A Disruptive Approach to the Characterization of mAb Charge Variants During Process Development

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Global vision and core purpose

To be the leading and most trusted global Contract Development and Manufacturing Organization partner in the biopharmaceutical industry.

Partners for *Life*

Advancing tomorrow's medicines

Global locations

4
Technologies
Microbial
Mammalian
Cell & Gene Therapy and Viral Vaccines

~4,000
Employees
worldwide, and growing

19
Licenses
for commercial manufacturing

$7.4B
In investments announced
in recent years

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End-to-End CDMO

Pre-clinical	Phase I, II	Phase III	Regulatory approval / launch

Pre-clinical development

- Expression Studies
- Strain Development
- Cell Line Development
- Process Invention
- Pre-Clinical Drug Substance
- Pre-Clinical Drug Product

Early phase clinical

- Process Development
- Process Optimization
- Analytical Development
- Formulation Development
- cGMP Drug Substance
- cGMP Drug Product
- DS/DP Stability

Late phase clinical

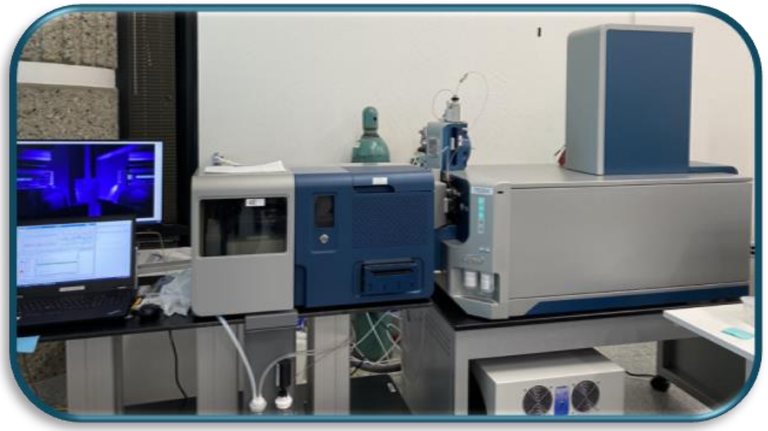
- Process Characterization
- Process Validation
- Analytical Method Validation
- Formulation Optimization
- cGMP Drug Substance
- cGMP Drug Product
- DS/DP Stability

Commercial production

- cGMP Drug Substance
- cGMP Drug Product
- Finished Goods (ALP)
- Post-Approval Activities
- Product Life Cycle Management

Introduction

- Charge variant encompass the majority of a mAb's heterogeneity
- Traditional approaches to characterization of the charged isoforms of mAbs are extremely laborious
- The Intabio icIEF-MS system coupled with the SCIEX ZenoTOF 7600 system (high-resolution mass spectrometer) offers a unique and disruptive combination of charge heterogeneity analysis and molecular mass characterization in one process.
- The present study highlights rapid characterization of charge variant species of biotherapeutic mAb cell culture process development samples using the research breadboard Intabio icIEF-MS system.



*Breadboard design

Antibody therapeutics 101

- Monoclonal antibody (mAbs) are one of the most common protein-based biotherapeutics
  - 141 mAbs approved or are in regulatory review in the EU or US
  - 2019: Companies are currently sponsoring clinical studies of more than 570 mAbs
  - 2022: 135 antibody therapeutics are in late-stage clinical studies, 61 cancer, 54 non-cancer and 20 COVID-19 indications

https://www.antibodysociety.org/resources/approved-antibodies/
mAbs 2022, Vol. 14, NO. 1, e2014296

mAb Heterogeneity

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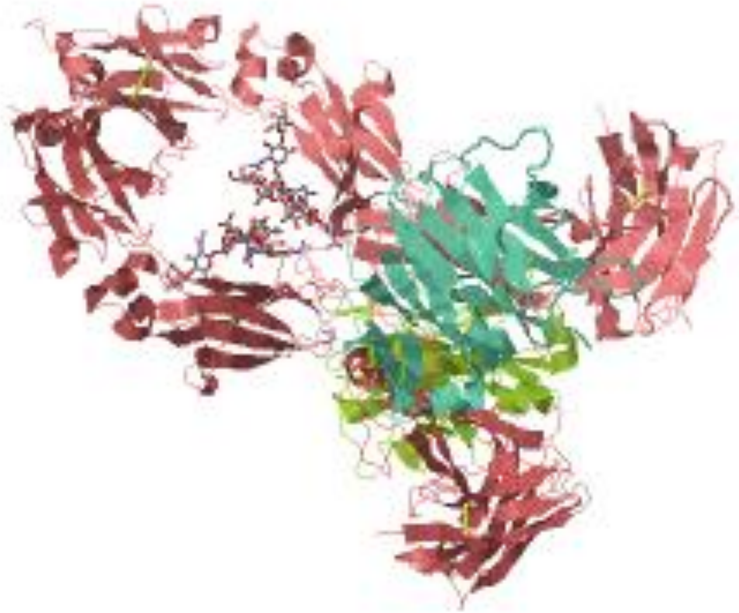
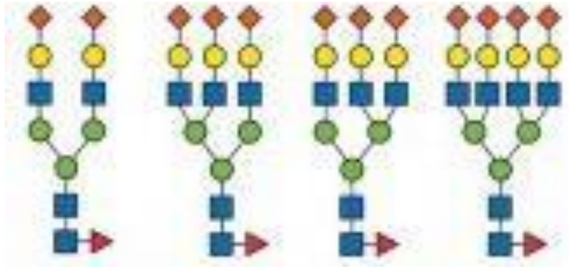
Acidic Variants

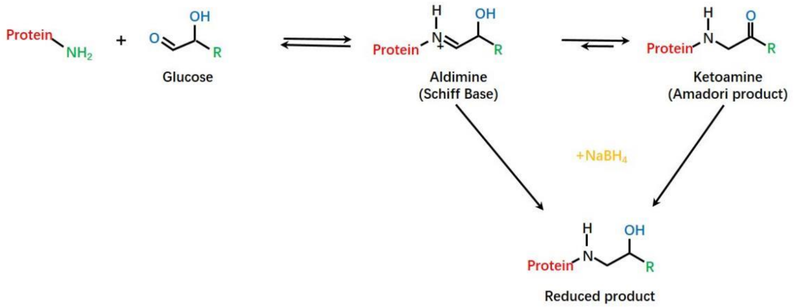
- Sialylated glycoforms
- Deamidation
- Trisulfide
- Reduced disulfides
- Glycation of basic lysine residues



Basic Variants

- C-terminal lysine truncation
- N-terminal pyroglutamate
- Succinimide
- Oxidized species
- Aglycosylated species

