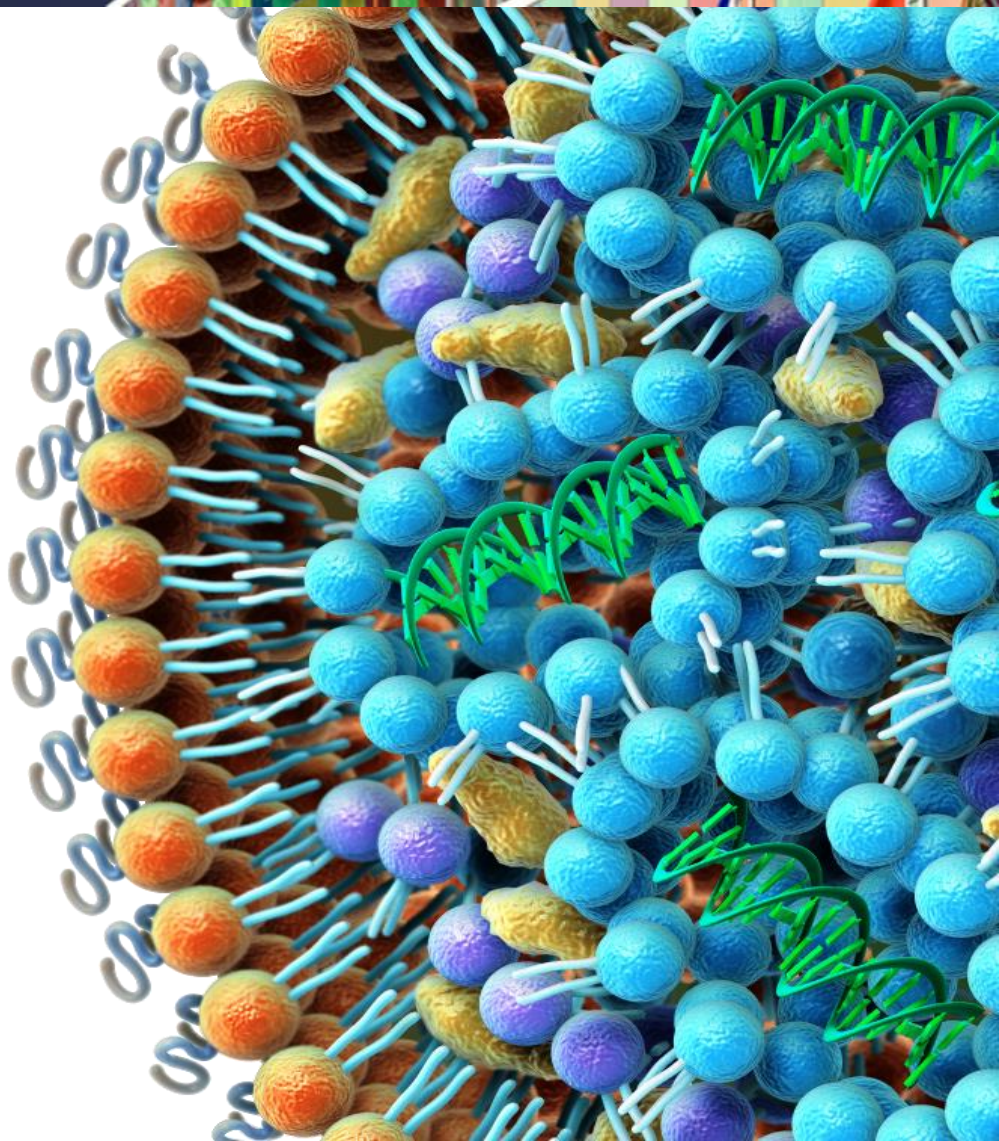
CRS 2022 Annual Meeting & Expo
Advanced Delivery Science
July 11 – 15, 2022 | Montreal Congress Center, Montreal Canada

Accelerating the Development of RNA-Based Medicines Using the Genomic Medicine Toolkit: A Case Study on Developing a COVID-19 mRNA-LNP Vaccine

Serra Gürcan, PhD
Martin Rabel, PhD, Pharmacist

12th July 2022



PRECISION NANOSYSTEMS
Create Transformative Medicines™

Our Mission

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

©2022 Precision NanoSystems Inc.

We are part of the Danaher Life Sciences family

Beckman Coulter Life Sciences · cytiva · idbs · IDT Integrated DNA Technologies · Leica Microsystems

Molecular Devices · Pall · phenomenex · Precision NanoSystems · SCIEX · aldevron

©2022 Precision NanoSystems Inc.

A Global Leader in Nanoparticle Solutions

PRECISION NANOSYSTEMS
Create Transformative Medicines™

Our Headquarters

Leading
End-to-End Nanoparticle Solutions Provider

150+
Contract Service Projects Supported

700+
Systems Placed

Universal
Application Across Payloads, Delivery Technologies & Drug Products

5
Nanomedicine Innovation Network Centers

225+
Cumulative Publications in Peer Reviewed Journals

2
cGMP Manufacturing Lines Under Development in Vancouver

~125
Patents Granted or Pending for Proprietary Technology Platforms

©2022 Precision NanoSystems Inc.

1

Genomic Medicines are the Future: The Genomic Medicine Toolkit

PRECISION NANOSYSTEMS
Create Transformative Medicines™

©2022 Precision NanoSystems Inc.

Genomic Medicines are the Future

PRECISION NANOSYSTEMS
Create Transformative Medicines™

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

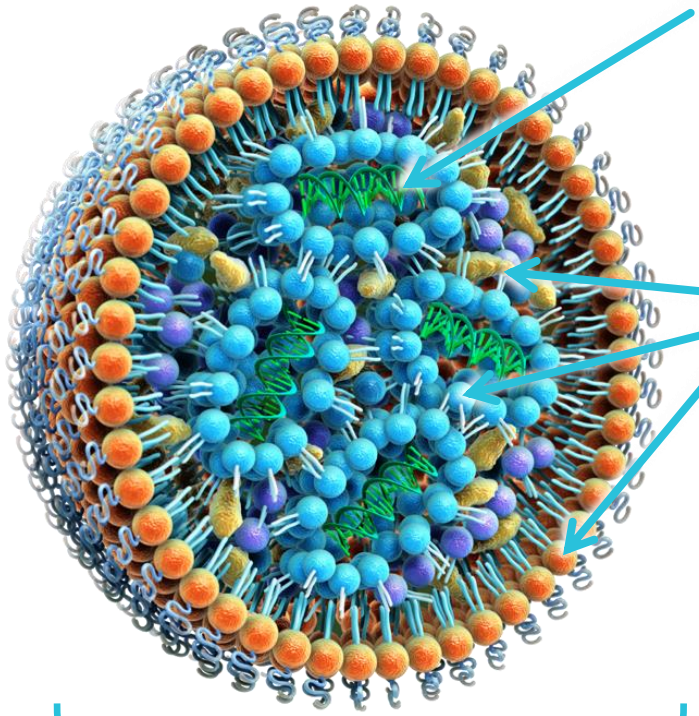
Limitless Possibilities

©2022 Precision NanoSystems Inc.

Genomic Medicines = Nucleic Acid + Delivery

PRECISION NANOSYSTEMS
Create Transformative Medicines™

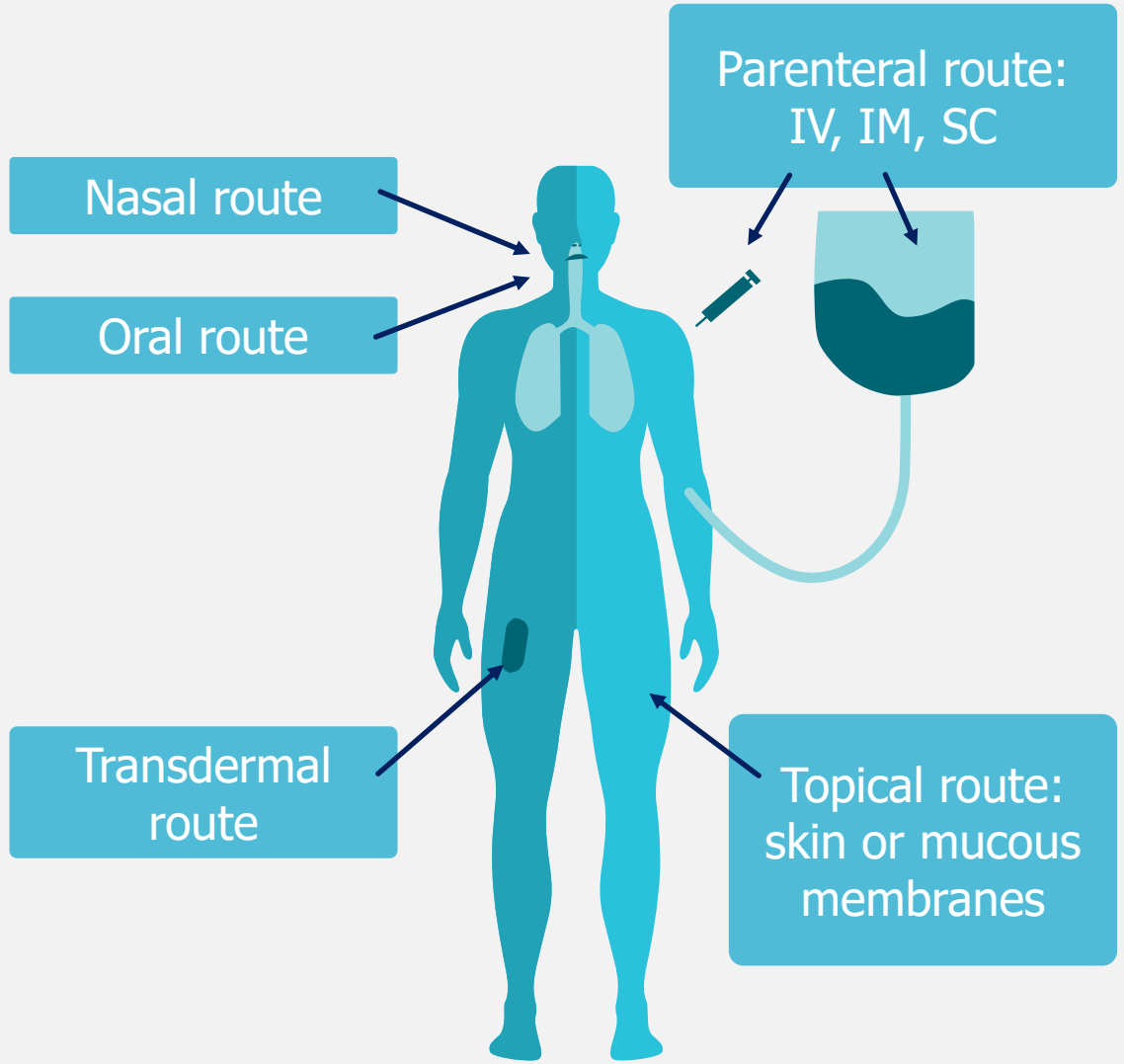
RNA & DNA are large molecules that require nanoparticle delivery technologies to get into tissues and cells.



RNA or DNA
Active Pharmaceutical Ingredient (API)

Synthetic Lipids or Polymers
Delivery technologies (excipients)

Drug Product
(10 – 1000 um)



©2022 Precision NanoSystems Inc.

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

©2022 Precision NanoSystems Inc.

Full Stack of Technology to Enable the Genomic Medicine Revolution

PRECISION NANOSYSTEMS
Create Transformative Medicines™

Genomic Medicine Toolkit

Disease Target

Biological insights can identify target gene(s) driving disease

Genetic Payload Platform

Proprietary self-amplifying mRNA (SAM) to express specific proteins, including antigens used in RNA vaccines against COVID-19

RNA/DNA can also silence or edit target gene(s)

GenVoy™ Delivery Platform

Lipid nanoparticles (LNP), derived from a proprietary lipid library, that protect and deliver nucleic acids (RNA, DNA, derivatives) to target cells

Rapidly develop at lab scale and seamless translation to the clinic

NanoAssemblr® Manufacturing Platform

Proprietary, scalable, continuous flow, and single-use microfluidic mixing technology for controlled and precise nanoparticle encapsulated genetic medicine development & manufacturing

Produce the best drugs — faster, easier, and with the least risk possible — from µL lab scale to GMP scale

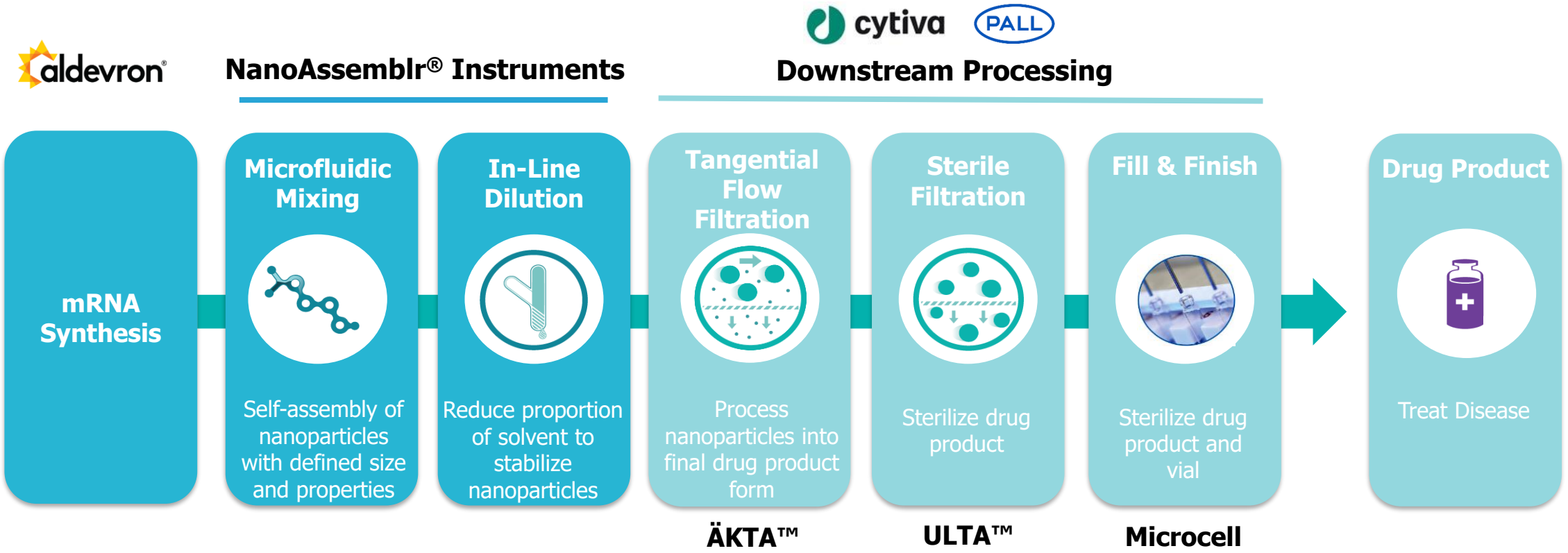
Drug Development Expertise

Leverage world-leading expertise in LNPs and genetic medicine development

©2022 Precision NanoSystems Inc.

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

©2022 Precision NanoSystems Inc.

2

Genetic Payload Platform: PNI-RNA Technology

©2022 Precision NanoSystems Inc.

Full Stack of Technology to Enable the Genomic Medicine Revolution

PRECISION NANOSYSTEMS
Create Transformative Medicines™

Genomic Medicine Toolkit

Disease Target

Biological insights can identify target gene(s) driving disease

Genetic Payload Platform

Proprietary self-amplifying mRNA (SAM) to express specific proteins, including antigens used in RNA vaccines against COVID-19

RNA/DNA can also silence or edit target gene(s)

GenVoy™ Delivery Platform

Lipid nanoparticles (LNP), derived from a proprietary lipid library, that protect and deliver nucleic acids (RNA, DNA, derivatives) to target cells

Rapidly develop at lab scale and seamless translation to the clinic

NanoAssemblr® Manufacturing Platform

Proprietary, scalable, continuous flow, and single-use microfluidic mixing technology for controlled and precise nanoparticle encapsulated genetic medicine development & manufacturing

Produce the best drugs — faster, easier, and with the least risk possible — from µL lab scale to GMP scale

Drug Development Expertise

Leverage world-leading expertise in LNPs and genetic medicine development

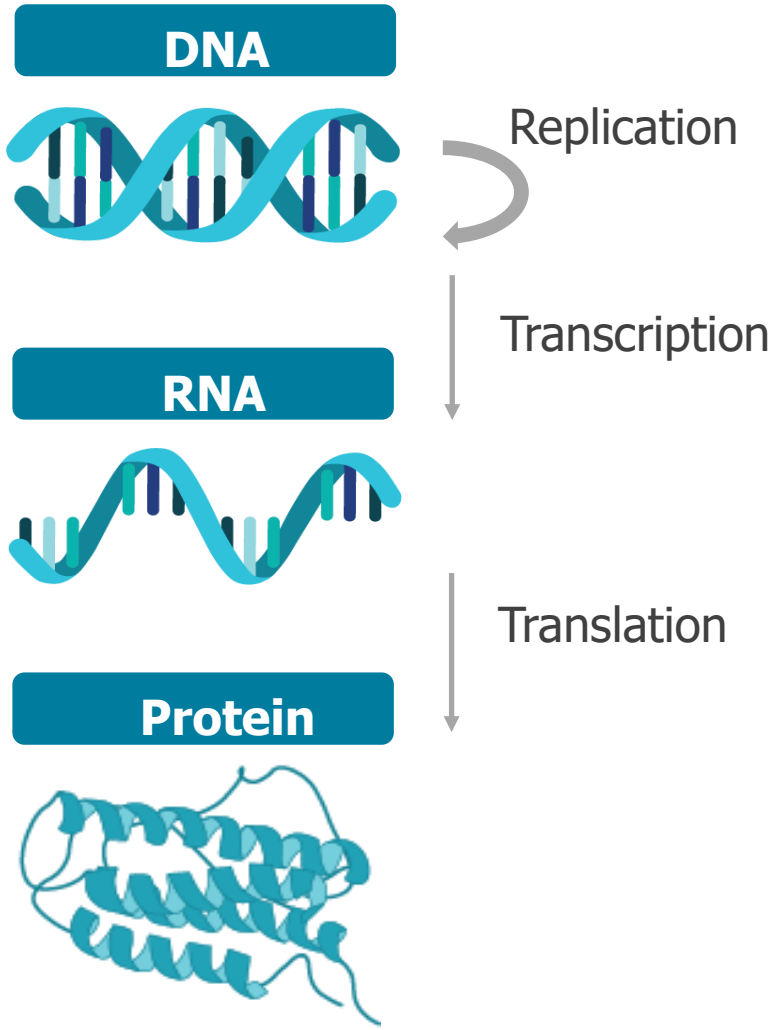
©2022 Precision NanoSystems Inc.

DNA or RNA?

