Hongene Biotech

Chemoenzymatic Ligation: Advancing Next-Generation Manufacturing Technologies for siRNA and sgRNA

David Butler, Chief Technology Officer

TIDES Europe, Basel, Tuesday November 11th,2025.

Disclosures

- Dr. Butler is an employee of Hongene Biotech Corporation and serves on the board of directors of Akte Therapeutics

Presentation overview

- Introduction
- Chemoenzymatic ligation for siRNA synthesis
  - Case study: Divalent exNA siRNA
  - Controlling the cost of manufacturing
  - Case study: Crude-to-purified (C-to-P)
  - Engineered thermostable T4 RNA ligase
  - Chemoenzymatic ligation technology now supporting siRNA clinical development
- CMC regulatory considerations
- Conclusions

Introduction

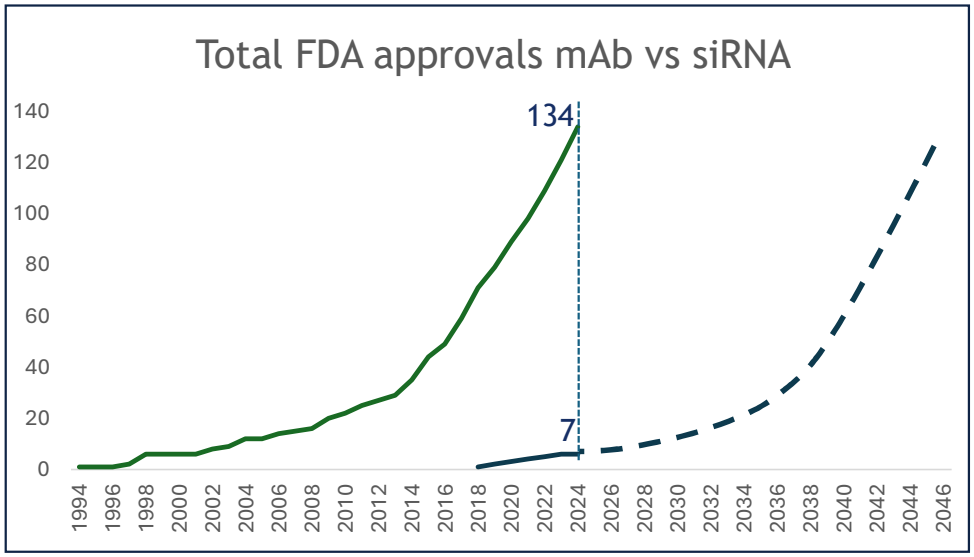
Market potential of siRNA drugs

Market demand for siRNA drugs and CMC challenges expected to grow significantly over the next decade

Cardiovascular disease area	Lead siRNA drug	Target	Phase	Possible approval
Hypercholesterolemia	Inclisiran	PCSK9	4	Approved
Hypertriglyceridemia	Plozasiran	APOC3	3	~2025
Elevated Lp(a)	Olpasiran	LPA	3	~2027
Hypertension	Zilebesiran	AGT	2	~2030
NASH/MASH	Rapirosiran	HSD	2	~2030
Obesity	*Multiple*	INHBE	1	2030s

- Several disease areas have potential to reach >10M US patients
- inclisiran + 4 could be approved by 2030
- Research suggests >10 t API required by early 2030s

siRNA is in its infancy in its life trajectory as a therapeutic drug modality



The Antibody Society. Therapeutic monoclonal antibodies approved or in regulatory review. (Aug 2024); Antibody therapeutics product data - The Antibody Society

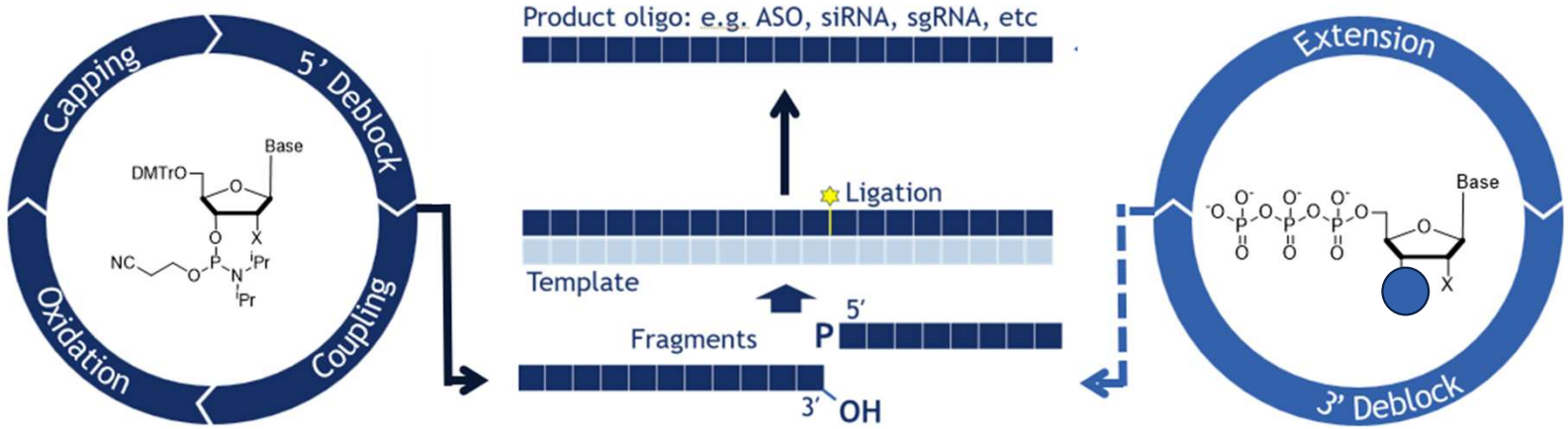
Three generations of oligonucleotide synthesis technology

	GENERATION 1	
Raw materials	Phosphoramidites	
Development status	>40 years, current paradigm	
Product purity	Lower	
Product yield	Lower	
Sustainability	>3,000 kg RM/kg API[1]	

GENERATION 3	
NTPs, 3'-protected NTPs, enzymes	
Early[2,3,4,5]	
Enzymes need to be engineered	
Enzymes need to be engineered	
Best (aqueous)	

1. B. Andrews et al, *JOC*, 2021; 2. E.R. Moody et al, *Science*, 2023; 3. N. Sabat et al, *Front Chem*, 2023; 4. D. Wiegand et al, *Nat Biotechnol*, 2024; 5. S. Forget et al, *NAR*, 2025.

Three generations of oligonucleotide synthesis technology

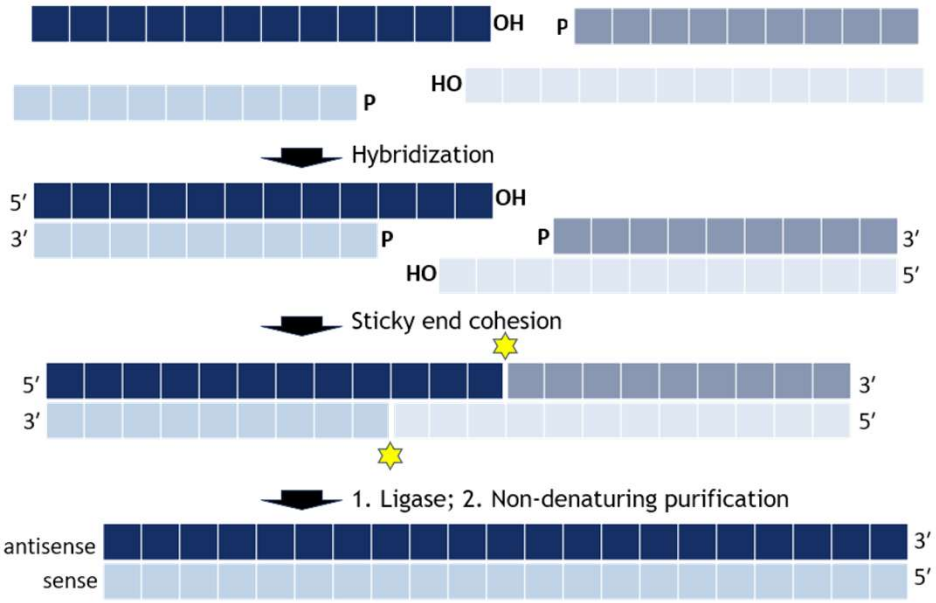


	GENERATION 1		GENERATION 2		GENERATION 3	
Raw materials	Phosphoramidites		Oligonucleotide fragments		NTPs, 3'-protected NTPs, enzymes	
Development status	>40 years, current paradigm		Ready for manufacturing		Very early[2,3,4,5]	
Product purity	Lower		Higher		Enzymes need to be engineered	
Product yield	Lower		Higher		Enzymes need to be engineered	
Sustainability	>3,000 kg RM/kg API[1]		Better (partly aqueous)		Best (aqueous)	

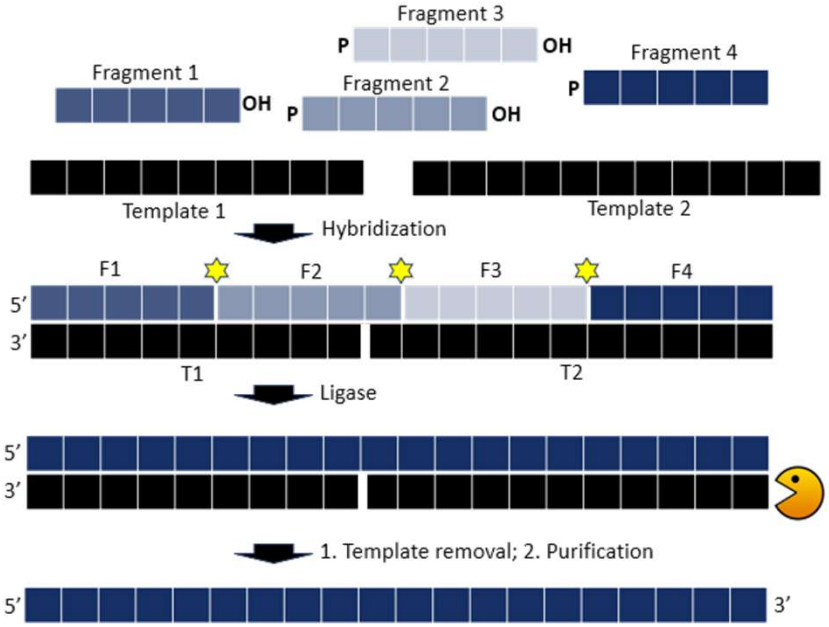
1. B. Andrews et al, *JOC*, 2021; 2. E.R. Moody et al, *Science*, 2023; 3. N. Sabat et al, *Front Chem*, 2023; 4. D. Wiegand et al, *Nat Biotechnol*, 2024; 5. S. Forget et al, *NAR*, 2025.

