Oligonucleotide CMC for Sustainable and Scalable Enzymatic siRNA Manufacturing

Alison Moore, CTO

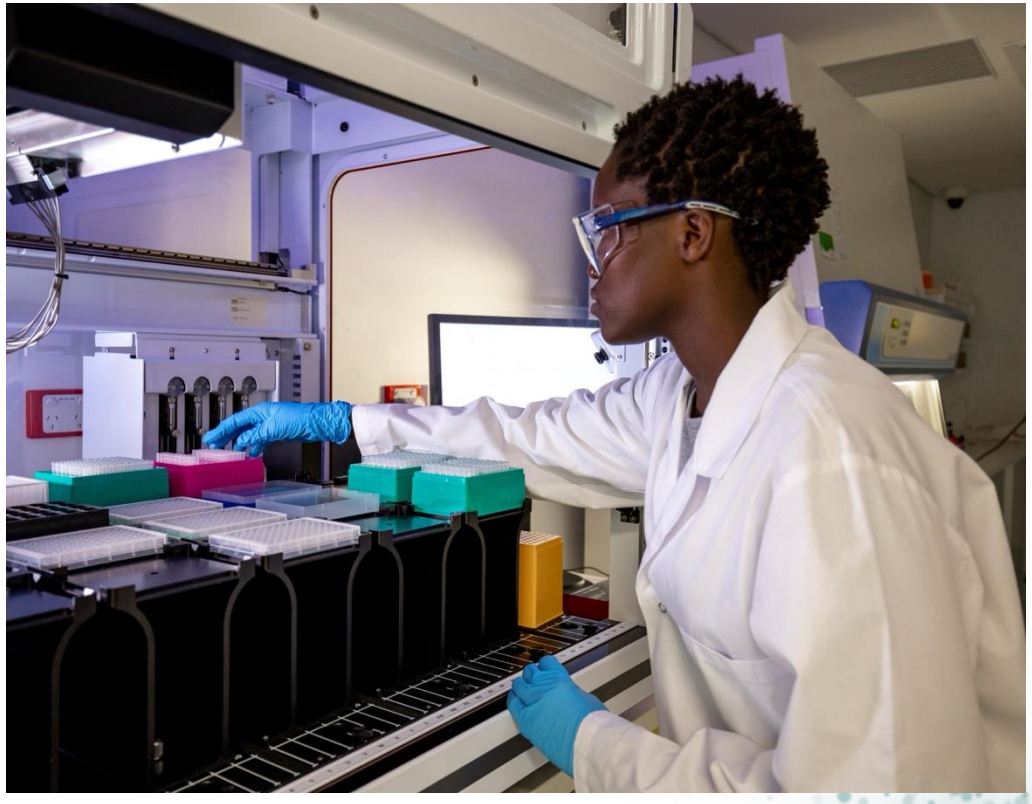
Derek Gauntlett, Head of ECO Process Development

CODEXIS®

TIDES US 2025

Codexis: Your Technology Enabler for Complex Therapeutics

- Headquartered in California, USA
- **22+ years** of enzyme expertise
- **16+ commercialized therapies using Codexis enzymes**
  - **PAXLOVID™, Januvia®, Janumet®**
- Pioneering **enzymatic RNA manufacturing** through 2 proven methods with our ECO Synthesis™ Manufacturing Platform
  - dsRNA fragment ligation
  - Sequential enzymatic synthesis



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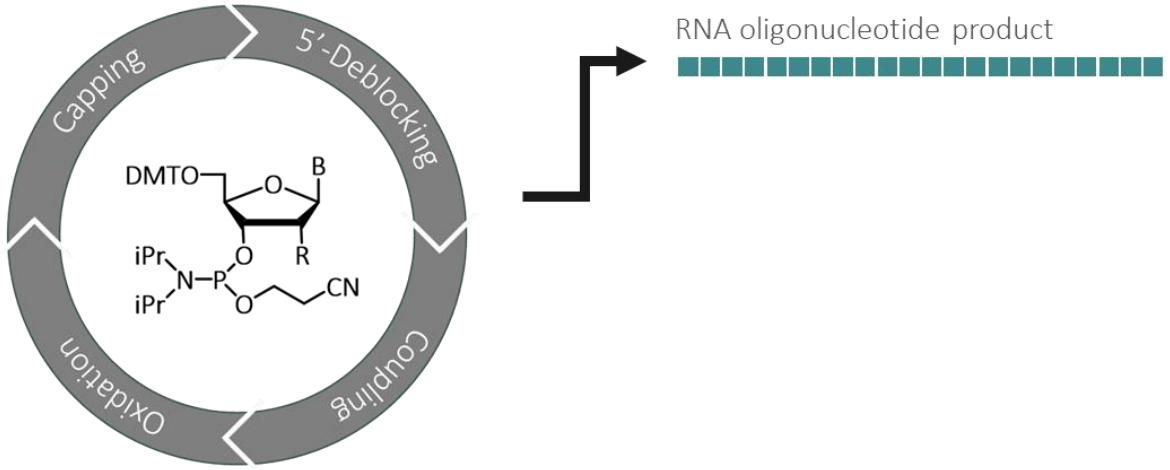
RNA Synthesis – An Evolving Manufacturing Landscape



	Phosphoramidite Chemistry
Development status	>40 years; mature w/ incremental improvements
Product quality	High
Product yield	High
ESG	>3000 kg organic solvent/kg API