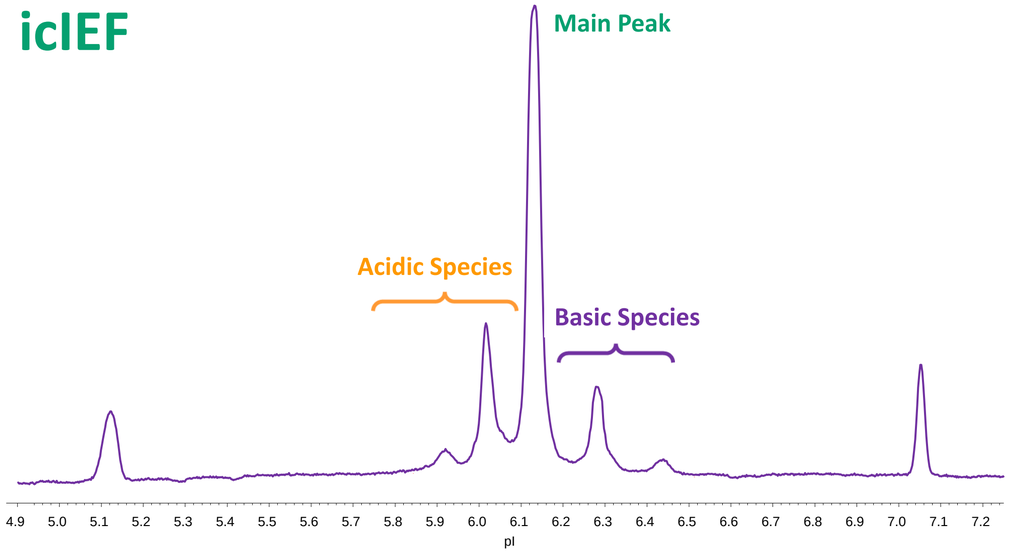




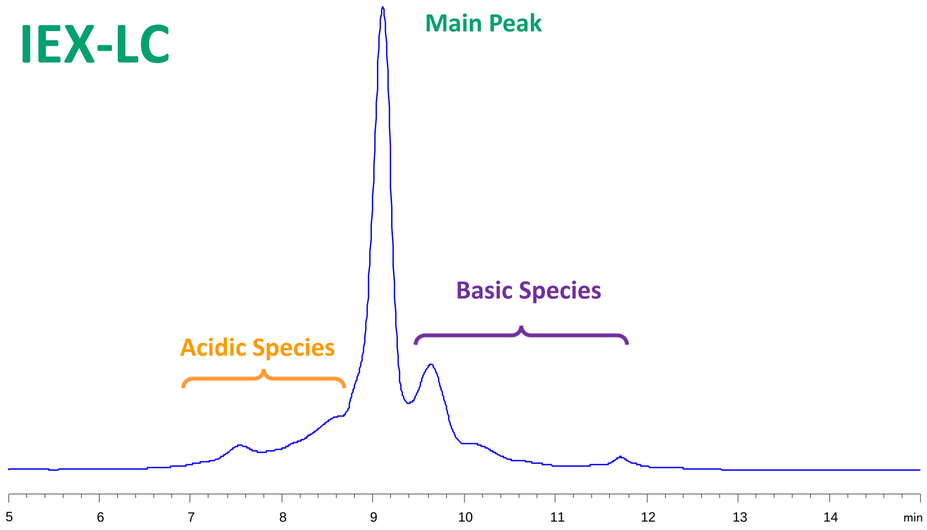
mAb Charge variant analysis

- Key component to understanding the heterogeneity of a mAb's physicochemical properties
  - Methods are used across the entire development cycle
  - mAb main peak and acidic/basic components are often reported as critical quality attributes to ensure patient safety and process consistency
- Charge variants typically monitored using icIEF or IEX-LC

icIEF

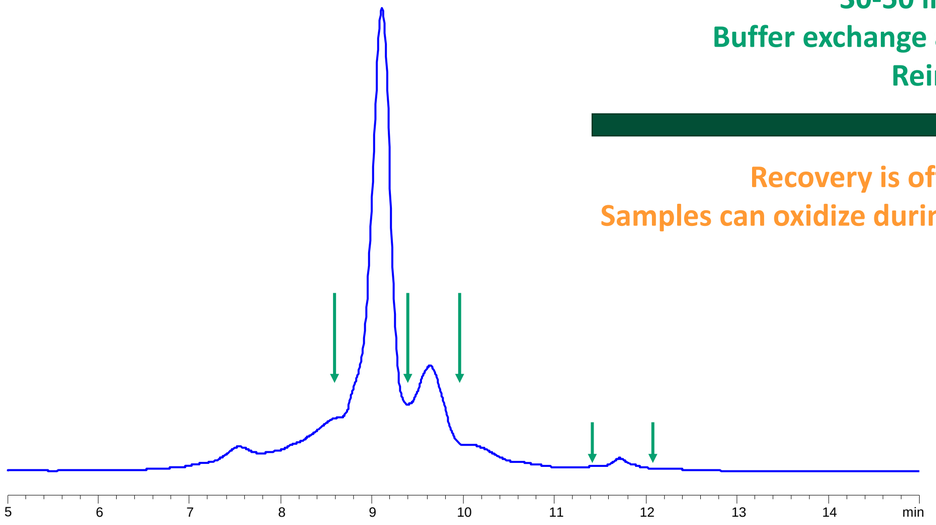


IEX-LC



mAb charge variant analysis

- For early-stage projects, prior knowledge is often utilized to risk assess the need for more in-depth characterization
- Traditional approaches to characterize charge variant species requires laborious LC fractionation
  - Difficult to obtain high purity fractionated species
  - LC separation may not be directly representative of the icIEF profile
    - Requires additional development of an orthogonal IEX-LC method and peak correlation
  - Sample fractionation/concentration often introduces artifacts

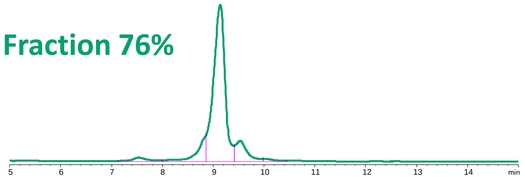


Arrows representation where peaks were collected

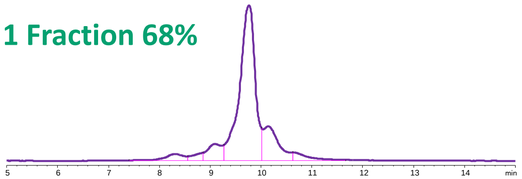
Maximize IEX load without impacting resolution
30-50 injections
Buffer exchange and concentration
Reinject

Recovery is often low (~50%)
Samples can oxidize during exchange concentration

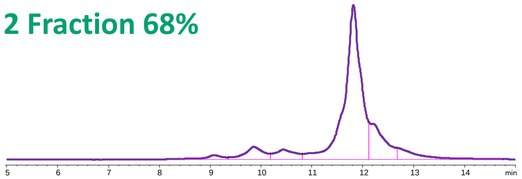
Main Peak Fraction 76%



Basic Peak 1 Fraction 68%

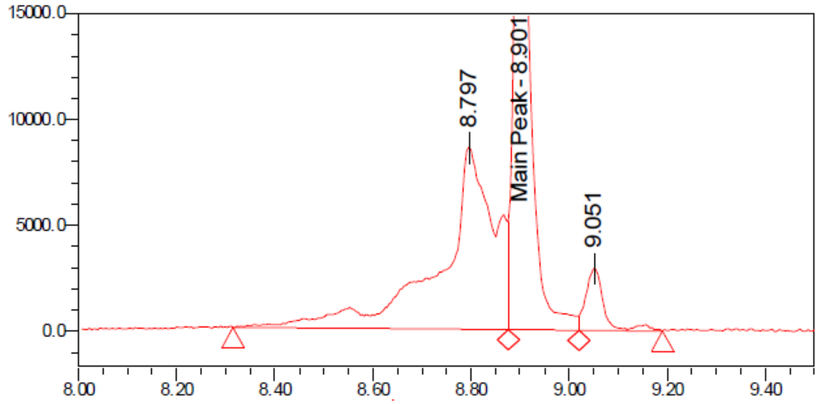


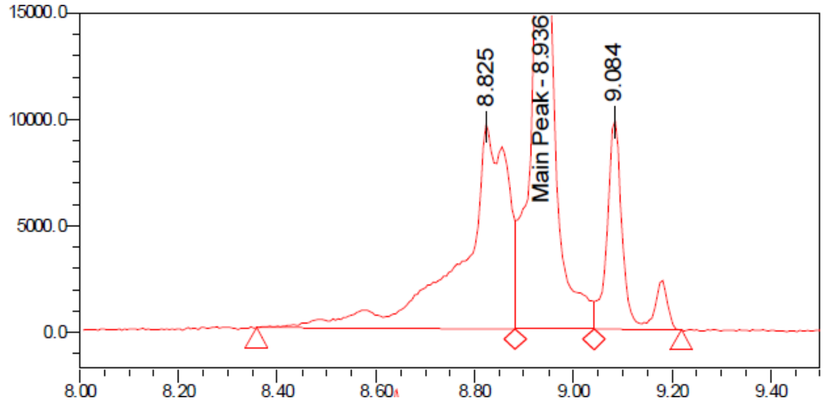
Basic Peak 2 Fraction 68%



High throughput mAb cell line development and process development

- mAb cell line development performed in Ambr15 micro-bioreactor with 48 parallel conditions
- mAb process development executed in 24 run DOE formats using Ambr250 mini-bioreactors
- icIEF is used as the primary tool for rapid evaluation of charge variants
- Data are evaluated for ratios of acidic and basic variants





