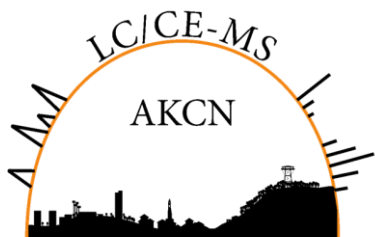
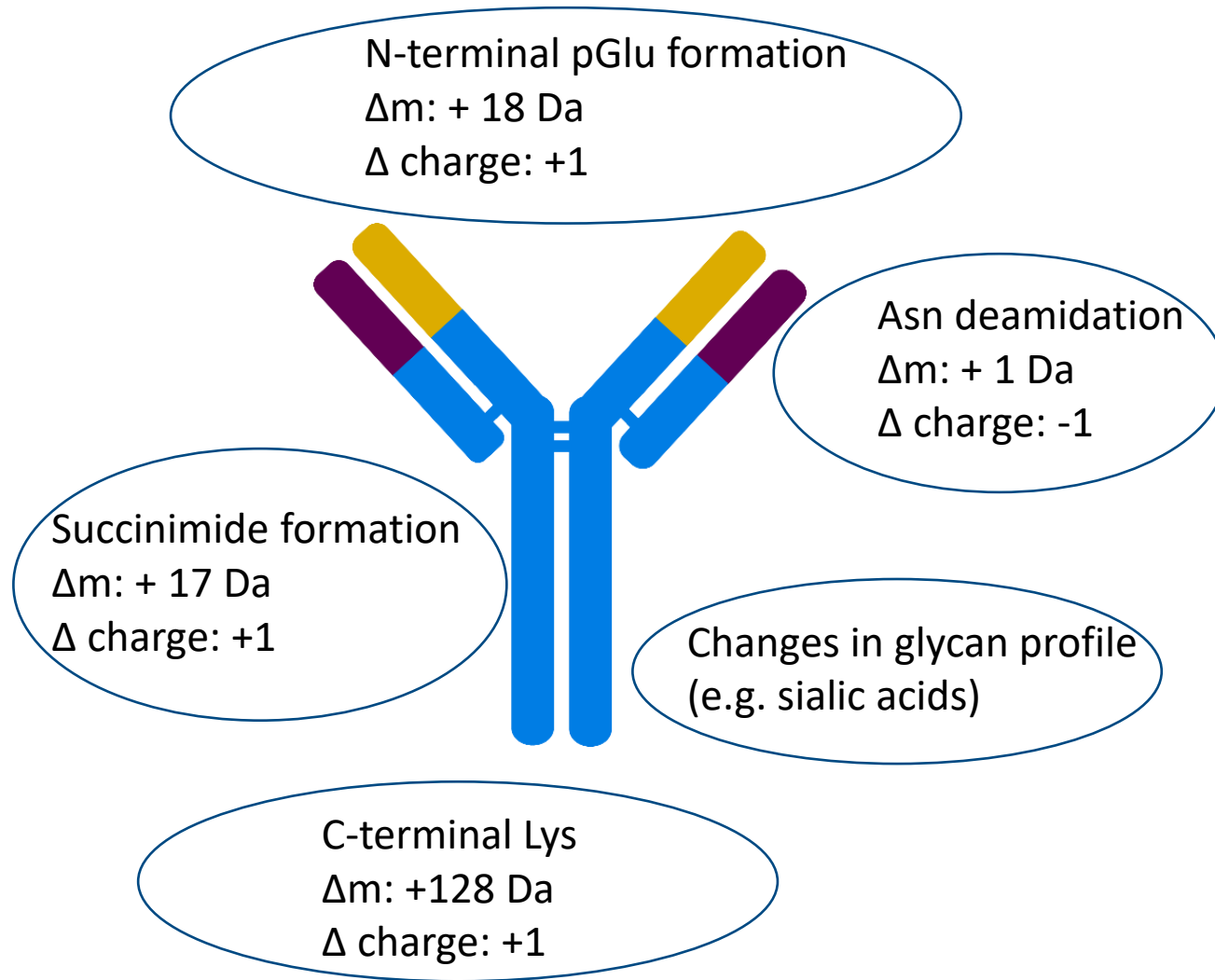


Mass Spectrometric Characterization of iCIEF-separated Antibody Charge Variants

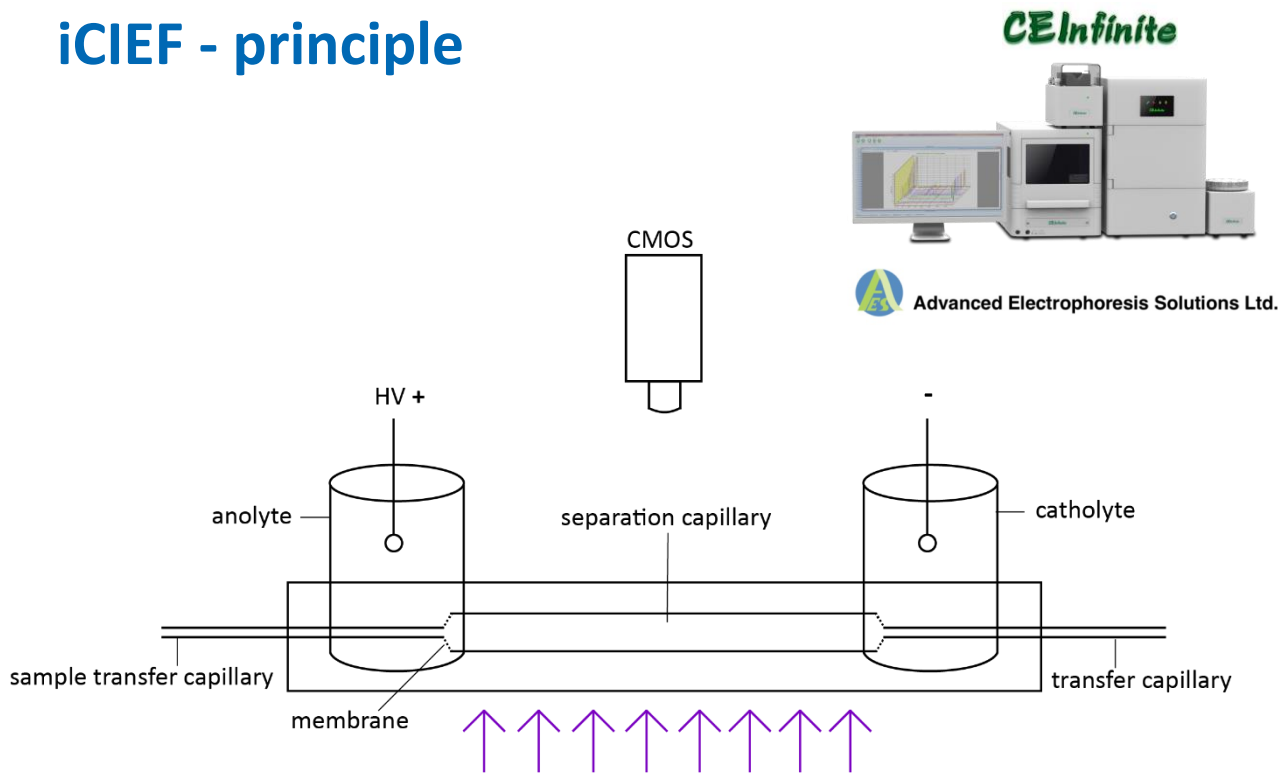
Johannes Schlecht, Tiemin Huang, Martin Donker, Gerard Rozing,
Steffen Kiessig, Bernd Moritz, Christian Neusüß

