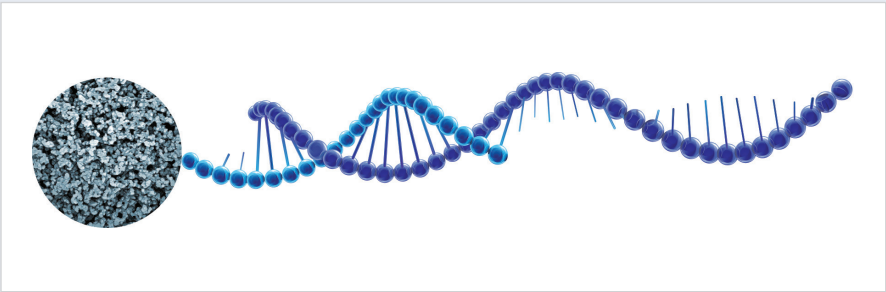
A Shot in the Arm for mRNA Vaccines

Standard methods of preparing mRNA require multiple purification steps, sometimes-costly single-use resins, and various chemicals. At the commercial scale, such methodology is impractical; at the vaccine scale (hundreds of millions of doses per annum), it is impossible.



Large pore bead with increased binding capacity for the efficient capture of mRNA.

The concept of mRNA therapy has gone from concept to realistic prospect with remarkable speed, but not everyone was taken by surprise. Scott Zobbi (Senior Business Development Manager, Thermo Fisher Scientific) first saw a significant increase in research into RNA therapeutics several years ago, particularly with regard to using mRNA as an alternative to recombinant proteins. But the really exciting development, according to Zobbi, is the application of mRNA to vaccination – not only in cancer immunotherapy, but also for infectious diseases.

In fact, mRNA vaccination constitutes an entirely new immunization modality. Administration of mRNA rather than protein antigens induces the body to produce antigenic proteins internally, eliminating some of the more expensive and time-consuming components of traditional vaccine manufacture. Similarly, the mRNA approach permits more rapid and responsive vaccine development: a simple RNA sequence change will quickly accommodate viral strains that have mutated away from the original vaccine, and entirely new vaccines can be promptly developed when dealing with a novel virus pandemic, such as COVID-19.

Broad commercial application of mRNA vaccines, however, assumes availability of appropriate supporting technology; for example, cost-effective, scalable mRNA purification methods. As Kelly Flook (Senior Product Manager, Purification Products, Thermo Fisher Scientific) puts it: "The move towards mRNA therapeutics and vaccines demands methods to achieve high purity mRNA in as few steps as possible, at the lowest cost possible."

Cleaner capture at lower cost

Flook explains that there are a number of deficiencies with existing methods. "Usually, mRNA is purified with ion pair reversed-phase chromatography, or similar techniques, which rely on ion-pairing reagents," she says. Reagent disposal and elimination of toxins from the drug product comes at a cost – a cost that becomes significant during scale up. "Manufacture of mRNA for COVID-19 mass vaccination would involve the production of millions of doses – hundreds of grams of mRNA – and purification steps requiring hundreds of liters of resin," says Flook. "Costs would be higher still if it turned out that each vaccine course required multiple shots, or if annual vaccinations were necessary. The industry needs a scalable product that can efficiently accommodate such demand."

How did Thermo Fisher Scientific address the need? "It wasn't always straightforward," says Zobbi, who notes that a critical part of the process was screening the different base beads that Thermo Fisher Scientific uses for its affinity capture products. "We found the best performance was provided by our POROS polystyrene divinylbenzene bead at the largest pore size, which wasn't the obvious answer when we started out," he says.

Similarly, the length of the poly-T capture ligand required optimization to ensure efficient capture of the mRNAs of interest. "Eventually, we found a 25-mer poly-T to be ideal, but this took a lot of work. For example, we had to test various coupling chemistries to ensure the poly-T behaved appropriately in the resin," says Zobbi. "Similarly, a balance had to be found between maximum mRNA capture and the quantity of poly-T ligand on the resin. We want to capture as much mRNA as possible, but increasing the poly-T concentration beyond a certain point does not provide an economically defensible increase in yield."

Flook notes how each aspect of the development process was driven by demand: "We optimized raw materials characteristics to ensure the resulting product fitted the market need – efficient, scalable mRNA purification." The outcome of the development process is the latest addition to the Thermo Scientific POROS family of products, Oligo(dT) 25 Affinity Resin. Comprising base beads with a

hydrophilic coating, formed from a highly robust, structurally rigid backbone, the Oligo(dT) resin permits sample loading in high salt solution – to favor mRNA-polyT annealing – and elution with reduced salt buffer or water. Key advantages of the system include:

- Efficient use of space and material – the polymer's structural attributes enable dense column packing
- Ligand stability at extremes of temperature (70 oC) and pH permit column clean-up and re-use over multiple cycles, thereby saving material costs
- Hydrophilic bead coating resists non-specific binding, thereby reducing purification cost and complexity
- Elimination of toxic reagents reduces operating risk, as well as cost and complexity of waste disposal
- Universal approach (applicable to all mRNAs) enables manufacturers to apply a single platform to all mRNA products in their portfolios, thereby saving development time
- Easy to use, simple to scale-up; cost savings become highly significant at commercial scale

And Thermo Fisher Scientific's clients back up these claims (see The Customer View). Flook adds, "Customers have reported plasmid DNA removal to below detectable limits, and binding capacities of 5 mg RNA per mL of resin for a 4000 base pair mRNA, which is excellent!"

Opportunity knocks

Manufacturers have a unique opportunity to take advantage of the cost and time advantages associated with Oligo(dT) – and the adoption process is simple. "We are always happy to have conversations with clients regarding optimization and scale-up," says Zobbi. Those who wish to evaluate the product can benefit from the technical expertise of Thermo Fisher Scientific's field sales force, which routinely provides clients with detailed support during process development and scale-up. "Our field-based application support team has global reach and can assist with the complete range of Thermo products for mRNA preparation," says Flook.

In conclusion, Zobbi adds, "We welcome new collaboration partners, and are very familiar with the variety of needs they may have."

Pharmaceutical Grade Reagent. For Manufacturing and Laboratory Use Only.

The Customer View

AmpTec manufactures pharmaceutical grade nucleic acids for diagnostic and therapeutic applications. We asked CEO, Peter Scheinert, for his views on the evolution of mRNA manufacture.

AmpTec has been manufacturing nucleic acids for fifteen years; today, the growth in mRNA applications – such as cancer immunotherapy and genome editing – is one of our strongest drivers. But we are particularly excited about the field of mRNA vaccines.

Standard vaccine production involves time-consuming steps, such as virus propagation and antibody generation. By contrast, the mRNA approach – injecting viral antigen mRNA – gets the body to produce the vaccine. It's much more flexible than standard vaccine approaches in that we can rapidly and easily modulate mRNA to reflect new mutations or to respond to new viral threats. But this speed and flexibility demands equivalently rapid and robust purification methods. Routine HPLC methods are associated with toxic reagents – requiring specialized ventilation and waste disposal systems – and scale-up difficulties. Our search for better upscaling solutions led us to a partnership with Thermo Fisher and the opportunity to work with them on the development of the Oligo(dT) resin, an easy-to-handle product that gives excellent mRNA yield. And it works equally well with all mRNAs; efficiency is unaffected by sequence or length. Furthermore, it binds the poly-A tail, and so returns full-length mRNAs, not truncated RNAs, which simplifies purification. Finally, it uses toxin-free reagents, thereby reducing method costs and complexity.

In brief, there is a dramatic and continuing increase in large-scale mRNA production, with mRNA vaccines being a key driver. We believe Oligo(dT) will be a critical manufacturing tool, not least for COVID-19 vaccine trials. Furthermore, the ease of use of this product, and the absence of toxic reagents, means that it can be used in any lab without any special safety or clean-up requirements. AmpTec is now assessing Oligo(dT) in large-scale processes, and I am confident that it will become our standard large-scale purification option. I think it's a fantastic tool!