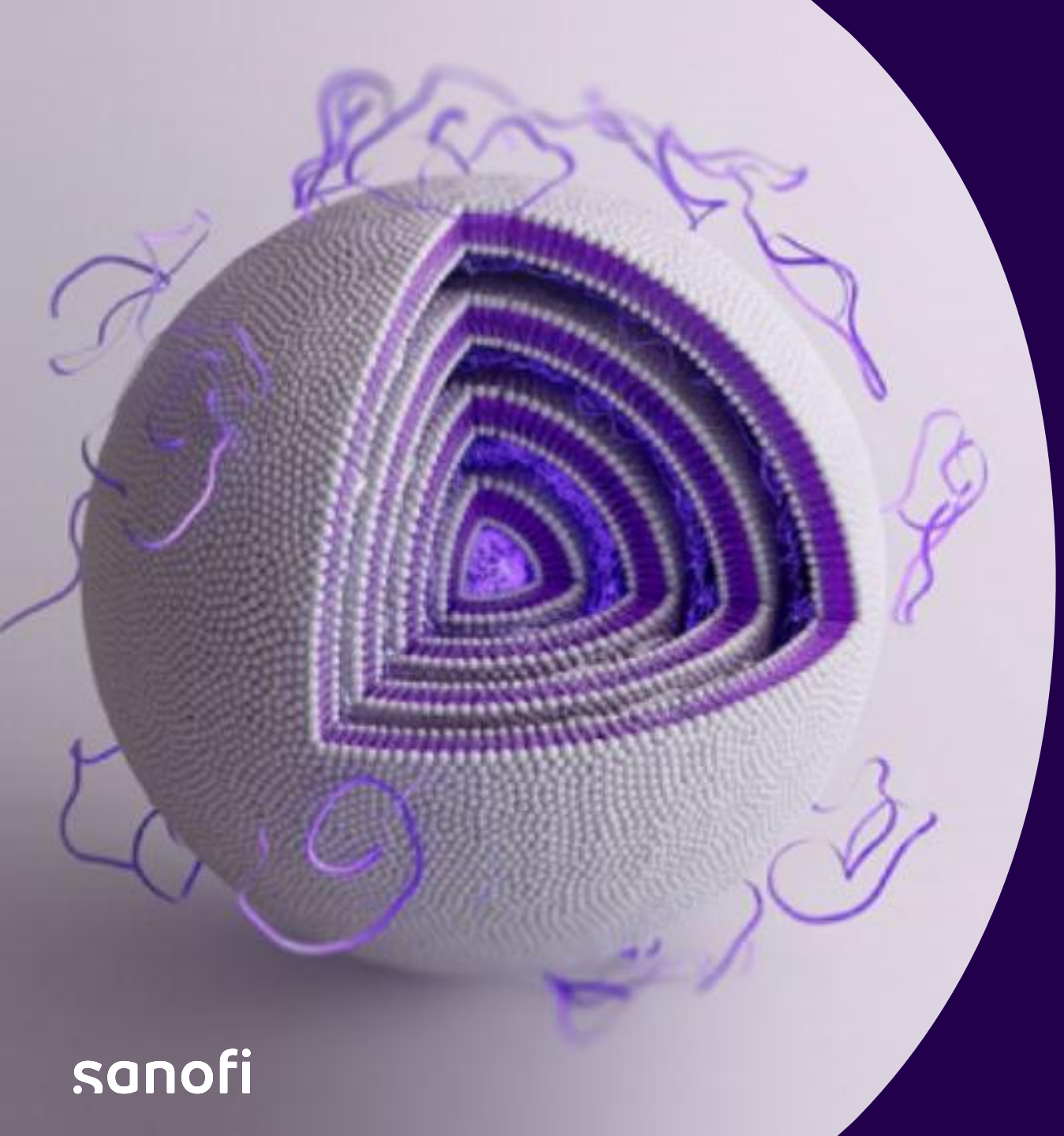

Development of mRNA-LNP manufacturing process platform

LNP formation unit operation

Sumit Luthra

CMC Drug Product Development, mRNA CoE, Sanofi, Waltham, MA

Hourdel et al International Journal of Pharmaceutics, 672, 2025

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Introduction and Overview

Historical Development

•From "magic bullets" concept to modern delivery systems
•mRNA discovery (1961) to successful LNP encapsulation (1970s)
•First commercial LNP-mRNA vaccines: 2020 (COVID-19)

