AWP 2-4 – Polymer Chemistry

Polymers in Life Science – D) Gene Delivery

University of Potsdam

Matthias Hartlieb

Summer term 2025

A fraction of the slides copied or adapted with curtesy of Dr. Anja Träger (FSU Jena)

Overview

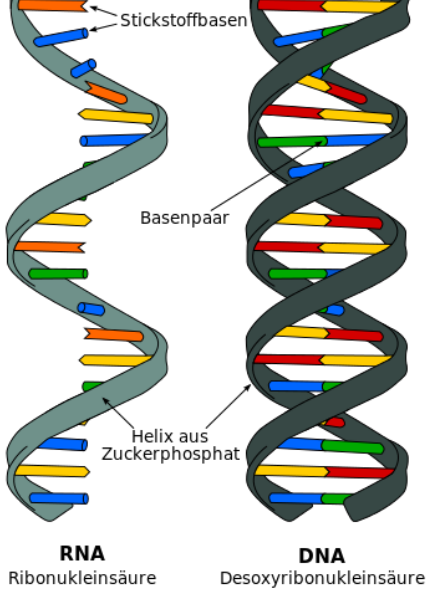
- DNA/RNA as a drug
- Gene delivery - concept
- Polymers in gene delivery
- Polyplexes and analytics
- Biological assays

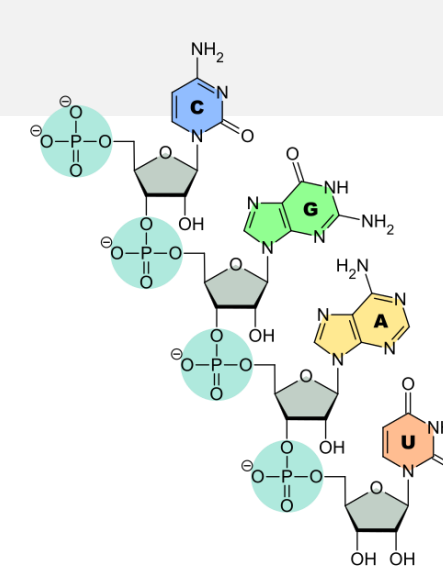
Learning objectives

- Understand how nuclei acid-based biomolecules can be used in therapy
- Be able to discuss the differences between regular drug delivery and gene delivery
- Know about the hurdles for gene delivery (particular on a cellular level) and be able to suggest ways to overcome them
- Know some examples of polymers that can be used for gene delivery and how they form polyplexes
- Be ablet o suggest ways to characterize polyplexes and their properties

Gene transport

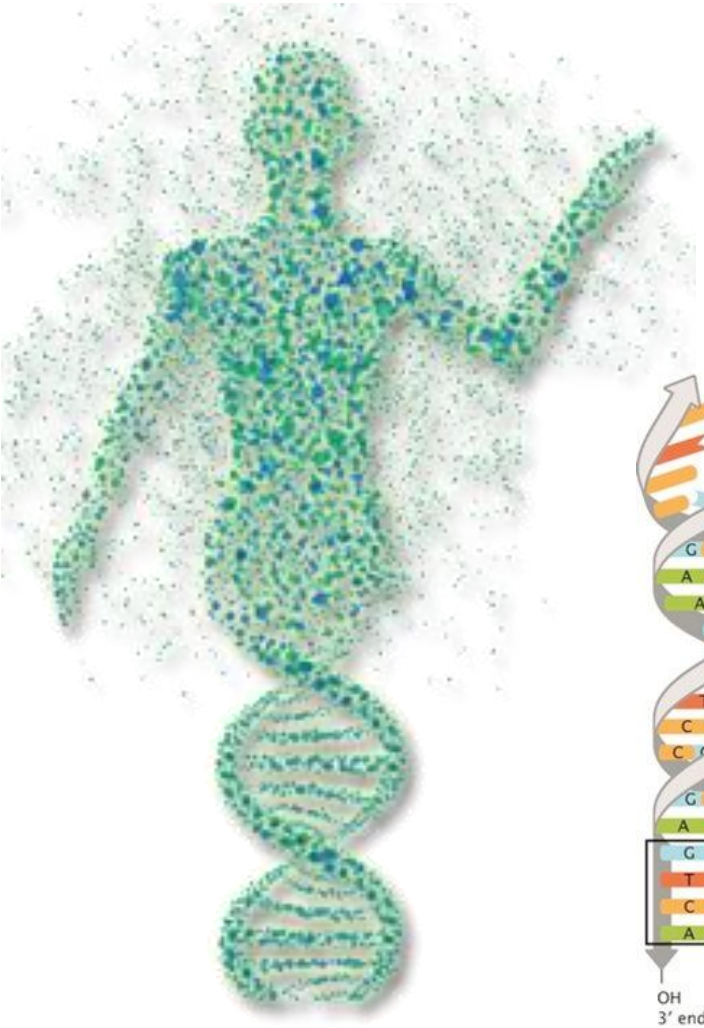
- Delivery of genetic material into cells
- DNA has to reach the nucleus (reprogramming of the cell)
- RNA has to reach the cytosol (protein production or inhibition)
- Gene therapy is promising for many issues:
  - Genetic or auto immune diseases
  - Cancer therapy
  - **vaccinations** BIONTECH
- Problems:
  - D/RNA is instable (Degradation by enzymes)
  - D/RNA is negatively charged (no uptake from cells)

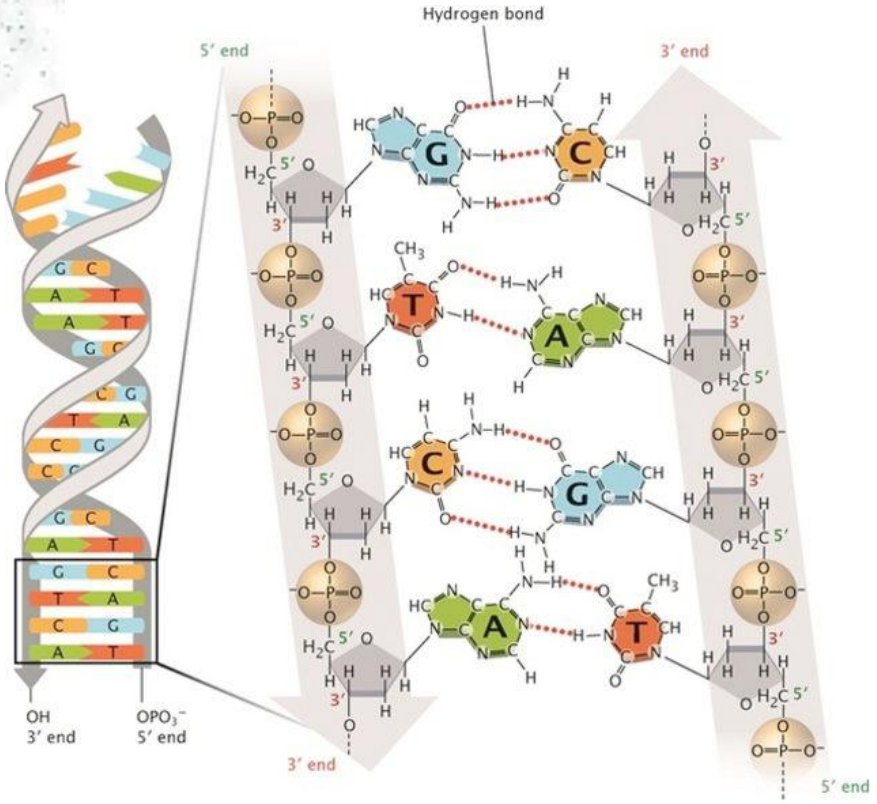


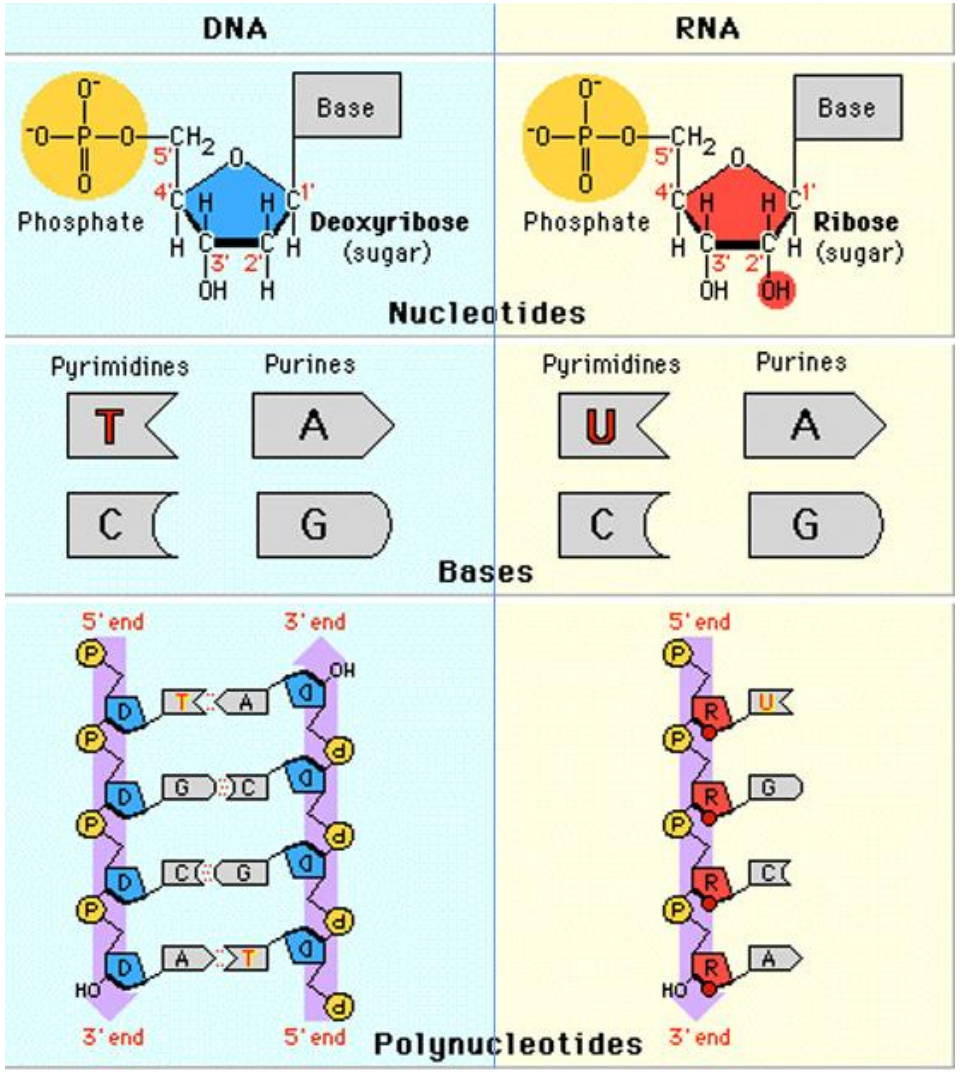


Nobel Prize for Chemistry 2020
Emmanuelle Charpentier and Jennifer A. Doudna
„for the development of a method for genome editing“.

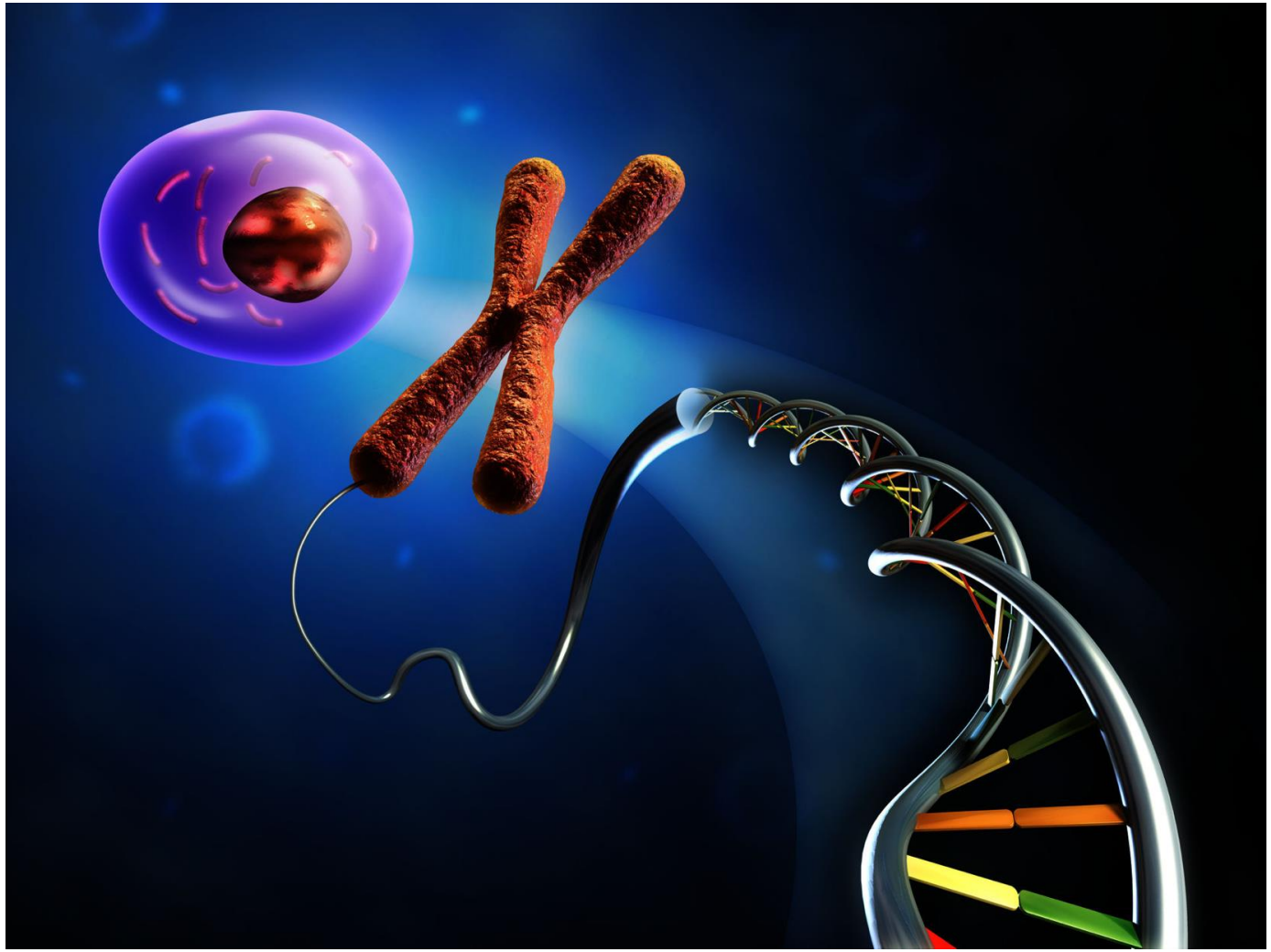
Genetic material: biological complexity with chemical simplicity (?)





