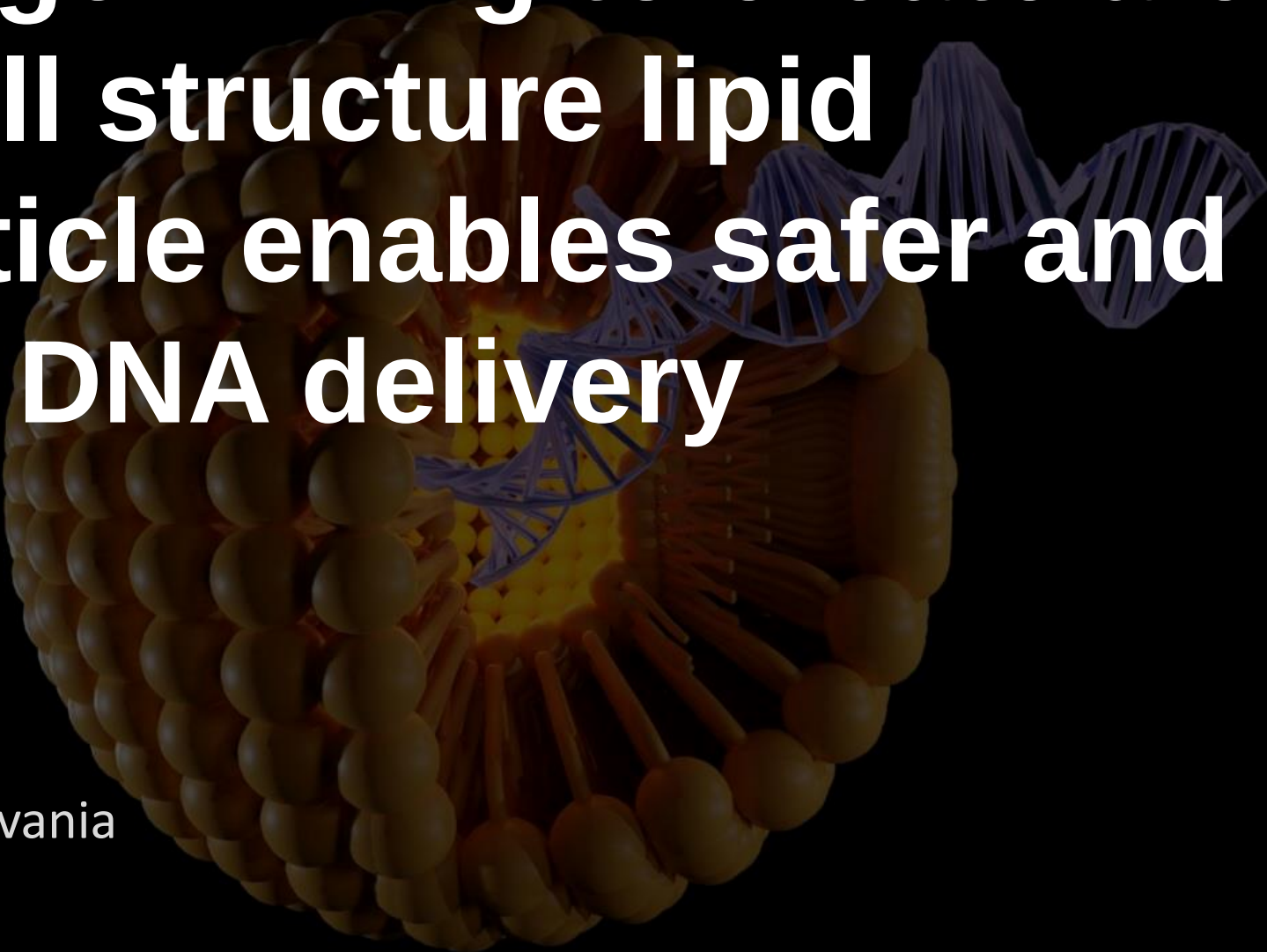


Multi-stage mixing to create a core-then-shell structure lipid nanoparticle enables safer and more effective DNA delivery



Jia Nong

University of Pennsylvania

Controlled Release Society Annual Meeting and Exposition, 7.17.2025

Lipid Nanoparticles (LNPs): A Game-Changer

1

Development

Rapid formulation by mixing lipids and nucleic acid

2

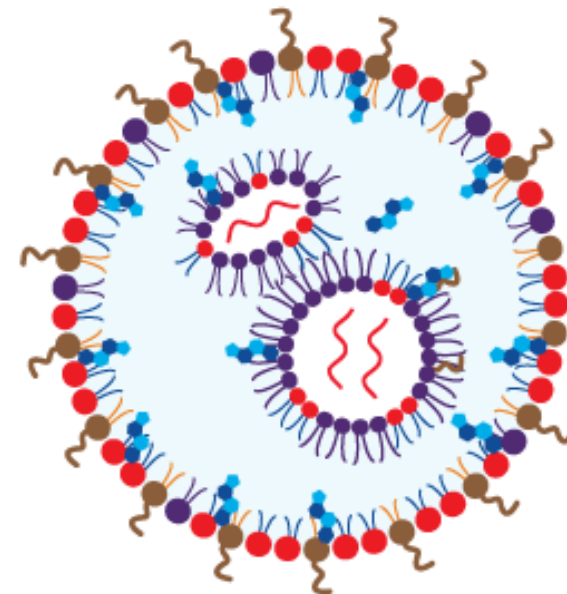
Delivery

Efficient cellular uptake of nucleic acid and transfection

3

Potential

Applicable for various diseases



Ionizable cationic lipids



PEGylated lipids



Phospholipids



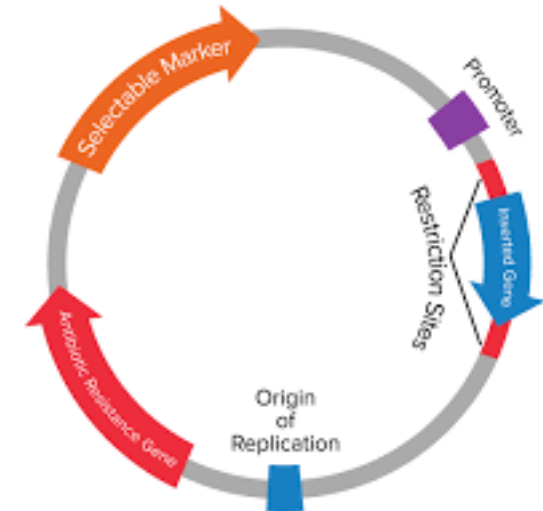
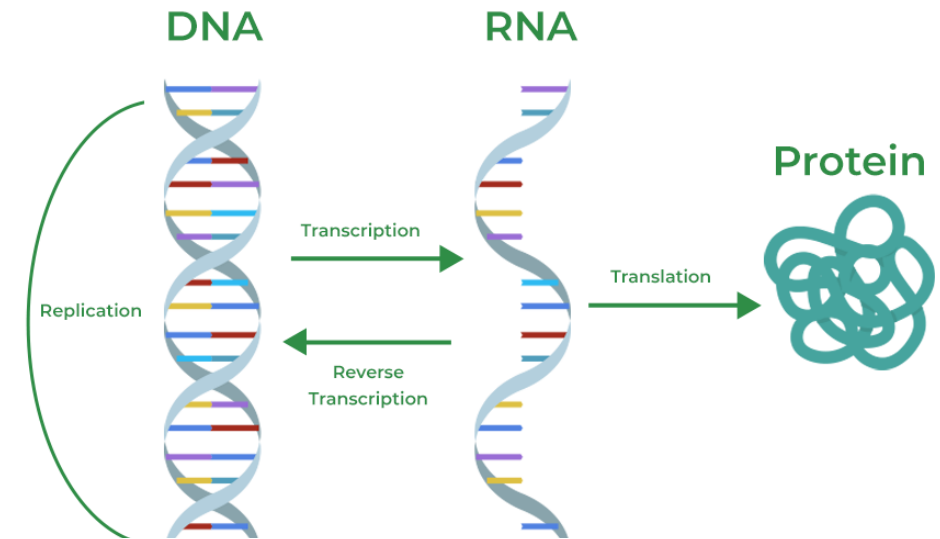
Sterol lipids



Cargo

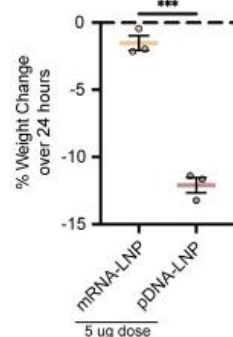
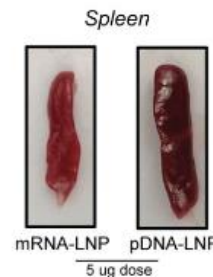
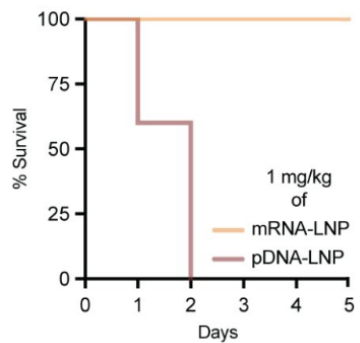
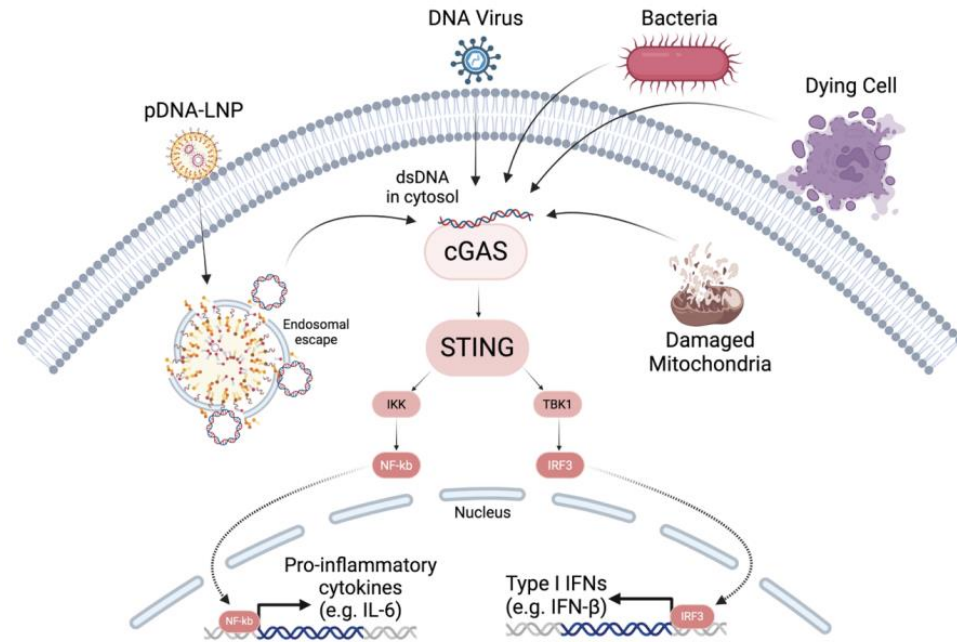
DNA's benefits over mRNA