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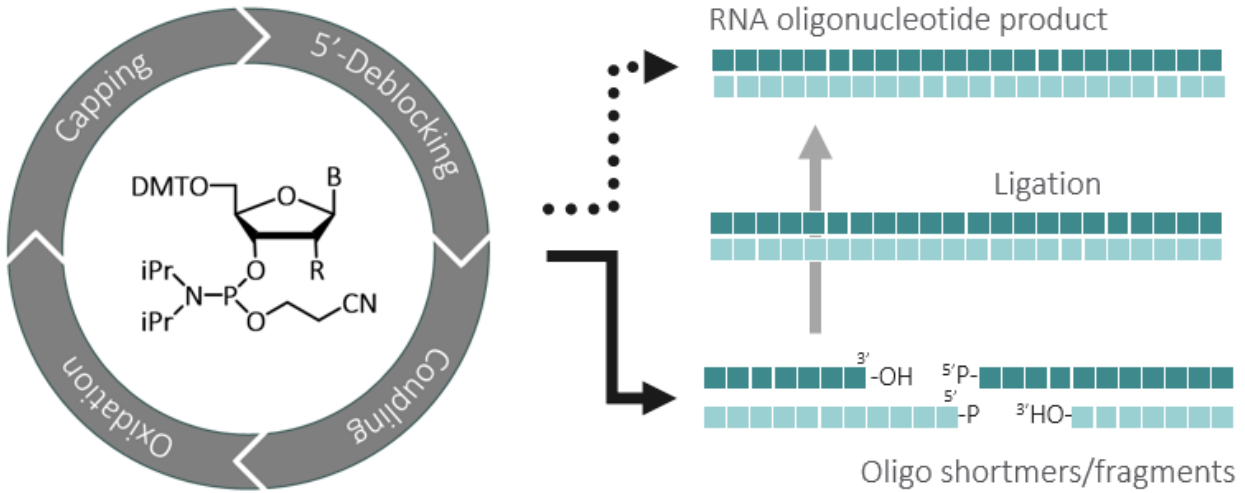
RNA Synthesis – An Evolving Manufacturing Landscape



	Phosphoramidite Chemistry	Enzymatic Ligation
Development status	>40 years; mature w/ incremental improvements	Ready for manufacturing
Product quality	High	High
Product yield	High	Higher
ESG	>3000 kg organic solvent/kg API	Partially aqueous

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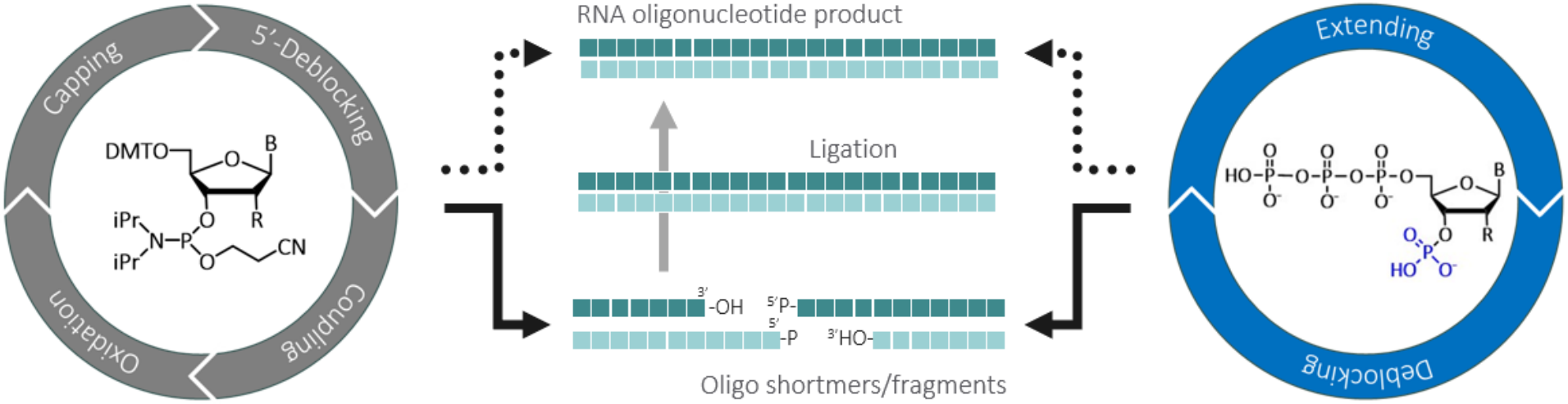
RNA Synthesis – An Evolving Manufacturing Landscape



	Phosphoramidite Chemistry	Enzymatic Ligation	Enzymatic Sequential Synthesis
Development status	>40 years; mature w/ incremental improvements	Ready for manufacturing today	Operational at 10-100g with path to GMP manufacturing
Product quality	High	High	High
Product yield	High	Higher	Higher
ESG	>3000 kg organic solvent/kg API	Partially aqueous / organic	Fully aqueous

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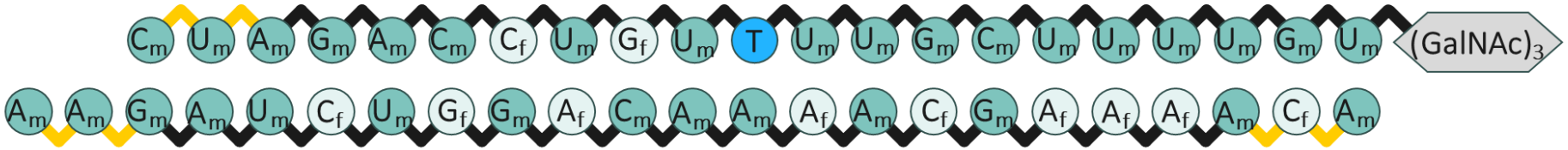
ECO Synthesis™ Technology Provides High Performance and Versatility

- First successful enzymatic synthesis of Inclisiran® – a commercial therapeutic asset
- Routes 2-4 produced siRNA therapeutic assets of equal quality and yield



Route 1:

Fully enzymatic, sequential synthesis using ECO Synthesis for both strands & incorporation of targeting moiety

Route 2:

Oligo fragments synthesized by SPOS chemical method & ligated

Route 3:

Oligo fragments synthesized by both chemical or enzymatic methods & ligated

Route 4:

Oligo fragments synthesized by ECO Synthesis & ligated

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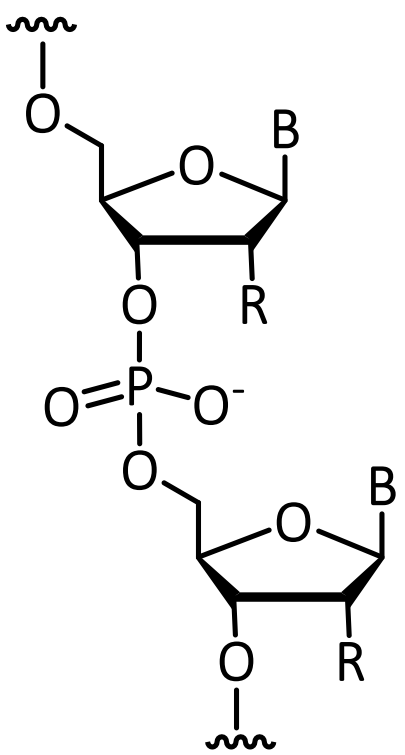
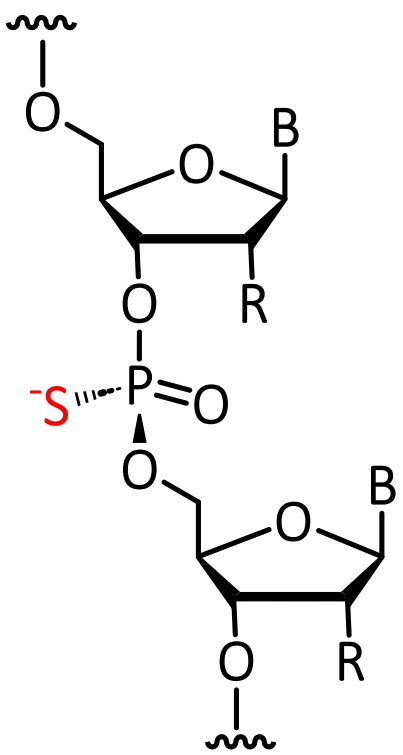
ECO Synthesis Technology can offer Stereochemical Control

Stereochemistry can impact efficacy, half-life, and toxicity of therapeutic oligos

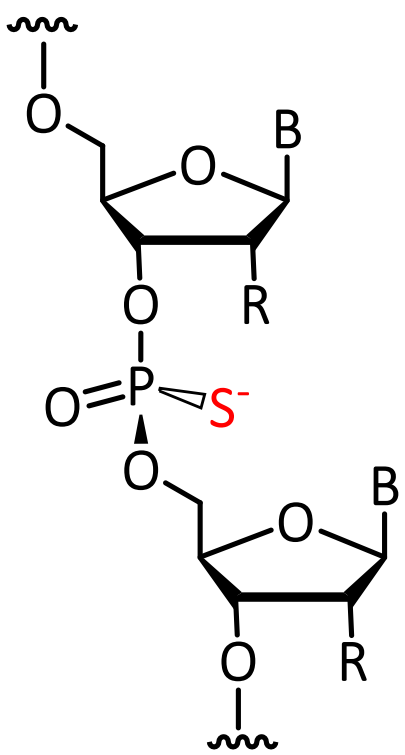


Achiral

R = H, OH, OMe, F, etc.



Rp



Sp

TIDES talk in the Exhibit Hall at 10:10am on '*Comparative Analysis of Diastereomeric Distribution in siRNA Synthesis: PAC vs Enzymatic Synthesis*'

Sakamuri, et al. *ChemBioChem* **2020**. 21, 1304-1308; Jahns, et al. *Nuc. Acids Res*. **2022**. 50,3 1221-1240; Liu, et al. *Nuc. Acids Res*. **2023**. 51, 9, 4126-4147

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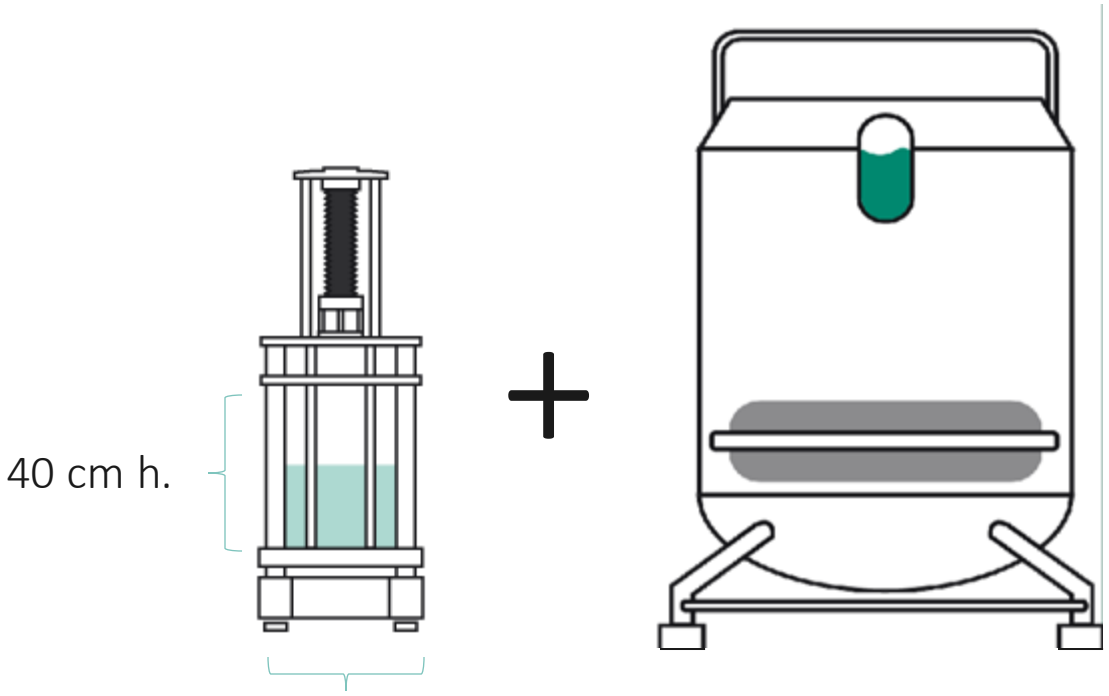
ECO Synthesis Technology using Immobilized Enzymes can Deliver Highly Scalable Processes

