Enabling icIEF-MS Characterization of Charge Isoforms for Biotherapeutic Products

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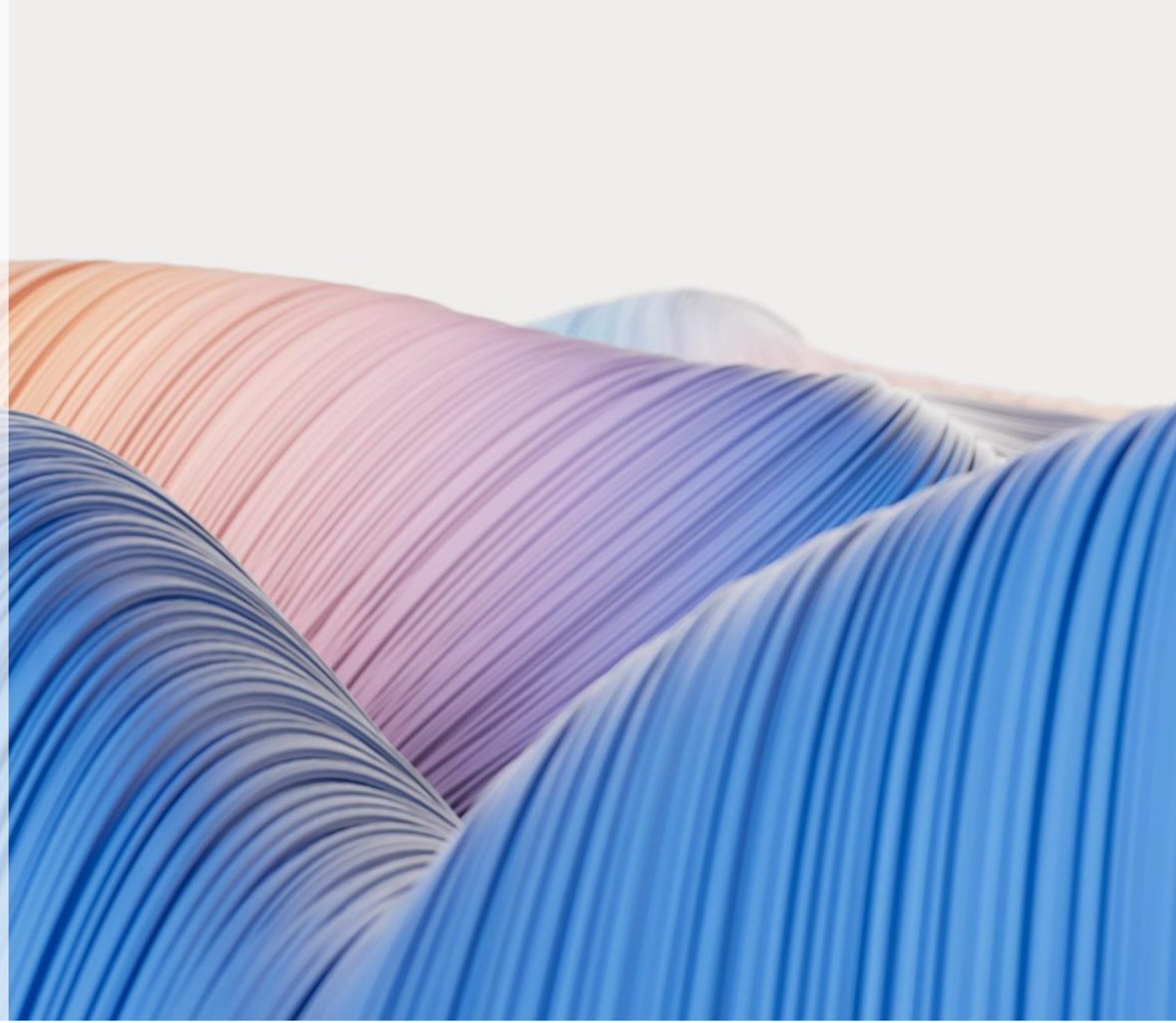
CE Pharm 2024



Agenda

- icIEF for Release verses IEX
- Overview of icIEF-MS Technology
  - Rapid development of icIEF-MS platforms
    - Intabio/ZenoTOF 7600 direct coupling
    - MauriceFlex fractionation with subsequent MS analysis
- Enabling icIEF-MS Characterization of Charge Isoforms and Implementing towards Biotherapeutic Portfolio Support
  - Case study with a complex protein using MauriceFlex
  - Case studies with mAb, bispecific, complex protein, AAV using Intabio/ZenoTOF 7600

icIEF (imaged capillary Iso-Electric Focusing)

- The pI-based charge analysis has been used for charge heterogeneity determination and quantitation to support release, stability testing and product control of quality attributes including identity, purity, and PTM characterization
- **Why icIEF verses the Traditional IEX for Release?**

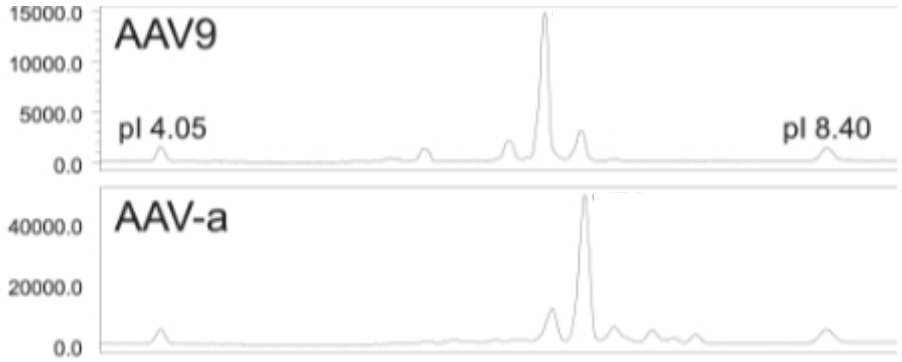
Method	Pro	Con
icIEF	• Platform method for release and stability • Minimal protein-specific method development • Robust and high throughput • Minimal sample consumption	• Direct characterization of charge isoforms not possible • Collection of charge isoforms for further characterization not possible
IEX-HPLC	• After optimization, profiles can be similar to that of icIEF • Allows characterization directly by MS or via fractions	• Need for more method development. usually has less resolution • May not be robust for a routine release method • Not high throughput • Multiple modes of separation

Why is Peak Identification Needed for icIEF?

Pfizer has a portfolio of divergent and complex modalities

AAV

For AAV, capsid deamidation is a CQA. An icIEF method was able to be developed; however, it is unclear which peaks represent which capsid proteins and/or acidic species thereof



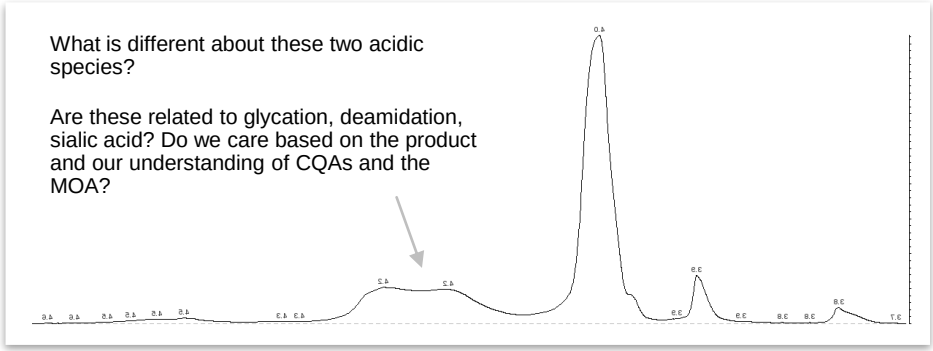
Assigning icIEF peaks was a complex, multiyear process and required significant work

MT-MCD paper
doi.org/10.1016/j.omtm.2023.03.002

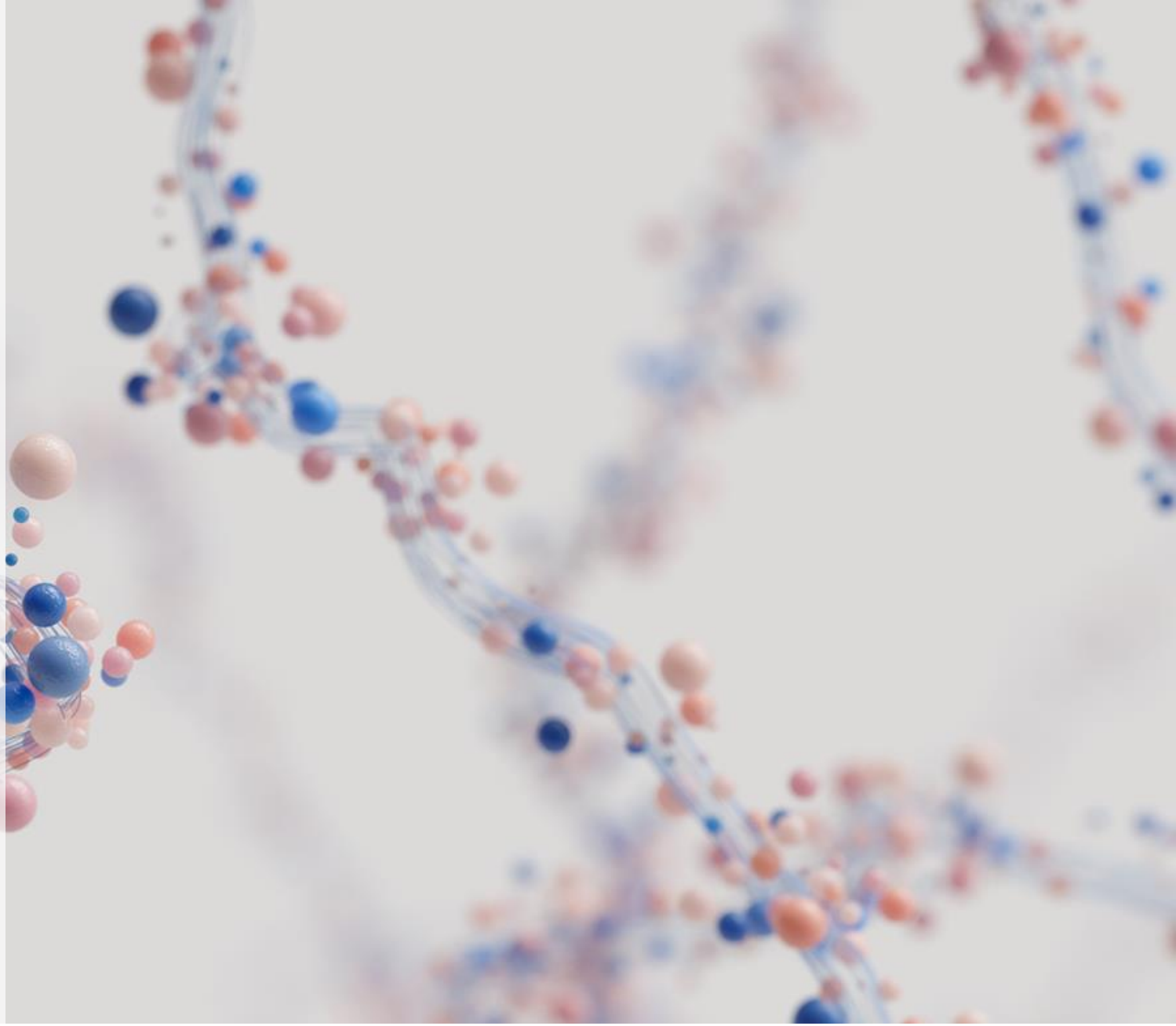
- Development of RP-HPLC method and MS characterization (not robust)
- Fractionation of RP-HPLC peaks and analysis by icIEF method (VP stability issues)
- Generation and analysis of capsid mutants
- IEX-HPLC method was not developed

…Finally, Identification was achieved! Project teams had already made decision to validate MAM during elapsed time.

Peak assignment can be important, even for simpler molecules!



Overview of icIEF-MS Technology



Characterization of icIEF Charged Species

Unable to characterize charge variants – requires orthogonal assay e.g. IEX-MS

- Indirect methods for peak identification
  - Enzymatic treatment
  - IEX online/fractions-MS characterization
- Assume charge variant identity
  - Based on platform knowledge

Focus of today's presentation

Rapid development of icIEF-MS platforms – Bridge the gap and provide peak ID

Two icIEF-MS systems

- **Intabio icIEF/ZenoTOF 7600 – SCIEX**
- **MauriceFlex – ProteinSimple, BioTechne**

- Others
  - CE Infinite – Advanced Electrophoresis Solutions
  - ZipChip CE-MS– 908 Devices
  - BioSummit™ CVA cIEF-MS – CMP Scientific Corp

Overview of the Two Novel icIEF-MS Platforms

MauriceFlex

Pro

- Consistent icIEF profile as release procedure
- Allows for fractionation of icIEF peaks and subsequent characterization with MS or potency assays

Con

- Direct characterization of charge isoforms not possible
- May require molecule/modality specific method development for mobilization

Intabio/ Zeno TOF 7600

Pro

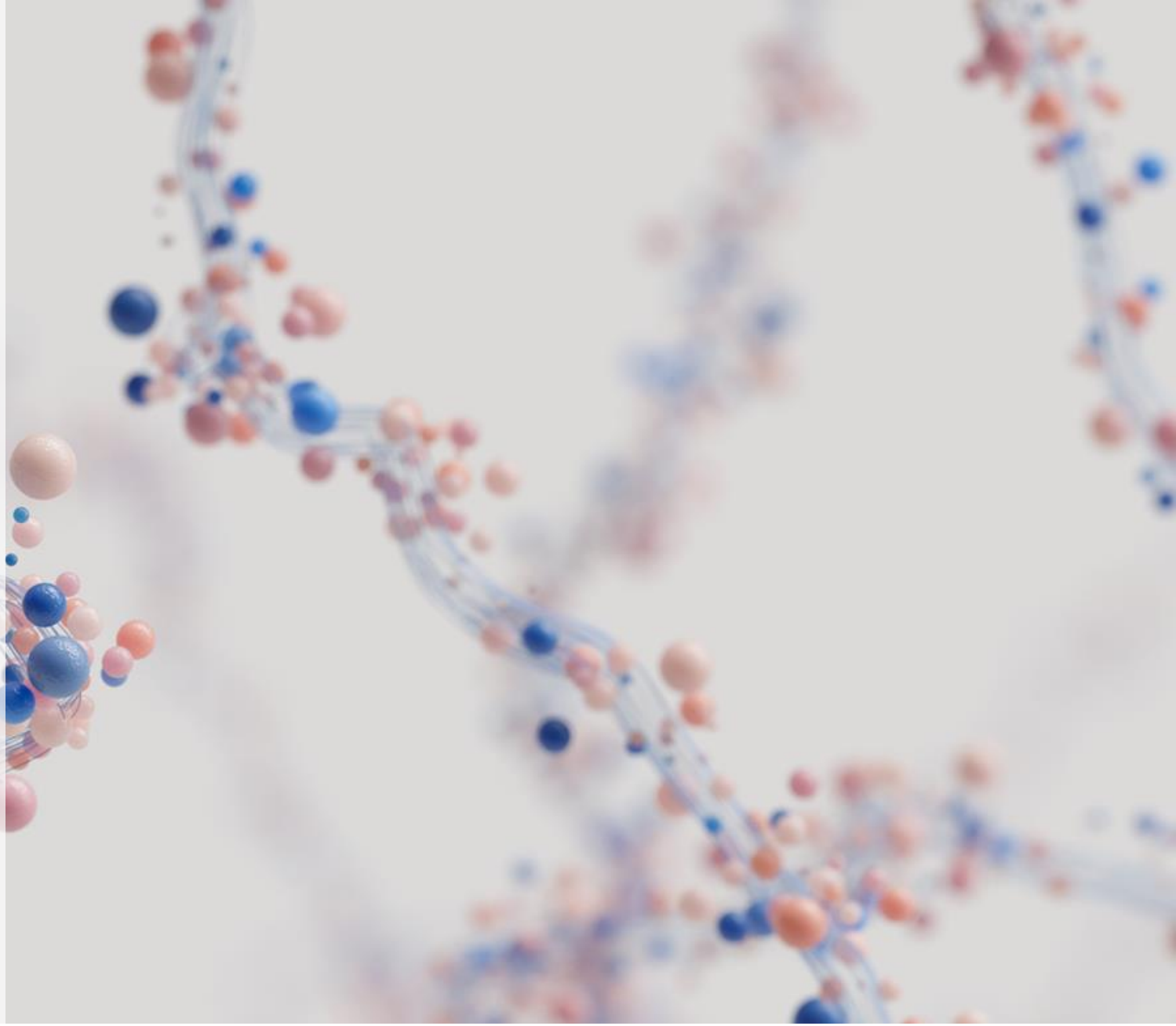
- Consistent icIEF profile as release procedure
- Allows characterization directly by MS
- Rapid and robust

