Engineer Lipid Nanoparticle Hydrophobicity to Modulate Nano-Bio Interface and Enable Tissue-Selective mRNA Delivery

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CRS 2025 ANNUAL MEETING & EXPOSITION
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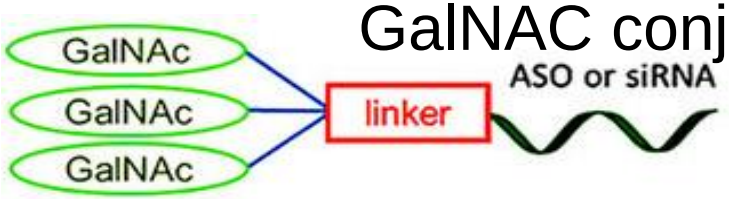
JULY 13-18, 2025

NEXT-GENERATION DELIVERY INNOVATIONS

A need to increase the extra-hepatic delivery of RNA therapeutics

Non-viral gene therapies and vaccines

Lipid nanoparticle



GalNAC conjugation

COMIRNATY® (COVID-19 Vaccine, mRNA)

spikevax™ COVID-19 Vaccine, mRNA

MRESVIA™ (Respiratory Syncytial Virus Vaccine, mRNA)

Hepatic delivery

onpattro® siRNA

GIVLAARI® (givosiran) injection for subcutaneous use 189 mg/mL

amvuttra® (vutrisiran) injection 25 mg/0.5 mL

OXLUMO® (lumasiran) injection 94.5 mg/0.5 mL

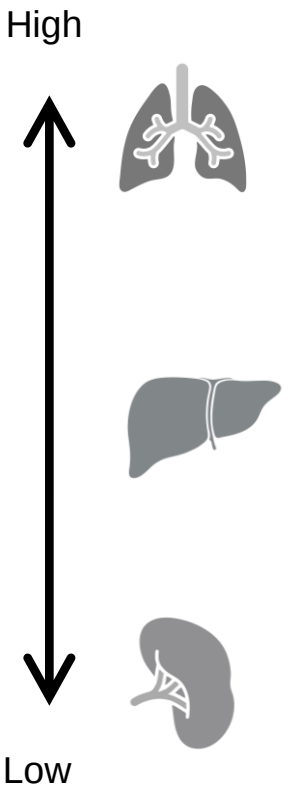
rivfloza®

NTLA-2001 (Phase III) mRNA/gRNA

LEQVIO® (inclisiran) injection 284 mg/1.5 mL

Qfitlia™

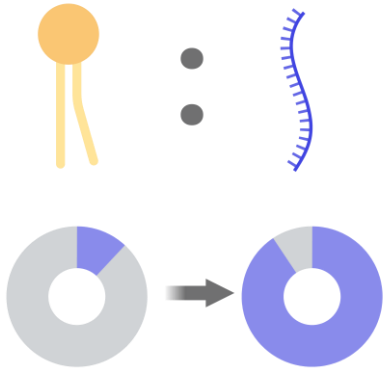
Status quo: Apparent pKa-mediated extra-hepatic delivery



Apparent pKa

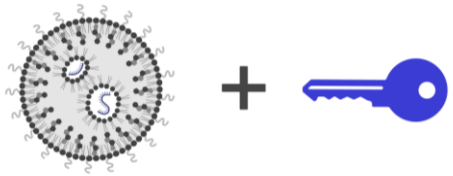
The apparent pKa provides information about the overall charge state of a nanoparticle

Tune N:P ratio



LM Kranz, et. Al Nature volume 534 (2016)

Add SORT LNPs



Nat Nanotechnol. 2020; 15(4): 313–320.

The structure of lipid tails affect LNP's extrahepatic delivery

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Enhancing spleen-targeted mRNA delivery with branched biodegradable tails in lipid nanoparticles†

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Article

Multiarm-Assisted Design of Dendron-like Degradable Ionizable Lipids Facilitates Systemic mRNA Delivery to the Spleen

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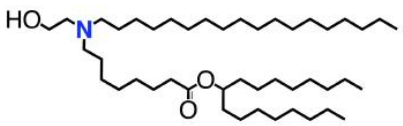
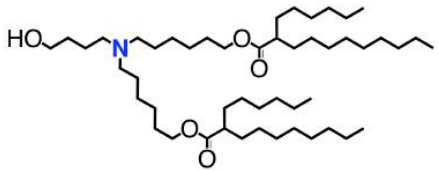
NEXT-GENERATION
DELIVERY INNOVATIONS

Unpublished data

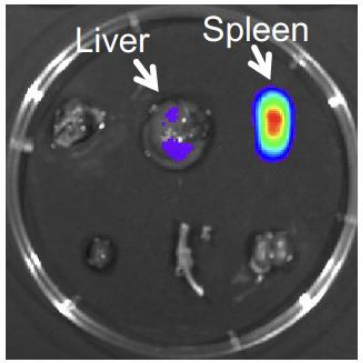
The structure of lipid tails affect LNP's extrahepatic delivery

