

Freeze drying to enable the long term storage of mRNA lipid nanoparticles

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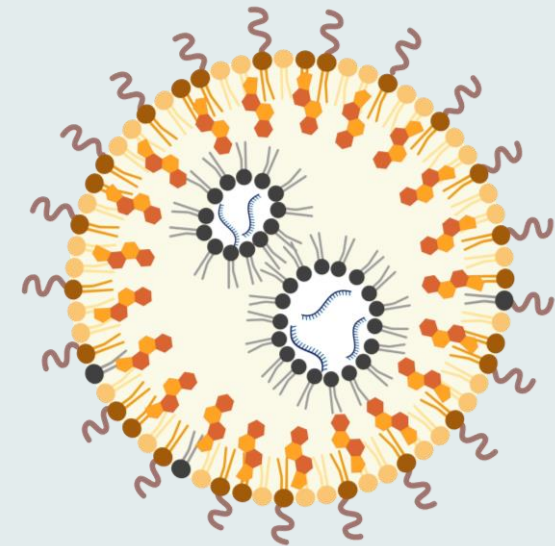
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Introduction

mRNA degradation

- ✓ pH hydrolysis
- ✓ nuclease-activity
- ✓ lipid:mRNA-adducts

lipid oxidation and hydrolysis

- ✓ H₂O₂ impurities

Freezing
degradation rate ↓

↓

Freeze-drying
dehydration



DMG-PEG (1,5 mol%)



DSPC (10 mol%)



Cholesterol (38,5 mol%)

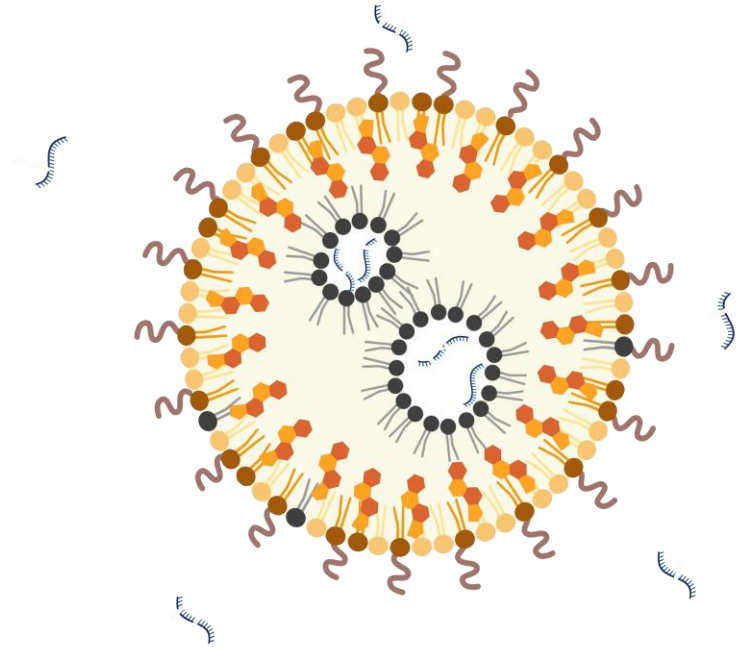


Ionizable lipid C12-200 (50 mol%)

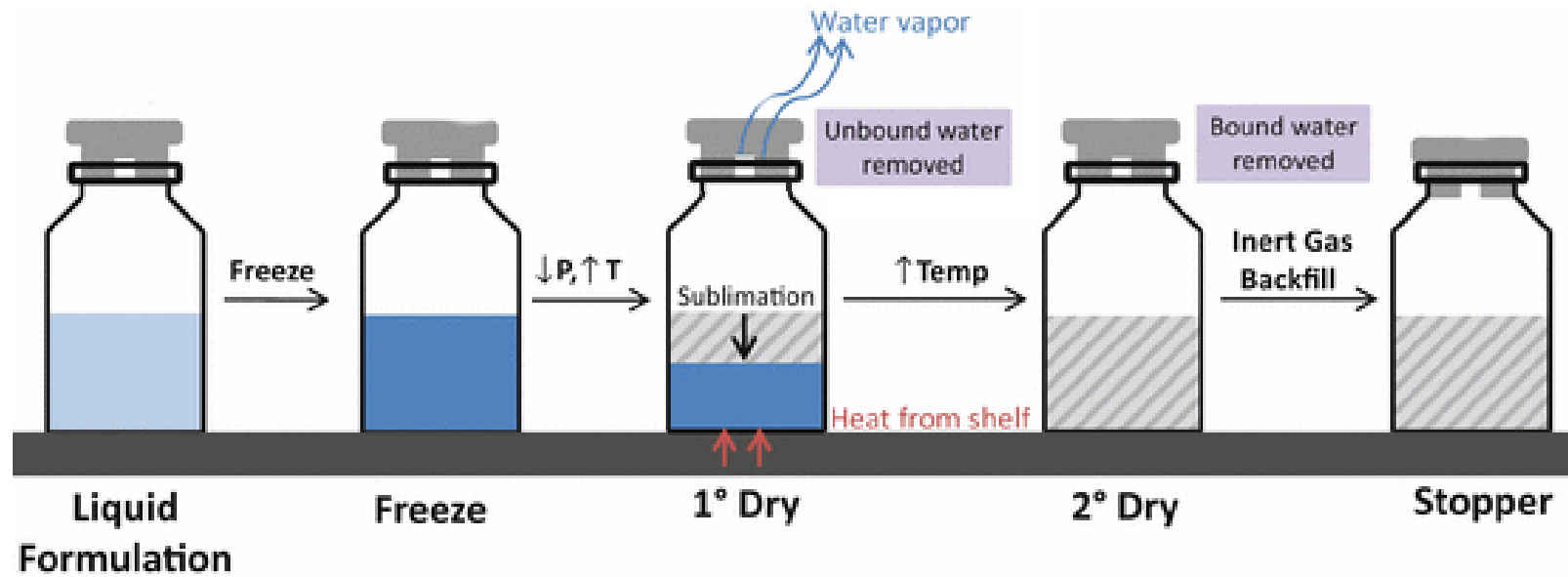


N¹-Methylpseudouridine substituted mRNA

→ reporter mRNA (luciferase/eGFP)

