Structural Investigation of Lipid Nanoparticles is key for Successful mRNA Delivery

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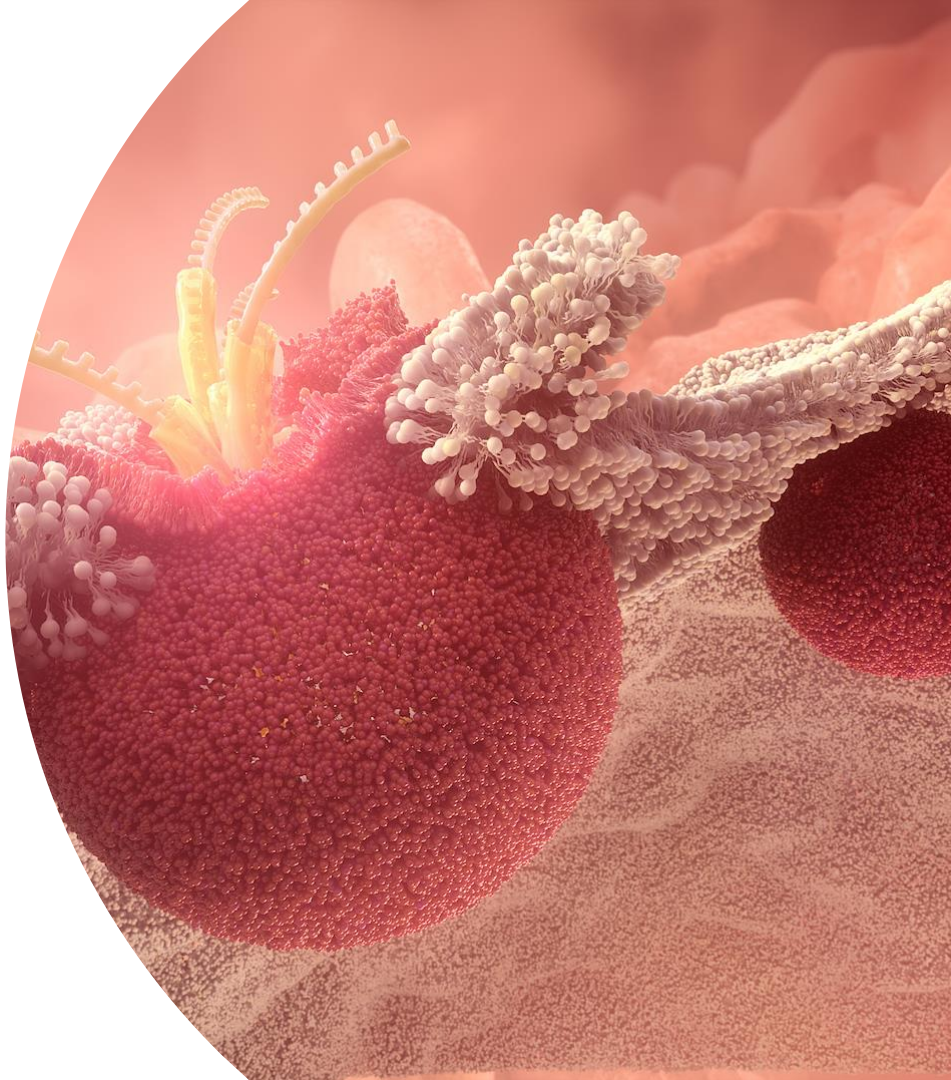
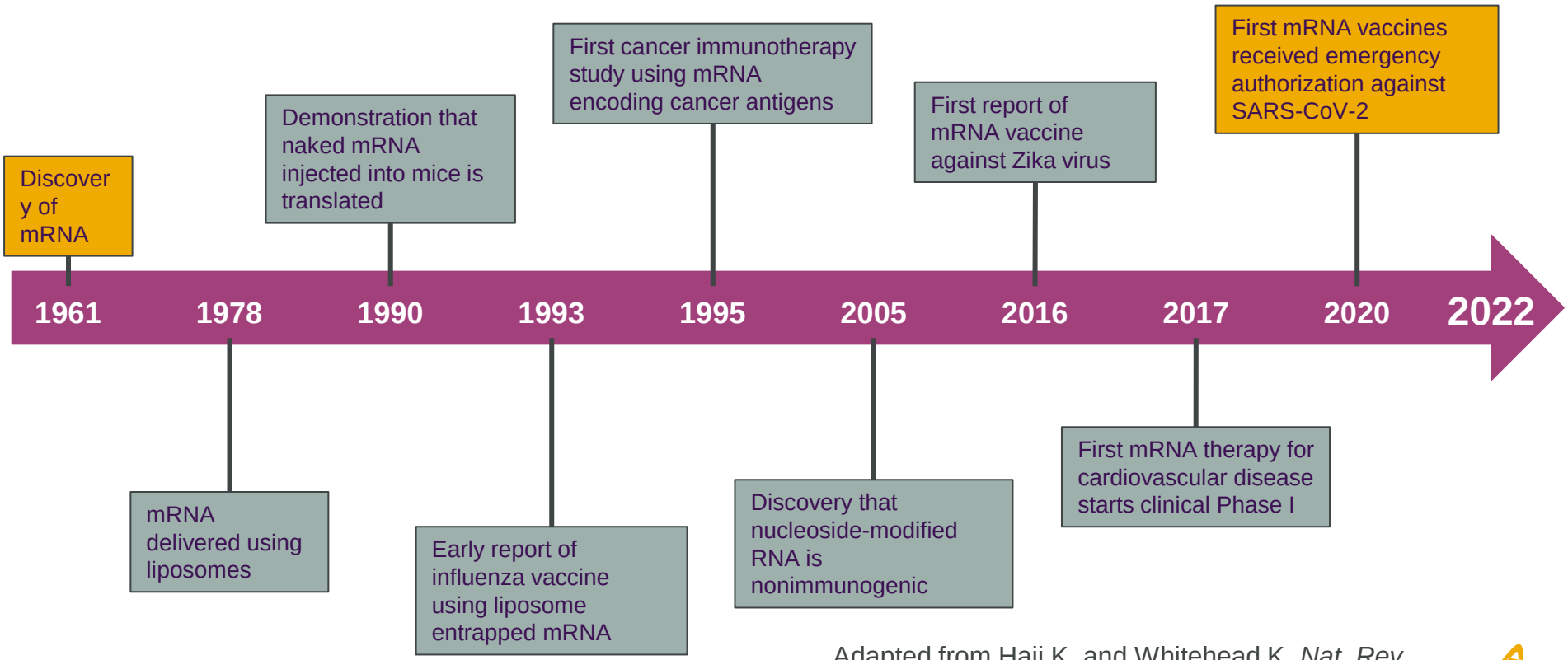


Timeline of key advances for mRNA therapeutics

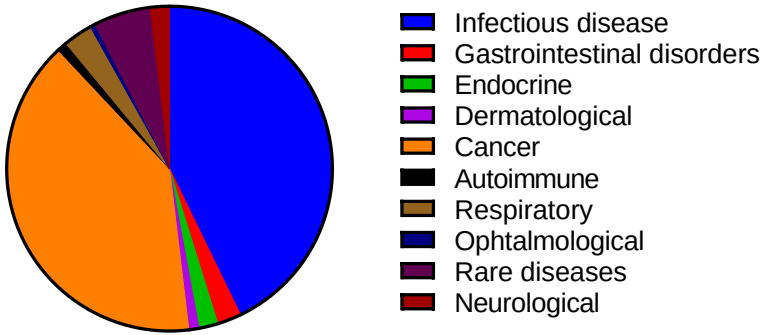


Adapted from Hajj K. and Whitehead K. *Nat. Rev.* **2017**

Looking at the future of RNA based therapies

How does the future looks for RNA therapies?

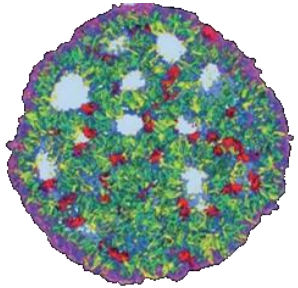
Clinical trials of RNA medicines and RNA-transfected cell therapies for disease condition (Adapted from Webb et al. Mol. Pharmaceutics 2022)



In 2021, 75 new clinical trials were registered using mRNA as the modality according to clinicaltrials.gov

What is enabling RNA therapies coming this far?

LNPs: cationic ionizable lipid (CIL), cholesterol (Chol), distearoylphosphatidylcholine (DSPC) and a poly(ethylene glycol lipid).

