MICHIGAN STATE UNIVERSITY

Automated and high-resolution clEF-MS methods for delineation of proteoforms and protein complexes

Michigan State University
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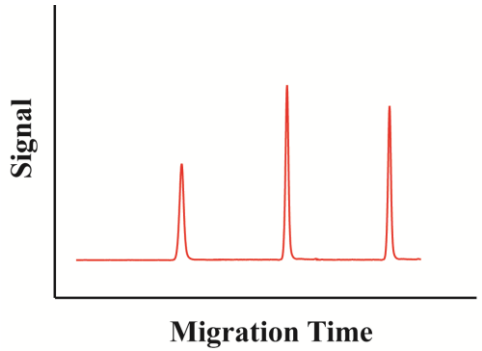
CE Pharm 2021

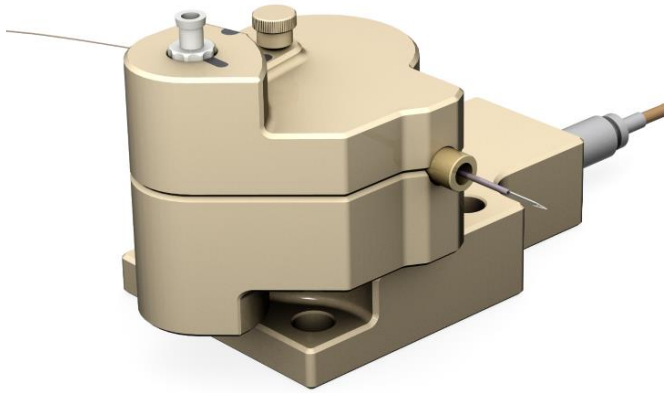
Capillary isoelectric focusing (cIEF)

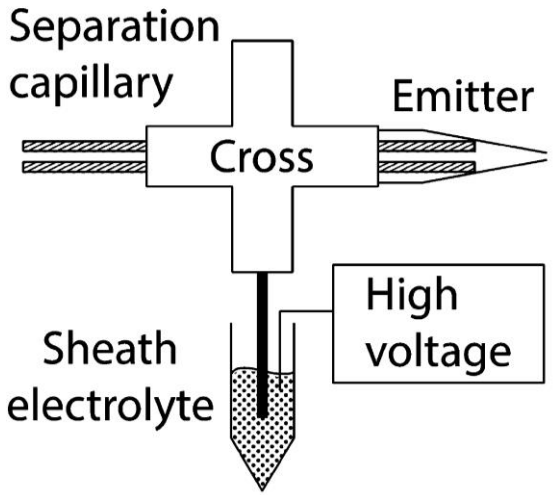


- Separation based on analytes' pIs
- 50-100 µm i.d. capillary with neutral coatings (linear polyacrylamide)
- Features
  - Ultrahigh resolution for protein separation (ΔpI: 0.001)
  - Concentrate and separate analytes simultaneously
  - High sample loading capacity (µL)
  - Accurate pI information of proteoforms