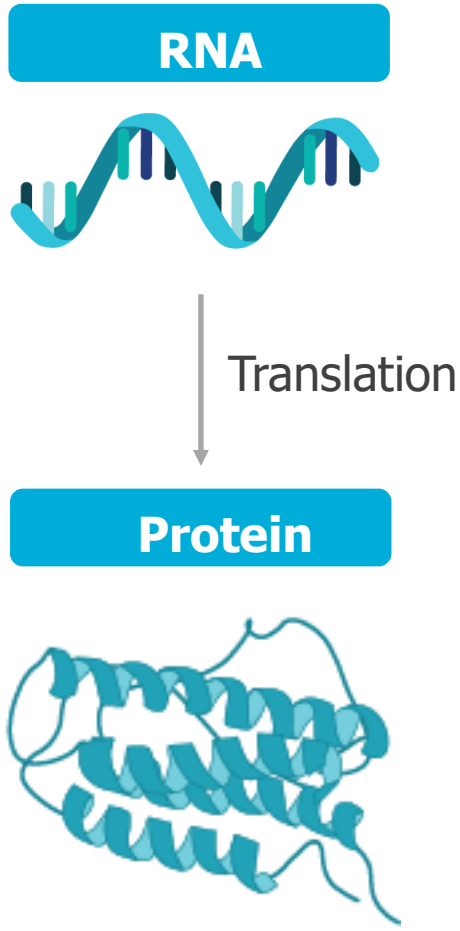
PRECISION NANOSYSTEMS
Create Transformative Medicines™

Genetic Payload Platform

DNA Vaccine



RNA Vaccine

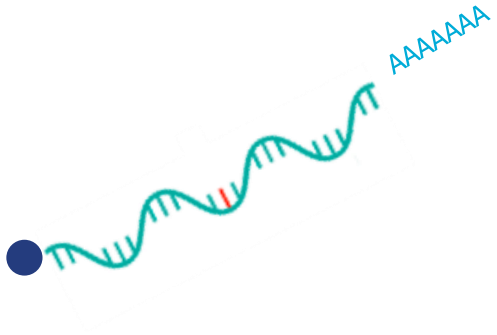


©2022 Precision NanoSystems Inc.

Not All mRNA is Created Equal

PRECISION NANOSYSTEMS
Create Transformative Medicines™

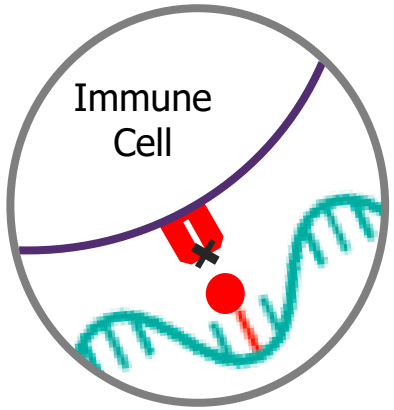
Genetic Payload Platform



Unmodified mRNA

Enhanced stability and translation by adding structural motifs at 3′ and 5′ locations

CUREVAC

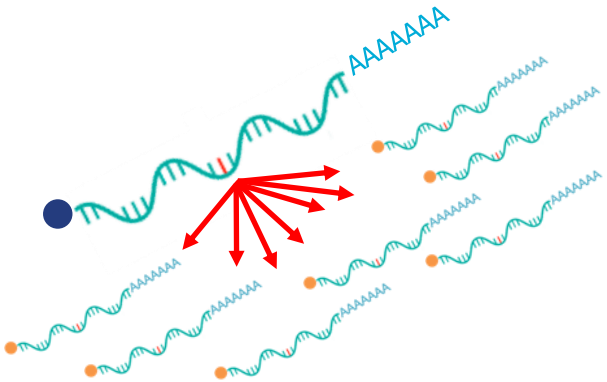


Base-Modified mRNA

Drives RNA translation, base modification leading to reduced innate immune response

moderna
messenger therapeutics

BIONTECH | Pfizer



Self-Amplifying mRNA (saRNA)

Contains genes of alphavirus that encode the non-structural proteins that replicate RNA and act as "adjuvant"

Imperial College London

ARCTURUS therapeutics

PRECISION NANOSYSTEMS

©2022 Precision NanoSystems Inc.

Why saRNA?

Potential to be 10x – 100x more potent than mRNA vaccines

1

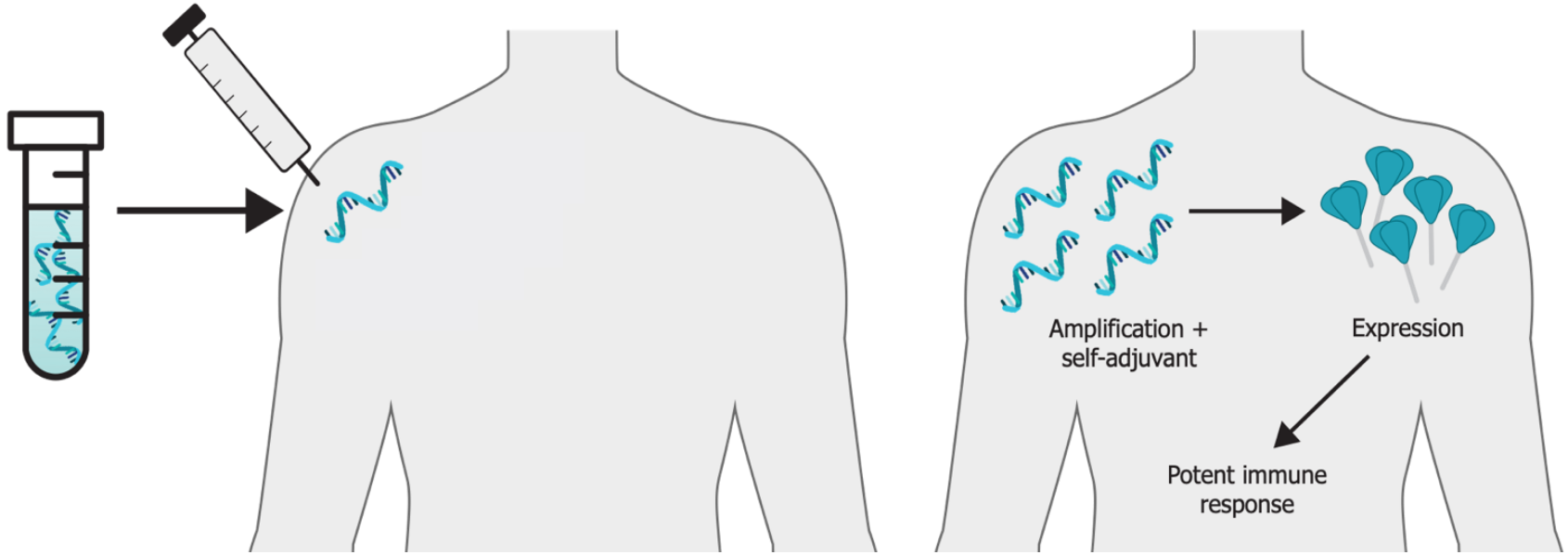
saRNA is enzymatically synthesized *in vitro* from a DNA template

2

saRNA is delivered in an LNP formulation, to cytoplasm of host cells

3

saRNA self-amplifies, produces multiple copies of mRNA in the target cells and expresses vaccine antigen



- Self-amplifying RNA encodes nonstructural proteins of the alpha virus that are translated into replicases that make many more copies
- saRNA can reduce doses by a factor of 100 thus reducing manufacturing burden

©2022 Precision NanoSystems Inc.

3

Lipid Nanoparticles for RNA delivery: GenVoy™ Delivery Platform

PRECISION NANOSYSTEMS
Create Transformative Medicines™

©2022 Precision NanoSystems Inc.

Full Stack of Technology to Enable the Genomic Medicine Revolution

PRECISION NANOSYSTEMS
Create Transformative Medicines™

Genomic Medicine Toolkit

Disease Target

Biological insights can identify target gene(s) driving disease

Genetic Payload Platform

Proprietary self-amplifying mRNA (SAM) to express specific proteins, including antigens used in RNA vaccines against COVID-19

RNA/DNA can also silence or edit target gene(s)

GenVoy™ Delivery Platform

Lipid nanoparticles (LNP), derived from a proprietary lipid library, that protect and deliver nucleic acids (RNA, DNA, derivatives) to target cells

Rapidly develop at lab scale and seamless translation to the clinic

NanoAssemblr® Manufacturing Platform

Proprietary, scalable, continuous flow, and single-use microfluidic mixing technology for controlled and precise nanoparticle encapsulated genetic medicine development & manufacturing

Produce the best drugs — faster, easier, and with the least risk possible — from µL lab scale to GMP scale

Drug Development Expertise

Leverage world-leading expertise in LNPs and genetic medicine development

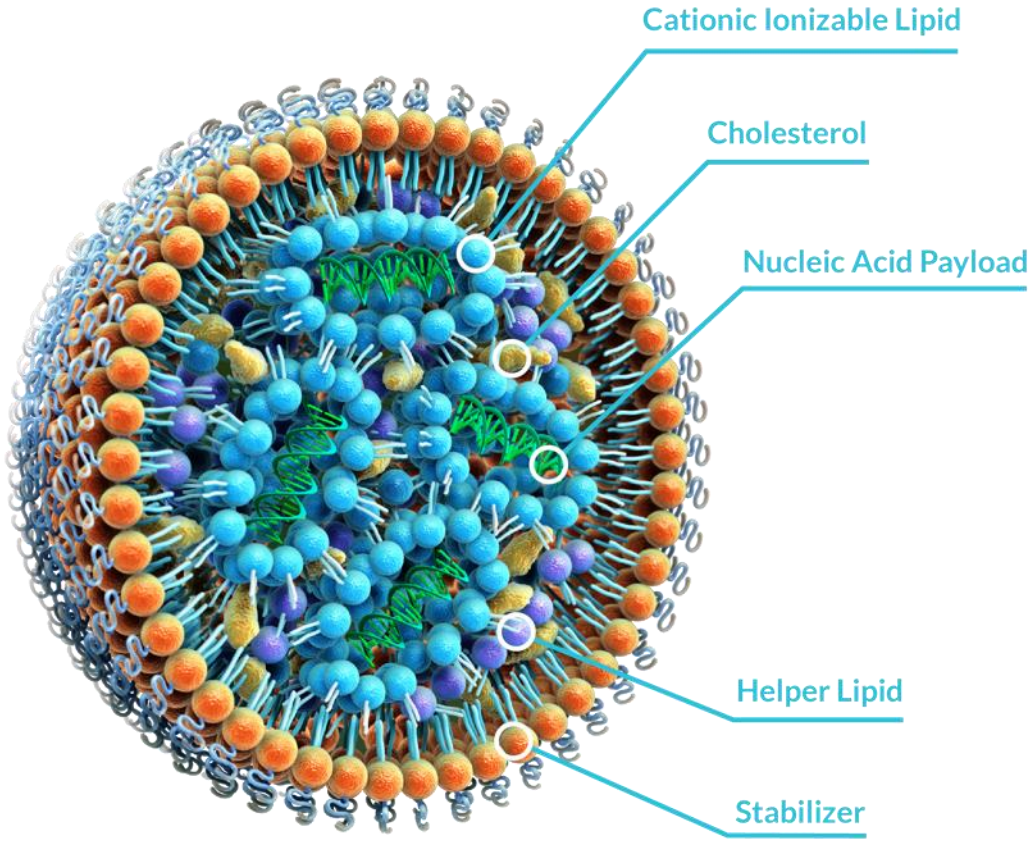
©2022 Precision NanoSystems Inc.

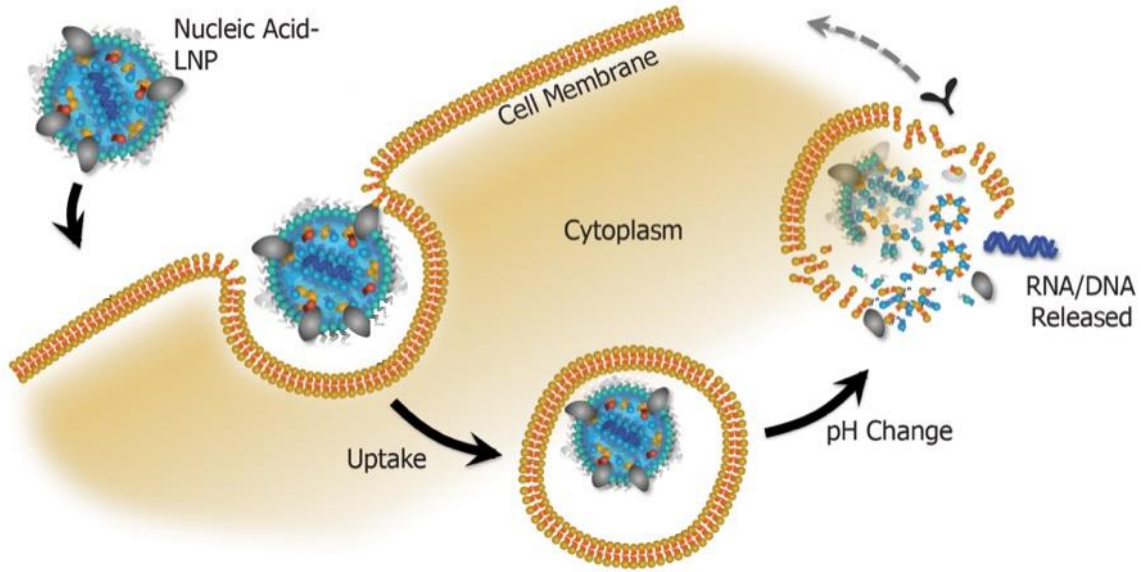
How is RNA Delivered?

PRECISION NANOSYSTEMS
Create Transformative Medicines™

Lipid nanoparticles (LNPs) are the most clinically advanced non-viral gene delivery system.

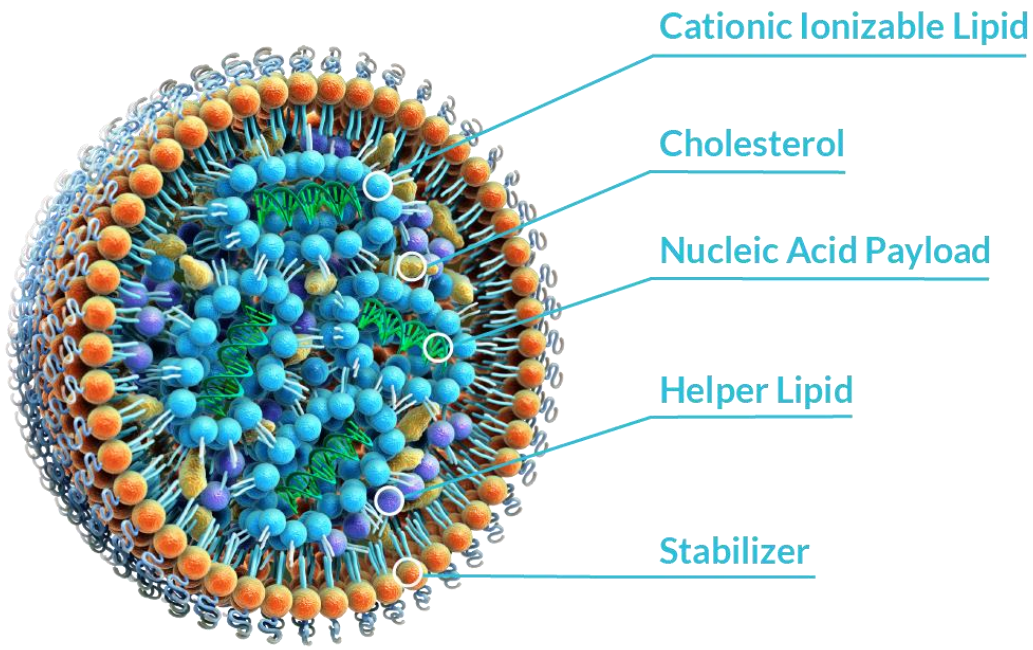
GenVoy Delivery Platform





©2022 Precision NanoSystems Inc.

Why PNI's GenVoy™ LNP Delivery Platform?



Lipid Nanoparticle (LNP) with genetic payload in green

PNI has developed a diverse structural and functional Ionizable lipid library for LNP development:

- PNI engineers LNP specifically for the intended use:
  - Vaccine LNP are engineered to be immunogenic and for intramuscular administration
  - Cell Therapy LNP are engineered for ex vivo applications and optimized for culture media
  - Gene Therapy LNP are engineered to mitigate immune response & for intravenous administration
- **POC data sets available for proprietary lipids enabling Cell Therapy, Protein Replacement therapy, and Vaccine applications**

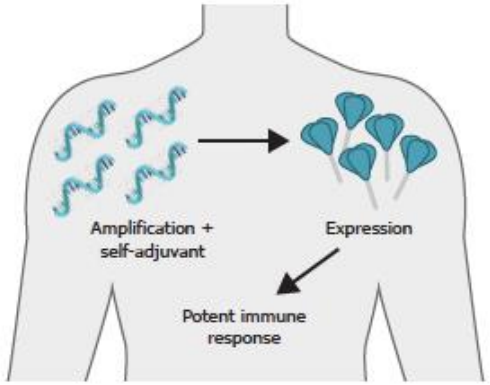
- PNI proprietary lipids are covered by PNI lipid patents/patent applications.

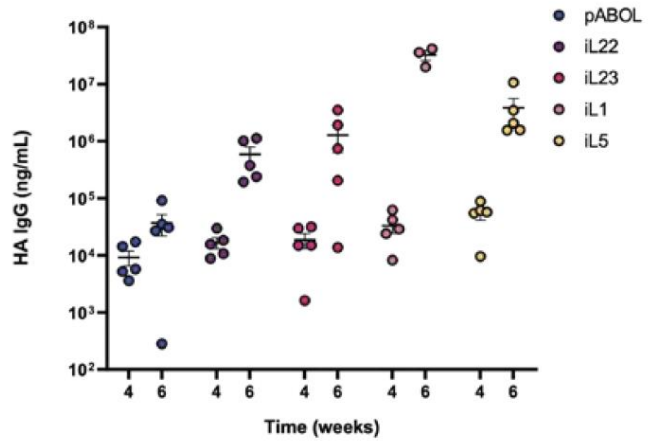
©2022 Precision NanoSystems Inc.

