Electrophoretic analysis of

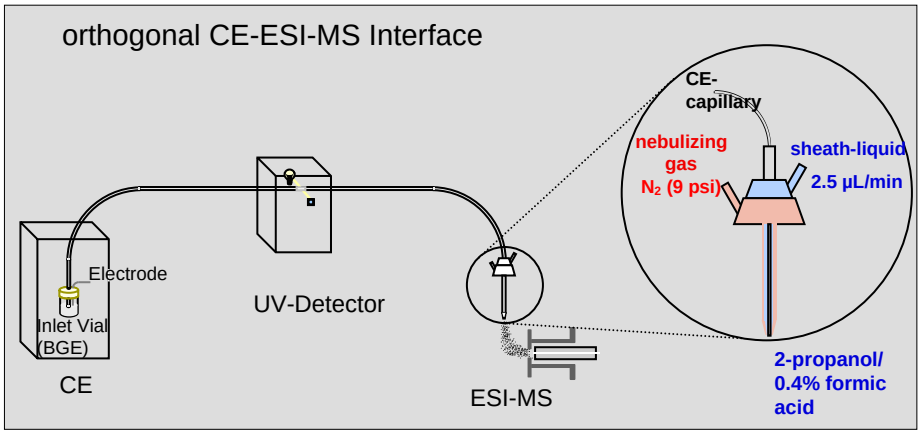
Glycoproteins	Glcopeptides	Glycans
CZE Low surface/volume ratio Reduced adsorption No hydrophobic adsorption Mild BGE conditions (no ACH, EtOH etc.) **2D-GE** Glycoproteomics High Peak capacity Separation by pI	**CZE** Faster than HPLC No memory	**CZE** Faster than HPLC No memory Derivatization needed

CZE-MS/MS Instrumentation

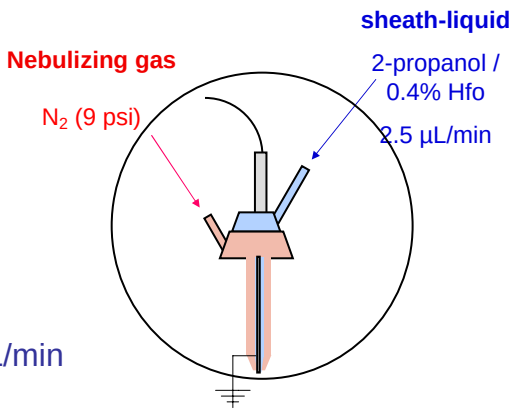
ESI
MALDI

CZE-ESI-MS

Quadrupol Ion Trap (Bruker Esquire 3000+)



CZE-ESI-MS
triple tube sprayer

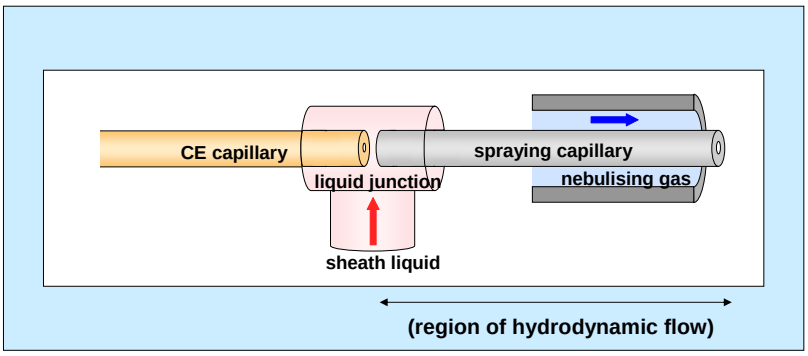


commercially available:

micro-sheath-flow: 2-4 µL/min

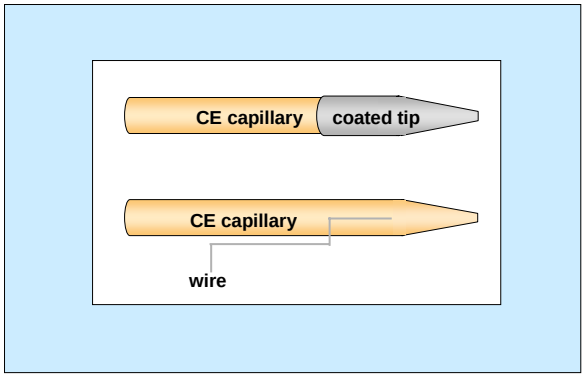
CZE-ESI - Interface

liquid junction



CZE-ESI - Interface

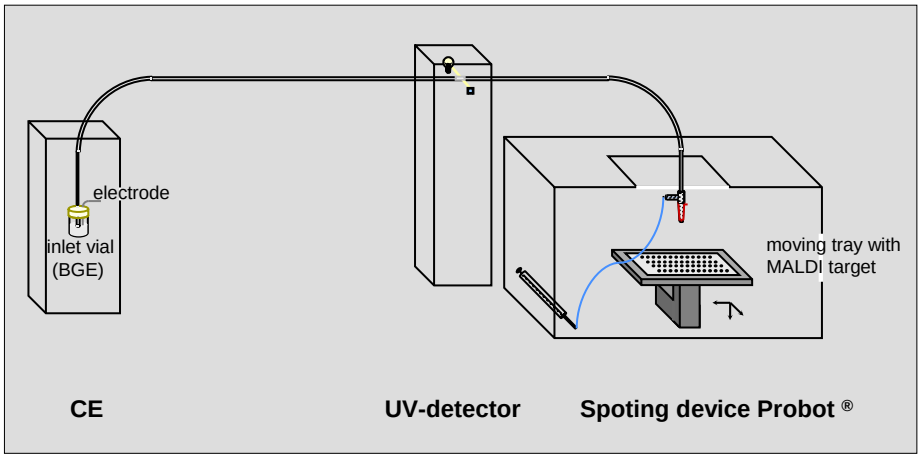
Sheathless / EOF driven



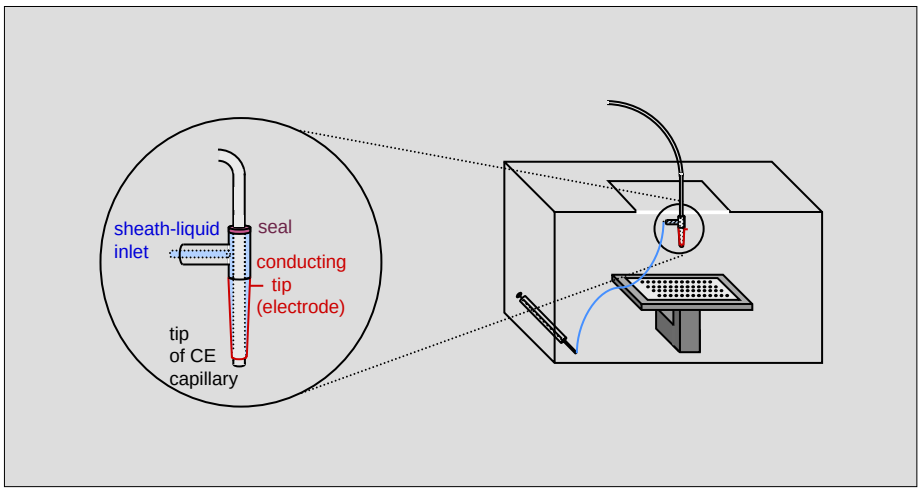
J. Peter-Katalinic; A. Zamfir

CZE-MALDI-MS

automatic sheath-flow assisted sampling device: Probot®

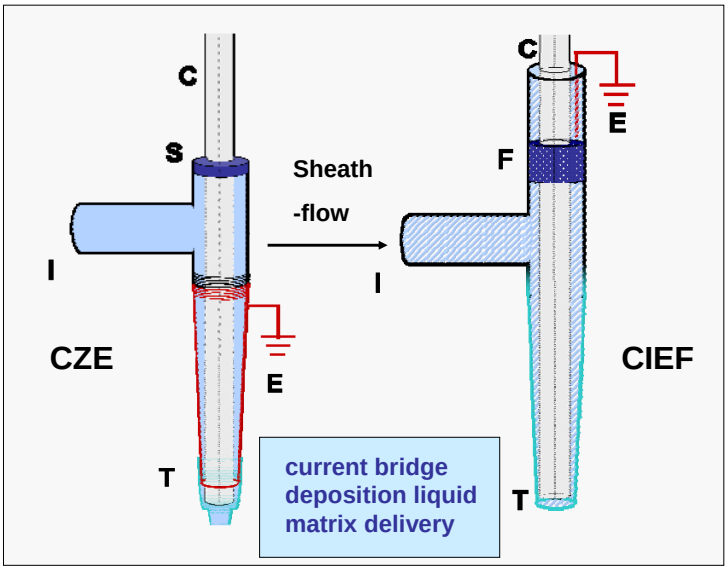


CZE-MALDI-MS



CE-MALDI-MS

Sheath flow assisted analyte deposition



S.Amon, A.Plematl, A.Rizzi *Electrophoresis* (2006)

Sheath flow assisted analyte deposition

spotting
1-2 spots/min
sheath flow: 0.3 µL/spot
matrix flow: 0.4 µL/spot

CZE → **sheath liquid**
BGE

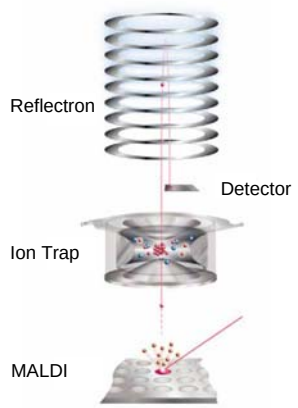
