

# Characterization and Monitoring of mAb Charge Variants via Online IEX-MS

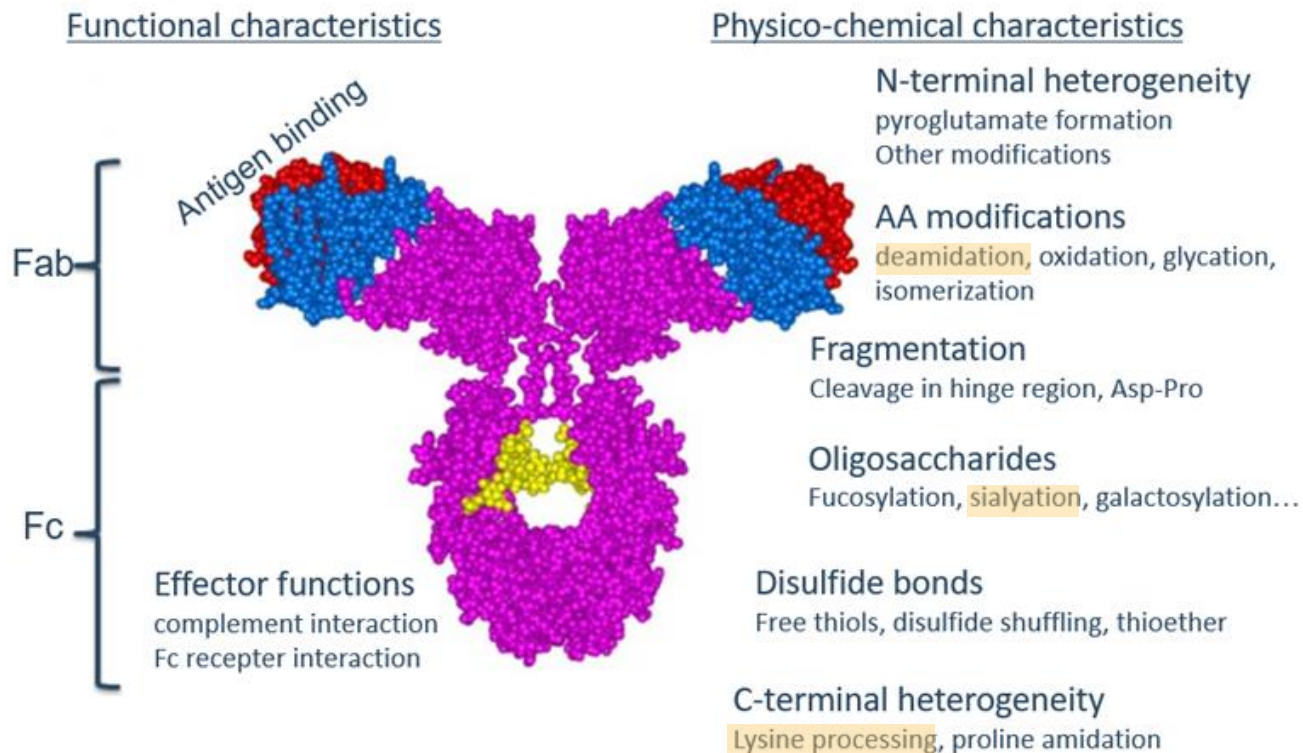
**Samantha Ippoliti**

**Waters MS User Meeting  
ASMS 2019  
Atlanta, GA  
June 1, 2019**

# Agenda

1. mAb CQA Characterization
2. Reverse Phase vs Ion Exchange Chromatography
3. Online IEX-MS: Considerations for Method Development
4. Case Studies
5. Summary and Q & A

# Quality Attributes to Consider – Biotherapeutic Monoclonal Antibodies (mAb)

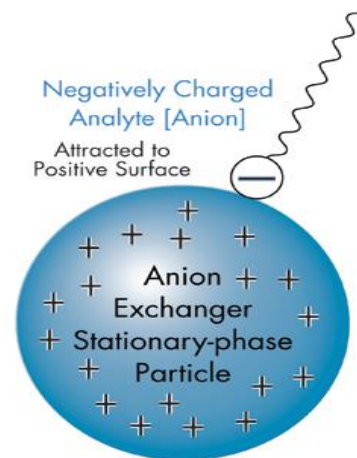
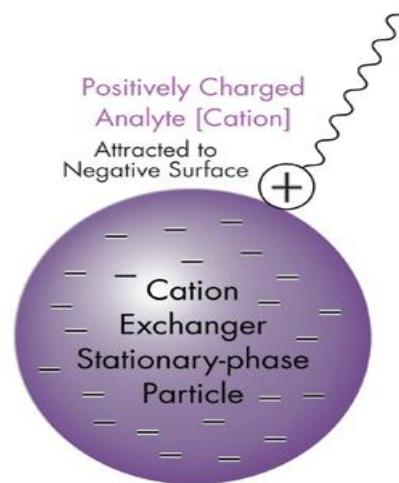


# Reverse Phase vs Ion Exchange Chromatography

	Reverse Phase (RP)	Ion Exchange (IEX)
Separation Based On...	Hydrophobicity	Protein Surface Charge
Conditions	Denaturing	Native
Common Variants Separated	<ul style="list-style-type: none"> <li>• Oxidation</li> <li>• Clipping (LMW)</li> </ul>	<ul style="list-style-type: none"> <li>• Deamidation</li> <li>• Sialic acid &amp; other N-glycan variants</li> <li>• C-terminal lysine variants</li> <li>• Sulfation/phosphorylation</li> </ul>
Advantages	Easy MS-compatibility	Ability to collect fractions and test functionality of variants
Gaps	<ul style="list-style-type: none"> <li>• Method destroys folding and functionality</li> <li>• Cannot separate deamidation / isomerization species or N-glycan variants</li> </ul>	<ul style="list-style-type: none"> <li>• Traditional IEX mobile phases are not MS-compatible</li> <li>• Tedious fraction collection is required for simple peak identification</li> </ul>

## Ion Exchange Chromatography

- Separation according to differences in their **surface charge**
- Most mAbs have  $pI > 7$ , so cation exchange (CEX) is more common
- CEX separation strategies:
  - **pH gradient** (increase of pH, altering protein surface charge)
  - **Salt gradient** (increase ionic strength, altering protein binding to stationary phase)



