Industry Case Studies on reducing turn around times for product quality analysis using next generation CE-SDS technology

Chelsea Leonce[1], Theresa Merlino[2], Kristen Nields[1], Bob Hepler[1], Hirsh Nanda[1]

[1]CEED | API Proteins
Discovery, Product Development & Supply | Janssen Research & Development, LLC
[2]Analytical Development| Process Analytical Sciences | API Proteins
Discovery, Product Development & Supply | Janssen Research & Development, LLC

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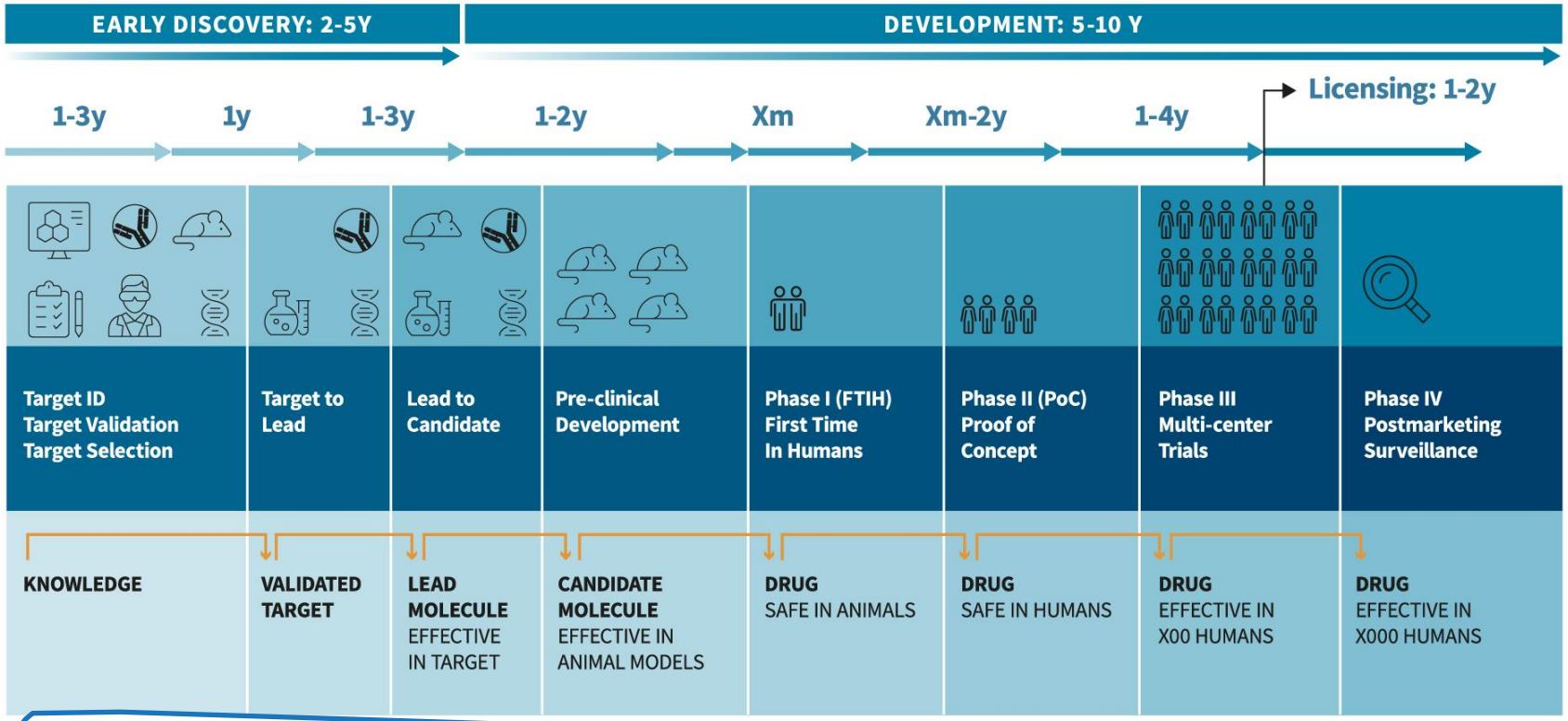
Outline

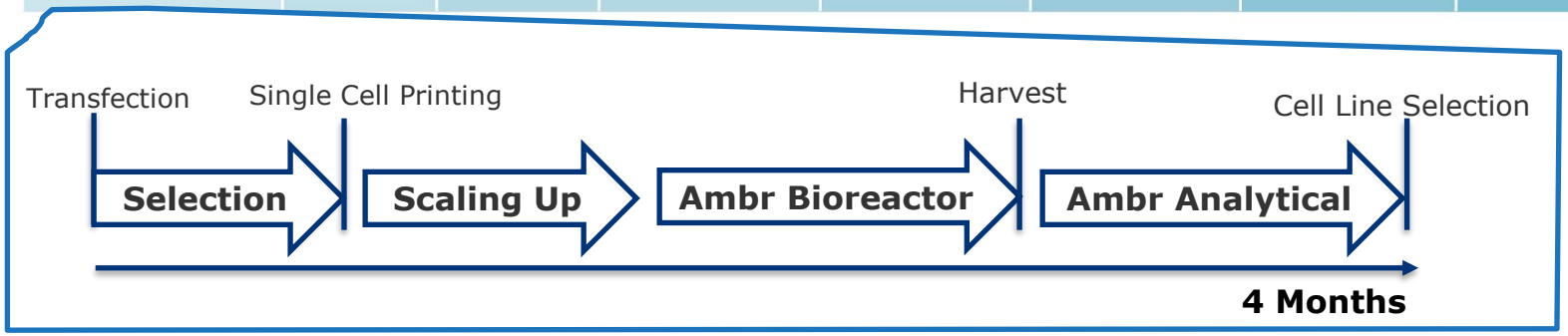
- Janssen Pharmaceuticals: Who we are
- The drug discovery process
  - Role of capillary electrophoresis sodium dodecyl sulfate (CE-SDS) in Drug development
- Challenges with CE-SDS technology
  - Closing the reliability and robustness gap of CE-SDS
  - Pushing the envelope of CE-SDS - Traditional vs Lightning methods
- Case Studies
  - Springhouse
  - Malvern
- Conclusions
- Acknowledgments

Who we are

- **Janssen Pharmaceuticals**
  - Cell Engineering and Early Development group in Drug Product and Development Sciences (DPDS)
  - Analytical Development (AD) in Process Analytical Sciences
- Our goal is to use orthogonal methods to develop cell lines with high titer and high product quality
  - Large molecule drug products
  - Evaluate reduced and non-reduced purity to identify low and high molecular weight product-related impurities and potential HCPs
  - Track molecular integrity and stability
- **This Data is critical to the timely selection and development of new drug products for Janssen**

The drug discovery process





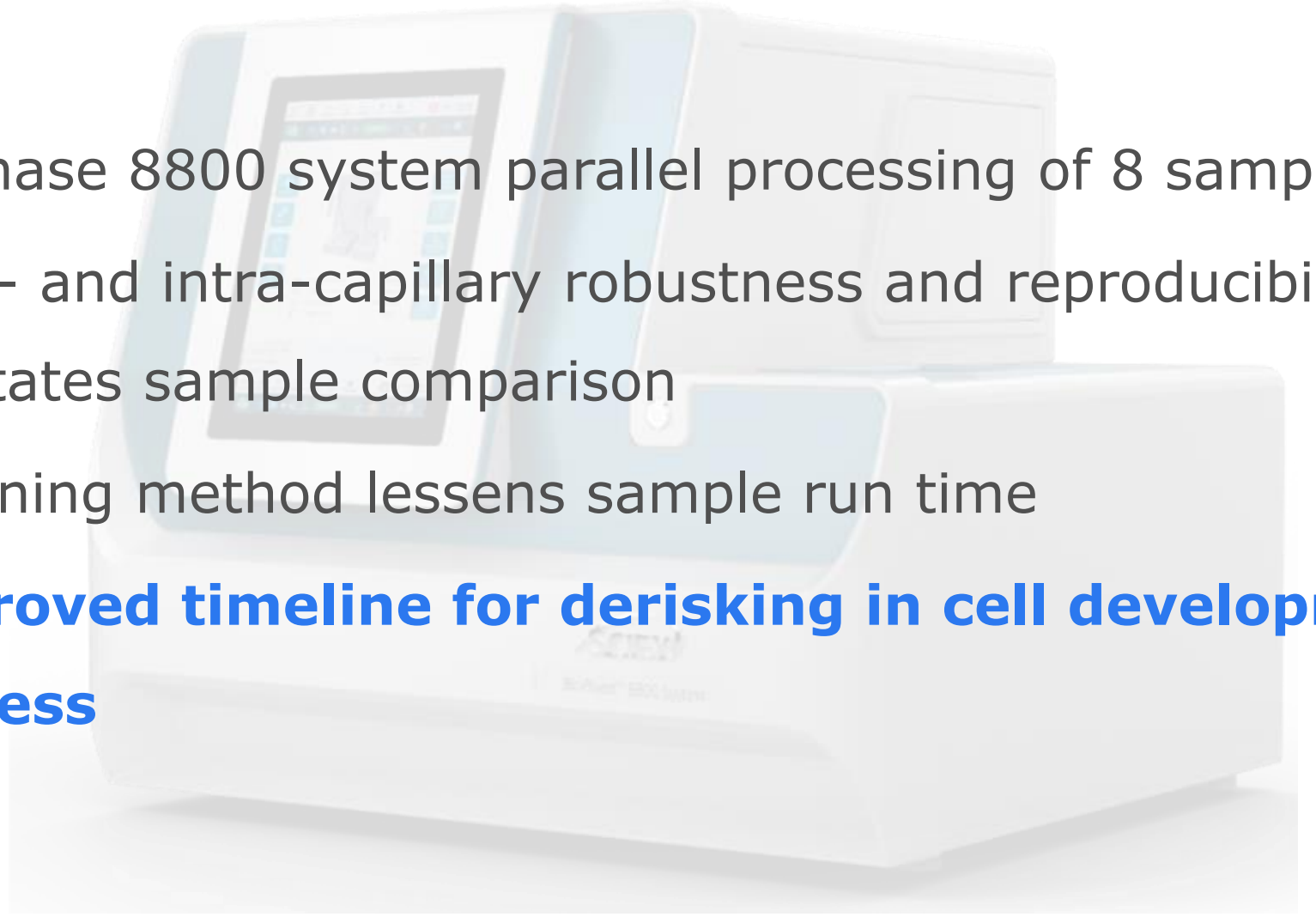
Role of capillary electrophoresis sodium dodecyl sulfate (CE-SDS) in Drug development

- **Cell Line Selection and Process Development**
  - Evaluating multiple cell lines for product quality and titer
  - Characterization of product quality in order to
    - Determine developability for clinical manufacturing
    - Determine Product quality for multiple clones
    - Pick the best clone and back up clone for cell banking
  - Characterization of Low molecular and high molecular weight species to determine yield and purity for downstream manufacturing
  - Late development evaluation of cell line stability
  - Determining the best conditions for cell growth, target protein yield, and maintenance of critical quality attributes
  - Evaluating how purification processes affect cell lines
  - Determining effect of formulations on selected cell lines

Challenges with capillary and microfluidic cSDS technology

- Capillary
  - Long run times
  - Need for increased efficiency due to increased number of molecules coming through the pipeline
- Microfluidic
  - Lack of reproducibility and robustness
- **Need to make more informed decisions in a quicker time frame**
- **Ensure reproducibility across all samples while ensuring the same data quality**

