WCBP 2023
Waters Technical Seminar

Nick Pittman

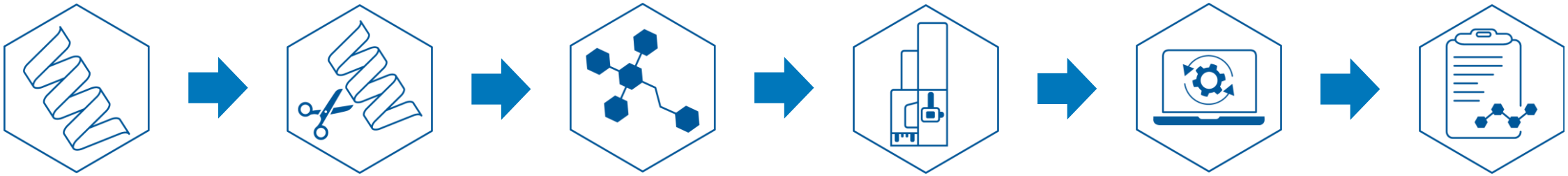
Marketing Manager, Global Biopharmaceutical Business

26th January

Protein Digestion

Quick, Clean, Complete

Challenges with Protein Digestion



Protein digestions are plagued by *long digestion times and performance trade-offs*

Productivity and data analysis can be hampered by messy maps with various non-tryptic peaks

What if you had reproducible, efficient digestions that deliver high sequence coverage and low baseline noise?

Quick, Clean, Complete

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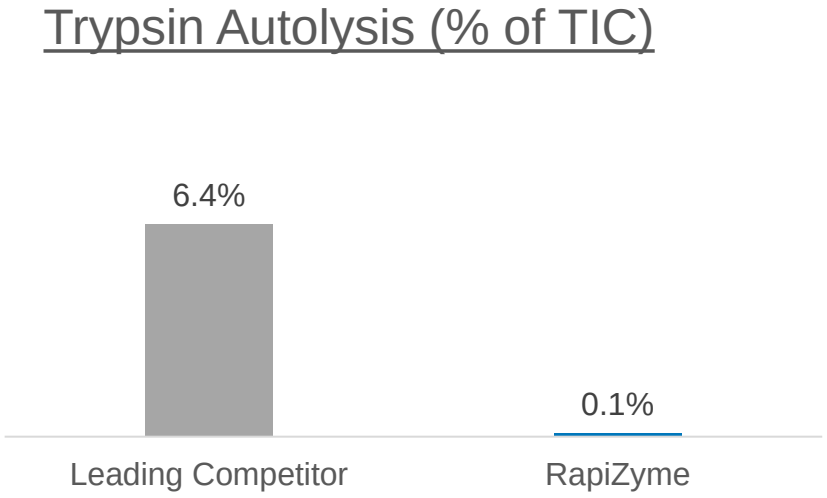
MS-Grade Trypsin

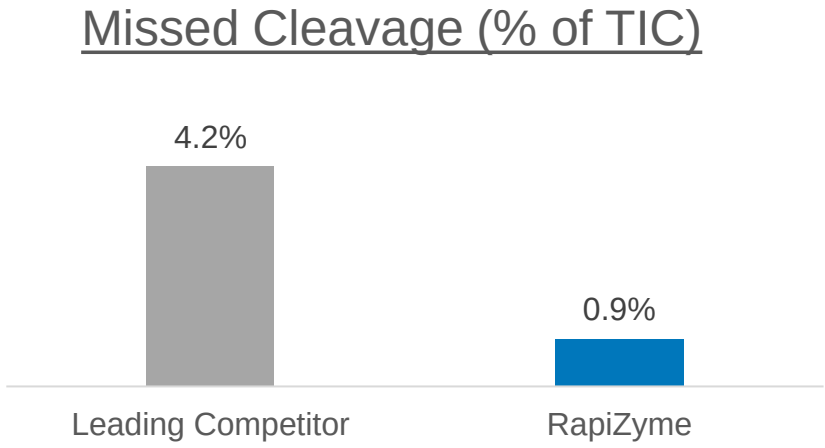
Coming February 2023…

QUICK | Tired of long sample prep times?

High enzyme ratios (1:5) are faster, but typically lead to high autolysis. RapiZyme trypsin's unique autolysis resistance and low missed cleavage unlocks ***efficient 30-minute digestions*** vs. standard 3-hour digestion (using 1:20)

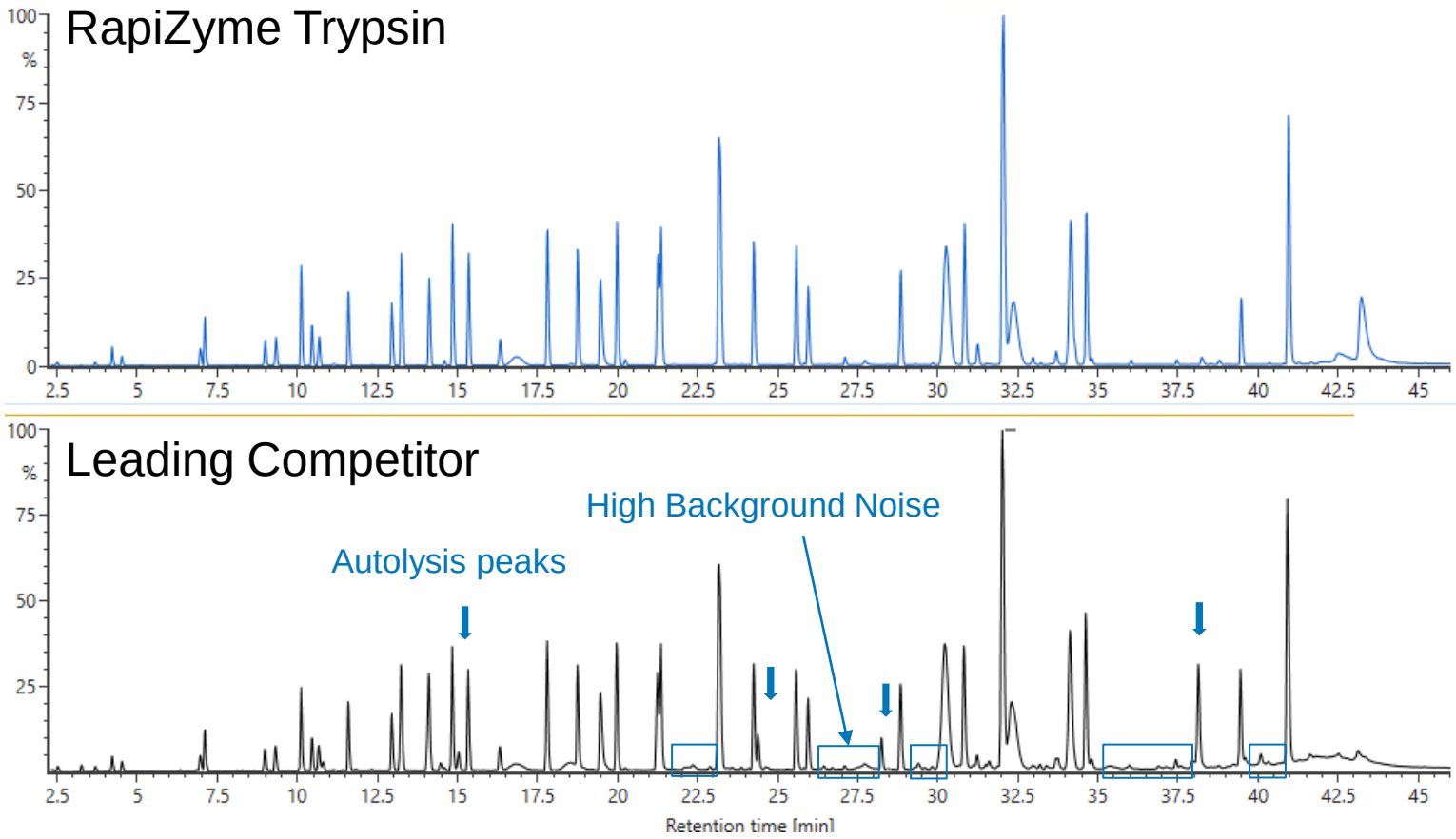
Remicade™ digestion, 1:5 Enzyme: Protein Ratio, 30 min.

