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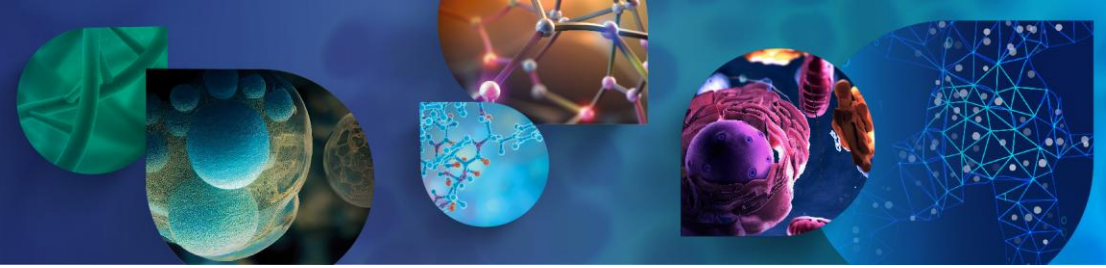


Next Generation LNP Pharmaceuticals Analytics: Intact Chromatography for Comprehensive

10th November 2025

Tristan Kovačič

SARTORIUS

BIA separations

Session Description and Objectives

- Current understanding and requirements for the characterization of LNPs.
- Innovations made with monolithic chromatography.
- Learn about the challenges of LNP CMC.
- Learn about innovations in this space.

Challenges

Be Early in Establishing Appropriate CMC during Development and Expand your Analytical Toolkit

- Key missing points:
  - Multiple cargo formulations
  - Proper identification of covalent imuprities
  - Downfalls of certain methods for complex formulations (e.g. Ribogreen and DLS)
  - Quantification of LNPs conjugated with targeting ligands
  - LNP stability in blood

Quality	Attribute	Method
Identity	RNA identification	Sanger Sequencing PCR
	Identity of lipids	RP-LC-CAD
Content	RNA concentration/RNA encapsulation efficiency	RiboGreen
	Lipid content	RP-LC-CAD
Integrity	LNP size and polydispersity	DLS
	RNA size and integrity	CGE
Purity	DP related impurities – aggregate quantitation	SEC-LC
	DP related impurities – percentage of fragmented mRNA	IP-RP-LC
Potency	Expression	Cell-based assay
Safety	Endotoxin	USP <85>
	Sterility	USP <71>
Other	Appearance	USP <790>
	Residual solvents	USP <467>
	Osmolality	USP <785>
	Subvisible particles	USP <787>
	Extractable volume	USP <1>
	Container closure integrity	USP <1207>
	pH	USP <791>

Covalent impurities: RNA-lipid adducts

- Covalent impurities formed as degradati products
- Side reactions occur between lipids and/or their impurities and nucleic acids forming inactive species.
- Multiple possible chemical mechanisms:
  - Aldehyde from oxidized tertiary amine
  - Aldehyde from peroxides
  - Nucleophilic attack to esters
  - Oxidation of double bonds

Kovačič et al. 2025

nature reviews chemistry
https://doi.org/10.1038/s41570-025-00763-x

Perspective

Check for updates

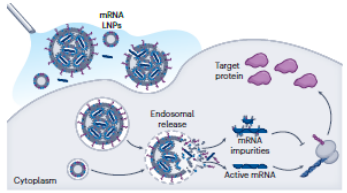
The impact of chemical reactivity on the quality and stability of RNA–LNP pharmaceuticals

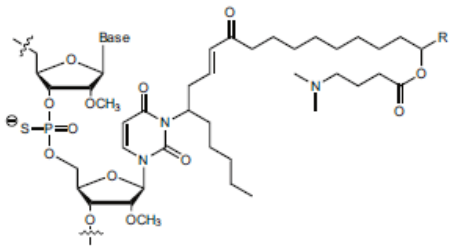
Tristan Kovačič[1,2,3,4,5,6], Heinrich Haas[7,8], Lior Stotsky-Oterin[2,3,4,5], Aleš Štrancar[6], Urban Bren[1,9,10] & Dan Peer[2,3,4,5]

