Testing thousands of nanoparticles *in vivo*

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I co-founded GuideTx, which was acquired by Beam.

I am an advisor to GV.

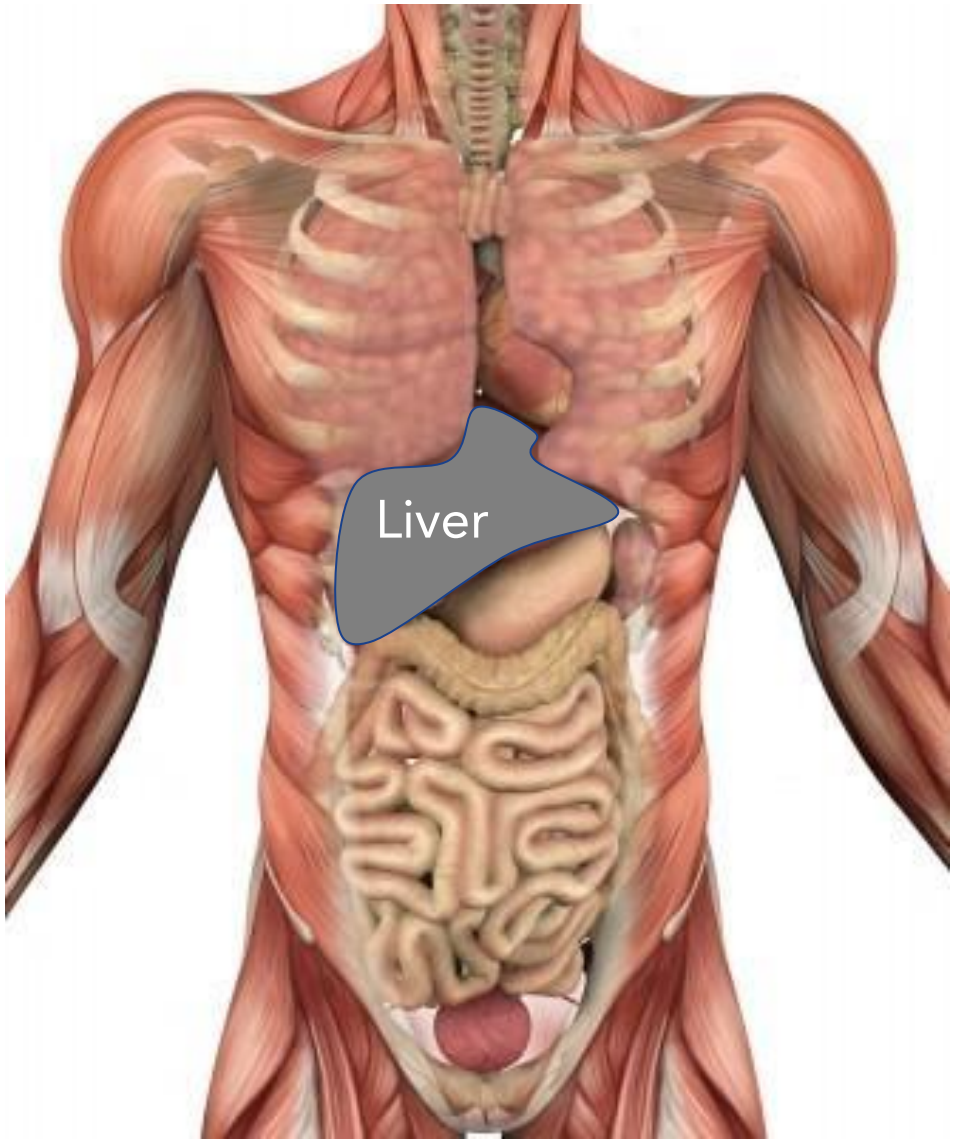
What have we learned?

Many lipids are unexplored.

Keep LNP structure as simple as possible.

You find what you look for, so run predictive assays.

Non-hepatocyte delivery will be important



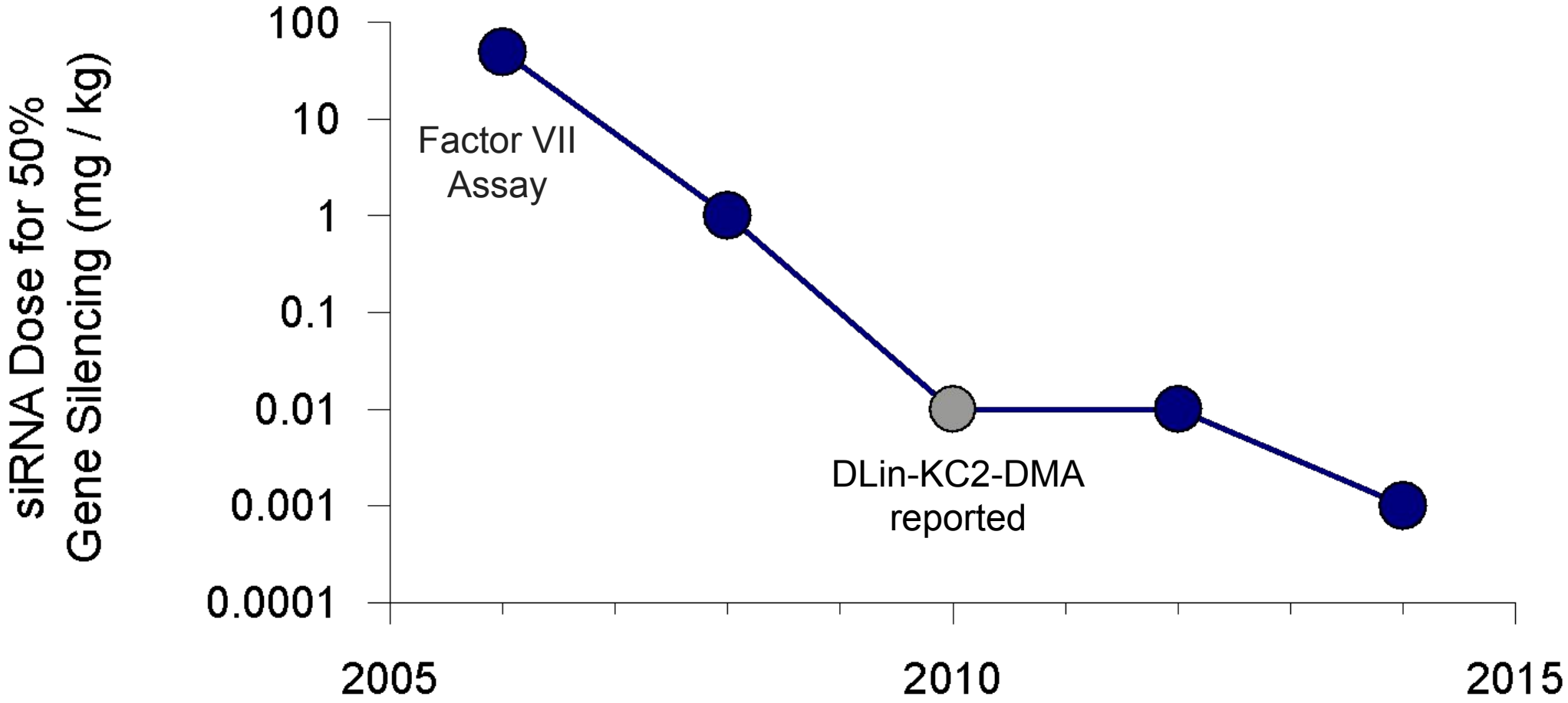
Liver physiology promotes delivery

The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs

The regulatory approval of Onpattro, a lipid nanoparticle-based short interfering RNA drug for the treatment of polyneuropathies induced by hereditary transthyretin amyloidosis, paves the way for clinical development of many nucleic acid-based therapies enabled by nanoparticle delivery.

Akinc A et al., *Nat Nano* 2019.

The field looked for hepatocyte delivery for a decade



History lesson from hepatocyte RNA drugs:

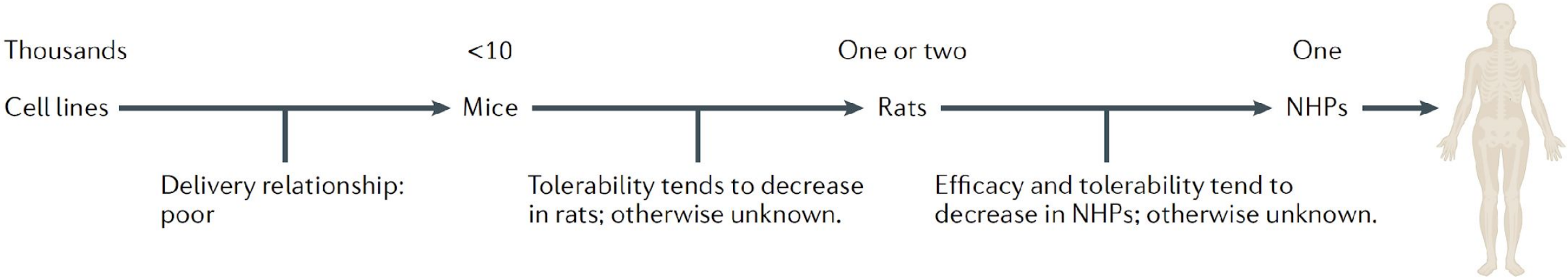
By iteratively quantifying how hundreds of nanoparticles delivered RNA to hepatocytes *in vivo*, the field found clinically relevant delivery to hepatocytes.

Our hypothesis:

By iteratively quantifying how thousands of nanoparticles deliver RNA to any combination of desired cell types *in vivo*, you may find clinically relevant delivery to other cell types.

Is this an assay problem?

We study / optimize the nanoparticle discovery pipeline



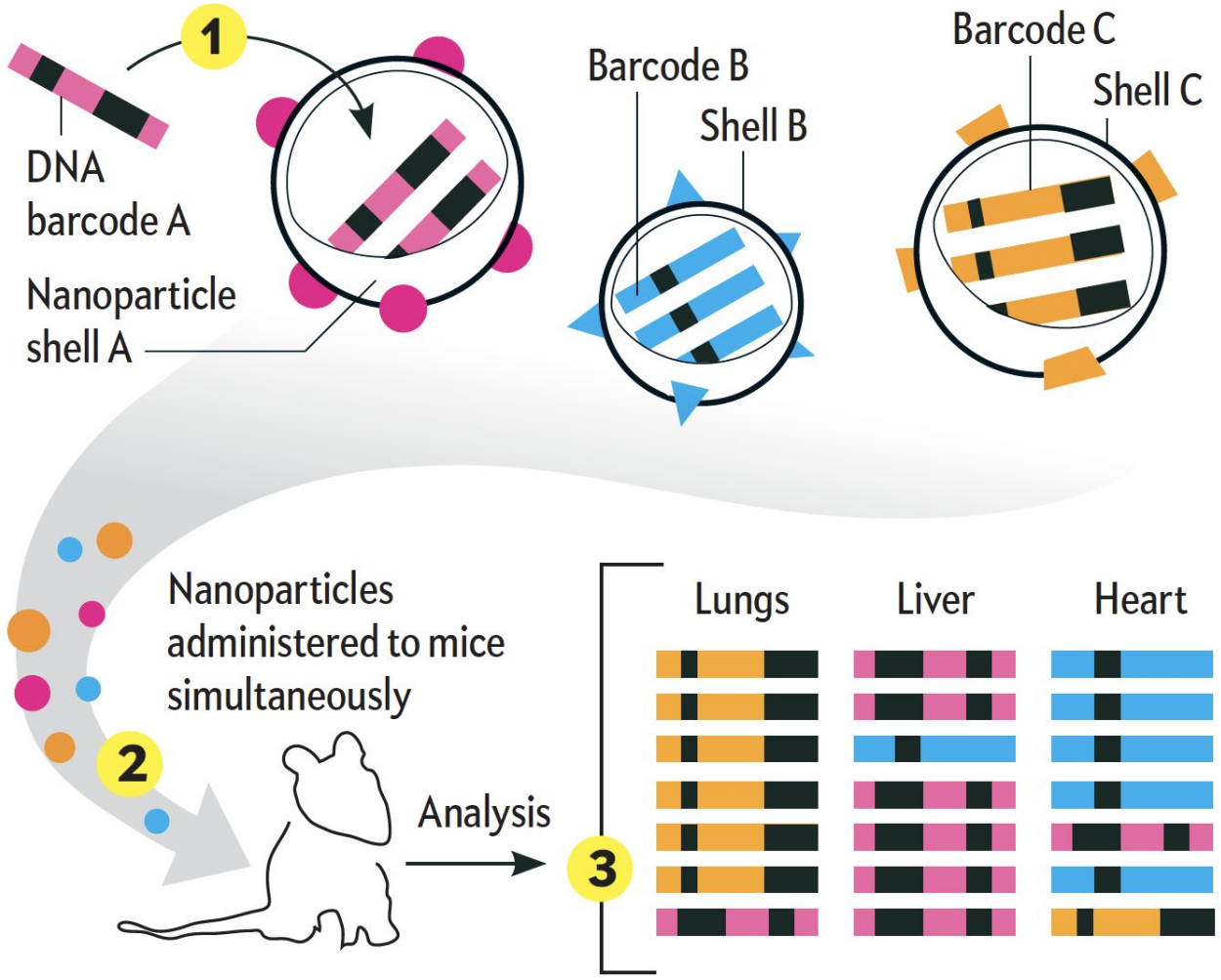
Paunovska, Loughrey, Dahlman, *Nat Rev Genetics* 2022.

