

High Throughput Application of High Resolution LC-MS for Upstream and Downstream Biotherapeutics Process Development

Daniella Cohen
Senior Scientist, SystImmune Inc.

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Outline

- SysImmune Introduction
- Overview of our Process Development
- LC-MS to support CLD, Upstream and Downstream Process Development
- Summary

SystImmune Introduction

- Founded in 2014
- Located near Seattle, WA, USA
- Subsidiary of Biokin Pharma headquartered in Chengdu (China)
- 25+ scientific staff
- Discover antibodies through own antibody discovery platform
- Develop multi-specific antibodies with focus in immuno-oncology



Supporting Research and Process Development with State of The Art Mass Spectrometry

Objective:

- Speed up analytical turnaround times
- Reduce high-cost outsourcing of mass spectrometry analyses
- Improve quality of analytical results to meet higher standards
- Flexibility in MS experimental design
- MS results are included in CLD, UPD and DPD data package

Outcome:

- Confirm CQAs of drug candidates
- Confirm expected products
- Automated UNIFI software aids in speeding data analysis
- Support research and process development programs

**Waters XEVO G2 XS QTOF with
UNIFI scientific information system**

The Challenge in Monoclonal Antibody Analytics

Trends that increase the number of mAb samples

Increase Mab candidate #s

Cell culture recovery and screening automation

QbD guidelines require Mab QA monitoring

mAb Workflow

Drug Discovery

- Target identification and validation
- Mab generation

Preclinical Dev.

- Cell line development
- Clone screening
- Clone selection

Clinical Dev.

- Cell culture
- Purification process

Pre-commercialization

- Formulation
- Lot release tests
- Stability studies

Post-commercialization

- Product improvements
- Patents
- Biobetters

mAb analytics

Product titer

Purity/impurities

Product identification

- Intact mass
- Sequence converge

Quality

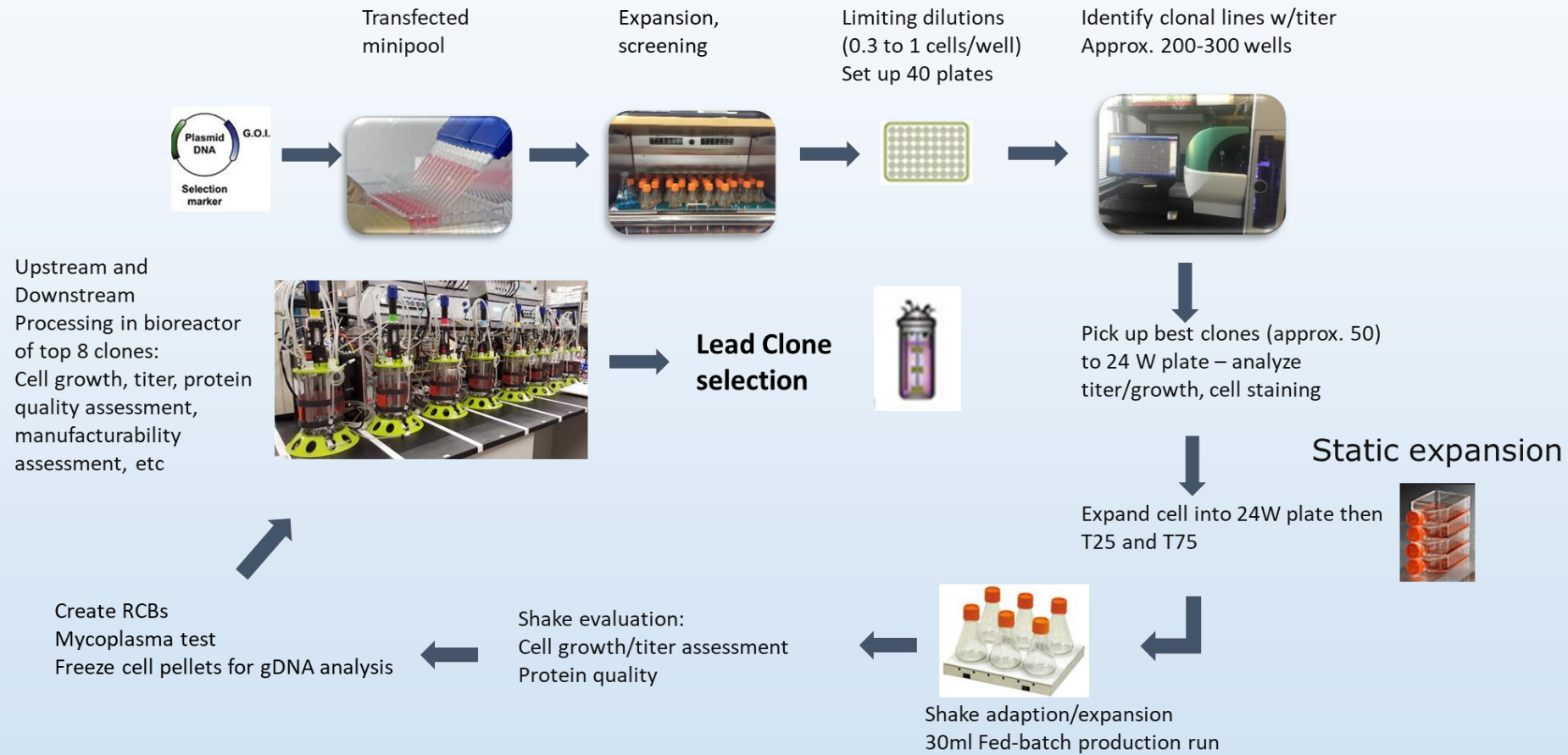
- Charge variation
- Aggregates
- Modifications
- Fragments

DMPK/Metabolite

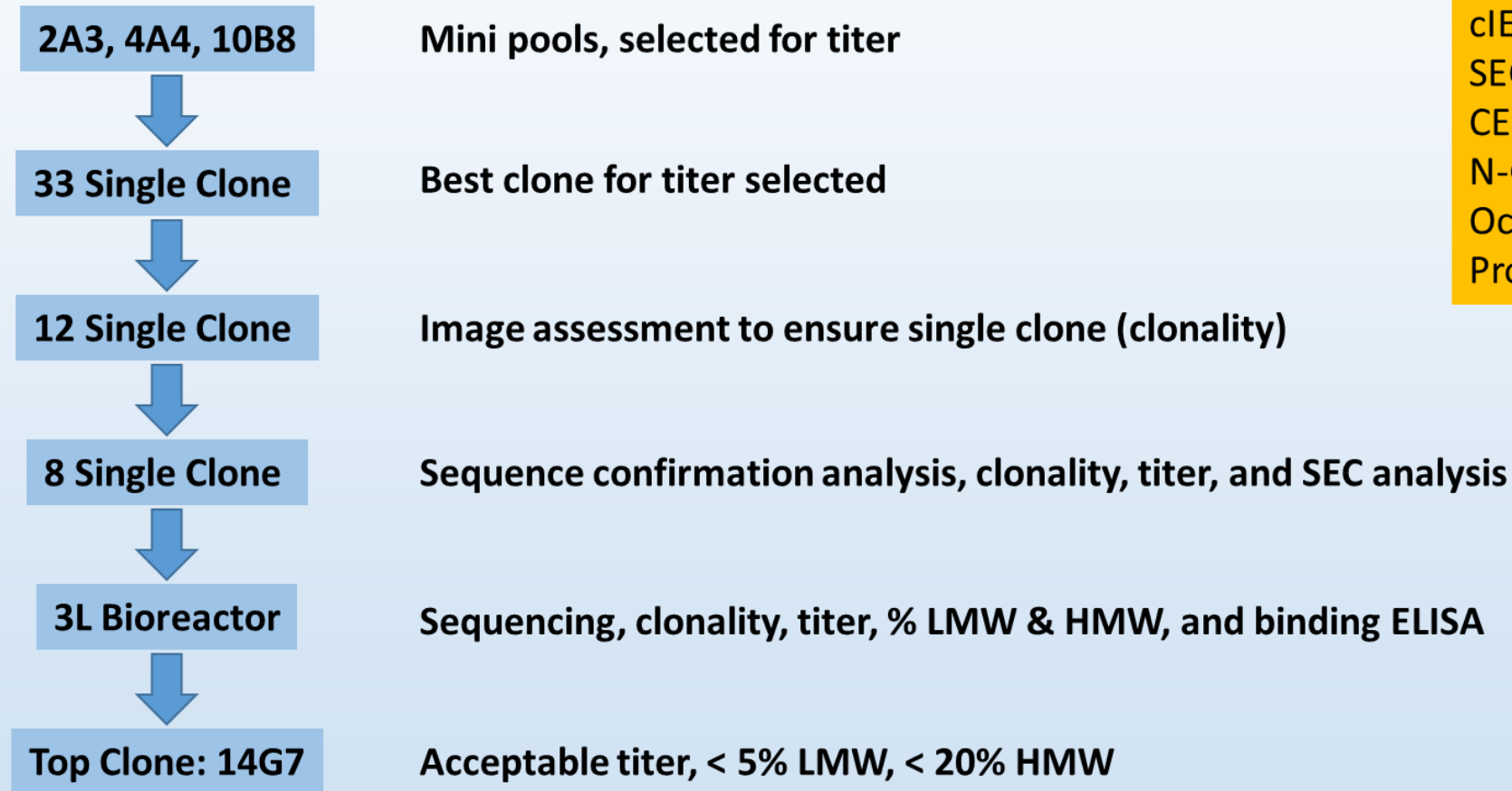
Glycans

Goal of analytical team: speed, throughput and productivity

Overview of Cell Line Development Process



Summary of Cell Line Development & Clone Selection for project X



cIEF / IEX – charge variants
SEC- HMW
CE-SDS – LMW & NG
N-Glycan – Glycosylation
Octet – Binding
Protein Tm - Stability

1. CLD and Upstream Support- by Intact and Reduced LC-MS

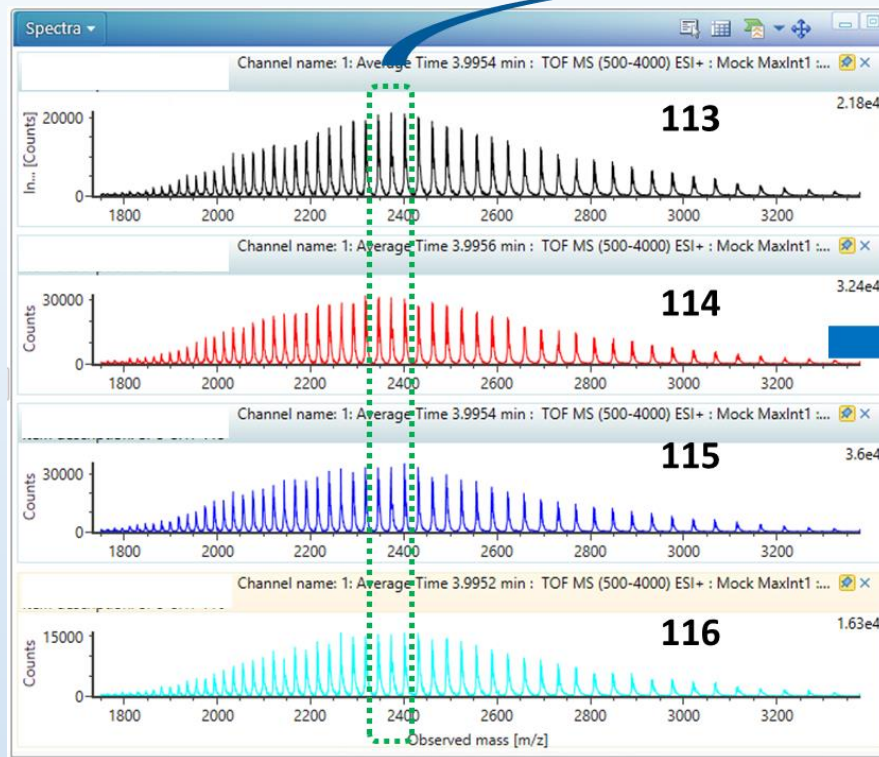
Project X Clone Selection Samples

| Bioreactor # | Single Clone name | Sample Concentration (mg/mL) |
|--------------|-------------------|------------------------------|
| 109 | Clone 1 | 7.22 |
| 110 | Clone 2 | 7.35 |
| 111 | Clone 3 | 6.85 |
| 112 | Clone 4 | 7.25 |
| 113 | Clone 5 | 7.35 |
| 114 | Clone 6 | 7.95 |
| 115 | Clone 7 | 7.96 |
| 116 | Clone 8 | 7.31 |

