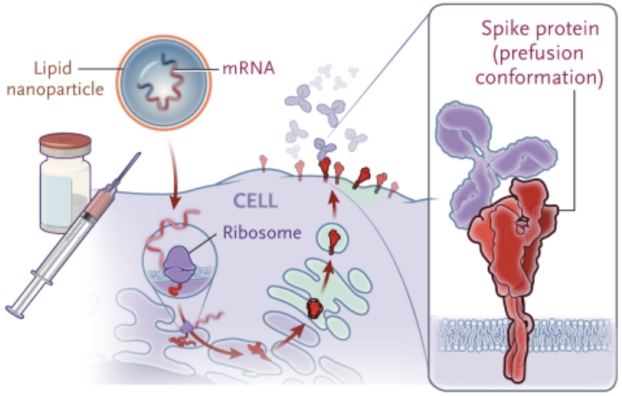
Lipid Nanoparticles for Overcoming Biological Barriers to mRNA Delivery

Michael J. Mitchell, PhD
Associate Professor of Bioengineering, University of Pennsylvania
LNP Delivery Systems Group Leader, Penn Institute for RNA Innovation
mitchell-lab.seas.upenn.edu
@MJMitchell_Lab

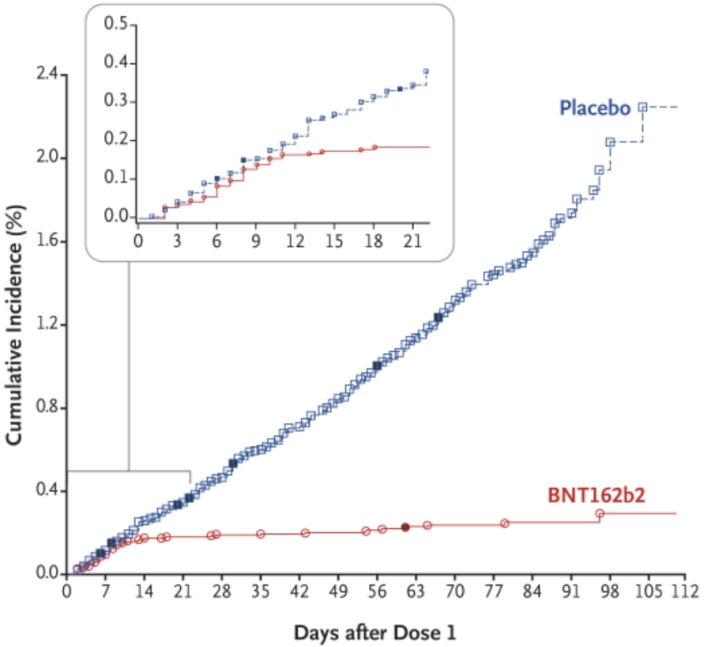
Institute for RNA Innovation

Penn Engineering

Lipid Nanoparticle (LNP) COVID-19 mRNA Vaccines

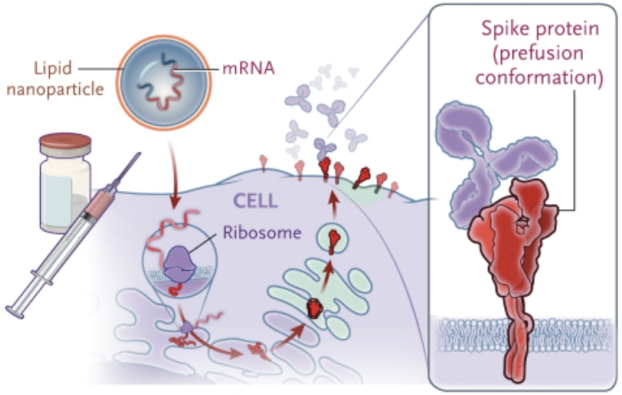


Interdisciplinary Science Feat: RNA and Nanoparticles



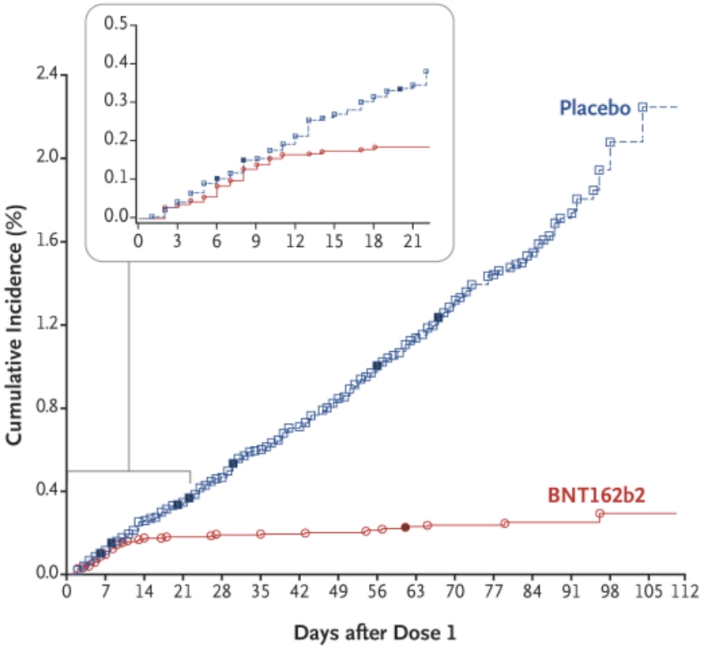
F.P. Polack et al. *New England Journal of Medicine* (2020).