We optimize the pipeline with DNA barcoded LNPs

These assays are:

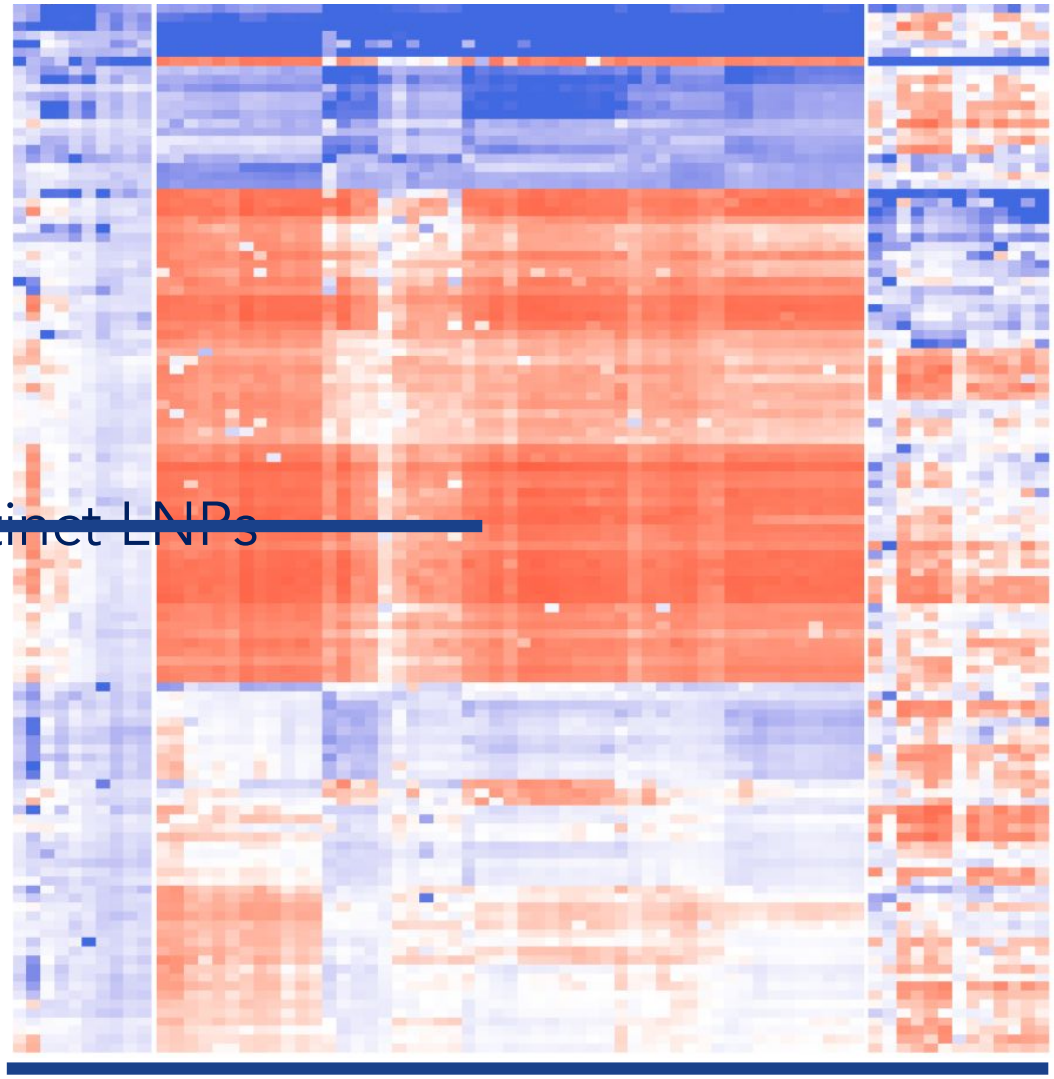
(1) High throughput (up to ~300 LNPs / mouse)
(2) *In vivo* (mice)
(3) Functional (mRNA □ protein, siRNA □ silencing protein)
(4) Iterative (data from LNPs 1-200 inform LNPs 201-400)

We label individual LNPs with barcodes, then pool / administer



Dahlman, *Scientific American* 2019.

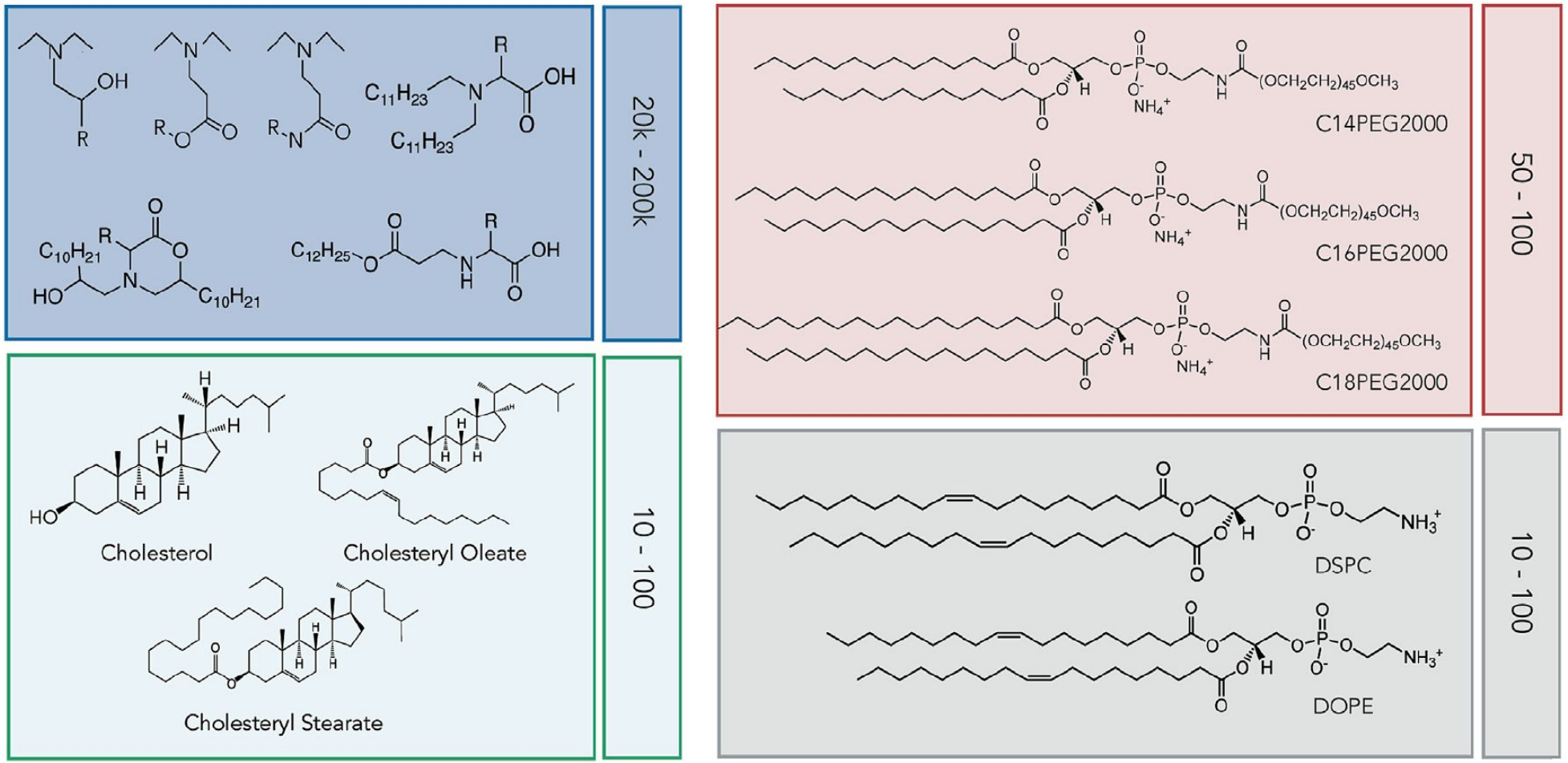
We study many LNPs / animal



~~Chemically Distinct LNPs~~

Tissues and cell types

Testing many LNPs matters: chemical space is very large

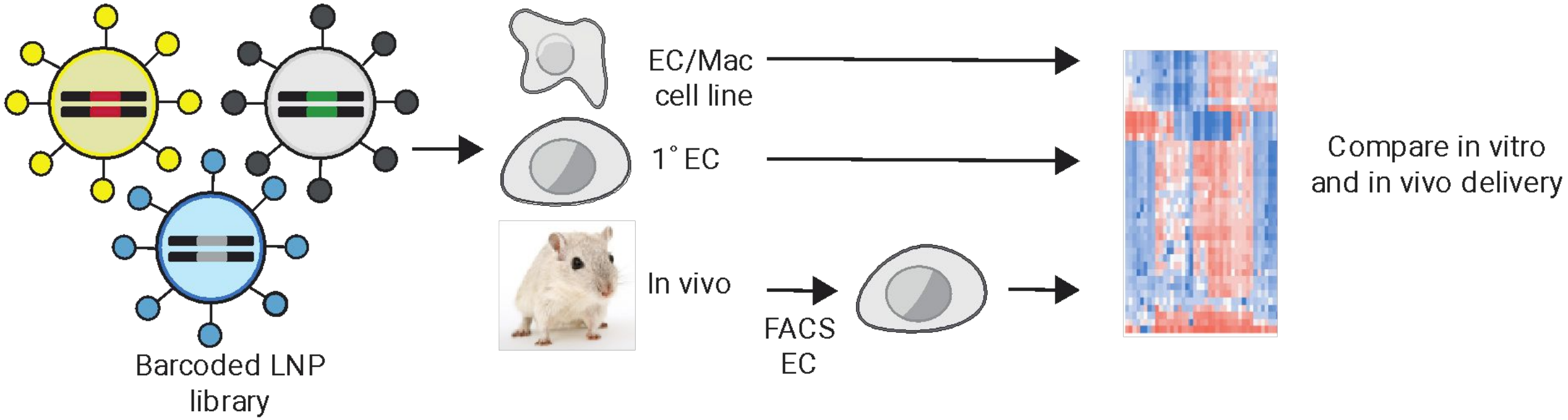


Lokugamage et al., *Current Opinion BME* 2018.