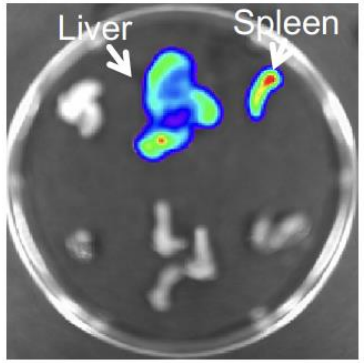


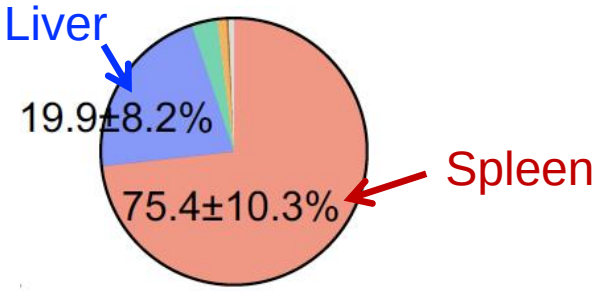
SM-102
Apparent pKa = 7.17±0.05

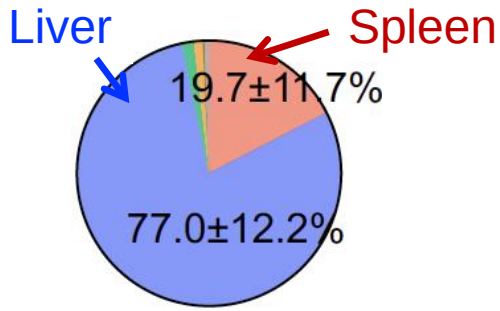


ALC-0315
Apparent pKa = 6.44±0.02



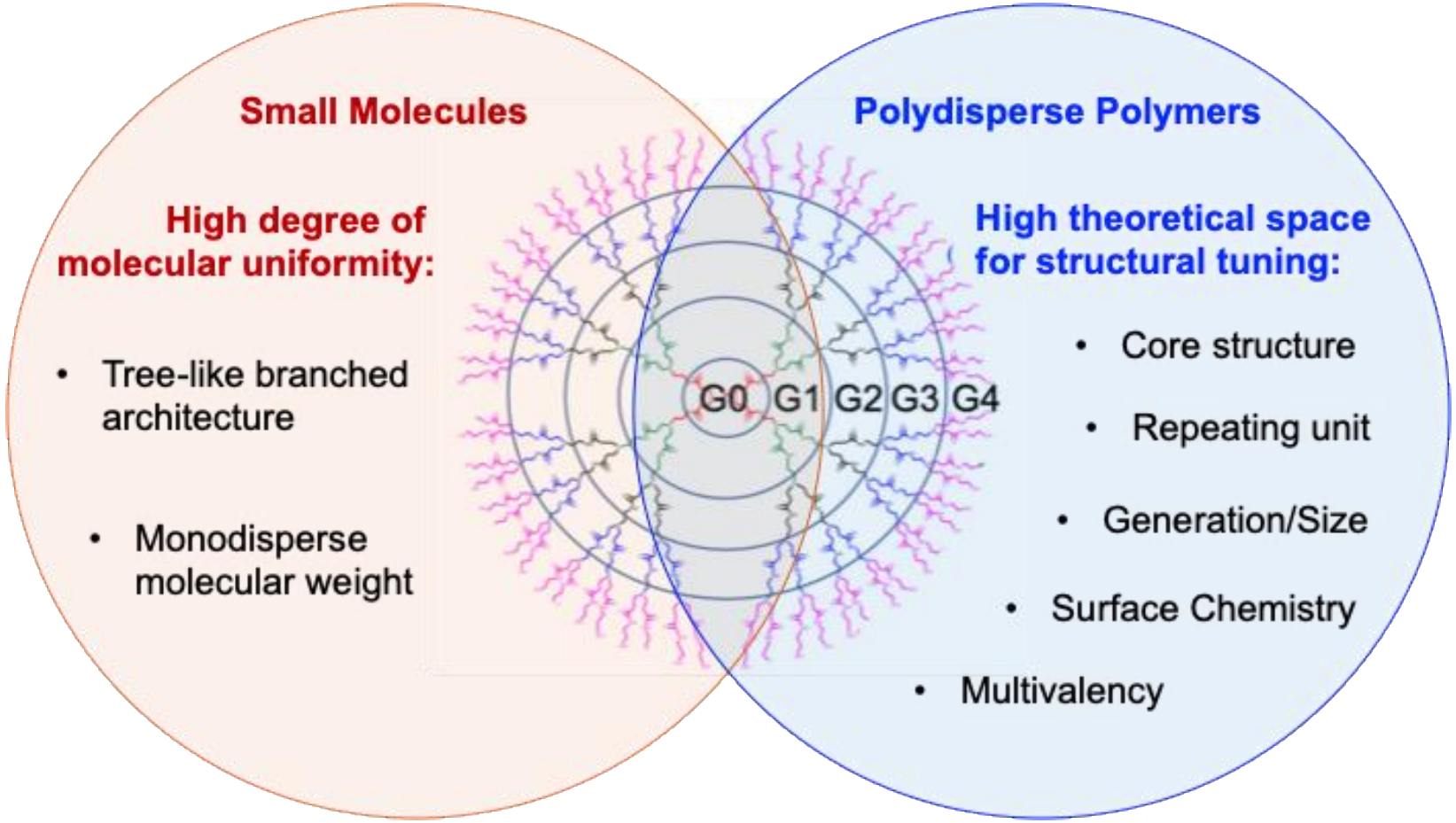






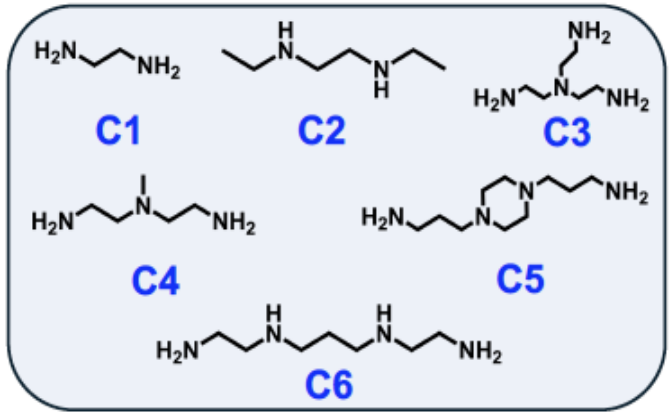
- Alternative mechanism might affect LNP's tissue tropism

Construct a library of structurally diverse dendrimer-based ionizable lipid (dIL)



Construct a library of structurally diverse dendrimer-based ionizable lipid (dIL)

