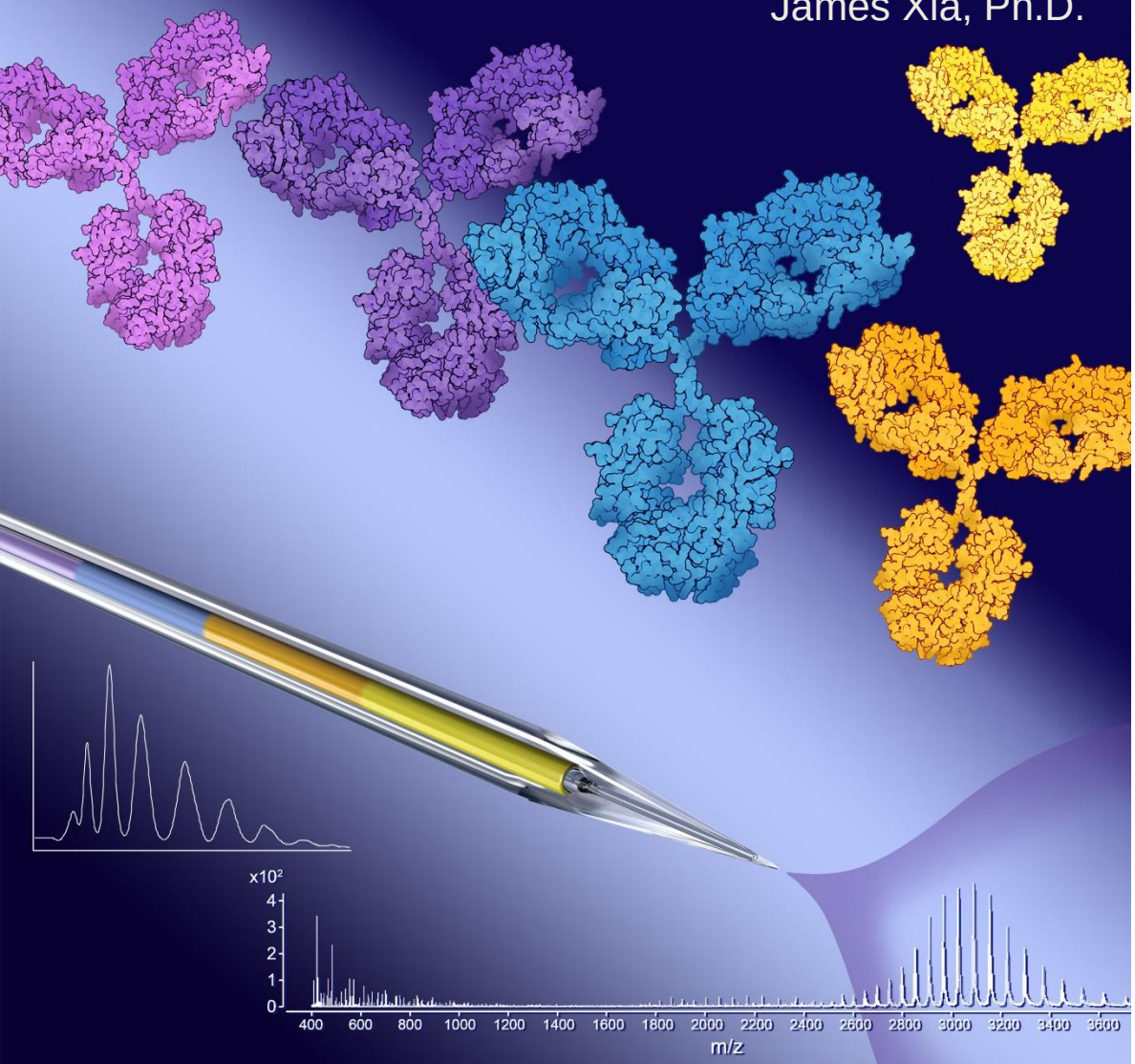
Capillary Electrophoresis - Mass Spectrometry

Transforming Biotherapeutics Quality Assessment

James Xia, Ph.D.



EMASS-II

7100 CE **6545XT Q-TOF**

July 12, 2018

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Common IEF-based Charge Variants Analysis Techniques

Isoelectric Focusing Gel

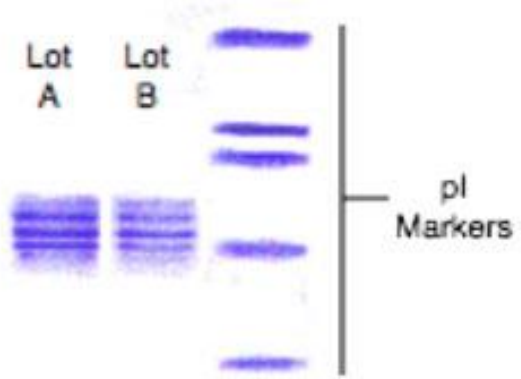
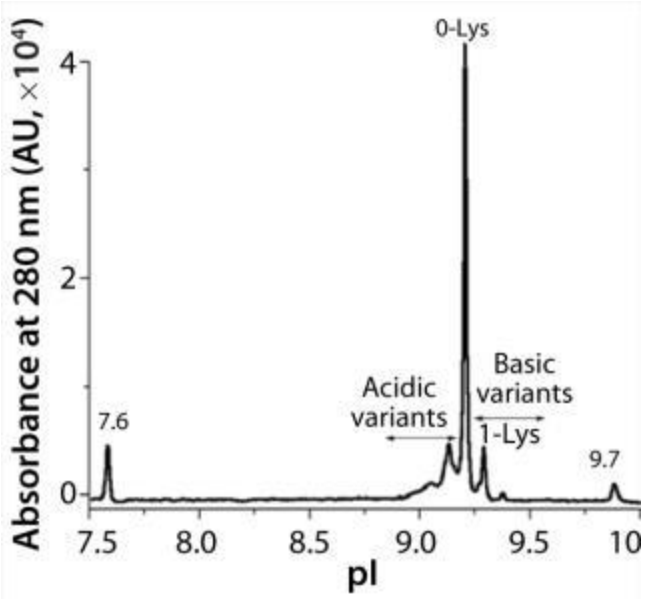


Figure courtesy of Antibody Solutions.
www.antibody.com

Imaged Capillary Isoelectric Focusing



http://www.bioprocessintl.com/analytical/product-characterization/imaged-capillary-isoelectric-focusing-for-charge-variant-analysis-of-biopharmaceuticals-323451/

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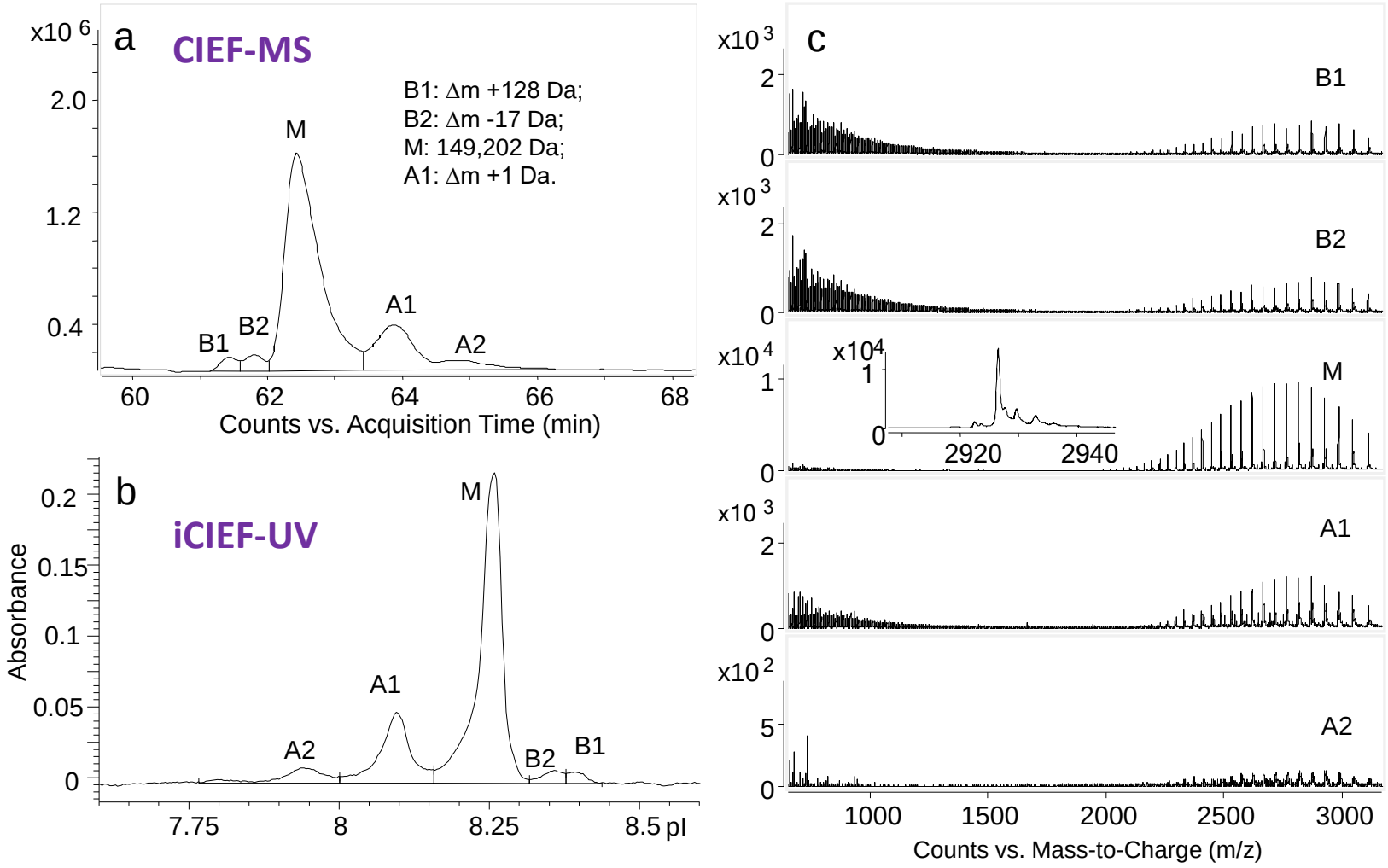
Bevacizumab cIEF-MS and icIEF-UV results



2018. Analytical Chemistry. Jun Dai, et al. CIEF-MS Method for the Separation and Online Characterization of Intact Monoclonal Antibody Charge Variants.

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Fully automated cIEF-MS for IgG charge variants analysis

analytical chemistry
Article
pubs.acs.org/ac

Capillary Isoelectric Focusing-Mass Spectrometry Method for the Separation and Online Characterization of Intact Monoclonal Antibody Charge Variants

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Supporting Information

ABSTRACT: We report a new online capillary isoelectric focusing-mass spectrometry (CIEF-MS) method for monoclonal antibody (mAb) charge variant analysis using an electrokinetically pumped sheath-flow nanospray ion source and a time-of-flight MS with pressure-assisted chemical mobilization. To develop a successful, reliable CIEF-MS method for mAb, we have selected and optimized many critical, interrelating reagents and parameters that include (1) MS-friendly anolyte and catholyte; (2) a glycerol enhanced sample mixture that reduced non-CIEF electrophoretic mobility and band broadening; (3) ampholyte selected for balancing resolution and MS sensitivity; (4) sheath liquid composition optimized for efficient focusing, mobilization, and electrospray ionization; (5) judiciously selected CIEF running parameters including injection amount, field strength, and applied pressure. The fundamental premise of CIEF was well maintained as verified by the linear correlation (R^2 = 0.99) between p*I* values and migration time using a mixture of p*I* markers. In addition, the charge variant profiles of trastuzumab, bevacizumab, infliximab, and cetuximab, obtained using this CIEF-MS method, were corroborated by imaged CIEF-UV (iCIEF-UV) analyses. The relative standard deviations (RSD) of absolute migration time of p*I* markers were all less than 5% (n = 4). Triplicate analyses of bevacizumab showed RSD less than 1% for relative migration time to an internal standard and RSD of 7% for absolute MS peak area. Moreover, the antibody charge variants were characterized using the online intact MS data. To the best of our knowledge, this is the first time that direct online MS detection and characterization were achieved for mAb charge variants resolved by CIEF as indicated by a well-established linear pH gradient and correlated CIEF-UV charge variant profiles.

https://pubs.acs.org/doi/abs/10.1021/acs.analchem.7b04608

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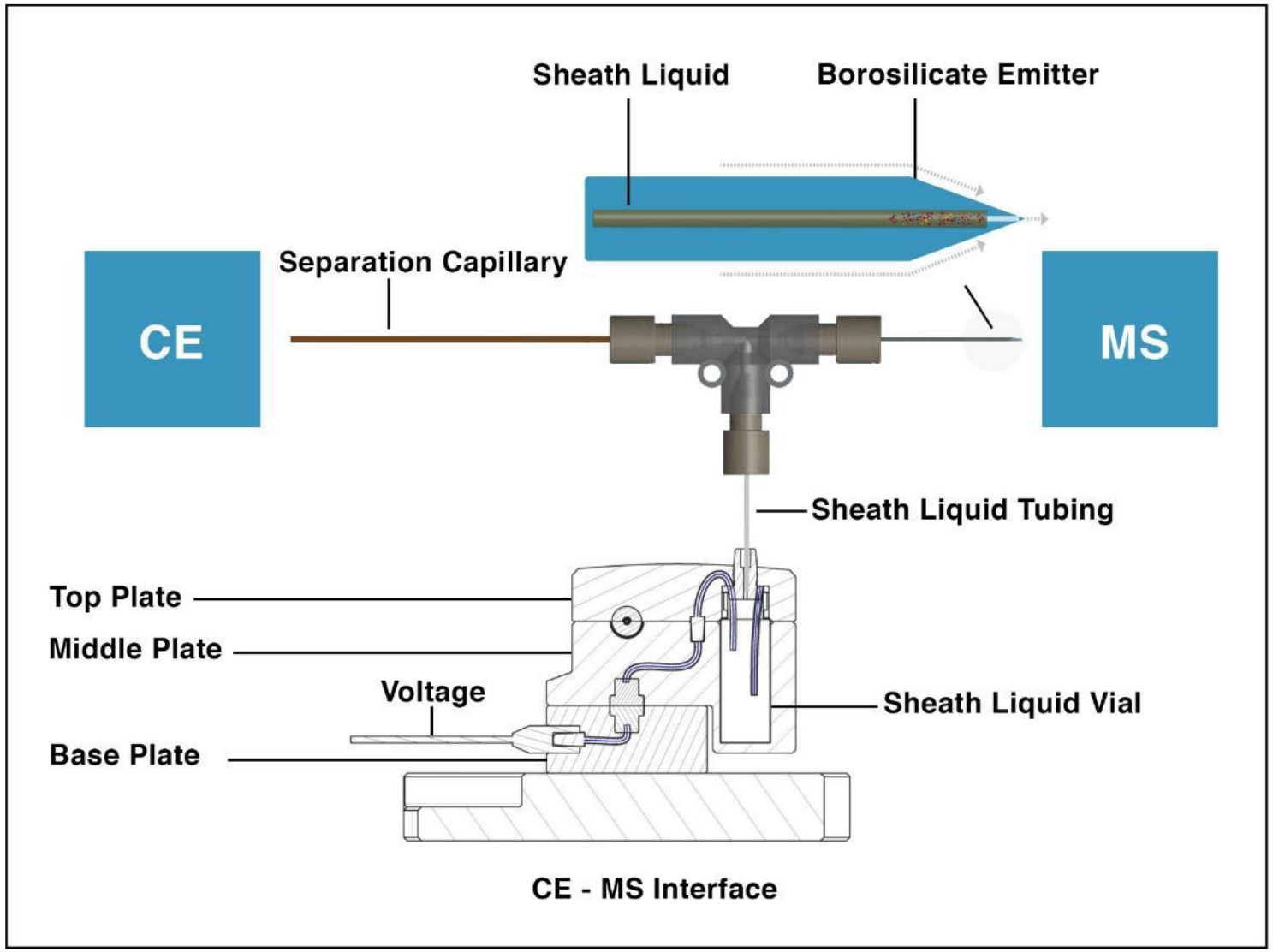
Electrokinetically pumped sheath-flow nanospray