- Duration: mRNA decays in hours, DNA-LNPs could provide expression for 6 months per dose. Opening up opportunities for *chronic* diseases.
- Promoters: cell-type-specific promoters and promoters that can be regulated by small molecule drugs like doxycycline

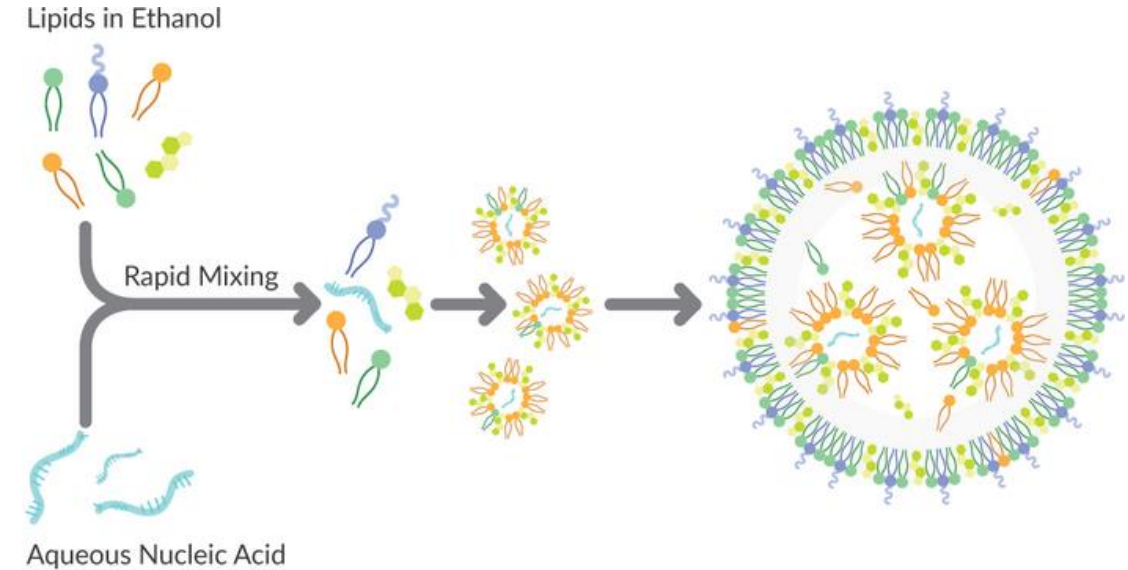


Problems of delivery DNA

- Toxicity



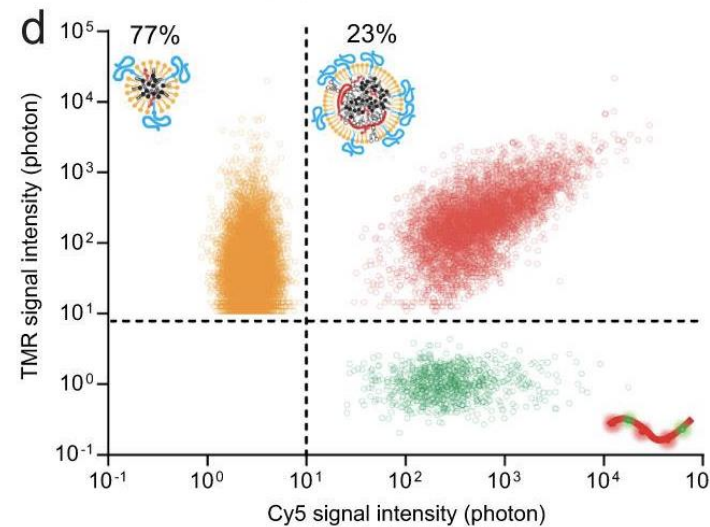
- Low transfection using LNPs



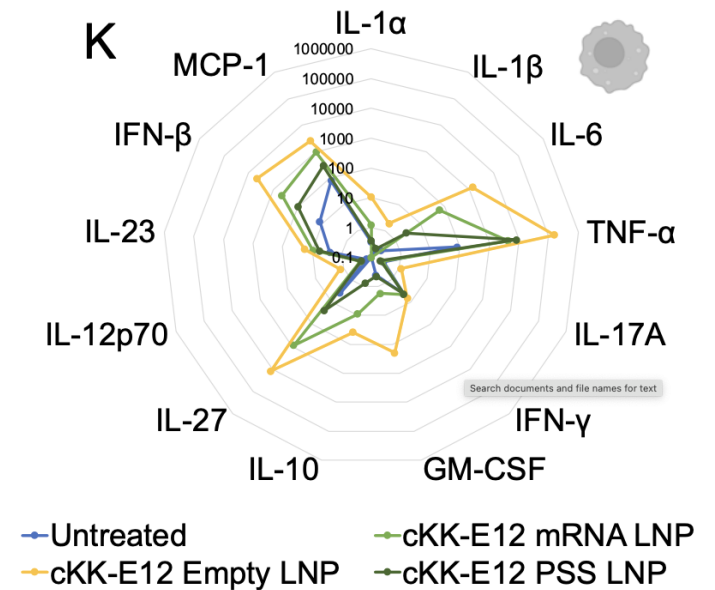
Challenges in LNP Nucleic Acid Delivery

1. Empty LNP without payload

- More than half of formulated LNPs are empty
- Empty LNPs are more immunogenic and cytotoxic



