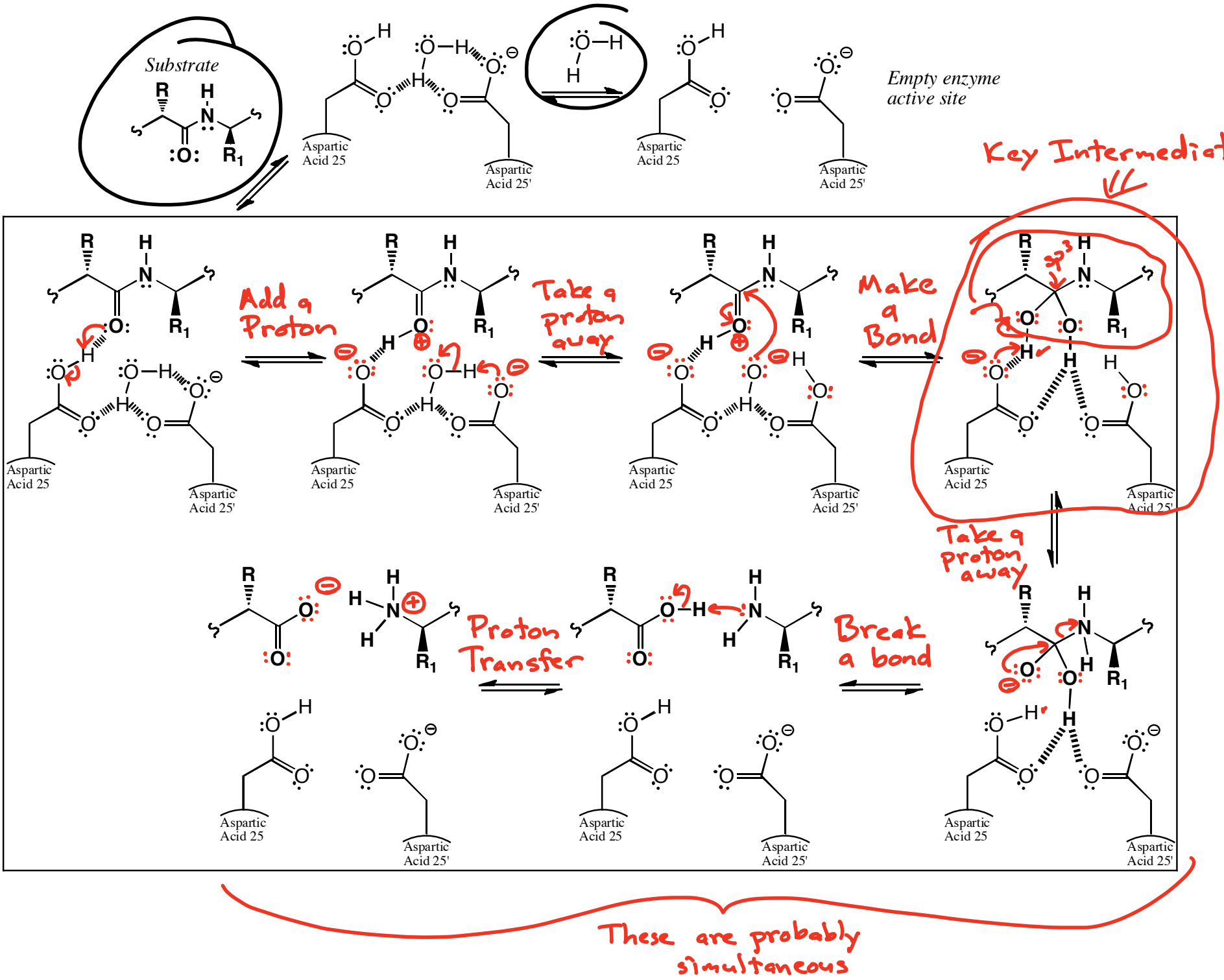
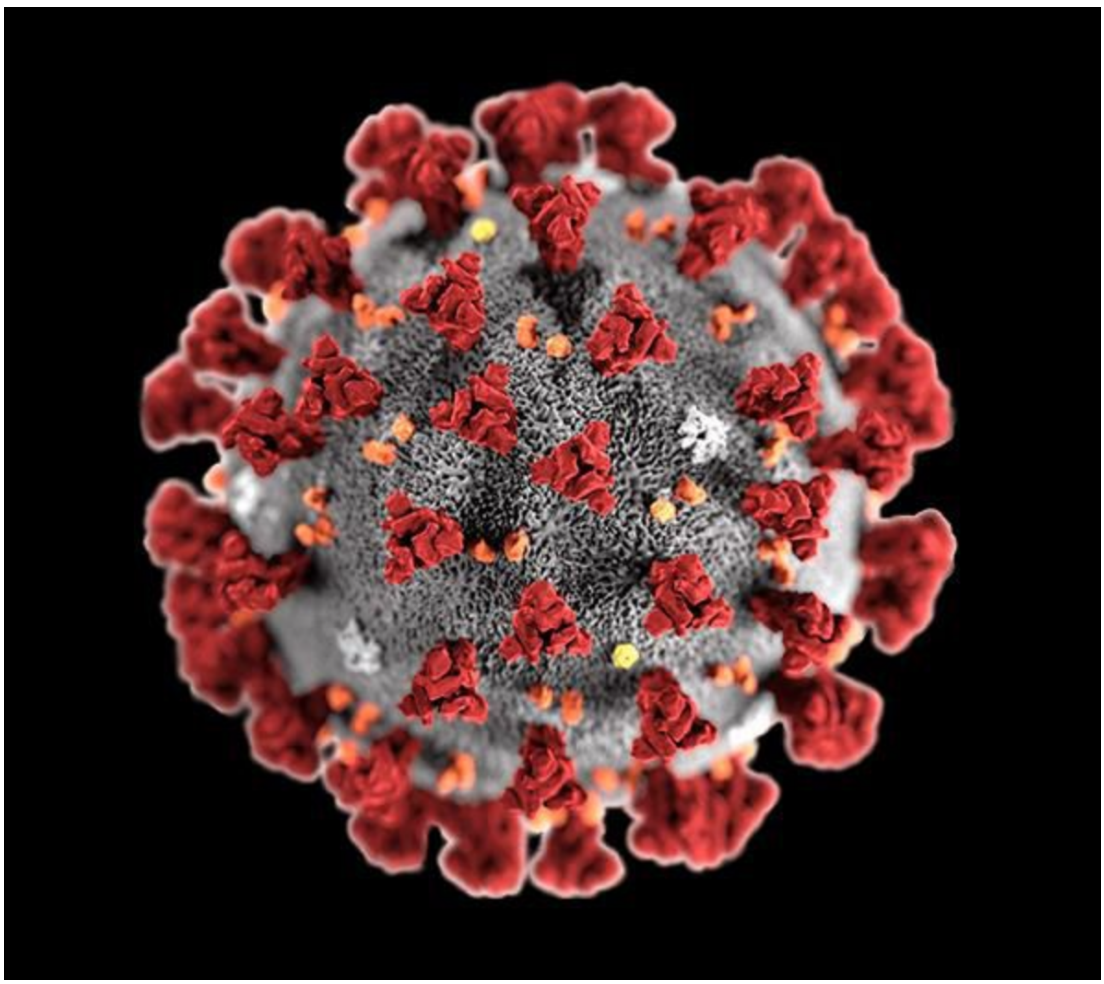