Hongene's chemoenzymatic ligation platform processes

1. siRNA
Sticky end ligation[1-4]



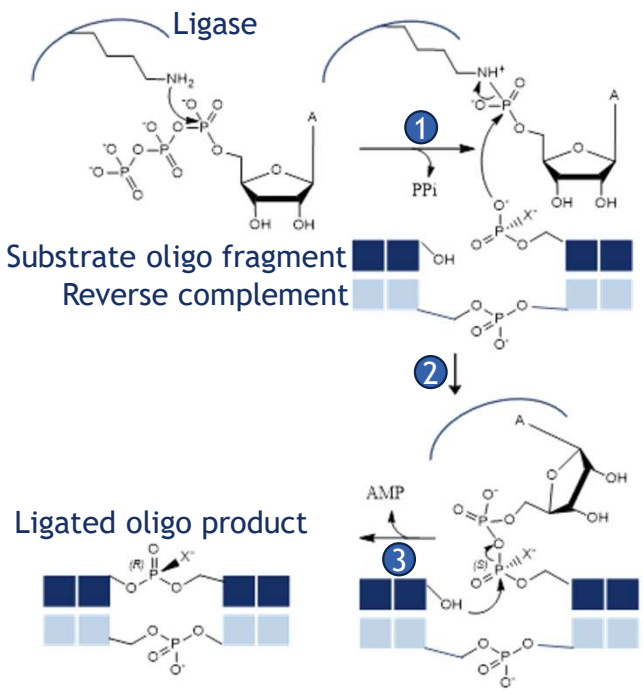
2. sgRNA
Splinted (template) ligation[5-7]



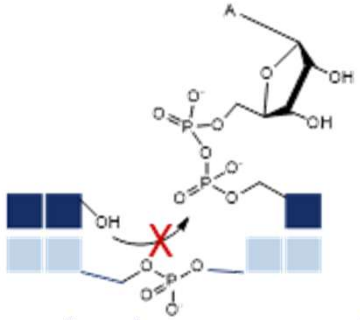
1. H.G. Khorana, *Pure Appl Chem*, 1968; 2. A. Sosic et al, *Bioconj Chem*, 2014; 3. S. Paul et al, *ACS Chem Biol*, 2023; 4. S. Kajimoto et al, *AEM*, 2022; 5. M. Moore et al, *Methods Enzymol*, 2000; 6. N.Sabat et al, Nat Commun, 2024; 7. Bigatti et al, *OPRD*, 2025.

Mechanism and properties of ligation reaction

Reaction mechanism[1]



Important properties of ligation



- Some shortmers are rejected

- Fidelity (specificity)
- Efficiency (yield)
- Kinetics (rate)

} Process optimization: Buffer, temp, time, conc, fragment length, enzyme engineering

- Prochiral 5'-thiophosate → *R*p PS stereochemistry in product[2]

1. J. Nandakumar et al, *Cell*, 2006; 2. F. R. Bryant et al, *Biochemistry*, 1982.

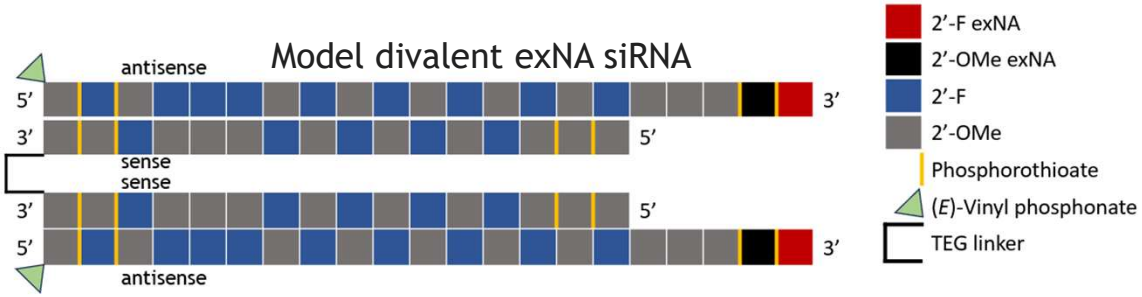
Chemoenzymatic ligation for siRNA synthesis

Ligation case study
Divalent exNA siRNA

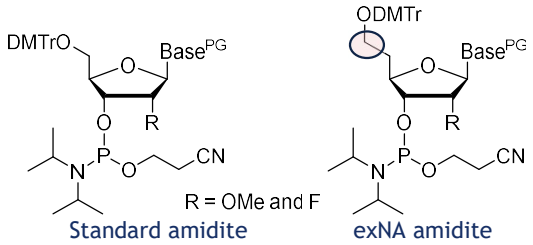
Ligation case study: Divalent exNA siRNA

Background

- UMass has invented divalent siRNAs with enhanced efficacy. These contain exNA chemistry[1]
- Scientists at Broad, UMass and Harvard recently identified a divalent siRNA clinical candidate for Prion disease[2]
- Hongene manufactured this clinical candidate using SPOS for the clinical trial
- We were curious as to whether divalent siRNA chemistry was amenable to chemoenzymatic ligation
- A model divalent siRNA construct was selected for PoC studies



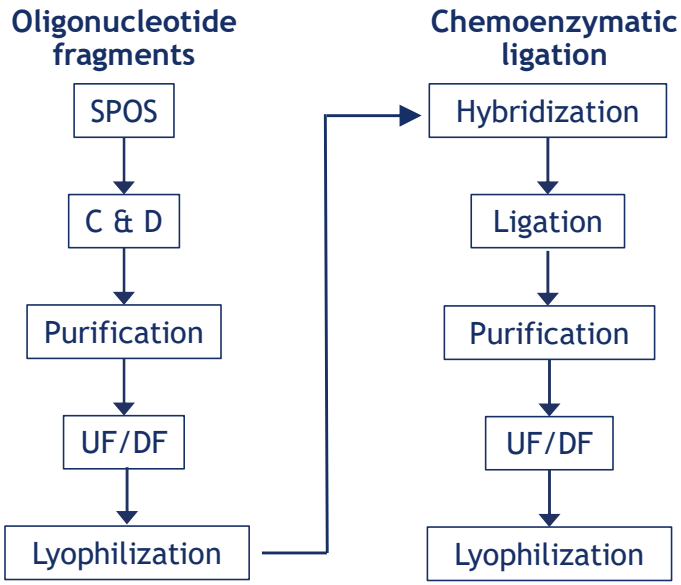
Novel exNA phosphoramidite Starting Materials scaled for GMP oligonucleotide CDMO services

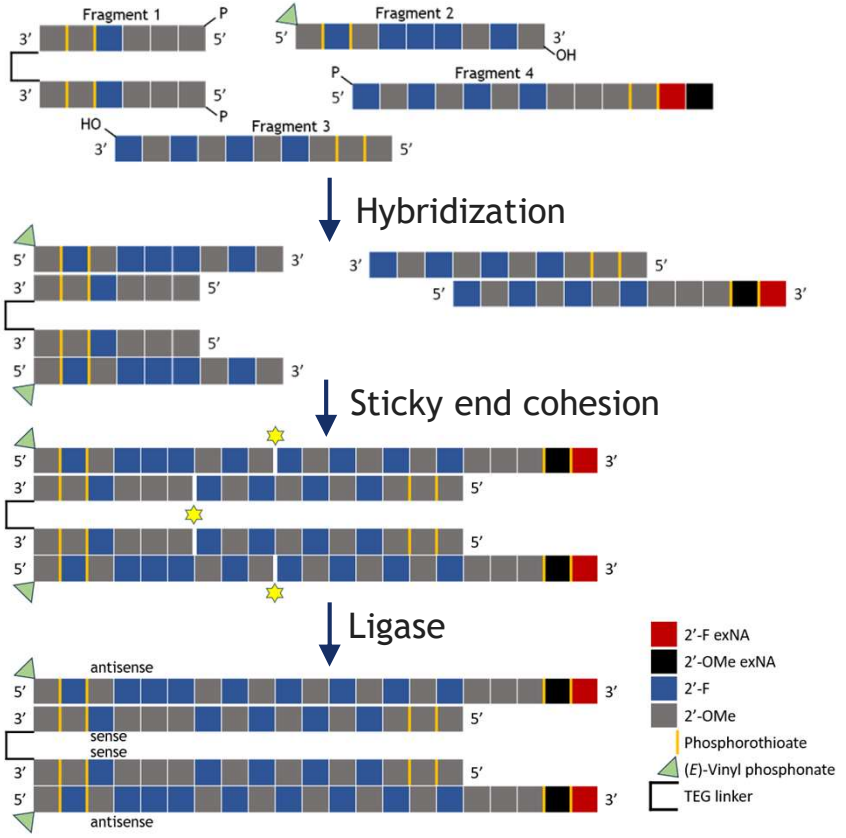


1. K. Yamada et al, *Nat Biotechnol*. 2025; J.E. Gentile et al, *bioXriv*, 2024.

Divalent siRNA: Chemoenzymatic process design

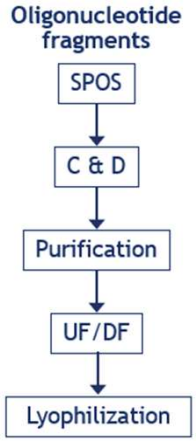
Purified fragments and purified siRNA (P-to-P)

