A TECHNOLOGY GUIDE

FOR SELF-AMPLIFYING mRNA VACCINES

DNASCRIPT | DDN EXPLORING DRUG DISCOVERY AND DEVELOPMENT

Top considerations for generating self-amplifying mRNA vaccines

BY VINAY MENON, PHD

The technology behind mRNA vaccines has been in development since the 1990s, but it has recently gained popularity since the COVID-19 vaccines became the first mRNA vaccines approved for human use. In the shots developed by Pfizer-BioNTech, Moderna, Johnson & Johnson, and others, patients receive an mRNA strand that encodes the SARS-CoV-2 spike protein, packaged within a lipid nanoparticle. Once inside cells, the mRNA is translated into the spike protein, which triggers an immune response in the host. While these vaccines were incredibly effective, this technology typically necessitates booster shots and larger dosages to maintain host immunity.

A new generation of self-amplifying RNA (saRNA) vaccines aims to solve this problem by carrying the code for a replicase enzyme that amplifies the saRNA within cells. Compared to conventional mRNA vaccines, saRNA vaccines are significantly larger and more complex. They typically contain sequences for the antigen of interest, also known as the subgenome, four non-structural proteins (nSP) that form the replicase, a 5′ cap, untranslated regions (UTRs) at both the 5′ and 3′ ends, and a 3′ poly(A) tail, resulting in a molecule roughly 9 to 12 kilobases in length. Once delivered into cells, the replicase catalyzes the production of many copies of the original saRNA molecule, which continues to produce the antigen of interest.

To develop effective saRNA vaccines, researchers should consider the following key aspects.

1. saRNA design

The RNA molecule itself is typically derived from the genome of single-stranded RNA viruses. Researchers most commonly use Venezuelan equine encephalitis virus and other negative strand alphaviruses, although they have also used positive strand flaviviruses, such as West Nile virus and Dengue virus, and other viral genera for saRNA development. The choice of the viral vector impacts how the saRNA molecule will interact with the host cell. Researchers typically modify the viral vector by replacing viral structural genes with genes encoding the antigen of interest to prevent the production of infectious viral particles (1).

In recent years, researchers have developed several design variations for the saRNA molecule. One group identified six nsP mutations that could enhance subgenome expression, while another created a split replicon system, where the replicase and gene of interest are on separate RNA molecules. Additionally, researchers introduced a new system using trans-amplifying RNA, where a second RNA molecule provides the missing replicase function, which demonstrated similar efficiency to traditional systems in mouse models (2).

2. saRNA production

Researchers can synthesize saRNA molecules from a linear DNA template in a cell-free in vitro transcription (IVT) reaction. Several commercial IVT kits are available to produce 0.1 to 8.5 milligrams of RNA within 30 minutes. Key components in an IVT reaction include a high affinity promoter such as T7, a ribonucleotide triphosphate (rNTP) mix, RNAse inhibitors, pyrophosphatase, magnesium ions, and dithiothreitol. To protect the mRNA strand from enzymatic degradation in the cytoplasm, researchers typically encode a 3′ poly(A) tail directly in the linear DNA template or add it after RNA purification using enzymes like E. coli poly(A) polymerase (3,4). They also add the 5′ cap, which initiates transcription and protects from nuclease degradation, enzymatically using a commercially available vaccinia virus-derived capping enzyme along with guanosine triphosphate (GTP) and S-adenosylmethionine as a methyl source. These capping reactions are highly efficient and proceed in about an hour (3).

Following saRNA production via IVT, the next step is its purification. Due to its large size and polar nature, purifying saRNA molecules is challenging, especially at an industrial scale. For smaller molecules (≤ 3 megadaltons), conventional techniques such as size-exclusion chromatography (SEC) and high-performance liquid chromatography (HPLC) might suffice. For larger molecules, however, researchers typically precipitate the molecule out of cold ethanol with the addition of lithium chloride. Scaling up saRNA purification for applications beyond routine preclinical studies remains an active area of research but could rely on techniques such as tangential flow filtration, SEC, affinity chromatography, or ion-exchange chromatography (2).