CIEF → **sheath liquid**
50mM NH_3

matrix solution
10µg/µl THAP +
di-ammonium hydrogen
citrate in MeOH/H_20, 5/1

MALDI-MS

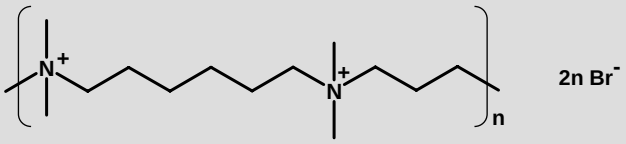
MS^1
MALDI-linear TOF
(Kratos AXIMA LNR)

MS^2 , MS^3
MALDI-IonTrap-RTOF
(Kratos AXIMA QIT)



cationic coating

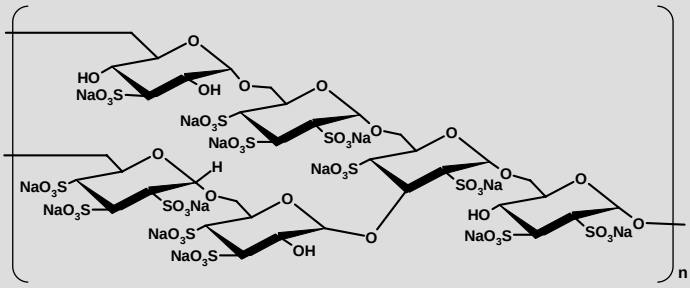
Hexadimethrinbromid, HDMB



- permanently positive charged coating
- strong anodic (reversed) EOF

anionic coating

Dextransulfat, DS



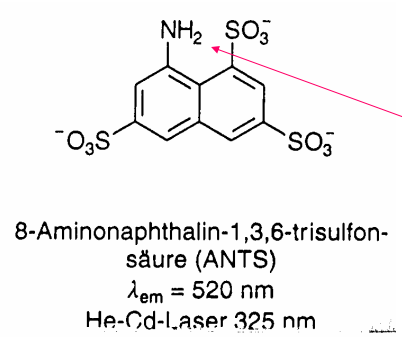
SMIL (successive multiple ionic-polymer layer)

- permanently negatively charged coating
- strong cathodic EOF

Glycans after Derivatization

Labeling for LIF

8-Aminonaphthaline-1,3,6-trisulfonic acid (ANTS)