Automated cIEF-MS

EMASS-II CE-MS interface

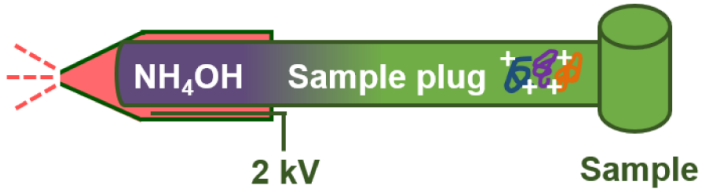




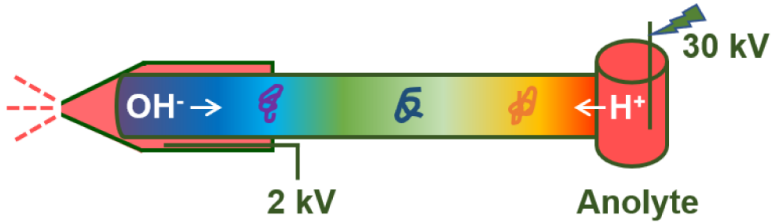
Automated cIEF-MS/MS method

"Sandwich" sample injection configuration

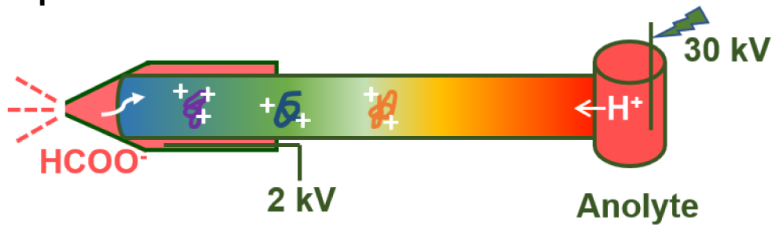
Step 1. Catholyte and sample injection



Step 2. Focusing based on isoelectric point

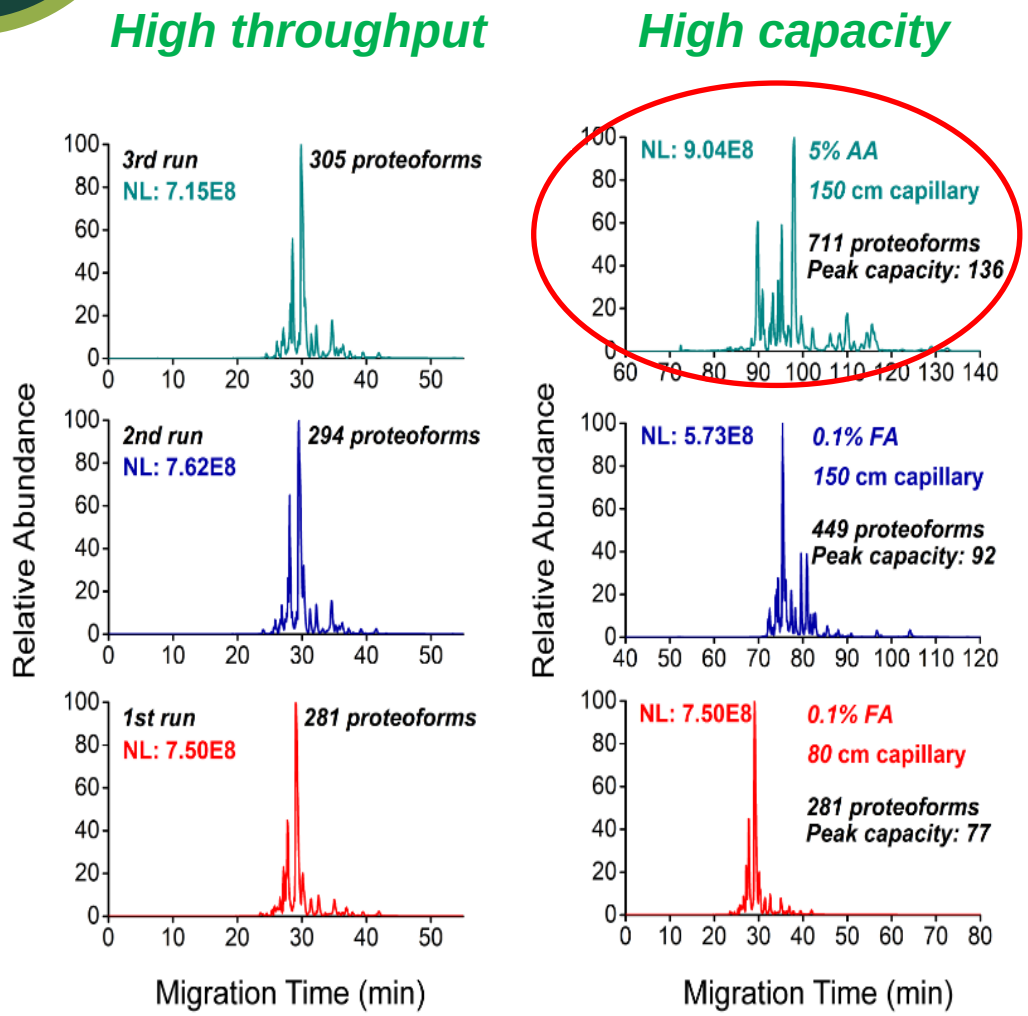


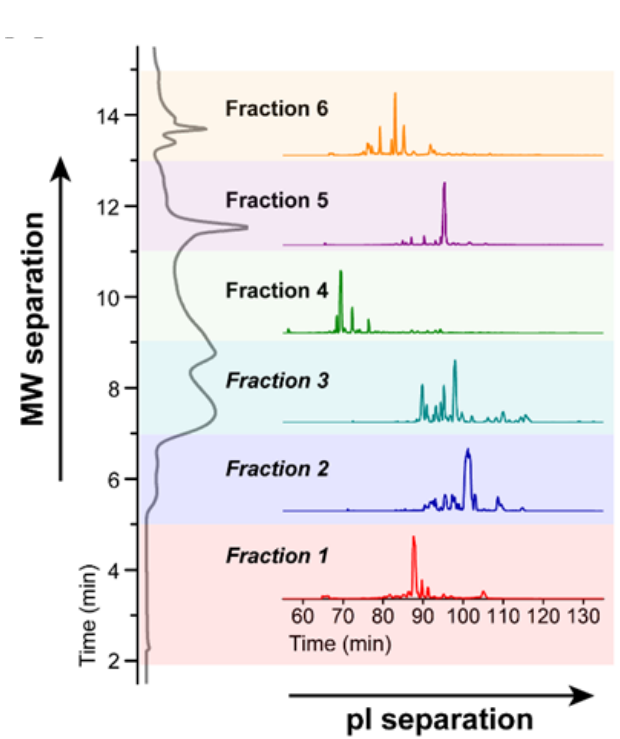
Step 3. Chemical mobilization

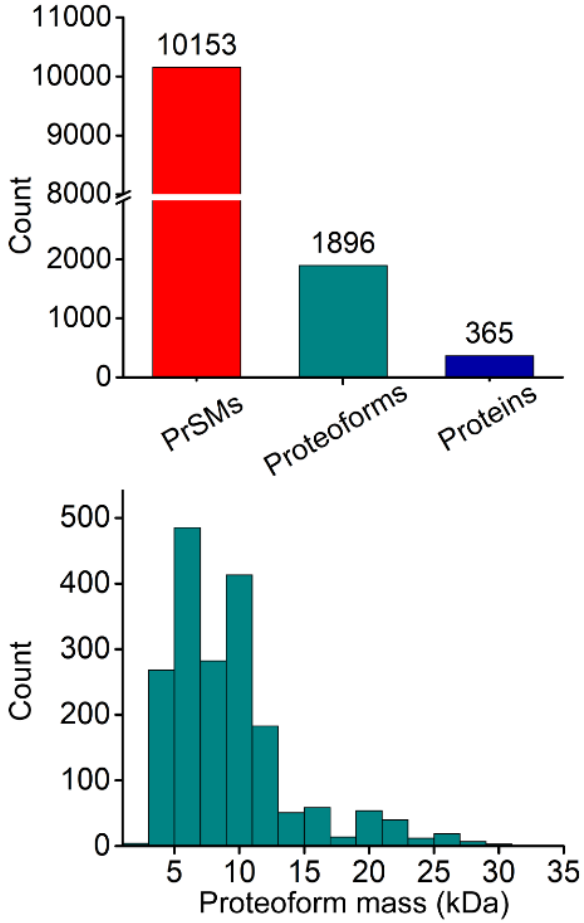


Sun, L., et al., 2015. Journal of proteome research, 14(5), pp.2312-2321. Tian *et al.* Anal Chem 2020, 92, 15890.