Closing the reliability and robustness gap of CE-SDS



- BioPhase 8800 system parallel processing of 8 samples
- Inter- and intra-capillary robustness and reproducibility facilitates sample comparison
- Lightning method lessens sample run time
- **Improved timeline for derisking in cell development process**

Pushing the envelope of CE-SDS - traditional vs lightning methods

Method	Traditional	Lightning
Base	80 psi 2 min	80 psi 2 min
Base	20 psi 5 min	
Acid	20 psi 5 min	50 psi 2 min
Water	20 psi 3 min	50 psi 1 min
Gel	80 psi 10 min	80 psi 4 min
Run	35 min (NR)/25 min (R) at 15 kV	35 min (NR)/25 min (R) at 15 kV

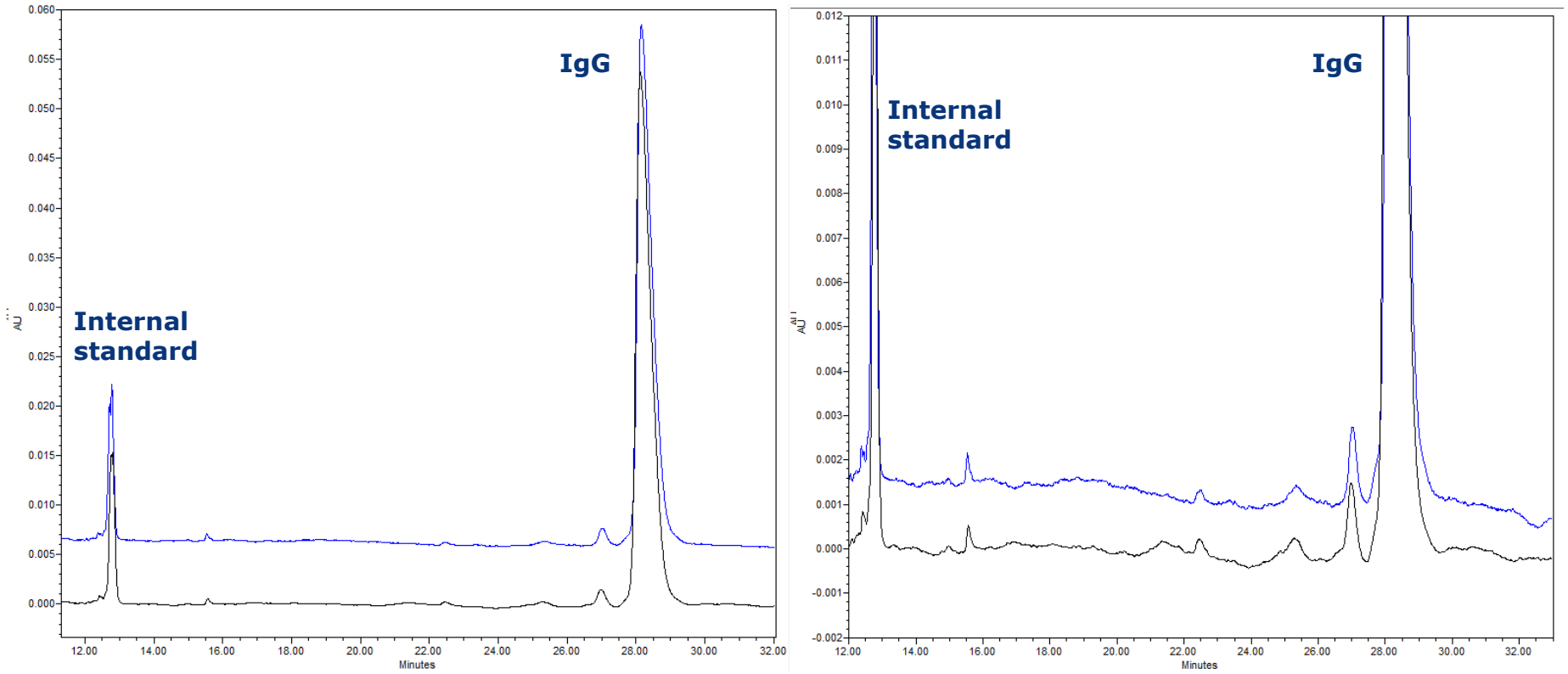
Traditional vs lightning methods timetable

Method type	Sample type	Capillary conditioning time (in minutes)	Separation time (in minutes)	Total cycle time (in minutes)	Throughput in samples per Hour
Traditional	Reduced	25	25	50	9
	Non-reduced	25	35	60	8
Lightning	Reduced	**9**	25	**34**	**14**
	Non-reduced	**9**	35	**44**	**11**

Conditioning time reduced by 64%

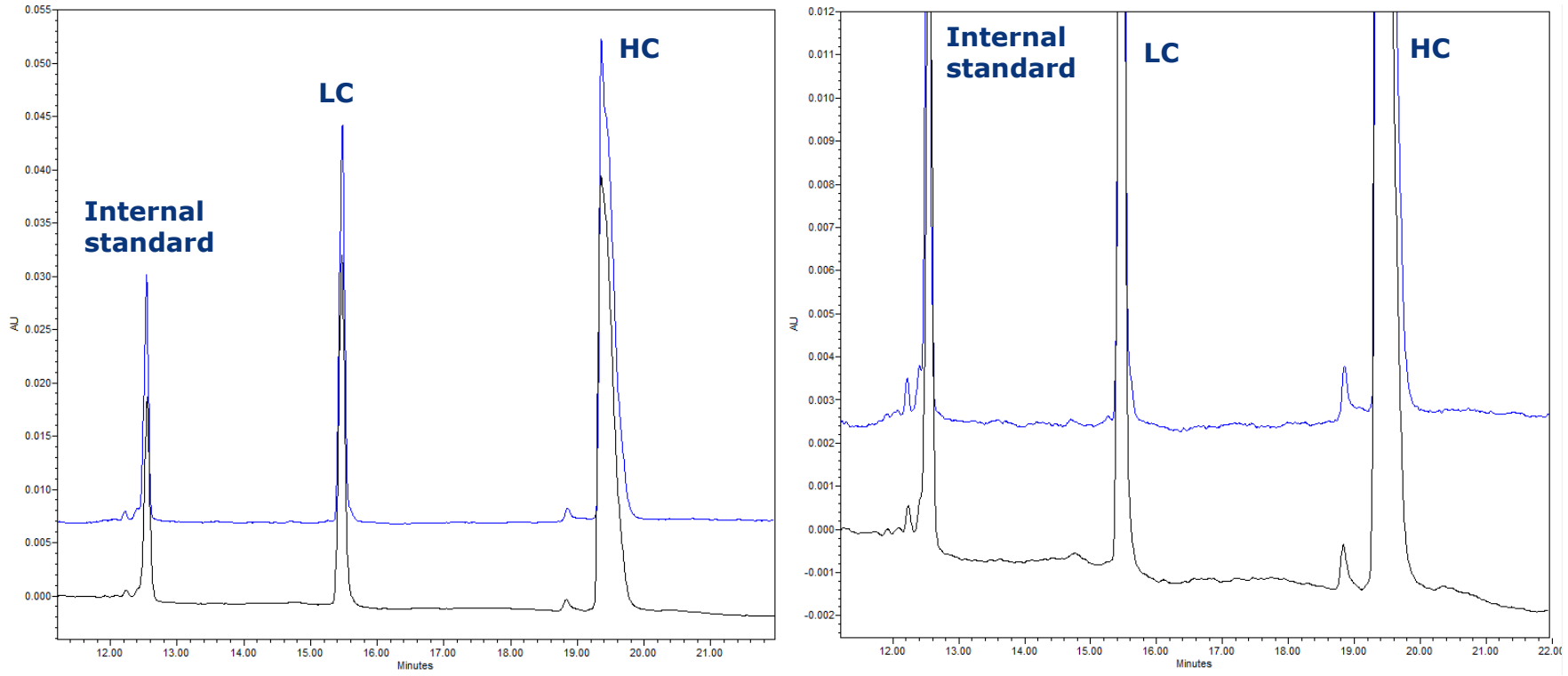
Case Study 1: Springhouse

Lightning method maintains separation efficiency in non-reduced antibody



Blue trace: lightning method; Black trace: traditional method

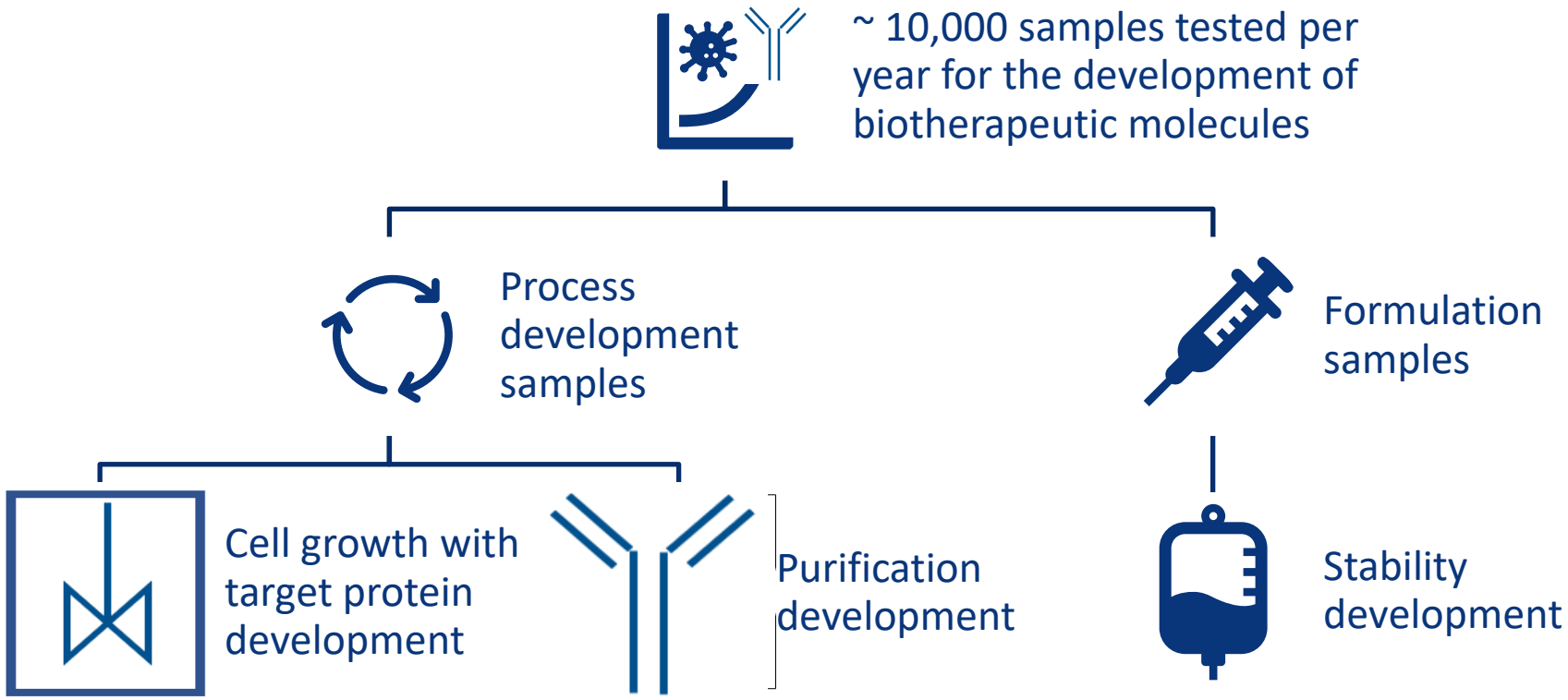
Lightning method maintains separation efficiency in reduced antibody



