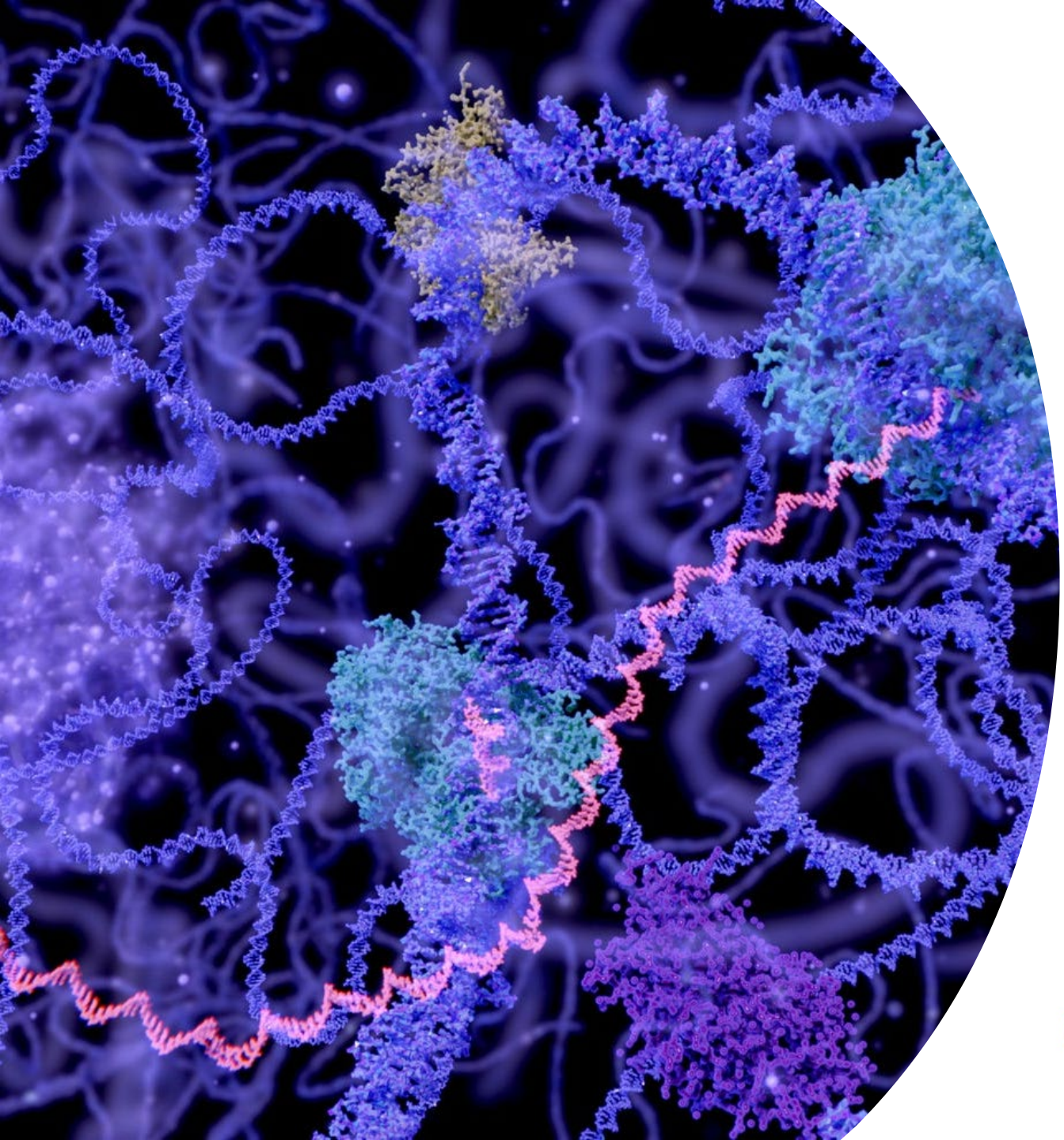


LNP mRNA Drug product Continuous Manufacturing: Leveraging single vial Freeze drying unit Technology to improve Drug Product cycle time and Stability

Florent PERAL

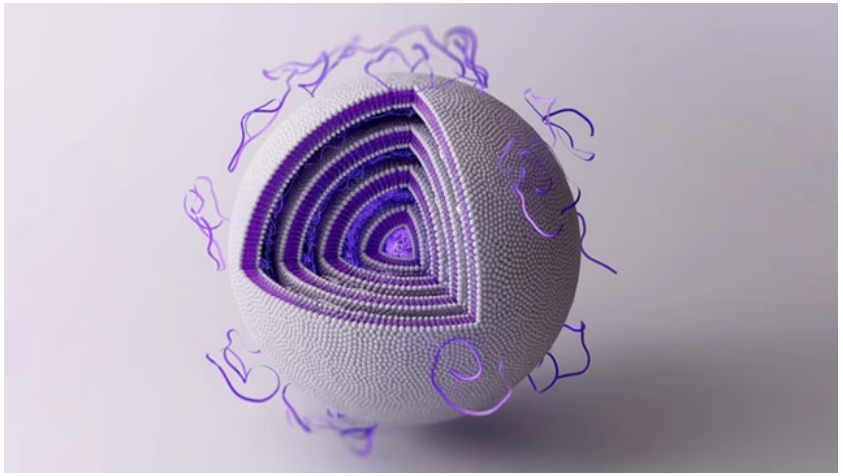
GMP Investigations, Sanofi, Waltham (MA)

26 February 2025

9th Annual CCP Summit

Commercializing Continuous Processing In Pharma

Contents



• Together, we chase the *miracles* of science •

Disclosures:

This work was financially supported by Sanofi.

Florent PERAL is a Sanofi employee and may hold shares and/or stock options in the company.

Assumptions are Florent PERAL’s view and may not reflect Sanofi position on technical interpretation

Sanofi *Vaccines Profile* 2024

500 million people
vaccinated annually with our vaccines worldwide

A world leader
in influenza and pediatrics vaccines

Ambition to deliver *8 Vaccines phase 3 programs readouts by 2028*

Aiming to save *330 tons* of plastic per year and achieve *carbon neutrality by 2030*

€8.3 billions in sales in 2024

Our vaccines are available in about *150 countries*

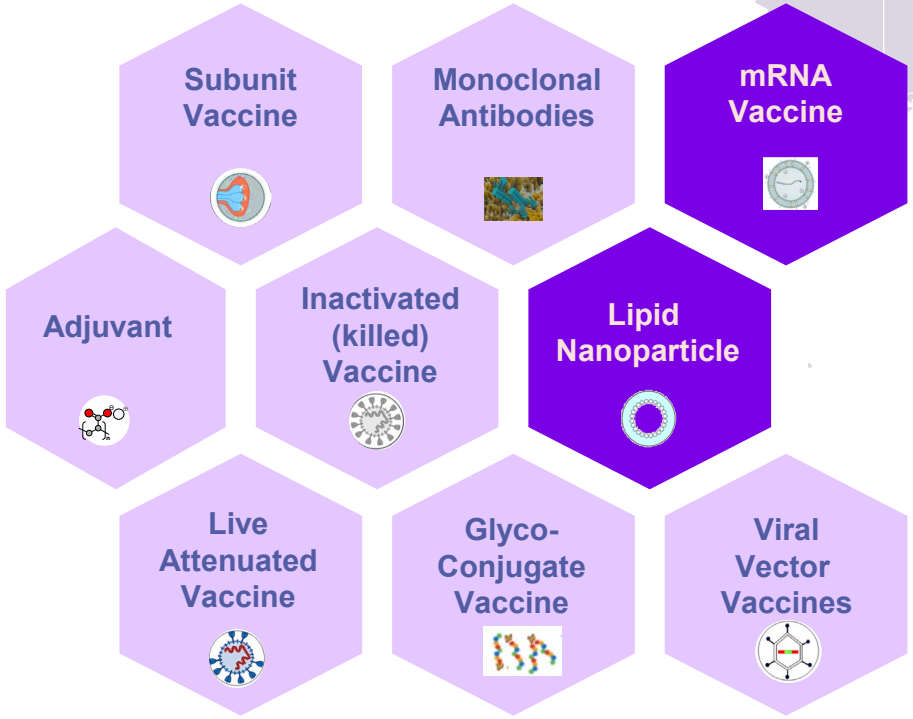
Infectious Diseases We Offer Protection Against