Image of EMASS-II CE-MS interface was adapted from CMP Scientific http://www.cmpscientific.com/

Automated cIEF-MS/MS for TDP of *E. coli*





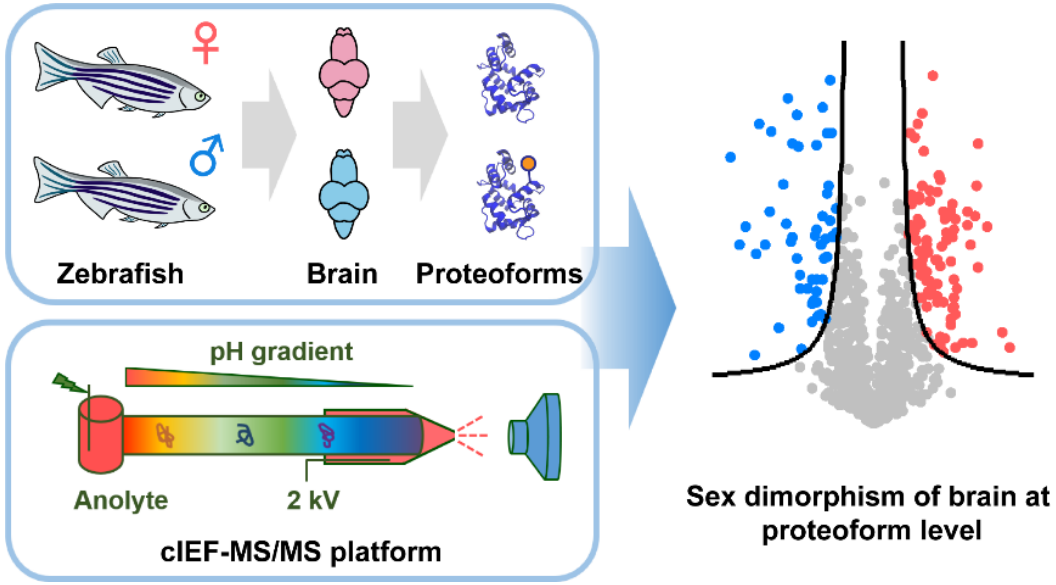


The data represents the first TDP dataset using cIEF-MS/MS

Tian *et al.* Anal Chem 2020, 92, 15890.

Quantitative TDP of zebrafish male and female brains

- Sexual dimorphism of brains: *the expression of sex chromosome genes and effects of hormones secreted from gonads*.
- **No TDP studies have been done to study the sex dimorphism.**
- Zebrafish is an important model organism in developmental biology for both embryogenesis studies and drug development.

