Revolutionizing Nucleic Acid Synthesis with Engineered Enzymes

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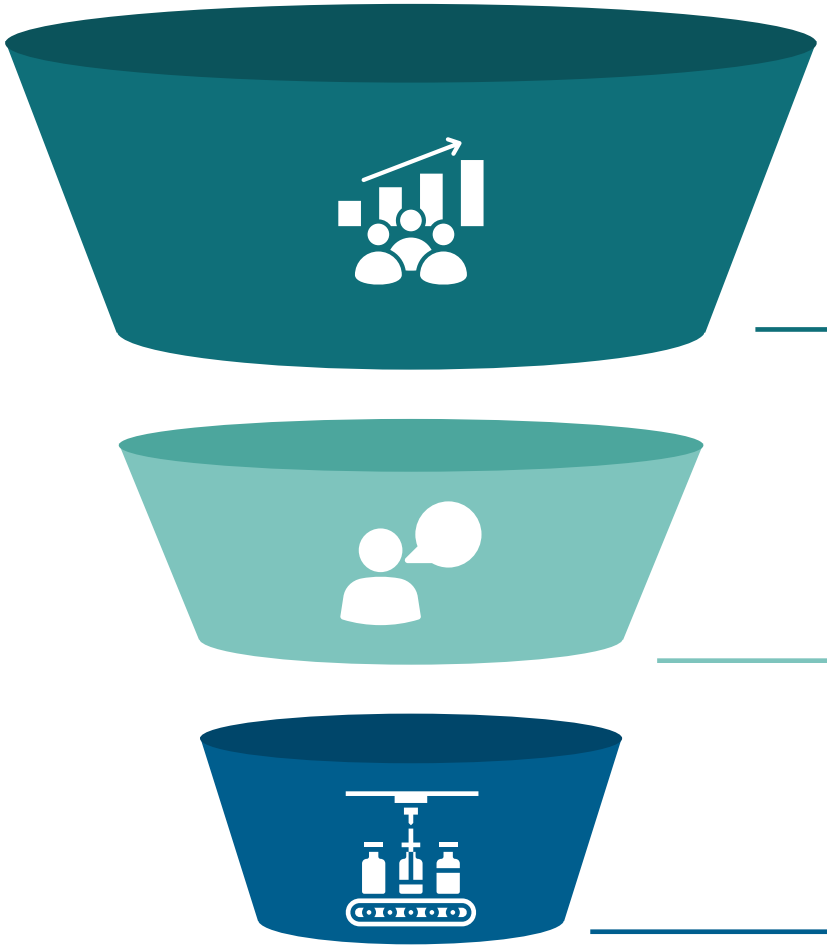
ECO Synthesis™ Platform: Positioned to Deliver in RNAi Market

RNAi Demand is Coming



Chemical synthesis (phosphoramidite chemistry) alone **cannot meet projected future wave of demand for RNAi therapeutics**

Customers are asking us for a **scalable, more sustainable enzymatic solution** to complement chemical synthesis

1 kg of siRNA requires ~ 1000 kg of MeCN (BioSpring)

PMI per nucleotide added ~200 kg/kg (*Org. Proc. Dev,* **2021**, *86*, 1, 49–61)

Codexis positioned to deliver based on **20-year history of enzyme engineering** and **directly relevant Pharmaceutical Manufacturing commercial expertise**

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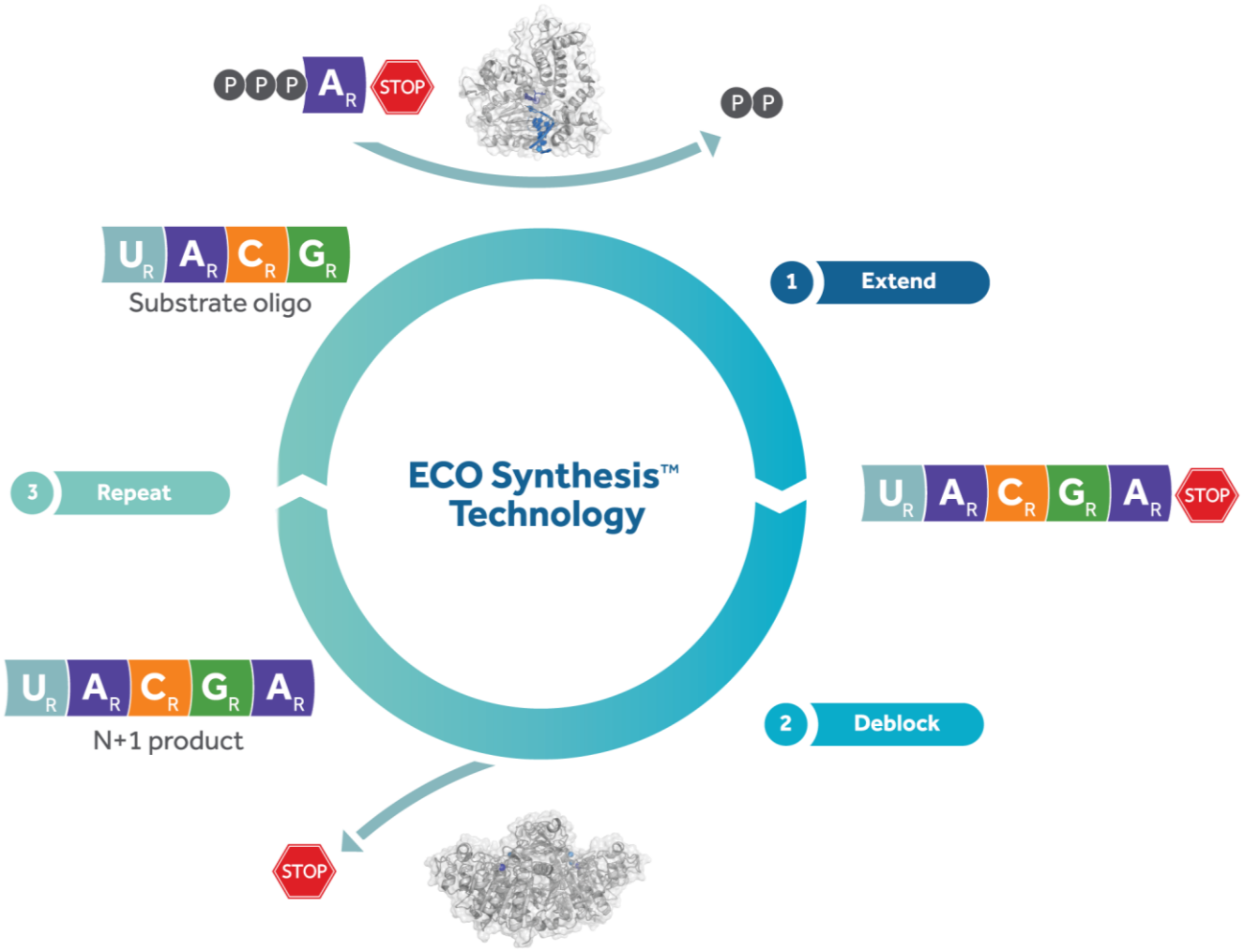
Codexis ECO Synthesis™ Technology



