

**biotechne**<sup>®</sup>

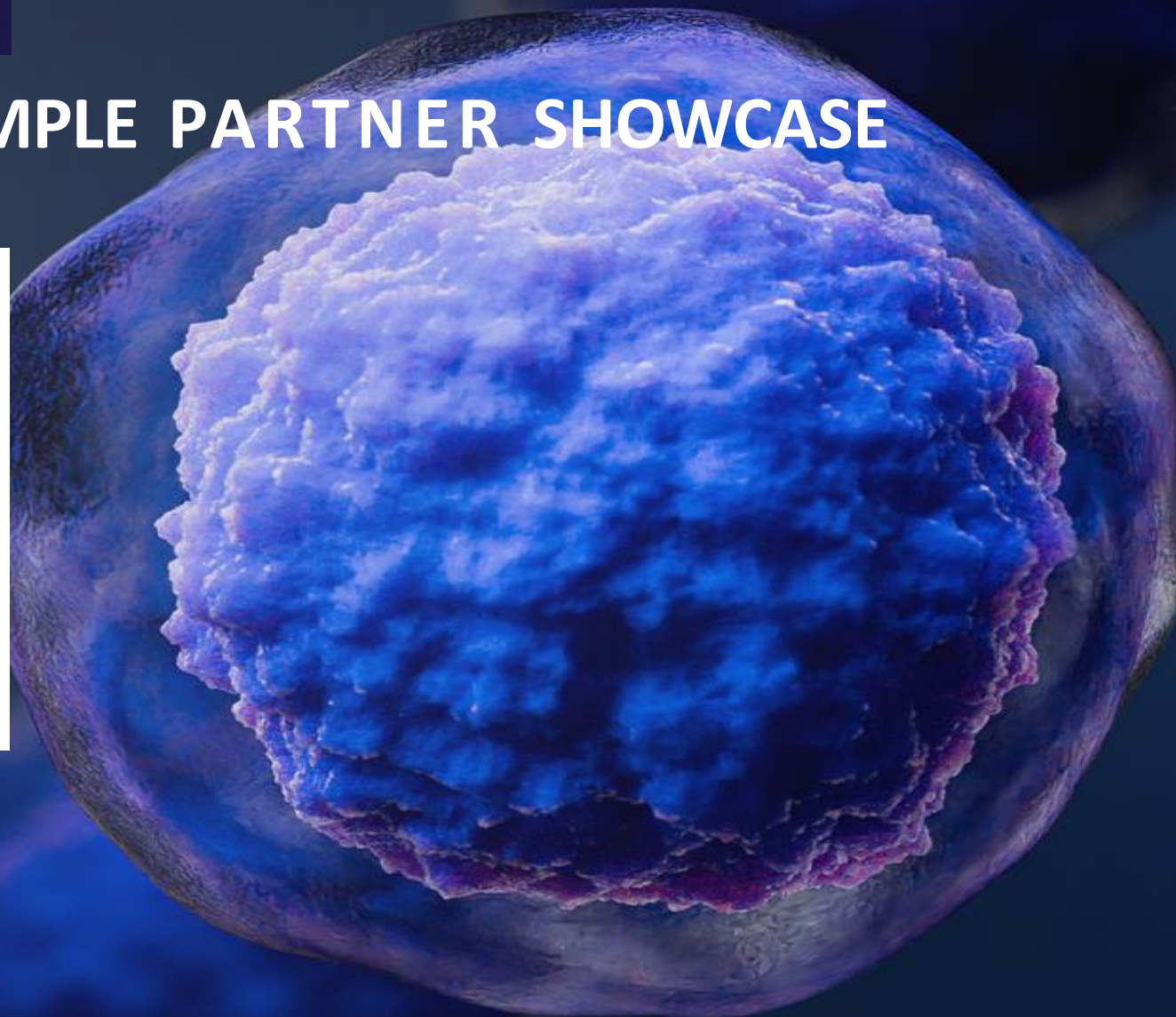
# PROTEINSIMPLE PARTNER SHOWCASE



CELEBRATING TWENTY-FIVE  
YEARS *of* ICE INNOVATION

Ed Chase, Senior Director

CE Pharm 2024



# CELEBRATING YEARS OF ICE INNOVATION

*Thank you....*



- for your support and friendship
- for making the iCE the gold standard for cIEF
  - The technology
  - Superior application support (Jiaqi, Chris, Will)
  - iCE Platform method – 2006 Xiaoping He, Pfizer

# CELEBRATING YEARS OF ICE INNOVATION

Test Bed 1997



First Sale 1999 Coulter



# CELEBRATING YEARS OF ICE INNOVATION



**1999**

- 2005 CE Pharm 2 iCE podium presentations
- 2006 1<sup>st</sup> iCE platform assay presentation (Xiaoping He)
- 2006 iCE280 gold standard for charge



