Enzymatic Oligonucleotide Synthesis

Process Flow and Substance Impurity Profile

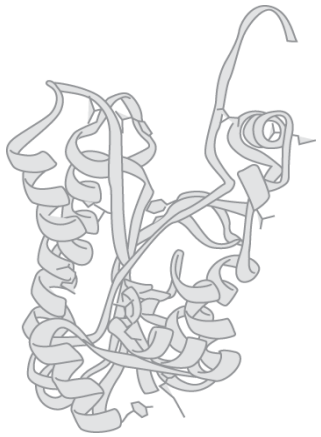
May 14th 2024

CODEXIS®

Catalyzing Innovation Through Engineered Enzymes

Powerful Underlying Science

CodeEvolver® directed evolution platform underpins exquisite enzyme engineering capabilities

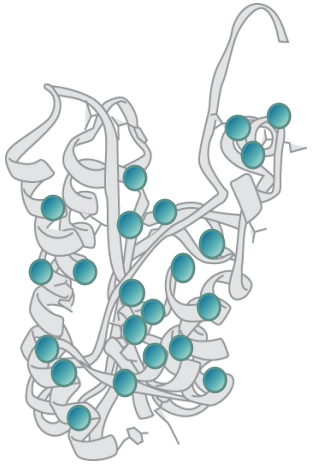


Enzymes from Nature

CodeEvolver®

Deep Technical Expertise

20+ years evolving enzymes supporting **small molecule API production and life science applications**



Highly differentiated products

Focus Areas

Custom Solutions for Biocatalysis

Over 50 commercialized enzymes supporting small molecule API production

Potential Game-Changing Technology

ECO Synthesis™ manufacturing platform **poised to disrupt the RNAi synthesis market**

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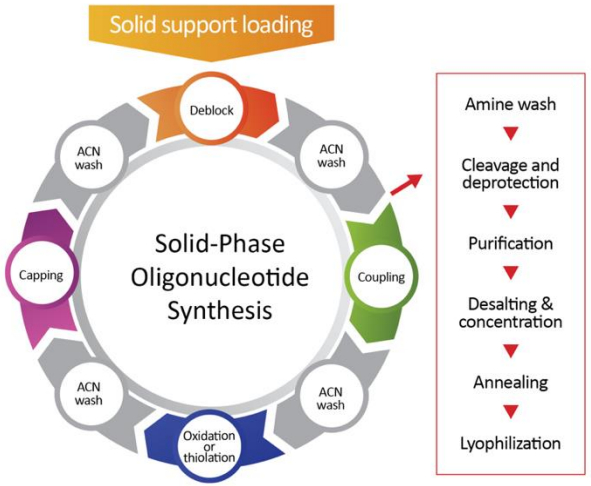
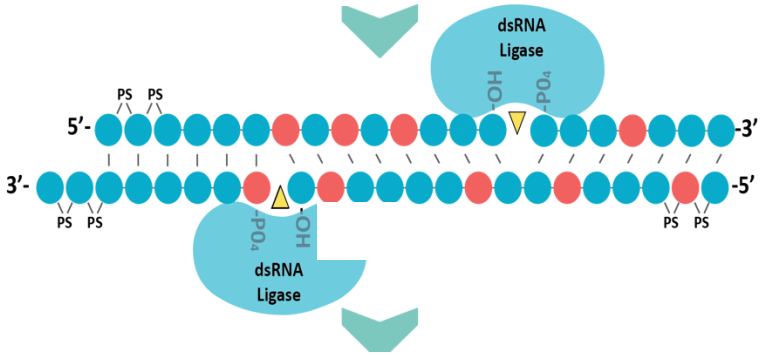
Enzymatic Solutions for siRNA Synthesis are Increasing

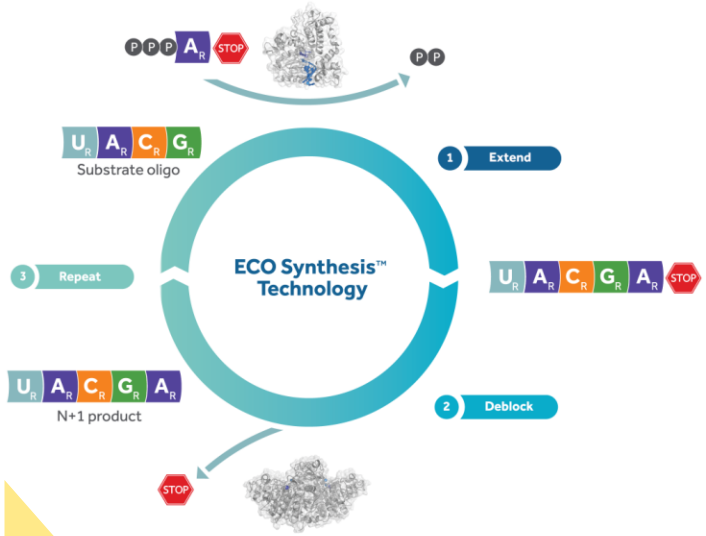
Chemical



Hybrid



Enzymatic



Traditional siRNA Synthesis using Phosphoramidite Chemistry (PAC)

PAC fragments + Ligation

ECO Synthesis™ (Enzyme-Catalyzed Oligonucleotide Synthesis) Manufacturing Platform

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ECO Synthesis™ Manufacturing Platform: Process Flow and Impurity Profile

Spotlight Presentation Luncheon 1

Derek Gauntlett, MBA, Director, ECO Process Development

Two Enzymatic Approaches for Large-scale siRNA Synthesis

TIDES Talks in the Exhibit Hall

Thursday, 16 May 2024 10:10 - 10:20 AM

Mathew Miller, PhD, Director, Life Sciences & RNA Technology

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ECO Synthesis™ Manufacturing Platform