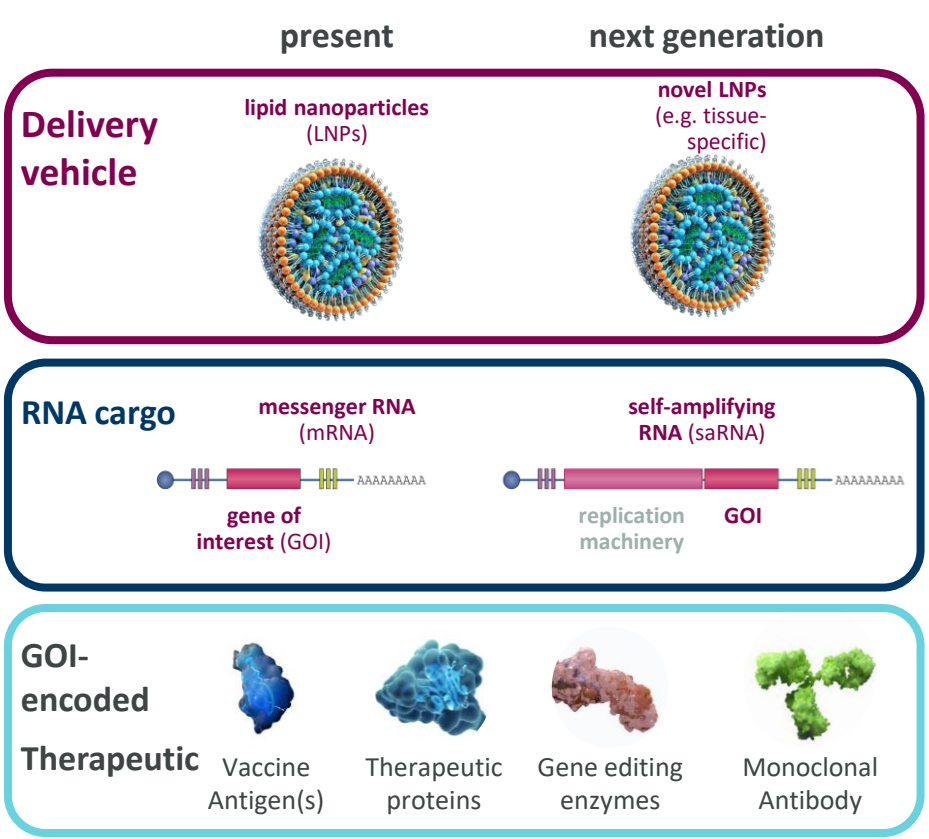


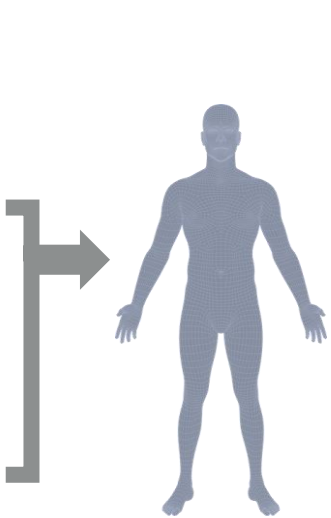
Representation of LNP containing siRNA based on molecular simulations (Rozmanov et al. Faraday Discussions 2014)

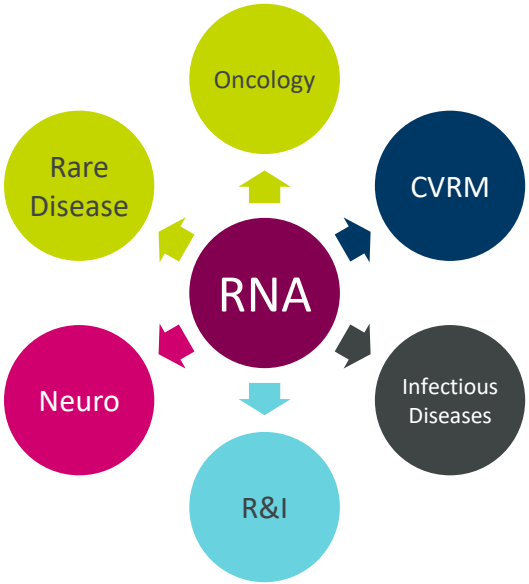
1 *siRNA* product and 2 *mRNA* vaccines have been approved using LNPs

onpattro® (patisiran) lipid complex injection

LNP-delivered RNA therapeutics are potentially transformative across a number of disease indications



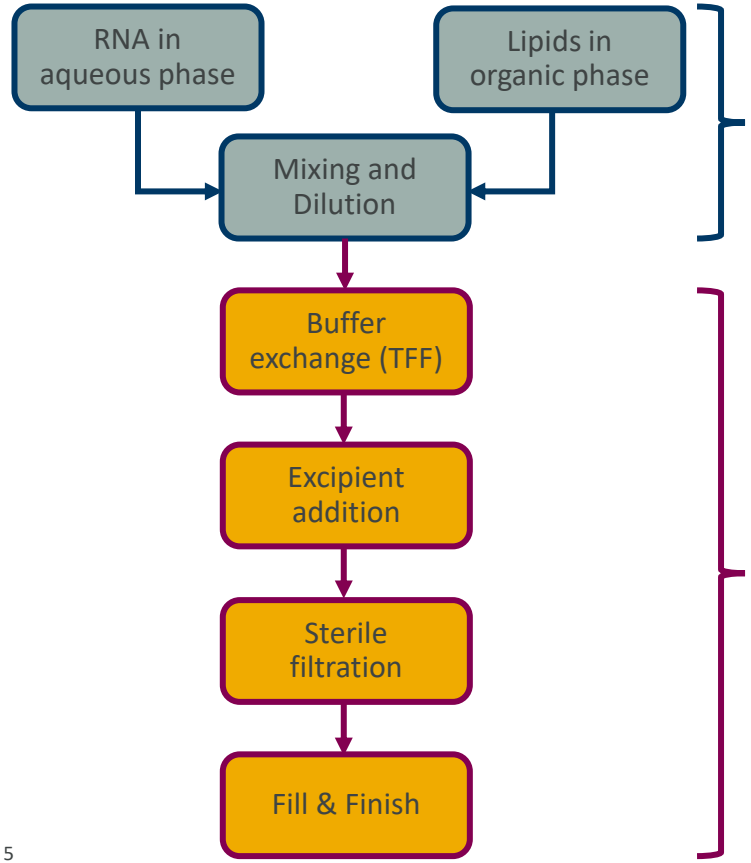




- Patients produce tailored biotherapeutics within their bodies
- Broad application across therapeutic indications

Slide courtesy of my colleagues Lennart Lindfors, James Button, Jason Laliberte and Grzegorz Sienski

Developing a robust manufacturing process is key for success



Mixing

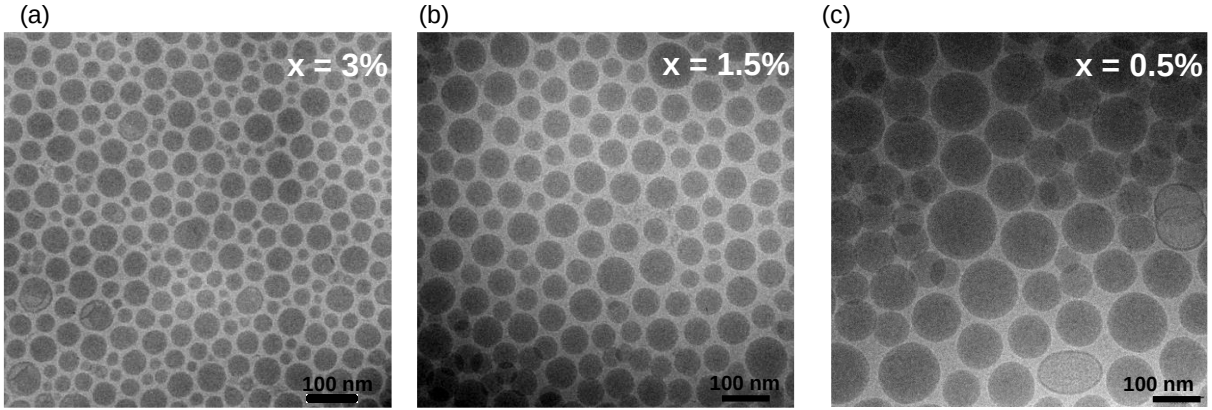
- Mixing geometry (e.g. T-junction vs microfluidics)