Core = ○



Different number and types of amines

↓

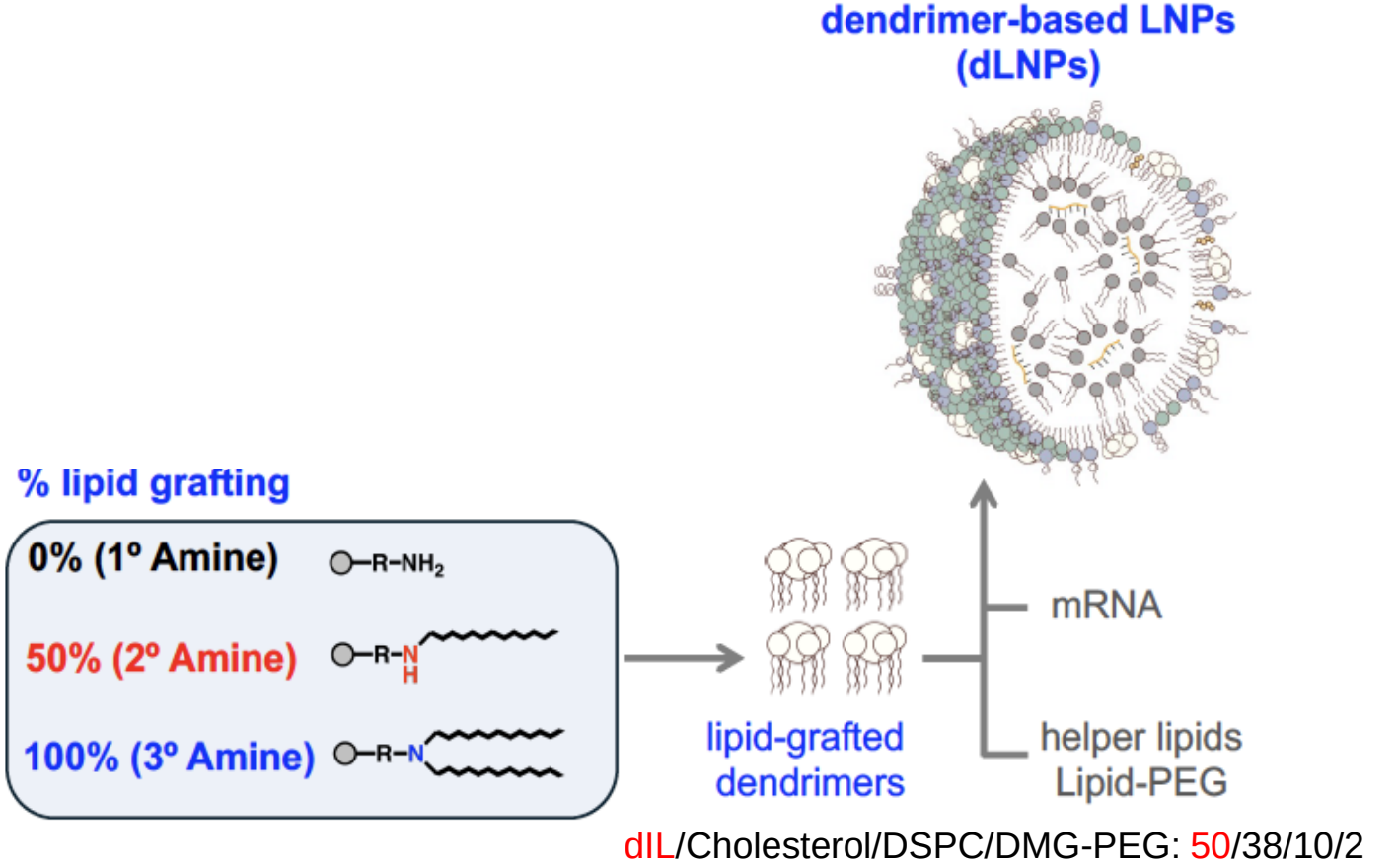
Different charge/ ionizable status

Different number and types of lipid tails

↓

Different hydrophobicity/ lipophilicity

Design of dendrimer-based LNP (dLNP) library with diverse structural properties

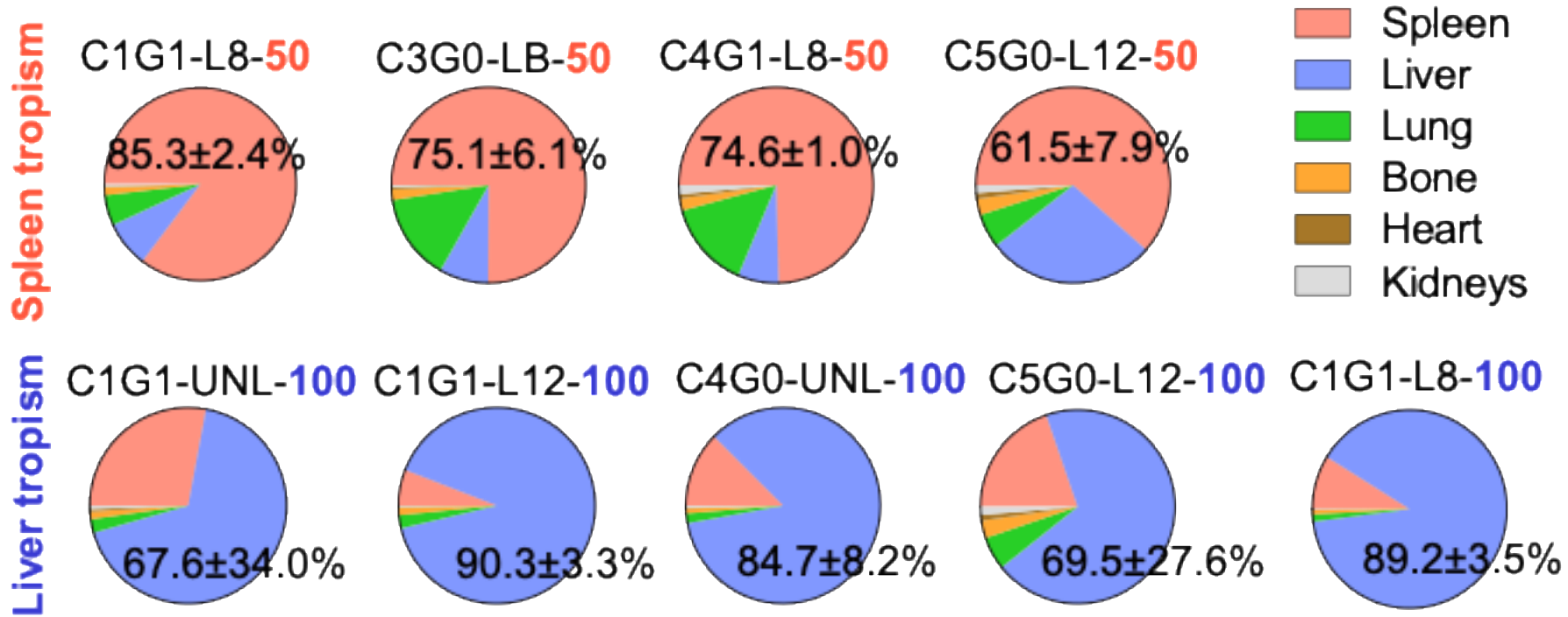


dIL/Cholesterol/DSPC/DMG-PEG: 50/38/10/2

dIL	dLNPs
Charge/ ionizable status	Apparent pKa
Different hydrophobicity/ lipophilicity	Hydrophobicity/ Lipophilicity

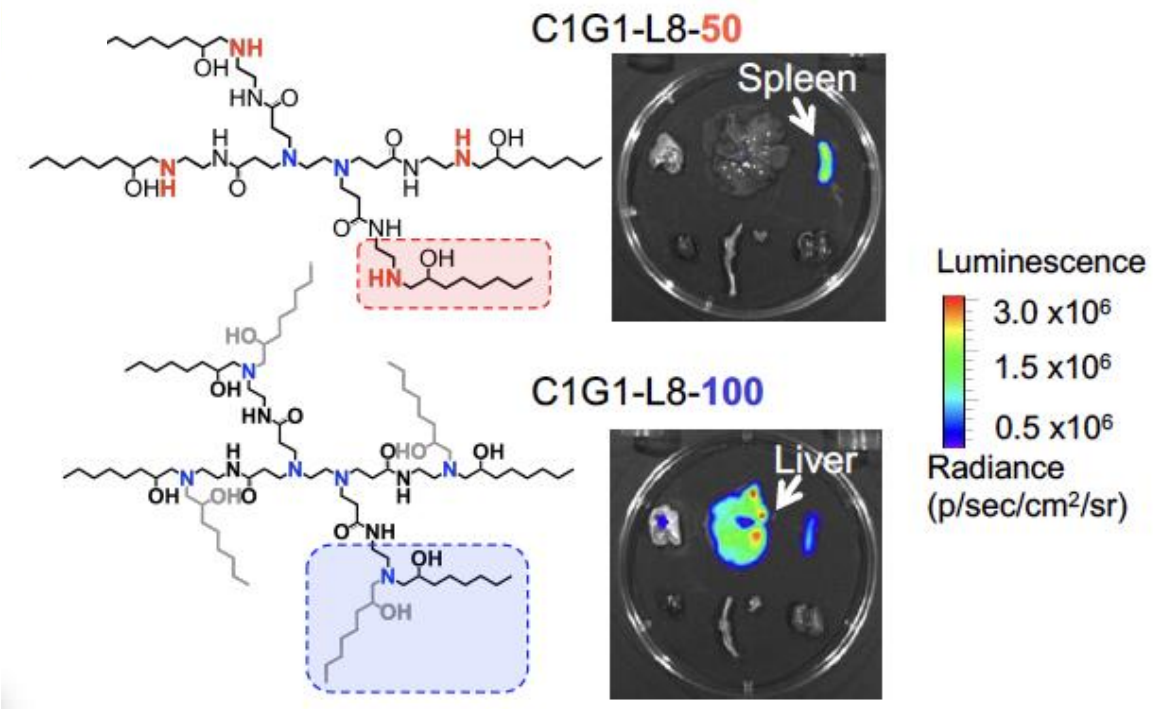
- Size
- Zeta-potential
- Polydispersity index
- Encapsulation efficiency

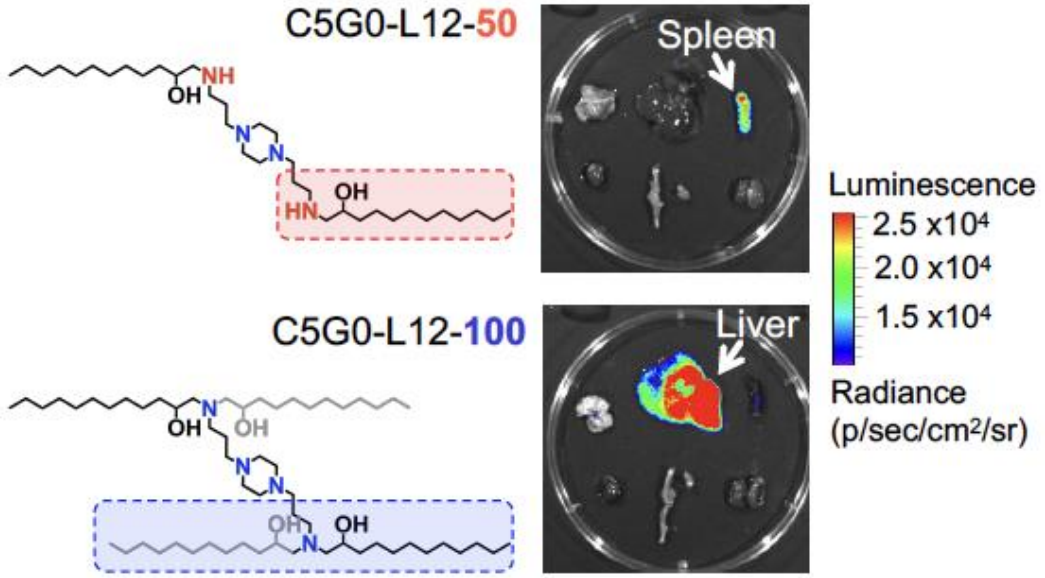
dLNP tissue-selectivity is associated with the percentage of lipid grafting



Unpublished data

Sister formulations with different lipid grafting levels showed distinct tissue tropism





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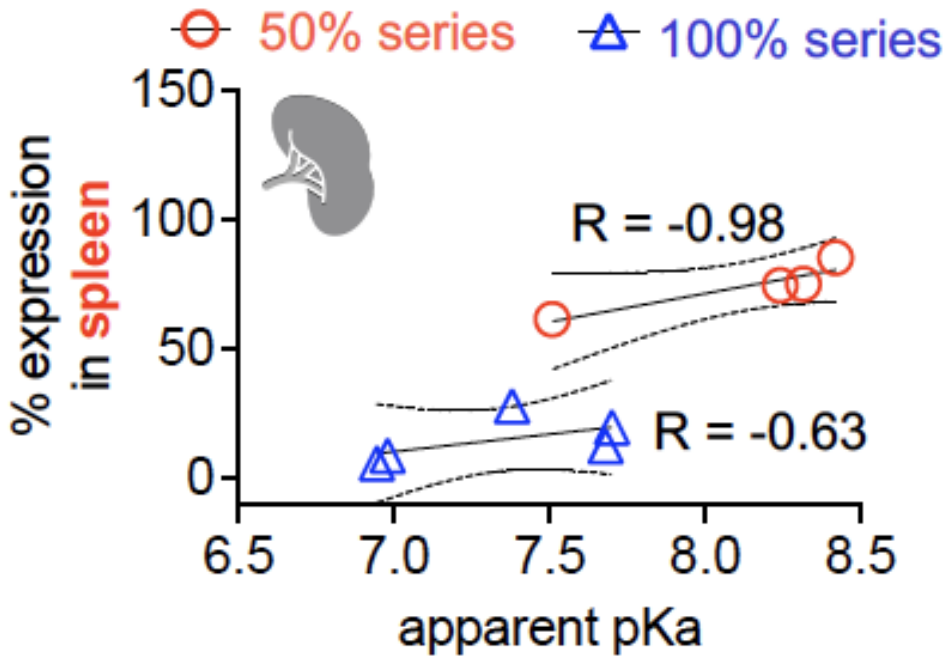
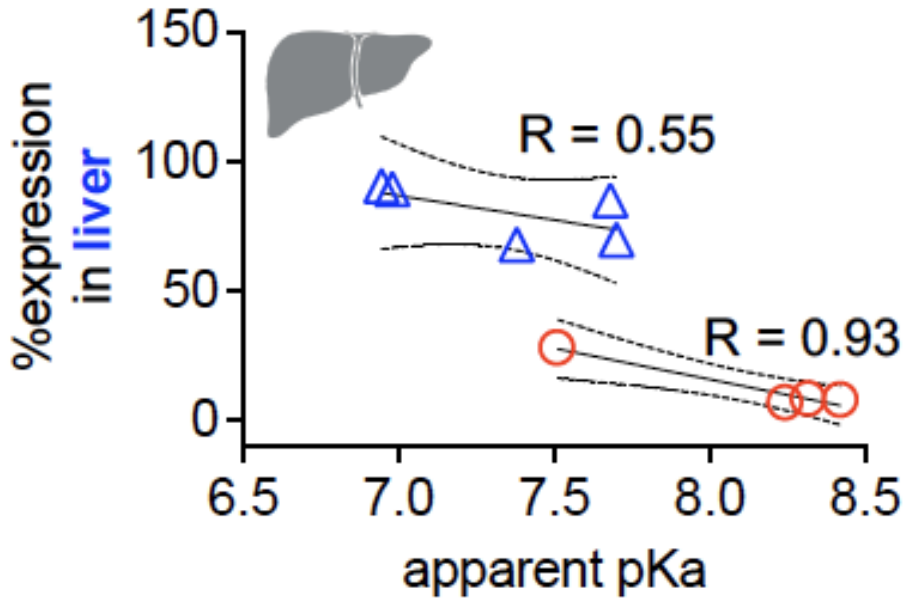
The percentage of lipid grating and the apparent pKa synergistically regulate the dLNP tissue tropism





