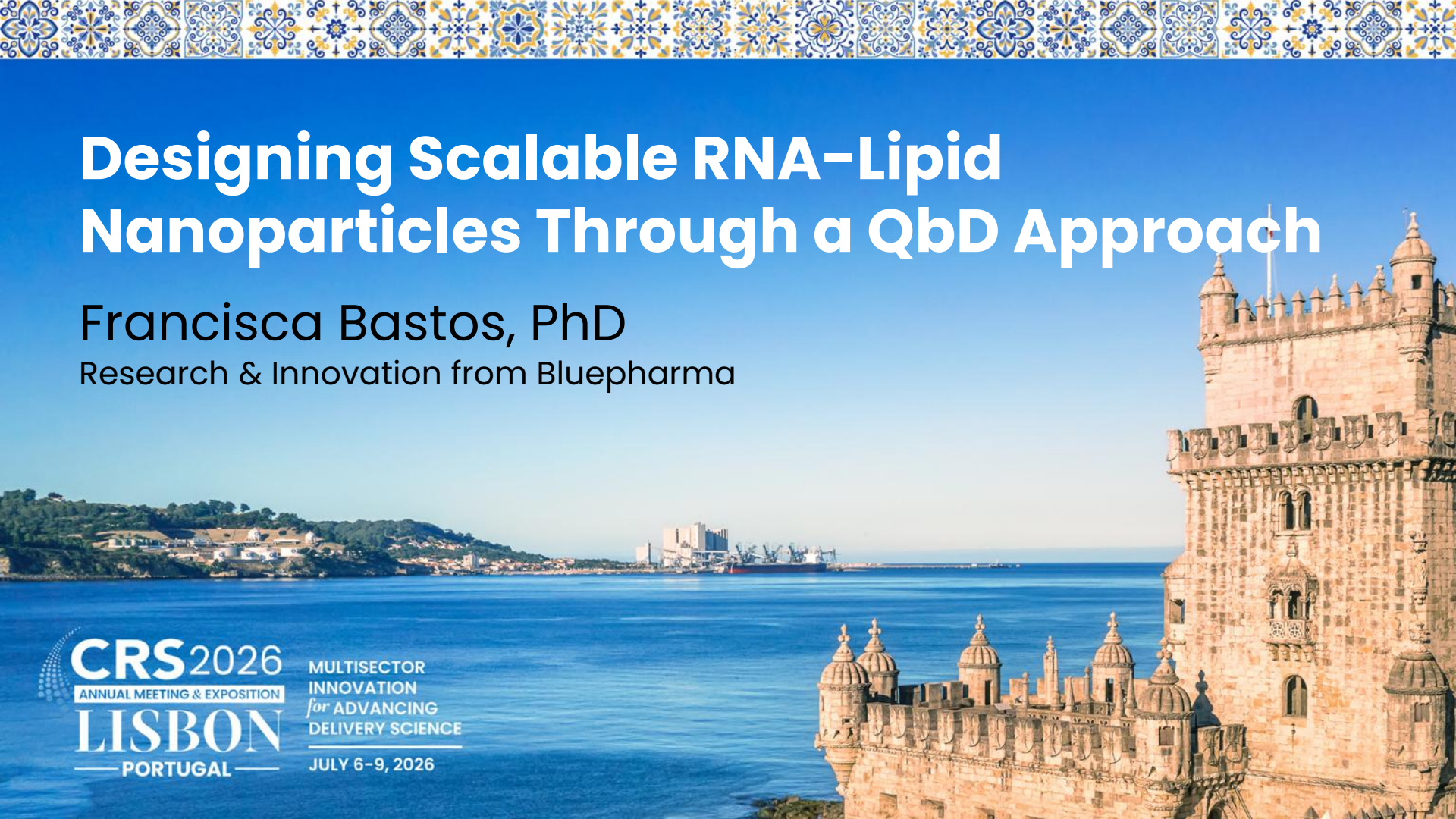
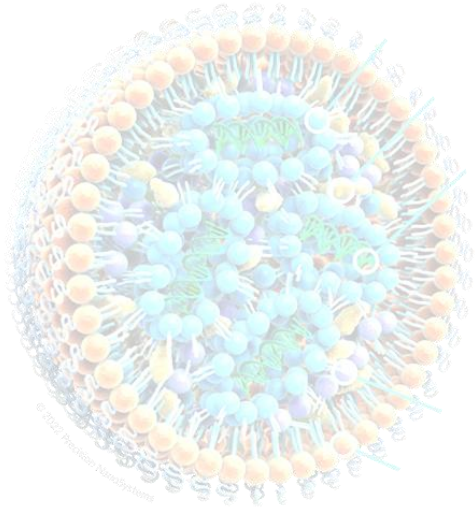
Designing Scalable RNA-Lipid Nanoparticles Through a QbD Approach

Francisca Bastos, PhD
Research & Innovation from Bluepharma



Outline

1. **Background & Study Rationale**
   - CInTech Project Overview
   - Relevance of RNA therapeutics & LNPs
2. **Objectives & QbD Framework**
   - Process Optimization for LNP-mRNA Platform
   - Novel Ionizable Lipid Evaluation
3. **Additional Experimental Work**
   - Lipids Screening Strategy
   - RNA modalities screening
4. **Key Findings & Conclusions**



Background · CInTech Project Overview

Technological Hub for Innovation, Translation and Industrialization of Complex Injectable Drugs

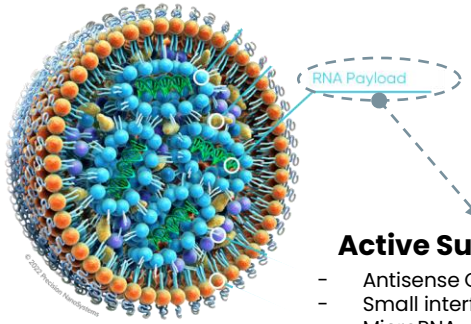
Research & Innovation
Center

Development & **scale-up**
Center

Highly specialized GMP
Center

Background · RNA Modalities

LNP as innovative technological platform designed to **efficient protect and deliver RNA molecules**



Active Substance:

- Antisense Oligonucleotide
- Small interfering RNA
- MicroRNA
- Messenger RNA
- Self-amplifying RNA
- CRISPR/Cas9 (gRNA)
- Circular RNA
- Others…

Advantages

Protective effect (*i.e.,* RNases, clearance, immune system)

Lower Systemic Toxicity

Improvement of pharmacokinetic profile

Controlled and Sustained Release

Targeted delivery of RNA molecules

Therapeutic Areas

➢ **Increasing the expression of a given protein** (*e.g.*: mRNA, saRNA, circRNA)
- → Protein Replacement Therapy
- → Vaccination (Infectious Diseases/ Cancer)
- → Cancer Immunotherapy (*e.g.* CAR-T; mAbs)
- → Regenerative Medicine

➢ **Knocking out/down targeted genes/ modulate genes processing** (*e.g.*: ASO, siRNA, miRNA)
- → Genetic Disorders
- → Cancer
- → Metabolic/ Cardiovascular Disorders
- → Eye/ Skin Disorders