Lipid Nanoparticle (LNP) COVID-19 mRNA Vaccines



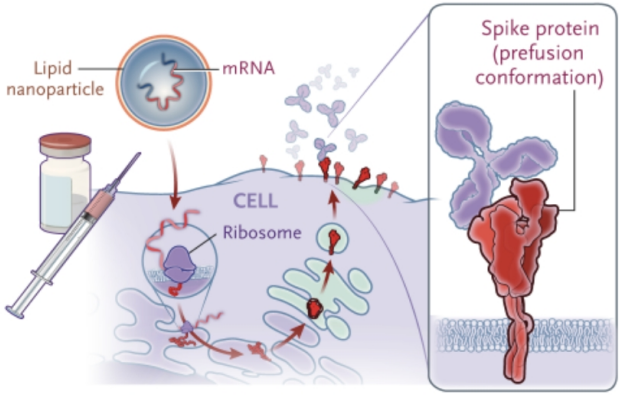
Interdisciplinary Science Feat: RNA and Nanoparticles

And Many Others!



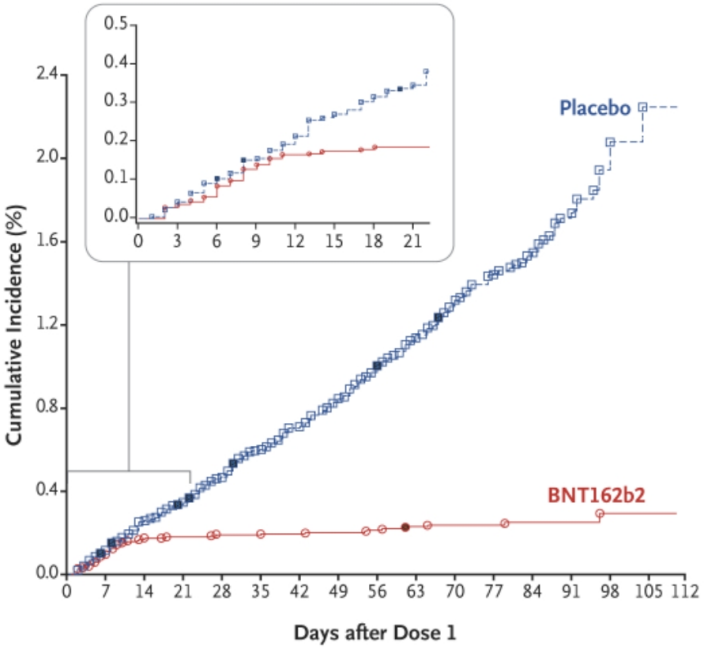
F.P. Polack et al. *New England Journal of Medicine* (2020).

Lipid Nanoparticle (LNP) COVID-19 mRNA Vaccines



Interdisciplinary Science Feat: RNA and Nanoparticles

And Many Others!



Can mRNA LNPs revolutionize pharmaceuticals? Key bottleneck: delivery, new nanotechnology

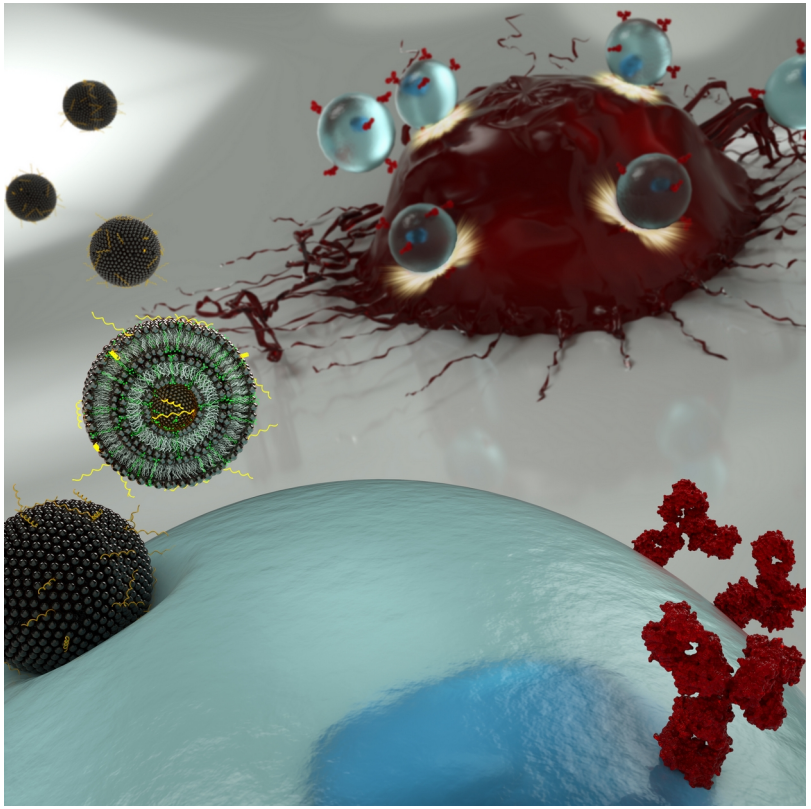
F.P. Polack et al. *New England Journal of Medicine* (2020).