Key Topics

•LNP Structure and Size Considerations
•Nanoprecipitation Process
•Manufacturing Methods
•Mixing Technologies
•Formulation & Process Parameters
•Scale-up Challenges

LNP Structure and Size Considerations

Composition: Four key lipid components

•Ionizable lipid (IL): mRNA binding, endosomal escape

•Phospholipid: structural integrity, membrane fluidity

•Cholesterol: membrane stability, fusion

•PEGylated lipid: size control, steric barrier

Structures

•Multilamellar

•Dense core (most common)

•Bleb structure

Size : Importance & Dependent Effects

•Must be reasonably small for sterilizing filtration

•Affects biodistribution and immune response

•<10 nm: rapid elimination

•20-50 nm: direct lymph node targeting

•>150-200 nm: trapped at injection site

•85-100 nm: optimal immune activity

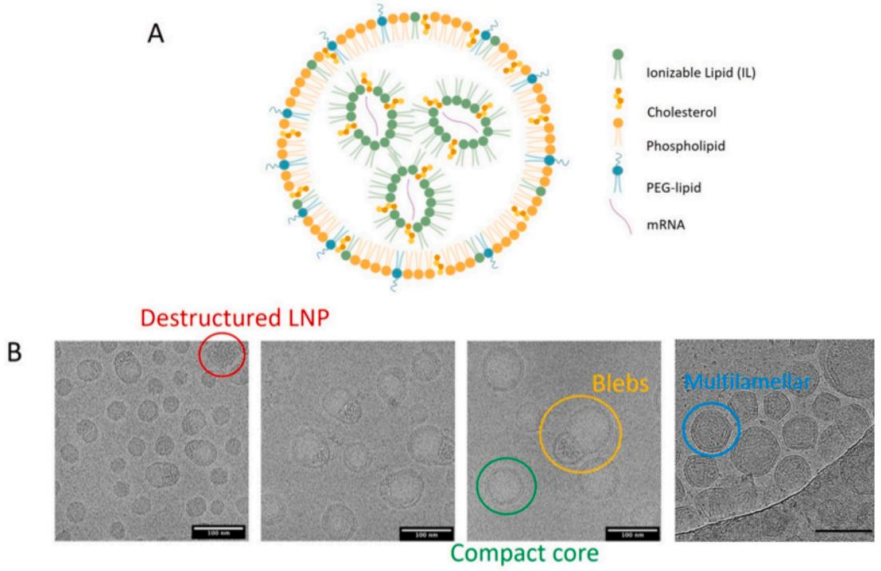
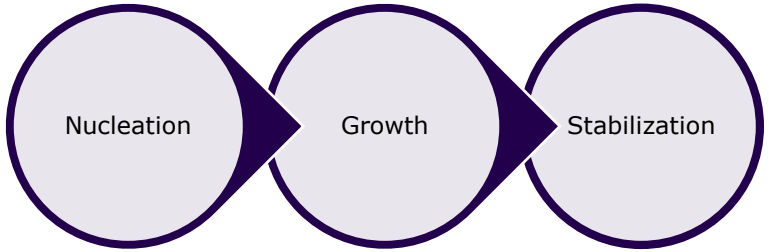


Fig. 1. Schematic description of an LNP structure (A) and cryo-TEM image of LNPs (B) (Eygeris et al., 2020; O'Brien Laramy et al., 2023).

Nanoprecipitation Process & Influencing Factors



- **Nucleation:** Lipids reach supersaturation and form solid nuclei
- **Growth:** Condensation and coagulation of lipids around nuclei
- **Stabilization:** Through PEGylated lipids and electrostatic repulsion

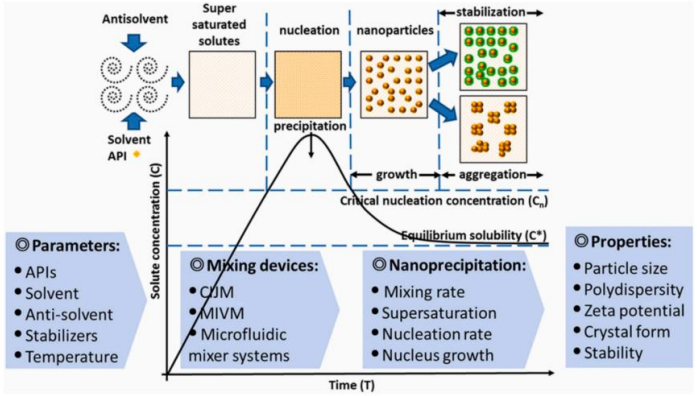


Fig. 2. Schematic representation of the nanoprecipitation process steps (Tao et al., 2019).

- **Mixing time:** Faster mixing -> smaller LNPs
- **Aqueous phase fraction:** Higher fraction -> higher supersaturation -> smaller LNPs
- **Temperature:** Higher temperature ? higher lipid solubility -> larger LNPs
- **Solvent-antisolvent miscibility:** Must be miscible for effective nucleation
- **mRNA electrostatic interactions:** Negative charge facilitates association with ionizable lipids

Manufacturing Methods Comparison

Batch Processes

•Thin lipidic film evaporation

•Organic phase injection

•Reverse phase evaporation

•Emulsion-diffusion

•Limitations:

- Poor reproducibility
- Scale-up challenges
- Low encapsulation efficiency (30-40%)

Continuous Processes

•Microfluidic processes (lab scale)

•Large-scale mixers (industrial scale)

•Advantages:

- Better control of parameters
- Improved scalability
- Higher encapsulation efficiency
- Monodisperse nanoparticles

Mixing Technologies: From Micro to Large Scale

Parameter	Microfluidic Mixers	Large-Scale Mixers
Common types	Staggered Herringbone, Hydro Flow Focusing, T-mixers	T-shaped, Static, Chaotic mixers
Flow regime	Laminar (Re < 2000)	Laminar or turbulent
Mixing mechanism	Diffusion at liquid-liquid interface	Convection, turbulent mixing
Scale	Lab/small batches	Clinical/industrial
Scalability	Limited	Good

Fluid Mixing Characterization & Optimization

Characterization Methods

•Experimental:

- Passive tracers (dyes, fluorophores)
- Particle Image Velocimetry (PIV)
- Laser-Induced Fluorescence (LIF)

•Numerical:

- Computational Fluid Dynamics (CFD)
- Mixing index calculations

Geometric Optimization

•Critical features:

- Mixing chamber design
- Shape of downstream part
- Inclination angle of inlet flows

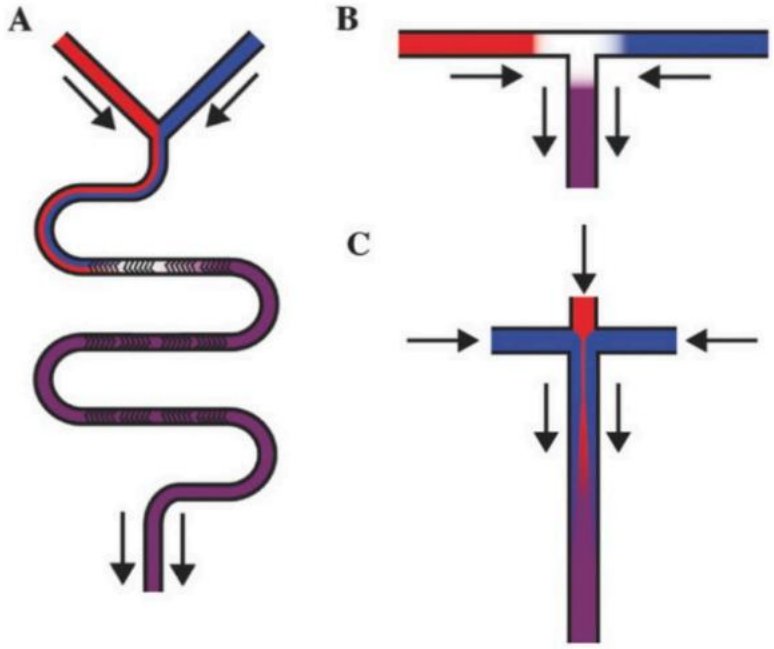


Fig. 3. Schematic representation of most used micromixers: (A) SHM, (B) T mixer, (C) HFF. The red phase corresponds to the organic phase and the blue phase corresponds to the aqueous one (Evers et al., 2018).

Formulation Parameters

Organic Phase

•Ionizable lipid concentration (N/P ratio)
- Controls encapsulation rate
- Critical for mRNA binding

•Organic solvent type and fraction
- Must be ≥80% for efficient nanoprecipitation
- Different solvents affect particle size

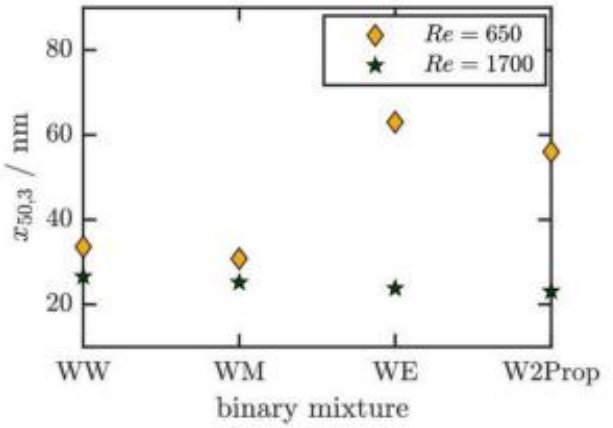


Fig. 7. Evolution of the simulated mean particle size as a function of the solvent nature at two Reynolds numbers (Schikarski et al., 2023).

Aqueous Phase

•pH effects
- Lower pH → higher encapsulation efficiency
- Higher pH → increased LNP size

•Buffer type
- Affects cell activity and LNP properties
- Citrate buffer: larger LNPs but better mRNA protection

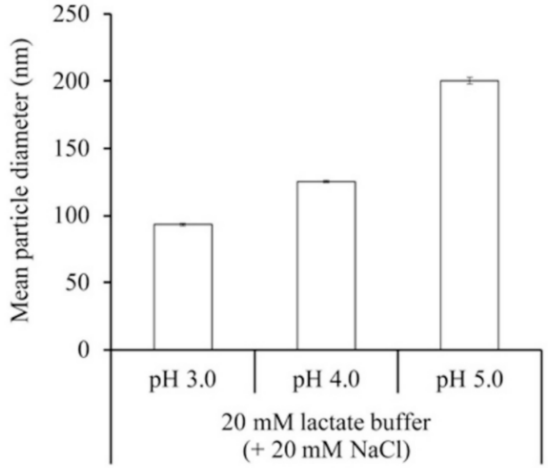


Fig. 8. Impact of the pH of the aqueous phase on the LNP size (Nakamura et al., 2022).

Process Parameters & Scale-up Challenges

Flow Rate Effects

•Higher flow rate → lower mixing time → smaller LNPs

•Reynolds number determines mixing regime

•Transition to turbulent flow (Re ≈ 1900) reduces polydispersity

•Size plateau reached at turbulent transition

Scale-up Challenges

•Maintaining consistent mixing quality

•Different mass transfer between scales

•Hydrodynamic control at larger scales

•High throughput while maintaining quality

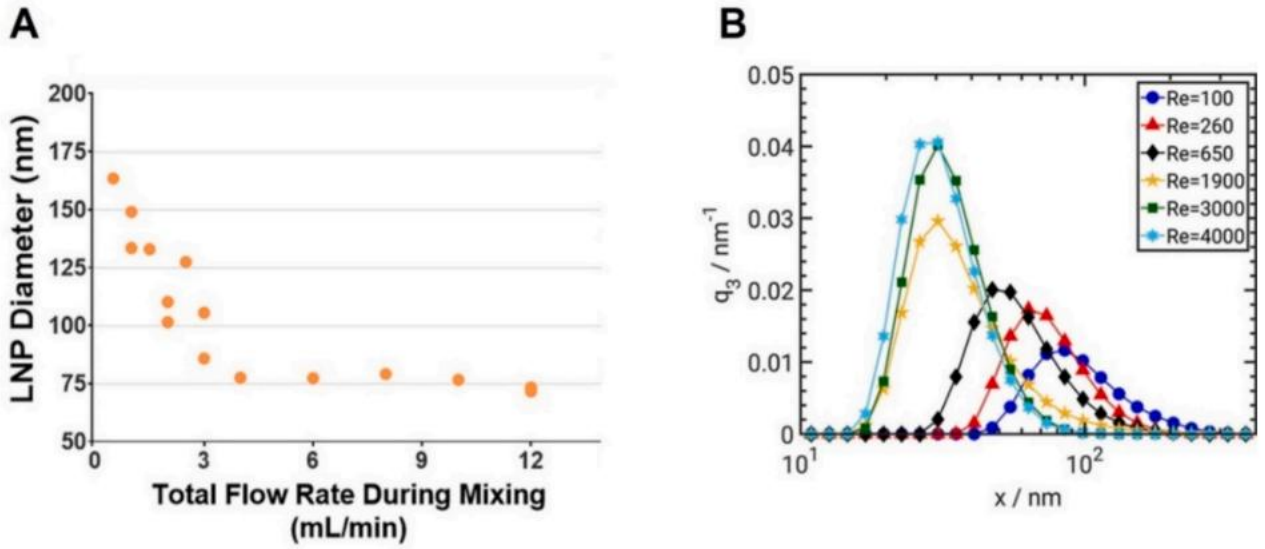


Fig. 15. (A) LNP size evolution as a function of the total flow rate (Hassett et al., 2021), (B) LNP size distribution evolution as a function of the Reynolds number (q_3 is the mass-weighted density) (Schikarski et al., 2019).

Systematic Optimization & Conclusion

Formulation parameters

- Optimize lipid composition
- Tune N/P ratio
- Select appropriate buffer and pH
- Determine optimal phase ratio

Process parameters

- Design mixing geometry
- Set appropriate flow rates
- Control temperature
- Establish scale-up strategy

Questions?

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Sumit Luthra is a Sanofi Employee and may hold shares and/or stock options in the company

Back-ups (Previously disclosed slides)

mRNA product manufacturing *multi-steps* description

CMC

Plasmid manufacturing

- Cell bank
- Fermentation
- pDNA purification & linearization

Drug Substance manufacturing

Linearized DNA template

- In-vitro transcription / capping
- mRNA purification

LNP Bulk Drug Product (bDP) formulation

- Lipids
- mRNA
- LNP formulation
- LNP purification

Drug Product manufacturing

- LNP bDP
- Fill & Finish

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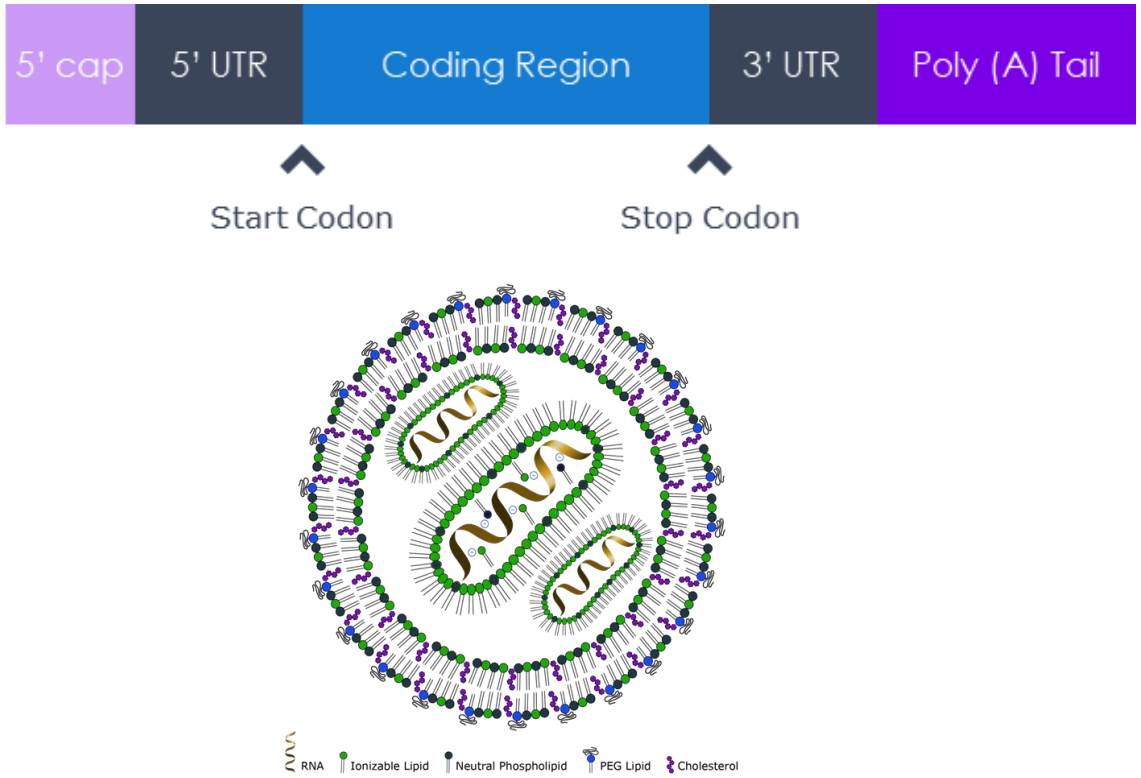
Sanofi's mRNA Center of Excellence focus on platform *innovation* & program *acceleration* of vaccines and therapeutics