AT Europe 2020



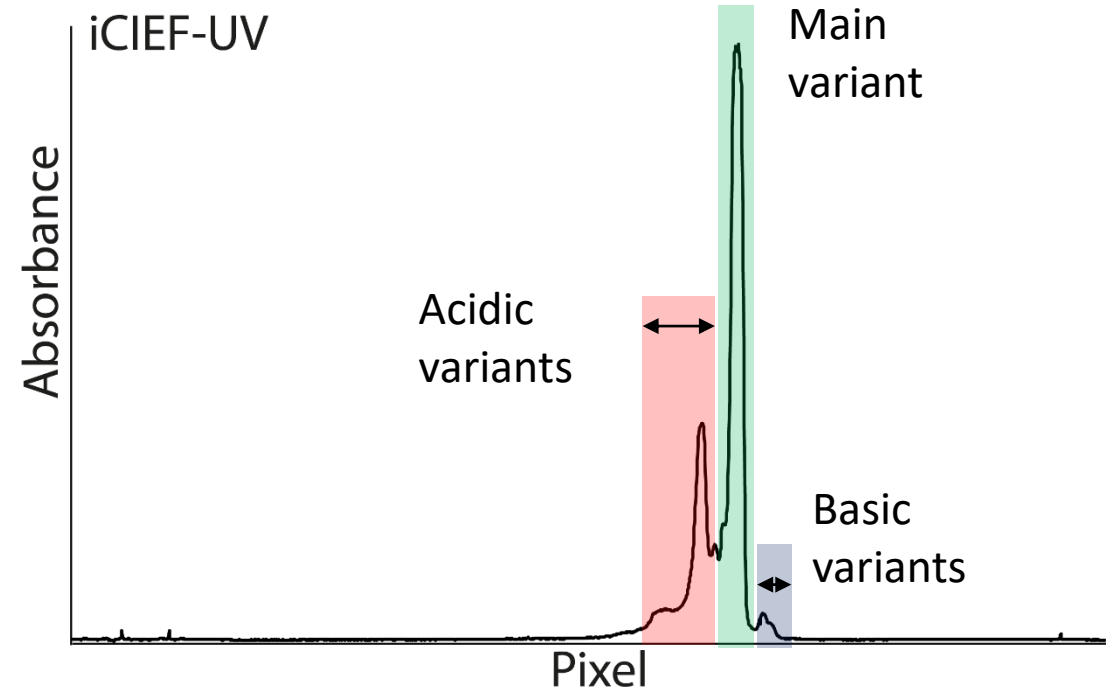


- Recombinant mAb heterogeneity due to manufacturing process, PTMs and during storage (degradation)
- Various mAb charge variants possible
 - can influence binding efficiency
 - impact on therapeutic effect
- Analytical techniques for charge variant separation
 - IEC
 - CZE (EACA)
 - IEF/CIEF/iCIEF



- Whole column imaging detection
- No mobilization step required
- High loadability of iCIEF cartridge (100 μm – 320 μm ID)
- Preparative system, fraction collection or ESI-MS

- Separation capillary: 50 mm
- (sample) transfer capillary: 50 μm (ID)
- Neutral (AD) or FC coated capillaries