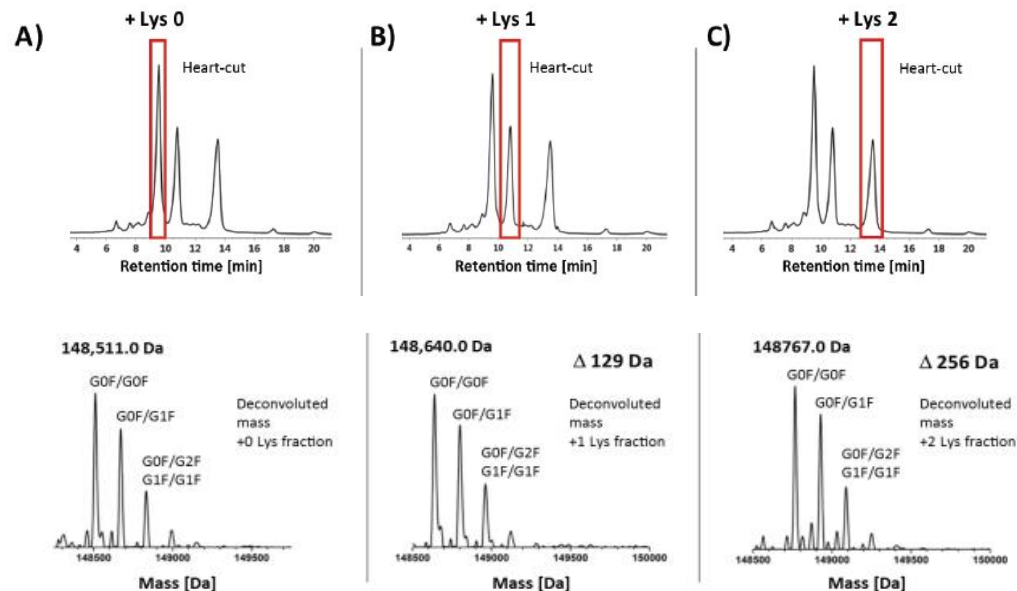
# Charge Variant Identification with Traditional IEX

- Fraction Collection & MS Analysis
  - Intact or subunit analysis
  - Peptide Mapping

## ■ 2D-LC/MS


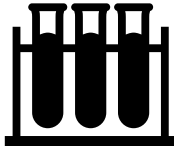




Acquity WFMA



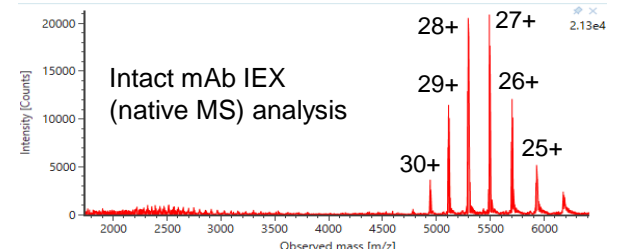
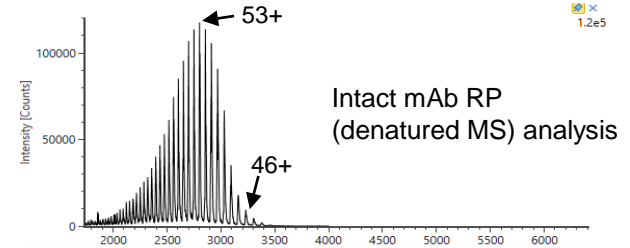
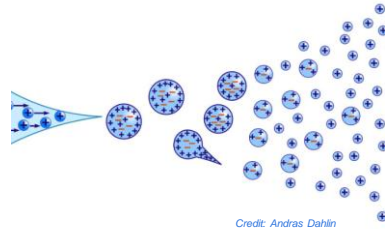
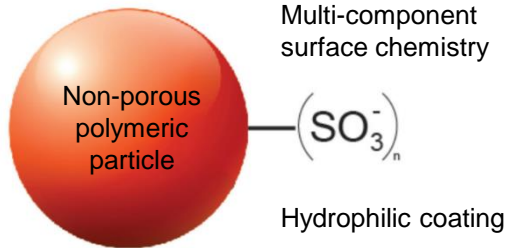
Birdsall, R. et al "Characterization of Biotherapeutics: ACQUITY UPLC H-Class Bio with 2D Part 2 of 3: Rendering a Viable Interface for IEX with ESI-MS Analysis"

## Why Should We Couple IEX to MS Directly?

- Enable quick decision-making 
- Reduce the need for tedious fraction collection 
- Easily distinguish desired product from impurities 
- Avoid complex 2D-LC setups 

# Considerations for Online IEX-MS

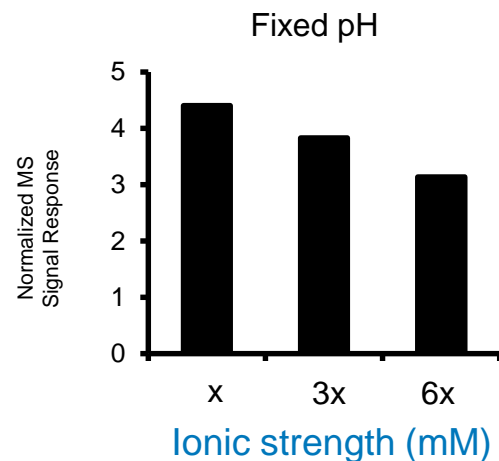
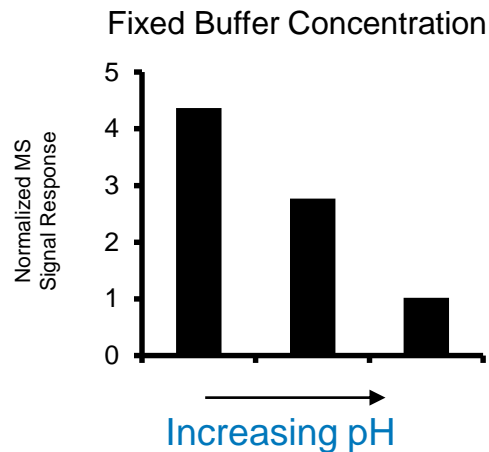
- Optimal chromatographic separation with volatile salts allowing for ESI ionization
  - BioResolve SCX column
  - Ammonium-based dual salt / pH gradient mechanism for optimal separation
  - Native MS analysis