Standard Equipment



Sequential ECO Synthesis Platform*

Maximum Batch Size (theoretical)	5x + increase
Number of Synthesis runs	>75% decrease
Production Time (months)	>50% decrease
CapEx for facility build	>70% decrease

*Comparison vs. phosphoramidite chemistry, modeled based on inclisiran sequence, 2 trains, 24/7 operation, facility capacity ~1,000 kg per year

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Sequential ECO Synthesis Performance Continues to Improve

Process Metric	TIDES EU 2024 -> TIDES US 2025
Coupling Efficiency	Constant at >98.5%
Synthesis Concentration	Constant at 6mM
Oligo Synthesis Recovery	Increased by from 25 to 75%
Avg. Cycle Time (h)	Decreased by ~24%
PS->PO Impurity (%)	Decreased by >75% to <1.5% per strand
N-X Impurity (%)	Constant at <10% per strand
N+X Impurity (%)	Decreased by 5% at <6% per strand

Values based on syntheses of 21mer Sense and 23mer Antisense strands

Sequential ECO Synthesis™ results in yields of ≥30 g/L siRNA

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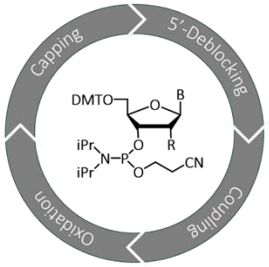
Immobilized dsRNA Ligase can Deliver Clinical Quality siRNA

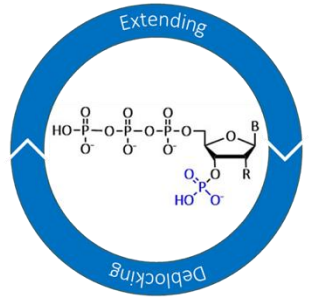
Route for Oligo Synthesis

Fragment Workup

Triplicate Ligation

Ligation Characterization





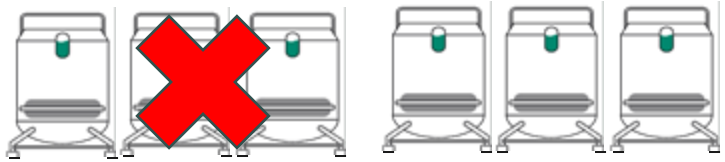
Crude

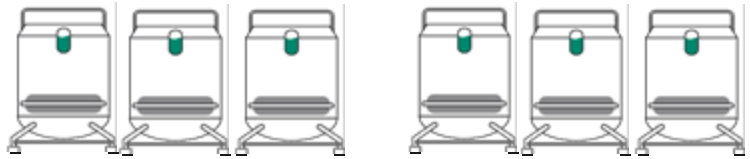
AEX Purified

Crude

AEX Purified

*





Immobilized Ligase

Purity & Impurity by MS, Duplex Purity by SEC and Residual Ligase

* SPOS crude fragments not of sufficient quality (<75% purity per fragment) for comparable assessment

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Ligation Fragment Design for Optimal Ligation Performance

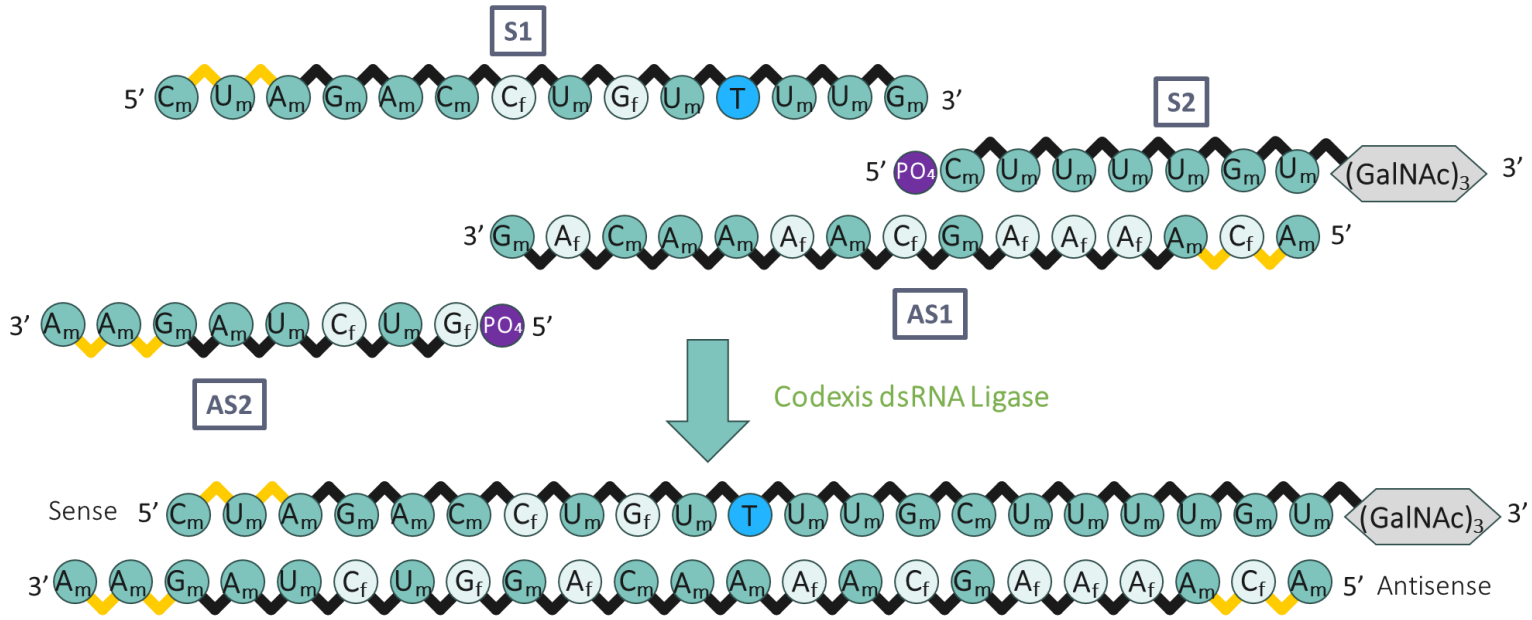
Two nick / four-fragment approach used for the generation of Inclisiran®