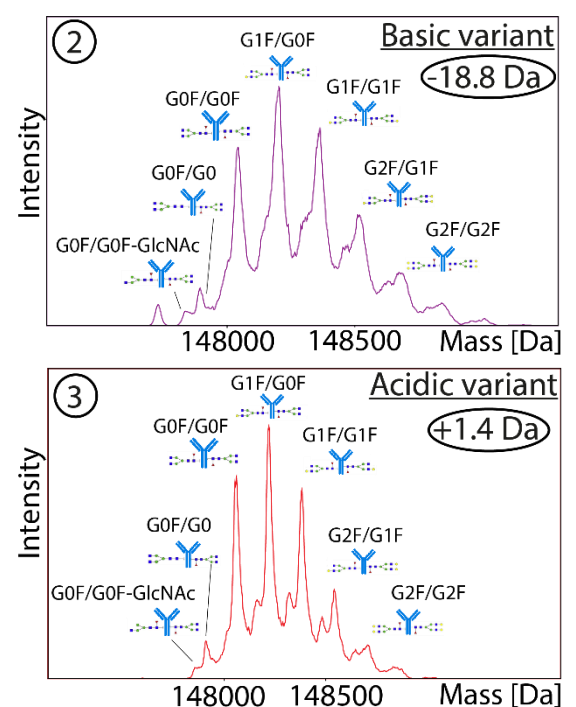
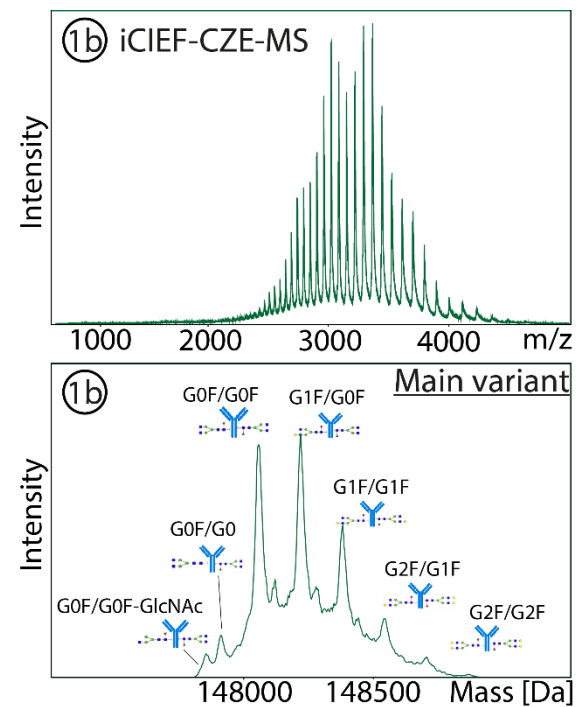
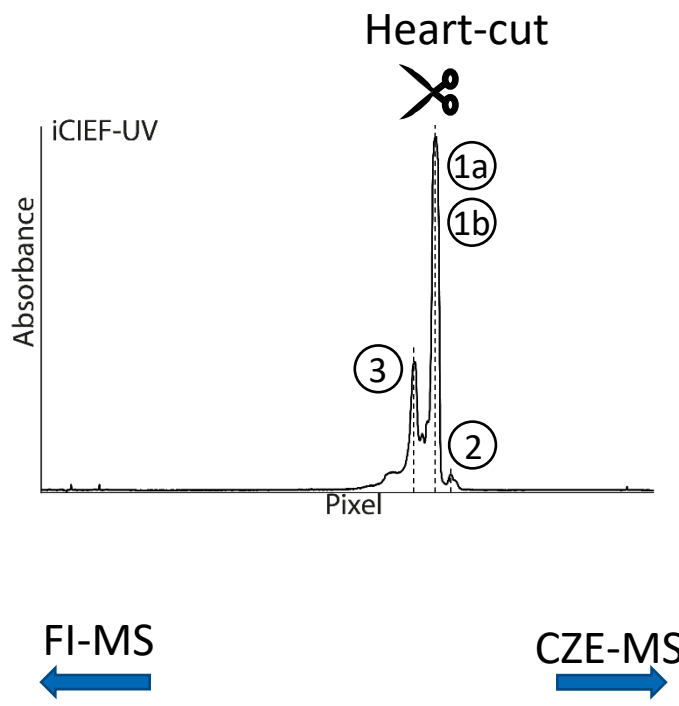
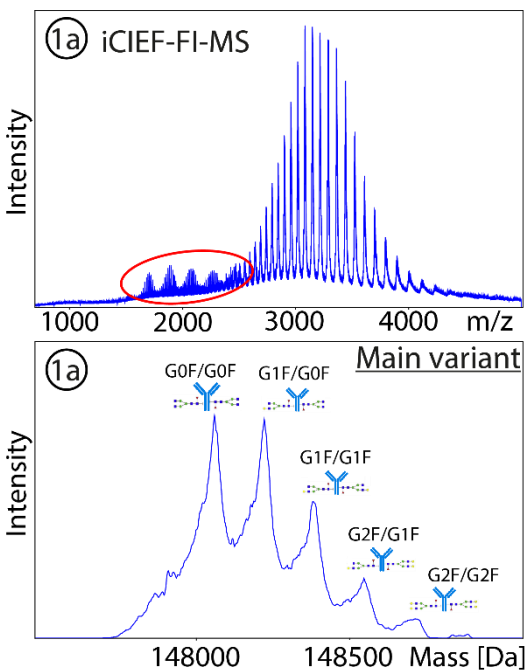
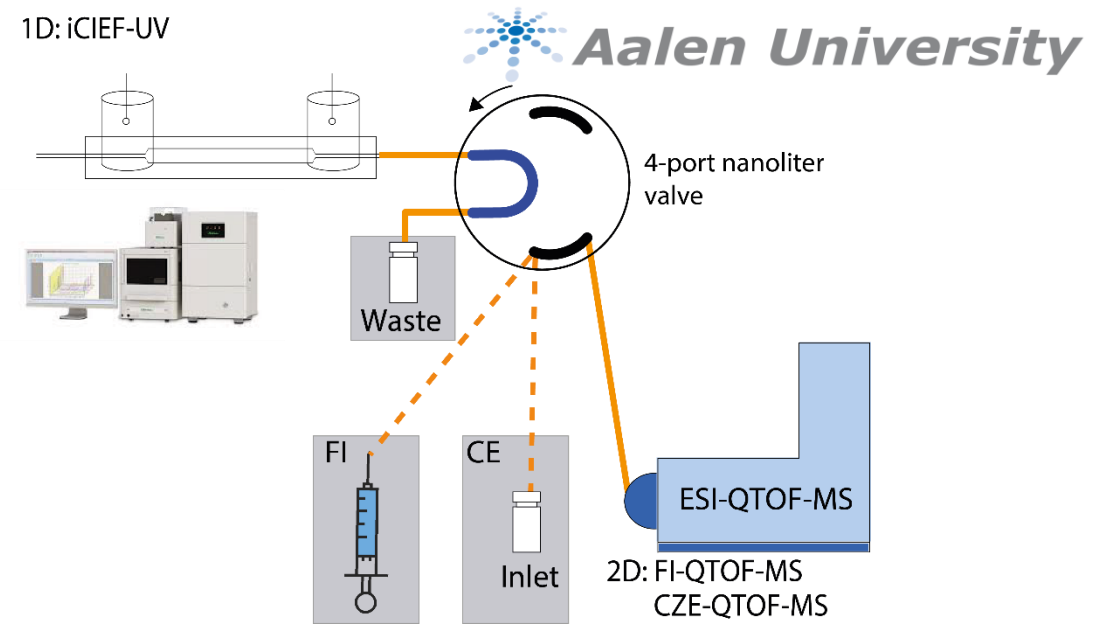