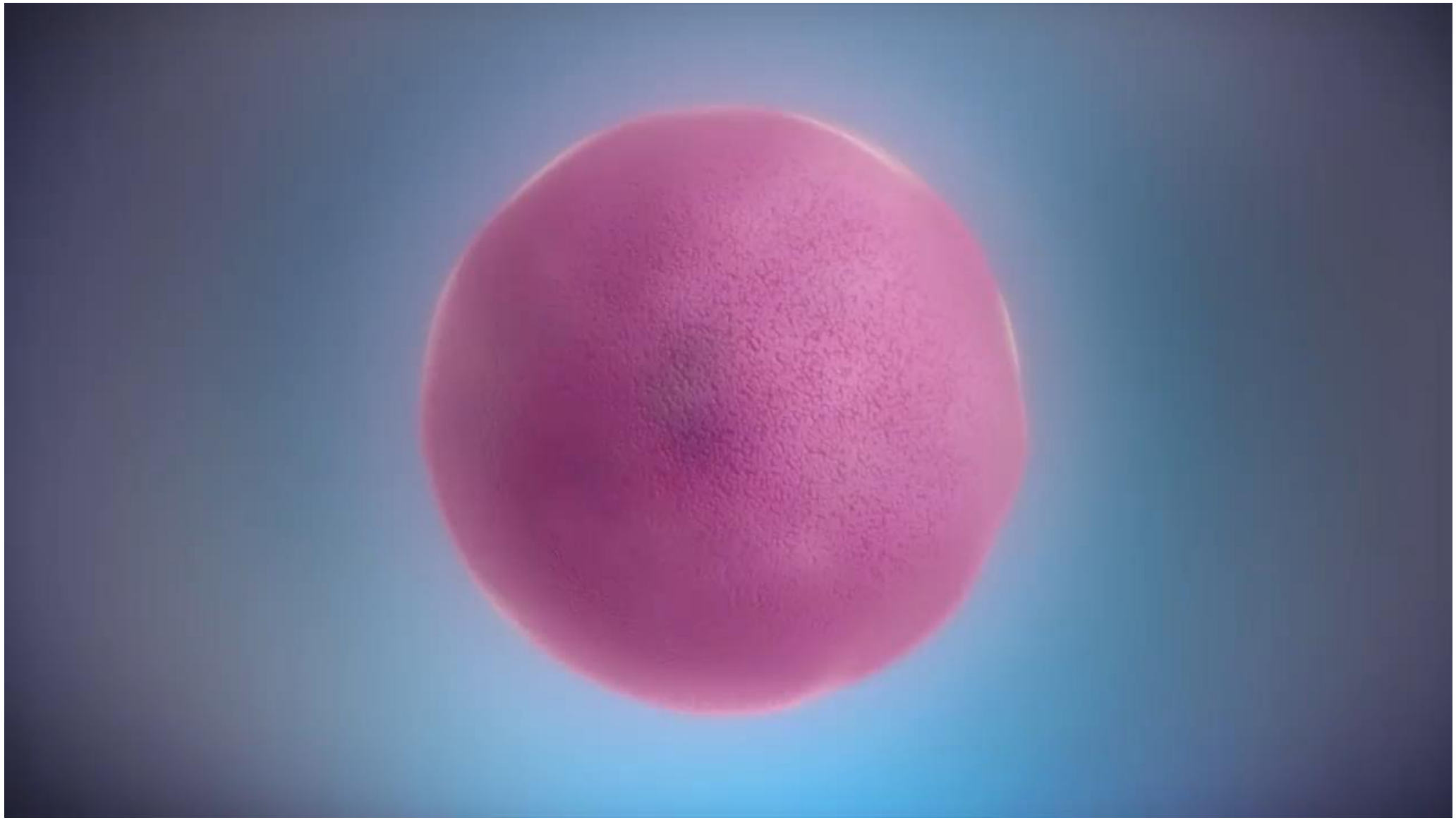


Genetic diseases



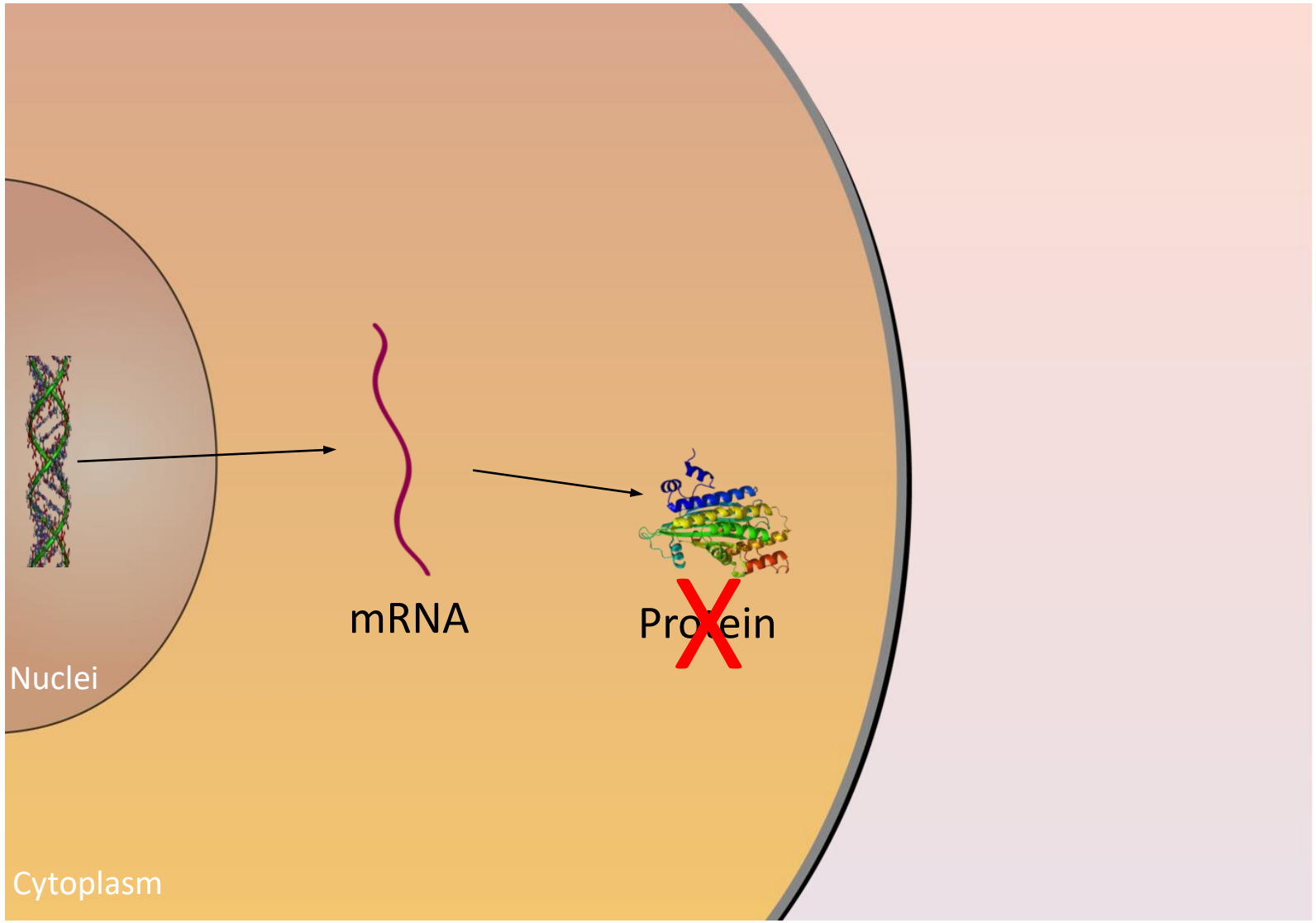
- Cystic fibrosis
- beta thalassemia
- Autosomal dominant diseases
- Neurofibromatosis type 1 & 2
- Tuberous cerebral sclerosis
- von Hippel-Lindau syndrome
- Sturge-Weber Syndrome
- Klippel-Trenaunay Syndrome
- osteogenesis imperfecta
- Duchenne Muscular Dystrophy Type
- Muscular dystrophy type Becker
- Haemophilia
- Familial hypophosphatemia
- Incontinentia pigmenti Bloch-Sulzberger
- Rett syndrome

Gentherapie – Was ist das?



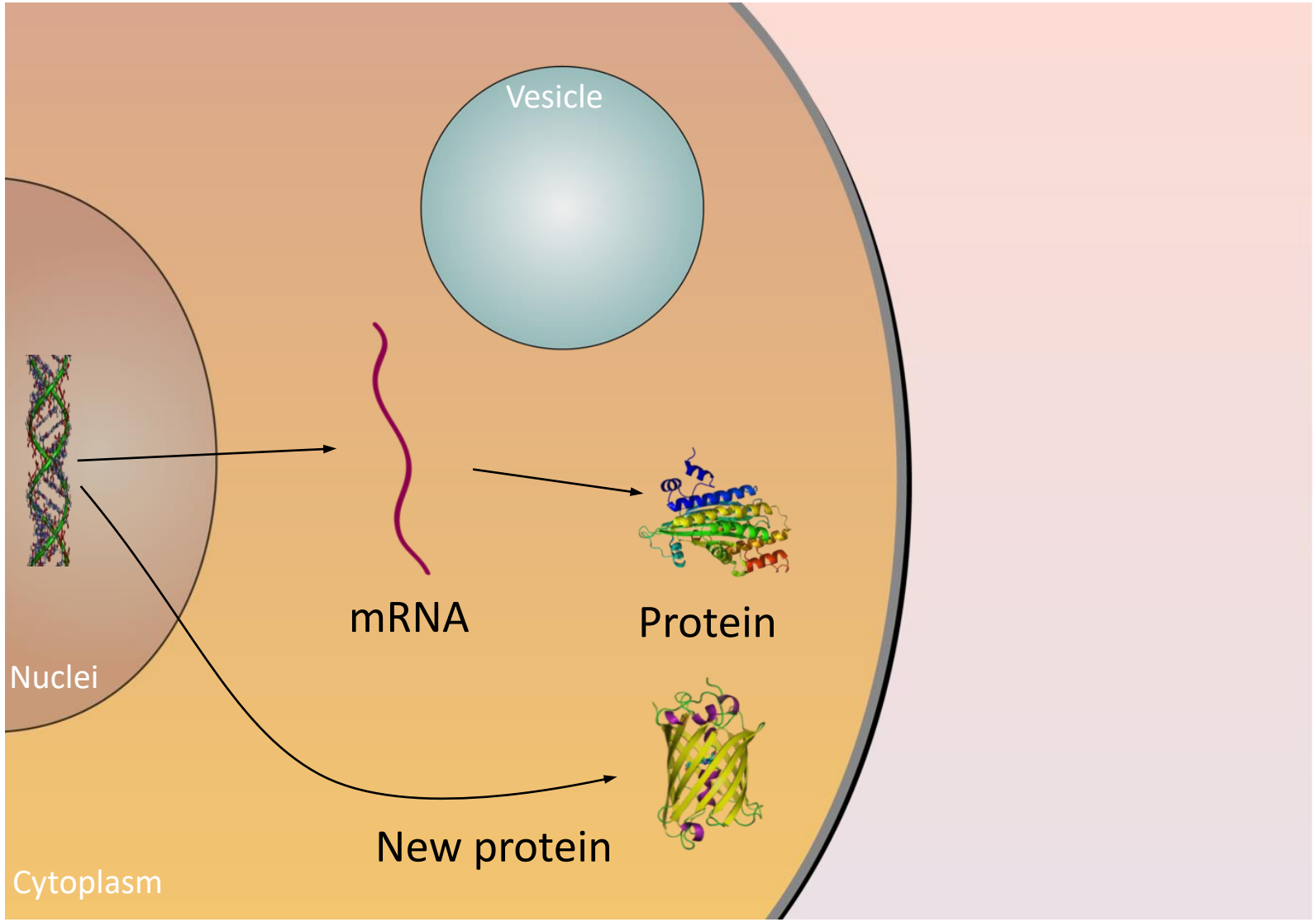
https://www.youtube.com/watch?v=gG7uCskUOrA

Gene delivery – What else?