You find what you look for: *in vitro / in vivo,* mouse vs. NHP, and route of administration

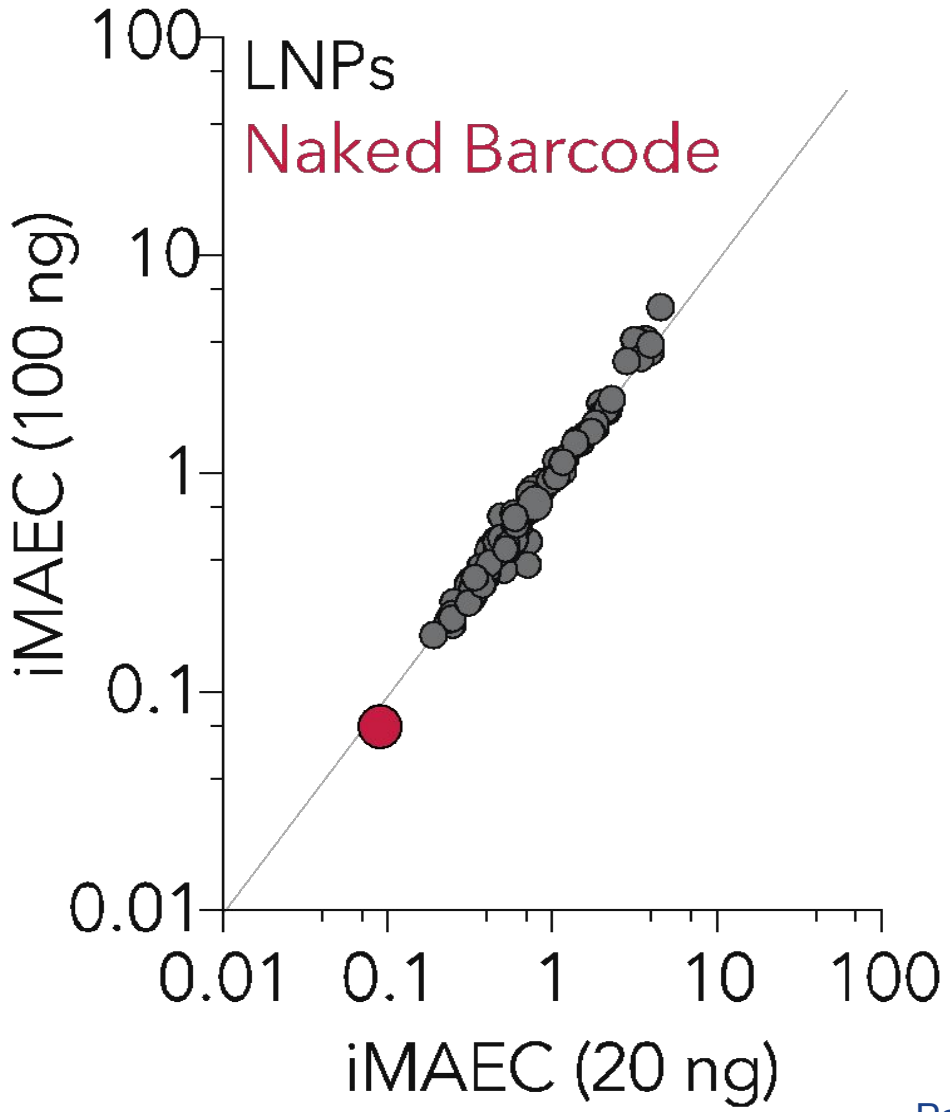
LNPs are typically screened *in vitro*

Does *in vitro* delivery predict *in vivo* delivery?



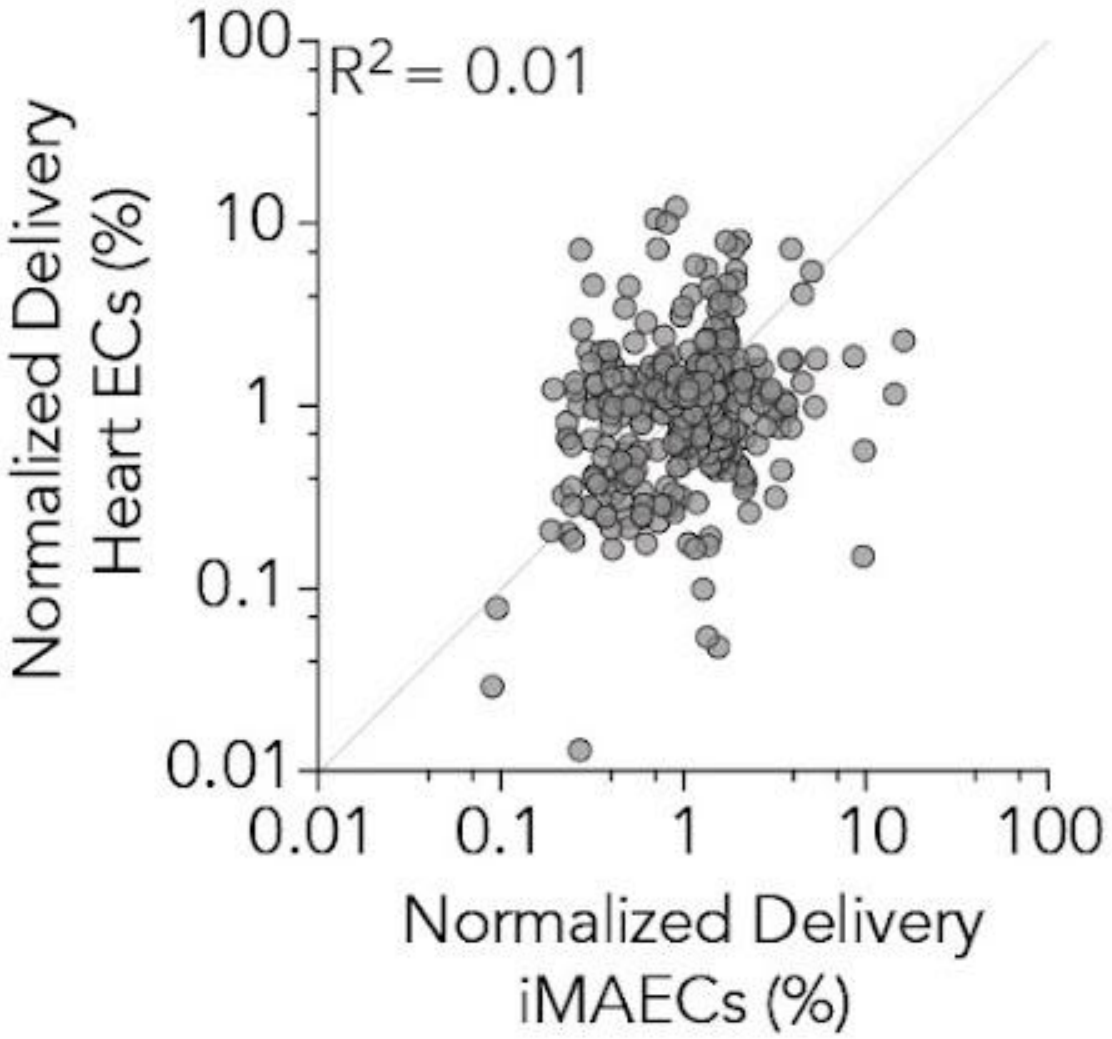
Paunovska*, Sago*, et al., *Nano Letters* 2018.

Control: *in vitro* delivery predicts *in vitro* delivery



Paunovska*, Sago*, et al., *Nano Letters* 2018.

In vitro delivery does not predict *in vivo* delivery



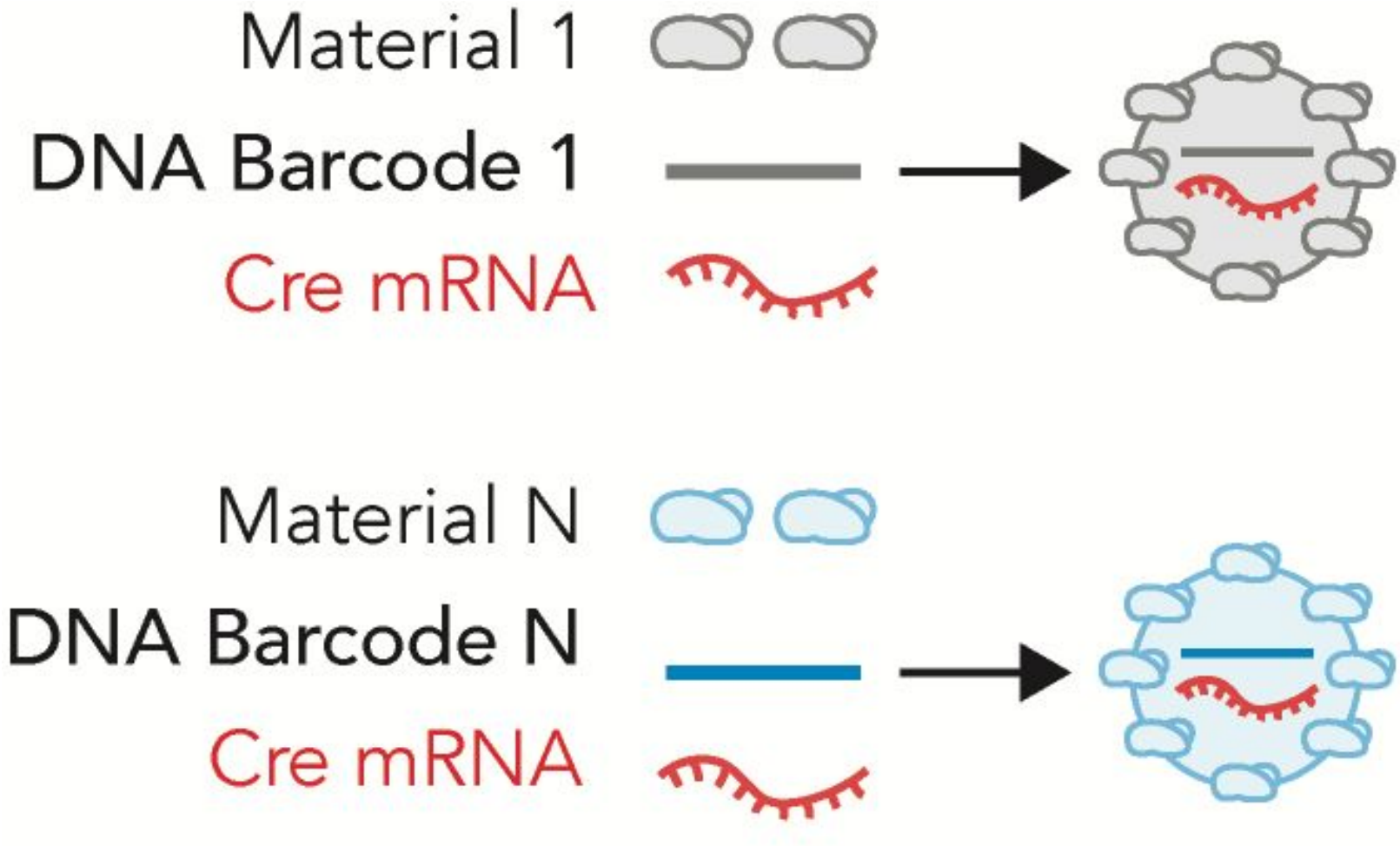
Paunovska*, Sago*, et al., *Nano Letters* 2018.

This is an opportunity to meaningfully improve LNP discovery

We designed assays to readout functional delivery

Sago et al., *PNAS* 2018.

We can screen for Cre mRNA delivery *in vivo*

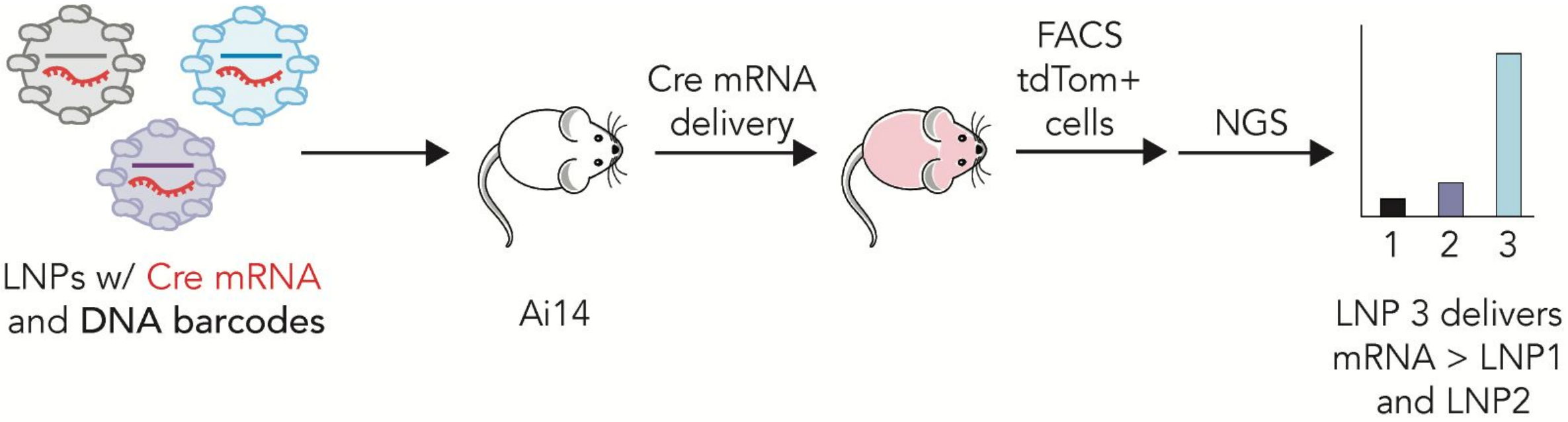


Sago et al., *PNAS* 2018.

