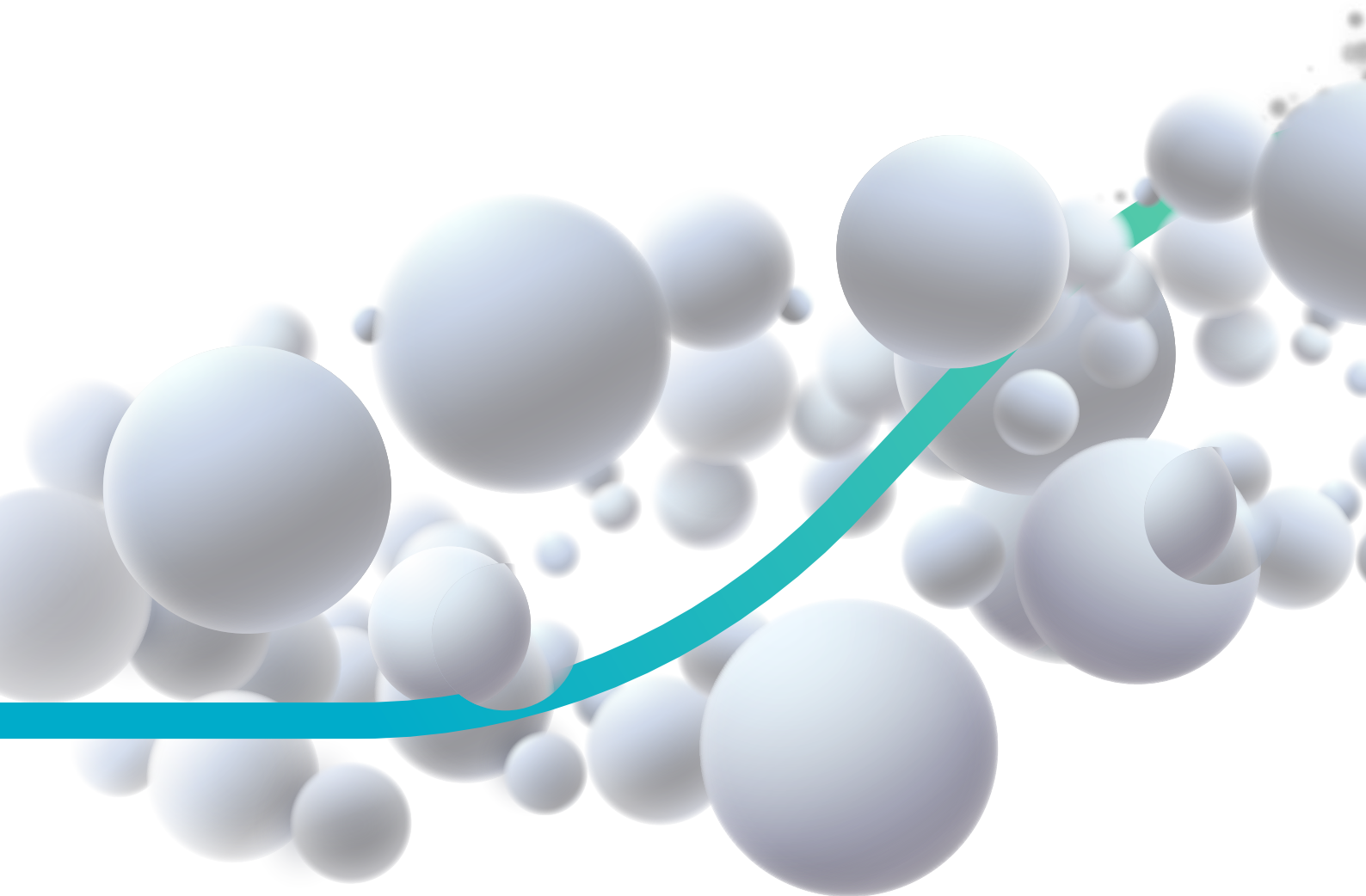


Unlocking the Future of RNA Manufacturing:

A Guide to Enzymatic Synthesis



The Rise of RNA Therapeutics

RNA-based therapies have emerged as a true game-changer in medicine, holding incredible promise for tackling a wide range of diseases — from rare diseases to large indications like cardiovascular disease. These innovative treatments harness the power of RNA to precisely target and modify cellular processes. One prominent example is RNA interference (RNAi), a biological mechanism where small RNA molecules silence specific genes, effectively turning off the production of disease-causing proteins.