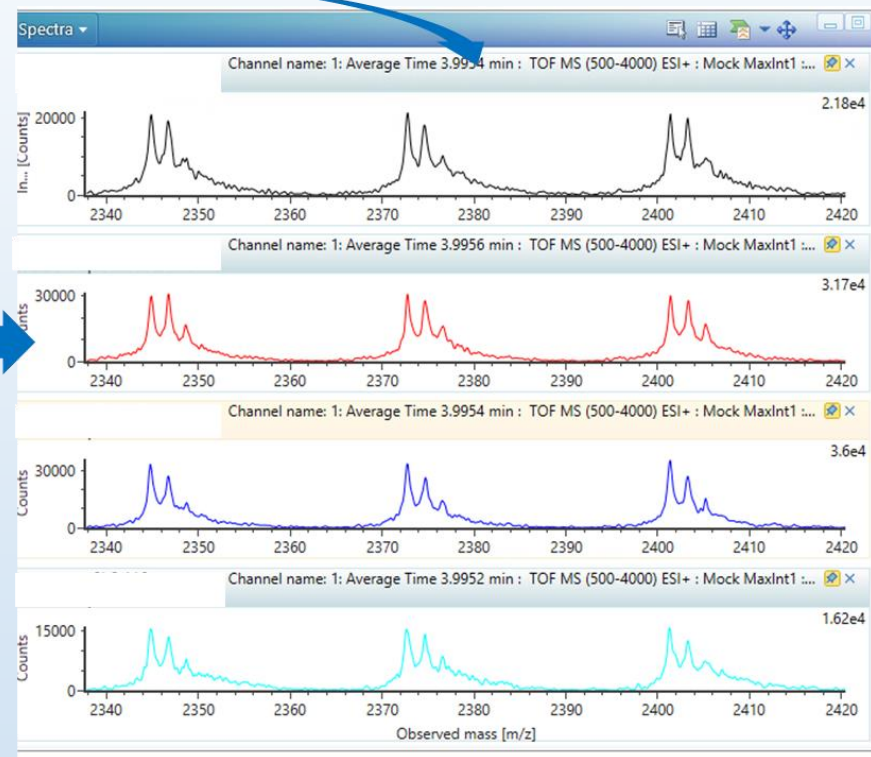
Questions: Clone quality

- **Goal:** Rapid examination of LC and HC masses and comparison of heavy chain glycoforms
- **Method:** Characterize the clone by intact LC-MS experiment and reduced LC-MS
- **All 8 experiments can be prepared, collected, processed, and reported within a single day!**

Intact LC-MS Bioreactors 113-114

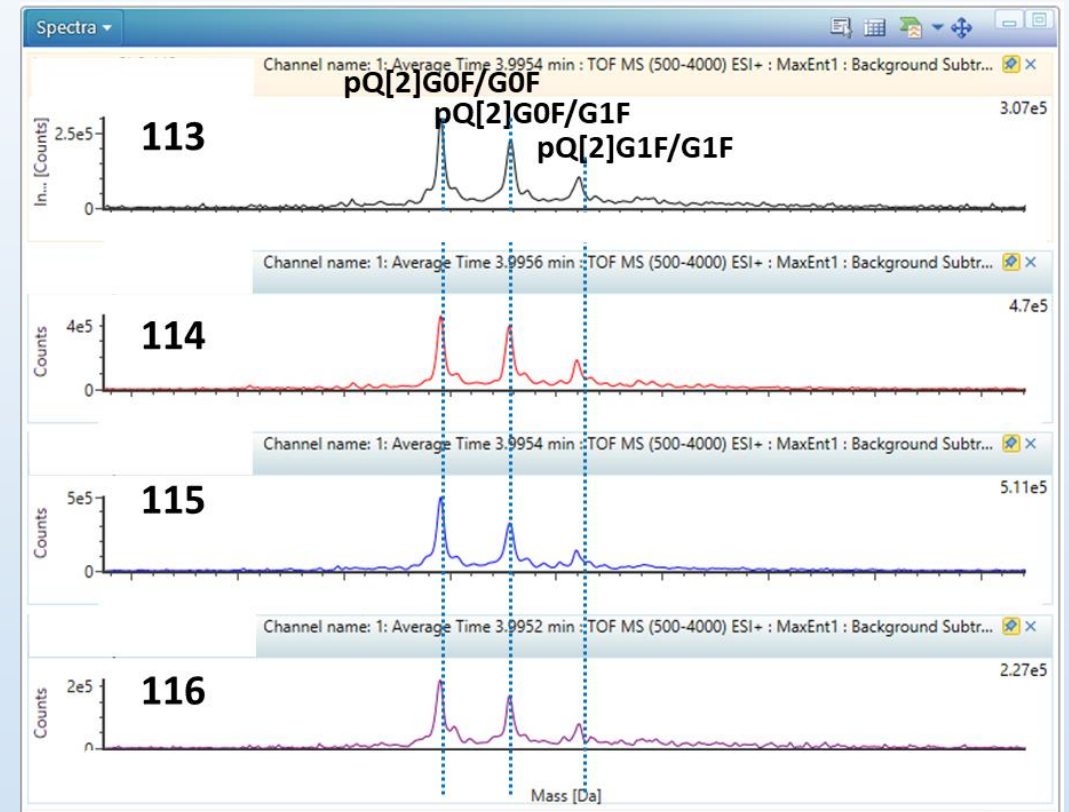
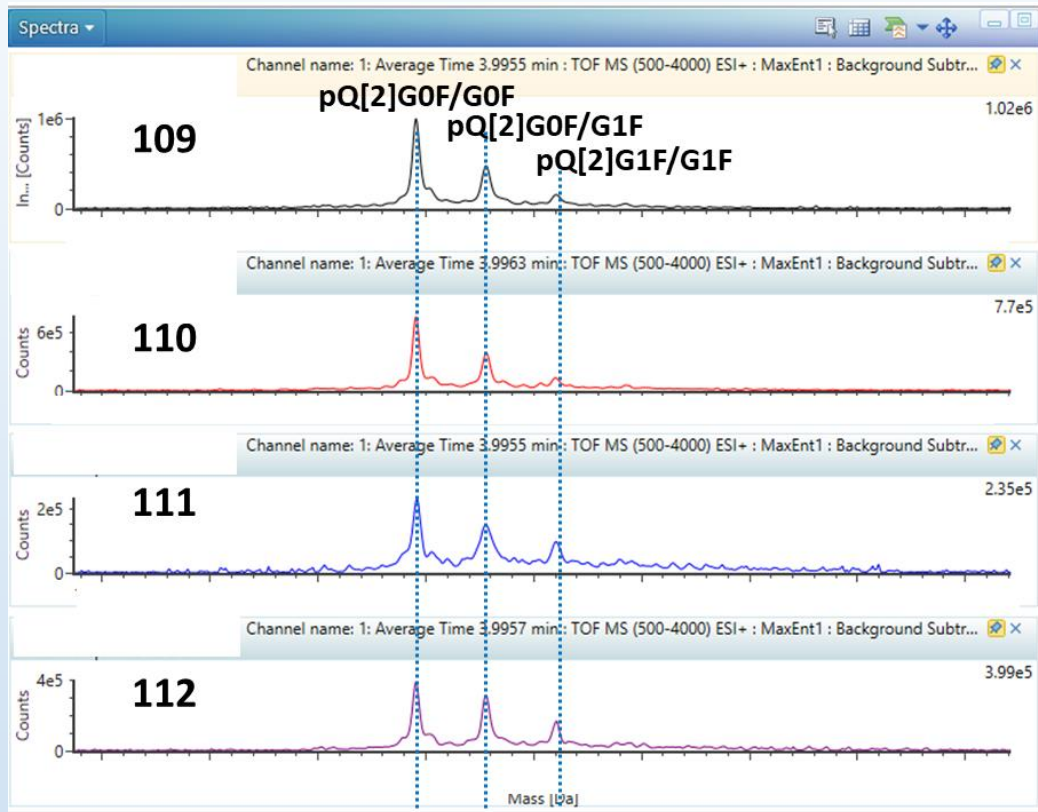


**Summed MS
(Combined MS Spectrum)**



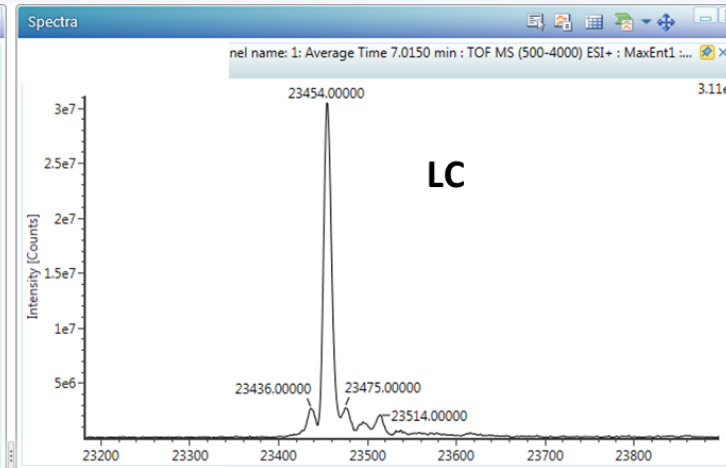
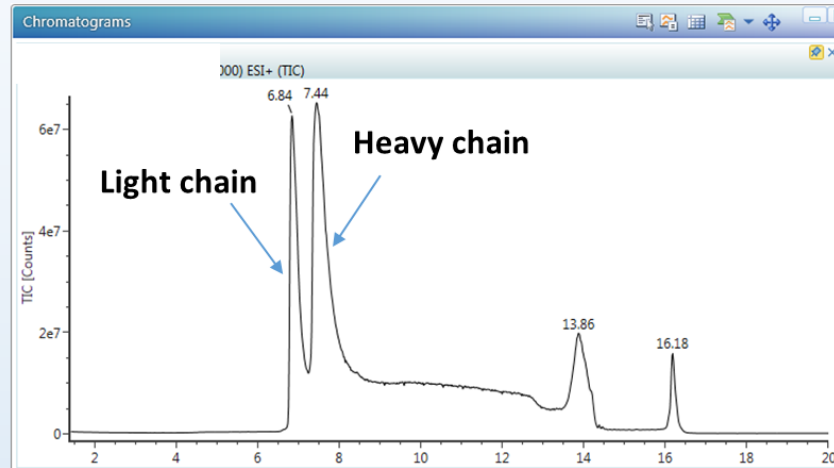
**Summed MS
(Enhanced View)**

