UCI University of California, Irvine

R&D Vaccine Center

mRNA Lipid Nanoparticles: Past, Present & Future

150 Years of Vaccine Science

Questions From the mRNA LNP Vaccine Hesitent Public

Q1: Is the mRNA Lipid Nanoparticle Vaccine Safe ?

A: Yes we've been working on it for 35 years, it's been administered to humans, and it is safe.

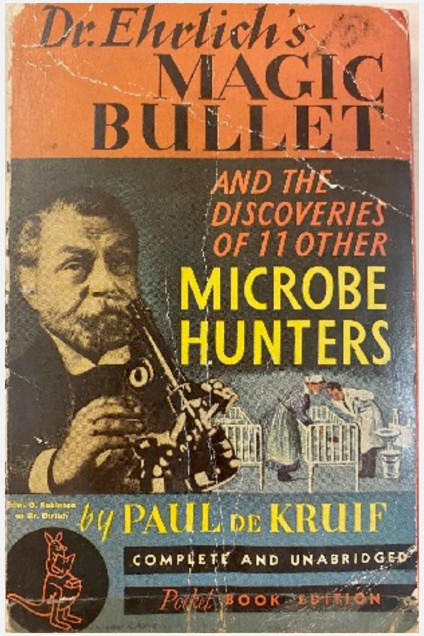
Q2: If you've been working on it for 35 years, why did it take so long ?

A: ???

Outline

- **Vaccine History – Timeline**
  - Deploying and Adopting Scientific Discovery Takes Time
- **Bangham's Magic Bullets (Ehrlich)**
  - Lipid bilayer membranes (Thompson)
- **Lipoplex**
  - Synthetic self-assembling gene delivery systems
- **Nucleic Acid Vaccines**
  - How good are they?
- **What's next?**

Copyright 1926



https://archive.org/details/in.ernet.dli.2015.221187

"Charite" https://www.netflix.com/ca/title/80178971

Epidemics of the Past

430 B.C.	Plague of Athens
160 A.D.	Plague of Antinonine
542 A.D.	Plague of Justinian
1340 A.D.	The Medieval Plague
1500 A.D.	Plague of the Incas
1665 A.D.	Great Plague of London
1793 A.D.	Yellow Fever
1832 A.D.	Cholera
1918 A.D.	Influenza
20-21st Century	Ebola, HIV, Swine Flu, Chikungunya, Zika

"The impact of vaccination on the health of the world's peoples is hard to exaggerate. With the exception of safe water, no other modality has had such a major effect on mortality reduction and population growth."

Susan and Stanley Plotkin
A Short History of Vaccination, in *Vaccines* 1st Edition, 1988

Stanley Plotkin

Emeritus Professor of Pediatrics, Perelman School of Medicine, University of Pennsylvania

To my Vaccine History slide deck Stanley replied,

"I hope you will forgive me for saying that I think your analysis is oversimplified."

49 Vaccines from 1798 – 2020

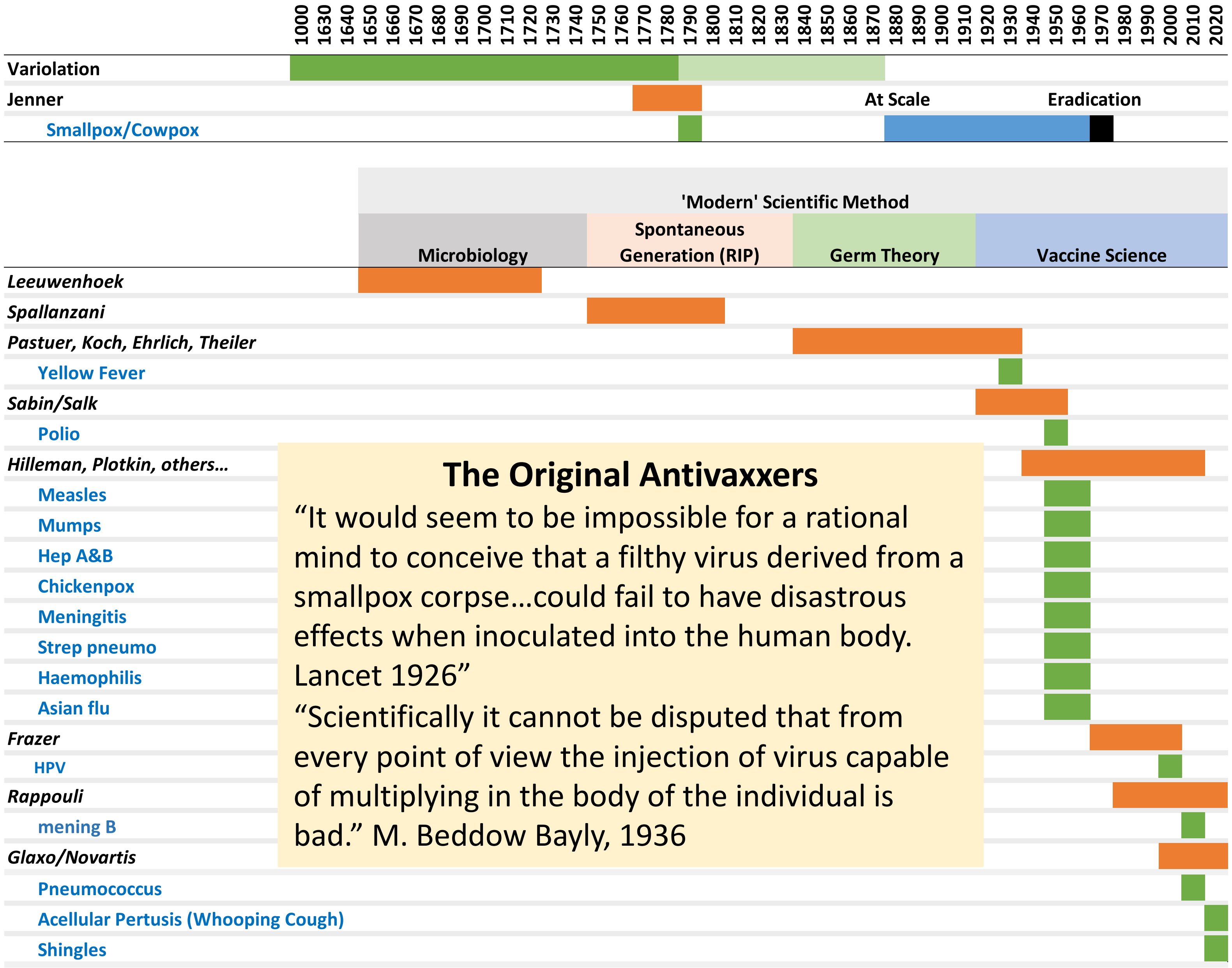
each with its own history

#	Year	Vaccine
1	1798	Smallpox (*live*)
2	1885	Rabies (*live*)
3	1886	Typhoid (*inactivated*)
4	1896	Cholera (*inactivated*)
5	1897	Plague (*inactivated*)
6	1923	Diphtheria (*toxoid*)
7	1924	Tetanus (*toxoid*)
8	1926	Pertussis (*inactivated*)
9	1927	TB (*live*)
10	1935	Yellow Fever (*live*)
11	1936	Influenza (*inactivated*)
12	1938	Typhus (*inactivated*)
13	1955	Polio (*inactivated*)
14	1963	Polio (*live, oral*)
15	1963	Measles (*live*)

#	Year	Vaccine
16	1967	Mumps (*live*)
17	1969	Rubella (*live*)
18	1970	Anthrax (*protein*)
19	1974	Meningococcal PS
20	1977	Pneumococcal PS
21	1980	Adenovirus (*live*)
22	1980	Rabies (*cell culture*)
23	1981	Tick encephalitis (*inactivated*)
24	1981	Hep B (*plasma*)
25	1985	Haemophilus (polysacc.)
26	1986	Hep B (*recombinant*)
27	1987	Haemophilus (conjugate)
28	1989	Typhoid (*live*)
29	1991	Cholera (recomb. Toxin)
30	1992	Japanese Encephalitis (*inact.*)

#	Year	Vaccine
31	1993	Varicella (*live*)
32	1994	Cholera (live)
33	1994	Typhoid (polysacc.)
34	1995	Pneumococcal (PS)
35	1996	Acellular Pertussis (protein)
36	1996	Hep A (inactivated)
37	1998	Lyme (OspA protein)
38	1999	Rotavirus (reassortants)
39	1999	Meningococcal (conjugate)
40	2000	Pneumococcal(conjugate)
41	200-	Live attenuate Flu
42	200-	Rotavirus
43	200-	Meningococcal A/C/W/Y
44	200-	HPV
45	200-	Zoster (live and subunit)
46	200-	Japanese Encephalitis (Vero)
47	200-	Meningococcal Group B
48	200-	Oral Cholera
49	2020	RTS,S Malaria

Vaccine History Timeline - *Adopting Scientific Discovery Takes Time – Why?*



The Original Antivaxxers

“It would seem to be impossible for a rational mind to conceive that a filthy virus derived from a smallpox corpse…could fail to have disastrous effects when inoculated into the human body. Lancet 1926”

“Scientifically it cannot be disputed that from every point of view the injection of virus capable of multiplying in the body of the individual is bad.” M. Beddow Bayly, 1936

Convincing Professional and Lay Communities – *takes time*

- Public Belief and Acceptance
  - 1992 Pope Paul II vindicated Galileo for heresy 350 years earlier
  - Nucleic Acid vaccination isn’t ‘Cold Fusion’

Archaic Manufacturing – *takes time*

- ‘Vaccine Farms’
- Safety – Vaccinees and Manufacturing Personnel
- Scale - 300 million chicken eggs

Foundations of Modern Vaccine and Lipid Nanoparticle Science

Foundations of Vaccine and Lipid Nanoparticle Science

A Personal Timeline

1885 - 1915

Pasture, Koch, Ehrlich – Germ Theory, Antibodies, Antibiotics, Attenuated, Organism Vaccines

1950-75

Salk/Sabin Polio Vaccine

Maurice Hilleman –>

9 of 12 recommended Vaccines for children

1965

Alec Bangham – 'Liposomes'

'Magic Bullets'

1978

Tom Thompson – Lipid Vesicle Biophysics, University of Virginia

Tom Thompson 1926 - 2021

1982

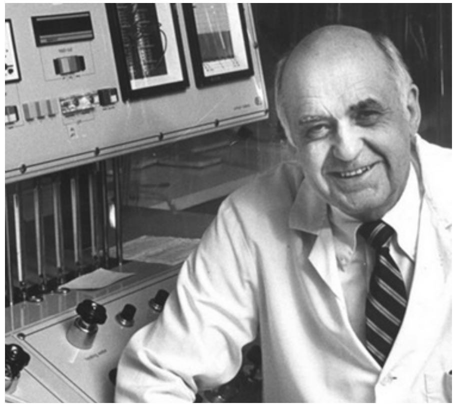
Tony Allison – Vaccine Science, squalene nanoemulsion adjuvant (Addavax), Syntex Research

1988

Felgner – Vical

DNA/RNA Vaccines

Merck sponsored research



Maurice Hilleman

2002

UC Irvine -> Vaccine Science

• Hydrodynamic Properties of lipid vesicles

Proc. Natl. Acad. Sci. USA
Vol. 75, No. 1, pp. 308–310, January 1978
Biophysics

Geometric packing constraints in egg phosphatidylcholine vesicles*

(inner and outer monolayers of vesicle bilayers/transmembrane asymmetry)

C. HUANG† AND J. T. MASON

Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, Virginia 22901

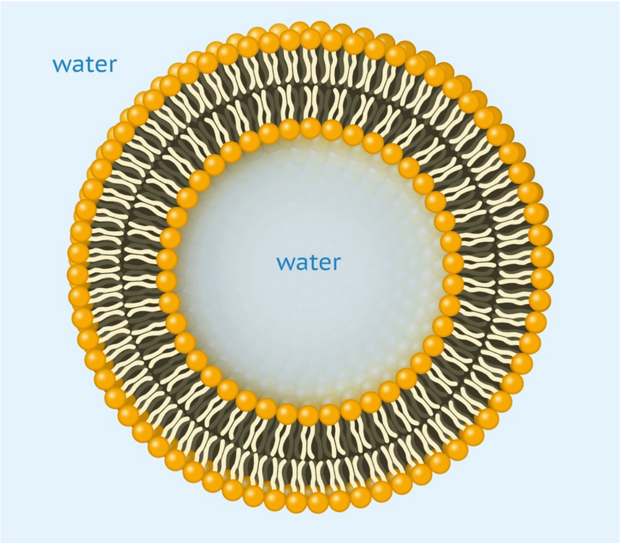
Communicated by Manfred Eigen, October 17, 1977

20 nm Small Unilamellar Vescicle (SUV)
2,500 lipid molecules
Molecular Weight = 1.9 million Kd

Table 2. Geometric packing parameters for egg phosphatidylcholine vesicles

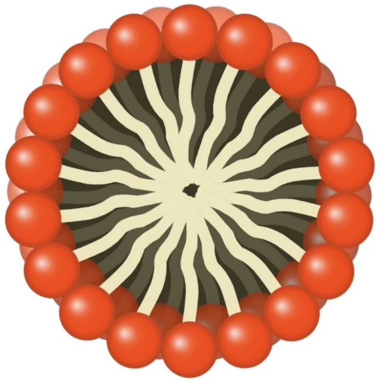
Parameter	Values
Radial parameters	R_A = 62 Å; R_B = 78 Å; R_C = 99 Å
Outer monolayer thickness	$(R_C - R_B)$ = 21 Å
Inner monolayer thickness	$(R_B - R_A)$ = 16 Å
Number of lipid molecules in each monolayer	n_o = 1658; n_i = 790
Volume per lipid molecule	$V_o = V_i$ = 1253 Å^3
Surface area per lipid head group	Outer, 74 Å^2; inner, 61 Å^2
Acyl chain cross section at R_B	Outer, 46 Å^2; inner, 97 Å^2
Anhydrous bilayer thickness	$(R_C - R_A)$ = 37 Å

Small Unilamellar Liposome (SUV)

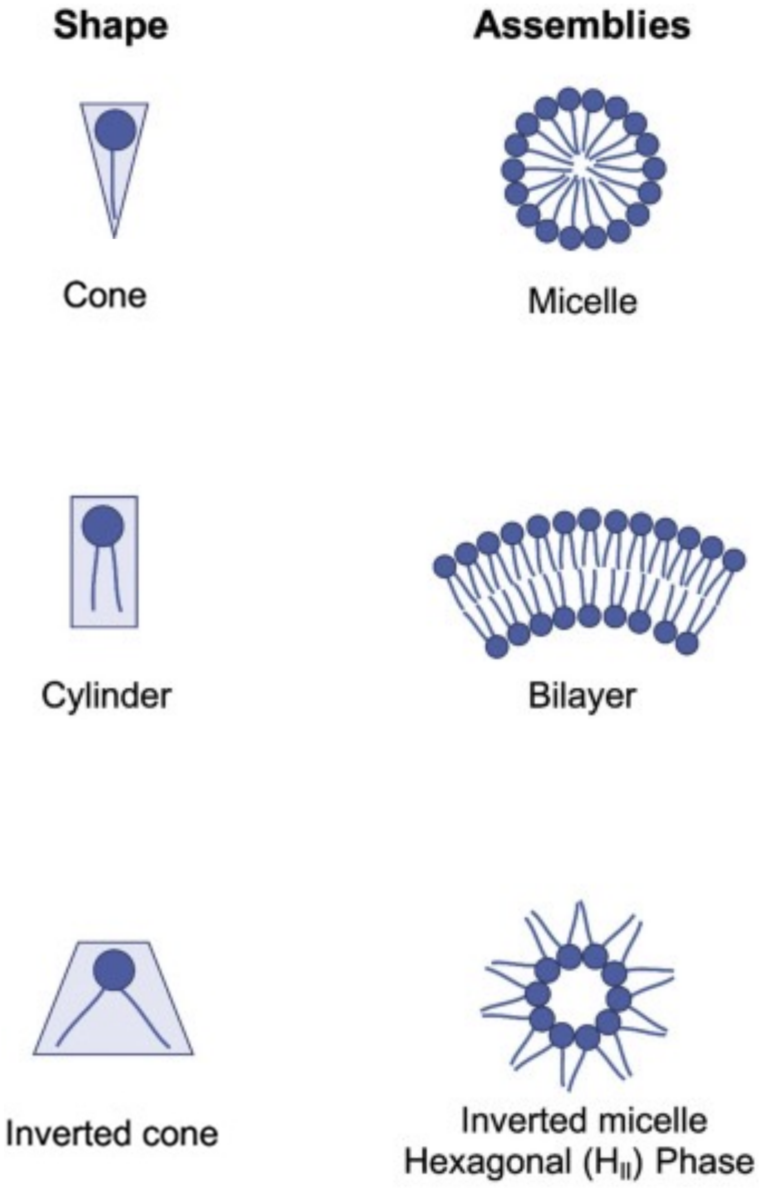


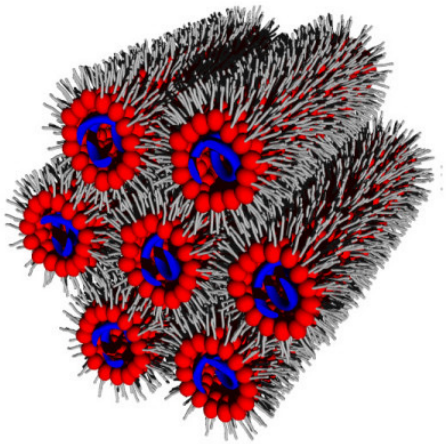
Lipid Shape determines Nanoparticle structure

A self-assembling system whose structure is determined by the shape and physical properties of monomers

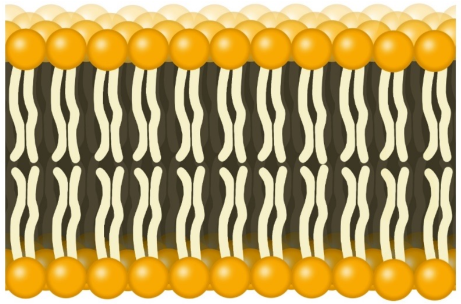


lipid micelle





inverted micelle Hex II phase

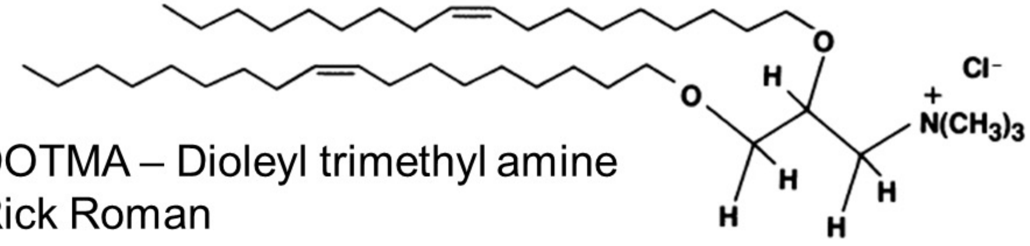


lipid bilayer

No Positivley Charged Lipids In Nature

Gene and Vaccine Science at Syntex

- In 1982 there were no positively charge lipid molecules that could form stable bilayers



DOTMA – Dioleyl trimethyl amine
Rick Roman

- The First Stable Cationic Bilayer Vesicles
  - Drug delivery vehicle
  - Cell penetration enhancer
  - Vaccine formulation
  - **Nucleic acid delivery**

Tony Allison 1925 – 2014

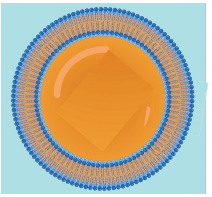
Syntex Adjuvant Formulation 1 (SAF-1) squalene O/W emulsion (Addavax) became, Novartis MF59

MF59-adjuvanted H1N1 pandemic influenza vaccine (Focetria® and Celtura®)

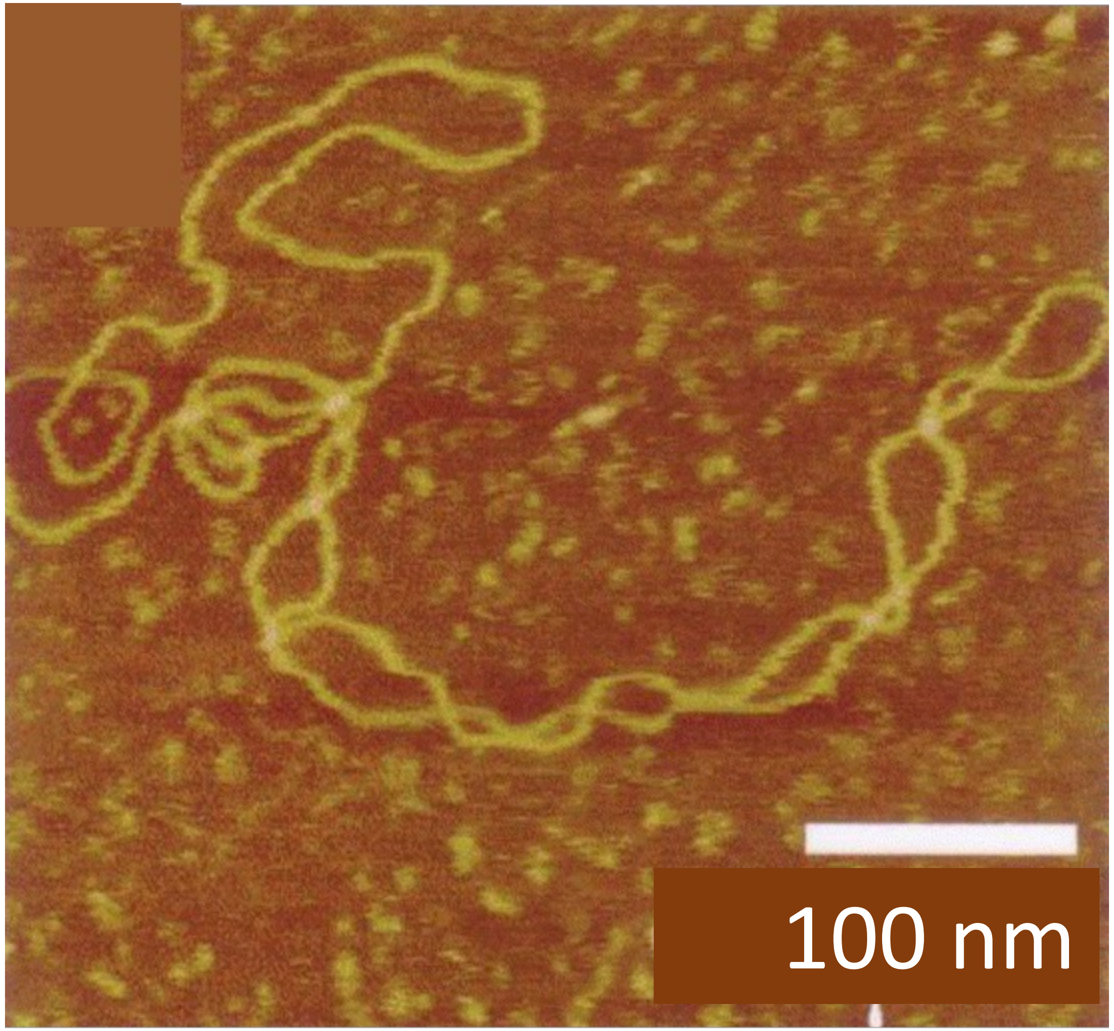
Virus-like Liposomes for Gene Delivery ?