Con

- Collection of charged isoforms for further characterization not possible

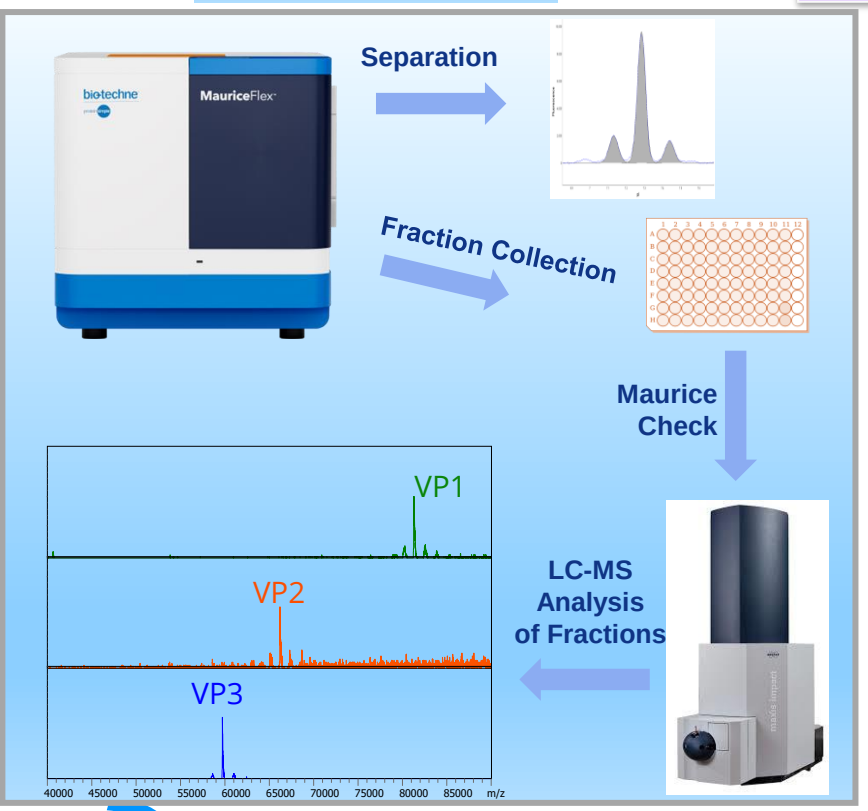
Enabling icIEF-MS Characterization of Charge Isoforms

- icIEF-LC/MS offline via fractionation of MauriceFlex
- icIEF-UV/MS online coupling with Intaibo/Zeno TOF 7600



icIEF-MS Workflows with Two Systems

- **MauriceFlex**



Sample Preparation

- Aligned with release icIEF method

icIEF Separation and Mobilization

INTABIO

MS Detection

- Sciex 7600

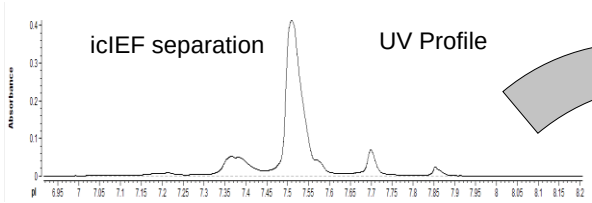
Alignment between UV and MS Data

- Intabio Software

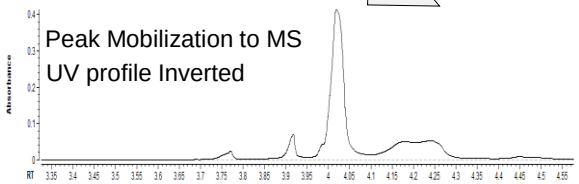
Data Processing

- Biologics Explorer

- **Intabio/Zeno TOF 7600**

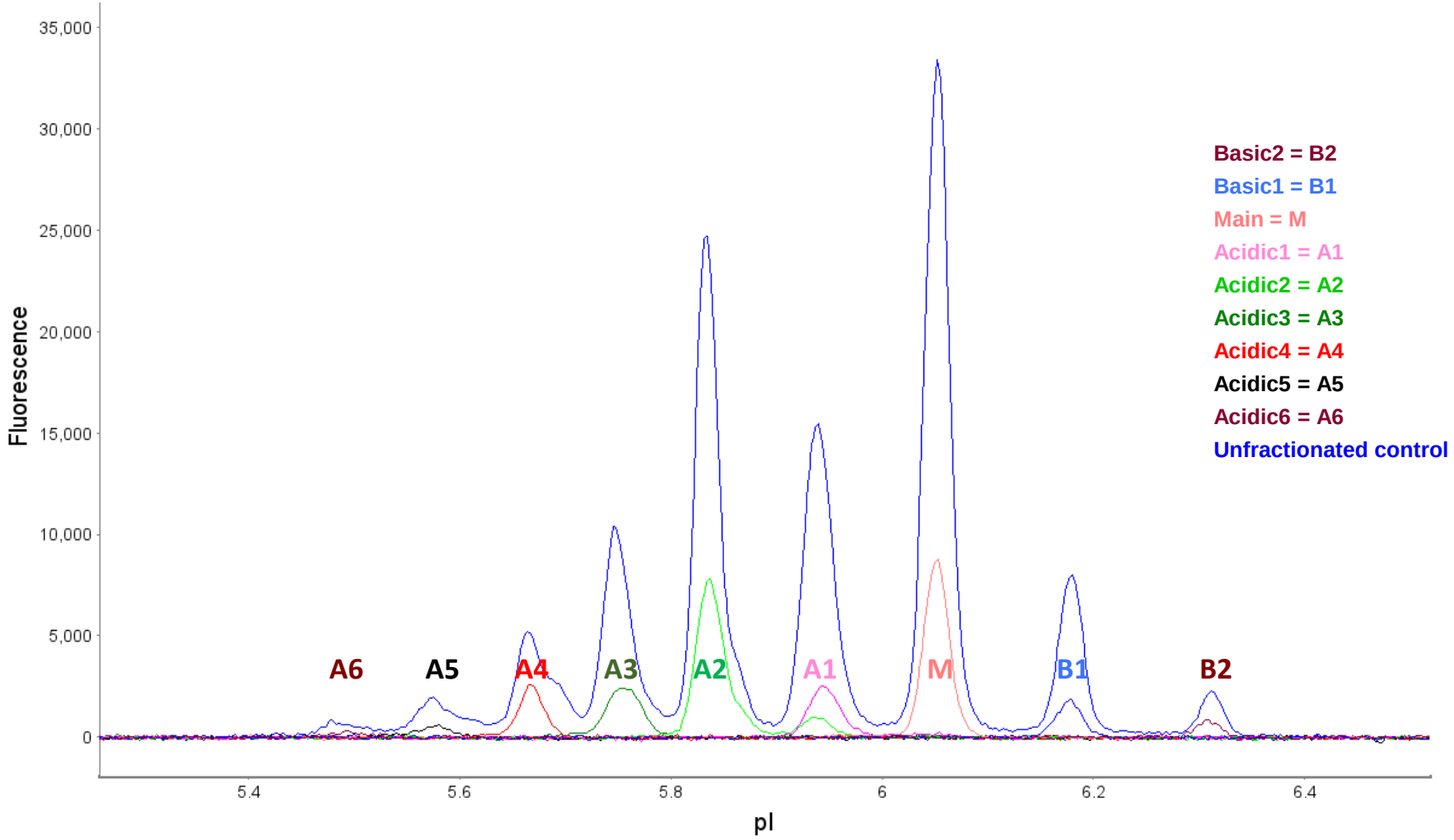


Chromatogram Inverted

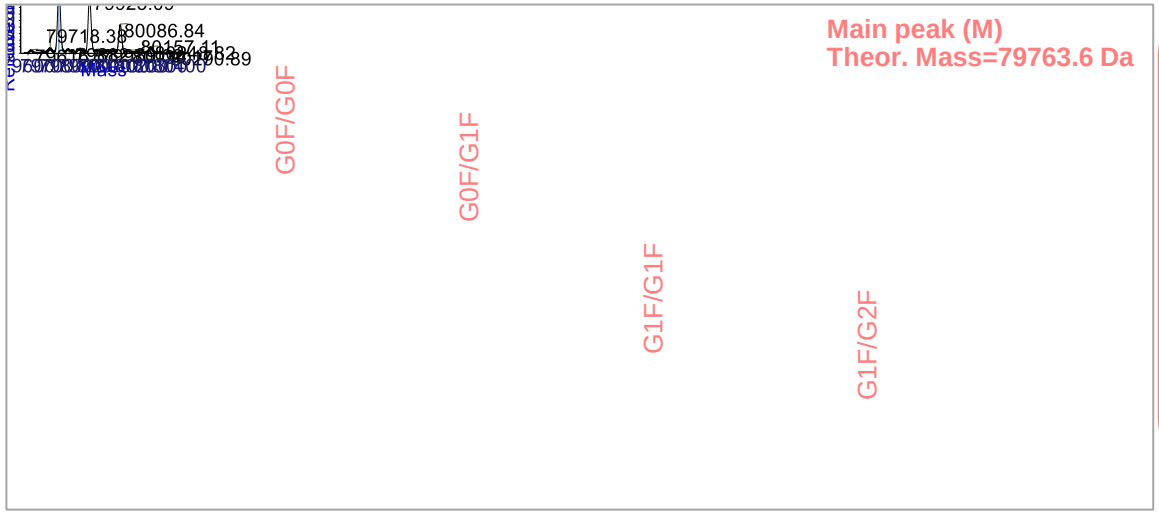


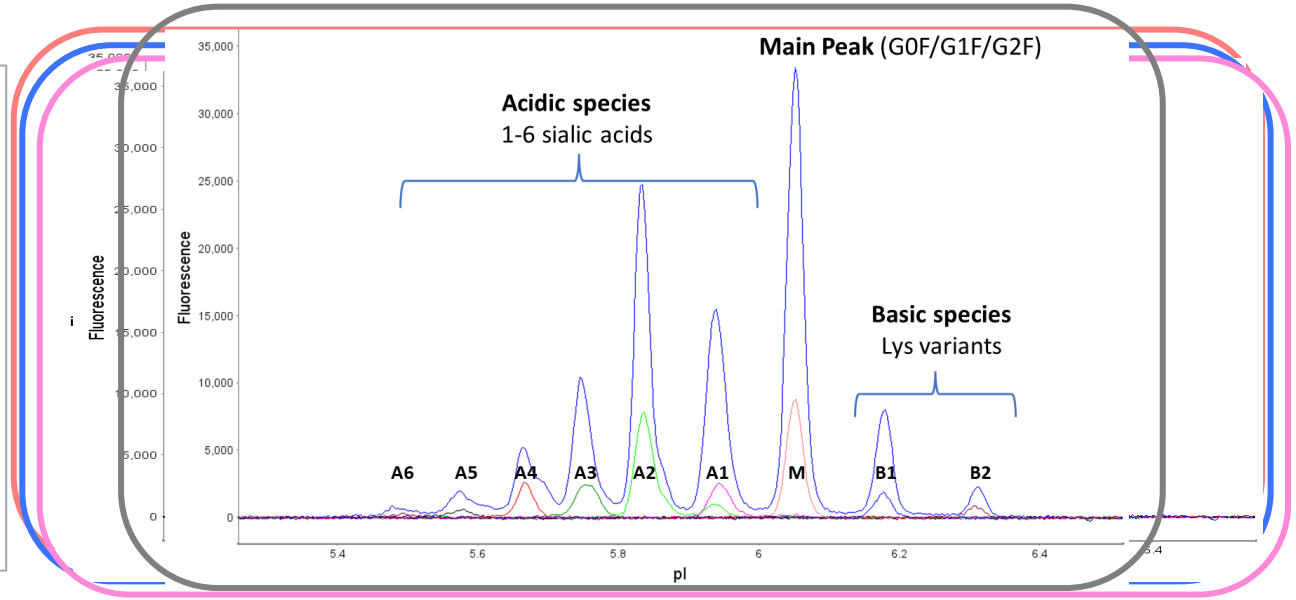
An Example of Internal Complex Protein on MauriceFlex

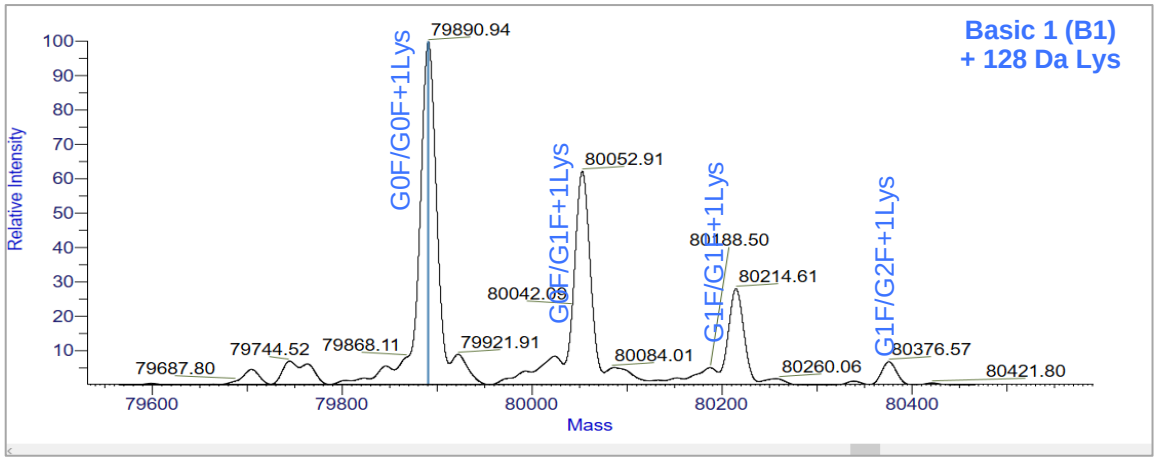
E-gram overlay of fraction samples with unfractionated control: showing high purity charge species

