Simplifying Progress

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

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Introduction

The mRNA-LNP production process involves four key steps: in most of the cases expressing plasmid DNA in *E. coli,* linearizing and purifying the pDNA, synthesizing mRNA through in-vitro transcription, purifying the mRNA, and encapsulating nucleic acids into lipid nanoparticles (LNPs) for effective delivery of mRNA vaccines and therapies. There are, however, several challenges that need to be addressed regarding their manufacturing and characterization: correct formation and integrity of the drug product, sufficient recovery during purification while maintaining functionality, and producing a well-purified and thoroughly characterized product. To introduce fine-tuned separation and obtain uniform and functional product, purification using CIM® monolithic columns was developed.
RNA was transcribed *in vitro* from pDNA and both were purified using CIM monolithic columns, enhancing the quality of encapsulation process. The purification of the encapsulated nucleic acid-LNP product is currently streamlined at large scales with TFF. To enhance separation capabilities, a chromatography purification method for the RNA-LNP product was developed using CIM monolithic columns. Throughout this process, PATfix® analytics monitored all quality attributes. For LNP characterization, the PATfix LNP Switcher Platform was employed to assess product quality, including encapsulation efficiency, encapsulated RNA quantity and purity, and the presence of RNA-lipid adducts. Additionally, lipid ratios, cell-based assay for RNA-LNP activity and size measurements (NTA, DLS) were determined.

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.

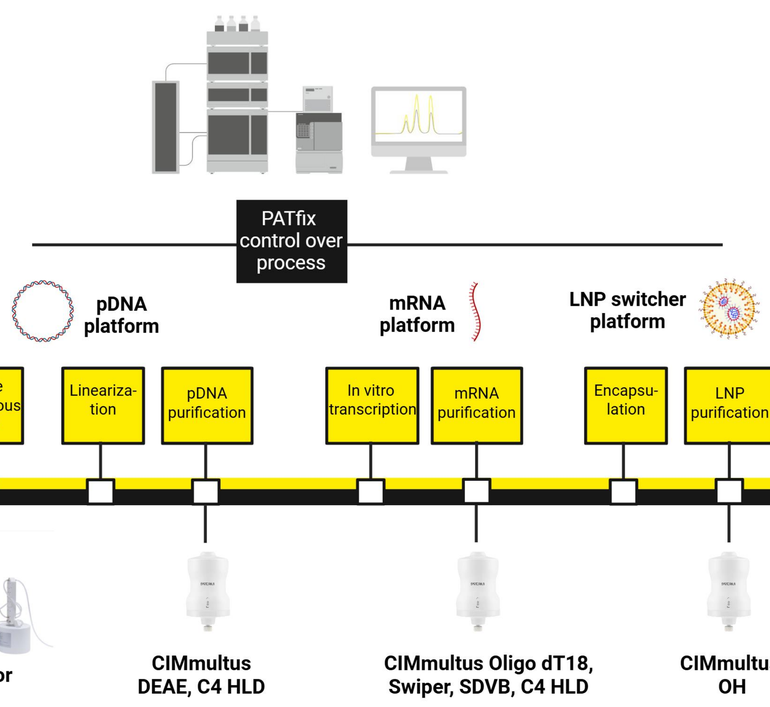


Figure 1: Schematic diagram of end-to-end manufacturing process from pDNA production to LNP purification.

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

The Alkalizator is an automated system for in-line lysis, tailored for pDNA production following *E. coli* expression. It features a closed single-use loop and sophisticated mixing control, efficiently handling 10–50 kg of resuspended cells daily in GMP settings, reducing pDNA degradation risks from uncontrolled alkaline lysis and mechanical stress. Purification utilizes a platform process with chromatography and tangential flow filtration to ensure high purity and formulation of plasmid DNA, such as supercoiled pDNA for transfection or linear pDNA for in vitro transcription. Chromatography involves two stages: anion exchange chromatography (CIM DEAE) to eliminate process impurities, and hydrophobic chromatography (CIM C4 HLD) to refine the sample by removing residual contaminants, enzyme, endotoxins, and unwanted plasmid DNA isoforms.