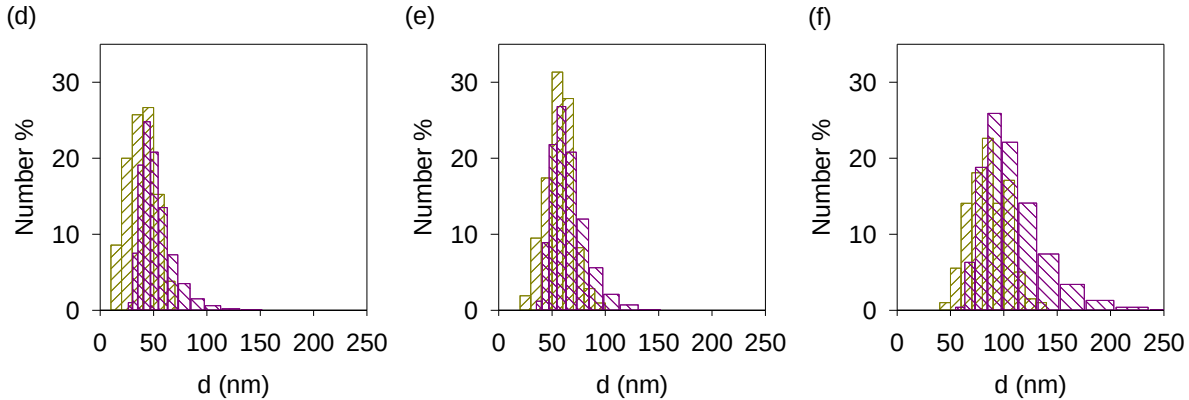
- Speed of mixing
- Concentrations and volumes
- Aqueous/organic mix ratio
- In-line dilution

Downstream Process

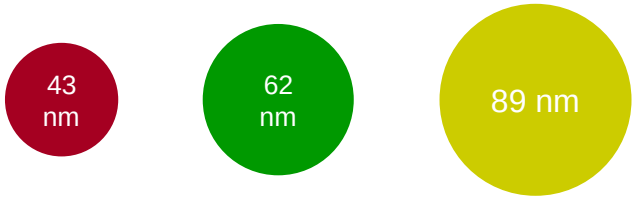
- Exchange from mixing composition to final buffer using typically TFF. Critical parameters as flow rate/shear effect, time and compatibility with filters needs to be addressed
- Concentration adjustment
- Addition of excipients for stability
- Sterile filtration
- Lyophilization if required, including reconstitution
- Fill-finish
- Pack, label and distribution of RNA-LNP drug product

Controlling the size of the LNPs





Standard approach:
CIL:Chol:DSPC:PEG-lipid in a 50:40-x:10:x mole ratio.



Size distributions: DLS number distribution vs. Cryo-TEM distribution

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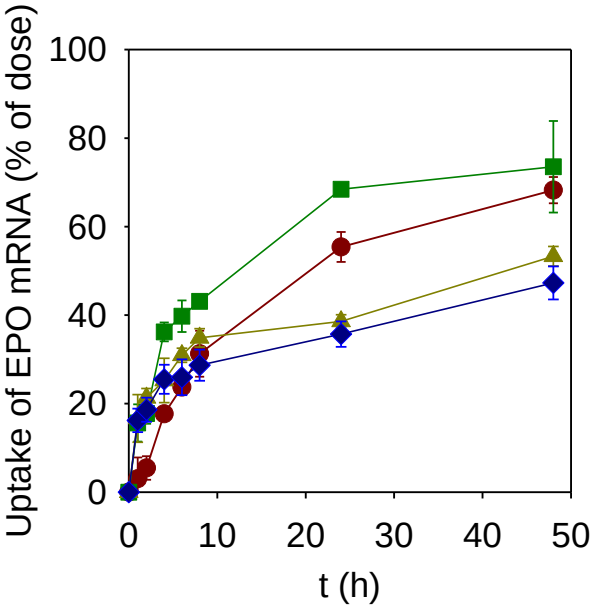
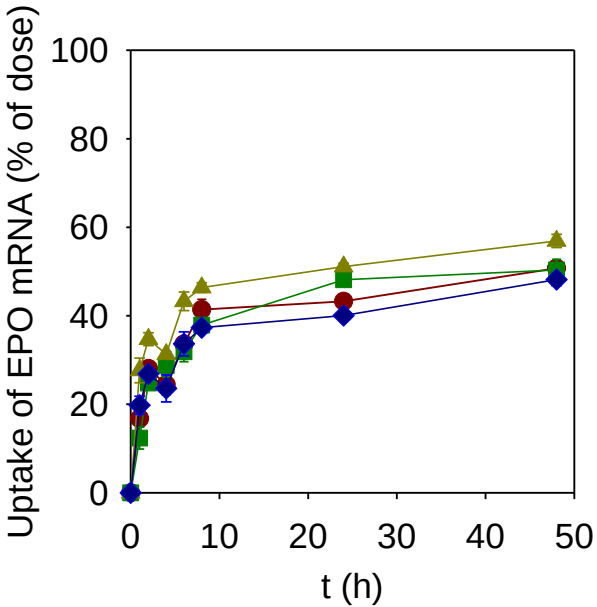
In vitro uptake of LNPs of different size

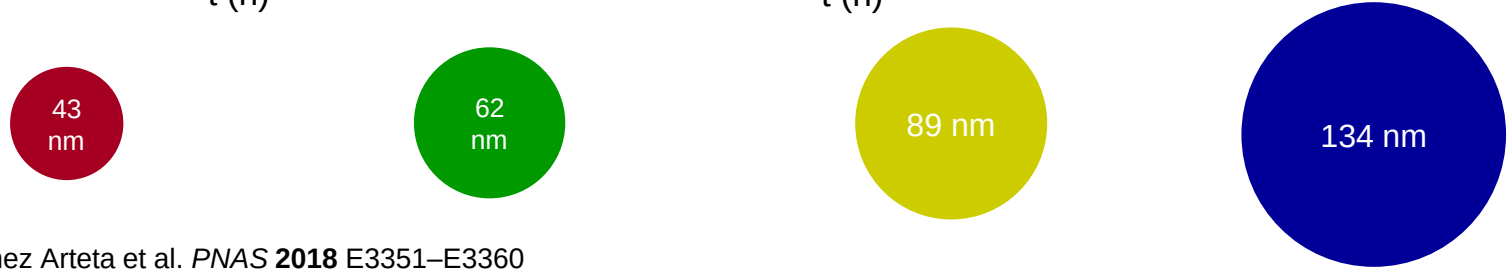
LNP uptake in human adipocytes



LNP uptake in iPSC derived hepatocytes



Tracking of LNPs containing ^{3}H labelled DSPC.



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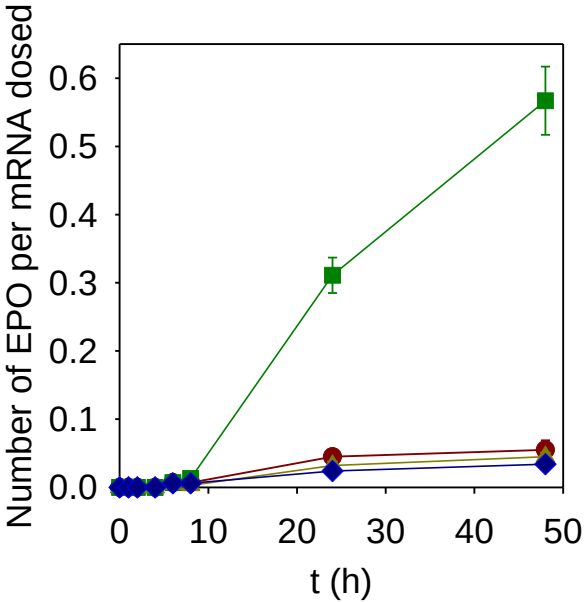
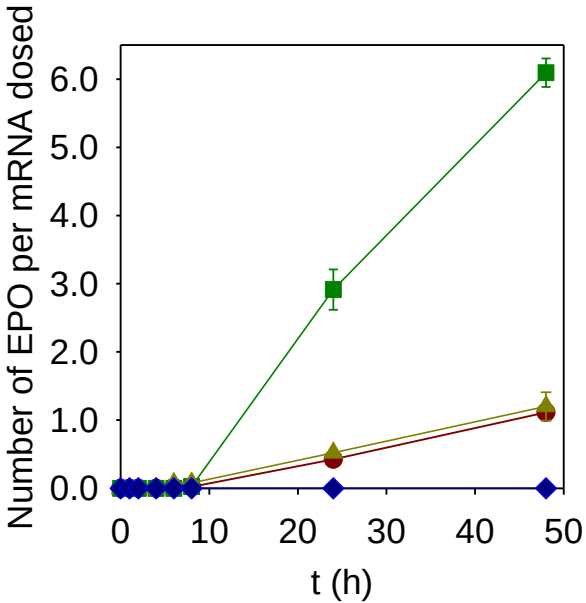
Expression of EPO mRNA *in vitro* for LNPs of different size

Protein expression in adipocytes



Protein expression in hepatocytes



Why do LNPs of 62 nm have a higher transfection efficacy?