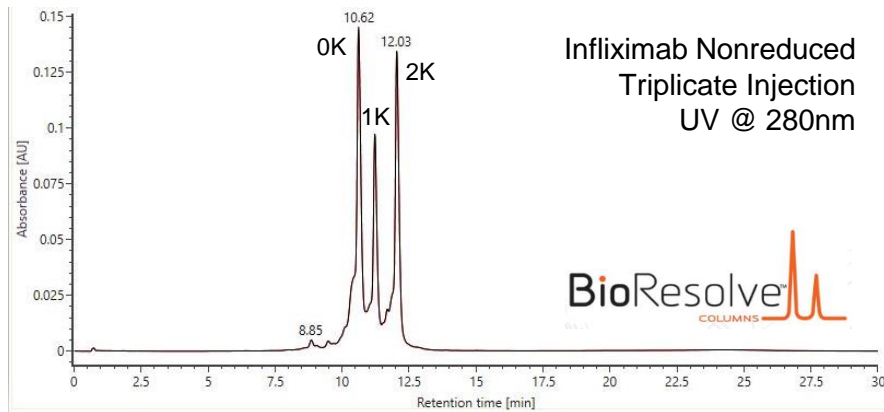
# SEC-MS to Study pH and Ionic Strength Effect on MS Signal

## *Intact NIST mAb*

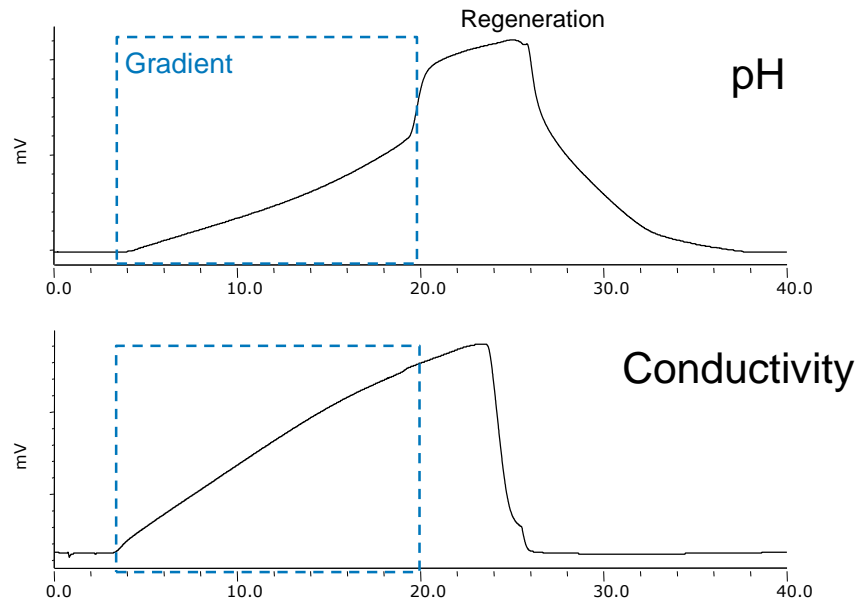


- SEC-MS to observe MS response vs. mobile phase pH and ionic strength
- pH impacts signal more than increases in ionic strength, yet it is still desirable to reduce salt content to not overburden the MS

## A Robust and Reproducible IEX Method



- Column: BioResolve SCX, 3 $\mu$ m, 2.1 x 50 mm @ 30 °C
- MP: Ammonium-based dual salt/pH gradient
- Flow Rate: 0.1 mL/min
- Injection Volume: 5-10  $\mu$ g on column



Online pH & conductivity traces confirm desired linear gradient

# Is The Separation Generic?

Column: BioResolve SCX 3 $\mu$ m, 2.1 x 100 mm @ 30 °C

Mobile Phases: Ammonium-based dual salt / pH gradient

Flow Rate: 0.1 mL/min

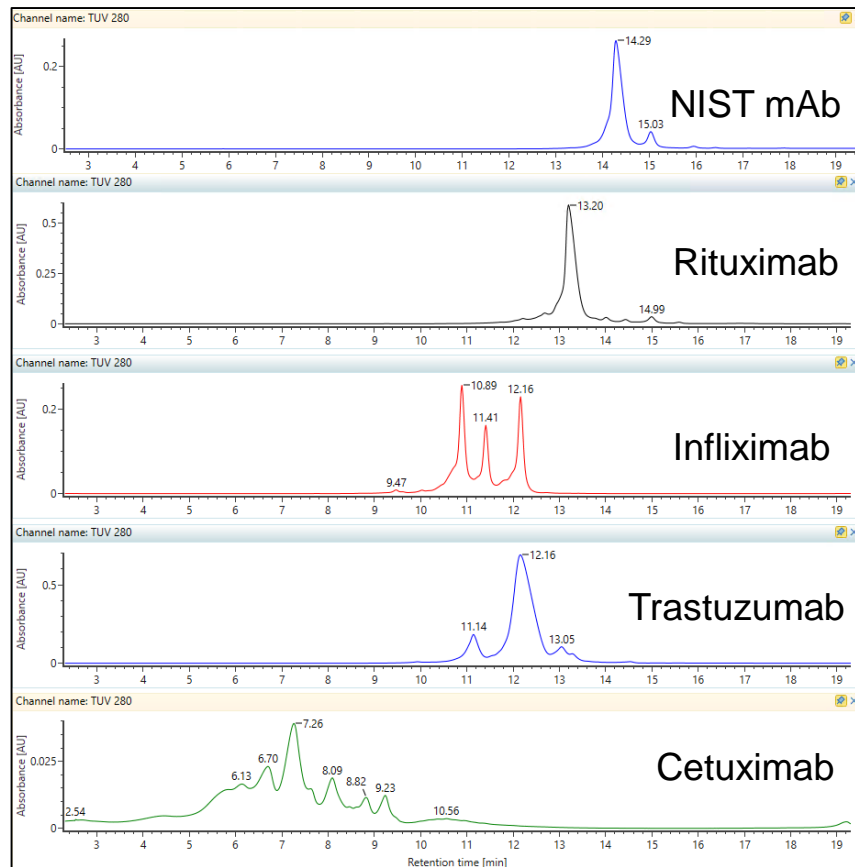
Gradient: 40-98% B in 20 min



One single gradient works for a wide range of mAbs!