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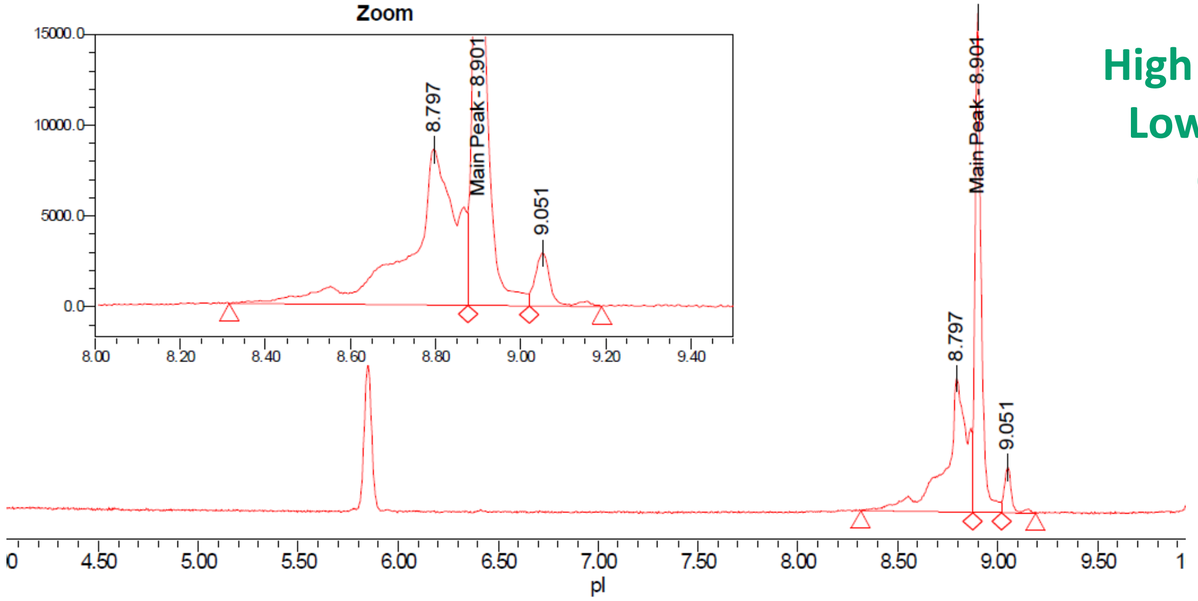
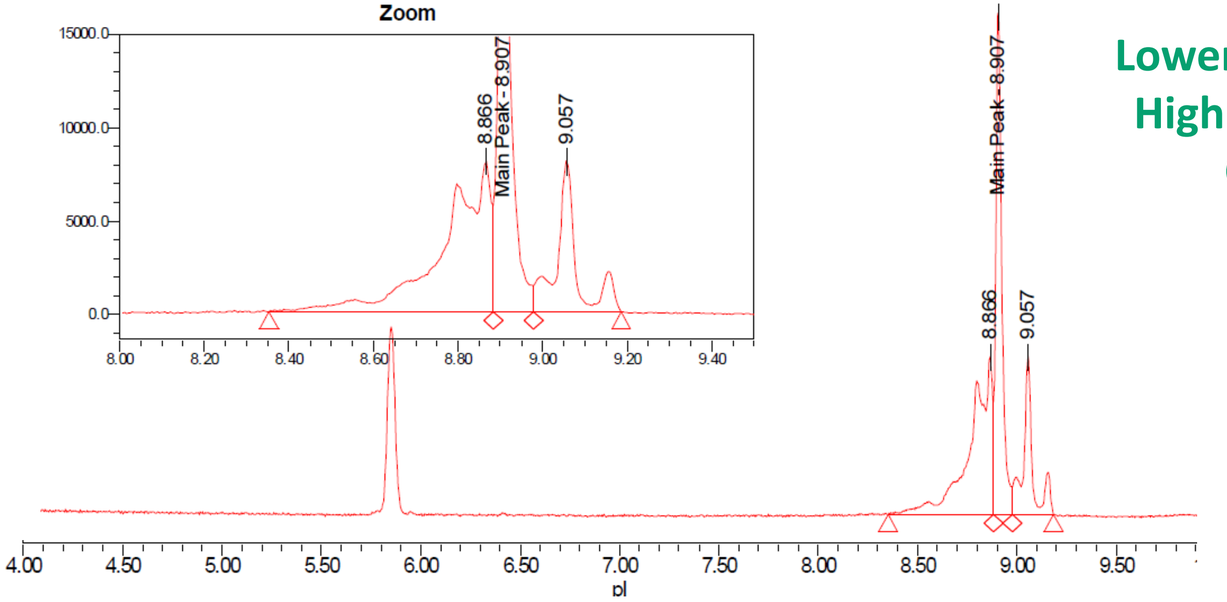
When unexpected results are observed...



High Acidic (50%)
Low Basic (4%)
Content



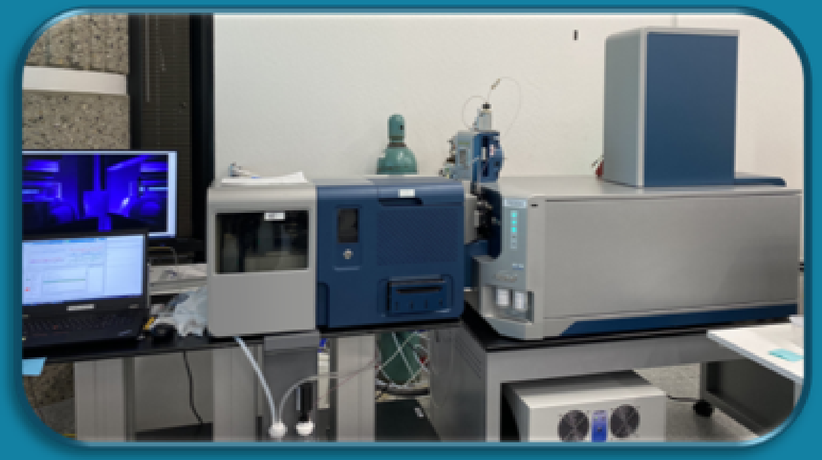
Lower Acidic (38%)
High Basic (18%)
Content

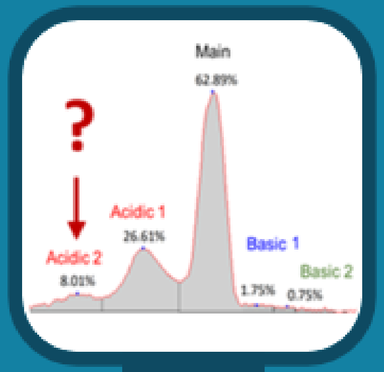
How to approach the identification of these variant species?

- *Assume from prior knowledge?*
- *Pace of cell line development doesn't align with in-depth fractionation at this stage of development*
- *What is really needed is something in our analytical toolbox to rapidly characterize these species.*

Intabio iclEF-MS system

Up to 30 minutes per sample*





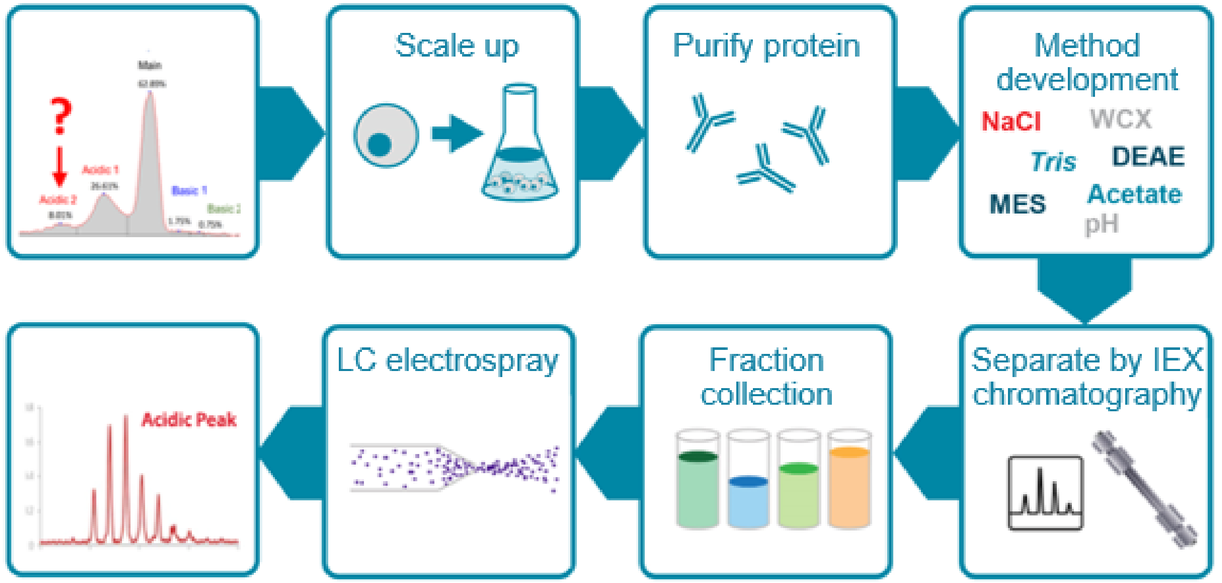
Characterization by MS

*Data from research breadboard

vs.