The Problem with Virosome Synthesis:
Low DNA Encapsulation Efficiency

Liposome
Internal
Diameter



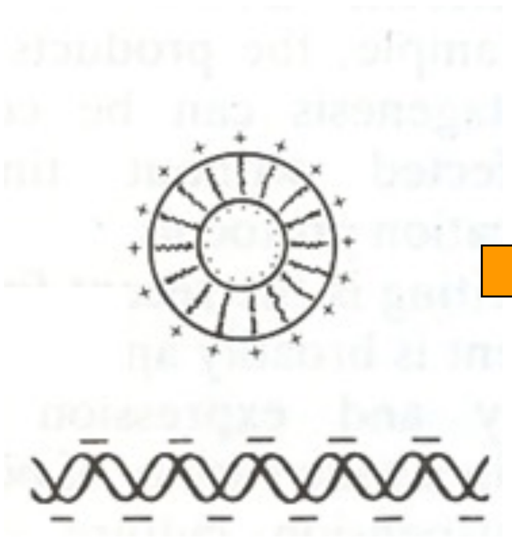
70 nm

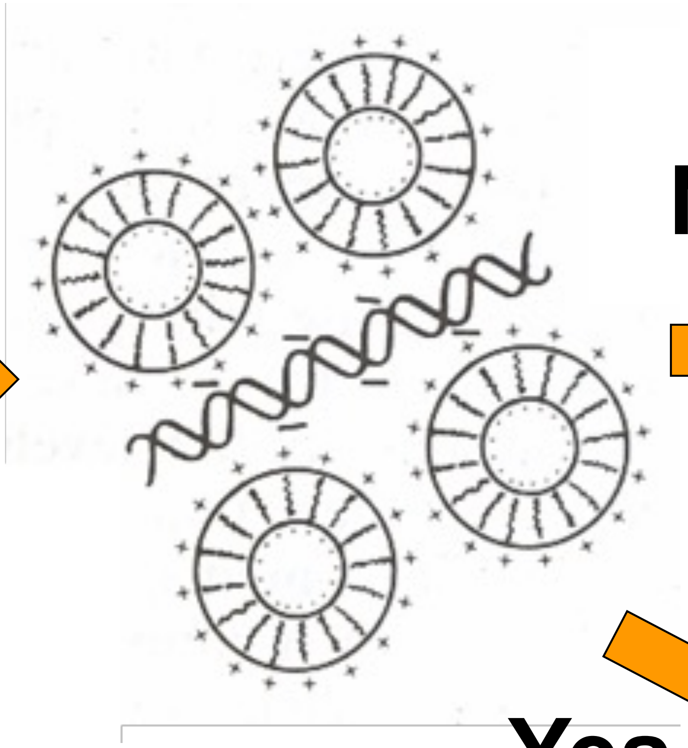


5,000 Base Pair Plasmid
→ 1,700 nm long

Can Cationic Lipid Vesicles capture nucleic acid?

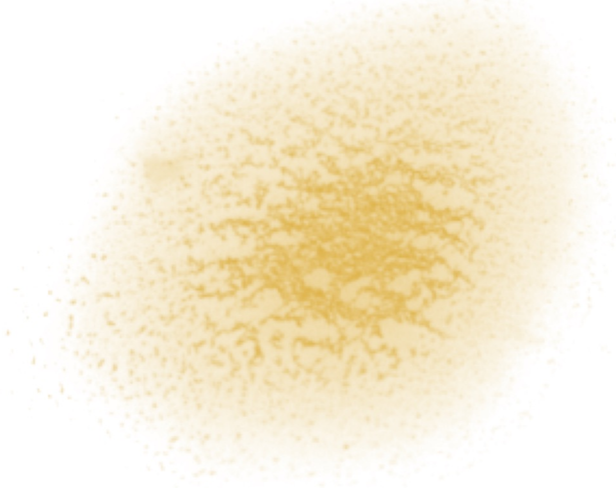
Make Virosomes by self-assembly rather then encapsulation





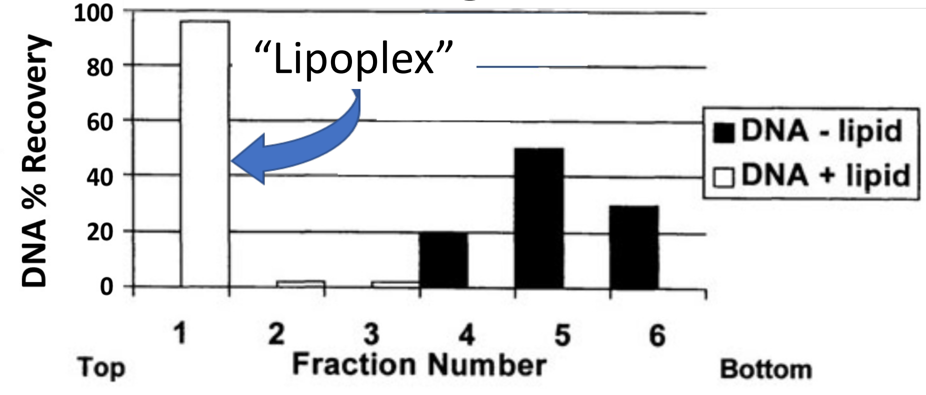
No ?

Surprise #1
No Aggregation



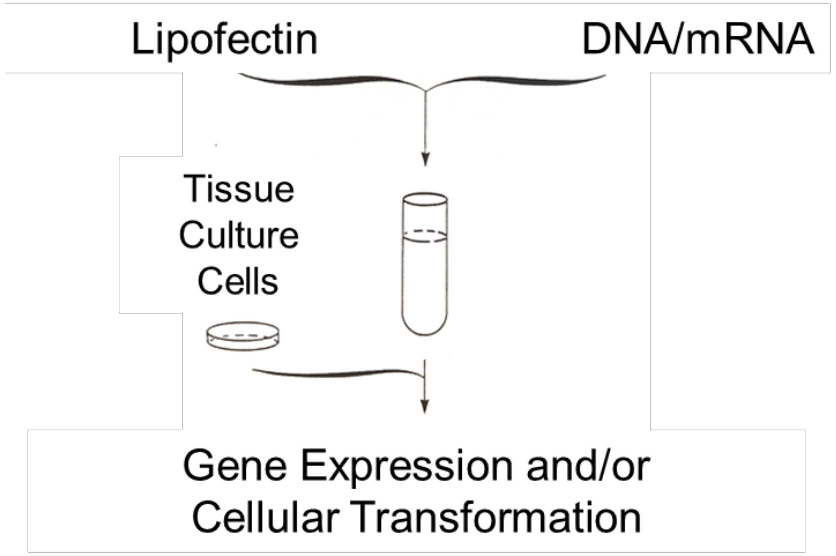
Yes

Centrifugation

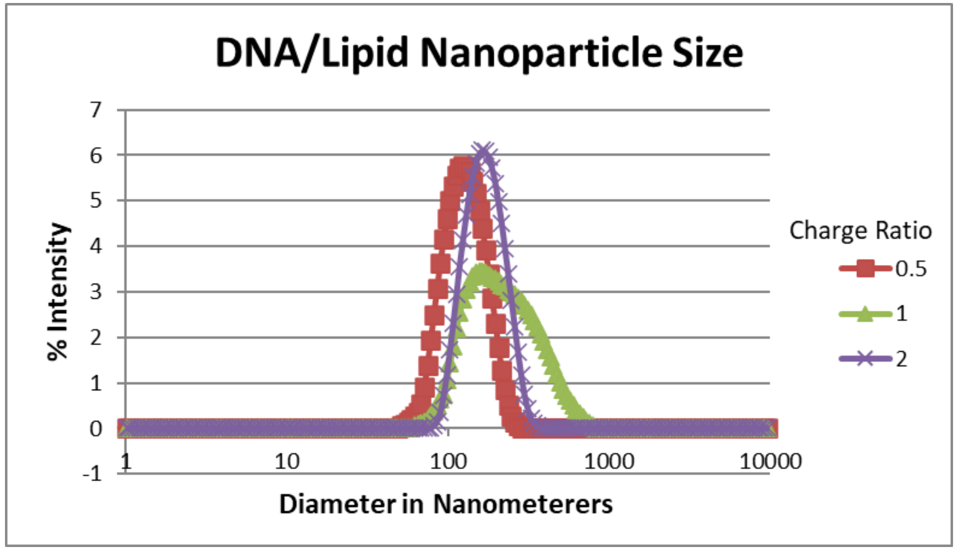


Surprise #2: It worked right away to deliver and express functional plasmid DNA and mRNA genes into living cells
Genetic Reprograming ~1985

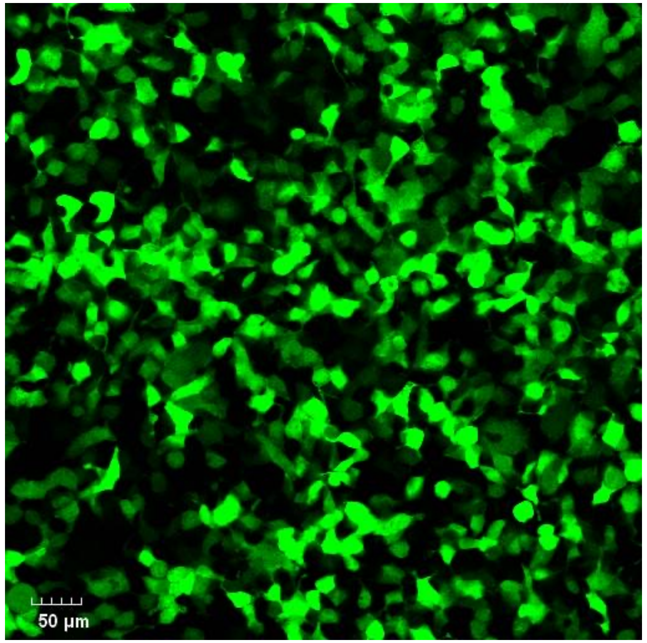
Lipofection Reagent
Convenient Genetic Reprograming of Cells in the Laboratory



90 – 150 nm Lipid Nanoparticles



Green Fluorescent Protein Expression in Cultured Cells after '*Lipofectin*'

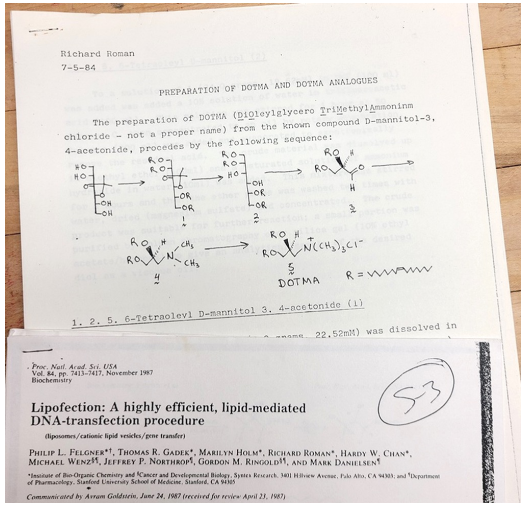


DOTMA patent

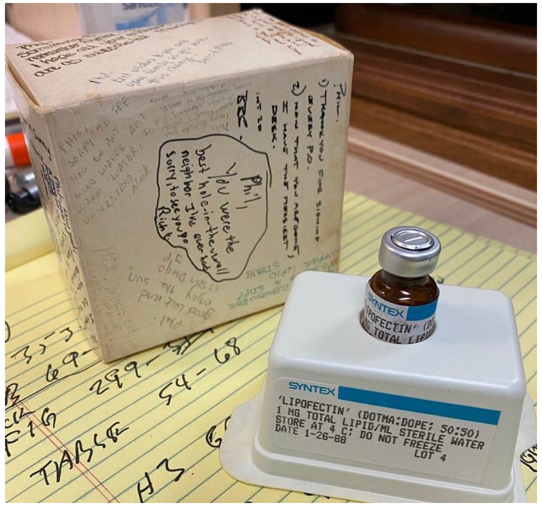
Surprise #3: Lipofectin is a phenomenally successful product

Value - $300M/year for 30 years

1984
DOTMA Synthesis



1987
Lipofectin Reagent



United States Patent [19] — **Eppstein et al.**

[11] **Patent Number:** 4,897,355

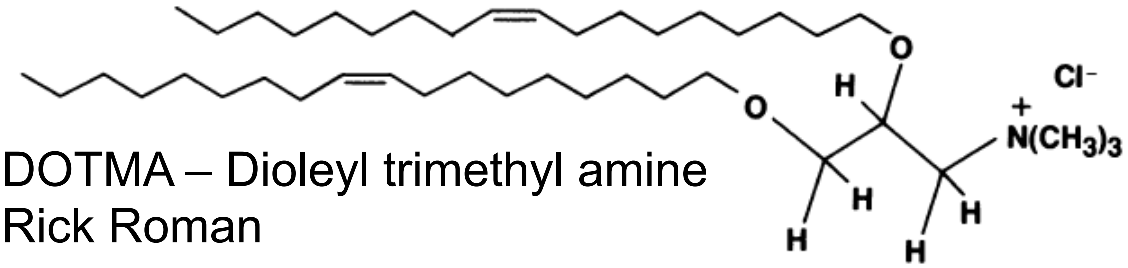
[45] **Date of Patent:** Jan. 30, 1990

N[ω,(ω-1)-DIALKYLOXY]- AND N-[ω,(ω-1)-DIALKENYLOXY]-ALK-1-YL-N,N,N-TETRASUBSTITUTED AMMONIUM LIPIDS AND USES THEREFOR

Inventors: **Deborah A. Eppstein**, Menlo Park; **Philip L. Felgner**, Los Altos; **Thomas R. Gadek**, Oakland; **Gordon H. Jones**, Cupertino, all of Calif.; **Richard B. Roman**, Fairhope, Ala.

Assignee: **Syntex (U.S.A.) Inc.**, Palo Alto, Calif.

Filed: **Oct. 29, 1987**



DOTMA – Dioleyl trimethyl amine
Rick Roman

https://www.romangoshoes.com/video.html

DNA/RNA Lipofection In Vivo

- **Surprise #4:** Syntex - 1987
  - "No, we can't support it. Gene Therapy is for the year 2020", Hardy Chan, Dir. Mol Biol
- Started Vical Inc.

Surprise #5: The in vivo study worked the first time

Jon Wolff
University Wisconsin
1956-2020

Volume 12 Number 18 1984 — Nucleic Acids Research

Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter

D.A.Melton, P.A.Krieg, M.R.Rebagliati, T.Maniatis, K.Zinn and M.R.Green

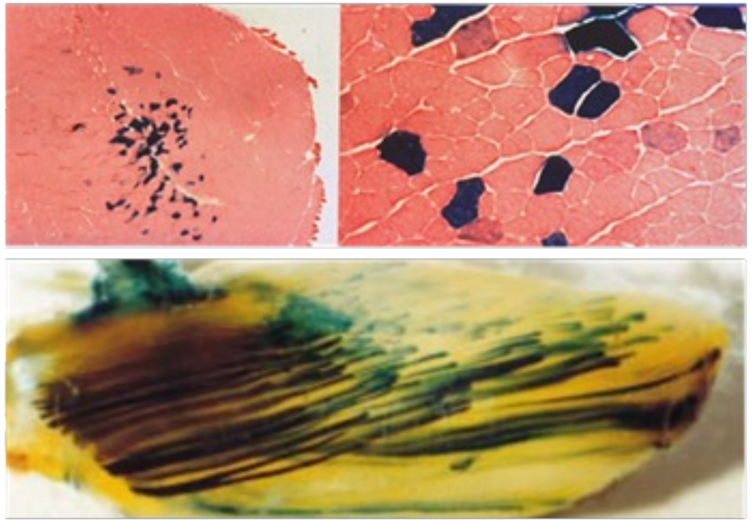
Doug Melton
Harvard
mRNA Synthesis & Expression in Oocytes

1990 – Naked Gene Transfer

Reprint Series 23 March 1990, Volume 247, pp. 1465-1468 — SCIENCE

Direct Gene Transfer into Mouse Muscle in Vivo

JON A. WOLFF,* ROBERT W. MALONE, PHILLIP WILLIAMS, WANG CHONG, GYULA ACSADI, AGNES JANI, PHILIP L. FELGNER



Merck & Co, License
Maurice Hilleman

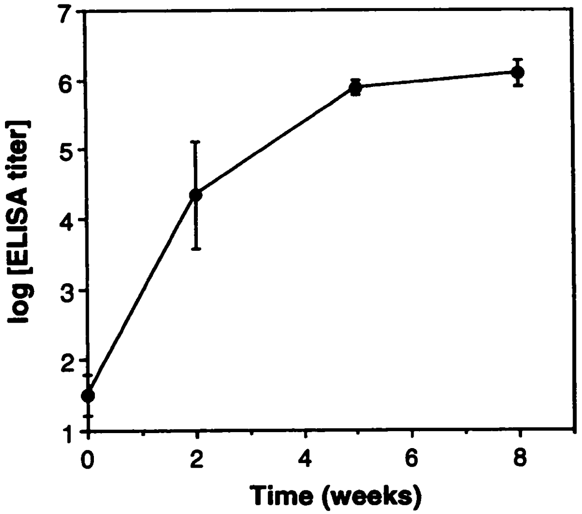
Nucleic Acid Vaccines
Surprise #6: It worked the first time

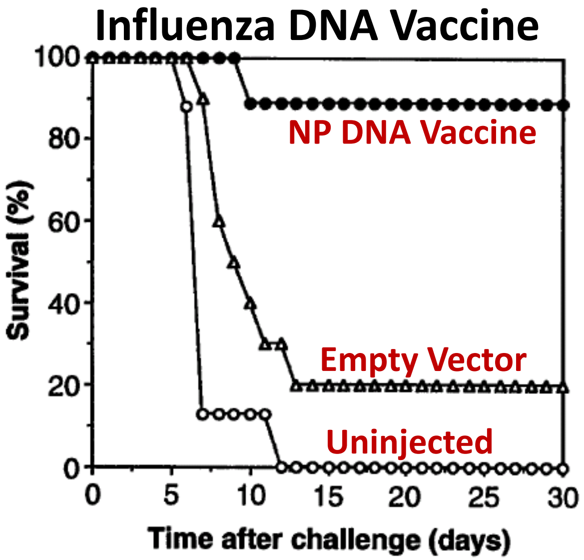
Jeff Ulmer
TechImmune LLC

Nancy Haigwood
Oregon Health & Science Univ.

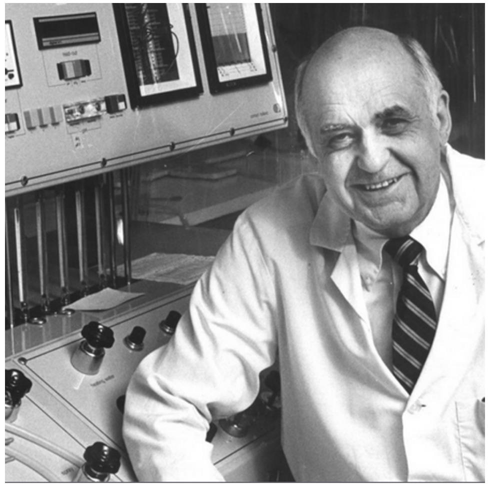
Heterologous Protection Against Influenza by Injection of DNA Encoding a Viral Protein

Jeffrey B. Ulmer,* John J. Donnelly,* Suezanne E. Parker, Gary H. Rhodes, Philip L. Felgner, V. J. Dwarki, Stanislaw H. Gromkowski, R. Randall Deck, Corrille M. DeWitt, Arthur Friedman, Linda A. Hawe, Karen R. Leander, Douglas Martinez, Helen C. Perry, John W. Shiver, Donna L. Montgomery, Margaret A. Liu†





"This is one of the most exciting things in modern vaccinology", 1993

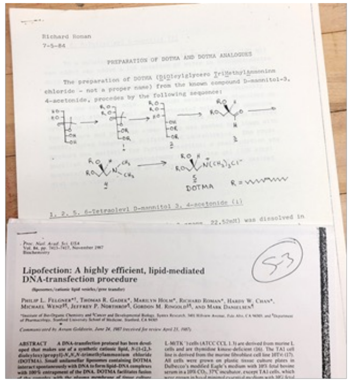


Maurice Hilleman 1919 – 2005
Merck Sharp & Dohme
Vaccines for Measles, mumps, hepatitis A & B, chickenpox, meningitides, Strep pneumo, Haemophilis, and Asian flu

Origins of the 'Secret Sauce' LNP that makes the mRNA vaccine great!