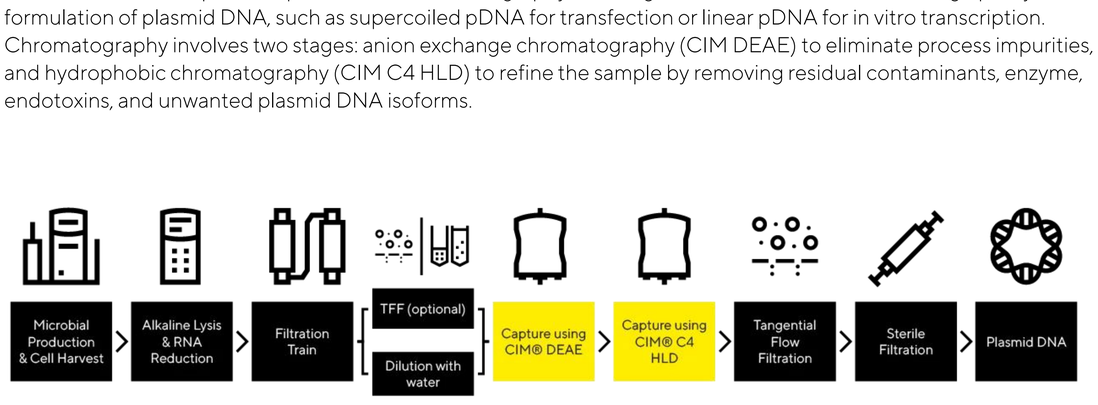


Figure 2: pDNA production and purification scheme.

3. mRNA: IVT and preparation of purified payloads

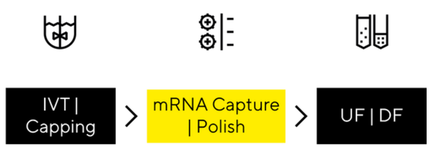


Figure 3: IVT and mRNA purifcation scheme.

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 separation capabilities, a chromatography purification method for the RNA-LNP product was developed using CIM monolithic columns. Efficient binding of LNPs is achieved during the load phase and release during the elution phase. Ethanol and formulation buffer removal are achieved during the load and the wash phase. Additionally, free RNA is removed in the flow through and can be regenerated for following encapsulation. Separation of particle subpopulations is achieved by buffer gradient of the chromatographic run, resulting in high functioning and uniform particles. During the cleaning in-place (CIP) phase the column is cleaned, but no encapsulated RNA is lost during that step. PATfix Semi prep MALS used to efficiently monitor particle elution.

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

5. PATfix LNP Switcher platform: Two-dimensional column setup for LNP characterization beyond the existing methods

The two monolithic columns are connected in sequence and separated by valve switching. In the first phase, the CIMac OH 0.1 mL Analytical Column, which utilizes hydrophobic interaction chromatography with large 6 µm channels, is employed to separate lipid nanoparticles (LNPs) from free nucleic acids. The size of the LNPs is determined using a multi-angle light scattering (MALS) detector. In the second stage, the CIMac SDVB 0.1 mL Analytical Column, which employs reverse phase chromatography combined with an ion-pairing reagent and features 2 µm channels, is used to assess both free and encapsulated nucleic acids, thereby determining encapsulation efficiency.

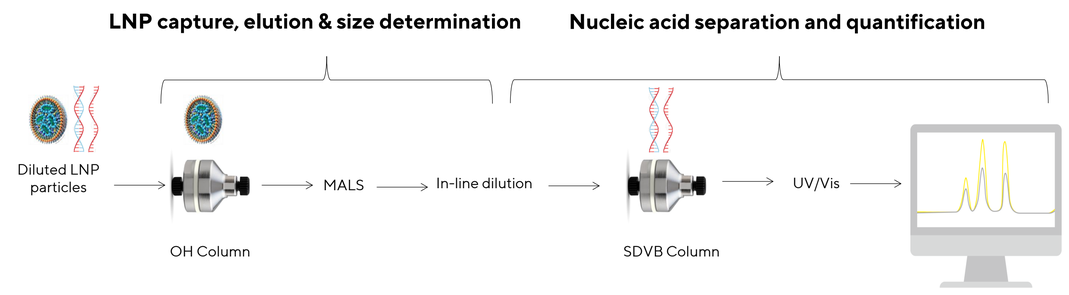


Figure 5: Simplified presentation of PATfix LNP Switcher platform.