PNI GenVoy™ LNPs Enable Delivery for Key Applications in Genomic Medicine

PRECISION NANOSYSTEMS
Create Transformative Medicines™

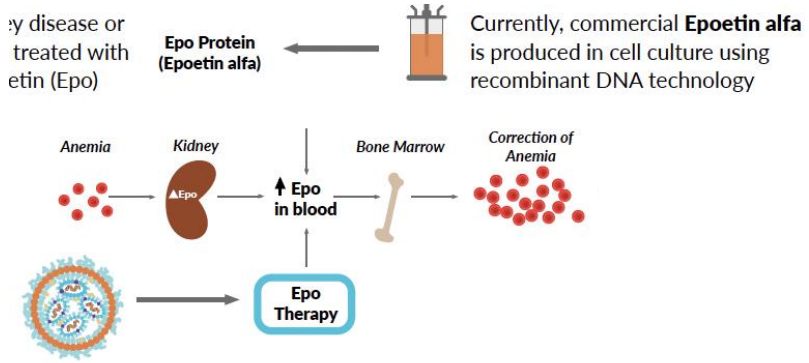
Vaccines

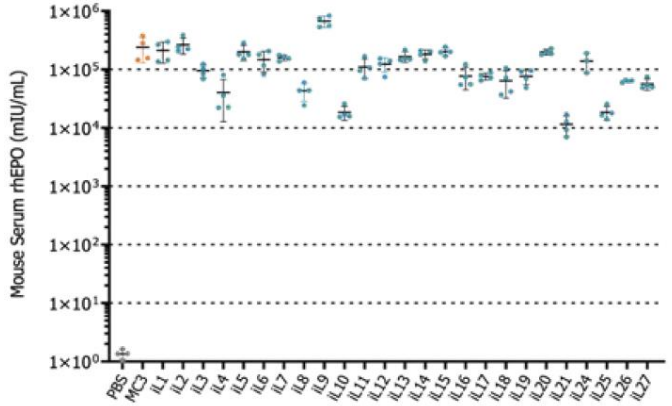




HA IgG Generation in Mice

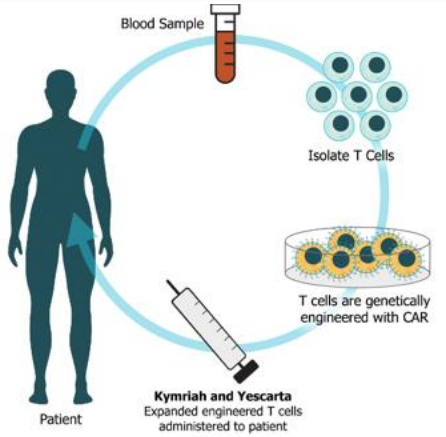
Gene Therapy

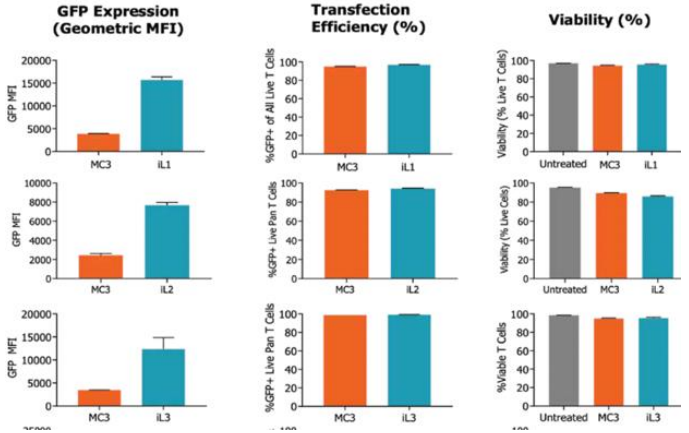




Epo Production in Mice

Cell Therapy (ex vivo)





GFP Expression in Primary Human T Cells

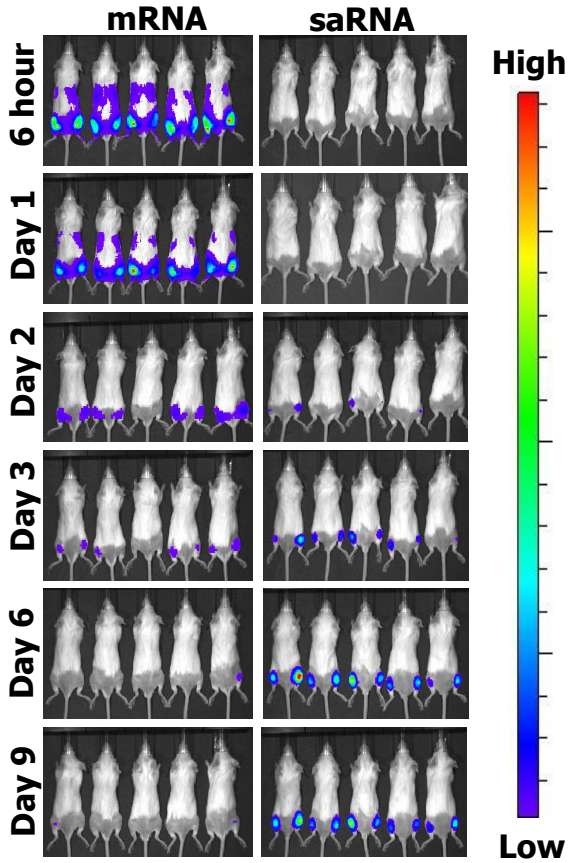
©2022 Precision NanoSystems Inc.

PNI GenVoy™ LNPs for Vaccine Development

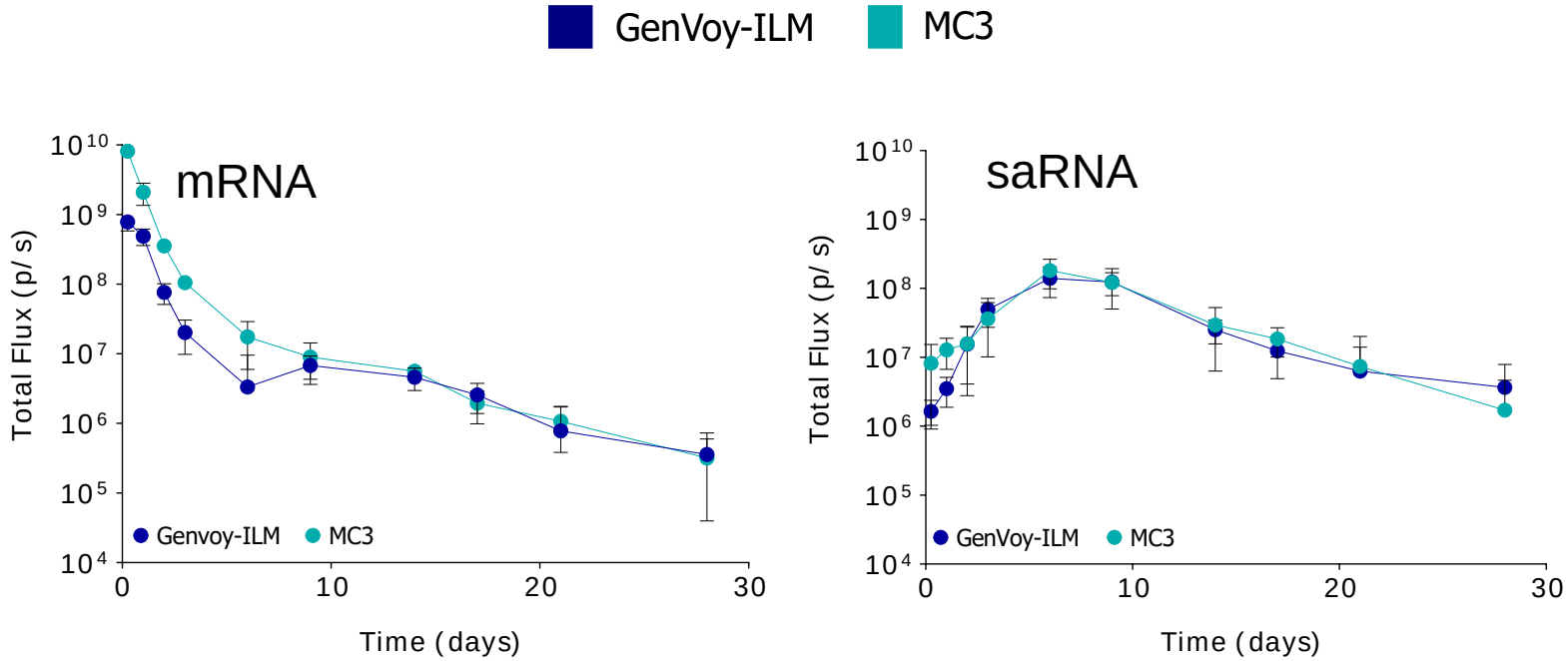
PRECISION NANOSYSTEMS
Create Transformative Medicines™

GenVoy-ILM™ LNPs are an Effective *In Vivo* Delivery Vehicle for Both mRNA and saRNA

A



B

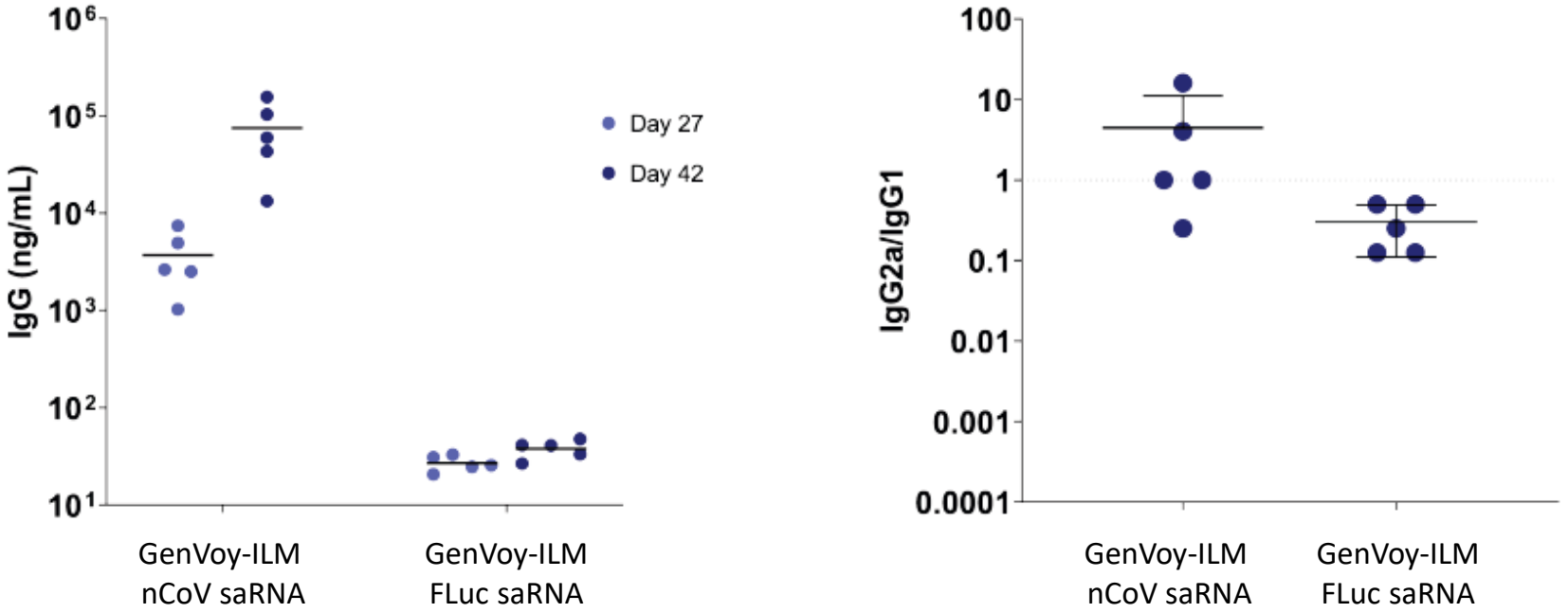


GenVoy-ILM and MC3 LNPs were prepared with 0.1 mol% DiD, encapsulating mRNA (5 µg/leg) or saRNA (1 µg/leg) encoding for FLuc. Female BALB/c mice (n=5) were injected IM with LNPs, and protein expression was determined using luminescence imaging (IVIS® Spectrum) over 28 days. Mice were injected IP with D-luciferin (150mg/kg) 15 minutes before imaging. (A) shows representative luminescence images of mice injected with GenVoy-ILM LNPs over 9 days. (B) shows the change in luminescence (total flux p/s) over 28 days post-IM injection with LNPs containing mRNA (left) and saRNA (right). Results are shown as the mean ± SD.

©2022 Precision NanoSystems Inc.

PNI GenVoy™ LNPs for Vaccine Development

GenVoy-ILM LNPs elicited IgG response against SARS-CoV-2 spike protein in mice with a skew towards IgG2a indicative of Th1 response.



Also studied:

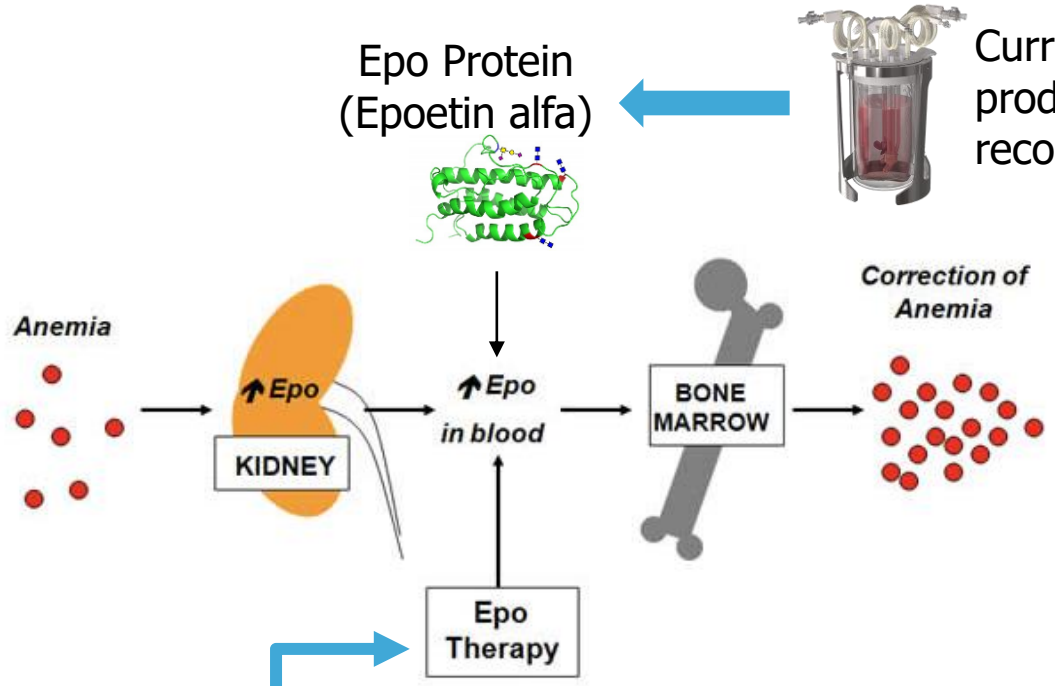
- GFP saRNA vs mRNA dose response in HEK293 cells
- FLuc saRNA vs mRNA expression and clearance in mice following IM injection

©2022 Precision NanoSystems Inc.

PNI GenVoy™ LNPs for Gene Therapy Applications

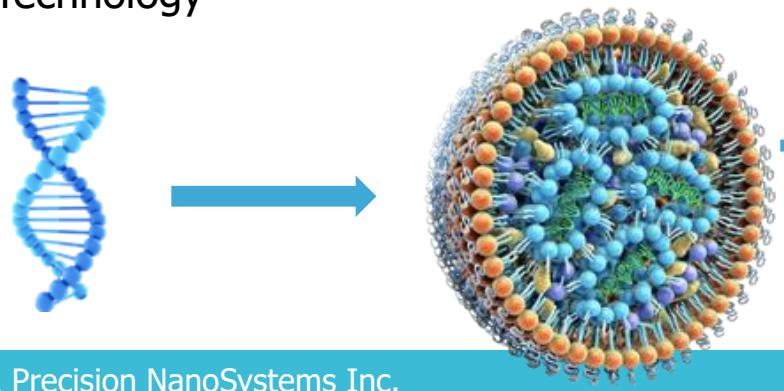
We developed, formulated and scaled up a model messenger RNA therapeutic

Anemia caused by kidney disease or cancer chemotherapy is treated with recombinant erythropoietin (Epo)



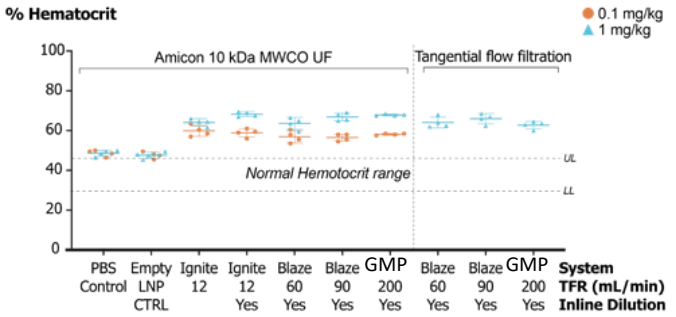
Currently, commercial **Epoetin alfa** is produced in cell culture using recombinant DNA technology

1. We encoded human Epo in mRNA (850 bp), packaged it in a non-viral carrier using NxGen Technology



2. The LNP delivers the mRNA to liver cells which express EPO protein, which stimulates red blood cell production

3. We observed an increase in red blood cell production in mice with consistent results across scales and NanoAssemblr instruments



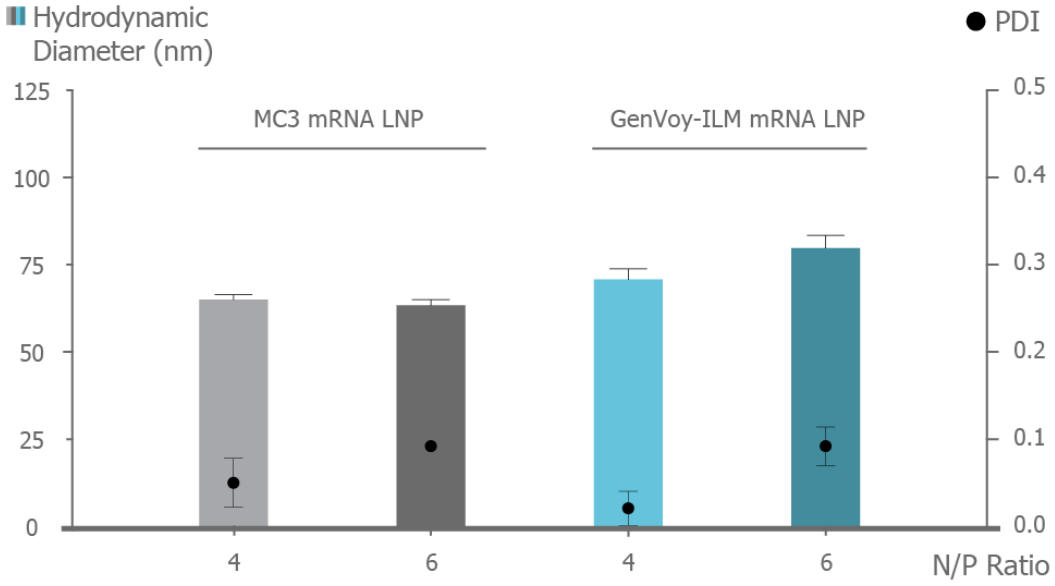
©2022 Precision NanoSystems Inc.

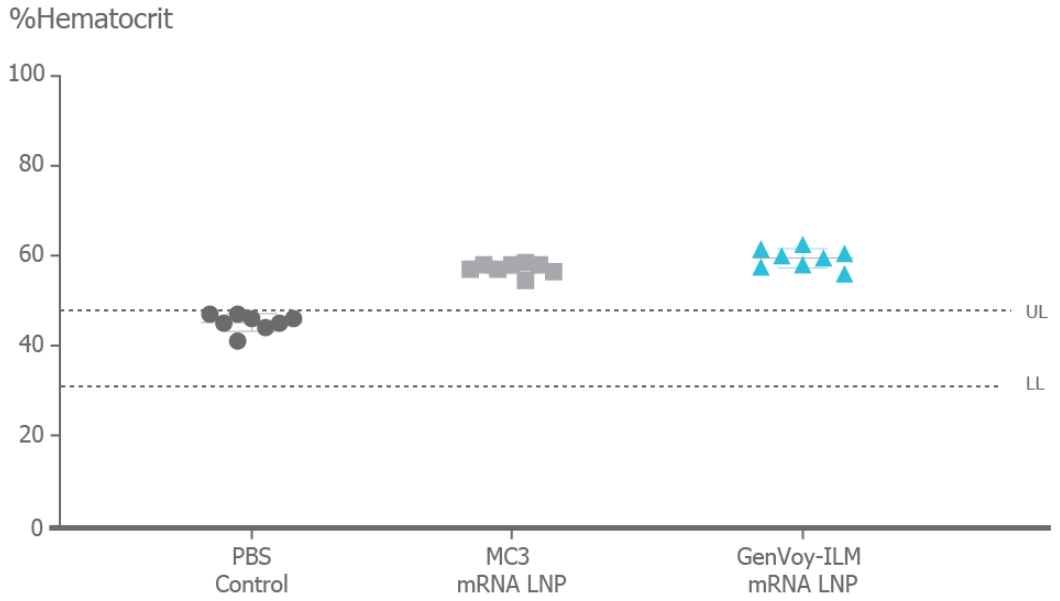
PNI GenVoy™ LNPs for Gene Therapy Applications

PRECISION NANOSYSTEMS
Create Transformative Medicines™

GenVoy-ILM mRNA-LNP showed similar biophysical and biological behaviour when compared to mRNA-LNP containing the ionizable cationic lipid, MC3, which is used in the FDA approved RNA-LNP Onpattro®.