Abstract

Lipid nanoparticles (LNPs) are the most established platform for delivery of mRNA payloads. Their tunability and streamlined manufacturing facilitated an unprecedentedly rapid scale-up during the COVID-19 pandemic. However, being multicomponent, complex systems also poses a challenge of controlling their quality and safety. Analytical checkpoints need to be established to characterize LNPs on multiple levels during development and commercialization. This Perspective centres on the chemical reactivity and purity of mRNA–LNP components, which need to be addressed as raw materials, drug substance, excipients, and the fully formed and stored product. Herein, we describe such appropriate orthogonal analytics to design and analyse LNP formulations. For such novel biopharmaceuticals, better controls that go beyond the current analytical workflow and address the nuanced chemical stability, which helps ensure reproducibility, stability and safety, need to be established.

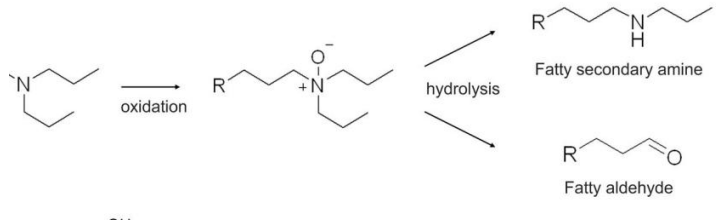
Sections

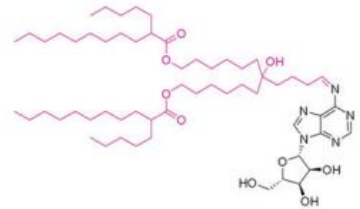




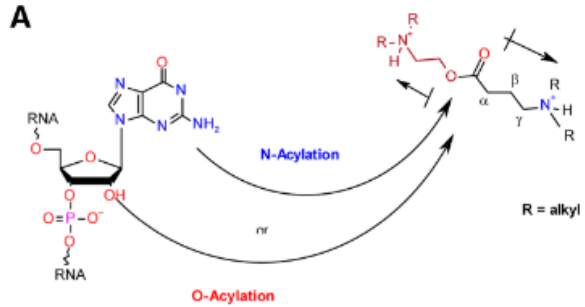
RNA-lipid adduct

Estabrook et al. 2025





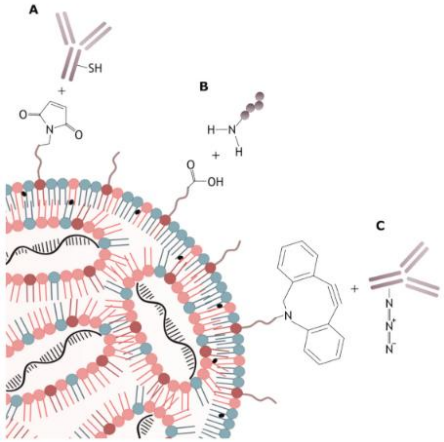
Packer et al., 2021
Hashiba et al., 2024.