Substrate
Aspartic Acid 25
Aspartic Acid 25'
Empty enzyme active site
Key Intermediate
Add a Proton
Take a proton away
Make a Bond
Take a proton away
Proton Transfer
Break a bond
These are probably simultaneous



New UT cases

The University of Texas said two people in its community, an unnamed student and the Dean of Undergraduate Studies Brent Iverson, had tested positive for the virus. It is unclear if the pair are included in Travis County's count of confirmed cases.

CORONAVIRUS

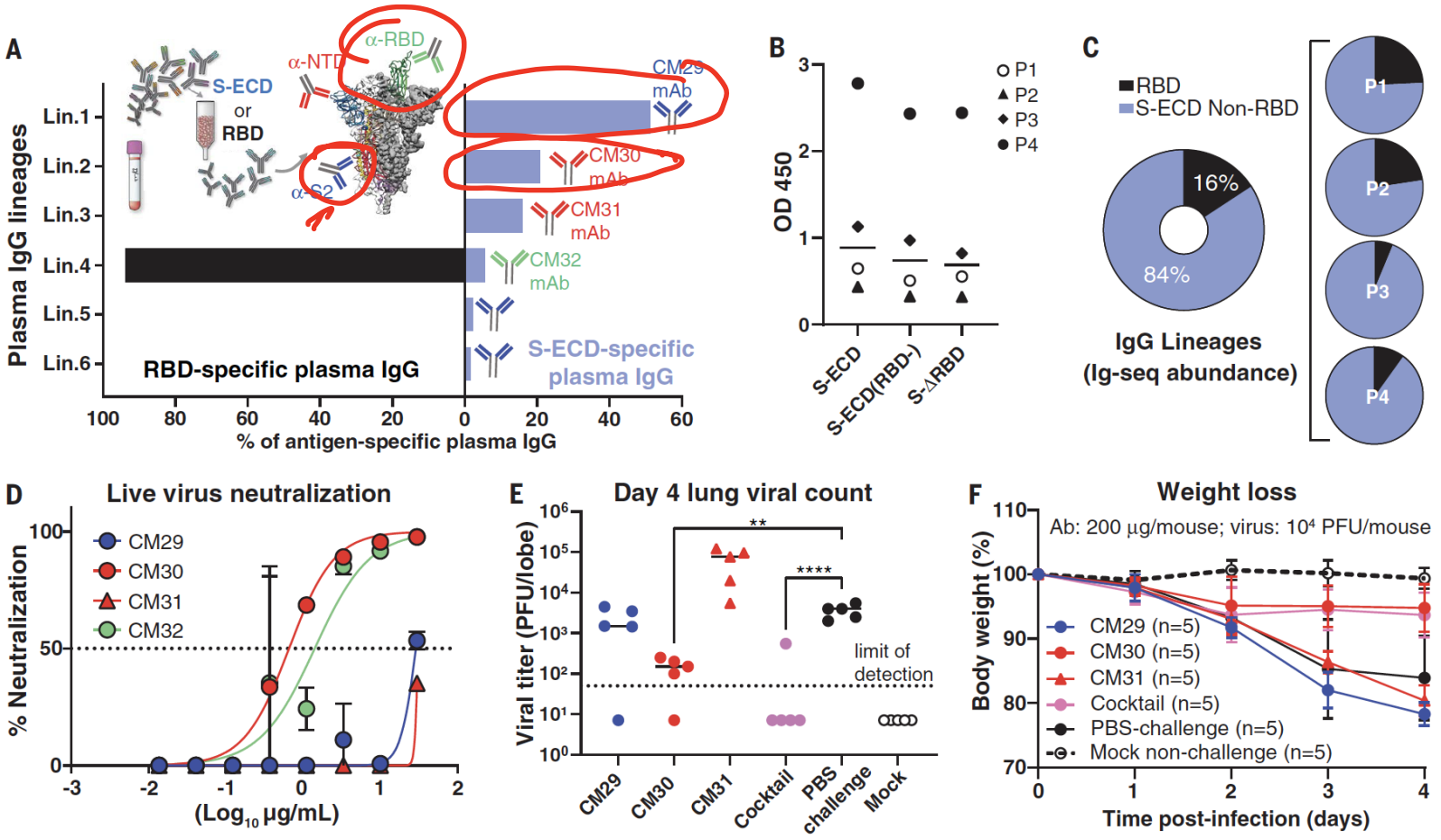
Prevalent, protective, and convergent IgG recognition of SARS-CoV-2 non-RBD spike epitopes

