Challenges & Perspectives in the Manufacture of Synthetic sgRNA

Cell and Gene Therapy Products 2023 Symposium

Joe Guiles, PhD
Head of Chemical Development
26 June 2023

Agilent
Trusted Answers

Agilent Technologies

Nucleic Acid Solutions Division (NASD)

Two GMP facilities located in
Boulder and Frederick, CO

Disclaimer

This presentation reflects the views of the author and should not be construed to represent Agilent Technologies views.

Agenda

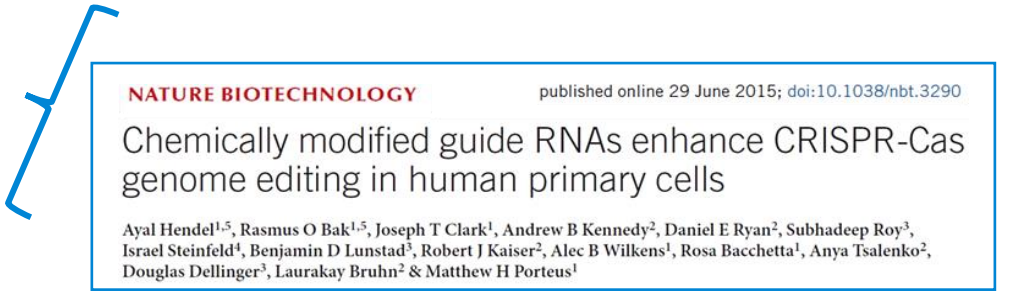
- Types and utility of sgRNA
- Process challenges of synthetic guides
- sgRNA manufacturing process
- Case study data of sgRNA (impurity profile, purity outcomes, impurity characterization)
- In Process Control Challenges & Analogies to THX Oligonucleotides
- Summary & perspectives & moving forward

Types of sgRNA and Utility

- Nucleic Acid Synthetic Oligomers – Oligonucleotides & sgRNA
  - Both act in a sequence-specific manner to exert genetic mechanism
  - Both utilize chemical modification to stabilize
    - Sugar & PO4 focused

- sgRNA Composition
  - sgRNA is 2-part function guide
  - 40 - 120 nucleotides
  - 12,000 - 36,000 mw (small big molecule)

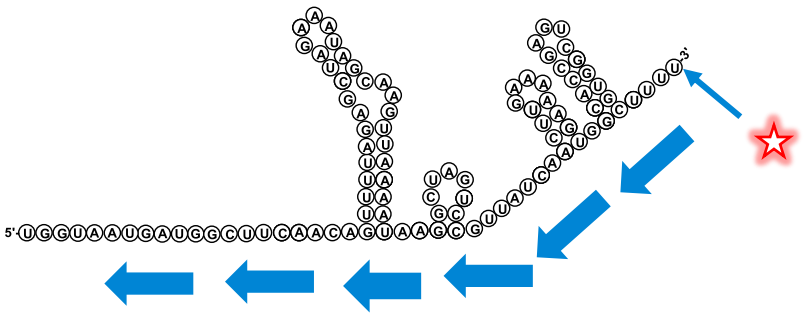
- sgRNA Utility
  - Nucleotide and base editing
  - Gene knockout, interference, integration, activation