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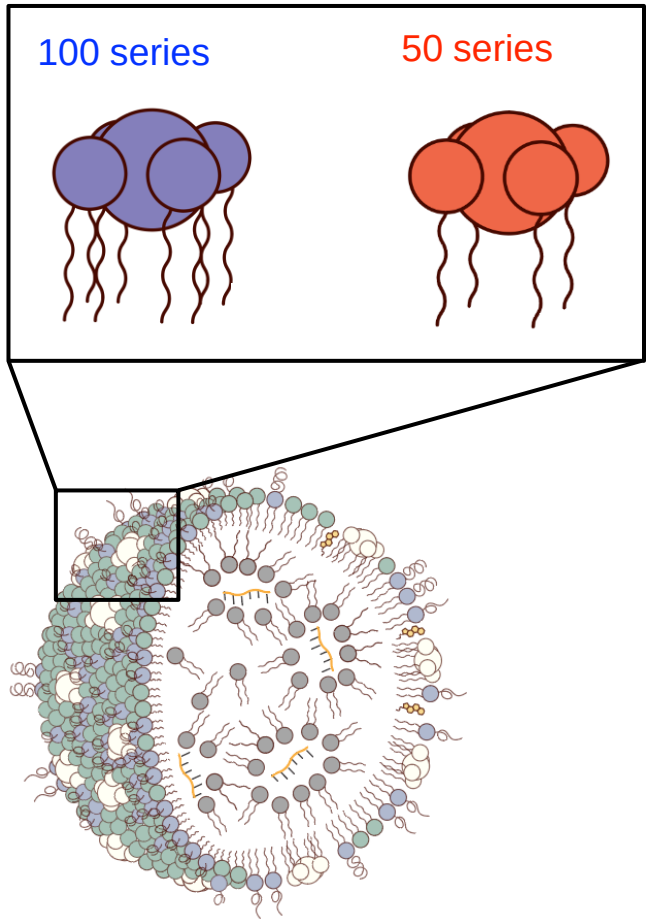
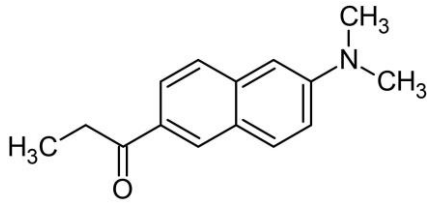
The percentage of lipid grating affects the hydrophobicity/lipophilicity of the dLNPs

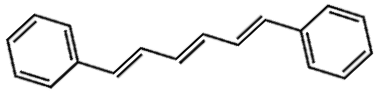


Lipid-grafted dendrimer accounts for 50% of the lipid component (molar ratio) in dLNP

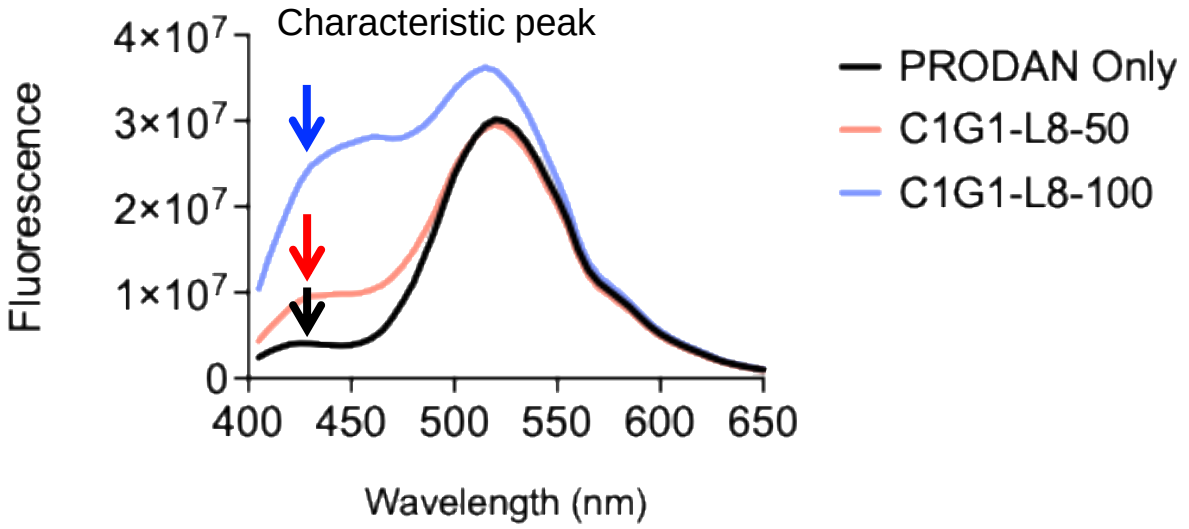
Probe LNP lipophilicity using hydrophobic fluorescent dyes