GenVoy Delivery Platform



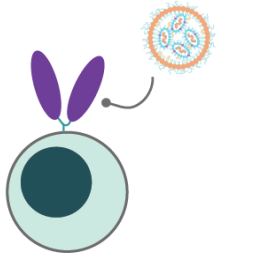


©2022 Precision NanoSystems Inc.

PNI GenVoy™ LNPs for Cell Therapy Development

PRECISION NANOSYSTEMS
Create Transformative Medicines™

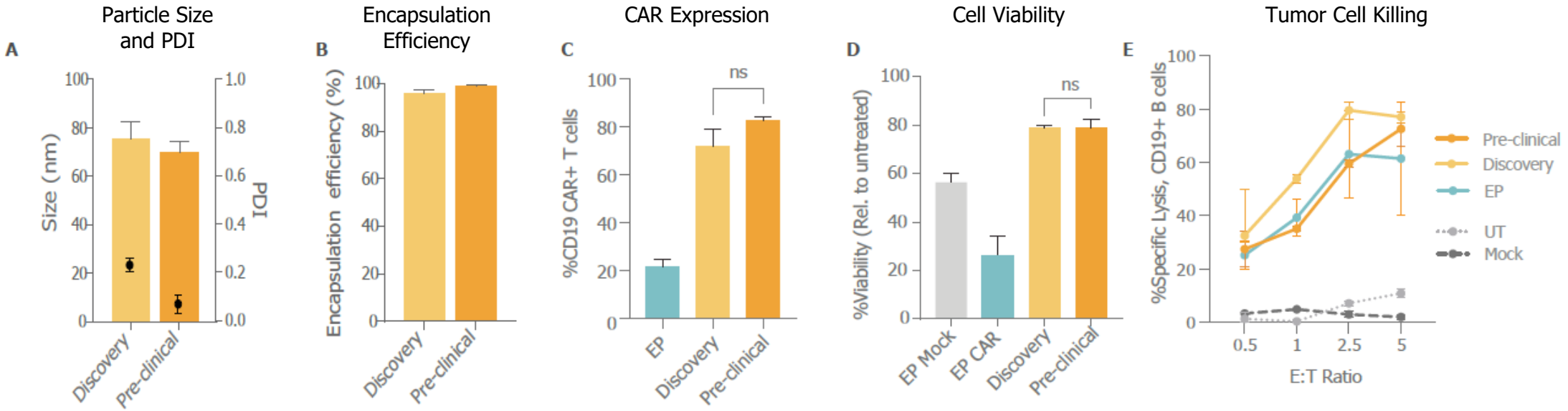
Enable Gene Editing and Delivery in Human Primary T Cells using Lipid Nanoparticles: Seamlessly Scalable from Discovery to Preclinical



Discovery
Sufficient RNA-LNP
for ~10 million CAR T

New

Preclinical
Sufficient RNA-LNP for
~700 million CAR T



©2022 Precision NanoSystems Inc.

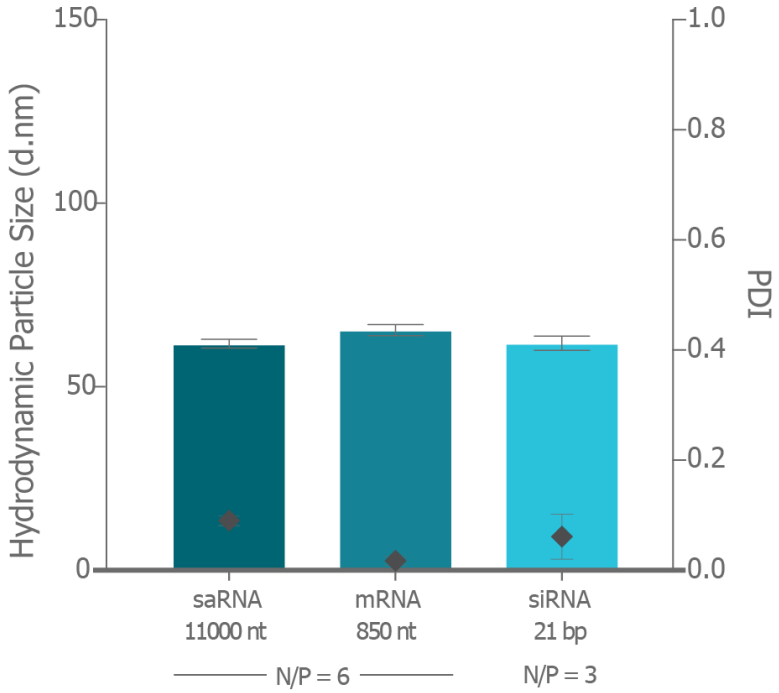
PNI GenVoy™ LNPs Can Encapsulate a Variety of Payloads

PRECISION NANOSYSTEMS
Create Transformative Medicines™

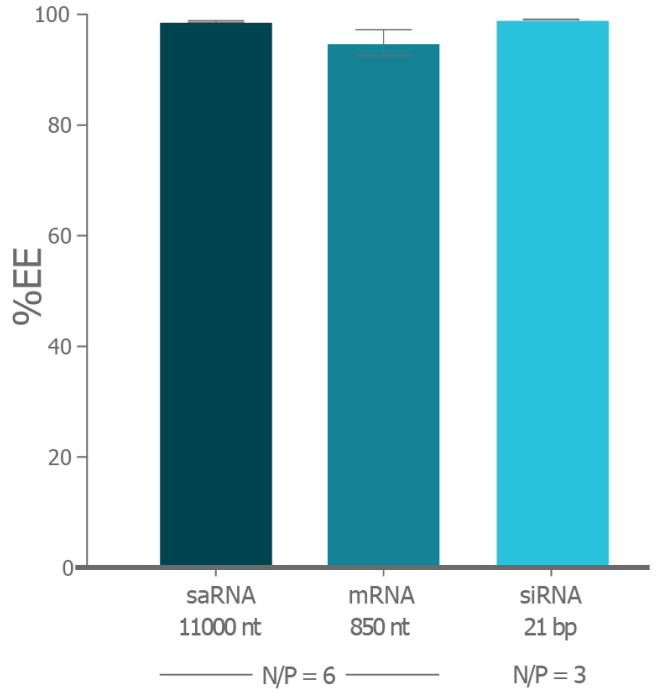
GenVoy-ILM allows encapsulation, delivery, and manufacturing of nearly any encoded antigen including large sequences over 10 kb.

GenVoy Delivery Platform

LNP size unaffected by RNA payload size



Encapsulation efficiency unaffected by RNA payload size



Formulations were made using GenVoy-ILM™ reagent on the NanoAssemblr™ Ignite® (Total Flow Rate = 12 mL/min). Particles were then diluted, concentrated and had buffer exchanged. The subsequent particles were sterile filtered using 0.2 µm filters. The particle size and polydispersity (PDI) were determined by DLS (Malvern ZetaSizer). Encapsulation efficiency was measured using a Ribogreen-based RNA assay.

©2022 Precision NanoSystems Inc.

4

RNA - Lipid Nanoparticle Assembly using Microfluidics

PRECISION NANOSYSTEMS
Create Transformative Medicines™

©2022 Precision NanoSystems Inc.

Full Stack of Technology to Enable the Genomic Medicine Revolution

PRECISION NANOSYSTEMS
Create Transformative Medicines™

Genomic Medicine Toolkit

Disease Target

Biological insights can identify target gene(s) driving disease

Genetic Payload Platform

Proprietary self-amplifying mRNA (SAM) to express specific proteins, including antigens used in RNA vaccines against COVID-19

RNA/DNA can also silence or edit target gene(s)

GenVoy™ Delivery Platform

Lipid nanoparticles (LNP), derived from a proprietary lipid library, that protect and deliver nucleic acids (RNA, DNA, derivatives) to target cells

Rapidly develop at lab scale and seamless translation to the clinic

NanoAssemblr® Manufacturing Platform

Proprietary, scalable, continuous flow, and single-use microfluidic mixing technology for controlled and precise nanoparticle encapsulated genetic medicine development & manufacturing

Produce the best drugs — faster, easier, and with the least risk possible — from µL lab scale to GMP scale

Drug Development Expertise

Leverage world-leading expertise in LNPs and genetic medicine development

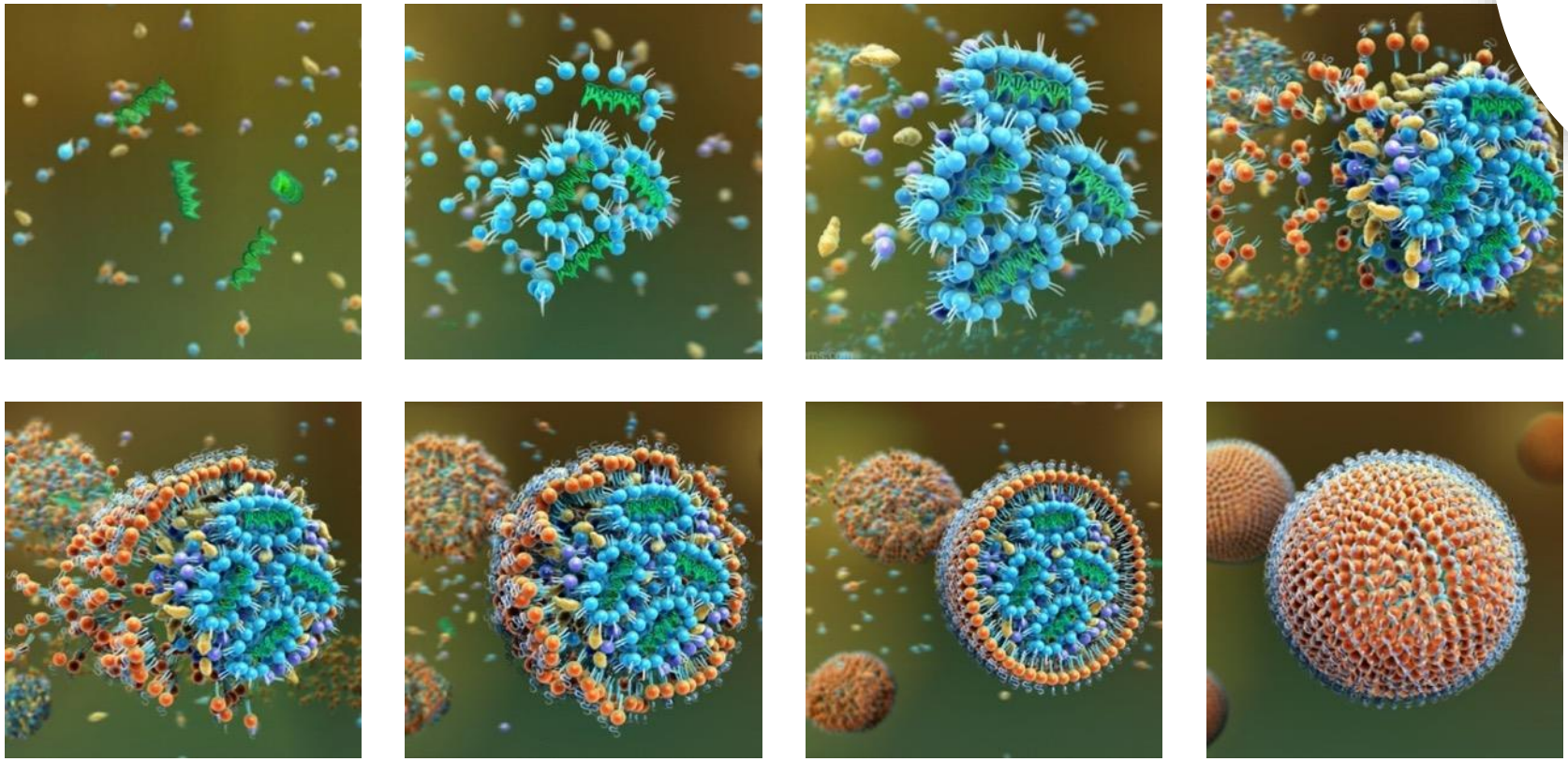
©2022 Precision NanoSystems Inc.

Bottom-Up Assembly Using Microfluidics

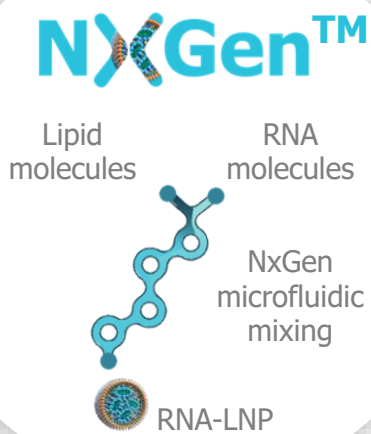
PRECISION NANOSYSTEMS
Create Transformative Medicines™

NanoAssemblr Manufacturing Platform

Optimal Nanoparticles are Achieved by Controlling the Self-Assembly Process



NXGen™



©2022 Precision NanoSystems Inc.

Unprecedented Performance and Capabilities That Are Uniquely Addressing Key Manufacturing Pain Points and Bottlenecks

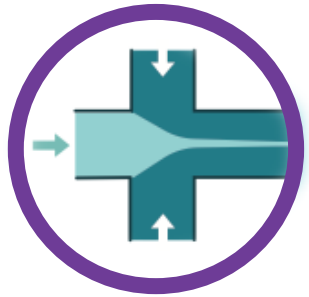
PRECISION NANOSYSTEMS
Create Transformative Medicines™

Sonication/Extrusion

- − Limited applications
- − Difficult to reproduce
- − Harsh process conditions
- − Difficult to scale

T-tube and Impingement Jet Macromixing

- − Limited applications
- − Difficult to reproduce
- − Not suited for rapid development
- \+ Gentler process conditions
- \+ Demonstrated scale-up for limited applications



Other Microfluidic Approaches

- − Challenges scaling up
- − Not designed for specific nanoparticle manufacturing
- \+ Expanded applications
- \+ Reproducible
- \+ Non-turbulent process conditions
- \+ Suited to small volume formulations

NxGen Microfluidics

- \+ Easy to scale
- \+ Broad range of applications
- \+ Potential multi-mixer integration opens possibilities
- \+ Reproducible
- \+ Non-turbulent process conditions
- \+ Compatible with series mixing and other complex architectures

PRECISION NANOSYSTEMS
Create Transformative Medicines™

PNI's Second Generation Technology

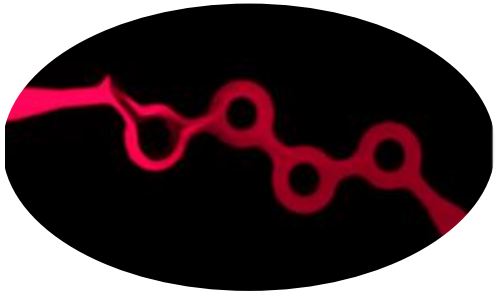
PNI/Pall NxGen technology comes with freedom of usage (IP protected)

©2022 Precision NanoSystems Inc.

Uniform Scalable Nanoparticle Manufacture Using NxGen

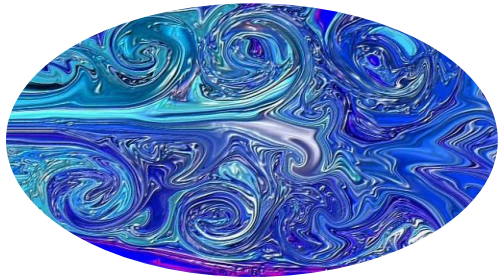
PRECISION NANOSYSTEMS
Create Transformative Medicines™

PNI's NxGen Time-Invariant Mixing

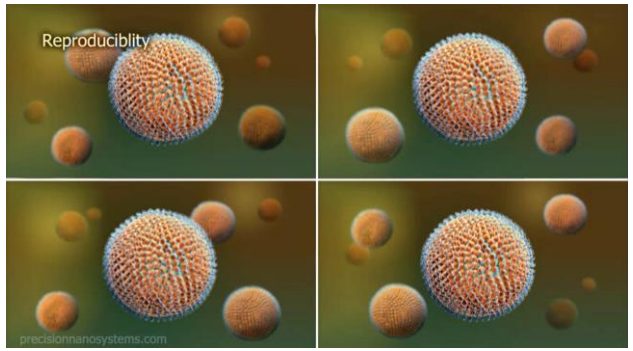


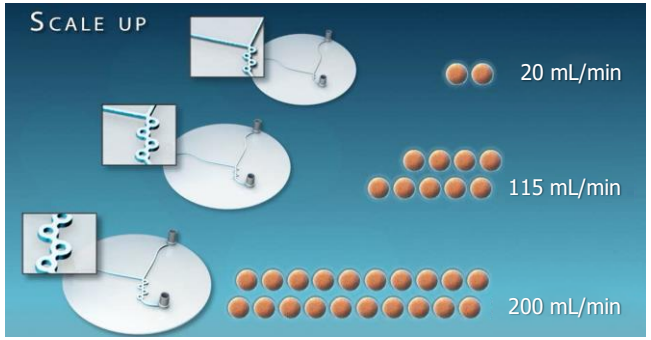
Mixing and particle formation consistent over time

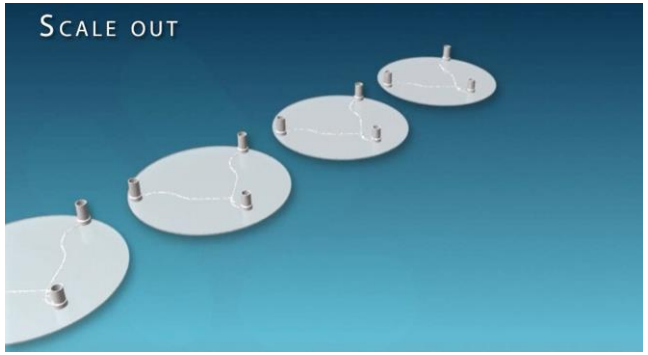
Unsteady Turbulent Mixing (Non-PNI Mixing Method)

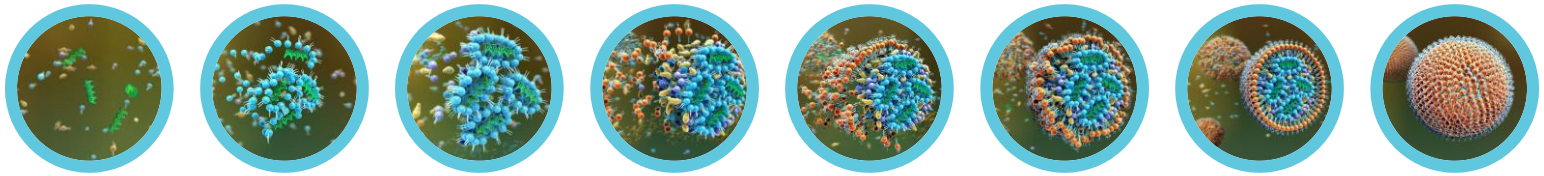


Mixing conditions constantly changing over time









©2022 Precision NanoSystems Inc.