Li, *Nat Comm*, 2022

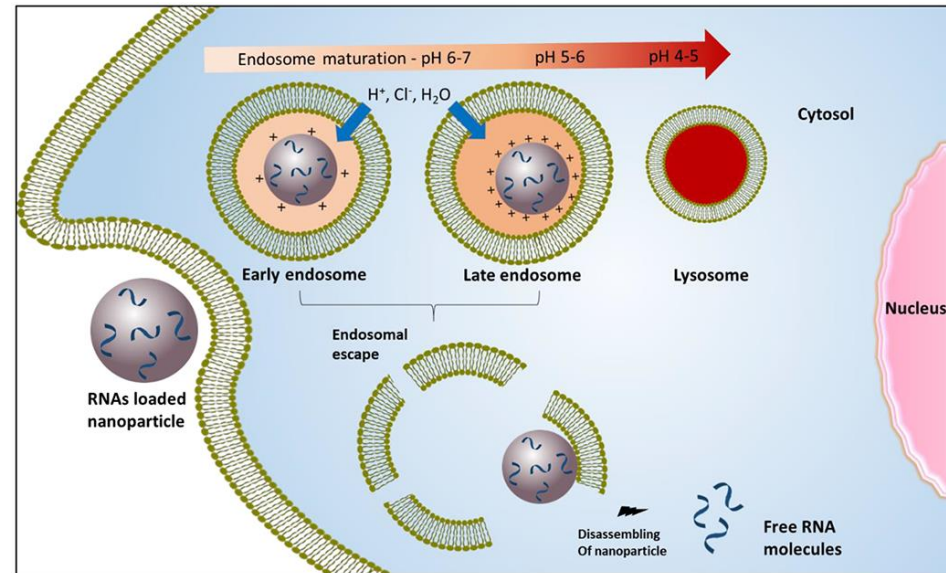


Omo-Lamai*, Wang*, Patel*, *Nature Nano*, 2024

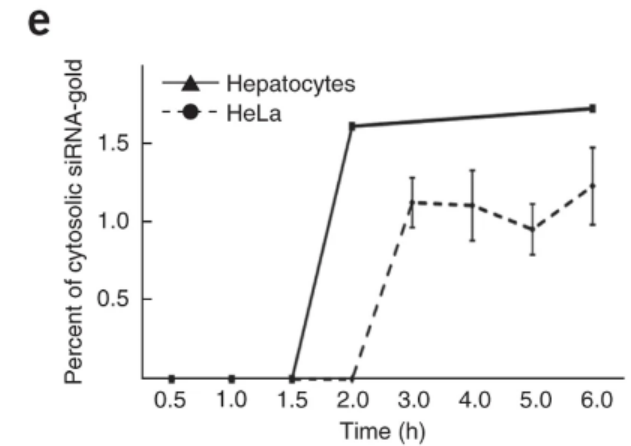
Challenges in LNP Nucleic Acid Delivery

2. Low Endosome Escape Rate

- <2% of RNA payload can escape endosome and enter cytosol



Patel, *Trends Pharmacol Sci*, 2021

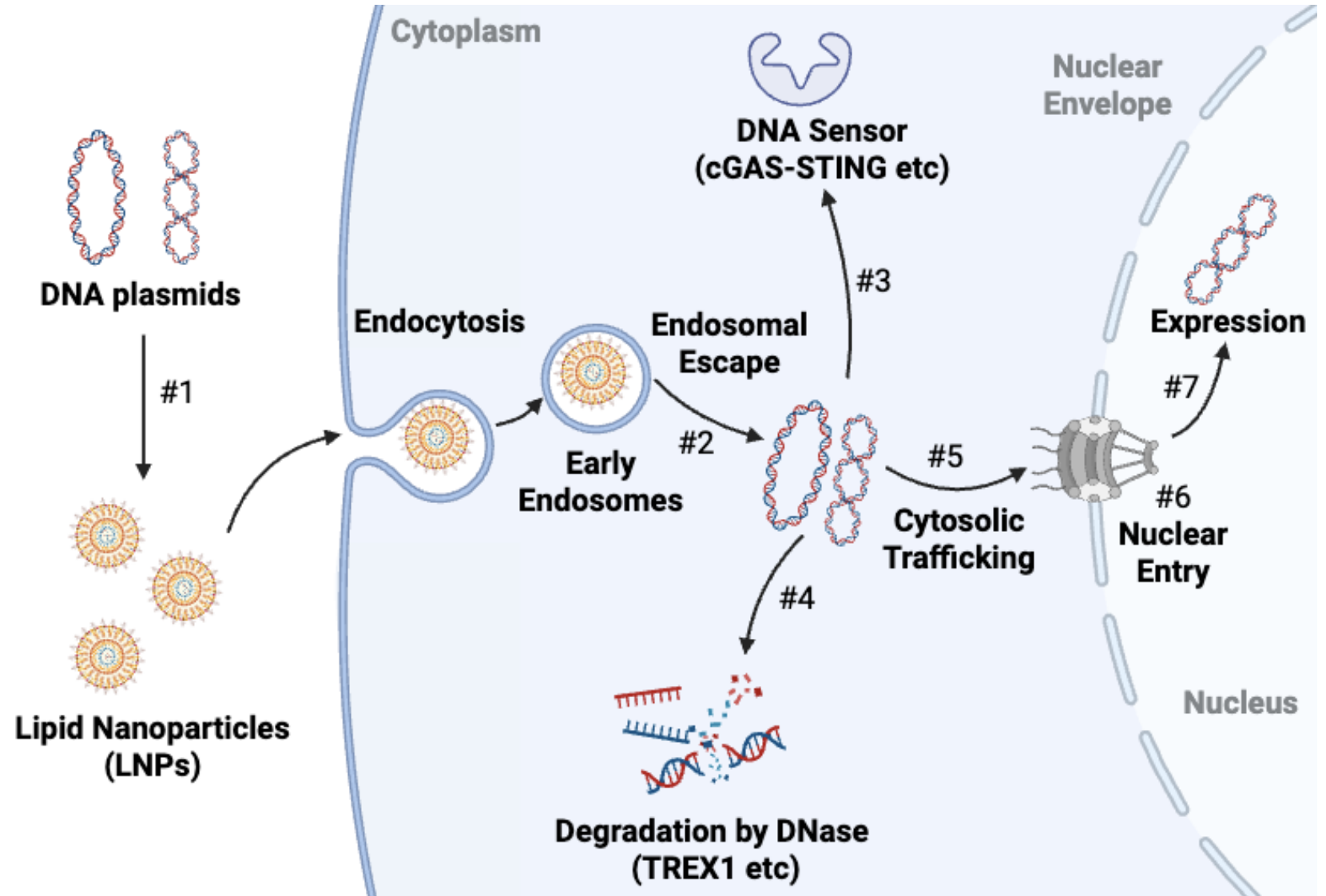


Gilleron, *Nat Biotech*, 2013

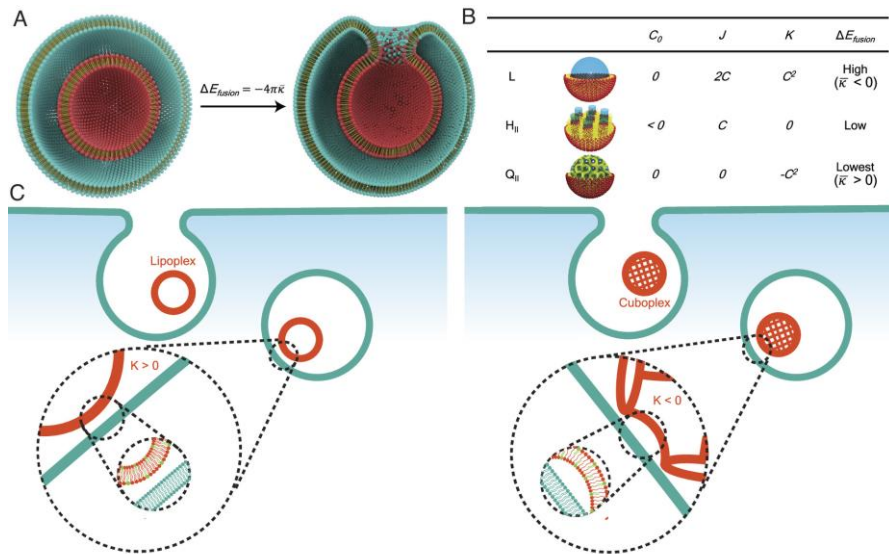
Challenges in LNP Nucleic Acid Delivery

3. DNA delivery barriers

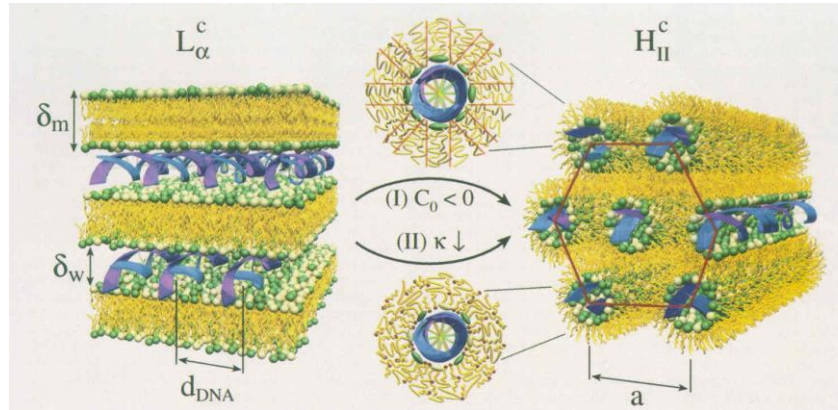
- DNA is much larger than RNA
- Cytosolic release and stability



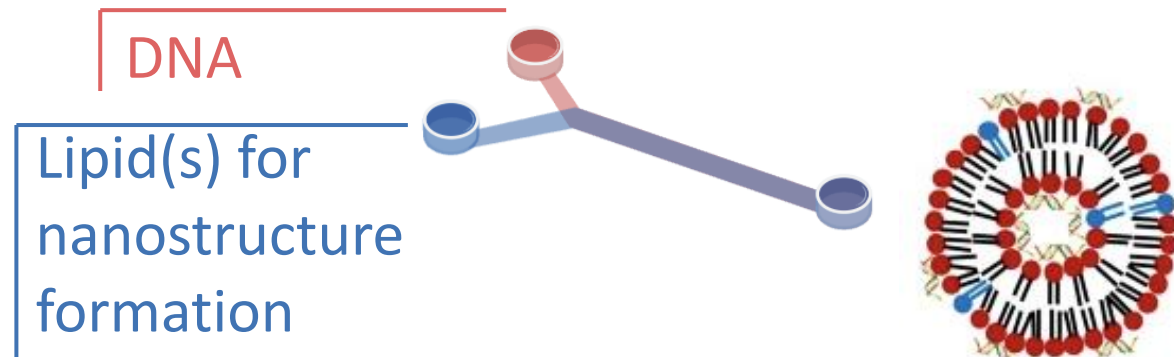
Nanostructure of RNA-LNP affects endosome membrane fusion efficiency



Li, et al, *PNAS*, 2023



Koltover et al, *Science*, 1998

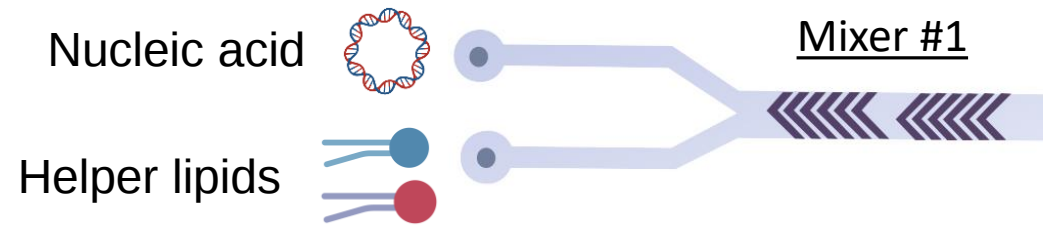


Unstable lipoplex

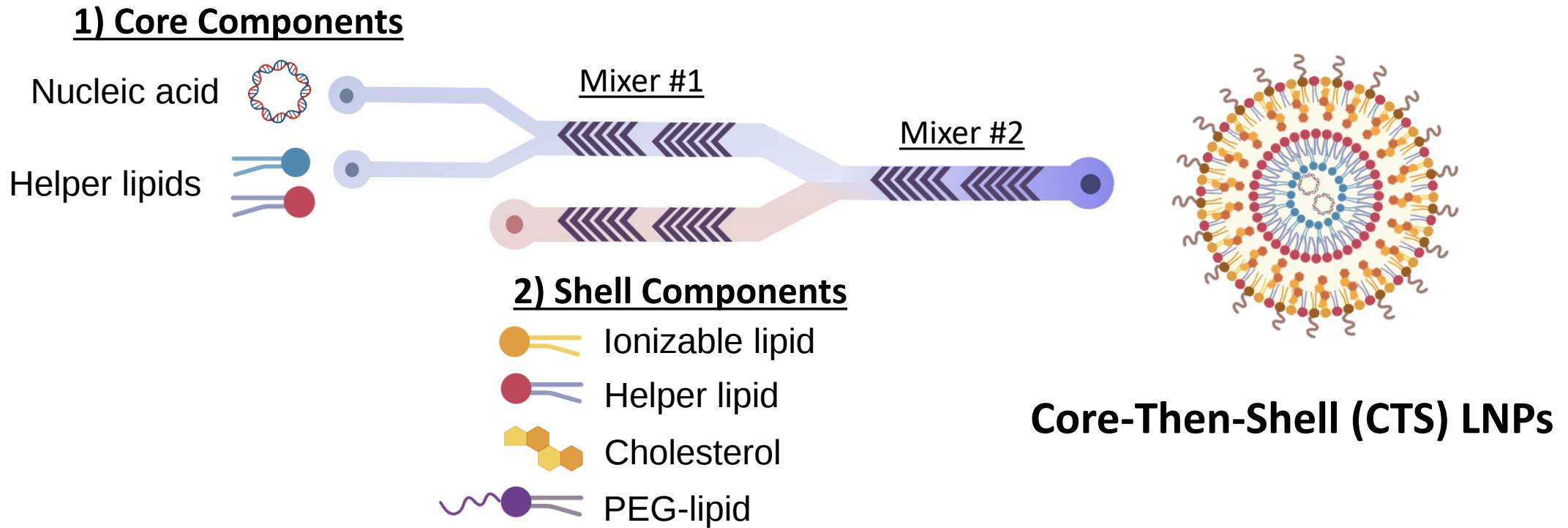
- Increasing size as soon as mixing
- DNA exposure for immune response

Design of Multi-Staging Mixer (MSM)