PRODAN assay



DPH

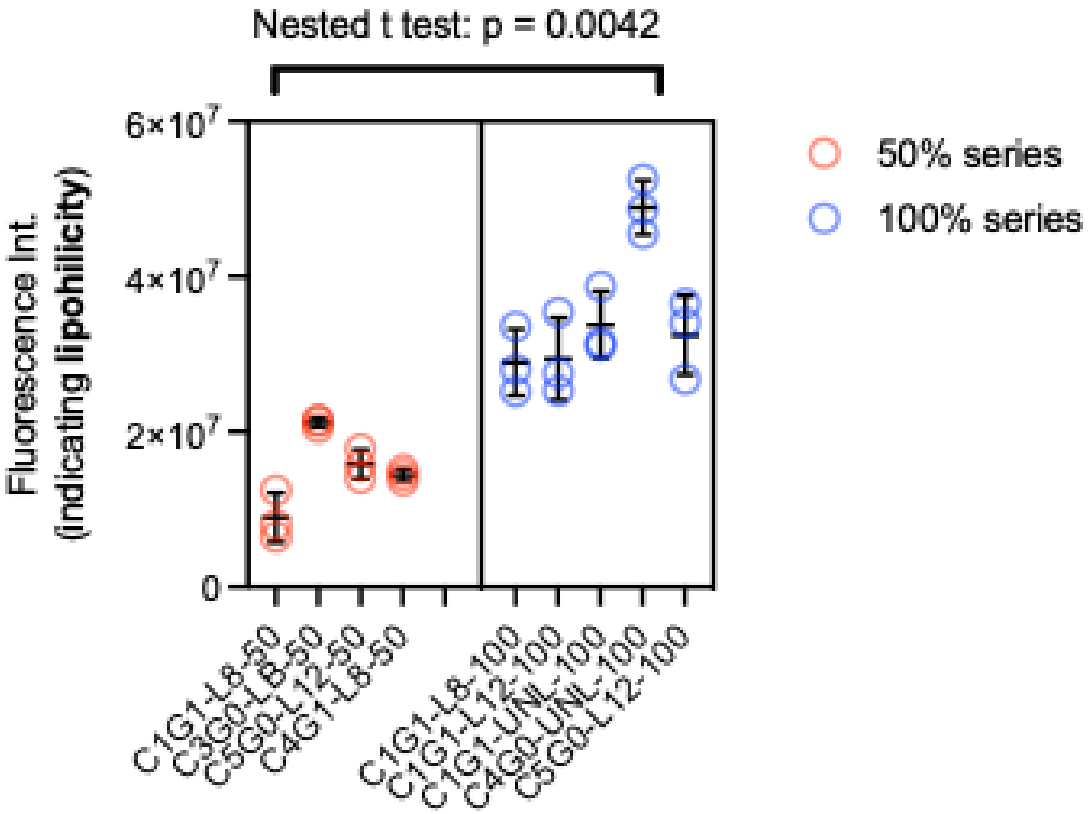


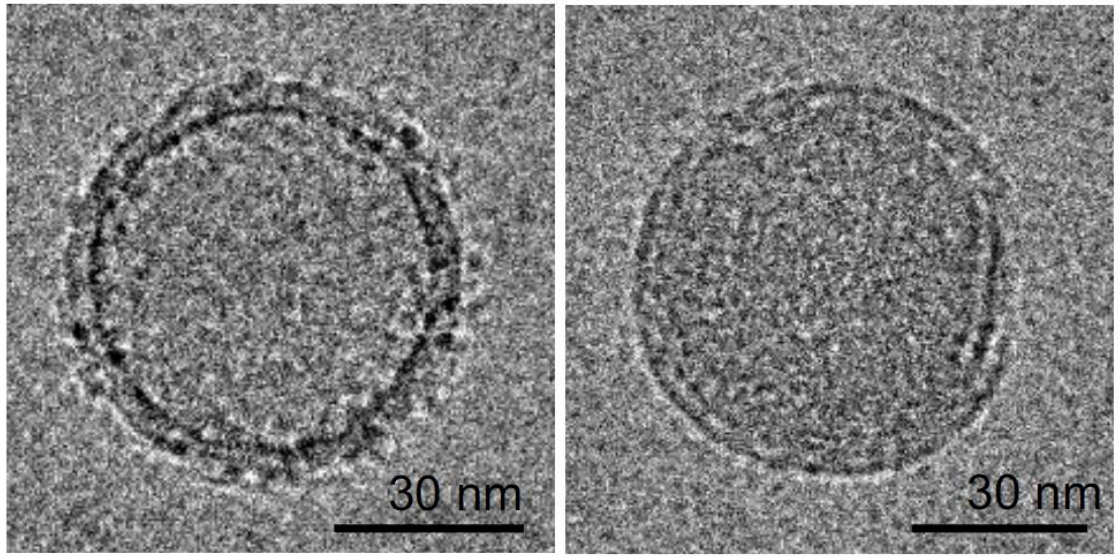
Unpublished data

- The surface hydrophobicity of a nanoparticle has significant effects on its stability, biological absorption, transport, and distribution

The percentage of lipid grating affects the hydrophobicity/lipophilicity of the dLNPs

dLNP lipophilicity
based on **PRODAN assay**

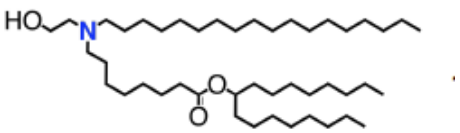




C1G1-L8-50 C1G1-L8-100

Unpublished data

The percentage of lipid grating affects the hydrophobicity/lipophilicity of the dLNPs



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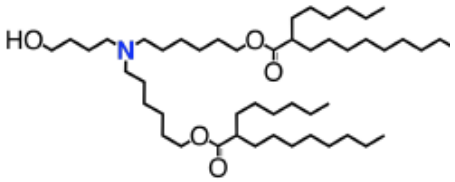

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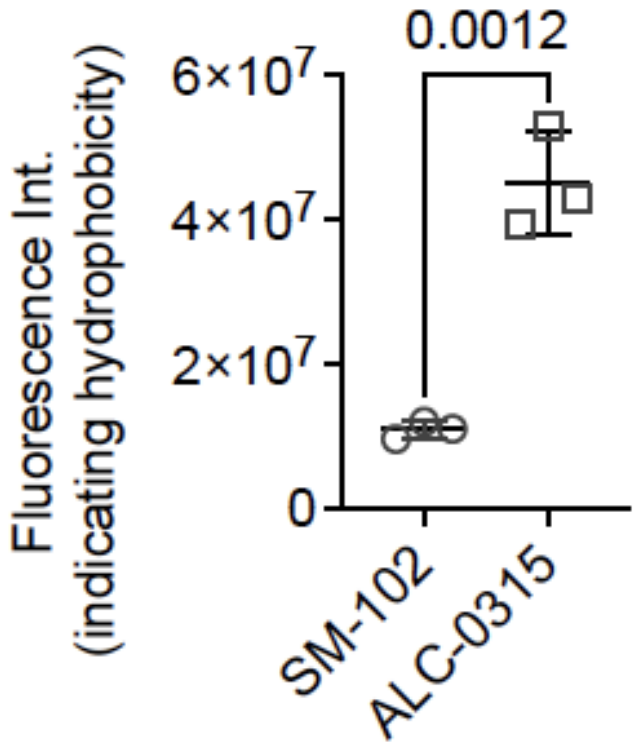
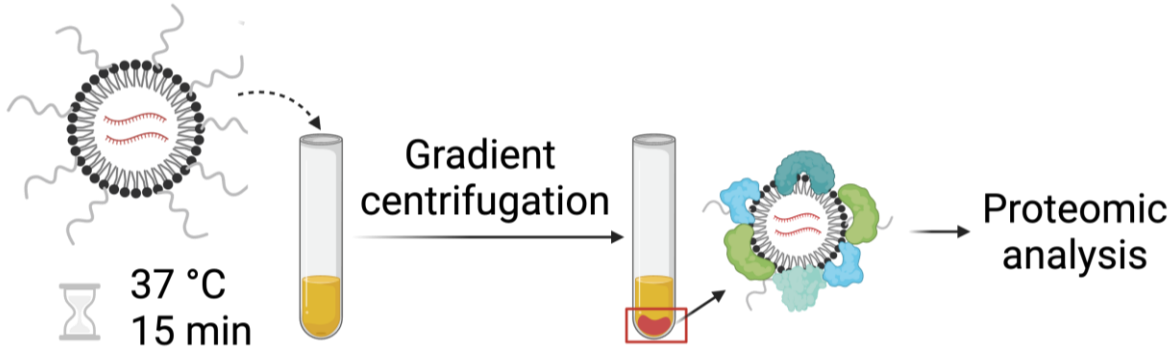


Unpublished data

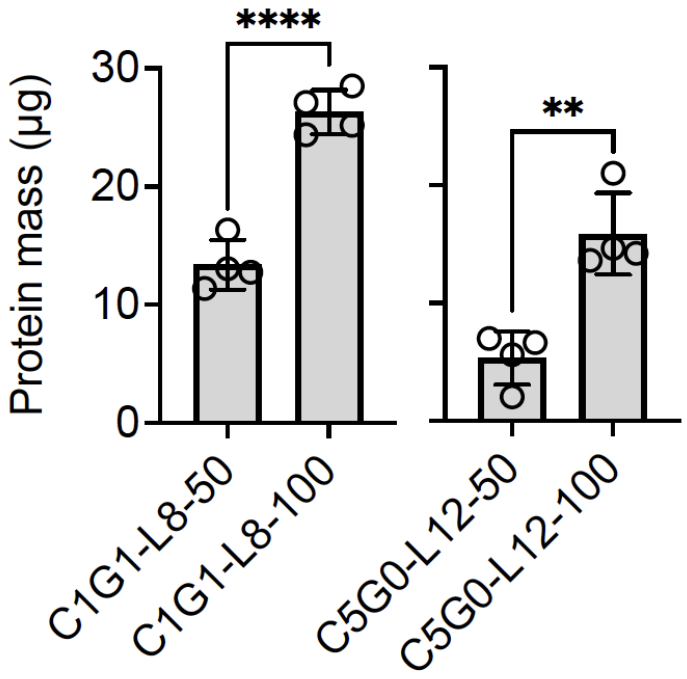
No difference in the **relative abundance** of Apos for both 50% and 100% series dLNPs