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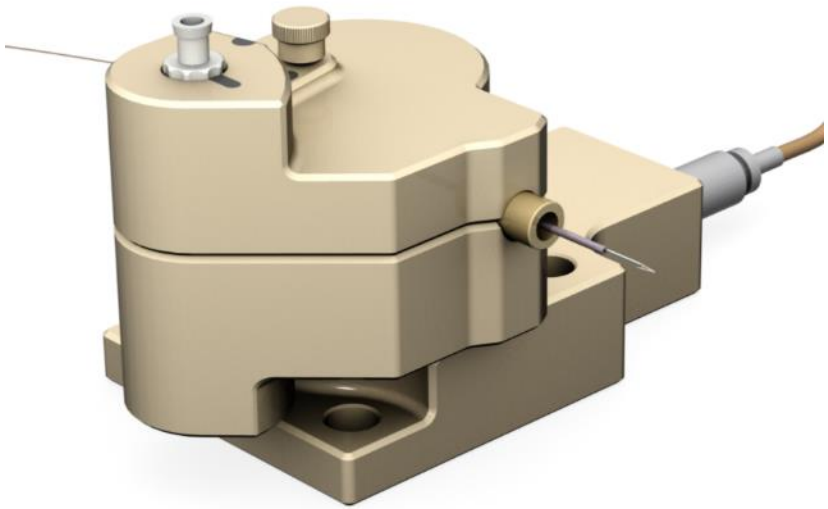
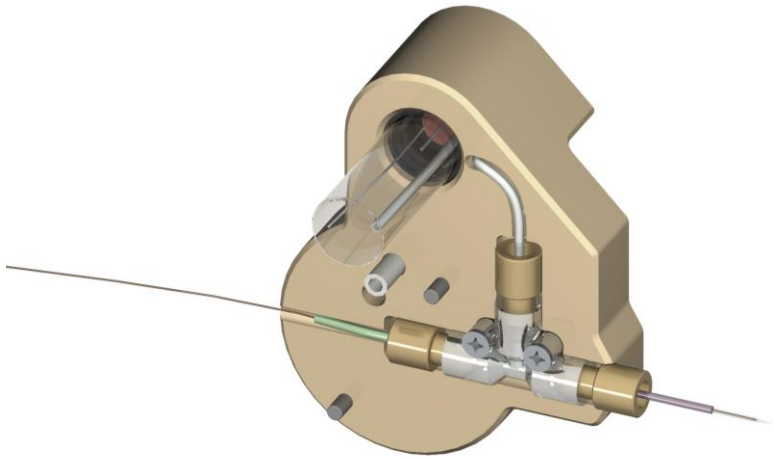
CMP Scientific EMASS-II CE-MS Technology





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CMP EMASS-II ion source for Agilent mass specs

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Agilent 7100 - CMP EMASS-II – Agilent 6545 Q-TOF

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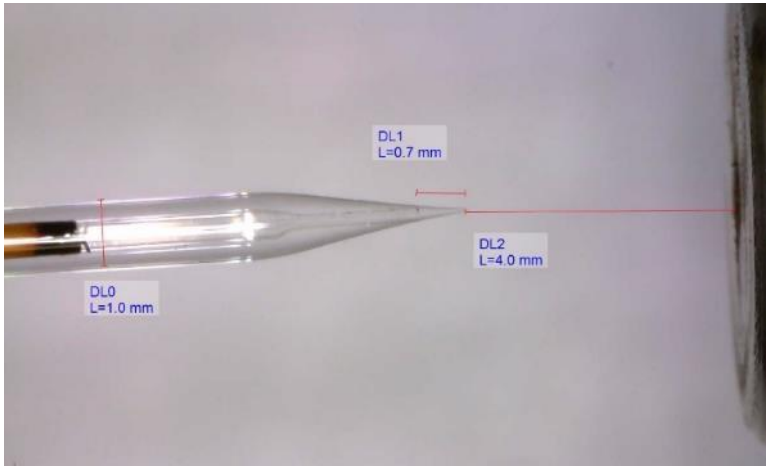
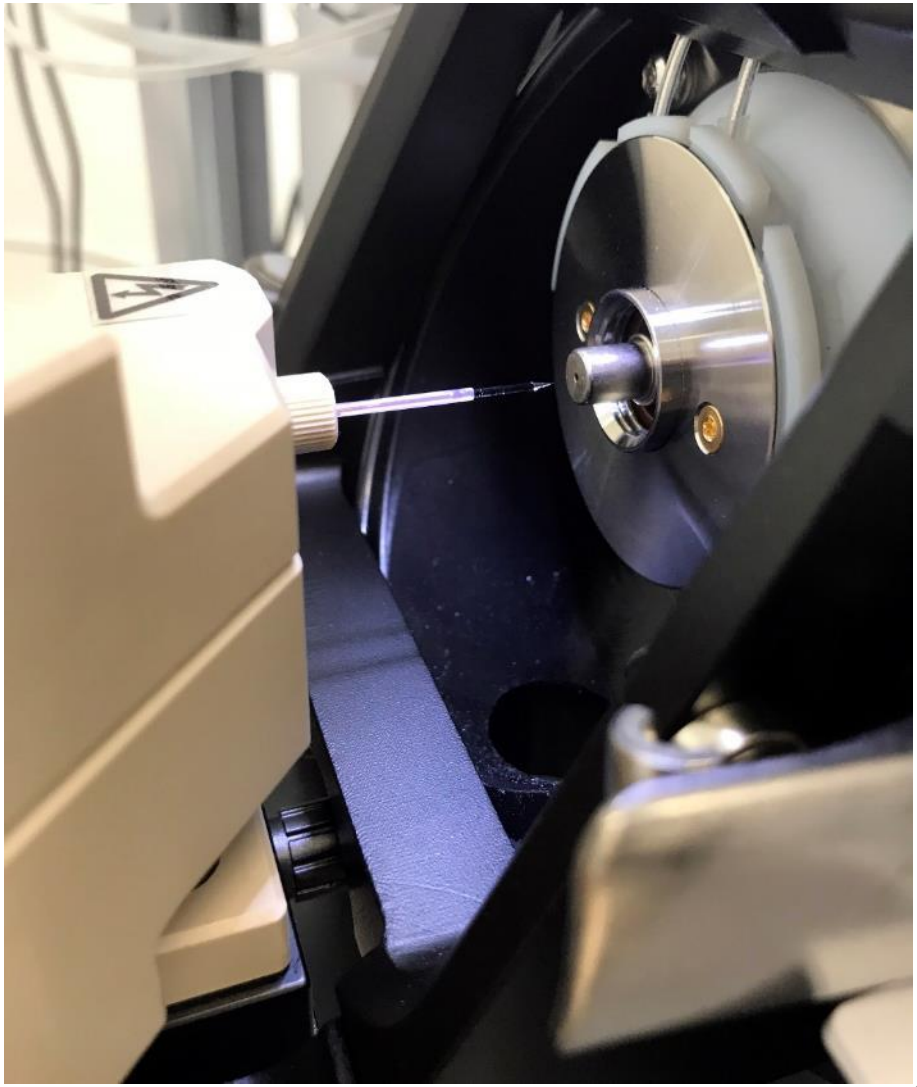
EMASS-II CE-MS Technology On Agilent MS





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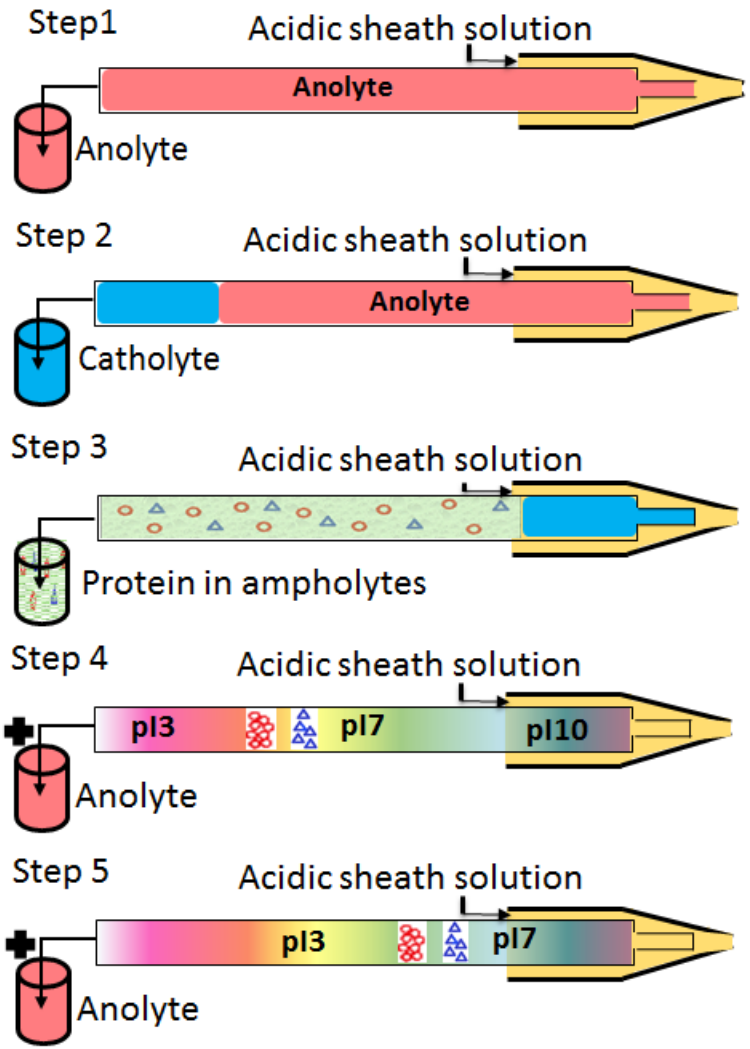
Schematic flow of the fully automated cIEF-MS method



Solution	Components
Sheath liquid	20% acetic acid, 20% acetonitrile
Catholyte	0.2 N NH_4OH, 15% glycerol
Sample solution	1-1.5% Pharmalyte®, 15% glycerol
Anolyte	1% formic acid, 15% glycerol

2018. Analytical Chemistry. Jun Dai, et al. CIEF-MS Method for the Separation and Online Characterization of Intact Monoclonal Antibody Charge Variants.

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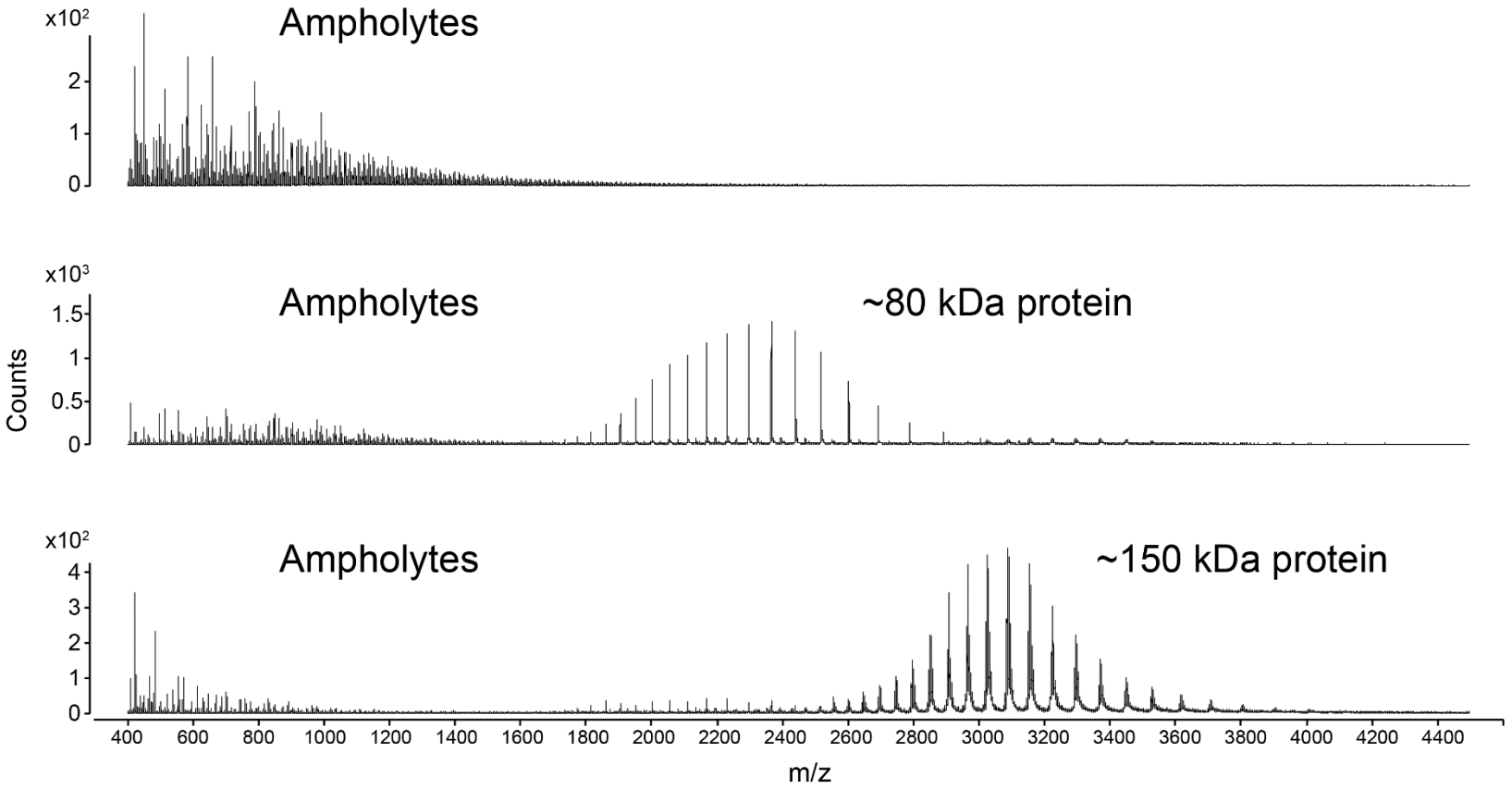
MS spectra of proteins in the presence of ampholyte



2018. Analytical Chemistry. Jun Dai, et al. CIEF-MS Method for the Separation and Online Characterization of Intact Monoclonal Antibody Charge Variants.