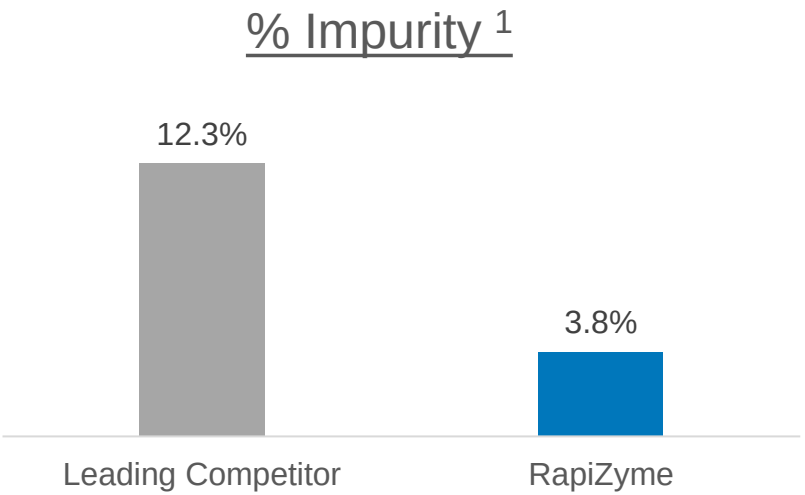




Digestion details: 37 °C, pH 7.5. N=6 per enzyme, 2 batches of each enzyme, 3 digestion replicates per batch.

CLEAN | Looking for noise-free maps for quick and easy data analysis?



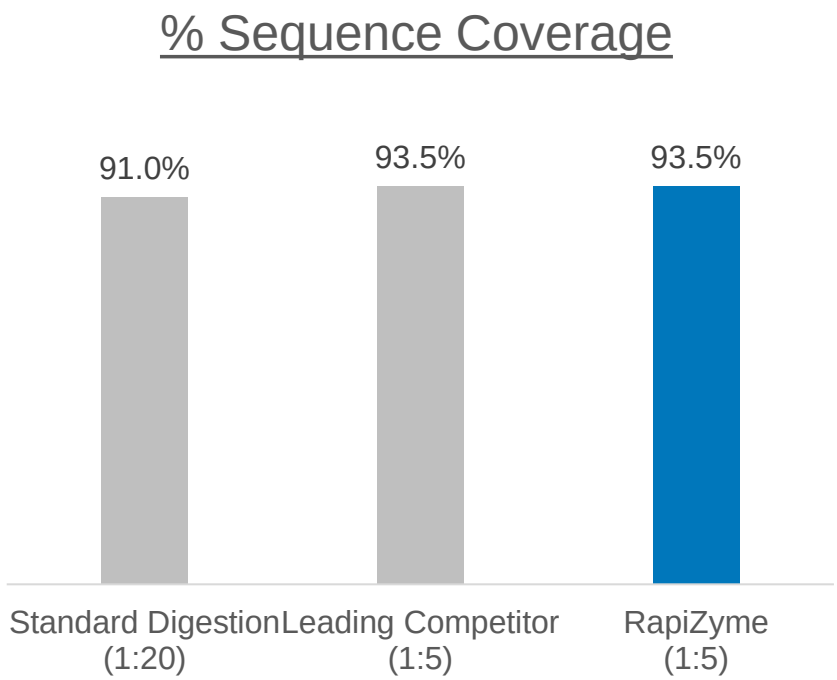


Critical decisions are made distinguishing the smallest changes in a peptide map. Proceed quickly and confidently with **exquisite baselines** free from noise

1. % total TIC area associated with autolysis, unmatched, non-specific, and missed cleavage peak area. N=6 digestion replicates, 2 batches of each enzyme, 3 digestion replicates per batch. Chromatogram is of 1:5 Enzyme:Protein ratio digestions of Remicade, 30 minutes, 37 °C, pH 7.5.

COMPLETE | Need to ensure full identification and characterization?

