Quality requirements for nanomedicines: which role for the European Pharmacopoeia?

7-8 June 2022

mRNA Vaccines Session

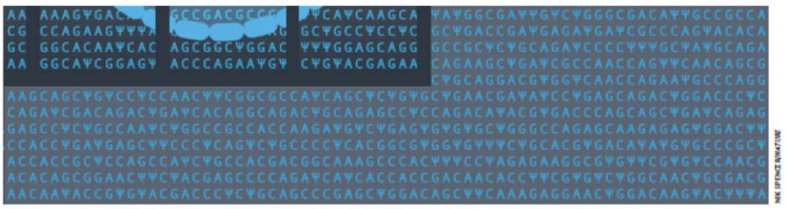
Moderator:
Gerrit Borchard, Chair of the Non-Biological Complex Drugs (NBC) Working Party, University of Geneva, Switzerland

Keynote lecture: The science behind mRNA vaccines

EDQM Strasbourg 7/6-2022

Camilla Foged, Full Professor, Vaccine Design and Delivery, Department of Pharmacy

UNIVERSITY OF COPENHAGEN

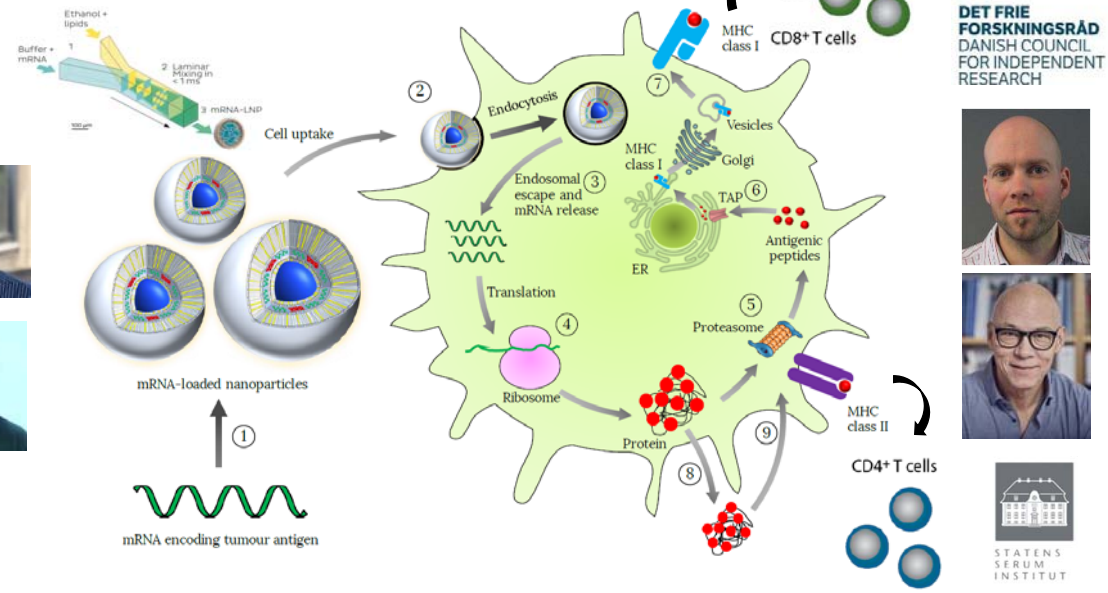


The RNA sequence used in the COVID-19 vaccine developed by Pfizer and BioNTech (Ψ is a modified form of the uridine nucleotide, U).

THE TANGLED HISTORY OF MRNA VACCINES

Hundreds of scientists had worked on mRNA vaccines for decades before the coronavirus pandemic brought a breakthrough. By Elie Dolgin

Next-generation mRNA vaccines



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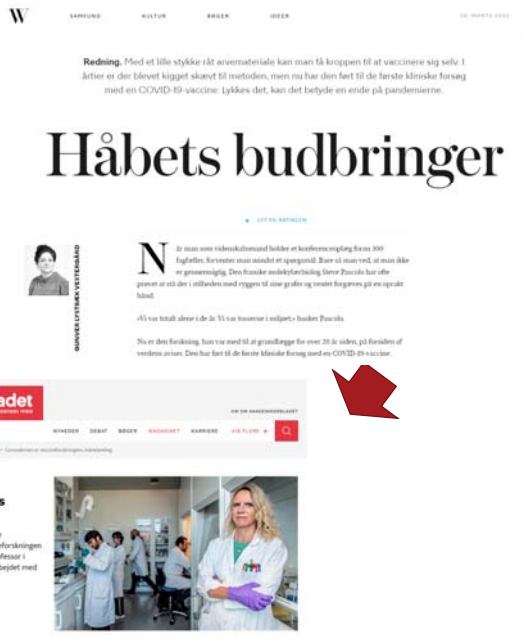
Review

Opportunities and Challenges in the Delivery of mRNA-Based Vaccines

Abishek Wadhwa, Anas Aljabbari, Abhijeet Lokras, Camilla Foged and Aneesh Thakur *

Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen Ø, Denmark; abishek.wadhwa@sund.ku.dk (A.W.); anas.aljabbar@sund.ku.dk (A.A.); abhijeet.lokras@sund.ku.dk (A.L.); camilla.foged@sund.ku.dk (C.F.)
* Correspondence: aneesh.thakur@sund.ku.dk; Tel.: + 45-3533-3938; Fax: +45-3533-6001

Received: 28 December 2019; Accepted: 26 January 2020; Published: 28 January 2020



Redning. Med et lille stykke råt arvemateriale kan man få kroppen til at vaccinere sig selv. I årtier er der blevet kigget skævt til metoden, men nu har den fået til de første kliniske forsøg med en COVID-19-vaccine. Lykkes det, kan det betyde en ende på pandemierne.

Håbets budbringer

Akademikerbladet

Coronakrisen er vaccineforskningens månelanding

USA's måneprogram gav store tekniske landvindinger. Tilsvarende med vaccineforskningen under coronakrisen. Biokemiker og professor i vaccineforskning Camilla Foged har arbejdet med vacciner i 21 år.

SUNDHEDSSTYRELSEN

Dato 17-06-2021

Sagsnr. 05-0600-914

Vedr. vaccination af børn på 12-15 år

SUNDHEDSSTYRELSEN

Dato 25-11-2021

Sagsnr. 05-0601-1671

Revaccination mod COVID-19 for personer over 18 år

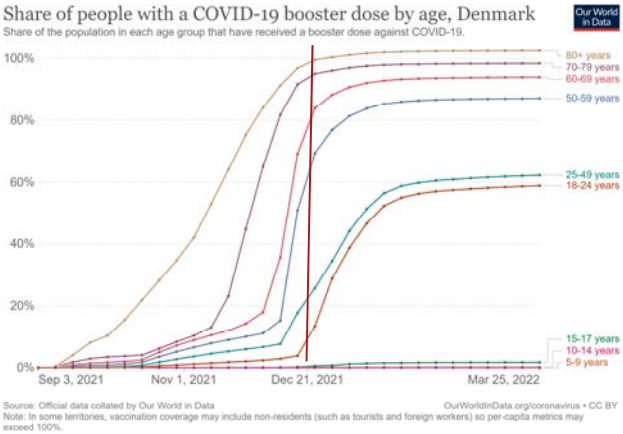
SUNDHEDSSTYRELSEN

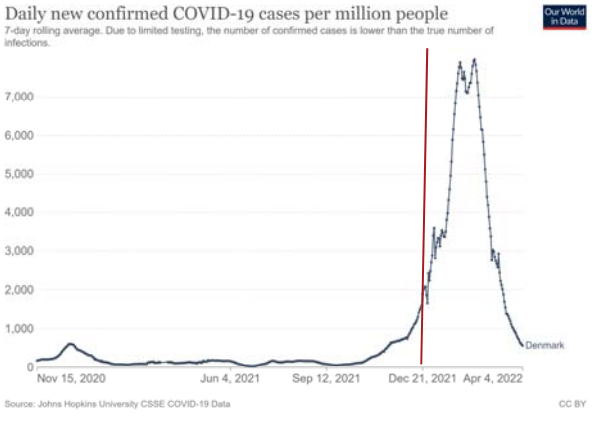
Dato 06-12-2021

Sagsnr. 05-0600-1260

COVID-19 vaccination af børn på 5-11 år

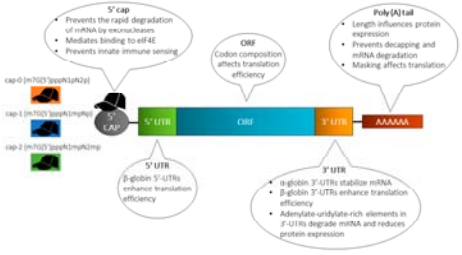
Booster vaccination and COVID-19 cases in Denmark



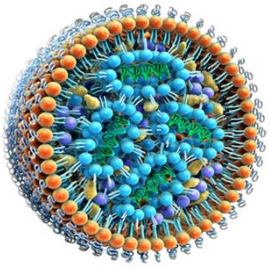


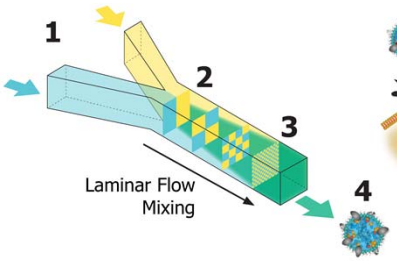
mRNA vaccines: 60 years of research in enabling technologies

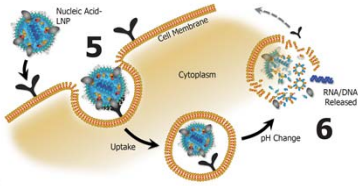
- mRNA stabilisation
- Delivery system: Lipid nanoparticles (LNPs)
- Scalable LNP manufacturing: Microfluidics



Wadhwa *et al.*, Pharmaceutics 2020







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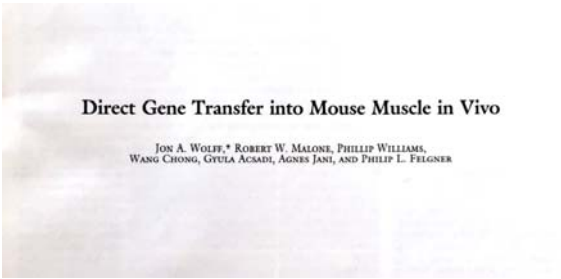
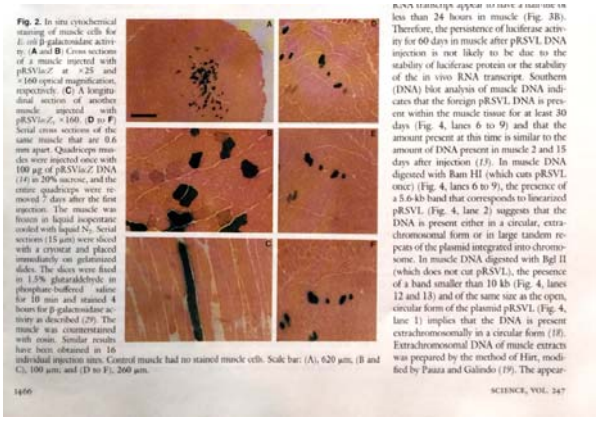
mRNA as a drug – the pioneer experiments

