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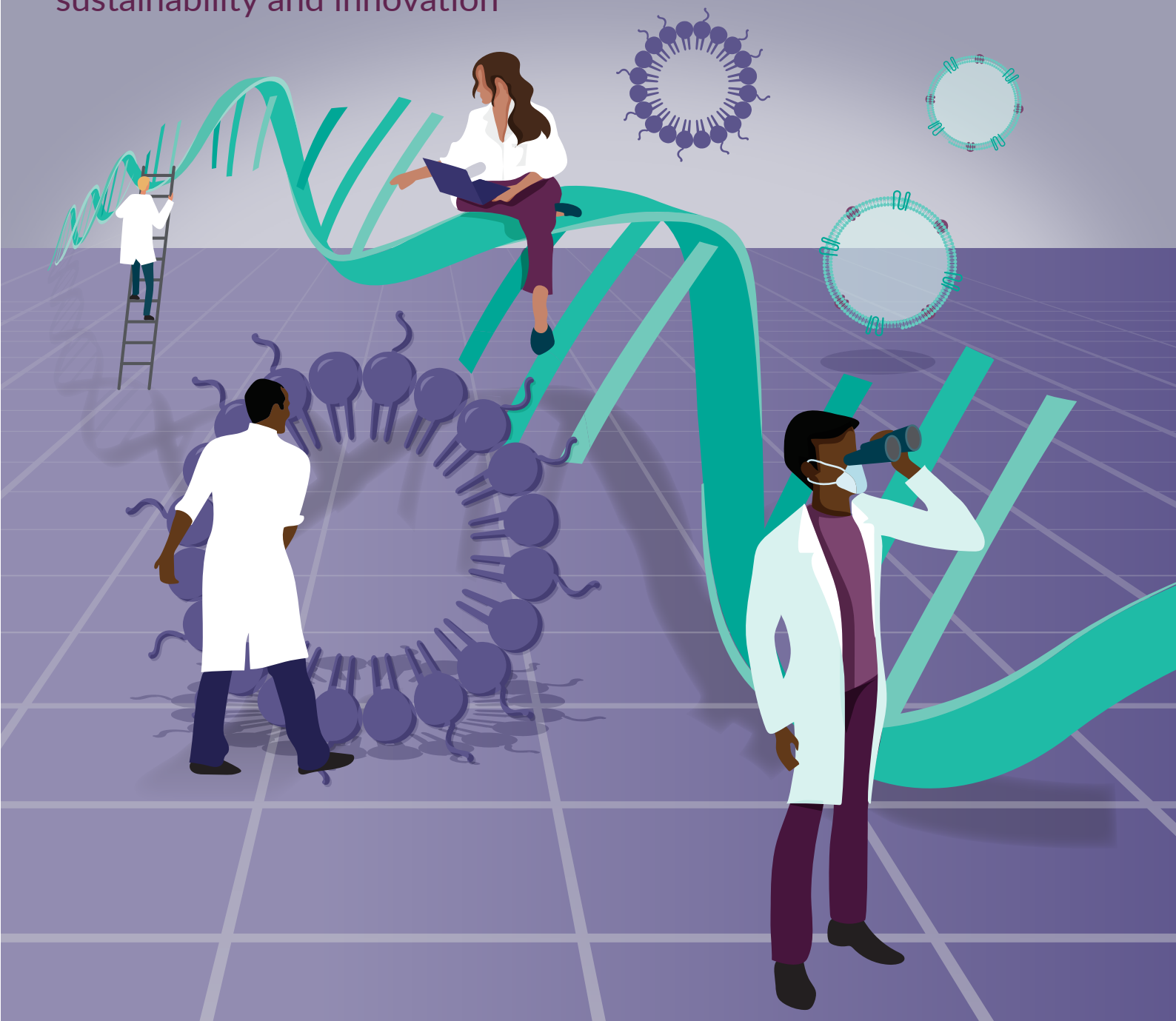
# NUCLEIC ACID INSIGHTS

