Charge heterogeneity characterization and peak identification of complex protein therapeutics using the SCIEX Intabio ZT icIEF-MS system

Rick Linkous, Mingjie Cui, Rodrigo Vargas Eguez, Trust Razunguzwa, Kristin SchultzKuszak

Analytical Sciences, Biopharmaceutical Development, AstraZeneca, Gaithersburg, Maryland, USA

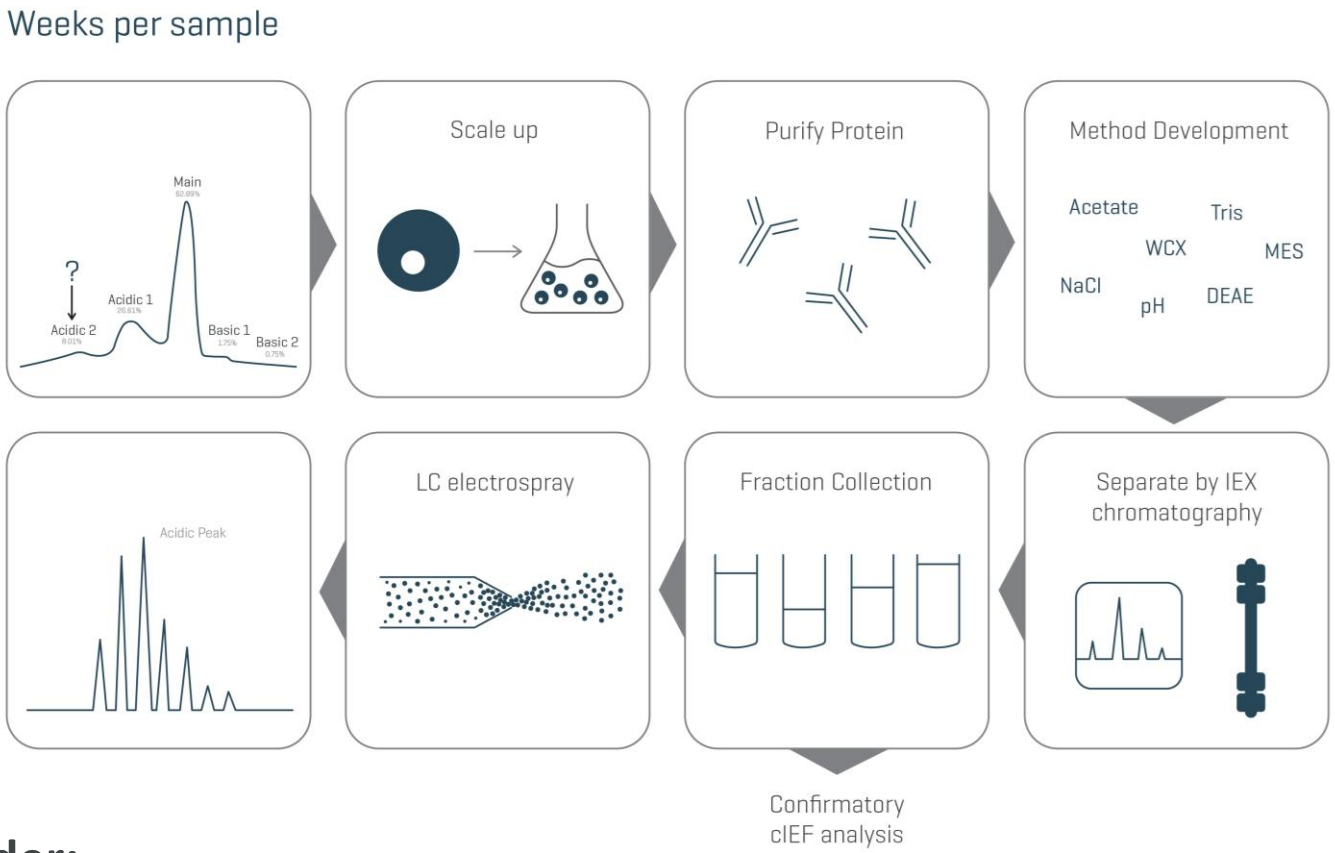
CASSS CE Pharm, 28-Sep-2026



Charge Heterogeneity Assessments Play a Critical Role in Product Development

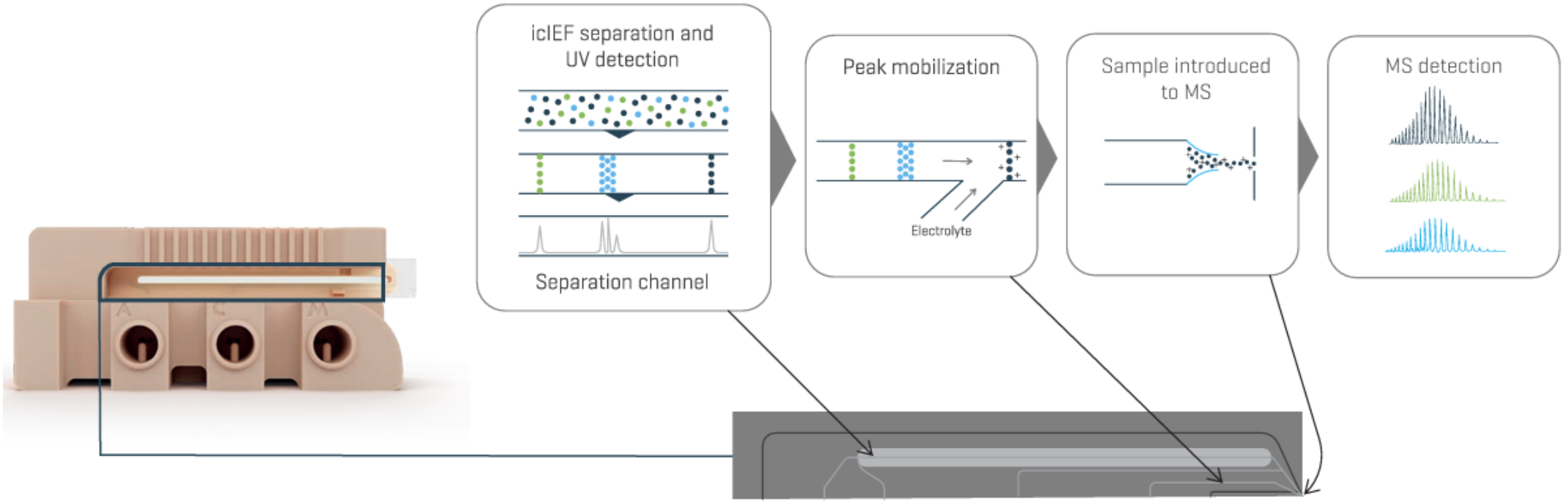
- Charge heterogeneity is present in most biopharmaceutical protein products
  - The pKas of the constituent amino acids along with post translation modifications impart charge
  - In some instances, critical quality attributes are monitored by these methods (i.e. deamidations, sialyation, Fab isomerization, ADC conjugation, fragmentation…)
  - Charge heterogeneity methods give a snapshot into process consistency
- Isolation and identification of charge variants is an important part of product characterization and manufacturing control strategy development
- Current solutions:
  - Multiple IEC-HPLC fractionation then pooled
  - Multiple Maurice Flex for cIEF fractionation then pooled
  - Native IEC-MS
  - CZE-MS
  - cIEF-MS

What Can We Do When icIEF Does Not Connect to MS?



- **Issues to consider:**
  - Sample abundance
  - Sample stability
  - Turnaround time for the characterization
  - MS method resolution and sensitivity

Sciex's IntabioZT Microfluidic Chip icIEF-UV/MS



icIEF separation
Real-time UV absorbance imaging

~30-min sample analysis

High-sensitivity and high-resolution MS data from ZenoTOF 7600 system

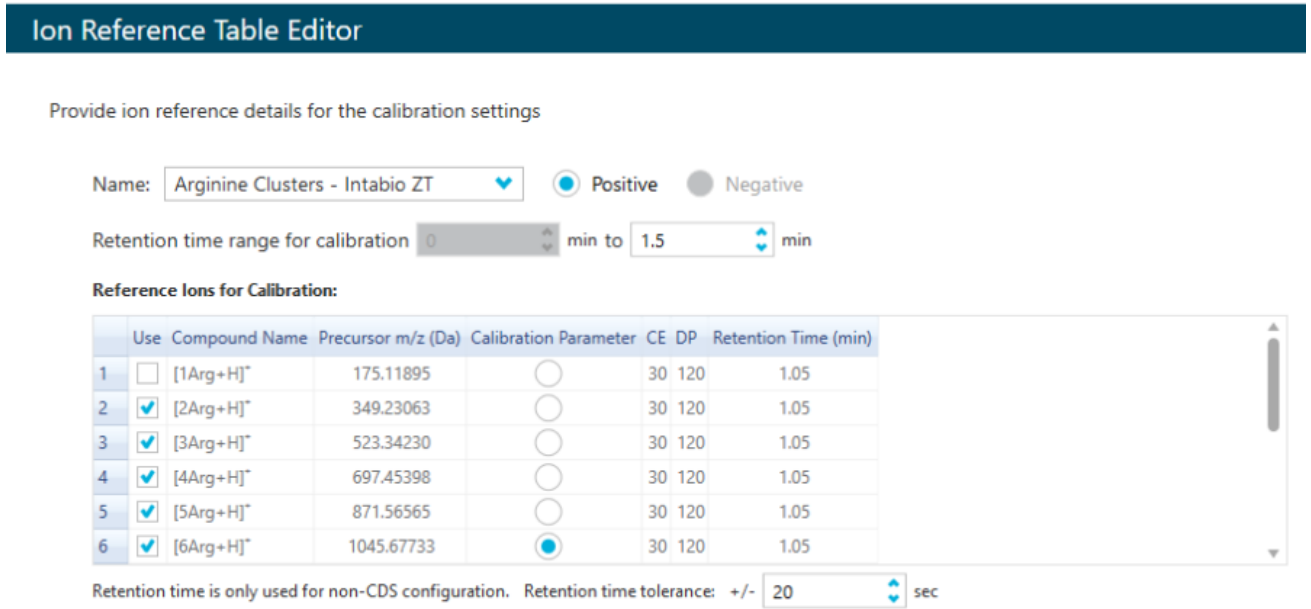
Biologics Explorer software for data analysis

Sample preparation and method for IntabioZT

- Most samples use pharmalytes 3% 5-8, 3% 8-10.5. Formamide is used for denaturing if needed. All samples desalted before master mix
- Voltages: 1500 V 1 min, 3000 V 2 min, 4500 V 4 min focusing, followed by 3500 V 10 min mobilization to MS
- Used both Arginine and Iminodiacetic Acid (IDA) as anodic and cathodic blocker
  - Arginine also used for MS calibration
- Default ZenoTOF Intabio intact mass setting for MS acquisition (mass envelope adjustment if needed)

MS calibration

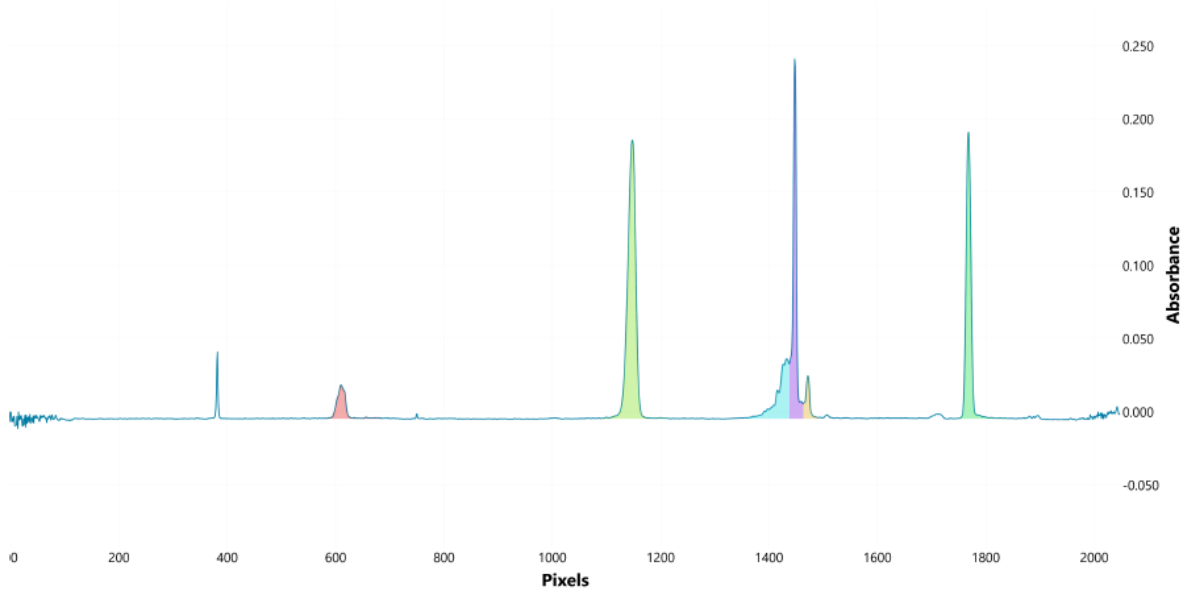
- Mass calibration by Arginine cluster (349-1045 m/z, Intabio cathodic blocker) in first 1.5 min of every single injection.
- <5 ppm for all major components in the on-site testing



