# Reflections on mRNA-LNP Systems for New Biologics

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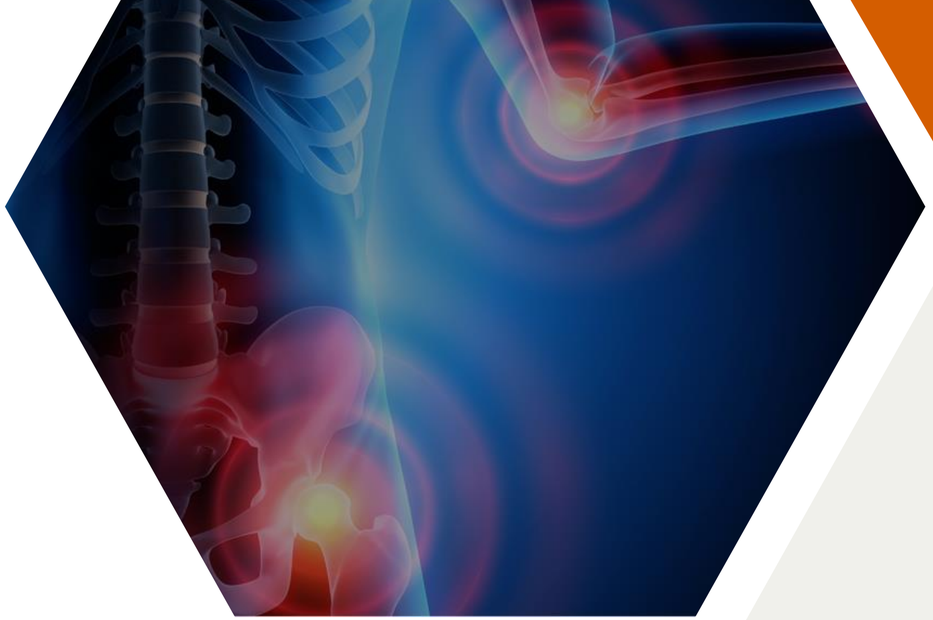
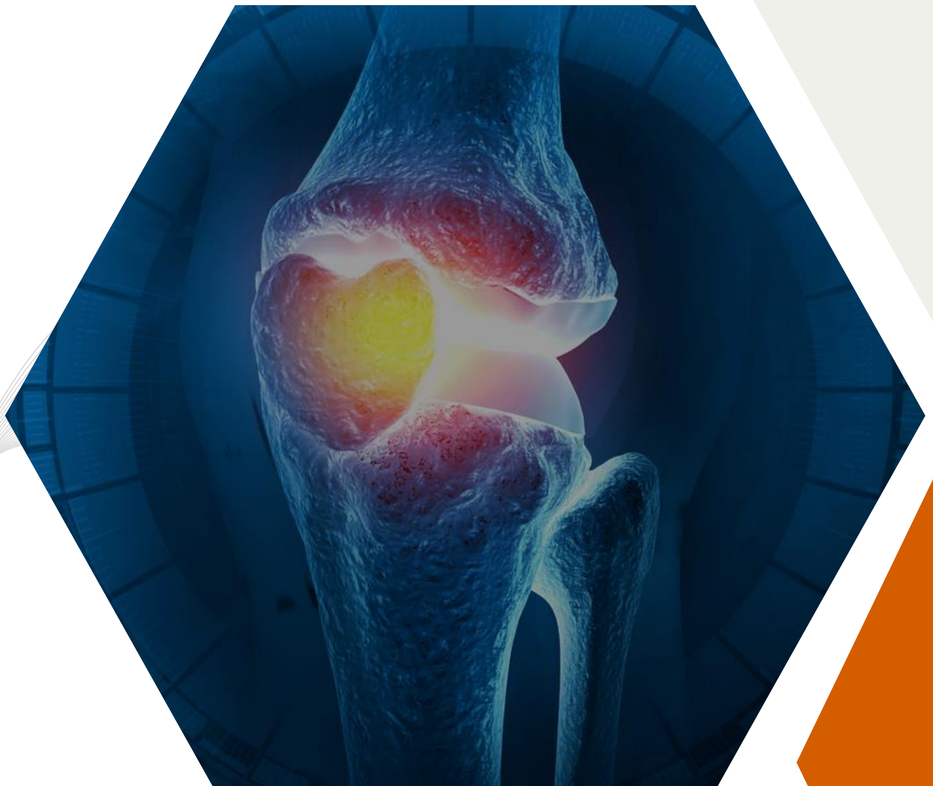
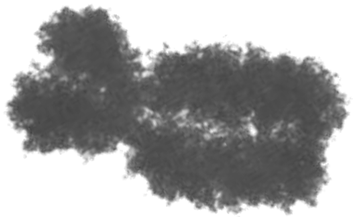
13 September 2024





# mRNA: An Instruction Molecule in Biology – Now in Vaccines and Medicines

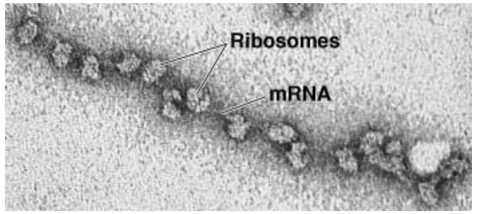
## DNA



**DNA stores** genetic information

*Transcription*

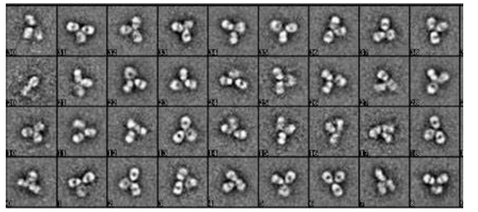
## messenger RNA




**mRNA instructs** cells in protein synthesis

*Translation*

## Protein



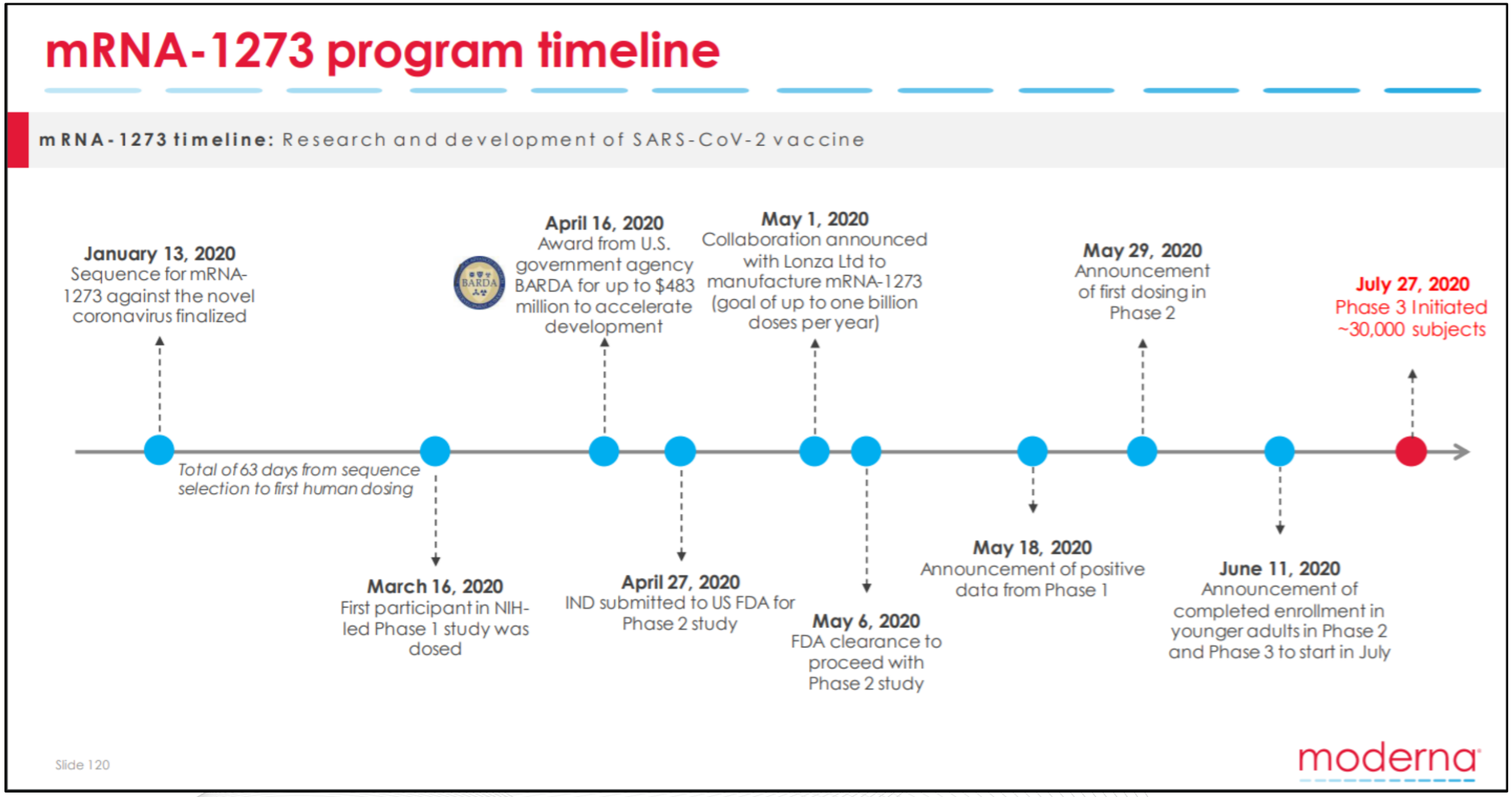
**Proteins act:** structure, signaling, metabolism, catalysis, pathogen defense