- Anolyte: 80 mM H_3PO_4
- Catholyte: 100 mM NaOH

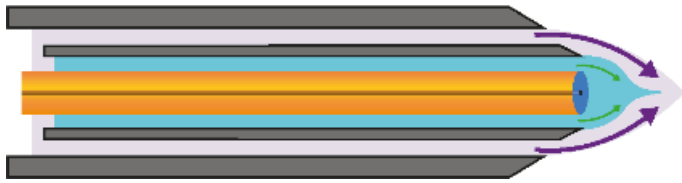
- Focusing step: 1 kV (1 min), 2 kV (2 min), 3 kV (9 min)
- Mobilization: 3 kV / 30 - 120 nL/min

2D-iCIEF-CZE/FI-MS

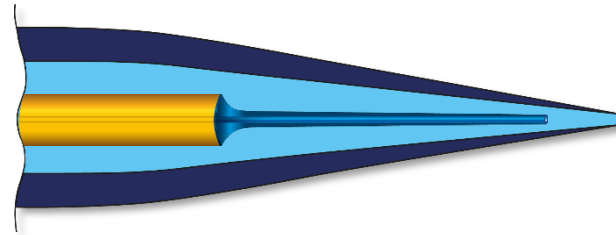
- Valve-based heart-cut 2D approach
 - Flow-injection (FI)-MS or CZE-MS
- Characterization of mAb charge variants



- Why online iCIEF-MS coupling?
 - Simplify instrumental setup and MS characterization of charge variant profile
- iCIEF sample composition: H₂O, ampholytes, mAb
 - Direct ESI approach of intact proteins is limited
 - Organic modifier required for efficient ionization
- How to add an organic modifier between iCIEF and MS?
- Maintenance of separation during mobilization?

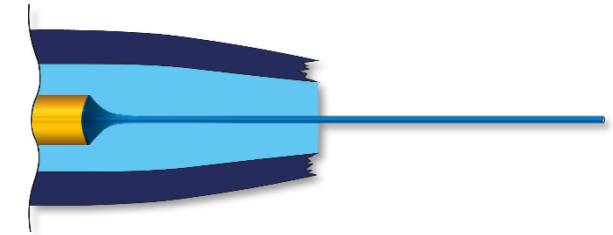


Co-axial sheath liquid interface (Agilent)



Nano flow sheath liquid interface (nano SL) (comm. by CMP Scientific)
Hsieh_1999 RCM; Wojcik_2010 RCM