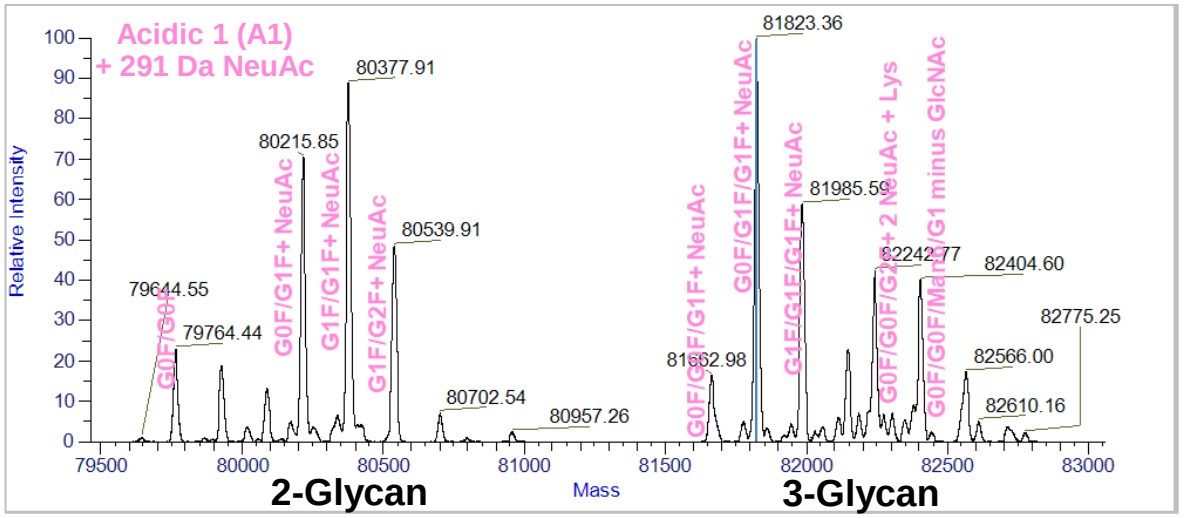


Peak Identity Confirmation of Fraction Samples by LC-MS

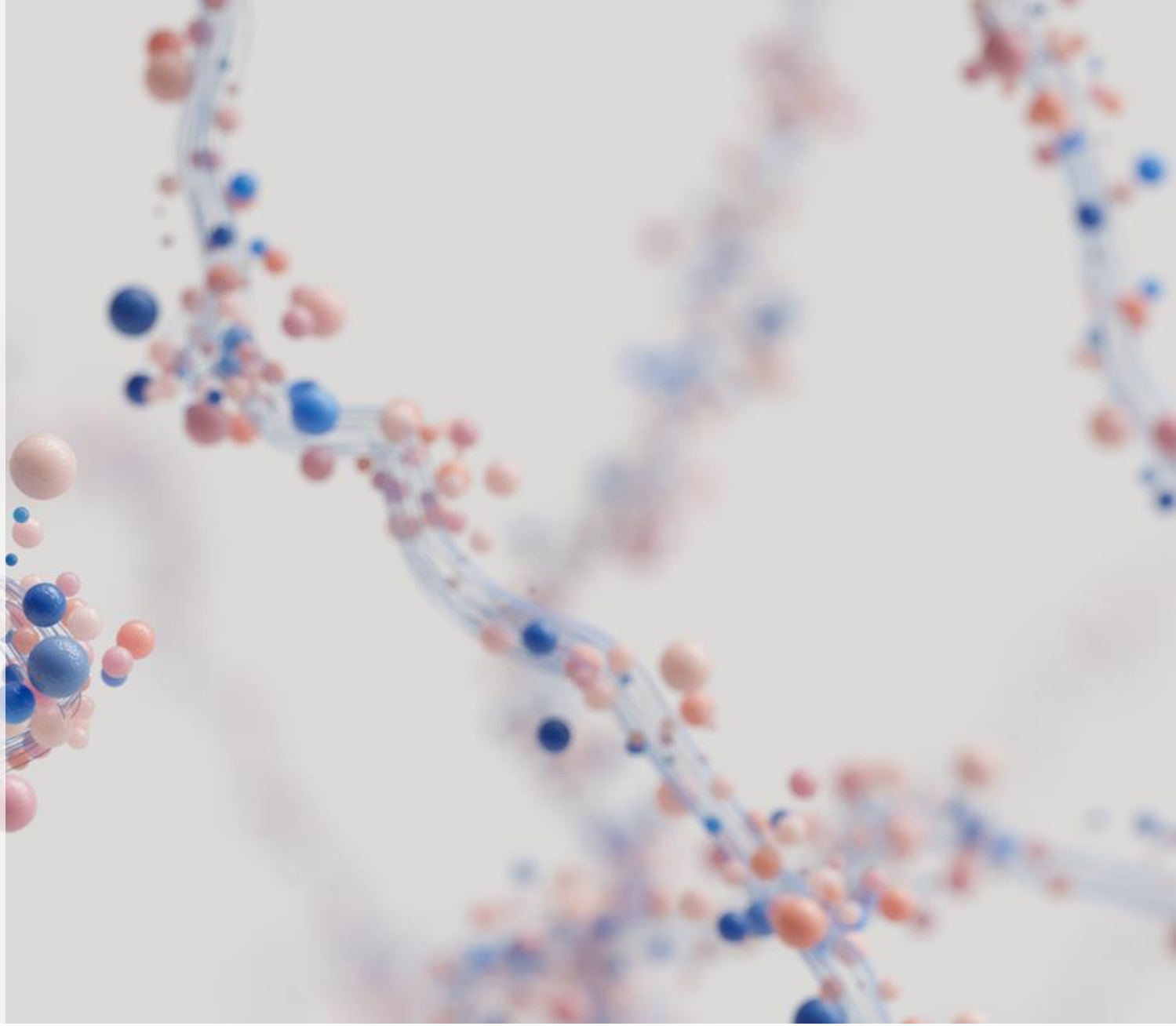






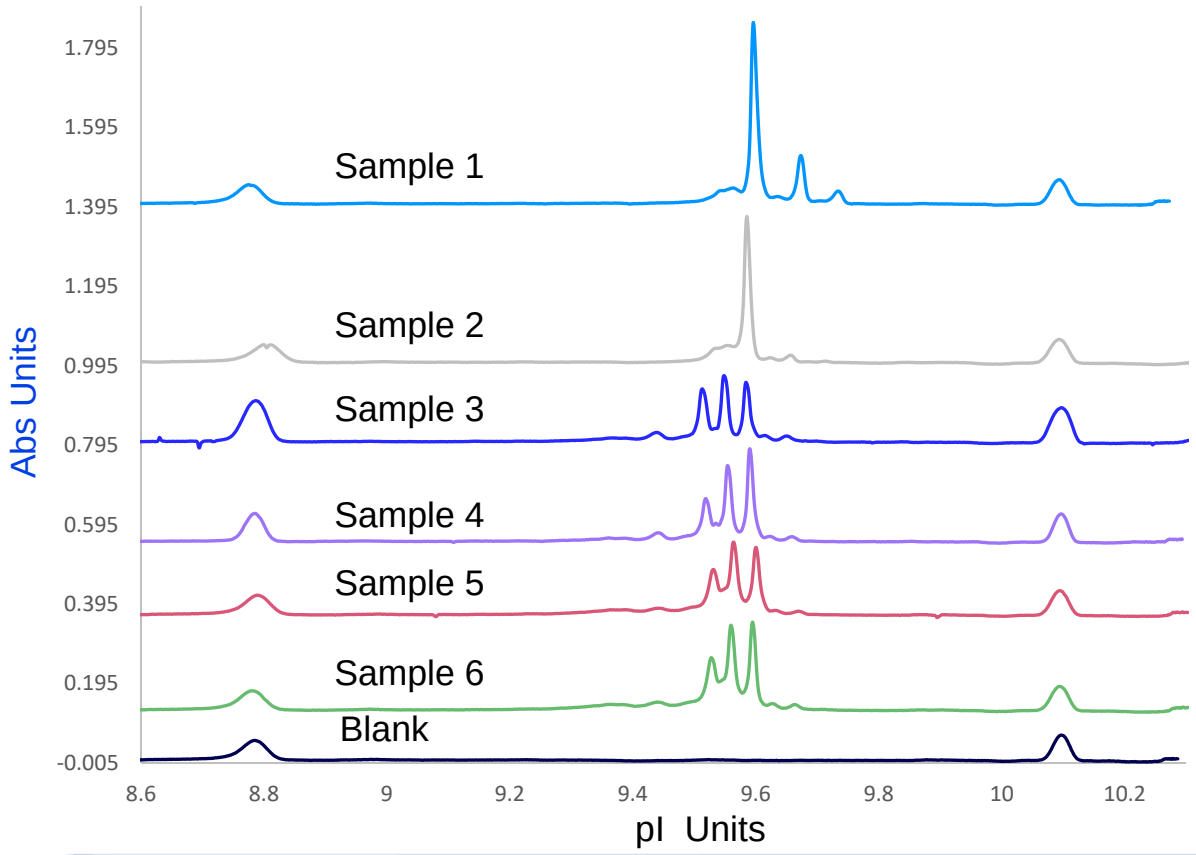


Introducing Intabio-icIEF for BTx Characterization

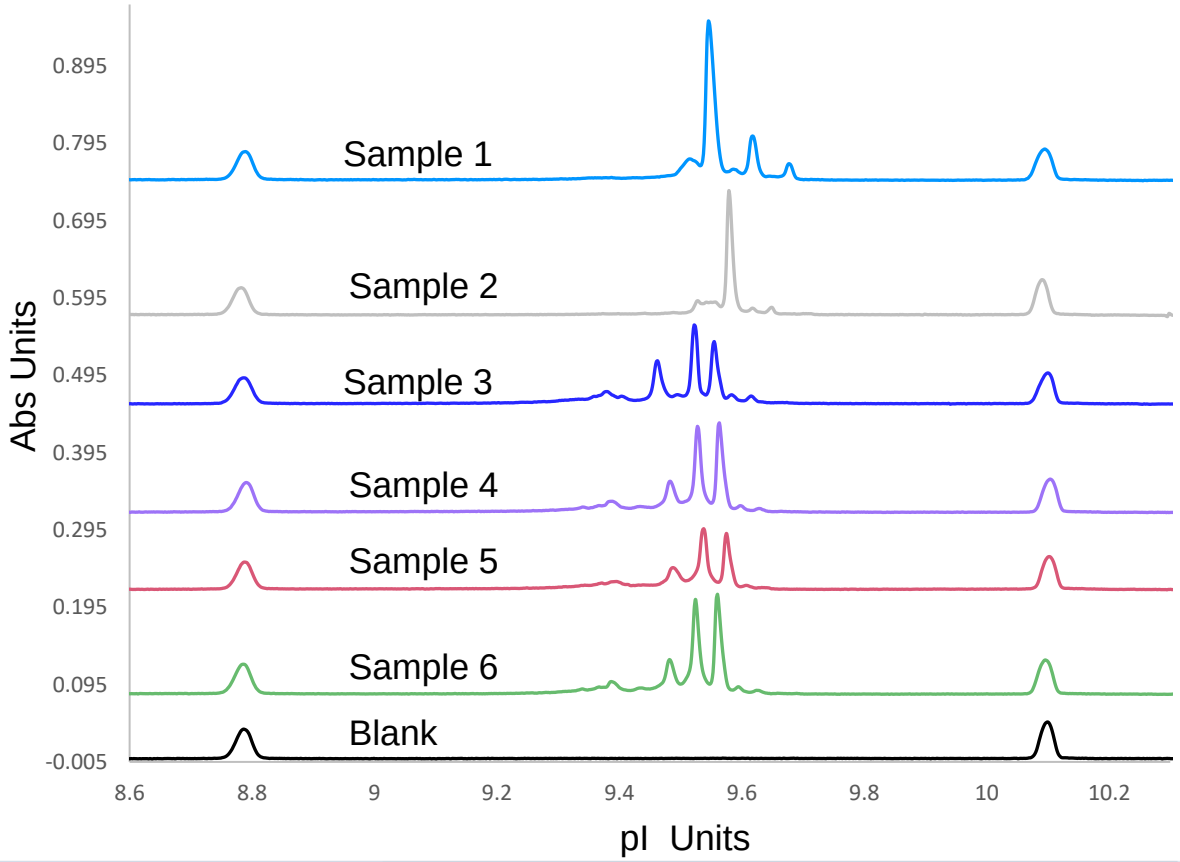


Intabio icIEF-UV Charge Profile Compared with Pfizer Internal icIEF

Pfizer Internal icIEF UV Profiles

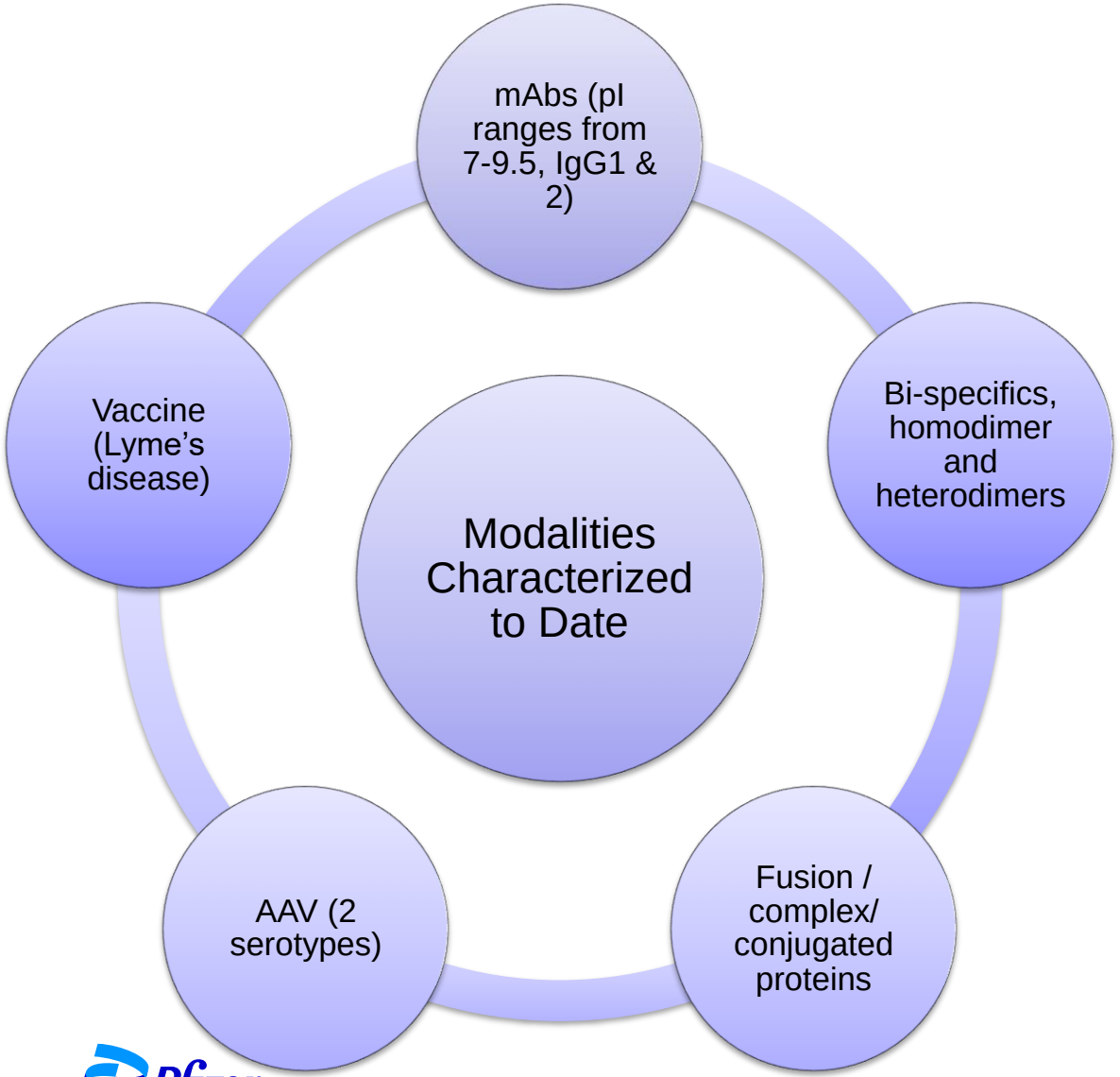


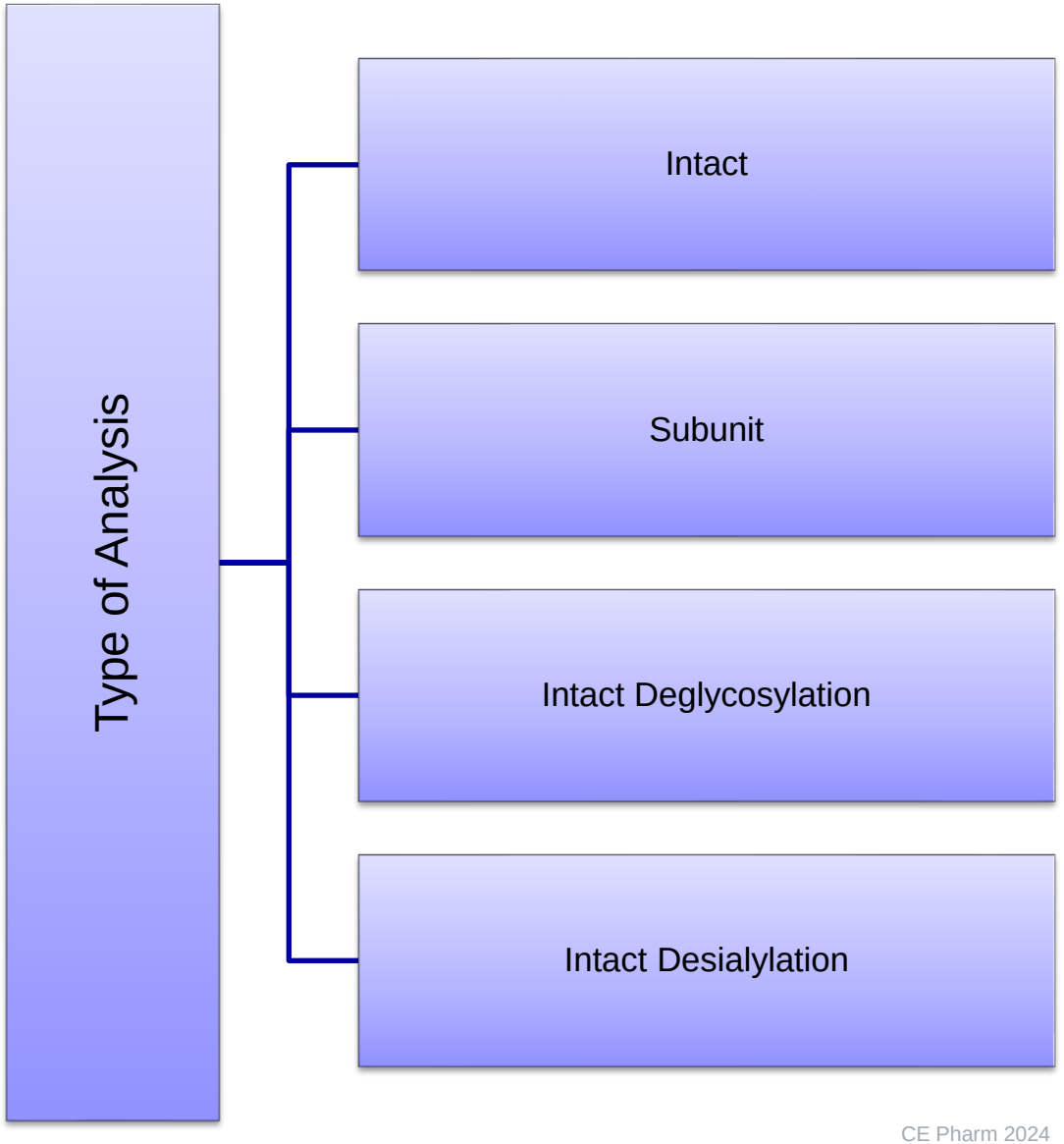
Intabio icIEF UV Profiles



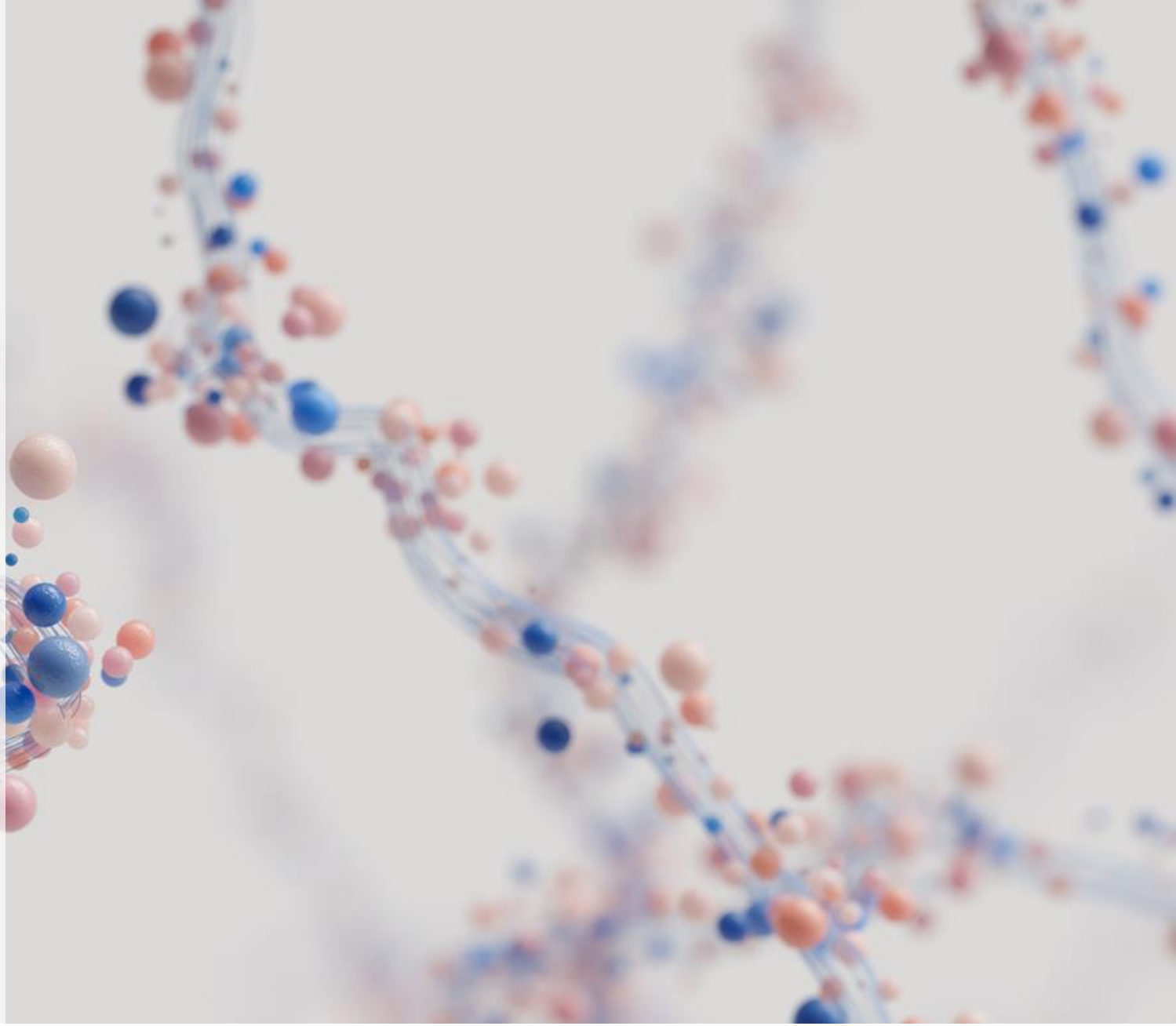
UV profiles are aligned between traditional icIEF separations and the Intabio icIEF separation

Overview of Work to Date





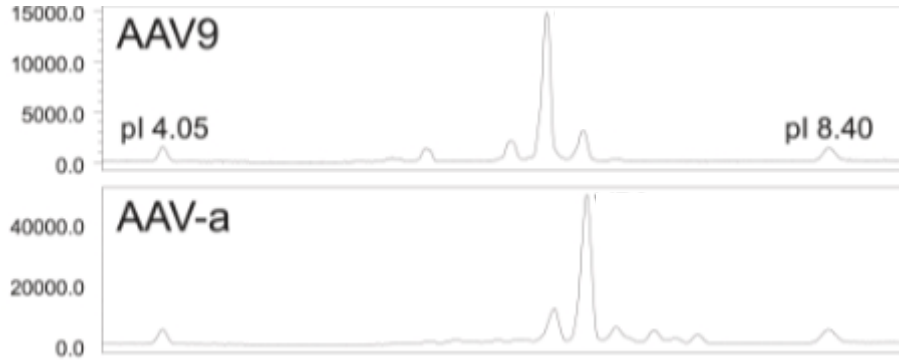
Revisiting the AAV Story: Application of Intabio Workflow to AAV



Intabio Analysis of AAV

For AAV, capsid deamidation is a CQA. An icIEF method was able to be developed; however, it is unclear which peaks represent which capsid proteins and/or acidic species thereof