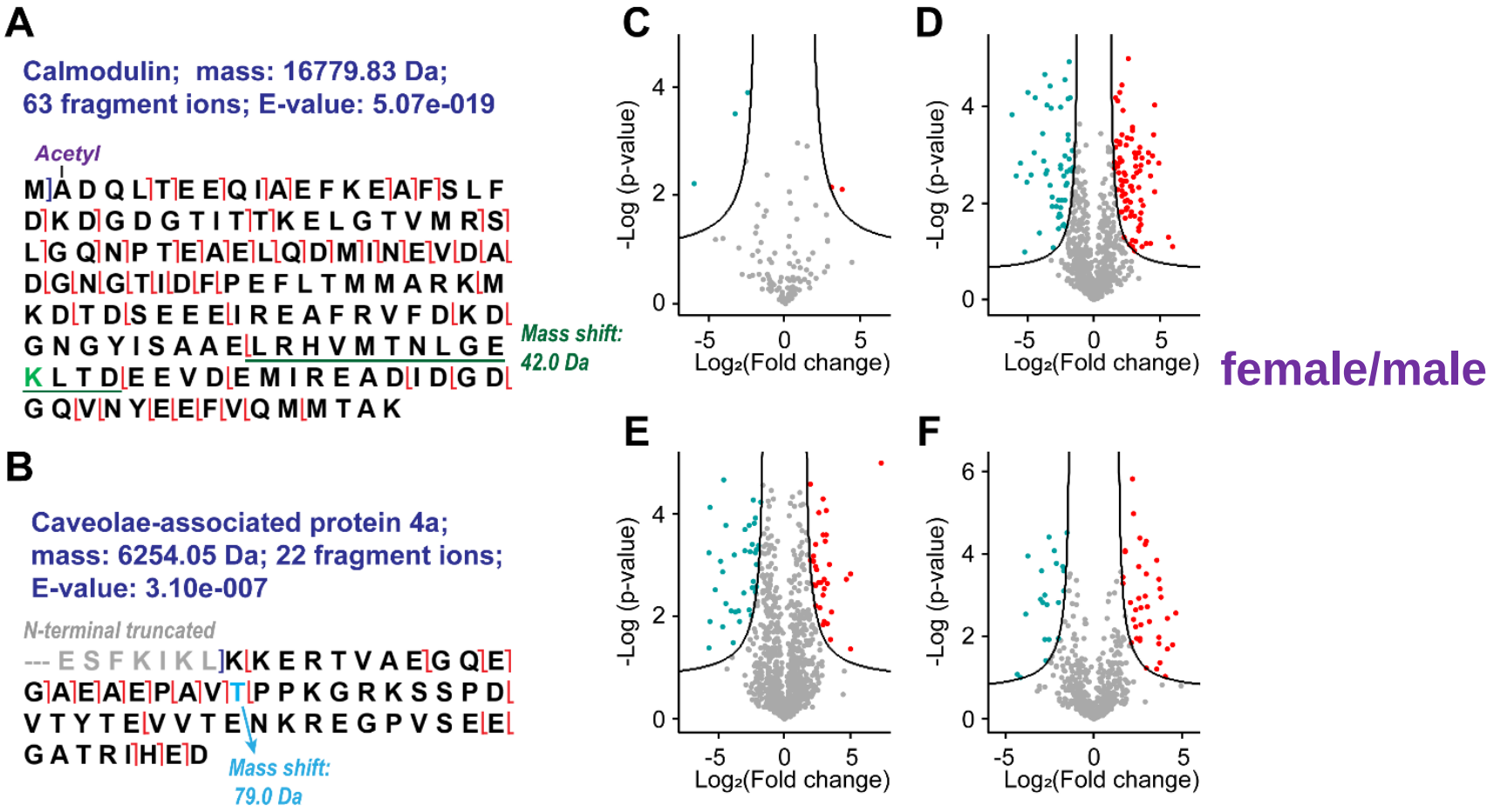


Label-free quantification (LFQ)

TopPIC software from
Dr. Xiaowen Liu's group
(Tulane University)

Tian *et al.* Anal Chem 2020, 92, 15890.