Fast Identification of Nanoparticle Delivery (FIND)

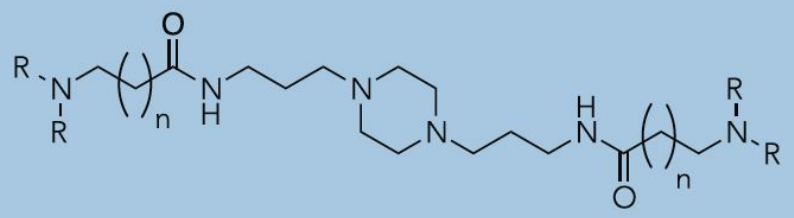
Control: Lox – Stop – Lox - tdTomato

Control: Lox - tdTomato

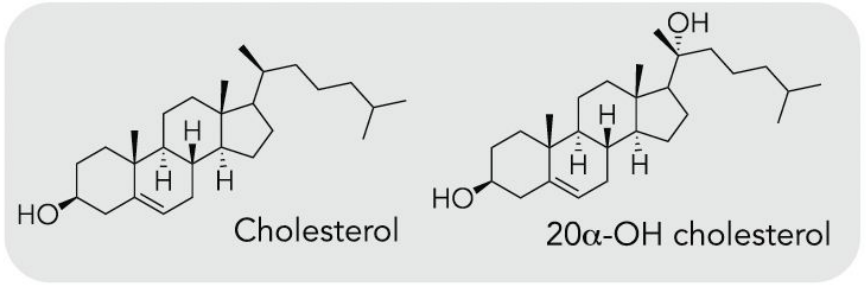


Sago et al., *PNAS* 2018.

FIND identified piperazine-based LNPs for immune delivery



Scaffold	Tail ID	R tails
PPZ-A (n = 1)	10	Me
	11	Me
	12	Me
	18-2Z	Me
PPZ-B (n = 2)	10	Me
	11	Me
	12	Me
	18-2Z	Me

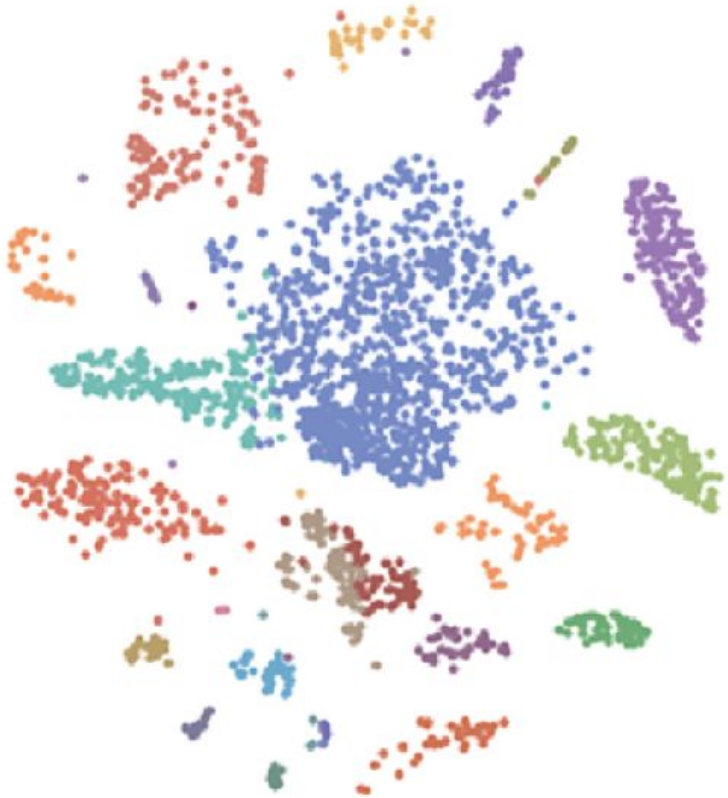


$C_{14}PEG_{2K}$ $C_{18}PEG_{2K}$ DOPE

Molar Ratio				
Ionizable Lipid	30	35	45	50
Cholesterol	30	46.5	39.5	35
PEG-Lipid	1	2.5	2.5	2.5
Helper Lipid	39	16	13	12.5

Ni H, Hatit MCZ, et al., *Nat Communications 2022*.

FIND also identified LNPs that deliver across lung



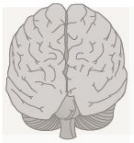
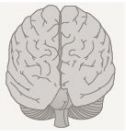
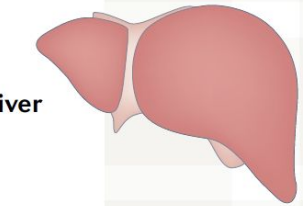
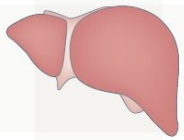
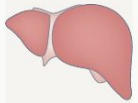
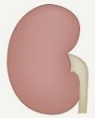
Cell type

Delivered mRNA

Radmand A et al., *Nano Letters 2023*.

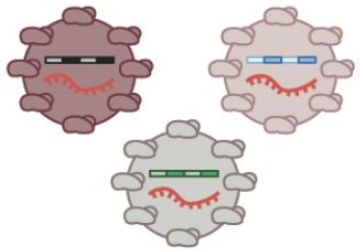
You find what you look for: *in vitro / in vivo,* mouse vs. NHP, and route of administration

The species probably matters

	Mice	% of bw	Rats	% of bw	NHPs	% of bw	Humans	% of bw
Brain		2.1		0.9		1.4		1.9
Heart		0.6		0.4		0.4		0.4
Lung		0.7		0.5		0.6		1.0
Liver		4.5		3.0		2.2		2.0
Spleen		0.4		0.2		0.1		0.2
Kidney		1.4		0.8		0.4		0.3

Paunovska, Loughrey, Dahlman, *Nat Rev Genetics* 2022.

We built a species-agnostic barcoding system



Nanoparticle N carries
Barcode N, aVHH mRNA

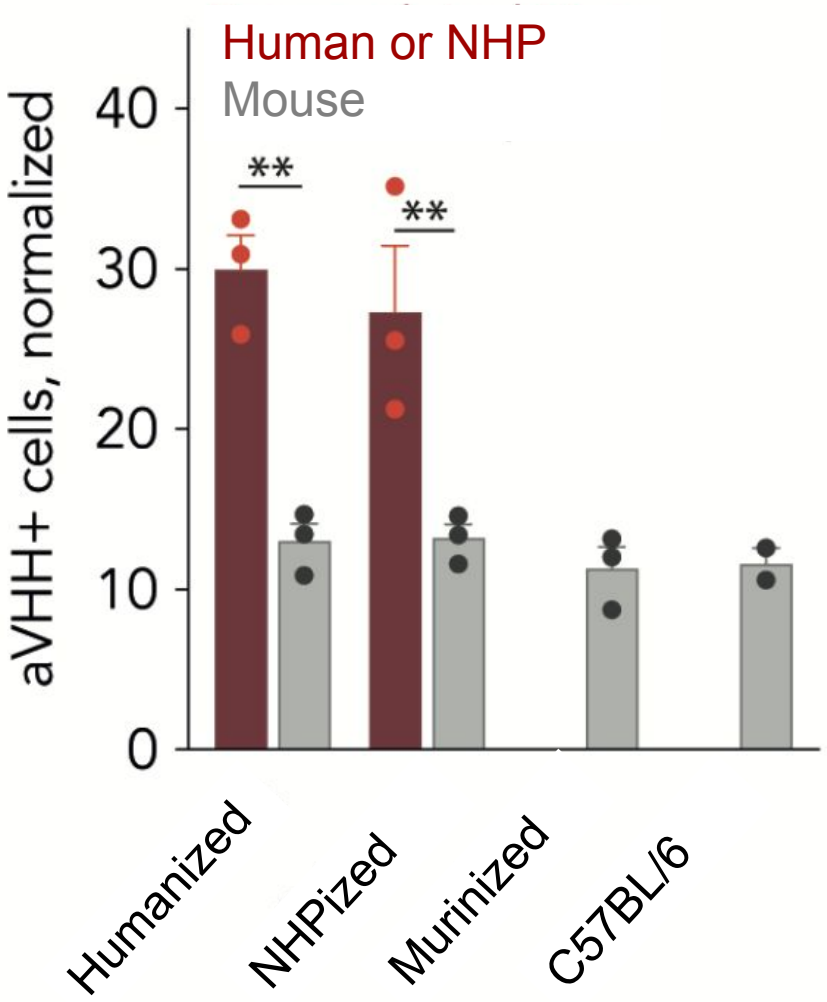
Hepatocyte
repopulated mice

Yecuris FRG® KO on C57Bl/6	 Human	 Primate	 Murine
Repopulated Hep. FACS anti-human CD47^{+}	Humanized/H	Primatized/P	
Resident Hep. FACS CD31^{-}CD45^{-}	Humanized/M	Primatized/M	Murinized/M

Common mouse strains	 C57BL/6J	 BALB/cJ	 NZB/BlNJ
Resident Hep. FACS CD31^{-}CD45^{-}	BL/6	Balb/C	NZB

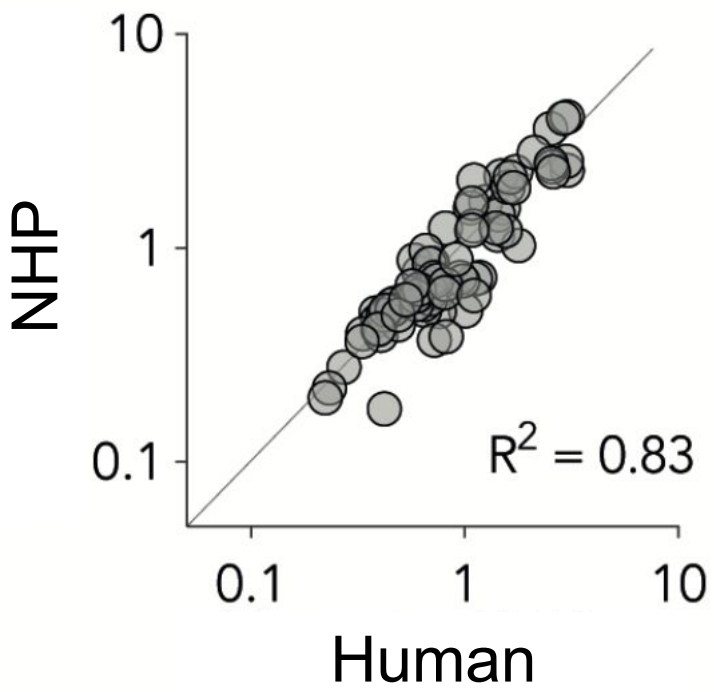
Hatit*, Lokugamage*, Dobrowolski*, et al., *Nat Nano* 2022.