**2012**

- iCE3 replaces iCE280
- Improved reproducibility *plus* Alcott and Prince autosampler improvements



**2016**

- Maurice adds CE-SDS ... Turbo cartridge 5X speed
- Adds icIEF Native fluorescence (4x Sensitivity)
- Empower and Chromeleon
- **Platform instrument technology for future needs**



**2023**

- MauriceFlex - Full Maurice capabilities (icIEF + CE-SDS)
- Adds icIEF charge variant fractionation
- Offline for peptide mapping, etc.
- MS agnostic - use LC or ZipChip as an MS interface

© 2024 Bio-Techne®. All rights reserved.

© 2024 Bio-Techne®. All rights reserved.

# CELEBRATING YEARS OF ICE INNOVATION

*Technical Seminar: Wednesday at noon*

Recent Applications and Collaborations using the MauriceFlex Platform,  
including a new Rapid icIEF method  
Presenter: Chris Heger, ProteinSimple



icIEF fractionation of an ADC by MauriceFlex and Characterization of  
Charge Variants  
Presenter: Amreen Jonas, Mersana Therapeutics



Evolution of the J&J Capillary Isoelectric Focusing Platform Technology  
from the iCE3 to the Maurice  
Presenter: Chris Cammarata, Johnson & Johnson



# CELEBRATING YEARS OF ICE INNOVATION

## *Scientific Program Presentations*

Charge Variant Microheterogeneity Explored via icIEF for the Analysis of Complex Biopharmaceuticals and Biosimilarity Assessment

Presenter: Sara Carillo, NIBRT

Wednesday September 18 @1:45 pm – 2:10 pm

icIEF-MS Characterization of Charge Isoforms for Biotherapeutic Products

Presenter: Xiaoping Z. He, Pfizer

Wednesday September 18 @3:00 pm – 3:25 pm

# CELEBRATING YEARS OF ICE INNOVATION

## *Poster Presentations Tuesday and Wednesday*

Evaluation of preparative icIEF with MS offline coupling for the analysis of charge variants of monoclonal antibodies

Antonia Wittmann, Yannick Wilke, Hermann Wätzig // TU Braunschweig, Braunschweig, Germany

Presenter: Antonia Wittmann

Evaluation of iCE3 and Maurice Comparability in Support of the iCE3-to-Maurice Transition for Monoclonal Antibody Charge Variant Analysis

Presenter: Huilin Yang // Regeneron, Tarrytown, NY, USA

Optimizing a Charge Variant Method for an Engineered Antibody-Like Molecule 1: Overcoming Challenges to a Highly Basic pI Molecule and cIEF Method-Induced Changes

Misa Mai, Deepika Gollapudi, Chendi Niu, Xiaoyu Chen // AstraZeneca, Gaithersburg, MD, USA

Presenter: Misa Mai

# CELEBRATING YEARS OF ICE INNOVATION

## *More Poster Presentations*

Charge Variant Fractionation of Biotherapeutics Using MauriceFlex™

Jiaqi Wu, Srinivasa Rao, Will McElroy, and Chris Heger // ProteinSimple, a Bio-Techne brand, San Jose, CA 95134

Presenter: Jiaqi Wu

Development of the SupersonicIEF™ Method for the Maurice™ Platform

Will McElroy, Chris Heger // ProteinSimple, a Bio-Techne brand, San Jose, CA 95134

Presenter: Will McElroy

Therapeutic Protein Charge Variant Characterization with Intact Mass & Peptide Mapping Following Microgram Preparative Capillary Isoelectric Focusing Electrophoresis Fractionation

Thomas Menneteau<sup>1</sup>, Claire Butré<sup>1</sup>, Géry Van Vyncht<sup>1</sup>, Arnaud Delobel<sup>1</sup>, Marion Galaup<sup>2</sup>, Kristell Lebozec<sup>2</sup>, Laurence Talbot<sup>3</sup>, Kefei Wang<sup>4</sup> // 1Quality Assistance, Thuin, Belgium; 2Acticor Biotech, Paris, France; 3Bio-Techne, Noyal Châtillon sur Seiche, France; 4Bio-Techne, San Jose, CA, United States

