Lipid Nanoparticles Formulated with Polypeptide-Cholesterol for Efficient *in Vivo* mRNA Delivery

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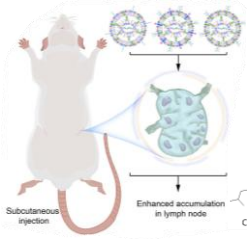
INTRODUCTION



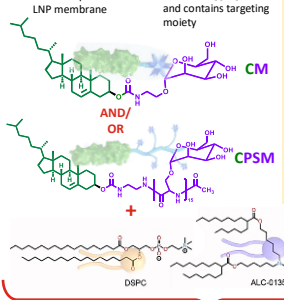
Conventional LNPs:
- Lack active targeting → low APC accumulation
- High doses required due to inefficient delivery to APCs
- Liver accumulation potentially inducing hepatoxicity

Mannosylated LNPs:
- Targeted accumulation in lymph nodes
- Minimal off-target delivery to the liver
- Enhanced uptake by dendritic cells

DESIGN & METHODOLOGY

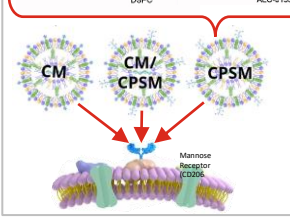
Hydrophobic — Anchors lipid to the LNP membrane

Hydrophilic — Prevents aggregation and contains targeting moiety



Mannose serves a dual purpose

1. Maintains colloidal stability of LNPs by providing hydrophilicity
2. Enhances mRNA delivery efficiency to cells overexpressing mannose receptors (e.g. dendritic cells)

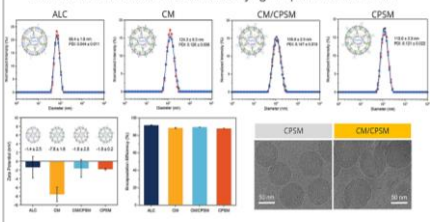


LNP screening and in vitro studies were performed to investigate:
1. Physiochemical properties
2. Cellular uptake
3. Endosomal escape
4. mRNA transfection
5. Cytocompatibility

RESULTS & DISCUSSIONS

3 LNPs stood out after *in vitro* screening

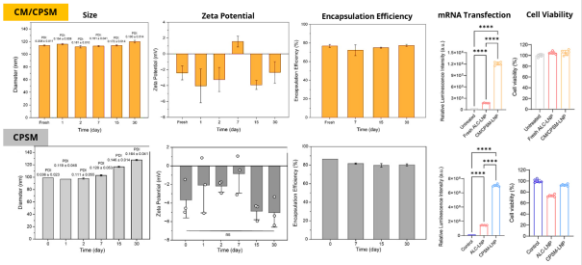
35 different mole ratios were screened: varying component's mole ratios



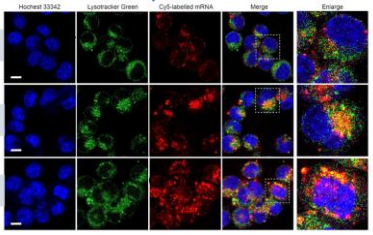
Physiochemical properties of optimal LNPs after screening:
- Size: <130 nm
- Zeta Potential: within ±10mV
- Encapsulation efficiency (EE): >85%

Mole Ratios	ALC0 315	DSPC	Chol	PEG	CM	CPSM
ALC	46.3	9.4	42.7	1.6	0	0
CM	Kept Constant		32.7	0	11.6	0
CM/CPSM			27.8	0	15.0	1.4
CPSM			42.7	0	0	1.4

Mannosylated LNPs Retain Stability and Enhance Transfection After 4 Weeks of Storage at 4°C



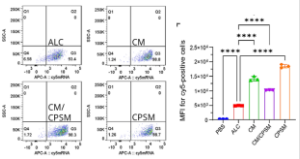
Successful endosomal escape



- **Colocalization analysis** was performed using **red (Cy5-mRNA)** and **green (lysotracker)** fluorescence.
- **After 6 hours** of incubation with CPSM-LNP or CM/CPSM-LNP, Cy5-mRNA **separated** from lysotracker signal.
- The separation indicates endosomal escape and cytoplasmic delivery of mRNA.

Mannosylated LNPs led to more cy5-positive DC2.4 cells

- LNPs encapsulated with **Cy-5 labelled** mRNA
- The Cy5 intensity of DC2.4 cells was **significantly higher** when incubated **with mannosylated LNPs** than with ALC-LNPs

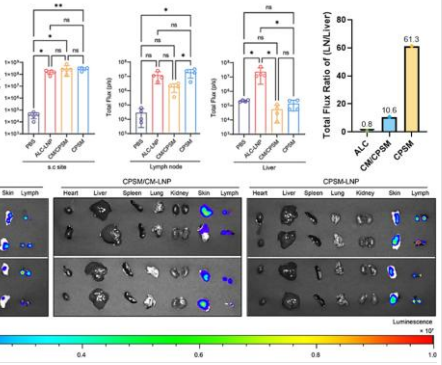


Mannosylated LNPs Preferentially deliver mRNA to lymph nodes

Whole-body imaging 6hrs post injection, followed by **ex vivo biodistribution**

The luminescence intensity of mannosylated LNPs at **skin** and **lymph nodes** was **comparable to ALC**

Significantly lower luminescence intensity **in** the **liver** for mannosylated LNPs as compared to ALC



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2. J.-Y. Zeng, S. Lingesh, N. D/O B. Krishnan, B. Seow, M. Liu, Q. Chen, Y. Y. Yang. *Small Methods* **2025**, 2401712

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CONCLUSION

- **Two amphiphilic lipids** were successfully synthesized to **incorporate mannose** into LNP formulations.
- Nanosized LNPs with narrow size distribution and high colloidal stability
- **Mannosylated LNPs** exhibited **enhanced uptake** in **dendritic cells** compared to ALC-LNPs.
- **Mannosylated LNPs maintained** physicochemical and mRNA transfection **stability** for up to **one month**, comparable to ALC-LNPs.
- **Mannosylated LNPs** demonstrated **preferential** accumulation in **lymph nodes** with **minimal liver uptake**.