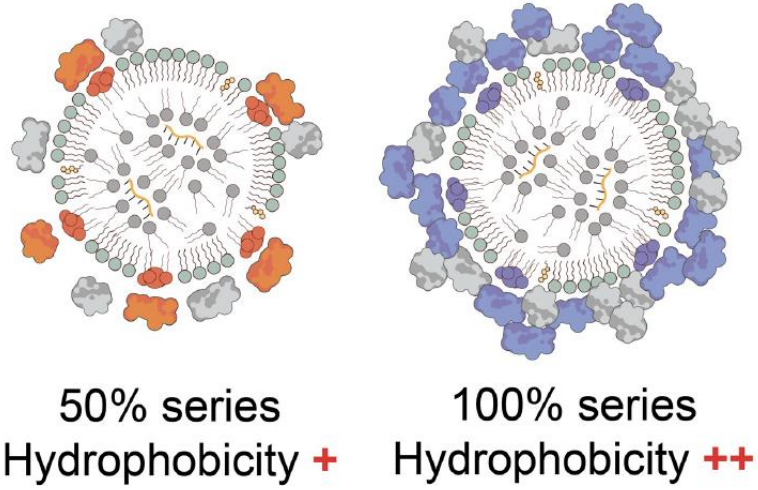


Protein	Abundance (%) C1G1-L8-100	Abundance (%) C1G1-L8-50	Functional Class
Apolipoprotein A-I	**21.5**	**21.4**	**Apolipoprotein**
Vitronectin	16.5	20.9	Tissue Leakage
Albumin	14.3	10.5	Other

The hydrophobicity of dLNPs affects the quantity of plasma protein adsorption

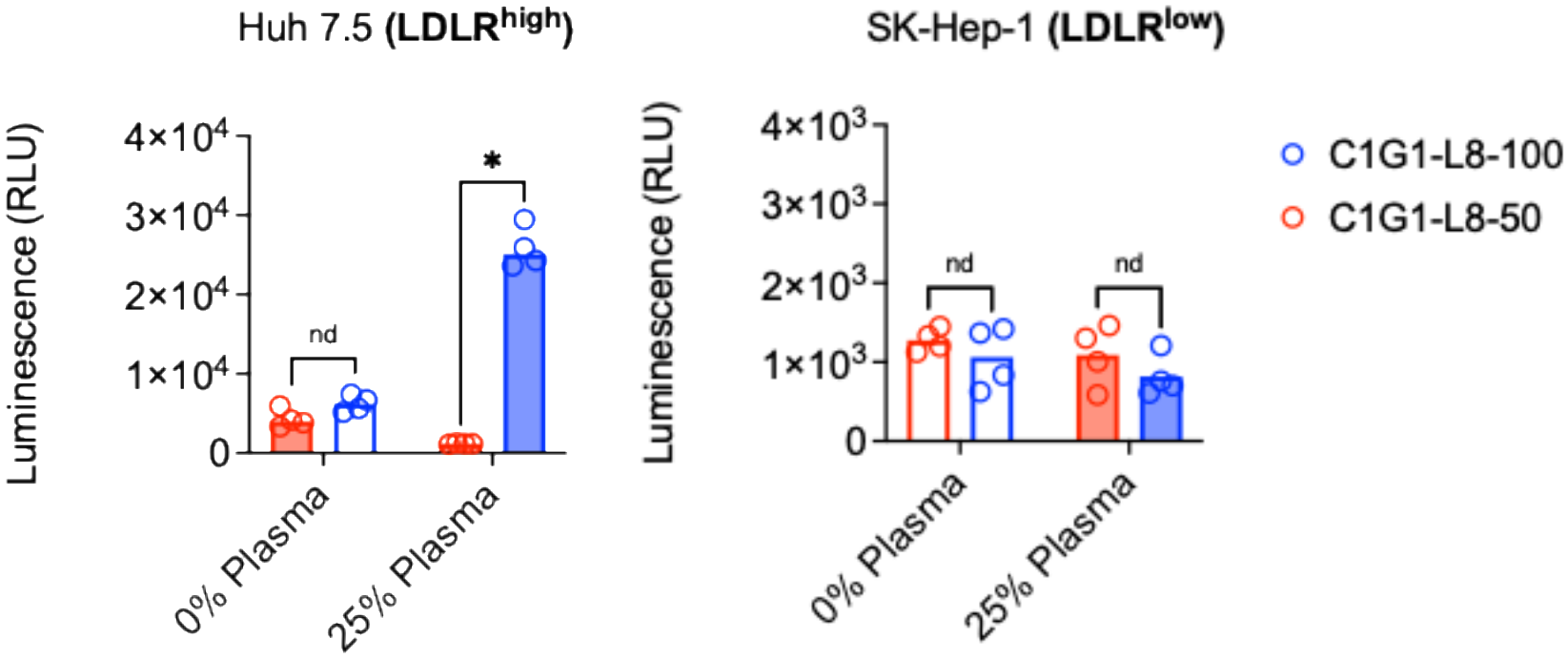
The **absolute abundance** of Apos is higher in 100% series than 50% series dLNPs

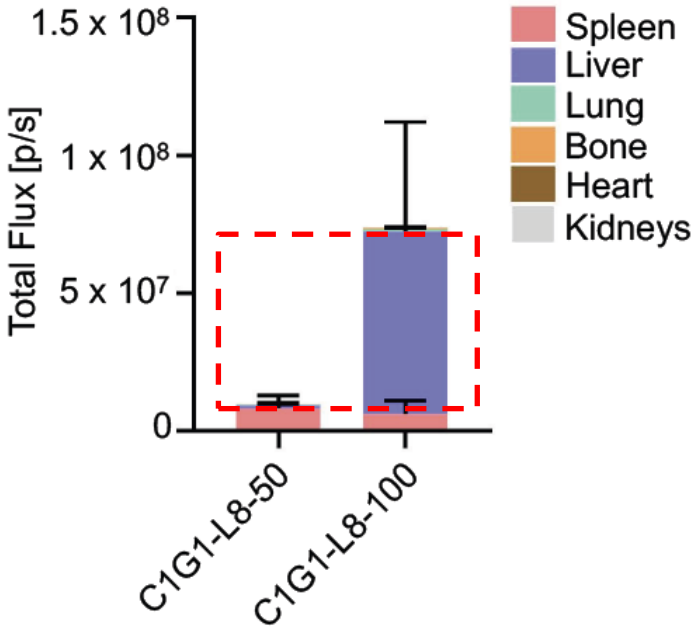




Unpublished data

The liver hepatocyte uptake of dLNP is dependent on the **Apos adsorption** and **LDLR density**