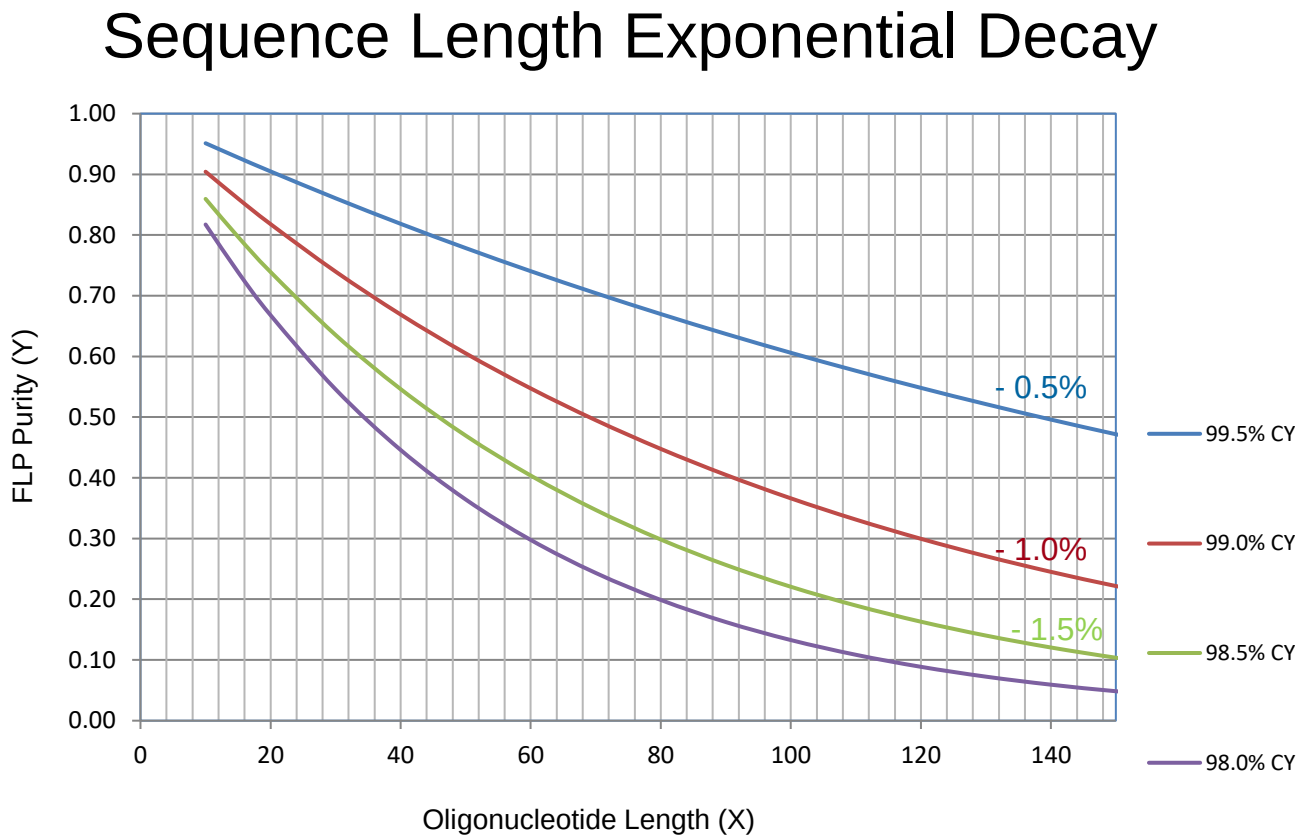
*Finn, J.D. et.al. Cell Rep. 2018 Feb 27;22(9):2227-2235

*ICH 2022 March guidance for Gene Editing

Process Challenges from sgRNA Length & Properties



Purity Y = $(\%CE)^X$



PURITY FACTORS = Sequence Composition, Sequence Length & Coupling Efficiency

sgRNA Length

Purity Y = $(\%CE)^X$

X (# nucleotides)	%CE	Theoretical % Purity after Synthesis
40	99	67
100	99	37
120	99	30

- Nucleic acid synthesis has high fidelity = high coupling efficiency
- Build up low % impurities as the oligo (chain) extends
- Longer the chain the more total impurities
- Reduction (control) in the % of impurities by downstream purification
- PRIs (product related impurities) are close in structure, purging control
- Length based crude purity – translates to final purity outcome
- Universal purity requirement not realistic