**Before the 2020s, no mRNA pharmaceutical products had been commercialized**

COVID-19

# Global pandemic, a clarion call for mRNA vaccine development

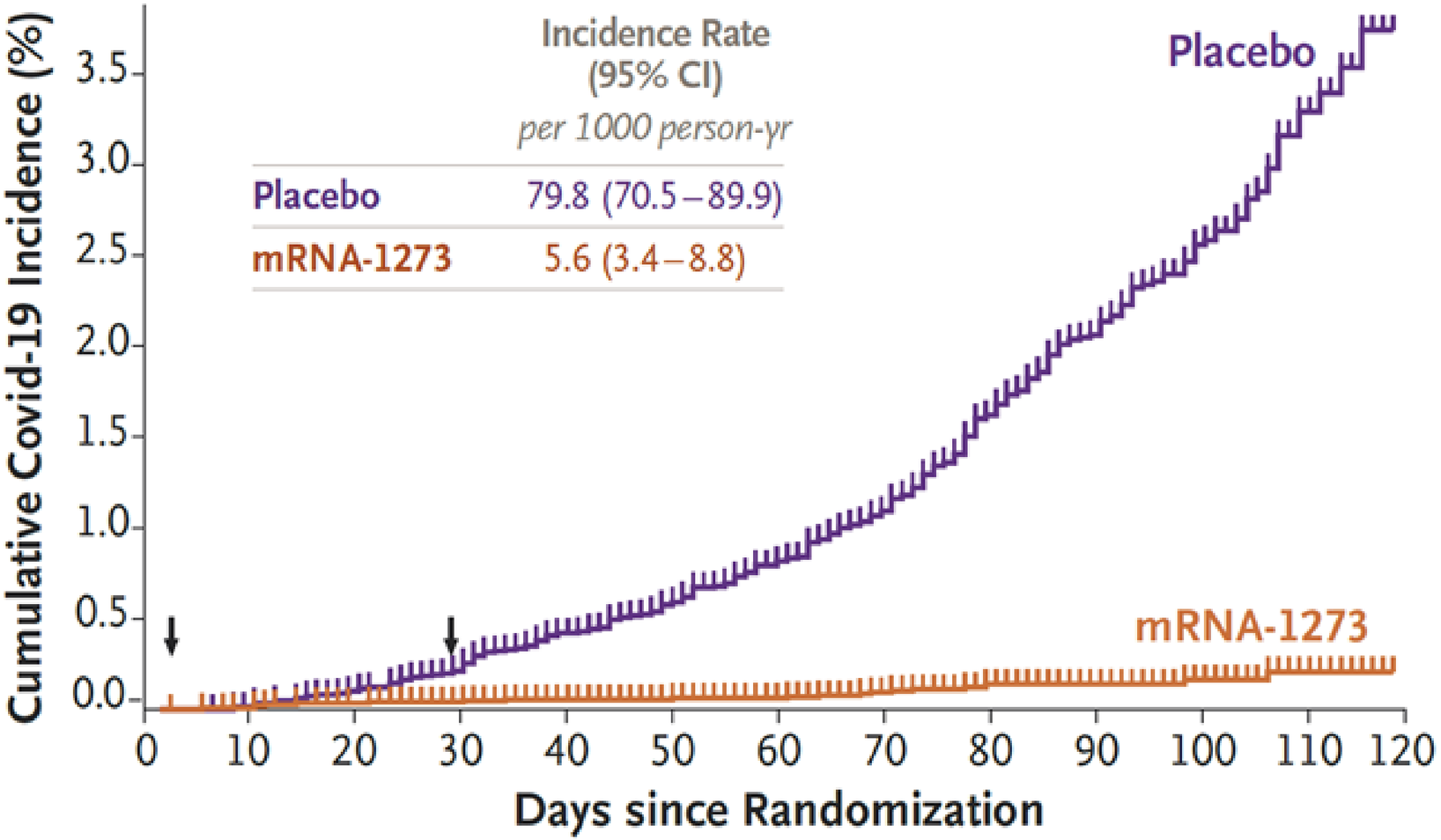
# The Race to Make an mRNA Vaccine for Covid19




**Speedy response** made possible by years of preparation by many people in a wide range of areas, including:

- ✓ Sequence design, bioinformatics
- ✓ Infectious disease research with NIH, other collaborators
- ✓ Clinical, biostatistics
- ✓ mRNA scale-up
- ✓ Delivery science, biology
- ✓ LNP design and scale-up
- ✓ Operations, Quality, Logistics

# The Race to Make an mRNA Vaccine for COVID-19




**Speedy response** made possible by years of preparation by many people in a wide range of areas.

95% efficacy in first Phase 3 studies. Sequence adjusted multiple times since, to adapt vaccine to mutants causing infection and disease.

Baden et al. *N. Engl. J. Med.* 384:403-416 (2021)

Pfizer timeline with BioNTech vaccine 2020-2022

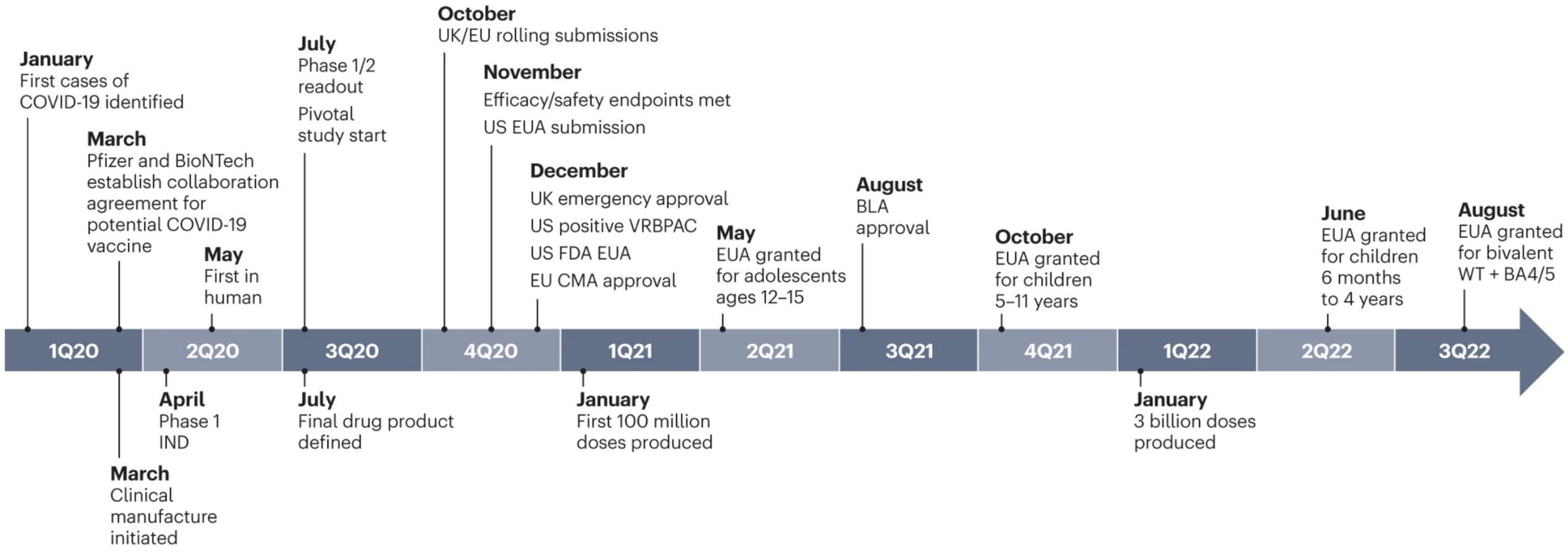
# Making Comirnaty Rapidly Available at Scale