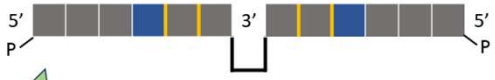


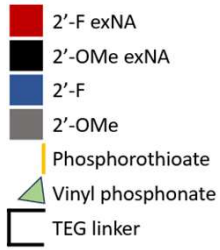


Divalent siRNA: Control of oligonucleotide fragment purity

IPRP-HPLC/MS



Fragment	Construct	L	Purity	Yield
1		12	96.2%	26.3%*
2		9	95.4%	48.2%
3		10	96.0%	42.5%
4		12	93.6%	30.2%



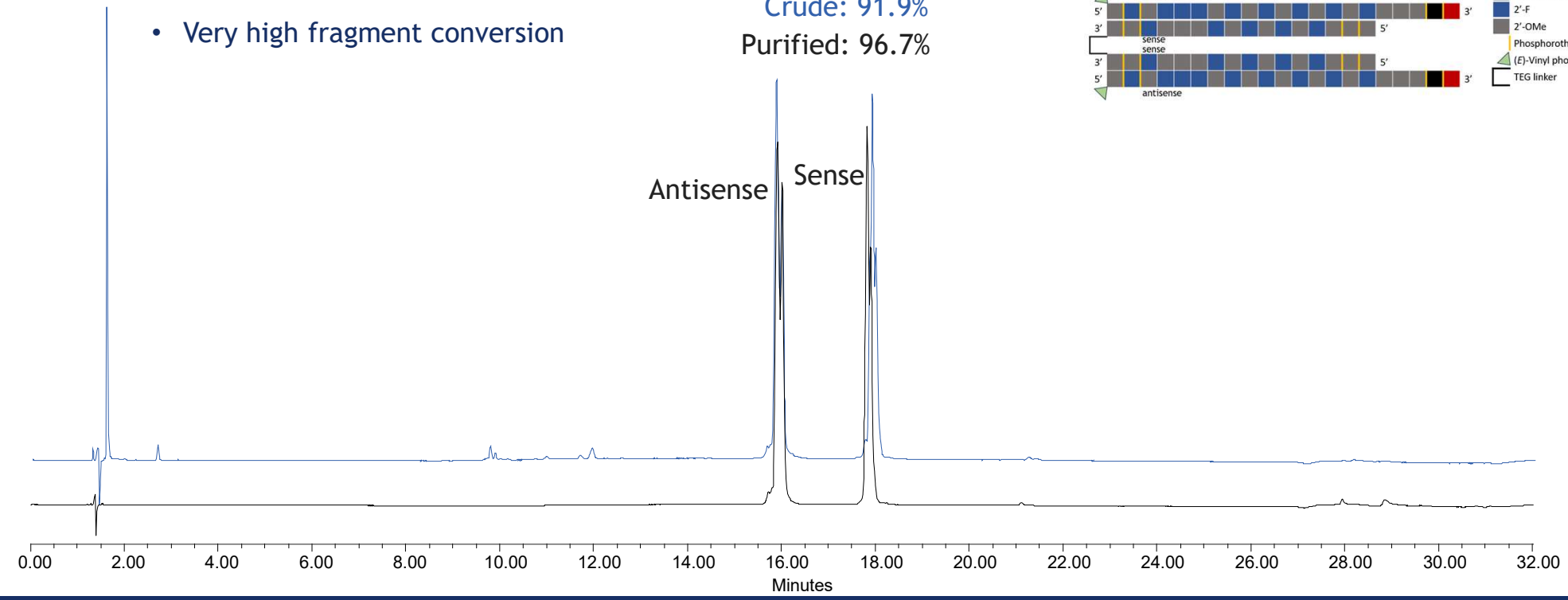
Yields based on theoretical MEC. Purity measured by IPRP-HPLC. *Lowest value used to calculate final siRNA % yield.

Divalent siRNA: Control of purity and impurities

IPRP-HPLC/MS

- Very high fragment conversion

Crude: 91.9%
Purified: 96.7%



19% yield

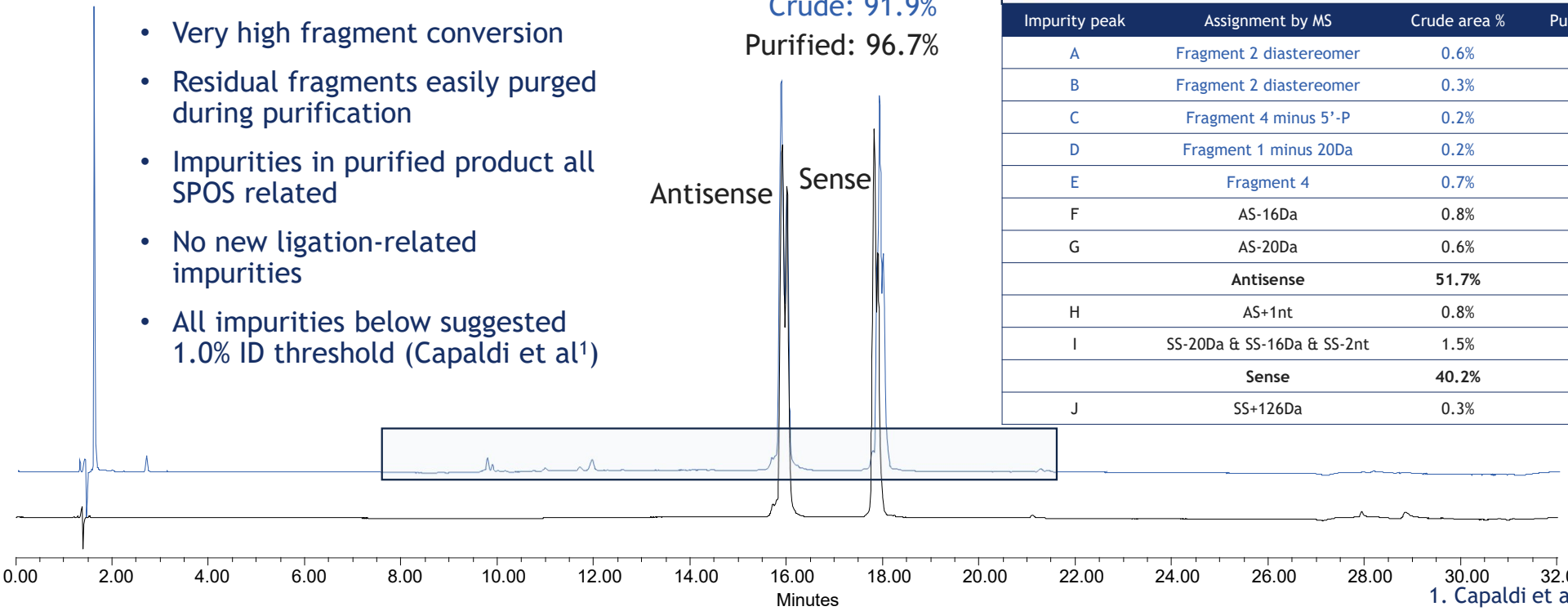
Divalent siRNA: Control of purity and impurities

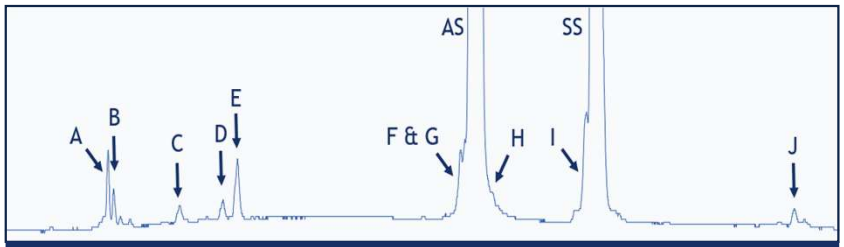
IPRP-HPLC/MS

- Very high fragment conversion
- Residual fragments easily purged during purification
- Impurities in purified product all SPOS related
- No new ligation-related impurities
- All impurities below suggested 1.0% ID threshold (Capaldi et al[1])

Crude: 91.9%

Purified: 96.7%



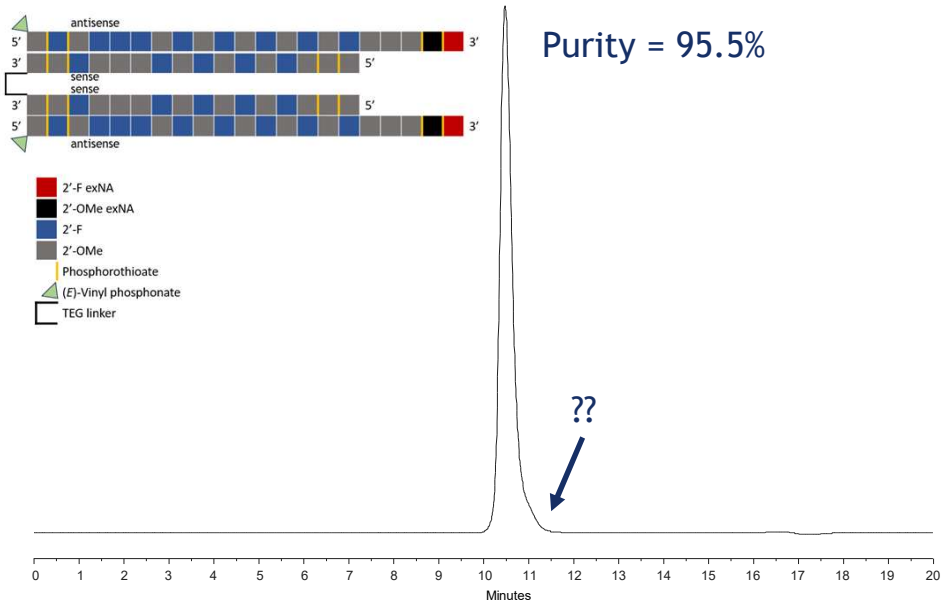


Impurity peak	Assignment by MS	Crude area %	Purified area %
A	Fragment 2 diastereomer	0.6%	N.D.
B	Fragment 2 diastereomer	0.3%	N.D.
C	Fragment 4 minus 5'-P	0.2%	N.D.
D	Fragment 1 minus 20Da	0.2%	N.D.
E	Fragment 4	0.7%	N.D.
F	AS-16Da	0.8%	0.8%
G	AS-20Da	0.6%	0.6%
	Antisense	**51.7%**	**52.9%**
H	AS+1nt	0.8%	0.5%
I	SS-20Da & SS-16Da & SS-2nt	1.5%	0.2%
	Sense	**40.2%**	**43.8%**
J	SS+126Da	0.3%	0.3%

