UNIVERSITE PARIS-SACLAY | FACULTÉ DE PHARMACIE

GENE THERAPY
NON VIRAL VECTORS

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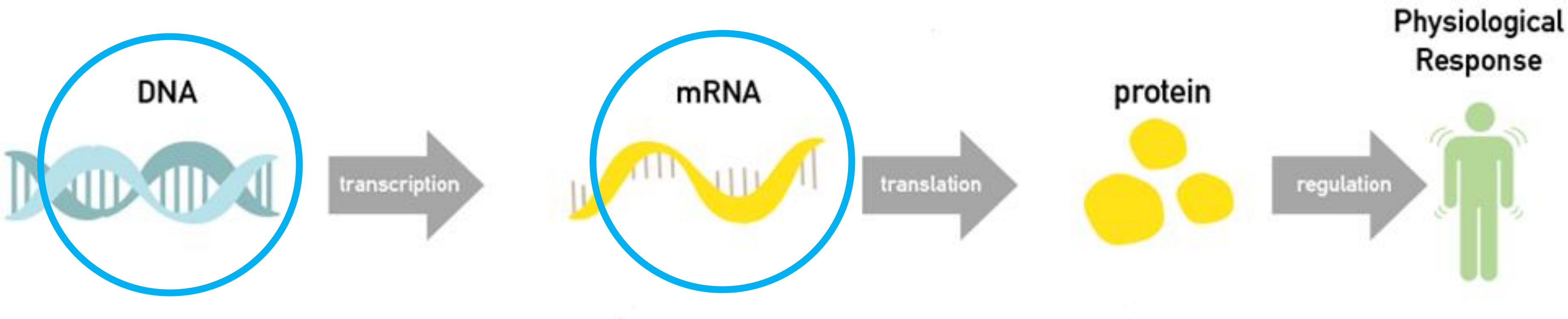
CNRS | INSTITUT GALIEN PARIS-SUD

- Add DNA
- Add mRNA
- React with protein



- Remove DNA
- Replace DNA

- Destroy mRNA (prevent translation)
- Modify mRNA (modify translation)

Natural barriers?

Figure from http://www.bonac.com/global/en/nucleic/about/

Natural barriers
Physiological barriers

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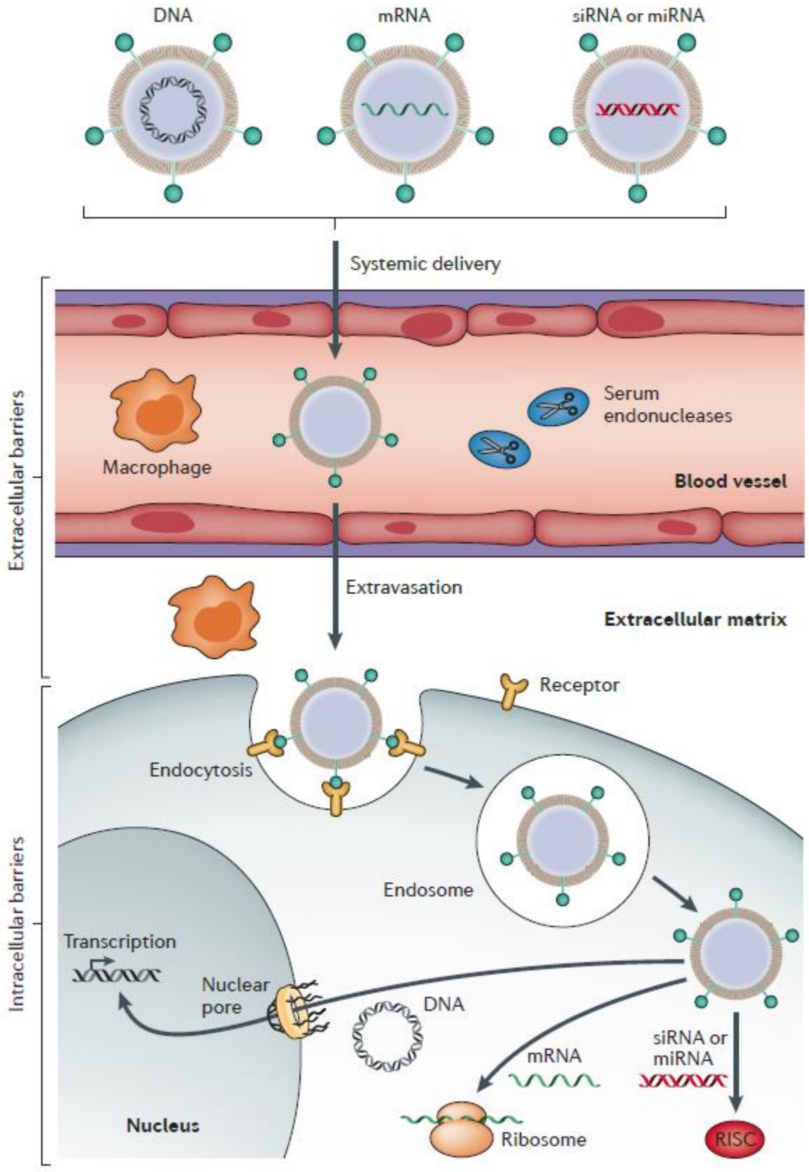
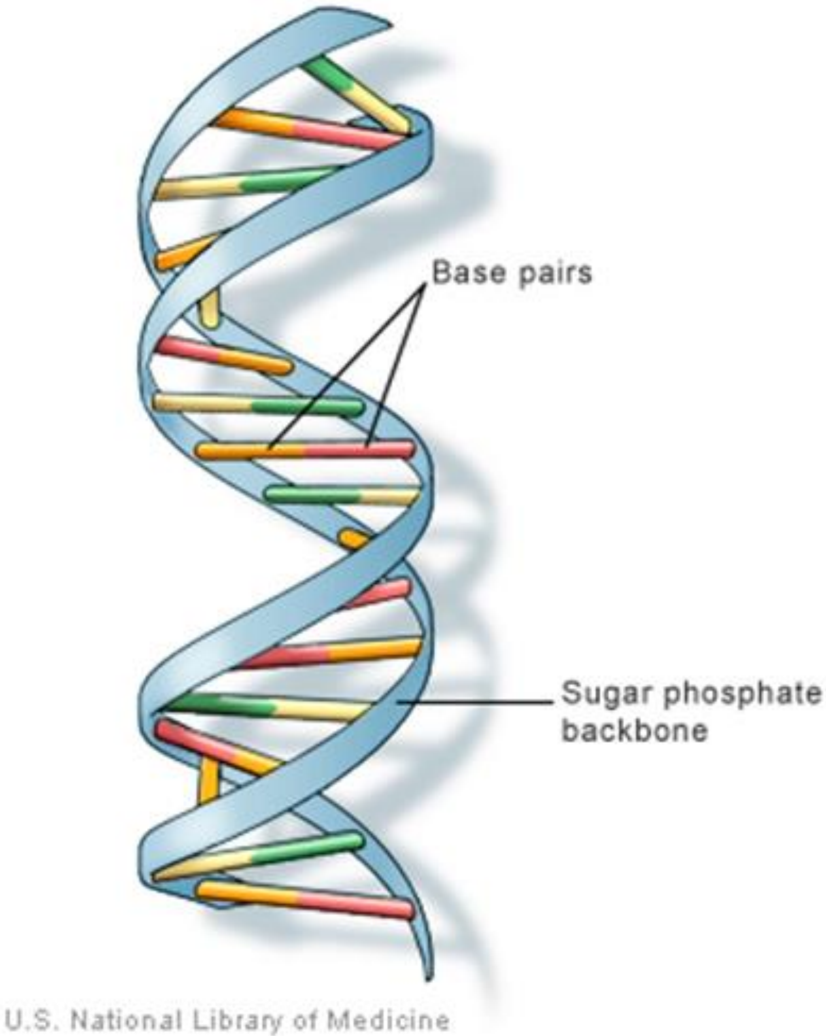
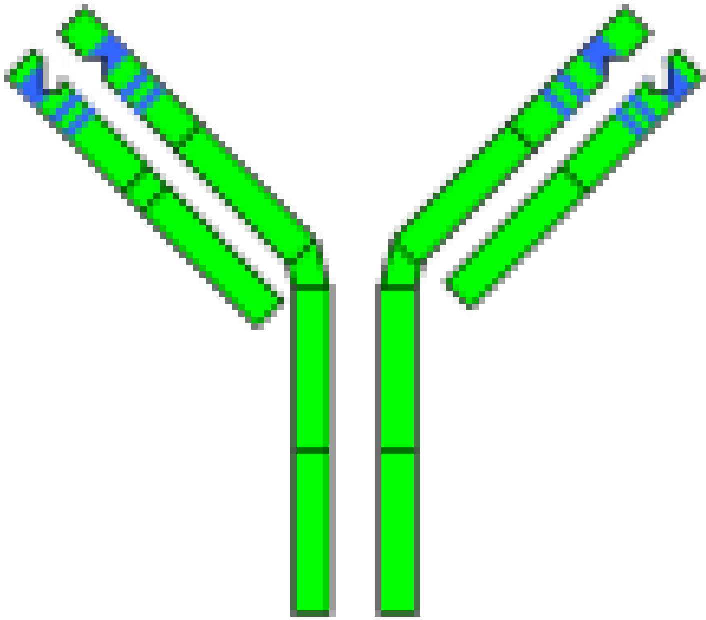


