



# A Briefing on the Evolution of Nucleic Acid Vaccines

By JF Trembl

The University of Kansas Biotechnology program emphasizes the importance of teaching students to critically evaluate and clearly communicate complex scientific research. Each year, students work in small groups to interpret and present primary research articles arranged chronologically around a common theme. This format allows the class to trace how a field develops over time while giving students repeated practice identifying the key questions, evidence, and implications hidden within complex studies.

This year, we examined the development of nucleic acid vaccines, from early studies of genetic immunization to recent advances in self-amplifying RNA, lipid nanoparticle design, antigen engineering, personalized cancer vaccines, and tolerogenic mRNA therapies. Students followed the field's scientific trail, examining how each new discovery refined the strategy, delivery, and control of mRNA-based medicine. For their final project, students selected a recent publication related to the course theme and prepared a review explaining how the study fits into the larger scientific dossier. The following reviews represent exemplary work completed by students in the course.

## The Replication Directive: A Briefing on the Rise of Self-Amplifying RNA Vaccines

By Yenny Feng\* and JF Trembl\*†

Vaccines are the quintessential form of immunotherapy, with mRNA vaccines evolving into the dominant form of new products over the past four decades. Despite their success, a major challenge remains achieving long-term stability. Even though individual vaccine doses are relatively small, large-scale outbreaks, such as the recent SARS-CoV-2 pandemic, led to limitations in global distribution. The number of doses that could be transported and stored simultaneously at low temperature was insufficient compared to the extent of demand. In their 2025 Nature article, Voigt *et al.* investigated a novel strategy to improve RNA vaccine durability by engineering a self-amplifying RNA (saRNA) platform designed to maintain stability at room temperature, offering a promising path towards more scalable and resilient COVID-19 vaccination.<sup>1</sup>

Voigt *et al.* developed a saRNA vaccine targeting SARS-CoV-2 that induces a strong immune response in mice. This vaccine is formulated with a nanostructured lipid carrier (NLC), making it thermostable and more effective. Researchers showed that

the vaccine not only produced cell-mediated immunity, but also established long-lived antibody-secreting plasma cells. Although the study only demonstrated effectiveness in mice, Voigt *et al.* believe it is an important advance in RNA vaccine technology. Lower dose and thermostability are particularly important for low-income areas, where resources are scarce.<sup>1</sup> This approach is important not only for the SARS-CoV-2 pandemic but also for future outbreaks or pandemics.

Across decades, nucleic acid vaccines have undergone continuous enhancement, from DNA encoding proteins to lipid-encapsulated mRNA products.<sup>2</sup> Early *in vivo* studies showed that injecting DNA expression vectors encoding enzymes such as chloramphenicol acetyltransferase, luciferase, and  $\beta$ -galactosidase into mouse muscle resulted in gene expression without any viral vector (reviewed by Wolff *et al.*)<sup>2</sup> Building on this, plasmid DNA encoding influenza hemagglutinin (HA) was shown to enter host cells, replicate, drive HA antigen expression, and induce strong humoral responses.<sup>3</sup> Parallel work by Tang *et al.*

showed that particle bombardment (gene gun) delivery in skin cells produces clustered reporter gene expression and higher antibody titers with less DNA, highlighting the importance of delivery methods.<sup>4</sup> Extending this work beyond influenza, scientists explored gene inoculation of Human Immunodeficiency Virus (HIV) envelope DNA in mice, demonstrating that DNA vaccination can generate both humoral and cellular immunity against non-influenza pathogens.<sup>5</sup>

In addition to DNA vaccines, mRNA vaccines were also explored. Hoerr *et al.*, investigated the efficiency of RNA-based vaccines encoding for  $\beta$ -galactosidase ( $\beta$ -gal). Results showed that unmodified naked and protamine-encapsulated mRNA could induce antigen-specific CTL and antibody responses across multiple mouse haplotypes (H-2b, H-2d, and H-2k).<sup>6</sup> In contrast, Martinon *et al.* demonstrated that liposome-encapsulated mRNA encoding viral NP could drive strong CTL responses and antigen expression *in vivo*; however, this was tested in a single mouse haplotype (H-2d).<sup>7</sup> Later studies validated the effectiveness of sequence-optimized mRNA vaccines encoding the HA influenza virus, showing high neutralizing antibody titers and complete protection in mouse challenge models.<sup>8</sup>

Kariko *et al.* noticed that the incorporation of modified nucleosides such as pseudouridine, which are present in mammalian RNA but not in prokaryotic nucleic acids, resulted in reduced TLR-mediated cyto-