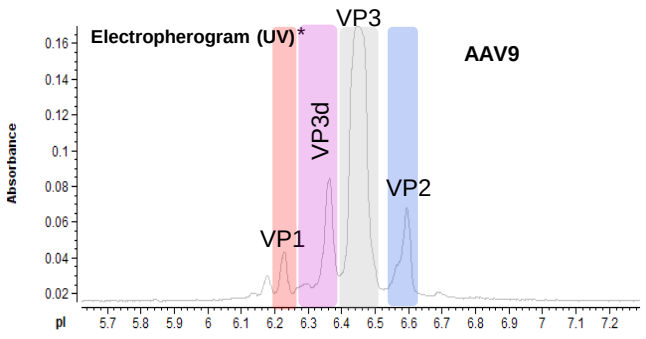
AAV

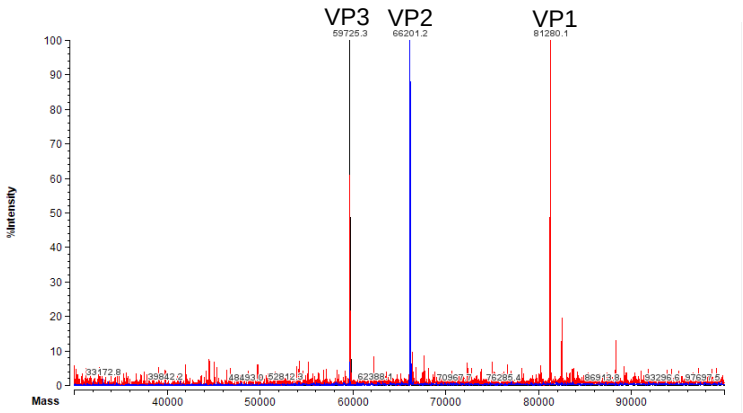


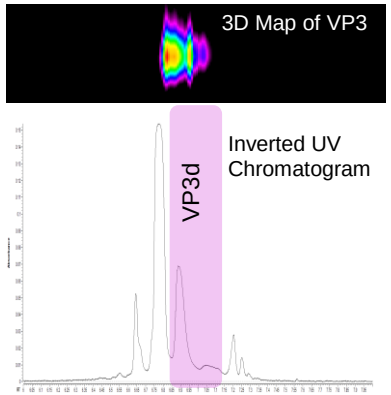
Assigning peaks was a complex, multiyear process (He et al. Methods and Clinical Development, 2023)

- RP-HPLC/MS method development characterization (not robust)
- Fractionation and subsequent analysis icIEF (VP stability issues)
- Generation and analysis of capsid mutants
- IEX-HPLC method was not developed

Project teams had already made progressed with an alternate complex analytical method during elapsed time.



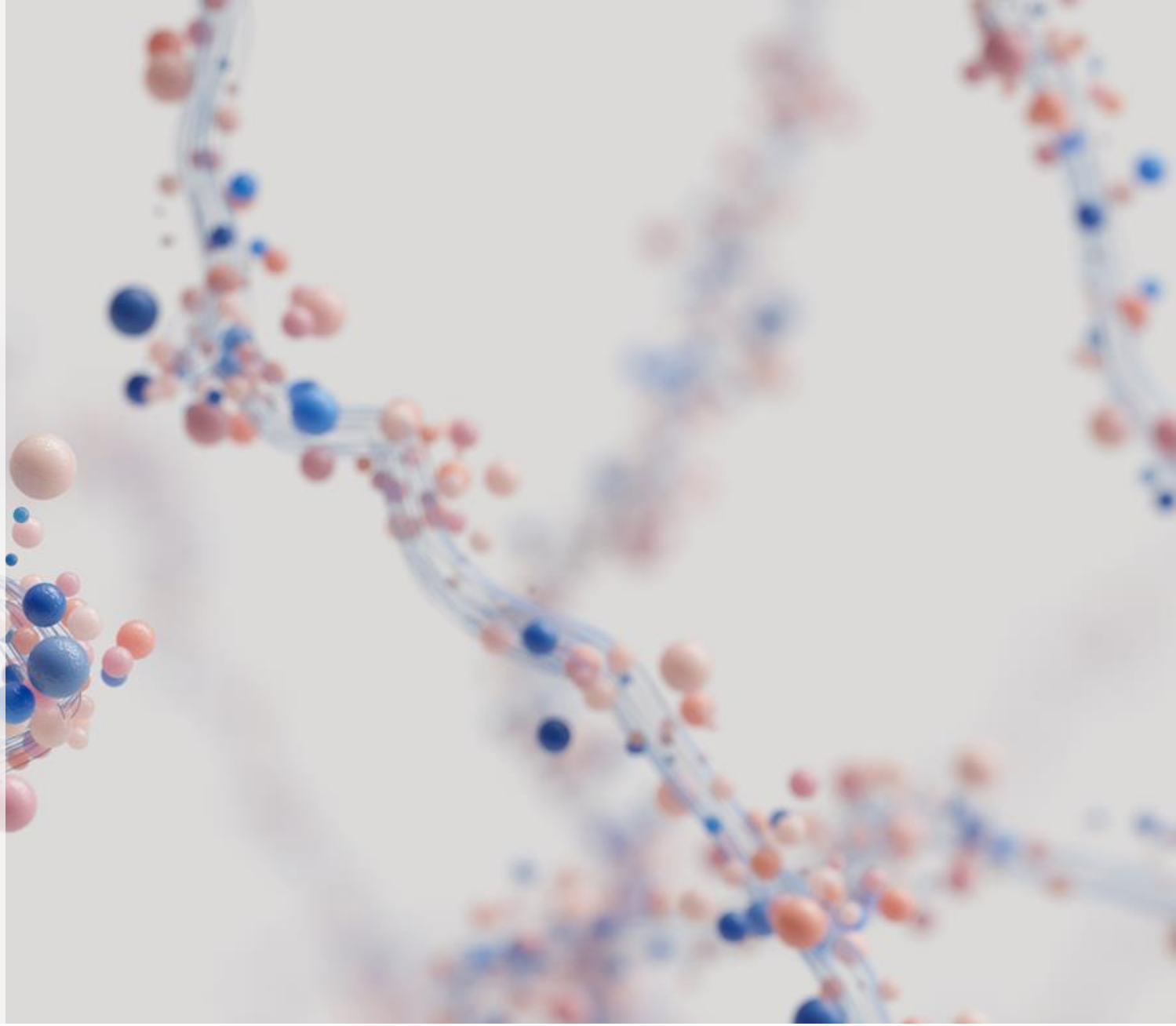




- icIEF profile replicated on Intabio
- VP1, VP2, and VP3 peaks easily identified
- 3D map shows acidic VP3 related peaks
- **Access to Intabio system could have impacted project team strategy regarding acidic species**