Enzyme Catalyzed Oligonucleotide Synthesis

Core process:
Oligonucleotide synthesis by iterative, single nucleotide extension

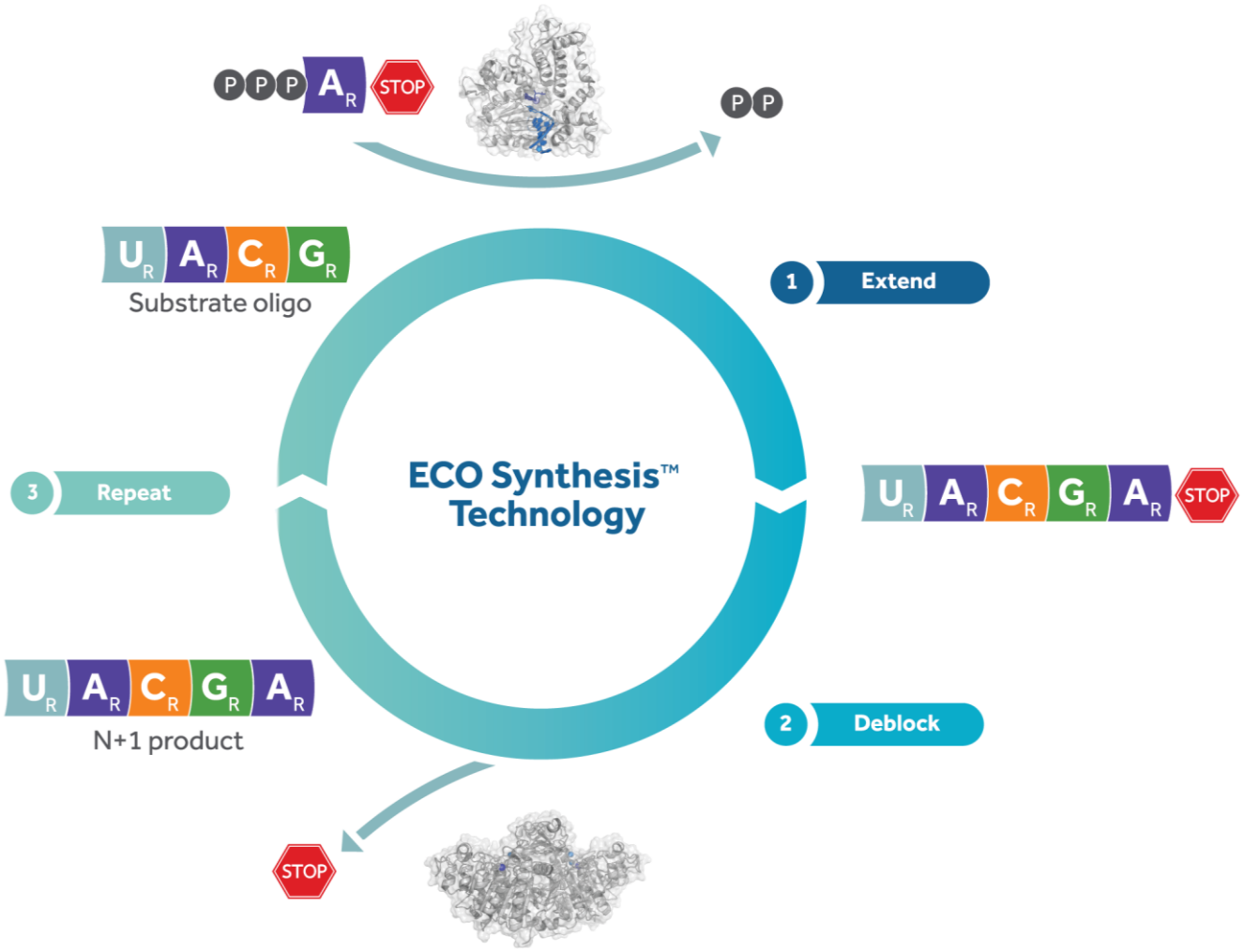
- **Extend:** Controlled, enzymatic addition of modified, 3'-phosphate blocked ribonucleotides
- **Deblock:** Enzymatic cleavage of 3'-phosphate blocking group & excess ribonucleotides