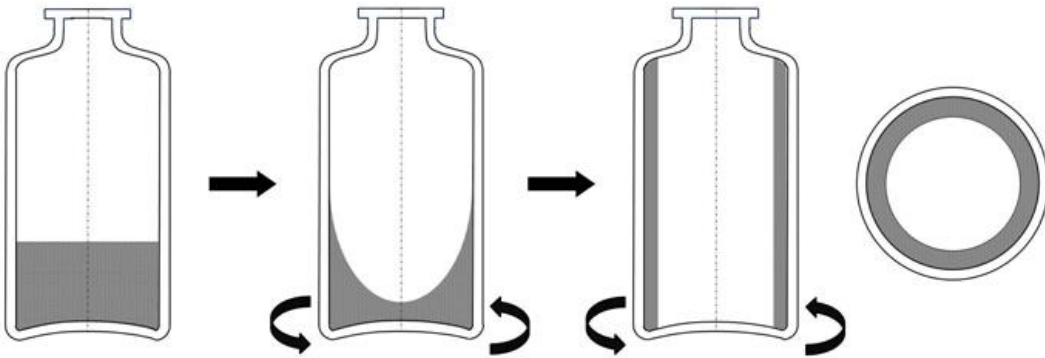


(Classical) Freeze-drying



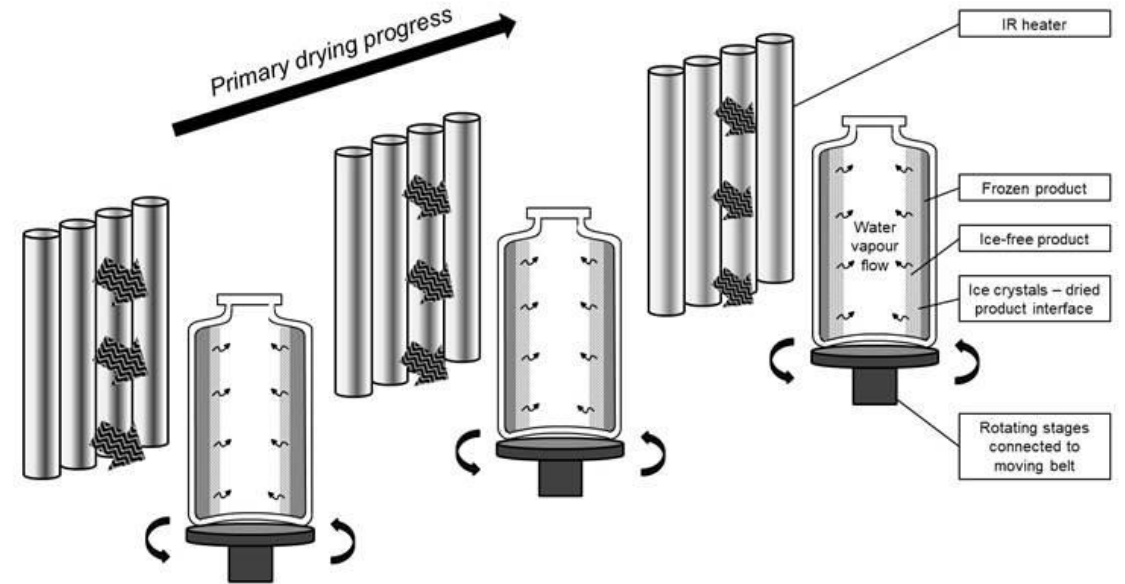
Continuous freeze-drying

Spin freezing



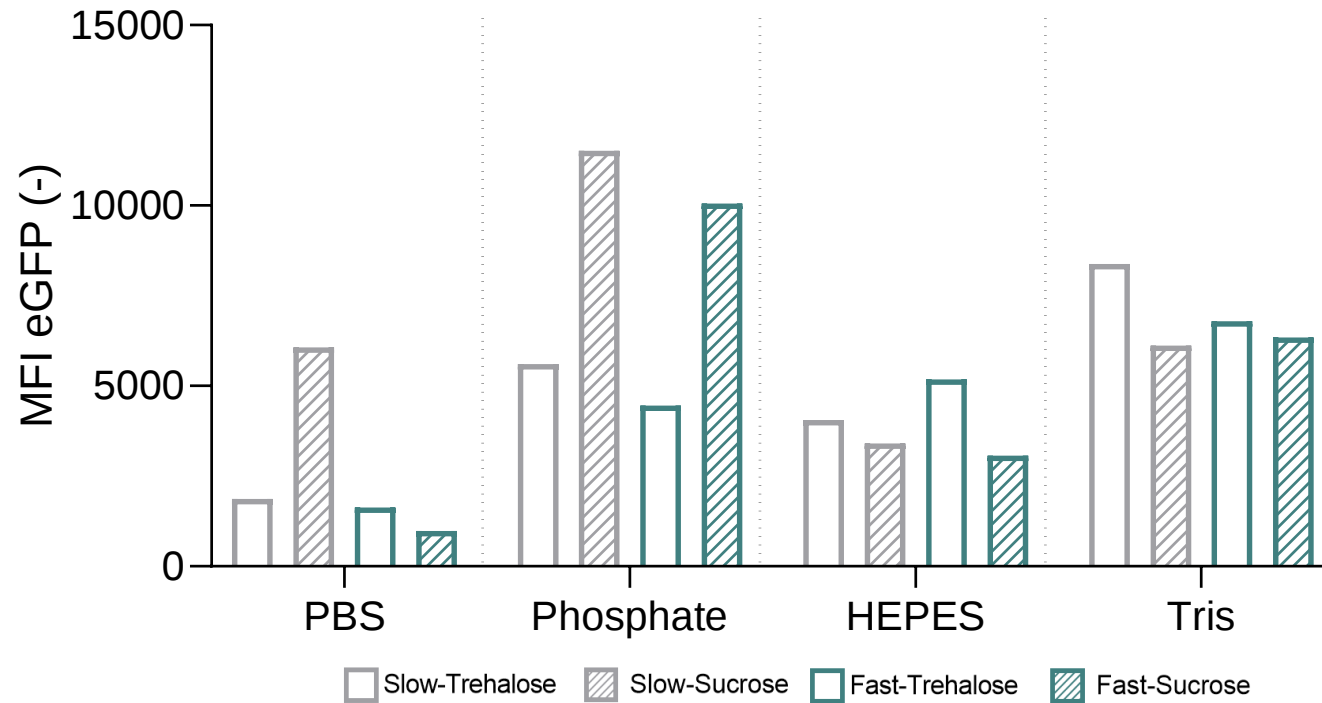
- Vials rotated along longitudinal axis
