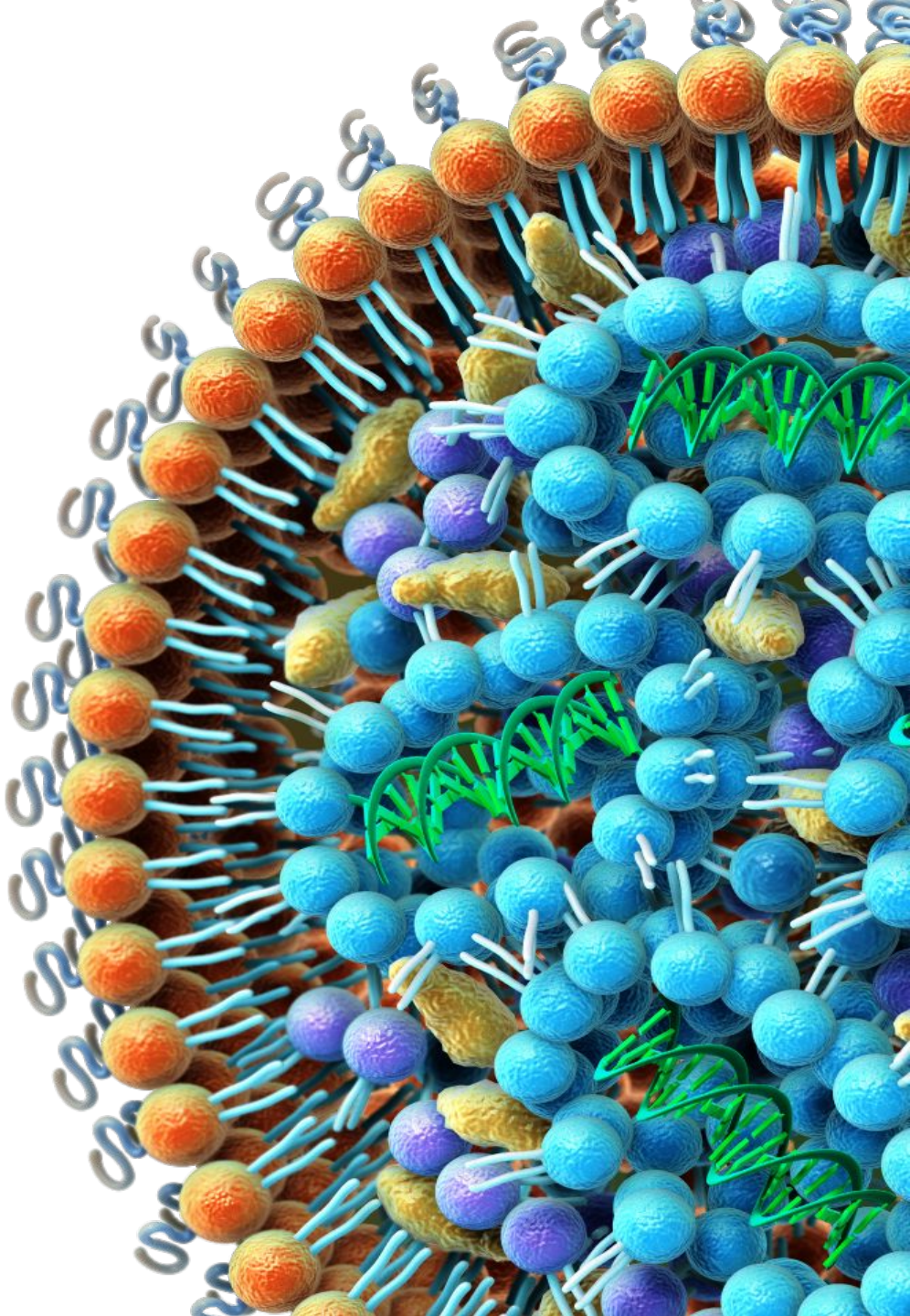
Accelerate the Preclinical Development of RNA Vaccines using GenVoy-ILM™, a Novel Lipid Nanoparticle Reagent.