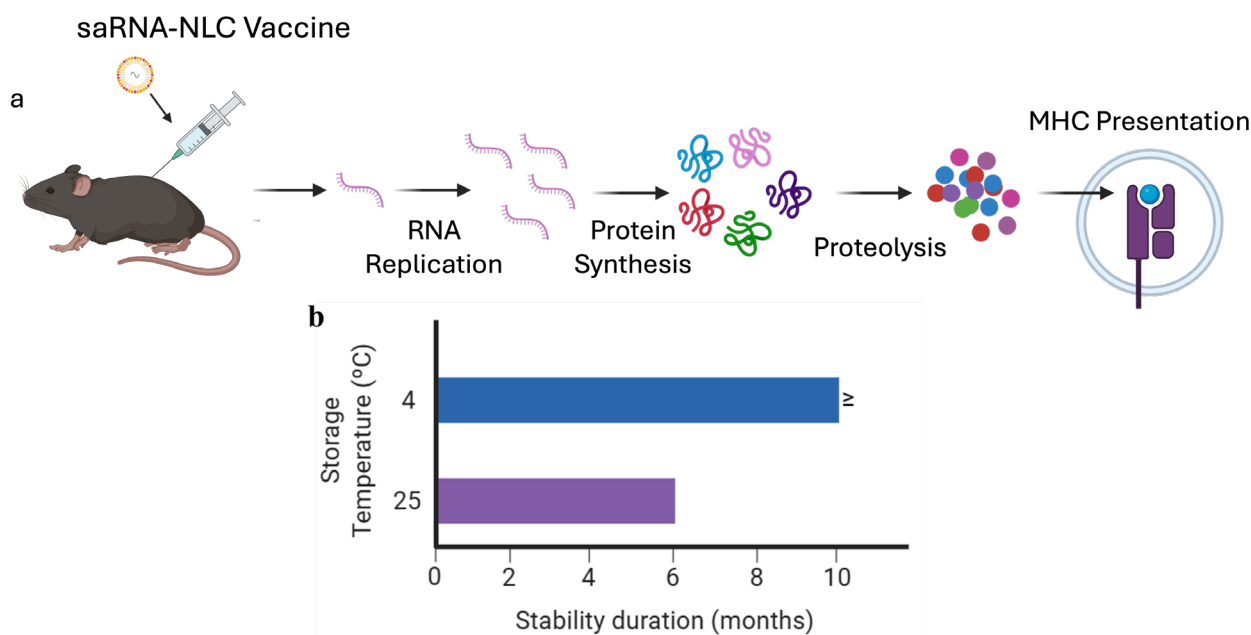
kine production and prevented innate immune overactivation.<sup>9</sup> A second challenge that arose was the strong immune activation triggered at the injection site, which limited tolerability. To address this, Hassett *et al.* evaluated different lipid nanoparticle (LNP) chemistries, confirming that LNP composition is the key determinant of both potency and tolerability.<sup>10</sup>

Hassett *et al.*'s findings were timely with the advent of the SARS-CoV-2 pandemic. Nucleoside-modified mRNA encapsulated in LNPs generated strong immunity against SARS-CoV-2 after a single immunization.<sup>11</sup> This approach also increased *in vivo* expression while minimizing innate immune activation, establishing that chemical modification was essential for achieving

antigen production without excessive inflammation.<sup>12</sup> The potency and immunogenicity were determined by the structure of the ionizable lipid within LNPs, revealing that small chemical changes in lipid design produce large-scale differences in expression, cytokine induction, and tolerability.<sup>13</sup> Recently, Voigt *et al.* introduced a COVID-19 saRNA vaccine. The major advance of this study was the development of a thermostable saRNA vaccine formulated with a nanostructured lipid carrier (NLC).<sup>1</sup> Unlike conventional LNP-mRNA vaccines, which require ultra-cold storage, this platform maintains long-term stability at room temperature (**Fig. 1B**), addressing one of the most persistent barriers to global vaccine distribution.<sup>14</sup> The authors showed that

lyophilized saRNA-NLC remains chemically intact and biologically potent for at least six months at room temperature, and even longer under refrigerated conditions.<sup>1</sup> This represents an essential improvement over current mRNA vaccines, which rapidly lose activity outside cold-chain conditions.

saRNA vaccines induce strong neutralizing antibody responses and Th1 cellular responses, demonstrating that thermostability does not compromise immunogenicity.<sup>1</sup> This design enabled high antigen expression at extremely low doses, allowing the RNA to replicate within the cell (**Fig. 1A**).<sup>1</sup> This effect is vital for a next pandemic response, where manufacturing capacity and cold storage can limit global access. The



**Figure 1 | Mechanism and stability of a self-amplifying RNA (saRNA) vaccine formulated with a nanostructured lipid carrier.** (a) Illustration of low-dose saRNA-NLC vaccine initiating immune activation. After intramuscular injection, the saRNA enters host cells where it undergoes intracellular RNA replication, producing multiple copies of the antigen-encoding RNA. The amplified RNA translates into the viral spike protein. (b) Long-term stability of the saRNA-NLC formulation. Created in BioRender. Feng, Y & Treml, J. (2026) <https://BioRender.com/b6w5qjk>

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study also showed cross-variant neutralization, with measurable activity against multiple SARS-CoV/2 variants, such as alpha, beta, and delta strains, suggesting that the platform remains functional even when the virus evolved.<sup>1</sup>

Overall, the work emphasized manufacturability and adaptability. The NLC formulation is simpler to produce than traditional LNPs, making it more suitable for low-resource settings. By combining saRNA, thermostable formulation, and broad immune activation, this study demonstrates a vaccine platform designed not only for potency but also for global accessibility. It represents a shift from focusing only on immunogenicity to addressing real-world challenges that determine whether vaccines can reach the populations that need them most.

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