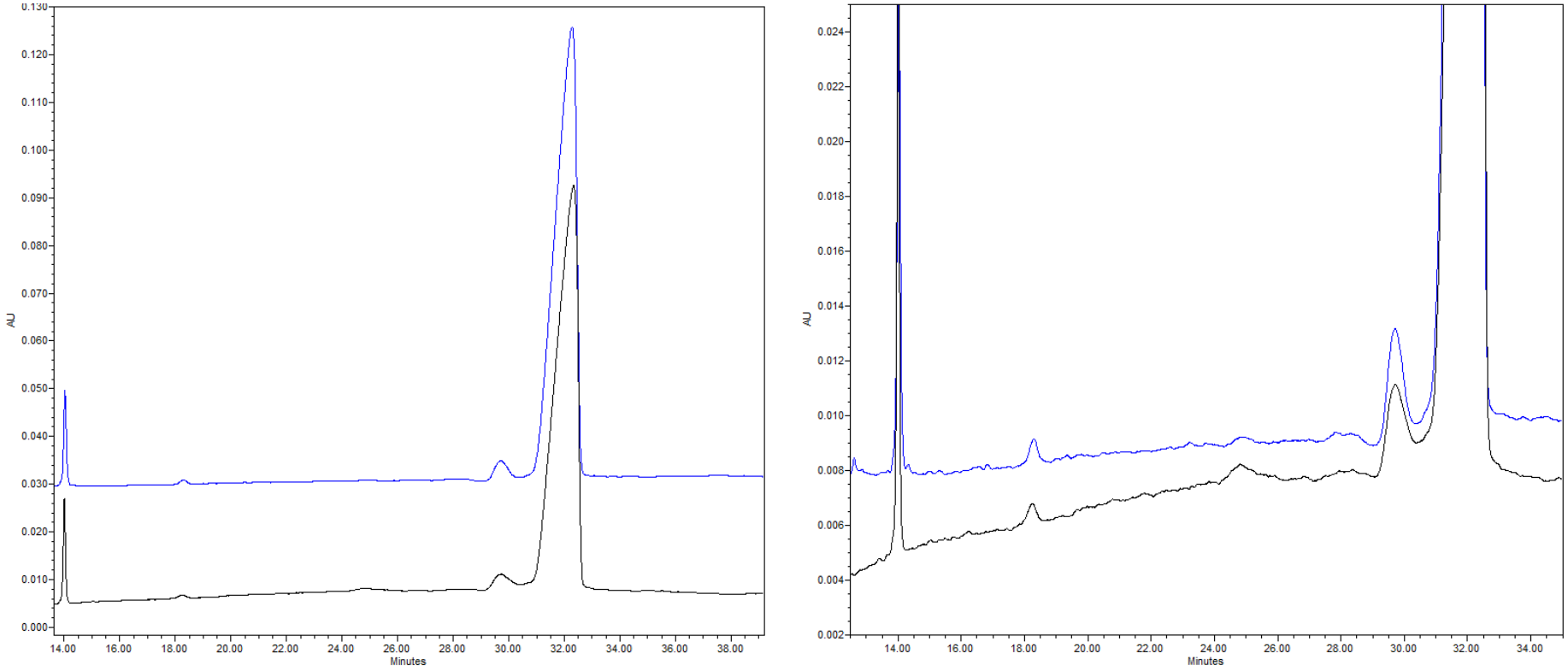
Blue trace: lightning method; Black trace: traditional method

Case study 2: Malvern

Evaluating CE-SDS for reproducibility and reliability for high throughput analysis

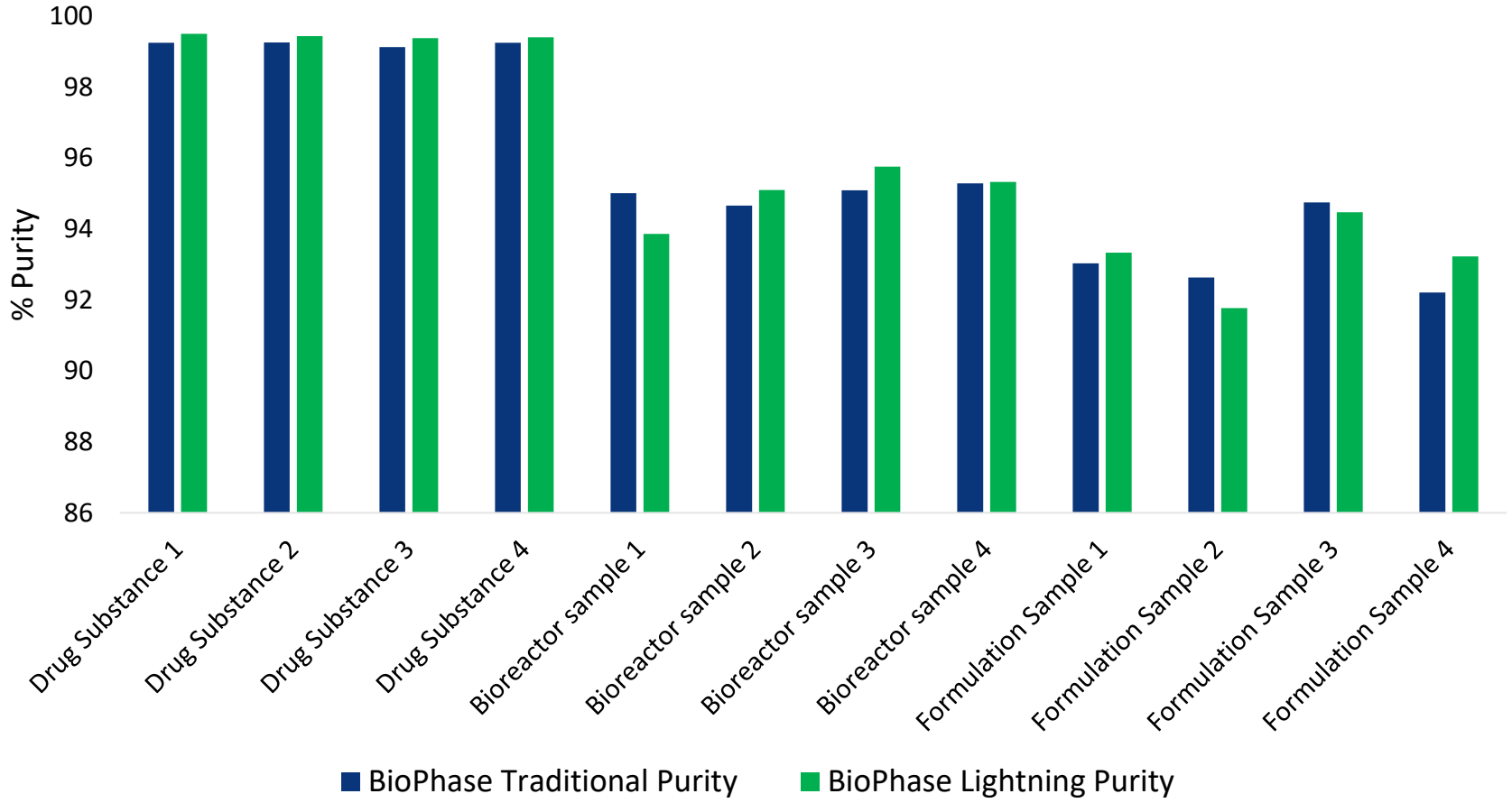


Representative e-grams of a non-reduced mAb CE-SDS overlays of traditional vs lightning methods

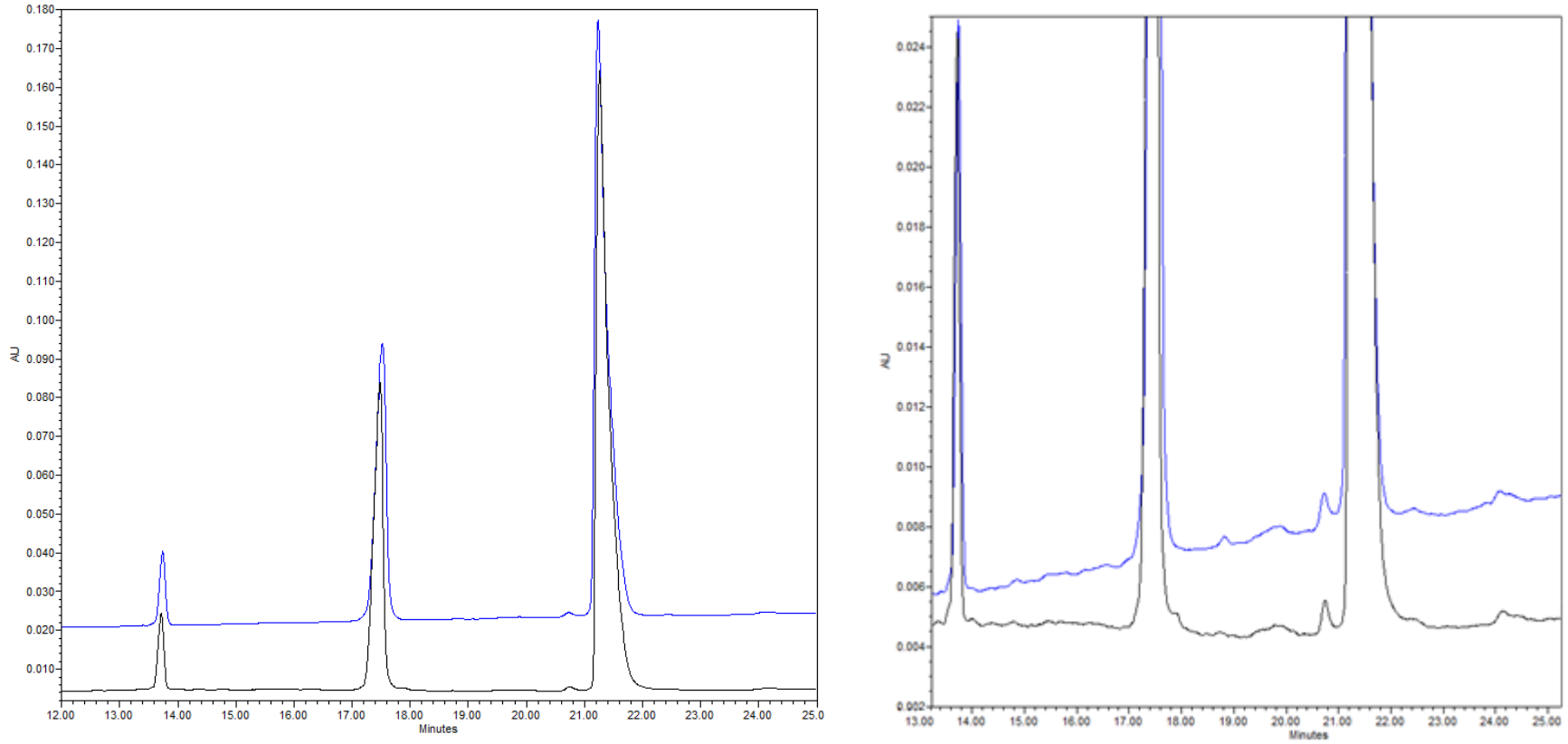


Blue trace: traditional method; Black trace: lightning method

Non-reduced CE-SDS traditional vs lightning methods on the BioPhase 8800 system shows comparable data



