Non-viral Delivery of Prime Editing (PE): Analytical Tools for Testing and Characterizing PE RNAs-LNP and Case Studies

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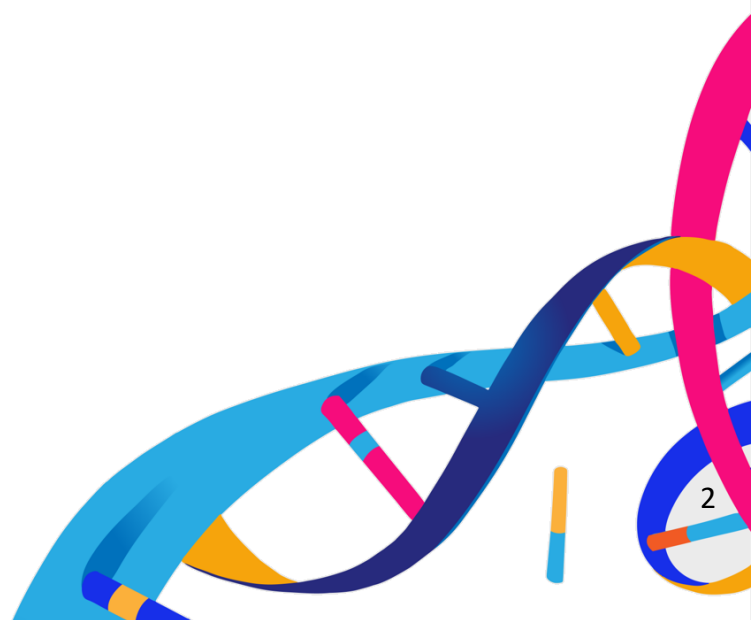
On behalf of the team at Prime Medicine

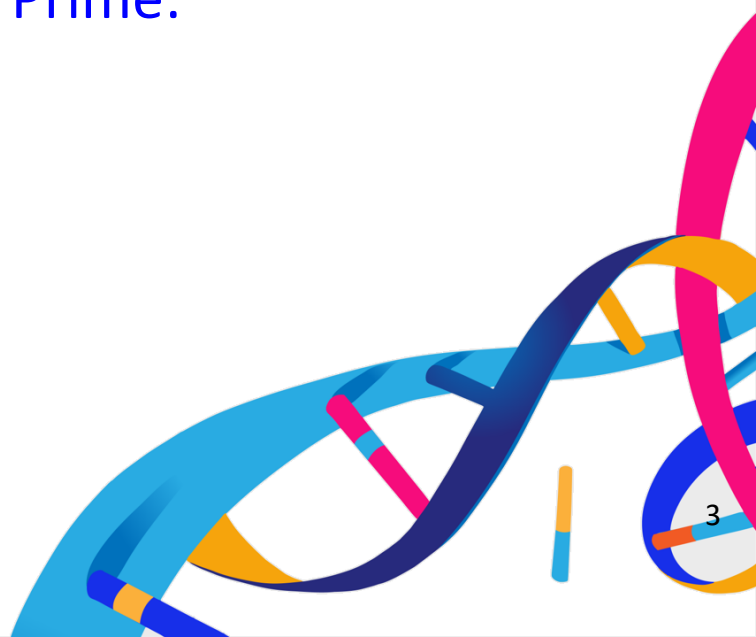
Disclosures

- Minjia Wang declares he is a current employee of Prime Medicine, Inc. and owns equity in Prime Medicine



Agenda

1. Gene therapy using LNP delivery of Prime Editing (PE)
2. LNP characteristics and attributes
3. Analytical methods available for PE LNP drug products
4. Analytical assays supporting LNP drug products developed by Prime:
    a. LNP lipid component ratio analysis
    b. LNP payload integrity and component ratio analysis