Natural barriers
DNA / RNA Biochemistry



NIH

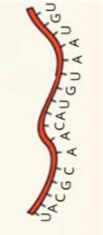
Natural barriers
DNA / RNA Biochemistry



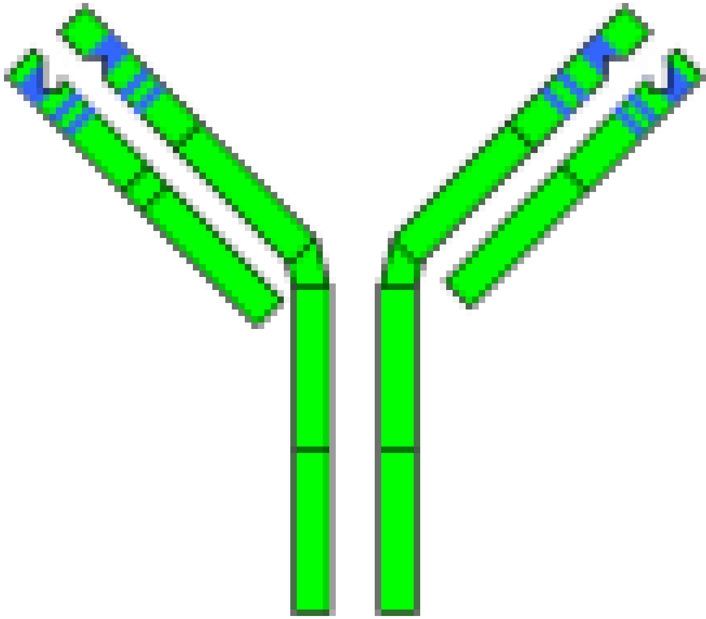
Aspirin 0,18 kda | RNA ? | Antibodies 150 kda