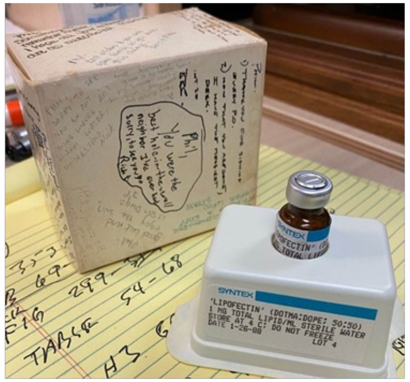
The UCI Lipid Nanoparticle Legacy

1984 DOTMA Synthesis
1st Lipid Nanoparticle

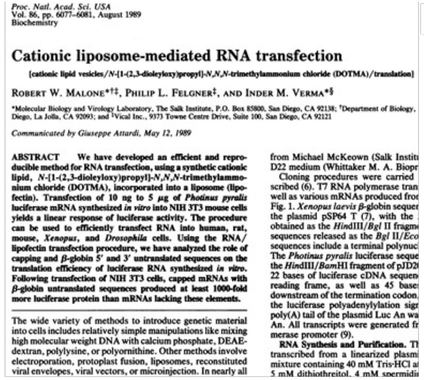


https://www.romangoshoes.com/

1987 – Lipofectin Reagent
$300 million Annual Sales



1989 – RNA Transfection
1st mRNA LNP


Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 6077–6081, August 1989
Biochemistry

Cationic liposome-mediated RNA transfection

Robert W. Malone, Philip L. Felgner, and Inder M. Verma

Communicated by Giuseppe Attardi, May 12, 1989

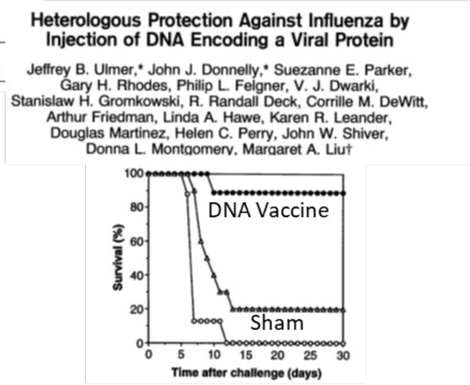
1990 – Naked Gene Transfer
1st In Vivo Gene Transfer


Science

Direct Gene Transfer into Mouse Muscle in Vivo

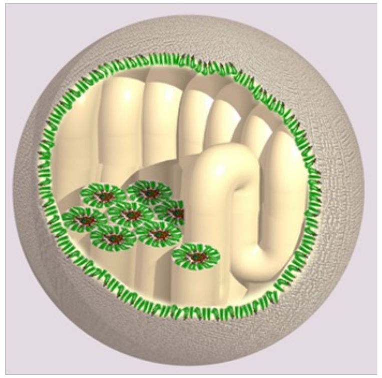
Jon A. Wolff,* Robert W. Malone, Phillip Williams, Wang Chong, Gyula Acsadi, Agnes Jani, Philip L. Felgner

1993 – Naked DNA Vaccine
1st Nucleic Acid Vaccines


Heterologous Protection Against Influenza by Injection of DNA Encoding a Viral Protein

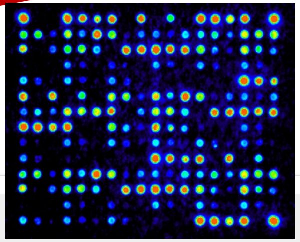
Jeffrey B. Ulmer,* John J. Donnelly,* Suezanne E. Parker, Gary H. Rhodes, Philip L. Felgner, V. J. Dwarki, Stanislaw H. Gromkowski, R. Randall Deck, Corrille M. DeWitt, Arthur Friedman, Linda A. Hawe, Karen R. Leander, Douglas Martinez, Helen C. Perry, John W. Shiver, Donna L. Montgomery, Margaret A. Liu†

1995 – Lipoplex "Secret Sauce'
LNP Structure



Time period

Coronavirus
Antigen
Microarray



1987 Vical Founded

1984 First DOTMA Synthesis
1990 DOTMA Patent Issued

1990 Direct Gene Transfer into Mouse Skeletal Muscle

1996 Delivery of exogenous DNA sequences in a mammal Patent issued

2010 Moderna

2016 DOTMA RNA Adjuvant Published

1982 1984 1986 1988 1990 1992 1994 1996 1998 2000 2002 2004 2006 2008 2010 2012 2014 2016 2018 2020

1987 Lipofection Published

1989 First RNA transfection published

1993 Influenza Nucleic Acid Vaccine

1992 Inovio

2000 CureVac

2008 BioNtech

2020 COVID mRNA Vaccine

17th LIPOSOME RESEARCH DAYS 2022

University of British Columbia, Vancouver, Canada

Alec Bangham Award

KEYSTONE SYMPOSIA

SEPTEMBER 06 - 08, 2023 | VIRTUAL | TO BE DETERMINED

NEXT-GENERATION NANOMEDICINE

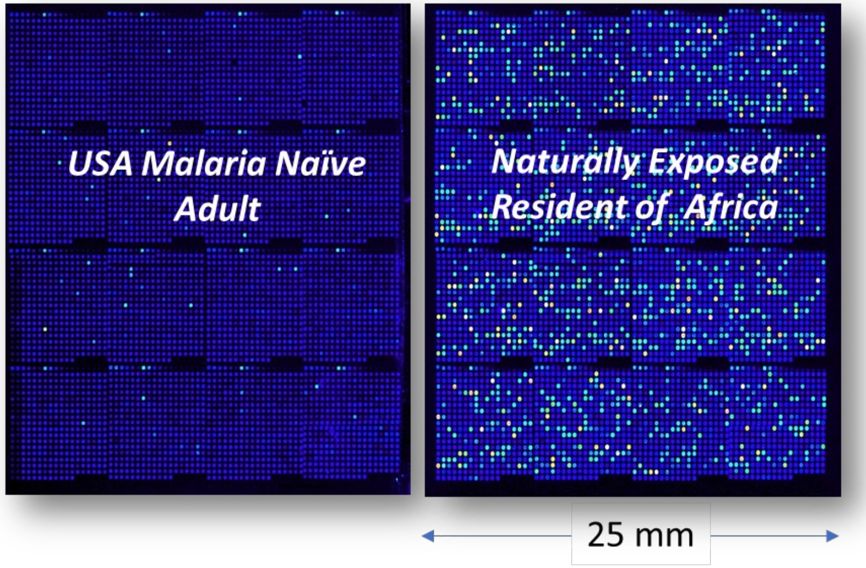
Organizers: Philip Felgner, Anna Blakney, and Norbert Pardi

UCI Vaccine Antigen Discovery – *2002*

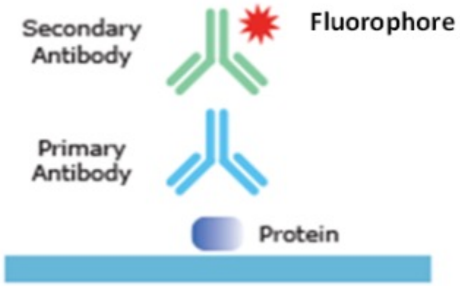
COVID Serosurveillance in Orange County – *2020*

Immunologic Imprinting with Proteome Microarrays

Example:
***P falciparum* Proteome Microarray – 5,200 Proteins**



Serological Testing Solid Phase ELISA with Microarrays
(requires 1 μl of blood)



38 Infectious Agents

100,000 arrays printed

80,000 sera Probed

Inventory of Cloned Genes, Proteins & Microarrays

		Category	Total # proteins	# completed
Retroviruses	HIV 1&2 (5 subtype, 4 clades)		83	74
Papilloma	HPV viruses (11 types)		88	88
Orthopoxviruses	3 types	A	260	260
Herpes Viruses	HSV 1&2, VZV, EBV		300	270
Flaviviruses	WNV, Dengue, YF, SLE, JE	A	50	50
Alphaviruses	Chikungunya	C	10	10
Bacteria	*Brucella melitensis*	B	3,194	3,190
	Chlamydia trachomatis		911	900
	Chlamydia muridarum		911	900
	Mycobacterium tuberculosis	C	3,990	3,899
	Francisella tularensis	A	1,933	1,874
	Coxiella burnetii	B	2,065	2,000
	Borrelia burgdorferi		1,600	1,400
	Burkholderia pseudomallei	B	5,728	1,400
	Leptospira interrogans		3,658	3,658
	Salmonella enterica Typhi	B	4,318	4000
	Orientia tsutsugamushi	C	1,400	1,400
	Rickettsia ricketsii	B	900	900
	Bartonella henselae	B	1,400	1,400
	Enteric toxogenic E. coli		9,000	9,000
	Streptococcus penumonia		6,000	6,000
	Staphylococcus aureus (MRSA)		2,628	2,509
	Vibrio cholera		4000	4000
	Clostridium difficile		4,000	40
Parasites	*Plasmodium falciparum*		8500	5,722
	Plasmodium vivax		5,300	2,200
	Schistosoma mansoni / japonicum		9,000	300
	Toxoplasma gondii		12,000	2,600
	Necator americanus		12,000	600
	Trypanosoma cruzi		15,099	240
	Trypanosoma brucei		8,529	214
Human	*Autoimmune array*		21,000	1,800
	Endometriosis		"	"
	Ovarian, Breast & Pancreas Cancer		"	"
Total			127,623	>60,000

Benchtop Microarray Assay for Serological Testing

Serological Testing with Coronavirus Antigen Microarrays (COVAM)

Printing Probing Imaging Analysis

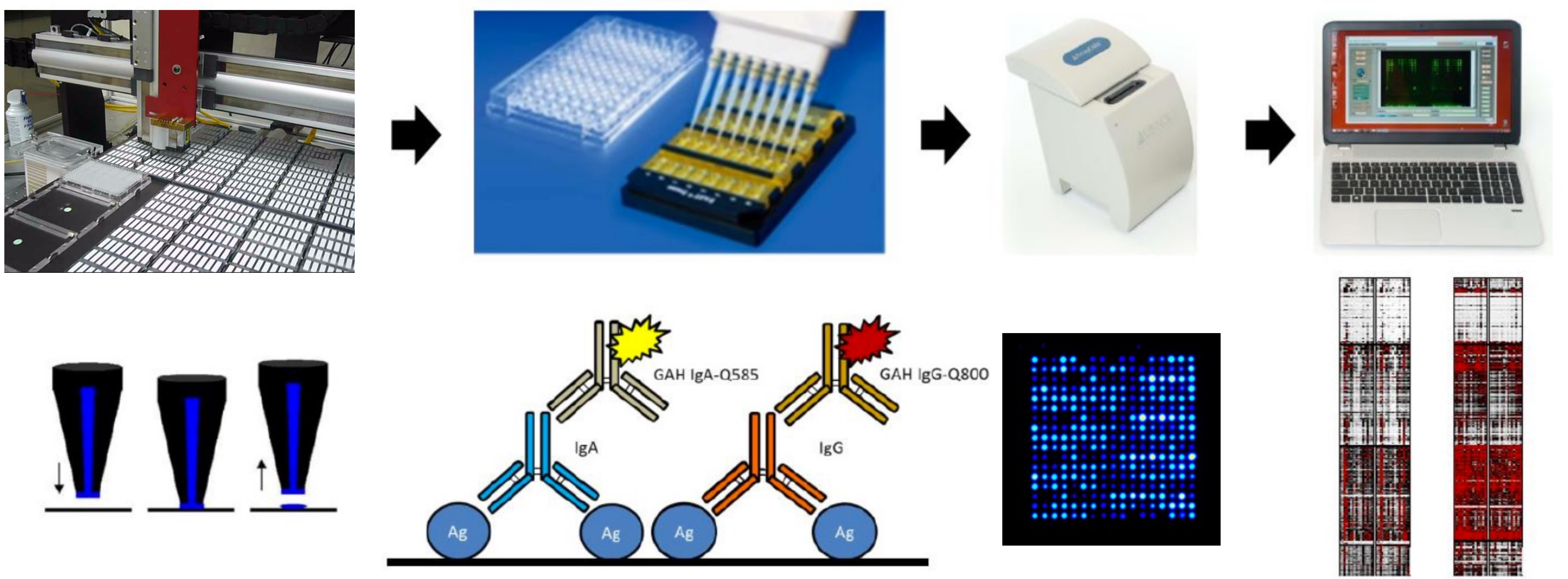


Figure 1. Schematic diagram illustrating the steps for printing, sample probing, array imaging and analysis of data from the CoVAM (*Khan S. et al., bioRxiv, 2020*).

Finger Stick Blood

Computational Analysis

Respiratory Virus Antigen Microarray – *'DARPA Dormatory Cohort - 2019'*

67 Antigens Printed in Quadruplicate

21 CoV-2/SARS/MERS

Virus	Subtype	Strain	Protein
CoV	Beta	SARS-CoV-2	NP
CoV	Beta	SARS-CoV-2	S1-RBD
CoV	Beta	SARS-CoV-2	S1
CoV	Beta	SARS-CoV-2	S1
CoV	Beta	SARS-CoV-2	S1
CoV	Beta	SARS-CoV-2	S2
CoV	Beta	SARS-CoV-2	S1+S2
CoV	Beta	SARS	PLpro
CoV	Beta	SARS	S1-RBD
CoV	Beta	SARS	S1-RBD
CoV	Beta	SARS	S1
CoV	Beta	SARS	NP
CoV	Beta	MERS	NP
CoV	Beta	MERS	S1-RBD
CoV	Beta	MERS	S1-RBD
CoV	Beta	MERS	S1-RBD
CoV	Beta	MERS	S1-RBD
CoV	Beta	MERS	S1
CoV	Beta	MERS	S1
CoV	Beta	MERS	S1+S2
CoV	Beta	MERS	S2

12 Common CoV

Virus	Subtype	Strain	Protein
CoV	Alpha	NL63	S1
CoV	Alpha	NL63	S1+S2
CoV	Alpha	229E	S1
CoV	Alpha	229E	S1+S2
CoV	Beta	HKU1	S1
CoV	Beta	HKU1	S1
CoV	Beta	HKU1	S1+S2
CoV	Beta	HKU1	HE
CoV	Beta	HKU23-368F	NP
CoV	Beta	OC43	S1
CoV	Beta	OC43	S1+S2
CoV	Beta	OC43	HE

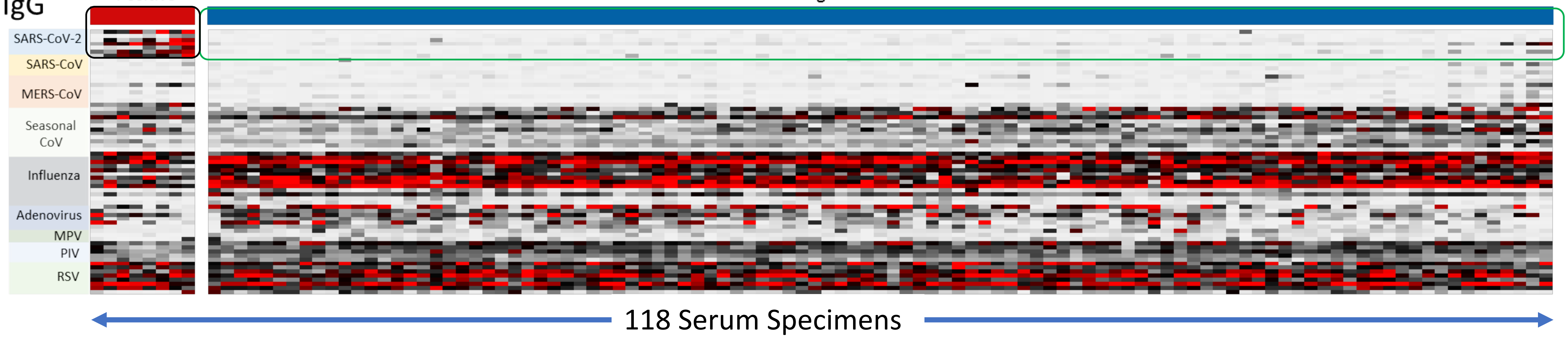
34 Other Respiratory Viruses RSV/MPV/PIV/Adeno/Flu

Virus	Subtype	Strain	Protein
RSV	A	LA2-94/2013	F
RSV	A	LA2-94/2013	G
RSV	A	A2	F
RSV	A	rsb1734	G
RSV	A	RSS-2	F
RSV	B	TH-10526/2014	F
RSV	B	TH-10526/2014	G
RSV	B	B1	G
MPV	A	PER/CFI0320/2010/A	G
MPV	B	PER/CFI0466/2010/B	G
MPV	B	PER/CFI0320/2010/A	F
PIV	1	12O3	F
PIV	1	12O3	H
PIV	3	USA/10991B/2010	H
PIV	4	hPIV-4b/10-H2/2016	F
PIV	4	hPIV-4b/10-H2/2016	H

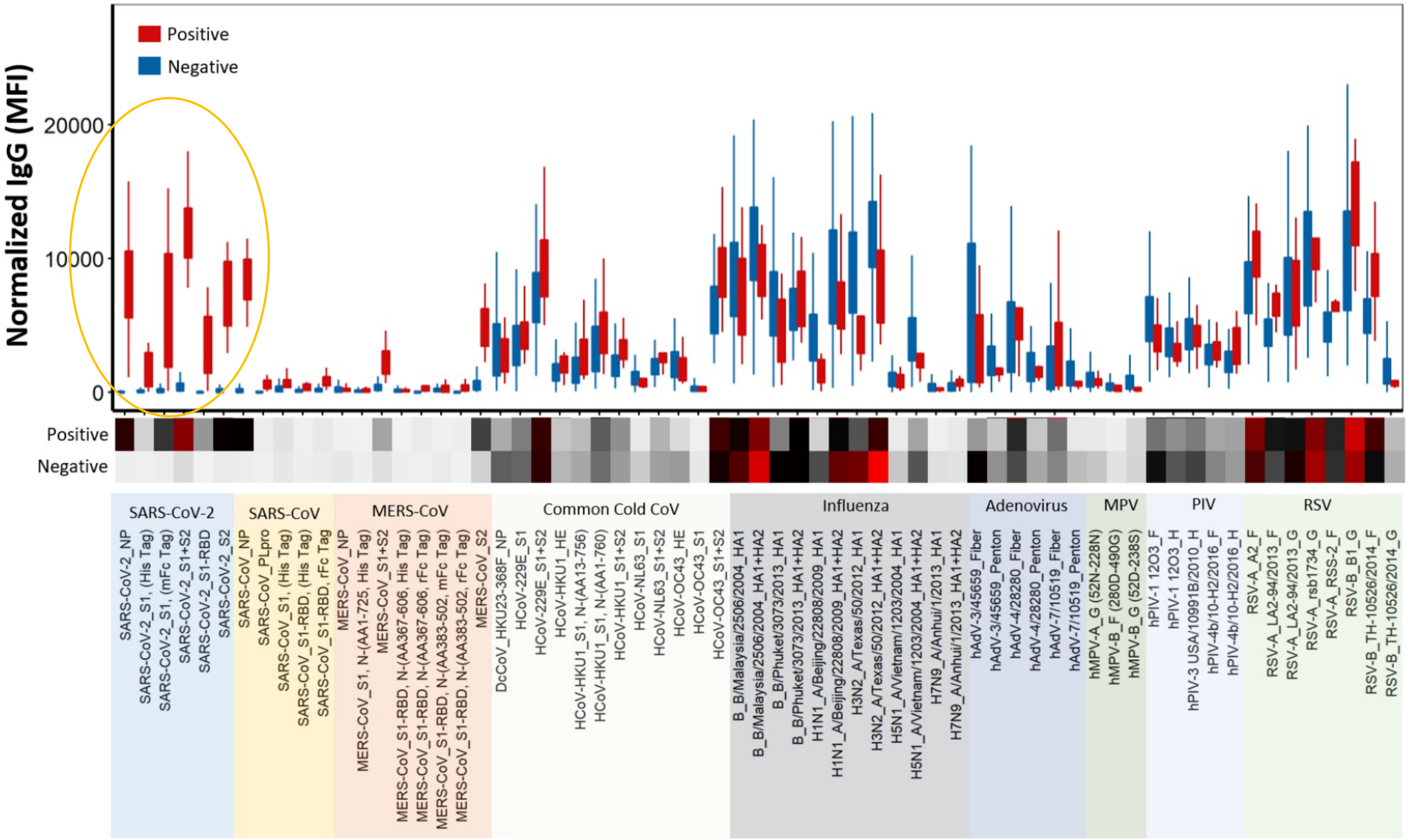
Virus	Subtype	Strain	Protein
Adeno	3	hAdV-3/46659	Fiber
Adeno	3	hAdV-3/46659	Penton
Adeno	4	hAdV-4/28280	Fiber
Adeno	4	hAdV-4/28280	Penton
Adeno	7	Adeno7 10519	Fiber
Adeno	7	Adeno7 10519	Penton
Flu	H1N1	A/Beijing/22808/2009	HA1
Flu	H1N1	A/Beijing/22808/2009	HA1+HA2
Flu	H3N2	A/Texas/50/2012	HA1
Flu	H3N2	A/Texas/50/2012	HA1+HA2
Flu	B	B/Malaysia/2506/2004	HA1
Flu	B	B/Malaysia/2506/2004	HA1+HA2
Flu	B	B/Phuket/3073/2013	HA1
Flu	B	B/Phuket/3073/2013	HA1+HA2
Flu	H5N1	A/Vietnam/1203/2004	HA1
Flu	H5N1	A/Vietnam/1203/2004	HA1+HA2
Flu	H7N9	A/Anhui/1/2013	HA1
Flu	H7N9	A/Anhui/1/2013	HA1+HA2

PCR Confirmed (8)
Positive

Pre CoV (110)
Negative



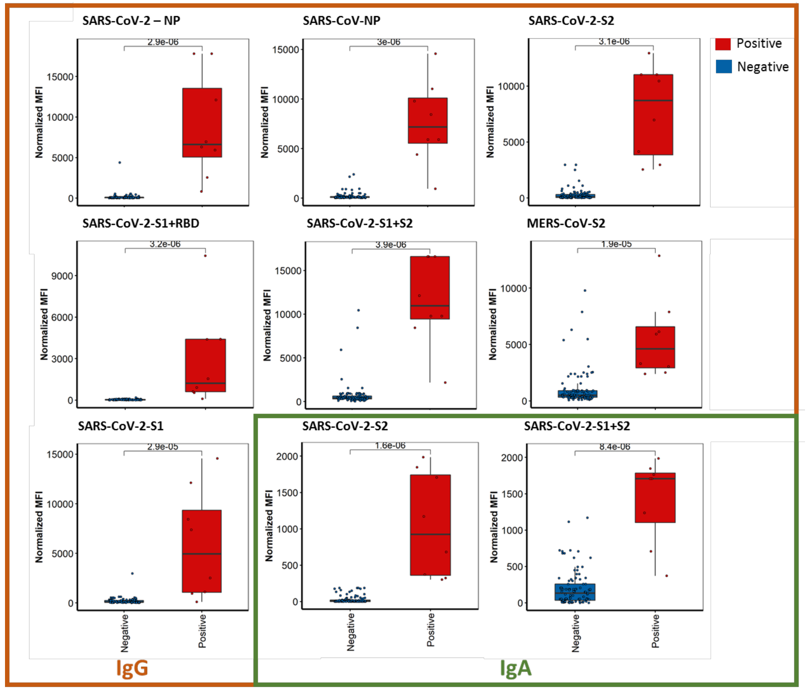
Coronavirus Antigen Microarray - COVAM

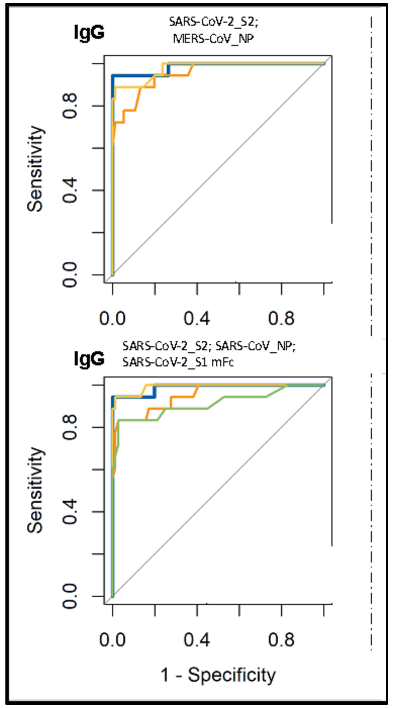


Why Microarray Testing for COVID-19?