Presenter: Kefei Wang



# CELEBRATING YEARS OF ICE INNOVATION

## Application Notes – 12 months

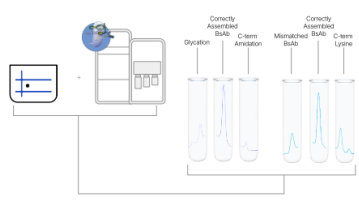
- Comparing Charge Variants: Innovator vs Biosimilar Using the MauriceFlex System & Mass Spectrometry
- Optimize Your icIEF Fractionation Method on the MauriceFlex System with Simple Western pI Markers
- icIEF and CE-SDS protocol for Mosunetuzumab
- Streamlining Data Analysis: Introducing the Maurice Chromeleon Driver Kit
- Is That Milk? Use CE-SDS on Maurice and Find Out!
- AAV Thermostability Assessment with Micro-Flow Imaging

**biotechne** In collaboration with **GENOVS**

### Comparing Charge Variants: Innovator vs Biosimilar Using the MauriceFlex System & Mass Spectrometry

**Abstract**

This application note demonstrates an efficient workflow for the characterization of charge variants of a therapeutic bispecific antibody (BsAb) and a research-grade biosimilar. Imaged capillary isoelectric focusing (icIEF) on the MauriceFlex™ system was used to separate and collect various charge isoforms, which were treated with FABRICATOR® for further digestion prior to LC-MS analysis. A comparison of the results highlighted critical differences between the innovator and biosimilar, including the presence of incorrectly paired species.



**biotechne** Protocol

### Mosunetuzumab

**Introduction**

Mosunetuzumab (brand name Lunsumio™) is a bispecific antibody (BsAb) used to treat follicular lymphoma. It binds to two different targets: CD20 on B-cells and CD3 on T-cells. The simultaneous binding of Mosunetuzumab to two different targets ultimately leads to the death of cancerous B-cells. Developed by Genentech, Mosunetuzumab was approved as a therapeutic by the FDA in December 2022.

**Maurice icIEF Method**

**Sample Preparation**  
The innovator drug was procured from Genentech. The Mosunetuzumab biosimilar (MOSUN) was procured from IchorBio. The samples were diluted to a final concentration of 0.1 mg/ml in the sample solution.

**Ampholyte solution**  
BRT (Broad Range 3 to 7)  
N2 (Narrow Range 8 to 10.5)  
5 mM arginine  
These reagents are provided in the Maurice icIEF Method Development Kit (CE-MSD501-1).

**pI markers**  
705 (PIL0101-010)  
95 (PIL0101-017)

**Running conditions**  
70 minutes at 1200 V then 12 minutes at 3000 V.

**Imaging**  
Absorbance and native fluorescence.

**Data Analysis**  
Compass for ICE, Version 4.0.0.

**Results**

Figure 1 shows representative electropherograms of the innovator and biosimilar, detected with absorbance using the Maurice icIEF method. The acidic, main, and basic peaks are clearly separated, with their percent peak area and reproducibility captured in Table 1.

Similarly, native fluorescence (NF) detection of the samples is shown in Figure 2, with the results listed in Table 2. Advantages of NF on the Maurice system include 6X higher sensitivity than absorbance, therefore suitable for analyzing lower amounts of analyte, and generating quieter baselines, which simplify method optimization and transfer!



**biotechne** Application Note

### Streamlining Data Analysis: Introducing the Maurice Chromeleon Driver Kit

**Table of Contents**

Introduction	1
Material and Methods	2
Results	3
icIEF	4
Turbo CE-SDS	6
CE-SDS Plus	8
Conclusion	10

**Introduction**

The new Maurice Chromeleon Driver Kit gives you direct control of your Maurice™ system with Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS). Whether you need a centralized data management system for your lab or are simply looking for familiar software for your Maurice instrument, you can now collect and analyze imaged icIEF (icIEF) and CE-SDS data using Chromeleon CDS.

To demonstrate the power of the Maurice Chromeleon Driver Software, this application note compares results between Compass for ICE, the Maurice system's default software, and Chromeleon CDS. For both icIEF (also referred to as "icIEF" interchangeably in this document) and CE-SDS, data are presented on the following quantitative aspects:

- Reproducibility
- Linearity
- Limit of detection (LOD)