Natural barriers
DNA / RNA Biochemistry

Aspirin 0,18 kda

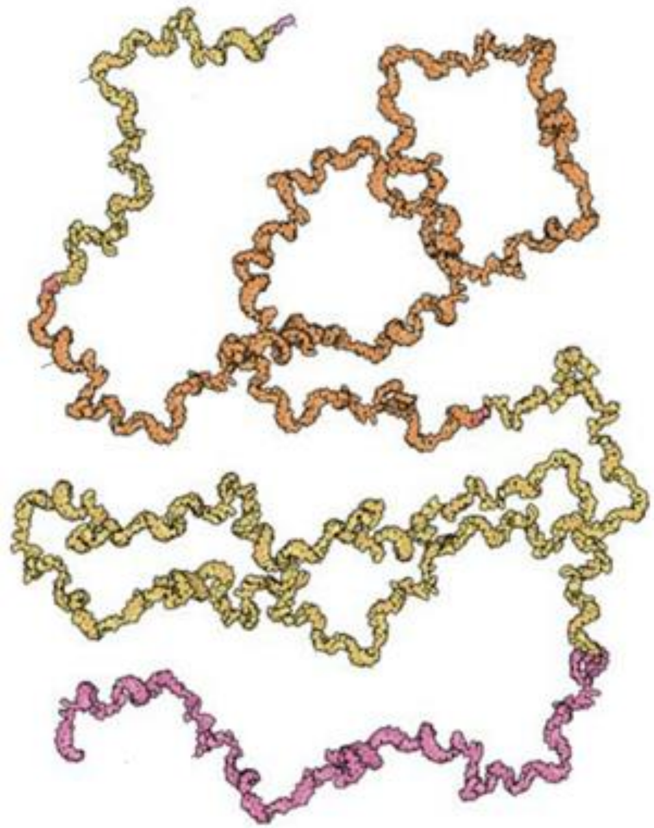


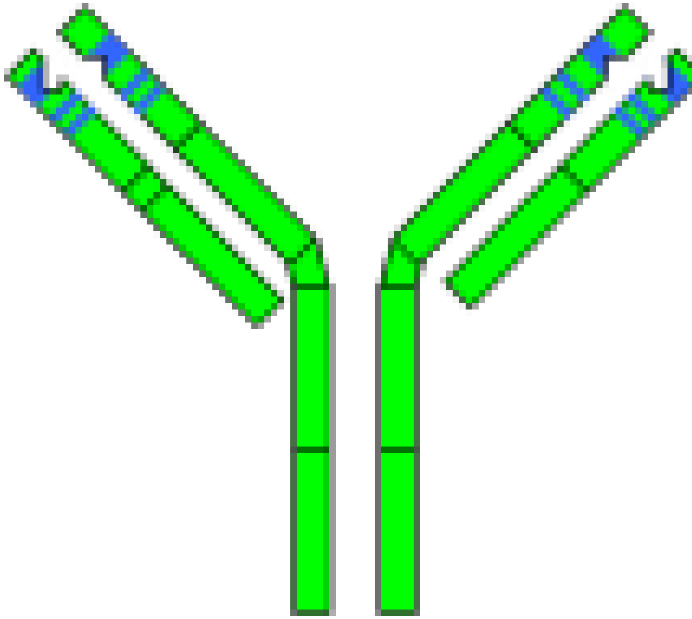
iRNA ~10 *kDa*



Antibodies 150 kda

Acides nucléiques à visées thérapeutiques



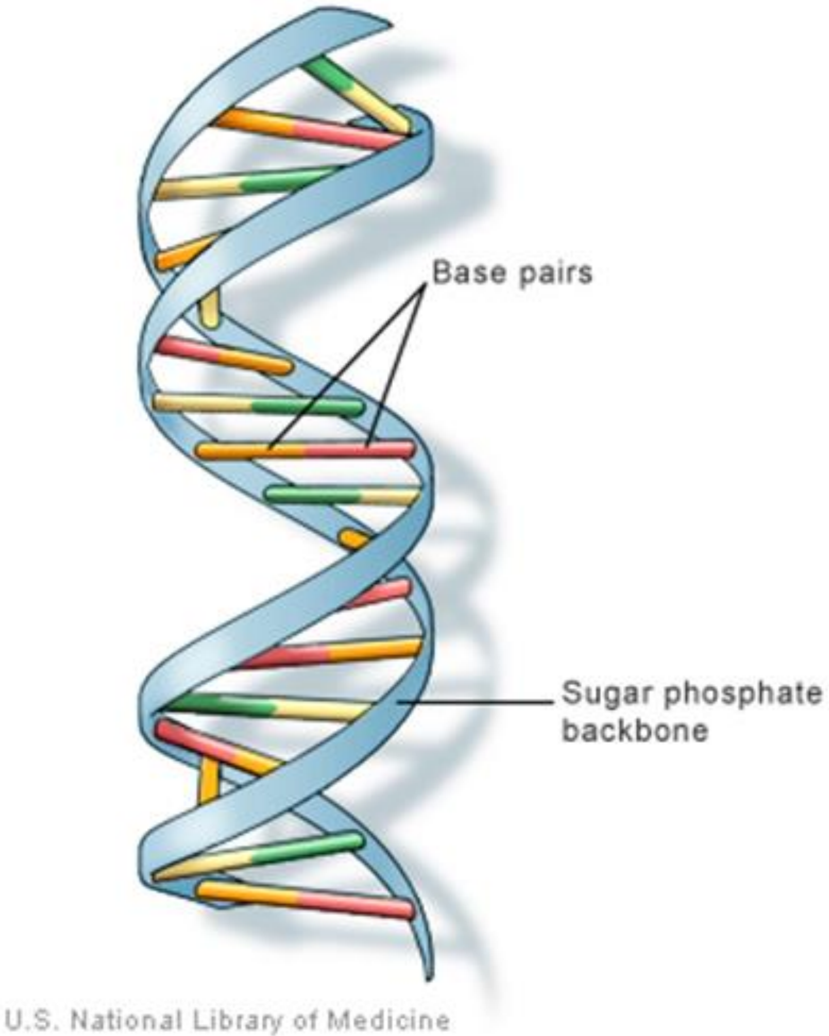


Aspirine 0,18 kda

mRNA ~400 *kDa*

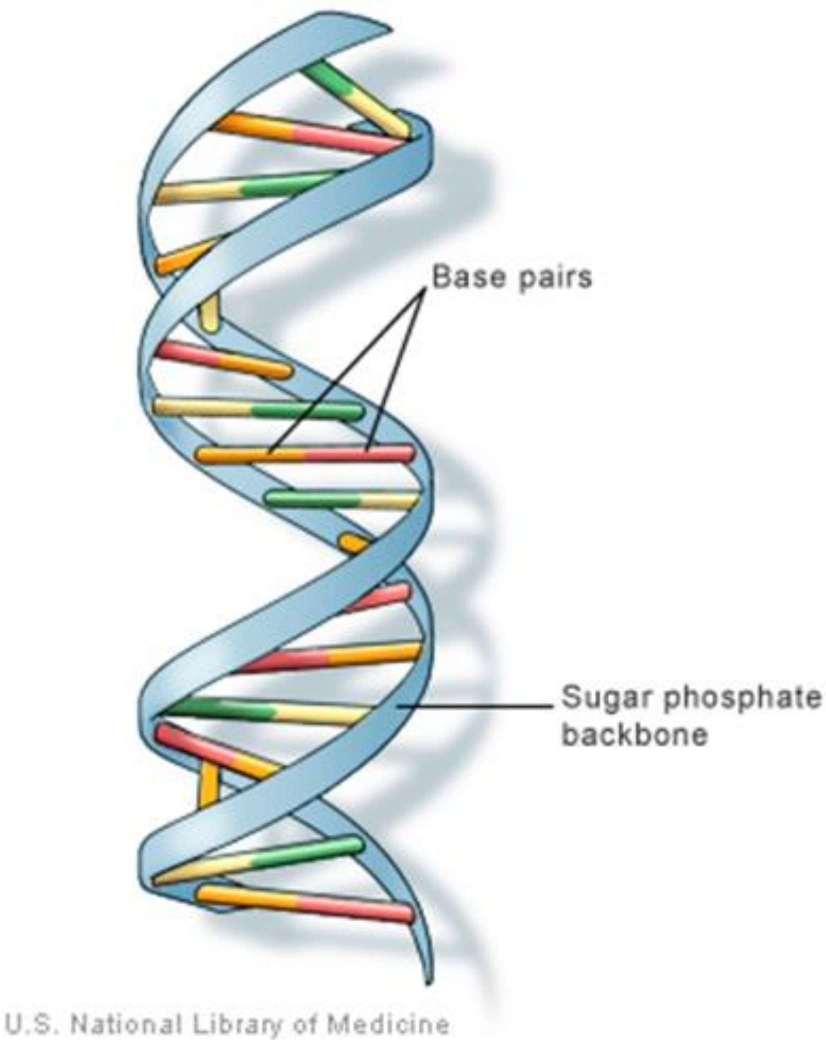
Antibodies 150 kda

Natural barriers
DNA / RNA Biochemistry



- Low stability
- High molecular weight
- Negative charge
- Hydrophilicity

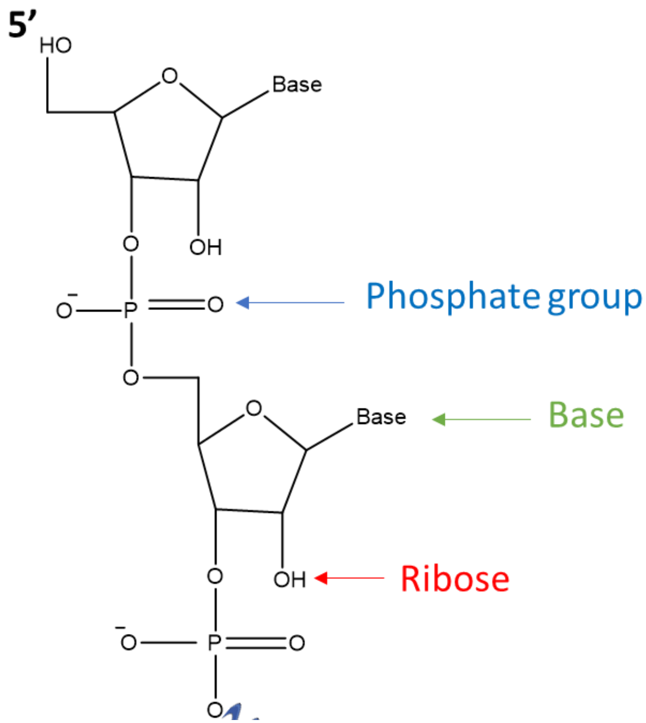
Natural barriers
DNA / RNA Biochemistry



- Low stability
- High molecular weight
- Negative charge
- Hydrophilicity

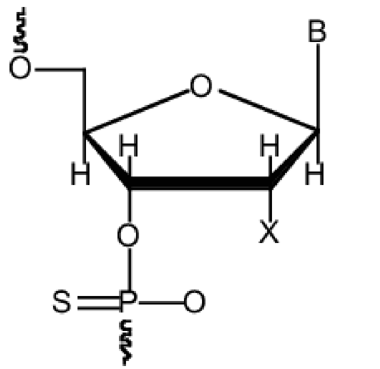
→ Need modification and/ or vectorisation!

NIH

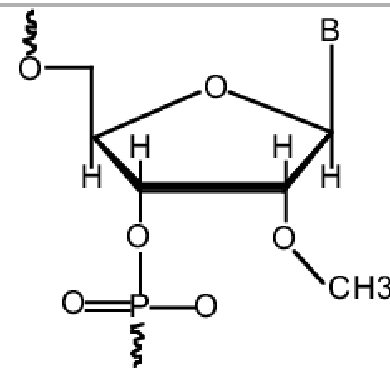

5'
HO
O
Base
O
OH
O
P
O
Phosphate group
O
O
Base
Base
O
OH
Ribose
O
O
P
O
O

DNA / RNA Biochemistry

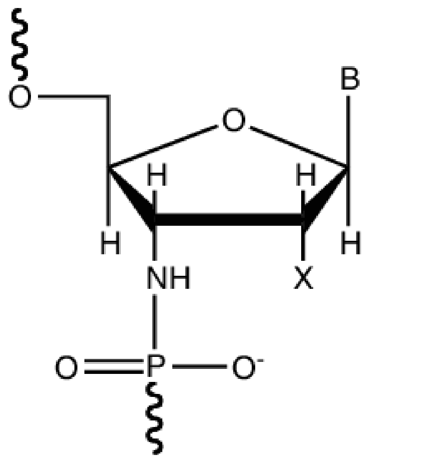
BACKBONE STRUCTURE | **SUGAR RING**



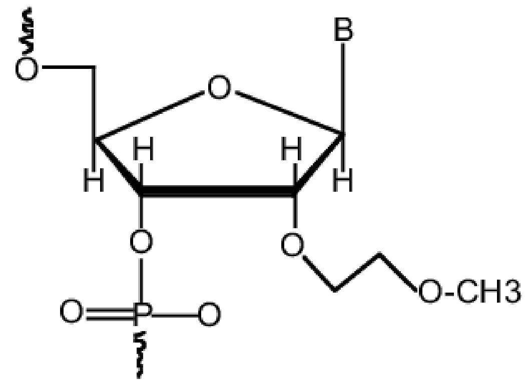
Phosphorothioate (PS)



2′*O*-Methyl (2′*O*-Me)



N′3 Phosphoramidate (NP)



2′*O*-Metoxyethyl (MOE)

Duarte Moreno DOI:10.3389/fchem.2014.00087

Vectors

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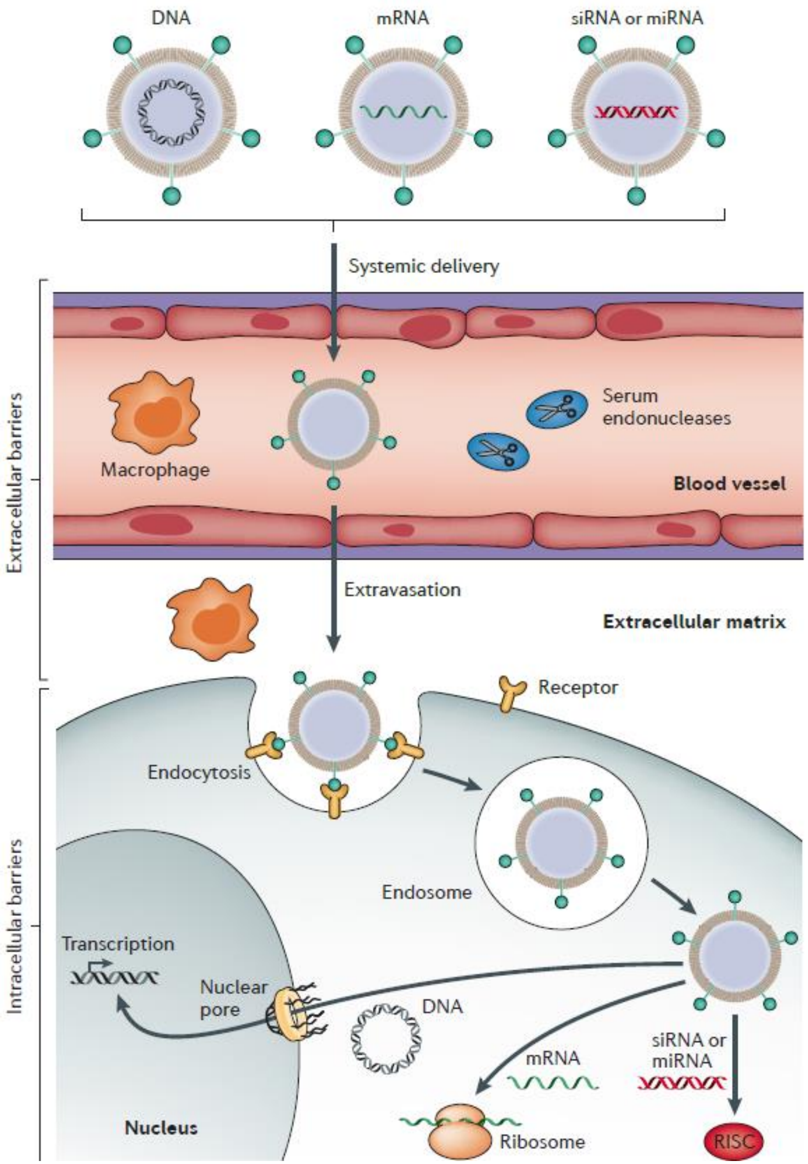
Viral Vectors

Strengths

- High transfection efficiency
- Natural tropism (ability to infect different cells)
- Evolved mechanisms for endosomal escape
- Natural transportation mechanism of DNA into nucleus

Weaknesses

- Strong immune reactions against viral proteins prohibit multiple administrations
- Possibility of chromosomal insertion and protooncogene activation
- Complicated synthesis process
- Limitation on gene size
- Toxicity, contamination of live virus



Design criteria

?