Mitchell Lab: LNPs that Deliver Nucleic Acids into Target Cells and Tissues *In Vivo, Ex Vivo, In Utero*

Cancer Immunotherapy

Lipid Nanoparticles (LNPs)

Genetic Engineering of Immune Cells in Body

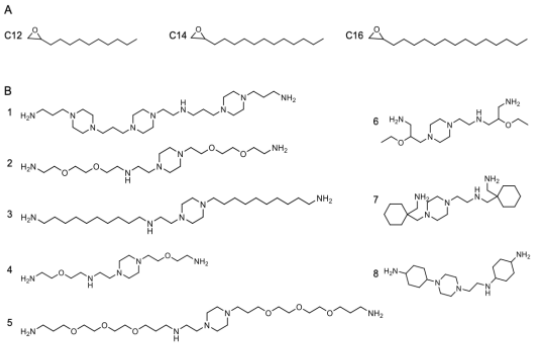


LNPs for mRNA delivery to a range of cells and tissues:

- Immune cells
- Stem cells
- Brain
- Heart
- Kidney
- Lung
- Liver
- Tumors
- Bone, bone marrow
- Placenta
- Fetus

Mitchell Lab: LNP Toolbox for Nucleic Acid Delivery

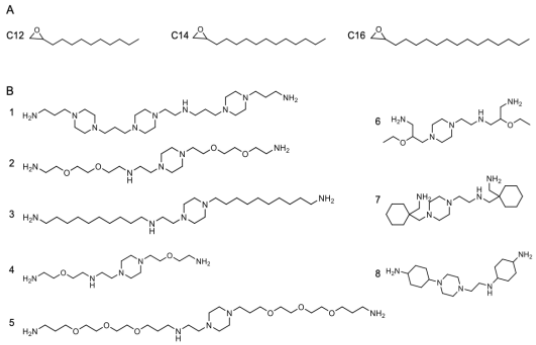
Combinatorial Chemistry
Synthesize Ionizable Lipids and Polymers (>1000)



X. Han et al, *Nature Nanotechnology* (2023)
X. Han et al, *Nature Communications* (2023)
R.S. Riley et al. *Science Advances* (2021)

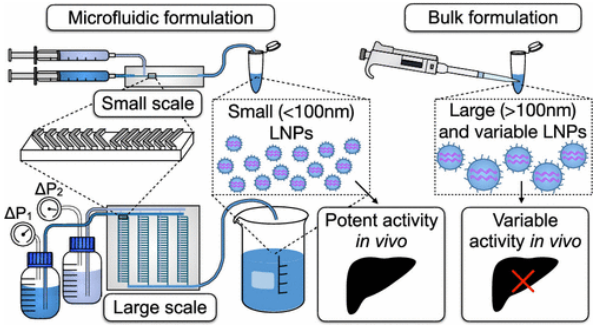
Mitchell Lab: LNP Toolbox for Nucleic Acid Delivery

Combinatorial Chemistry
Synthesize Ionizable Lipids and Polymers (>1000)



X. Han et al, *Nature Nanotechnology* (2023)
X. Han et al, *Nature Communications* (2023)
R.S. Riley et al. *Science Advances* (2021)

Microfluidics for Controlled LNP Formulation

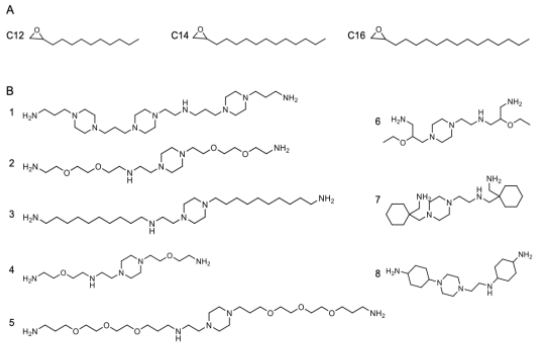


S.J. Shepherd et al. *Nano Letters* (2021)
S.J. Shepherd et al. *PNAS* (2023)

Han, Zhang, Mitchell et al. *Nature Communications* (2021)

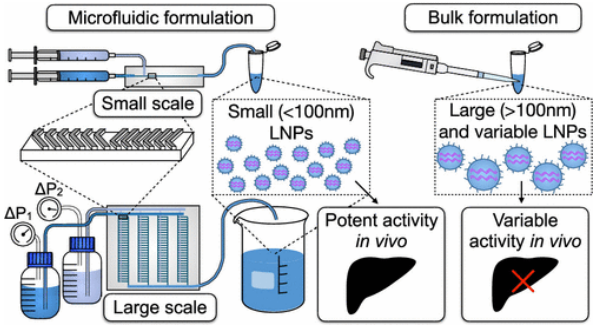
Mitchell Lab: LNP Toolbox for Nucleic Acid Delivery

Combinatorial Chemistry Synthesize Ionizable Lipids and Polymers (>1000)



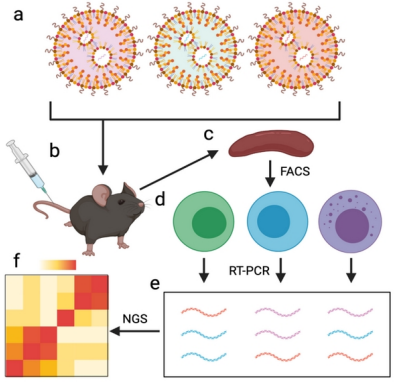
X. Han et al, *Nature Nanotechnology* (2023)
X. Han et al, *Nature Communications* (2023)
R.S. Riley et al. *Science Advances* (2021)