Current workflow for charge variant peak ID

Weeks per sample



Confirmatory clEF analysis

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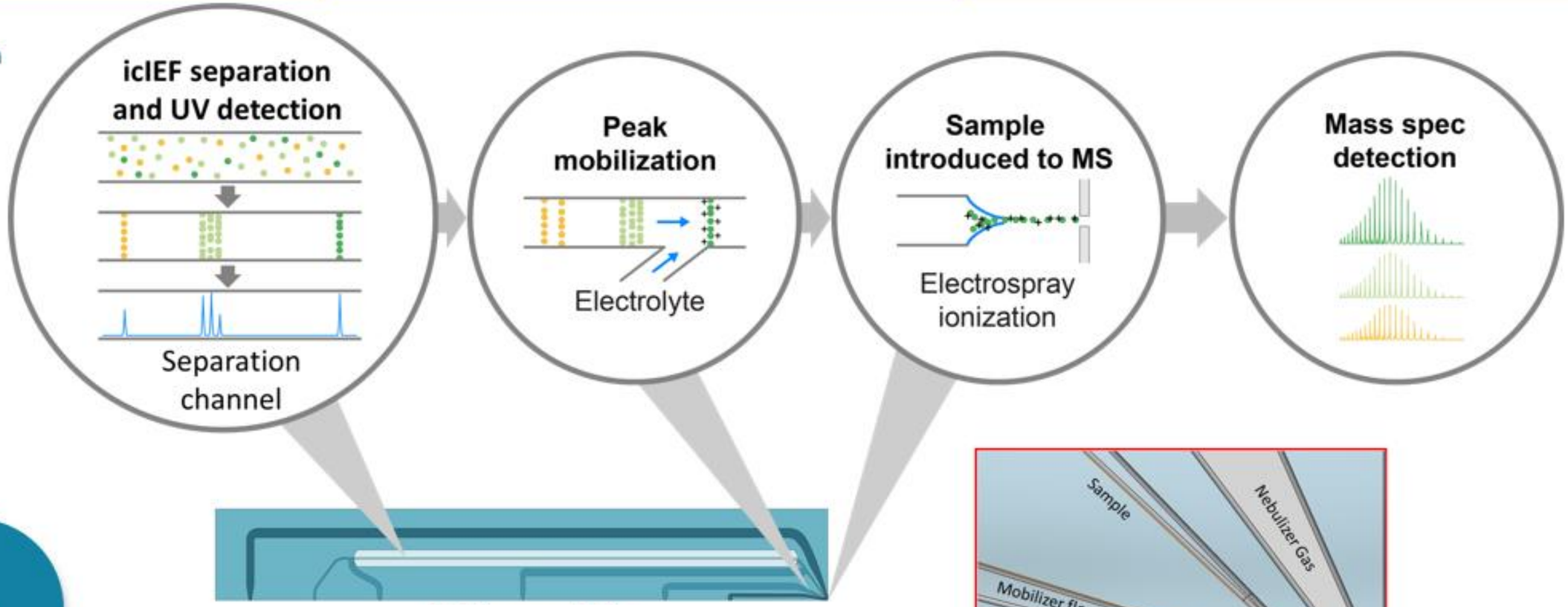
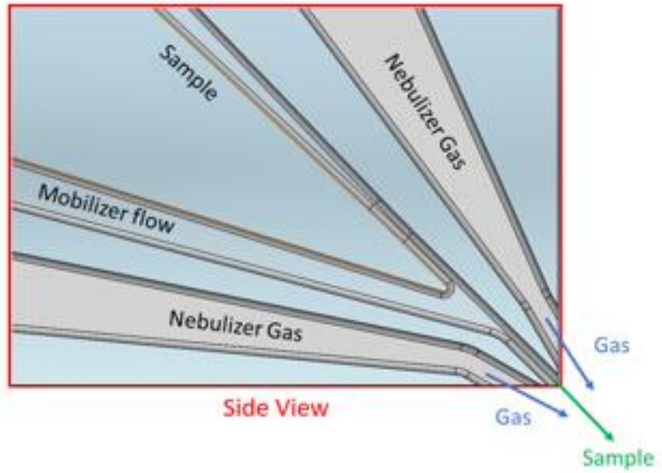
Microfluidic chip-based integrated icIEF-MS technology integrates key analytical functions

- cIEF separation
- Real-time UV absorbance imaging



30-min run time per sample

Microfluidic chip schematic



*Data collected from research breadboard

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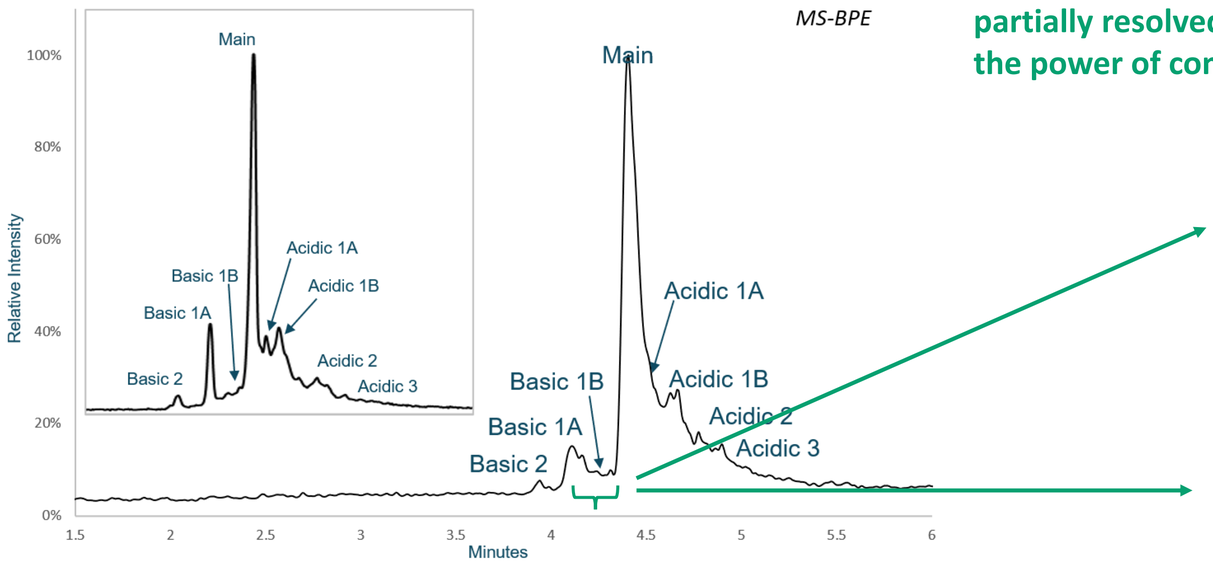
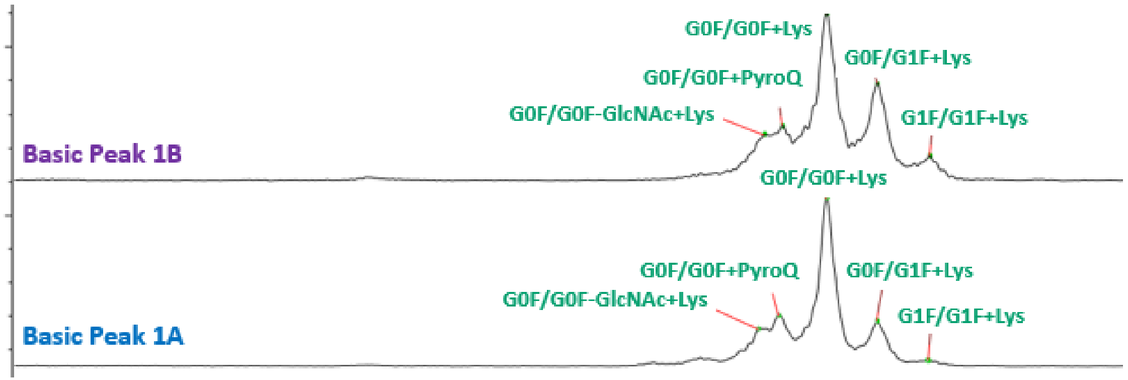
Data generated on research breadboard Intabio icIEF-MS system and ZenoTOF 7600 system