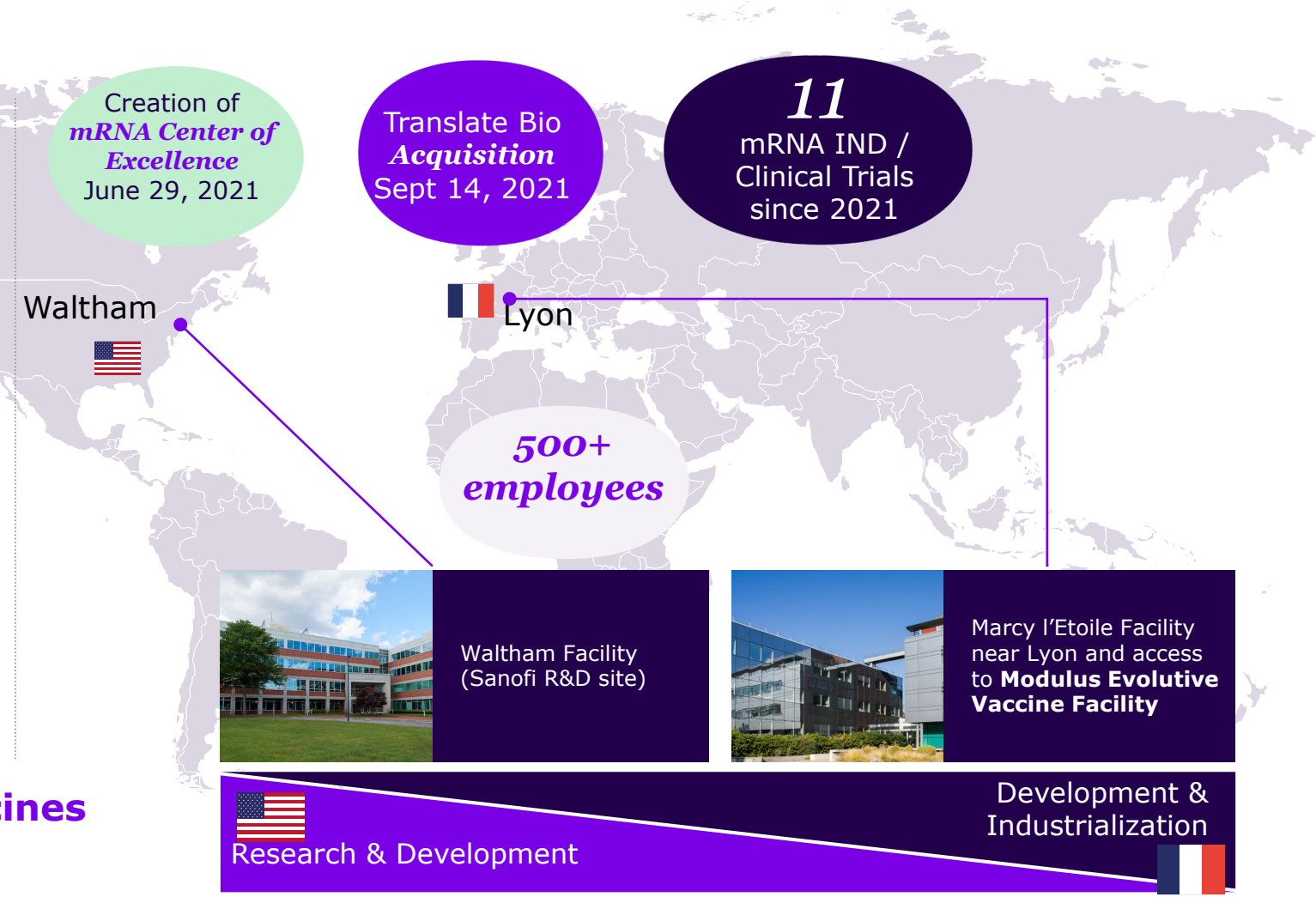
Dengue	Diphtheria	Haemophilus influenzae type b infections (Hib)
Hepatitis A	Hepatitis B	Meningococcal meningitis
Pertussis	Poliomyelitis	Rabies
Tetanus	Typhoid fever	Yellow fever

https://www.sanofi.us/en/your-health/vaccines

Sanofi's full range of modality platforms for bacterial and viral vaccines

mRNA modality platform added and mRNA CoE established

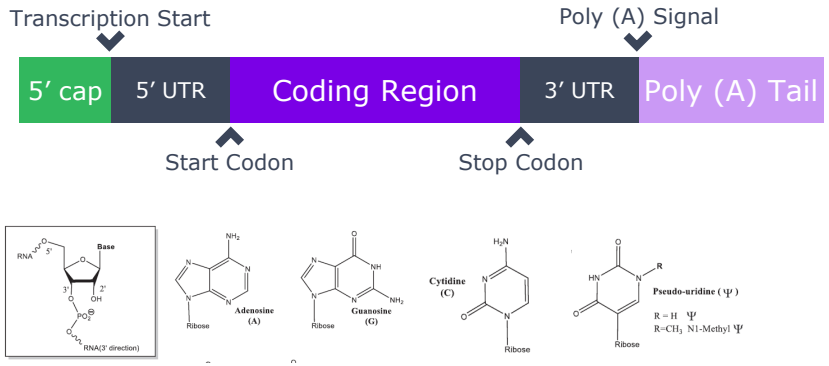




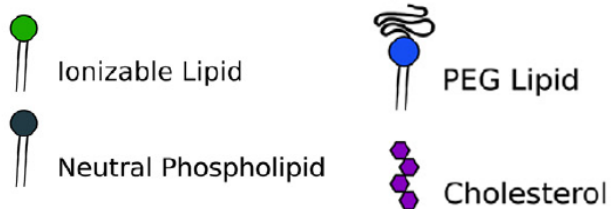
Broad range of established and new vaccines modalities platforms

LNP-mRNA – *New therapeutics modalities composition & constraints*

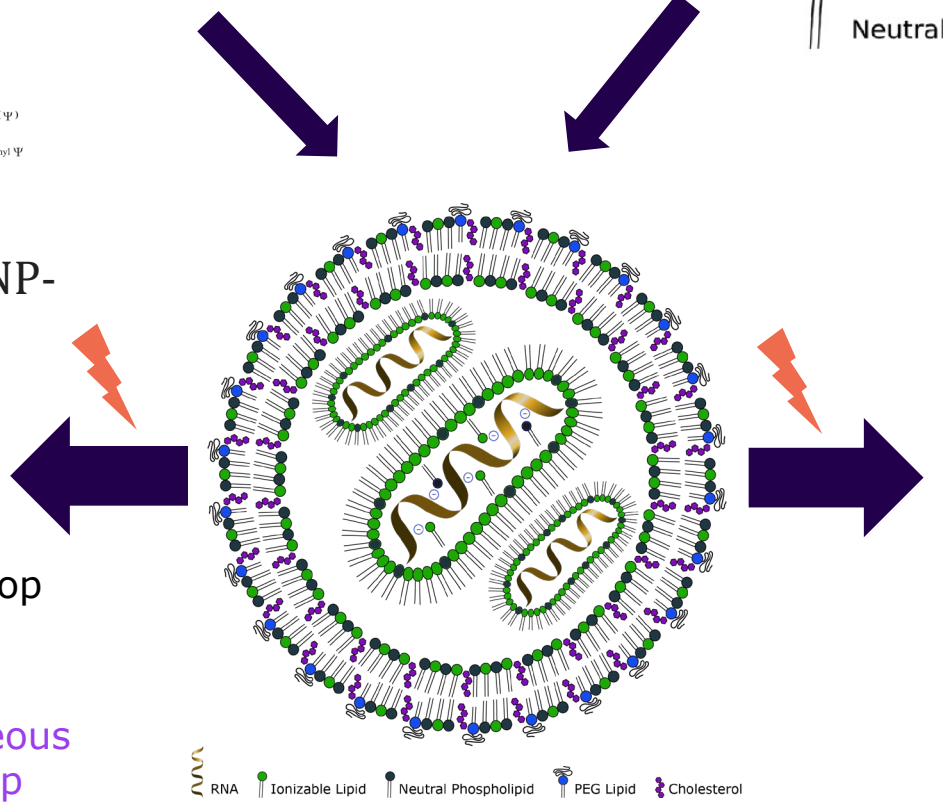
mRNA is the active ingredient



Lipids are the buildings blocks for delivery vehicle to cells



Poor long-term stability profile of LNP-mRNA in aqueous solution



Physical degradations:

Fusion, aggregation & Encapsulation drop

=> Main degradation pathway are aqueous form related and freeze Drying may help overcome long term storage issues related to chemical degradation

Chemical degradations:

mRNA degradation (fragmentation, mRNA-lipid adducts)

Lipids degradation (hydrolysis, oxidation)

Quality by Design for enabling RNA platform production processes

Simon Daniel, Zoltán Kis, Cleo Kontoravdi, and Nilay Shah

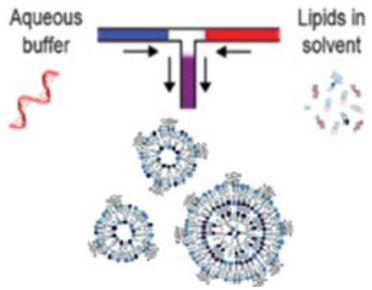
The Storage and In-Use Stability of mRNA Vaccines and Therapeutics: Not A Cold Case

Erik Oude Blenke[a], Eivor Örnskov[a], Christian Schöneich[b], Gunilla A. Nilsson[a], David B. Volkin[b,c], Enrico Mastrobattista[d], Örn Almarsson[e,f], Daan J.A. Crommelin[d,*]

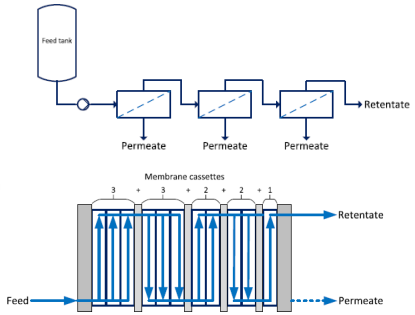
LNP-mRNA – *From Self Assembly to DP – A good process candidate for CM*

Continuous Manufacturing E2E integration – High Level flow illustration

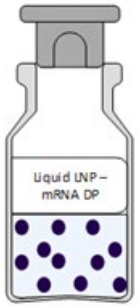
1- Self Assembly



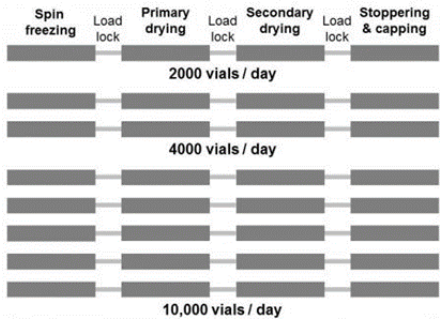
2- Purification – Formulation



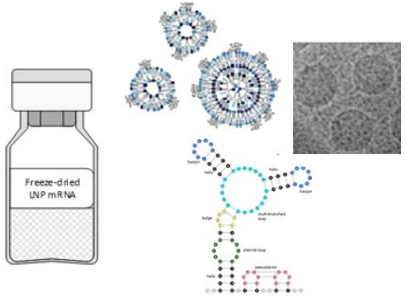
3- Filling in vials



4- Single Vial Drying



5- Storage / Reconstitution & Injection



PAT - Retroloop
LNP Size – DLS
RNA content – UV
Lipids content - Turbidity

PAT – *Monitoring*

Temperature monitoring / vial level
Real Time Stability Prediction (Models)

mRNA API In Aqueous

Lipids in EtOH

Mixing – LNP Self Assembly

SPTFF Purification / Formulation

Frontal Filtration

Filling in vial

Single Vial Freeze Drying

DP Storage

Class C/D operation (closed single use assemblies)

Class B/A operations (Isolator – Sterility Insurance)

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Single pass tangential flow filtration: Critical operational variables, fouling and main current applications

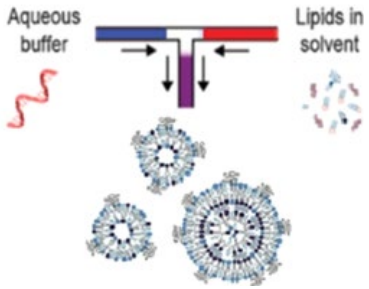
Emil Madsen, Johann Kaiser, Ulrich Krühne, Manuel Pinelo *

Department of Chemical and Biochemical Engineering, Technical University of Denmark (DTU), Søltofts Plads 227, Kgs. Lyngby 2800, Denmark

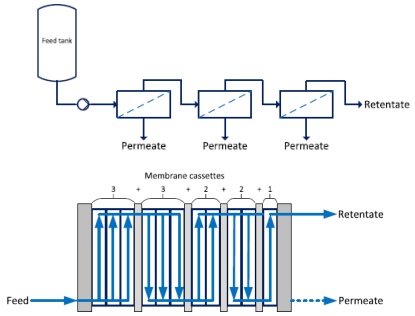
LNP-mRNA – *From Self Assembly to DP – A good process candidate for CM*

Continuous Manufacturing E2E integration – Sized for the final user needs

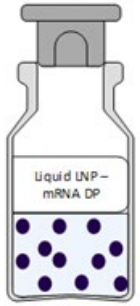
1- Self Assembly



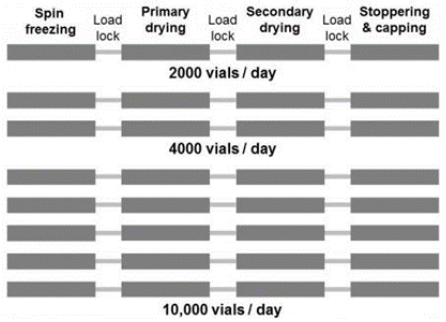
2- Purification – Formulation



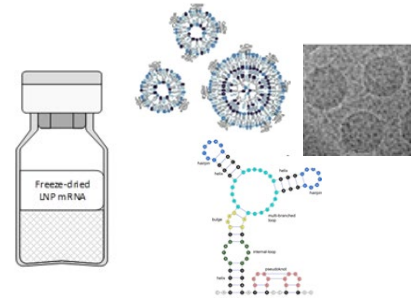
3- Filling in vials



4- Single Vial Drying



5- Storage / Reconstitution & Injection



CM of LNP-mRNA drugs could be sized (Volume / Process flow) to differents therapeutic Target Product Profile

Therapeutic modality	Volume needed	Route of Administration	Number of vials
Personalized Drug (Ex. Melanoma / Pancreatic Cancer)	0.5 – 2 g RNA	IV – mg/kg	≈ 1-20 / patient – One drug=One patient
Semi Personalized Cancer Drug (Ex. Common Tumor Antigens – CAR-T)	2 – 20g RNA	IV – mg/kg	≈ 1-5 / patient – Hundreds of patients
Therapeutics Vaccines (Ex. Gene Editing for CVD)	20 g – 100g RNA	IV – mg/kg	≈ 1-10 / patient – Thousands of patients
Infectious Vaccine (Ex. Influenza / COVID)	> 100 g RNA	IM - ug dose	1 / patient – Millions of patients / years

mRNA Vaccine Induced Immune Response in Pancreatic Cancer trial

Verve Therapeutics Pipeline Progress

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The future of genetic medicines delivered via targeted lipid nanoparticles to leukocytes