NIST mAb

- Used as system test
- UV profile as expected

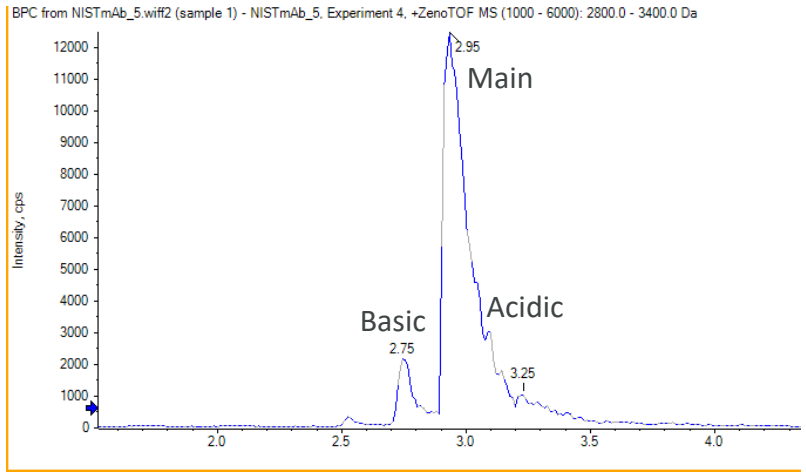
Run: 20250610 - NISTmAb_Test
Injection # 1 NIST mAB



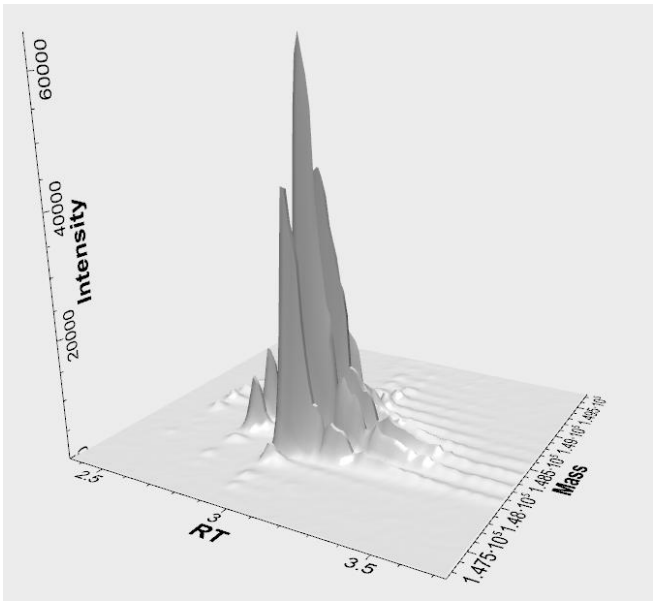
Peak Table

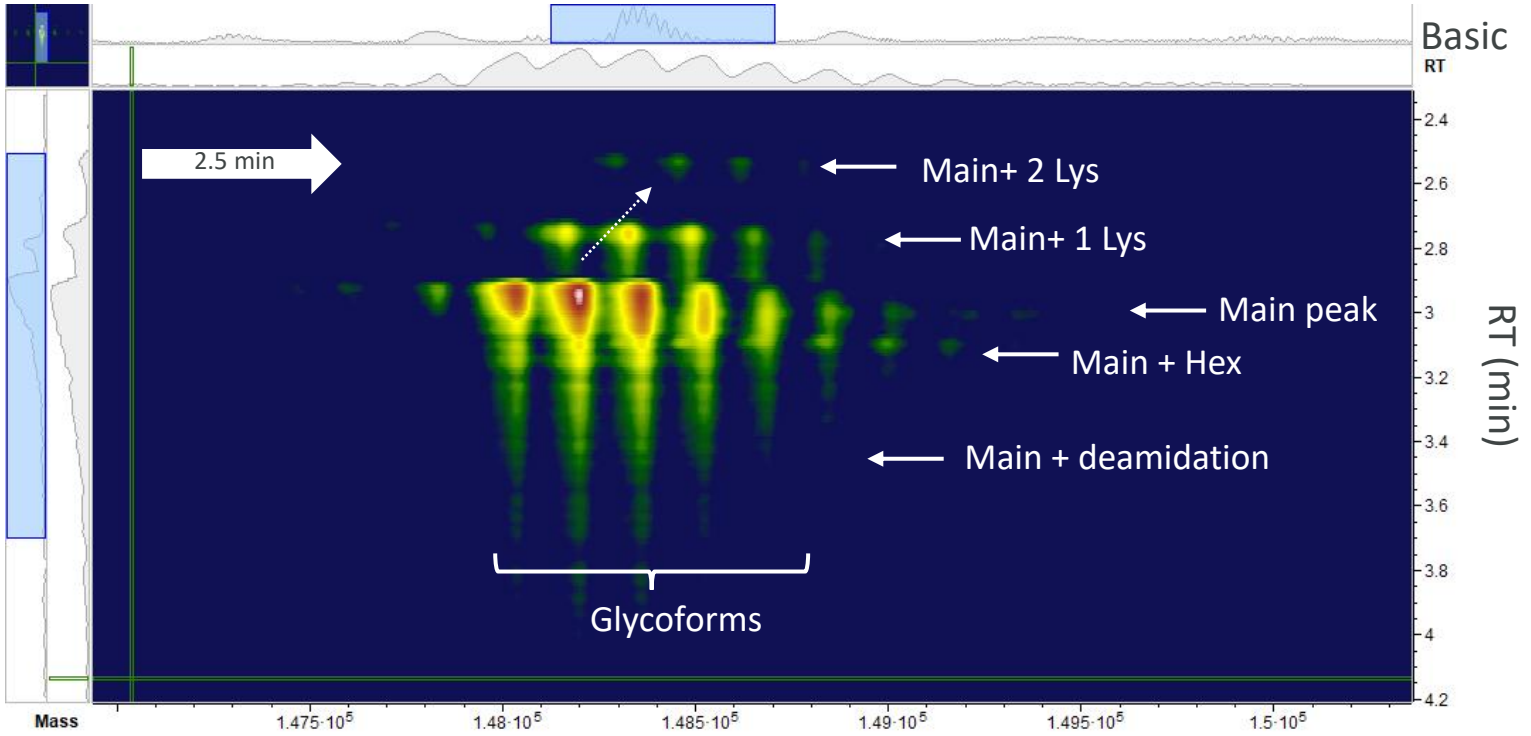
Peak Name	Pixel	pI	Height	Area	FWHM	Resolution	S/N	Start Pixel	End Pixel
-	610	7.0203	0.0182	0.4247	17.52	18.974	70.35	583	683
8.4 Marker	1148	8.4	0.1855	3.2849	15.84	8.926	582.33	1090	1215
-	1431	9.1258	0.036	0.9569	21.46	0.68	124.98	1358	1437
Main Peak	1447	9.1668	0.2409	1.9303	6.24	2.07	751.9	1437	1463
-	1471	9.2283	0.0244	0.2704	7.4	20.233	89.28	1463	1493
9.99 Marker	1768	9.99	0.191	2.0783	9.87		599.24	1746	1842

Mass spec BPE, deconvoluted 2D heat map and 3D

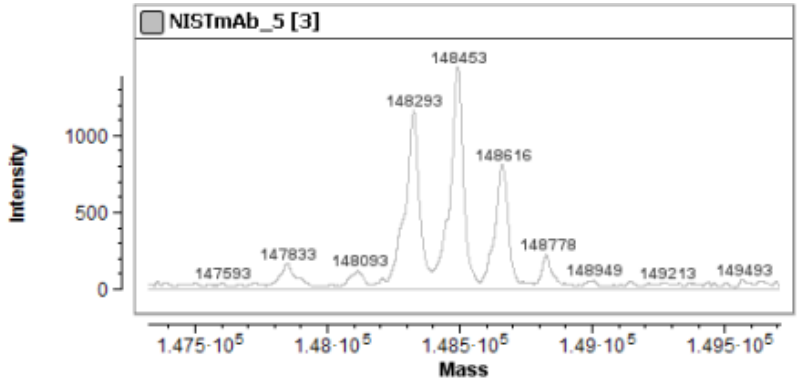


Base peak electropherogram

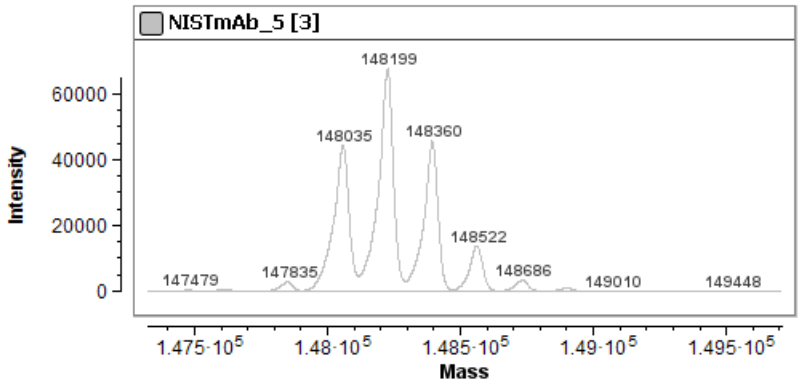




Molecular weight (Da)



Sensitivity at 2.5 min

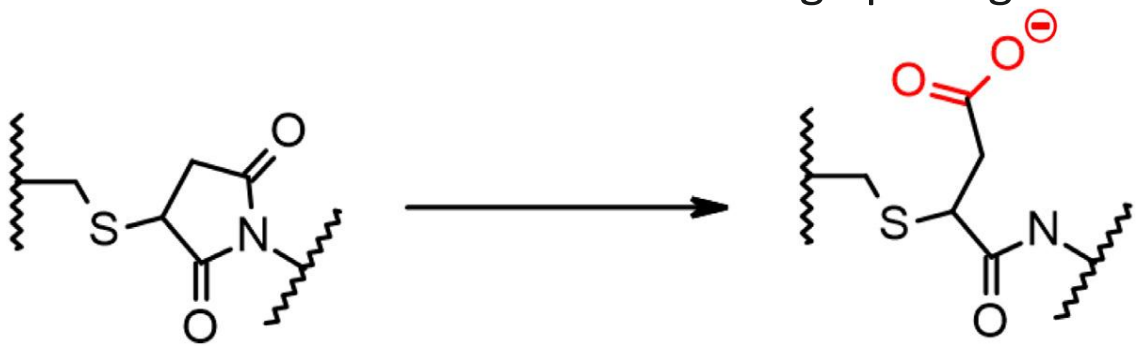


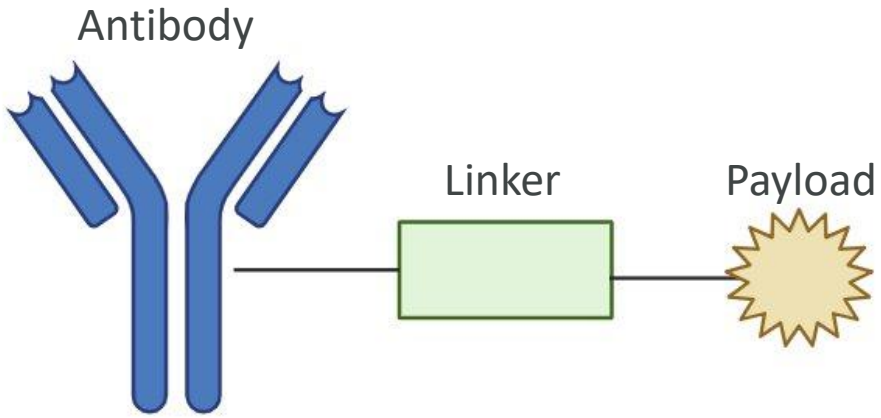
Main peak at 2.9 min

ADCs- Amazingly Difficult Charge (analysis)

- Many antibody drug conjugate (ADC) drugs on the market and in clinical trials are conjugated at a cysteine residue and contain a succinimide-thioether functional group
- Charge heterogeneity profiles are heavily convoluted by contributions from the lactone and succinimide rings in the linker-payload structures
  - Both Ion Exchange Chromatography and Capillary Isoelectric Focusing yield complex profiles
  - Lot-to-lot variability of ring opening events due to in-process hold time variability muddies profile comparisons
  - Ring hydrolysis happens quickly in vivo, so levels at release are not critical quality attributes