1. Capaldi et al, *NAT*, 2017.

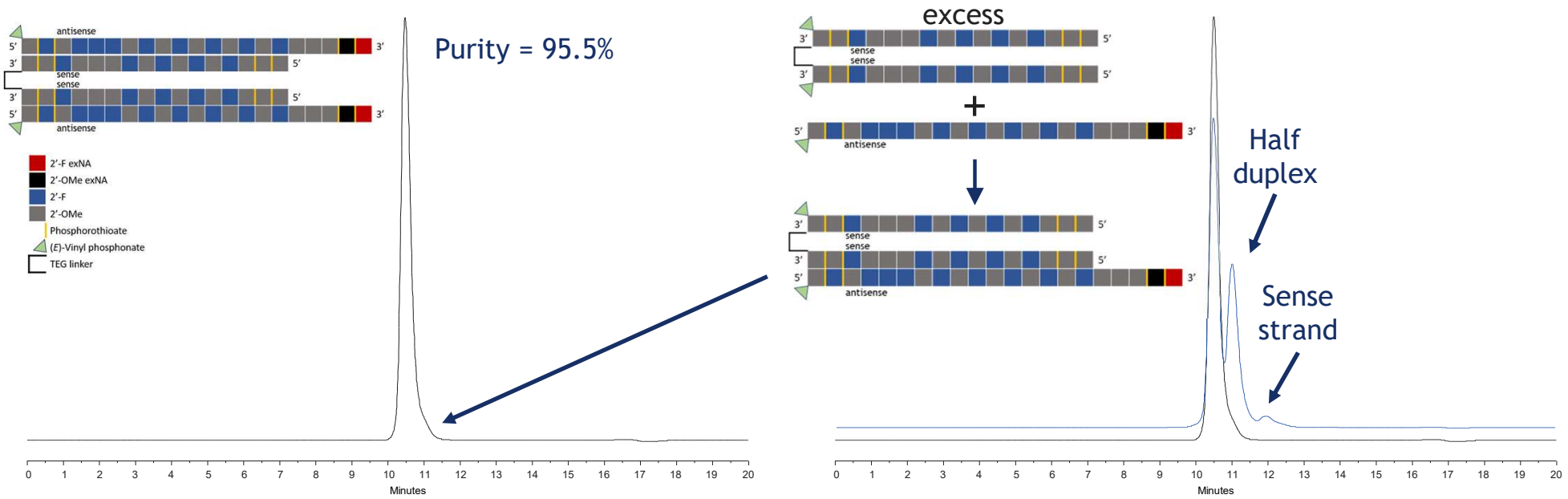
Divalent siRNA: Control of duplex impurities

Non-denaturing size exclusion chromatography



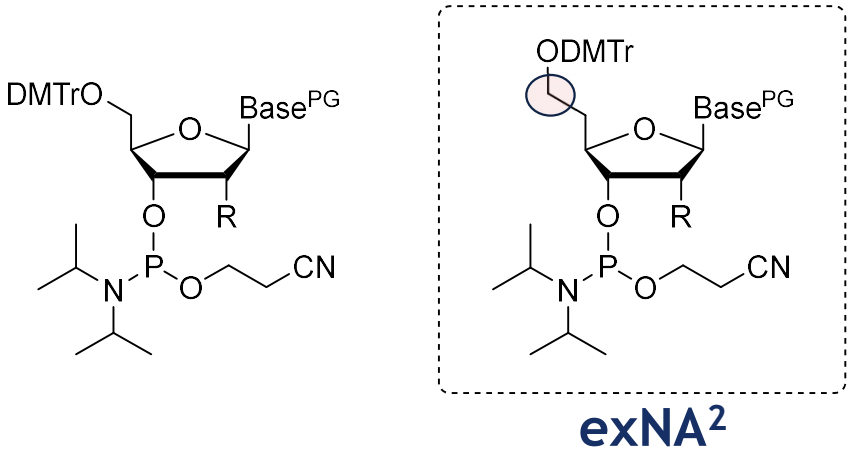
Divalent siRNA: Control of duplex impurities

Non-denaturing size exclusion chromatography



Hongene is licensed to sell exNA monomers and oligonucleotides!

Supporting the research market[1]



exNA[2]

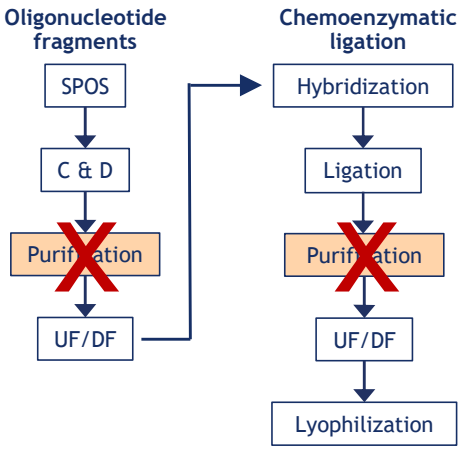
Download the exNA flyer

1. Hongene to supply exNA oligonucleotide technology under new licensing deal | Hongene. 2. K. Yamada et al, *Nat Biotechnol*, 2025.

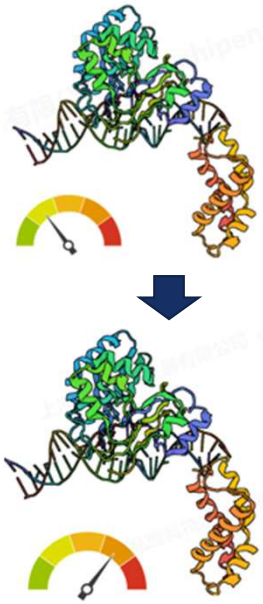
Controlling the cost of manufacturing

Driving down the cost of manufacturing

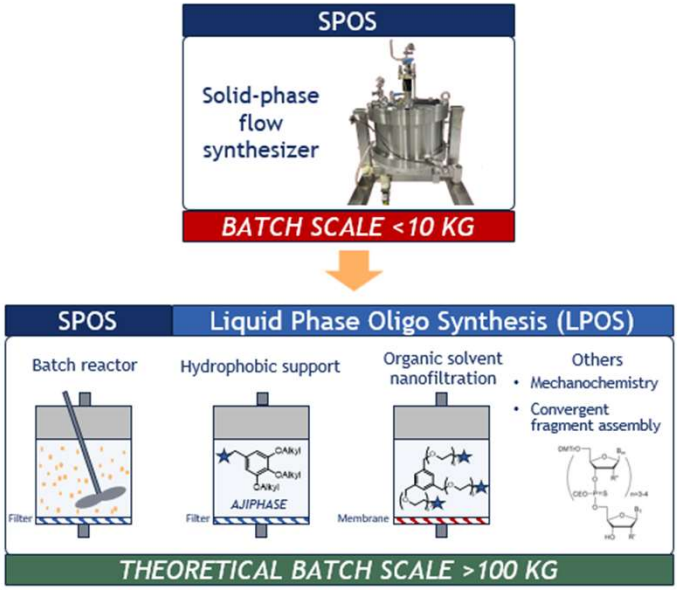
Three key levers



1. Removal of chromatography



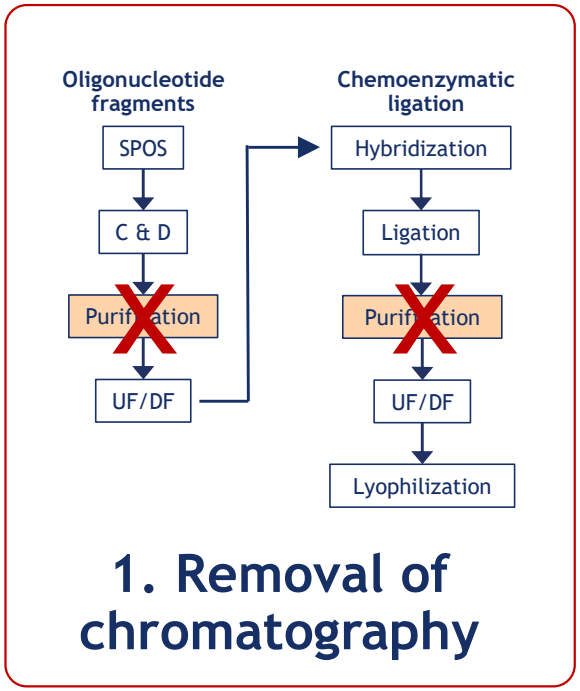
2. Engineered enzymes



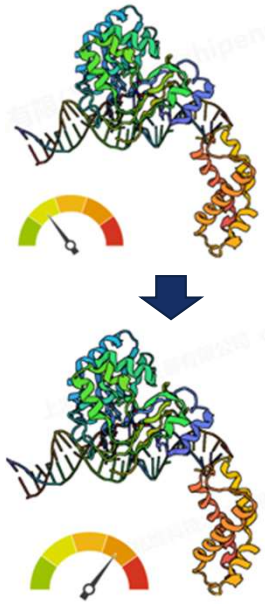
3. Improved fragment manufacturing technologies