Deliver **confident** *protein identification* **in efficient 30-minute** *digestions, while* **maintaining high-sequence coverage**



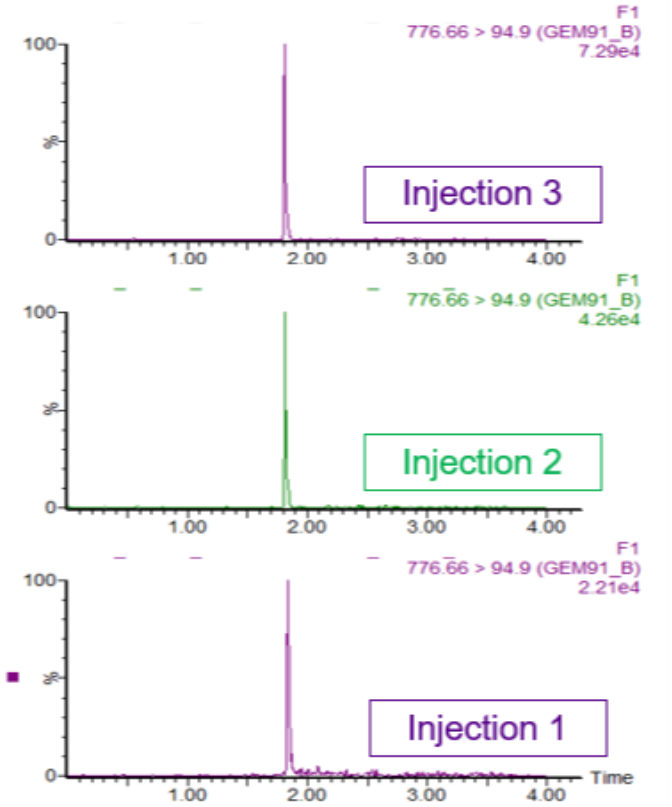
1:5, 30 Minutes Digestions: Remicade™ digestion. Average of n=6 per enzyme, 2 batches for each enzyme, 3 digestion replicates per batch, 37 °C, pH 7.5

Standard Digestion: 1:20 enzyme:protein ratio, 3 hours, 37 °C, pH 7.5, leading competitor enzyme, 1 batch, 1 digestion replicate

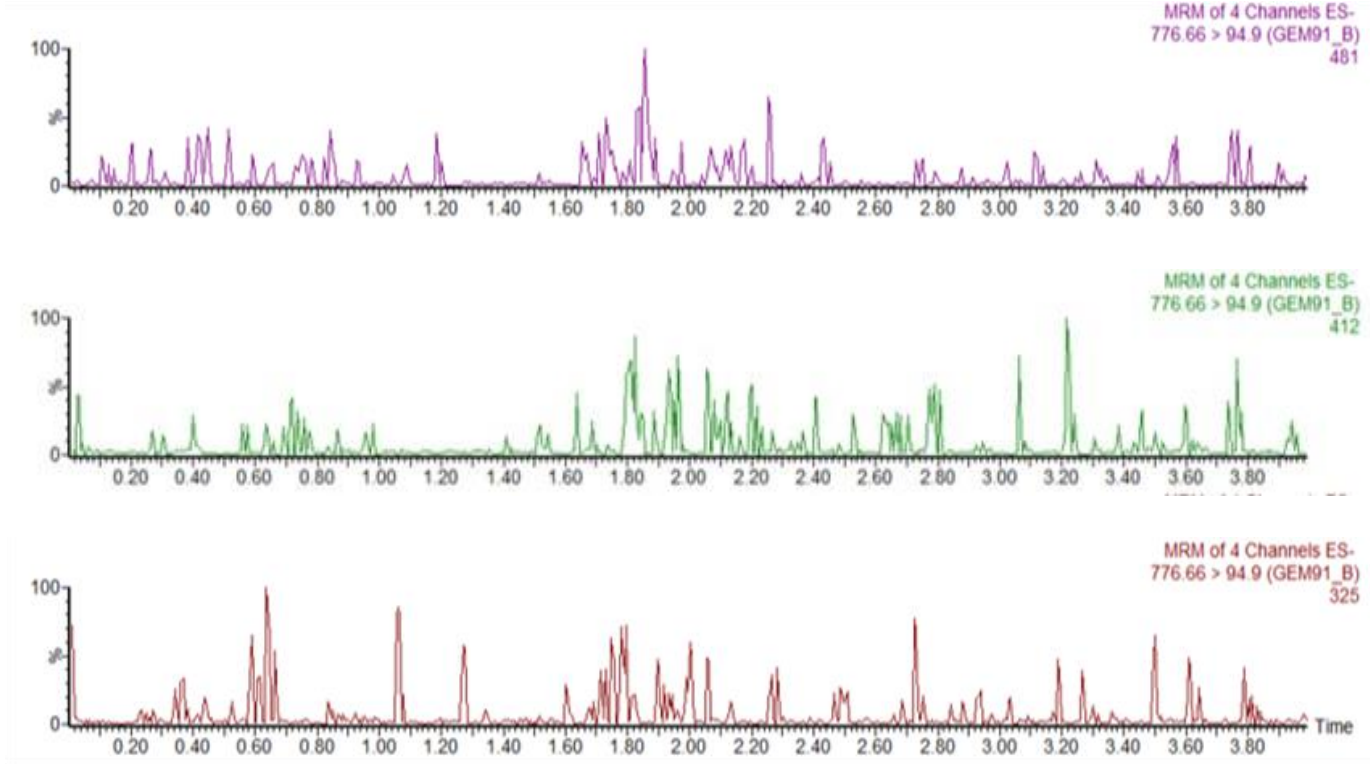
How Can You Ensure Maximum Confidence in Your Results?

What Happens When You DON'T Have Confidence in Your Results?

You inject a sample and expect this….



But see this….



Where is it?

Molecules Can Bind to Surfaces

Non-Specific Binding (NSB), Non-Specific Adsorption (NSA) can be a problem

- Any binding or adsorption that was NOT intended
- Molecules tend to adhere to **any** exposed surfaces
- **Any** chemical interaction can be the source of binding, but especially:
  - Polarity-based interactions (e.g., hydrophobic attraction)
  - Ionic interactions (e.g., coulombic attraction)