… **Repeat**

Supply processes:

- Enzyme cascade for synthesis of modified, 3'-phosphate blocked ribonucleotides (NQPs)
- Enzymatic synthesis of starter oligonucleotide

Codexis ECO Synthesis™ Technology - Key Platform Traits



Enzyme Performance

- High incorporation efficiency
- Robustness & manufacturability

Scalable & Economical Manufacturing

- High volumetric productivity
  - “Oligo in solution, enzyme immobilized”
- Controlled addition of monomers
  - Low impurity production
- Smaller infrastructure/facilities footprint
- Established reagents supply

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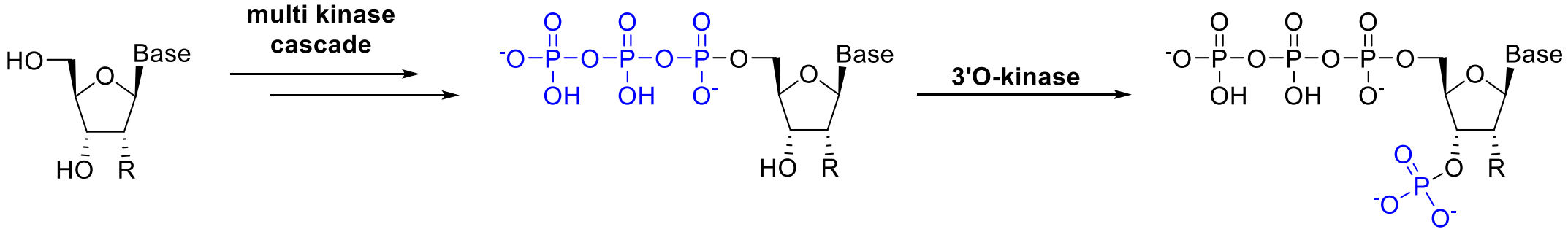
Supplying Critical NQP Reagents For ECO Synthesis™ Platform

Building a “one-pot, two-step” enzyme cascade with engineered kinases



Targeted Key Performance Indicators

Robustness/Manufacturability - soluble expression & stability

Productivity - >98% conversion at millimolar substrate concentration

Substrate Tolerance - accepts ribonucleotides with 2' modified sugars & phosphorothioate backbone modifications

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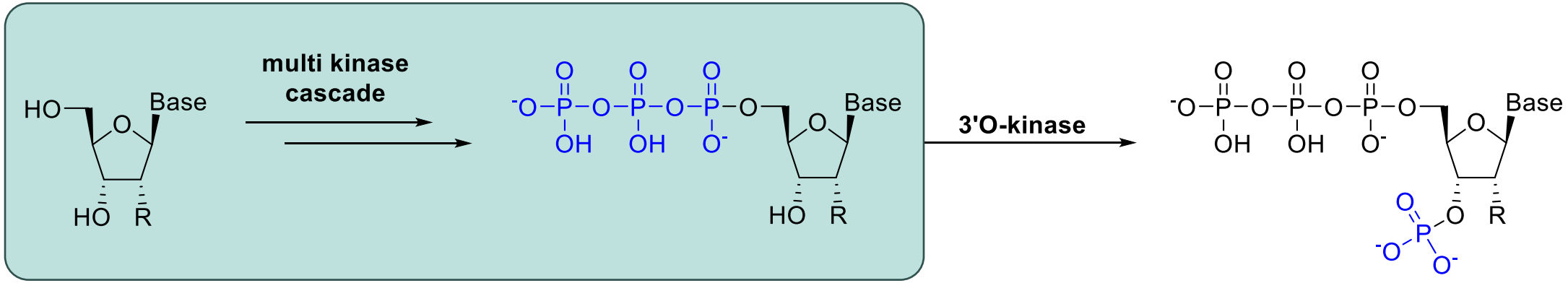

Step 1: N→NTP conversion via three consecutive phosphorylation steps, using three kinases

Initial (wild type) kinases

	A	C	G	U
2'-OH	**99**	**22**	**1**	**7**
2'-dF	**64**	**0**	**0**	**0**
2'-OMe	**0**	**0**	**0**	**0**

	A	C	G	U
2'-OH	**98**	**96**	**93**	**91**
2'-dF	**93**	**19**	**93**	**29**
2'-OMe	**55**	**0**	**0**	**0**

Current engineered kinases

Percent conversion for individual nucleosides to the corresponding NTPs

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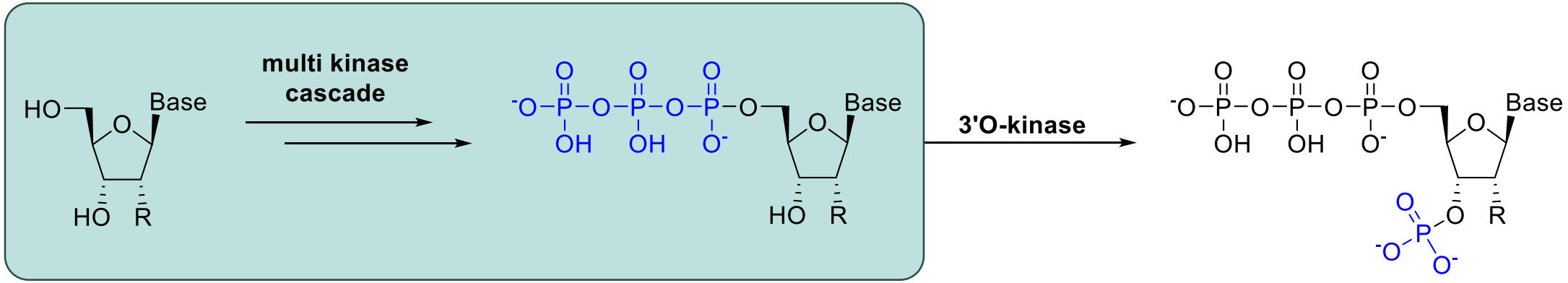
Supplying Critical NQP Reagents For ECO Synthesis™ Platform