The therapeutic potential of these revolutionary medicines is reflected in the burgeoning RNA therapeutics market, projected to reach \$18 billion by 2030, growing at a CAGR of 5.6% from 2023 [1]. This growth is driven by the increasing prevalence of chronic diseases and rising R&D investments into RNA-based drug modalities targeting a broader range of therapeutic areas, including those where existing small-molecule drugs have proven less effective [2]. This expansion includes applications in large patient populations, like cardiovascular drugs, which will require manufacturing in the multi-metric ton range. Increased demand requires highly efficient and scalable manufacturing processes.

Challenges of Traditional RNA Manufacturing Methods

As RNA therapeutics expand into larger indications and more complex formats, manufacturing demands have intensified. The ability to deliver high-purity RNA constructs at scale — quickly, cost-effectively, and sustainably — has become a defining challenge for therapeutic developers and manufacturers alike.

What is PAC?

Phosphoramidite chemistry (PAC) has long served as the foundation of oligonucleotide synthesis. This solid-phase oligonucleotide synthesis (SPOS) stepwise process builds RNA sequences one nucleotide at a time through chemical coupling reactions. It's a reliable platform for constructing a wide range of RNA sequences and remains the industry standard across clinical-stage RNA synthesis.

Despite its utility, PAC struggles to efficiently deliver commercial-scale RNA manufacturing for large indications. PAC processes face several constraints:

- **Limited synthesis scalability**, with yields typically peaking at just <7 kg per synthesis
- **Low yield with increasing length**, due to stepwise inefficiencies
- **High infrastructure burden**, including extensive solvent use and specialized equipment
- **Capital-intensive scale-up**, exemplified by Agilent's \$725 million investment to expand capacity for traditional RNA manufacturing methods [3]
- **Sustainability shortfalls**, driven by high solvent consumption and chemical waste
- **Chemical complexity**, such as the use of harsh reagents and protecting groups, can introduce side reactions and structurally diverse impurities. These include Class IV impurities, which are typically challenging or impossible to remove by current purification methodologies. According to EMA draft guidance, these impurities require qualification if present above the 1.5% threshold and should ideally be minimized through process optimization rather than preclinical testing [4].

While incremental innovations such as liquid-phase synthesis (LPOS) and solvent recycling have improved PAC's attractiveness for manufacturing, they do not entirely address the technology's limitations. As the industry evolves toward broader indications and larger-volume production, these challenges have driven interest in alternative approaches that promise greater scalability, flexibility, and sustainability.



The Alternative Solution: Enzymatic Synthesis

The limitations of traditional PAC methods prompted a focus on enzymatic manufacturing approaches as a next-generation solution for RNA therapeutics. Unlike stepwise chemical synthesis, enzymatic RNA synthesis leverages engineered enzymes to construct oligonucleotides under mild, aqueous conditions — offering a fundamentally different approach to process design, scalability, safety and sustainability.

Instead of relying on harsh reagents and protecting group chemistry, enzymatic workflows eliminate many of the chemical complexities that contribute to yield loss, impurity formation,

and solvent waste. With fewer steps and a solution-phase format, these platforms support more modular, scalable manufacturing, accommodating both short and long RNA constructs.

As demand for RNA therapeutics continues to rise, the industry looks for innovative approaches that can overcome the constraints of current processes.