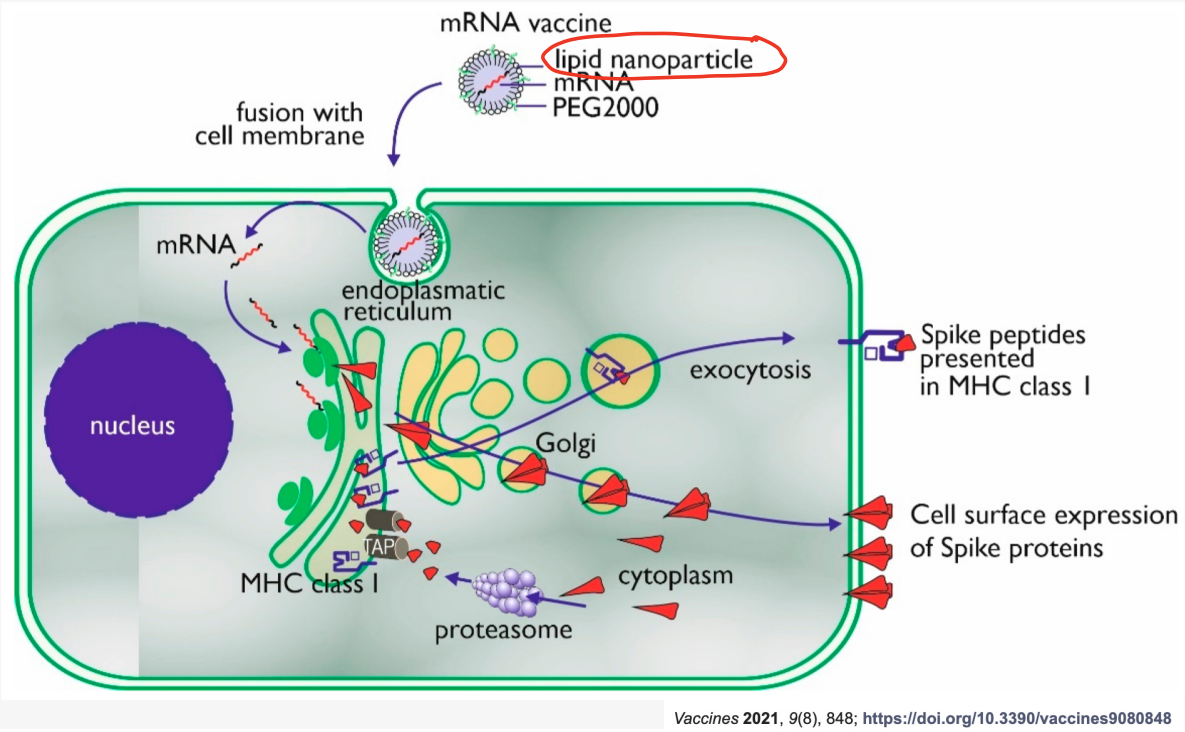
William N. Voss[1], Yixuan J. Hou[2,#], Nicole V. Johnson[1,#], George Delidakis[3], Jin Eyun Kim[4], Kamyab Javanmardi[1], Andrew P. Horton[1], Foteini Bartzoka[1], Chelsea J. Paresi[5], Yuri Tanno[3], Chia-Wei Chou[1], Shawn A. Abbasi[6], Whitney Pickens[1], Katia George[1], Daniel R. Boutz[1,7], Dalton M. Towers[3], Jonathan R. McDaniel[8], Daniel Billick[1], Jule Goike[1], Lori Rowe[9,10], Dhwani Batra[9], Jan Pohl[9], Justin Lee[9], Shivaprakash Gangappa[11], Suryaprakash Sambhara[11], Michelle Gadush[12], Nianshuang Wang[1], Maria D. Person[12], Brent L. Iverson[5], Jimmy D. Gollihar[1,7,13], John M. Dye[6], Andrew S. Herbert[6], Ilya J. Finkelstein[1], Ralph S. Baric[2,14], Jason S. McLellan[1], George Georgiou[1,3,4,15], Jason J. Lavinder[1,3]*, Gregory C. Ippolito[1,13,15]*

Voss *et al.*, *Science* **372**, 1108–1112 (2021) 4 June 2021

CORONAVIRUS

Prevalent, protective, and convergent IgG recognition of SARS-CoV-2 non-RBD spike epitopes