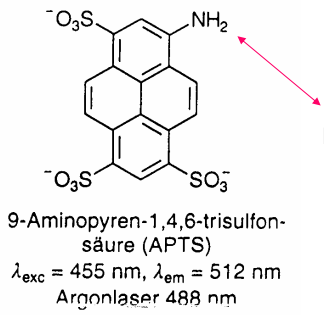
Reductive Amination

325 (He/Cd) / 520

Net negative charge: → electrophoretic migration; negative ion mode MS LIF-detection

Labeling for LIF

9-Aminopyrene-1,4,6-trisulfonic acid
(APTS)



Reductive Amination

488 (Ar) / 512

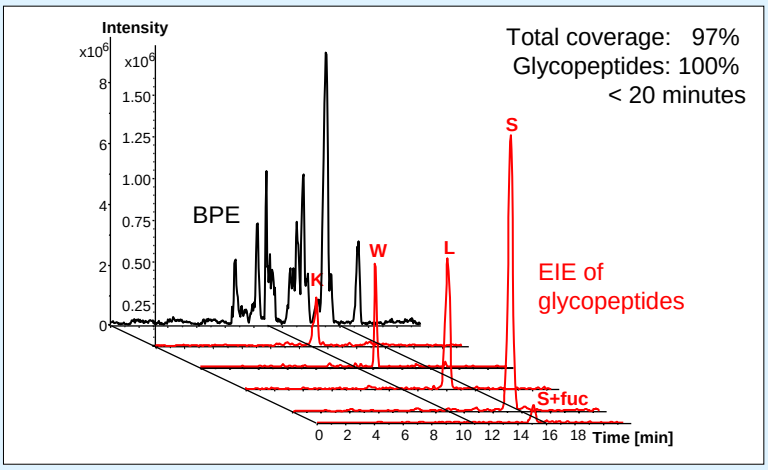
Separation according

- charge
  (number of SA, sulfate, phosphate)
- size
  (size of antenna, branching, linkage position)
- complexation constant with borate
  (epimeric sugars)

Glycopeptides

Analysis of peptides/gylcopeptides

CZE-ESI-MS sheath-liquid interface

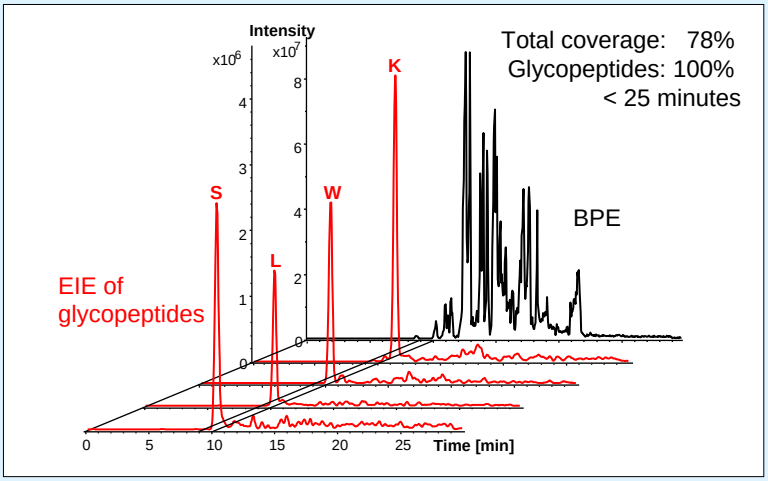


Antithrombin; pH 8,0, DS coated capillary → cathodic EOF

S.Amon, A.Plematl, A.Rizzi *Electrophoresis* (2006)