Derek Gauntlett

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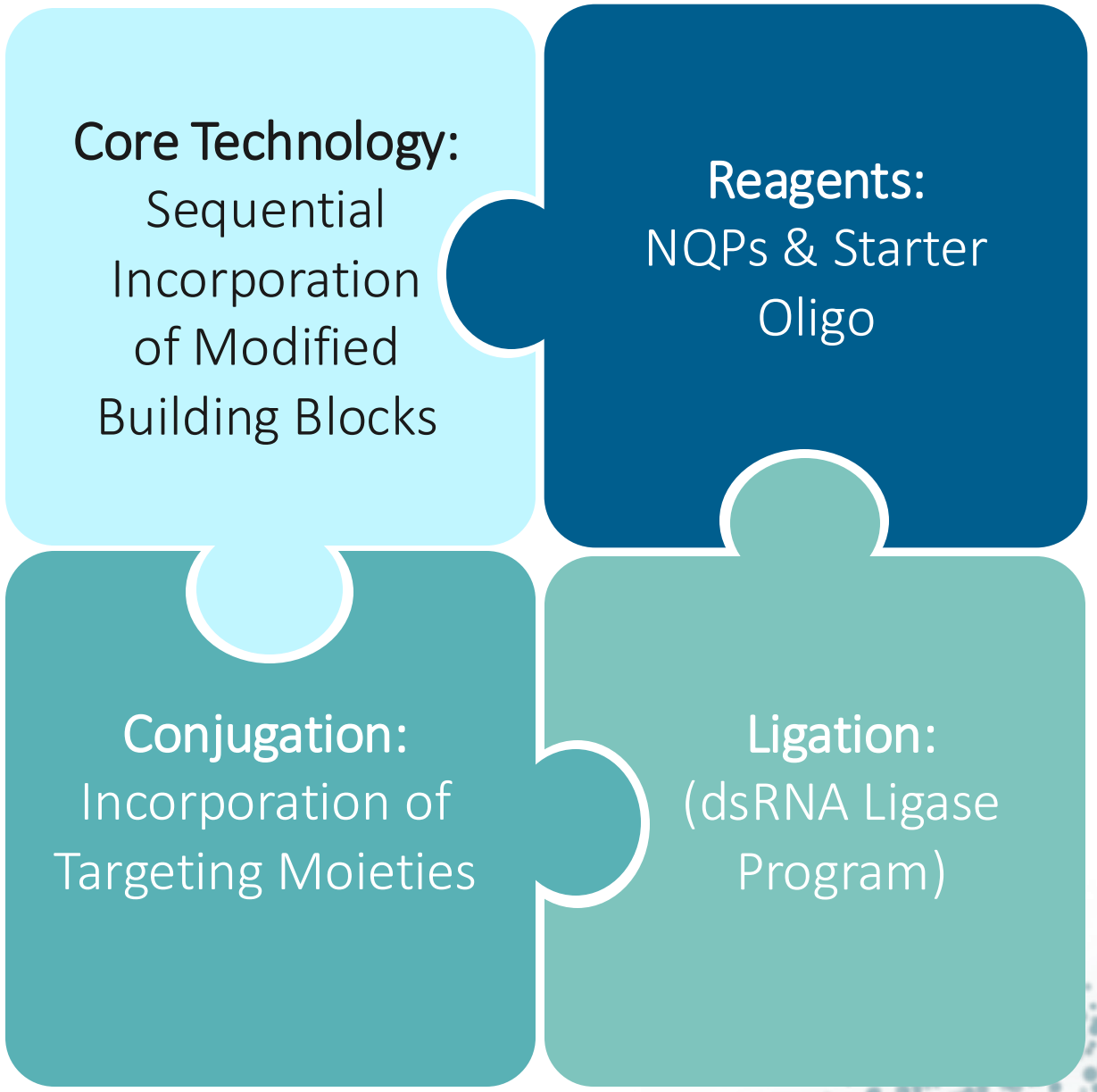
ECO Synthesis™ Manufacturing Platform: Modularity for Maximum Versatility



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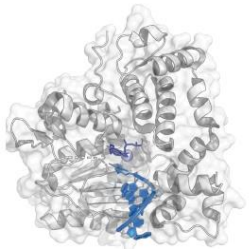
ECO Synthesis™ Manufacturing Platform – Key Elements

Starter Oligo

- Substrate from which synthesis initiates
- Synthesized via phosphoramidite chemistry (PAC) or enzymatically

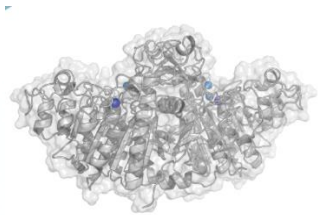
Nucleotide Quad Phosphate (NQP)

- Modified nucleotide monomers with blocking group
  - Phosphoramidite analog
- Synthesized chemically or enzymatically