Verbeke et al Nanotoday
https://doi.org/10.1016/j.nantod.2019.100766
Yin et al Nature Reviews Genetics 2014

viral and non-viral vectors
Some design criteria

- Packaging of large DNA/RNA quantities
- Easy administration
- Serum stability
- Targetability to specific cell types
- Inexpensive synthesis
- Easy purification
- Robustness/stability

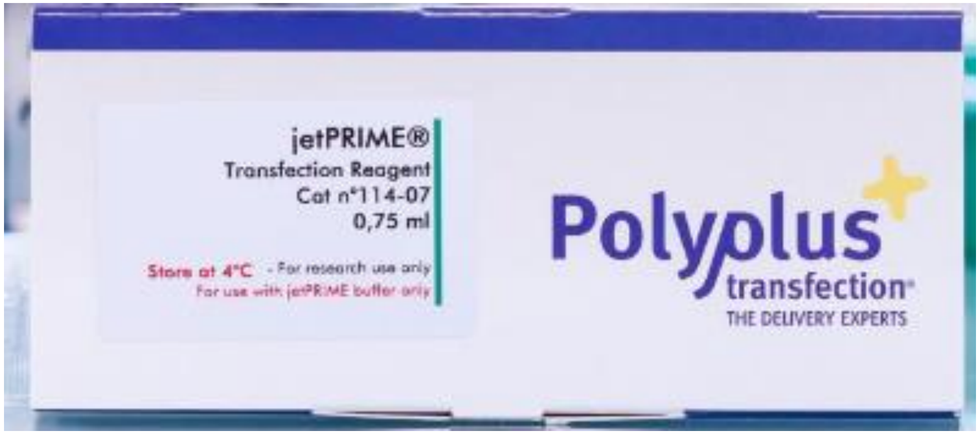
- Cell Internalization
- Endolysosomal escape
- Nuclear transport
- Efficient unpackaging
- Infection of non-dividing cells
- Safety
- Non-toxic
- Non-immunogenic
- Non-pathogenic

Non-viral vectors

Université Paris-Saclay
Faculté de Pharmacie

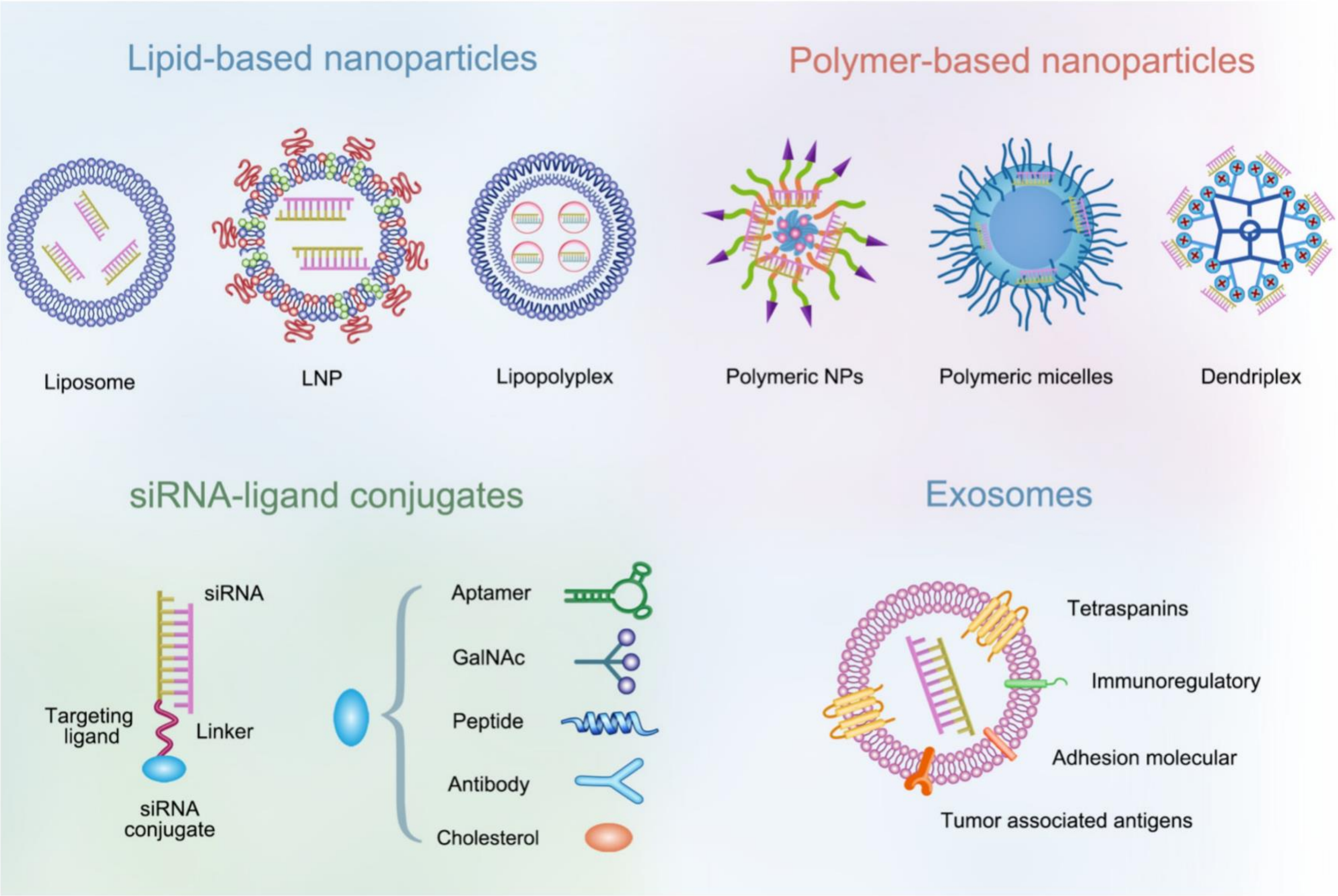
Huang https://doi.org/10.3390/pharmaceutics14081586

Non-viral vectors



Invitrogen / Polyplus

Non-viral vectors



Lipid NanoParticles

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Ionisable lipid

% molar ratio : 30-50%

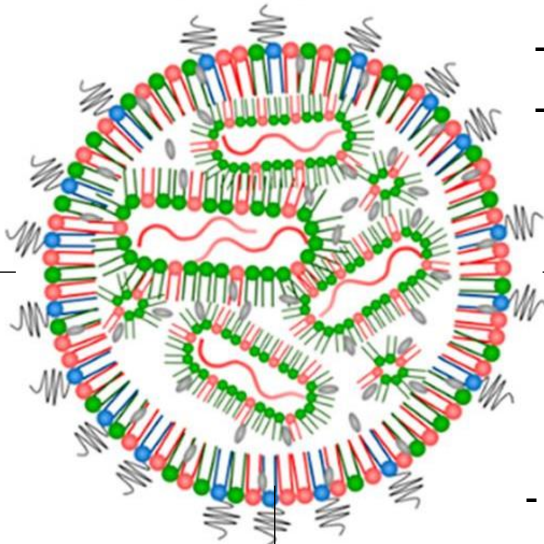
- pKa <7, usually around 6
- Electrostatic interactions with RNA
- Involved in endosomal escape

Cholesterol

% molar ratio : 30-50%

-Stability and membrane fluidity

-Helps cellular internalization



Helper lipid

% molar ratio : 10-30%

-Influence LNP structure

-Involved in endosomal escape

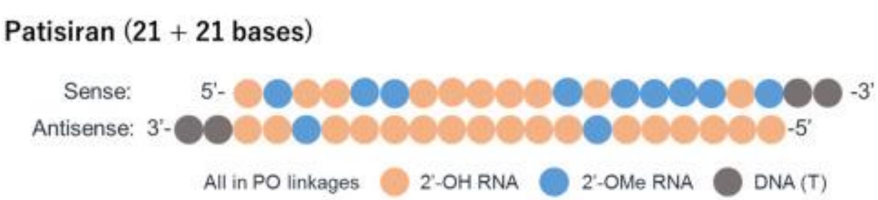
- Surface protein interaction

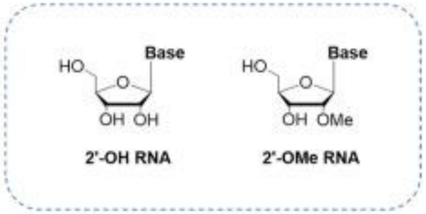
→ *in vivo* behavior ?

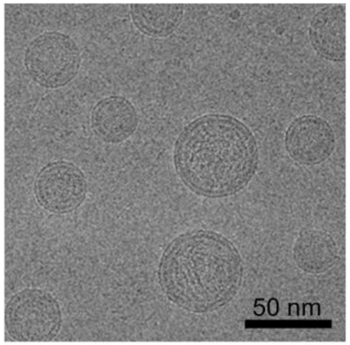
PEGylated lipid

% molar ratio : <2%

- Promotes colloidal stability
- Prevents protein aggregation
- Modulate size, transfection efficiency