Vaccines **2021**, *9*(8), 848; **https://doi.org/10.3390/vaccines9080848**

Molecular Therapy
Original Article

A Novel Amino Lipid Series for mRNA Delivery: Improved Endosomal Escape and Sustained Pharmacology and Safety in Non-human Primates

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The success of mRNA-based therapies depends on the availability of a safe and efficient delivery vehicle. Lipid nanoparticles have been identified as a viable option. However, there are concerns whether an acceptable tolerability profile for chronic dosing can be achieved. The efficiency and tolerability of lipid nanoparticles has been attributed to the amino lipid. Therefore, we developed a new series of amino lipids that address this concern. Clear structure-activity relationships were developed that resulted in a new amino lipid that affords efficient mRNA delivery in rodent and primate models with optimal pharmacokinetics. A 1-month toxicology evaluation in rat and non-human primate demonstrated no adverse events with the new lipid nanoparticle system. Mechanistic studies demonstrate that the improved efficiency can be attributed to increased endosomal escape. This effort has resulted in the first example of the ability to safely repeat dose mRNA-containing lipid nanoparticles in non-human primate at therapeutically relevant levels.

INTRODUCTION

mRNA-based therapies have the potential to revolutionize the way we treat diseases. The surging interest in mRNA as a drug modality stems from the potential to deliver transmembrane and intracellular proteins, targets that standard biologics are unable to access due to their inability to cross the cell membrane.[1] One major challenge to making mRNA-based therapies a reality is the identification of an optimal delivery vehicle. Due to its large size, chemical instability, and potential immunogenicity, mRNA requires a delivery vehicle that can offer protection from endo- and exo-nucleases, as well as shield the cargo from immune sentinels. Lipid nanoparticles (LNPs) have been identified as a leading option in this regard.[2] Moderna Therapeutics has recently validated this approach by demonstrating safe and effective delivery of an mRNA-based vaccine formulated in LNPs.[3]

Key performance criteria for an LNP delivery system are to maximize cellular uptake and enable efficient release of mRNA from the endosome. At the same time, the LNP must provide a stable drug product and be able to be dosed safely at therapeutically relevant levels. LNPs are multi-component systems that typically consist of an amino lipid, phospholipid, cholesterol, and a PEG-lipid.[2] Each component is required for aspects of efficient delivery of the nucleic acid cargo and stability of the particle. The key component thought to drive cellular uptake, endosomal escape, and tolerability is the amino lipid. Cholesterol and the PEG-lipid contribute to the stability of the drug product both *in vivo* and on the shelf, while the phospholipid provides additional fusogenicity to the LNP, thus helping to drive endosomal escape and rendering the nucleic acid bioavailable in the cytosol of cells.

Several amino lipid series have been developed for oligonucleotide delivery over the past couple of decades.[4] The literature highlights direct links between the structure of the amino lipid and the resultant delivery efficiency and tolerability of the LNP. The amino lipid MC3 (DLin-MC3-DMA) is the most clinically advanced oligonucleotide delivery system, as siRNA formulated in MC3-based LNPs has progressed to phase III for the treatment of transthyretin-mediated amyloidosis.[5,6] More recently, literature reports have demonstrated the effectiveness of MC3-based LNPs to deliver mRNA.[7] LNPs of this class are quickly opsonized by apolipoprotein E (ApoE) when delivered intravenously (i.v.), which enables cellular uptake into hepatocytes by the low-density lipoprotein receptor (LDLr).[8] Concerns remain that MC3's long tissue half-life could contribute to unfavorable side effects hindering its use for chronic therapies.[9] In addition, LNPs can induce activation of the immune system resulting in complement activation-related pseudoallergy (CARPA), an acute immunological response that can lead to anaphylactic-like shock.[10]

To unleash the potential of mRNA therapies for humans, we required a class of LNPs with increased delivery efficiency along with a metabolic and toxicity profile that would enable chronic dosing in humans.

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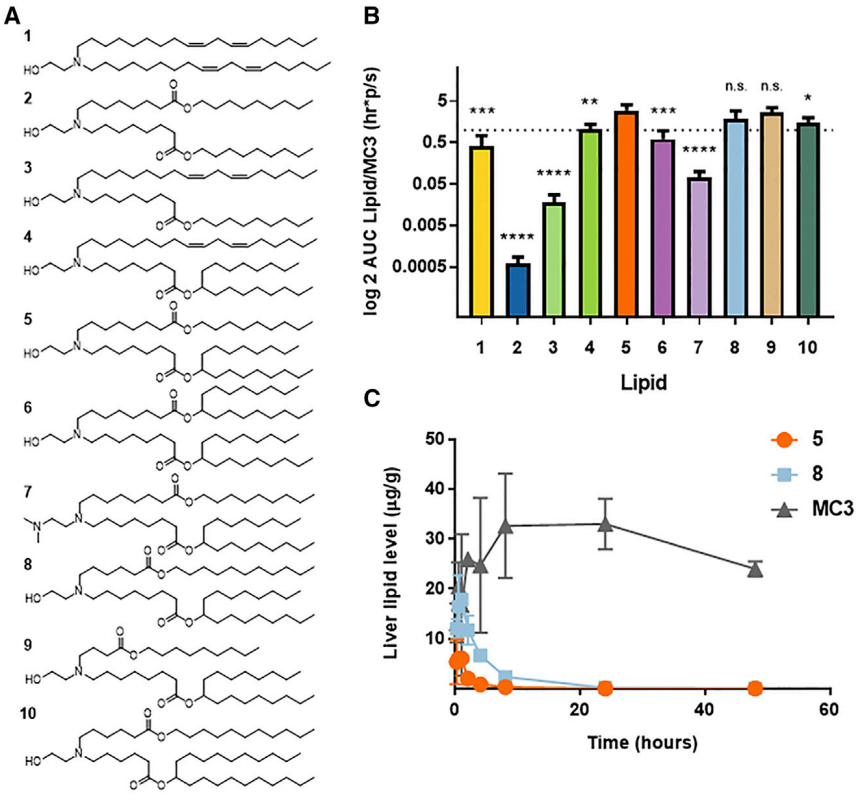