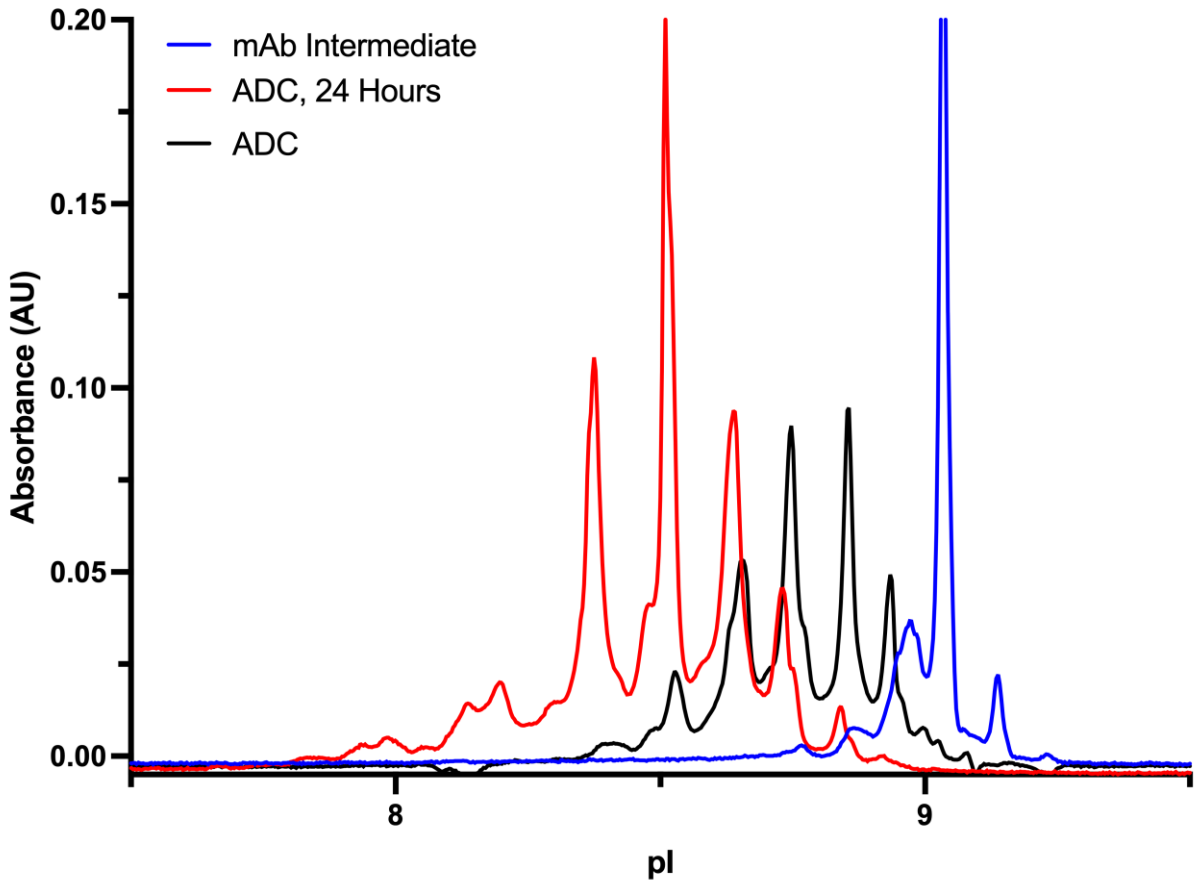
Irreversible succinimide-thioether ring opening:



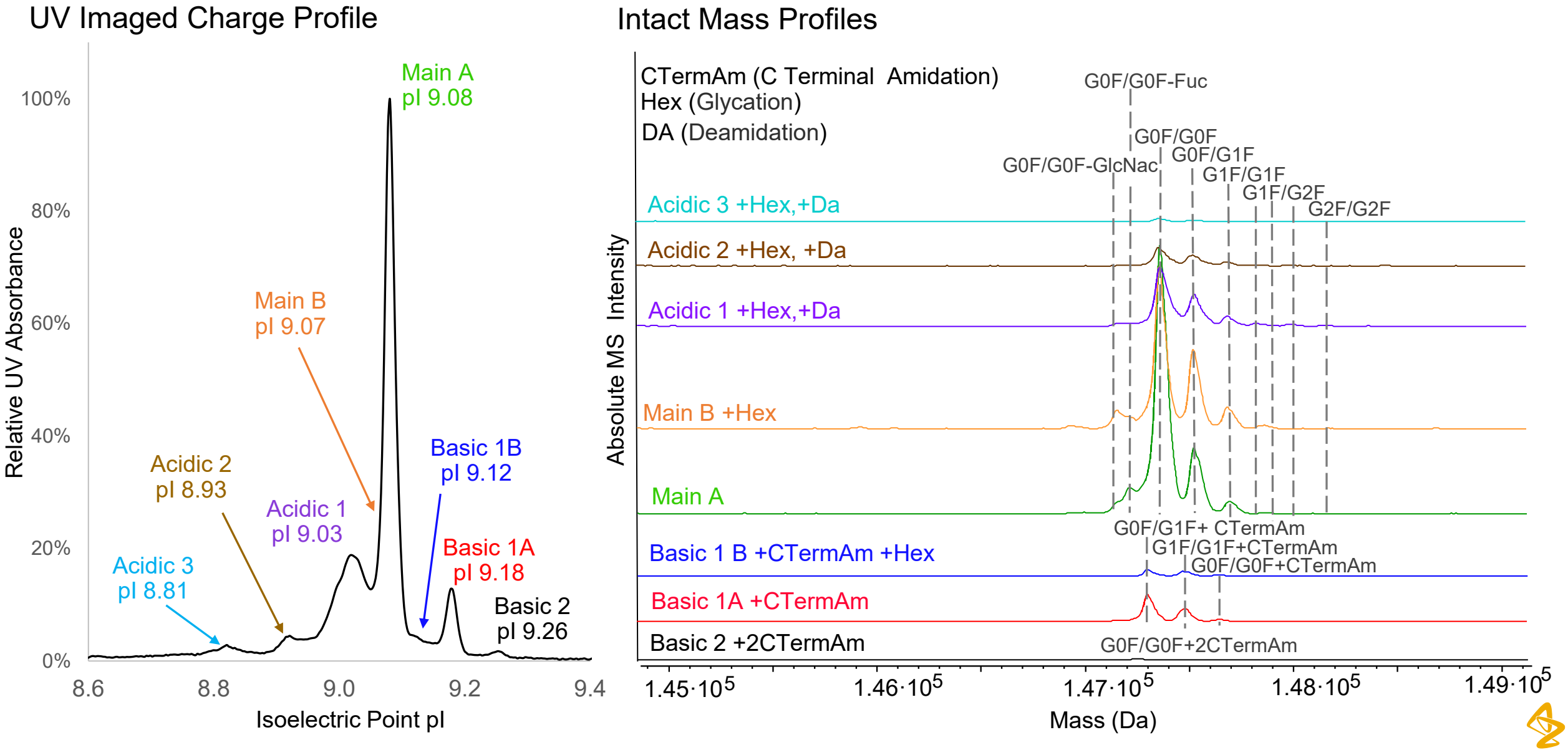


ADC Molecule

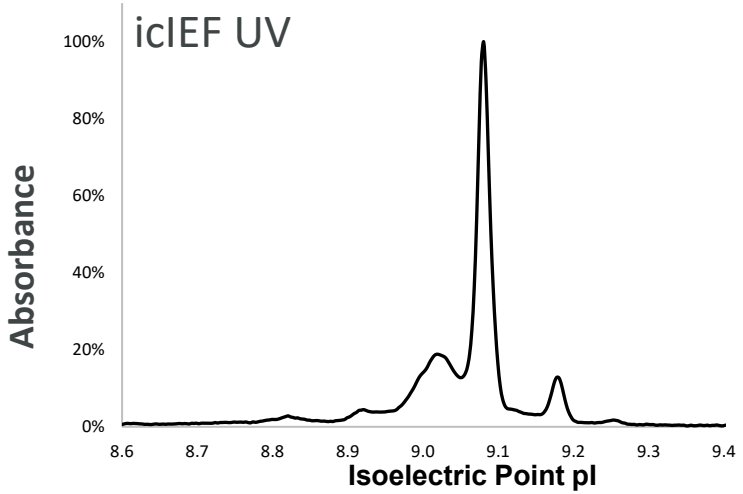
- cIEF profile visibly consistent with previous data
- Pharmalytes in Master Mix cause large acidic shift due to ring opening
  - Limits ADC injection sequence length

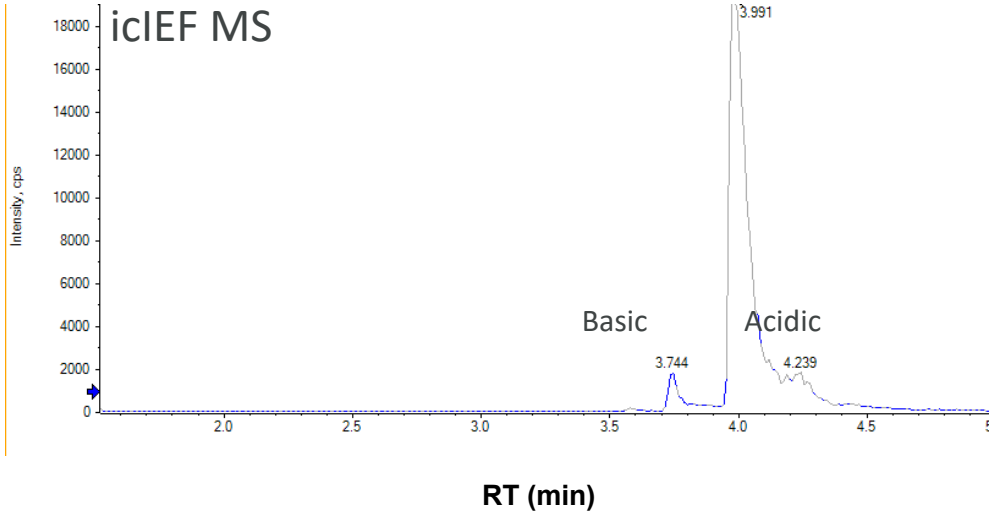


mAb Intermediate Charge/Mass Identification: Normal Monoclonal Antibody Quality Attributes (2023 demo)