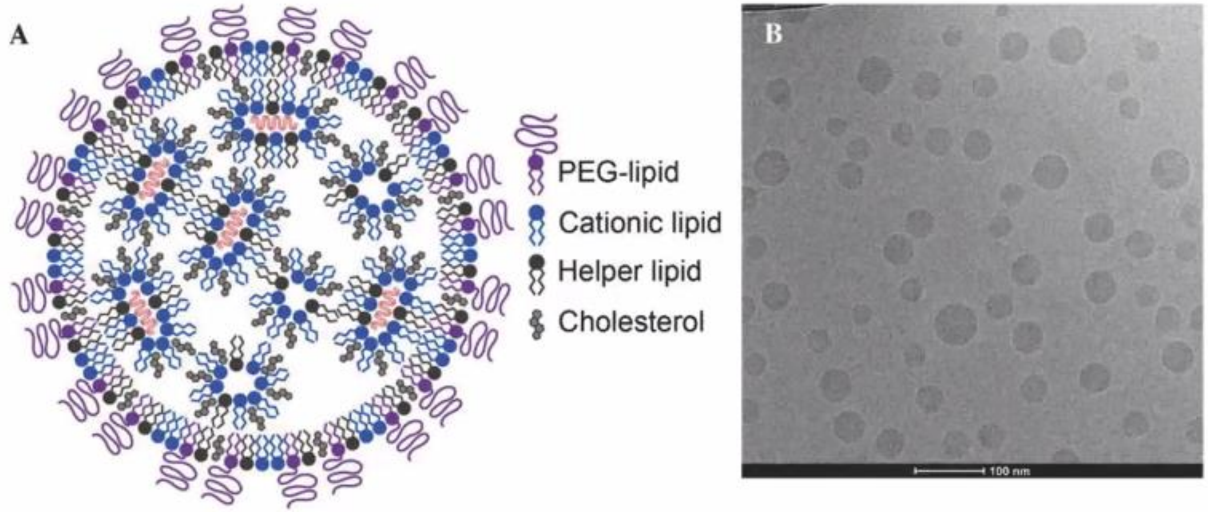




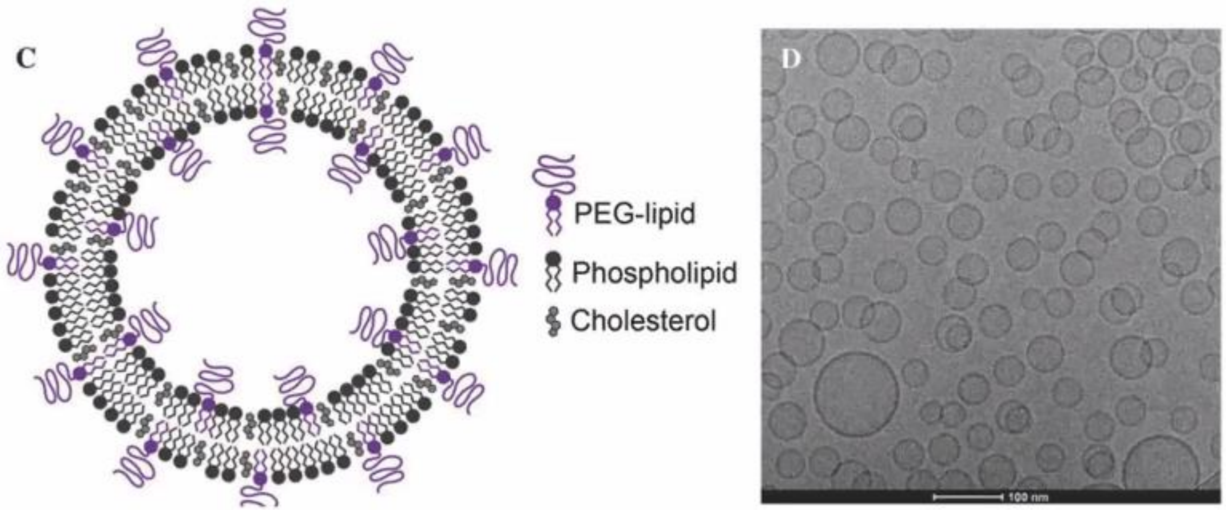


Lipid NanoParticles

lipid nanoparticle



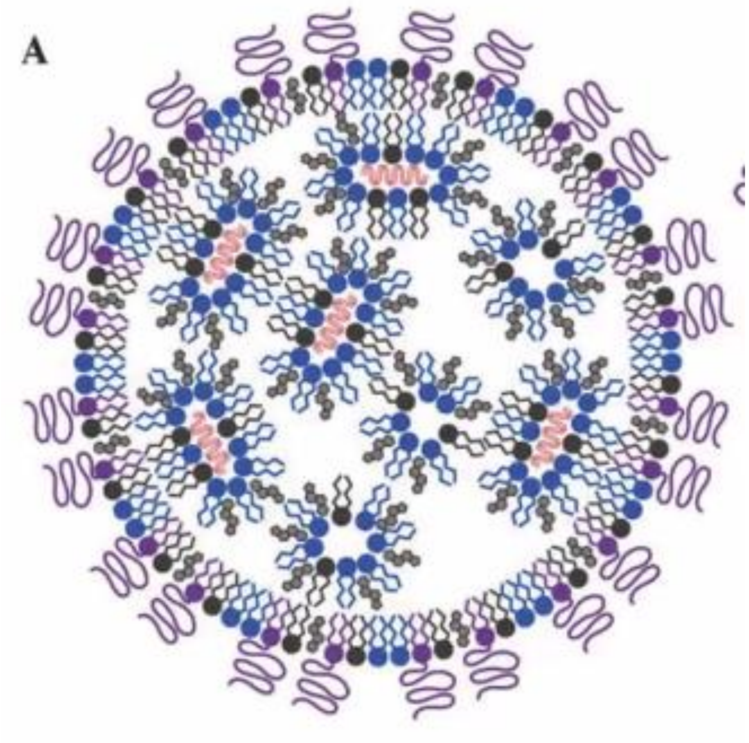
liposome

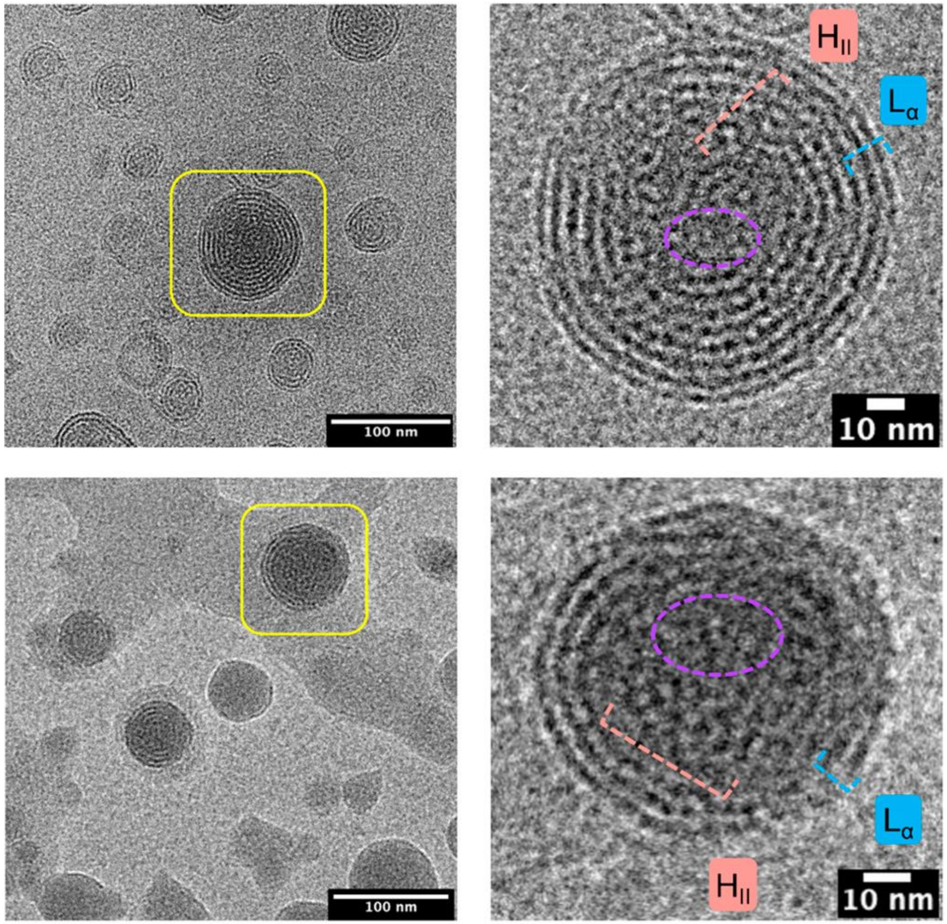


ACS Nano **2018**, *12*, 4787−4795

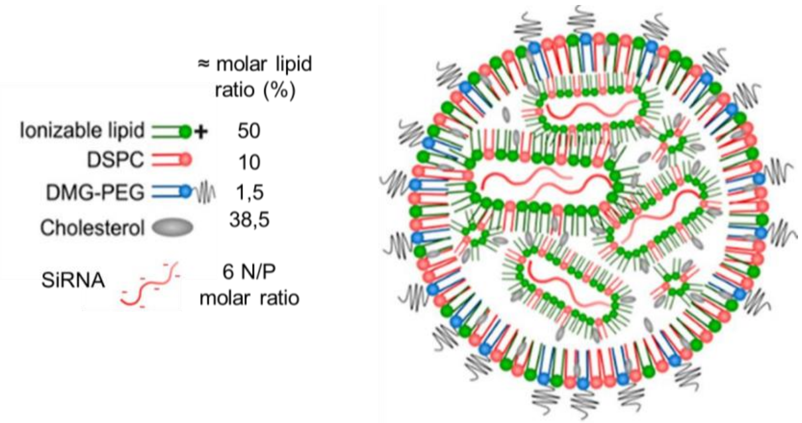
http://nano.petrcigler.cz

Lipid NanoParticles



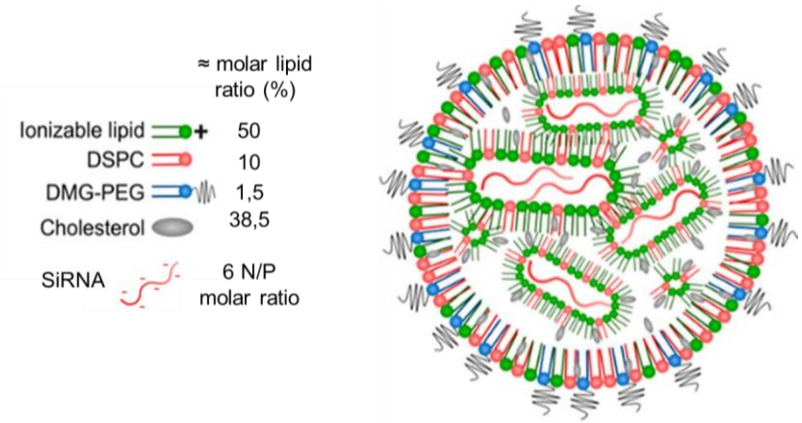


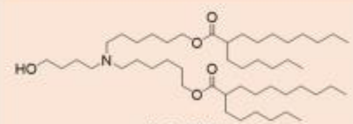
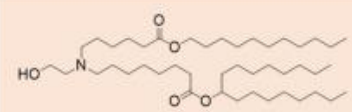
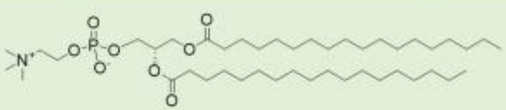
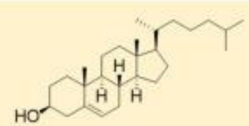
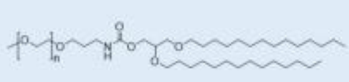
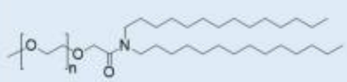
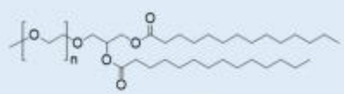
mRNA LNP formulation