Driving down the cost of manufacturing

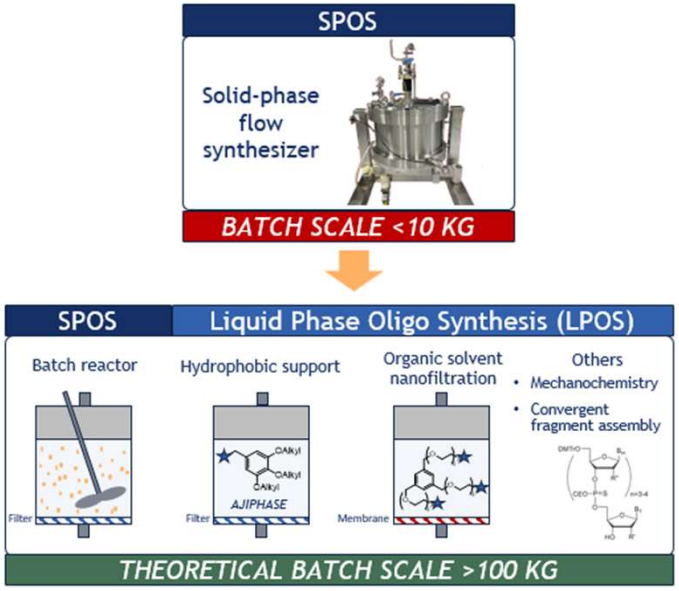
Three key levers



1. Removal of chromatography



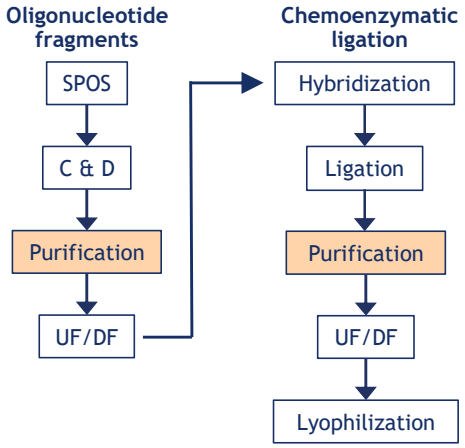
2. Engineered enzymes



3. Improved fragment manufacturing technologies

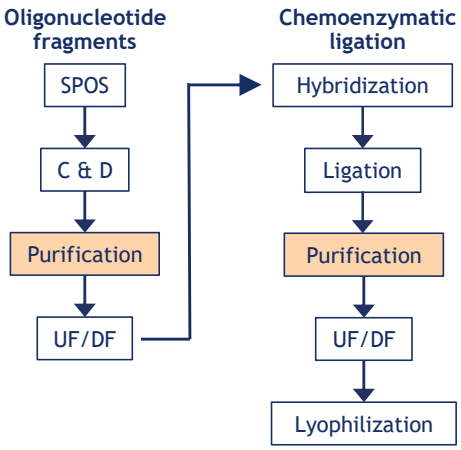
Removal of chromatography for cost control

Purified to Purified (P-to-P)

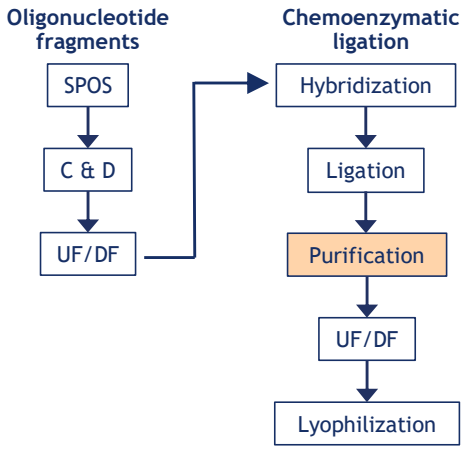


Removal of chromatography for cost control

Purified to Purified (P-to-P)

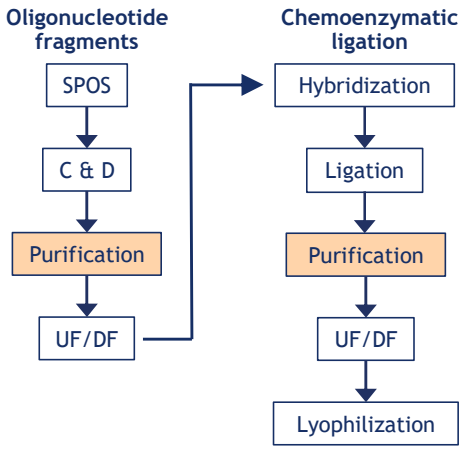


Crude to Purified (C-to-P)

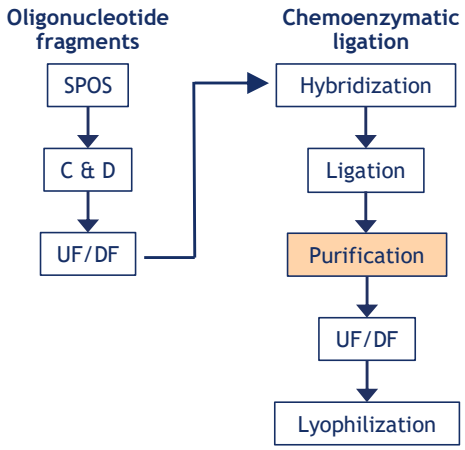


Removal of chromatography for cost control

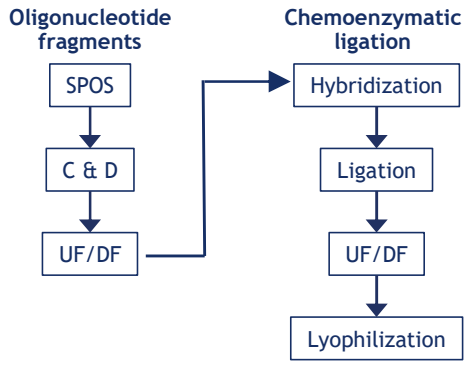
Purified to Purified (P-to-P)



Crude to Purified (C-to-P)



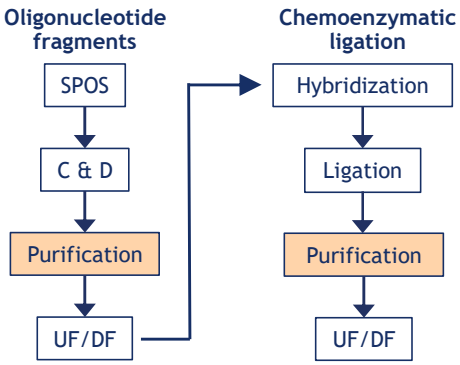
Crude to Crude (C-to-C)



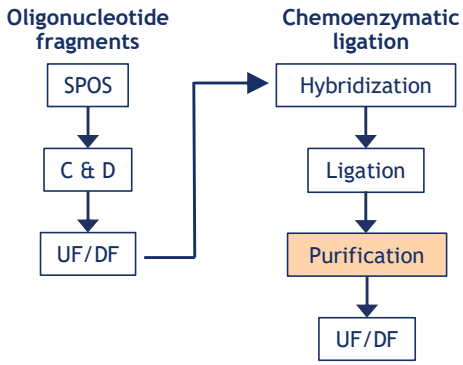
Under active investigation

Removal of chromatography *and lyophilization* for cost control

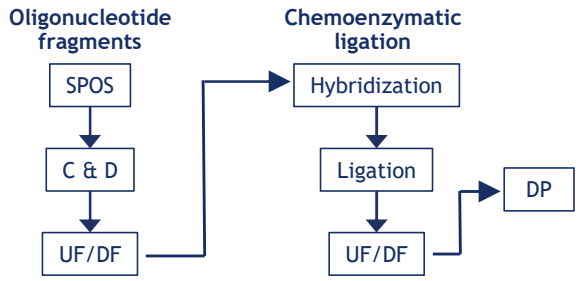
Purified to Purified (P-to-P)



Crude to Purified (C-to-P)



Crude to Crude (C-to-C)

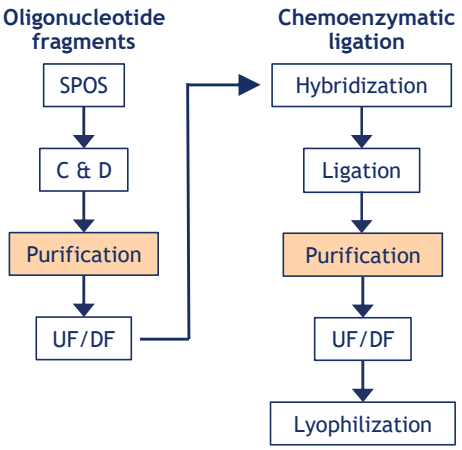


Optimal process for cost-effective large-volume manufacturing

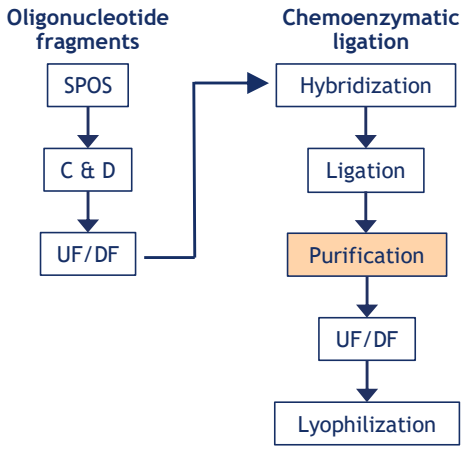
Crude-to-purified (C-to-P) siRNA case study

Removal of chromatography for cost control

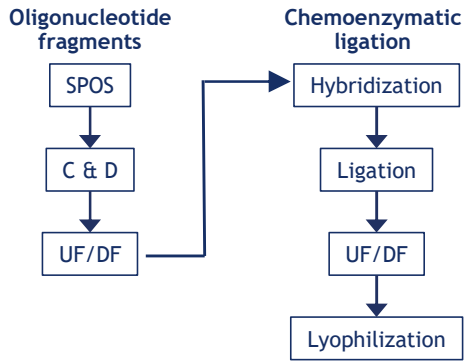
Purified to Purified (P-to-P)



Crude to Purified (C-to-P)



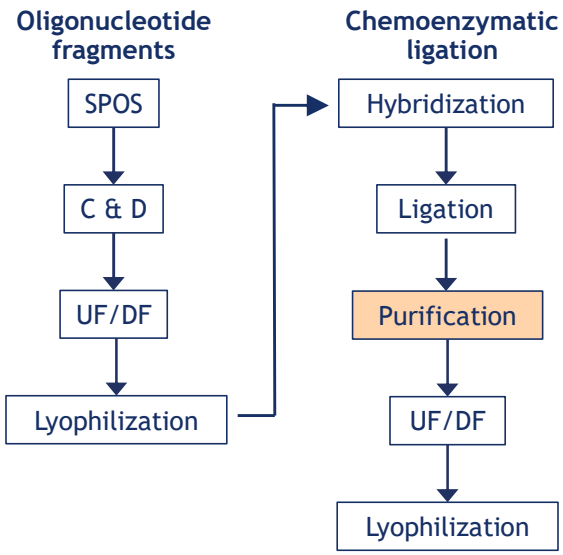
Crude to Crude (C-to-C)

