NanoPulse: A Scalable Micromixing Method for Robust and Reproducible RNA-LNP Formulation Across All Scales

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ABSTRACT

Nanomedicine landscape:
- Growing interest in RNA-LNP therapies, with proven clinical impact.

Translational bottleneck:
- Lack of unified technology to scale LNP production from early-stage R&D to clinical production.
- No single formulation technology ensures identical LNPs across all scales.

NanoPulse technology:
- Only technology that fully covers the whole development pipeline from µL-scale screening to continuous large-scale production.
- First micromixer delivering consistent mixing – thus LNP characteristics, across all scales (µL → L) and with different cargos.

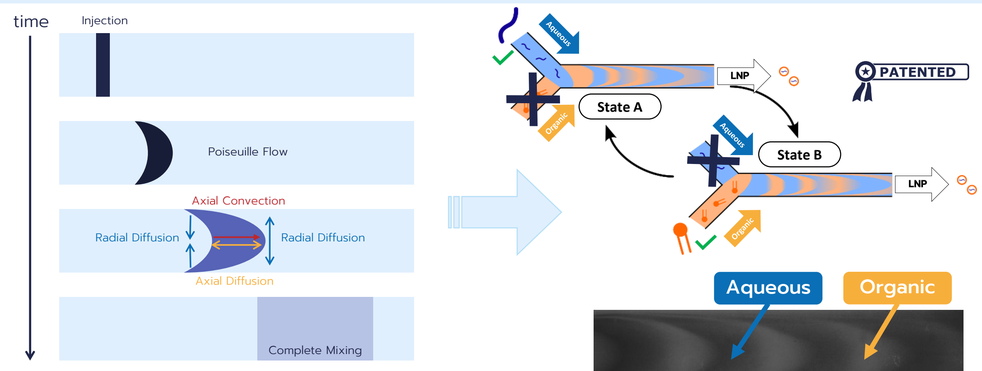
TECHNICAL BOTTLENECKS

1. **Small-scale gap:** IJMs and other large-scale mixers struggle to handle discovery volumes (< 1 mL).
2. **Large-scale gap:** Microfluidics face throughput limits and are prone to aggregation; therefore, are unsuitable for clinical production.
3. **Platform switching & scale-up:** Transitions between methods or platforms alter critical quality attributes (CQAs), requiring re-optimization and re-validation, slowing development.

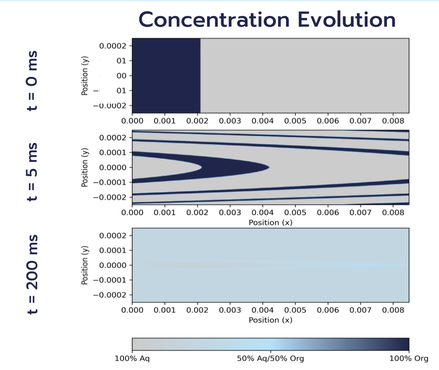
Staggered Herringbone Mixer (SHM) vs NanoPulse after up to 10 mins of continuous LNP formulation

1. NanoPulse Technology

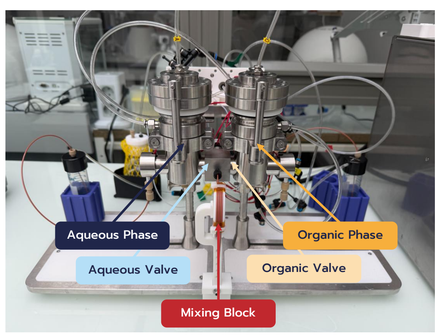
- **Mixing principle: Sequential injection** of the organic phase (lipids in solvent) and the aqueous phase (with or without cargo, e.g., RNA, DNA) ensures controlled LNP formulation **(Figure A)**. Mixing efficiency validated via **semi-Lagrangian CFD simulations (Figure B)**.
- **Operational control:** Total Flow Rate (TFR), Flow Rate Ratio (FRR), and valve-injection frequency set via the controller **(Figure C)**.
- **Scalability:** Reproducible LNP characteristics from screening to clinical-scale production (**goal:** 200 µL up to 100+ L).
- **Additional advantages: Aggregation-free** operation and **seamless transition** from discovery to production without platform switching; reducing regulatory complexity, accelerating process development, minimizing development costs and risks, and ensuring consistent efficacy.