Analysis of peptides/gylcopeptides

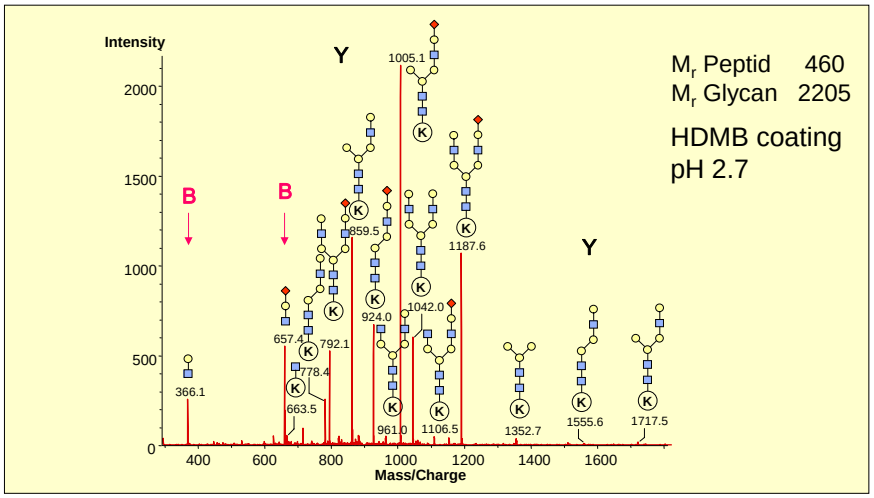
CZE-ESI-MS sheath-liquid interface



Antithrombin; pH 2,7, HDMB coated capillary → anodic EOF

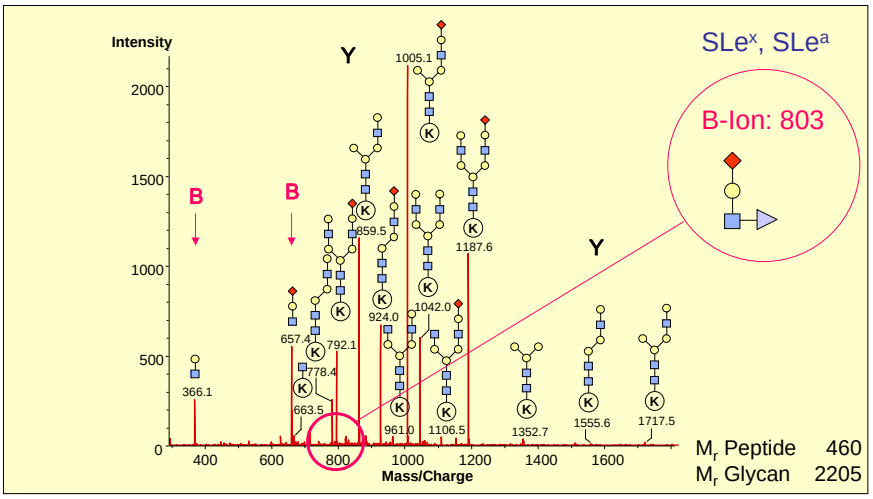
S.Amon, A.Plematl, A.Rizzi *Electrophoresis* (2006)

MS² with low energy CID yields primarily **Y** ions
Some **B** ions are useful for automatic assignment as glycopeptide