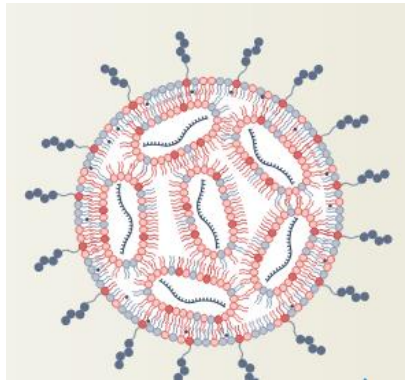


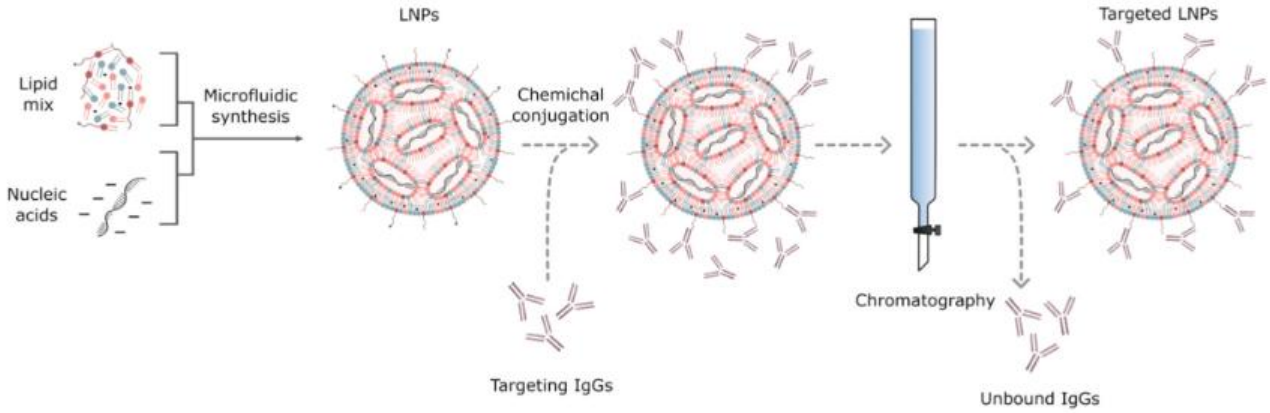
Peronin et al. 2025

Going Beyond the 4-lipid Component LNP Introduces Immense Additional Complexity

- Using targeting ligands to achieve active targeting of tissues with lipid nanoparticles
- Recently, more attention has been gained with Capstan Tx novel *in vivo* CAR-T cell generation
- Chemically conjugating an LNP to a targeting ligand (antibody or their fragments, peptide, aptamers, small molecule ligands, …) introduces immense complexity into the system:
  - Unconjugated ligands
  - Unconjugated LNPs
  - Leftover reactive groups
  - Improper orientations of ligands
  - Too much or too little ligands conjugated
  - Heterogeneity particles with different amount of surface ligands