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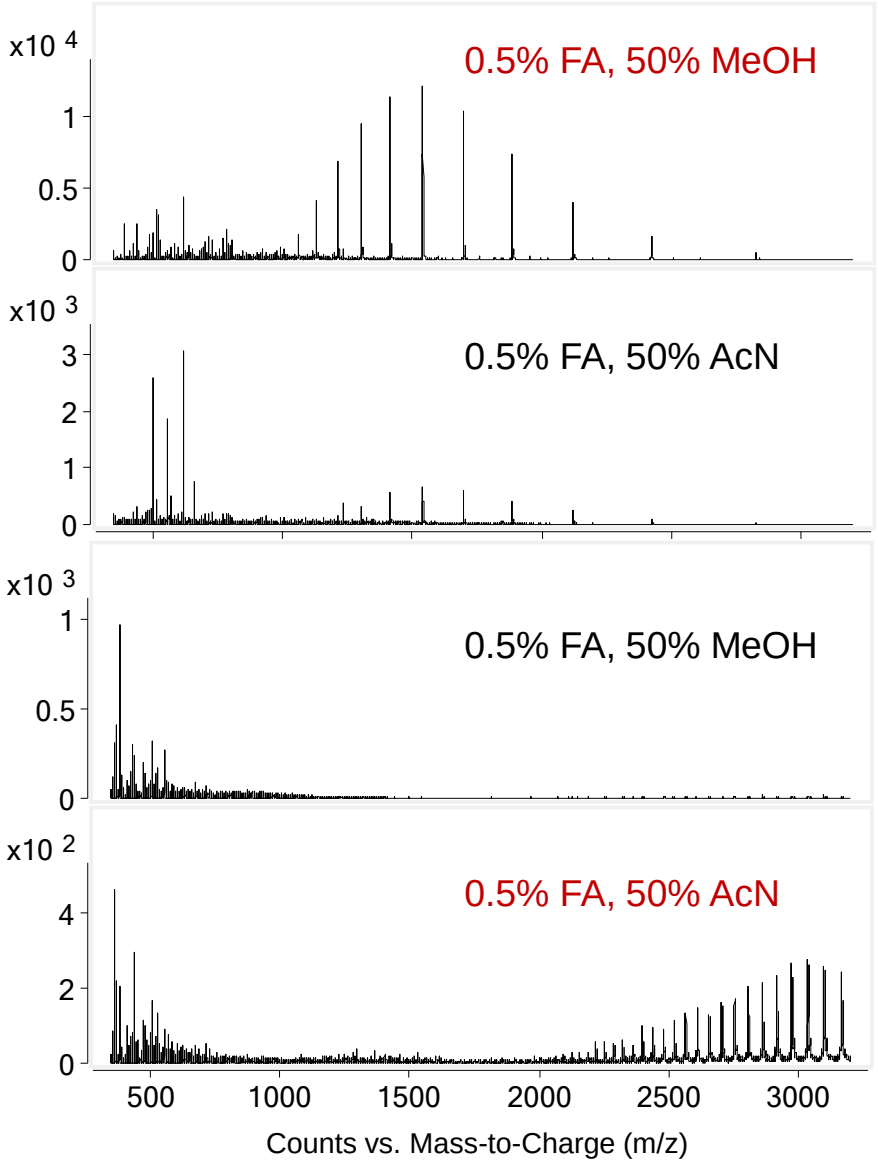
Sheath solution optimization for cIEF-MS



Myoglobin

(17 kDa)

IgG

(150 kDa)

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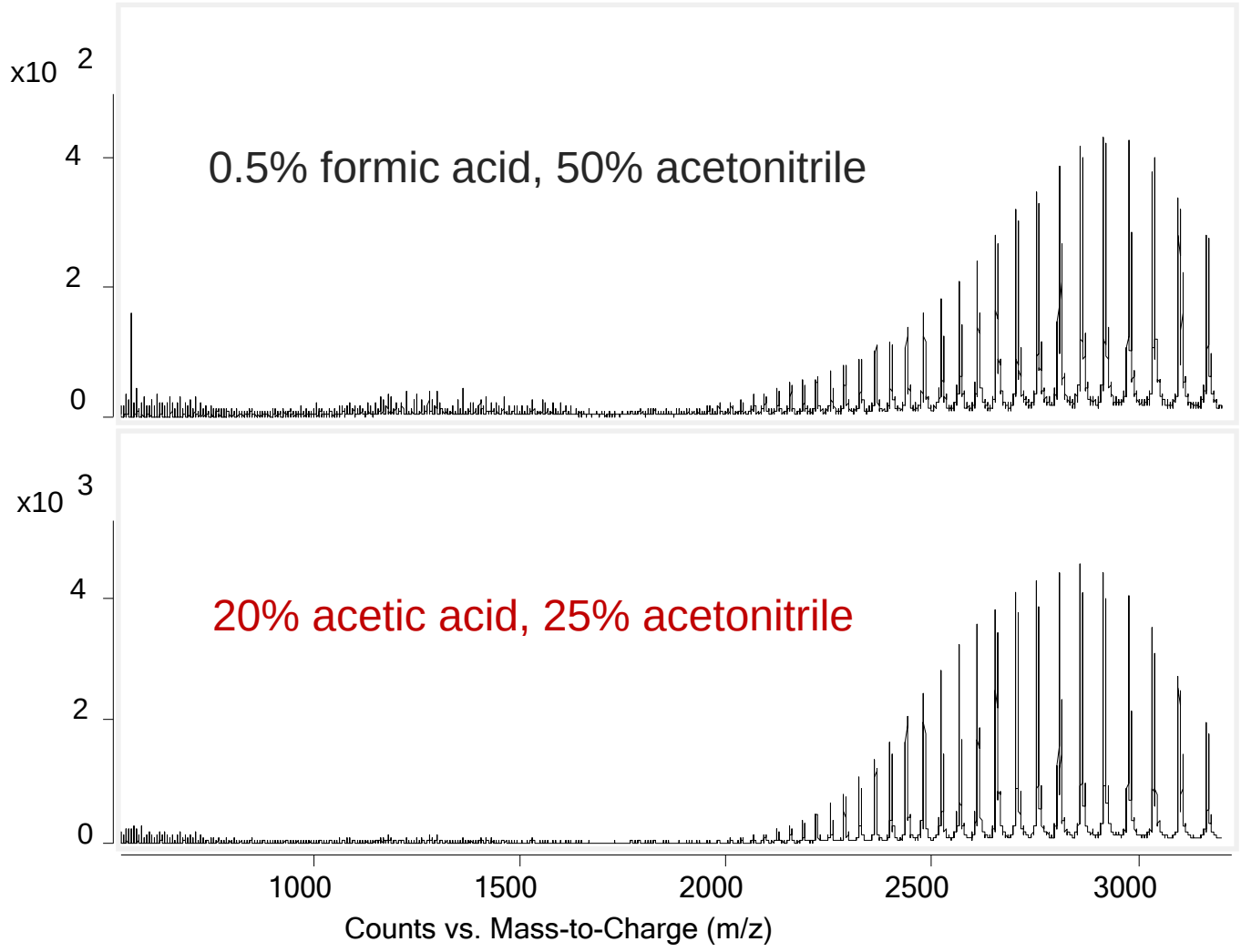
Sheath solution optimization for IgG cIEF-MS analysis



2018. Analytical Chemistry. Jun Dai, et al. CIEF-MS Method for the Separation and Online Characterization of Intact Monoclonal Antibody Charge Variants.

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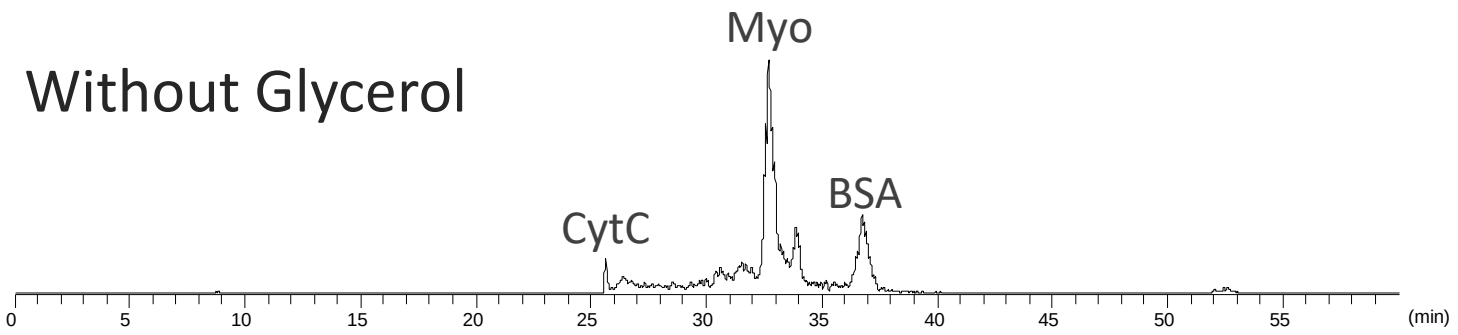
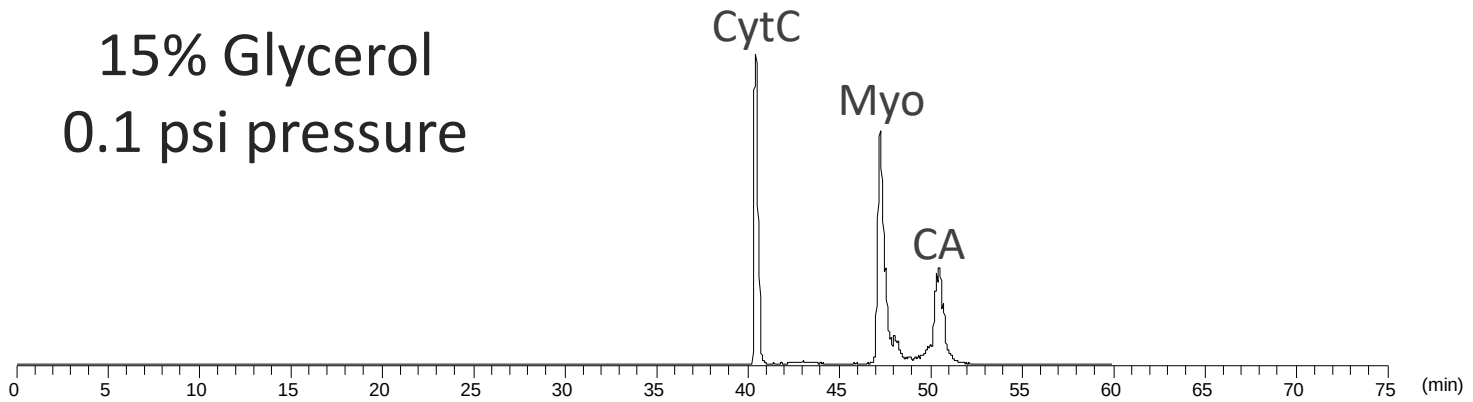
Glycerol is important for focusing and optimal resolution





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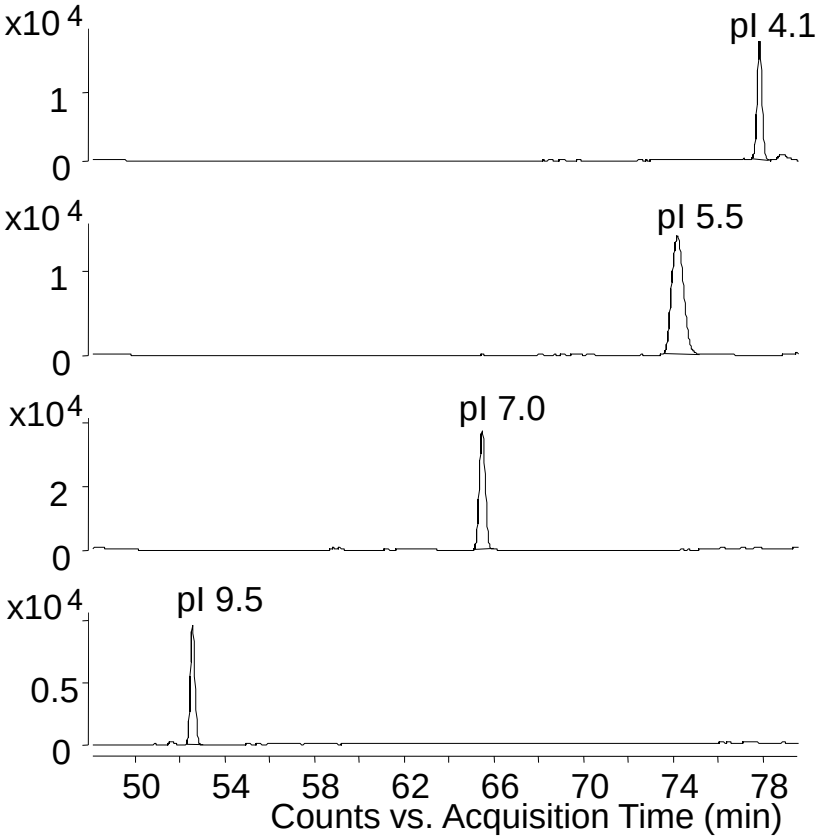
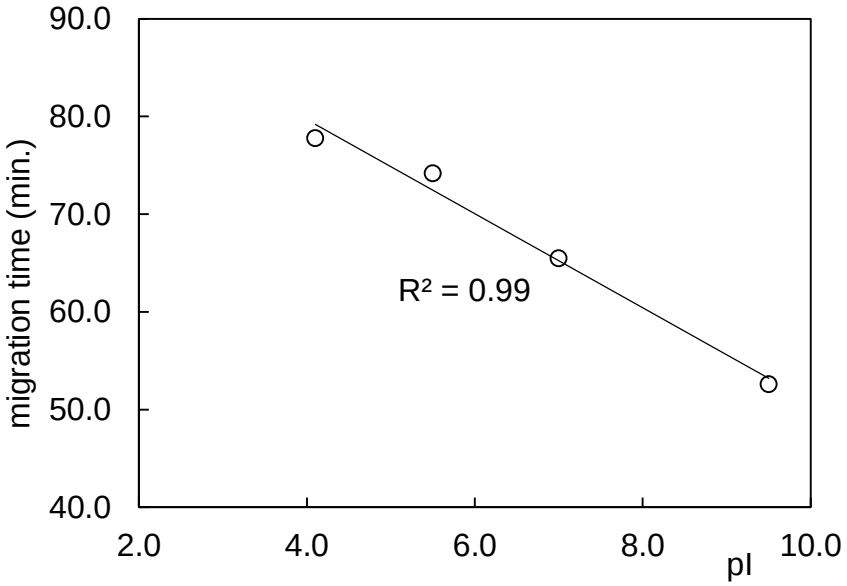
cIEF-MS of pI markers under standard conditions