Microfluidics for Controlled LNP Formulation



S.J. Shepherd et al. *Nano Letters* (2021)
S.J. Shepherd et al. *PNAS* (2023)

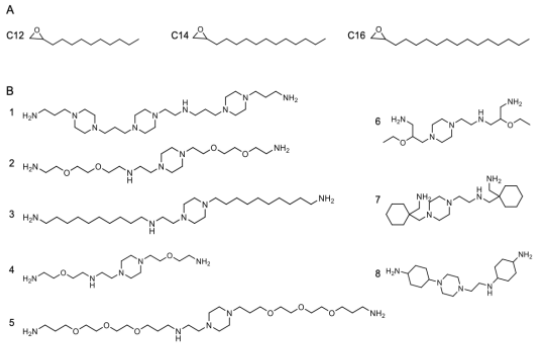
Molecular Barcoding and DOE for High-Throughput *In Vivo* Screening and Analysis



P.P.G. Guimaraes et al. *PNAS* (2013)
R. El-Mayta et al. *Biomaterials Science* (2021)
P.P.G. Guimaraes et al. *J Control Release* (2019)

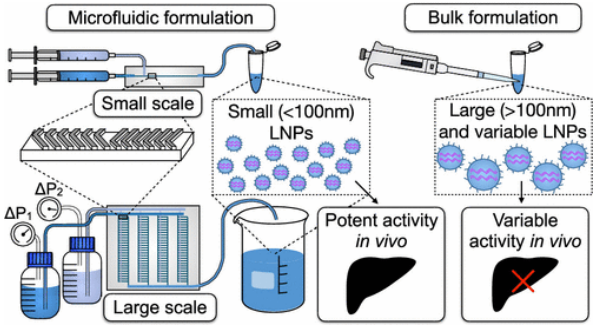
Mitchell Lab: LNP Toolbox for Nucleic Acid Delivery

Combinatorial Chemistry
Synthesize Ionizable Lipids and Polymers (>1000)



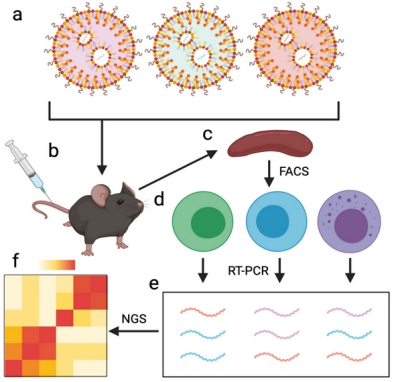
X. Han et al, *Nature Nanotechnology* (2023)
X. Han et al, *Nature Communications* (2023)
R.S. Riley et al. *Science Advances* (2021)

Microfluidics for Controlled LNP Formulation



S.J. Shepherd et al. *Nano Letters* (2021)
S.J. Shepherd et al. *PNAS* (2023)

Molecular Barcoding and DOE for High-Throughput *In Vivo* Screening and Analysis

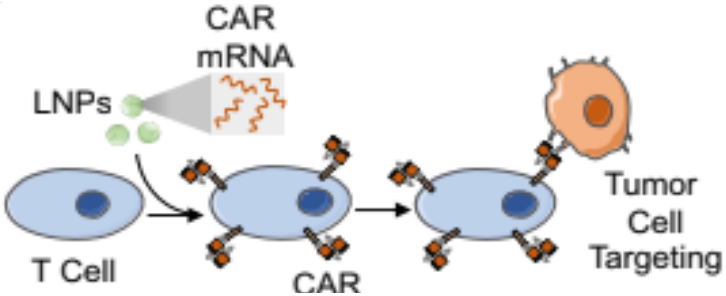


P.P.G. Guimaraes et al. *PNAS* (2013)
R. El-Mayta et al. *Biomaterials Science* (2021)
P.P.G. Guimaraes et al. *J Control Release* (2019)

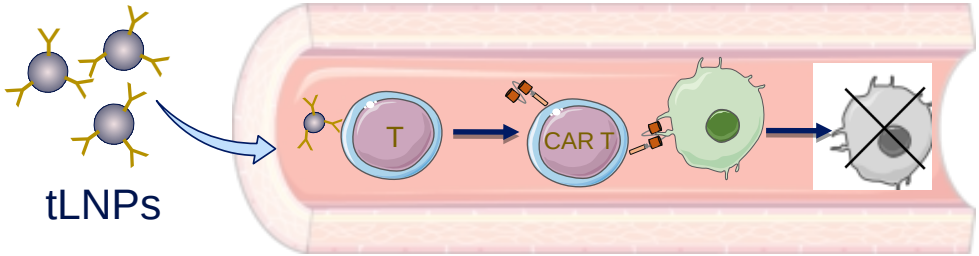
Accelerate the design and identification of LNPs for unlocking delivery to new cells and tissues