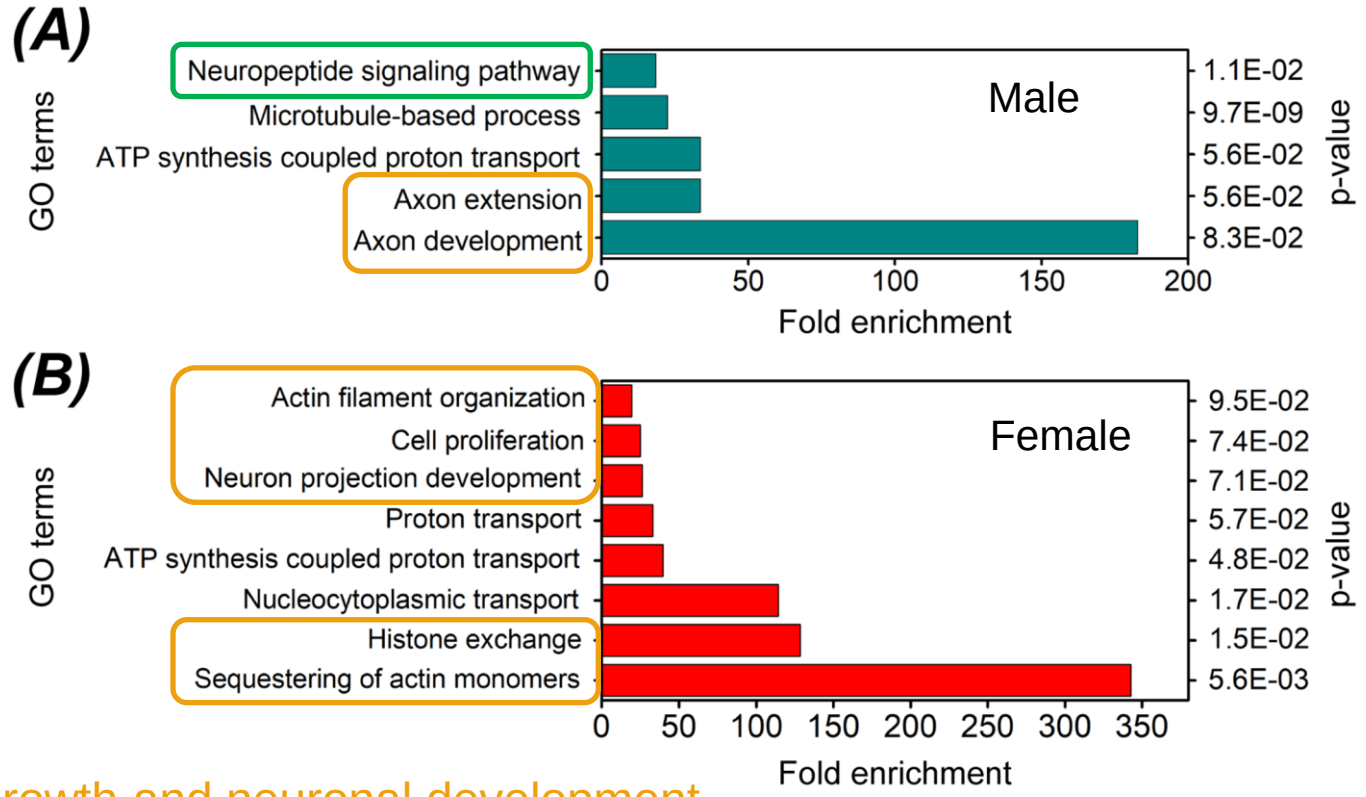
LFQ of zebrafish brain proteoforms



263 proteoforms showed statistically significant difference in abundance.

Tian *et al.* Anal Chem 2020, 92, 15890.

GO enrichment analysis of differentially expressed proteoforms



Peptide hormones

***POMC* and *PDYN* (pro-opiomelanocortin and prodynorphin)**

Neurite outgrowth and neuronal development

Automated cIEF-MS for monoclonal antibody (mAb) charge variants

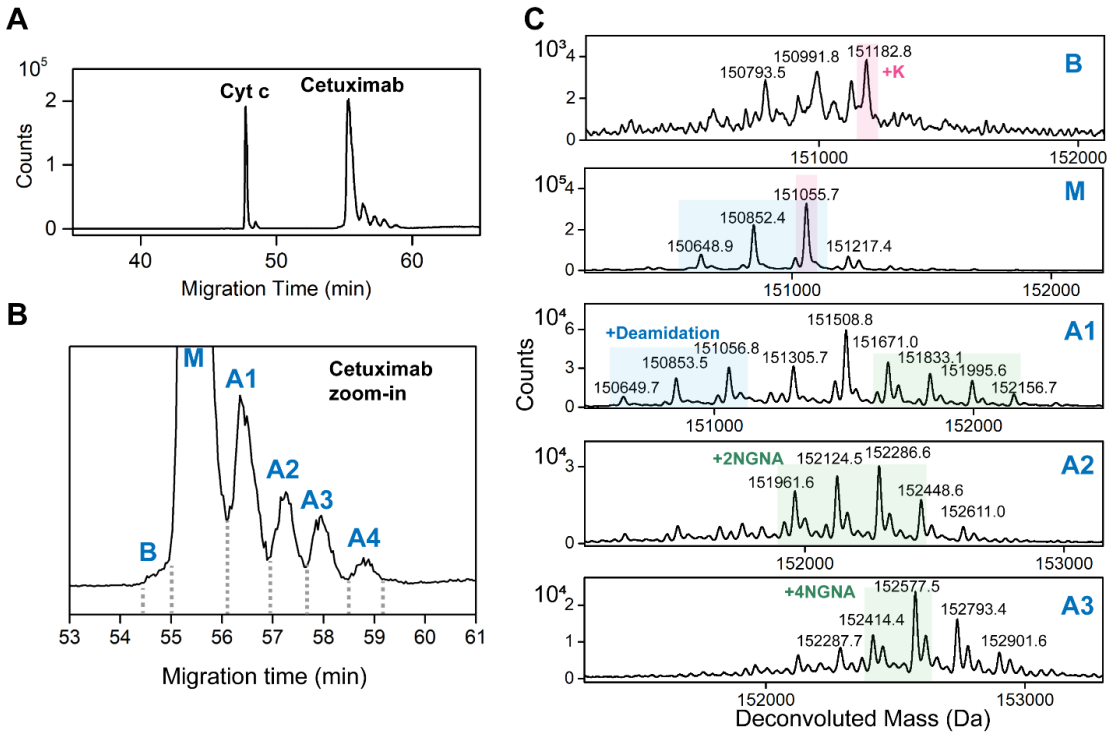
- cIEF-MS is a powerful tool for characterizing mAb charge variants.
  - High-resolution separation.
  - automated operation.
  - much better identification of charge variants than the traditionally used cIEF-UV.

