Nanopharmazeutika für die Gentherapie

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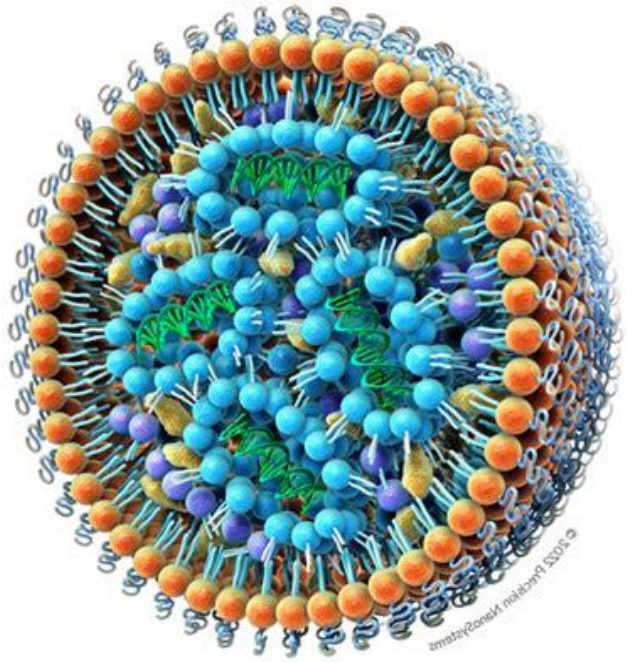
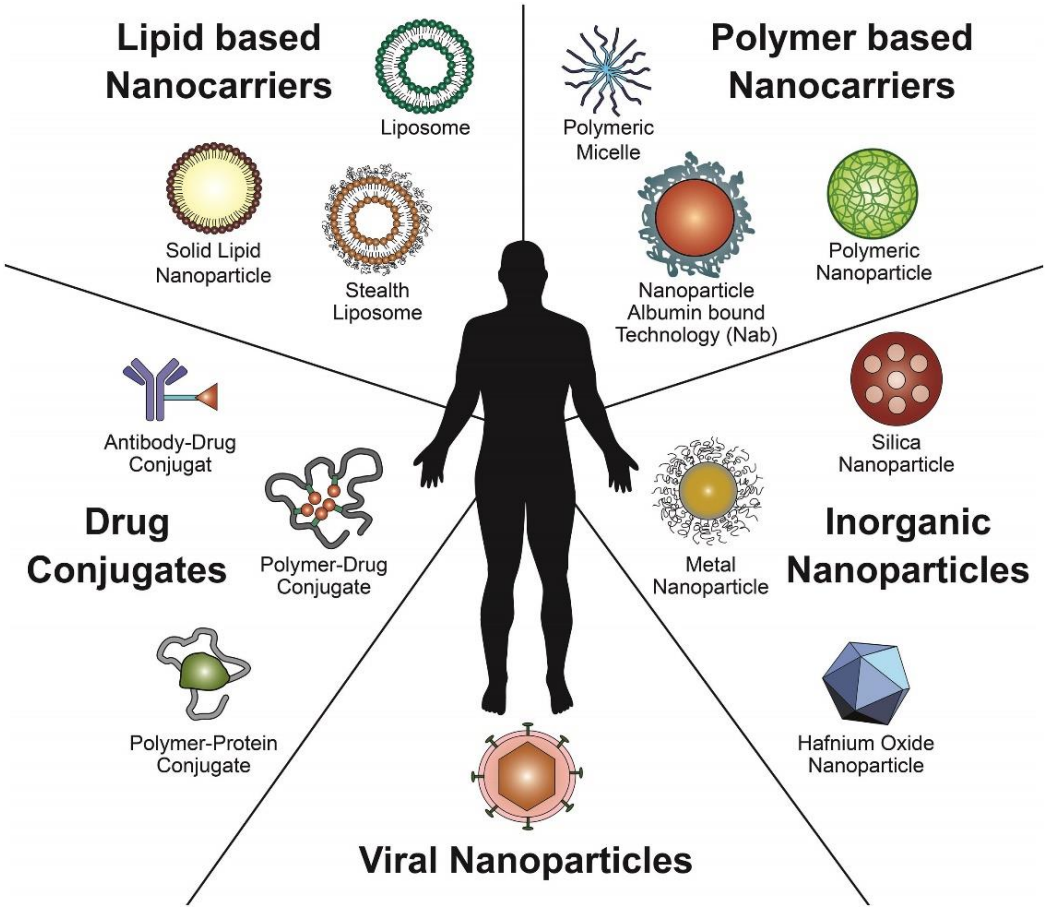