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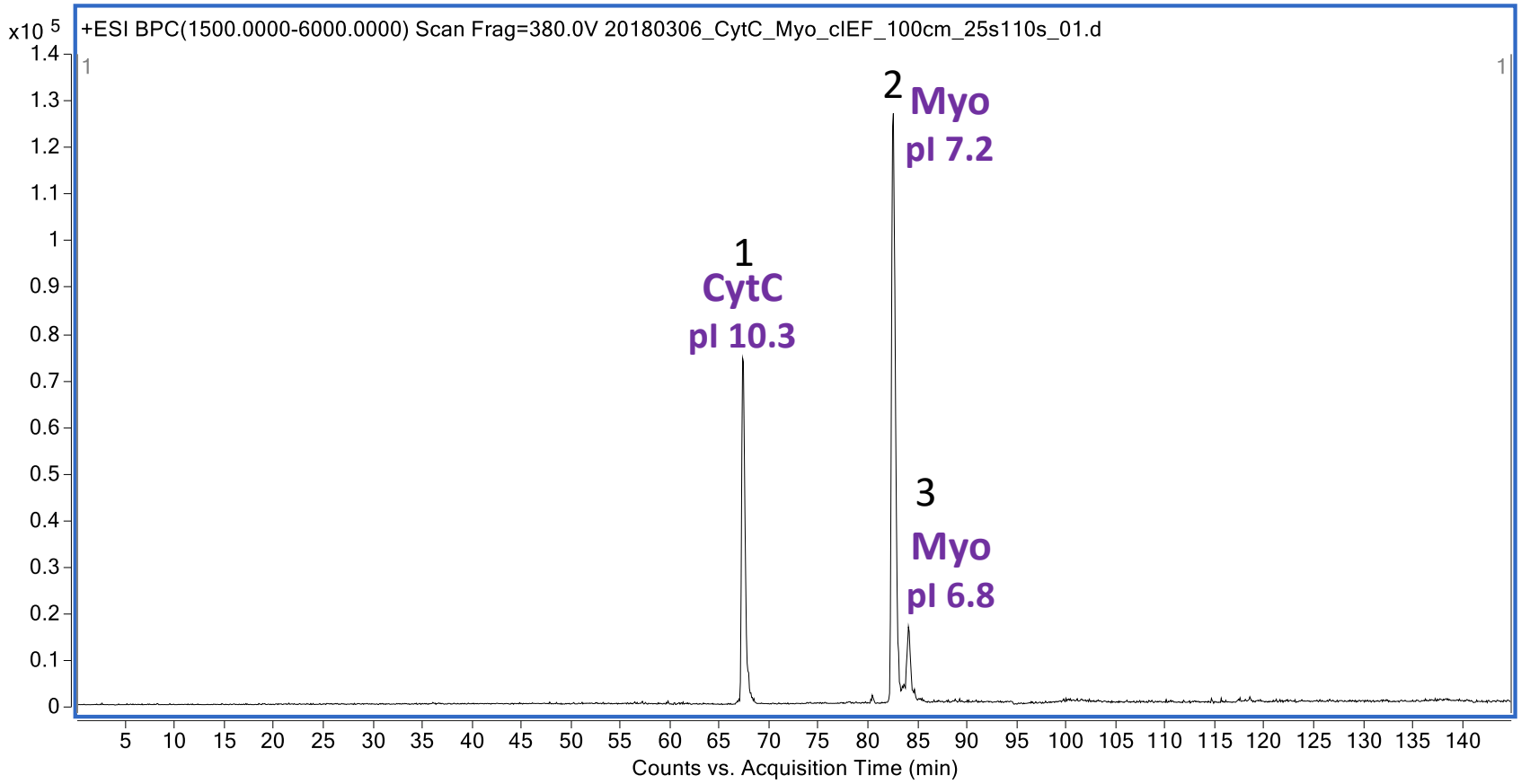
cIEF-MS on a 100 cm PS1 capillary



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cIEF-MS and icIEF analysis of mAb molecules

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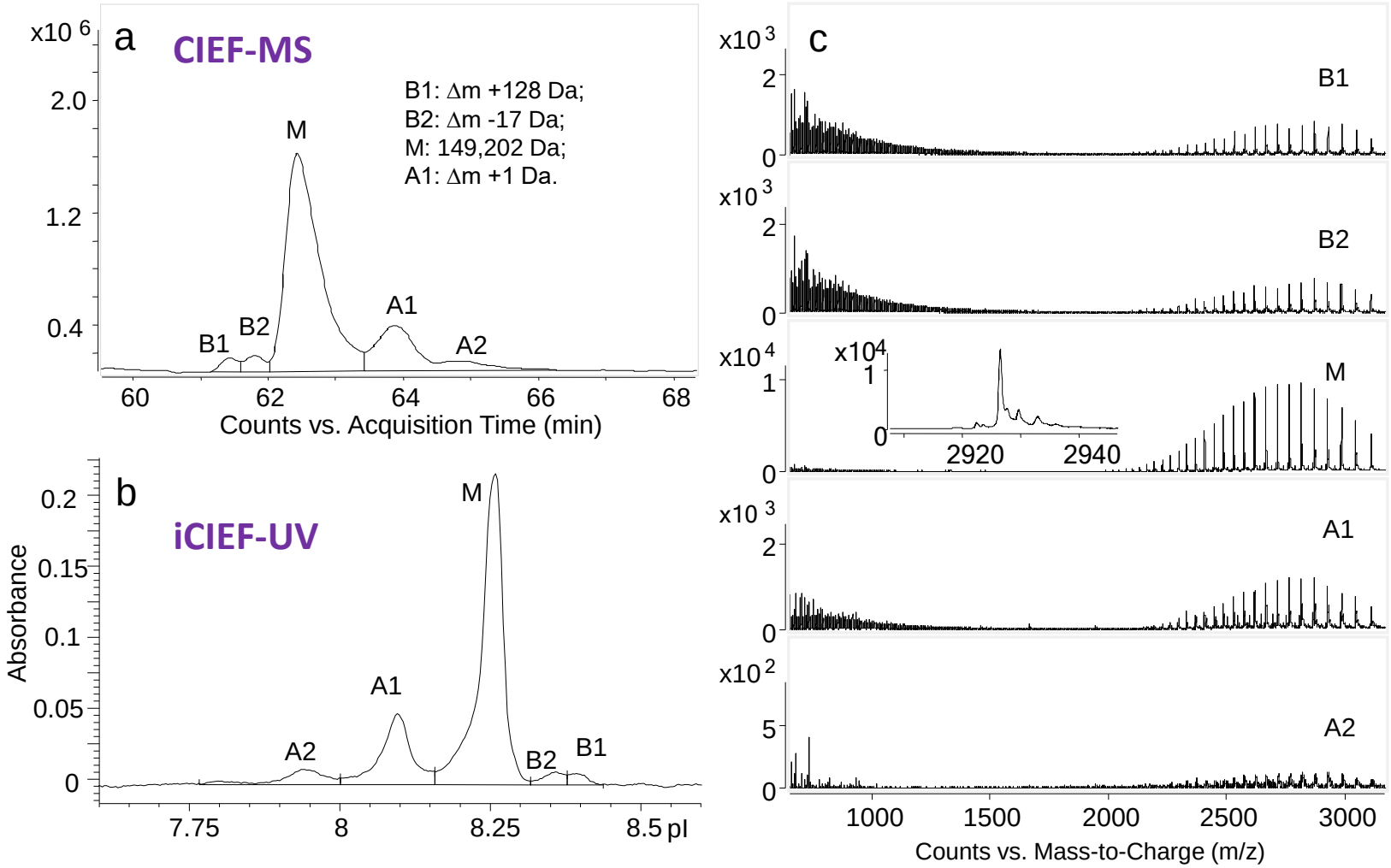
Bevacizumab cIEF-MS and icIEF-UV results



2018. Analytical Chemistry. Jun Dai, et al. CIEF-MS Method for the Separation and Online Characterization of Intact Monoclonal Antibody Charge Variants.

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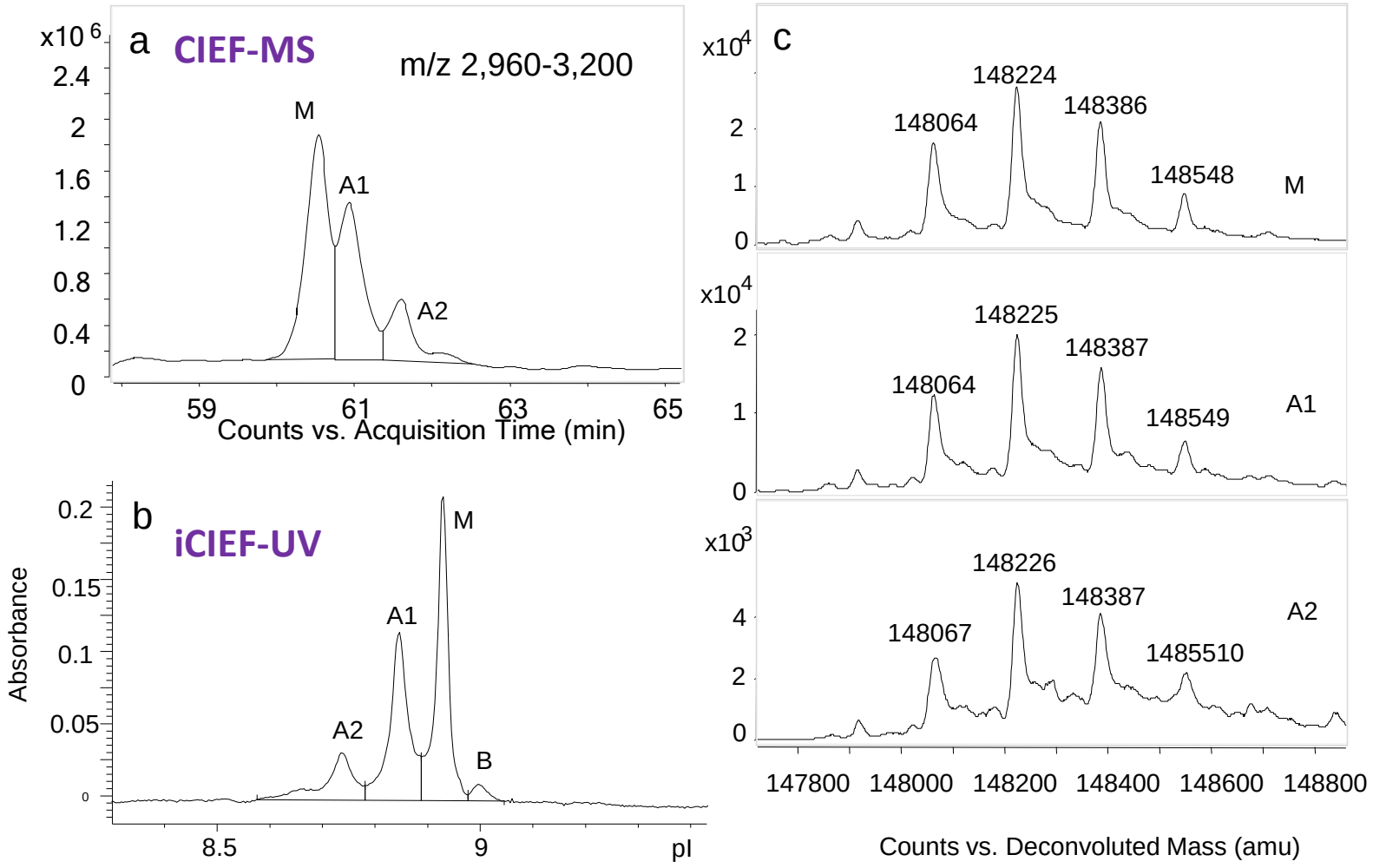
Trastuzumab cIEF-MS analysis in comparison with icIEF-UV



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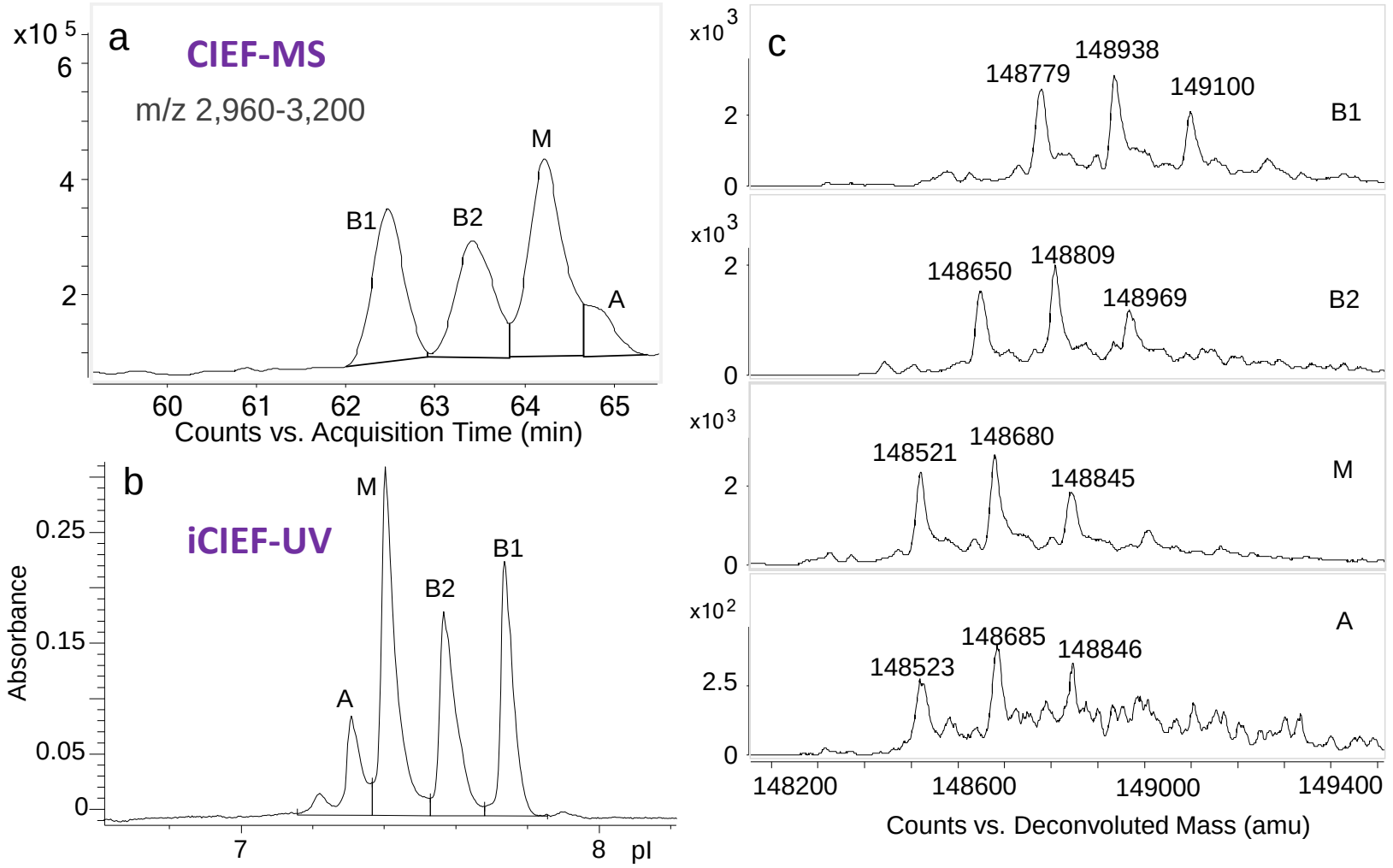
Infliximab cIEF-MS analysis in comparison with icIEF-UV



2018. Analytical Chemistry. Jun Dai, et al. CIEF-MS Method for the Separation and Online Characterization of Intact Monoclonal Antibody Charge Variants.

