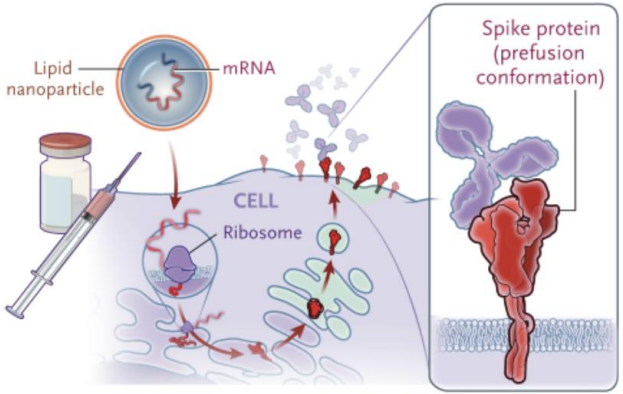
# mRNA Lipid Nanoparticles for Treating Disease Before Birth

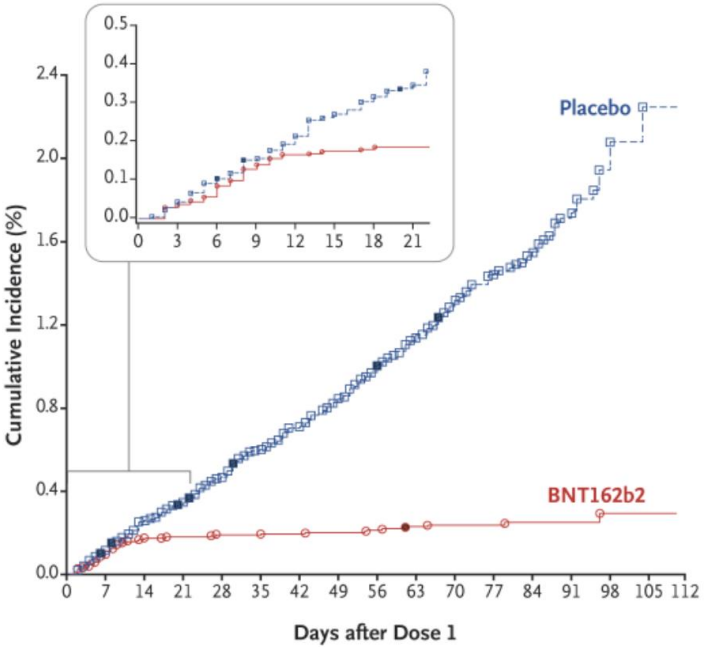
**Michael J. Mitchell, PhD**
Skirkanich Assistant Professor of Innovation
Department of Bioengineering, University of Pennsylvania
mitchell-lab.seas.upenn.edu
@MJMitchell_Lab

Penn Engineering

# Lipid Nanoparticle (LNP) COVID-19 mRNA Vaccines



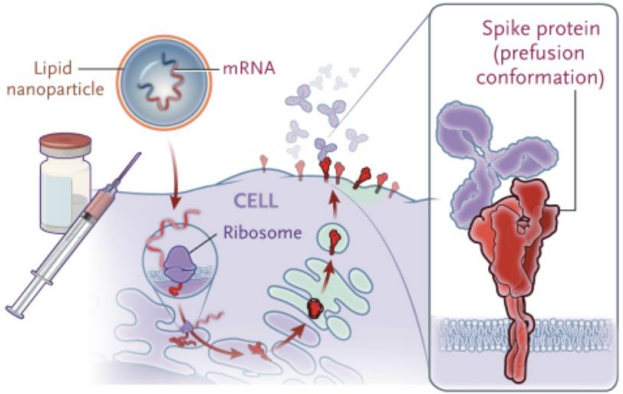

Interdisciplinary Science Feat: RNA and LNPs





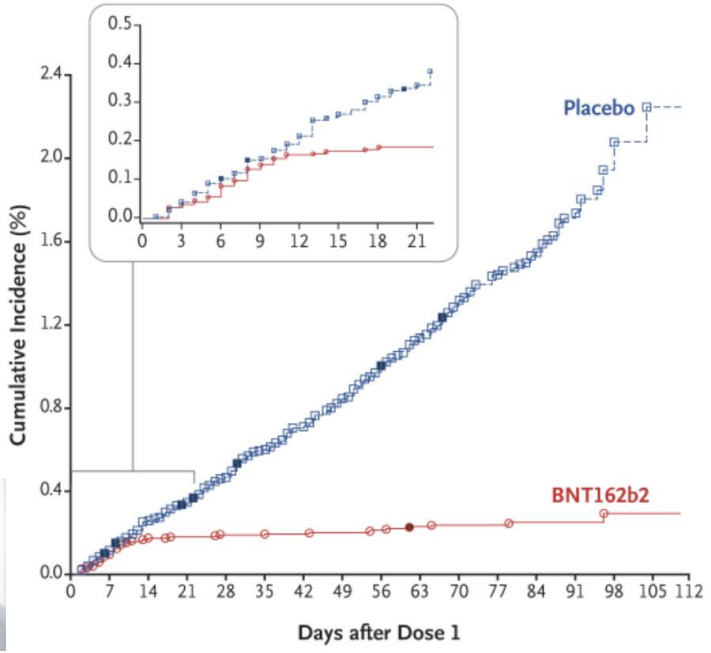

F.P. Polack et al. *New England Journal of Medicine* (2020).

# Lipid Nanoparticle (LNP) COVID-19 mRNA Vaccines




Interdisciplinary Science Feat: RNA and LNPs

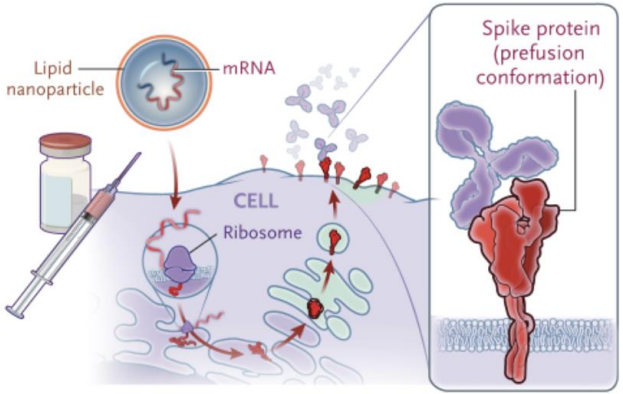
And Many Others!





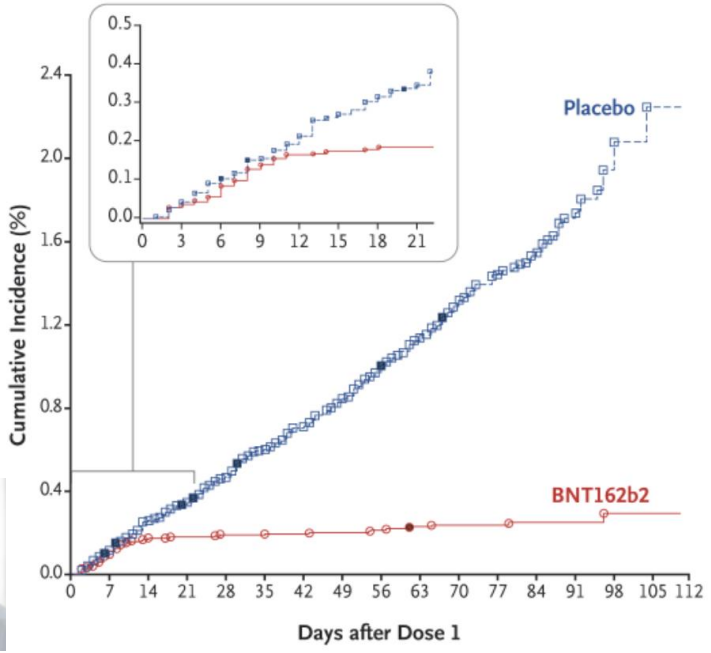

F.P. Polack et al. *New England Journal of Medicine* (2020).

# Lipid Nanoparticle (LNP) COVID-19 mRNA Vaccines




Interdisciplinary Science Feat: RNA and LNPs

And Many Others!




**Can mRNA LNP tech revolutionize pharmaceuticals? Key bottleneck: materials for delivery**



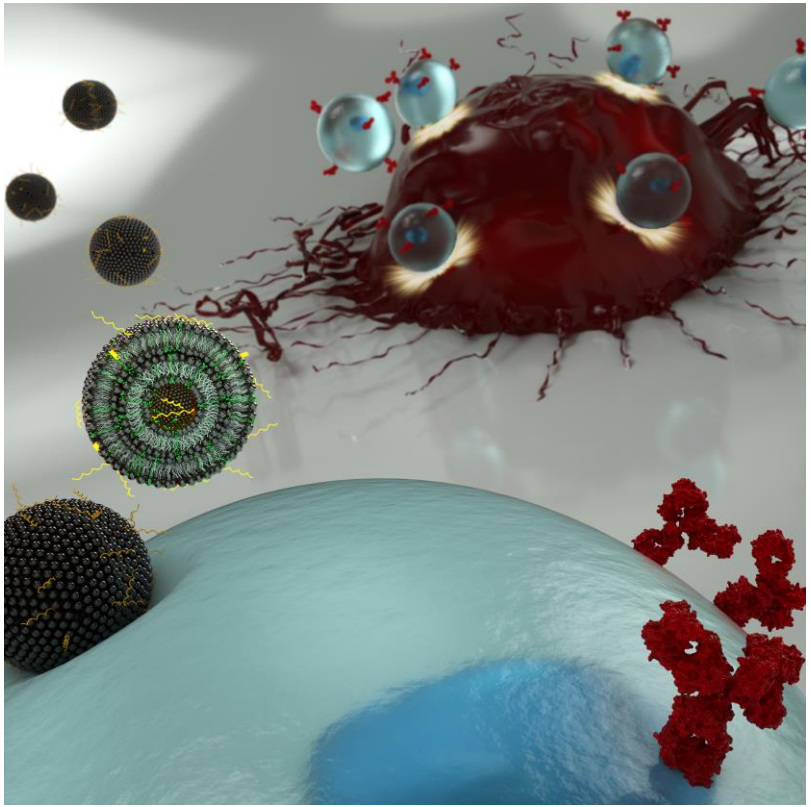
F.P. Polack et al. *New England Journal of Medicine* (2020).