- Further improvement in stability and separation resolution is needed.

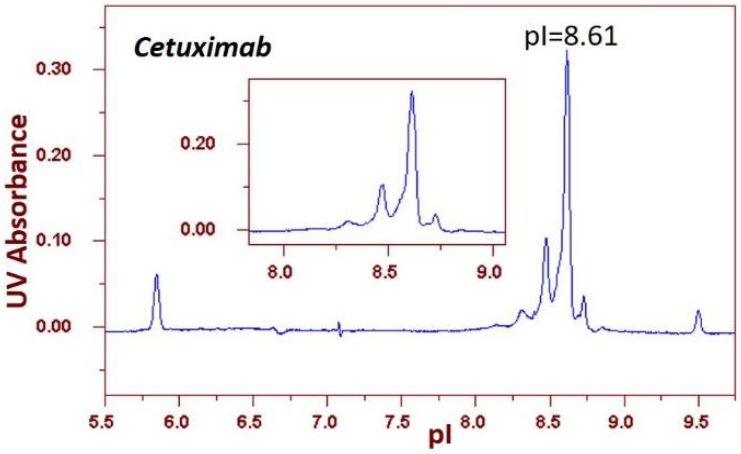
- Our work:
  - Bettered the quality of linear polyacrylamide (LPA) capillary coating.
  - Reduced catholyte pH to 10.
  - Systematically optimized the cIEF-MS separation conditions for characterizing of mAbs.

Charge variants of cetuximab

cIEF-MS



cIEF-UV



Cartridge: FC-Coated cIEF Capillary Cartridge, ProteinSimple Catalog No. 101701. Pharmalyte: Pharmalyte 3-10. Final prepared sample: 0.8 M Urea, 0.28% methyl cellulose, 4% carrier ampholytes, 0.2% pI 5.85 marker, 0.2% pI 9.50 marker, ~1.0 mg/mL protein. Focusing period 1: 1500V for 1 min; Focusing period 2: 3000V for 8 min. UV detection wavelength: 280 nm. System: iCE3 (ProteinSimple).

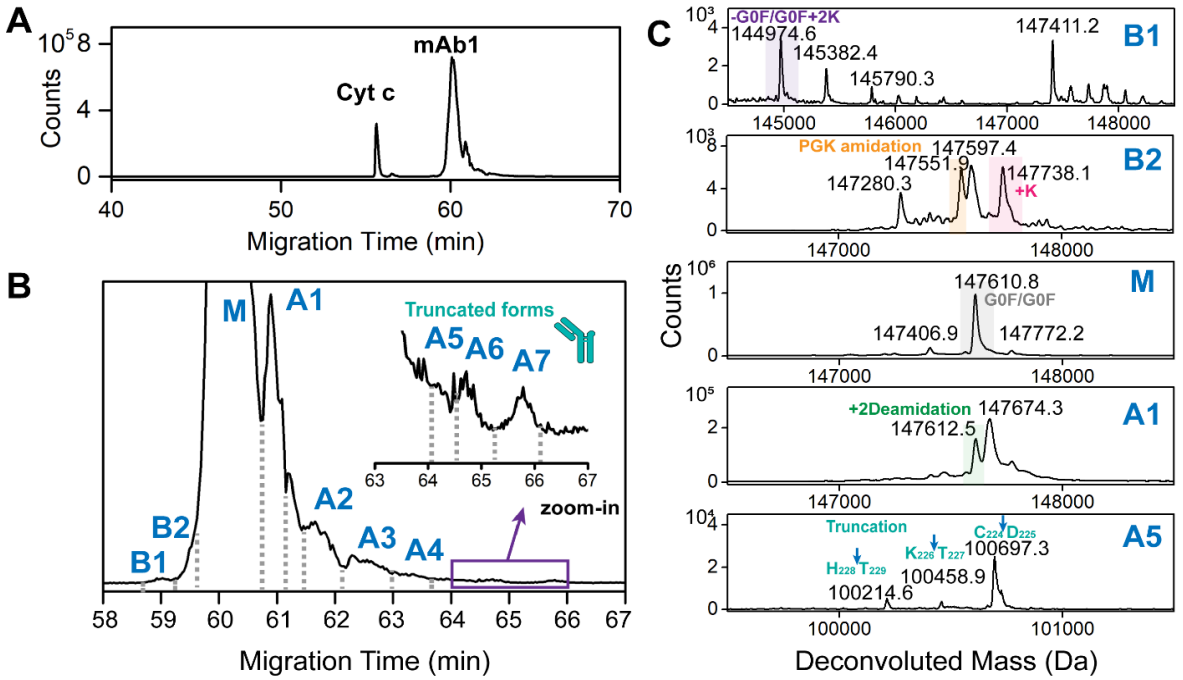
N-glycolyl neuraminic acid (NGNA)