1) Core Components

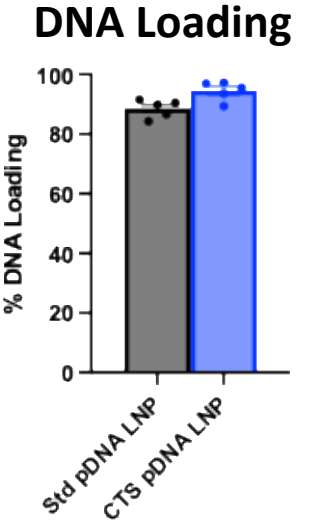
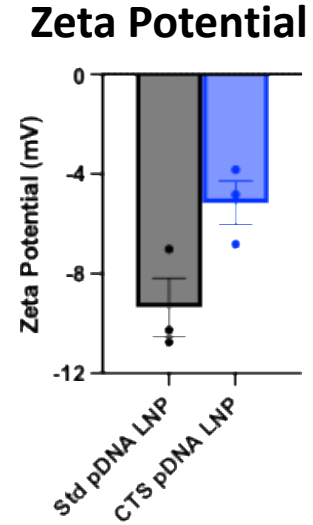
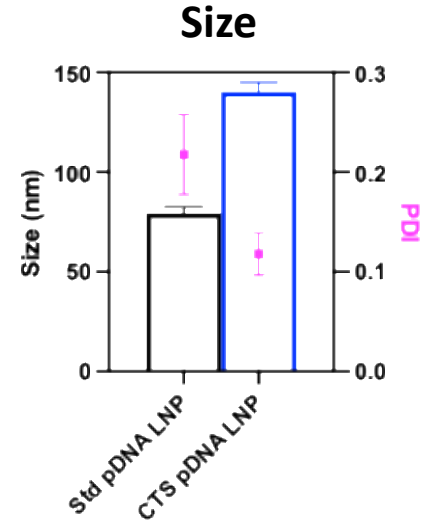
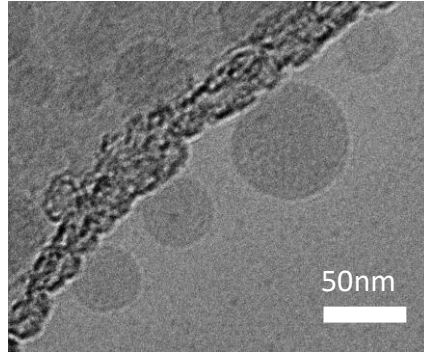
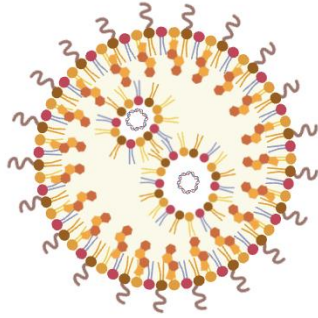


Design of Multi-Staging Mixer (MSM)

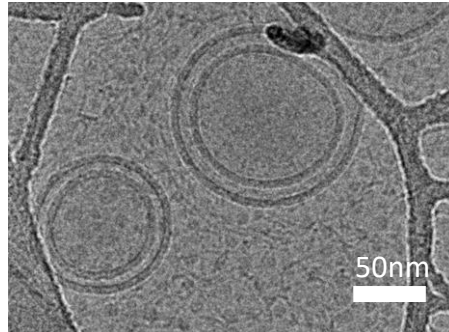
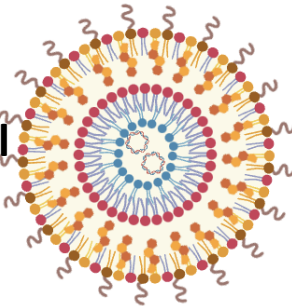


Unique core-shell structure of CTS LNP

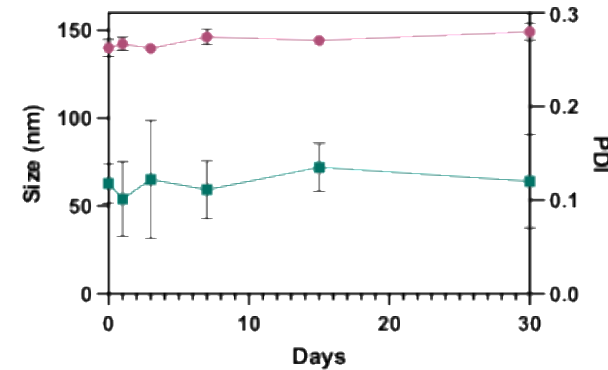
Std LNP



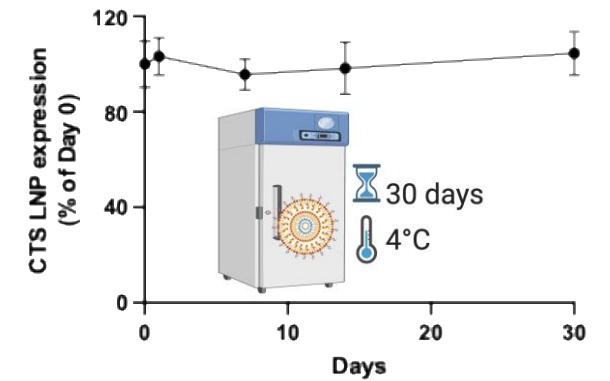
Core-Then-Shell
(CTS) LNP



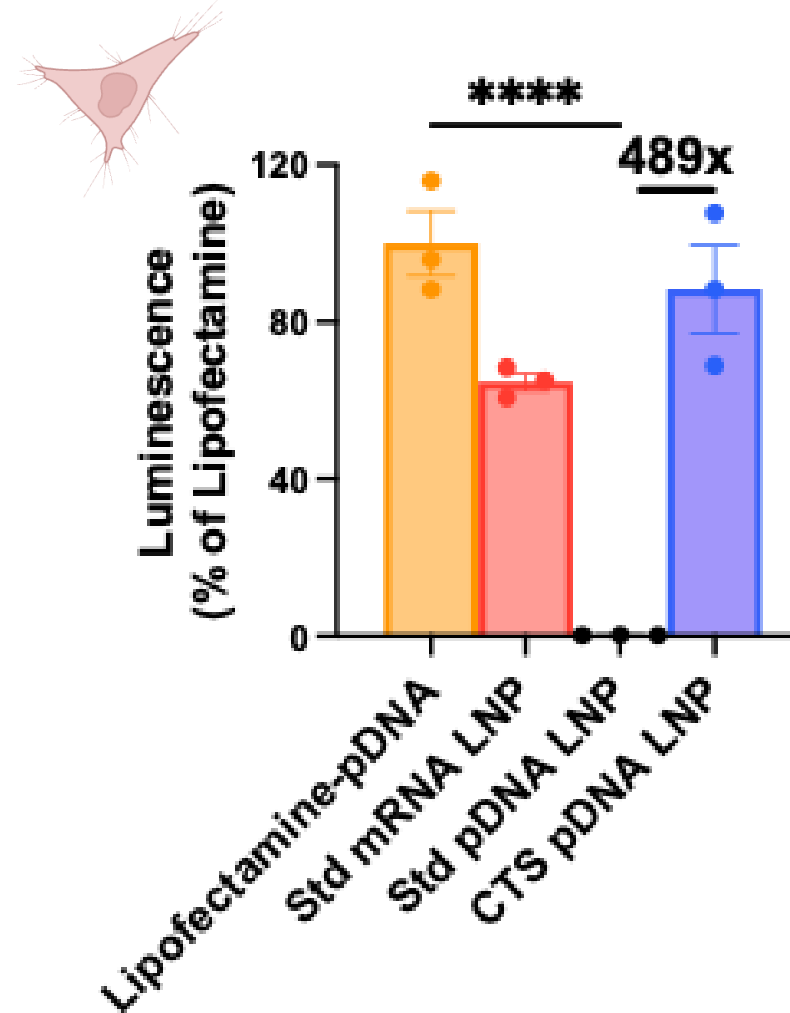
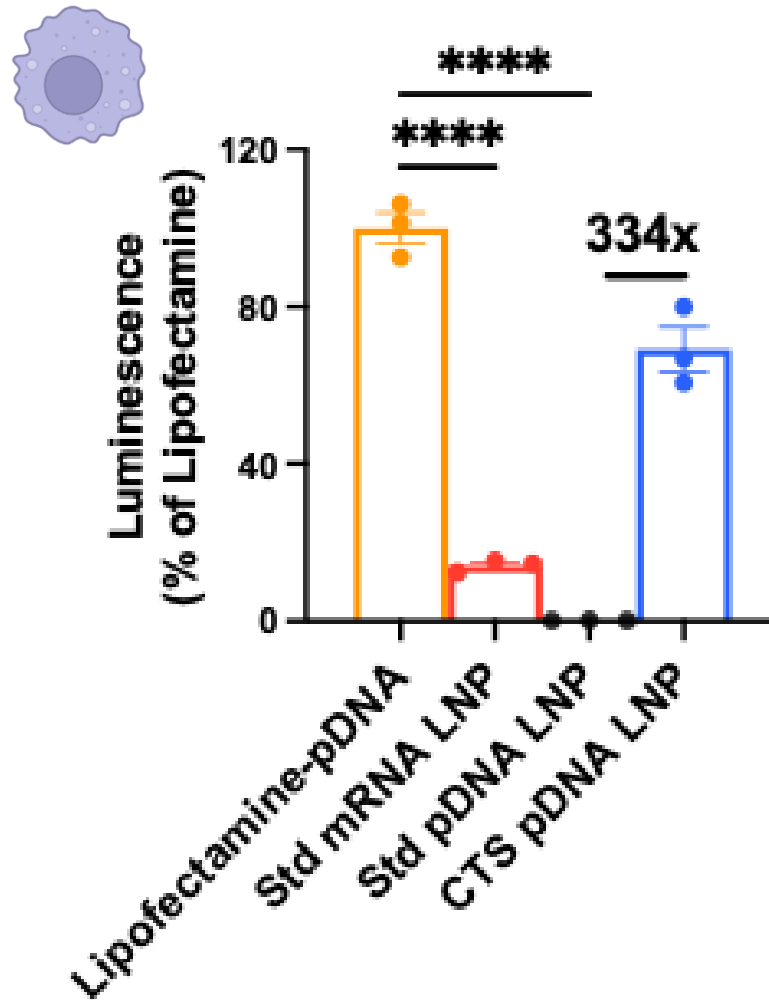
Size Stability