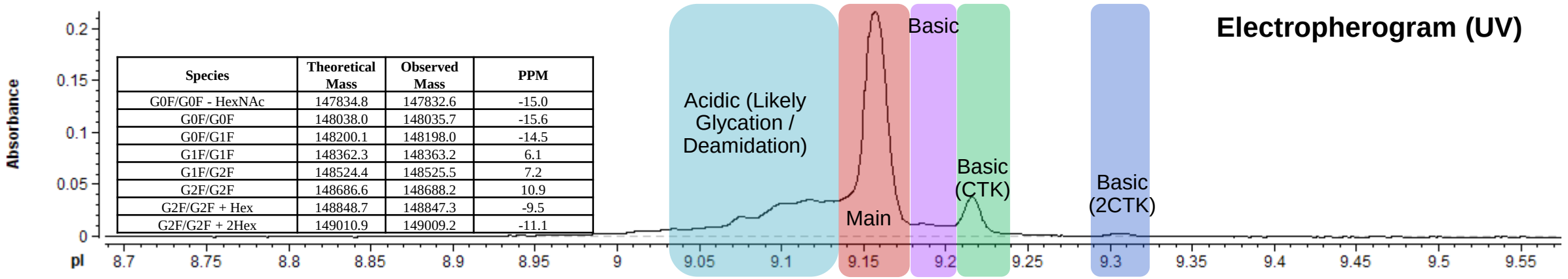
* Different sample / prep compared to historical profile

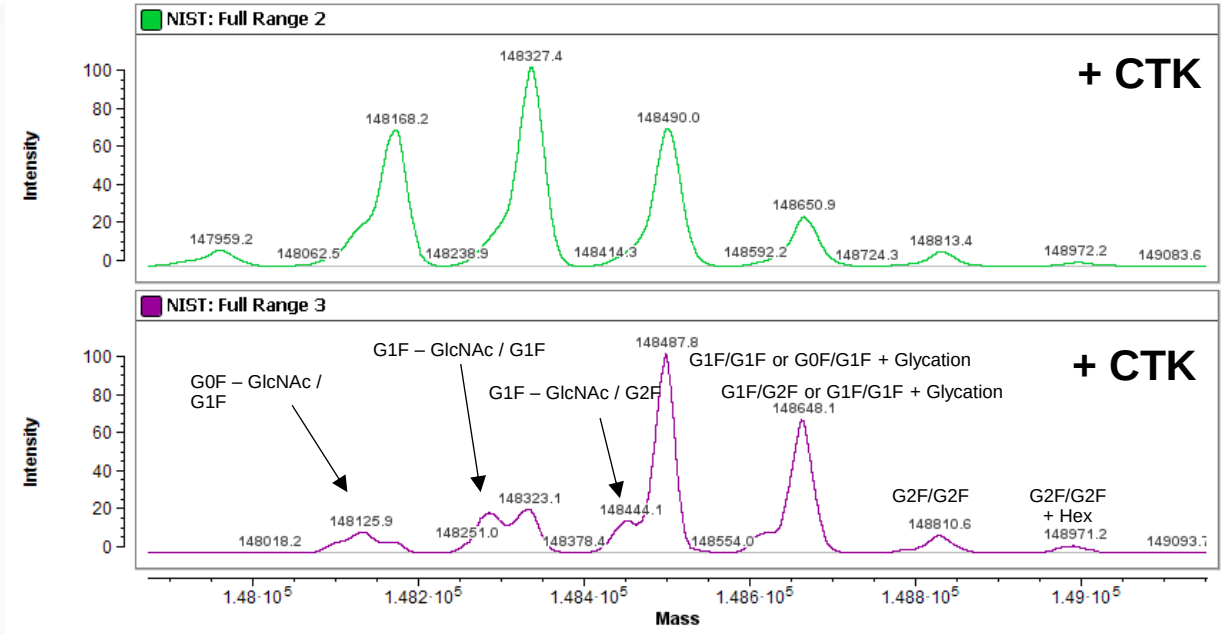
Streamlined Analysis of the mAbs using the Intabio



Example of NIST mAb Data Processing

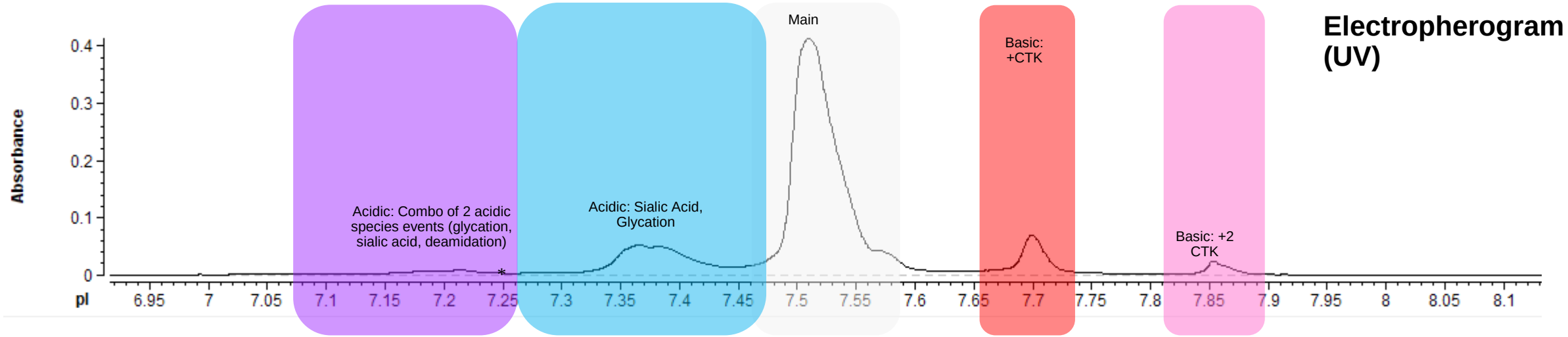
Species	Theoretical Mass	Observed Mass	PPM
G0F/G0F - HexNAc	147834.8	147832.6	-15.0
G0F/G0F	148038.0	148035.7	-15.6
G0F/G1F	148200.1	148198.0	-14.5
G1F/G1F	148362.3	148363.2	6.1
G1F/G2F	148524.4	148525.5	7.2
G2F/G2F	148686.6	148688.2	10.9
G2F/G2F + Hex	148848.7	148847.3	-9.5
G2F/G2F + 2Hex	149010.9	149009.2	-11.1

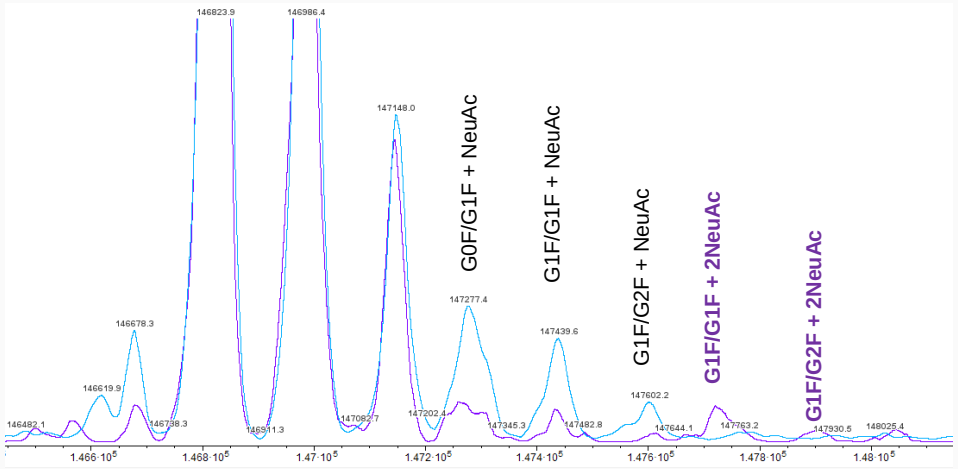




- Same species identified between Intabio data and in literature
  - Peak resolution of G0F – GlcNAc is not as good on Intabio dataset, but is fit for purpose for intended need
- Presence of + C-Terminal Lysine and 2 C-Terminal Lysines can be easily identified
- Broad acidic species indicative of hexose addition, likely due to glycation. Some signal could also be linked to deamidation.
- pI can be impacted by specific glycoform (G0F-GlcNAc) and there can be some combination of acidic and basic modifications that can complicate categorization

Example of Pfizer mAb Data Processing

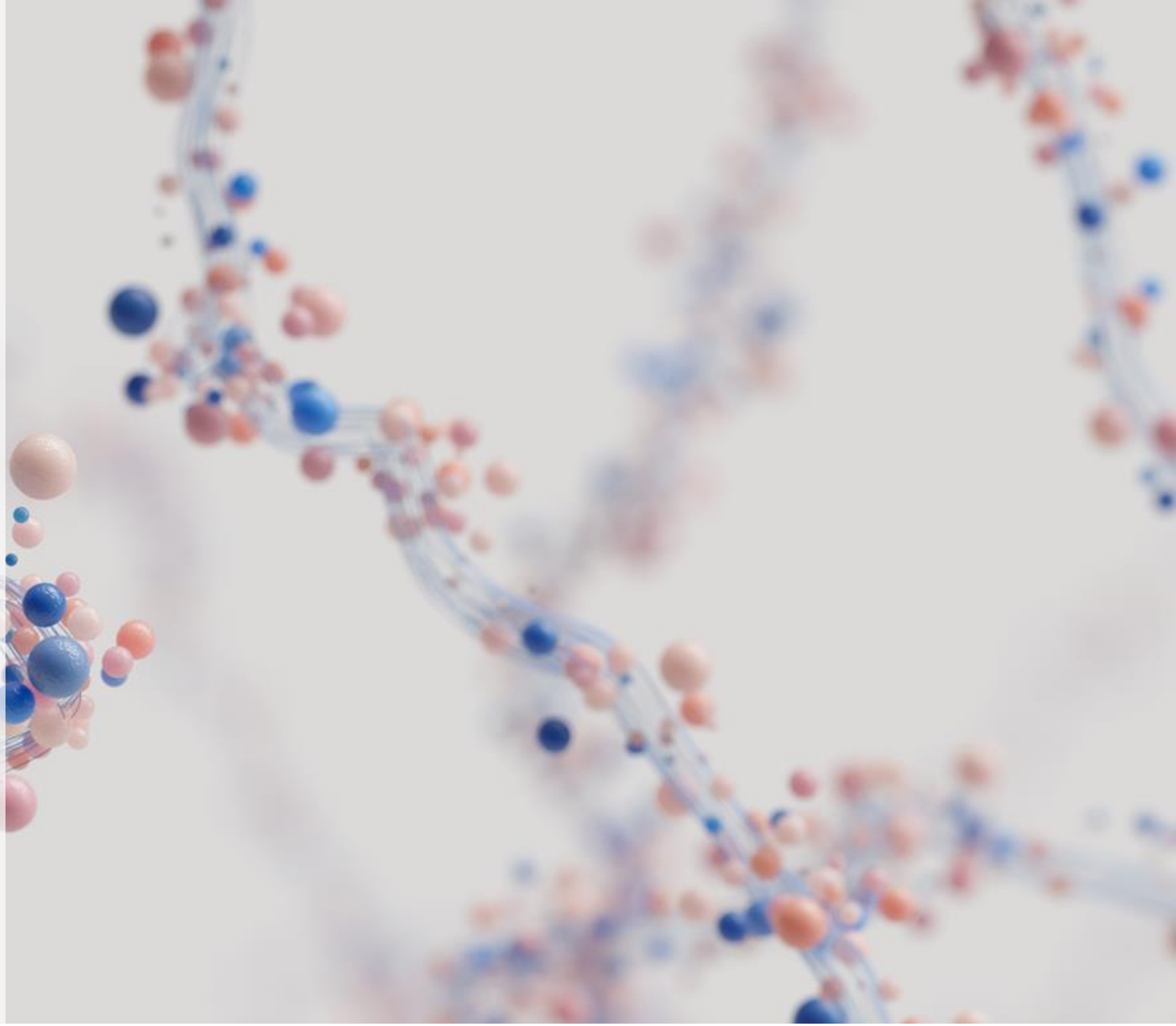




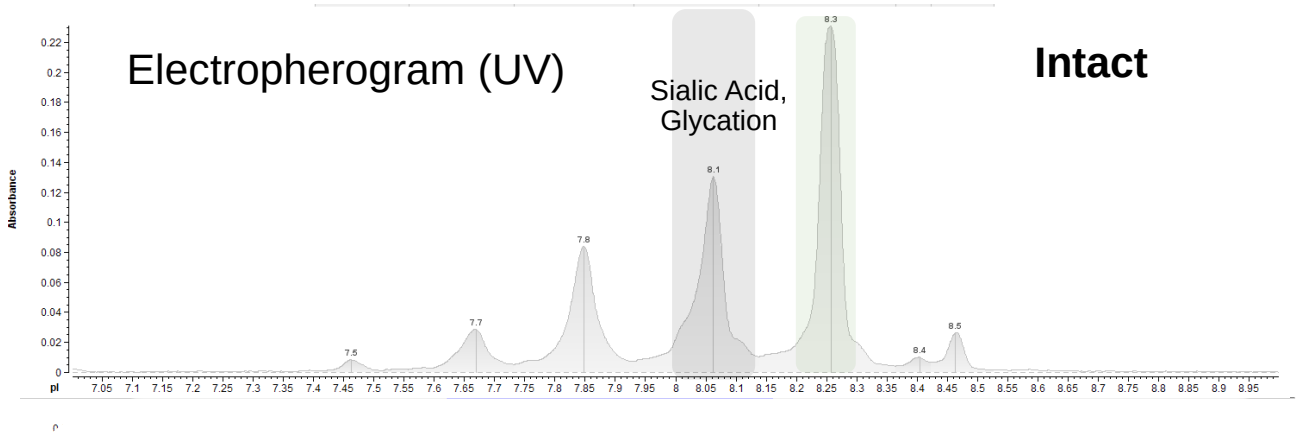
- Main species for internal mAb can be easily identified in Intabio data with good mass accuracy
- Basic species can be easily identified as + C-Terminal Lysine and 2 C-Terminal Lysines
- Acidic species 1 likely represents glycation, deamidation and sialic acid
- Acidic species 2 represents combination of acidic species events (glycation, sialic acid, deamidation)