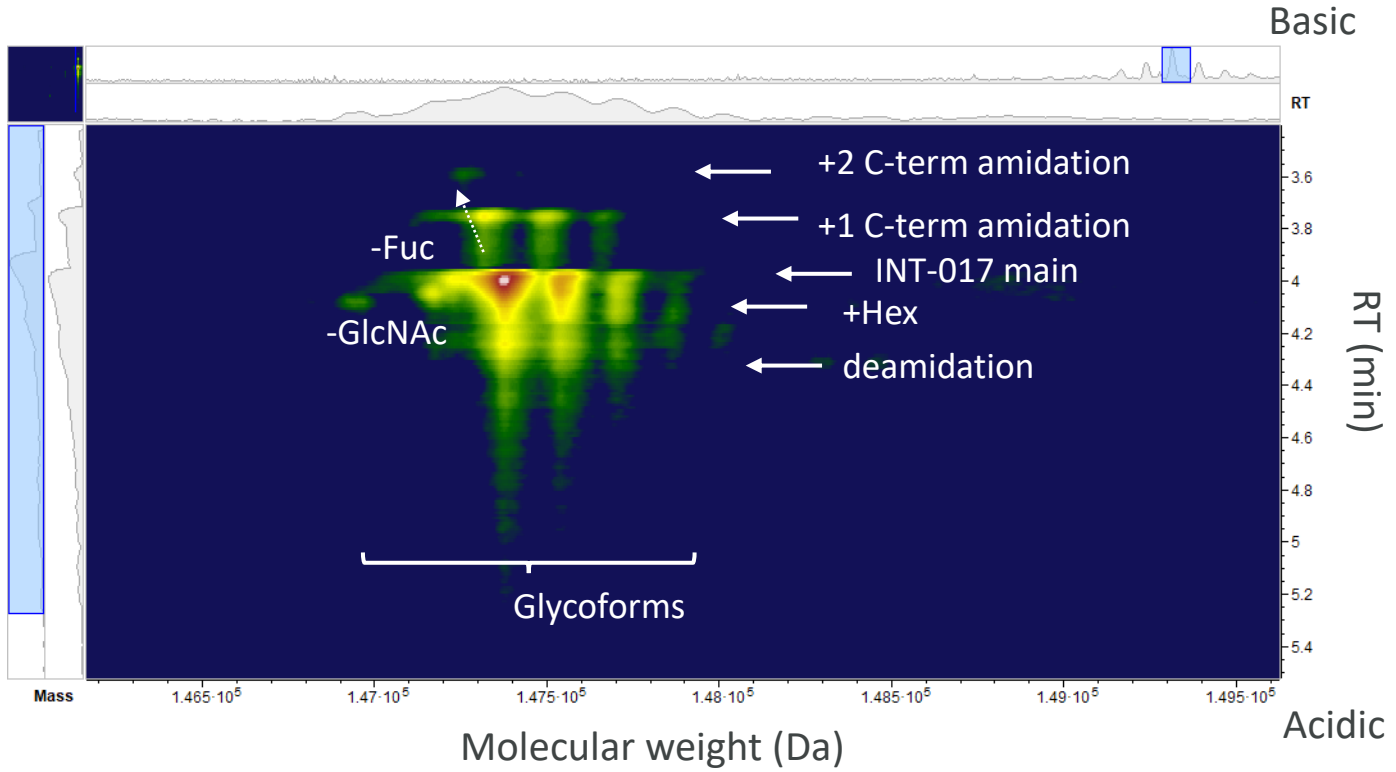


Antibody intermediate

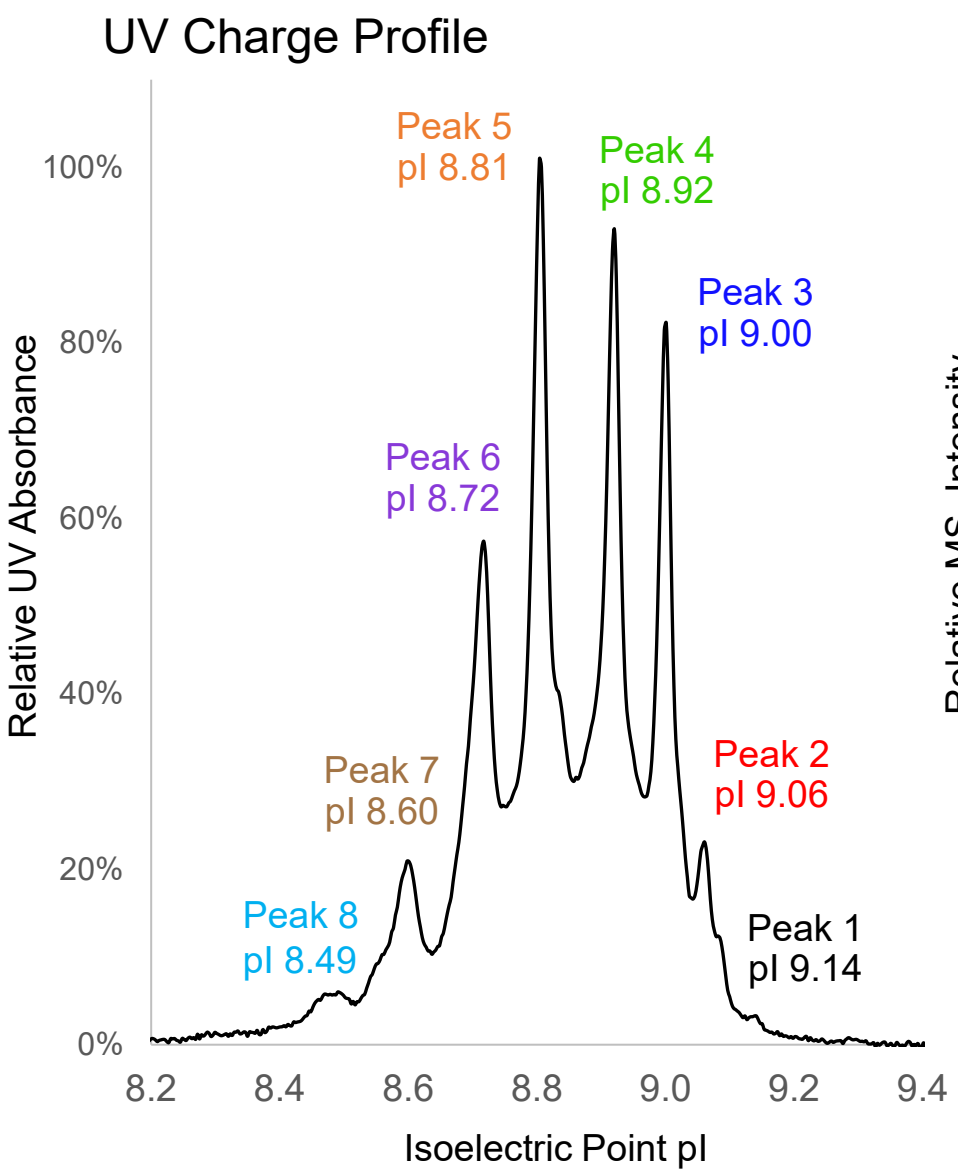


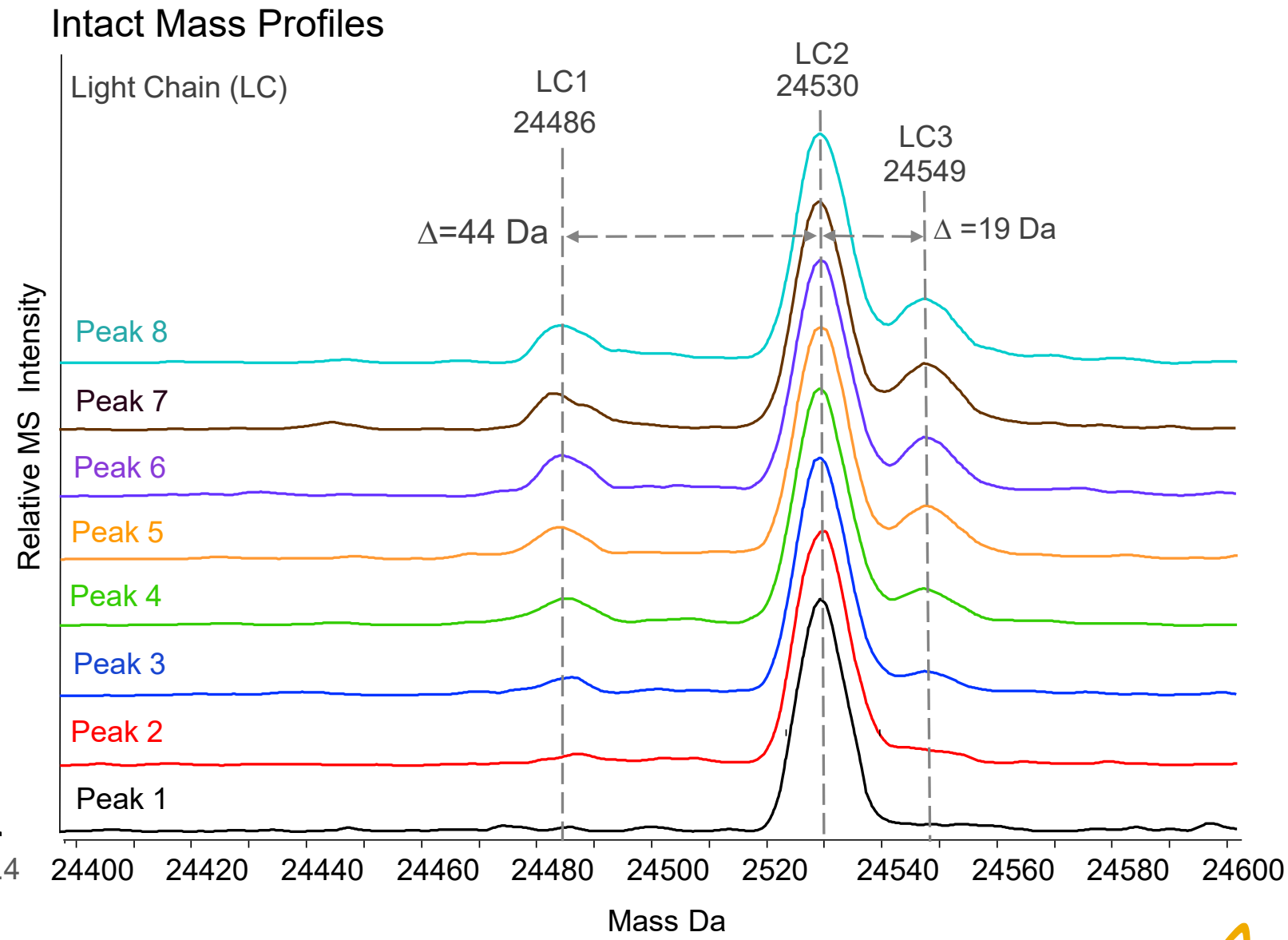


- Peak IDs reproduced from early demo results

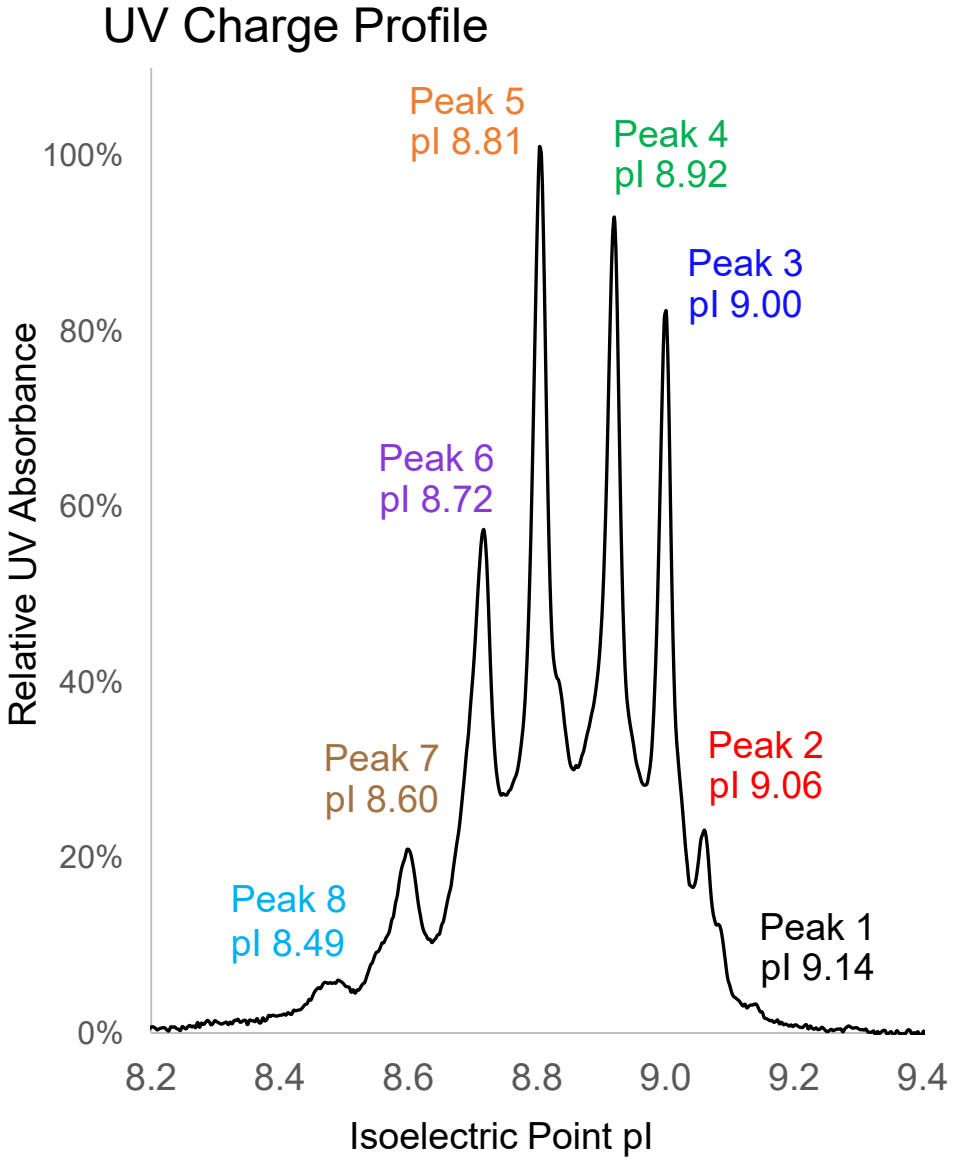


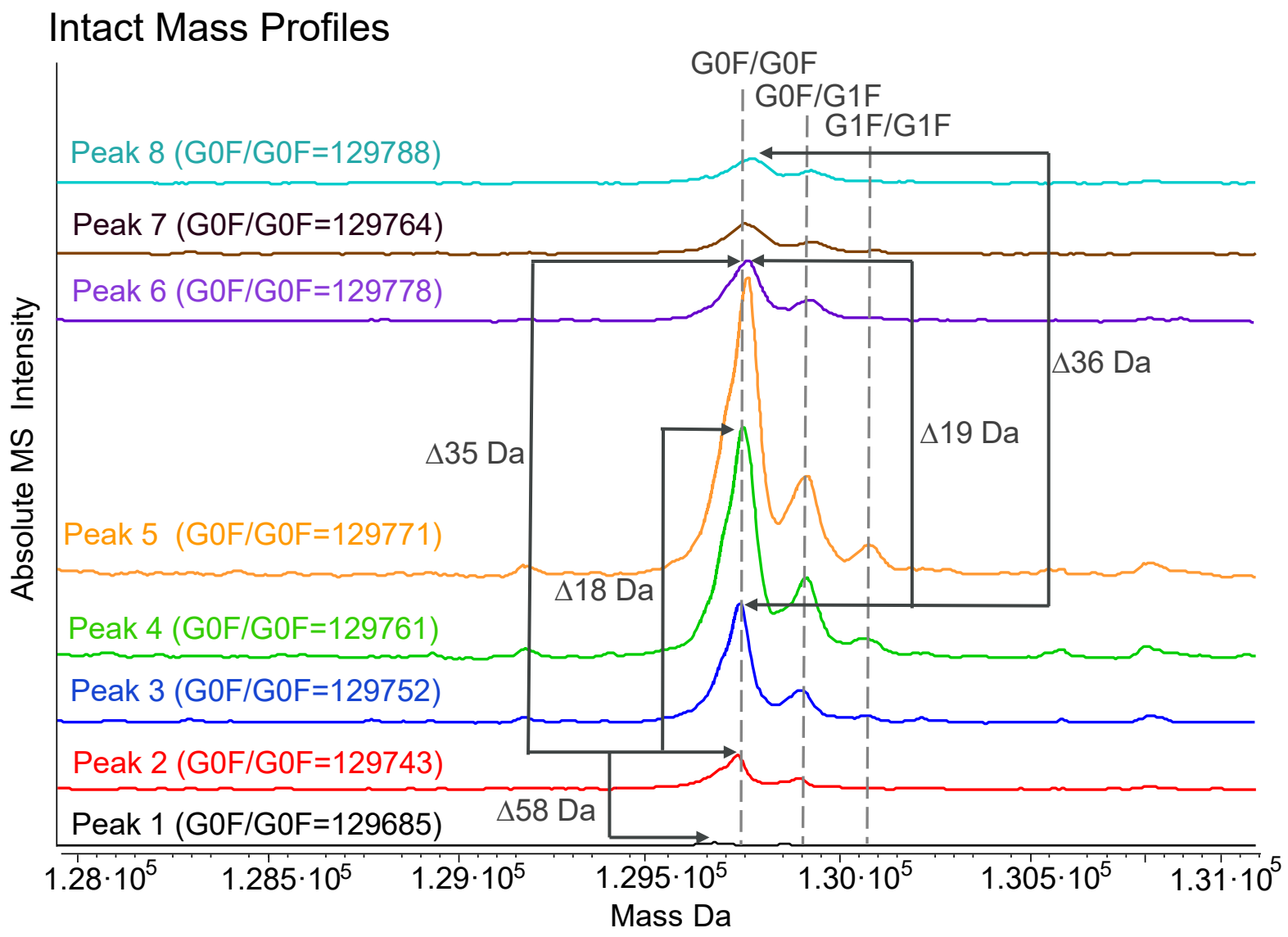
ADC Light Chain Charge/Mass Isoforms Show Shifts Related to payload modifications





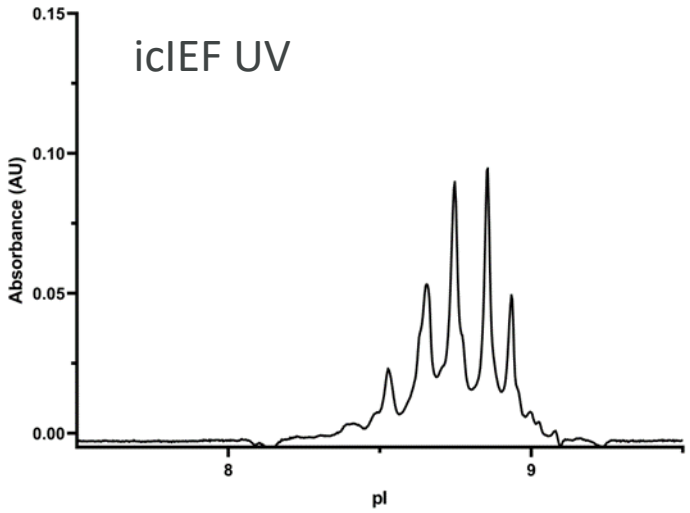