Expression Stability

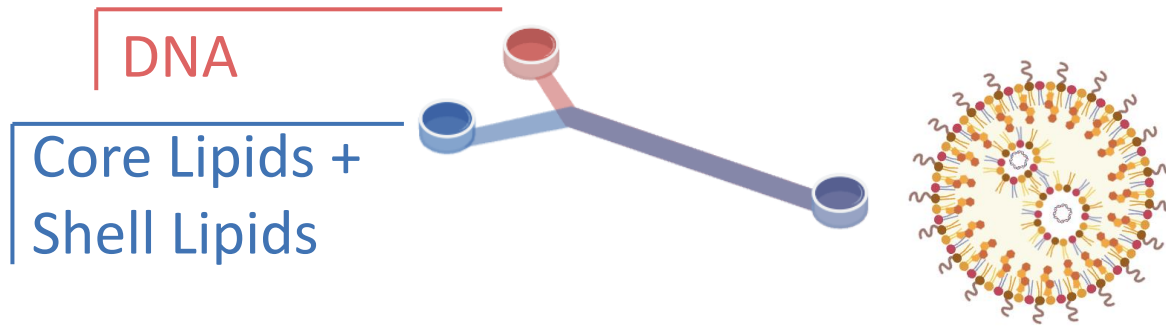


CTS LNPs improve DNA expression in vitro

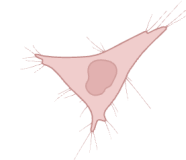
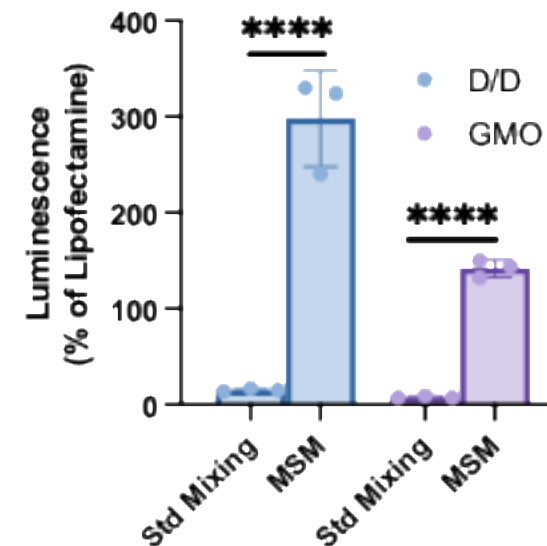
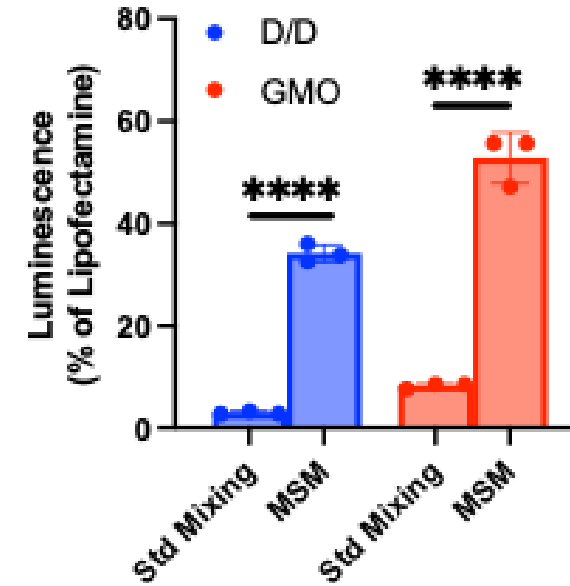
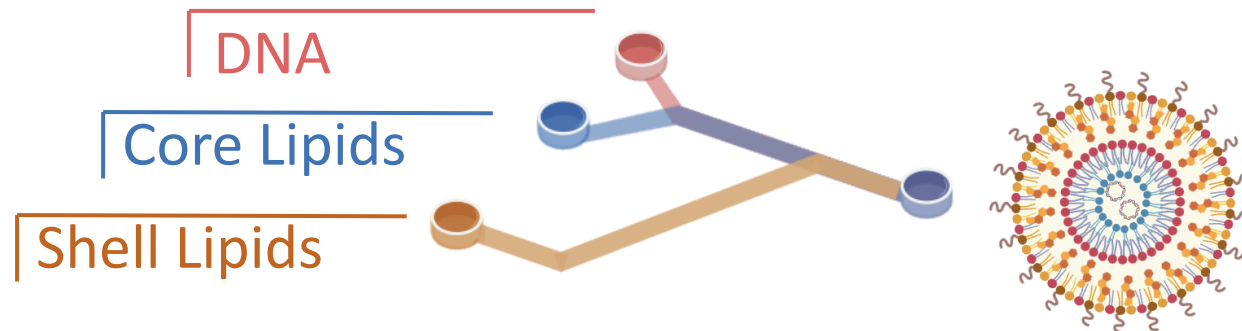


Internal core structure is critical for improved transfection

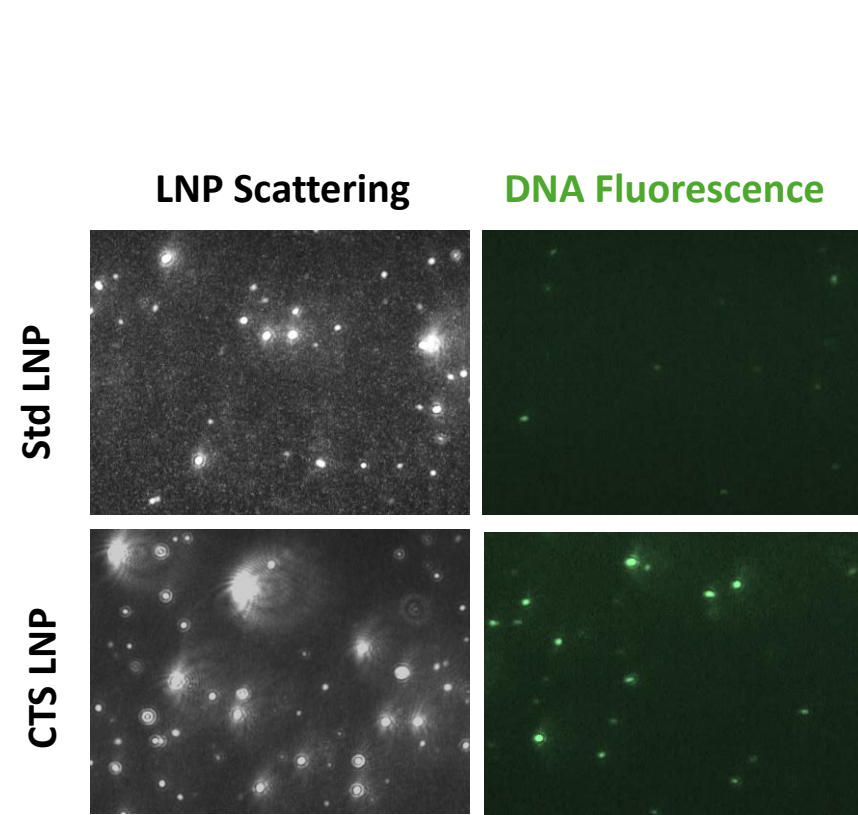
Standard Bulk Mixing



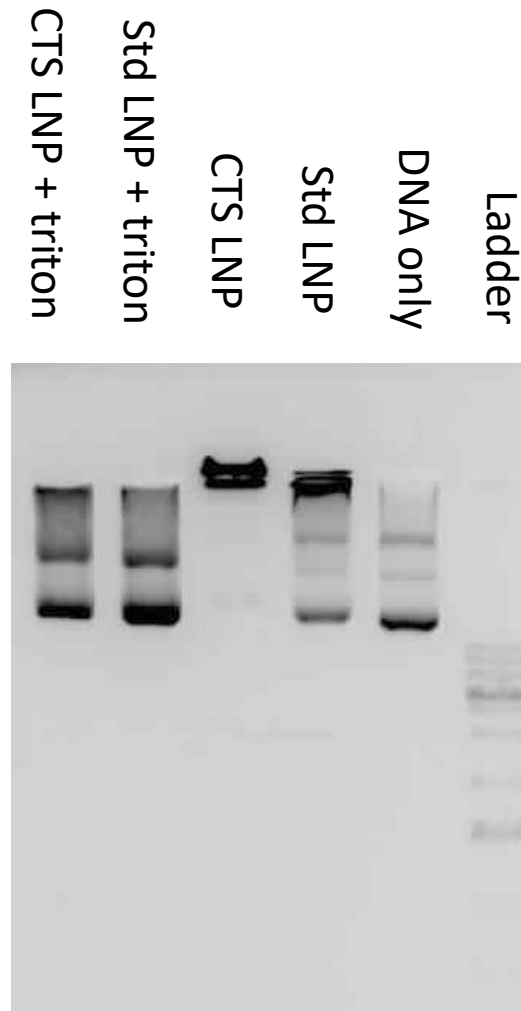
Multi-Staging Mixing (MSM)



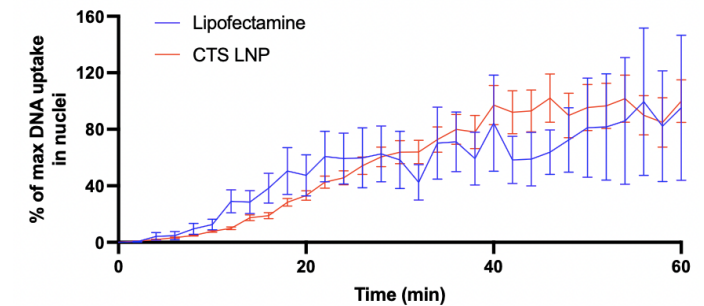
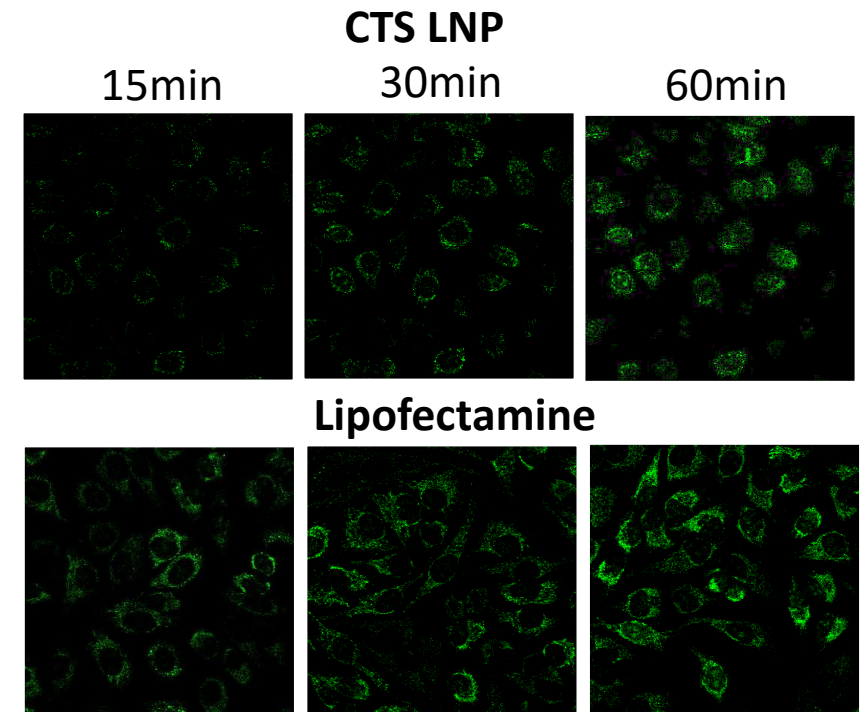
Biological Mechanism of better CTS LNP transfection