The Codexis Solution: Addressing Scalability Through a Strategic Approach

Codexis brings its expertise in enzyme engineering to the forefront of enzymatic RNA manufacturing through the ECO Synthesis™ Manufacturing Platform — a flexible, enzyme-enabled system that addresses the limitations of traditional PAC synthesis. This platform uses highly engineered enzymes for both sequential synthesis of RNA therapeutics and enzymatic ligation, supporting scalable, modular production of full-length RNA therapeutics.

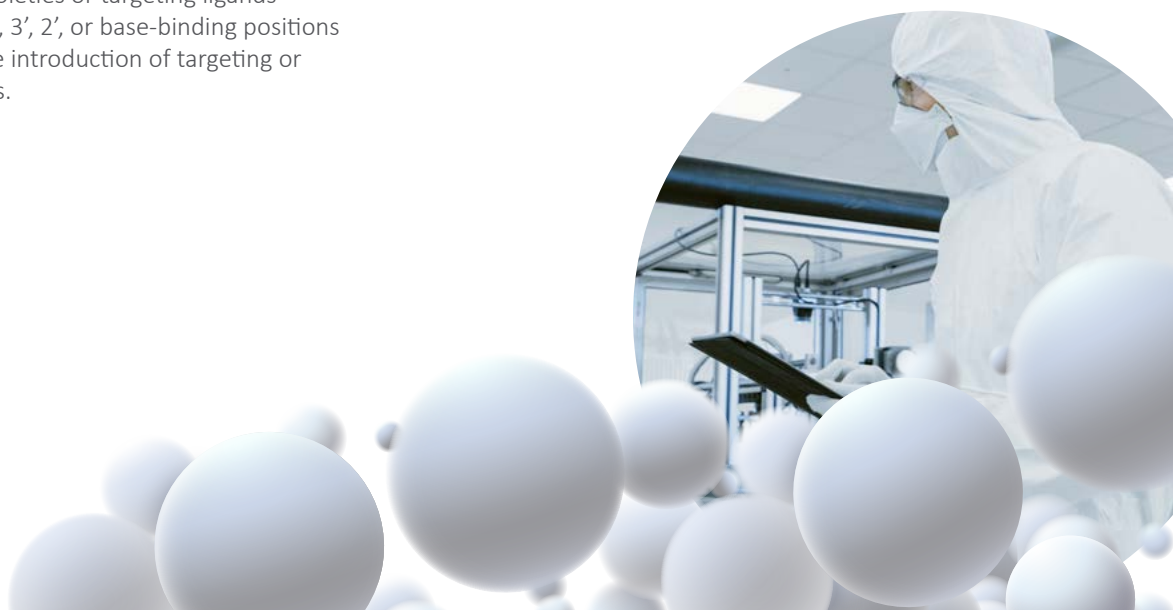
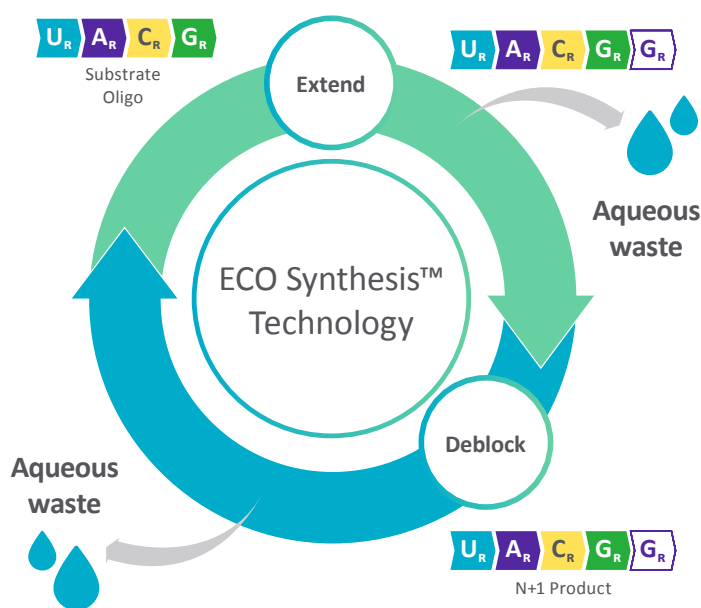
Built on the company's deep expertise in protein engineering and process development, ECO Synthesis supports two distinct approaches to RNA synthesis: fully sequential enzymatic synthesis and ligation-based assembly. These options can be used independently or in combination, depending on the therapeutic format and manufacturing needs.