ADC Heavy-Heavy-Light Charge/Mass Isoforms Show Shifts Related payload modifications

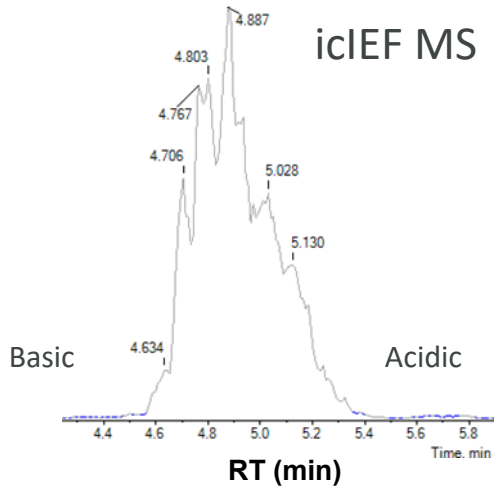


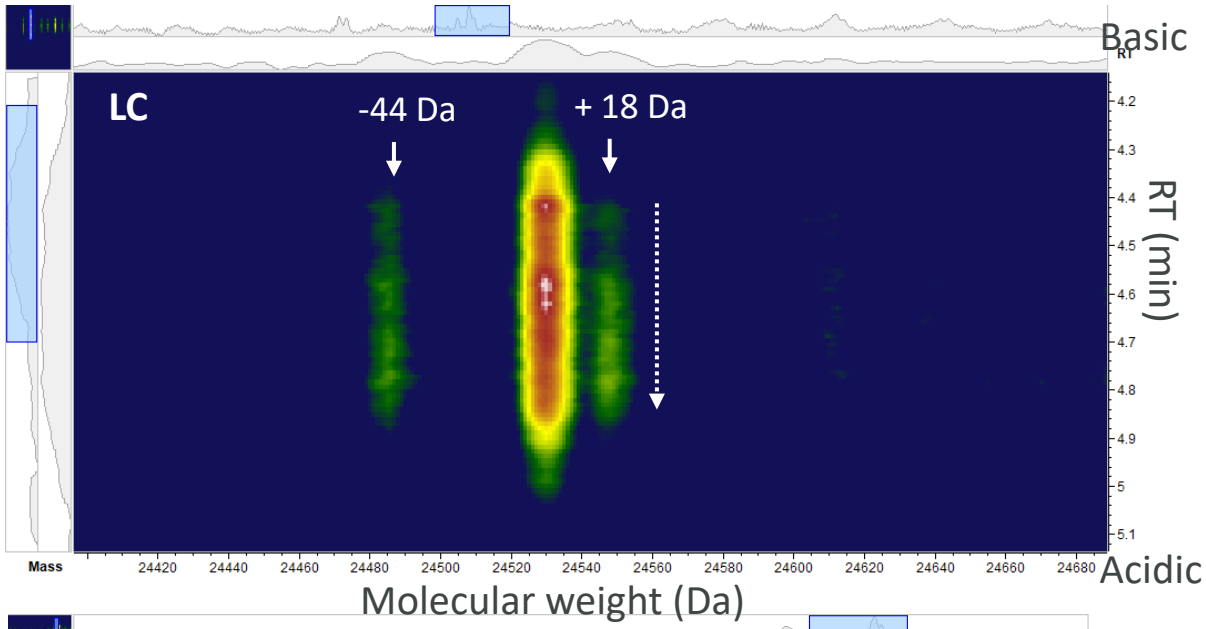


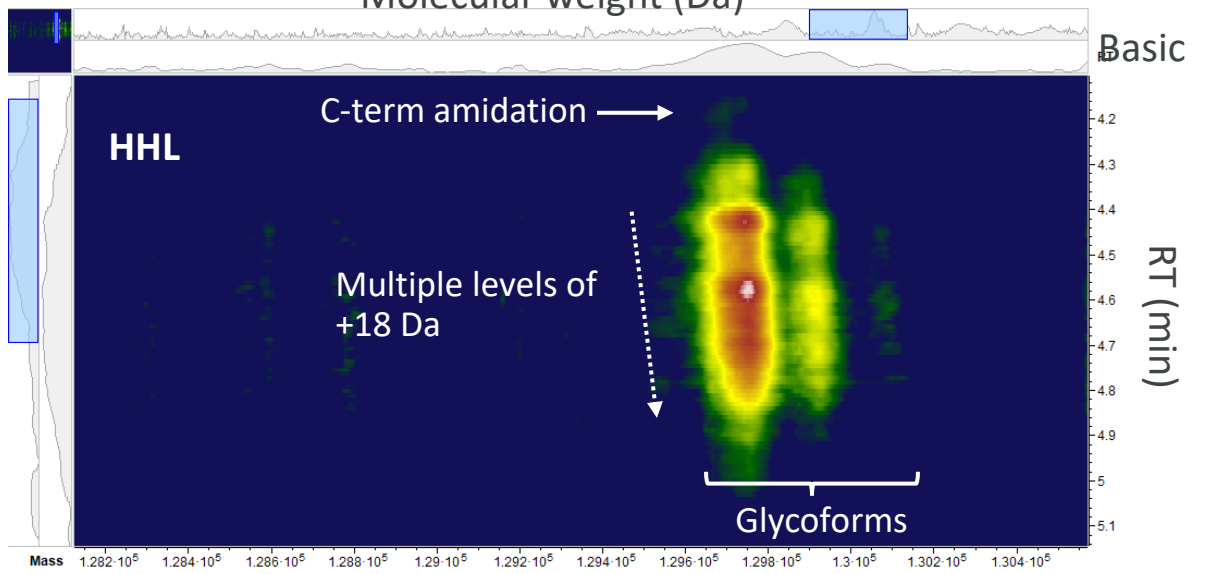
ADC Charge/Mass Identification

- Peak IDs reproduced from early demo results
- Able to isolate data before ring opening
- Have successfully applied method to multiple ADCs and Bioconjugate molecules