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

**Cancer Immunotherapy**

**Lipid Nanoparticles (LNPs)**

**Genetic Engineering of Immune Cells in Body**

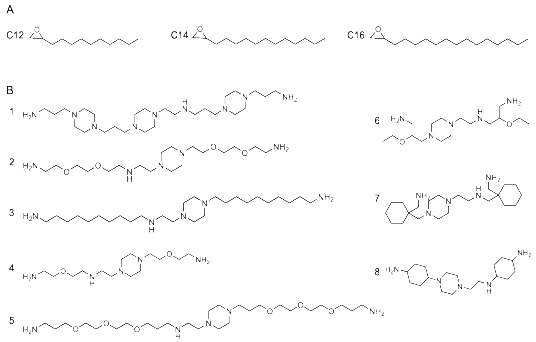


**Delivery Technologies Beyond Vaccines to Target:**

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

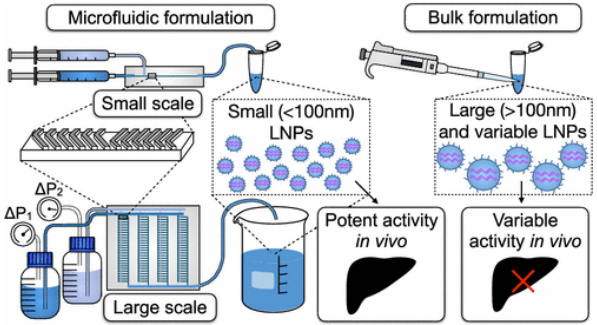
# Mitchell Lab: LNP Toolbox for Nucleic Acid Delivery

**Combinatorial Chemistry**
Synthesize Ionizable Lipids and Polymers



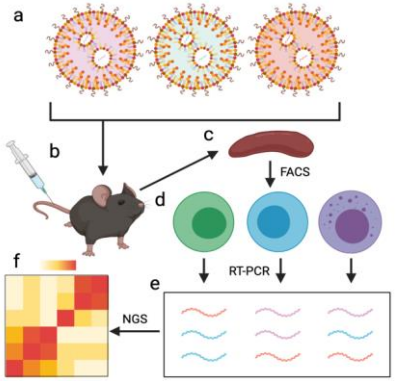

R.S. Riley et al. *Science Advances* (2021)
M.M. Billingsley et al. *Nano Letters* (2020)

**Microfluidics** for Controlled LNP Formulation




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

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




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

**Accelerate the design and identification of LNPs for unlocking delivery to new cell and tissue types**

# LNPs for *In Utero* Therapy – Treating Disease Before Birth

## Bill Peranteau (CHOP)

**Can we use genome editing to edit out deadly mutations *in utero* before a baby is born?**

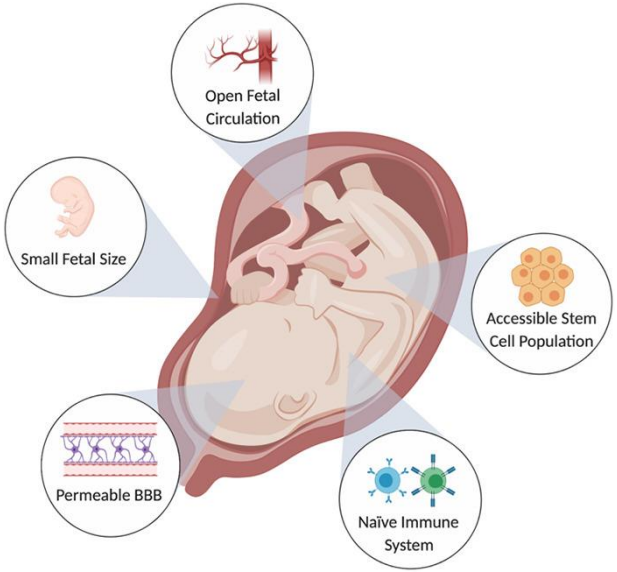


Palanki, Pereanteau, Mitchell. *Adv Drug Deliv Rev* (2021)

# LNPs for *In Utero* Therapy – Treating Disease Before Birth

## Bill Peranteau (CHOP)

**Can we use genome editing to edit out deadly mutations *in utero* before a baby is born?**

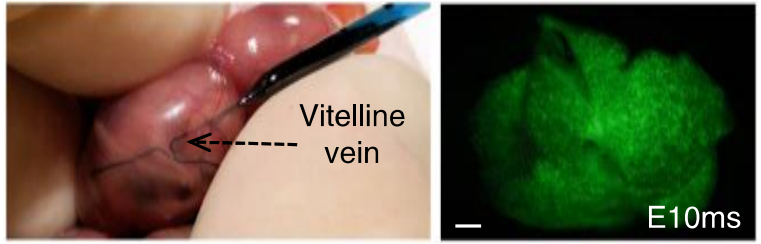



**Benefits of *in utero* delivery:**

- Uptake into progenitor cells during rapid cell division
- Circumvents adverse immune responses due to the immature immune system
- Allows for treatment prior to the onset of irreversible pathology

# *In Utero* CRISPR-Mediated Therapeutic Editing of Metabolic Genes

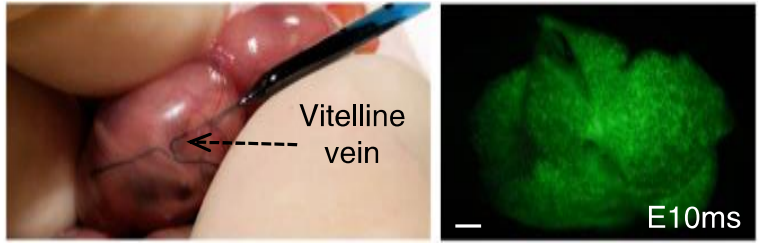
Adenoviral vectors transfect hepatocytes *in utero* on embryonic day 16 (E16)



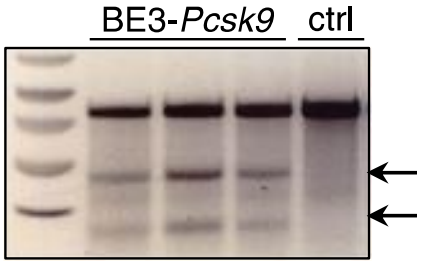

- **Hereditary tyrosinemia 1:** results in mutated Fah gene, lethal liver failure upon birth
- Introducing nonsense mutation in Hpd gene *in utero* permanently knocks out gene function, rescues lethal liver failure
- ***In utero* editing can potentially prevent death of newborns with deadly mutations**

# *In Utero* CRISPR-Mediated Therapeutic Editing of Metabolic Genes

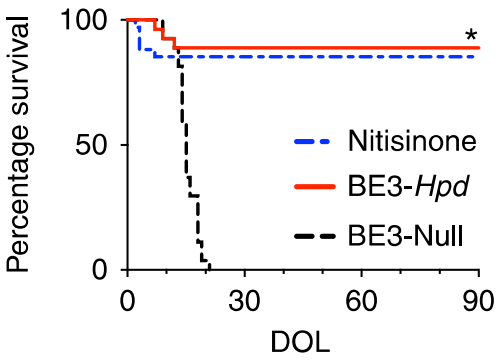
Adenoviral vectors transfect hepatocytes *in utero* on embryonic day 16 (E16)




Newborn mice exhibit editing of Pcsk9, reduced cholesterol levels




- **Hereditary tyrosinemia 1:** results in mutated Fah gene, lethal liver failure upon birth
- Introducing nonsense mutation in Hpd gene *in utero* permanently knocks out gene function, rescues lethal liver failure
- ***In utero* editing can potentially prevent death of newborns with deadly mutations**

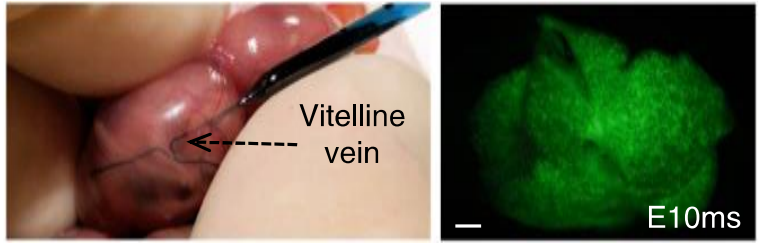




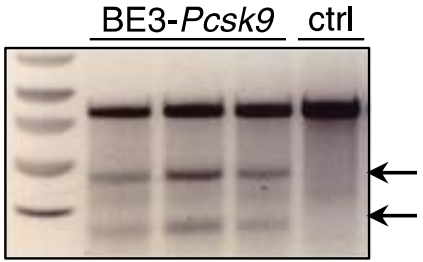

Rossidis, Musunuru, Peranteau et al. *Nature Medicine* (2018)

# *In Utero* CRISPR-Mediated Therapeutic Editing of Metabolic Genes

Adenoviral vectors transfect hepatocytes *in utero* on embryonic day 16 (E16)




Newborn mice exhibit editing of Pcsk9, reduced cholesterol levels



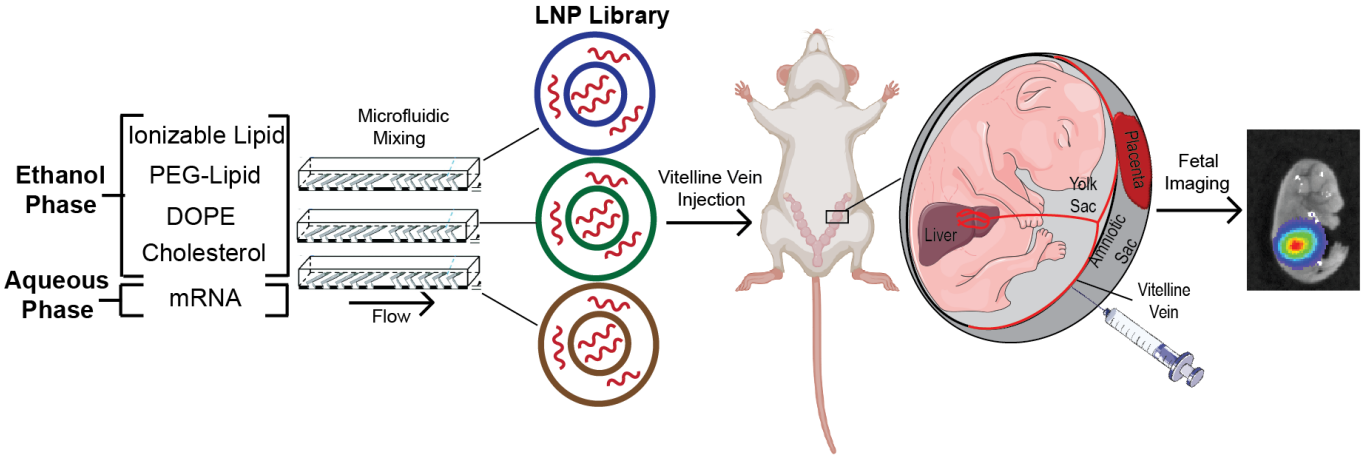

**Challenges:**

- Base editing constructs are large (~5.1kb) cannot be delivered using AAVs (~4.7kb capacity)
- Adenoviral (Ad) vectors are used as POC
- **Ad vectors are limited for clinical translation, due to potential adverse host immune responses, systemic toxicity**



Rossidis, Musunuru, Peranteau et al. *Nature Medicine* (2018)