Small molecules

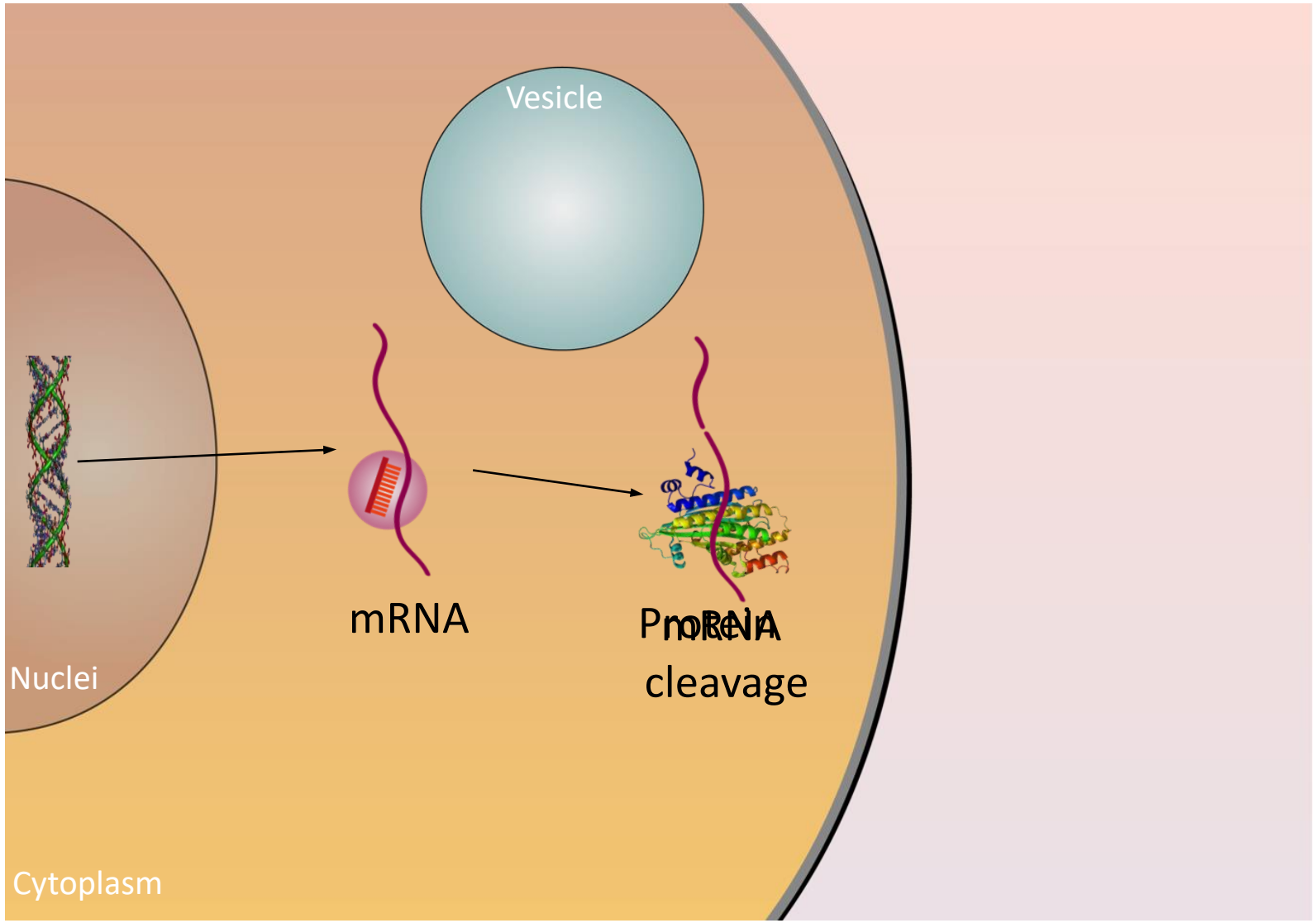
Known gene delivery



Small molecules

Plasmid DNA

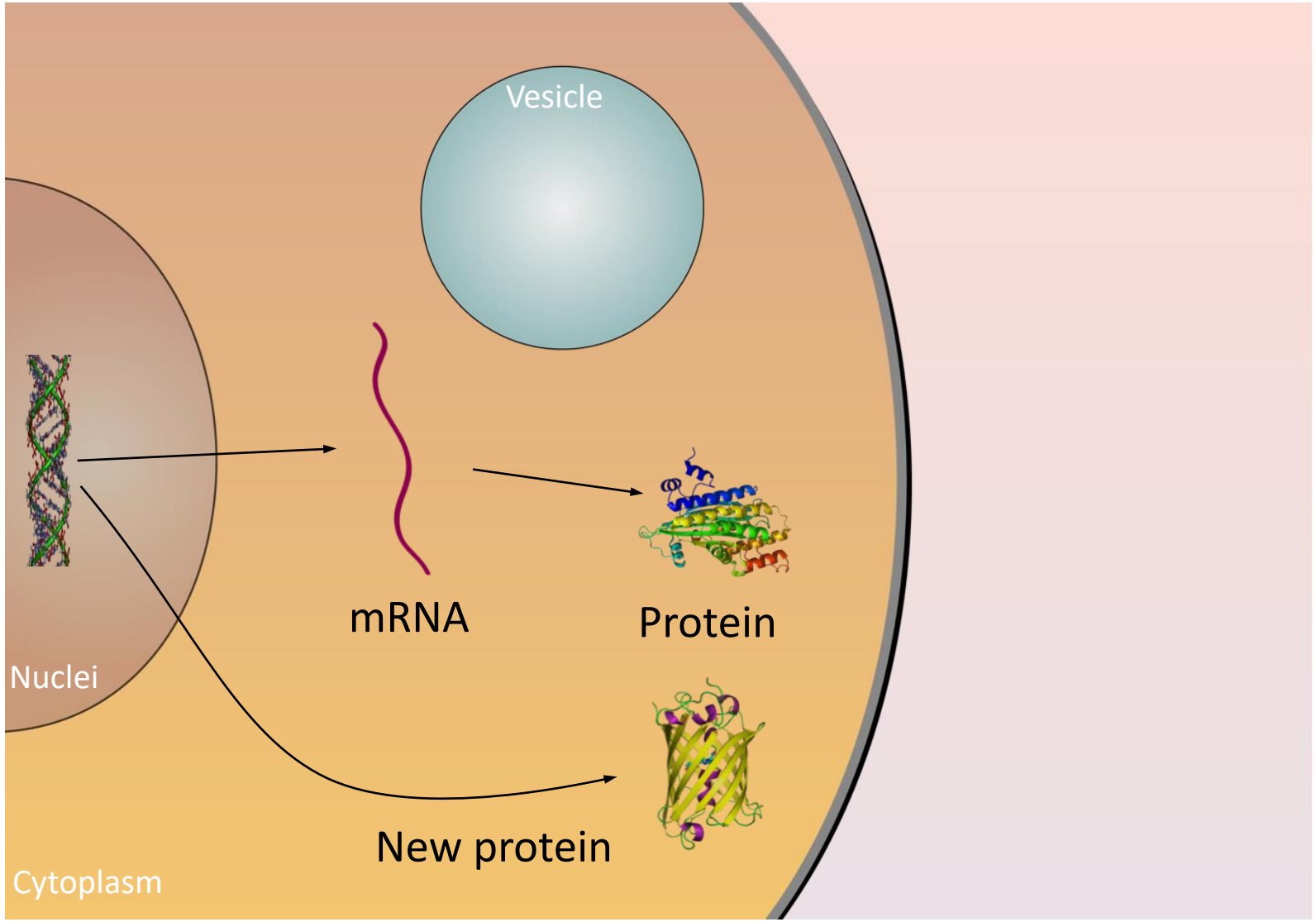
RNAi



- Small molecules
- Plasmid DNA
- siRNA

- Targeted delivery *in vitro*
- Immune cells *in vitro*

CRISPR/Cas

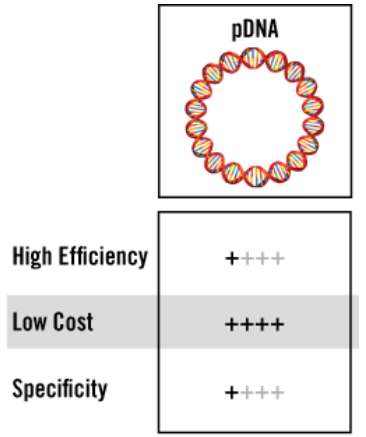


Small molecules

Plasmid DNA

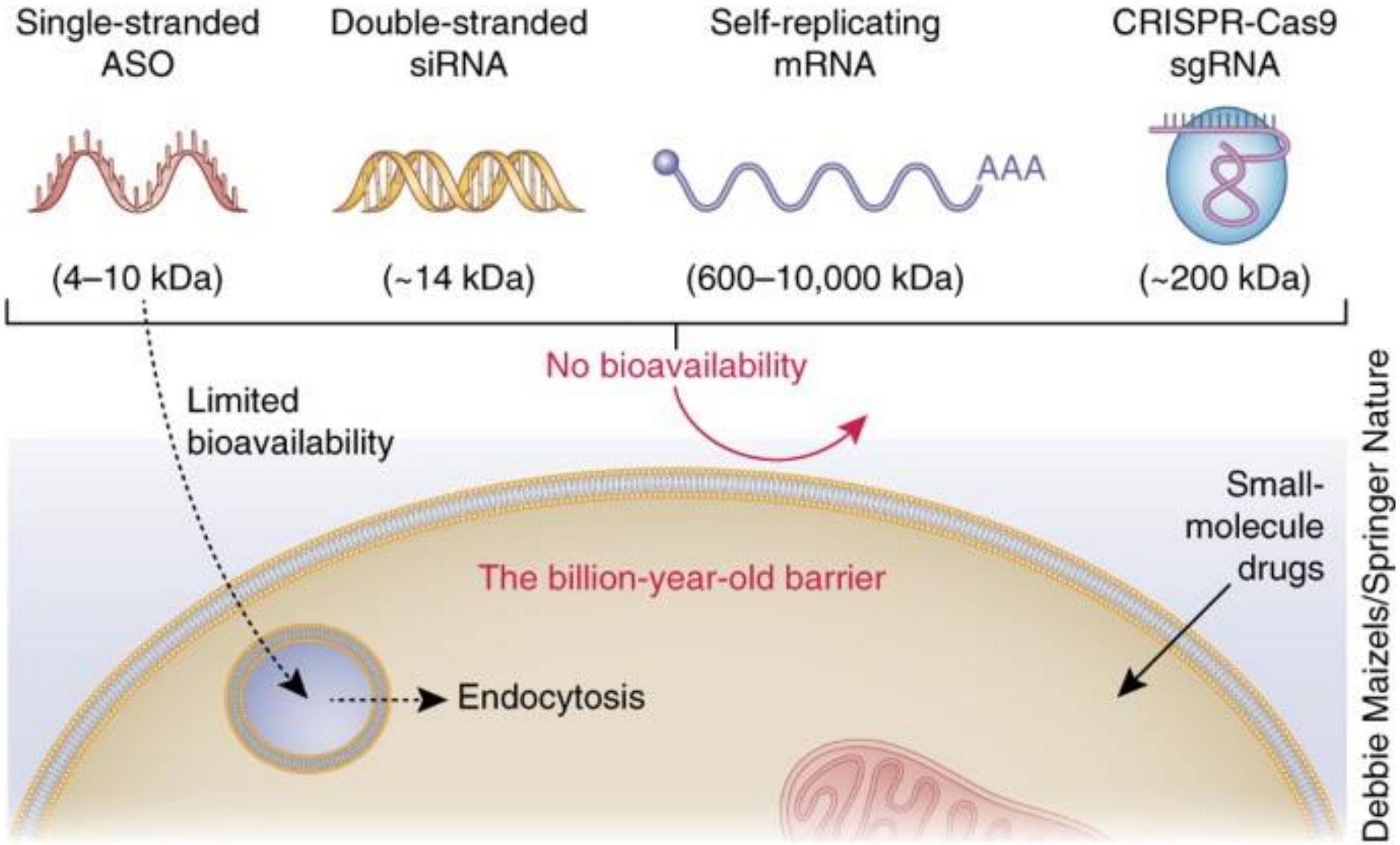
siRNA

pDNA + gRNA

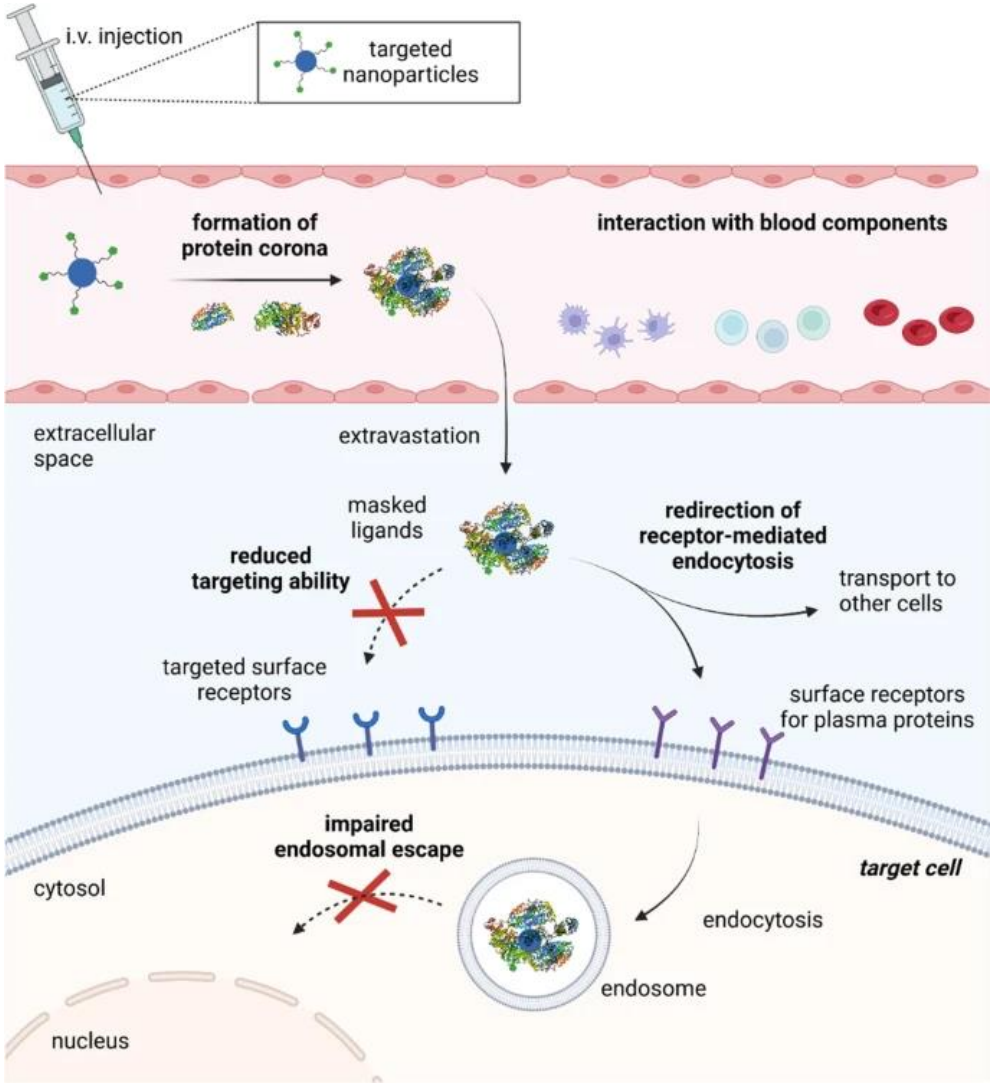


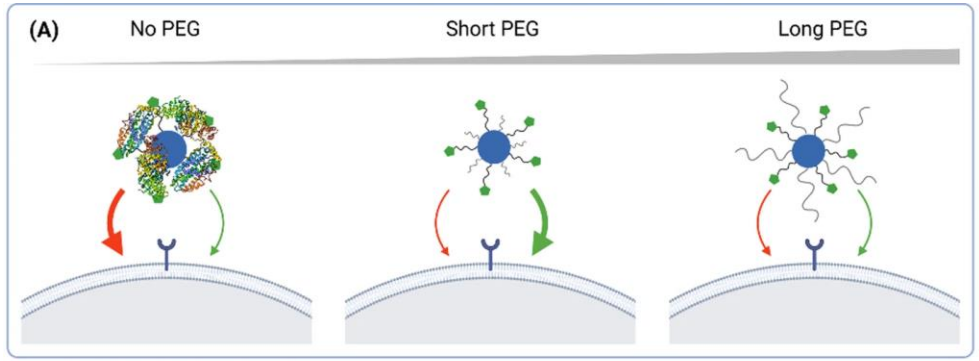