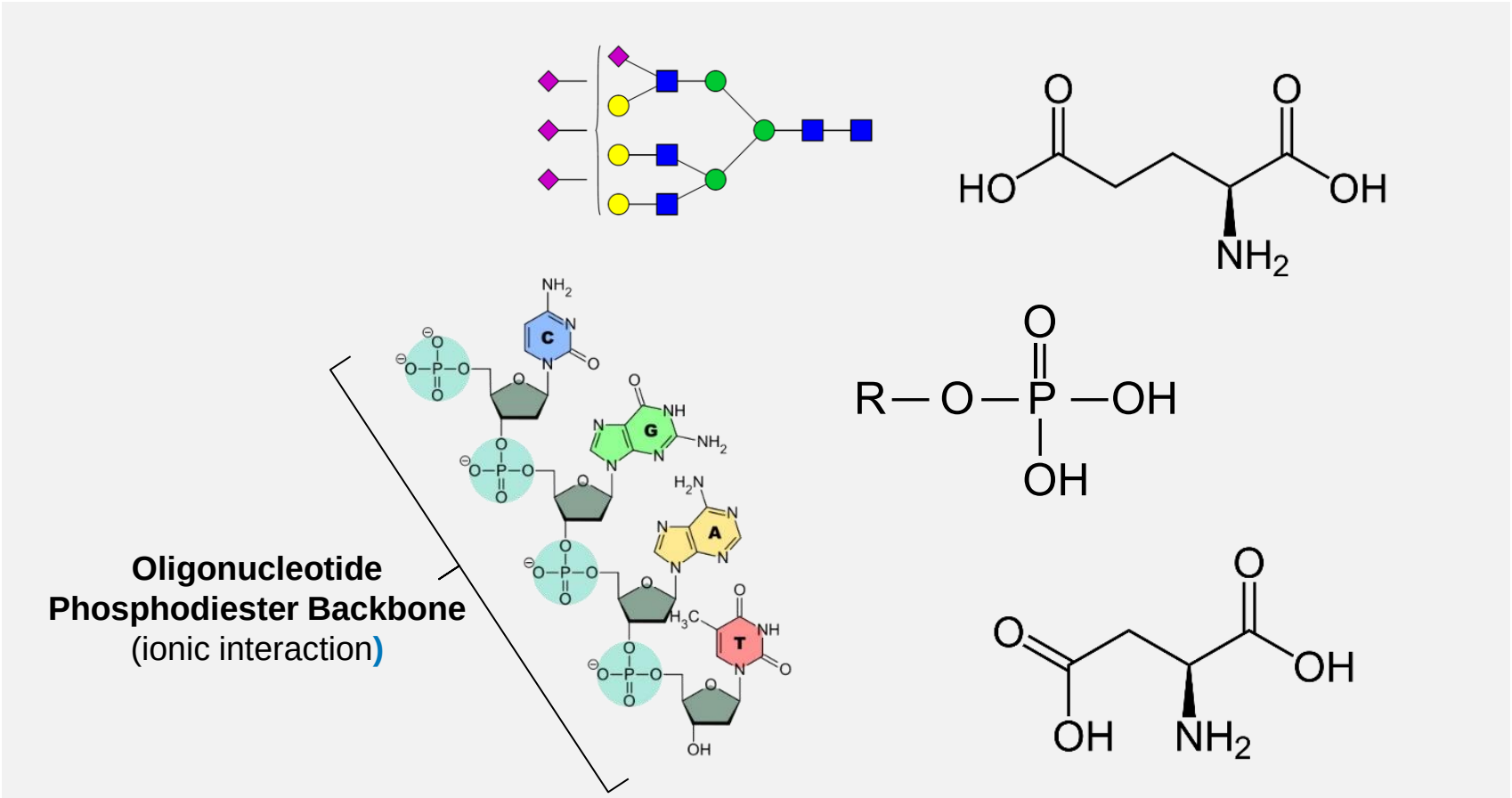
To suppress binding losses of known interactions…

- Avoid interaction between the surface and analytes
- Maintain the environment so that interactions are not strong

Some LC Separations are More Challenging Than Others

Biomolecular Analytes Prone to Metal Interactions

- Acidic & Phosphorylated Peptides & Proteins
- Nucleotides
- Oligonucleotides
- Sialylated glycans
- Glycoproteins…



(Photo Credit : Sponk (talk)/Wikimedia Commons)

A Better Solution from Waters R&D

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MAXPEAK™ HIGH PERFORMANCE SURFACES

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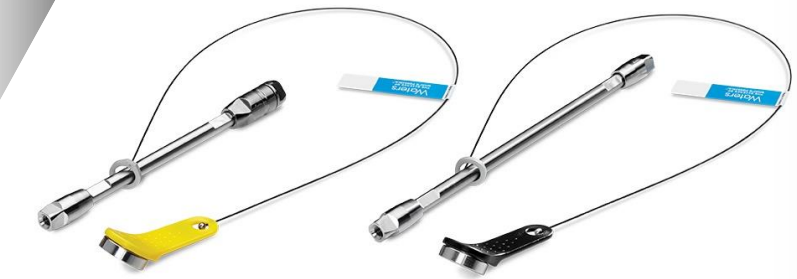
QuanRecovery™ WITH MAXPEAK HPS™

Acquity™ PREMIER

COLUMNS AND SYSTEMS

with MAXPEAK™ HIGH PERFORMANCE SURFACES



Analytical Tools & Solutions for Bioseparations

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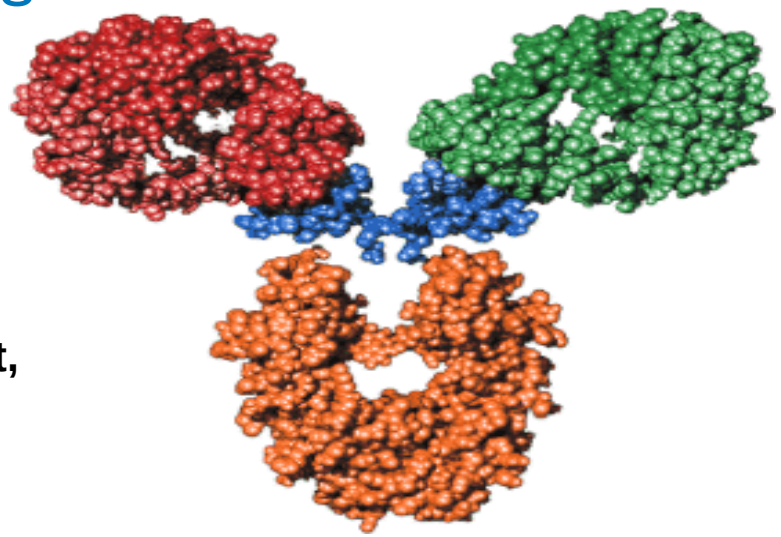
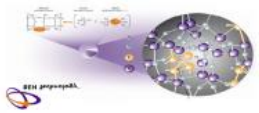
Understanding Primary Structure



Process & Product Impurities
Host Cell Protein
Excipients Analysis (e.g., Polysorbates)

Purity, Content, Glycosylation

Higher Order Structure
HDX-MS
SEC-MALS



Reversed Phase	Peptide Mapping	Amino Acid Analysis	Hydrophobic Interaction	Size Exclusion	Glycan HILIC/ Mixed Mode	Ion Exchange
▪ Separate oxidized, fragmented species ▪ Intact mass ▪ Reduced mAb characterization	▪ mAb Identity ▪ PTM characterization ▪ Disulfides ▪ MAM	▪ Understanding molar absorptivity ▪ Protein concentration ▪ Quantitative AAA	▪ Separate based on hydrophobicity under non denaturing conditions	▪ Separation based on size of molecule ▪ Dimers/aggregation ▪ High molecular weight ▪ Low molecular weight ▪ Clips, SEC-MALS	▪ Glycosylation pattern ▪ Released N-Glycan ▪ Orthogonal methods for intact, subunit, glycopeptide mapping	▪ Separation based on charge ▪ Monitor deamidation, sialylation, pyroglutamate
MAXPEAK™ HIGH PERFORMANCE SURFACES	MAXPEAK™ HIGH PERFORMANCE SURFACES			MAXPEAK™ HIGH PERFORMANCE SURFACES	MAXPEAK™ HIGH PERFORMANCE SURFACES	

More Information and Additional Educational Assets

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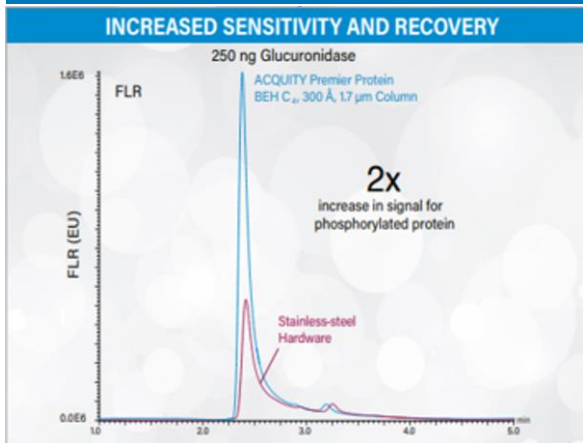
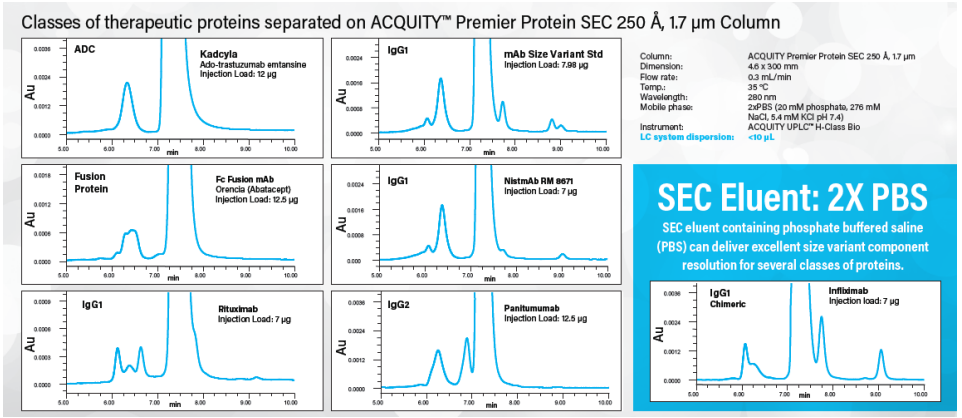
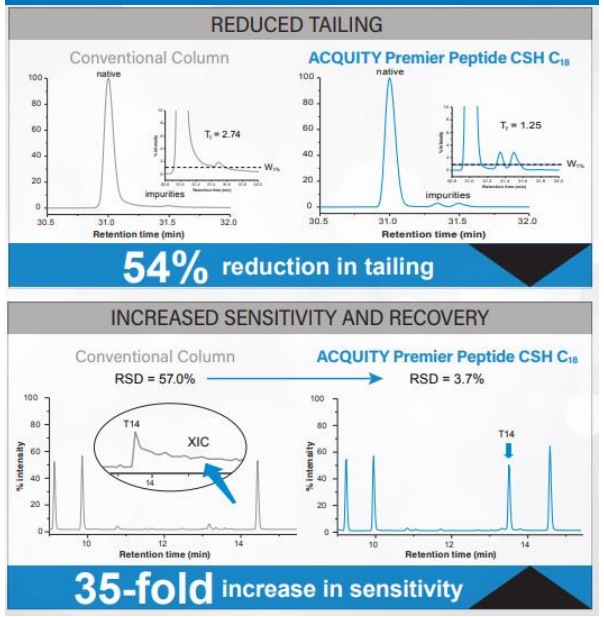
Protein Premier