Extension Enzyme

- Engineered to incorporate 2’-modified NQPs



Deblocking Enzyme

- Engineered to remove blocking group

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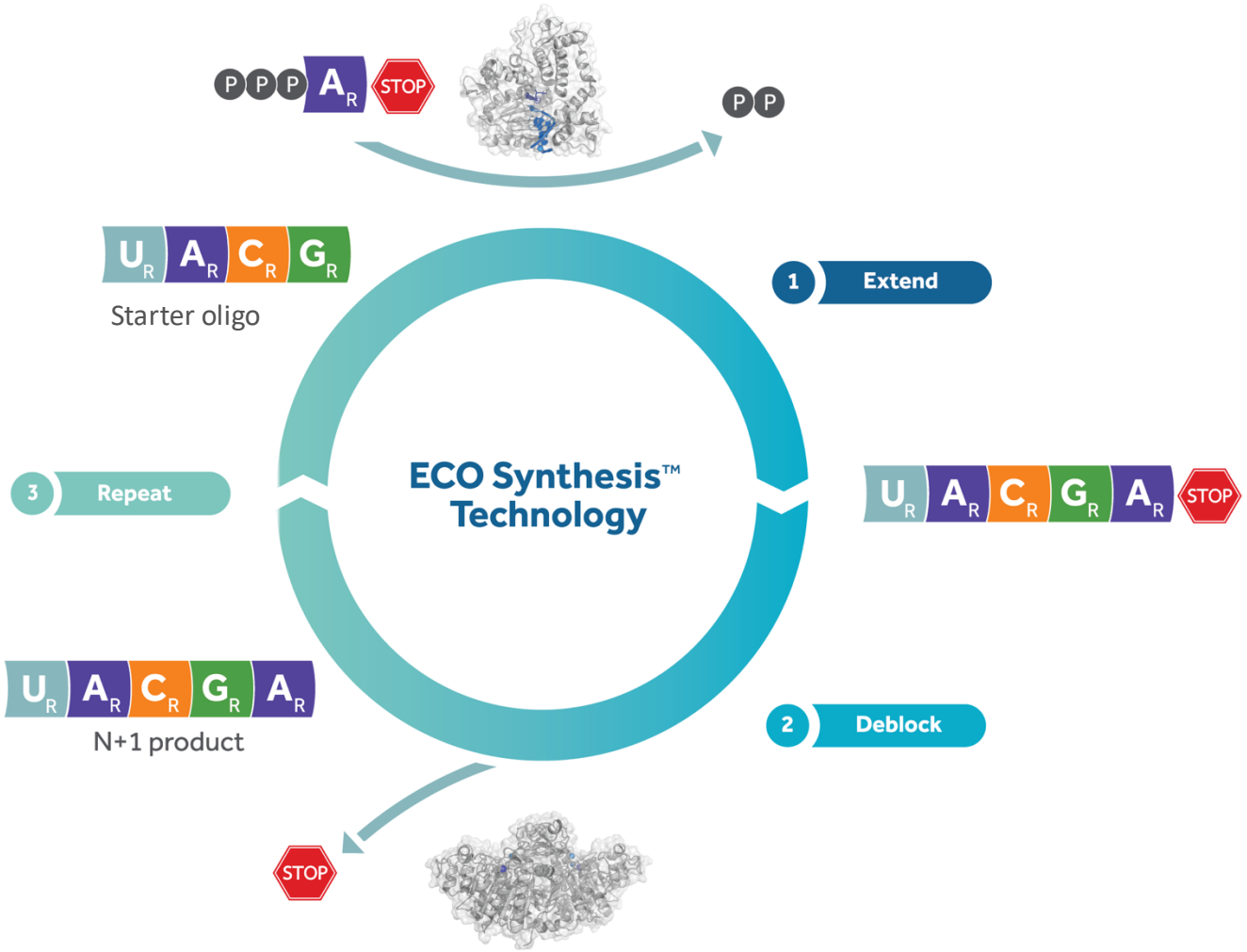
ECO Synthesis™ Manufacturing Platform - Overview



Key Performance Indicators for ECO enzymes:

- Non-native Substrates
  - Engineered for modified nucleotides
- Promiscuity
  - Minimal bias on 300k+ possible substrates
- Productivity
  - Coupling efficiency ~99% in hours
  - Minimal substrate & product inhibition
- Robustness
  - Stability
  - Manufacturability