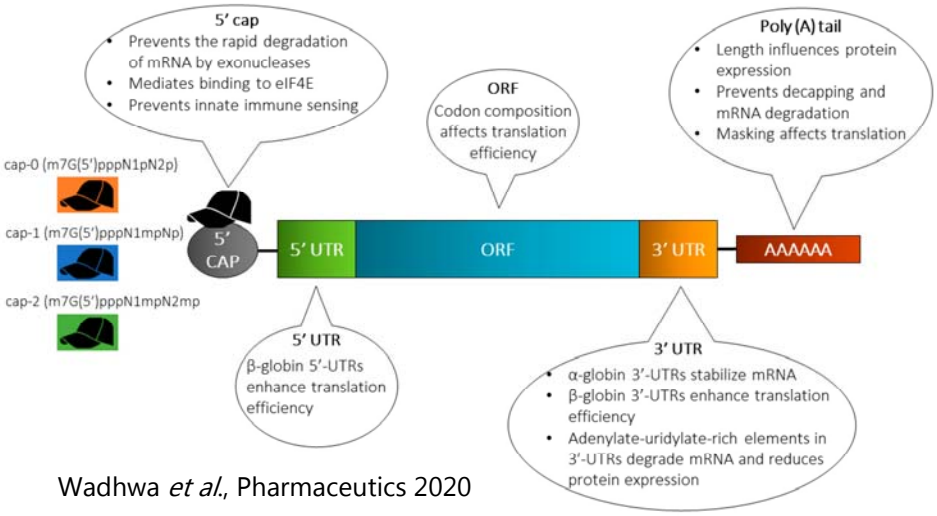


Direct Gene Transfer into Mouse Muscle in Vivo

JON A. WOLFF,* ROBERT W. MALONE, PHILLIP WILLIAMS, WANG CHONG, GYULA ACSADI, AGNES JANI, AND PHILIP L. FELGNER

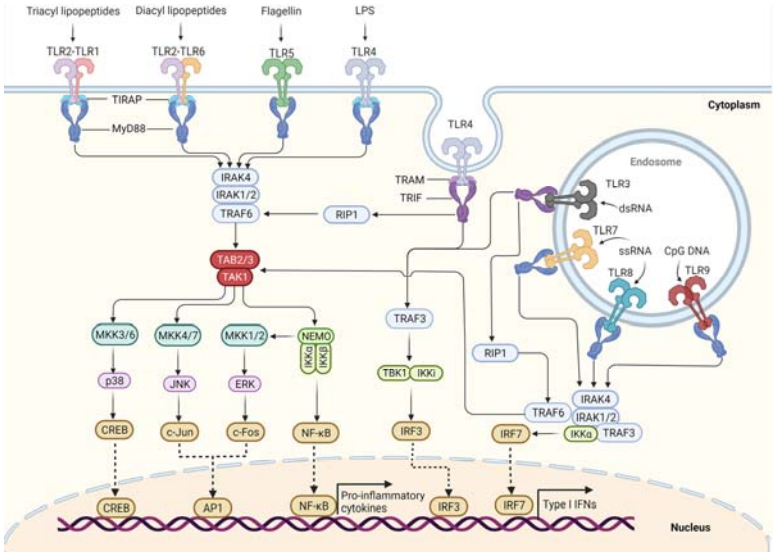


mRNA as a drug: Chemical and enzymatic stabilization



Wadhwa *et al.*, Pharmaceutics 2020

Stimulation of the innate immune system



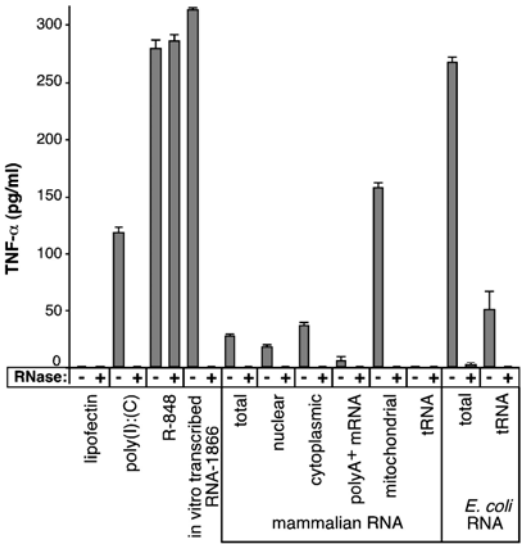
mRNA as a drug: Modulating mRNA immunogenicity

Immunity, Vol. 23, 165–175, August, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.immuni.2005.06.008

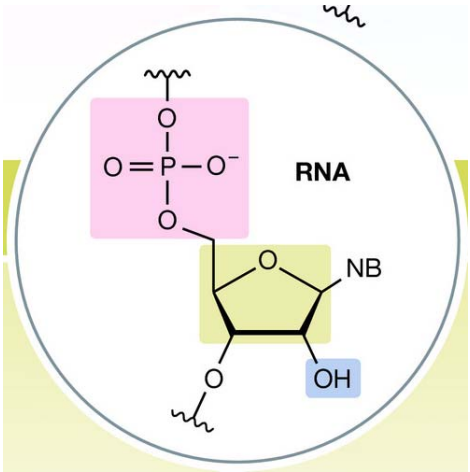
Suppression of RNA Recognition by Toll-like Receptors: The Impact of Nucleoside Modification and the Evolutionary Origin of RNA

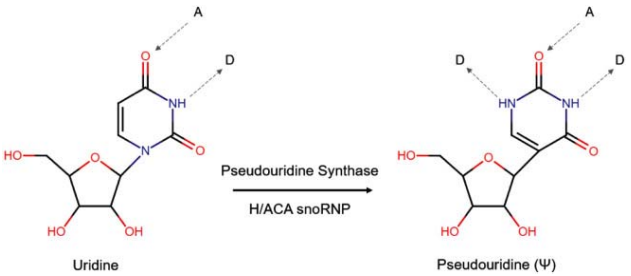
Katalin Karikó,[1,*] Michael Buckstein,[2] Houping Ni,[2] and Drew Weissman[2]
[1]Department of Neurosurgery
[2]Department of Medicine
University of Pennsylvania School of Medicine
Philadelphia, Pennsylvania 19104

thetic antiviral compound R-848 (Jurk et al., 2
a natural ligand has not been identified.
It has been known for decades that select
and RNA molecules have the unique property
vate the immune system. It was discovered
cently that secretion of interferon in response
is mediated by unmethylated CpG motifs acti



Enhancing mRNA stability by chemical modification





Delivery of oligonucleotide-based therapeutics: challenges and opportunities
EMBO Mol Med, Volume: 13, Issue: 4, First published: 06 April 2021, DOI: (10.15252/emmm.202013243)

How to get mRNA into cells?

Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 6077–6081, August 1989
Biochemistry

Cationic liposome-mediated RNA transfection

[cationic lipid vesicles/*N*-[1-(2,3-dioleyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride (DOTMA)/translation]

ROBERT W. MALONE*†‡, PHILIP L. FELGNER‡, AND INDER M. VERMA*§

*Molecular Biology and Virology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, CA 92138; †Department of Biology, University of California–San Diego, La Jolla, CA 92093; and ‡Vical Inc., 9373 Towne Centre Drive, Suite 100, San Diego, CA 92121

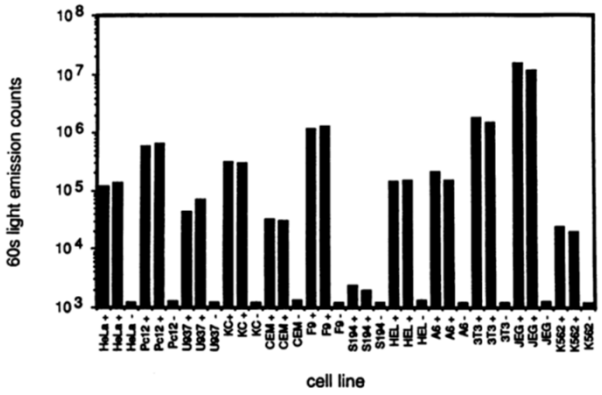
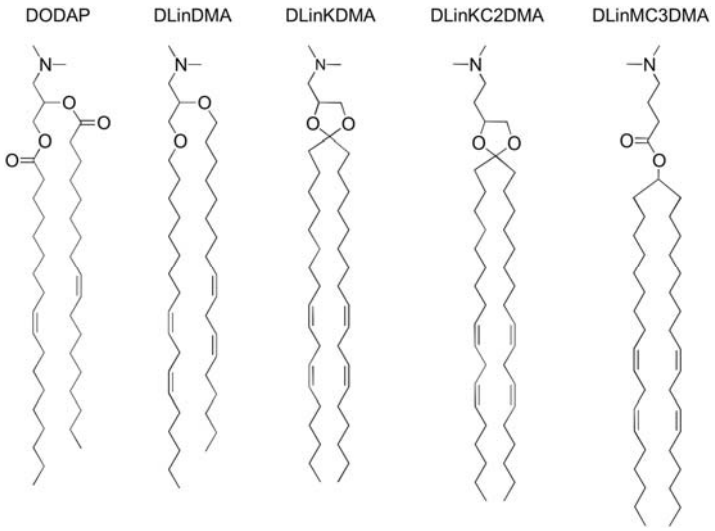
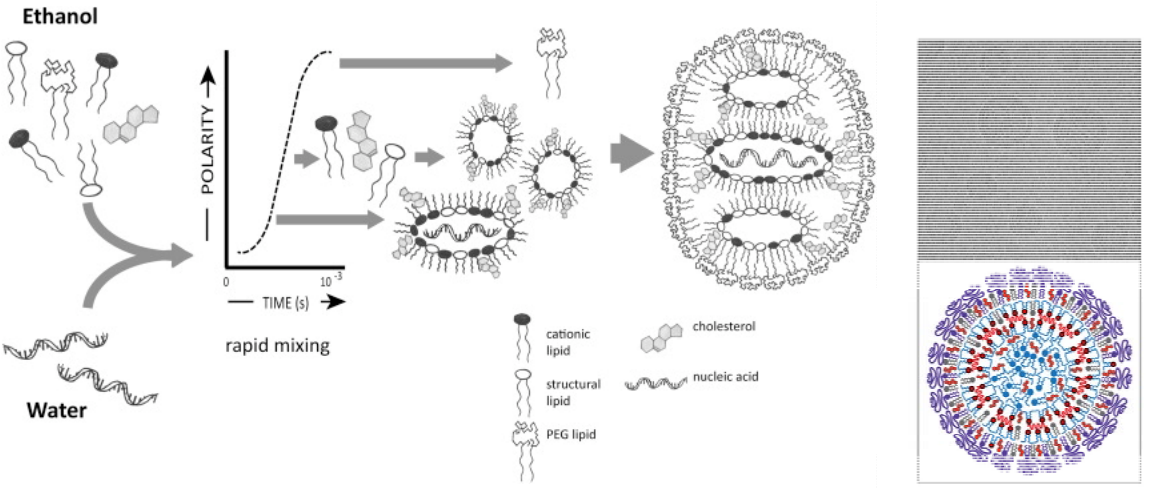


FIG. 4. RNA transfection in a variety of cell types. Fifty micrograms of lipofectin liposomes was used to transfect various cell lines either with (+) or without (−) 20 μg of mRNA (Cap-βg Luc βg An). Lysates were prepared 8 hr after addition of lipofectin and were analyzed for luciferase specific activity as before.

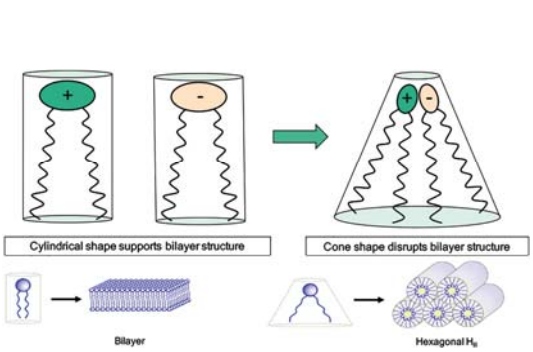
Ionizable lipids

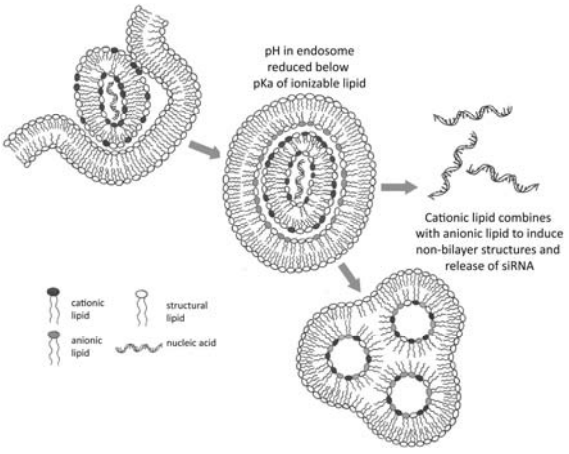


Loading of LNPs with oligonucleotides

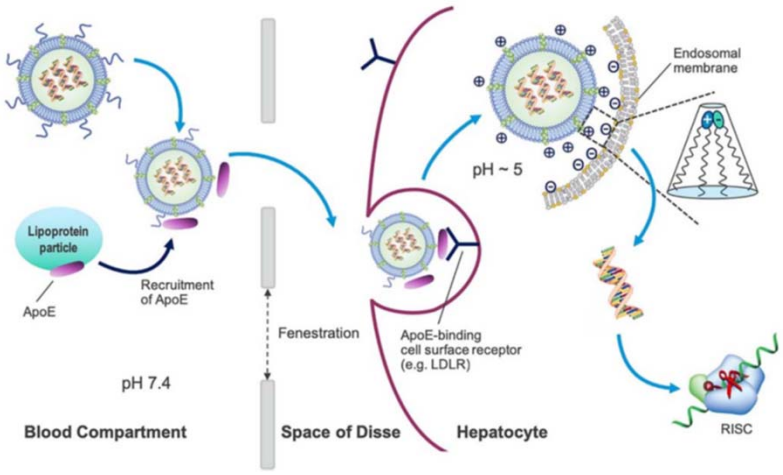


Mechanism of endosomal escape: The molecular shape hypothesis





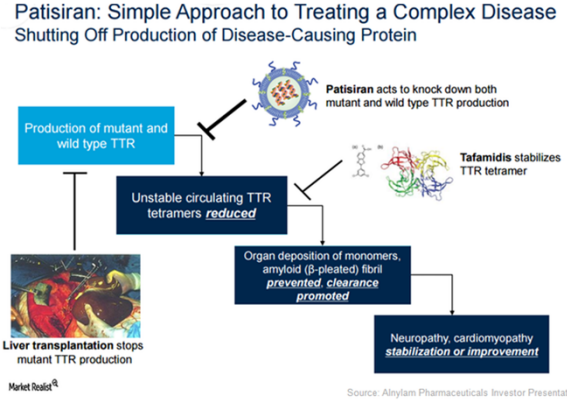
Active hepatocyte LDL receptor targeting of siRNA via ApoE binding to LNPs