Fully Sequential Synthesis

The sequential synthesis approach uses a cyclic, enzymatic process to construct RNA strands one nucleotide at a time in a controlled and scalable manner. The workflow consists of the following steps:

- **Enzyme immobilization:** Engineered enzymes are immobilized on solid supports to enable solution-phase synthesis.
- **Extension:** A packed bed reactor containing immobilized polymerase has oligonucleotide substrate and nucleotide quad phosphate (NQP) flow through it. The extended product is protected on the 3' end with a phosphate. This enables controlled, directional extension of the sequence from 5' to 3'.
- **Deblocking:** The extended oligonucleotide is passed through a second packed-bed reactor containing immobilized phosphatase, which removes blocking groups and deactivates residual NQP to prepare the strand for the next cycle.
- **Desalting:** After synthesis, ultrafiltration and diafiltration steps remove small molecules, leaving the target oligonucleotide ready for downstream processing.
- **Conjugation:** Functional moieties or targeting ligands can be conjugated to the 5', 3', 2', or base-binding positions of the RNA, allowing for the introduction of targeting or delivery-enhancing features.

This approach offers a modular, green synthesis approach for RNA sequences with chemically modified nucleotides, all while operating under mild, aqueous conditions.



Ligation-based Assembly

For siRNA, duplexed or chemically complex RNA constructs, ECO Synthesis enables ligation-based assembly of multiple short single-stranded RNA fragments into the desired final therapeutic. These short single-stranded RNA (ssRNA) fragments may be produced via sequential enzymatic synthesis or traditional phosphoramidite chemistry (PAC) and are subsequently joined using engineered dsRNA ligases.

This method allows:

- **Parallel synthesis** and purification of shorter fragments, as needed
- **Controlled assembly** into complex double-stranded RNA (dsRNA) constructs
- **Potential for improved purity** and yield due to reduced cumulative side reactions

By enabling high-efficiency ligation, this workflow enhances scalability and reduces manufacturing complexity and cost.

Selecting and Optimizing Ligation Strategies

The performance of a ligation-based synthesis workflow depends not only on the design of the fragments, but also on the selectivity properties of the ligase enzyme. Codexis tailors ligase development to the specific requirements of each RNA format — such as synthesizing siRNA and antisense oligonucleotides (ASOs) — with plans to extend this capability to additional constructs like guide RNA (gRNA) in the near future. Key factors include manufacturability of the ligases, compatibility with modified nucleotides, selectivity for designed sequence junctions, and the ability to operate efficiently across a range of process conditions, including temperature, pH, and buffer composition.

By combining enzyme engineering with ligation process development, Codexis ensures that each ligase performs optimally under RNA manufacturing conditions — improving ligation efficiency, RNA yields, minimizing side products, and enabling scalable modular RNA assembly.

Enzyme Engineering and Customization: CodeEvolver

Both workflows are enabled by Codexis' CodeEvolver® enzyme engineering platform, which has been used to develop tailored enzymes optimized for RNA manufacturing. These enzymes deliver high substrate specificity, robust catalytic performance across a range of process conditions, and tolerance for chemically modified bases and high-concentration substrates — supporting scalable, GMP-ready production.

Customized ligases are developed to meet the demands of different RNA modalities and process environments, enabling reliable ligation even with chemically modified junctions or challenging structural features.

Advantages of ECO Synthesis Platform for RNA Synthesis

The ECO Synthesis platform delivers a range of advantages grounded in enzyme-enabled efficiency, process adaptability, and commercial scalability. By integrating Codexis's enzyme engineering expertise with platform-ready manufacturing workflows, ECO Synthesis supports both performance optimization and long-term operational value across a variety of RNA constructs.