mRNA-LNP model*

- Influenza
- Respiratory syncytial virus
- Chlamydia
- Additional infectious diseases targets
- *....and therapeutics*

***Daniel et al, TIBTEC 2022**

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mRNA-LNP platform need to evolve to address diverse therapeutic area needs

	1st gen mRNA	*2nd gen mRNA*	*3rd gen mRNA*
Immunogenicity	✓ High	✓ High	✓ High
Reactogenicity	— Moderate to high	✓ Tolerability profile in line with established vaccines	✓ Tolerability profile in line with established vaccines
Thermostability	— ~1 month shelf life (2-8°C)	✓ Lyophilized or 9 – 12 months fully liquid	✓ Lyophilized or 9 – 12 months fully liquid
Duration of expression	— 1 – 3 days	— 1 – 3 days	✓ Extended half-life
Targeting	— -	— -	✓ Efficient cell & organ specificity
	Applicable to pandemic market	Required profile for general vaccine markets	Optimal profile for therapeutics

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mRNA/LNP specific *Quality Attributes* linked to composition and function in *CMC platform* development

mRNA:

Identity
Sequence integrity
% 5’ capping efficiency
3’ Poly(A) tail length & level

Activity (protein expression)

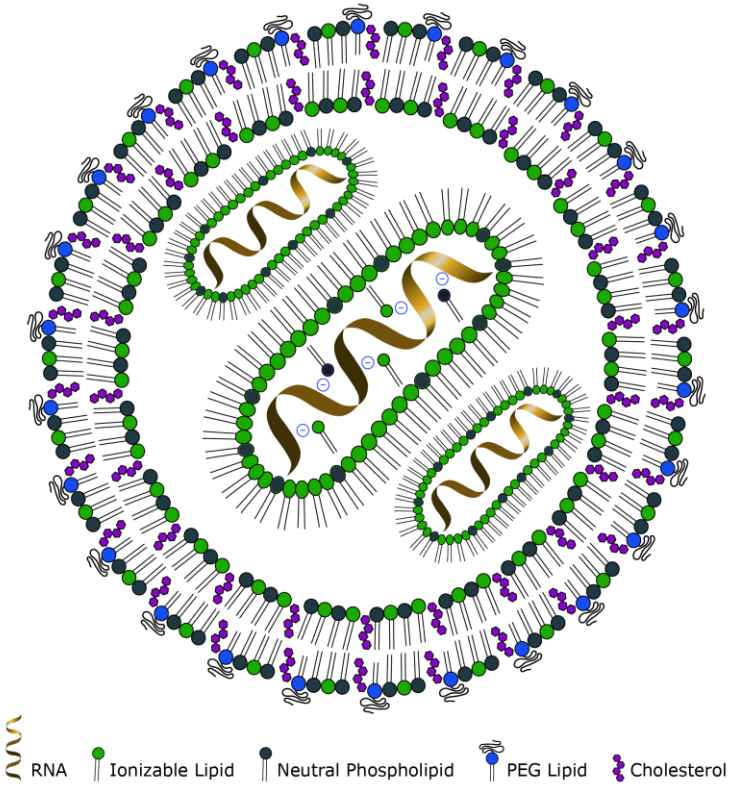
mRNA impurities

mRNA related substances *(untailed, dsRNA, abortive transcripts, fragments, aggregates..)*

Process related impurities *(pDNA, enzymes, NTPs,..)*

Lipids components

- Identity
- Total and individual Content
- Impurities



Lipid Nano Particles:

- Size & distribution
- Surface charge
- Morphology
- % mRNA encapsulated
- Total mRNA content
- Individual mRNA content (multivalent)

LNP impurities

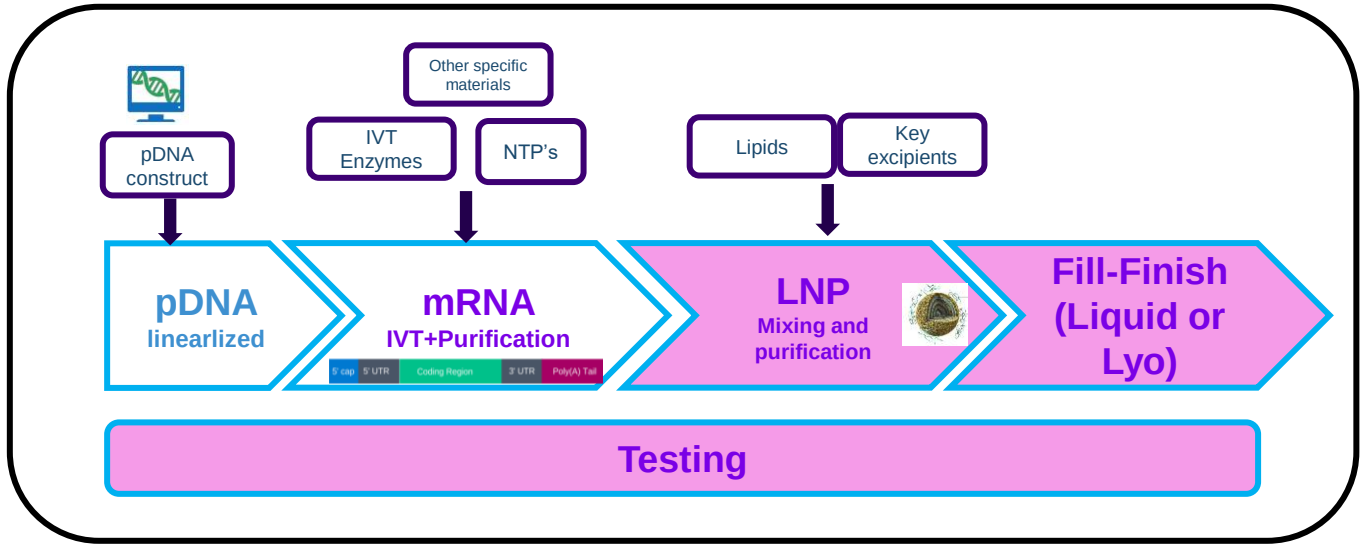
- *mRNA-lipid adduct*
- *Lipid degradants*
- *Process related impurities (Ethanol, RNAse..)*

Activity:

- Protein expression (individual)

USP Draft Guidelines available- Analytical Procedures for mRNA Vaccine Quality

E2E Manufacturing Process & *dosage forms* considerations early in *CMC platform* development