Gylcosylation-analysis of glycopeptides

CZE-ESI-MS²



Antithrombin (human), tryptic digest

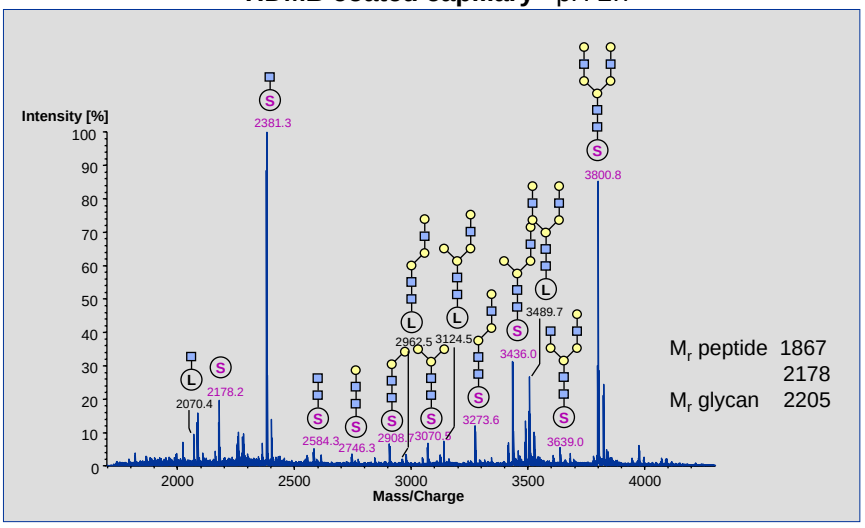
CZE-ESI-MS/MS

Interesting alternative to

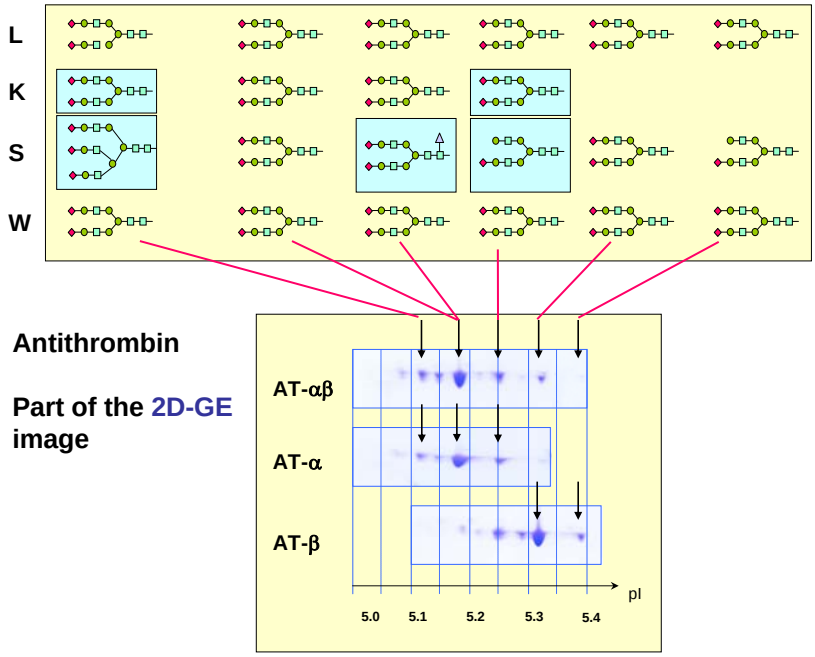
HPLC-ESI-MS/MS

- speed of analysis
- selectivity
- sensitivity
- MS^2 compatibility

Antithrombin (Human)
tryptic digest
CZE-MALDI-MS²
HDMB coated capillary pH 2.7



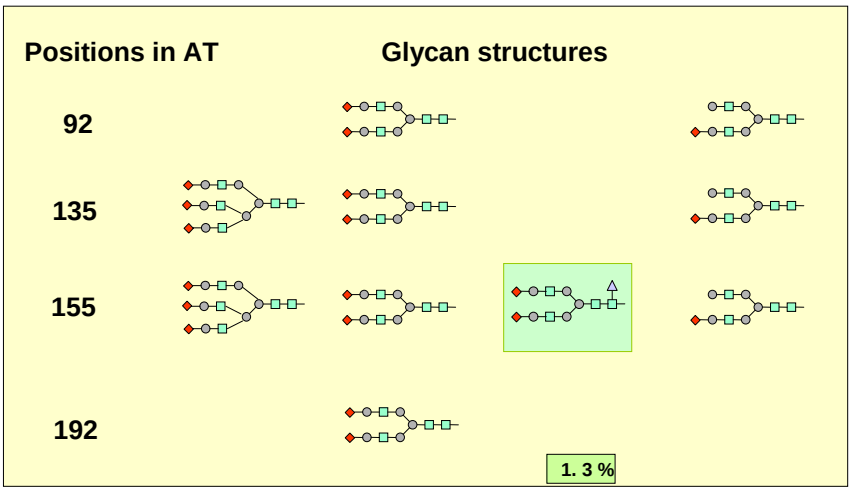
Glycoproteins



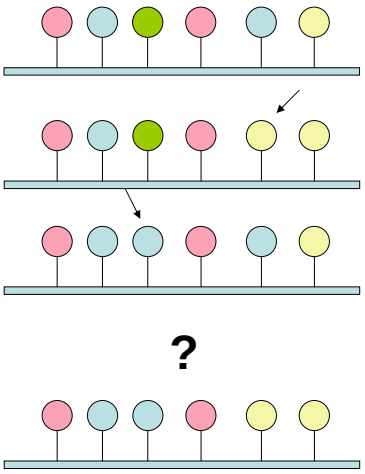
Antithrombin

Part of the 2D-GE image

Analysis of pooled plasma:
site-spezific glycosylation variability
→ sequential trimming of glycans