The value of this platform approach becomes most evident when looking at the outcomes it enables across key manufacturing performance areas:

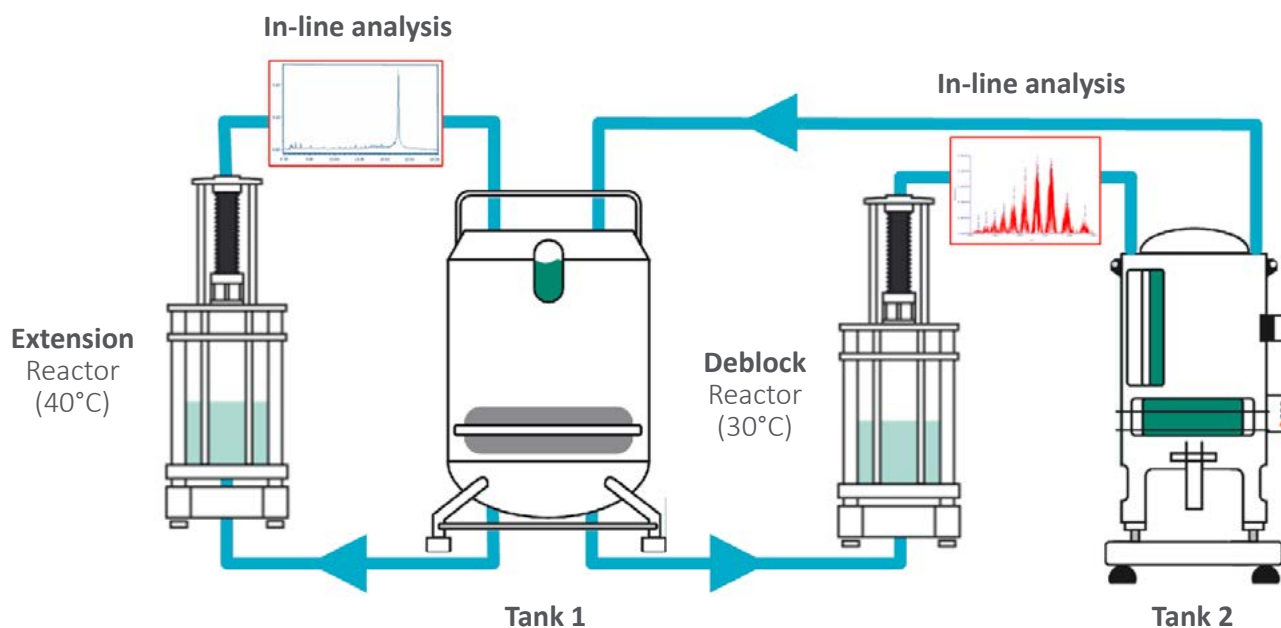
Scalability

Traditional chemical synthesis methods face inherent scale limitations due to cumulative yield loss, complex solvent handling, and infrastructure-intensive processes. Enzymatic synthesis sidesteps many of these constraints. The ECO Synthesis platform has capability to scale beyond 10 kg per run, exceeding the output of many optimized PAC workflows.

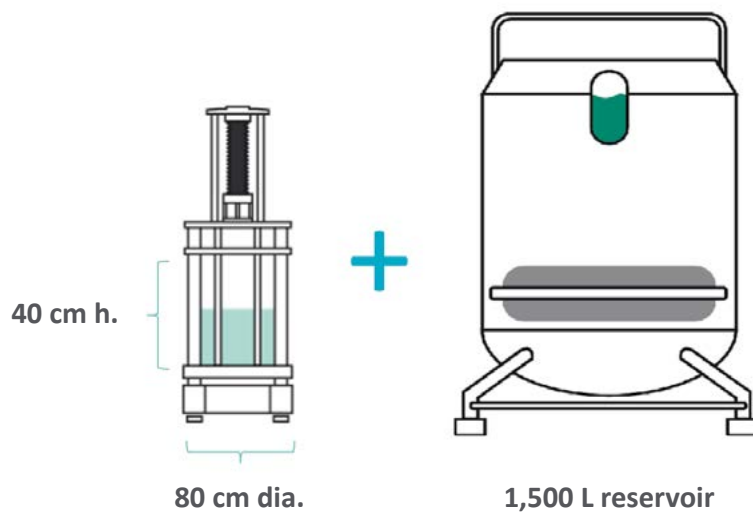
Codexis evolved enzymes maintain performance at high substrate concentrations and support robust activity across the diverse oligonucleotide sequence space. These properties, coupled with simplified reaction conditions and modular design, enable more predictable scale-up and support tech transfer into GMP environments.