- Frozen product spreads across wall: surface area x4-5

Drying step



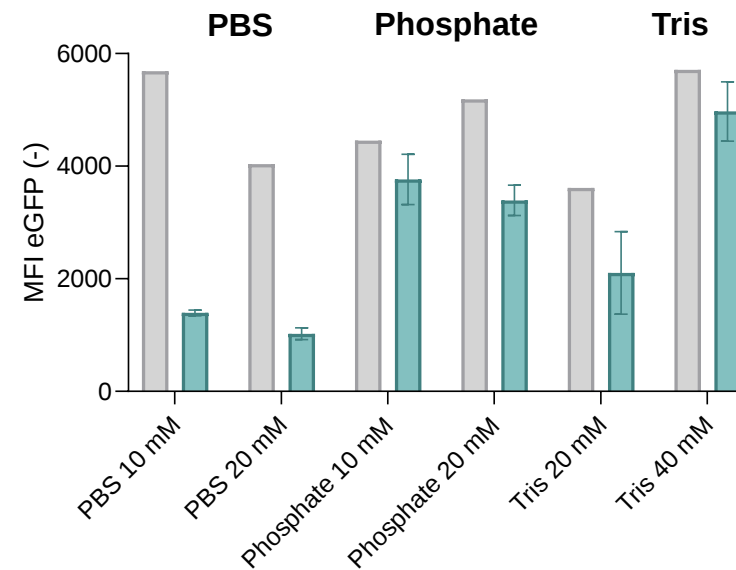
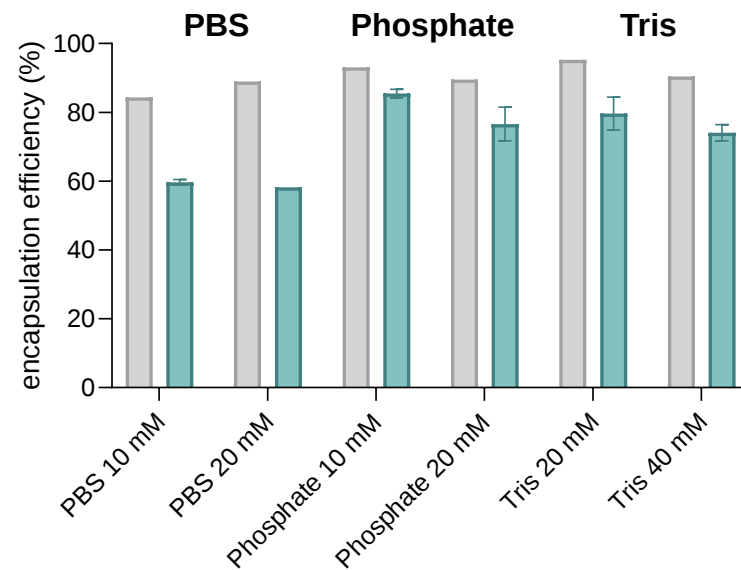
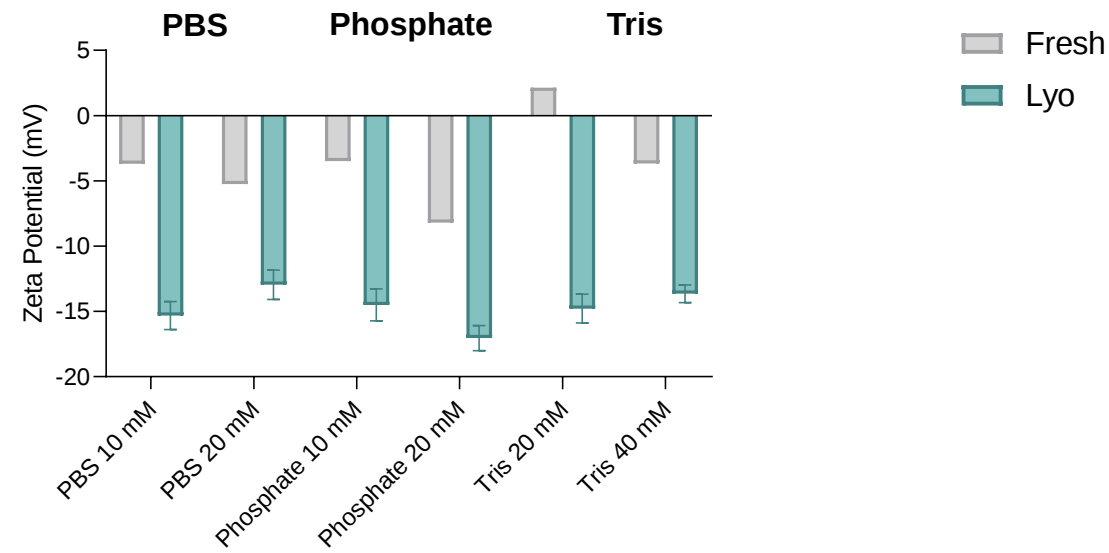
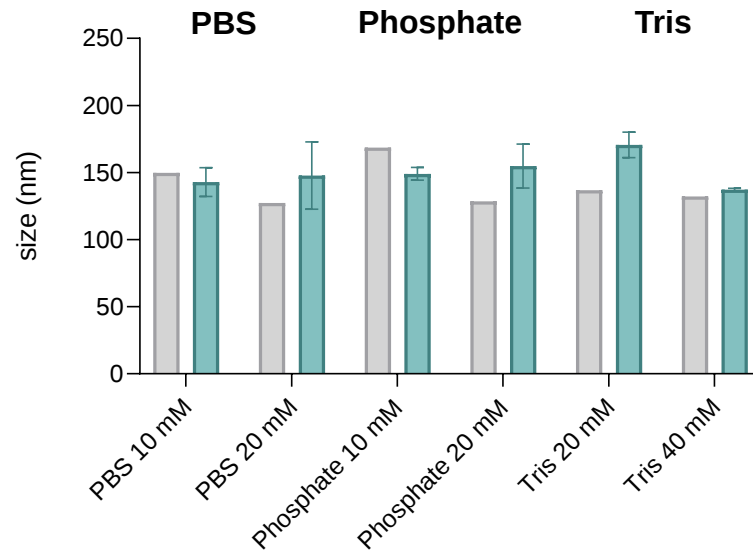
- Increased surface area → drastic reduction drying time

