Simplifying Progress

End-To-End Process for Improved Purity, Combined With Innovative Analytics of LNP-Based Therapeutics

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Introduction

Advancing LNP-based genetic medicines faces significant downstream bottlenecks. To secure structural integrity and functional recovery, we developed an optimized end-to-end workflow:

PURIFICATION: CIM monolithic columns purify pDNA, mRNA, and the formulated LNPs. Unlike conventional TFF, this chromatographic approach enables separation of free nucleic acids and particle subpopulations, minimizes shear stress, yielding higher payload activity and narrower size distributions.

ANALYTICS: The PATfix LNP Switcher Platform monitors Critical Quality Attributes, quantifying encapsulation efficiency, payload purity, and RNA-lipid adducts alongside orthogonal profiling. This integrated approach streamlines formulation development, yielding actionable data and highly pure, robust LNPs.

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1. End-to-End manufacturing process controlled by PATfix

Scheme below (Figure 1) represent intensified process from pDNA production in *E.coli* to encapsulated RNA-LNP.

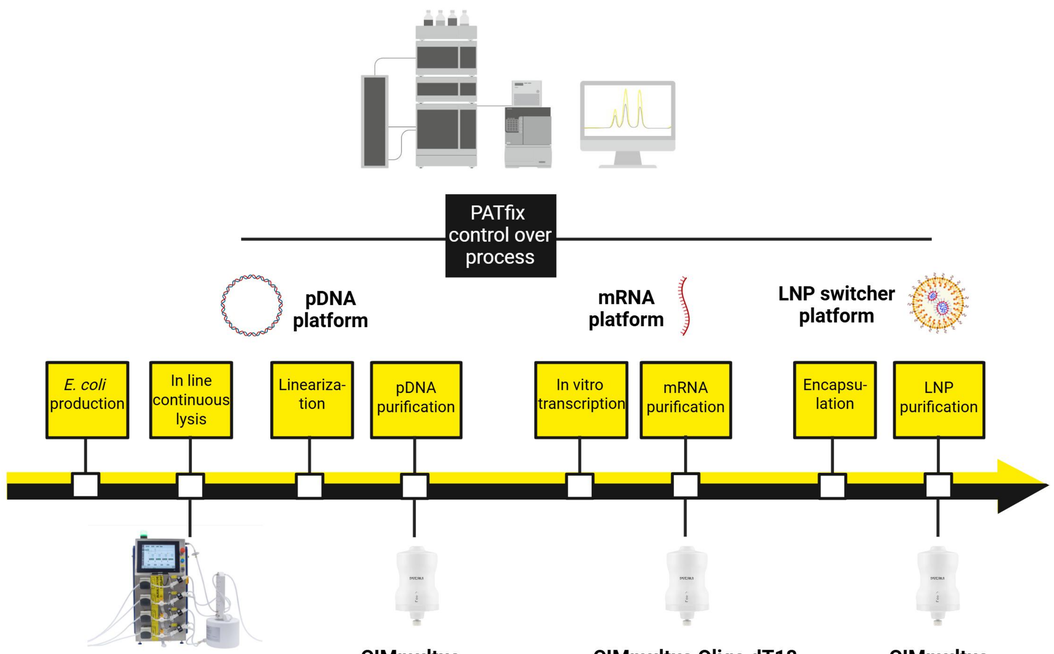


Figure 1: Schematic diagram of end-to-end manufacturing process from pDNA production to LNP purification.

2. pDNA: from in-line lysis to purified pDNA

AUTOMATED pDNA LYSIS

The Alkalizator is an automated, closed single-use system for in-line lysis following E. coli harvest. With sophisticated mixing control, it efficiently processes 10–50 kg of resuspended cells daily in GMP settings, improving lysis and floc separation and maximizing pDNA recovery.

FLEXIBLE PURIFICATION PLATFORM

Downstream purification integrates chromatography and/or tangential flow filtration (TFF) to yield highly pure supercoiled pDNA (for transfection) or linear pDNA (for IVT). The two-stage chromatographic process includes:

- Capture: CIM DEAE anion exchange eliminates bulk process impurities.
- Polishing: CIM C4 HLD hydrophobic chromatography removes residual contaminants, enzymes, endotoxins, and unwanted pDNA isoforms.

Figure 2: pDNA production and purification scheme.

3. mRNA: IVT and preparation of purified payloads

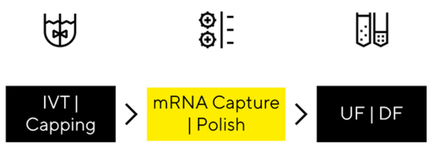


Figure 3: IVT and mRNA purifcation scheme.

After purifying linear pDNA, the template is transcribed in vitro to produce mRNA. mRNA is captured using either an Oligo dT18 column for polyadenylated mRNA or a Swiper column for all RNA. Polishing is done with an SDVB column for dsRNA removal or a C4 HLD column under hydrophobic conditions. This system supports various RNA types, including mRNA, saRNA, and emerging forms like circular and long non-coding RNA.