The NanoAssemblr Manufacturing Platform
Scalable Solutions from Research Through to Commercial

PRECISION NANOSYSTEMS
Create Transformative Medicines™

Discovery	Early Preclinical		Late Preclinical	Early Phase Clinical	Commercial
Spark™ Rapid screening, in vitro studies	**Ignite™** Formulation development, early PD	**Ignite+™** Extending capabilities, de-risking larger systems, early PD	**Blaze™** Process development, CMC, larger animal studies	**GMP** Able to produce clinical trial batches through Phase 1 and low volume applications under cGMP conditions	**Commercial Scale** Modular production system for commercial MFG under cGMP conditions with scalable number of mixing modules to meet throughput requirements

Existing preclinical systems to accelerate drug development through de-risking MFG runs at bench scale

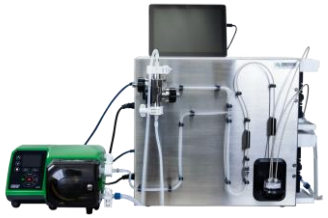
NanoAssemblr® Systems

Spark NxGen

NxGen

NxGen
NxGen 500

NxGen 500



NxGen 500

Meets all safety and controlled space requirements when working with solvents including 21 CFR part 11 compliance.

NanoAssemblr® Consumables

Up to 250 µl

Up to 20 mL
Up to 20 mL/min*

Up to 60 mL
Up to 200 mL/min*

Up to 10 L
Up to 115 mL/min*

Up to 200 mL/min*

Up to 1.6 L/min*

*Pre-dilution flow rates

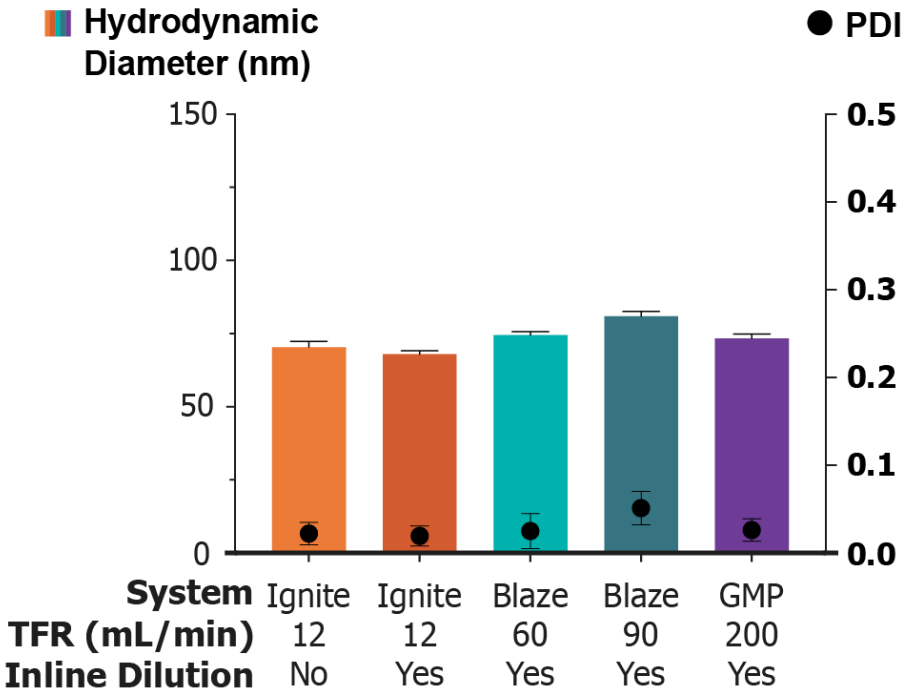
©2022 Precision NanoSystems Inc.

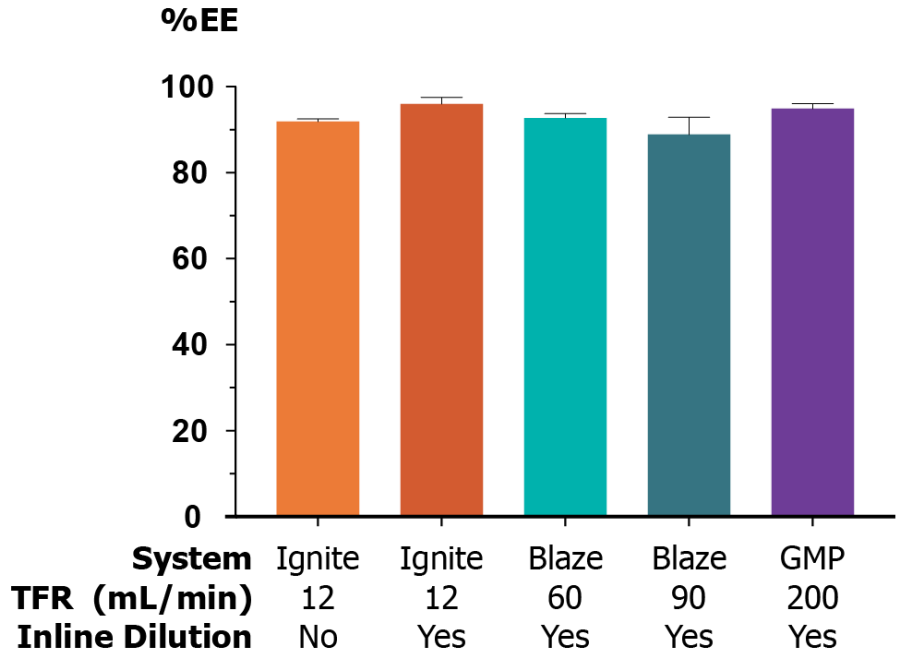
Scale from Preclinical to Commercial Manufacture with Consistent Quality

PRECISION NANOSYSTEMS
Create Transformative Medicines™

NanoAssemblr Manufacturing Platform

PNI's NanoAssemblr® technology allows LNP formulations to be scaled from pre-clinical volumes through to GMP manufacture





The mRNA-LNPs were prepared from the GenVoy-ILM reagent and Epo-encoded mRNA using NanoAssemblr® Ignite, Blaze and GMP systems. The Epo mRNA-LNP were diluted, purified and concentrated. The final Epo mRNA-LNPs were sterile-filtered using 0.2 µm filters. Size and polydispersity (PDI) were determined by DLS. Encapsulation efficiency was measured using a RiboGreen-based RNA assay.

©2022 Precision NanoSystems Inc.

5

Analytical Requirements to Assess RNA-LNPs

©2022 Precision NanoSystems Inc.

Full Stack of Technology to Enable the Genomic Medicine Revolution

PRECISION NANOSYSTEMS
Create Transformative Medicines™

Genomic Medicine Toolkit

Disease Target

Biological insights can identify target gene(s) driving disease

Genetic Payload Platform

Proprietary self-amplifying mRNA (SAM) to express specific proteins, including antigens used in RNA vaccines against COVID-19

RNA/DNA can also silence or edit target gene(s)

GenVoy™ Delivery Platform

Lipid nanoparticles (LNP), derived from a proprietary lipid library, that protect and deliver nucleic acids (RNA, DNA, derivatives) to target cells

Rapidly develop at lab scale and seamless translation to the clinic

NanoAssemblr® Manufacturing Platform

Proprietary, scalable, continuous flow, and single-use microfluidic mixing technology for controlled and precise nanoparticle encapsulated genetic medicine development & manufacturing

Produce the best drugs — faster, easier, and with the least risk possible — from µL lab scale to GMP scale

Drug Development Expertise

Leverage world-leading expertise in LNPs and genetic medicine development

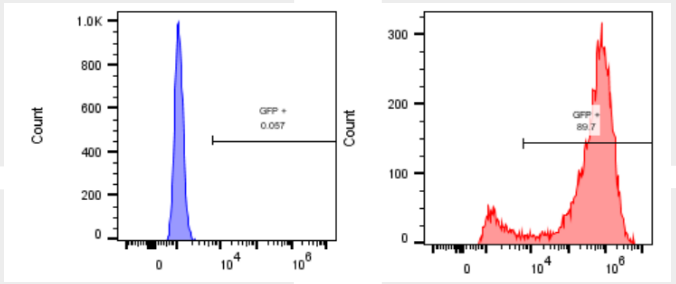
©2022 Precision NanoSystems Inc.

mRNA-LNP Vaccines Are Analytically Complex Drugs

PRECISION NANOSYSTEMS
Create Transformative Medicines™

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

RNA Quality
- Quantity
- Integrity
- Structure



***In Vitro* Potency Assay**

Payload Design
- Sequence
- Production
- Biology

Encapsulation
- %EE
- Location of RNA

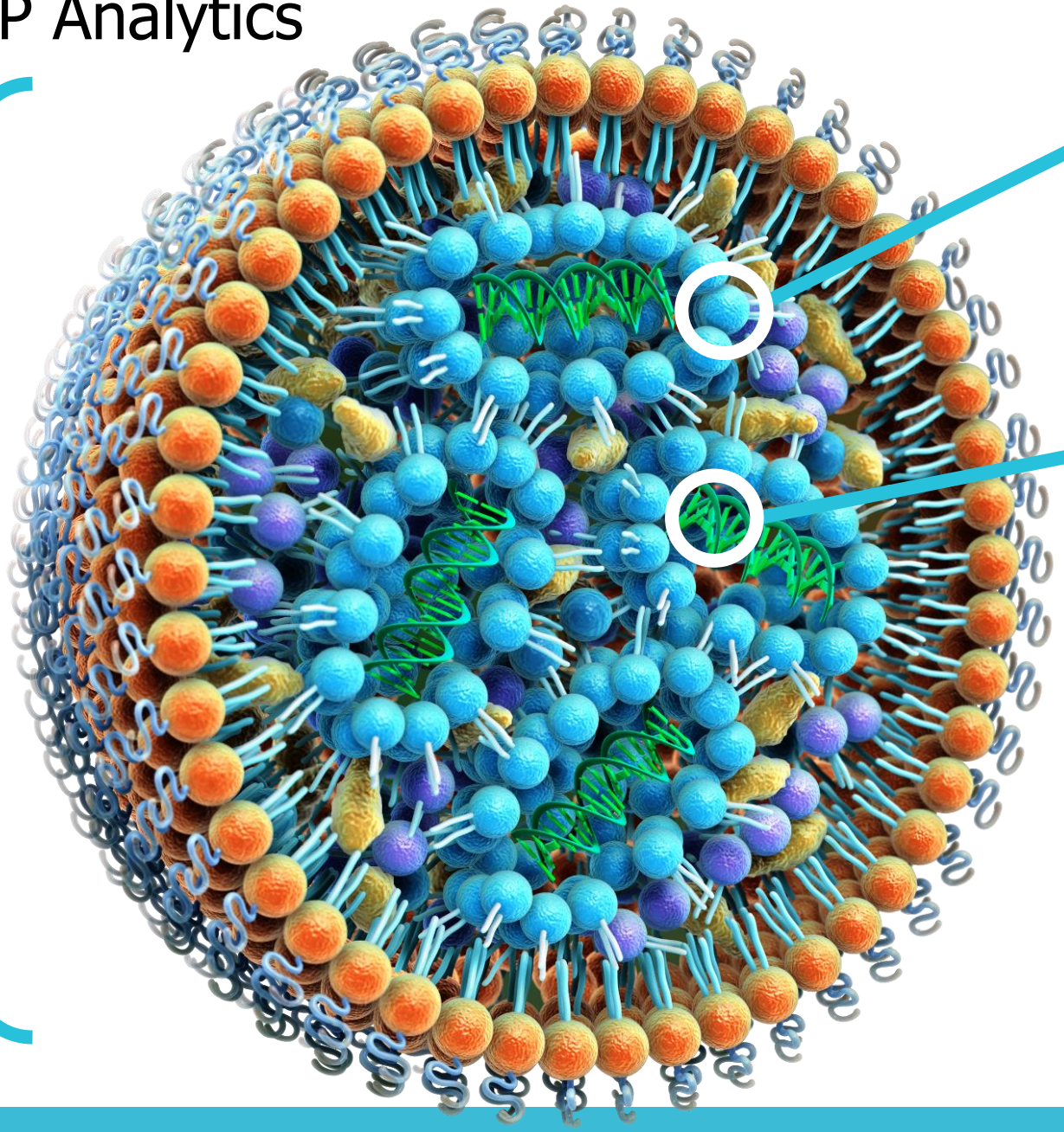
phenomenex
BECKMAN COULTER
SCIEX
MOLECULAR DEVICES

©2022 Precision NanoSystems Inc.

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

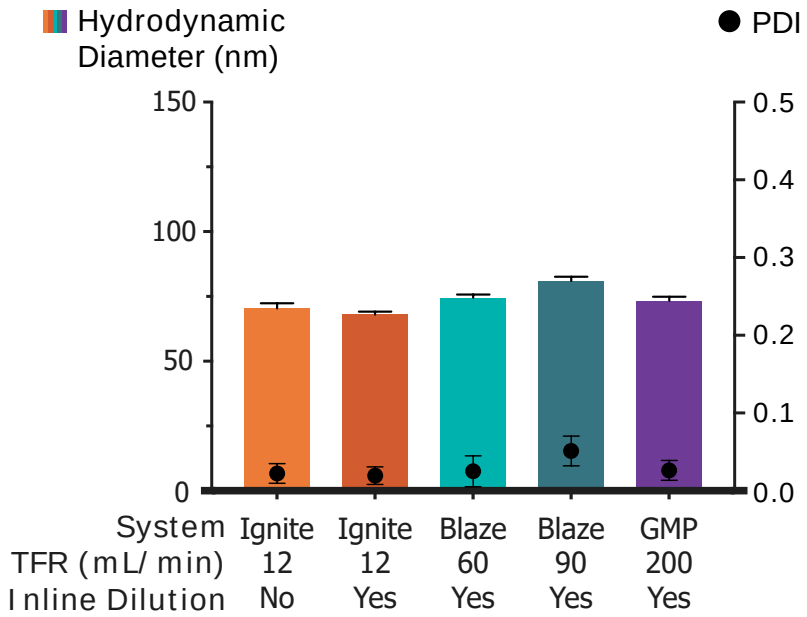
- RiboGreen/PicoGreen
- BioAnalyzer
- IPRP-UPLC-UV
- LC-MS

©2022 Precision NanoSystems Inc.

Analytics: Particle size, Distribution, Zeta Potential

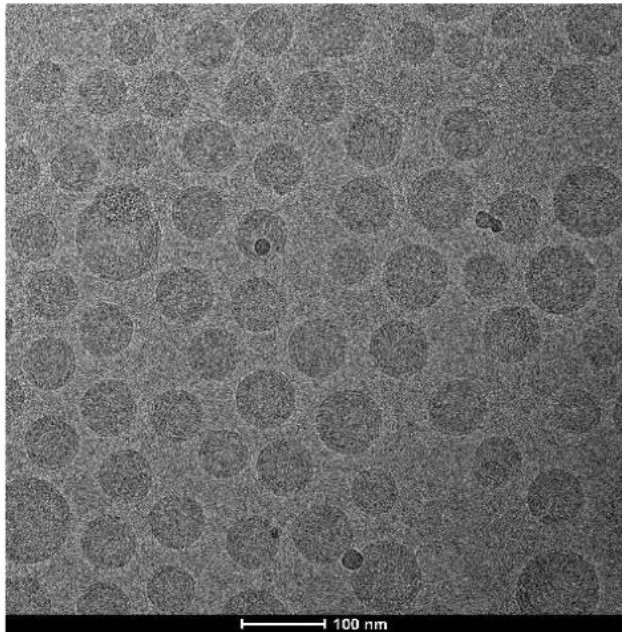
Dynamic Light Scattering

- Study size distribution
- Calculates hydrodynamic radius based on scattering intensity



Cryo-TEM

- Particle morphology
- Electron beam on flash-frozen samples

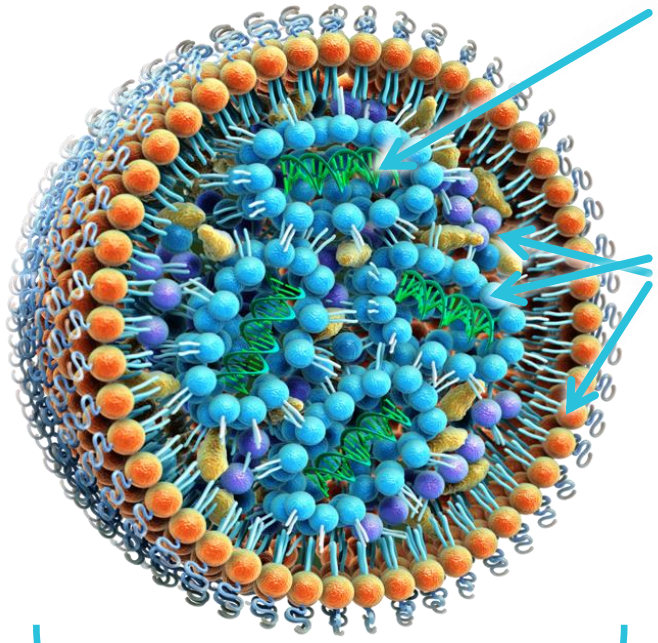


©2022 Precision NanoSystems Inc.

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



©2022 Precision NanoSystems Inc.

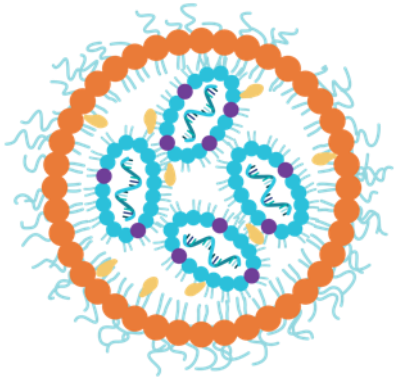
Analysis of mRNA Encapsulation

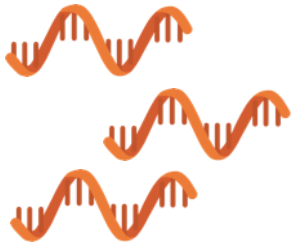
PRECISION NANOSYSTEMS
Create Transformative Medicines™

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

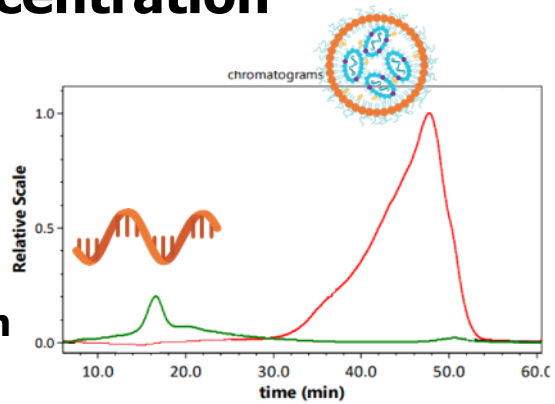
Encapsulated mRNA:
- **Low immunogenicity**
- **Protected from Nucleases**
- **High transfectability**

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



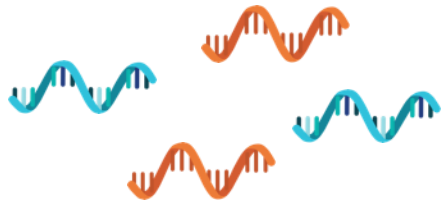


Separation



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

Disruption



'Total mRNA'

Fluorescent assays (E.g. Ribogreen™)

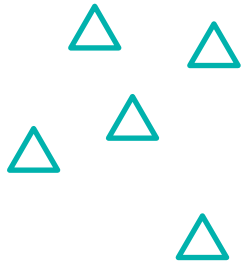
In general, therapeutic efficacy of LNP formulations improves with increased mRNA % encapsulation efficiency (%EE)

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

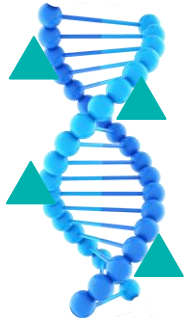
©2022 Precision NanoSystems Inc.