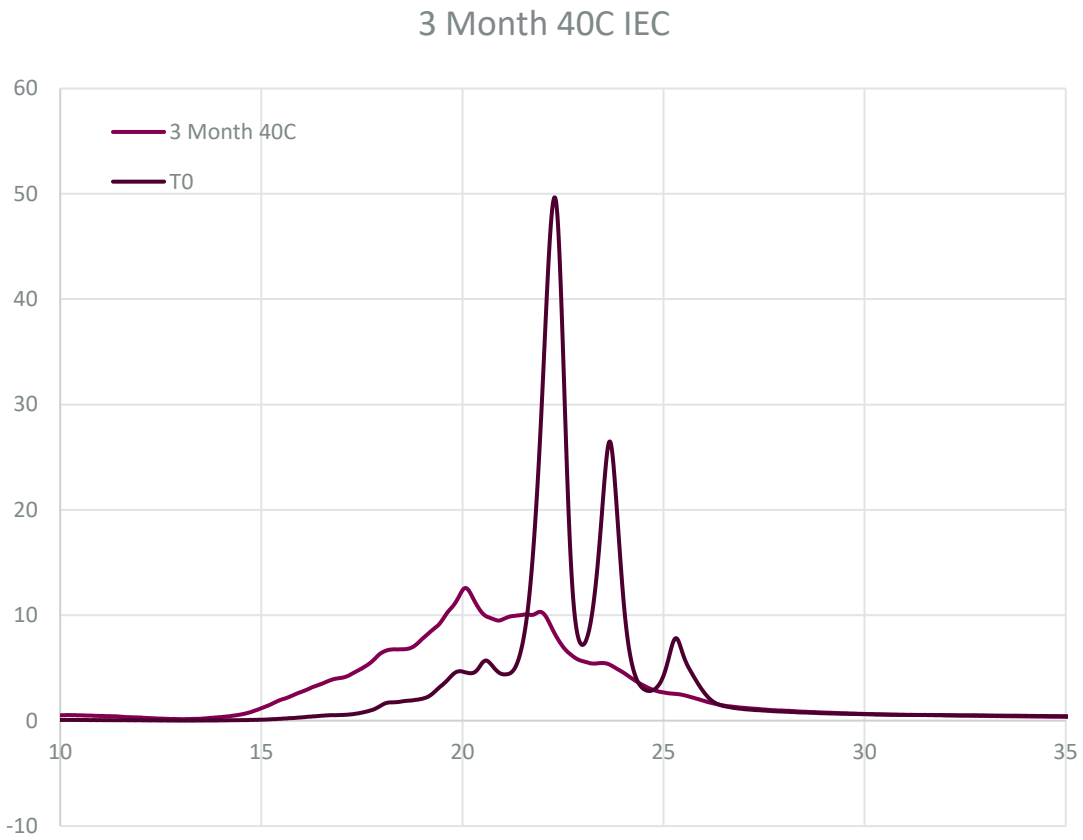


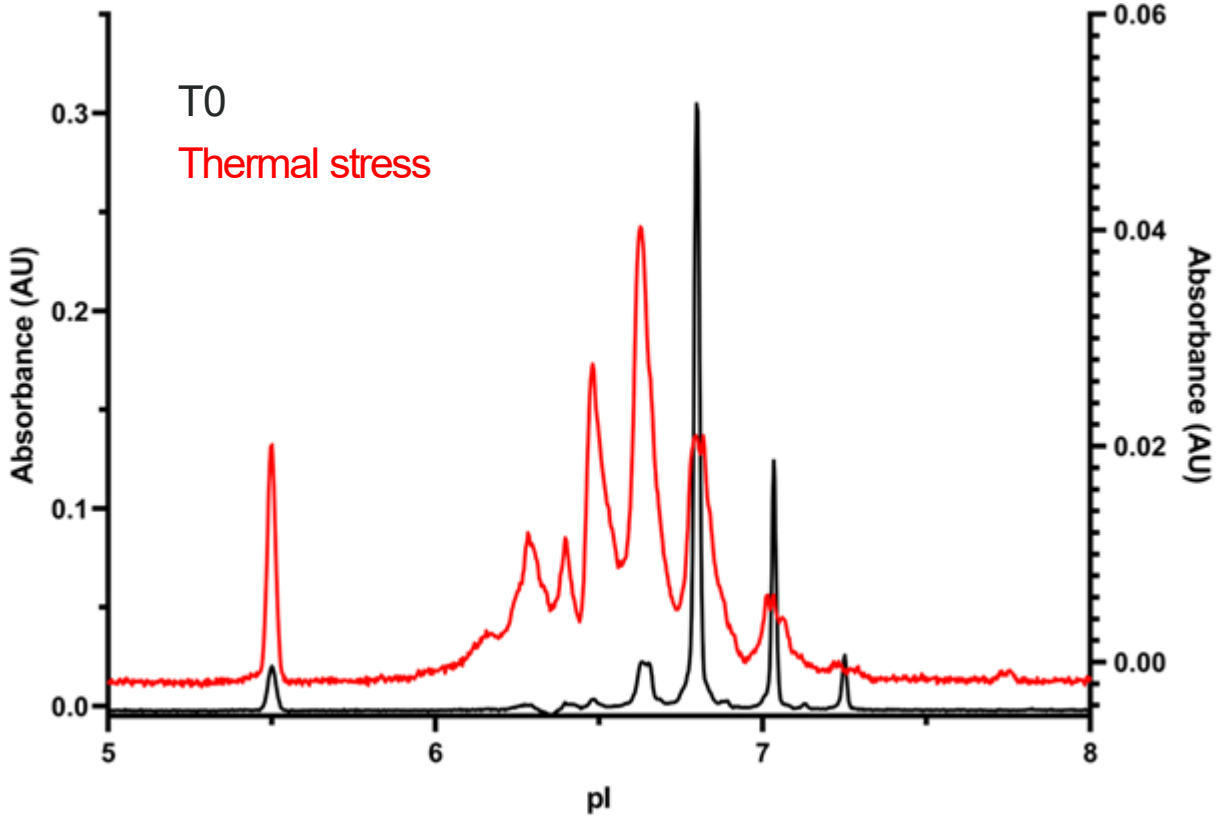




Bispecific T-Cell Engager IEC issues

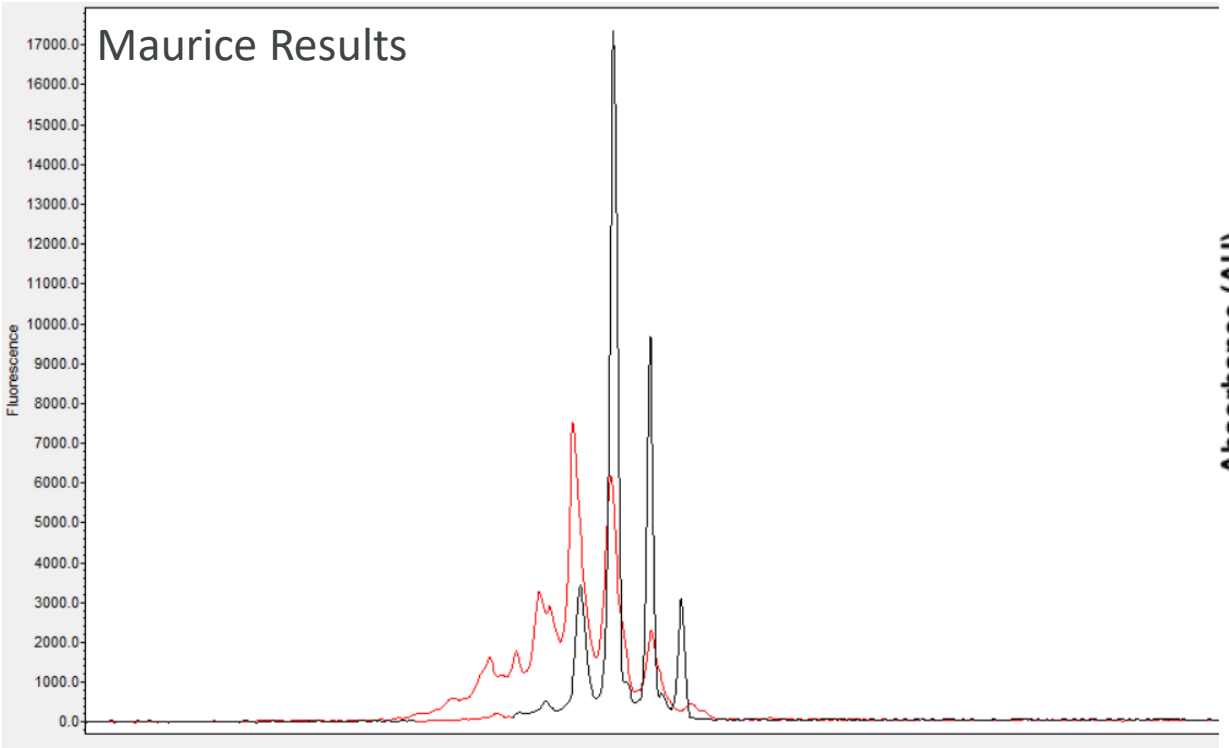
- Thermal stressed material had poor separation on IEC, material could not be effectively fractionated

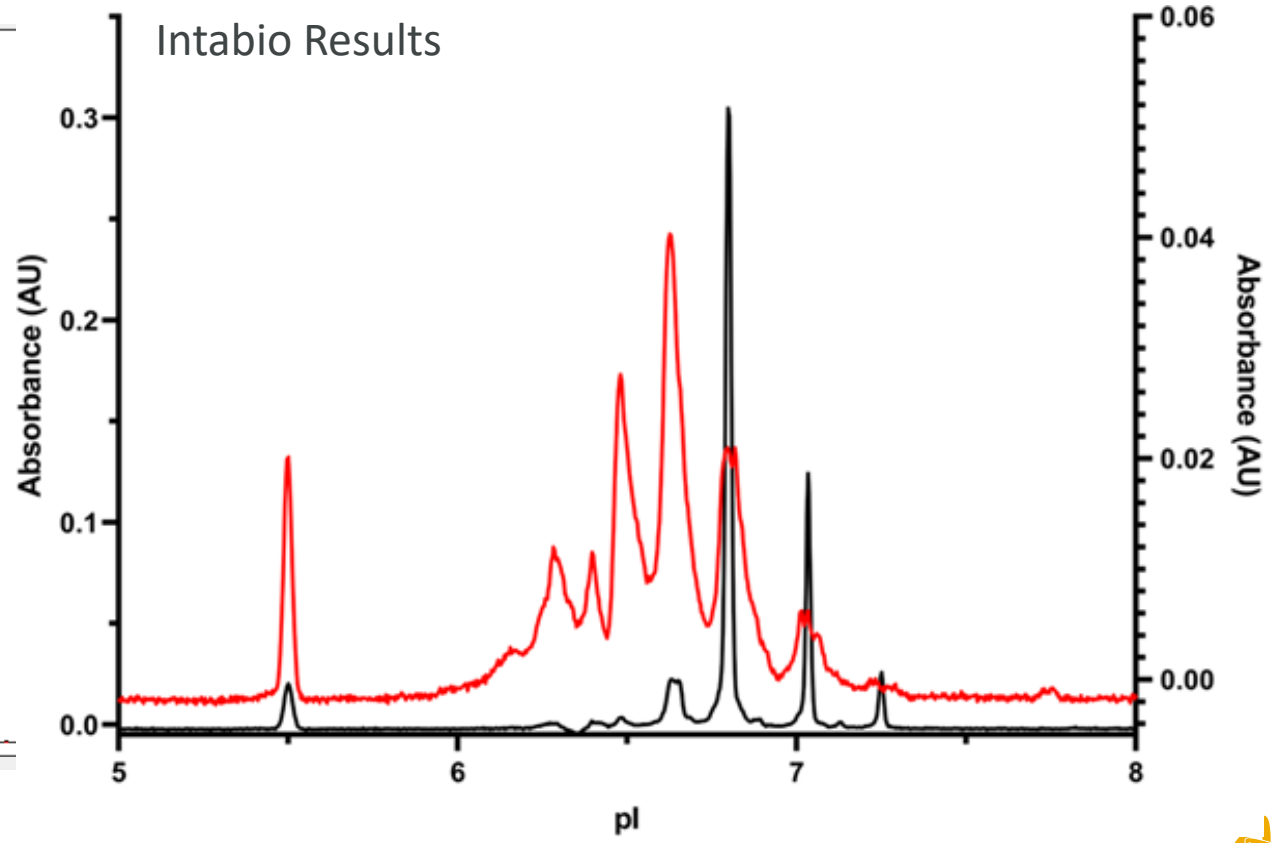




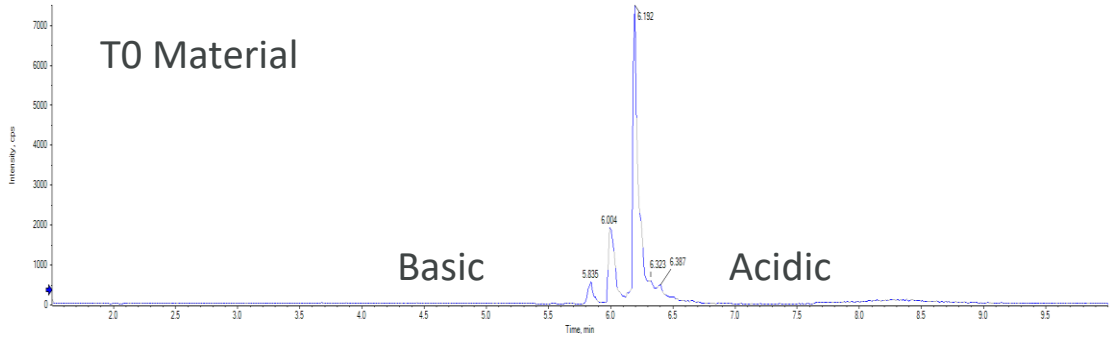
Bispecific T-Cell Engager

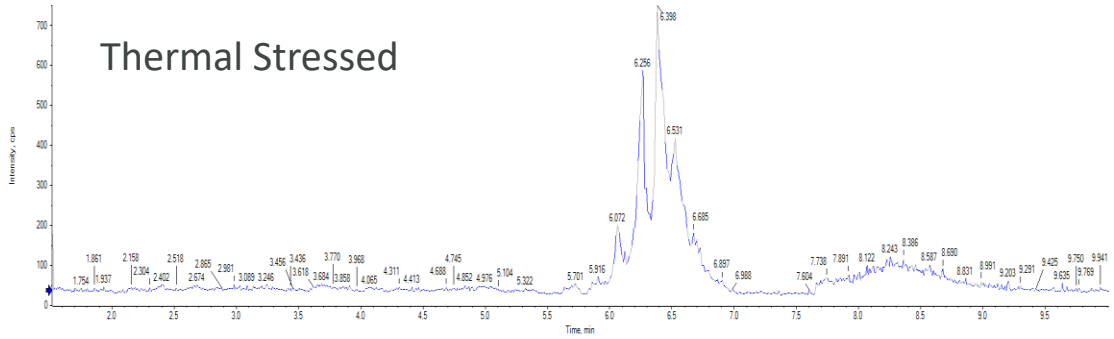
- Acidic shift consistent with Maurice results
- Resolution maintained during mobilization