Comparison of 8 Bioreactors - Deconvoluted Masses



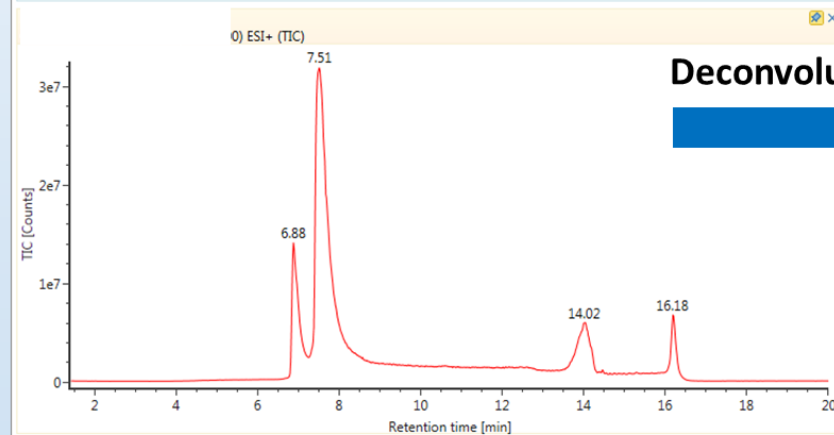
Comparison of Light Chain R109 and R110

#109

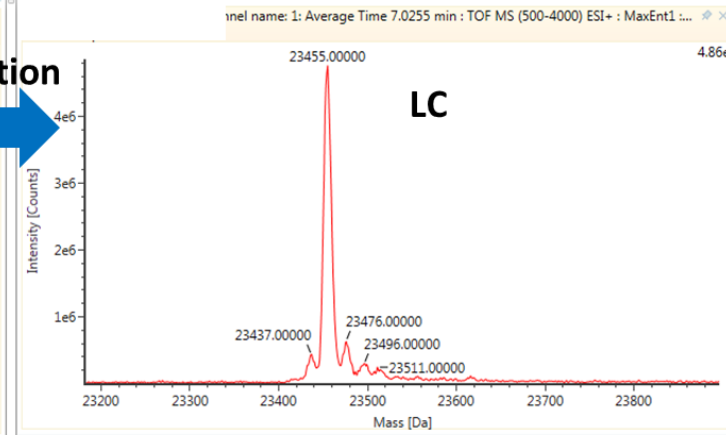


#109

#110

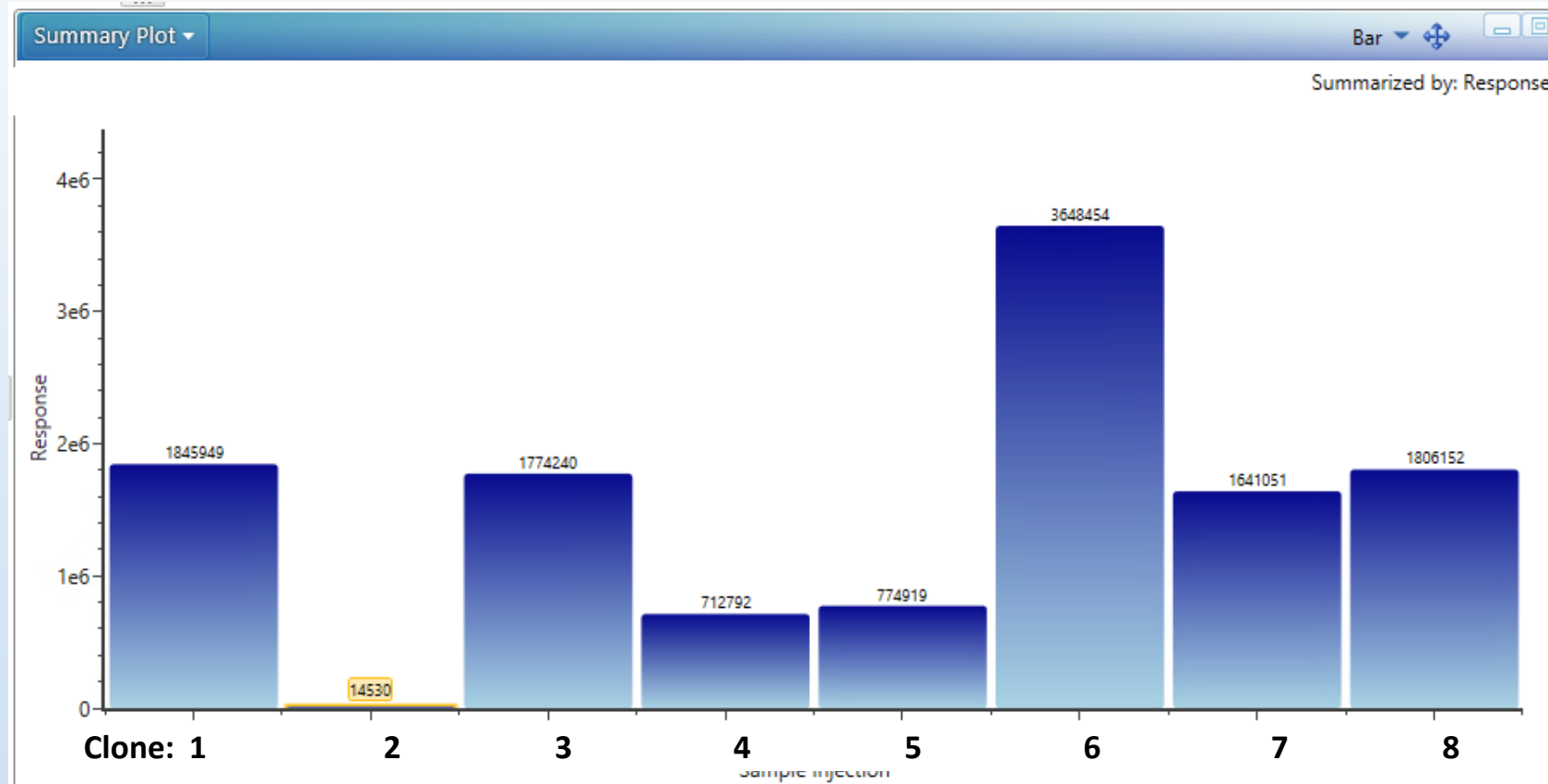


Deconvolution

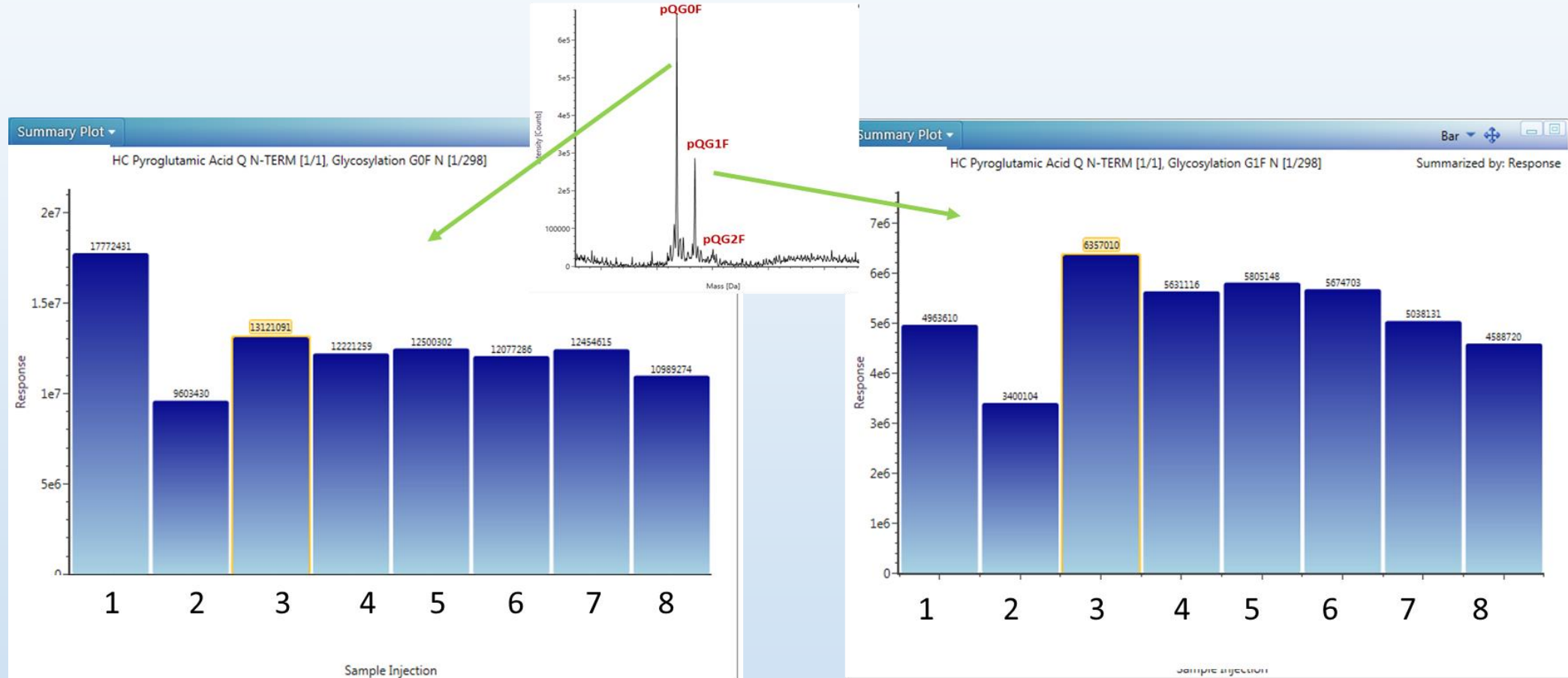


#110

Reduced LC-MS: Component plot summary for LC response

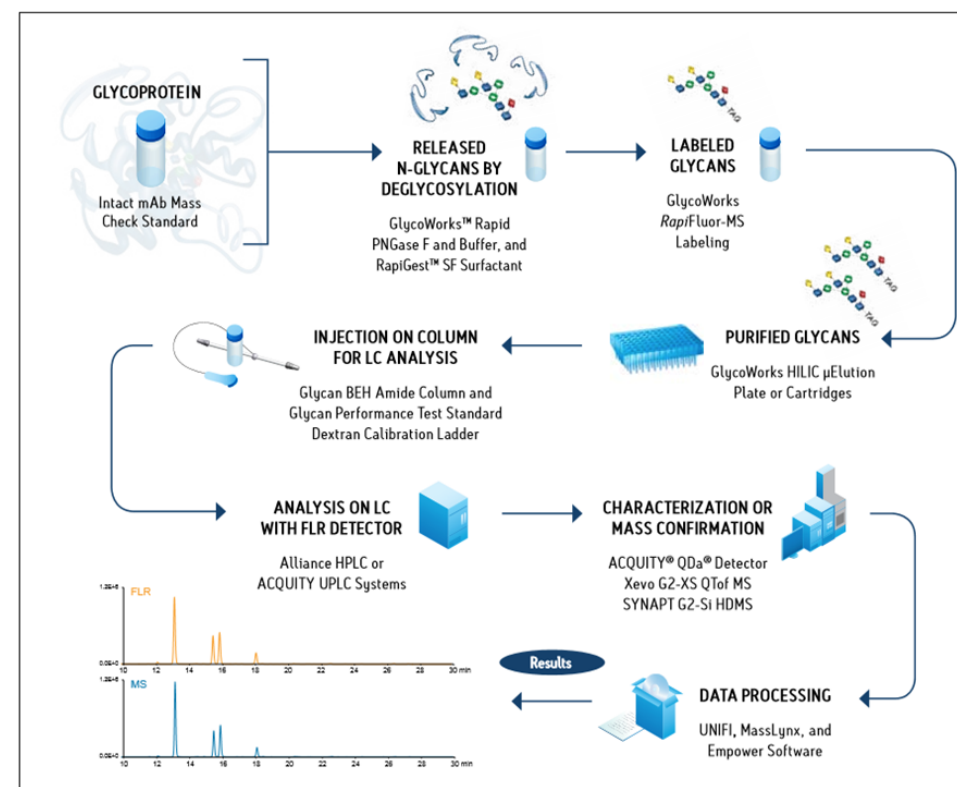
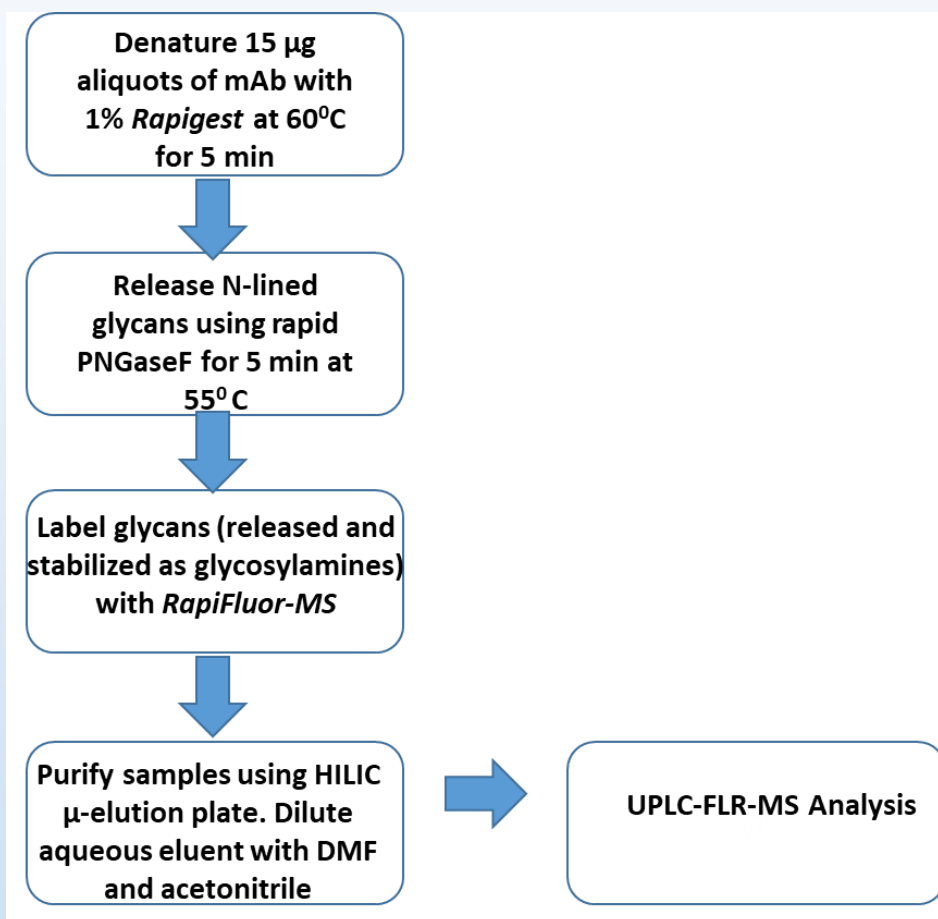


Reduced LC-MS: Component plot summary for pQHC+G0F and pQHC+G1F responses



2. CLD and Upstream support- by released glycan LC-FLR-MS

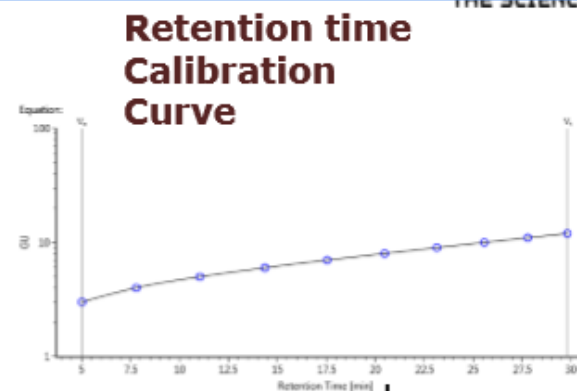
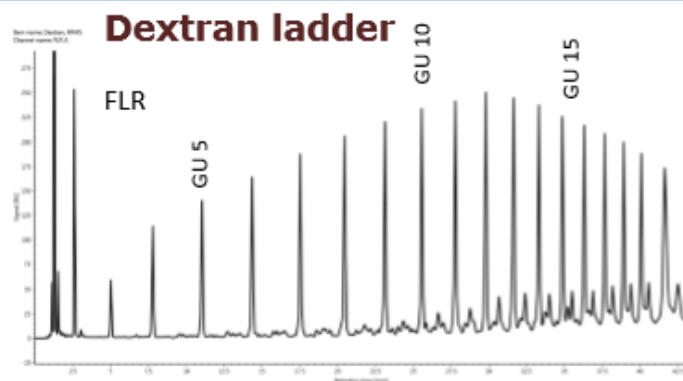
Workflow for the Rapid Preparation of N-glycans Using the RapiFluor-MS N-Glycan Kit



Glycan FLR + MS Confirmation Workflow

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®



