908devices

ZipChip Applications

8/17/2021

Agenda

- What is ZipChip and Overview of Components
- ZipChip for Biotherapeutics Characterization
- ZipChip – How it works
- Summary & questions

ZipChip Components

What is ZipChip?

ZipChip Hardware

- Light weight
- Separation device
- Auto sync & alignment with MS

Thermo Fisher Scientific

Sciex

Bruker

The Chips

- CE-ESI on a chip
- Application optimized
- 125 or 250 runs per chip

High Resolution

High Speed (wicked fast)

Reagent Kits

- All necessary chemicals
- Application optimized

Intact Antibody Kit

Metabolites Kit

Peptides Kit

Native Kit

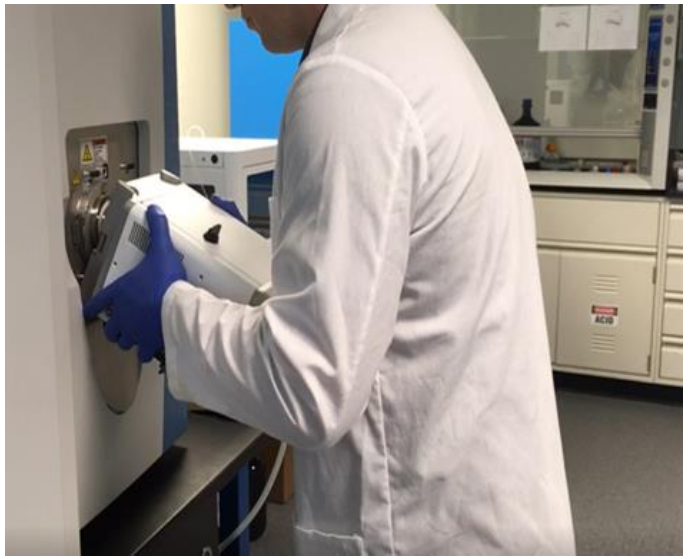
Charge Variant TOF Kit

Hardware - ZipChip Interface

ZipChip Interface

Light weight device

Quick and Simple Installation



Mounts easily and quickly to the MS. Synchronizes all electrical and fluidic connections

Installation to analysis in under 30 minutes

Hardware - Autosampler

Full Automation

- Two Sample Tray Positions
  - > 48 vial & 96/384 well plate compatible
- Tray Cooling
- 10, 20, or 40 µL sample transfer volumes

Simply load samples, press go, and walk away.

Chips – The Heart of the Technology

	ZipChip HS/HSX/HSN/HSB	ZipChip HR/HRX/HRN/HRB
Channel Length	10 cm channel	22 cm channel
Separation Type	Rapid Separations	High Resolution Separations
Separation Use	High Throughput	Thorough Characterization
Common Applications	Small Molecules Rapid Protein Mass	Intact Protein Analysis Peptide Mapping/Proteomics

Mix & match Chips and Kits to quickly achieve optimal results

Reagent Kits – Simple to use

BGE (BackGround Electrolyte)

- Is the liquid used to fill the channels – “mobile phase”
- Is what drives separation of analytes
- Is comprised of:
  - > Conductive mixture of water and a charge carrier
  - > An Organic modifier

BGE Kit	pH
Metabolite	2.2
Peptide	2.4
Antibody	3.3
Native	5.5
Charge Variant TOF	5.5

Target molecules must be *positively charged* at the pH of your chosen analysis conditions.

Simple workflow for multiple applications

Prepare, separate, & analyze in under 4 minutes

Pick your application

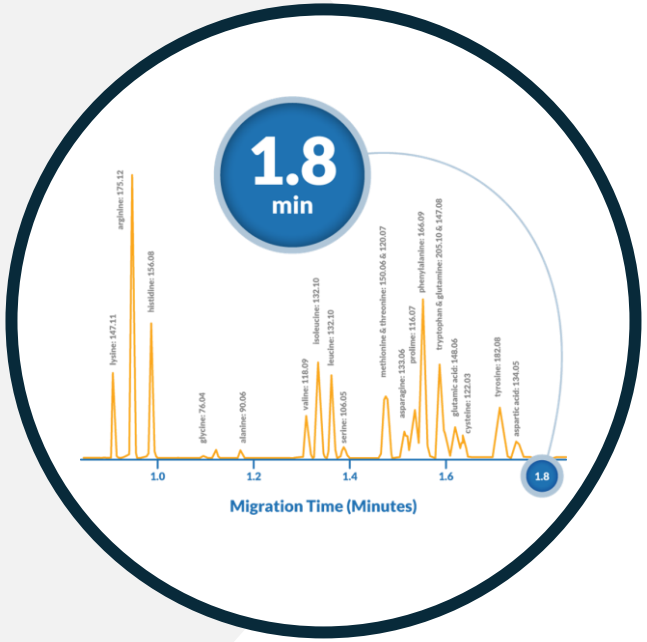
- Biotherapeutics
- Metabolomics
- Proteomics

Pick your kit

- Metabolites
- Peptides
- Intact Antibodies
- Native

Pick your ZipChip

- ZipChip HR
- ZipChip HS



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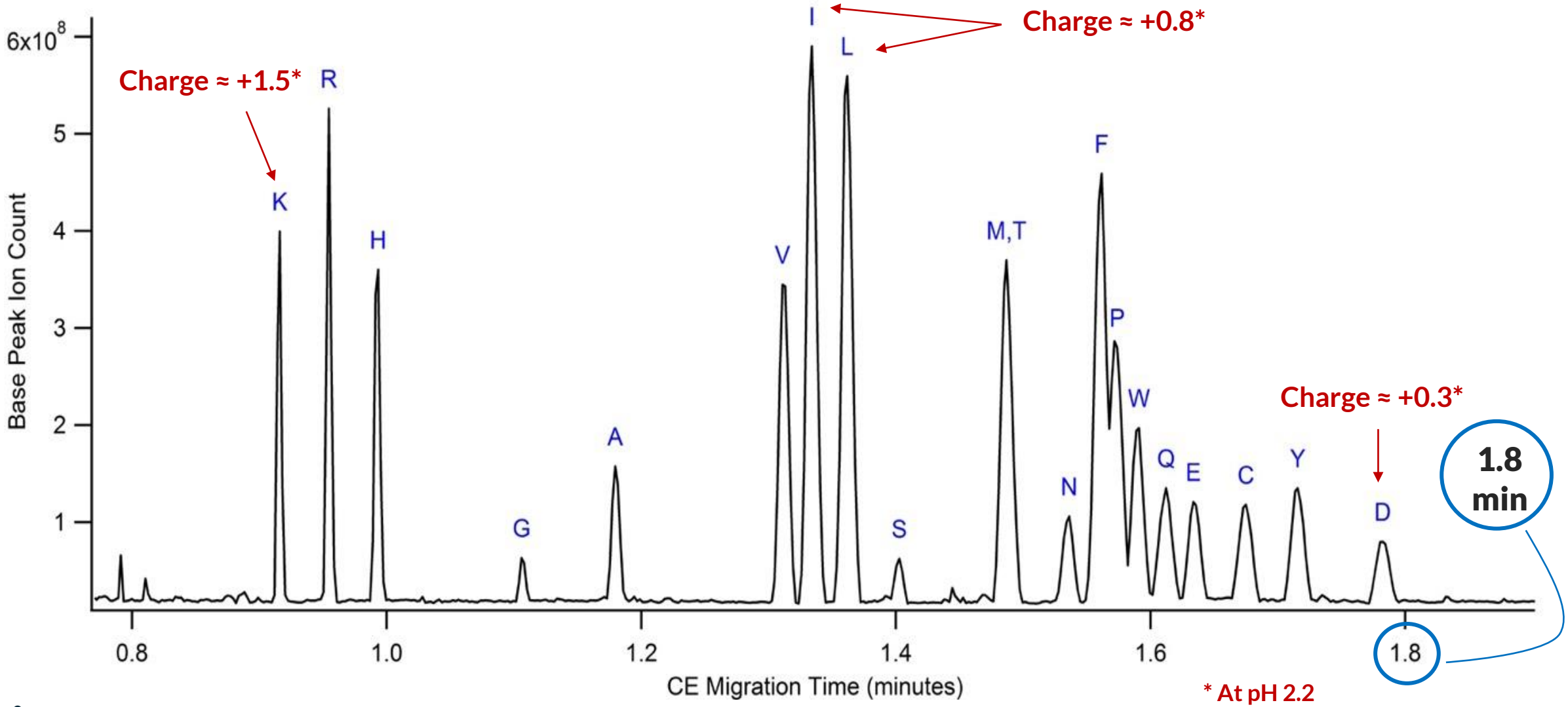
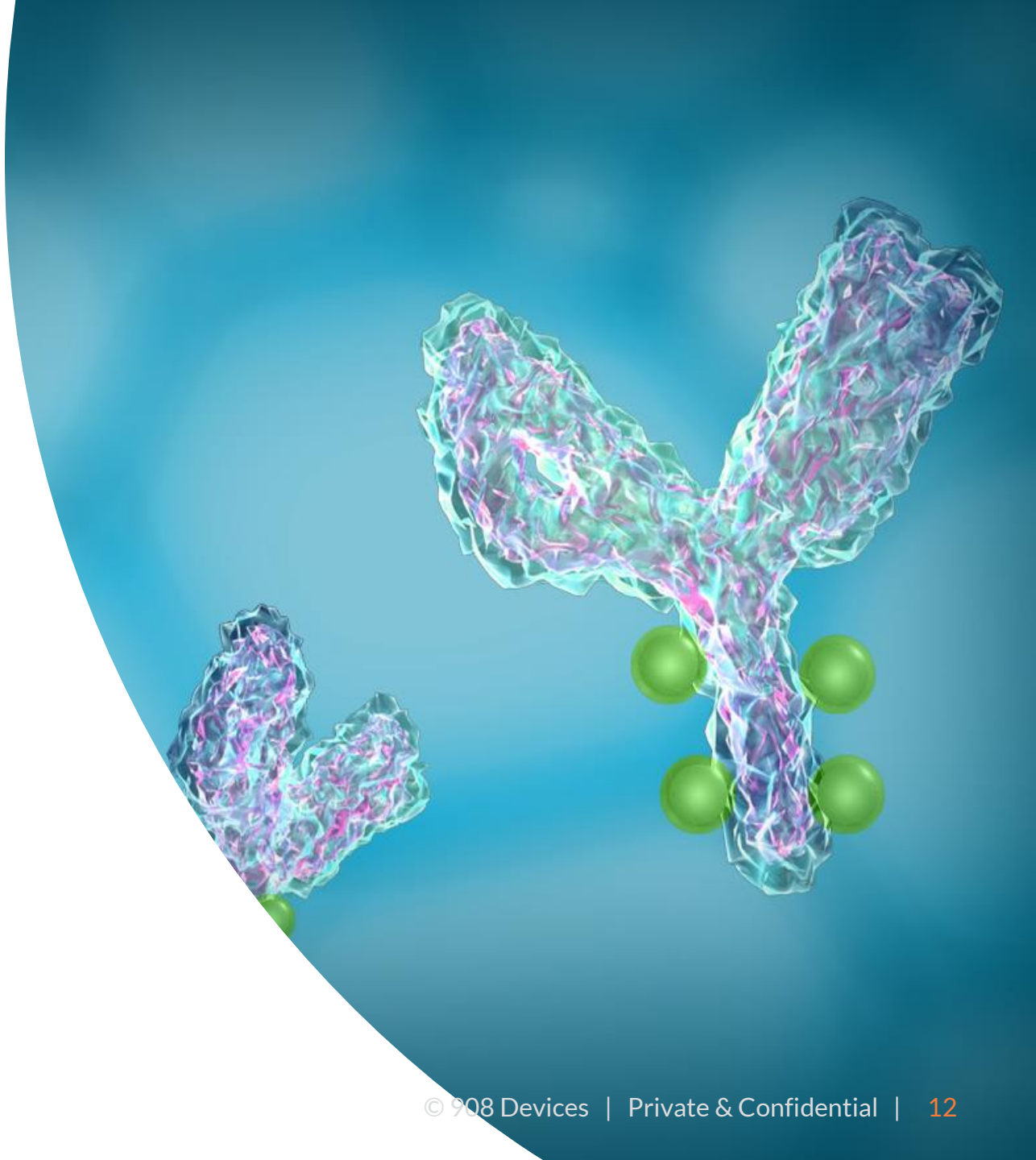
ZipChip – Fast Separation of Positive Analytes