4. LNP: Robust, high recovery process that yields improved particles quality and consistency

To enhance downstream processing, a chromatographic purification method for the RNA-LNP product was developed using CIM monolithic columns. This approach bypasses time-consuming dialysis by achieving simultaneous ethanol and buffer removal during the load and wash phases. Efficient LNP binding directs unencapsulated free RNA into the flow-through for recovery and subsequent encapsulation. During elution, a precise buffer gradient resolves particle subpopulations, yielding highly uniform, functional LNPs. Crucially, particles elute in small, concentrated volumes. This eliminates secondary concentration steps like centrifugation, accelerating the process, preventing additional shear stress, and minimizing product loss. The procedure concludes with a cleaning-in-place (CIP) phase regenerating the column without sacrificing encapsulated RNA yield, while PATfix Semi-prep MALS provides continuous monitoring of particle elution.

Figure 4: LNP purification chromatogram from CIMmultus OH monolithic column.

5. PATfix LNP Switcher: 2D Column Setup Beyond Standard Methods for Multi-Cargo LNP Characterization

Two monolithic columns are connected in sequence by valve switching on the PATfix LNP Switcher Platform to generate two complementary readouts for RNA-LNP characterization: in the first phase, the CIMac OH 0.1 mL Analytical Column, based on hydrophobic interaction chromatography with large 6 µm channels, separates lipid nanoparticles (LNPs) from free nucleic acids and determines LNP size via multi-angle light scattering (MALS); in the second phase, the CIMac SDVB 0.1 mL Analytical Column, using reverse phase chromatography with an ion-pairing reagent and 2 µm channels, quantifies both free and encapsulated nucleic acids to determine encapsulation efficiency, encapsulated RNA amount and purity, and RNA-lipid adducts, enabling multi-cargo analysis in a single formulation for quality control of CAR-T, CRISPR, and combination vaccine drug products.

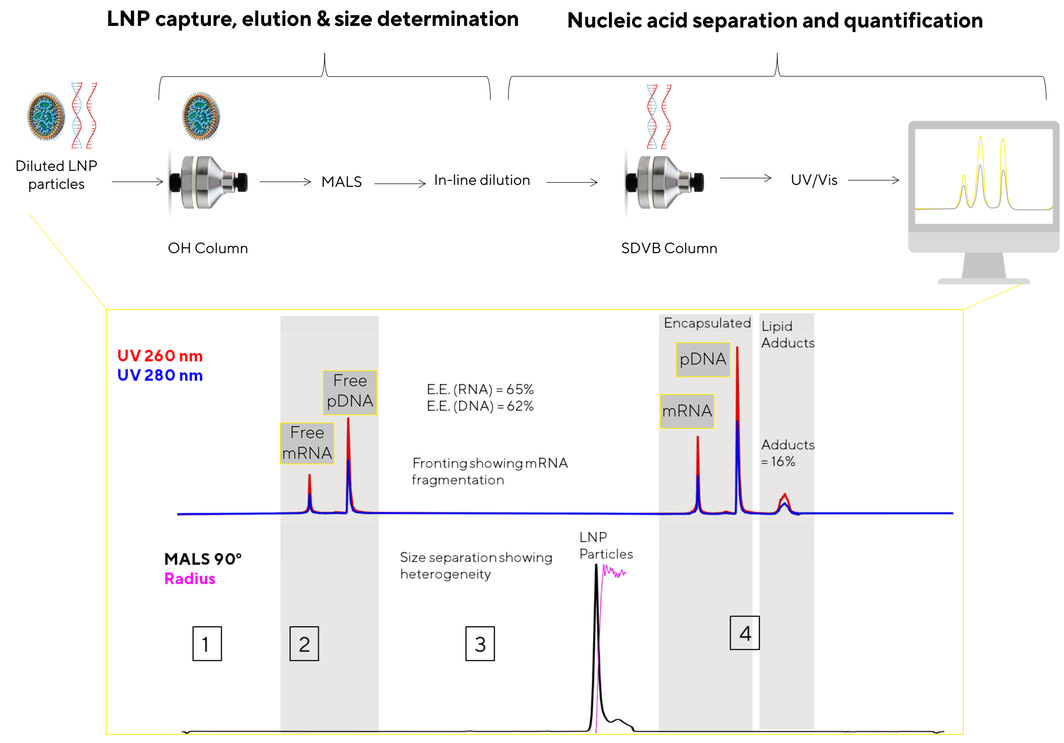
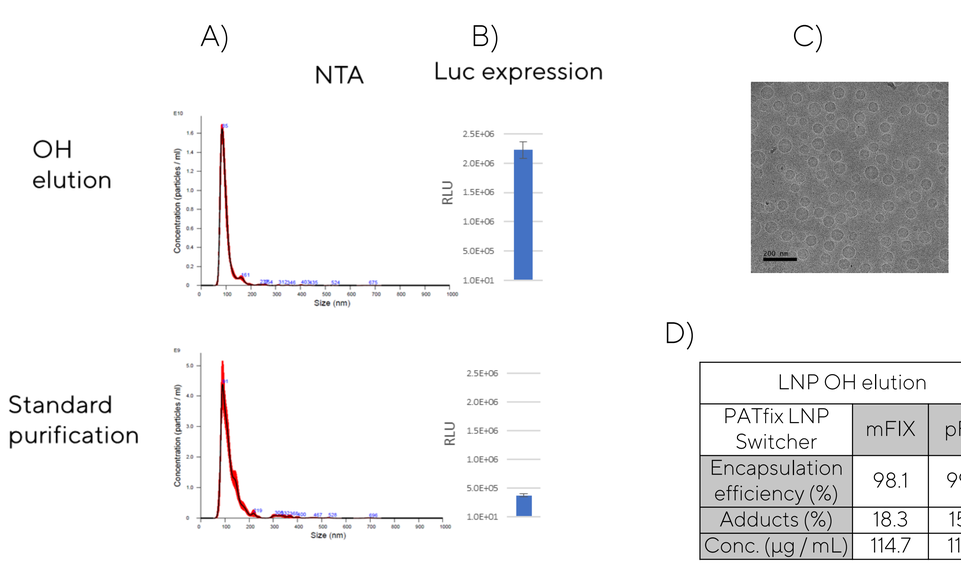