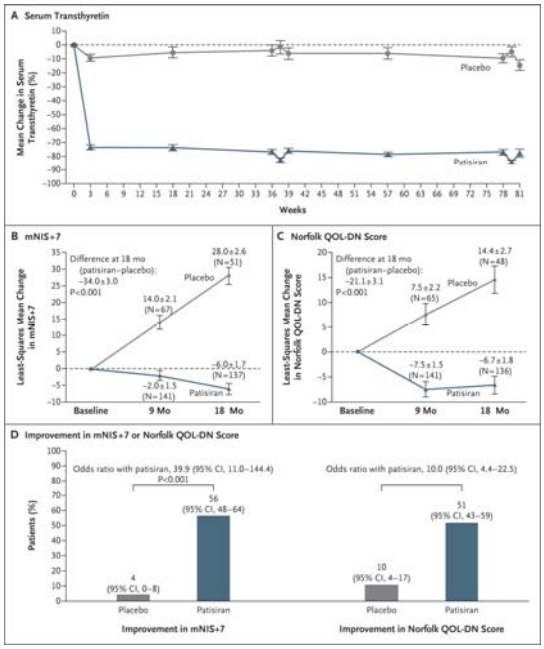


Akinc *et al.* 2019. Nat Nanotechnol

Patisiran

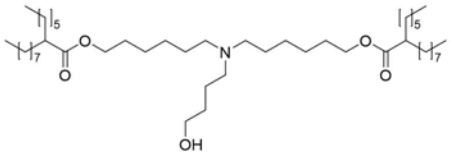
- IV infusion once every third week
- Pre-medication with cocktail of anti-inflammatory drugs





Adams *et al.* N Eng J Med 2018

Ionizable cationic lipids in mRNA vaccines



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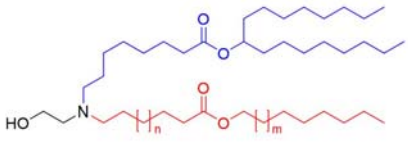
ALC-0315 (Acuitas Therapeutics; used by BioNTech)



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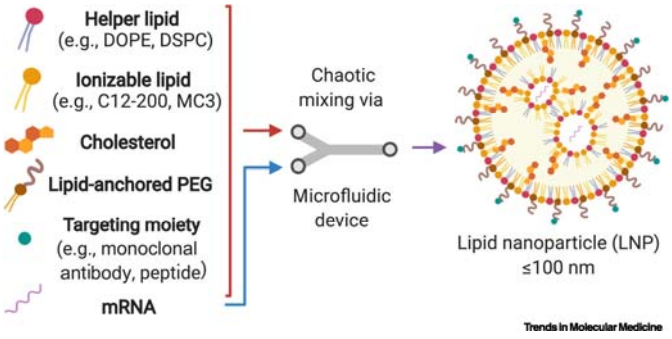
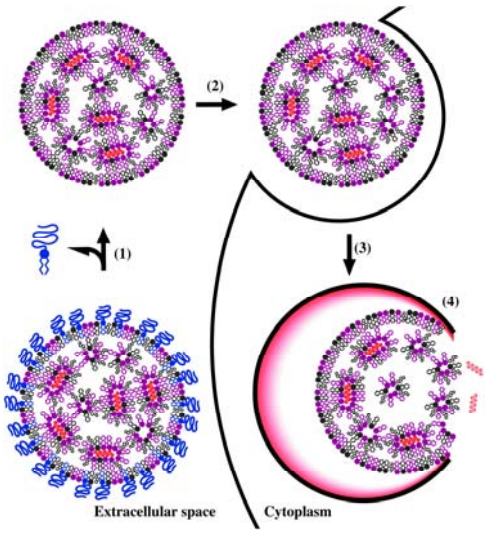
n = 1 ; m = 2 : SM-102 (Moderna Lipid H))
n = 3 ; m = 1 : Moderna Lipid 5

Lipid nanoparticles (LNPs)

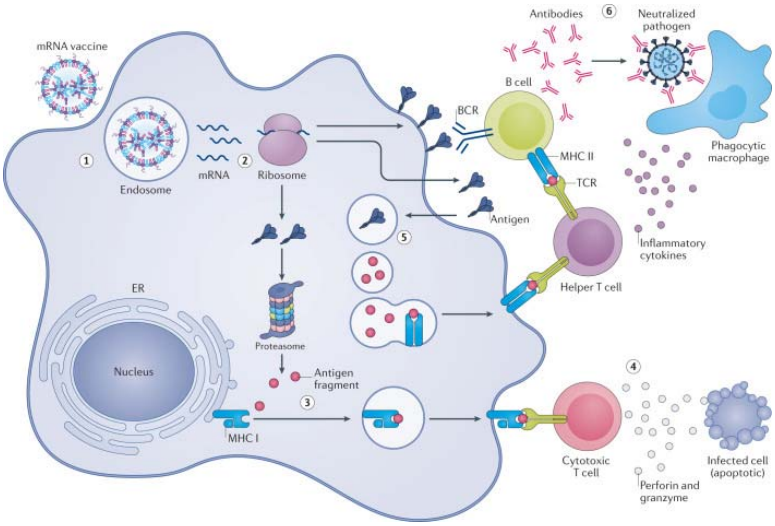


Swingle *et al.* Trends in Molecular Medicine 2021



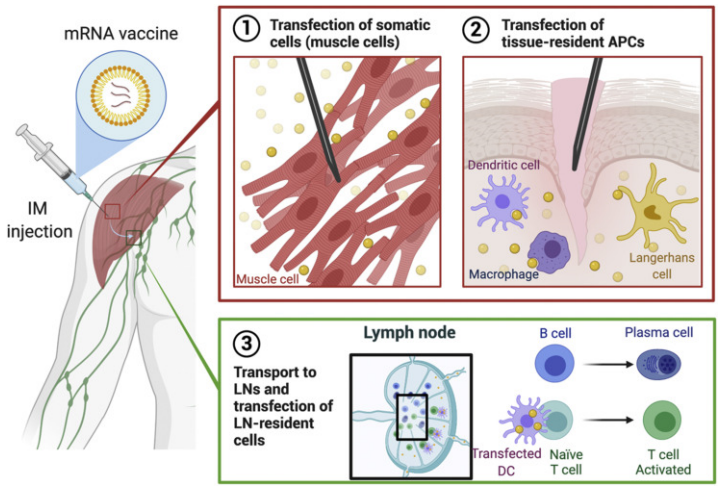
Kulkarni *et al.* Nucleic Acid Ther. 2018

mRNA vaccines – mechanism of action



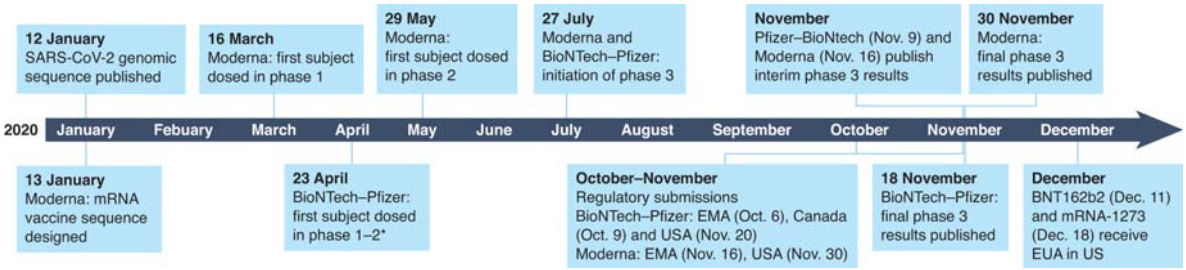
Chaudhary *et al.* Nat Rev Drug Discov. 2021

Modes of action - intramuscularly administered mRNA vaccines



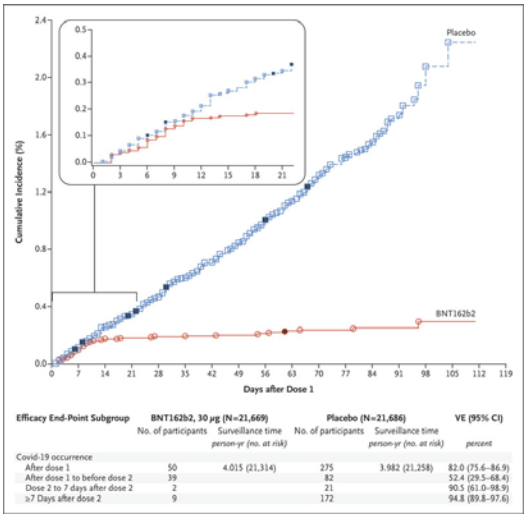
Kim *et al.* Advanced Drug Delivery Reviews, 2021

Rapid development of mRNA vaccines against SARS-CoV-2.

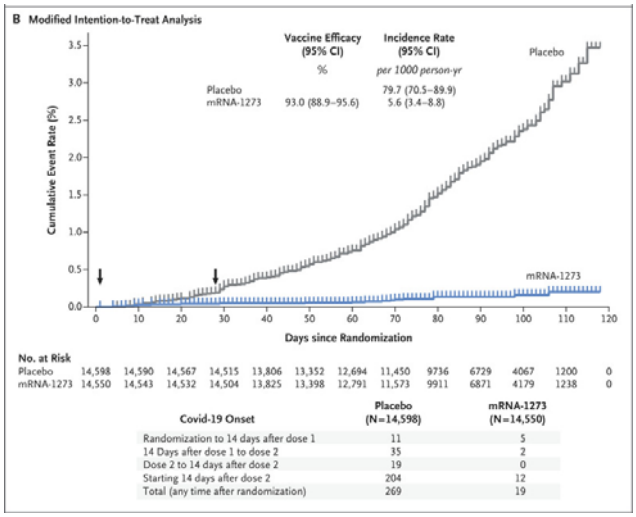


Barbier *et al.*, Nature Biotechnology, 2022

Efficacy – phase III

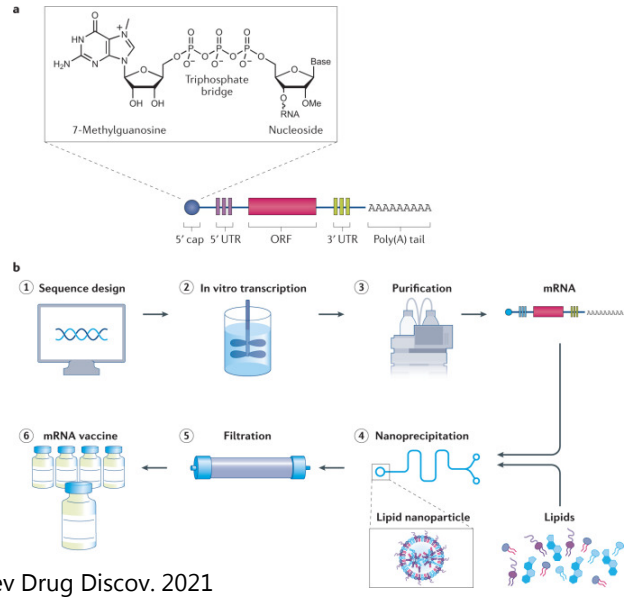


Efficacy End-Point Subgroup	BNT162b2, 30 μg (N=21,669) No. of participants	Surveillance time person-yr (no. at risk)	Placebo (N=21,686) No. of participants	Surveillance time person-yr (no. at risk)	VE (95% CI) percent
Covid-19 occurrence					
After dose 1	50	4.015 (21,314)	275	3.982 (21,258)	82.0 (75.6–86.9)
After dose 1 to before dose 2	39		82		52.4 (29.5–68.4)
Dose 2 to 7 days after dose 2	2		21		90.5 (61.0–98.9)
≥7 Days after dose 2	9		172		94.8 (89.8–97.6)



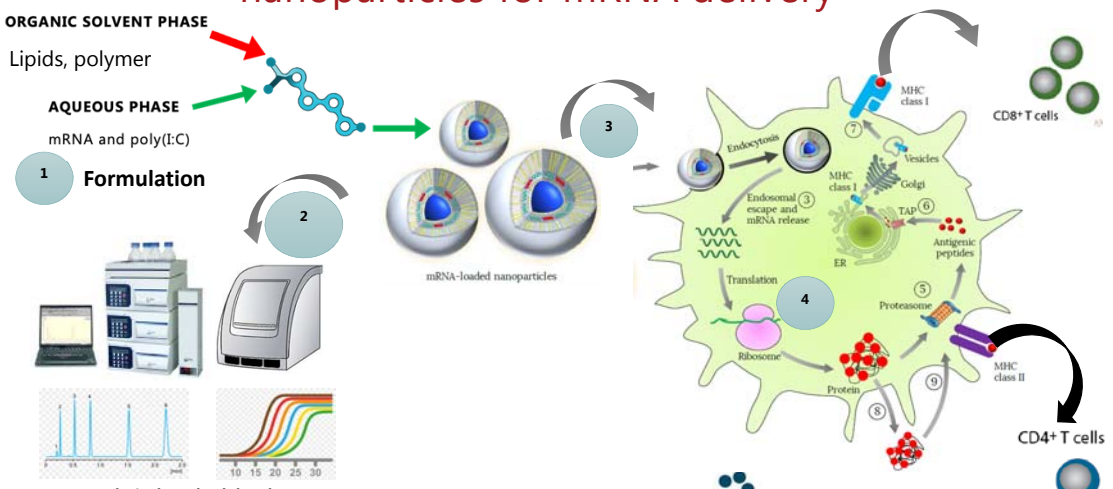
Covid-19 Onset	Placebo (N=14,598)	mRNA-1273 (N=14,550)
Randomization to 14 days after dose 1	11	5
14 Days after dose 1 to dose 2	35	2
Dose 2 to 14 days after dose 2	19	0
Starting 14 days after dose 2	204	12
Total (any time after randomization)	269	19

mRNA vaccine manufacturing: Speed and flexibility



Chaudhary *et al.* Nat Rev Drug Discov. 2021

The long road toward designing lipid-polymer hybrid nanoparticles for mRNA delivery



Analytical method development
Simultaneous quantitation of mRNA and poly(I:C) (HPLC – UV)
Quantitation of mRNA in macrophages (qPCR)
Lipids (HPLC – ELSD)

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Acknowledgements

Akash Chakravarty

Aneesh Thakur

Vaccine Design and Delivery Group

Abhijeet Girish Lokras

Henrik Franzyk

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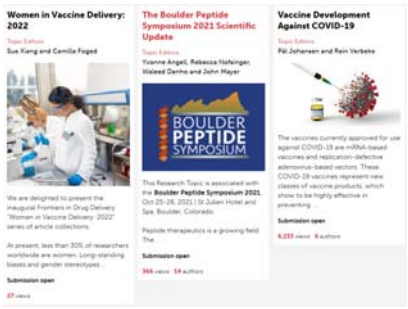
The **Vaccine Delivery** section publishes high-quality reports on basic, translational and clinical research on vaccine delivery.