## Fig. 1: Timelines for Comirnaty development.

From: Delivering 3 billion doses of Comirnaty in 2021

Warne et al. *Nat. Biotech.* (2023)
https://www.nature.com/articles/s41587-022-01643-1

**Clinical and regulatory timeline**



Active ingredient in BNT162b2

# 4284 Nucleotides, Mol.wt 1.4M Da

World Health Organization Messenger RNA Encoding the Full-Length SARS-CoV-2 Spike Glycoprotein.

Available online: https://web.archive.org/web/20210105162941/https://mednet-communities.net/inn/db/media/docs/11889.doc

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Ψ = 1-methyl-3'-pseudouridylyl
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# Three Other mRNA Covid19 Vaccine Developments of Note

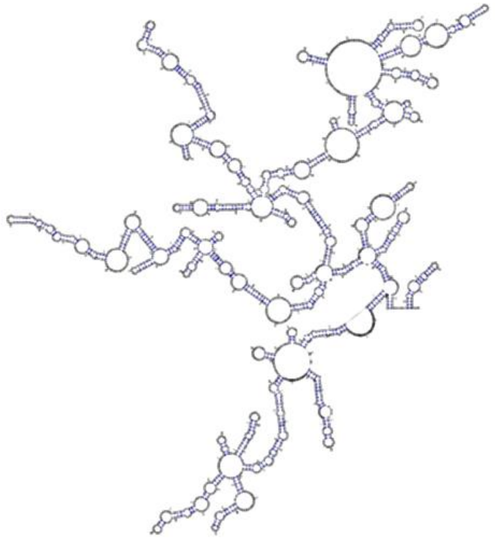
1. **CureVac**: first-generation vaccine candidate (CVnCoV), using unmodified mRNA, showed **47%** protection against symptomatic Covid19 in late-stage trials .

2. **Monash**: **RBD-targeted** Covid19 vaccine POC showed promise as an **alternative** to pre-fusion spike protein constructs used by Moderna and BioNTech/Pfizer.

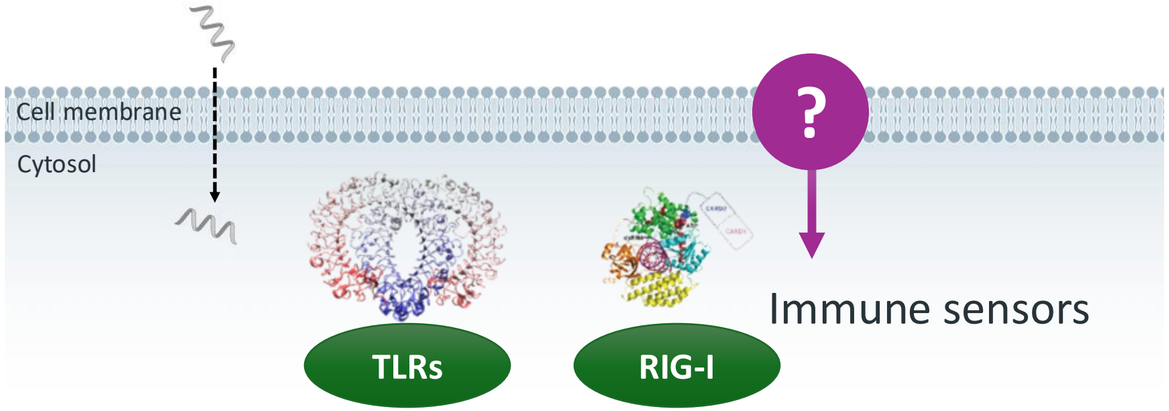
3. **Arcturus ARCT-154**: First commercial example of self-amplifying RNA vaccine, provided in **lyophilized format**, **2-8 ºC storage**, with involvement of CSL. Approved in Japan.

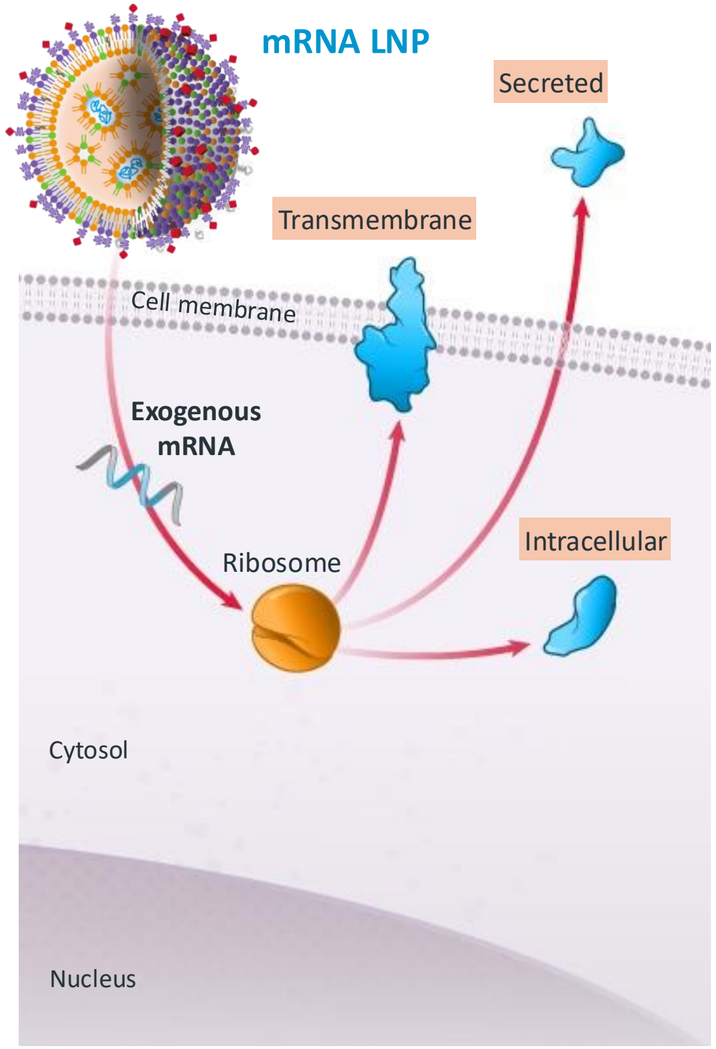
- All use LNP delivery, different lipid combination in each case
- Low doses, 100 ug mRNA or less, ARCT-154 most potent
- Booster shots required for best protection
- Sequence updates feasible for mutant coverage

# The Challenge of Delivery



- ‘Naked’ mRNA uptake into cells is saturable, limited efficiency
- Immunogenicity and RNase activity limit efficiency further
- **Delivery systems are critical** to success