Biotherapeutic Applications

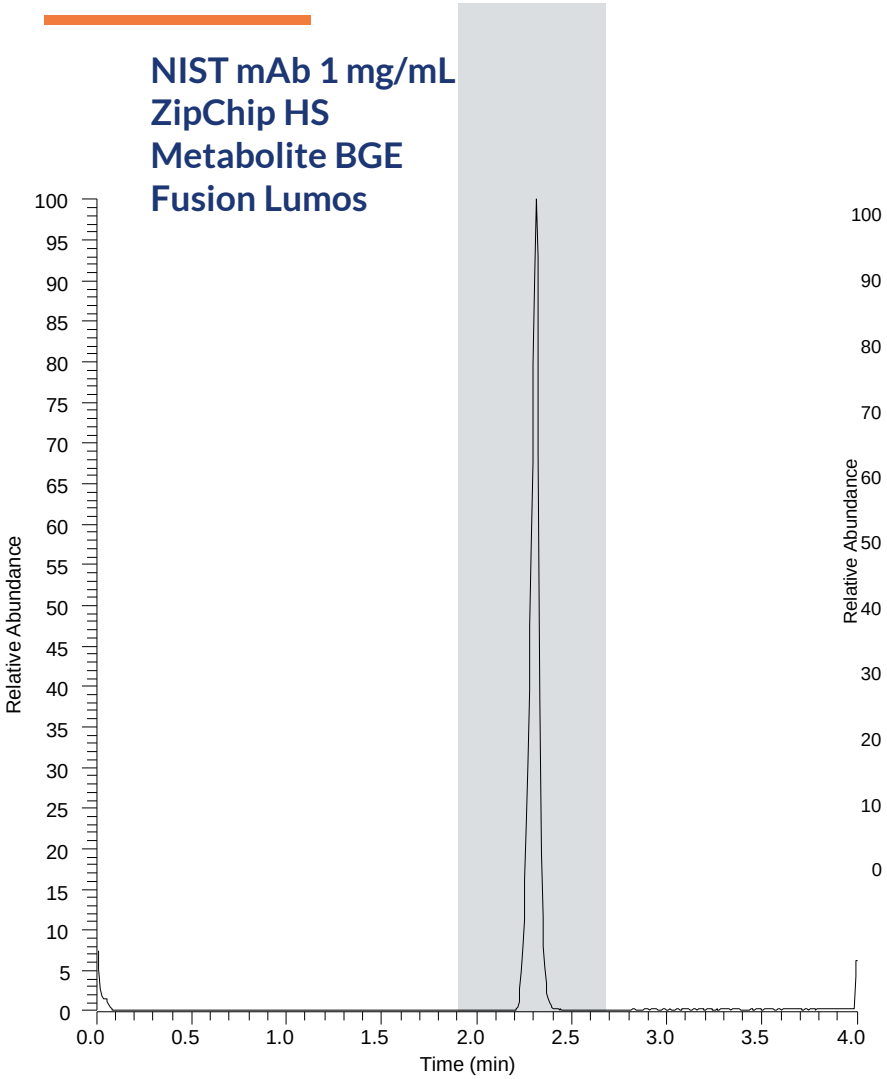
Biotherapeutic Applications

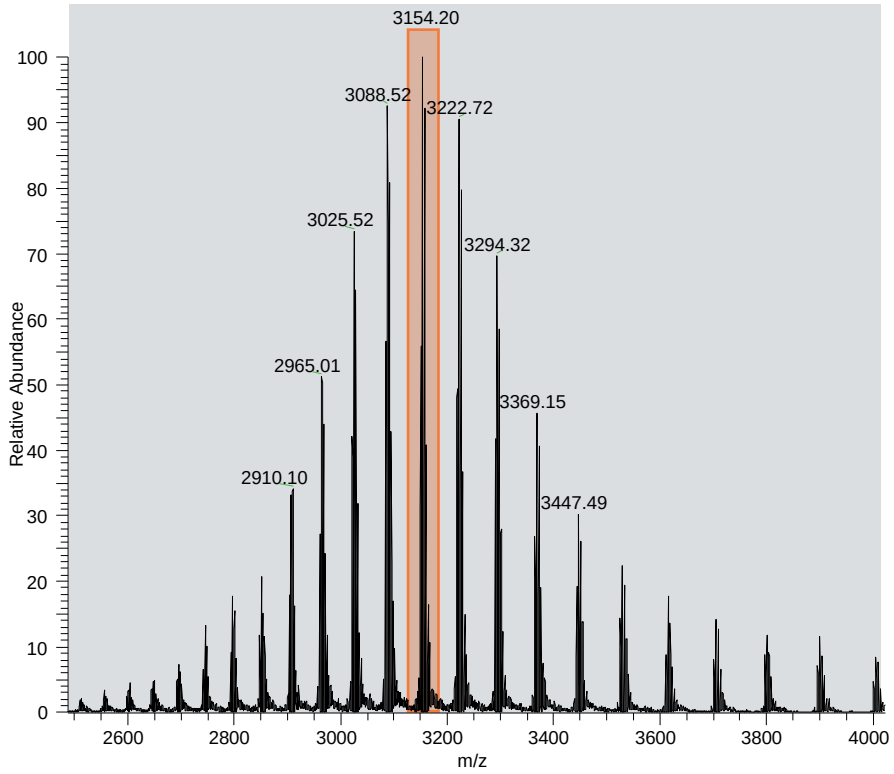
- Intact determination of charge heterogeneity, mass, and glycoform information in a single analysis
- Fully native MS mAb characterization
- Reduced and Subunit analysis
- Peptide Mapping
- Direct analysis from bioreactors
- Small Molecules
- Oligonucleotides



Intact Denatured Antibody Analysis

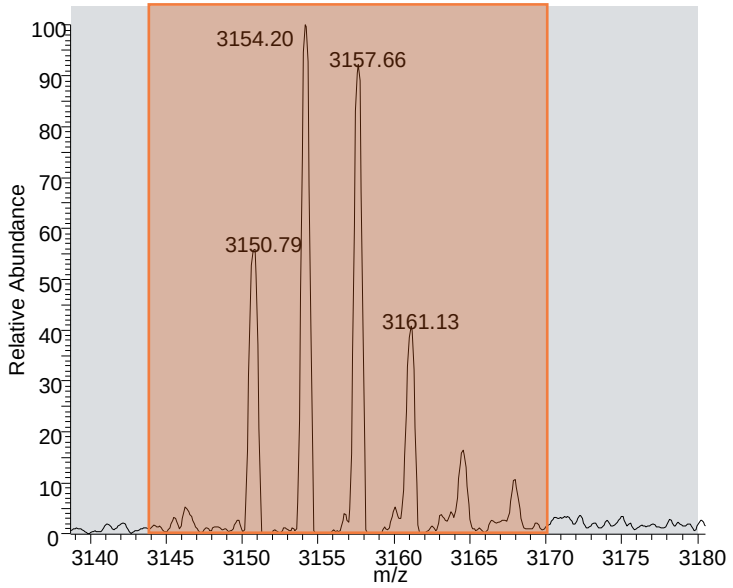
NIST mAb 1 mg/mL
ZipChip HS
Metabolite BGE
Fusion Lumos





mAb unfolds during analysis yielding higher charge states and no separation of charge variants

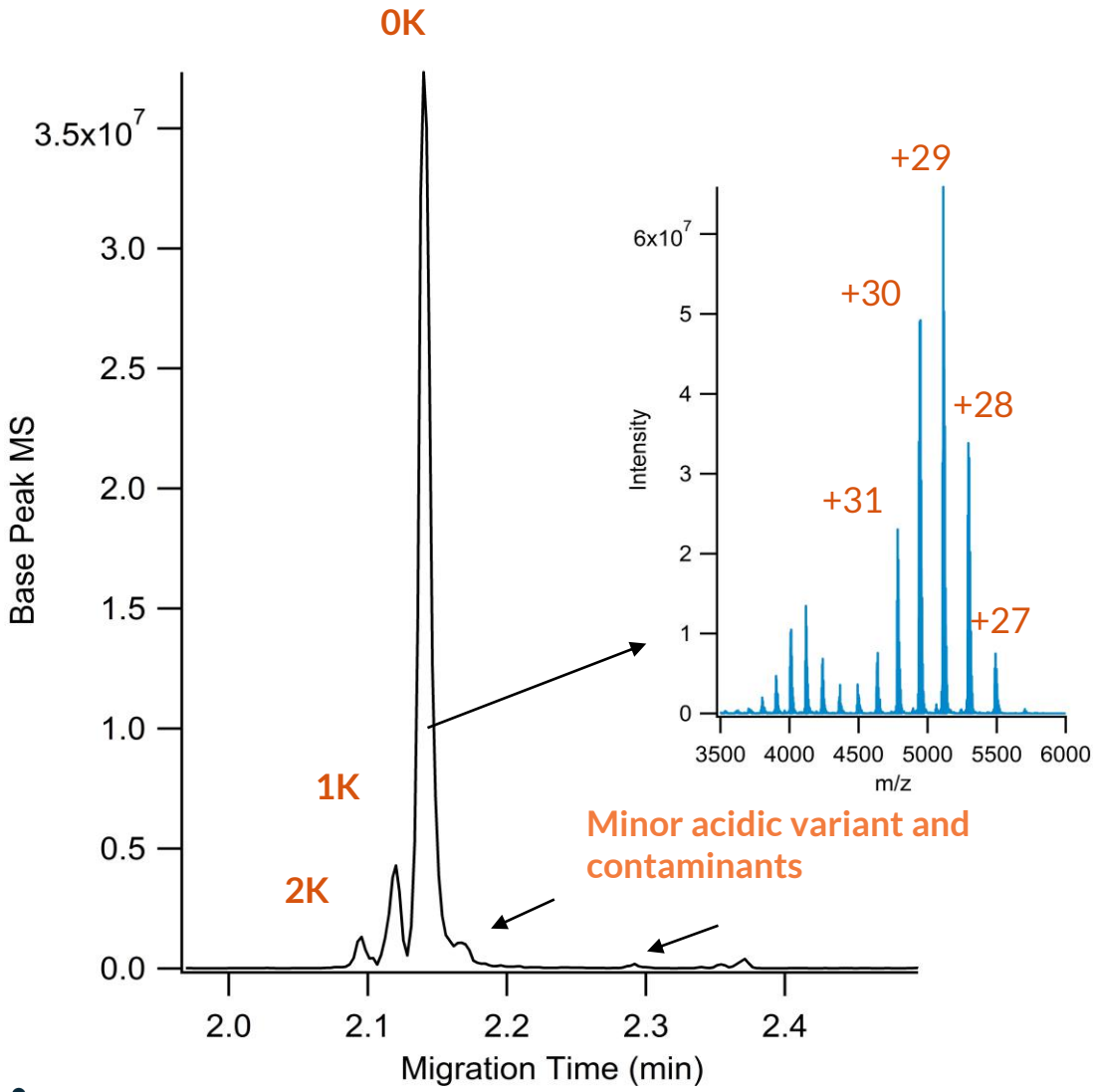
+47 charge state



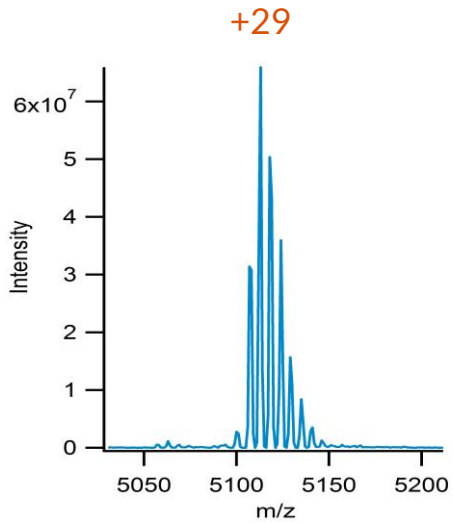
Accurate mass of most abundant variants, but less abundant variants may be lost

Intact Near-Native Antibody Analysis

Simultaneously assess charge heterogeneity, mass and glycoforms



Minor acidic variant and contaminants



Glycoform resolution within charge states

NIST mAb 0.5 mg/mL*
ZipChip HR
Intact Antibody BGE
Thermo Exactive Plus EMR

*The NIST mAb was diluted directly from formulation buffer for analysis.

Multiple charge variants and contaminants are separated and identified by their change in mobility as well as the mass determined from the MS data.