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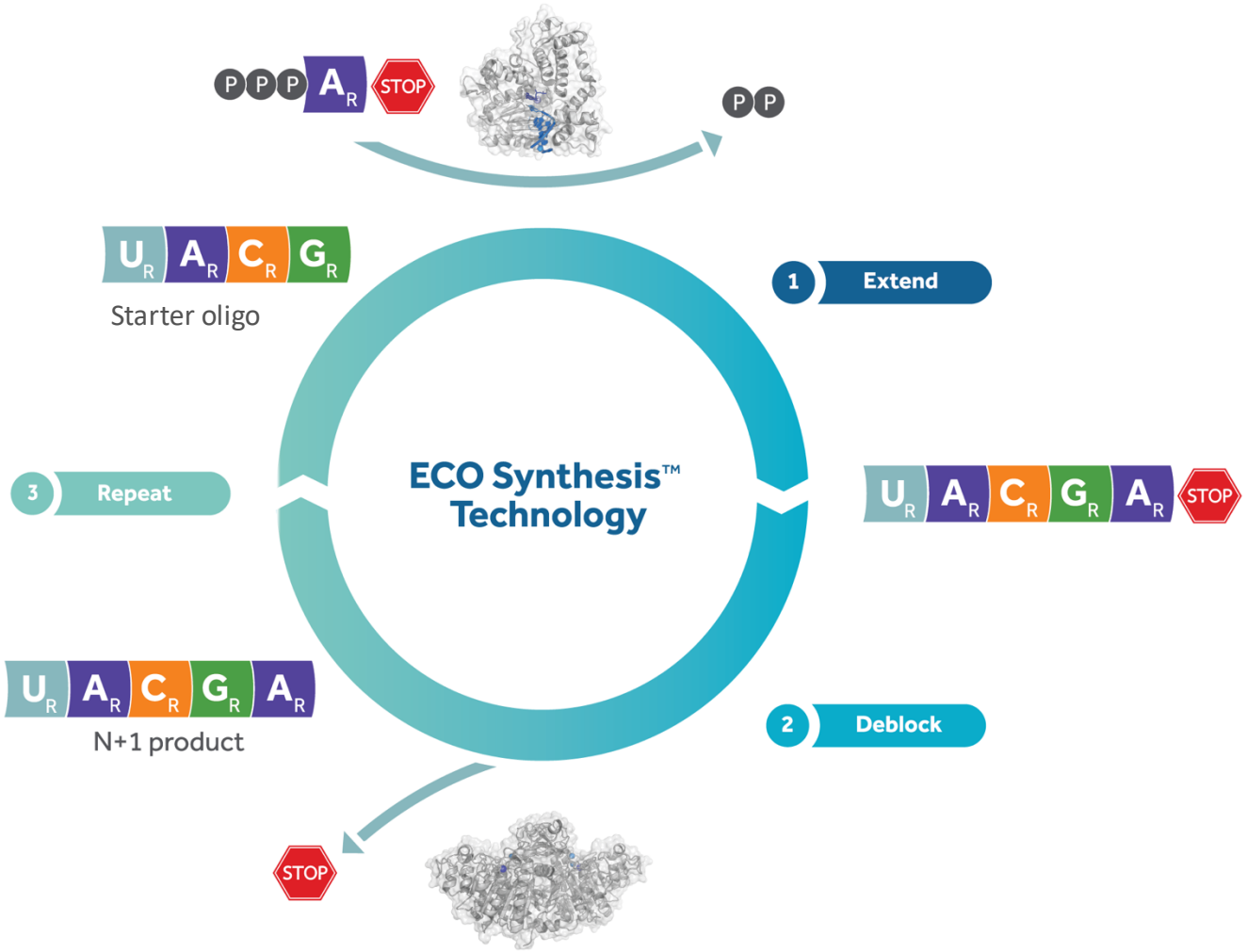
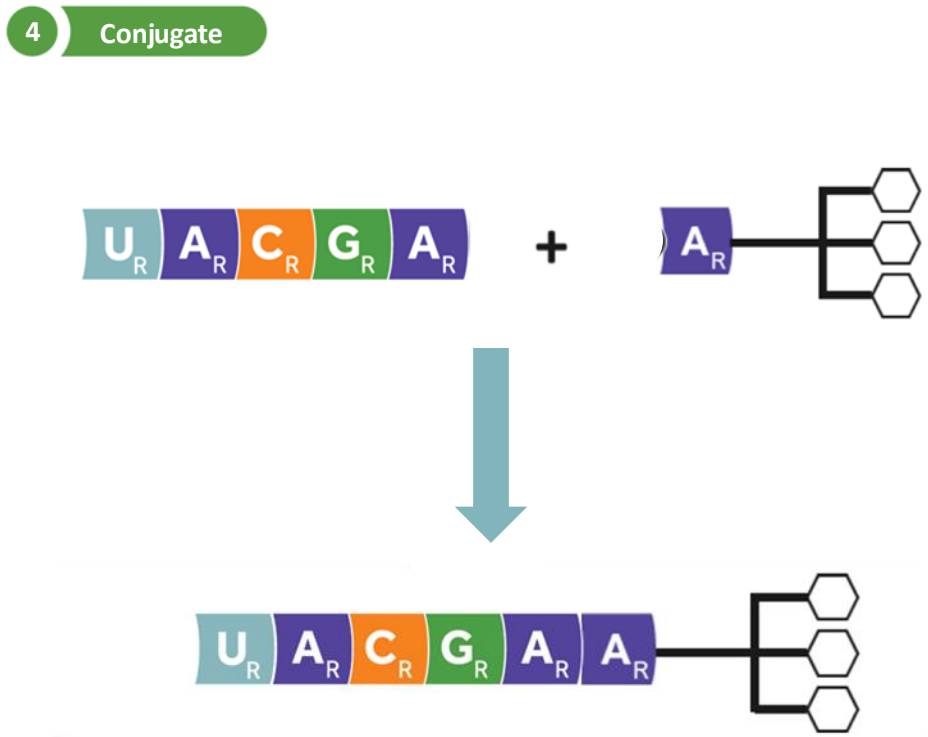
Targeting Moieties for Therapeutic Relevance

ECO Synthesis™ Manufacturing Platform Core Cycle



Enzymatic conjugation of targeting moieties



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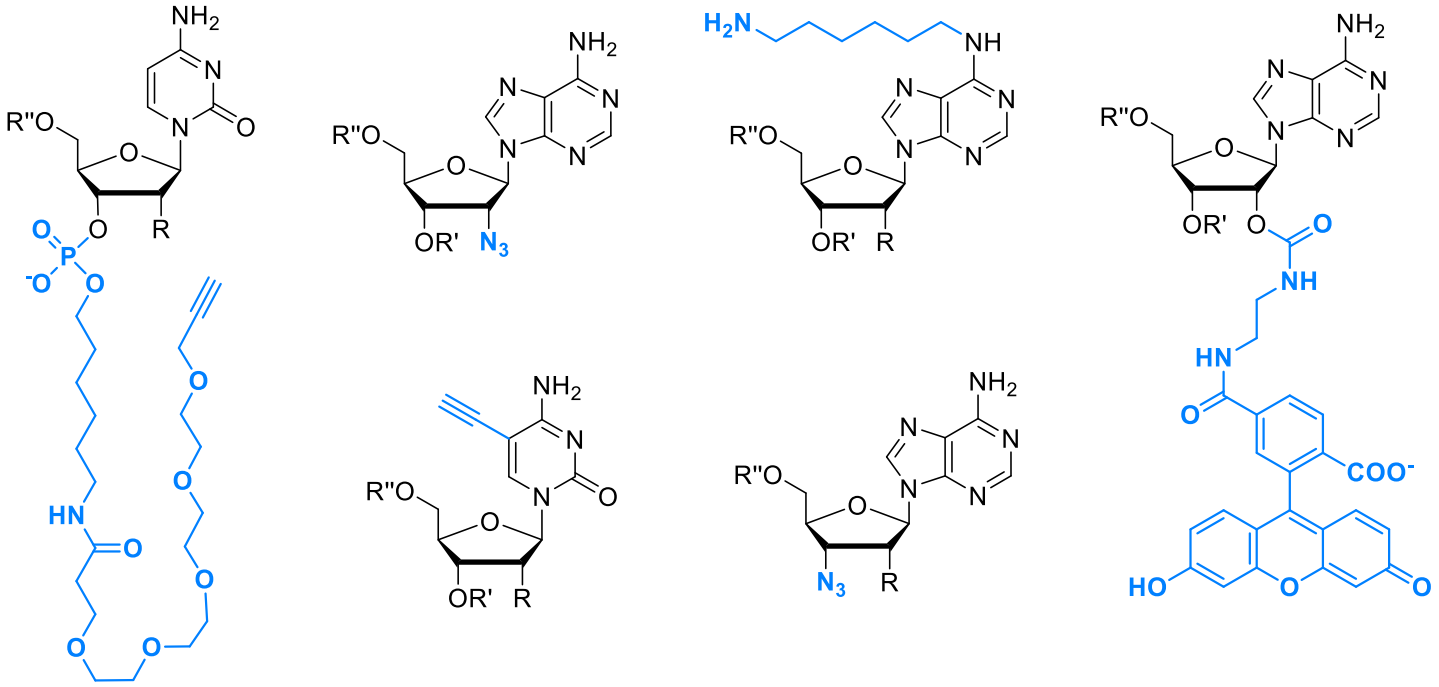
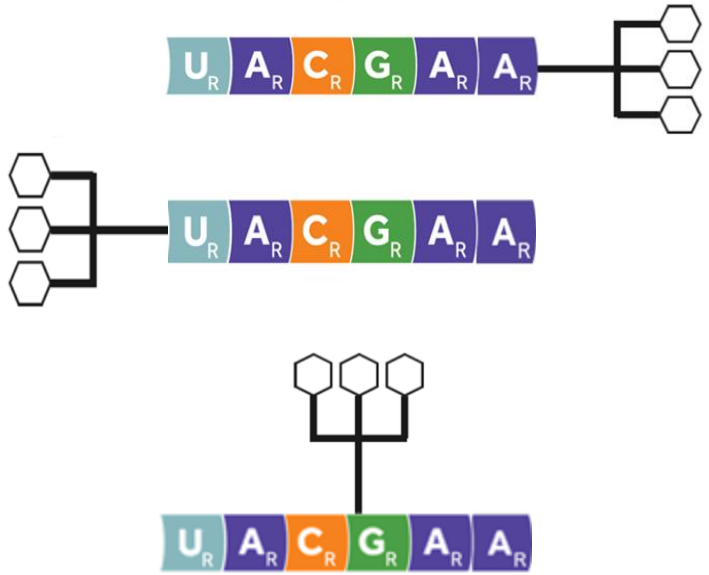
Targeting Moieties for Therapeutic Relevance