Lipid Nanoparticles (LNP) – A Versatile Delivery Method

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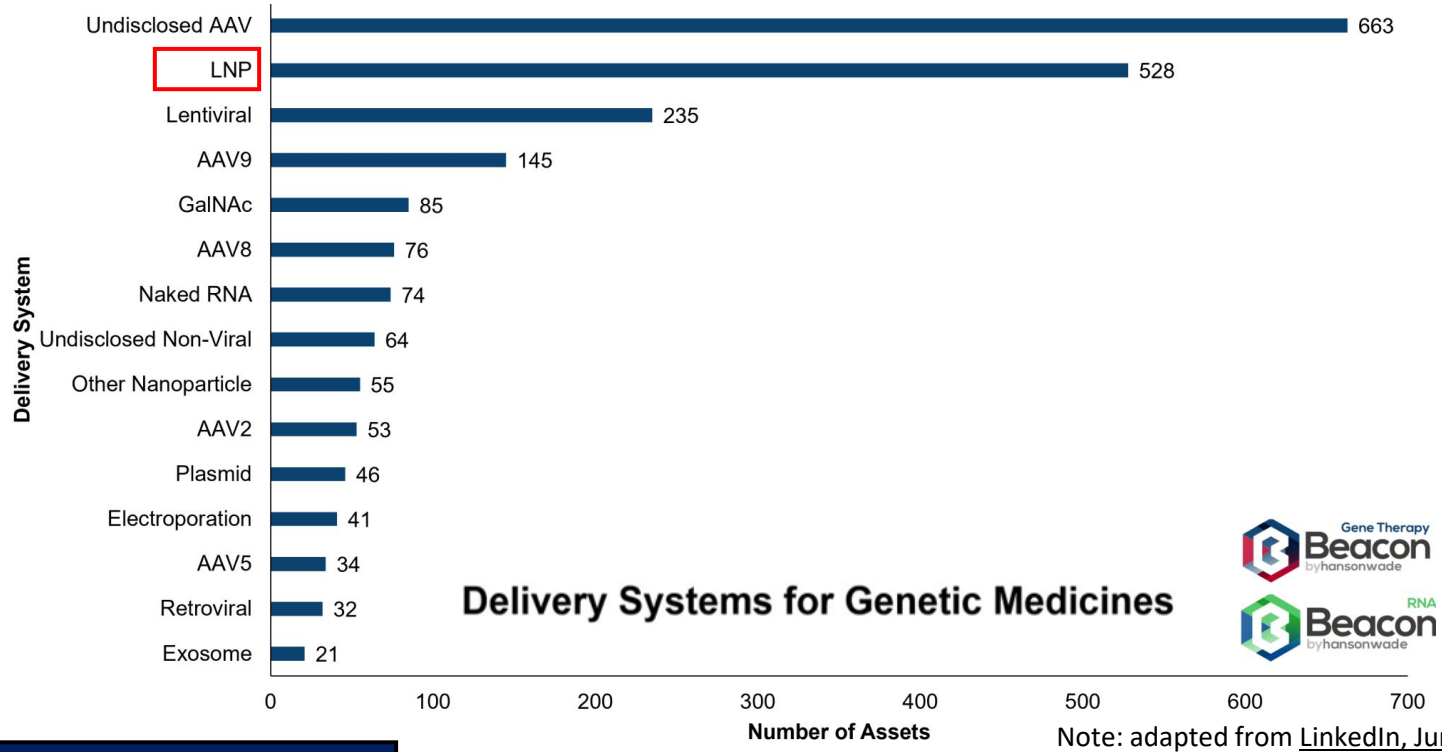
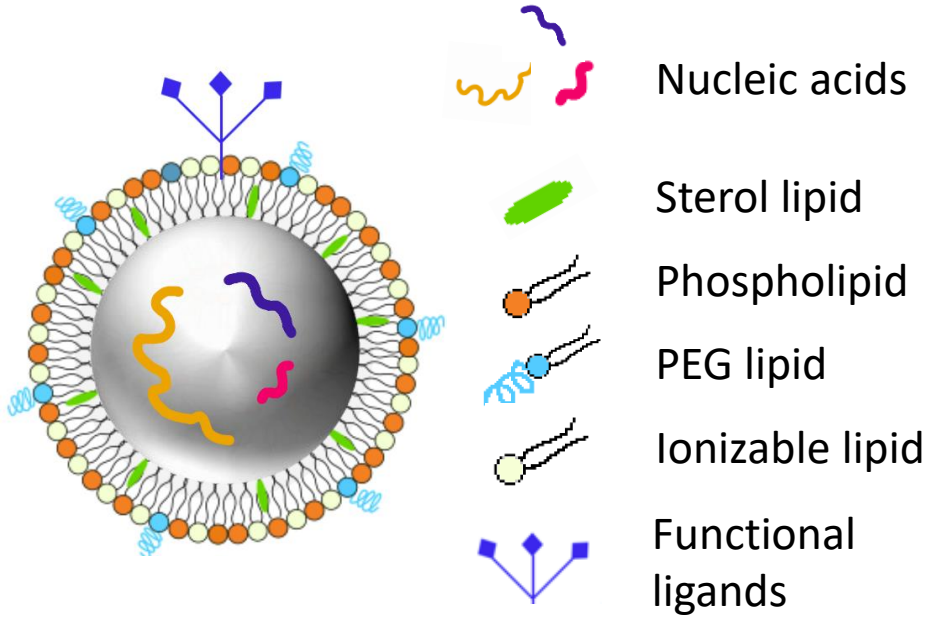
- LNP with ionizable lipid could effectively encapsulate nucleic acids with increased stability, enable CRISPR-based gene editing both *ex vivo* and *in vivo*

- Major advantages: tunable, tissue/cell targeting potential, versatile, scalable, and no genome integration compared with viral gene delivery





Note: adapted from LinkedIn, June 2023 posted by Beacon

Prime Editing: A Precise Gene Editing Technology That Is Programmable for Both Search and Replace