- Species are differentiated and identified without the need for lengthy and laborious protein scale-up and fractionation
- Scan rate of 2 Hz enables 30 TOF scans to be performed within 15 seconds; allowing detection and identification of two closely adjacent peaks
- Differences in relative abundance between Basic Peak 1A and Basic Peak 1B is clearly differentiated
- Separation, the scan rate of acquisition and MS sensitivity optimized for separation and peak identification



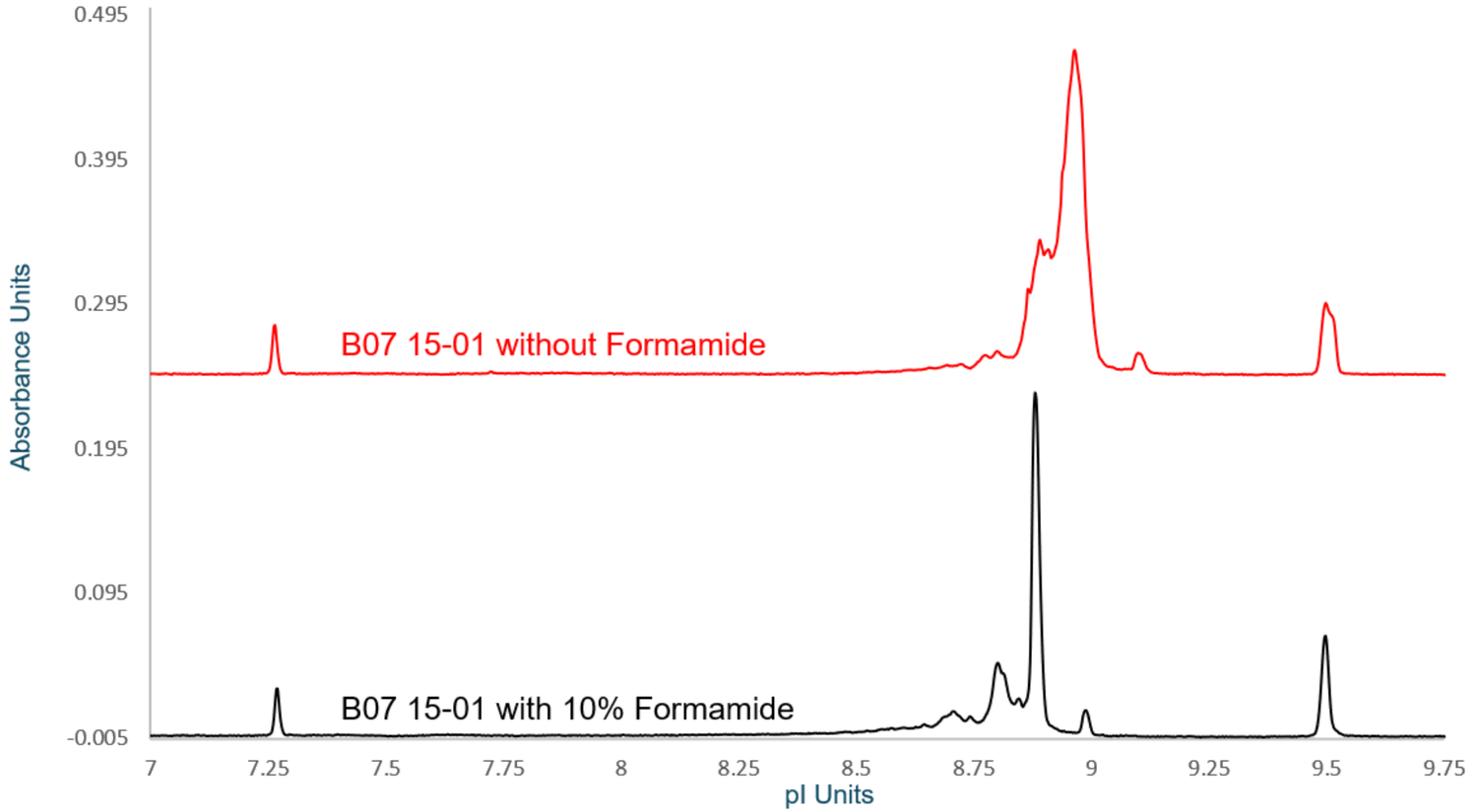
Differences in the intact mass profiles show identification of compositional differences between partially resolved basic peak 1A and 1B highlighting the power of combining icIEF and MS identification



*Data collected from research breadboard

icIEF-MS analysis parameters

- 3% Pharmalyte 8 to 10.5 and 1% Pharmalyte 3 to 10
- *Methylcellulose removed to enable ESI*
- 10.0 mM Arginine added as 2.5 mM Iminodiacetic Acid – *speed up focusing and inhibit ITP decay of pH gradient*
- 10% DI Formamide – *added instead of urea to maintain solubility during focusing because urea can impact electrospray ionization*
- pI estimated with pI 7.27 and 9.50 peptide markers
- **Focusing time 6.5 Min**
  - 1500 V 1 Min
  - 3000V 1 Min
  - 4500V 4.5 Min
- **Mobilization time 10 Min at 3500V**
- **ESI Tip 5500V**



*Data collected from research breadboard

How does icIEF-MS compare to icIEF on the Maurice?

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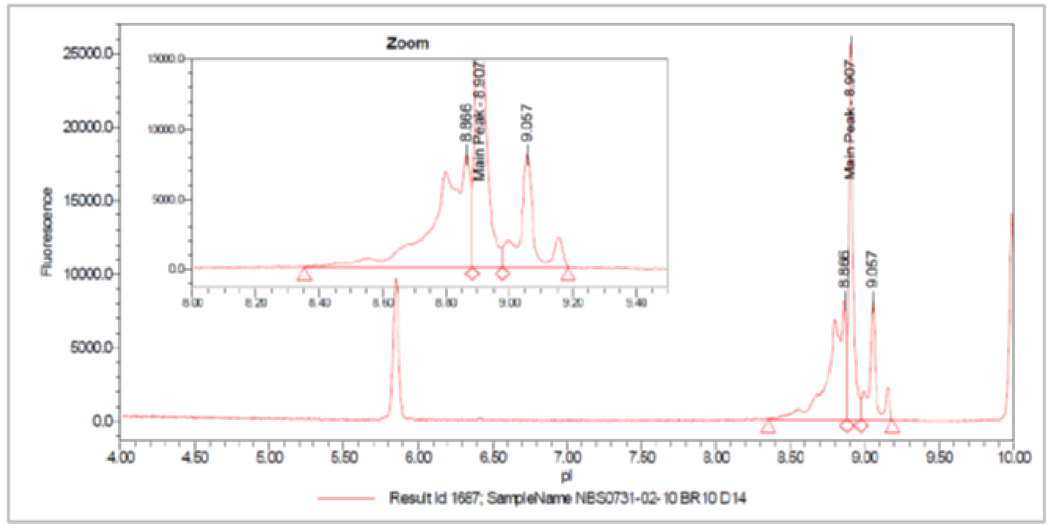
icIEF with Fluorescence Detection on Maurice (inset)