sgRNA Manufacturing Overview – Purity Outcome

Synthesis

Cleavage & Deprotection

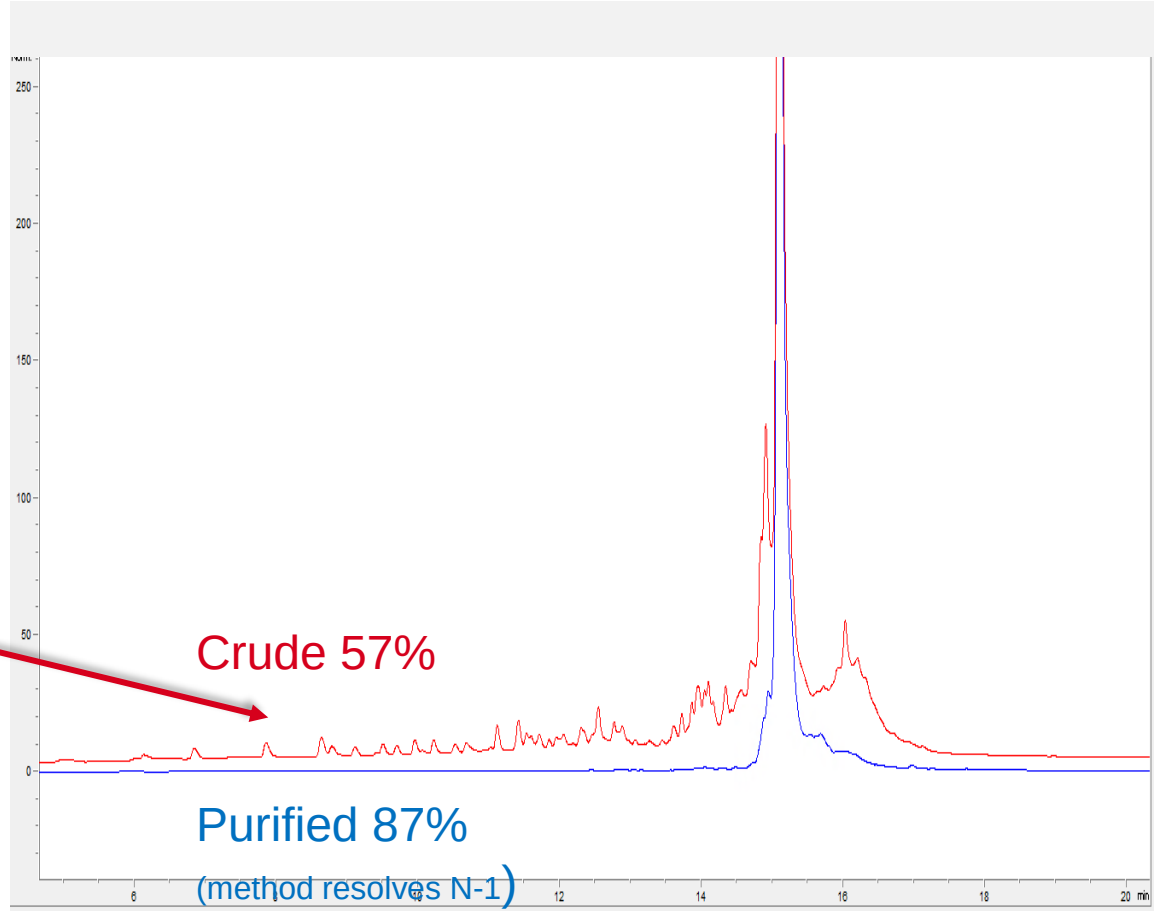
Ultrafiltration

Purification

Ultrafiltration