- Tens to hundreds of antigens can be probed in a single test.
- Highly sensitive and specific.
- Can provide serological testing on a large scale.
- Can monitor antibody levels to many viruses simultaneously.
- Plasma samples can be stored for long periods of time and easily shipped.
- <u>The data informs containment, mitigation & vaccine development.</u>

Coronavirus Antigen Microarray – COVAM – Sensitivity & Specificity





Antibody	Antigen	Coefficient	Intercept	Cutoff	Sensitivity	Specificity
IgG	NP	-0.000577	-11.0079	0.634	95.5%	100%
	S1+S2	0.000191				
	RBD(rFc)	0.006581				
IgM	NP	-0.000429	-18.2677	0.689	90.9%	100%
	S1(His)	0.009209				
	S1-RBD	0.002402				

<u>Finger Stick Blood Collections</u>

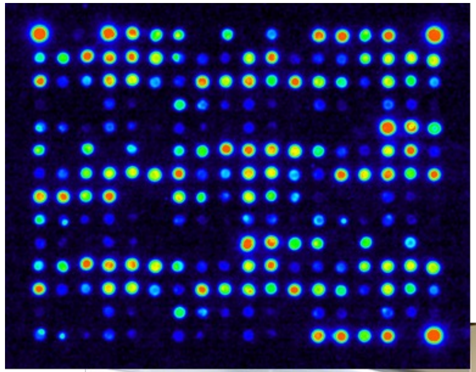
- 1,400 UCIMC Healthcare Workers
  - 10% Overall Seroprevalence
  - 14% Positive ICU Nurses
- 3,500 Orange County Residents
  - 10% Overall Seroprevalence
  - Granularity associated with demographics
- 1,400 UCIMC Longitudinal Study
- 8,000 Orange County Residents – December
- Up to 1,000 specimens / week
- 10,000 Individual Reports sent by QR code to each donor

Orange County Seroservailence Study

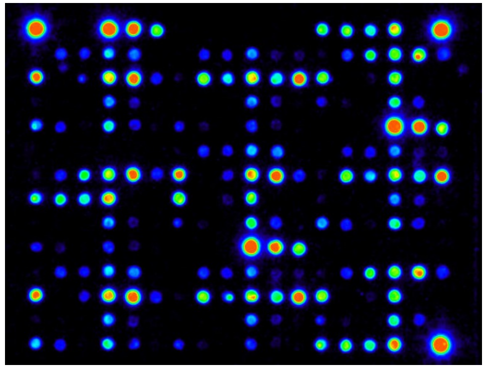
Monitoring Naturally Acquired Herd Immunity – *2020*

Virus	#	COVAM 4 Antigen Content
SARS-CoV-2	1	Nucleocapsid-His
	2	Spike S1 + S2-His
	3	Spike S2 ECD-His
	4	Spike S1-mFc
	5	Spike S1-His (Bac)
	6	Spike S1-His (HEK)
	7	Spike RBD-mFc
	8	Spike RBD-rFc
	9	Spike RBD-His (Bac)
	10	Spike RBD-His (HEK)
SARS	11	Nucleocapsid-His
	12	Spike S1-His
	13	Spike RBD-His
	14	Spike RBD-rFc
MERS	15	Nucleocapsid-His
	16	Spike S1+S2 ECD-His
	17	Spike RBD-rFc
Common CoV	18	HKU23-Nucleocapsid-His
	19	229E- Spike S1-His
	20	229E- Spike S1+S2 ECD-His
	21	HKU1-Nucleocapsid-His
	22	HKU1-Spike S1-His
	23	HKU1-Spike S1+S2 ECD-His
	24	NL63-Nucleocapsid-His
	25	NL63-Spike S1-His
	26	NL63-Spike S1+S2 ECD-His
	27	OC43-HA esterase-His
	28	OC43-Nucleocapsid-His
	29	OC43-Spike S1+S2 ECD-His
Influenza	30	B/Malaysia-HA1-His
	31	B/Malaysia-HA0-His
	32	B/PHUKET-HA1-His
	33	B/PHUKET-HA0- His
	34	A/Beijing-H1N1-HA1-His
	35	A/Beijing-H1N1-HA0- His
	36	A/Texas-H3N2-HA1-His
	37	A/Texas-H3N2-HA0-His

CoV Positive



Pre-CoV Negative



COVAM
Coronavirus
Antigen
Microarray

Fingerstick
Blood
Collection

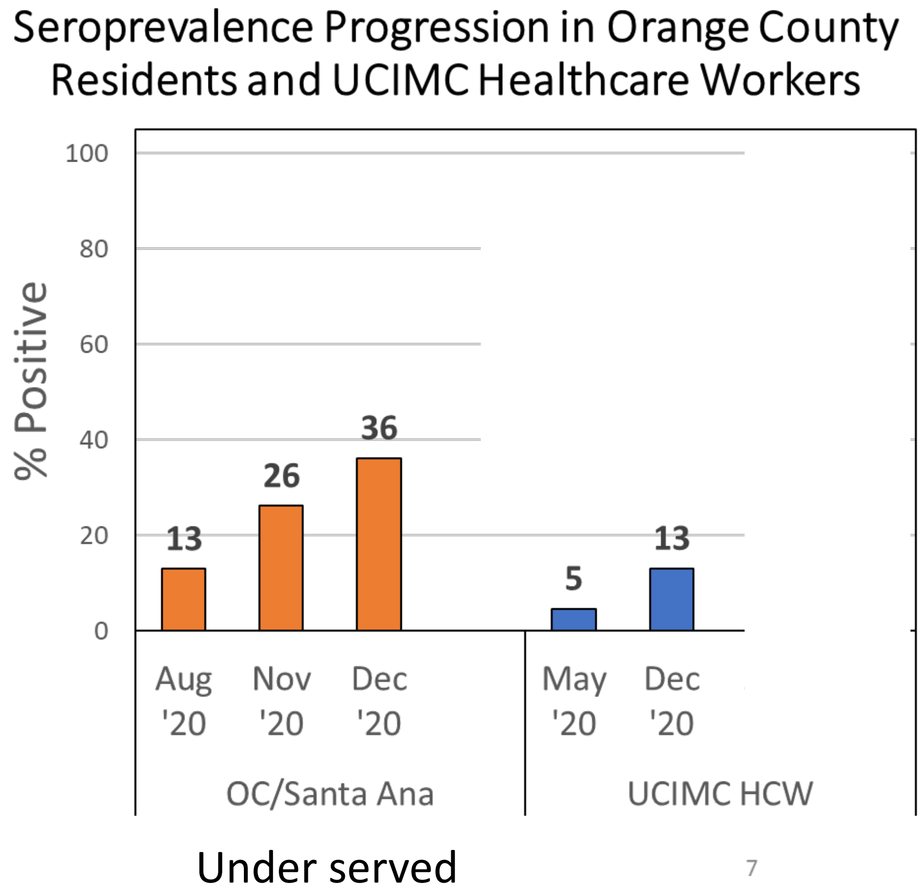
Measurements

Virus	Antigen #
SARS-CoV-2	10
SARS	4
MERS	3
Common CoV	12
Influenza A/B	8
Total	37
Triplicate	111

Samples

Collection	**Date**

Orange County Serosurveillance Study
Monitoring Naturally Acquired Herd Immunity – *2020*

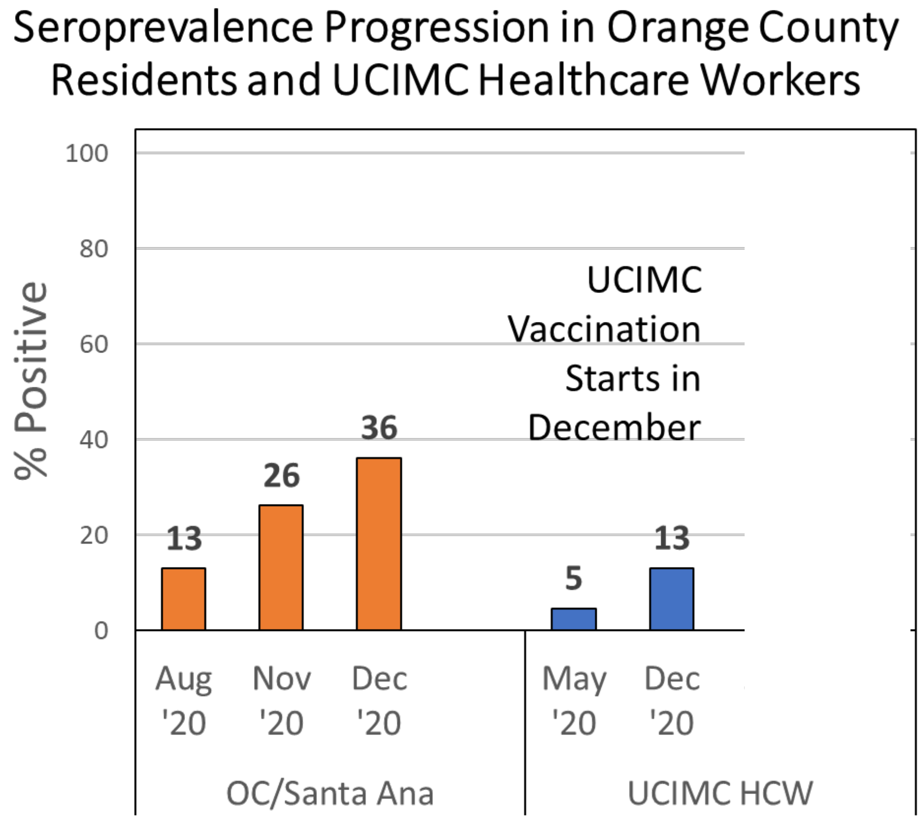


Under served Community within OC

Samples

Collection	**Number**	**Date**
Orange County	3,500	July '20
Santa Ana	3,000	Dec '20
UCI Healthcare Workers	1,400	May '20
UCI Healthcare Workers	313	Dec '20
UCIMC Biorepository	598	2020
Total	**8,811**	

Orange County Sero surveillance Study
Monitoring Naturally Acquired Herd Immunity – *2020*

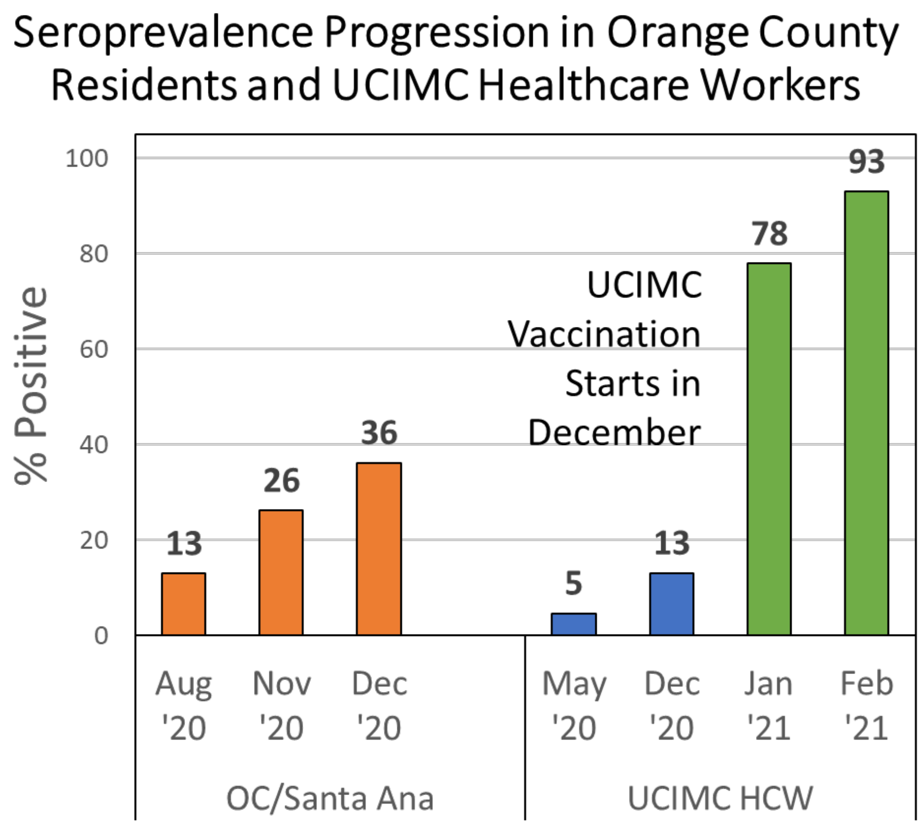


Samples

Collection	**Number**	**Date**
Orange County	3,500	July '20
Santa Ana	3,000	Dec '20
UCI Healthcare Workers	1,400	May '20
UCI Healthcare Workers	313	Dec '20
UCIMC Biorepository	598	2020
Total	**8,811**	
UCI Healthcare Vaccinees	140	Jan ’21
UCI Healthcare Vaccinees	560	Feb ’21
Total	**700**	

Orange County Sero surveillance Study

Monitoring Vaccine Induced 'Herd' Immunity – *2021*



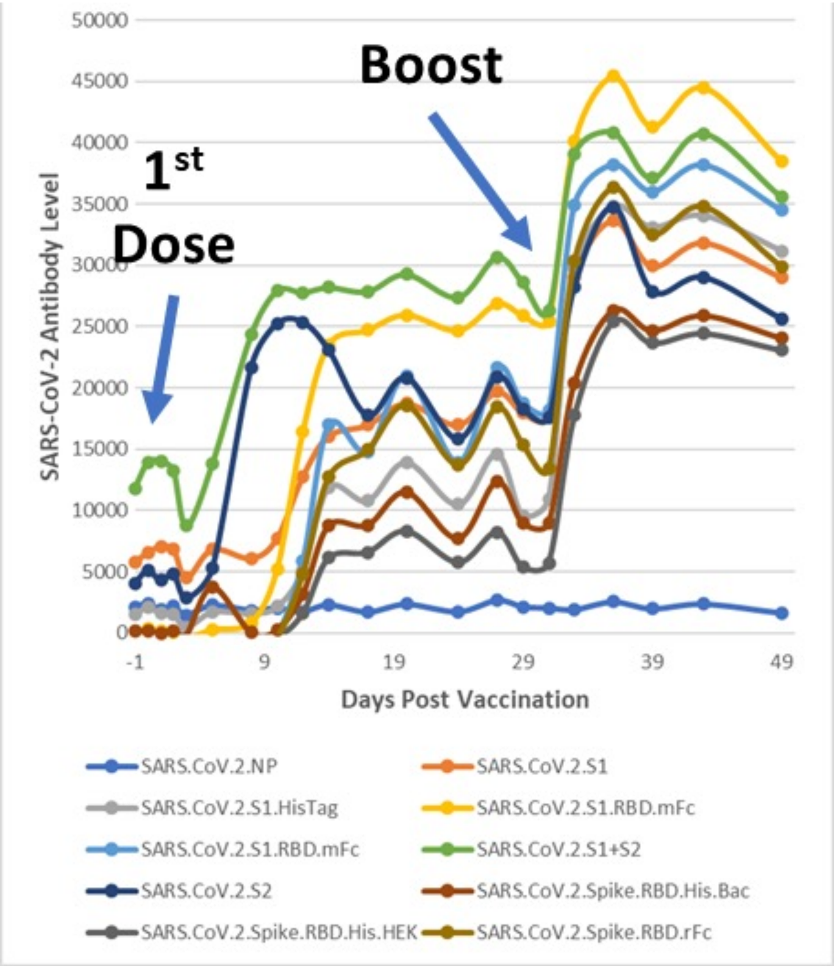
Samples

Collection	Number	Date
Orange County	3,500	July '20
Santa Ana	3,000	Dec '20
UCI Healthcare Workers	1,400	May '20
UCI Healthcare Workers	313	Dec '20
UCIMC Biorepository	598	2020
Total	**8,811**	
UCI Healthcare Vaccinees	140	Jan '21
UCI Healthcare Vaccinees	560	Feb '21
Total	**700**	

Orange County Sero surveillance Study

Monitoring Vaccine Induced 'Herd' Immunity – *2021*

mRNA Vaccine Ab Response Induced in Indiviuals

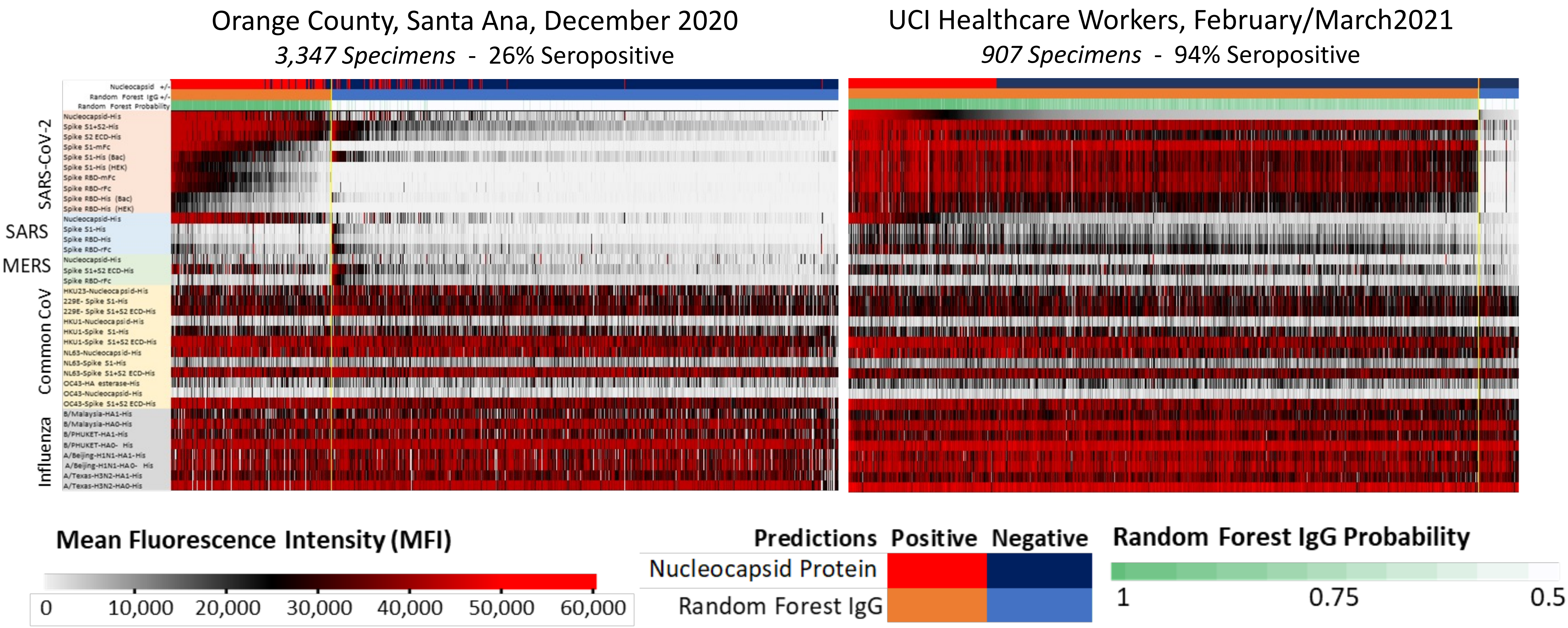


Samples

Collection	Number	Date
Orange County	3,500	July '20
Santa Ana	3,000	Dec '20
UCI Healthcare Workers	1,400	May '20
UCI Healthcare Workers	313	Dec '20
UCIMC Biorepository	598	2020
Total	**8,811**	
UCI Healthcare Vaccinees	140	Jan '21
UCI Healthcare Vaccinees	560	Feb '21
Total	**700**	