ECO Synthesis Production Eliminates Critical Scale Barrier



“Off the Shelf” Equipment



ECO Synthesis vs Traditional Chemistry: Potential Impact

	Phosphoramidite Chemistry		ECO Synthesis Platform
Maximum Synthesis Batch Size (kg)	~6-7	→	5x + increase
Number of Syntheses	~150	→	>75% decrease
Production Time (months)	~12-22	→	>50% decrease
Capex Required	~\$500-700M+	→	>70% decrease

Purity

High purity is essential for therapeutic-grade RNA. Both sequential and ligation-based enzymatic workflows reduce the formation of byproducts compared to chemical methods, improving product quality. Specifically, enzymatic approaches have been shown to reduce Class IV impurities, a critical quality attribute for RNA therapeutics.

In ligation-based workflows, the use of short, highly purified RNA fragments, in conjunction with immobilized Codexis dsRNA ligase, contributes to cleaner final assemblies with demonstrated ability to remove purification of duplex materials significantly improving volumetric productivity.

Sustainability

Sustainability is increasingly central to RNA manufacturing strategy. Traditional PAC relies on flammable and toxic solvents such as acetonitrile and toluene, contributing to waste and increasing the complexity of safe handling and disposal.

ECO Synthesis technology eliminates many of these challenges. The aqueous workflow removes the need for solvent processes, reducing overall chemical waste. By enabling greener production with no organic solvents during synthesis, combined with milder conditions, ECO Synthesis supports environmental goals without compromising quality or throughput.

By adopting enzyme-based solutions, the biopharmaceutical industry can move towards a more sustainable and environmentally friendly approach to RNA manufacturing, contributing to a healthier planet and a more responsible future for medicine.

Downstream Processing

The efficiency of enzymatic synthesis can reduce downstream purification requirements. In particular, ligation-based assembly can start from cleaner inputs, reducing the accumulation of byproducts and simplifying final purification steps.

In some cases, particularly for ligation-based synthesis, the number and intensity of purification steps can be reduced depending on product quality requirements. This flexibility allows manufacturers to adjust purification strategies to meet specifications without overengineering the process — saving time, materials and cost.

Production Timelines

Enzymatic synthesis can reduce production timelines by enabling shorter, more efficient workflows. Ligation-based approaches support parallel synthesis of shorter fragments, which can then be assembled into full-length RNA, enabling flexible modular process design and supporting faster manufacturing cycles.

ECO Synthesis enables a streamlined two-step cycle for sequential synthesis (extension and deblocking), performed under mild, aqueous conditions. While individual cycle times may be longer than PAC presently, the ability to run larger batches and produce higher material outputs per run can offset this difference, supporting more efficient manufacturing at scale.

The Future of RNA Manufacturing

To realize the full potential of RNA therapies in transforming the treatment of a wide range of diseases, the industry must embrace a future focused on innovation, collaboration, and sustainability in manufacturing. This means adopting novel approaches like enzyme engineering and fostering partnerships that leverage cutting-edge technologies to overcome existing limitations. As the demand for RNA therapeutics grows, so does the need for efficient, scalable, and environmentally responsible manufacturing processes.

Partnering For Success in RNA Manufacturing

The Value of Collaboration

In the rapidly evolving field of RNA therapeutics, collaboration between technology providers and drug developers is essential for driving innovation and accelerating the development of new treatments. By partnering with specialized technology providers, drug developers can leverage industry-leading expertise and resources to overcome the challenges of RNA manufacturing and accelerate their path to market.