Building a “one-pot, two-step” enzyme cascade with engineered kinases



Step 1: N→NTP conversion via three consecutive phosphorylation steps, using three kinases

Status: NTP Forming Cascade

- Full base promiscuity & emerging activity for 2’-modified nucleosides
- Operational at process-relevant substrate concentration
- ***Engineering on track to deliver full base and 2’-modification promiscuity for NTP formation***

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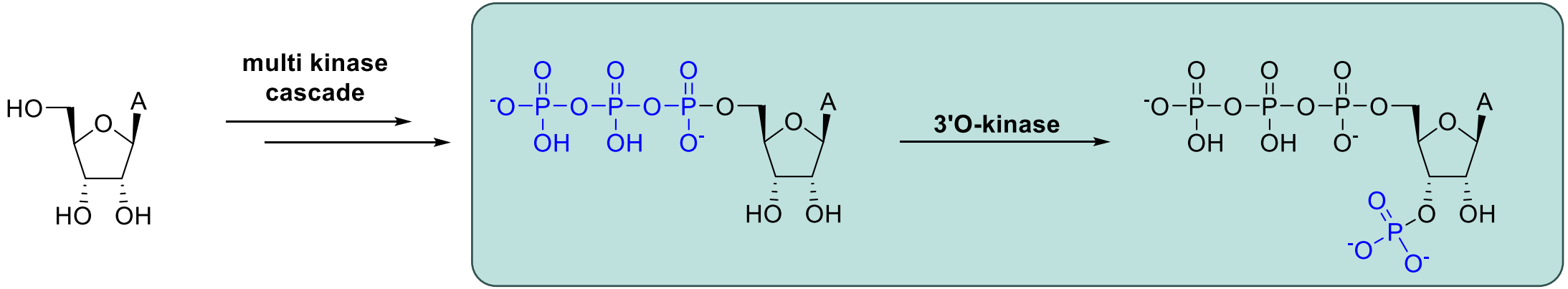
Supplying Critical NQP Reagents For ECO Synthesis™ Platform

Building a “one-pot, two-step” enzyme cascade with engineered kinases



Step 2: NTP→NQP percent conversion with current 3’-O kinase

Initial (wild type) kinase

	A	C	G	U
2'-OH	**2**	**0**	**0**	**0**
2'-dF	**0**	**0**	**0**	**0**
2'-OMe	**0**	**0**	**0**	**0**

	A	C	G	U
2'-OH	**99**	**18**	**38**	**36**
2'-dF	**12**	**0**	**0**	**0**
2'-OMe	**0**	**0**	**0**	**0**

Current engineered kinase

Percent conversion for individual NTPs to the corresponding NQPs

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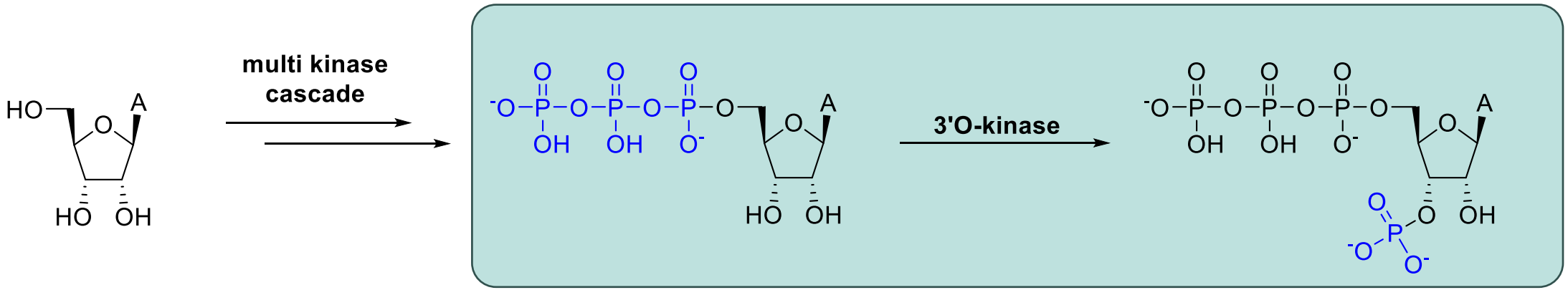
Supplying Critical NQP Reagents For ECO Synthesis™ Platform

Building a “one-pot, two-step” enzyme cascade with engineered kinases



Step 2: NTP→NQP percent conversion with current 3’-O kinase

Status: 3’-O-kinase engineering

- Engineering challenge as desired activity is non-natural (& potentially cytotoxic to expression host)
- Break through from initial strict A selectivity
- ***Ongoing engineering of 3’O-kinase starting to deliver activity on 2’-modification for NQP formation***