Contact Point Nano
Webinar vom 20.11.2023

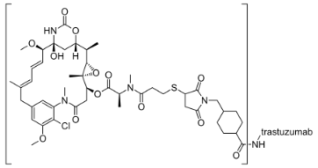
University of Basel

Particulate drug carriers in pharmaceutical sciences



Moderna/Pfizer mRNA-LNP vaccines

Aerosil: Fumed silica
(thixotropic; anti-caking)



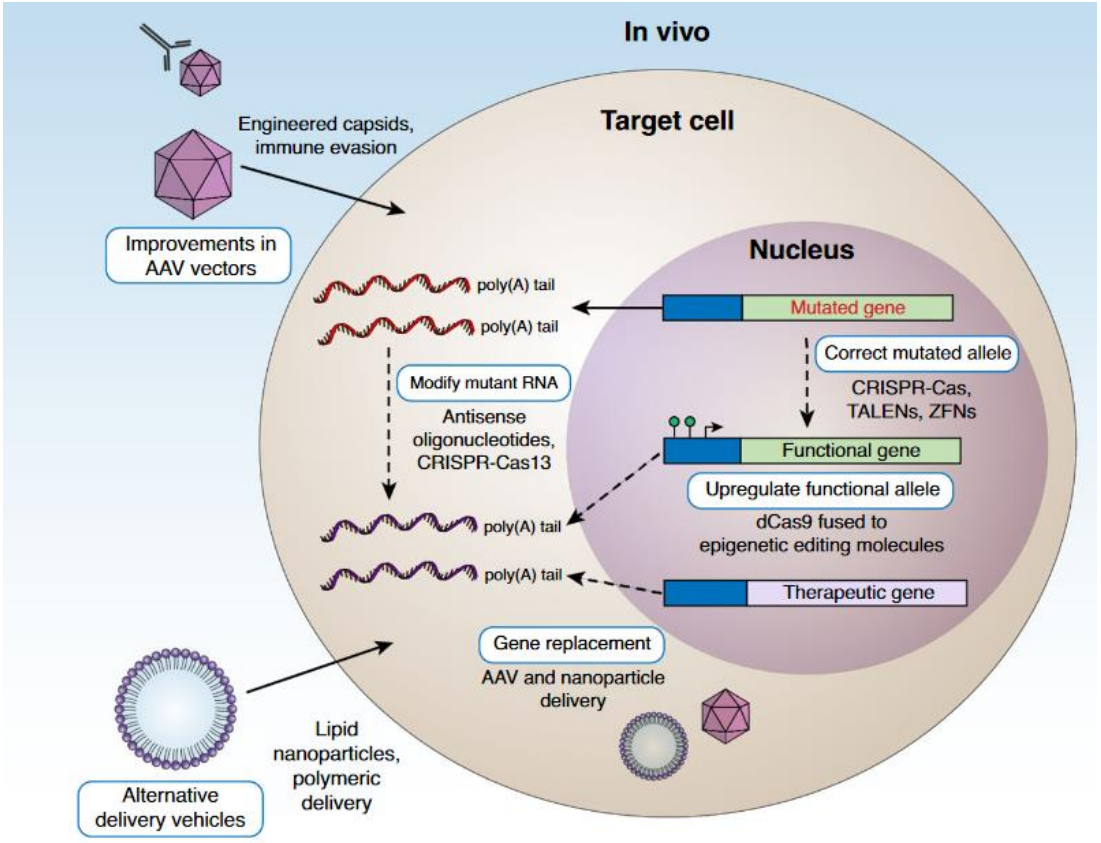
Trastuzumab emtansine

Abraxane (cancer):
nanoparticle albumin–bound paclitaxel

Doxil: pegylated liposomes
(cancer; doxorubicin)

Wicki et al., Journal of Controlled Release, 2015

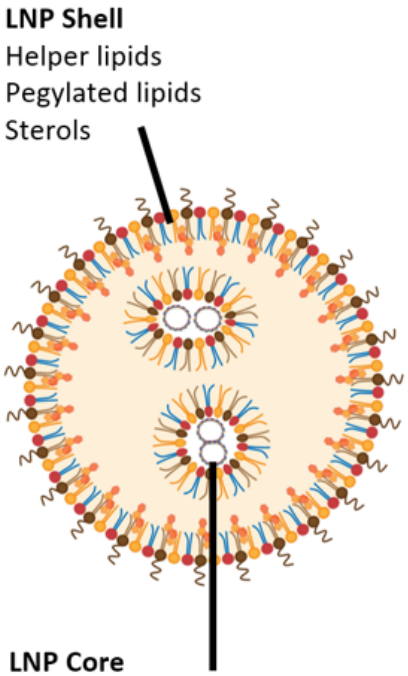
Introduction: Why gene therapy?



Nature Comm. (2020) 11:5820

1. Correct a mutated gene (e.g. CRISPR/Cas9)
2. Gene replacement (DNA or mRNA level)
3. Inactivate mutant RNA (RNAi)
4. RNA editing (e.g. ADAR, CRISPR/Cas13)

Lipid nanoparticles (LNPs)



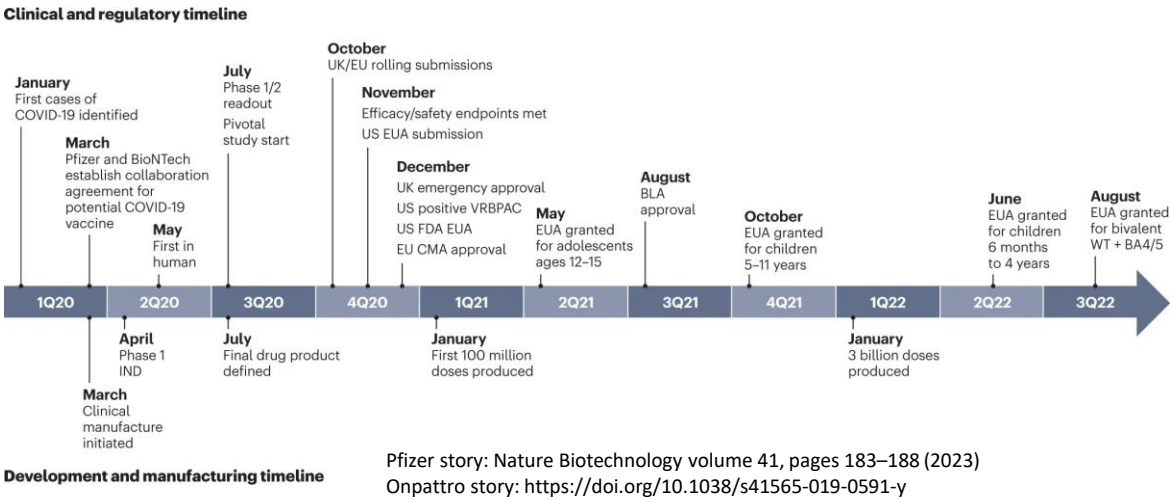
Component	Function	Examples	
Sterols **Cholesterol**	Membrane fluidity Fusiogenic properties	Cholesterol β-Sitosterol	**38%**
Helper lipids	Structural component Fusiogenic properties	Phosphatidylserine, Phosphatidylethanolamine, Phosphatidylcholine	**10%**
Ionizable lipids	DNA condensation Endosomal escape	DODMA, DOTAP, KC2, MC3, ALC-0315, SM-102	**50%**
Pegylated lipids	Steric stabilization Reduced opsonization	DSPE-PEG (C18) DMPE-PEG (C14) DMG-PEG (C14)	**1.5%**
Cargo (pDNA) **mRNA, siRNA**	Transgene delivery	Nanovector pDNA coding for ASS	**N/P 6**

Schematic representation of lipid nanoparticle (MF-DNA-LNP) structure, composition, and the proposed roles of the different constituents.

N/P ratio = the ratio of moles of the amine groups of cationic polymers to those of the phosphate ones of nucleic acids

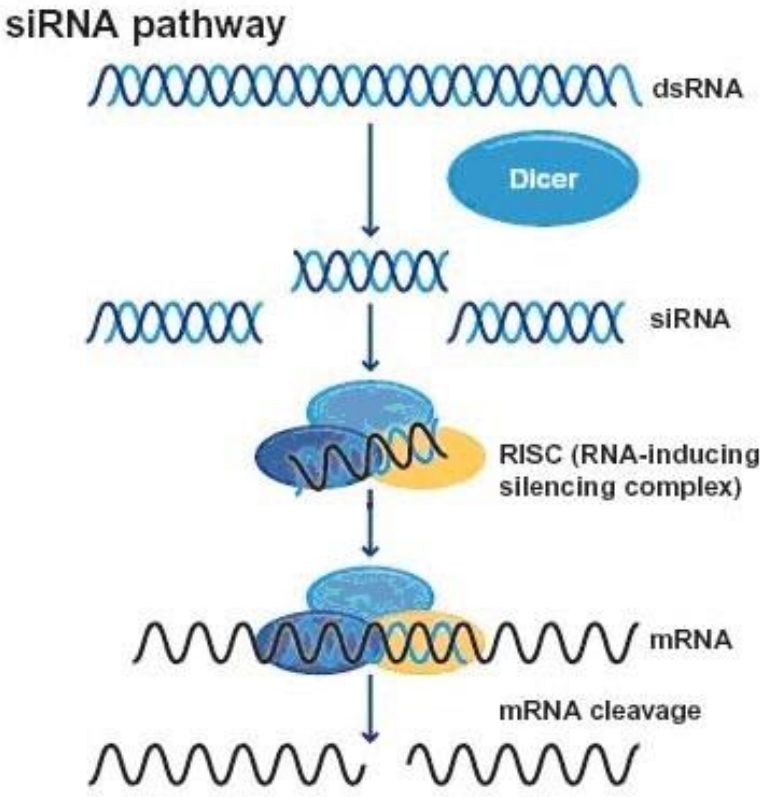
First example: Vaccination (Covid-19)

- Idea: “Reprogram” cells of the body to produce their own vaccine
- Induction of short acting (!) effects
- 2018: Onpattro (Patisiran) as first FDA approved LNP drug. siRNA approach to treat rare hereditary transthyretin-mediated amyloidosis.
- 2020: FDA approved mRNA based corona vaccines.
  e.g. Pfizer: 7 mo from the first reported case to pivotal trial, 11 mo to FDA Emergency Authorization.
  3 billion doses by end 2021.



Pfizer story: Nature Biotechnology volume 41, pages 183–188 (2023)
Onpattro story: https://doi.org/10.1038/s41565-019-0591-y
The history of RNA vaccines: https://www.nature.com/articles/d41586-021-02483-w

siRNA based therapeutics

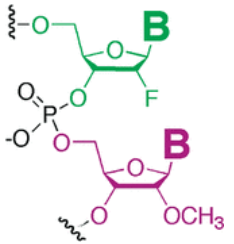


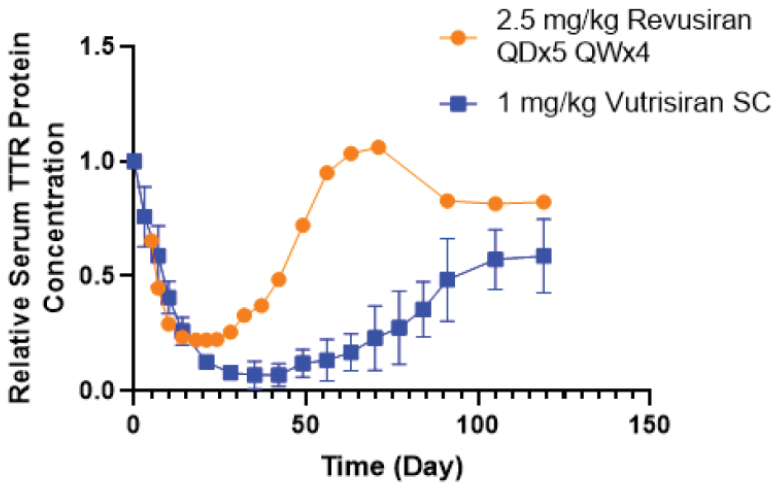
Chemically stabilized siRNA

5'- GGGUAAAUACAUUCUUCAU -3'
3'- CCCAUUUAUGUAAGAAGUA -5'

5'-P-GGGUAAAUACAUUCUUCAU -3'
3'- CCCAUUUAUGUAAGAAGUA-P-5'

N = 2'-OH; N = 2'-F; N = 2'-O-methyl





Patisiran:
IV infusion every 3 weeks &
dexamethasone 10 mg oral &
acetaminophen (500 mg) &
IV H1 and H2 blocker

Duration of activity correlates with metabolic stability of the siRNA.

DOI: 10.1124/dmd.121.000428

Second example: Urea cycle disease citrullinemia

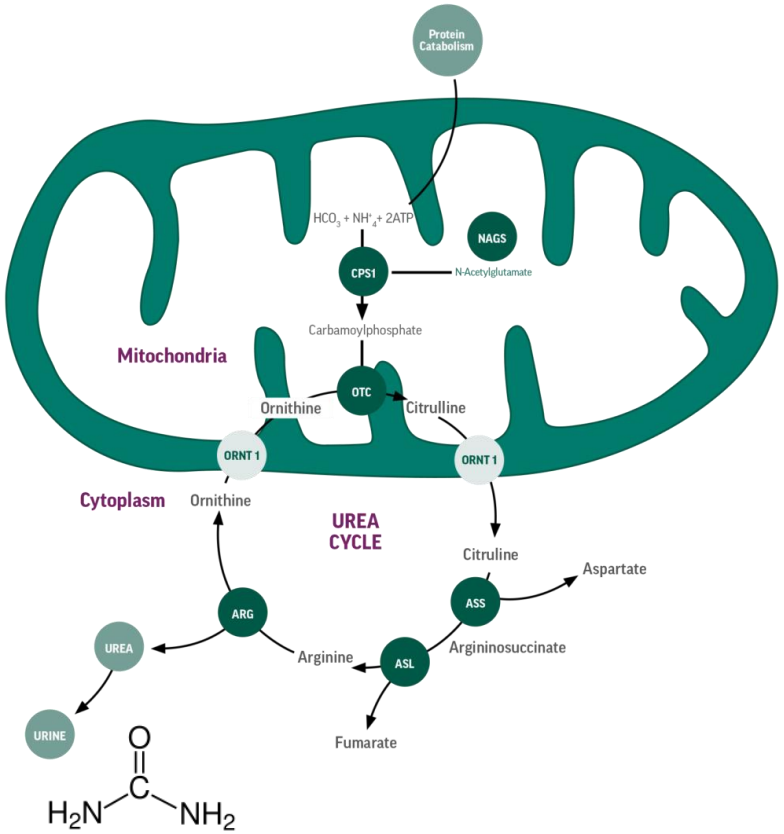
Idea: Induce long-acting effects to replace defective enzymes / transporters

Type 1: Defective ASS1 gene (argininosuccinate synthetase **ASS**)
- 1:57'000 birth, autosomal recessive, early onset
- Life-threatening accumulation of ammonia in plasma
- Lifelong diet > liver transplantation
- ASS1 expression in hepatocytes
- More than 130 affected gene loci

Type 2: Defective SLC25A13 (ORNT1)
(mitochondrial transporter citrin)
- 1:200'000 birth, adulthood
- Often Japanese man affected

Our vision:

- **Gene REPLACEMENT therapy to induce LONG-LASTING gene expression (5-10% of WT levels)**
- **Well tolerated and safe**

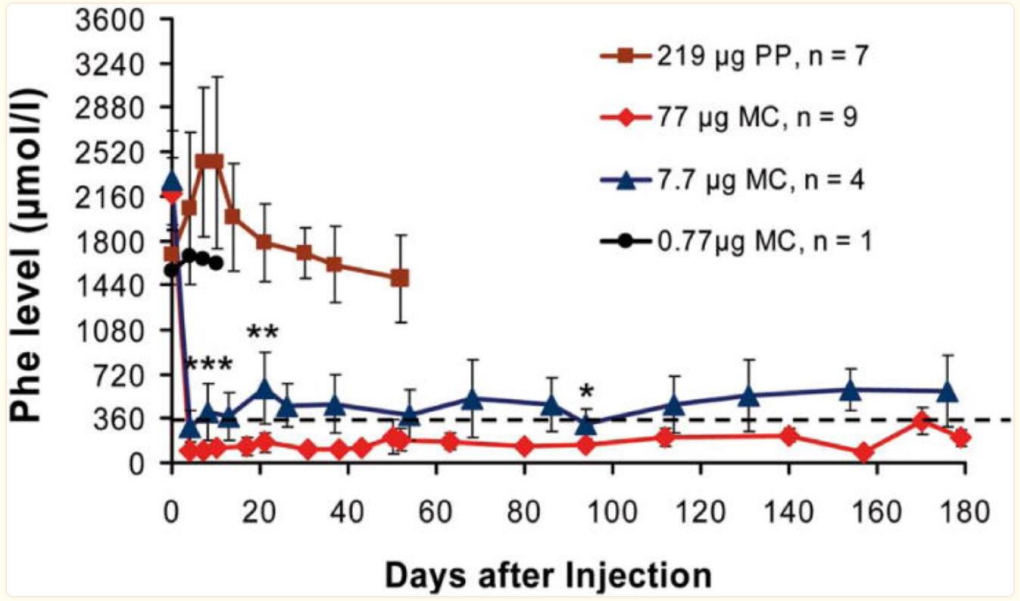


DNA versus RNA

Nanovector DNA as an alternative to RNA based gene delivery:

- DNA expression plasmid (0.45 kb backbone)
- Scaffold/matrix attachment region (S/MAR) motifs to mediate episomal maintenance and replication in mitotically active cells
- Superior IV tolerability of DNA versus RNA

→ **Lifetime expression of a transgene in a PKU mouse model**



Long-term correction of hyperphenylalaninemia in PKU (*Pah*^{enu2}) mice after delivery of MC-DNA vectors expressing the Pah-cDNA from the liver-specific P3 promoter. Vectors were delivered to the liver of adult PKU mice by a single HTV injection. Viecelli et al. Hepatology. 2014 Sep; 60(3): 1035–1043.

BB = 450 bp
T = GFP-2A-Puro splice
S/MAR = 800 bp

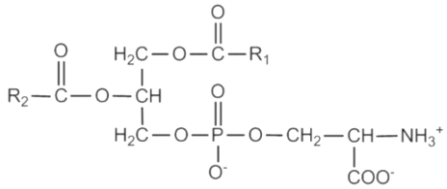
Bozza *et al.*, *Sci. Adv.* 2021; **7** : eabf1333

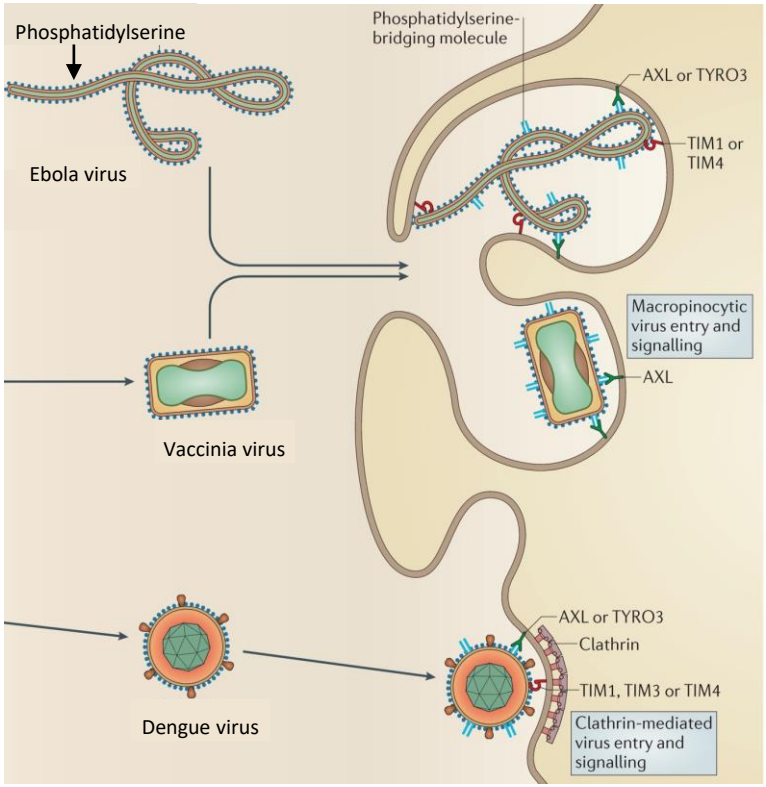
Design of biomimetic inspired LNPs

Claudia Lotter

Working hypothesis:

Modification of the helper lipid composition of LNPs and their combination with versatile and multifunctional lipid-polymer constructs offer a considerable potential for improvement.





Viruses (i.e., Ebola, Vaccinia, Dengue) have phosphatidylserine **(PS)** in their envelope

- **Facilitates host cell infection (cellular uptake)**

Goal:

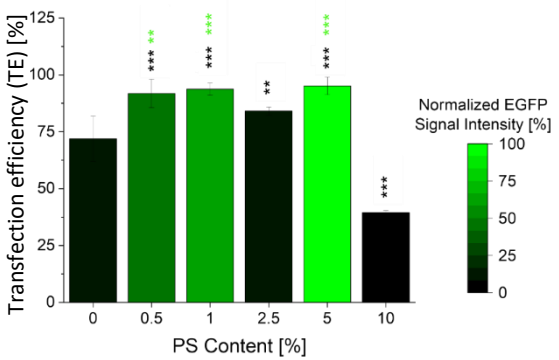
Development of biomimetic inspired LNP formulations using viral lipids (i.e., **PS**)

Eur J Pharm Biopharm. 2022. 172:134-143.

PS-LNPs show enhanced *in vitro* potency

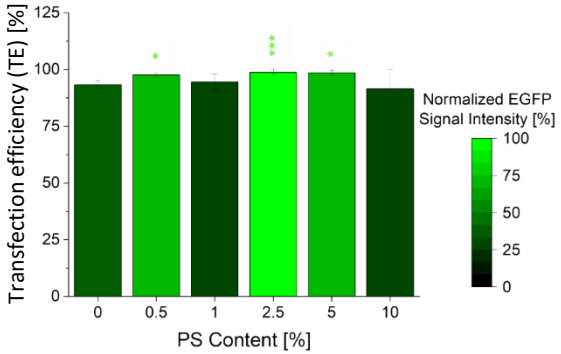
In vitro transfection (eGFP) of hepatocellular carcinoma HuH-7 cells
Transfection efficacy: Number (%) of transfected cells
Transfection potency: Fluorescent signal intensity

280 ng **pDNA, 48 h**



- Lead for pDNA: **5% PS**
- 30% increased TE
- 85% higher eGFP signal intensity

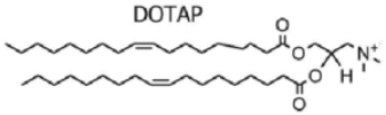
280 ng **mRNA, 24 h**



- Lead for mRNA: **2.5% PS**
- Comparable TE
- 75% higher eGFP signal intensity

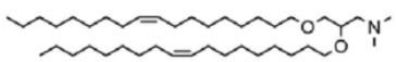
Permanent cationic lipids

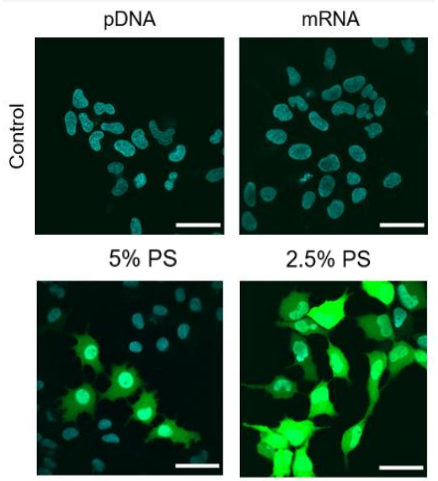
DOTAP



Ionizable cationic lipids

DODMA



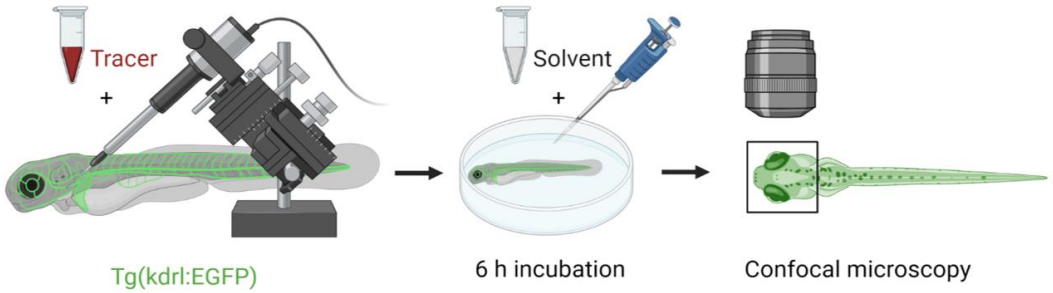


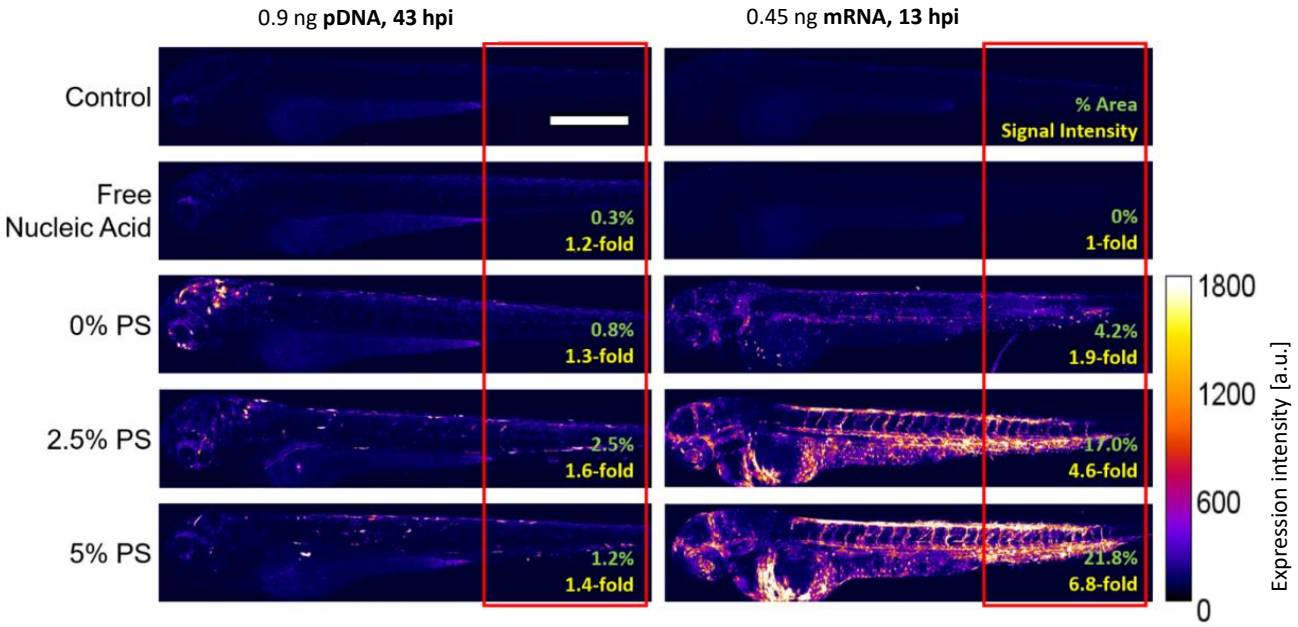
- Combination of PS with LNPs leads to increased transfection efficacy and potency *in vitro*
- Strongest effect using **2.5-5% PS**

N/P = 10; cationic lipid:cholesterol:DOPC:DMPE-PEG$_{2000}$ = 50:39:10:1; 0.28 µg nucleic acid mL^{-1}

PS-LNPs show enhanced *in vivo* potency

Zebrafish larvae (Danio rerio) as an *in vivo* vertebrate screening model
Intravenous injection of 1-3nl of fluorescent LNPs via *Duct of Cuvier*





Combination of PS with LNPs leads to increased transfection potency.

In vitro results confirmed *in vivo*.

Summary

Gene delivery offers promising therapeutic options

Lipid nanoparticles (LNPs) as a multifunctional drug delivery vehicle

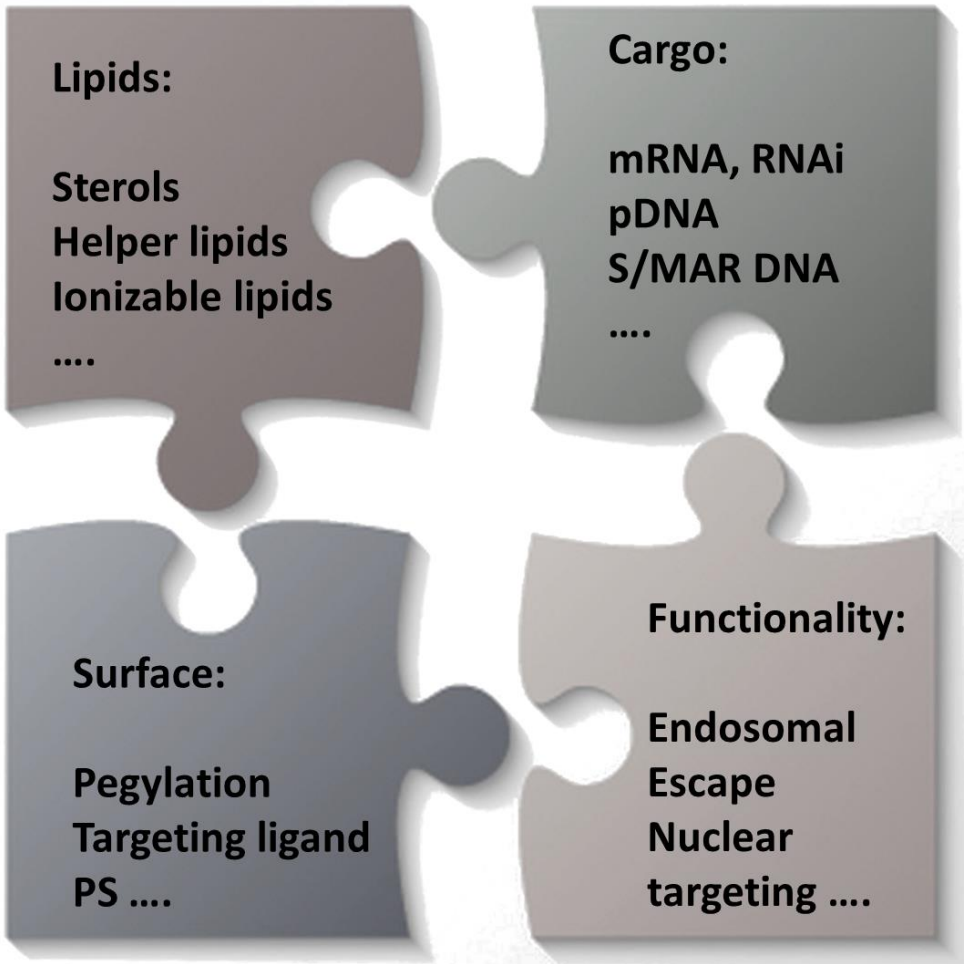
Transfection of **somatic cells only**, no integration into genome

PS promotes cellular uptake

mRNA mediates short duration effects

DNA mediates long duration effects

Our dream and vision:
Treatment options for a disease such as citrullinemia



Thank you for your attention!

Swiss National Science Foundation

LipoidStiftung

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Swiss Centre for Applied Human Toxicology