Making CAR T Cells and Macrophages in the Body via mRNA LNPs

Ex Vivo CAR T Cell Engineering



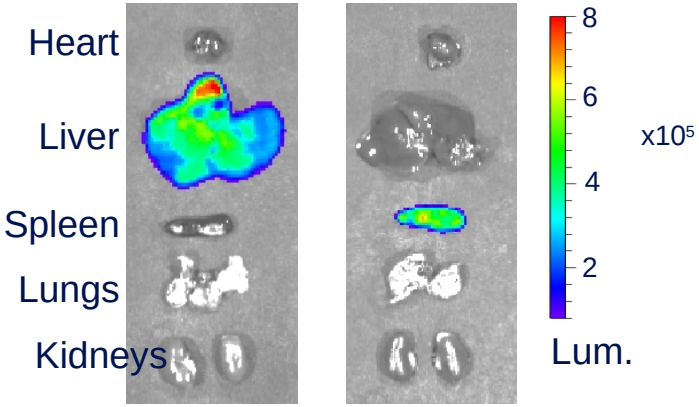
In Situ CAR T Cell Engineering



***In situ* benefit: injectable particle approach to potentially bypass laborious *ex vivo* CAR T cell engineering**

Making CAR T Cells and Macrophages in the Body via mRNA LNPs

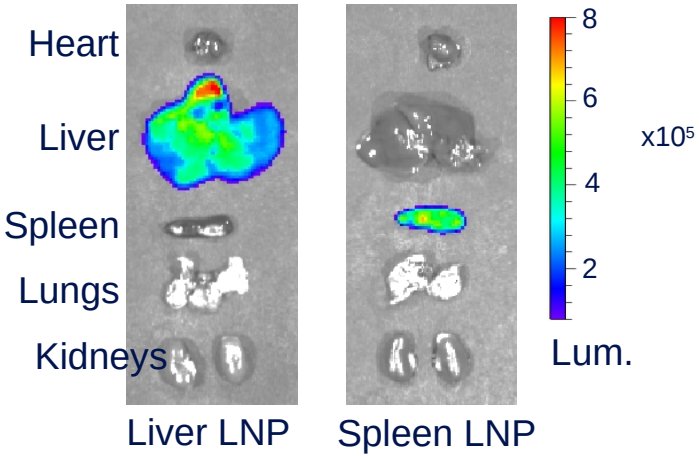
LNPs Targeting Immune-Rich Spleen



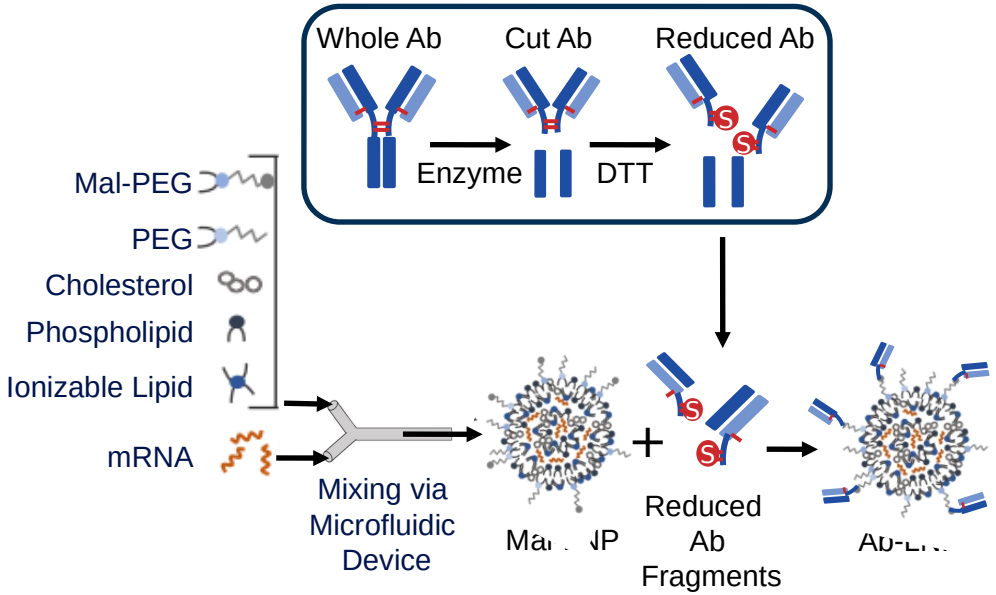
Billingsley, June, Mitchell et al. *Accepted*

Making CAR T Cells and Macrophages in the Body via mRNA LNPs

LNPs Targeting Immune-Rich Spleen



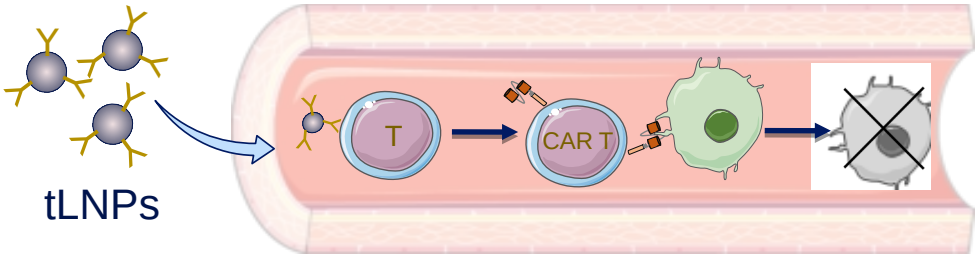
LNP Targeting via Functionalization with Antibody Fragments



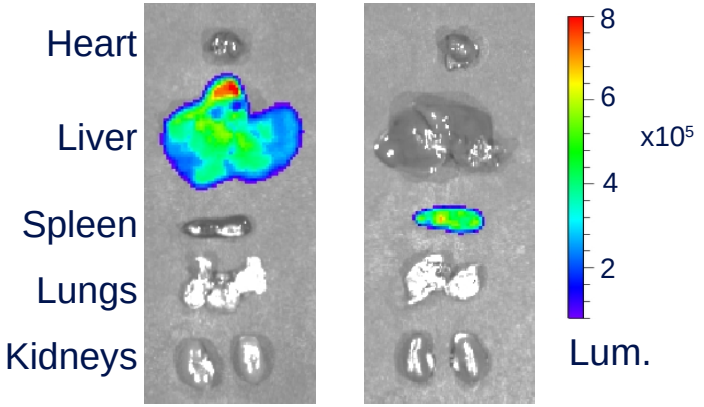
Billingsley, June, Mitchell et al. *Accepted*