- Particular delivery strategies required *in vitro* and *in vivo*

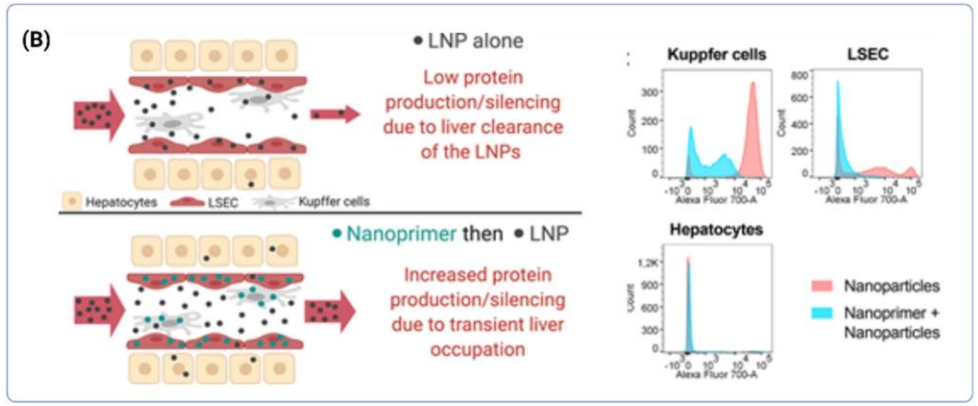
Types of NA-baed payload

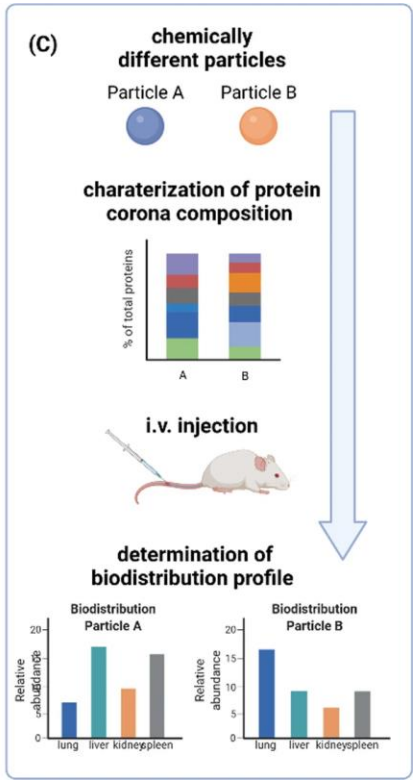


Blood – transport media with limitations



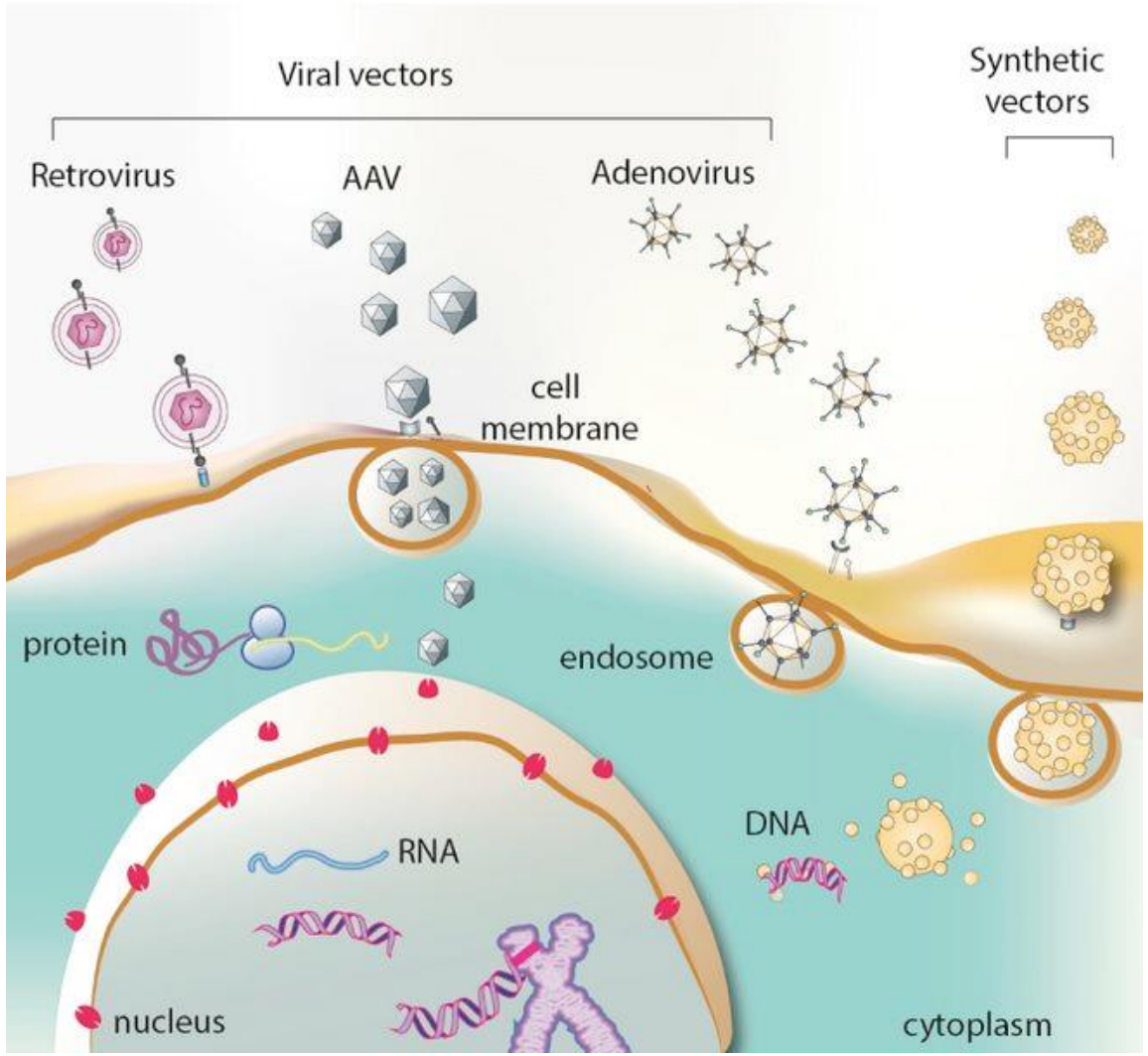






R. C. Steffens, E. Wagner, "Directing the Way—Receptor and Chemical Targeting Strategies for Nucleic Acid Delivery", *Pharmaceutical Research* **2023**, *40*, 47-76.

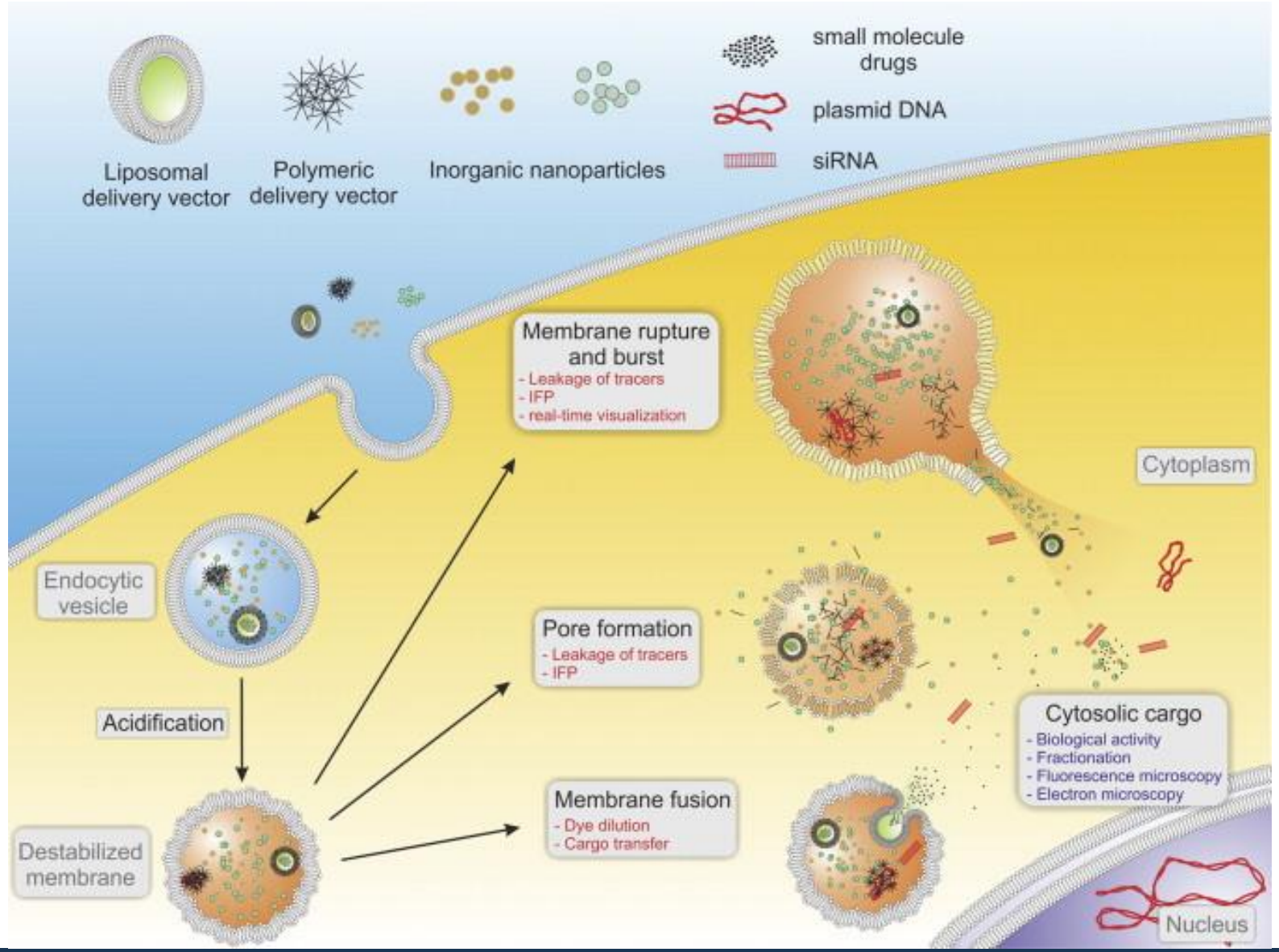
Vectors are required…



Gene Delivery systems should:

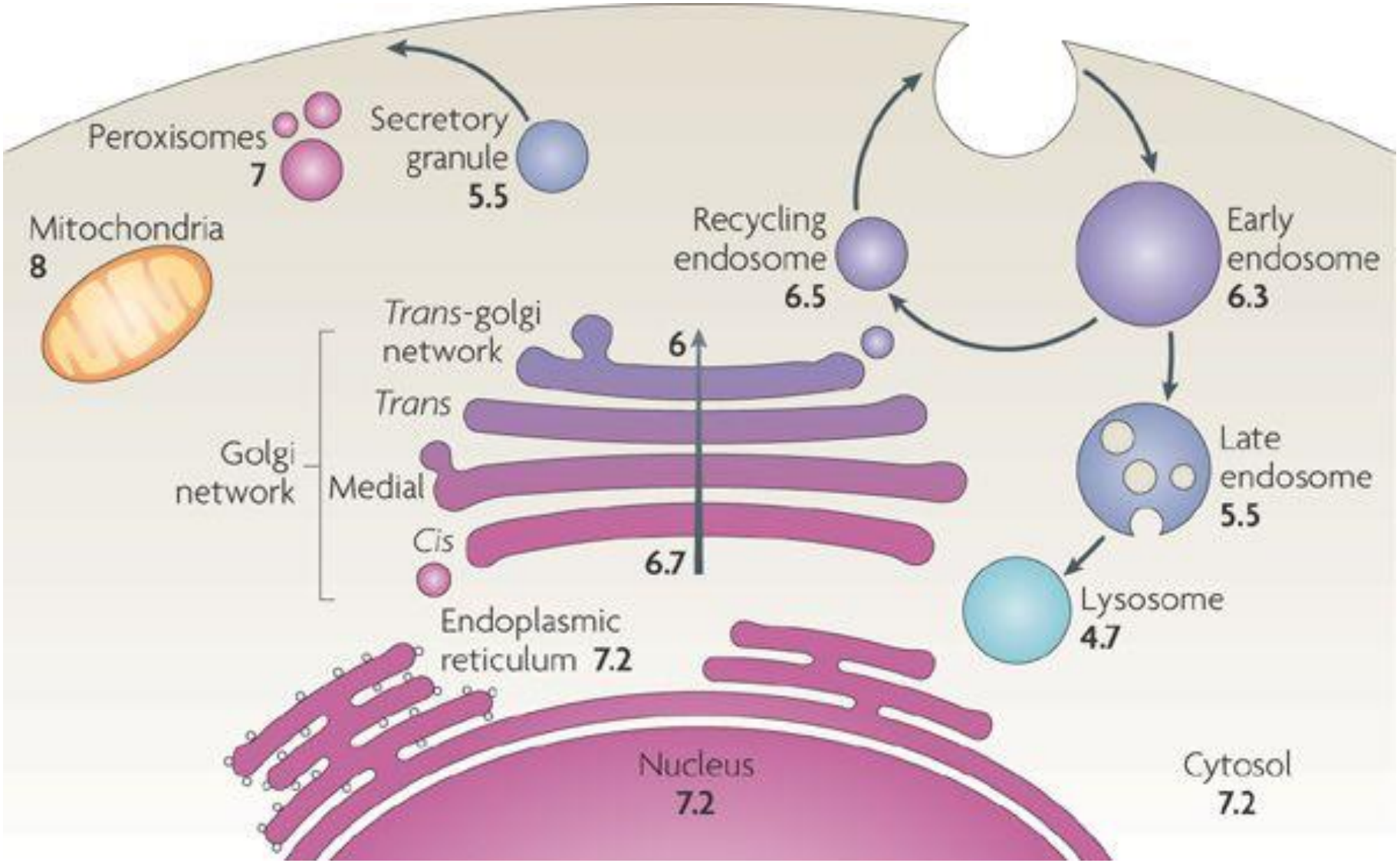
(i) protect nucleic acids against degradation by blood and interstitial nucleases,
(ii) promote internalization of the genetic material into target cells
(iii) release the nucleic acids once inside the cell to the correct site
(iv) should be effective, specific, long-lasting, safe, easy to use and as inexpensive as possible

Endosomal release mechanisms



Doi: 10.1016/j.nantod.2014.04.011

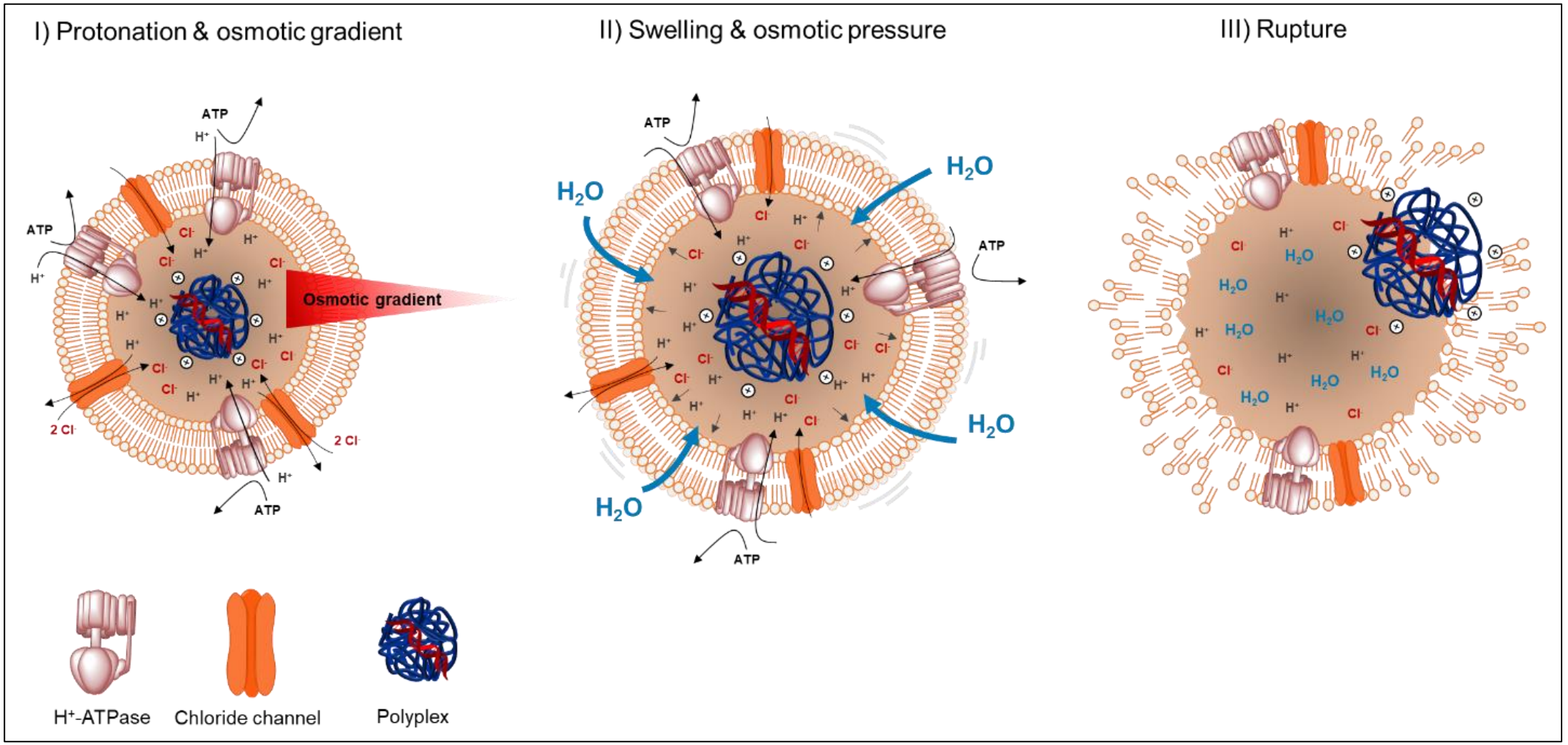
Cellular vesicular trafficking



The pH of individual cellular organelles and compartments in a prototypical mammalian cell. The values were collected from various sources. The mitochondrial pH refers to the matrix, that is, the space contained by the inner mitochondrial membrane. Early endosomes refer to the sorting endosomal compartment. The pH of the multivesicular late endosome refers to the bulk luminal fluid; the pH of the fluid contained by the internal vesicles might differ.