Natalie Orr, PhD
Product Development Scientist

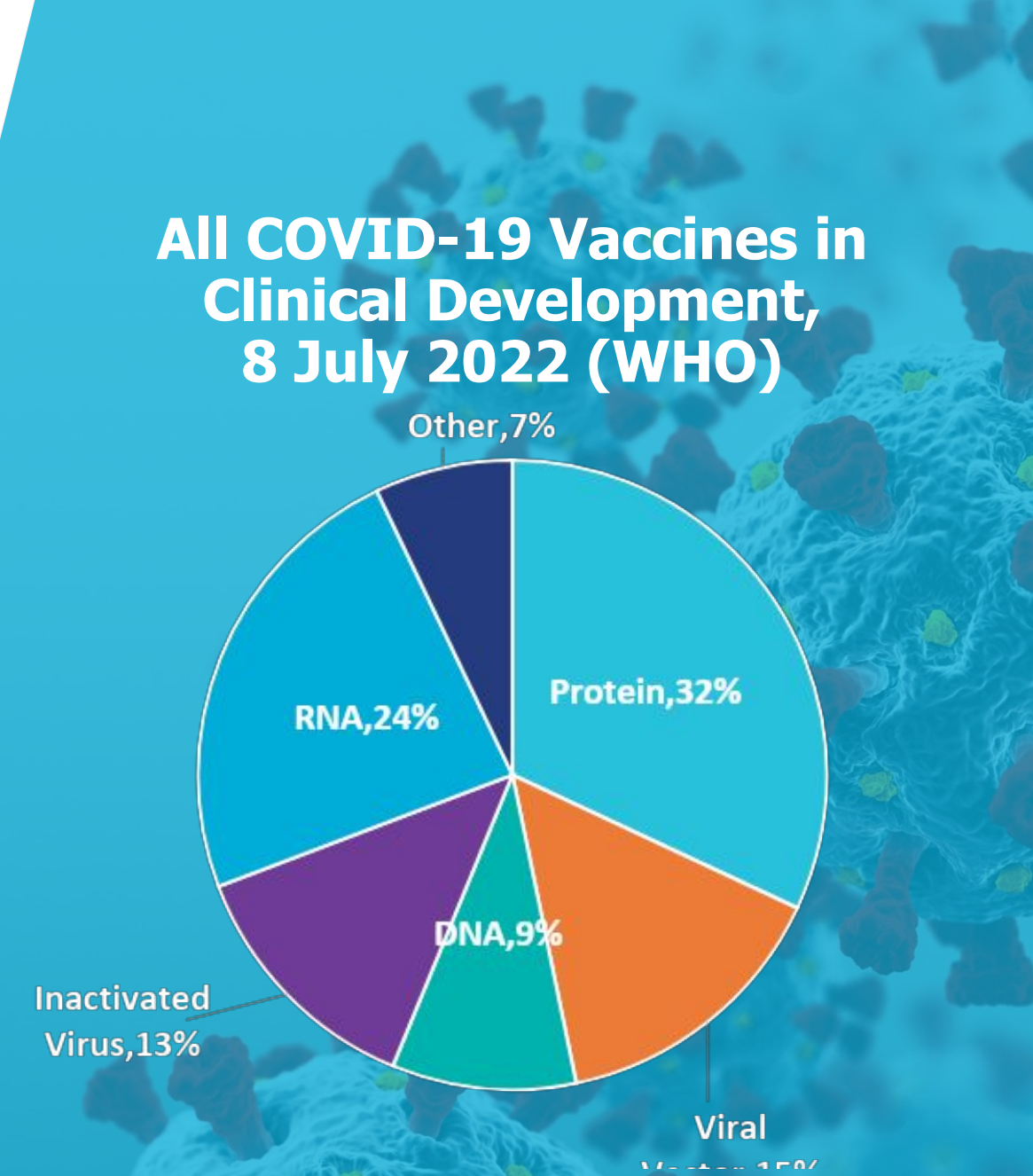
13th July 2022

PRECISION NANOSYSTEMS
Create Transformative Medicines™



RNA Vaccines

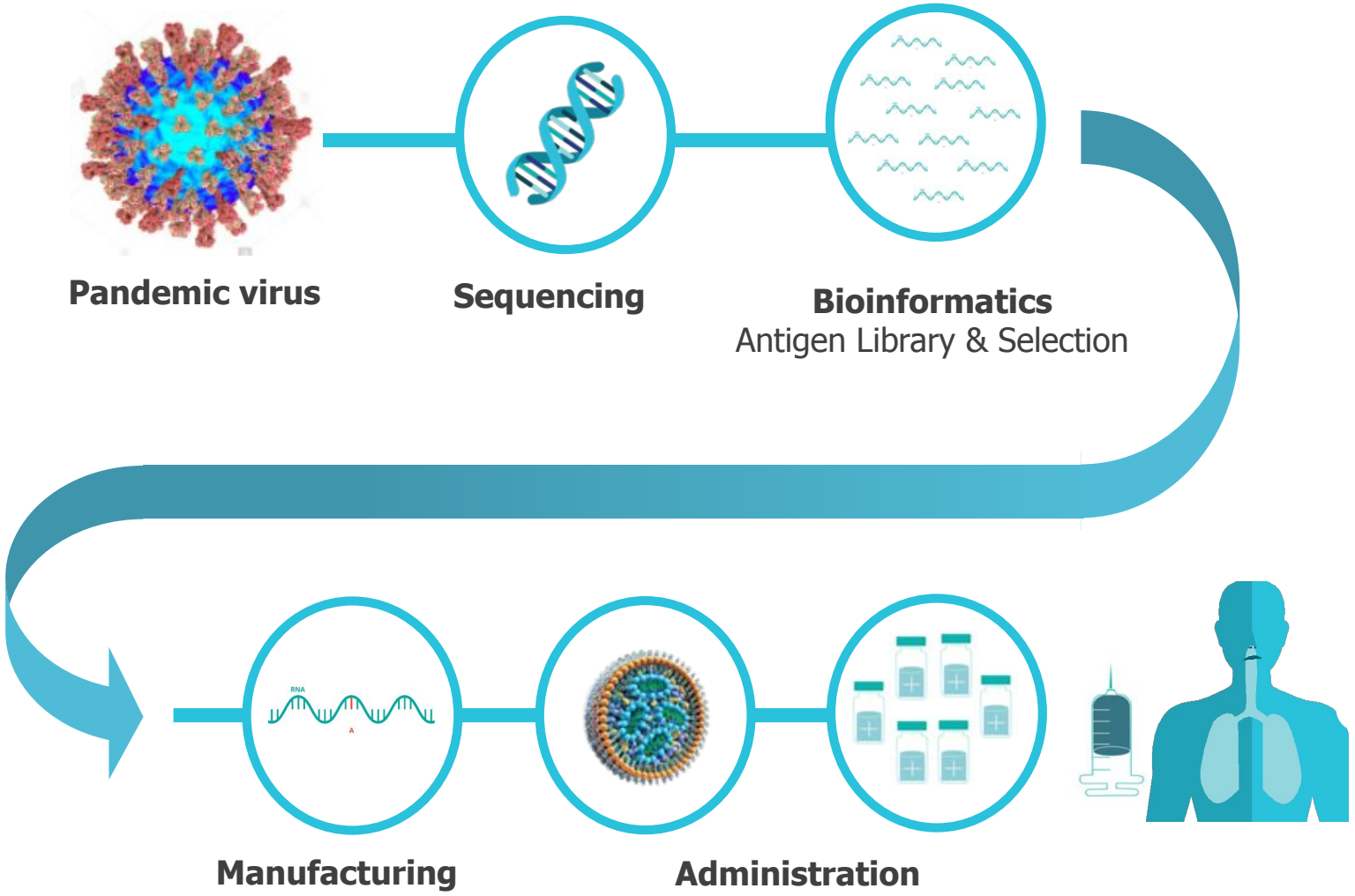
- RNA vaccines have been under investigation for 30 years.
- The first two FDA-approved vaccines for COVID-19 use **mRNA LNP technology.**
- As of July 8th, 2022, there are 169 COVID-19 vaccines in clinical development; another 198 are in preclinical development.
- 24% of COVID vaccines in clinical development are RNA-based.



- Dolgin, E. The tangled history of mRNA vaccines. *Nature*, 597(7876): 318-324 (2020)

©2022 Precision NanoSystems Inc.

mRNA LNP Technology for Rapid Response



- Moderna: Vaccine development was initiated after the SARS-CoV-2 genome was posted on January 10, 2020.
- Manufacture and delivery of clinical trials material was completed within 45 days.
- The first trial participants were vaccinated on March 16, 2020, just **66 days** after the genomic sequence of the virus was posted.

- Ulmer et al. Vaccines on demand: Science fiction or a future reality. *Expert Opin Drug Discov*, 10(2):101-106 (2015)
- Corbett et al. SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. *Nature*, *586*(7830): 567-571 (2020)

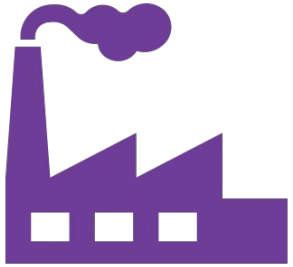
©2022 Precision NanoSystems Inc.