Ligation Reaction Conditions

Parameter	Condition
Fragment Concentration (mM)	2
Duplex Concentration (g/L)	30
Codexis dsRNA Ligase	Immobilized
Buffer Composition	Aqueous
Incubation Temperature (°C)	33
Incubation Time (h)	4

Fragment Design

- Optimized fragment design for ligation using Codexis Machine Learning - guided fragment design



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Starting Fragment Purity for Each Chemistry Route

Fragment Workup	AS1 (15mer) % Purity	AS2 (8mer) % Purity	S1 (14mer) % Purity	S2 (7mer + L96) % Purity
ECO Crude	88.6	93.5	89.6	88.3
SPOS Purified	88.9	93.2	97.7	91.9
ECO Purified	89.8	94.9	97.4	95.8

Fragment purity table – Purity data from denaturing IP-RP LC-UV-MS

- ECO crude fragments resulted in a 99% coupling efficiency from synthesis.
- ECO crude fragments were purified to match the source SPOS purified fragment purity.
- SPOS crude fragments were sourced but they were not of sufficient quality for use <75% purity per fragment.

Crude RNA Fragments can Generate High-Quality siRNA Duplex using AEX Purification Post Ligation

Ligation Pathway	Conversion (%)	Sense Strand (%)	Antisense Strand (%)	Crude Duplex Purity (%)	Residual Ligase (PPM)	Notable Impurities
ECO Crude	>92	33.4	49.1	77.9	< LOD [2.5ppm]	AS1, S1, S2, N-1 & N+1, PS/PO

Crude Duplex purified via AEX chromatography

Ligation Pathway	Conversion (%)	Sense Strand (%)	Antisense Strand (%)	Duplex Purity (%)	Residual Ligase (PPM)	Notable Impurities
ECO Crude	N/A	40.1	53.2	98.3	< LOD [2.5ppm]	S -mC, AS +mG, PS/PO

~70% purification yield when purifying crude duplex with a starting purity of ~78%

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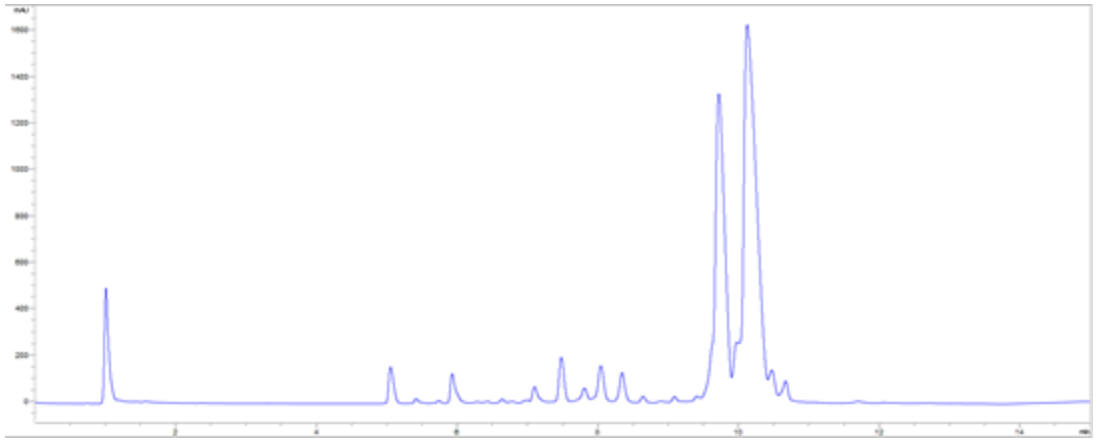
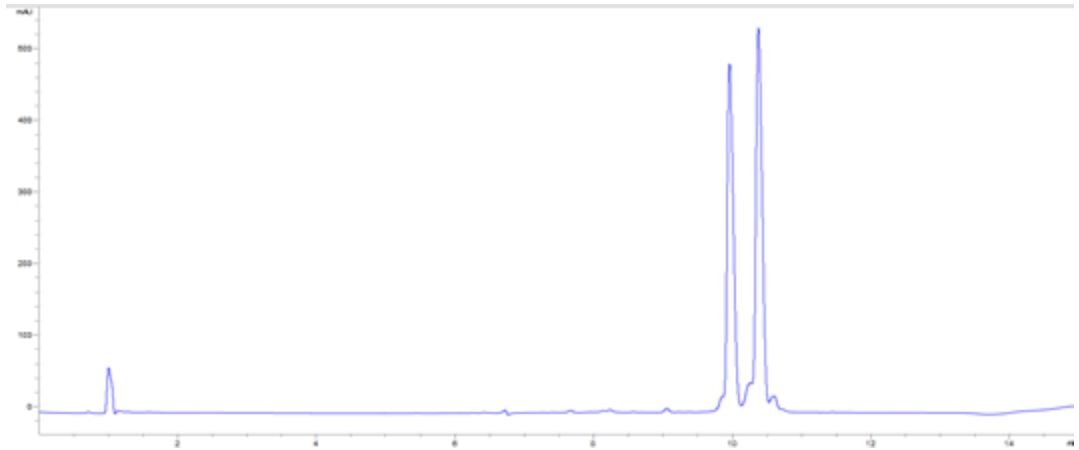
Crude ECO Synthesis Fragments can Skip Fragment Purification