Representative e-grams of a reduced mAb CE-SDS overlays of traditional vs lightning methods



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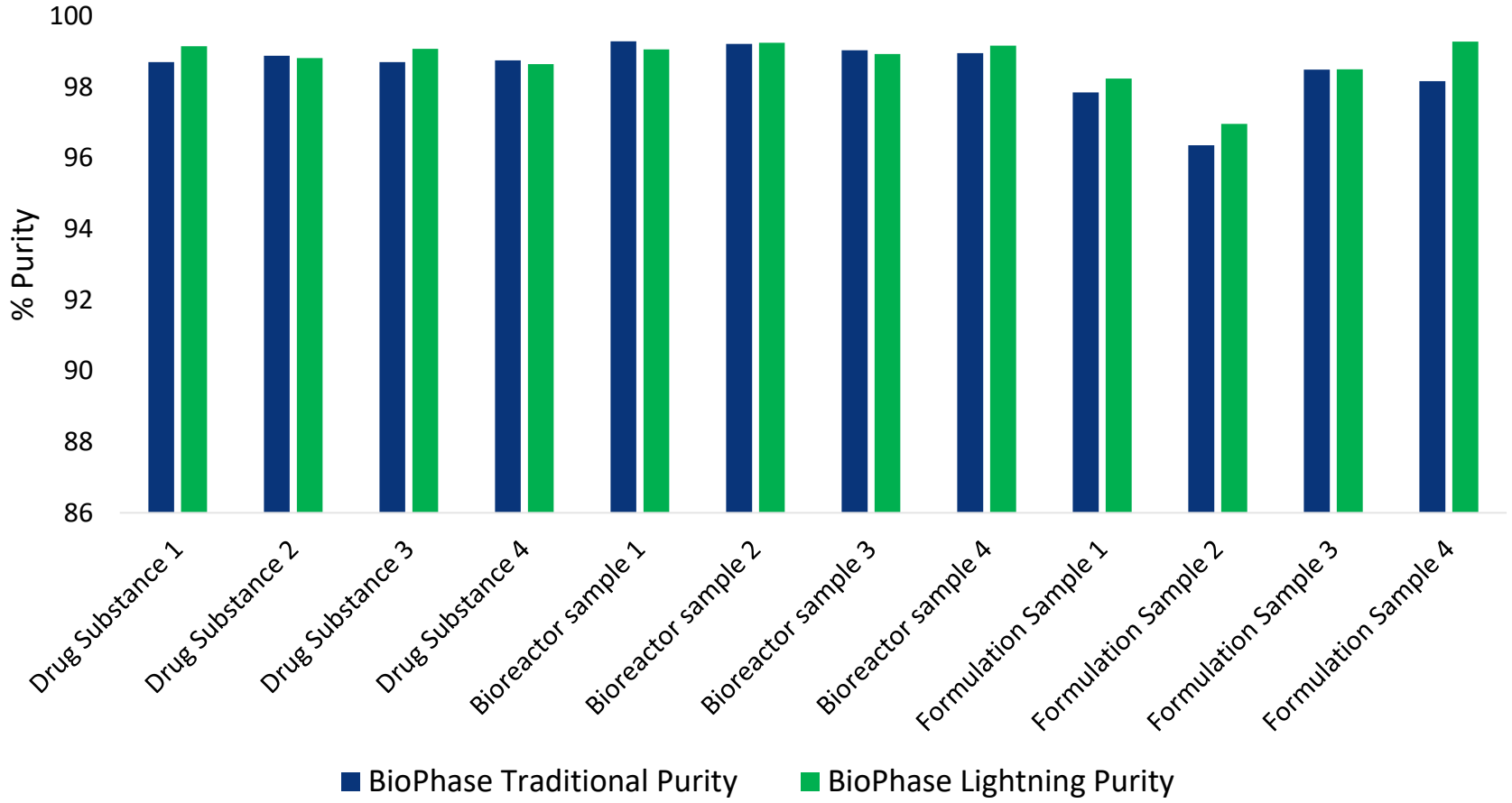
Reduced CE-SDS traditional vs lightning methods on the BioPhase 8800 system shows comparable data



Conclusions

- BioPhase 8800 allows for faster cell line selection and process development
- Lightning method reduces conditioning time by 64%
- Good equivalency across drug substances build confidence in the lightning method

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Please, hold you’re questions until the end

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

Jason Barker[1], Scott Mack[2], Maggie A. Ostrowski[2], Greg Adams[1]

[1]Fujifilm Diosynth Biotechnologies USA
[2]SCIEX

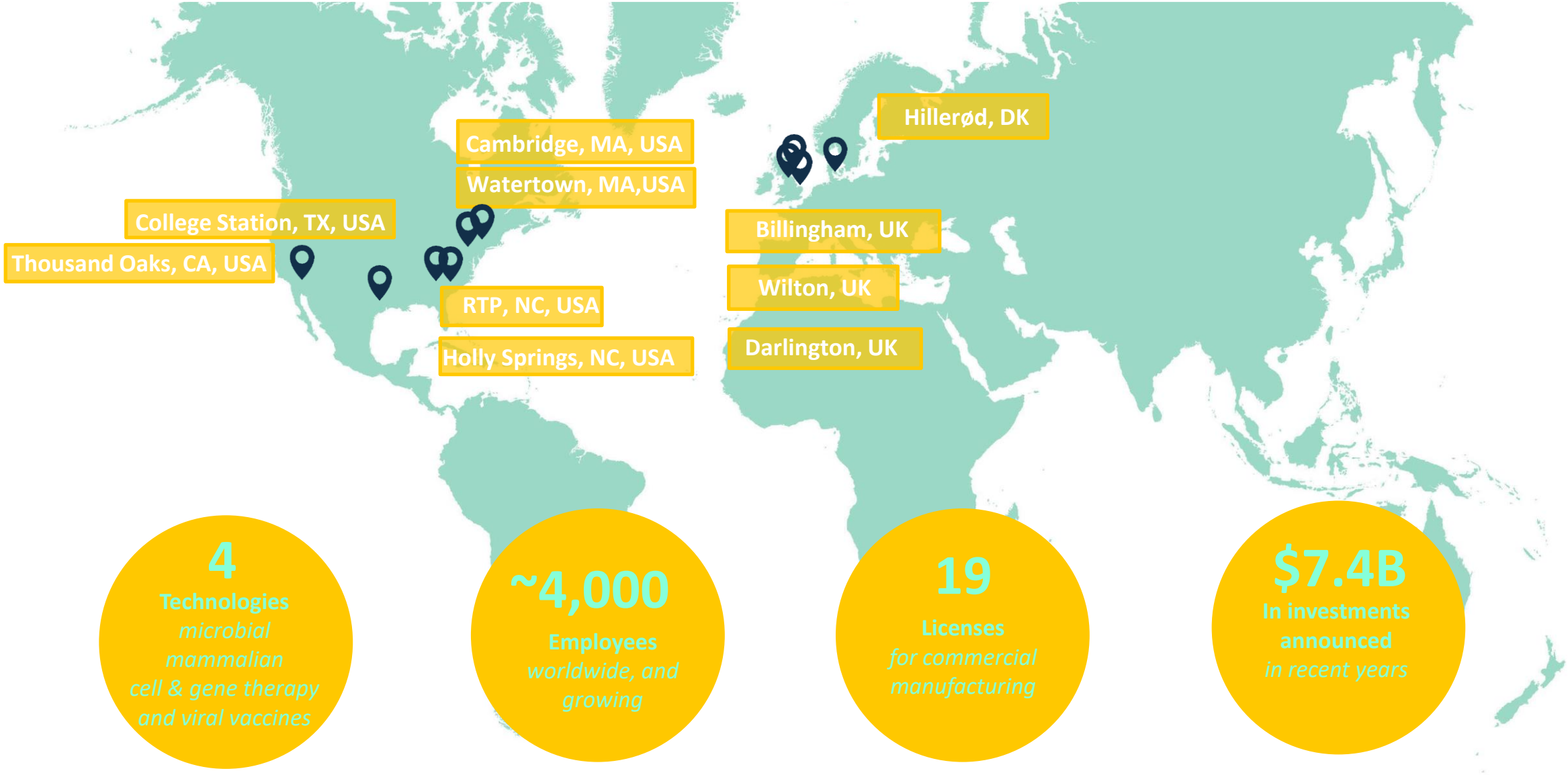
Global vision and core purpose

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



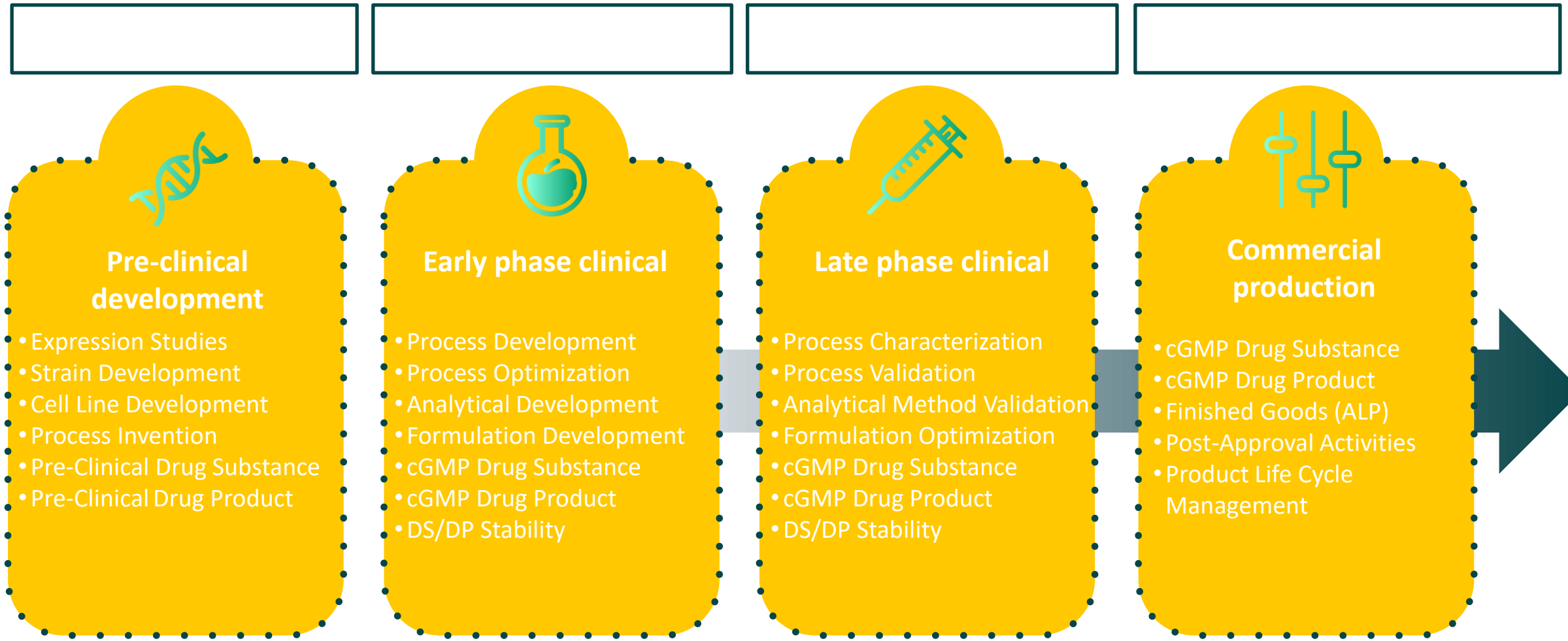
4
Technologies
microbial
mammalian
cell & gene therapy
and viral vaccines