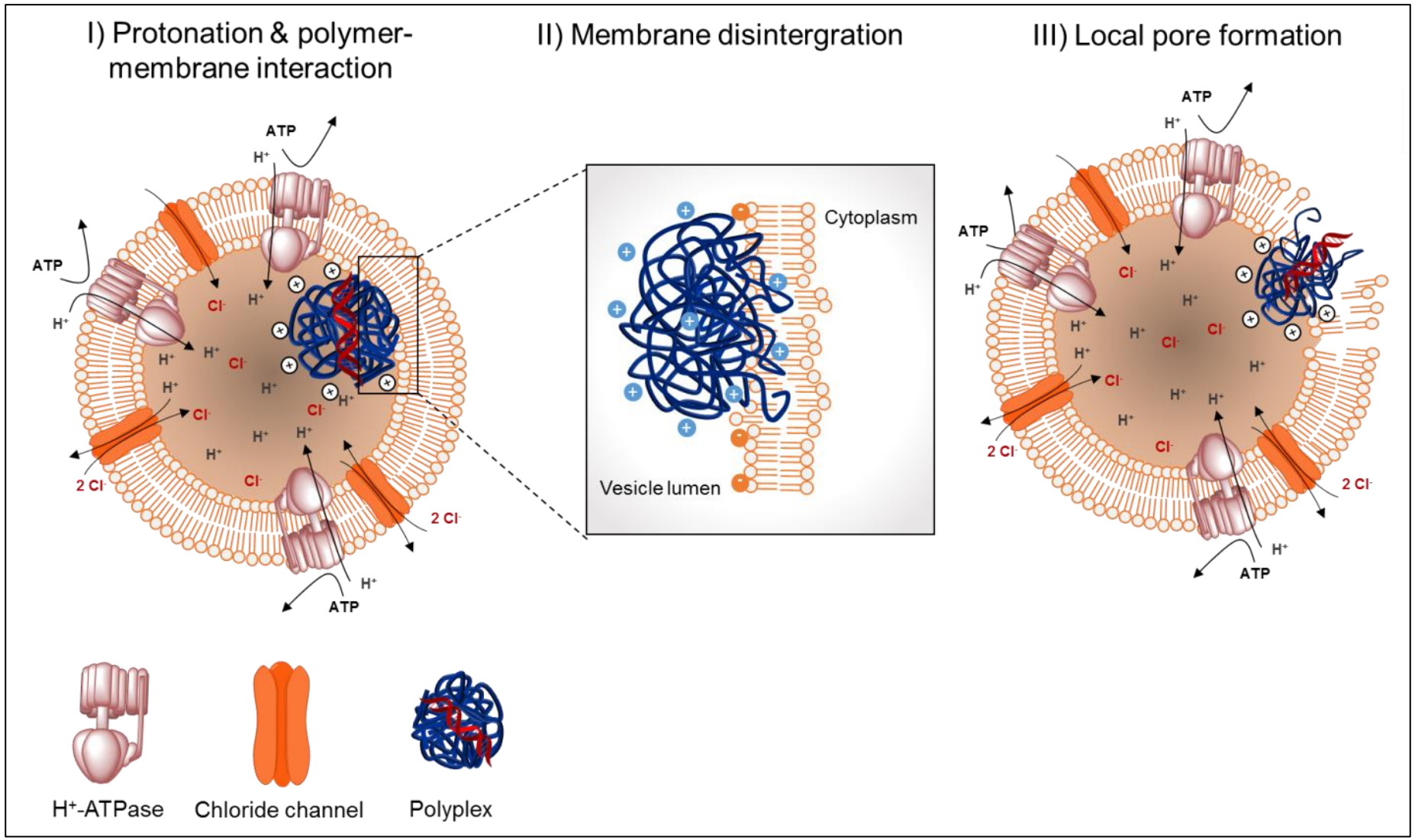
doi: 10.1038/nrm2820

Proton Sponge



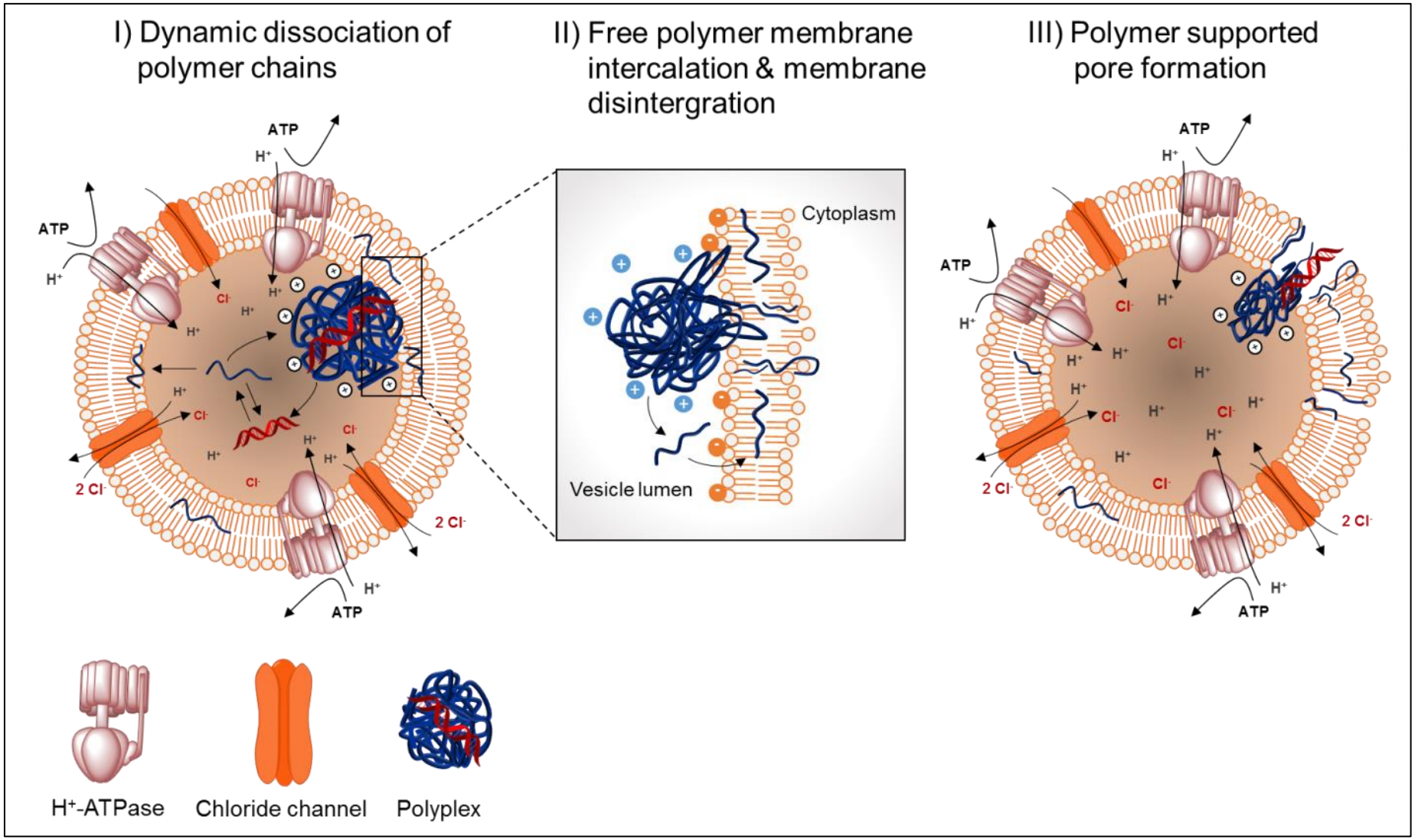
Doi: 10.1039/C8TB00967H

Pore formation 1



Doi: 10.1039/C8TB00967H

Pore formation 2



Doi: 10.1039/C8TB00967H

Why using genetic material as drug? Pros and Cons

Advantages

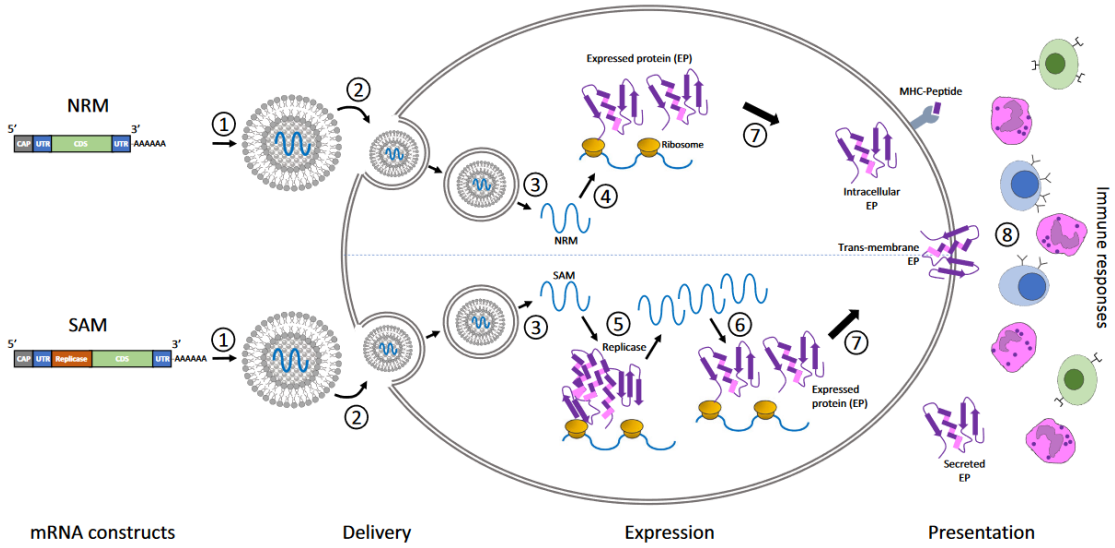
- Personalized medicine
- Specificity
- Origin, other diseases

Disadvantages/Limitations

- More sensitive, instable
- Prize
- Scepticism, new, long-term side effects?
- Less established, novel companies
- Upscale, special chemistry and tools
- Dosage/sufficient amount
- Stability, application routes

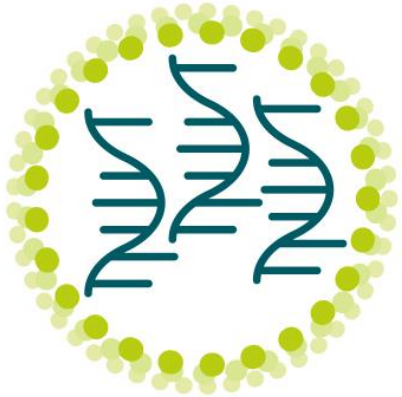
Moderna/Biontech CoViD-Impfungen: mRNA Delivery

- Genetic material in LNP
- Induces production of proteins (in this case Spike Protein of SARS-CoV2)
- Encodes display on cell surface
- Immune system can build antibodies

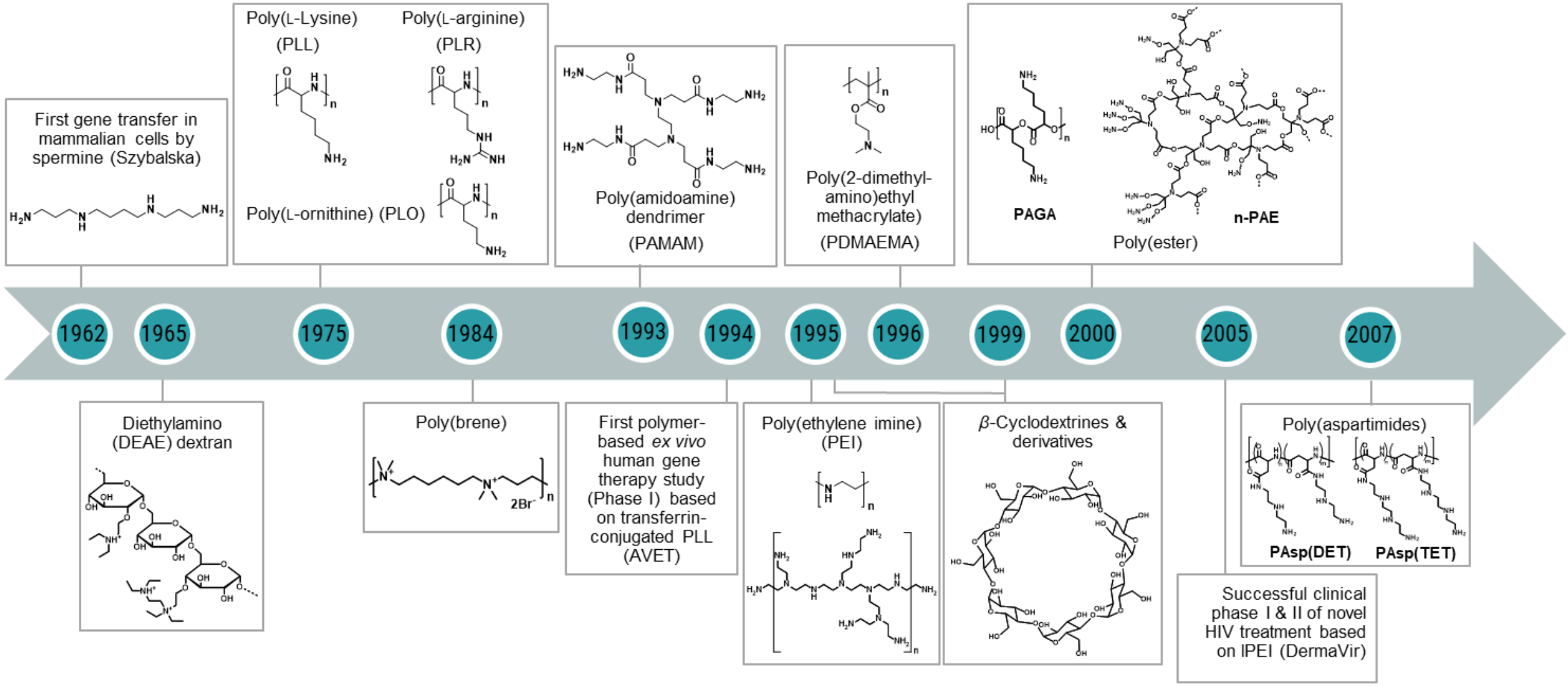


Jackson, N.A.C., Kester, K.E., Casimiro, D. *et al.* The promise of mRNA vaccines: a biotech and industrial perspective. *npj Vaccines* **5,** 11 (2020)

- mRNA is degraded rapidly (t½ ~ 10 h)
- Genes are not altered, transfected cells die
- No remaining foreign genetic material iin the body (other than the usually)