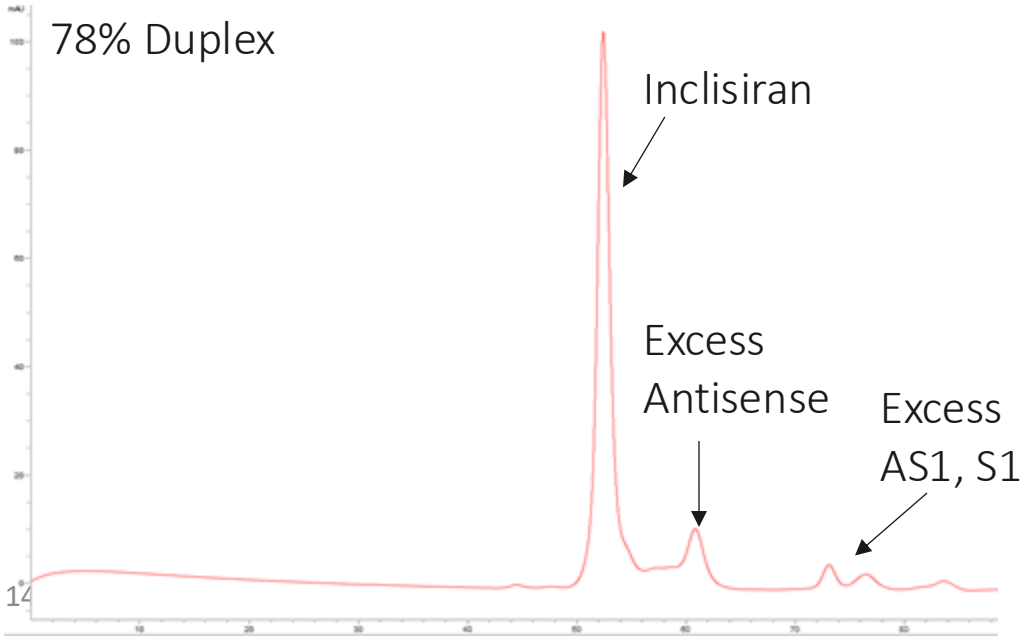
Crude Duplex



AEX

Purified Duplex





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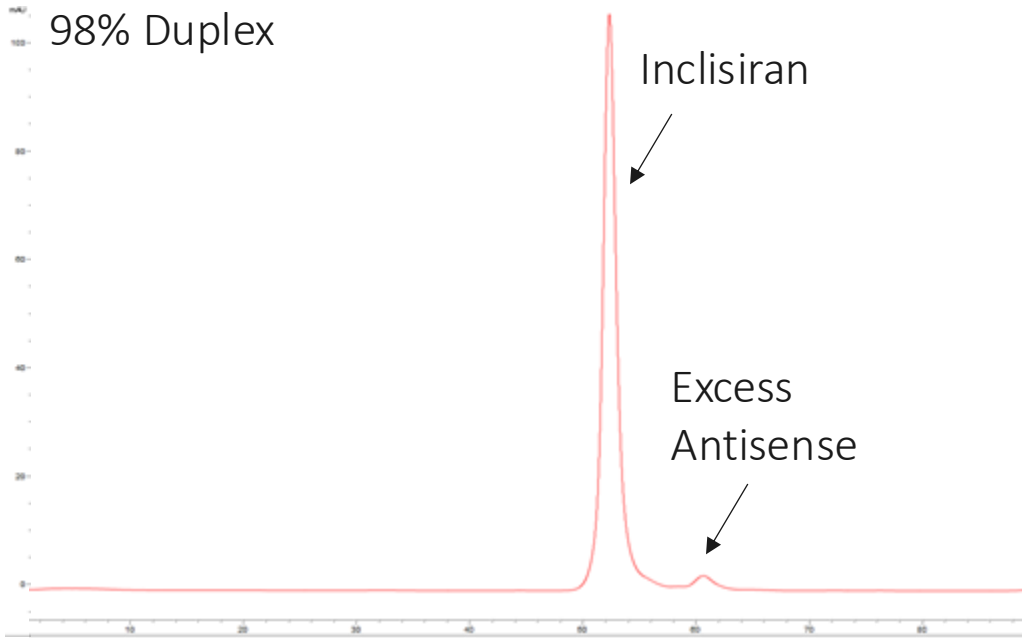
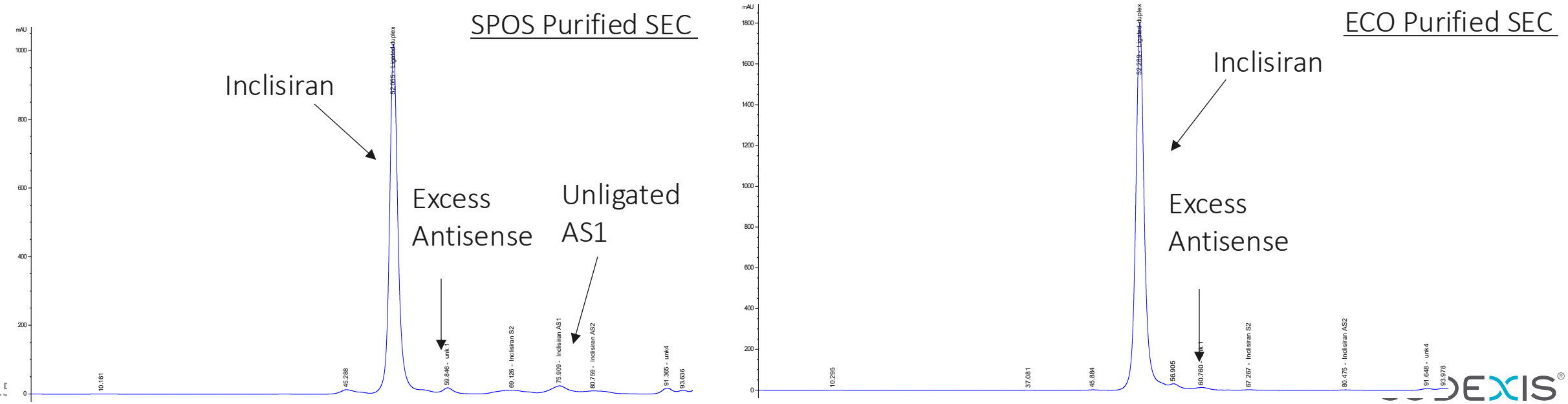


