains e.g. DOPC



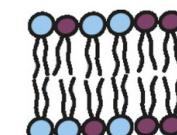
bilayer formed of unsaturated lipid chains with charged headgroups e.g. DOPG



mix together charged and neutral headgroups e.g. DOPG and DOPC



non-bilayer-forming lipids
lipids with negative spontaneous curvature, such as DOPE, do not form bilayers



non-bilayer forming lipids are mixed with bilayer-forming lipids e.g. DOPE mixed with DOPC

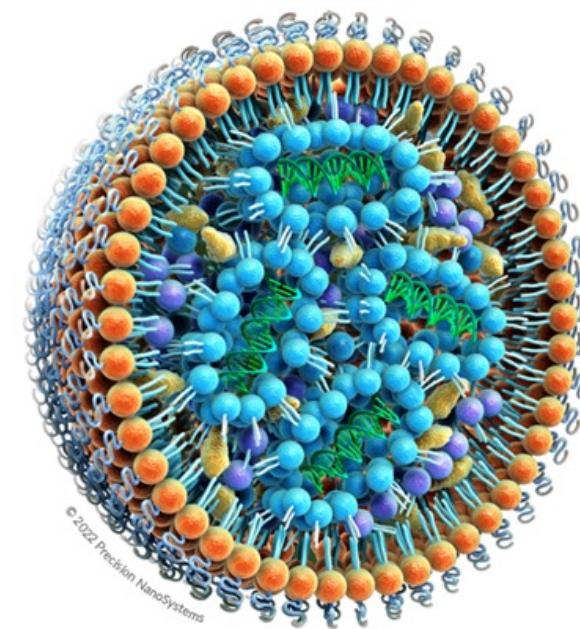
Lipid nanoparticles (LNPs)

- **Lipid nanoparticles (LNPs)** are the most clinically advanced non-viral gene delivery system
- safely and effectively deliver nucleic acids, overcoming a major barrier preventing the development and use of genetic medicines.
- gene editing, rapid vaccine development, immuno-oncology and treatment of rare genetic and undruggable diseases; all of which are usually **hindered by nucleic acid delivery inefficiency**.

LNPs offer many advantages over previous lipid-based nucleic acid delivery systems including:

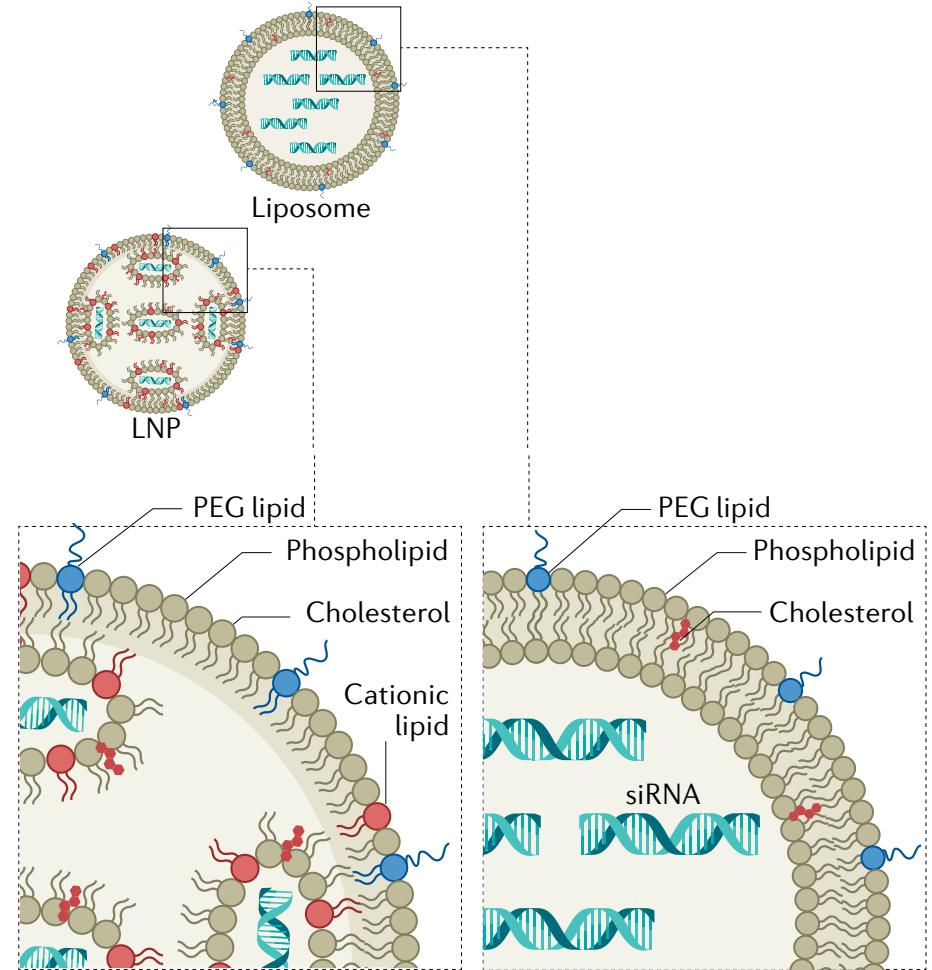
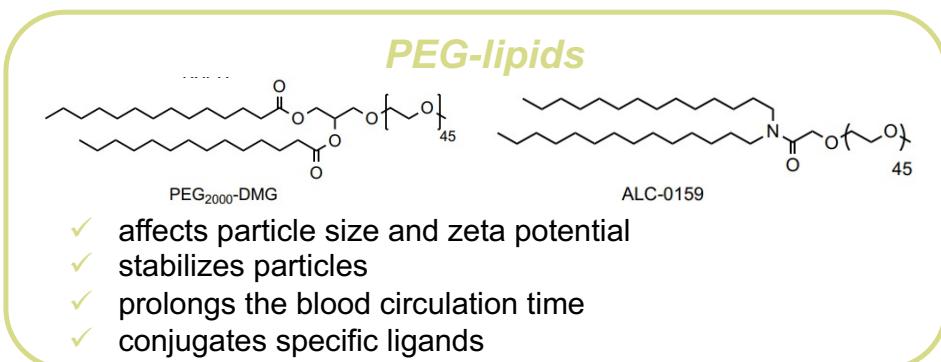
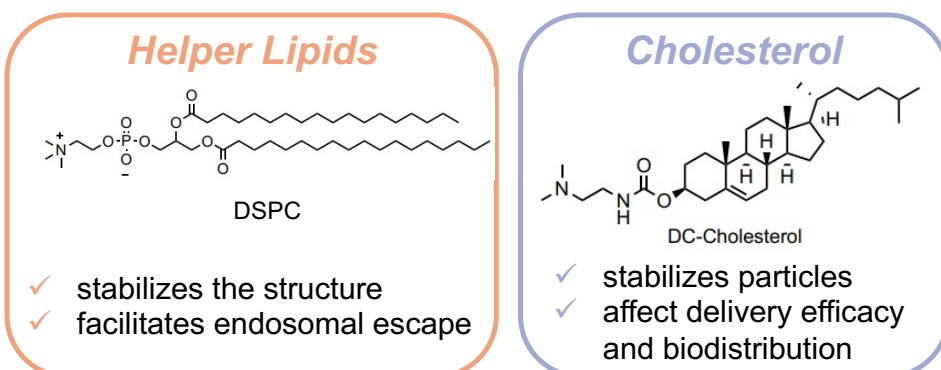
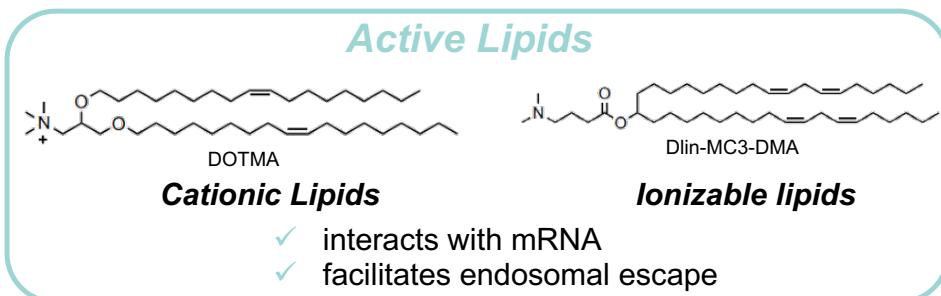
- High nucleic acid encapsulation efficiency and potent transfection
- Improved penetration into tissues to deliver therapeutics
- Low cytotoxicity and immunogenicity

The first RNAi drug uses LNPs and was approved by FDA in 2018.