~4,000
Employees
worldwide, and growing

19
Licenses
for commercial manufacturing

$7.4B
In investments announced
in recent years

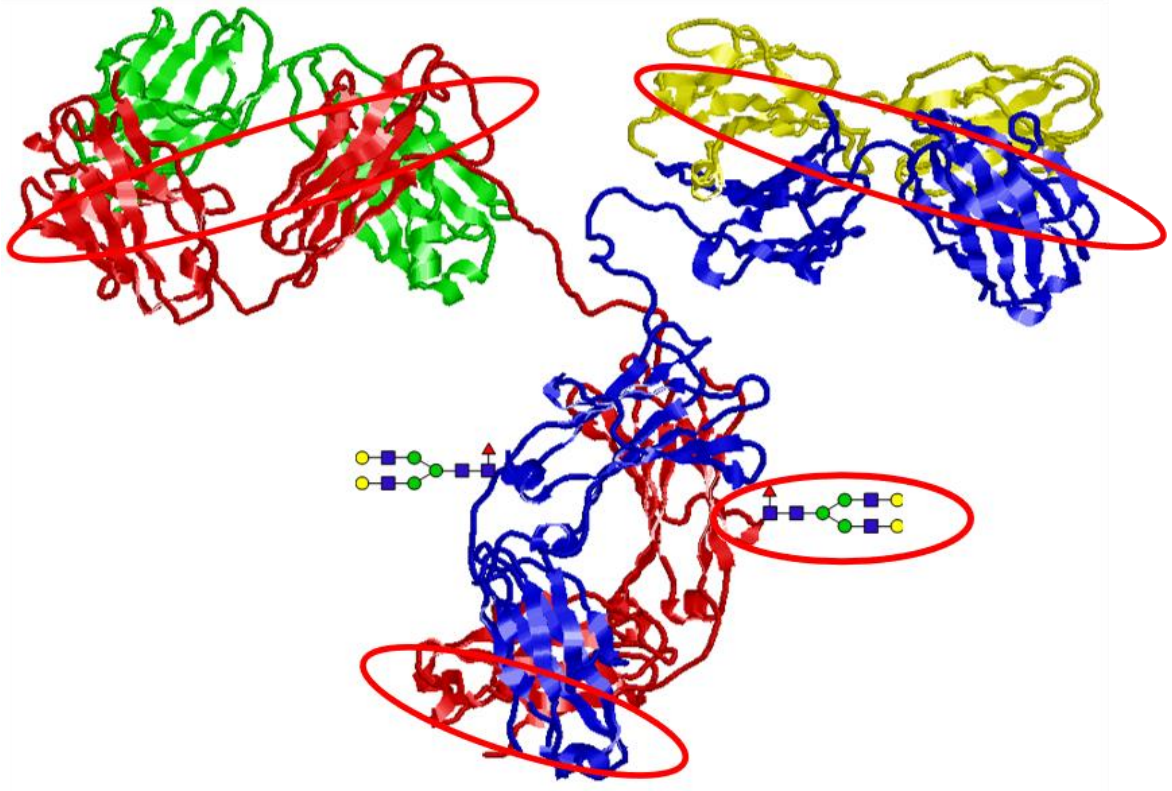
End-to-End CDMO



Introduction

- Charge variant analysis of mAbs is a key component to understanding the heterogeneity of a molecule's physicochemical properties to support the entire development cycle as well as a critical regulatory/quality requirement to ensure patient safety and process consistency.
- The Fujifilm Diosynth platform approach to monitoring charge variants during the development process is icIEF.
- Traditional approaches to characterization of the charged isoforms of mAbs requires laborious fractionation of the species and subsequent LC/MS analysis which may not offer sufficient resolution, may not be directly representative of the icIEF profile and may introduce artifacts.
- The Intabio icIEF-MS system coupled with the 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 demonstrates analysis of biotherapeutic mAb cell culture process development samples to represent characterization of charge variant species by the research breadboard Intabio icIEF-MS system.

mAb analysis 101 – charge variants



Acidic variants

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

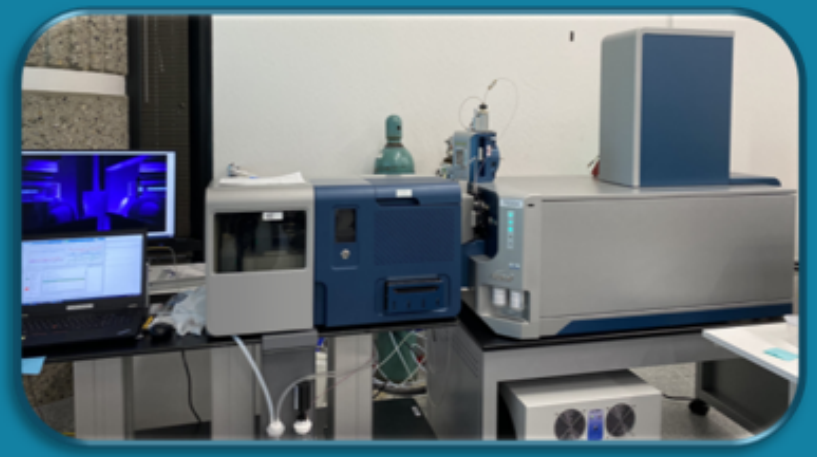
Basic variants

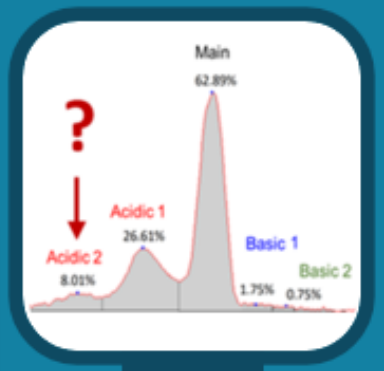
- C-Terminal Lysine Truncation
- N-terminal pyroglutamate
- Succinimide
- Oxidized species
- Aglycosylated species

- *Imaged capillary isoelectric focusing (icIEF) is the Fujifilm Diosynth mAb platform charge variant assay*
- *Automated Protein A purification miniprep is used to enable analysis of upstream cell culture samples*

Intabio iclEF-MS system

Up to 30 minutes per sample*





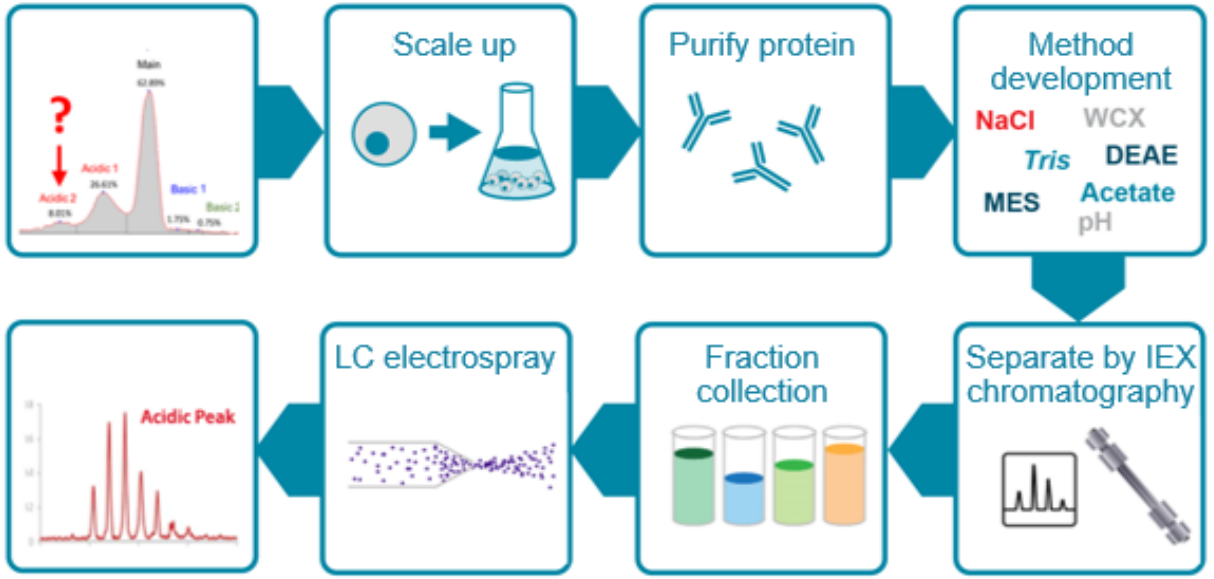
Characterization by MS

*Data from research breadboard

vs.

Current workflow for charge variant peak ID

Weeks per sample



Confirmatory clEF analysis

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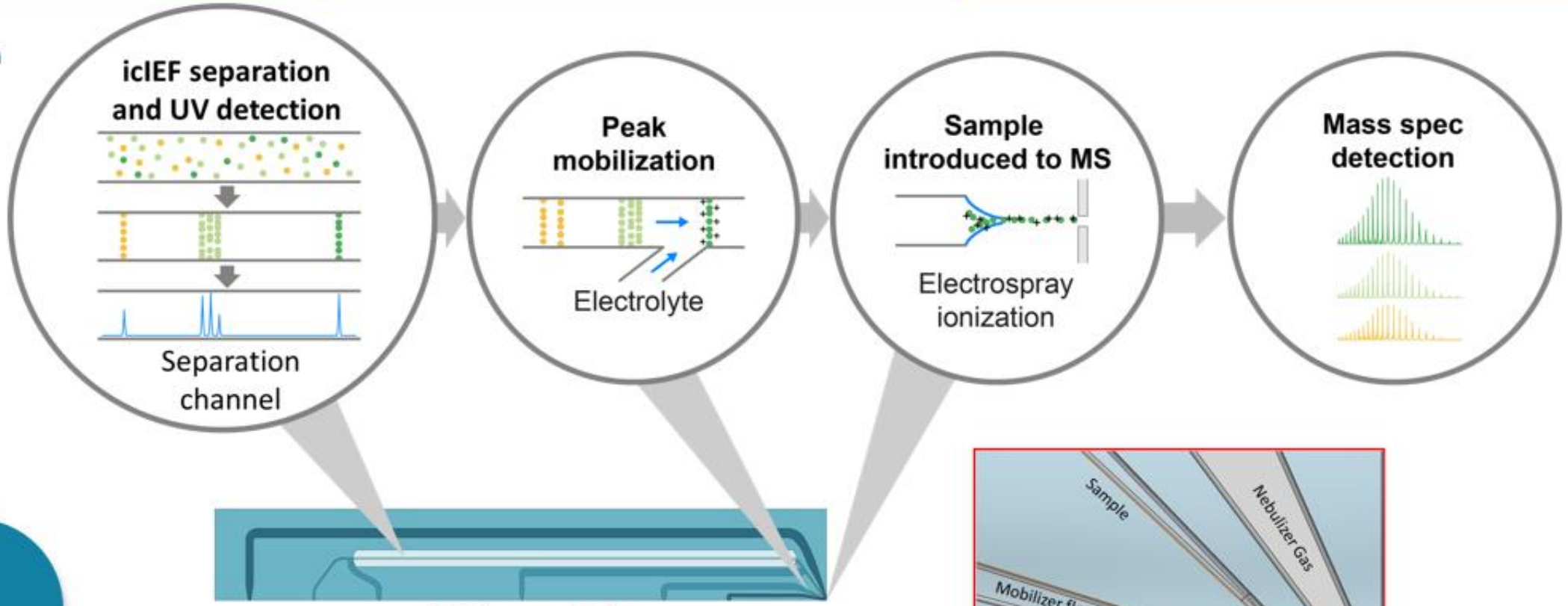
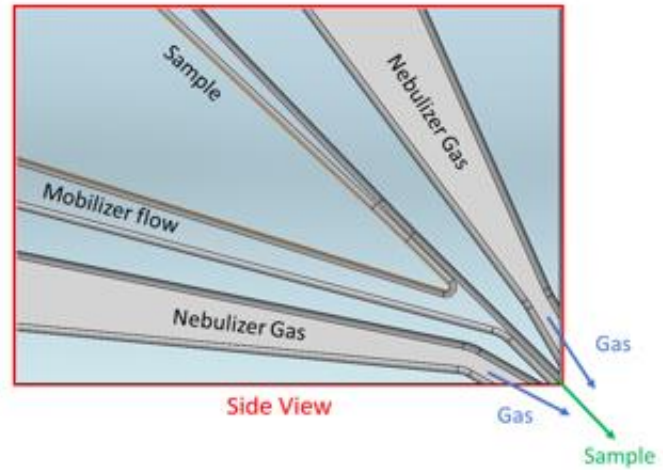
Microfluidic chip-based integrated icIEF-MS technology integrates key analytical functions