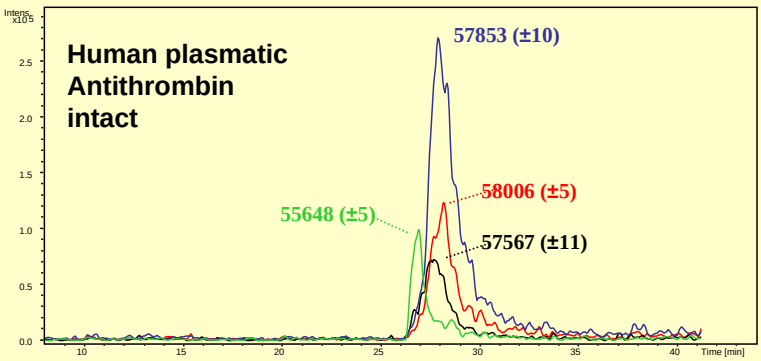
Combination of modifications in single protein isoforms



Analysis of the total number of modifications and the combination of variants needs the analysis of the intact glycoprotein

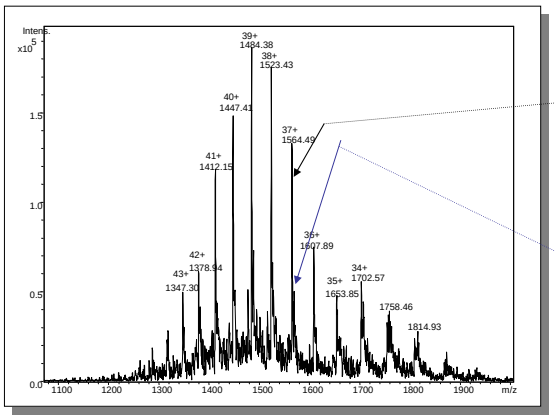
- CZE-MS/MS analysis of the intact glycoprotein helps to minimize adsorption problems
- Good relative quantitation possible, because of comparable ionization yields for glycoforms

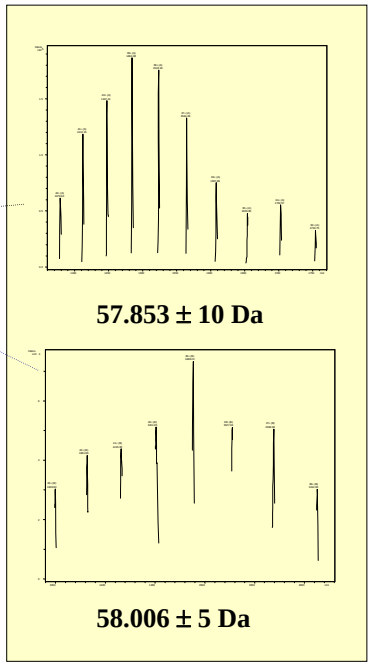
CZE-ESI-MS
QIT-analyzer



Extracted Ion Electropherogram: 4 selected mass traces

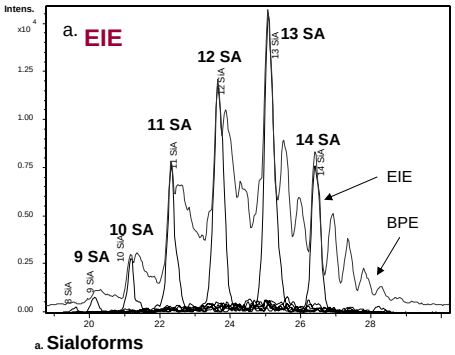
Positive-Ion ESI Mass-Spectra
Time-window 28 - 30.5 min