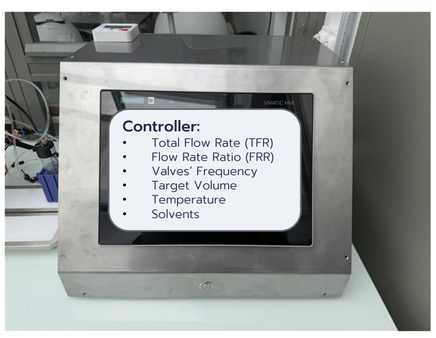


A NanoPulse Technology



B CFD Simulations





C NanoPulse Prototype

3. Experimental Results

Results in rotating disk format

Efficient formulation (Figure 1):
- mRNA-LNPs visualized at high (167 Hz) and low (12.5 Hz) frequencies using **Cryo-TEM**.
- Well-formed and intact LNPs with electron-dense internal structure at both frequencies, **consistent with successful RNA-LNP assembly**.

Continuous operation (Figure 2):
- **Stable LNP size & PDI** during extended runs, measured using DLS.

Identical RNA-LNP quality at all scales (Figure 3):
- **No significant changes** in size, PDI, EE%, potency, cell transfection, or protein expression, from 0.25 to 60 mL.

Pulse frequency effect (Figure 4):
- Direct impact on LNP size, aligning with NanoPulse's **theoretical model and simulation results**.
- **Higher injection frequency → Smaller LNP size**.
- **Matching microfluidics** (TAMARA) particle sizes by tuning injection frequency.

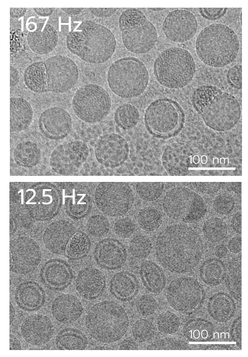


Figure 1

Adapted to sensitive cargoes (Figure 5):
- Efficient formulation and robust biological performance of saRNA at low and high TFRs.
- Pulse frequency **does not impact** significantly mRNA or saRNA-LNPs potency.
- Protein expression is **comparable to standard microfluidics** (TAMARA) production.

Figure 1
Cryo-TEM of mRNA-LNPs
TFR: 12 mL/min, FRR: 3:1, [Lipid]: 5 mg/mL, N/P: 6:1
ALC0315:DSPC:Cholesterol:DMG-PEG200 (50:10:38.5:1.5)

Figure 2
Size & PDI of empty LNPs
TFR: 36 mL/min, Frequency: 17 Hz, FRR: 3:1, [Lipid]: 2 mg/mL, V: 4 L
SPC:Cholesterol:DMG-PEG2000 (59:40:1)

Figure 3
siRNA-LNP CQA consistency from 250 µL to 60 mL
TFR: 20 mL/min, Frequency: 17 Hz, FRR: 3:1, [Lipid]: 5 mg/mL
ALC0315:DSPC:Cholesterol:DMG-PEG2000 (50:10:38.5:1.5)

Figure 4
Impact of frequency on siRNA-LNP size & PDI
TFR: 20 mL/min, FRR: 3:1, [Lipid]: 5 mg/mL
SM102:DSPC:Cholesterol:DMG-PEG200 (50:10:38.5:1.5)

Figure 5
Effect of RNA modality (saRNA vs mRNA) and TFR on eGFP protein expression level in HeLa cells
(Confidential composition)

HIGHLIGHTS

With **NanoPulse**, we can guarantee:

- ✓ **Quality:** Consistent LNP CQAs across all runs.
- ✓ **Scalability:** 0.25 mL to continuous production in a single platform.
- ✓ **Continuous production:** Agglomeration-free operation, even after long production times.
- ✓ **Formulation flexibility:** Different RNA-LNPs could be produced.
- ✓ **State-of-the-art performance:** Successfully benchmarked against microfluidic technologies.

CONCLUSION

- **Controlled LNP formulation** via **sequential injection** of organic and aqueous phases, ensuring **efficient mixing**, validated by **CFD simulations**.
- **Tunable parameters** (TFR, FRR, frequency) for precise **LNP size control**.
- **Seamless scale-up** from low-volume research to high-volume continuous production using a single device.
- **Reproducible, aggregation-free, volume-independent** LNP formulation.

FUTURE DIRECTIONS

- Expand dataset to **validate** preliminary results.
- Refine the **correlation** between simulation models and experimental outcomes.
- Characterize **CQAs across all scales** (morphology, in-vivo performance).
- Combine with **downstream processing** tools (dilution, TFF, etc.).
- Proceed with a NanoPulse **GMP-ready** formulation platform.

inside Therapeutics in collaboration with: Thermo Fisher Scientific, université angers, INSERM 1066 - CNRS 6021 MiNt MICRO & NANOMEDECINES TRANSLATIONNELLES, ART ARNm

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