3. Delivery systems

After researchers transcribe, process, and purify the saRNA molecule, they package it into an appropriate delivery system. Because of saRNA's large size and negative charge, delivering naked saRNA has limited success. Researchers commonly deliver saRNA vaccines in vivo through both viral and non-viral systems. However, delivery through viral replicon particles can trigger anti-vector immunity (5). Alternative formulations are non-viral delivery systems like polymeric nanoparticles, lipid nanoparticles, or cationic nanoemulsions, which impart a positive charge to protect the saRNA from degradation while not triggering immunogenic responses (2). *In vivo* delivery using non-viral systems has successfully elicited robust host immunity against a wide range of viruses, parasites, and cancers with doses as low as 10 nanograms of saRNA.

Some clever innovations in this area have included the development of a temperature-controllable saRNA that replicates at 33°C, ideal for intradermal administration. In another study, researchers used a synthetic, bioreducible lipid as the delivery vehicle for a SARS-CoV-2-targeting saRNA vaccine, mitigating the biocompatibility issue surrounding other non-viral delivery systems such as polyethylenimine. Scientists were also able to omit the delivery vehicle entirely by delivering DNA that encodes the saRNA in what is called a DNA-launched self-amplifying RNA replicon (DREP). As a DNA-based technology, DREP is inherently more stable than naked saRNA, while producing a robust immune response (6).

4. Improving saRNA stability and efficiency

Since the saRNA technology is still in its infancy, many studies for improving stability and transcription and translation efficiency center around modifying the structural elements, such as UTRs, the poly(A) tail, and the 5′ cap. For example, researchers have replaced the 5′ cap with an anti-reverse cap analog, a modified version of the traditional 5′ cap, which improved RNA transcription four-fold, while adding α- or β-globins to the UTR demonstrated improved RNA stability and translation efficiency. Researchers also found that the length of the poly(A) tail (up to 120 residues) is directly correlated with translation efficiency and protein production (7), underscoring the importance of fine-tuning the poly(A) tail to optimize saRNA-based vaccines and therapies.

Promising horizons

Despite facing challenges related to purification and immunogenic by-products, saRNA vaccines have shown significant promise as therapeutics for a number of infectious diseases and cancers. Beyond improving current COVID-19 vaccines, researchers have tested saRNA vaccines against other global health epidemics, such as human immunodeficiency virus 1 (8). The recent approval of the first saRNA vaccine, ARCT-154, in Japan, which could elicit robust antibody responses at much lower doses compared to conventional mRNA vaccines, highlights the exciting utility and possibilities of this technology for future vaccine and therapeutic development (9).