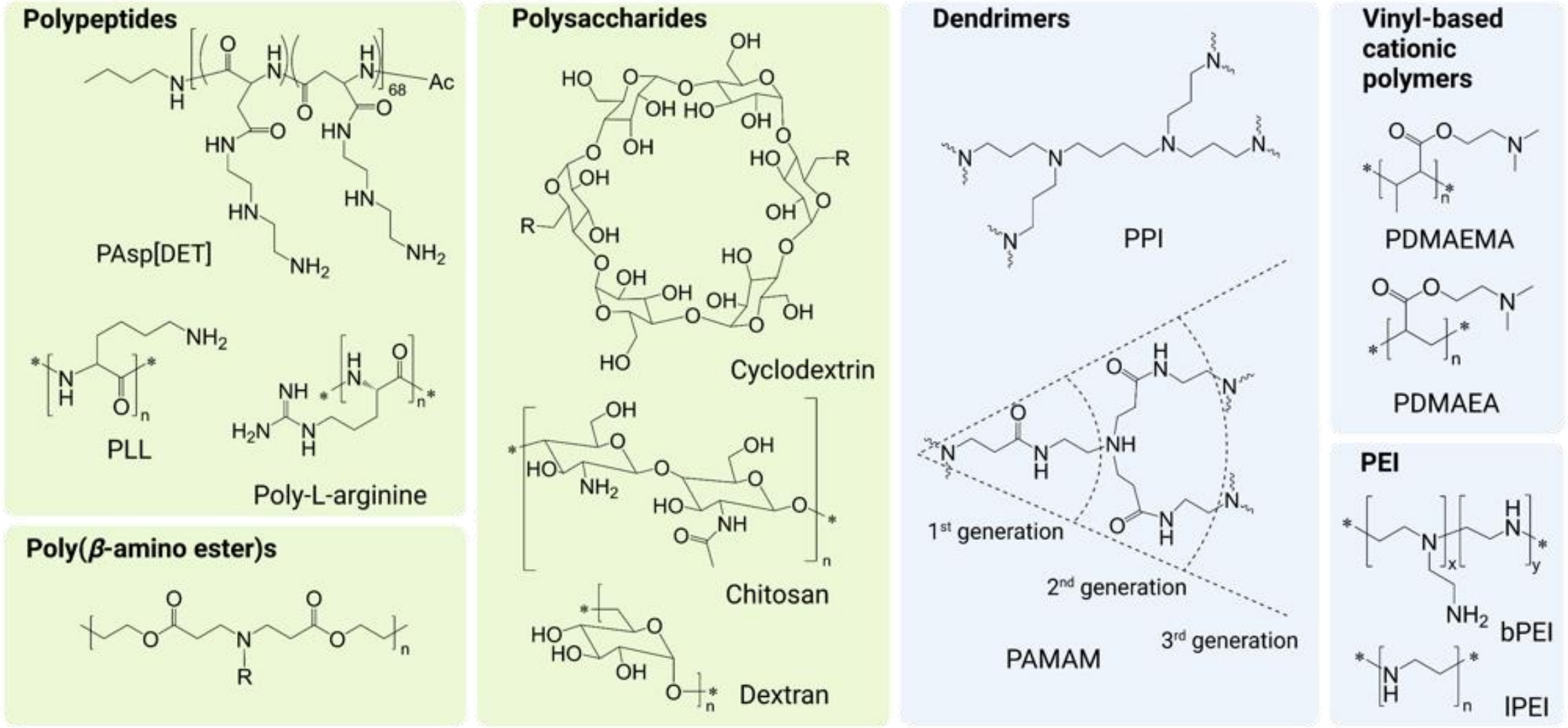


Polymers for gene delivery



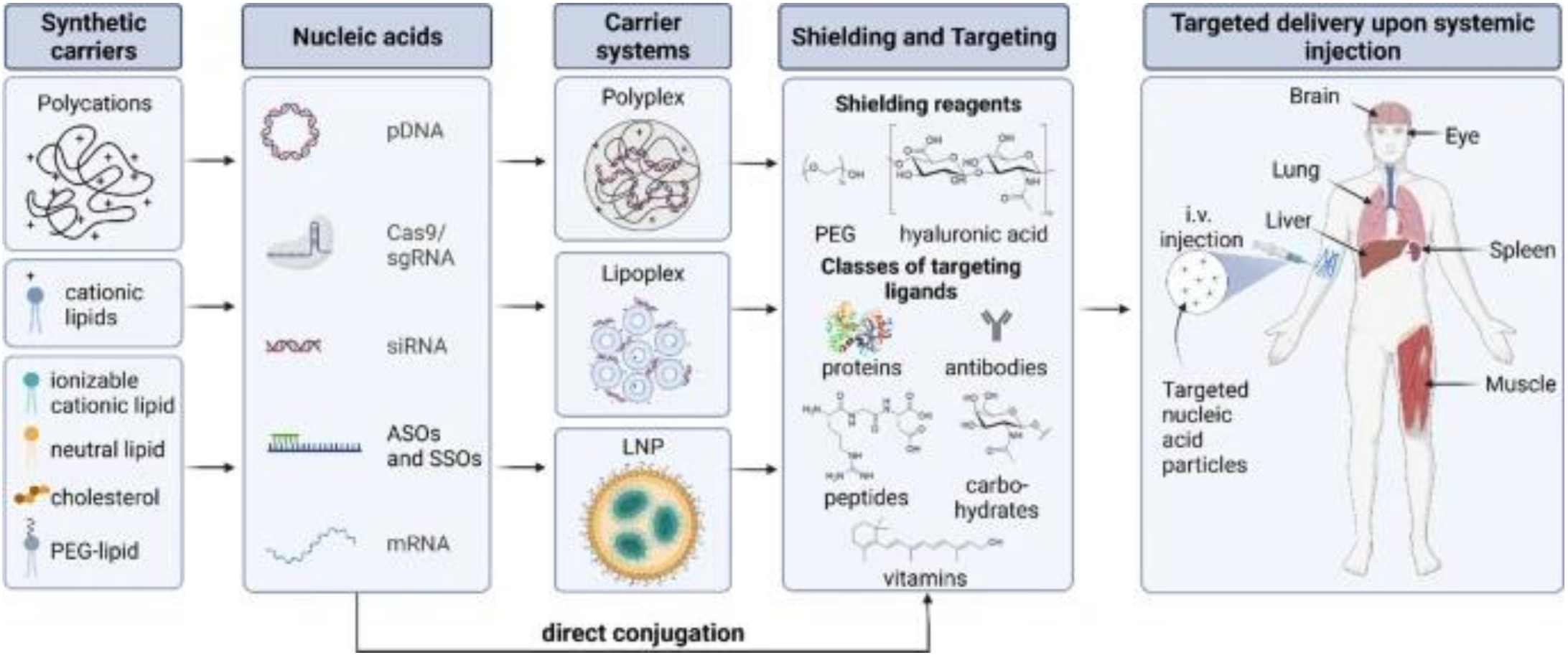
DOI:10.1039/C8TB00967H

Polymer-based Tools

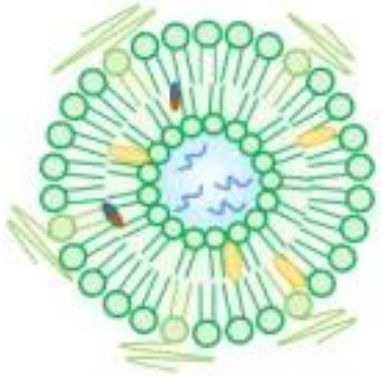
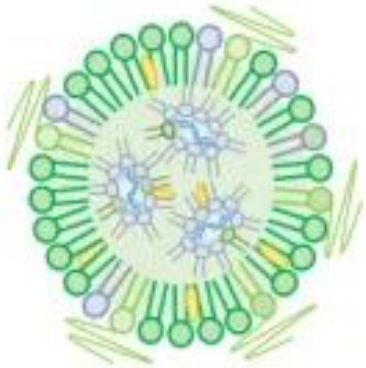
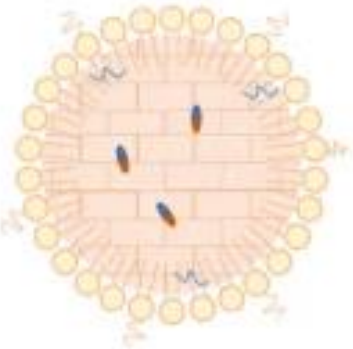


L. S. Reichel, A. Traeger, Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 1-17.

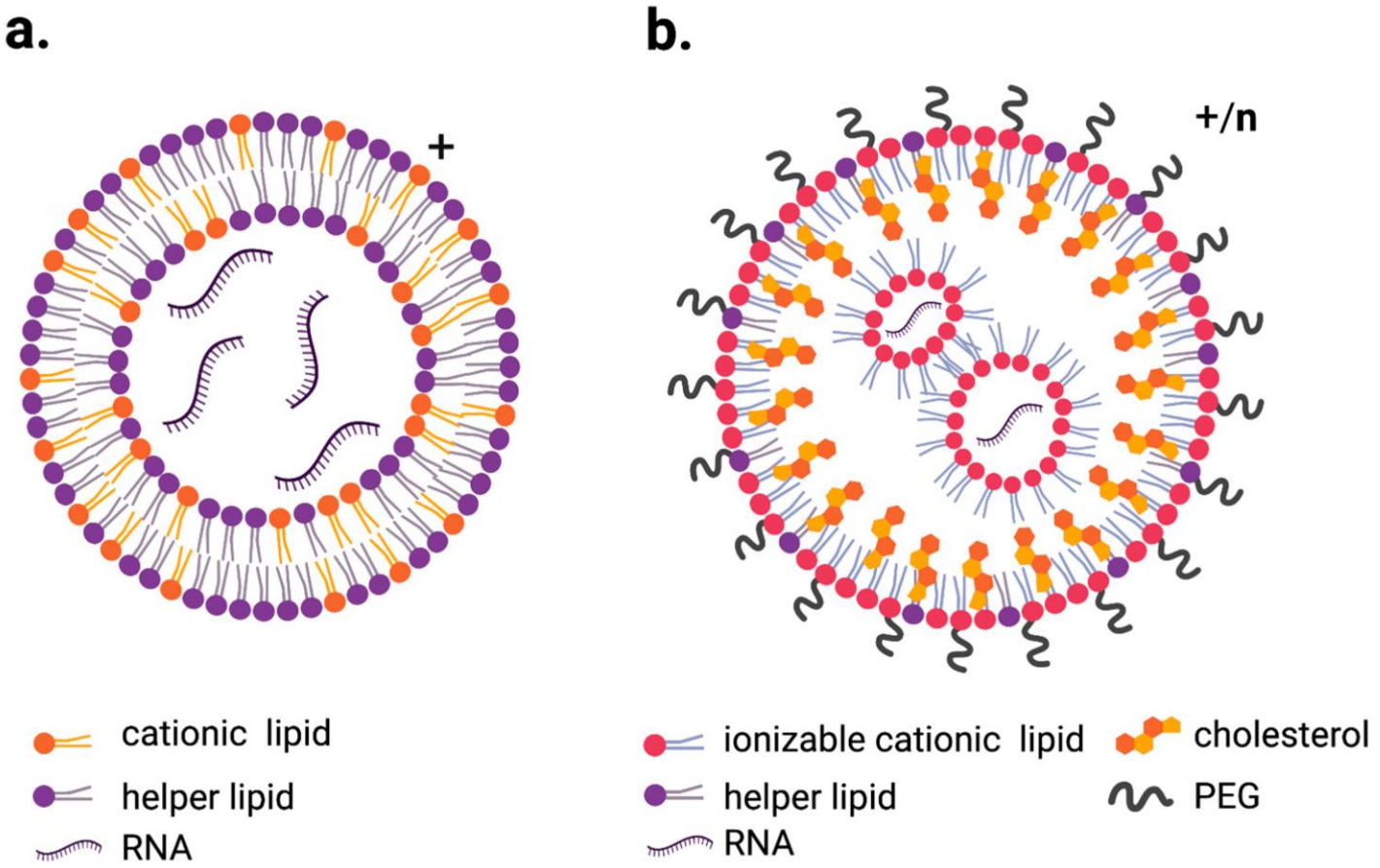
Non-viral carrier design



SLNP vs. LNPs

Name	Liposomes	Lipid Nanoparticle (LNP)	Solid Lipid Nanoparticle (SLN)	Nanostructured Lipid Carrier (NLC)
				
Shell	Bilayer	Monolayer	Surfactant	Surfactant
Core	Aqueous	Reverse micelles	Solid Lipids	Solid and liquid lipids
Load	Hydrophobic and/or hydrophilic small molecules	Nucleic/ Oligonucleotides	Hydrophobic and/or hydrophilic small molecules	Hydrophobic and/or hydrophilic small molecules

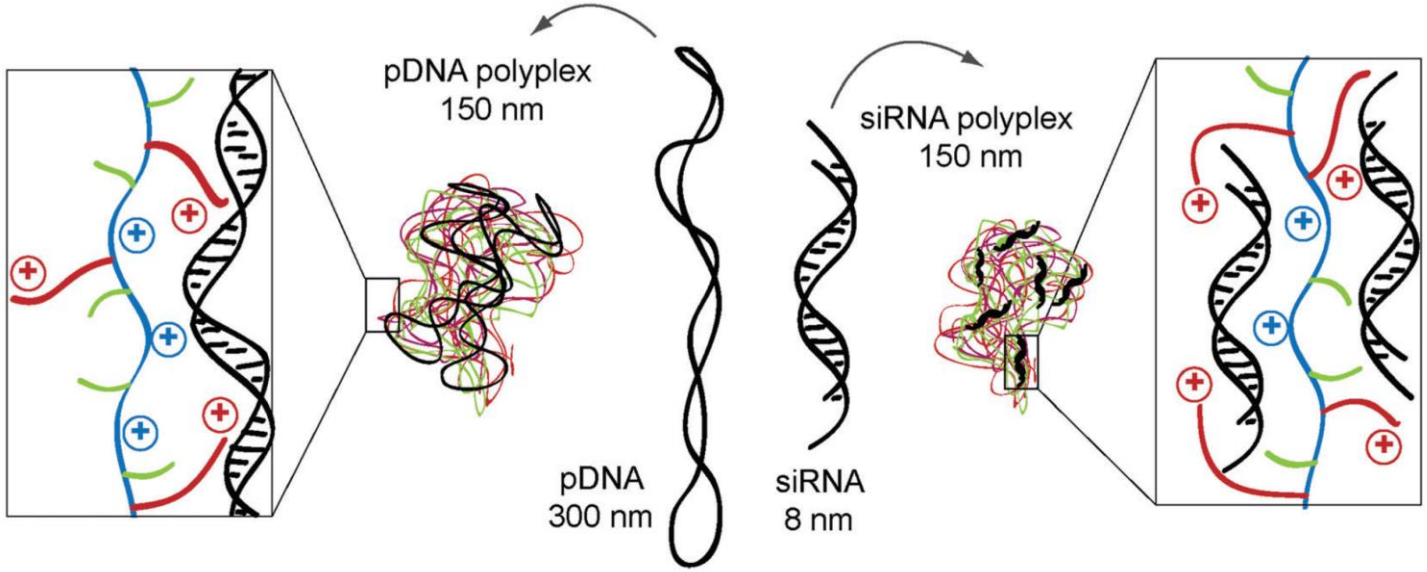
Liposomes vs. LNPs



M. M. Żak, L. Zangi, in *Pharmaceutics, Vol. 13*, **2021**.

Polymeric vectors: Polyplexes

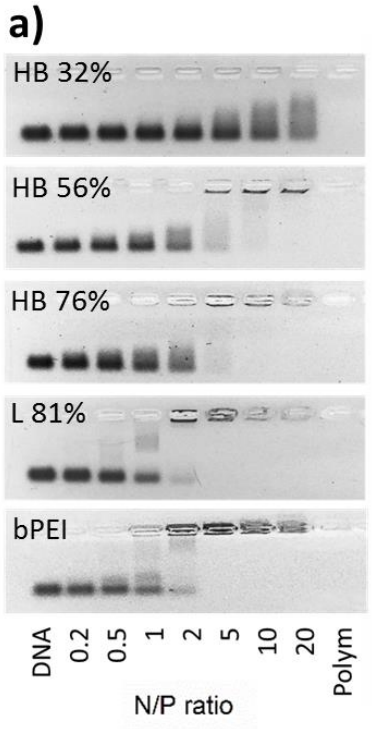
- Poly interelectrolyte complex = polyplex
- Positively charged polymer condenses negatively charged DNA/RNA
- Important parameter: ratio of positive to negative charge (N/P) [Nitrogen/Phosphor]
- Different types of NA require different vectors