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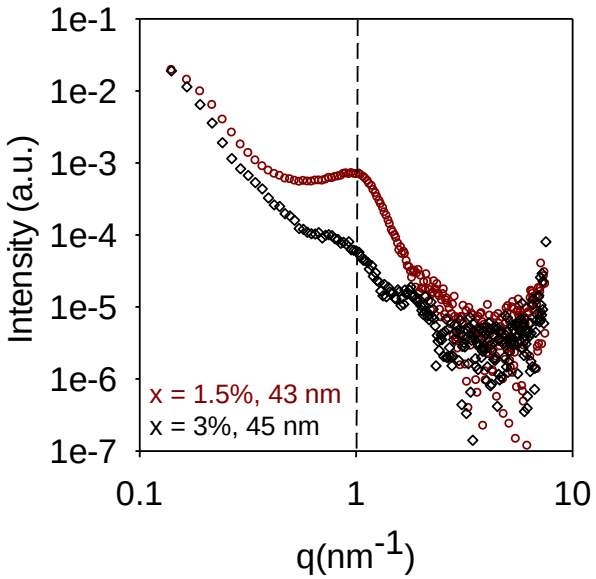
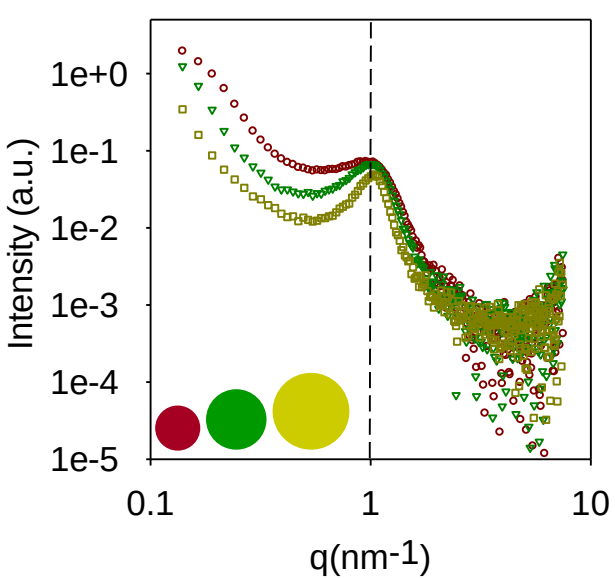
LNPs containing mRNA have a structured core

mRNA-LNPs have a “structured core” with a 6 nm correlation distance.

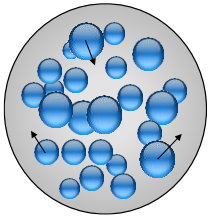
SAXS of mRNA LNPs vs. *Empty LNPs*



SAXS of mRNA LNPs of different sizes

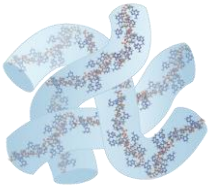


- Inverted micellar phase? (Literature)



- Onion?

- Wormlike micelles?



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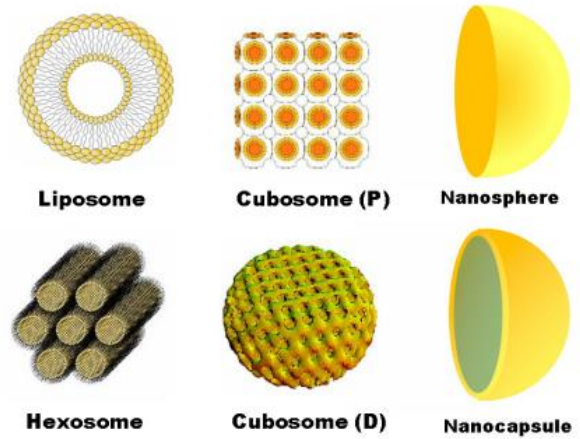
Why do we care about the structure of LNPs?

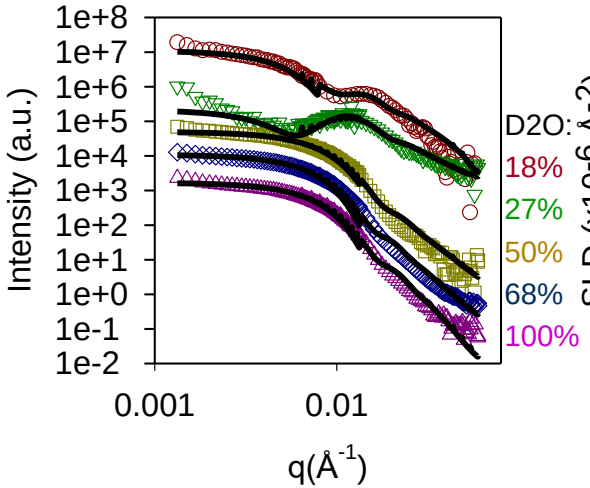
- LNP transfection efficacy is very low, 1-2% (Gilleron et al. (2013) *Nat. Biotech.* 31:638-646)
- Which type of structures will facilitate endosomal escape?



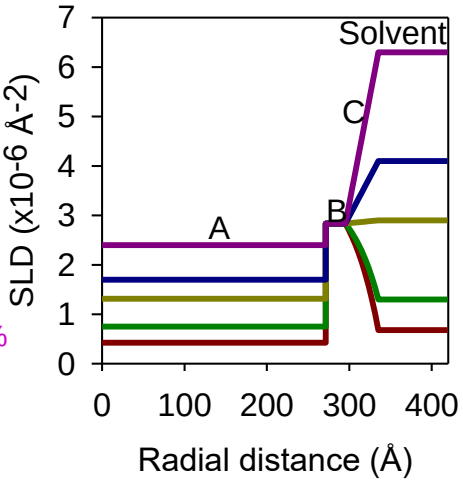
Géral et al., (2013) *Pharmaceutics* 5:126-167

Location of lipids within the LNPs obtained by SANS

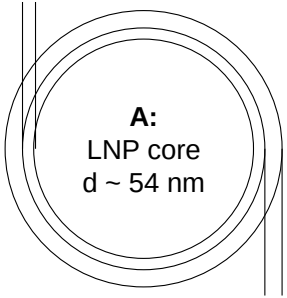
SANS of LNPs with deuterated DSPC and Chol in buffer with different H_2O/D_2O ratio



SLD profiles corresponding to the fits to the core-shell model



B: Lipid monolayer (enriched in DSPC): δ~2.4 nm



C: PEG_{2000} layer in mushroom configuration: L~4 nm

Schematic representation of the lipid distribution in the LNPs

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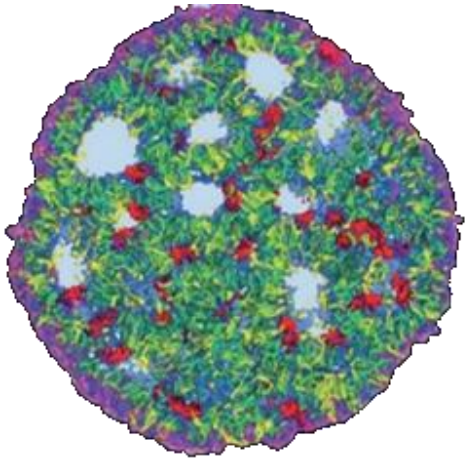
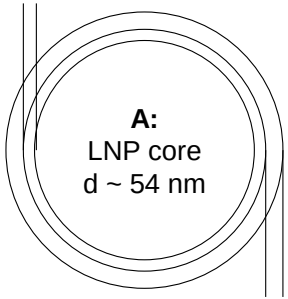
Location of lipids within the LNPs: Comparison with previous models



Representation of an LNP containing siRNA : CIL, Chol, DSPC and PEG-lipid. Based on molecular simulations
(Rozmanov et al. Faraday Discussions 2014)

Schematic representation of the lipid distribution in the LNPs

B: Lipid monolayer (enriched in DSPC): δ~2.4 nm



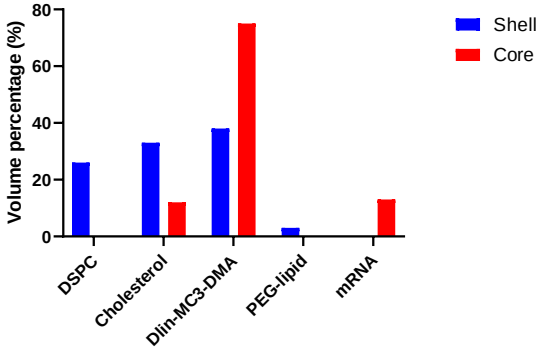
C: PEG_{2000} layer in mushroom configuration: L~4 nm