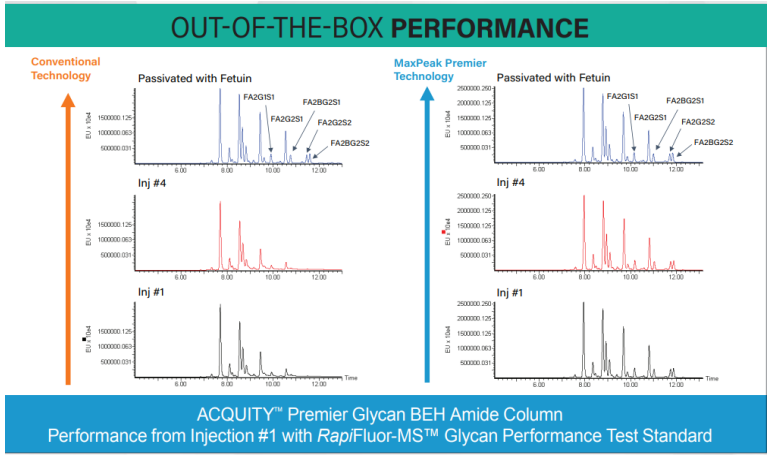
SEC Premier

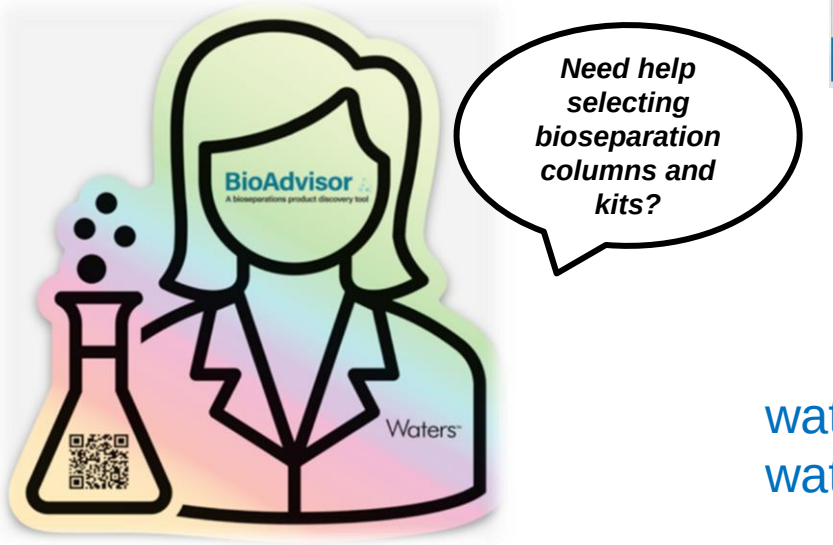


Peptide Premier



Glycan Premier





MAXPEAK™ PREMIER

waters.com/MaxPeakColumns
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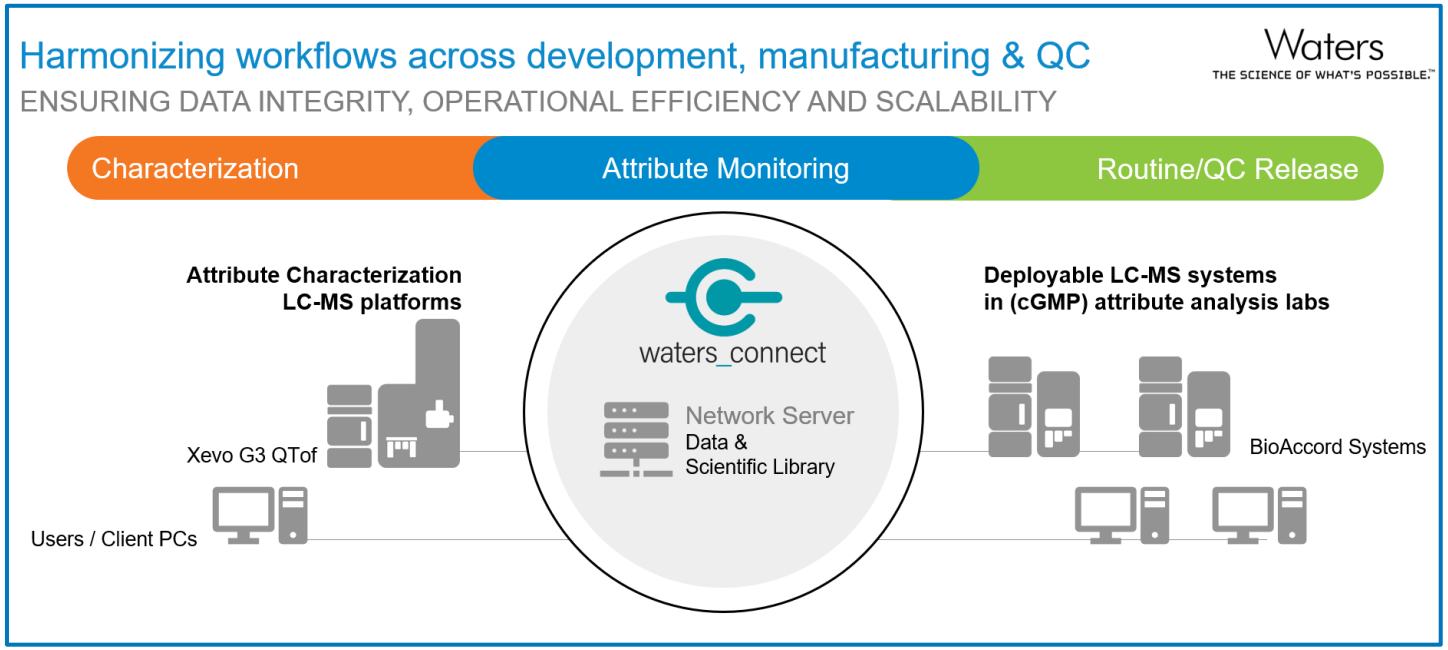
Waters @ WCBP 2023



Booth #1

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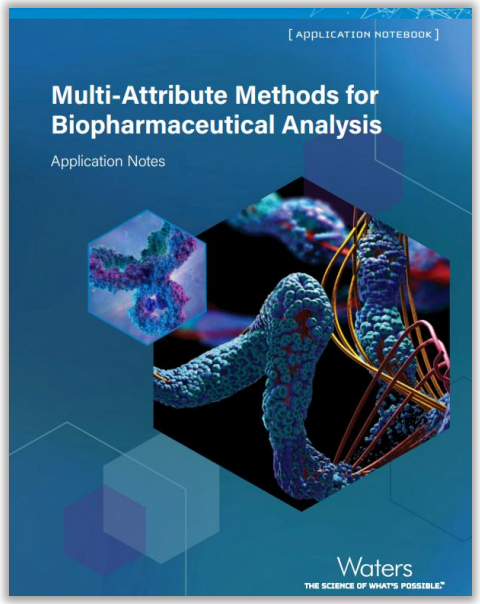
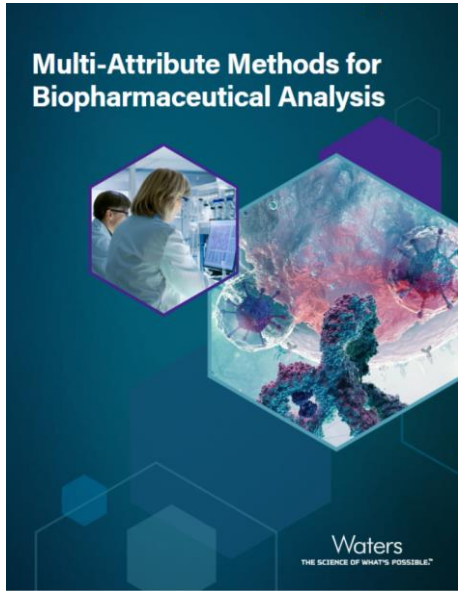
New Literature