1. Better DNA packaging and less empty LNPs

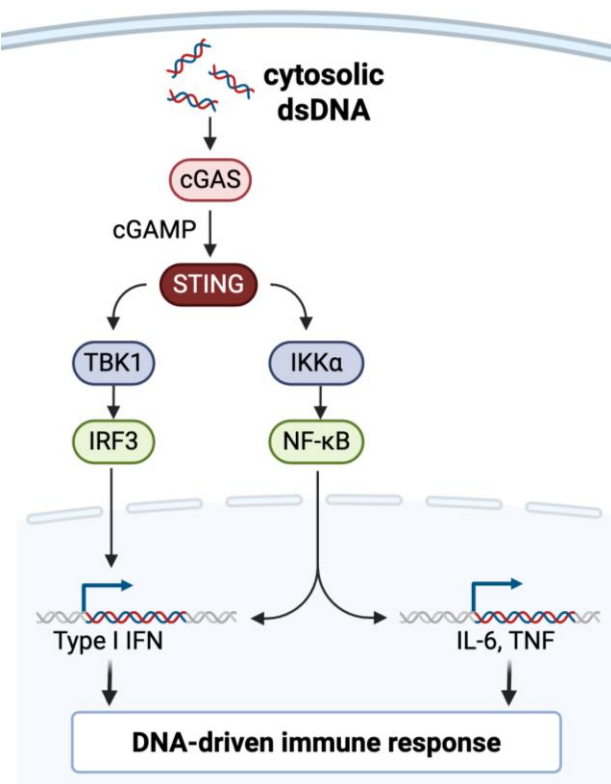


2. Better cytosolic protection of DNA from degradation

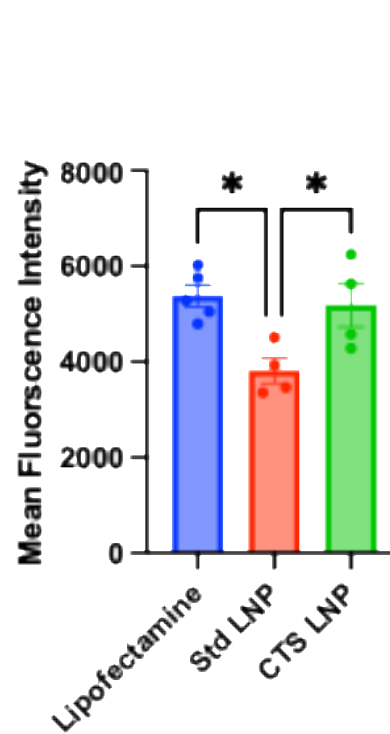
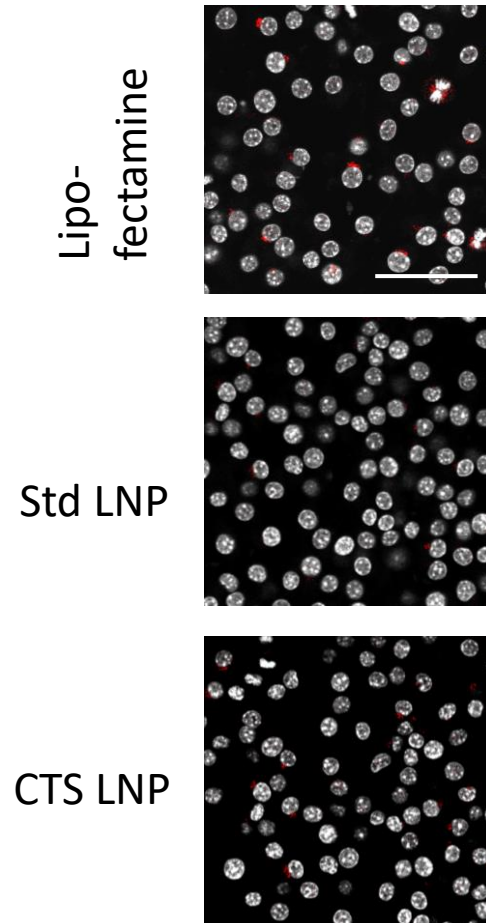


3. Comparable nuclear transport as lipofectamine

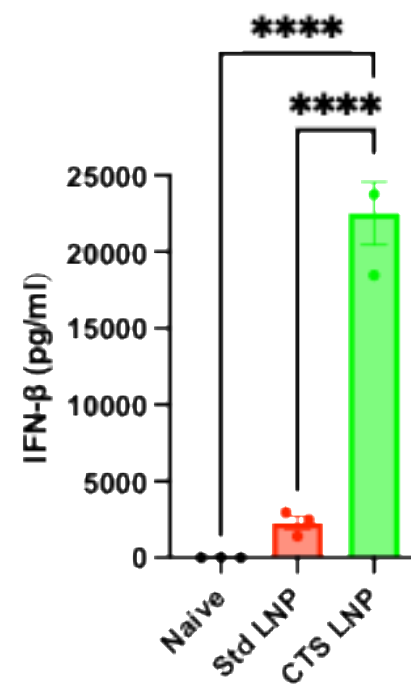
However, more DNA delivery leads to more toxicity



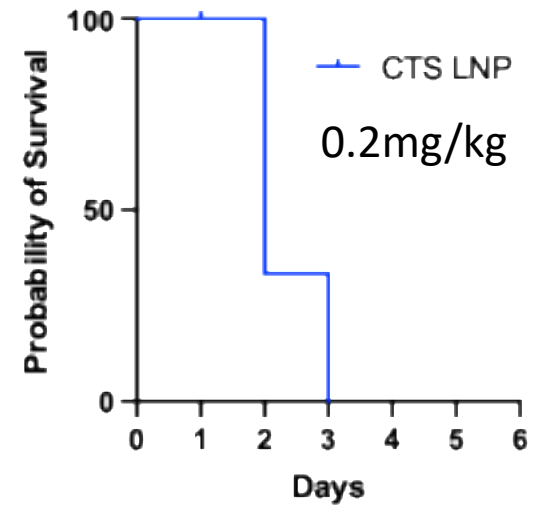
Phospho-Sting Quantification



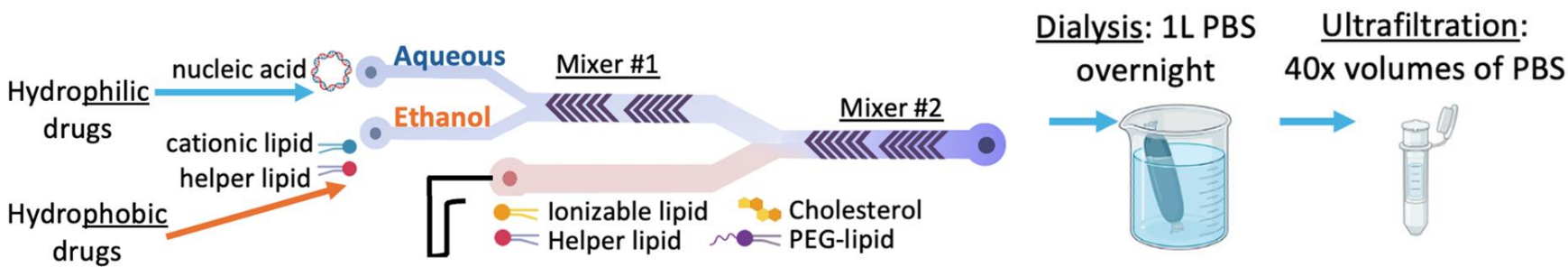
IFN-beta



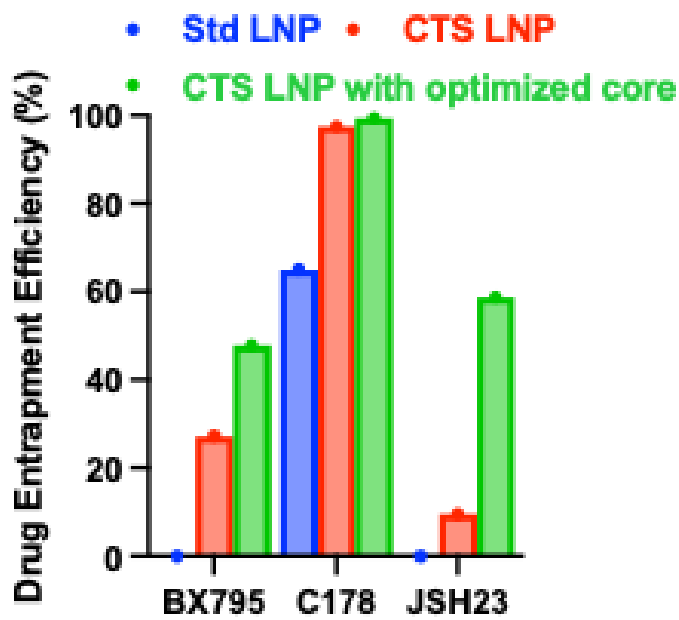
Survival



CTS LNPs are capable to load small molecule drugs

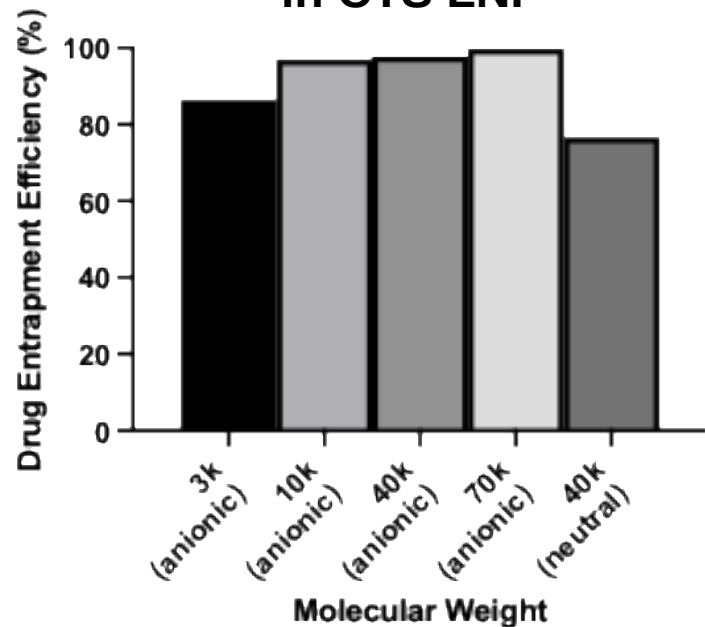


Drug loading of STING pathway inhibitors

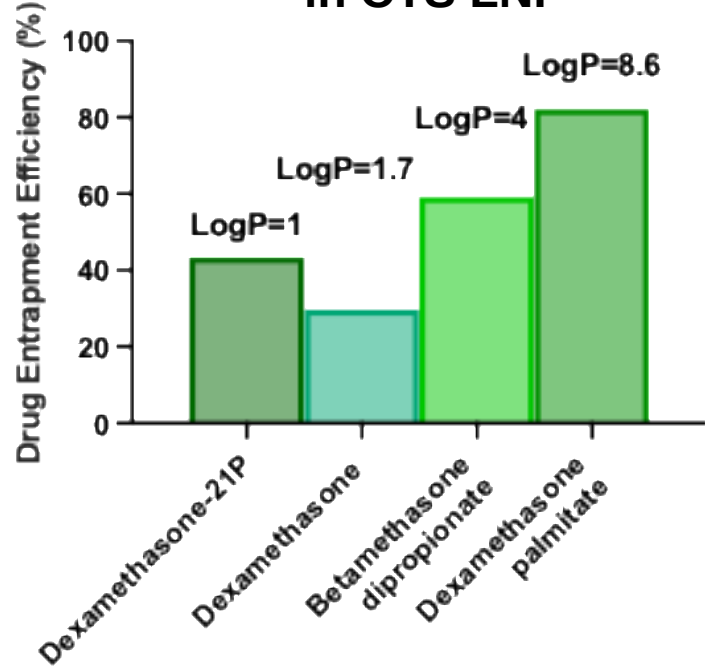


BX-795: TBK1 inhibitor (Mw: 591g/mol)
C-178: Sting inhibitor (Mw: 322g/mol)
JSH23: NFkb inhibitor (Mw: 240g/mol)