A. LNP core:

- Cationic ionizable lipid (CIL)
- Cholesterol (CHOL)
- 24% water
- mRNA

Final lipid distribution was quantified by formulating LNPs with multiple deuteration levels:

- **DSPC (d83)**
- **Dlin-MC3-DMA (d62)**
- **Cholesterol (“Match-out”)**



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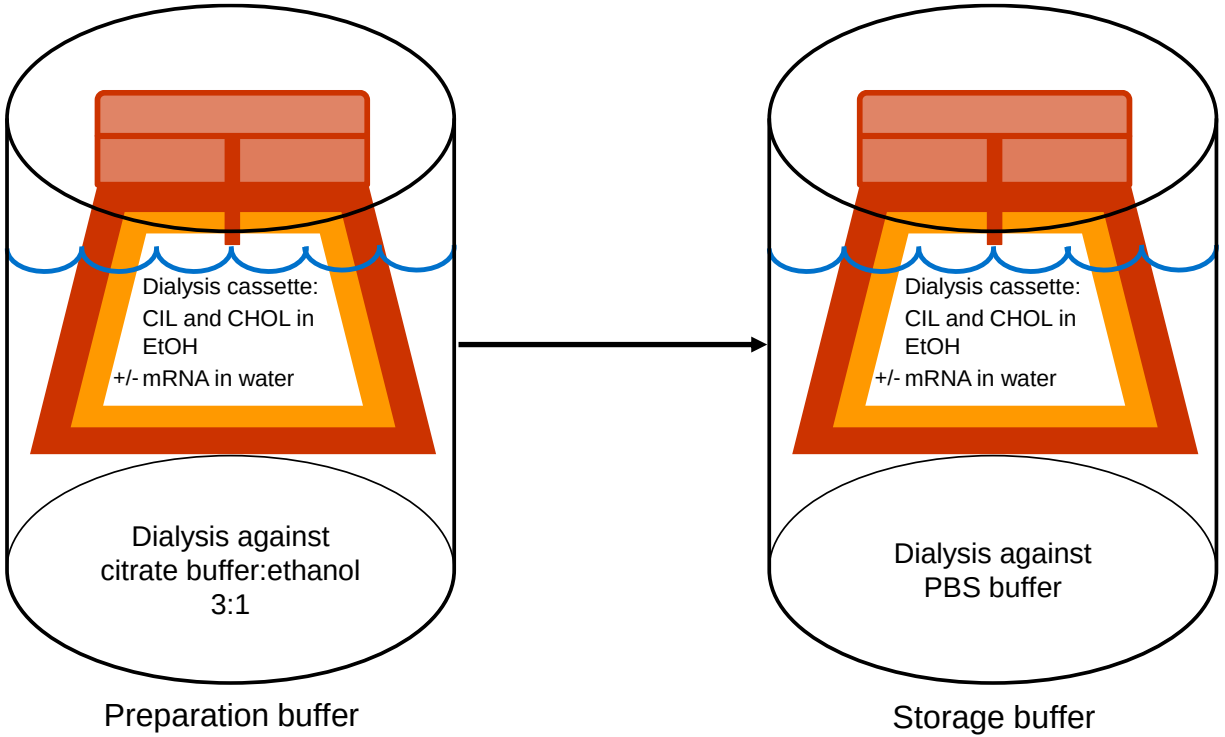
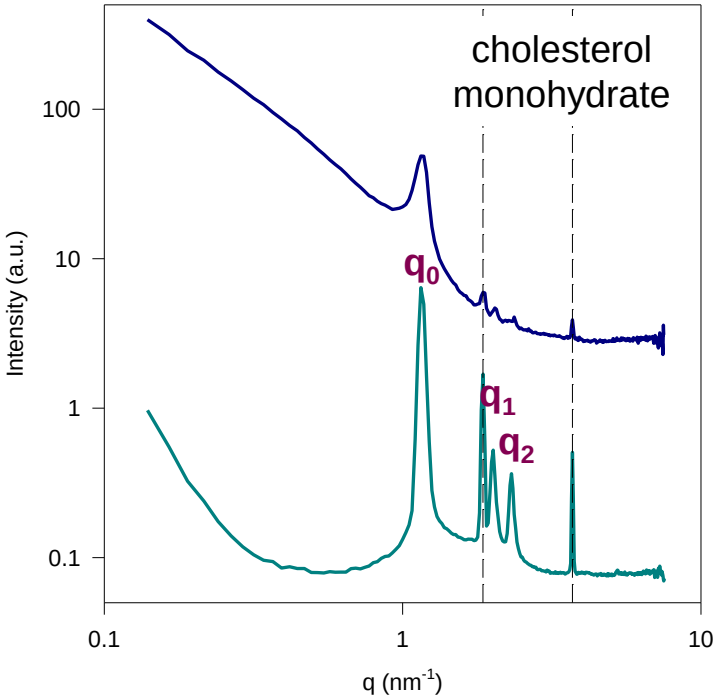
Sebastiani et al. *ACS Nano* **2021** 15, 6709–6722

Further exploration of the LNP core



SAXS of the core phase: citrate:ethanol 3:1 phase

Small angle x-ray scattering (SAXS) for empty and polyA LNP bulk phases (pH 3, 25% EtOH)



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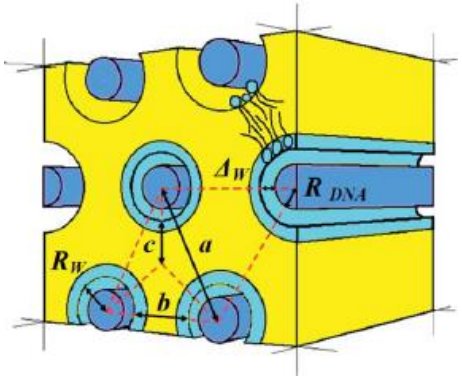
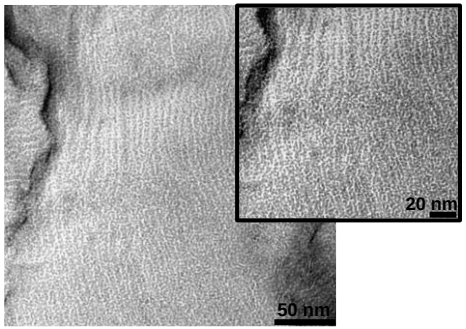


Schematic representation of a reversed hexagonal phase structure. (Bilalov *et al.* Soft Matter 2011)



Freeze fracture micrograph of the LNP core phase in citrate:ethanol 3:1 phase

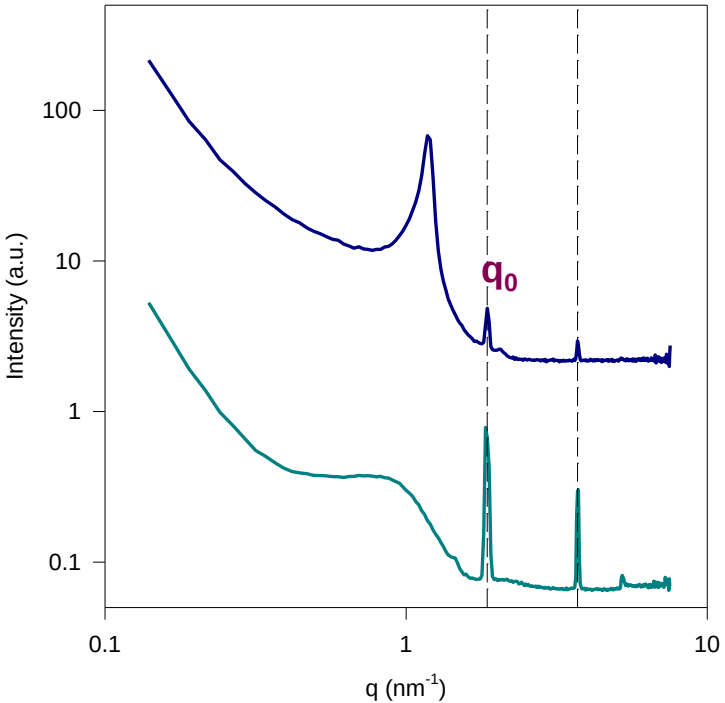
Reversed hexagonal phase (water or water/RNA rigid cylinders):

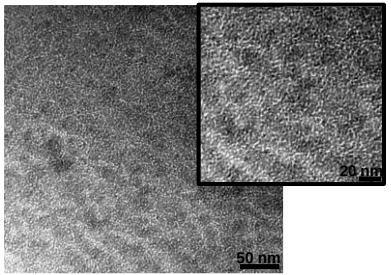
- $q_1=\sqrt{3}*q_0$
- $q_2=\sqrt{4}*q_0$

Center-center distance a=6.2 nm

SAXS of the core phase: PBS buffer and comparison with LNPs

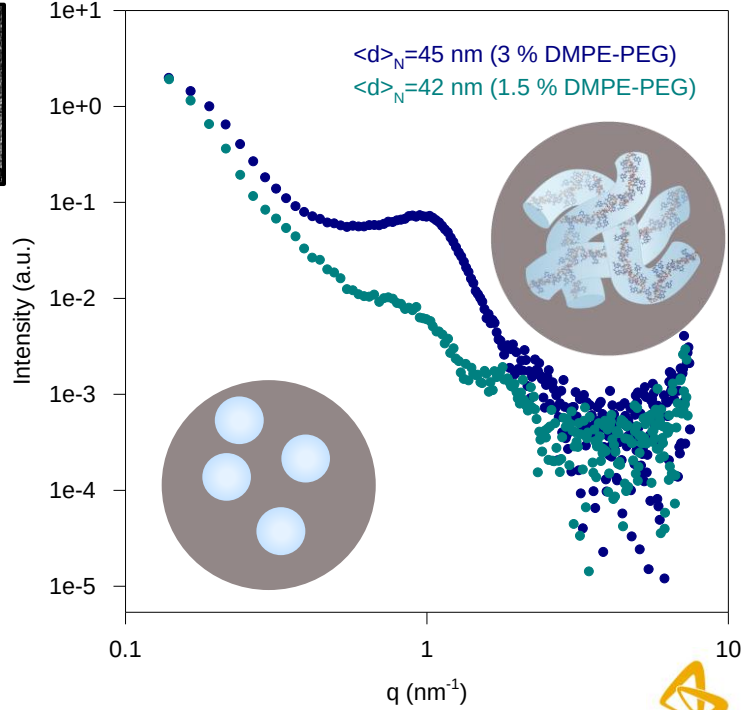
Small angle x-ray scattering (SAXS) for empty and polyA LNP bulk phases (pH 7.4)





Freeze fracture micrograph of the LNP core phase in PBS

Small angle x-ray scattering (SAXS) of mRNA LNPs vs empty LNPs



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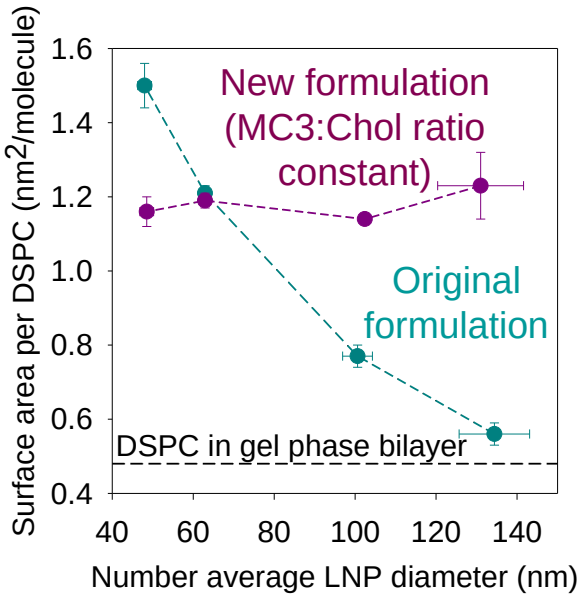
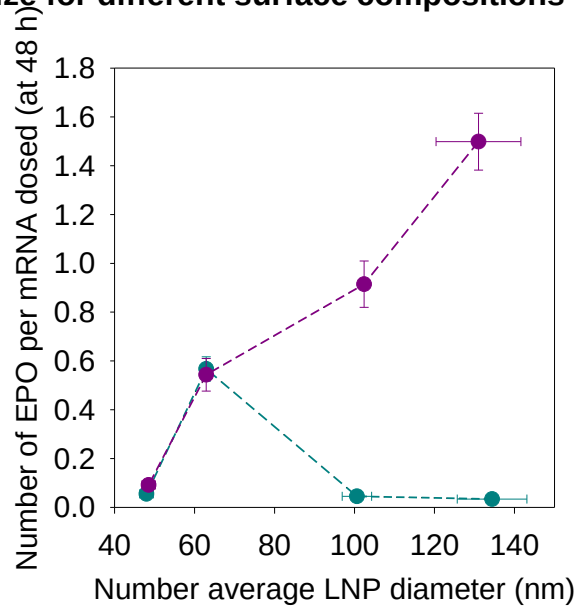
Reprograming cell protein production by modifying LNPs surface

Area per DSPC as a function of size for different LNP formulations



Protein expression as a function of the LNP size for different surface compositions

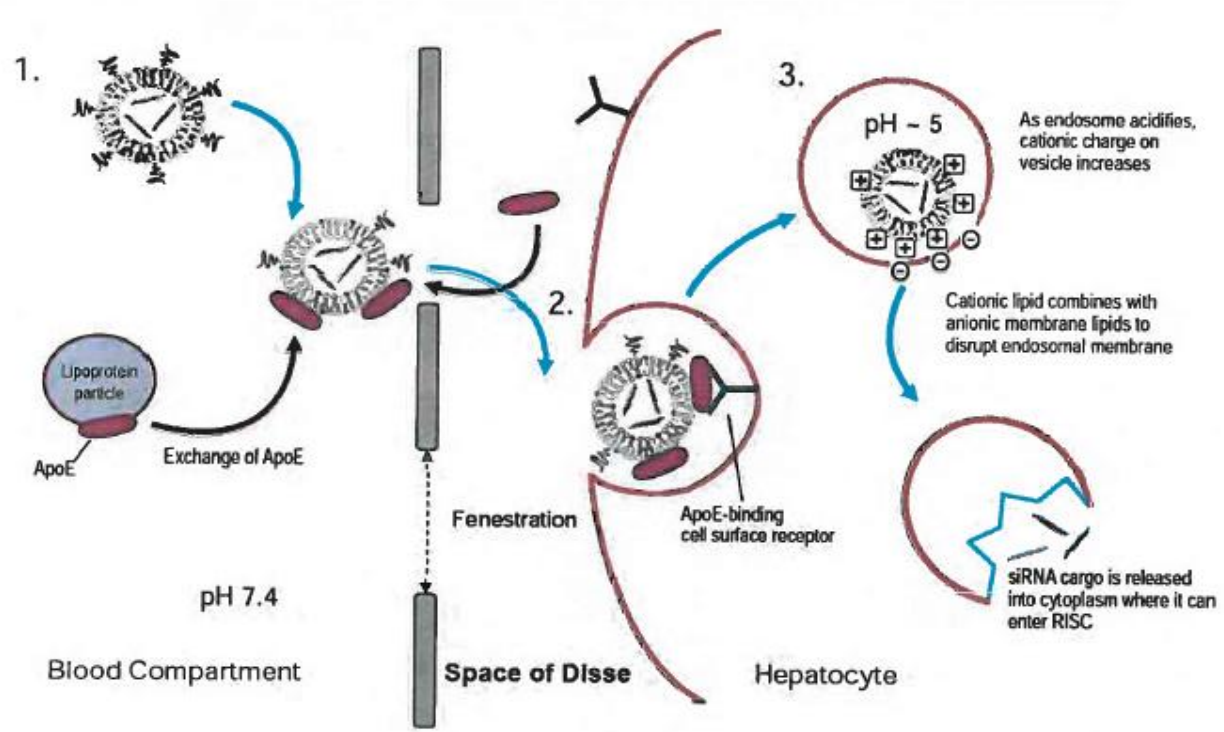


"One shoe size doesn't fit all"

Knowing the size and type of feet allows a rational design

Yanez Arteta et al. *PNAS* 2018, 115, E3351

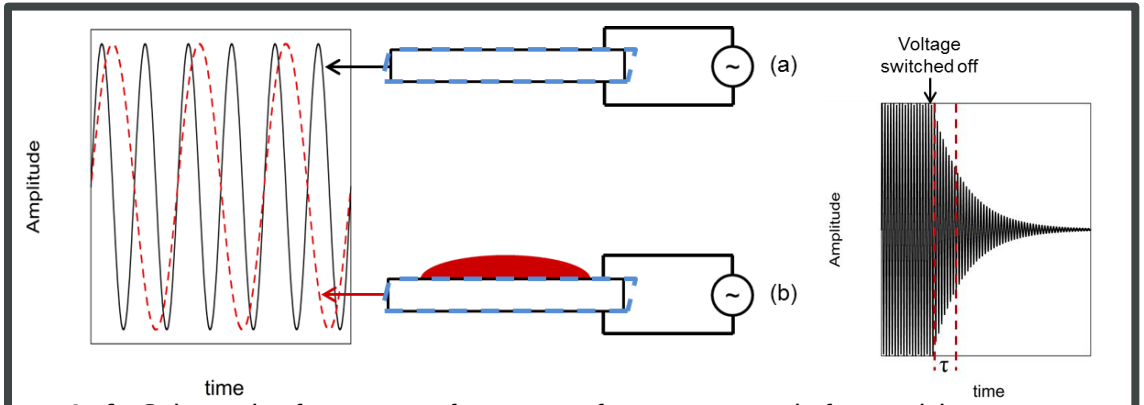
LNPs proposed mechanism of action for IV delivery to hepatocytes