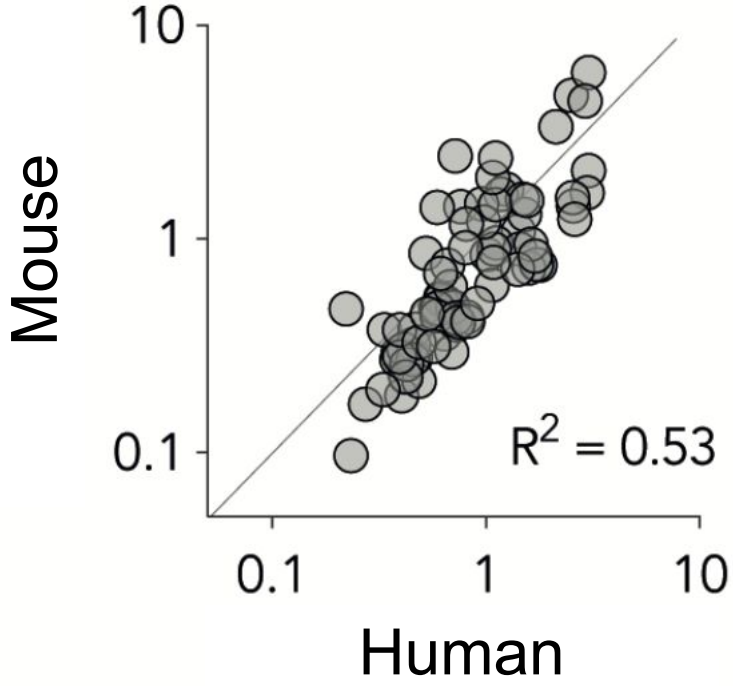
We observed species-dependent delivery



Hatit*, Lokugamage*, Dobrowolski*, et al., *Nat Nano* 2022.

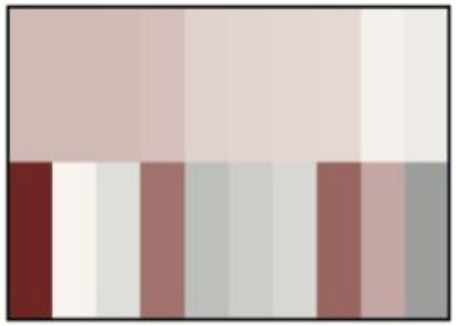
Delivery in primate cells predict human > mice





Hatit*, Lokugamage*, Dobrowolski*, et al., *Nat Nano* 2022.

Mouse LNP readouts can give false positives / negatives

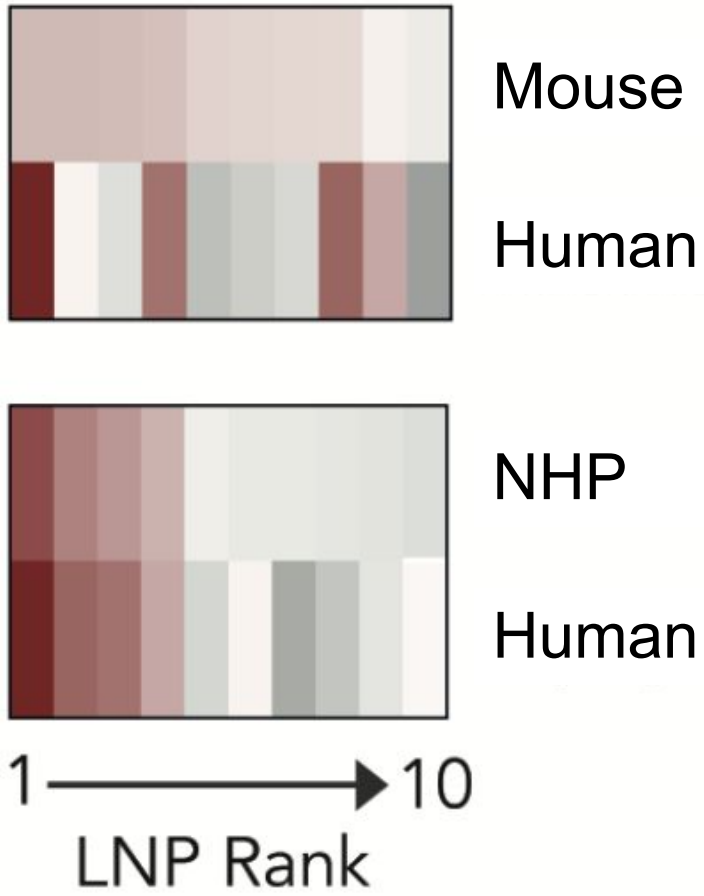


Mouse

Human

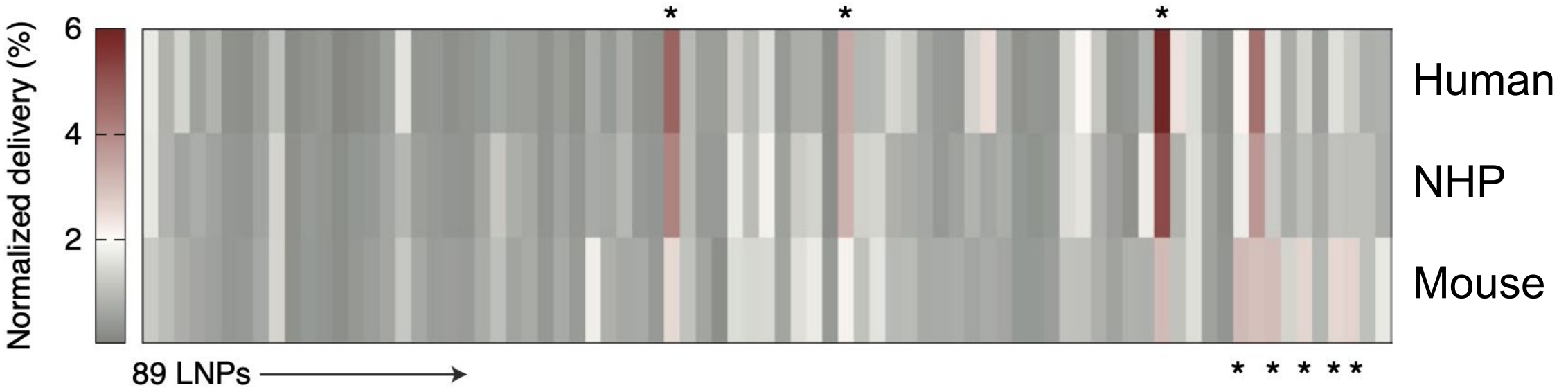
Hatit*, Lokugamage*, Dobrowolski*, et al., *Nat Nano* 2022.

Mouse LNP readouts can give false positives / negatives



Hatit*, Lokugamage*, Dobrowolski*, et al., *Nat Nano* 2022.

Mouse gives you false positives and false negatives



Hatit*, Lokugamage*, Dobrowolski*, et al., *Nat Nano* 2022.

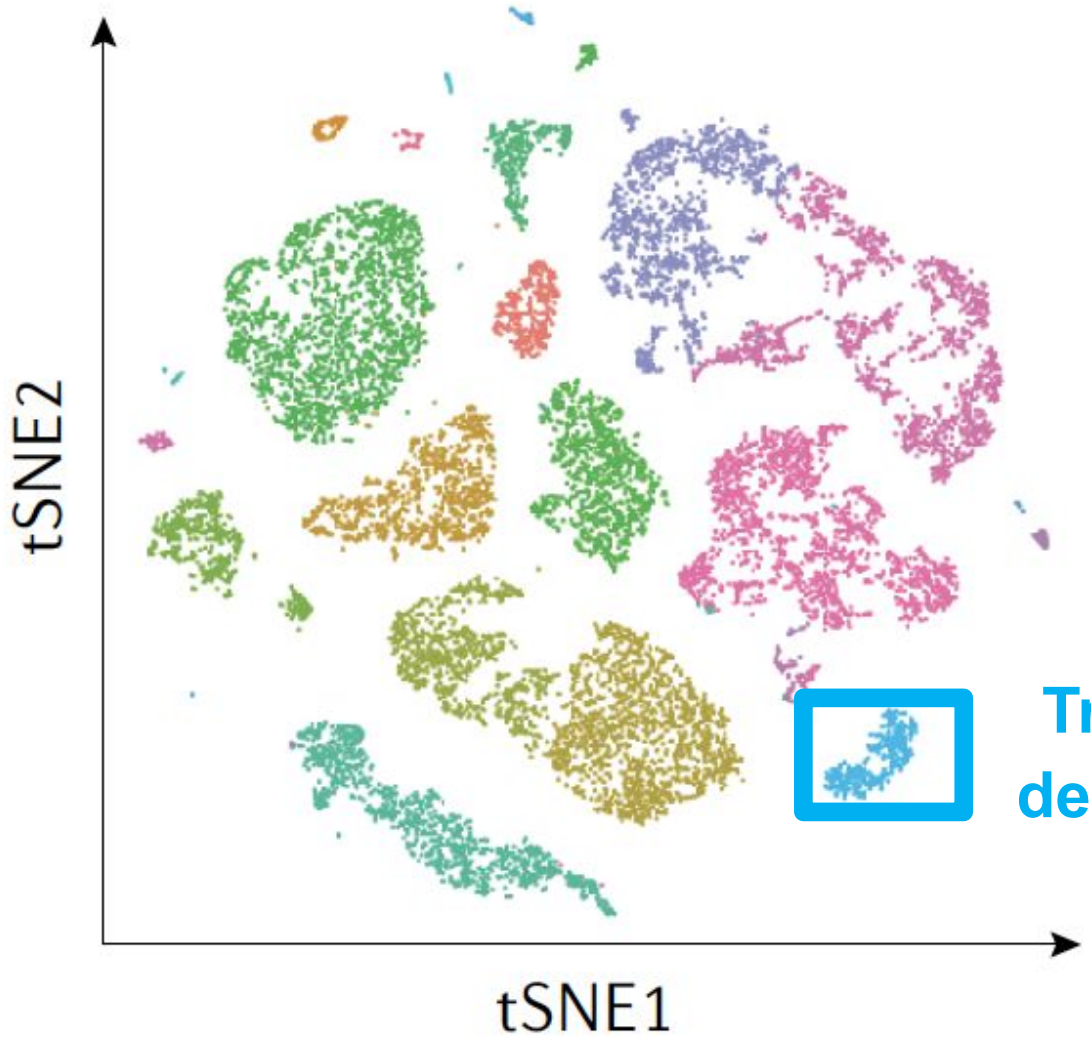
An ideal LNP screen would tell you…

1) Where did (many) LNPs go?

2) Where did (many) LNPs work?

… and it would do so with single cell resolution.

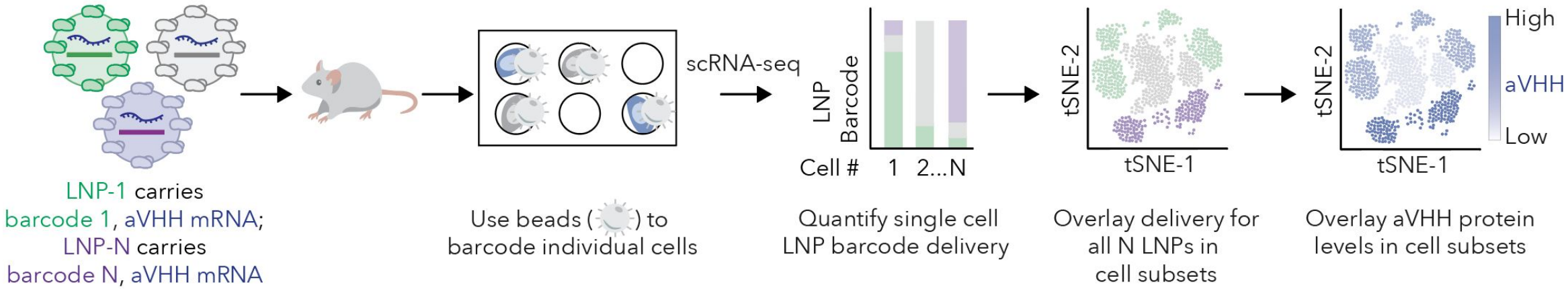
Single-cell RNA-sequencing gives you cell identity



Transcriptionally defined cell type A

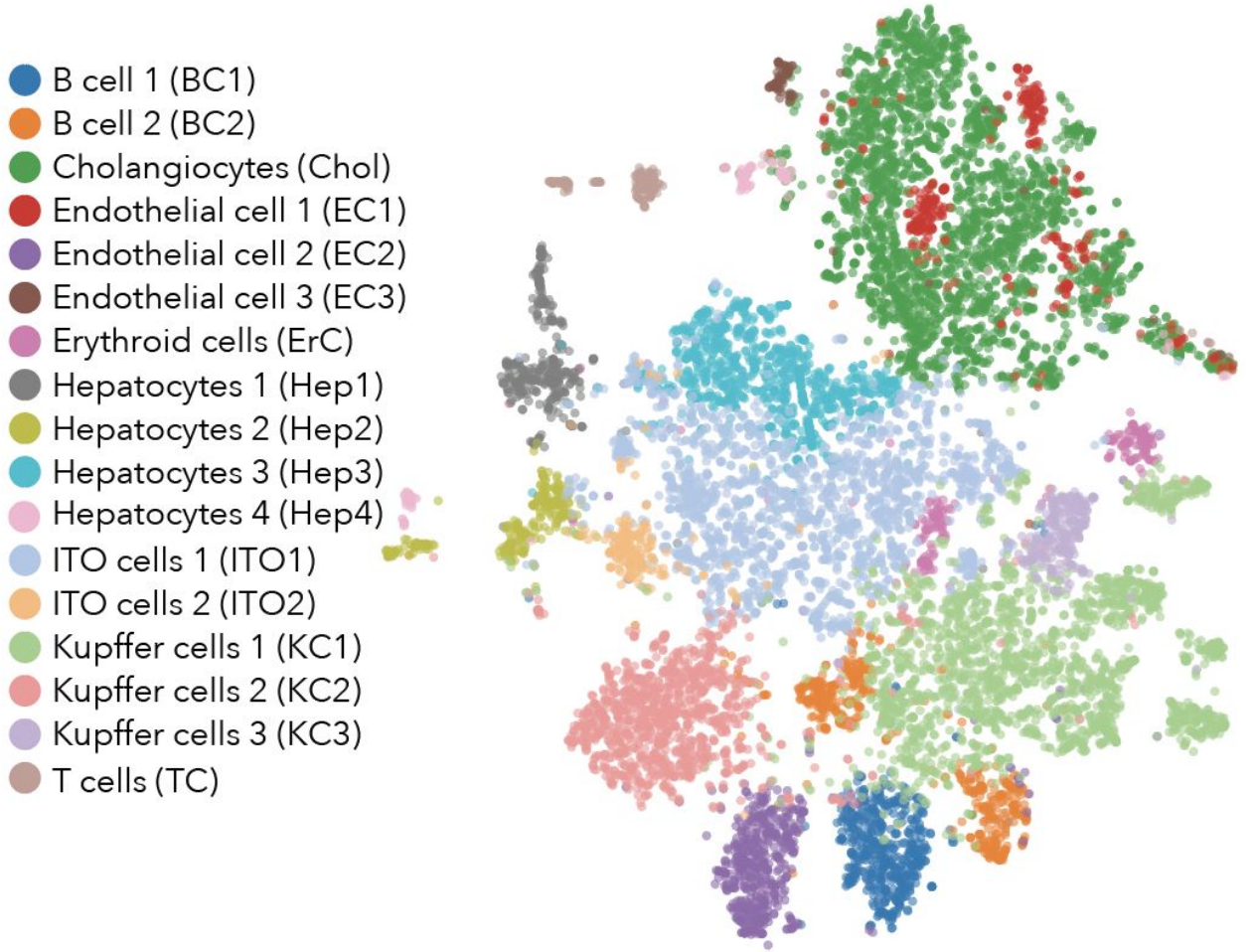
Stuart, Satija, *Nat Rev Genetics* 2019.

SENT-seq gives you barcode, protein, transcriptome



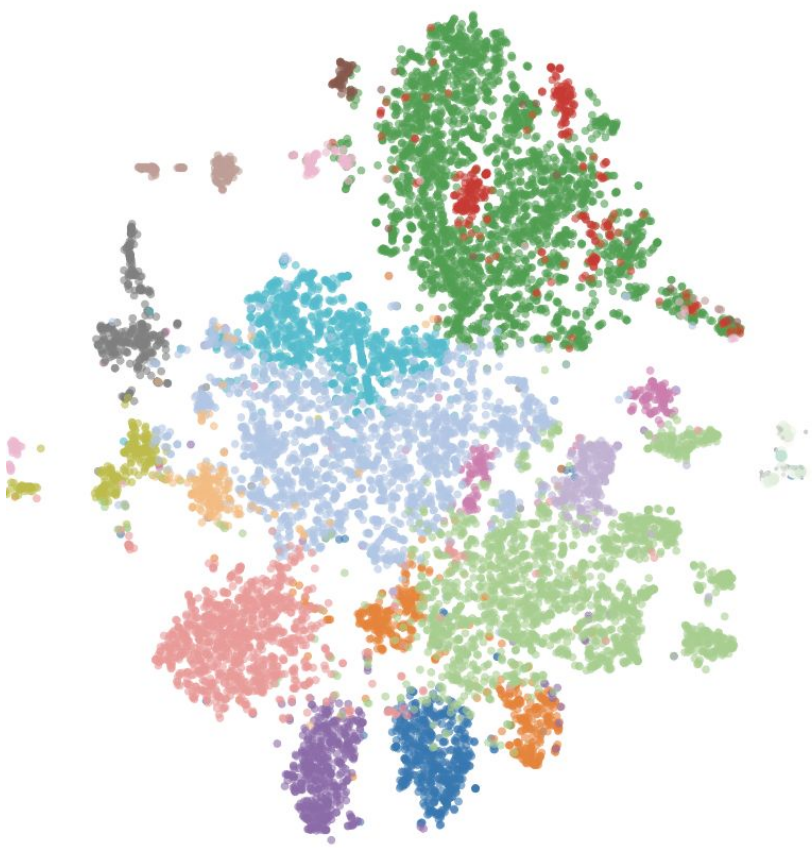
Dobrowolski*, Paunovska*, et al., *Nat Nano* 2022.

scRNA-seq = transcriptionally defined cell subtypes

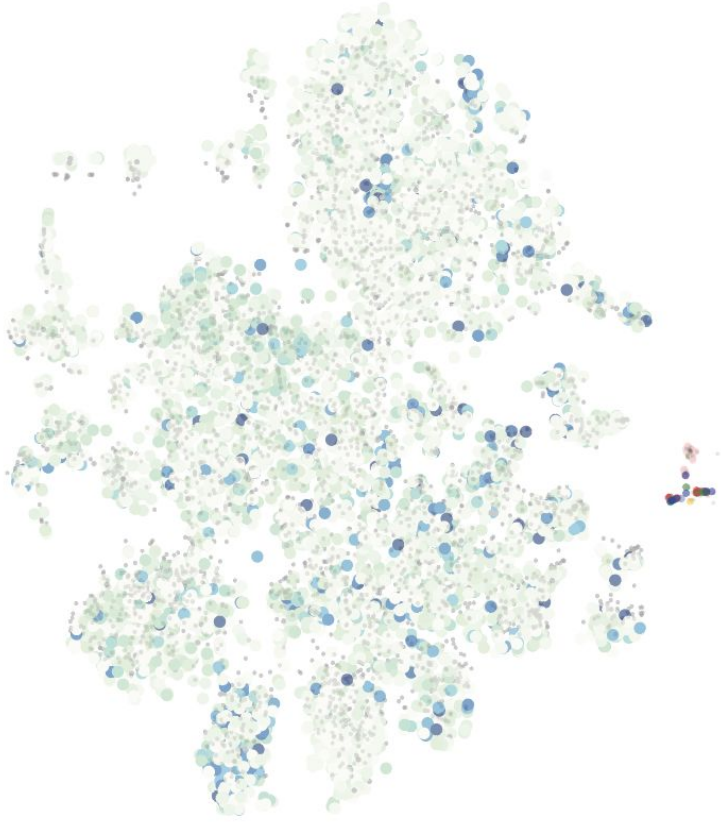


Dobrowolski*, Paunovska*, et al., *Nat Nano* 2022.

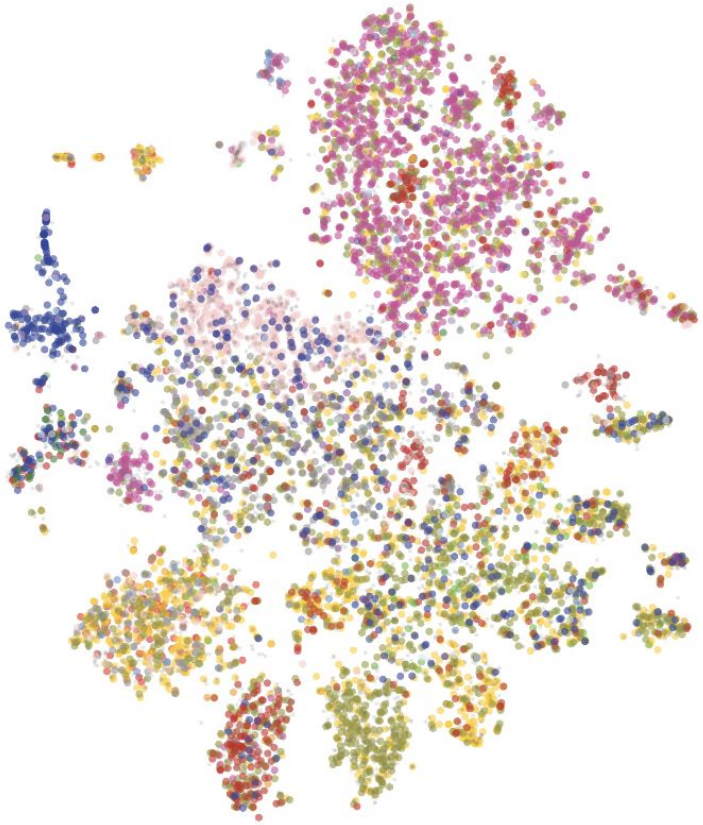
Overlay protein delivery and barcodes onto single cells



Cell type



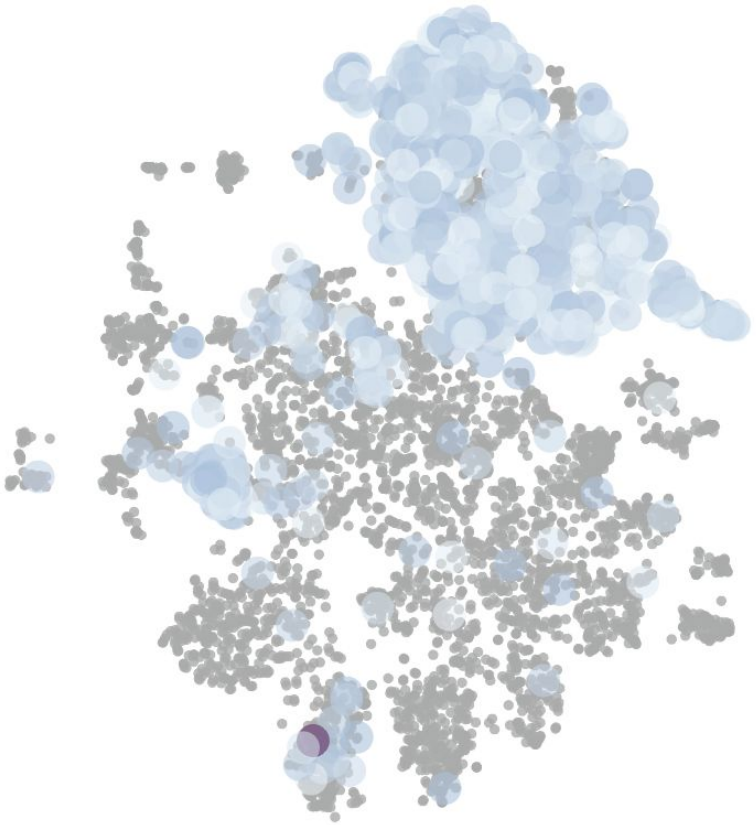
Protein delivery



Barcodes

Dobrowolski*, Paunovska*, et al., *Nat Nano* 2022.

This lets us measure LNP tropism in any single cell(s) you want

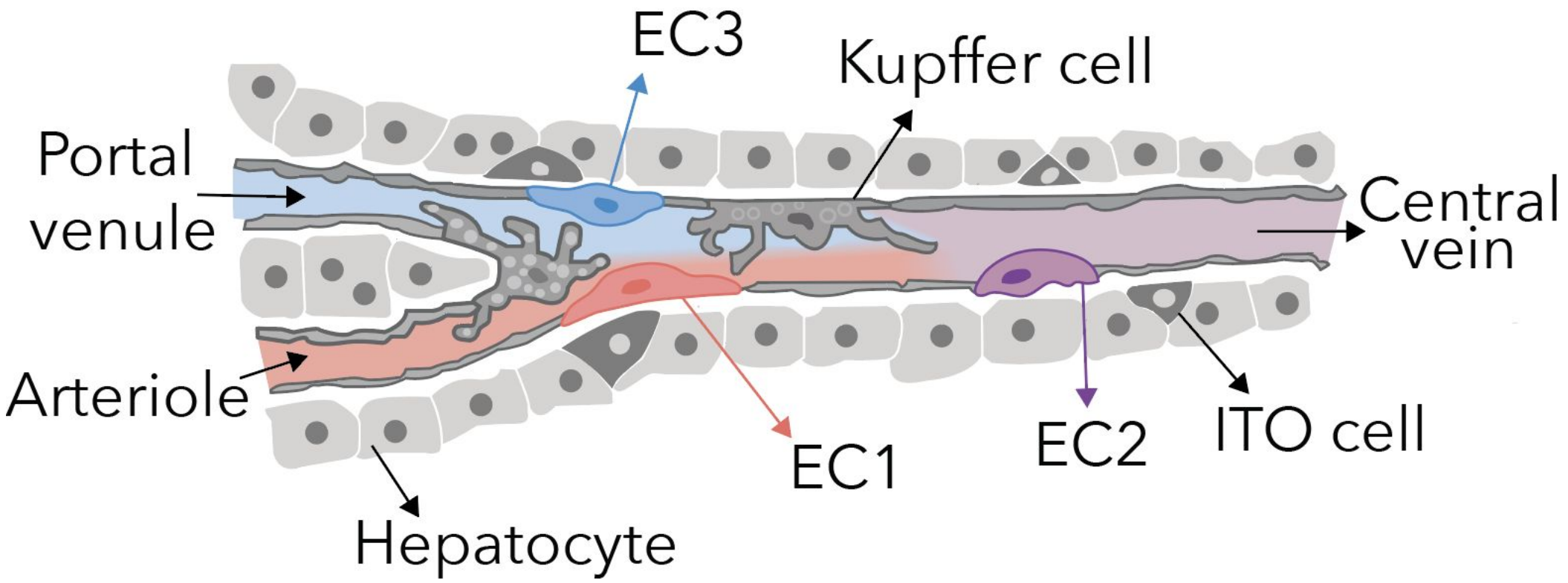


LNP-10

LNP-12

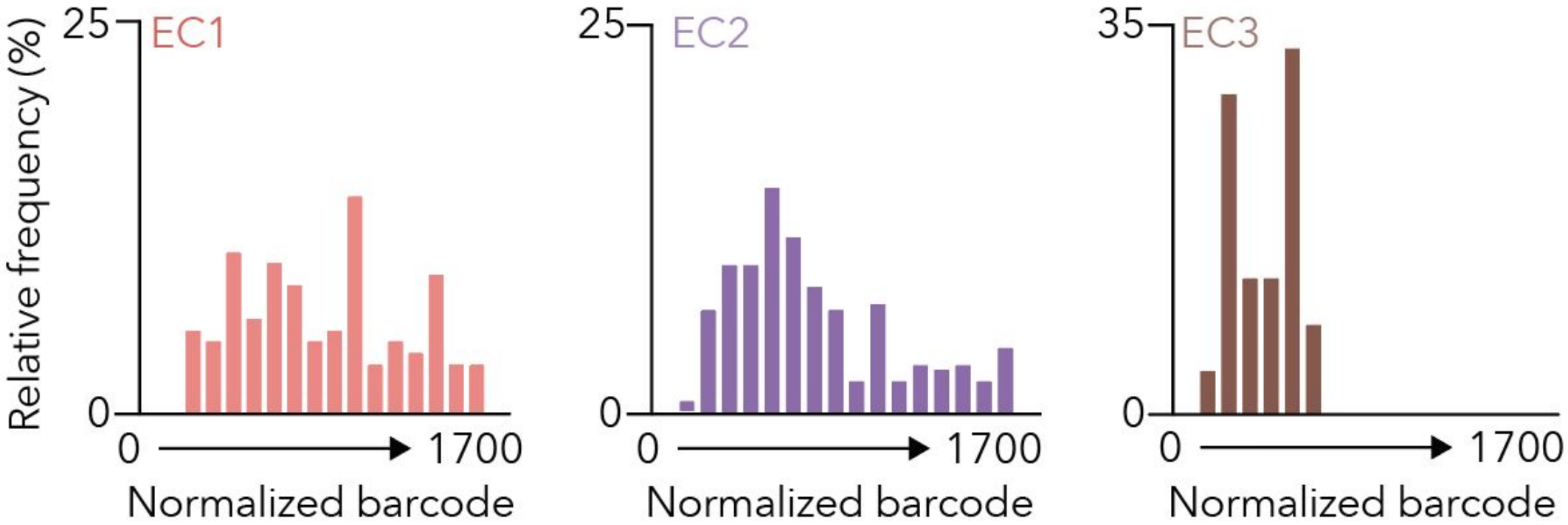
Dobrowolski*, Paunovska*, et al., *Nat Nano* 2022.

Looking at on / off-target in cell subtypes



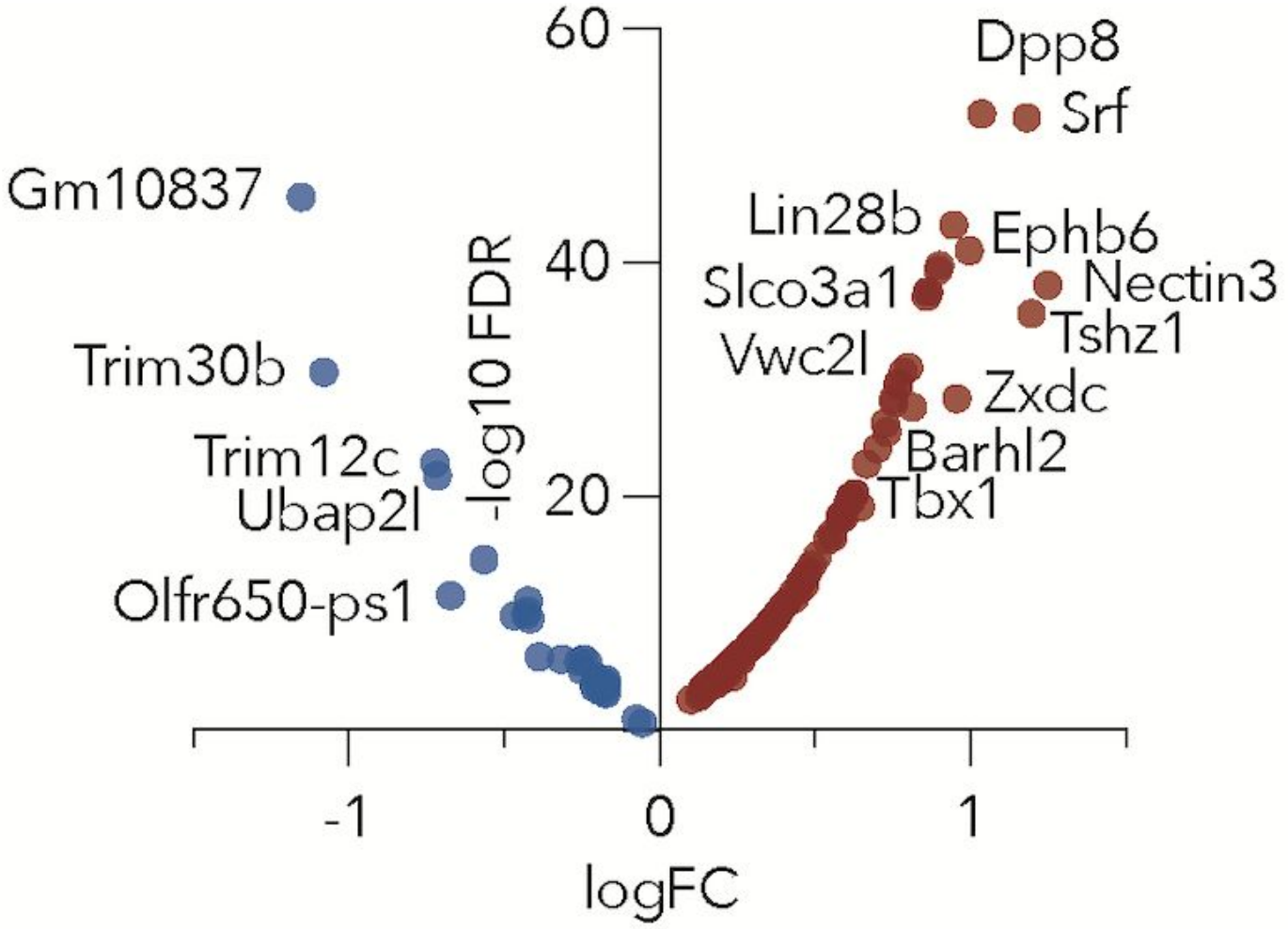
Dobrowolski*, Paunovska*, et al., *Nat Nano* 2022.

We can measure delivery distributions in single cells



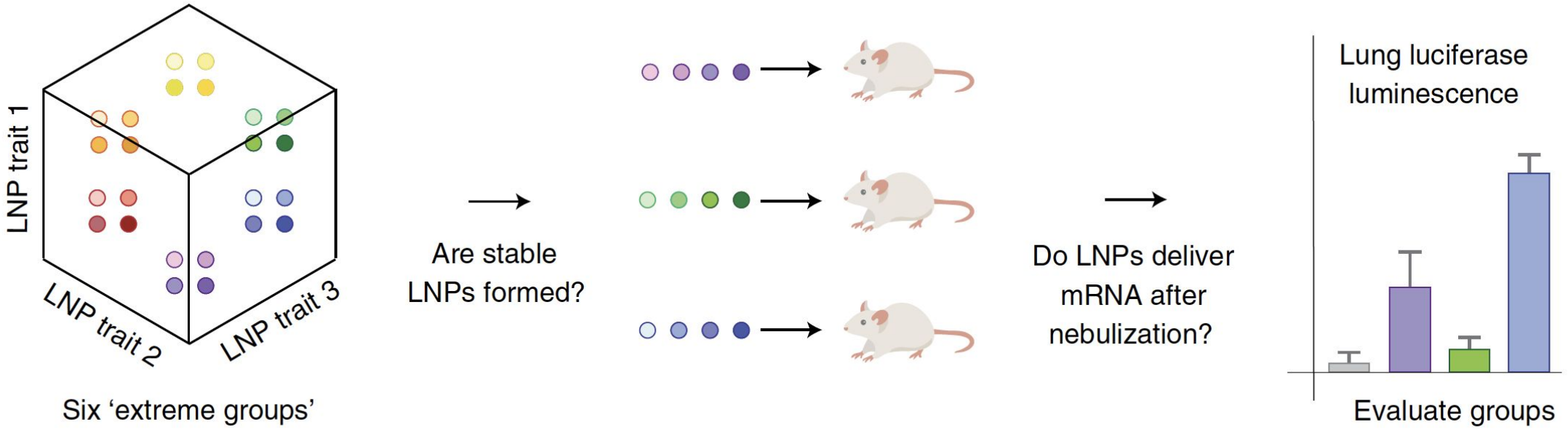
Dobrowolski*, Paunovska*, et al., *Nat Nano* 2022.

Transcriptional signatures in LNP^{High} vs LNP^{Low} cells



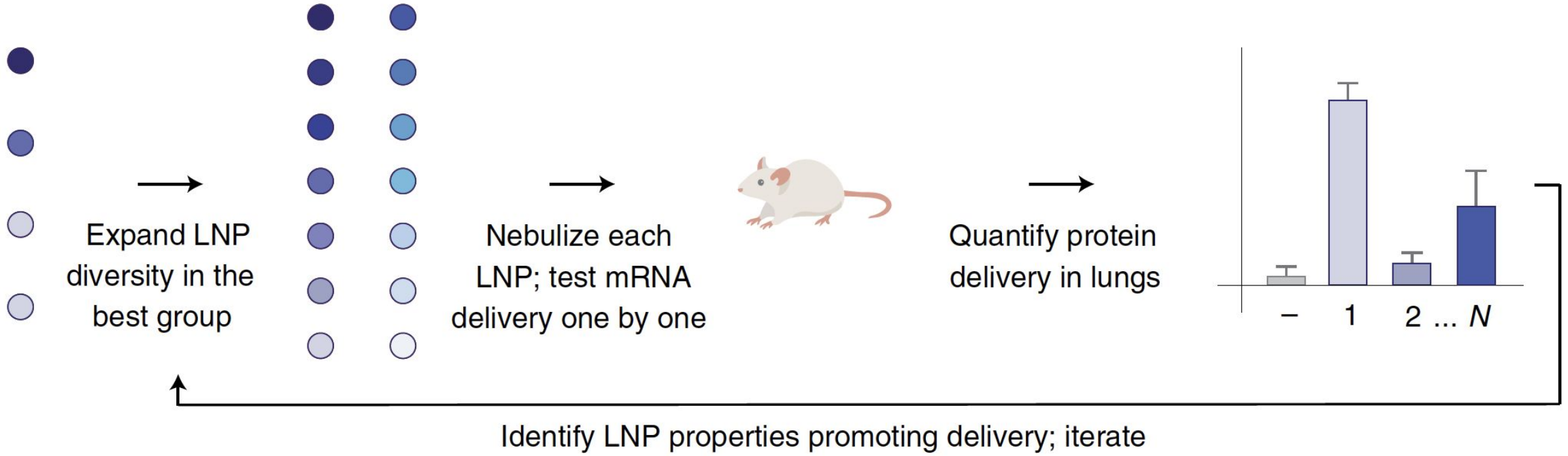
Dobrowolski*, Paunovska*, et al., *Nat Nano* 2022.

Finally, we screen across wide chemical spaces



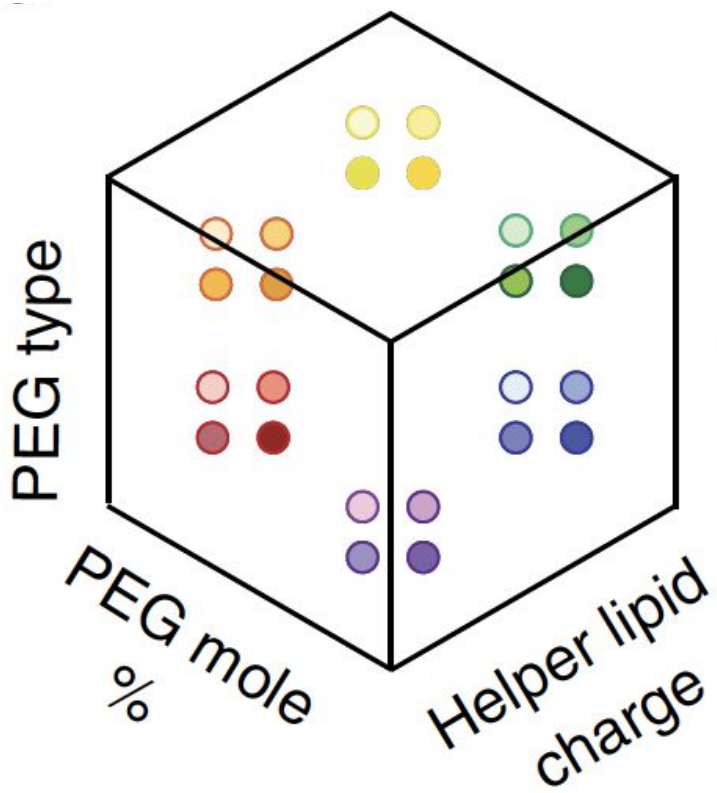
Lokugamage*, Vanover*, Beyersdorf*, Hatit*, et al., *Nat BME* 2022.

After a wide LNP screen, we “zoom in”



Lokugamage*, Vanover*, Beyersdorf*, Hatit*, et al., *Nat BME* 2022.

This approach led us to nebulization LNP design rules



110 LNPs formulated

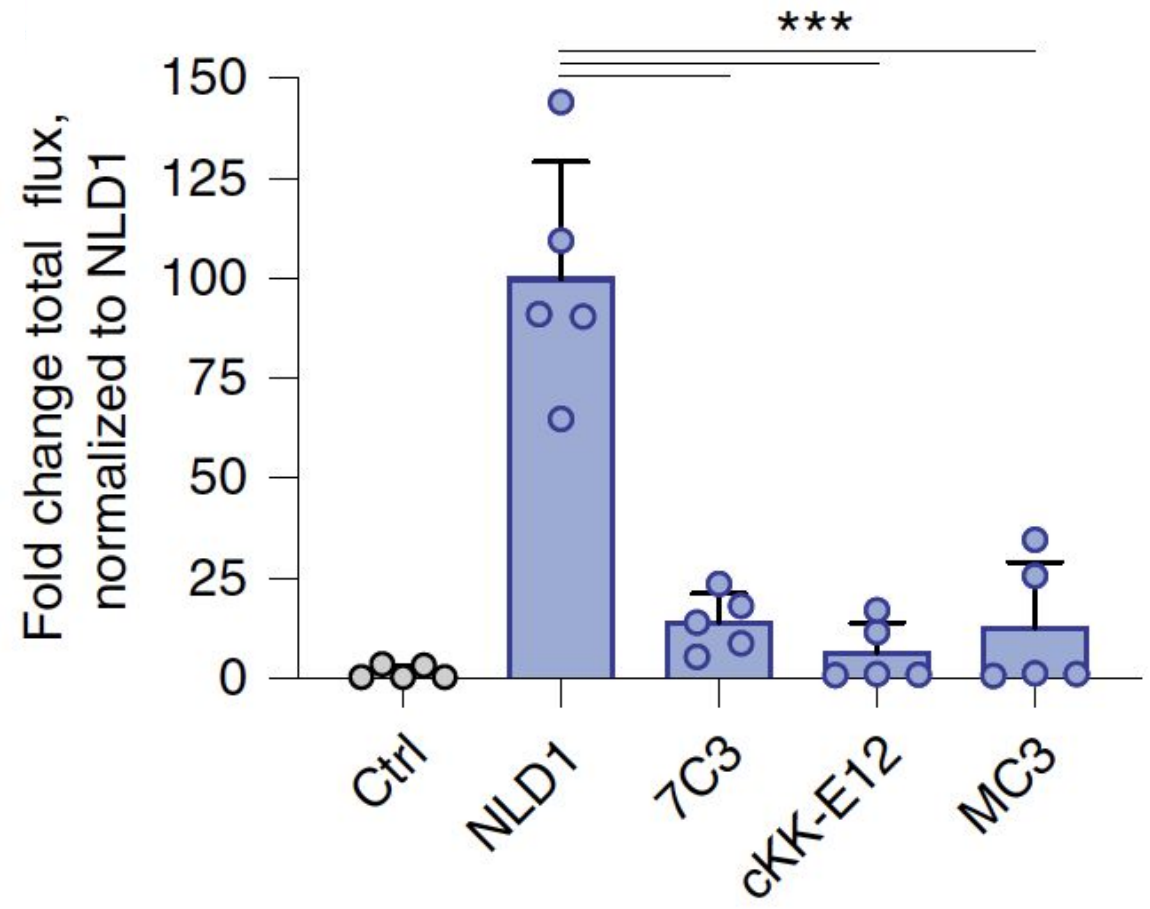
54 nebulized/in vivo

PEG is important for nebulized lung LNP delivery

1) PEG is necessary for LNP stability
2) Helper lipids and PEG interact
   (a) Neutral lipid + low PEG
   (b) Cationic lipid + high PEG

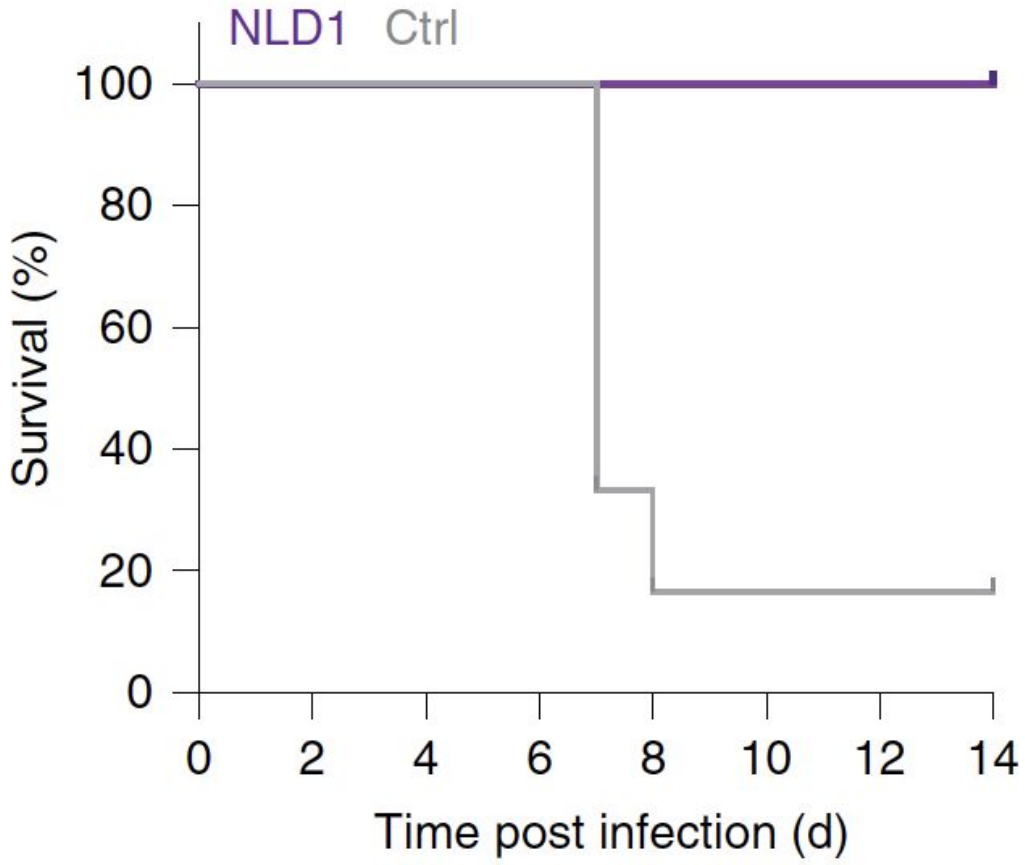
Lokugamage*, Vanover*, Beyersdorf*, Hatit*, et al., *Nat BME* 2022.

Nebulized LNPs designs is not the same as systemic LNPs



Lokugamage*, Vanover*, Beyersdorf*, Hatit*, et al., *Nat BME* 2022.

NLD1 can deliver mRNA protecting against fatal influenza



Lokugamage*, Vanover*, Beyersdorf*, Hatit*, et al., *Nat BME* 2022.

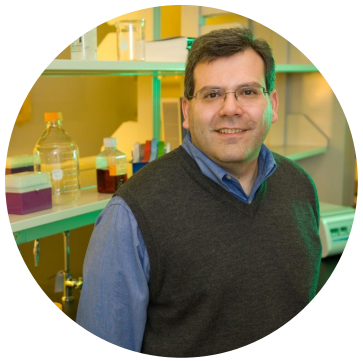
What have we learned?

Most lipids are unexplored.

Keep LNP structure as simple as possible.

You find what you look for, so run predictive assays.

Sincere thanks to collaborators, funders, trainees!



Phil Santangelo

Eric Sorscher

Hanjoong Jo

Jeffrey Glenn

Kalina Paunovska

Melissa Lokugamage

Curtis Dobrowolski

Marine Hatit

NIH The Common Fund

NIH NIGMS

NIH NIDCR

NIH NIAID

DARPA

CMT Research Foundation

Two additional delivery papers

nature chemistry

Article

https://doi.org/10.1038/s41557-023-01138-9

Nanoparticle stereochemistry-dependent endocytic processing improves in vivo mRNA delivery

nature biomedical engineering

ARTICLES

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