Enzymatic conjugation of targeting moieties

4 Conjugate

5’ -> 3’



ECO Synthesis™ manufacturing platform enables quantitative incorporation of modified nucleotides with reactive and sterically demanding bulky side chains at 5’, 3’ and intra oligonucleotide.

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Chemical Synthesis vs. ECO Synthesis™ Manufacturing Platform

Phosphoramidite Chemistry		ECO Synthesis™ Manufacturing Platform
Limited Scalability • Best suited for bench top scale due to limited, single-digit kilogram batch sizes • Capacity will be challenged to support future demand	→	**Scalable** ✓ Enzymes and flow-based process ✓ Dual reactor setup enables larger batch sizes compared to PAC
Toxic Solvent Use • Requires large volumes of inorganic solvent (acetonitrile) with high disposal costs • Likely future supply chain limitations & price volatility	→	**Reduced Waste** ✓ Aqueous reactions ✓ Significantly decreases chemical waste streams ✓ Path to enzymatically created monomers
Low Purity • Inefficient for longer RNAs • Significant impurities from complex protection / deprotections	→	**High Purity** ✓ Higher purity driven by milder conditions during synthesis
High Cost • High-cost infrastructure investment & raw materials • High purification costs • Expensive waste disposal	→	**Valuable Economics** ✓ De-bottlenecks current supply constraints with increased scale & efficiency ✓ Integrates with existing manufacturing facilities that have purification capabilities

Source: Codexis Market Research 2023; design features of ECO Synthesis™ manufacturing platform

Progress Achieved on the ECO Synthesis™ Manufacturing Platform

Demonstrating the Power of Combing Enzyme Engineering & Process Development in One Year

May 2023
TIDES USA

Incorporation efficiency: **~88%**

FLP purity at N+4 (max. 4) = **~60%**

Accepting mod. nucleotides: **2'-OMe, 2'-F**

Nov 2023
TIDES EU

Incorporation efficiency: **~92%**

FLP purity at N+4 (max. 6) = **72%**

Accepting mod. nucleotides: **2'-OMe, 2'-F, PS**

Dec 2023

Gram-scale synthesis (N+6 w/ fully modified nucleotides)

May 2024
TIDES USA

Incorporation efficiency: **>98%**

FLP purity at N+4 (max. 14) = **92.7%**

Accepting mod. nucleotides: **2'-OMe, 2'-F, PS, 2'-H, "others"**

Conjugation chemistry

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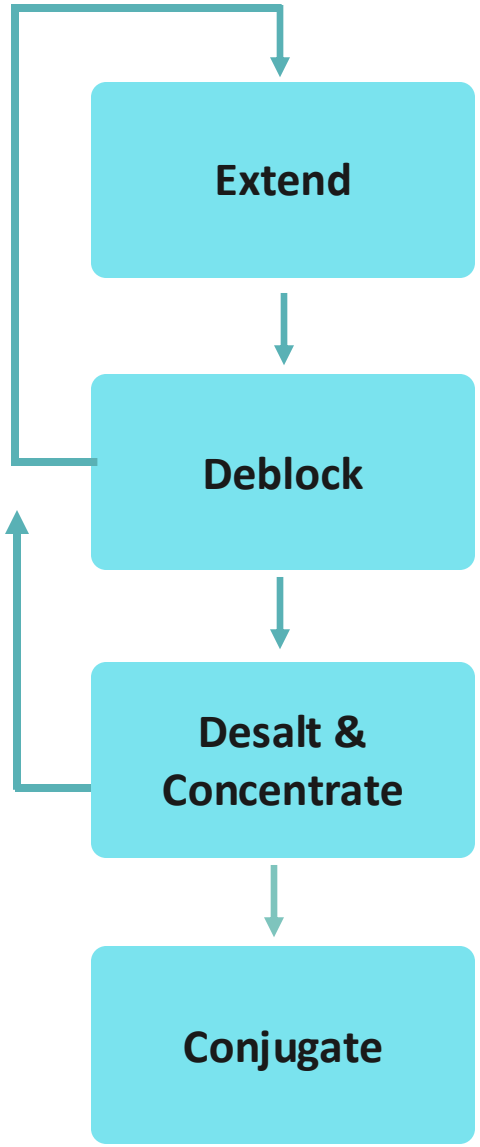
ECO Synthesis™ Current Process Overview: 4-Step Unit Operation



