“Advancing Targeted LNP Platforms”

Analytical Challenges and Strategies in Development and Characterization

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EDITAS MEDICINE

CASSS 2025 - CONSULTANTS' NETWORK - LIPID NANOPARTICLES

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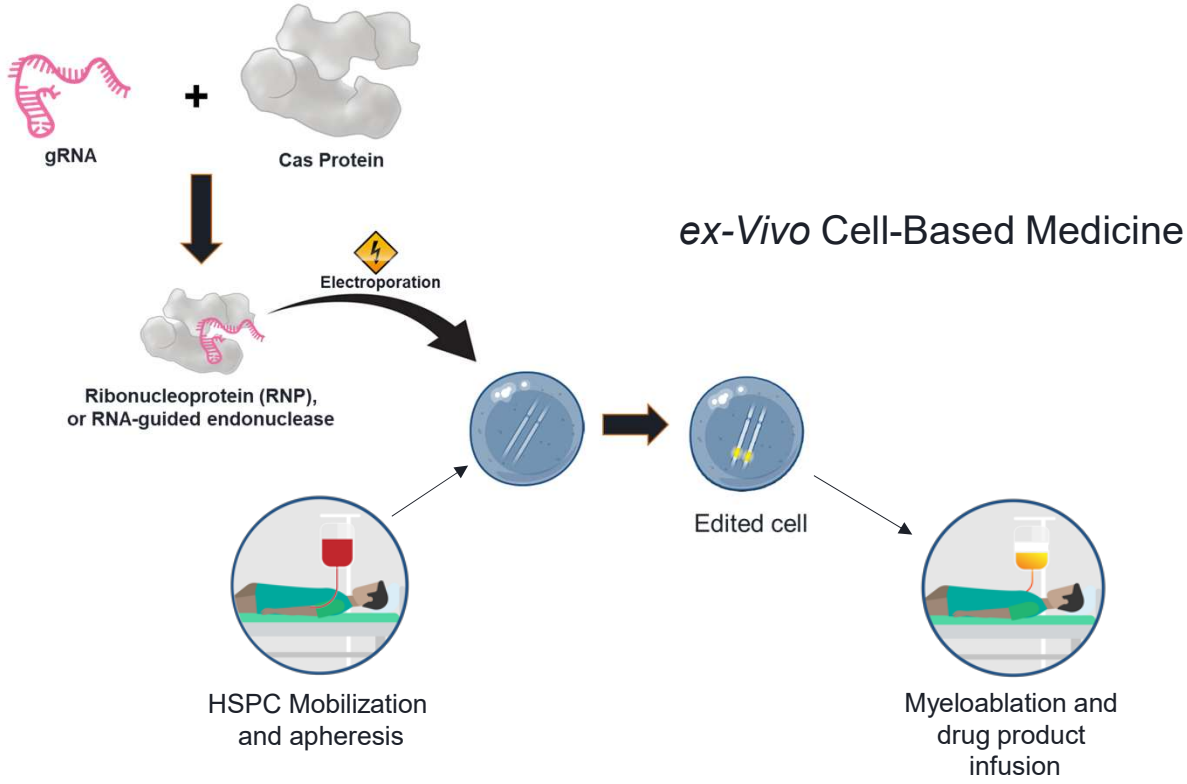
Disclosure

- I am an employee and shareholder of Editas Medicine, Inc.

CRISPR Application for Gene Editing

CRISPR is a gene editing technology derived from the bacterial adaptive immune system that can revise, remove, and replace genes in a highly targeted manner using a ribonucleoprotein (RNP).

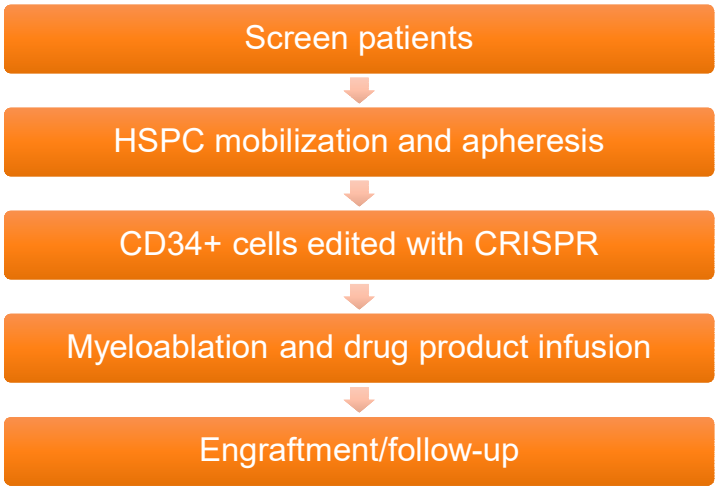
In a therapeutic context, CRISPR is used to edit disease-related genes:



HSPC: hematopoietic stem and progenitor cell

In Vivo Gene Editing: Transformative Potential

Ex Vivo (Autologous) Therapies



In Vivo Therapies

Patient is infused with drug product to edit their HSPCs *in vivo* and then discharged home

ex vivo	in vivo
• Significant cell processing and hospital infrastructure • Difficult to scale up and very expensive	• One manufacturing process for many patients • Simpler, scalable and lower COGs
• Multiple hospital stays for patients • Side effects of hospitalization and depletion steps	• Potentially single administration
• Not readily exportable to multiple international markets	• Exportable worldwide

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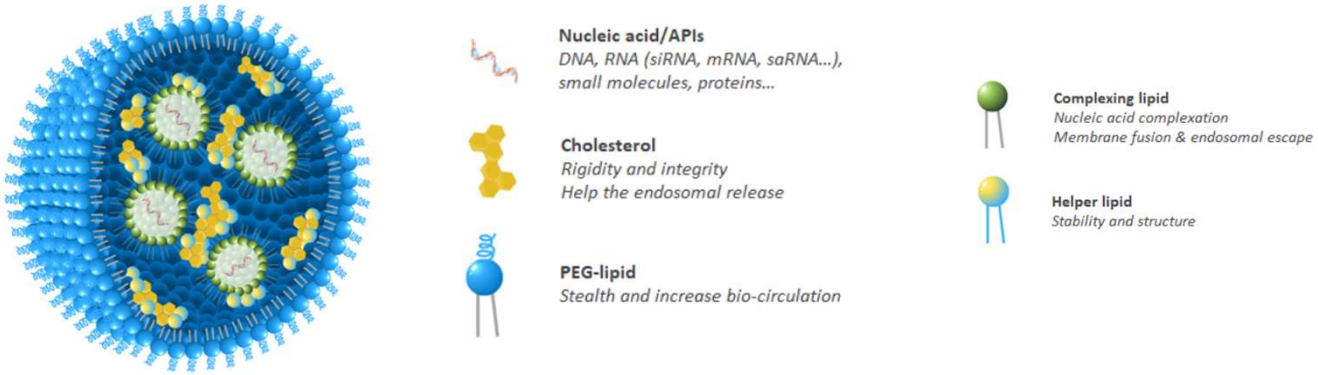
Lipid Nanoparticles: Delivery Vehicle for in vivo Gene Editing



- **Efficient delivery:** Protect cargo from rapid degradation and help them get into cells.
- **Biocompatibility:** lipids (fats), generally well tolerated by the body.
- **Controlled release:** structure can be tweaked so the cargo is released at the right time and place.
- **Scalability:** Can be produced cell-free and scaled relatively quickly for new therapeutic designs (e.g., vaccines)
- **Versatility:** Deliver multiple components together (e.g., Cas9 mRNA + gRNA in one package)
- **Low immunogenicity:** Lower immunogenicity compared to viral vectors
- **Non-viral:** avoids permanent genome integration risk of viral vectors

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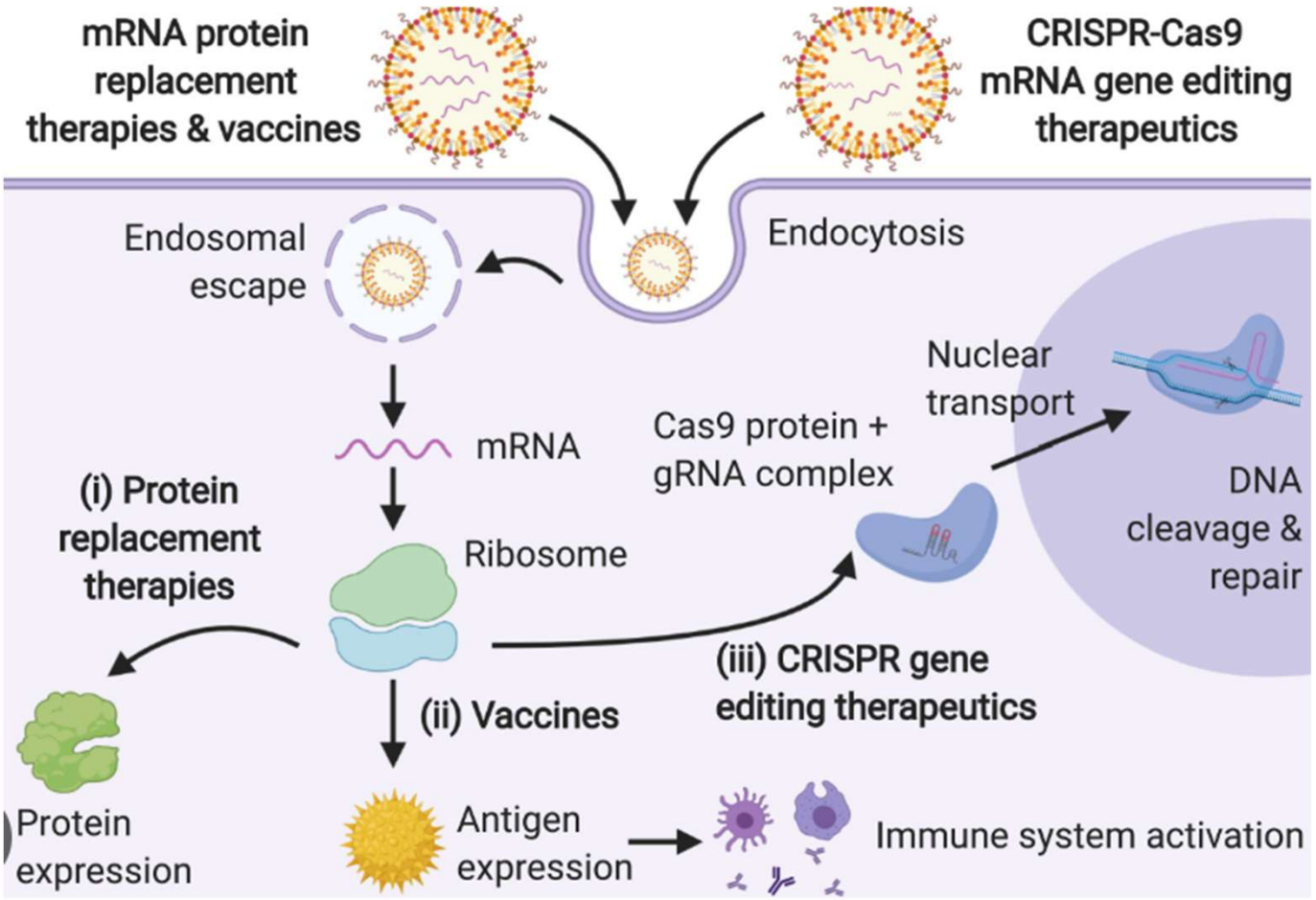
OzBiosciences (2025) https://ozbiosciences.com/340-lipid-nanoparticle-lnp.

LNP Therapeutic Delivery Applications



Swingle et al. (2021) Trends in Molecular Medicine 27(6), P616-617.

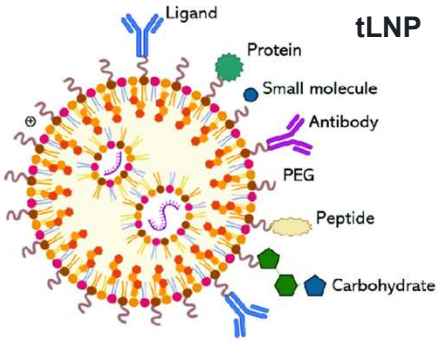
tLNPs for Extrahepatic Targeting

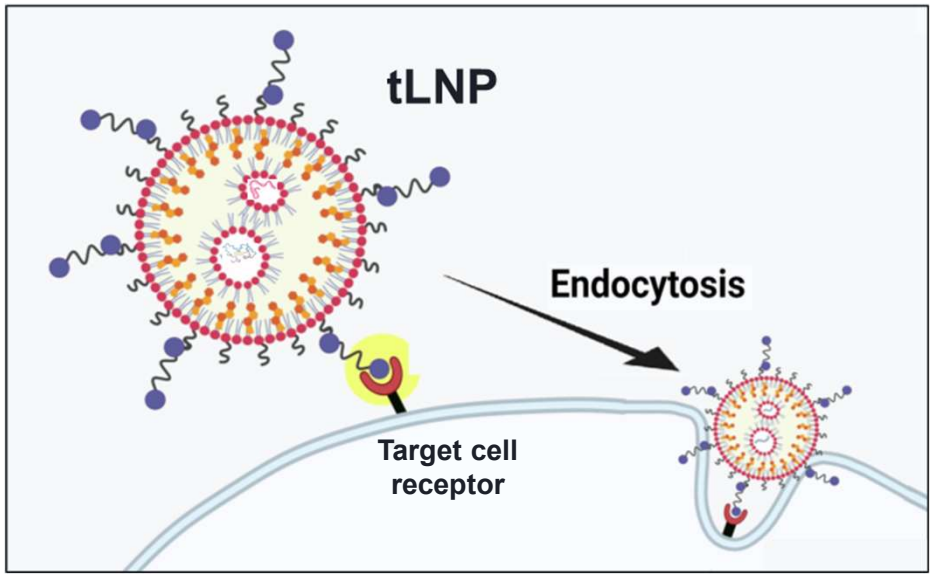
- LNPs shine for **liver-targeted** GE therapies (biology & design) (metabolic & rare genetic liver disorders)
- Many disease targets require extrahepatic delivery (e.g., to bone marrow, muscle, brain, or lungs)

One solution: decorating LNPs with ligands that bind to specific receptors on target cells

- Requires optimization of:
  - Linkers, ligand affinity & density
  - tLNP assembly/conjugation chemistry)

- Requires development of tLNP-specific analytics for:
  - Targeting components
  - Conjugation efficiency, binder density
  - Binding competence of targeting moiety

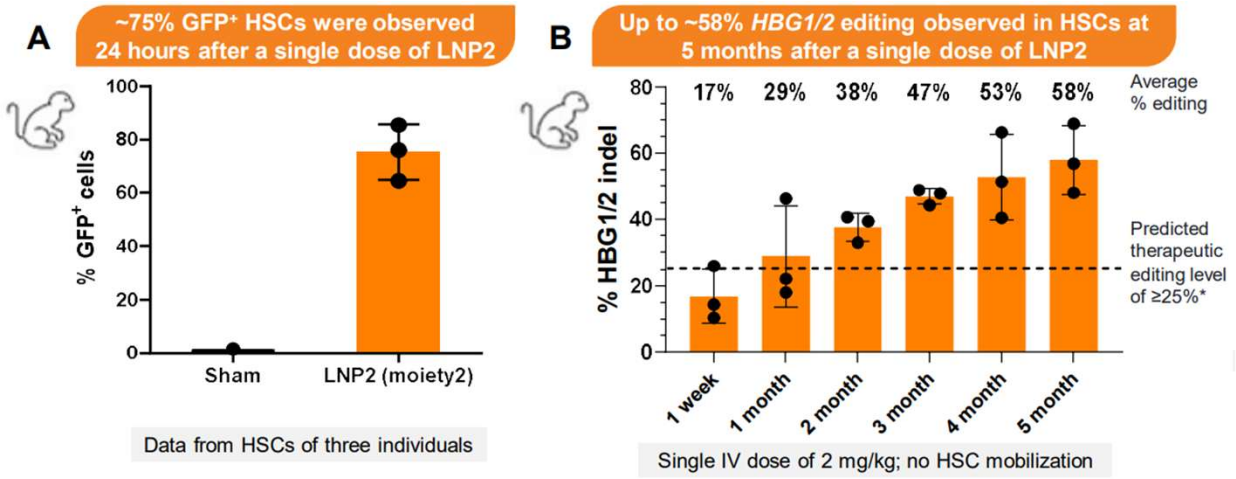


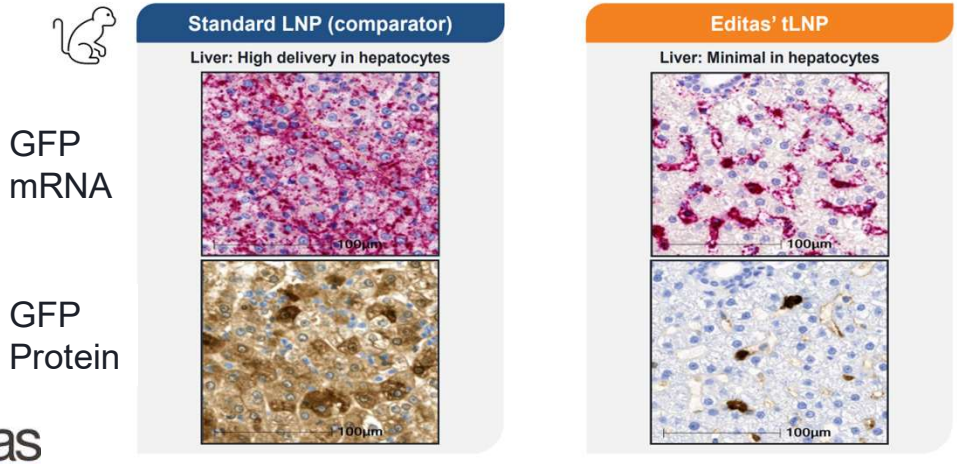


Dinh et al. (2024) Applied Nano 5, 143-161

tLNP Delivery – Recent Progress

tLNP delivery in NHP enables in vivo HBG1/2 promoter editing for β-hemoglobinopathies

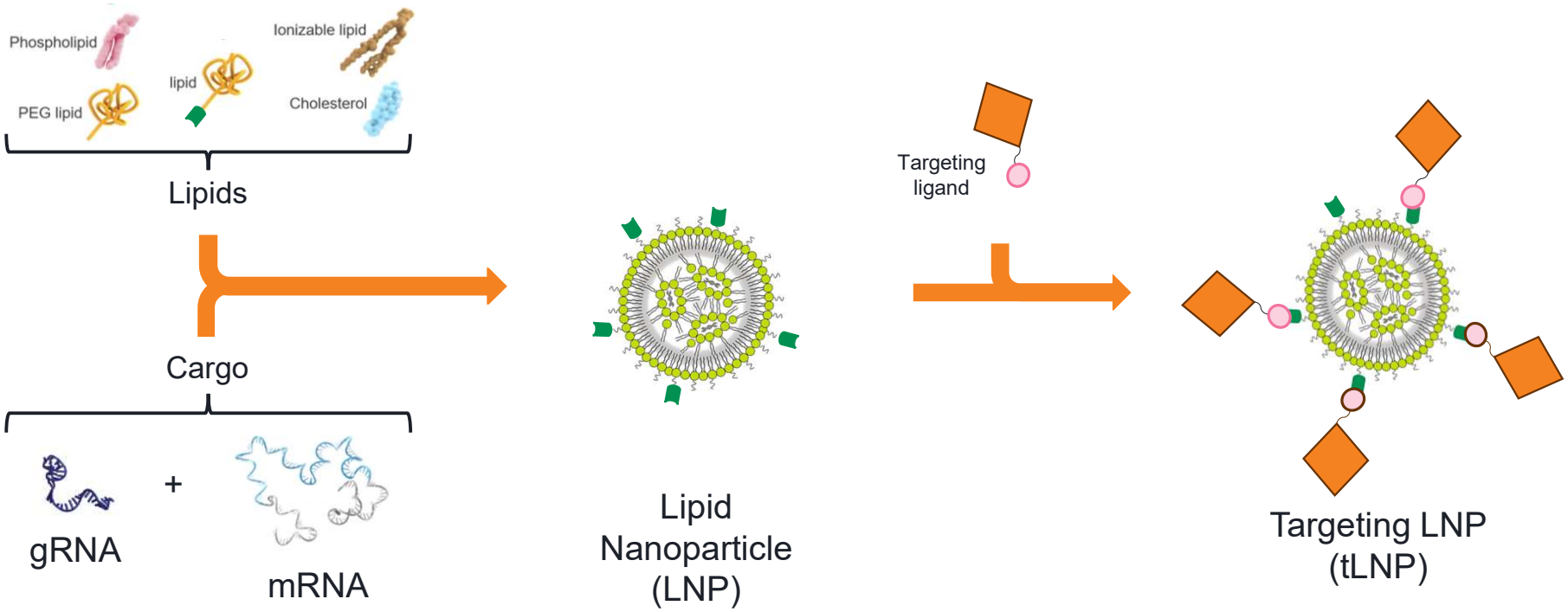




Biodistribution in NHPs shows significant de-targeting of the liver vs. standard LNPs

Gupta EHA 2025

tLNP Analytics – a Broad, Complex Landscape



tLNP: complex assembly of many components which all need characterization, in-process and/or release testing

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Regulatory Considerations

Limited Guidance

- This field is new & rapidly advancing
  - we are building the plane as we are trying to fly it
  - we need to work together with the agencies, opportunity to advise/educate, establish credibility
- Specific analytical guidance not yet in the ICH guidelines, but some guidance available
  - WHO Guidance for Vaccines
  - Human Gene Therapy Products Incorporating Human Genome Editing (Draft Guidance, MAR2022)
  - Potency Tests for Cellular and Gene Therapy Products (JAN2011)
- Consortia/white papers
  - DIA Consortium for oligonucleotides (Dan Capaldi et al.)
  - USP guidance for mRNA/LNP (v2 out June 2023)
  - BioPhorum guidance for mRNA/LNP (v1 out July 2023)
  - NIST Gene Editing Consortium

WHO, World Health Organization

Cargo Analytics

pDNA

Purity	A_{260}/A_{280}, CE, PAGE
Concentration	A_{260}
% SC	CE, HPLC

Linear pDNA

Linearization efficiency	CE

mRNA

Identity	PCR, Sanger, NGS
Purity % Full Length % capped polyA n, dist	 CE, HPLC HPLC-MS HPLC-MS
Impurity DNA temp. NTP Enzymes Solvents dsRNA Endotoxin	 PCR AEX ELISA GC-FID ELISA LAL
Potency	Case-by-case
Strength	UV/Vis, PCR

gRNA

Identity	Sanger, NGS LC/MS
Purity % FLP	 IP-RP-HPLC
Impurity % impurities Solvents Endotoxin	 IP-RP-HPLC GC-FID LAL
Activity	Spectroscopy
Strength	UV/Vis

AEX: Anion exchange chromatography
ELISA: enzyme-linked immunosorbent assay
GC-FID: Gas chromatography-flame ionization detection
LAL: Limulus amebocyte lysate

CE: capillary electrophoresis
PAGE: Polyacrylamide gel electrophoresis
HPLC: high performance liquid chromatography
IP-RP: Ion-pairing reverse phase

PCR: Polymerase chain reaction
NGS: Next-generation sequencing
MS: mass spectrometry
UV/Vis: Ultra-violet/visible

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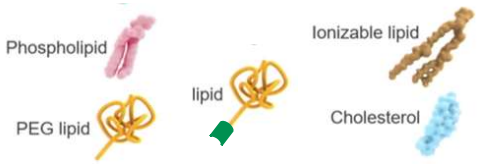
Lipids and Targeting Ligand Analytics



Lipids

Identity	HPLC-CAD, HPLC-MS LAL
Purity	
Impurity %impurities Endotoxin	
Strength	

Ligand

Identity	MS
Purity	HPLC
Impurity %impurities Endotoxin	HPLC LAL
Binding affinity	Case-by-case
Strength	UV/Vis

CAD: charged aerosol detection

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tLNP Analytics

Attribute Category	Attribute	Method
General	Appearance	Visual Evaluation
	pH	USP <791>
	Osmolality	USP <785>
	Sub-Visible Particles	USP <788>
	Extractable volume	USP <1>
Safety	Endotoxin	USP <85>
	Sterility	USP <71>
Identity	mRNA Identity	Sequencing
	gRNA Identity	Sequencing
	Ligand Identity	HPLC-MS
	Lipid Identity	HPLC-CAD

Attribute Category	Attribute	Method
Content / Strength	Total RNA Concentration	Ribogreen
	Total Lipid Concentration	HPLC-CAD
	gRNA/mRNA Ratio	IP-RP-HPLC
Purity	%Encapsulation	Ribogreen
	mRNA Integrity/Purity	CE
	gRNA Integrity/Purity	IP-RP-HPLC
	Zeta potential	ELS
	Particle Size	DLS
	Polydispersity	DLS
	Residual Solvents	GC-FID
	Unconjugated Ligand	HPLC-MS
	Conjugation Efficiency	HPLC-MS
Potency	Binding Competency	TBD
	Biochemical Activity in Target Cells	TBD

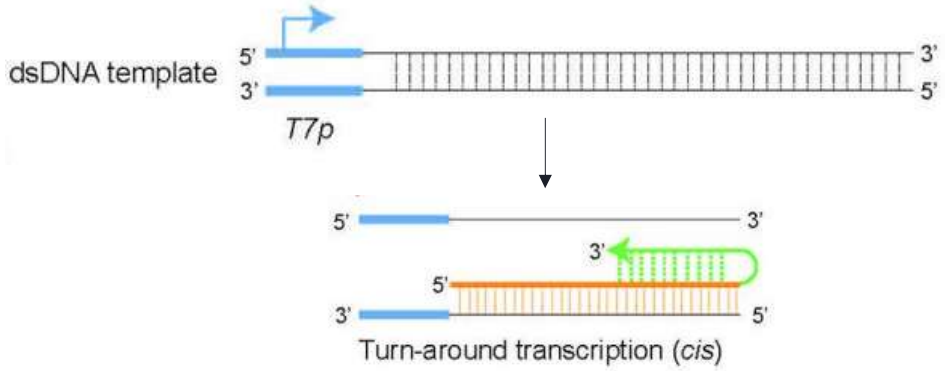
ELS: electrophoretic light scattering
DLS: Dynamic light scattering

tLNP Analytical Challenges & Opportunities

Double-stranded RNA Quantitation

Background

- dsRNA is immunogenic to cells and is undesirable in therapeutics.
- dsRNA is an impurity/by-product of IVT
  - Turn-around transcription generated late in transcription reaction
  - Turn-around transcription products are difficult to remove chromatographically
- Amount of dsRNA generated by in vitro transcription varies greatly based on:
  - template sequence and length
  - enzyme concentrations
  - buffer conditions
- Reduction during transcription is better than downstream purification
- Accurate quantitation of dsRNA is needed



Assay Options:

- Dot blot
- Ab assays (ELISA)
- Acridine orange assays
- HTRF – based assays
- MDA5-ATPase assay
- Cell-based assays (activation of immune response)
  - TLR or IFN receptor-based

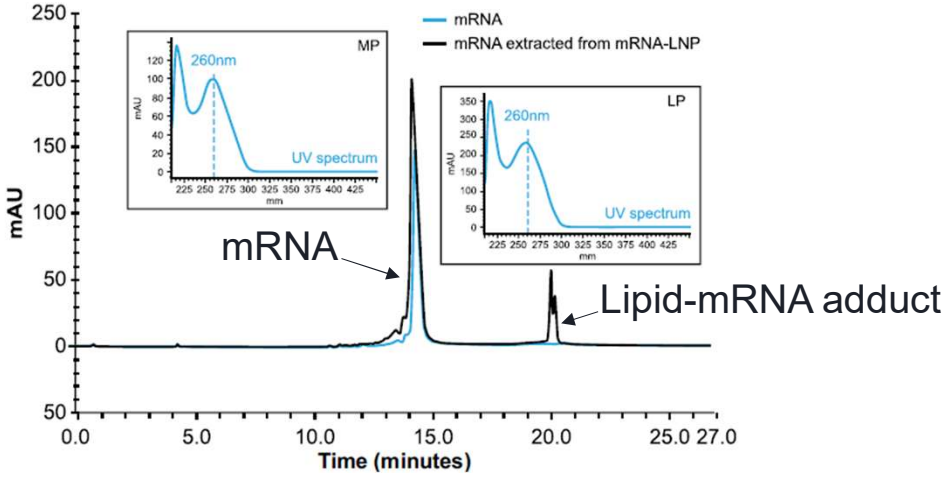
https://www.genengnews.com/resources/understanding-and-overcoming-the-immune-response-from-synthetic-mrnas-2/

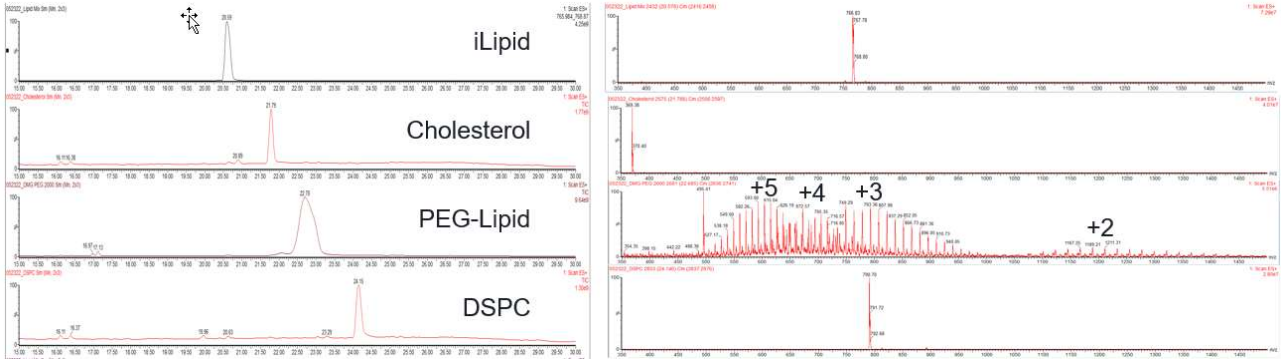
HTRF: Homogeneous Time Resolved Fluorescence
TLR: Toll-like receptor
IFN: interferon
IVT: in-vitro transcription

Lipid Impurity/Degradant Quantitation

Background

- The Moderna story (Packer et al., 2021)
  - Formation of covalent lipid-mRNA adduct from lipid impurity
- Upfront impurity/degradant characterization via LC/MS (and/or LC-CAD)





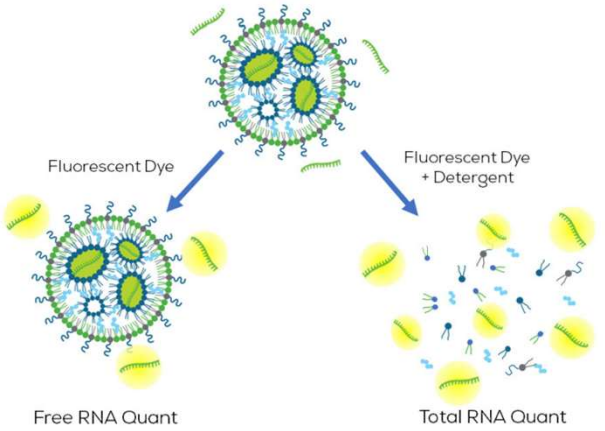
LC-ELSD/CAD and MS analysis of lipids

Packer et al. (2021) Nature Comms. 12, 6777.

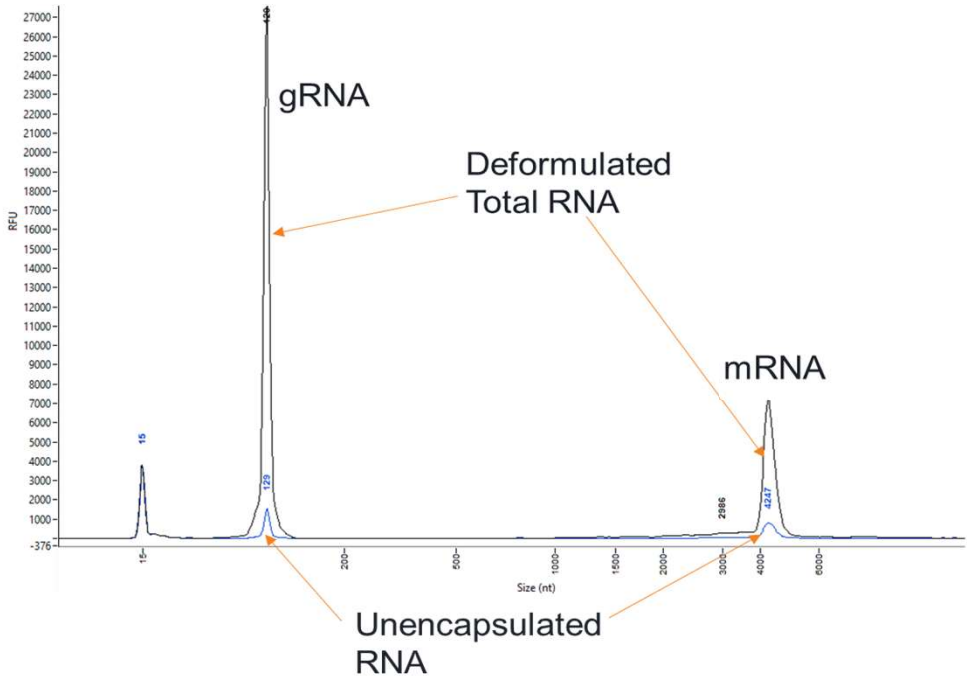
Encapsulation Efficiency (EE) and RNA ratio (CRISPR Cargo)

Background

- Current standard is the Ribogreen assay
- Fluorescent dye that binds nucleic acid
- Measure intact LNP to get Free RNA, then deformulate to get Total RNA
  - Encapsulated RNA = Total RNA – Free RNA
  - %EE = Encapsulated RNA/Total RNA
- Does not tease out gRNA from mRNA (CRISPR)
  - Can be done via UPLC or CE



Capillary electrophoresis allows to differentiate between % encapsulation of gRNA and mRNA



https://www.unchainedlabs.com/wp-content/uploads/2024/05/Skip-the-mess-with-dye-free-LNP-payload-quant-on-Stunner_RevA.pdf

Potency Determination

Potency Assays

- Four levels to measure potency
  - editing (on target)
    - Genomic sequencing
  - mRNA
    - RNA-seq, Fluorescent In Situ Hybridization (FISH)
  - protein
    - MS or epitope-based assays (e.g., ELISAs)
  - phenotype
    - Case-specific assays
    - e.g., for sickle cell therapies, reduction of RBC sickling
    - Regulators typically prefer this data, but not always feasible (MOA not understood and/or assay doesn't exist)
- Develop phase-appropriate assay

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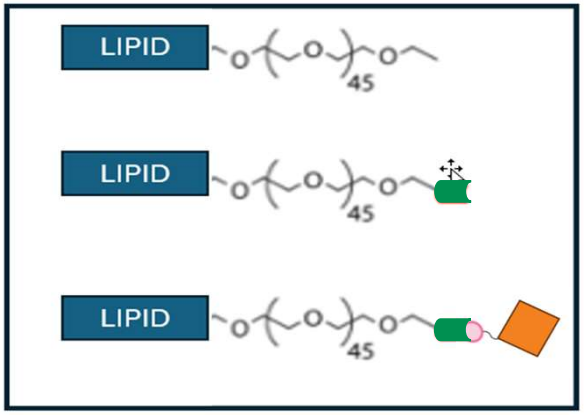
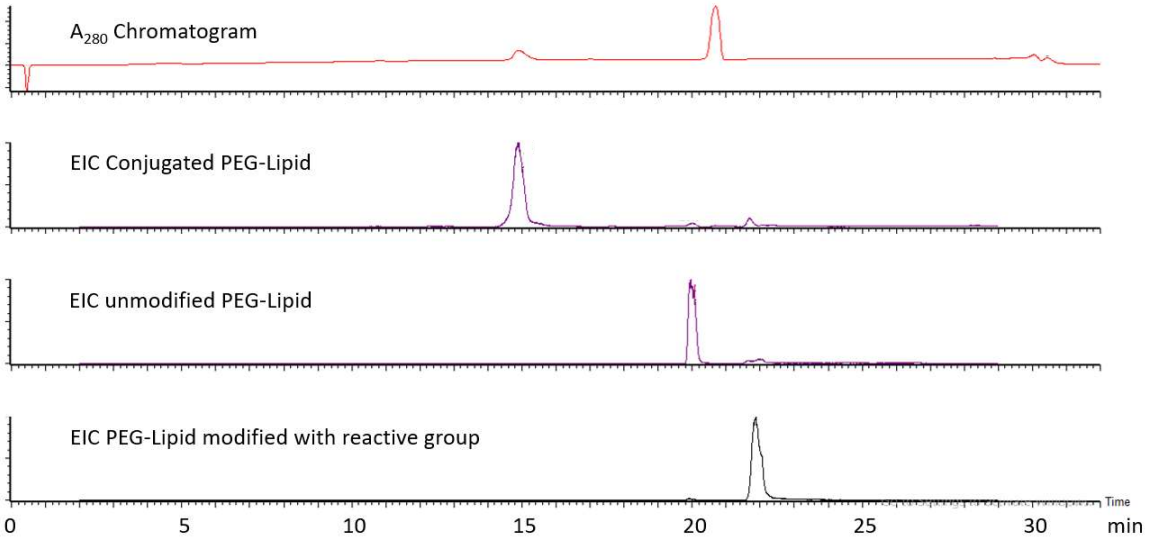
Giannoukos et al. (2018) BMC Genomics 19(1), 212.

Ligand Conjugation Efficiency

Characterization of Conjugation

- LCMS assays for % conjugation (may require a de-formulation step)
  - Pre-conjugation:
    - Unmodified PEG lipid
    - Modified PEG lipid for conjugation reaction
  - Post-conjugation:
    - Unmodified PEG lipid
    - Modified PEG lipid for conjugation reaction
    - Conjugated PEG lipid
    - Also, residual unconjugated moiety (impurity)
  - Note:
    - LC/MS method gives average over distribution
    - Is more granular data needed?



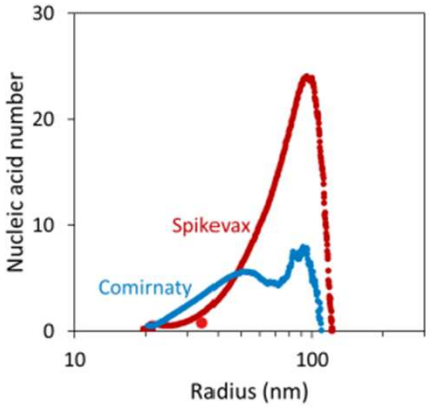


Wolk (2024) OPT Congress

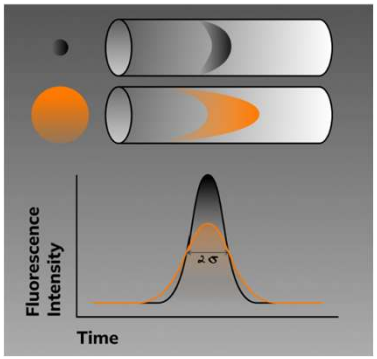
tLNP Areas for Additional Development - 1

Development of LNP Assays

- Size distribution
  - AF4-MALS for more granular size-based analysis (content vs. size)
  - TRPS: tunable resistive pulse sensing (true statistical size distribution)
- Assessment of empty LNPs
  - AF4 –MALS
- Binder density
  - NanoFCM
  - FIDA/NanoTemper (average binder density)
- Binding competency
  - FIDA/NanoTemper



Average number of mRNA per LNP as a function of LNP size by aF4-MALS

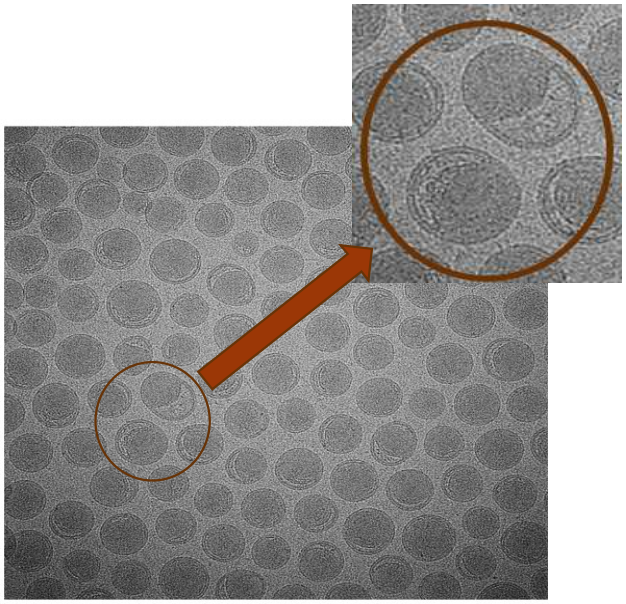


FIDA: binding detected as change in hydrodynamic radius

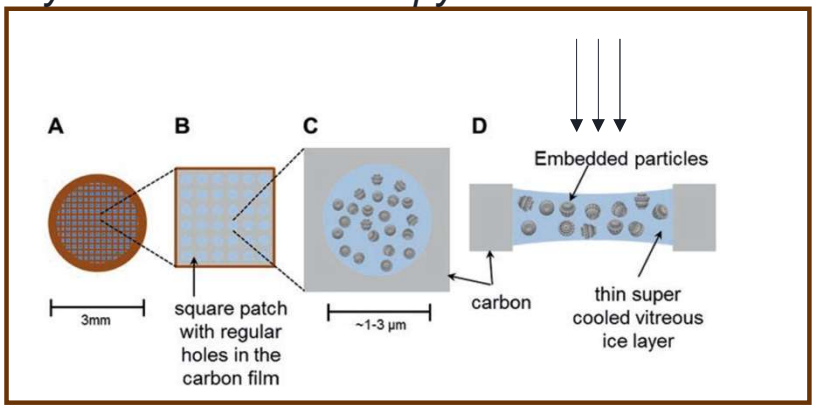
Kurnik Waters App Note AN2615
FIDA: flow induced dispersion analysis (www.fidabio.com)
AF4 - asymmetric flow field flow fractionation

tLNPs: Areas for Additional Development - 2 Morphology Assessment

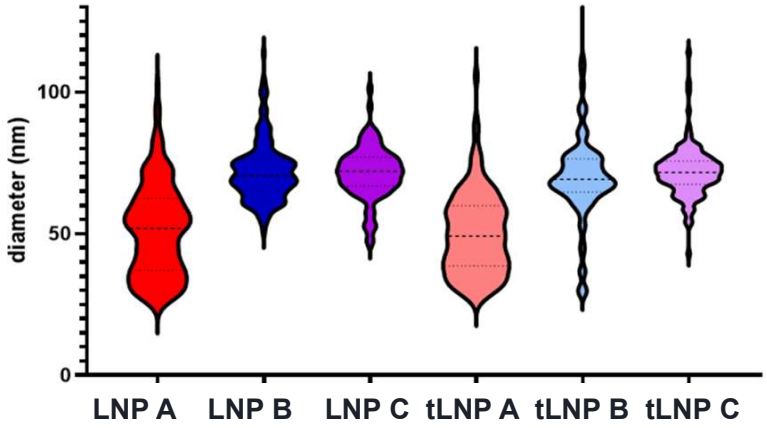
Shape/Morphology
(e.g., elliptical shapes, blebbing)



Cryo-Electron Microscopy



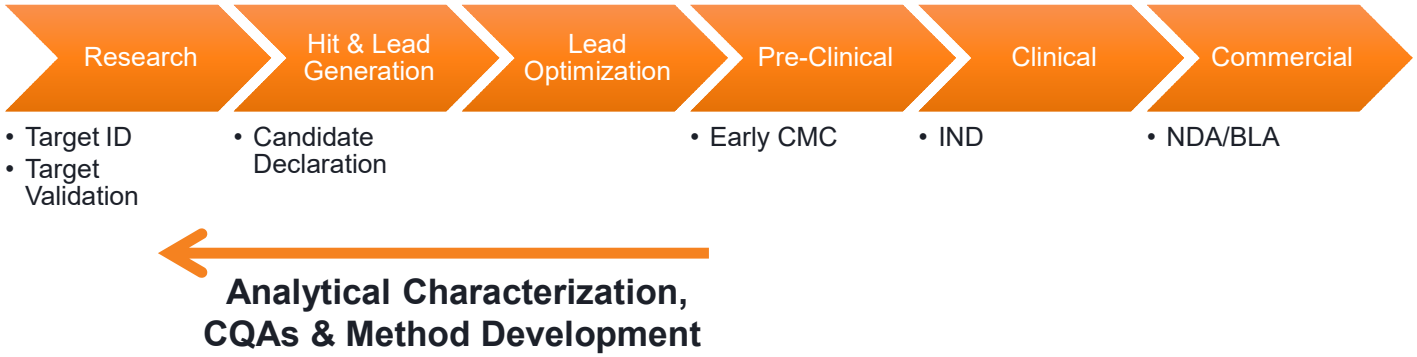
Changes Across Size Distribution



Orlova et al. 2017. Methods in Molecular Biology, Quentin et al. 2018. Journal of Molecular Medicine. Hok Yan Chen et al. Adv. Mat. 2023 Wolk (2024) mRNA QC and Compliance Summit

Final Thoughts

1. Analytical front-loading:
   - Early method development & qualification
   - Thorough product characterization
   - Critical quality attribute (CQA) definition and monitoring
   - Risk assessment and mitigation
   - Process understanding & efficiency in scale-up
   - Regulatory alignment (data packages)



2. As an emerging modality, tLNPs lack widely established analytical capabilities across most CTOs/CDMOs.

Acknowledgments

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- Alexis Burns
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Thank you for your attention!

Questions?