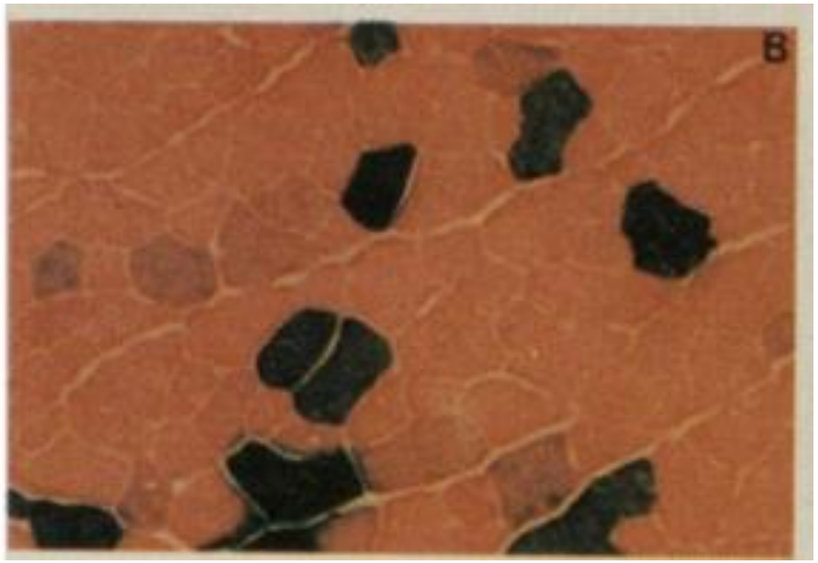
# Protein Expression *In Vitro* and *In Vivo* from Exogenous mRNA Transcripts

› Science. 1990 Mar 23;247(4949 Pt 1):1465-8. doi: 10.1126/science.1690918.

## Direct gene transfer into mouse muscle in vivo

J A Wolff [1], R W Malone, P Williams, W Chong, G Acsadi, A Jani, P L Felgner

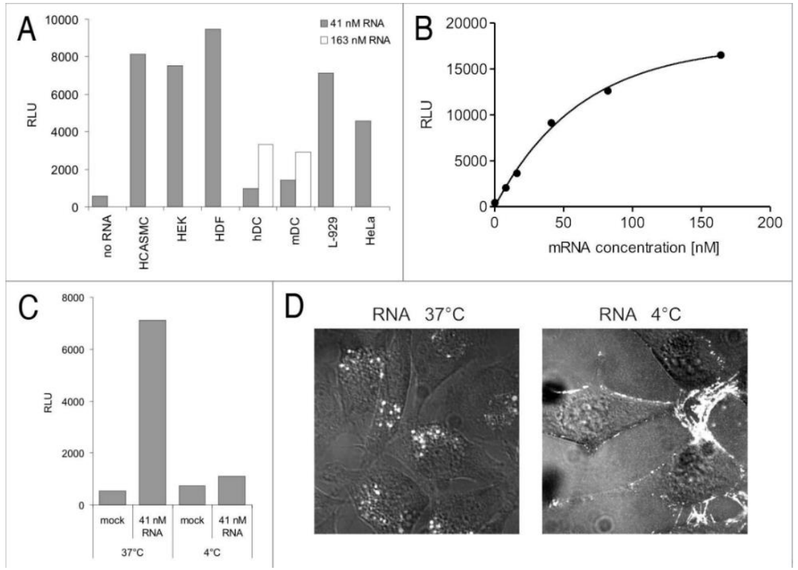
*"... no special delivery system was required ..."*




› RNA Biol. 2011 Jul-Aug;8(4):627-36. doi: 10.4161/rna.8.4.15394. Epub 2011 Jul 1.

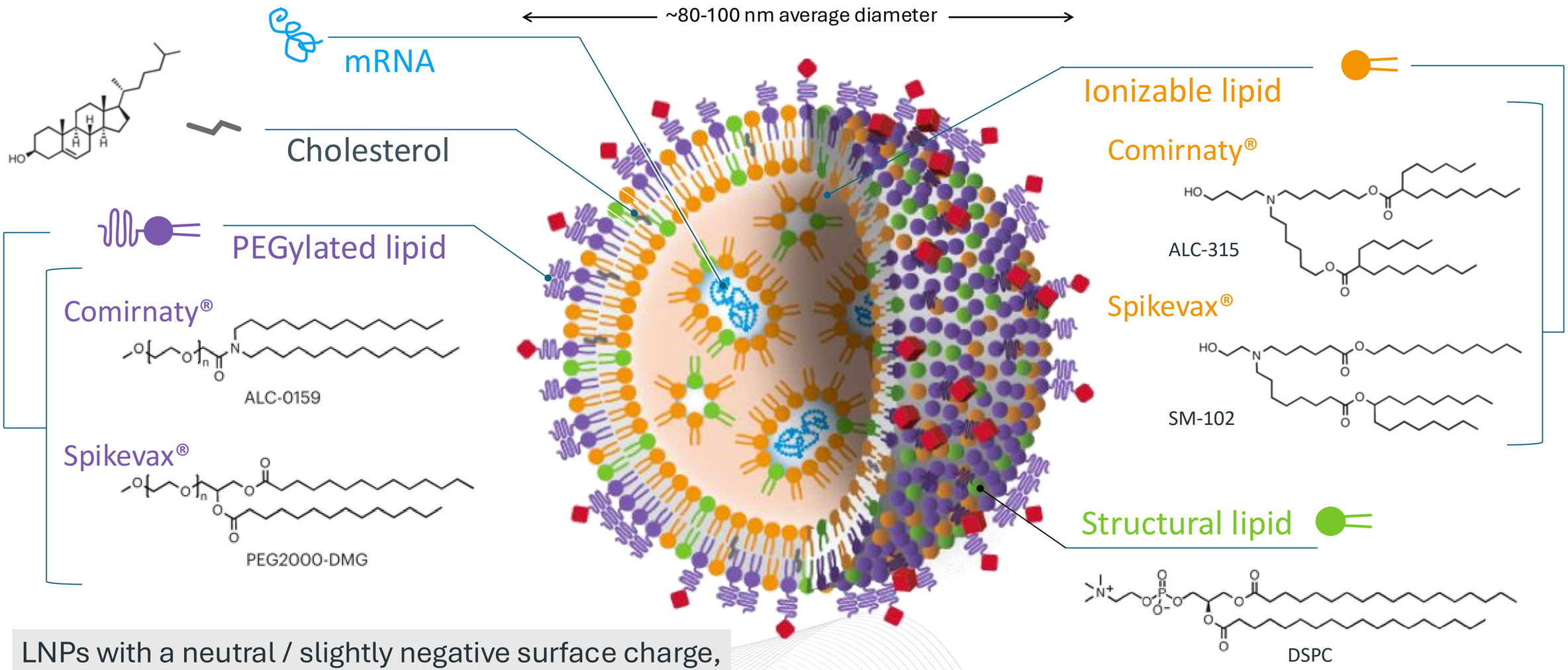
## Protein expression from exogenous mRNA: uptake by receptor-mediated endocytosis and trafficking via the lysosomal pathway

Christina Lorenz [1], Mariola Fotin-Mleczek, Günter Roth, Christina Becker, Thanh Chau Dam, Wouter P R Verdurmen, Roland Brock, Jochen Probst, Thomas Schlake