Benefits of RNA Vaccines

PRECISION NANOSYSTEMS
Create Transformative Medicines™

Speed of development

Safety



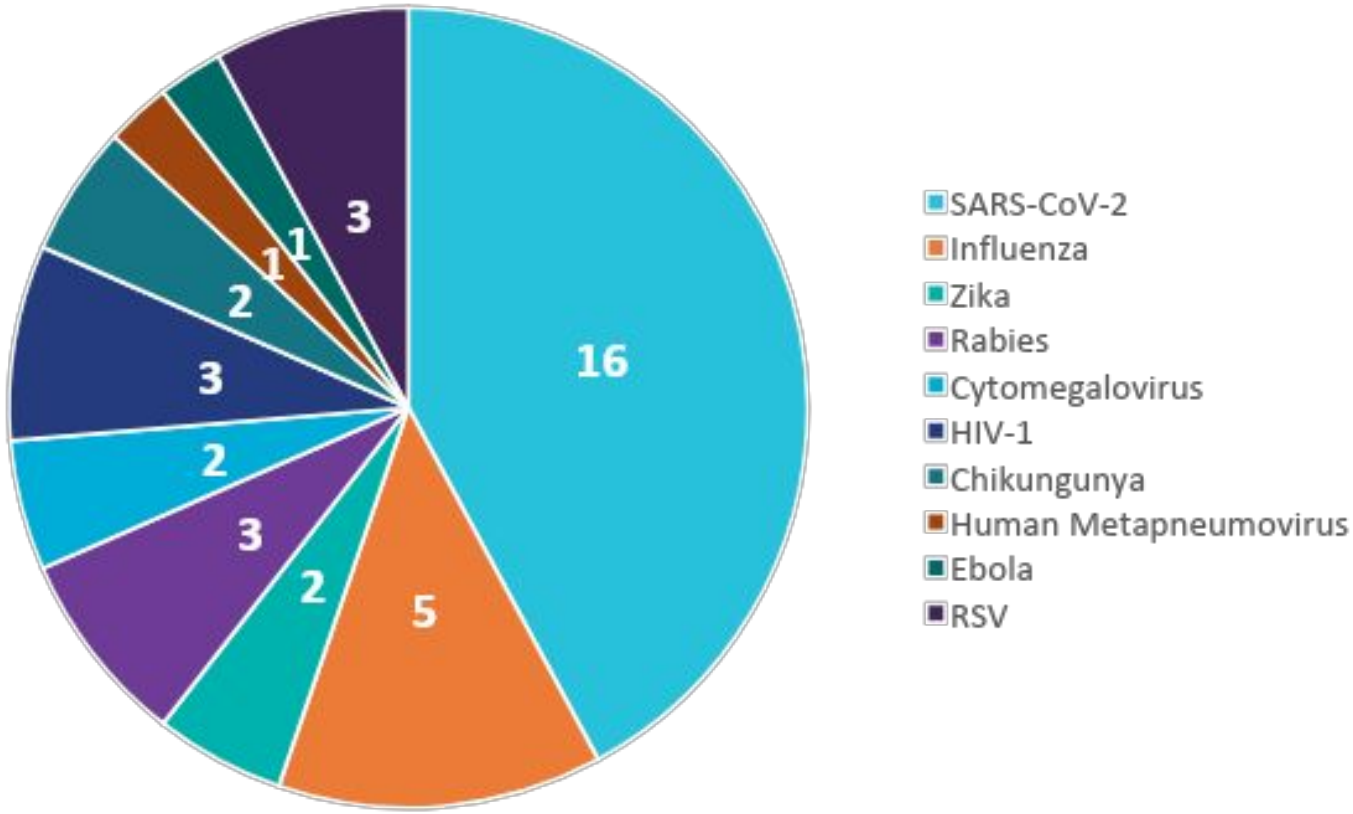
Manufacturability

Cost effective

Can encode any antigen

©2022 Precision NanoSystems Inc.

Clinical Trials of mRNA vaccines beyond COVID-19

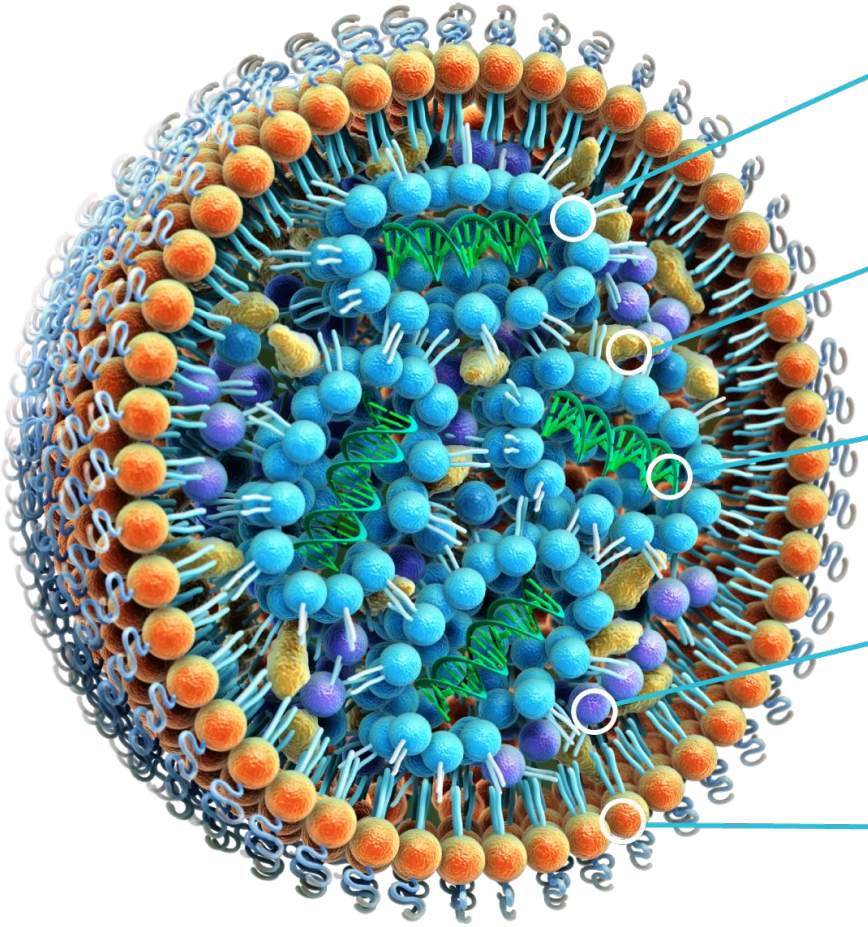


Chaudhary, N., Weissman, D., & Whitehead, K. A. mRNA vaccines for infectious diseases: principles, delivery and clinical translation. *Nature Reviews Drug Discovery*, *20*(11), 817-838 (2021)

©2022 Precision NanoSystems Inc.

Delivery of RNA – Lipid Nanoparticles

PRECISION NANOSYSTEMS
Create Transformative Medicines™



Ionizable Cationic Lipid

- Ensures high encapsulation efficiency
- Responsible for payload release

Cholesterol

- Mimics LDL → Uptake

Nucleic Acid Payload

- Payload

Helper Lipid

- Structural

Stabilizer

- Stabilizer

©2022 Precision NanoSystems Inc.

Vaccines Toolkit

PRECISION NANOSYSTEMS
Create Transformative Medicines™

Vaccine Toolkit

Disease Target

Mutations can be engineered into the antigen code to improve vaccine effectiveness.

Genetic Payload Platform

Nucleic acid that encode the antigen sequence along with RNA replication machinery.

GenVoy™ Delivery Platform

Enables a synthetic vaccine without cell line packaging, replication-competent virus and anti-vector immunity.

NanoAssemblr® Manufacturing Patform

Control of LNP formation that influences physico-chemical properties and biological activity.

Drug Development Expertise

End-to-end vaccine development expertise.

Recognized experts in RNA-LNP development.

©2022 Precision NanoSystems Inc.

Aim

To investigate GenVoy-ILM's utility as an easy-to-use lipid nanoparticle (LNP) reagent mix that accelerates the pre-clinical development of RNA vaccines.

©2022 Precision NanoSystems Inc.

LNP Composition

LNP composition of GenVoy-ILM™ and a MC3 control LNP formulation, compared to clinically relevant RNA products.

Lipid Component (mol %)	GenVoy-ILM™	MC3 Control	Onpattro patisiran (siRNA)	Moderna mRNA vaccine	CureVac mRNA vaccine candidate
Ionizable lipid	50	50 (Dlin-MC3-DMA)	50 (Dlin-MC3-DMA)	50	50
DSPC	10	10	10	10	10
Cholesterol	37.5	38.5	38.5	38.5	38.5
Stabilizer	2.5	1.5 *	1.5 *	1.5 *	1.5 *
N/P ratio	6	6	3	6	6