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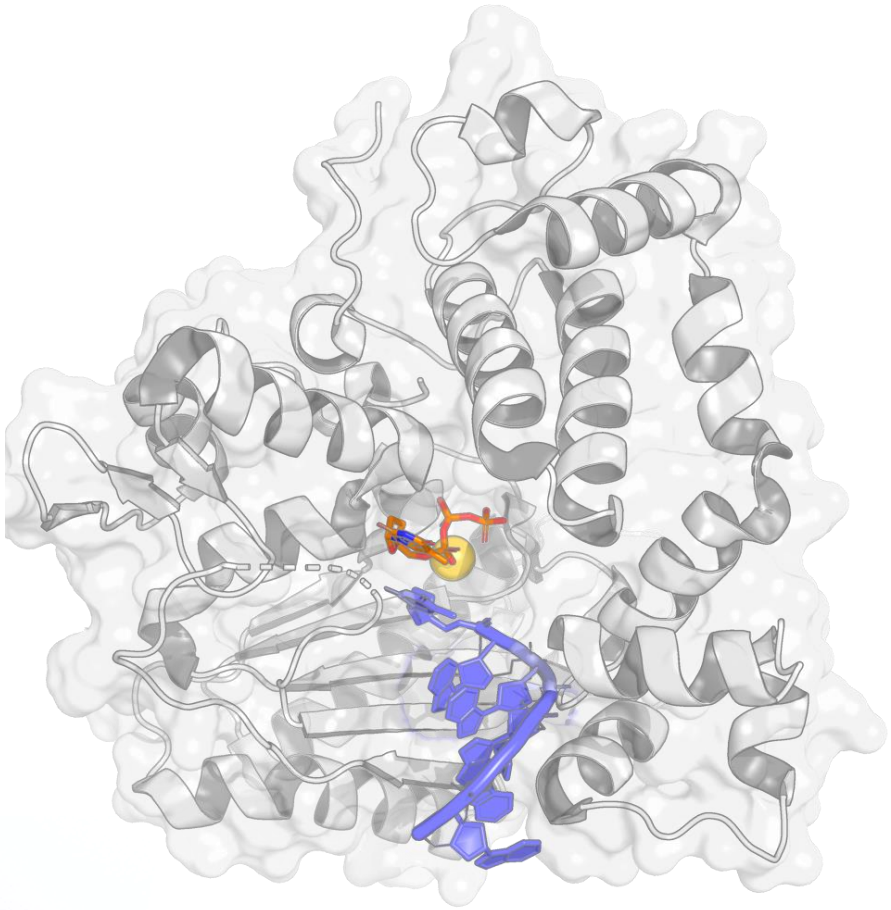
Terminal Deoxynucleotidyl Transferase (TdT)

A high engineered enzyme for catalyzing the extension reaction



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Targeted Key Performance Indicators

Robustness/Manufacturability - soluble expression & stability

Productivity - >99% conversion at millimolar substrate concentration

Substrate Tolerance - accepts ribonucleotides with 2'- and 3'-modifications & backbone phosphorothioate modifications

Promiscuity – minimal oligonucleotide sequence bias

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Engineering Terminal Deoxynucleotidyl Transferase (TdT)

Progressive performance improvements in oligonucleotide synthesis

3'-Terminus Sequence	NQP	Starting TdT	Iterative Rounds of Evolution						
AmC*mA*mG	mATP-3P	1	1	1	15	35	32	34	46
mAmAmAfG		2	2	2	2	2	3	4	58
mAmAfGmA		0	0	0	1	9	65	77	73
mAfGmUfG		0	0	0	0	0	0	0	39
…									
fGmUfGmU	mUTP-3P	0	0	0	1	31	48	67	68
mUfCmUfC		0	0	0	0	2	1	5	48
mUfCmAmU		0	0	0	3	55	35	56	78
fCmAmUmC		0	0	0	1	12	72	75	75
…									
mCmUmUmA	fATP-3P	0	0	0	2	10	76	65	68
mAmAmA(MOE)A		1	1	2	2	2	14	39	73
…									
mCmCmU*mU	mCTP-3P	0	0	0	2	9	47	66	68
	*mUTP-3P	0	0	0	0	1	33	54	69
	mGTP-3P	0	0	0	1	15	50	72	75
…									
*fA*fAfGmA	mCTP-3P	0	0	0	35	34	13	35	44
	*mUTP-3P	0	0	0	2	8	2	19	32
	mGTP-3P	0	0	0	35	41	20	42	52
…									

September 2023

Increased % incorporation efficiency

- One of the most complex evolution projects undertaken by Codexis to date
- Enabling synthesis of fully modified RNA oligonucleotides (2’-OMe, 2’-F, Phosphorothioate)

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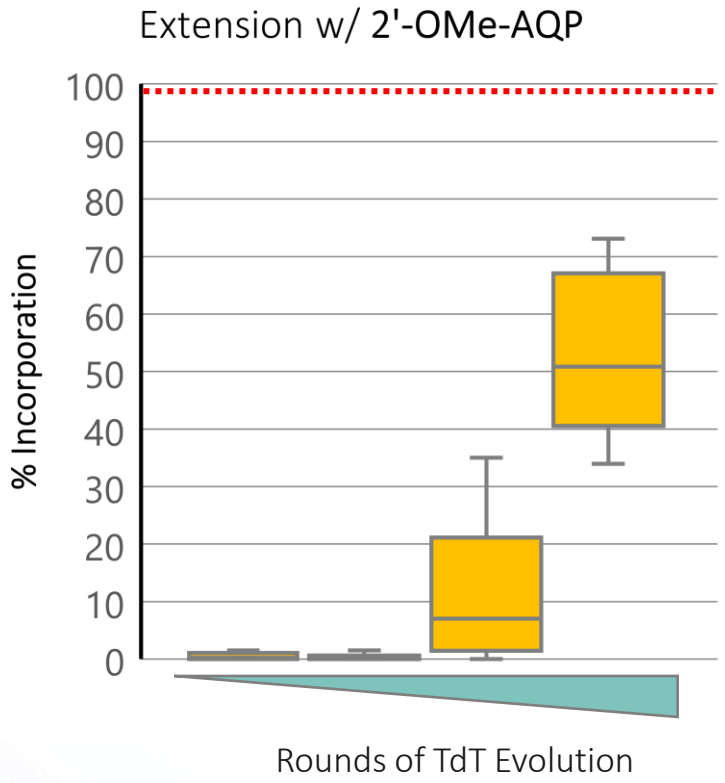
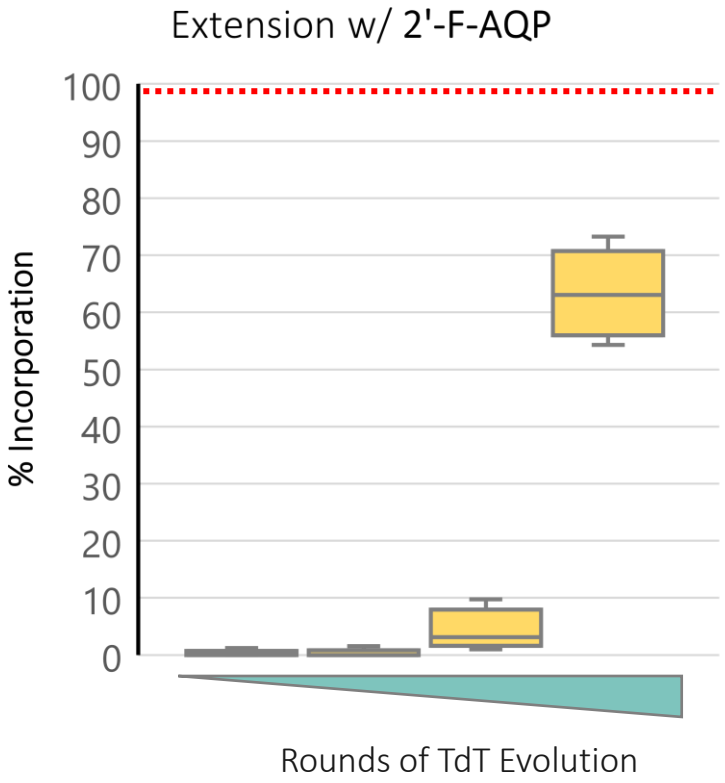
Engineering Terminal Deoxynucleotidyl Transferase (TdT)

Progressive performance improvements in oligonucleotide synthesis





- Incorporation efficiency (N+1) of each NQP measured with 5-8 fully modified oligonucleotide substrates
- Included oligo modifications : 2’-OMe, 2’-F, Phosphorothioate)
- **Current process conditions**:
- [oligo] = mM; [NQP] = 2-fold eq
- **Ongoing enzyme engineering & process development**

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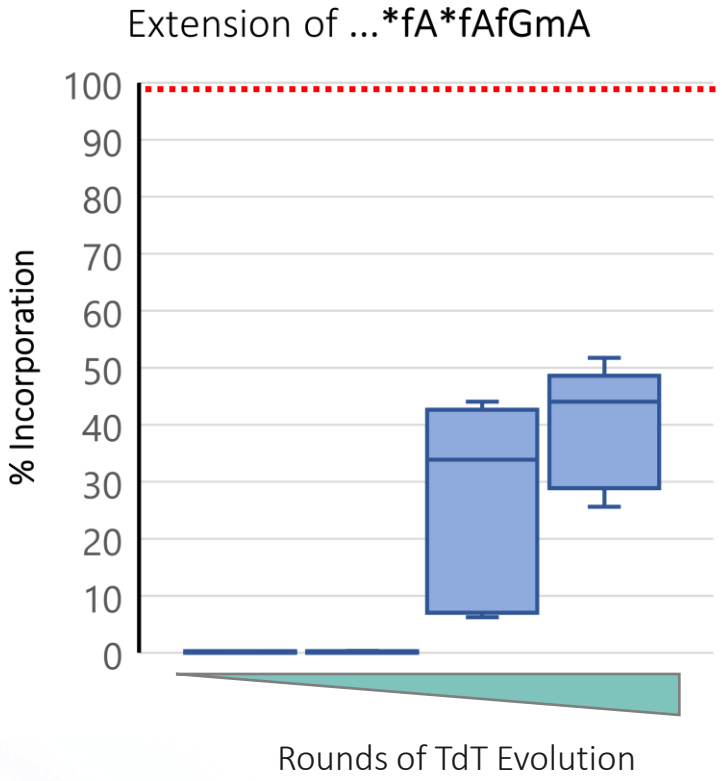
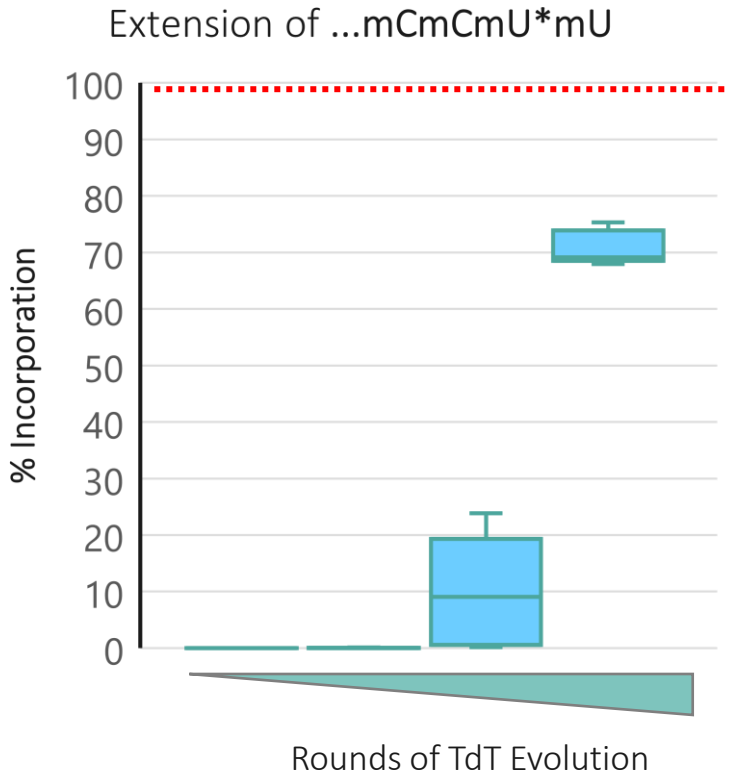
Engineering Terminal Deoxynucleotidyl Transferase (TdT)