Orange County Serosurveillance Study

Monitoring Vaccine Induced ‘Herd’ Immunity – *2020*

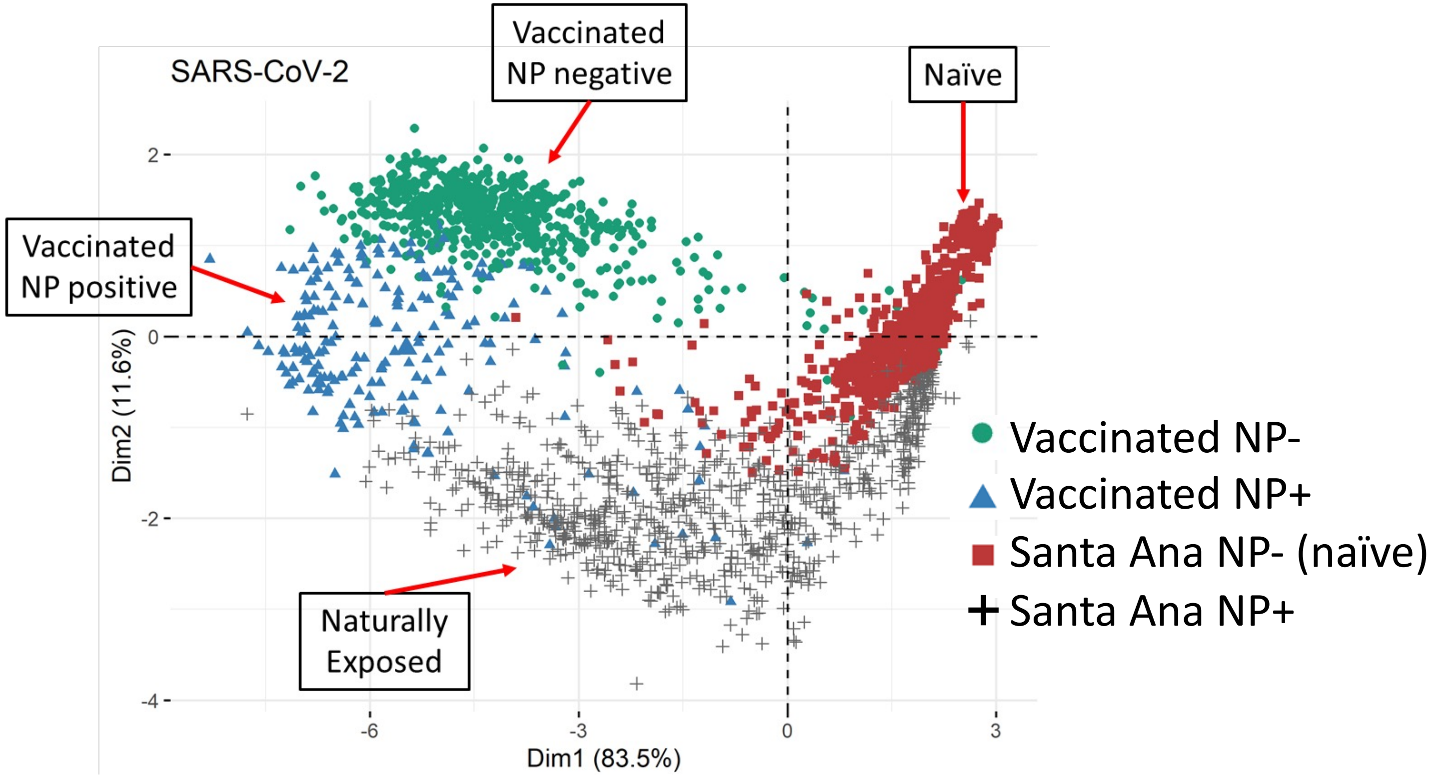


The COVID mRNA Vaccine is Spectacular !

Orange County Sero surveillance Study

Monitoring Vaccine Induced 'Herd' Immunity – *2021*

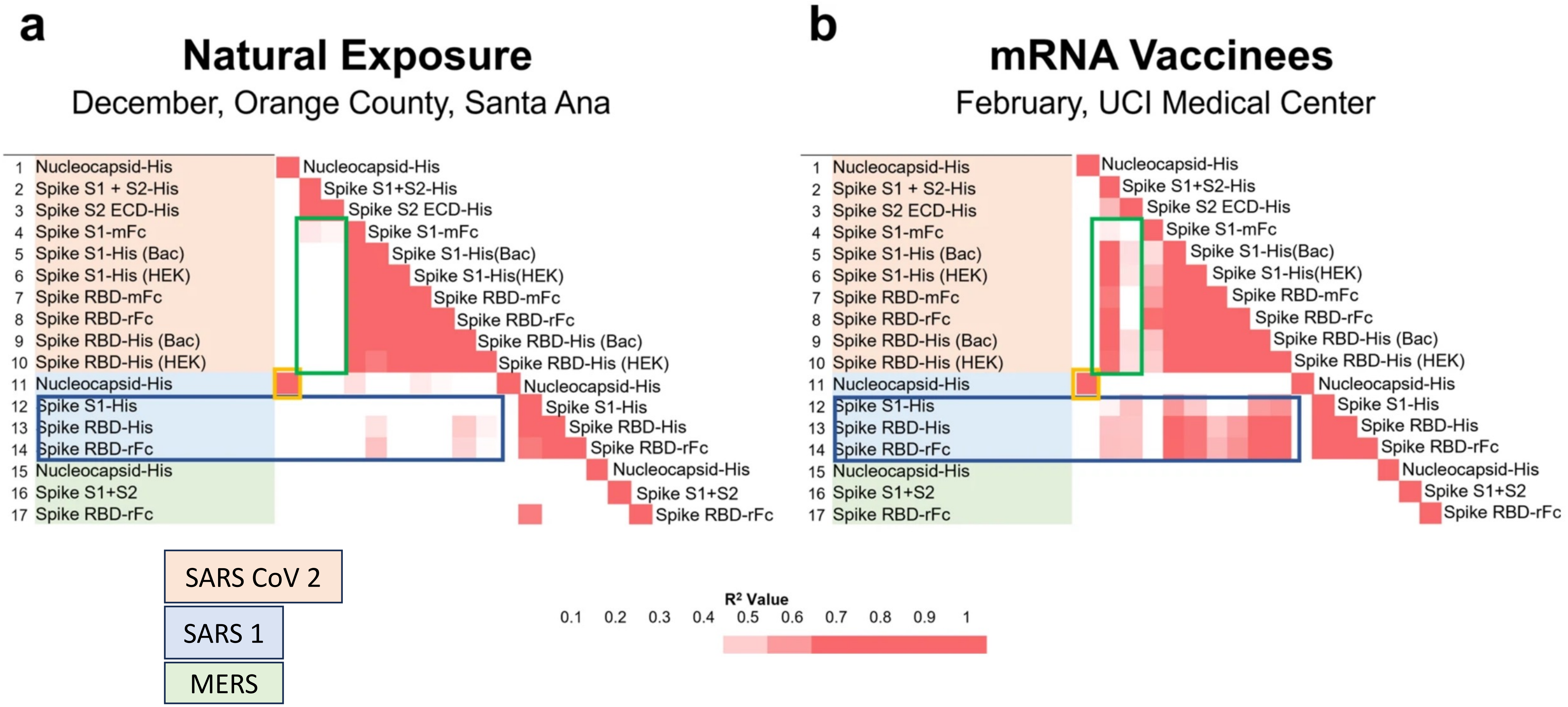
Compare
- Naïve vs. Naturally Exposed
- Vaccinated vs. Naturally Exposed
- Naïve/Vaccinated vs. Naturally Exposed/Vaccinated



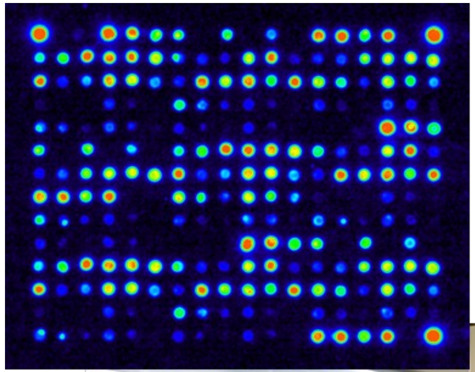
Summary
- 26% natural exposure positive in OC
- >90% HCW positive in February
- RBD not well recognized after natural exposure
- RBD dominant after mRNA vaccination
- 98% of the seropositive population in Santa Ana did not report to a clinic
- Everybody also has Ab against Common CoV and Influenza virus

Correlation matrix:

Comparing cross reactivity of Natural Exposure to mRNA Vaccination

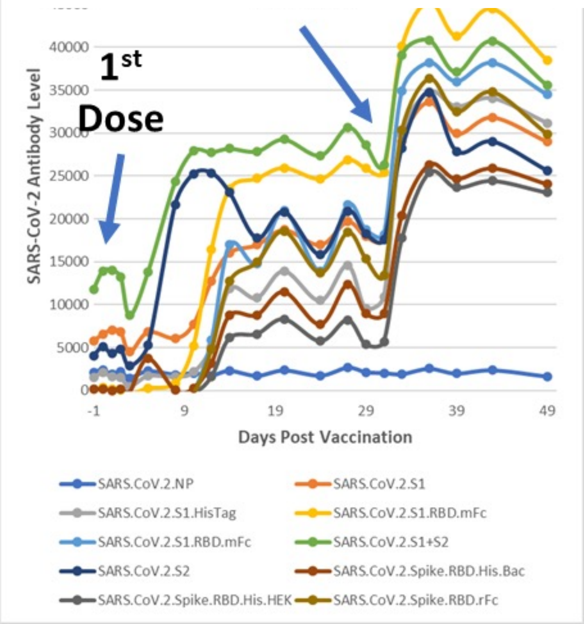


Summary: COVID mRNA Vaccine Induced Immunity

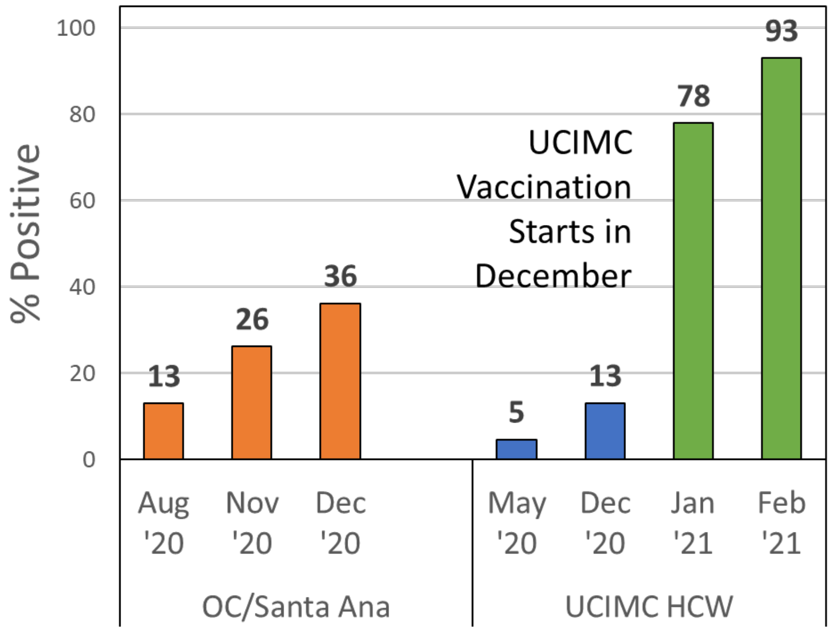


COVAM
Coronavirus
Antigen
Microarray

mRNA Vaccine Ab Response in 1 Person

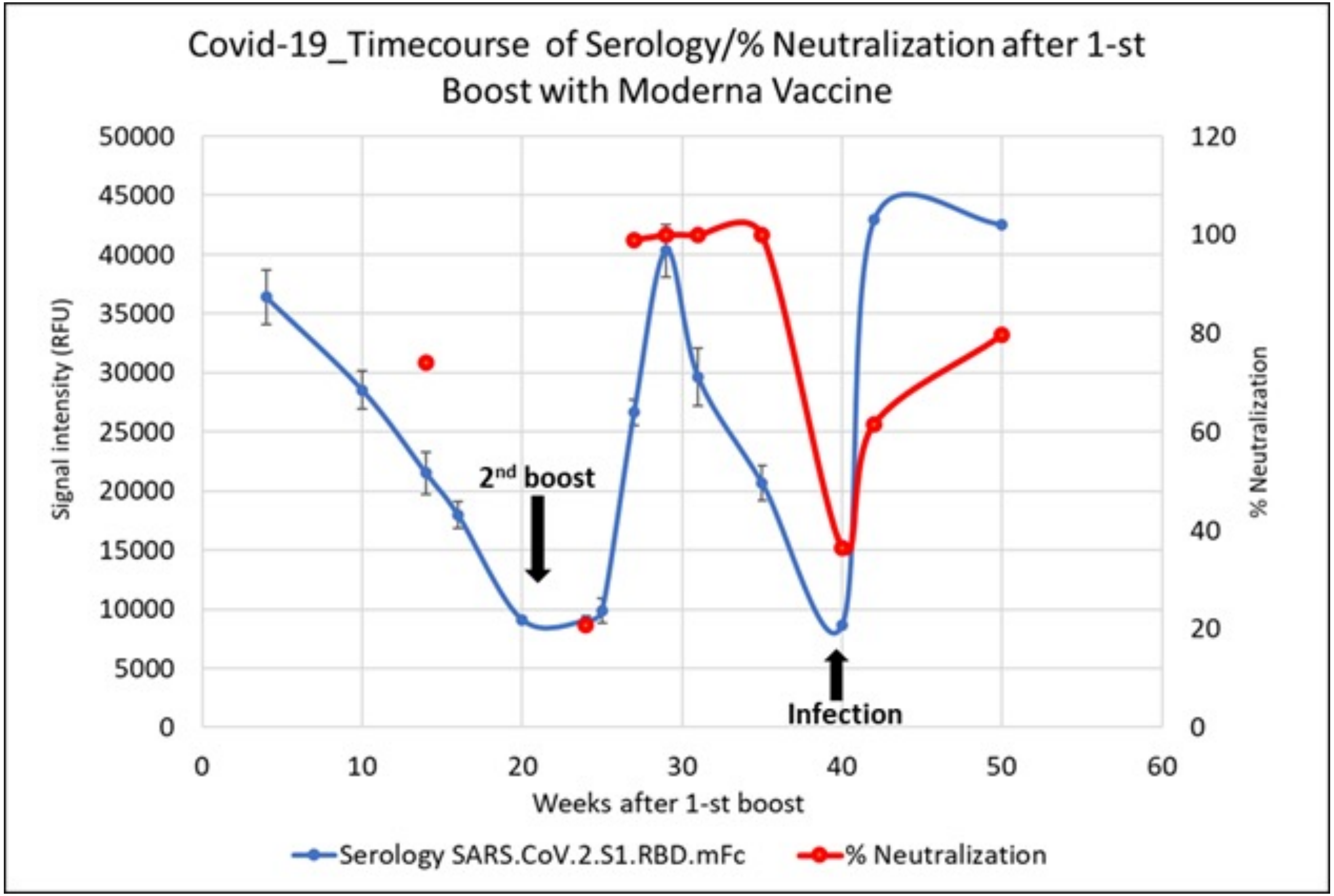


Seroprevalence Progression in Orange County Residents and UCIMC Healthcare Workers



Public and Personalized Medicine Need to Know:

- Could I have immunity to the COVID 19 virus ?
- How long does it last?
- Do I need the vaccine if I had COVID ?
- Can I go to work yet ?
- Which Vaccine is Better ?
- When do I need another shot ?


Covid-19_Timecourse of Serology/% Neutralization after 1-st Boost with Moderna Vaccine
Signal intensity (RFU)
% Neutralization
Weeks after 1-st boost
2nd boost
Infection
0
5000
10000
15000
20000
25000
30000
35000
40000
45000
50000
0
20
40
60
80
100
120
0
10
20
30
40
50
60
Serology SARS.CoV.2.S1.RBD.mFc
% Neutralization

Acknowledgements

Program in Public Health

- Bernadette Boden-Albala
- Daniel Parker

Vaccine R&D Team

- Aarti Jain
- Rafael Assis

UCI Medical Center

- Sebastian Schubl
- Cesar Figueroa
- Erika Linmey-Lasso

Student recruits and volunteers

UCI University of California, Irvine

Vaccine R&D Center

Felgner Lab
Rie Nakajima
Jiin Felgner
Li Liang
Sharon Jan
Al Jasinskas
Anthony Gregory
Aarti Jain
Rafael De Assis
Emily Silzel

Huw Davies Lab
Egest Pone
Jenny Davies
Michael Hwang
Kim Nguyen

Lisa Wagar Lab
Jenna Kastenschmidt
Suhas Sureshchandra
Mahina Mitul
Zach Wagoner
Andrew Sorn
Aviv Benchorin

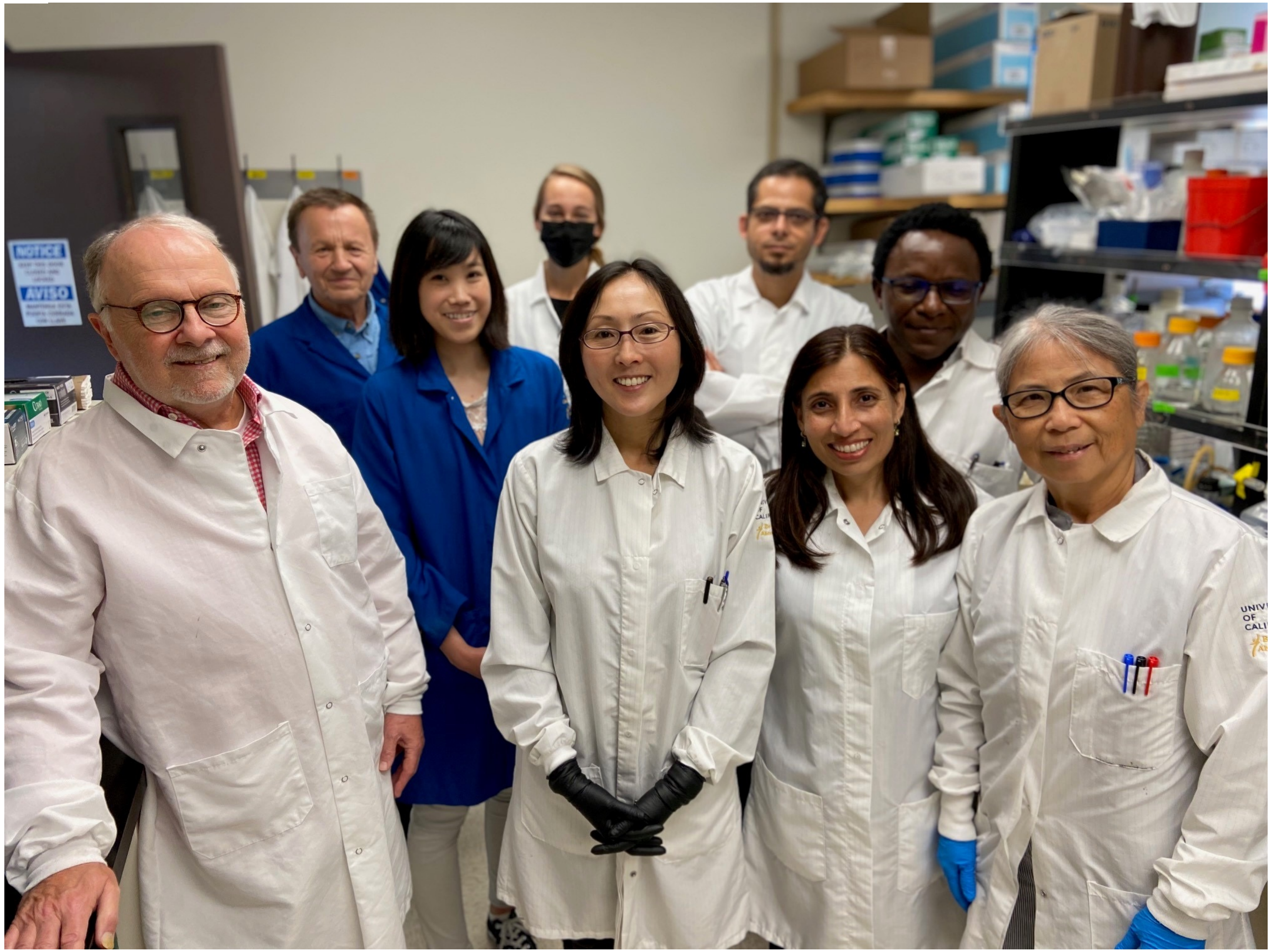
Funding

DTRA

NIH National Institutes of Health

DARPA

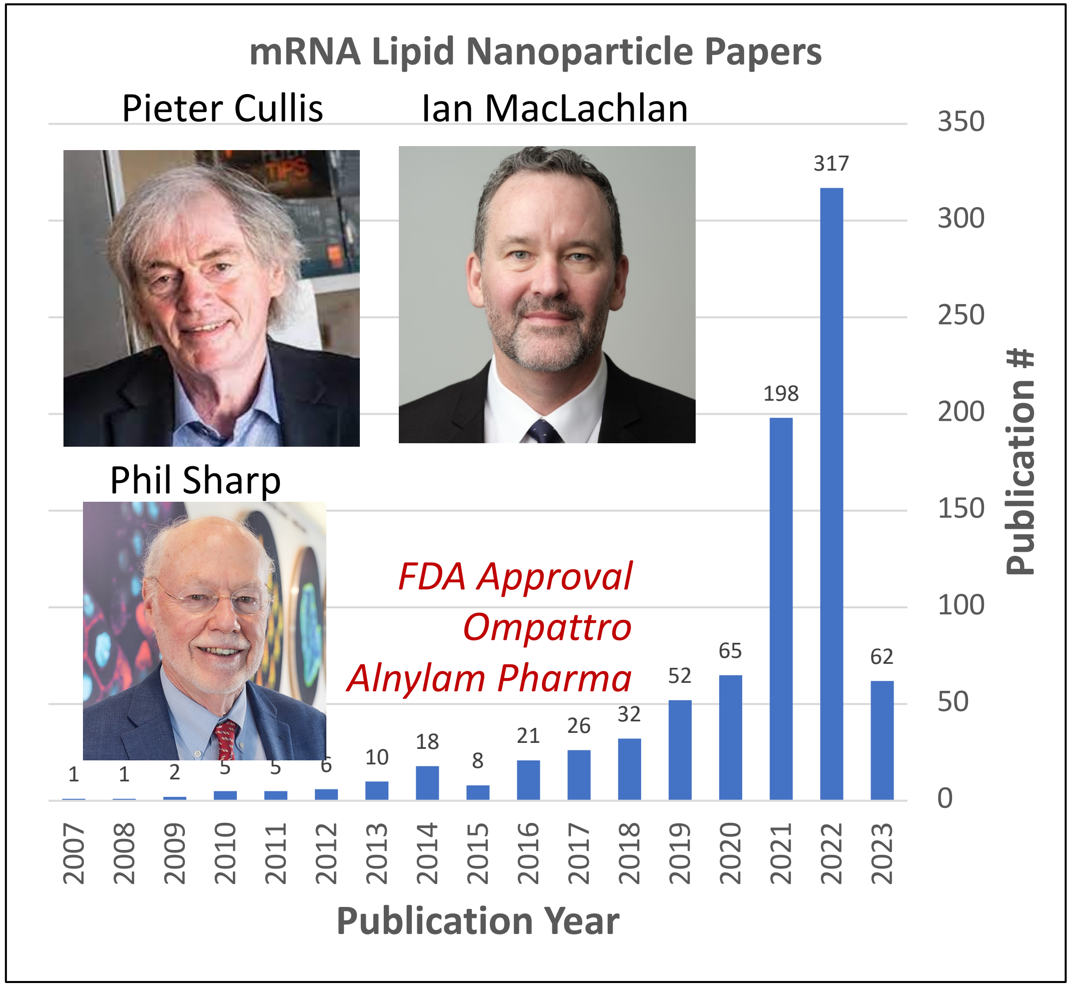
Polaris

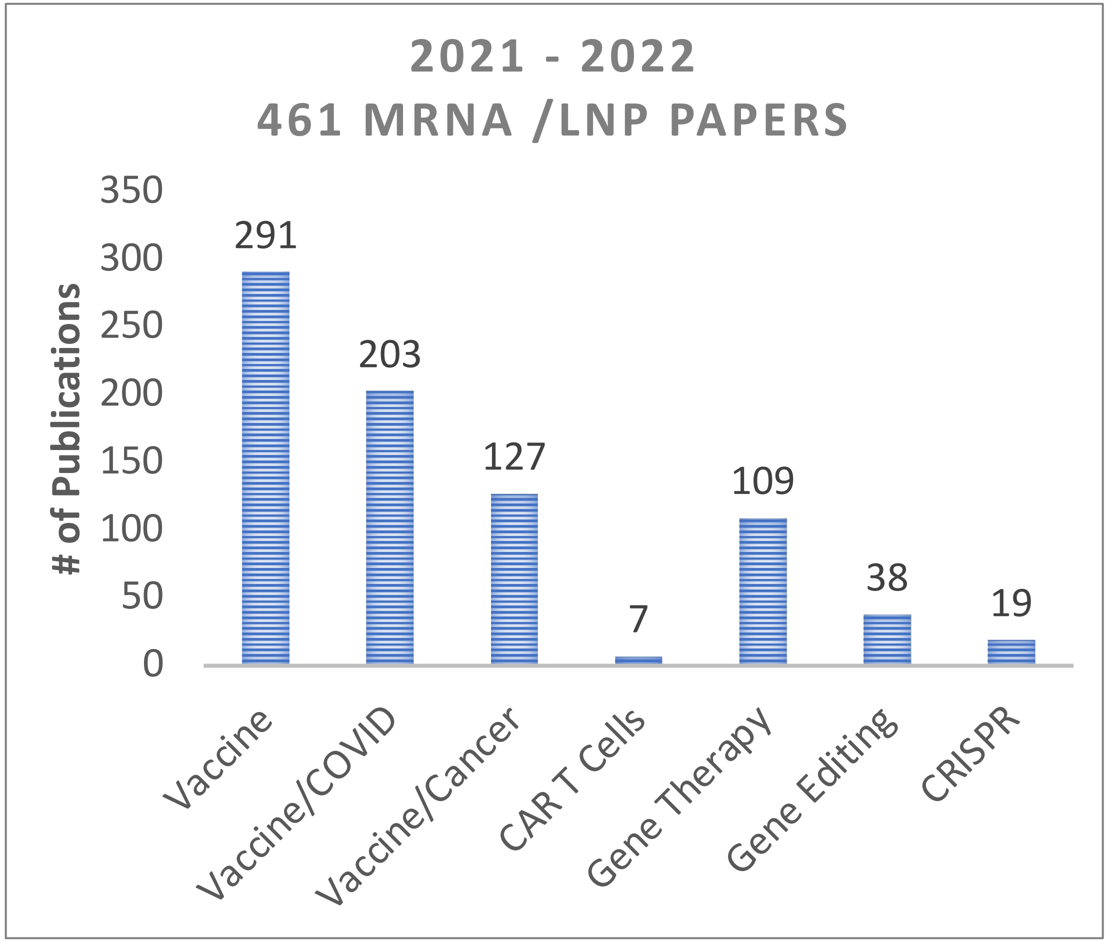


Vaccine Science in the 151st Year

What's Next ?

Lipid Nanoparticle Publication History

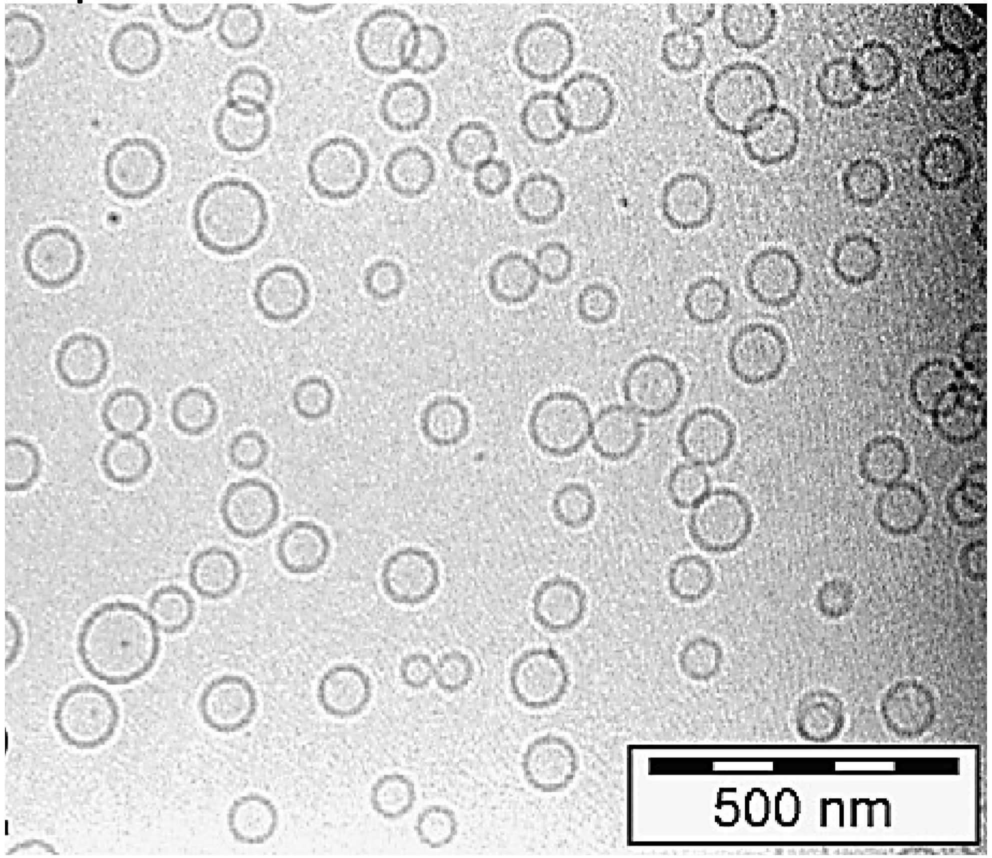




Ompattro – LNP siRNA for hereditary transthyretin amyloidosis

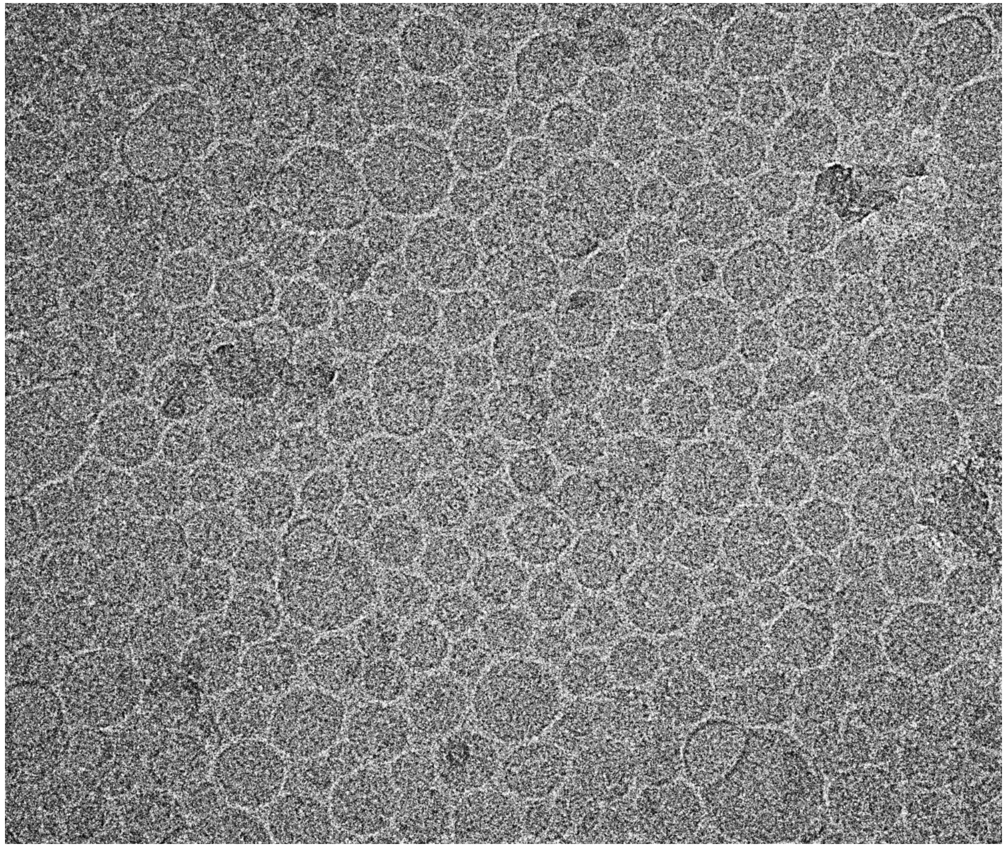
What is a Lipid Nanoparticle ?
Lipid Vesicles → mRNA Lipid Nanoparticles

Lipid Vesicles – Charlottesville **1978**



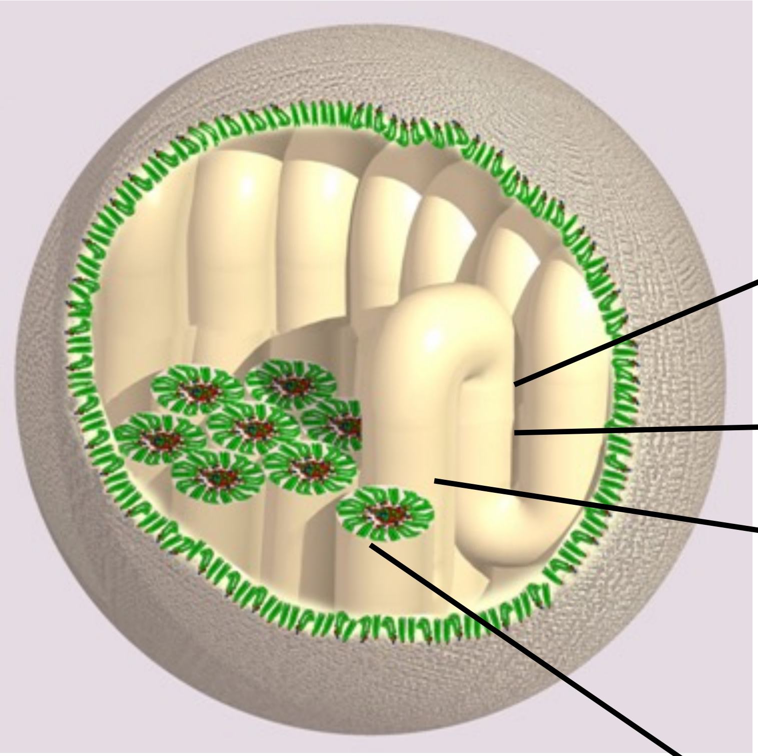
Nothing Inside

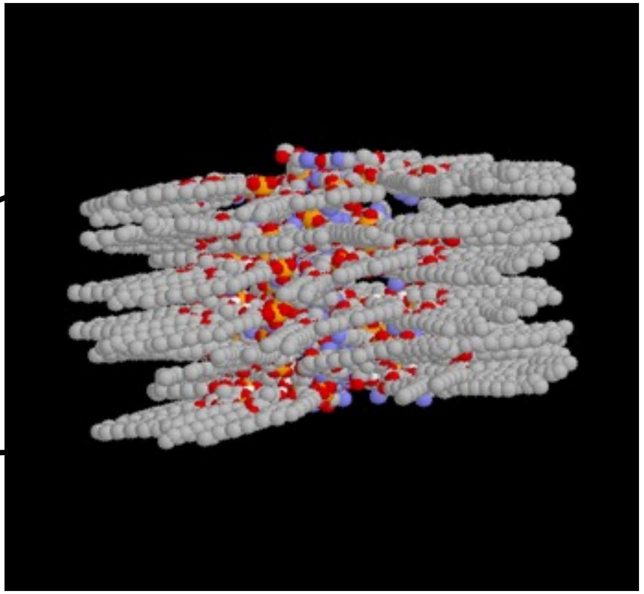
mRNA Lipid Nanoparticles – UCI **2021**

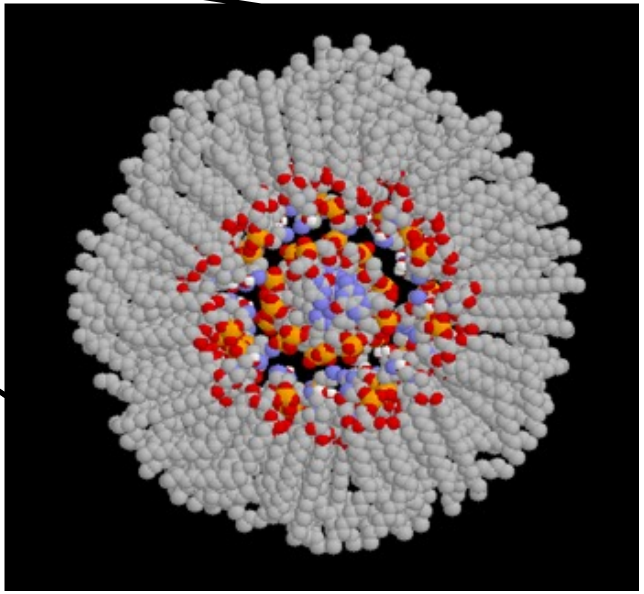


Packed with mRNA

Close-packed Plasmid Lipoplex - Recap





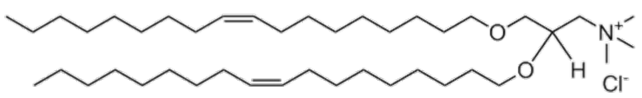
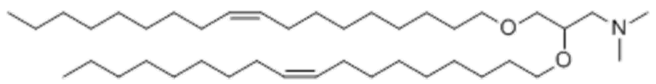
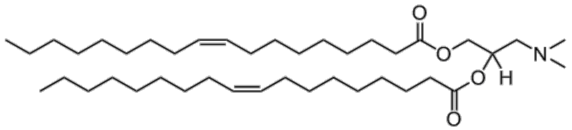
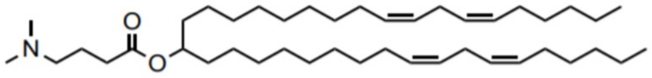
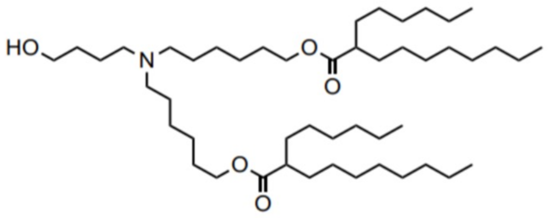
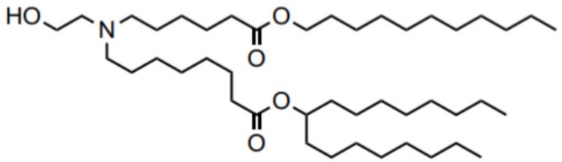


5,000 bp Plasmid

16,000 molecules DOTMA

16,000 molecules DOPE

Cationic Lipids

Lipid name	**Structure**	**pKa (by TNS)**	**Note**
DOTMA		NA (permanent cationic lipid)	
DODMA		6.59	
DODAP		5.62	
DLin-MC3-DMA		6.57	Used in Onpattro siRNA
ALC-0315		6.09	Used in Pfzier mRNA vaccine
SM-102		6.75	Used in Moderna mRNA vaccine

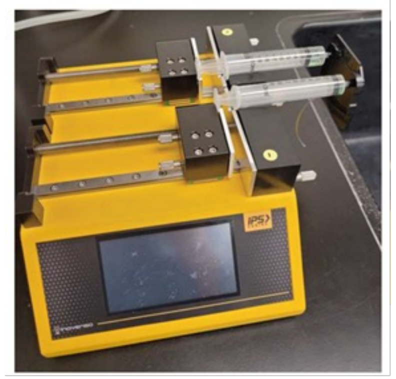
mRNA LNP self assembly instrument

Ignite (commercial system)

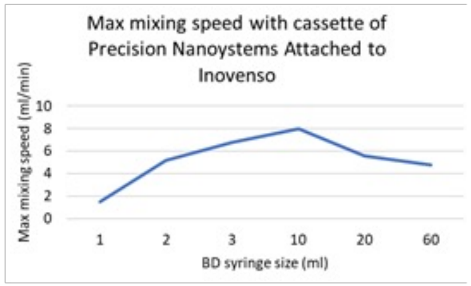
Dual syringe pump (in-house research use system)

Fabricated from Inovenso Pump, an inexpensive dual-syringe pump

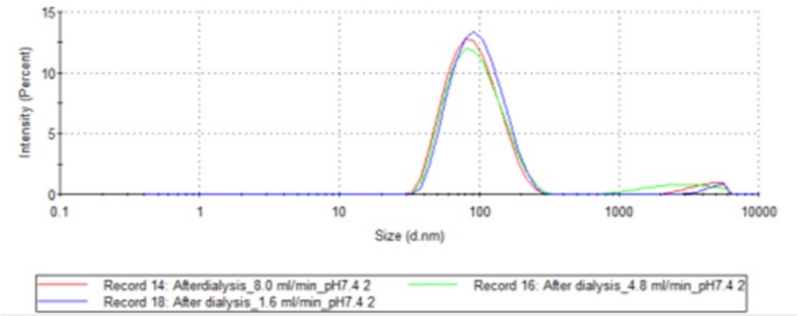
A Syringe Pump



B Max flow rate of different syringes



C LNP Size at different flow rates



	Ignite	In-house pump
Max flow rate	20 ml/min	8 ml/min
mRNA Encapsulation	95~100%	99%
LNP Particle size (d.nm)	70~80nm	86~91 nm
LNP Polydispersity Index	0.1~0.25	0.2~0.25
LNP Batch scale	Up to 20 ml	Up to 80 ml

In-house syringe pump produced mRNA LNPs with comparable quality.

mRNA LNP characterization

Particle sizing distribution (PSD) by Malvern sizer

Polydispersity Index (PdI) by Malvern sizer

Zeta potential (ZP) by Malvern zeta sizer

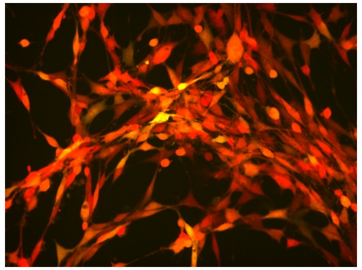
mRNA encapsulation by Ribogreen assay

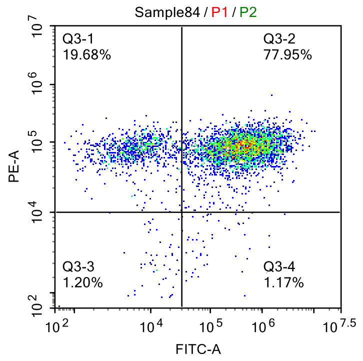
Functional protein expression by in vitro transfection

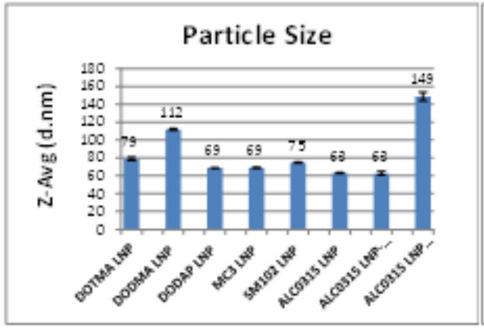
LNP Formulations Screen Example

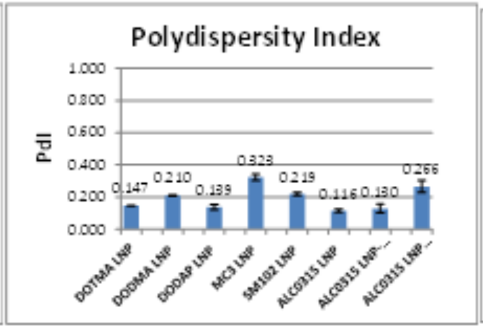
#	LNP	mRNA
1	DOTMA	Unmodified spike mRNA
2	DODMA	Unmodified spike mRNA
3	DODAP	Unmodified spike mRNA
4	MC3	Unmodified spike mRNA
5	SM102	Unmodified spike mRNA
6	ALC0315	Unmodified spike mRNA
7	ALC0315	Modified spike mRNA
8	ALC0315	None

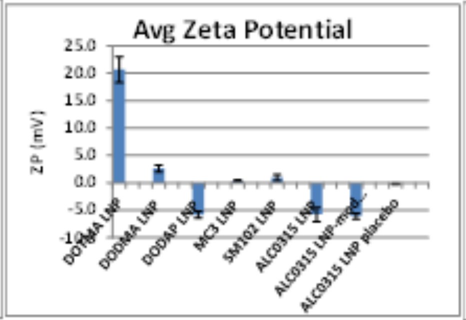
Transfection

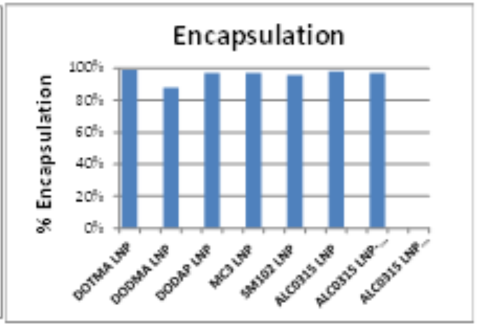






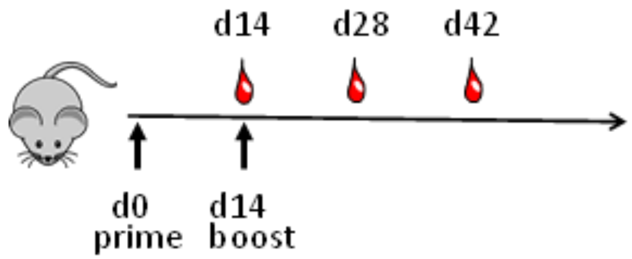






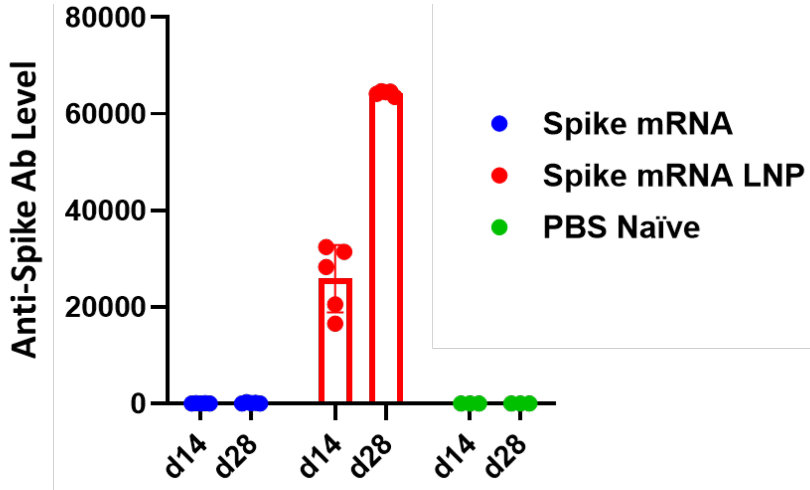
Spike mRNA/LNP Immunogentcy

In Vivo Immunization

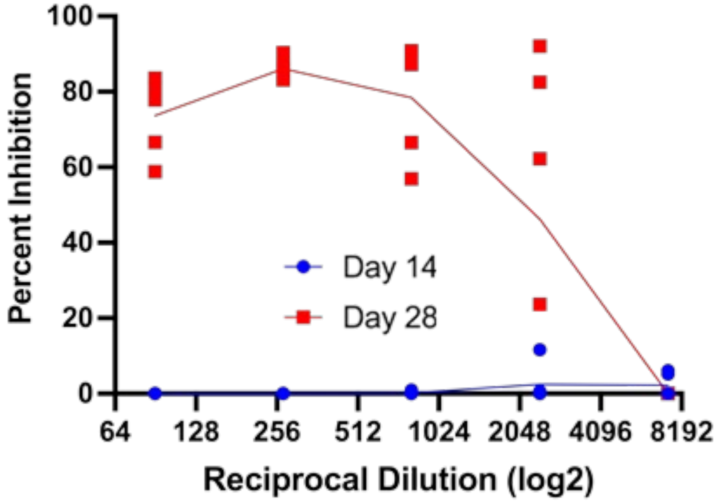


Antibody Levels

BioNTech Formulation ALC0315 Ionizable Lipid



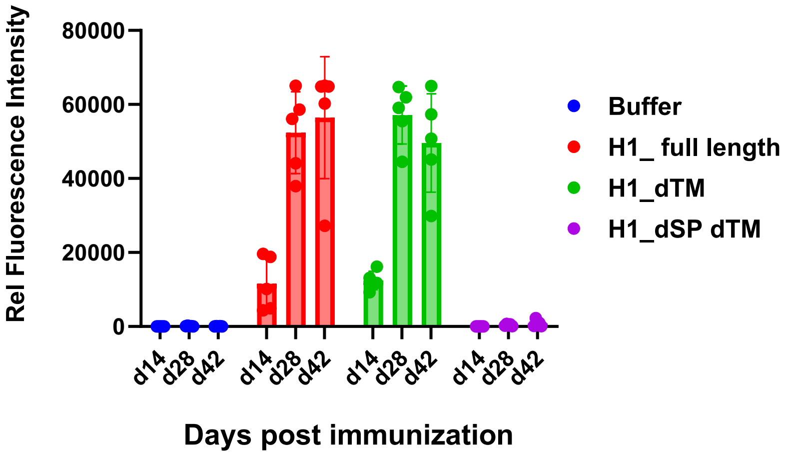
Virus Neutalization Assay



Secretory Signal and Transmembrane Domain Deletion Variants

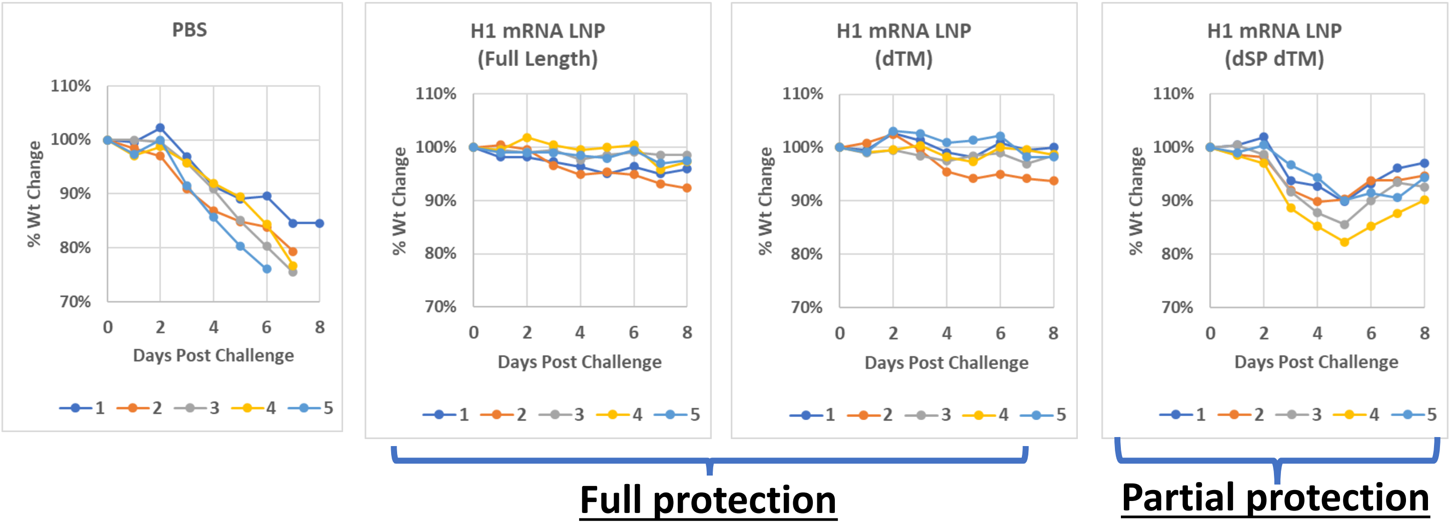
H1N1 LNPs immunization schedule

Hemagglutinin LNPs Ab levels
mRNA deletion variants



H1N1 virus challenge

50ul i.n. H1N1 grown in eggs, 10^3 TCID50/ml

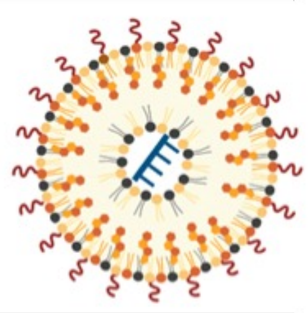


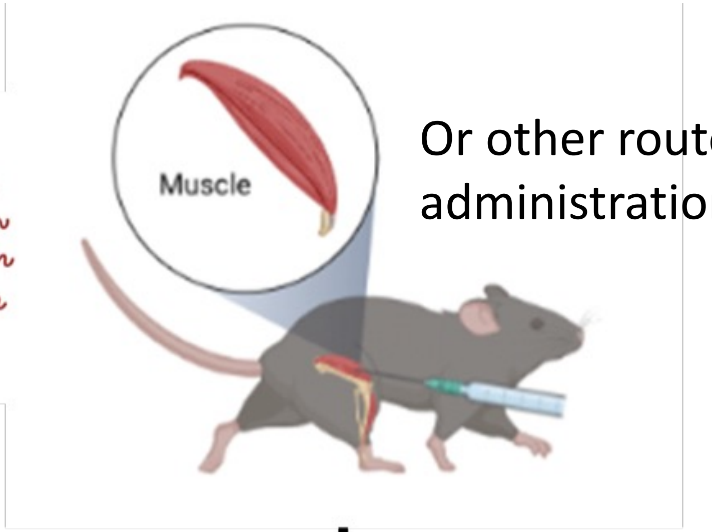
LNPs immunization study of H1 mRNA deletion variants. (A) H1N1 LNPs Immunization Schedule. (B) total IgG response time course in BALB/c mice. (C) Protection efficacy upon H1N1 virus challenge.

H1N1 virus challenge (cont'd)

- Both full length H1 mRNA and secreted H1 mRNA (dTM version) LNP conferred full protection.
- The intracellular H1 mRNA (dSP-dTM) LNP conferred partial protection in the absence of production of antigen specific IgG
- The partial protection is possibly mediated by CD8 cells since more weight loss was observed when anti-CD8 Mab was administered before and during challenge

Gene Editing with the Cre/Lox System

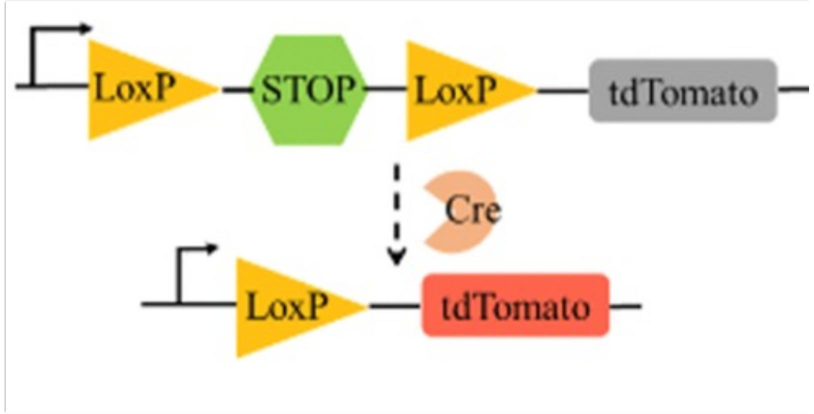




Or other routes of administration

D0 Inject

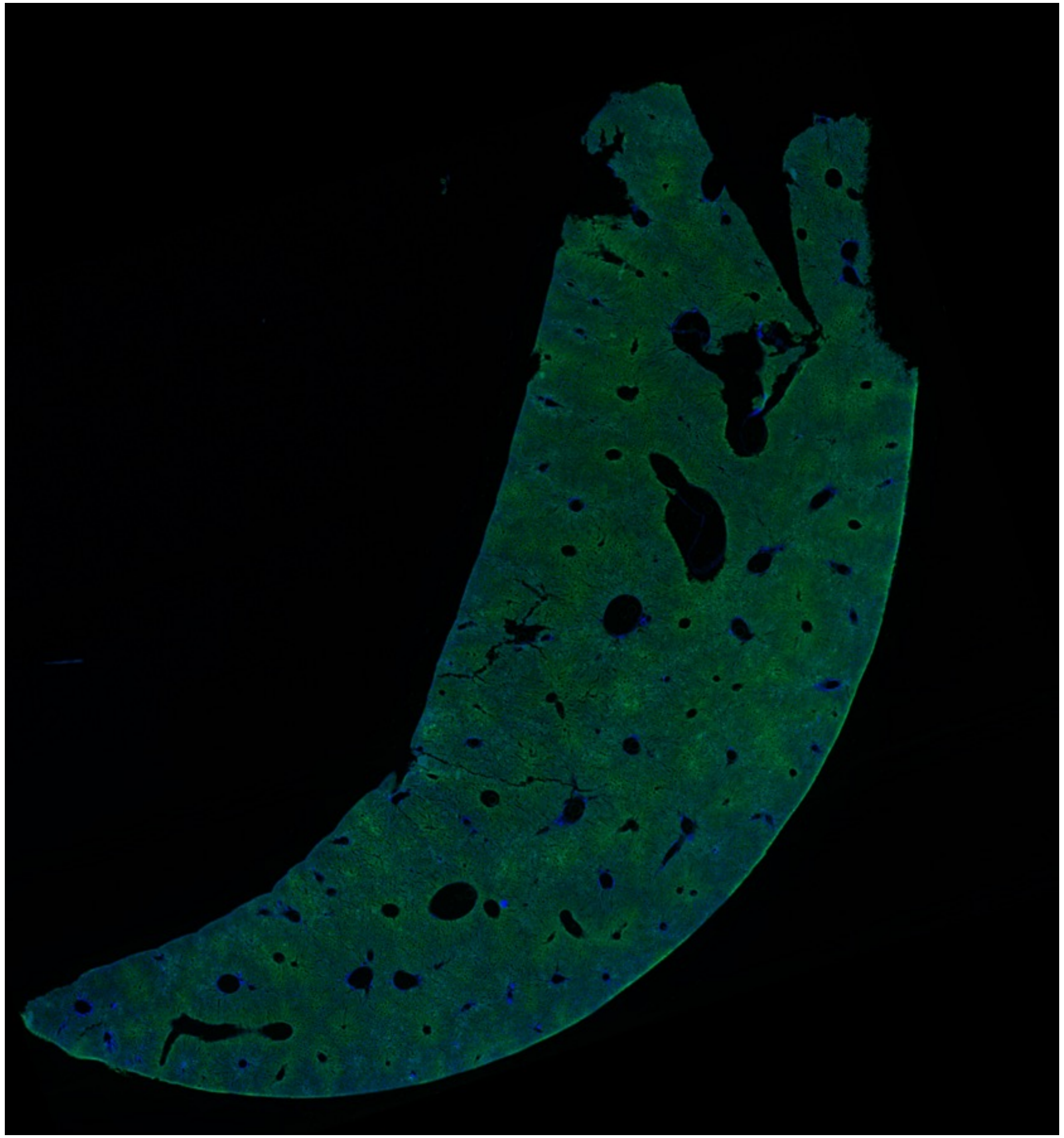
D10 Harvest



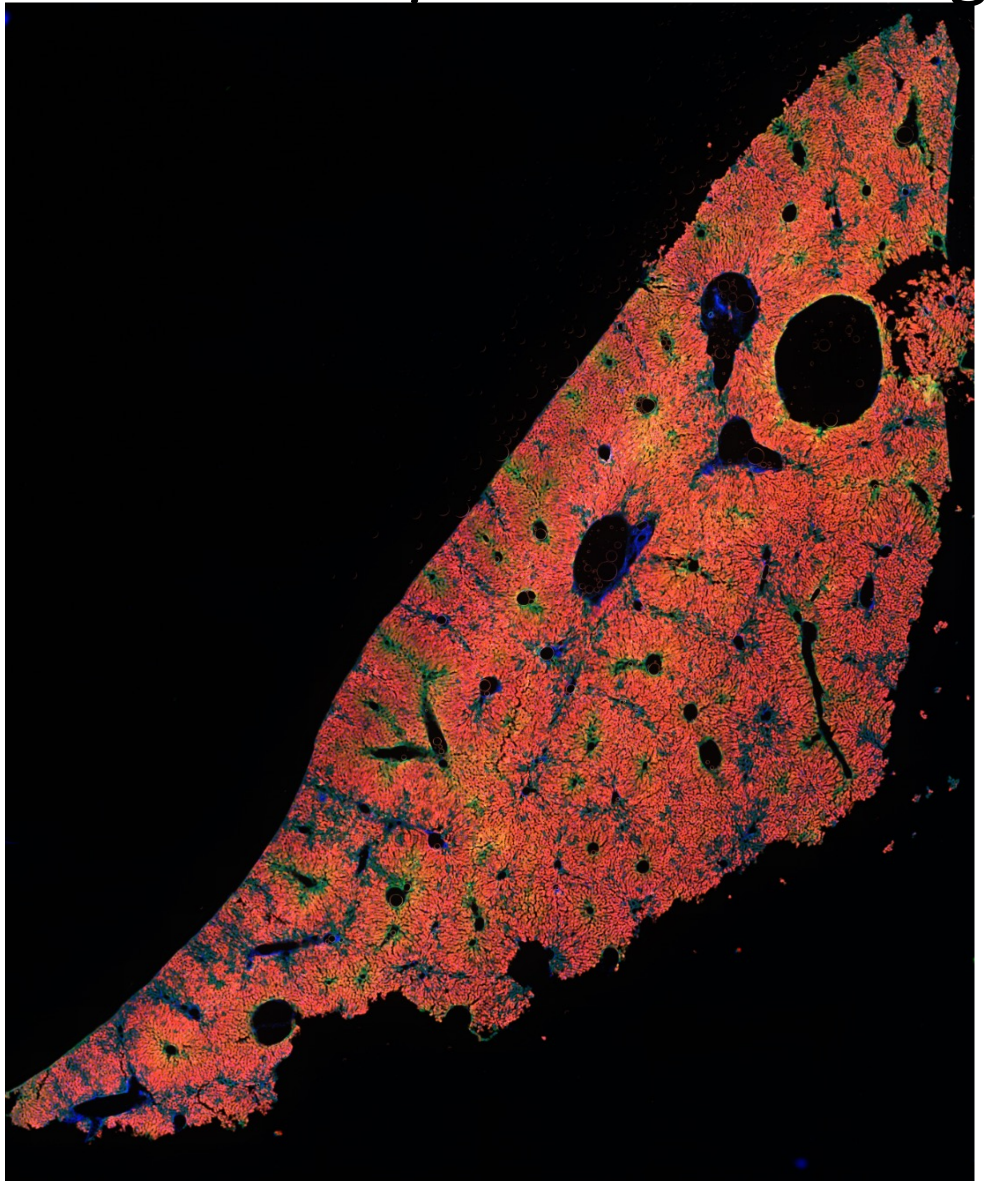
- AiG tdTomato
- CRE mRNA LNP
- ALC 0315
- 50 microliters
- 100 ug/ml or 280 ug/ml RNA

