



The mRNA That Came In from the Cold: Foiling Aldehyde Sabotage in Lipid Nanoparticles

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Nucleic acid vaccines have received increasing research attention over the past 40 years.¹ Traditional vaccines often require lengthy development, manufacturing, and regulatory timelines. Many traditional vaccines use live-attenuated or inactivated viruses, or selected viral proteins, to elicit protective immune responses. Although these approaches are effective, developing, manufacturing, and approving a new vaccine can require considerable time and resources that are all in short supply during a global pandemic such as that caused by the SARS-CoV-2 virus. The COVID-19 pandemic accelerated the development and widespread adoption of mRNA vaccine technology.

In the late 1980s and early 1990s, researchers began investigating whether nonreplicating DNA and RNA could direct protein production *in vivo*. Direct injection of gene-encoding mRNA or DNA into mouse skeletal muscle produced detectable protein without a specialized delivery system. Subsequent studies showed that vaccination by this method elicited antigen-specific immune responses.²⁻⁵ However, unmodified mRNA could activate innate immune pathways, causing inflammation and limiting mRNA stability, translation, and the desired antigen-specific response.^{6,7} Karikó and Weissman addressed this problem by incorporating modified nucleosides that reduced innate recognition of the mRNA while preserving translation of the encoded antigen.^{8,9}

As researchers improved the stability and immunological properties of mRNA, attention shifted toward delivery systems that could protect the molecule and promote efficient cellular uptake. Initial work focused on liposomes as delivery vehicles that could protect mRNA from degradation.^{10,11} Lipid nanoparticles (LNPs) gained attention because they could protect mRNA, promote cellular uptake, and improve delivery efficiency.¹²

Together, these advances established mRNA as a promising vaccine platform

capable of generating effective and durable immune responses. When the COVID-19 pandemic emerged, decades of prior research made it possible to develop and manufacture mRNA vaccines rapidly.¹³⁻¹⁶ However, one major issue remained: the limited thermostability of mRNA vaccines, a challenge Hashiba et al. sought to improve with their research published in *Communications Biology* in 2024.¹⁷ Pandemic responses require the rapid production of vaccines that remain stable and effective during storage and distribution. Early formulations of some COVID-19 mRNA vaccines required ultralow-temperature storage to limit mRNA degradation, complicating transportation and creating barriers for healthcare facilities without specialized freezers. Hashiba et al. designed an LNP capable of maintaining mRNA stability at 4 °C, a major step forward in making vaccines accessible for all. The researchers developed an LNP formulation containing the ionizable lipid CL15F. They compared this CL15F formulation with formulations containing CL4F, ALC-0315, or SM-102. To assess delivery efficiency, the researchers measured expression of luciferase-encoding mRNA. They found that the CL15F formulation produced greater levels of bioluminescence compared that containing CL4F. They also tested the CL15F formulation's ability to deliver vaccine that resulted in antigen response in mice, and found it was significantly stronger than the two clinical lipids. These results indicated that the CL15F formulation performed at least as well as the comparison formulations in the measured delivery and immunogenicity assays.

To assess storage stability, the researchers gave separate groups of mice formulations that had been stored for 0, 1, 3, or 5 months at -80 °C or 4 °C. After five months at -80 °C, the CL15F and comparison formulations retained similar *in vivo* activity. After storage at 4 °C, however, only the CL15F formulation retained substantial activity through five months.

An additional factor affecting the shelf stability of mRNA vaccines is the formation of aldehyde-containing impurities within LNP formulations. These reactive impurities can interact with mRNA and reduce its translational activity by obstructing ribosome progression (See **Figure 1**). To compare aldehyde impurity levels in CL4F and CL15F formulations, researchers used a fluorescence assay based on NBD-H, which becomes fluorescent after reacting with aldehyde carbonyl groups. The assay showed that CL15F formulations contained significantly lower levels of aldehyde impurities than the CL4F formulation. This difference is important for long-term mRNA stability. Hashiba et al. also examined how aldehydes react with mRNA to form covalent adducts, in which an aldehyde-derived chemical group becomes attached to the mRNA. They found that higher aldehyde concentrations were associated with greater adduct formation and a corresponding loss of mRNA activity.

Despite several limitations, the study showed that LNP structure can influence the long-term thermostability of mRNA formulations. Not all CL15F-based formulations produced equivalent results, and the loss of mRNA activity could not be attributed entirely to aldehyde impurities. Nevertheless, the findings suggest that both lipid structure and aldehyde-containing impurities influence storage stability. These findings may guide future efforts to improve the stability and accessibility of mRNA vaccines and therapeutics.

Future mRNA therapies have the potential to treat cancer, genetic disorders, and autoimmune diseases. These treatments, however, cannot become broadly accessible care options until a stable and consistent way to store and transport them is found. Continued improvements in the storage stability and delivery of mRNA formulations will allow these types of treatments to reach all the people who need them, rather than remaining as novel ideas in deep-freeze.



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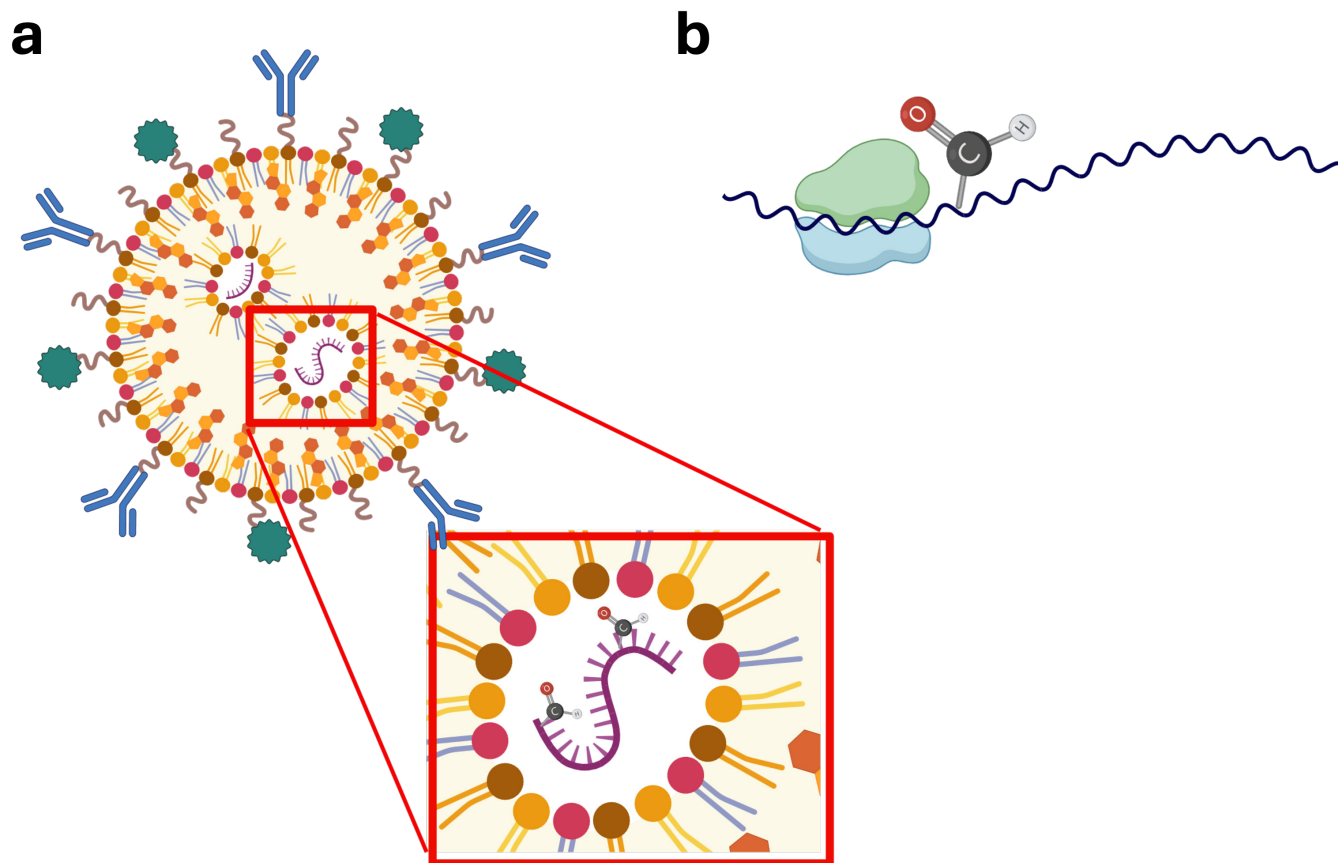


Figure 1 | Aldehyde Impurities Inhibiting Translation. a. An LNP-mRNA formulation with inset highlighting aldehyde impurities binding to the mRNA. b. An aldehyde residue binding to the mRNA in the coding sequence blocks translation by the ribosome. Created in BioRender. Theleman, A. & Trem1, J. (2026) <https://BioRender.com/c02h3wy>