Presentation focus

Dosage forms

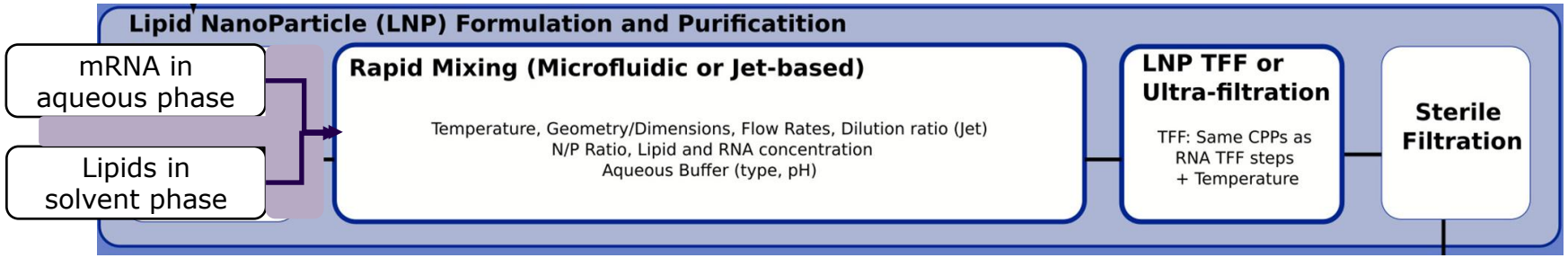
Frozen liquid vials or PFS
Lyophilized vials with diluent PFS
Fully liquid PFS
Micropellet, dry powder,…

LNP, Fill-finish and DP

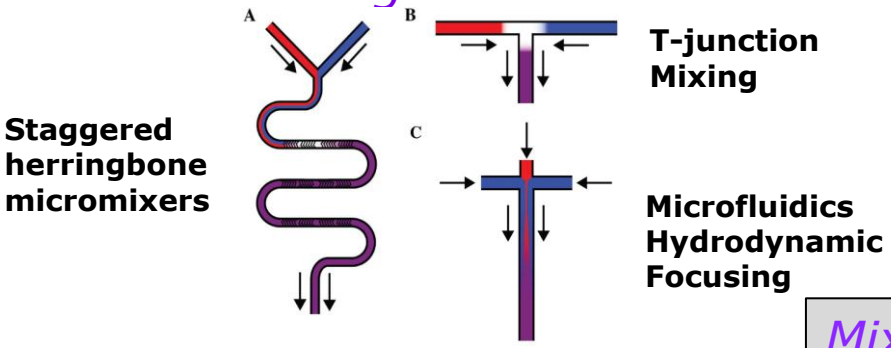
- LNP thermostability brings time/temperature constraints for well established fill-finish processes

- Drug device combination product requirement based on vaccine image to be incorporated early in development

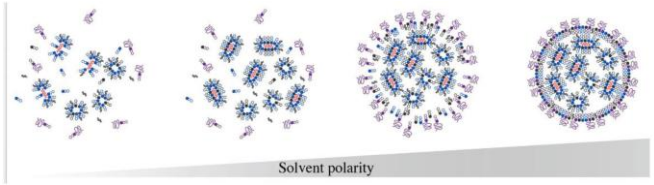
Manufacturing process of LNP



Mixing



Self-assembly of LNP



Mixing technology and scalability:
- scale-out (Parallelization): mainly for micromixers (laminar flow), it's still complex and challenging to reach large scale (>100L)
- scale-up: for turbulent mixers, can achieve large throughputs

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Evers et al, Small Methods 2018
Daniel et al, TIBTEC 2022

LNP specificity implications to process

LNP feature choices

- Lipid choice (cationic, helper,..)
- Lipid impurities
- Lipids molar ratio
- N/P ratio (lipid to mRNA)
- Number of mRNAs in LNP
- Thermostability requirement

Process implications

- Lipid solution composition
- mRNA solution composition
- Mixing technology- scale out vs scale up
- Input Concentrations
- Total flow rate and ratio
- Careful control of LNP microenvironment during purification
- Filterability
- F/T conditions
- Turbulent and laminar stress post-LNP formation
- Careful control of freeze-drying parameters
- Short mfg time to limit temp exposure
- Compatibility with manufacturing equipment and primary container

Attributes control

- Size and polydispersity
- Morphology
- Encapsulation efficiency
- Lipid content (total and individual)
- mRNA content (total and individual)
- mRNA integrity
- Aggregation
- mRNA-Lipid adduct
- Protein expression (activity)

Formulation and process design should address these challenges following QbD approach, considering
- Prior knowledge from mRNA-LNP platform, technical studies and process modelling tools
- Product specificities: mRNA loading and multivalency, Lipid changes, LNP design

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