Impact of buffer, lyoprotectant and freezing rate



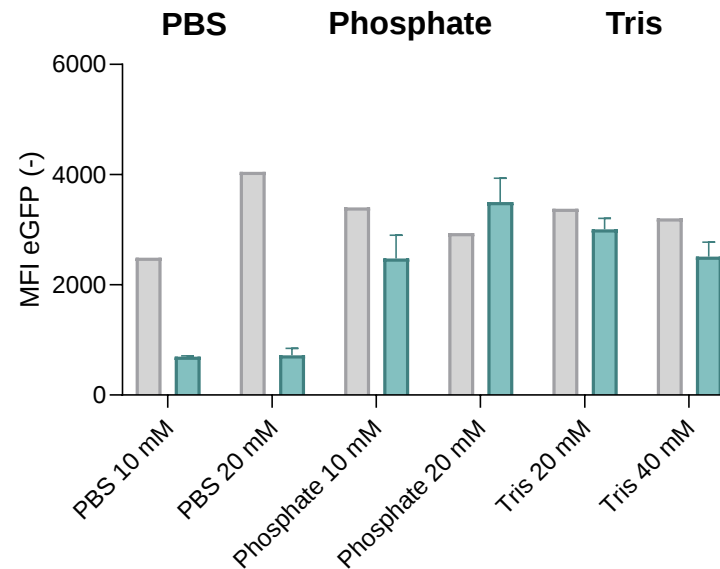
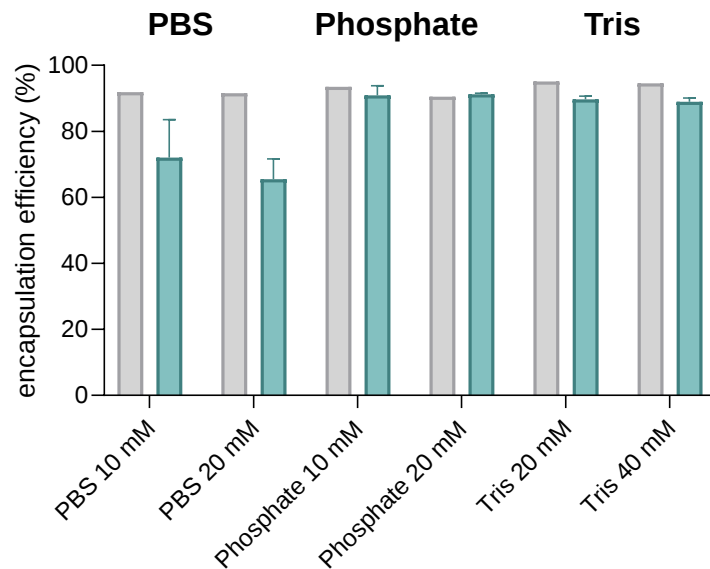
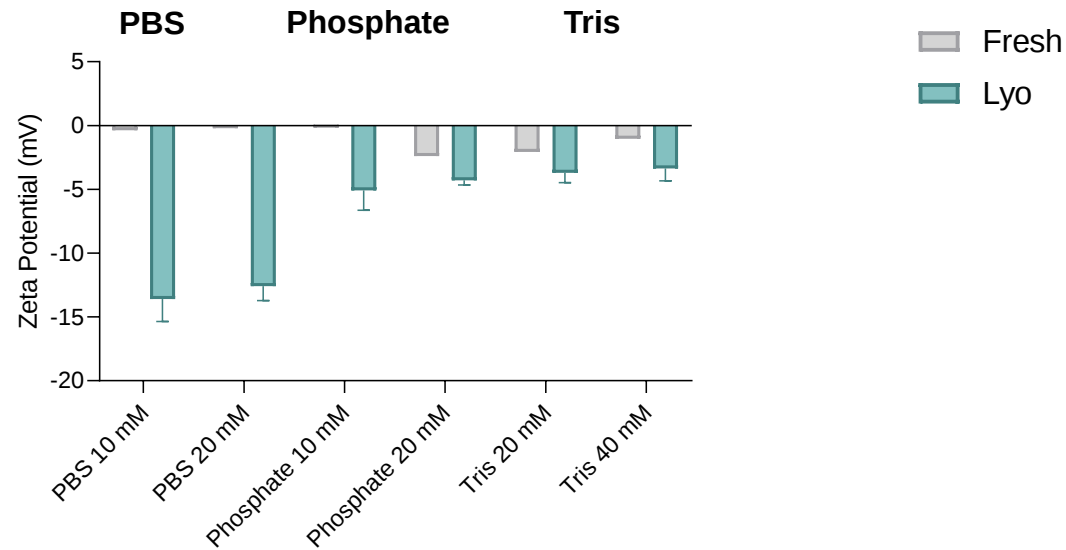
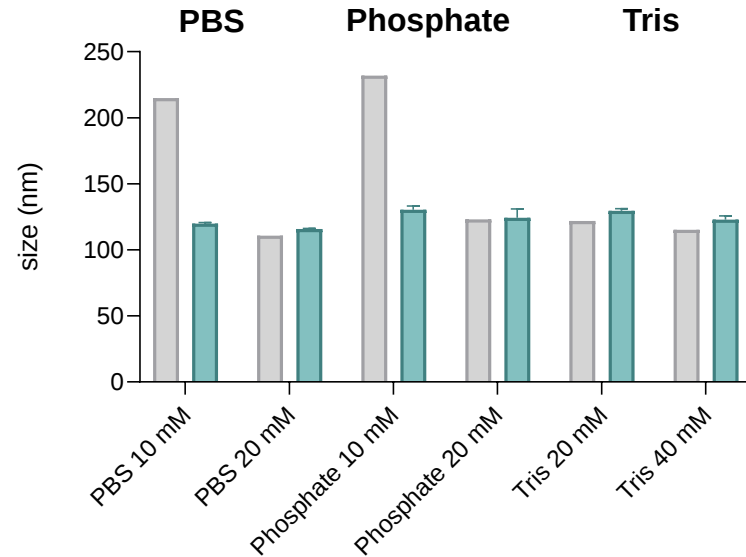
- ✓ Lyoprotectant concentration: 12,5 %
- ✓ LNP: NP ratio = 3
- ✓ eGFP mRNA LNPs

Impact of buffer (capacity) in mRNA LNPs with NP ratio of 3