Scientific Library Search

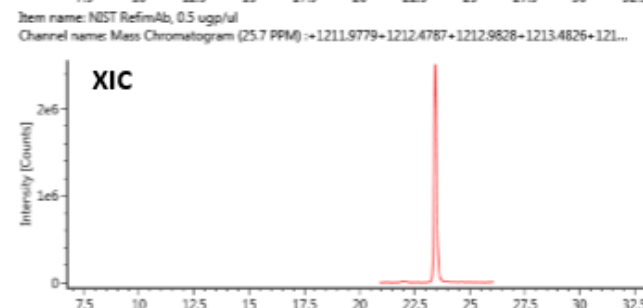
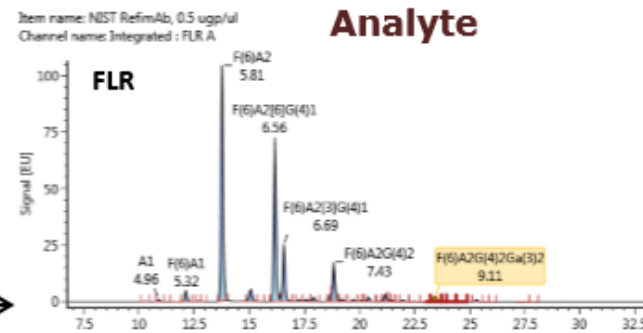
Library Search Results

| Component name | Structure | Expected GU | Δ GU | GU Std Dev | Expected m/z | Δ m/z | Mass Confirmed |
|----------------|-----------|-------------|--------|------------|--------------|-----------|-------------------------------------|
| 1 | | 9.1400 | 0.0268 | 0.0000 | 1109.5226 | -102.0554 | <input type="checkbox"/> |
| 2 | | 9.1733 | 0.0601 | 0.0200 | 1203.4648 | -0.0023 | <input checked="" type="checkbox"/> |
| 3 | | 9.3300 | 0.2168 | 0.0000 | 1211.8724 | -0.0055 | <input checked="" type="checkbox"/> |
| 4 | | 8.8900 | 0.2232 | 0.0000 | 1110.4328 | -103.5452 | <input type="checkbox"/> |

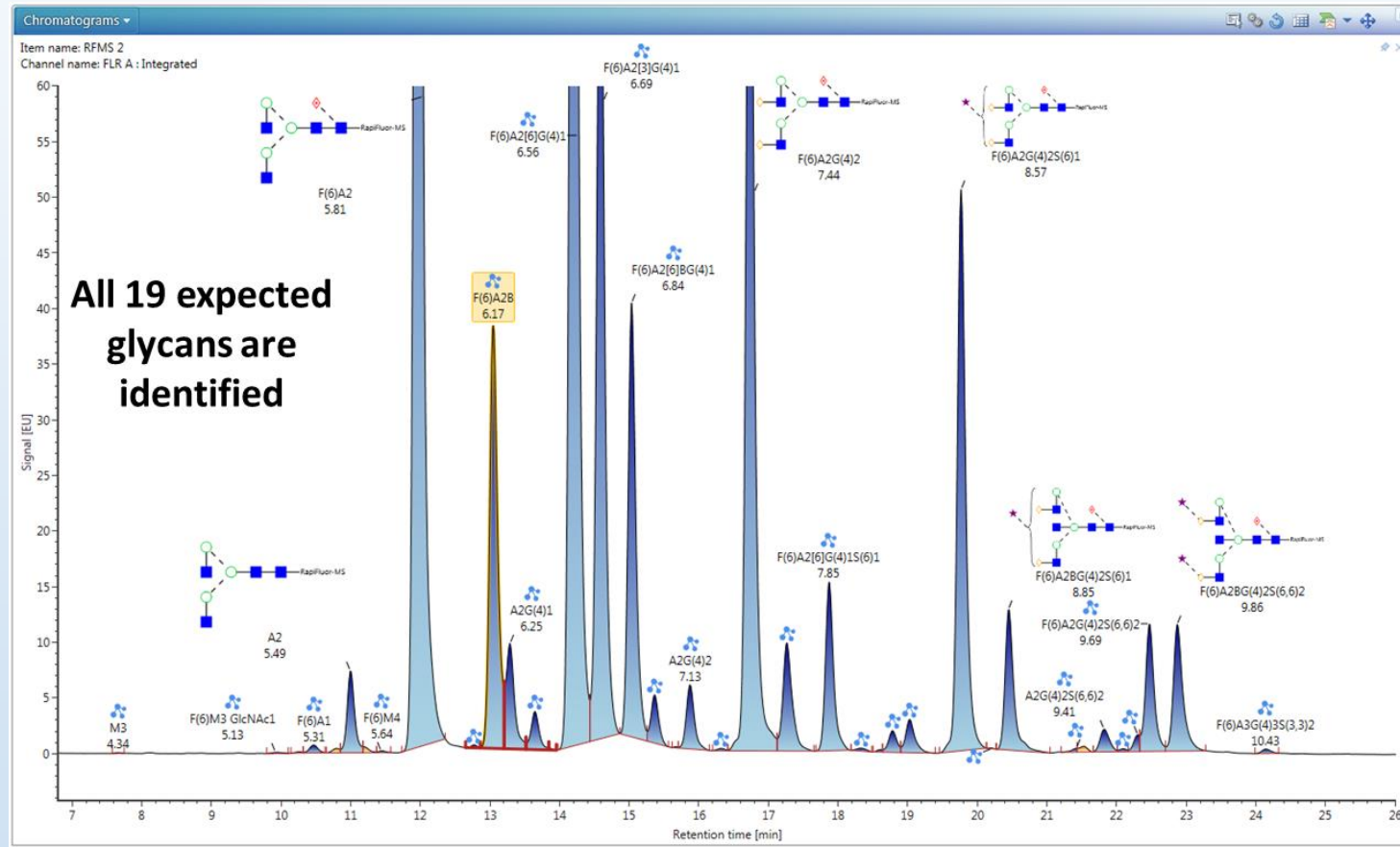
CFG and Oxford structures available.