- 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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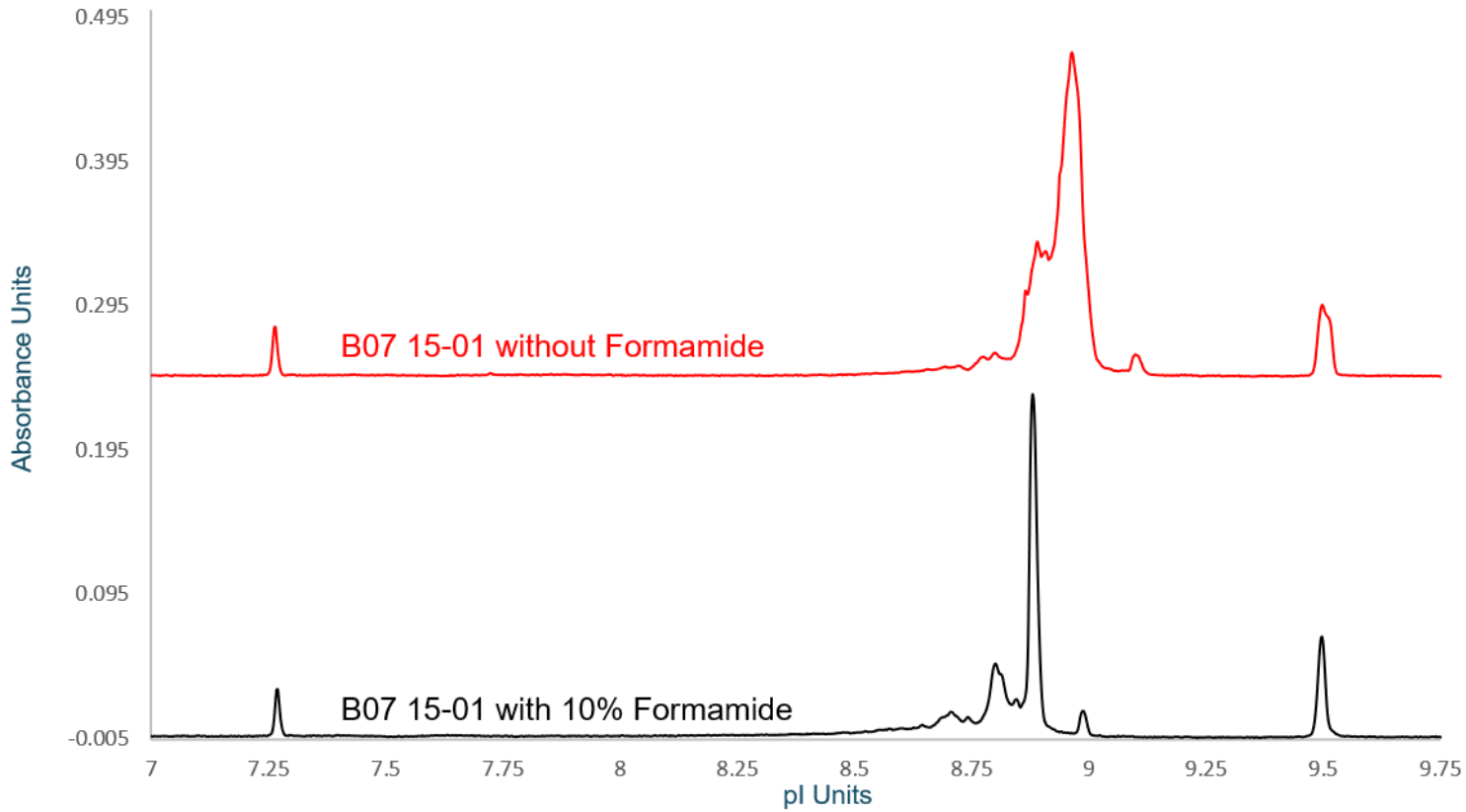
icIEF-MS analysis parameters

- 3% Pharmalyte 8 to 10.5 and 1% Pharmalyte 3 to 10
- 10.0 mM Arginine
- 2.5 mM Iminodiacetic Acid
- 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

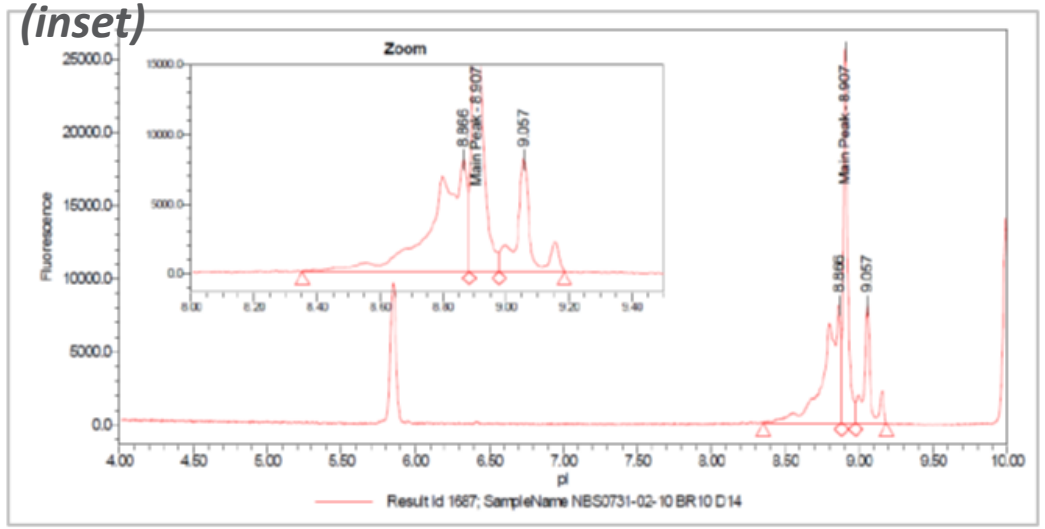


*Data collected from research breadboard

The $64,000,000 Question:
How does icIEF-MS compare to icIEF on the Maurice?

FUJIFILM Diosynth biotechnologies

icIEF with Fluorescence Detection on Maurice (inset)



icIEF-MS UV

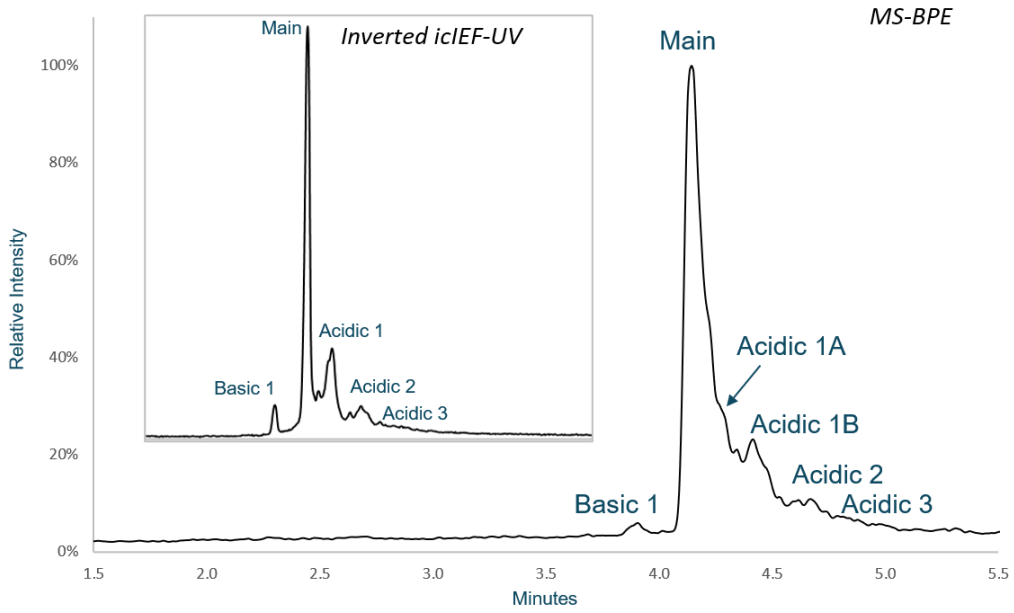
	icIEF (Maurice)	icIEF-MS UV
Main	pI = 8.91	pI = 8.86

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

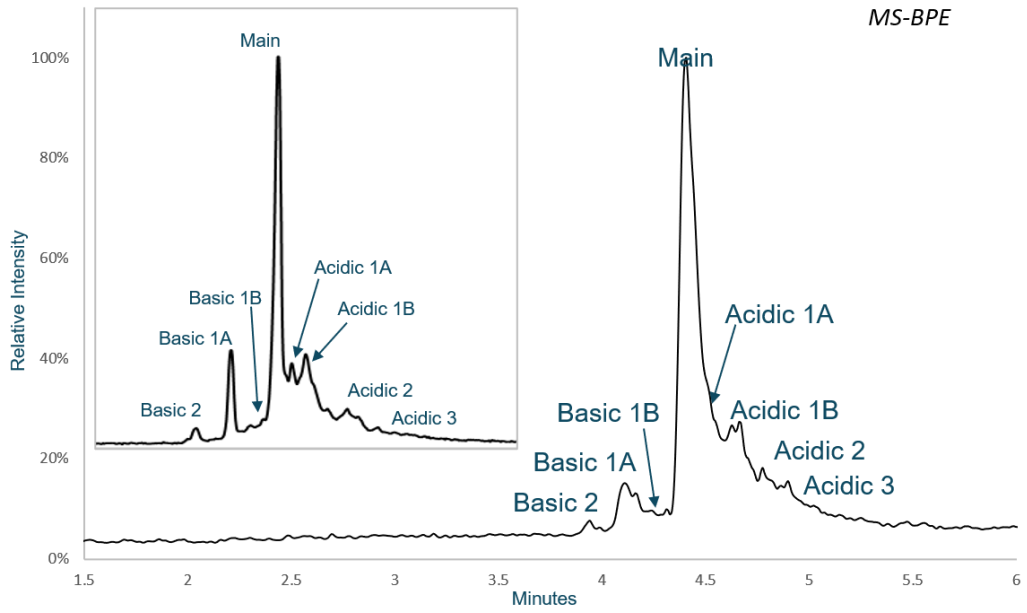
*Data collected from research breadboard