* Appears related to glycoform variability

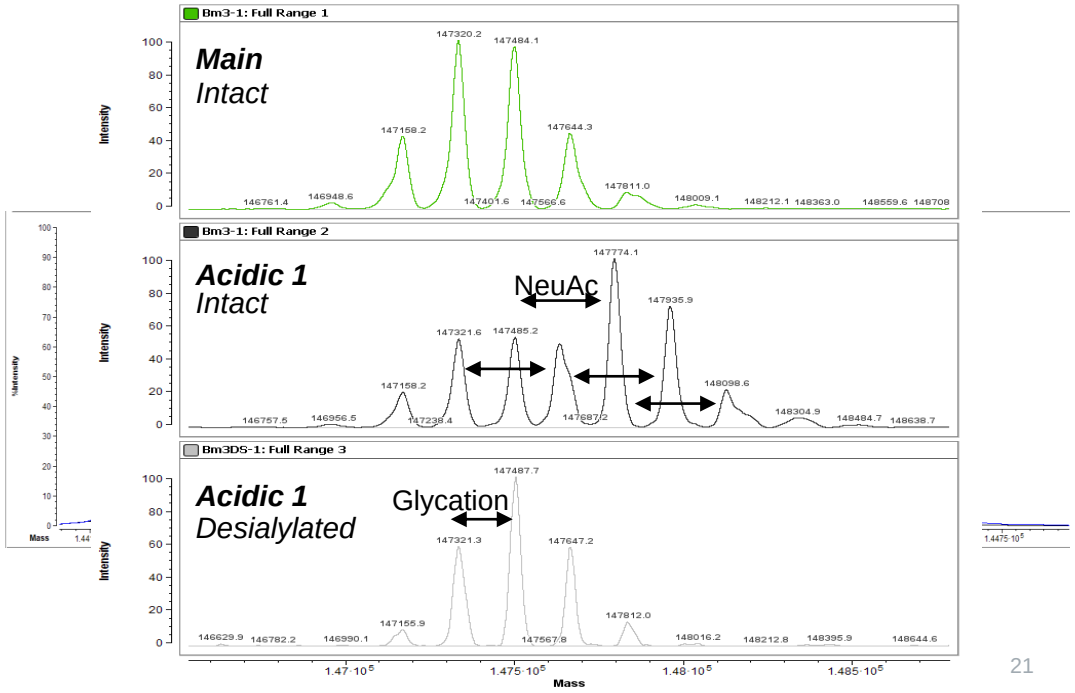
Analysis of Bispecifics with the Intabio and Combining icIEF-MS and Glycosidases



Analysis of Pfizer Bispecific



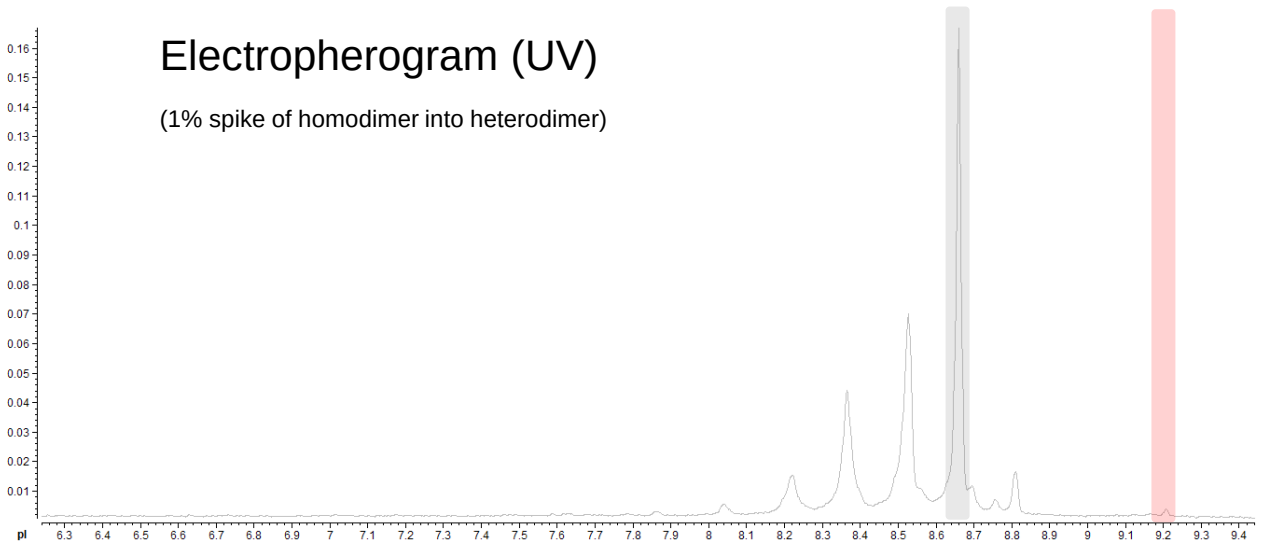
- Complex charge profile observed and glycosidase digestion could help elucidate species
- Basic species profile is not impacted by glycosidase reaction, suggesting that it is not linked to glycosylation/occupancy
- Basic species can be easily identified as + C-Terminal Lysine and Proline Amidation
- Acidic species 1 is related to sialic acid (portion removed by sialidase) and glycation
- Remaining acidic species largely linked to sialic acid (removed with sialidase)

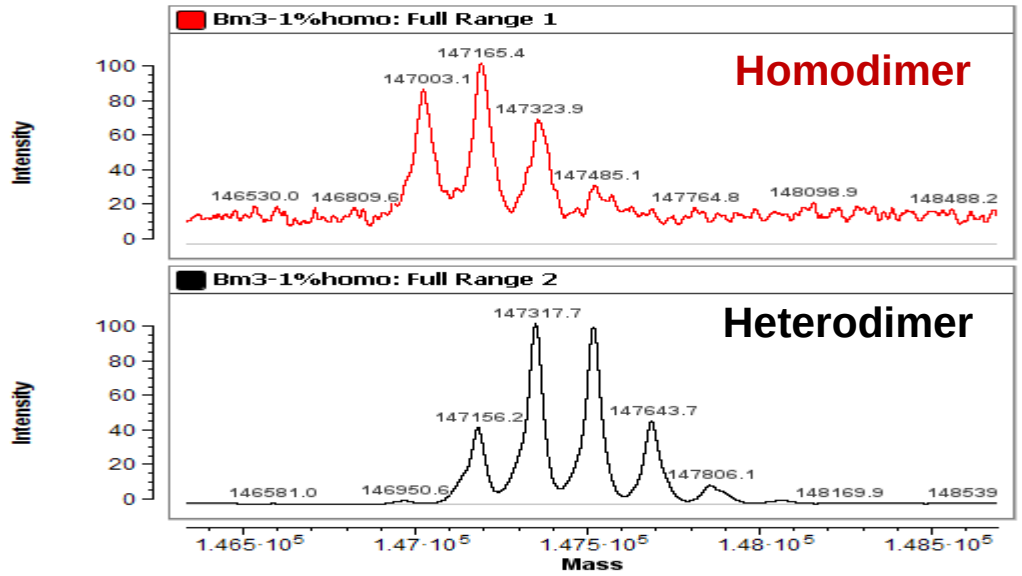


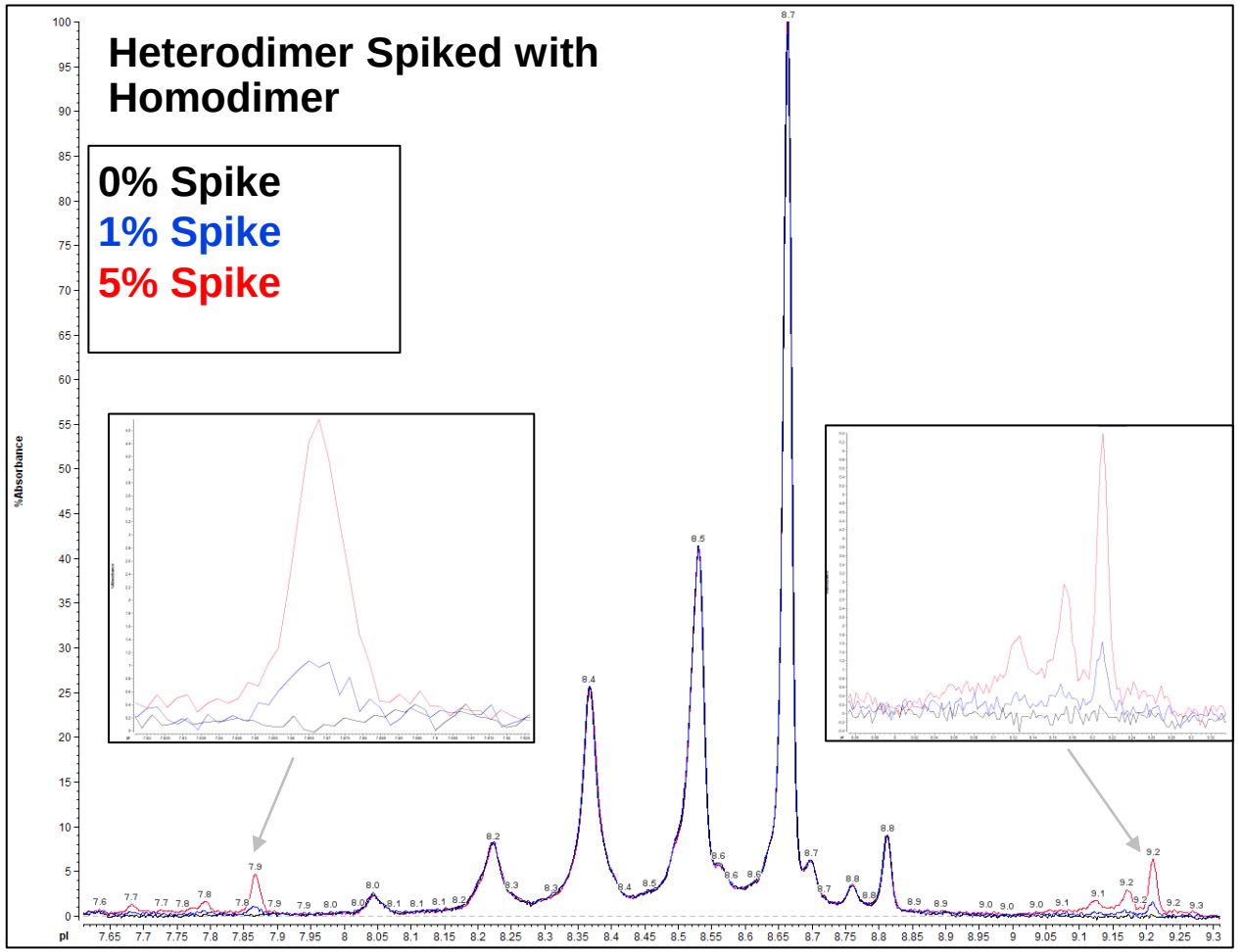
Assessment of Homodimers with icIEF

Electropherogram (UV)

(1% spike of homodimer into heterodimer)

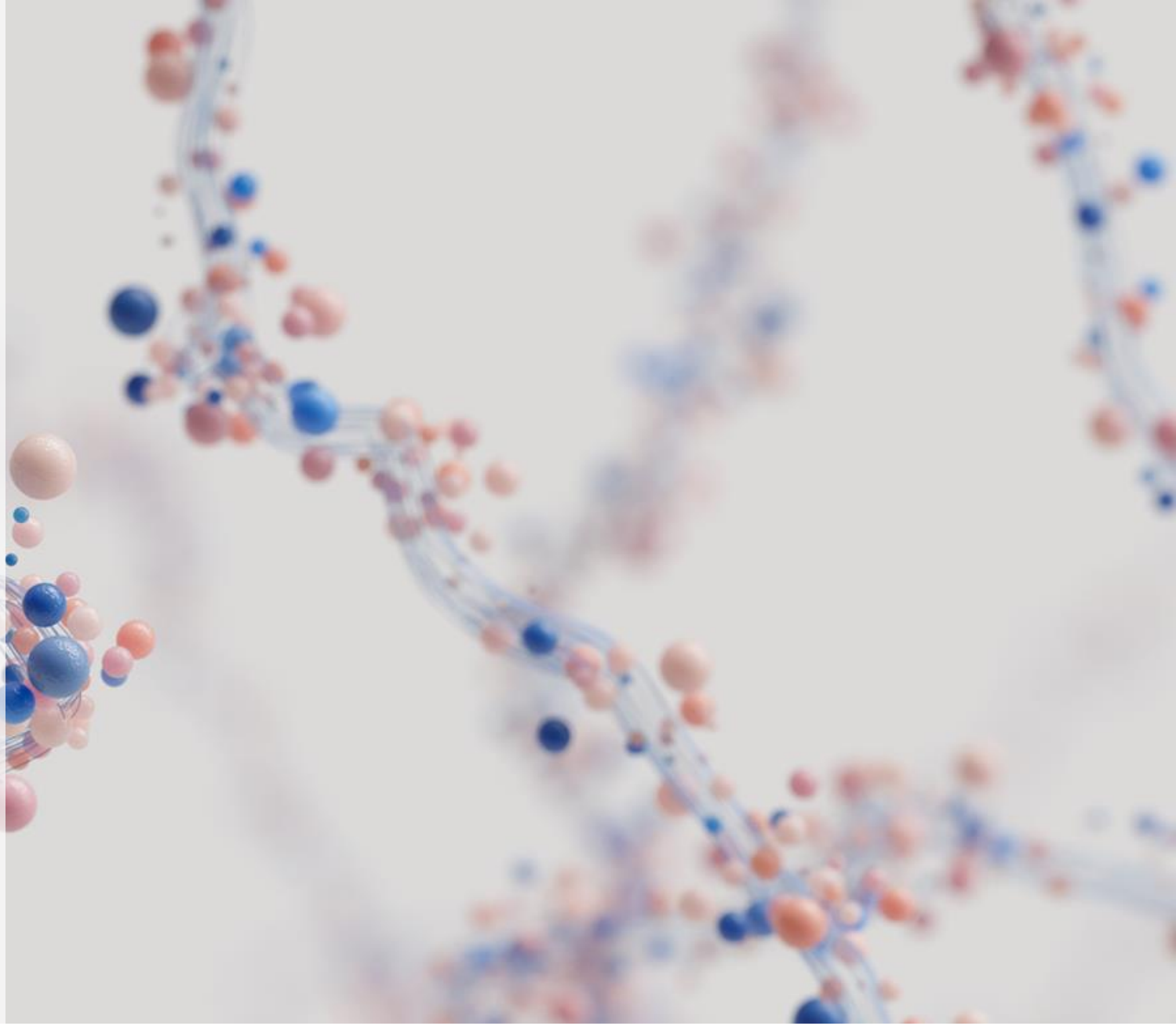






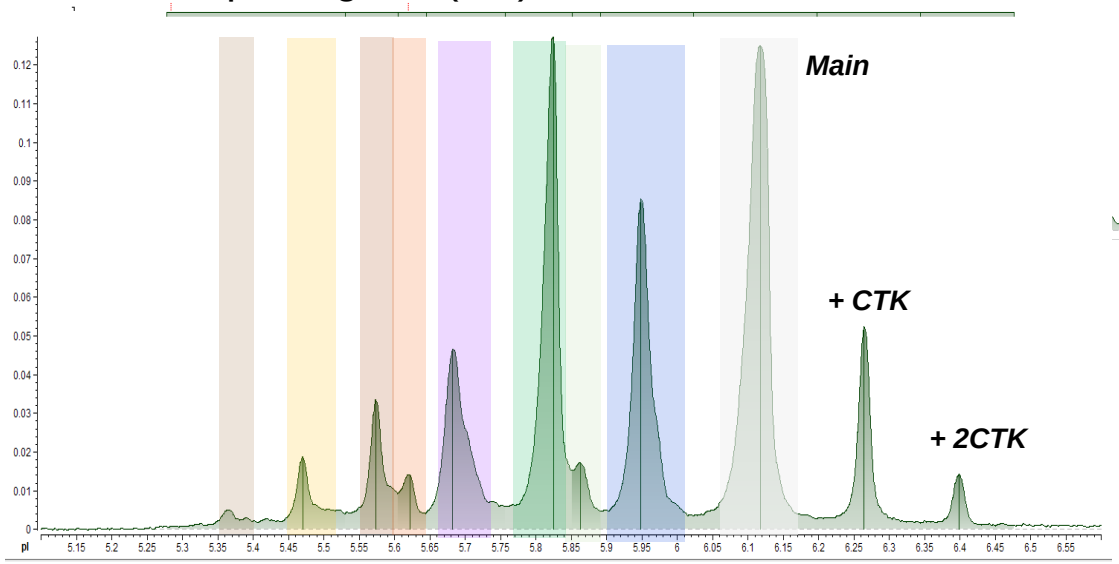
- Intabio system can identify and characterize the presence of **homodimers** in bi-specific species
- Spiking study confirmed the identification