Codexis is a collaborative partner that offers comprehensive solutions to address the challenges discussed earlier. With expertise in enzyme engineering and process optimization, Codexis enables drug developers to achieve higher yields, improved purity and faster production timelines while minimizing environmental impact.

Codexis' ECO Synthesis Innovation Lab

The ECO Synthesis Innovation Lab is a testament to Codexis' commitment to innovation and collaboration in the RNA space. This state-of-the-art facility enables customers to evaluate ECO Synthesis technology for generating siRNA compounds, addressing industry challenges with its cost-effective, scalable and sustainable solutions.

The Codexis ECO Synthesis Innovation Lab supports the development and optimization of RNA manufacturing workflows across both enzymatic ligation and sequential synthesis approaches. This includes downstream purification strategies and quality analytical methodologies to define critical process parameters and ensure reproducible product quality.

As part of its broader process development mandate, the lab also supports lead candidate ligation — helping partners identify and refine ligation strategies suited to their therapeutic format. These services include:

- **Ligase screening:** Identifying the optimal ligase for a specific application from Codexis's extensive library of engineered ligases.
- **Ligase optimization:** Tailoring ligases to meet specific needs, such as improved activity, stability, and substrate specificity.
- **Process optimization:** Optimizing reaction conditions and protocols to maximize ligation efficiency and yield.

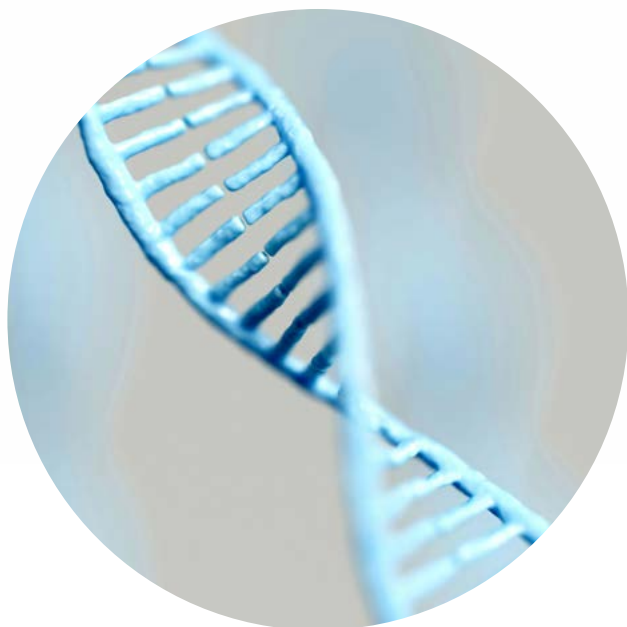
By leveraging Codexis' extensive library of engineered ligases and expertise in enzyme engineering, partners can achieve significant improvements in RNA manufacturing efficiency, leading to higher yields and scales, reduced costs and faster development timelines.

Advancing RNA Together

The landscape for RNA therapeutics is evolving rapidly, with expanding modalities, rising demand, and increasingly complex design challenges. As RNA programs mature, the need for scalable, high-quality, and environmentally sustainable manufacturing is more urgent than ever.

Codexis is meeting this moment with fit-for-purpose enzymatic solutions and a deep commitment to collaboration. From fully sequential synthesis to ligation-based assembly, the ECO Synthesis platform offers the tools, processes and support RNA developers need to overcome limitations and advance confidently toward commercial readiness.

Whether you're developing complex RNA constructs or optimizing for manufacturing scale, Codexis is ready to help — with enzyme-enabled solutions and the expertise to move you forward.



**Visit or contact us to discuss
your RNA Manufacturing goals**

Contact Us

Explore the latest advancements in RNA manufacturing and consider partnering with technology providers like Codexis to achieve your goals.

Together, we can accelerate the development of RNA therapies and shape the future of medicine.

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