icIEF-MS UV

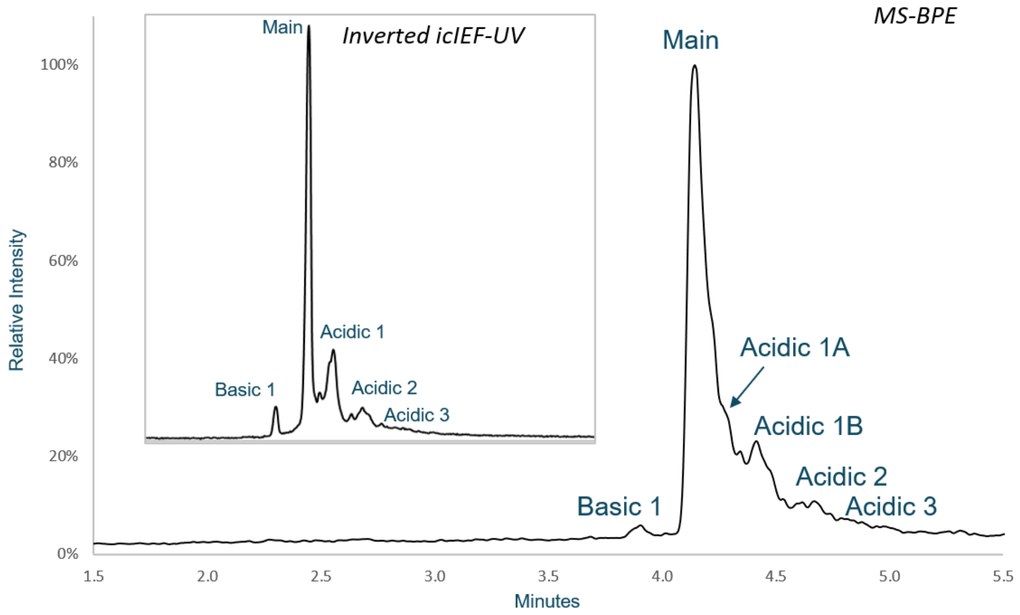
	icIEF (Maurice)	icIEF-MS UV
Main	pI = 8.91	pI = 8.86

	icIEF (Maurice)	icIEF-MS UV
Acidic	43.2%	44.7%
Main	38.6%	38.5%
Basic	18.2%	16.8%

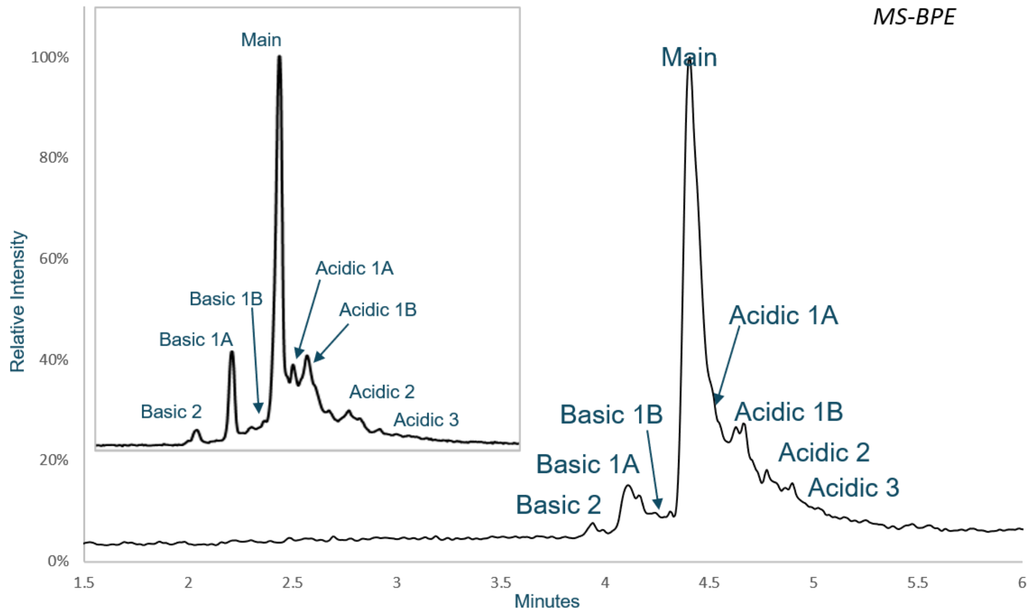
*Data collected from research breadboard

icIEF-MS UV analysis of cell culture development samples

	Low basic content (LDC)				High basic content (HBC)			
	B7 1501	B14 1503	B16 1504	B21 1506	B10 1502	B18 1505	B10 1601	B18 1602
Acidic	46.6%	49.9%	45.6%	46.0%	44.7%	43.0%	43.9%	42.3%
Main	48.3%	46.7%	50.5%	49.7%	38.5%	46.3%	38.1%	45.6%
Basic	5.1%	3.5%	4.0%	4.3%	16.8%	10.8%	18.1%	12.1%



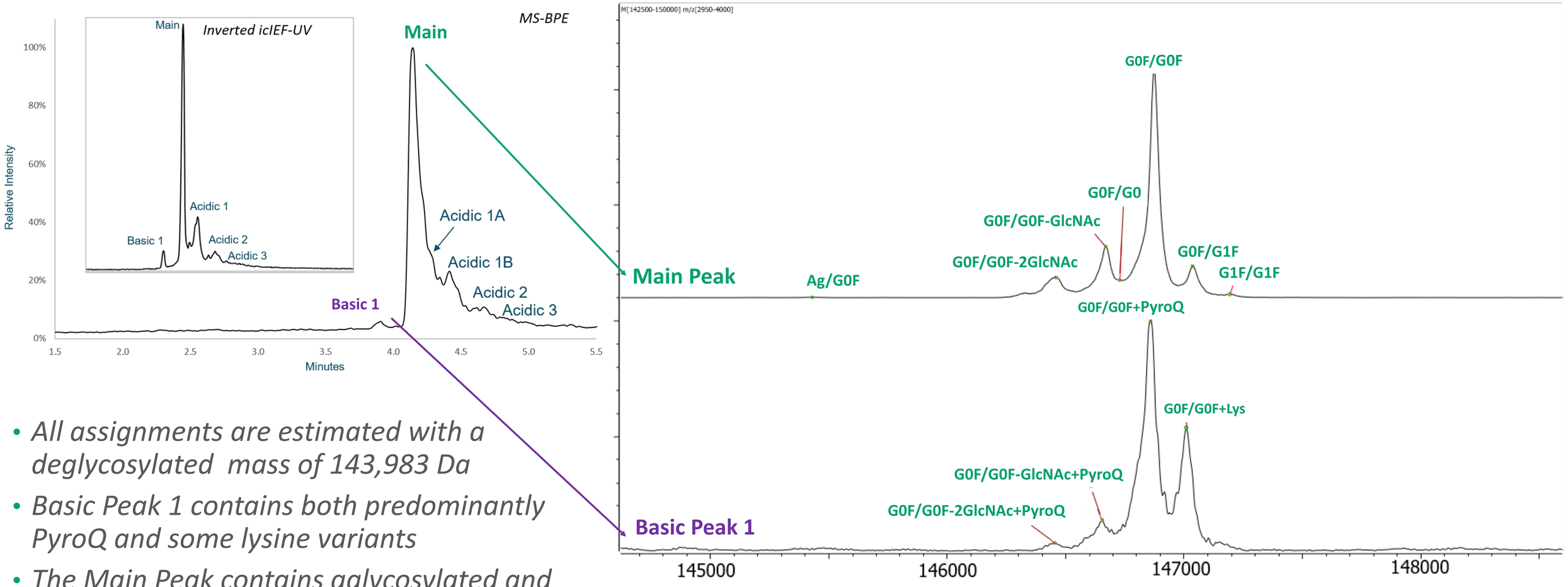
Low basic content sample B7 1501



High basic content sample B10 1502

*Data collected from research breadboard

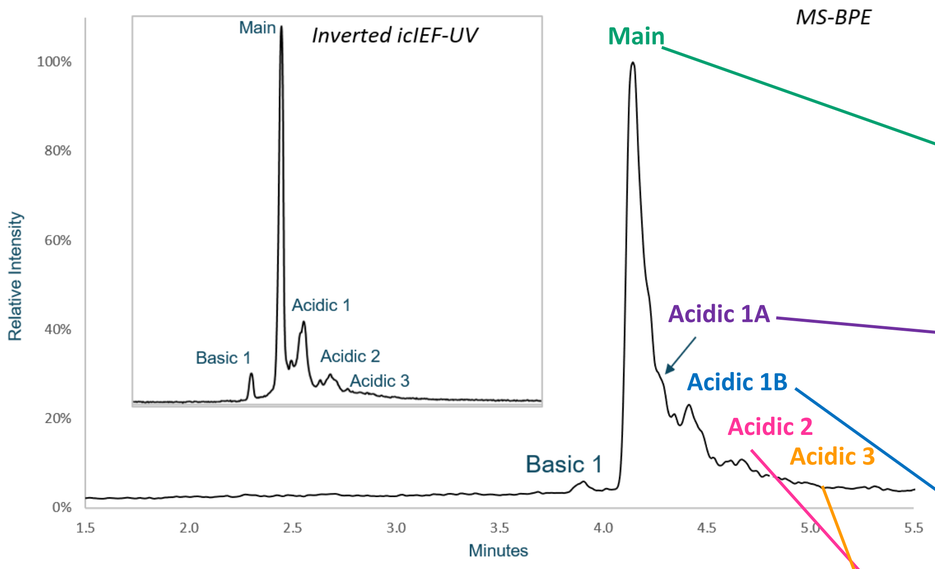
Low basic content samples: Basic variants and main species