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# Considerations for MS Signal Optimization

## NIST mAb, Vion IMS QTof

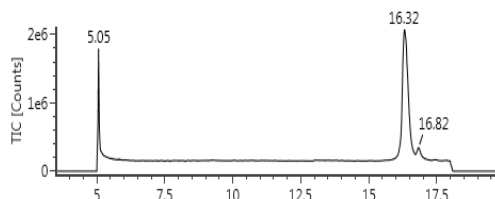
# Waters

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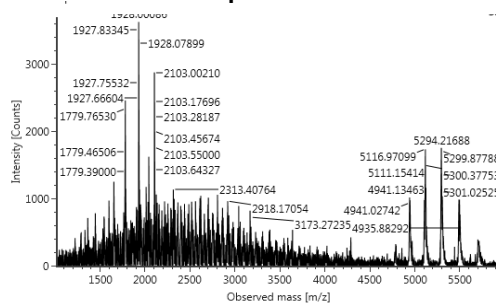
Parameter	Original	Optimized
Source temp.:	135 °C	120 °C
Desolvation temp.:	500 °C	350 °C
Cone gas flow:	300 L/h	100 L/h
Desolvation gas flow:	800 L/h	600 L/h

### Original settings

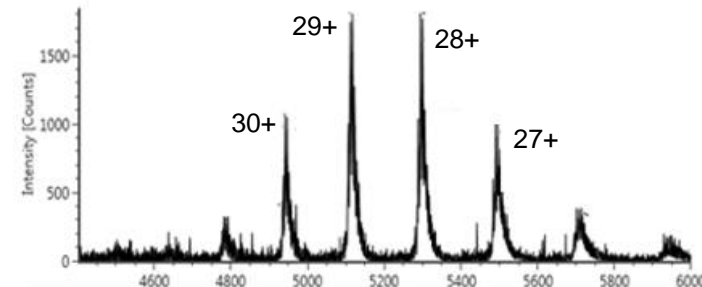
TIC



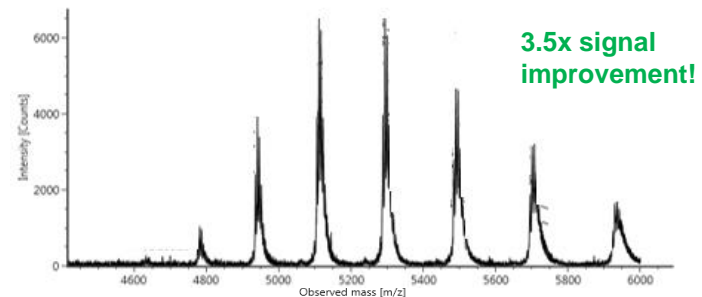
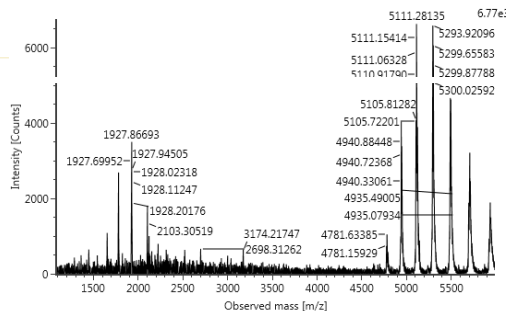
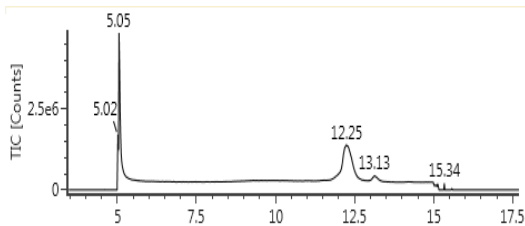
Combined Spectrum: Main Peak



Zoom m/z 4500-6000



### Optimized settings



# Considerations for MS Optimization

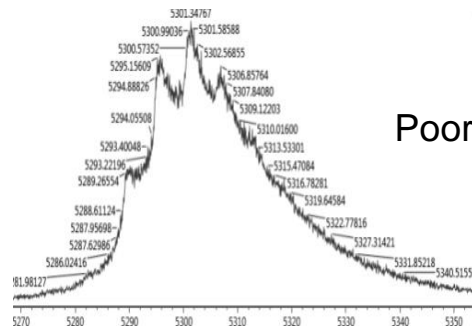
## *Quality of Reagents*

Quality of reagents and mobile phase preparation will have an impact...

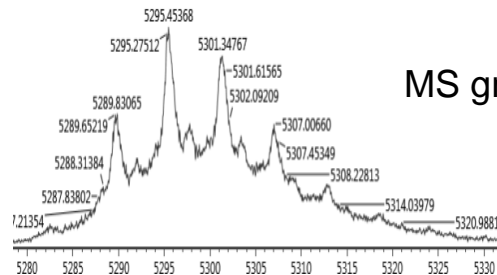
...on quality of results

...on cleanliness of the instrument

[Intact NIST mAb] <sup>28+</sup>



Poor quality reagents

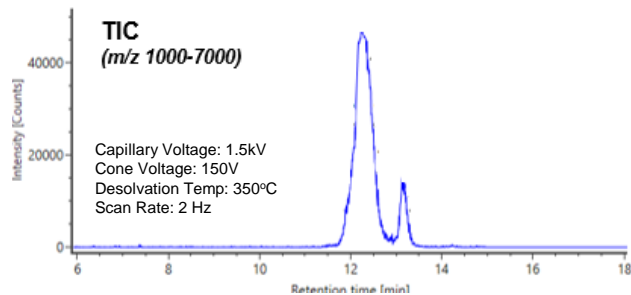
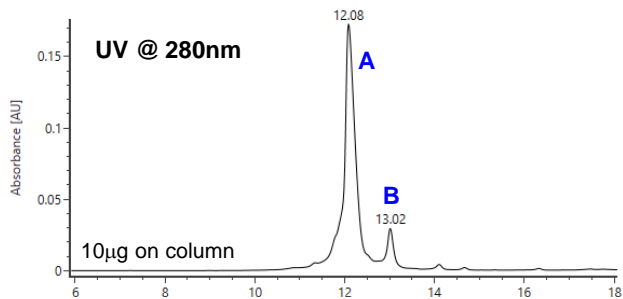