**Benefits of the Maurice Chromeleon Driver Kit:**

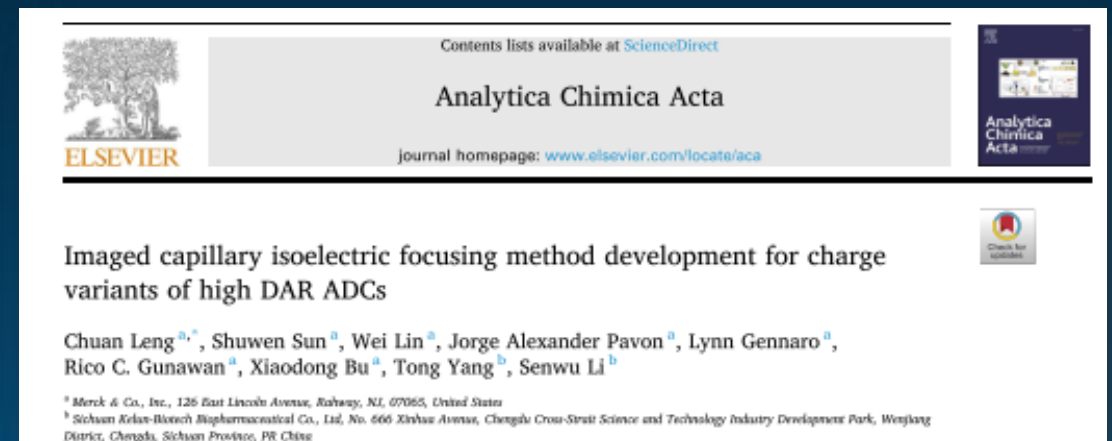
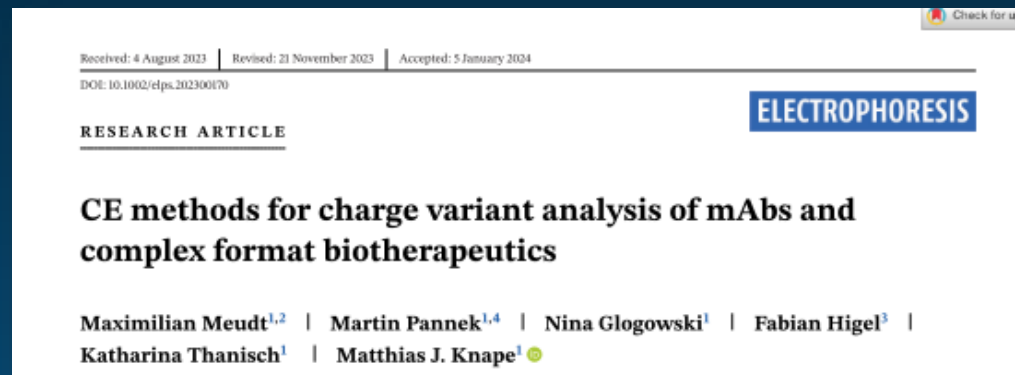
- You can now collect and analyze icIEF and CE-SDS data from the Maurice system using Chromeleon CDS.
- Data collected and analyzed on Chromeleon software is compatible to Compass for ICE.
- The Maurice Chromeleon Driver Software offers benefits such as pH recalibration, 21 CFR Part 11 compliance, data integrity, and seamless integration across labs.



# CELEBRATING YEARS OF ICE INNOVATION

## *Publications – 12 months*

- Imaged capillary isoelectric focusing method development for charge variants of high DAR ADCs
- Analytical Characterization for Similarity Assessment Between an Aflibercept Biosimilar SB15 and Reference Product (Eylea®)
- Characterizing Glycosylation of Adeno-Associated Virus Serotype 9 Capsid Proteins Generated from HEK293 Cells through Glycopeptide Mapping and Released Glycan Analysis
- Plant-Based Antibody Manufacturing
- CE methods for charge variant analysis of mAbs and complex format biotherapeutics



# CELEBRATING YEARS OF ICE INNOVATION

## *Applications Science Activities since 2019*

- 7 Peer-reviewed CE publications (5 published, 2 submitted, 1 in preparation)
- 1 Peer-reviewed CE review article
- 2 CE Book Chapters submitted
- 3 CE Patents – also invented SimpleSol™
- 62 Webinars and Tech Talks
- 50 Application Notes
- 28 CE Protocols
- 80 Conference Posters
- 1 CEPHARM Lifetime Achievement Award (Jiaqi Wu, Ph.D.)

# CELEBRATING YEARS OF ICE INNOVATION

## *The ProteinSimple team at CE Pharm*

- Chris Heger Director, Applications Science
- Ed Chase Senior Director, Strategic Solutions
- Jacklyn Huynh Senior Tradeshow & Event Specialist
- Jiaqi Wu Principal Scientist
- Kefei Wang Senior Product Manager
- Matilda Kapidani Biologics Sales Specialist San Diego
- Sherry Gregory Senior Sales Manager
- Will McElroy Staff Scientist, Applications Science

# CELEBRATING YEARS OF ICE INNOVATION

## *Speaking of Jiaqi*

- Co-inventor of iCE technology
- CE Pharm Lifetime Achievement Award
- Optimized assay conditions  
2005: 250 2017: 3,000 today: ??
- AKA the Godfather, the Chinese Elvis



CELEBRATING  YEARS OF ICE INNOVATION

***THANK YOU!***

Please stop by our booth  
and  
say hi to the team