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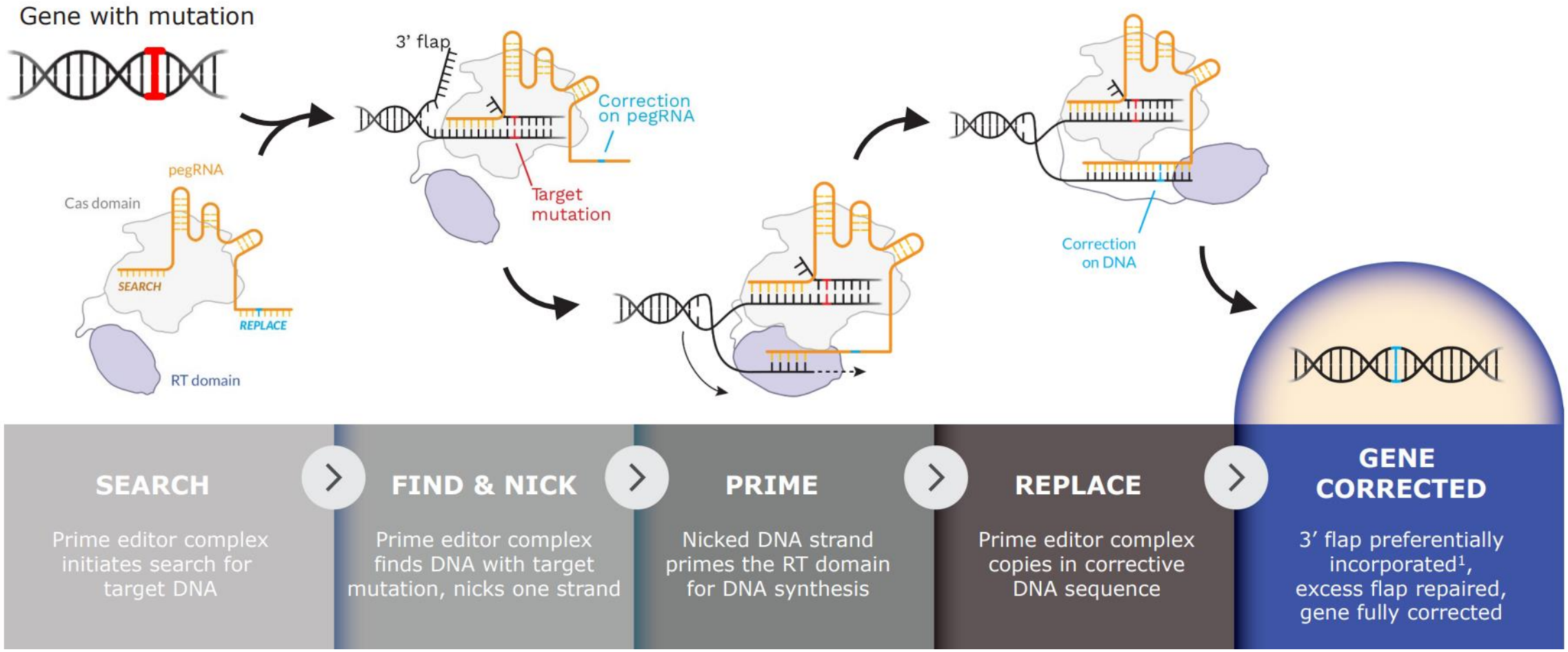
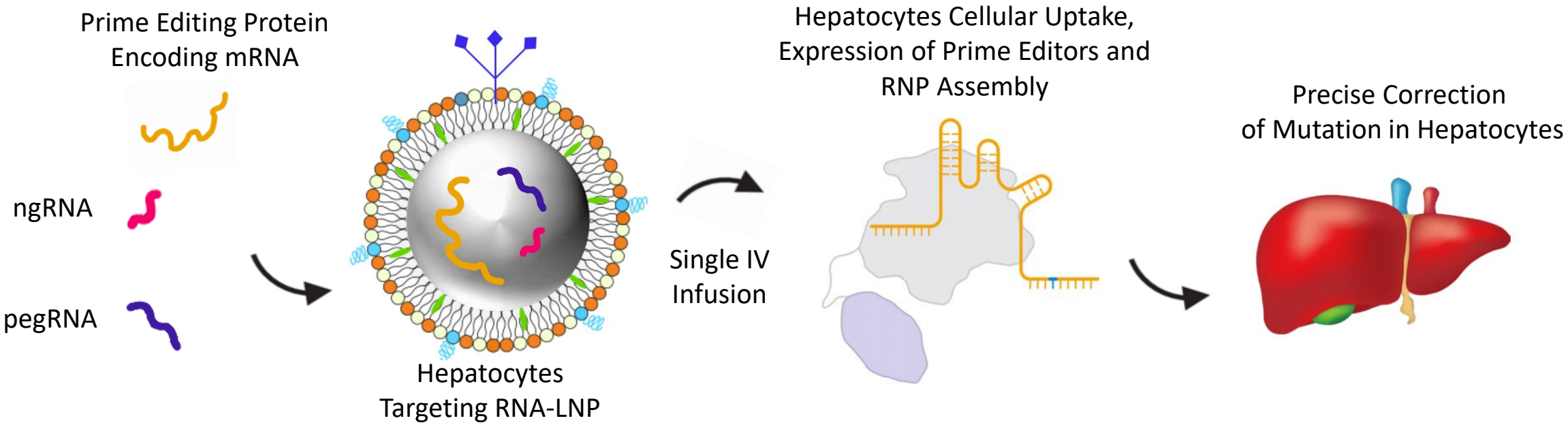
- Prime Editing can deliver a one-time curative therapy that has the potential to address a large percentage of genetic variants associated with disease



pegRNA: prime editing guide RNA; RT: reverse transcriptase; Cas: CRISPR associated protein;
Anzalone, et al (David R. Liu). Search-and-replace genome editing without double-strand breaks or donor DNA. Nature, 2019.