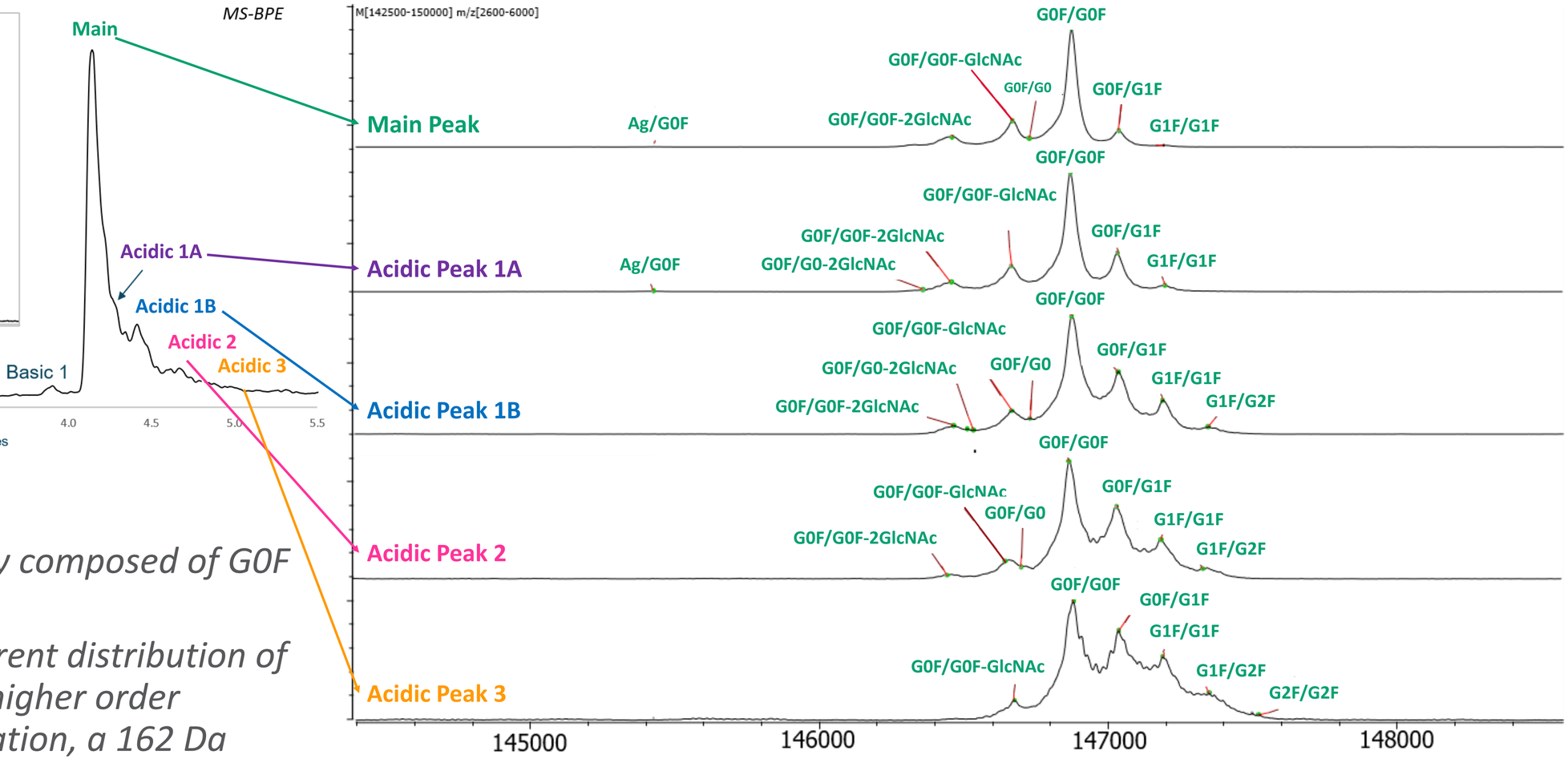


- *All assignments are estimated with a deglycosylated mass of 143,983 Da*
- *Basic Peak 1 contains both predominantly PyroQ and some lysine variants*
- *The Main Peak contains aglycosylated and complex glycans including –GlcNac and -Fuc*

*Data collected from research breadboard

Low basic content samples: Acidic variants

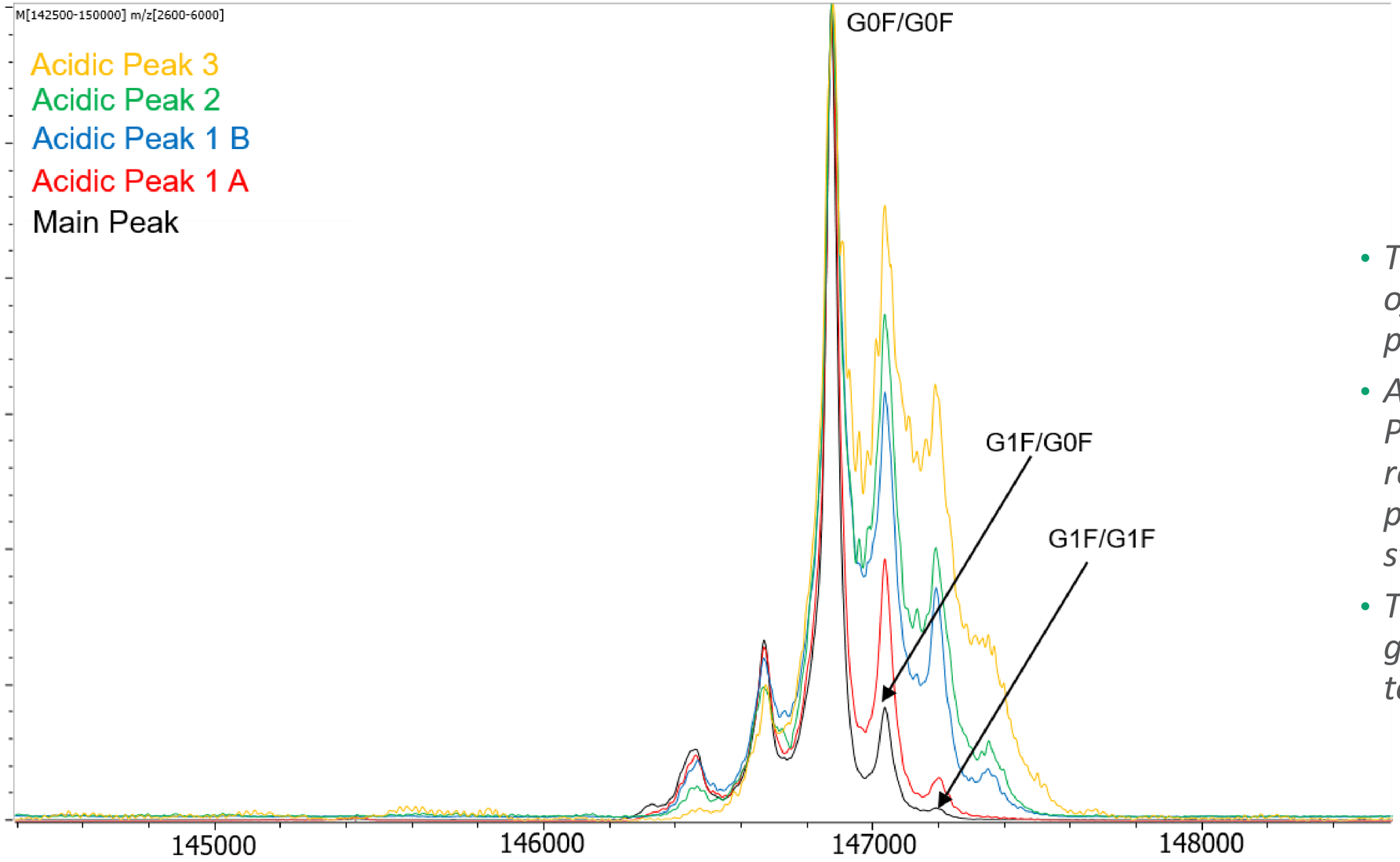




- *The Main Peak is primarily composed of G0F containing glycan pairs*
- *As pI decreases, the apparent distribution of the glycan pairs shifts to higher order structures indicating glycation, a 162 Da modification of lysine*