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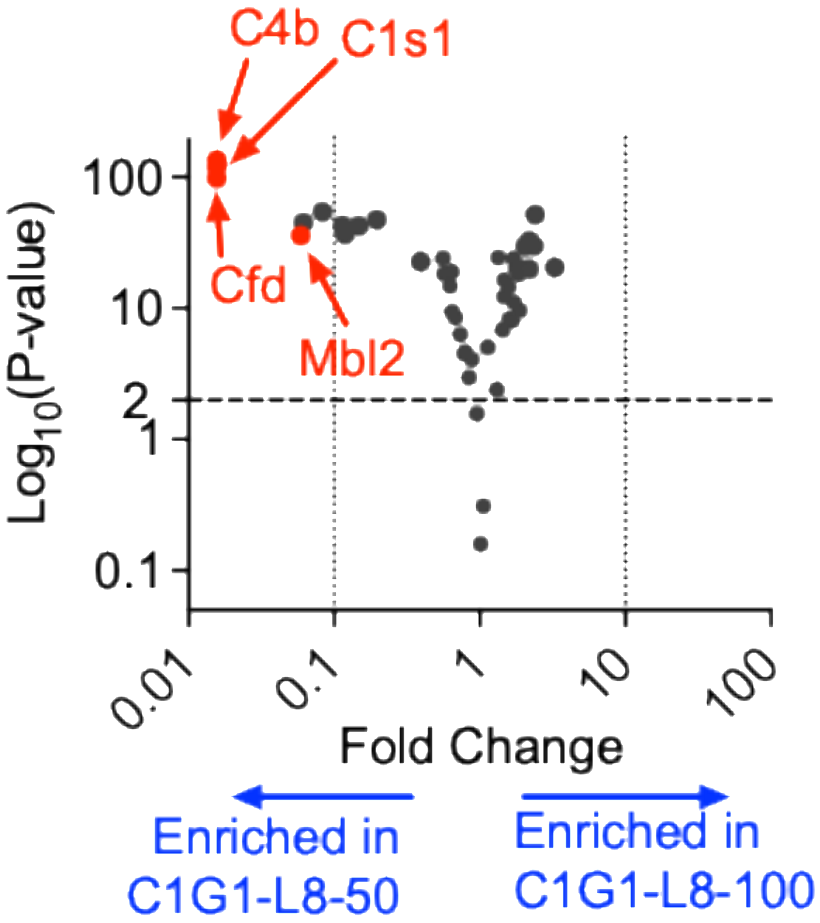
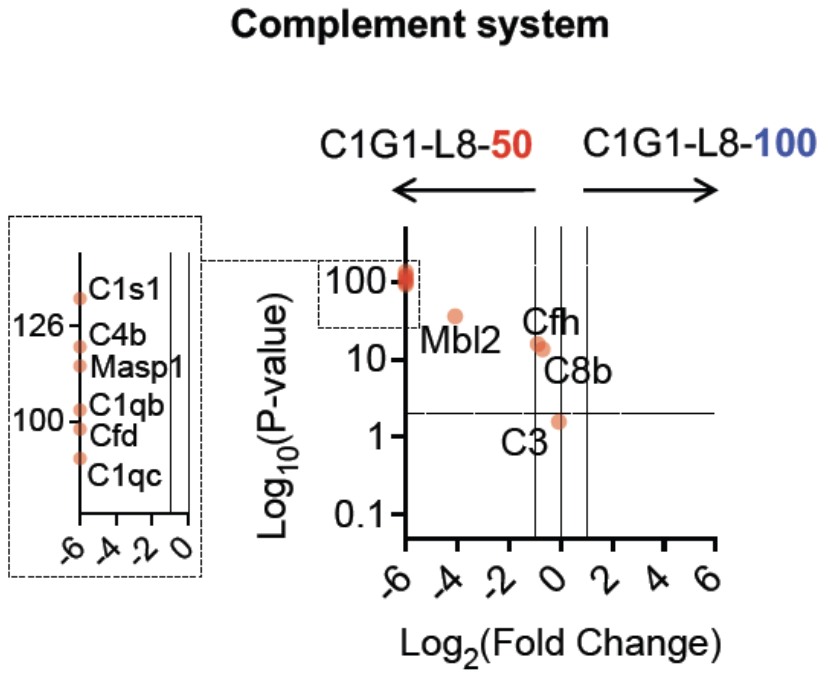
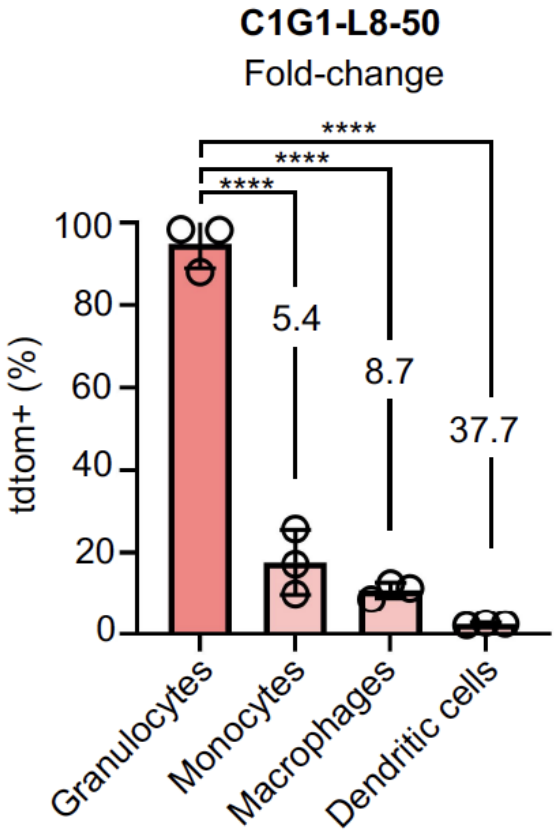
The 50% series dLNPs are associated with higher levels of complement proteins and immunoglobulins