Making CAR T Cells and Macrophages in the Body via mRNA LNPs

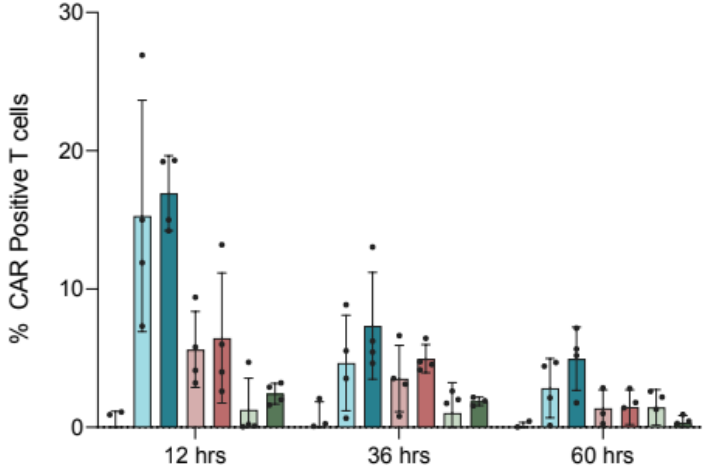
In Situ CAR T Cell Engineering



LNPs Targeting Immune-Rich Spleen



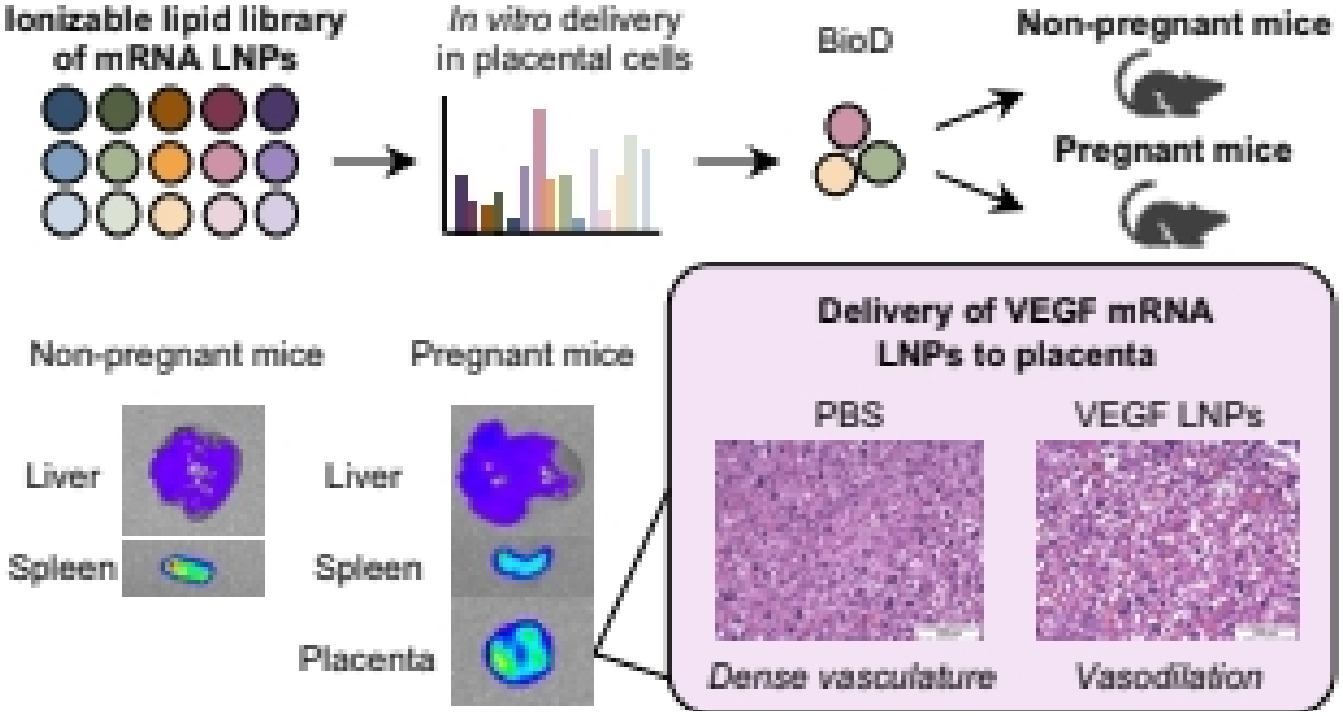
tLNPs for T Cell-Specific mRNA Delivery



Injectable LNP Therapeutic to Bypass Laborious *Ex Vivo* Cell Engineering

Billingsley, June, Mitchell et al. *Accepted*

Maternal Health: One Shot mRNA LNP Cure for Preeclampsia

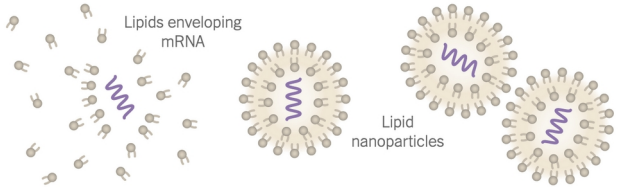


Kelsey Swingle
Penn BE PhD Student

Swingle, Weissman, Mitchell et al. *J Am Chem Soc* (2023)

Scalability Challenge of Formulating mRNA LNPs

A rack of 16 pumps precisely controls the flow of the mRNA and lipid solutions, then mixes them together to create lipid nanoparticles.