Deconvoluted spectra of isoforms →

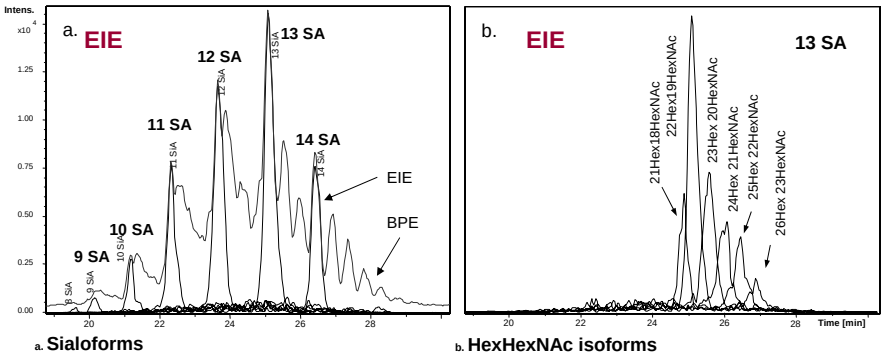
CZE-MS
oa TOF-analyzer
rec. human erythropoietin



(a) shows the EIE obtained for glycoforms with different number of silaic acids.

E. Balaguer et al.; Electrophoresis 2006: 2638-2650

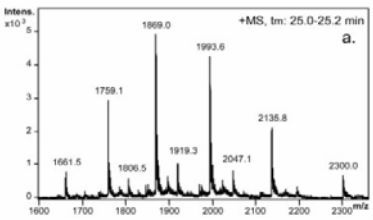
CZE-MS
oa TOF-analyzer
rec. human erythropoietin

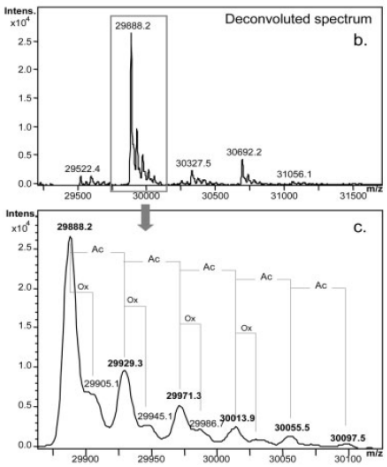


(b) shows the EIE obtained for glycoforms with different HexHexNAc content and identical silaic acid number.

E. Balaguer et al.; Electrophoresis 2006: 2638-2650

CZE-MS
TOF-analyzer
recombinant human erythropoietin





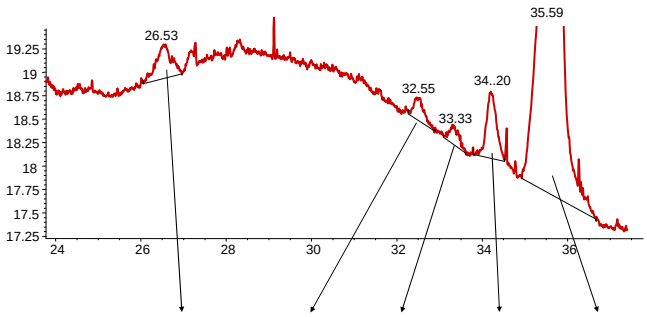
mass spectrum of time window 25.0–25.2 min
(a) Charge state distribution
(b) deconvoluted mass spectrum
(c) details of the deconvoluted spectra
(Ac, acetylation; Ox, oxidation).

E. Balaguer et al.; Electrophoresis 2006: 2638-2650

CZE with seeving gel – SDS/CGE

Analysis of intact IgG -non reduced

IGN-311-Batch 1



M_w (calc.)	**44**	**112**	**124**	**137**	**160**
	H	**HH**	**HHL**	**H_dH_dLL**	**HHLL**
rel. amount	**1.9%**	**0.7%**	**0.5%**	**2.4%**	**94.5%**

Special advantages of CZE

- **Fast and efficient separation**
- **Easy to miniaturize**
- Low surface / volume ration
  - →**Low degree of adsorption**
- **Rigorous cleaning possible**
- **Potential for multiplexing**

Special limitations of CZE

- **Limited concentration sensitivity**