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CMC Considerations for mRNA Vaccines

Romain Le Deun, M. Sc., Regulatory CMC International
EDQM Symposium, June 7, 2022

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This presentation contains forward-looking statements within the meaning of the Private Securities Litigation Reform Act of 1995, as amended, including statements regarding: the potential of the Company's mRNA platform and of mRNA as a potential new class of medicines; the advantages associated with the Company's mRNA platform and mRNA vaccines; the ability of the Company's manufacturing "kits" to enable scalability, consistency and reproducible production; efficiencies associated with the Company's manufacturing process; the potential for the establishment of collaborations with governments (including in Australia and Canada) to establish local manufacturing capabilities and long-terms supply agreements; the ability to customize pan-respiratory vaccines across time, demographics and geography; and the expected timing of the Company's clinical trials, including its Phase 3 study of its flu vaccine. In some cases, forward-looking statements can be identified by terminology such as "will," "may," "should," "could," "expects," "intends," "plans," "aims," "anticipates," "believes," "estimates," "predicts," "potential," "continue," or the negative of these terms or other comparable terminology, although not all forward-looking statements contain these words. The forward-looking statements in this presentation are neither promises nor guarantees, and you should not place undue reliance on these forward-looking statements because they involve known and unknown risks, uncertainties, and other factors, many of which are beyond Moderna's control and which could cause actual results to differ materially from those expressed or implied by these forward-looking statements. These risks, uncertainties, and other factors include, among others, those risks and uncertainties described under the heading "Risk Factors" in Moderna's most recent Annual Report on Form 10-K filed with the U.S. Securities and Exchange Commission (SEC) and in subsequent filings made by Moderna with the SEC, which are available on the SEC's website at www.sec.gov. Except as required by law, Moderna disclaims any intention or responsibility for updating or revising any forward-looking statements contained in this presentation in the event of new information, future developments or otherwise.

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Outline

- mRNA Platform Company
- Production Process Singularities
- Development of an Adapted Regulatory Framework
- Adapted QC Test Methods Panel
- Take Home Messages

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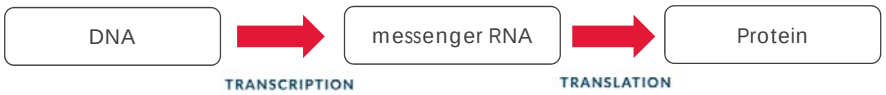
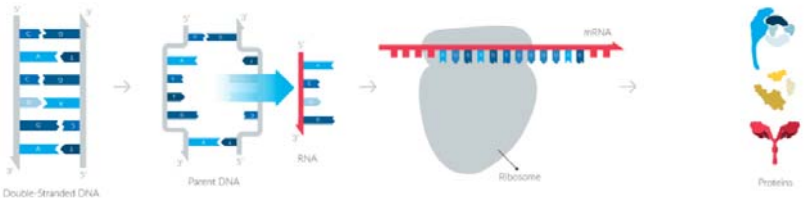
mRNA-based Drugs – Information Vehicles

Central Dogma of Molecular Biology





Hard Drive
Our genetic information

Software
mRNA reads off the DNA and instructs cells to make protein

Hardware
Basic building blocks of all cells in the body

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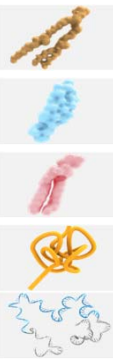
mRNA Encapsulation in Lipid Nanoparticles (LNPs)



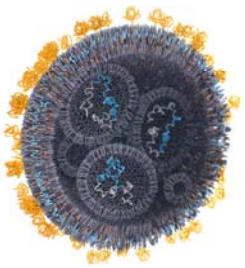
Ionizable Lipid

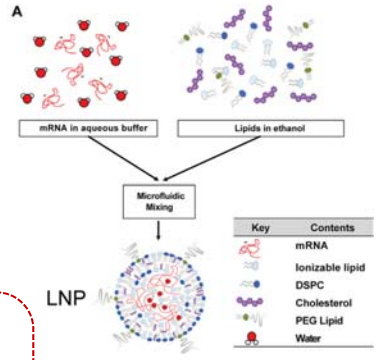
Sterol

Phospholipid

PEG lipid

mRNA





- Enhance stability *in vivo*
- Promote intracellular delivery and release
- Control immune recognition
- Guides cellular tropism

Richner, *et al.*, Modified mRNA Vaccines Protect against Zika Virus Infection; *Cell* (2017)

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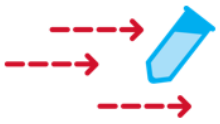
Advantages of mRNA Vaccines
Speed, Precision & Flexibility

Robust immune response

Cellular delivery of mRNA mimics natural viral infection

mRNA allows for rapid vaccine design



Single platform
↓
economies of scale

Time Cost Resources

Single facility for ALL vaccines

Ability to scale-up

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mRNA Technology Platform enabling Medicines Development

In 10 years we have…

Created the field of mRNA-LNP vaccines

…31 vaccines development programs
…22 respiratory vaccines clinical trials (RSV, Flu, CoV, hMPV, PIV)
…4 respiratory virus vaccine combinations
…4 Phase 3 programs by mid-2022 (CMV, RSV, Flu, COVID)

Created the field of mRNA-LNP therapeutics

… 15 therapeutics in development
… 8 in ongoing clinical trials
… Oncology, Rare Diseases, Cardiovascular, Autoimmune…

Manufactured >900 million doses of Moderna COVID Vaccine Spikevax in our first year, the majority from a single site in the US (~500,000 doses per employee)

~400 million people have received a dose of our medicine

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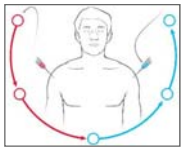
Extensive manufacturing history provides foundation of sequence-agnostic platform processing

	RESEARCH	PERSONALIZED VACCINE UNIT	CLINICAL	COMMERCIAL
				
# Unique Sequences	Tens of thousands	Hundreds	Dozens	Four
				
Scale Per lot	< 0.1 g	0.2 g	2 – 20 g	200 g

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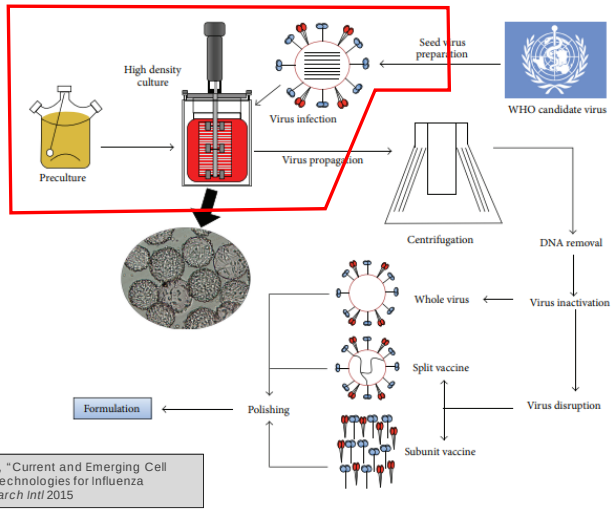
Traditional vaccine manufacturing are exposed to inherent biological complexity of production system.

Cell Based Inactivated Viral Vaccine Process



Milian, E. and Kamen, A., "Current and Emerging Cell Culture Manufacturing Technologies for Influenza Vaccines" *BioMed Research Intl* 2015

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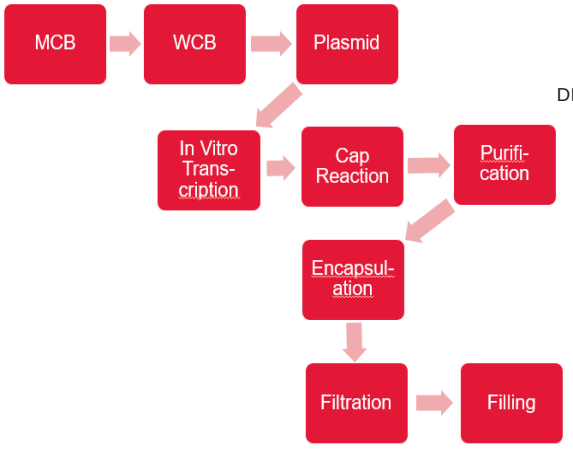
mRNA platform technology based on well-defined manufacturing process



Plasmid

Prokaryotic production system – DNA Template is process **Starting Material**

mRNA

Enzymatic *In Vitro* Transcription + Purification steps

mRNA-LNP

Microfluidic + Purification Steps

Drug Product

Standard Aseptic Fill / Finish

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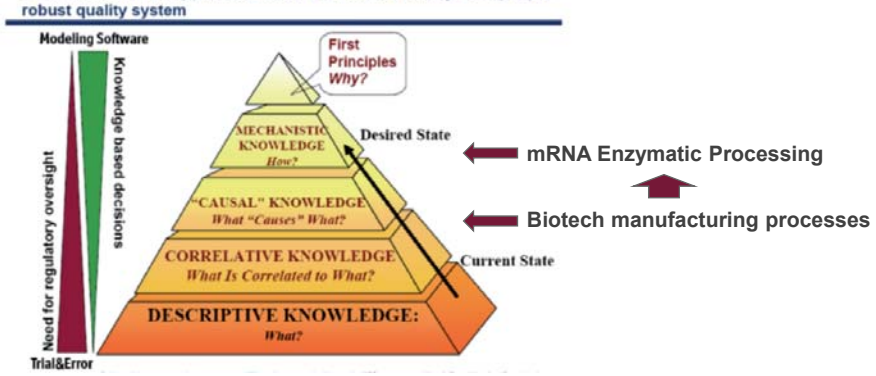
A Mature Technology Requiring Adapted Regulatory Oversight

Science- and Risk-Based Regulatory Decisions

Regulatory oversight can be tailored to reflect scientific rigor demonstrated in an application when it is realized through company's robust quality system



Source: 2008, Hussain, WHO quality-by-design-training-workshop-on-pharmaceutical-development.

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Regulatory Developments for mRNA Products Non-Exhaustive Overview

Regulations

- Falling under vaccine or ATMP (Gene Therapy) definition in the EU: Centralized Procedure.

Guidelines

- No RNA-specific guidance from FDA or EMA
- 2022 FDA Guidance Drug Products, Including Biological Products, that Contain Nanomaterials.
- 2021 WHO Guideline "Evaluation of the quality, safety and efficacy of messenger RNA vaccines for the prevention of infectious diseases: regulatory considerations".
- 2020 WHO Guidelines for assuring the quality, safety, and efficacy of plasmid DNA vaccines.
- 2018 FDA Guidance on Liposome Drug Products.

Standards

- ISO 22412:2017: Particle Size Analysis - Dynamic Light Scattering (DLS)
- ASTM E2859-11: Standard Guide for Size Measurement of Nanoparticles Using Atomic Force Microscopy

Pharmacopeias

- USP (Draft Guidelines) Analytical Procedures for mRNA Vaccine Quality *(To be expanded for DP)*
- USP project for mRNA Vaccine General Chapter .
- *USP plans for development of chapters for testing of molecular size, zeta potential, polydispersity of LNPs (as per Webinar dated March 2022).*

➢ ***Convergence of regulatory developments will facilitate mRNA platforms potential developments.***

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Regulatory Filings Integrating Compendial Requirements

Excipients
- Compendial grade excipients
  - Wfl, Sucrose, Tris. Cholesterol.
- TSE/BSE CEP as required.