Parameters for cIEF: 75 cm LPA-coated capillary, 30 cm catholyte plug, 45 cm sample plug, 0.8 mg/mL cetuximab, 0.05 mg/mL cytochrome c, 2% three-ampholyte mixture (pH range of 3-10, 5-8 and 8-10.5, ratio 1:2:4), 20 kV separation voltage, 10 mbar pressure applied at 20 min.

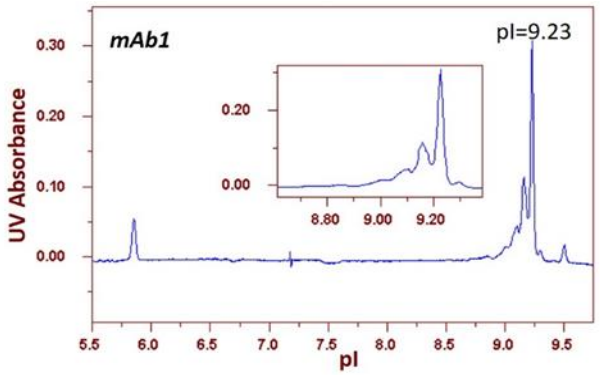
Unpublished data

Charge variants of mAb1

cIEF-MS



cIEF-UV



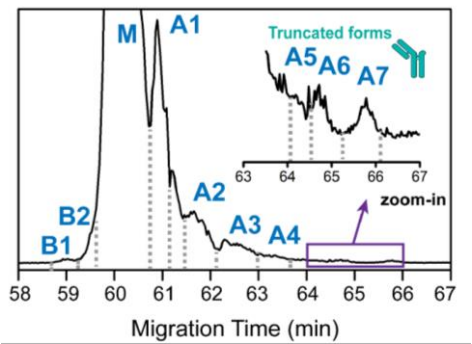
- Discovered ten charge variants with PTMs: PGK amidation, incomplete C-terminal lysine clipping, glycosylation, and deamination.
- Achieved much more complete analysis than the cIEF-UV.

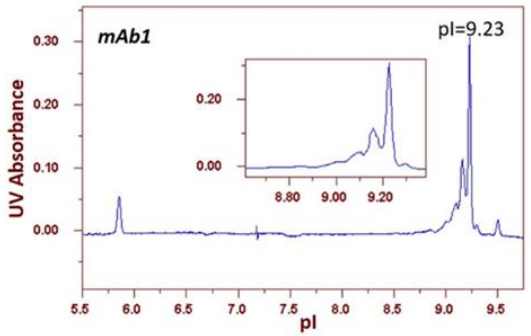
Unpublished data

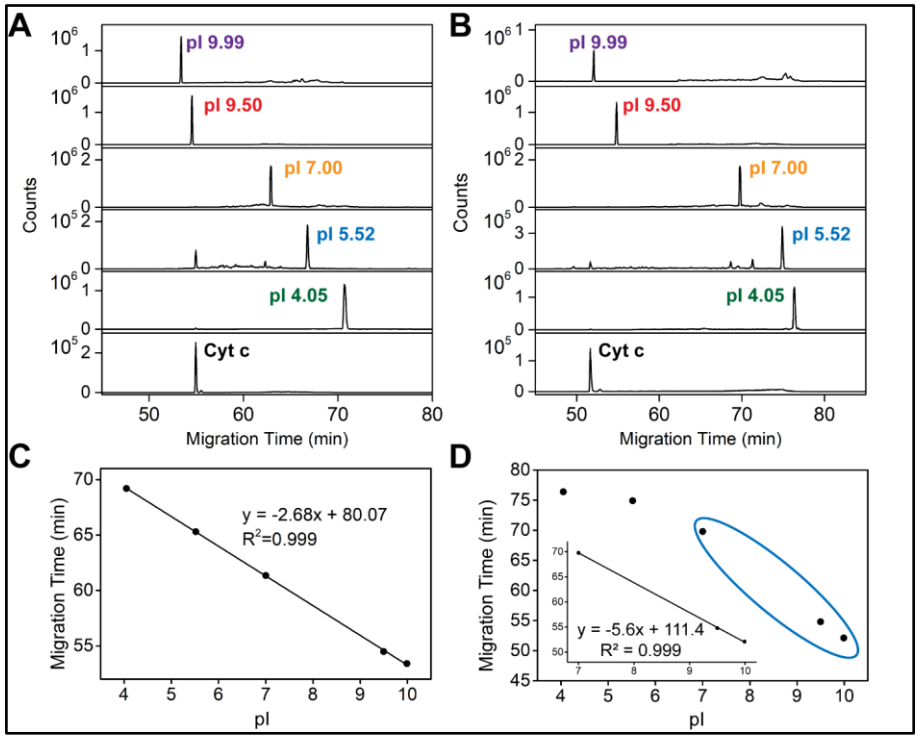
pI determination of mAb1 charge variants

