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Optimizing mRNA Purification

The revolution of mRNA-based therapeutics

mRNA manufacturing workflow

Tools to optimize mRNA purification

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Contents

The Revolution of mRNA-Based Therapeutics	4
Engineering the Future: Unraveling the Manufacturing Workflow of mRNA Therapeutics	10
Enabling mRNA-Based Therapeutics Development Through Efficient and Scalable mRNA Purification Methods	11
The mRNA Therapeutics Boom	12
New Bead Technology Enables Commercial-Scale mRNA Purification	17
Optimizing mRNA Purification Conditions by Using a High-Throughput Approach	19
Curated content to boost your mRNA purification process	20

Foreword

The potential of mRNA as a therapeutic tool has been recognized by researchers ever since its discovery in 1961. However, it was not until 2020, when mRNA vaccines for COVID-19 received approval, that the therapeutic promise of mRNA was fully realized.

Building on this success, researchers are today actively working on harnessing the power of mRNA to create new therapies for a broad range of medical conditions, including cancer, genetic disorders and other infectious diseases. As this field rapidly advances, mRNA therapies hold the promise of revolutionizing modern healthcare and opening new avenues for personalized and precise treatments.

Nevertheless, before these groundbreaking therapies can be scaled up and made available for patients, manufacturing processes need to be optimized. Solving this challenge is essential to maximize efficiency and ensure the production of adequate quantities of mRNA-based therapeutics for widespread application and accessibility.

This eBook reviews the history of mRNA-based therapeutics, examines the upstream and downstream steps in the manufacturing process of these products and presents innovative purification tools. Altogether, this eBook is an essential read for anyone interested in this revolutionary therapeutic tool.

The Revolution of mRNA-Based Therapeutics

Messenger RNA (mRNA) is a type of single-stranded ribonucleic acid that can be translated into functional proteins. Researchers have acknowledged mRNA's therapeutic potential since its discovery in 1961. However, it wasn't until 2020, when mRNA vaccines against COVID-19 were approved, that mRNA's therapeutic promise was finally fulfilled. Following this success, other mRNA therapies to treat a broad range of diseases are now under development. The first step in any mRNA-based therapy is the generation of a synthetic mRNA encoding the gene of interest using the *in vitro* transcription (IVT) process. This mRNA is usually encapsulated within a delivery system before being administered to the patient. Once inside a cell, the mRNA directs the translation of the desired protein (Figure 1).[1] This article summarizes the advances in mRNA research since its discovery, examines its therapeutic potential and discusses the future perspectives of the field.

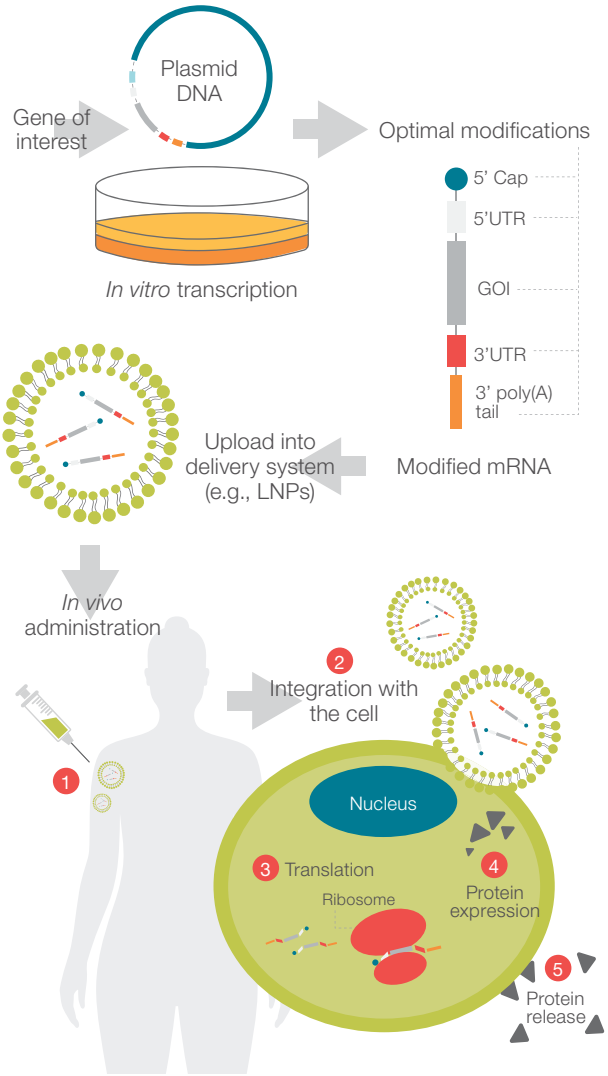

Figure 1. *Steps involved in mRNA-based therapies.*[1]

Six decades of research and development

Basic research (1961–1990)

The discoveries leading to the development of mRNA-based therapeutics began more than 60 years ago. Knowledge accumulated during the 1950s on the structure and function of DNA contributed to the discovery of mRNA in 1961 by two independent research groups.[2] The timeline in Figure 2 presents some of the milestones in mRNA research. For example, the development of mRNA delivery systems started in the same year as mRNA discovery.[3] Some years later, researchers successfully achieved *in vitro* transcription of mRNA in a mammalian cell-free system for the first

time.[4] Soon after, scientists successfully delivered mRNA into cells of different animal species, which led to protein translation and expression.[5,6] The commercialization of various materials for *in vitro* mRNA production began in the 1980s, making IVT more accessible and paving the way for numerous applications. Finally, in 1990, it was demonstrated that synthetic mRNA also leads to successful protein expression in *in vivo* models.[7] This milestone can be considered the end of the first phase in the development of mRNA-based therapeutics. Researchers now had the tools to produce mRNA *in vitro*, different methods for mRNA delivery and were able to successfully express proteins both *in vitro* and *in vivo*.

Exploring clinical applications (1990–2019)

Following this first phase, researchers in subsequent years used the knowledge accumulated to develop the first mRNA clinical applications (Figure 2).[8]

Protein replacement

The first study on mRNA-based protein replacement was reported in 1992. Researchers injected mRNA coding for vasopressin into the hypothalamus of rats unable to produce this protein – a condition that leads to diabetes insipidus. The injected mRNA was successfully translated

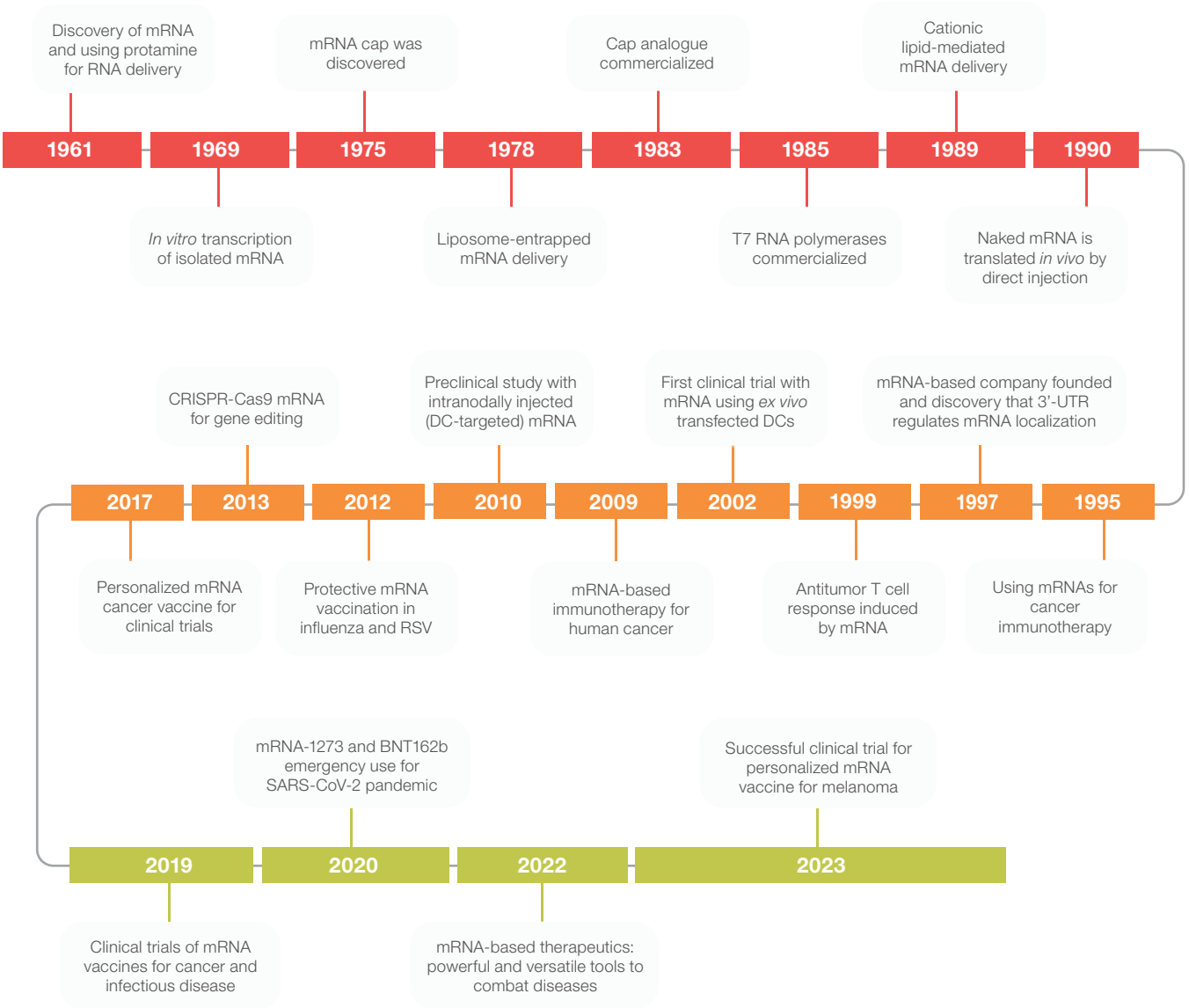

Figure 2. *Representative milestones in mRNA research and mRNA-based therapeutics development.*[8]

to vasopressin and temporarily reversed diabetes insipidus within hours.[9]

Cancer vaccines

In 1995, the use of mRNA as a cancer vaccine was evaluated for the first time.[10] This preclinical study demonstrated that intramuscular injection of naked RNA encoding carcinoembryonic antigen elicited antigen-specific antibody responses in mice. Another milestone in mRNA-based cancer therapy occurred in 1999 when researchers induced antitumor T-cell responses by mRNA injection *in vivo*.[11] In this case, mRNA coding for a human melanoma-associated antigen was encapsulated in liposomes and injected into mice's spleens, inducing both specific antibody and T-cell responses. Moreover, this injection delayed tumor growth and significantly prolonged survival.[11] These and other preclinical studies demonstrated the potential of mRNA-based tumor antigen vaccines for human cancer treatment.

In 2002, the first clinical trial for an mRNA-based cancer vaccine took place. Dendritic cells (DCs) transfected with mRNA encoding prostate-specific antigens (PSA) were injected into patients with metastatic prostate cancer. Following this treatment, patients displayed PSA-specific T-cell responses.[12] Similar results were obtained when metastatic melanoma patients were administered with protamine-protected mRNA coding for different tumor-associated antigens.[13] These studies confirmed that mRNA-based cancer vaccination was feasible and safe, encouraging further clinical investigation. In 2017, the first personalized mRNA vaccine was administered to patients with melanoma.[14] The vaccine induced specific immune responses, decreased the metastatic rate and prolonged the patients' progression-free survival.[14]

Vaccines for infectious diseases

The potential of mRNA-based prophylactic vaccines for infectious diseases was also explored. In 2012, two preclinical studies demonstrated the feasibility of mRNA-

Table 1. *Examples of mRNA-based therapeutics and their applications.*

Application	mRNA encoding	Examples
Prophylactic vaccines for infectious diseases	Pathogen-specific antigens	SARS-CoV-2 vaccines (approved) HIV (phase I clinical trial)[27] Influenza (phase III clinical trial)[28]
Cancer vaccines	Immunostimulant molecules Specific-tumor antigens	Solid tumors (phase I clinical trial)[29] Personalized vaccines for metastatic melanoma or epithelial cancer (phase II clinical trial)[30]
Protein replacement	Normal copy of the deficient/absent protein	Cystic fibrosis (phase II clinical trial)[31] Methylmalonic acidemia (phase II clinical trial)[32]
Antibody production	Antigen-specific antibodies	Bi-specific antibodies against cancer (preclinical)[33] Antibodies against Chikungunya virus (preclinical).[34]
Gene editing	CRISPR/Cas9 gene editing system	Transthyretin amyloidosis (phase I clinical trial)[35,36] Hereditary angioedema (phase II clinical trial)[37]

based protective vaccines for influenza and respiratory syncytial viruses.[15,16] In 2017, the first clinical trial for a prophylactic mRNA-based vaccine against rabies was performed.[17] This study served as a proof of concept and was followed by other trials in the subsequent years.

Gene editing

Yet another modality of mRNA-based therapeutic emerged in 2012 with the description of Cas9 as an RNA-guided endonuclease (RGEN).[18] The study described a family of endonucleases that use dual-RNAs for site-specific DNA cleavage and highlighted the potential of this system for RNA-programmable genome editing. The following year, it was shown that RGENs can be used to program genome editing in human cells.[19]

mRNA as a disruptive therapeutic technology (2019–present)

Since 2019, numerous clinical trials have been testing the efficacy and safety of mRNA-based vaccines for various types of cancer and infectious diseases.[20] The first approved therapeutics arrived in 2020, during the COVID-19 pandemic, with two mRNA vaccines against the SARS-CoV-2 virus (BNT162b2 and mRNA-1273).[21,22] These vaccines proved that mRNA is a powerful tool to combat disease and further boosted the number of clinical trials taking place. In April 2023, the results of a phase IIb clinical trial showed that a personalized mRNA-based cancer vaccine developed by Moderna, in combination with the immune checkpoint inhibitor pembrolizumab developed by Merck, cut the risks of recurrence or death by 44% in patients with high-risk melanoma.[23] Almost certainly, the next few years will witness the approval of new mRNA-based therapies.

mRNA-based therapeutics today

Currently, mRNA therapeutics fall into four basic categories: prophylactic vaccines, therapeutic vaccines, protein-encoding therapies (protein replacement and antibody production) and gene editing therapies (Table 1). There are hundreds of ongoing clinical trials investigating mRNA-based therapies for different types of diseases, including cancer, infectious diseases and genetic disorders.[24] All these applications require effective delivery of the mRNA to the target cells and different types of delivery systems have been developed to achieve this. At present, there are around 60 types of delivery systems, including various nanoparticles, polymers and viruses.[8,25] mRNA (naked or encapsulated in a delivery system) can be administered directly to the patient using different routes (e.g., intravenous, intramuscular, inhalation).[25] Yet, another strategy is to first transfect cells *ex vivo* (e.g., dendritic cells) and then inject these cells containing the therapeutic mRNA into the patient.[26]

Future perspectives

mRNA-based therapeutics are a novel and versatile alternative to traditional drugs and biologics, with enormous potential. They offer numerous advantages compared to other biologics. For example, mRNA overcomes the obstacles of post-translational modification, folding and assembly of protein-based therapies. The technology also allows for the delivery of "instructions" to synthesize multiple proteins (or proteins with multiple subunits) in a dose dependent manner. mRNA is safer than DNA-based therapies as it does not integrate into the host's genome, making the risk of insertion mutagenicity negligible. In addition, as mRNA itself is only transiently active, the burden to the host homeostasis is minimized. mRNA-based therapeutics are also more flexible, as the safety, efficacy, delivery and duration of action can be manipulated by altering the structure of the original molecule.[38]

Despite all these advantages, challenges still exist. For example, further research is needed to optimize delivery systems to improve mRNA delivery, activity and cell targeting.[1] This is particularly important for applications that need cell- or tissue-specific delivery. Improving mRNA stability (to avoid early degradation) and ensuring prolonged expression patterns (especially in chronic diseases requiring sustained protein expression) is also crucial.[1] Another important challenge to overcome is synthetic mRNA's high innate immunogenicity, which is undesired in applications such as protein-replacement therapies. Although modified nucleosides can suppress mRNA immunogenicity, this approach is expensive and imposes extra constraints in sequence design.[39] Hence, further research is needed to address this hurdle. Exploring the most appropriate administration route for each mRNA therapeutic is also essential to ensure the best results.[17]

The mRNA manufacturing process is cell-free, making it simpler, faster and cheaper to produce than other biologics. However, as it is still an emerging technology, manufacturers face a lack of clear and comprehensive regulatory guidance.[40] For instance, the impurity profile and critical quality attributes (CQAs) of mRNA-based products are not yet clearly defined – US Pharmacopeia has published some guidelines for CQAs and analytical techniques but there are no defined critical limits to any of the CQAs.[41] Moreover, establishing robust and scalable production processes to meet the growing demand for mRNA therapeutics is a priority in the industry. Process development and optimization to increase yield and quality, and to reduce costs, are still in their early stages. Researchers and manufacturers are actively exploring various approaches, such as improving mRNA synthesis methods and optimizing purification techniques. In addition, new formulations are being developed to over-come storage and shipping drawbacks.[40] Refining these processes and leveraging technological advancements are essential to ensure the accessibility and

widespread adoption of mRNA therapeutics for the benefit of patients worldwide.

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Engineering the Future: Unraveling the Manufacturing Workflow of

mRNA Therapeutics

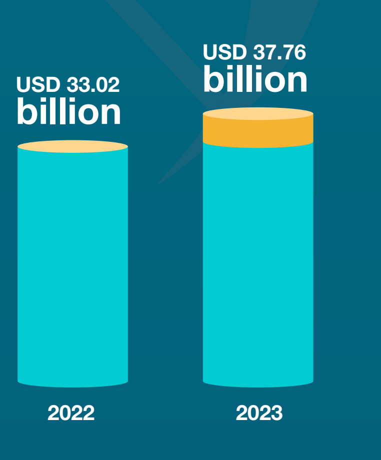
Breakthroughs in vaccine technology during the COVID-19 pandemic expanded the potential for mRNA-based therapeutics, opening the door to new disease treatment options. In this scenario, optimization of the mRNA manufacturing workflow became essential to sustain the increasing demand of these therapeutics.

This infographic provides valuable insights into the bioprocessing steps involved in the production of mRNA therapeutics.

Applications of mRNA therapeutics

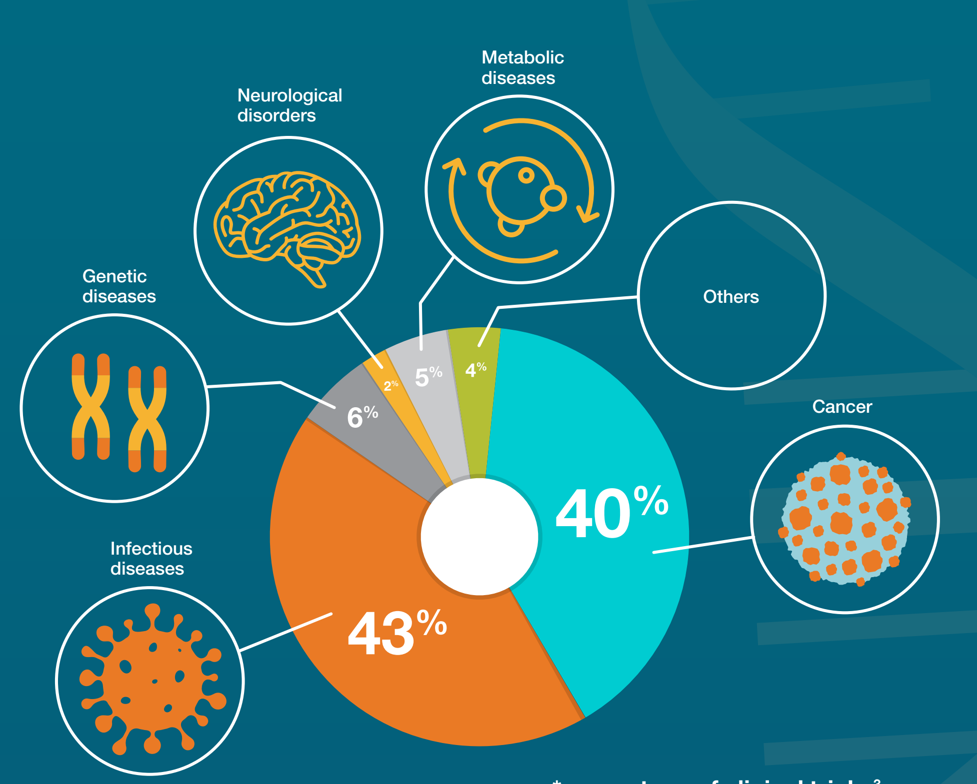
The field of mRNA-based therapeutics is rapidly expanding and projected to reach a global market size of USD 37.76 billion by 2030.[1] Various types of mRNA-based therapies are currently under development, including vaccines, mRNA-enhanced cell therapy, mRNA-based antibody production and mRNA-based protein replacements.[2] These innovative therapies hold the potential to treat a diverse range of diseases.

Global mRNA therapeutics market



market forecast to grow at a CAGR of 1.7%.

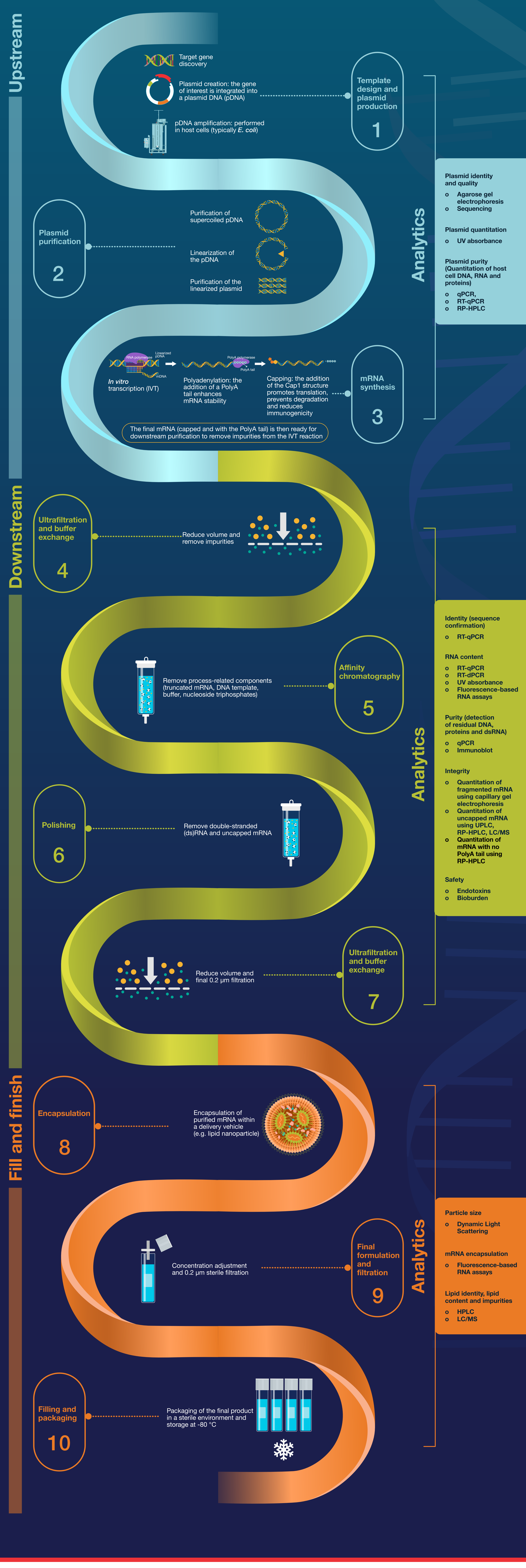
Applications



*percentage of clinical trials.[3]

How are mRNAs manufactured?

mRNA manufacturing begins with the production of plasmids containing a gene of interest. These plasmid templates are then used to synthesize the therapeutic mRNA through the process of *in vitro* transcription (IVT). Although IVT is a cell-free system, reaction mixtures may still contain impurities that can reduce translation efficiency and increase immunogenicity if delivered to the cells. Hence, impurities must be removed by chromatographic methods during downstream processing. The purified mRNA is encapsulated in a delivery vehicle to be administered to patients. A variety of technologies are employed throughout the bioprocessing workflow to ensure the quality and safety of the final therapeutic product.



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Enabling mRNA-Based Therapeutics Development Through Efficient and Scalable mRNA Purification Methods

The possibilities of mRNA-based therapeutics appear limitless, with a growing number of applications swiftly populating the clinical pipeline. As a result, the global market for these products is experiencing remarkable growth. To keep up with this surge in demand, the industry is actively exploring efficient and scalable manufacturing methods to support the commercial production of mRNA-based therapeutics.

A significant breakthrough in this field comes from the development of affinity resins that can bind mRNA through a simple AT base-pairing mechanism. These innovative resins serve as a purification platform for the purification of all mRNA molecules that contain a polyA tail.

Watch this webinar to learn more about the advantages of affinity capture of mRNA and how it maximizes the efficiency of mRNA purification.*

Watch Now

*webinar originally cited from BPI international

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The mRNA Therapeutics Boom

How to Simplify Workflows and Maximize the Efficiency of the mRNA Purification Process with POROS™ Oligo (dT)25 Affinity Resin.

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Scalable Purification of *In Vitro* Transcribed mRNA Accelerates mRNA-Based Therapy Development

mRNA is beautifully simple; it provides your body instructions. Analogous to computer code, mRNA programs the body to produce specific proteins giving this molecule utility in a myriad of therapeutic approaches, including vaccines against common and rare infectious diseases, oncology indications, and protein replacement treatments for genetic disorders. The wide diversity of mRNA-based therapeutic applications has led to increased interest in using synthetic mRNA.

In the early 90s, scientists demonstrated efficacy when using mRNA as a potential therapy.[1,2] But interest in antibodies, whose potential was more broadly accepted, took over in prominence. Today, mRNA

is back in focus with the general acknowledgment that these types of therapies do not target and manipulate genes and DNA.

To date, no mRNA-based therapy has been commercialized though some are in late-stage trials or approved for emergency use, such as vaccines targeting SARS-CoV-2. The vast majority of therapies in development apply to relatively small populations, thousands to hundreds of thousands of patients. Even mRNA-based cancer immunotherapies would serve a significantly smaller subset of patients than a global vaccination campaign.

Since preclinical and early clinical pipelines of most of these mRNA therapies only required a few liters of materials, traditional laboratory-scale approaches, such as precipitation, were leveraged for production.

"We are going to see mRNA therapies start to move quicker to the clinic," says Kelly Flook, PhD, Senior Product Manager, Purification Products, Thermo Fisher Scientific, "and a greater acceptance of protein replacement therapies and varied immunological approaches than are currently being evaluated."

Scaling-Up Options

The drive to rapidly develop a COVID vaccine put a focus on large-scale mRNA manufacturing. The limits of research-scale purification techniques were realized, and available purification methods became a bottleneck for commercialization.

To resolve this challenge, different options are under investigation. For example, scaling up reverse-phase chromatography is of interest. "It is scalable but not as efficient as an affinity approach in purifying the product and removing process impurities," says Flook. In addition, reverse-phase chromatography uses flammable solvents requiring the removal of detrimental post-purification impurities. Safety is a concern, as well as the necessity and expense of building a chemical manufacturing site to handle the solvents.

"Aqueous-based techniques, ion exchange, and affinity, are commonly used in research, and a similar solution is desirable for scale-up production of mRNA," says Flook. Process speed also plays an important role.

"A few years ago, as more companies began working on therapeutics in this space, we saw an increase in inquiries about large-scale mRNA purification," says Flook. "Most resins on the market were research-scale technology, such as our popular Dynabead option with a polyT on the surface. Initially, we provided custom resins until the momentum grew, and it made sense to develop a generic product. So we took the polyT technology and applied it to our bioprocessing POROS™ resins."

Producing mRNA

"RNA is made using a process called in vitro transcription (IVT). During the IVT process, DNA is converted to RNA," explains Venkata Indurthi, PhD, Vice President, Research and Development, Aldevron. "It is critical to get rid of all the impurities after the reaction is complete, including any residual raw materials, because they can trigger nonspecific immune responses."

Compared to DNA, RNA is fragile; harsh purification techniques are unsuitable. RNA also has secondary structures that can impact purification.

"There are multiple ways of purifying RNA, charge-based methods, precipitation-based methods, and others like hydrophobic interaction chromatography (HIC)," says Indurthi. "The specific advantage of the affinity oligo dT approach is that you can easily get rid of the impurities generated during IVT."

Affinity chromatography, a highly-scalable method, has earned its credits in the development of biologics, such as the use of Protein A for the purification of therapeutic antibodies and, more recently, anti-AAV resins in gene therapy workflows. An effective affinity purification step can help to simplify biomolecule downstream processing, reduce the number of purification steps, and lower the overall cost of goods in biotherapeutic manufacturing.

Thermo Fisher's new affinity-based mRNA chroma-

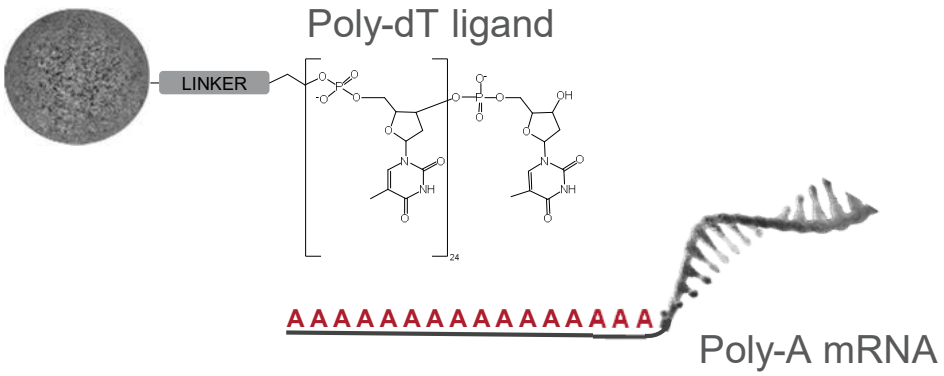

Fig. 1: POROS Oligo (dT)25 affinity resin consists of a polydeoxythymidine (dT-25) ligand attached to a 50µm rigid, porous bead through a proprietary linker. The poly-dT ligand allows binding with poly-A tailed mRNA molecules through AT base pairing.

tography resin, POROS Oligo (dT)25, was specifically developed for the scalable purification and isolation of mRNA from the IVT manufacturing processes.

"We worked closely with our customers to develop the resin, including AmpTec, a leading RNA CRO, in Europe," says Flook. "They were tasked to develop a scalable, efficient method for manufacturing mRNA that would allow them to take on large-scale vaccine manufacturing."

The Oligo dT Affinity Approach

POROS Oligo (dT)25 is based on POROS resin technology, a poly(styrene-divinylbenzene) base bead coated with a proprietary functional hydrophilic coating to reduce nonspecific binding. A dT-25–poly-deoxythymidine ligand is attached to the bead surface.

Since every mRNA has a polyA tail for molecular stability, the resin is a platform solution. "Across a range of mRNA sizes and constructs, you get equivalent recovery, purity, and yield," says Flook. "The size or sequence does not matter; the resin can be used to purify anything that has a polyA tail."

Use of the resin is straightforward; the polyT ligand on the bead binds to the polyA tail of the mRNA. In brief, hydrogen bonding occurs as salt neutralizes the backbone of the mRNA and the polyT, allowing flushing and removal of the non-bound IVT components. After the salt is removed, the hydrogen bonds break, and the polyA containing mRNA is eluted.

Typically, this affinity approach is used at the beginning of the purification scheme to remove process-related impurities, such as DNA templates, nucleotides, enzymes and buffer components, and other constituents such as mRNA without a polyA tail.

In some cases, a product-related impurity can result from IVT, such as double-stranded RNA (hairpin) or another undesirable species that has a polyA tail. "Then we suggest adding a second polishing step with ion exchange or HIC. The loop-back double-stranded effect can also be engineered out during the IVT process," says Flook. "Another optional way to use the resin is downstream as a final polishing step or for buffer exchange. You can elute in water and formulate directly from that."

Depending on the application, mRNA will vary in size and design of the backbone. "As a CDMO, Aldevron has been supplying RNA for a couple of years at all quality grades from RUO to GMP, and we are continuing to invest heavily in the space," says Indurthi. "RNA has a 5′ UTR and a 3′UTR and varying sequences.

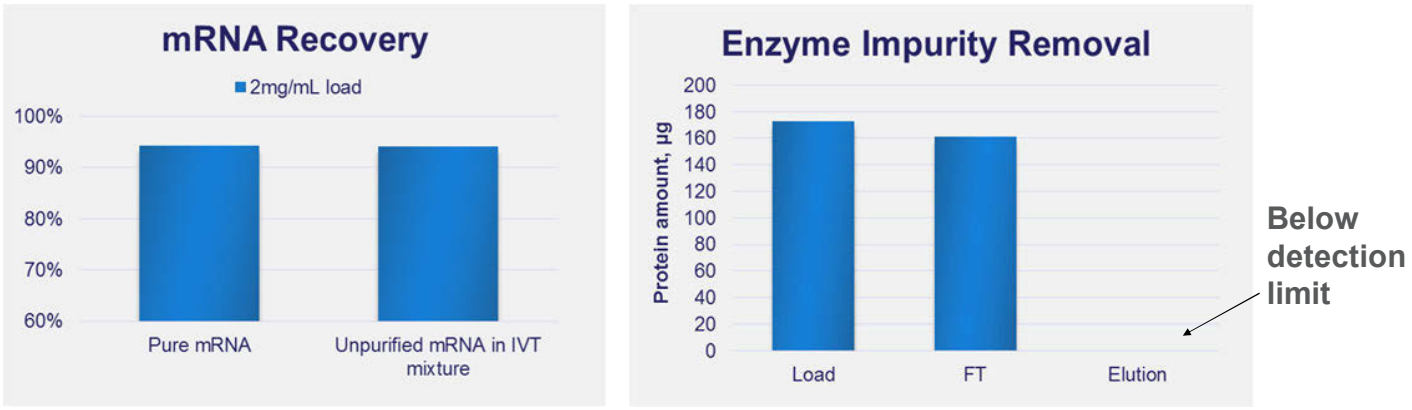

Fig. 2: High recovery and purity independent of sample type used. Recovery of mRNA from pure mRNA and unpurified IVT mixture, showed no differences (left). Amount of protein was determined in load, flow through (FT) and elution pools (right). No proteins were detected in the elution pool, indicating excellent impurity removal of IVT mixture products.

We do not control the design, and different designs can trigger complexity in the purification process."

The POROS Oligo (dT)25 resin, as a purification platform, can be cross applied to different constructs, emphasizes Flook. The resin addresses the current challenges involved with large-scale mRNA purification for potential clinical use by simplifying the downstream process, increasing purity and yield, and allowing for scale-up without the use of toxic chemicals.

Validating the POROS Oligo (dT)25 Resin

Standard mRNA contains between 1000-5000 nucleotides, which is the size the resin was designed to optimally operate in. Optimized conditions can maximize the binding capacity, even for larger RNAs, to achieve a more efficient purification process.

"From a binding perspective, we have seen customers achieving up to 5mg/ml of 4000 bp mRNA," says Flook. "This is significantly higher than what you would see with some of the research products."

A standardized experiment looked at the binding capacity of three different sizes of mRNA without optimizing conditions for each independently. Size did not impact recovery even with samples straight from an IVT mixture. Low nonspecific binding and the affinity approach only allow polyA species to bind. Recovery rates are greater than 90% and, in most cases, greater than 95%. Adjusting the column size according to need makes the process flexible and scalable.

From a purity perspective, evaluation of the proteinaceous load showed that primarily enzymes from the IVT mixture are seen in the flow-through but not detected within the elution peak. "If we analyze the fractions from the elution peak starting with about 17% product-related impurities, a reverse-phase spin column slightly reduces it to 13%," says Flook.

"All of the process-related components are removed with the POROS Oligo (dT)25 resin, and we see a significant reduction in product-related impurities," continues Flook. "This means our affinity resin does a much better job of removing non polyA species than reverse phase." All remaining product-related impurities are polyadenylated, as expected.

Overall, the POROS Oligo (dT)25 resin demonstrated efficient elution at different load concentrations and excellent recovery with high purity regardless of sample type.

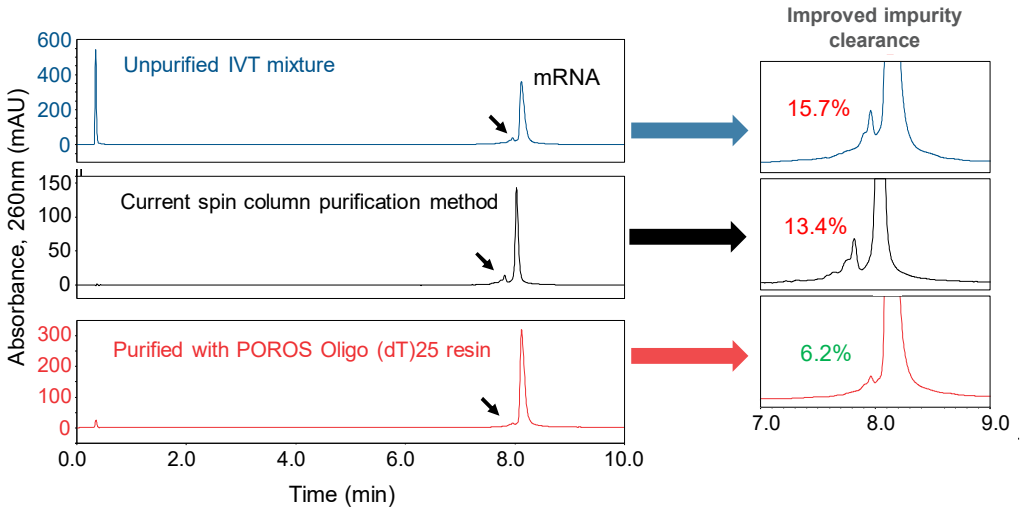

Fig. 3: Efficient removal of impurities compared to the spin column method. HPLC analysis of unpurified IVT mixture, spin column purified mRNA and mRNA purified with POROS Oligo (dT)25 resin.

"In reverse-phase, as the column is reused, the impurities start to follow the column, and that can change the column's effectiveness," says Flook. With POROS Oligo (dT)25 resin, the impurities are removed without first binding them to the column, rendering the number of impurities irrelevant. The resin is reusable and base stable for in-place sanitization.

"Each mRNA is designed for a specific application; the Cadillac version of purification is not always required. We have different purification strategies for different applications," says Indurthi. "As an example, if mRNA is being used for a vaccine, you do not need to get rid of certain impurities, but if you are making RNA for therapeutic use, you do because they will trigger an unwanted immune response."

In general, oligo dT purification can be used as a stand-alone purification. "We have seen pure RNA using this approach; it is a better way. The purer the end product, the less you need to get a response," says Indurthi. "Secondary structure, size, and other factors affect the recovery, not the resin itself."

"With all of the oligo dT resins we have tested, we observed that POROS Oligo (dT)25 has a very high binding capacity and provides the ability to purify larger mRNAs as compared to other products," adds Indurthi. "It works really well for our applications and will be added to our repertoire for mRNA production."

Looking to the Future

The COVID mRNA vaccines are expanding the already significant interest in the RNA space and taken mRNA manufacturing to a new level. Going forward, the biggest bottlenecks will be the DNA templates and the enzymes needed for synthesis. Luckily, the boom has also catapulted development activity in resins, nucleotides, and enzymes.

Hurdles still remain for mRNA therapeutics for different indications. One of the challenges is how to get the mRNA to the right cells, especially when targeting specific cancers. "We are going to see a lot of development around delivery systems," says Flook.

However, these challenges will not change the molecule's current trajectory. mRNA therapeutics are poised to become an important element in the healthcare landscape in the coming years. ■

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New Bead Technology Enables Commercial-Scale mRNA Purification

Affinity chromatography beads designed specifically for mRNA and FPLC eliminate toxic chemicals and purify tens of grams, removing a key bottleneck

By Gail Dutton

mRNA-based therapies catapulted to the forefront of public consciousness in the form of vaccines against the SARS-CoV-2 virus. After that success, mRNA therapeutics are now being developed for an ever-growing number of indications and applications that include cancer, cystic fibrosis, and infectious diseases, as well as gene and stem cell therapies based upon either gene replacement or gene editing.

However, the purification bottleneck must be solved before these therapies can be scaled up and produced in adequate quantities for clinical trials or commercialization.

"Traditionally, small-scale tools and products have been used to purify mRNA, such as reverse-phase high-performance liquid chromatography (HPLC), precipitation, and in some cases, cellulose-based chromatography," says Sirat Sikka, field application scientist at **Thermo Fisher Scientific** (Thermo Fisher). Those methods can be used to purify a few grams of mRNA and are adequate for bench work and some applications. Scale-up for clinical trials and commercialization, however, requires the ability to purify tens of grams or even tens of kilograms of mRNA.

"At Thermo Fisher, we understood the importance of mRNA and knew, even before the pandemic, that mRNA would be widely used," Sikka recalls. Since then, scientific and trade journals alike have cited mRNA therapeutics and vaccines as disruptive advances that can change the future of medicine and ease of manufacturing, and the ability to target pathways that otherwise are undruggable. Industry analyst Research and Markets predicts the global segment for mRNA therapeutics will grow from $46.7 billion in 2021 to $101.3 billion by 2026. That's a compound annual growth rate of 16.8%. (https://www.researchandmarkets.com/reports/5441159/mrna-therapeutics-and-global-markets-2021-2026).

Relieving the bottleneck

To be ready for such rapid growth in mRNA development, Thermo Fisher began developing a new affinity chromatography resin to isolate and purify mRNA long before the technology became a "household word." The team sought to develop a resin enabling improved recovery, increased purity, and enhanced reproducibility.

The POROS™ Oligo (dT)25 Affinity Resin—the resulting product—is a 50 µm poly(styrene-co-divinylbenzene) cross-linked porous bead functionalized with deoxythymidine (dT) strands that bind to mRNA via the poly-A tail (a chain of adenine nucleotides) that is on the three-prime end of all mRNA molecules.

One of the challenges is the size of mRNA. It is a large molecule —20 to 50 nm or greater in size that varies with construct length and solution composition—so there can be limitation to diffusion through the chromatography media and, therefore, hindrance to mass transfer, Sikka explains. Because the POROS™ beads have large throughpores the surface area available for interaction between the resin and mRNA molecule is increased leading to higher capacity. The large pores also result in a reduced mass transfer resistance, which helps to improve process efficiency and productivity.

"The POROS Oligo (dT)25 Affinity Resin minimizes the need to deal with organic solvents that are often used with HPLC systems," Sikka continues. "Using organic solvents in large volumes becomes an issue for manufacturing." She cites safety concerns regarding solvent disposal as well as the need to retrofit facilities to deal with them.

Instead of using toxic chemicals, after mRNA synthesis the column is loaded "with mRNA plus salt (for example, NaCl)." This neutralizes the negative charges on the RNA molecules so the poly-A tail can bind with the dT strands on the beads. Then, she says, "Elution can be performed using a low-conductivity buffer, or even water in some cases." Impurities and salt ions are washed away. With the sodium removed, the negative charges on poly-dT and the poly-A tail repel each other, freeing the purified mRNA and generating a recovery, typically above 90%, depending on the elution buffer and mRNA construct size.

From bench to manufacturing

The POROS Oligo (dT)25 Affinity Resin is designed for scalable purification processes,

so it is used to pack fast protein liquid chromatography (FPLC) columns. "The columns can be packed to multiple column size according to customers' needs, based on their process development and optimization," Sikka says. "We also have small-volume prepacked columns and Robocolumns. The 1 mL and 5 mL prepacked columns could be used with HPLC if needed, but that would only allow customers to purify very small sample volumes, may require re-plumbing and is usually not ideal for process development, so switching to FPLC is preferred. What customers mostly are looking for when they choose this resin is to scale-up purification, so they use it with FPLC systems."

Sikka says this affinity resin is a good option for scientists interested in developing a platform process that can be implemented for a variety of mRNA constructs. One of the benefits of using mRNA is that the same construct backbone could potentially be used to express different proteins. As a result, scientists can potentially use a platform process for multiple mRNA programs.

While researchers may switch out the gene of interest, "they could still be working with mRNA of comparable sizes," Sikka explains. "For example, depending on the protein they are trying to express, if the size range of all the constructs is between 4000 to 6000 bases, they could use this as the first capture step and develop a platform process." Working with much larger mRNA, such as self-amplifying could require some additional development.

As a platform technology, the first purification step with POROS Oligo (dT)25 would remove digested DNA template, nucleotides, enzymes, and buffer components. This could be the only step in the process before concentration and buffer exchange. If needed, a second chromatography step can be developed with POROS™ hydrophobic interaction chromatography (HIC) or anion exchange chromatography (AEX) resins to remove double-stranded RNA and uncapped or residual incomplete RNA transcripts.

"Starting with this resin during the research and discovery phase lets scientists continue using the same purification resin all the way to commercial manufacturing," she says. "This also eases the process of transitioning from one mRNA construct to another of similar size."

As she elaborates, "Once used in a process, the resin is already in the system and accepted by the customer's quality team." Additionally, scientists needn't redevelop their purification steps during each phase of scale-up, which minimizes the need to onboard a variety of chemicals and solutions or develop different buffer compositions, thus accelerating process development and reducing time to market. The resins are also reusable, which reduces the cost of goods.

"Importantly, POROS Oligo (dT)25 Affinity Resin beads are available for GMP production, and we provide the regulatory support package," Sikka says.

Transitioning to a new bead

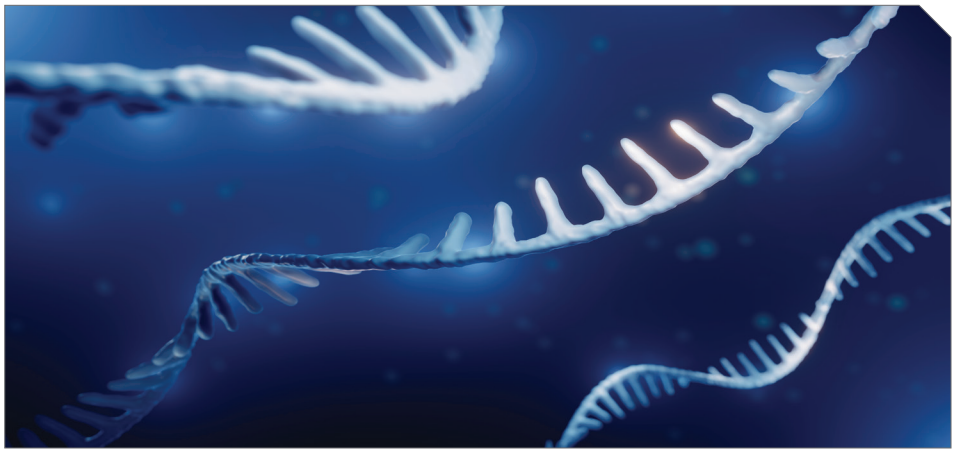
Switching to the POROS Oligo (dT)25 Affinity Resin is just a matter of ordering the prepacked columns if customers already use FPLC.

"A lot of our customers, however, are still at the research scale and are interested in scaling up," Sikka points out. "They don't necessarily have FPLC systems, and are trying to understand their options."

In those instances, she recommends ordering loose POROS Oligo (dT)25 Affinity Resin, which can be used in spin columns or microfuge tubes in a batch mode. If that works well for their purposes, they may consider investing in an FPLC for further purification optimization and scale-up.

"Thermo Fisher is very focused on the modern day," Sikka says, with solutions that address current and emerging purification challenges. Today, that means an intense focus on mRNA purification.

As interest in mRNA therapeutics continues to increase, the company's R&D is focusing on understanding the complexity associated with purifying self-amplifying mRNA, removal of product related impurities such as double-stranded RNA and abortive transcripts, and use POROS Oligo (dT)25 Affinity Resin and other technologies to resolve existing and emerging challenges. GEN



Before mRNA-based therapeutics can be produced in adequate quantities for clinical trials and commercial distribution, it will be necessary to remove a key bottleneck: mRNA purification. Existing methods usually purify just a few grams of mRNA, not the tens of grams or even the tens of kilograms needed. To improve mRNA purification, Thermo Fisher has developed the POROS Oligo (dT)25 Affinity Resin. Unlike alternate purification approaches, chromatography with a bead-based resin has excellent scalability. Notably, the resin selectively captures mRNA via the polyadenylated tail using simple salt and water purification steps. Artur Plawgo/Getty Images

Optimizing mRNA Purification Conditions by Using a High-Throughput Approach

The advancement of chromatography solutions for purifying mRNA is of utmost importance in optimizing the manufacturing process of mRNA-based therapeutics and meeting the growing demand for these products.

Affinity resins capable of specifically binding mRNA offer a valuable tool that helps to address the selectivity and capacity requirements for the large-scale manufacturing of mRNA therapeutics.

Watch this webinar to discover how you can implement a simplified mRNA purification workflow and enhance the efficiency of affinity chromatography in the mRNA manufacturing process. Don’t miss this opportunity to discover the latest techniques and strategies to improve mRNA purification for therapeutic applications.

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