Therapeutic Approach: LNP-mediated Targeting Delivery of Prime Editing to Liver

- Prime Medicine is developing a universal liver targeting RNA-LNP delivery platform that has the potential to expedite development of Prime Editing therapeutics
- Prime Editing can be used to new liver indication by just swapping the guide RNAs in the modular universal LNP and leveraging certain CMC, off-target and PharmTox packages across programs
- LNP co-delivers 3 Prime Editing components: pegRNA, ngRNA, and PE encoding mRNA



RNP: ribonucleoprotein; IV: intravenous; DP: drug product; ngRNA: nicking guide RNA; PE: prime editing;

Current Prime Medicine Pipeline Aims to Leverages the Versatility and Breadth of Prime Editing

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- Universal liver targeting RNA-LNP delivery platform to be used for different liver indications

STRATEGIC CATEGORY	TARGET TISSUE	INDICATION	DELIVERY	DISCOVERY	LEAD OPTIMIZATION	IND-ENABLING	Phase 1/2
IMMEDIATE	BLOOD	Chronic Granulomatous Disease	*ex vivo*				
		Fanconi Anemia	*ex vivo*				
	LIVER	Wilson's Disease	LNP				
		Glycogen Storage Disease 1b	LNP				
	EYE	Retinitis Pigmentosa/Rhodopsin	AAV				
		Retinitis Pigmentosa/Usher Syndrome	AAV				
	EAR	Usher Syndrome Type 3	AAV				
		Non-Syndromic Hearing Loss – GJB2	AAV				
DIFFERENTIATION: REPEAT EXPANSION DISEASES	NEURO-MUSCULAR	Friedreich's Ataxia	AAV				
		Myotonic Dystrophy Type 1	viral/non-viral				
		Amyotrophic Lateral Sclerosis	viral/non-viral				
		Oculopharyngeal Muscular Dystrophy	LNP				
		Fragile X Syndrome	viral/non-viral				
		Huntington's Disease	TBD				
	EYE	Fuchs' Endothelial Corneal Dystrophy	viral/non-viral				
DIFFERENTIATION: OTHER	MUSCLE	Duchenne Muscular Dystrophy	AAV				
	LUNG	Cystic Fibrosis	LNP				
PARTNERED PROGRAMS	BLOOD	Sickle Cell Disease (Beam Therapeutics)	*ex vivo*				

Analytical Assays Used for LNP Delivered PE Drug Product to the Liver

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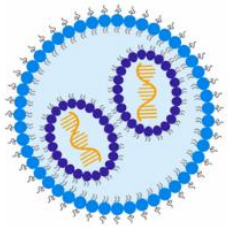
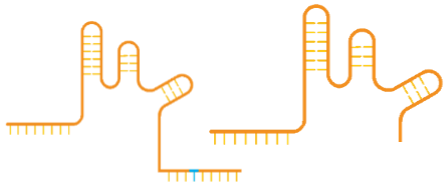
LNP delivered PE DP to liver



Safety/ General	Identity	Strength
Purity	Impurity	Potency

pegRNA/ngRNA



Safety/ General	Identity	Strength
Purity	Impurity	Potency

PE mRNA

Safety/ General	Identity	Strength
Purity	Impurity	Potency

- Battery of assays required for release, stability and characterization
- Many assays for gRNA, mRNA, and DP are developed as platform assays to support multiple programs
- Product specific potency assays are being developed early, where possible, to streamline CMC and clinical data correlation

Lipid Nanoparticles (LNP) – Characteristics and Analytical Tools

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- Important attributes allow for the characterization of LNP based therapeutics

LNP specific attributes:

- Morphology (CryoTEM, SAXS, SANS, etc.)
- Biophysical: size and PDI (DLS, FFF/SEC-MALS, nano flow cytometer, etc.), surface charge, pKa
- Payload encapsulation efficiency
- Lipid (**LC-MS/ELSD/CAD**) and cargo (**IP-RP-LC-UV**, **CGE**/FA, NGS/ddPCR) ID, integrity, quantity

Other attributes:

- Safety: endotoxin, sterility, etc.
- Process related impurities
- Potency/functional activity
- Compendial: appearance, osmolarity, pH, etc.
- Stability

TEM: Transmission electron microscopy; DLS: Dynamic light scattering; SAXS: Small-angle X-ray scattering
SANS: Small-angle neutron scattering; SEC-MALS: Size exclusion chromatography-multi-angle light scattering;

PDI: Polydispersity; FA: Fragment analyzer; CAD: Charged aerosol detector
ELSD: Evaporative light scattering detector; NGS: Next gen sequencing

LNP Composition/Lipid Ratio Analysis Using RP-UPLC-ELSD/MS

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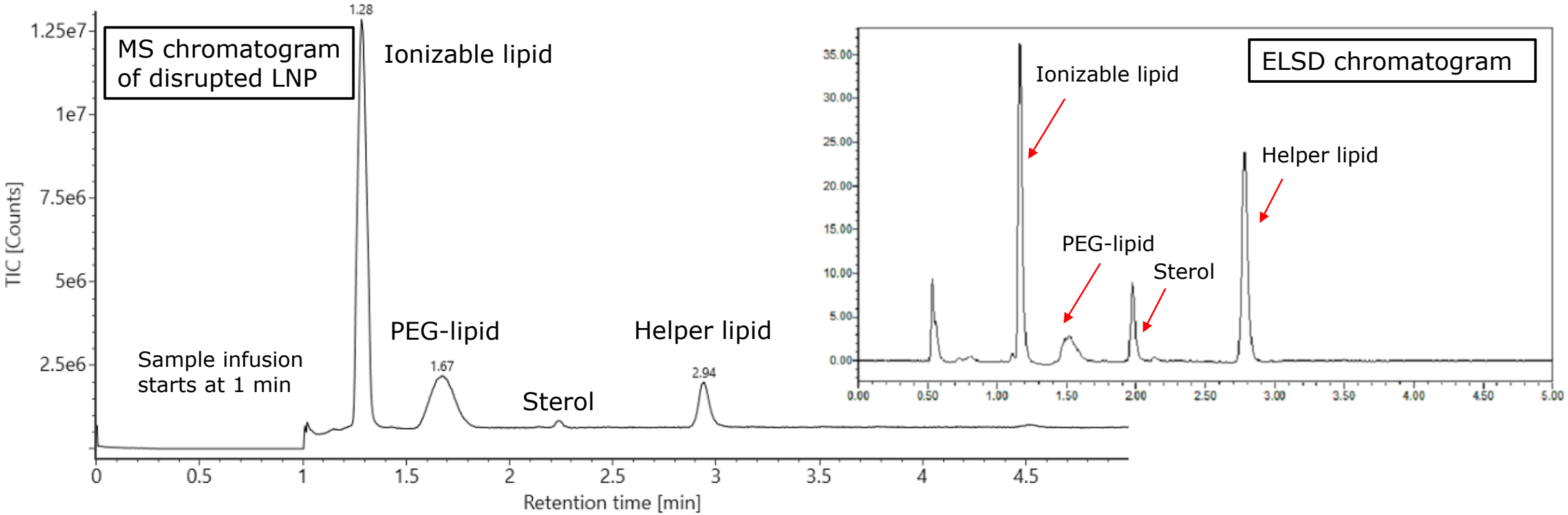
- Important parameters: diversity in lipid components, chromatography mode, sample preparation, etc.
- Fast (5 min run), sensitive, and robust reversed-phased (RP) LC method using both ELSD and MS (Tof).
- Well resolved peaks without matrix interference.