# mRNA LNPs for Treating Disease Before Birth (*In Utero*)

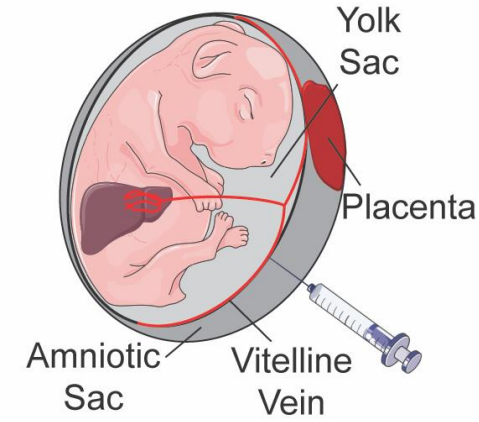



**Can we engineer mRNA LNPs to edit out deadly mutations *in utero* before a child is born?**

**Dr. Rachel Riley**
NIH Postdoctoral Fellow, Penn BE
Now: Assistant Professor of BME, Rowan U

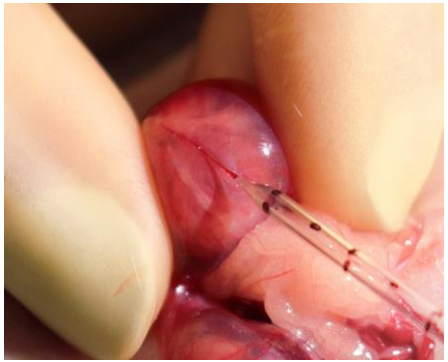
**Dr. Bill Peranteau**
Children's Hospital of Philadelphia (CHOP)

# mRNA LNPs for Treating Disease Before Birth (*In Utero*)




**Benefits of *in utero* delivery**

- Uptake into progenitor cells during rapid cell division
- Immature immune system
- Circumvent adverse immune responses
- Allows for treatment prior to the onset of irreversible pathology



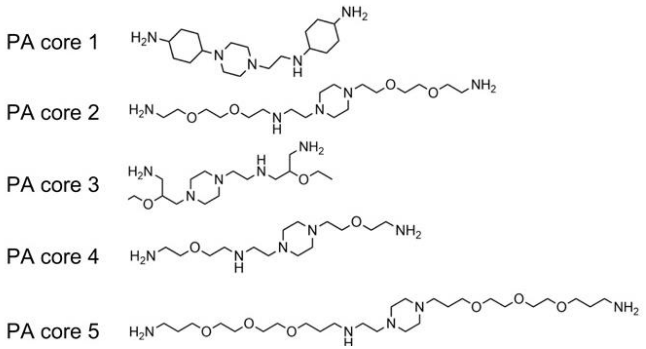
**Routes of administration**

- Vitelline vein injection: liver, kidney, spleen, heart
- Intra-amniotic injection: lungs, GI system
- Intraventricular injection: CNS delivery

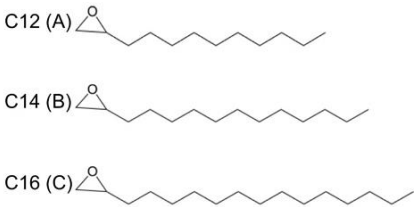
# mRNA LNPs for Treating Disease Before Birth (*In Utero*)

## Ionizable Lipid Combinatorial Library

Amine cores:

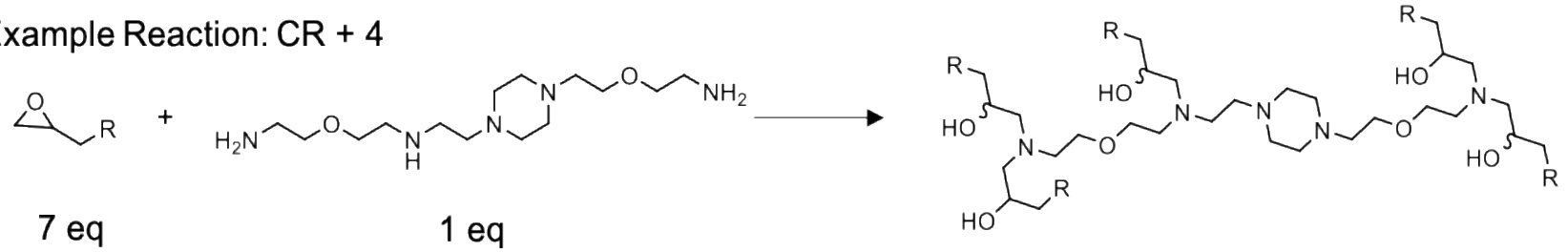



Alkyl tails:



# High-Throughput Synthesis of Ionizable Lipid-Like Materials to Engineer LNPs

Example Reaction: CR + 4



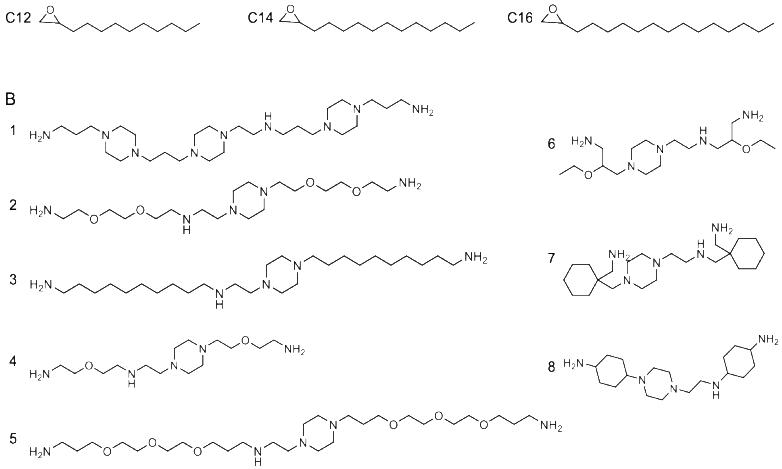

**Synthesis of ionizable lipid-like biomaterials**

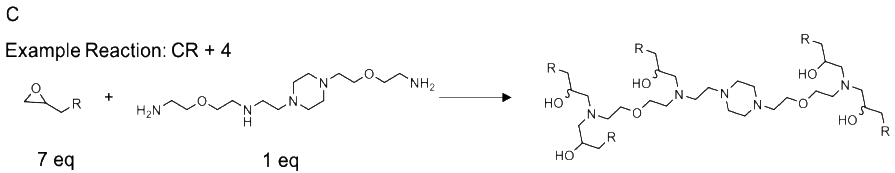
- Facile Michael addition chemistry – high heat, stir
- Reactions done in absence of solvents, catalysts
- Eliminates need for chemical protection/deprotection steps
- Minimal purification, concentration
- Therefore → enables high-throughput synthesis




Billingsley, Singh, Ravikumar, June, Mitchell, *Nano Letters*, 2020
Billingsley, Hamilton, June, Mitchell, *Nano Letters*, 2021

# High-Throughput Synthesis of Ionizable Lipid-Like Materials to Engineer LNPs



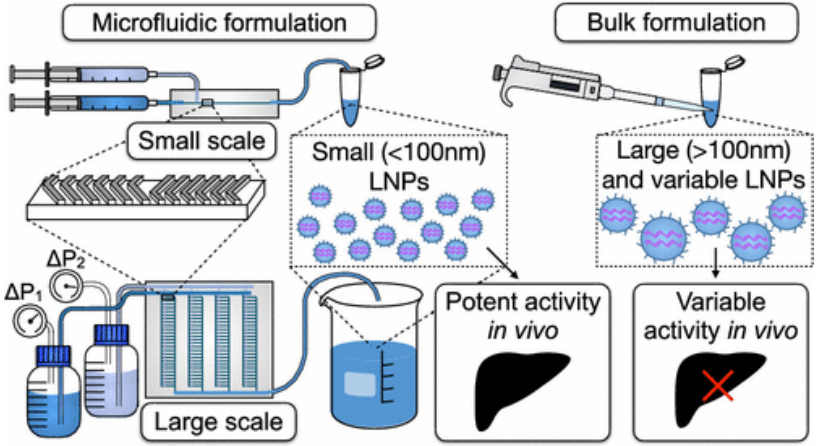

## Generation of large libraries of materials

- Addition of amines with alkyl-acrylates or alkyl-acrylamides
- Lipid-like materials can be formed with 1-7 alkyl tails
- Structurally diverse




Billingsley, Singh, Ravikumar, June, Mitchell, *Nano Letters*, 2020
Billingsley, Hamilton, June, Mitchell, *Nano Letters*, 2021

# Microfluidics for Controlled LNP Formulation





## Advantages

- Rapid (seconds) assembly and formulation of RNA and lipids into LNPs
- Smaller (~60-90 nm), low PDI LNPs
- PDMS or silicon and glass-based devices
- Small and **large-scale** (> 1L/hr) production of LNPs

**Sarah "Scaleup" Shepherd**
Penn BE PhD Student

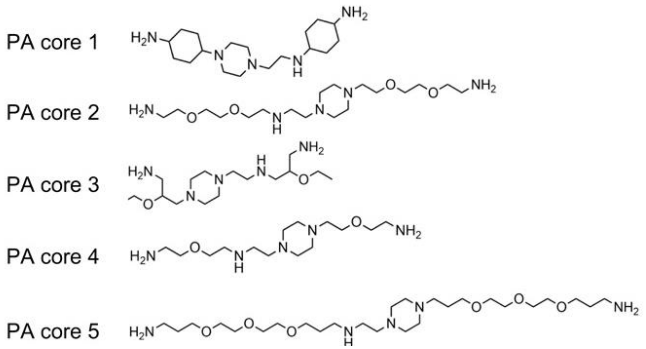


S.J. Shepherd, Issadore, Mitchell et al, *Nano Letters*, 2021

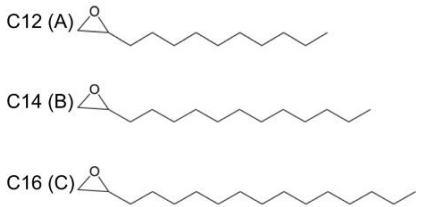
# mRNA LNPs for Treating Disease Before Birth (*In Utero*)

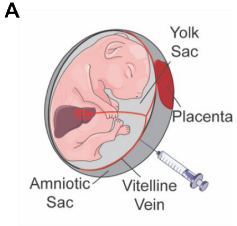
## Ionizable Lipid Combinatorial Library

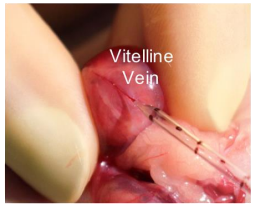
Amine cores:

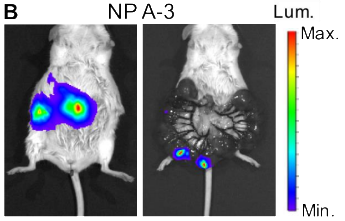



Alkyl tails:



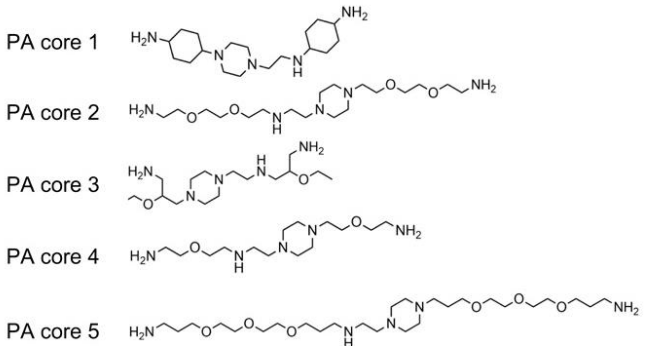



Riley, Billingsley, Peranteau, Mitchell et al. *Science Advances* (2021)

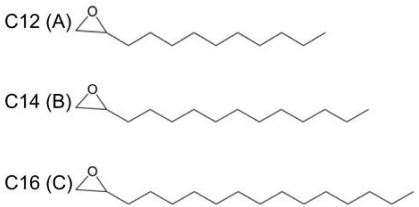
# mRNA LNPs for Treating Disease Before Birth (*In Utero*)

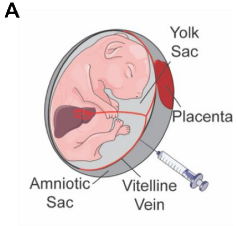
## Ionizable Lipid Combinatorial Library

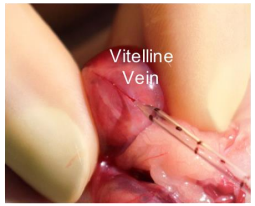
Amine cores:

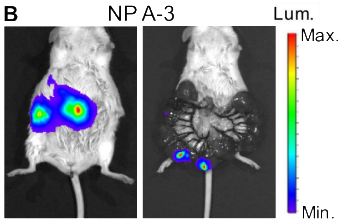


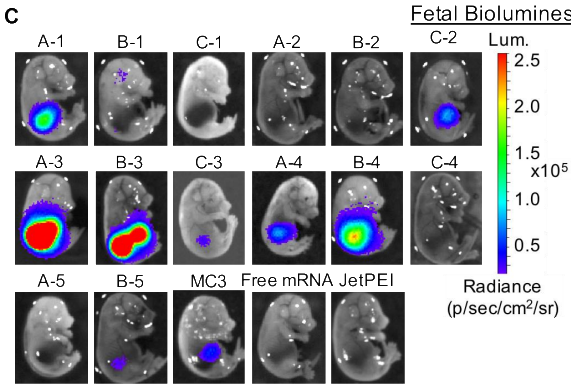

Alkyl tails:

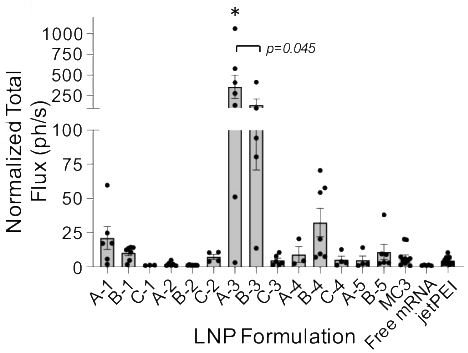


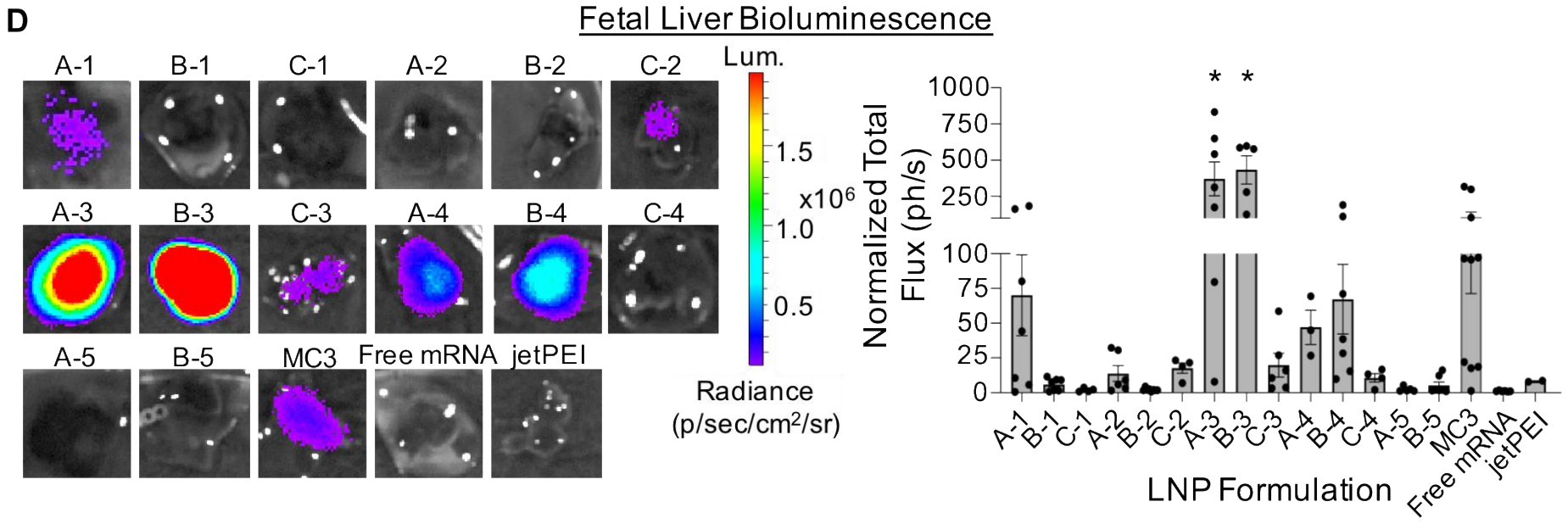




**Identification of new LNPs for *in utero* mRNA delivery that outperform gold-standard LNPs**

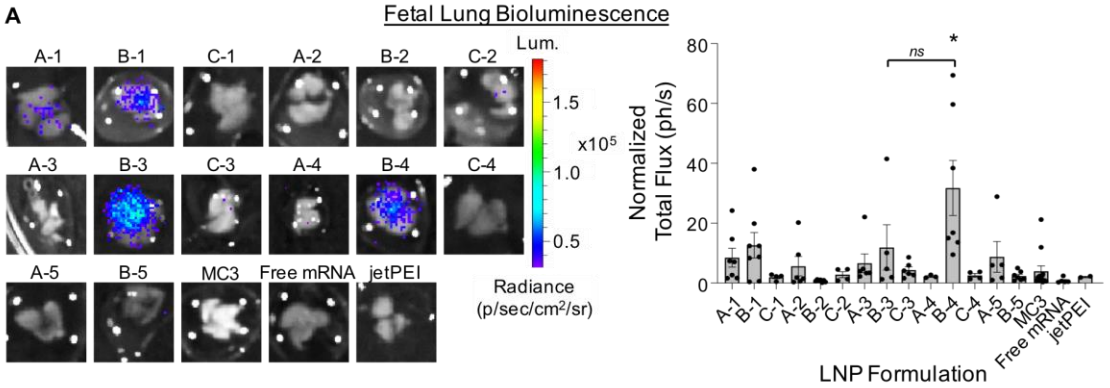


Riley, Billingsley, Peranteau, Mitchell et al. *Science Advances* (2021)

# mRNA LNPs Primarily Deliver to Fetal Liver



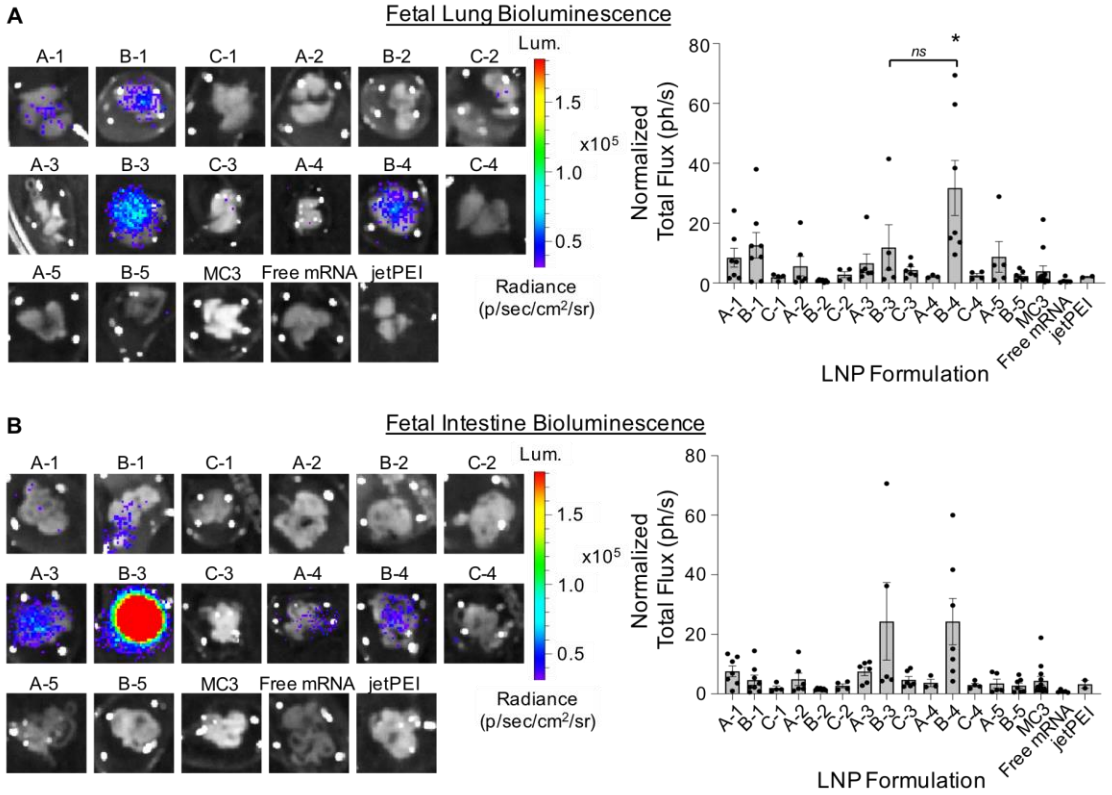
# *In Utero* mRNA LNP Delivery to Fetal Lung and Intestine






Riley, Billingsley, Peranteau, Mitchell et al. *Science Advances* (2021)