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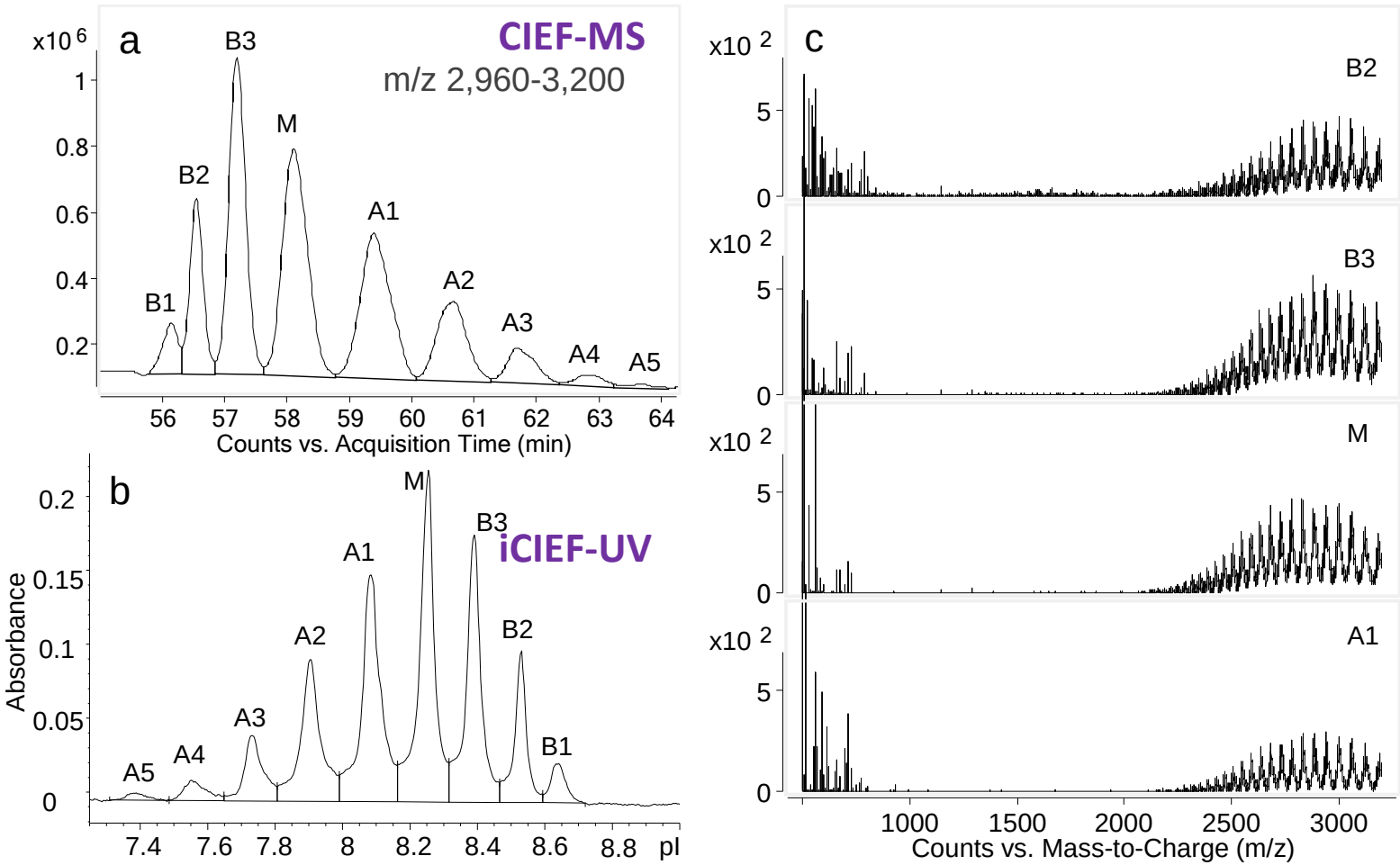
Cetuximab cIEF-MS analysis in comparison with icIEF-UV



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cIEF-MS analysis of Daratumumab

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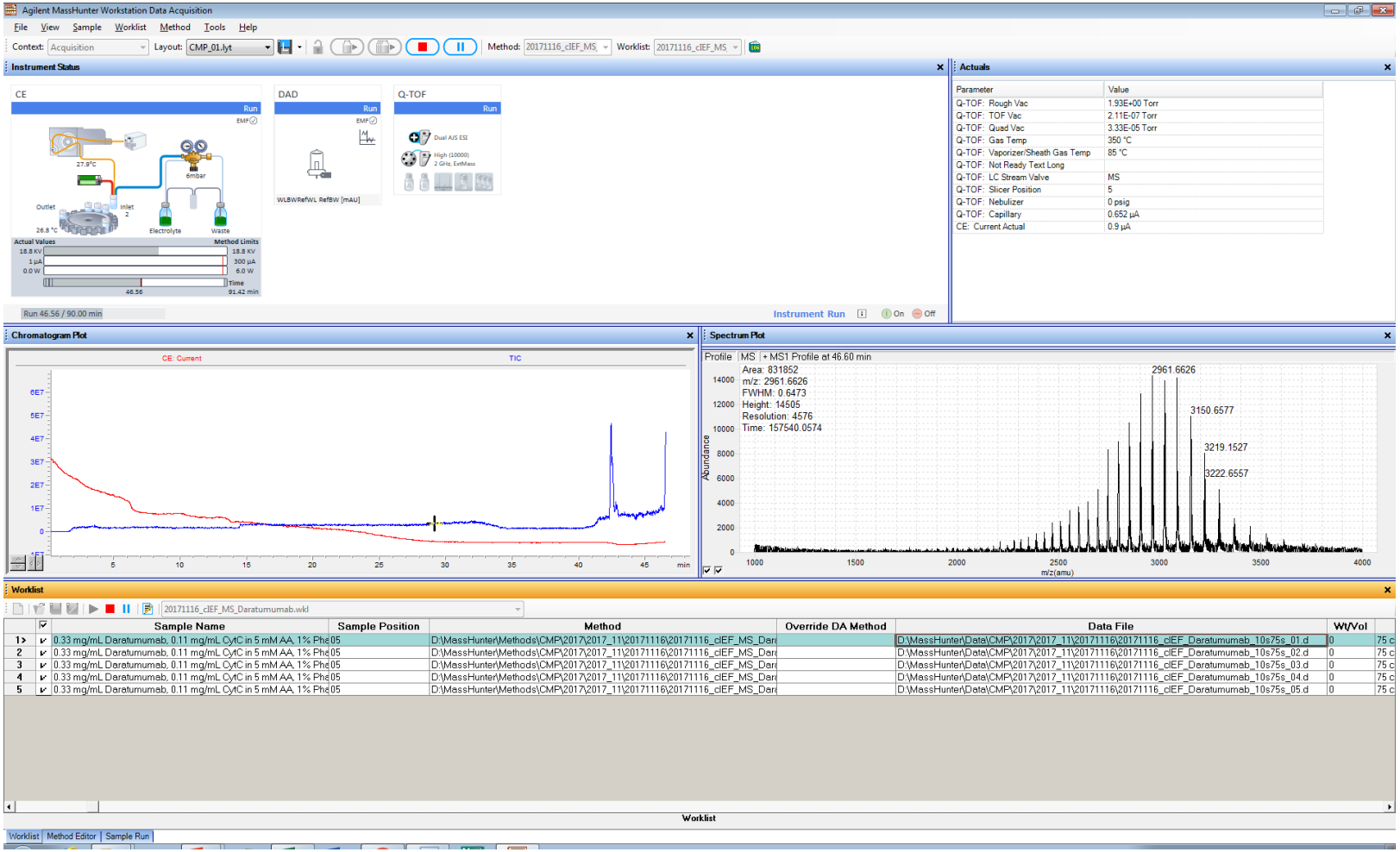
cIEF-MS analysis of Daratumumab



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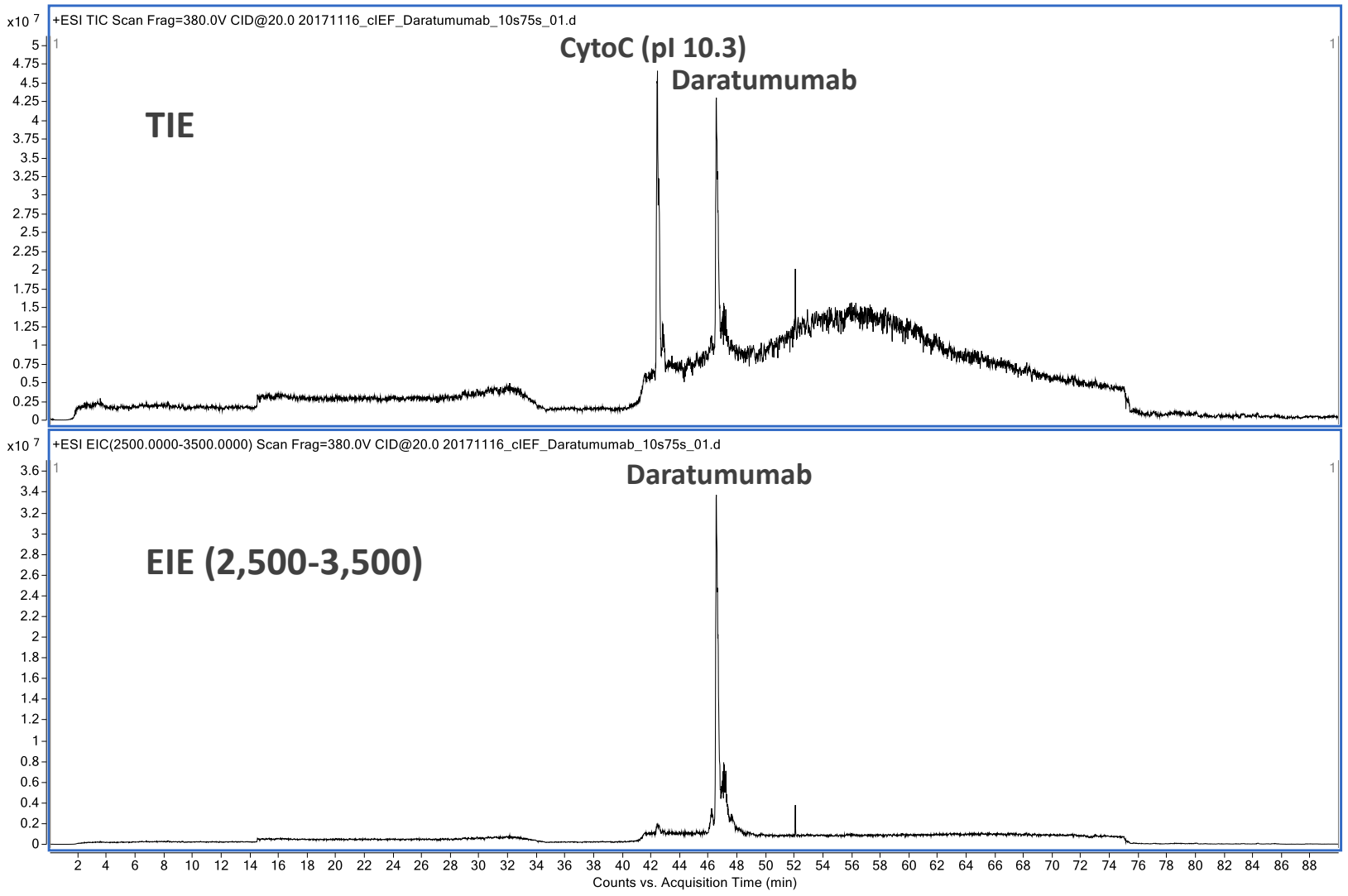
cIEF-MS analysis of Daratumumab



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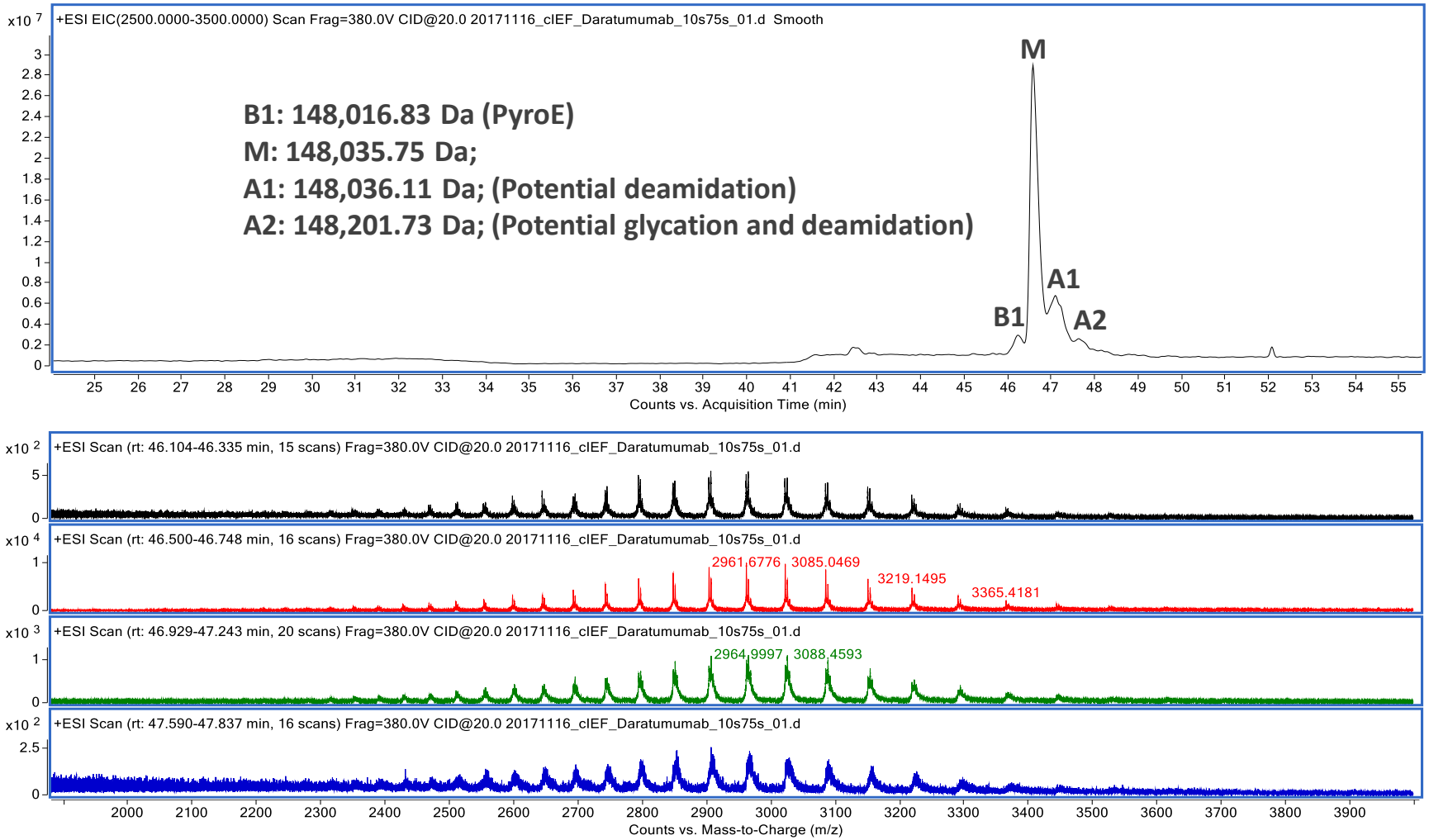
cIEF-MS analysis of Daratumumab