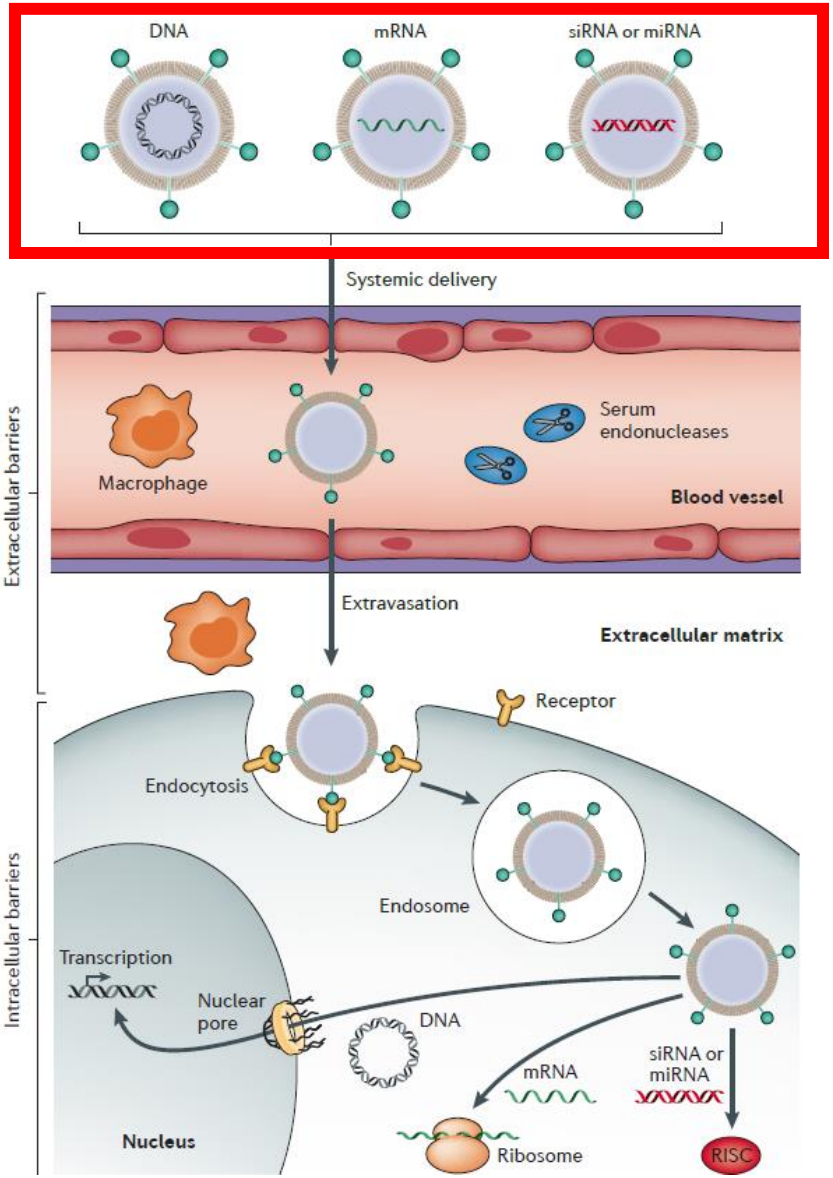
Active ingredient (Company)	Patisiran (Alnylam) Onpattro	Tozinameran (Pfizer/BioNTech) Comirnaty	Elasomeran (Moderna) Spikevax

mRNA LNP formulation



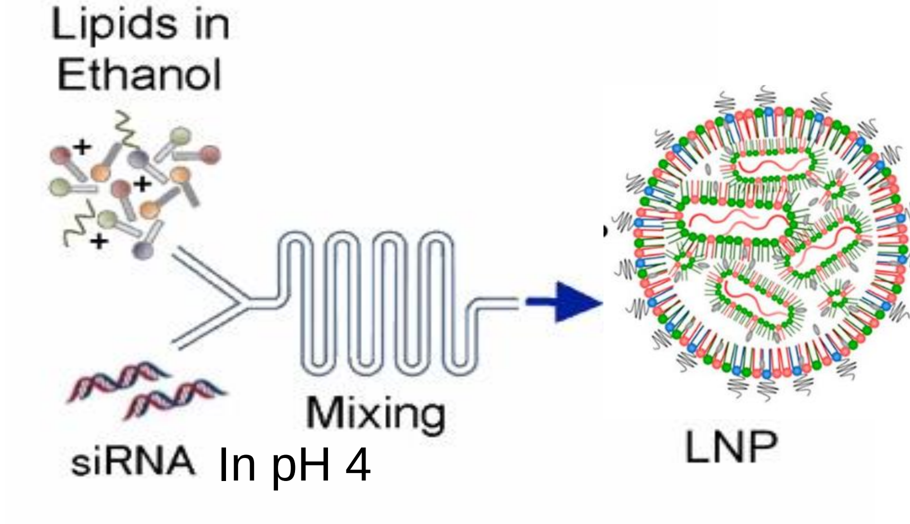
Active ingredient (Company)	Patisiran Onpattro (Alnylam)	Tozinameran Comirnaty (Pfizer/BioNTech)	Elasomeran Spikevax (Moderna)
Ionizable lipid	 DLin-MC3-DMA	 ALC-0315	 SM-102
Phospholipid	 DSPC		
Sterol	 Cholesterol		
PEG-lipid	 PEG_{2000}-C-DMG	 ALC-0159	 PEG_{2000}-DMG