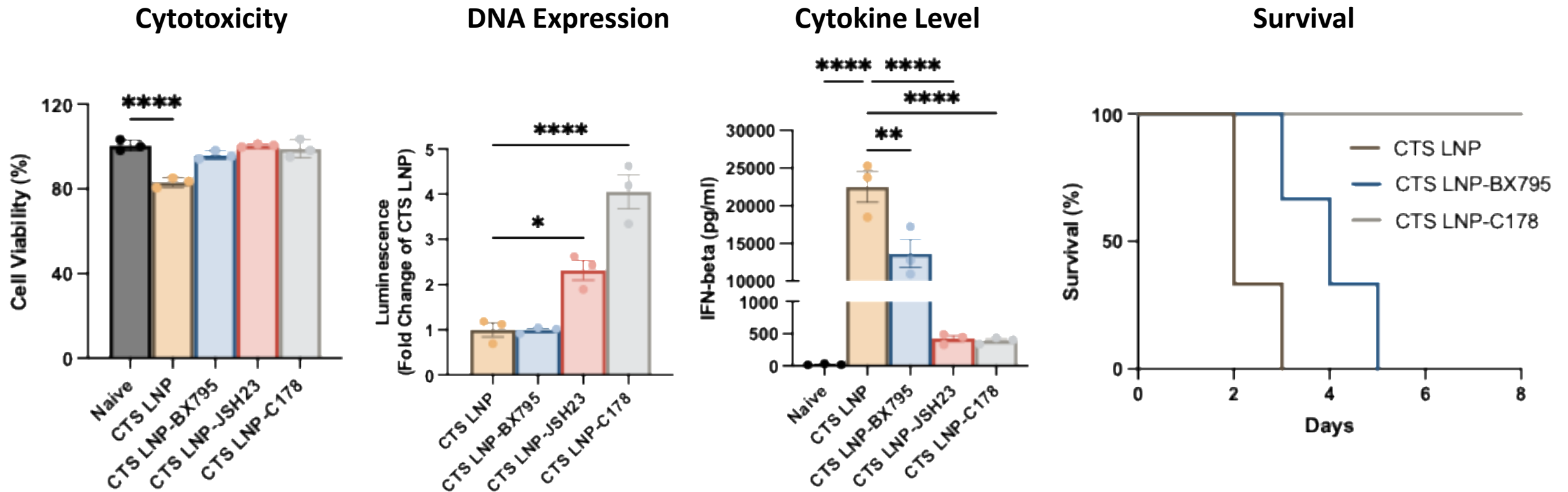
Drug loading of Dextran in CTS LNP



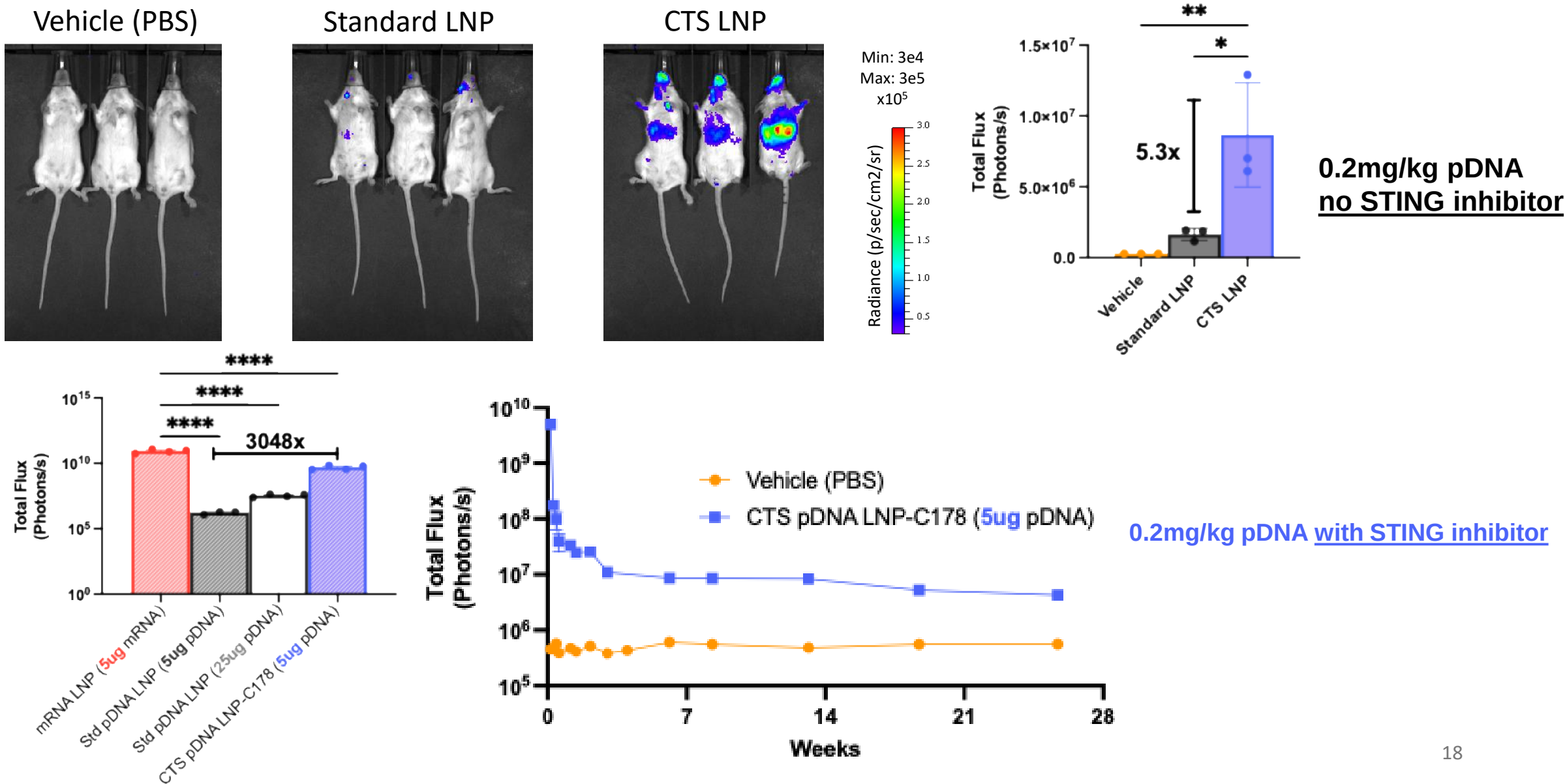
Drug loading of Dex in CTS LNP



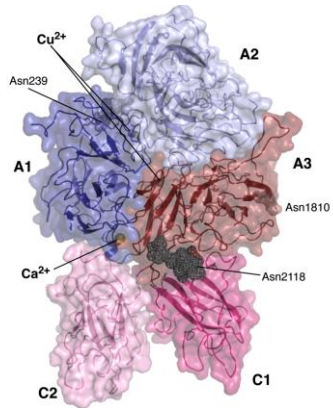
Loading inhibitors into CTS LNPs reduce inflammation, maintain expression and improve survival



CTS LNPs enhance DNA expression in vivo



CTS LNPs treating hemophilia A



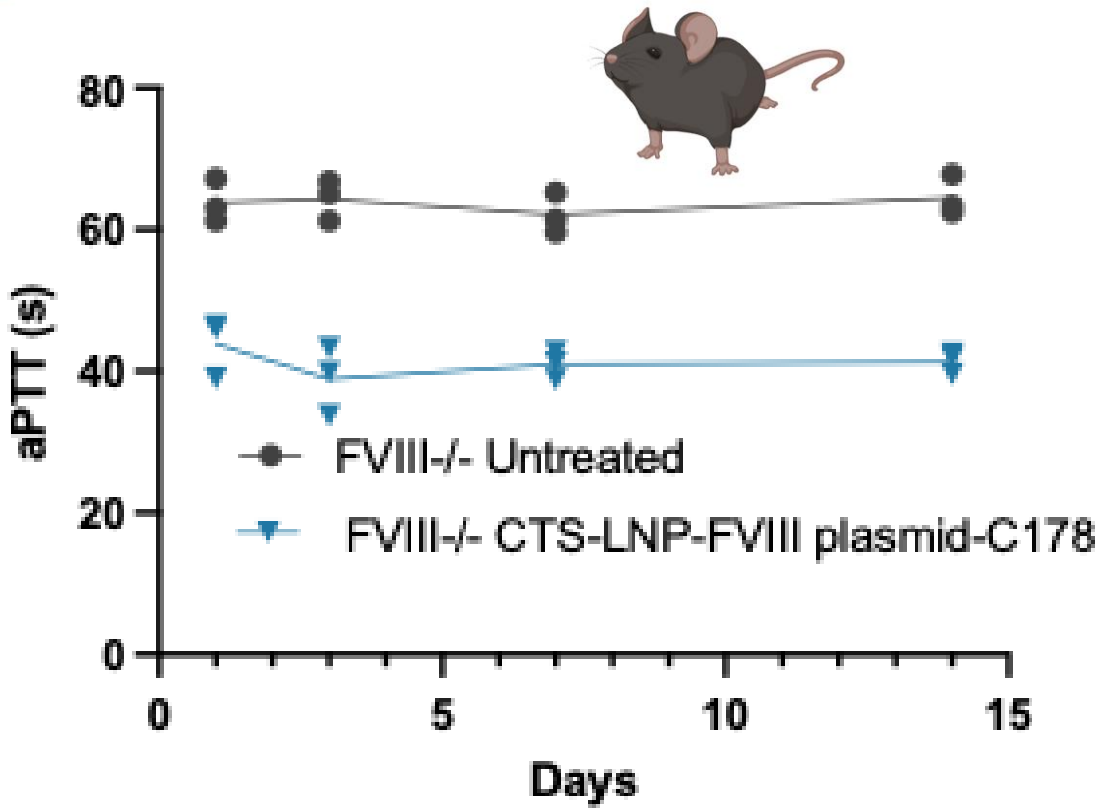
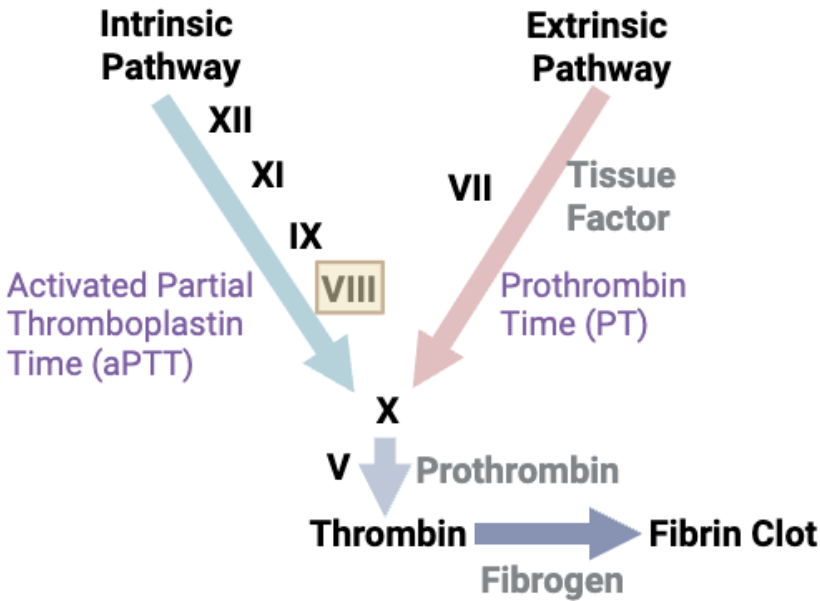
6.9 kb CDS
330 kDa protein