Analyte



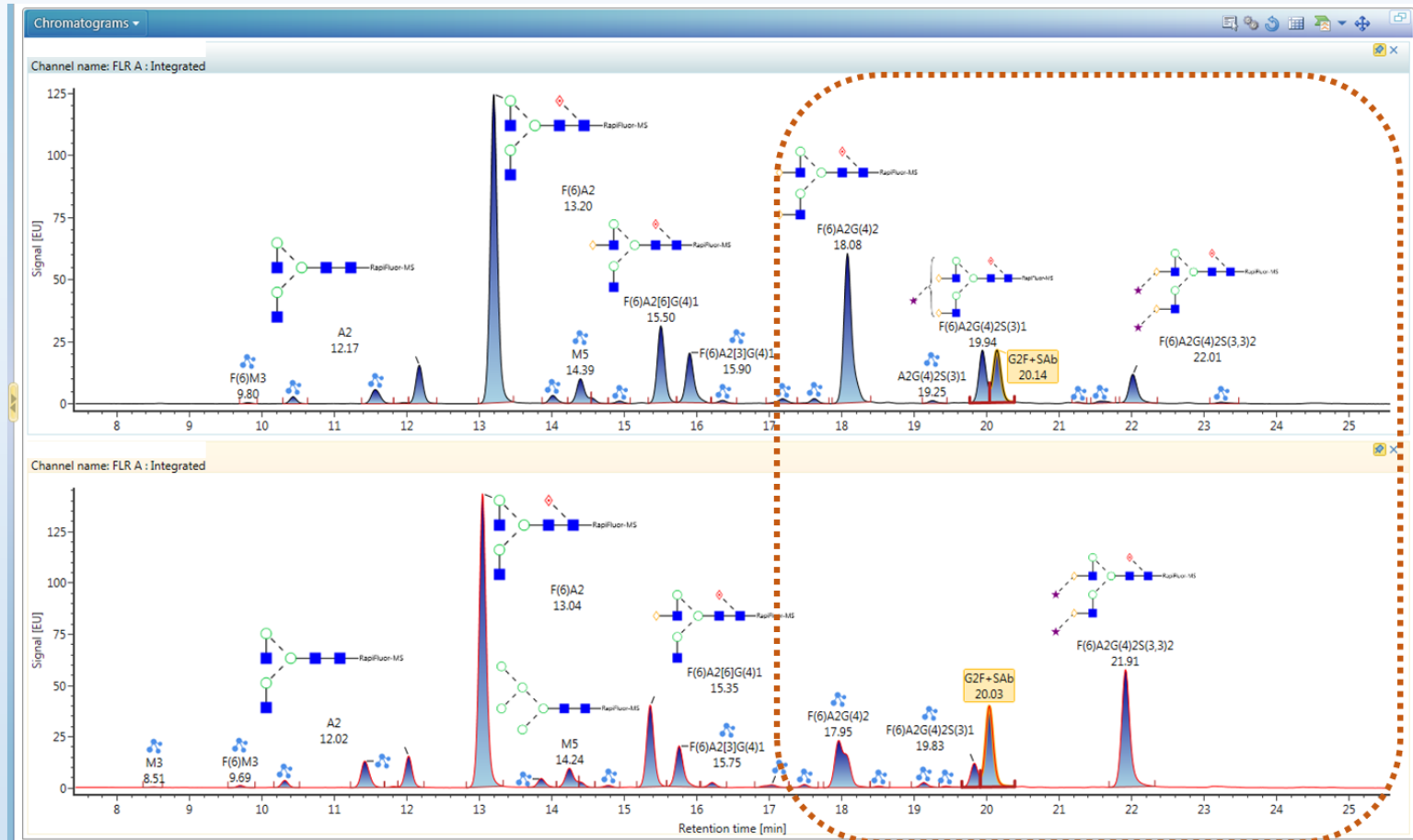
Waters RFMS mAb Identified Glycans using UNIFI



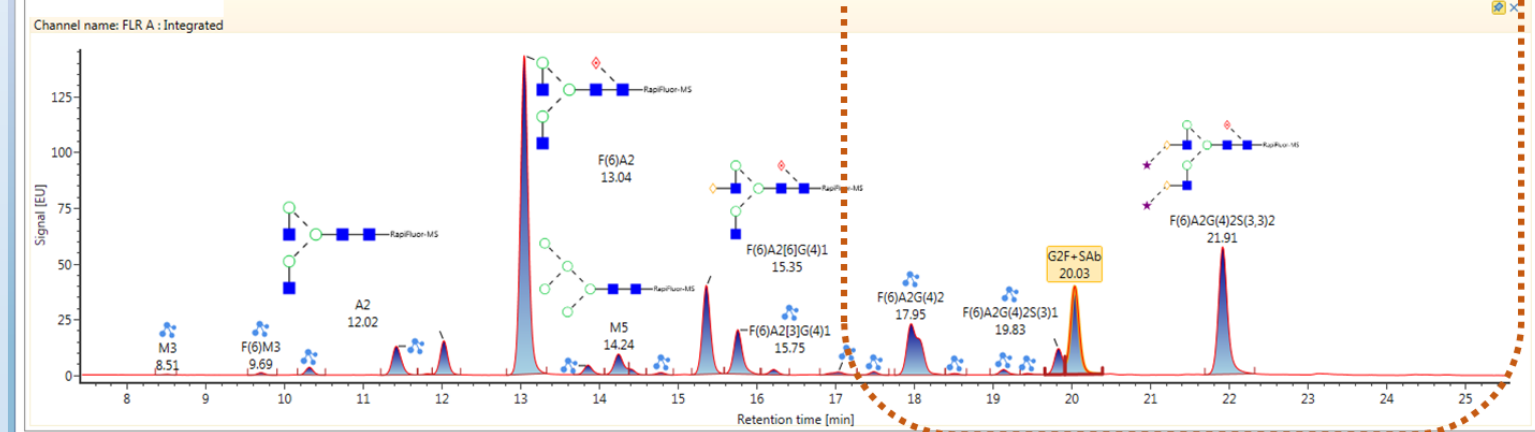
| # | Glycan Oxford | Alternative name |
|----|---------------|------------------|
| 1 | A2 | G0 |
| 2 | FA2 | G0F |
| 3 | FA2B | G0F+GN |
| 4 | A2G1 | G1 |
| 5 | A2G1 | G1 |
| 6 | FA2G1 | G1F |
| 7 | FA2G1 | G1F |
| 8 | FA2BG1 | G1F+GN |
| 9 | FA2BG1 | G1F+GN |
| 10 | A2G2 | G2 |
| 11 | FA2G2 | G2F |
| 12 | FA2BG2 | G2F+GN |
| 13 | FA2G1S1 | G1F+SA |
| 14 | A2G2S1 | G2+SA |
| 15 | FA2G2S1 | G2F+SA |
| 16 | FA2BG2S1 | G2F+GN+SA |
| 17 | A2G2S2 | G2+SA |
| 18 | FA2G2S2 | G2F+2SA |
| 19 | FA2BG2S2 | G2F+GN+2SA |