## CONTENT PILLAR

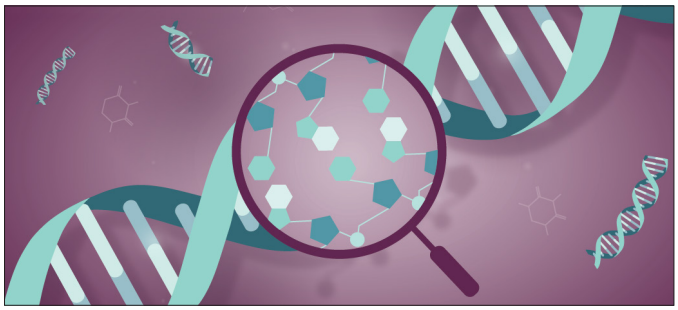
Oligonucleotides

## Upstream/synthesis: sustainability and innovation



NUCLEIC ACID INSIGHTS

## CONTENTS VOLUME 2 · ISSUE 5



### OLIGONUCLEOTIDES
Upstream/synthesis: sustainability and innovation



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# Oligonucleotide manufacturing innovation: challenges, risks, and opportunities

Barrie Cassey

Oligonucleotides represent a rapidly advancing class of therapeutic molecules with the potential to treat a broad range of diseases, including those previously considered untreatable. Despite the promise of these therapies, large-scale manufacturing presents significant challenges related to cost, scalability, and environmental sustainability. Current solid-phase phosphoramidite synthesis, while well established, is limited in batch size and efficiency, making it difficult to meet the growing demand for oligonucleotide-based drugs. Innovative approaches such as solution-phase synthesis, fragment assembly, and enzymatic synthesis offer potential solutions to these limitations, enabling higher yields, reduced waste, and improved process sustainability. This article explores the key technological advances and obstacles in oligonucleotide manufacturing, including purification complexities, the environmental impact of production, and the evolving role of stereochemistry in therapeutic efficacy. As the field progresses, a diversified manufacturing landscape incorporating multiple methodologies will be essential to ensuring the scalability and accessibility of oligonucleotide therapies while addressing sustainability concerns.

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Oligonucleotides are a rapidly growing, novel class of therapeutic molecule for the treatment of a wide variety of diseases. Their ability to interact with our cells' protein expression mechanisms gives them a unique ability to treat diseases that have previously been considered untreatable by traditional small molecule therapeutics. There are currently 20 approved oligonucleotide drugs on the market with almost 60% of these approved within the last 5 years. Whilst the majority of oligonucleotides have been licensed for the treatment of rare diseases with relatively small patient populations, the approval of cholesterol-lowering Leqvio® (inclisiran) in 2020 heralded a new era for oligonucleotide therapies targeting large patient populations. The current global clinical pipeline is heavily populated with drugs with the potential

to treat tens and hundreds of thousands of patients, meaning a potential need to significantly increase supply.

Oligonucleotides have a number of modes of action, the most well-known of which is gene silencing—preventing the production of a disease-associated protein—achieved through inducing the degradation of the messenger ribonucleic acid (mRNA) molecule for the protein or blocking the translation of the protein from the mRNA molecule. However, they can also act to influence gene-splicing—promoting the production of a particular version of a protein, or to up-regulate the expression of a target protein. The *in vivo* properties of oligonucleotides can be improved by introducing various chemical modifications to the RNA structure. These modifications can improve cellular uptake, enhance binding efficiency, and increase resistance to degradation by normal cellular mechanisms.

The current standard process for the manufacture of oligonucleotides utilizes phosphoramidite chemistry on a solid support. This technology is well understood and has served the industry well for the last 40 years. The flexibility of immobilized, flow-through column synthesis allows the production of a wide range of oligonucleotides from a single manufacturing platform, making the development of a manufacturing process and supply of material relatively quick. However, the changing environment around oligonucleotides is presenting significant challenges in scale of manufacture, cost, and environmental sustainability to this well-established technology.

Due to the physical constraints of operating a process in a flow-through column reactor, the largest synthesis scale is around 20 kg, but operating at half that scale is more common. During process development and supply of product for rare disease indications, a relatively small batch size is appropriate, even ideal. However, with the introduction of oligonucleotide therapeutics to treat large patient population indications, a small batch size becomes a critical issue. Aside from manufacturing time needed for repeated synthesis and purification of small batches, the associated intensive analytical testing and quality assurance activities to release each batch become crippling. To supply 1,000 kg of purified oligonucleotide could easily require the production of over 100 batches.