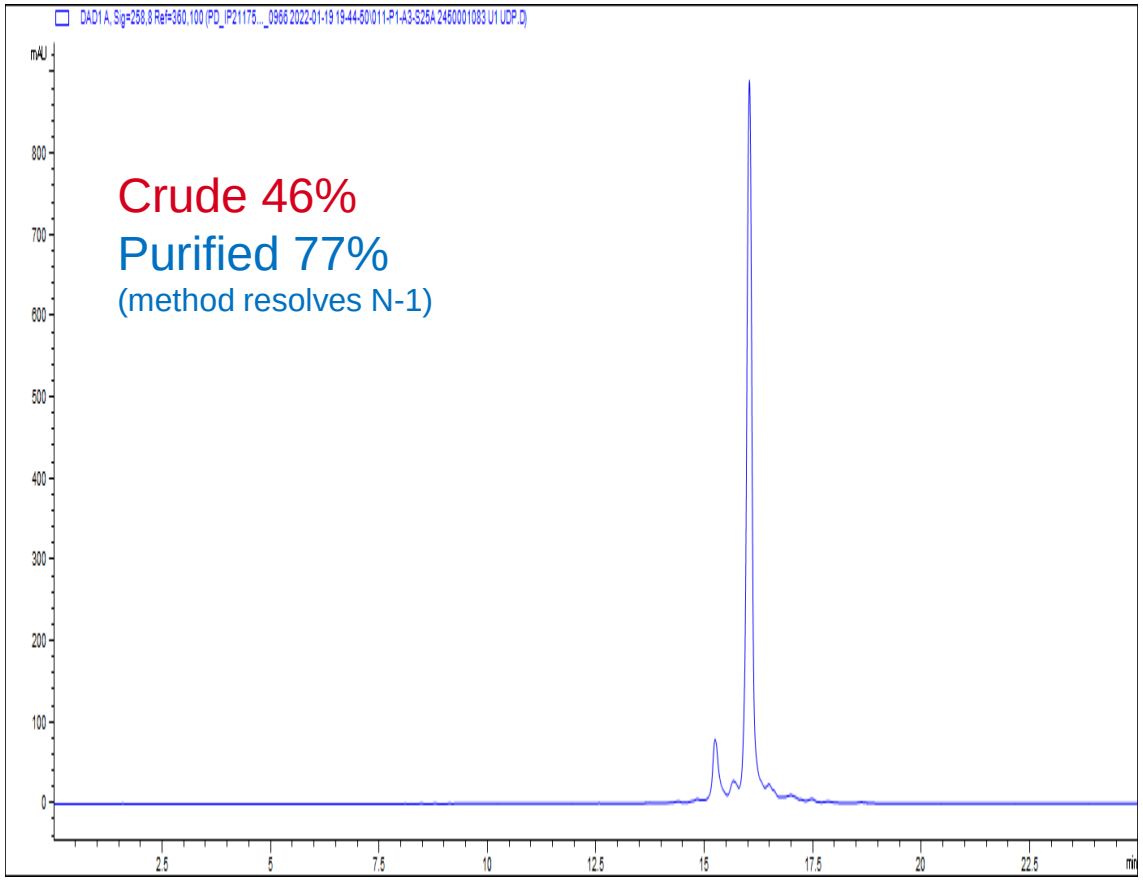
Lyophilize

~70 mer guide



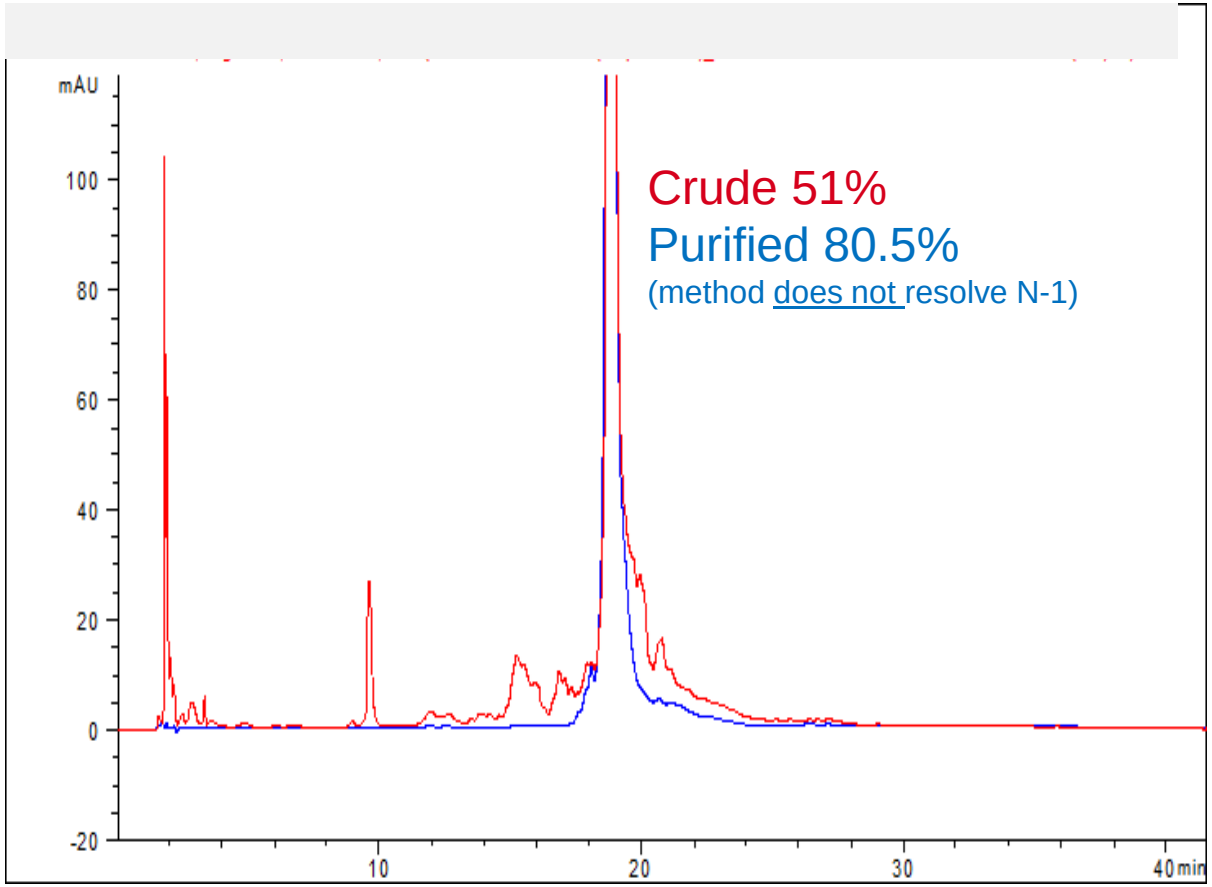
Light vs. Heavy Modified Guides – 100 mers

Light Modified Guide (~100 mer)



Crude 46%
Purified 77%
(method resolves N-1)

Heavy Modified Guide 100 mer



Crude 51%
Purified 80.5%
(method does not resolve N-1)