- Immobilized polymerase to elongate the substrate in a controlled manner.
  - Fixed packed bed reactor with co-immobilized enzymes on resin
  - Recirculation flow model
  - One reservoir system
- Immobilized phosphatase to remove blocking group from substrate and NQP.
  - Fixed packed bed reactor with immobilized enzyme on resin.
  - Linear flow model
  - Two reservoir system
- Ultra filtration diafiltration (UFDF) to increase concentration and desalt
  - Desalt only performed as needed - compound dependent.
  - TMP Flux model
  - Cassette style system
- Enzyme to add conjugation moieties in batch
  - Batch reactor system

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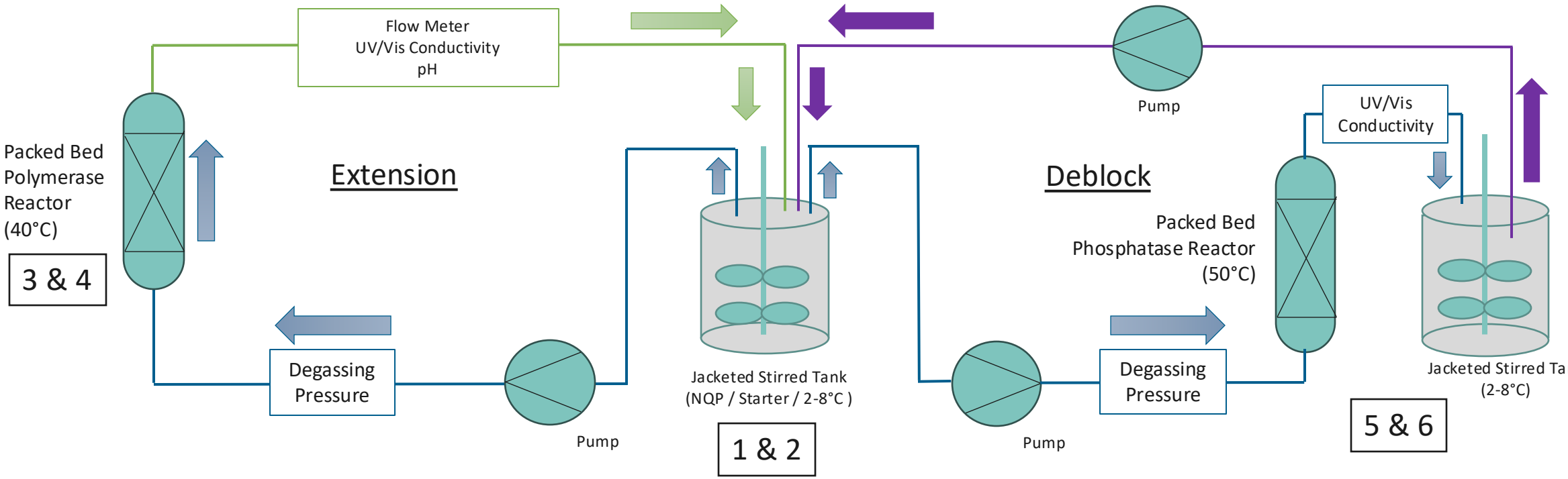
ECO Synthesis™ Liquid Flow Cycle – Current Research Scale



Key Process Conditions

1. **Scale:** 2mM (Starter) / 1.5 e.q. NQP
2. **Volume Start:** 9 mLs
3. **Extension Residence Time:** 0.10 hours
4. **Extension Column Size**: 3g resin / column
5. **Deblock Column Size:** 3g resin / column
6. **Deblock Residence Time:** 0.10 hours

Note: UFDF performed using 1kDa MWCO cassette at 5th and 13th extensions

Process conditions are not finalized and are under optimization

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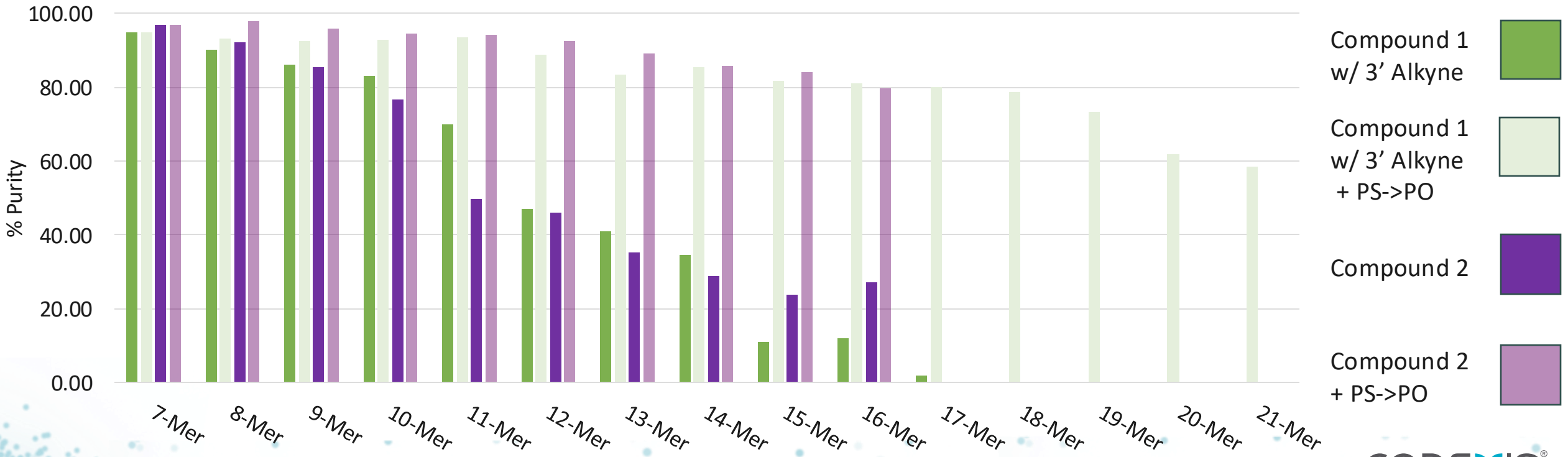
ECO Synthesis™ - Process by the Numbers

- Two fully 2'-modified RNA oligonucleotides were synthesized using ECO Synthesis™ manufacturing platform demonstrating the technology's capability to synthesize different sequences.