When the lipids come into contact with the naked strands of mRNA, electric charge pulls them together in a nanosecond. The mRNA is enveloped in several layers of lipids, forming an oily, protective vaccine particle.

Synchronizing eight pairs of pumps is not an ideal solution, but Pfizer engineers chose to scale up existing technology instead of trying to build a larger, unproven type of precision mixing device.

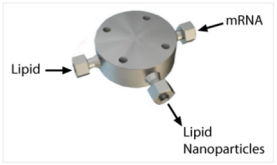
The newly made vaccine is filtered to remove the ethanol, concentrated and filtered again to remove any impurities, and finally sterilized.

Knauer develops Impingement Jet Mixing Technology for the production of mRNA-filled nanoparticles.

APRIL 20, 2021 HPLC SYSTEMS

Knauer has developed a technique for mass-producing lipid nanoparticles (LNPs) using impingement jet mixing technology (IJM). LPNs have been shown to be a suitable delivery form for the development of mRNA-based vaccines against Corona, and is at present being used to produce millions of vaccination doses a week by Pfizer. This technique has been shown to form a stable basis for the administration of active ingredients such as RNA, mRNA, siRNA, or DNA-based APIs.

The impingement jet technology uses a mixer which forces lipids into a small chamber under pressure, against an opposite flow of active ingredient, such as mRNA, also forced in under pressure. The active nanoparticles that are produced flow out of the third outlet.



Impingement Jet Mixer

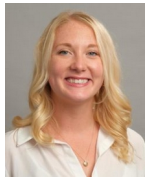
The Knauer pumps used for mass-producing LNPs is the BlueShadow 80P; either the 100, 250 or 500 ml/min models, all with titanium fluid paths. Multiples of these pumps can be utilized at the same time, depending upon the output required. Knauer's IJM skid for LNP production incorporates sixteen 8oP pumps, eight for the lipid injection and eight for the mRNA injection.

BlueShadow Pump 80P

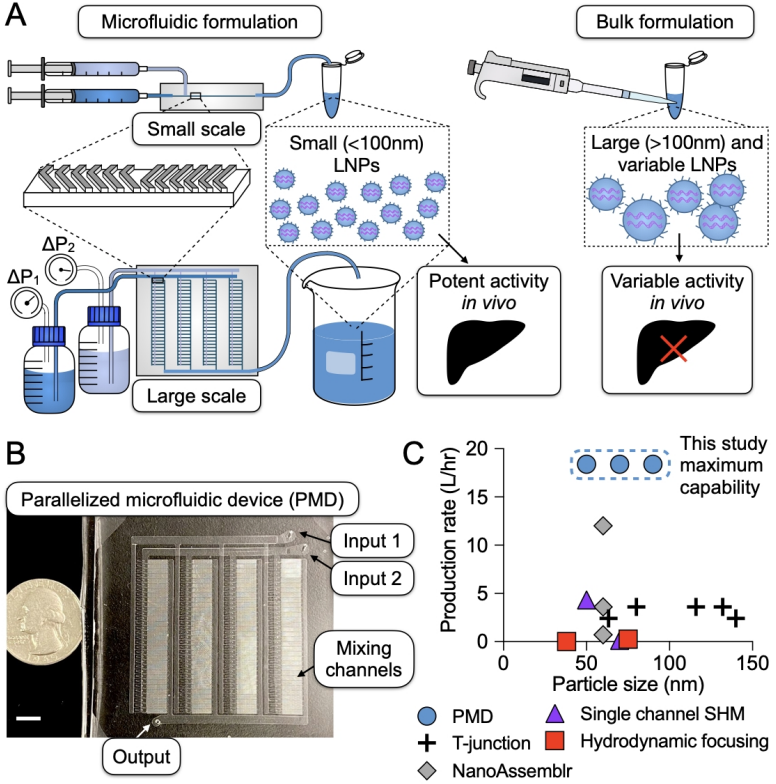
Can we develop new tech to formulate mRNA LNP vaccines and therapeutics at industrial scale?

Parallelized Microfluidic Tech to Scaleup mRNA LNPs

- Microfluidics: small, homogeneous LNPs
- Challenge: not scalable, pressure issues
- Bulk mixing: used in industry, results in larger, heterogeneous LNPs
- Filtration required, large number of empty LNPs
- **Solution: parallelized microfluidic device (PMD) for clinical scale LNP formulations**