Analysis of a Complex Internal Protein



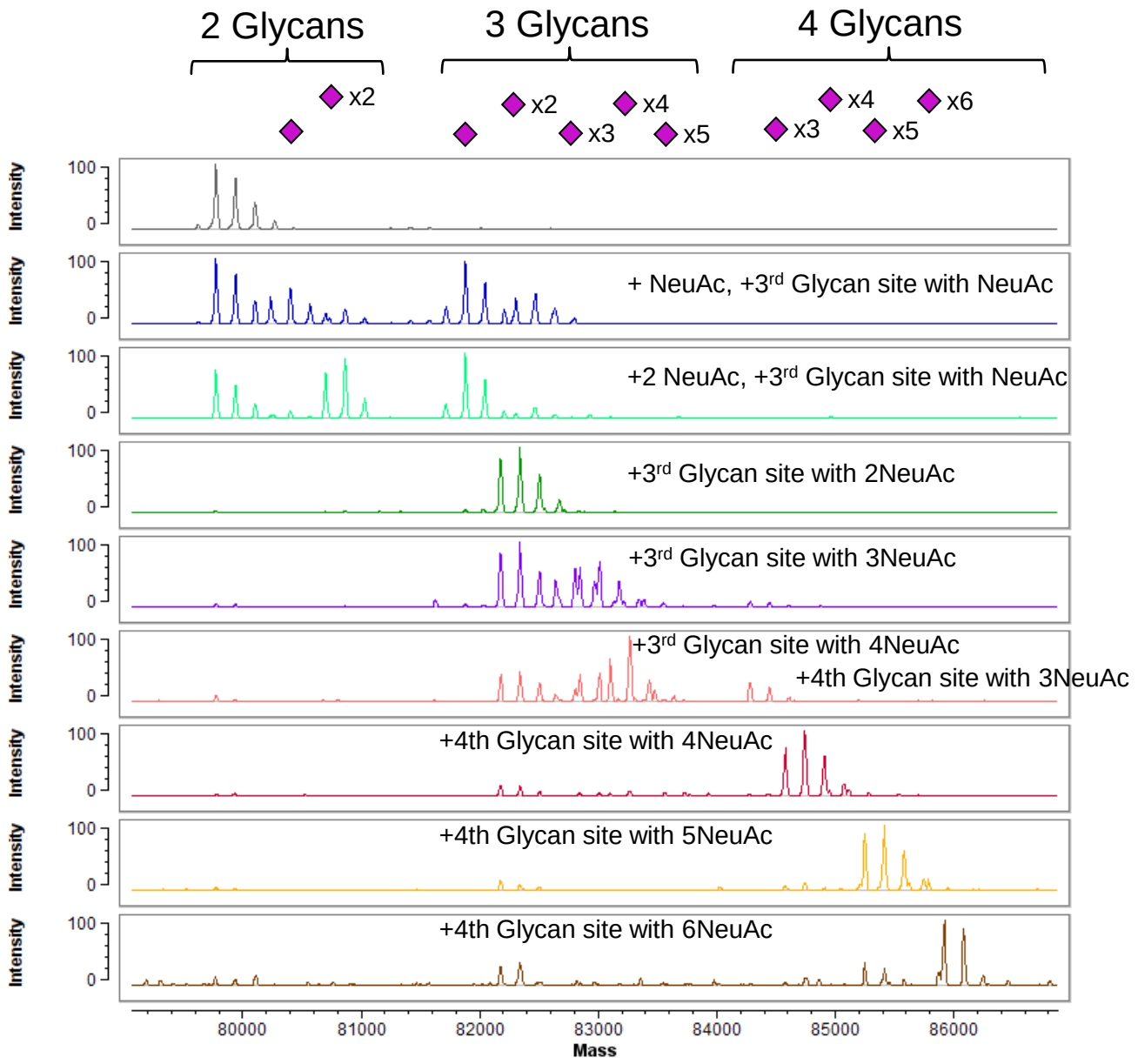
Analysis of a Complex Protein

Electropherogram (UV)

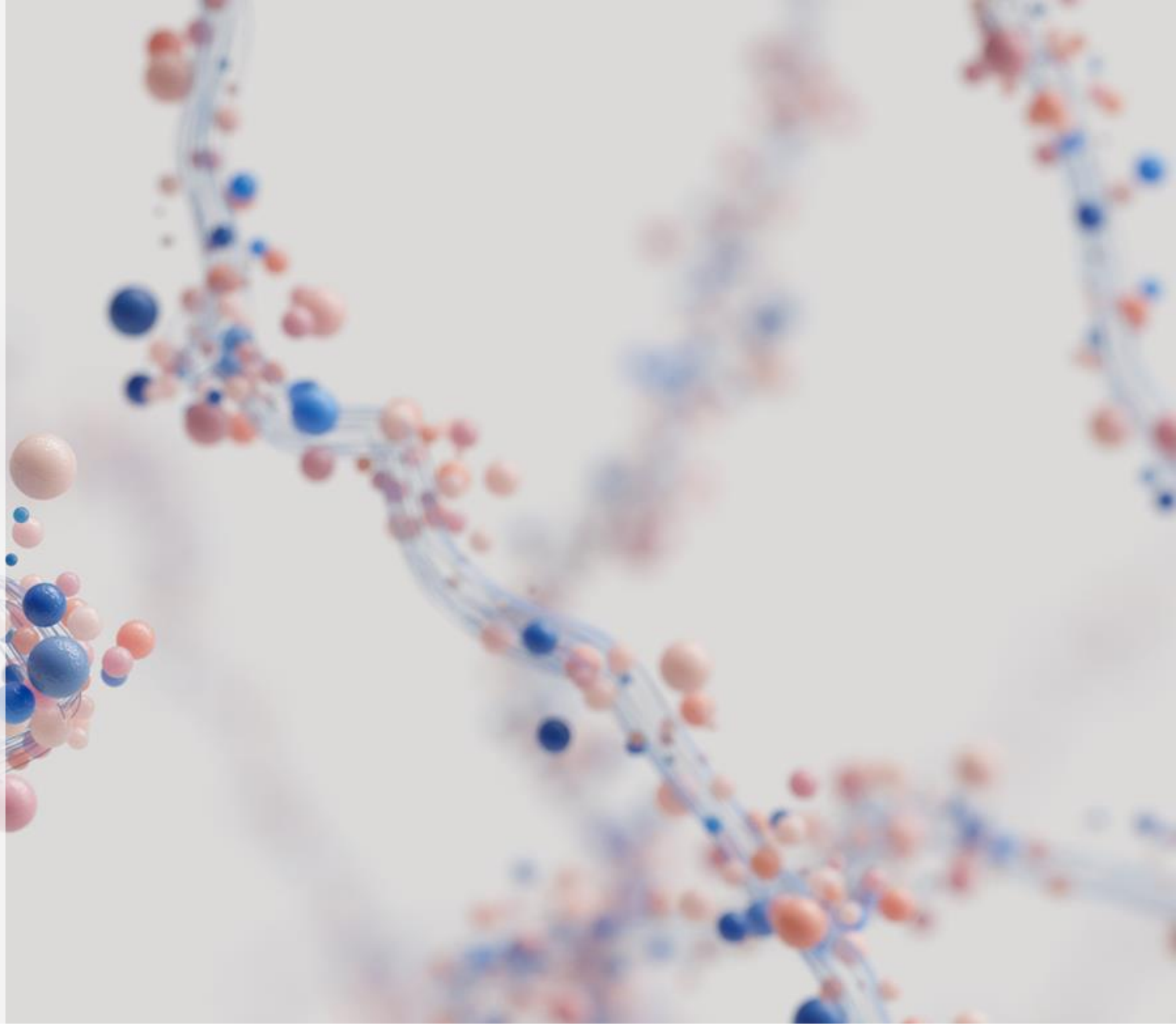


- For complex molecules it may not be apparent which peak is the main species, other than by pI
- Sialidase helps to clarify main peak
- Analysis of acidic species reveals additional sialic acid

  And glycoforms



Beyond icIEF Characterization: How icIEF-MS can Bolster Understanding of MS Data

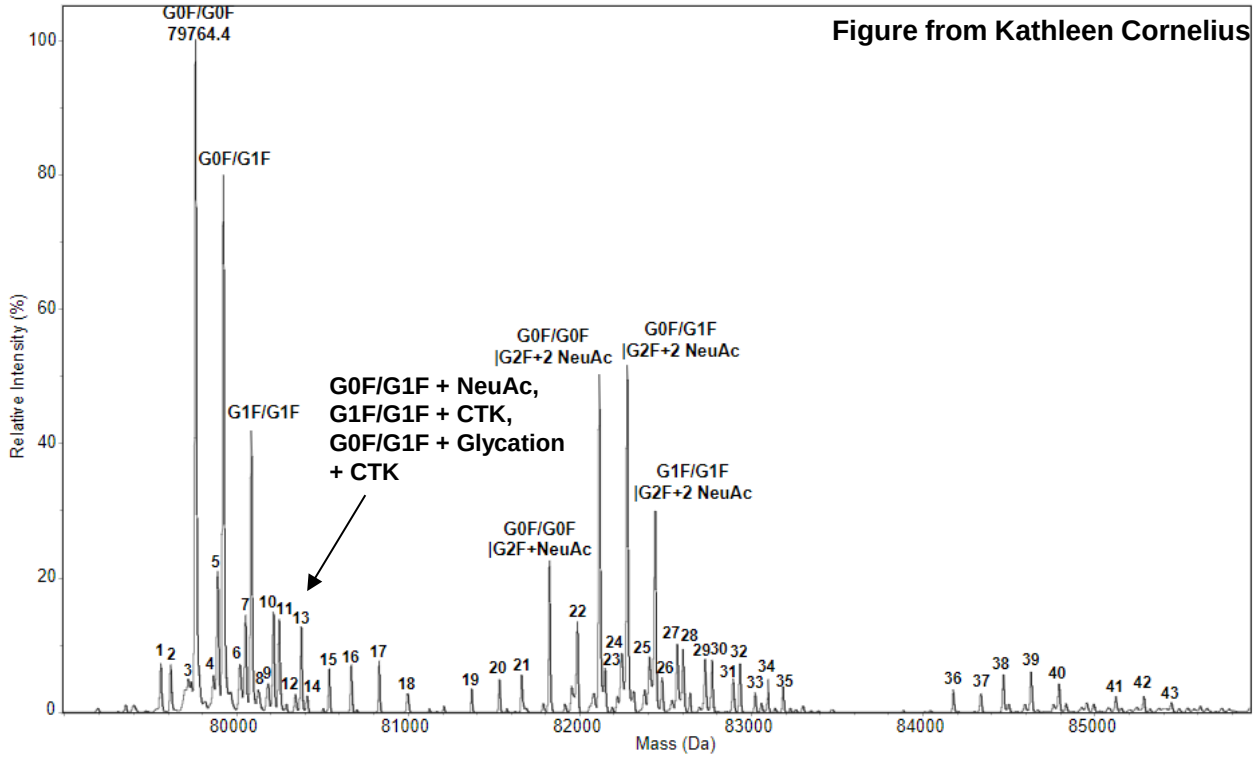


icIEF-MS Data can Aid in Mass Assignments

Intact mass analysis can have challenges differentiating modification combinations

C-Terminal Lysine 128 Da	C-Terminal Lysine 128 Da	Sialic Acid 291 Da
+ Galactose 162 Da	+ Glycation 162 Da	

MS ~ 290 Da ~ 290 Da ~ 291 Da



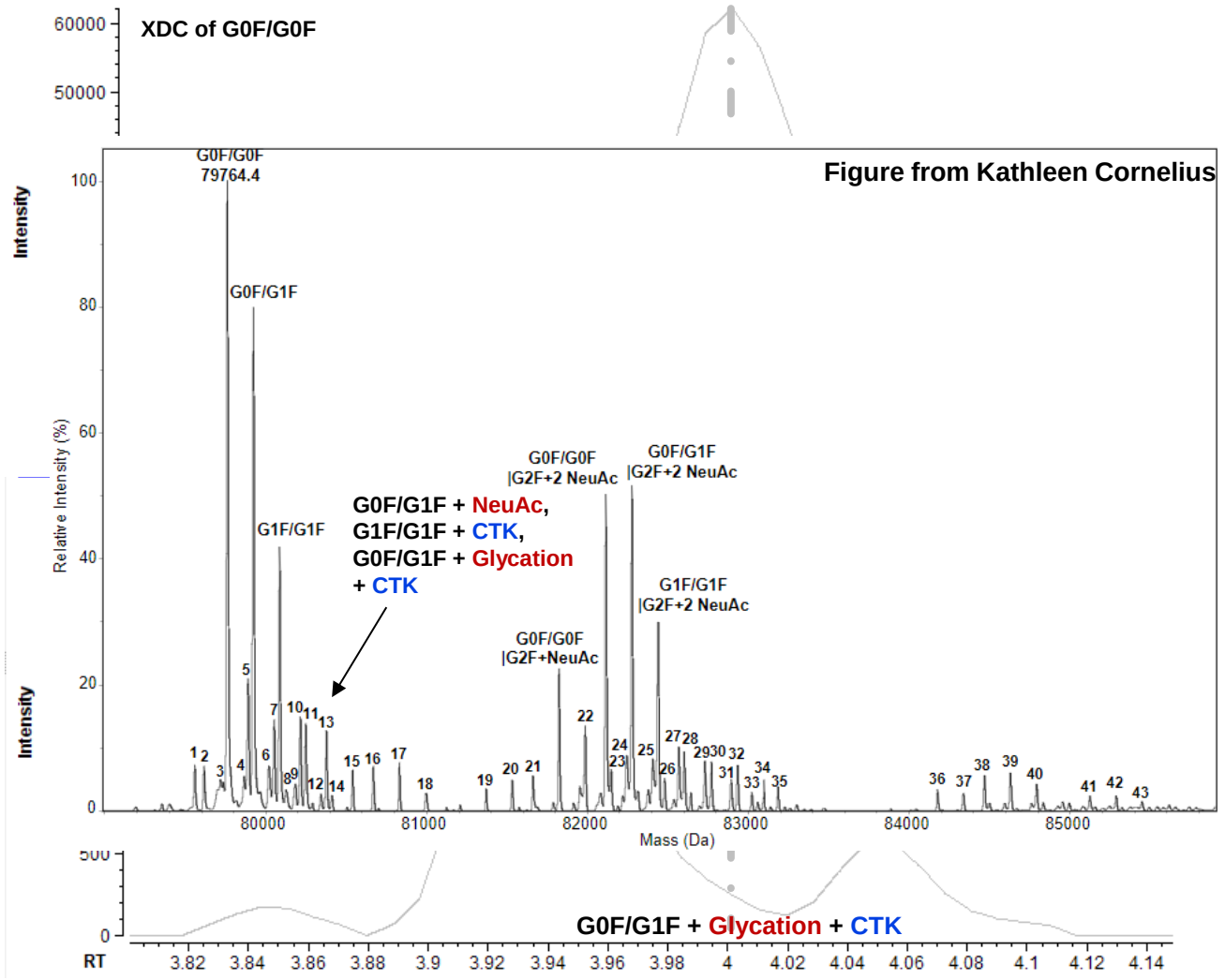
- Combinations in modifications can result in highly similar masses that are difficult to distinguish by mass alone