* $DMG\text{-}PEG_{2000}$

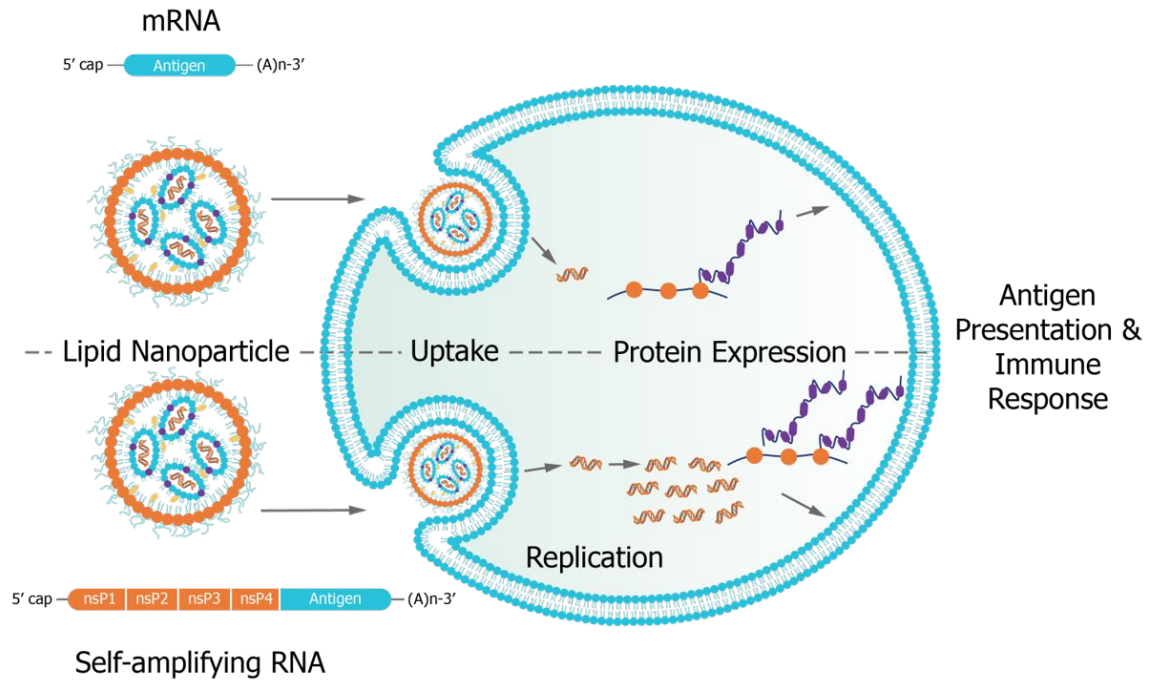
Schoenmaker, Linde, et al. "mRNA-lipid nanoparticle COVID-19 vaccines: structure and stability." *International Journal of Pharmaceutics* 601 (2021): 120586.

©2022 Precision NanoSystems Inc.

Nucleic Acid Details

Details of nucleic acid constructs.

RNA	Chain length
PolyA	2-10 nucleotides
Cleancap® FLuc mRNA (5moU)	1929 nucleotides
Cleancap® eGFP mRNA	996 nucleotides
PNI FLuc saRNA	8900 nucleotides
PNI eGFP saRNA	8463 nucleotides
PNI nCoV saRNA	11560 nucleotides



mRNA

Each mRNA molecule results in the translation of an antigen molecule.

Self-amplifying mRNA (saRNA)

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

©2022 Precision NanoSystems Inc.

Workflow: GenVoy-ILM™ and Ignite™ for Vaccine Applications

PRECISION NANOSYSTEMS
Create Transformative Medicines™

Produce lead RNA LNP candidates

RNA LNPs encapsulating different mRNA and saRNA constructs were prepared using GenVoy-ILM™ and a NanoAssemblr® Ignite™ and characterized.

Determine *in vitro* activity

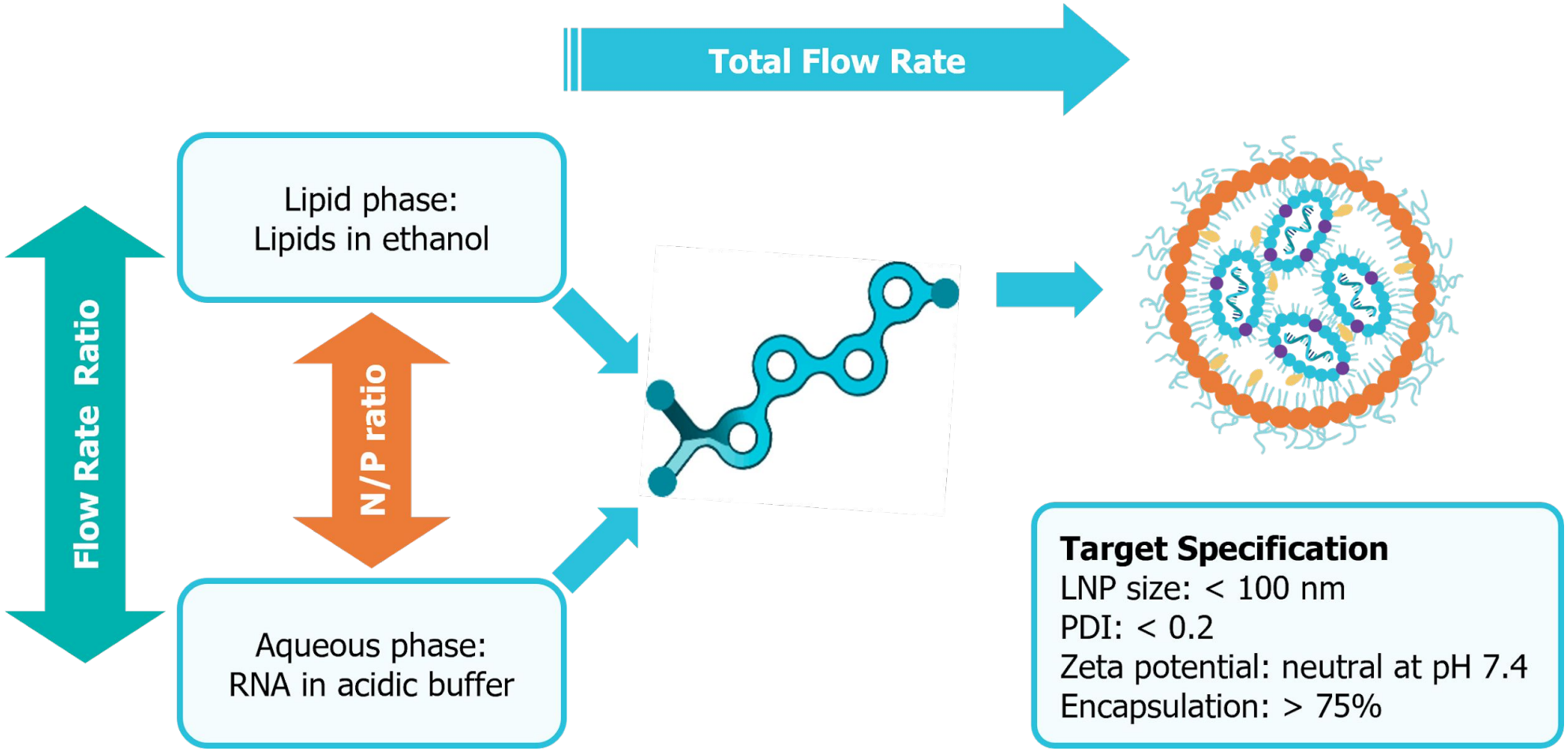
In vitro toxicity and protein expression were assessed in HEK293 cells.

Determine *in vivo* activity

In vivo distribution and protein expression were assessed by bioimaging; immunogenicity studies were performed using a saRNA encoding for SARS-CoV-2 spike protein.

©2022 Precision NanoSystems Inc.