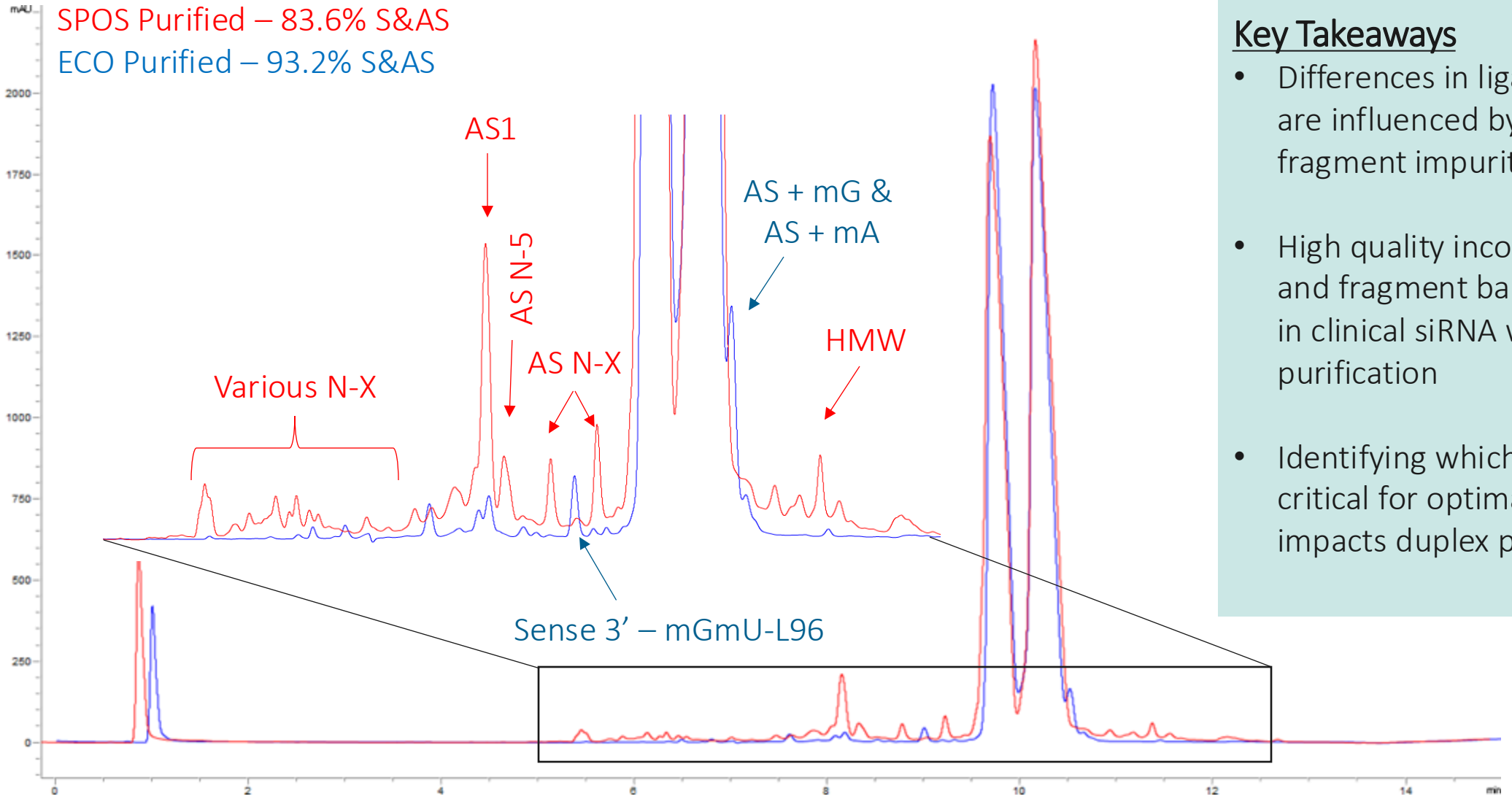
High Purity RNA Fragments Can Result in High-Quality siRNA Duplex without further Purification

Ligation Pathway	Conversion (%)	Sense Strand (%)	Antisense Strand (%)	Crude Duplex Purity (%)	Residual Ligase (PPM)	Notable Impurities (IP-RP-UV-MS)
SPOS Purified	>95	35.4	48.2	92.1	< LOD [2.5ppm]	AS1, S – mC
ECO Purified	>98	43.8	49.4	97.9	< LOD [2.5ppm]	AS +mG, AS +mA, AS +fA
Method		**Denaturing IP-RP-UV-MS**		**Non-denaturing SEC**	**LC/MSMS**	



Nondenaturing SEC chromatograms UV260nm

Starting Fragment Impurity Profile is a Critical Quality Attribute for Ligation



Key Takeaways

- Differences in ligation efficiency are influenced by the incoming fragment impurity profile.
- High quality incoming fragments and fragment balancing can result in clinical siRNA without purification
- Identifying which impurities are critical for optimal ligation directly impacts duplex purity.