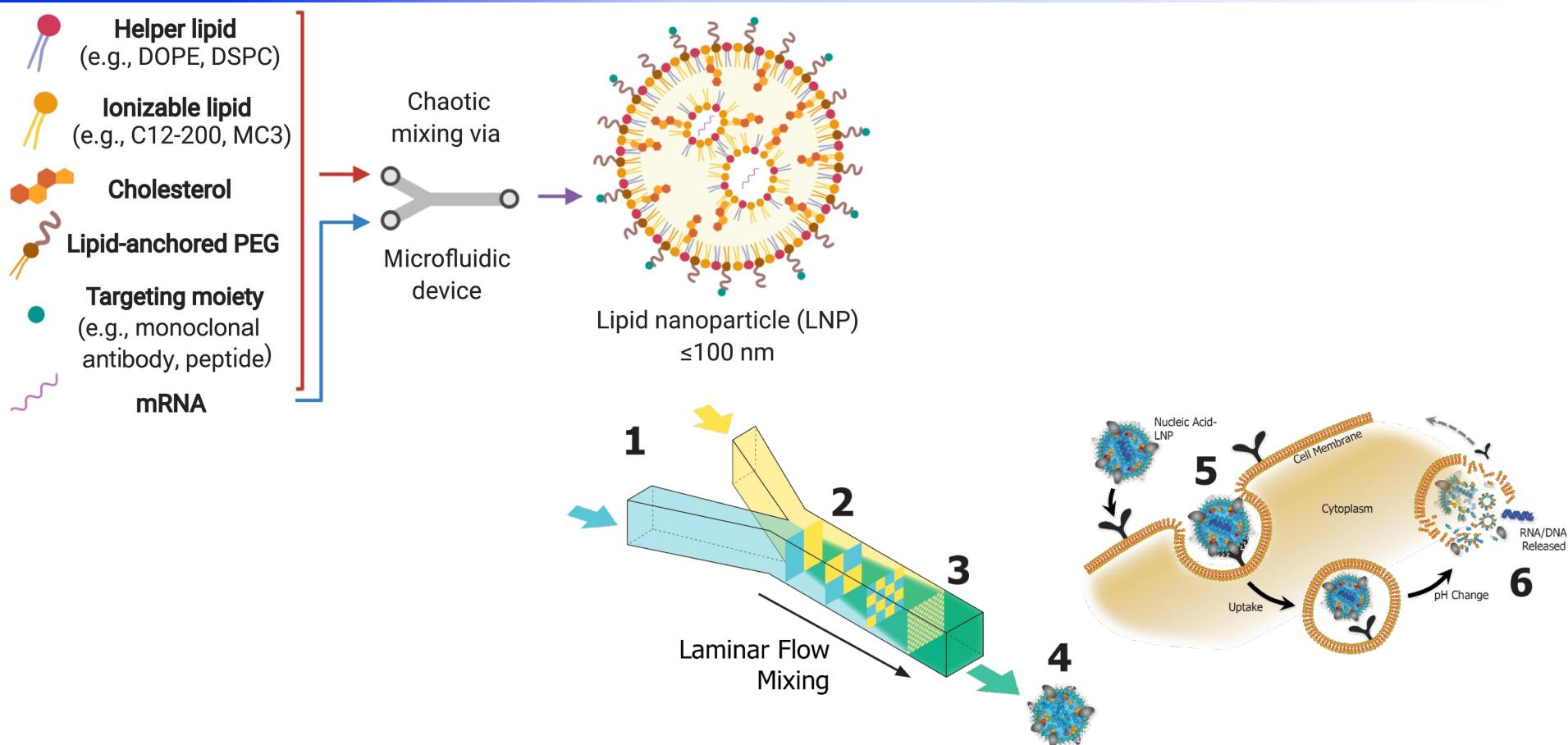


LNPs - Composition

➤ LNPs – 4 major components



LNPs - Preparation

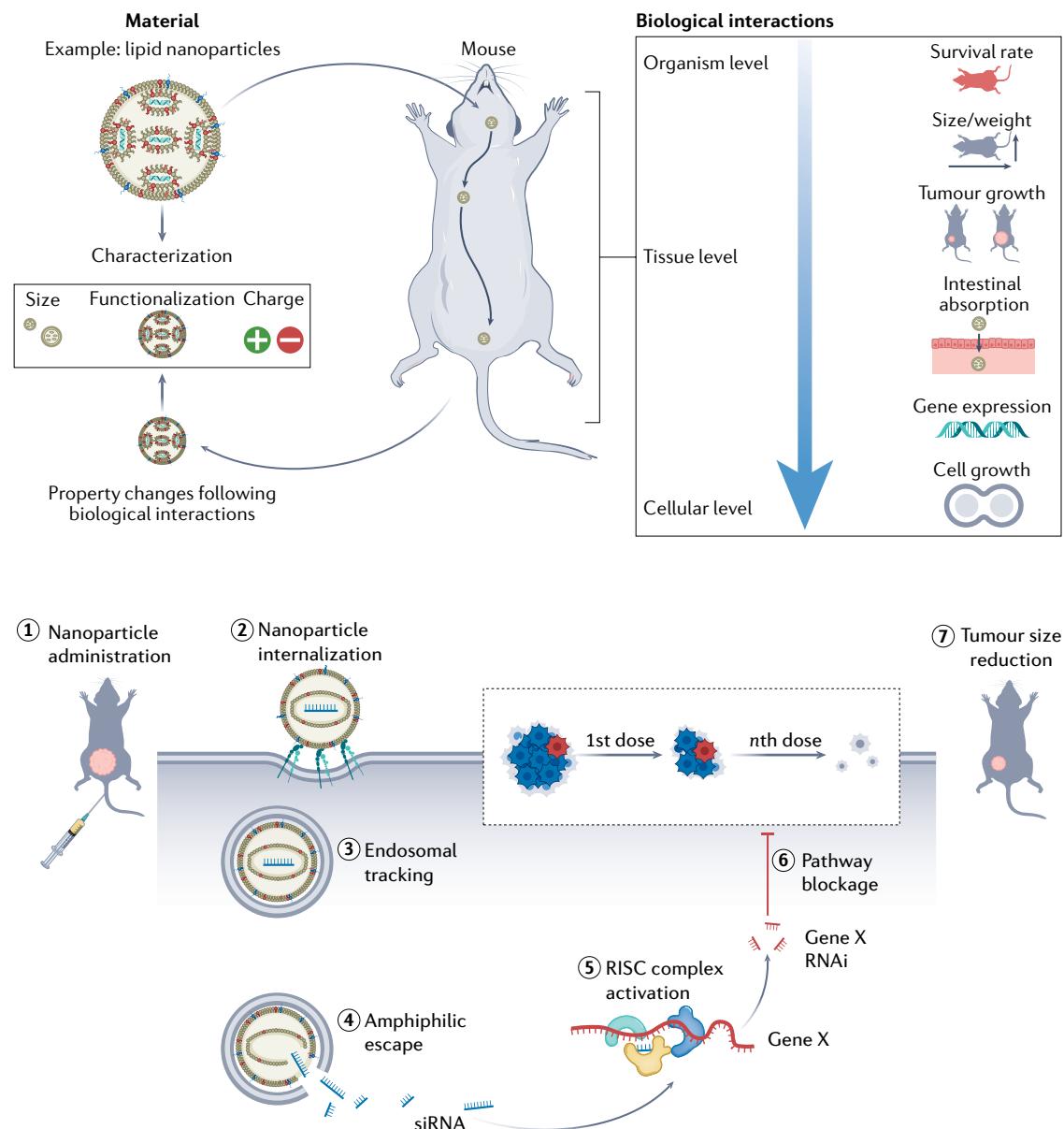


- 1) An organic solvent containing dissolved lipids and an aqueous solution containing nucleic acids are injected into the two inlet channels of the cartridge.
- 2) Under laminar flow, the two solutions do not immediately mix, but microscopic features engineered into the channel cause the two fluids to intermingle in a controlled and reproducible way.
- 3) Within a millisecond, the two fluids are completely mixed, causing a change in solvent polarity that triggers the self-assembly of lipid nanoparticles loaded with nucleic acids.

LNPs-guided nucleic acid delivery

Nucleic acid-loaded LNP delivery in cancer therapies.