LNP Composition/Lipid Ratio Analysis Using RP-UPLC-ELSD/MS

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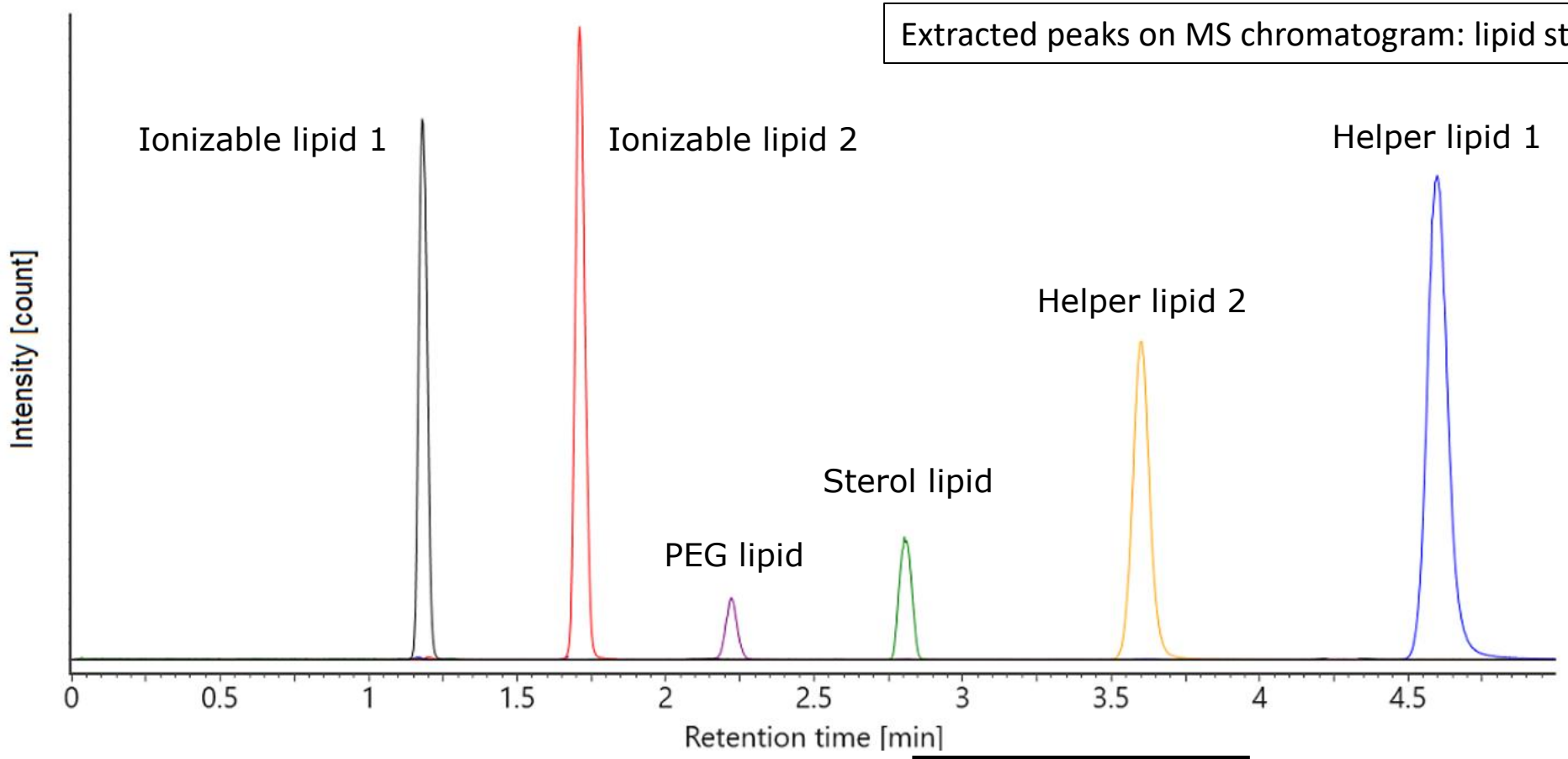
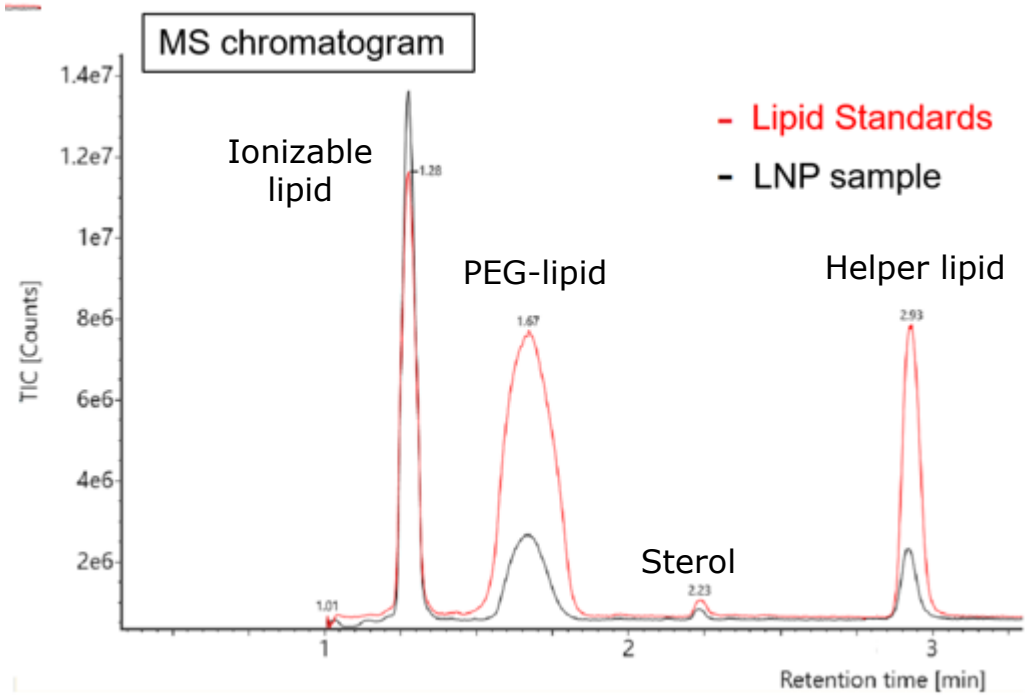
- Optimized LC conditions that can easily accommodate different lipid components, ratios, and concentrations in different LNP samples