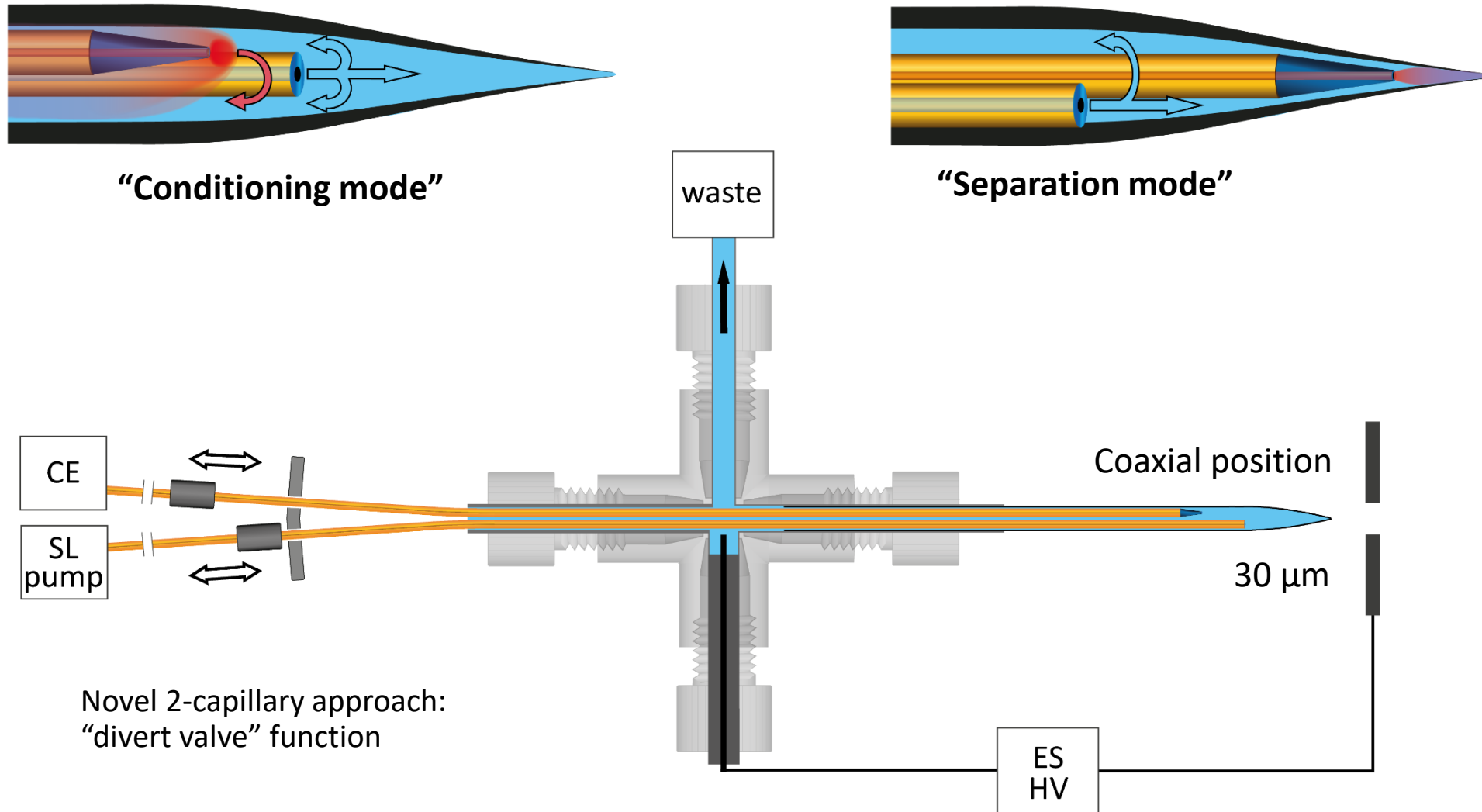
Sensitivity improvement: 10x-40x



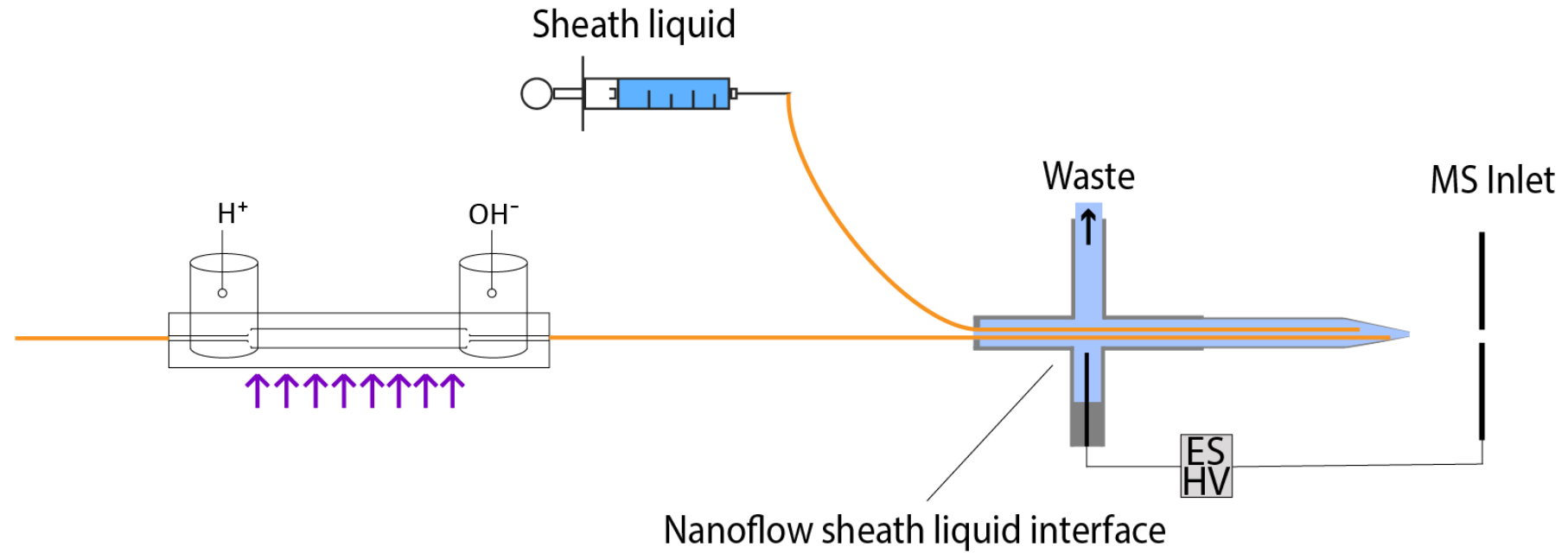
Sheathless porous tip interface (comm. by Sciex)
Moini_2007 AC

Sensitivity improvement: 10x-40x

[Höcker et al. Anal. Bioanal. Chem. 2018]



[Höcker, Kniermann, Meixner and Neusüß, Electrophoresis 2020]