Quality Control
- Standard compendial test methods for
  - Appearance, pH, Osmolality, sub-visible particles, Container Content, Bacterial Endotoxins, Bioburden, Sterility

Raw Materials
- Compendial grade raw materials
  - salts, acid, base

- ***Potential for development of new monographs to cover mRNA platform standard reagents (e.g. Nucleotides) or general texts for new test methods.***
- ***Requires convergence of Pharmacopeias for a practical adoption across platforms.***

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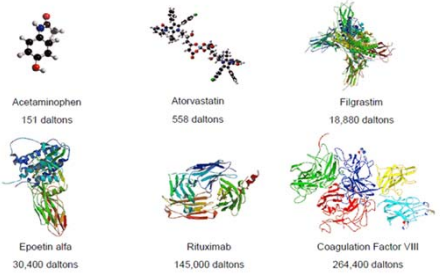
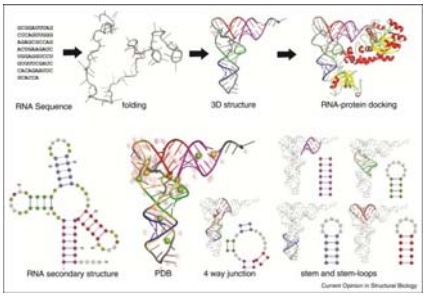
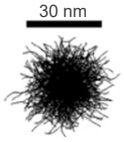
mRNA's Chemical and Structural Diversity Requires New Assay Development and Innovation





MW for a 1000 nt mRNA is >300 kDa



975nt mRNA in water pH 7.4
100 configuration overlay
Gopal et al. RNA 2014

Increased molecular mass and complexity

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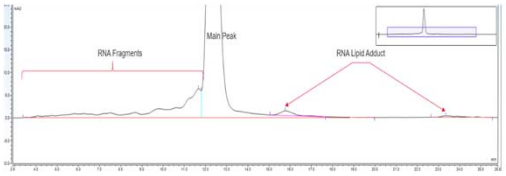
mRNA CQAs Well Characterized and Understood

A cap structure is required at the 5' end for an mRNA to be translated

A polyA tail is required at the 3' end for an mRNA to be translated

mRNA must be intact to be translated – degradants lack either a cap at the 5' end or the polyA tail at the 3' end and would therefore not be translated (i.e., degradants do NOT generate truncated proteins)

The coding region of the mRNA must be correct to ensure that the intended protein is expressed



- Purity is the area percent of the main peak, which represents full length mRNA in the product.
- Impurities are reported as area percent.
  - Pre-main peak contains RNA fragments
  - Post-main peak RNA lipid adduct, nucleotides being modified by lipid impurities or degradants.

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USP Analytical Development Focus

Table1: Quality Attributes for mRNA Drug Substance

Quality	Attribute	Method
Identity	Sequence confirmation	Next generation sequencing (NGS)
		Sanger sequencing
		Reverse Transcriptase – PCR
Content	RNA content	RT-qPCR and RT-dPCR, Ultraviolet Spectroscopy
Integrity	Percentage of intact mRNA and fragment mRNA	Capillary gel electrophoresis
	5' cap	IP-RP-HPLC
	3' poly(A)	RP-HPLC
	mRNA integrity	Gel electrophoresis
Purity	Product related impurities - dsRNA	Immunoblot
	Residual DNA template	qPCR
Safety	Endotoxin	USP <85>
	Bioburden	USP <61>, <62>, <1115>
	Sterility	USP <71>
Other	Appearance	USP <1>, <790>
	pH	USP <791>

Source: Analytical Procedures for mRNA Vaccine Quality (Draft Guidelines).

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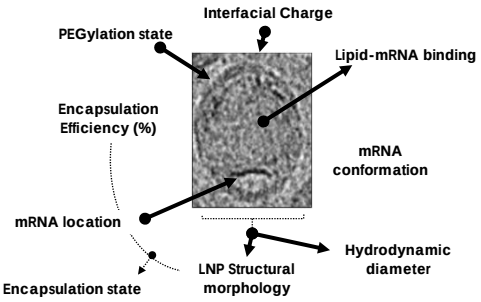
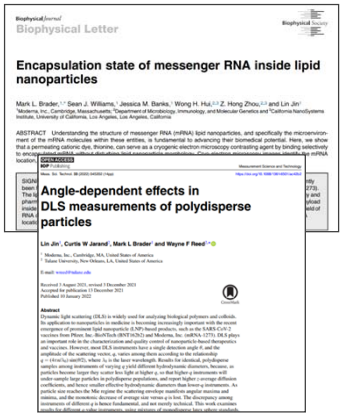
To be complemented for mRNA-LNP – biophysical attributes characterization




Biophysical Letter

Encapsulation state of messenger RNA inside lipid nanoparticles

Angle-dependent effects in DLS measurements of polydisperse particles

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Comprehensive Analytics For mRNA-LNP Characterization

Sequence
- Sanger and NGS sequencing*
- Oligo sequencing and mapping via LC/MS/MS
- Nucleotides and nucleoside analysis by LC or LC/MS/MS

Biochemical and Biophysical Properties
- HPLC Size exclusion with Multi-Angle Light Scattering (MALLS)*, ion exchange, ion paring and affinity
- Differential Scanning Calorimetry (DSC)
- Dynamic Light Scattering (DLS)*, fluorescence spectroscopy
- Flow microscopy*, Cryogenic electron microscopy (CryoEM)*, and atomic force microscope (AFM)*

High Order Structure
- Circular Dichroism (CD), Fourier-transform infrared spectroscopy (FTIR), and Small angle X-ray scattering (SAXs)

Function*
- Cell free translation and cell-based assay
- In vivo expression (multiple species)

Process and product related Impurities
- Residual proteins and solvents
- Residual DNA
- dsRNA*
- RNA fragments and RNA lipid adducts*

*Methods not covered by general EP text for method of analysis.

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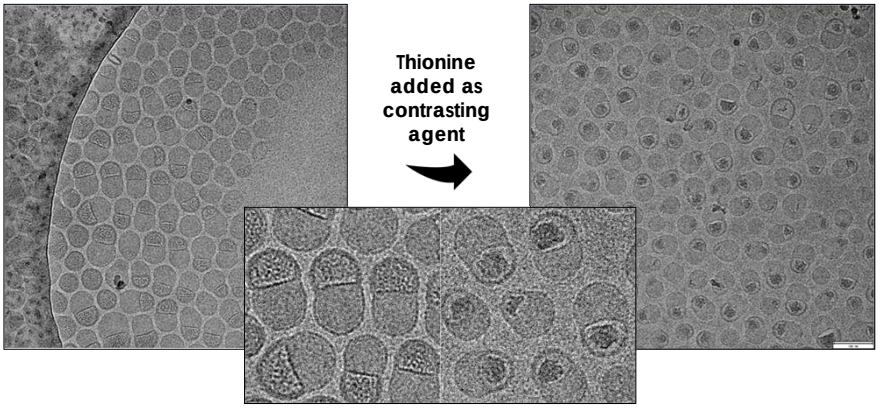
Visualizing mRNA *inside* prototypical LNPs using Cryo-EM



Mark L. Brader, Sean J. Williams, Jessica M. Banks, Wong H. Hui, Z. Hong Zhou, Lin Jin, Encapsulation state of messenger RNA inside lipid nanoparticles, Biophysical Journal, 2021

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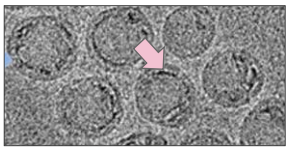
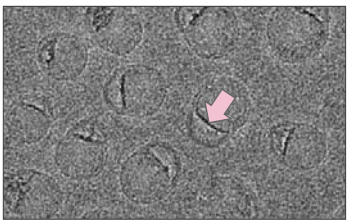
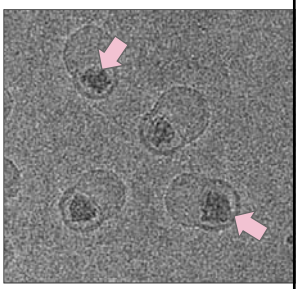
Characterization of mRNA Encapsulation State Inside Lipid Nanoparticles



Lipid-associated mRNA



Poised to transition into bleb



mRNA fully transitioned into bleb aqueous environment

- Thionine is an mRNA binding dye permeable through LNP which selectively bind encapsulated mRNA.
- By applying thionine to mRNA-LNP samples, mRNA is highlighted in Cryo-EM images.
- This combination of dye-binding and cryo-electron microscopy pinpoints the mRNA location, providing new insights into its encapsulation state and chemical microenvironment to advance biophysical studies.

Mark L. Brader, Sean J. Williams, Jessica M. Banks, Wong H. Hui, Z. Hong Zhou, Lin Jin, Encapsulation state of messenger RNA inside lipid nanoparticles, Biophysical Journal, 2021

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Control of Novel Lipid Modified- mRNA Species from mRNA -LNP System

- **A novel lipid modified- mRNA species was discovered** in formulated mRNA -LNP system
- **Industry gold standard techniques** (CE and SEC) **could not detect** this impurity
- **Both NGS and oligonucleotide mapping** showed an identical profile between the MP and LP, **suggesting very low abundance of non-site-specific modifications**.
- **Nucleoside profiling revealed several abundant mass-to-charge (m/z)** values that were exclusively found in the isolated LP.
- **MS/MS** revealed that the unique m/z are **lipid-modified nucleosides** (modified on the nucleobase).
- **Reaction modeling studies** was performed to identify lipid **contributors to adduct formation** (data not shown)- **electrophilic/oxidative impurities** originating from the ionizable cationic lipid were **identified as reactants**, resulting in adduct formation

Source: A novel mechanism for the loss of mRNA activity in lipid nanoparticle delivery systems. Packer M, et al. Nat Commun. 2021.

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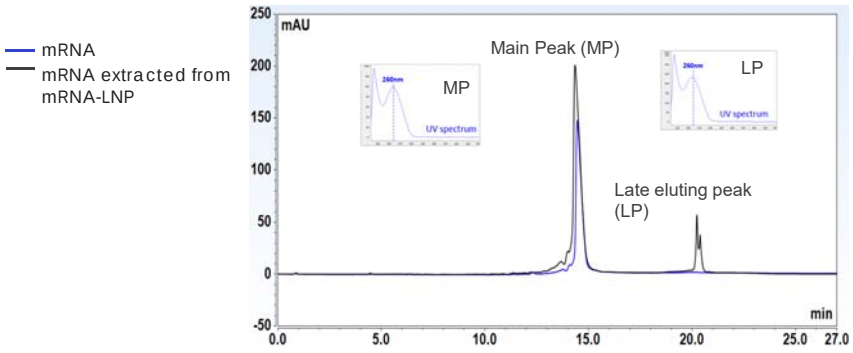
mRNA Extracted From the Drug Product

When Characterized by RP-IP Showed an Additional Late Eluting Peak



- LP and MP had the same maximum absorbance at 260 nm – **confirming RNA-related population**
- Cell free/in-vitro expression assay revealed that **LP showed little to no expression**

Source: A novel mechanism for the loss of mRNA activity in lipid nanoparticle delivery systems. Packer M, et al. Nat Commun. 2021.

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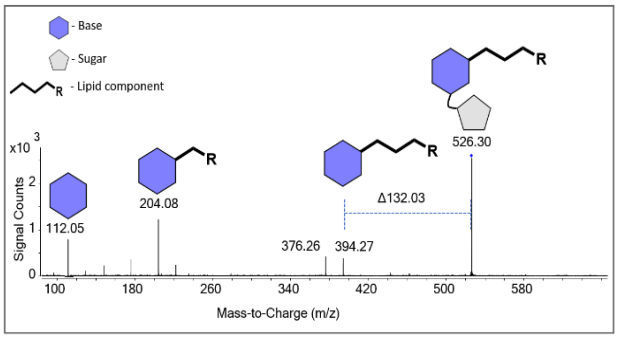
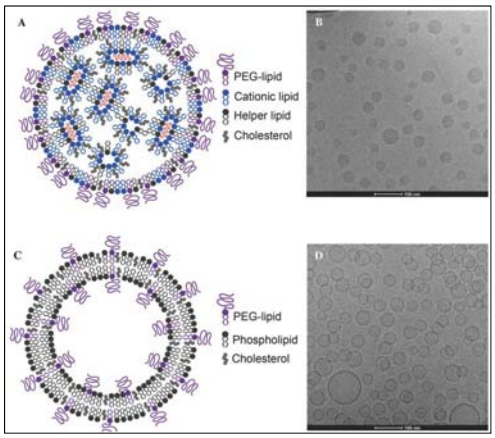
MS/MS Analysis Revealed That the Unique m/z Observed in LP are Lipid-Modified Nucleosides

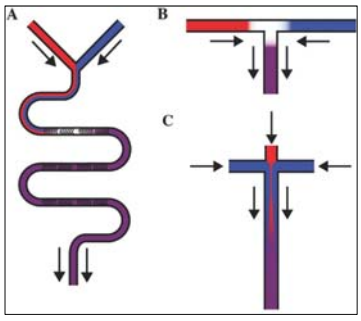


- The fragment ion of m/z 112.05 corresponds to the exact mass of protonated cytosine (nucleobase).
- The characteristic neutral mass loss of 132 Da, (ribose loss) indicates that the ribose is not modified.