icIEF-MS Data can Aid in Mass Assignments

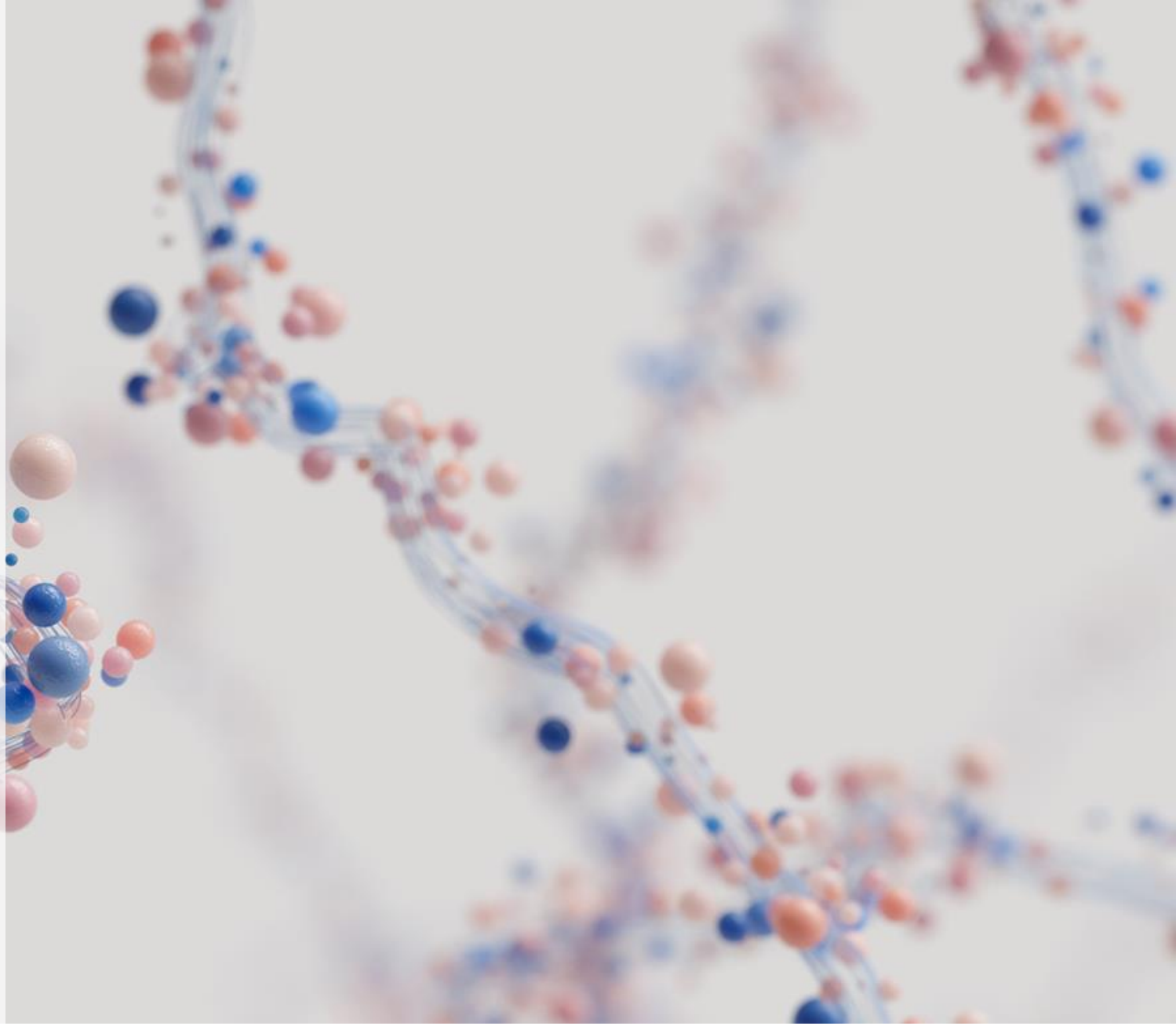
Intact mass analysis can have challenges differentiating modification combinations

C-Terminal Lysine (Basic)	C-Terminal Lysine (Basic)	Sialic Acid (Acidic)
+ Galactose (Neutral)	+ Glycation (Acidic)	
MS ~ 290 Da	~ 290 Da	~ 291 Da
icIEF Basic	**Main**	**Acidic**

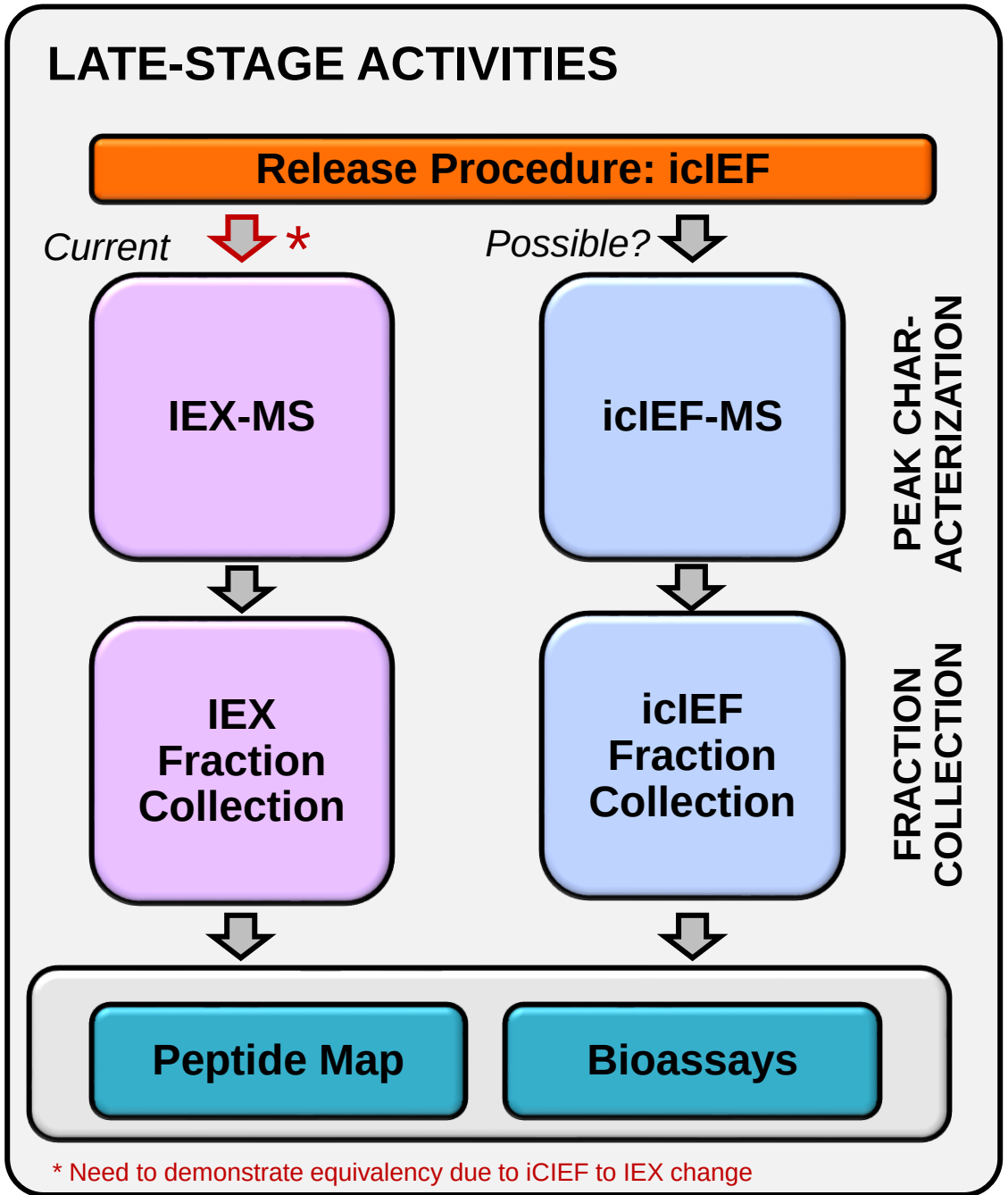
- Combinations in modifications can result in highly similar masses that are difficult to distinguish by mass alone
- Having charge variant data provides a separate separation dimension beyond mass to properly assign species



How Could This Technology Fit into BTx Development?



Charge Variant Characterization Roadmap



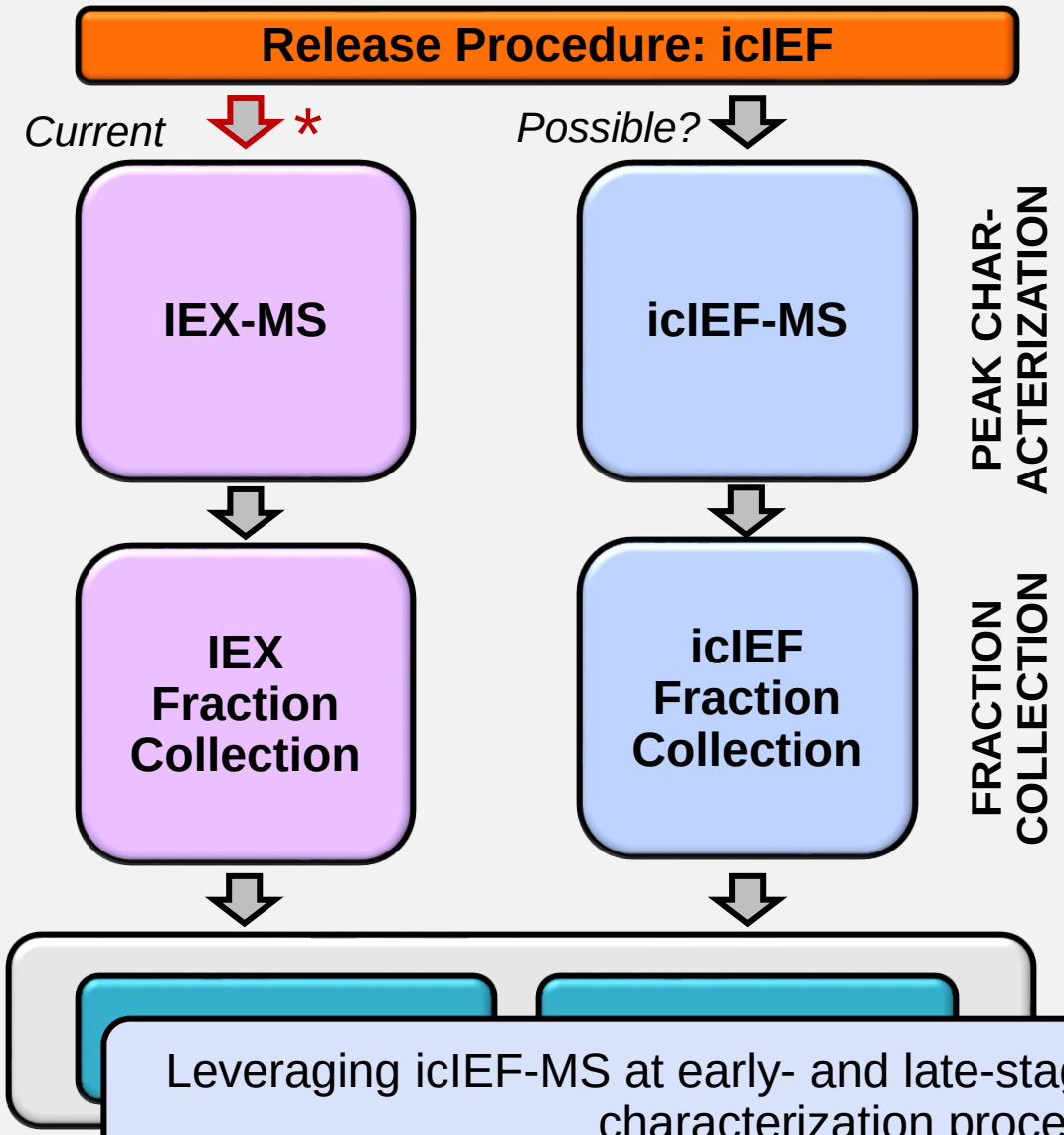
Start with the End in Mind – Peptide map and bioassay data are typically needed to assess attribute and criticality

- IEX is conventional approach
- icIEF fractionation (Maurice Flex) could be used as mobilization is non-denaturing

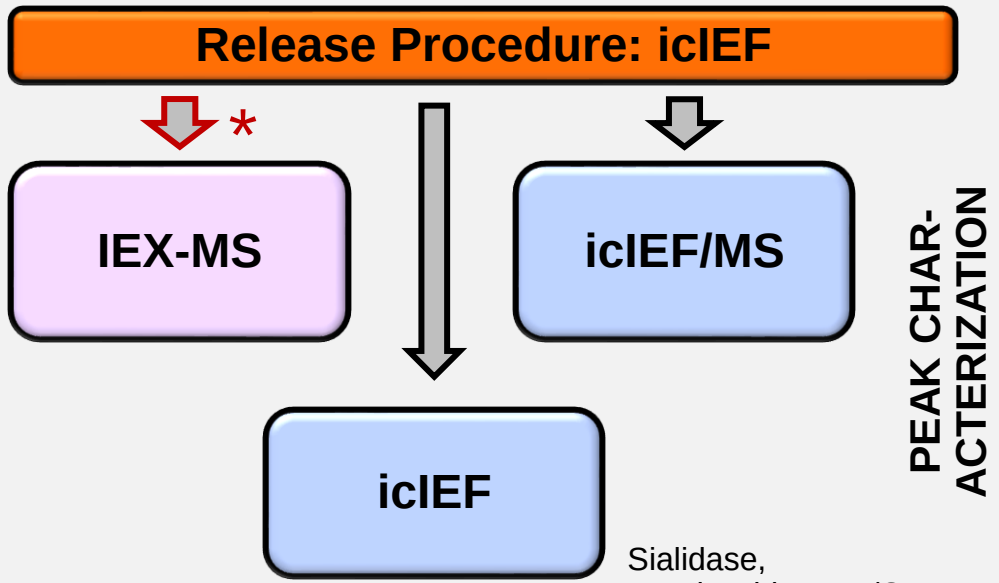
Use of icIEF fractionation, if feasible, would allow consistency with release procedure and subsequent characterization

Charge Variant Characterization Roadmap

LATE-STAGE ACTIVITIES



EARLY-STAGE ACTIVITIES



- Method can be transferred directly from release icIEF method and mirrored exactly – no need to develop IEX method
- Having this data is essential to ensure comprehensive product understanding

Leveraging icIEF-MS at early- and late-stage steps ensures continuity between release procedure and characterization procedures and can streamline development

* Nee

Conclusions

- icIEF-MS technology has been developed to support diverse Biotherapeutic modalities for charge heterogeneity determination to monitor and control charge associated product quality attributes
  - Proof of concept data have been generated for across modalities, including heavily glycosylated proteins, bispecifics, mAbs, fusion proteins and AAV products
  - Attributing to the high resolution of cIEF, very low-level modifications can be observed
  - Facilitate early understanding of icIEF profile and support investigations of charge variants
- Path forward: implementing the technology to support BTx research portfolio provides
  - Early stage: direct MS characterization for icIEF charged species in release or stability test
  - Late stage: icIEF-MS charge variant data align icIEF release results with MS peak identity confirmation for BLA filing

Method	Pro	Con
icIEF (iCE)	• Platform method for release and stability, with limited project-specific development needed • Robust and high throughput • Minimal sample consumption	~~• No direct characterization of charge isoforms~~ ~~• Collection of charge isoforms for further characterization not possible~~

Acknowledgement

Pfizer Team

- Xiaoping He
- Sisi Huang
- Thomas Powers
- Melissa Anderson
- Courtney Sloan
- John Orlet
- Tom Lerch

- Justin Sperry
- Andrew Dawdy
- Jim Mo

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Pfizer Project Support

- Kathleen Cornelius
- Jacky Smith
- Melissa Ly
- Leah Wang
- Wenqin Ni
- Richard Jerome
- Suzane DeMarco
- Kyle Paquette
- Aimee Nicol