	Data 1			Data 2			Data 3				
	t1	t2	pI	t1	t2	pI	t1	t2	pI	Mean	SD
M	59.6	57	9.15	53.3	56.9	9.17	56.3	56.9	9.17	9.16	0.01
A1	60.5	57.9	9	54.2	57.8	9.01	57.2	57.8	9.01	9.01	0.01
A2	61.4	58.8	8.85	55.2	58.8	8.85	58.2	58.8	8.85	8.85	0.00
A3	62.1	59.5	8.73	56	59.6	8.71	59	59.6	8.71	8.72	0.01

Main peak pI:
9.23 (UV) vs. 9.16 (MS)



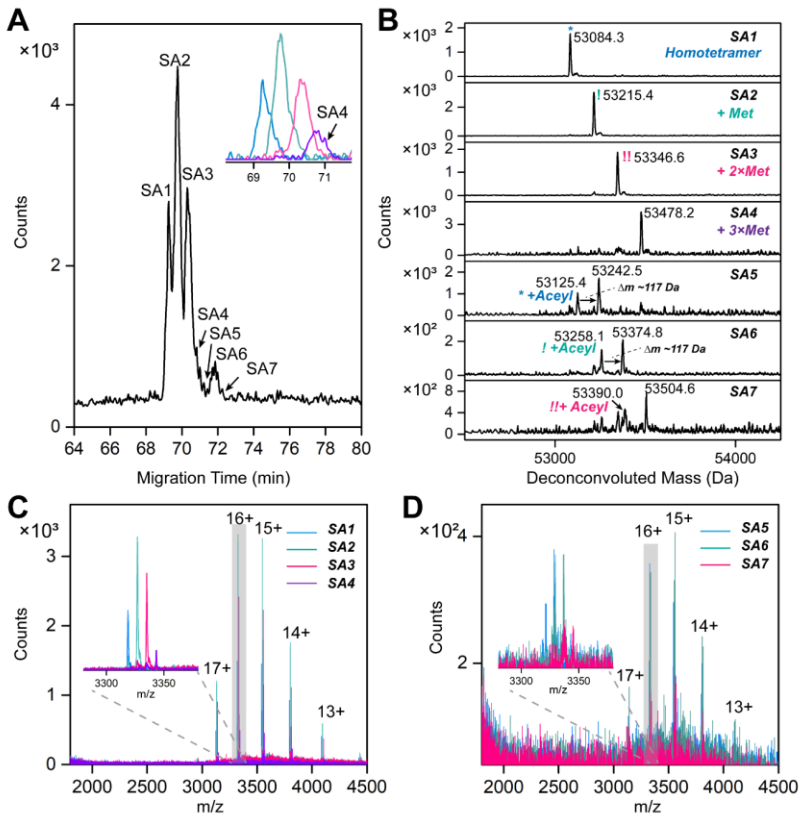




- Achieved reproducible measurements of pIs of charge variants (cyto.c for normalization)
- UV and MS data agreed well.
- Provide a useful approach for studying how PTMs influence pIs of proteoforms in TDP.

Unpublished data

Automated and native cIEF-MS for protein complexes



- Streptavidin (SA): Homo-tetramer
- High-resolution separations of seven proteoform complexes of SA.
- Determined the pIs of SA homo-tetramers and studied how sequence variations and acetylation influence the pI of SA homo-tetramer.
- Provide a useful tool for native top-down MS delineation of protein complexes.

Collaboration with Abbvie (Dr. Linjie Han)

SA variants	Normalized migration time (min)			pI			pI
	Run#1	Run#2	Run#3	Run#1	Run#2	Run#3	(Mean±STD)
SA1	62.82	62.60	62.70	7.36	7.36	7.40	7.37±0.02
SA2	63.09	62.84	62.90	7.26	7.29	7.33	7.29±0.03
SA3	63.38	63.26	63.22	7.16	7.16	7.22	7.18±0.03
SA4	63.67	63.43	63.50	7.07	7.06	7.12	7.08±0.04
SA+acetylation	64.14	63.73	63.90	6.90	6.94	6.99	6.94±0.04

Unpublished data

Acknowledgments

- **Advisor:**
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- **Collaborator:**
  Dr. Linjie Han (Abbvie)

- **Group members:**
  - **Qianjie Wang**
  - **Qianyi Wang**
  - **Jorge Colonrosado**
  - **Fei Fang**

Support: