
Rapid Characterization of Charge Isoforms of New Modalities by iCIEF-MS

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SCIEX

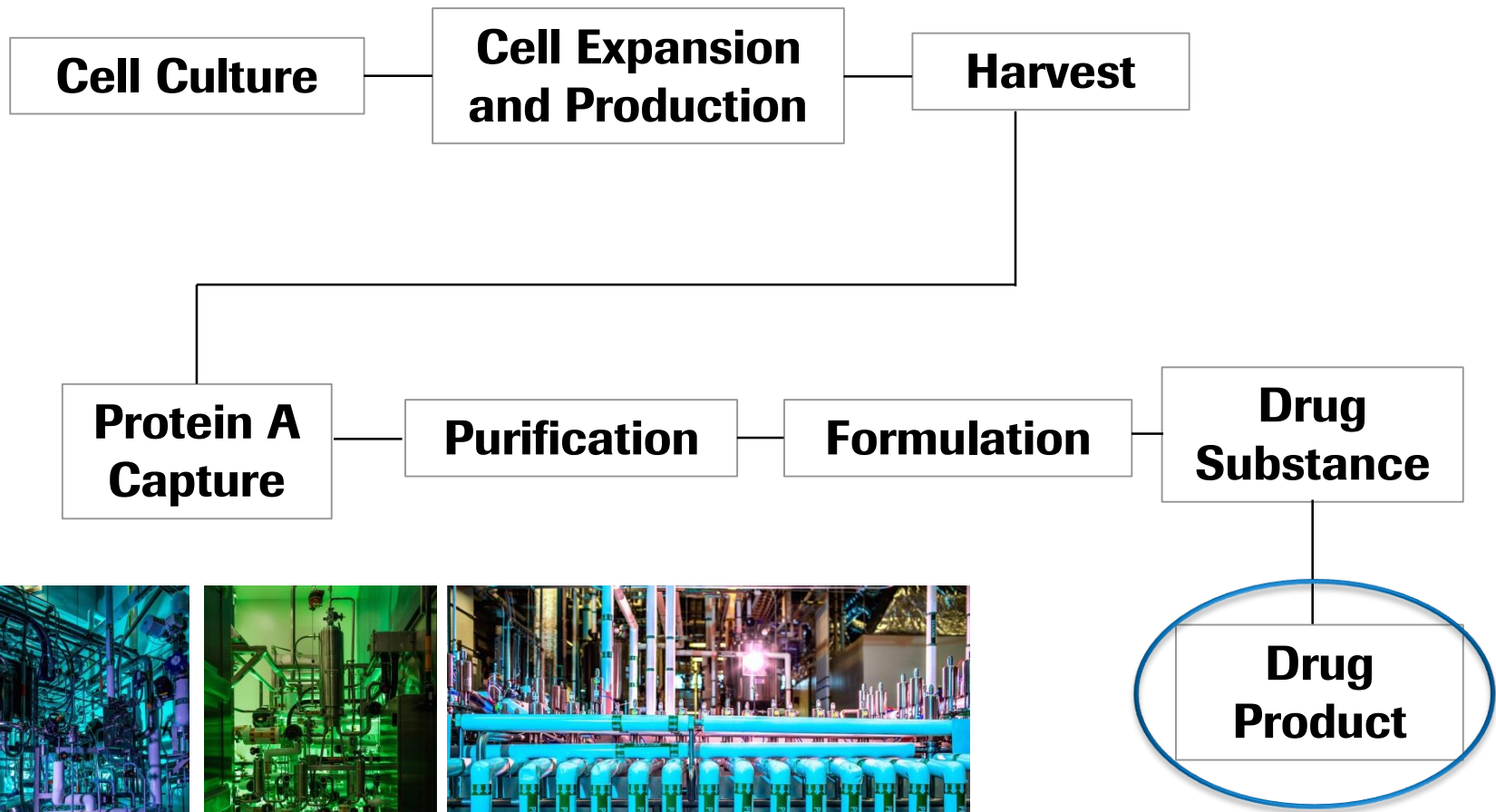
CE Pharm 2021

NIMBL The National Institute for
Innovation in Manufacturing
Biopharmaceuticals

The SCIEX logo, featuring the word "SCIEX" in a bold, blue, sans-serif font with a stylized blue and white swoosh above the letters.

Now part of SCIEX
 **INTABIO**

Biologics Manufacturing Process

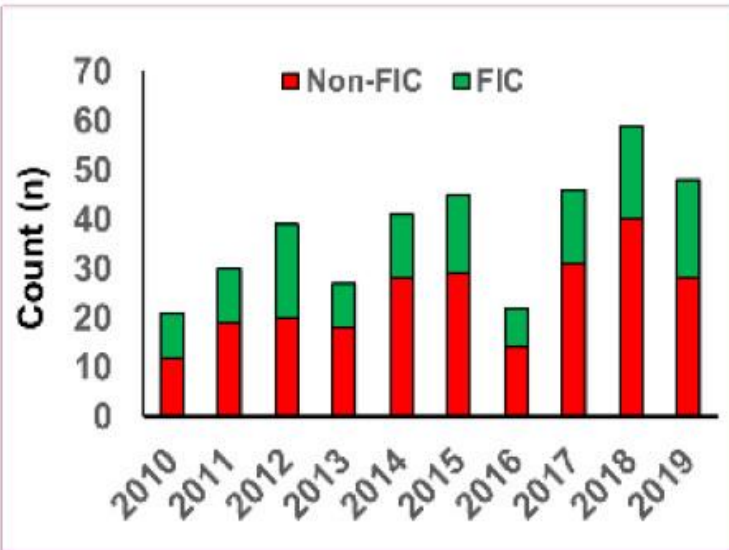


**Fast, Efficacious
and Safe to
Patients**

New Modalities are rapidly progressing...

2010-2019 FDA Approved Drugs

- 378 novel drugs and 27 biosimilars approved
- Oncology leads approvals (25%), followed by infection (15%) and CNS (11%)
- Most oral drugs adhere to Ro5
- New modalities (ADC, ASO, siRNA) gain traction
- FIC drugs continue to make up significant numbers of approvals

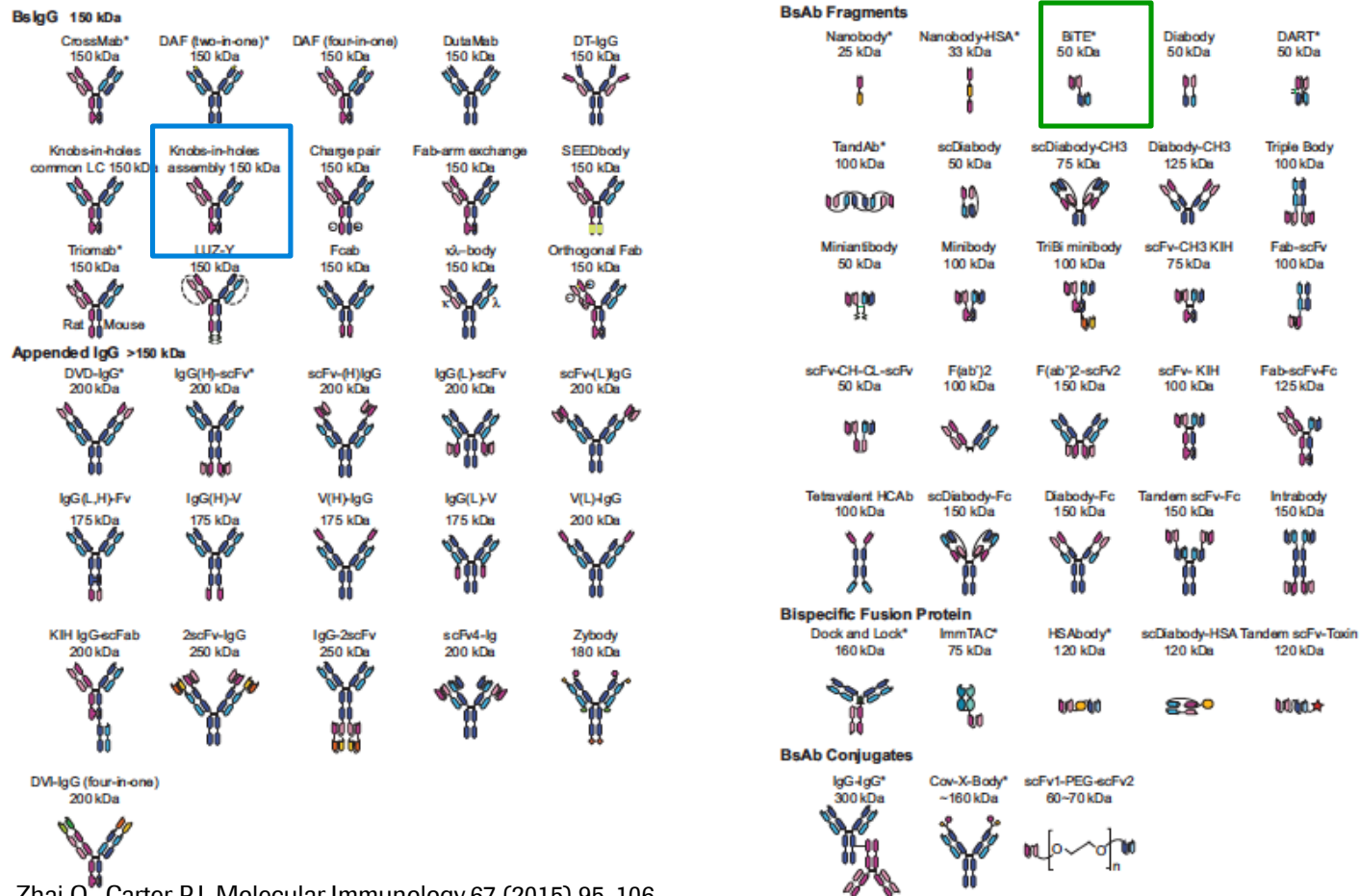


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*for the **patients** with unmet medical needs*

Bispecifics are Becoming the Next-Generation Biologics

- **Three** bispecifics are **FDA-approved** for therapy (Rybrevant, Blincyto, Hemlibra)
- > 100 are in clinical development
- > 80 different formats (5 distinct groups)

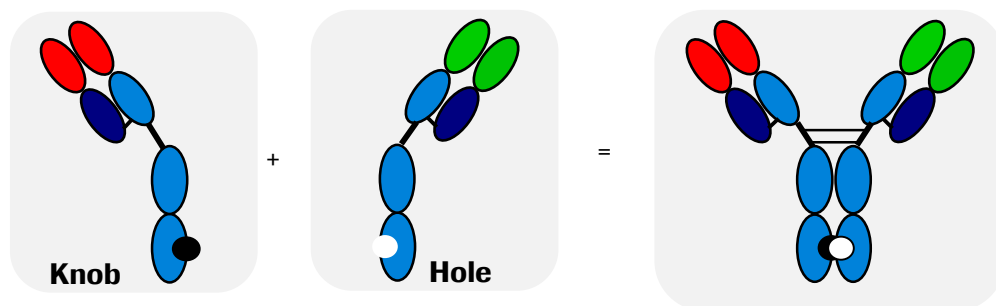
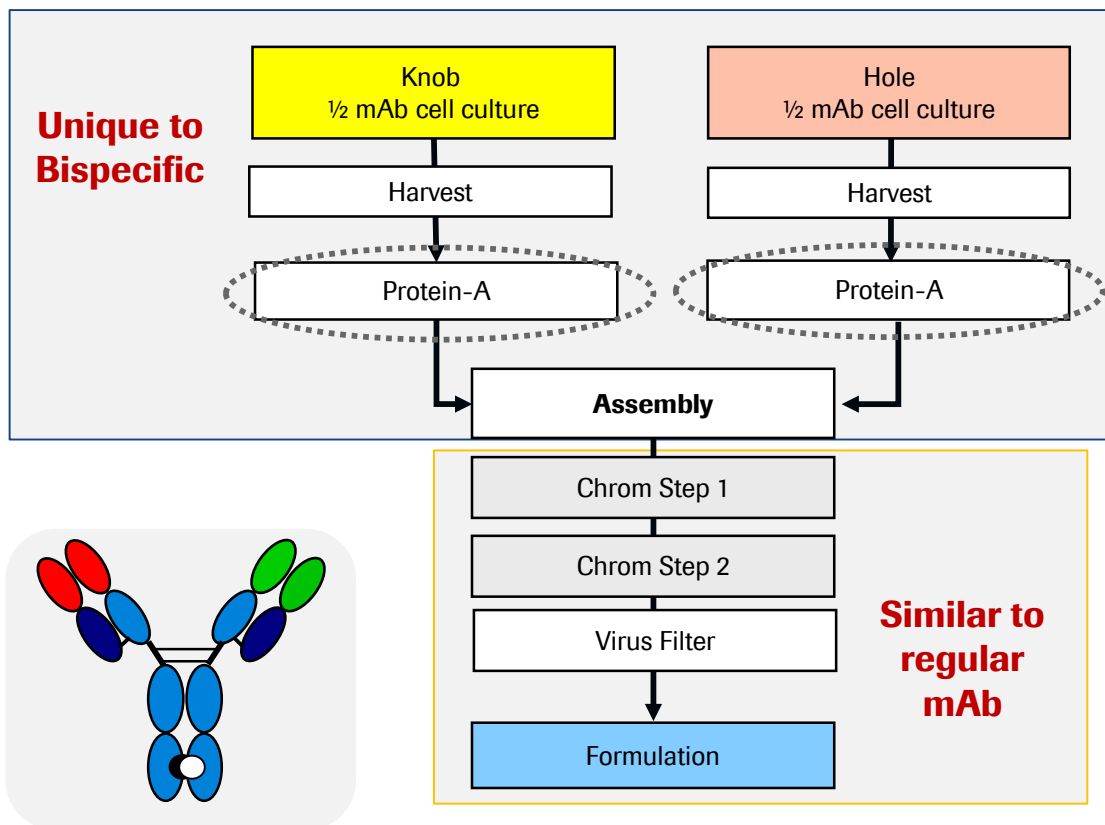


Knob-into-Hole Bispecific



Unique to Bispecifics:

- Two cell fermentation
- Assembly step



Two distinct half antibodies dimerized *via* Knob-into-Hole technology.

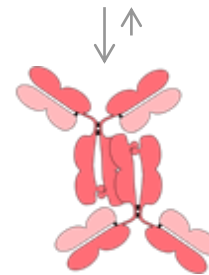
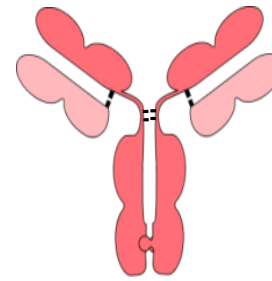
Challenges in Analytics of Bispecifics



- Product quality assessment of **two different fermentation products**
- Unique **assembly step added complexity** to the process
- **Peculiar product and process-related variants** incite early characterization

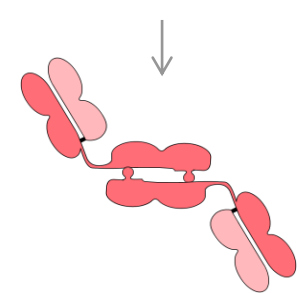
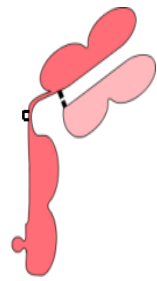
KiH Bispecific Fermentation Products

Homodimer (covalent)
~150kD



Aggregates
>150kD

Half-mAb
~75kD



Homodimer (non-covalent)
~150kD

Quality Attributes and Control System

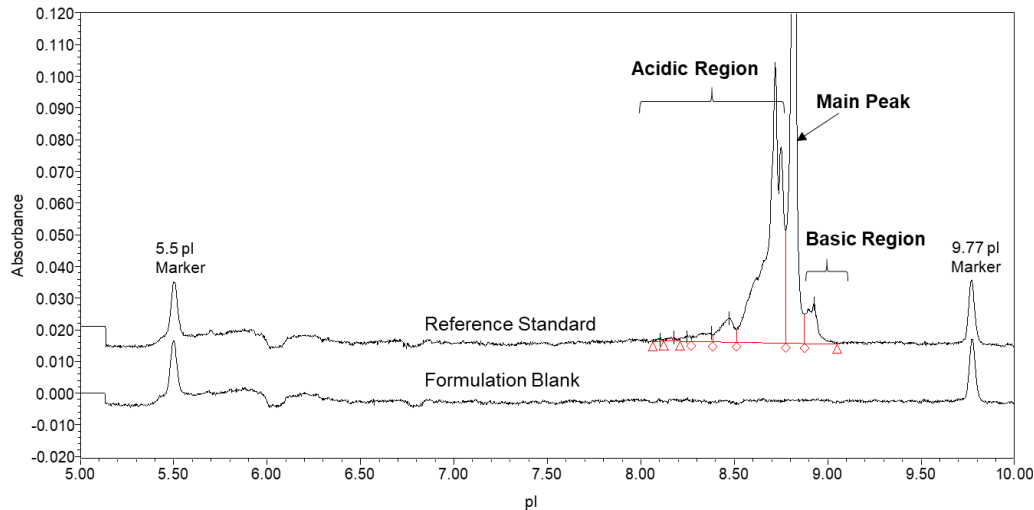
| Category | Quality Attributes | Control System Methods |
|------------------|--|-------------------------|
| Charge | Deamidation, Glycation, Proline Amidation, C-terminal Lysine | iCIEF, IEC |
| Size | HMW and LMW forms | SEC, CE-SDS |
| Sequence Variant | Sequence variants | LC-MS/MS |
| Oxidation | CDR and Fc oxidation | Peptide Map |
| Cysteine related | Free thiol, trisulfide, disulfide scrambling | Non-reduced peptide map |



iCIEF is a Reliable Method for Charge Heterogeneity



- Provides a critical quality measure of “purity”
- Used for lot release, stability and extended characterization



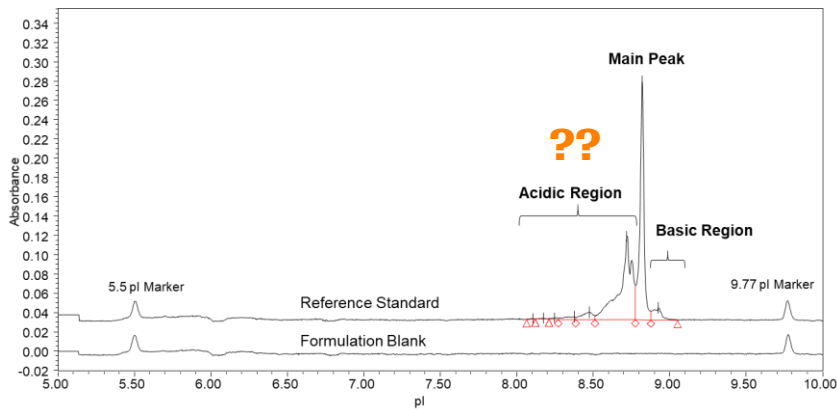
| PRODUCT ACCEPTANCE REQUIREMENT / CERTIFICATE OF ANALYSIS | | | |
|--|-------------------|------------------------------|--------|
| Filtered Bulk for Storage | | Code: _____ | |
| Concentration: 10 mg/mL | | Lot Number: _____ | |
| TEST CODE | TEST NAME | ACCEPTANCE CRITERIA | RESULT |
| | CE-SDS (NR) | ≥ 85.0% Main Peak | _____ |
| | | ≤ 15.0% Sum of LMW Forms | _____ |
| | | Report X.X% Sum of HMW Forms | _____ |
| | CE-SDS (R) | ≥ 85.0% Sum of LC and HC | _____ |
| | Imaged cIEF (CpB) | ≥ 30.0% Main Peak | _____ |
| | | ≤ 62.0% Acidic Region | _____ |
| | | ≤ 16.0% Basic Region | _____ |

- Separation based on isoelectric point (pI)
- pH gradient is provided by a mixture of ampholytes
- Proteins are focused through the ampholyte medium until they reach a net zero charge state at their isoelectric point
- Apparent pI values can be approximated using two internal markers

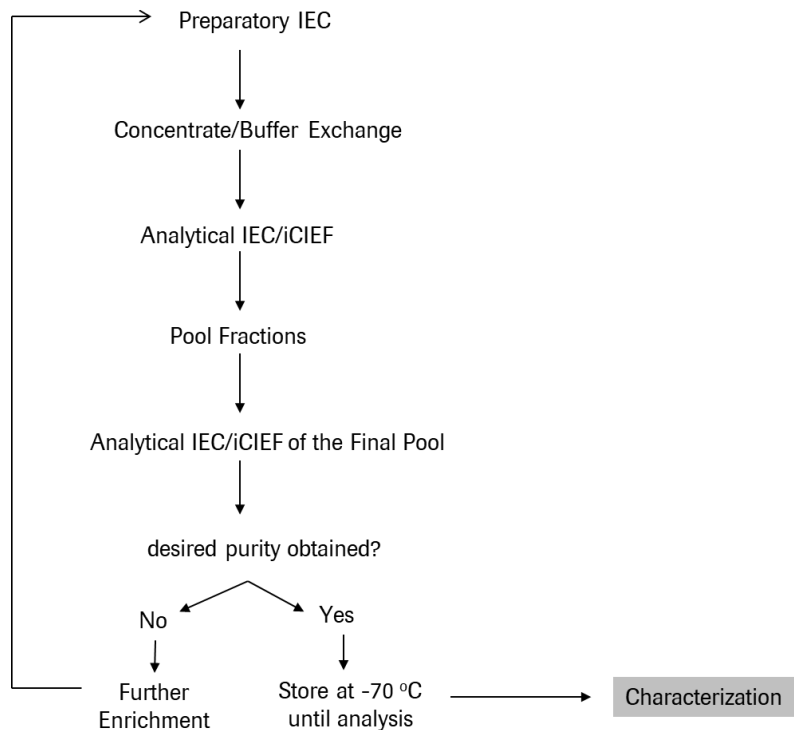
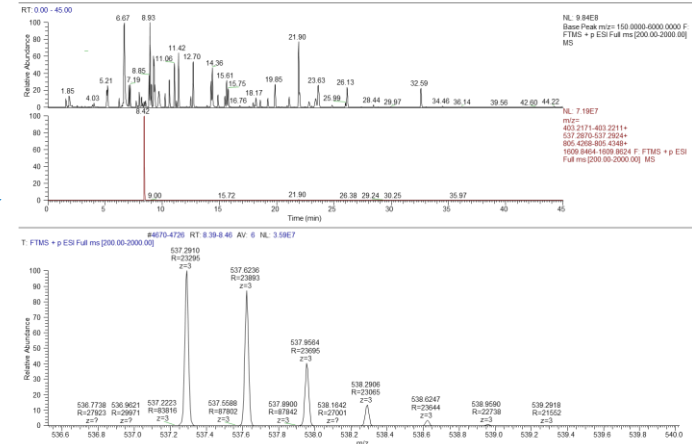
Challenges with Identification of Charge Isoforms



Separation



Identification



- **Days/weeks** spent on development, scale-up, LC-MS analysis
- **Huge sample** amount requirement for scale-up
- Potential **artifacts** induced from the isolation process can complicate data interpretation
- Necessitate both IEC/iCIEF and bridge back to LC-MS data

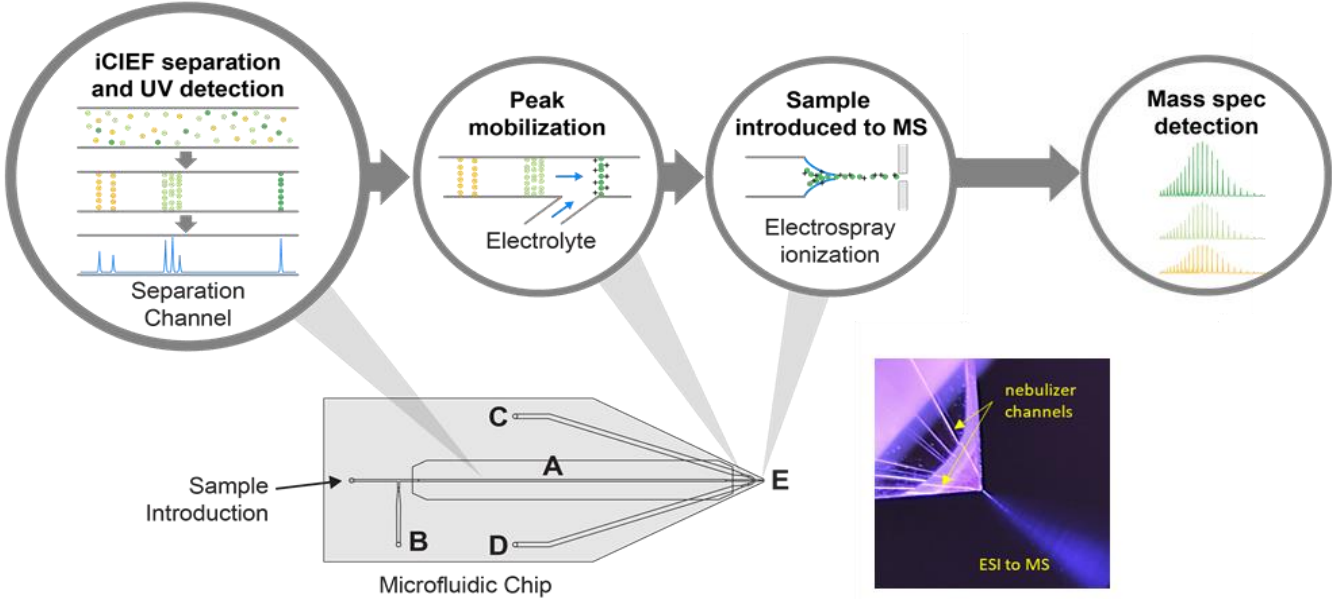
NIIMBL Project: Intabio Blaze™ Microchip System for Real-Time Characterization of Intact Biopharmaceuticals

- To align this Intabio Blaze™ technology with the most urgent needs of biopharma
 1. Verify and validate Blaze™ assay performance on partner samples
 2. Identify data analysis features and develop an MS adaptor to facilitate the efficient integration of Blaze™ into the biopharmaceutical analytical workflow
 3. Assessment for in-line Bioreactor Analytics Workshop
 - Genentech bispecific samples* analyzed:
 1. Basic pH stressed
 2. Main peak IEC fraction
- *previously characterized by traditional methods

Intabio Blaze™ iCIEF – MS System

Key Benefits:

- **Integrated system – separation, quantitation and MS analysis**
- **Identification/characterization in minutes**
- **iCIEF is similar to release method**



Intabio technology presentation:

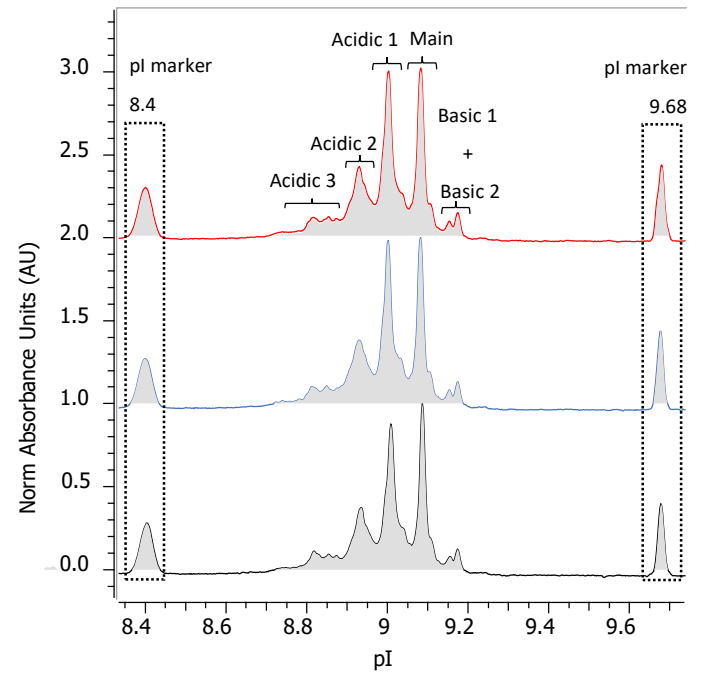
Sept 16th, 10:15am PST

Title: Technology Innovations to Enable Rapid, Comprehensive Charge Variant Characterization of Biotherapeutics by Microfluidic Chip-Based iCIEF-MS

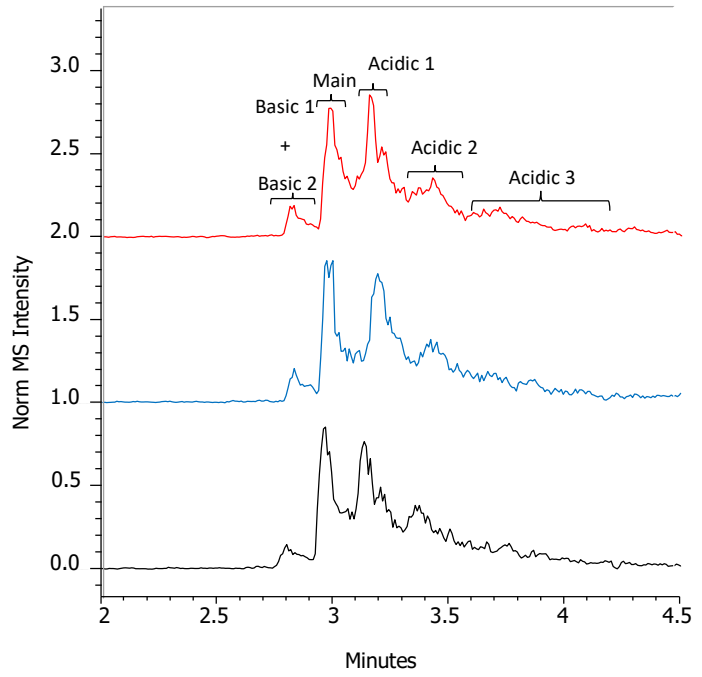
Presenter: Scott Mack, Intabio/SCIEX

Good Repeatability

UV Trace at 280 nm from iCIEF-MS



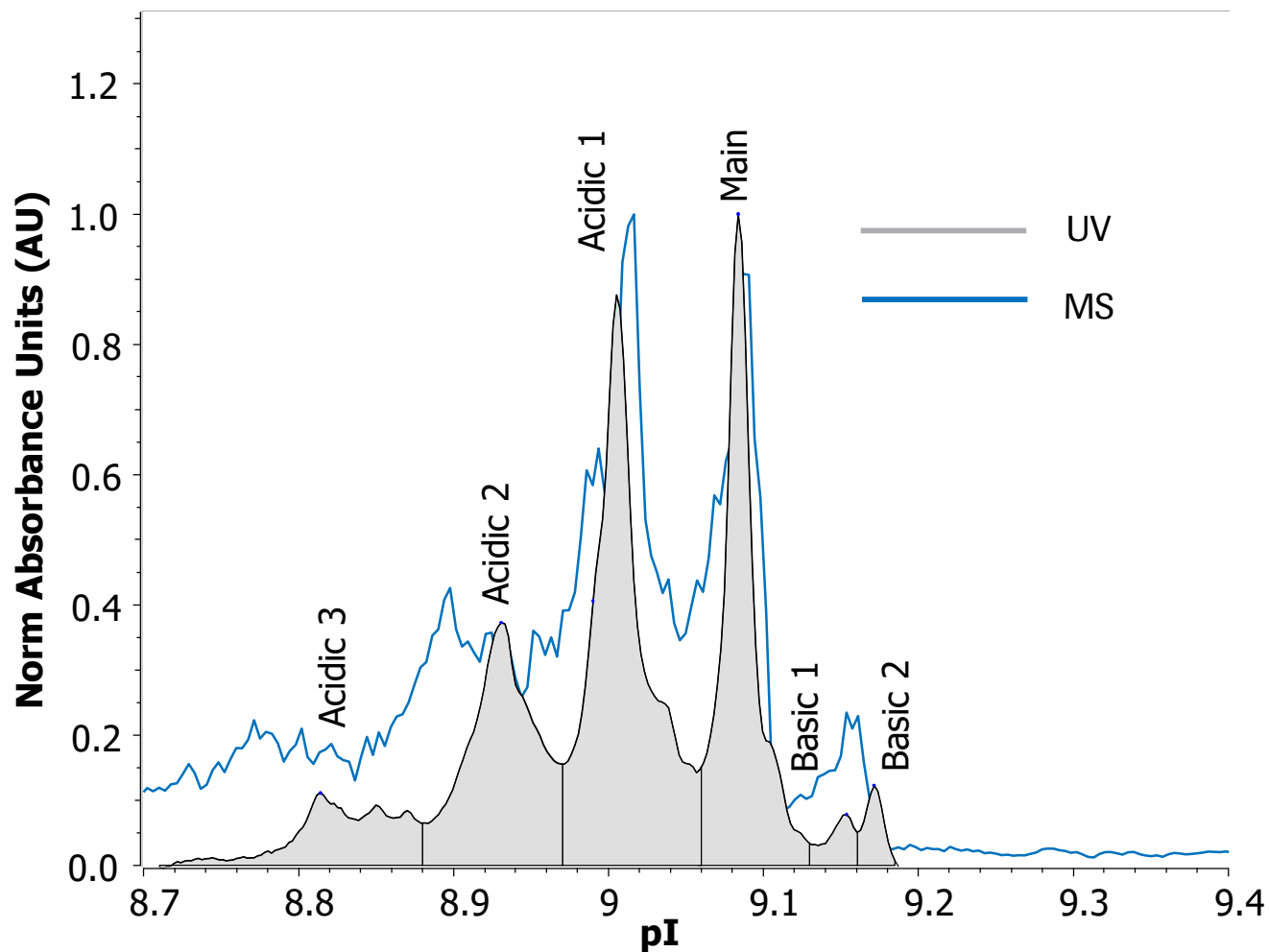
MS (Base Peak) from iCIEF-MS



| Peak Label | Average pI | %CV of pI | Average Relative Area % | %CV of Peak Area |
|------------|------------|-----------|-------------------------|------------------|
| Basic 2 | 9.17 | 0.06 | 2.0 | 6.7 |
| Basic 1 | 9.15 | 0.00 | 1.5 | 7.2 |
| Main | 9.08 | 0.00 | 27.7 | 1.1 |
| Acidic 1 | 9.00 | 0.06 | 37.5 | 0.9 |
| Acidic 2 | 8.93 | 0.00 | 22.2 | 1.5 |
| Acidic 3 | 8.84 | 0.26 | 9.1 | 2.6 |

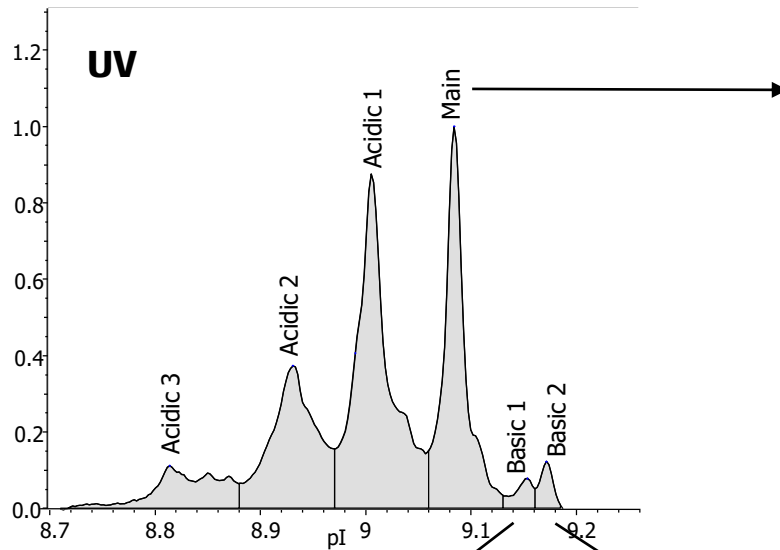
% CV < ~7 % (n = 3)

Consistent Profiles Between iCIEF (UV) and Mirror Image of MS BPE for Confident Peak ID

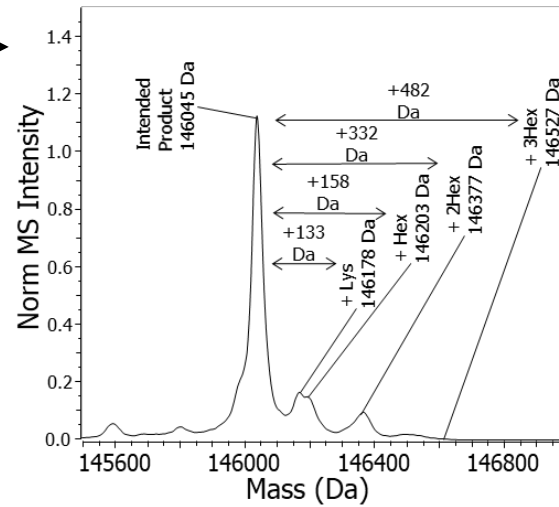


MS BPE = MS Base Peak Electropherogram

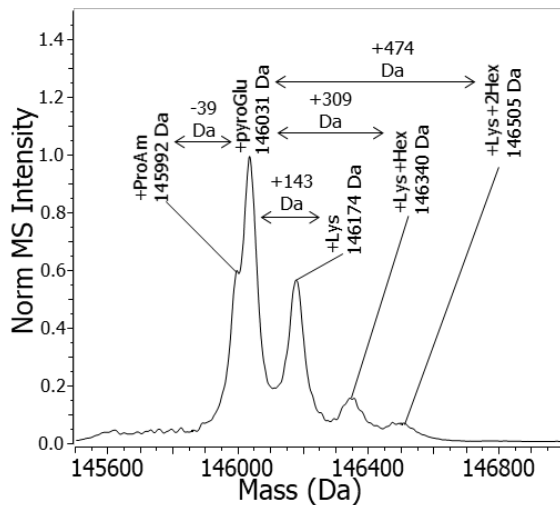
iCIEF-MS Analysis of a Bispecific Stressed Sample



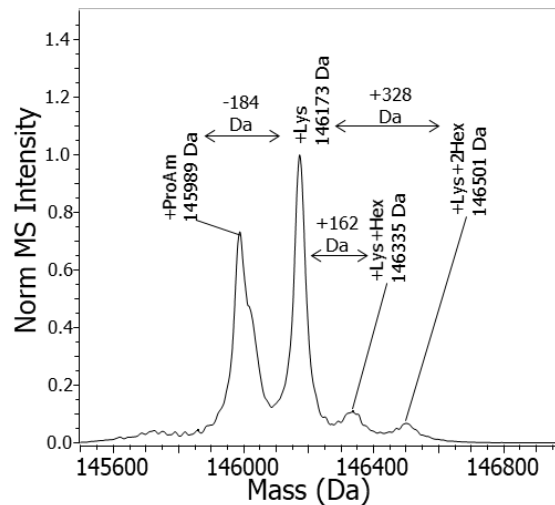
MS – Main Peak



MS – Basic 1

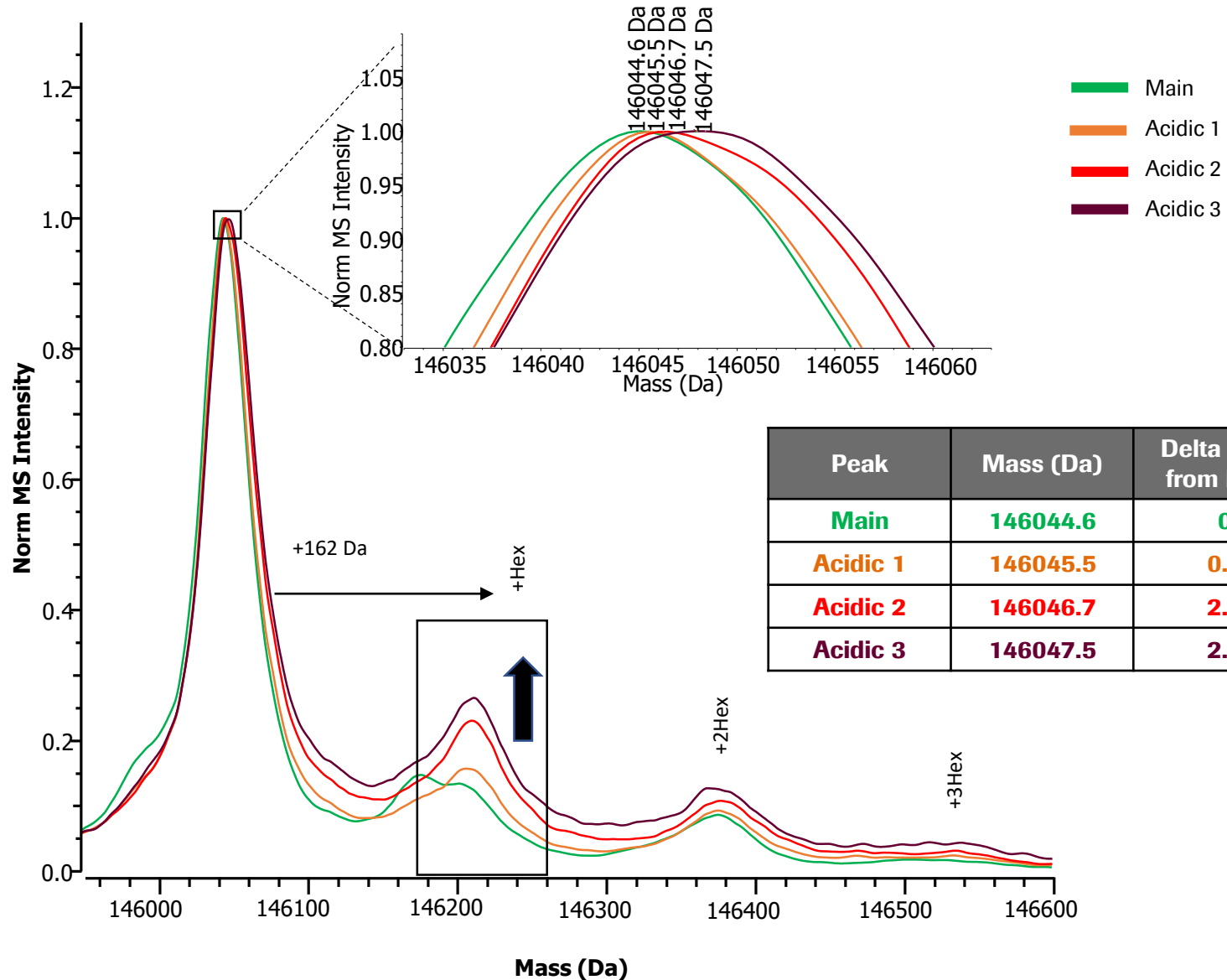


MS – Basic 2

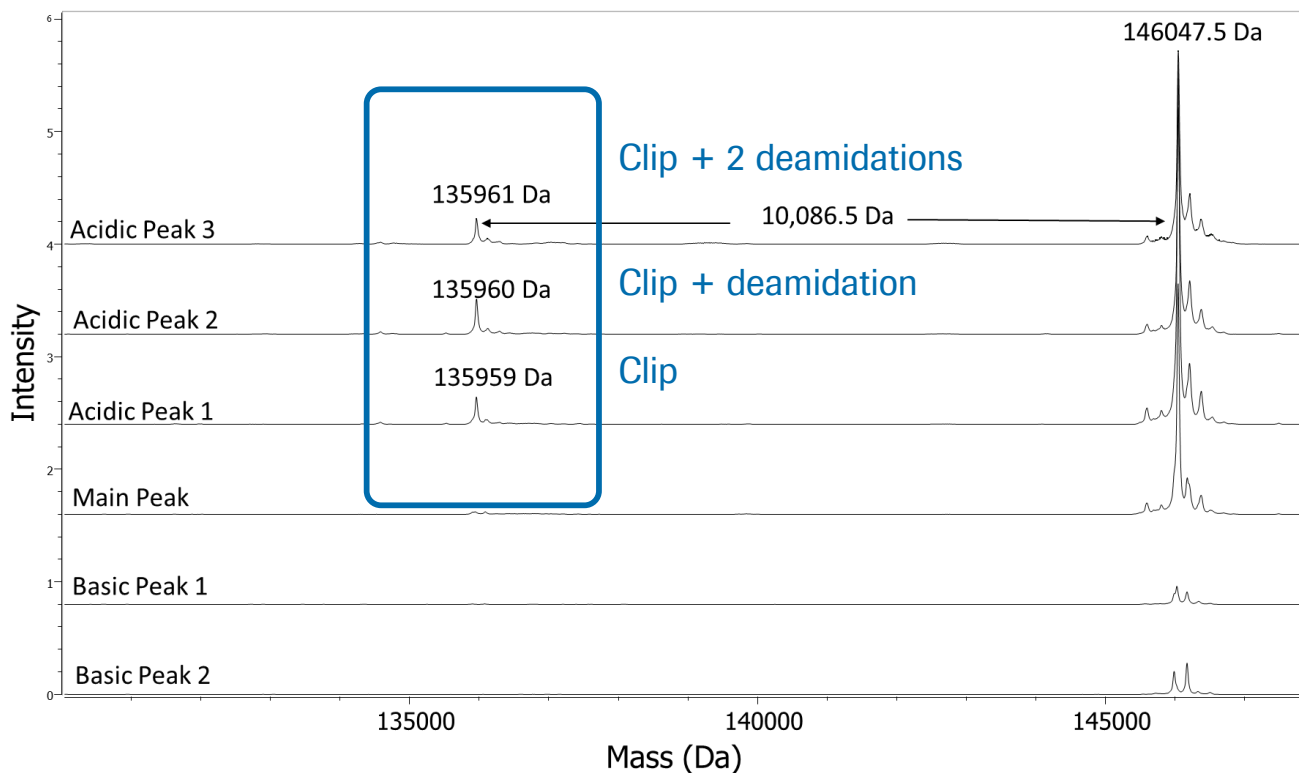


- Rapid ID even for low level peaks
- Combination of PTMs detected and identified
- Separation, quantitation and characterization in a single run analysis

Mass Spectra of Main and Acidic Peaks Identify Deamidation and Glycation

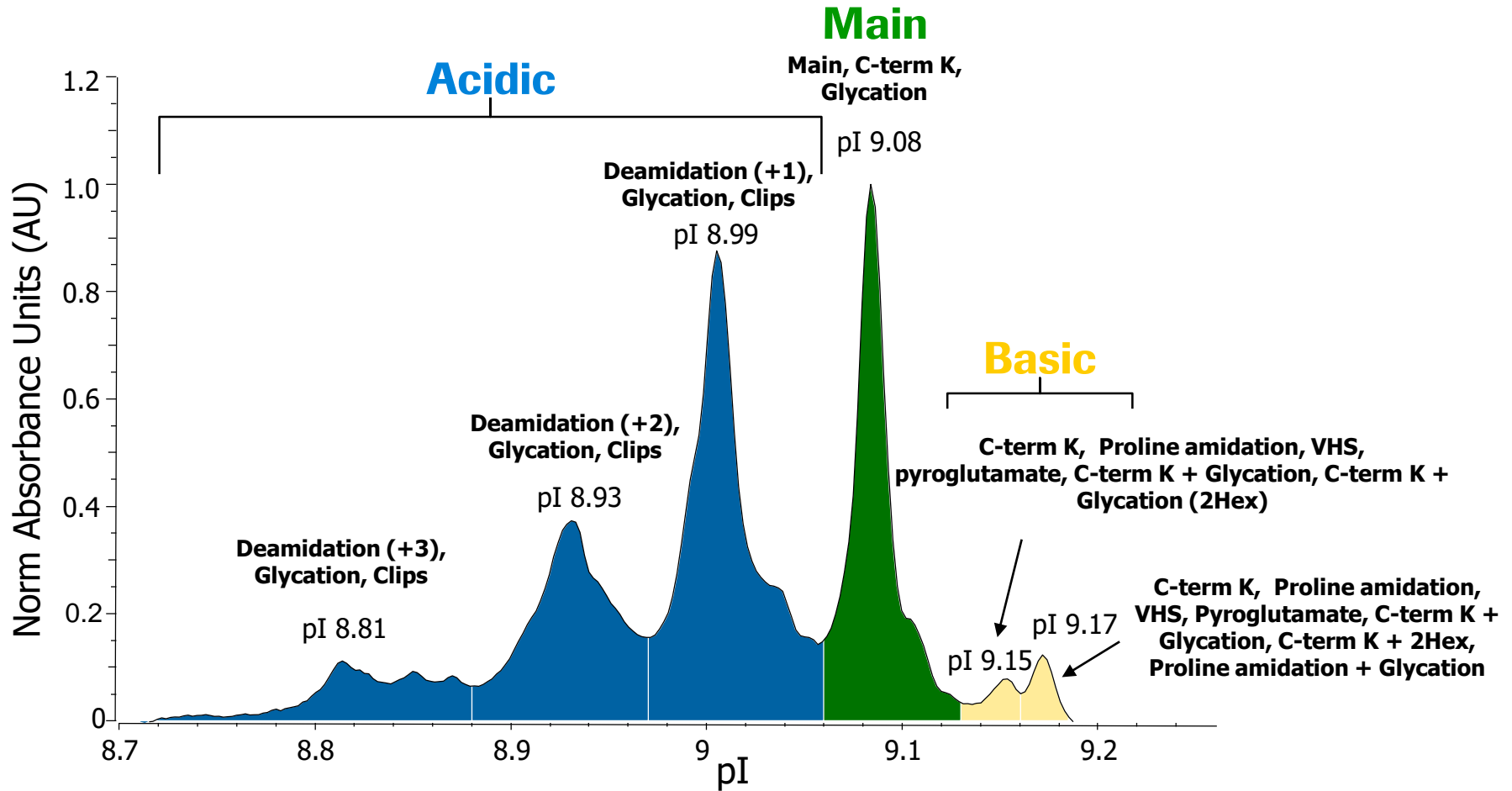


Deamidated Clips Measured by iCIEF-MS in Acidic Peaks



Clips and their charge variants are also separated and detected by Intabio iCIEF-MS

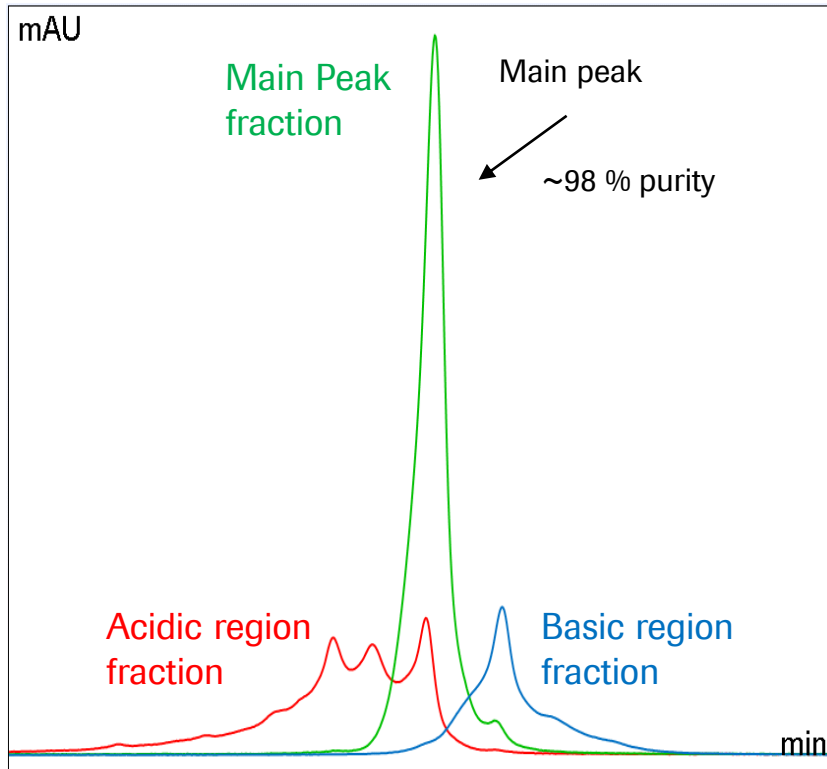
Comprehensive Characterization of Charge Heterogeneity in a Single iCIEF-MS Analysis



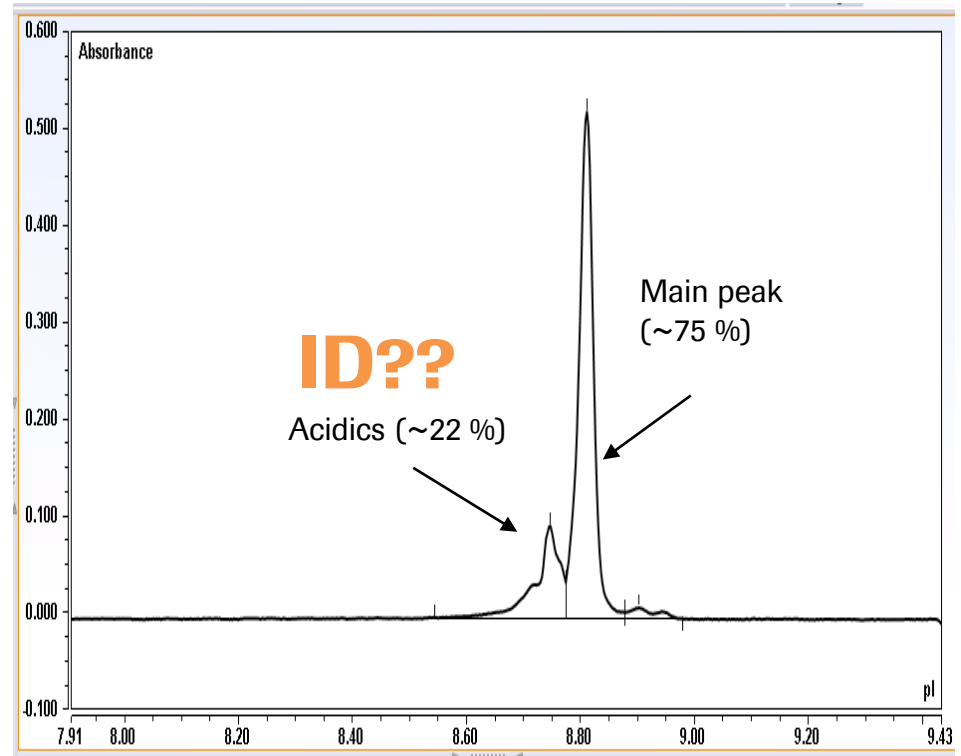
Annotated iCIEF electropherogram of basic pH stressed bispecific sample

Isolated Main Peak Fraction (IEC vs iCIEF)