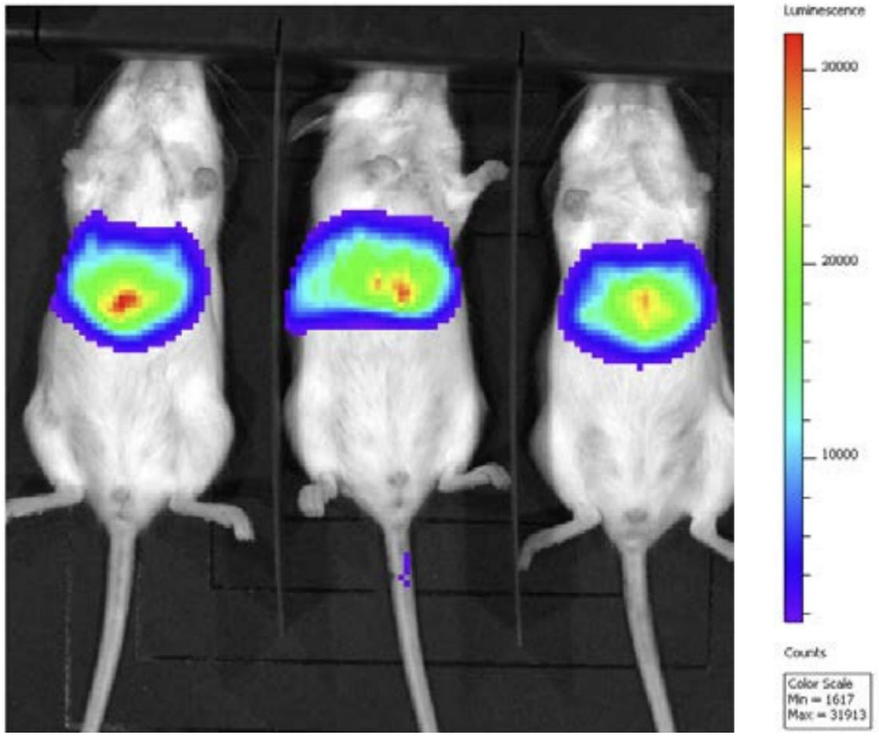

**Inefficient, saturable, not practical for human vaccines and therapeutics**

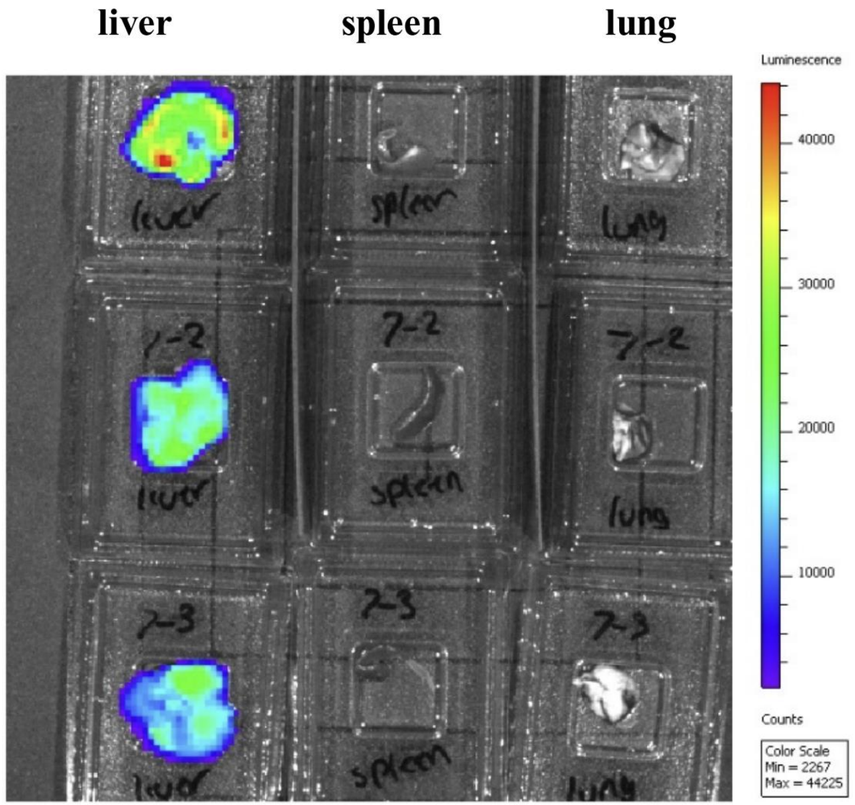
# Key Components in First mRNA Vaccine Lipid Nanoparticle (LNP) Products




LNPs with a neutral / slightly negative surface charge, deriving colloidal stability from PEG-lipid

# IV Delivery of mRNA-LNPs: Protein Expression in Liver




Luminescence after IV dosing of **LNPs with mRNA encoding luciferase enzyme** (luciferin substrate dosed separately):

Whole body and isolated organs, hours post mRNA-LNP dose

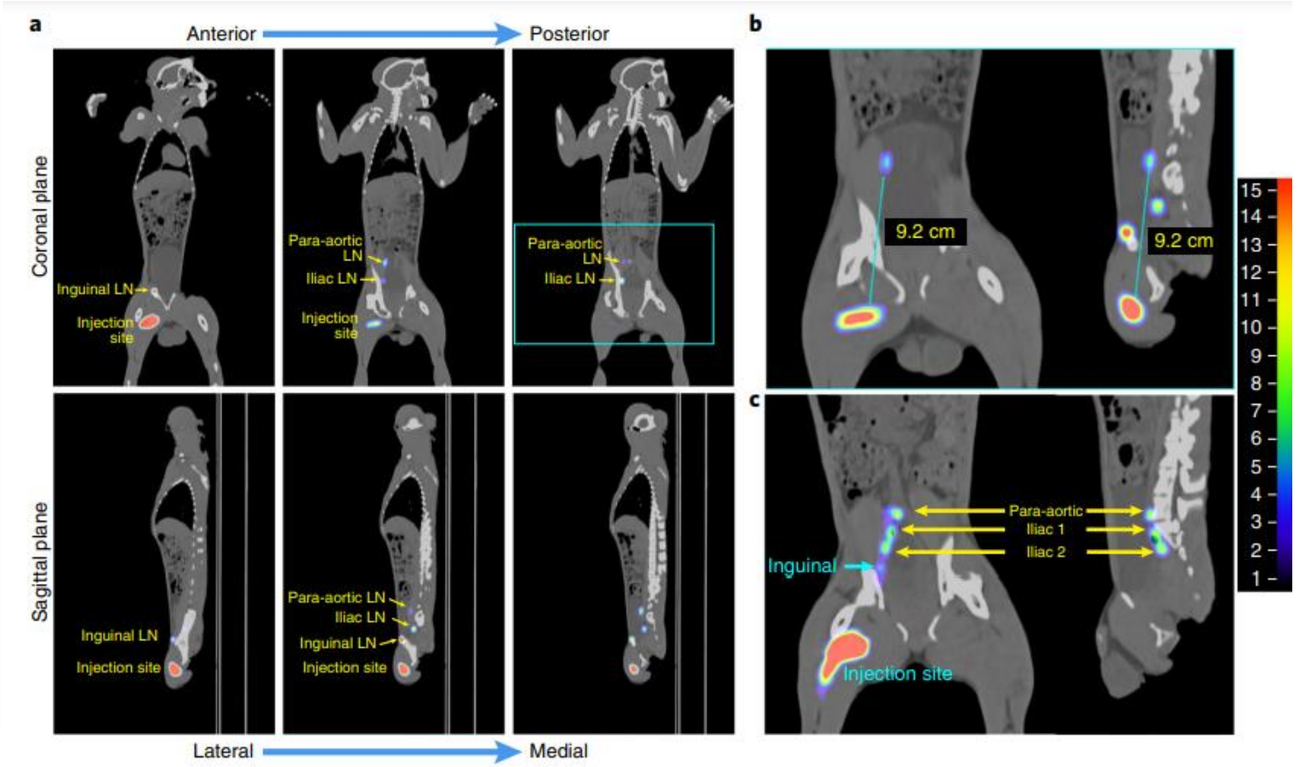
Liver is the main organ target of LNPs: ApoE/LDLr interaction and uptake mechanism

Sabnis et al. *Molecular Therapy* 26:1509-1519 (2018)

# Tracking the Fate of mRNA Vaccine to Local Lymphnodes *In Vivo*

- PET-CT and NIR imaging, labelled mRNA
- Transport from IM injection site to lymph nodes observed
- APCs largely taking up and translating the mRNA