Comparison Between Two Clones for Project Y

Clone 1



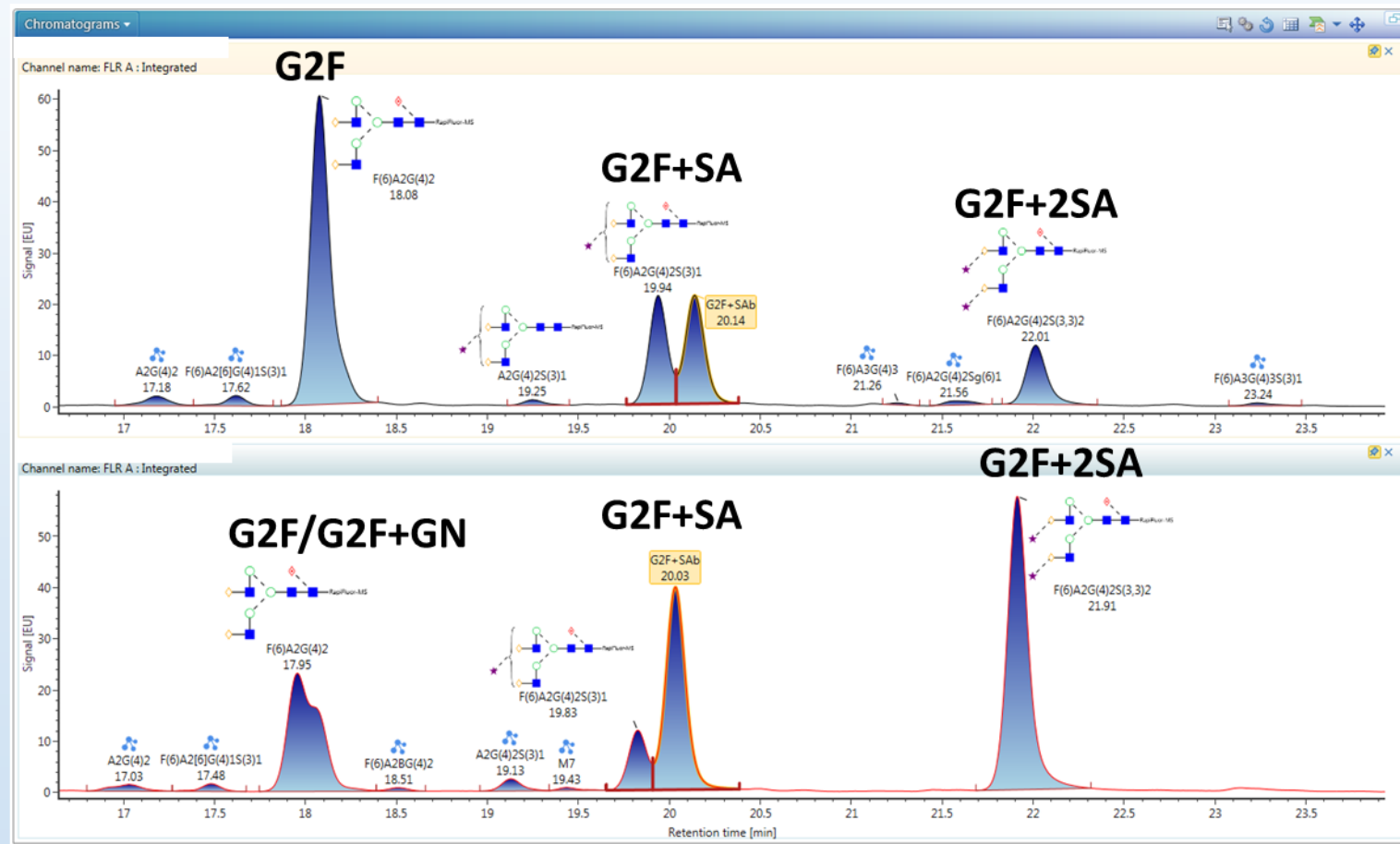
Clone 2



Comparison Between Two Clones for Project Y

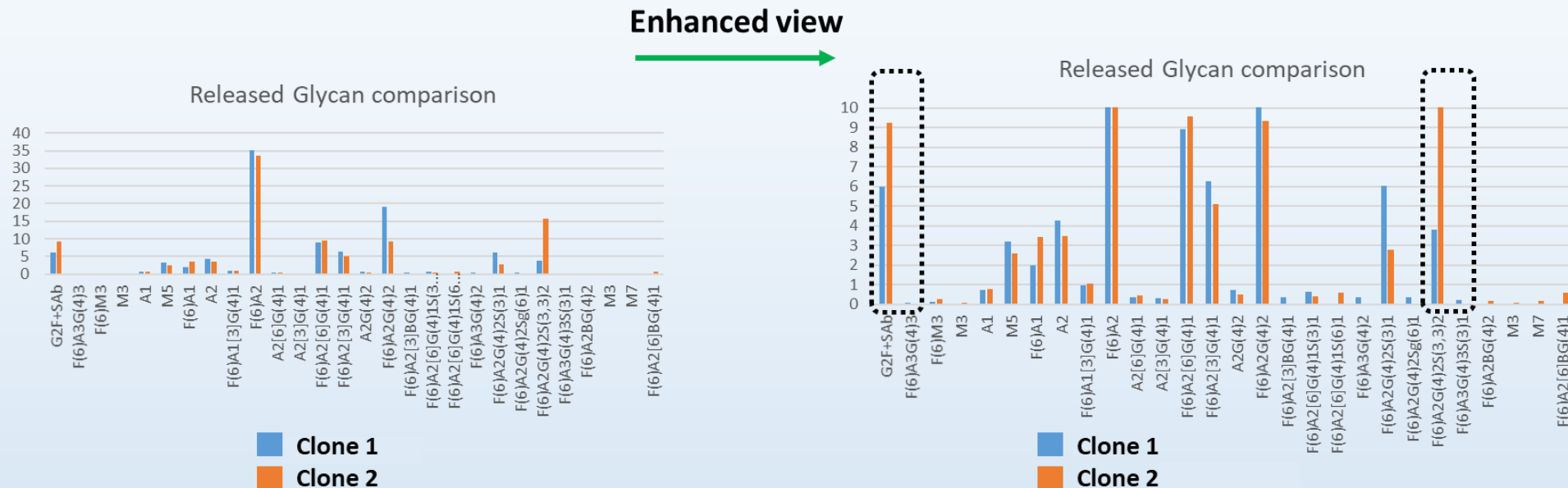
17-23 min

Clone 1



Clone 2

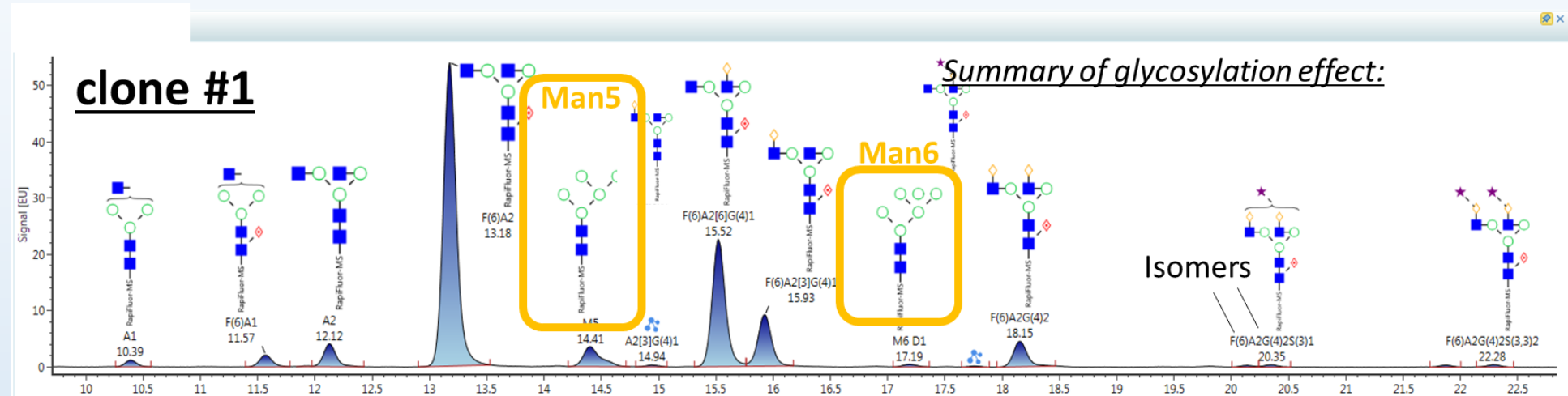
Comparison Between Two Clones for Project Y



Clone 2 expressed increased amounts of sialylated glycans G2F+SA as well as G2F+2SA, G2F+GN and MAN7

| Glycan | Clone 1 | Clone 2 |
|---------|---------|---------|
| G2F+SA | 6.01 | 9.24 |
| G2F+2SA | 3.79 | 15.62 |

Overlay of Project Y Single Clone Selection



Mannose species

High Man effect:

- Enhanced antibody-dependent-cell-mediated-cytotoxicity (ADCC)
- Faster clearance rate

Table II. Impact of Fc glycans on safety/immunogenicity, biologic activity/efficacy and clearance (PK/PD)

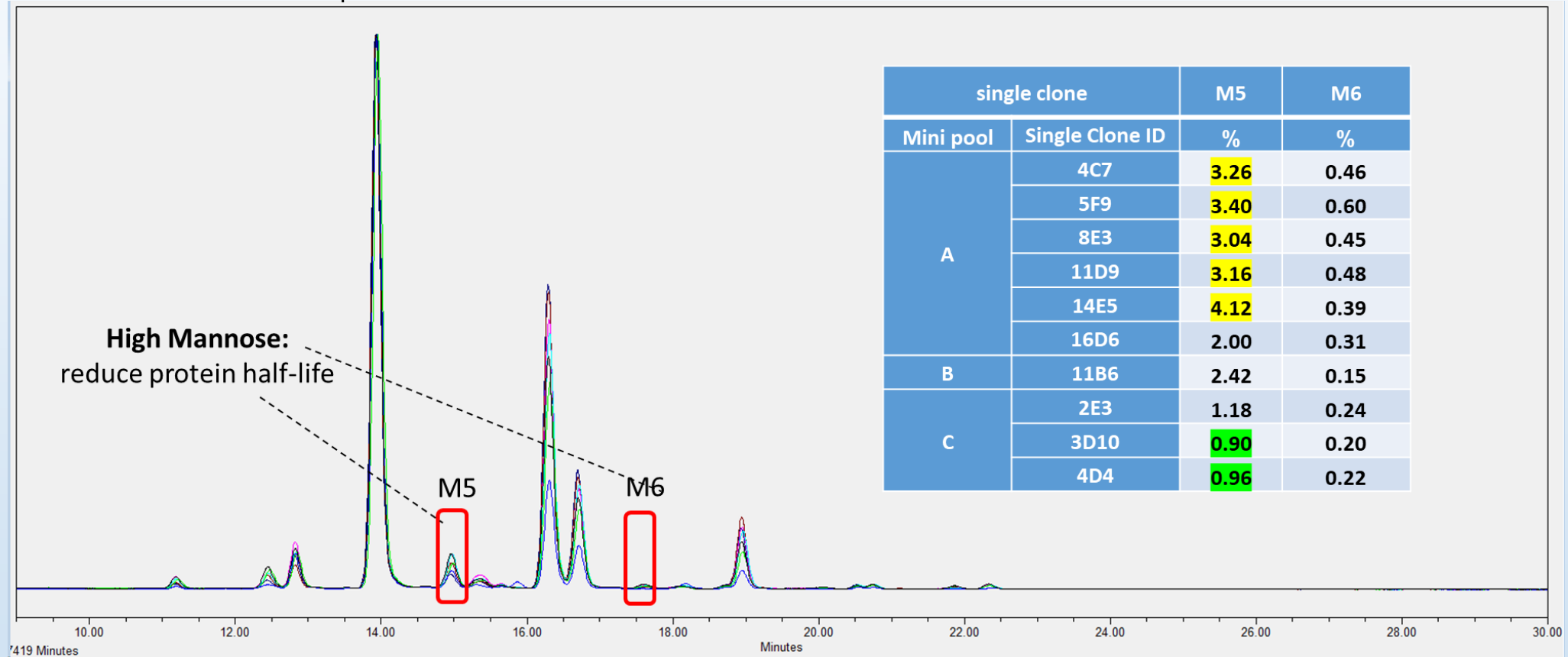
| Glycan species | Safety/immunogenicity | Biologic activity/efficacy | Clearance (PK/PD) |
|---|-----------------------|----------------------------|-------------------|
| Galactose | Unknown | + | Unknown |
| α 1,3-galactose | — | Unknown | Unknown |
| Fucose | (—) | ++ | Unknown |
| Disecting GlcNAc | (—) | + | Unknown |
| High mannose | Unknown | + | — |
| NANA | Unknown | (—) | + |
| NGNA | — | (—) | + |
| β 1,2-Xylose/ α 1,3-Fucose | — | Unknown | Unknown |
| NGHC | Unknown | — | (—) |

+ Positive impact; — negative impact; ++ high positive impact; — high negative impact; (+/—) potential impact.

https://www.researchgate.net/publication/280998644_Fc_glycans_of_therapeutic_antibodies_as_critical_quality_attributes_CQAs