Adapted from U. Elia [D.Peers] et al ACS Nano 2021



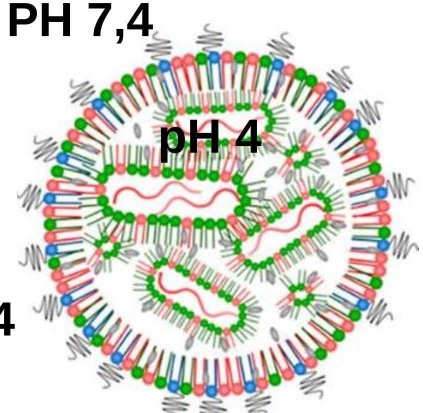
LNP

2) mixing



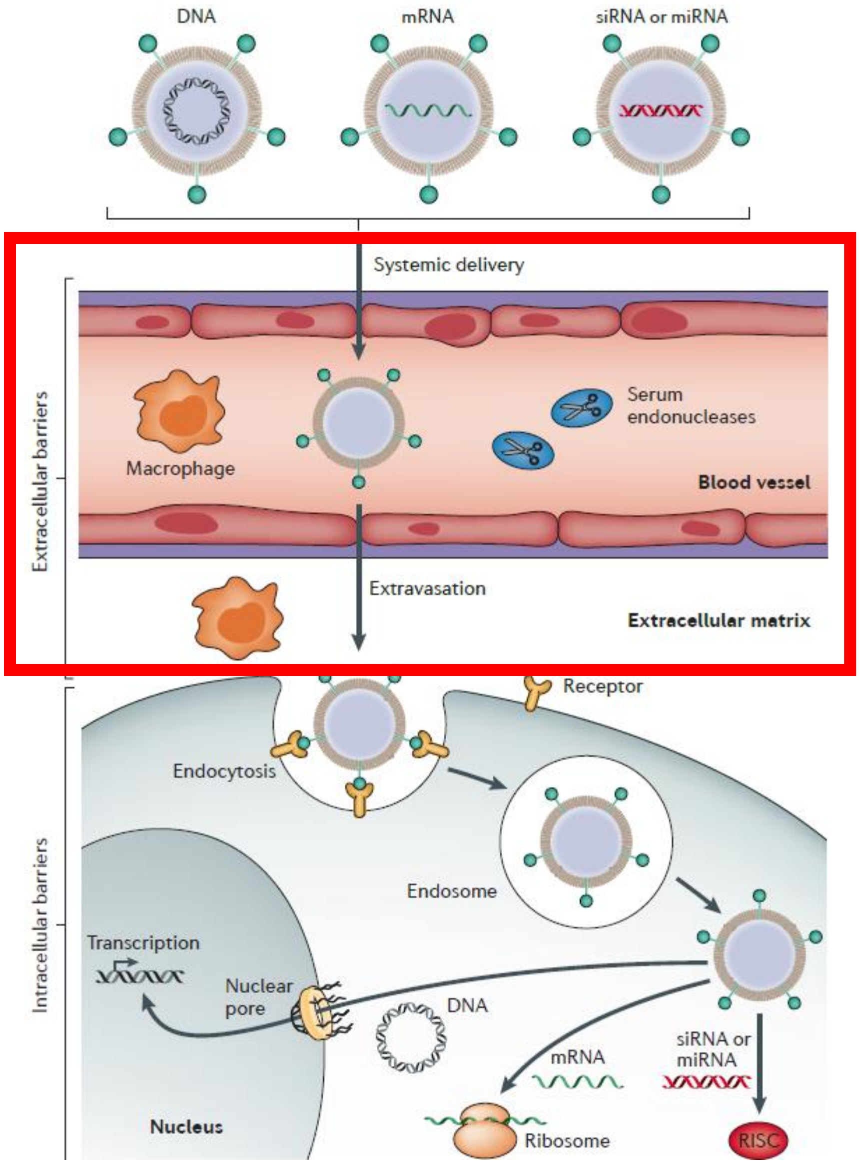
2) dialyse PH 7,4

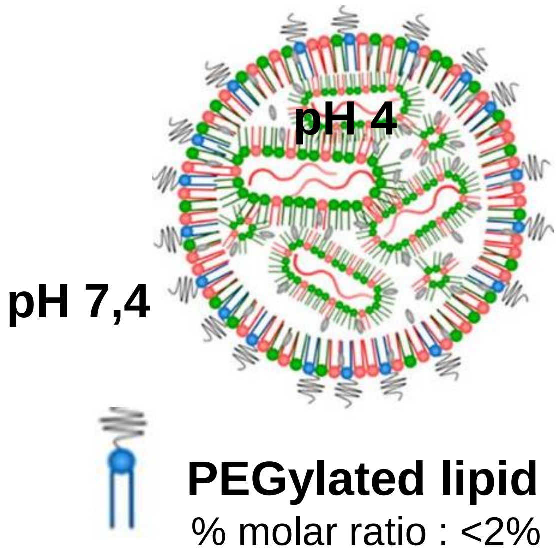
pH 7,4



Mizrahy S. et al. Molecular Therapy 2017

Natural barriers
Physiological barriers

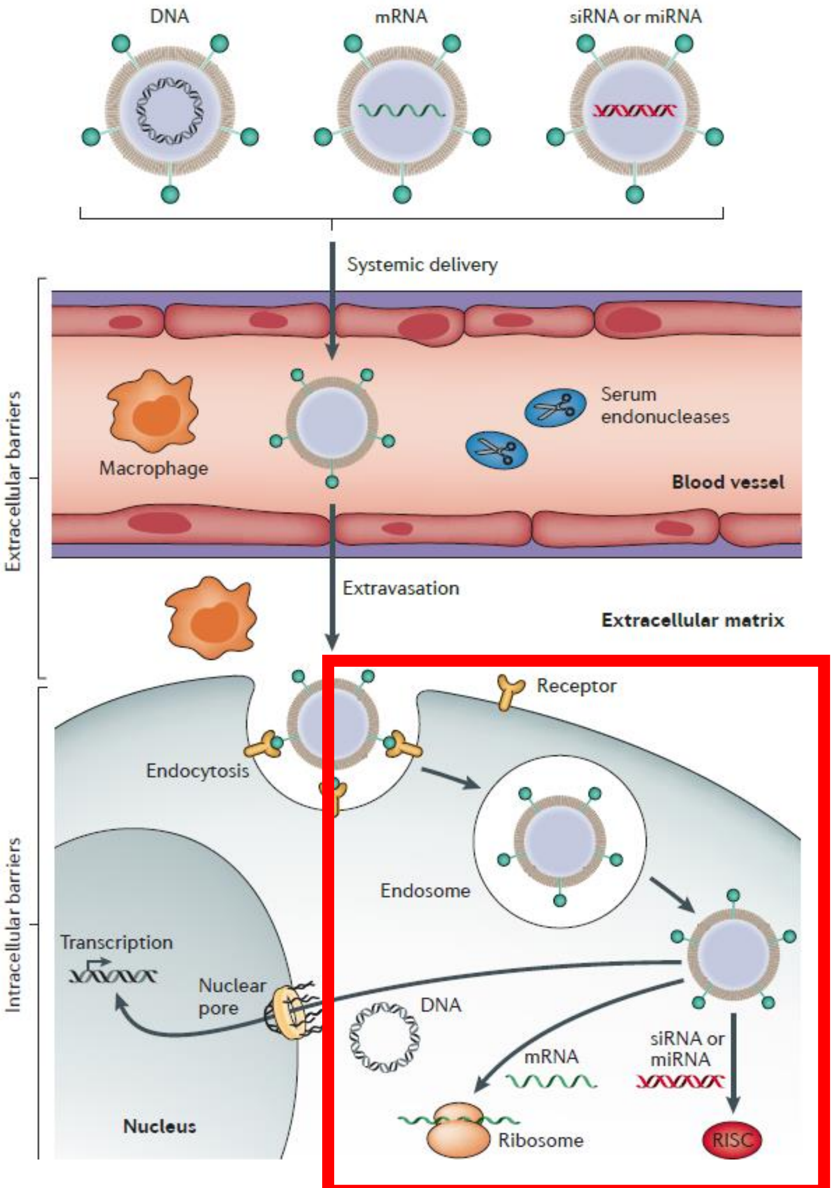




PEGylated lipid
% molar ratio : <2%

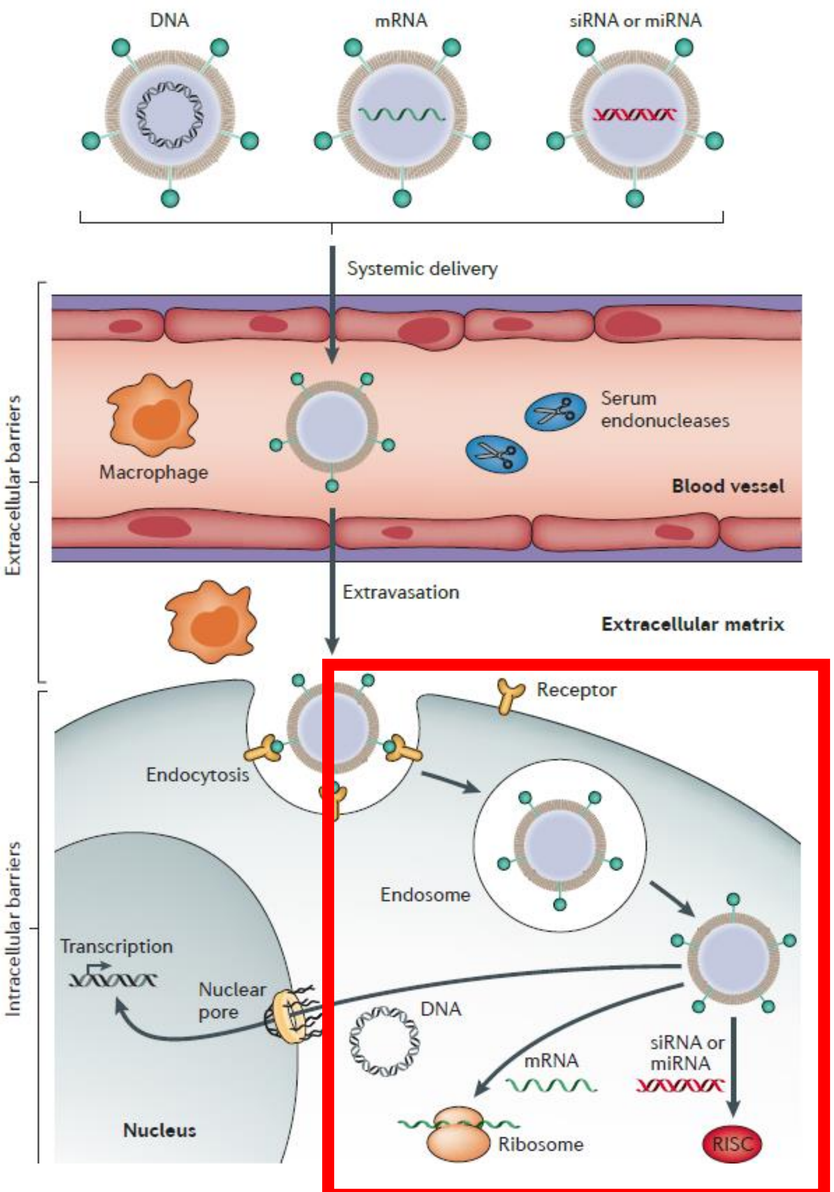
- Promotes colloidal stability
- Prevents protein aggregation
- Modulate size, transfection efficiency

Natural barriers
Physiological barriers

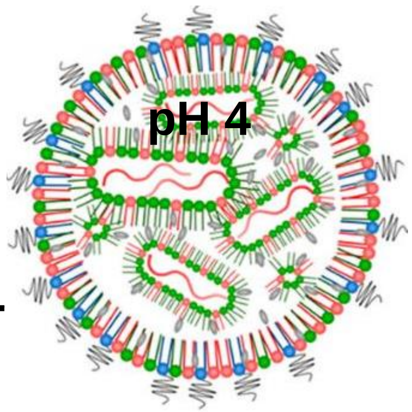


Yin et al Nature Reviews Genetics 2014

Natural barriers
Endosomal escape



- Ionizable Cationic Lipids: pKa 6.2–6.5



~~pH 7,4~~

pH 5

Natural barriers
Endosomal escape

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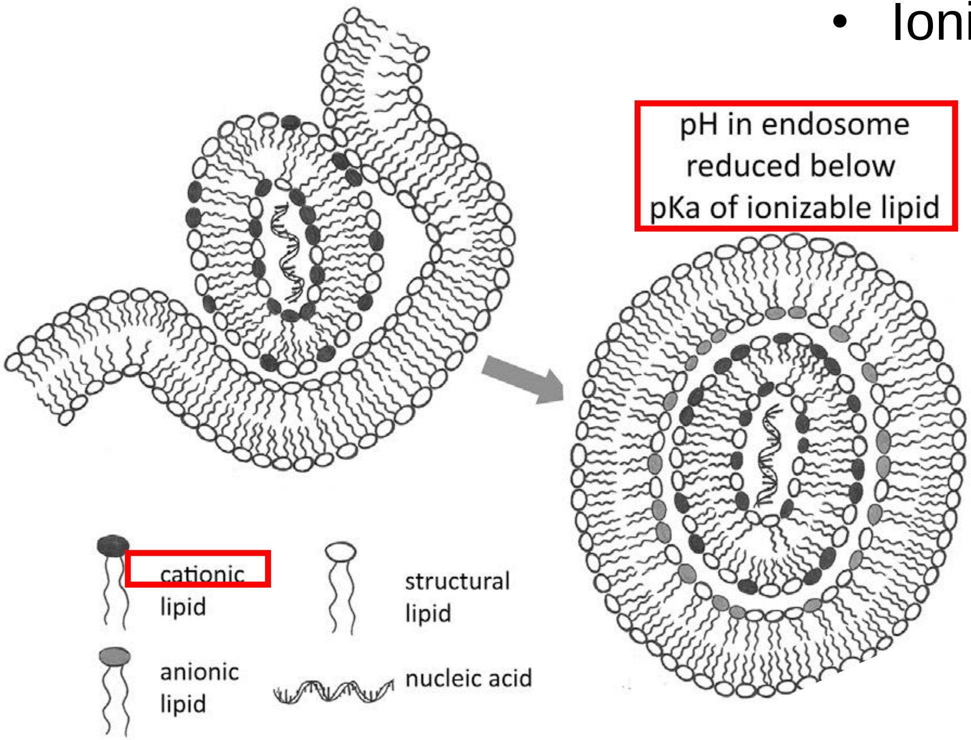