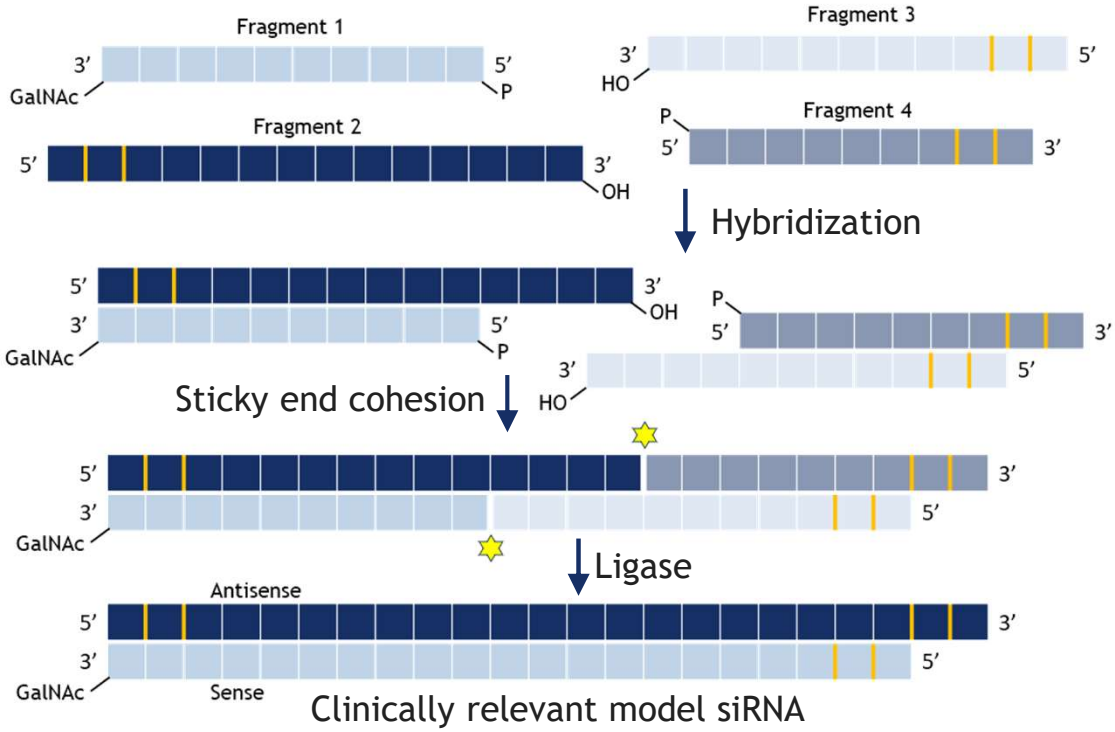


Under active investigation

C-to-P siRNA: Chemoenzymatic process design

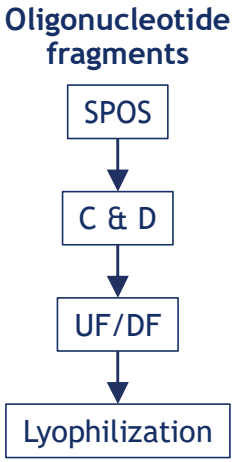
Crude fragments → purified siRNA



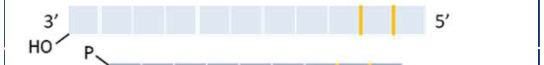


C-to-P siRNA: Control of oligonucleotide fragment purity

IPRP-HPLC/MS



- Average fragment yields
  - Average for this C-to-P study = 68%
  - Compared to P-to-P ~40% (previous case studies)

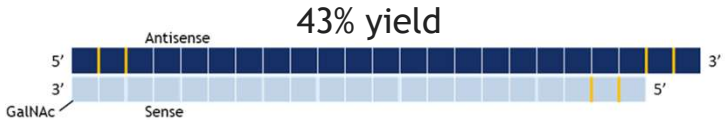
Fragment	Construct	L	Purity	Yield
1		10	88.4%	61.9%
2		14	90.6%	79.0%
3		11	94.0%	61.8%*
4		9	94.0%	69.3%

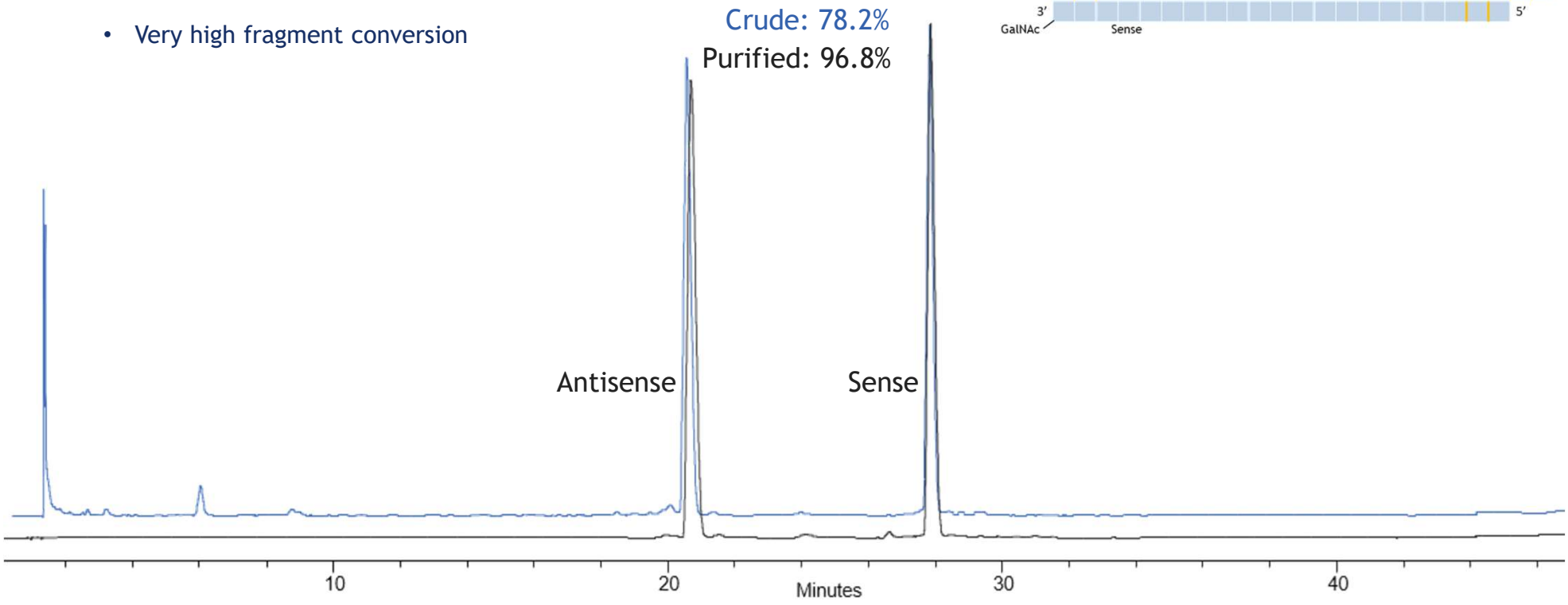
Yields based on theoretical MEC. Purity measured by IPRP-HPLC. *Lowest value used to calculate final siRNA % yield.

C-to-P siRNA: Control of purity and impurities

IPRP-HPLC/MS of duplex

- Very high fragment conversion

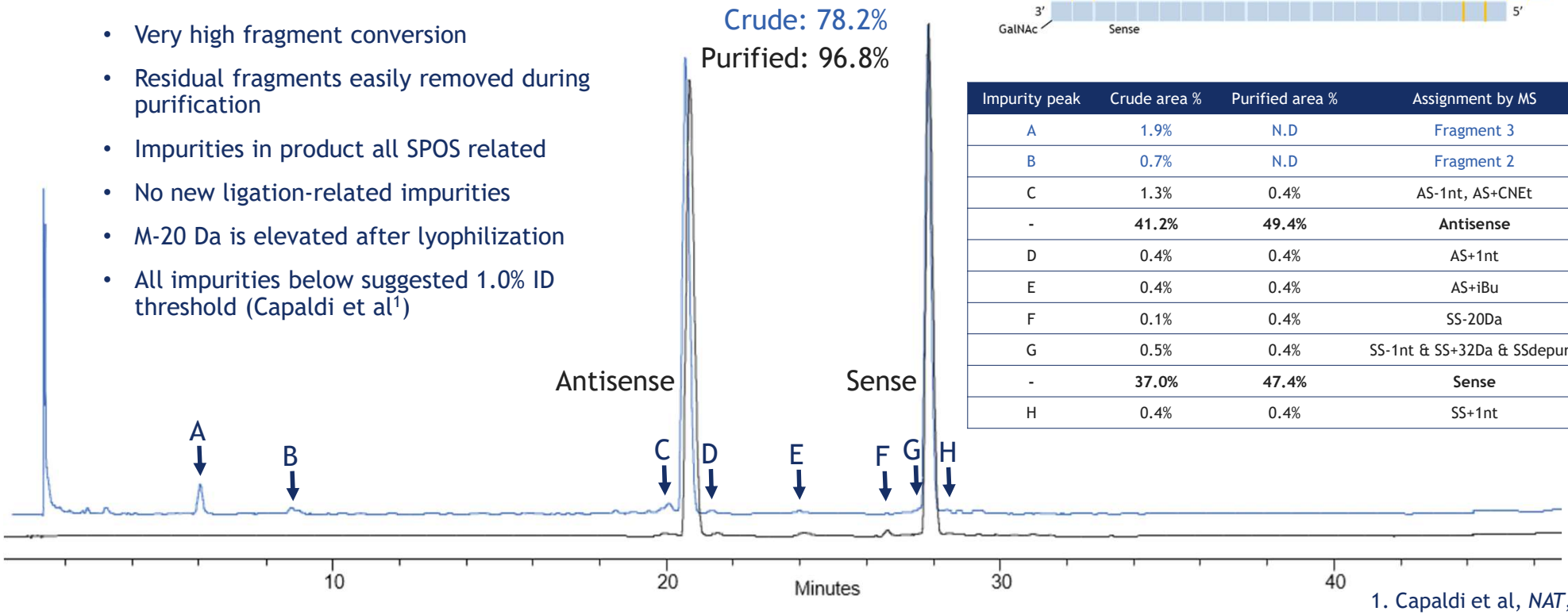


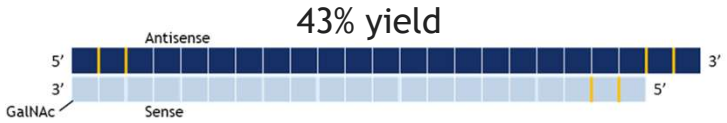


C-to-P siRNA: Control of purity and impurities

IPRP-HPLC/MS of duplex

- Very high fragment conversion
- Residual fragments easily removed during purification
- Impurities in product all SPOS related
- No new ligation-related impurities
- M-20 Da is elevated after lyophilization
- All impurities below suggested 1.0% ID threshold (Capaldi et al[1])