Figure 1. Optimization of Efficiency and Clearance of Amino Lipid

(A) Structures of amino lipids. (B) Whole-body luciferase bioluminescence AUC of novel LNPs versus MC3 LNPs, measured in CD-1 mice (n = 6 at 3 and 6 hr, n = 3 at 24 hr), 0.5 mg/kg dose firefly luciferase (ffLuc) mRNA, i.v. bolus, error bars indicate SD of the ratio of novel lipid AUC versus MC3 AUC. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, n.s. = not statistically significant. (C) Parent amino lipid levels measured in liver tissue from Sprague-Dawley rats (n = 3 per time point), 0.2 mg/kg dose hEPO mRNA, mean ± SD, p < 0.05 for lipids 5 and 8 AUC relative to MC3.

We took a rational medicinal chemistry approach to amino lipid optimization aiming to identify structural motifs that provide chemical stability, optimal tissue clearance, and mRNA delivery efficiency. Our initial rodent screens led to the identification of a lead lipid with good delivery efficiency and pharmacokinetics. The lead LNP was profiled further in non-human primate for efficiency of delivery after single and repeat dosing. Finally, the optimized LNPs were evaluated in 1-month repeat dose toxicity studies in rat and non-human primate.

RESULTS

Initial screening of a broad chemical space identified ethanolamine as an amino lipid head group that could effectively drive mRNA encapsulation and provide LNPs with superior physiochemical properties. Combining the ethanolamine head group with di-linoleic lipid tails (lipid 1; Figure 1; Table 1) generated an LNP with high encapsulation of luciferase mRNA, small particle size, and low polydispersity index (PDI). The LNP with lipid 1 had a surface pK_a (apparent value for the particle) in the range that has been shown to be optimal for siRNA delivery.[11,12] To evaluate the efficiency of the new amino lipids, LNPs using the novel lipids were tested *in vivo* in mice using firefly luciferase mRNA as a reporter. An MC3 LNP was included as a control in each experiment, enabling us to compare LNPs from experiment to experiment. Measured luciferase activity also enabled us to determine protein bio-distribution. i.v. delivery of 0.5 mg/kg (mRNA dose level) of lipid 1-based LNPs to mice resulted in luciferase activity two-fold lower than an MC3 LNP control (Figure 1B). Whole-body imaging clearly demonstrated that the majority of protein expression was localized in the liver (Figure S1). We found that the lipid had similar clearance to MC3 from liver tissue with 66% of the original dose remaining in liver tissue of mice 24 hr post-dose (Table 1).

To improve tissue clearance, we introduced ester linkages in the lipid tails (lipids 2 and 3; Figure 1A), which are reported to trigger metabolism by esterases *in vivo*.[13] This has been shown to be a viable strategy to improve lipid clearance in a MC3-based lipid structure.[9] First, we established that the lipids were chemically stable by measuring ethanol stability at room temperature and 37°C (Table S1). We observed less than 1% change in purity for all lipids tested. LNPs formed with lipid 2 were significantly larger with a surface pK_a > 7 (Table 1). Removal of one ester (lipid 3) afforded LNPs with improved physiochemical characteristics and lower LNP surface pK_a. *In vivo*, neither lipid demonstrated efficient mRNA delivery (Figure 1B); however, we did observe rapid tissue clearance, with no lipid detected at 24 hr (Table 1).

Improvement in protein expression was observed when a secondary ester was introduced (lipid 4; Figures 1A and 1B). We observed equivalent expression to MC3 LNPs, but the clearance rate was slower than lipids 2 and 3 (67% lipid remaining; Table 1). Replacement of the linoleic tail with a primary ester-containing lipid tail (lipid 5; Figure 1A) provided increased expression (3-fold higher than MC3; Figure 1B) and optimal tissue clearance (no lipid detected at 24 hr; Table 1). To further increase expression an additional secondary ester was introduced (6), but this resulted in a lowering of the surface pK_a to 6.00 and lower luciferase activity. In addition, the lipid had a significantly slower tissue clearance with 68% remaining at 24 hr.

With an optimal lipid tail structure identified we re-visited the ethanolamine head group. Lipid 7 is one representative example (Figure 1A) where the alcohol functionality is replaced with a dimethylamine. This generated an LNP with comparable physiochemical properties, but complete loss of delivery efficiency (Figure 1B).