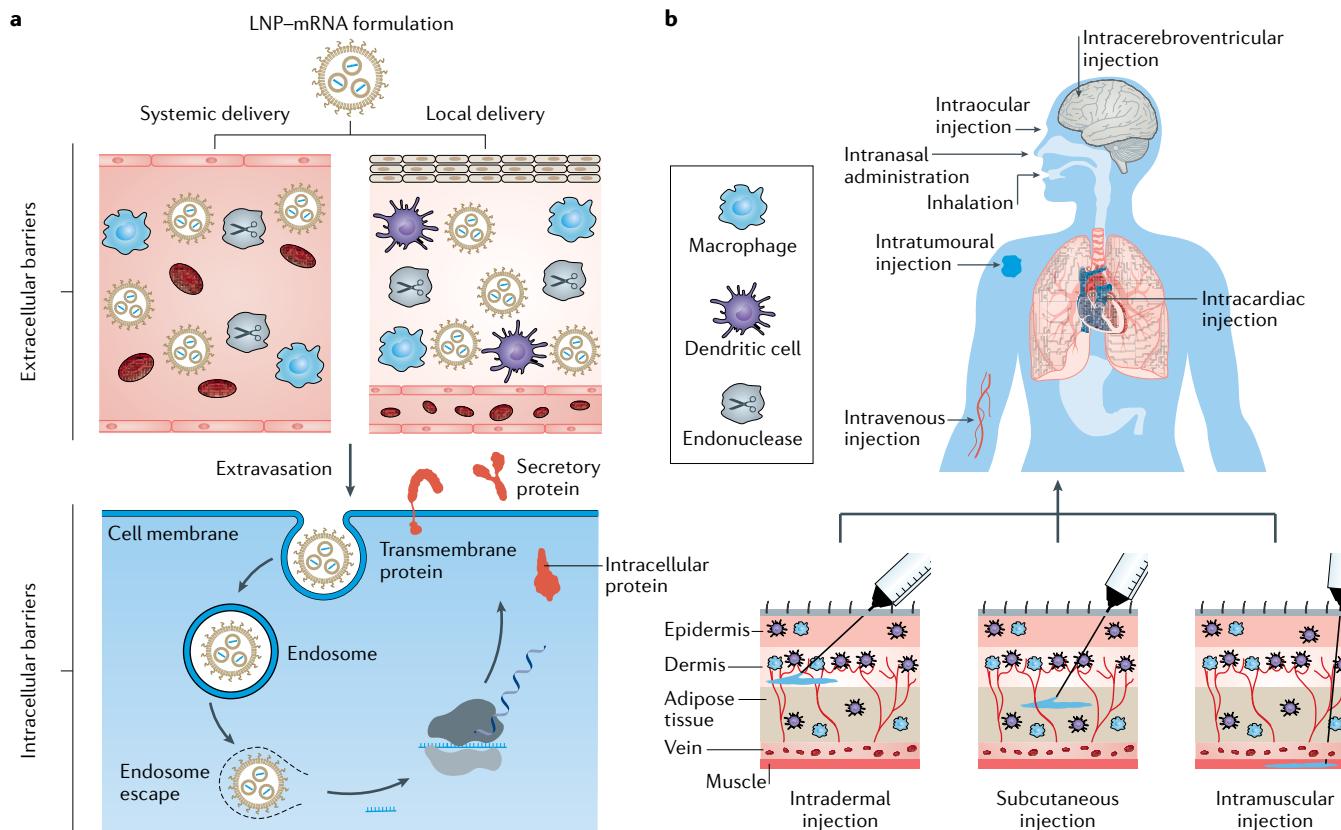
- Nucleic acid-loaded LNP formulations are administered *in vivo*
- LNP intrinsic features - determine their biological behaviour at organism, tissue and cellular levels.
- Upon successful LNP administration (1), internalization (2) and siRNA release/escape (3–4), gene silencing (5–6) and subsequent tumour size reduction (7) are achieved
- RNA-induced silencing complex ; RNAi, RNA interference; siRNA, small interfering RNA.