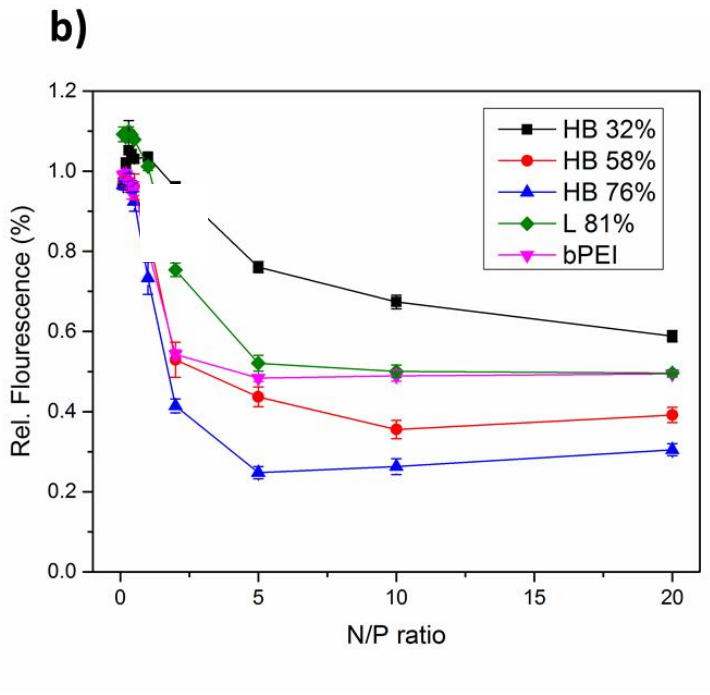
T. Bus, C. Englert, M. Reifarth, P. Borchers, M. Hartlieb, A. Vollrath, S. Hoeppener, A. Traeger, U. S. Schubert, *J. Mater. Chem. B*, **2016**, *5*, 1258-1274.

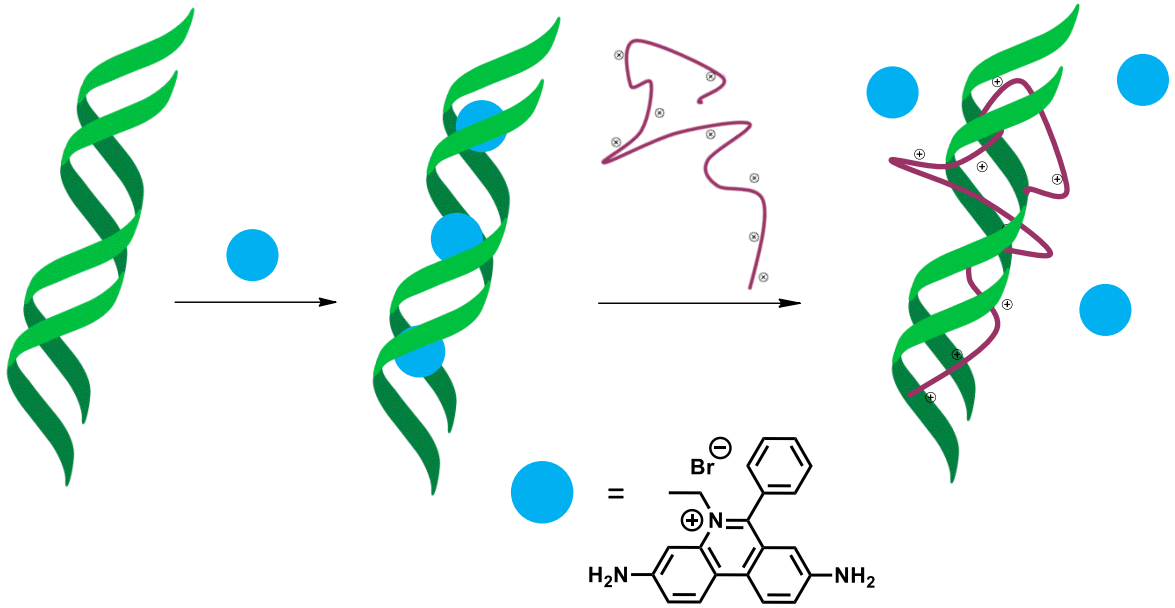
Analytic: binding

Gel electrophoresis



Ethidium-Bromide Assay





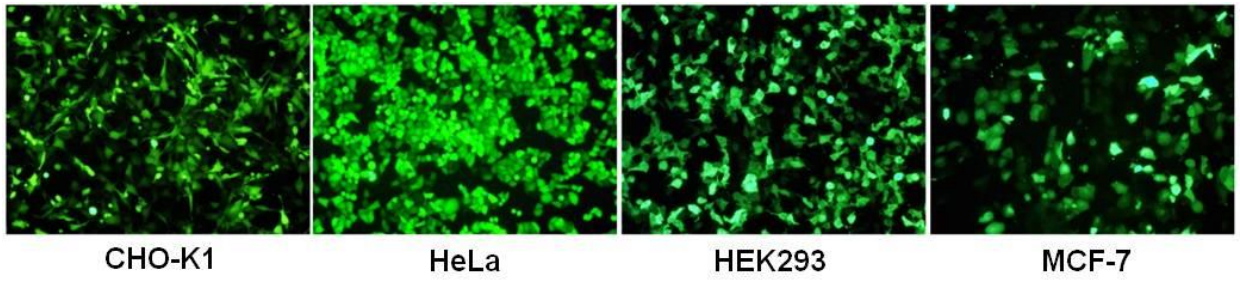
- Also important: size (+distribution)
  - DLS
  - Microscopy techniques (AFM, SEM, TEM, ..)

A. B. Cook, R. Peltier, J. Zhang, P. Gurnani, J. Tanaka, J. A. Burns, R. Dallmann, M. Hartlieb, S. Perrier, *Polym. Chem.*, **2019**,*10,* 1202-1212.

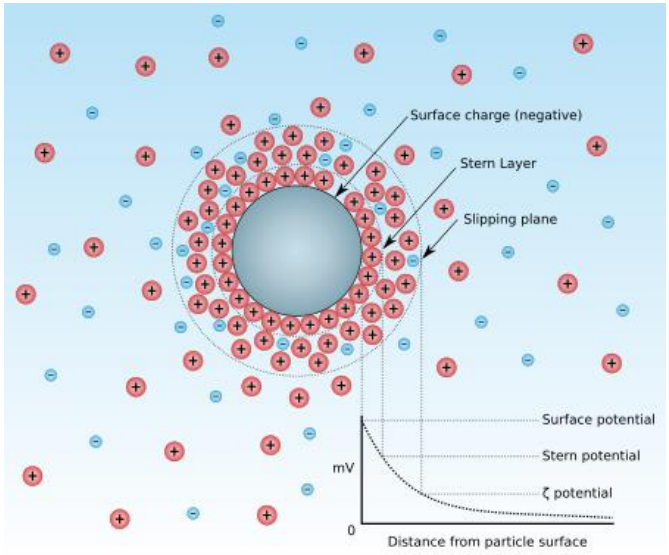
Analytics: polyplexes+ biological

- Polyplexes should be slightly positively charged (better uptake)
- Increased charge leads to pronounced toxicity
- Size and distribution need to be in a range for endocytosis

- Bioactivity
  - Cellular uptake (Fluorescence assisted cell sorting (FACS))
  - Toxicity (cyto/hemo tox Assays)
  - Efficient release = transfection (→ reporter with e.g. GFP [green fluorescence protein] expression)



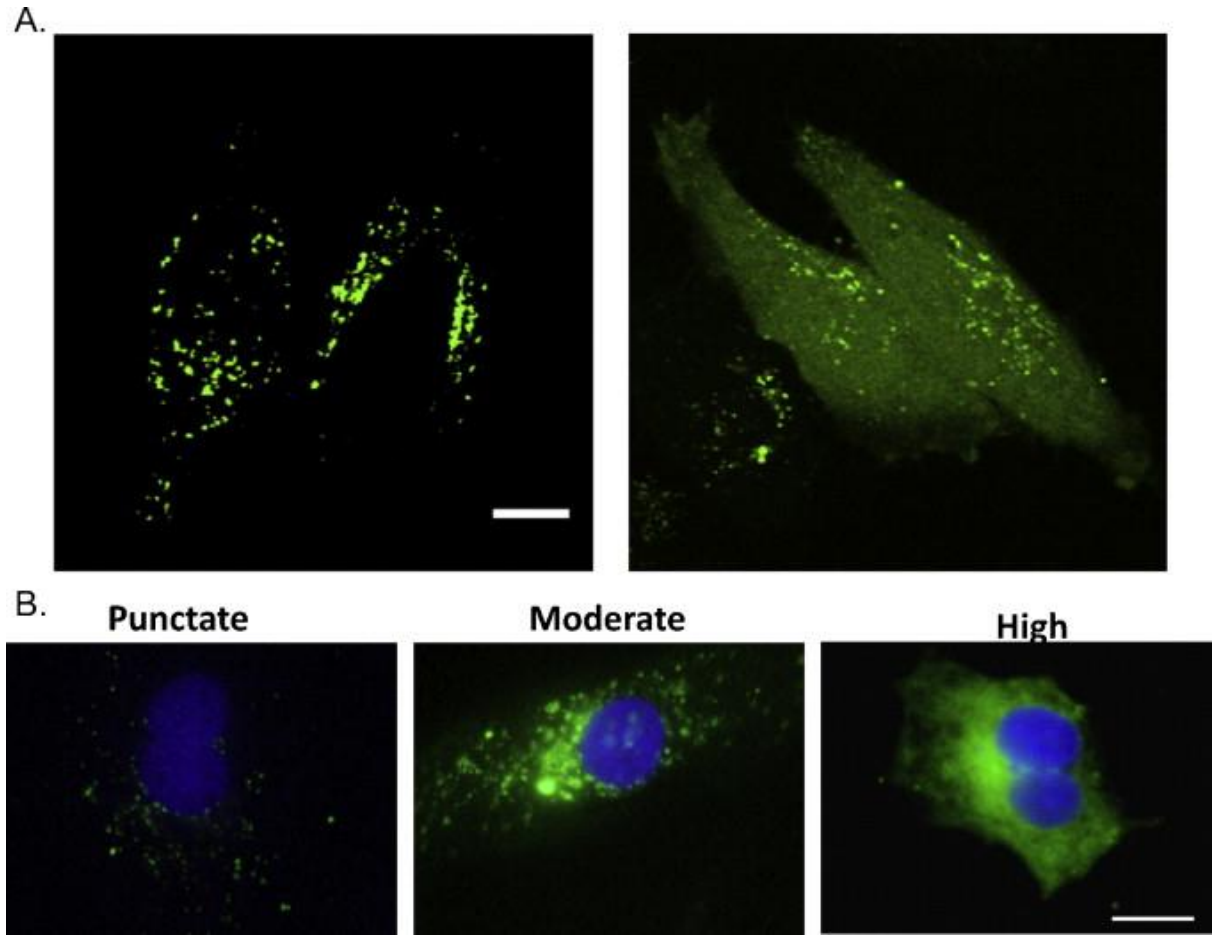
https://www.abpbio.com/product/lipofectmax/



https://de.wikipedia.org/wiki/Zeta-Potential

https://de.wikipedia.org/wiki/Grün_fluoreszierendes_Protein

Visualization of release: intracellular tracking



(A) The intracellular fluorescence profile (IFP) of 3 kDa dextrans(green fluorescence), illustrating the difference between a punctate pattern (sequestered cargo; left) and diffuse staining (cytosolic cargo; right). Scale bar 10 µm. Reprinted from [53], © 2012, with permission from Elsevier. (B) IFP of Qdots (green fluorescence), visually classified as a punctate pattern, moderate release and a high amount of release. Blue color indicates the nucleus. Scale bar 50 µm.

Take-home message

- **Gene therapy** hold promises for issues not addressable by regular pharmaceuticals
- There have been rapid advances in **NA-based technology** in the past decades
- The **delivery of NA-based drugs** to cells/tissue has obstacles different to “regular” drug delivery
- Among other materials, **cationic polymers** can be used as means to condense NA to polyplexes and delivery them to their target
- The **properties of polyplexes** are essential for their efficacy and **biological assays** can be used to determine activity