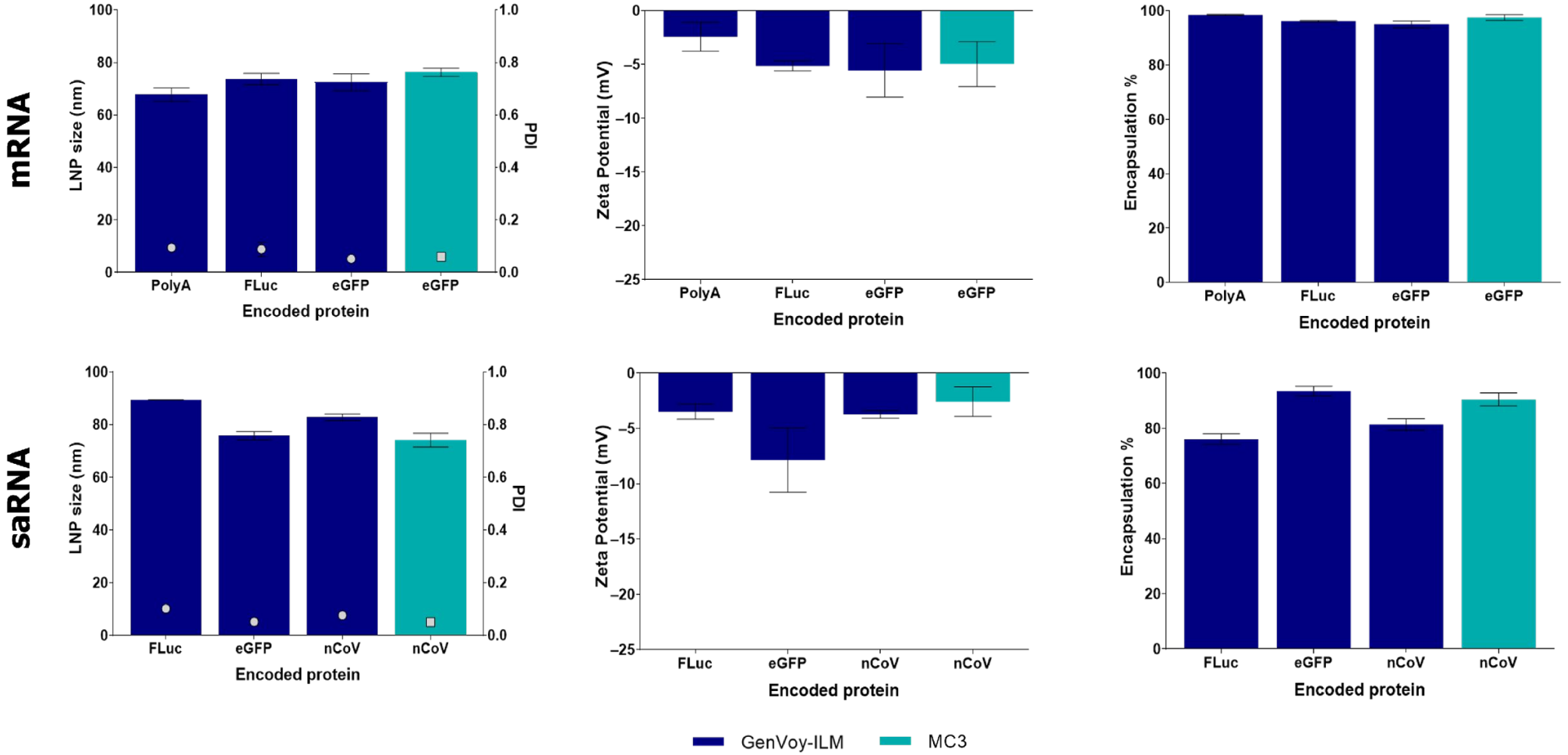
Optimization Experiments



©2022 Precision NanoSystems Inc.

GenVoy-ILM™ LNPs can Encapsulate Both mRNA and saRNA While Retaining the Target LNP Characteristics of an RNA Vaccine

PRECISION NANOSYSTEMS
Create Transformative Medicines™

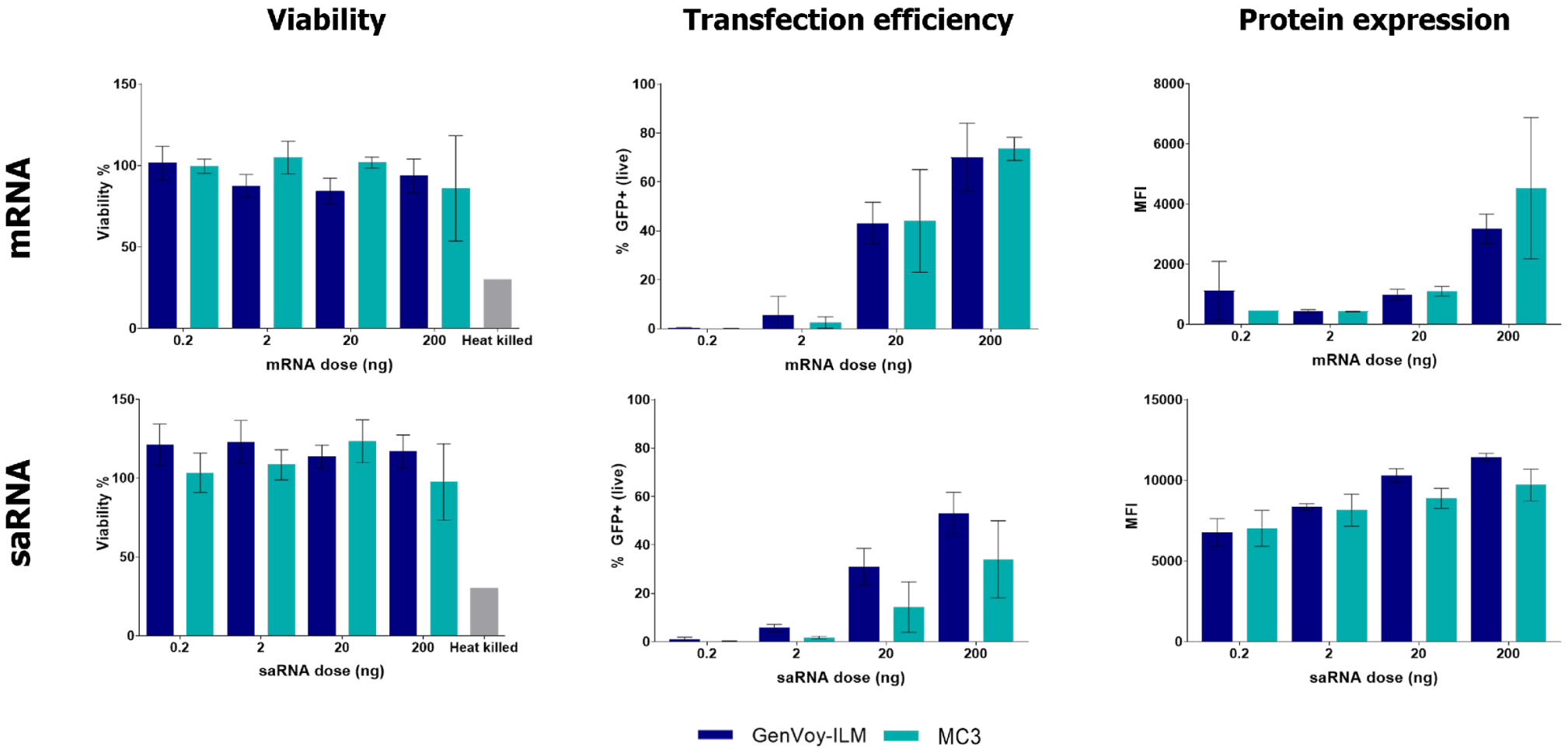


Physicochemical characteristics of LNPs prepared with GenVoy-ILM or an MC3 control lipid mix, encapsulating either mRNA and saRNA encoding for different proteins. LNPs were prepared using the Nanoassemblr® Ignite™ at a flow rate of 20 mL/min, flow rate ratio of 3:1, with an N/P ratio of 6. Data is presented as the mean ± SD of 3 batches.

©2022 Precision NanoSystems Inc.

GenVoy-ILM™ LNPs are an Effective *In Vitro* Delivery Vehicle for Both mRNA and saRNA with Titratable Protein Expression and No Toxicity

PRECISION NANOSYSTEMS
Create Transformative Medicines™



HEK293 cells were treated overnight with GenVoy-ILM and MC3 LNPs containing either mRNA or saRNA encoding for eGFP, at doses of 0.2-200 ng. Cell viability, transfection efficiency and eGFP expression were determined using flow cytometry. Untreated cells were used as controls. Data is presented as the mean ± SD from 3 batches.