Compound	Number of Extensions	RNA Starter Length	Added RNA Base Types	Avg. Coupling Efficiency (%)	Avg. Coupling Time (H)	Avg. Deblock Time (H)
Compound 1 w/ 3' Alkyne	14	7	2'F / 2'OMe	98	11.1	2.5
Compound 2	11	7	2'F / 2'OMe	98	6.0	2.5



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ECO Synthesis™ Impurity Profile MS - Compound 1 w/ 3’ Alkyne

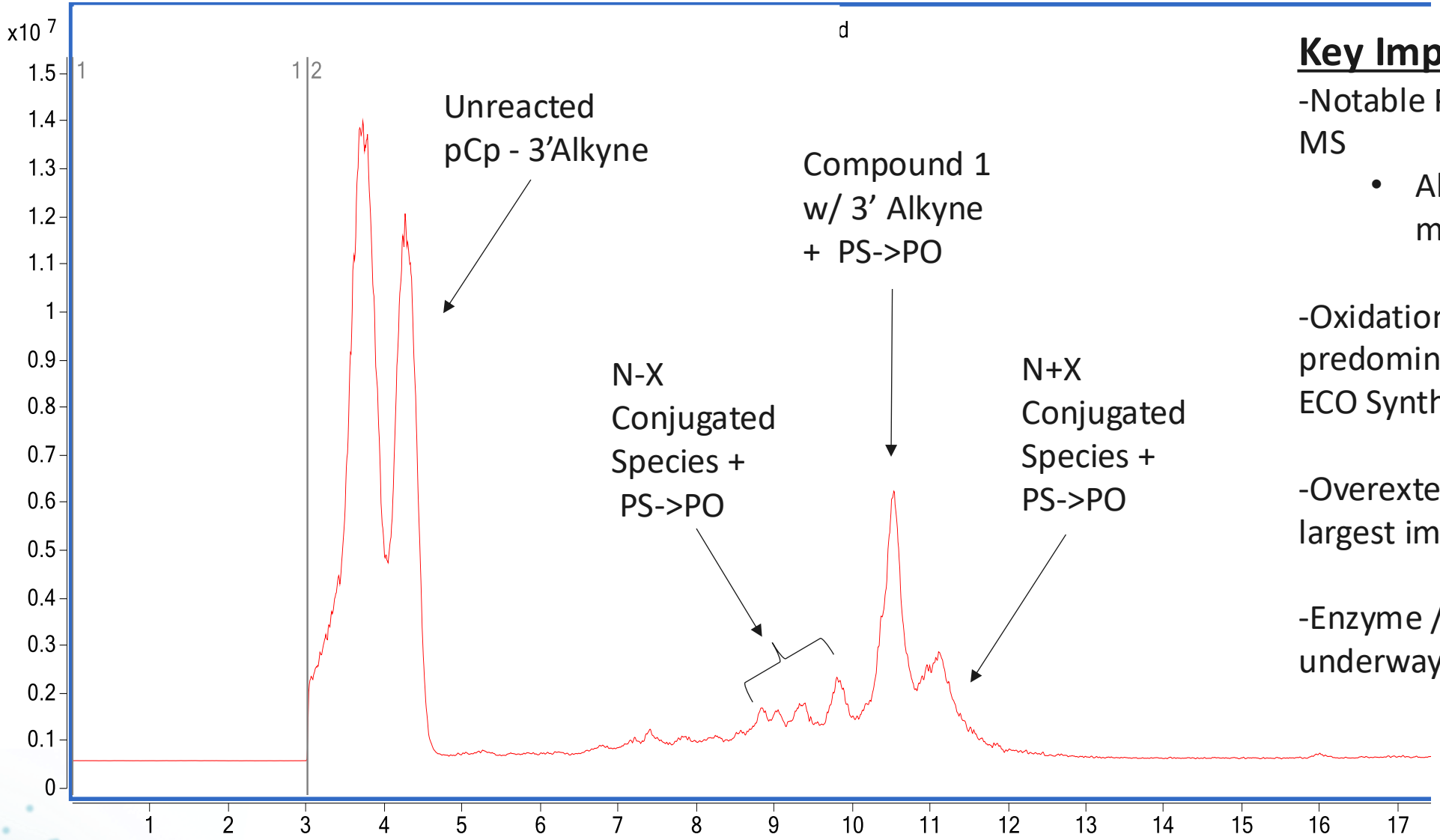

Figure. TIC of Compound 1 w/ 3’Alkyne

Key Impurity Takeaways

-Notable PAC impurities **not** present by MS

- Abasic, acetyl, IBU, cNet, and methylation

-Oxidation of PS->PO is the predominate impurity generated using ECO Synthesis™ today.

-Overextension products are second largest impurity present.

-Enzyme / Process fixes are currently underway for both sets of impurities.