Figure 6: A simplified presentation of PATfix LNP Switcher platform with an LNP Switcher chromatogram showing analysis of with co-encapsulated cargo of 2 different payloads: mRNA and pDNA.

6. The monolith process produces more uniform particles with higher activity than standard purification

Purified LNP were analyzed for particle heterogeneity by Nanoparticle tracking analysis (NTA) and Cryo-TEM for visualizing particles. Activity of encapsulated payload (in this case Luciferase mRNA) was measured in cell-based assay using HEK293 cell line.



D)

LNP OH elution		
PATfix LNP Switcher	mFIX	pFIX
Encapsulation efficiency (%)	98.1	99.9
Adducts (%)	18.3	15.5
Conc. (µg / mL)	114.7	118.1

Figure 7: Comparison of standard purification (TFF) and OH elution. A) Particles size distribution by NTA, B) Cell based assay for Luciferase expression, C) Cryo-TEM of LNP eluted from CIMmultus OH column, D) PATfix LNP Switcher result for LNP OH elution (mRNA and pDNA dual payload).

7. Advanced process analytics for active targeted LNPs

Targeted lipid nanoparticles (tLNPs) enable cell- or tissue-specific delivery of genetic medicines but need better analytics to confirm ligand conjugation and sample homogeneity. Here we show that monolithic columns can be used to monitor the conjugation reaction and to distinguish between targeted and untargeted particles.

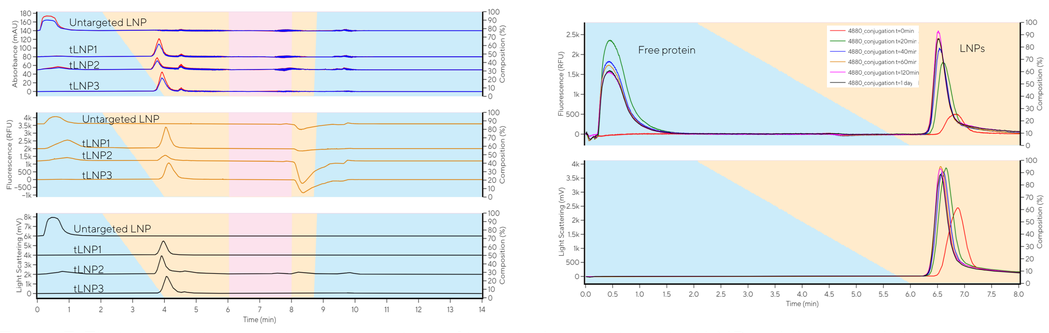


Figure 7: First chromatogram shows separation of targeted and untargeted LNPs, while second chromatogram shows monitoring consumption of free proteins to show formation of tLNPs.

Conclusions

- End-to-End Manufacturing Process:
  - Covers the entire manufacturing/purification from pDNA to mRNA and LNP.
  - Showcases diverse strategies for preparation and purification at each manufacturing step.
- CIMmultus Chromatographic Columns:
  - Ensure scalable, high-resolution purification and maximum functional recovery across the entire process.
- Monitoring and Control:
  - Critical checkpoints are maintained to the highest standards.
  - Utilizes the PATfix system for real-time process analytics and control.
- Enhanced Product Quality and Process Performance:
  - Achieves high purity while significantly reducing the number of operational steps.
  - Minimizes additional shear stress on LNPs, directly improving particle integrity and functional yield.
  - Facilitates the development of safe and effective RNA-LNP-based therapies.
- Promising Solutions for Multi Nucleic Acid Delivery:
  - Offers innovative approaches for delivering multiple nucleic acids effectively.

References

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