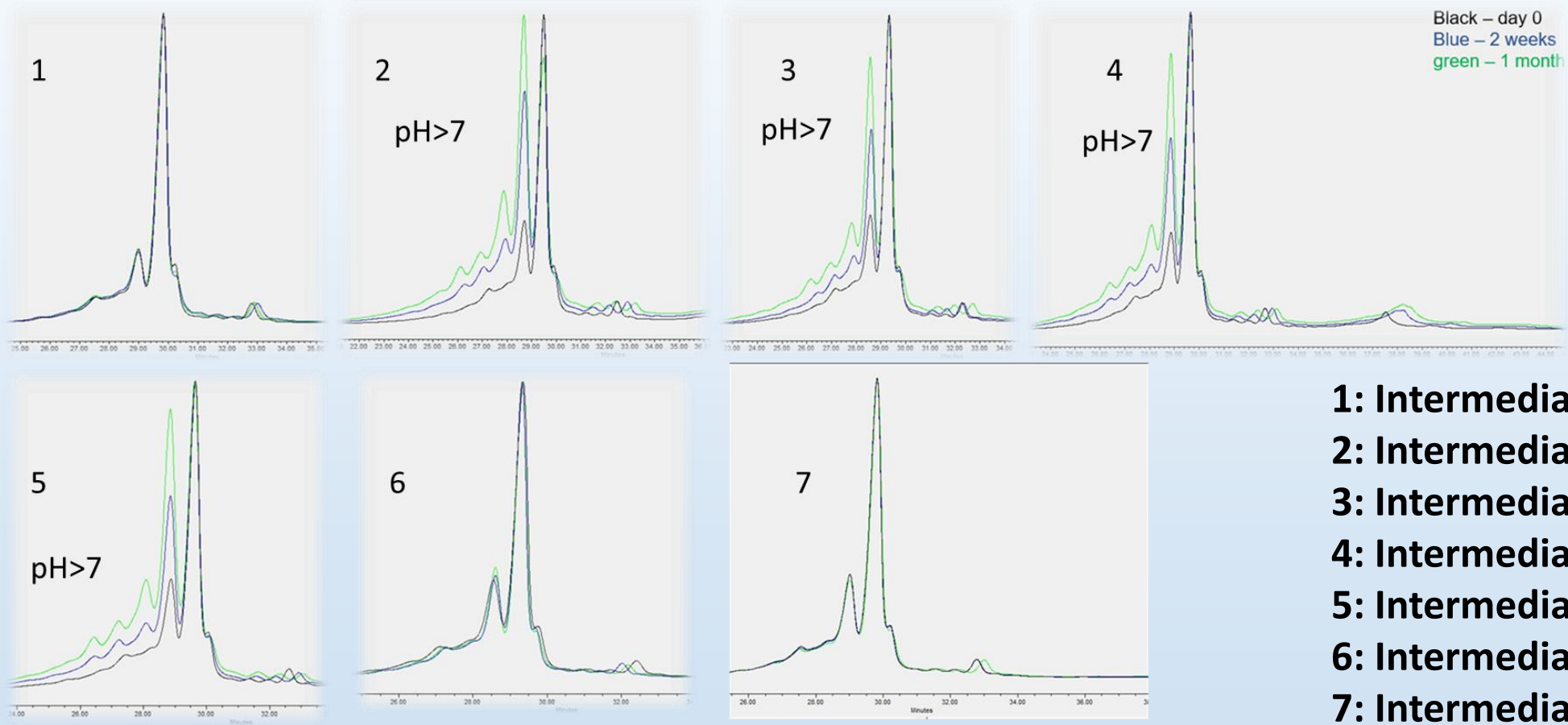
N-Glycan overlay

- All clones from Mini pool C has low mannose

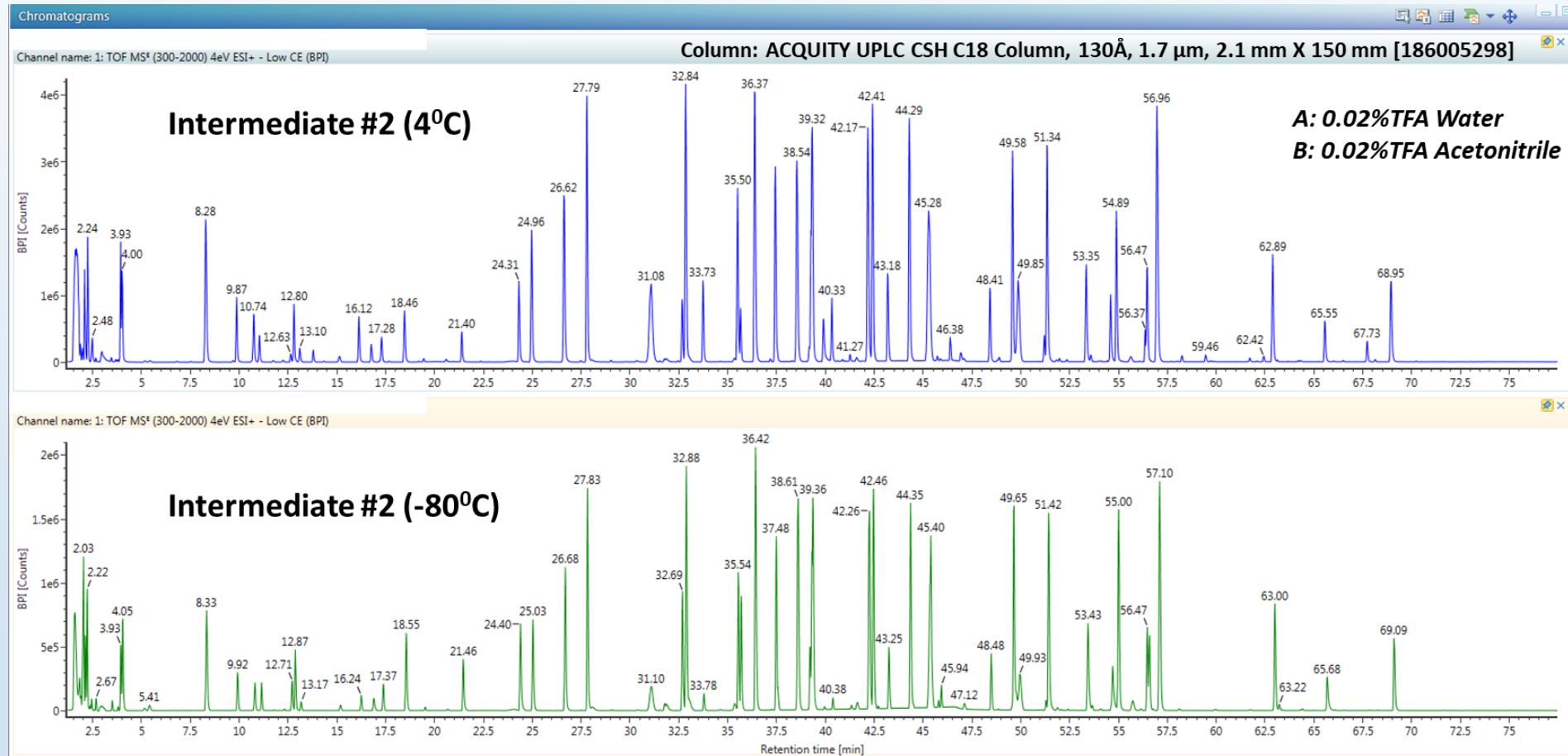


3. Downstream support- by peptide mapping

IEX data @40C, acidic group increased

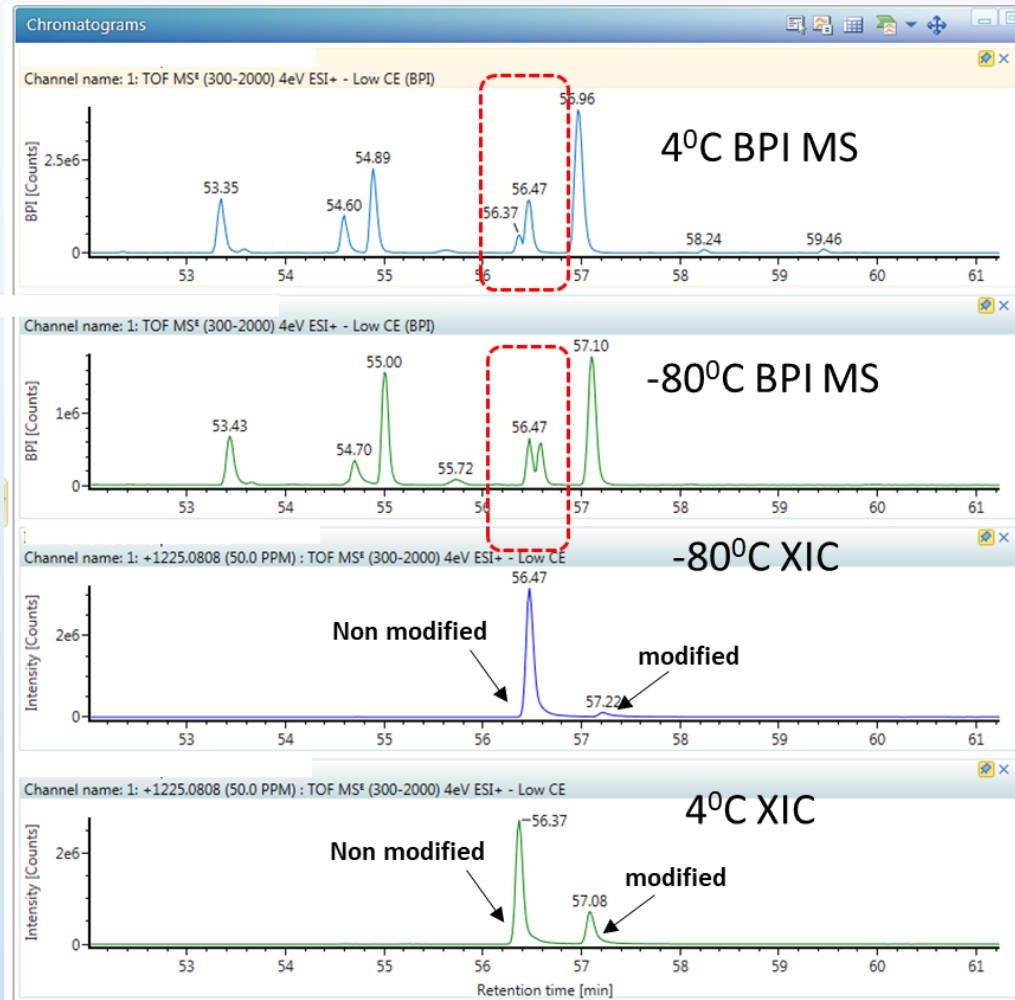


Stability of Downstream Intermediate by Reduced Peptide Mapping



Heavy Chain Peptide X:

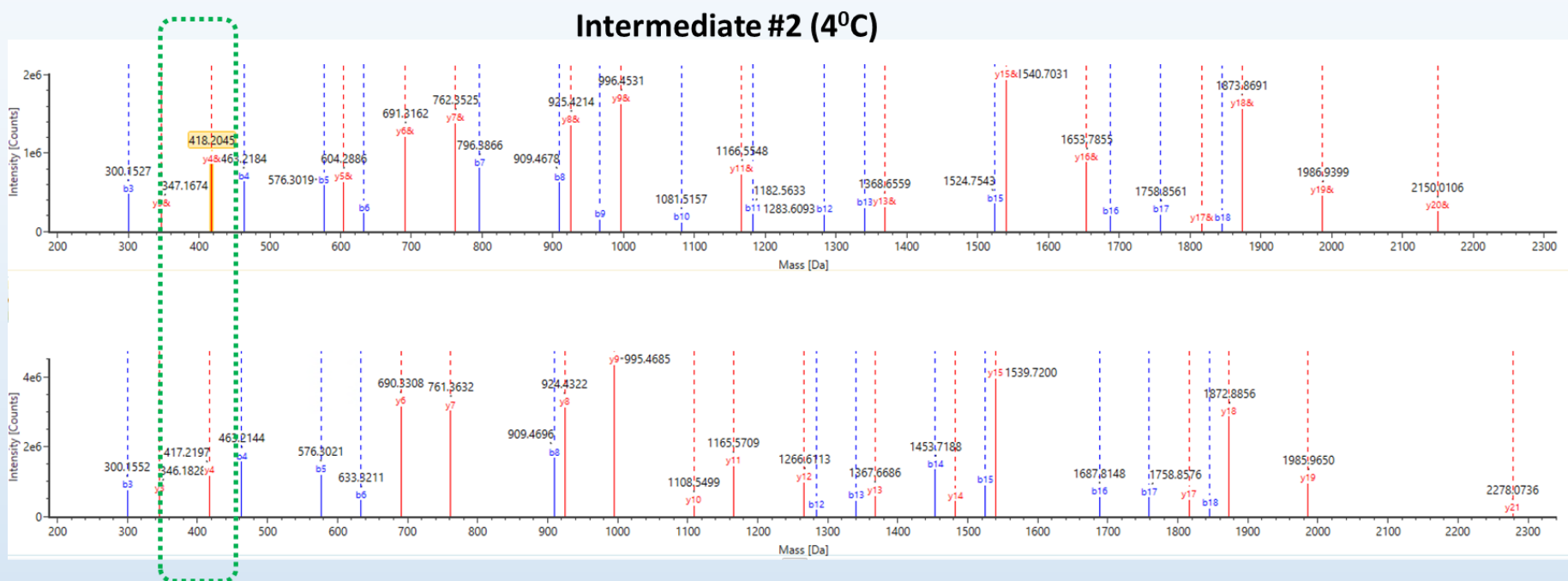
$N_{xxx}G$



| Peptide | %Deamid N_{xxx} Intermediate #2 -80°C | %Deamid N_{xxx} Intermediate #2 4°C |
|------------|--|--|
| $N_{xxx}G$ | 9.3 | 49.13 |