Lindsay et al. *Nat. Biomed. Eng.* 3(5):371-380 (2019)



# Lipid Nanoparticles are Dynamic: A Blessing and a Curse

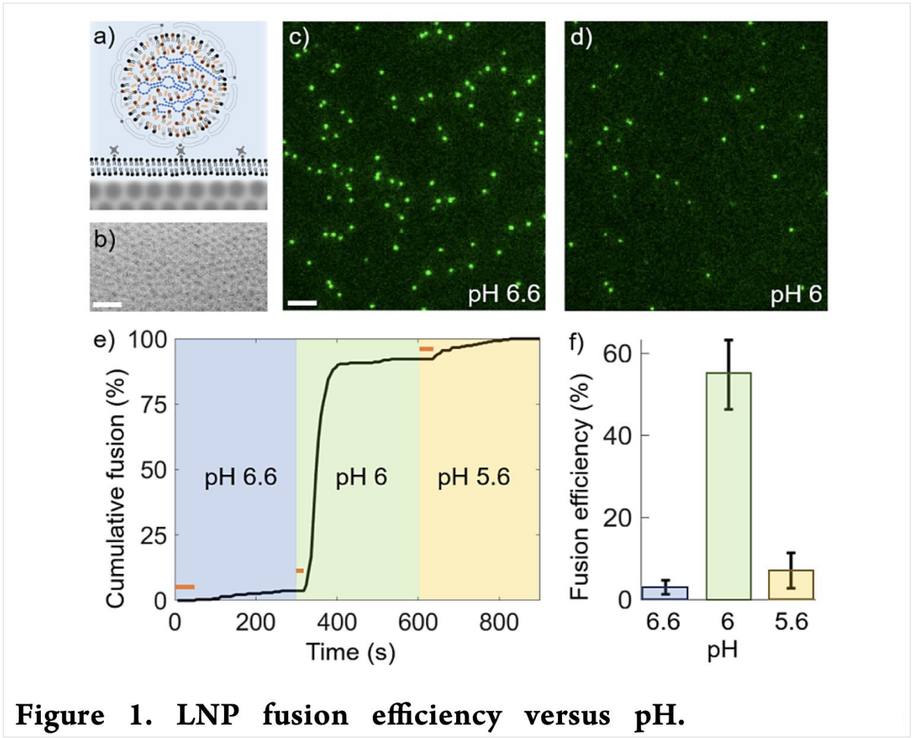
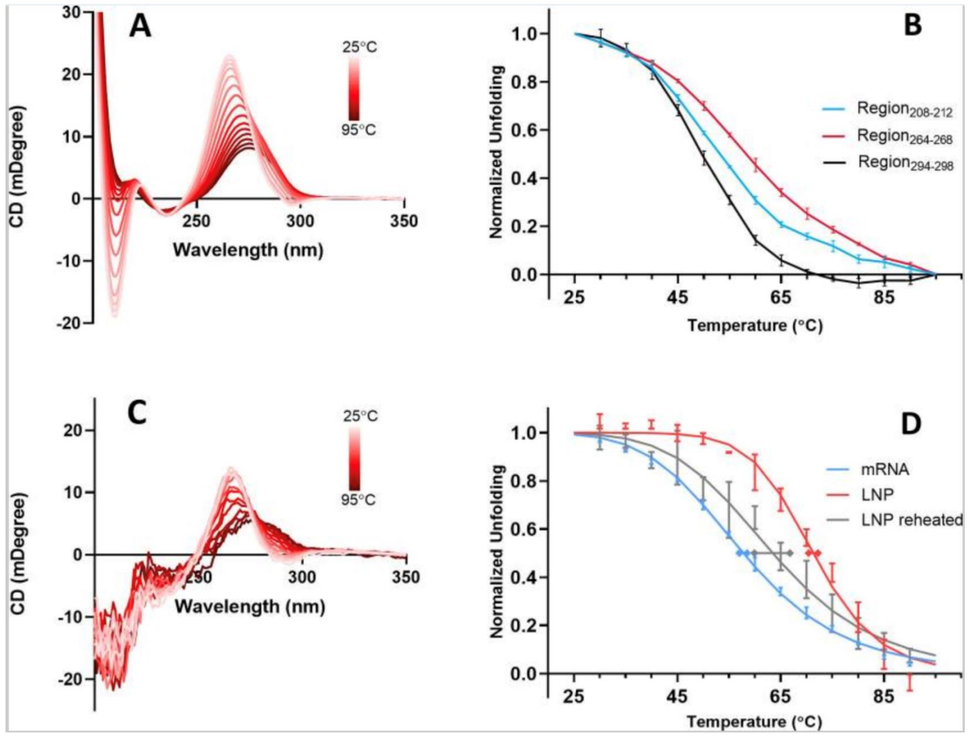



**Figure 1. LNP fusion efficiency versus pH.**

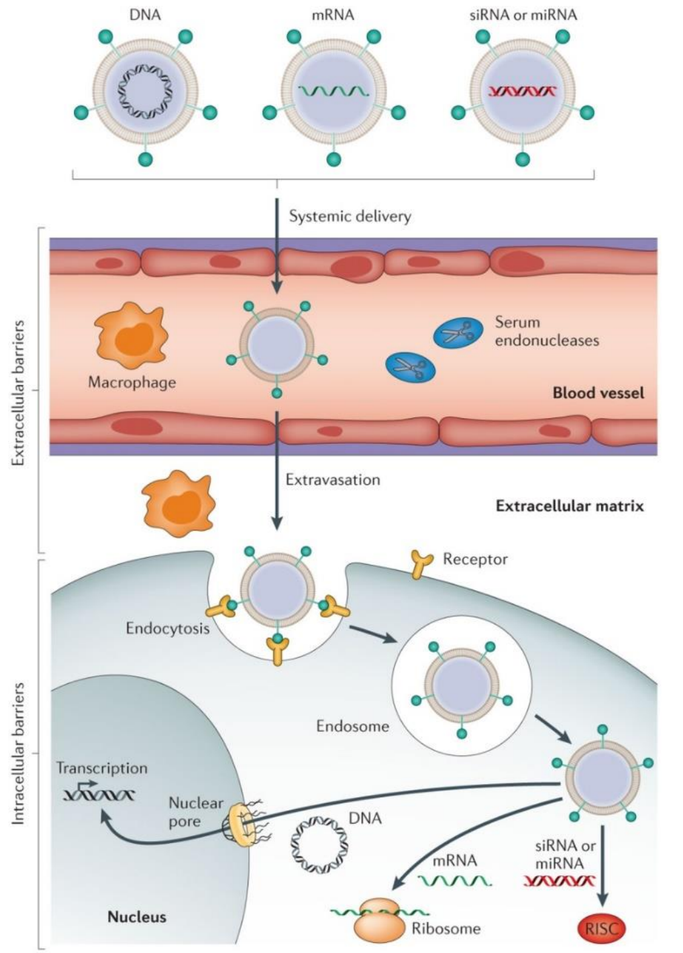
Aliakbarinodehi et al. *ACS Nano*. 18(34):22989 (2024)




Kloczewiak et al. *Mol. Pharm*. 19(7):2022 (2022)