Source: A novel mechanism for the loss of mRNA activity in lipid nanoparticle delivery systems. Packer M, et al. Nat Commun. 2021.

Lipid Nanoparticle Preparation and Structure





Evers,*et al.*, State-of-the-Art Design and Rapid-Mixing Production Techniques of Lipid Nanoparticles for Nucleic Acid Delivery *Small Methods* (2018)

Take Home Messages

- mRNA-LNP platforms have the potential to support the development of a new “information vehicle” class of medicinal products.
- To the difference of vaccines / recombinant proteins manufactured through biological production system, it is based on simpler and well characterized enzymatic (IVT) manufacturing process.
- Although the mRNA technology supported recent innovative developments; it is already supported by a mature manufacturing platform technology.
- Convergence of requirements for Characterization and Quality Control will help to the harmonized control of future mRNA-LNP based products.
- Quality control of mRNA-LNP based products can integrate standard methods but each unique formulation / process can lead to different product conformation and process related impurities.

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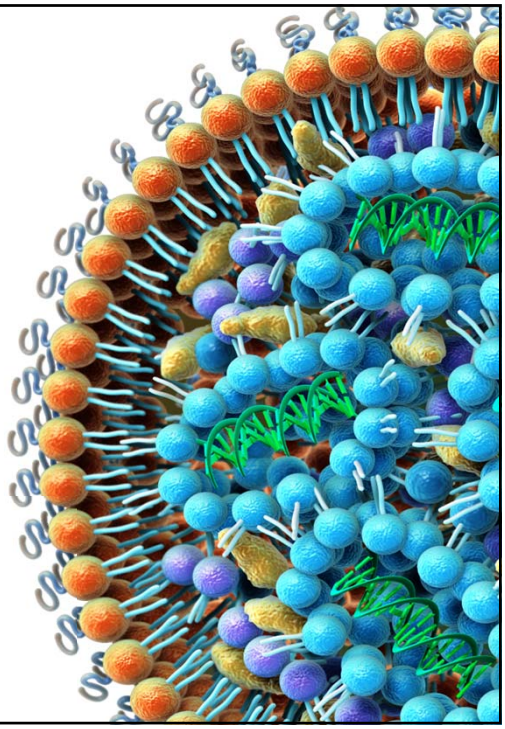
EDQM: Quality requirements for nanomedicines: what role should the European Pharmacopoeia play?

Manufacturing Consideration for RNA-based Nanomedicines: Assembly, Downstream Processing and Analytics

Martin Rabel, PhD, Pharmacist

07th June 2022



PRECISION NANOSYSTEMS
Create Transformative Medicines™

edqm
European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

COUNCIL OF EUROPE
CONSEIL DE L'EUROPE

PRECISION NANOSYSTEMS

Our Mission

To accelerate the creation of transformative medicine that significantly impacts human well being.

1

Genomic Medicines are the Future

Genomic Medicines are the Future

Treating Disease at Its Fundamental Level

Silence Genes	Express Genes		Edit Genes
			
siRNA & other non-coding	Messenger RNA	DNA	CRISPR & other gene editing

Target Any Gene Any Way

Designed Not Discovered

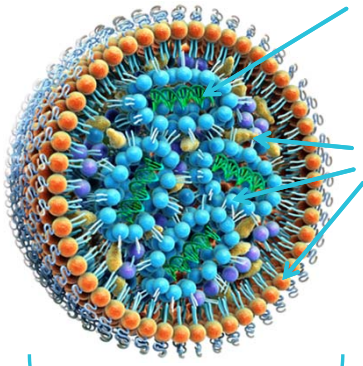
Validated & Ready for Mainstream

Manufacturable

Limitless Possibilities

Genomic Medicines = Nucleic Acid + Delivery

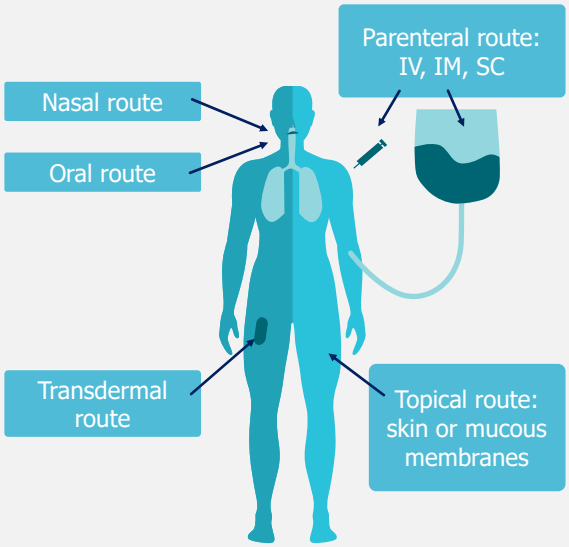
RNA & DNA are large molecules that require nanoparticle delivery technologies to get into tissues and cells.



RNA or DNA
Active Pharmaceutical Ingredient (API)

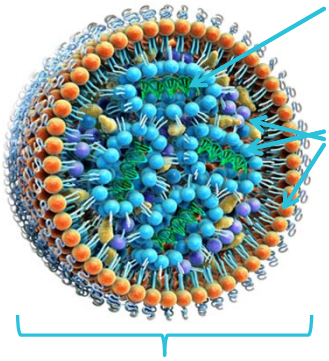
Synthetic Lipids or Polymers
Delivery technologies (excipients)

Drug Product
(10 – 1000 um)



Factors That Influence the Delivery of Nanoparticles

- RNA & DNA are large molecules that require delivery technologies that protect and traffic them to the site of disease
- LNPs allow delivery of larger mRNAs and co-delivery of multiple RNAs that can enable new therapeutic mechanisms
- **A vast library of lipids** can be mixed, matched and modified to deliver a variety of nucleic acid APIs to different targets in the body.
- **Critical quality attributes** of the LNPs affect safety, efficacy and biodistribution



RNA or DNA
Active Pharmaceutical Ingredient (API)

Synthetic Lipids or Polymers
Delivery technologies (excipients)

Drug Product
(10 – 1000 um)

Pillars of a Successful Genomic Medicine

PRECISION NANOSYSTEMS
Create Transformative Medicines™

PNI Capabilities

Target

Identify specific gene targets and need to silence, express, or edit

Payload— Genetic API

Choose modality appropriate for target modulation

Delivery— Nanoparticle

Protect, transport, and release API into target cells

Manufacturing

Scalable production for all stages of development

2

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RNA - Lipid Nanoparticle Assembly using Microfluidics

Process Development Requirements of RNA-LNP

RNA LNP Formulation

Lipid composition
Molar ratio
N/P ratio
Drug substance

Downstream Processing

Buffers
Buffer exchange (TFF)
Sterile filtration

Biological Assay*

In vitro/in vivo activity
Toxicity
PK/BD

Particle Formation
(microfluidic parameters)

Flow rate ratio
Total flow rates
Dilution rates

Analytics

Analytical methods for lipids, RNA, LNP, endotoxin, osmolality, etc.
Target spec's—materials & DP

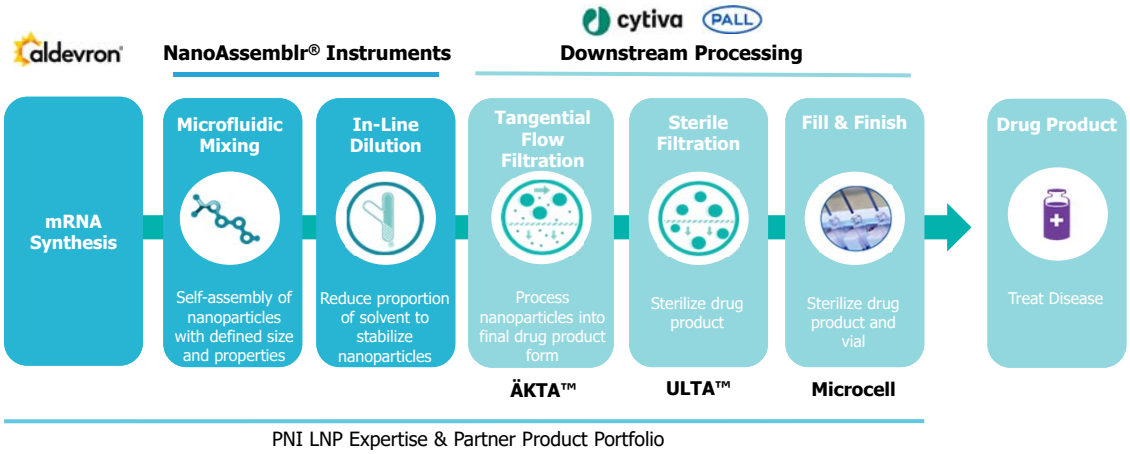
Initial Stability

In-process
Long-term storage

***consider adding a benchmark formulation**

End-to-End Manufacturing of Genomic Medicines

Enabling (bio)pharma companies and CDMOs with no technology access fees or royalties associated with PNI instruments



PNI LNP Expertise & Partner Product Portfolio

The Evolution of Manufacturing Methods for Nanomedicines

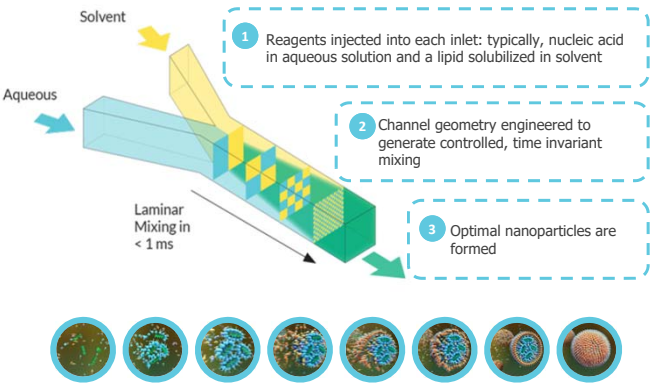
High Energy Techniques	In-Line Macromixing	Microfluidic Approaches	NxGen Microfluidics
		- Some scaling challenges remain	+ Easy to Scale
		- Mixers are difficult to make	+ Mixers are Easy to make
- Limited applications	- Limited applications	+ Expanded Applications	+ Potential multi-mixer integration opens possibilities
- Difficult to reproduce	- Difficult to reproduce	+ Reproducible	+ Reproducible
- Harsh process conditions	+ Gentler process conditions	+ Non-turbulent process conditions	+ Non-turbulent process conditions
- Difficult to scale	+ Improved Scalability		

Precise - Non-turbulent particle formation to ensure the most reproducible results for a wide range of nanoparticle types

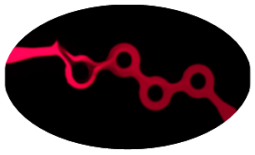
Scalable - More than 25X single mixer throughput simplifies scaling up while maintaining particle quality and batch-to-batch reproducibility

Innovative - Platform designed to rapidly take ideas to patients

PNI Microfluidics Are the Optimal Way to Manufacture Nanomedicines

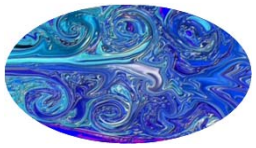


PNI's NxGen Time Invariant Mixing



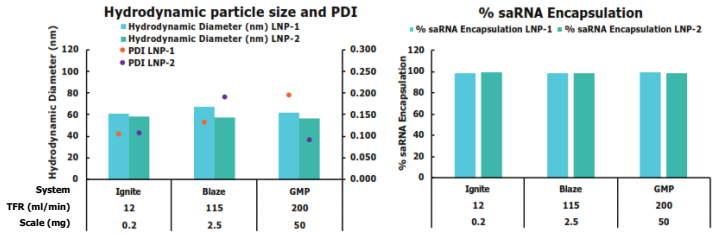
Mixing and particle formation unchanged over time

Unsteady Turbulent Mixing (Non-PNI Mixing Method)



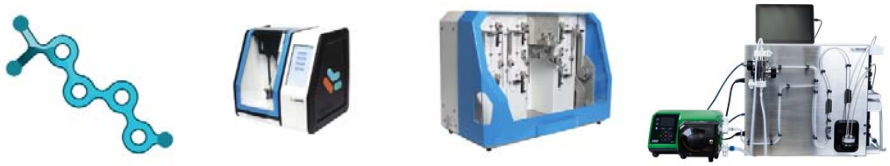
Mixing conditions constantly changing over time

Self-Amplifying RNA-LNPs Have Equivalent Size, PDI and Encapsulation Across Scales (Ignite-Blaze-GMP)



- SARS-CoV-2 self-amplifying RNA-LNP made with PNI proprietary ionizable lipid had similar Critical Quality Attributes (CQAs) such as size (~60 nm), polydispersity (<0.2) and encapsulation efficiency (>90%) across all scales tested with two different LNP compositions.