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IgG Subunits Analysis (CZE-MS): Low Carryover

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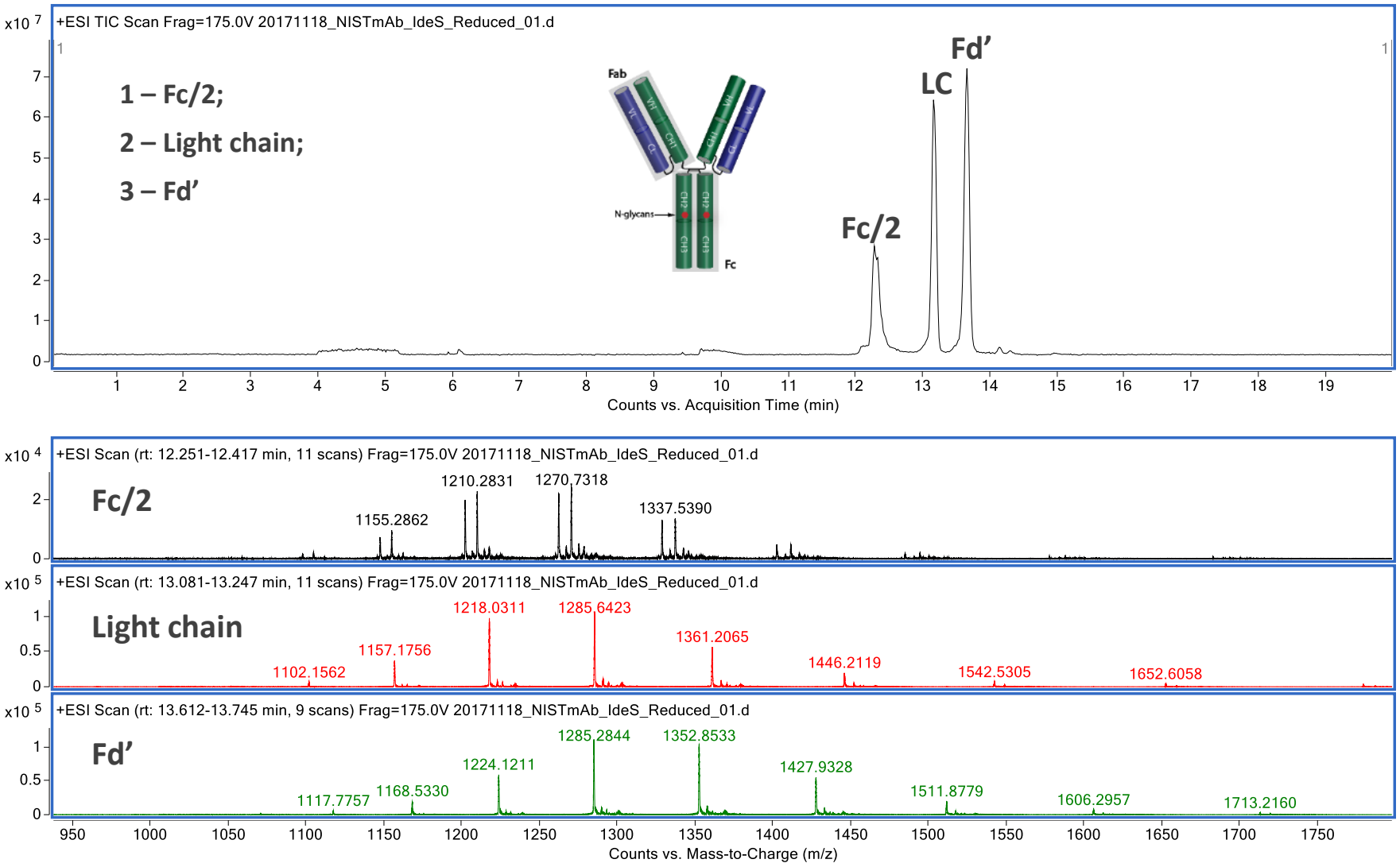
Reduced IdeS digest of NISTmAb



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IgG Intact Mass Analysis: On-line Desalting

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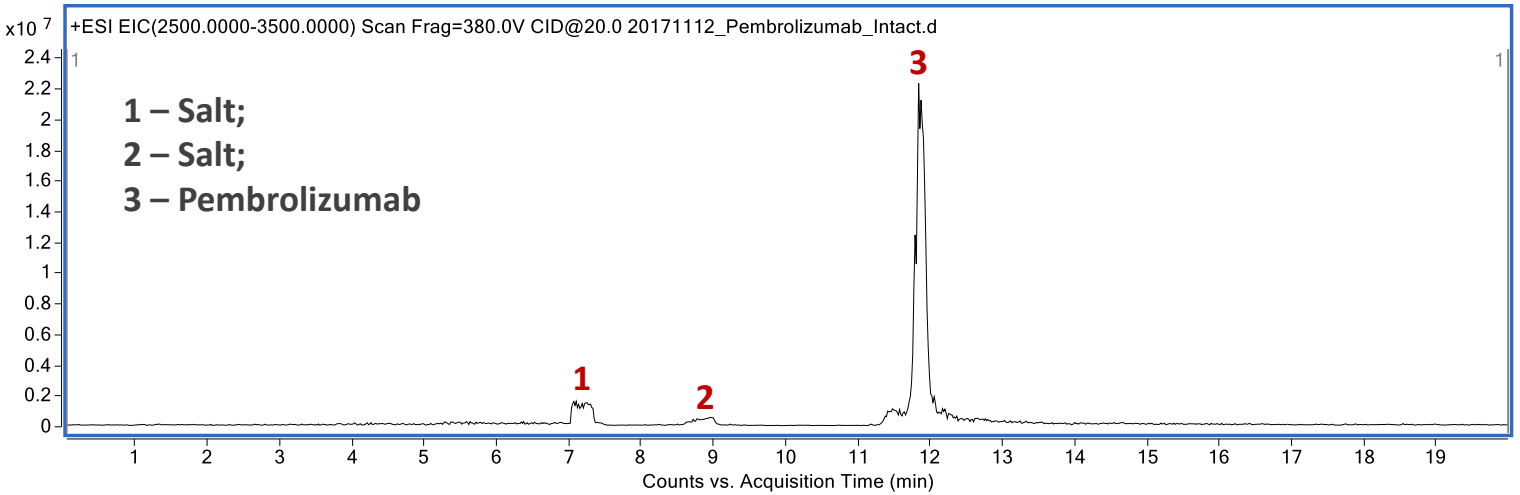
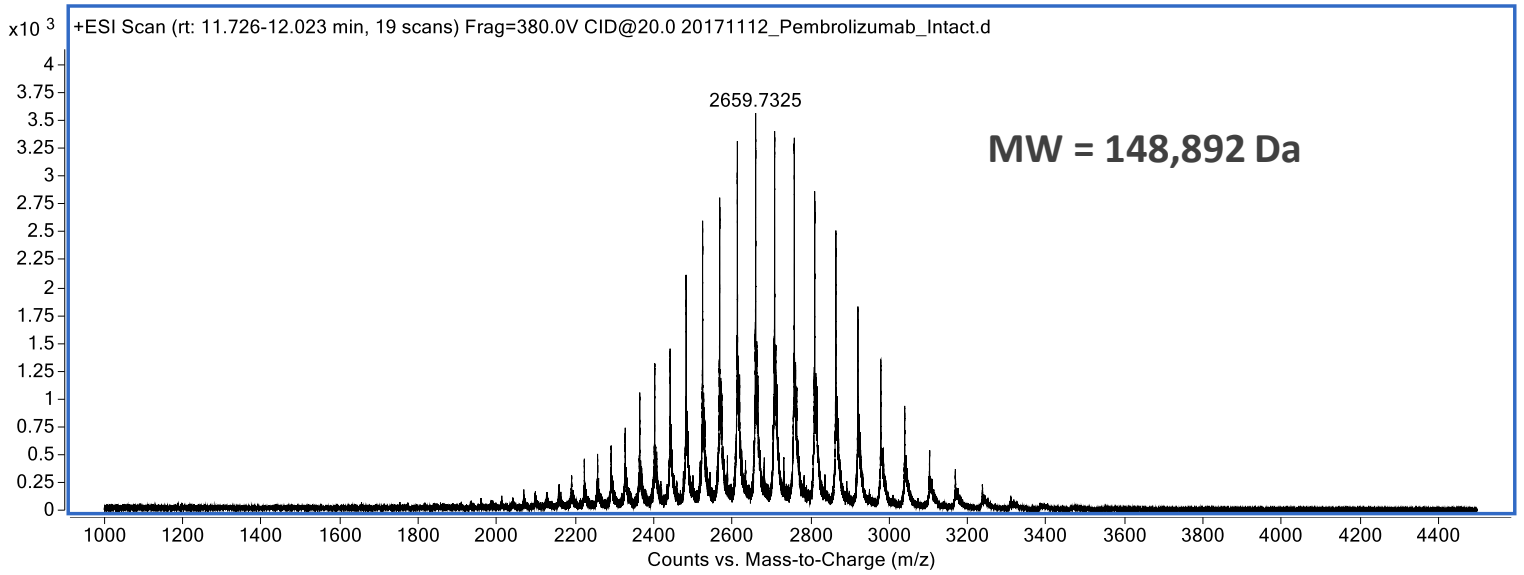
Intact mass analysis of Pembrolizumab





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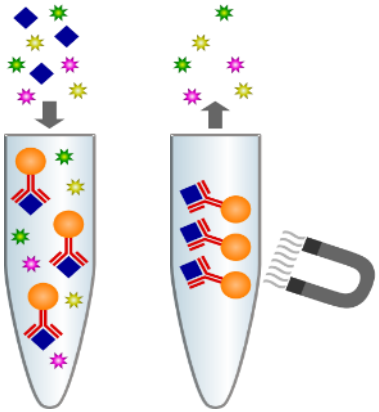

Immunoaffinity Capture - CE-MS: Preclinical ADME

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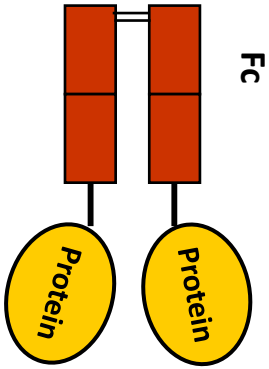
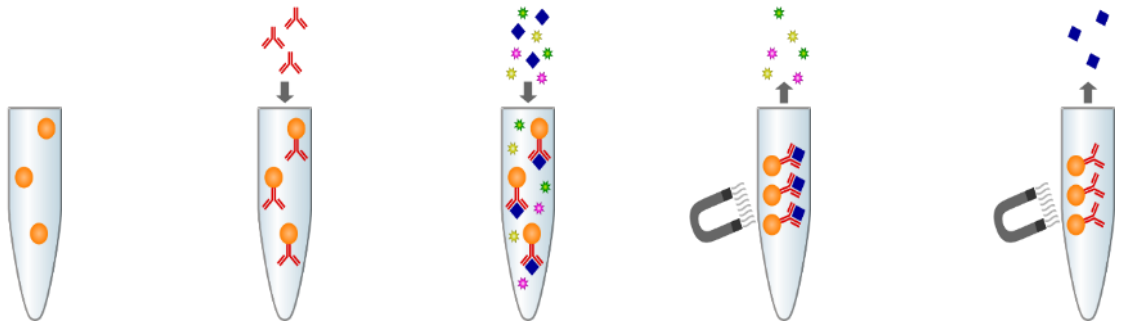
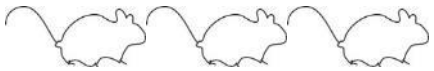
Which Fc-fusion constructs are more stable *in vivo*?





A Fc – Linker – Protein Fc-protein WT

B Fc – Linker – Protein Fc-protein RG mutation



Mice dosed with Fc-protein WT

Mice dosed with Fc-protein RG mutation

1h 8h 24h 48h 72h 96h

Post-dosing Serum

1h 8h 24h 48h 72h 96h

Immunoaffinity capture

Immunoaffinity capture

CE-MS

CE-MS

Courtesy of Amgen Inc. South San Francisco PKDM group.