Innovations to increase the synthesis scale possible in a column reactor are being developed, but a fundamental shift in technology would make scale-up of manufacturing significantly simpler. The most obvious approach is to switch synthesis from immobilized on a solid support to solution phase where batch size would only be constrained by confidence in committing a large quantity of valuable raw materials to a single batch. However, the nature of the current synthetic approach of sequential addition of nucleotides creates significant challenges for solution phase synthesis. Multiple solution phase processes are in development; however, the fundamental issue is the requirement to 'clean up' the reaction solution between cycles of nucleotide addition. Precipitation of the growing oligonucleotide chain between addition cycles and phase transfer between cycles have been developed as techniques. More recently, the application of solvent-stable nanofiltration membranes to allow used reagents to be washed from the reaction medium has been identified and is currently being developed through a collaborative effort involving UK government and industry support.

In addition to the scale challenge for synthesis, there is a similar challenge for purification. Current chemical synthesis requires 50–150 chemical reactions to generate the oligonucleotide, depending on the nature of the molecule. Consequently, the purity of the unpurified oligonucleotide is significantly lower than that needed for a pharmaceutical application. Further,

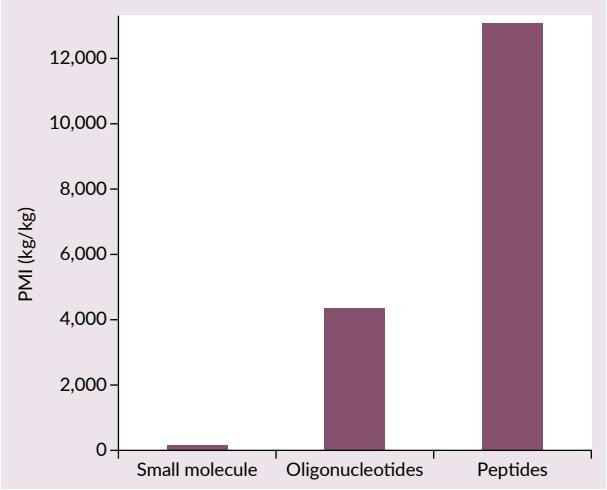
the product of synthesis contains a wide range of impurities with a number very closely related to the target molecule making purification challenging. The standard approach of column chromatography (anion exchange, reverse phase, HILIC, or a combination) is effective but time-consuming, low-yielding, and scale-constrained.

In addition to the challenge of manufacturing scale, increasing demand for oligonucleotides is amplifying the issue of the environmental impact of oligonucleotide manufacture. A 2021 ACS Green Chemistry Institute Pharmaceutical Roundtable study collected data on several oligonucleotides in development or commercial supply from several companies. The assessment used the relatively crude but useful measure of process mass intensity (PMI) to compare modalities. PMI estimates the number of kilograms of materials consumed to manufacture 1 kg of the product. Whilst the range was reasonably broad, the average process mass intensity (kg materials consumed in the manufacture of a kg of product) was 4300 kg/kg, which compares to an average value of 243 kg/kg determined when the same study was undertaken for traditional 'small molecule' pharmaceuticals [1,2]. Whilst investment in complex waste handling processes, water treatment facilities, and (multiple different) solvent recovery processes can be implemented to mitigate the quantity of waste, these processes are only viable at a significant scale of manufacture, complex and expensive to install, and expensive and energy consuming to operate. The fundamental solution to this issue is to develop processes that reduce water, solvent, and reagent consumption; deconvolute processes to simplify solvent recovery/recycling; and increase product yields. **Figure 1** shows the average PMI for traditional small molecule pharmaceuticals [1], oligonucleotides [2], and peptides [3].