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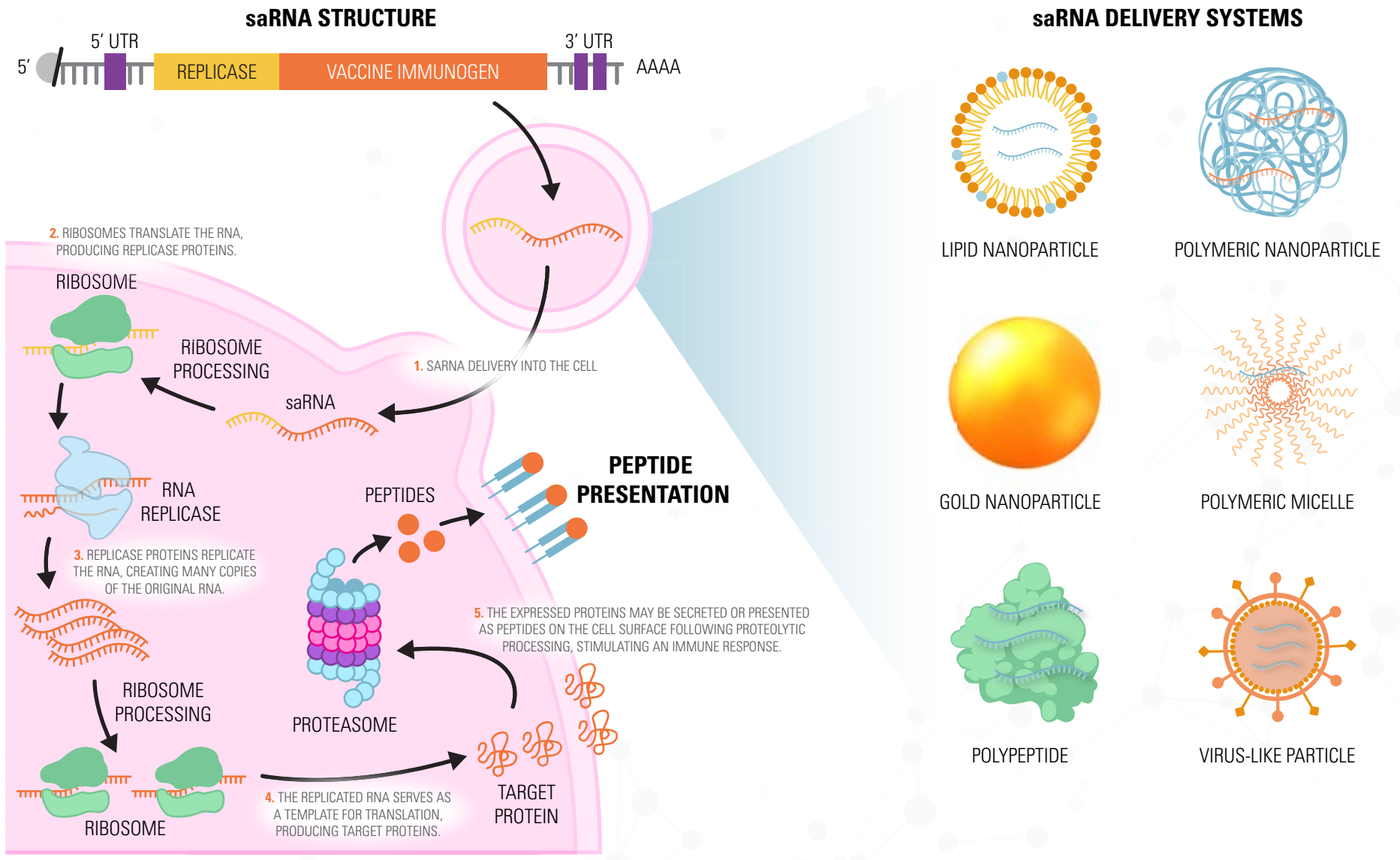
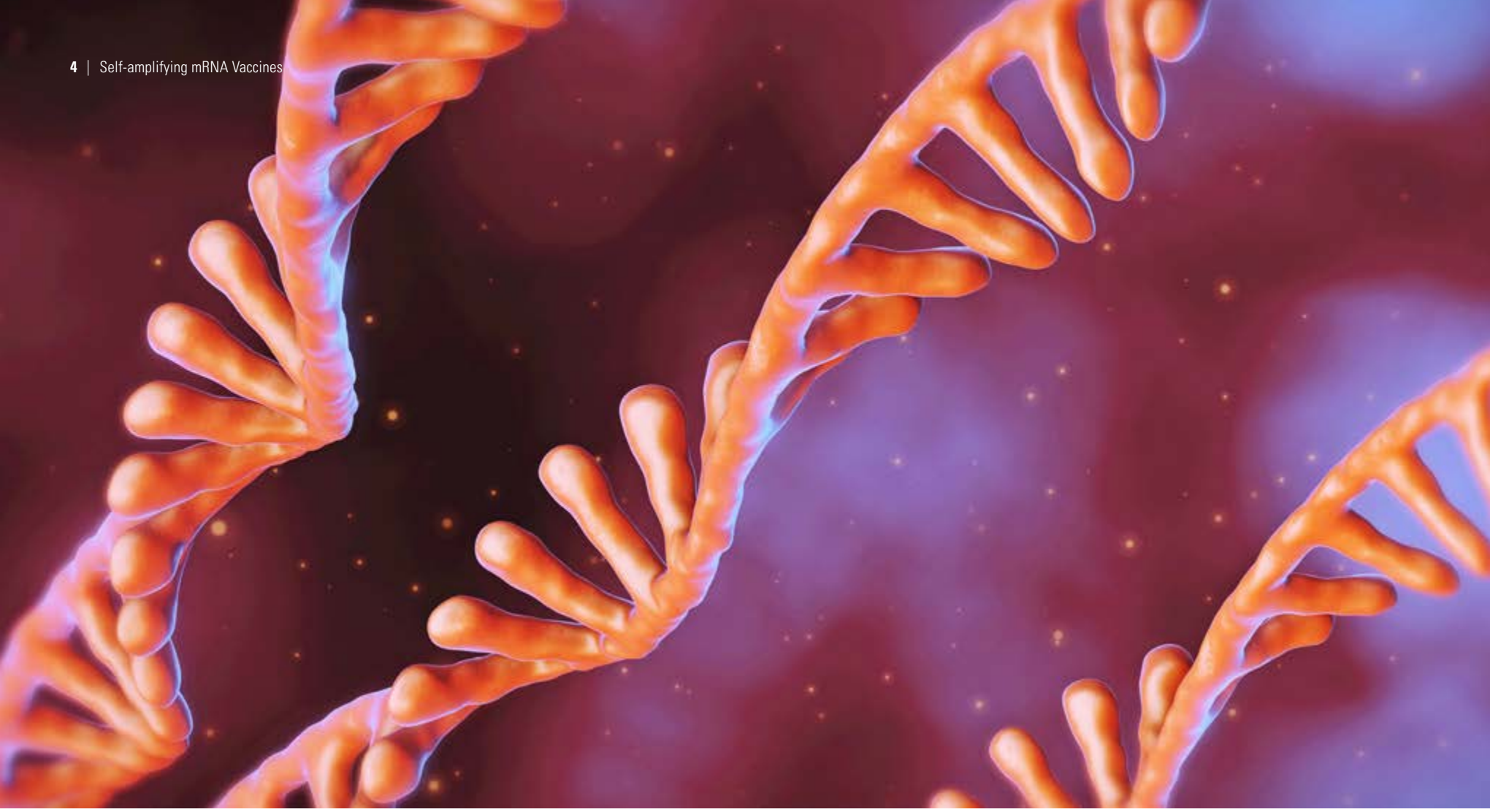


Essential steps and materials for developing saRNA vaccines

Step	Description	Materials needed
1. RNA construct design	Design the self-amplifying RNA with a replicase gene and antigen coding sequence.	- Target antigen gene sequence - Replicase gene - 5′ and 3′ UTRs for stability and efficient translation - 5′ cap - 3′ poly(A) tail
2. RNA synthesis and purification	Synthesize the self-amplifying RNA construct in vitro and purify the synthesized RNA to remove impurities and unwanted byproducts.	- IVT system (promoter, rNTPs, RNAse inhibitors, pyrophosphatase, magnesium ions, and dithiothreitol) - RNA purification kits - SEC, HPLC, affinity chromatography, ion-exchange chromatography
3. Encapsulation in delivery system	Package the saRNA into lipid nanoparticles or other suitable delivery systems for efficient cell entry.	- Lipid nanoparticles (e.g., 1,2-distearoyl-sn-glycero-3-phosphocholine, cholesterol), polymeric nanoparticles (e.g., polyethyleneimine, dioleoyl-3-trimethylammonium propane) , or other delivery vehicles - Solvents (e.g., ethanol, chloroform for lipid dissolution)
4. Quality control	Ensure that the final product is free from contaminants and has high RNA integrity.	- Dynamic light scattering system for size measurement - Zeta potential analyzer for charge measurement - UV-Vis spectrophotometer for RNA concentration - RNA integrity assay (e.g., gel electrophoresis, Nanodrop)
5. Preclinical testing	Conduct animal studies to evaluate immunogenicity, safety, and efficacy.	- Animal models (e.g., mice, non-human primates) - Immune response assays (e.g., ELISA, flow cytometry)
6. Scale-up and production	Scale up RNA synthesis and delivery system formulation for large-scale vaccine production.	- Bioreactors for RNA synthesis - Industrial-scale purification and formulation equipment
7. Clinical trials	Test the vaccine in human clinical trials to evaluate safety and efficacy.	- Clinical trial protocols and infrastructure - Regulatory approvals (e.g., FDA, EMA)

This table outlines the steps and materials required to create and develop saRNA vaccines. The exact materials can vary depending on the specific design of the vaccine, the target antigen, and the chosen delivery system.