Dana Tarab-Ravski [a,b,c,d], Lior Stotsky-Oterin [a,b,c,d], Aviad Elisha [a,b,c,d], Govinda Reddy Kundoor [a,b,c,d], Srinivas Ramishetti [e], Inbal Hazan-Halevy [a,b,c,d], Heinrich Haas [e,f], Dan Peer [a,b,c,d,*]

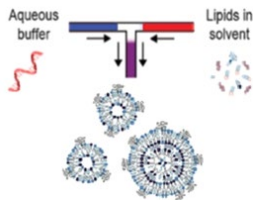
sa-mRNA influenza vaccine raises a higher and more durable immune response than mRNA vaccine in preclinical models

Cheng Chang, Harsh Patel, Annette Ferrari, Tina Scalzo, Daniel Petkov, Howard Xu, Evan Rossignol, Giuseppe Palladino, Yingxia Wen *

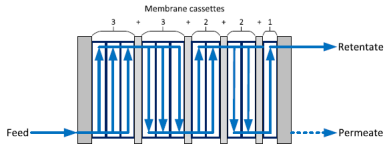
CSL R&D, Waltham, MA, USA

LNP-mRNA – *Process Integration* - *Self Assembly to bulk LNP*

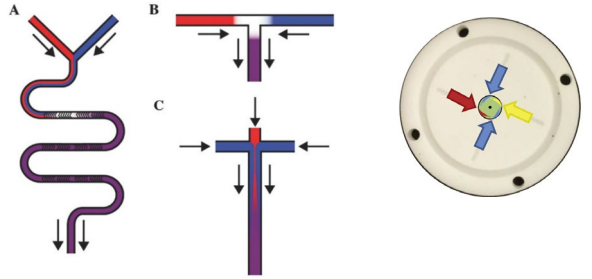
1- Self Assembly



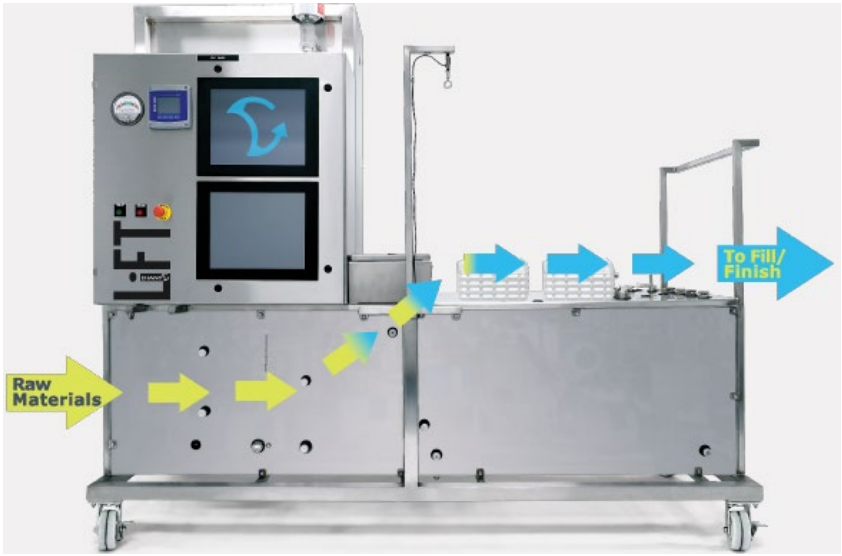
2- Purification – Formulation



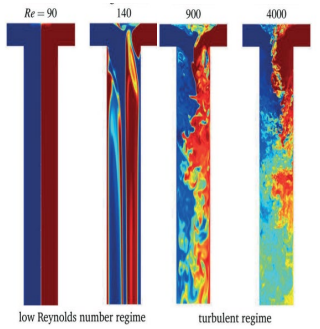
Mixing property and quality is key for LNP self Assembly
Differents mixer can be used to achieve adapted mixing behavior

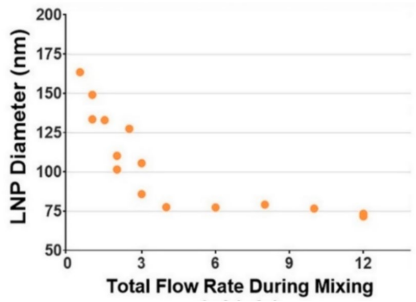


Companies are working on equipment's solution for E2E LNP manufacturing integration - Including PAT

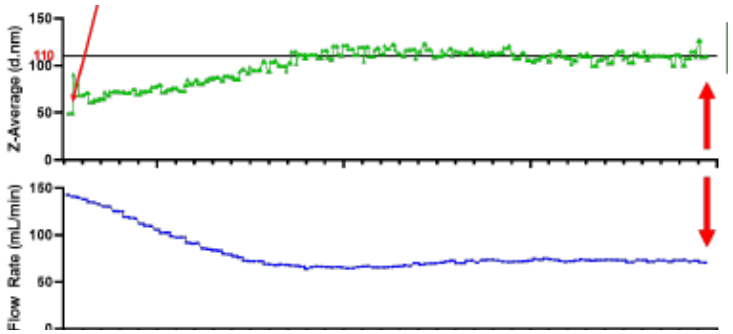


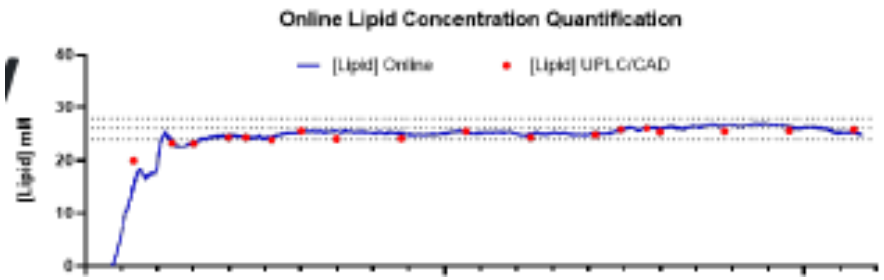
Leveraging CFD / PAT as Digital twin solution





LNP size /RNA & Lipid content can be monitored in line





Review

Overview on LNP-mRNA encapsulation unit operation: Mixing technologies, scalability, and influence of formulation & process parameters on physico-chemical characteristics

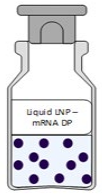
Laurine Hourdel [a,b,*], Noureddine Lebaz [b], Florent Peral [a], Manon Ripoll [a], Stéphanie Briançon [b], Fethi Bensaid [a], Sumit Luthra [a], Claudia Cogné [b]

sanofi DIANT® Tailored Uniformity • Streamlined Control • Optimal Yield

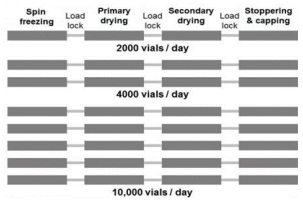
Lipid Nanoparticle Manufacturing | LNP | DIANT

LNP-mRNA – *Process Integration from Fill&Finish – Lyophilisation and DP Storage*

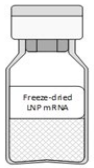
3- Filling in vials

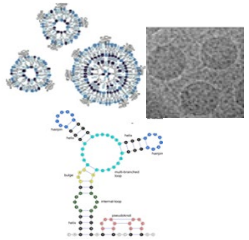


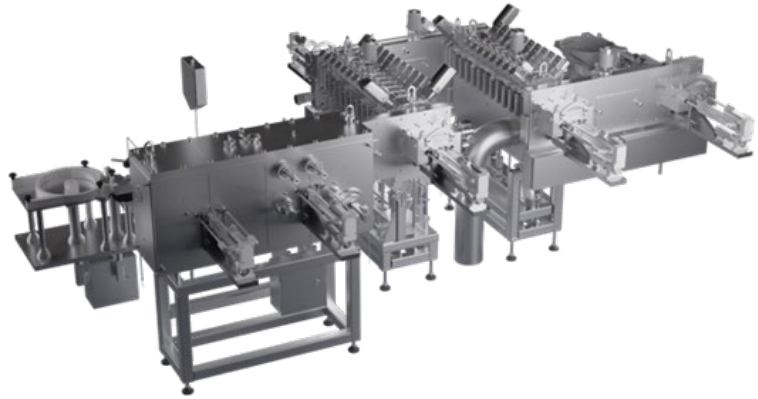
4- Single Vial Drying



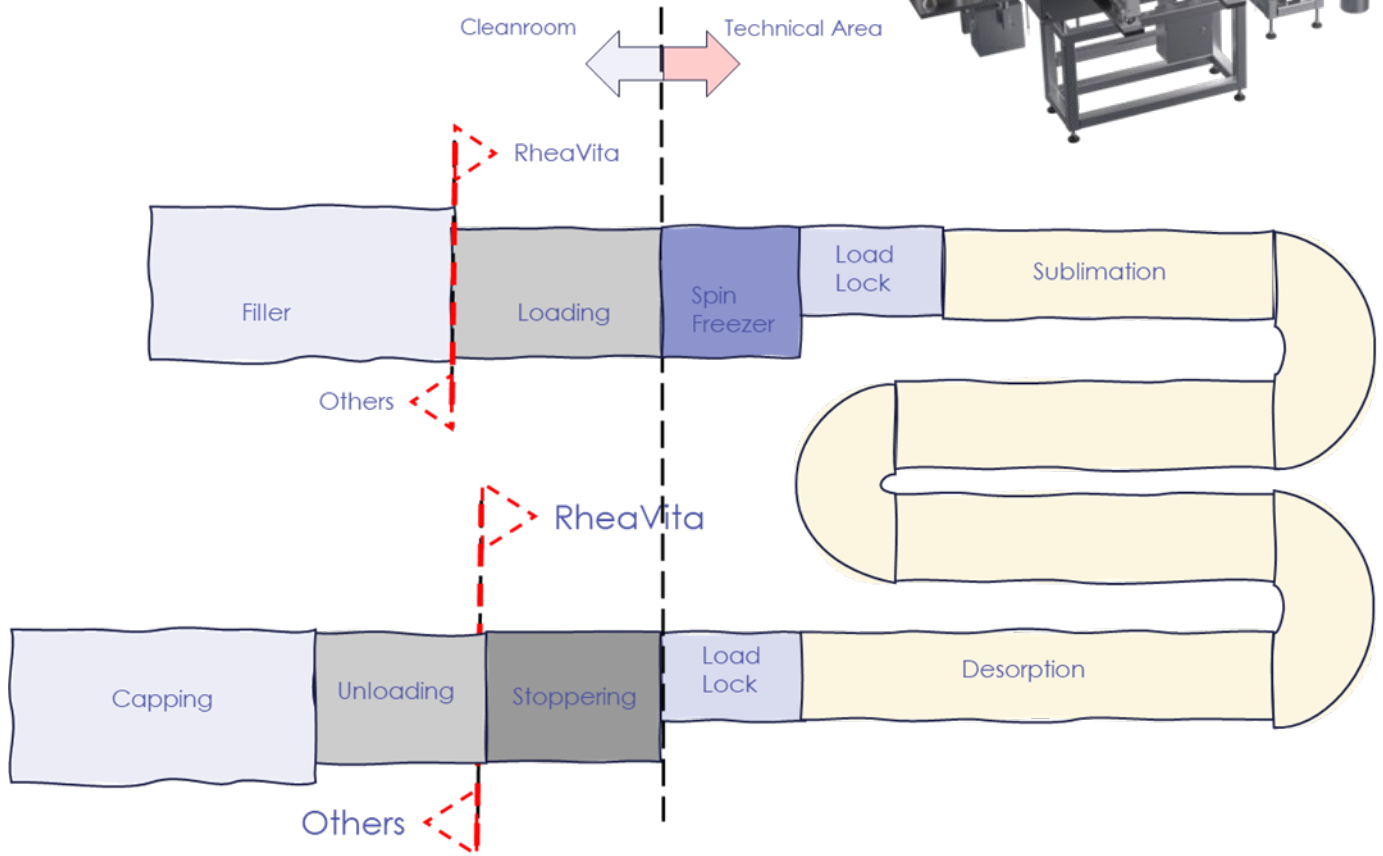
5- Storage / Reconstitution & Injection







Companies are working on equipment's solution for E2E filling and single vial freeze drying integration - Including PAT

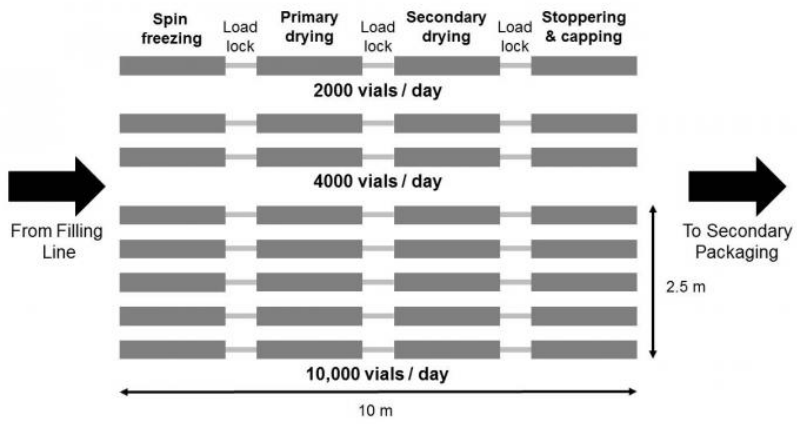


RheaVita – Continuous Freeze-Drying

Use case – *Single vial freeze dryiong – Technology Presentation*

- **Main Steps *of conventional Lyo conserved* :**
  - Freezing step : Spin vial product freezing using N2 gaz
  - Sublimation : Infra Red Assisted sublimation under vacuum and monitored via thermal imaging
  - Secondary drying : Infra Red +30°C under vacuum and monitored via thermal imaging
  - Global cycle time : of 2h to 3h (vs > 24h for standard batch mode)

- **Operational conditions**
  - From R&D scale to "industrial" the scale up is mainly a scale out
  - Constraints / limitation : Sterility, operations ect



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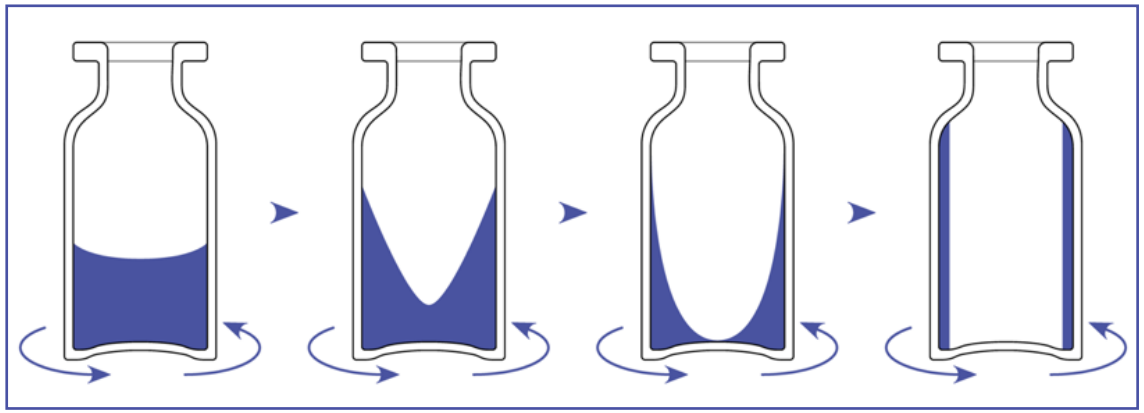
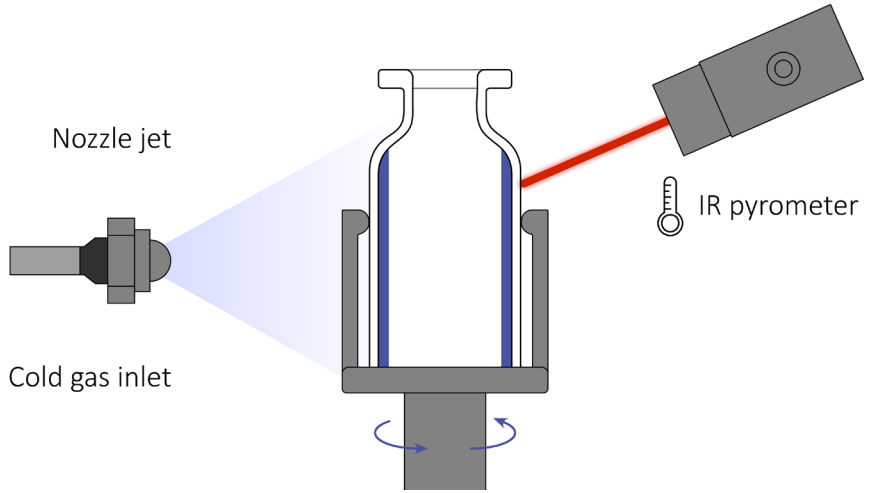
RheaVita

RheaVita – Continuous Freeze-Drying

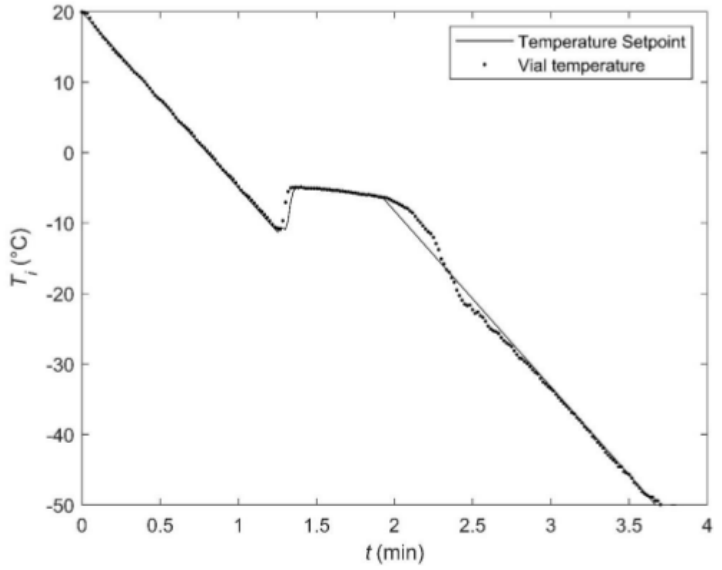
Use case – *Single vial freeze drying – Freezing Technology*

- **Freezing :**
  - Freezing step : Spin vial product freezing using N2 gaz





  - Rotation 3000 rpm
  - A thin product layer spread over the entire inner vial wall is formed
  - Cooling rate takes place at 5°C/min to 50°C/min as monitored by thermal imaging up to -50°C



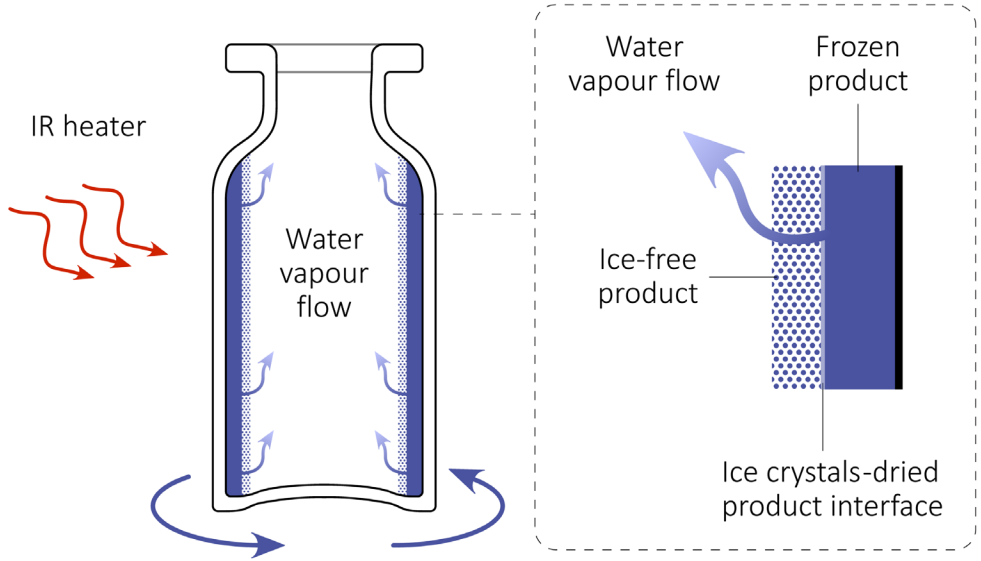
Optimization of continuous spin-freeze-drying: The role of spin-freezing on quality attributes and drying efficiency of a model peptide formulation

Zarah Schaal [a,b], Pieter-Jan Van Bockstal [a], Joris Lammens [a], Julian H. Lenger [c], Adrian P. Funke [d], Stefan C. Schneid [c], Hristo L. Svilenov [e,1], Thomas De Beer [a,b,*]

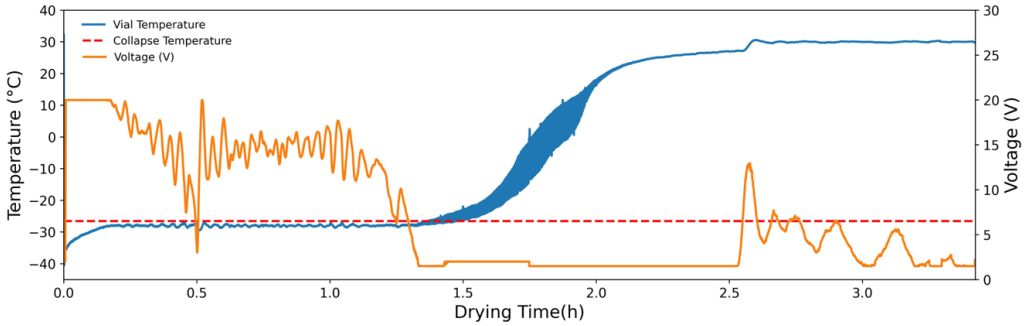
RheaVita – Continuous Freeze-Drying

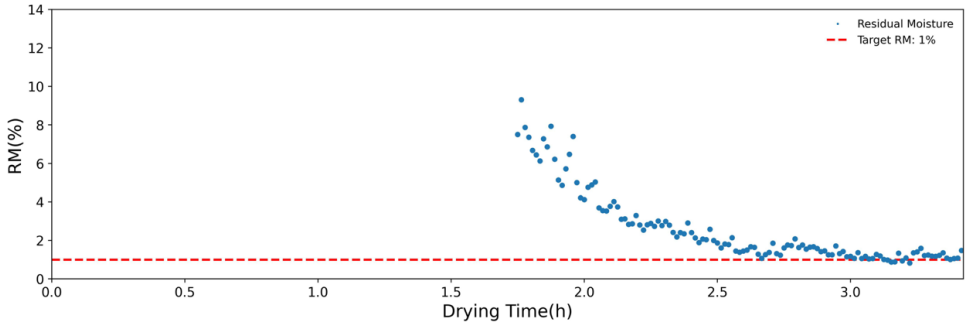
Use case – *Single vial freeze drying – Drying & PAT Technologies*

- **Drying Principle :**
  - Drying at 8 Pa (80 uBars) pressure
  - Energy supply towards the surface of the spin frozen vial via infrared (IR) radiation
  - Spin frozen vial slowly rotating (10 rpm) in front of an individual IR heater
  - IR Heater set at a specific temperature



- **PAT Monitoring :**
  - **Freezing :**
    - Thermal Imaging :
      - Product Temperature
  - **Primary & Secondary Drying:**
    - Thermal imaging:
      - Product temperature at sublimation front
      - Primary and secondary drying endpoint
    - NIR spectroscopy:
      - Drying progress/ Residual moisture