Analytical IEC

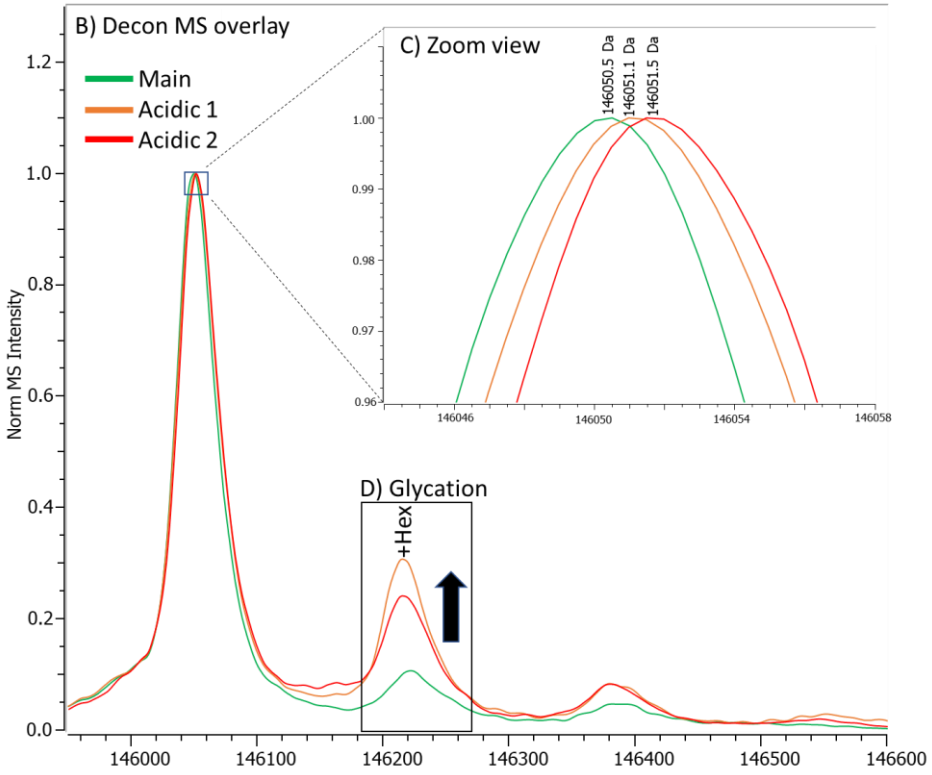
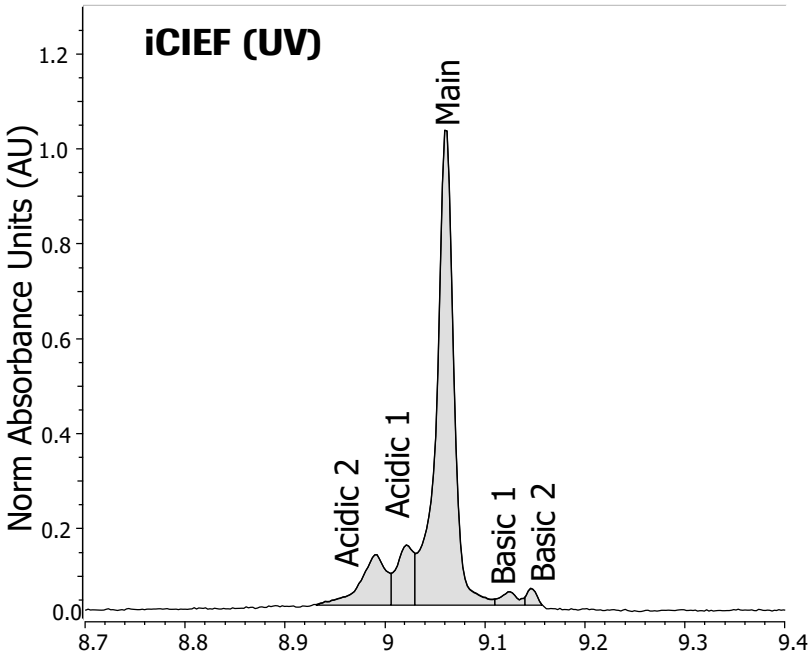


Imaged CIEF



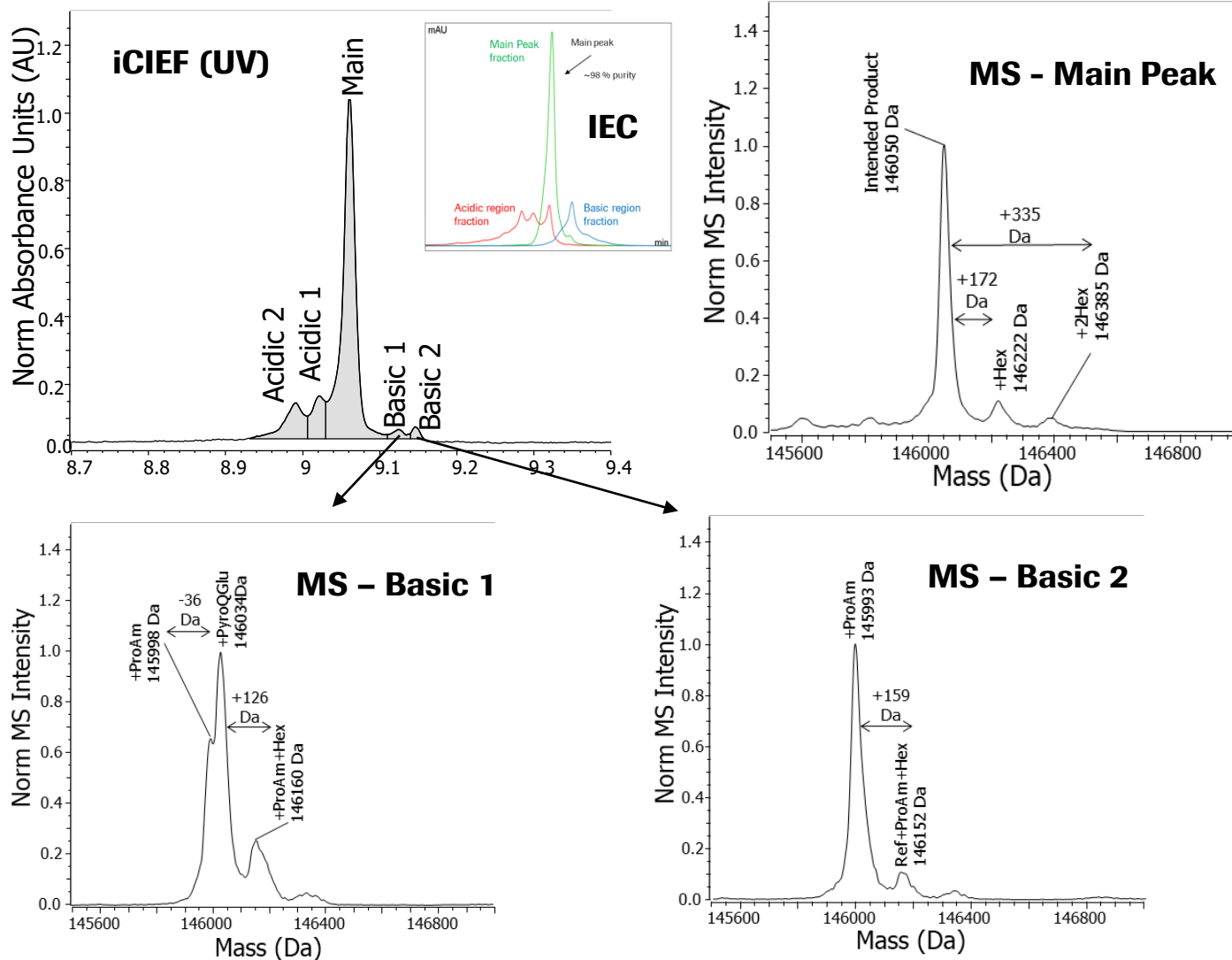
- IEC and iCIEF have fundamentally different separation mechanisms
 - Protein-column interaction cause adsorption in IEC
- Imaged CIEF can resolve charge isoforms that IEC cannot
 - Intabio Blaze™ system can help identify these isoforms

iCIEF-MS Analysis of Main Peak Fraction



Acidics in the main peak fraction primarily comprise of glycation and deamidation

iCIEF-MS Analysis of Main Peak Fraction



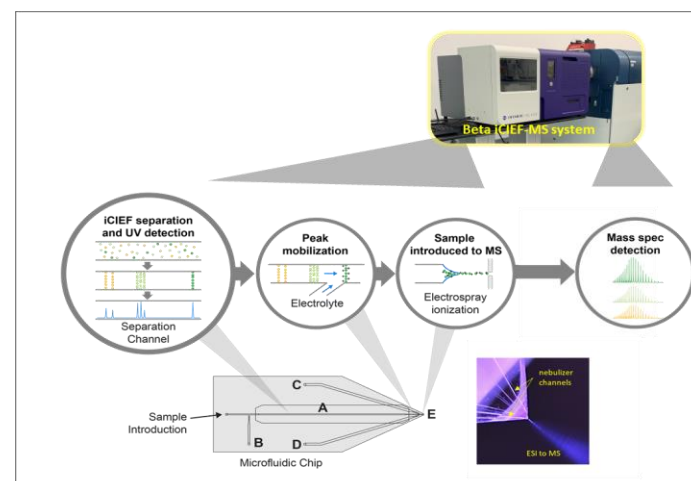
- In-depth and extensive characterization of a purified sample even for very low abundant attributes

Summary and Outlook

- iCIEF-MS as a new analytical tool for a rapid charge isoform characterization of bispecifics
- This new analytical platform yields comprehensive analysis providing UV quantitation, peak identification and pI values
- Deamidation events with a small mass difference of ~ 1 Da were resolved and characterized at the intact level
- Difficult to detect combinations of PTMs were detected and identified

Benefit of iCIEF-MS system:

- Speeds up decisions on bioprocess development and production (e.g. clone selection, process validation, product quality assessment)



Acknowledgments

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Doing now what patients need next