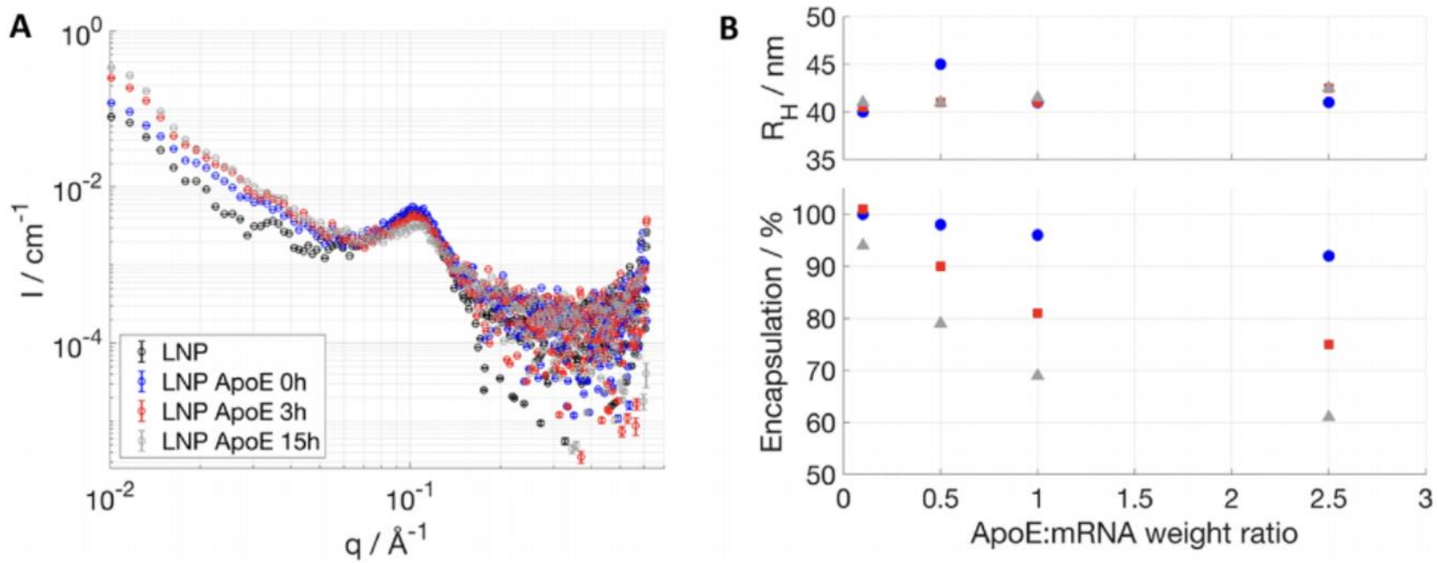
Influence of pH, temperature, membrane contact, are among factors that make LNPs work in biology - but these interactions and dynamics make **long-term storage outside of cold storage challenging**

# Example of Induced Changes of Surface and Internal Particle




Yin et al. *Nature Rev. Genetics* 15:541–555 (2014)

**ApoE binding** induces dynamics of the surface and core, reduced encapsulation with increased protein adsorbate




Sebastiani et al. *ACS Nano* 15:6709-6722 (2021)

A degree of short-range order exists in mRNA-LNP particles
Induced changes in contact with biological matrices

# Summary of mRNA-LNP Knowledge and Questions Remaining

**What we have learned** about structure:

- LNPs have discernible structural short-range order, and are dynamic in their nature
- Variable stoichiometry of mRNA/particle
- DSPC/PEG dominated surfaces
- Water content inside particles is ~20% V/V
- PEG lipid acts as steric stabilizer, dissociates from LNPs in vivo
- Structural changes of LNP occur *in vivo*, inside as well as outside, upon protein adsorption
- pH changes and contact with membranes have profound structural effects on LNP

**Some of the many questions** remaining:

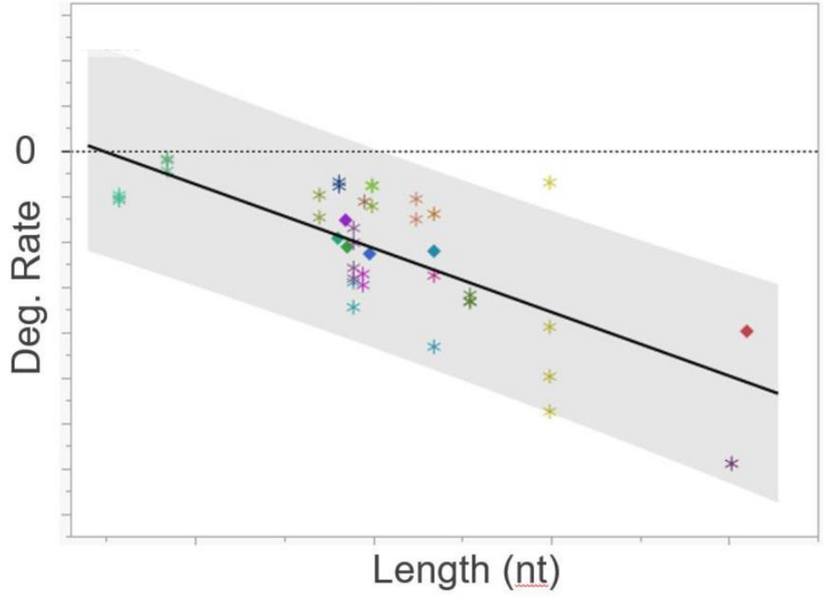
- *How to best balance dynamics relative to timing of delivery in the body?*
- *What factors stand in the way of translating mRNA-LNP activity, e.g. in vitro-in vivo disconnect*
- *Improved control of mRNA stoichiometry and order, how and how important?*
- *Do we need to start reporting data for % empty LNP? E.g. AAV products*
- *Improvements for non-vaccine delivery needs? E.g. mitigating inflammation, immune responses*
- *What is the upper limit of thermal stability achievable for mRNA-LNP systems?*

**“We are ignorant, but to a high level of sophistication”** Prof. Vladimir Prelog (1906-1998)

# Product Stability Challenges with mRNA-LNPs

- mRNA-LNPs are not thermally stable, long-term room-temp profile elusive
- Freezer storage limits product profiles, raises cost and distribution issues
- As liquid, limiting factors include:
  - Chemical integrity of mRNA
  - Physical stability of LNPs
- As lyophilized powder, higher temps:
  + Improved chemical stability of mRNA
  - Physical instability of LNP distribution, in-process and on reconstitution
- Simultaneous *"blocking-and-tackling"* with purity controls, scavengers, etc.

Strand stability decreases with size of mRNA:




Longer mRNAs have a faster degradation rate and hence shorter shelf-life

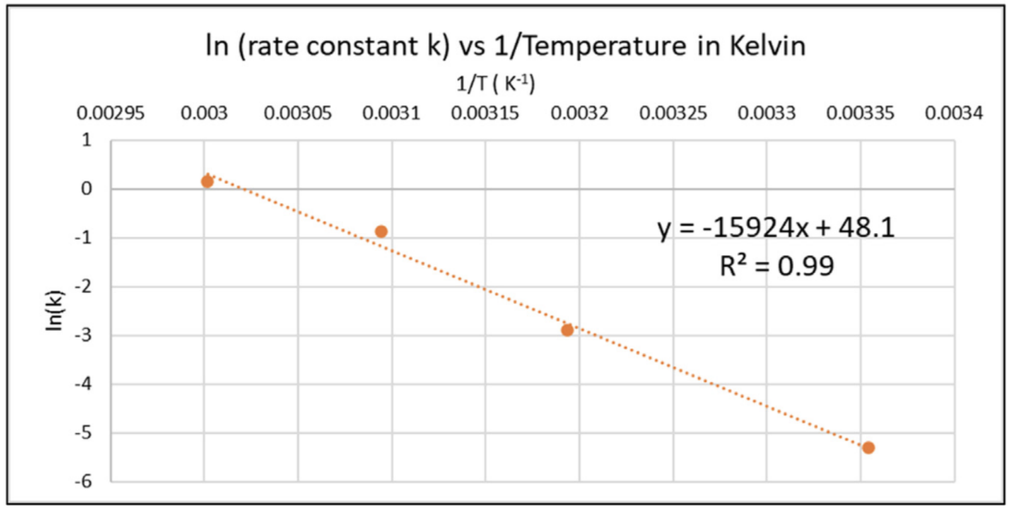


moderna

# mRNA Thermal Stability: Inverse Correlation with Transcript Size

*U. Chheda et al. / Journal of Pharmaceutical Sciences 00 (2023) 1–9*

**A** **4000 NT transcript, Arrhenius plot**

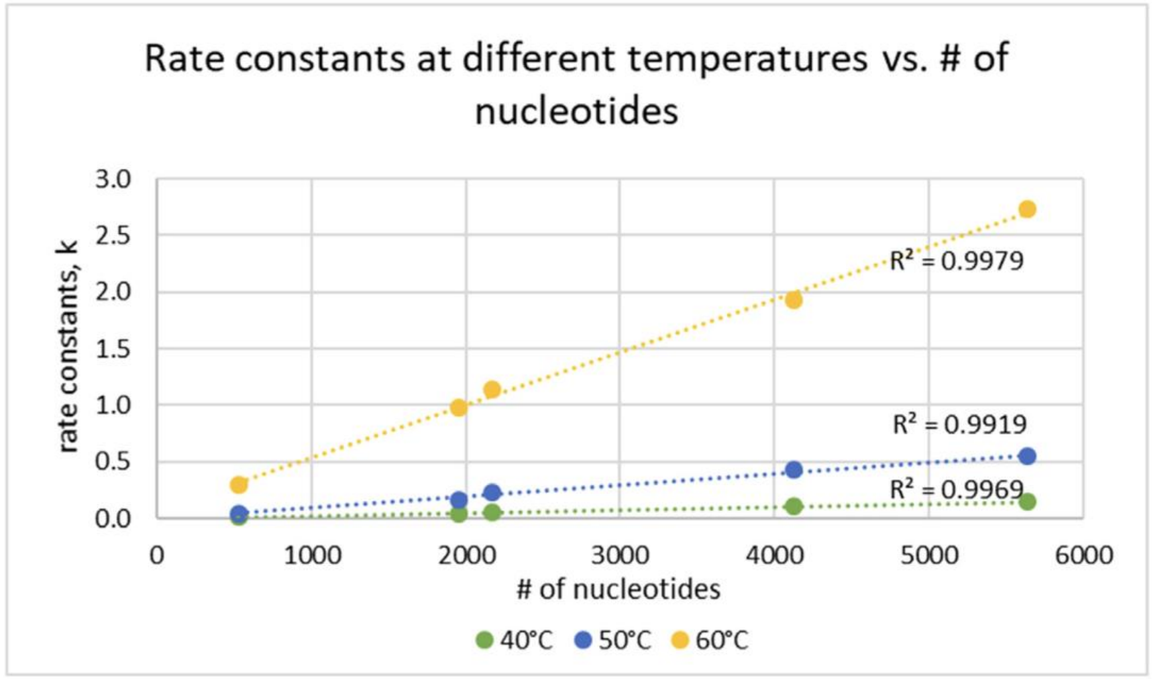



**B**

| Temperature (°C) | Half-life (days) |
|---|---|
| 25 | 140.0 |
| 40 | 12.5 |
| 50 | 1.6 |
| 60 | 0.6 |

Study of mRNA only, not accounting for effects of LNP encapsulation on chemical stability

**A**




**B**

| Length | Half-life (days) | | |
|---|---|---|---|
| | 40°C | 50°C | 60°C |
| 528 | 49 | 14 | 2.3 |
| 1953 | 14 | 4 | 0.7 |
| 2172 | 11 | 3 | 0.6 |
| 4122 | 7 | 1.6 | 0.4 |
| 5640 | 5 | 1.3 | 0.3 |

Therapeutics require **multi-year half-lives at 25 ºC** to justify RT storage labels. **Retaining >90% of drug intact** for adequate potency means it is more appropriate to think in terms of $t_{90}$ than $t_{50}$ ($t_{1/2}$).

# Prospects and Approaches for Room-Temperature Stable mRNA Products

## Prospects

Review published in early 2023, basis of WHO dialog that spring

Journal of Pharmaceutical Sciences

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REVIEW REVIEW | ARTICLES IN PRESS

The storage and in-use stability of mRNA vaccines and therapeutics: Not a cold case

Erik Oude Blenke • Eivor Örnskov •
Christian Schöneich • ...
Enrico Mastrobattista • Örn Almarsson •
Daan J.A. Crommelin • Show all authors

Open Access • Published: November 06, 2022 •

DOI: https://doi.org/10.1016/j.xphs.2022.11.001

## Approaches

- "Block-and-tackle" for liquid stability, LNP and other NPs
- Lyophilization of LNPs, now practiced extensively
- Alternatives, e.g. drying, assembly of kits for "at-site" LNPs

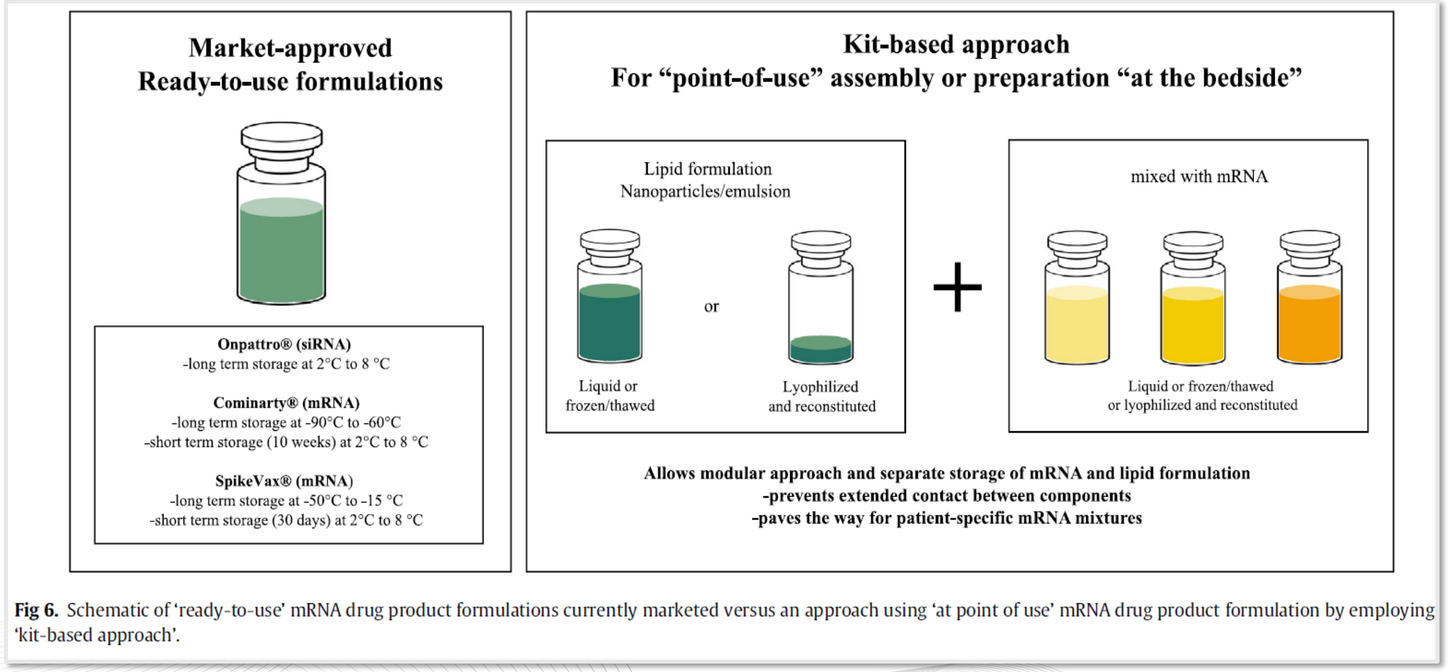


**Fig 6.** Schematic of 'ready-to-use' mRNA drug product formulations currently marketed versus an approach using 'at point of use' mRNA drug product formulation by employing 'kit-based approach'.

# Product Stability and WW Use Outlook

Based on a 2023 WHO expert meeting:
Unleashing mRNA in products worldwide depends our collective ability to address many factors, including…

- Enhancing thermal stability of mRNA products, *via*…
- Mechanistic knowledge and control of chemistry, supramolecular structure
- Performance-based purity controls for all contact materials
- Ingenuity in process design, production and supply for the warmest and most remote places where people live
- Continuous attention to cost optimization

It will take time and effort… **it's still only the beginning**