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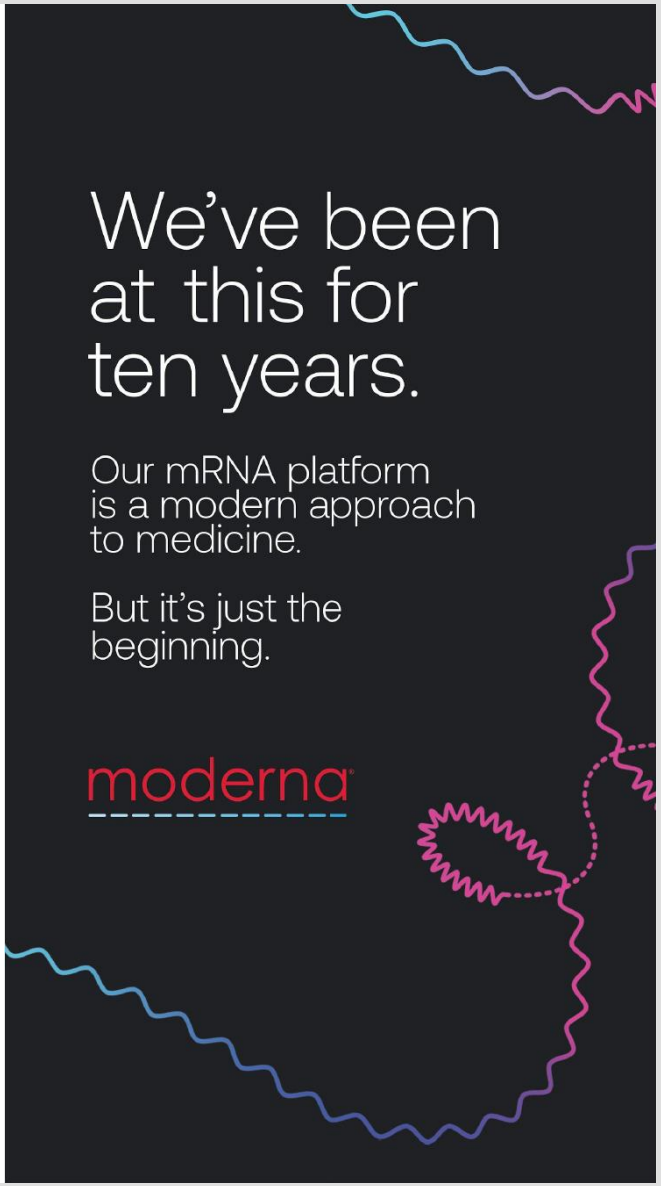
Fourth Annual Science Day

May 27, 2021



Forward-looking statements and Disclaimer

This presentation contains forward-looking statements within the meaning of the Private Securities Litigation Reform Act of 1995, as amended, including statements regarding: the potential for mRNA as a new class of medicines and the product opportunity, the probability of technical success, research and development timelines and capital efficiency; the Company's investments in the development of additional medicines, advancing new development candidates into the clinic, and the invention of new nucleic acid technologies; efforts to scale the Company, optimize processes and the Company's competitive advantages; the Company's strategy for combatting COVID-19, including processes for monitoring emerging variants and waning immunity; the potential for new epidemic waves associated with SARS-CoV-2 and the emergence of variants of concern; factors that the Company evaluates and tracks in identifying variants of concern, and processes for developing and testing vaccines that are specific to those variants; timelines for identifying and developing vaccines in response to variants of concern; strategies for modeling viral escape; the applicability of lessons learned from SARS-CoV-2 to combatting other illnesses and developing vaccines against other diseases; the ability to optimize codons and mRNA structures to increase total protein outputs; the potential for the Company to develop processes for controlling protein expression by modifying ribosomal loads; the ability to impact translational efficiency of mRNA by deformulating lipid nanoparticles ("LNPs"); the potential for slow lipid component release to adversely impact functional mRNA delivery; the Company's ability to engineer LNPs capable of accessing difficult-to-transfect primary cells with efficient endosomal escape and high functional mRNA delivery; the potential for delivery of mRNA to hematopoietic stem and progenitor cells ("HSPC") in vivo; methods of detecting and interrogating HSPC in vivo; and the potential for delivering LNPs to hematopoietic stem cells in vivo. In some cases, forward-looking statements can be identified by terminology such as "will," "may," "should," "could," "expects," "intends," "plans," "aims," "anticipates," "believes," "estimates," "predicts," "potential," "continue," or the negative of these terms or other comparable terminology, although not all forward-looking statements contain these words. The forward-looking statements in this presentation are neither promises nor guarantees, and you should not place undue reliance on these forward-looking statements because they involve known and unknown risks, uncertainties, and other factors, many of which are beyond Moderna's control and which could cause actual results to differ materially from those expressed or implied by these forward-looking statements. These risks, uncertainties, and other factors include, among others: the fact that there has never been a commercial product utilizing mRNA technology approved for use; the fact that the rapid response technology in use by Moderna is still being developed and implemented; the safety, tolerability and efficacy profile of the Moderna COVID-19 Vaccine observed to date may change adversely in ongoing analyses of trial data or subsequent to commercialization; the Moderna COVID-19 Vaccine may prove less effective against variants of the SARS-CoV-2 virus, or the Company may be unsuccessful in developing future versions of its vaccine against these variants; despite having ongoing interactions with the FDA or other regulatory agencies, the FDA or such other regulatory agencies may not agree with the Company's regulatory approval strategies, components of our filings, such as clinical trial designs, conduct and methodologies, or the sufficiency of data submitted; Moderna may encounter delays in meeting manufacturing or supply timelines or disruptions in its distribution plans for the Moderna COVID-19 Vaccine; whether and when any biologics license applications and/or additional emergency use authorization applications may be filed in various jurisdictions and ultimately approved by regulatory authorities; potential adverse impacts due to the global COVID-19 pandemic such as delays in regulatory review, manufacturing and clinical trials, supply chain interruptions, adverse effects on healthcare systems and disruption of the global economy; and those other risks and uncertainties described under the heading "Risk Factors" in Moderna's most recent Annual Report on Form 10-K filed with the U.S. Securities and Exchange Commission (SEC) and in subsequent filings made by Moderna with the SEC, which are available on the SEC's website at www.sec.gov. 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Moderna COVID-19 Vaccine: Authorized Use & Important Safety Information

Authorized Use in the United States:

The Moderna COVID-19 Vaccine has not been approved or licensed by the US Food and Drug Administration (FDA), but has been authorized for emergency use by FDA, under an Emergency Use Authorization (EUA), to prevent Coronavirus Disease 2019 (COVID-19) for use in individuals 18 years of age and older. There is no FDA-approved vaccine to prevent COVID-19.

The EUA for the Moderna COVID-19 Vaccine is in effect for the duration of the COVID-19 EUA declaration justifying emergency use of the product, unless the declaration is terminated or the authorization is revoked sooner.

Important Safety Information:

- Do not administer the Moderna COVID-19 Vaccine to individuals with a known history of severe allergic reaction (e.g., anaphylaxis) to any component of the Moderna COVID-19 Vaccine.
- Appropriate medical treatment to manage immediate allergic reactions must be immediately available in the event an acute anaphylactic reaction occurs following administration of the Moderna COVID-19 Vaccine. Monitor Moderna COVID-19 Vaccine recipients for the occurrence of immediate adverse reactions according to theCenters for Disease Control and Prevention guidelines (https://www.cdc.gov/vaccines/covid-19/).
- Immunocompromised persons, including individuals receiving immunosuppressive therapy, may have a diminished response to the Moderna COVID-19 Vaccine.
- The Moderna COVID-19 Vaccine may not protect all vaccine recipients.
- Adverse reactions reported in a clinical trial following administration of the Moderna COVID-19 Vaccine include pain at the injection site, fatigue, headache, myalgia, arthralgia, chills, nausea/vomiting, axillary swelling/tenderness, fever, swelling at the injection site, and erythema at the injection site. Additional adverse reactions, some of which may be serious, may become apparent with more widespread use of the Moderna COVID-19 Vaccine. Severe allergic reactions, including anaphylaxis, have been reported following administration of the Moderna COVID-19 Vaccine during mass vaccination outside of clinical trials.
- Available data on Moderna COVID-19 Vaccine administered to pregnant women are insufficient to inform vaccine-associated risks in pregnancy. Data are not available to assess the effects of Moderna COVID-19 Vaccine on the breastfed infant or on milk production/excretion.
- There are no data available on the interchangeability of the Moderna COVID-19 Vaccine with other COVID-19 vaccines to complete the vaccination series. Individuals who have received one dose of Moderna COVID-19 Vaccine should receive a second dose of Moderna COVID-19 Vaccine to complete the vaccination series.
- Additional adverse reactions, some of which may be serious, may become apparent with more widespread use of the Moderna COVID-19 Vaccine.
- Vaccination providers must complete and submit reports to VAERS online at https://vaers.hhs.gov/reportevent.html. For further assistance with reporting to VAERS, call 1-800-822-7967. The reports should include the words " Moderna COVID- 19 Vaccine EUA " in the description section of the report.

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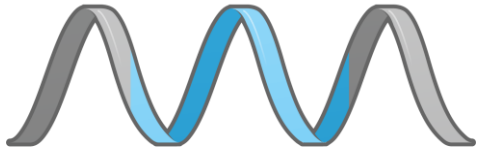
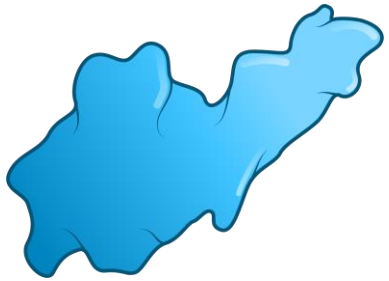
Central dogma of molecular biology

DNA → **messenger RNA** → **Protein**





Storage

DNA stores instructions for proteins in the nucleus

Software

mRNA is a temporary set of instructions for cells to make a protein; mRNA is made using DNA

Applications

Proteins form the basis of life by performing the functions required by every cell; proteins are made using mRNA

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Our mission

To deliver on the promise of mRNA science to create a new generation of transformative medicines for patients.

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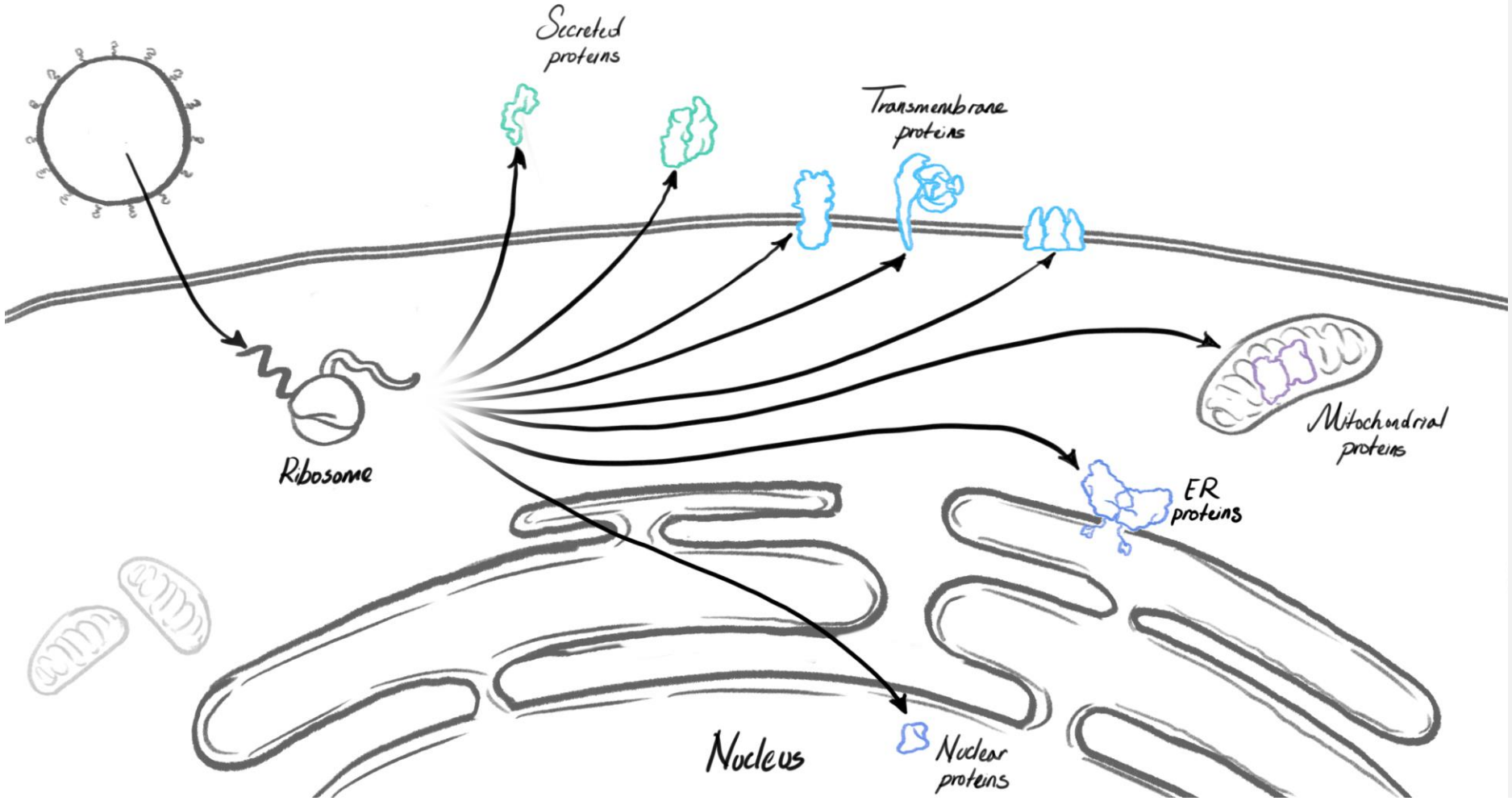
mRNA as a new class of medicines



1. Large product opportunity
2. Higher probability of technical success
3. Accelerated research and development timelines
4. Greater capital efficiency over time vs. recombinant technology

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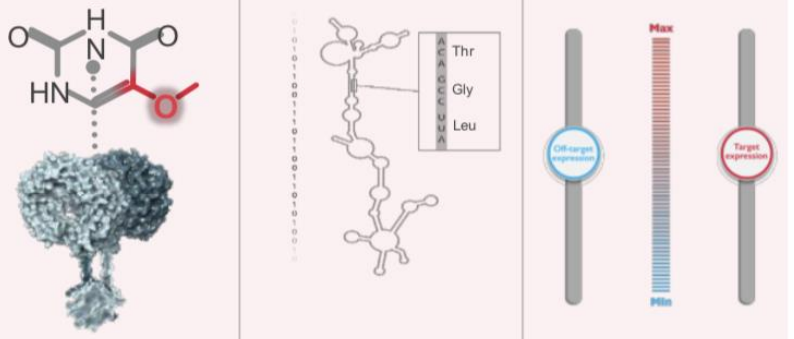
Our platform

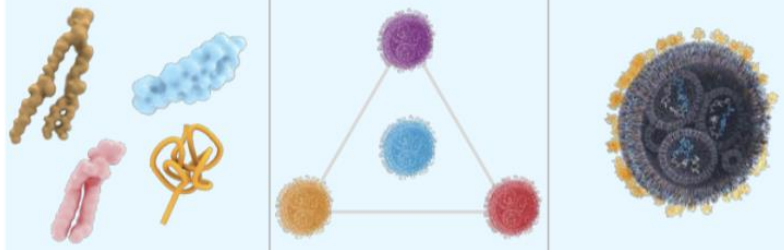
mRNA

- Chemistry
- Sequence engineering
- Targeting elements



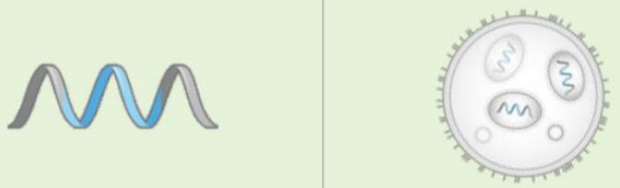
Delivery

- Chemistry
- Composition
- Surface properties



Manufacturing Process

- mRNA
- LNP



Fully Integrated mRNA-LNP Company

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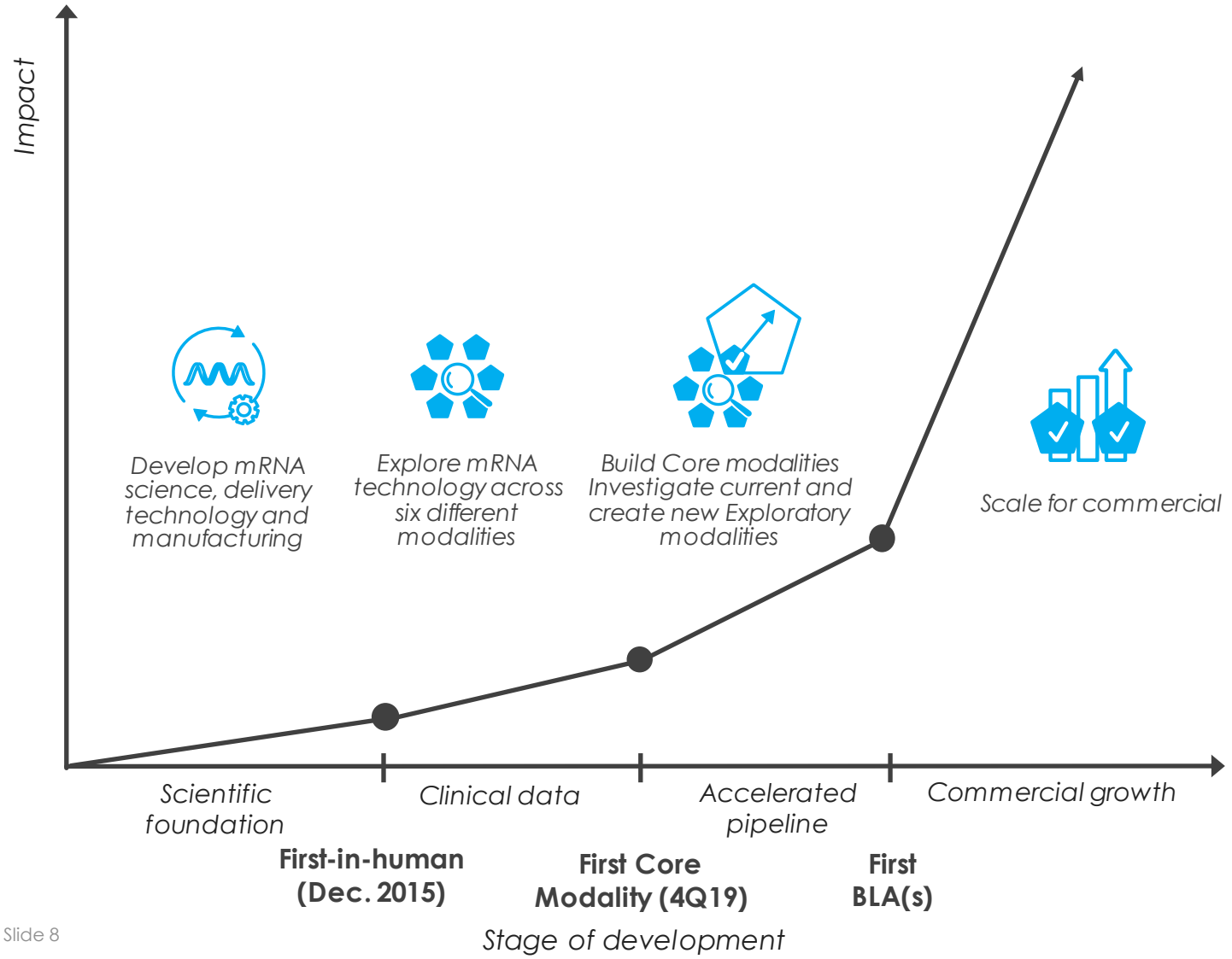
Our commitment to be the best at mRNA science is core to who we are



We have advanced mRNA science to enable our current development pipeline

Developing transformative medicines **requires investments:**

- For the long-term
- At scale
- With the right team

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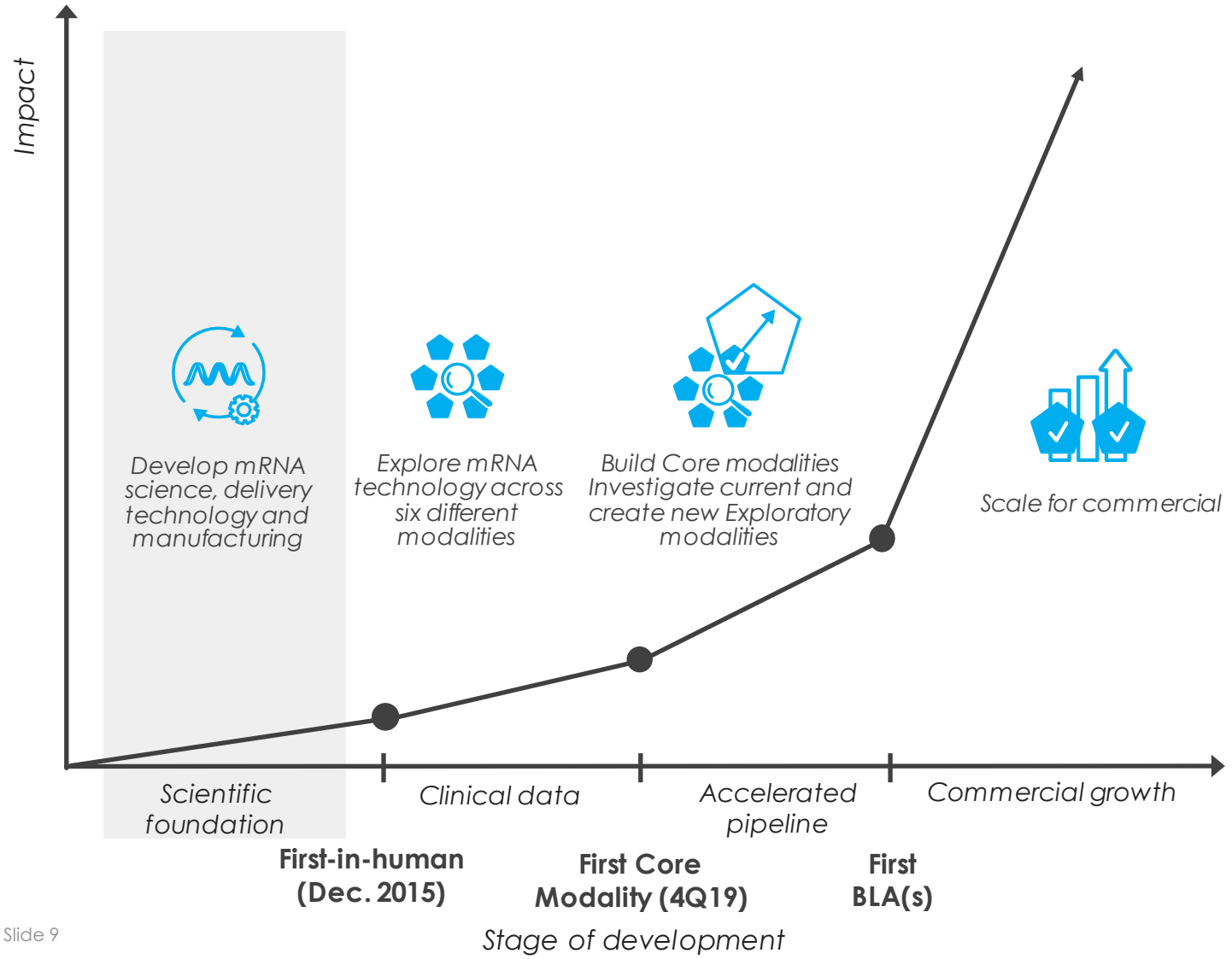
Since inception our investments in platform research advanced mRNA science and established a scientific foundation



We have advanced mRNA science to enable our current development pipeline

Establishing our scientific foundation **requires investments in:**

- Platform research
- Digital resources
- Teams

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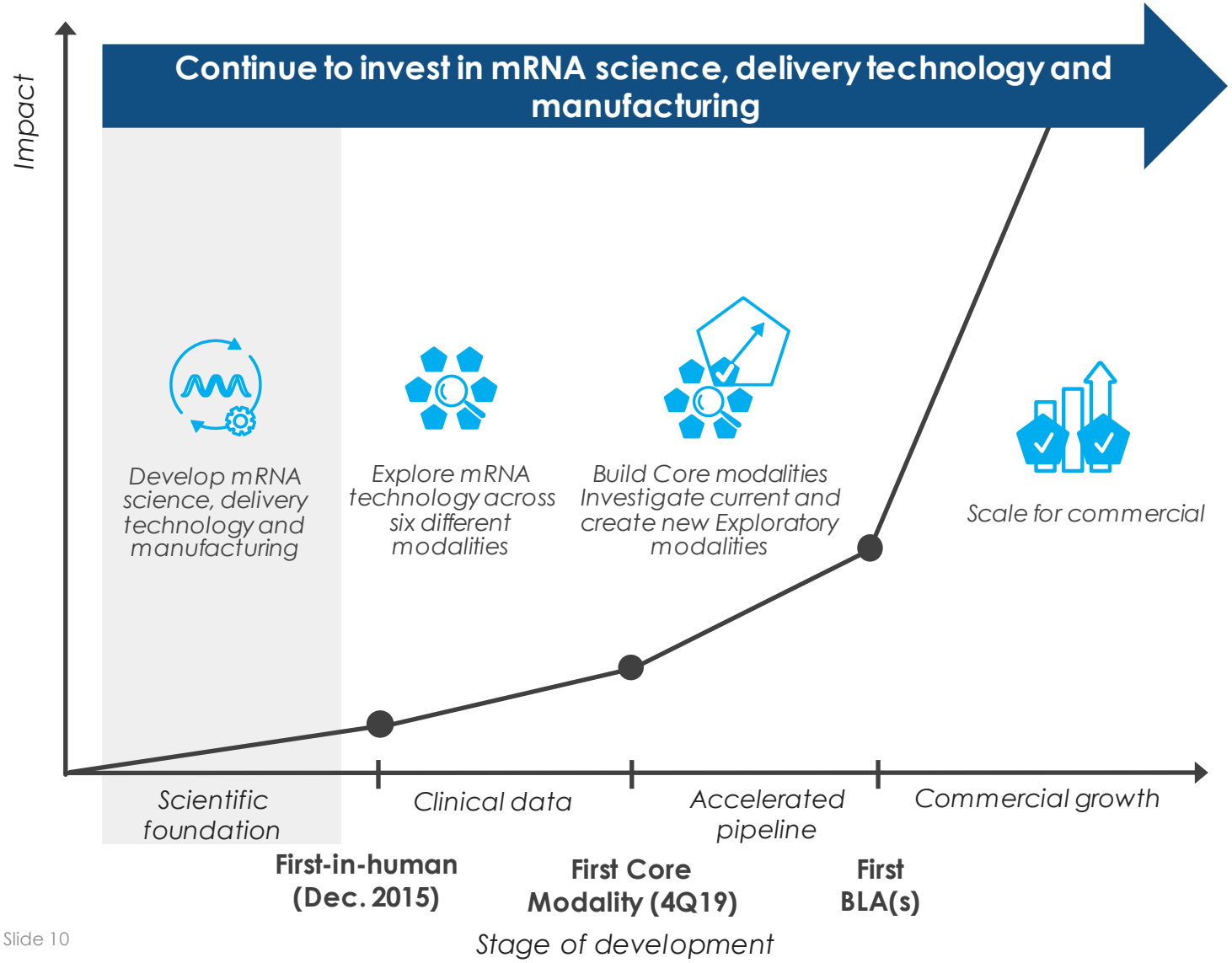
We are a leading mRNA platform and continue to invest like it's the beginning of the journey



We have advanced mRNA science to enable our current development pipeline

We believe continuing to invest in mRNA science **will enable:**

- Additional transformative medicines coming to market
- New development candidates entering the clinic
- Invention of new nucleic acid technologies

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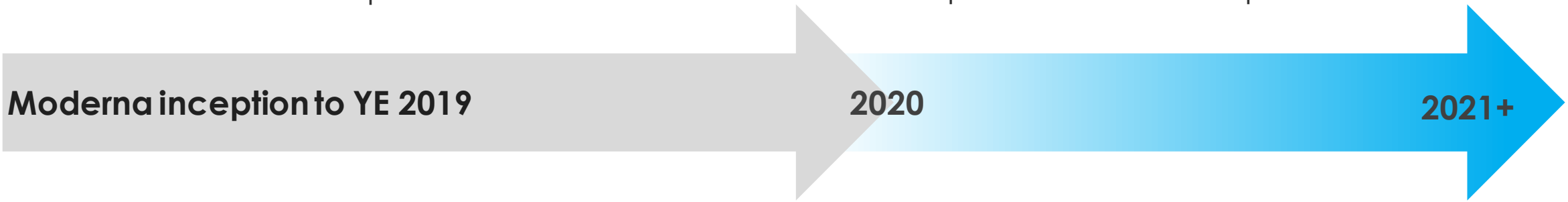
Investments in platform research allowed Moderna to be ready to respond to COVID-19

Invested heavily in research, technical development and manufacturing for our pipeline of 20+ mRNA vaccines and therapeutics

Focus on scale of clinical development and manufacturing to respond to COVID-19 pandemic



…and **investments into platform research and mRNA science** are helping us get there

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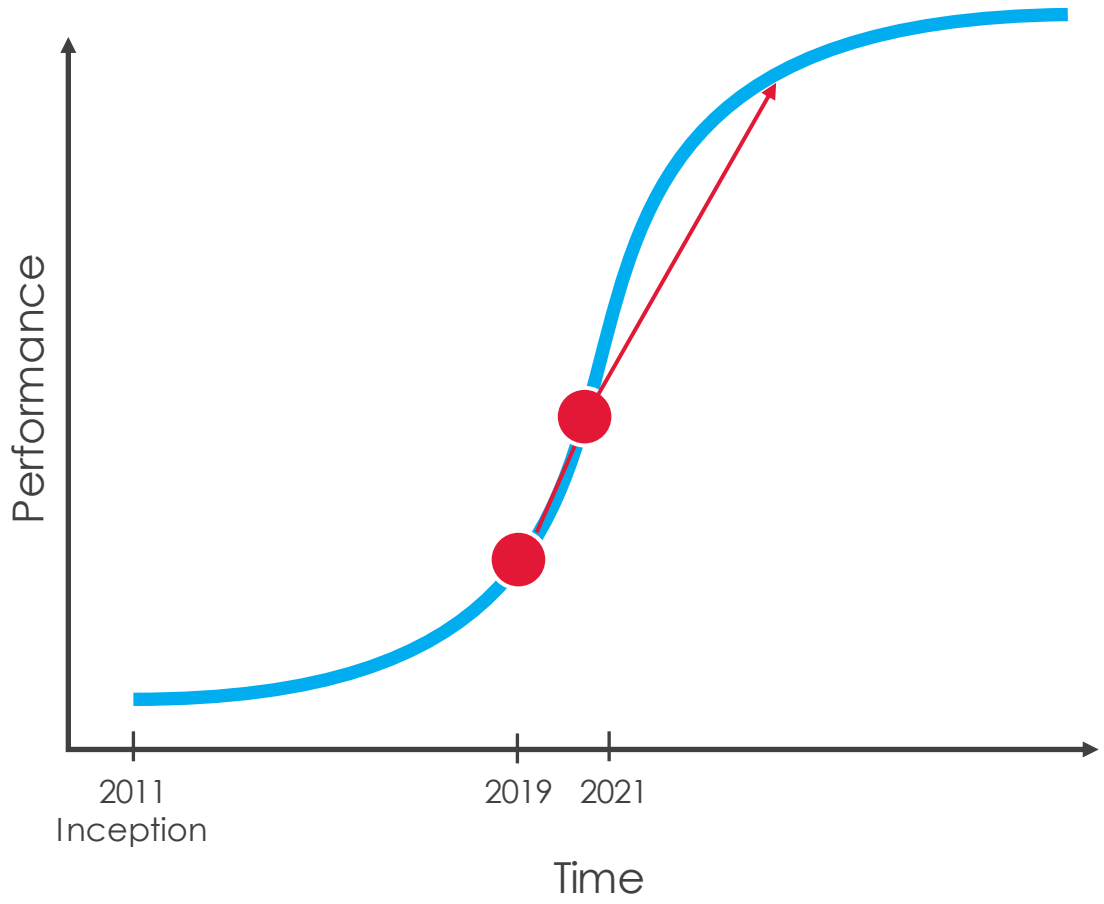
Our commitment to be the best at mRNA science is core to who we are: A 20-year journey



- **10x mentality**: We know our process works and aim to scale it out to other programs
- **Process optimization**: Use data analytics, machine learning, AI and robotics
- **Moderna's competitive advantages:**
  - Platform research investments for 10+ years
  - Infrastructure (digital backbone + manufacturing plants)
  - Structured data collection and ability to leverage it
- **Largest scale in the mRNA industry**

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Agenda

Introduction	**Stéphane Bancel**, CEO **Melissa Moore, Ph.D.**, CSO, Platform Research
Tracking, assessing and predicting SARS-CoV-2 variants of concern	**Melissa Moore, Ph.D.**, CSO, Platform Research **Guillaume Stewart-Jones, Ph.D.** **Wei Zheng, Ph.D.**
mRNA Engineering: Optimizing Ribosome Load	**David Reid, Ph.D.**
Coffee Break	
Understanding and engineering intracellular events affecting LNP performance	**Melissa Moore, Ph.D.**, CSO, Platform Research
In vivo mRNA delivery to Hematopoietic Stem and Progenitor Cells	**David Alvarez, Ph.D.**
Closing remarks	**Stephen Hoge, M.D.**, President
Q&A	

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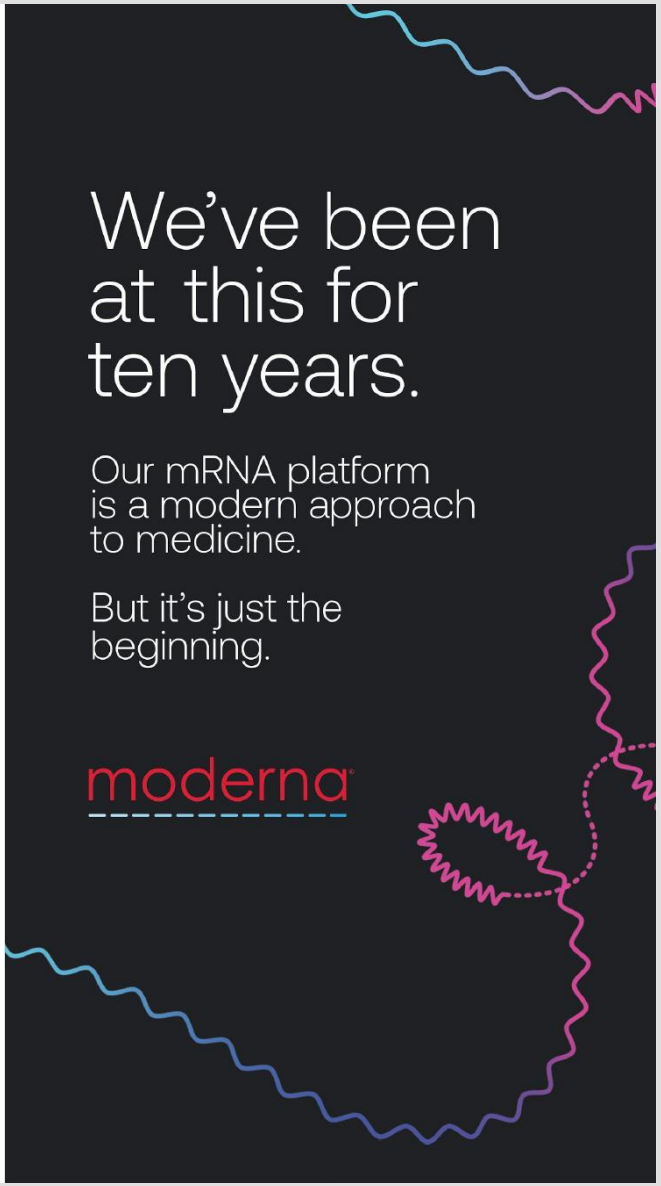
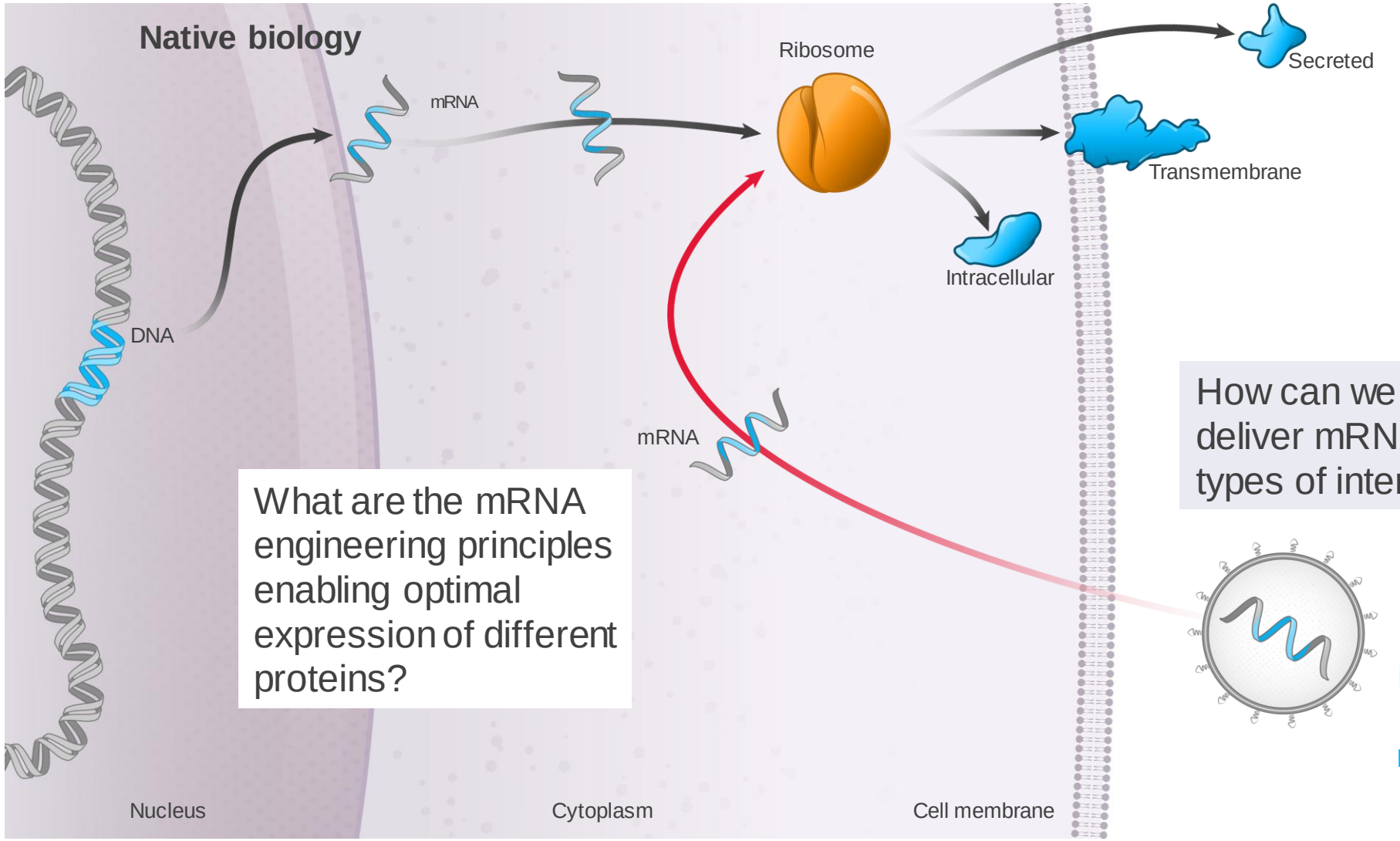


Platform Technologies: mRNA engineering and delivery systems

Platform Research

mRNA engineering and delivery technology discovery



Moderna mRNA medicine

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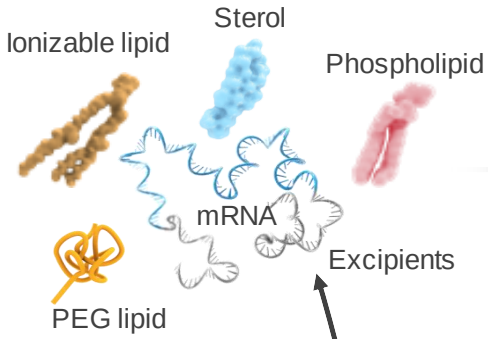
Engineering mRNA medicines

Delivery systems

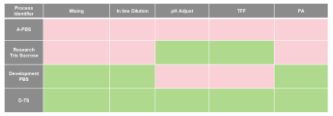
Components



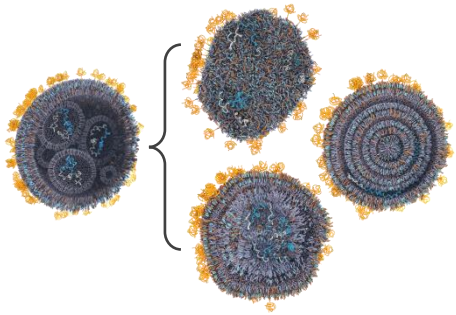
Composition & Process

Δ molecules
Δ composition

Δ process



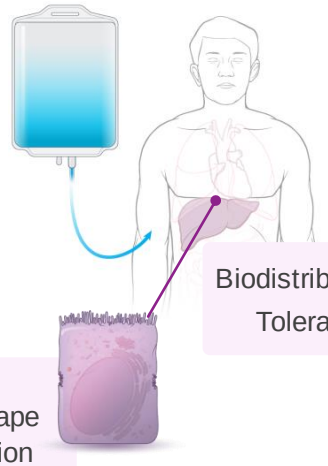
Structure



Function

Chemical stability
Physical stability

Therapeutic effect

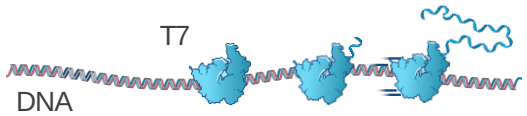


Biodistribution
Tolerability

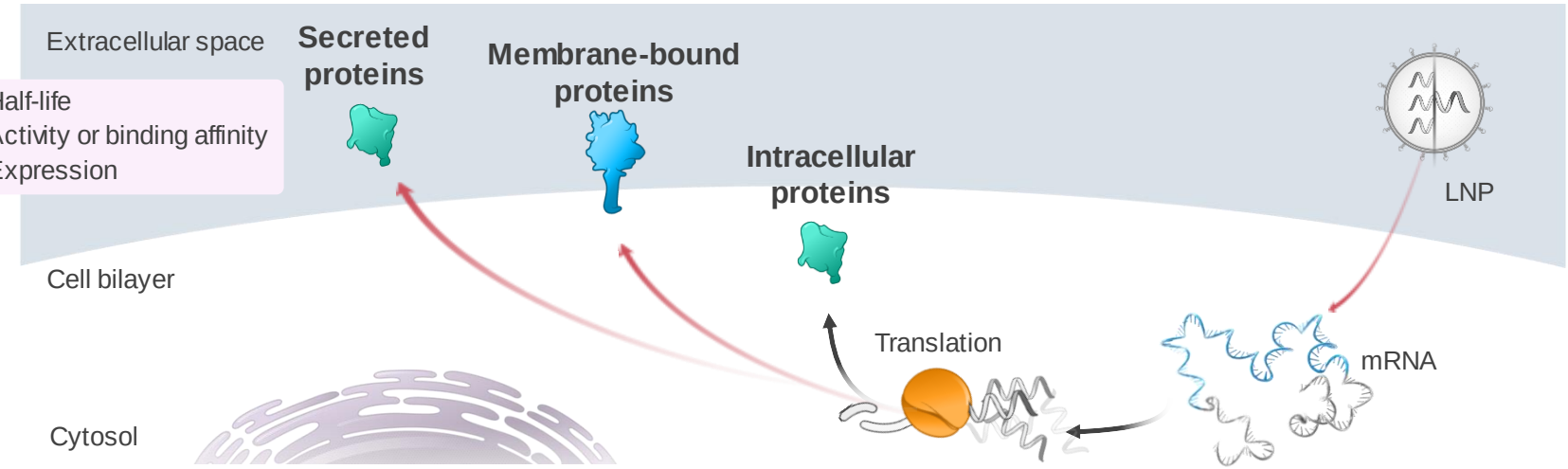
Cellular uptake
Endosomal escape
Protein expression

Proteins

Manufacturing enzymes



RNA quality
RNA fidelity
Protein stability
Capping efficiency



Half-life
Activity or binding affinity
Expression

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Acknowledgements

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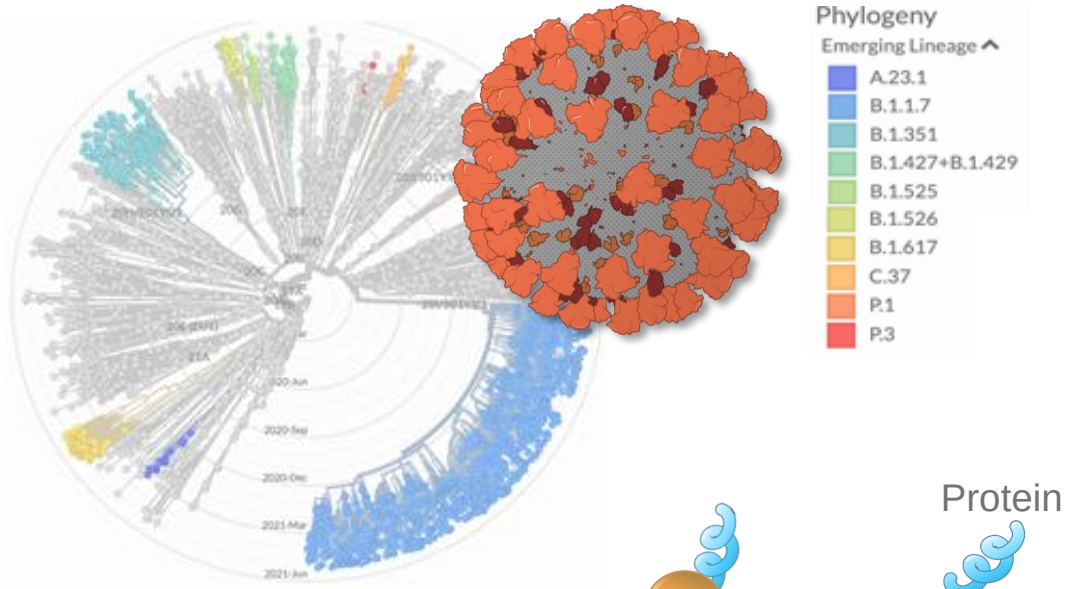
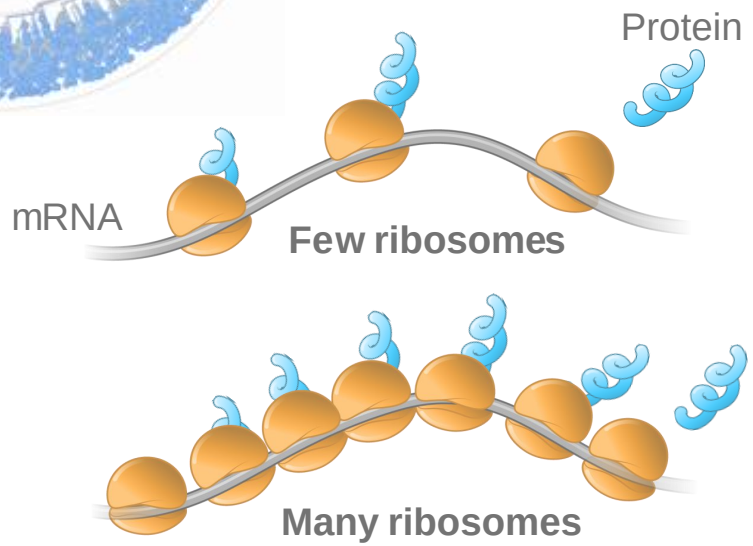
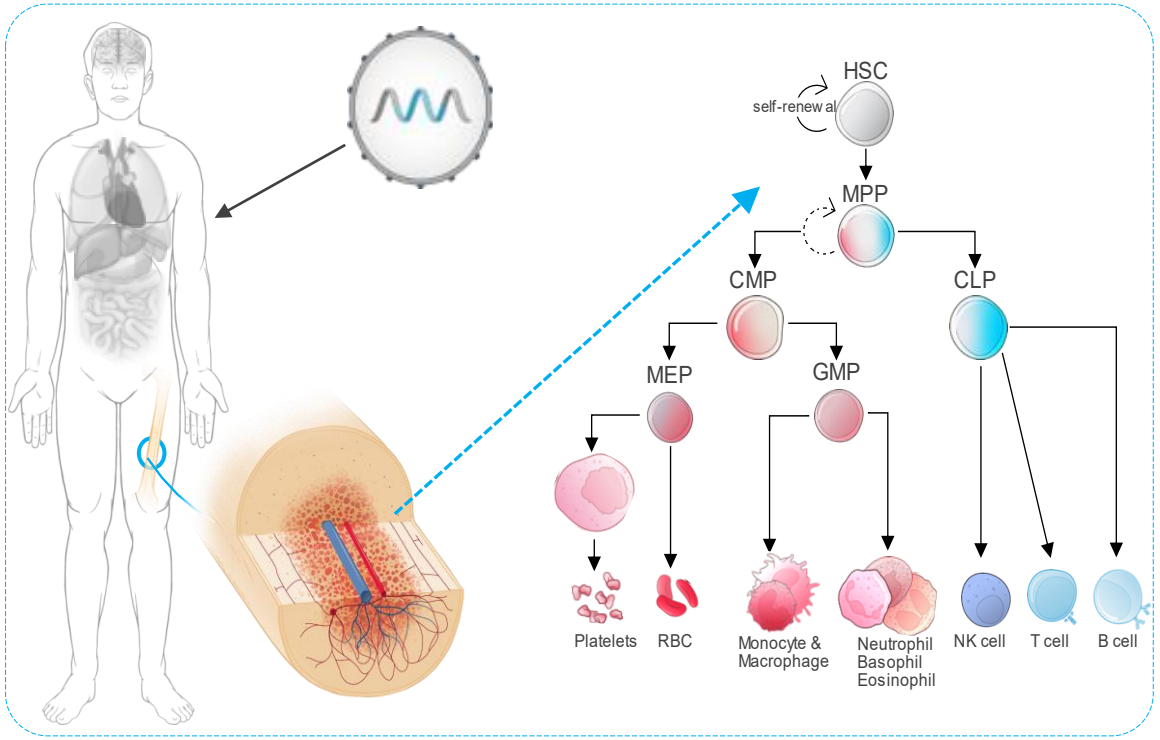
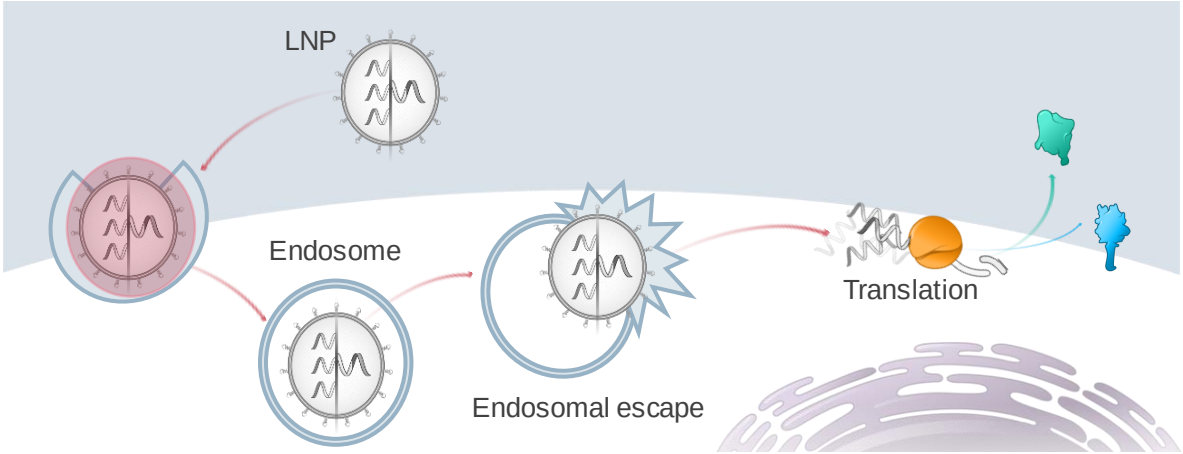
Today's agenda









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Our strategy for combating COVID-19

Focus of today's presentation

Stay ahead of SARS-CoV-2

1. Closely monitor **emerging Variants of Concern** and **waning immunity**
2. Rapidly move to **update vaccine** for VoCs with breakthrough potential
3. Partner with governments to **ensure access** to the most *up-to-date* boosters

…with Coronaviruses the question is 'when', not 'if'

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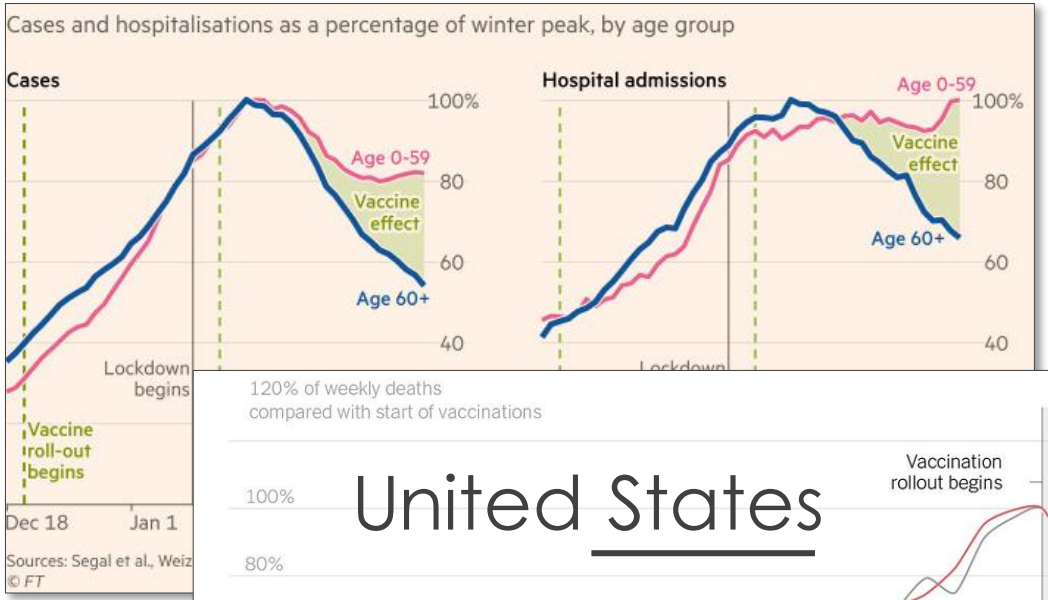
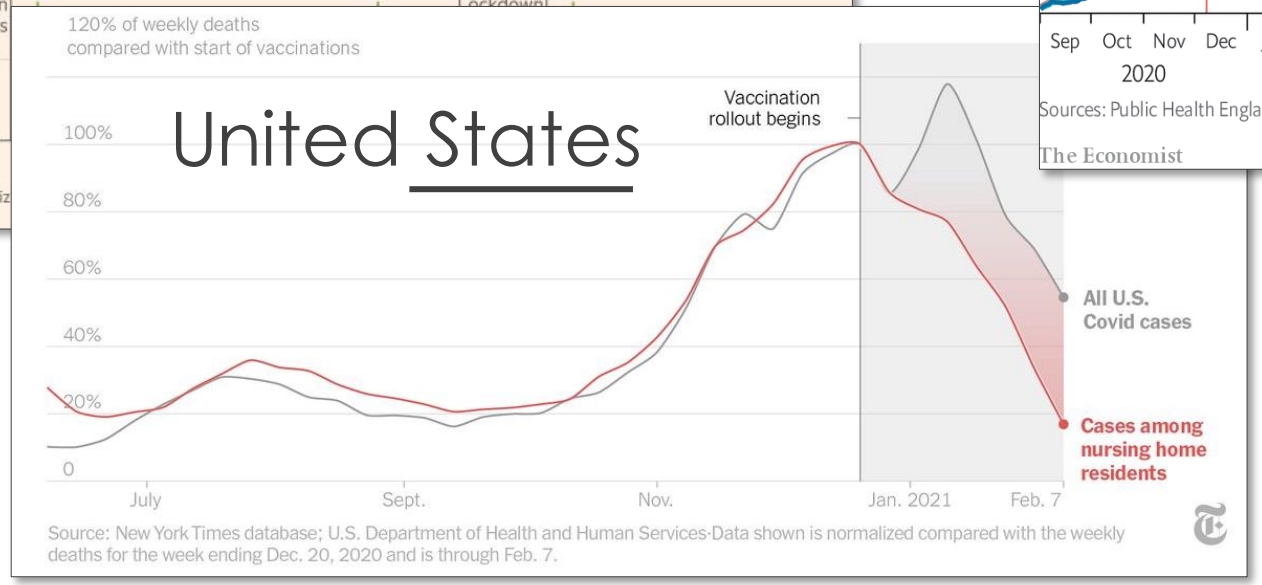
Early evidence that vaccines are having an impact

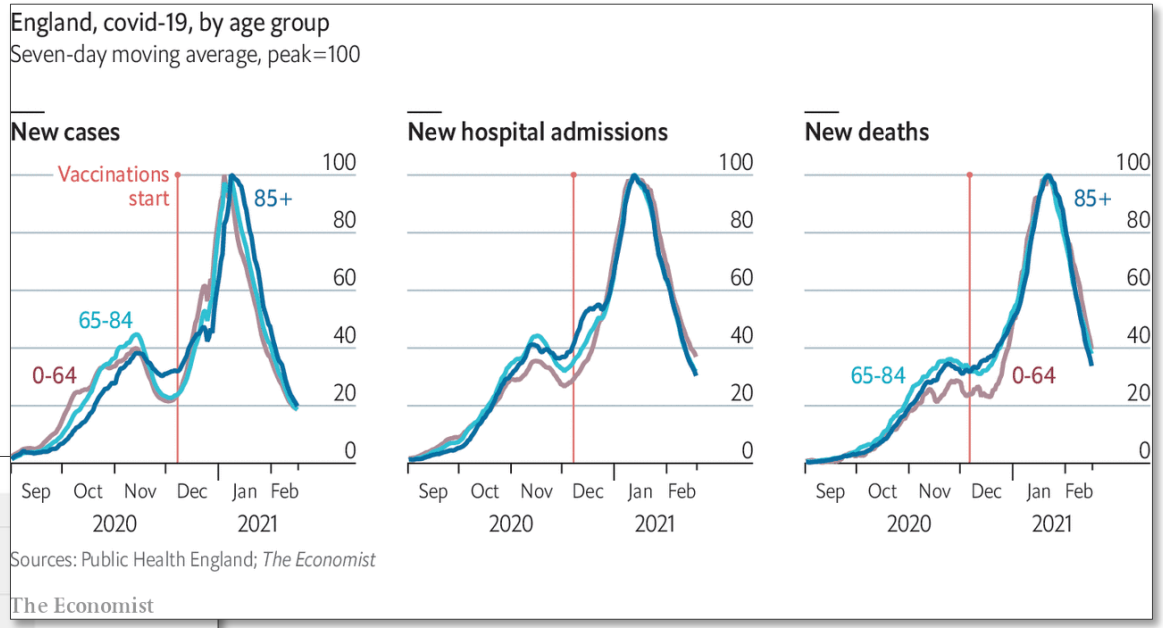
Israel



United States



England



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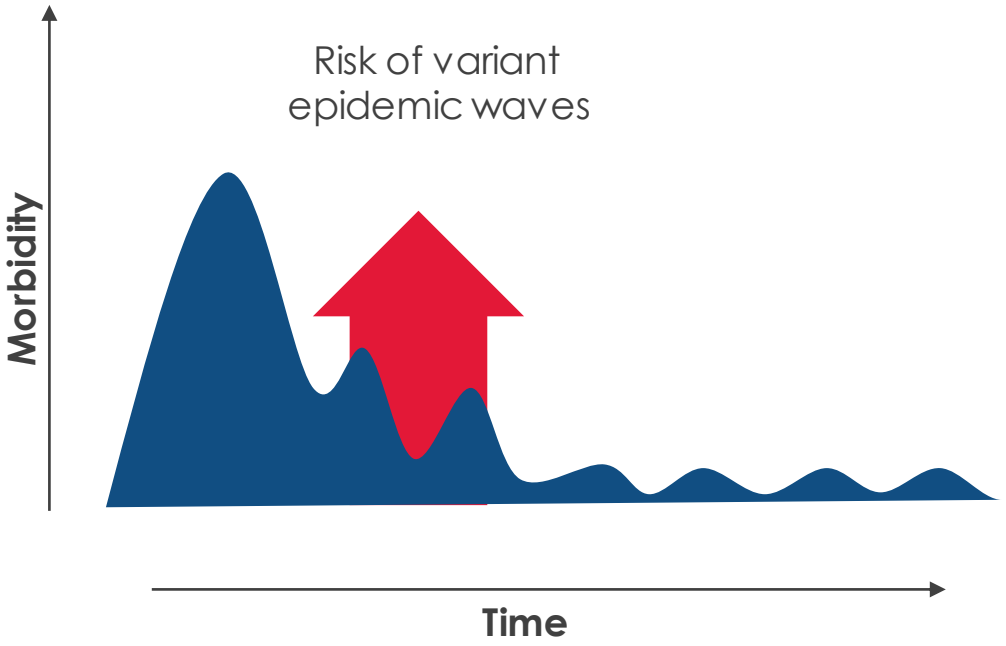
But new epidemic waves are undoubtedly on the way



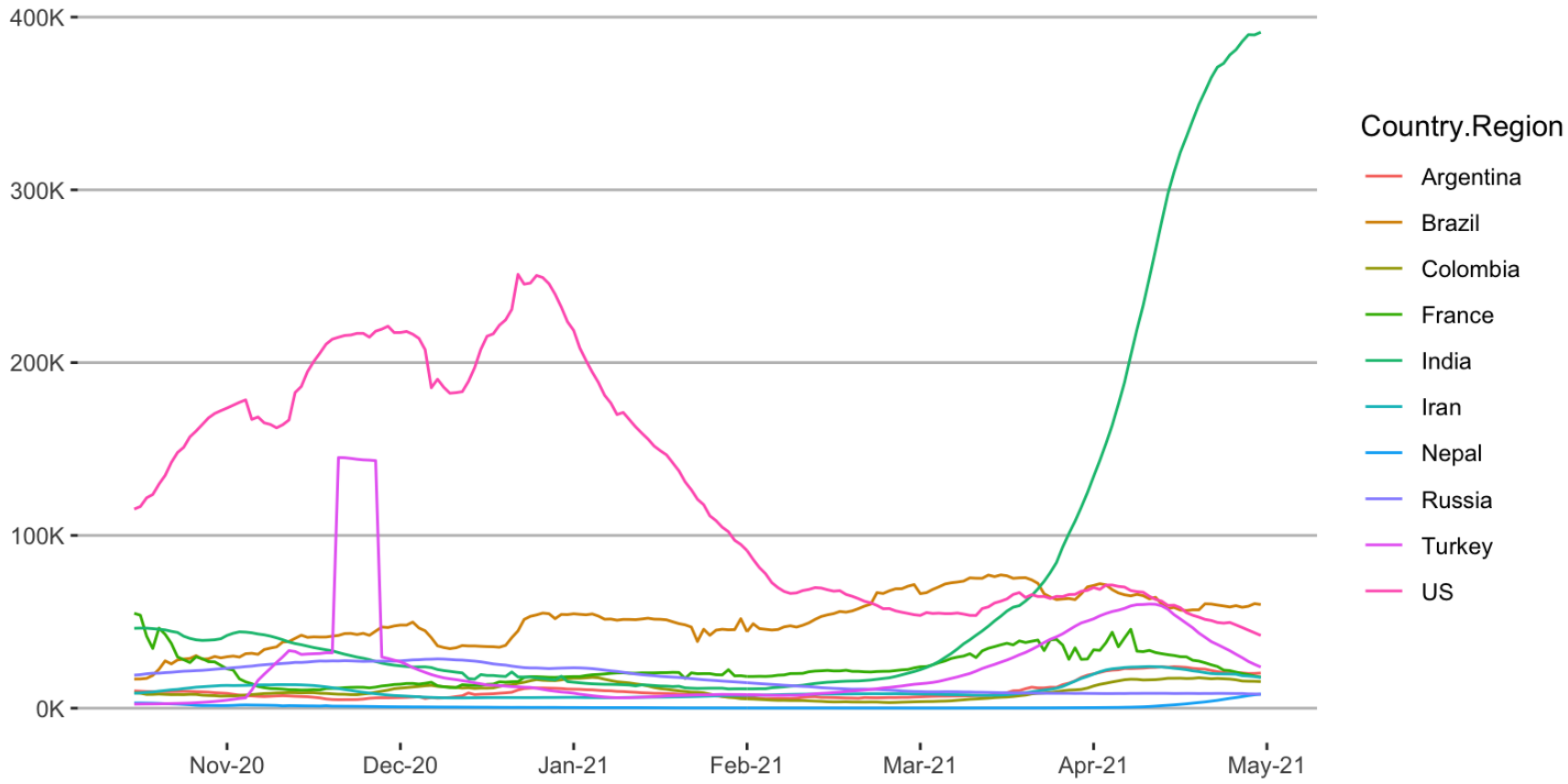
- Lower neutralizing titers against key VoCs suggest a risk of reduced duration of protection post-vaccination
- Increased transmissibility of VoC could increase the exposure risks in high-risk populations
- Immune pressure and selection are just beginning

Tegally , Houriyah et al. “Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa” (22 Dec 2020) https://www.medrxiv.org/content/10.1101/2020.12.21.20248640v1.full
Wang, C et al. “COVID-19 in early 2021: current status and looking forward,” *Sig Transduct Target Ther* (08 Mar. 2021), https://doi.org/10.1038/s41392-021-00527-1

But new epidemic waves are undoubtedly on the way

DAILY CONFIRMED NEW CASES (7-DAY MOVING AVERAGE)

Outbreak evolution for the currently most affected countries



Source: Johns Hopkins University, New COVID-19 Cases Worldwide

Major Variant Families

- B1.617
- P.1 P.3
- B1.427+B1.429
- B1.351

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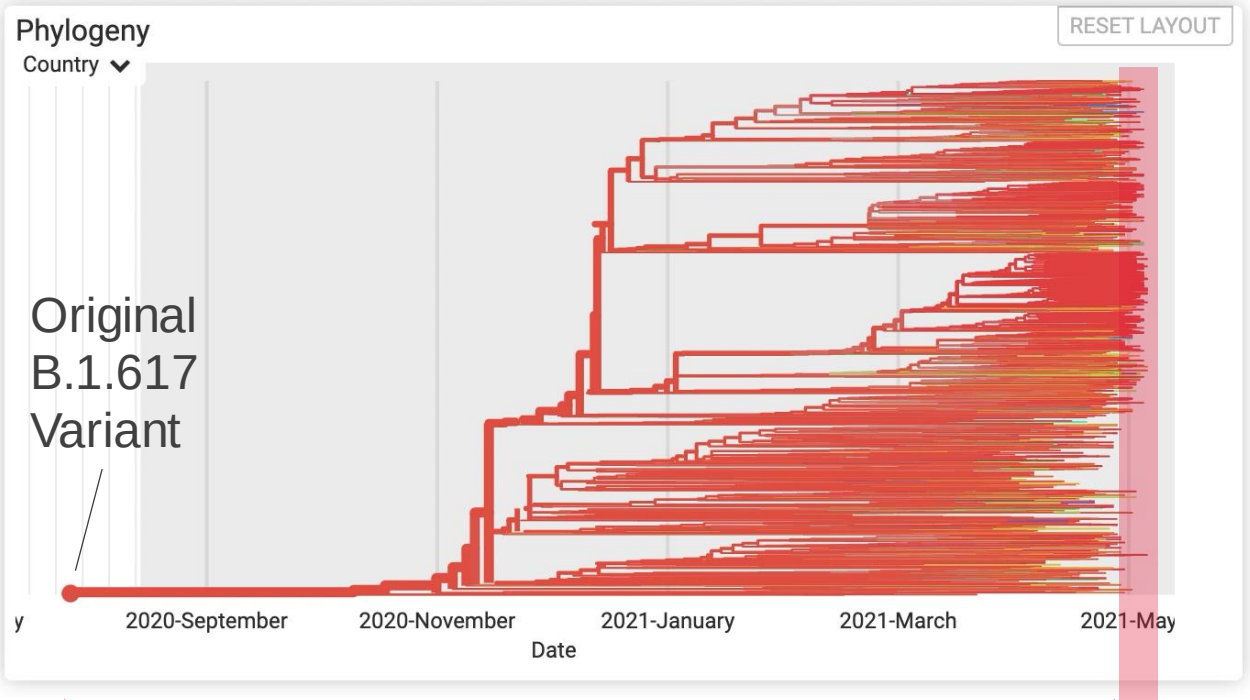
A recent example: The B.1.617 variant family



Nextstrain

B.1.617 Variant Family

>800 spike protein haplotypes

Each named "variant" spawns a family of individual "haplotypes", with each haplotype containing a unique combination of mutations.

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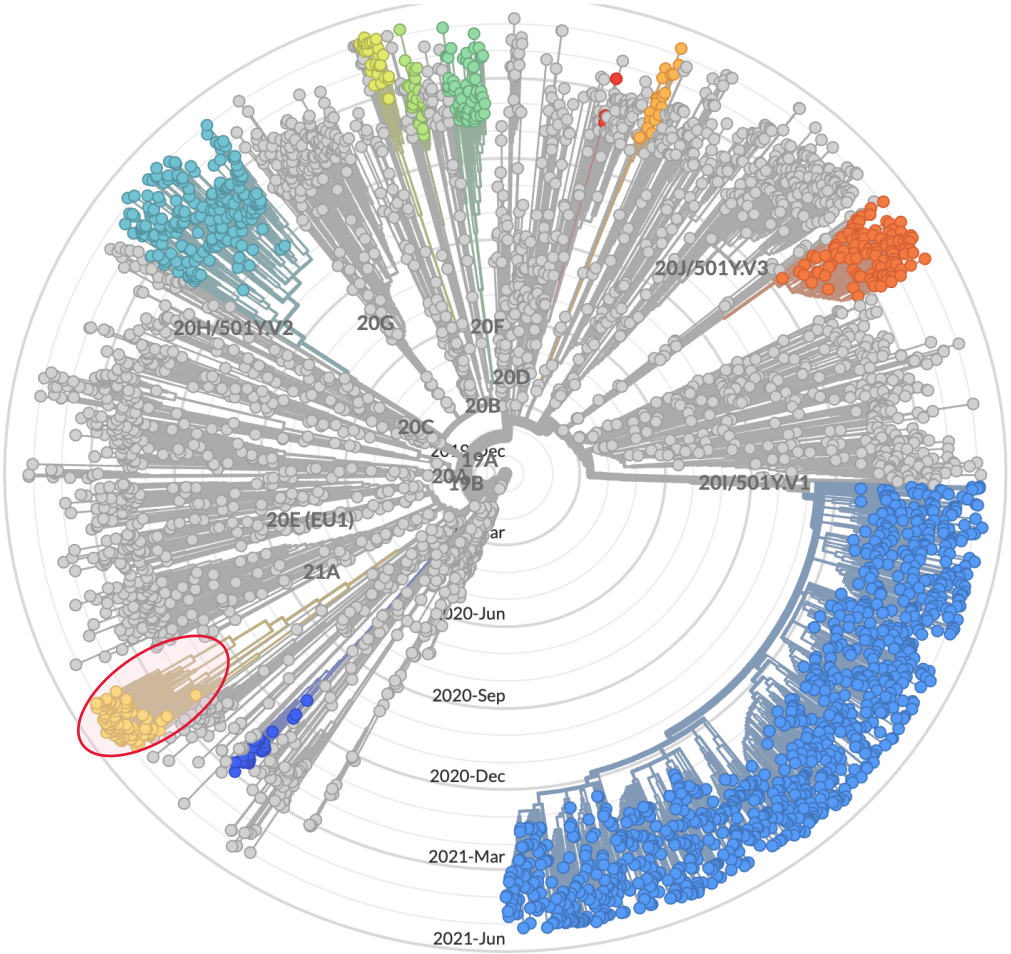
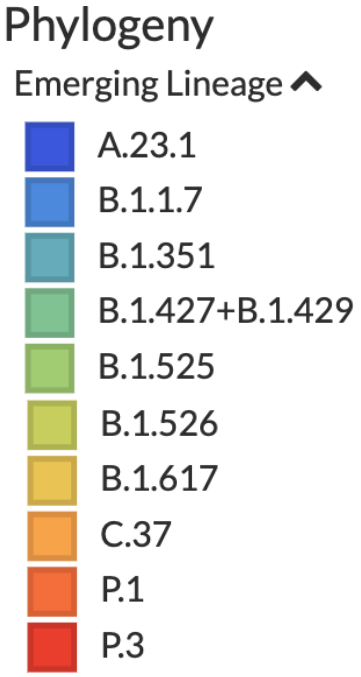
What Moderna needs to know





- Which sets of mutations will enable the virus to break through our current vaccine?
- Which exact combinations of mutations (i.e., individual haplotypes) should we incorporate into new booster vaccines?

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Variant families can be classified according to risk profiles

CDC Variant Classification System

	Immunity from prior infection or vaccination ↓	Transmissibility ↑	Disease severity ↑	Susceptibility to treatment ↓	Diagnostic detection ↓
Variant of Interest **(VoI)**	association	predicted	predicted	predicted	predicted
Variant of Concern **(VoC)**	significant	evidence	evidence	evidence	evidence
Variant of High Consequence **(VoHC)**	significant	evidence	increased hospitalizations	significantly reduced	demonstrated failure

CDC, SARS-CoV-2 Variant Classifications and Definitions,

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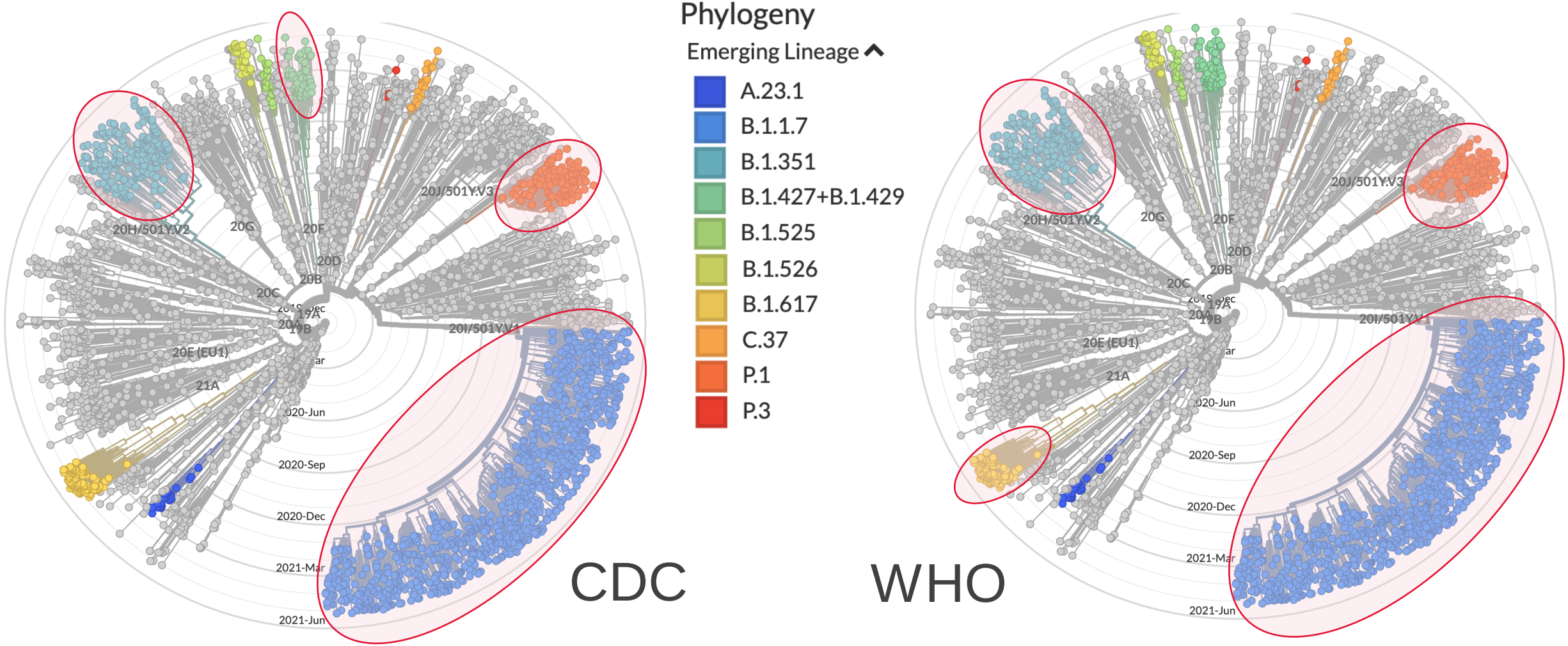
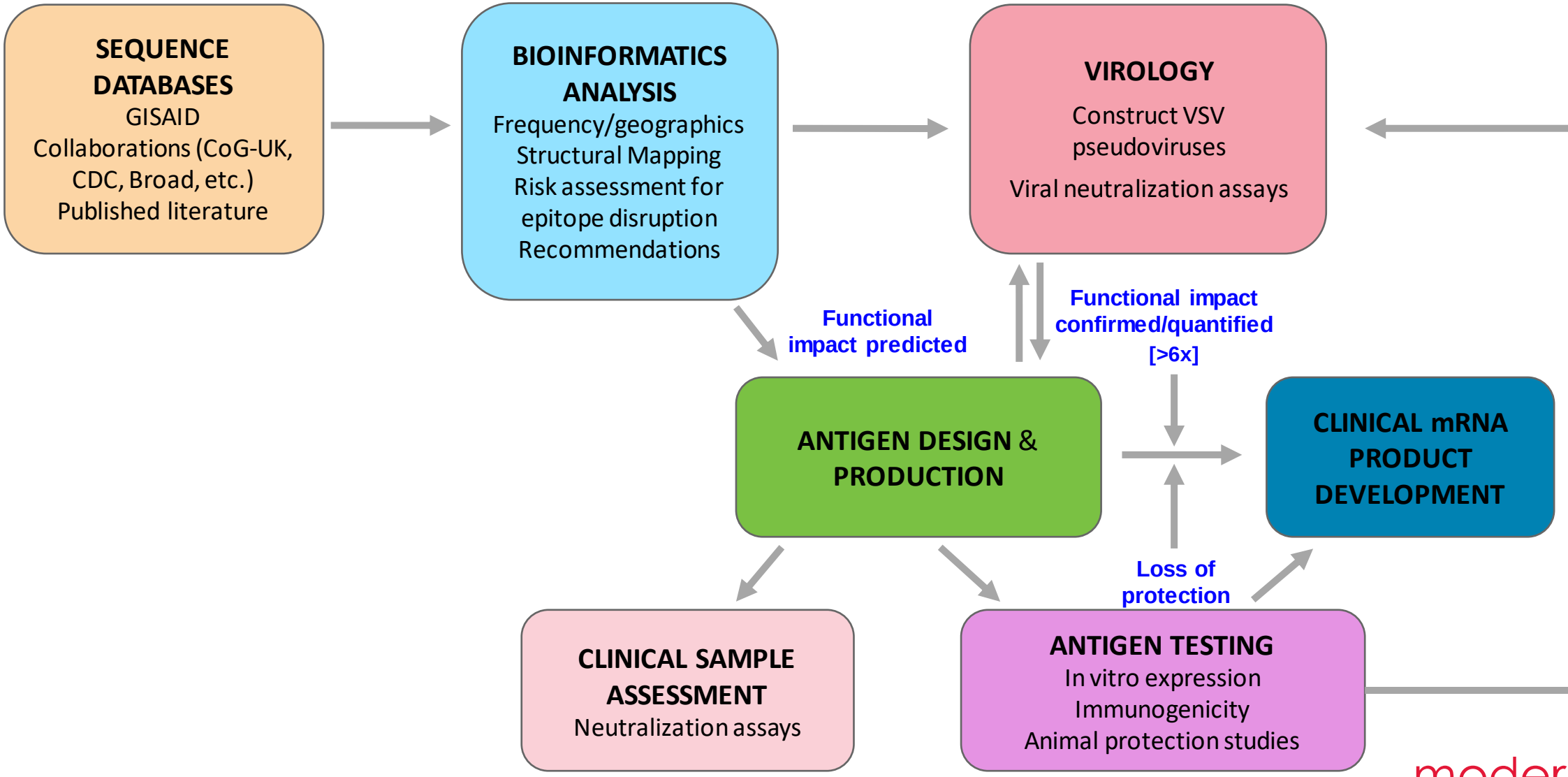
CDC and WHO have identified different VoCs



How does Moderna decide?

- Building a comprehensive set of pre-clinical tools to assess potential of VoCs to break through our current vaccines
- Constantly creating and testing new vaccines
- Building sophisticated in-house computational tools to get out ahead of the virus

Moderna's current workflow to rapidly evaluate potential mRNA-1273 escape variants



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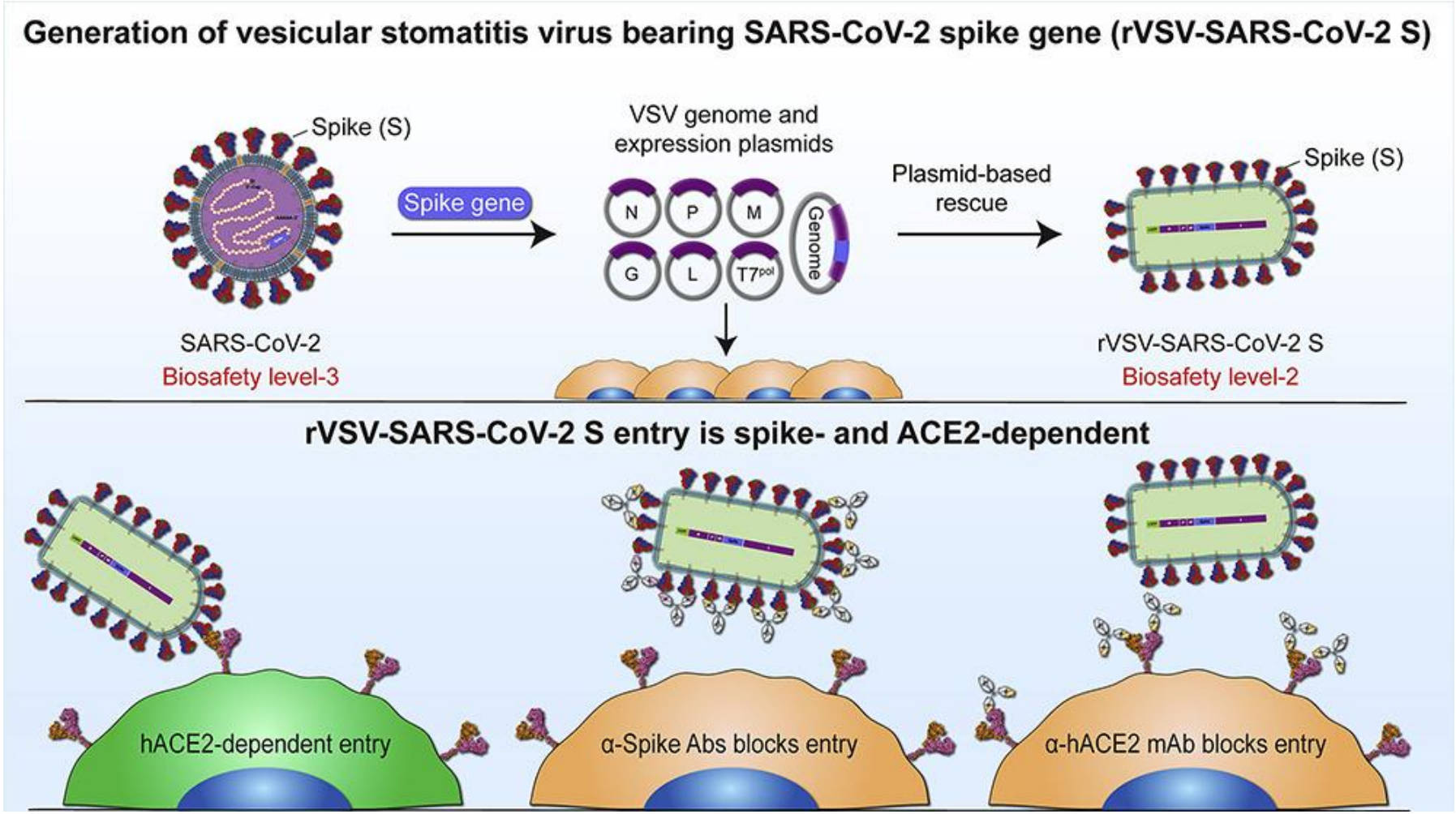
VSV pseudoviruses enable safe and effective assessment of antibody neutralization activity



Dieterle … Jangra (2020) Cell Host and Microbe

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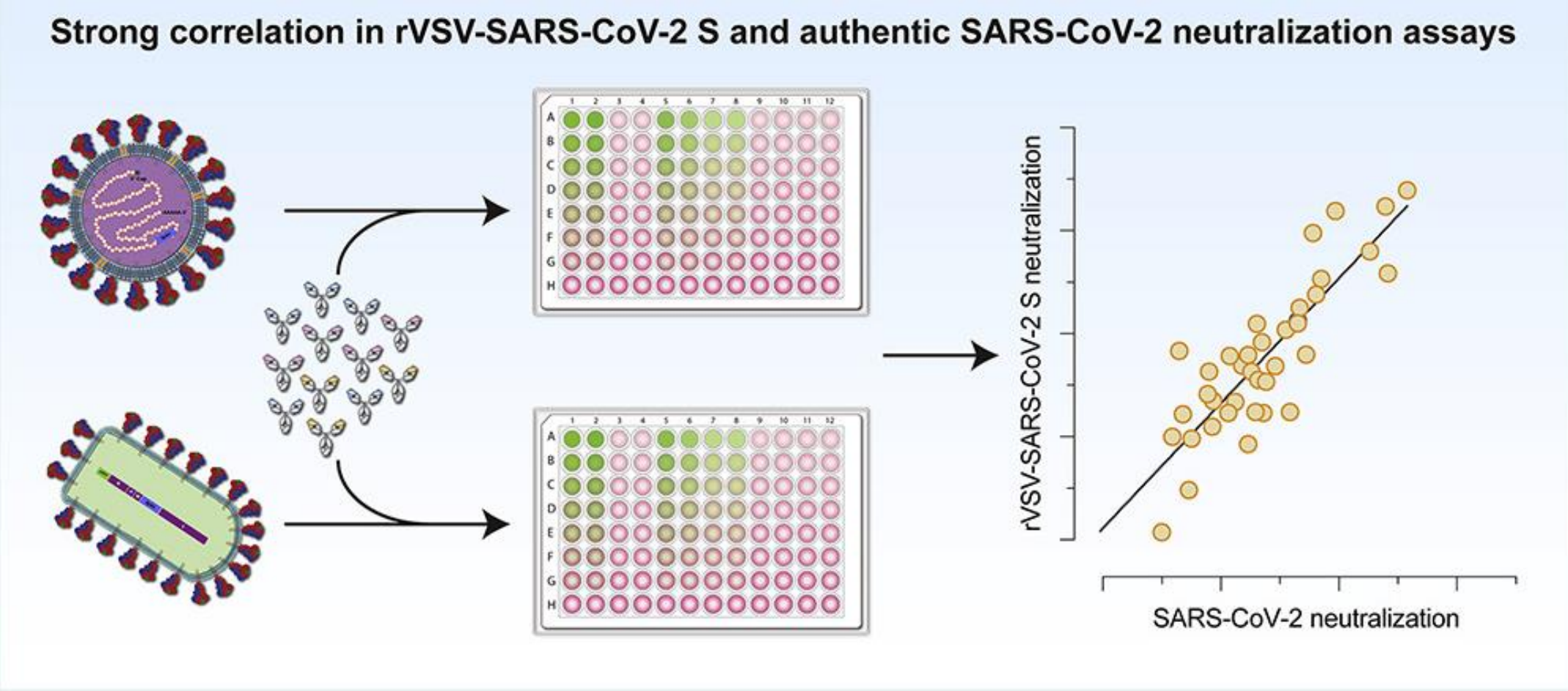
VSV pseudoviruses enable safe and effective assessment of antibody neutralization activity



Dieterle … Jangra (2020) Cell Host and Microbe

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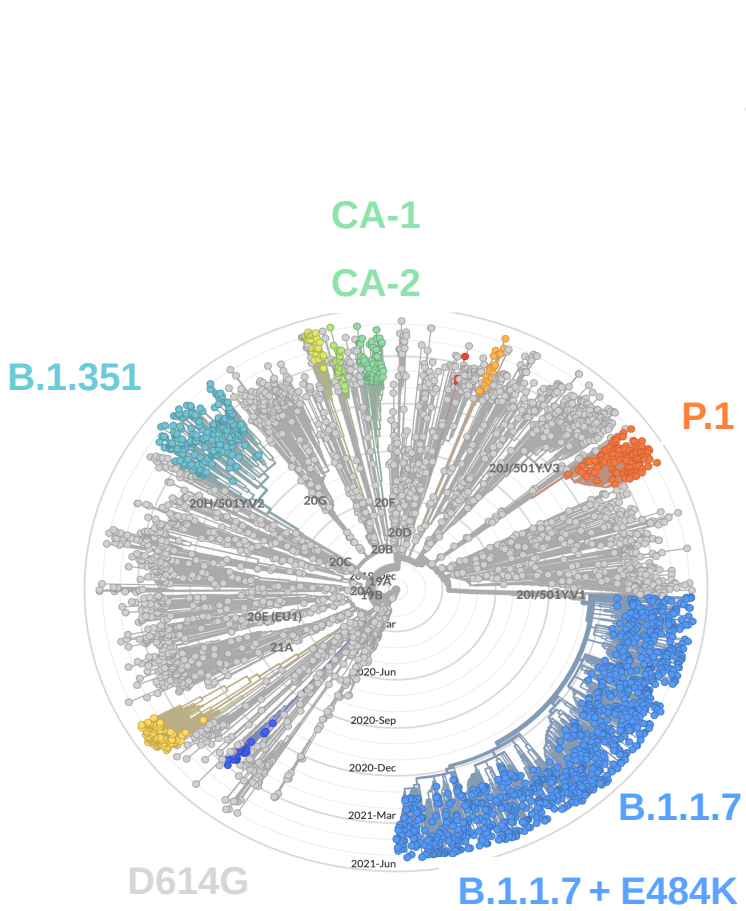
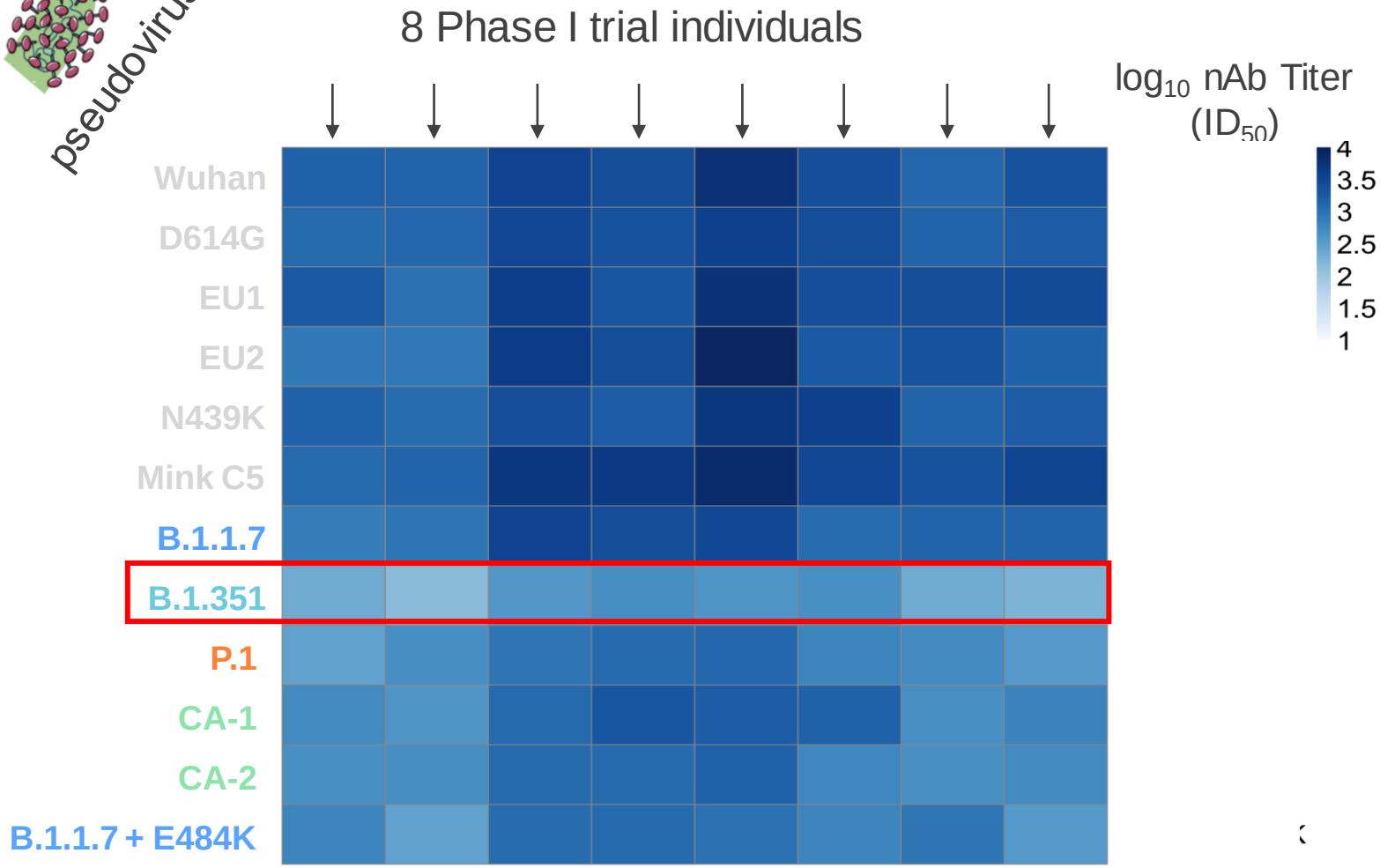
Our pseudovirus neutralization data revealed that B.1.351 was a Variant of Concern





Wu … Edwards (2021) NEJM

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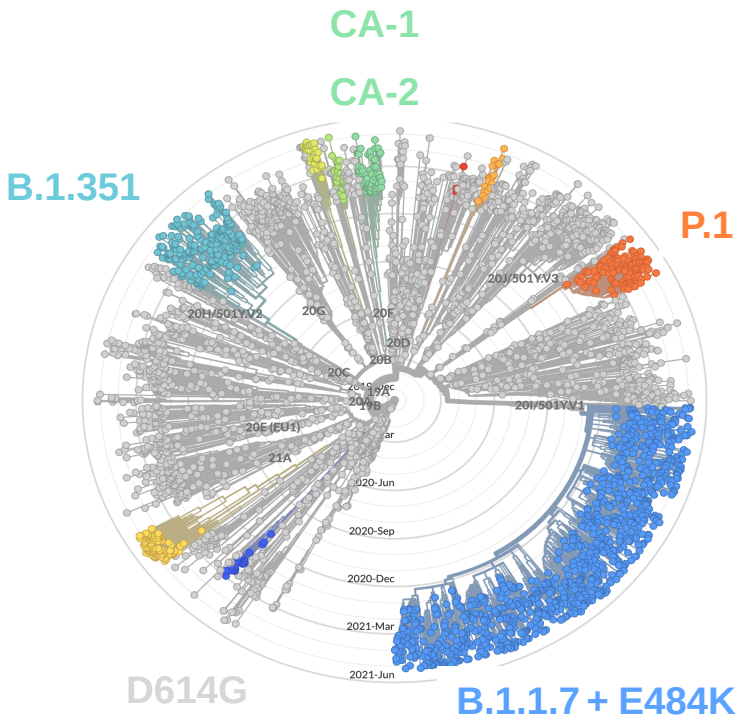
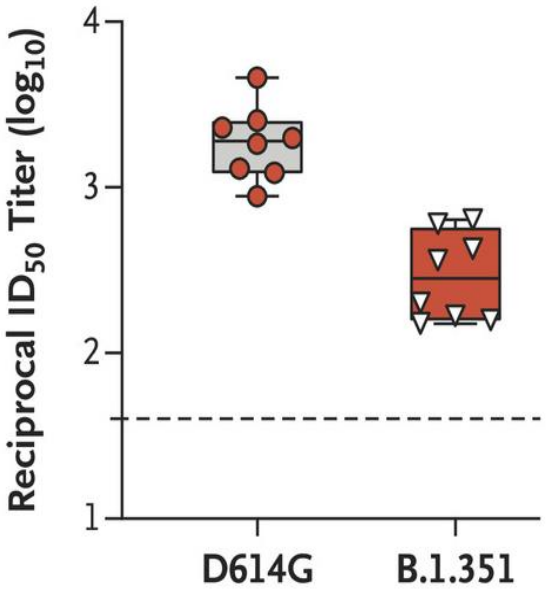
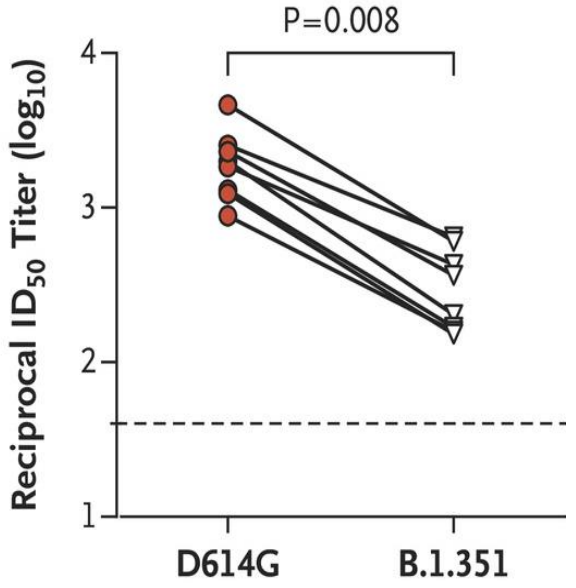
Our pseudovirus neutralization data revealed that B.1.351 was a Variant of Concern







Wu … Edwards (2021) *NEJM*

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So we created a new vaccine, mRNA-1273.351

mRNA-1273	L	D	D	L	A	L	R	K	E	N	D	A
AA position in Spike Protein	18	80	215	242	243	244	246	417	484	501	614	701
mRNA1273.351	F	A	G	-	-	-	I	N	K	Y	G	V

Wu .. Edwards (2021) bioRxiv Preprint

- D614G
- B.1.351
- CAL.20C
- P.1
- B.1.526
- A.23.1
- B.1.525
- B.1.1.7
- B.1.1.7 + E484K
- B.1.617.1

# Mice per group	Prime (Day 1) & 2nd (Day 22) Dose	Sera Collection to be tested for Neutralization
4	Placebo	Day 36
8	mRNA-1273 (1μg)	
8	mRNA-1273.351 (1μg)	

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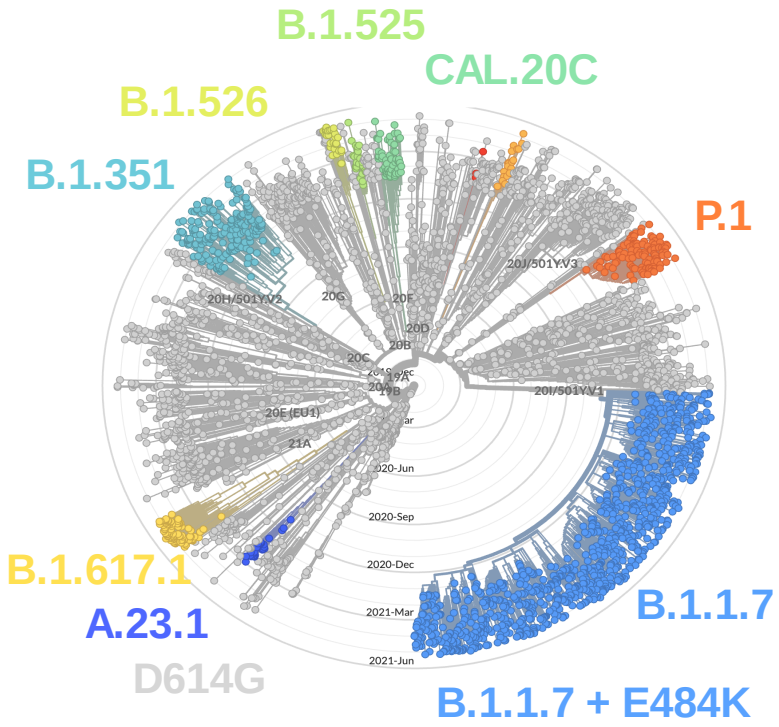
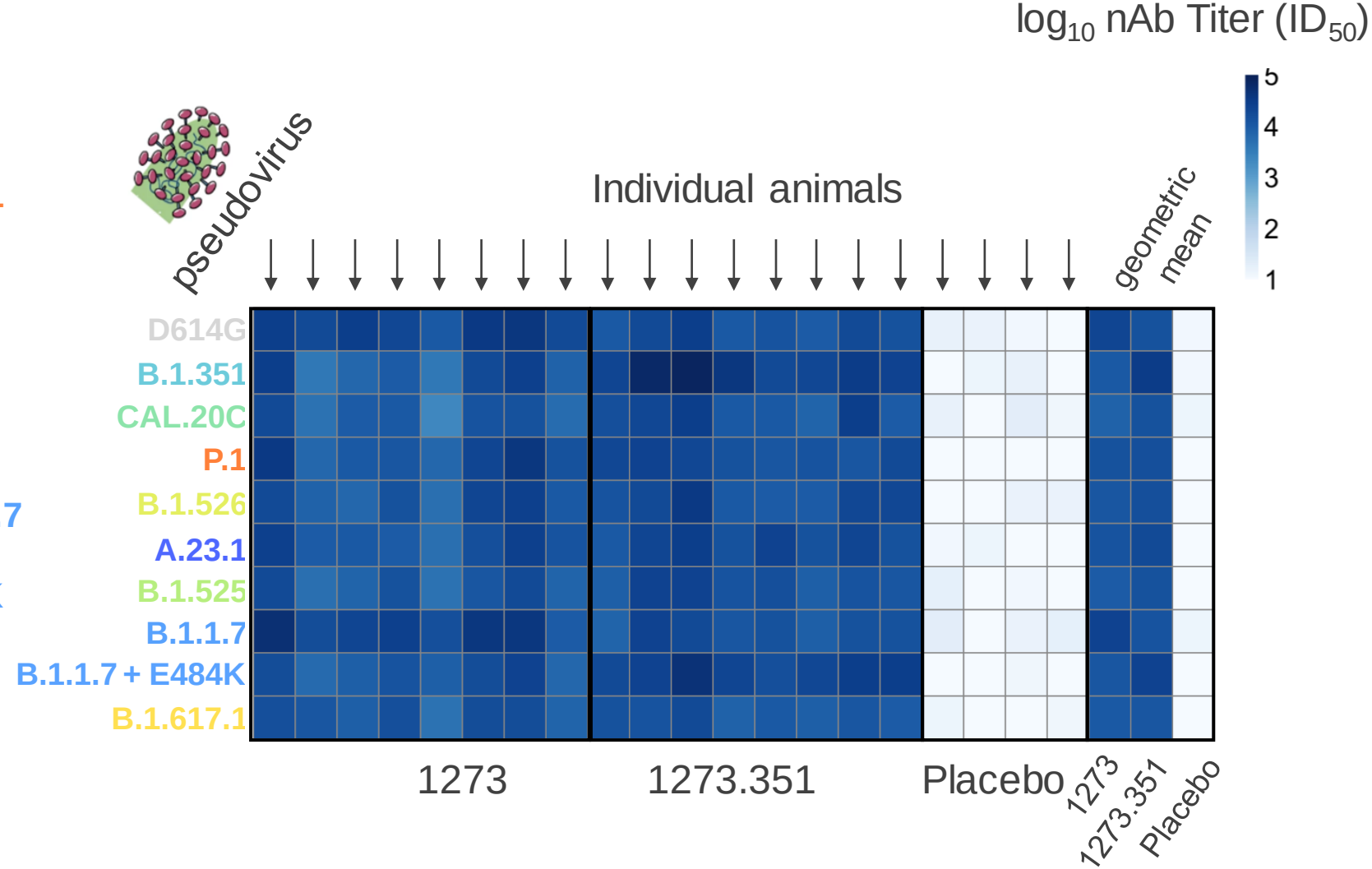
mRNA-1273.351 and mRNA-1273 vaccines elicit neutralizing antibodies against a panel of SARS-CoV-2 variants





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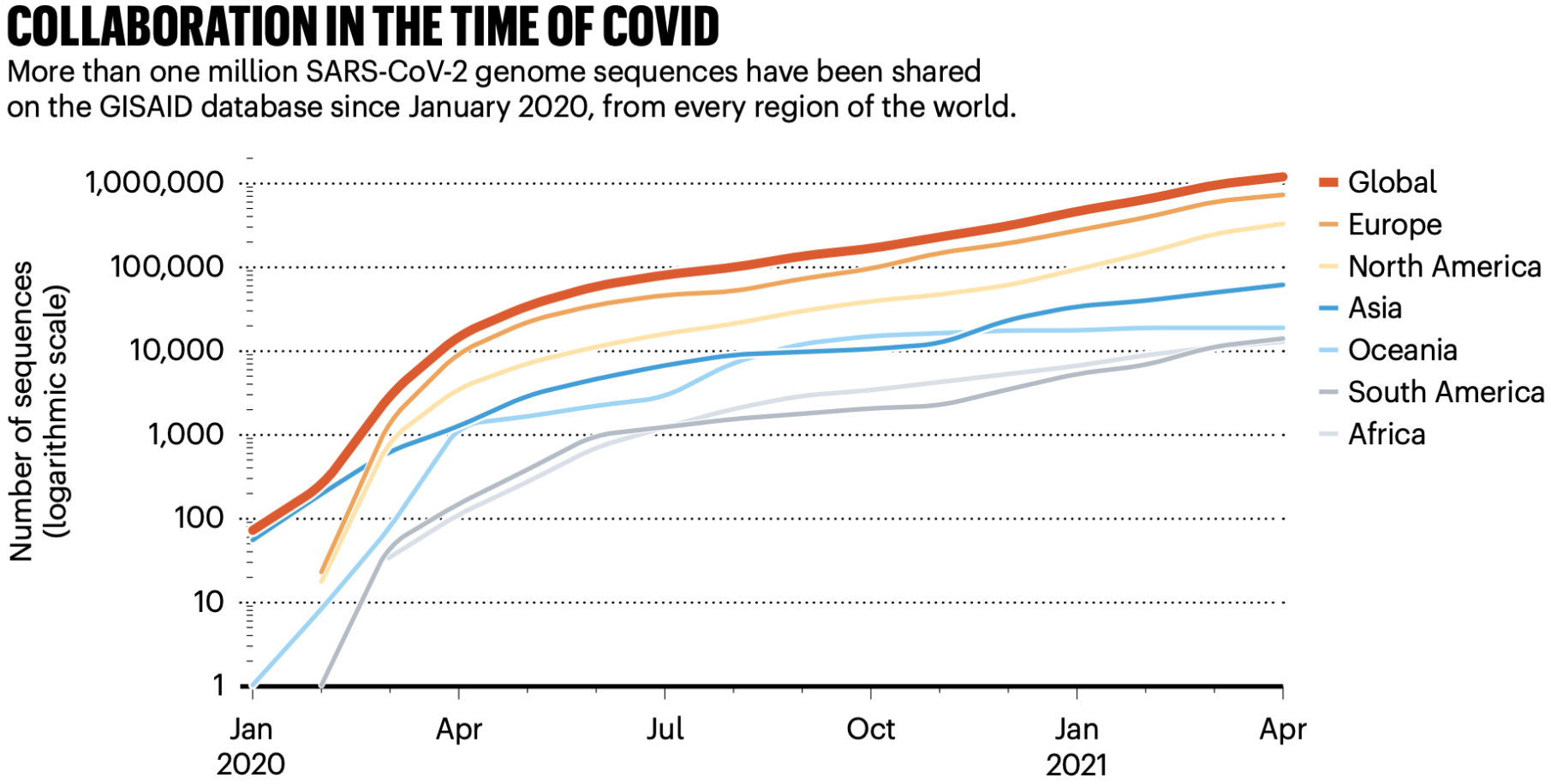
How can we do even better?

- The required suite of pre-clinical activities take considerable time and resources
  - *Shortest time from VoI identification through pseudovirus production to experimental VoC confirmation with neutralization assays is 2-3 months.*
- But new viral variants are constantly emerging in real time
- How can we speed this up? Can we use computational methods to characterize variants as soon as the sequences are deposited?

SARS-CoV-2 sequences are being generated, aggregated and shared on an unprecedented scale (>1.5 M to date)



Nature **593**, 21 (2021) *doi:* https://doi.org/10.1038/d41586-021-01069-w (data source: GISAID)

GISAID

Global
Initiative on
Shared
Avian
Influenza
Data

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Moderna CoView app enables our scientists to rapidly sift through the sequences as they appear

Filter for geograp… mutations of inter…

Continent

Africa Asia Europe North America

Country (overrides Continent if not blar…

Region (overrides Country if not blank)

Lineage

ALL

Currently selected mutations:

()

Escape Risk Scores: NTD = 0, RBD = 0, …

Known Variant

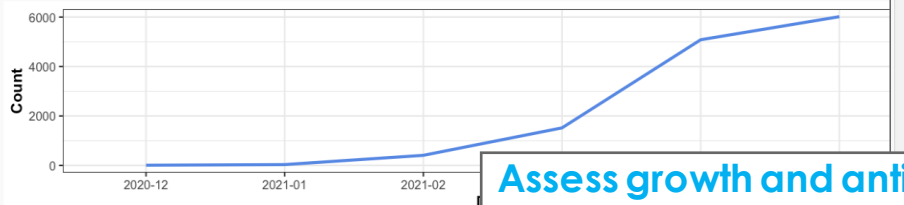
(None selected)

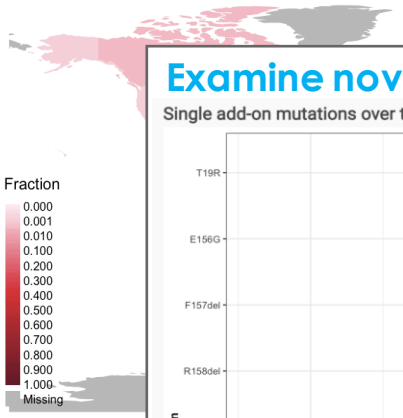
Additional Mutations

D614G

Track global variant prevalence

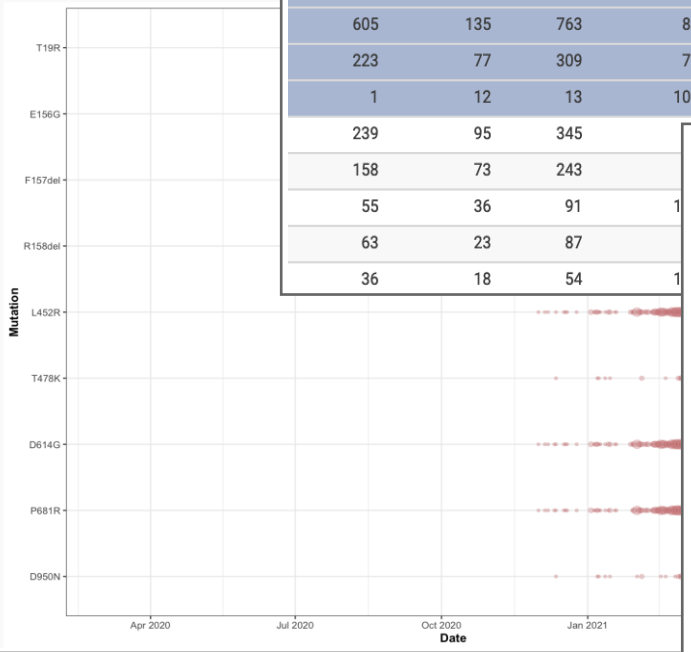
Cumulative mutation combination occurrence over time:





Examine novel…

Single add-on mutations over time:

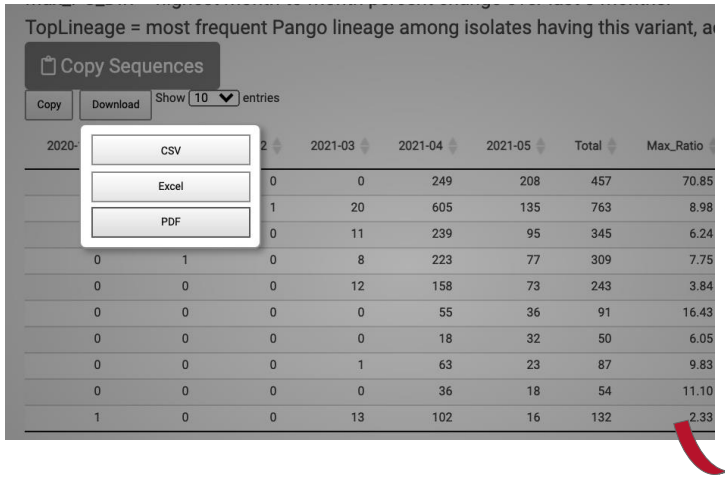


Map mutations to spike protein structure

Assess growth and antibody escape for mutation combinations

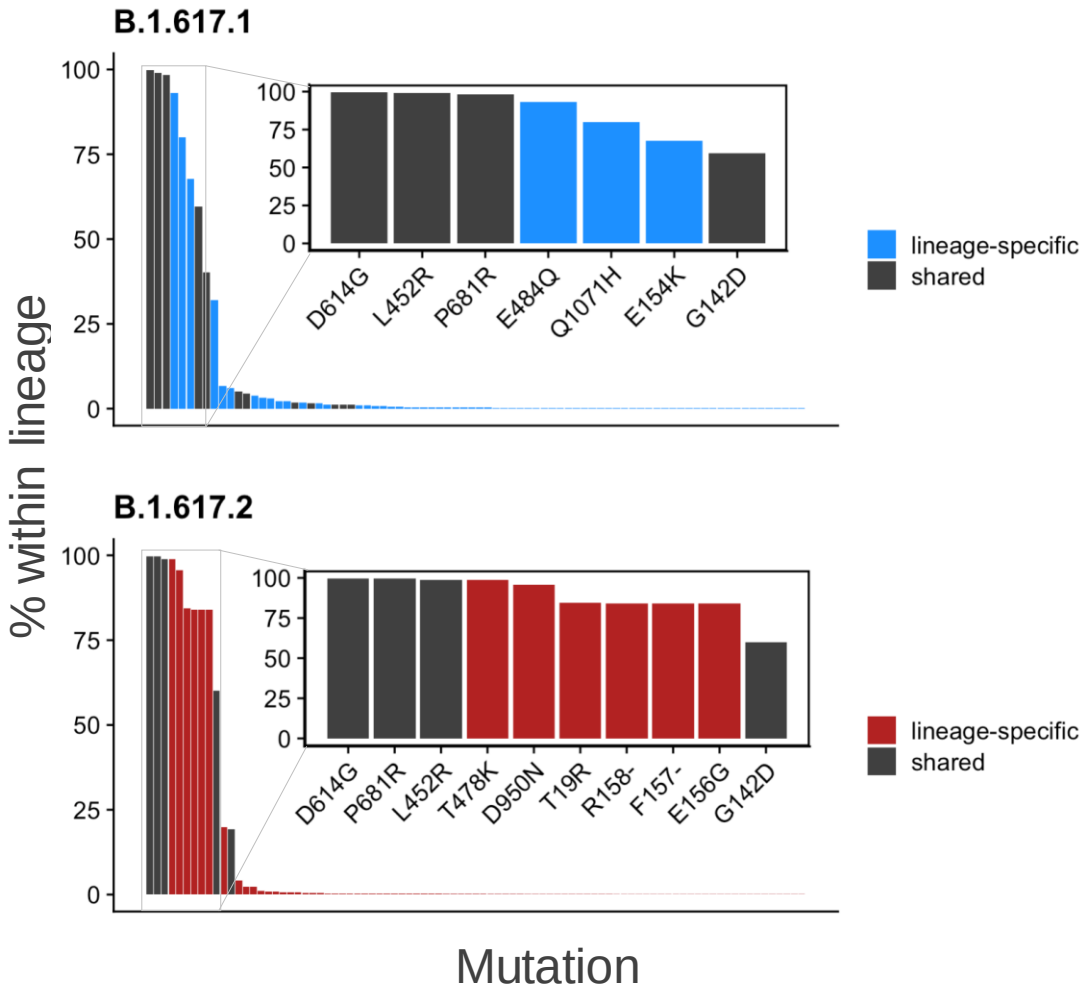
2021-04	2021-05	Total	Max_Ratio	Max_PC_Diff	TopLineage	risk_NTD	risk_RBD	risk_All	Variant
18	32	50	6.05	2.90	B.1.617.2	3	2	5	T19R T95I G142D E156G F157del R158del L216F L452R T478K D614G P681R D95
249	208	457	70.85	15.30	B.1.617.2	2	2	4	T19R T95I G142D E156G F157del R158del L452R T478K D614G P681R D950N
605	135	763	8.98	15.20	B.1.617.2	2	2	4	T19R G142D E156G F157del R158del L452R T478K D614G P681R D950N
223	77	309	7.75	5.50	B.1.617.2	2	2	4	T19R G142D E156G F157del R158del A222V L452R T478K D614G P681R D950N
1	12	13	10.80	1.30	B.1.617.2	2	2	4	T19R E156G F157del R158del L216F L452R T478K D614G P681R D950N G1124V
239	95	345							
158	73	243							
55	36	91							
63	23	87							
36	18	54							

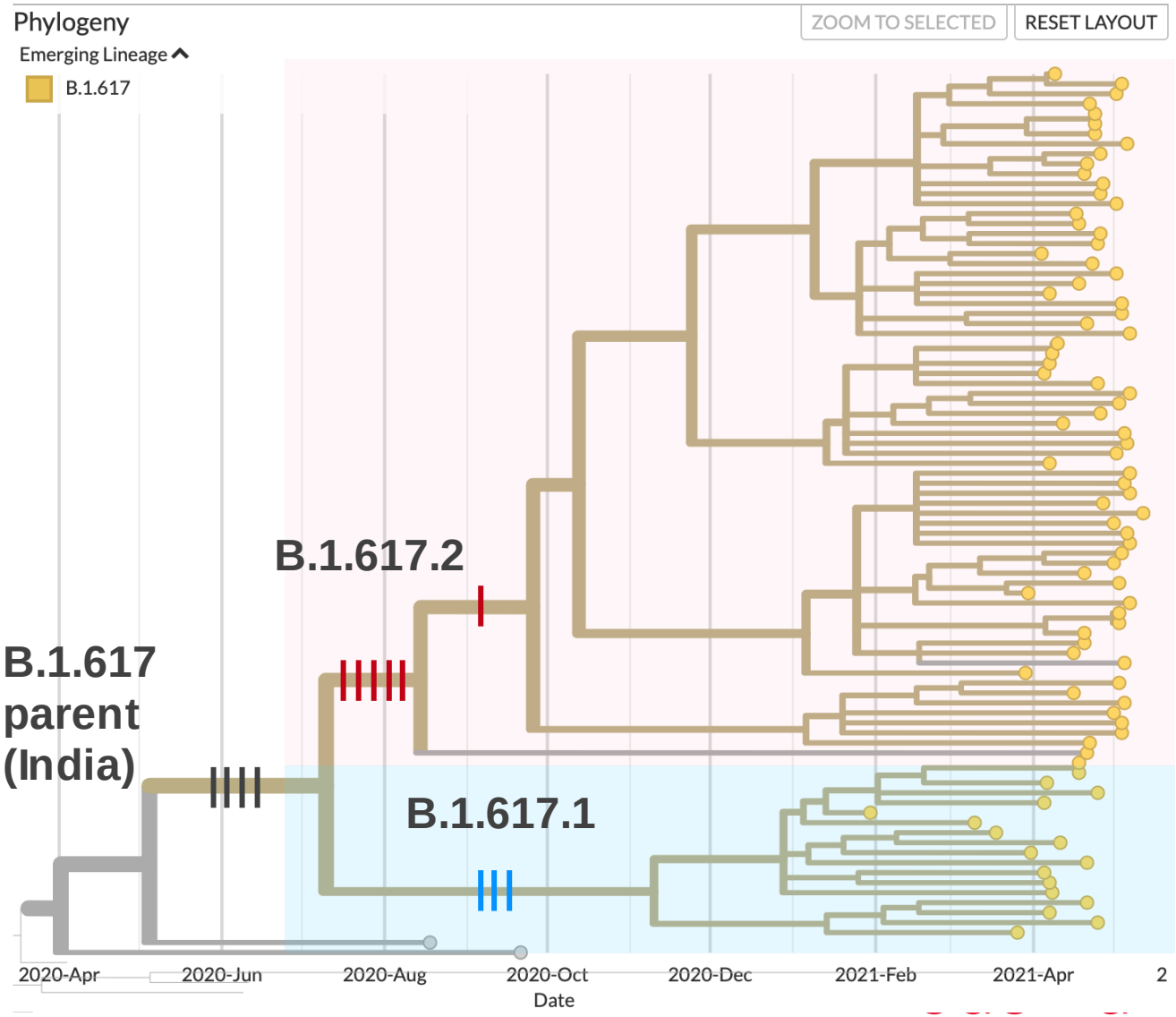
Output filtered datasets for further investigation



DIAMOND AGE DATA SCIENCE

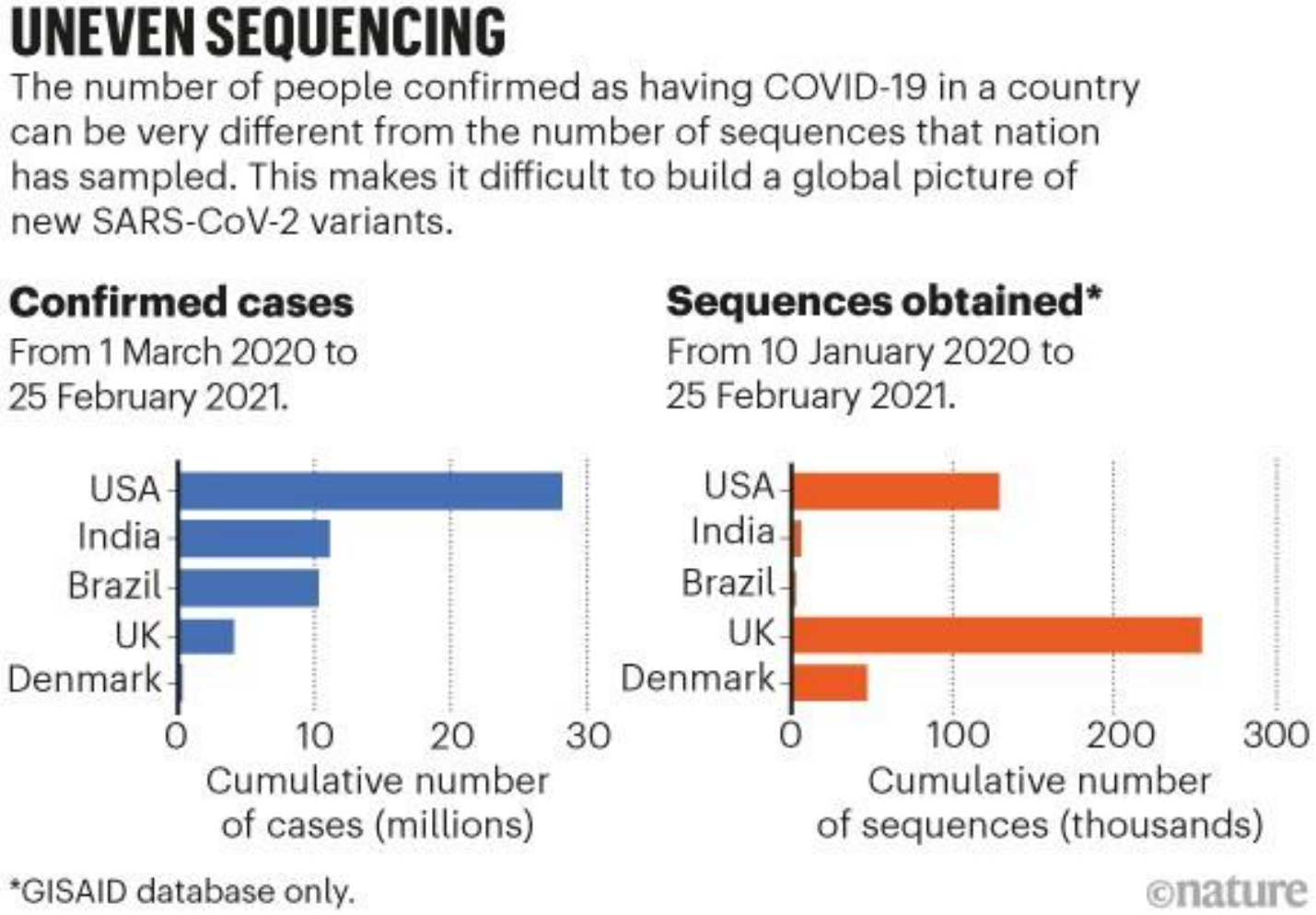
Selecting B.1.617 sequences for further investigation





data source: GISAID

But different countries have different sampling depths



Hodcraft .. Dessimoz (2021) Nature

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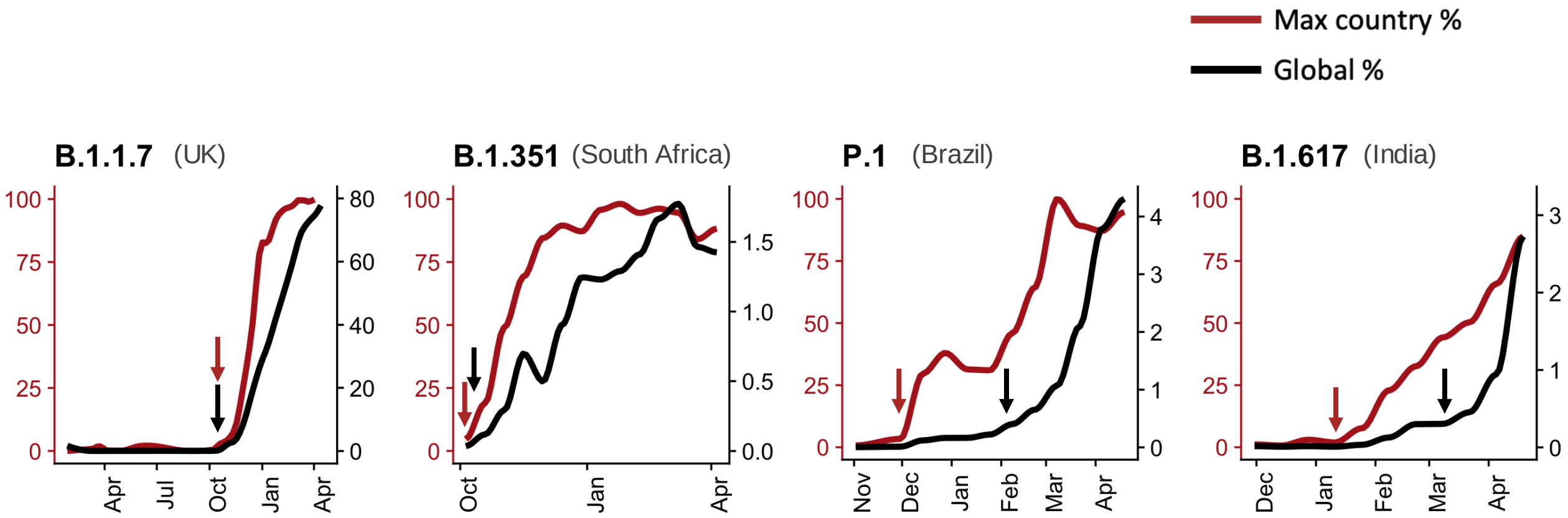
Tracking prevalence over time at the country level



Data source: GISAID

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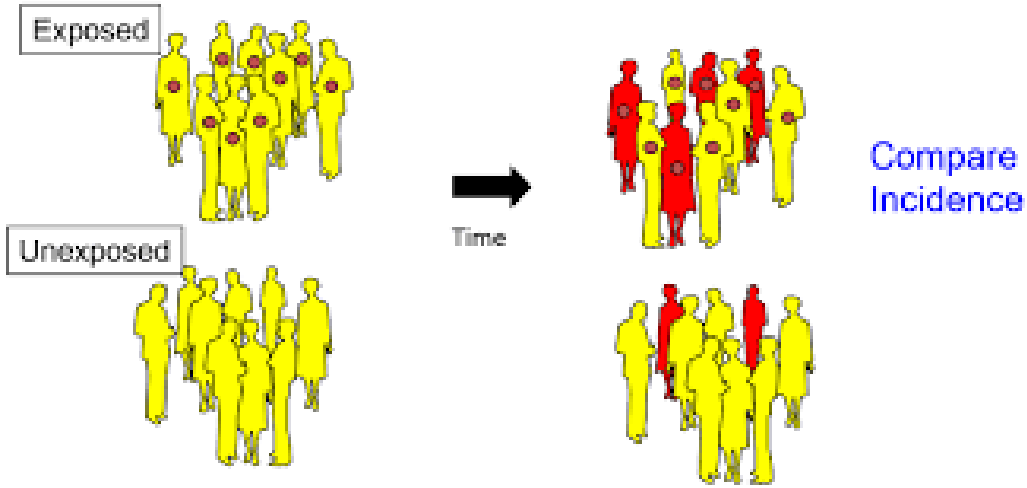
We're also tracking variants from our clinical and epidemiology studies

Data Generation

- Sequence SARS-CoV-2 from all positives detected in clinical trials and long-term effectiveness study
- Sequence SARS-CoV-2 from positive specimens detected by national pharmacy chains or other national collaborations (under feasibility assessment)

Goal

- Link vaccination status, SARS-CoV-2 sequence and clinical information



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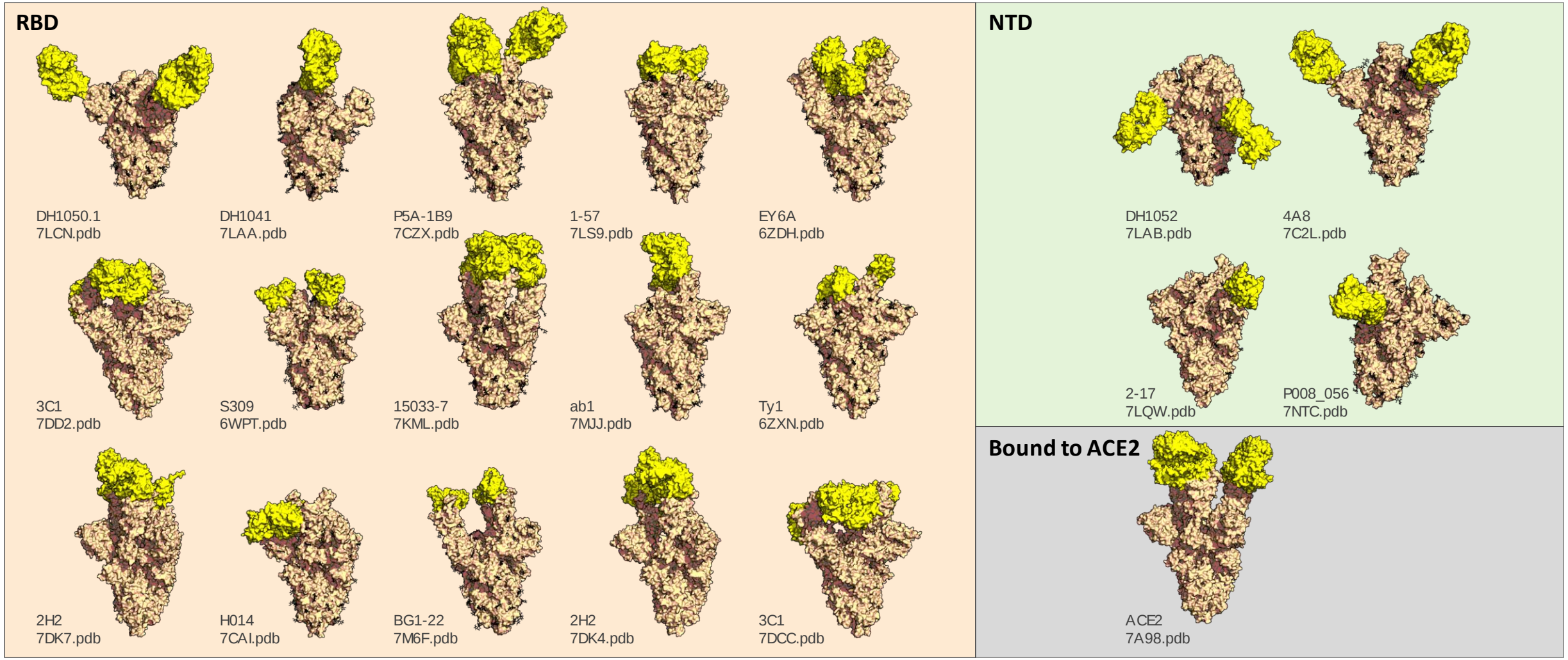
Many structures have been generated of the spike trimer complexed with individual antibodies



data source: PDB

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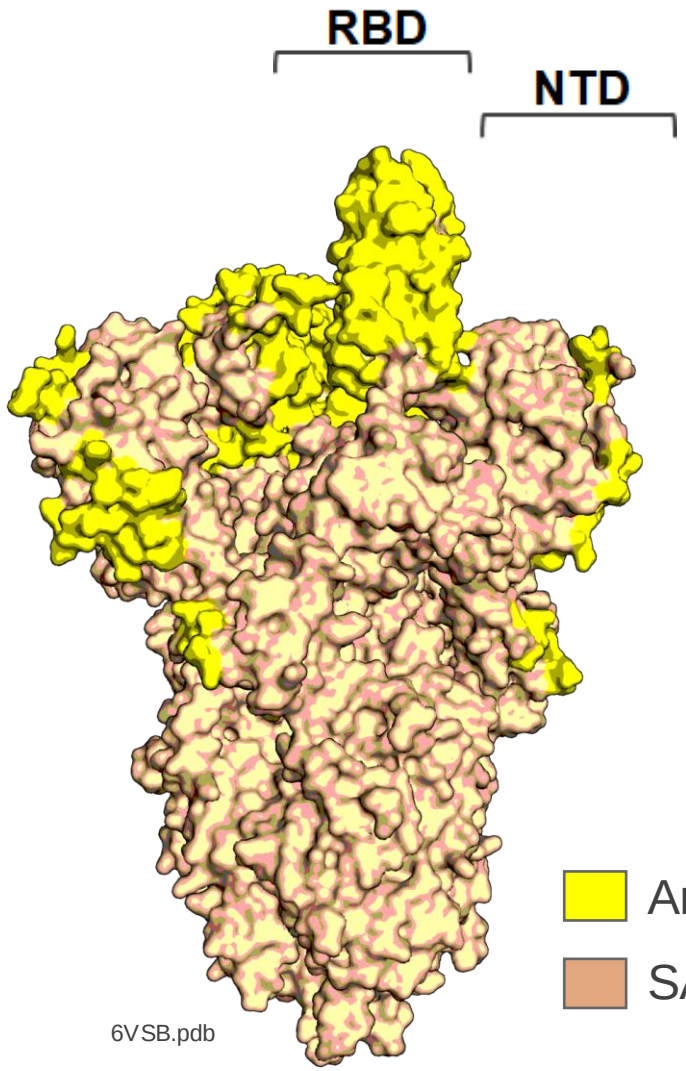
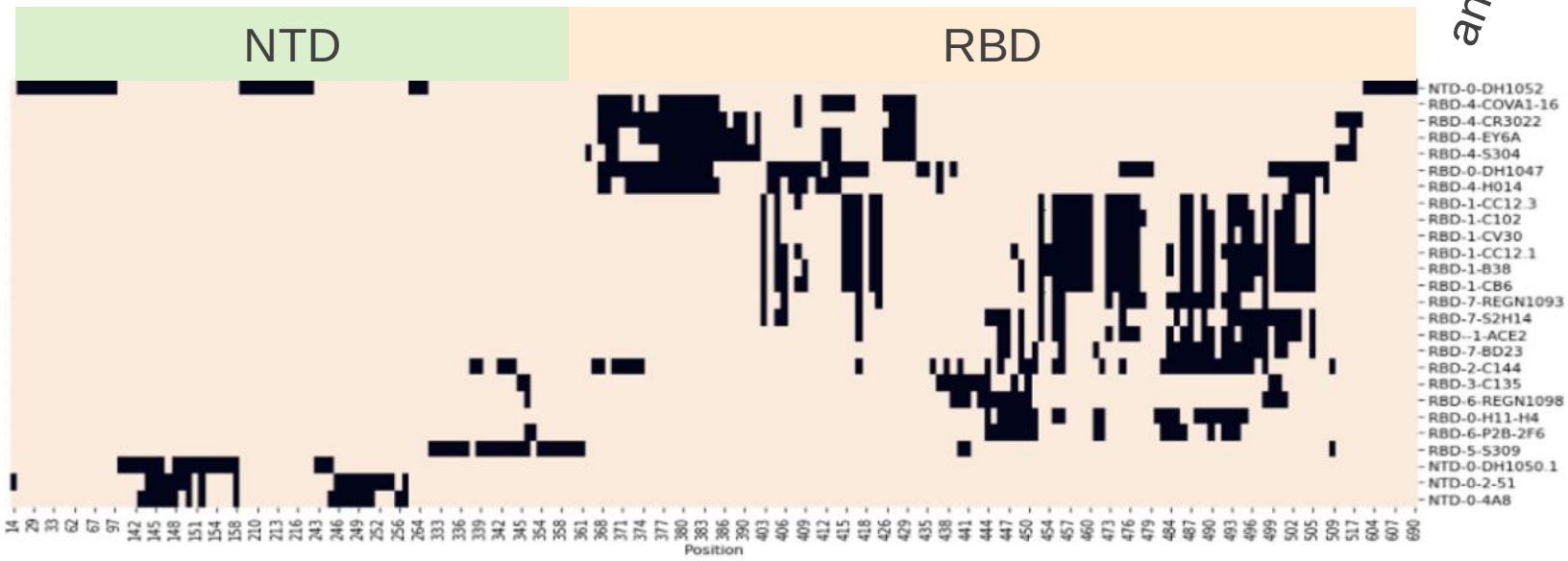
Structural mapping of neutralizing antibody contact sites reveal NTD and RBD as sites of potential vaccine escape



Antibody contact surface

SARS-CoV-2 Spike trimer

Neutralizing epitope contact map



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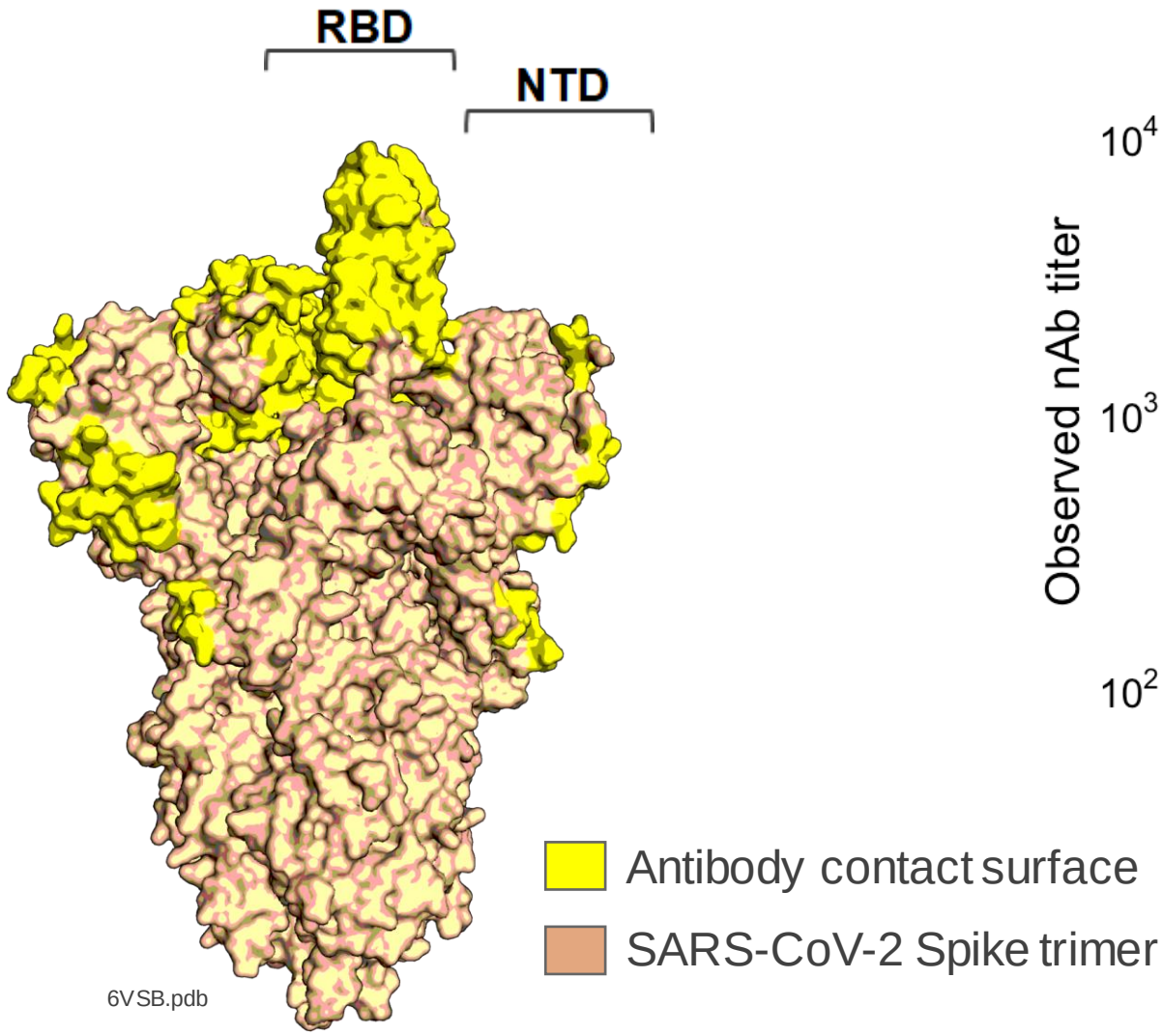
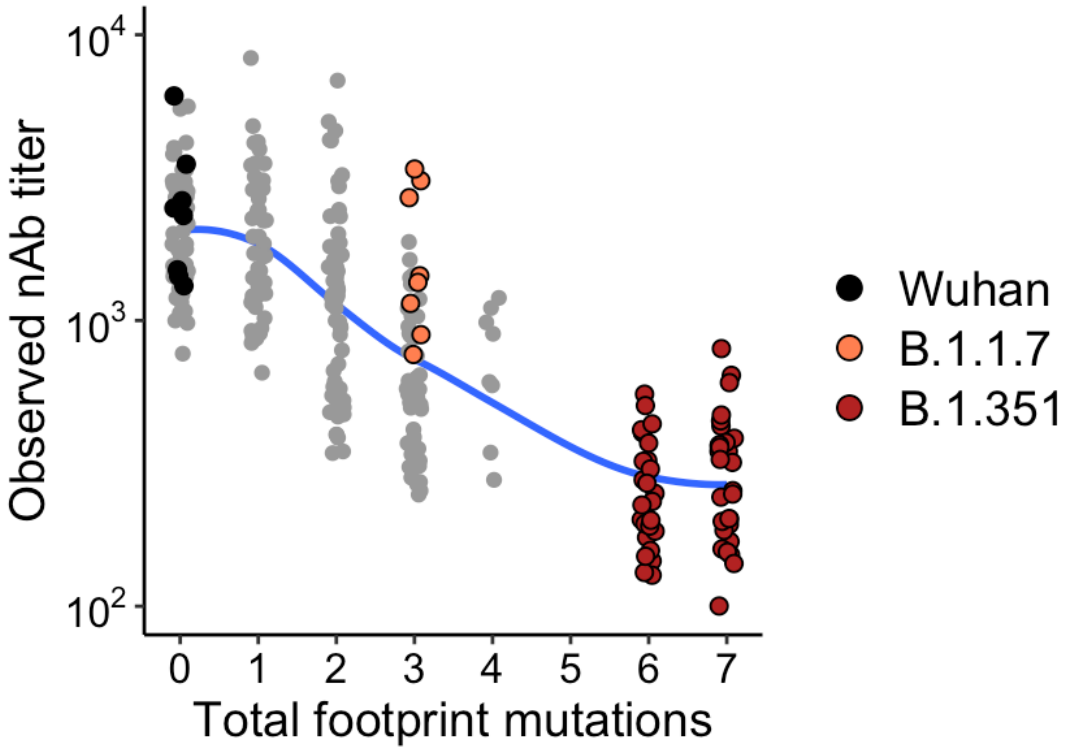
Scoring mutations using the antibody contact map can predict antibody escape





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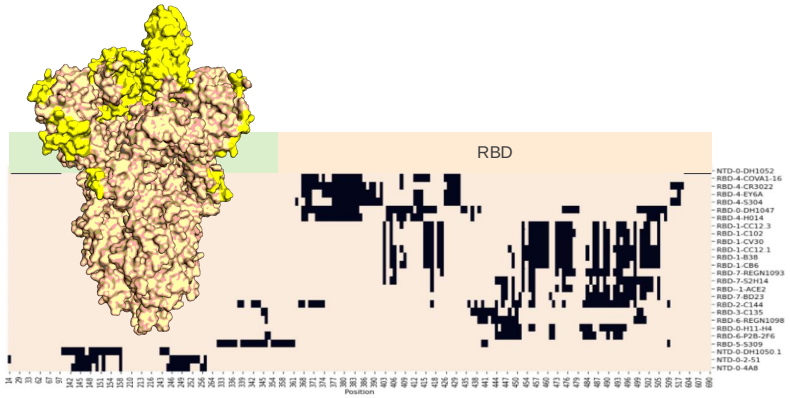
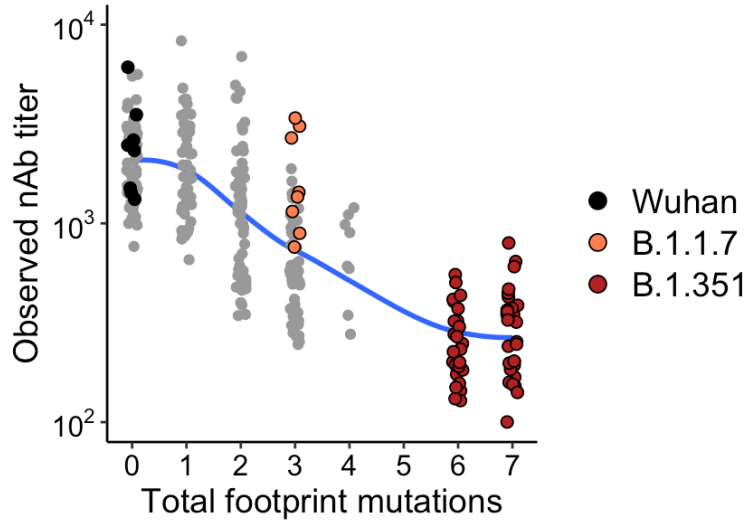
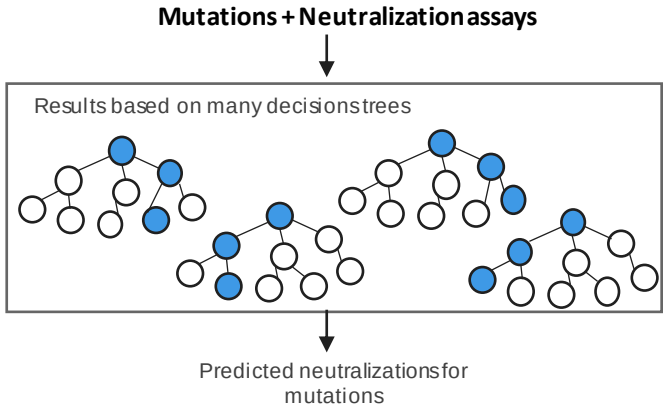
More strategies for modeling viral escape

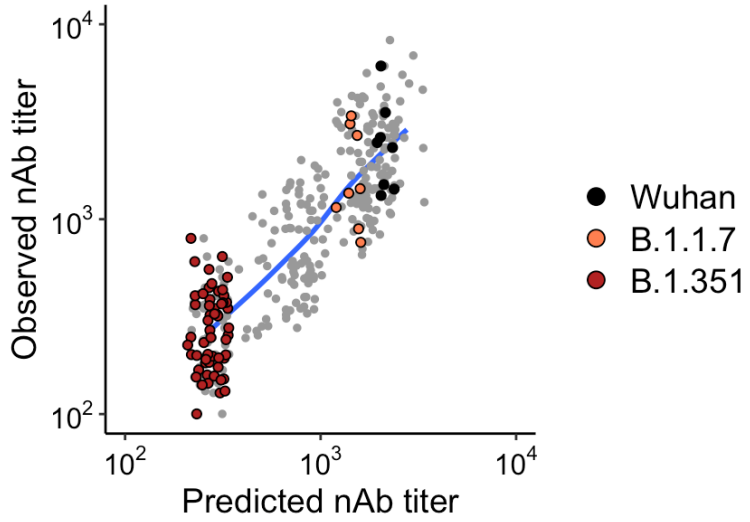
Antibody footprint model



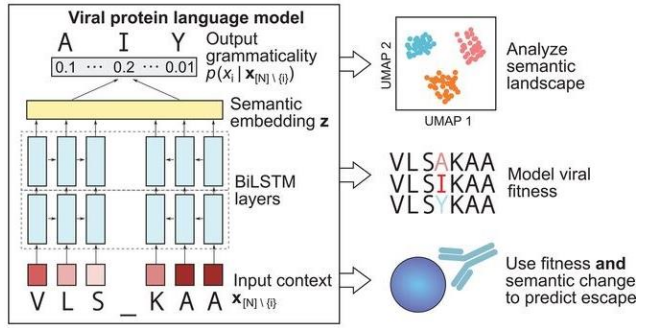


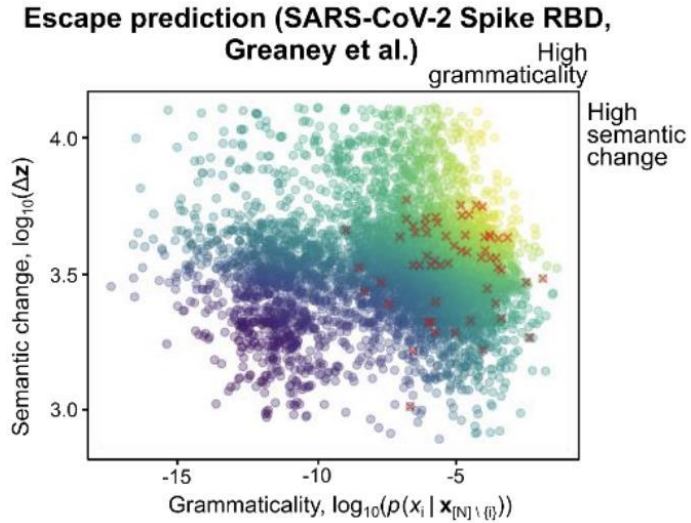
Supervised machine learning





Natural language model





Hie, Zhong, Berger, Bryson (2021) Science

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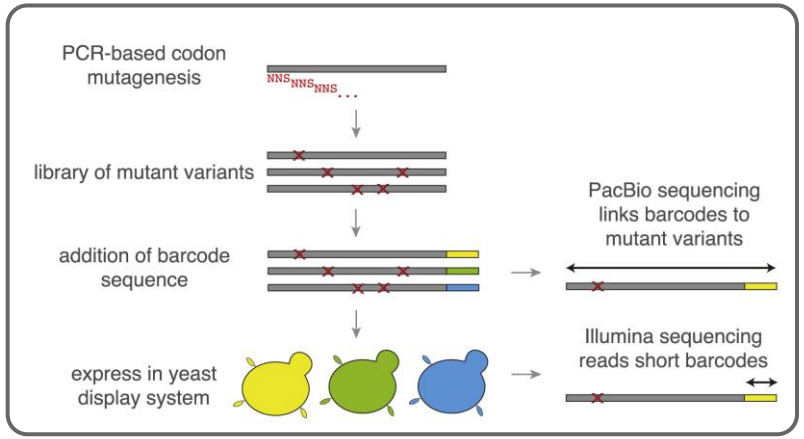
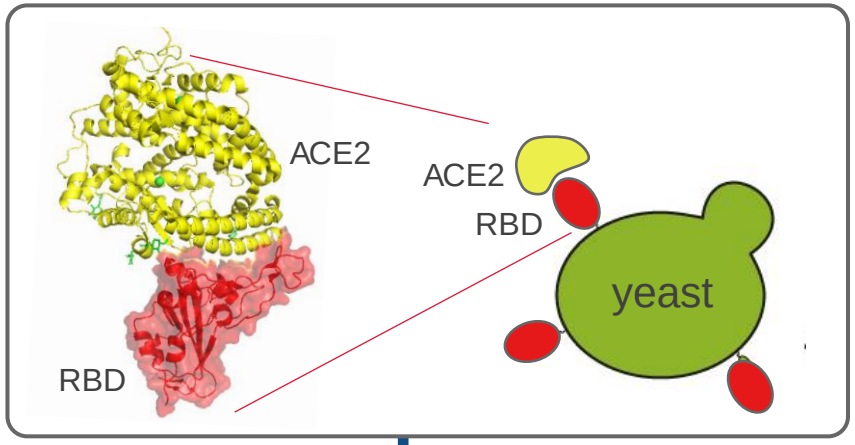
Deep mutational scanning (DMS): Comprehensive mutation-function maps by selection and deep sequencing

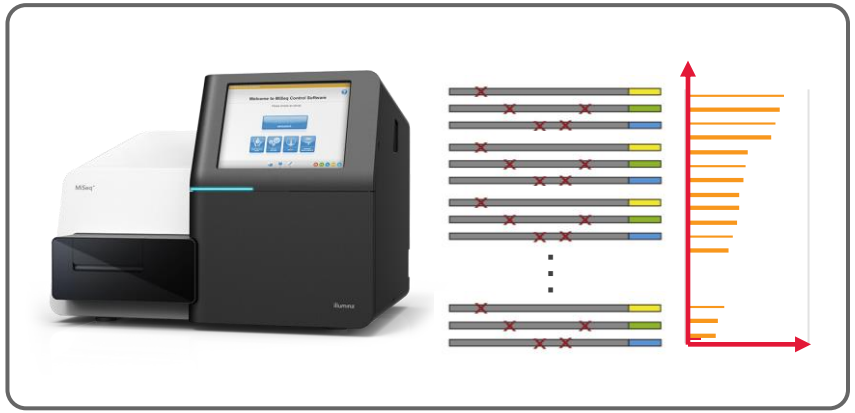
1 Build comprehensive (yeast display) library of mutants



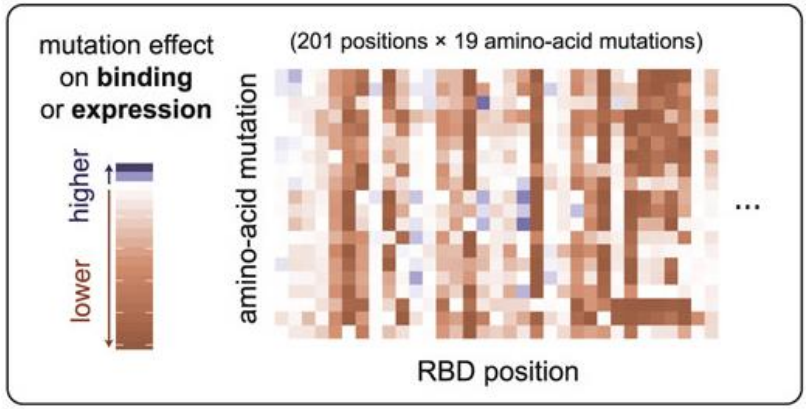
2 Execute selection experiment (e.g., with cell sorting)



3 Deep sequence the results, obtain enrichment counts



4 Convert enrichment counts to map of mutation effects



Starr… Bloom (2020) *Cell*

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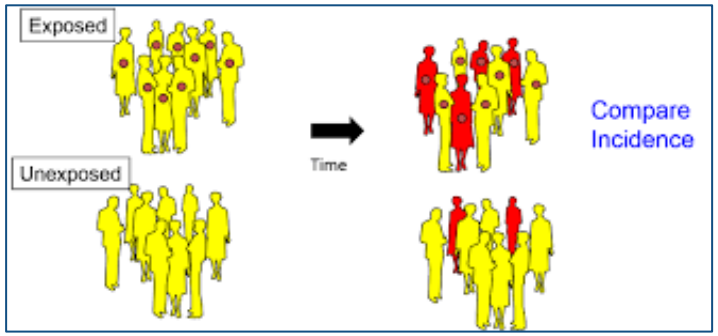
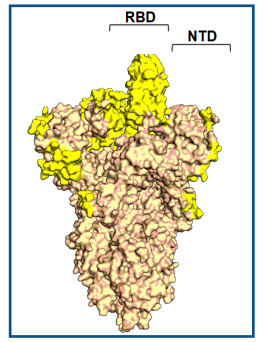
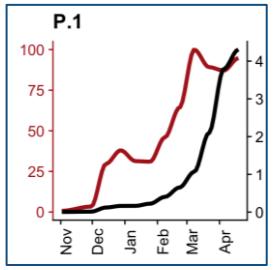
We are working to integrate these information using AI and machine learning to predict escape risk



Breakthrough sequences from clinical trial and RWD

Spike-antibody/ACE2 contacts

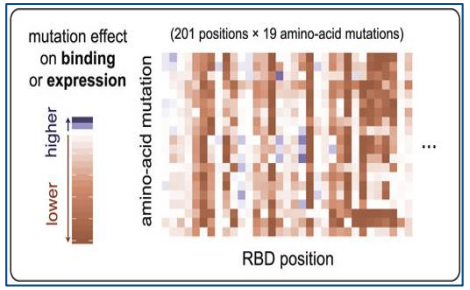


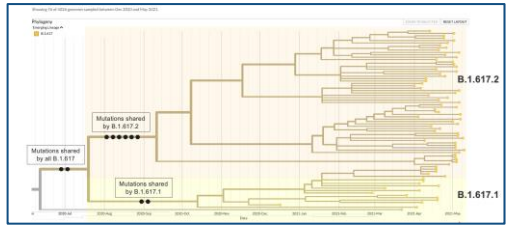


Time tracking

Statistical Model of SARS-CoV-2 Escape Risk

Spike protein biophysical/DMS data

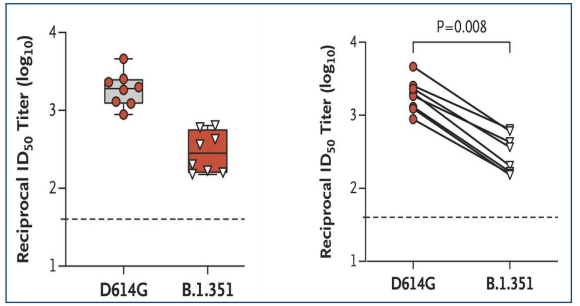




Spike mutations and lineage info

Next-generation vaccines

Pseudovirus neutralization data



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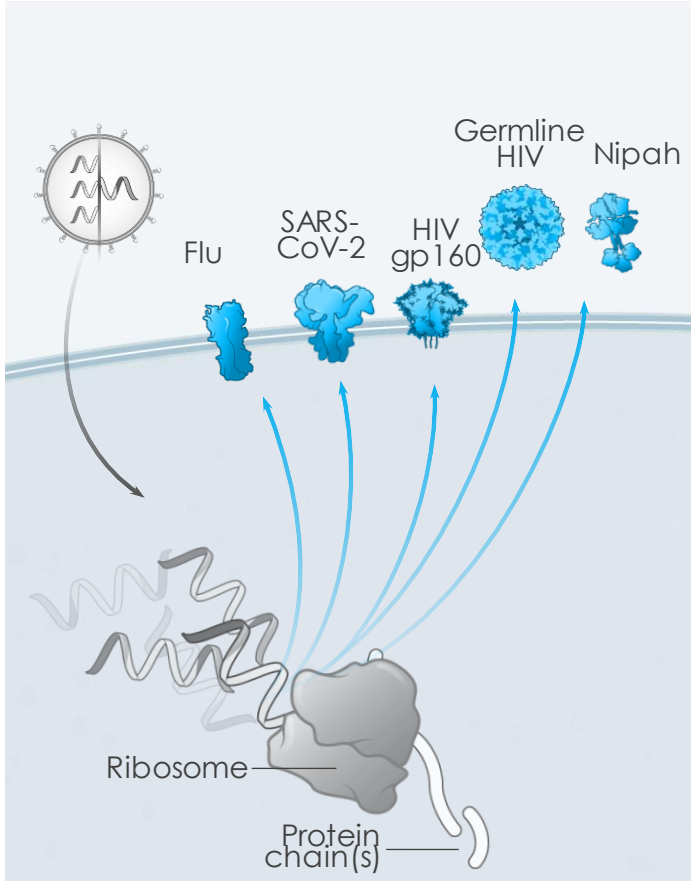
Our learnings in fighting SARS-CoV-2 will improve future readiness for other rapidly evolving viruses

Rapidly evolving viruses	**Moderna programs** *(disclosed)*	**Clinical data against pathogen**
• Influenza	mRNA-1851, -1010/20/30	✓
• SARS-CoV-2	mRNA-1273, -1283	✓
• HIV	mRNA-1644, -1574	
• Nipah	mRNA-1215	
• Endemic human coronavirus		
• Rhinovirus (HRV)		



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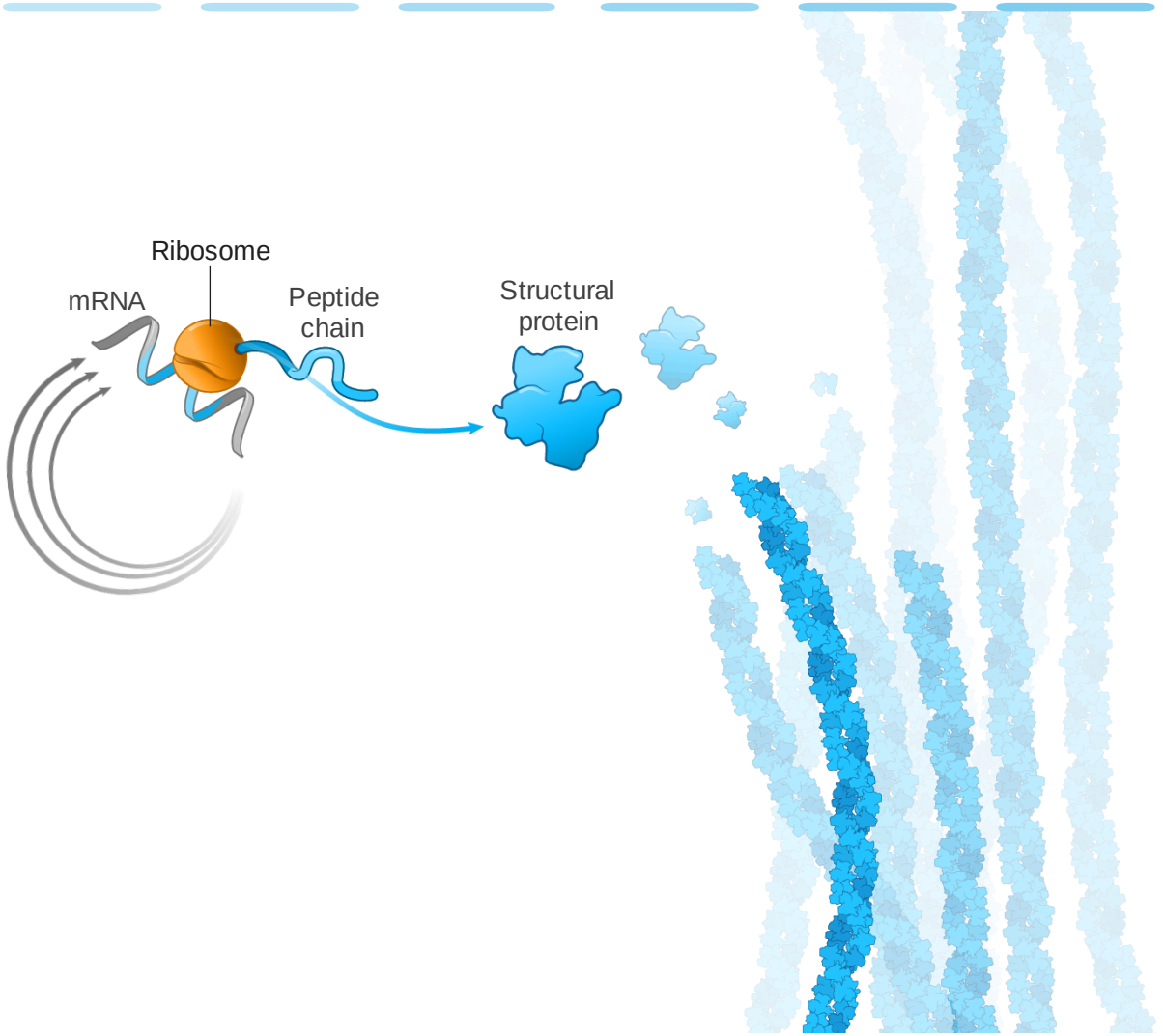
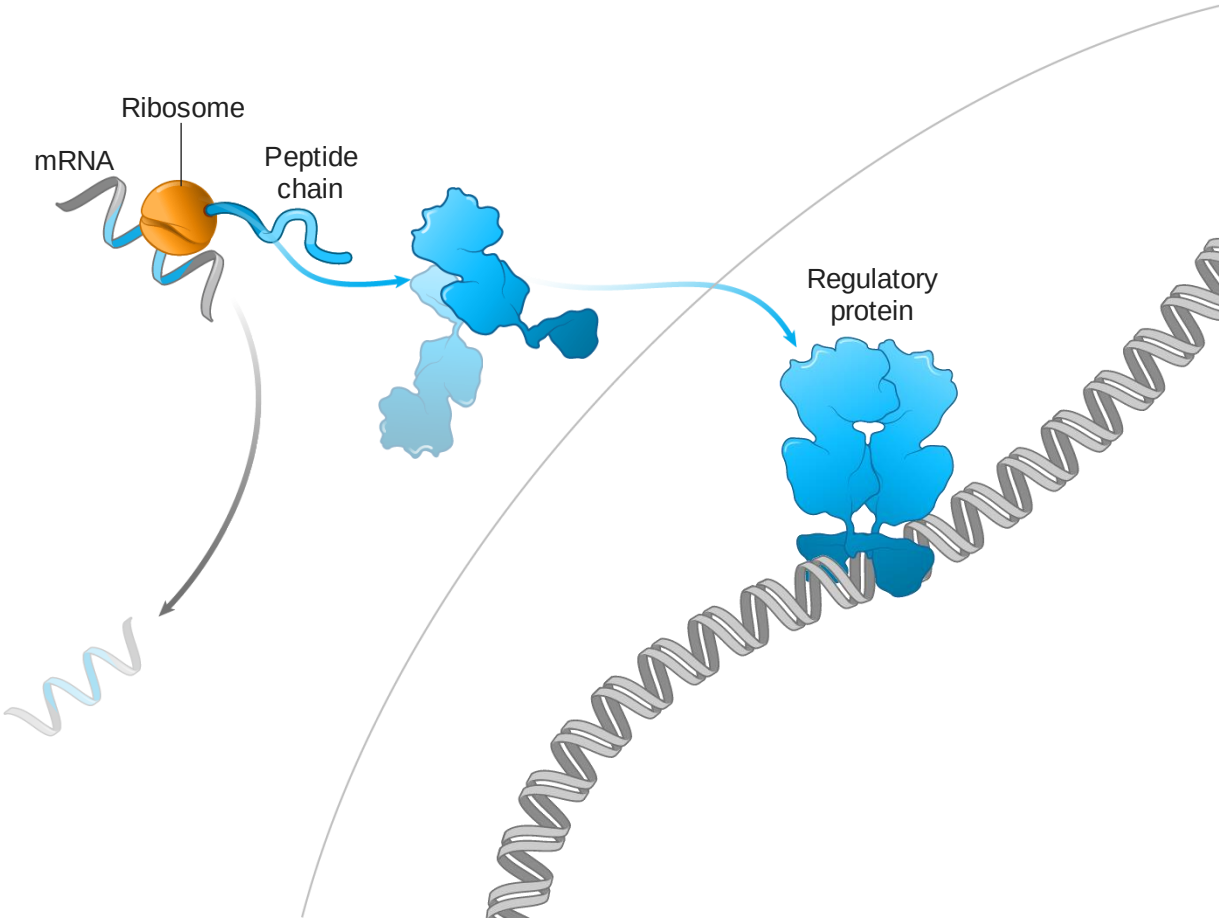
mRNA Sequence Engineering

Achieving optimal performance of mRNA





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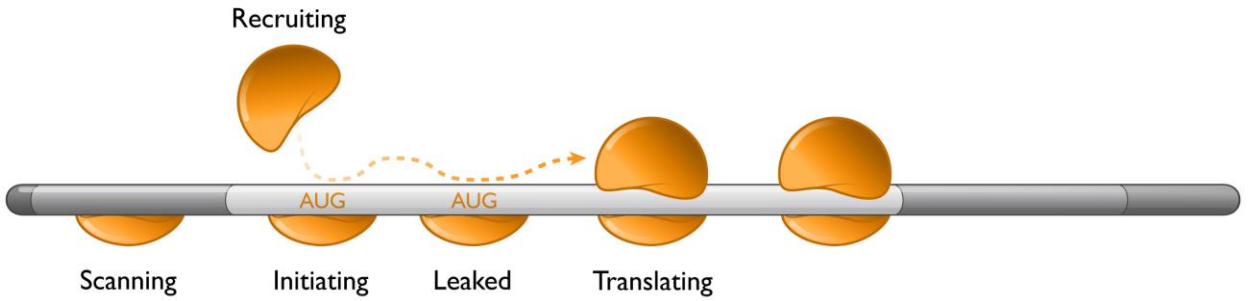
Previous translation stories

2018

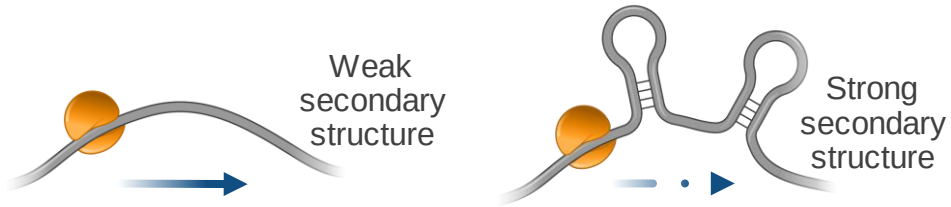
Leaky scanning



2019

Codon optimality

mRNA secondary structure



mRNA structure regulates protein expression through changes in functional half-life

David M. Mauger[a], B. Joseph Cabral[a,1], Vladimir Presnyak[a], Stephen V. Su[a,2], David W. Reid[a], Brooke Goodman[a], Kristian Link[a], Nikhil Khatwani[a,3], John Reynders[a,4], Melissa J. Moore[a,5], and Iain J. McFadyen[a,6]

[a]Platform Research, Moderna, Inc., Cambridge, MA 02139

Mauger … McFadyen (2019) PNAS

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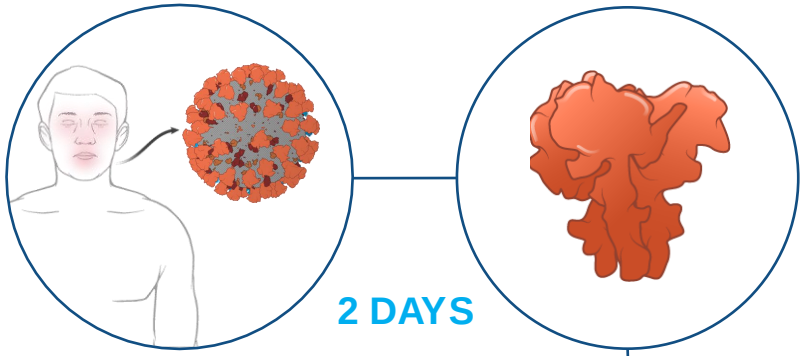
Accelerated research and development timeline

Example: SARS-CoV-2 (mRNA-1273)

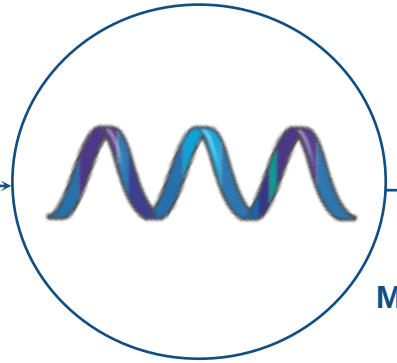
Isolation/Sequencing
Viral sequence isolated from infected patient

Antigen Design
Spike protein designed to elicit robust immune response



2 DAYS

mRNA Design

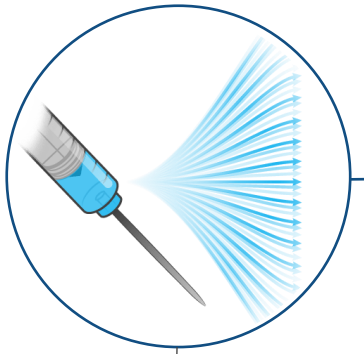


42 DAYS

Vaccine Manufacturing + Quality Testing

9 MONTHS

Clinical Trials



EUA

mRNA-1273

Total Time: 11 MONTHS

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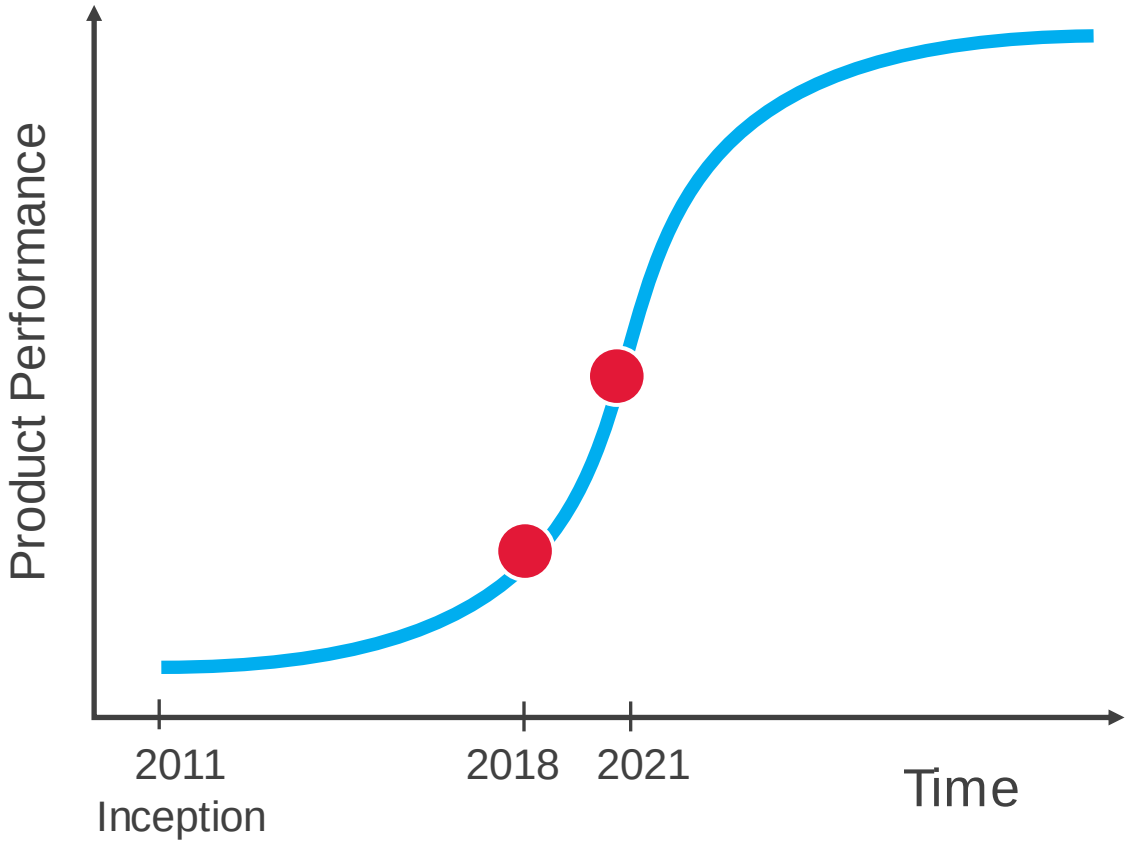
We're playing a 20 year game



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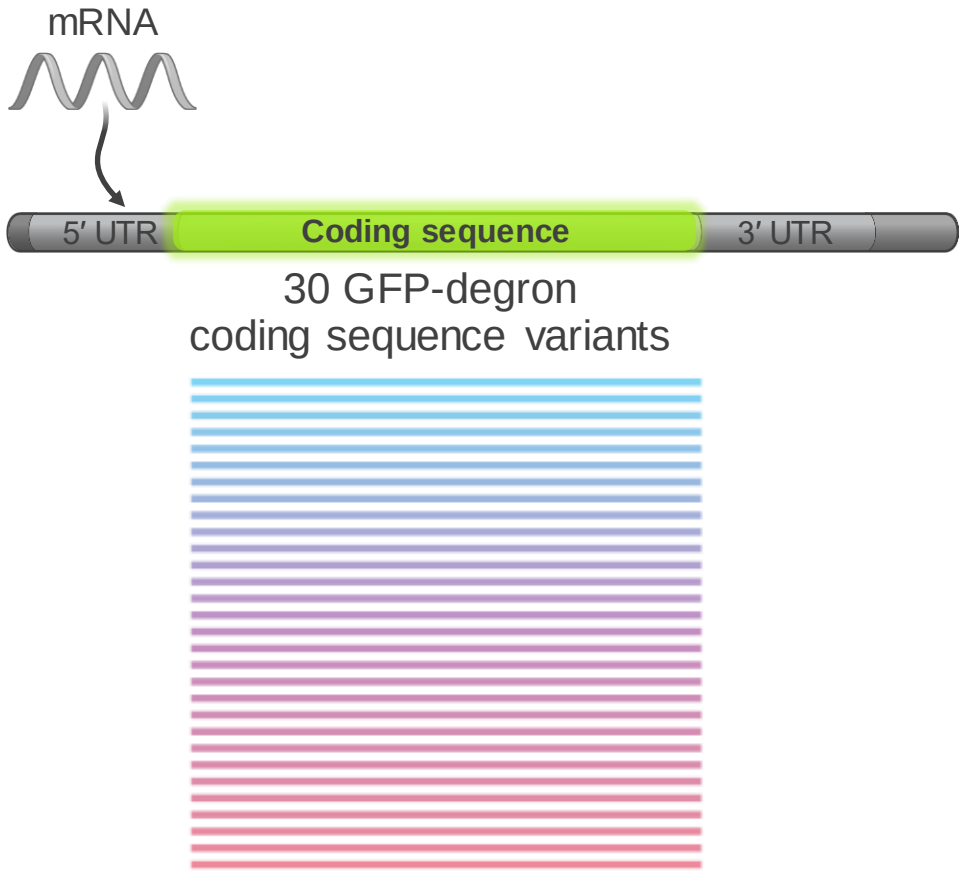
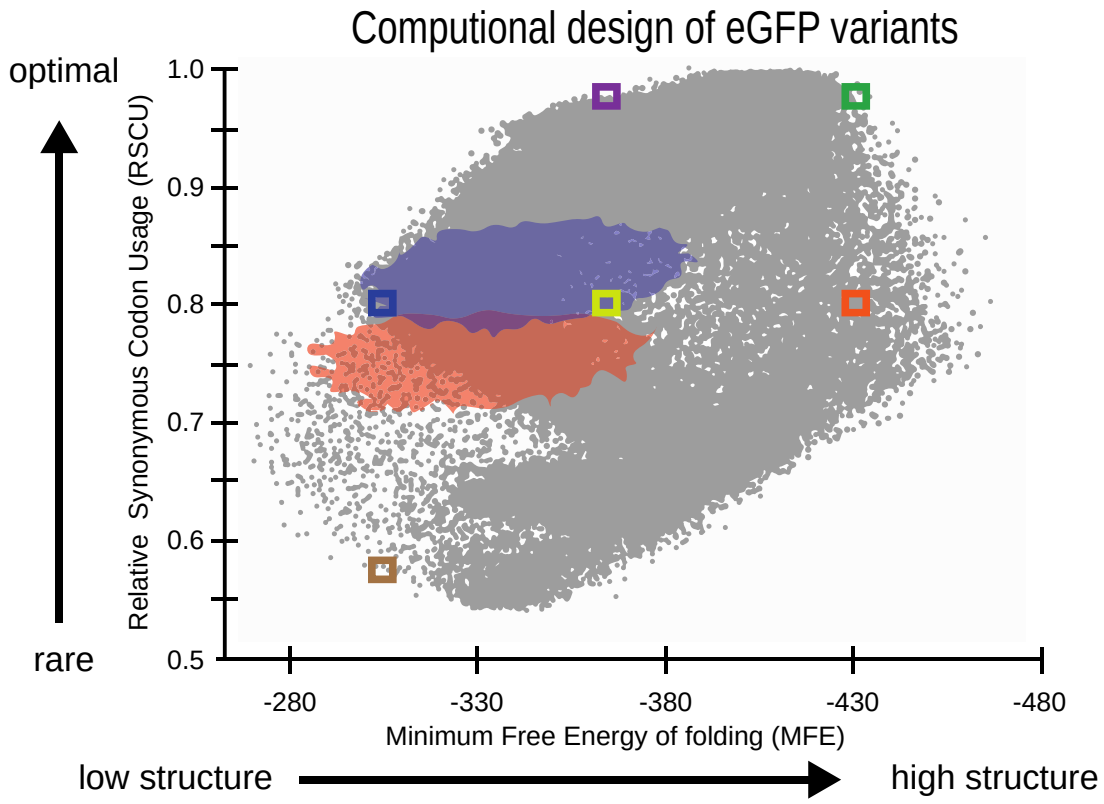
Both codon optimality and mRNA structure both contribute to total protein output





Read more about these mRNA sequences and mRNA half-life:
Mauger… McFadyen. *Proceedings of the National Academy of Sciences* 2019

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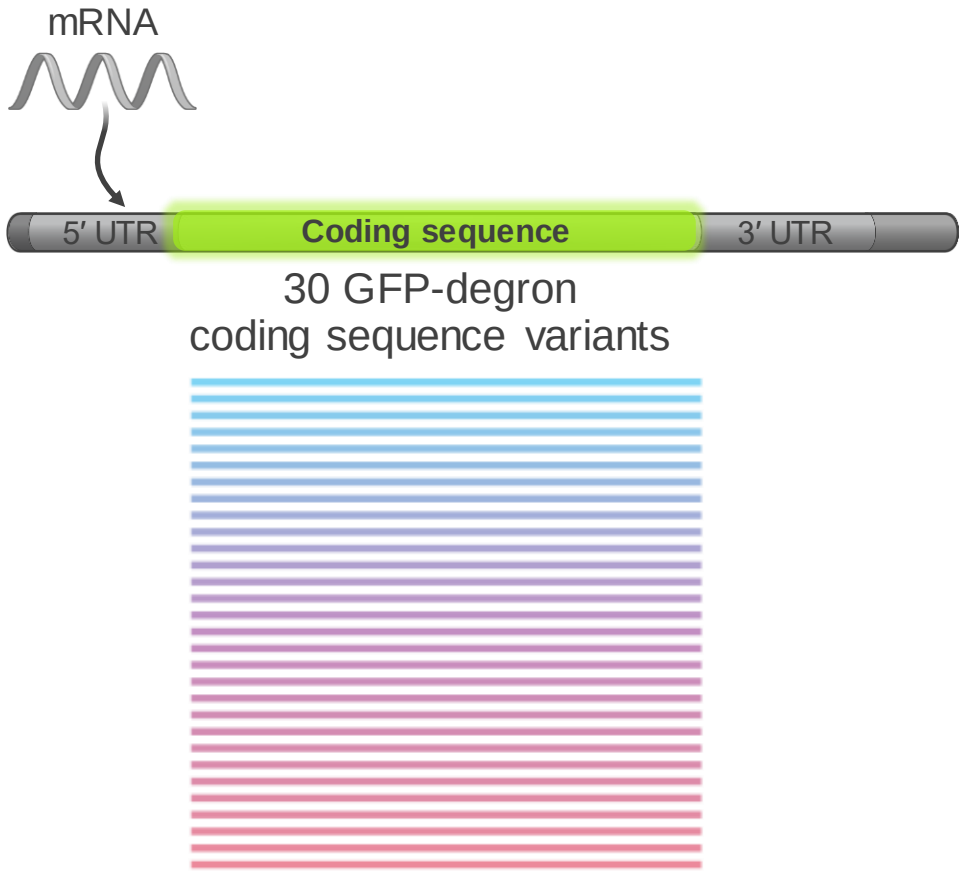
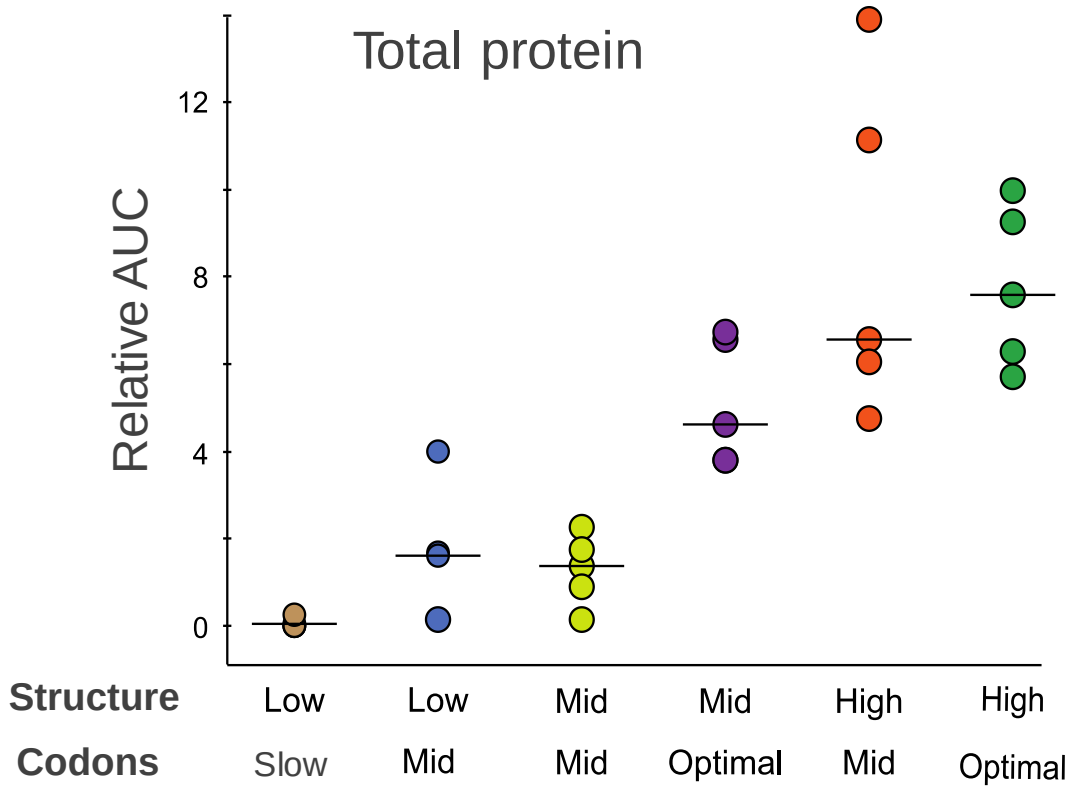
Both codon optimality and mRNA structure both contribute to total protein output





Read more about these mRNA sequences and mRNA half-life:
Mauger… McFadyen. *Proceedings of the National Academy of Sciences* 2019

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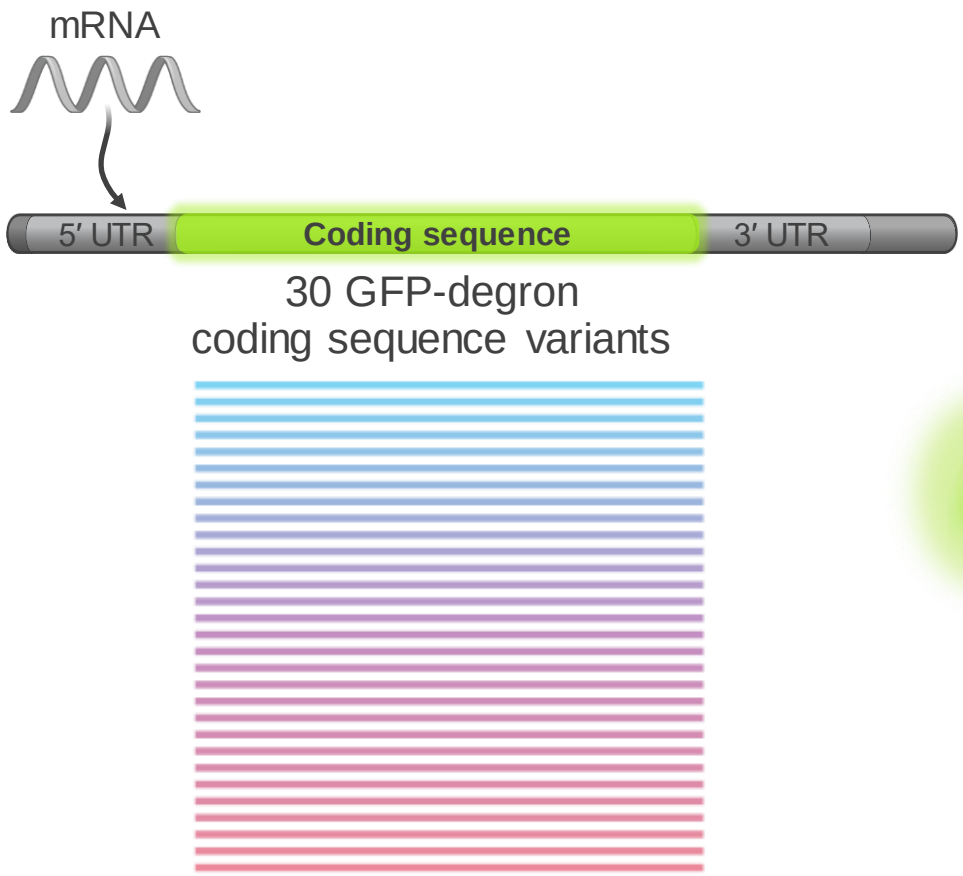
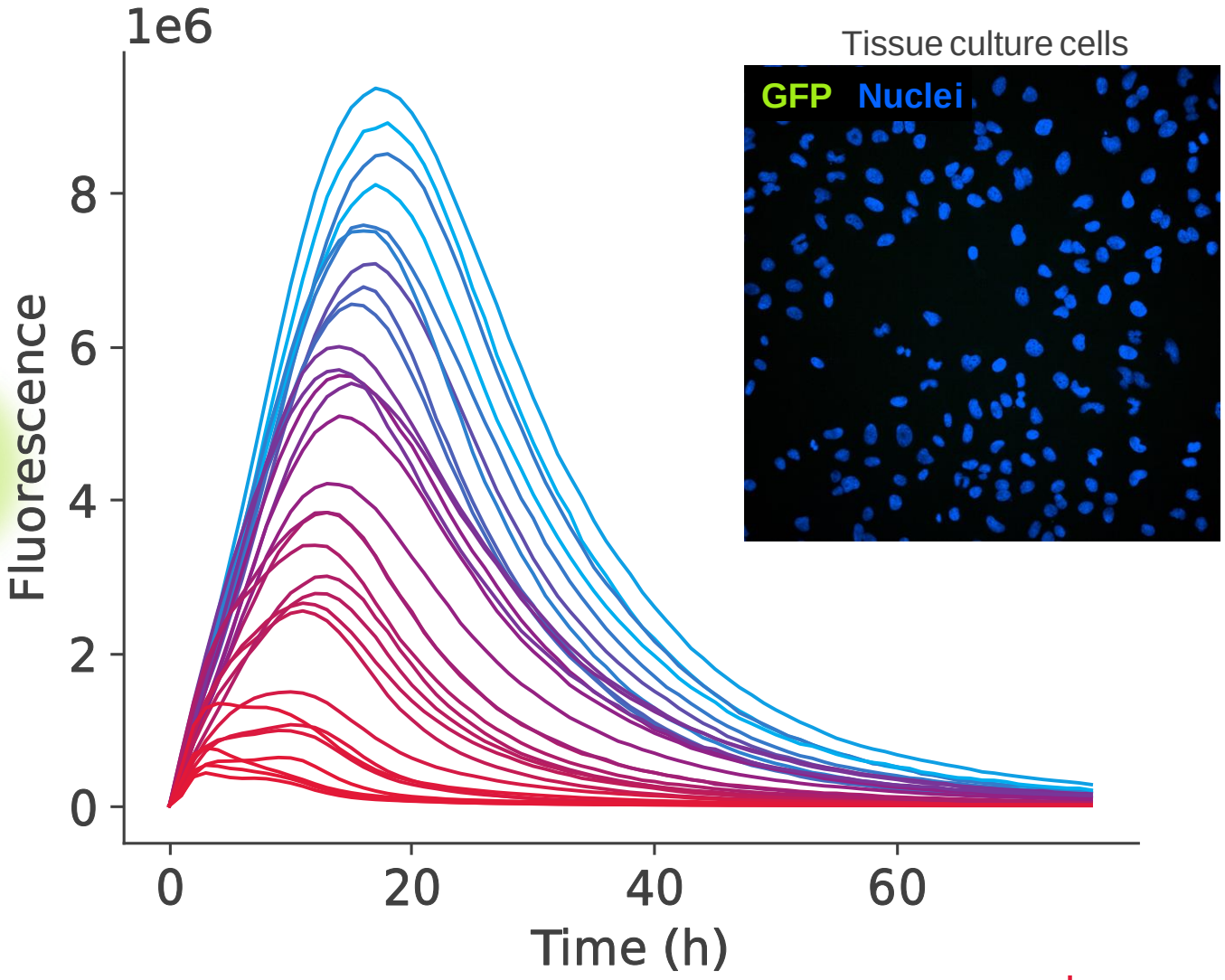
mRNA Engineering: Optimizing Ribosome Load

Different mRNA sequences make different amounts of protein





Read more about these mRNA sequences and mRNA half-life:
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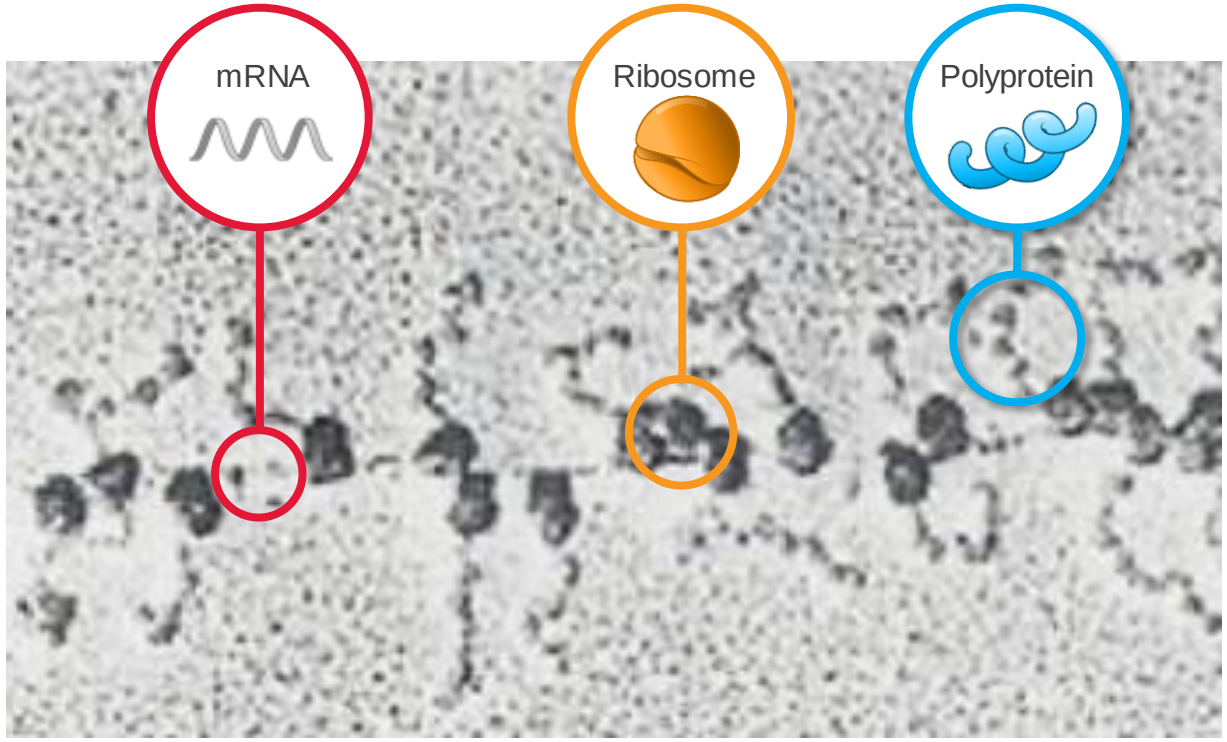
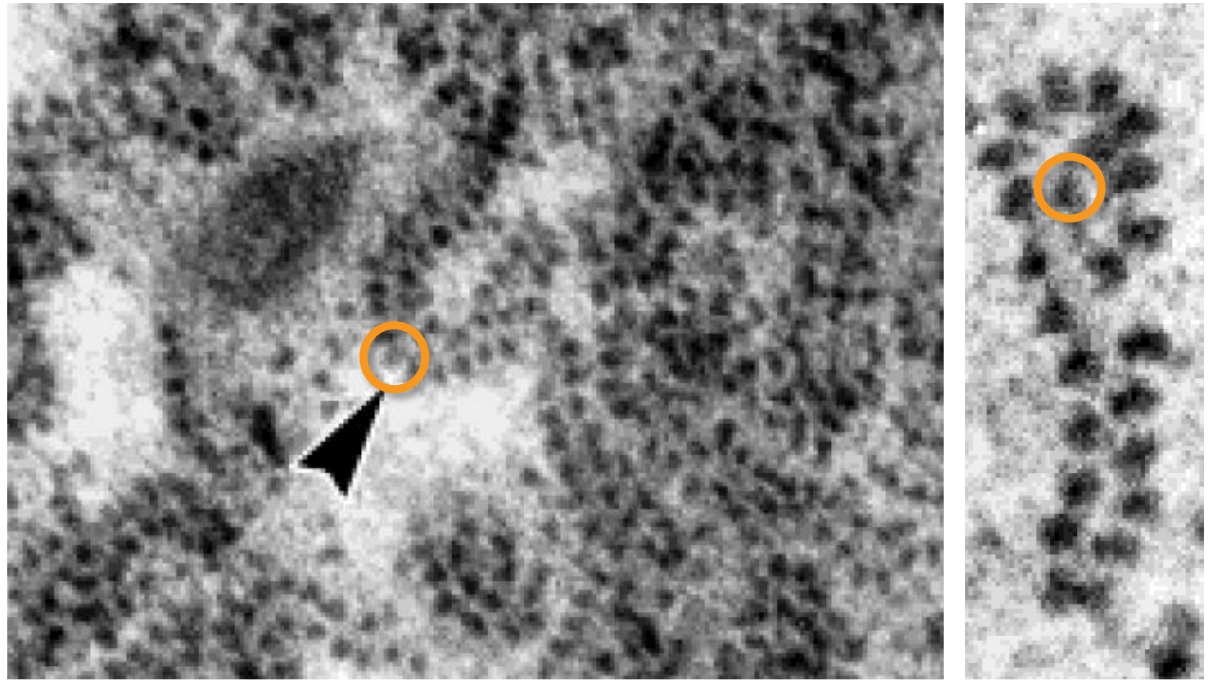
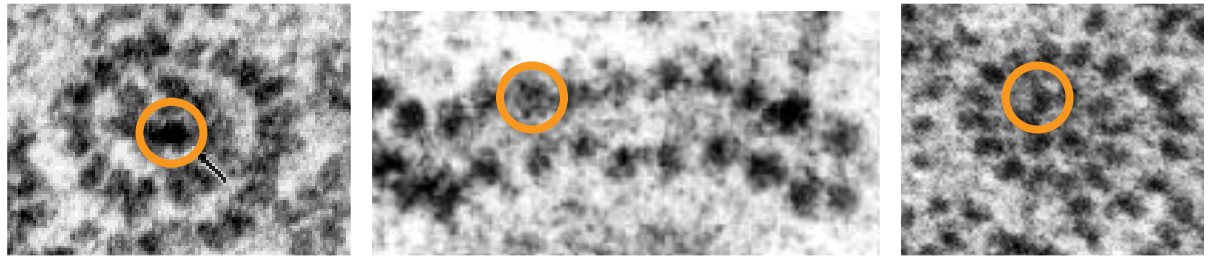
A single mRNA is translated by many ribosomes



▲Modified from Kiseleva (1989) FEBS Letters



Modified from Christensen (1999) Anatomical Rec ►



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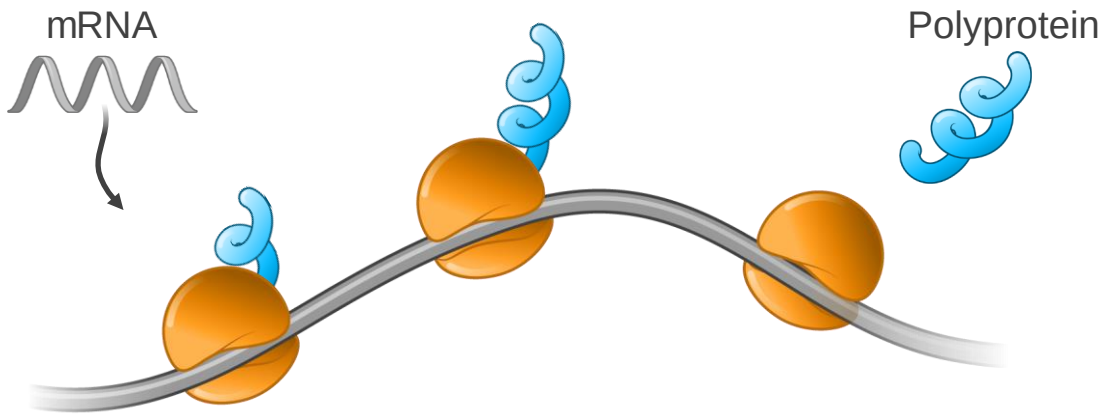
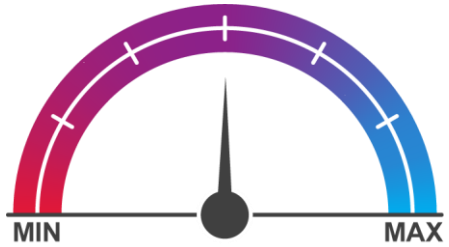
Relating translation to mRNA function





1. How does **translation** relate to protein output?
2. How do these learnings apply to **mRNA drugs**?
3. Can we **develop levers** that influence ribosome behavior to control mRNA function?

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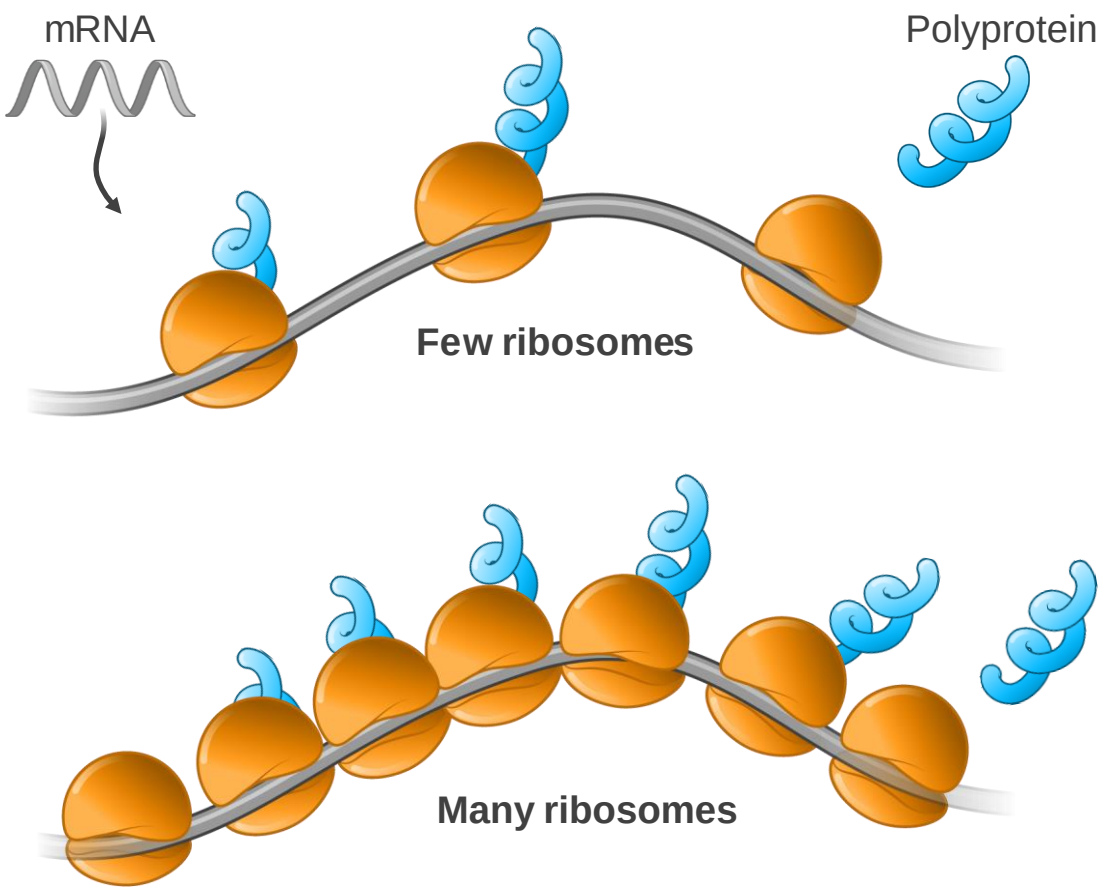
How does ribosome load relate to protein output?



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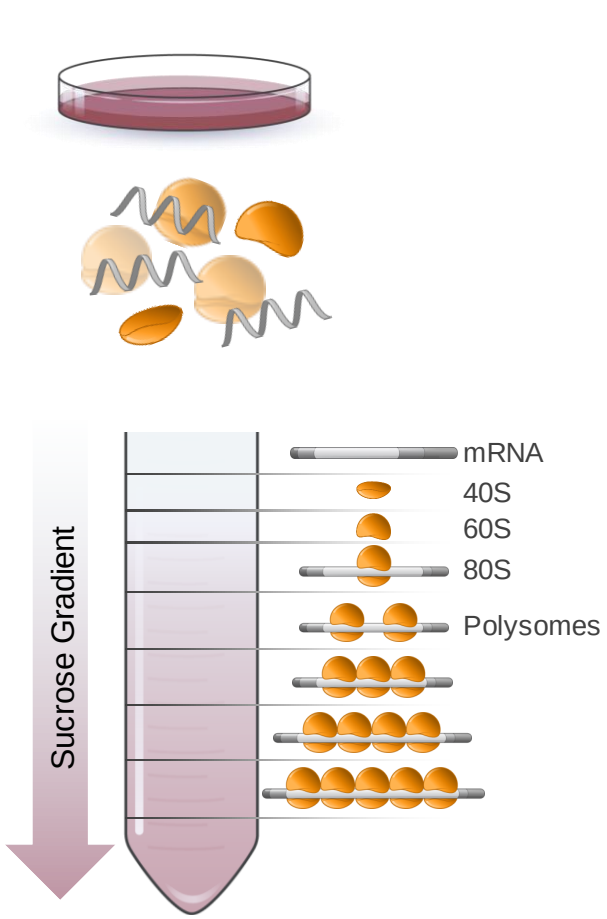
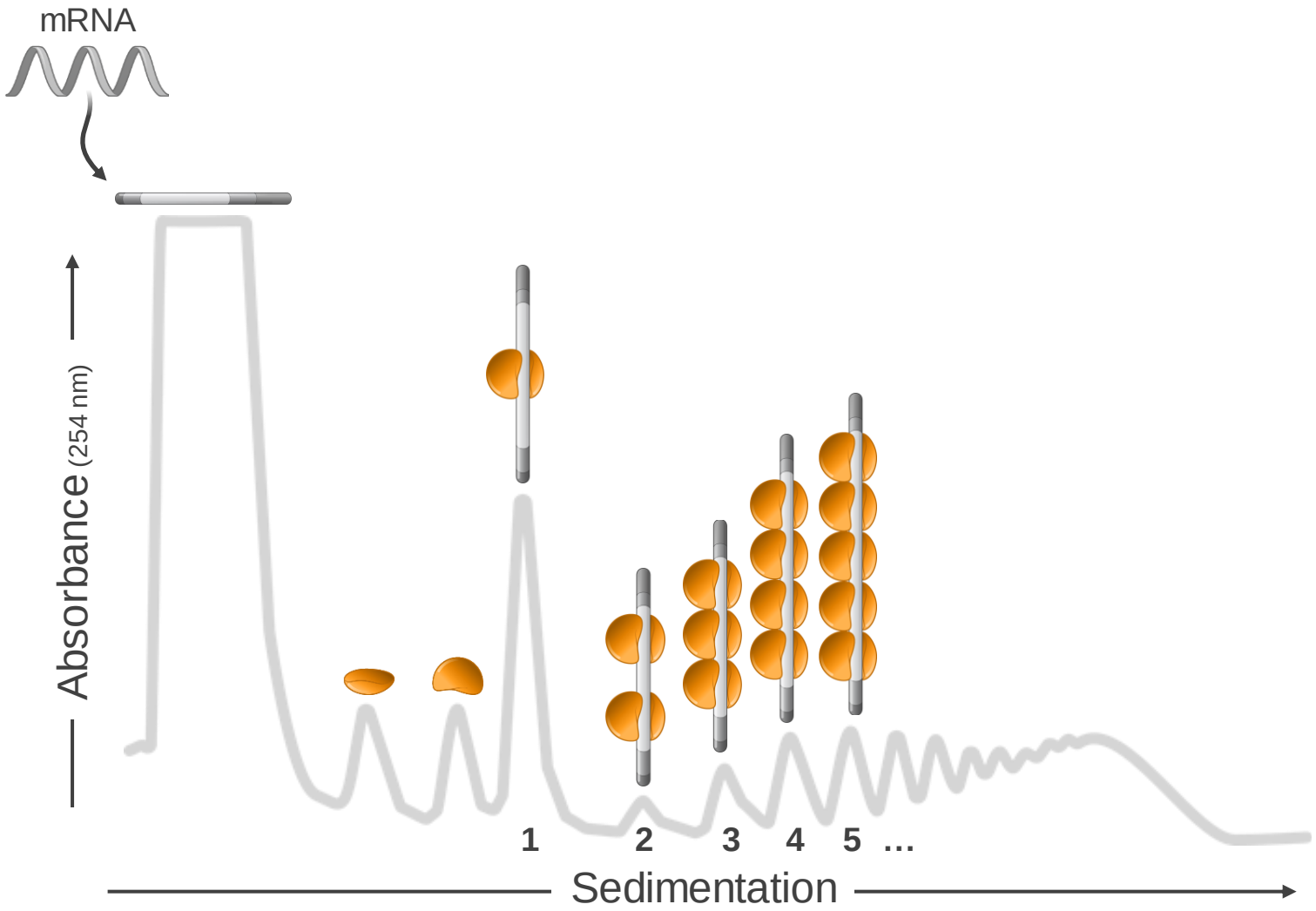
Measuring ribosome load: Polysome profiling





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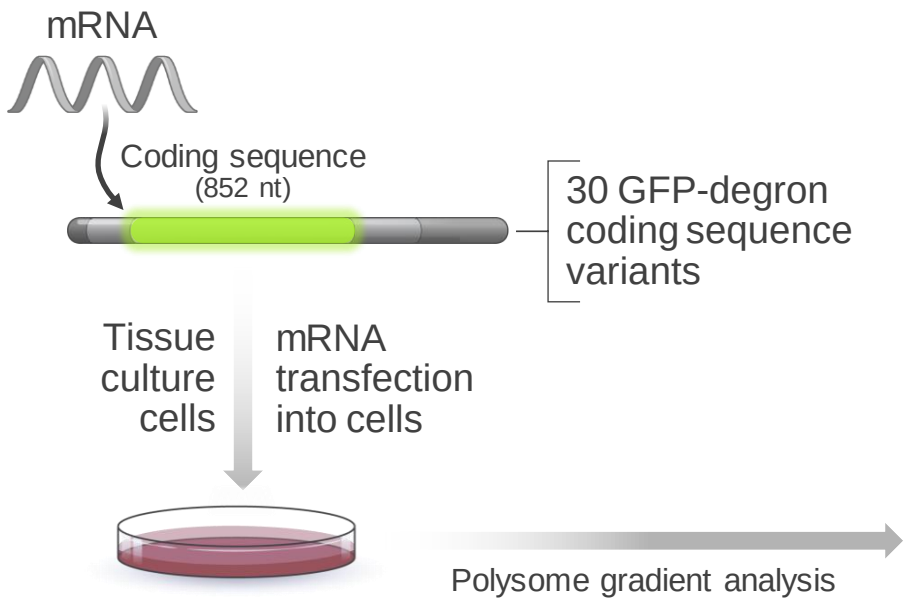
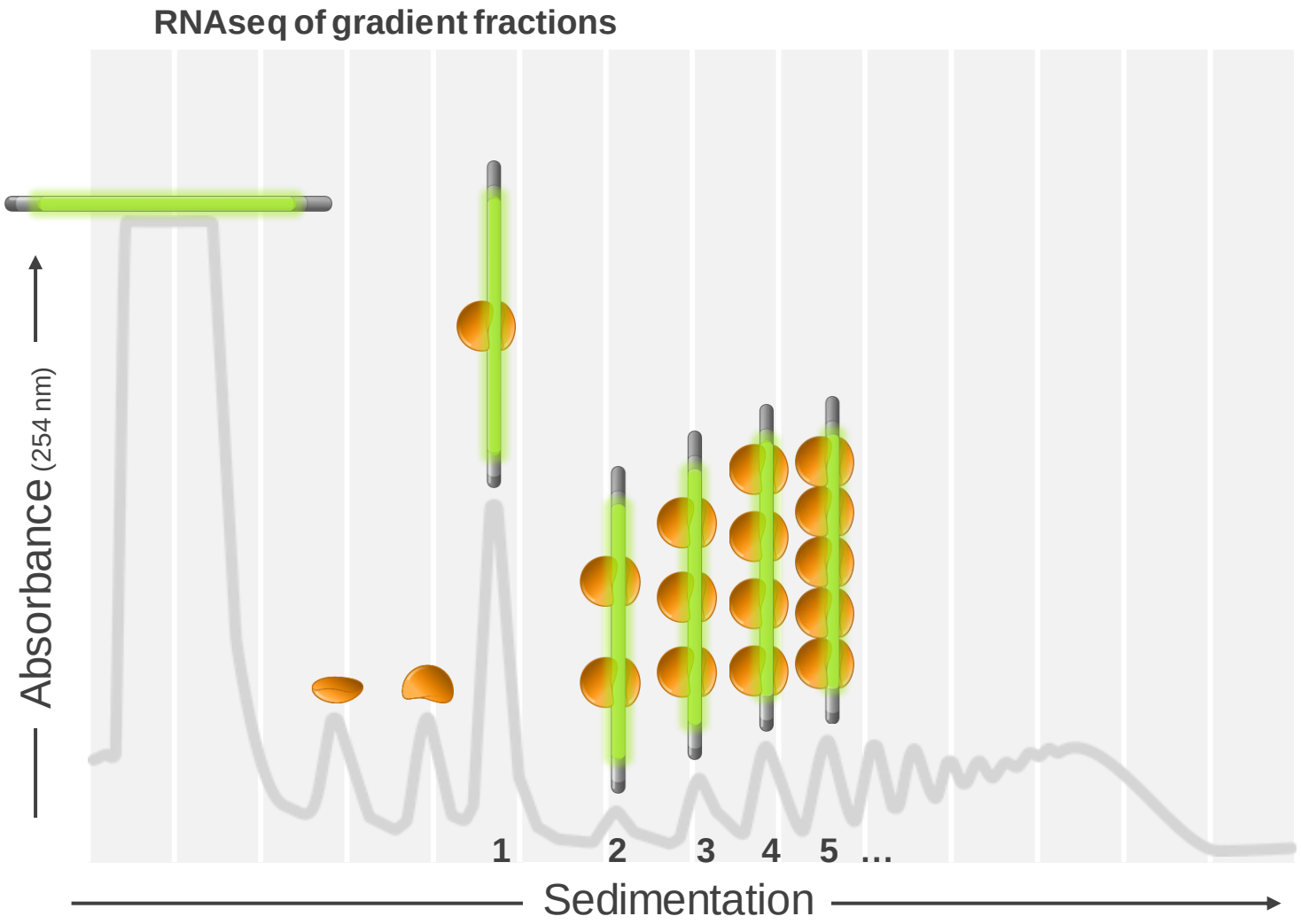
Ribosome densities vary among mRNA sequence variants





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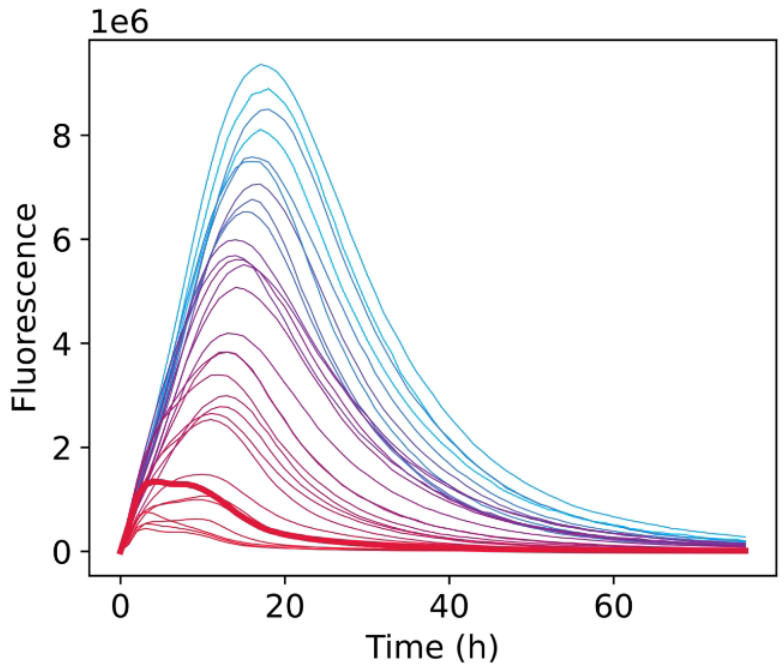
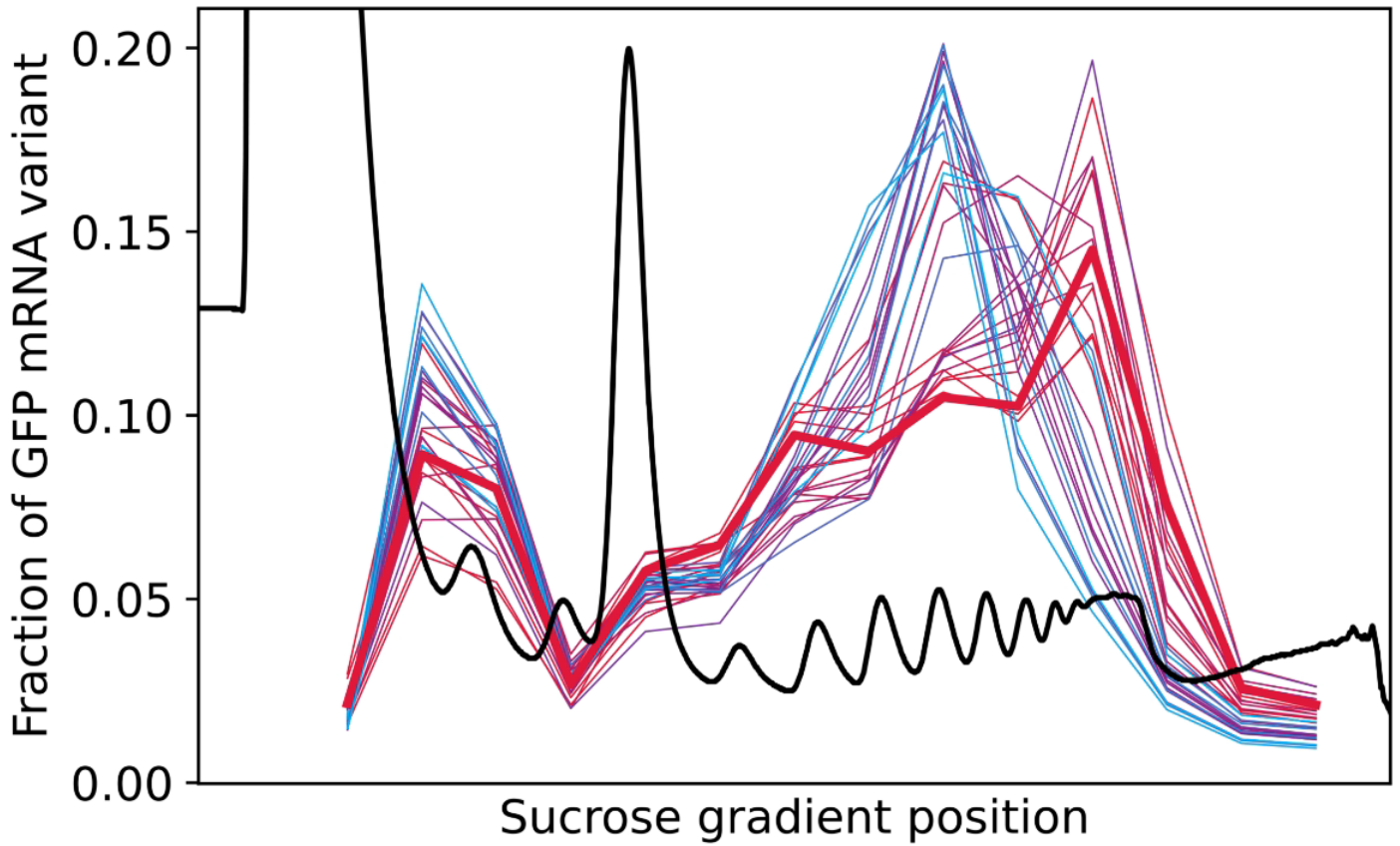
Distinct polysome profiles among mRNA sequence variants





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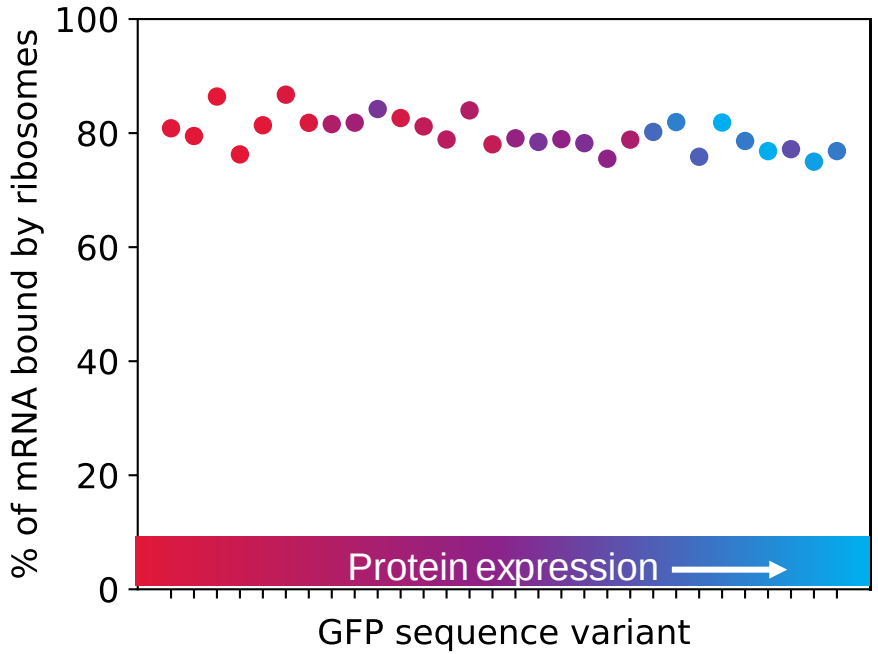
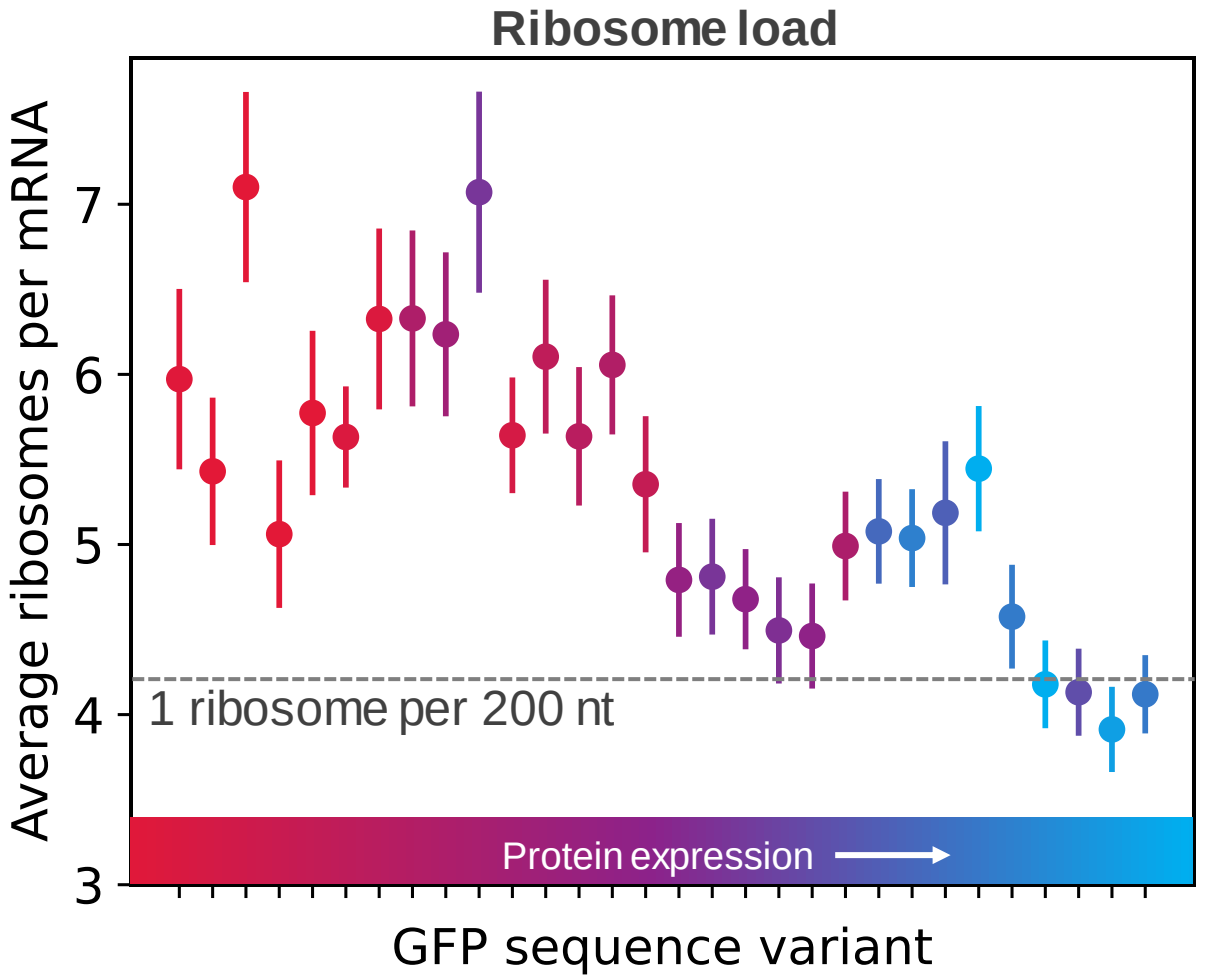
Protein output is *negatively* correlated with ribosome load





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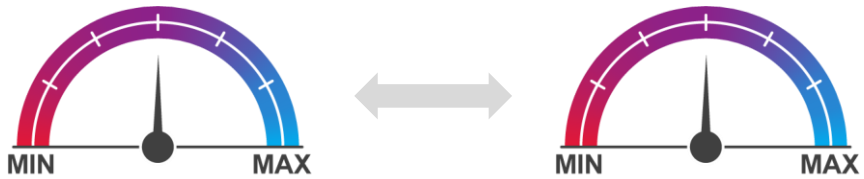
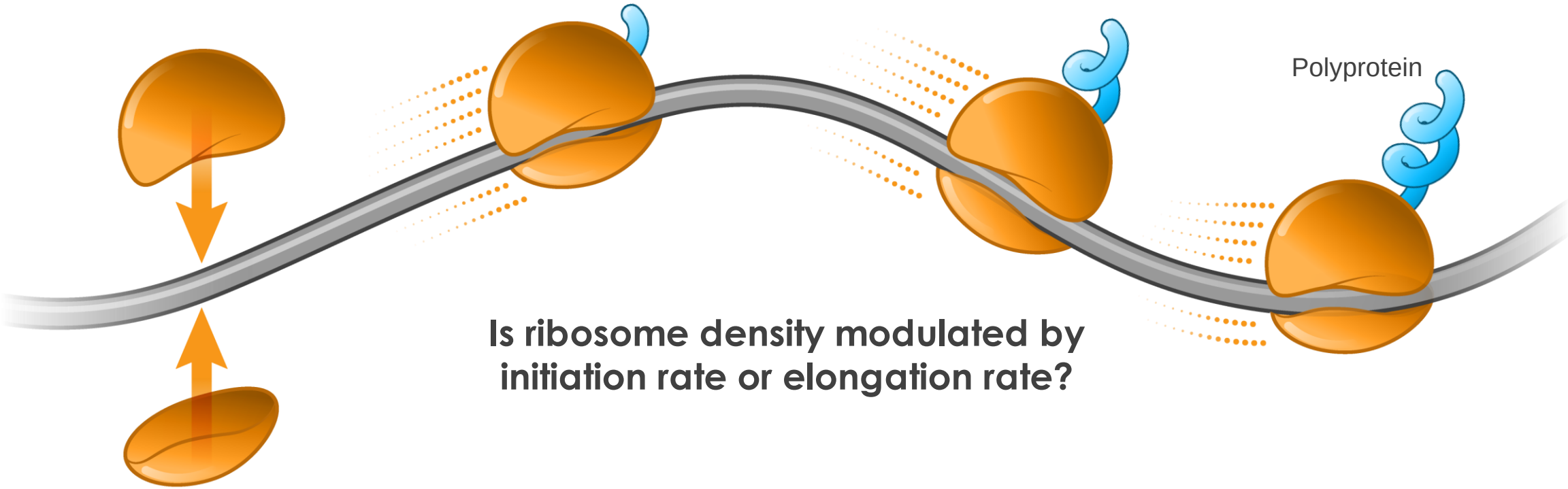
How can we control ribosome density?





Is ribosome density modulated by initiation rate or elongation rate?

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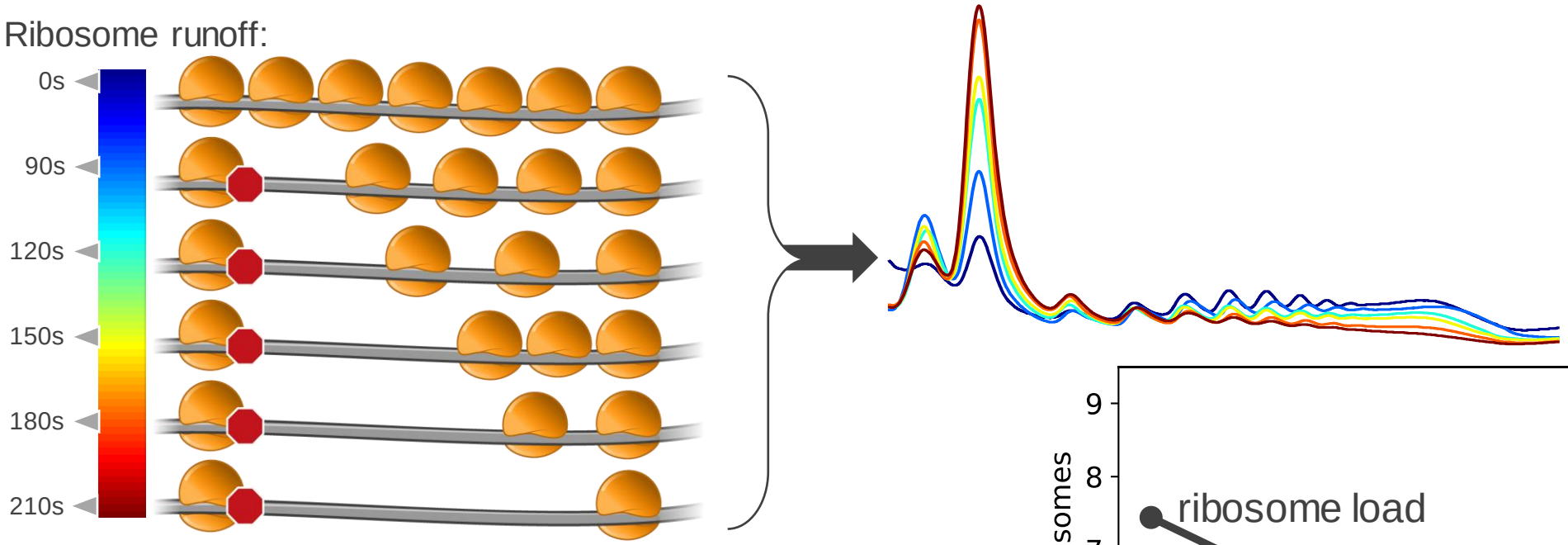
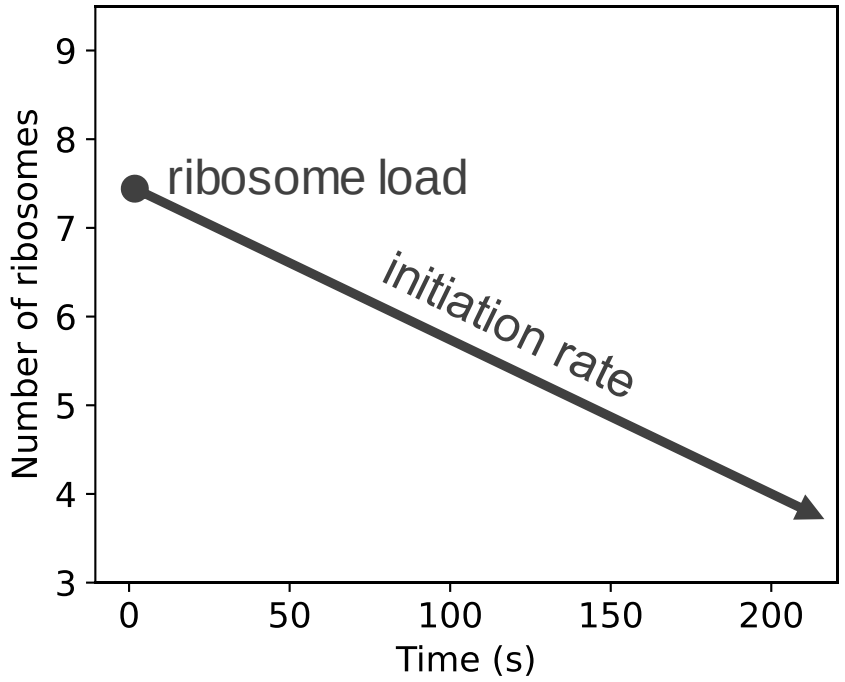
Measuring initiation and elongation rates

Ribosome runoff:



$$\text{ribosome load}\ \left(\frac{\text{ribosomes}}{\text{codon}}\right) = \frac{\text{initiation rate}\ \left(\frac{\text{ribosomes}}{\text{time}}\right)}{\text{elongation rate}\ \left(\frac{\text{codons}}{\text{time}}\right)}$$



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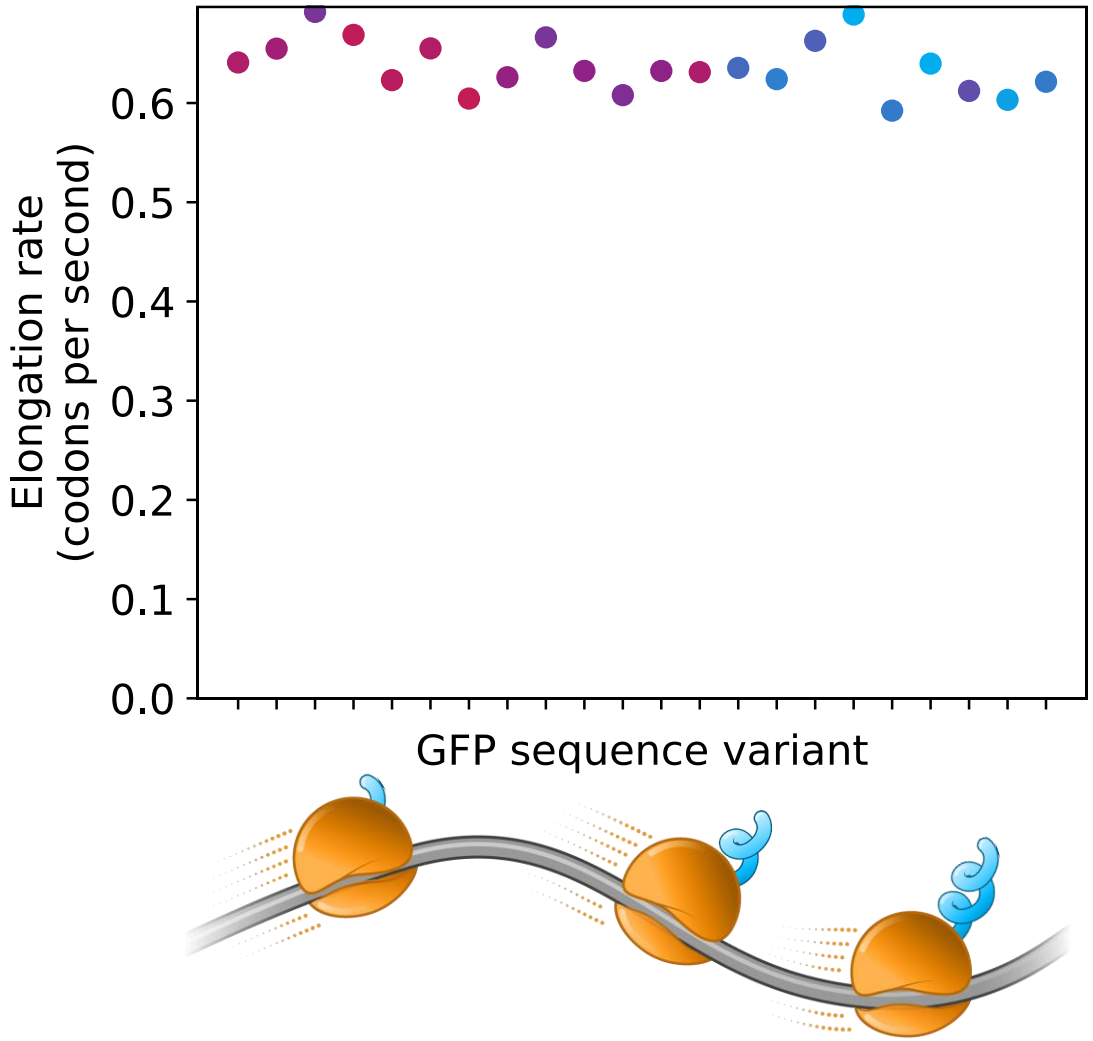
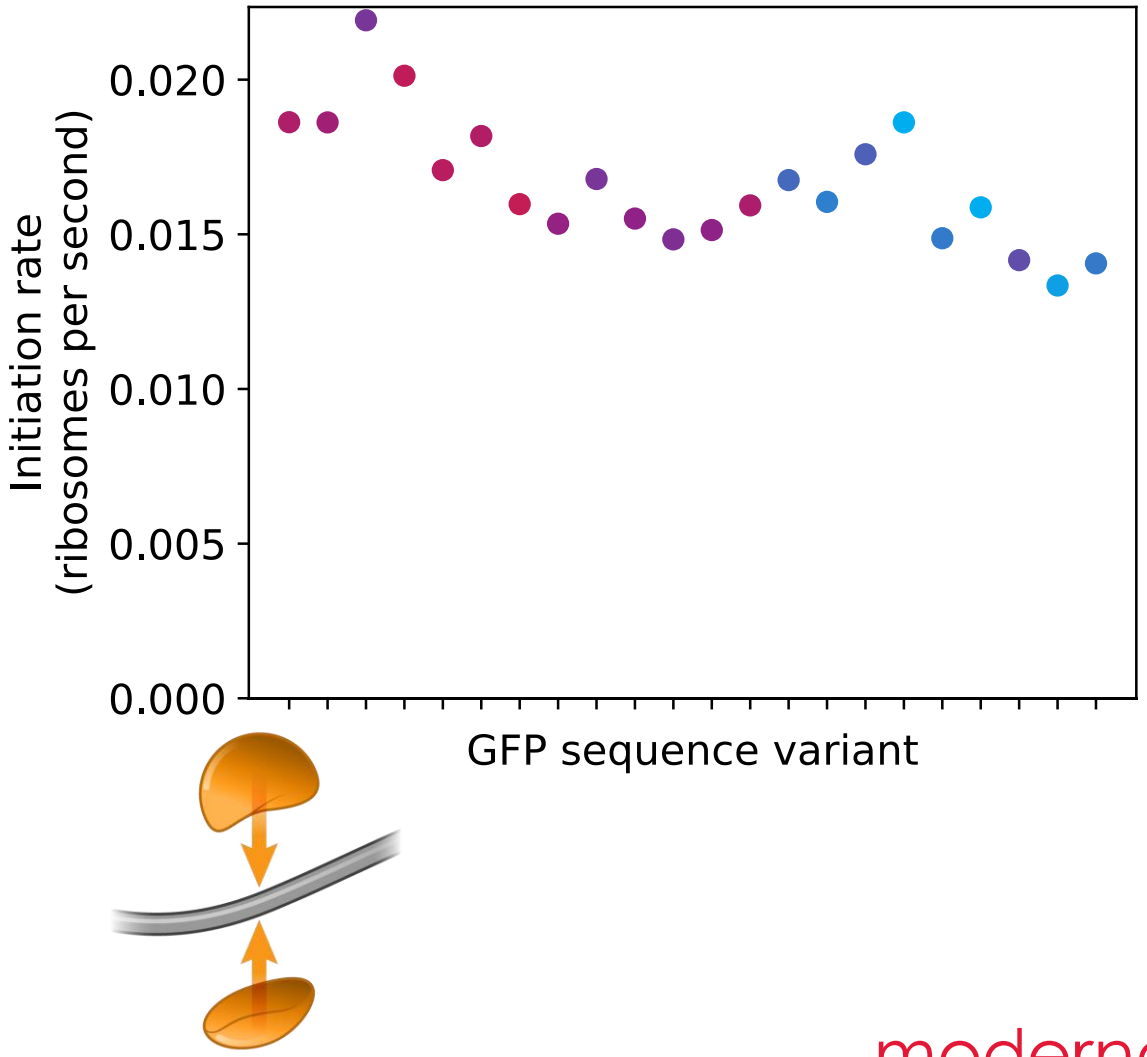
Quantification of elongation and initiation rates





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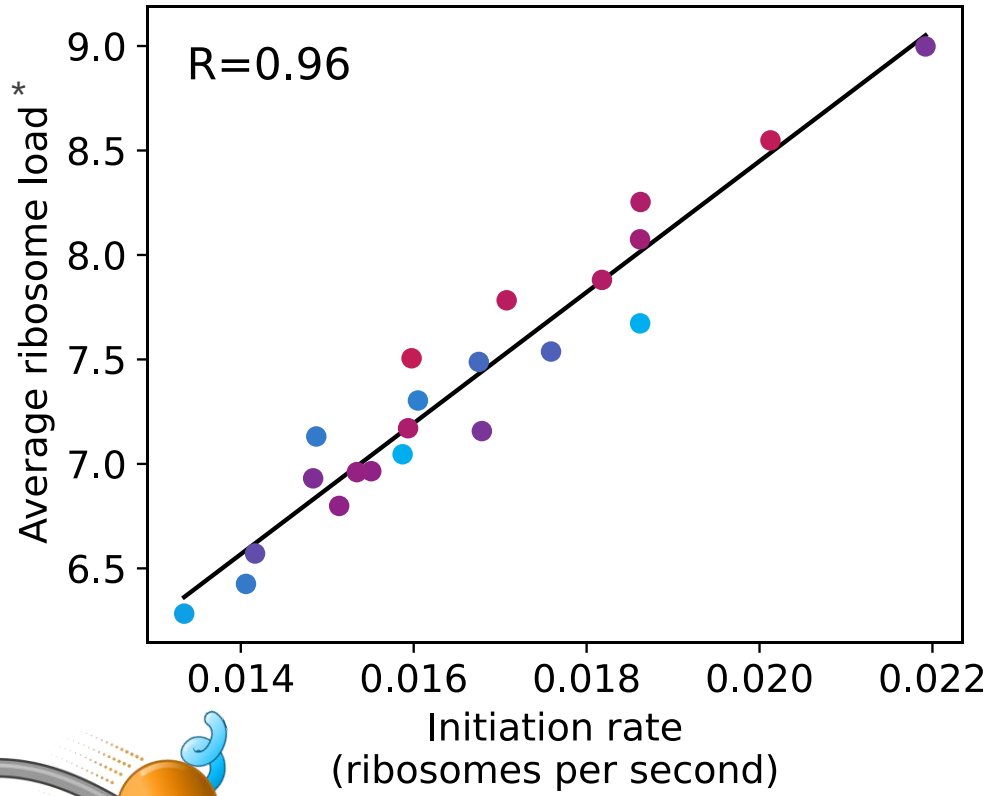
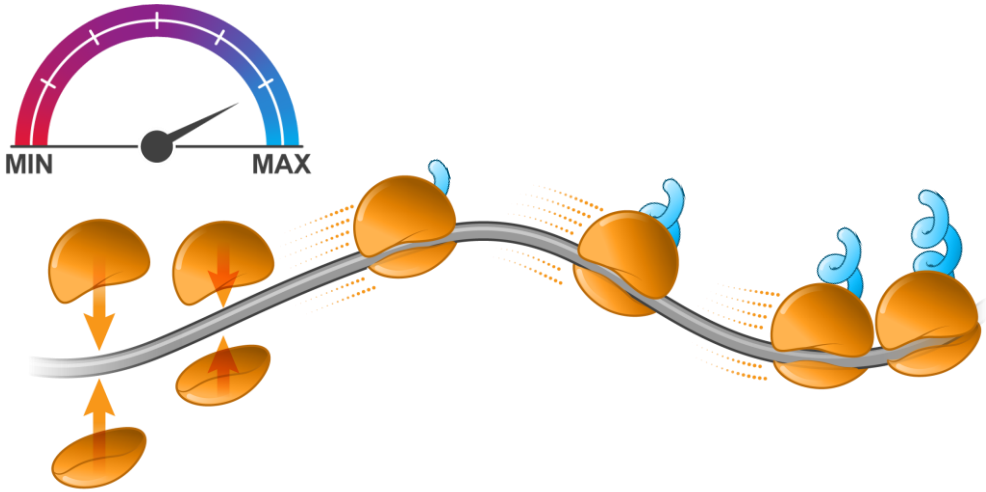
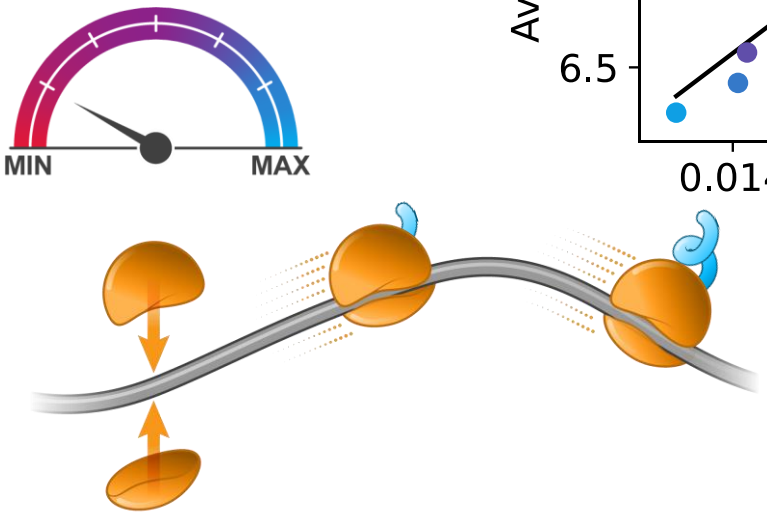
Ribosome load is driven by initiation rate







*Runoff-based ribosome load calculations do not consider non-translating mRNA, so values are higher

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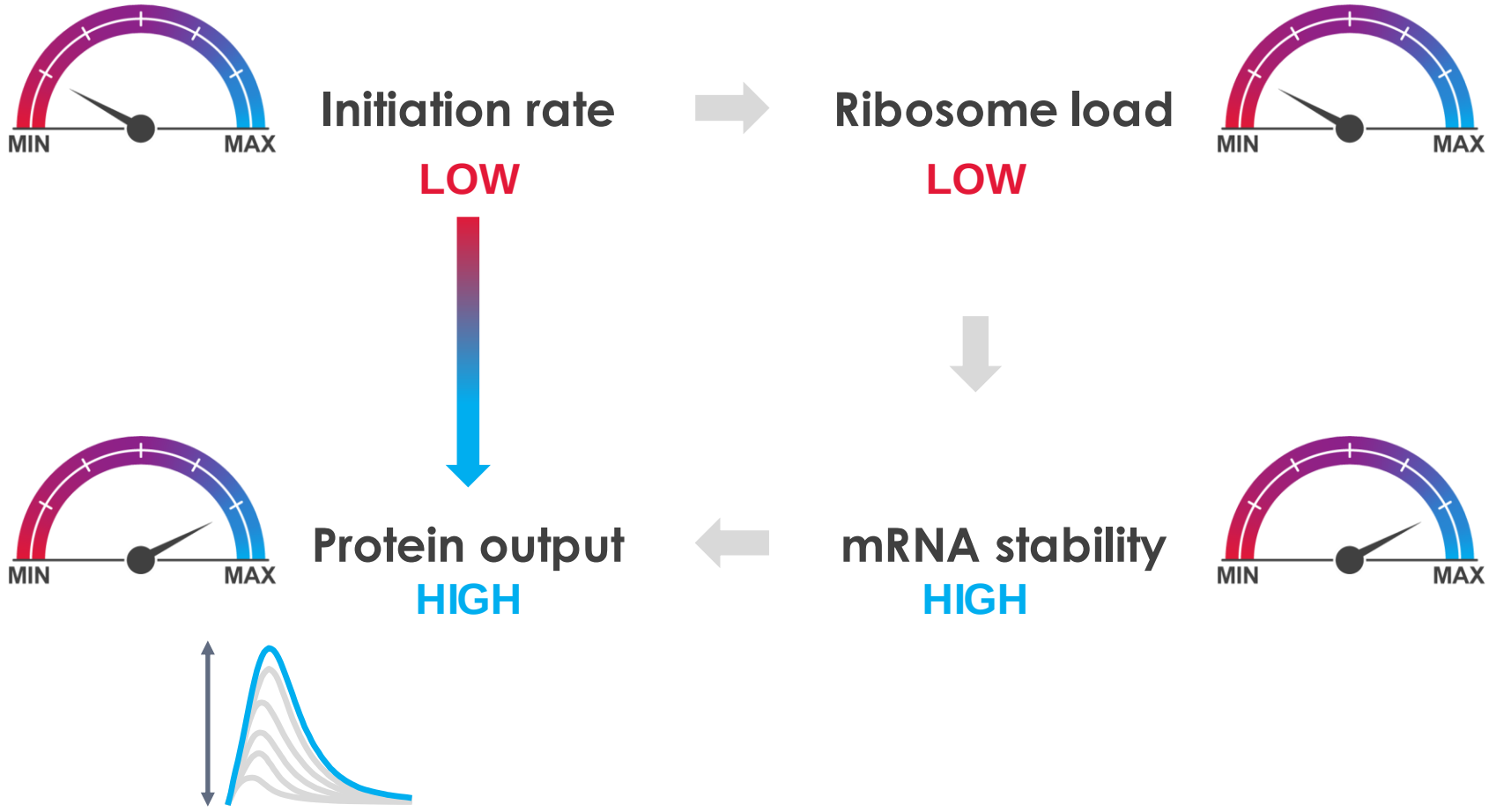
Linking translation initiation rates to protein output



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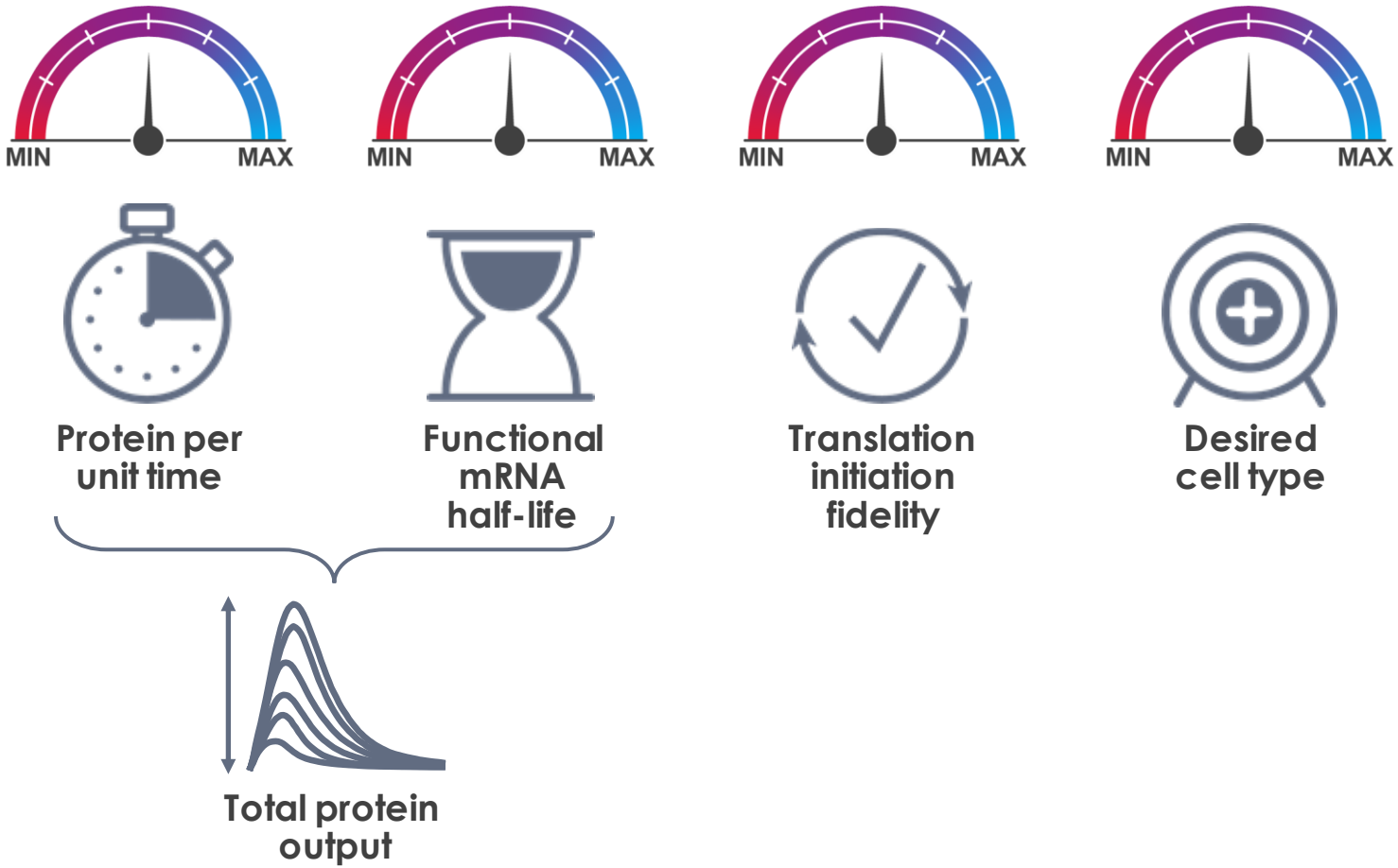
Fit-for-purpose mRNA engineering



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Agenda

Introduction	**Stéphane Bancel**, CEO **Melissa Moore, Ph.D.**, CSO, Platform Research
Tracking, assessing and predicting SARS-CoV-2 variants of concern	**Melissa Moore, Ph.D.**, CSO, Platform Research **Guillaume Stewart-Jones, Ph.D.** **Wei Zheng, Ph.D.**
mRNA Engineering: Optimizing Ribosome Load	**David Reid, Ph.D.**
Coffee Break	
Understanding and engineering intracellular events affecting LNP performance	**Melissa Moore, Ph.D.**, CSO, Platform Research
In vivo mRNA delivery to Hematopoietic Stem and Progenitor Cells	**David Alvarez, Ph.D.**
Closing remarks	**Stephen Hoge, M.D.**, President
Q&A	

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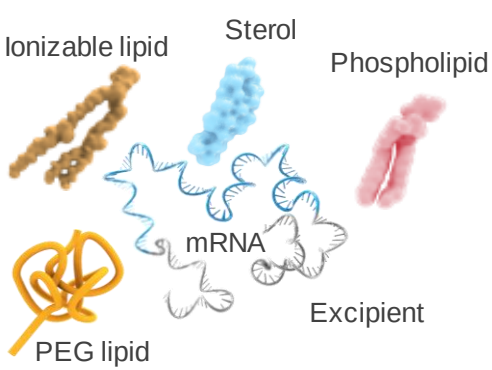
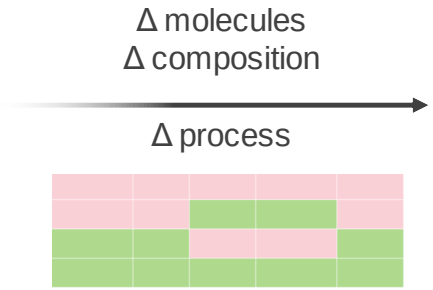
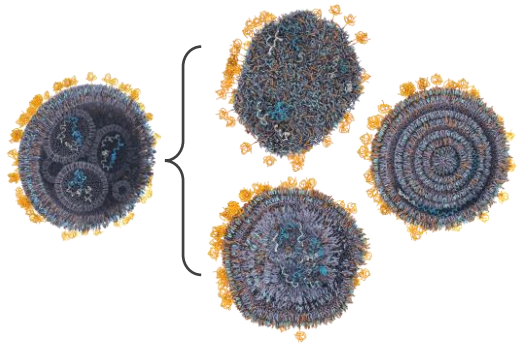
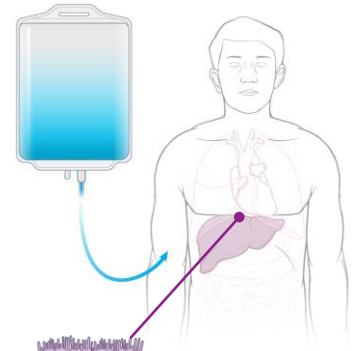
Considerations for LNP engineering

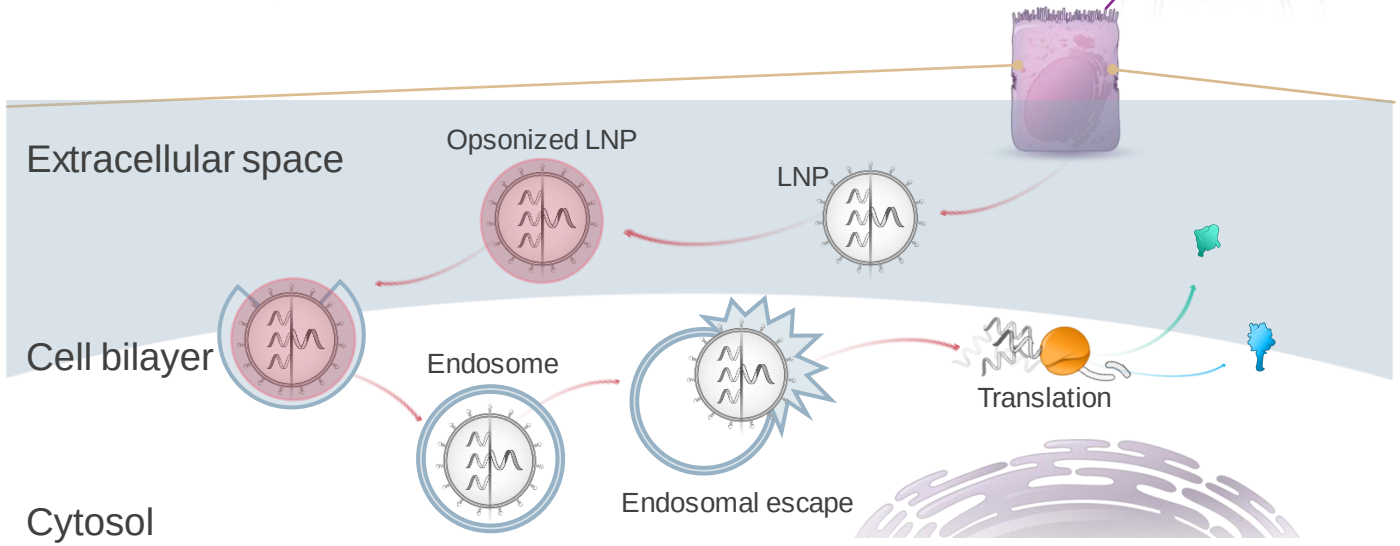
Components | **Composition & Process** | **Structure** | **Stability** | **Routes of Administration**











Each new route of administration (ROI) or target cell type requires consideration and optimization of all parameters

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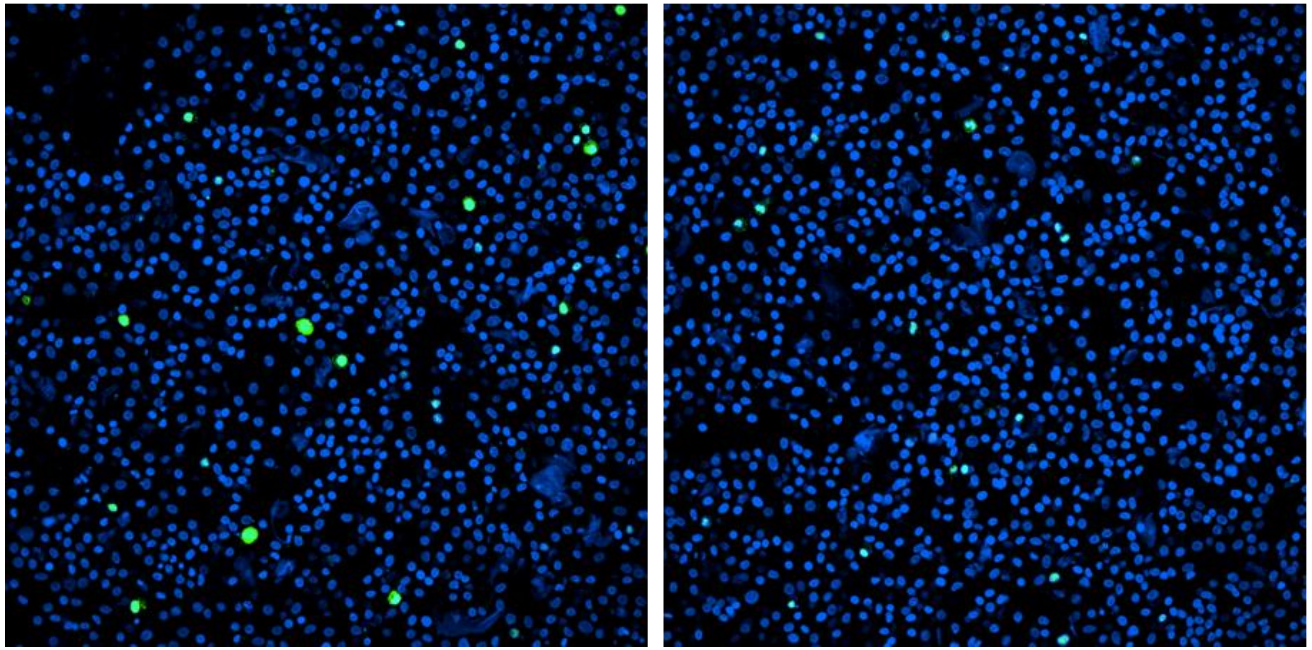
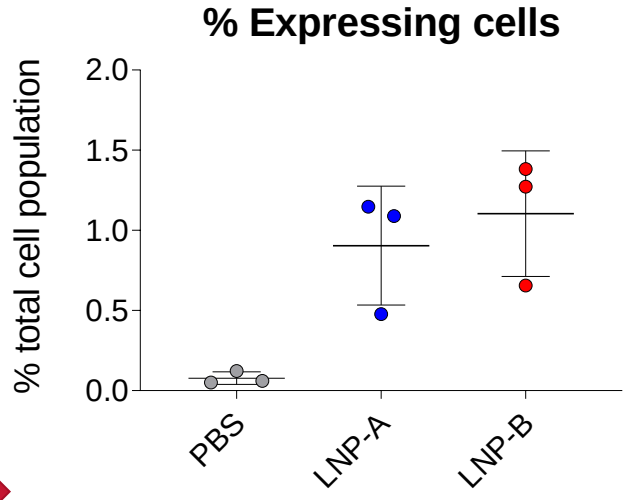
Understanding and engineering intracellular events affecting LNP performance

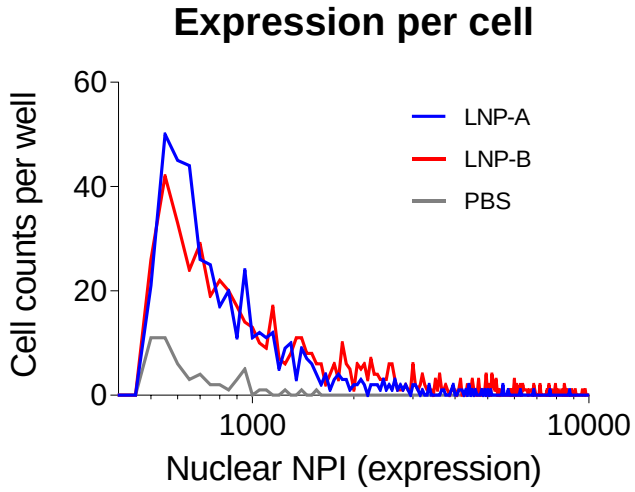
Two LNPs with similar overall transfection efficiency…

LNP-A **LNP-B**



Blue: Nuclei (DAPI)
Green: Reporter protein IHC





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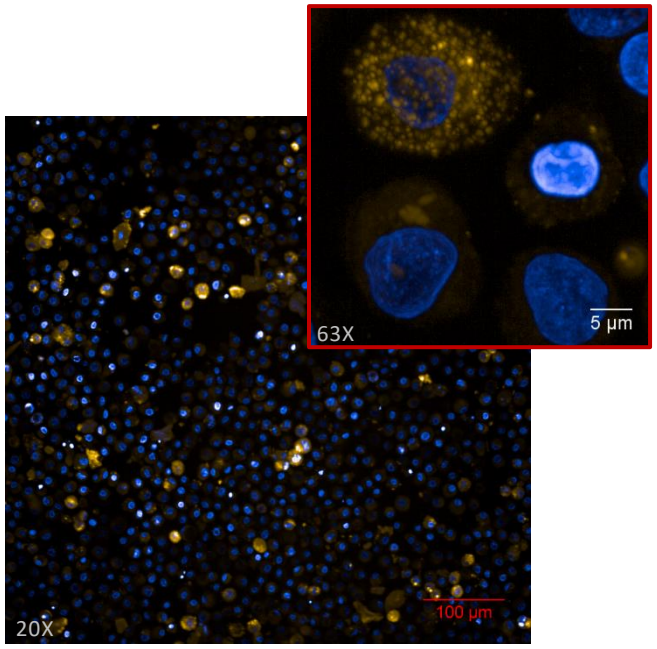
…but very different efficiencies of cellular uptake

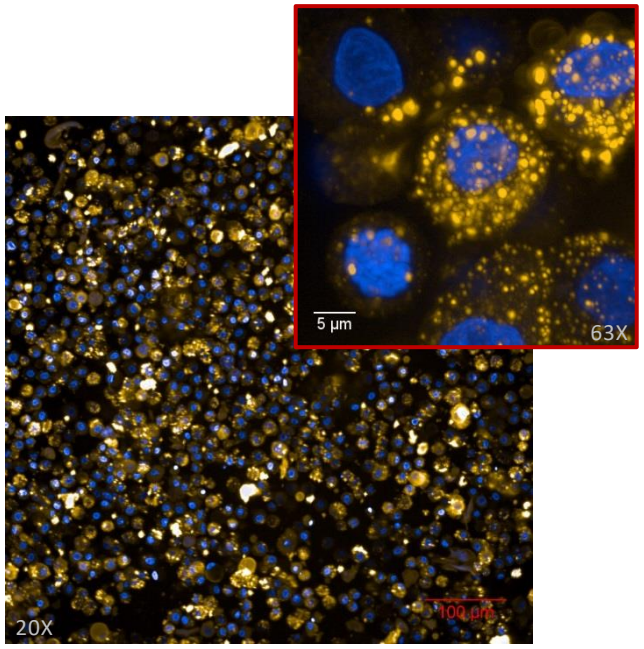
LNP-A

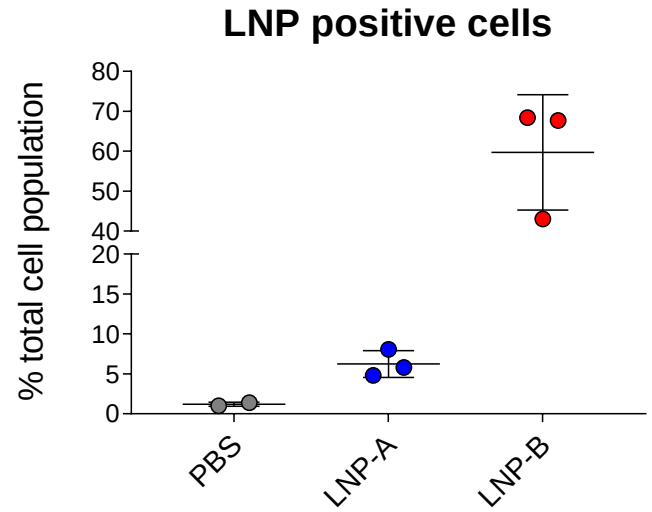
- Limited cells taking up LNPs
- Fewer LNPs per cell

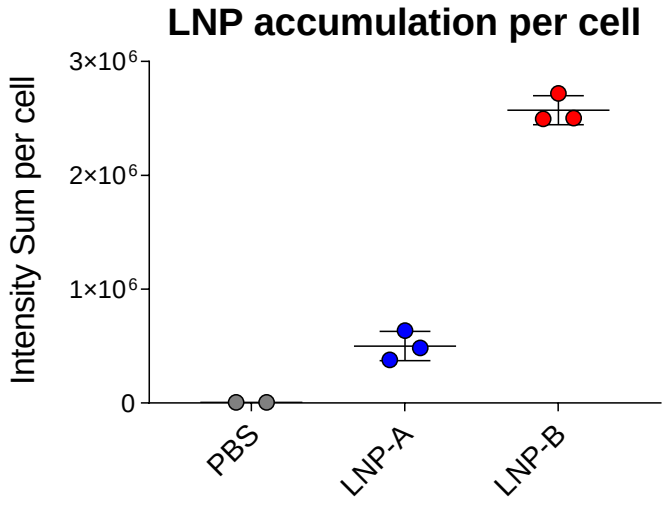


LNP-B

- More cells taking up LNPs
- More LNPs per cell







Blue: Nuclei (DAPI)
Yellow: Fluorescent LNP (Rhodamine)

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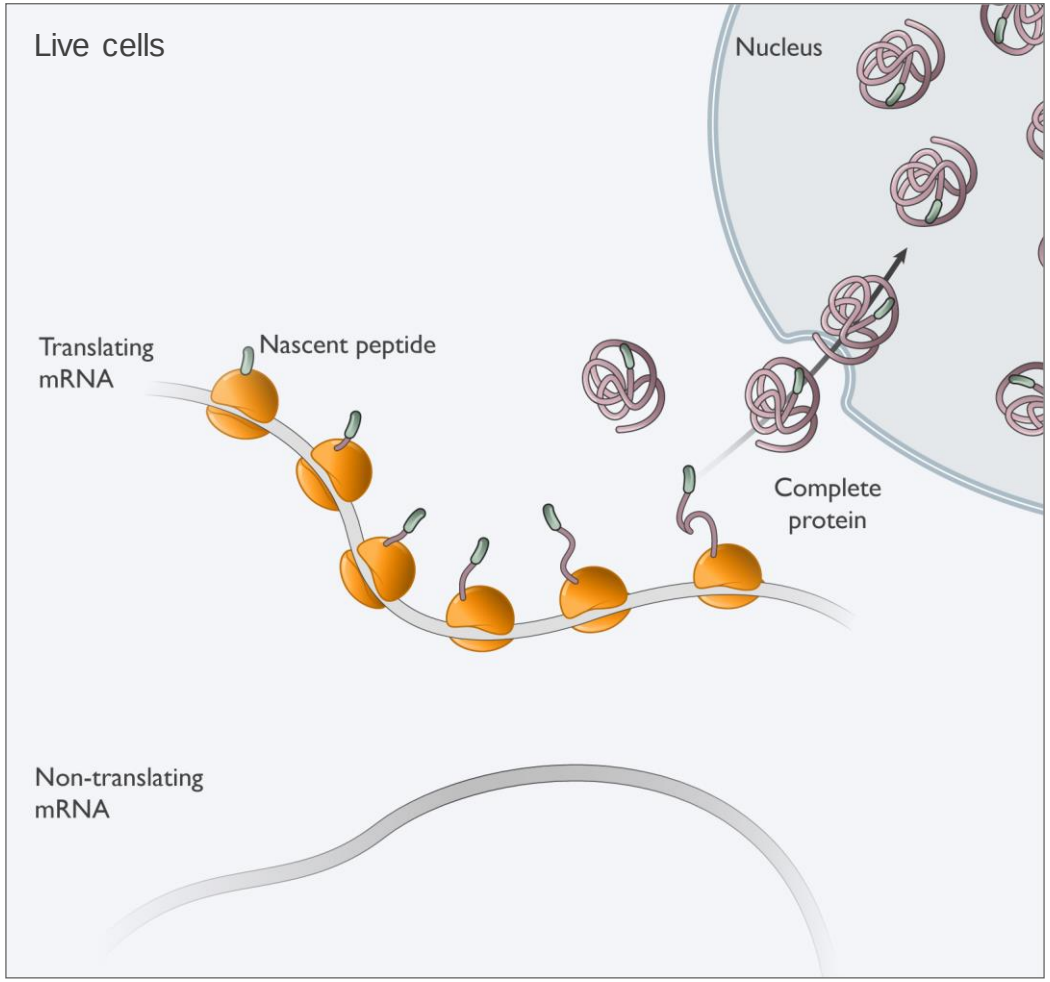
Nascent Peptide Imaging (NPI) allows us to both count cytoplasmic mRNAs and assess their translational activity



Adapted from Yan …..Tanenbaum (2016) *Cell*

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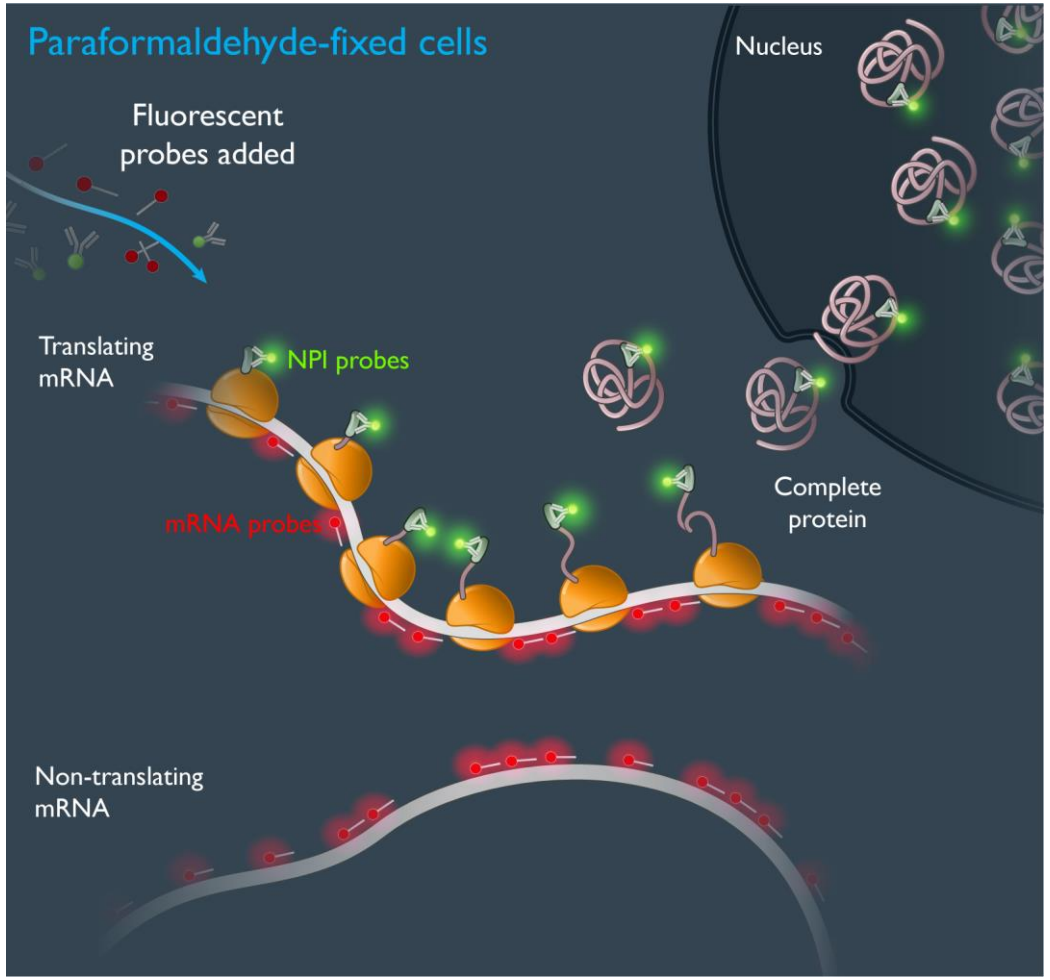

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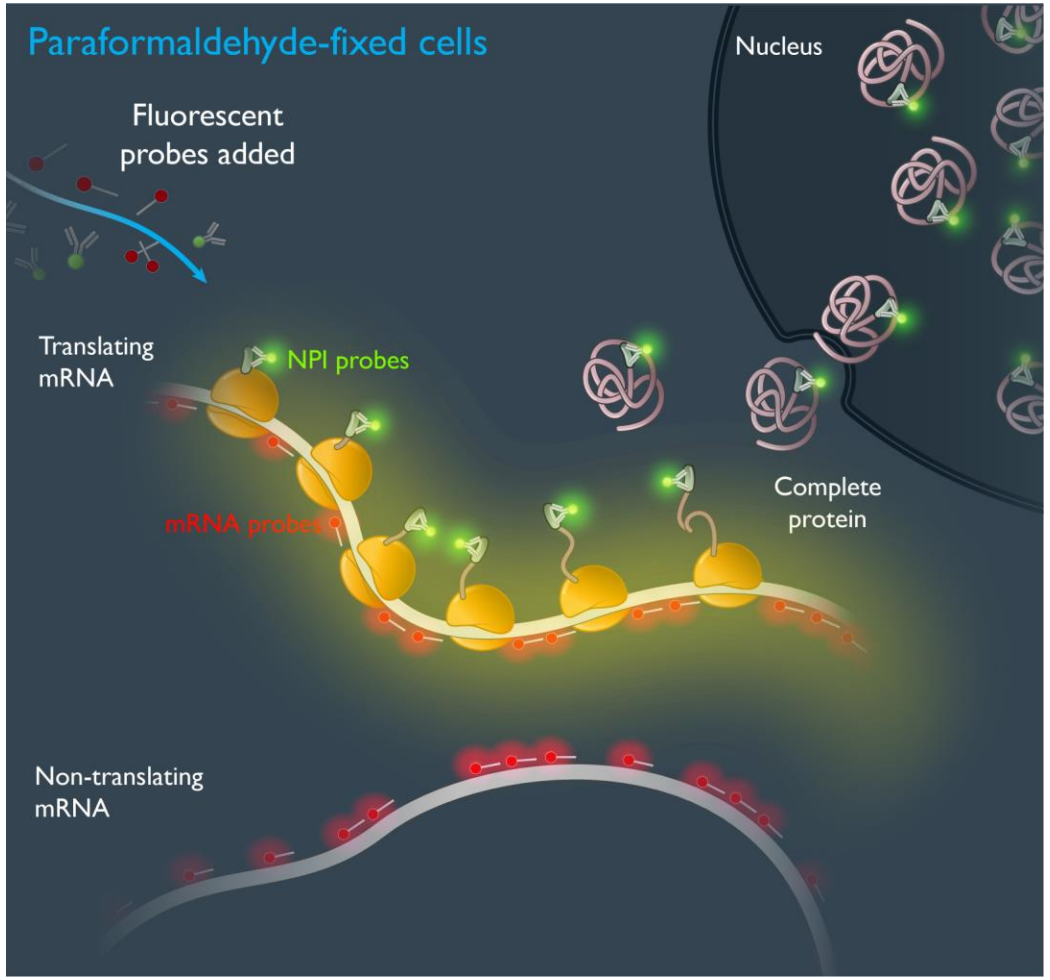

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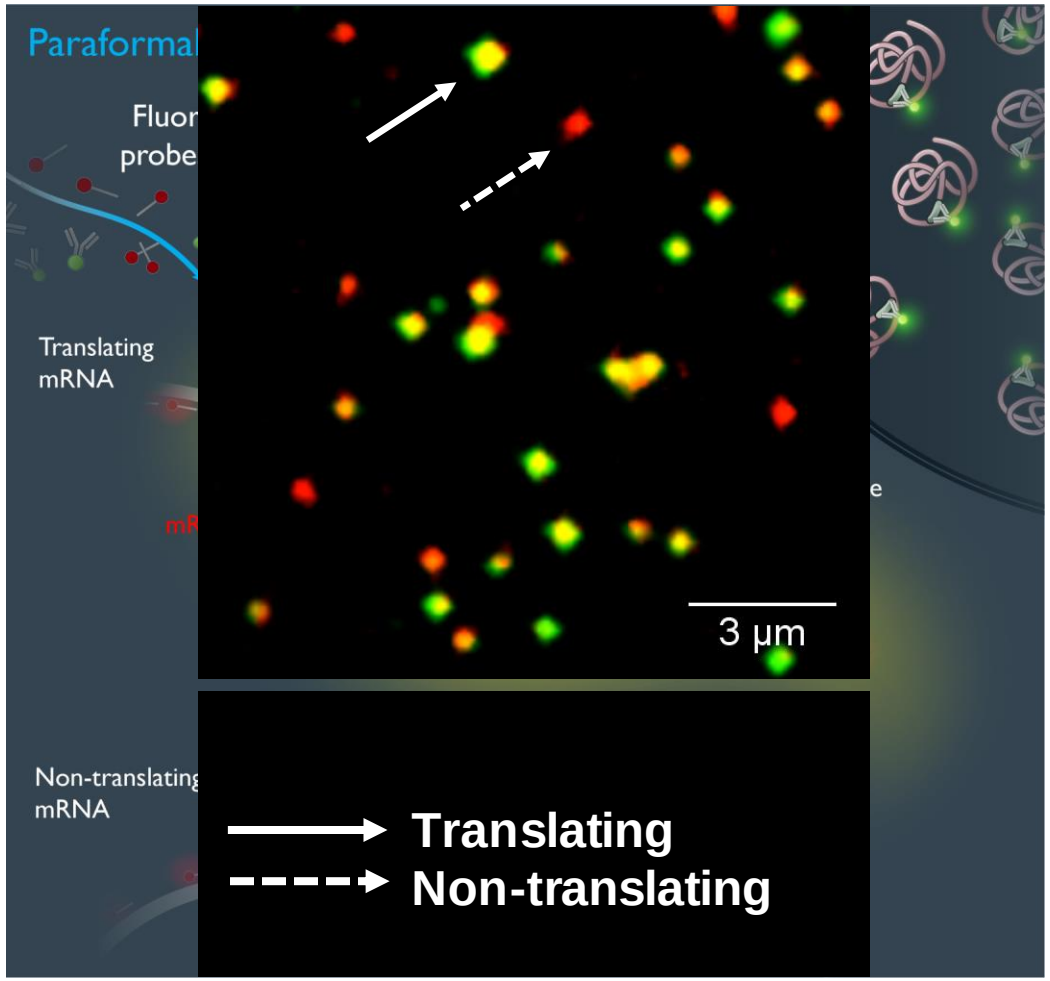

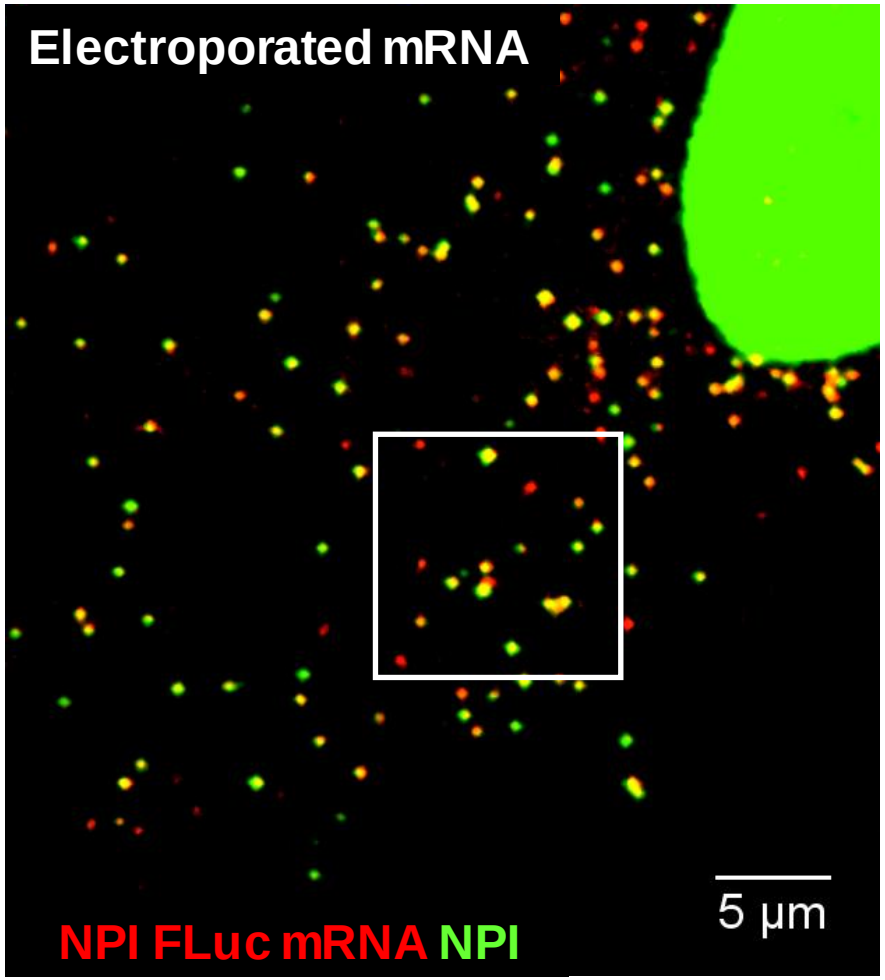

Adapted from Yan …..Tanenbaum (2016) *Cell*

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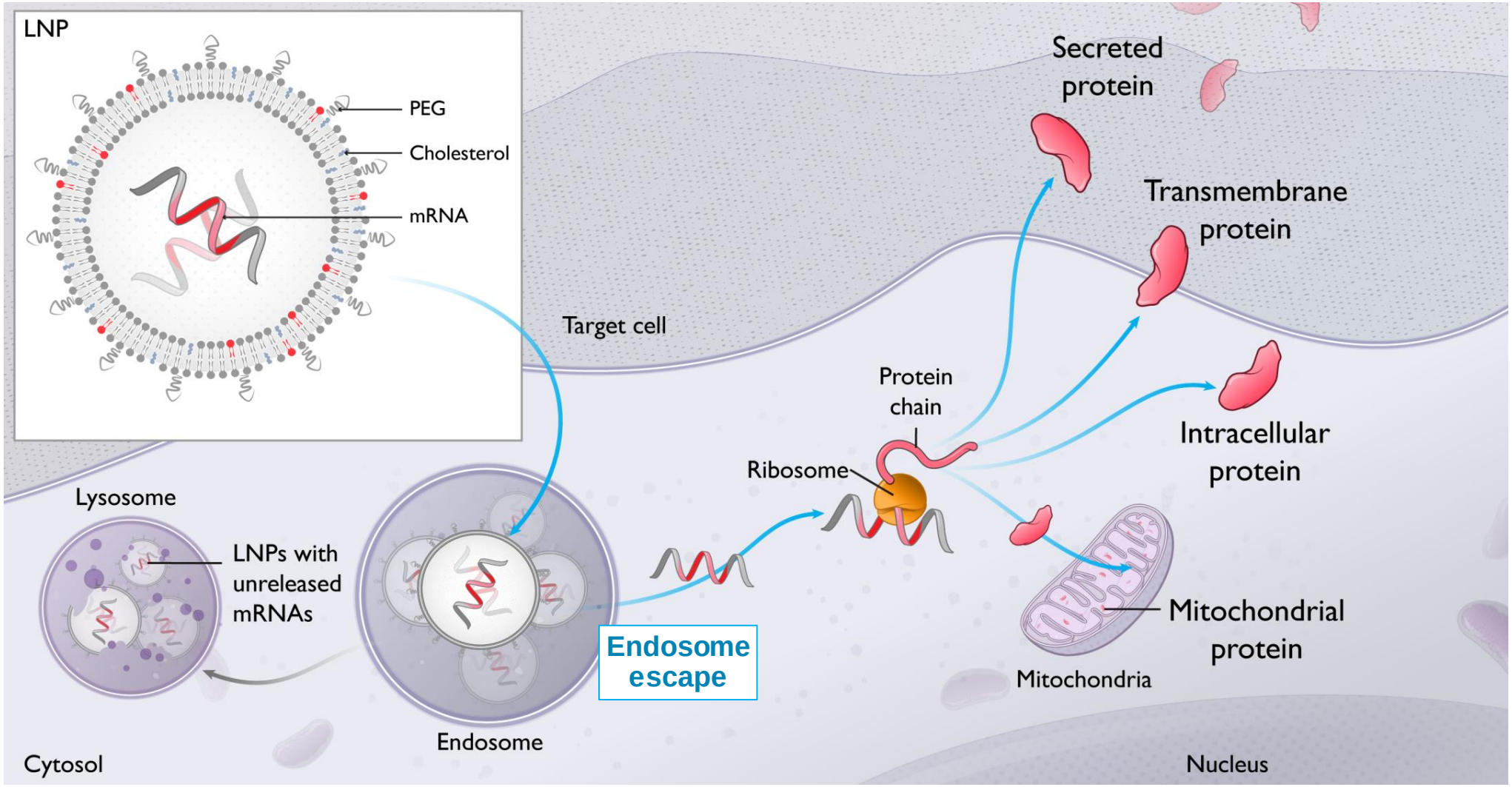
How efficient is endosome escape?



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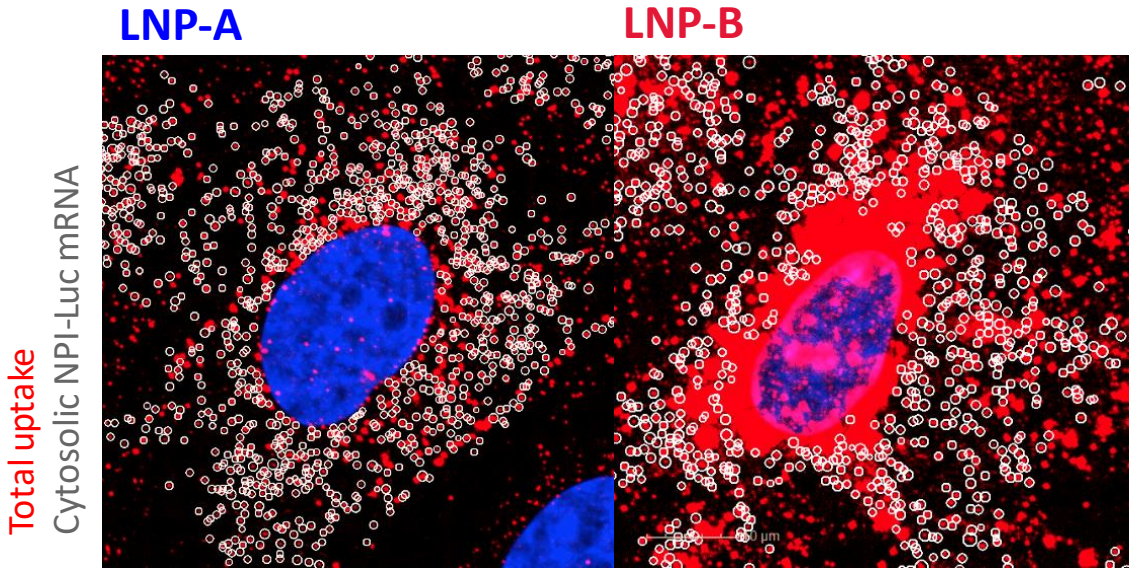
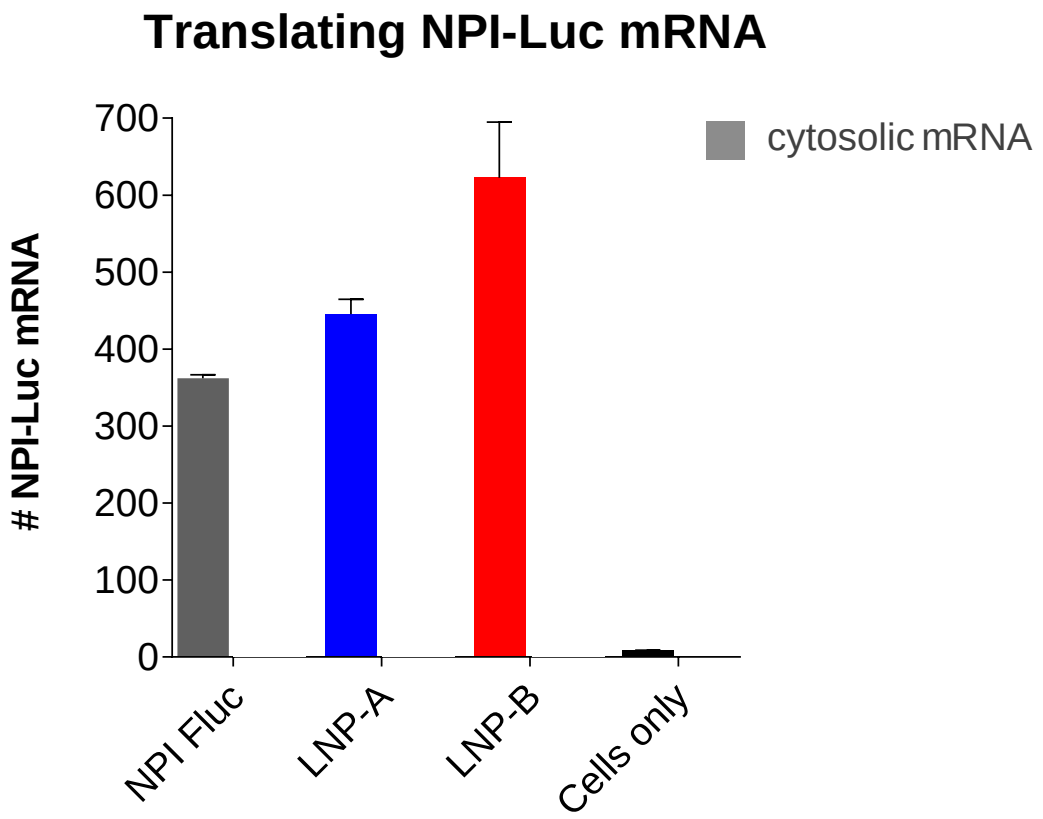
Endosome escape was not the problem…





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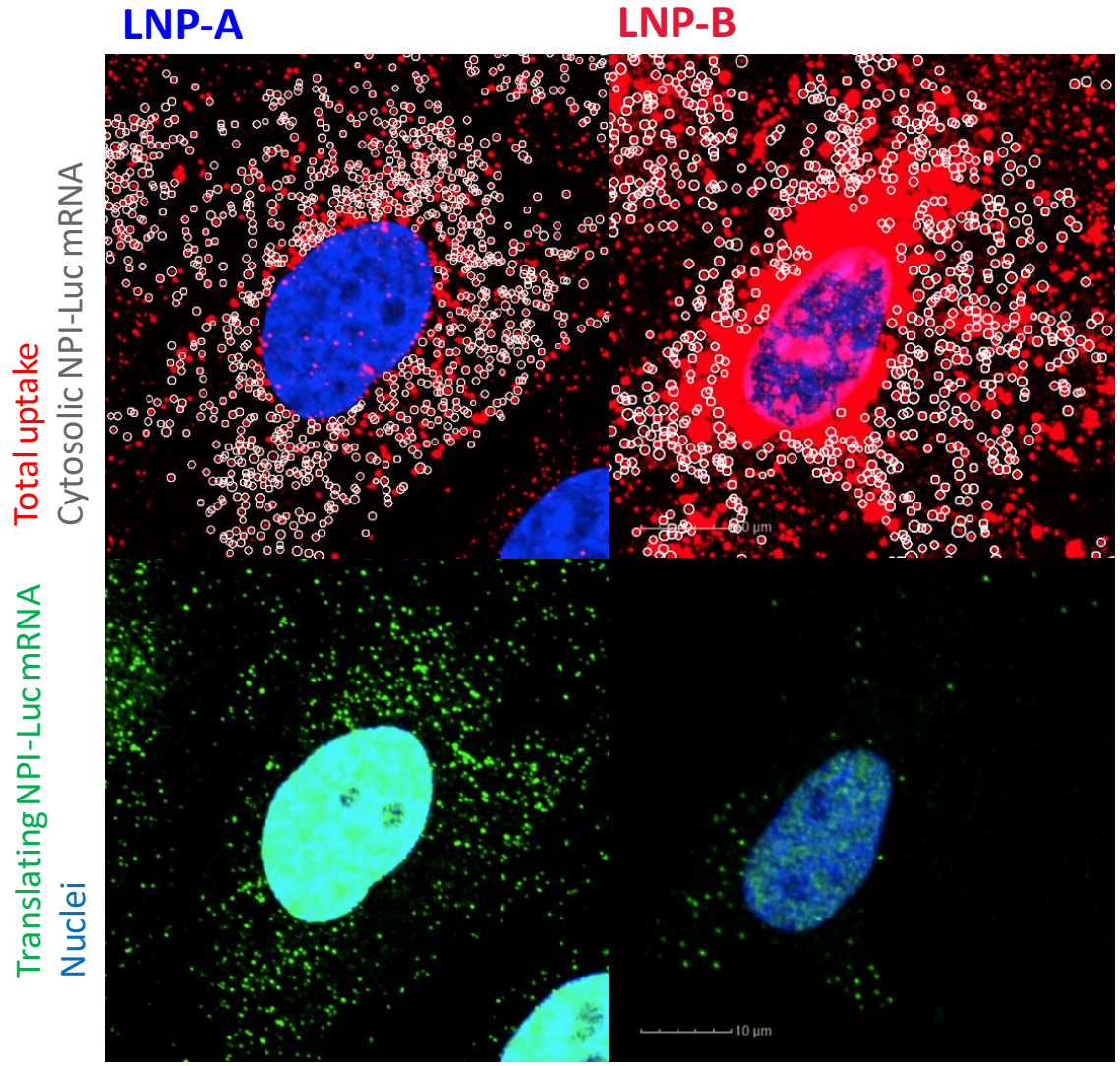
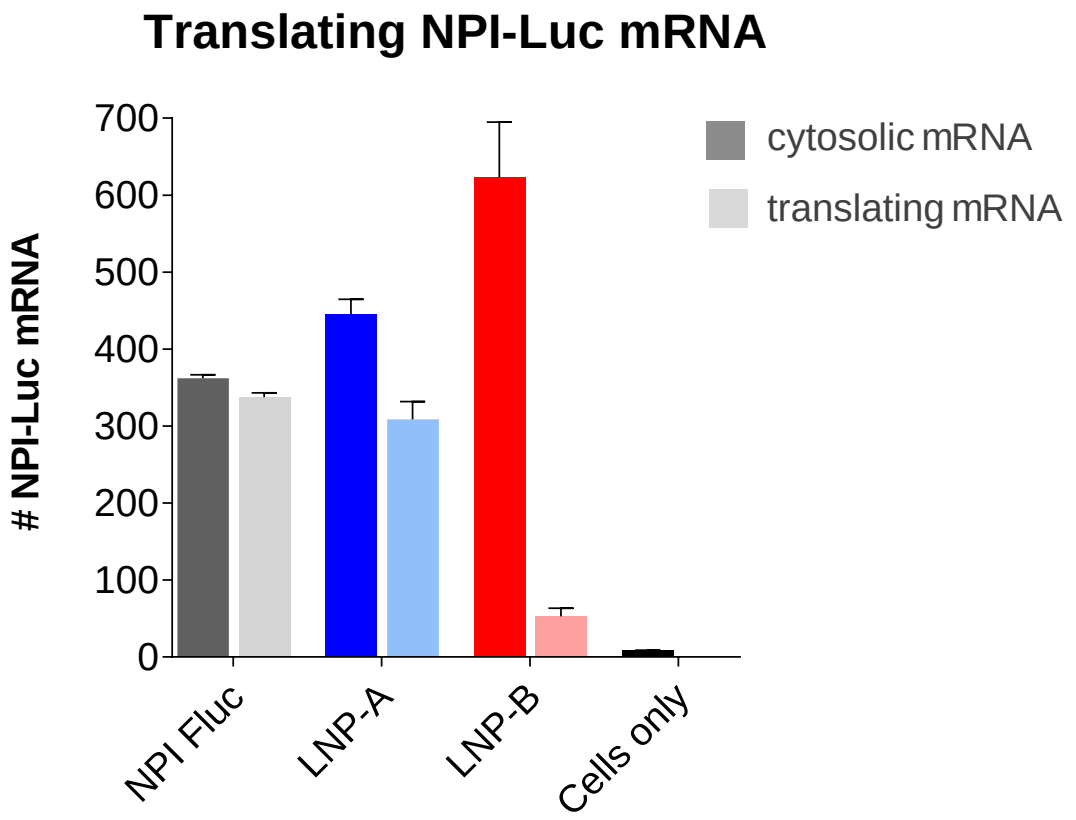
…but once in the cytoplasm, very few mRNA molecules were translationally active





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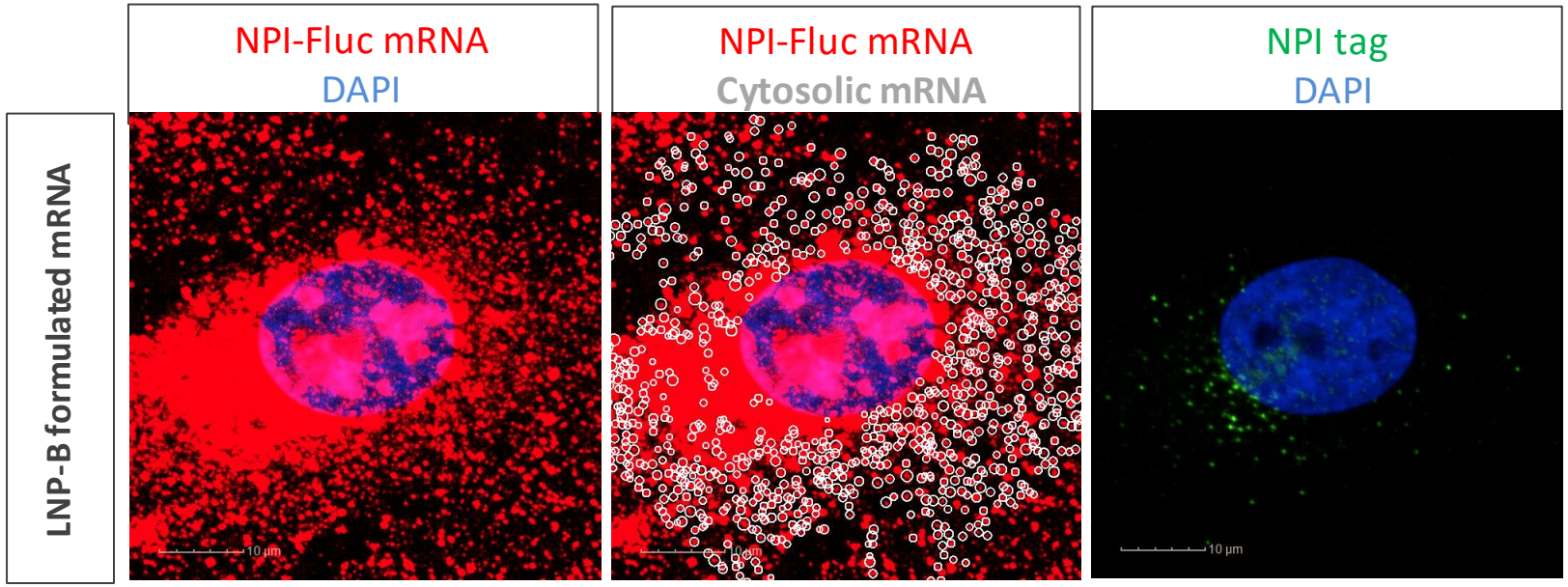
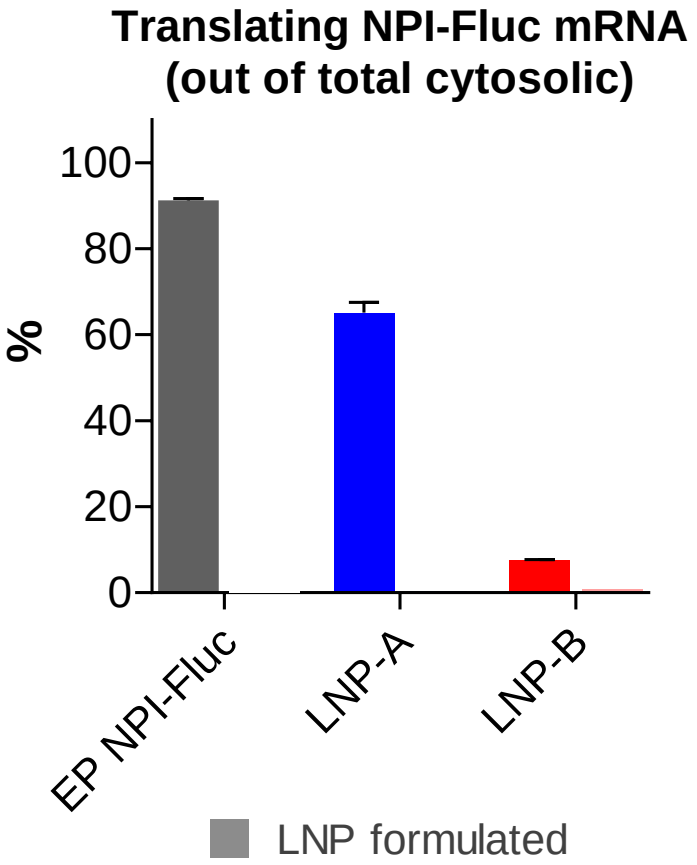
Deformulation rescues translation efficiency





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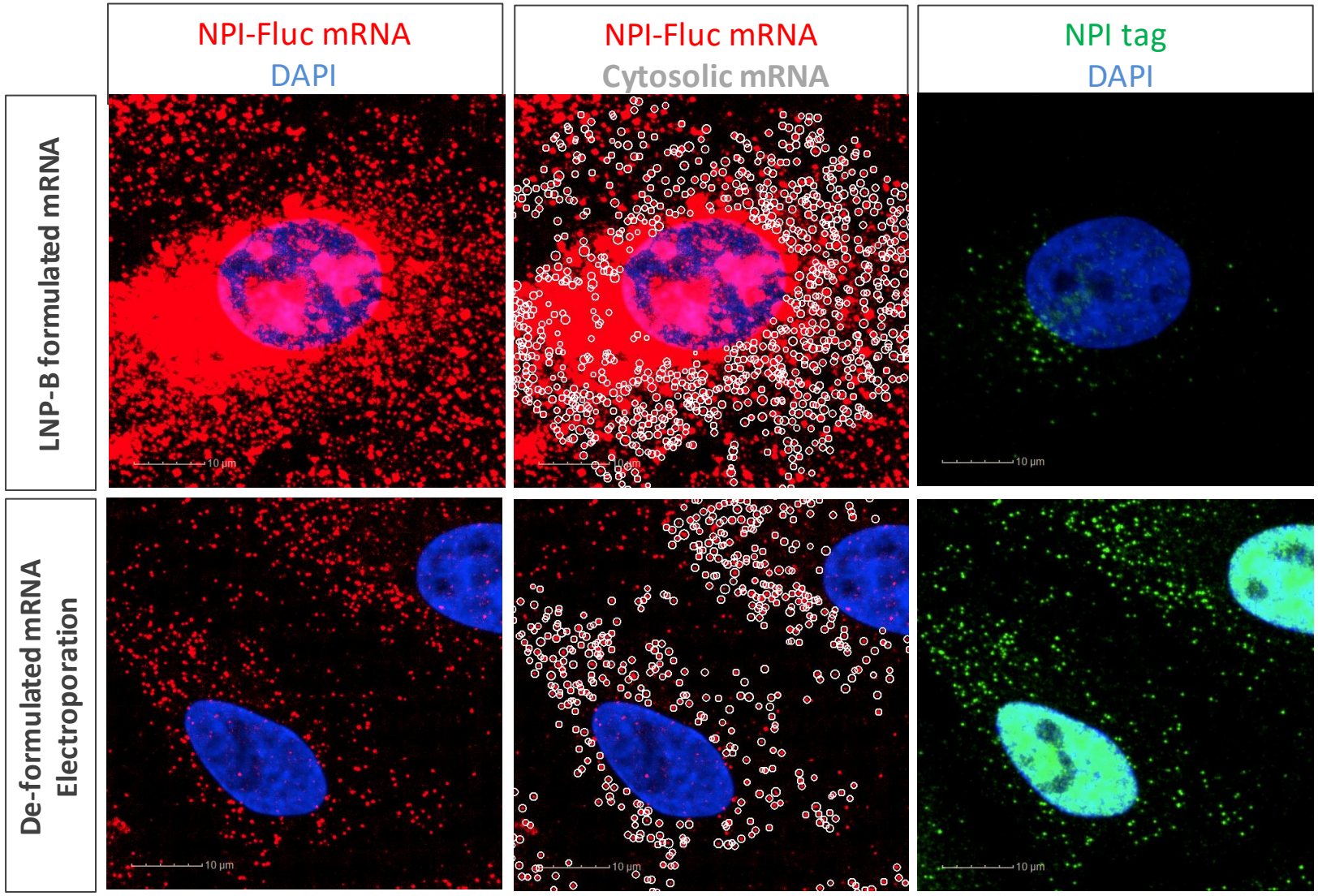
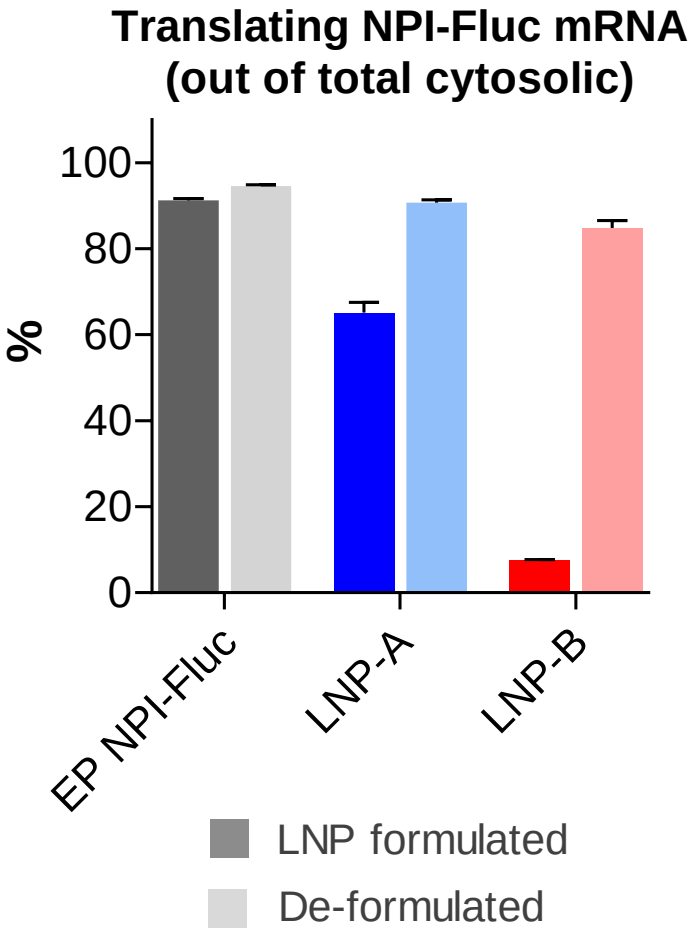
Deformulation rescues translation efficiency





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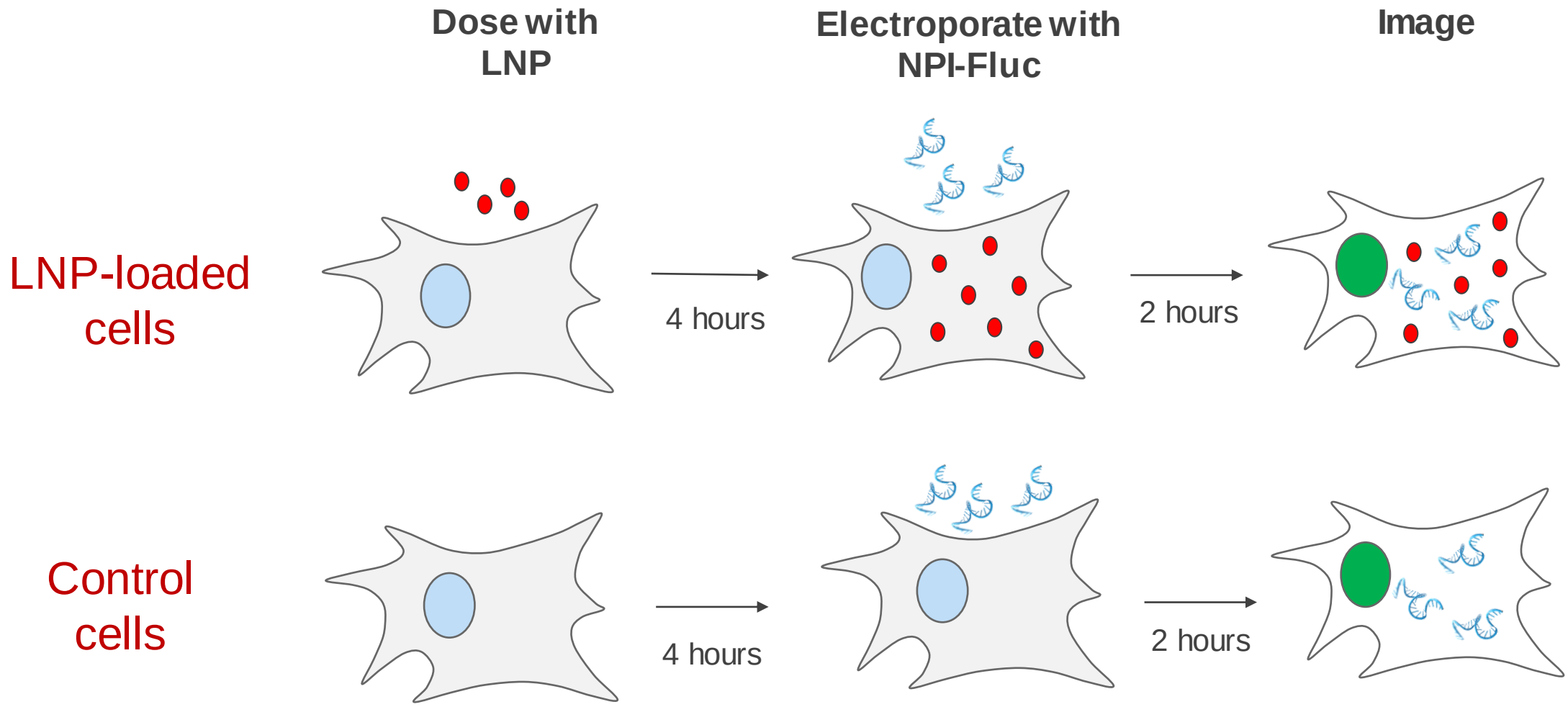
Testing the impact of LNP uptake on global translation



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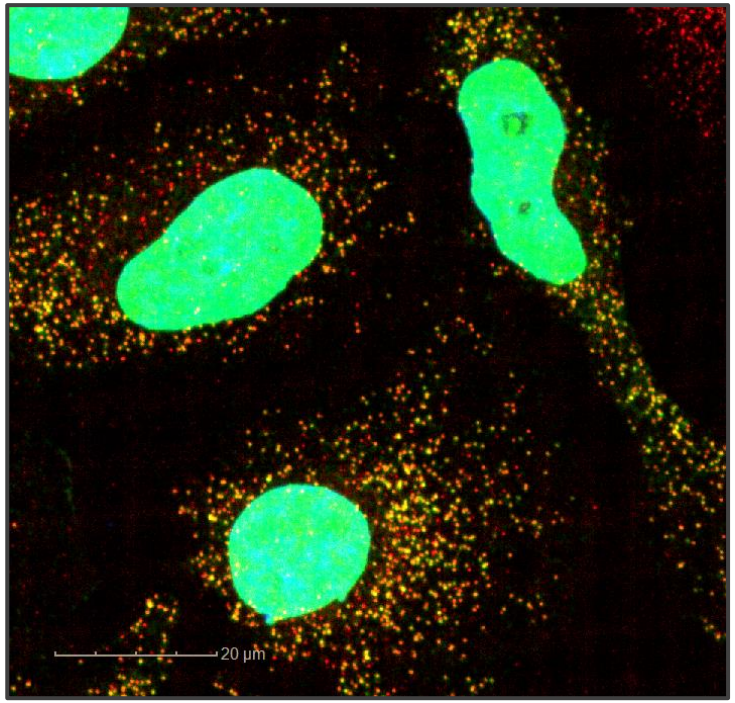
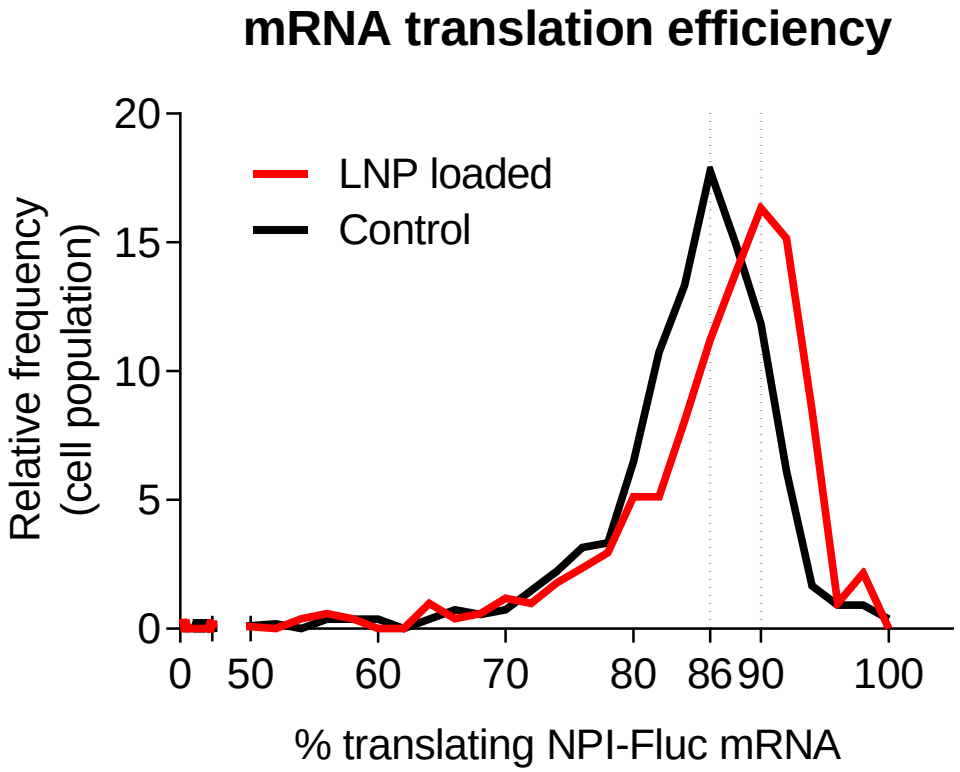
LNP-B does not inhibit global translation

LNP-B loaded cells



NPI FLuc mRNA
NPI Protein



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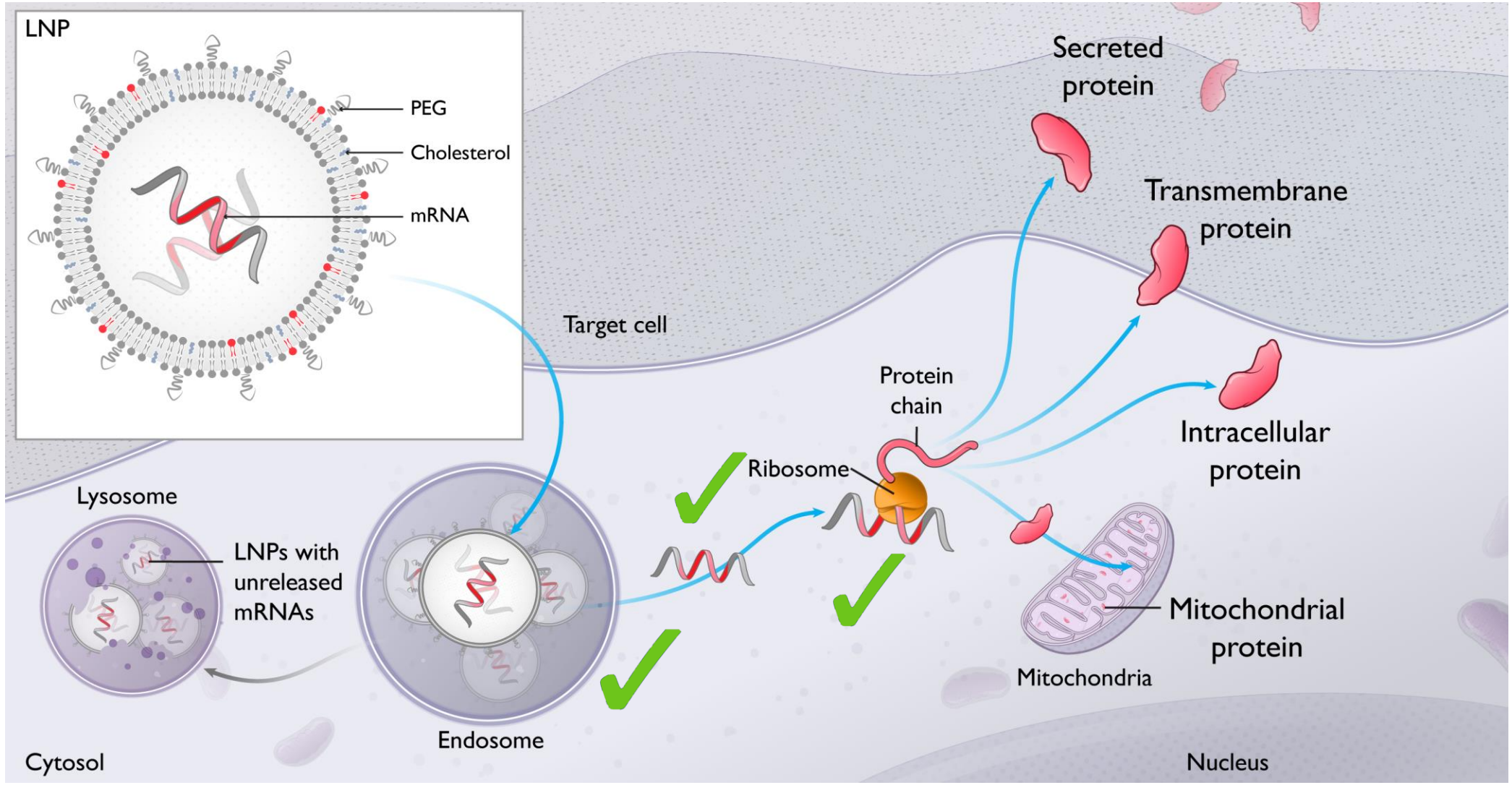
So what was the problem?



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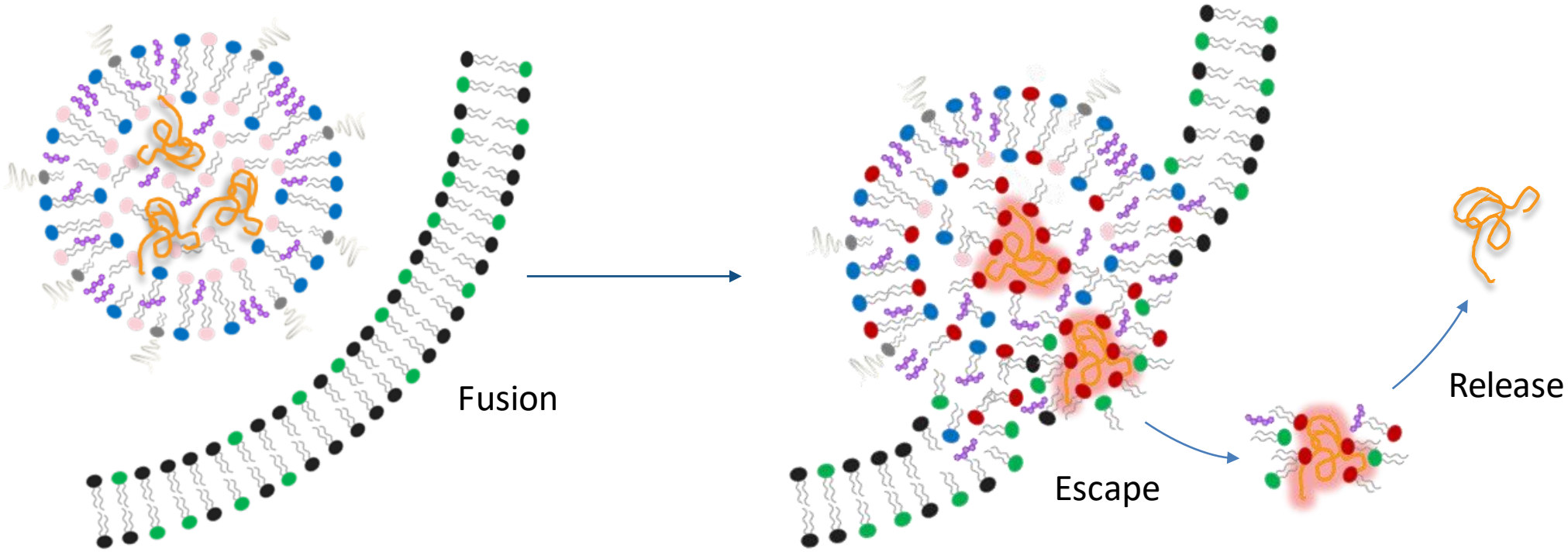
Was LNP-B simply too "sticky"?



Adapted from Degors IMS et al, *Acc. Chem. Res. 2019, 52, 1750−1760*

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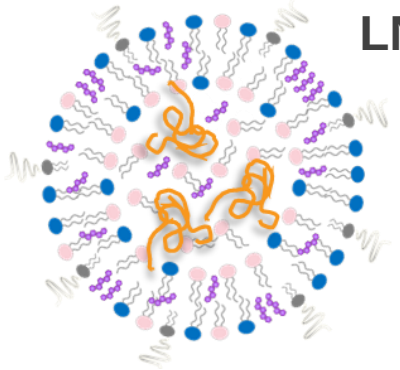
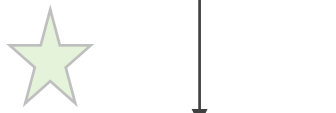
Ribogreen assay measures accessible RNA

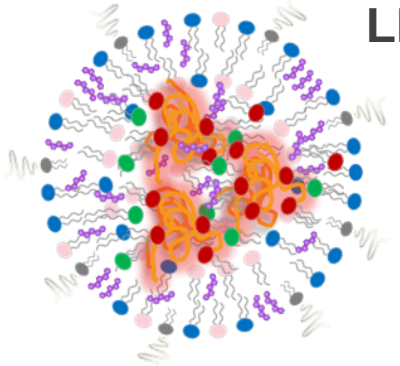


LNP-A

Detergent +
Ribogreen Dye

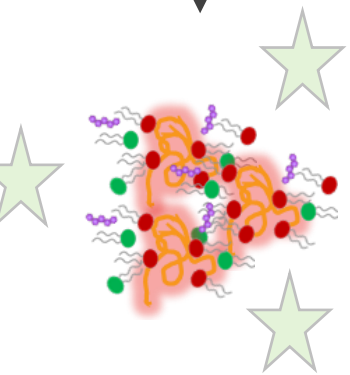


fluorescence

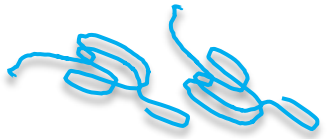


LNP-B

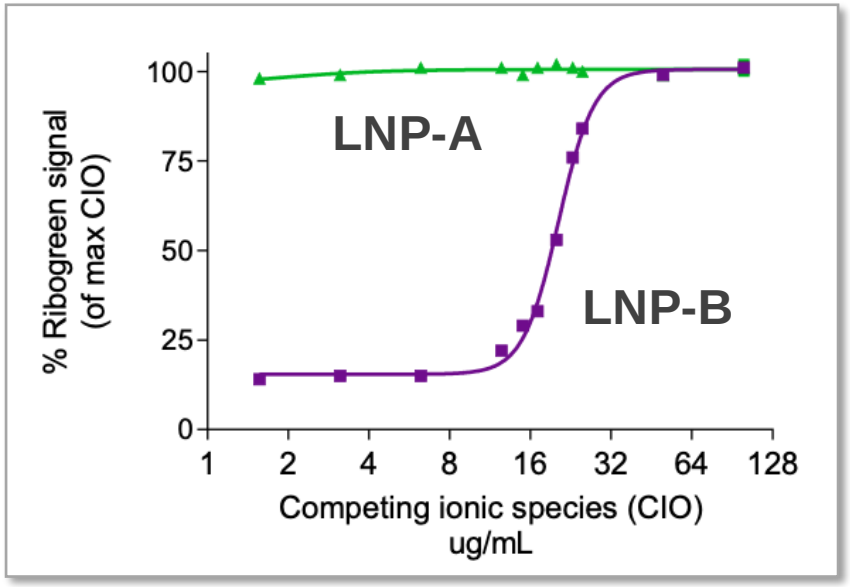
Detergent +
Ribogreen Dye



Titrate in competing
anionic species



fluorescence



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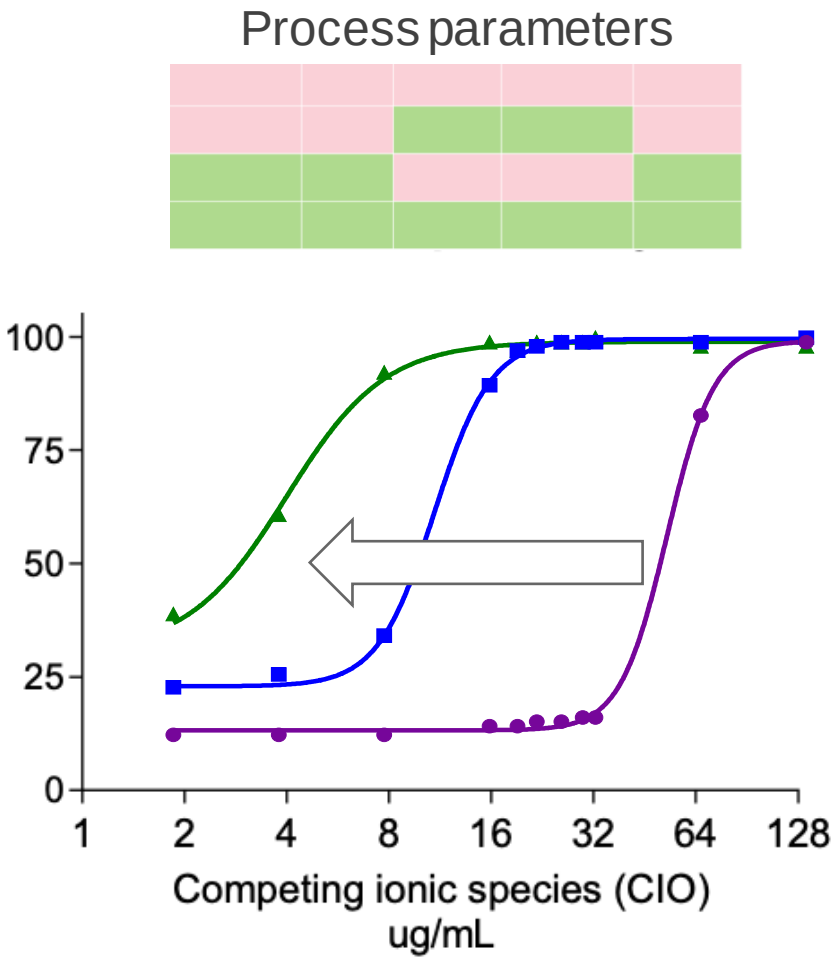
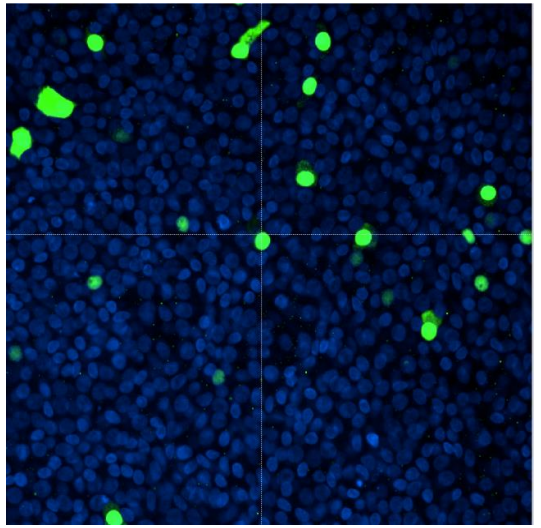
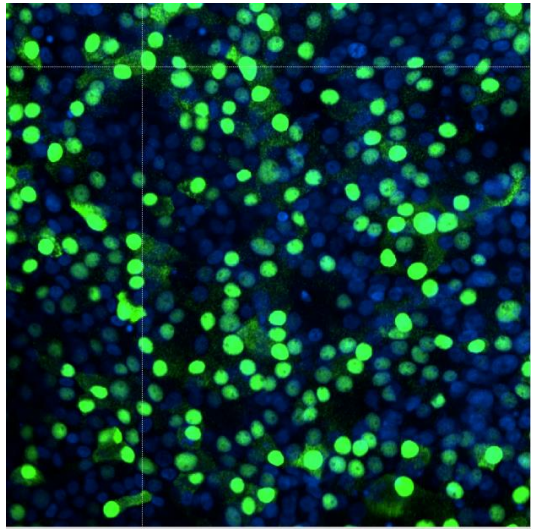
LNP-B "stickiness" could be mitigated by varying LNP assembly process parameters



LNP-B



LNP-C



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Summary

- Single molecule imaging is an invaluable tool for dissecting intracellular events affecting functional mRNA delivery
- In addition to endosomal escape, slow lipid component release can adversely impact functional mRNA delivery
- Our mechanistic elucidation of intracellular events has allowed us to engineer LNPs capable of accessing difficult-to-transfect primary cells with efficient endosomal escape and high functional mRNA delivery

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In vivo mRNA delivery to Hematopoietic Stem and Progenitor Cells

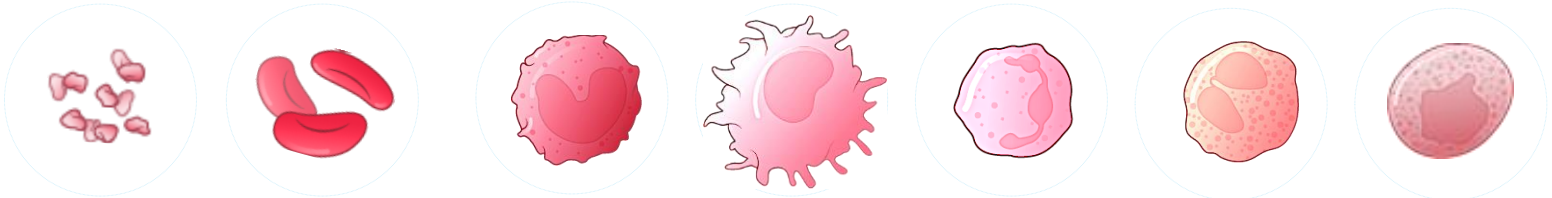
The Hematopoietic Lineage

Clotting & Gas Exchange

Innate Immune System
Our body's first line of defense

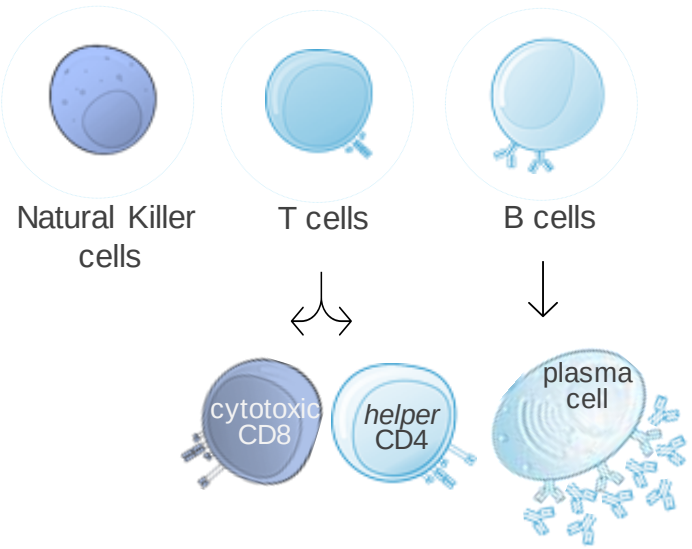
Adaptive Immune System
Immunity and memory

Myeloid cells



platelets | red blood cells | monocytes | macrophages | neutrophils | eosinophils | basophils

Lymphoid cells



Natural Killer cells | T cells | B cells

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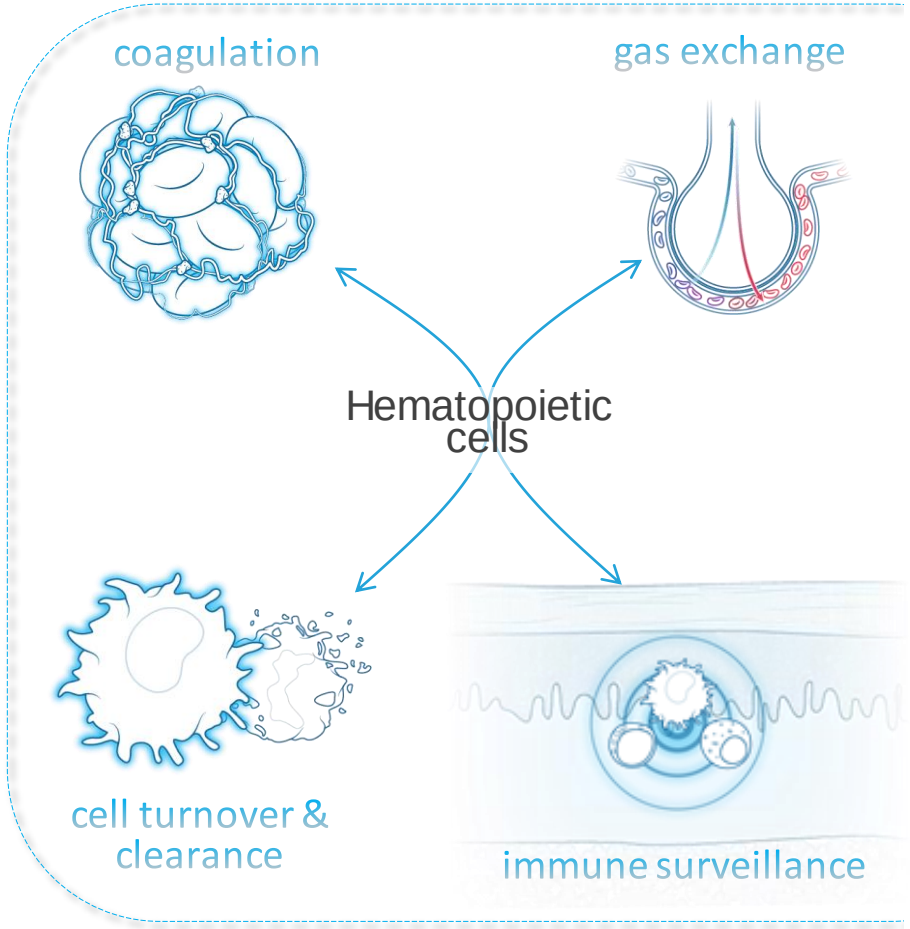
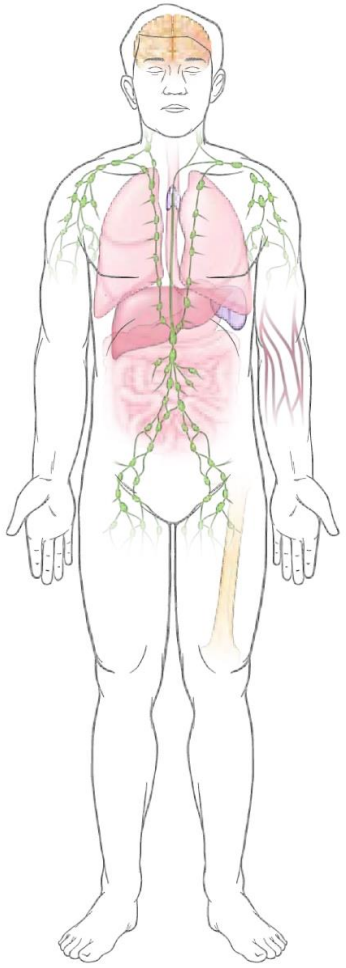
The Hematopoietic Lineage in Health & Physiology

Health & Physiology





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The Hematopoietic Lineage in Inflammation & Disease

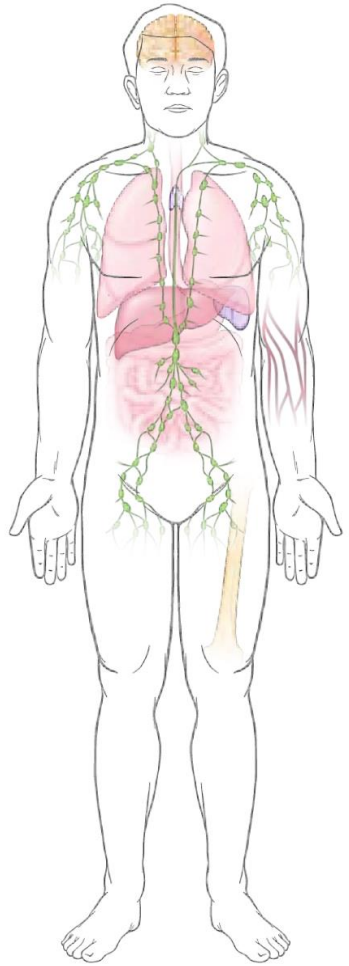
Health & Physiology

coagulation

gas exchange

cell turnover & clearance

immune surveillance



Inflammation & Disease

Hematologic malignancies

type 1 diabetes

atherosclerosis

Hematopoietic cells

demyelination in MS

Asthma & Allergy

Inflammatory bowel disease

Rheumatoid arthritis

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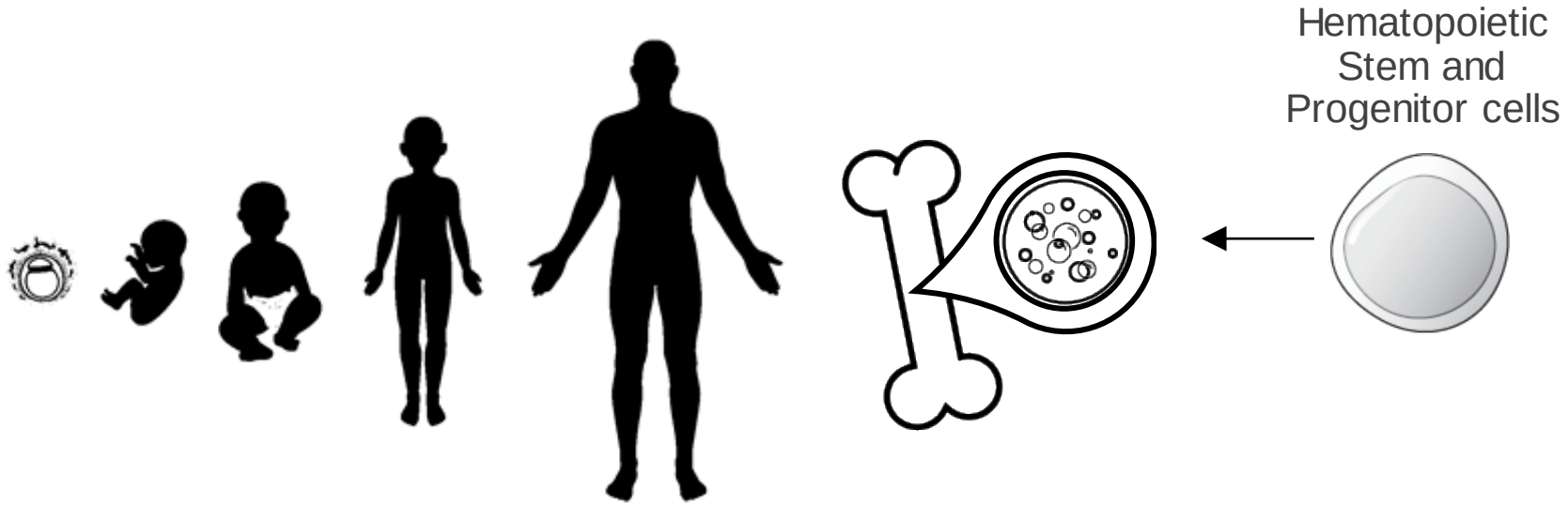
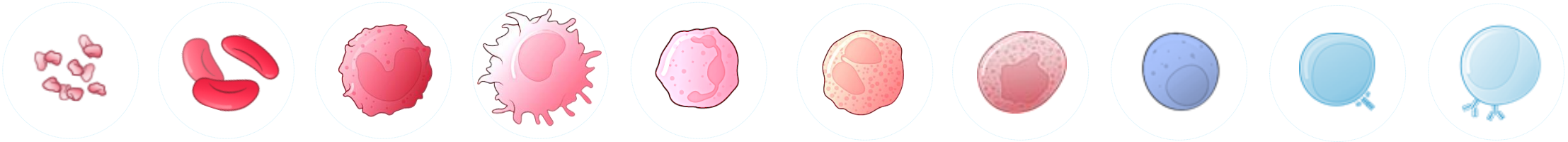
Origins of the Hematopoietic Lineage

Hematopoiesis

- from *Greek haima- ("blood") and -poiesis ("to produce")*





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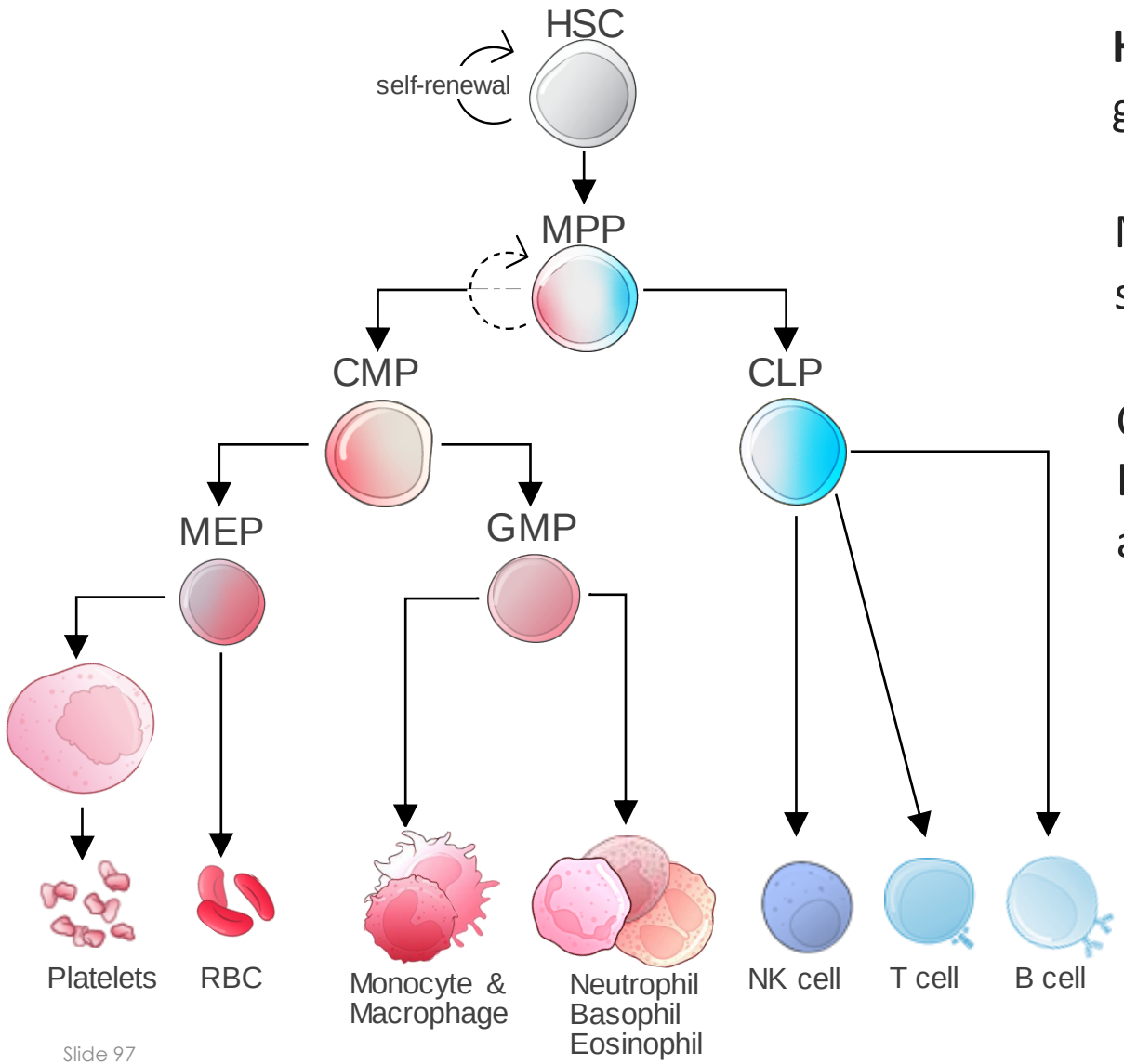
Stem cells at the root of Hematopoiesis



HSC have long-term self-renewal and pluripotency, which give rise to entire the hematopoietic system over a lifetime.

Multipotent Progenitors (**MPP**) are pluripotent with limited self-renewal that can sustain hematopoiesis at steady state.

Common Myeloid Progenitors (**CMP**) and Common Lymphoid Progenitors (**CLP**) differentiate into myeloid and lymphoid lineages, respectively.

Committed precursor cells further specialize into lineage-committed cells, which become restricted to individual cell lineages

Hematopoietic Stem & Progenitor Cells

HSPC

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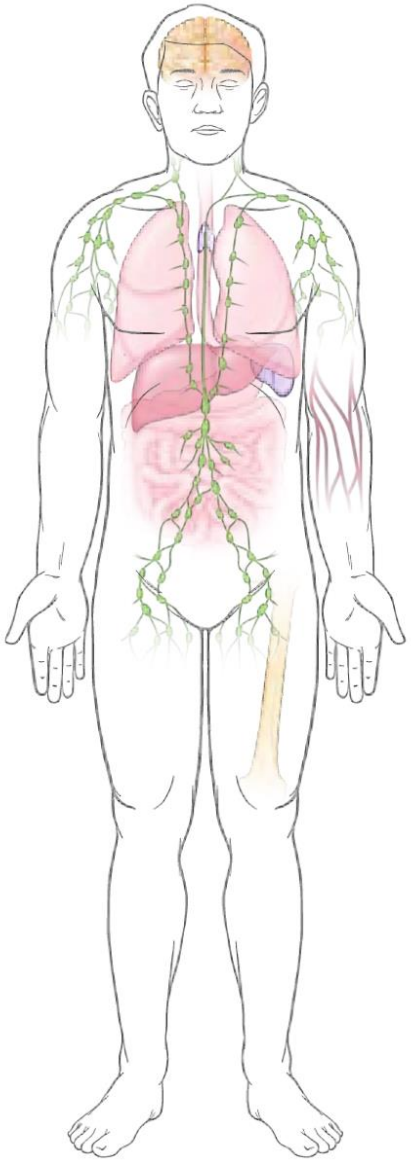
Hematopoiesis by the numbers

~30 trillion cells in the adult human body



Among hematopoietic cells:

~84% red blood cells

~5% platelets

~11% white blood cells

~90% are of hematopoietic origin

Lee-Six … Campbell (2018) *Nature*

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Hematopoiesis: Supply and Demand

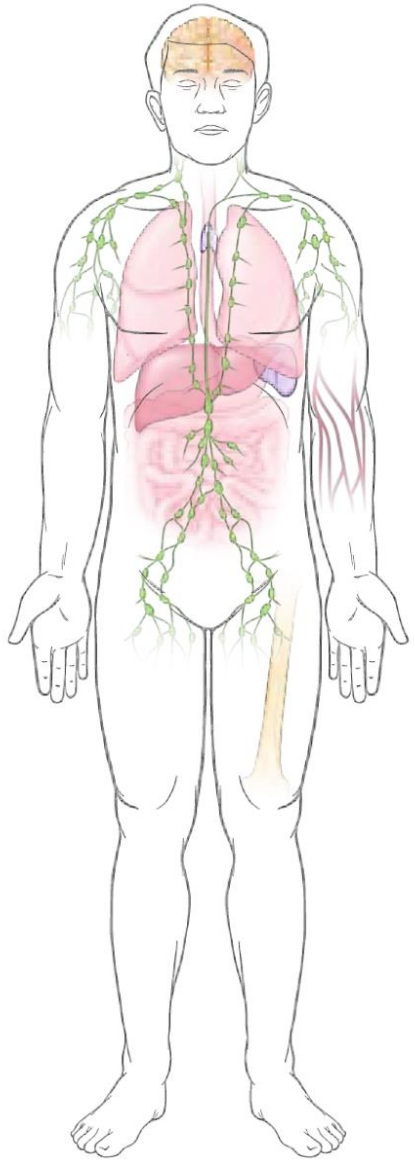
Total turnover rate:

~330 billion

cells per day

~3 million

blood cells per second



50,000-200,000 HSC

actively making white blood cells

Frequency of HSC in the bone marrow

1 in 10,000

Sender … Milo (2021) *Nature Medicine*
Szilvassy … Eaves (1990) *PNAS*
Mcculloch … Till (1960) *Radiation Research*

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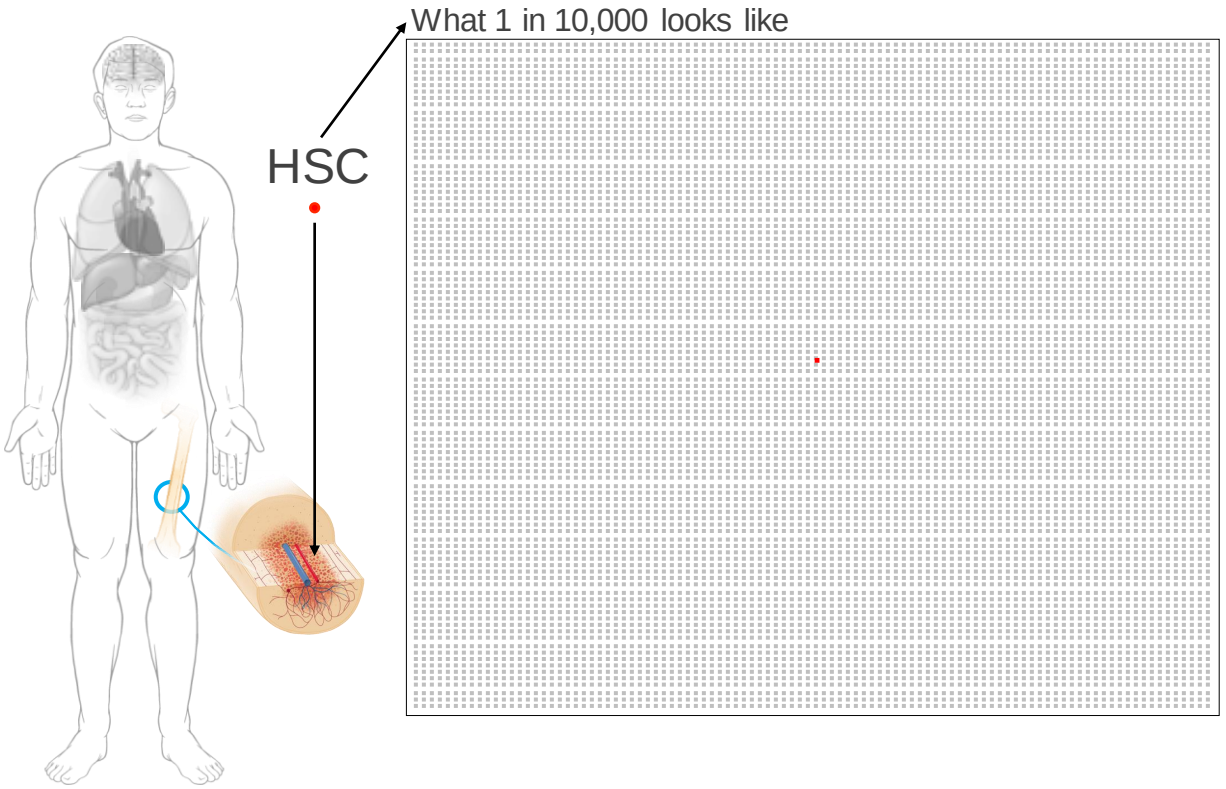
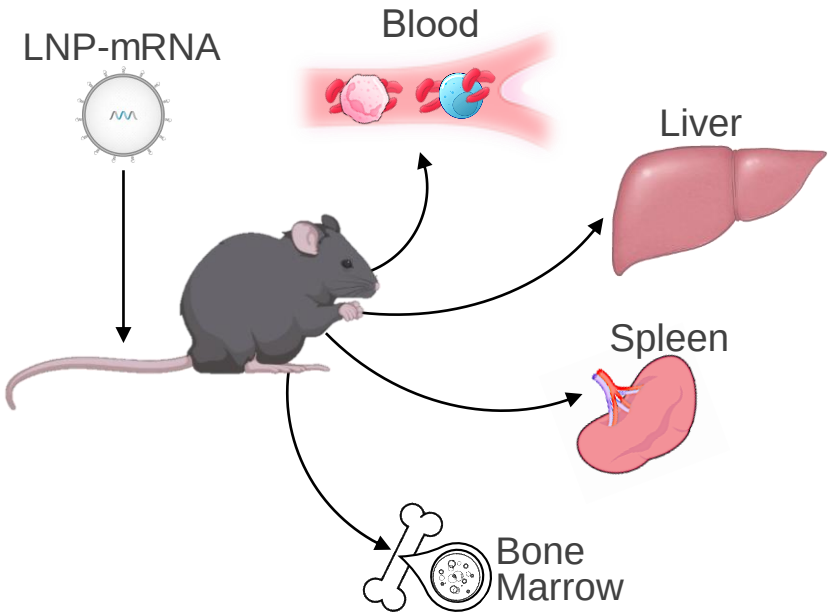
Can we deliver mRNA to HSPCs in vivo?



Scavenging and Sequestration of systemic LNP by circulating blood cells, the liver, and the spleen



created using BioRender

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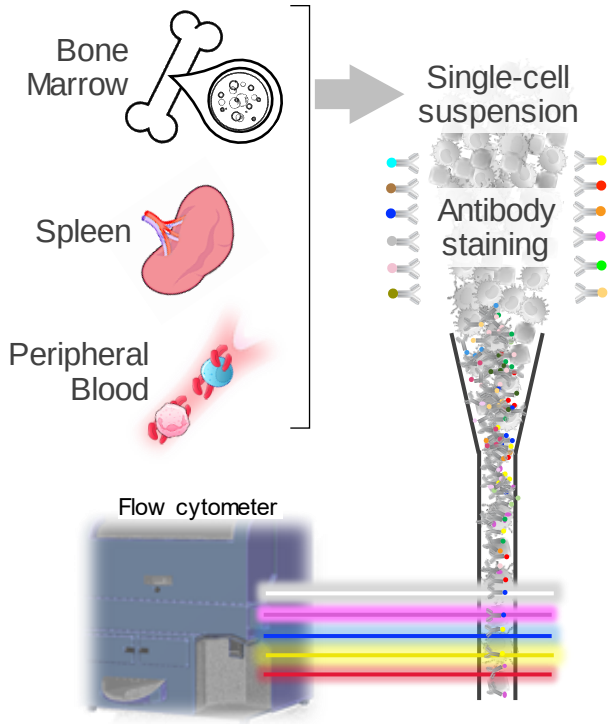
Detection and interrogation of HSPC in vivo

High-throughput multiparameter flow cytometry to interrogate rare HSPCs



- Multiplexing with up to **15** fluorescent labels we can comprehensively phenotype and quantify rare cells
- Interrogate cells at a rate of **10,000 cells per second**
- Accumulate data on over **10 million** cells per sample, and **billions** of cells across studies

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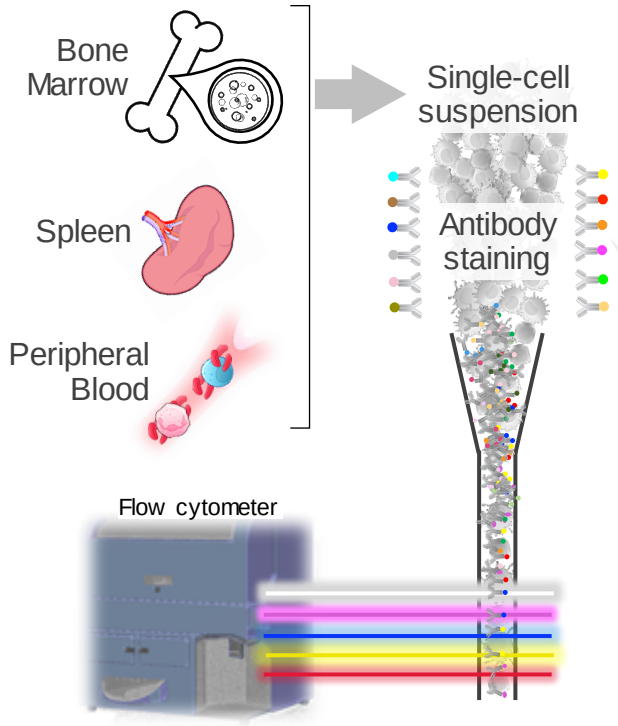
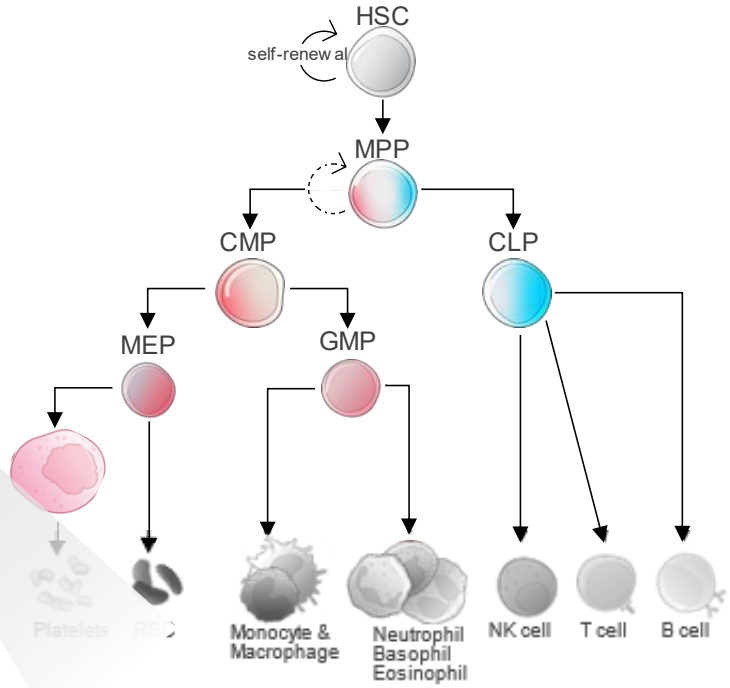
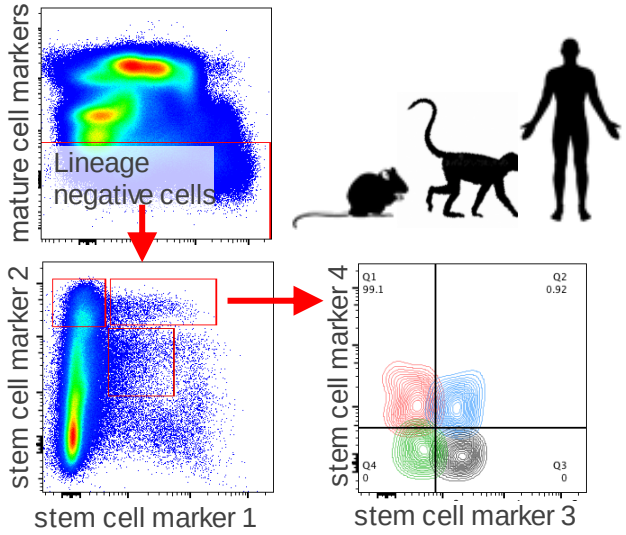
Detection and interrogation of HSPC in vivo

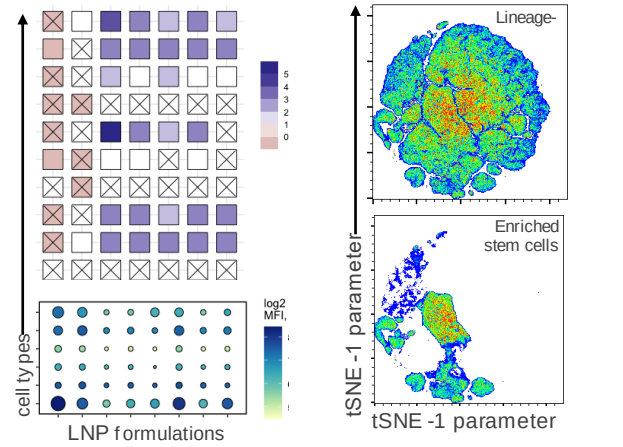
High-throughput multiparameter flow cytometry to interrogate rare HSPCs





Exclusion of mature hematopoietic lineages





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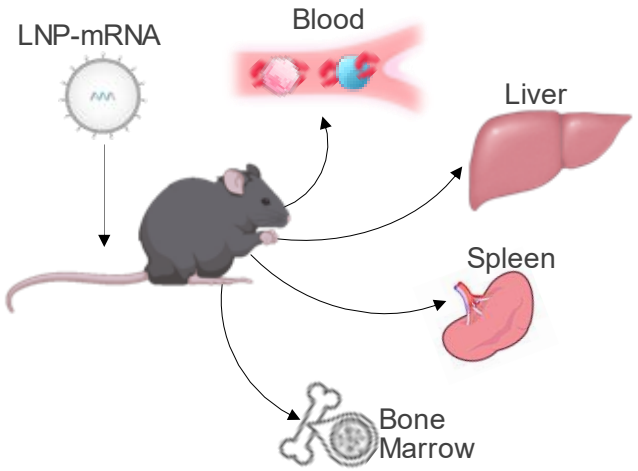
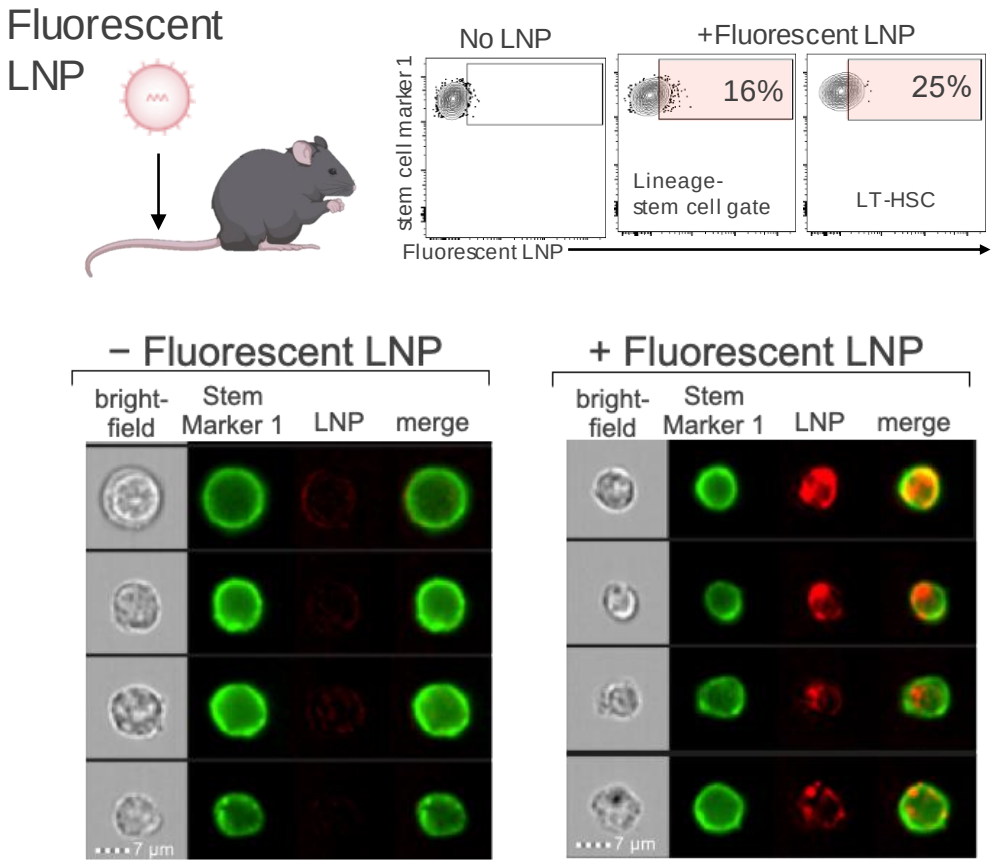
Can we deliver LNP to Hematopoietic Stem Cells in vivo?

Scavenging & Sequestration



Evidence of LNP uptake or association with BM HSPCs?



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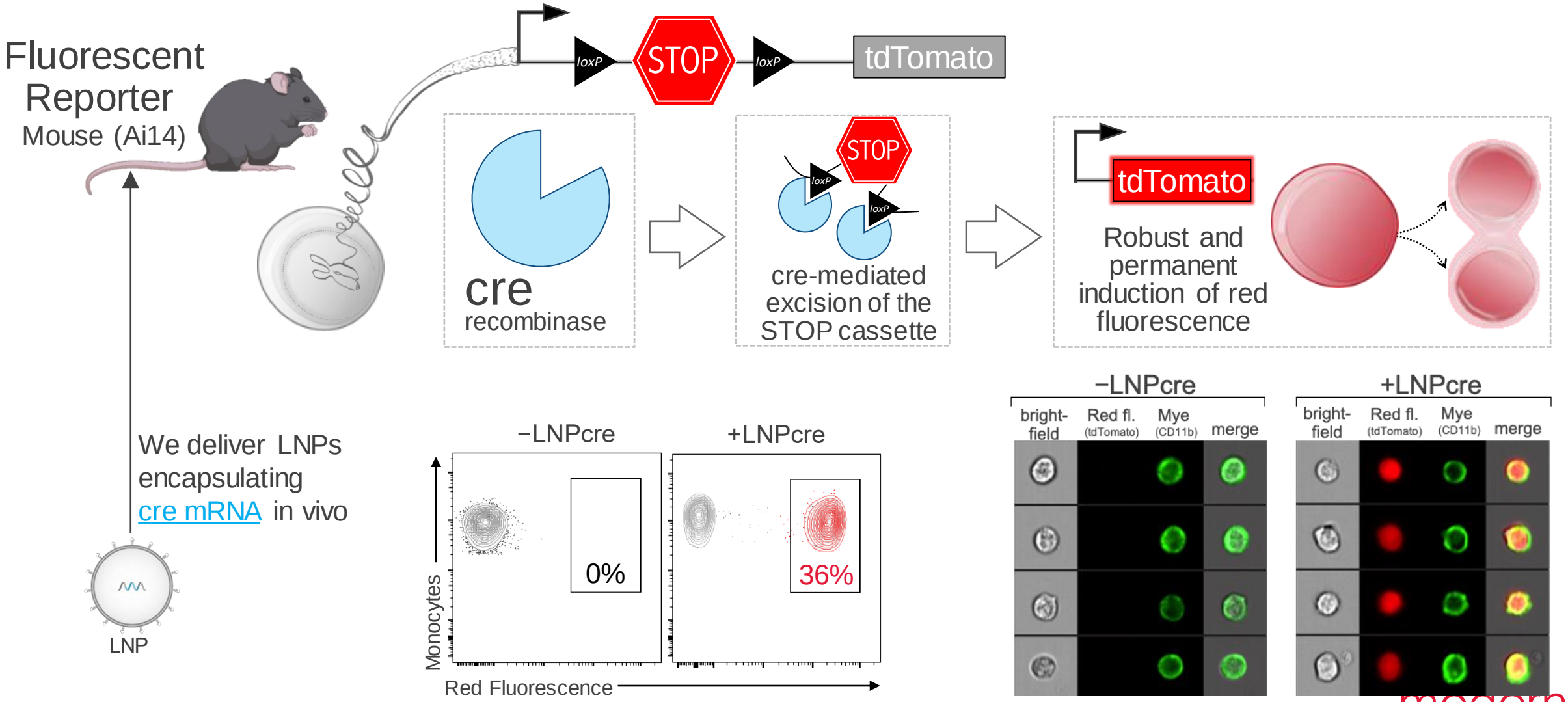
Ai14 mice contain a silent red fluorescent protein gene that can be permanently activated by Cre recombinase



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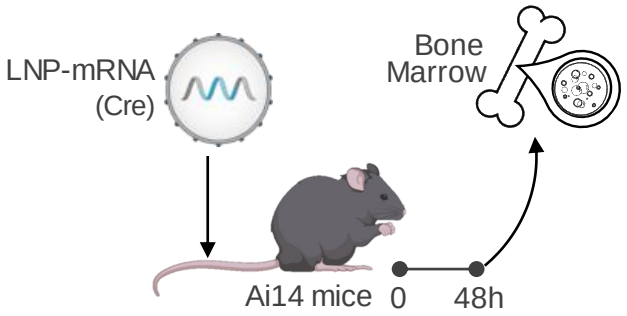
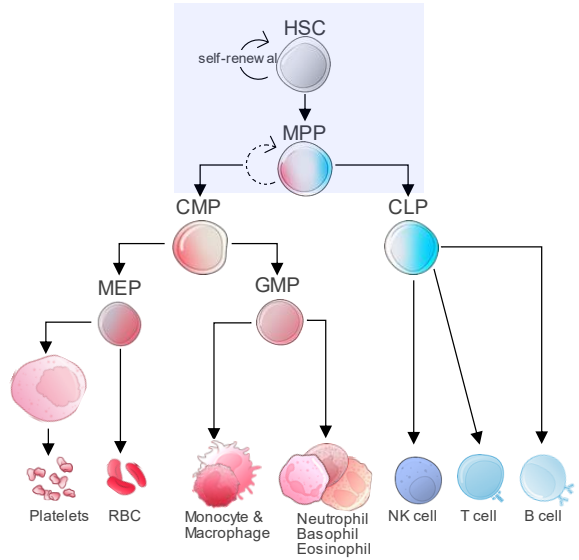
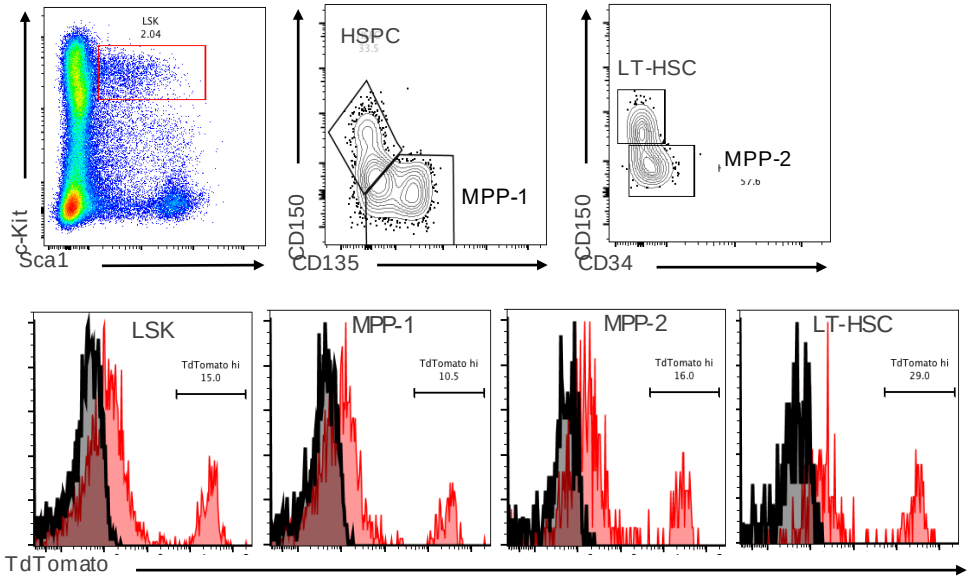
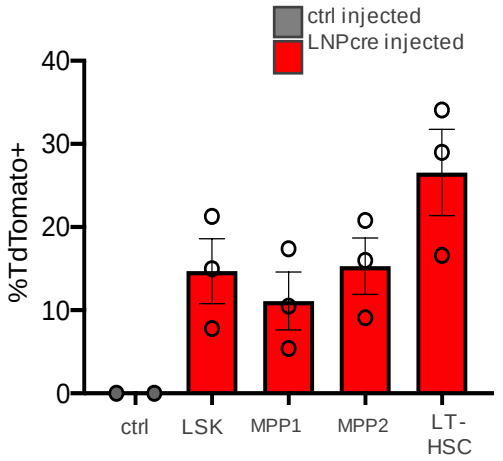
Evidence of mRNA delivery to HSPCs in vivo: Reporter Induction

- Flow cytometry Analysis of Stem Cell Populations following LNPcre delivery in vivo









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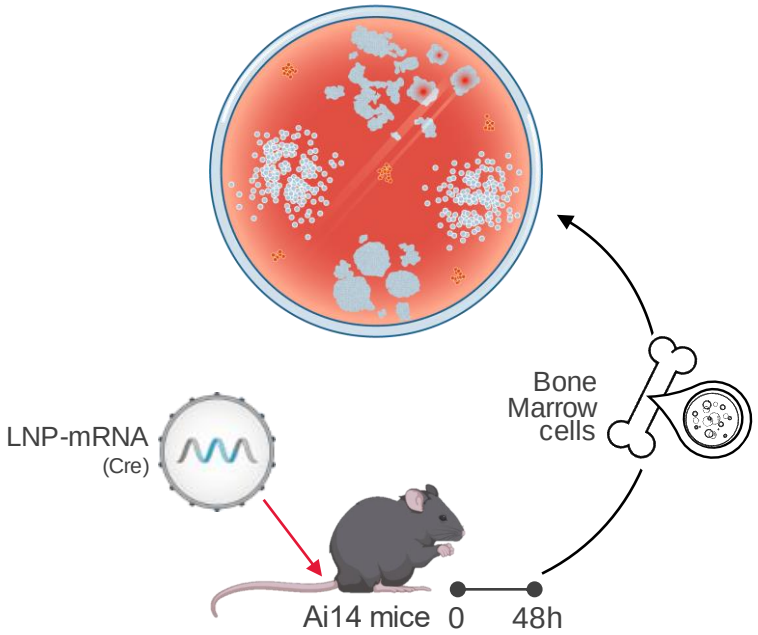
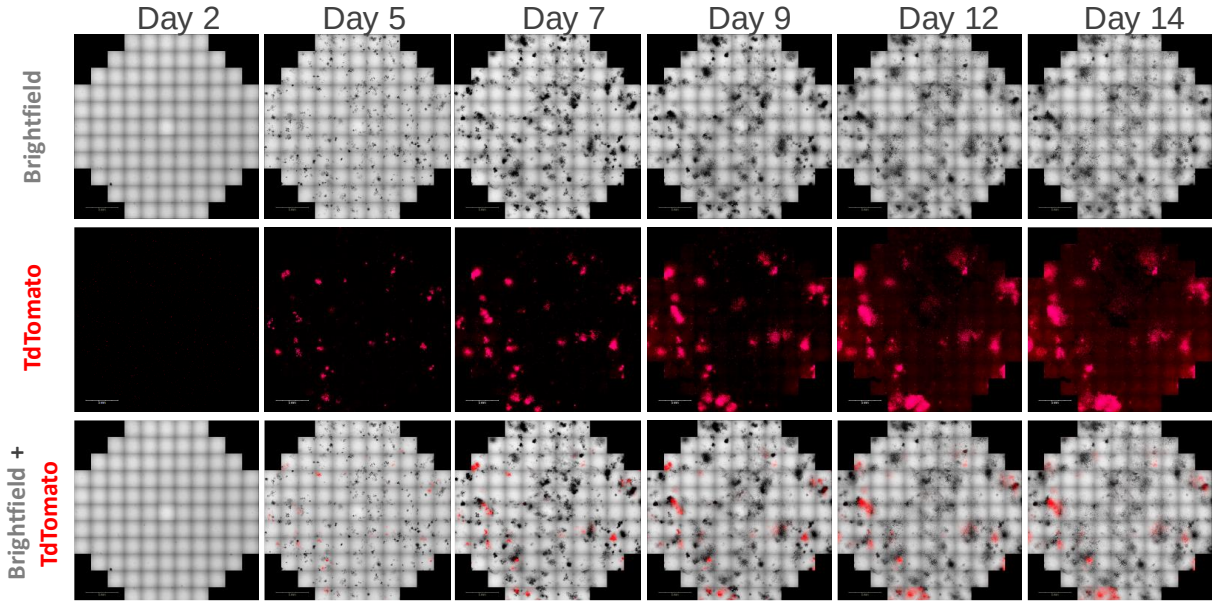
Evidence of mRNA delivery to HSPCs in vivo: Proliferative Capacity

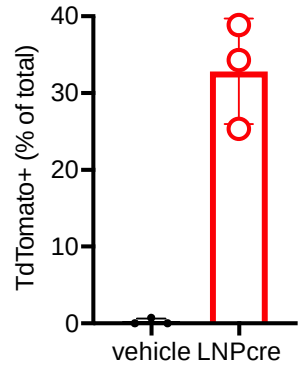
Testing for proliferative potential in transfected-bone marrow cells:

- Colony Forming Unit (CFU) Assays

Stem cells exhibit proliferative potential & can give rise to colonies when grown in growth factor rich-media







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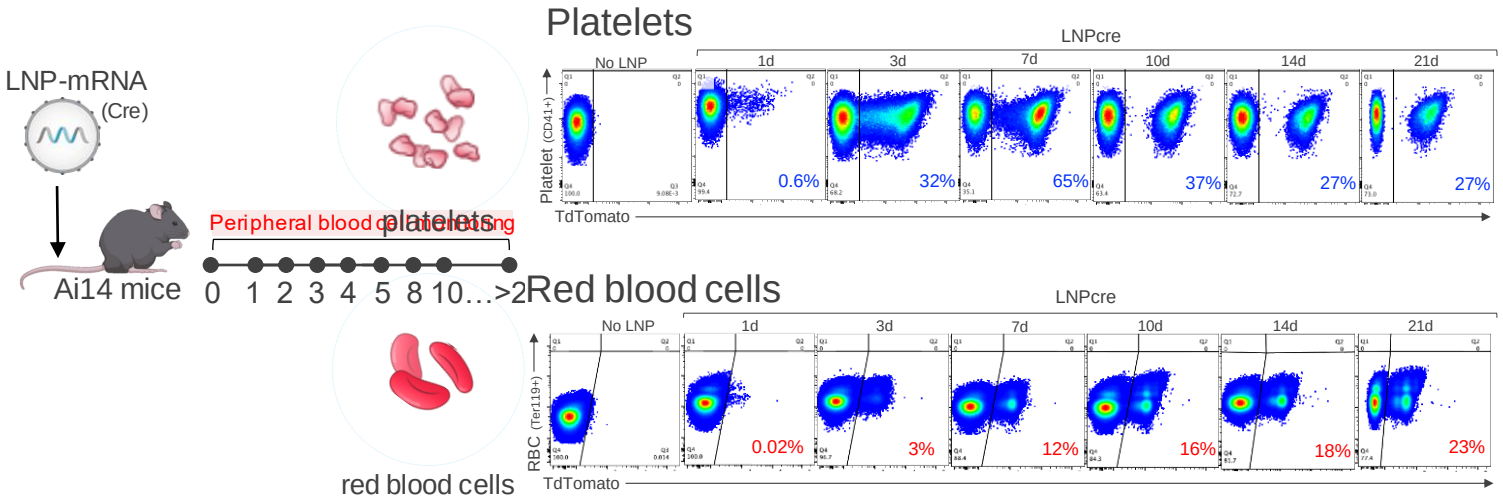
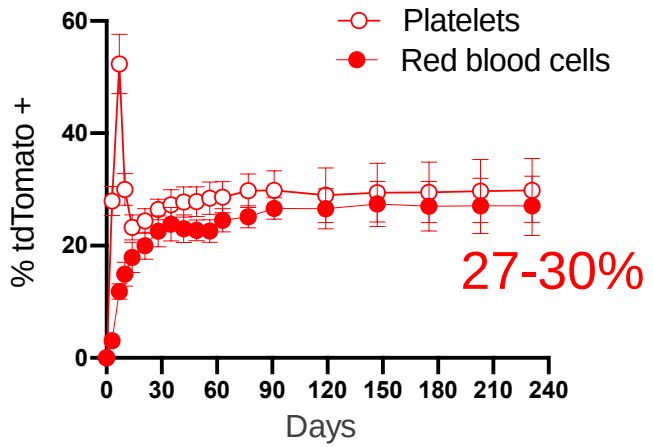
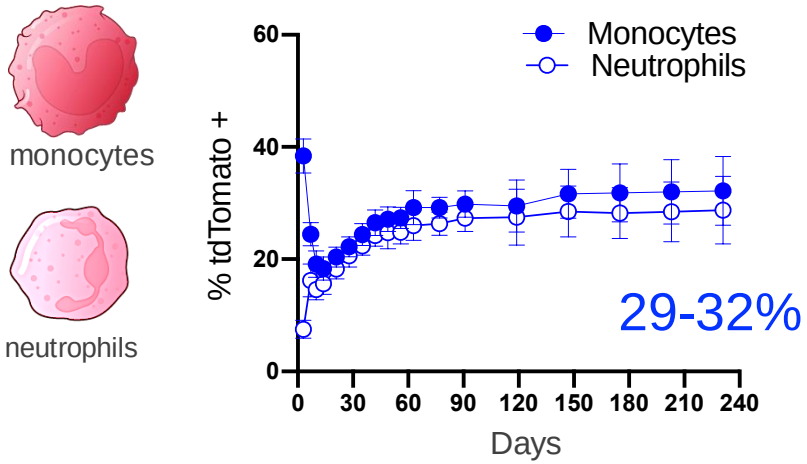
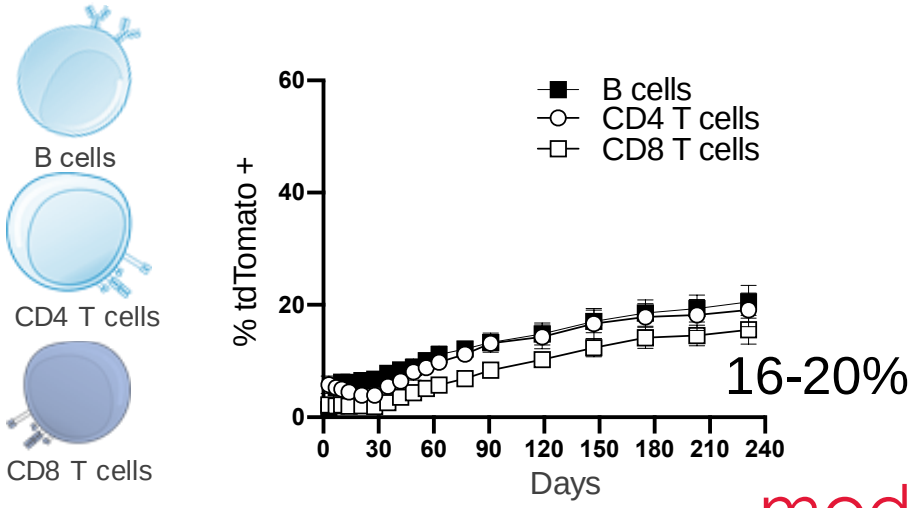
Evidence of mRNA delivery to HSPCs in vivo: Lineage Tracing









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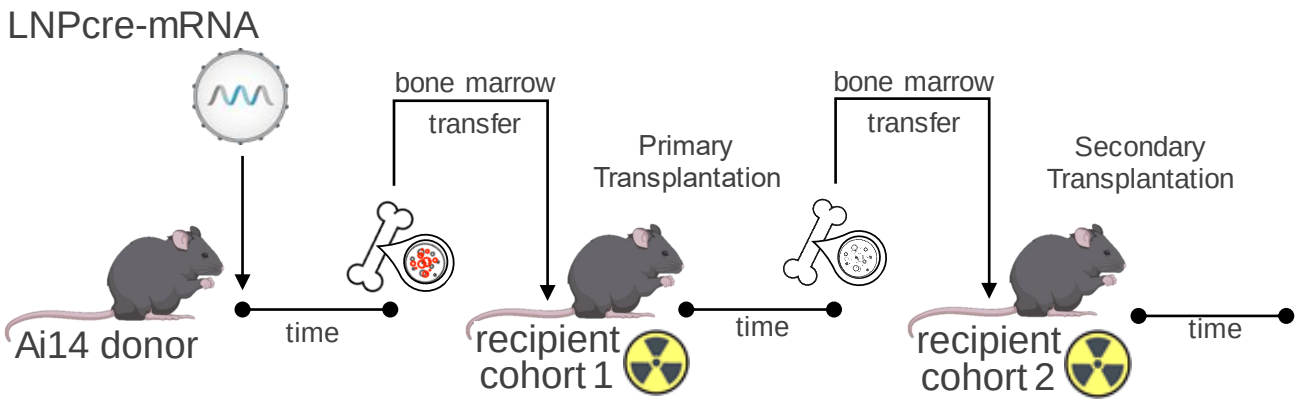
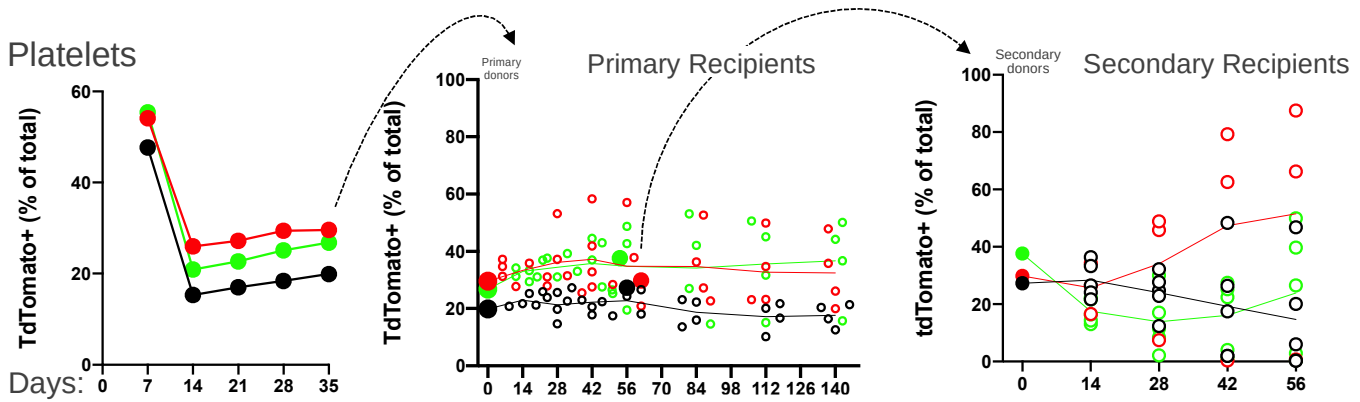
Evidence of mRNA delivery to HSPCs in vivo: Serial Transplantation



- Engraftment success
- Look for red fluorescence in all downstream hematopoietic lineages



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Recipients

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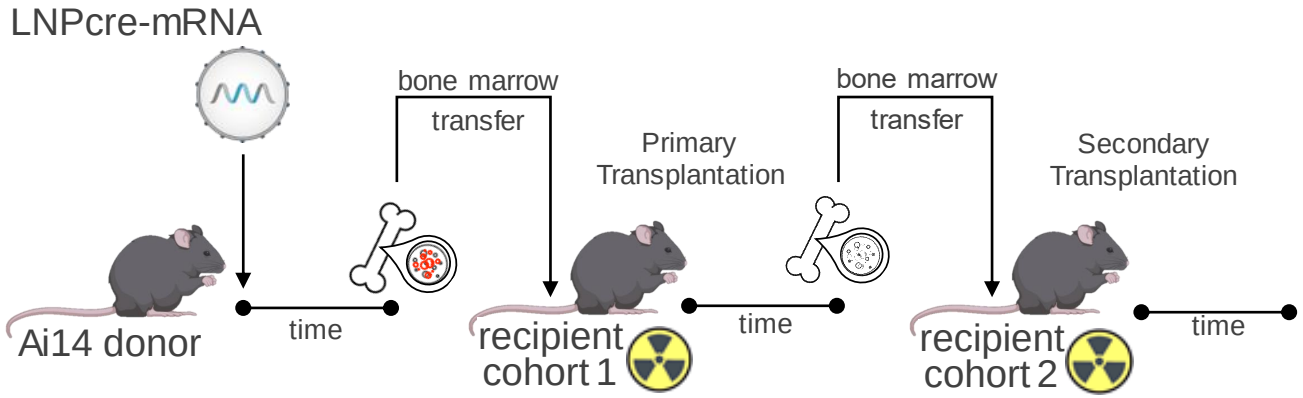
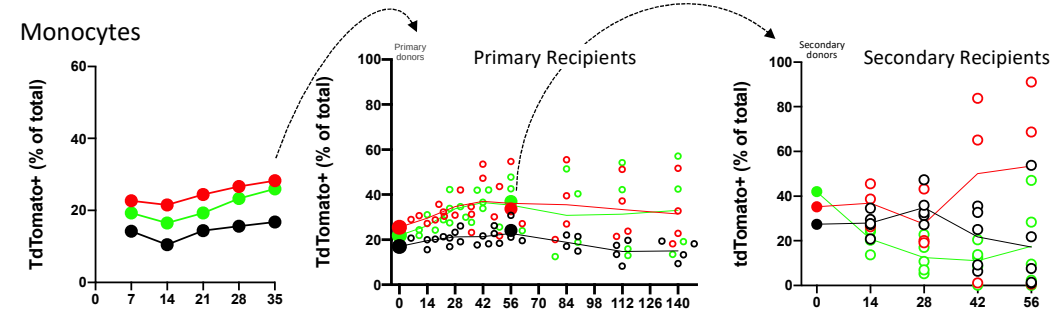
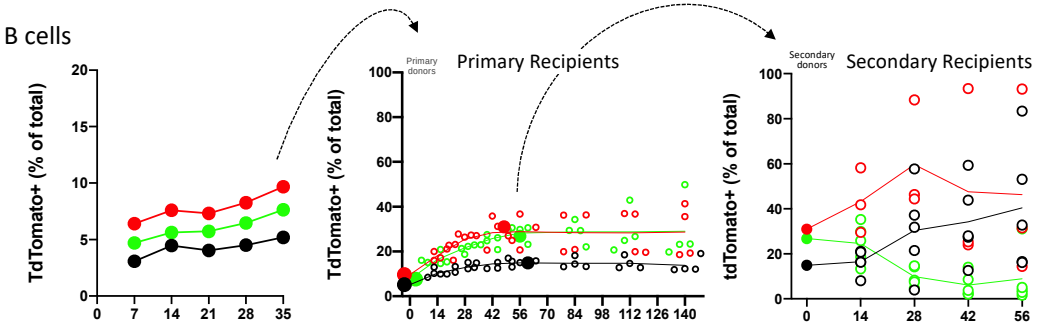
Evidence of mRNA delivery to HSPC in vivo: LT-HSC transfection







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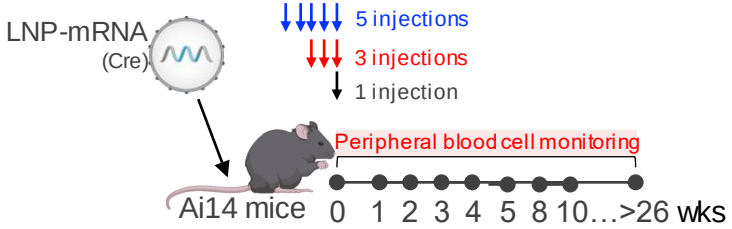
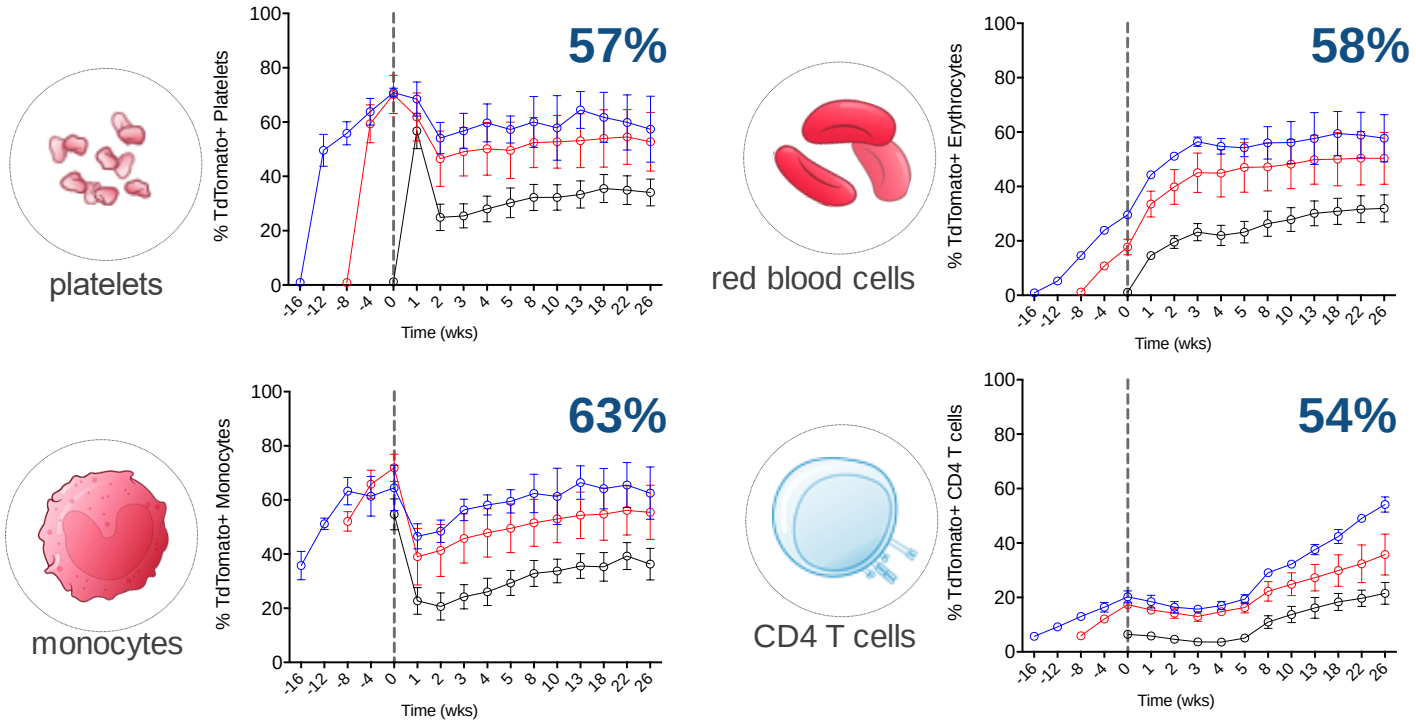
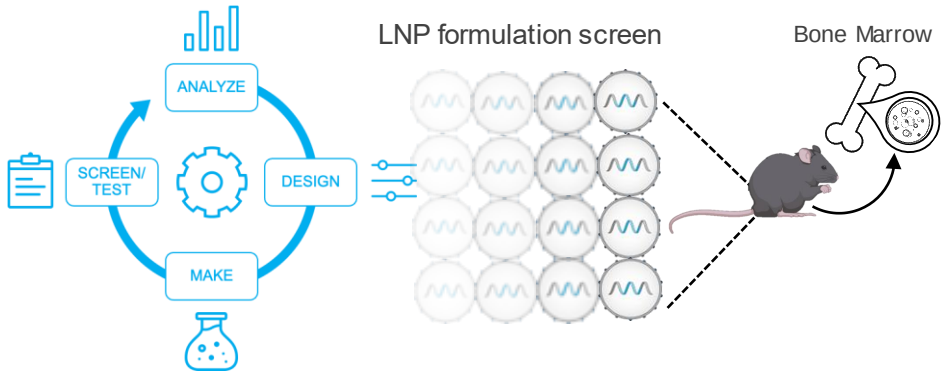
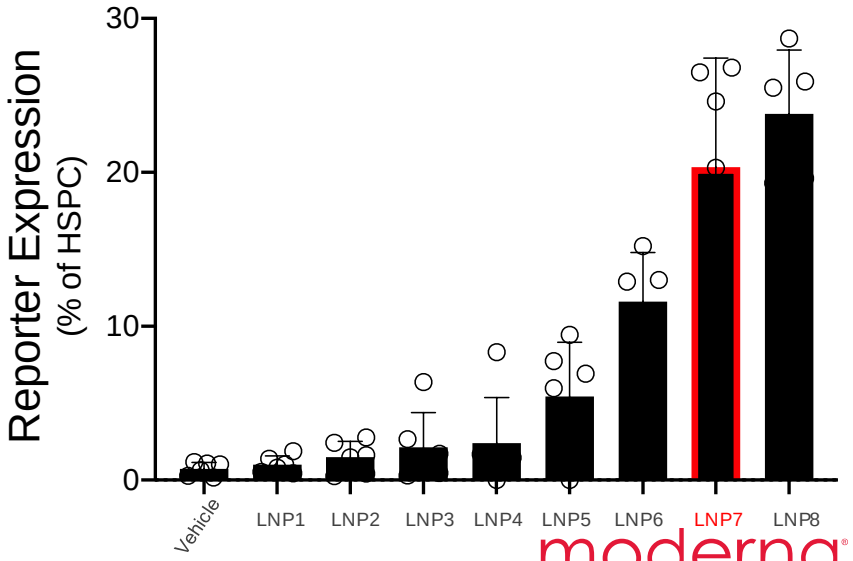
Enhancing mRNA delivery to HSPC through repeat dosing and different LNP formulations





- LNP Design, Formulation, and Screening

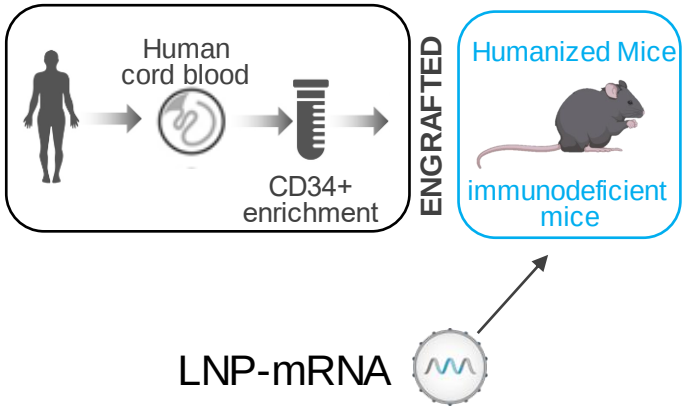


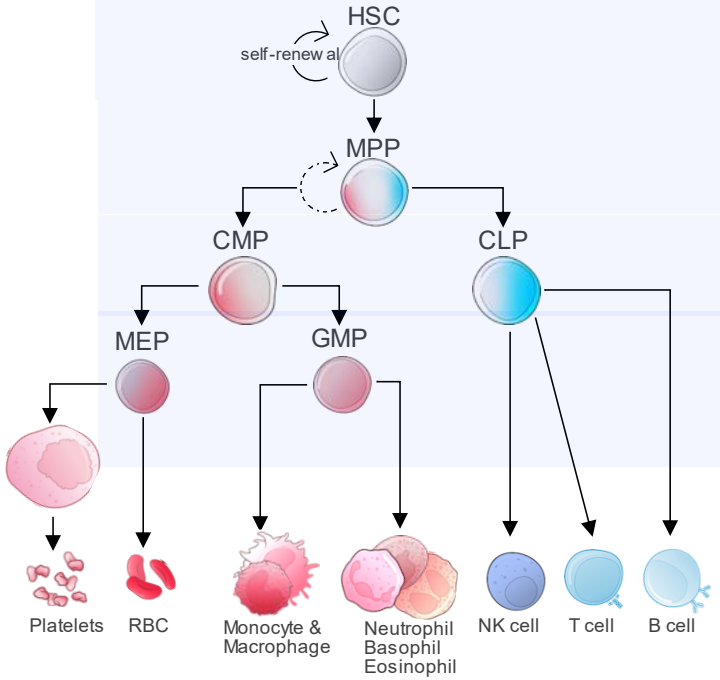


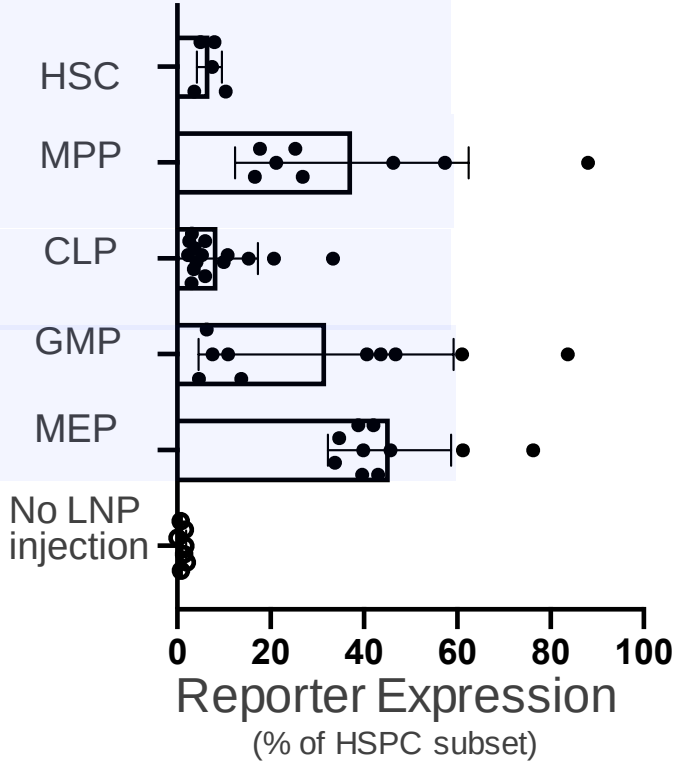
mRNA Delivery to Human HSPC

Can we deliver LNP-mRNA to human HSPC ?

Using humanized mice to study human cell populations in vivo







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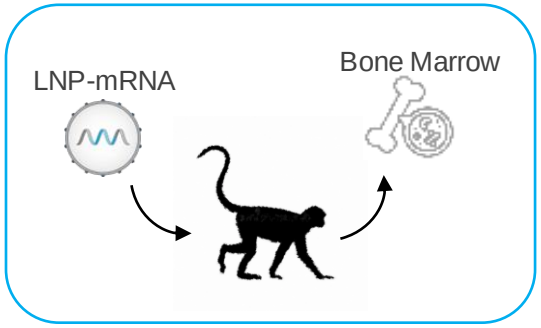
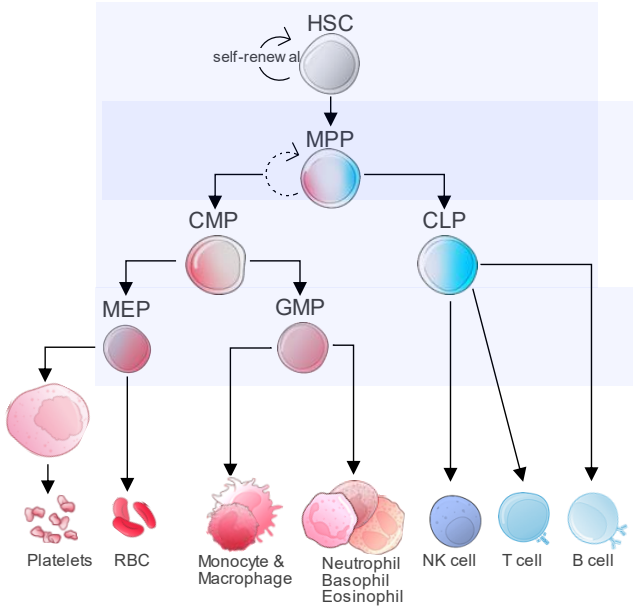
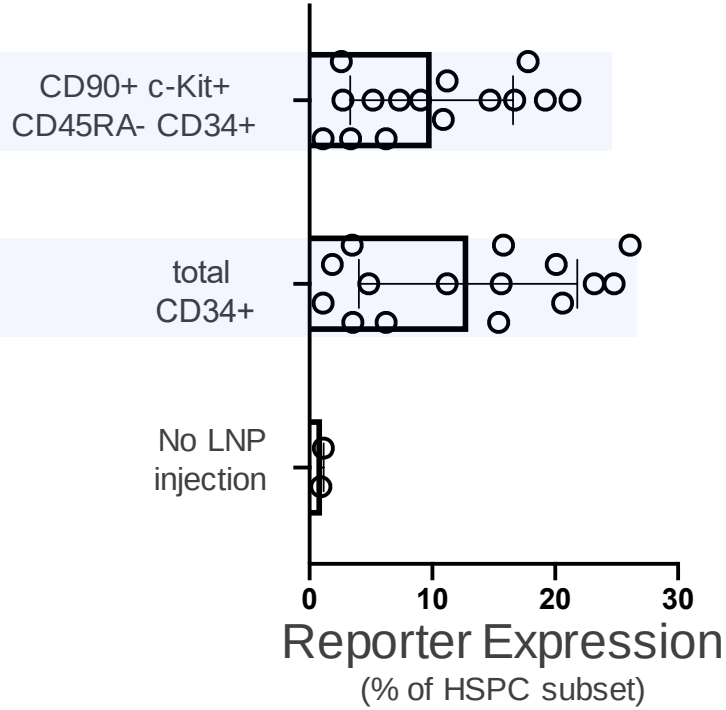
mRNA Delivery to HSPC in Non-human Primates

Can we deliver LNP-mRNA to Non-Human Primate HSPC in vivo?







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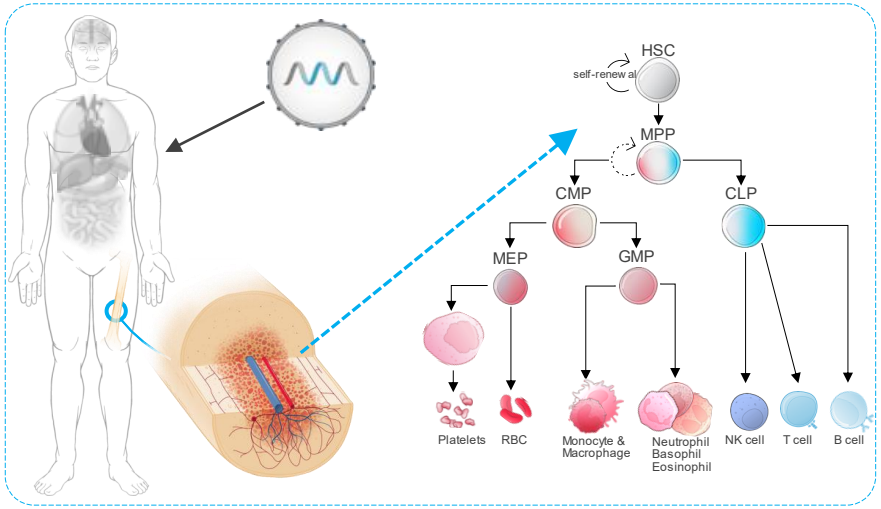
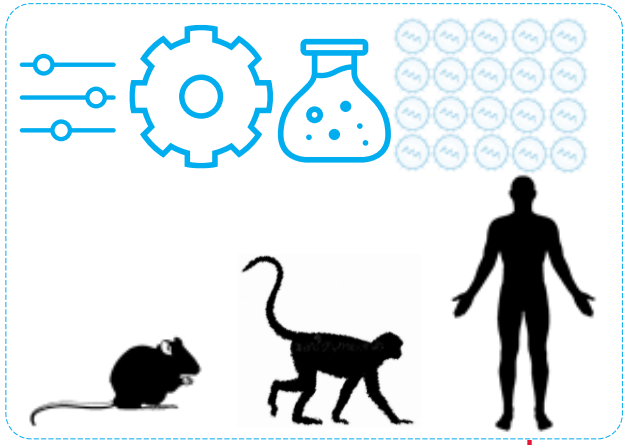
Summary

- We have established in vivo mRNA delivery to the bone marrow, leading to HSPC transfection and long-term modulation of all hematopoietic lineages



- Different LNP formulations and repeat dosing can enhance transfection of mouse and NHP bone marrow HSPC, as well as human HSPC in humanized-mouse model systems



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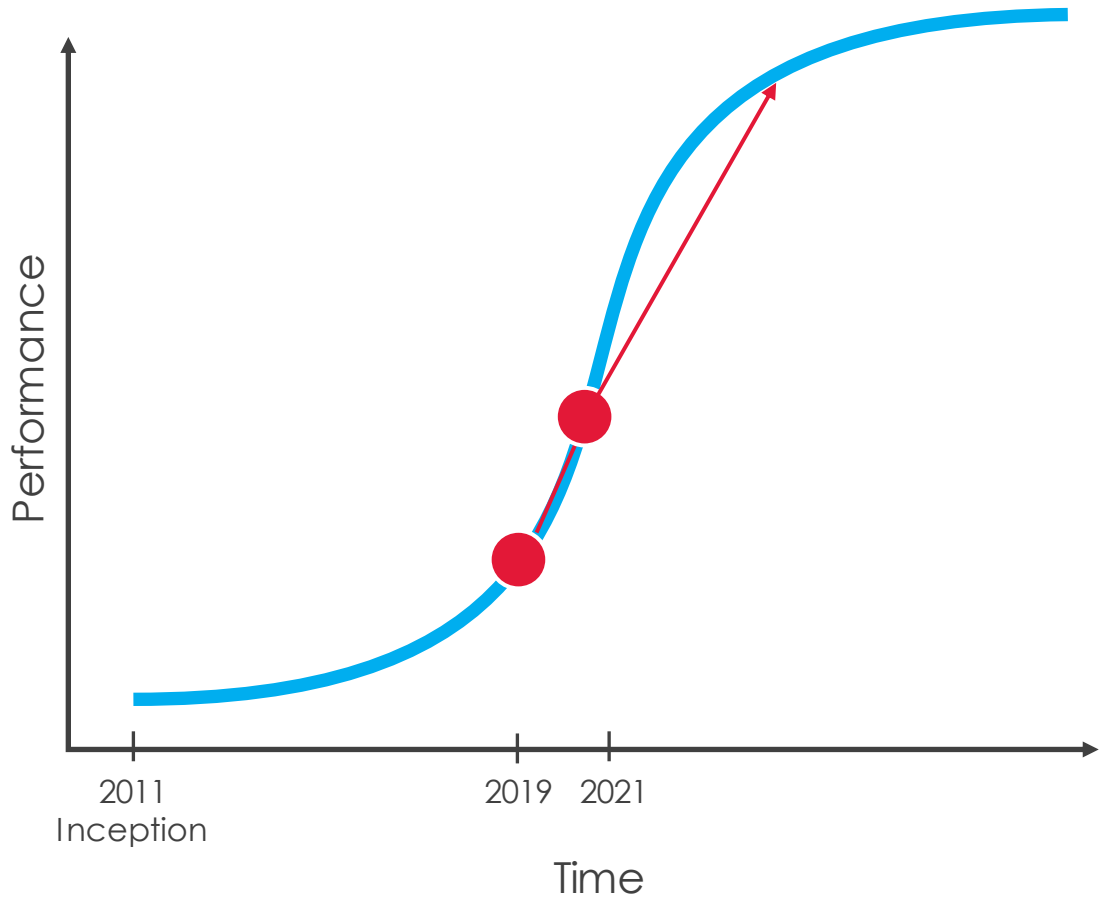
Our commitment to be the best at mRNA science is core to who we are: A 20-year journey



- **10x mentality**: We know our process works and aim to scale it out to other programs
- **Process optimization**: Use data analytics, machine learning, AI and robotics
- **Moderna's competitive advantages:**
  - Platform research investments for 10+ years
  - Infrastructure (digital backbone + manufacturing plants)
  - Structured data collection and ability to leverage it
- **Largest scale in the mRNA industry**

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Q&A