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%



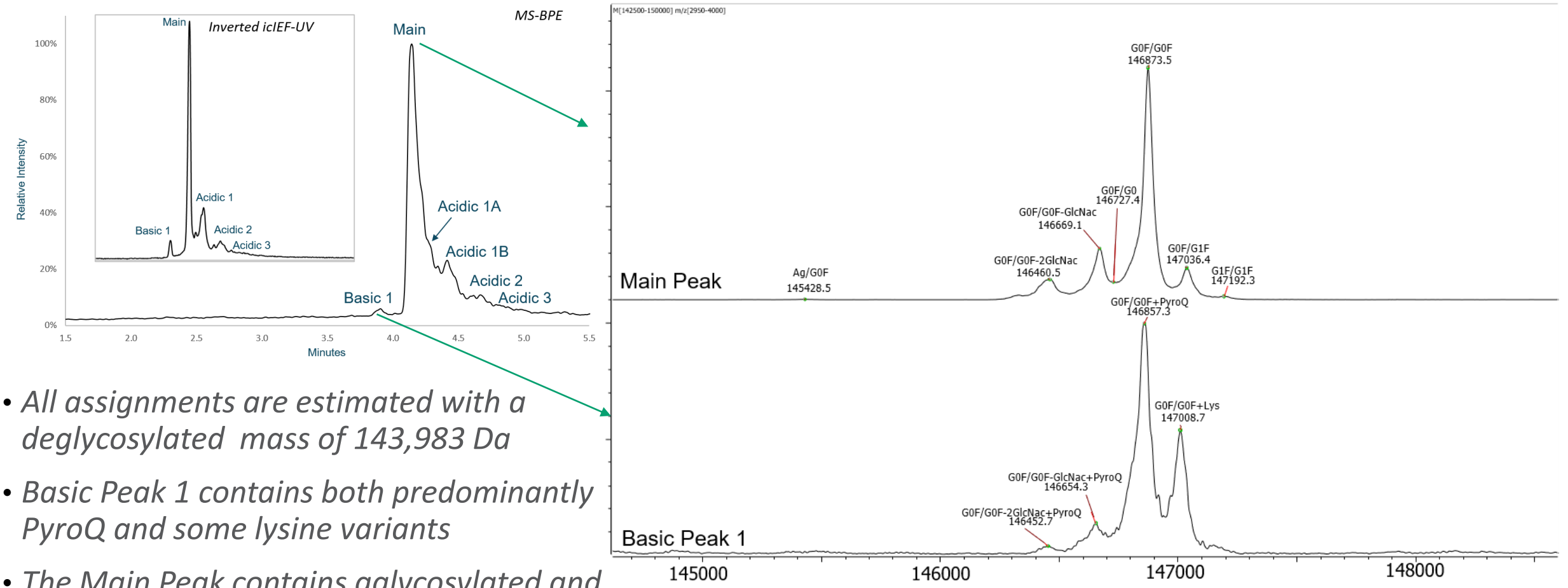
Low basic content sample B7 1501



High basic content sample B10 1502

*Data collected from research breadboard

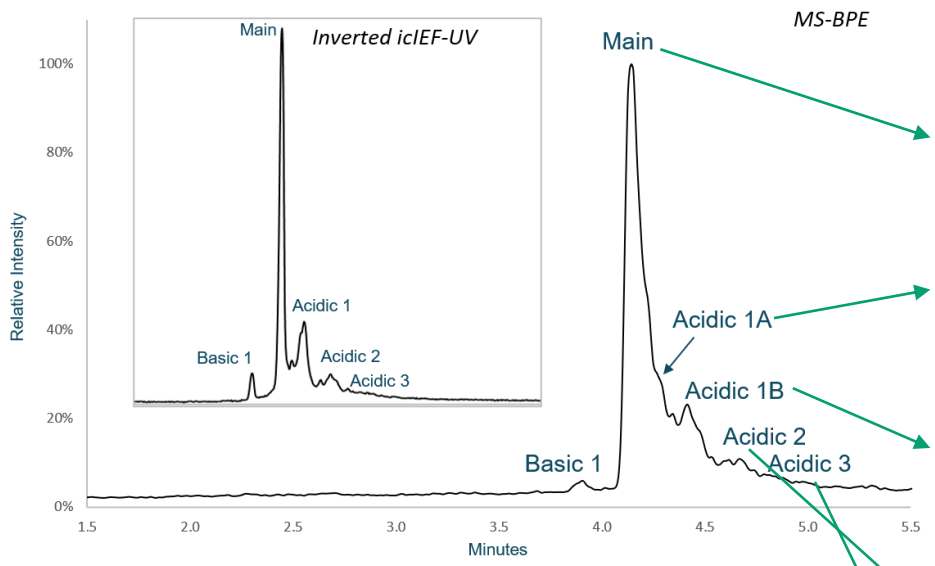
Low basic content samples: basic variants and main species

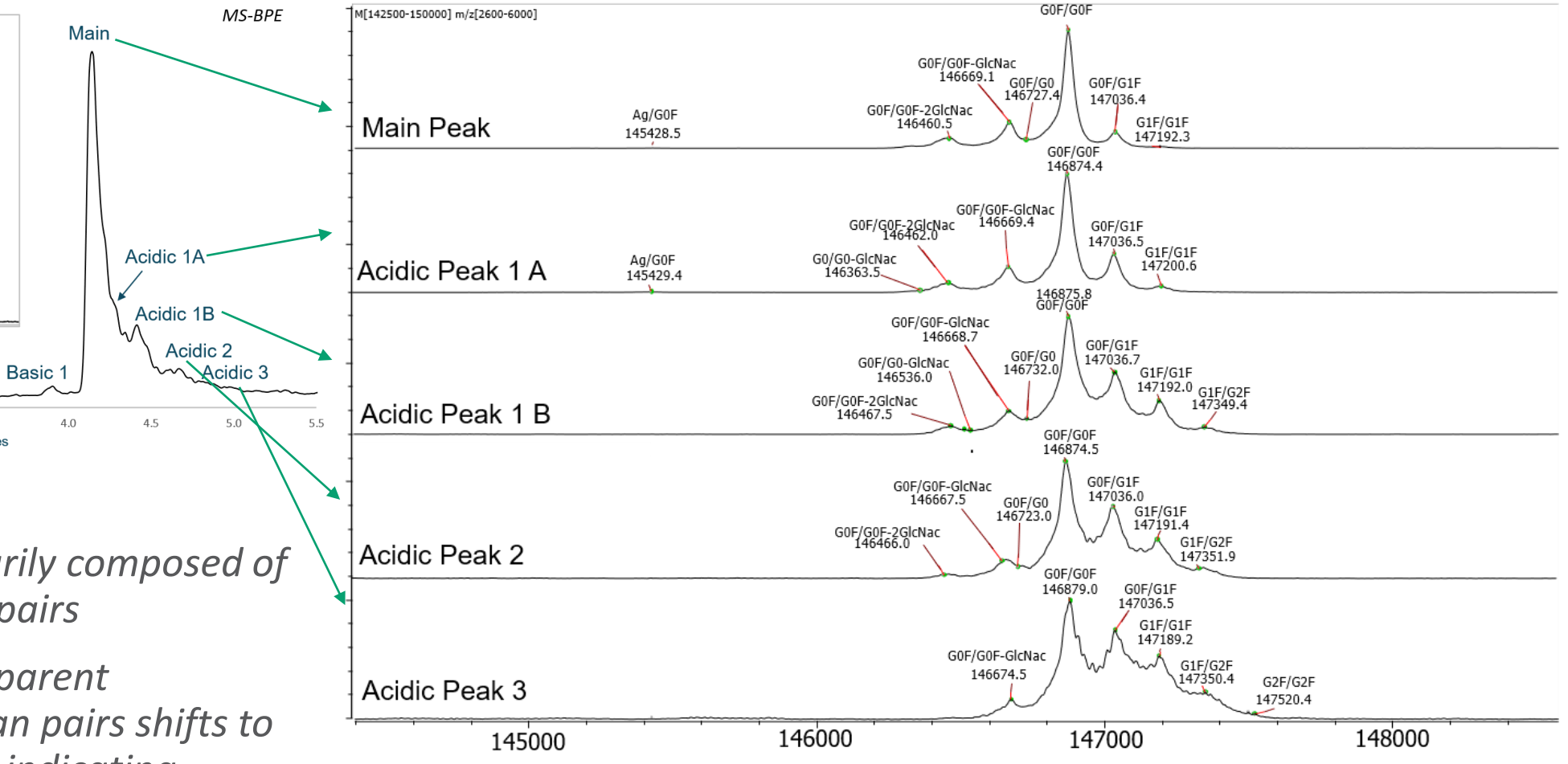


- *All assignments are estimated with a deglycosylated mass of 143,983 Da*
- *Basic Peak 1 contains both predominantly PyroQ and some lysine variants*
- *The Main Peak contains aglycosylated and complex glycans including –GlcNac and -Fuc*

*Data collected from research breadboard

Low basic content samples: acidic variants

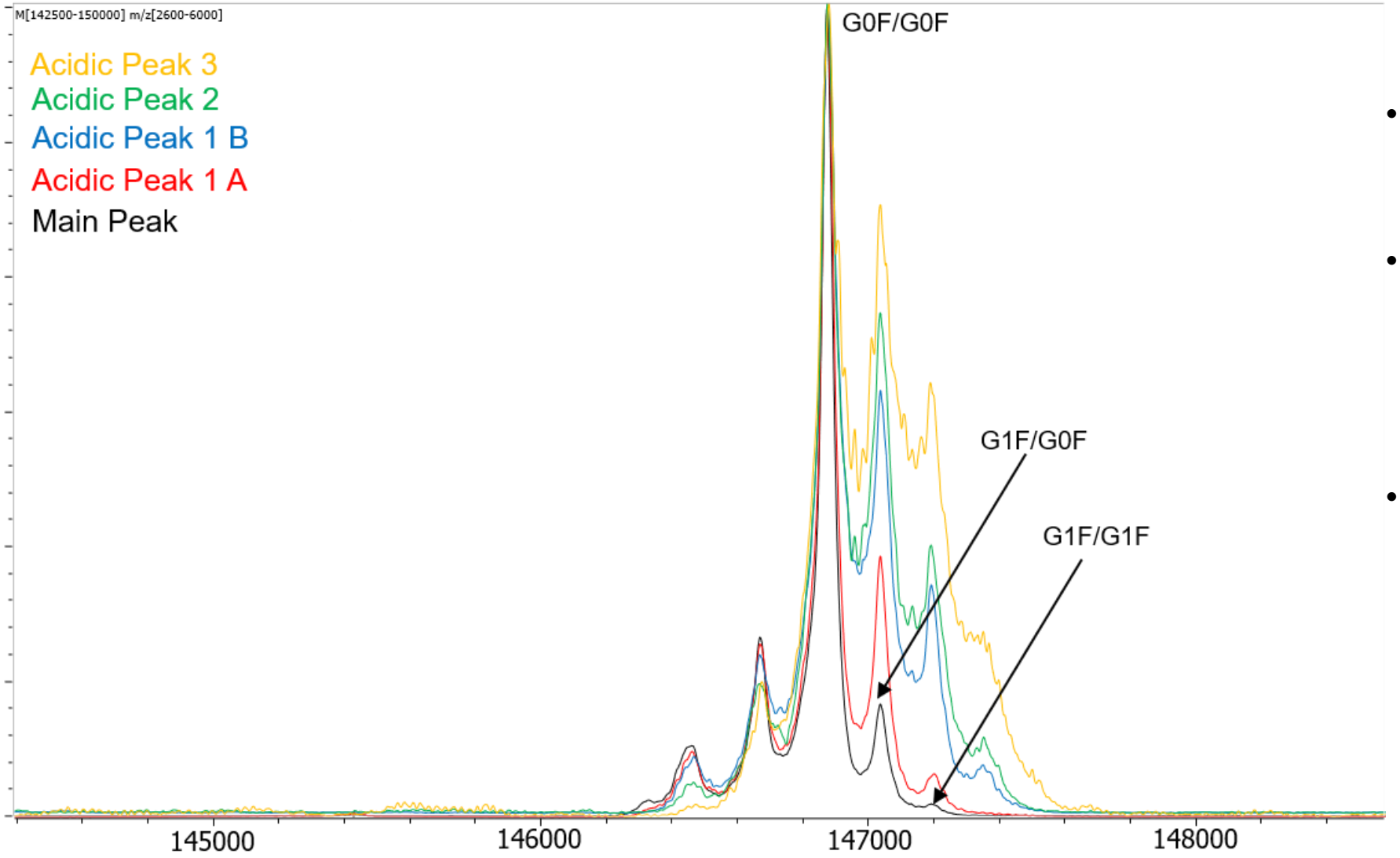




- *The Main Peak is primarily composed of G0F-containing glycan pairs*
- *As pI decreases, the apparent distribution of the glycan pairs shifts to higher order structures indicating glycation, a 162 Da modification of lysine*