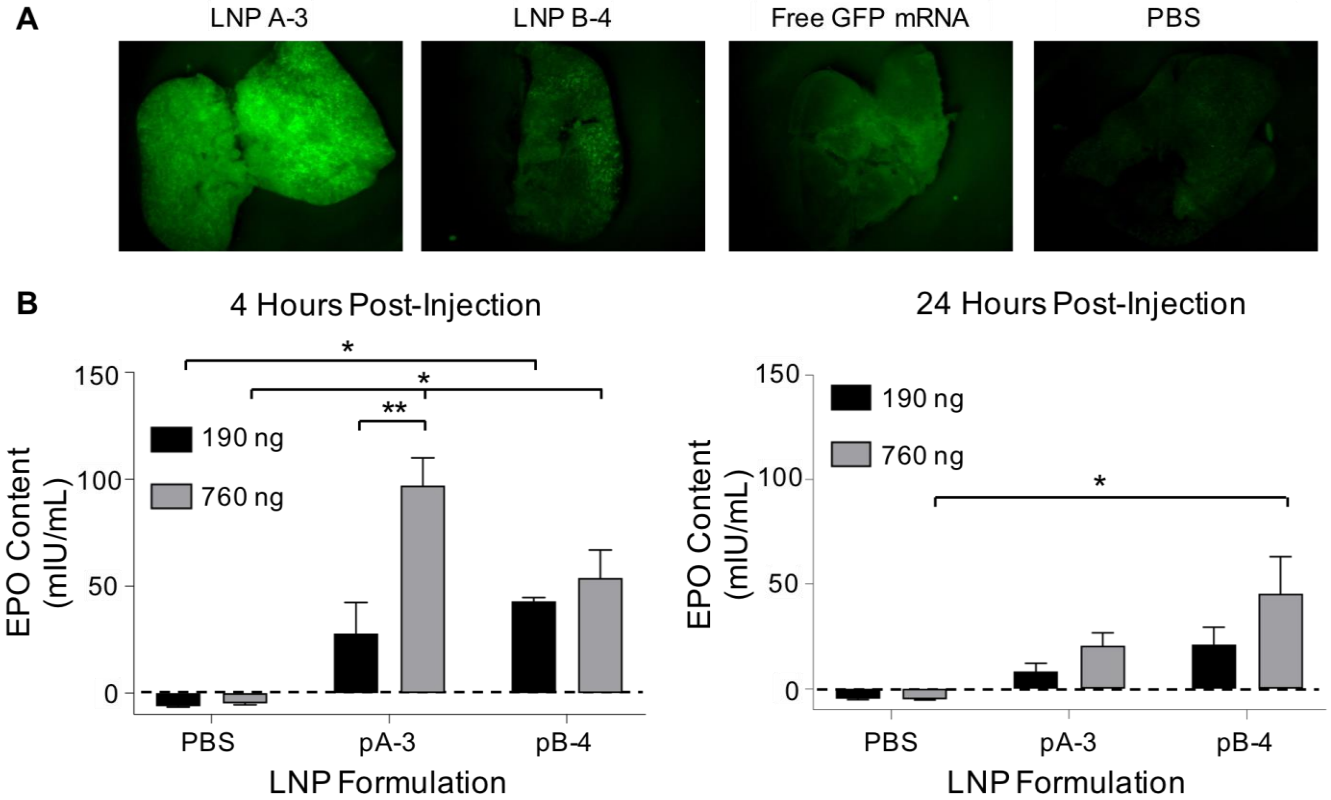
# *In Utero* mRNA LNP Delivery to Fetal Lung and Intestine






Riley, Billingsley, Peranteau, Mitchell et al. *Science Advances* (2021)

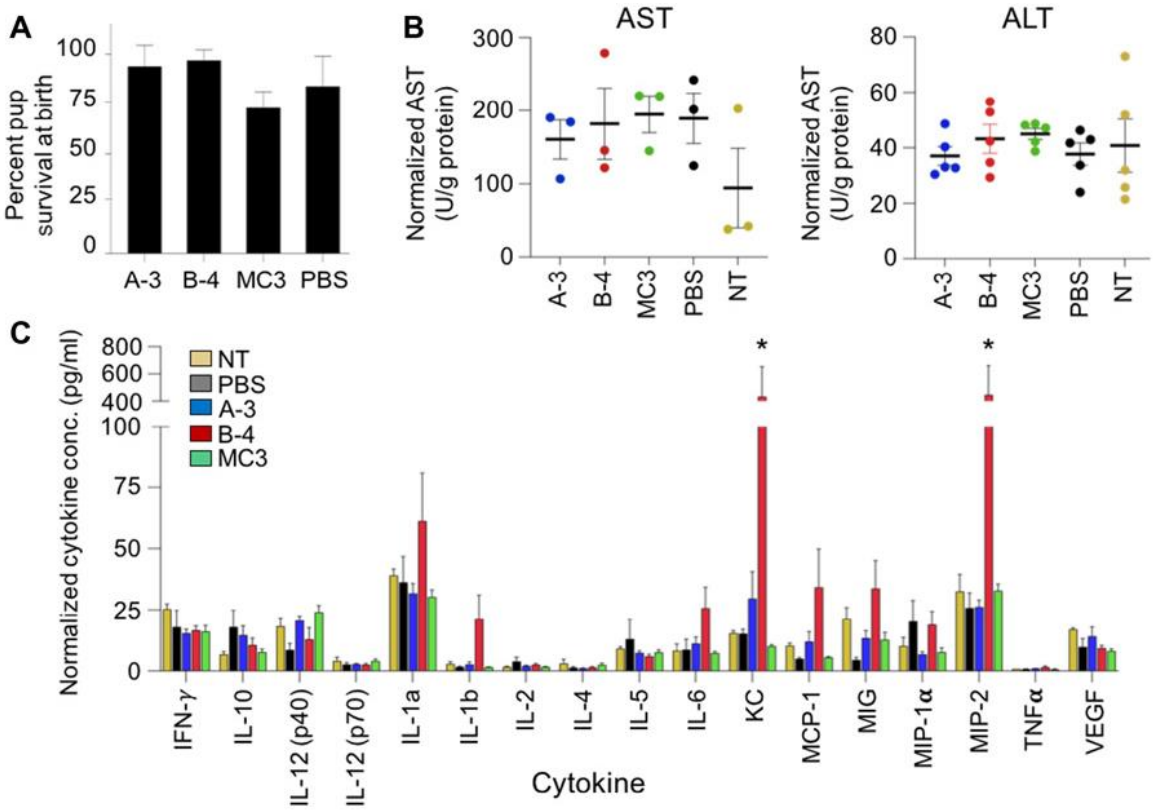
# LNPs Deliver Erythropoietin mRNA *In Utero* as a Model Therapeutic






Riley, Billingsley, Peranteau, Mitchell et al. *Science Advances* (2021)

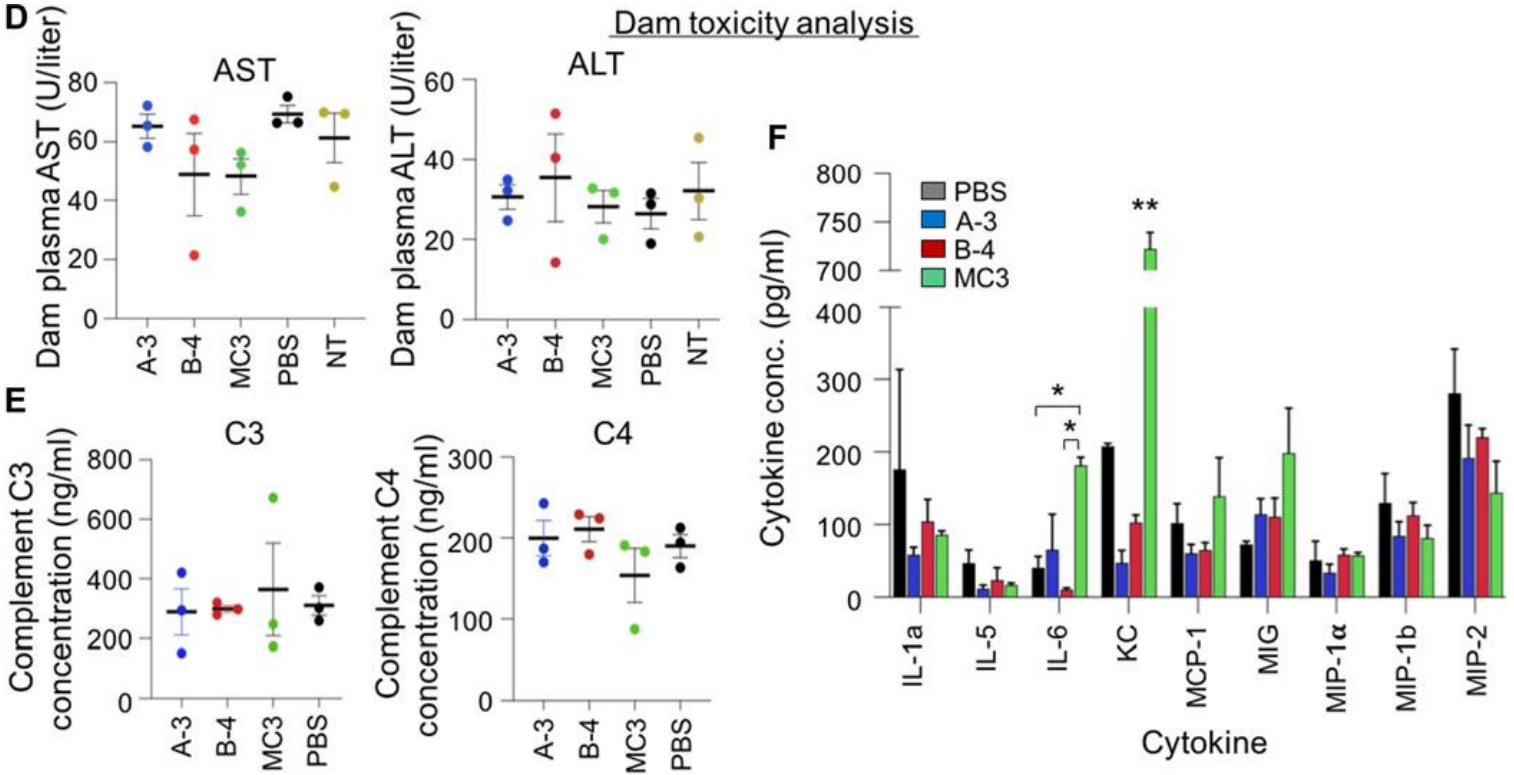
# LNPs Well Tolerated by Fetus and Dam






Riley, Billingsley, Peranteau, Mitchell et al. *Science Advances* (2021)

# LNPs Well Tolerated by Fetus and Dam

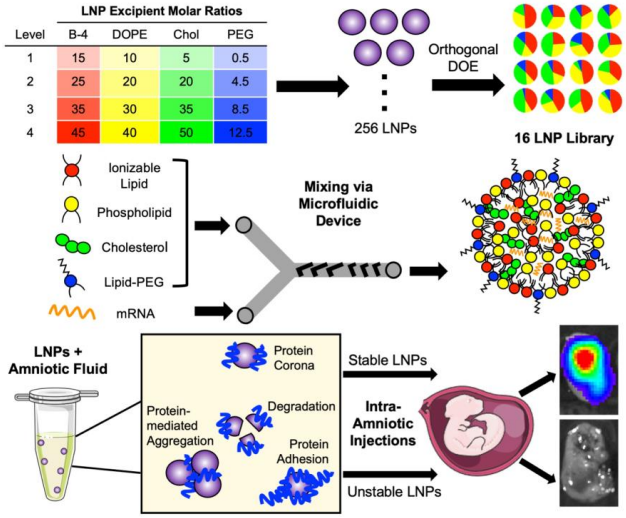





Riley, Billingsley, Peranteau, Mitchell et al. *Science Advances* (2021)

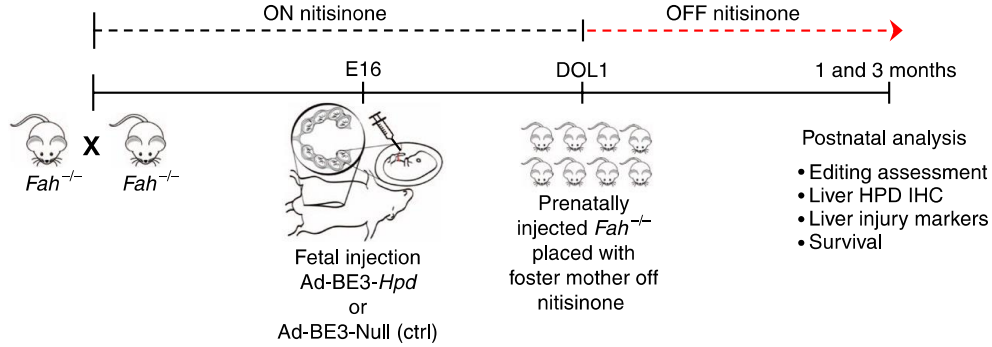
# Intra-amniotic *In Utero* mRNA Delivery and Gene Editing

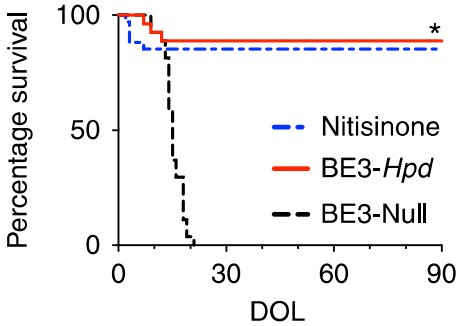
## Intra-amniotic mRNA LNP Delivery




**Kelsey Swingle**
Penn BE PhD Student

## *In Utero* Gene Editing



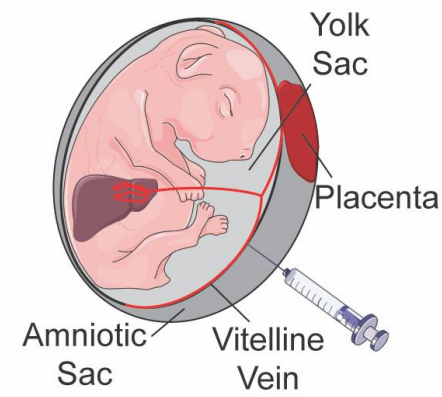

**Ongoing studies in neonatal and *in utero* gene editing of liver, lung, brain**




Rossidis, Peranteau et al. *Nature Medicine* (2021)
Swingle, Billingsley, Mitchell et al. *J Controlled Release* (2022)

# Intra-amniotic *In Utero* mRNA Delivery

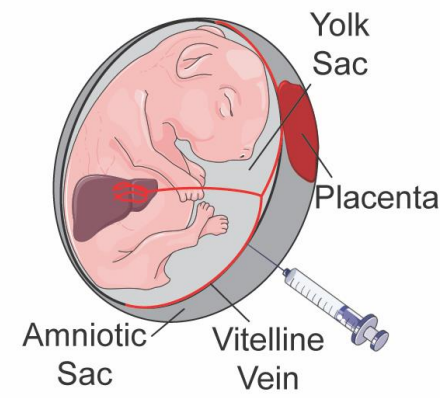
**Intra-amniotic Delivery**




**Kelsey Swingle**
Penn BE PhD Student

# Intra-amniotic *In Utero* mRNA Delivery

## Intra-amniotic Delivery




**Kelsey Swingle**
Penn BE PhD Student

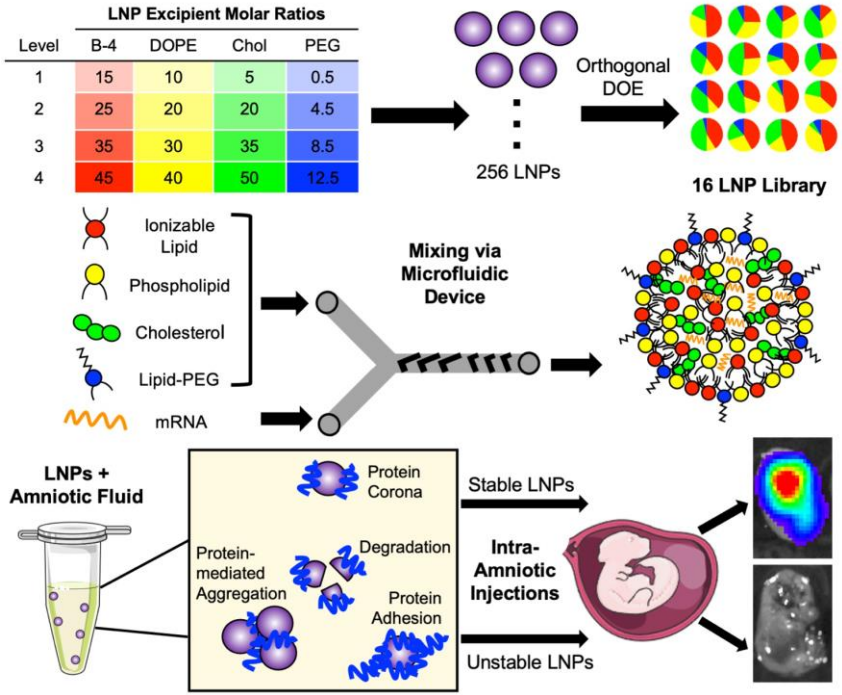
## Amniotic Fluid LNP Injections

## Advantages

- Clinically translatable route of delivery
- Minimally invasive, image guided
- Protein replacement and enzyme therapeutics
- LNP ingestion by fetus
- Access to lungs, stomach, GI tract

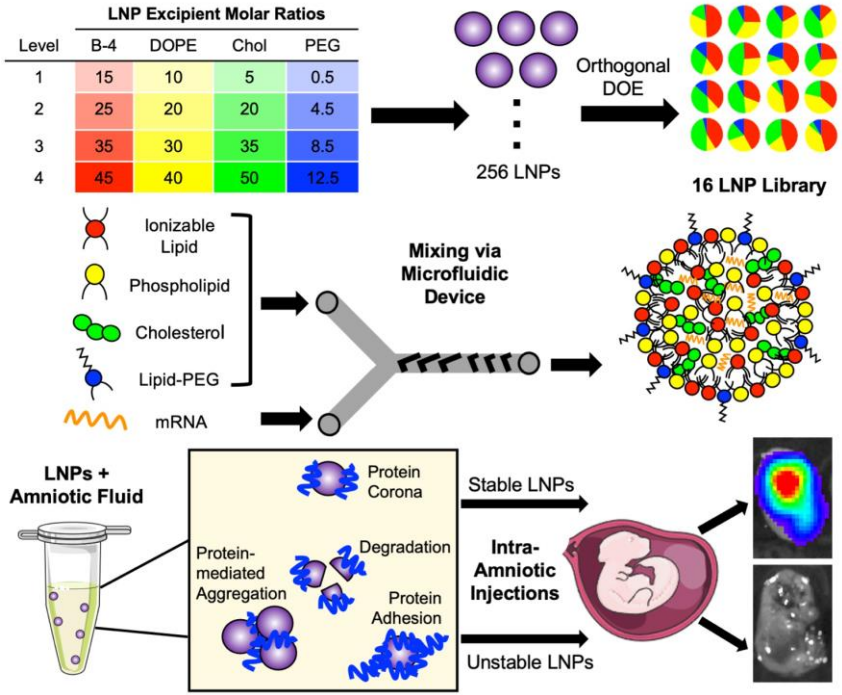
# Intra-amniotic *In Utero* mRNA Delivery

## *Ex Utero* Amniotic Fluid LNP Stability

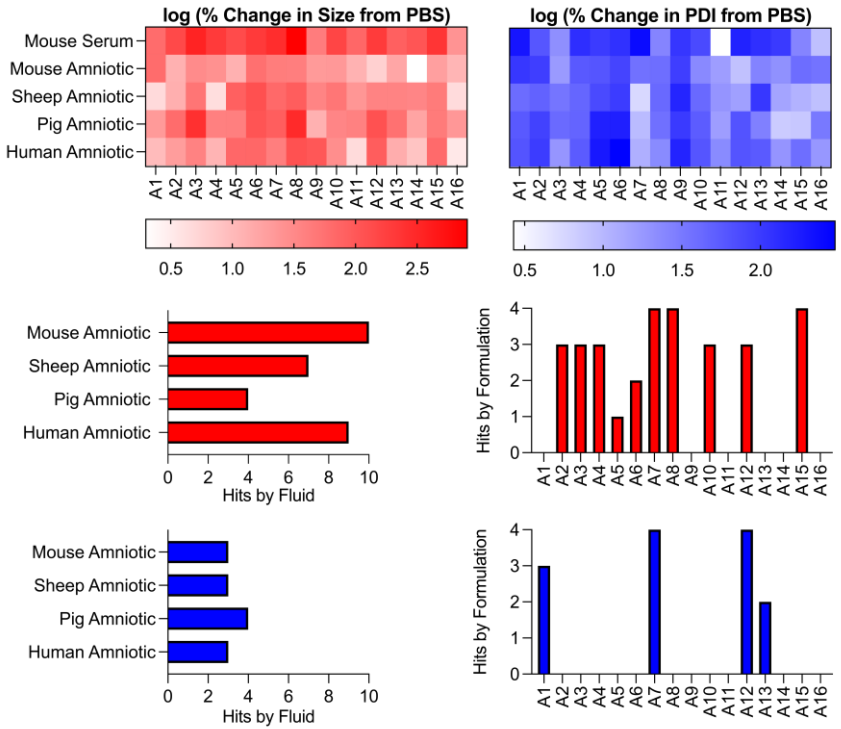


# Intra-amniotic *In Utero* mRNA Delivery

## *Ex Utero* Amniotic Fluid LNP Stability




## LNPs More Stable in Amniotic Fluid than Serum

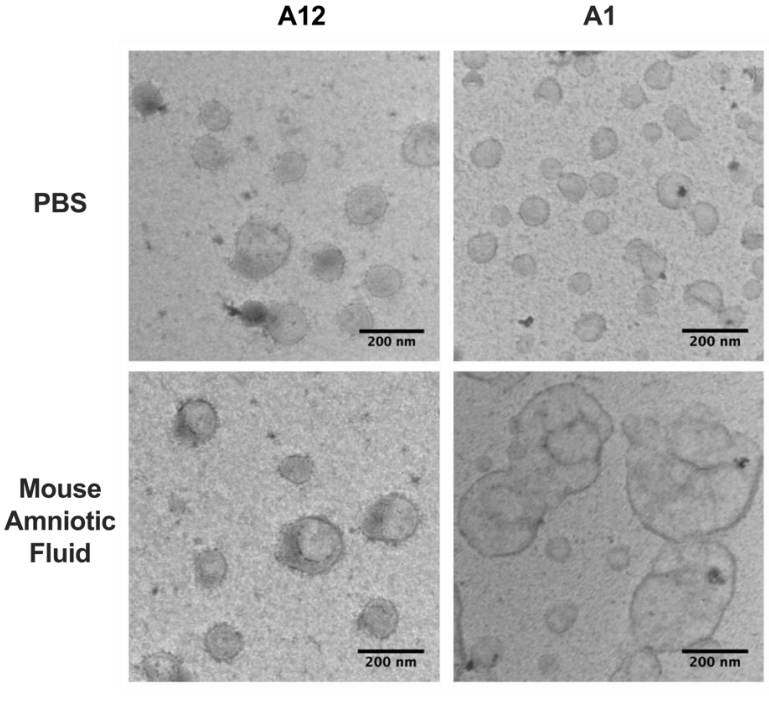





Swingle, Billingsley, Mitchell et al. *J Controlled Release* (2022)

# *Ex Utero* Assay Identifies LNPs for *In Utero* mRNA Delivery

## TEM of mRNA LNPs in Amniotic Fluid

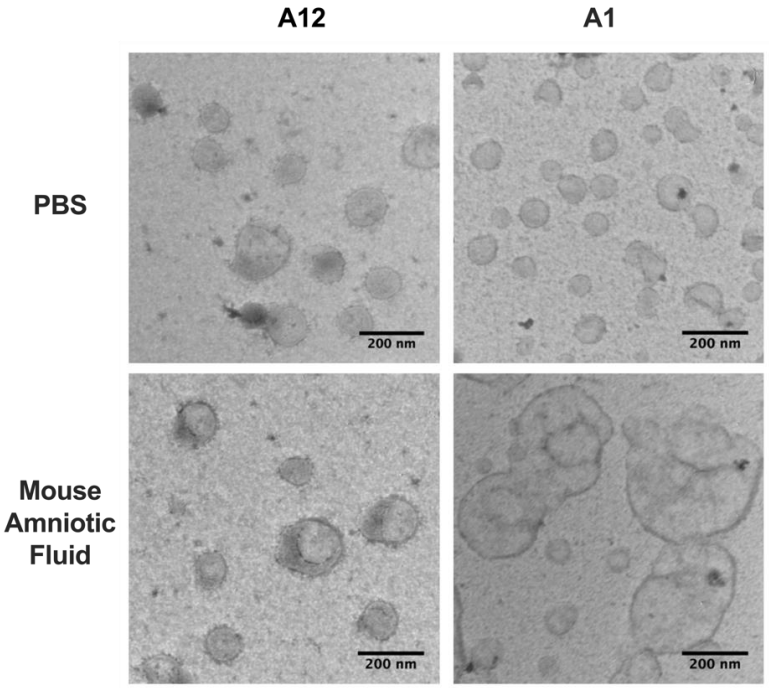




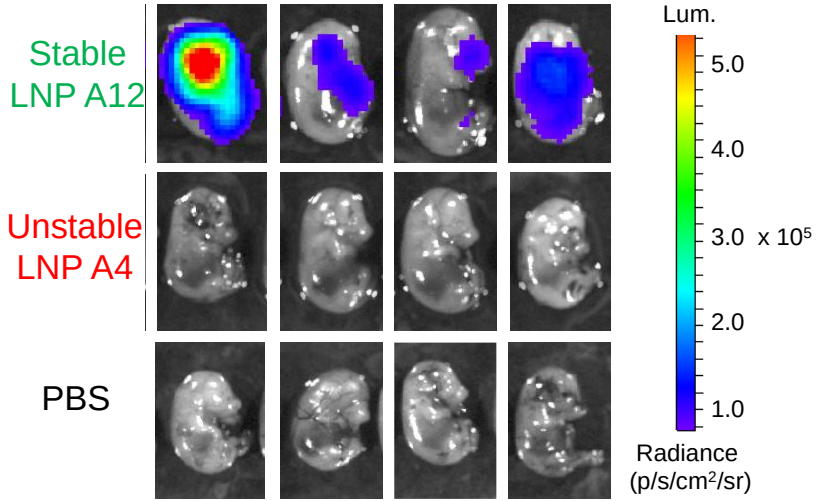

Swingle, Billingsley, Mitchell et al. *J Controlled Release* (2022)

# *Ex Utero* Assay Identifies LNPs for *In Utero* mRNA Delivery

## TEM of mRNA LNPs in Amniotic Fluid



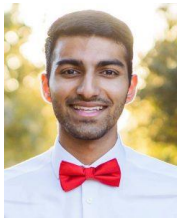

## mRNA Delivery via Amniotic Fluid-Stabilized LNPs



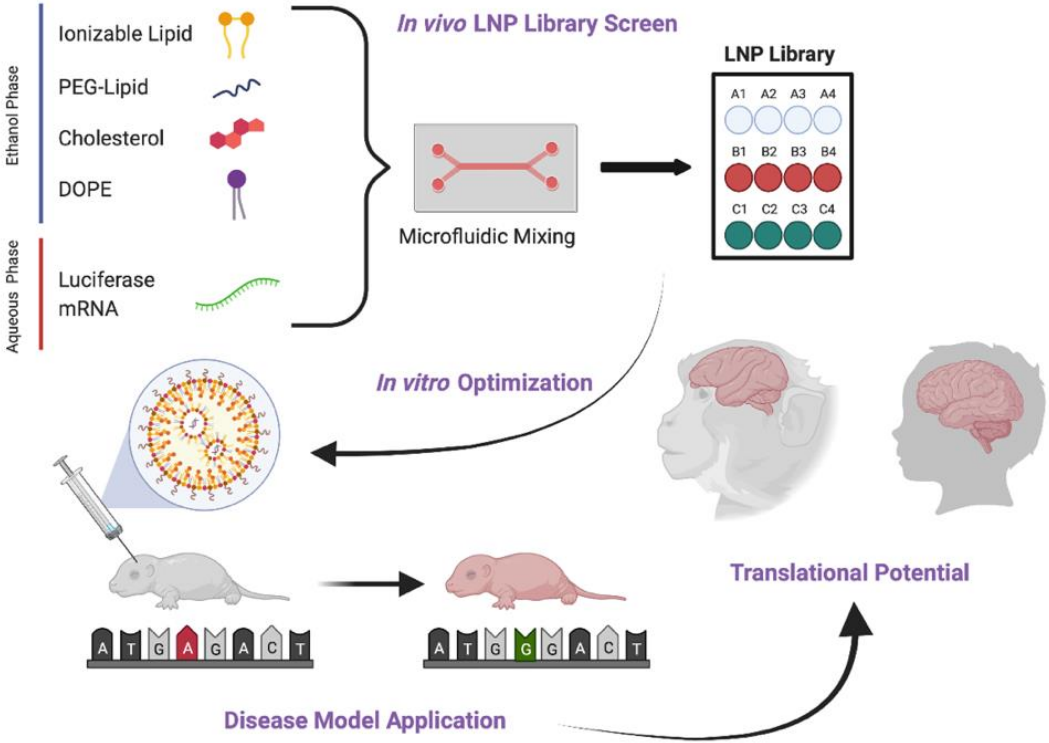



Swingle, Billingsley, Mitchell et al. *J Controlled Release* (2022)

# LNP Base Editing of the Brain *In Utero* and in Neonates



**Rohan Palanki**
Penn BE MD PhD Student

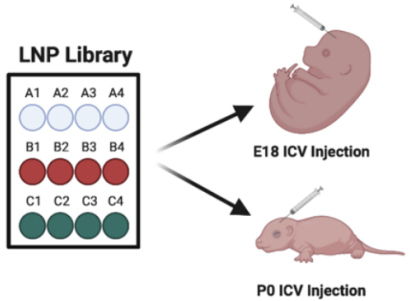


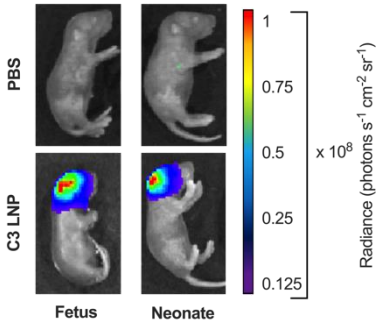



Palanki, Peranteau, Mitchell et al. *Submitted*

# LNP Base Editing of the Brain *In Utero* and in Neonates

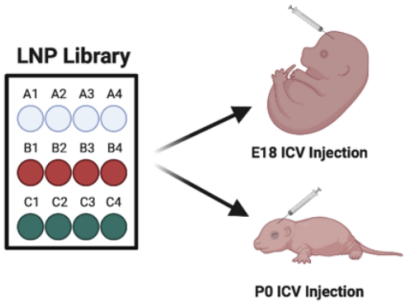
## ICV mRNA LNP Delivery to Brain

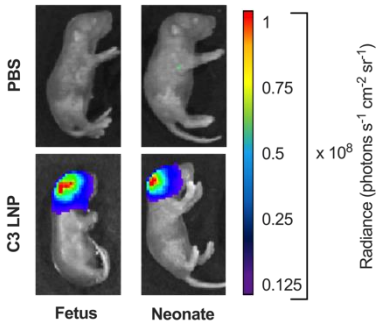


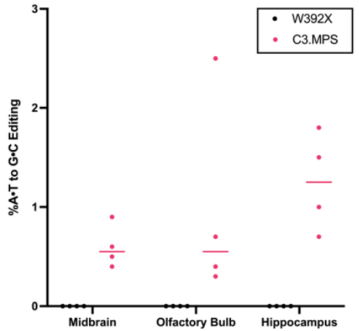
# LNP Base Editing of the Brain *In Utero* and in Neonates

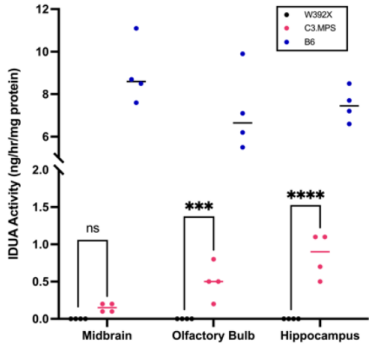
## ICV mRNA LNP Delivery to Brain



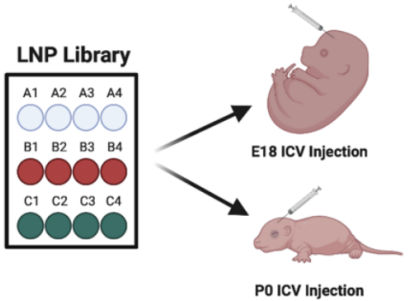

## *In Utero* Base Editing in Brain Increased IDUA Enzyme Activity

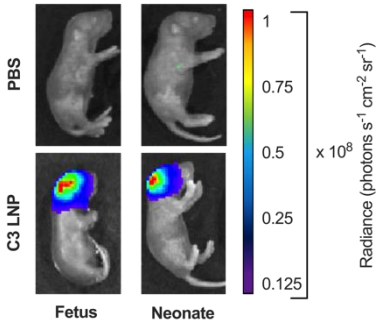


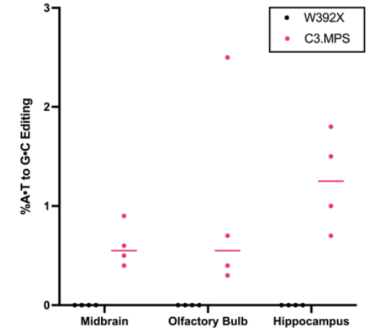
# LNP Base Editing of the Brain *In Utero* and in Neonates

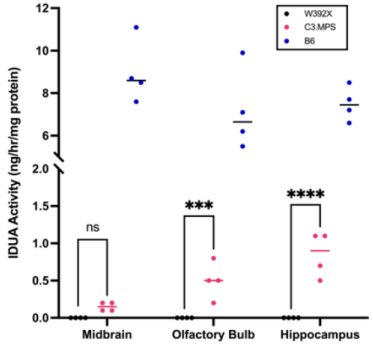
## ICV mRNA LNP Delivery to Brain



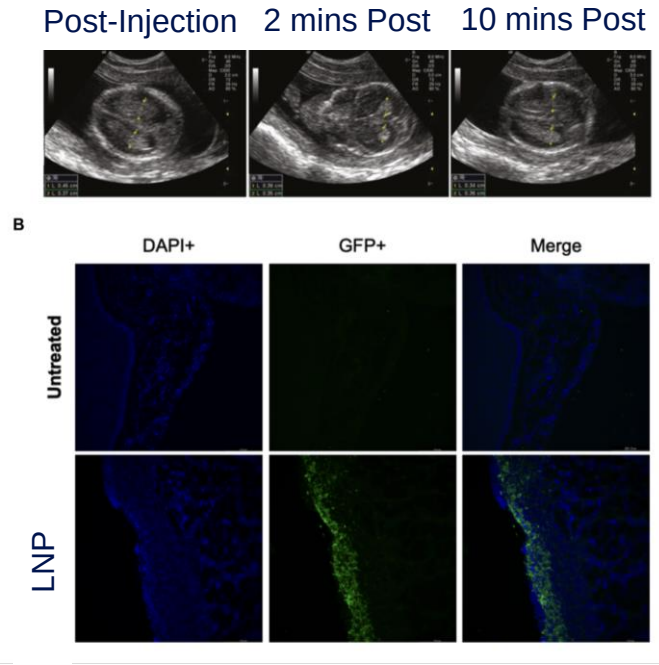




## *In Utero* Base Editing in Brain Increased IDUA Enzyme Activity



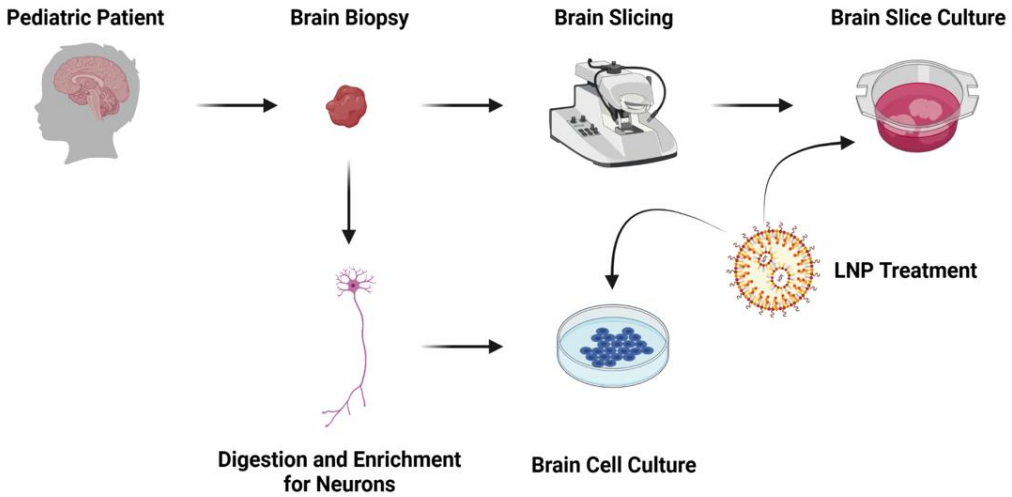




## *In Utero* mRNA LNP Brain Delivery in NHPs



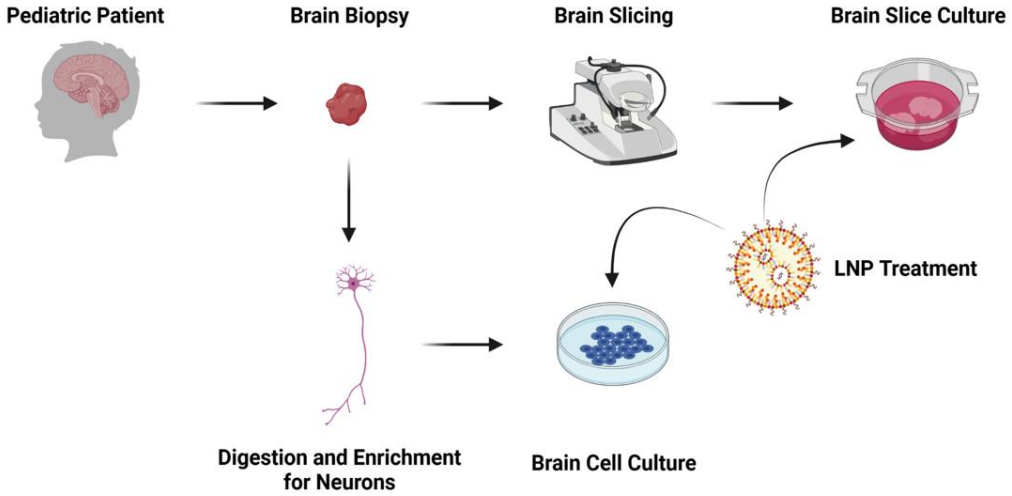
# LNP Base Editing in Human Brain Tissue

## mRNA/gRNA LNP Delivery to Human Brain Tissue

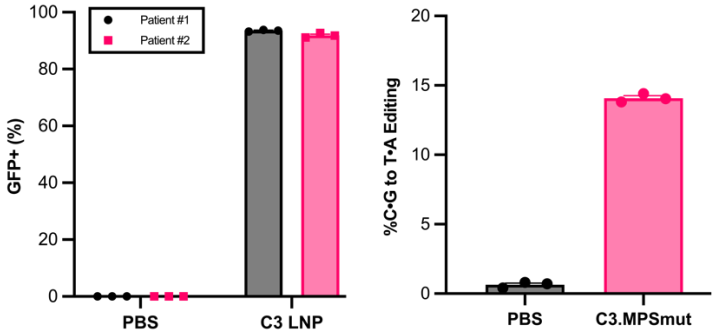


# LNP Base Editing in Human Brain Tissue

## mRNA/gRNA LNP Delivery to Human Brain Tissue




## LNP mRNA and Base Editing in Human Brain Tissue



# Conclusions

- **Many opportunities for mRNA LNP therapeutics for precision beyond vaccines, many will require new materials**
- **LNPs can be engineered to deliver mRNA before birth (*in utero*)**
- **Intra-amniotic delivery: *ex utero* stability assays in pig, sheep, and human amniotic fluid identify new LNPs for intra-amniotic delivery**
- **Gene editing: ICV injections enable potent mRNA/gRNA LNP gene editing in brain**
- **Translation: mRNA delivery *in utero* in NHPs, gene editing in human tissue samples**

# Acknowledgements


**Mitchell Lab**

Rachel Riley, PhD
Rui Zhang, PhD
Pedro Guimaraes, PhD
Jingya Qin, PhD
Ningqiang Gong, PhD
Xuexiang Han, PhD
Lulu Xue, PhD
Marshall Padilla, PhD
Maggie Billingsley
Alvin Mukalel
Sarah Shepherd
Rebecca Haley
Chris Figueroa-Espada
Rohan Palanki
Alex Hamilton
Kelsey Swingle
Kamila Butowska
Ann Metzloff
Hannah Geisler
Hannah Safford
Ajay Thatte
Hanwen Zhang
Rakan El-Mayta
Ella Atsavapranee
Xisha Huang
Hanwen Zhang
Rakan El-Mayta
Ella Atsavapranee
Andres Hubsch
Seth Thayumanavan
Savan Patel
Emily Kim
Caitlin Frazee
Jacqueline Li
Matthew Jester
Kaitlin Mrksich
Aditi Ghalsasi

**Collaborators**

Drew Weissman, MD PhD
Carl June, MD
Jim Wilson, MD PhD
William Peranteau, PhD
Mohamad-Gabriel Alameh, PhD





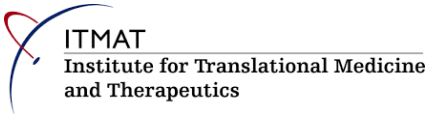
















https://mitchell-lab.seas.upenn.edu

# Questions?

**Mitchell Lab**

Rachel Riley, PhD
Rui Zhang, PhD
Pedro Guimaraes, PhD
Jingya Qin, PhD
Ningqiang Gong, PhD
Xuexiang Han, PhD
Lulu Xue, PhD
Marshall Padilla, PhD
Maggie Billingsley
Alvin Mukalel
Sarah Shepherd
Rebecca Haley
Chris Figueroa-Espada
Rohan Palanki
Alex Hamilton
Kelsey Swingle
Kamila Butowska
Ann Metzloff
Hannah Geisler
Hannah Safford
Ajay Thatte
Hanwen Zhang
Rakan El-Mayta
Ella Atsavapranee
Xisha Huang
Hanwen Zhang
Rakan El-Mayta
Ella Atsavapranee
Andres Hubsch
Seth Thayumanavan
Savan Patel
Emily Kim
Caitlin Frazee
Jacqueline Li
Matthew Jester
Kaitlin Mrksich
Aditi Ghalsasi

**Collaborators**

Drew Weissman, MD PhD
Carl June, MD
Jim Wilson, MD PhD
William Peranteau, PhD
Mohamad-Gabriel Alameh, PhD


Email: mjmitch@seas.upenn.edu