MS grade reagents

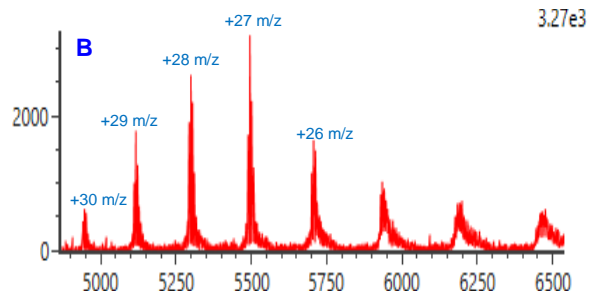
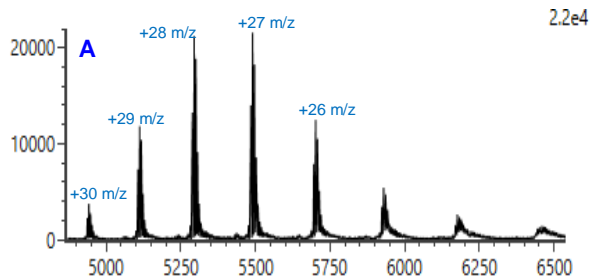
# NIST mAb C-terminal Lysine Variant Evaluation on BioAccord System



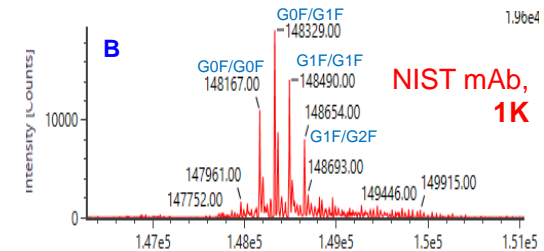
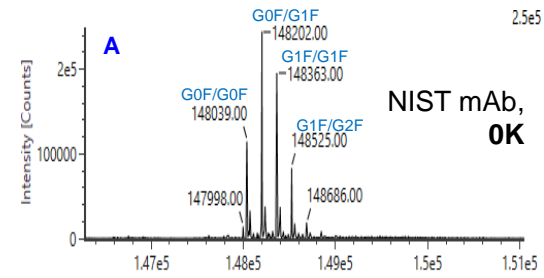
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Combined MS Spectrum of Selected Peaks



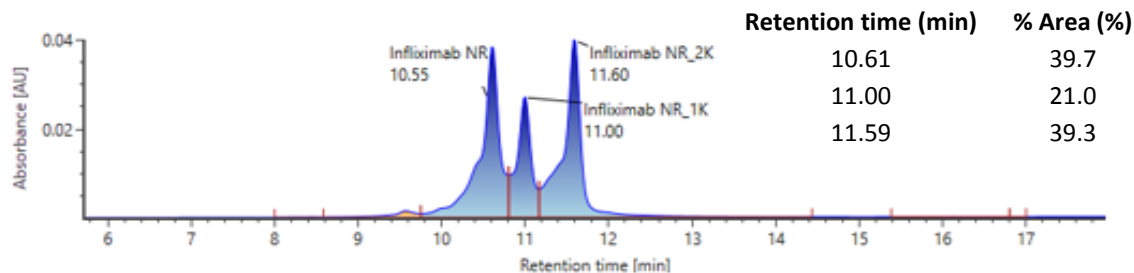
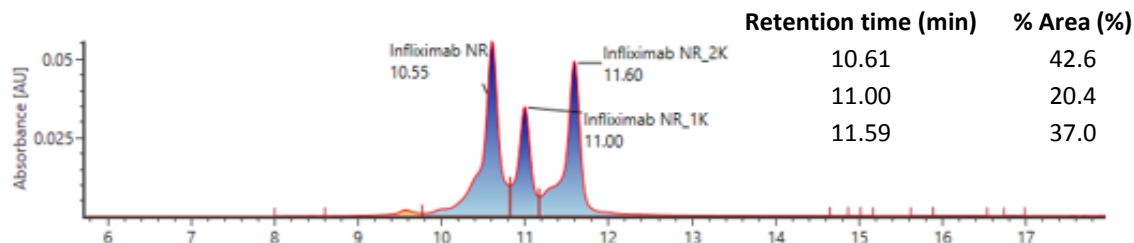
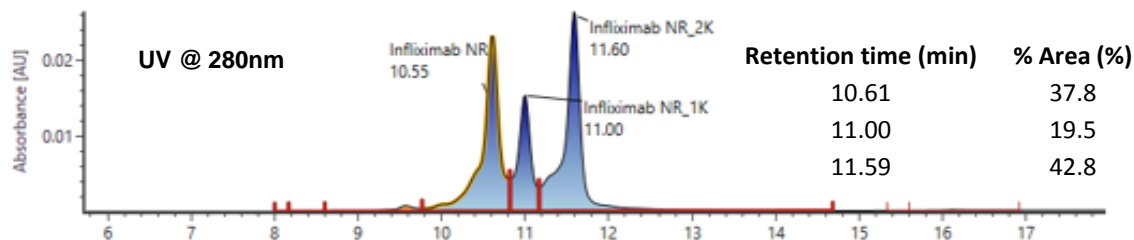
MaxEnt1 Deconvolution



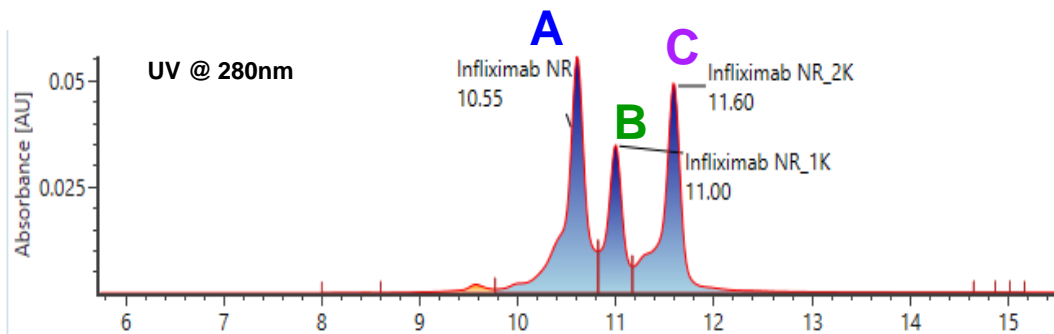
Expected Mass: 148,036.6 Da (G0F/G0F, 2 x pQ1)

# Case Study 1: Infliximab Biosimilar Analysis

## UV integration for quantitative comparison charge variant profiles

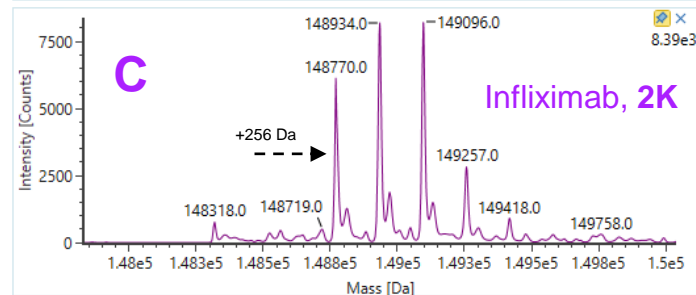
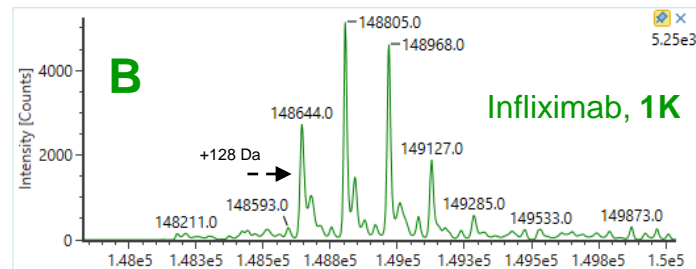
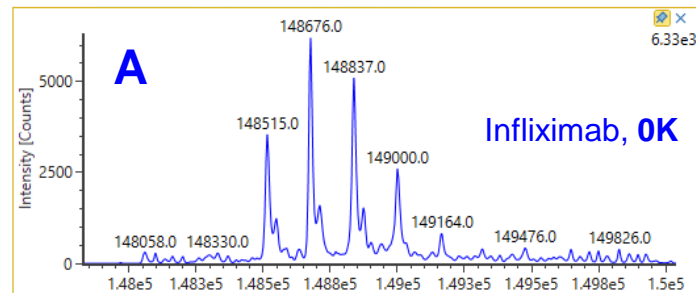


## Case Study 1: Infliximab Biosimilar Analysis



Qualitative online MS analysis to confirm species or investigate differences

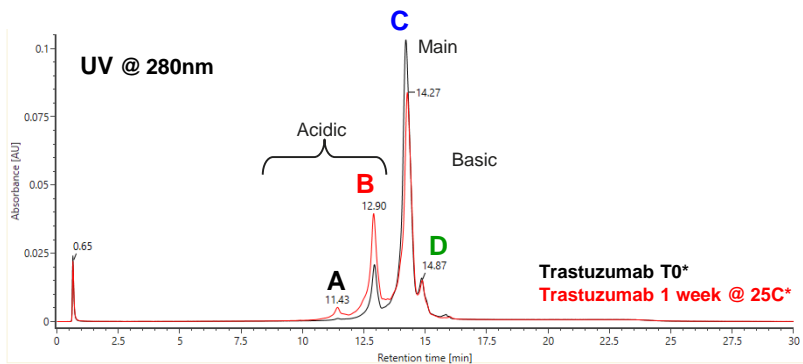
### MaxEnt1 Deconvolution





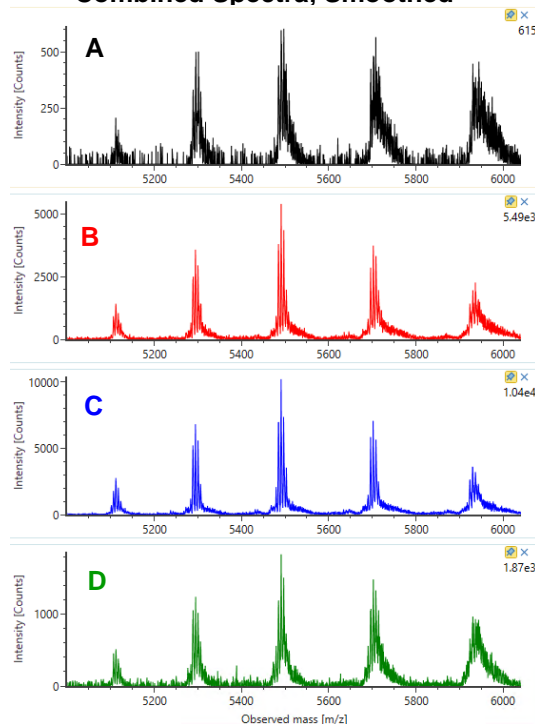
# Case Study 2: Forced Degradation Study

## Trastuzumab, pH 8.0 Stress

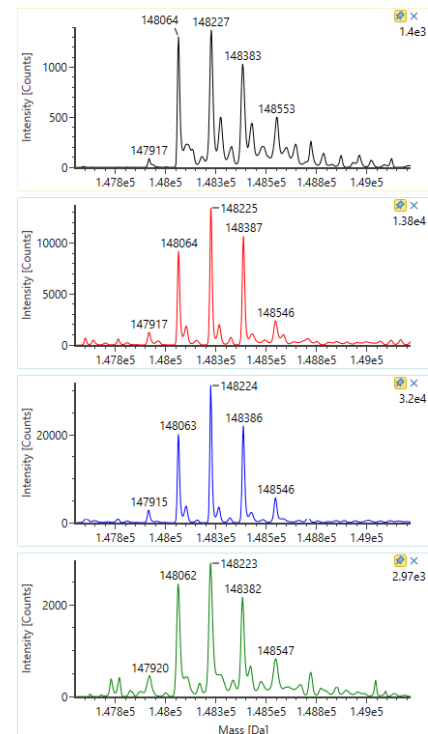


	Acidic	Main	Basic
T0	14.0%	74.7%	11.3%
1wk25C	32.7%	58.3%	9.0%
$\Delta$	<b>+18.7%</b>	<b>-16.4%</b>	<b>-2.3%</b>

**Combined Spectra, Smoothed**



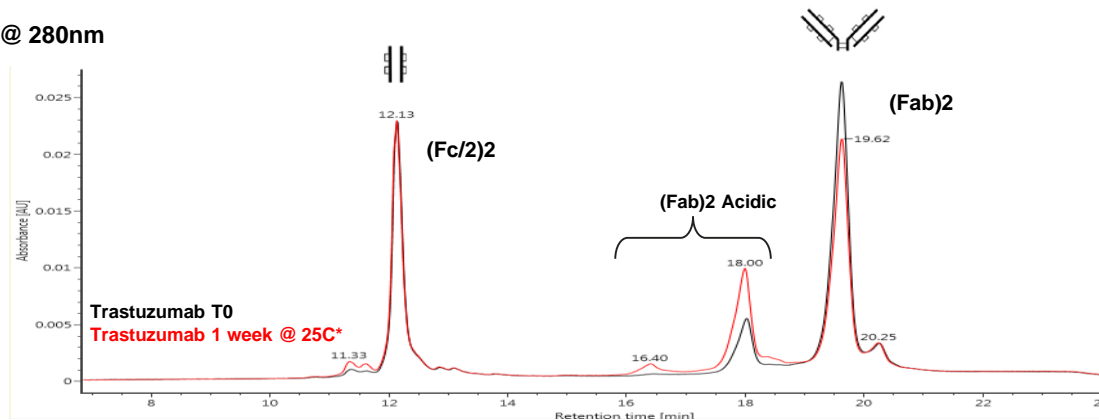
**MaxEnt1 deconvolution**



# Forced Degradation Study

## Trastuzumab: IdeS Digested Samples

UV @ 280nm



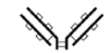
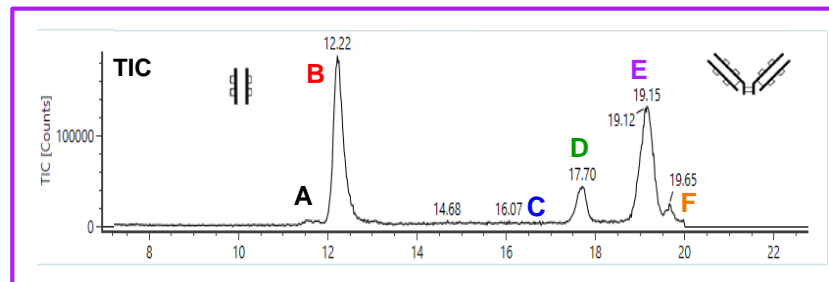
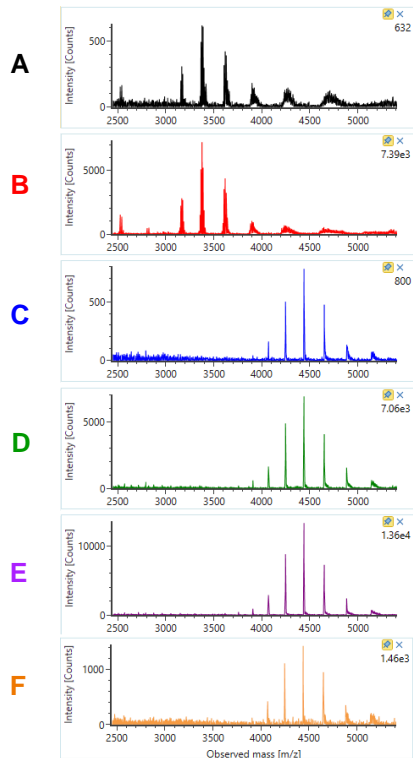
- Further localize the increase in acidic variants to the Fab region
- Good correlation to intact mAb analysis via UV integration
- IdeS digest analysis gives better mass accuracy and greater confidence in assignments

(Fc/2)2	Acidic	Main	Basic
T0	7.1%	84.4%	8.5%
1wk25C	11.6%	81.2%	7.2%
$\Delta$	<b>+4.5%</b>	<b>-3.2%</b>	<b>-1.3%</b>

(Fab)2	Acidic	Main	Basic
T0	20.9%	70.3%	8.9%
1wk25C	34.9%	56.4%	8.7%
$\Delta$	<b>+14.1%</b>	<b>-13.8%</b>	<b>-0.2%</b>