*Data collected from research breadboard

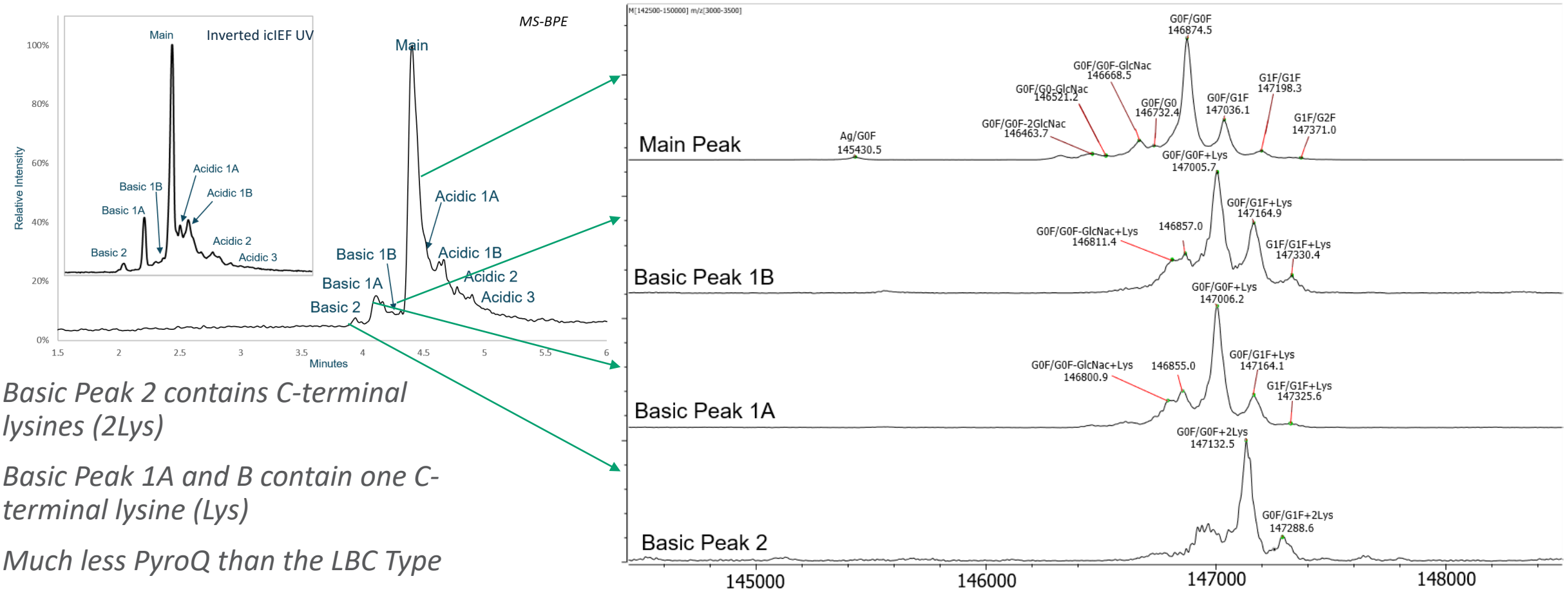
Acidic variants are mostly glycated species



- *The complex glycan structure of the Main Peak is predominately G0F/G0F*
- *As pI decreases from Acidic Peak 1A to Acidic Peak 3 the relative abundances of glycan pairs shifts to higher mass*
- *The pI-dependent shift in the glycoprofile is most likely due to glycated lysines – a 162 addition of a hexose sugar to the lysine residue.*

*Data collected from research breadboard

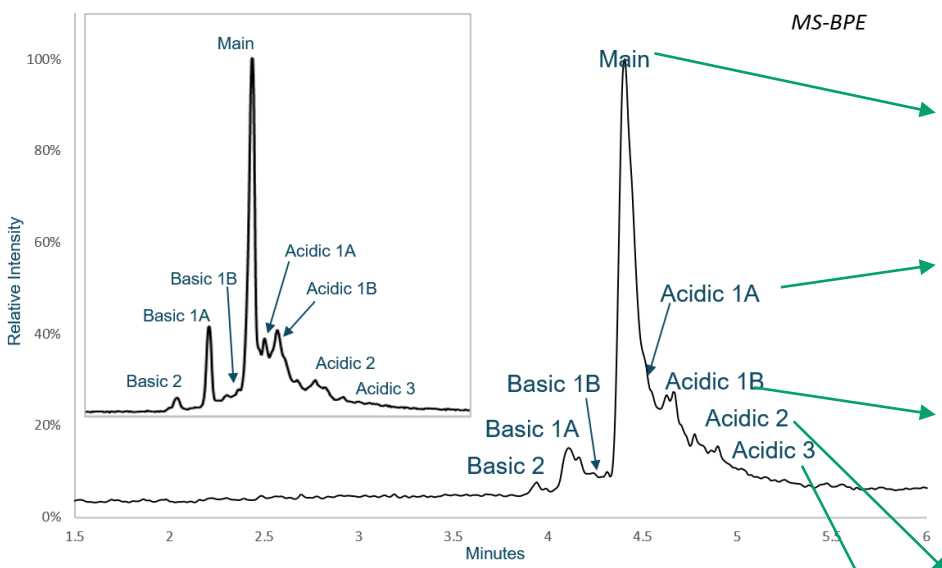
High basic content samples: basic variants and main species

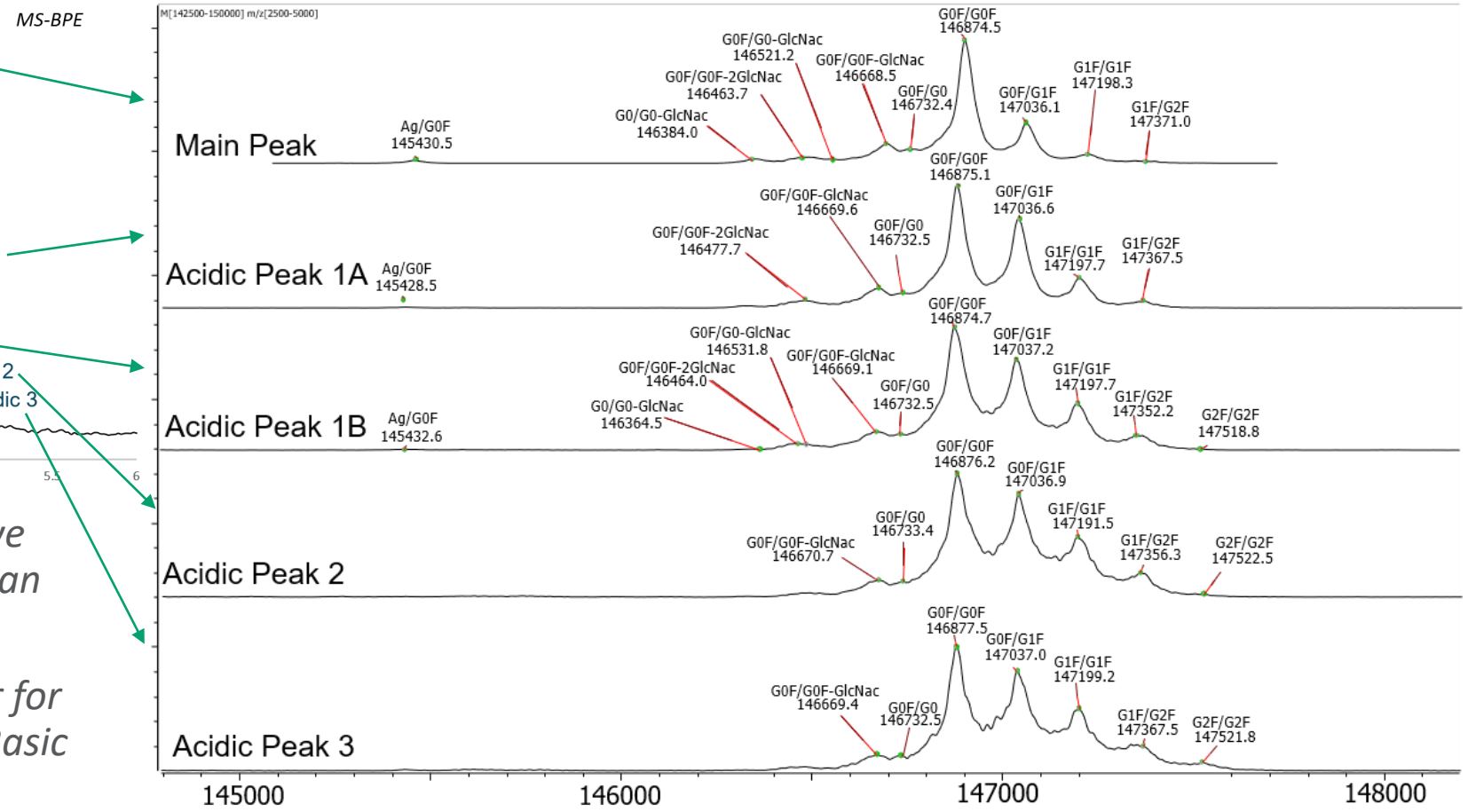


- *Basic Peak 2 contains C-terminal lysines (2Lys)*
- *Basic Peak 1A and B contain one C-terminal lysine (Lys)*
- *Much less PyroQ than the LBC Type*
- *The change in apparent relative abundances in glycan pairs indicates Basic Peak 1B has convoluted glycation*

*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*

*Data collected from research breadboard

Conclusions

- The research breadboard Intabio icIEF-MS system coupled with the ZenoTOF 7600 system enabled the characterization of charge variants from 8 cell-culture samples in less than a day. A traditional fraction collection approach would have taken multiple weeks, with the possibility of introducing sample stability/handling artifacts during the process.
- Comparable icIEF profiles were observed with the separation on the research breadboard Intabio
  icIEF-MS system and traditional icIEF separation 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.
- Biopharmaceutical product development is all about product knowledge, whether you are writing a regulatory submission, investigating unexpected results, or performing extended characterization. The research breadboard Intabio icIEF-MS system is an emerging tool with the potential to dramatically simplify charge variant characterization.