Note: Samples loaded at a high concentration to elucidate low level impurities

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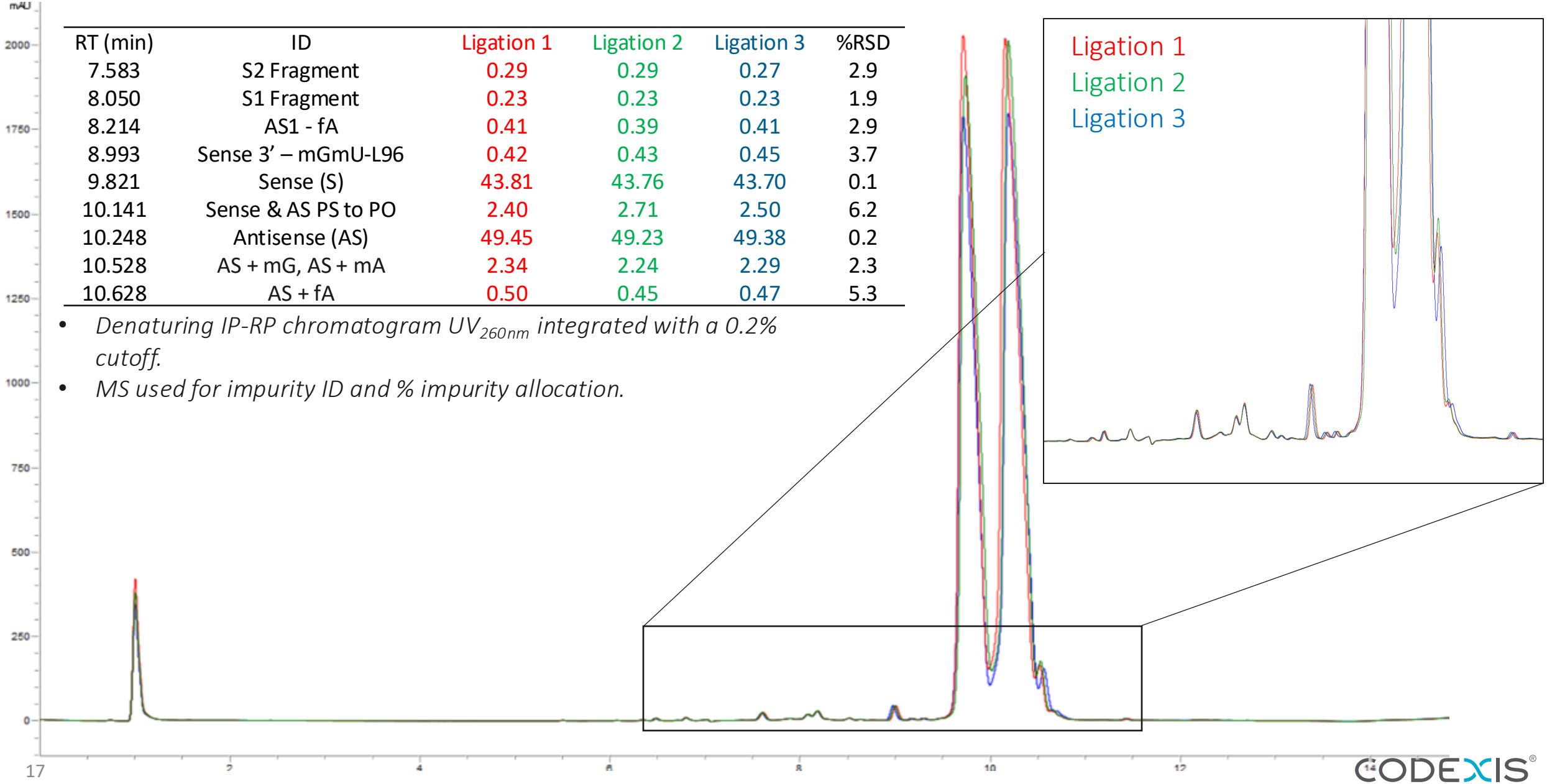
Ligation using ECO Synthesis Fragments is Reproducible

RT (min)	ID	Ligation 1	Ligation 2	Ligation 3	%RSD
7.583	S2 Fragment	0.29	0.29	0.27	2.9
8.050	S1 Fragment	0.23	0.23	0.23	1.9
8.214	AS1 - fA	0.41	0.39	0.41	2.9
8.993	Sense 3' – mGmU-L96	0.42	0.43	0.45	3.7
9.821	Sense (S)	43.81	43.76	43.70	0.1
10.141	Sense & AS PS to PO	2.40	2.71	2.50	6.2
10.248	Antisense (AS)	49.45	49.23	49.38	0.2
10.528	AS + mG, AS + mA	2.34	2.24	2.29	2.3
10.628	AS + fA	0.50	0.45	0.47	5.3

- *Denaturing IP-RP chromatogram UV_{260nm} integrated with a 0.2% cutoff.*
- *MS used for impurity ID and % impurity allocation.*



Note: Samples loaded at a high concentration to elucidate low level impurities

Immobilized Ligation can Significantly Simplify siRNA Manufacturing

Process Unit Operations	Traditional Synthesis	Crude Fragment Ligation with Duplex Purification	High Purity Fragment Ligation without Duplex Purification
Synthesis	2	4	4
Oligonucleotide Purification	2	1	0
Ultrafiltration / Diafiltration	2	1	1
Annealing	1	0	0

Process simplification results in volumetric productivity gains when using ECO Synthesis technology

CODEXIS®

ECO Synthesis Technologies deliver dramatic improvements in siRNA manufacturing

1. Immobilizing dsRNA ligase removes residual ligase and simplifies downstream processing

2. Fragment quality and stoichiometry are critical quality attributes for ligation

3. dsRNA ligation is reproducible and can result in high purity and high yield siRNA

4. ECO Synthesis technologies can enable simple, large scale production of siRNA

5. Codexis will continue to build industrializable, high quality, production processes using ECO Synthesis and will be meeting with FDA to discuss in June

What's next for ECO Synthesis Manufacturing Platform

- Continued process development with demonstrated scale up (2H 2025)
  - Drive bench scale to larger scale productions for both sequential and ligation siRNA processes
  - Evaluate batch to batch variability from incoming fragments and enzyme lots
  - Further decrease cycle times using sequential synthesis, and gain volumetric productivity improvements

Why Partner with Codexis?

Expert siRNA manufacturing drives next-gen enzymatic synthesis adoption

The partnership to enable your innovations

The ECO Synthesis Innovation Lab discovers what's possible

The technology to scale sustainably

Achieve greater scale through a greener route

The expertise to power your project

Our 22+ years of expertise accelerates process development for enzymatic siRNA manufacturing

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Questions and thank you

- TIDES talk in the Exhibit Hall at 10:10 on

Comparative Analysis of Diastereomeric Distribution in siRNA Synthesis: PAC vs Enzymatic Synthesis

- Visit us at booth 619