➢ **Gene editing** (*e.g.* CRISPR/Cas9)
- → Genetic Disorders

Background · Lipid Nanoparticles

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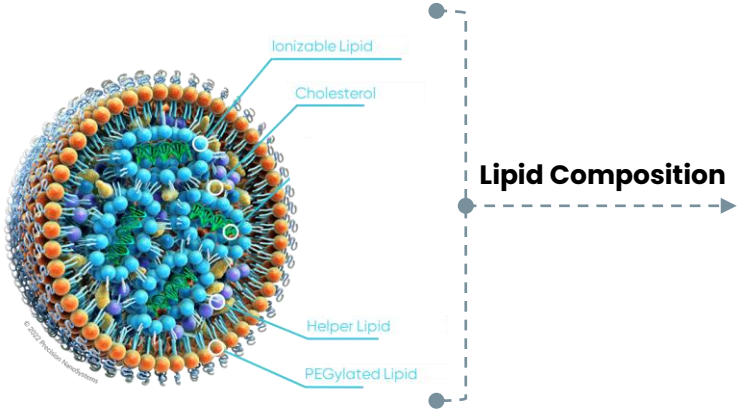


Lipid Composition

Ionizable Lipid

- **Interact with RNA molecules** (allowing encapsulation)
- **Reduce** the extent of **opsonization**
- Stimulate intracellular **release from endosomes**

Cholesterol

- Allow the formation of an **organized and rigid lipid layer**
- **Stabilize the LNP**
- Influence the **lipid packing**, **membrane fluidity**, and **permeability** of the layer

Helper Lipid

- Allow the **formation** of an **organized and rigid lipid bilayer**
- **Stabilize the LNP**
- May **assist in endosomal escape**
- **Can influence** target organ specificity

PEG-derivatized Lipid

- **Prevent destabilization** and **aggregation** of the LNPs
- **Reduce the** extent of **opsonization** (leading to an increase of the LNP blood circulation time)

Study Rationale · LNP-mRNA

Effects of the CIntech Project and Bluepharma's Strategic Approach

Potential Innovation Opportunities

Delivery System

- Inclusion of **targeting moieties**
- Co-encapsulation of **responsive** agents
- Development of **Hybrid systems** (*e.g.* Lipid–Polymer Hybrids)

Therapeutic Indication

- Innovative and more effective **oncology** treatments

RNA Molecule

- **Co-delivery** of two or more types of RNA molecules (*e.g.* siRNA + mRNA; mRNA + gRNA)

Formulation

- **Lyophilization**
- Use of different **excipients** in the formulations to increase the stability

Value Proposition

Library of Novel Ionizable Lipid-like Molecules

- **Ionizable character** for RNA encapsulation & endosomal escape
- **Customizable** properties/structures
- **Biocompatible** & **Biodegradable**

Novel Lipid Nanoparticle-based Platform

- **Customizable** nanoparticle physico-chemical properties
- Tailored for different **administration pathways**
- Tailored for different **RNA types**

Fast, simple & reproducible manufacturing process based on **Microfluidics**

Possibility of **IP protection**

Objectives

2. Formulation Optimization with Novel IL

⚙️ QbD Formulation
🧪 Characterization & Pre-Stability
🔬 In Vitro

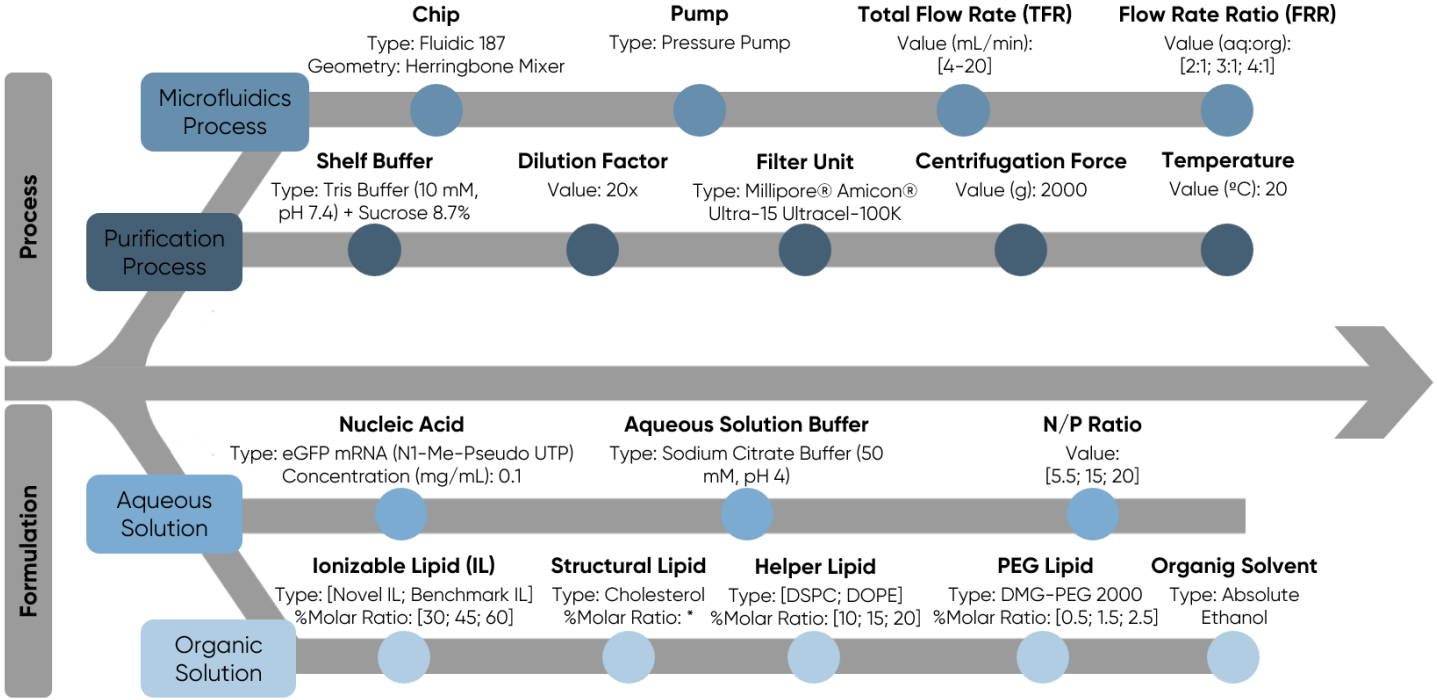
1. Manufacturing Process Optimization

⚙️ QbD Process
🧪 Characterization & Pre-Stability
🔬 In Vitro & In Vivo
📈 Scale-up

3. Lipids & RNA Modalities Screening

🧪 Characterization
🔬 In Vitro & In Vivo

QbD-driven LNP-mRNA Optimization • Ishikawa Diagram



Target LNP-mRNA

Z-Average (d.nm): [50-150]

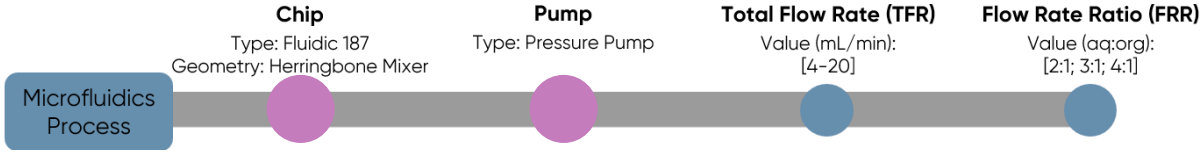
PdI: <0.2

Encapsulate (%): >95

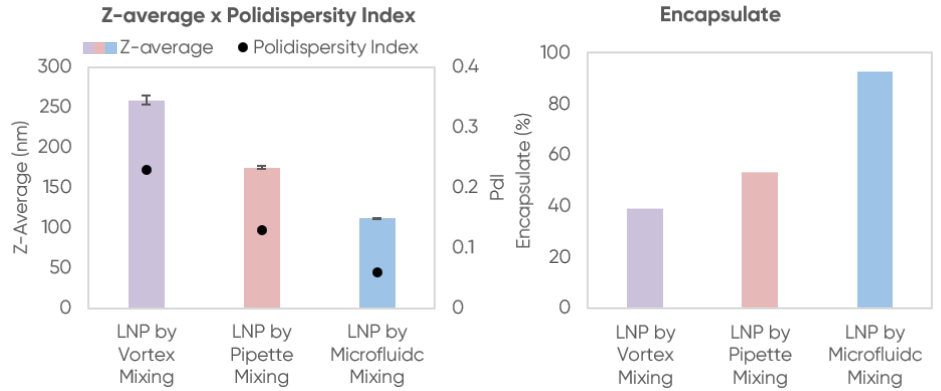
*The molar percentage of the structural lipid corresponds to the remaining fraction necessary to ensure that the total molar contribution of all lipids equals 100%

QbD-driven LNP-mRNA Optimization • Process Selection

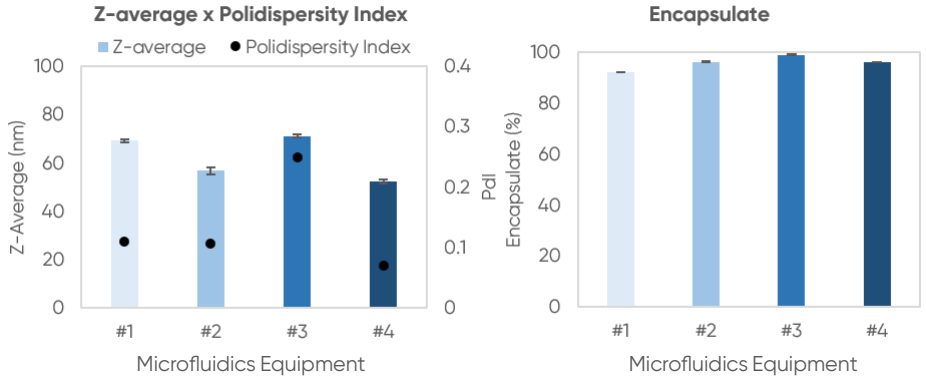
Comparative analysis of LNP-mRNA manufacturing processes & equipment



Manufacturing Processes



Microfluidics Equipment



QbD-driven LNP-mRNA Optimization · Process Optimization

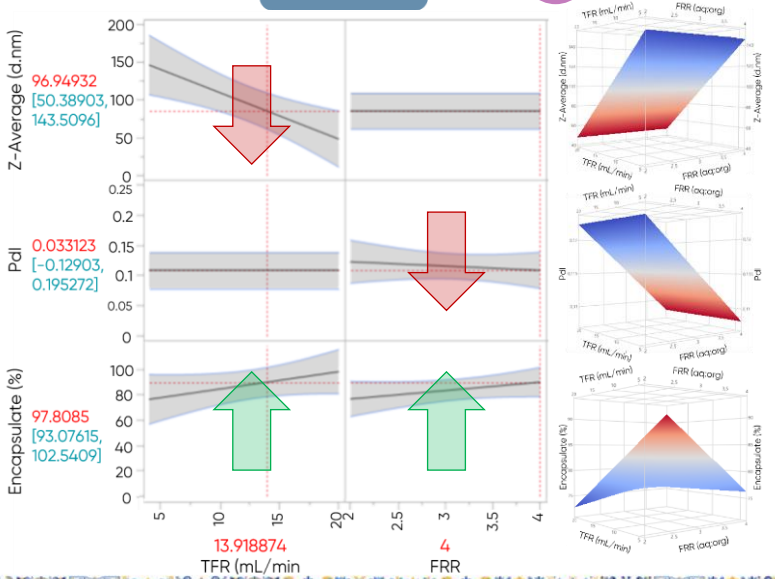
Chip
Type: Fluidic 187
Geometry: Herringbone Mixer

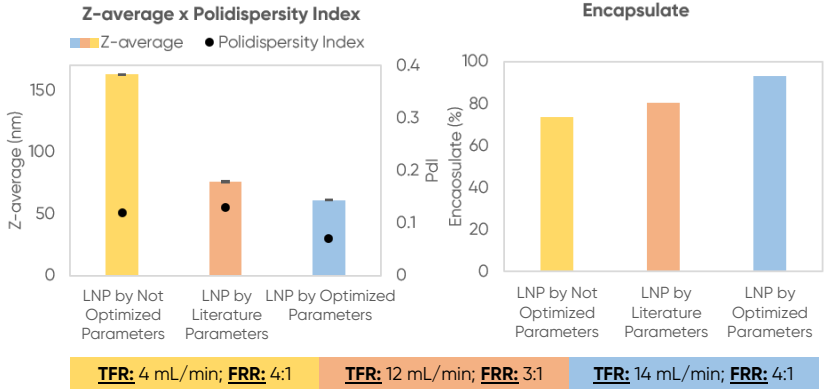
Pump
Type: Pressure Pump

Total Flow Rate (TFR)
Value (mL/min):
[4-20]

Flow Rate Ratio (FRR)
Value (aq:org):
[2:1; 3:1; 4:1]

Microfluidics Process





TFR: 4 mL/min; **FRR:** 4:1 | **TFR:** 12 mL/min; **FRR:** 3:1 | **TFR:** 14 mL/min; **FRR:** 4:1

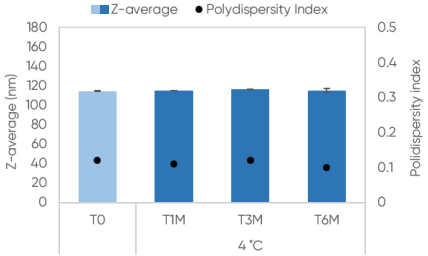
QbD-guided microfluidic process optimization yields smaller, more homogeneous LNPs with higher encapsulate

QbD-driven LNP-mRNA Optimization · Process Optimization

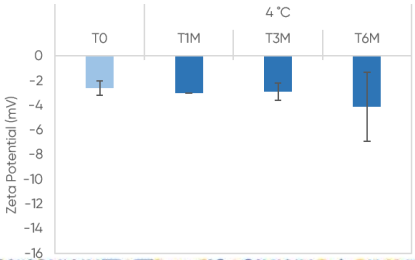
Extended Physicochemical Characterization & Pre-Stability

LNP Attributes

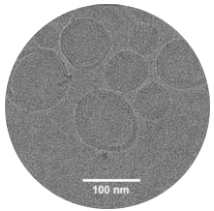
Z-average x Polidispersity Index



Zeta Potential



Morphology (cryo-TEM)



Residual Solvents

< 0.03 %

Particle Concentration

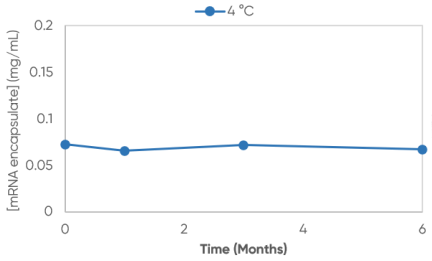
(1.20±0,02)×10¹²

Lipids Assay

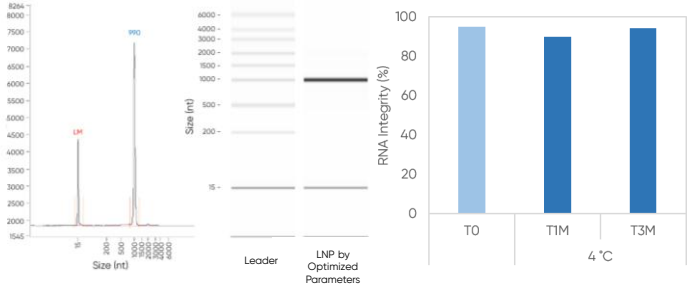
[Chol] = 0.376 mg/mL
[DSPC] = 0.211 mg/mL
[DMG-PEG] = 0.106 mg/mL

mRNA Attributes

RNA Encapsulated



RNA Integrity



LNPs produced by the DoE-optimized microfluidic process maintained physicochemical stability for at least 6 months at 4 °C

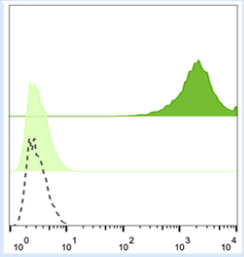
QbD-driven LNP-mRNA Optimization · Process Optimization

In Vitro & *In Vivo* Performance

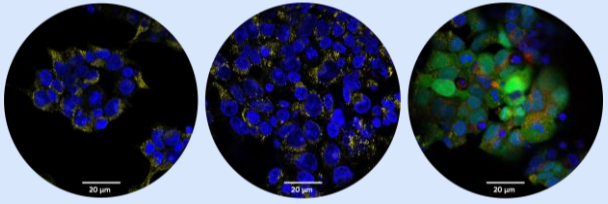
In Vitro Performance

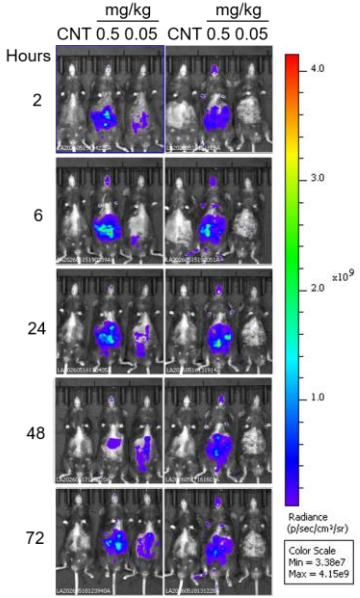
Flow Cytometry

	% eGFP⁺ Cells	MFI
Negative Control	0.7	3.2
Free eGFP mRNA	0.8	3.3
LNPs(eGFP mRNA)	98.7	1776.6

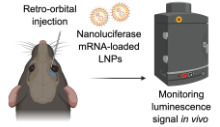


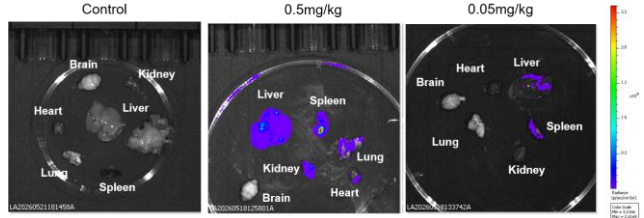
Confocal Microscopy

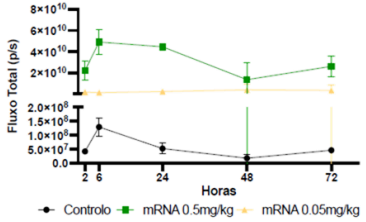


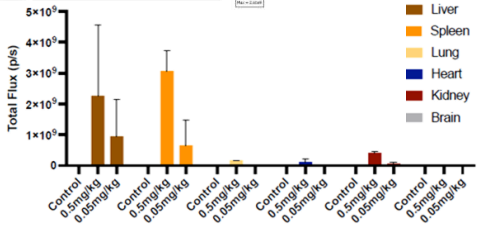


In Vivo Performance







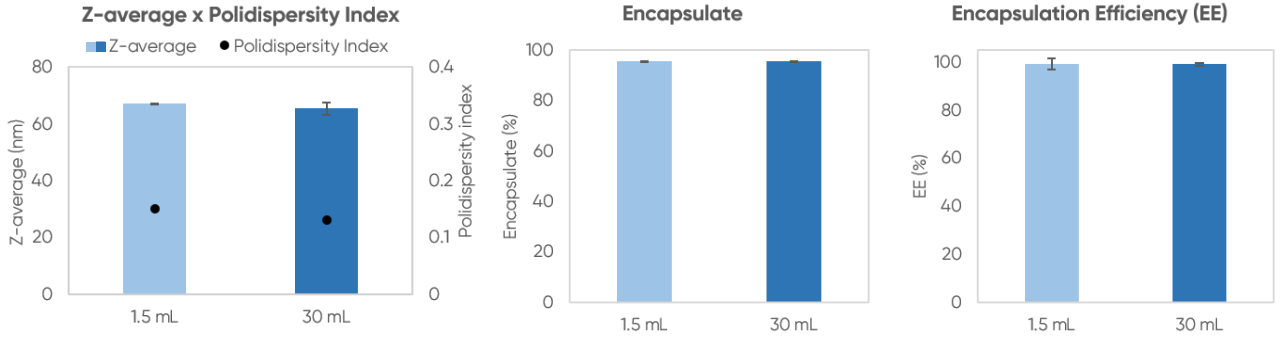


Main Conclusions

- → Rapid onset of protein expression after systemic LNP delivery
- → Clear **dose–response relationship** with **liver and spleen targeting**
- → Suitable for **short-term**, **high-intensity expression** applications

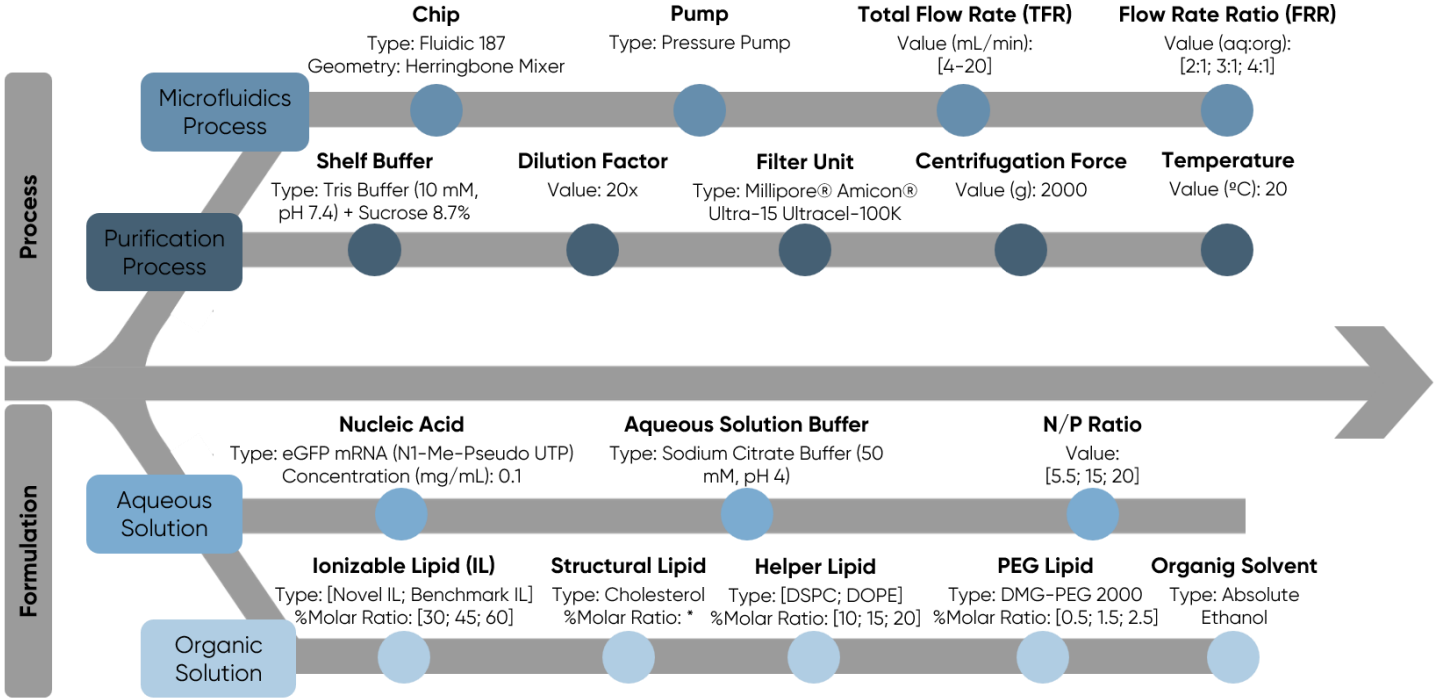
QbD-driven LNP-mRNA Optimization · Process Optimization

20-Fold Scale-Up



The DoE-optimized microfluidic process **preserved LNP physicochemical properties** and ***in vitro*** **activity** following a **20-fold scale-up**

QbD-driven LNP-mRNA Optimization • Ishikawa Diagram



Target LNP-mRNA

Z-Average (d.nm): [50-150]

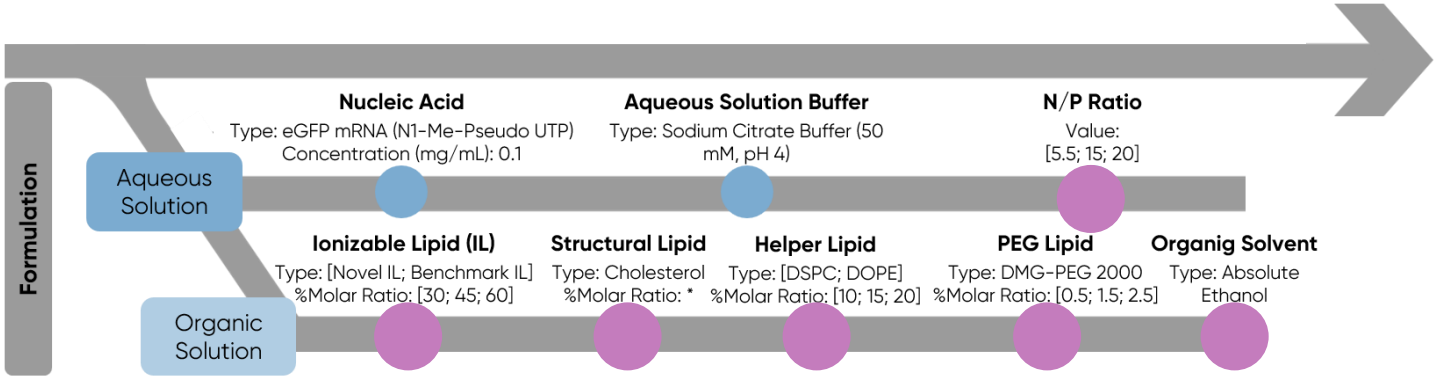
PdI: <0.2

Encapsulate (%): >95

*The molar percentage of the structural lipid corresponds to the remaining fraction necessary to ensure that the total molar contribution of all lipids equals 100%

QbD-driven LNP-mRNA Optimization • Formulation Optimization

DoE Factors & Responses



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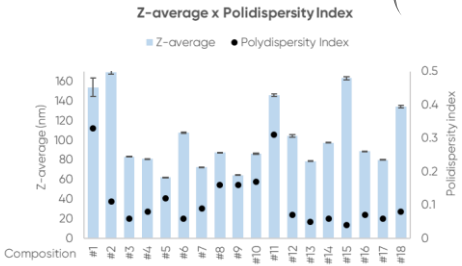
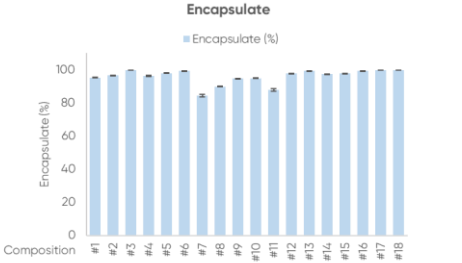
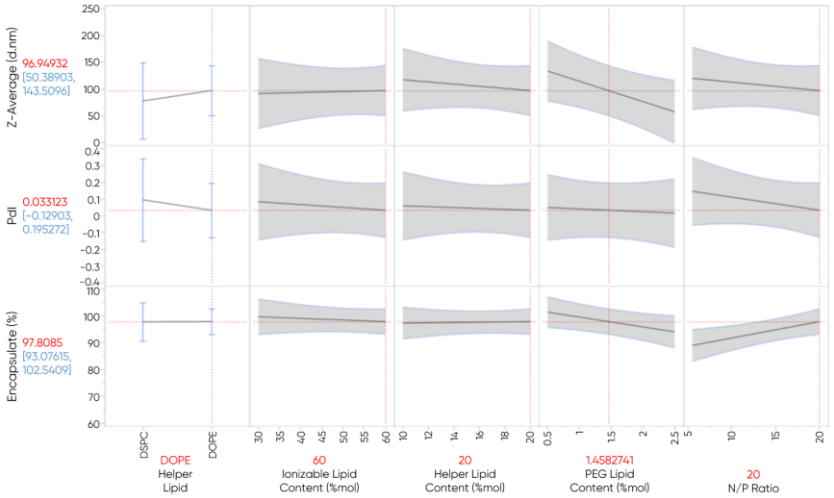
QbD-driven LNP-mRNA Optimization · Formulation Optimization

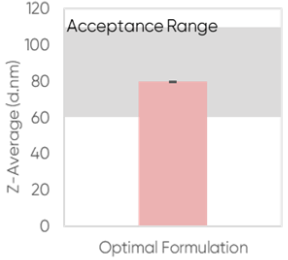
DoE-driven selection of optimal LNP compositions based on physicochemical properties

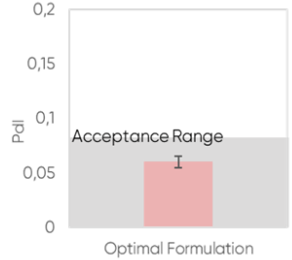
Validation of DoE prediction profiler

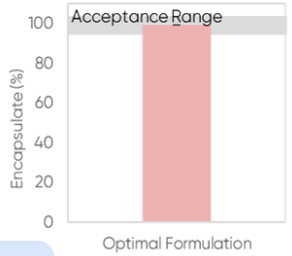








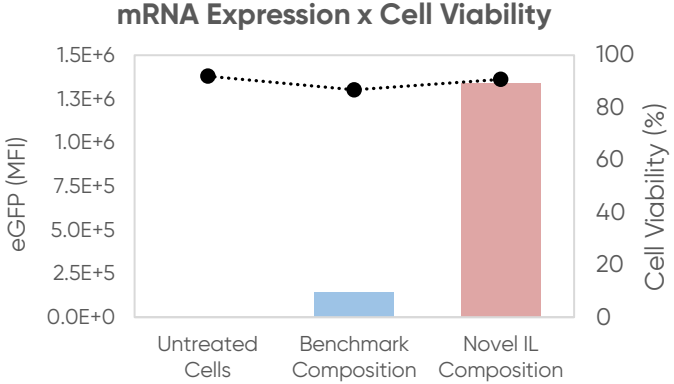




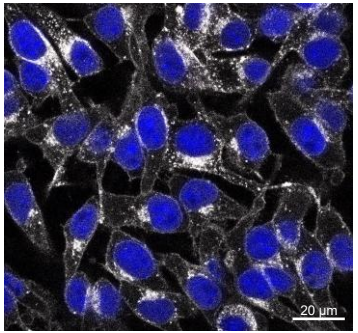
QbD-driven DoE enables **predictive optimization of formulation parameters** for LNP–mRNA systems with a novel ionizable lipid

QbD-driven LNP-mRNA Optimization • Formulation Optimization

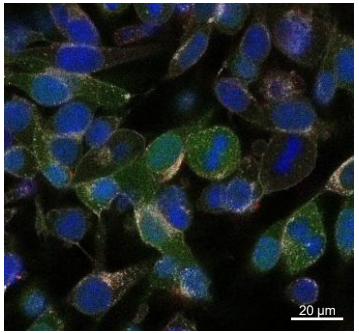
In Vitro Performance



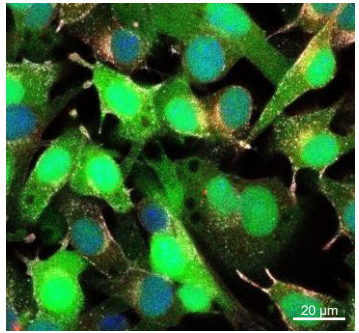
Untreated Cells



Benchmark Composition



Novel IL Composition

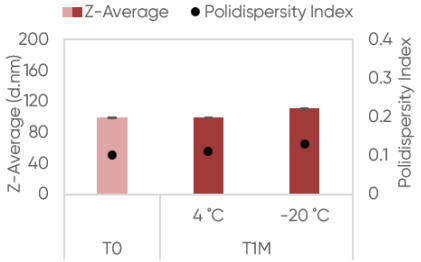


QbD-optimized LNP-mRNA with the **novel ionizable lipid enhance *in vitro* performance** compared to commercial counterparts

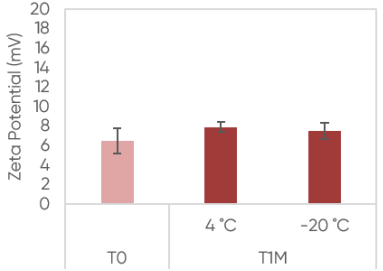
QbD-driven LNP-mRNA Optimization • Formulation Optimization

Pre-Stability Study

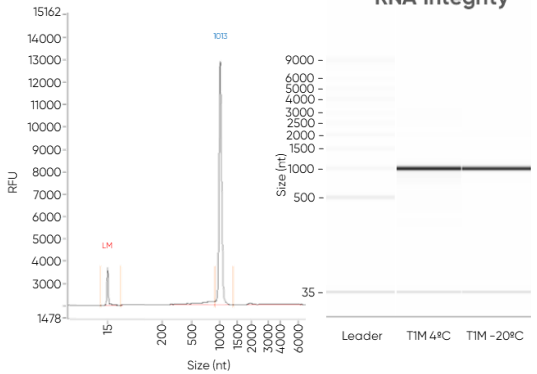
Z-Average x Polidispersity Index

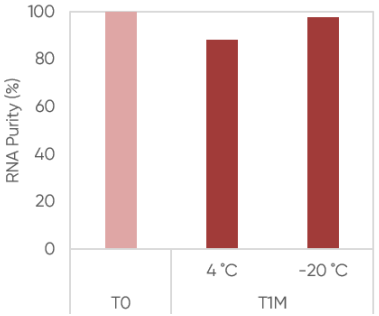


Zeta Potential

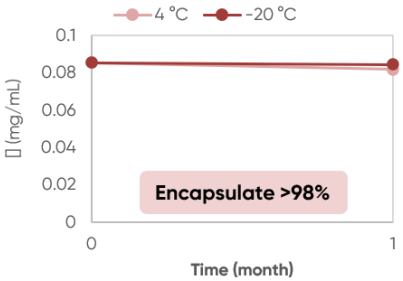


RNA Integrity

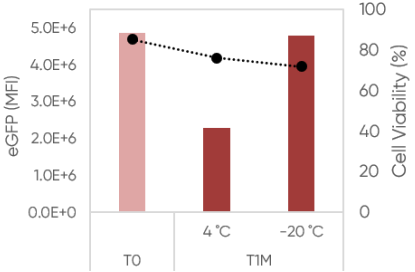




[RNA Encapsulated]



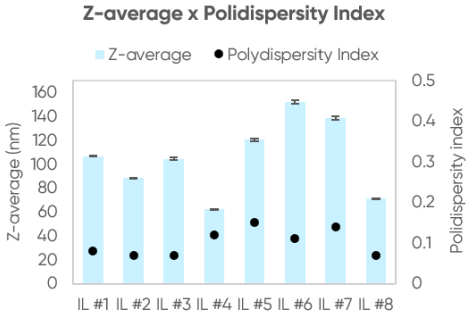
mRNA Expression x Cell Viability

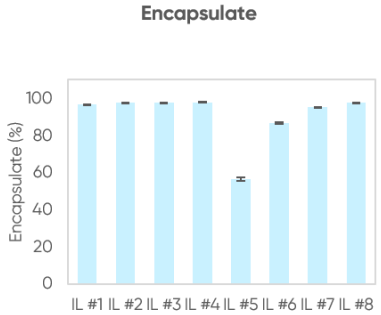


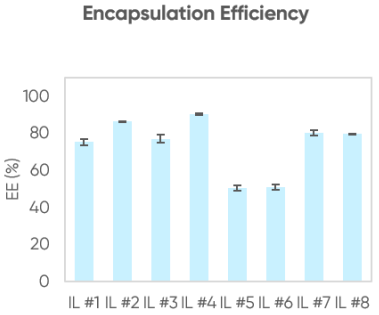
LNP–mRNA formulated with a **novel ionizable lipid** remains **stable at -20 °C** for at least **one month**

Screening of Ionizable and Helper Lipids • Additional Studies

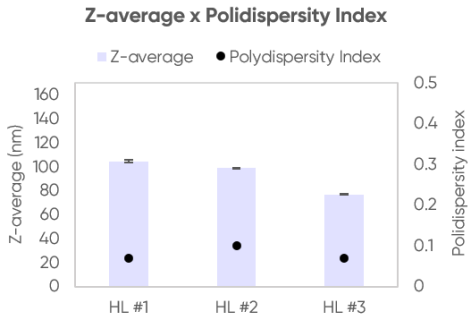
Ionizable Lipids (IL)

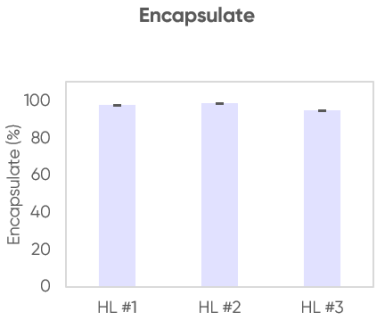


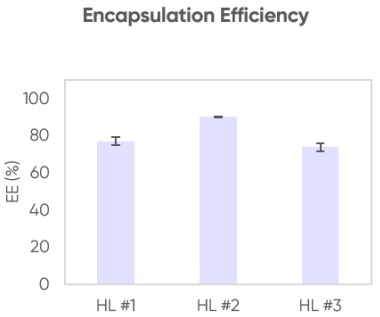




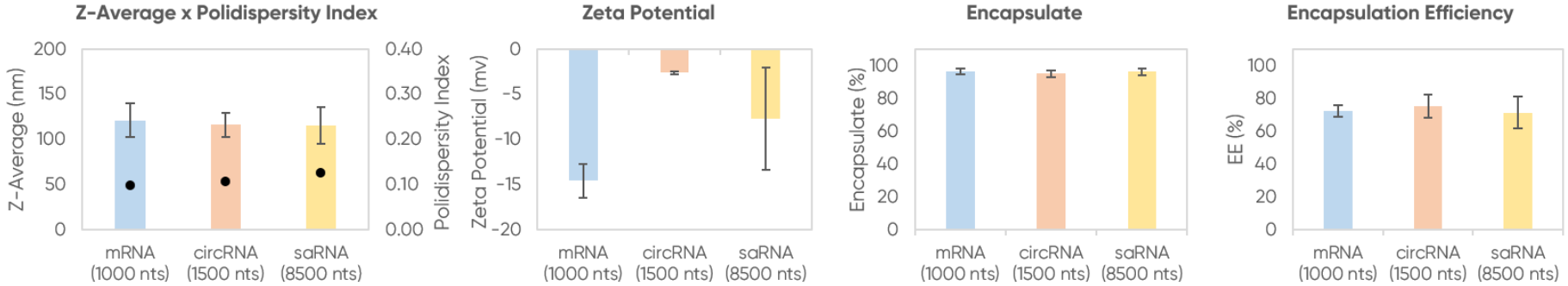
Helper Lipids (HL)







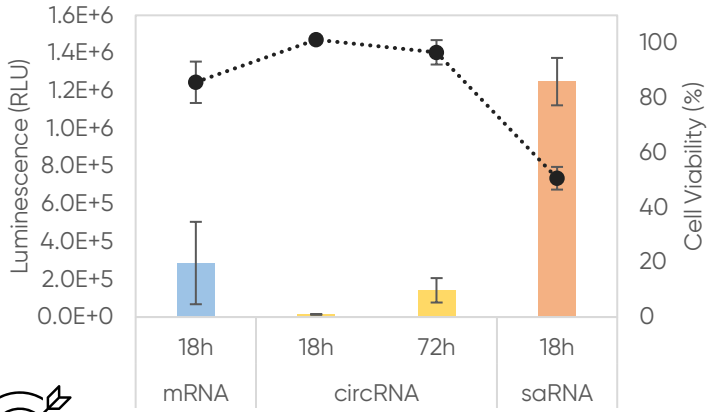
Evaluation of Different RNA Modalities • Additional Studies



LNPs exhibit **similar physicochemical properties** when encapsulating RNA payloads of different lengths and structures

Evaluation of Different RNA Modalities · Additional Tests

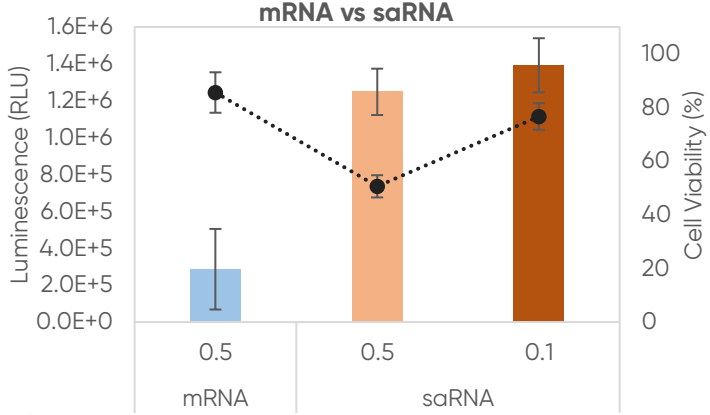
Expression & Viability Across RNA Modalities



saRNA maximizes potency but **reduces viability**

mRNA and **circRNA preserve viability**, with **circRNA** requiring **longer incubation** to reach expression levels comparable to mRNA

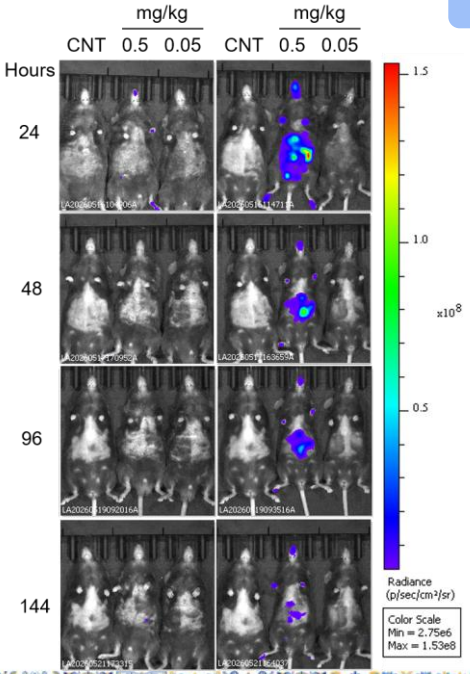
Dose-Dependent Expression & Viability: mRNA vs saRNA

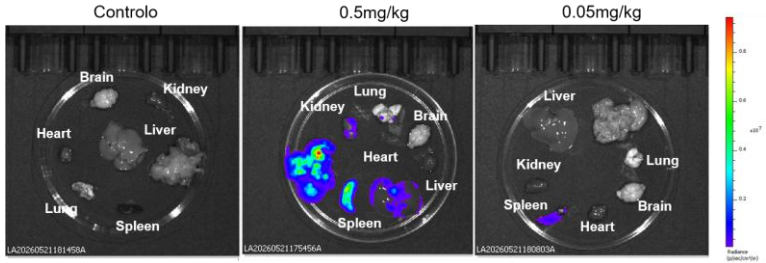


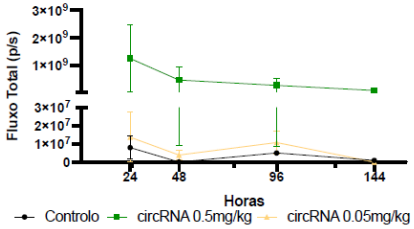
saRNA delivers **~5× higher expression** at **~5× lower doses** than mRNA, while maintaining comparable cell viability

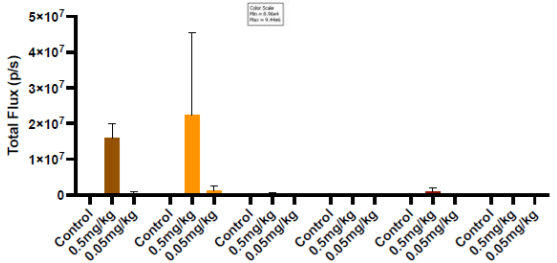
Evaluation of Different RNA Modalities · Additional Tests

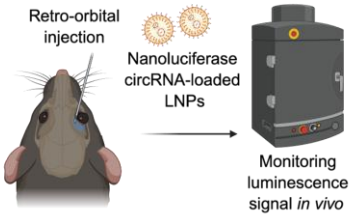
circRNA *In Vitro* Performance









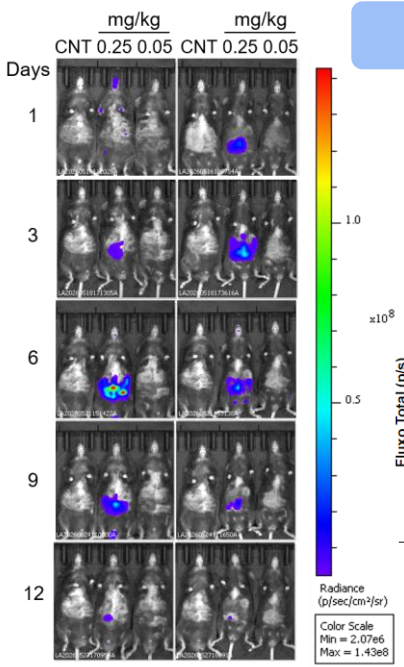


Liver
Spleen
Lung
Heart
Kidney
Brain

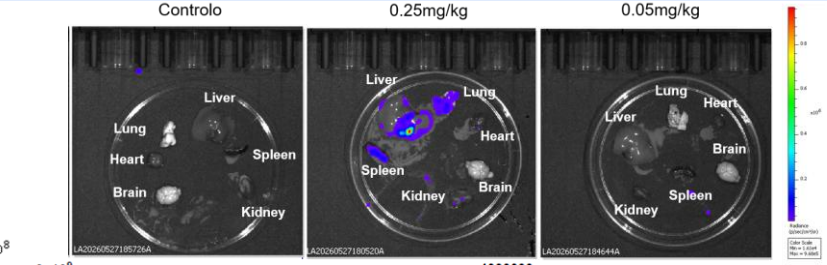
Main Conclusions

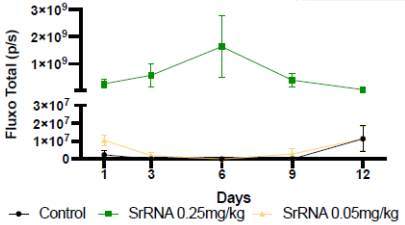
- → Stable, **dose-dependent** NanoLuc expression with predominant **liver and spleen targeting**
- → Provides a balance between **expression duration and stability**

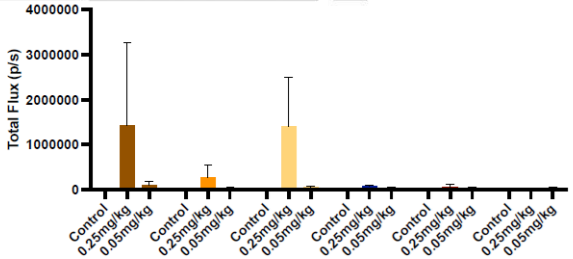
Evaluation of Different RNA Modalities · Additional Tests

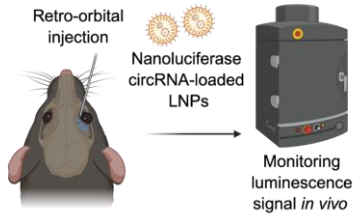


saRNA/srRNA *In Vitro* Performance









- Liver
- Spleen
- Lung
- Heart
- Kidney
- Brain

Main Conclusions

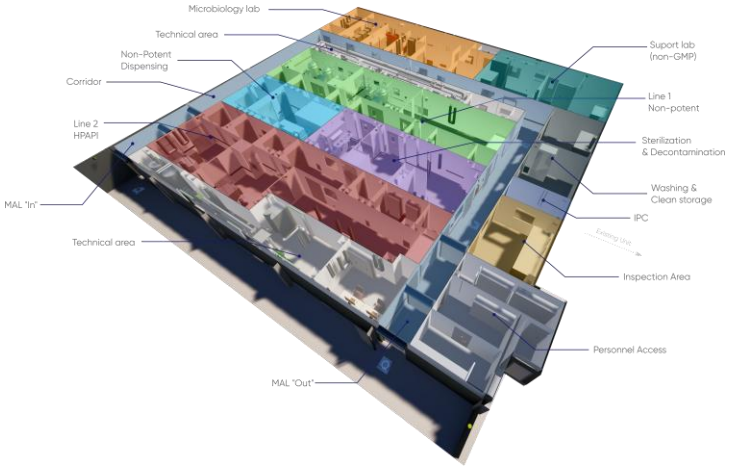
- → **Prolonged** and **sustained** protein expression *in vivo*
- → **Strong dose–response** with **liver**- and **spleen**-preferential distribution
- → Ideal for **long-lasting therapeutic** expression

Key Findings & Conclusions

- **QbD**- and **DoE**-driven optimization enabled the **rational design of LNP–mRNA systems and precise control of key attributes**
- **20× scale-up preserved physicochemical properties and *in vitro* activity**, confirming process robustness and industrial relevance
- **Novel ionizable lipid** seems to **significantly improve *in vitro* performance** compared to benchmark formulations
- Optimized formulations exhibited **pre-stability** for **up to 1 month at 4 °C and/or -20 °C**
- The platform demonstrated **high versatility**, with consistent performance across different lipid compositions and multiple RNA modalities

Next Steps

- Evaluate the **novel ionizable lipid *in vivo*** to assess performance, safety, and delivery efficiency
- Assess **long-term** formulation **stability**
- Further **optimize and expand RNA modalities**, broadening applicability across different therapeutic approaches
- Develop and scale up the production process under **GMP conditions**
- New **manufacturing unit for GMP production of injectables**



Acknowledgments

Bluepharma's Research & Innovation Team

Tumor Microenvironment and Targeted Therapies Group
Vectors, Gene and Cell Therapy Group

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