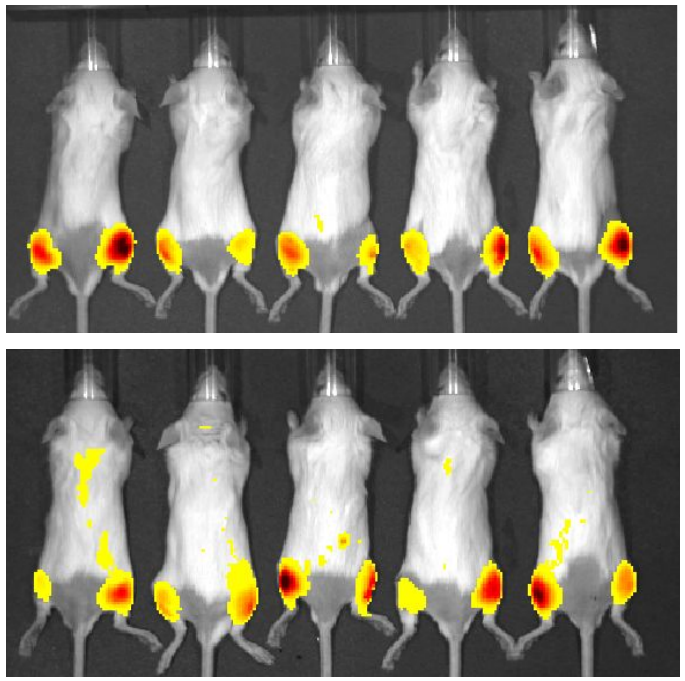
©2022 Precision NanoSystems Inc.

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

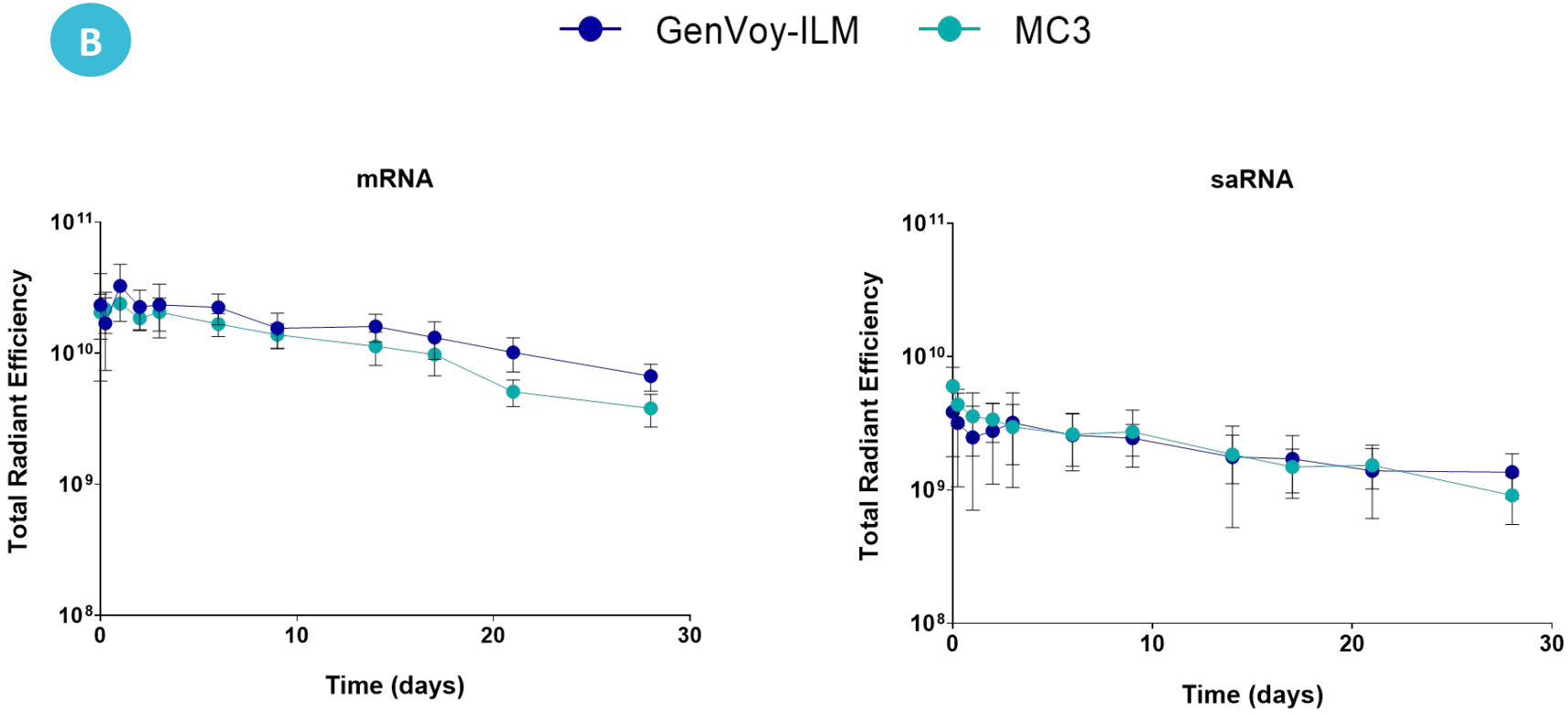
PRECISION NANOSYSTEMS
Create Transformative Medicines™

A

GenVoy-ILM LNPs



B



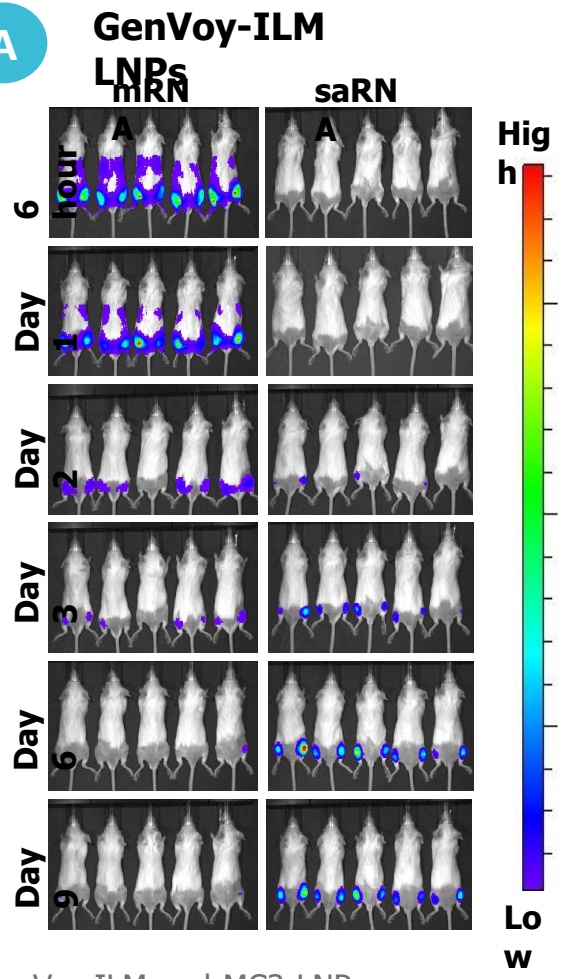
GenVoy-ILM and MC3 LNPs were prepared with 0.1 mol% DiD, encapsulating either mRNA (5 µg/leg) or saRNA (1 µg/leg) encoding for FLuc. Female BALB/c mice (n=5) were injected IM with LNPs, and distribution was determined using fluorescence imaging (IVIS® Spectrum). (A) shows representative fluorescence images of mice immediately post-IM injection with GenVoy-ILM LNPs. (B) shows the change in fluorescence (total radiant efficiency) over 28 days post-IM injection with LNPs encapsulating mRNA (left) and saRNA (right). Results are shown as the mean ± SD.