In Process Control Challenges

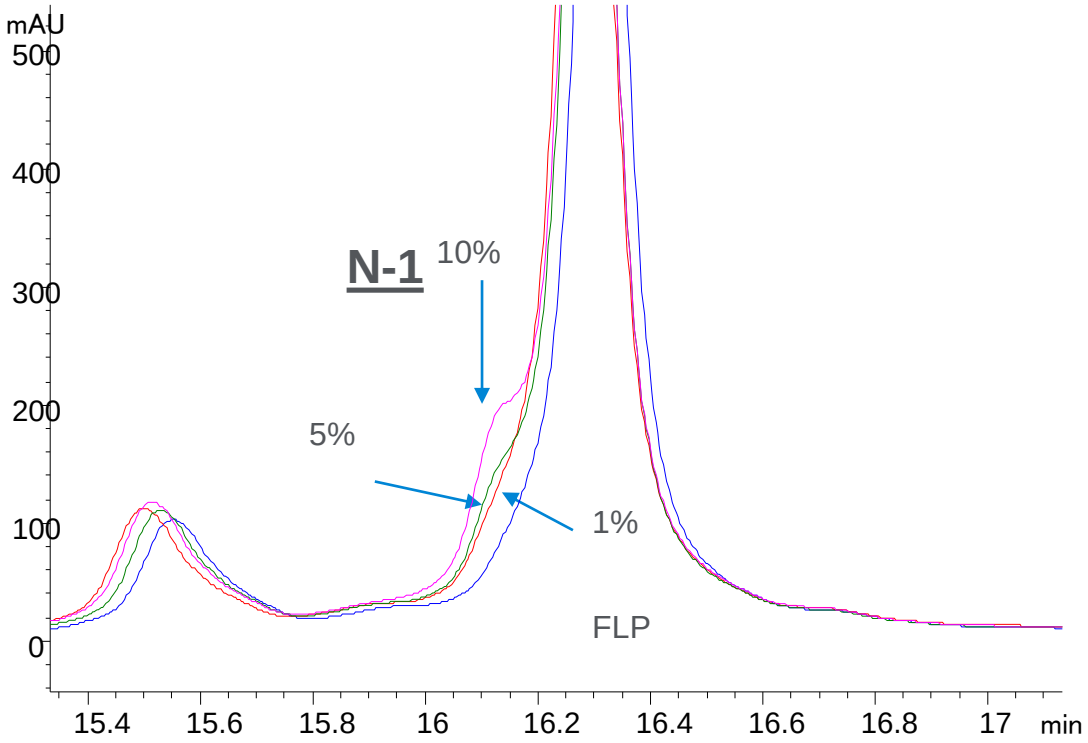
Commonalities with THX Oligos

- sgRNA is a complex small big molecule:
  - Full characterization is challenging, more challenging than a THX oligo
  - Large number of impurities
- Quantitation of overlapping impurities is challenging:
  - Banding strategies for peak families can be utilized/justified
- Chromatographic resolution with mass spectrometry sensitivity is challenging:
  - Orthogonal methods can be utilized to resolve overlapping peaks, final purity needs justification
  - Ion-pair reversed-phase chromatography (IP-RP) is the highest resolving method, and it limits overlapping peaks, so it's the method of choice for oligonucleotides* (must limit MS ion suppression in method development)

* Oligonucleotide API Sameness and Impurity Assessment Considerations, Zhang, D. FDA, CDER SBIA September 20, 2022, Day 1, Session 1B:

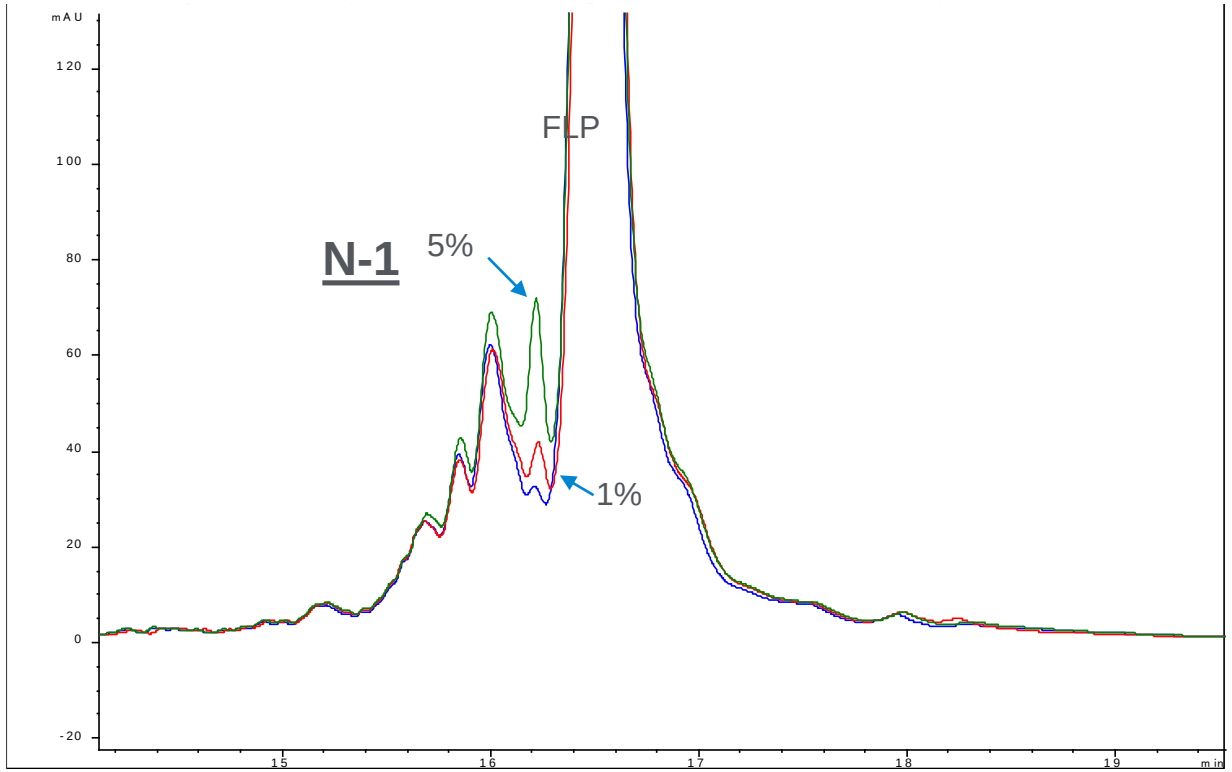
Chromatography Resolution (100 mer examples)