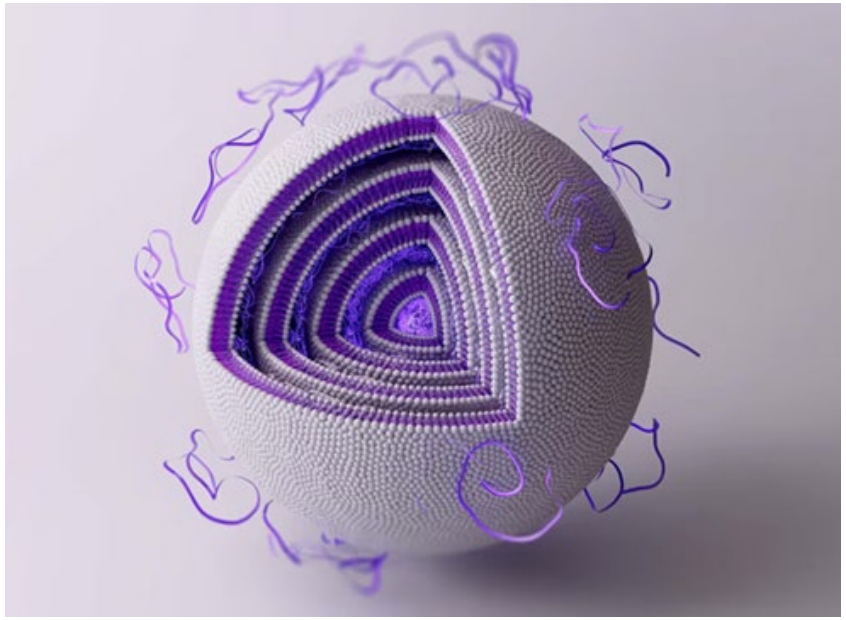


sanofi RheaVita RheaVita – Continuous Freeze-Drying

Use case – *Single vial freeze drying – Study design*

Freeze drying method

- Type I 2R vials
- Filling volume 0.5 mL
- Single vial Freeze drying pilot equipment (Rheavita)



Stability Control

Critical Quality Attribute	Method	Stability program				
		T0 Liquid	T0 Lyo	+ 5°C 2 Weeks	+25°C 2 Weeks	+37°C 2 Weeks
LNP Size	Light Scattering	X	X	X	X	X
RNA Content and EE%	Fluorescence	X	X	X	X	X
RNA integrity and Lipid adducts	Separative Chromatography	X	X	X	X	X

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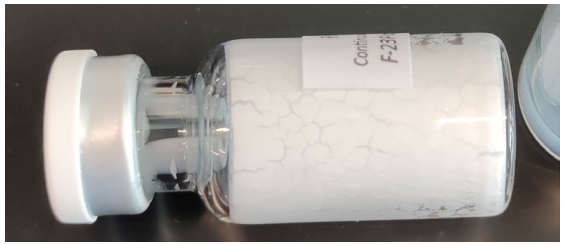
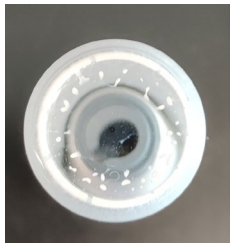
Use case – *Single vial freeze drying – Results*

Visual aspects :

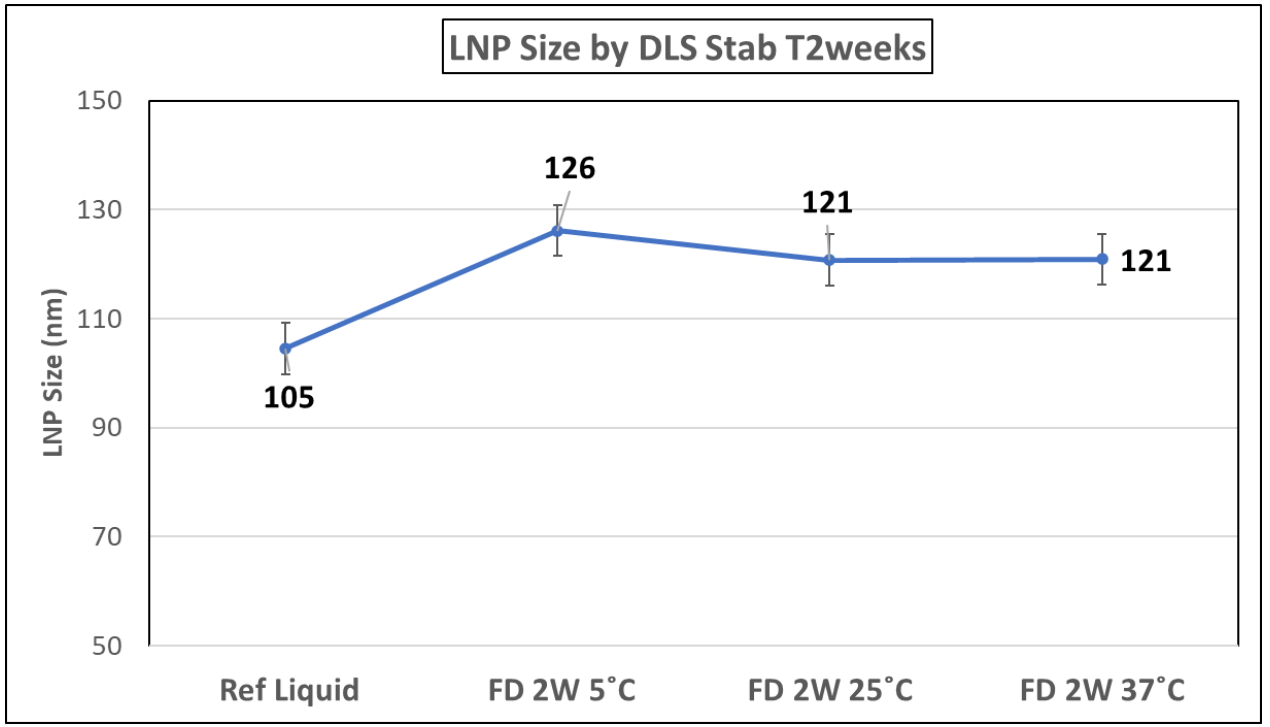
- Lyo Presentation appears to be elegant and homogeneous :
- Optimization needed for shrinkage
- Reconstitution is easy and quick (less than 10s)





LNP Size before and after the Single Vial freeze drying process

- Slight raise in LNP size after freeze drying process
- LNP size is stable for 2weeks stored at +37°C



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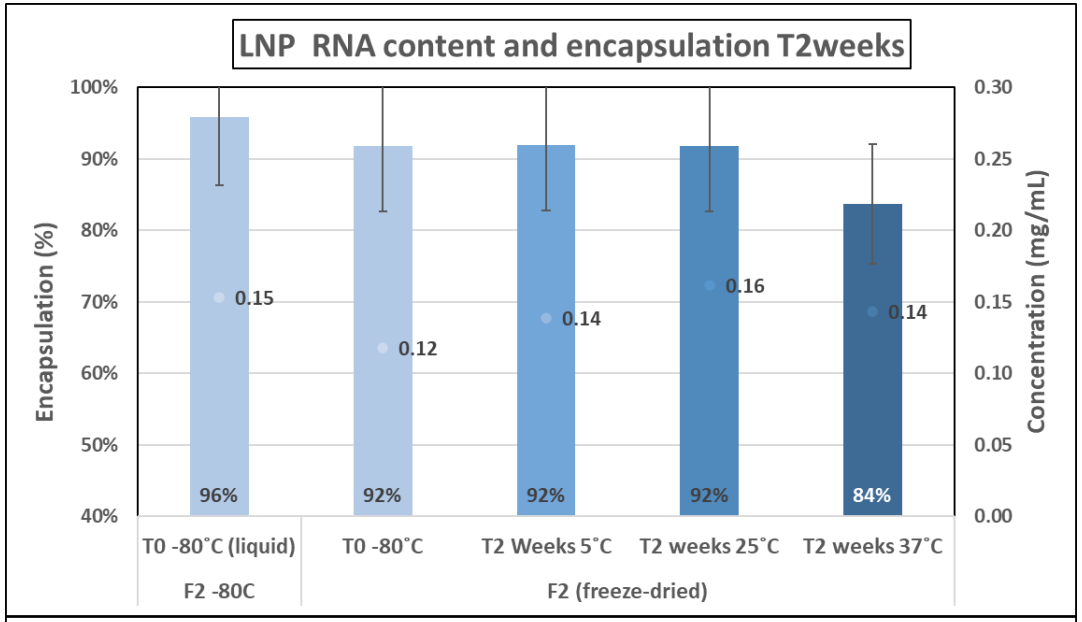
Use case – *Single vial freeze drying – Results*