Progressive performance improvements in oligonucleotide synthesis





- Incorporation efficiency (N+1) on each oligonucleotide measured with 5 modified NQPs
- Included NQP modifications : 2'-MeO, 2'-F, Phosphorothioate)
- **Current process conditions**:
- [oligo] = mM; [NQP] = 2-fold eq
- **Ongoing enzyme engineering & process development**

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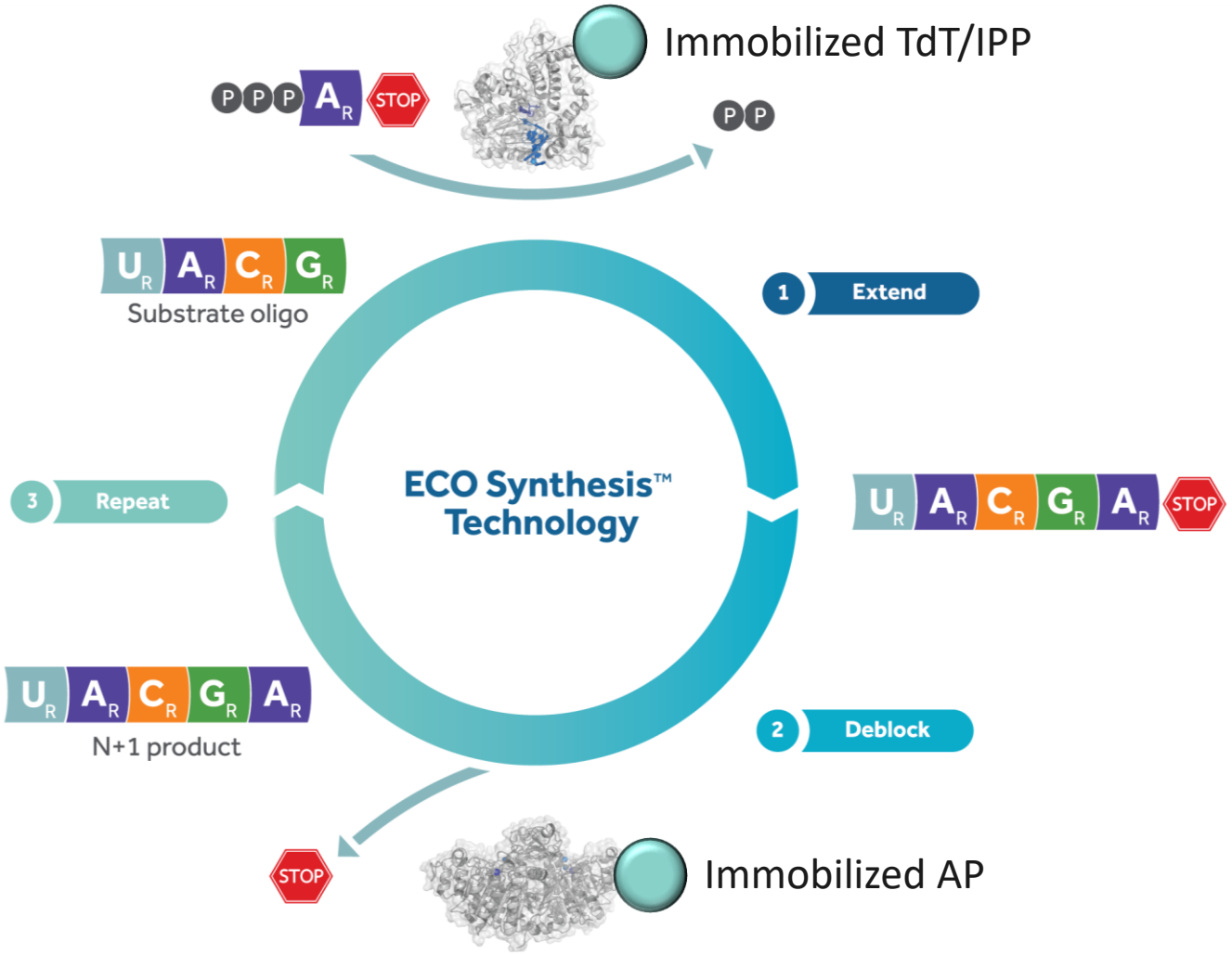
Enzyme Performance: A Highly Engineered TdT

% Incorporation efficiency of N+1 additions over multiple rounds of evolution

- Consistent improvement in oligo promiscuity and acceptance of 2’-modified NQP
- Consistent movement of screening conditions to the desired process relevant targets
- Further engineered to remove remaining biases and increase oligo concentration to > 1 mM on going.
- Next steps, show process relevance