Sarah "Scaleup" Shepherd, PhD
Penn BE PhD Student, Mitchell/Issadore Labs

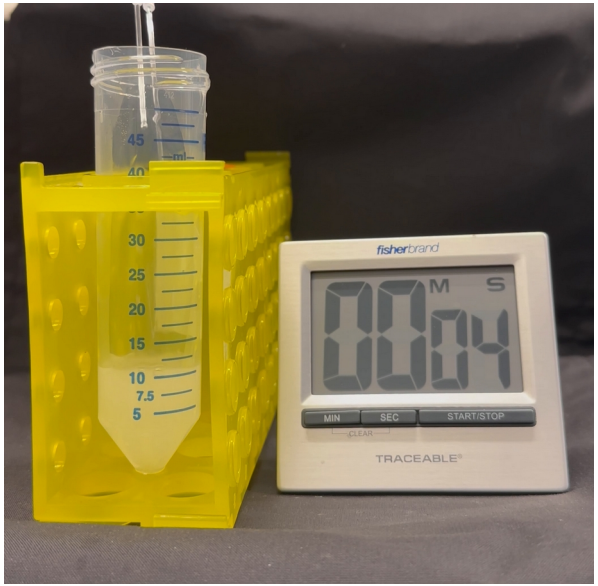


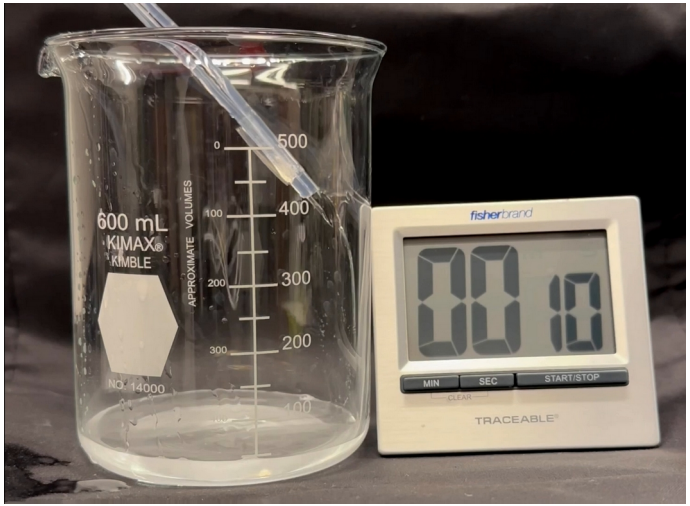
Shepherd, Issadore, Mitchell et al, *Nano Letters* (2021)
Shepherd, Issadore, Mitchell et al, *PNAS* (2023)

Formulation of LNPs at 15 L/hr with SCALAR 256x Chip

Experimental setup

Total volumetric throughput ~ 15 L/hr





New technology to address scaleup challenge of mRNA nanoparticle therapeutics

Acknowledgments

Mitchell Lab

Rachel Riley, PhD
Rui Zhang, PhD
Pedro Guimaraes, PhD
Ningqiang Gong, PhD
Xuexiang Han, PhD
Lulu Xue, PhD
Jingya Qin, PhD
Marshall Padilla, PhD
Dongyoon Kim, PhD
Junchao Xu, PhD
Il-Chul Yoon, PhD
Jeongeun Shin, PhD
Adele Ricciardi, MD PhD
Ye Zeng, PhD
Jinjin Wang, PhD
Maggie Billingsley, PhD
Zain Siddiqui, PhD
Qiangqiang Shi, PhD
Sarah Shepherd, PhD
Alvin Mukalel
Rebecca Haley
Chris Figueroa-Espada
Rohan Palanki
Alex Hamilton
Kelsey Swingle
Ann Metzloff
Hannah Geisler
Hannah Safford
Ajay Thatte
Emily Han
Andrew Hanna
Hannah Yamagata
Amanda Murray
Briyanna Hymms
Rakan El-Mayta
Ella Atsavapranee
Jingcheng Xu
Savan Patel
Emily Kim
Jacqueline Li
Kaitlin Mrksich
Aditi Ghalsasi
Ryann Joseph
Sridatta Teerdhala
Ben Nachod

Collaborators

Drew Weissman, MD PhD
Carl June, MD
Jim Wilson, MD PhD
David Issadore, PhD
Karin Wang, PhD
Fanxin Long, PhD
William Peranteau, PhD
Andrew Tsourkas, PhD

Acknowledgments

Mitchell Lab
Rachel Riley, PhD
Rui Zhang, PhD
Pedro Guimaraes, PhD
Ningqiang Gong, PhD
Xuexiang Han, PhD
Lulu Xue, PhD
Jingya Qin, PhD
Marshall Padilla, PhD
Dongyoon Kim, PhD
Junchao Xu, PhD
Il-Chul Yoon, PhD
Jeongeun Shin, PhD
Adele Ricciardi, MD PhD
Ye Zeng, PhD
Jinjin Wang, PhD
Maggie Billingsley, PhD
Zain Siddiqui, PhD
Qiangqiang Shi, PhD
Sarah Shepherd, PhD
Alvin Mukalel
Rebecca Haley
Chris Figueroa-Espada
Rohan Palanki
Alex Hamilton
Kelsey Swingle
Ann Metzloff
Hannah Geisler
Hannah Safford
Ajay Thatte
Emily Han
Andrew Hanna
Hannah Yamagata
Amanda Murray
Briyanna Hymms
Rakan El-Mayta
Ella Atsavapranee
Jingcheng Xu
Savan Patel
Emily Kim
Jacqueline Li
Kaitlin Mrksich
Aditi Ghalsasi
Ryann Joseph
Sridatta Teerdhala
Ben Nachod

Collaborators
Drew Weissman, MD PhD
Carl June, MD
Jim Wilson, MD PhD
David Issadore, PhD
Karin Wang, PhD
Fanxin Long, PhD
William Peranteau, PhD
Andrew Tsourkas, PhD

Email: mjmitch@seas.upenn.edu