# Forced Degradation Study Trastuzumab: IdeS Digested Samples

## Combined Spectra

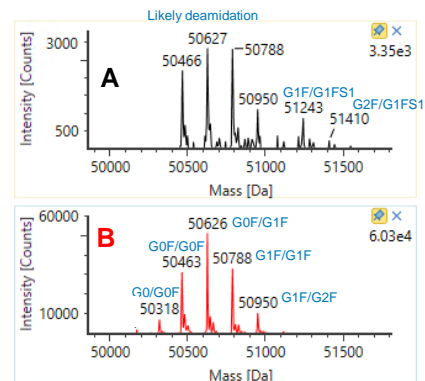


Expected mass:  
97,627.96 Da

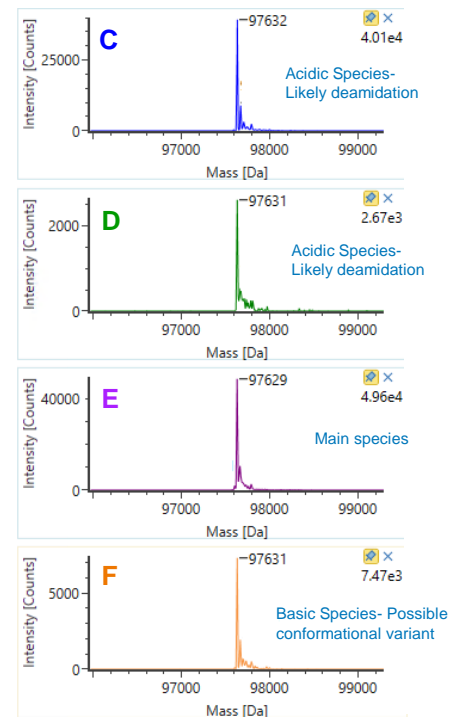


Expected mass (G0F/G0F):  
50,464.08 Da

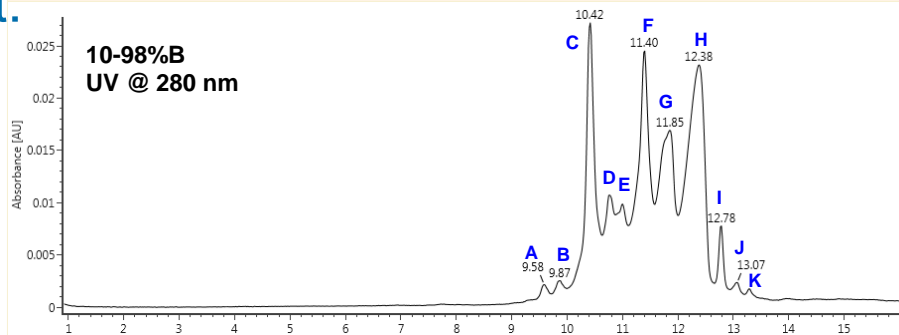
## MaxEnt1 deconvolution (Fc/2)2



## MaxEnt1 deconvolution (Fab)2



# Cetuximab IdeS Digest: Charge Variants

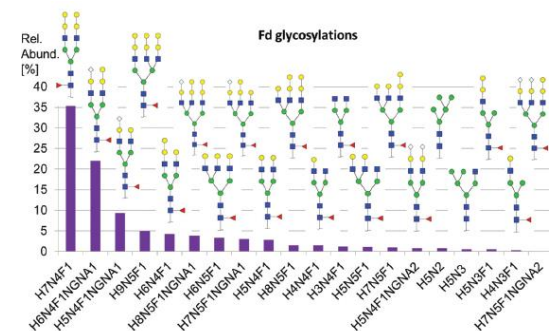


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System: Vion MS QToF  
Capillary Voltage: 3.0kV  
Cone Voltage: 150V  
Source Temperature: 120 °C  
Desolvation Gas: 350 °C  
Cone Gas flow: 100L/h  
Desolvation Gas flow: 600L/hr

Peak	(Fab)2 or (Fc/2)2	Species Detected
A,B	(Fc/2)2	G0F/G0F-G1F/G2F Deamidation
C	(Fc/2)2	G0F/G0F-G1F/G2F
D	(Fc/2)2	G0F/Man5; G1F/Man5; G0F/G0F, 1K Deamidation
E	(Fc/2)2	Man5/Man5
F	(Fc/2)2 & (Fab)2	Fc: G0F/G0F-G1F/G2F, 1K Fab: H6N4F1+NGNA / H6N4F1+NGNA; H6N4F1+NGNA / H8N5F1+NGNA
G	(Fc/2)2 & (Fab)2	Fc: G0F/Man5, 1K; G1F/Man5, 1K; Fab: H7N4F1/H6N4F1+NGNA; H9N5F1/H6N4F1+NGNA
H	(Fab)2	H7N4F1/H7N4F1; H7N4F1/H9N5F1
I	(Fc/2)2	G0F/G0F-G1F/G2F, 2K
J	(Fc/2)2	G0F/Man5, 2K; G1F/Man5, 2K
K	(Fc/2)2	Man5/Man5, 2K

Released Glycan analysis<sup>1</sup>: orthogonal support for IEX-MS peak assignments



1. [Ayoub, et al](#) mAbs, 5:5, 699-710 (2013)

H3N4F1 = G0F  
H4N4F1 = G1F  
H5N4F1 = G2F  
H5N2 = Man5

- IEX is used to monitor native state protein charge heterogeneity, and isolate charge variants for structural and functional analyses
- We have separated and identified mAb charge variants using IEX-MS with a combined salt (volatile) and pH gradient separation
- The ability of online IEX-MS to simplify charge variant characterization should reduce dependency on traditional fractionation-based workflows over time

## Acknowledgements

- Matt Lauber
- Ying Qing Yu
- Qi Wang
- Henry Shion
- Steve Koza
- Hua Yang
- Weibin Chen
- Min Du
- Bill Warren

*Thank you for your attention!  
Any questions, please ask!*



Tuesday Poster # T003: 10:30-11:30am & 12:30-2:30pm

“Online IEX-MS Characterization and Monitoring of mAb Charge Heterogeneity Using an Optimized Cation Exchange Resin and Compact TOF Mass Spectrometer”

# Backup Slides

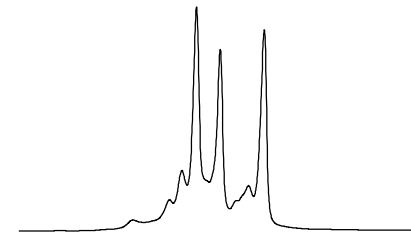
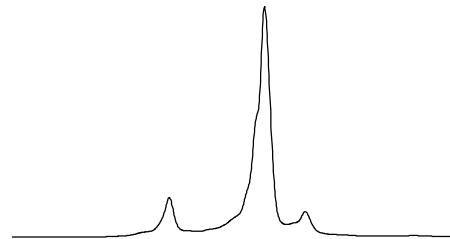
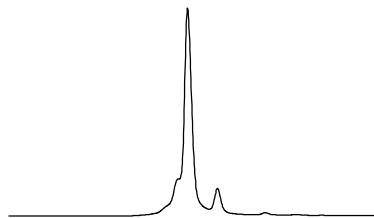
# Comparison of Profiles for Traditional and MS-Compatible IEX

NIST mAb

Trastuzumab

Infliximab

Non-Volatile  
Mobile Phases



Ammonium-based  
Volatile Mobile Phases