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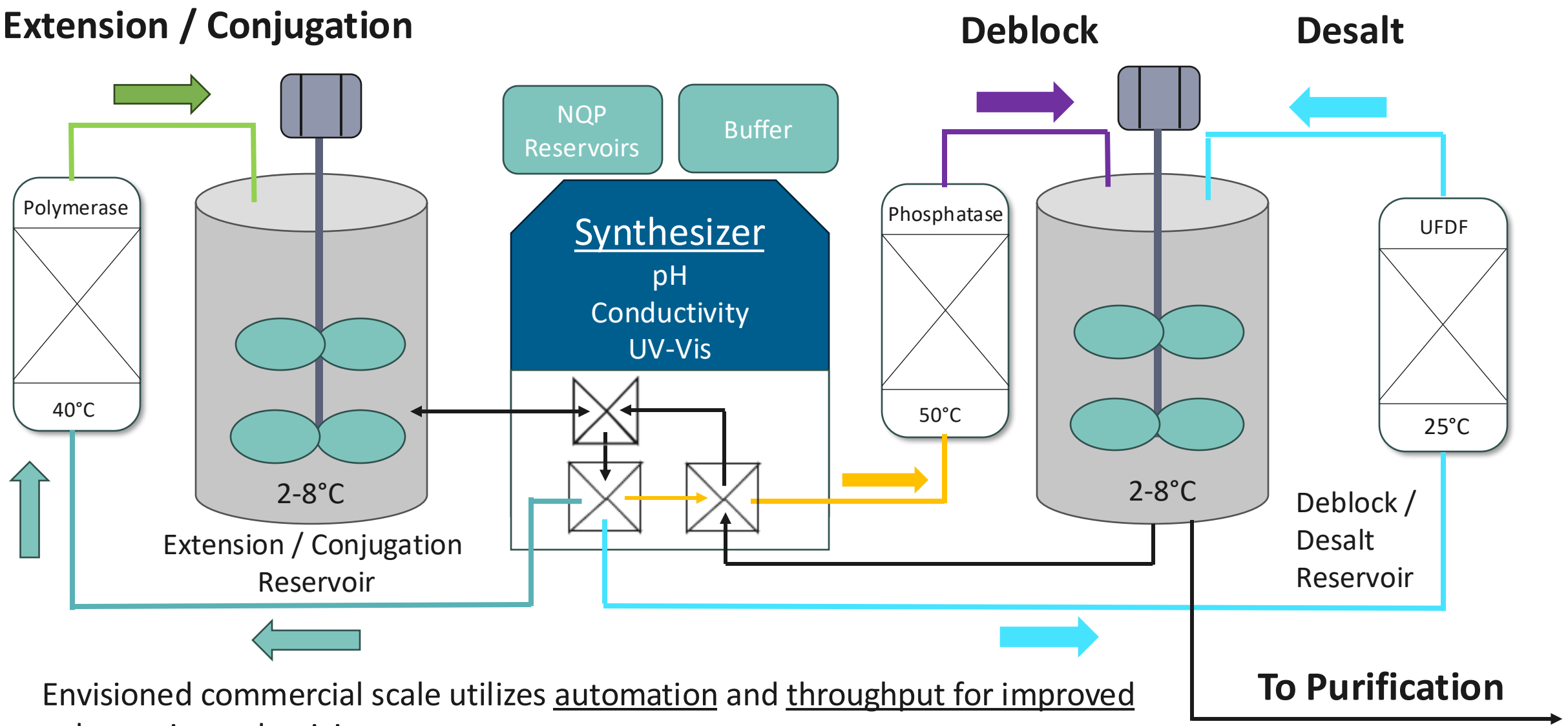
ECO Synthesis™ Process Design – Commercial Scale



Envisioned commercial scale utilizes automation and throughput for improved volumetric productivity.

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Forward Looking for ECO Synthesis™ Manufacturing Platform

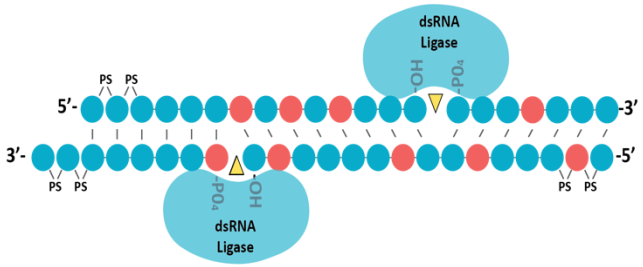
Codexis is Positioned for Significant ECO Synthesis™ Improvements in 2024

- ECO Synthesis™ manufacturing platform has made significant progress since TIDES USA in May 2023
- Continue process development
  - Holistic process design space created using a QbD approach to push >99% conversion efficiencies
  - Phosphorothioate oxidation control under sequential ECO Synthesis™ platform
  - Decreased coupling times for challenging sequences for volumetric productivity improvements
  - Pushing research scale towards fully automated commercial scale processes
  - Evaluate therapeutically relevant conjugate moieties
- Continue enzyme engineering

Codexis Center of Excellence for Enzymatic RNA Synthesis

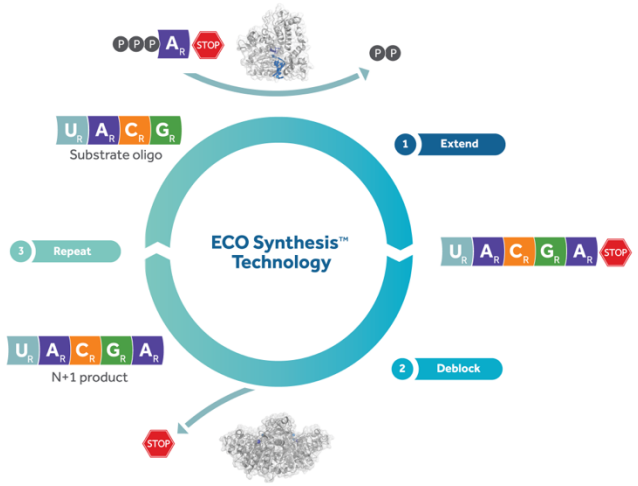
Your Pathway to Lower Cost and More Scalable RNA Manufacturing

Hybrid



RNA Ligase Services

Enzymatic



ECO Synthesis™ Manufacturing Platform

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Booth #628

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