©2022 Precision NanoSystems Inc.

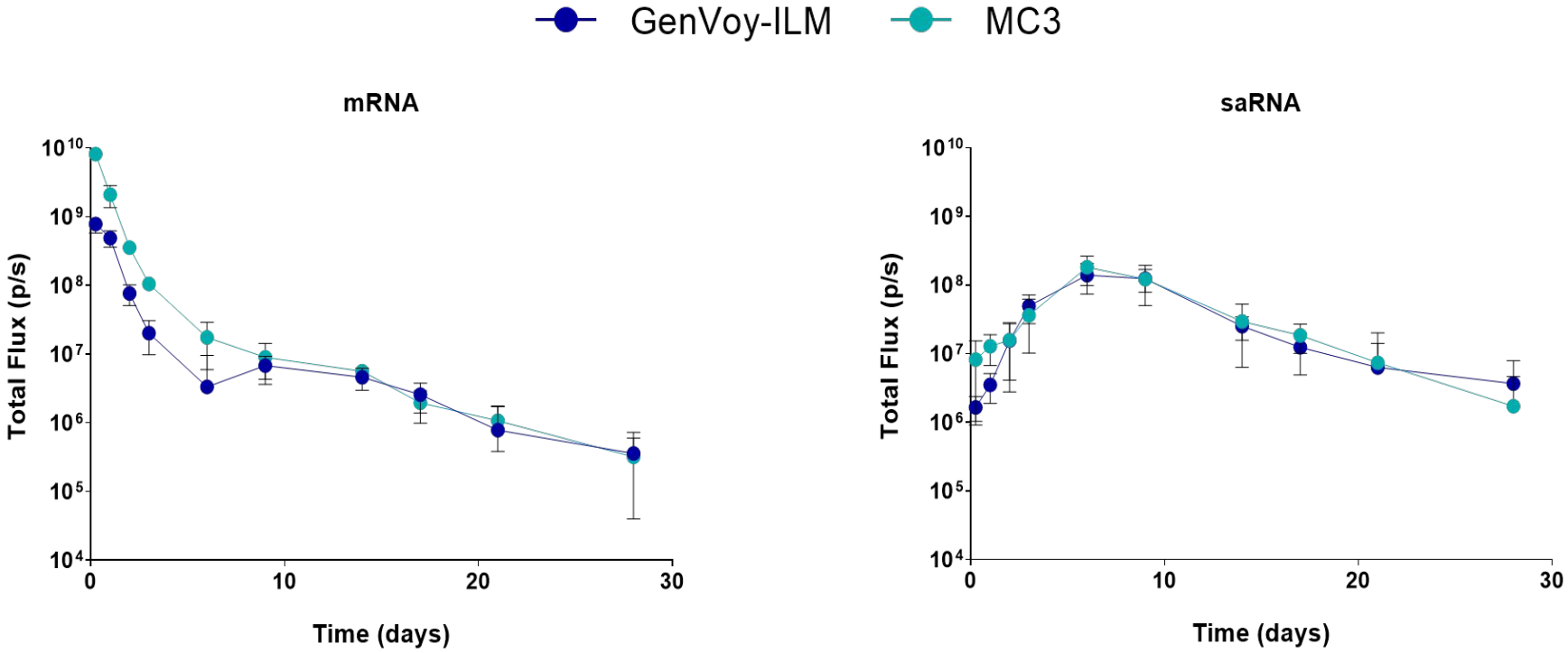
GenVoy-ILM™ LNPs are an Effective *In Vivo* Delivery Vehicle for Both mRNA and saRNA: Protein Expression