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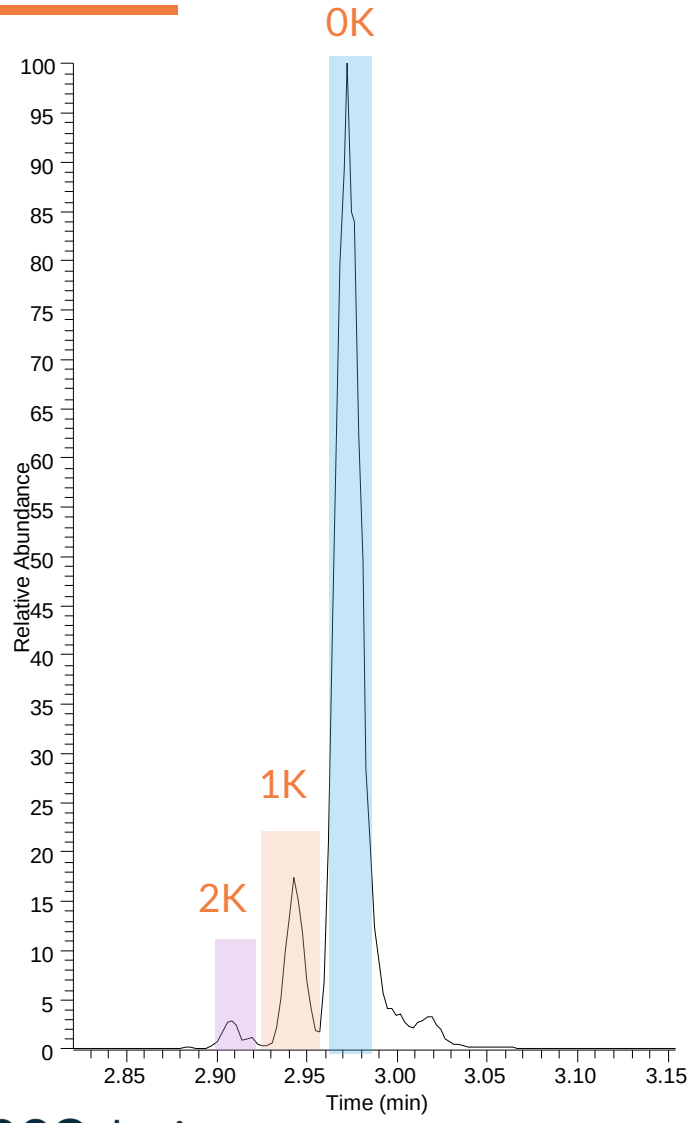
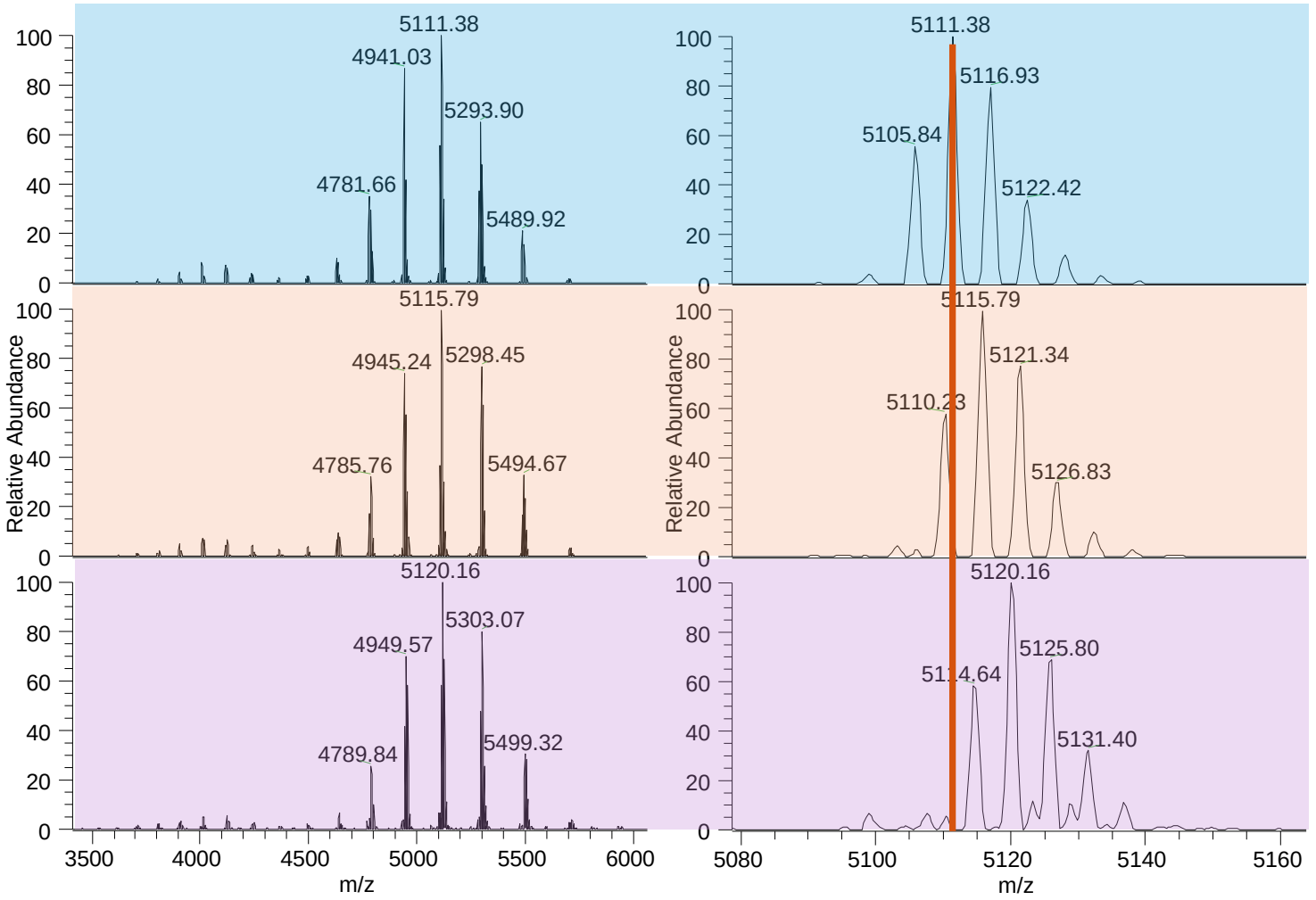
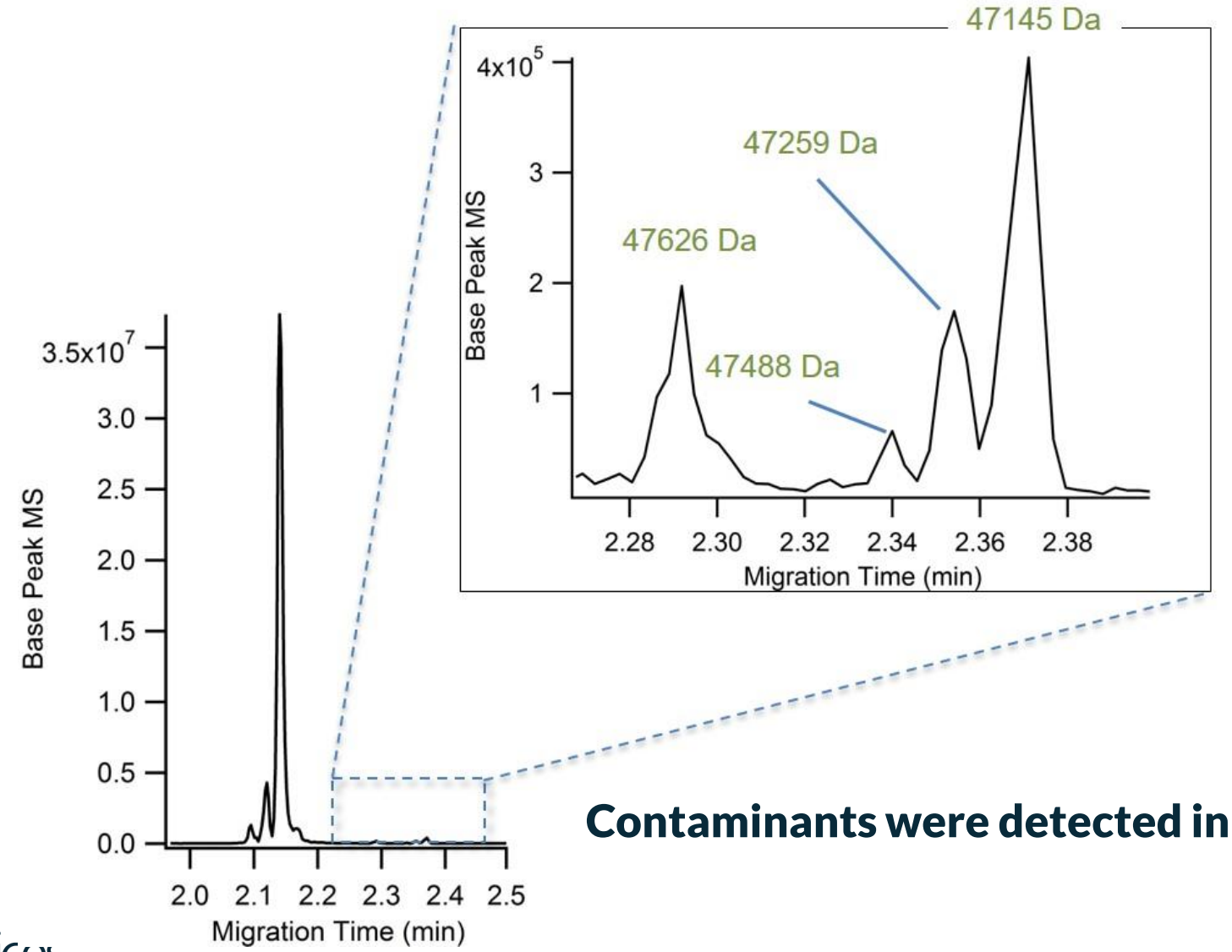
Intact Near-Native - Mass Shift



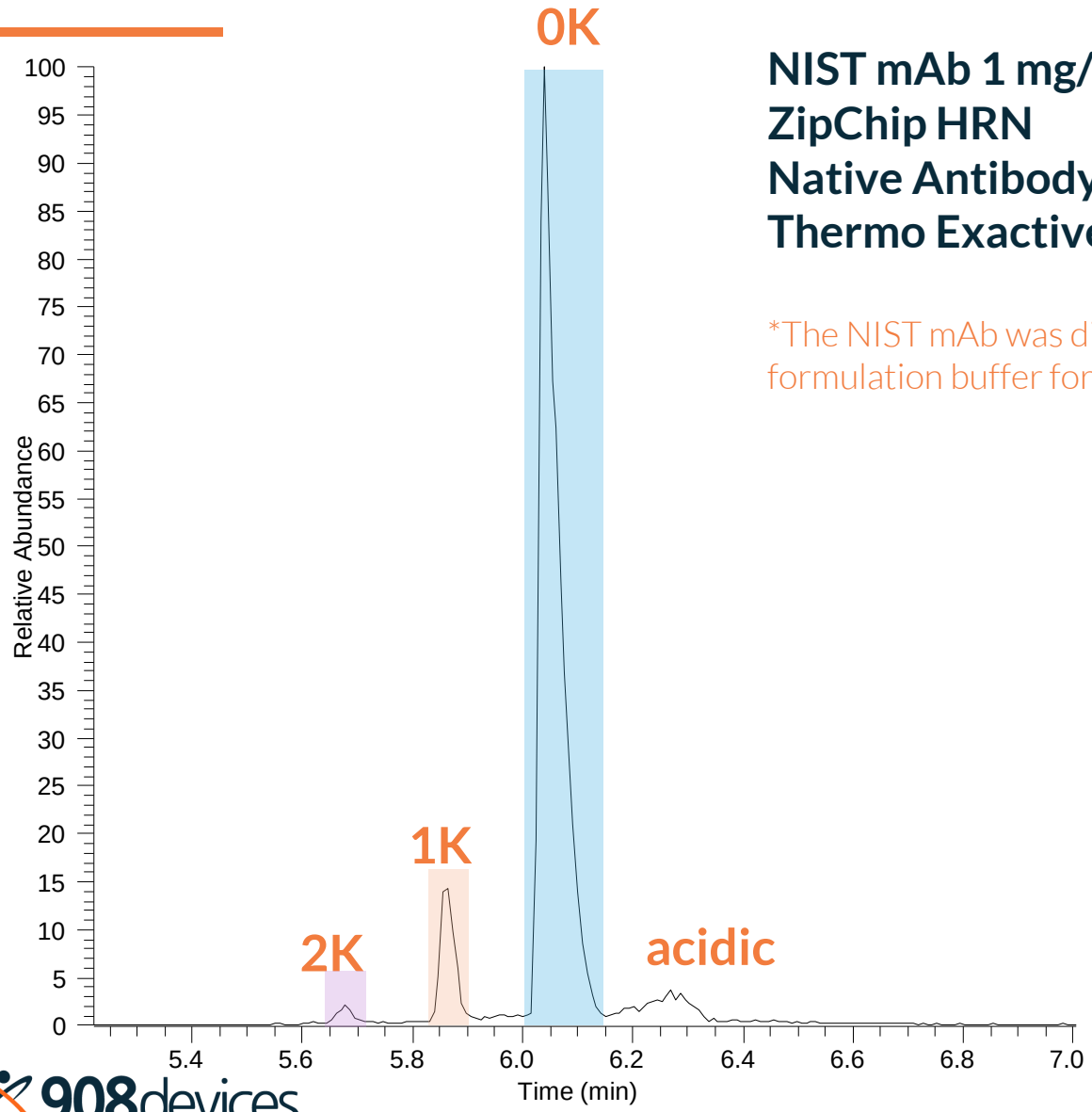


Resolving Power - Contaminants



Contaminants were detected in the NIST mAb formulation

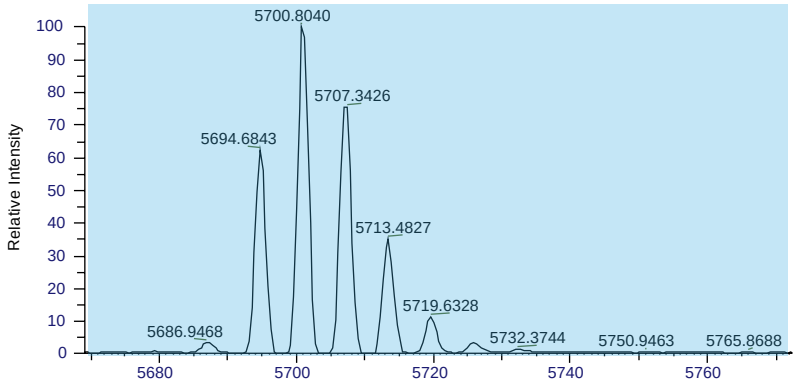
Native NIST mAb



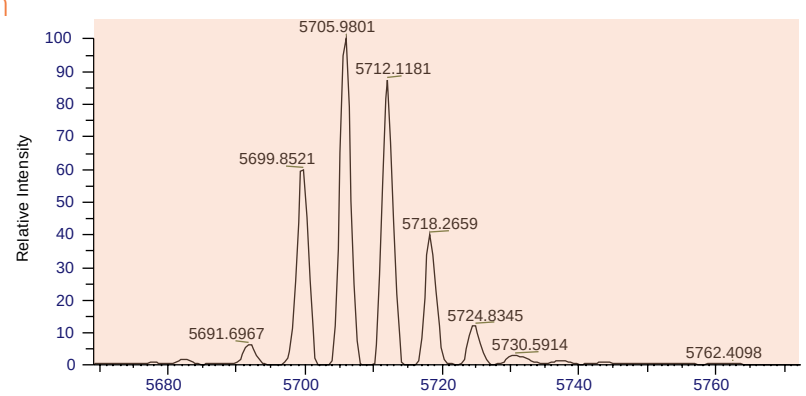
NIST mAb 1 mg/mL*
ZipChip HRN
Native Antibody BGE
Thermo Exactive Plus EMR

*The NIST mAb was diluted directly from formulation buffer for analysis.