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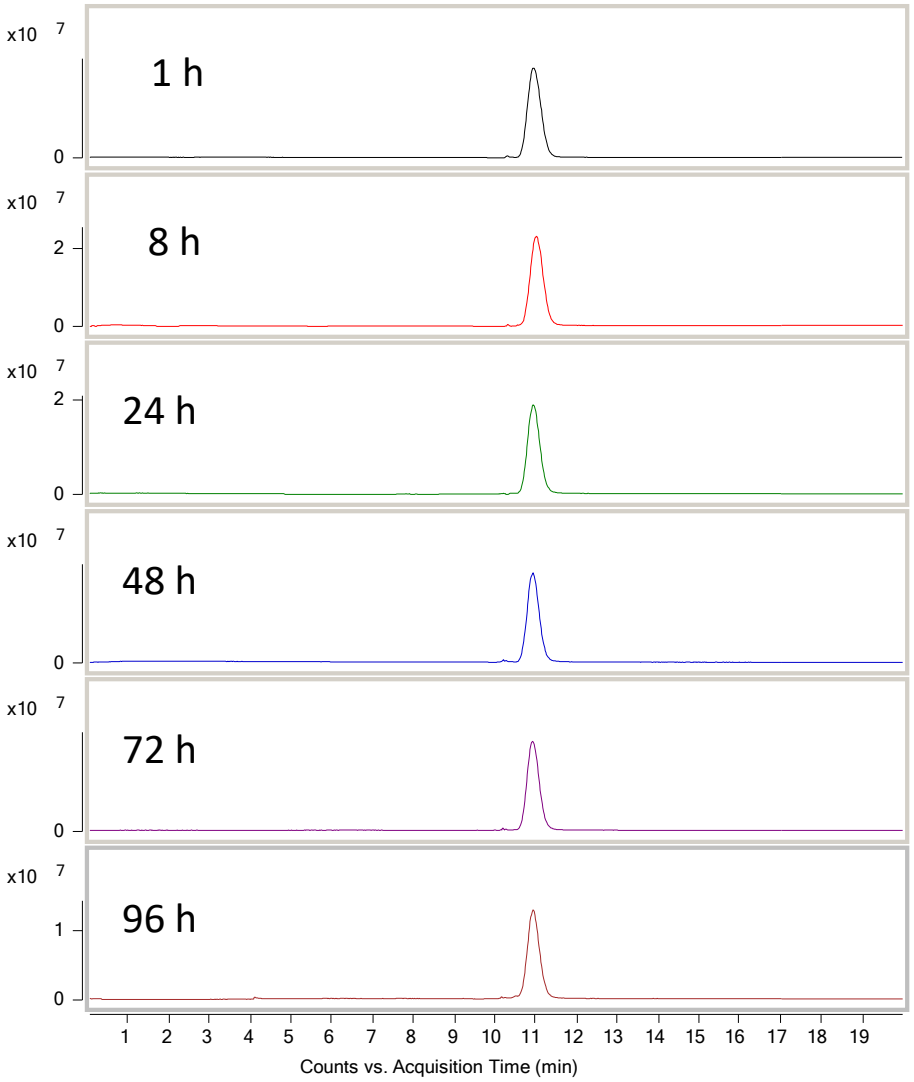
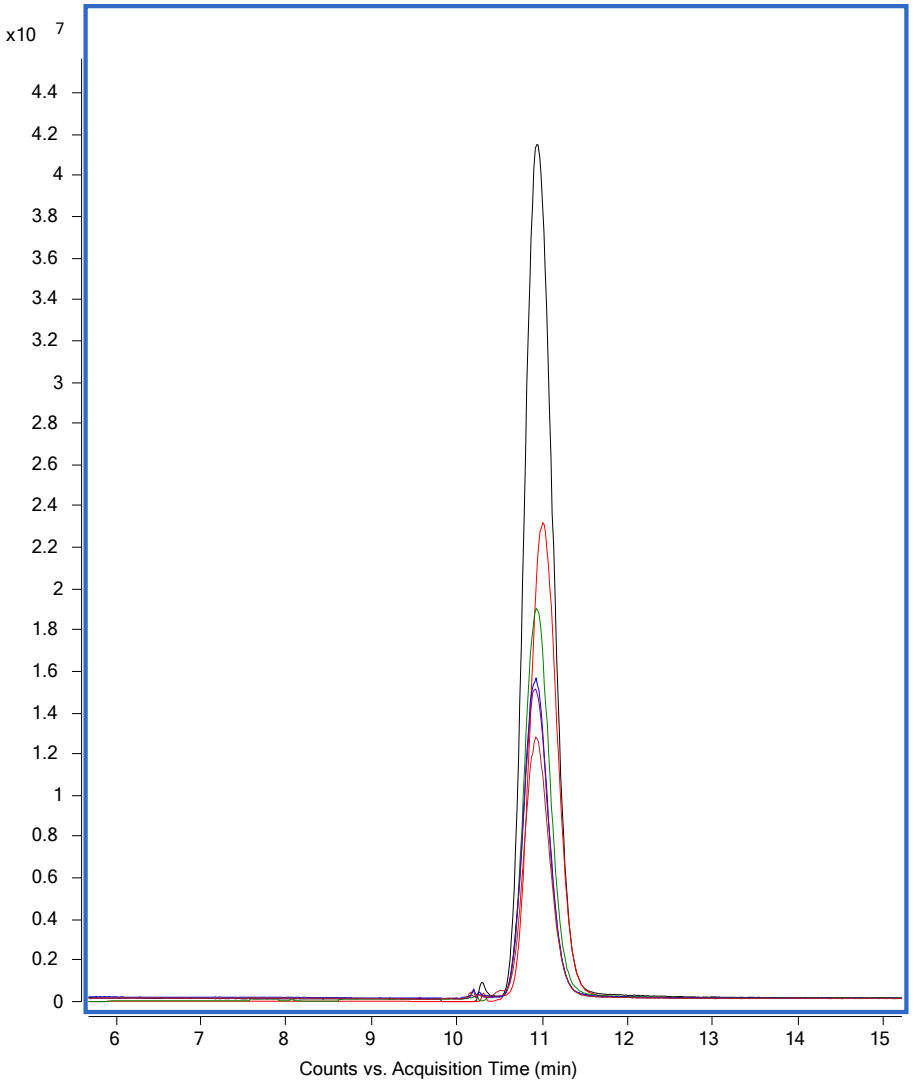
CE-MS results of Fc-fusion WT PK samples





Courtesy of Amgen Inc. South San Francisco PKDM group.

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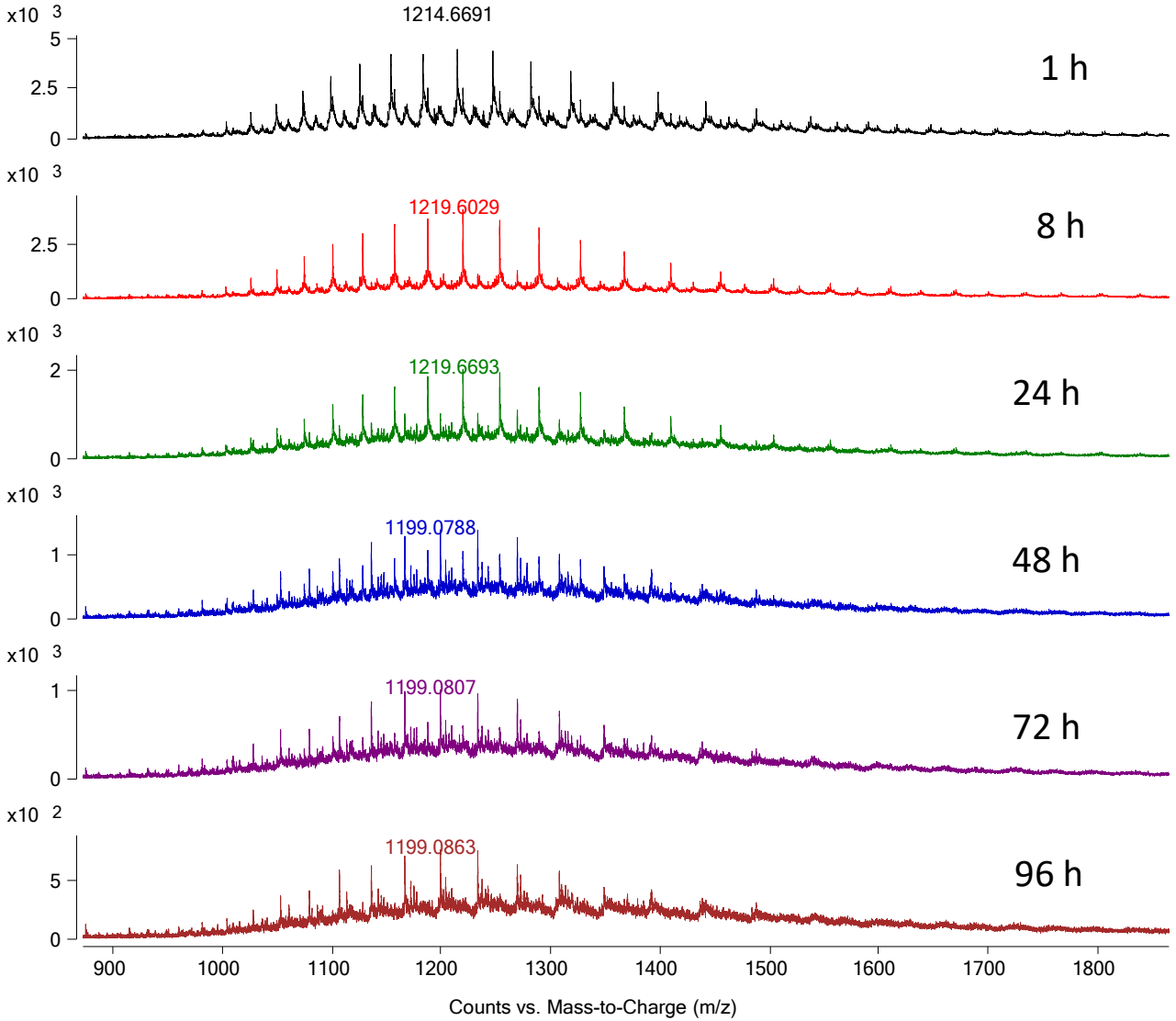
CE-MS results of Fc-fusion protein WT PK samples



Courtesy of Amgen Inc. South San Francisco PKDM group.

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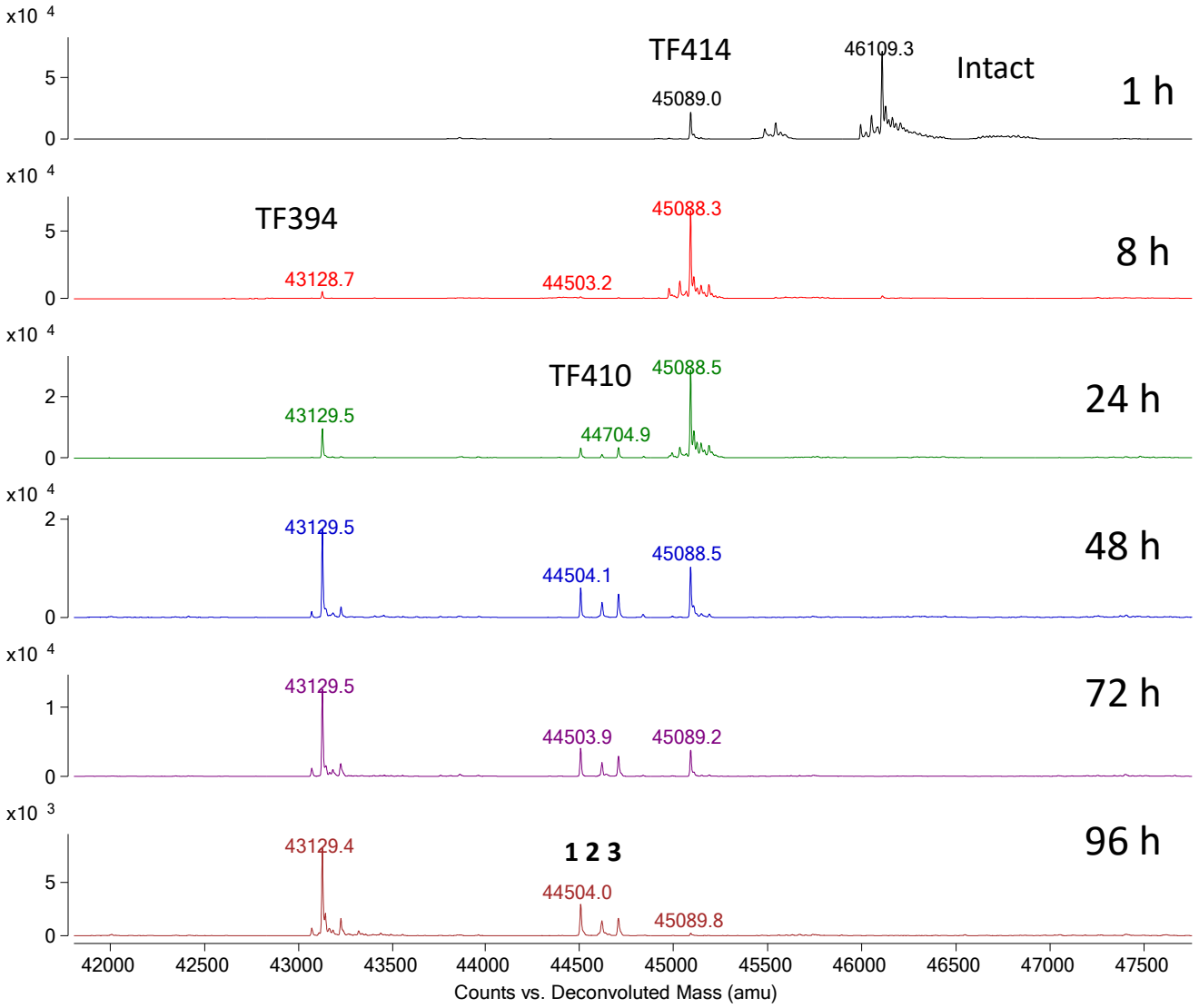
Identified species in Fc-protein WT PK samples



Courtesy of Amgen Inc. South San Francisco PKDM group.

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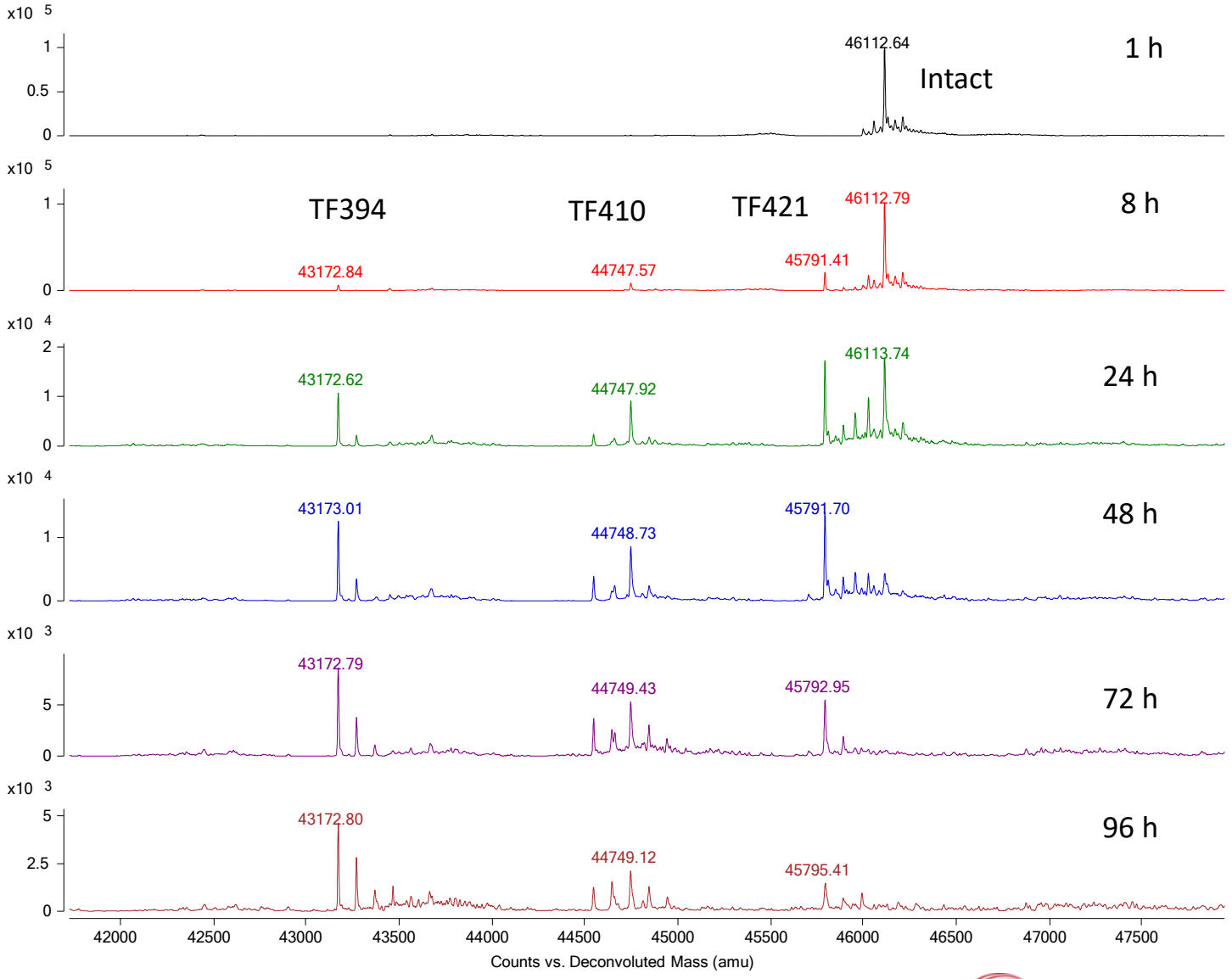
Identified species in Fc-protein RG mutation samples



Courtesy of Amgen Inc. South San Francisco PKDM group.