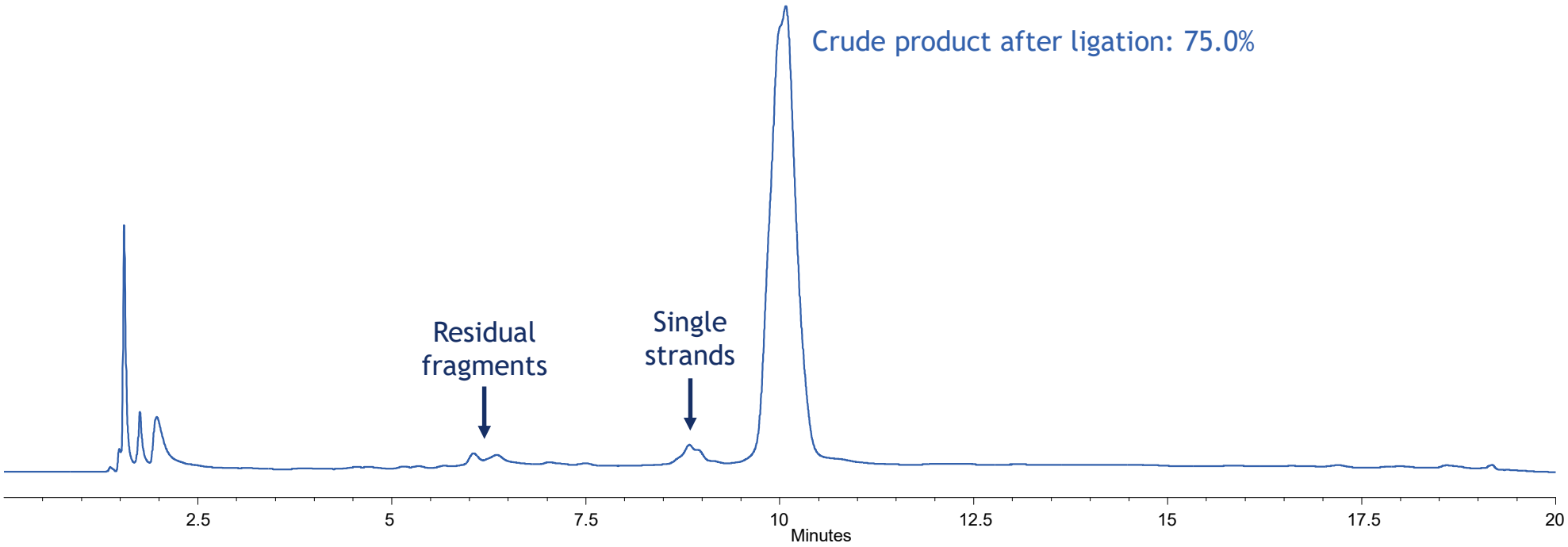


Impurity peak	Crude area %	Purified area %	Assignment by MS
A	1.9%	N.D	Fragment 3
B	0.7%	N.D	Fragment 2
C	1.3%	0.4%	AS-1nt, AS+CNEt
-	**41.2%**	**49.4%**	**Antisense**
D	0.4%	0.4%	AS+1nt
E	0.4%	0.4%	AS+iBu
F	0.1%	0.4%	SS-20Da
G	0.5%	0.4%	SS-1nt & SS+32Da & SSdepurA
-	**37.0%**	**47.4%**	**Sense**
H	0.4%	0.4%	SS+1nt

1. Capaldi et al, *NAT*, 2017.

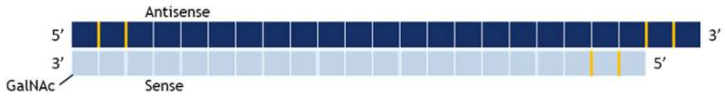
C-to-P siRNA: Control of duplex impurities

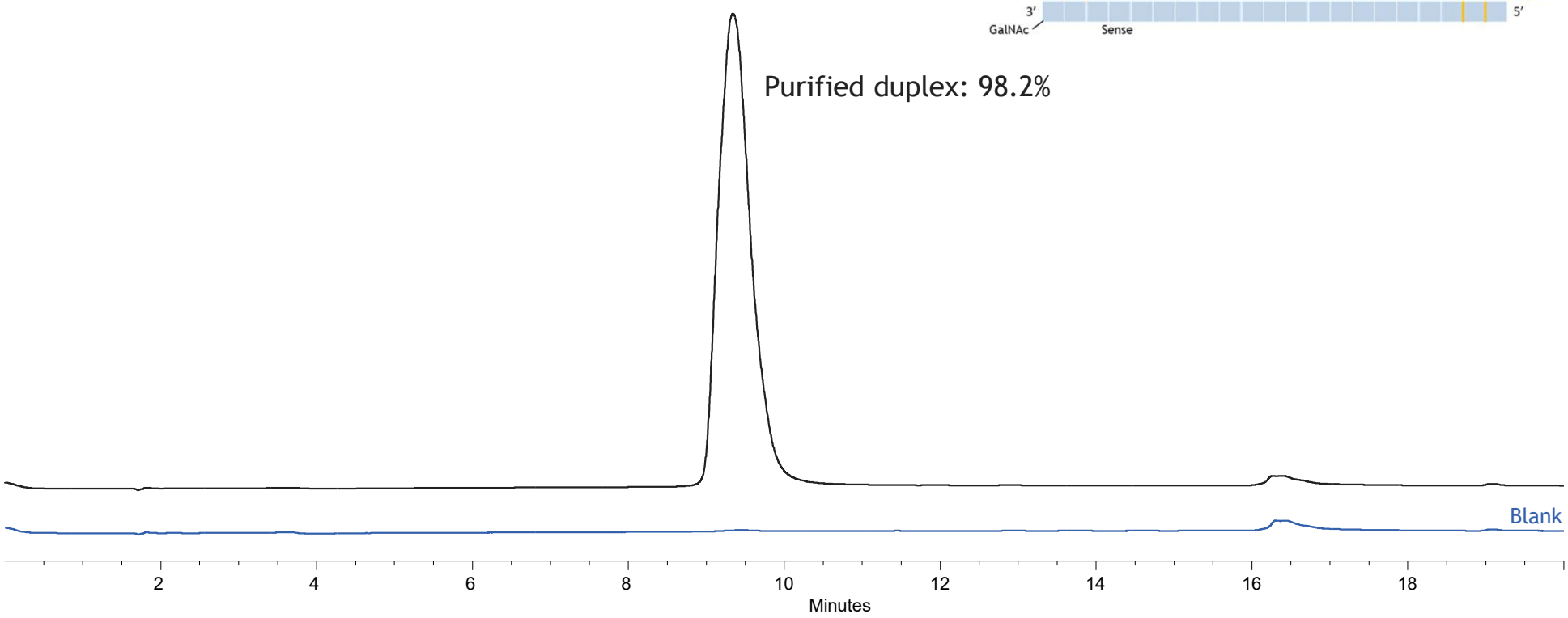
Non-denaturing reverse phase chromatography



C-to-P siRNA: Control of duplex purity

Non-denaturing reverse phase chromatography

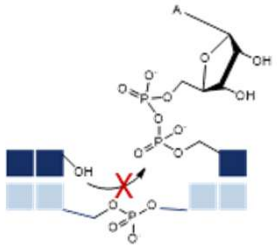




Oligonucleotide fragments and impurity control

Two reasons for improved purity for Gen 2 vs Gen 1

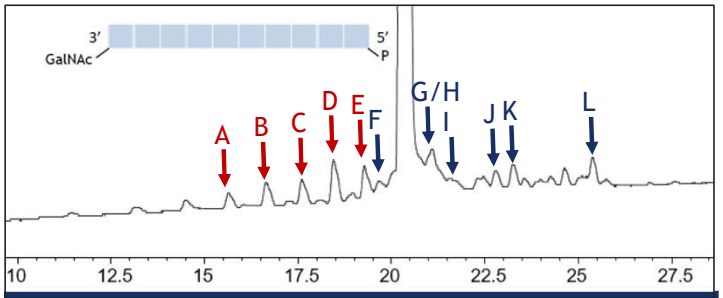
1. Some oligonucleotide fragment impurities aren't good substrates for ligase
   - These don't persist in product because they are purged during siRNA column chromatography



Many impurities in oligonucleotide fragments are purged
- Not substrates for ligase (e.g. 5′(N-1nt) or
- Inefficient hybridization

2. SPOS-related impurities such as N-1nt, N+1nt, PO, M-20Da, M-2Da, CNET, iBu, and GalNAc degradants reduced compared to full-length synthesis
   - Less opportunity to form and accumulate

Example: Fragment 1 impurities - IPRP-HPLC/MS



Impurity peak	RT (min)	UV area%	Detected in siRNA	Assignment
A	15.6	0.3	N	N-5
B	16.7	0.4	N	N-4
C	17.6	0.4	N	N-3
D	18.4	0.7	N	N-2
E	19.3	0.7	N	N-p
F	19.7	0.4	Y	N-1nt(internal)
-	**20.3**	**88.4**	**Y**	**Fragment 1**
G	21.1	1.1	Y	M+14Da, N+1nt
H	21.2	0.5	Y	M-GalNAc deg1
I	21.6	0.6	Y	M-GalNAc deg2
J	22.8	0.5	Y	M+iBu
K	23.3	0.6	Y	M+iBu
L	35.4	0.5	N	Branchmer