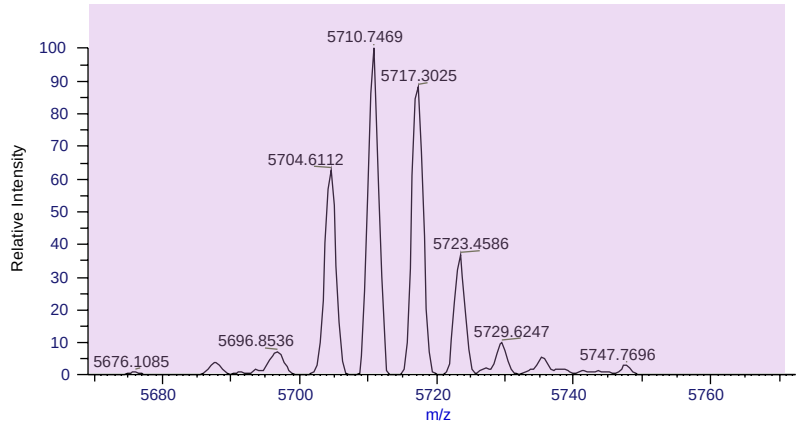
+26 Charge State



0K

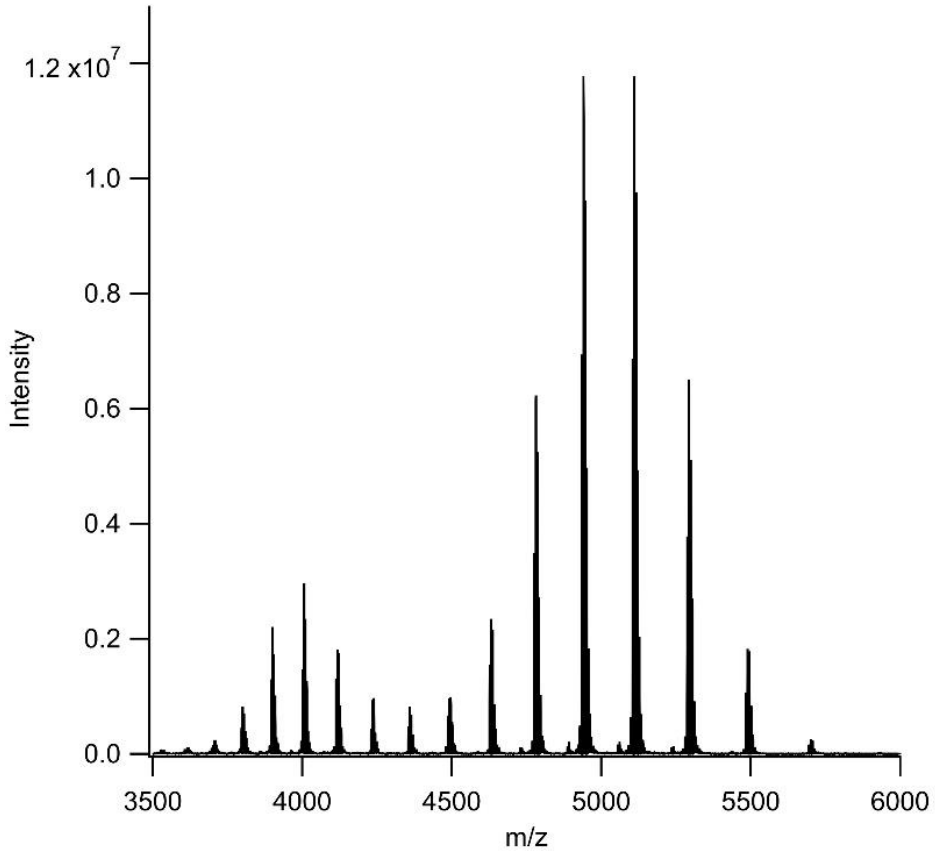


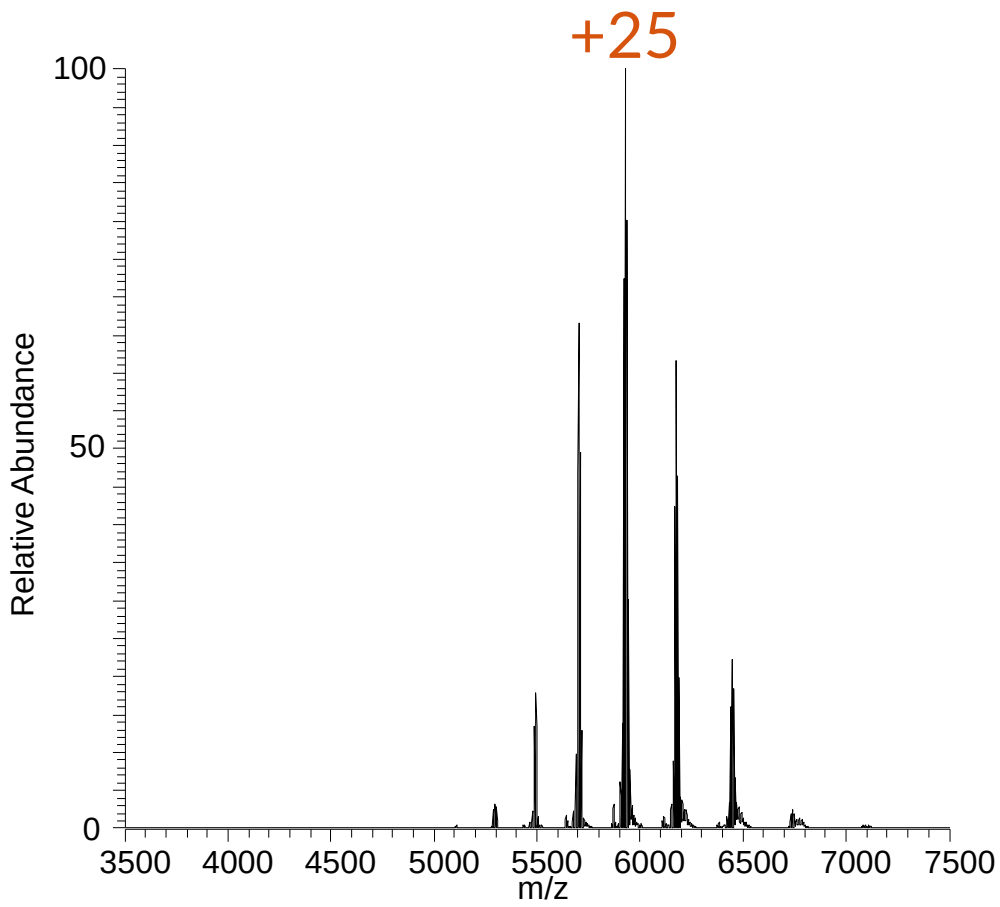
1K



2K

Native NIST mAb - Mass Spectra





Generate Fully Native Mass Spectra

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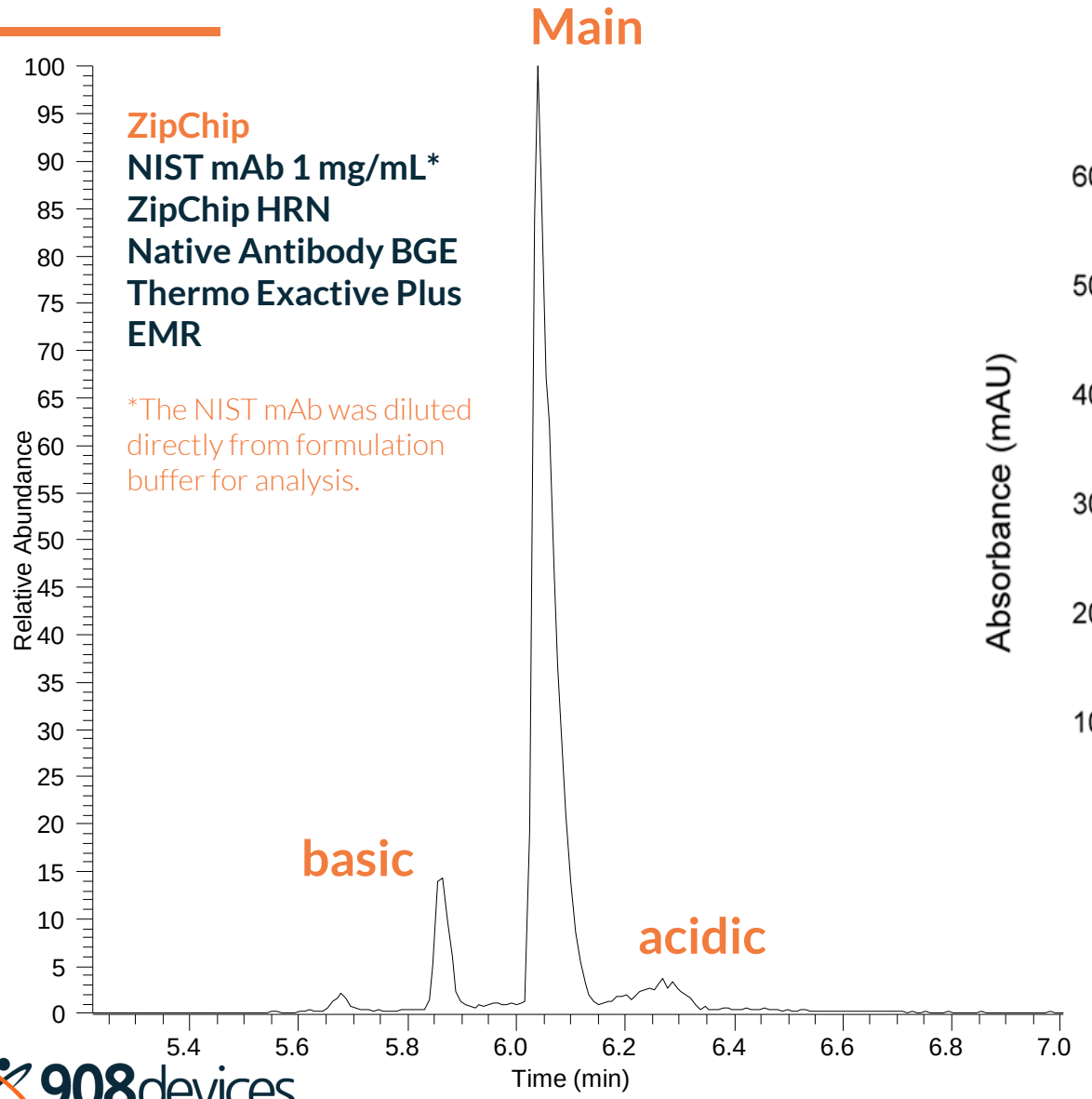
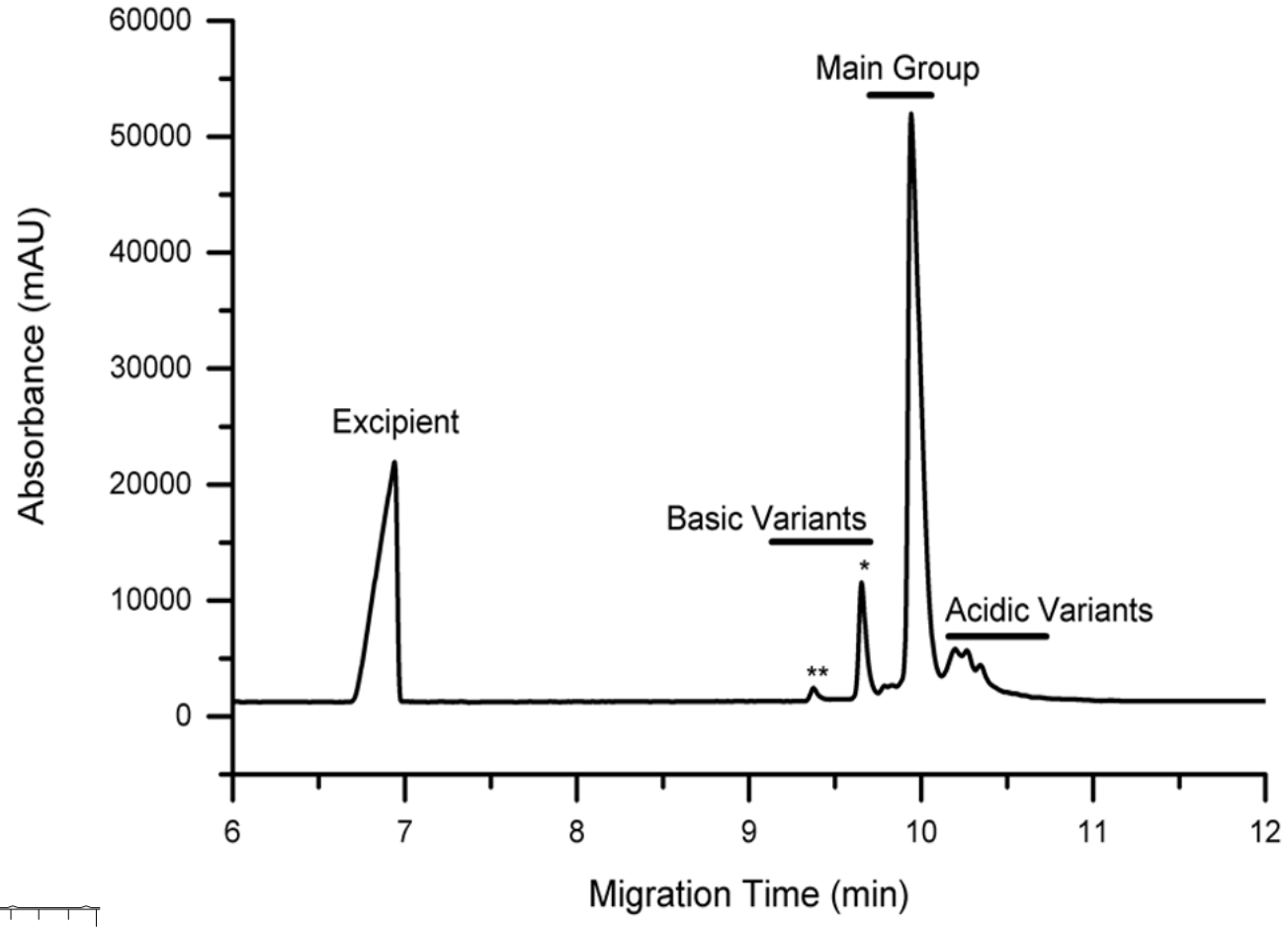
Orthogonal Method Comparison – NIST mAb



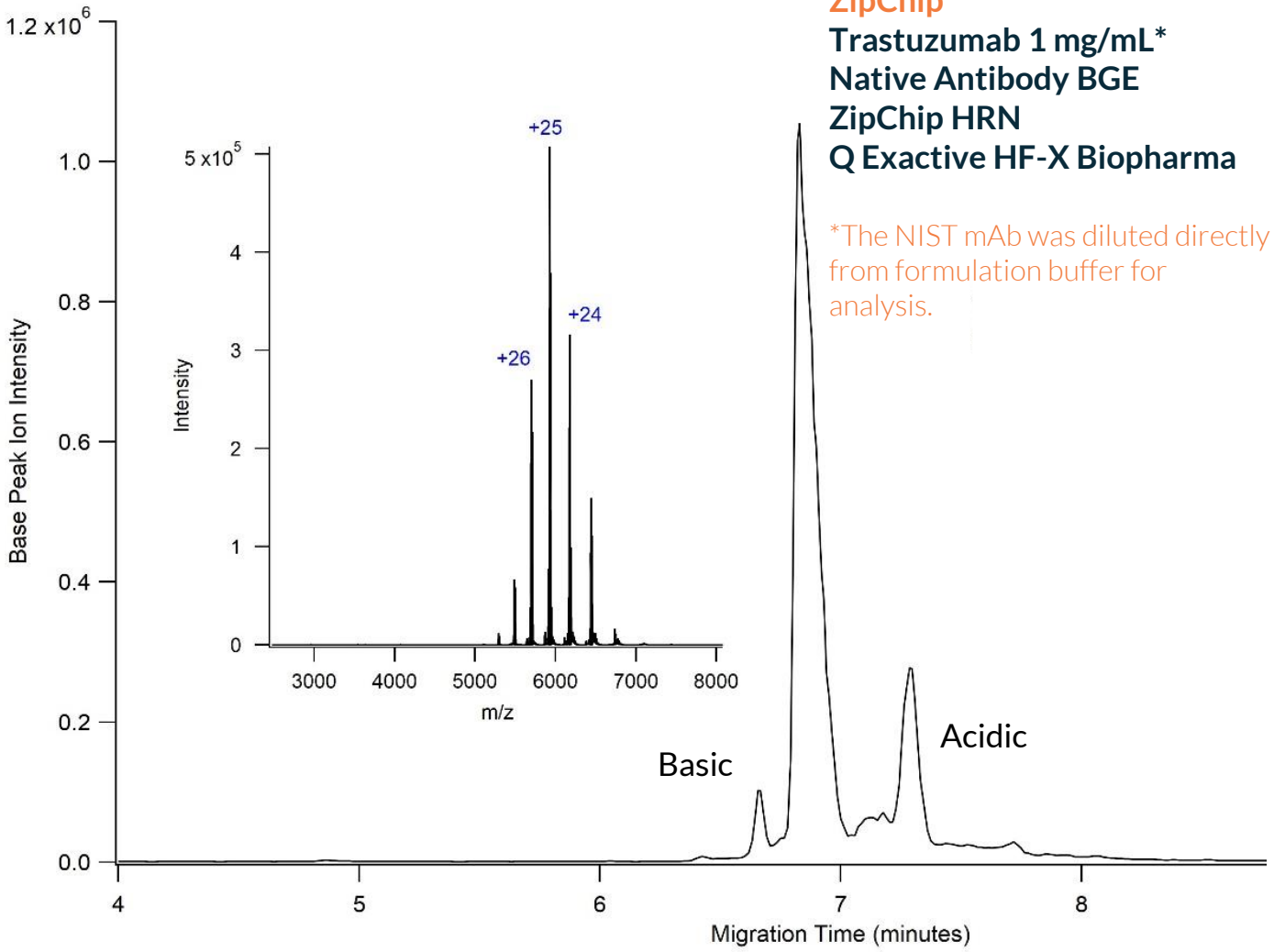
CZE charge variant analysis (UV absorbance detection) from the NIST Report of Investigation for the NIST mAb (RM 8671)

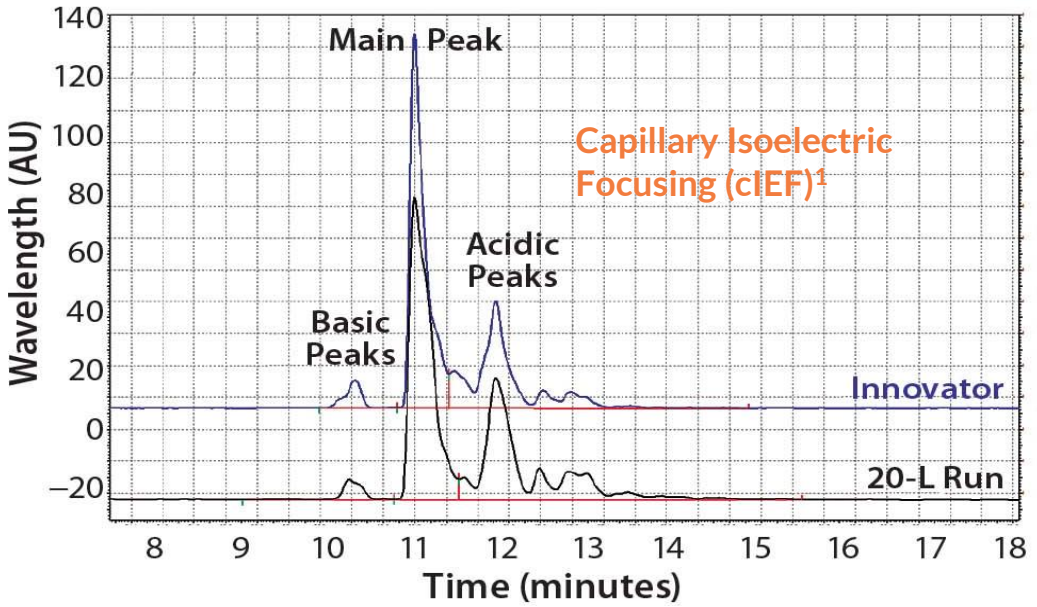


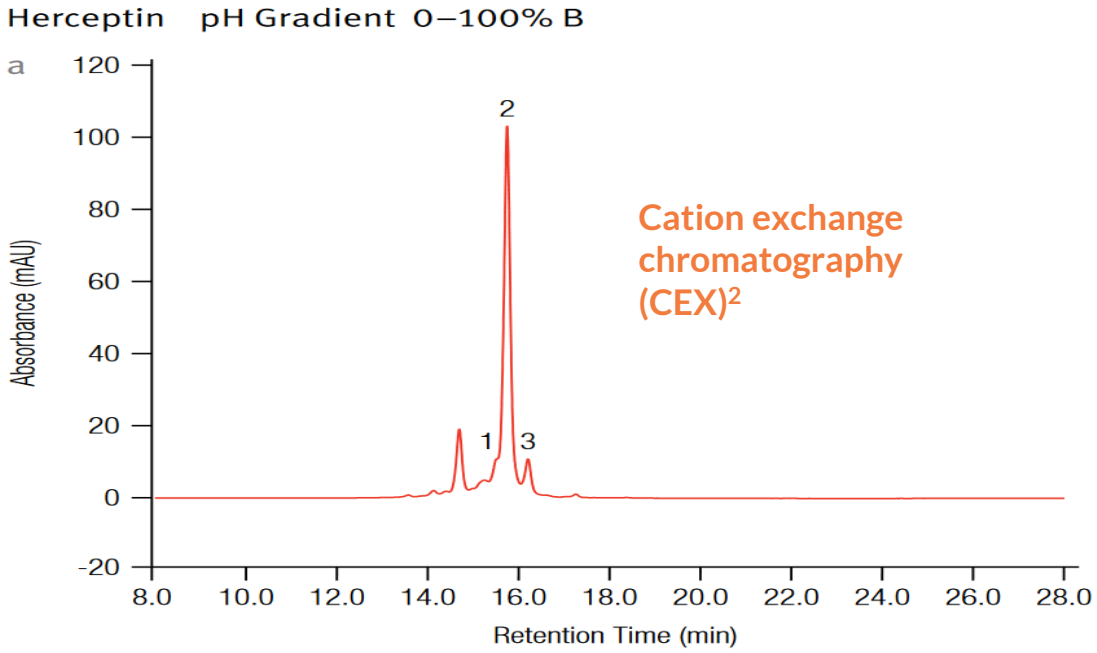
Method Comparison - Trastuzumab

ZipChip
Trastuzumab 1 mg/mL*
Native Antibody BGE
ZipChip HRN
Q Exactive HF-X Biopharma

*The NIST mAb was diluted directly from formulation buffer for analysis.







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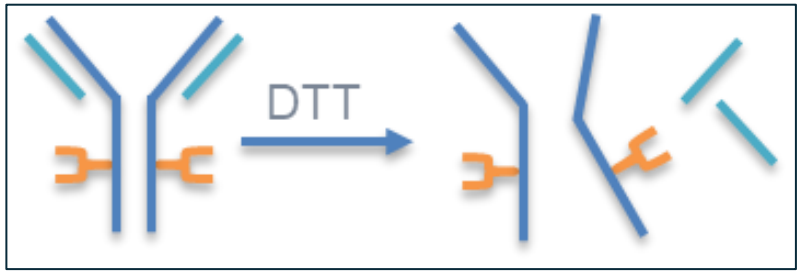
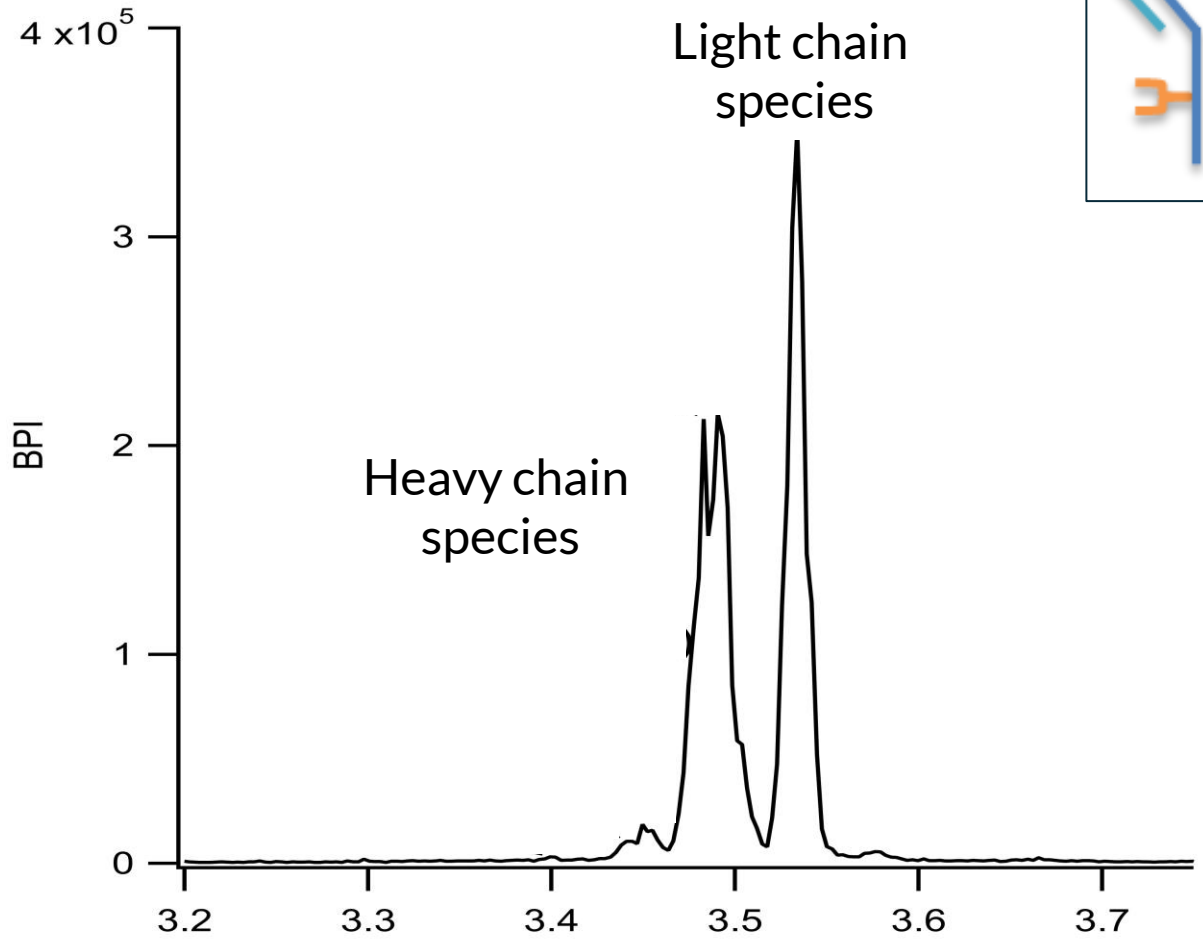
[1]Girod et al., Bioprocess International, April 2015
[2]Thermo Fisher Application Note No. 21092

Reduced NIST mAb





The light chain and heavy chain of the mAb separated in less than 4 minutes

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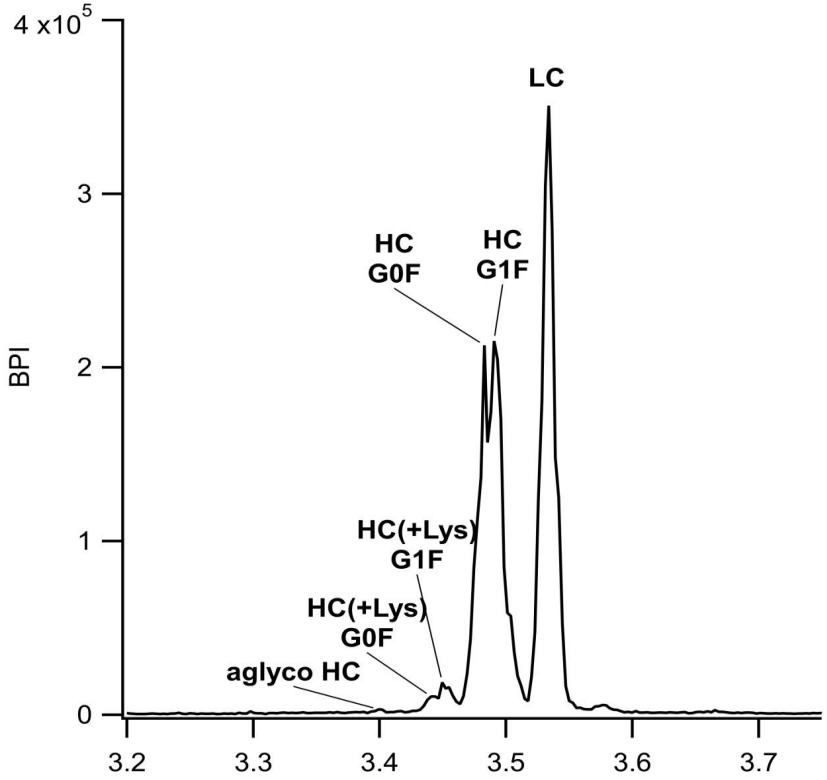
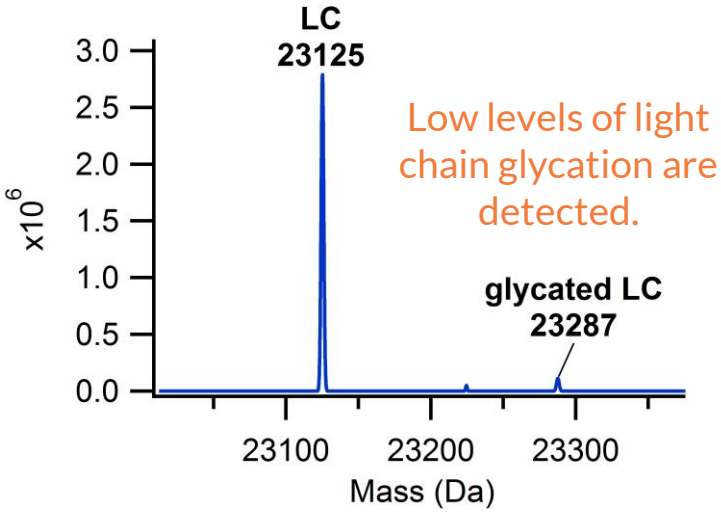
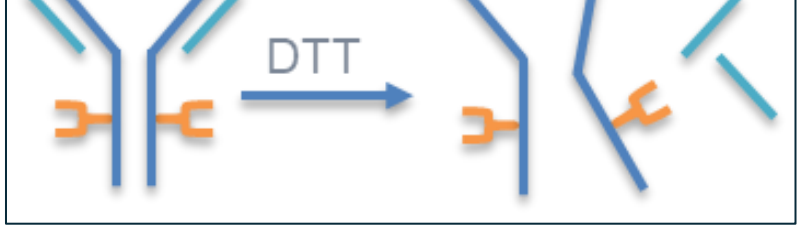
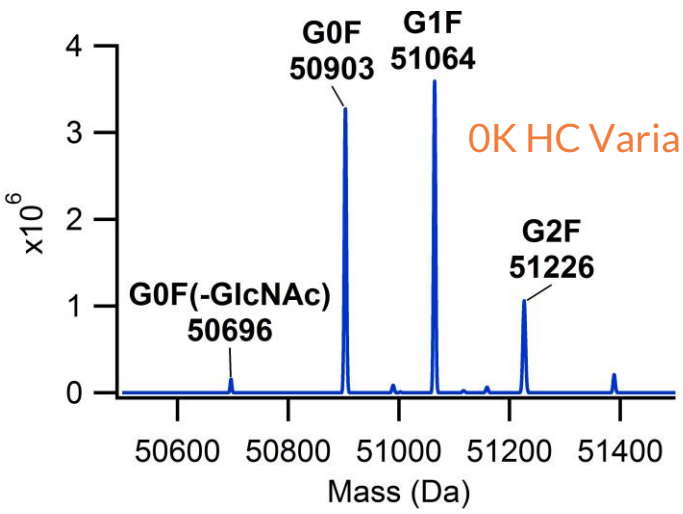
Reduced - Identification of Variants





Low levels of light chain glycation are detected.





0K HC Variant

Several different HC variants are detected in the separation and deconvoluted spectra

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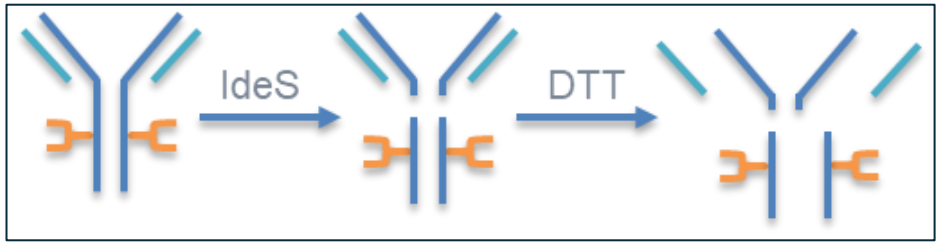
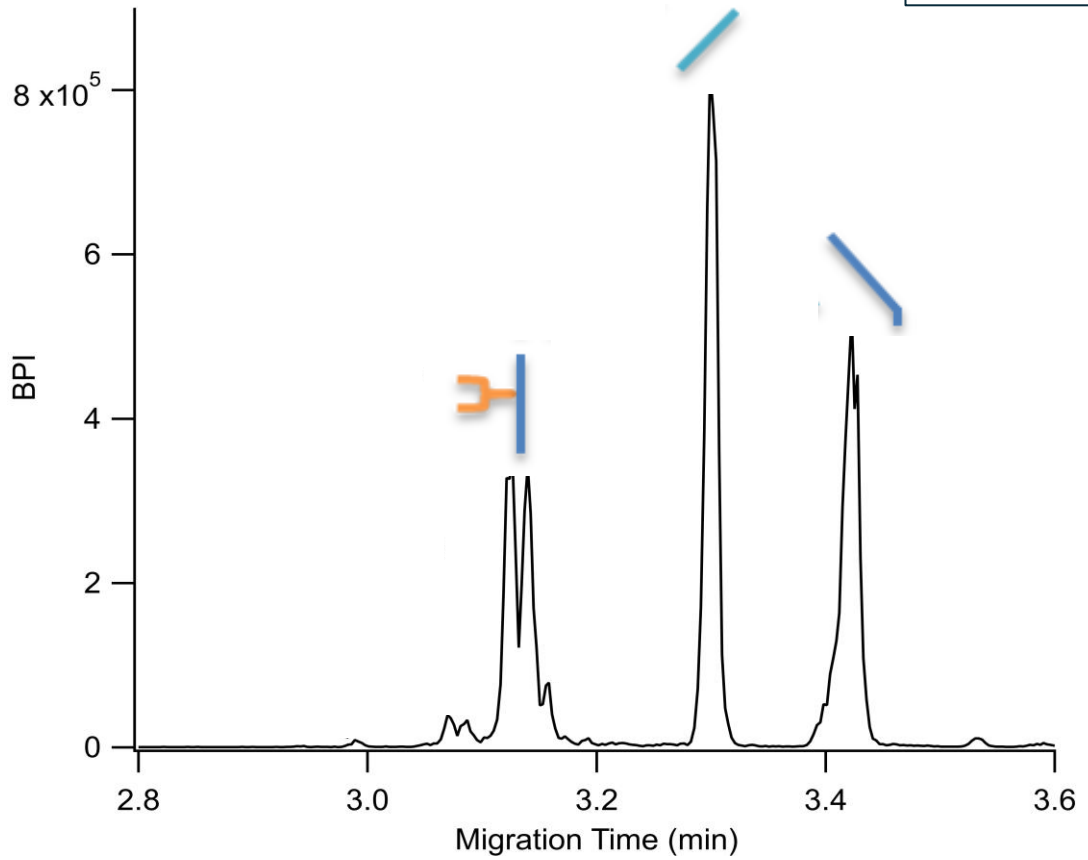
Subunit NIST mAb





Baseline resolution between mAb fragments achieved in less than 4 minutes

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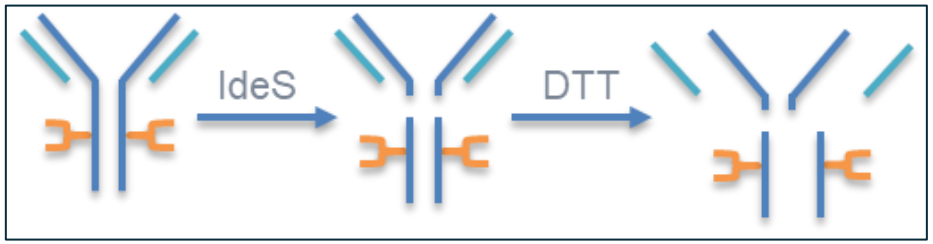
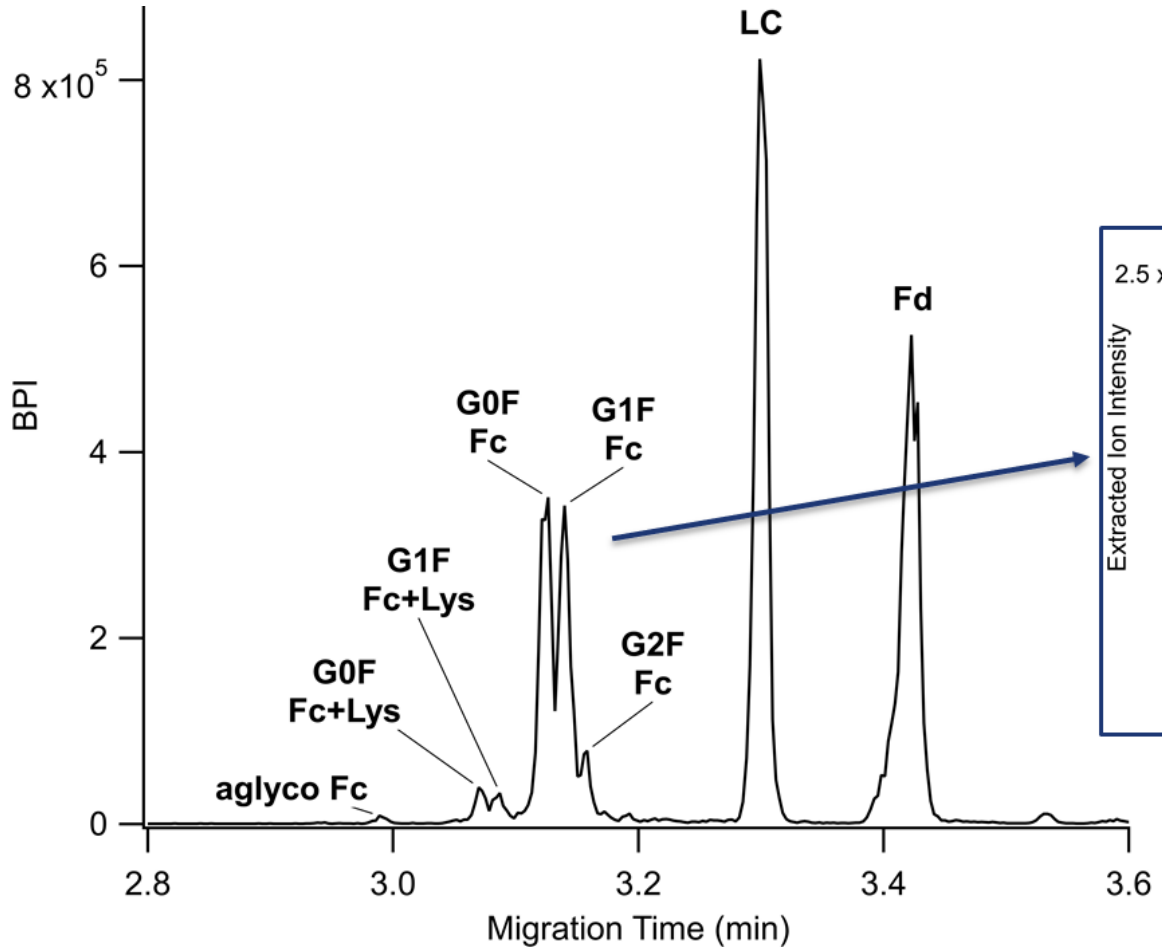
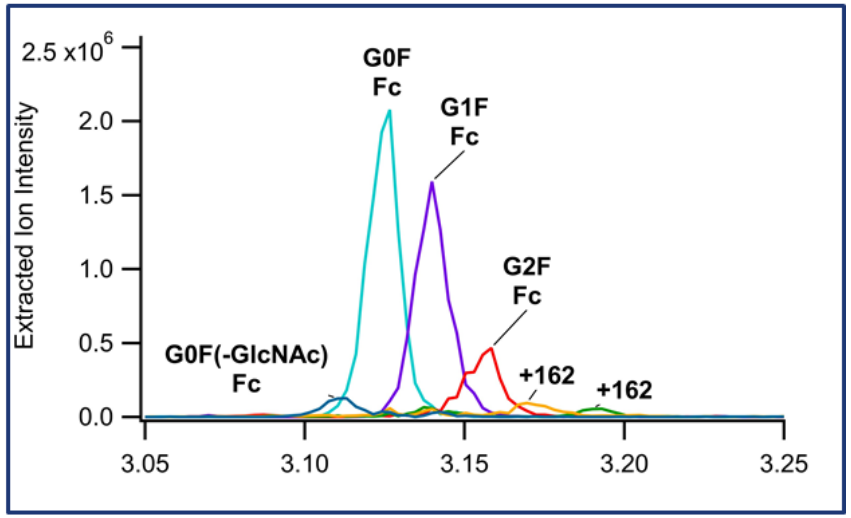
Subunit – Separation of Fc Glycoforms

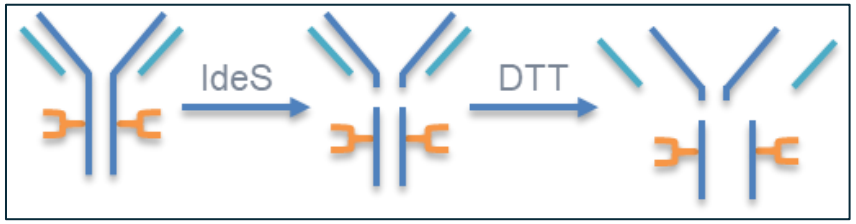


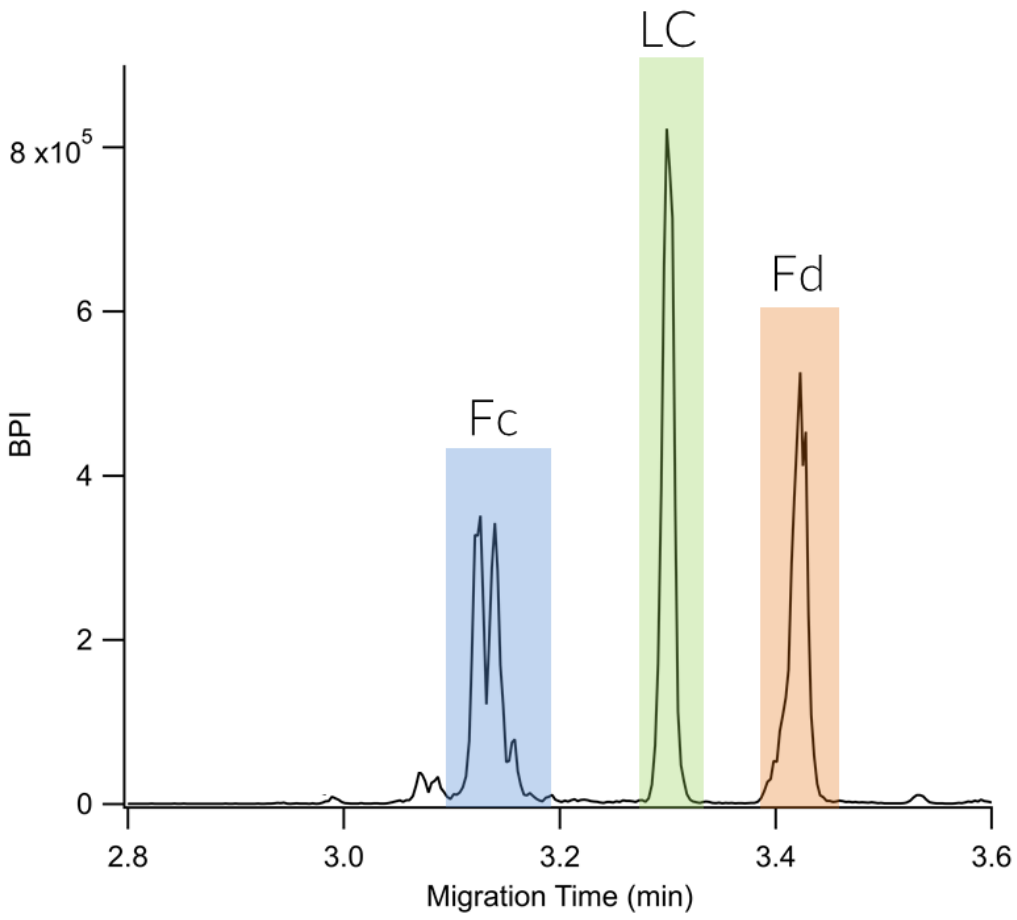


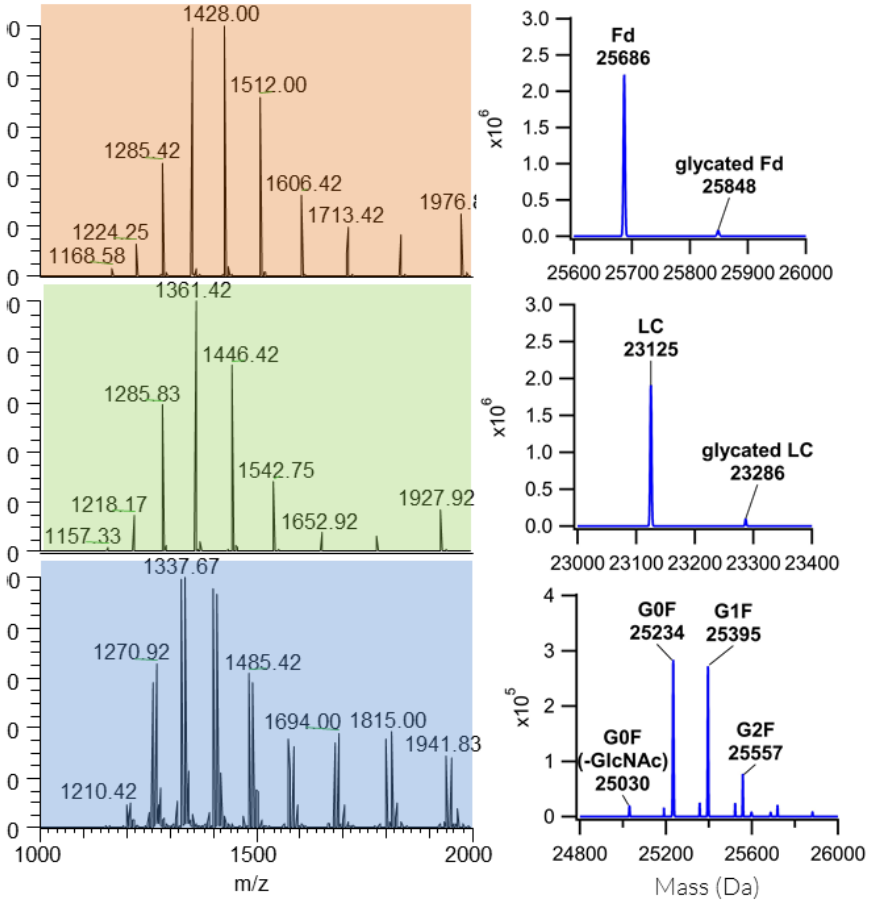


Achieving separation between different glycoforms of the Fc fragment >4 minutes

Subunit - Characterization







Observation of glycation on light chain and the FD region not observed at intact level

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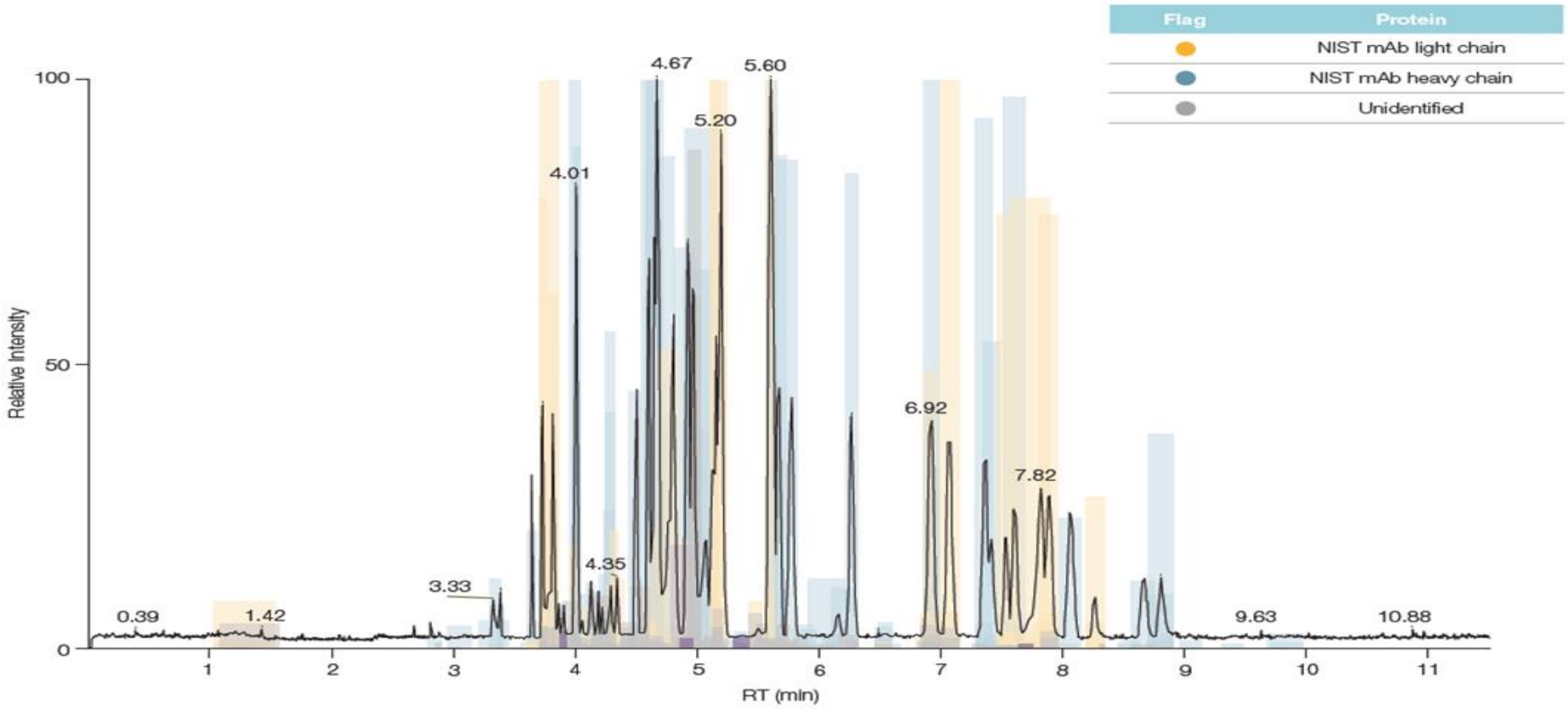
Peptide Mapping



Proteins	Number of MS Peaks	MS Peak Area	Sequence Coverage	Abundance (mol)
NSIT mAb light chain	141	26.4%	100.0%	41.67%
NSIT mAb heavy chain	339	60.5%	97.6%	56.35%
Unidentified	1441	12.6%		

Fast (≥5x reduction in run time) and 98% sequence coverage

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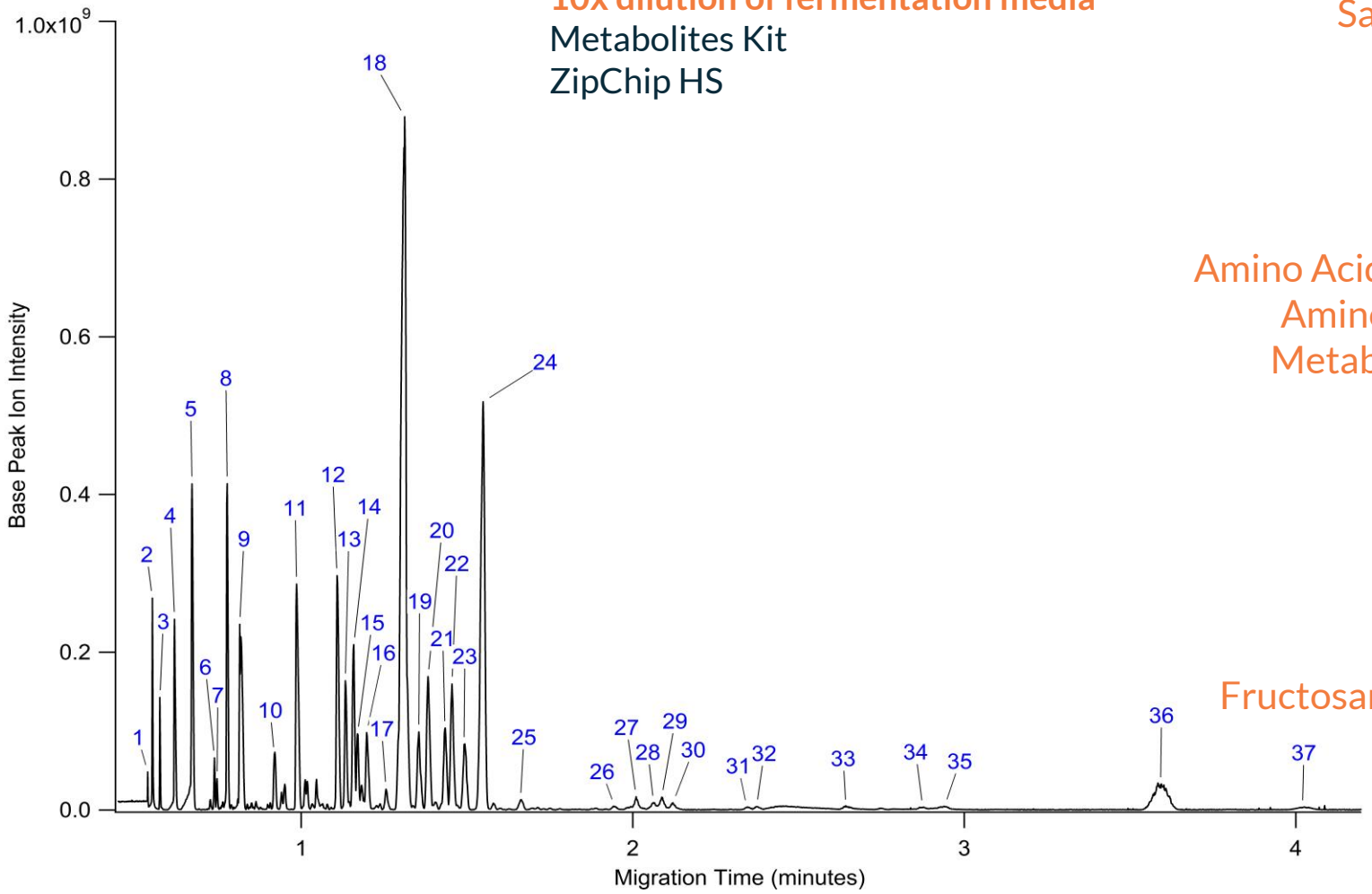
Growth Media Analysis

10x dilution of fermentation media
Metabolites Kit
ZipChip HS



Biogenic Amines, Salt Ions

Amino Acids and Amino Acid Metabolites

Fructosamines

Peak #	Migration Time (minutes)	m/z	Assignment
1	0.54	89.1081	putrescine
2	0.55	131.1296	agmatine
3	0.57	120.9813	calcium adducts
4	0.62	105.0036	salt adducts
5	0.67	104.1075	choline
6	0.74	147.1120	lysine
7	0.75	133.0976	ornithine
8	0.78	175.1184	arginine
9	0.82	156.0760	histidine
10	0.92	76.0398	glycine
11	0.99	90.0555	alanine
12	1.11	118.0861	valine
13	1.13	132.1025	isoleucine
14	1.16	132.1025	leucine
15	1.17	130.0865	pipecolic acid
16	1.20	244.0927	cytidine
17	1.26	120.0654	threonine
18	1.31	116.0711	proline
19	1.36	205.0962	tryptophan
20	1.38	148.0608	glutamic acid
21	1.43	268.1039	adenosine
22	1.45	182.0807	tryosine
23	1.49	134.0450	aspartic acid
24	1.55	118.0862	betaine
25	1.66	124.0399	picolinic acid
26	1.94	238.0923	N-(1-Deoxy-1-fructosyl)glycine
27	2.01	252.1082	N-(1-Deoxy-1-fructosyl)alanine
28	2.06	294.1534	N-(1-Deoxy-1-fructosyl)isoleucine
29	2.09	294.1534	N-(1-Deoxy-1-fructosyl)leucine
30	2.12	280.1374	N-(1-Deoxy-1-fructosyl)valine
31	2.35	268.1042	N-(1-Deoxy-1-fructosyl)serine
32	2.37	328.1379	N-(1-Deoxy-1-fructosyl)phenylalanine
33	2.64	295.1134	distichonic acid
34	2.87	268.1038	Deoxyguanosine
35	2.94	278.1243	N-(1-Deoxy-1-fructosyl)proline
36	3.58	284.0972	guanosine
37	4.03	137.0457	Hypoxanthine