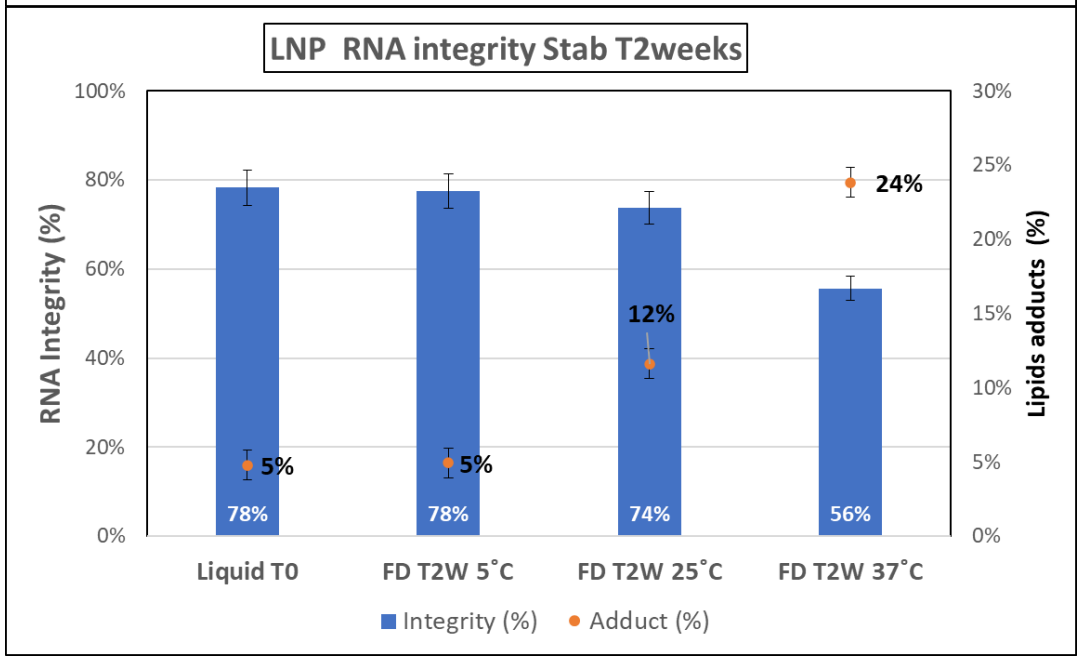
LNP Encapsulation Stability

- Loss EE % from -80˚C storage to -80˚C storage FD (4 %)
- No loss during storage +5˚C and 25˚C (92%)
- Loss after 2 weeks at 37˚C (8%)

mRNA Stability

- mRNA Stable stored 2 week +5˚C
- Decrease stability during storage +25˚C and 37˚C





Conclusion

As for many biodrugs, **the shift towards continuous bioprocessing** present a **transformative opportunity for LNP manufacturing**, offering potential improvements in:

- Sustainability, Efficiency, **Productivity, Facilities footprint and manufacturing costs**

LNP-mRNA modalities presents interesting potential for continuous manufacturing approaches:

- **Mixing process for LNP self-assembly is continuous by nature**
- Standard Filling process or **Innovative thermostability process can be connected**

Equipment engineering, automation, monitoring and controls will play a crucial role to implement these processes to supply lifesaving drugs worldwide.

While **Single Vials Freeze Drying approach** needs process and formulation optimization, it presents interests **in a global End to End Continuous manufacturing value chain** for sized process needs (Therapeutic target)

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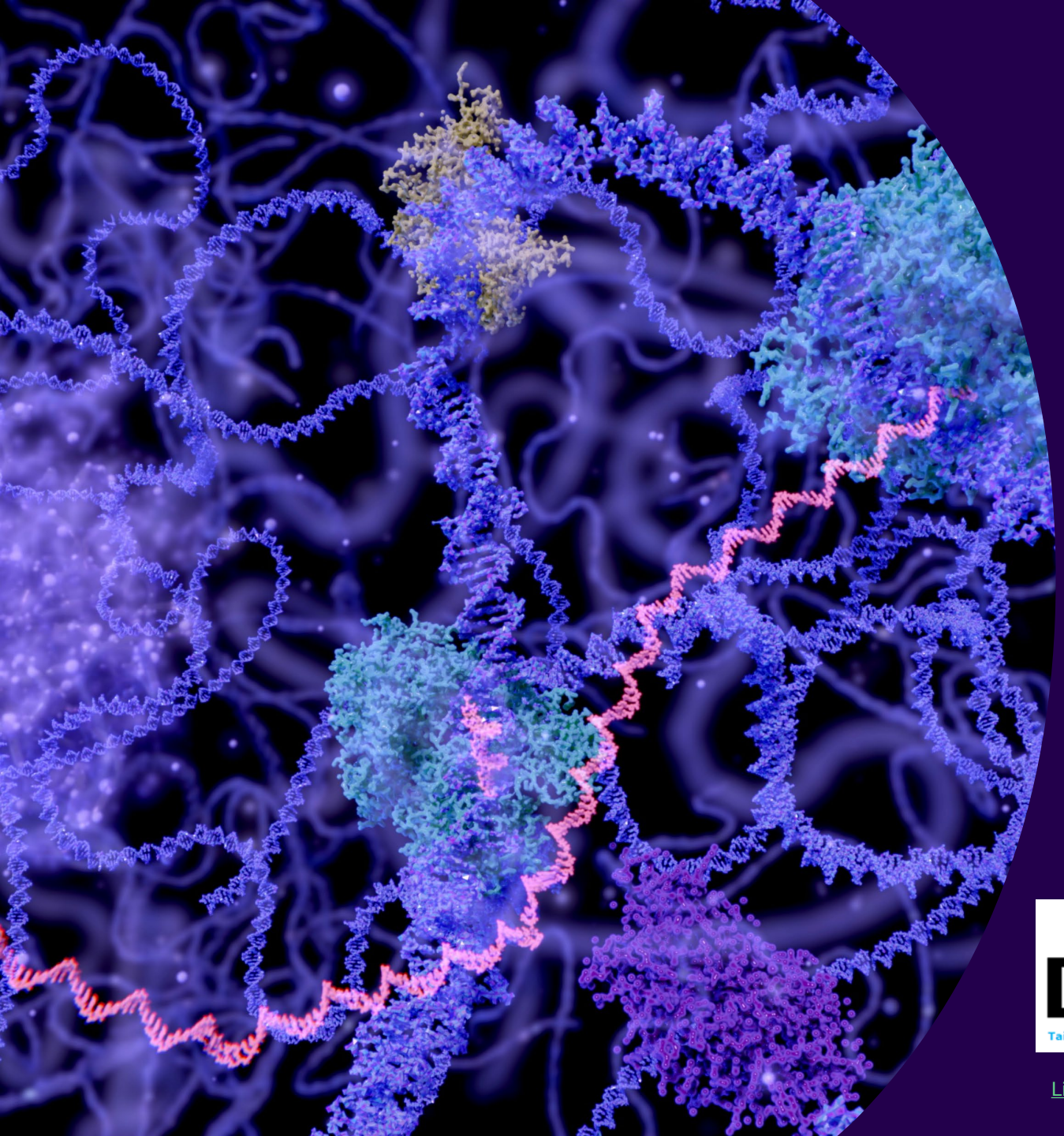

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Thank you

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RheaVita

RheaVita – Continuous Freeze-Drying