EXPERT ADVICE:
Printing a new path for self-amplifying RNA vaccine development

As researchers push the boundaries of RNA vaccine design, innovations in DNA synthesis are helping them overcome longstanding technical barriers to speed and complexity.

INTERVIEWED BY YUNING WANG, PHD

THE ABILITY TO REPROGRAM living cells using synthetic genetic material has transformed modern biotechnology. During the COVID-19 pandemic, mRNA vaccines demonstrated how quickly scientists could design and produce effective immunizations. Now, researchers are exploring new platforms such as self-amplifying RNA (saRNA), which can extend the duration of protein expression and reduce the amount of material needed per dose.

Developing saRNA vaccines, however, presents its own challenges. These RNA molecules are longer and more complex than standard mRNA constructs, requiring precise design and high-quality DNA templates. That complexity often slows down research and limits the pace of discovery.

Thomas Ybert, Chief Scientific Officer at DNA Script and a molecular biologist by training, has spent his career trying to speed things up. While working in the San Francisco Bay Area, he was struck by the difference in innovation speed between the tech and life sciences sectors. "The turnaround time for the innovation cycle in tech — the design, build, test loop — was incredibly fast," Ybert said. "In life sciences, it's incredibly slow and tedious."

To help close that gap, Ybert co-founded DNA Script to develop a faster, more flexible, and more accessible way to generate synthetic DNA. The company's core technology, enzymatic DNA synthesis (EDS), uses enzymes rather than harsh chemical reagents to build DNA sequences. This approach enables researchers to generate long, complex sequences with greater speed and accuracy right at the lab bench. With the EDS-powered benchtop instrument, the SYNTAX DNA printer, Ybert's team is working to remove a key bottleneck in RNA research and accelerate the development of advanced therapeutics.

What led you to the field of synthetic biology?

I'm a molecular biologist and yeast geneticist by training. I began with an engineering background in France and completed my PhD at Sanofi, where I worked on using yeast to manufacture monoclonal antibodies. From there, I shifted toward the field of sustainability, working on bio-jet fuel development through a collaboration between TotalEnergies and Amyris. That experience helped me realize the incredible potential of life sciences to address major global challenges, from health to energy, yet it was clear the field was vastly underfunded and underpowered compared to the tech world.

One reason for that, I believed, was the slow pace of biological innovation cycles. Unlike the fast turnaround in tech, biology is much slower to build, test, and iterate. That led me to the idea of programmable biology. Just as we can print a document on demand, why not print DNA? That vision inspired me to invent a DNA printer based on enzymatic synthesis, a technology that ultimately became the foundation for DNA Script.

What distinguishes saRNA from traditional mRNA at a construct design level?

Traditional mRNA vaccines deliver a short-lived RNA strand that gets translated into protein and then degrades. They work well but usually require high doses and may not last long in the body.

Self-amplifying RNA, or saRNA, carries extra genetic components that allow it to replicate itself inside the cell. This self-replication amplifies protein expression, meaning we can

CREDIT: THOMAS YBERT

Ybert is pioneering enzymatic DNA synthesis to accelerate RNA vaccine development.

"Instead of relying on *in vivo* systems like *E. coli*, which are slow, costly, and difficult to scale safely, we want researchers to access high-quality DNA and RNA in days, not weeks."

CREDIT: THOMAS YBERT

The SYNTAX System by DNA Script enables benchtop enzymatic DNA synthesis, allowing researchers to rapidly generate high-quality DNA templates for applications like saRNA vaccine development.

use a smaller initial dose and still achieve a strong, sustained response. It's especially promising for vaccines or therapies that benefit from longer-lasting protein expression and fewer administrations.

Structurally, saRNA contains everything mRNA does, including a 5' cap, untranslated regions (UTRs), the coding sequence or payload, and a poly(A) tail. But it also includes genes encoding non-structural proteins (NSPs) that drive replication, typically derived from alphaviruses. A subgenomic promoter also helps ensure proper expression of the payload. All of these additions increase the length and complexity of saRNA constructs. While mRNA is typically one to three kilobases long, saRNA ranges from 10 to 15 kilobases. That makes it much more difficult to synthesize, assemble, and deliver.

How do researchers typically design these complex saRNA constructs?

Most researchers use components from established libraries. The NSPs are usually derived from alphaviruses like Venezuelan equine encephalitis virus. These aren't designed from scratch. Instead, researchers choose from a menu of known elements and optimize them depending on where and how the saRNA will be used.

The main challenge comes in testing the right combination. It's a bit like screening promoter libraries. Researchers might combine different UTRs, NSPs, and payload sequences to see what works best. The longer the construct, the more difficult it becomes to assemble and test. Eventually, we may see AI tools that can help automate some of this optimization. But for now, it's a process that still involves trial and error.

What are the biggest hurdles researchers face when constructing saRNA?

First, capping is critical. It enables ribosome binding and successful translation. If the capping isn't efficient, the RNA won't express properly. Then there are the UTRs, which stabilize the RNA and influence how much protein gets made. The poly(A) tail also plays an important role in stabilizing the molecule and preventing degradation.

One interesting trend we're seeing is the demand for longer poly(A) tails. Some researchers are looking for tails with 300, 400, or even 600 adenines. They believe longer tails improve RNA stability and increase protein expression, but most traditional synthesis methods can't precisely control tail length at that scale.

The biggest bottleneck is often the DNA template needed for *in vitro* transcription (IVT). For standard mRNA, this isn't too hard. But saRNA templates are much larger and more complex. Conventional synthesis methods can take weeks to deliver them and sometimes fail entirely due to sequence complexity.

How does DNA Script's EDS technology help overcome these challenges?

Enzymatic DNA synthesis, or EDS, uses enzymes instead of chemical catalysts to build DNA one base at a time. Enzymes are more efficient and operate in aqueous conditions, which makes the process safer and more flexible.

Our technology lets researchers synthesize both short and long oligonucleotides with high fidelity. We can go up to 600 bases per oligo, and then assemble them into very large constructs, even up to 15 kilobases in size. That includes regions that are traditionally hard to synthesize, like GC-rich sequences or very long poly(A) tails. This approach helps researchers get the DNA templates they need for IVT without the delays and limitations of traditional synthesis. It's a faster and more reliable path from sequence design to functional RNA.

How much faster and easier is this process compared to traditional gene synthesis?

Traditional providers might take weeks or even months for a complex construct like a full-length saRNA template. With our EDS technology and the SYNTAX DNA printer, we've shrunk that to as little as 24–72 hours. That's a massive acceleration, especially valuable in situations like personalized cancer vaccine development, where time is critical.

The SYNTAX DNA printer is designed to be accessible, like a diagnostic machine in a clinical lab. The user uploads the DNA sequence and ensures the reagents are loaded. That's it. You don't need a PhD in organic chemistry. Our platform handles the complexity. It's like operating a regular printer, just for DNA.

How are researchers incorporating your platform into their RNA development workflows today?

Several major pharma and biopharma companies, including some involved in COVID-19 vaccine development, are already using SYNTAX to screen mRNA designs. They print oligos, assemble them into DNA templates, run IVT, and test expression — all on a fast design-build-test cycle. For future products, we aim to integrate the entire workflow — DNA synthesis, IVT, and potentially even formulation — into a fully synthetic, end-to-end system. That could make personalized cancer vaccines feasible at scale. Instead of relying on slow, expensive bacterial production and shipping across continents, labs could generate saRNA therapies on-site, quickly and safely.

What's next for your team?

We're working toward that vision of a fully integrated, synthetic workflow that can power applications like personalized cancer vaccines and rapid therapeutic development. Instead of relying on in vivo systems like *E. coli*, which are slow, costly, and difficult to scale safely, we want researchers to access high-quality DNA and RNA in days, not weeks.

We've come a long way, from a single scientist with an idea to a company with over 200 employees and multiple products on the market. Now we're building the next generation of tools to make biology programmable, scalable, and accessible.

This interview has been condensed and edited for clarity.