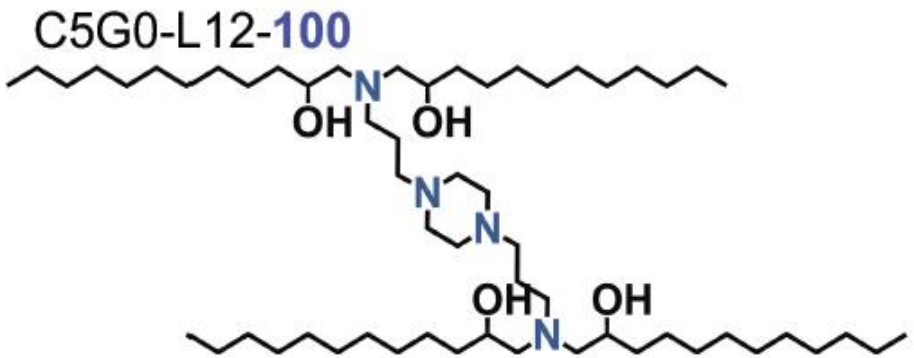


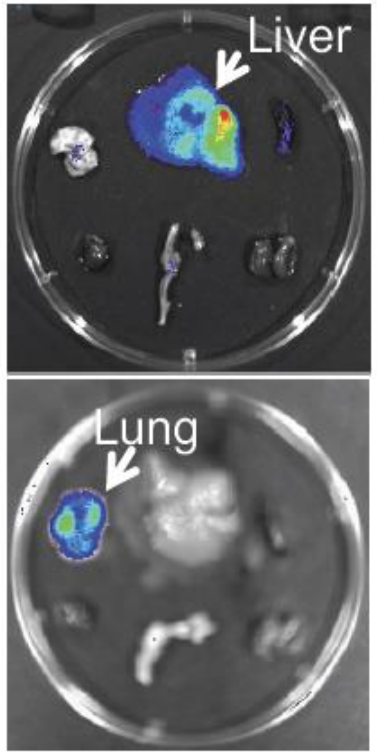


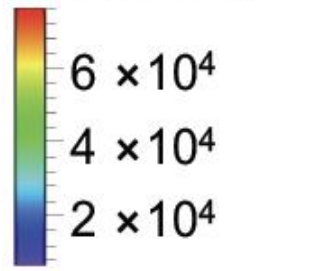


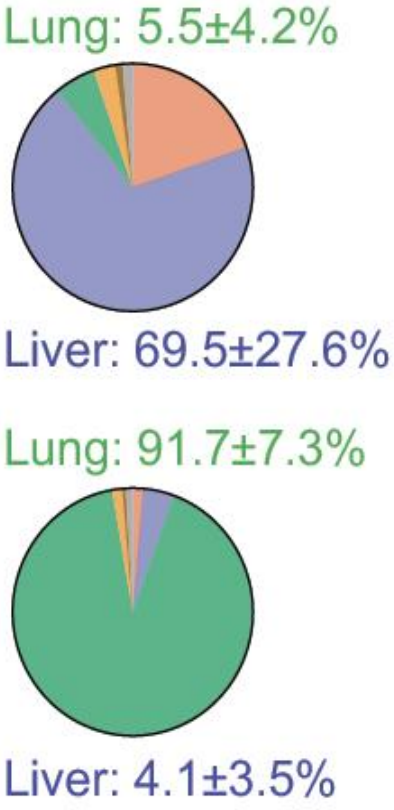
Unpublished data

Construct Quaternary amine (QA) dendrimers for lung-specific mRNA delivery



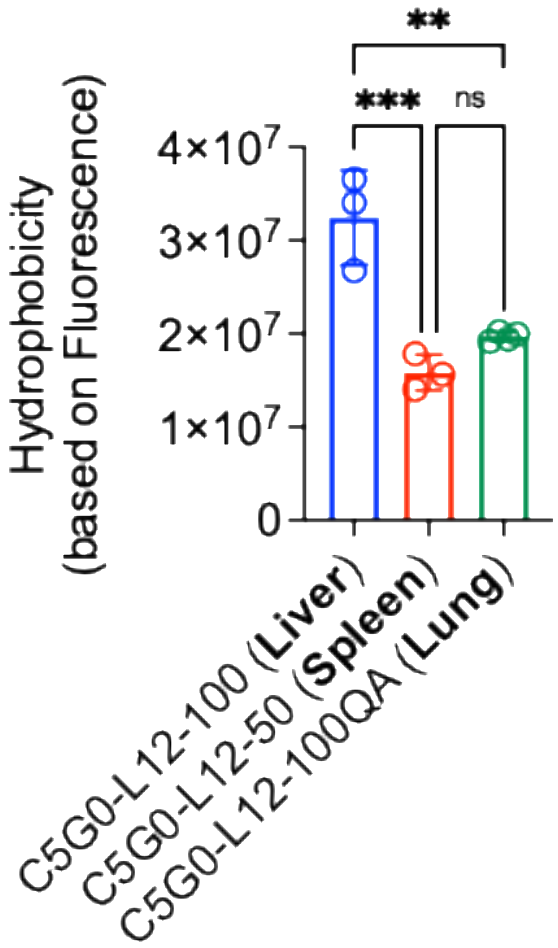


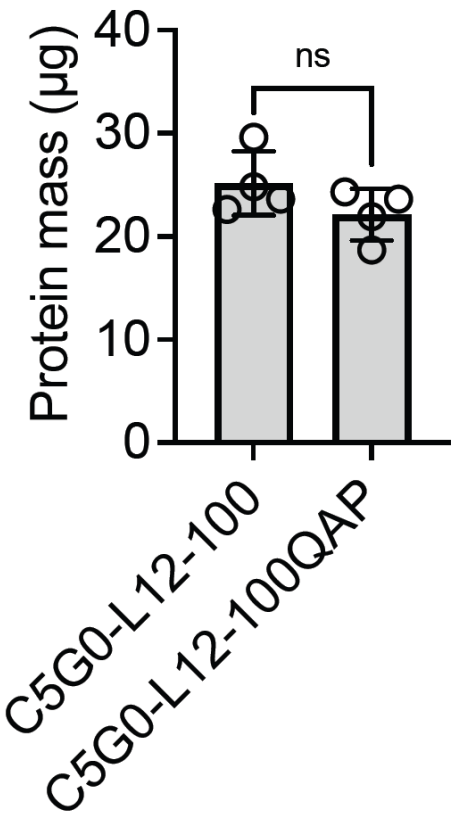


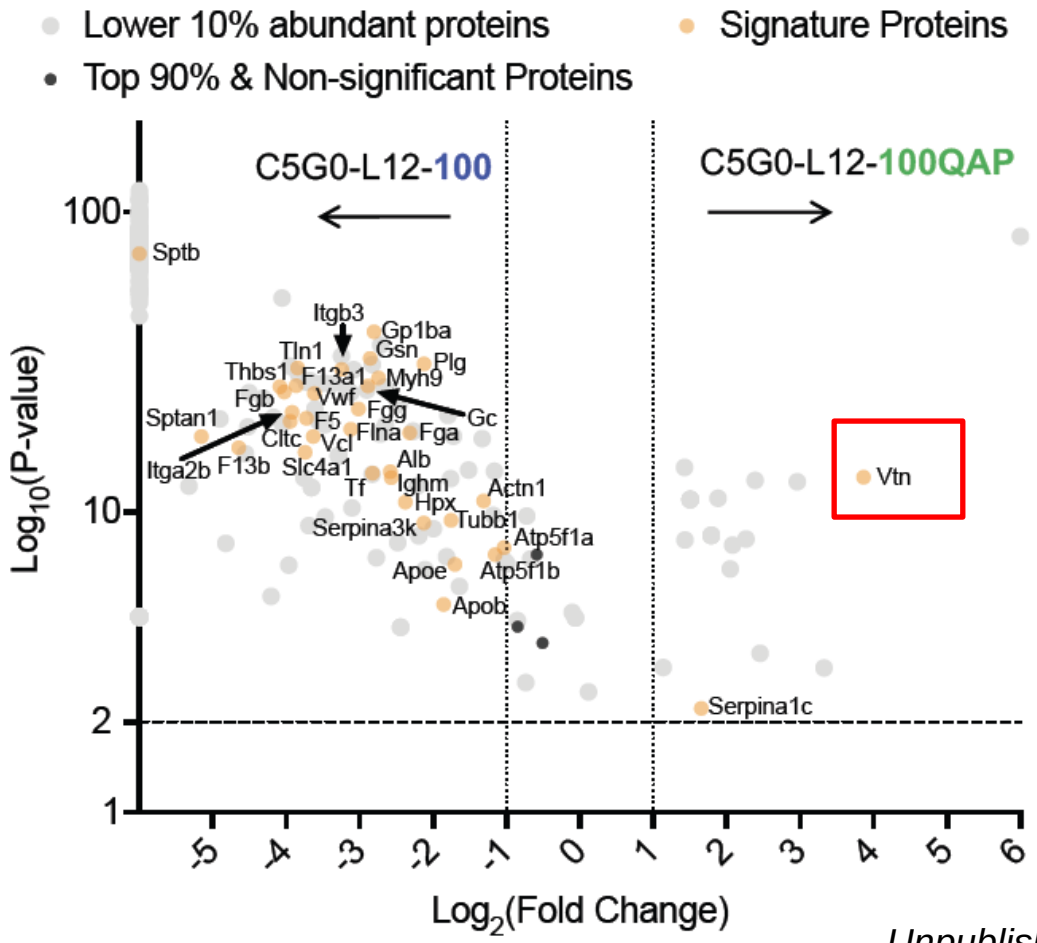


Unpublished data

QA series dLNP QA dendrimers enabling lung-specific mRNA delivery through a mechanism independent of total protein abundance.



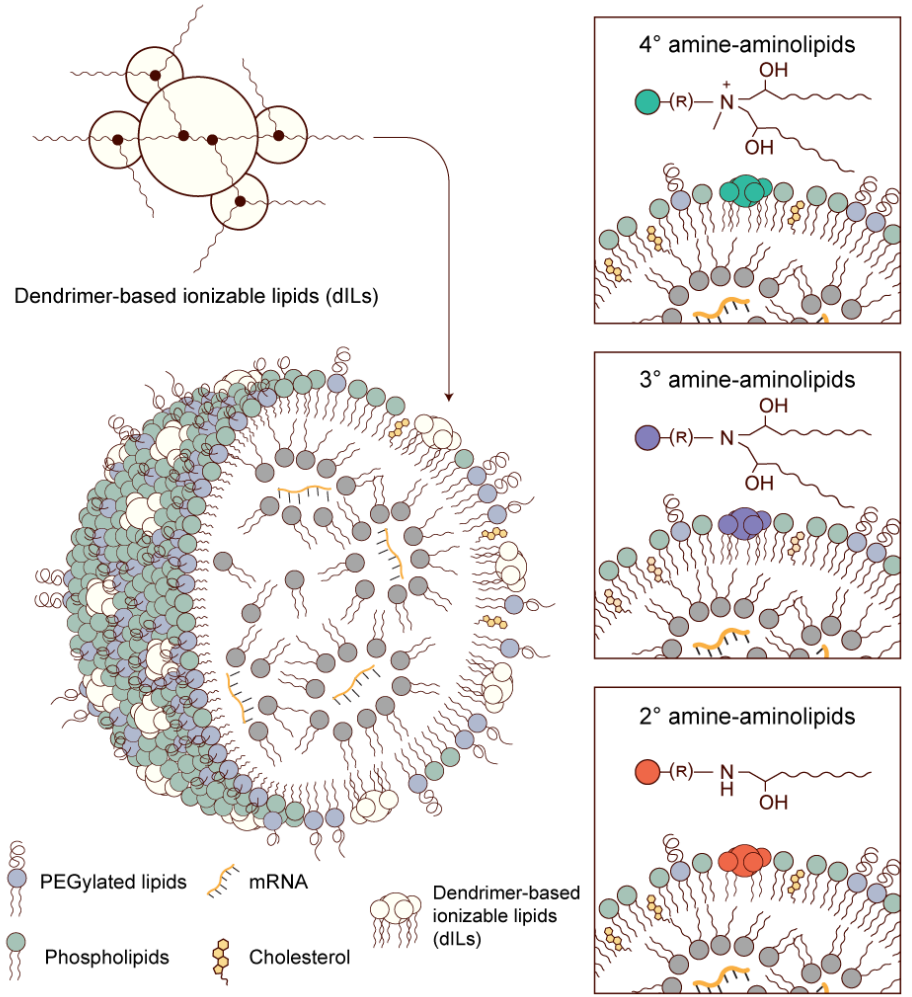




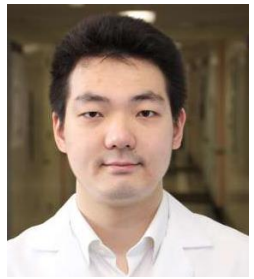
Unpublished data

Summary

- In vitro and in vivo screening identified 11 lead mRNA formulations with distinct tropism for liver, spleen, and lung;
- Lower lipid grafting on dendrimers reduces dLNP hydrophobicity and plasma protein adsorption, thereby limiting liver uptake
- Lower hydrophobicity also enriched complement proteins in the dLNP corona, promoting high splenic uptake
- QA modification of dendrimer reduced dLNP hydrophobicity and increased their apparent pKa.
- While overall protein adsorption was unchanged, the higher apparent pKa enriched vitronectin in the corona, promoting uptake by lung uptake.



Acknowledgement



Zijing Xu, M.S. **Kevon Jolly, B.S.**

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Dennis Feng, M.S.

Akanksha Ramadas Shanbhag, M.S.

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Jinmai Jiang, Ph.D.
Andrew A. Brock. BS

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IRG-21-139-01-IRG-02

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