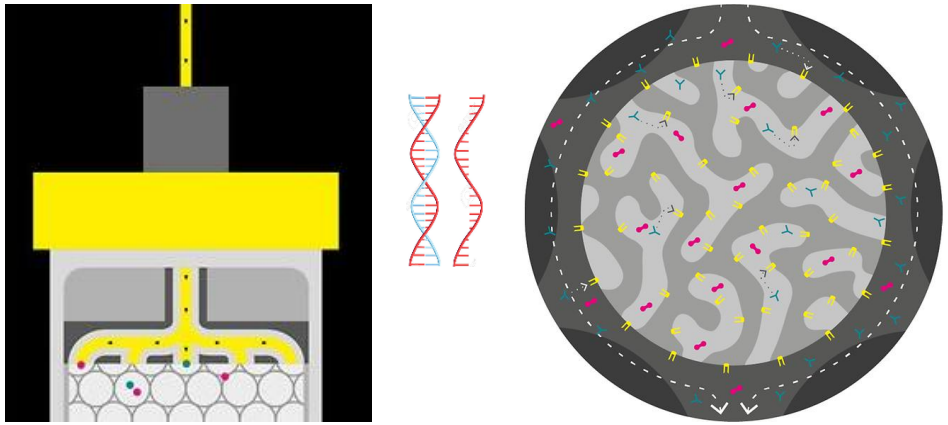


Tarab-Ravski *et al*., **JCR**, 2024
Kon *et al.,* **Nat Rev Clin Oncol**, 2023

Innovations

Traditional vs Monolith Approach

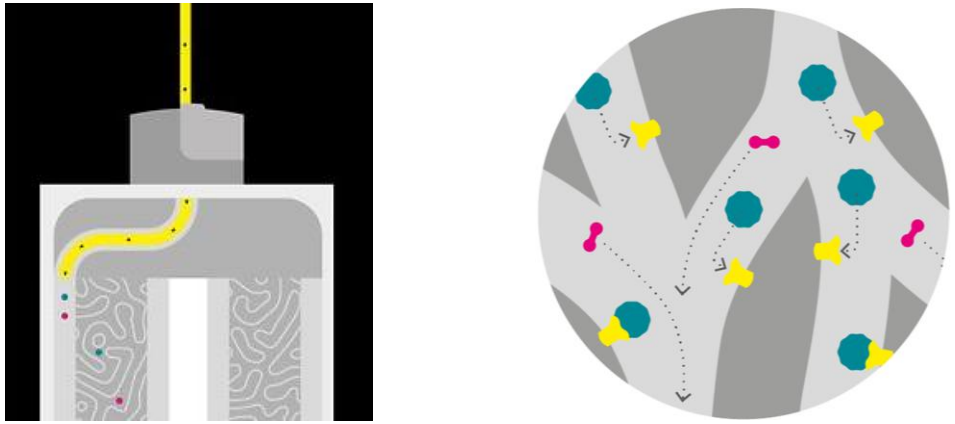
Porous Particle Media



Traditional approach:

- Packing required
- Diffusion limitations – slow process
- Dead-ends – entrapment of large biomolecules
- Lower DBC for larger biomolecules
- Higher backpressure – slower flow rate

Monolithic Columns



Monolith approach:

- Pre-packed and ready to use
- Convective mass transport (no diffusion) – fast process
- Homogeneous channels (no dead ends) – no entrapment
- High DBC for large molecules – the larger the better
- Shear-less process due to laminar flow – low backpressure

PATfix LNP Switcher

PATfix LNP Switcher enables:

- Direct injection of LNPs
- Online particle deformulation
- Determination of encapsulation efficiency
- Co-encapsulated cargo quantification
- Size and size distribution determination
- Determination of mRNA integrity
- Nucleic acid-lipid adduct quantification

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Analysis of lipid nanoparticles using two-dimensional chromatography: Simultaneous determination of encapsulation efficiency, nucleic acid integrity, and size of LNP formulations

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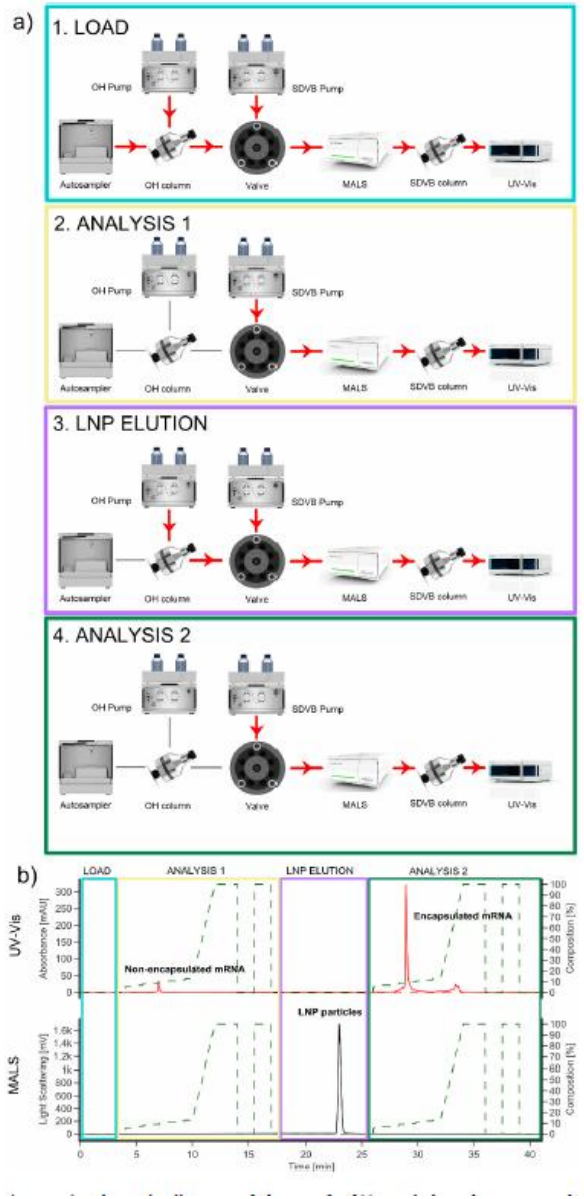
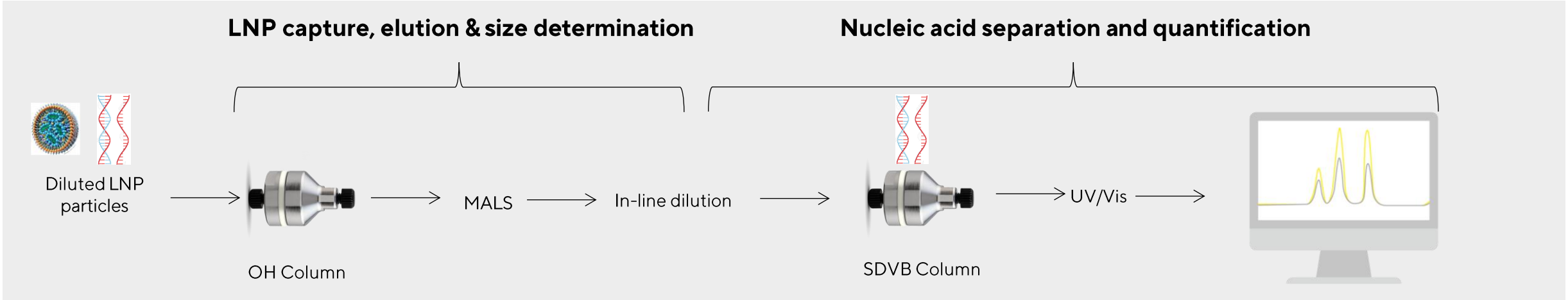


Two-dimensional Column Setup For LNP Characterization



CIMac OH 0.1 mL Analytical Column (6 µm)	CIMac SDVB 0.1 mL Analytical Column (2 µm)
▪ Separation of LNPs from free nucleic acid	▪ Separation of free and encapsulated nucleic acid
▪ HIC (hydrophobic interaction chromatography) column	▪ Reverse phase column
▪ Large 6 µm channel	▪ Used in combination with ion paring reagent

The two columns are connected in-line and separated through valve switching.

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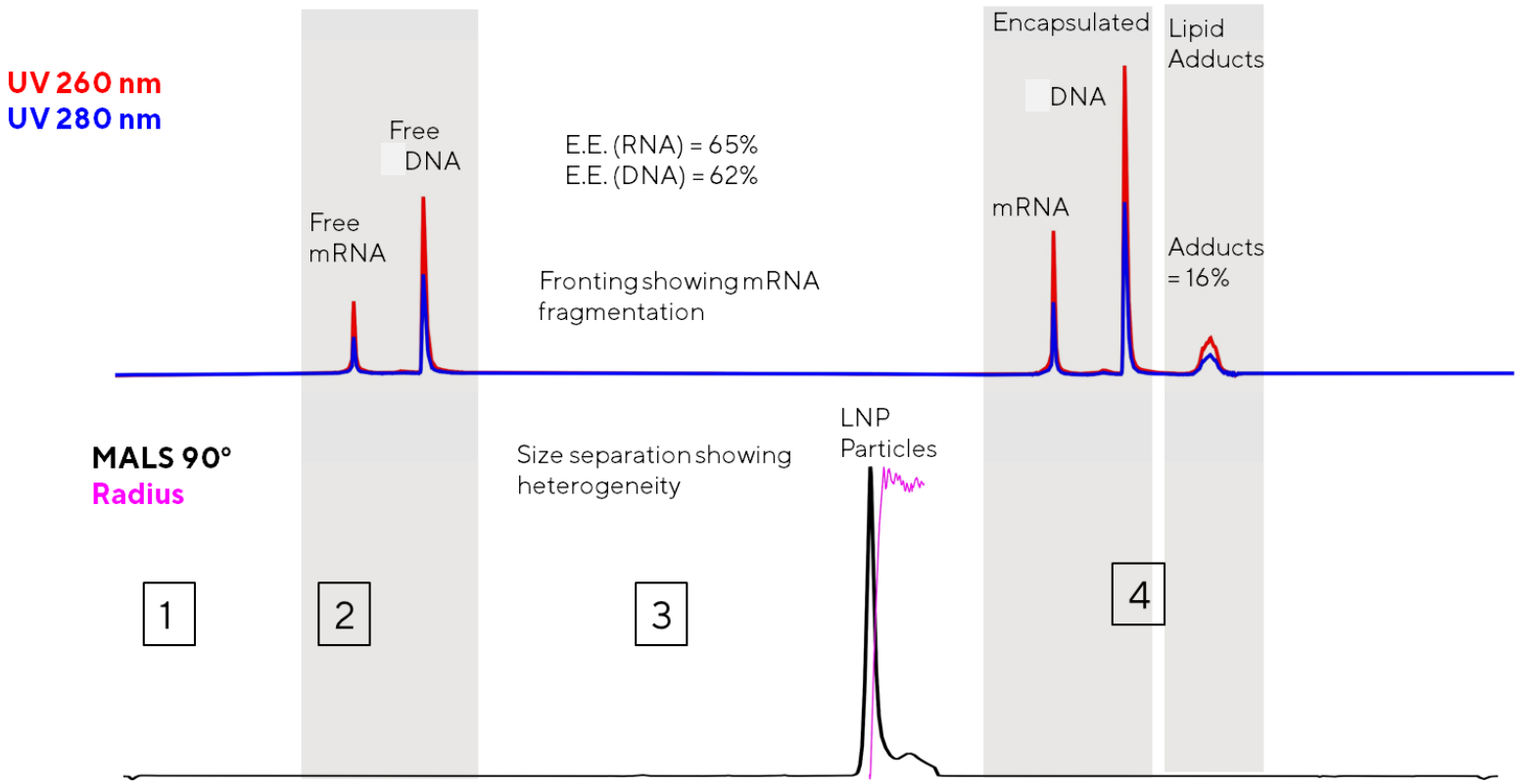
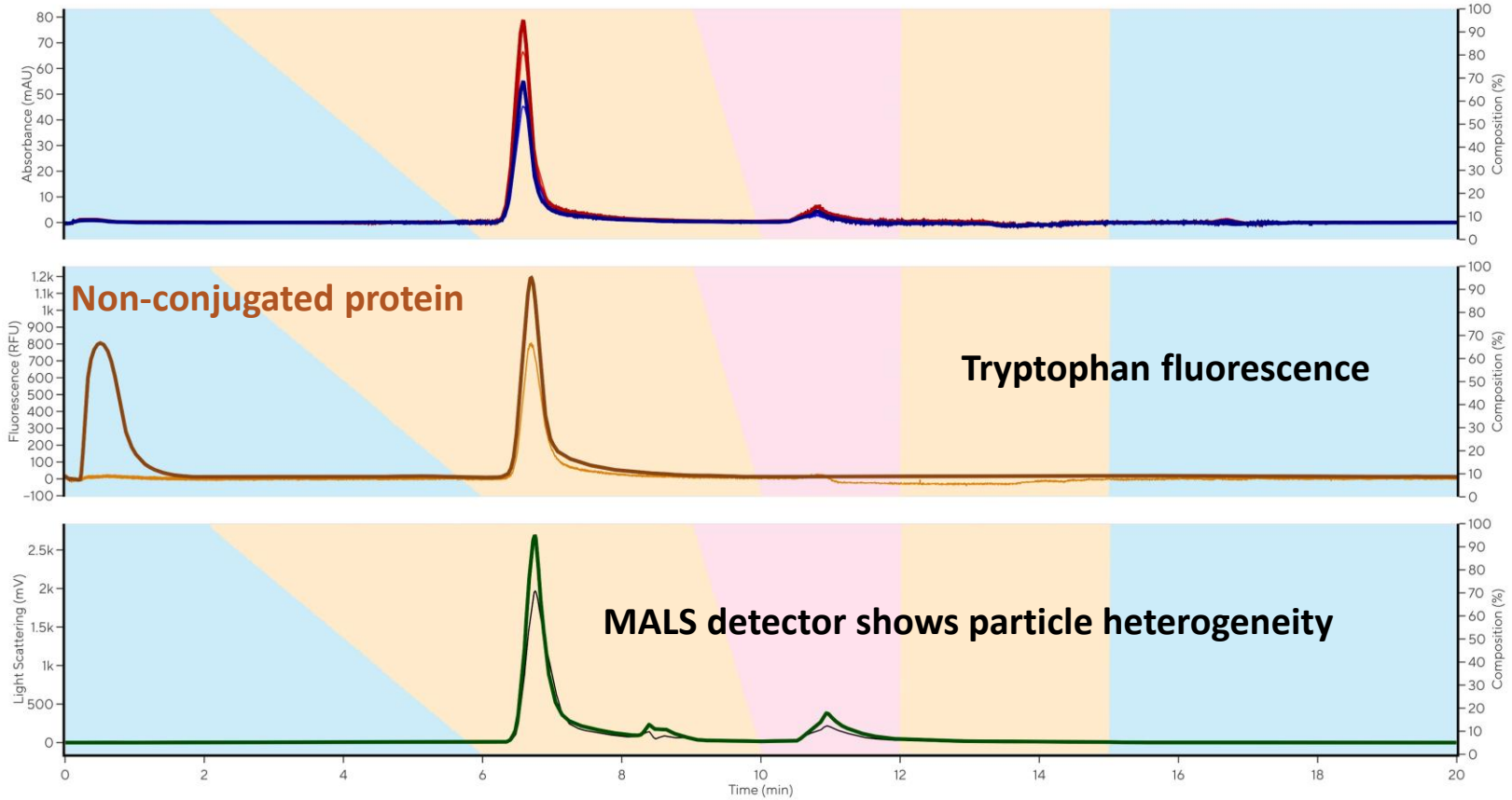
2D Chromatographic Set-up Enables Comprehensive Analytics of Multiple Cargo LNP formulations



- Separation and quantification of multiple-cargo formulations
  - Examples: CRISPR, CAR-T, combination vaccines
- Accurate detection of different nucleic acid species
- Encapsulation efficiency determination
- Heterogeneity assessment
- RNA-lipid adduct detection
- Fragmentation of RNA assessment

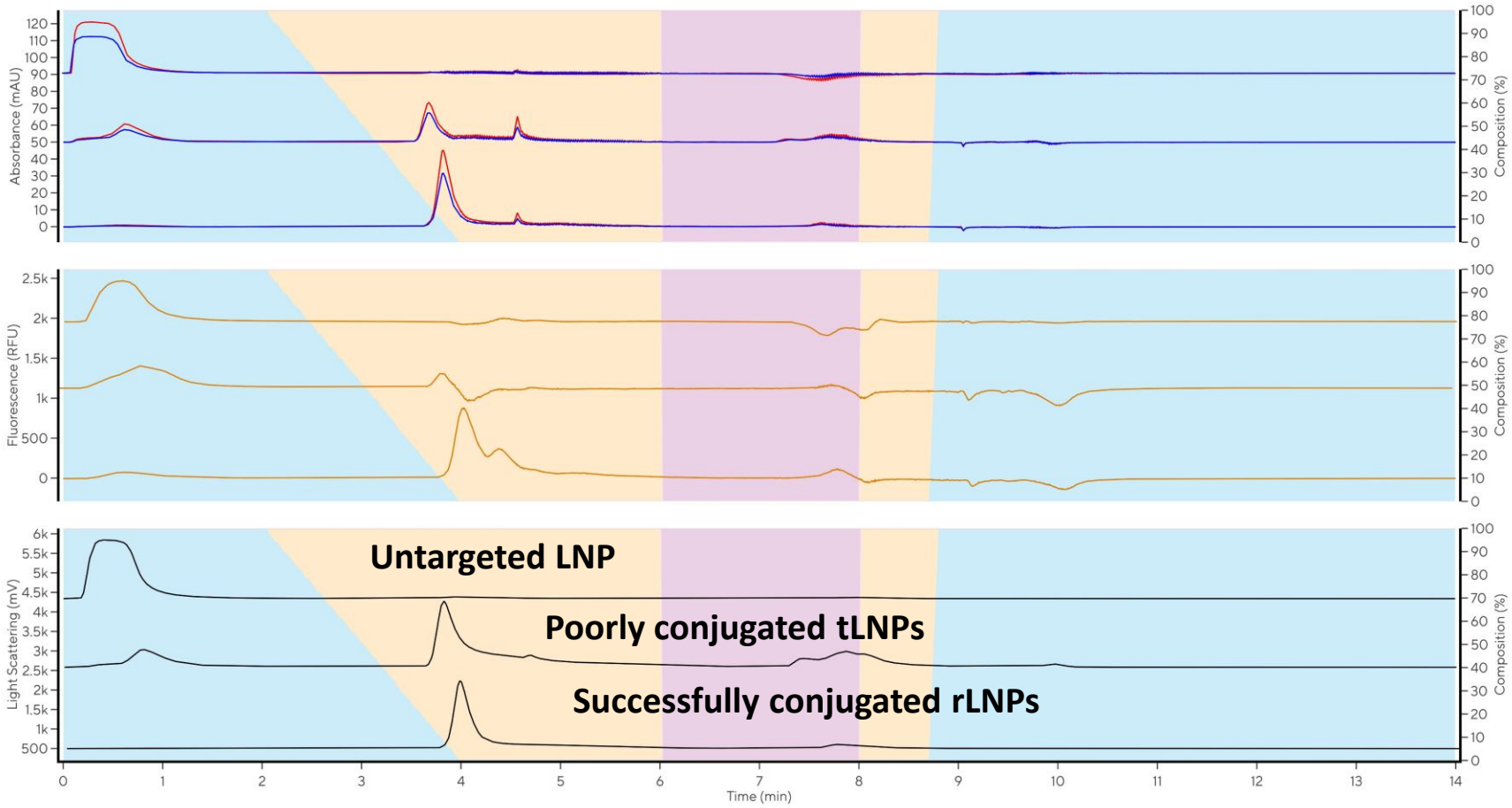
Addressing Complex tLNPs by Monitoring their Manufacturing

- Conjugation reactions are poorly controlled
- A HIC method was developed to separate free protein or its conjugate from particles
- Can be used for reaction monitoring, QC or purification



Separating tLNPs from LNPs on a Particle Level

- On particles level especially there are very limited methods
- An IEX method was developed to address this issue
- You can successfully separate conjugated from non-conjugated particles using this method with high recovery



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PC3 Process Development Viruses

Mojca Tajnik Sbaizero, Tjaša Leban, Ana Železnik, Maja Leskovec

PC2 Process Development mRNA/pDNA

Rok Sekirnik

PC7

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R&D

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Questions

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