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

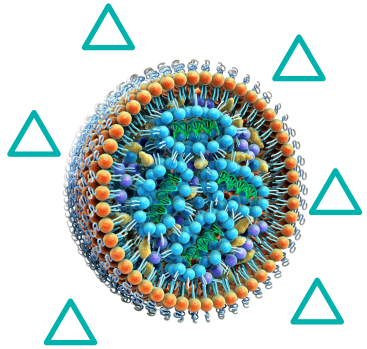


Unbound RiboGreen (low fluorescence)



RiboGreen bound to RNA (high fluorescence)

RiboGreen cannot bind to RNA encapsulated in LNP



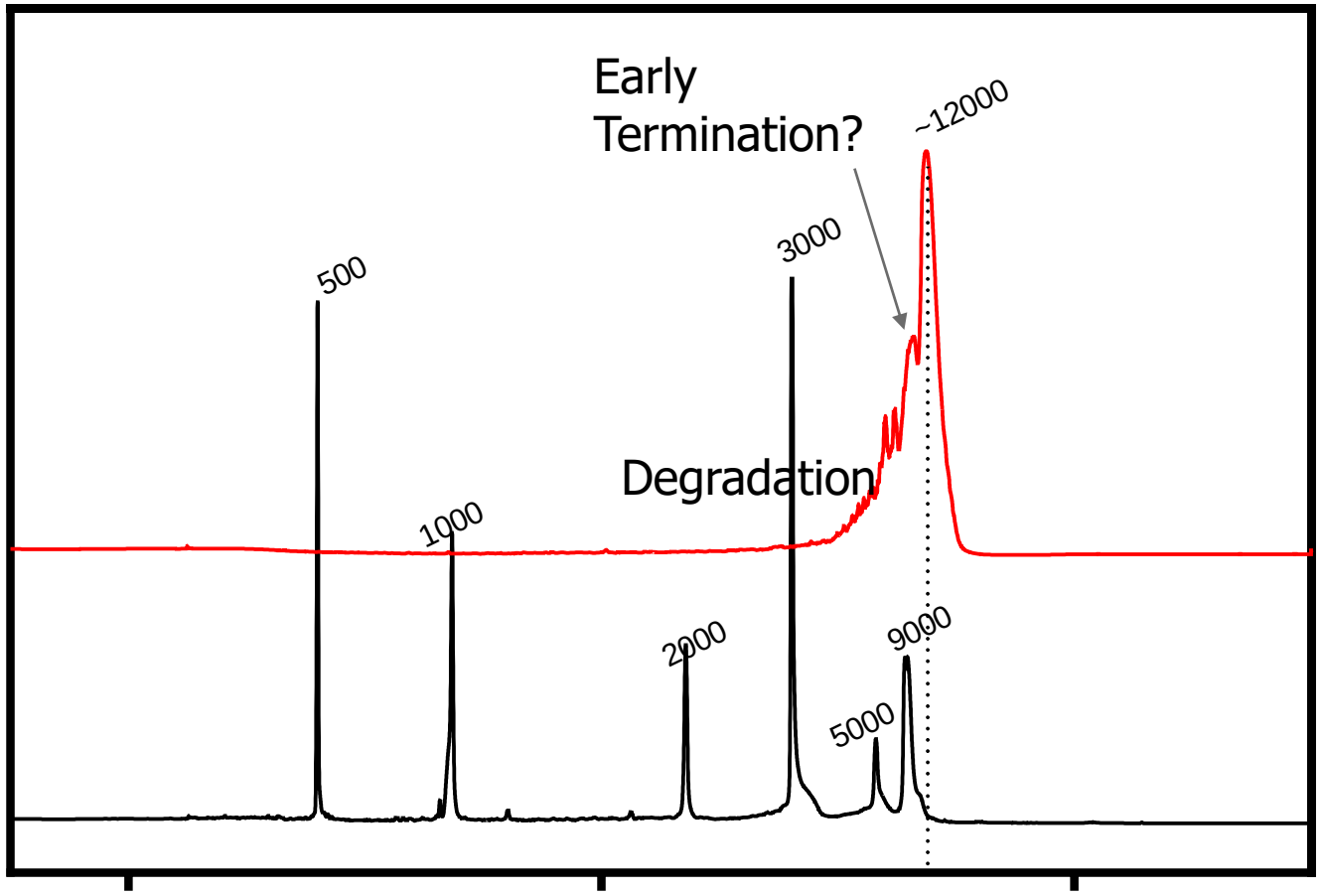
A modified protocol to release the RNA is required

©2022 Precision NanoSystems Inc.

Characterization of RNA: Integrity using Capillary Electrophoresis

Sciex PA800Plus CE

Custom method development



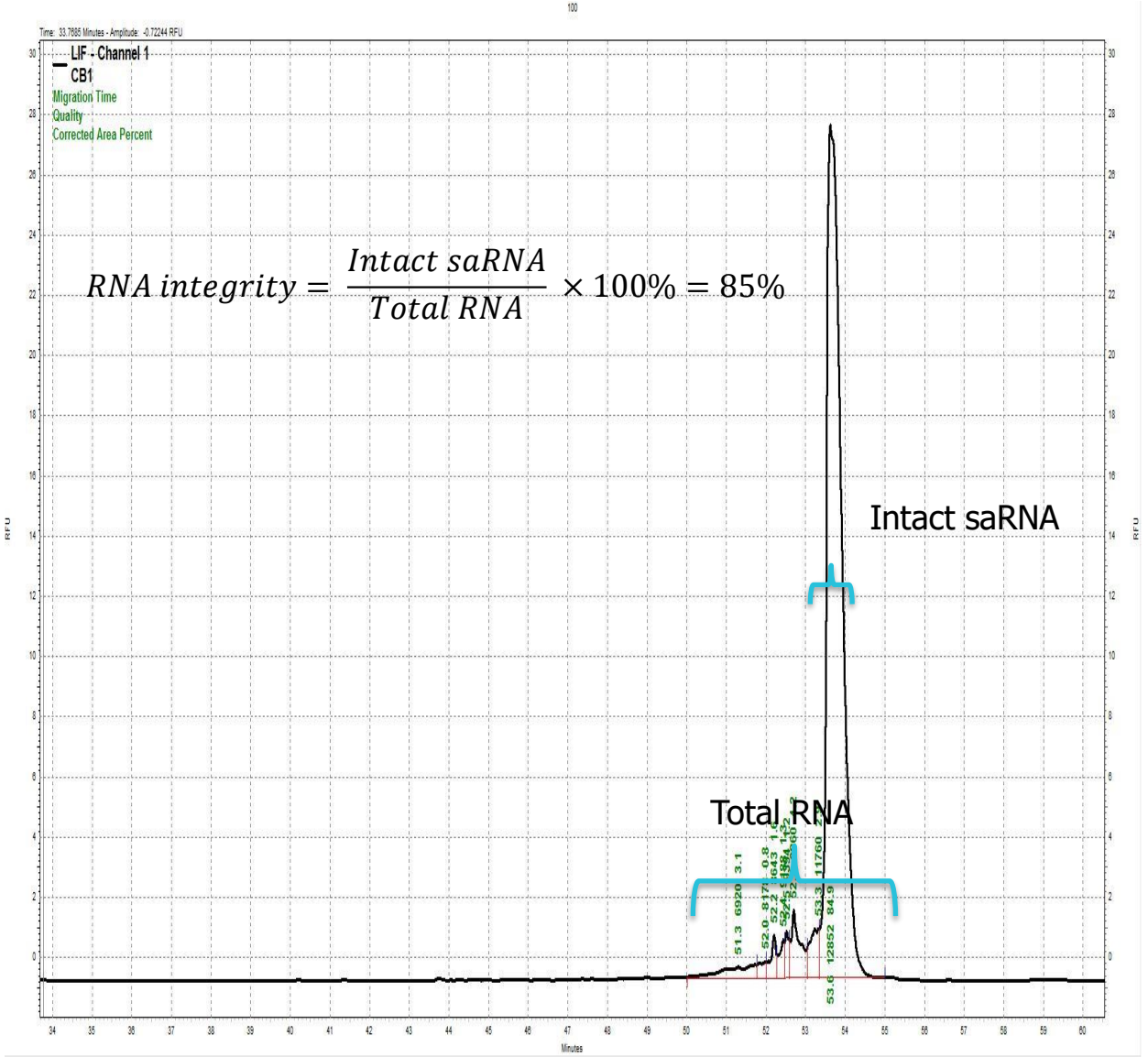
- RNA-LNP production can involve high shear forces, low pH and high temperatures resulting in degradation of mRNA

©2022 Precision NanoSystems Inc.

Characterization of RNA: Integrity using Capillary Electrophoresis

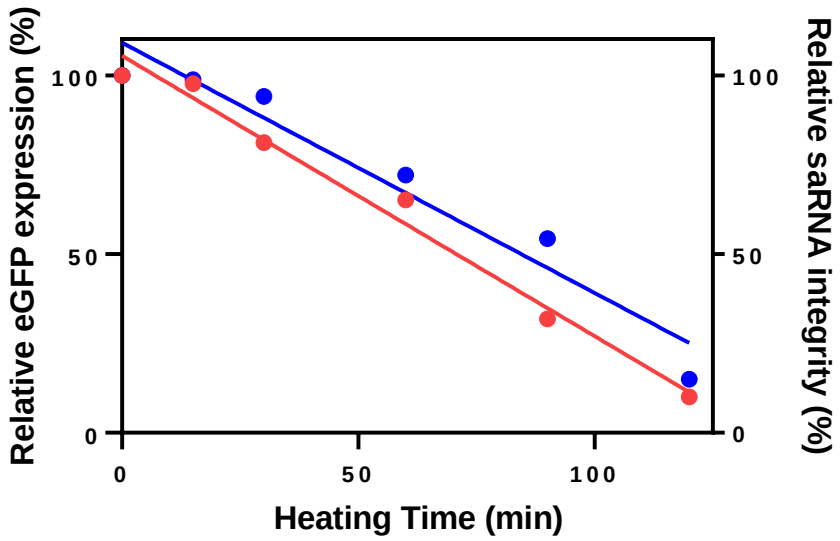
Resulting method provides:

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

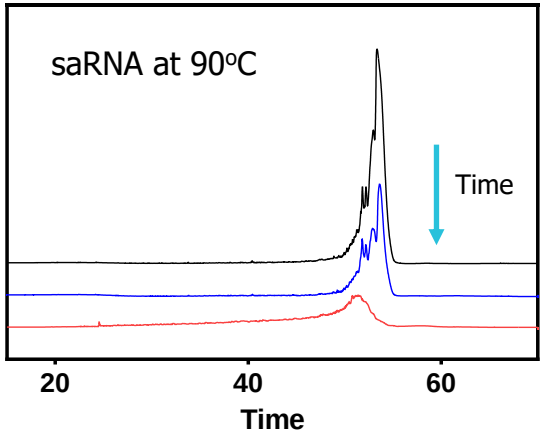


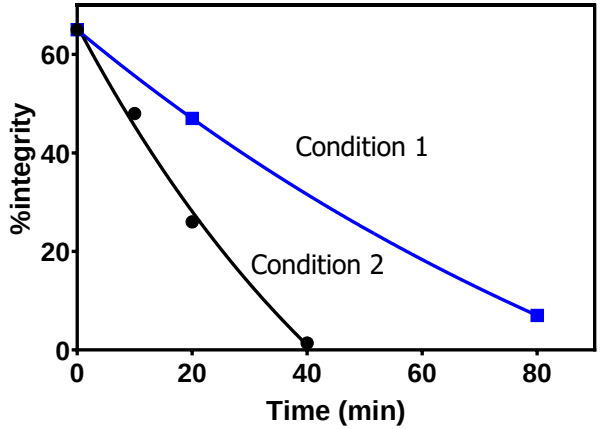
©2022 Precision NanoSystems Inc.

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

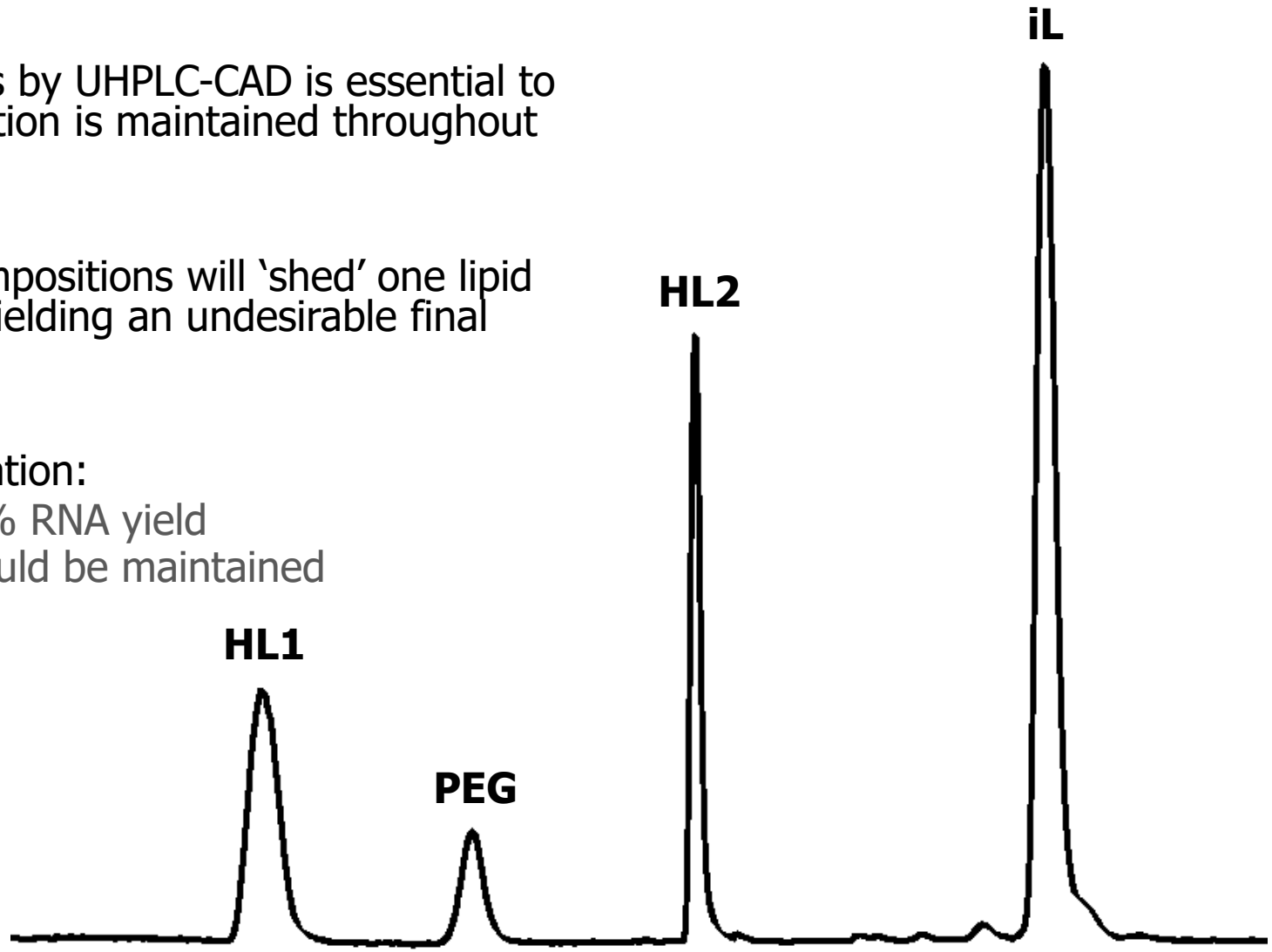




©2022 Precision NanoSystems Inc.

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

©2022 Precision NanoSystems Inc.

Analytical Methods Overview

PRECISION NANOSYSTEMS
Create Transformative Medicines™

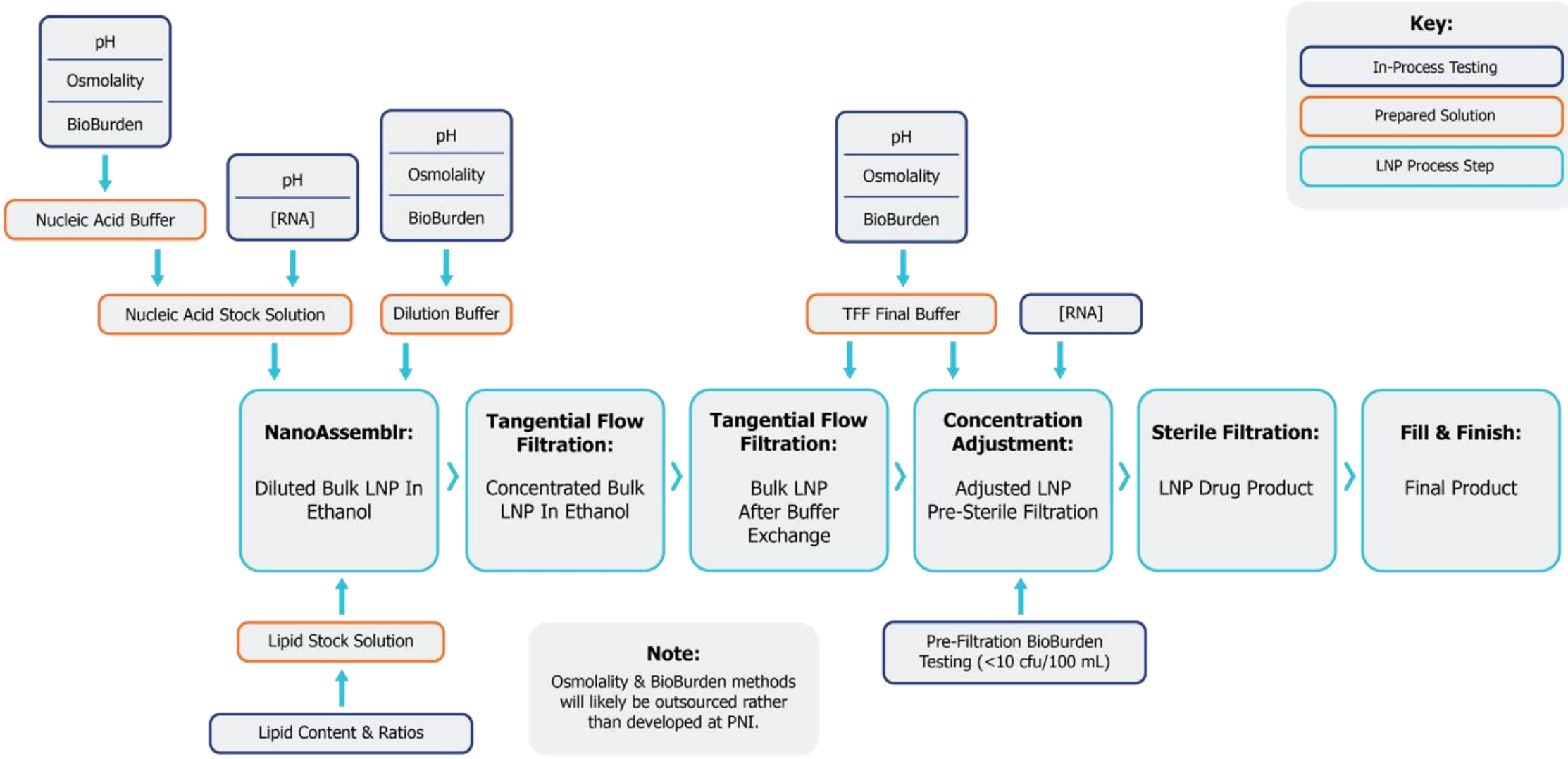
	Test	Method	Release Testing	In Process Testing
Analytical Requirements for Injectable Drugs (USP/Ph.Eur. methods available)	Appearance	Visual Inspection: USP <790> / Ph.Eur. 2.9.20	X	
	pH	Potentiometric: USP <791> / Ph.Eur 2.2.3	X	(X)*
	Osmolality	Freezing Point Depression: USP <785> / Ph.Eur. 2.2.35	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

©2022 Precision NanoSystems Inc.