MAM Application Notebook
Collation of Waters MAM material
for BioAccord & QDa



MAM eBook
Interviews with
subject matter experts

WCBP Posters

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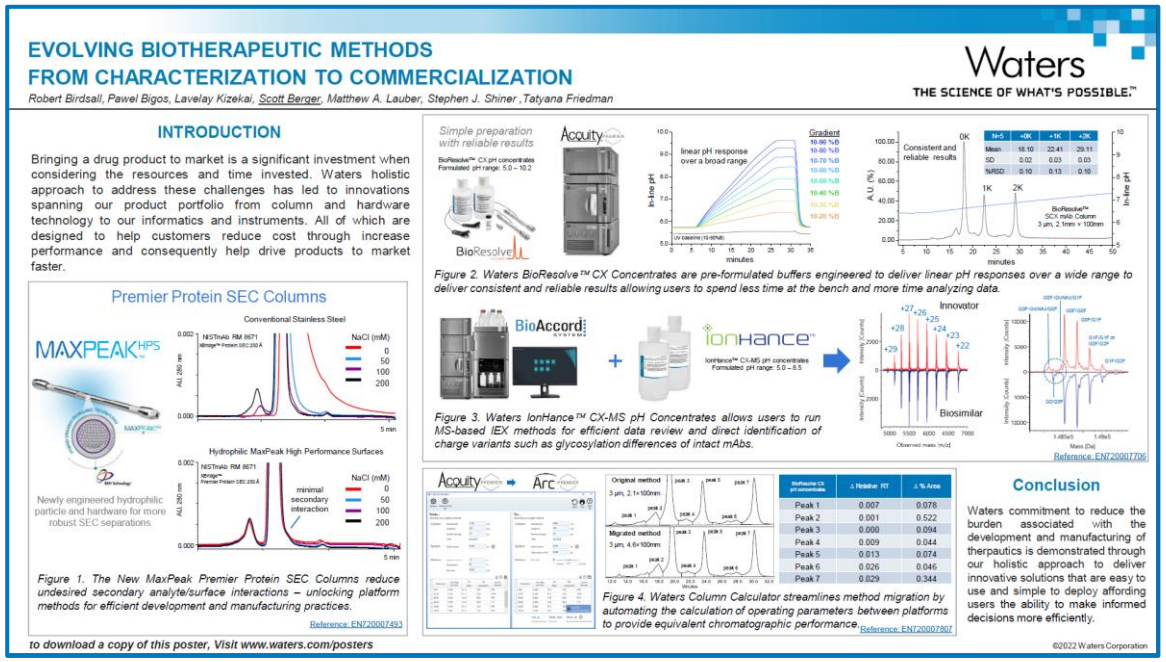

EVOLVING BIOTHERAPEUTIC METHODS
FROM CHARACTERIZATION TO COMMERCIALIZATION

Robert Birdsall, Pawel Bigos, Lavelay Kizekai, Scott Berger, Matthew A. Lauber, Stephen J. Shiner ,Tatyana Friedman

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INTRODUCTION

Bringing a drug product to market is a significant investment when considering the resources and time invested. Waters holistic approach to address these challenges has led to innovations spanning our product portfolio from column and hardware technology to our informatics and instruments. All of which are designed to help customers reduce cost through increase performance and consequently help drive products to market faster.

Premier Protein SEC Columns

Figure 1. The New MaxPeak Premier Protein SEC Columns reduce undesired secondary analyte/surface interactions – unlocking platform methods for efficient development and manufacturing practices.

Reference: ENT20007493

Figure 2. Waters BioResolve™ CX Concentrates are pre-formulated buffers engineered to deliver linear pH responses over a wide range to deliver consistent and reliable results allowing users to spend less time at the bench and more time analyzing data.

Figure 3. Waters IonHance™ CX-MS pH Concentrates allows users to run MS-based IEX methods for efficient data review and direct identification of charge variants such as glycosylation differences of intact mAbs.

Reference: ENT20007706

Figure 4. Waters Column Calculator streamlines method migration by automating the calculation of operating parameters between platforms to provide equivalent chromatographic performance.

Reference: ENT20007807

Conclusion

Waters commitment to reduce the burden associated with the development and manufacturing of therpautics is demonstrated through our holistic approach to deliver innovative solutions that are easy to use and simple to deploy affording users the ability to make informed decisions more efficiently.

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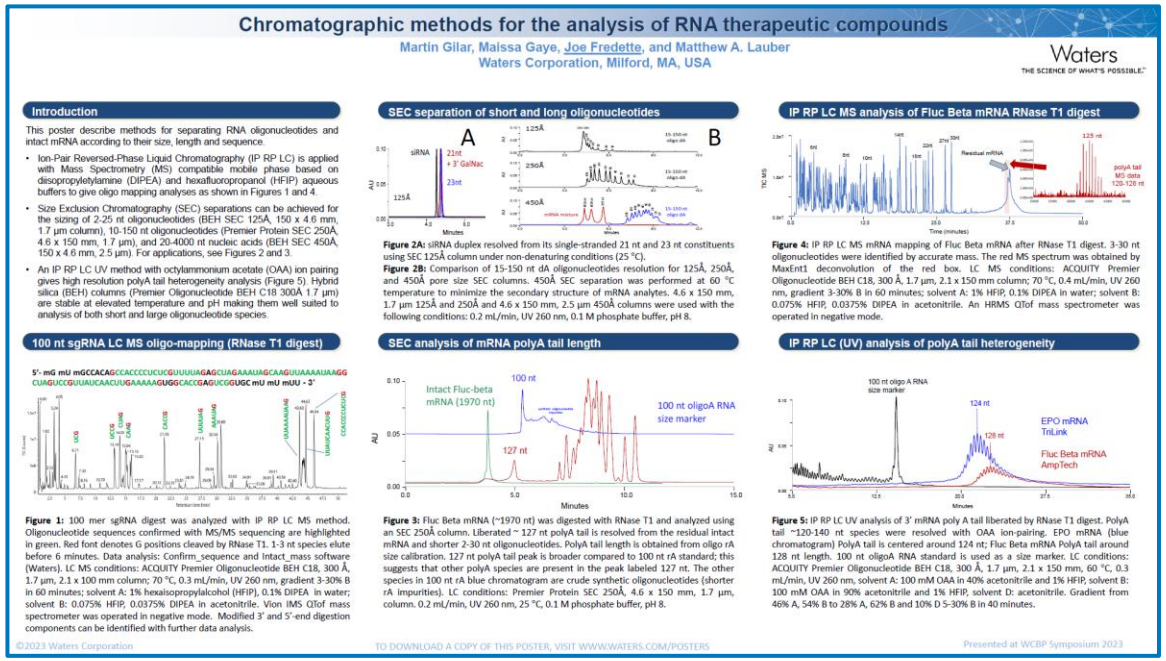
Evolving Biotherapeutic Methods from Characterization to Commercialization;
Robert Birdsall, ***Scott Berger***



Chromatographic methods for the analysis of RNA therapeutic compounds

Martin Gilar, Maissa Gaye, Joe Fredette, and Matthew A. Lauber
Waters Corporation, Milford, MA, USA

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Introduction

This poster describe methods for separating RNA oligonucleotides and intact mRNA according to their size, length and sequence.