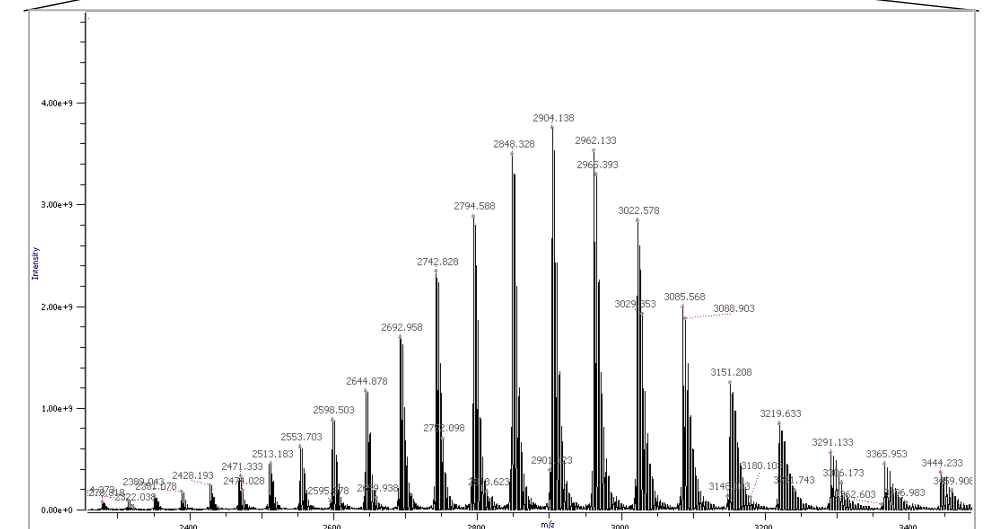
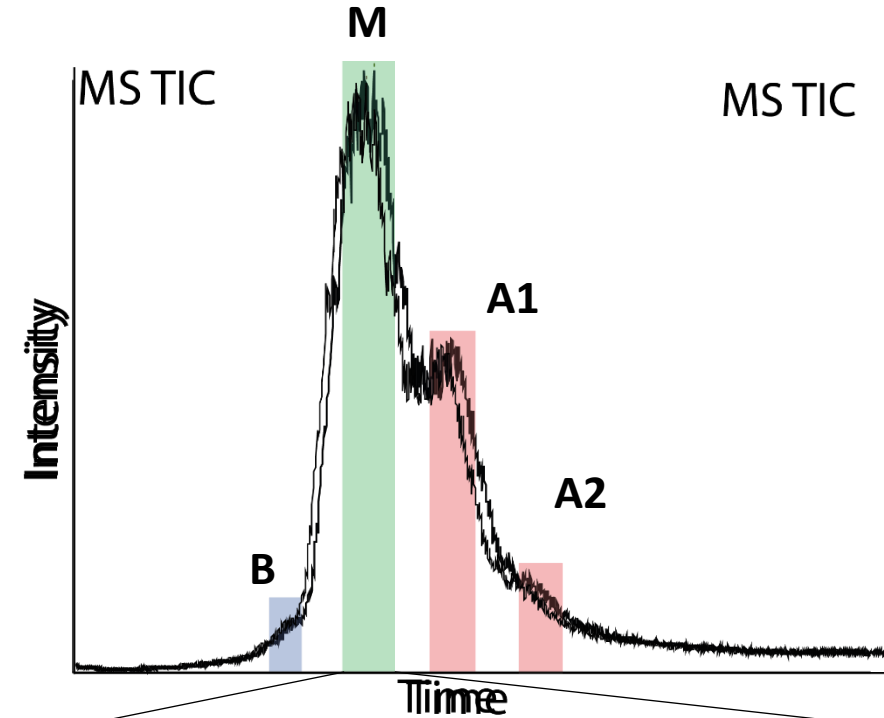
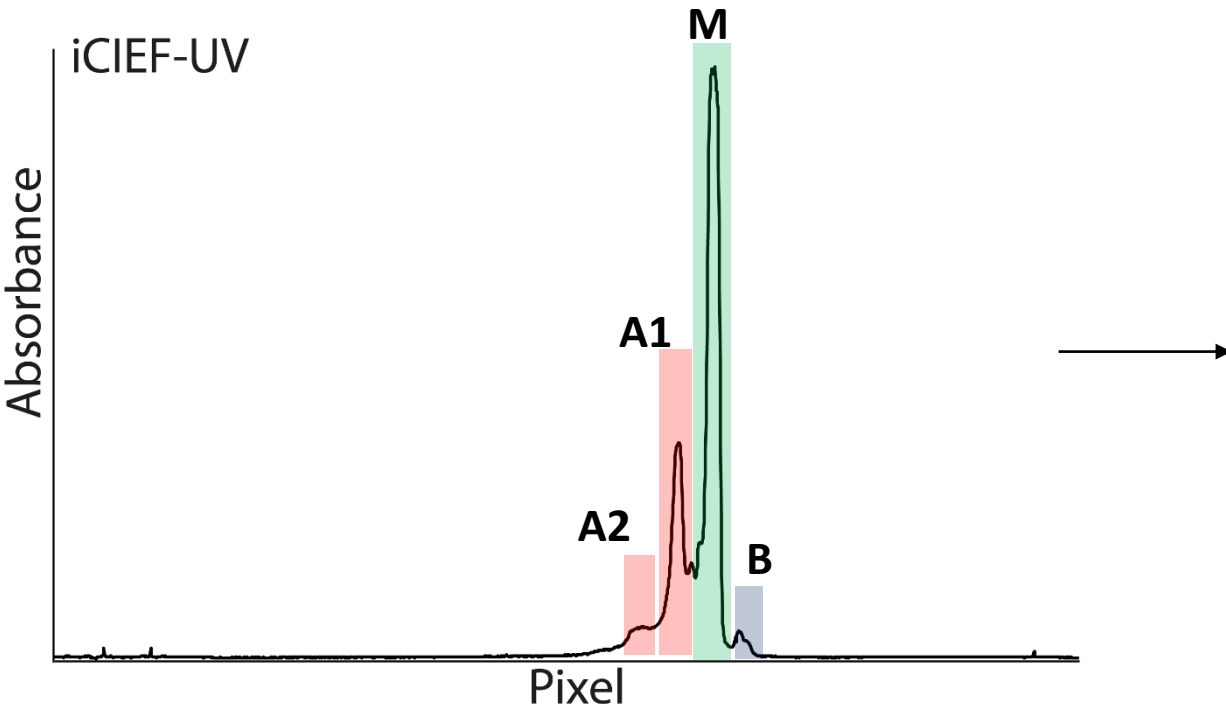