- ✓ High freezing rate
- ✓ Lyoprotectant: 12,5 % sucrose
- ✓ LNP: NP ratio = 3
- ✓ eGFP mRNA LNPs

Increasing NP-ratio to 6 avoids loss of encapsulated mRNA during freeze-drying

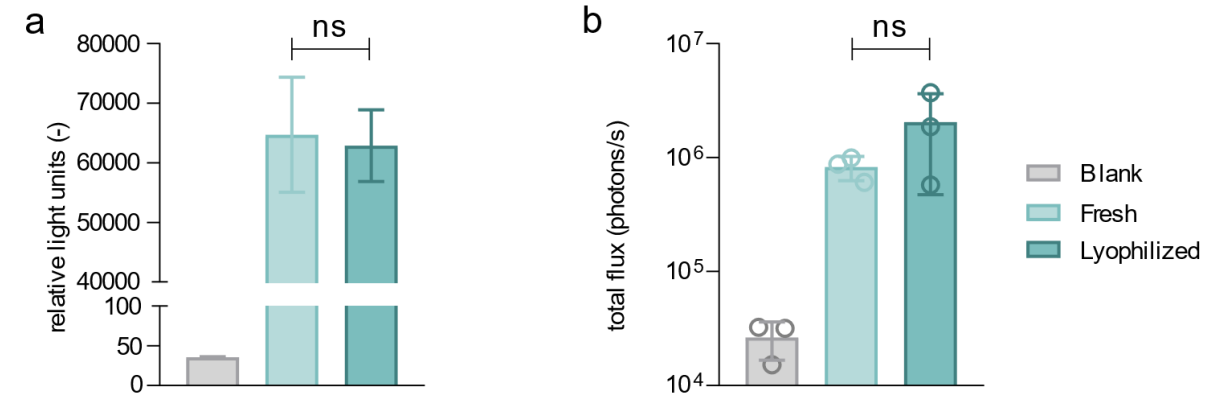


- ✓ High freezing rate
- ✓ Lyoprotectant: 12,5 % sucrose
- ✓ LNP: NP ratio = 6
- ✓ eGFP mRNA LNPs

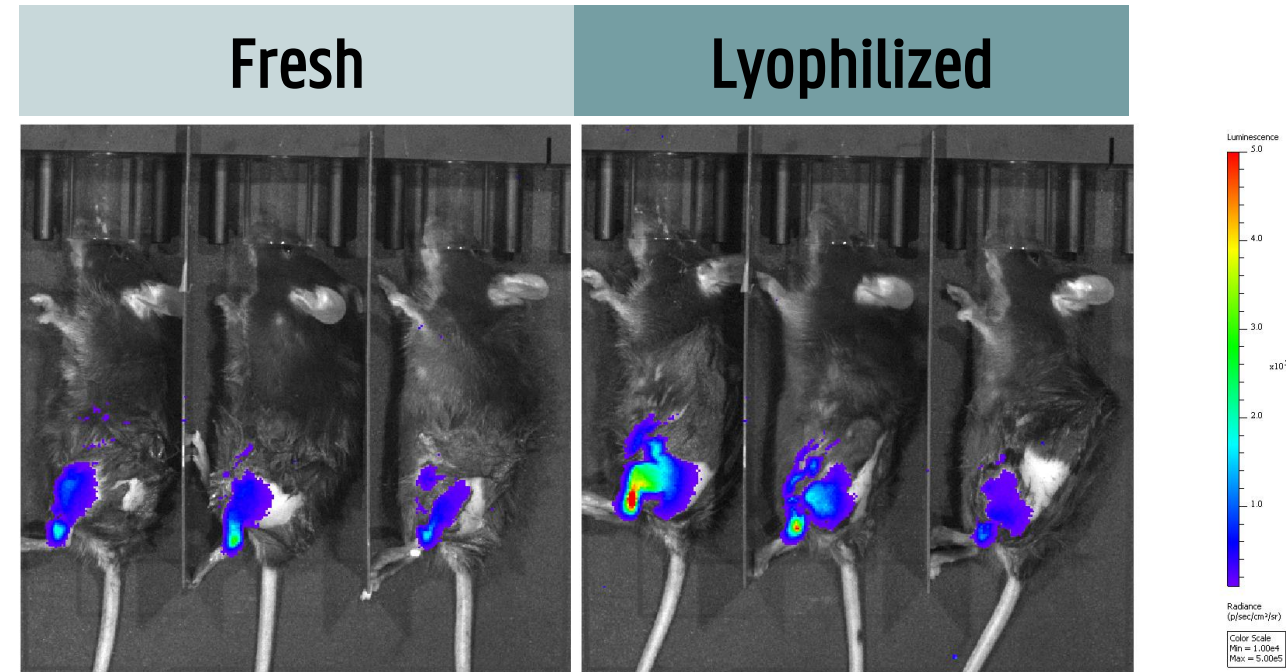
Lyophilized mRNA LNPs conserve transfection efficiency in vivo

In vitro HEK293T transfection

In vivo luciferase expression

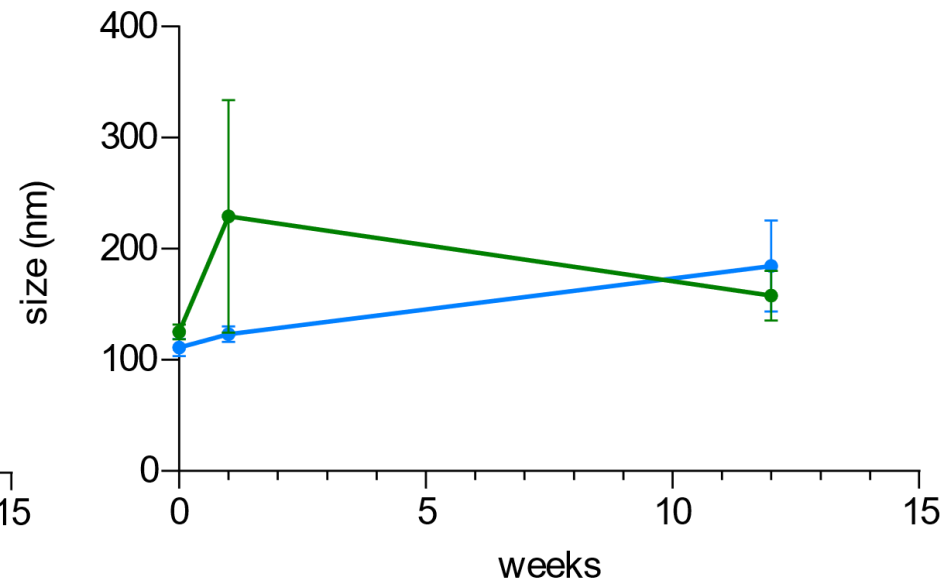
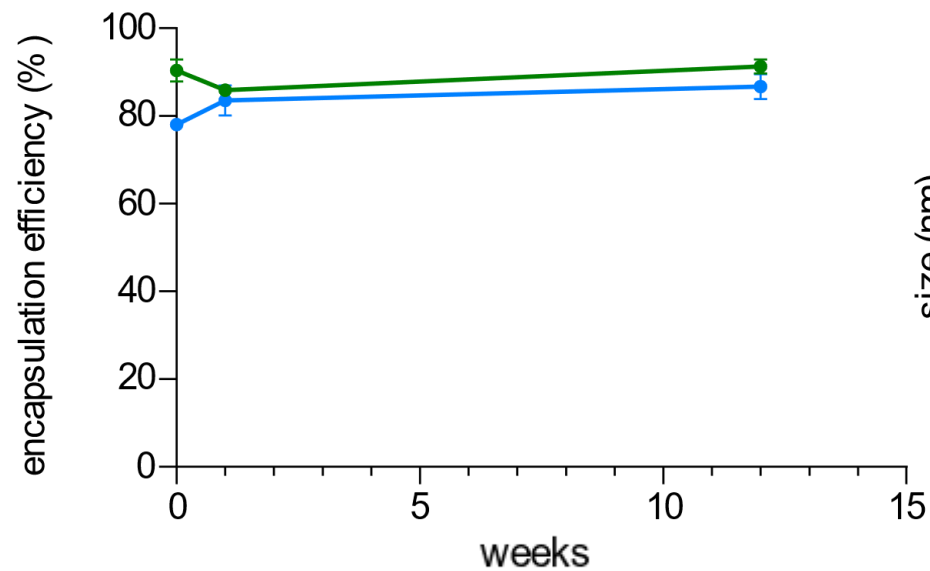
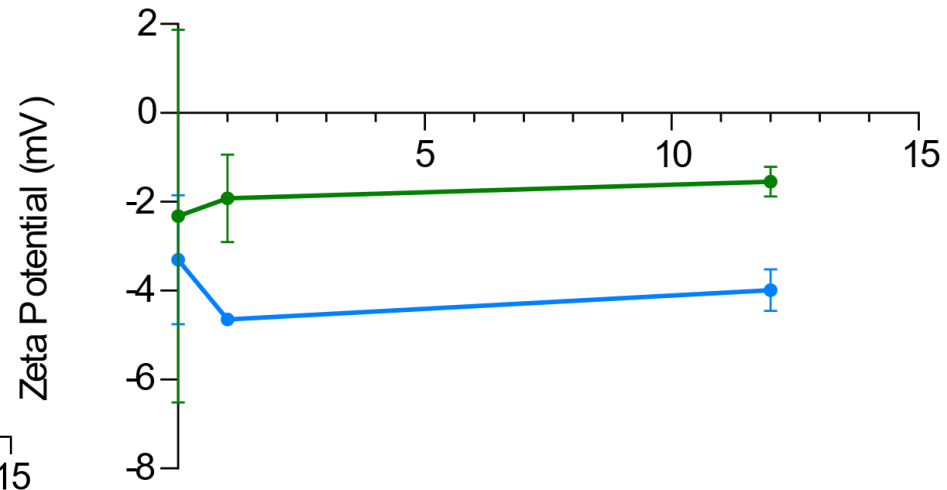
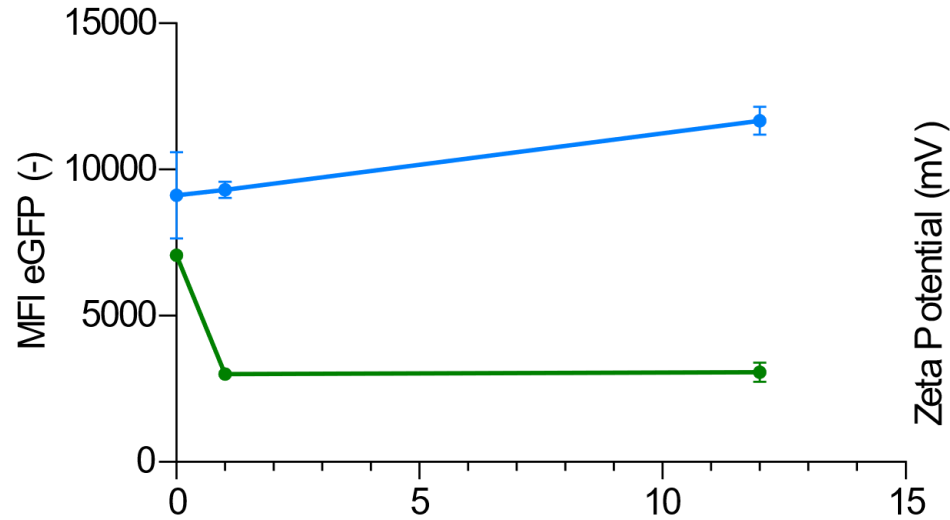


- ✓ Freezing: high freezing rate
- ✓ Tris 20 mM
- ✓ Lyoprotectant: 12,5 % sucrose
- ✓ LNP: NP ratio = 6



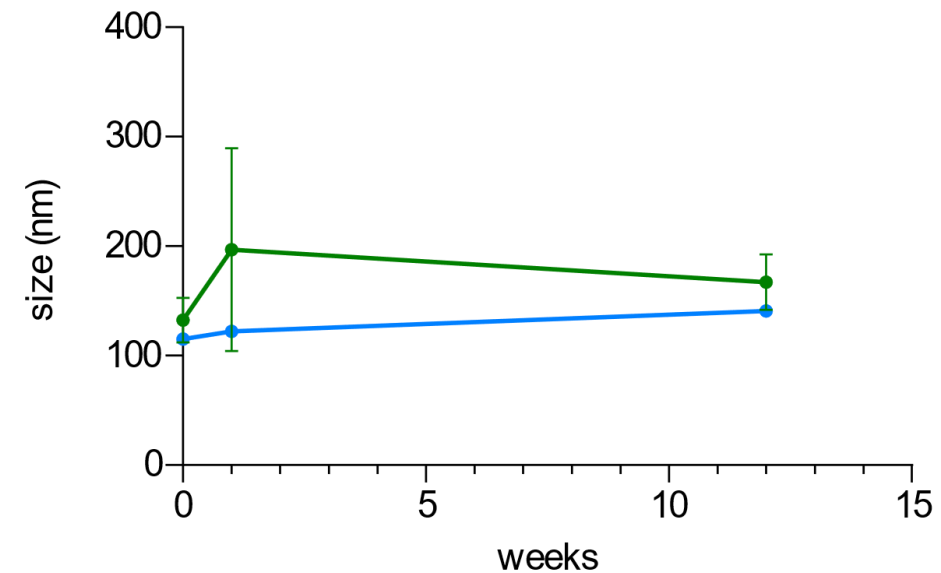
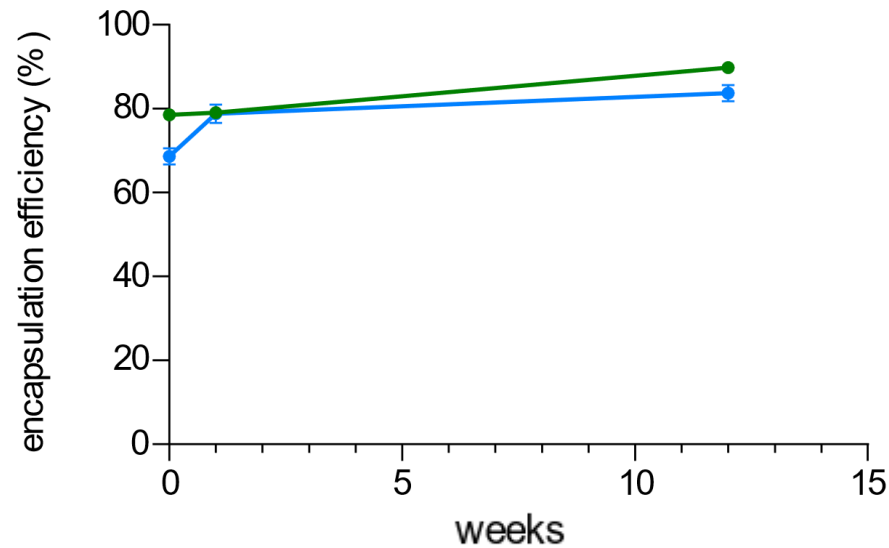
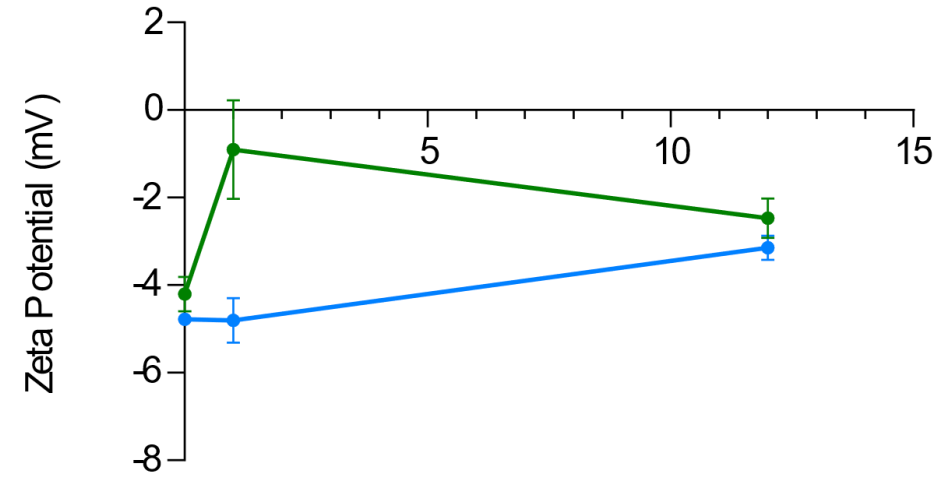
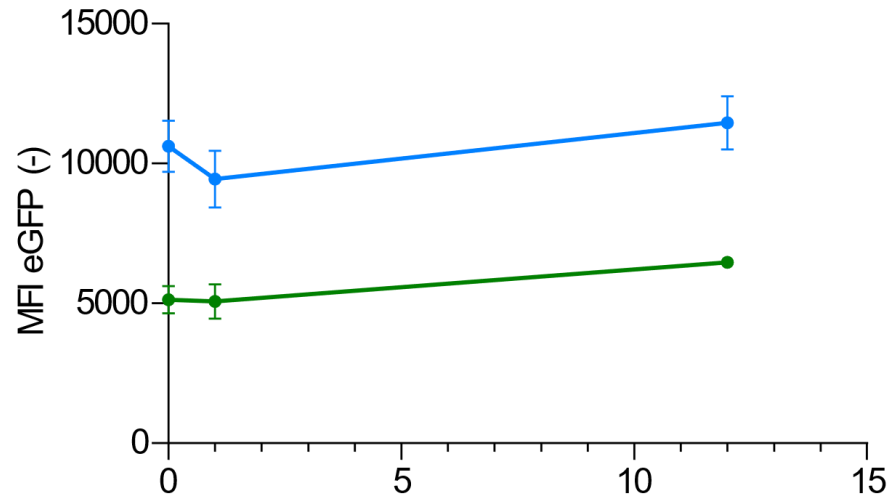
C57BL/6 mice i.m. injected with a dose corresponding with 3 μ g luciferase mRNA (n=3)

Stability of lyophilized mRNA LNPs at 4 °C



- ✓ Lyoprotectant: 12,5 % sucrose
- ✓ LNP: NP ratio = 6
- ✓ eGFP mRNA LNPs

Stability of frozen mRNA LNPs at -20 °C



- ✓ Lyoprotectant: 12,5 % sucrose
- ✓ LNP: NP ratio = 6
- ✓ eGFP mRNA LNPs

Conclusions

Continuous freeze-drying
based on spin freezing



mRNA expression *in vivo*
conserved after lyophilization



Buffer type and NP-ratio are
crucial parameters



Lyophilized LNPs tend to increase
in size, other characteristics
remain conserved

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Kom op
tegen Kanker