- Ion-Pair Reversed-Phase Liquid Chromatography (IP RP LC) is applied with Mass Spectrometry (MS) compatible mobile phase based on diisopropylethylamine (DIPEA) and hexafluoropropanol (HFIP) aqueous buffers to give oligo mapping analyses as shown in Figures 1 and 4.
- Size Exclusion Chromatography (SEC) separations can be achieved for the sizing of 2-25 nt oligonucleotides (BEH SEC 125Å, 150 x 4.6 mm, 1.7 µm column), 10-150 nt oligonucleotides (Premier Protein SEC 250Å, 4.6 x 150 mm, 1.7 µm), and 20-4000 nt nucleic acids (BEH SEC 450Å, 150 x 4.6 mm, 2.5 µm). For applications, see Figures 2 and 3.
- An IP RP LC UV method with octylammonium acetate (OAA) ion pairing gives high resolution polyA tail heterogeneity analysis (Figure 5). Hybrid silica (BEH) columns (Premier Oligonucleotide BEH C18 300Å 1.7 µm) are stable at elevated temperature and pH making them well suited to analysis of both short and large oligonucleotide species.

SEC separation of short and long oligonucleotides

Figure 2A: siRNA duplex resolved from its single-stranded 21 nt and 23 nt constituents using SEC 125Å column under non-denaturing conditions (25 °C).
Figure 2B: Comparison of 15-150 nt dA oligonucleotides resolution for 125Å, 250Å, and 450Å pore size SEC columns. 450Å SEC separation was performed at 60 °C temperature to minimize the secondary structure of mRNA analytes. 4.6 x 150 mm, 1.7 µm 125Å and 250Å and 4.6 x 150 mm, 2.5 µm 450Å columns were used with the following conditions: 0.2 mL/min, UV 260 nm, 0.1 M phosphate buffer, pH 8.

IP RP LC MS analysis of Fluc Beta mRNA RNase T1 digest

Figure 4: IP RP LC MS mRNA mapping of Fluc Beta mRNA after RNase T1 digest. 3-30 nt oligonucleotides were identified by accurate mass. The red MS spectrum was obtained by MaxEnt1 deconvolution of the red box. LC MS conditions: ACQUITY Premier Oligonucleotide BEH C18, 300 Å, 1.7 µm, 2.1 x 150 mm column; 70 °C, 0.4 mL/min, UV 260 nm, gradient 3-30% B in 60 minutes; solvent A: 1% HFIP, 0.1% DIPEA in water; solvent B: 0.075% HFIP, 0.0375% DIPEA in acetonitrile. An HRMS QTof mass spectrometer was operated in negative mode.

100 nt sgRNA LC MS oligo-mapping (RNase T1 digest)

Figure 1: 100 mer sgRNA digest was analyzed with IP RP LC MS method. Oligonucleotide sequences confirmed with MS/MS sequencing are highlighted in green. Red font denotes G positions cleaved by RNase T1. 1-3 nt species elute before 6 minutes. Data analysis: Confirm_sequence and Intact_mass software (Waters). LC MS conditions: ACQUITY Premier Oligonucleotide BEH C18, 300 Å, 1.7 µm, 2.1 x 100 mm column; 70 °C, 0.3 mL/min, UV 260 nm, gradient 3-30% B in 60 minutes; solvent A: 1% hexaisopropylalcohol (HFIP), 0.1% DIPEA in water; solvent B: 0.075% HFIP, 0.0375% DIPEA in acetonitrile. Vion IMS QTof mass spectrometer was operated in negative mode. Modified 3' and 5'-end digestion components can be identified with further data analysis.

SEC analysis of mRNA polyA tail length

Figure 3: Fluc Beta mRNA (~1970 nt) was digested with RNase T1 and analyzed using an SEC 250Å column. Liberated ~ 127 nt polyA tail is resolved from the residual intact mRNA and shorter 2-30 nt oligonucleotides. PolyA tail length is obtained from oligo rA size calibration. 127 nt polyA tail peak is broader compared to 100 nt rA standard; this suggests that other polyA species are present in the peak labeled 127 nt. The other species in 100 nt rA blue chromatogram are crude synthetic oligonucleotides (shorter rA impurities). LC conditions: Premier Protein SEC 250Å, 4.6 x 150 mm, 1.7 µm, column. 0.2 mL/min, UV 260 nm, 25 °C, 0.1 M phosphate buffer, pH 8.

IP RP LC (UV) analysis of polyA tail heterogeneity

Figure 5: IP RP LC UV analysis of 3' mRNA poly A tail liberated by RNase T1 digest. PolyA tail ~120-140 nt species were resolved with OAA ion-pairing. EPO mRNA (blue chromatogram) PolyA tail is centered around 124 nt; Fluc Beta mRNA PolyA tail around 128 nt length. 100 nt oligoA RNA standard is used as a size marker. LC conditions: ACQUITY Premier Oligonucleotide BEH C18, 300 Å, 1.7 µm, 2.1 x 150 mm, 60 °C, 0.3 mL/min, UV 260 nm, solvent A: 100 mM OAA in 40% acetonitrile and 1% HFIP, solvent B: 100 mM OAA in 90% acetonitrile and 1% HFIP, solvent D: acetonitrile. Gradient from 46% A, 54% B to 28% A, 62% B and 10% D 5-30% B in 40 minutes.

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Presented at WCBP Symposium 2023

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REGENERON

Implementation of a Qualified HRMS System in the QC Environment

Yuwei (Serena) Wu