6. Comprehensive analytics of multiple cargo LNP formulations for LNP-based therapies

For RNA-LNP characterization, the PATfix LNP Switcher Platform was employed to assess product quality, including encapsulation efficiency, encapsulated RNA quantity and purity, and the presence of RNA-lipid adducts. The platform enables the analysis of encapsulated multiple cargos within a single formulation, crucial for proper quality control of drug products for CAR-T, CRISPR and combination vaccine applications.

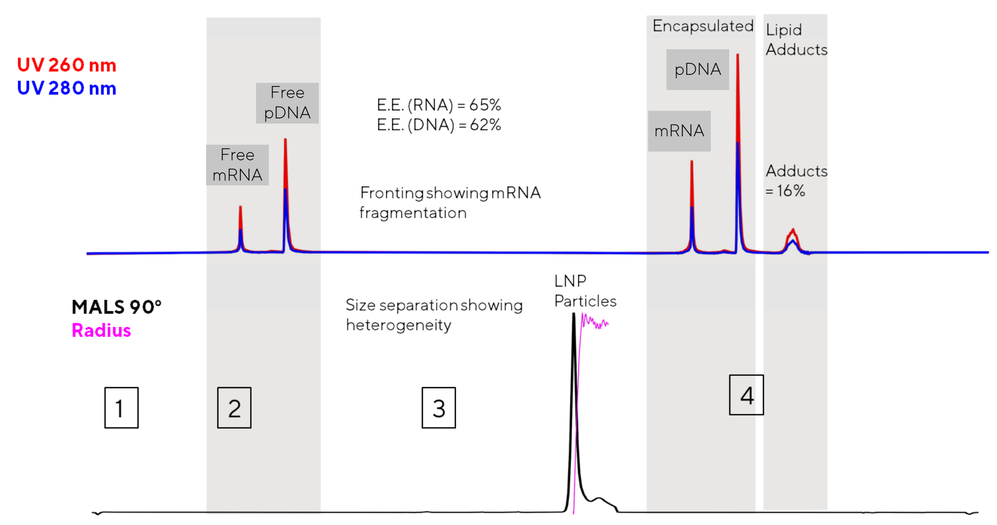
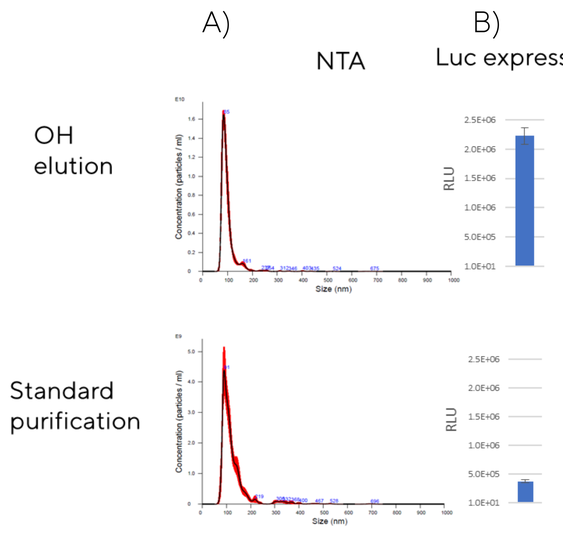


Figure 6: An LNP Switcher chromatogram showing analysis of an LNP with co-encapsulated cargo of 2 different payloads: mRNA and pDNA.

7. 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).

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:
  - Perfectly suited for these applications, ensuring optimal performance.
- 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:
  - Results in improved purification efficiency.
  - 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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