Structure-based design of prefusion-stabilized SARS-CoV-2 spikes

Ching-Lin Hsieh[1], Jory A. Goldsmith[1], Jeffrey M. Schaub[1], Andrea M. DiVenere[2], Hung-Che Kuo[1], Kamyab Javanmardi[1], Kevin C. Le[2], Daniel Wrapp[1], Alison G. Lee[1], Yutong Liu[2], Chia-Wei Chou[1], Patrick O. Byrne[1], Christy K. Hjorth[1], Nicole V. Johnson[1], John Ludes-Meyers[1], Annalee W. Nguyen[2], Juyeon Park[1], Nianshuang Wang[1], Dzifa Amengor[1], Jason J. Lavinder[1,2], Gregory C. Ippolito[1,3], Jennifer A. Maynard[2]*, Ilya J. Finkelstein[1,4]*, Jason S. McLellan[1]*

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The COVID-19 pandemic has led to accelerated efforts to develop therapeutics and vaccines. A key target of these efforts is the spike (S) protein, which is metastable and difficult to produce recombinantly. Here, we characterized 100 structure-guided spike designs and identified 26 individual substitutions that increased protein yields and stability. Testing combinations of beneficial substitutions resulted in the identification of HexaPro, a variant with six beneficial proline substitutions exhibiting ~10-fold higher expression than its parental construct and the ability to withstand heat stress, storage at room temperature, and three freeze-thaw cycles. A 3.2 Å-resolution cryo-EM structure of HexaPro confirmed that it retains the prefusion spike conformation. High-yield production of a stabilized prefusion spike protein will accelerate the development of vaccines and serological diagnostics for SARS-CoV-2.

Mol Ther. 2008 November ; 16(11): 1833–1840. doi:10.1038/mt.2008.200.

Incorporation of Pseudouridine Into mRNA Yields Superior Nonimmunogenic Vector With Increased Translational Capacity and Biological Stability

Katalin Karikó[1], **Hiromi Muramatsu**[1], **Frank A Welsh**[1], **János Ludwig**[2], **Hiroki Kato**[3], **Shizuo Akira**[3], and **Drew Weissman**[4]

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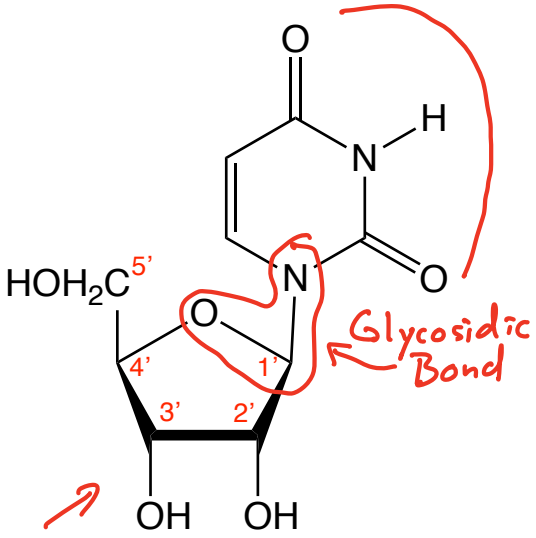
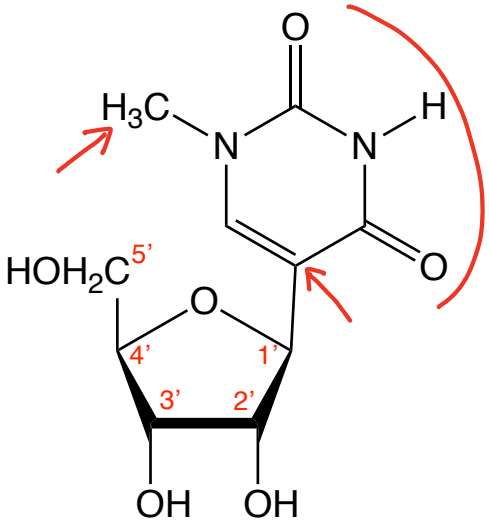
Abstract

In vitro–transcribed mRNAs encoding physiologically important proteins have considerable potential for therapeutic applications. However, in its present form, mRNA is unfeasible for clinical use because of its labile and immunogenic nature. Here, we investigated whether incorporation of naturally modified nucleotides into transcripts would confer enhanced biological properties to mRNA. We found that mRNAs containing pseudouridines have a higher translational capacity than unmodified mRNAs when tested in mammalian cells and lysates or administered intravenously into mice at 0.015–0.15 mg/kg doses. The delivered mRNA and the encoded protein could be detected in the spleen at 1, 4, and 24 hours after the injection, where both products were at significantly higher levels when pseudouridine-containing mRNA was administered. Even at higher doses, only the unmodified mRNA was immunogenic, inducing high serum levels of interferon-α (IFN-α). These findings indicate that nucleoside modification is an effective approach to enhance stability and translational capacity of mRNA while diminishing its immunogenicity *in vivo*. Improved properties conferred by pseudouridine make such mRNA a promising tool for both gene replacement and vaccination.



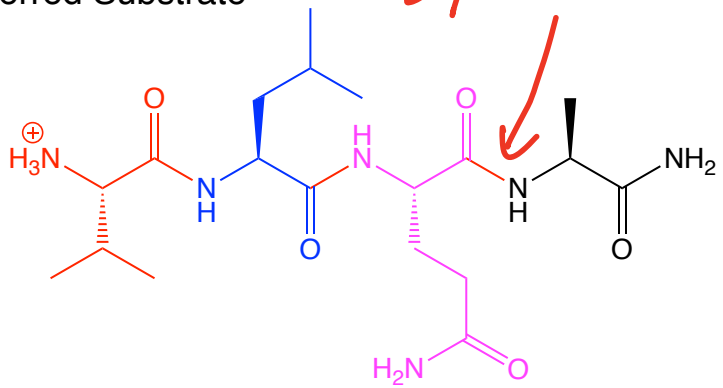
Uridine

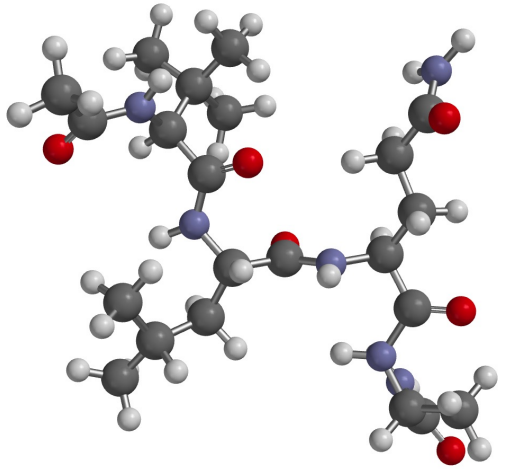


N1-methylpseudouridine
(m1Ψ)

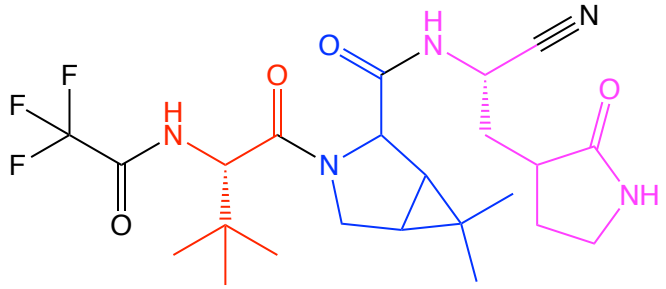
Preferred Substrate

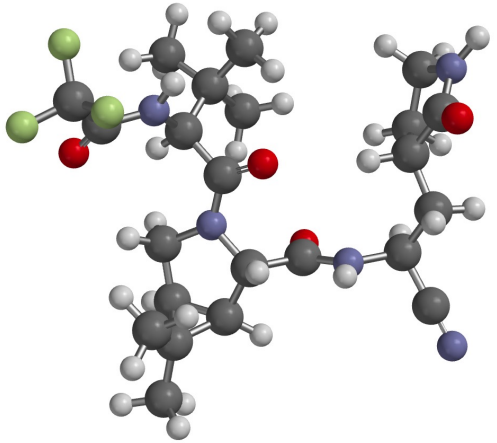
This bond is cleaved by the enzyme



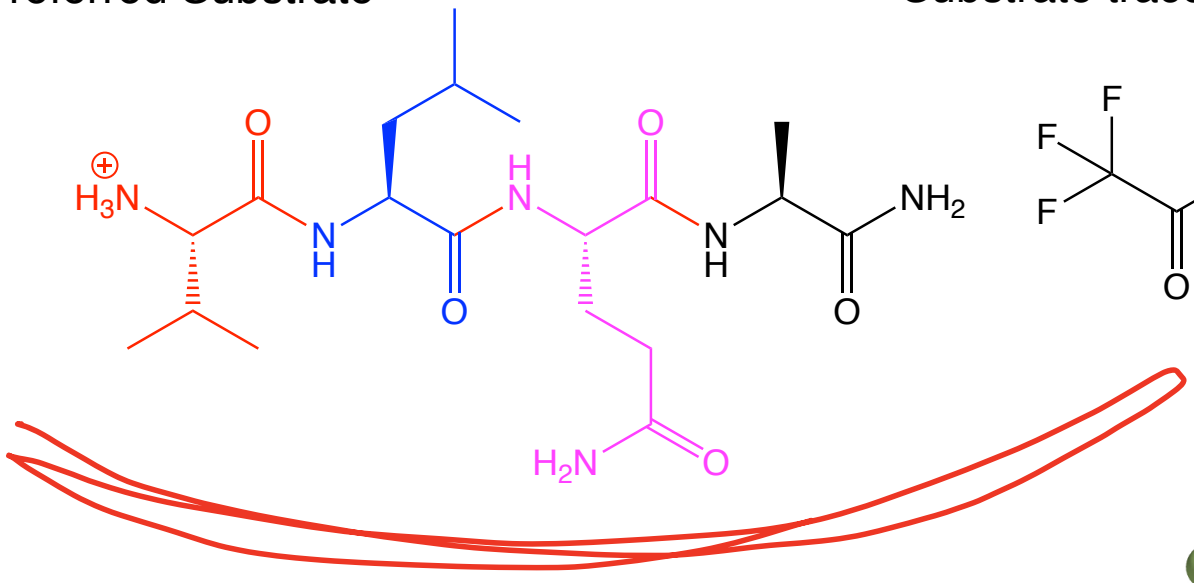


Paxlovid

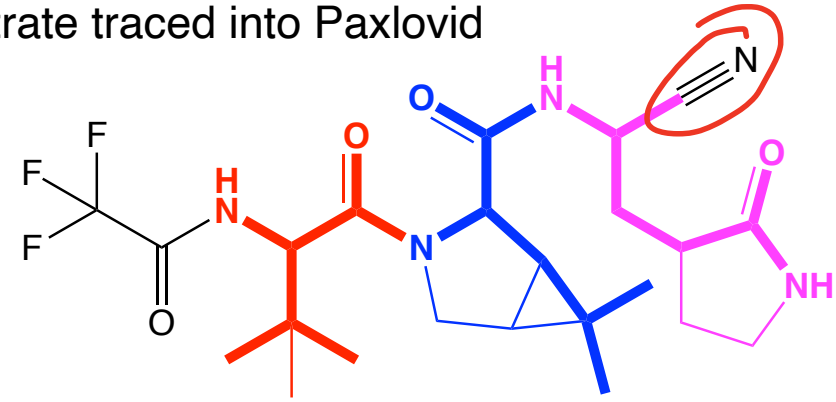




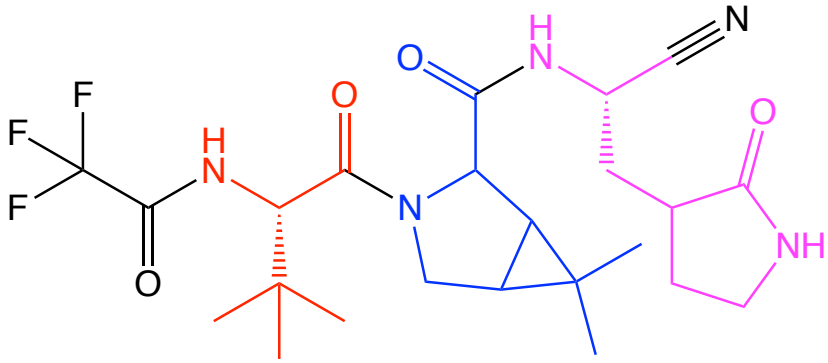
Preferred Substrate

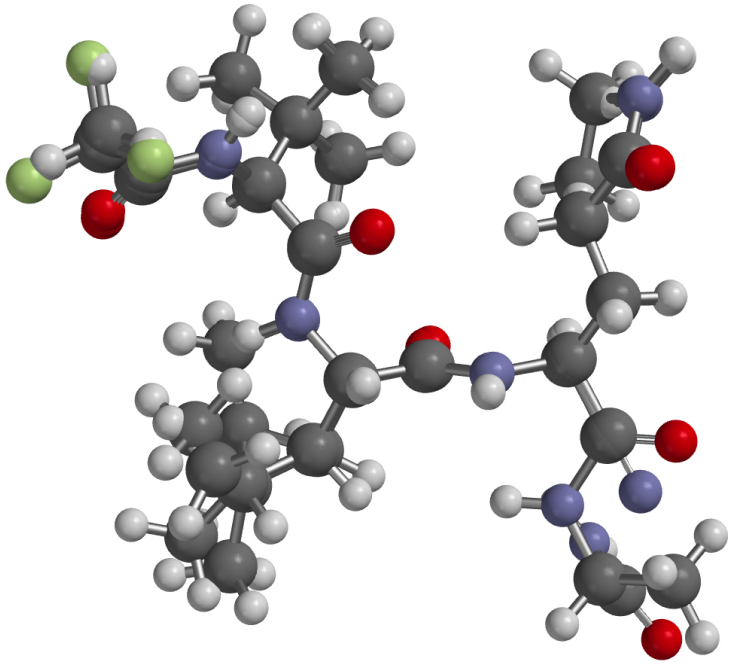


Substrate traced into Paxlovid



Paxlovid





<u>Organic Chemistry</u> is the study of carbon-containing molecules. This class has two points.

The first point of the class is to understand the organic chemistry of living systems. We will teach you how to think about and understand the most amazing molecules on the planet!!

You will learn how MRI scans work. 1/12/23

You will learn the basic principles of pharmaceutical science and how many drugs work. 1/19/23

You will learn about the special bond that holds carbohydrates such as glucose in six-membered rings, connects carbohydrate monomers together to make complex carbohydrate structures and is critical to DNA and RNA structure. 1/26/23

You will learn how soap is made from animal fat and how it works to keep us clean. 2/16/23

You will learn the important structural reason proteins, the most important molecular machines in our bodies, can support the chemistry of life. 2/14/23

You will learn how important antibiotics like penicillins work, including ones that make stable covalent bonds as part of their mode of action. 3/7/23

You will learn why carrots are orange and tomatoes are red. 3/28/23

You will learn the very cool reason that the DNA and RNA bases are entirely flat so they can stack in the double helix structure. 4/13/23

You will learn even more about why fentanyl is such a devastating part of the opioid problem and how Naloxone is an antidote for a fentanyl overdose. 4/18/23

You will learn even more details about why Magic Johnson is still alive, decades after contracting HIV, and how the same strategy is being used to fight COVID. 4/18/23

You will learn about the surprising chemical reason the Pfizer and Moderna mRNA vaccines elicit strong immune responses. 4/20/23

The second point of organic chemistry is the synthesis of complex molecules from simpler ones by making and breaking specific bonds, especially carbon-carbon bonds.

You will learn how carbon-metal bonds lead to new carbon-carbon bonds. 1/17/23

You will learn how most reactions of carbonyl compounds involve only the four common mechanistic elements operating in only a few common patterns. 1/17/23

You will learn how, by simply adding a catalytic amount of base like HO^- to aldehydes or ketones, you can make new carbon-carbon bonds, giving complicated and useful products. 2/28/23

You will learn a reaction that can convert vinegar and vodka into a common solvent. 2/16/23

You will learn why molecules with six-membered rings and alternating double bonds are stable. 3/30/23

You will learn a reaction that can turn model airplane glue into a powerful explosive. 4/18/23

Most important, you will develop powerful critical thinking skills:

1. You will learn how to look at a molecule and accurately predict which atoms will react to make new bonds, and which bonds will break during reactions.
2. You will learn how to analyze a complex molecule's structure so that you can predict ways to make it via multiple reactions starting with less complex starting molecules.

We want to hear from you!