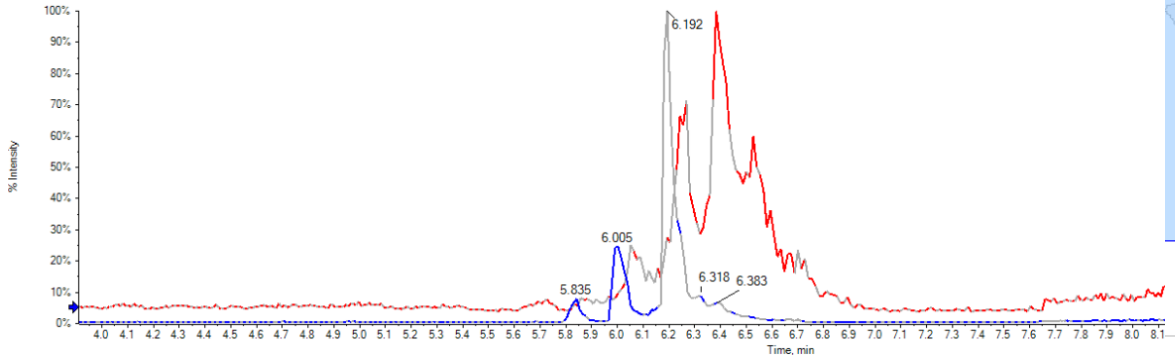


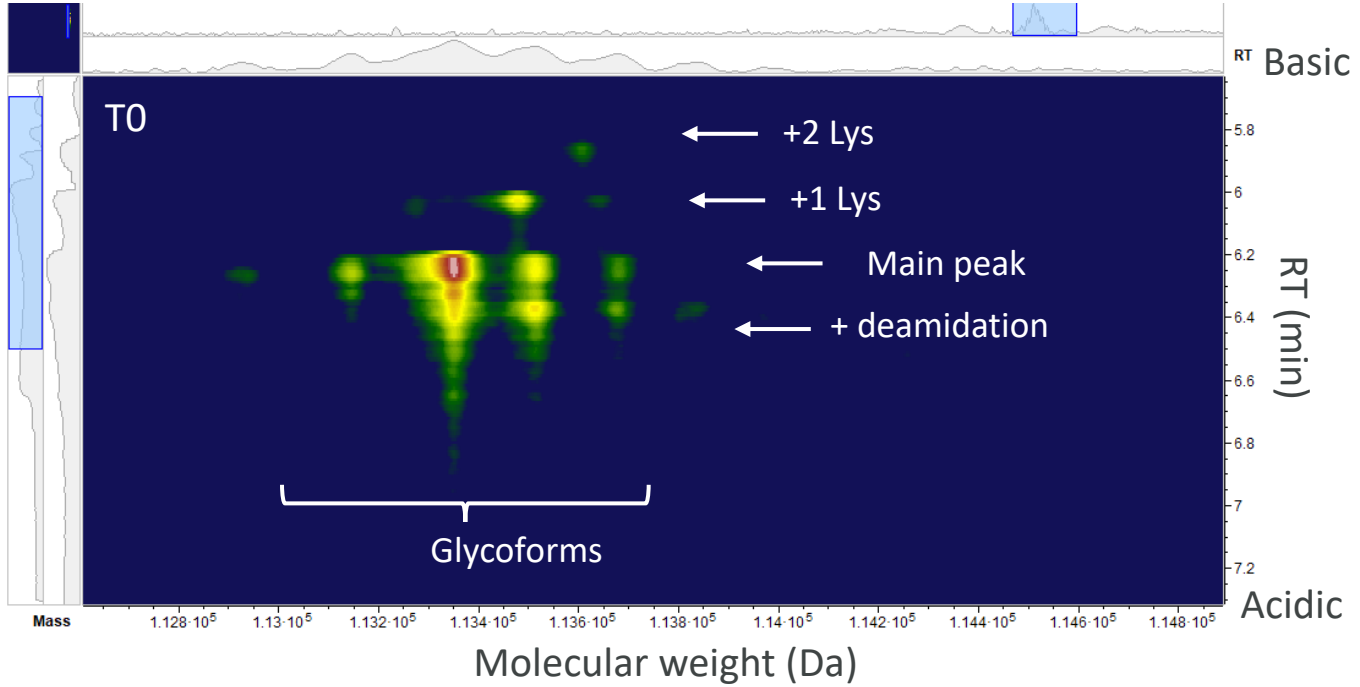


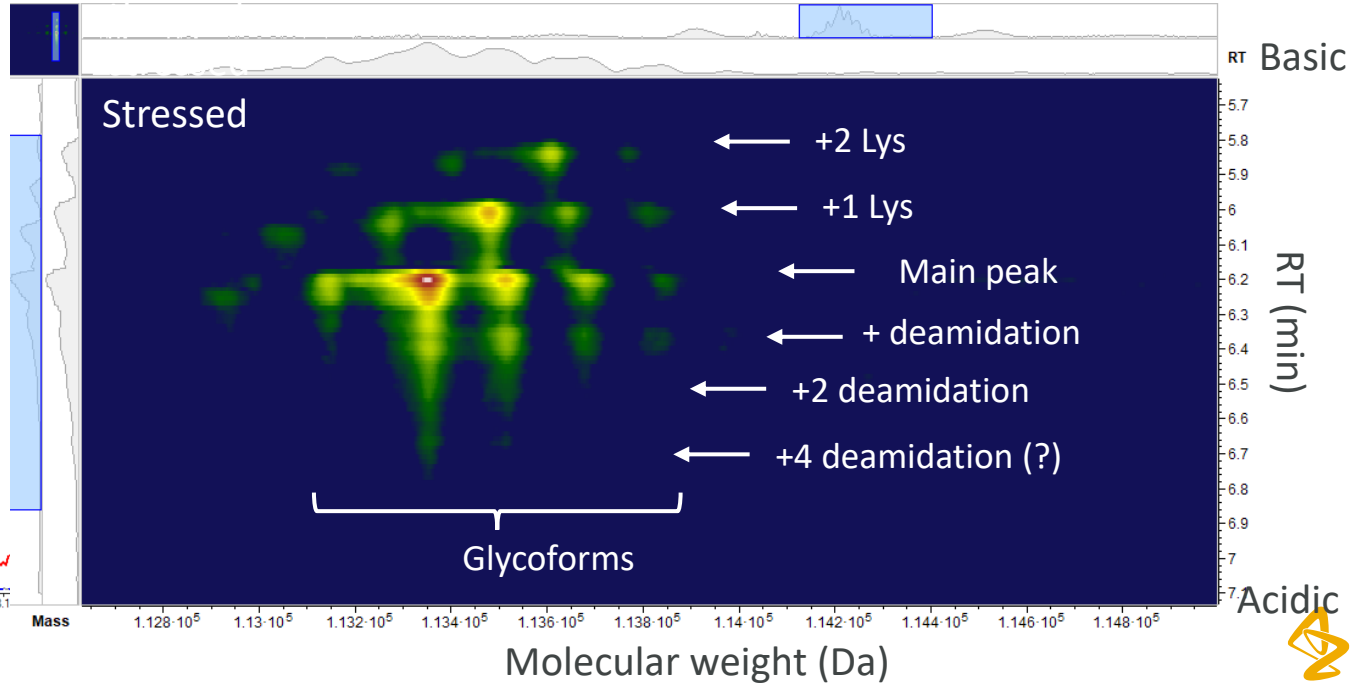
TCE MS BPE and cIEF peak ID







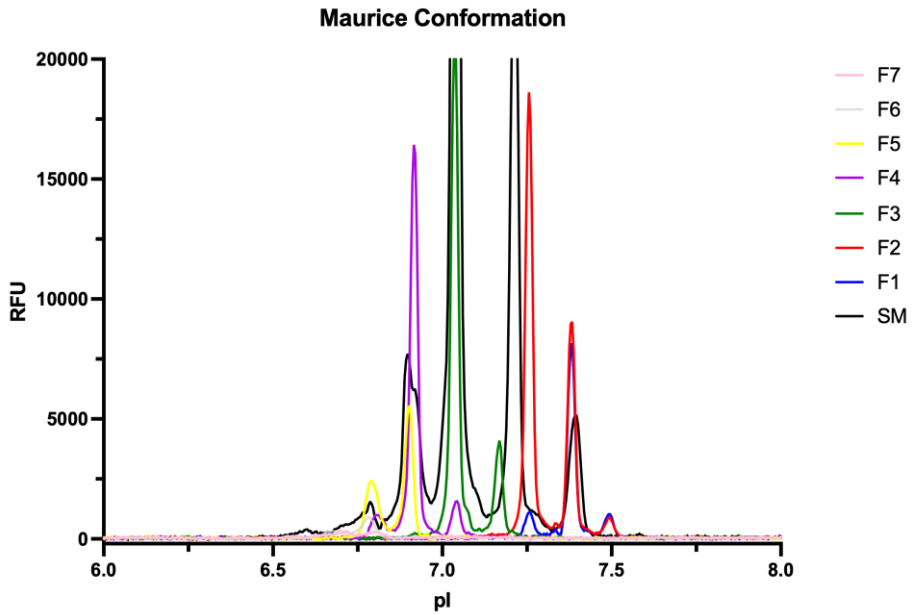


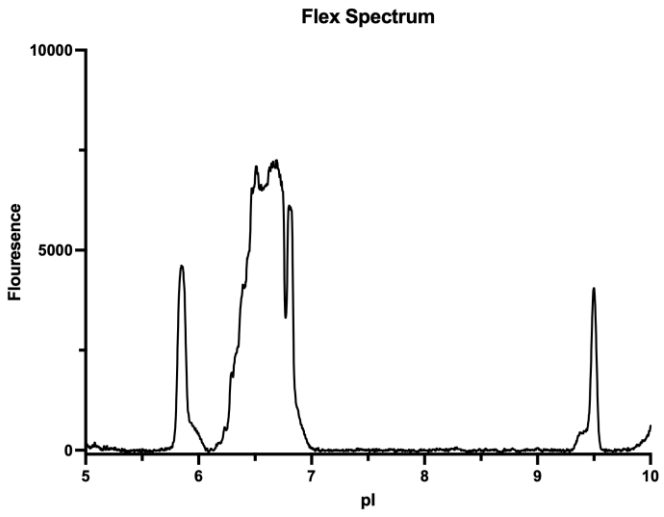


Maurice Flex Fractionation of TCE

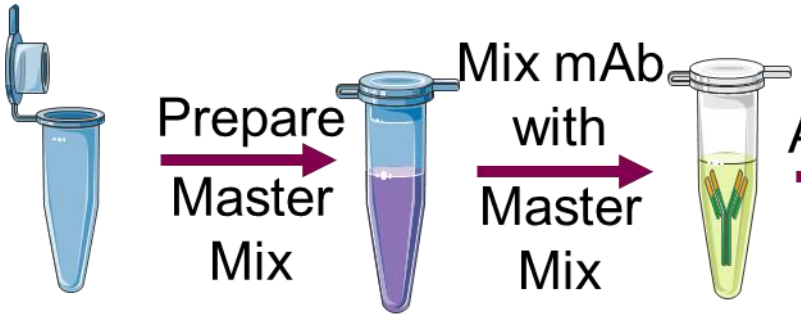
- Maurice Flex is an established cIEF fractionation method
- Direct comparison to release icIEF methods (same consumables and instrumentation)
- Able to load 4mg/mL sample into Flex
- Seen reproducible separation of TCE, no MS data due to limited resources