CEInfinite – Orbitrap Fusion Lumos



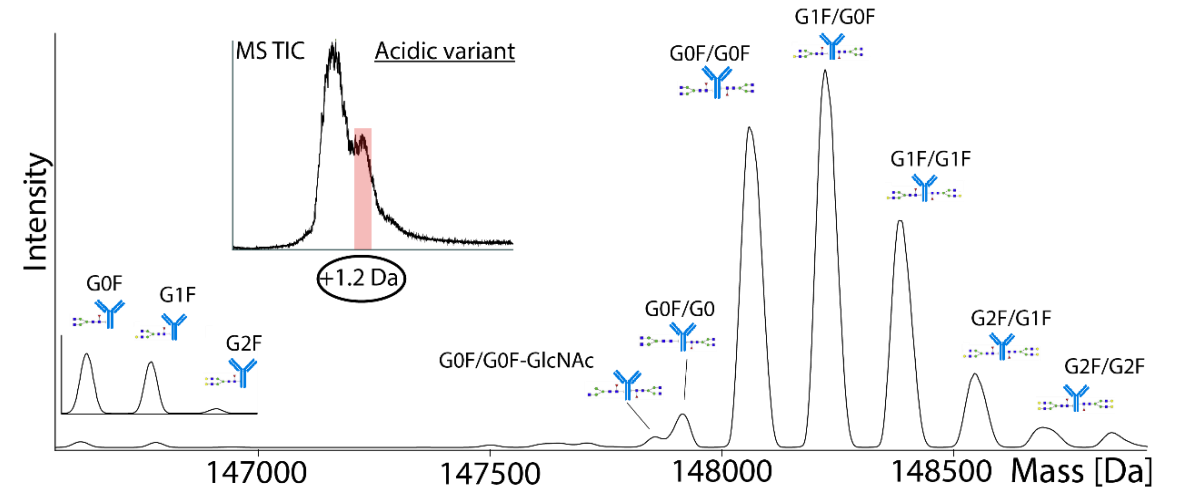
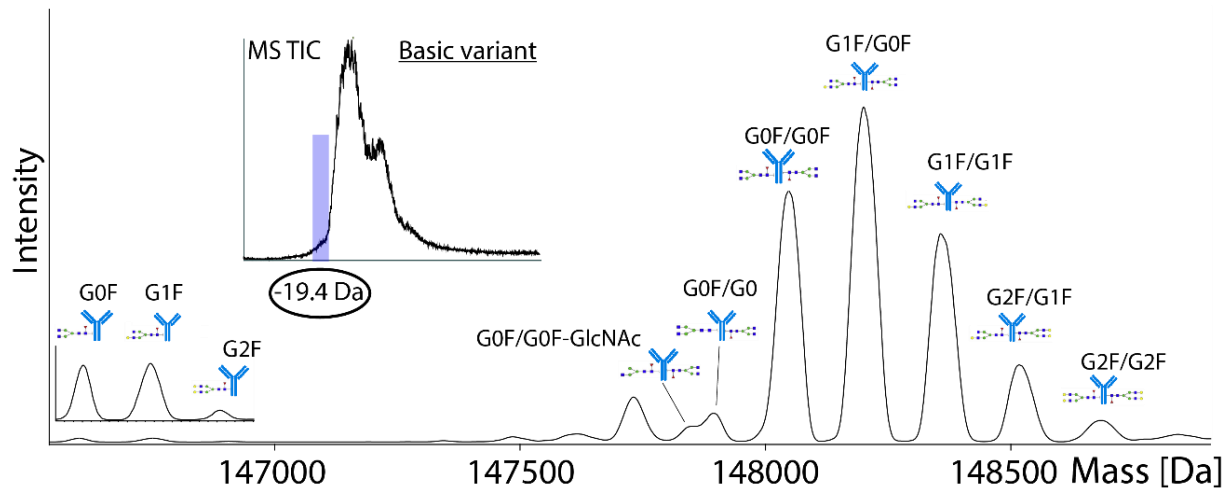
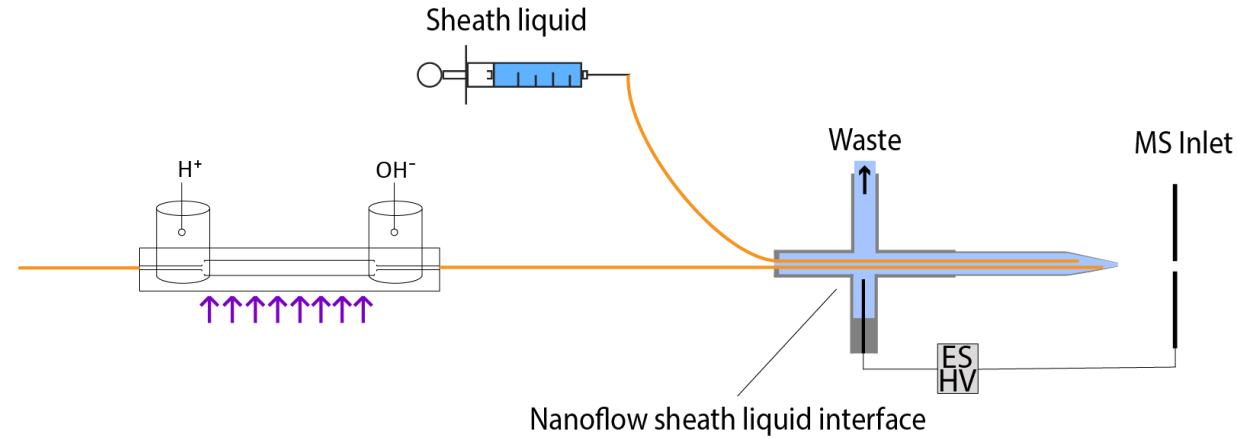
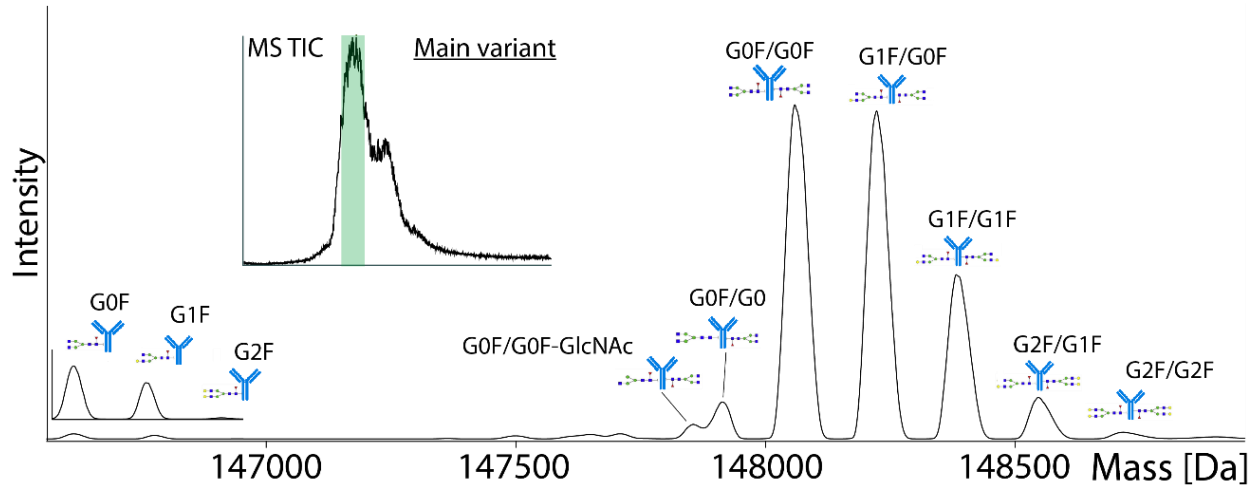
Electrospray emitter