Liver Ai9 TdTomato mouse – CRE mRNA/LNP Retroorbital Injection

Control

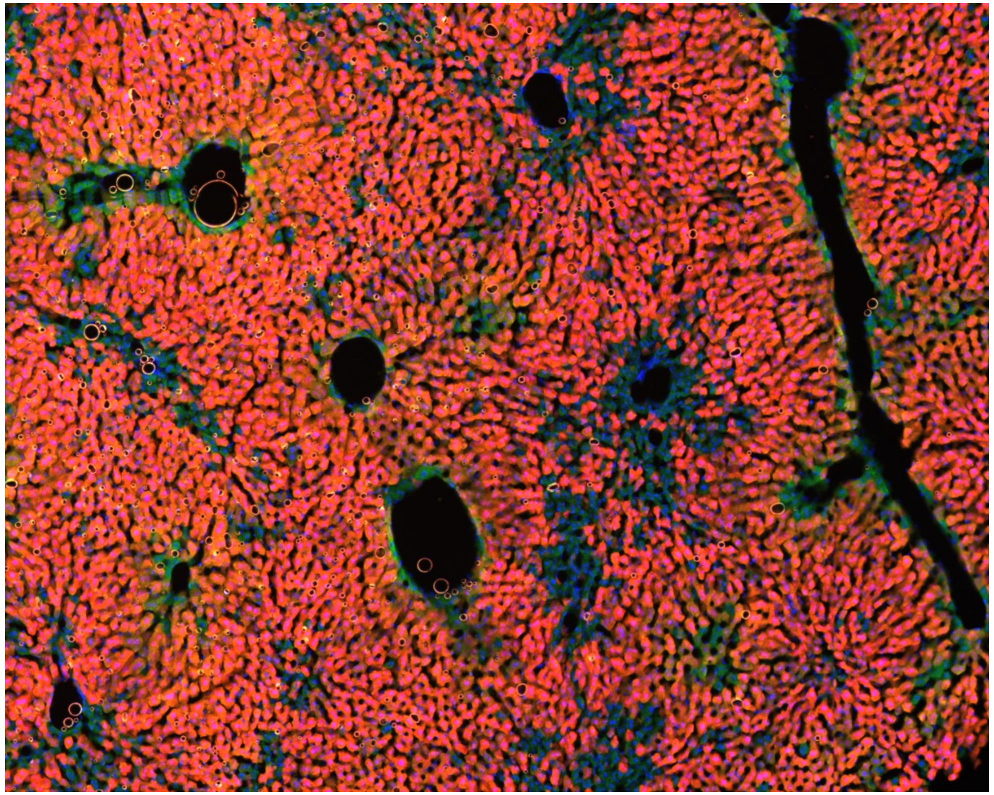


CRE mRNA/LNP LOX Editing

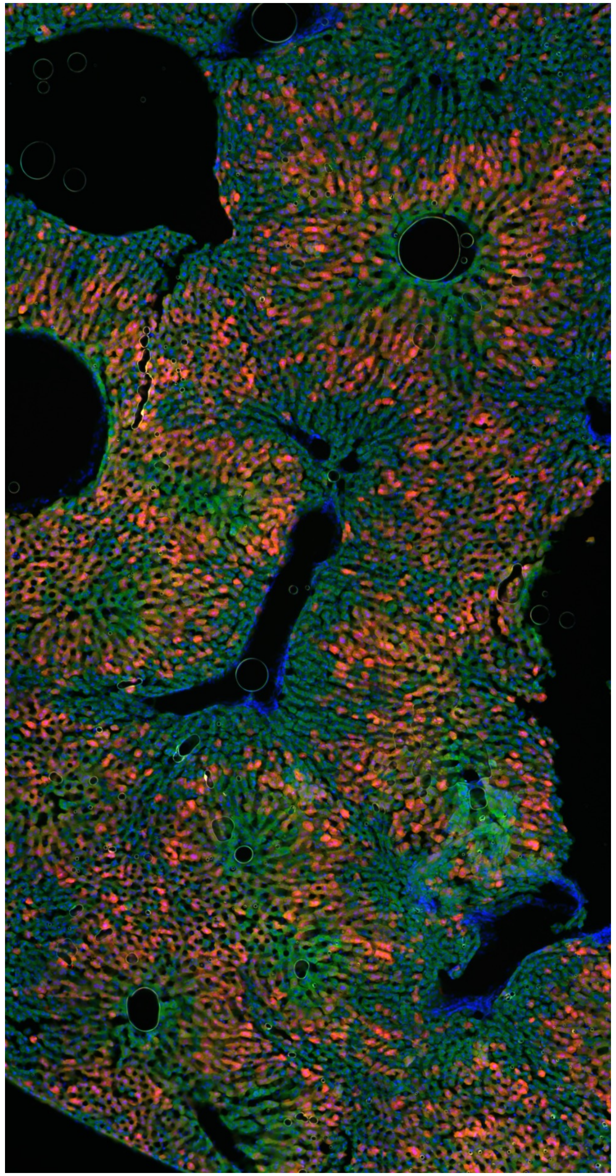


Liver

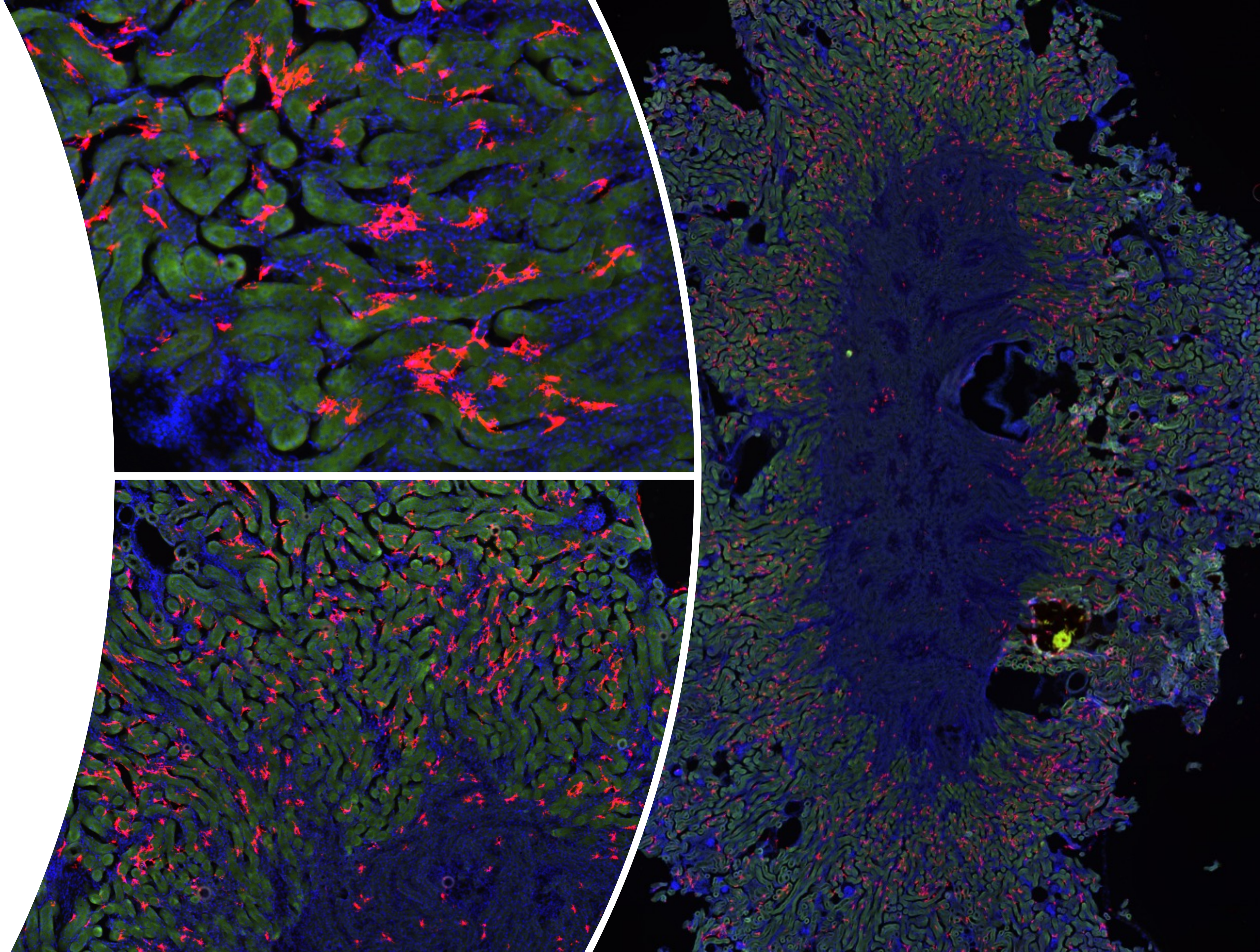
High Dose



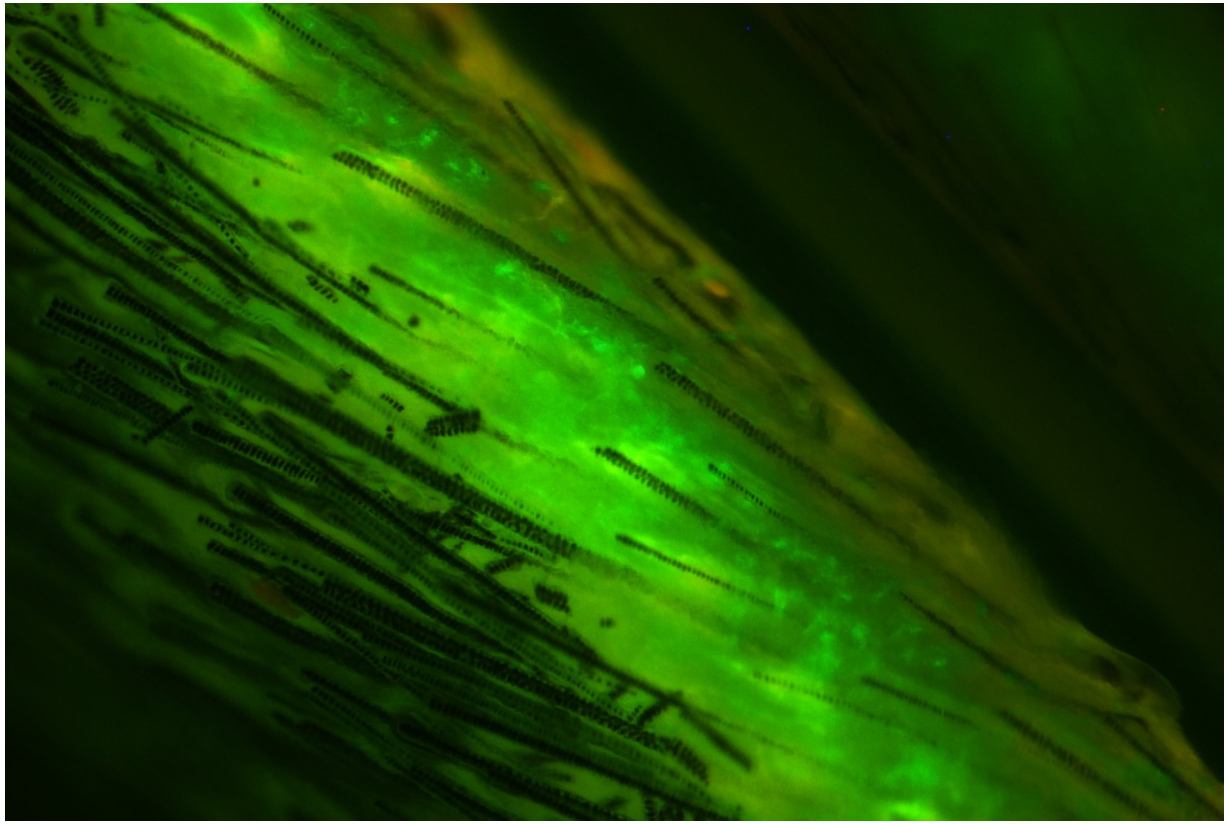
Low Dose

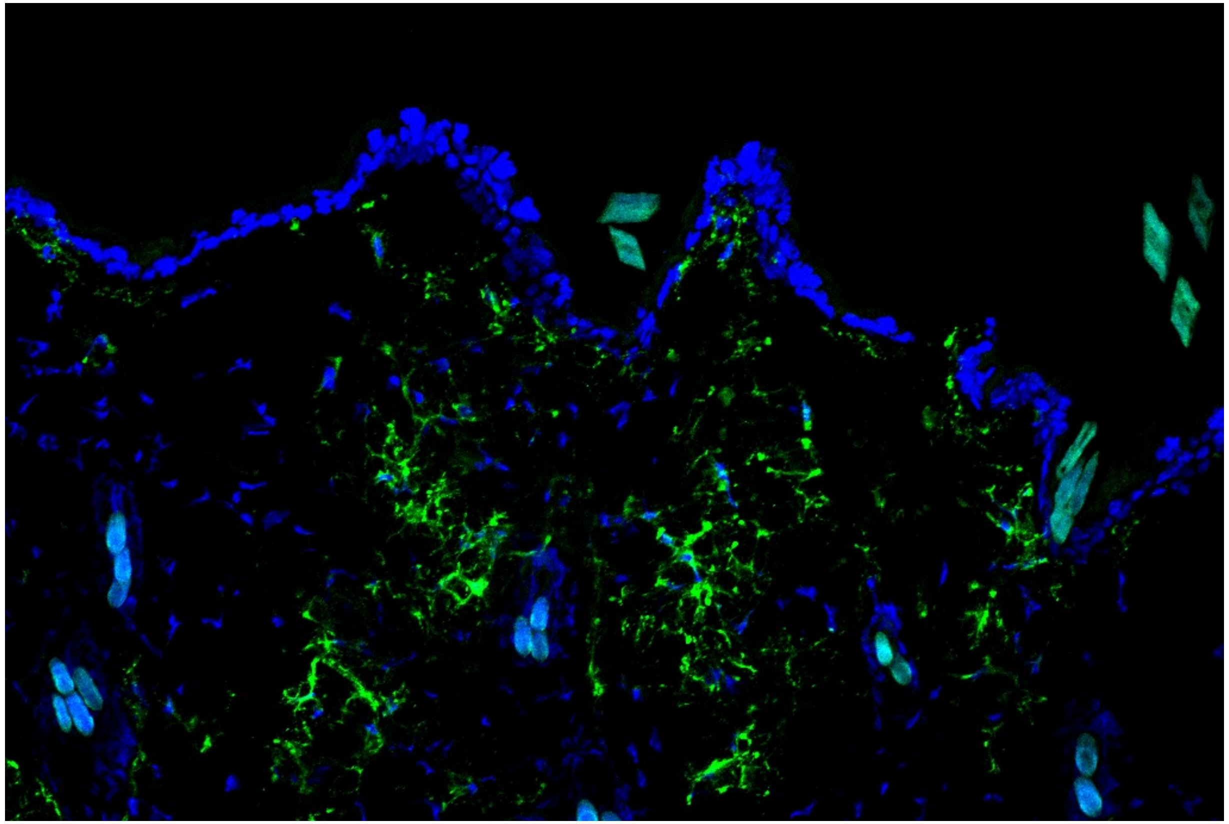


- **Kidney**



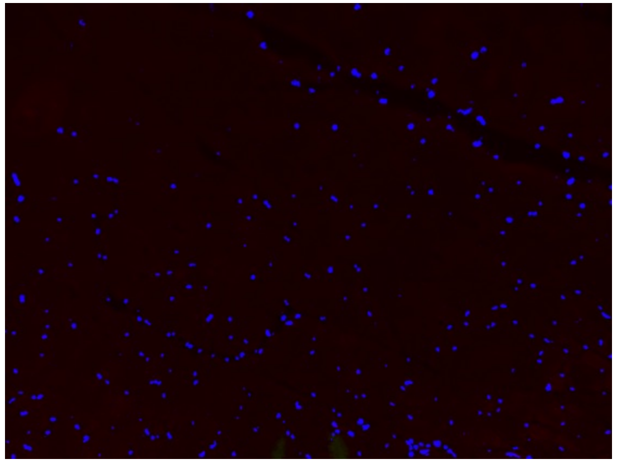
Cre/Lox GFP in Skin



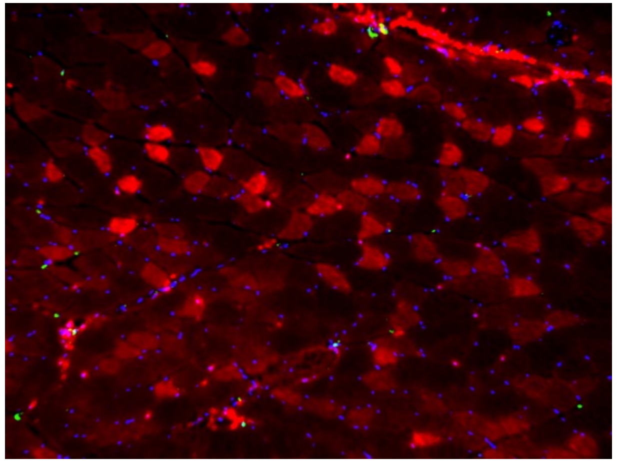


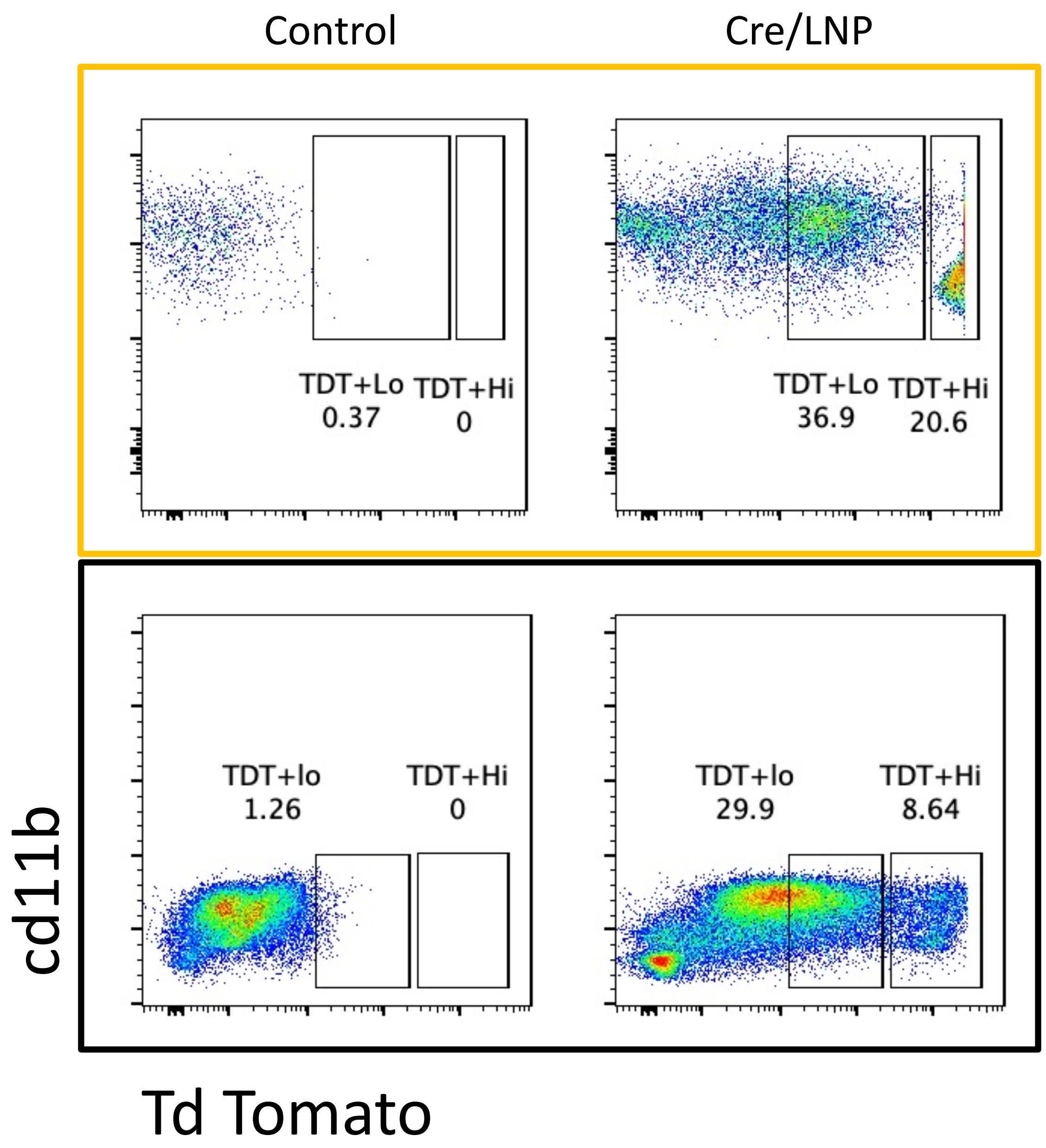
Intramuscular Cre/LNP : What are the target cells ?

Control



Cre / LNP



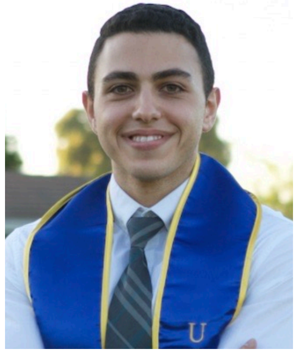


Macrophages

Dendritic Cells

Enily Silzel

Philip Farahat



Where to we go from here?

It is the Gene Therapy and Nucleic Acid Vaccine Revival

Vaccines for viruses, bacteria and parasites	• Replace traditional vaccines
Cancer treatment and prevention	• CAR T Cells
Gene editing to correct genetic disorders	• Sickle cell disease • Blindness
Protein Augmentation	• Hemophilia • Fabray's disease
Protein inhibition	• siRNA

The Washington Post

January 27, 2021

Stanley Plotkin

"… Say all you want about the horrors of this last year … We have accomplished more than I would have imagined possible…. It's astounding. It's thrilling. I was about to say it's miraculous, but that's not right. I don't believe in miracles. It might sound a little fancy, but I believe in science. I believe in our capacity to endure and overcome …"

UCI University of California, Irvine

Vaccine R&D Center

Felgner Lab
Rie Nakajima
Li Liang
Jiin Felgner
Sharon Jan
Al Jasinskas
Anthony Gregory
Aarti Jain
Rafael De Assis
Erwin Strasburg
Emily Silzel
Andy Yeromin
Jacob Laster

Huw Davies Lab
Jenny Davies
Egest Pone
Michael Hwang
Sarai Ochoa

Lisa Wagar Lab
Jenna Kastenschmidt
Suhas Sureshchandra
Mahina Mitul
Zach Wagoner
Andrew Sorn
Aviv Benchorin
Tim Yates

Funding

NIH National Institutes of Health

DTRA

Polaris

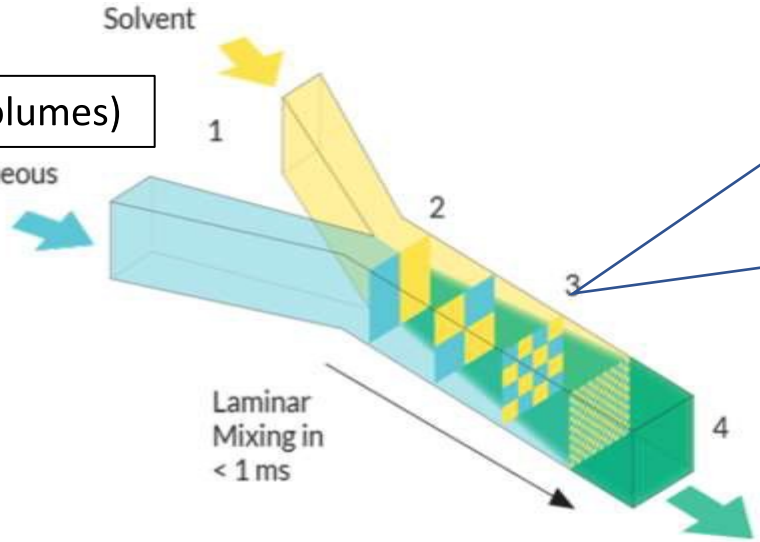
DARPA

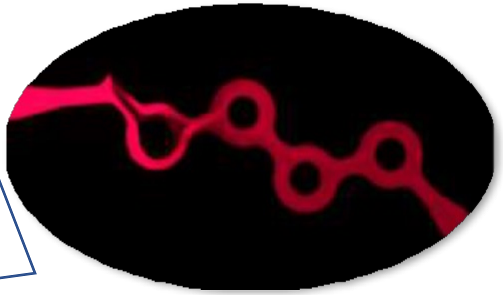
mRNA LNP self assembly by ‘Ignite Microfluidic’ Mixing

Enabling the World

Lipid mix in ethanol solvent (1 volume)

mRNA in pH 4 buffer (3 volumes)





Channel geometry

mRNA LNPs are formed

PRECISION
NANOSYSTEMS