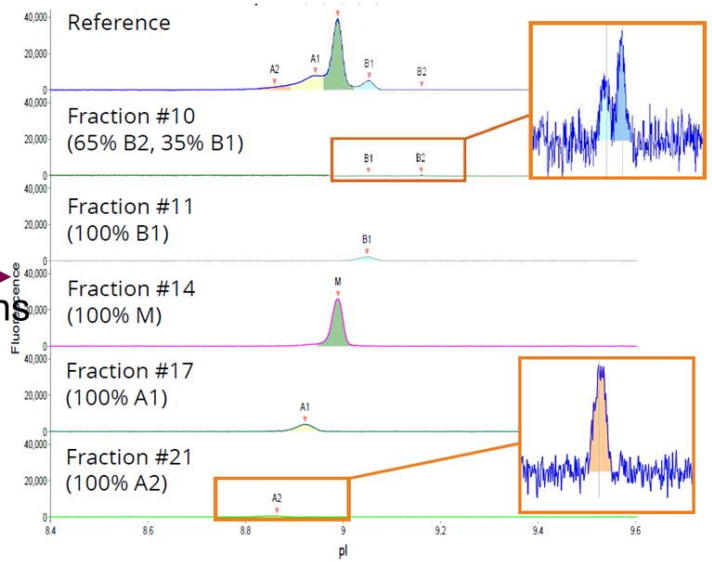
MauriceFlex™ Workflow



Analyze



Collect and
Verify Fractions

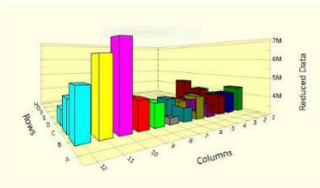


Samples verified using ZenoTOF 7600

2. Assess fraction plate with a plate reader at excitation 280 nm and emission 350 nm.

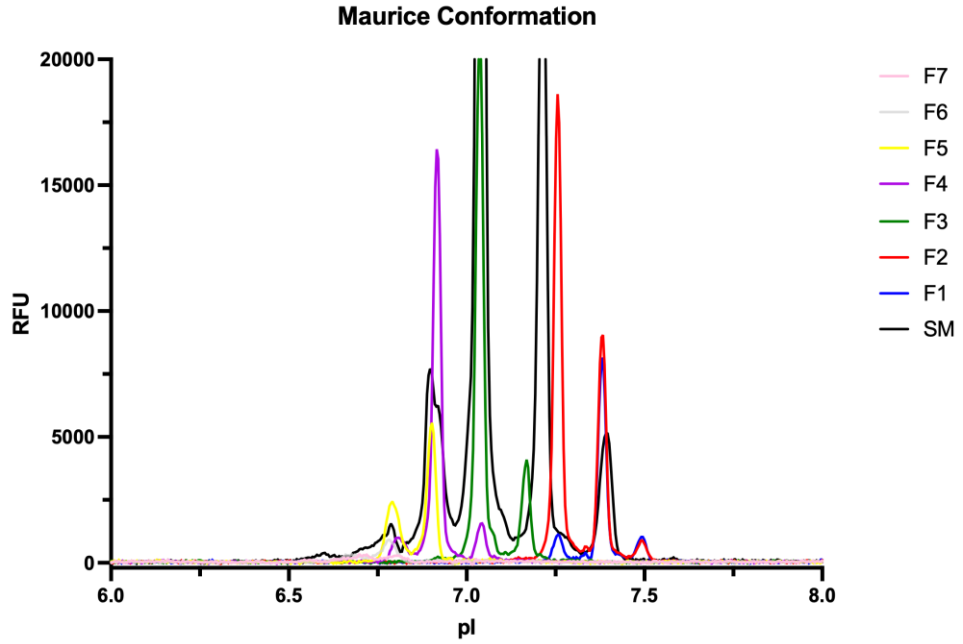


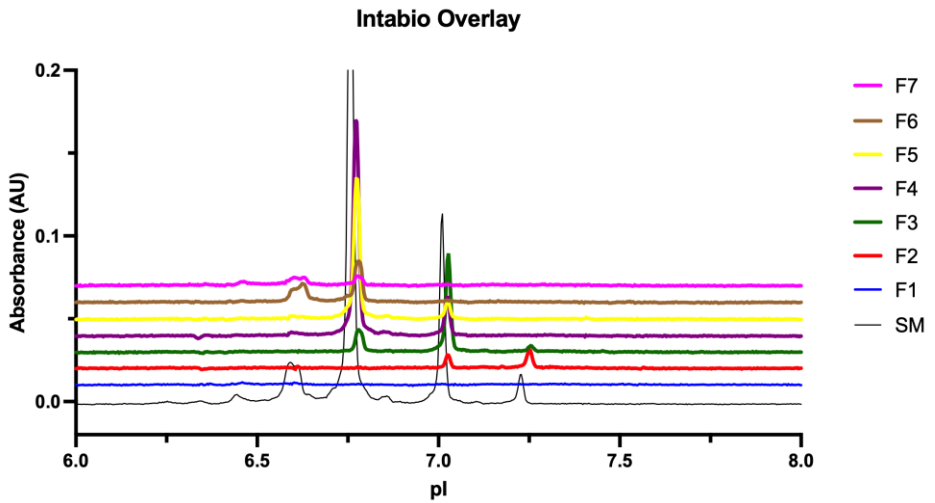
Recommendation: Collect from 7 wells before and after the main peak.



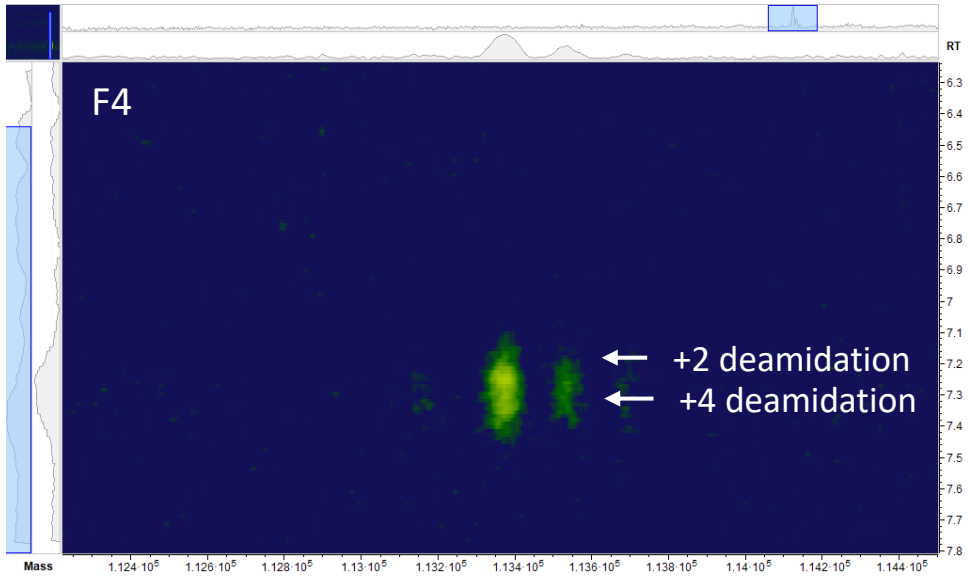
Confirmation of Masses on the Intabio ZT

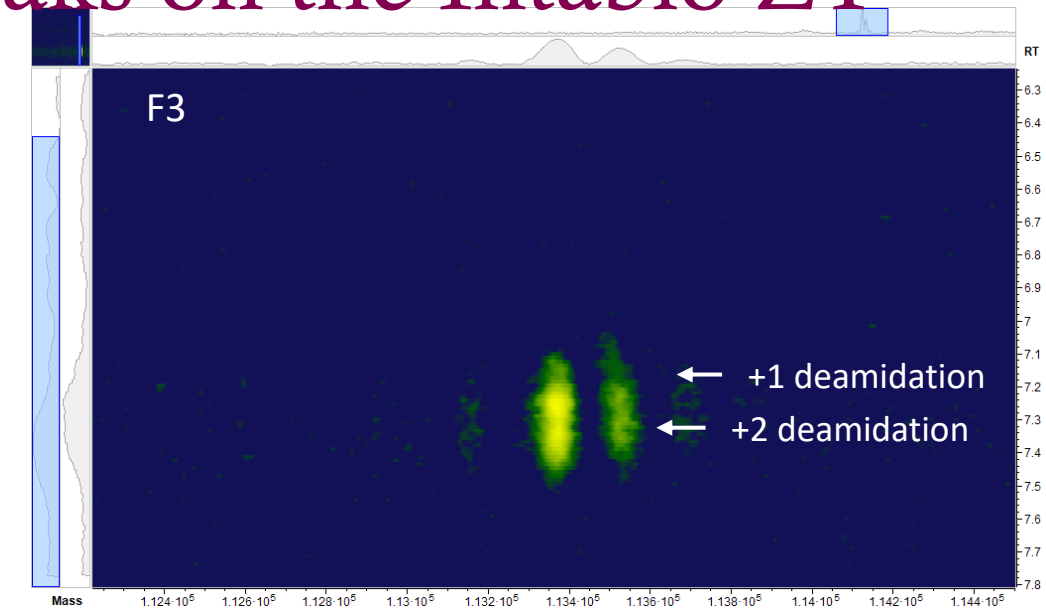
- Flex fractions reproduce Intabio Identification of starting material
- What is the benefit?
  - Simplifies Flex conformation for our labs
  - Decreases turnaround time and needed resources
- *Might* be able to leverage bioassay simultaneously, with high sample concentrations (~8 mg/mL Flex load)- more data to come





Confirmation of Stressed Peaks on the Intabio ZT

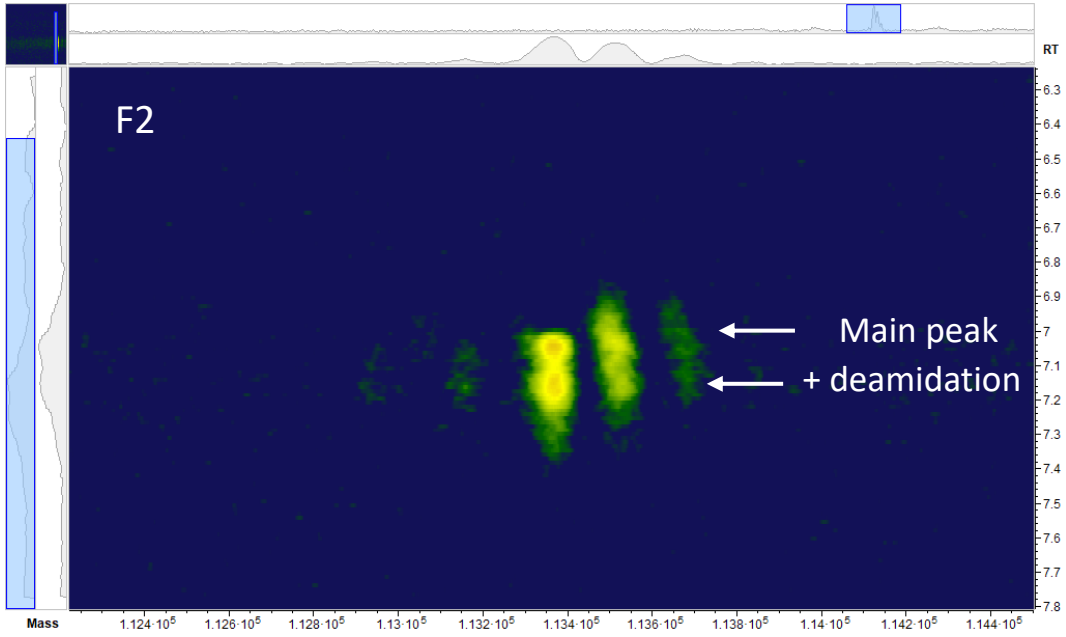


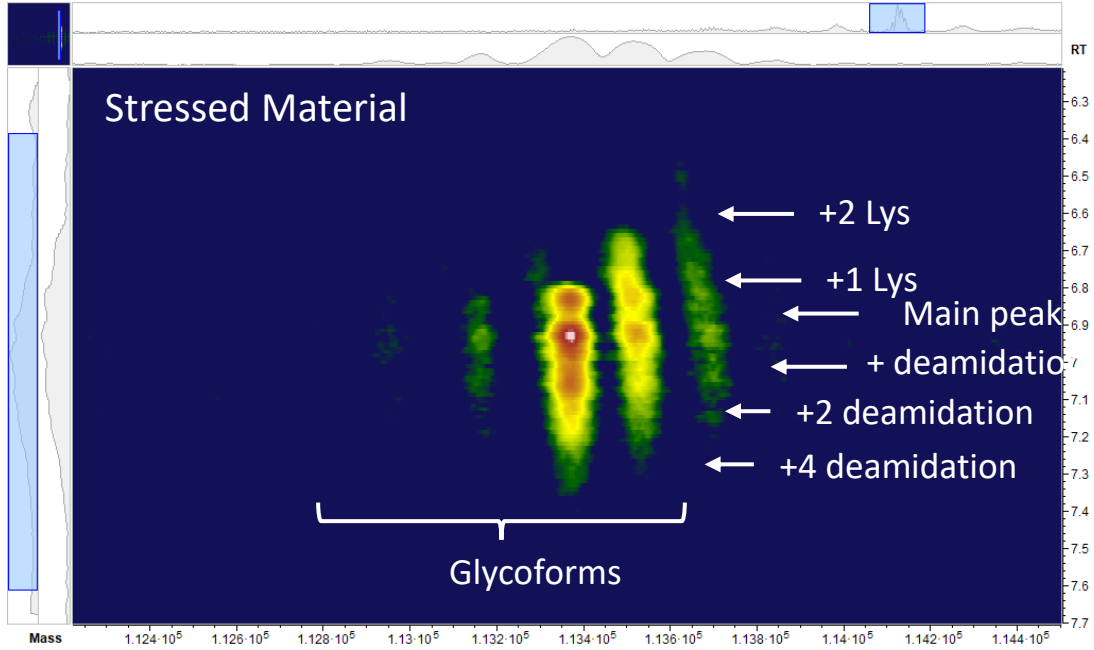


Basic

RT (min)

Acidic





Basic

RT (min)

Acidic

Molecular weight (Da)

Summary

- Maurice Flex is a powerful tool for cIEF fractionation and peak characterization
  - Previously shown Nanoflow timsTOF workstream
  - Can be fed into other CE-MS workflows for peak ID
- Intabio ZT microfluidic chip-based icIEF coupled to SCIEX ZenoTOF 7600 system (icIEF-MS) was able to characterize charge variants of multiple complex protein therapeutics, elucidating the quality attributes including glycosylation, deamidation, N/C-terminal modifications, linker-payloads, fragmentation…
- Due to the microfluidic chip design, Intabio ZT consumes far less material than the traditional LC and fractionation approach, and has fast turnaround time within 40 minutes per sample.
- icIEF-MS technology platforms improve product **understanding and manufacturing control strategy development**

Acknowledgements

- Mingjie Cui
- Wendy Lan
- Kristin Schultz Kuszak
- Rodrigo Vargas
- Trust Razunguzwa

- Moral Support:
  - Squanchi



Questions?



Confidentiality Notice

This file is private and may contain confidential and proprietary information. If you have received this file in error, please notify us and remove it from your system and note that you must not copy, distribute or take any action in reliance on it. Any unauthorized use or disclosure of the contents of this file is not permitted and may be unlawful.

AstraZeneca PLC, 1 Francis Crick Avenue, Cambridge Biomedical Campus, Cambridge, CB2 0AA, UK
+44(0)203 749 5000
www.astrazeneca.com