*Data collected from research breadboard

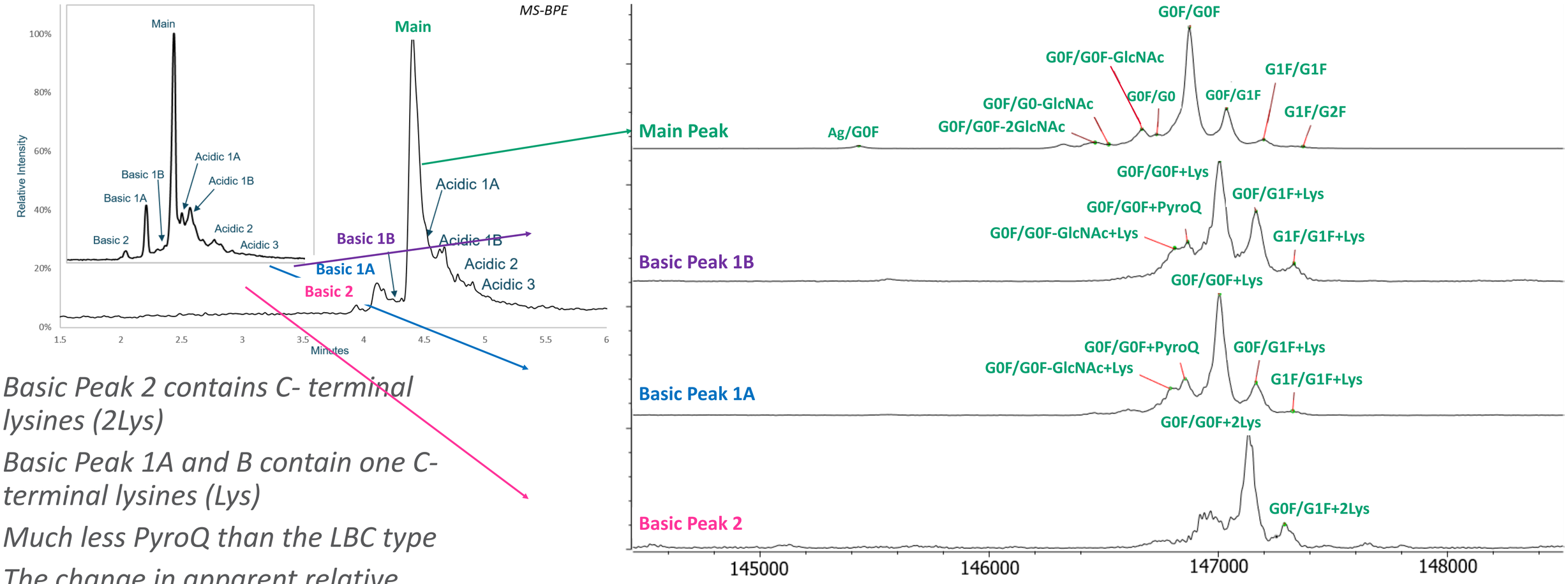
Acidic variants are mostly glycated species



- *The complex glycan structure of the main peak is predominately G0F/G0F*
- *As pI decreases form Acidic Peak 1A to Acidic Peak 3 the relative abundances of glycan pairs shifts to higher order structures*
- *The pI dependent shift in the glycoprofile is most likely due to glycated lysines*

*Data collected from research breadboard

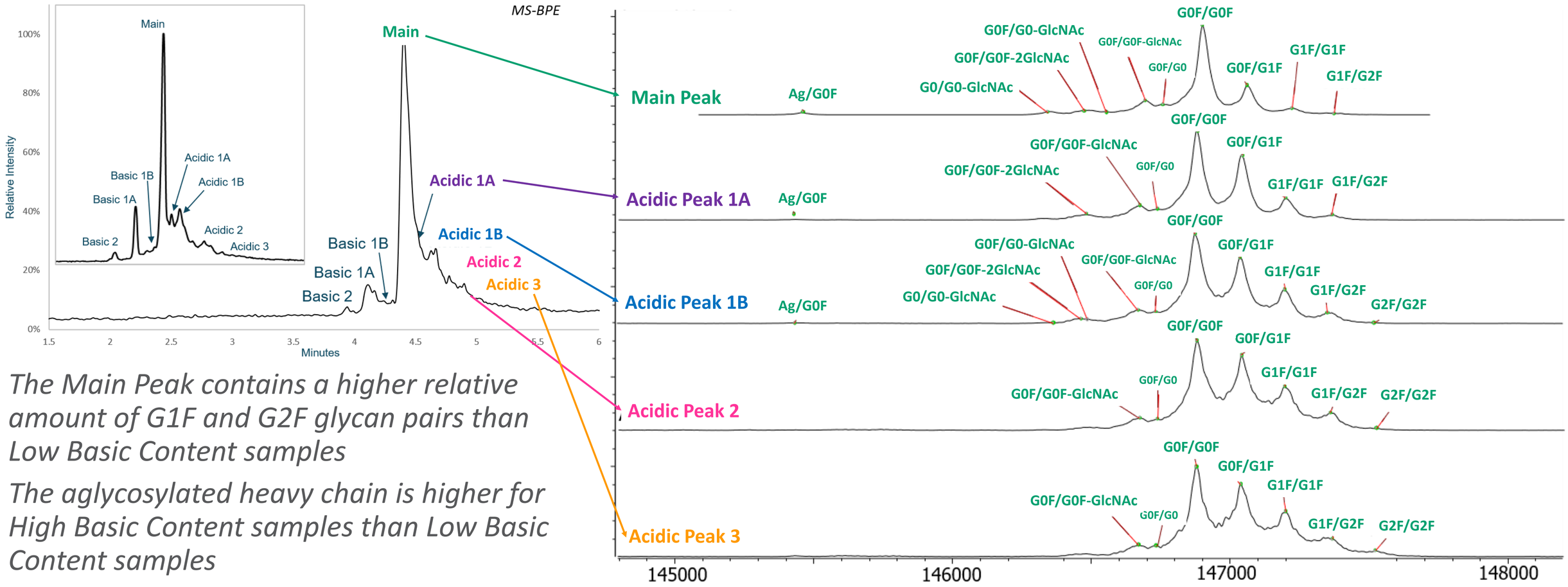
High basic content samples: Basic variants and main species



- *Basic Peak 2 contains C- terminal lysines (2Lys)*
- *Basic Peak 1A and B contain one C-terminal lysines (Lys)*
- *Much less PyroQ than the LBC type*
- *The change in apparent relative abundances in glycan pairs indicates Basic Peak 1B has convoluted glycation*

*Data collected from research breadboard

High basic content samples: Acidic variants



- *The Main Peak contains a higher relative amount of G1F and G2F glycan pairs than Low Basic Content samples*
- *The aglycosylated heavy chain is higher for High Basic Content samples than Low Basic Content samples*
- *As pI decreases the distribution of the glycan pairs shifts to higher order structures, indicating glycation (Hexose)*

*Data collected from research breadboard

Conclusions

- The research breadboard Intabio icIEF-MS system coupled with the ZenoTOF 7600 system enables the rapid characterization of charge variants of mAb cell culture cell line development/process development samples
  - A traditional fraction collection approach would have taken multiple weeks
- Comparable icIEF separation profiles were observed with the research breadboard Intabio icIEF-MS system and traditional icIEF using the Maurice
- Samples with lower basic species demonstrated slightly lower order N-linked complex glycan structure, greater C-terminal lysine processing, and higher pyroglutamate formation than higher basic species samples
- Acidic species observed in all samples were mostly attributed to higher levels of glycation products
- The research breadboard Intabio icIEF-MS system is an emerging tool with the potential to dramatically simplify charge variant characterization
- The power of knowledge in product characterization during biopharmaceutical product development is critical to the Fujifilm Diosynth Biotechnology capabilities

Acknowledgements

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