PRECISION NANOSYSTEMS
Create Transformative Medicines™

A



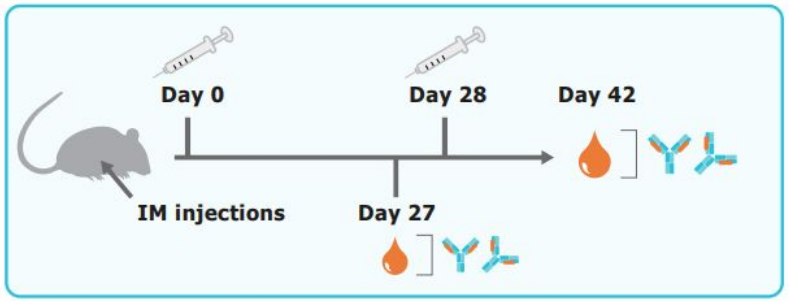
B

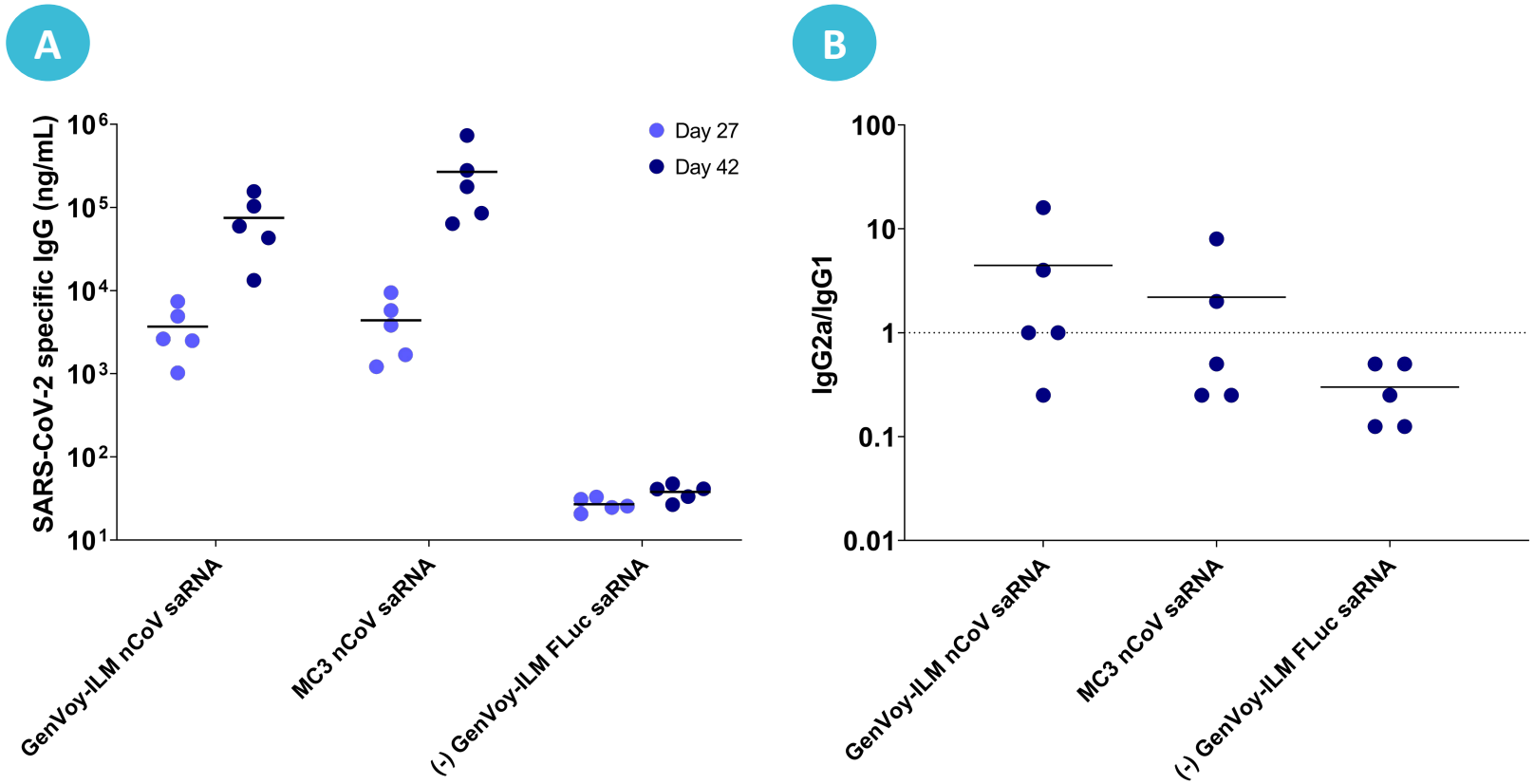


GenVoy-ILM and MC3 LNPs were prepared with 0.1 mol% DiD, encapsulating mRNA (5 µg/leg) or saRNA (1 µg/leg) encoding for FLuc. Female BALB/c mice (n=5) were injected IM with LNPs, and protein expression was determined using luminescence imaging (IVIS® Spectrum) over 28 days. Mice were injected IP with 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.

GenVoy-ILM™ LNPs Induce a Strong Immune Response Against a Target Antigen





GenVoy-ILM and MC3 LNPs were prepared encapsulating saRNA encoding for the full-length SARS-CoV-2 spike protein. GenVoy-ILM LNPs encapsulating Fluc saRNA were used as a negative control. Female BALB/c mice (n=5) were injected with 1 µg encapsulated saRNA on Day 0, with a booster dose delivered on Day 28. Tail bleeds were performed on Day 27, and mice were euthanized by cardiac puncture on Day 42. Serum samples were collected at both time points and the SARS-CoV-2 specific IgG levels were determined by quantitative ELISA, shown in graph (A). The ratio of IgG subtypes (IgG1 and IgG2a) were determined using Day 42 serum samples by semi-quantitative ELISA, shown in Graph (B). Results are shown as a scatter dot plot, with the solid line indicating the geometric mean.

©2022 Precision NanoSystems Inc.

Summary: GenVoy-ILM™ for Vaccine Applications

PRECISION NANOSYSTEMS
Create Transformative Medicines™

Proof-of-Concept Demonstrated for Vaccine Applications

GenVoy-ILM™ is validated for nucleic acid delivery in a vaccine application at the preclinical scale using LNPs formulated on the NanoAssemblr® Ignite™ instrument and cartridges.

Works Well Regardless of Payload Type and Size

GenVoy-ILM™ effectively encapsulates conventional mRNA and self-amplifying RNA (saRNA) of various lengths and designs.

Optimized to Accelerate Your Vaccine Research

Using the ready-to-go GenVoy-ILM™ and an optimized protocol gives you the confidence to move forward quickly and successfully with your proof-of-concept vaccine studies.

Scale from Discovery to Commercial Production

GenVoy-ILM™ and the proprietary lipid library can support your program from discovery all the way to the clinic using the same NxGen™ microfluidics technology across instrument platforms.

©2022 Precision NanoSystems Inc.

Acknowledgements

- Edward Grahame, PhD
- Yvonne Perrie, PhD
- Rita Zhao
- Suraj Abraham, PhD
- Sijo Chemmannur, PhD
- Anitha Thomas, PhD
- Sitalakshmi Thampatty
- Samuel Clarke, PhD
- Angela Zhang, PhD
- Andy Geall, PhD
- Perrie Research Group
- Reagents Product Development Team
- Delivery R&D Team

Grant Support

SMART:Scotland

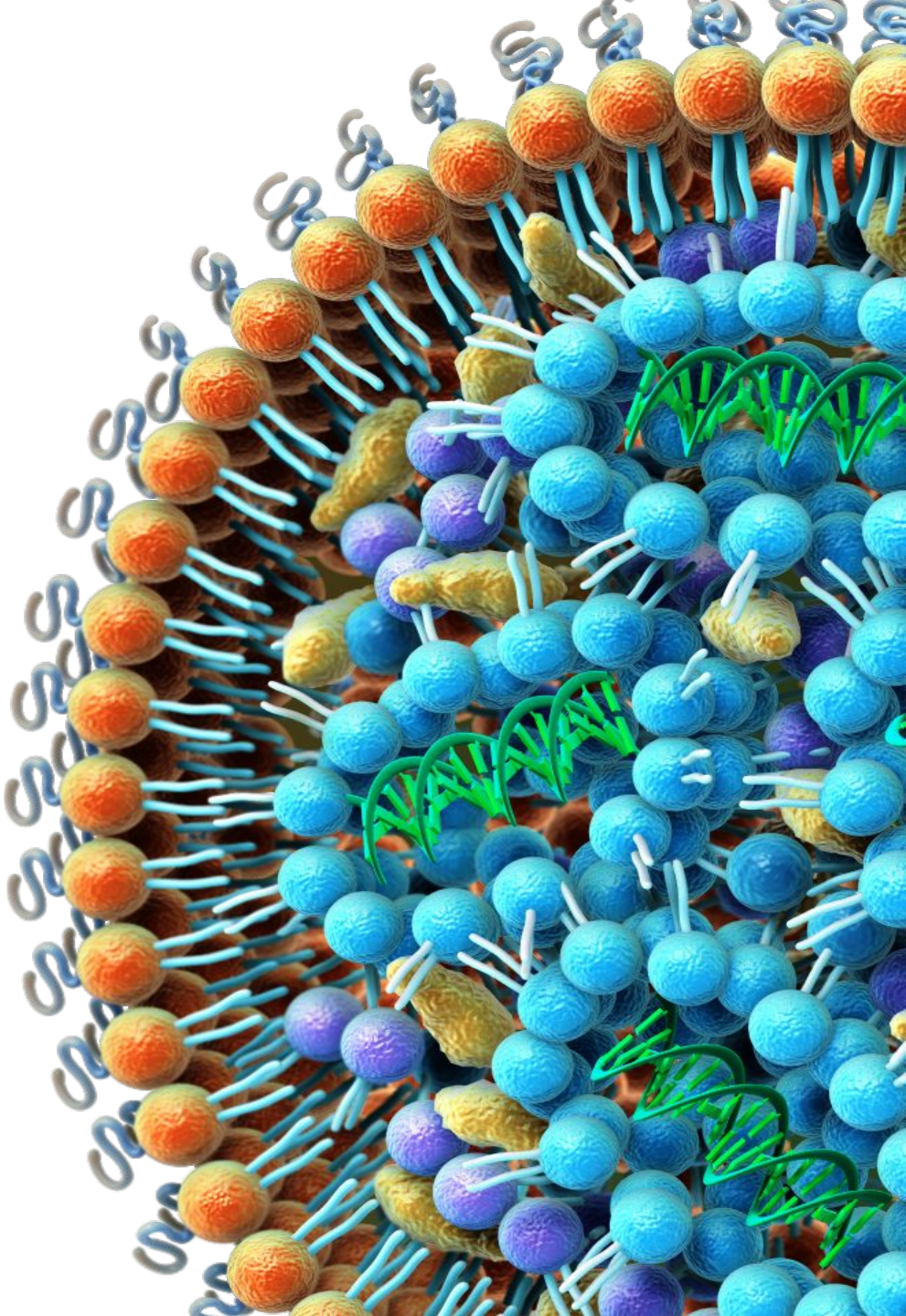
linkedin.com/company/precision-nanosystems-inc-

twitter.com/precisionnano

youtube.com/PrecisionNanoSystems

Thank you for listening!

Questions?



PRECISION NANOSYSTEMS

Create Transformative Medicines™