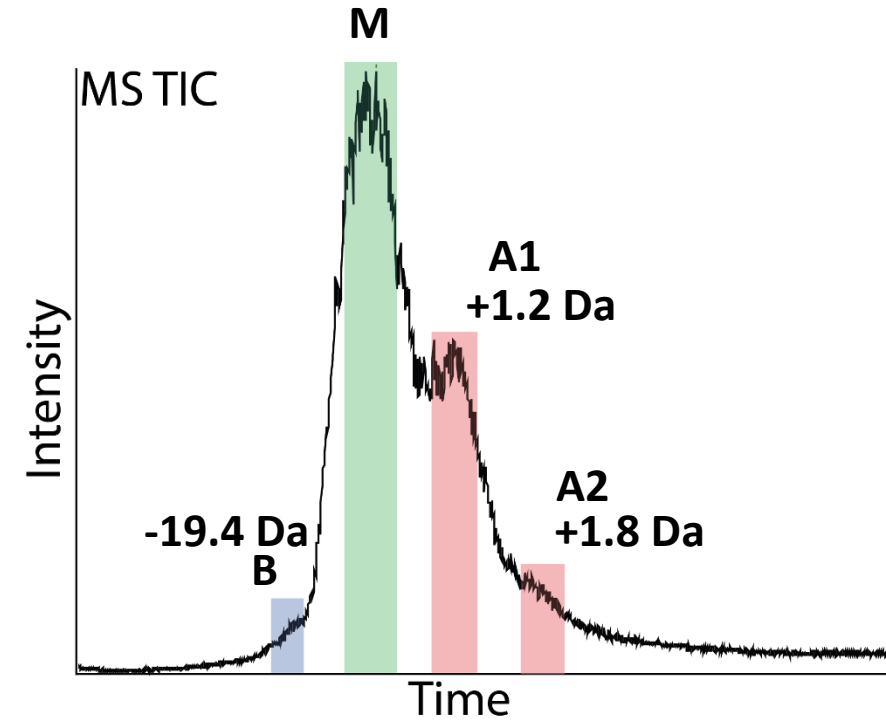
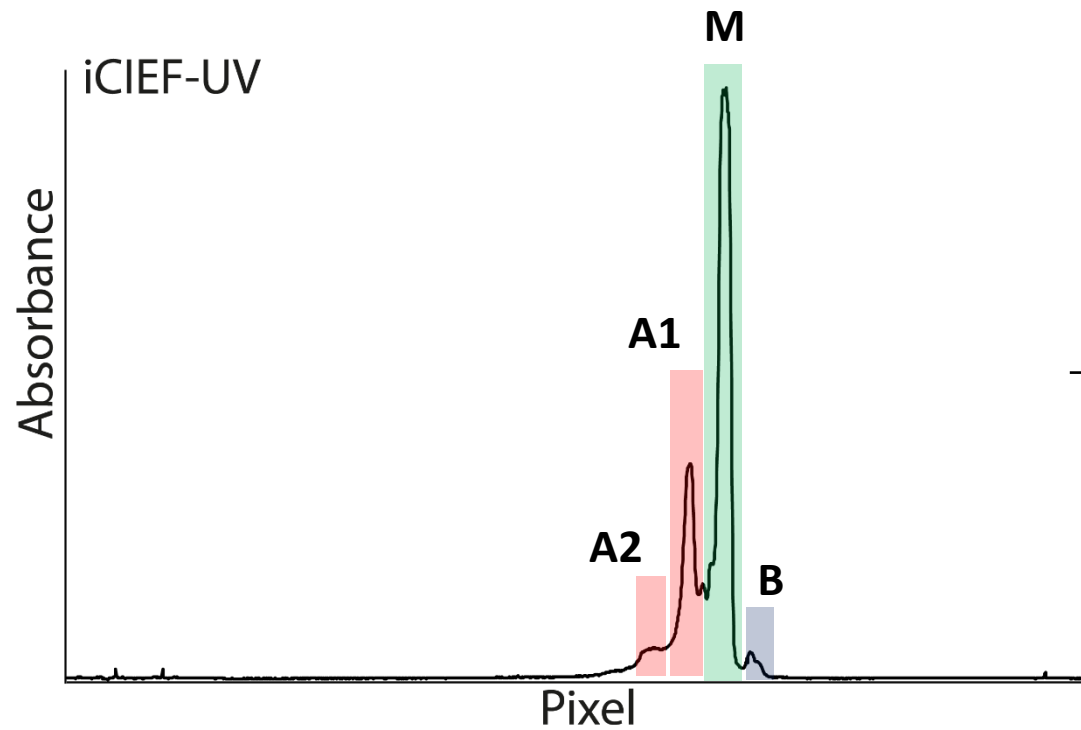
iCIEF-MS of Trastuzumab



- Trastuzumab: 2 mg/mL (20 μ L injected)
- Ampholytes: 0.5 % (v/v) Pharmalyte 3-10
1.5 % (v/v) Pharmalyte 8-10.5
- Sheath liquid: IPA:H₂O 1:1 + 1% (v/v) FA

iCIEF-MS of Trastuzumab





- Assignment of iCIEF-separated peaks to MS TIC profile possible
- Charge variants and minor glycoforms detectable
- Partial loss of separation during transfer

Modification	Δm [Da]
N -> D/isoD	0.98
Succinimide	17
E -> pE	18

- Successful online iCIEF-MS coupling with different MS instruments and ESI interfaces
- Nanoflow sheath liquid interface provides most promising approach for online iCIEF-MS mAb characterization
- Online iCIEF-MS enables charge variant profile characterization of mAbs
- Analysis of minor mAb charge variants and glycoforms feasible
- Maintenance of separation remains challenging

Tiemin Huang (Advanced Electrophoresis Solutions Ltd.)



Martin Donker (Isogen Life Science B.V.)



Group Prof. Neusüß (Aalen University)