- Deamidation is confirmed for peptide X on the heavy chain by MS/MS
- The %modified of N_{xxx} in the intermediate 2 sample held at 4°C was found to be ~4 times higher than in the intermediate 2 sample held at -80°C

MS/MS Confirmation for Deamidation



| Fragment ion | Fragment ion mass (Da) | Peak mass (Da) | Mass error (mDa) | Retention time (min) | Modifiers |
|--------------|------------------------|----------------|------------------|----------------------|-------------------|
| y4& | 418.2 | 418.2 | -0.01 | 57.15 | Deamidation N (1) |
| Fragment ion | Fragment ion mass (Da) | Peak mass (Da) | Mass error (mDa) | Retention time (min) | Modifiers |
| y4 | 417.2 | 417.2 | -0.711 | 56.41 | |

Summary of Deamidation and Oxidation Modifications

| Peptide | sequence | modification | site | chain | %Deamid intermediate 2 (4°C) | %Deamid intermediate 2 (-80°C) | %Deamid intermediate 2 control |
|---------|----------|--------------------|------------------|-------|------------------------------|--------------------------------|--------------------------------|
| | | | | HC | 2.85 | nd | 1.05 |
| | | | | LC | 0.32 | 0.22 | 0.74 |
| | | | | HC | 3.04 | 1.98 | 3.87 |
| | | | | HC | 0.27 | nd | 0.38 |
| | | | | HC | 0.4 | nd | 1.05 |
| | | Deamidation N [21] | N _{xxx} | HC | 49.13 | 9.38 | 7.86 |

| Peptide | sequence | modification | site | chain | %ox intermediate 2 (4°C) | %ox intermediate 2 (-80°C) | %ox intermediate 2 control |
|---------|----------|-----------------|------|-------|--------------------------|----------------------------|----------------------------|
| | | | | HC | 0.54 | nd | 1.08 |
| | | | | HC | 1.76 | 1.03 | 2.14 |
| T21 | DTLMISR | Oxidation M [4] | M253 | HC | 5.28 | 5.07 | 6.88 |
| | | | | HC | 0.3 | nd | 2.05 |
| | | | | HC | 0.4 | nd | 1.27 |

- The %modified of N_{xxx} on peptide X in the intermediate 2 held at 4°C was found to be ~4 times higher than in the intermediate 2 held at -80°C
- This is in agreement with IEX data which shows a decrease in main peak and an increase in acidic forms
- No major differences were detected for the oxidation modifications between the two intermediate 2 samples and control sample

4. Stability Indicating Peptide Map

Sample info for Stressed PTM

Oxidation condition:

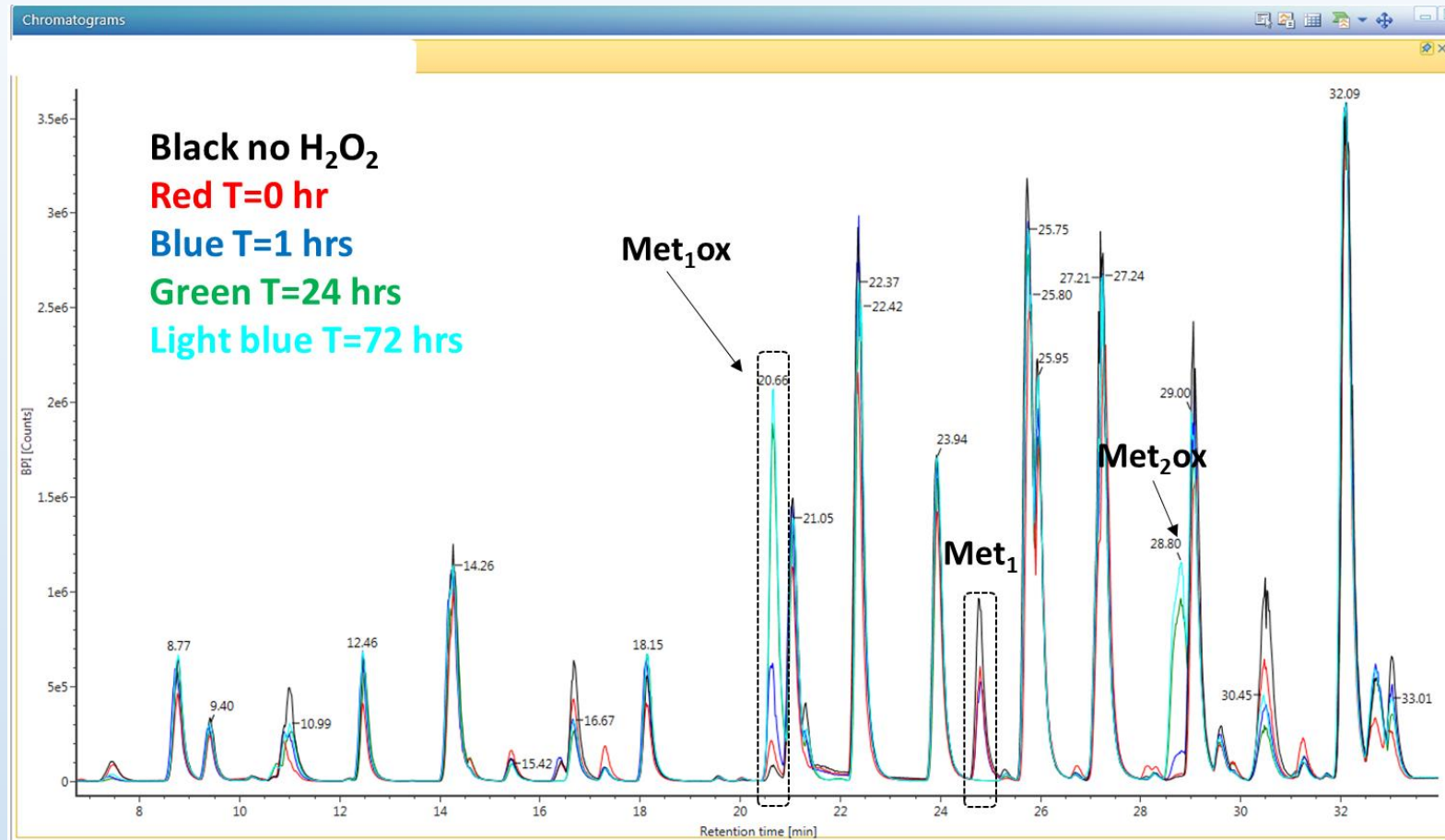
| Sample | PTM type | Condition | Incubation Temp (°C) | Exposure time (hr) |
|--------|-----------|-------------------------------|----------------------|--------------------|
| mAb1 | oxidation | H ₂ O ₂ | 25°C | 0 |
| mAb1 | oxidation | H ₂ O ₂ | 25°C | 2 |
| mAb1 | oxidation | H ₂ O ₂ | 25°C | 24 |
| mAb1 | oxidation | H ₂ O ₂ | 25°C | 72 |

Low and high pH conditions:

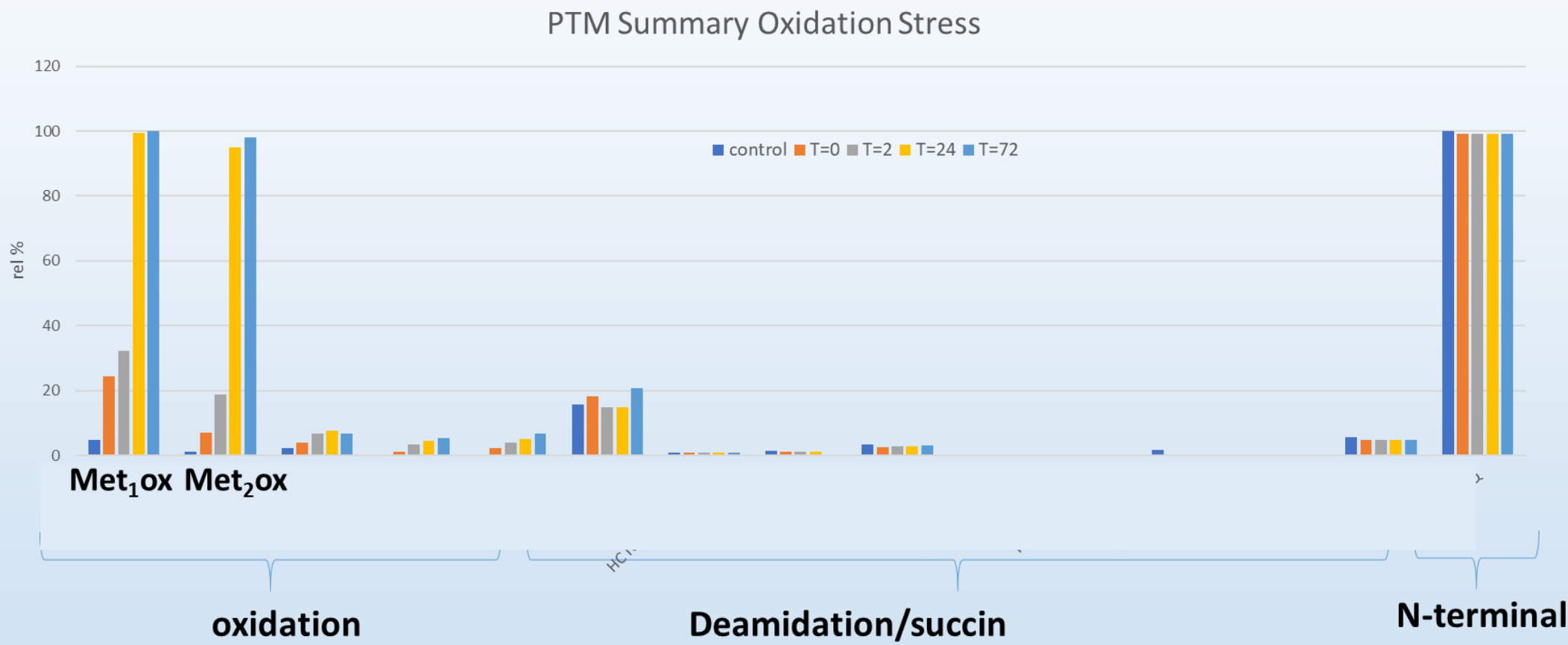
| Sample | PTM type | Condition | Condition | Incubation Temp (°C) | Exposure time (hr) |
|--------|-------------|-----------|-----------|----------------------|--------------------|
| mAb1 | Deamidation | pH9 | pH3 | 40°C | 0 |
| mAb1 | Deamidation | pH9 | pH3 | 40°C | 2 |
| mAb1 | Deamidation | pH9 | pH3 | 40°C | 24 |
| mAb1 | Deamidation | pH9 | pH3 | 40°C | 72 |

More PTMs were detected

Oxidation stress conditions (6-34 min)



Oxidation Stress Summary



Summary

- Waters G2 XS XEVO MS with UNIFI software is used routinely at SystImmune to support CLD, upstream and downstream programs
- Total cost per sample analysis is reduced
- Quality of analytical results improved to meet higher standards
- Decisions on product quality are made quickly and as a result the direction of the program is determined

Acknowledgments

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- Yi Zhu (CEO)
- Amanda Mak
- Weibin Zhou
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- Camilla Wang
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Waters

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- Mike Daly
- Justin Chang
- Jack Chacun
- James Movius
- William Alley