From Enzyme Engineering to Platform Development



Targeted Process Conditions

- Enzymes immobilized & oligos in solution
- Aqueous reaction system
- Substrates at millimolar concentration
- Elevated reaction temperature
- Cycle time per extension/deblocking: hours/NQP
- In-line monitoring of reaction progress

Platform performance

- Multiple consecutive cycles of oligonucleotide synthesis to produce a fully modified RNA sequence

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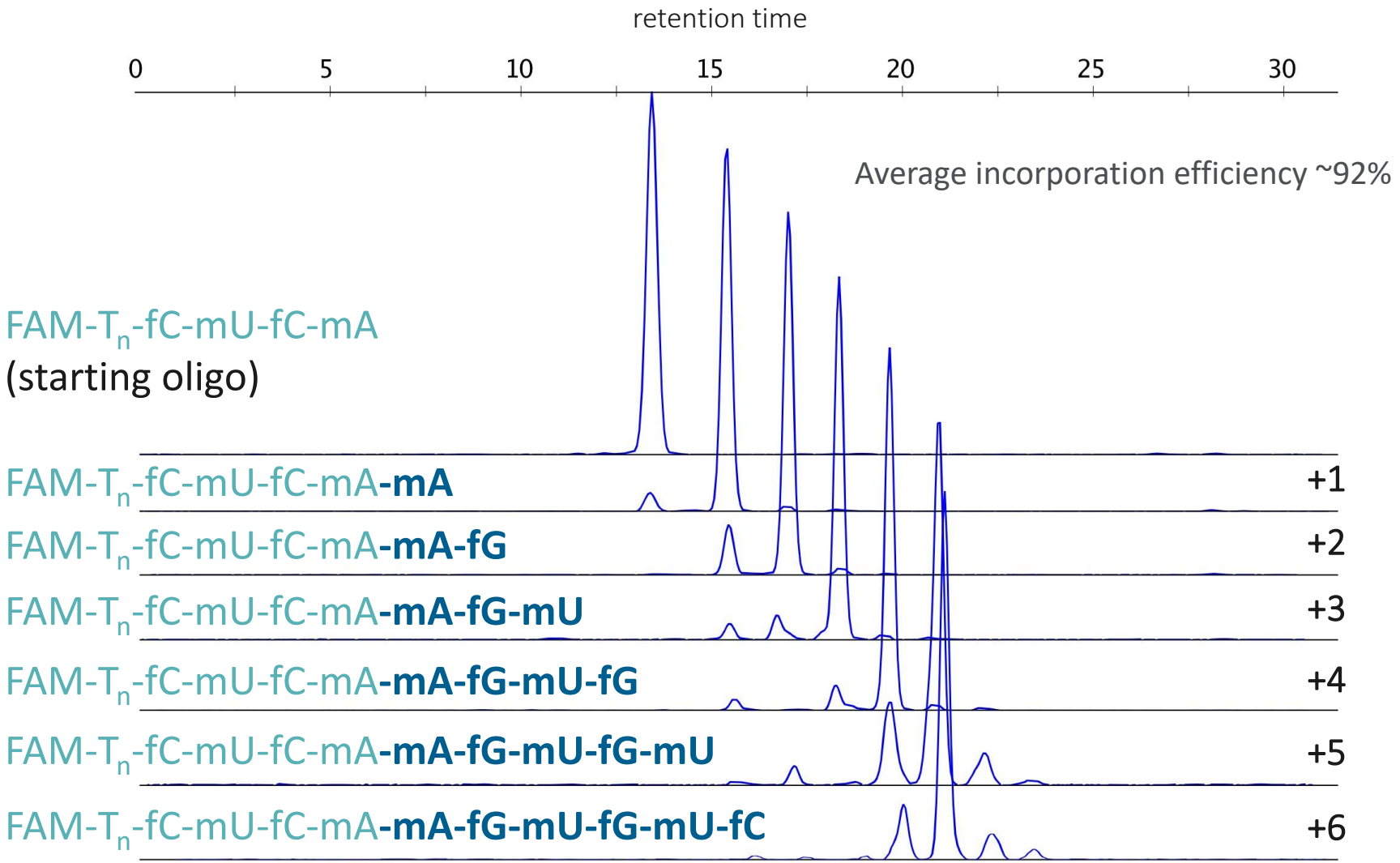
Iterative oligonucleotide synthesis w/ ECO Synthesis™ platform

Platform performance

- 6+ extension cycles
- **Process conditions:** oligo in solution, enzyme immobilized
- Extension with modified NQPs to yield fully modified RNA oligonucleotide
- **Further enzyme engineering & process optimization ongoing**



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ECO Synthesis™ Technology: Controlled addition of monomers

- **To our knowledge, the first ever multiple non-templated iterative extensions of an oligo in the solution phase mediated by highly engineered immobilized enzymes**
- 6 extensions in batch mode with FAM-labelled oligo with immobilized enzymes
- Next step, demonstration in packed bed immobilized enzymes with solution phase unlabeled oligos in a flow set-up

ECO Synthesis™ Technology: A Vision For RNA synthesis

Unlocking exciting new possibilities in RNAi therapeutics manufacturing

- Accessing 10 - 100 kg batch size for oligonucleotide synthesis
- Reduction in organic burden & operational/facility costs
- Mild reaction conditions, elimination of protection groups, quality improvements

Codexis' deliverables

- Oligonucleotide synthesis process developed and optimized for product yield and quality
- Enzymatic manufacturing & supply chain for critical reagents (NQPs)
- Robust, high-performance enzymes for core & supply processes of ECO Synthesis™ platform, leveraging our CodeEvolver® enzyme engineering technology

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Acknowledgements

ECO-Synthesis™ Technology - A company-wide cross functional collaboration

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