Summary of exemplary siRNAs and sgRNAs synthesized by ligation

Molecule	Oligonucleotide chemistry				Synthesis strategy[1]	Yield[2]	Purity[3]
	SS/AS	2′-Ribose mods	Backbone	GalNAc			
Inclisiran siRNA	21/23	2′-OMe, 2′-F, 2′-deoxy	PS/PO	✓	P-to-P	26%	97%
Divalent siRNA	16/21	2′-OMe, 2′-F, exNA, (*E*)-VP	PS/PO/TEG	x	P-to-P	19%	97%
C-to-P siRNA	21/23	2′-OMe, 2′-F	PS/PO	✓	C-to-P	43%	97%
sgRNA	100mer	2'-OMe, 2'-OH	PS/PO	X	Splinted	NR	96%
pegRNA	161mer	2'-OMe, 2'-OH	PS/PO	X	Splinted	NR	97%

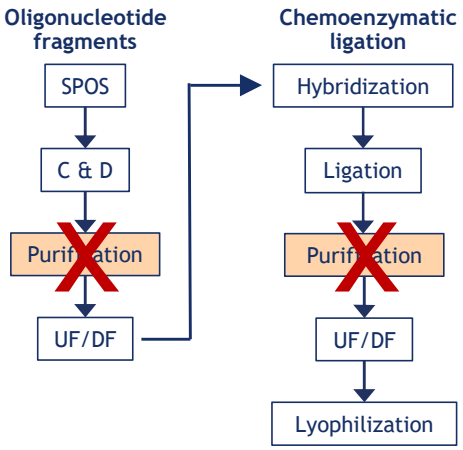
1. P-to-P = HPLC purified fragments and purified siRNA; C-to-P = UF/DF processed fragments and HPLC purified siRNA; 2. % Yields based on theoretical MEC, calculated from lowest yielding fragment; 3. Denaturing IPRP-UPLC method

- sgRNA 100mer and inclisiran case studies described previously (TIDES US, May 2025)

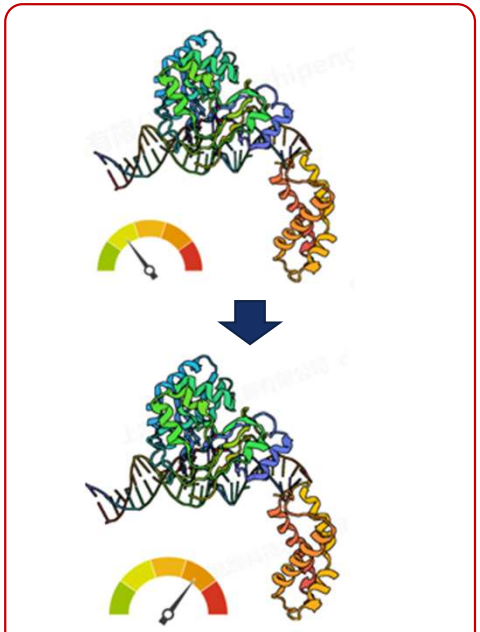
Engineered thermostable T4 RNA ligase

Driving down the cost of manufacturing

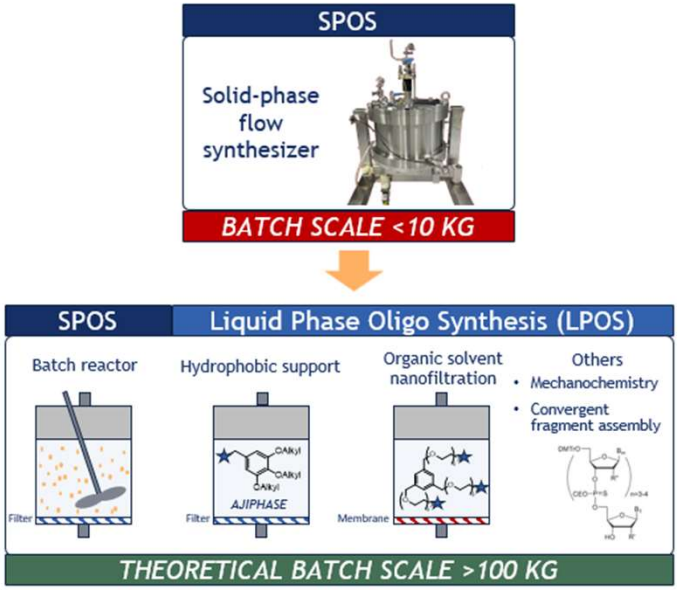
Three key levers



1. Removal of chromatography



2. Engineered enzymes

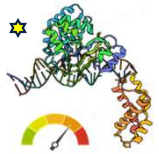


3. Improved fragment manufacturing technologies

Engineered thermostable T4 RNA ligase is highly active at 52°C

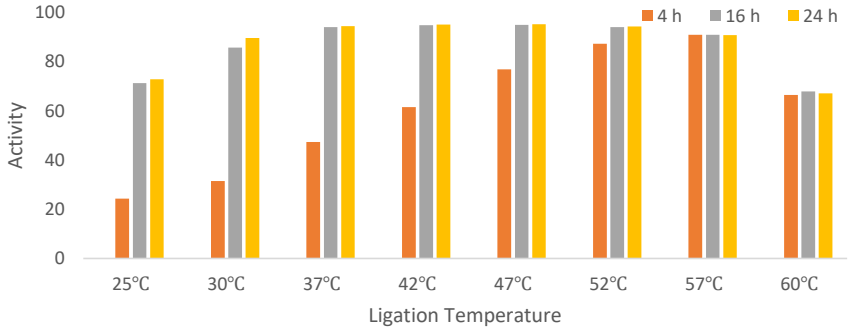
- Rationale for thermostable enzyme
  - Facilitates ligation at higher temperature
  - Reduced secondary structures
  - Enhanced ligase deselection of oligonucleotide fragment impurities
  - Mitigation of incorrectly ligated byproducts
  - Leverage C-to-C strategy further driving down cost

T4 mutant engineered to be maximally active between 52-57°C



T4 Mutant

Model siRNA



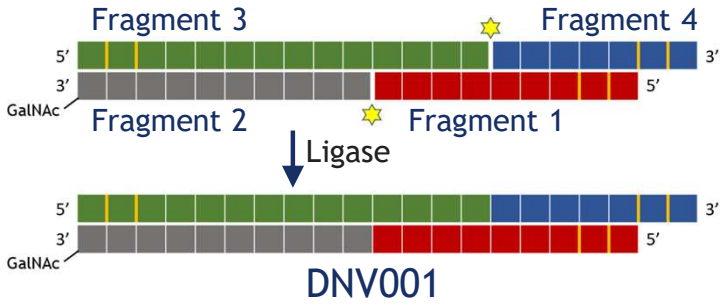
Data shared at OTS, Budapest, 2025. Chris Li: chris.li@hongene.com

Chemoenzymatic ligation technology now supporting siRNA clinical development

Chemoenzymatic technology now supporting siRNA clinical development[1]

Fragment	Length	Non-GMP C-to-P[1]	GMP batch P-to-P[1]
F1	9	96.1%	98.0%
F2	10	94.4%	96.3%
F3	14	90.1%	95.6%
F4	7	95.2%	98.5%

1. Denaturing IPRP-UPLC method



siRNA	GMP	Oligonucleotide chemistry				Synthesis strategy[1]	Yield	Purity[2]
		SS/AS	2′-Ribose modifications	Backbone	GalNAc			
DNV001	No	19/21	2′-OMe, 2′-F	PS/PO	✓	C-to-P	~960 g	95%
DNV001	Yes	19/21	2′-OMe, 2′-F	PS/PO	✓	P-to-P	~1,020 g	97%

1. P-to-P = HPLC purified fragments and purified siRNA; C-to-P = UF/DF processed fragments and HPLC purified siRNA; 2. Denaturing IPRP-UPLC method

1. Hongene Supports Clinical Advancement of siRNA DNV001 Using Proprietary Chemoenzymatic Ligation Platform

CMC regulatory considerations

CMC regulatory considerations

- Oligonucleotide fragments – will these be RSMs in the future?
- Control of phosphorothioate stereochemistry
  - HPLC and NMR can differentiate distributions
  - How does *P* stereochemistry compare for Gen 2 vs Gen 1?
- ID, purity, and impurity control
  - Denaturing HPLC/MS methods for Gen 2 to measure on the duplex directly
  - For Gen 1 a focus of control is sense and antisense
  - For Gen 2 a focus of control is oligonucleotide fragments
- Control of small molecule ligation reagents
- New test methods for residual enzyme for Gen 2 process

Conclusions

Conclusions

- Market demand for siRNA drugs is anticipated to grow significantly over the next decade
- Demand will be met by adoption of chemoenzymatic ligation technology
- Advantages are improved purity, yield, scalability, sustainability and cost
- Versatile technology, supporting complex siRNA, sgRNA, and other oligonucleotide products
- Priority for large volumes: Improve scalability and drive down cost of manufacturing
    1. Removal of chromatography: C-to-P and C-to-C strategies
    2. Engineered T4 RNA ligases expected to boost efficiency
    3. Better processes for oligonucleotide fragment manufacturing
- Successfully leveraged for GMP siRNA DS to support clinical development
- We see no regulatory hurdles prohibiting wider adoption of the technology

Acknowledgements

Hongene Oligo Team

- Jim Yang
- Frank Rao
- Wangqiyue Sun
- Hu Cheng
- Brian Lu
- Xiaomei Luo
- Chenlin Hua
- Hanqing Tang
- Mei Tang
- Chao Chen
- Yinchun Liu

Hongene Biocatalysis Team

- Minzhi Wei
- Min Guo
- Zaiji Xiong
- Juan Xia
- Zhaoyue Fan
- Yanxing Liu
- Yumai Lu
- Liang Jiang
- Jianxiong Liu

Hongene Biotech

Thank you!
Visit us at Booth 427

Download our brochures

Contact us