Extracted peaks on MS chromatogram: lipid standards mixture



LNP Composition/Lipid Ratio Analysis Using RP-UPLC-ELSD/MS

- Accurate quantification using both ELSD and MS detector for LNP lipid ratio analysis with high consistency to expected values

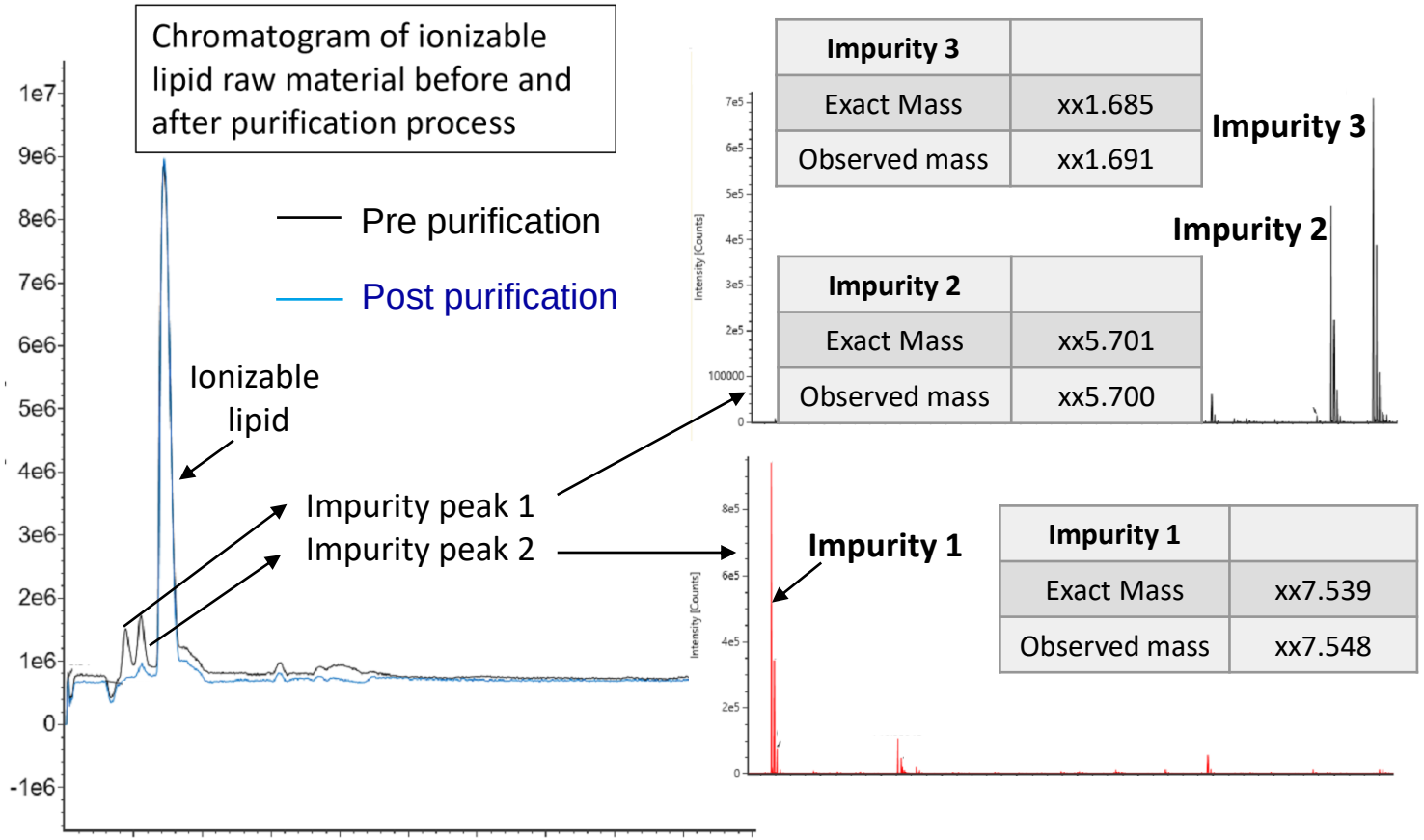


Representative lipid ratio analysis in disrupted LNP sample

Component	Ionizable lipid	Helper lipid	Sterol	PEG-lipid
Normalized expected lipid concentration (%)	100	100	100	100
Actual molar concentration as % of expected value	99.2	104.6	98.3	109.0

Impurity/Purity Analysis in Lipid Raw Material Using LC-MS

- Successful separation, structural identification, formation mechanism, and relative quantification of impurities in raw material (ionizable lipid) before and after purification



Impurity 3	
Exact Mass	xx1.685
Observed mass	xx1.691

Impurity 2	
Exact Mass	xx5.701
Observed mass	xx5.700

Impurity 1	
Exact Mass	xx7.539
Observed mass	xx7.548

Formation mechanism of impurities

Samples	Formation mechanisms
Impurity 1	Degradant of ester bond hydrolysis
Impurity 2	Process related byproduct
Impurity 3	Process related byproduct