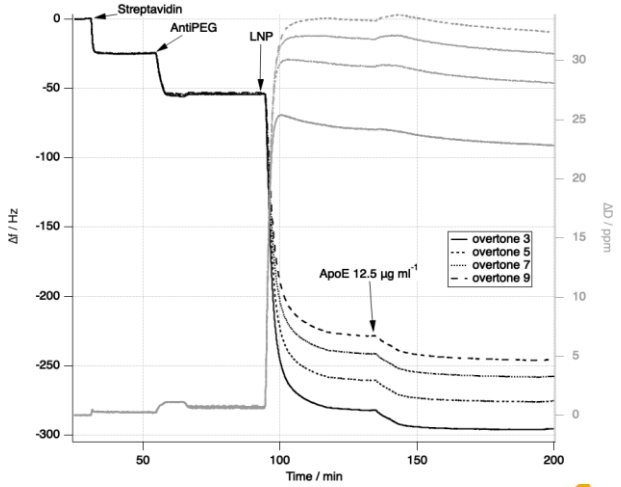
Akinc et al., (2010) *Mol. Ther* 18:1357-1364

The principle behind QCM-D

- **Q**uartz **C**rystal **M**icrobalance with **D**issipation monitoring is an acoustic technique
- It allows measuring the adsorbed mass on a solid substrate in real time with a sensitivity of 0.01 mg m^{-2}



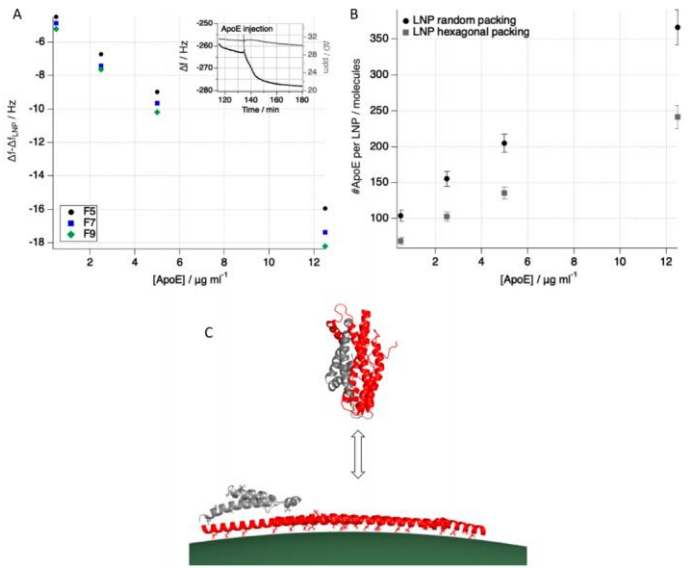
Left: Schematic of resonance frequency of a quartz crystal after applying an alternating current in the (a) absence and (b) presence of adsorbed layer
Right: Dissipation of energy after voltage is switched off gives information about the viscoelastic properties of the layer



Sebastiani et al. *ACS Nano* **2021** 15, 6709–6722

Developing a sensor for protein binding to LNPs

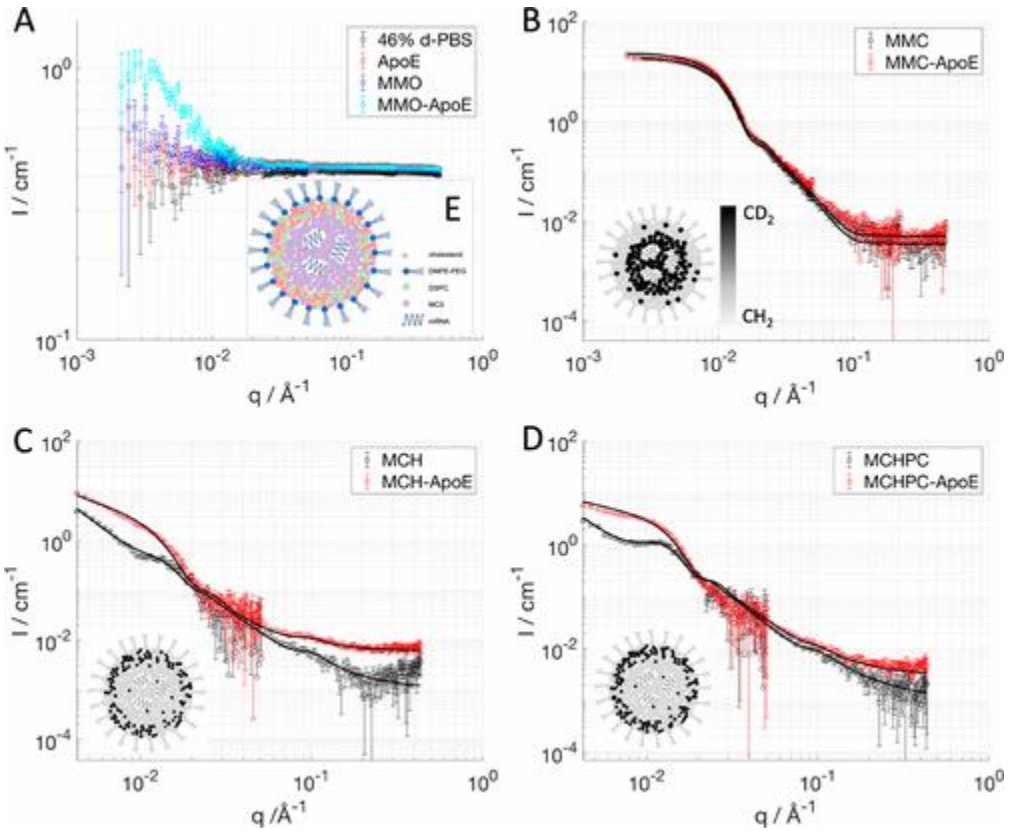
- Functionalized sensors have been developed to investigate the affinity of proteins to LNPs using QCM-D
- ApoE show the highest binding affinity while other proteins as ApoA and HSA show low or no binding
- The sensor is able to differentiate between LNPs of different composition



Sebastiani et al. *ACS Nano* **2021** 15, 6709–6722
Sebastiani et al. *JCIS* **2022** *610*, 766-774

SANS as a tool to characterize LNPs in the presence of proteins

- LNPs were incubated with ApoE for 3 h
- Isotopically labelled LNPs were designed to highlight LNP components
- Addition of ApoE shows clear changes in the LNP structure