Whilst there is an urgent need and many opportunities to improve the sustainability of oligonucleotide therapeutic manufacturing, evaluation of the planetary burden of manufacturing a kilogram of oligonucleotide versus a traditional small molecule therapeutic oversimplifies the comparison. The dosing regimen of oligonucleotide therapies is significantly different to traditional pharmaceuticals. Where traditional therapies often require medication to be taken multiple times per day, oligonucleotide therapies are commonly administered at a frequency of weeks, months, or even longer, and the dose of the active ingredient is often lower. Statins, the traditional small molecule therapy for the reduction of LDL cholesterol, are taken daily. In contrast, inclisiran, an oligonucleotide alternative, is administered twice yearly. Despite the PMI of an average oligonucleotide being 23-fold higher than an average small molecule, drug supply for a patient on statins may consume more raw materials than a patient on the oligonucleotide inclisiran. Whilst these analyses are very generic, exclude the drug formulation process, and rely on average PMI data, they show that the comparison at the patient level between the traditional therapies and oligonucleotide therapies with resource-consuming

**FIGURE 1**

Average PMI for traditional small molecule pharmaceuticals, oligonucleotides, and peptide.



manufacturing processes can be surprising. Further, when the opportunities to improve the sustainability profile of oligonucleotide therapies are delivered, the enormous potential of these life-changing new therapies can be realized, and the environmental impact of healthcare can be reduced.

## FRAGMENT ASSEMBLY

Assembly of oligonucleotides from fragments is of significant interest due to the opportunity to dramatically reduce the cost and, potentially, carbon footprint of synthesis. The process involves the synthesis of sub-sections of the overall oligonucleotide, ranging in size from 4–12 nucleotides, and assembly of the full-length oligonucleotide from these fragments. Multiple fragment assembly processes are in development either utilizing overlapping fragments to assemble double-stranded oligonucleotides, using short complementary strands to support the assembly of single-stranded oligonucleotides, or assembly on a reuseable template. One of the key challenges in a fragment assembly approach is to ensure the fragments assemble in the desired order, hence why many of the processes in development utilize the complementarity of a template or overlapping opposite strands to guide assembly.

The interest in fragment assembly approaches is driven by a non-linear relationship between production yield and length of oligonucleotides. As the length of an oligonucleotide increases, the quality of the synthesis product decreases and the challenge to purification increases, leading to an accelerating drop-off in yield. The concept of convergent synthesis is not a new concept to organic chemistry and is often employed in the synthesis of large, complex molecules—application of the idea to oligonucleotide synthesis offers interesting opportunities. Linear synthesis and purification of a 22-nucleotide-long oligonucleotide might be achieved with a yield of around 50%, whereas an 11-mer fragment might be produced with an approximately 80% yield. Even allowing for inefficient ligation, the overall efficiency of the fragment assembly process is superior to linear synthesis. However, the economic case for fragment assembly is not constrained to chemical yield—the overall efficiency of the process must be considered. When compared to full-length oligonucleotide synthesis, fragment production using current chemical synthesis technology under-utilizes the solid support and manufacturing infrastructure. Aside from quantities of phosphoramidites and some reagents consumed, the manufacture of a fragment and full-length oligonucleotide are almost identical. Resin support consumption, resin swelling solvent consumption, cleavage/deprotection reagent consumption, chromatography buffer consumption, and manufacturing time are all very similar, yet the full-length synthesis will generate at least double the product mass of oligonucleotide compared to the fragment. Further, fragment assembly is an additional step, requiring reagents, usually enzymes, and manufacturing plant time. Thus, whilst the chemical yield may be higher, the output from the manufacturing plant is lower.

The concept of convergent synthesis using fragments is still a relatively new one and multiple opportunities exist to improve fragment production productivity. A solid phase synthesis optimized for the production of a shorter fragment could utilize raw materials and reagents more efficiently and apply different solid supports with higher packing and loading densities to dramatically improve productivity from existing manufacturing equipment. Liquid phase processes and biocatalytic approaches also offer significant opportunity. Further, fragment ligation processes, through the nature of coupling two adjacent fragments, naturally eliminate the short- and long-chain impurities common

in unpurified oligonucleotides that are difficult to remove during chromatographic purification. This intrinsic effect, coupled with focused synthesis process development, creates the opportunity to eliminate the need for chromatographic purification, with the associated reduction in reagent consumption, analytical time, and manufacturing infrastructure.

## BIOCATALYSIS

Biocatalytic approaches are also being developed, with natural enzymes being engineered to enable the synthesis of therapeutic oligonucleotides. Operated in aqueous solution, these enzymatic approaches offer enormous potential for both increased batch scale and improved environmental sustainability. The approach most similar to the current chemical synthesis approach involves the use of enzymes (e.g., terminal deoxynucleotide transferase [TDT]) that add single nucleotides to an existing oligonucleotide chain, with the enzyme catalyzing the coupling rather than utilizing phosphoramidite chemistry. These untemplated approaches utilize 3′-protected nucleotides to prevent uncontrolled multiple additions and follow a similar cycle to the current chemical process with protected nucleotide addition followed by deprotection of the terminal nucleotide to allow addition of the next nucleotide. Consequently, untemplated enzymatic synthesis requires the same 'clean up' of the reaction solution between each coupling cycle, which comes with the same challenges as the chemical approach. Further, the efficiency of the enzymatic coupling is currently insufficient to create a high purity oligonucleotide that avoids the need for chromatographic purification.

An alternative biocatalytic approach utilizes a template to synthesize the oligonucleotide. In this system, a polymerase enzyme generates the complementary strand to a designed template, with the oligonucleotide strand freed from the template by the action of an endonuclease. Both enzymes are utilized in a 'one pot' system alongside an excess of unprotected nucleotide triphosphates, allowing the template to be used in the production of multiple oligonucleotides. This approach has its constraints and challenges: evolution of a polymerase to accept the full spectrum of 2′-modifications commonly deployed in therapeutic oligonucleotides, and the potential to accept new modified nucleotides created in the future. Also, the polymerase utilizes Watson-Crick base pairing to assemble the correct nucleobase sequence but cannot distinguish between different 2′-modified nucleotides, thus, different 2′-modified versions of nucleotides containing the same nucleobase cannot be guaranteed to be incorporated into the oligonucleotide in the required location. This constraint makes the use of templated enzymatic synthesis unlikely for full-length oligonucleotides but provides an excellent mechanism for the synthesis of shorter fragments. Despite these constraints, templated enzymatic synthesis offers an opportunity for completely aqueous, scalable, high-fidelity synthesis with significantly higher atom efficiency (due to the lack of multiple protecting groups) that can eliminate the requirement for expensive and time-consuming chromatographic purification and radically reduce the carbon footprint of oligonucleotide synthesis.

A potential switch to any biocatalytic synthesis approach poses an interesting supply chain challenge. Through rising demand for chemical synthesis of oligonucleotides, a well-established and expanding supply chain for the modified, chemically protected nucleoside phosphoramidites has been established. Further, the rapid expansion in demand for mRNA vaccines has established a supply chain for natural RNA nucleotide triphosphates. However, there is no established commercial supply chain for the various 2′-modified nucleotide

triphosphate starting materials that would be required to support a biocatalytic oligonucleotide synthesis approach.

## STEREOCHEMISTRY

The other significant difference between chemical and enzymatic synthesis of oligonucleotides is the stereochemistry of the resultant molecule. Whilst stereochemistry is not relevant to standard phosphate linkages between nucleotides, the widely utilized phosphorothioate (PS) linkage does introduce stereospecific conformations. Phosphoramidite chemistry-mediated synthesis is known to randomly generate either the Rp- or Sp-conformation and, whilst the ratio of Rp and Sp is influenced by the activating reagent used with the phosphoramidite, the resultant molecule is essentially stereo-random. However, enzymatic approaches result in stereo-pure oligonucleotides [4,5]. The current generally applied approach to the variability introduced through chemical synthesis is to ensure that, within reason, the diastereoisomer ratio present in the molecule used in preclinical and clinical safety and efficacy studies is preserved in the commercial product. Clearly, consistency of diastereoisomer ratio presents a challenge to switch existing oligonucleotide therapeutics from chemical to enzymatic synthesis. Repeating safety and efficacy studies to enable a switch would likely be prohibitively expensive and risky; however, efforts to enable enzymatic systems to deliver both Rp and Sp isomers have started.

Significantly, there has been recent progress toward relatively efficient chemical synthesis and purification of stereo-defined PS oligonucleotides, allowing more research into the biological effects of stereochemistry [4,5]. From this research, it is clear that chirality has a significant influence on the pharmacokinetics and pharmacodynamics of oligonucleotides, as might be expected due to their interaction with multiple biological systems and molecules. The relationship is complex with effects appearing to be sequence and target dependent, and the presence of Rp and Sp isomers in different parts of the molecule have differing beneficial effects. However, as understanding of the advantages of introducing controlled stereochemistry to oligonucleotide therapeutics increases, the demand for manufacturing processes that can efficiently produce stereospecific molecules will only increase, with enzymatic synthesis a highly attractive option.

## CONCLUSIONS

Whilst the increase in demand for oligonucleotides does create significant challenges for manufacturing, this is a 'nice problem to have' as it means the enormous potential benefit to human health of oligonucleotide therapeutics is being realized. Also, with increasing demand, manufacturing approaches that seem utterly unsuitable for small-scale supply start to become viable options. Liquid phase processes are more suited to larger manufacturing batches, with their economies of scale driving down costs and reduction in solvent consumption. Further, the requirement for larger quantities of individual oligonucleotides increases the viability of fragment assembly approaches. The option of fragment assembly also facilitates the use of enzymatic synthesis approaches, which would have a major impact on the environmental impact of oligonucleotide manufacture.

Whilst solid phase synthesis will remain as a core technology for the development and production of oligonucleotides, the future of oligonucleotide manufacture will involve a range of processes, tailored to the molecule and the scale of demand for it.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### AUTHORSHIP & CONFLICT OF INTEREST

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NUCLEIC ACID INSIGHTS

Meet the

# *Nucleic Acids Insights* Editorial Board

An integral part of the team that brings you *Nucleic Acid Insights* is our fantastic Editorial Advisory Board. This article is part of our 'Meet the EAB' series, created to showcase the leaders in the field who provide their time and expertise to help to steer the scope and focus of the journal.

## INTERVIEW

**Jeske Smink**, Senior Director, Head of Drug Substance, Silence Therapeutics

*Nucleic Acid Insights* 2025; 2(5), 95–97 · DOI: 10.18609/nuc.2025.015

## Q Can you tell us a bit about your current role?

We are developing small interfering RNA (siRNA) compounds for the treatment of patients across different indications, including cardiovascular, immunological, and rare diseases. In my role as Senior Director, Head of Drug Substance, I oversee the CMC development of early-stage projects, including process and analytical development, scale-up, and clinical product manufacturing of siRNA compounds. I also contribute to the quality sections of regulatory documentation. Additionally, I manage a team consisting of several CMC project leaders, starting material lead, and analytical chemists. I also support colleagues in the CMC department with Phase 2 and Phase 3 programs.

## Q How did your academic background and career path lead you to the nucleic acids space?

After several years in academic research, I decided to transition into the biotech industry and pharmaceutical development. I began my career at a biotech company specializing in tissue engineering, specifically focusing on cell therapy for cartilage regeneration. This

role aligned perfectly with my scientific background in cartilage and bone biology, and I was offered a position that marked my first step into the biotech industry.

I joined the company at an exciting time, as it was during the central market authorization process at the EMA to get approval of the product for the European market. Alongside a colleague, I was heading the R&D department and was involved in preclinical and quality studies of the cartilage cell transplant required for the regulatory filing. During my time at this company, I gained valuable knowledge about the quality aspects of biopharmaceuticals, GMP manufacturing, and regulatory requirements for drug development. This experience allowed me to take the next step and apply my knowledge to another therapeutic area.

I then joined a CDMO focused on biologics, including recombinant proteins and therapeutic antibodies. As a CMC Project Manager, I oversaw various customer projects within the production department. My responsibilities involved process and analytical development, scale-up, and large-scale production of drug substances for preclinical and clinical studies. This role deepened my understanding of the steps and requirements involved in the early stages of drug development.

After expanding my knowledge in the CMC aspects of biologics, I had the opportunity to join my current company as Head of Drug Substance. This role has been an exciting challenge, allowing me, as a biologist, to apply my CMC knowledge in cell therapies and biologics, to chemical products such as siRNA compounds.

## Q What are your top predictions for the next five years in the nucleic acids field, and what developments do you most hope to see?

I expect a shift towards new manufacturing technologies, such as liquid-phase synthesis, biocatalytic approaches, or combinations like blockmer synthesis via solid-phase synthesis followed by enzymatic ligation. This will enable the production and supply of larger quantities of oligonucleotides, required for broader indications. This represents a significant shift in the coming years, with more interest in addressing larger patient populations, rather than focusing solely on rare indications as therapeutic areas for oligonucleotides.

Currently, the majority of oligonucleotides are designed to target the liver, utilizing the well-established GalNAc delivery system. However, I expect there will be a greater focus on extrahepatic targeting over the next five years. I hope to see the first therapeutic compounds approved that target extrahepatic tissues, further strengthening oligonucleotides as effective, safe, and highly durable therapeutic modalities.

## Q What was your motivation for joining the board of *Nucleic Acid Insights*, and what do you most hope to see the journal achieve as we enter our second year?

*Nucleic Acid Insights* not only focuses on oligonucleotides but also encompasses all DNA and RNA-related therapeutics. Although these therapeutic modalities have been known for many years, they are finally emerging as promising new drugs. The most exciting part is that these types of modalities offer very different modes of action and can be used to

target a wide range of diseases. Despite their broad applications, they share certain commonalities that unify the field. For me, this represents an emerging area of novel therapeutics that will be a game changer in pharmaceutical development. I joined the editorial board because I am eager to see to the evolution of this exciting field.

# Precise mRNA purity assessment for accelerated therapeutic development and release testing

**Paul Mania**, Bioanalytics Applications Specialist, **Joe Ferraiolo**, Director, Bioanalytical Applications, Repligen

*Nucleic Acid Insights* 2025; 2(5), 41 · DOI: 10.18609/nuc.2025.009 · 

Traditional fixed-pathlength UV-Vis spectroscopy for nucleic acid analytics has limitations, including the need for sample dilution, which introduces variability and increased preparation time. Variable pathlength technology (VPT) is a UV-Vis-based spectroscopy technique that enables the measurement of multiple absorbance values through variable pathlengths, improving the accuracy and efficiency of nucleic acid analytics. Case studies explored in this poster demonstrate VPT's effectiveness in measuring pDNA and mRNA purity, highlighting its role in streamlining processes and enhancing product development.

## Advantages of VPT over traditional UV-Vis spectroscopy for nucleic acid analytics

Although fixed-pathlength UV-Vis spectroscopy techniques are commonly utilized for nucleic acid analytics, they have multiple limitations. For example, traditional spectrophotometers require sample dilution to ensure readings fall within the operational range, increasing preparation time and variability, leading to difficult method transfers.

In contrast, VPT offers key advantages, including the ability to measure multiple absorbance values without the need to blank the sample or obtain absolute absorbance values. In essence, VPT uses slope regression to convert measurements to standard pathlengths, removing the need for dilutions and reducing errors. VPT also drives better process control by providing real-time, high-quality, and reproducible data, and continuous monitoring.

VPT-based systems such as at-line SoloVPE® and in-line FlowVPX® have been applied across various applications, and several case studies were conducted to demonstrate the benefits of this technology for nucleic acid applications.

## Case study 1: Utilizing VPT to determine pDNA purity in human gene therapy products

Based on a study conducted in collaboration with Pfizer, which involved analysis of both internal and external human gene therapy products, SoloVPE can be utilized to accurately determine pDNA purity. 25 different levels of purity values were created, comparing the theoretical (NanoDrop) purity values with measurements taken using the SoloVPE system. The 260/280 ratios (R-values) were analyzed, and the differences between theoretical and observed values were recorded **(Table 1)**. It was demonstrated that the difference between theoretical and observed data was less than 2%, illustrating that SoloVPE can be used to obtain reliable pDNA purity ratio values.

## Case study 2: Advancing mRNA concentration measurement with VPT

Another study carried out in partnership with Aldevron focused on determining mRNA concentration and purity using the SoloVPE System. The mRNA molecule was formulated in three matrices: Tris-EDTA, water for injection, and sodium citrate. The slope was quantified at 260 nm and 280 nm in Abs/mm, with the values representing concentration magnitude to determine the mRNA purity **(Table 2)**. The results showed that the SoloVPE system is highly accurate compared to theoretical methods and enables rapid product-specific analysis with reduced sample handling, which streamlines the mRNA purity measurement process.

## Conclusion

In summary, the integration of VPT into platforms such as SoloVPE enhances nucleic acid analysis, streamlining the determination of pDNA purity, mRNA concentration, and other critical metrics. Overall, VPT-driven systems offer enhanced precision, reduced processing times, and improved process efficiency, making them valuable tools in advanced therapeutic development.

**Table 1.** The differences in pDNA purity ratios between theoretical (NanoDrop) and observed (SoloVPE) values.

| Level | Theoretical purity ratio | Observed purity ratio | Percentage difference (%) |
|---|---|---|---|
| 1 | 0.6259 | 0.62723 | 0.21 |
| 2 | 0.87087 | 0.90315 | −0.22 |
| 3 | 1.05311 | 1.06122 | 0.74 |
| 4 | 1.18483 | 1.17076 | −1.14 |
| 5 | 1.28451 | 1.28847 | 0.30 |
| 6 | 1.42528 | 1.42358 | −0.11 |
| 7 | 1.51996 | 1.52481 | 0.31 |
| 8 | 1.58798 | 1.58959 | 0.10 |
| 9 | 1.63927 | 1.65568 | 0.96 |
| 10 | 1.6793 | 1.67489 | −0.25 |
| 11 | 1.71134 | 1.69203 | −1.09 |
| 12 | 1.7377 | 1.71515 | −1.25 |
| 13 | 1.75964 | 1.73533 | −1.33 |

**Table 1 continued.**

| Level | Theoretical purity ratio | Observed purity ratio | Percentage difference (%) |
|---|---|---|---|
| 14 | 1.77821 | 1.77064 | −0.41 |
| 15 | 1.79418 | 1.81956 | 1.36 |
| 16 | 1.80804 | 1.77874 | −1.56 |
| 17 | 1.82026 | 1.80414 | −0.85 |
| 18 | 1.83094 | 1.81184 | −1.00 |
| 19 | 1.84046 | 1.82975 | −0.56 |
| 20 | 1.84902 | 1.85282 | 0.29 |
| 21 | 1.85672 | 1.84667 | −0.52 |
| 22 | 1.86028 | 1.88378 | 1.22 |
| 23 | 1.86365 | 1.85282 | −0.56 |
| 24 | 1.86692 | 1.84941 | −0.90 |
| 25 | 1.87 | 1.87147 | 0.08 |

**Table 2.** mRNA purity ratios using SoloVPE, and comparisons between theoretical and observed (SoloVPE) concentration values.

| Matrix | Level | $A_{260}$ concentration (mg/mL) | | Accuracy | Purity |
|---|---|---|---|---|---|
| | | Theoretical | SoloVPE | Percentage recovery to theoretical | $A_{260}/A_{280}$ |
| TE | 1 | 4.10 | 4.35 | 106.08 | 2.23 |
| | 2 | 2.05 | 2.21 | 107.99 | 2.26 |
| | 3 | 1.03 | 1.11 | 108.07 | 2.24 |
| | 4 | 0.51 | 0.55 | 108.12 | 2.25 |
| | 5 | 0.26 | 0.28 | 107.74 | 2.24 |
| WFI | 1 | 4.70 | 4.59 | 97.56 | 2.05 |
| | 2 | 2.35 | 2.39 | 101.75 | 2.07 |
| | 3 | 1.18 | 1.21 | 103.27 | 2.00 |
| | 4 | 0.59 | 0.61 | 103.55 | 1.93 |
| | 5 | 0.29 | 0.31 | 104.50 | 1.87 |
| Sodium citrate | 1 | 4.01 | 3.78 | 94.27 | 2.13 |
| | 2 | 2.01 | 1.91 | 95.07 | 2.12 |
| | 3 | 1.00 | 0.96 | 96.00 | 2.11 |
| | 4 | 0.50 | 0.49 | 96.98 | 2.10 |
| | 5 | 0.25 | 0.25 | 98.06 | 2.11 |