Sequence A



Unable to resolve N-1

Sequence B

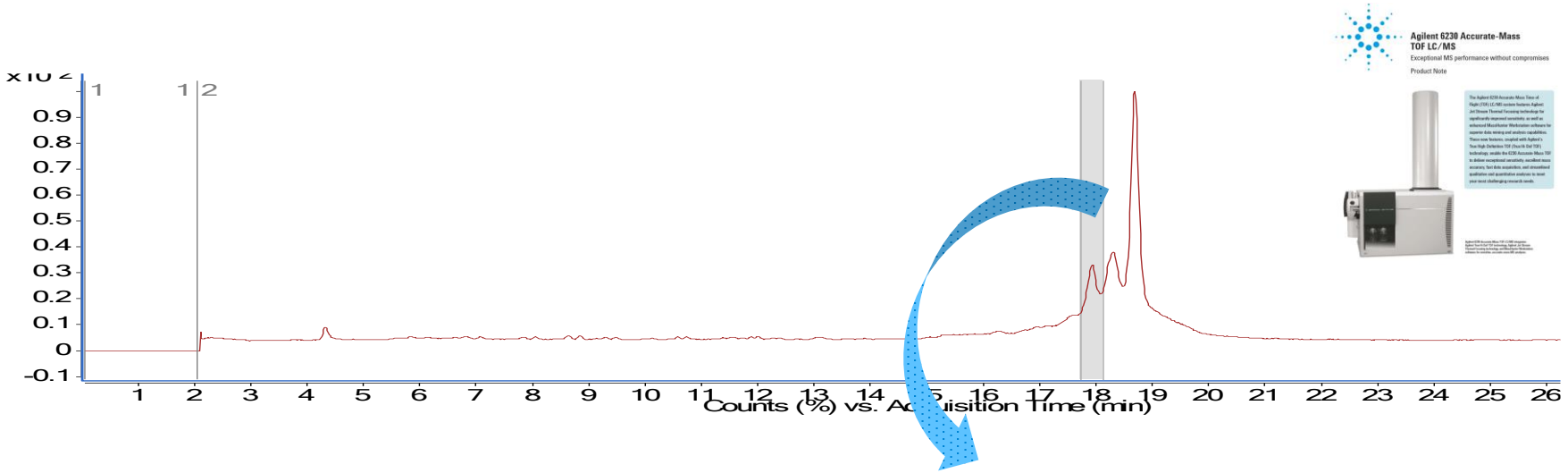


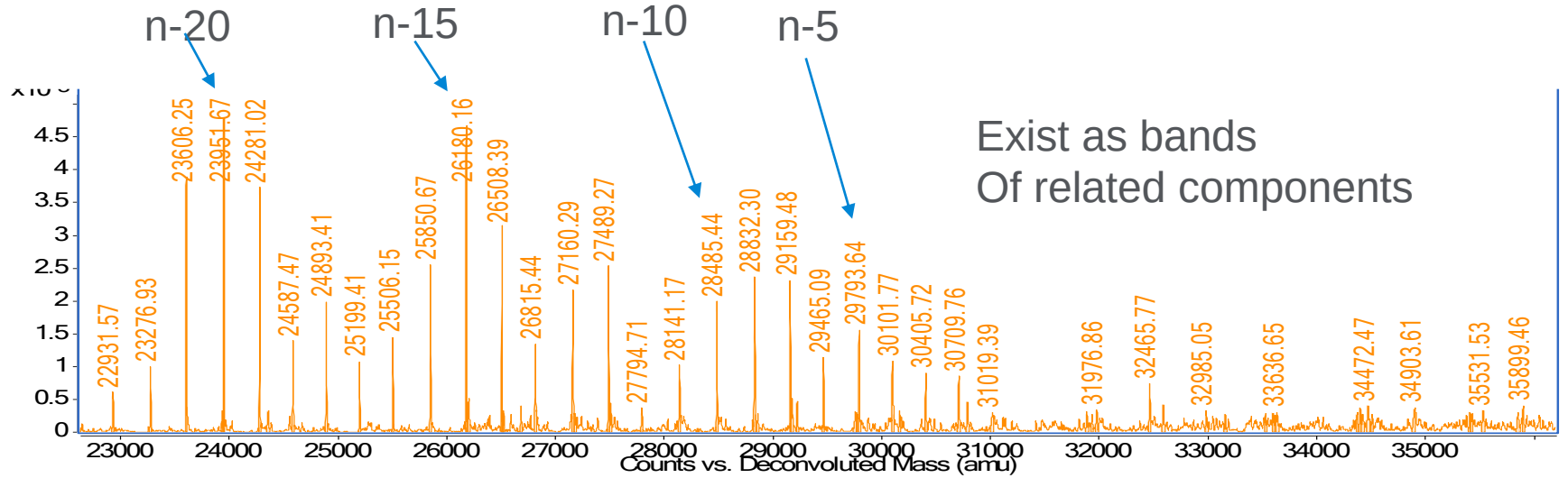
N-1 resolved

Resolution Factors = Sequence Composition & Length

Purity Method Development Approach

- Stage appropriate
- Scientifically sound
- Sequence specific
- Robust
- Stability indicating
- Compatible with MS detection





Exist as bands
Of related components

Summary and Perspectives of sgRNA Production

- Chemical synthesis of sgRNA utilizes highly similar manufacturing process to that of THX oligonucleotides
  - Automated and well understood, utilizes high quality RSM's/RM's, exponential decay
  - Purity outcome is dependent on length and composition of the sgRNA
- High resolving HPLC purity methods that are compatible with MS are possible
- Each sgRNA behaves differently and has variability in the resolution of impurities
- Final purity is sequence-dependent, each sequence varies… continuous improvements being focused on
- CDER guidance for THX oligos utilizes guidelines for LOD, LOQ, stage dependent specified limits that facilitates in process control and release quality
  - Purity and impurity control via in-process controls emphasis on sameness
  - Impurity characterization can be extensive but more challenging than THX oligo
  - Purity target or threshold has no guideline

Thank You

Topics for Roundtable

- This meeting is important to progress towards establishing science-based regulatory guidelines
- What are the considerations for purity guidelines ?
- Is a generic guideline appropriate?
- What are the other relevant CQA's and how do they compare to THX Oligonucleotides?
- What are the basis for specification design (process capabilities, method capabilities, stability data, safety/tox data ?
- Nucleotide sequence order verification, what are the technological options and their capabilities?