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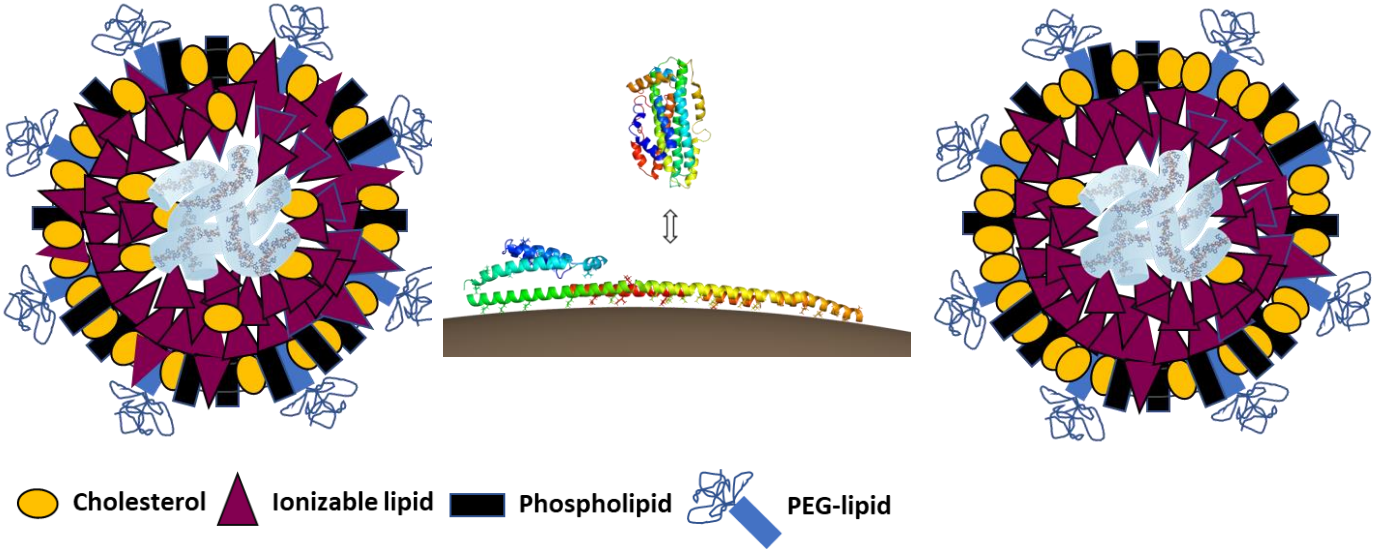
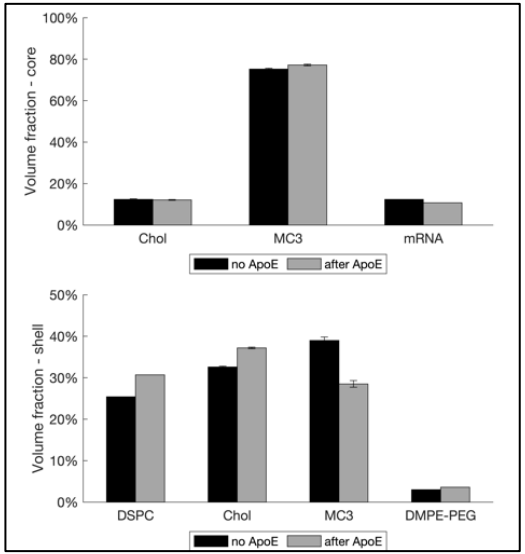
ApoE binding induces lipid redistribution

- ApoE binding leads to an increased cholesterol concentration in the LNP surface which seems to be accompanied by nanodomain formation.
- The surface nanostructure will play a role in the intracellular fate of LNPs.

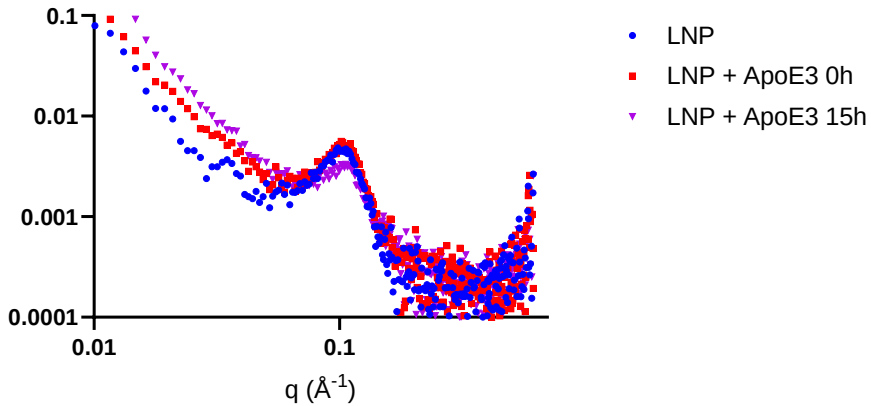


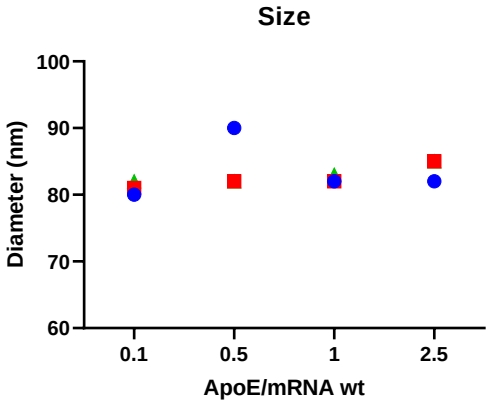


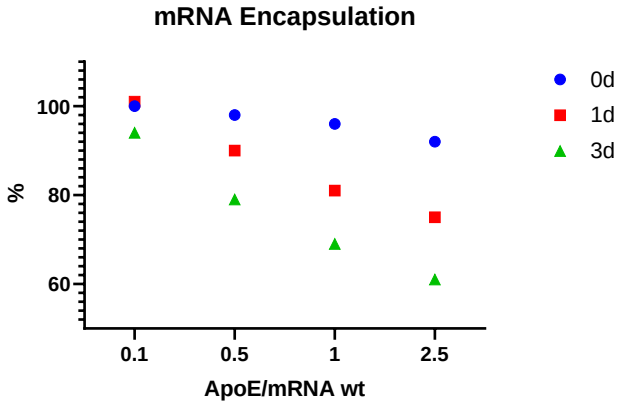
Sebastiani et al. ACS Nano **2021**, 15, 6709–6722

ApoE Binding induces changes in LNP core

- Incubation of ApoE with mRNA-LNPs does not affect the size measure by DLS, but it does affect mRNA encapsulation
- SAXS indicates a “less ordered” internal structure







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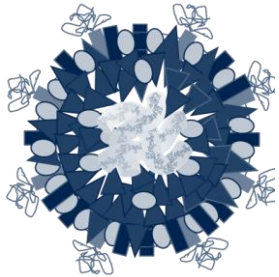
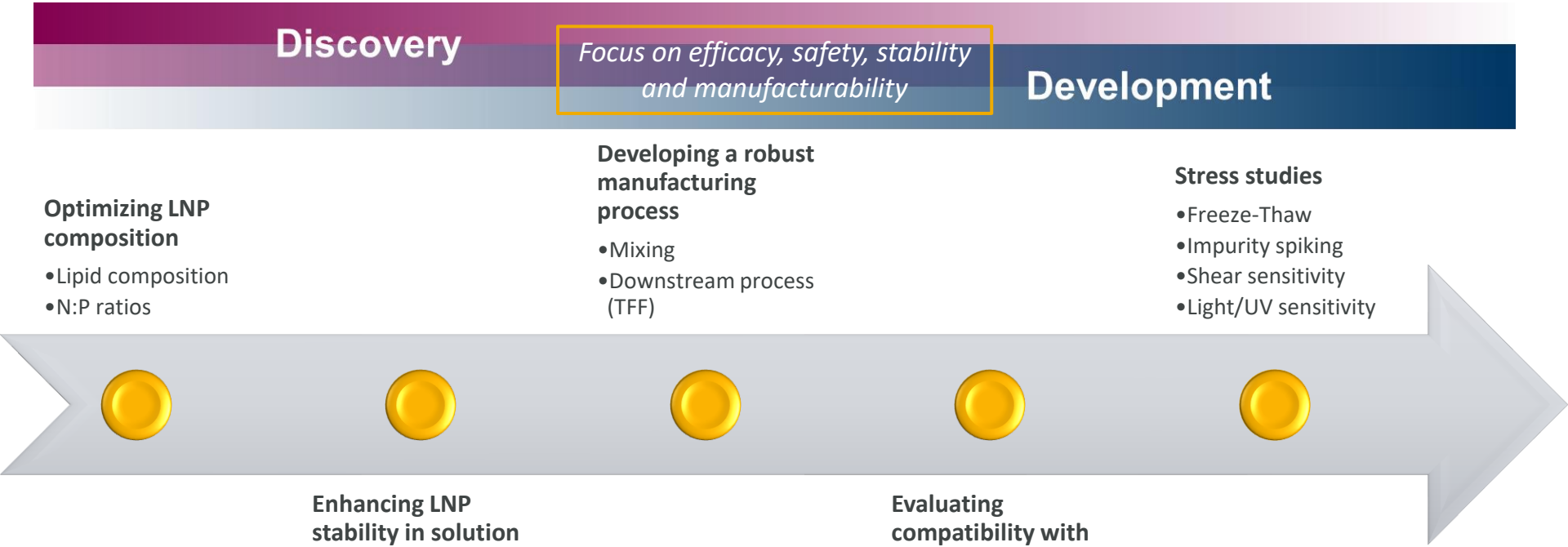
Summary



- LNPs are leading delivery vehicles for RNA therapy
- SANS is a powerful tool for characterization and development of mRNA-containing LNPs
- The transfection efficacy of LNPs containing mRNA is size and surface composition dependent
- Binding of ApoE induces lipid redistribution across mRNA-containing LNPs

Planning the formulation development strategy for a RNA-LNP



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Confidentiality Notice