Samples	Normalized impurity 1 concentration	Normalized impurity 2 concentration	Normalized impurity 3 concentration
Pre-purification	100 %	100 %	100 %
Post-purification	0%	13.3 %	22.8 %

RNAs Payload Analysis: Impurity Analysis with CGE and RNA Quantification/Ratio Analysis with IP-RP-HPLC-UV

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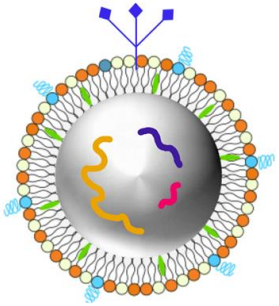
- Optimized workflow for sample preparation and payload analysis

RNA-LNP formulation



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Disruption of LNP

Mix, vortex and incubate

Capillary gel electrophoresis (CGE)

Ion paring-reversed phase-HPLC-UV (IP-RP-HPLC-UV)

High Resolution and Sensitivity Integrity Analysis of the Prime Editing Components in LNP with Capillary Gel Electrophoresis

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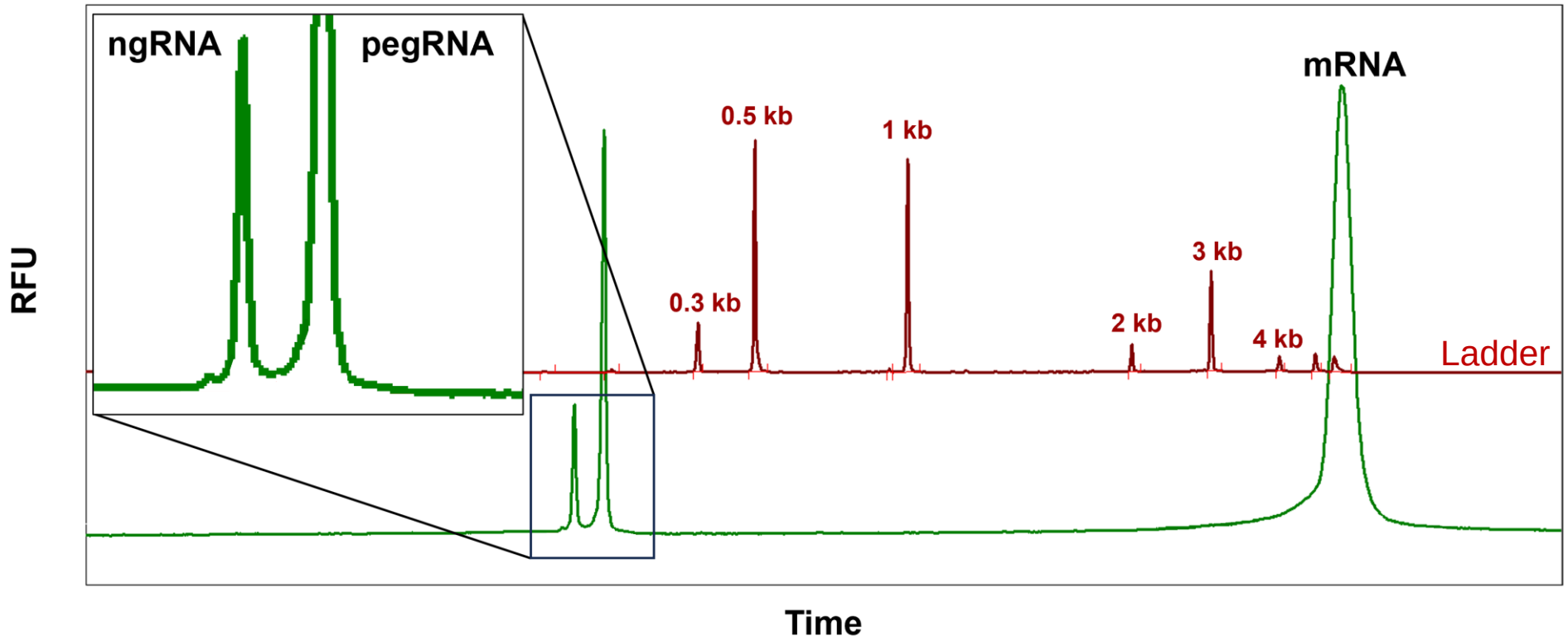
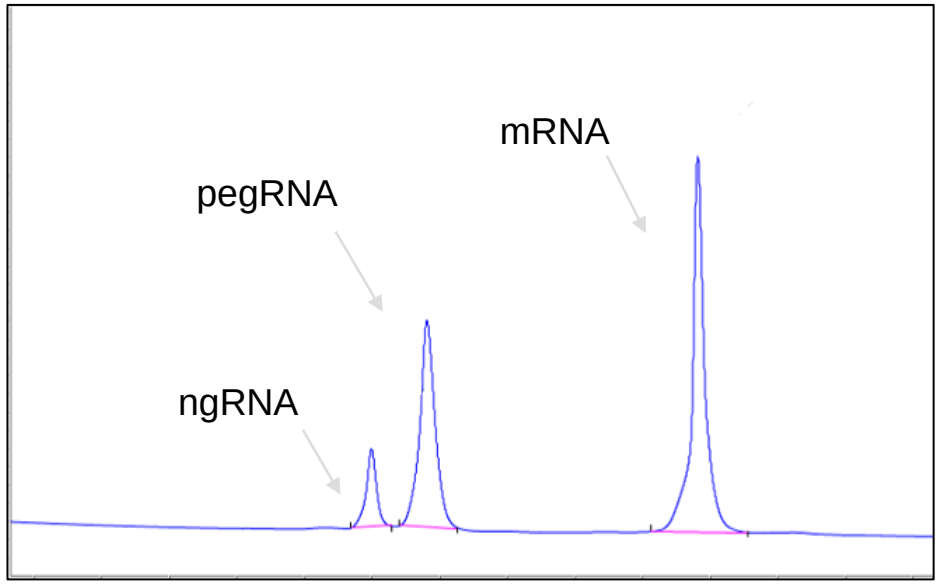
- Well resolved gRNA components (pegRNA and ngRNA) with ~ 20 n.t. difference by CGE
- Streamlined LNP disruption method, fluorescence dye selection, and optimized CGE run conditions enable high sensitivity integrity analysis in different LNP samples