RNA-LNP Production: In-Process Testing

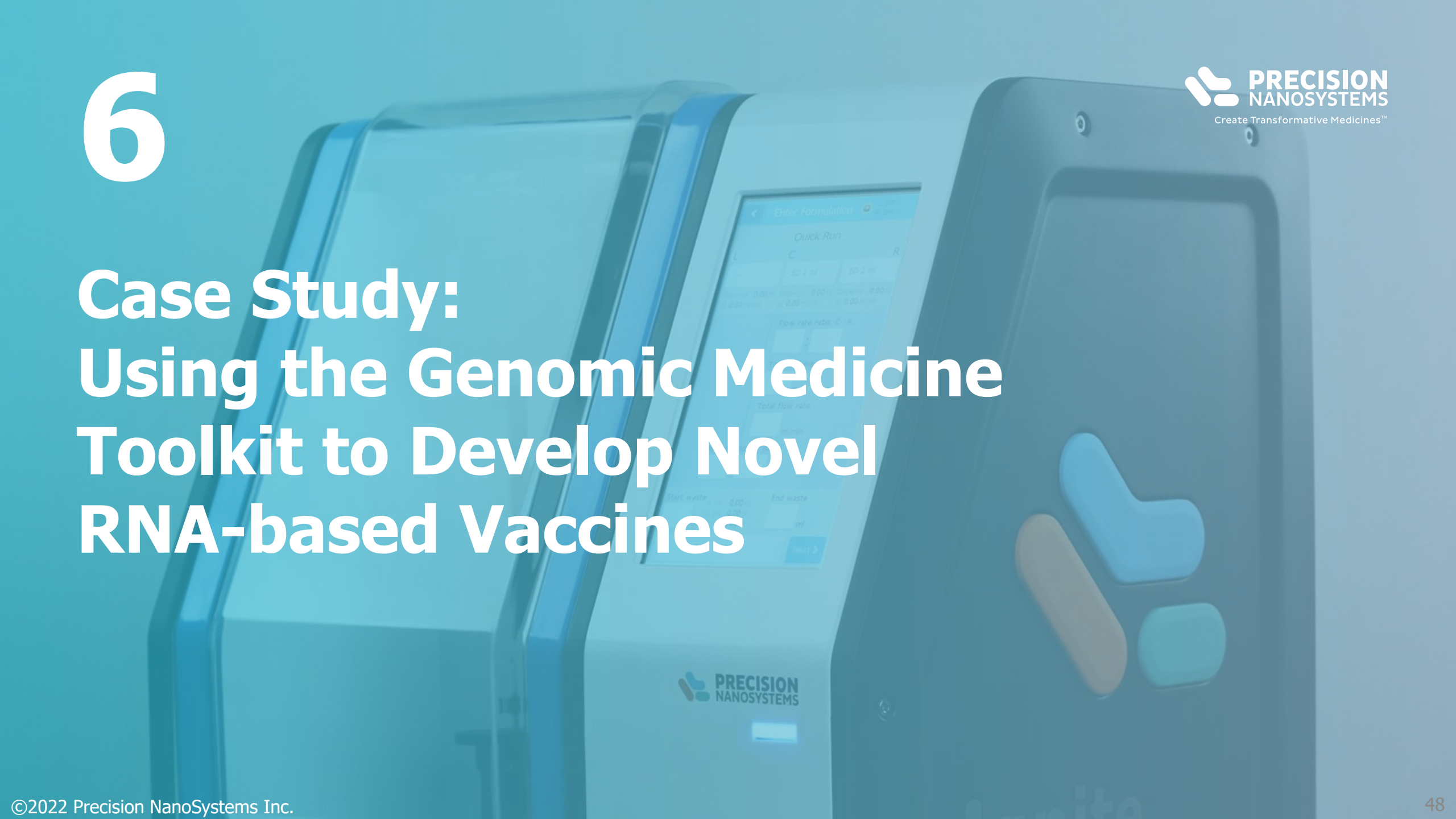
PRECISION NANOSYSTEMS
Create Transformative Medicines™



©2022 Precision NanoSystems Inc.

6

Case Study: Using the Genomic Medicine Toolkit to Develop Novel RNA-based Vaccines



PRECISION NANOSYSTEMS
Create Transformative Medicines™

©2022 Precision NanoSystems Inc.

Full Stack of Technology to Enable the Genomic Medicine Revolution

PRECISION NANOSYSTEMS
Create Transformative Medicines™

Genomic Medicine Toolkit

Disease Target

Biological insights can identify target gene(s) driving disease

Genetic Payload Platform

Proprietary self-amplifying mRNA (SAM) to express specific proteins, including antigens used in RNA vaccines against COVID-19

RNA/DNA can also silence or edit target gene(s)

GenVoy™ Delivery Platform

Lipid nanoparticles (LNP), derived from a proprietary lipid library, that protect and deliver nucleic acids (RNA, DNA, derivatives) to target cells

Rapidly develop at lab scale and seamless translation to the clinic

NanoAssemblr® Manufacturing Platform

Proprietary, scalable, continuous flow, and single-use microfluidic mixing technology for controlled and precise nanoparticle encapsulated genetic medicine development & manufacturing

Produce the best drugs — faster, easier, and with the least risk possible — from µL lab scale to GMP scale

Drug Development Expertise

Leverage world-leading expertise in LNPs and genetic medicine development

©2022 Precision NanoSystems Inc.

Key Pre-Clinical and Process Development Requirements for RNA-LNP Drug Product

PRECISION NANOSYSTEMS
Create Transformative Medicines™

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**

©2022 Precision NanoSystems Inc.

End-to-End Manufacturing of Genomic Medicines

PRECISION NANOSYSTEMS
Create Transformative Medicines™

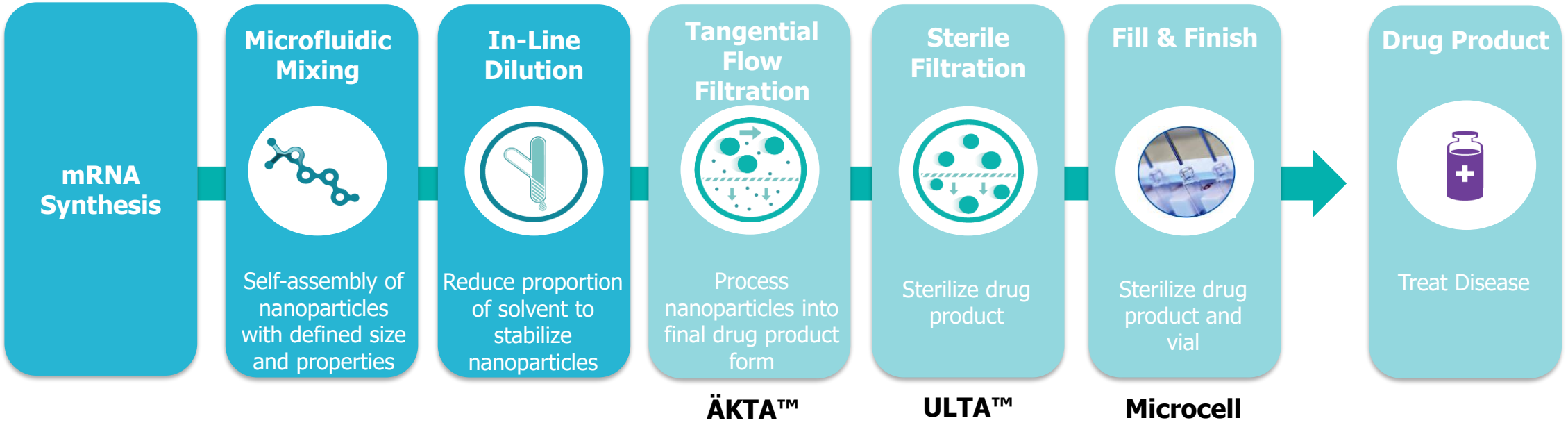
Enabling (bio)pharma companies and CDMOs with no technology access fees or royalties associated with PNI instruments

aldevron

NanoAssemblr® Instruments

cytiva PALL

Downstream Processing



PNI LNP Expertise & Partner Product Portfolio

©2022 Precision NanoSystems Inc.

PNI's Covid-19 saRNA-LNP Program

- In October 2020, PNI received $18.2 million from Canadian Strategic Innovation Fund (SIF) to develop cost-effective made-in-Canada COVID-19 self-amplifying RNA (saRNA) vaccine
- PNI also received a contribution of CAD $25.1 million through SIF to build a Genomic Medicine Biomanufacturing Centre with the goal of producing vaccines and other genetic medicines in Canada

STRATEGIC INNOVATION FUND

Q1 2021

SARS-CoV-2 *in vivo* and *in vitro:* Lead candidate selection

Q2 2021

Safety tox study initiation

Q4 2021

GMP Vaccine Manufacturing Capability

2022

Manufacture Clinical Vaccine Batch & Start Phase I/II clinical trial

Genomic Medicine Biomanufacturing Centre
Vancouver, Canada – Opening 2023

©2022 Precision NanoSystems Inc.

Process Development Objectives

PRECISION NANOSYSTEMS
Create Transformative Medicines™

- To scale up SARS-CoV-2 self-amplifying RNA-Lipid nanoparticle using PNI developed ionizable lipid and formulation from 0.2, to 50 mg scale using PNI's Manufacturing Platform (NanoAssemblr® Ignite™, Blaze™, GMP)
  - A phase I vaccine trial with saRNA-LNP requires 50 – 100 mg scale
- To optimize the down-stream processing (TFF/Sterile filtration)
  - TFF type, Material, MWCO
  - Processing buffers
  - TFF shear rate
  - TFF scalability
- To select a lead saRNA-LNP formulation and define a manufacturing process based on formulation activity, stability, scalability, repeatability and critical process parameters

©2022 Precision NanoSystems Inc.

Why saRNA?

Potential to be 10x – 100x more potent than mRNA vaccines

1

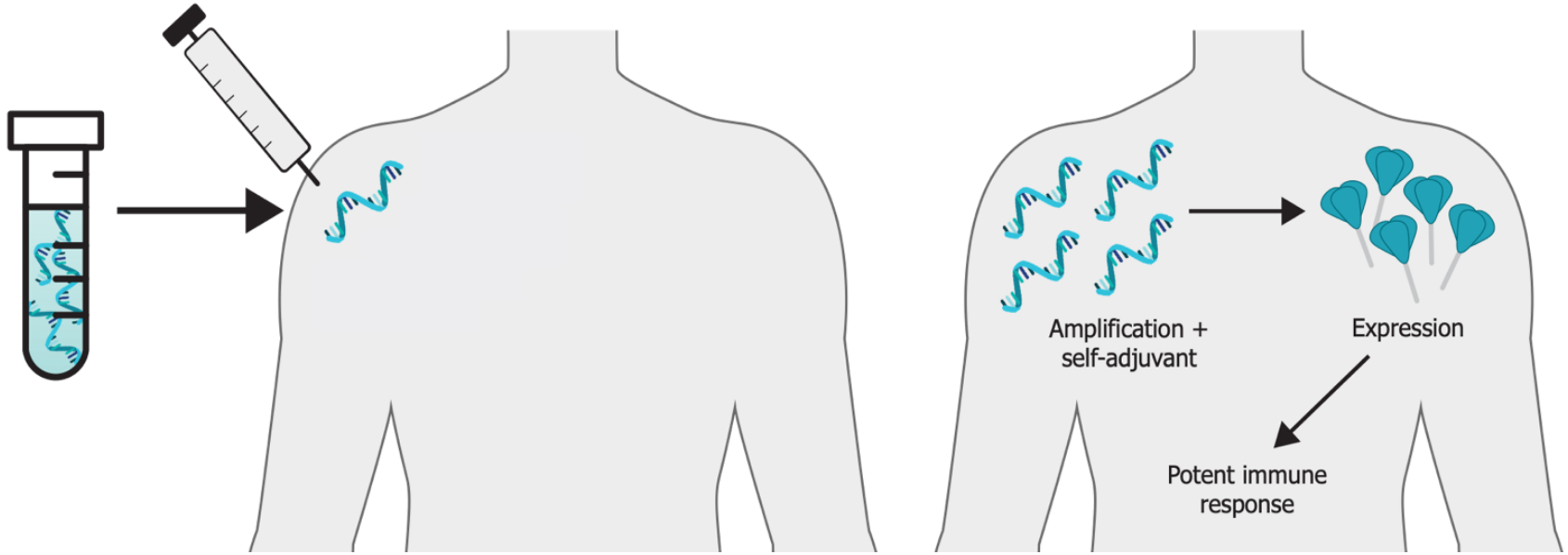
saRNA is enzymatically synthesized *in vitro* from a DNA template

2

saRNA is delivered in an LNP formulation, to cytoplasm of host cells

3

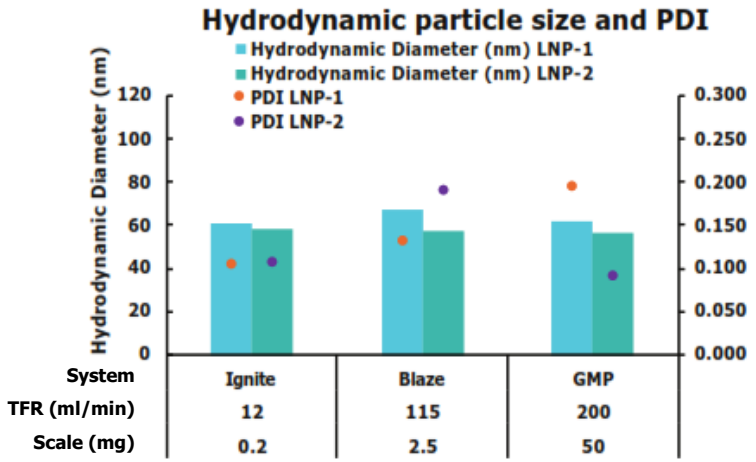
saRNA self-amplifies, produces multiple copies of mRNA in the target cells and expresses vaccine antigen

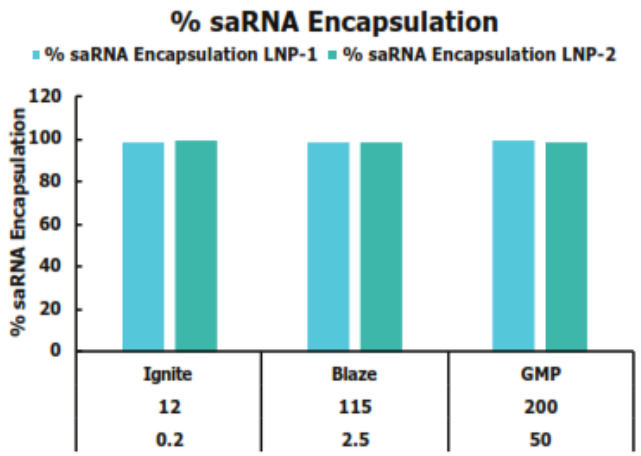


- Self-amplifying RNA encodes nonstructural proteins of the alpha virus that are translated into replicases that make many more copies
- saRNA can reduce doses by a factor of 100 thus reducing manufacturing burden

©2022 Precision NanoSystems Inc.

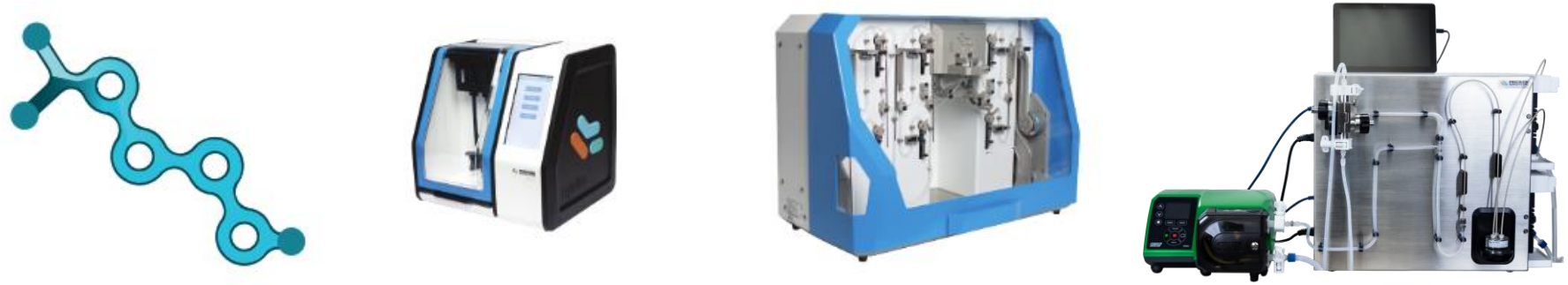
Self-Amplifying RNA-LNPs Have Equivalent Size, PDI and Encapsulation Across Scales (Ignite-Blaze-GMP)





- 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.

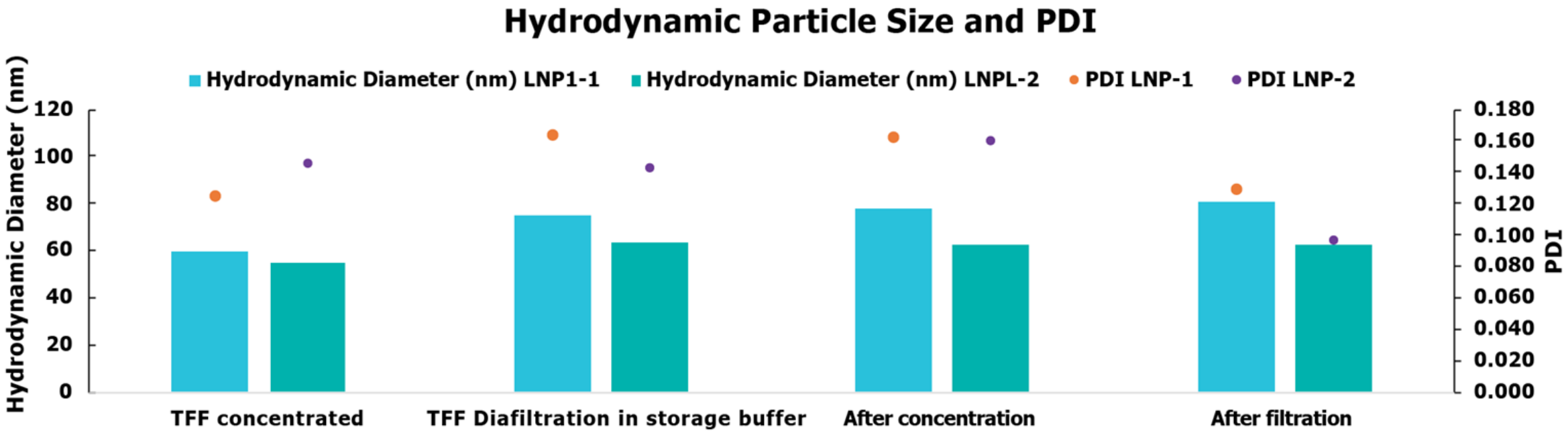
Scalability Across Platform was achieved for LNP-1 and LNP-2



Simplified Scale-up of mRNA-LNP Using NxGen™

©2022 Precision NanoSystems Inc.