Scalability Across Platform was achieved for LNP-1 and LNP-2

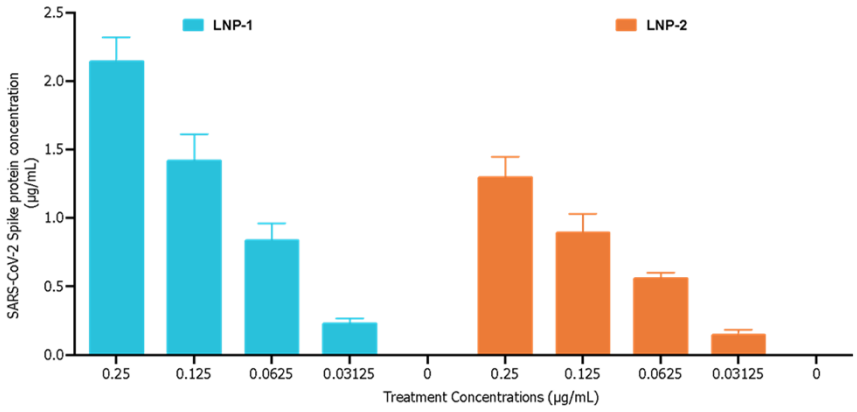


Simplified Scale-up of mRNA-LNP Using NxGen™

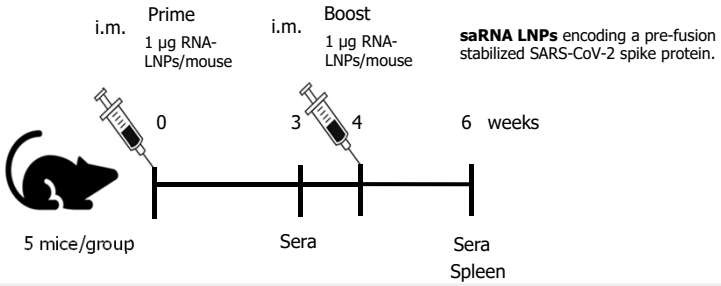
In Vitro Activity saRNA LNPs Using Two Novel Lipid Compositions

ELISA quantification of the SARS-CoV-2 spike protein expression in HEK-293 cells after transfection with saRNA LNPs

- Both LNPs showed an activity dose response *in vitro*
- LNP-1 is more active in-vitro than LNP-2



In Vivo Testing of saRNA-LNP Candidate Formulations

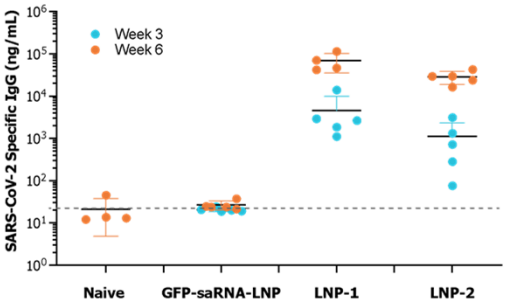


Sera →
- SARS CoV2 specific IgG ELISA
- Cytokine measurements/Neutralization assays

Spleen →
- Isolation of splenocytes
- *Ex vivo* restimulation with SARS-CoV-2 peptides
- Intracellular cytokine staining/Cytokine measurements

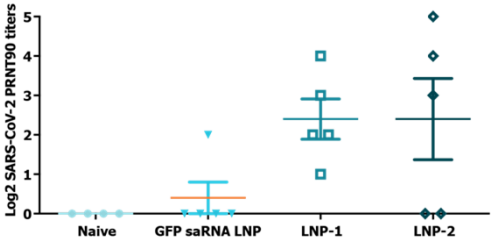
In Vivo Activity of saRNA-LNP with Novel Lipid Compositions

SARS-CoV-2 Specific Serum IgG Measurements at Week 3 & 6



Neutralizing antibodies against SARS-CoV-2

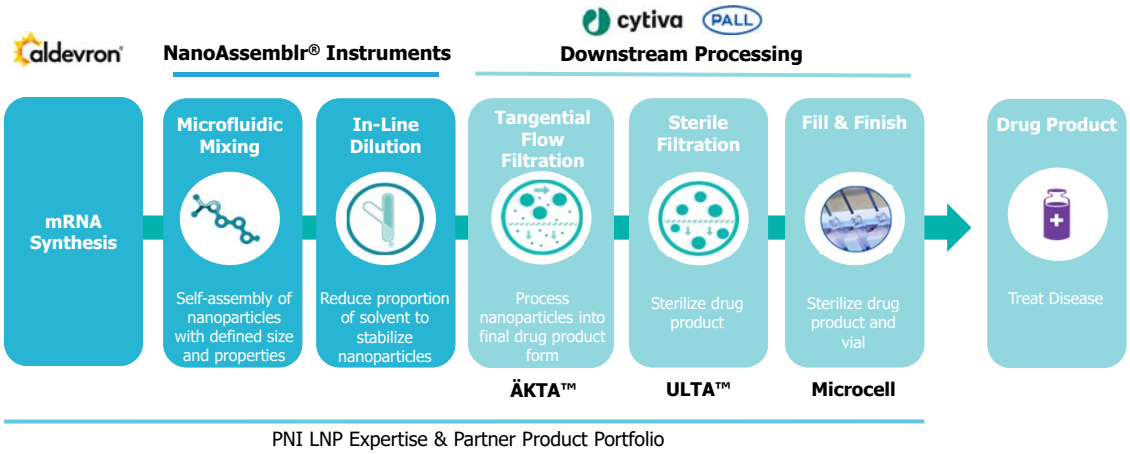
pRNT90 Values (SARS-CoV-2 real virus particles)



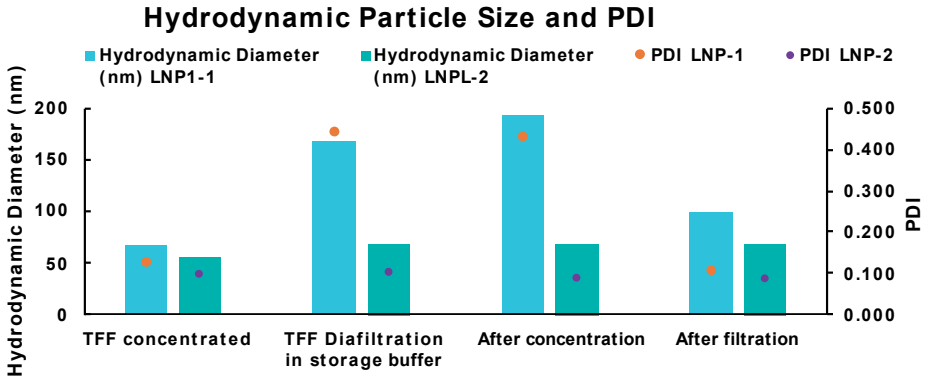
- Both LNP-1 and LNP-2 efficiently induced SARS CoV2 specific IgG response in mice
- As observed *in vitro,* LNP-1 showed slightly higher activity as compared to LNP-2
- Both LNP-1 and LNP-2 generated neutralizing antibodies against the SARS-CoV-2 virus
- Both LNP-1 and LNP-2 also showed effective cellular and humoral immune responses (data not shown)

End-to-End Manufacturing of Genomic Medicines

Enabling (bio)pharma companies and CDMOs with no technology access fees or royalties associated with PNI instruments



Need to Evaluate Scalability, Stability & Robustness of saRNA-LNP Candidates for the entire Manufacturing Process



- At the 50 mg scale LNP-1 showed particle size increase during down-stream processing (TFF)
- RNA encapsulation > 90% for both formulations for all processing steps
- LNP-2 particle size stable throughout TFF and was selected as the lead clinical formulation

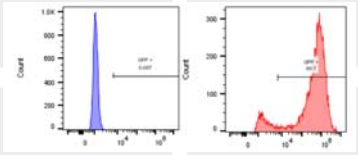
3

Analytical Requirements to Assess RNA-LNPs

Analytical Methods Are Critical for Developing RNA-LNP Medicines

LNP Composition
- Size, PDI, ζ-potential
- Stability
- Lipid composition
- Excipients

RNA Quality
- Quantity
- Integrity
- Structure



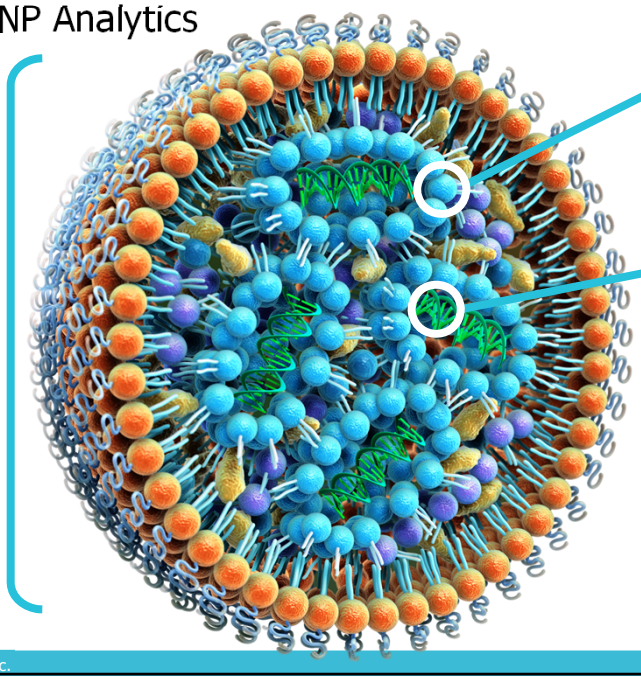
***In Vitro* Potency Assay:**
- Sum of all effects

Payload Design
- Sequence
- Production
- Biology

Encapsulation
- %EE
- Location of RNA

Common LNP Analytics

Whole Particle:
- Dynamic Light Scattering
- NTA
- Electron microscopy (cryo-TEM)
- Zeta potential

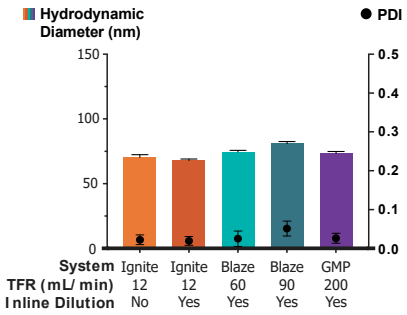


Lipid Components
- LC-MS
- UPLC-ELSD
- UHPLC-CAD

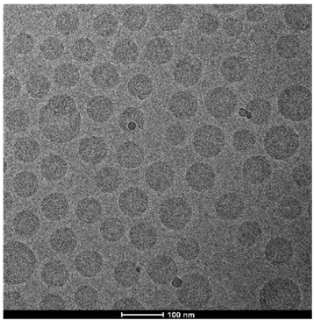
Nucleic Acid
- RiboGreen/PicoGreen
- BioAnalyzer
- IPRP-UPLC-UV
- LC-MS

Analytics: Particle size, Distribution, Zeta Potential

Dynamic Light Scattering
- Study size distribution
- Calculates hydrodynamic radius based on scattering intensity



Cryo-TEM
- Particle morphology
- Electron beam on flash-frozen samples

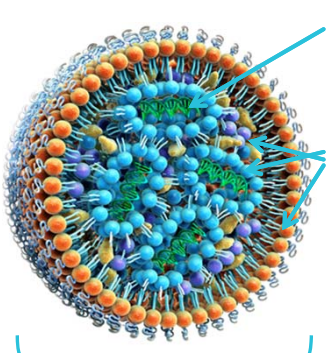


en.wikipedia.org

LNPs Pose a Challenge for RNA Characterization

mRNA-LNPs contain a highly ordered structure of lipids, RNA and excipients resulting in **unique analytical challenges**:

- Drug product is not in solution but is a suspension prone to aggregation, precipitation and sample losses when transferred
- High lipid/excipient concentration greatly impacts RNA assays (matrix effects, sample handling, light scattering, fluorescence quenching, etc.)
- Particle physical characteristics (size, PDI) impact assay efficacy
- Drug product may contain populations of encapsulated and free RNA
  - Extraction of RNA is often required
- mRNA is highly structured and prone to degradation



RNA or DNA
Active Pharmaceutical Ingredient (API)

Synthetic Lipids or Polymers
Delivery technologies (excipients)

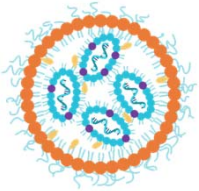
Drug Product
(10 – 1000 um)

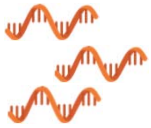
Analysis of mRNA Encapsulation

Therapeutic dose of drug product is largely defined by **concentration** of **encapsulated** nucleic acid API

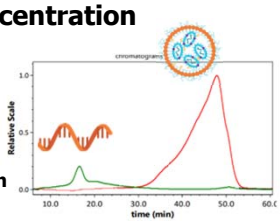
Encapsulated mRNA:
- **Low immunogenicity**
- **Protected from Nucleases**
- **High transfectability**

Free mRNA:
- **Immunogenic**
- **Unstable**
- **Poor Cellular Uptake**



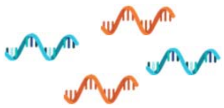


Separation



Size exclusion chromatography (SEC)
Field-Flow Fractionation (FFF)
Capillary Electrophoresis (CE)
Analytical Ultracentrifugation (AUC)