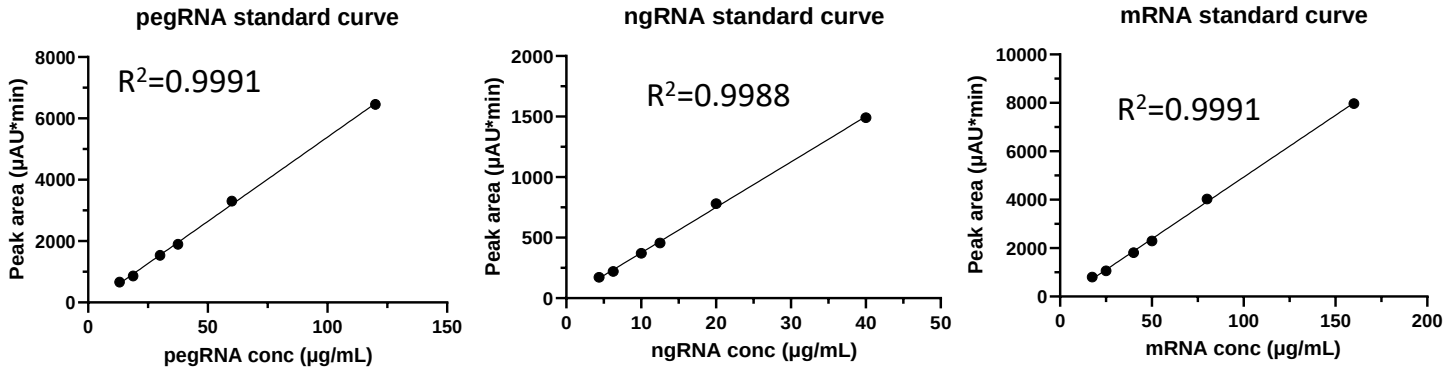


RNA Components Ratio/Quantification Analysis Using IP-RP-HPLC

- Optimized IP-RP-LC conditions can resolve different gRNA components with ~ 20 n.t. difference
- Calibration curves with good linearity for accurate RNA quantification in different LNP samples

UV chromatogram for disrupted RNA-LNP sample





Representative RNA ratio analysis in disrupted RNA-LNP sample

Component	pegRNA	ngRNA	mRNA	Total RNA
Normalized expected RNA concentration (%)	100	100	100	100
Actual RNA concentration as % of expected value	91.0	95.2	108.2	94.8

Summary

- Prime Editing is considered a third generation gene editing technology that has the potential to address a large percentage of genetic variants associated with disease
- Prime Medicine is developing a universal LNP-RNA platform to deliver Prime Editors to treat liver and metabolic diseases
- Prime Medicine has developed multiple platform analytical assays that can support different LNP programs
- Three platform analytical assays developed internally by Prime Medicine were shared to show the lipid and payload analysis of LNP drug products

THANK YOU!