In Process Data for saRNA-LNP Using Blaze (2.5 mg scale)



- At the 2.5 mg scale LNP-1 showed a slight particle size increase during the TFF process

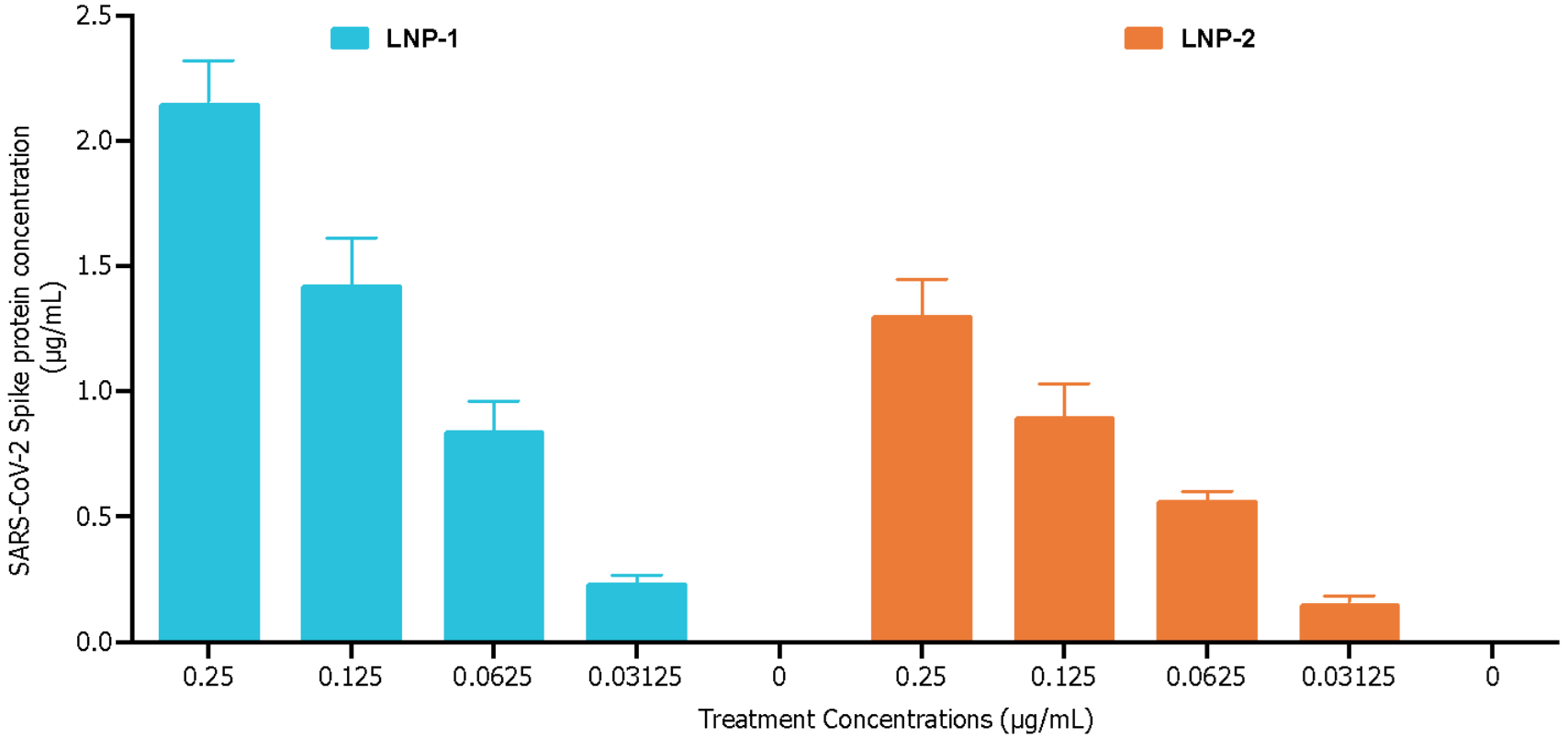
©2022 Precision NanoSystems Inc.

In Vitro Activity saRNA LNPs Using Two Novel Lipid Compositions

PRECISION NANOSYSTEMS
Create Transformative Medicines™

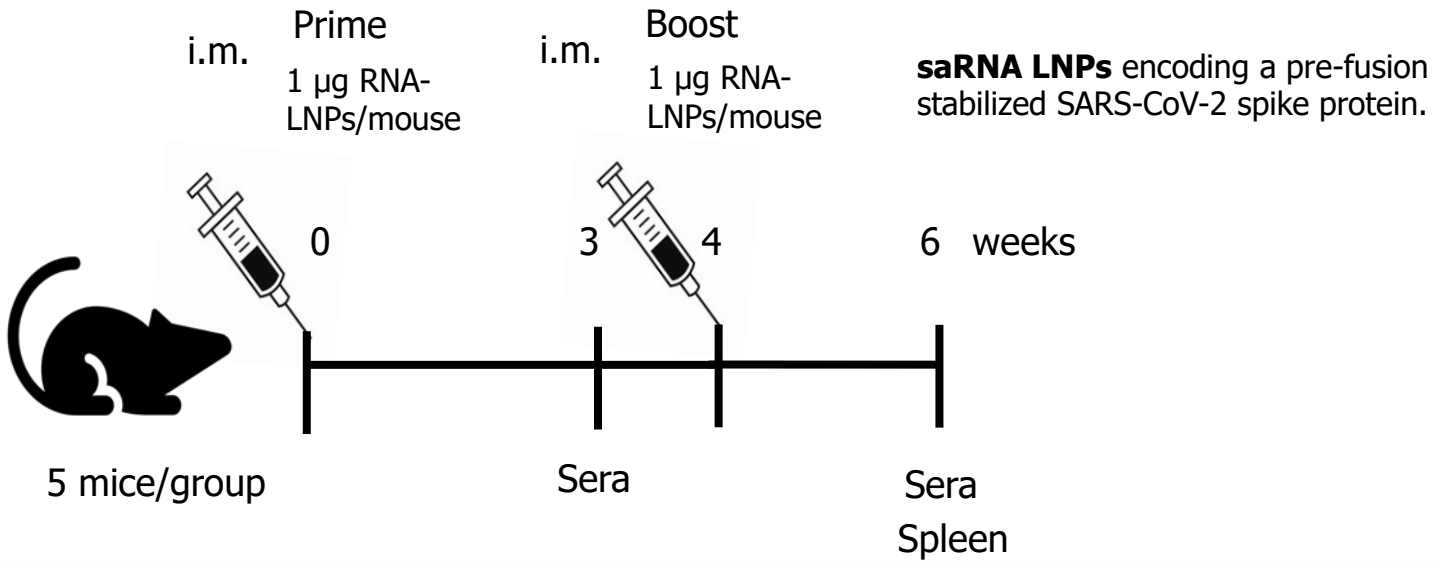
ELISA quantification of the SARS-CoV-2 spike protein expression in HEK-293 cells after transfection with saRNA LNPs

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



©2022 Precision NanoSystems Inc.

In Vivo Testing of saRNA LNPs



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

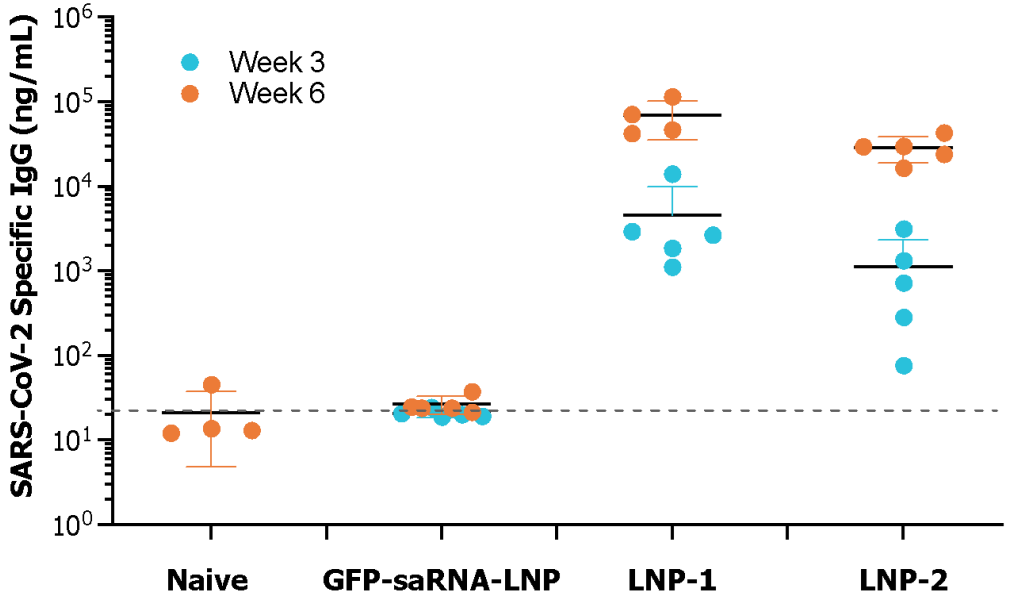
Spleen →
- Isolation of splenocytes
- *Ex vivo* restimulation with SARS-CoV-2 peptides
- Intracellular cytokine staining/Cytokine measurements

©2022 Precision NanoSystems Inc.

In Vivo Activity of saRNA LNPs Using Novel Lipid Compositions

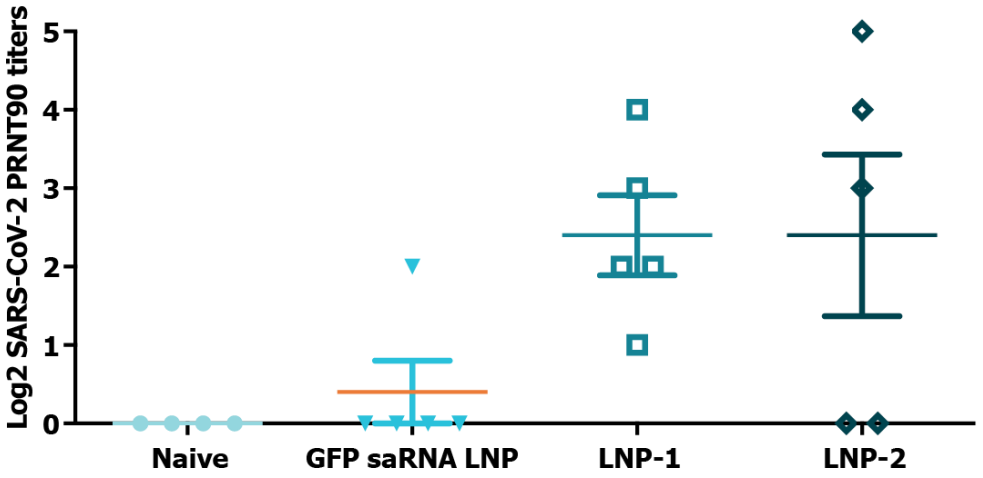
PRECISION NANOSYSTEMS
Create Transformative Medicines™

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



Neutralizing antibodies against SARS-CoV-2

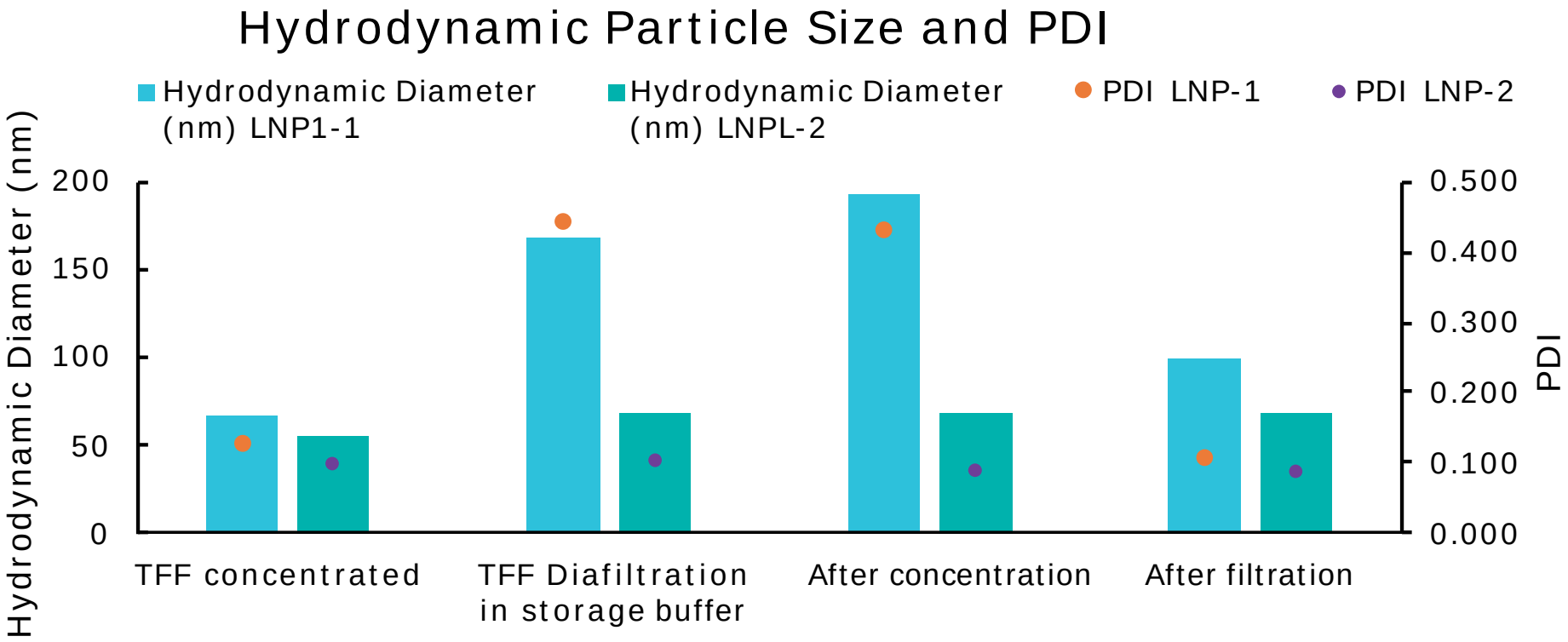
pRNT90 Values (SARS-CoV-2 real virus particles)



- 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)

©2022 Precision NanoSystems Inc.

LNP Composition Was Selected Based on Stability and Robustness During TFF Process

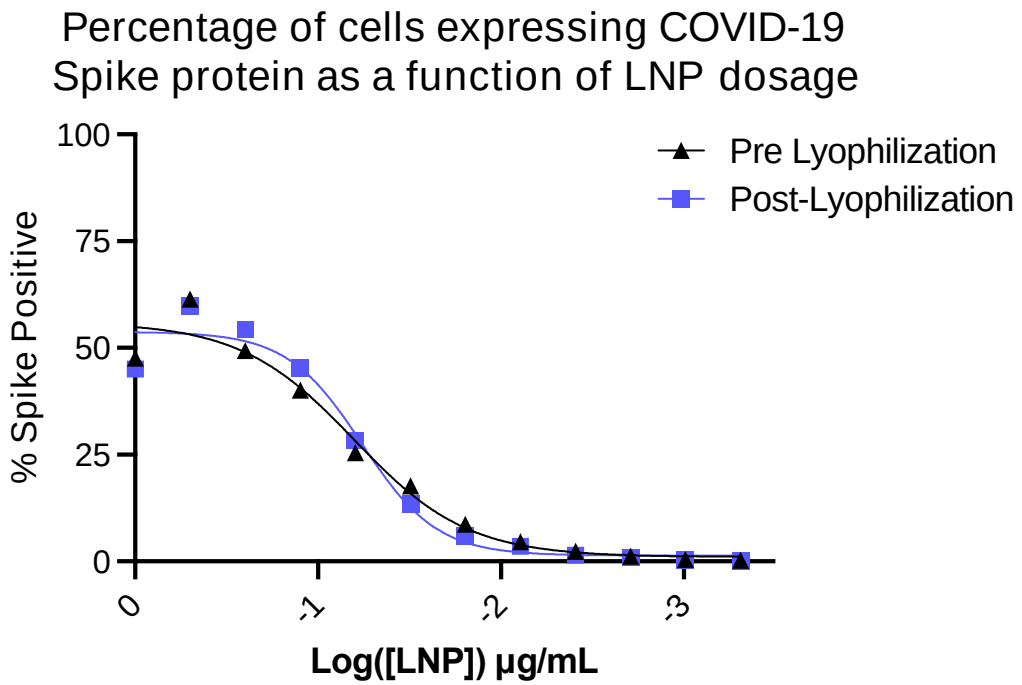


- At the 50 mg scale LNP-1 showed particle size increase during down-stream processing (TFF)
- RNA encapsulation > 90% for both formulations for all processing steps
- LNP-2 particle size stable throughout TFF and was selected as the lead clinical formulation

©2022 Precision NanoSystems Inc.

PNI's Lyophilized saRNA-LNP Vaccine Candidate Retains Activity

	Before Lyophilization	After Lyophilization
Size (d.nm)	71	89
PDI	0.074	0.09
% saRNA encapsulation	97	95
EC50 (ug/mL)	0.063	0.057



BHK 570 cells transfected in a 96-well plate with SARsCov-2-SARNA LNP in a dose response manner from 1 to 0.00049 ug/mL

- Similar particle characteristics and in vitro potency following lyophilization cycle

©2022 Precision NanoSystems Inc.

Acknowledgements

Funding:

- Government of Canada Strategic Innovation Fund

Collaborators:

- Dr. Robin Shattock & Team at Imperial College London
- Dr. Yvonne Perrie & Team at University of Strathclyde

PNI departments:

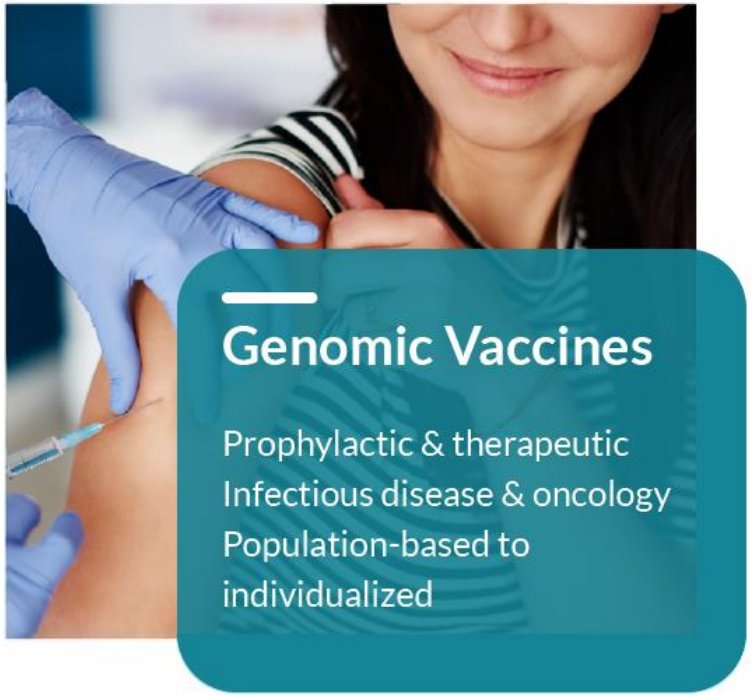
- Clinical Manufacturing
- Research
- Engineering & Operations
- Preclinical
- Process Development
- Analytical Development
- Quality Control
- Quality Assurance
- Project Management
- RNA Development Services
- Sales and Marketing

©2022 Precision NanoSystems Inc.

Accelerating Tomorrow's Genomic Medicines

From idea to approved medicine.

PRECISION NANOSYSTEMS
Create Transformative Medicines™



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

©2022 Precision NanoSystems Inc.

linkedin.com/company/precision-nanosystems-inc-

twitter.com/precisionnano

youtube.com/PrecisionNanoSystems

Thank you for listening!

Questions?

PRECISION NANOSYSTEMS

Create Transformative Medicines™