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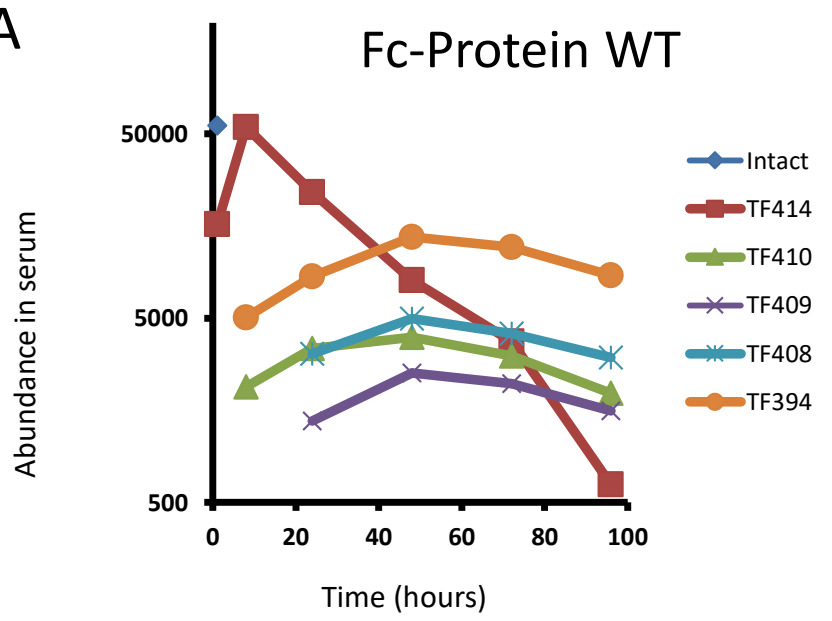
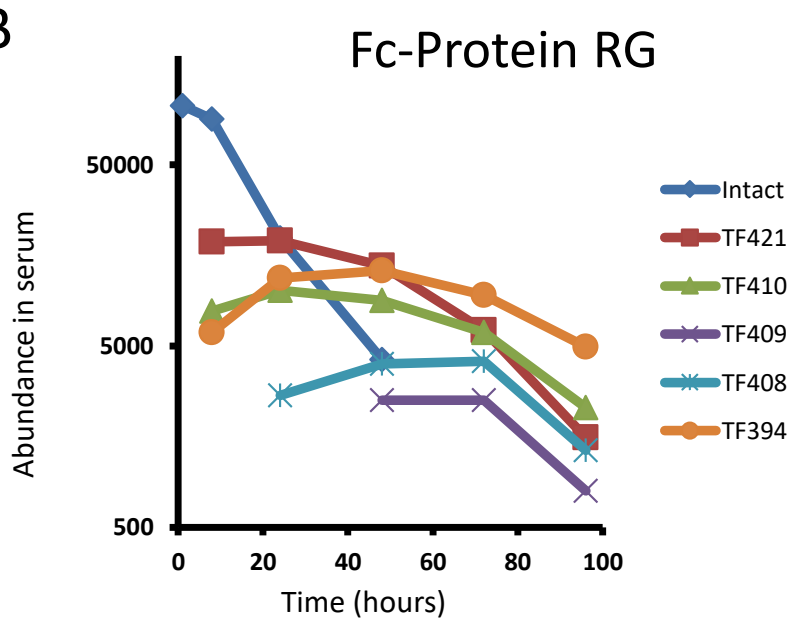
Fc-protein PK profiles plotted using peak abundance

A



B



Courtesy of Amgen Inc. South San Francisco PKDM group.

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Peptide Mapping of ADCs

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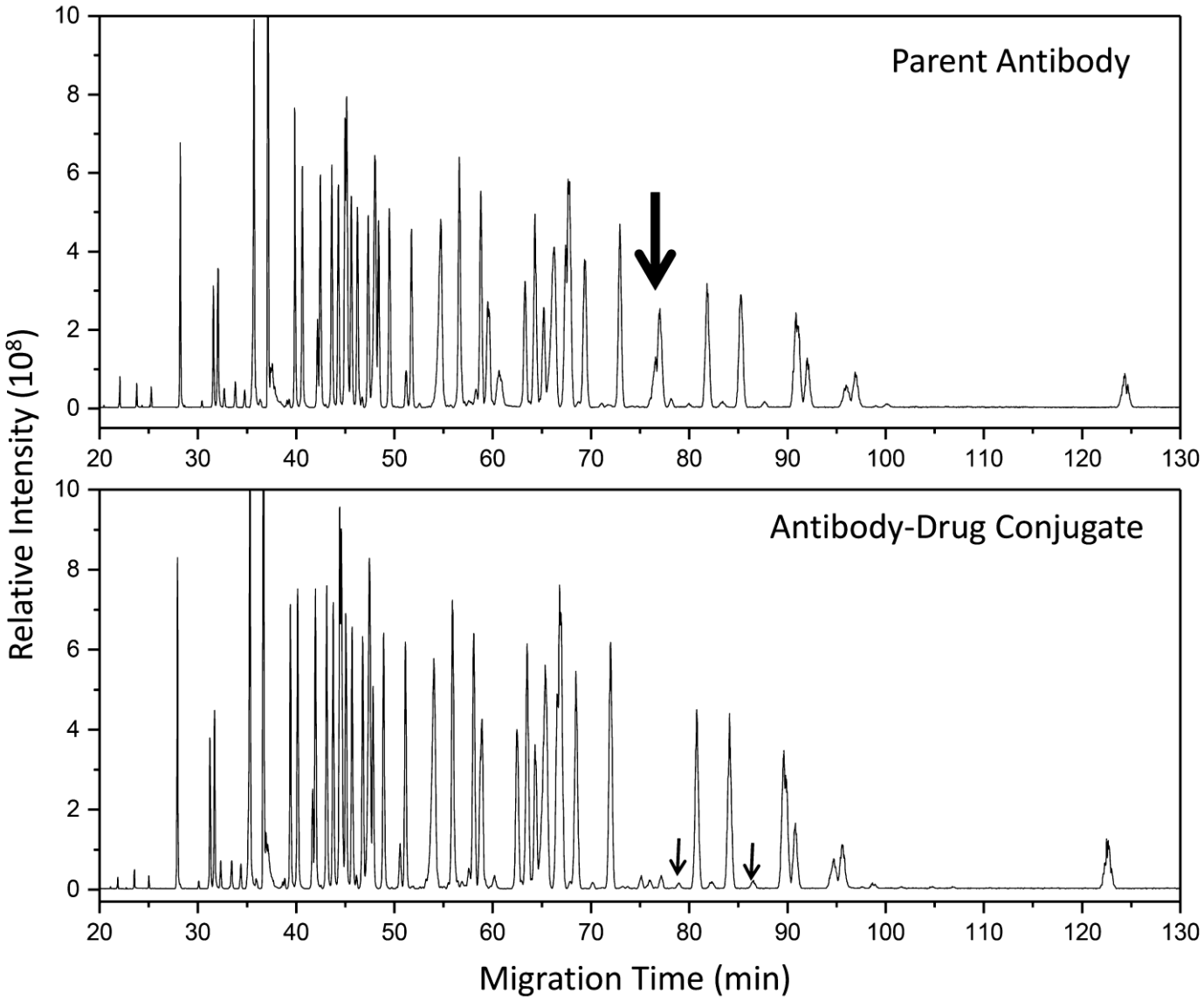
EMASS-II CE-MS Technology Enhances Peptide Mapping



2017. Analytical Chemistry. Oluwatosin Dada, et al. High-Resolution Capillary Zone Electrophoresis with Mass Spectrometry Peptide Mapping of Therapeutic Proteins: Peptide Recovery and Post-translational Modification Analysis in Monoclonal Antibodies and Antibody–Drug Conjugates

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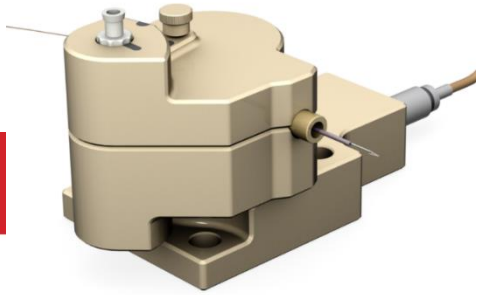
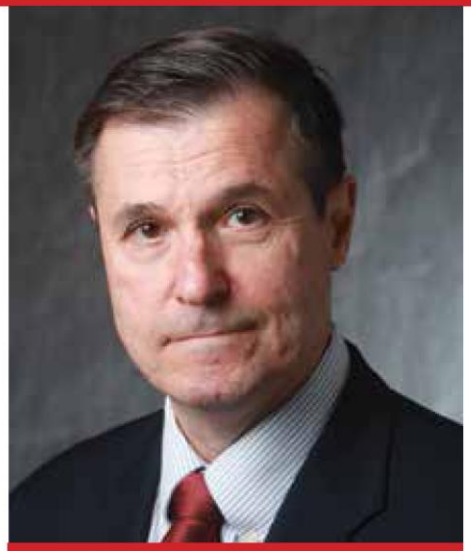
Other Applications



ask the expert



Professor Robert J. Linhardt

ASK THE EXPERT

DEVELOPMENTS IN CAPILLARY ELECTROPHORESIS MASS SPECTROMETRY (CE-MS) by Rachel Muenz

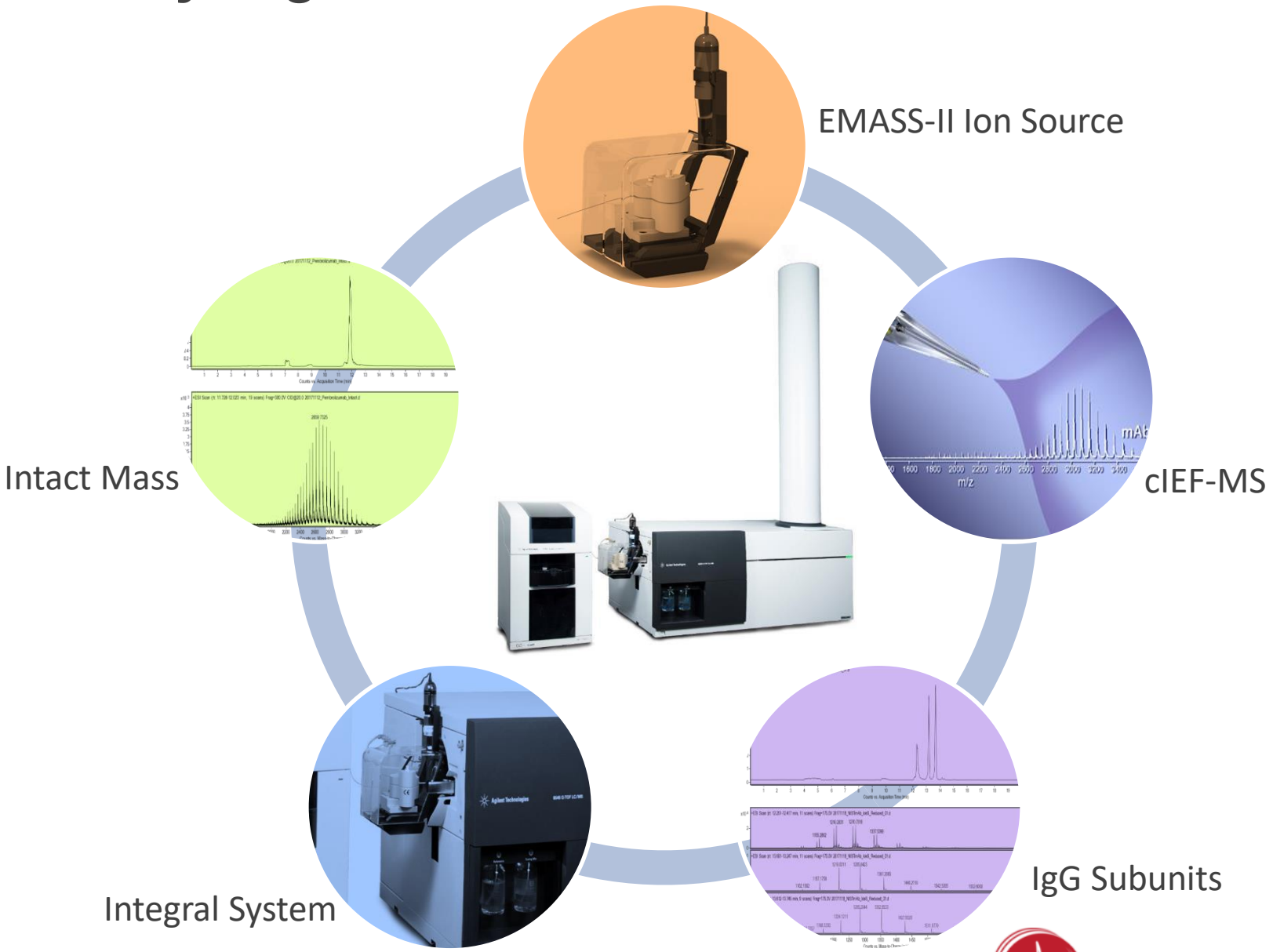
Professor Robert J. Linhardt received his PhD in chemistry from Johns Hopkins University in 1979 and did postdoctoral studies at MIT. He is the Anne and John Broadbent Jr. '59 Senior Constellation Chair in Biocatalysis and Metabolic Engineering at Rensselaer Polytechnic Institute. His research focuses on glycoscience and he is an expert on glycosaminoglycans. He has received multiple honors, including the ACS Isbell, Hudson, and Wolfrom Awards; the Volwiler Research Achievement Award; and the Scientific American 10. Dr. Linhardt is a fellow of the National Academy of Inventors, holds over 50 patents, and has authored over 800 research articles.

Q: Why is the interface so important?

A: Before this interface, it was very difficult to interface capillary electrophoresis and mass spectrometry. We think capillary electrophoresis is an excellent way to perform separations, but until we had a CE-MS interface that worked, we were unable to do these types of experiments.

http://www.labmanager.com/ask-the-expert/2017/09/developments-in-capillary-electrophoresis-mass-spec-ce-ms-#.W0V6ddJKiF4

Summary: Agilent-CMP CE-MS Workflow



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Discussion Period

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