Disruption



'Total mRNA'

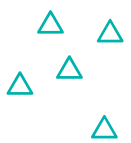
Fluorescent assays (E.g. Ribogreen™)

In general, therapeutic efficacy of LNP formulations improves with increased mRNA % encapsulation efficiency (%EE)

$$\%EE = \frac{Encapsulated\ mRNA}{Total\ mRNA} \times 100\%$$

Analytics: Encapsulation Efficiency

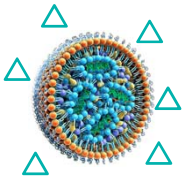
- RiboGreen is a dye that fluoresces when bound to RNA
- UV quantification is prone to interference from proteins and lipids
- Relatively standard assay that is readily available
- Linear over a wide concentration range



Unbound RiboGreen (low fluorescence)

RiboGreen bound to RNA (high fluorescence)

RiboGreen cannot bind to RNA encapsulated in LNP

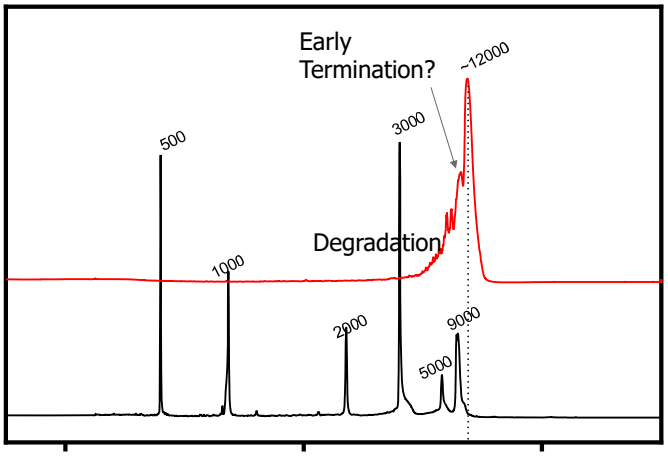


A modified protocol to release the RNA is required

Characterization of RNA: Integrity using Capillary Electrophoresis

Sciex PA800Plus CE

Custom method development

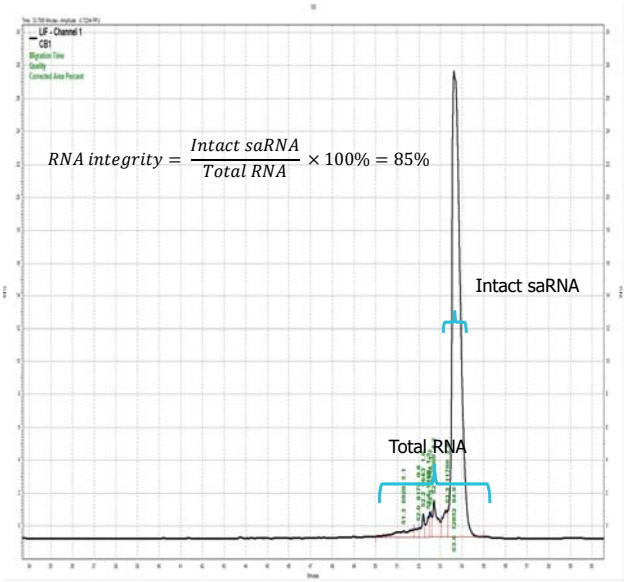


- RNA-LNP production can involve high shear forces, low pH and high temperatures resulting in degradation of mRNA

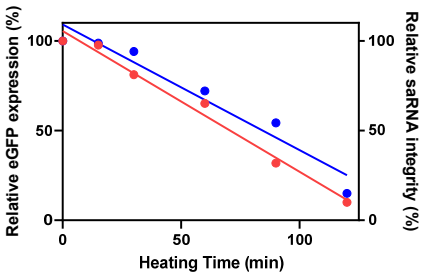
Characterization of RNA: Integrity using Capillary Electrophoresis

Resulting method provides:

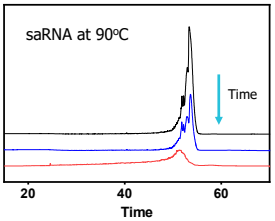
- Identity via sizing
- Concentration
- Resolution of impurities
- RNA integrity
- Stability profiling

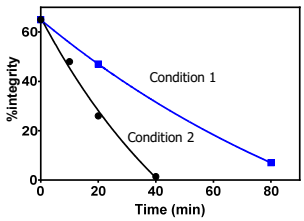


Impact of RNA Quality on Potency



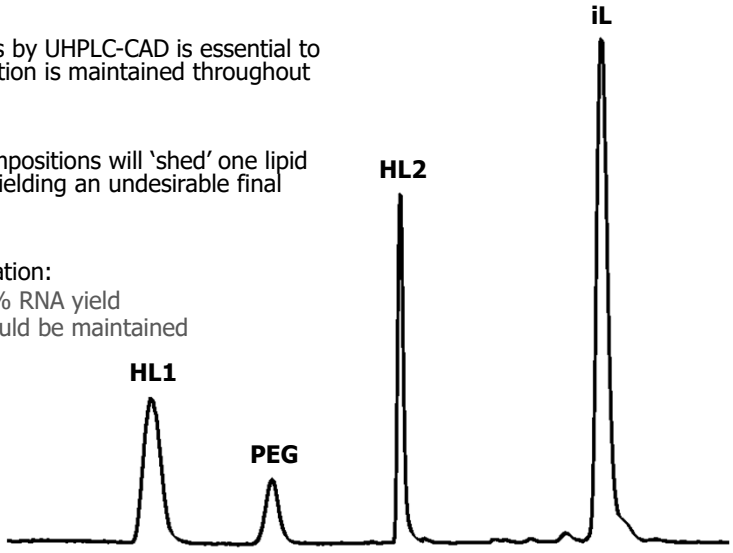
- eGPF sa-mRNA employed as a model to assess impact of sa-mRNA integrity on potency
- Integrity by CE correlates (r=0.92) very well with *in vitro* potency
- CE enables screening formulation conditions to optimize saRNA stability





Lipid Analysis Can Identify Process Issues

- Routine lipid analysis by UHPLC-CAD is essential to ensure LNP composition is maintained throughout formulation process
- Why? Some LNP compositions will 'shed' one lipid during formulation yielding an undesirable final composition
- Formulation optimization:
  - Target high % RNA yield
  - N:P ratio should be maintained



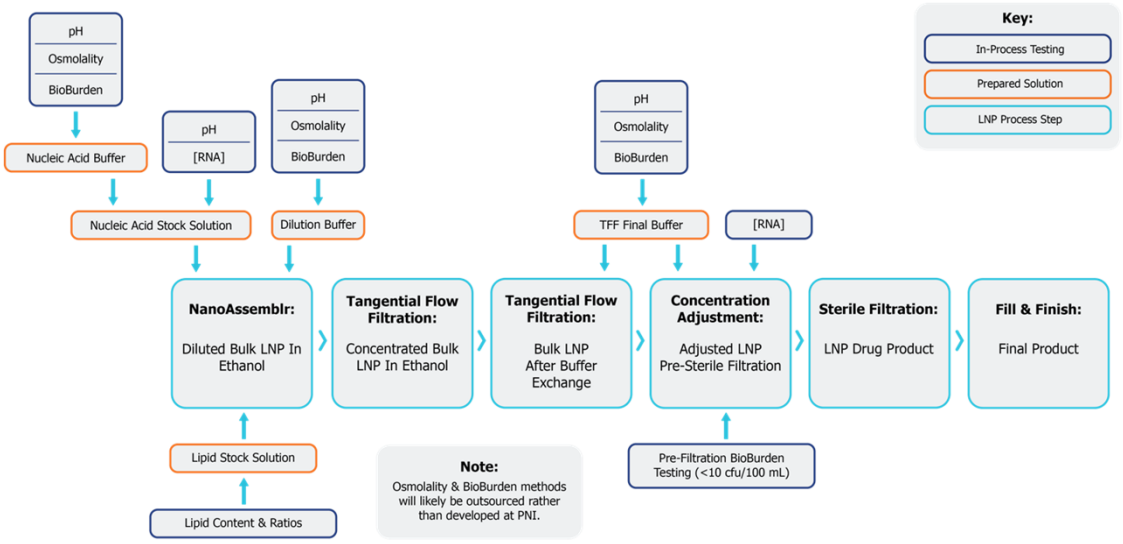
iL: ionizable lipid
HL1: helper lipid 1
HL2: helper lipid 2

Analytical Methods Overview

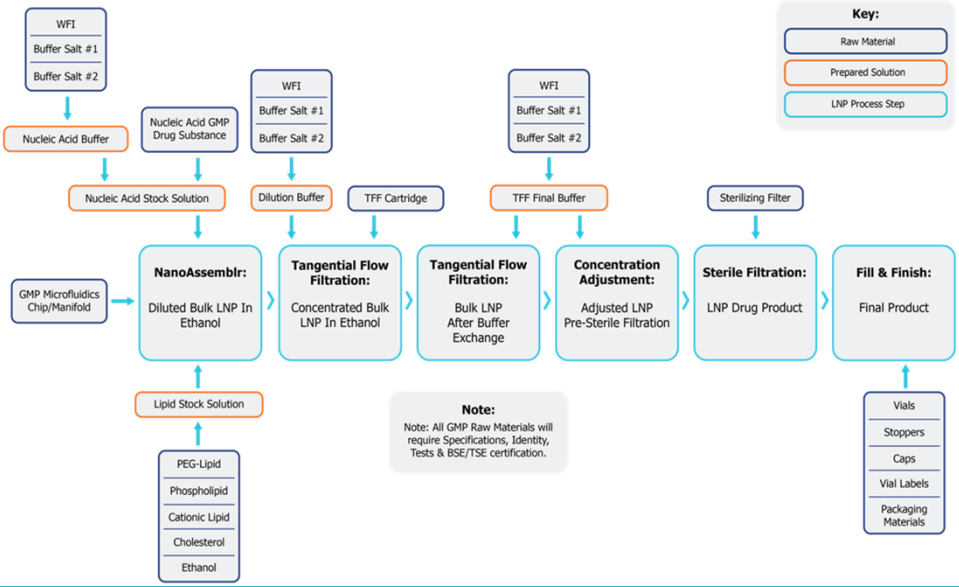
	Test	Method	Release Testing	In Process Testing
Analytical Requirements for Injectable Drugs (USP/Ph.Eur. methods available)	Appearance	Visual Inspection: USP <790> / Ph.Eur. 2.9.20	X	
	pH	Potentiometric: USP <791> / Ph.Eur 2.2.3	X	(X)*
	Osmolality	Freezing Point Depression: USP <785> / Ph.Eur. 2.2.35	X	(X)*
	Bacterial Endotoxins	USP<85> / Ph.Eur 2.6.14.	X	
	Sterility/BioBurden	USP<71> / Ph.Eur 2.6.1.	X	(X)*
	Particulate Matter	USP<788> / Ph.Eur 2.9.19.	X	
	Elemental Impurities	USP<233> / Ph.Eur 2.4.20	X	
LNP Specific Analytics (not available)	RNA Identity/Integrity	Capillary Electrophoresis or Bioanalyzer	X	X**
	Particle Size/PDI	Dynamic Light Scattering	X	X**
	RNA Content/Encapsulation	Ribogreen Assay	X	X**
	Lipid Content	UPLC-CAD	X	X**
	Lipid:RNA Ratio	Calculation	X	
	Potency Bioassay	In Vitro Assay	X	

*applied for all aqueous buffer systems **applied for all LNP processing steps

RNA-LNP Production: In-Process Testing

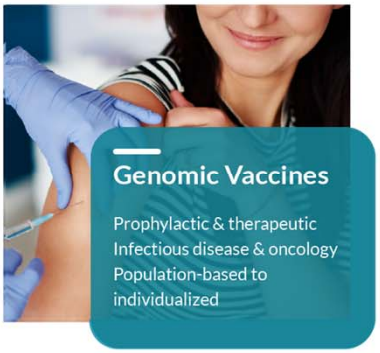


RNA-LNP Manufacturing: GMP Raw Materials



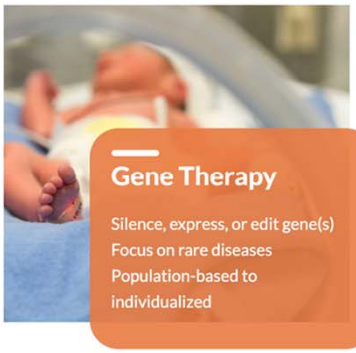
Accelerating Tomorrow's Genomic Medicines

From idea to approved medicine.



Genomic Vaccines

Prophylactic & therapeutic
Infectious disease & oncology
Population-based to individualized



Gene Therapy

Silence, express, or edit gene(s)
Focus on rare diseases
Population-based to individualized

Cell Therapy

Immune cells including T-Cells
Focus on oncology
Autologous & allogenic

These therapeutic modalities have broad application in the prevention and treatment of diseases including infectious diseases, rare diseases and cancer

Thank you for Listening!

Additional resources:

Website:
https://www.precisionnanosystems.com

Publications, posters, application notes, videos:
http://www.precisionnanosystems.com/resources

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