Natural barriers
Endosomal escape

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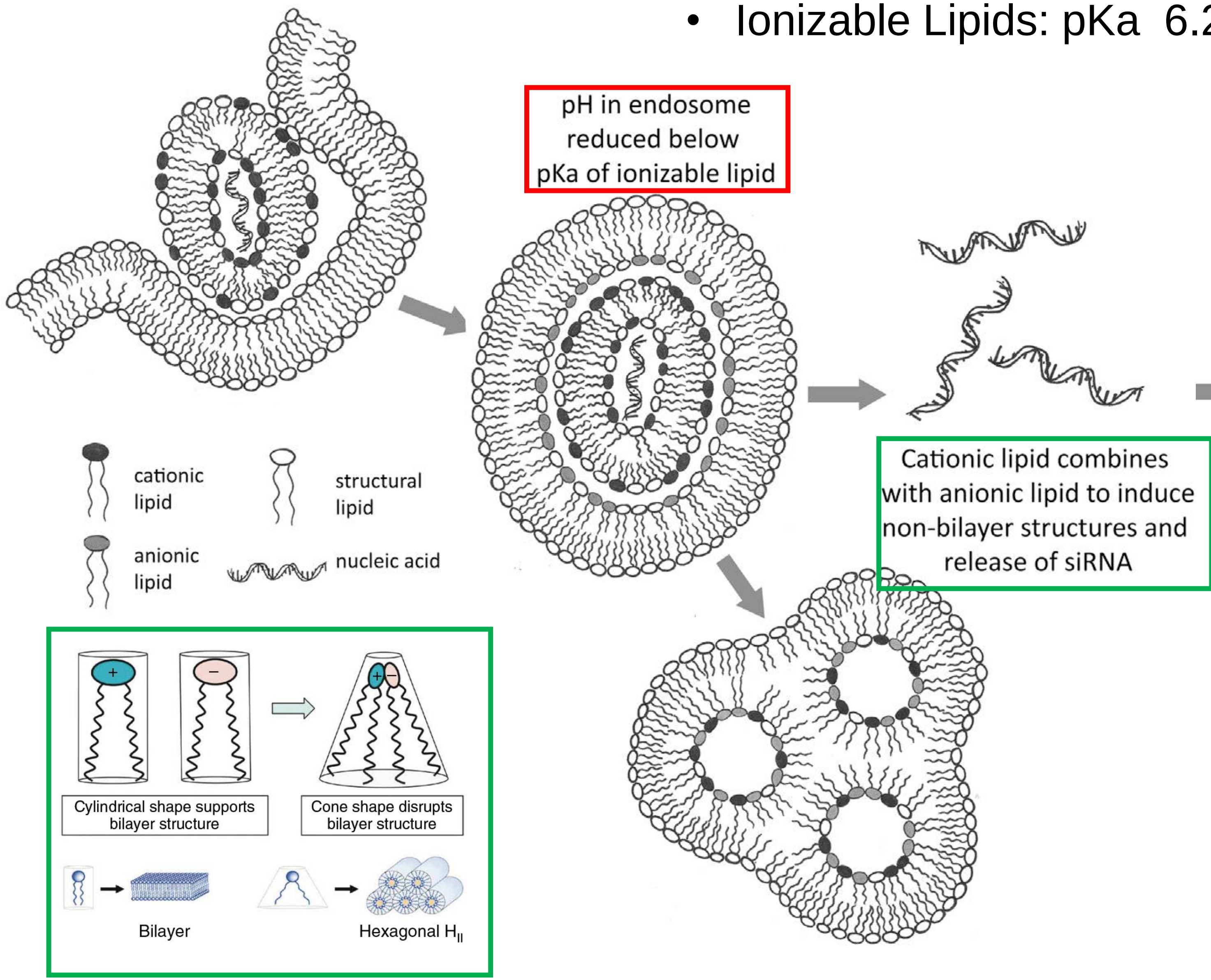

Effect

Cullis et al Mol Ther. 2017

Ionizable Lipids (pKa 6.2–6.5)

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Evolution of ionizable lipid during Patisiran development

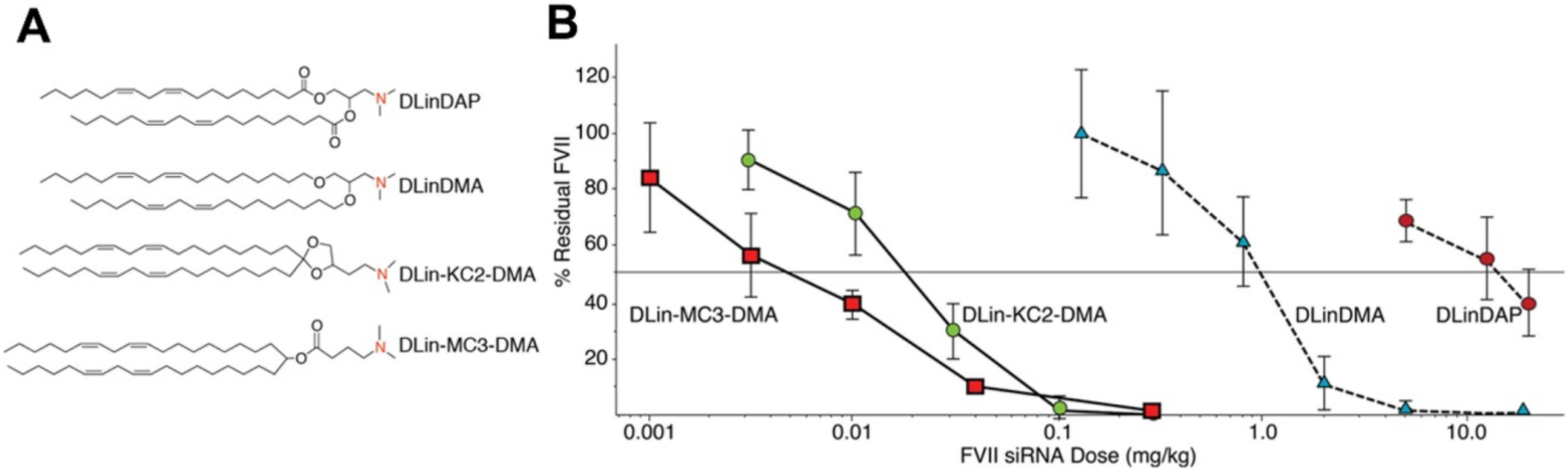

Figure 2. Evolution of ionizable cationic lipids enabling gene silencing *in vivo*. (A) DLinDMA, an ether-linked variant of DLinDAP, was the first lipid that enabled appreciable LNP-siRNA gene silencing activities *in vivo* following intravenous administration. Subsequent studies identified KC2 and MC3 lipids as potent successors with the optimal structure and pK_a necessary for siRNA delivery. (B) *In vivo* optimization of LNP-siRNA systems using the FVII mouse model. Over 300 species of ionizable cationic lipids were synthesized, formulated in LNP-siRNA, and screened in the FVII model. This led to the identification of the current "gold standard" lipid MC3, which resulted in improved LNP-siRNA potency by 4 orders of magnitude compared to DLinDAP, with no increase in toxicity. MC3 was subsequently employed in the patisiran formulation.[4,5,17]

Kulkarni [Cullis] et al ACR 2019

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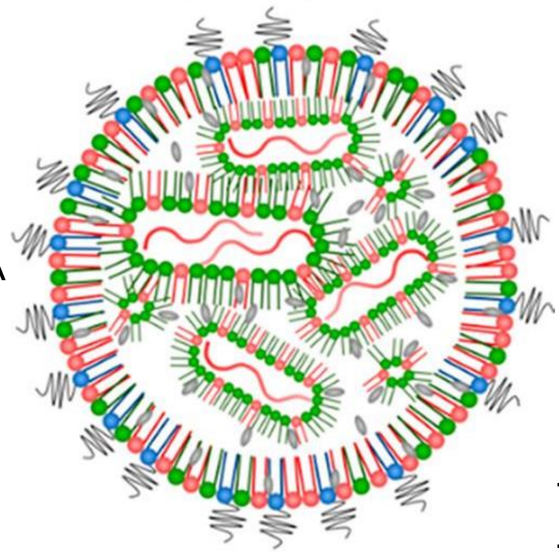

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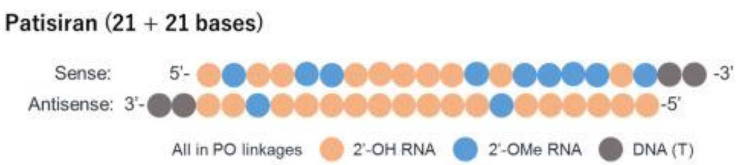

Organ/cell targeting
RNA half-life
off target effect
Price
Inflammation