Dr. Lindsey George



Dr. Ben Samelson-Jones

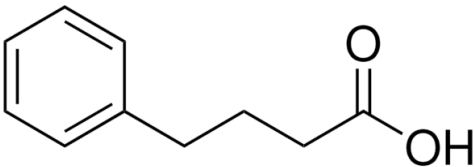


The Children's Hospital
of Philadelphia®

CTS LNPs treating genetic epilepsy

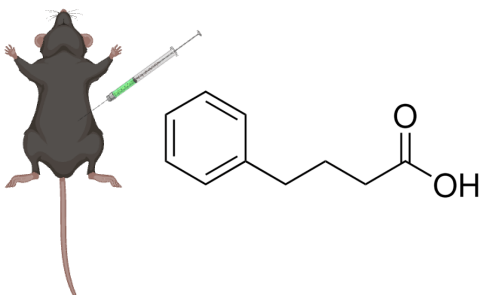
CTS LNP co-load mRNA and **4-Phenylbutyric acid**

- Effective in treating epilepsy in pediatric patients
- Significant GI problem when taking PBA orally

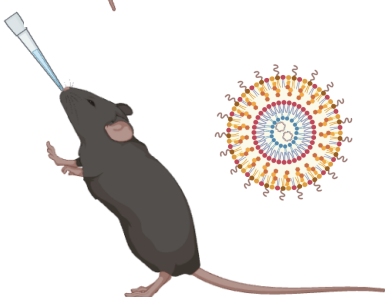


Dr. Jingqiong Kang

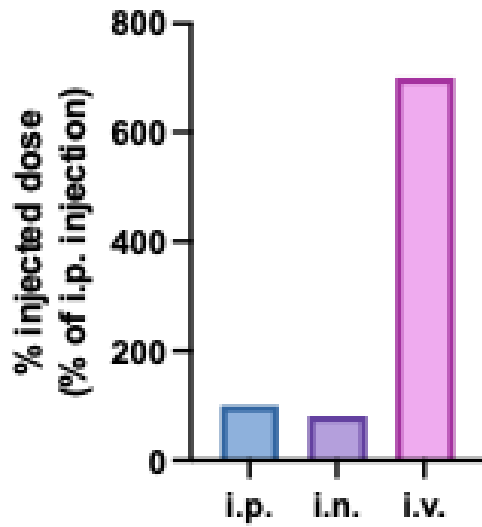
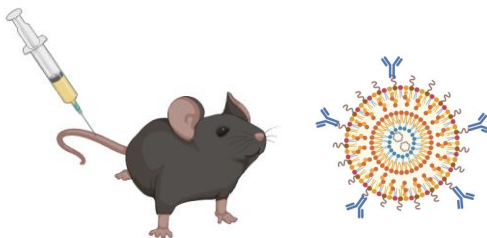
Free drug
intraperitoneal
injection



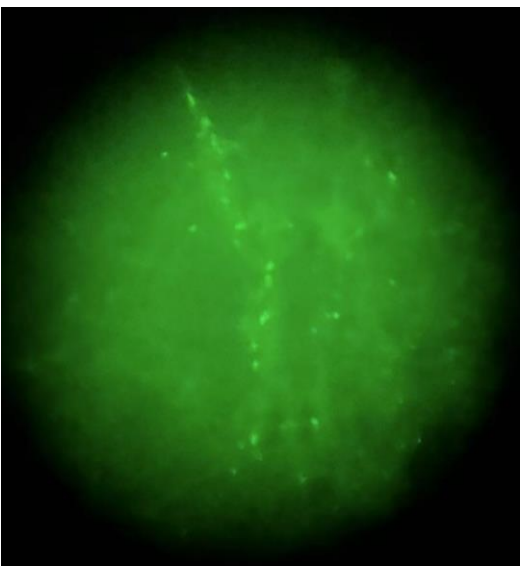
Bare LNP
intranasal delivery



BBB-targeted LNP
IV delivery



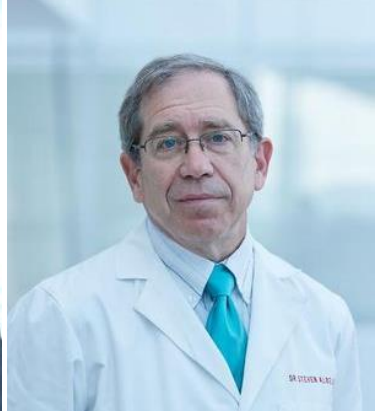
eGFP mRNA expression in brain
after i.n.



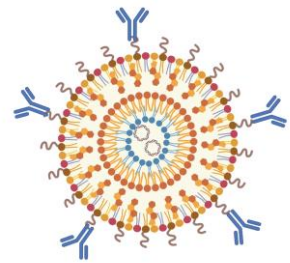
CTS LNPs loaded with functional protein enhance T cell transfection



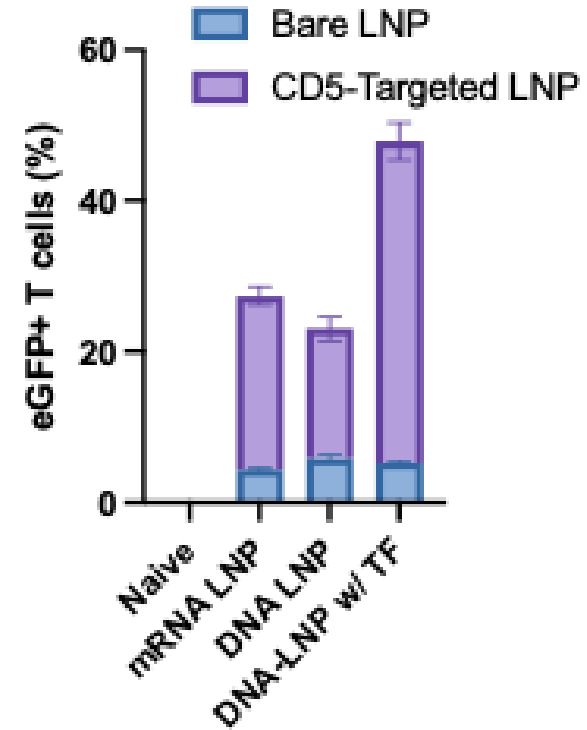
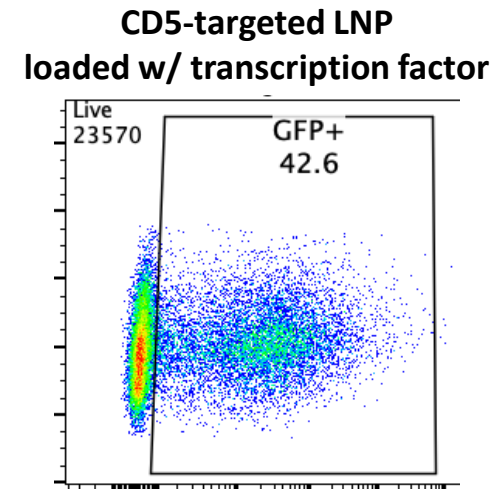
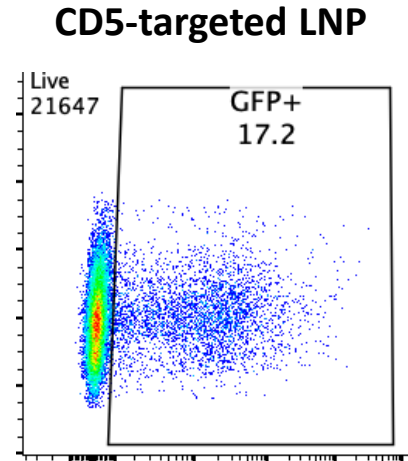
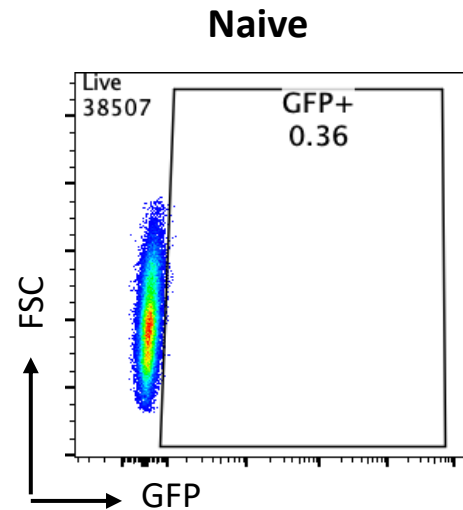
Dr. John Wherry



Dr. Steven Albelda



CD5-targeted
CTS LNP



Conclusion

- Microfluidic design controls over LNP assembly kinetics and enables unique core-shell particle architecture
- CTS structure massively improves DNA transfection efficiency by tackling multiple biological barriers
- CTS LNPs' capability of loading small molecule drugs and proteins greatly broaden its application
- This innovation opens new possibilities for treating numerous common chronic diseases

Acknowledgement



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Aleksa Milosavljevic
Sachchidanand Tiwari
Manthan Patel
Jichuan Wu

Leal Lab

Lining Zheng

Issadore Lab

Andrew Hanna
Gregory Datto

Kushol Gupta

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of Health