Delivery barriers for LNPs-mRNA formulations

Overcoming physiological barriers

- protected from nuclease degradation in physiological fluids
- evade the interception by the MPS and clearance by renal glomerular filtration post systemic administration
- need to reach target tissues, followed by internalization by target cells
- mRNA molecules must escape endosomes to reach the cytoplasm, where translation occurs



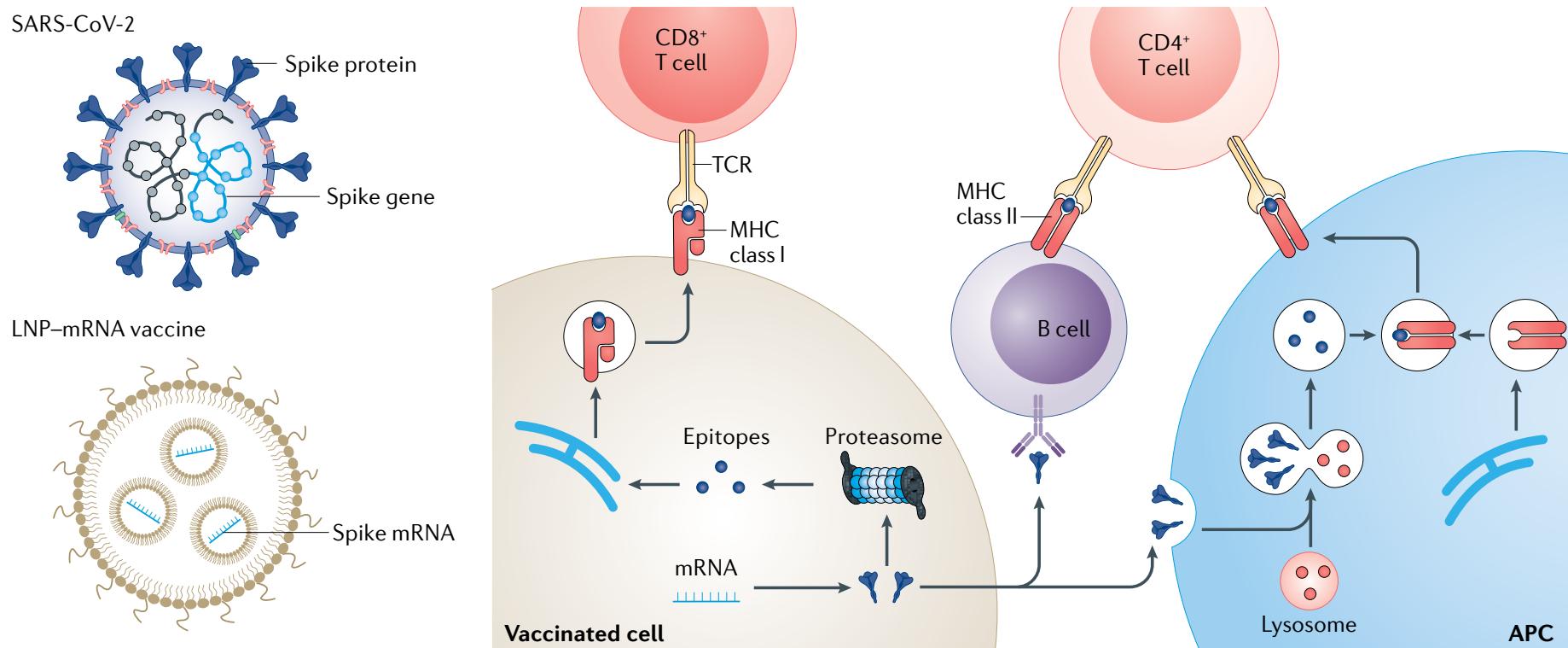
a | Physiological barriers for LNP–mRNA formulations post systemic and local delivery.

b | Administration routes for LNP–mRNA formulations

LNPs-mRNA as influenza vaccines

LNPs-mRNA vaccines

- LNP-mRNA vaccines are internalized by muscle cells and recruited APCs
- LNP-mRNA vaccines can centre draining lymph nodes, where naive T and B cells reside
- Spike antigens expressed in the cytoplasm are degraded by proteasomes, and MHC class I presents the epitopes to CD8⁺ T cells
- Spike antigens can be endocytosed by APCs, and are degraded in the lysosomes of APCs and presented by MHC II for CD4⁺ T cells. Also, secreted spike antigens can be internalized by B cell receptors and processed for presentation to CD4⁺ T cells by MHC class II



LNPs – Targeted delivery

Overcoming delivery barriers with LNPs

- Ionizable phospholipids have been developed to enhance the delivery of mRNA and sgRNA for gene editing by selective organ targeting and endosomal membrane destabilization.