Rapidly interrogate growth media components

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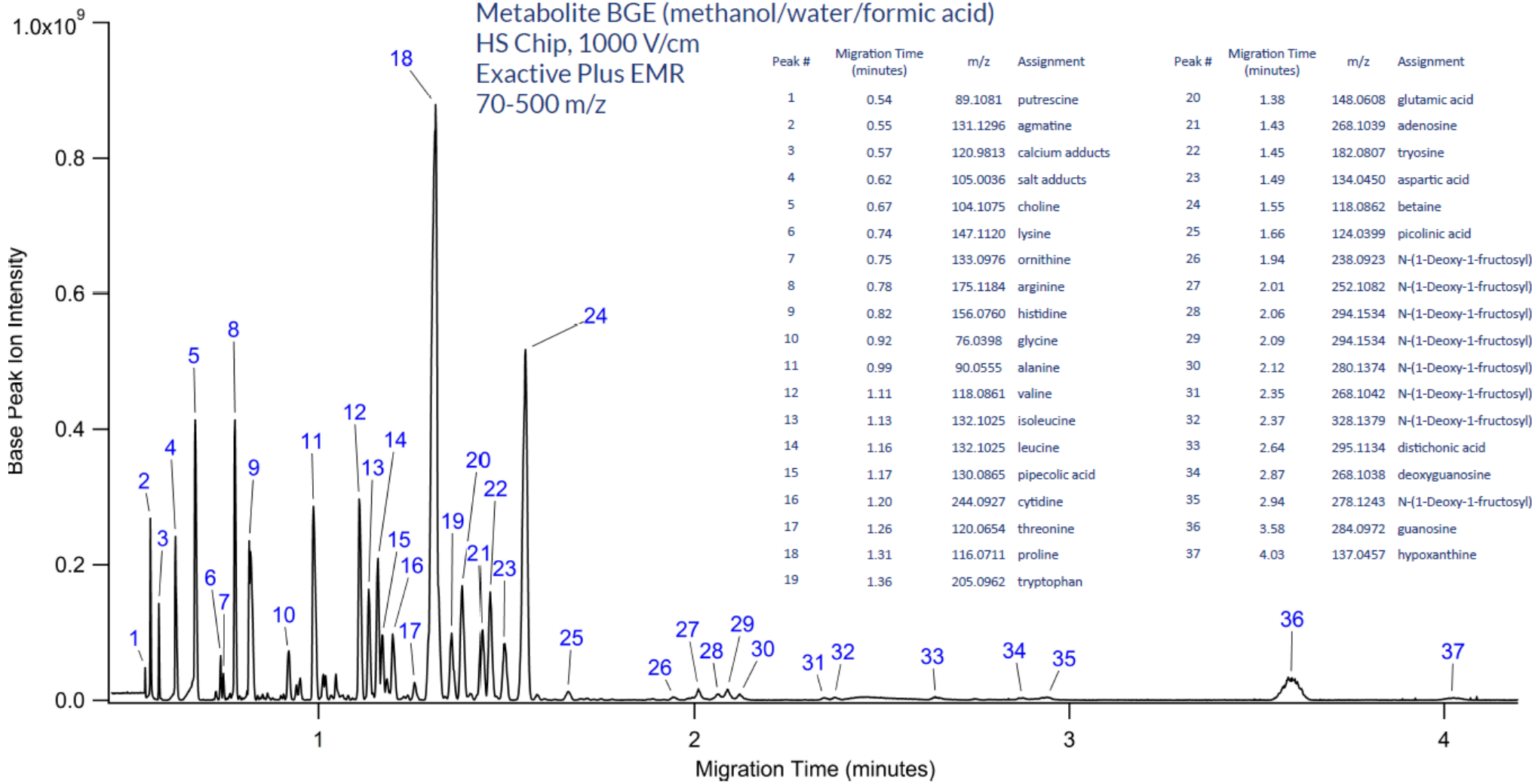
Beer Analysis

Beer diluted 10x with ZipChip metabolite kit sample diluent (2 nL injected)
Metabolite BGE (methanol/water/formic acid)
HS Chip, 1000 V/cm
Exactive Plus EMR
70-500 m/z



Peak #	Migration Time (minutes)	m/z	Assignment	Peak #	Migration Time (minutes)	m/z	Assignment
1	0.54	89.1081	putrescine	20	1.38	148.0608	glutamic acid
2	0.55	131.1296	agmatine	21	1.43	268.1039	adenosine
3	0.57	120.9813	calcium adducts	22	1.45	182.0807	tryosine
4	0.62	105.0036	salt adducts	23	1.49	134.0450	aspartic acid
5	0.67	104.1075	choline	24	1.55	118.0862	betaine
6	0.74	147.1120	lysine	25	1.66	124.0399	picolinic acid
7	0.75	133.0976	ornithine	26	1.94	238.0923	N-(1-Deoxy-1-fructosyl)glycine
8	0.78	175.1184	arginine	27	2.01	252.1082	N-(1-Deoxy-1-fructosyl)alanine
9	0.82	156.0760	histidine	28	2.06	294.1534	N-(1-Deoxy-1-fructosyl)isoleucine
10	0.92	76.0398	glycine	29	2.09	294.1534	N-(1-Deoxy-1-fructosyl)leucine
11	0.99	90.0555	alanine	30	2.12	280.1374	N-(1-Deoxy-1-fructosyl)valine
12	1.11	118.0861	valine	31	2.35	268.1042	N-(1-Deoxy-1-fructosyl)serine
13	1.13	132.1025	isoleucine	32	2.37	328.1379	N-(1-Deoxy-1-fructosyl)phenylalanine
14	1.16	132.1025	leucine	33	2.64	295.1134	distichonic acid
15	1.17	130.0865	pipecolic acid	34	2.87	268.1038	deoxyguanosine
16	1.20	244.0927	cytidine	35	2.94	278.1243	N-(1-Deoxy-1-fructosyl)proline
17	1.26	120.0654	threonine	36	3.58	284.0972	guanosine
18	1.31	116.0711	proline	37	4.03	137.0457	hypoxanthine
19	1.36	205.0962	tryptophan				

Complex Matrix – Minimal Sample Prep

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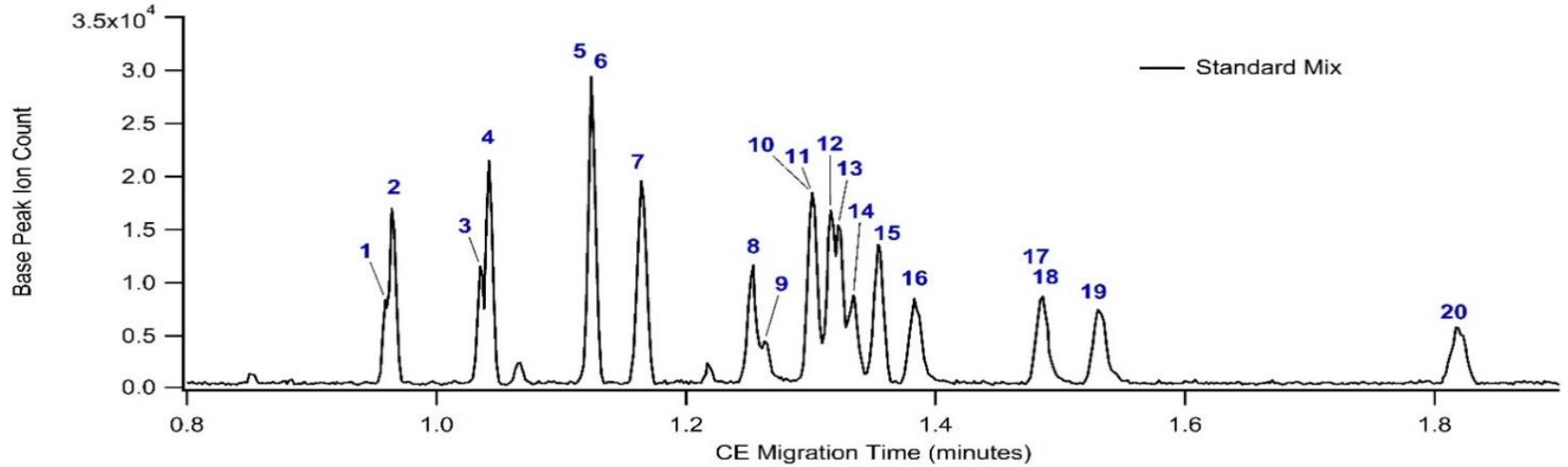
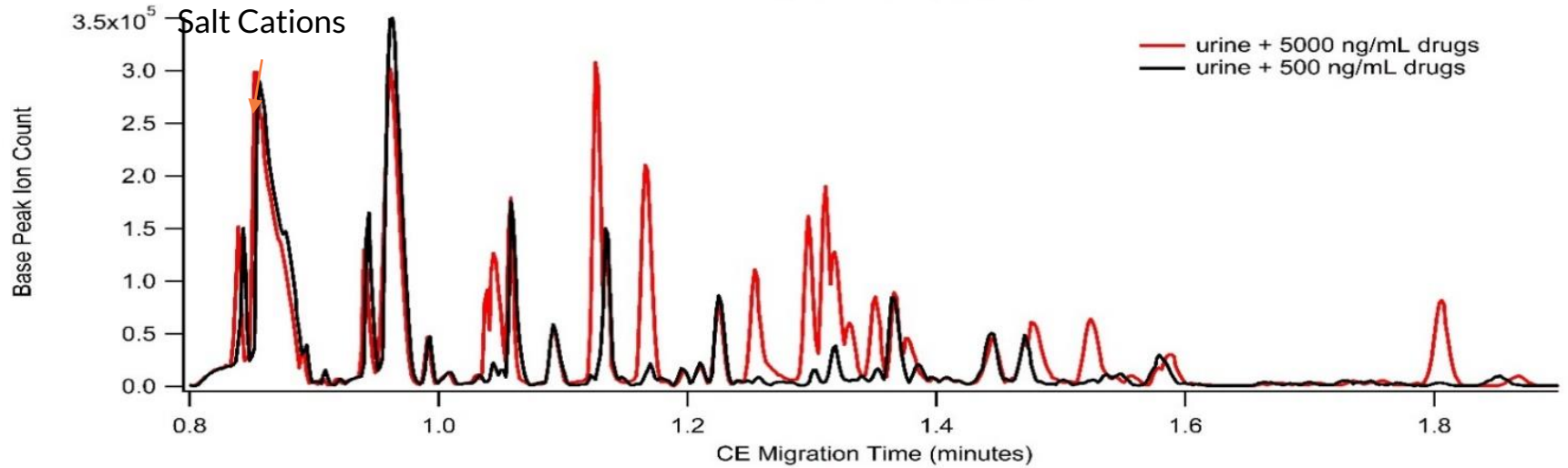
Drug Standards Spiked into Water and Urine





Peak #	Analyte	LOD (ng/mL)
1	(±)-Amphetamine	1.3
2	Phentermine	0.9
3	MDA	1.5
4	(±)-MDMA	0.6
5	(±)-Methamphetamine	2.1
6	(±)-MDEA	1.3
7	Meperidine	0.5
8	Codeine	1.9
10	Hydrocodone	1.2
11	Morphine	1.0
12	cis-Tramadol HCl	1.0
13	(±)-Methadone	10.0
14	Oxycodone	1.7
15	Hydromorphone	0.9
16	Oxymorphone	2.9
17	Naloxone	3.2
18	Fentanyl	2.5
19	Naltrexone	4.1
20	Buprenorphine	3.1

Low Level Detection in Urine

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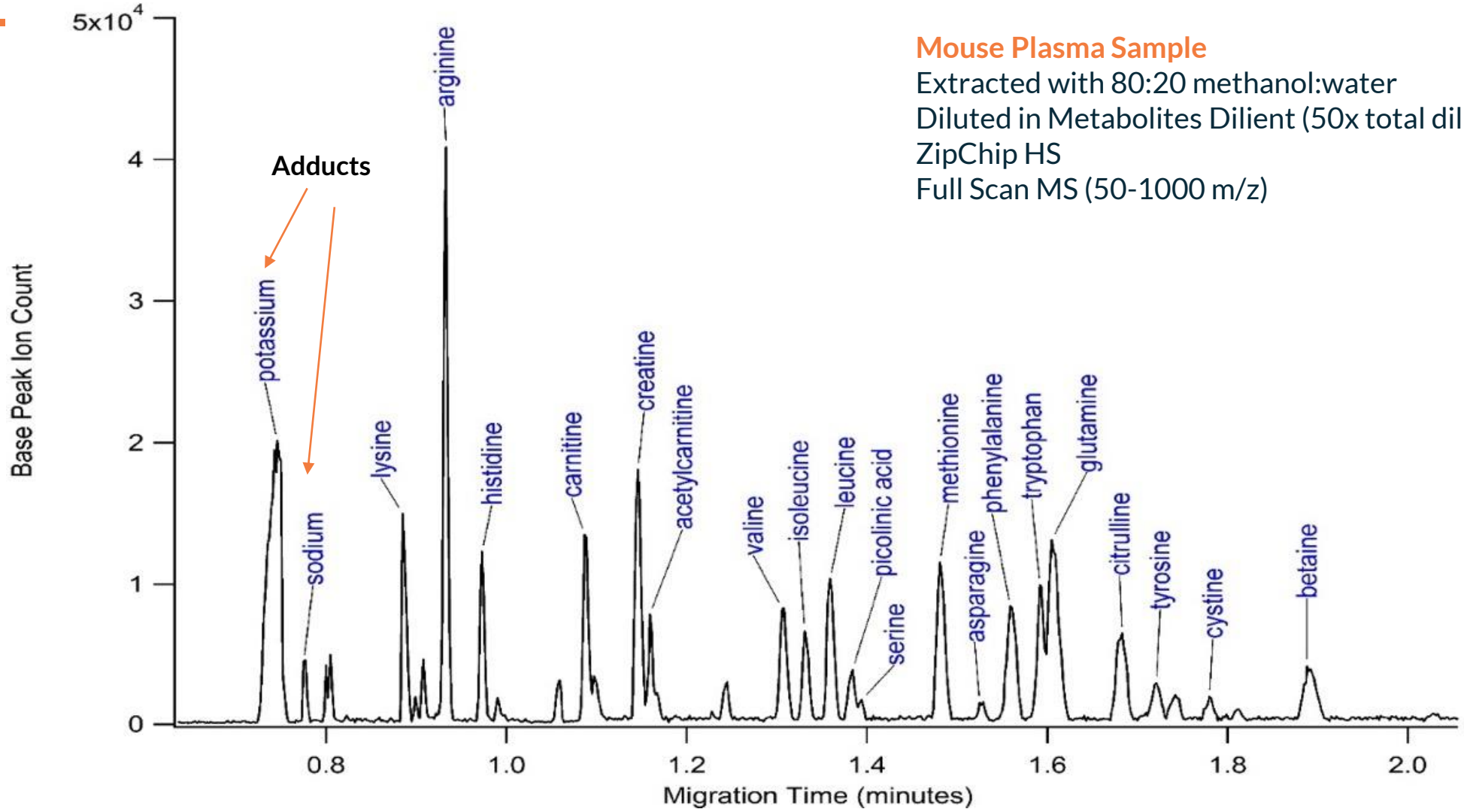
Metabolomics in Mouse Plasma



Mouse Plasma Sample
Extracted with 80:20 methanol:water
Diluted in Metabolites Dilient (50x total dilution)
ZipChip HS
Full Scan MS (50-1000 m/z)

Minimal Sample Preparation

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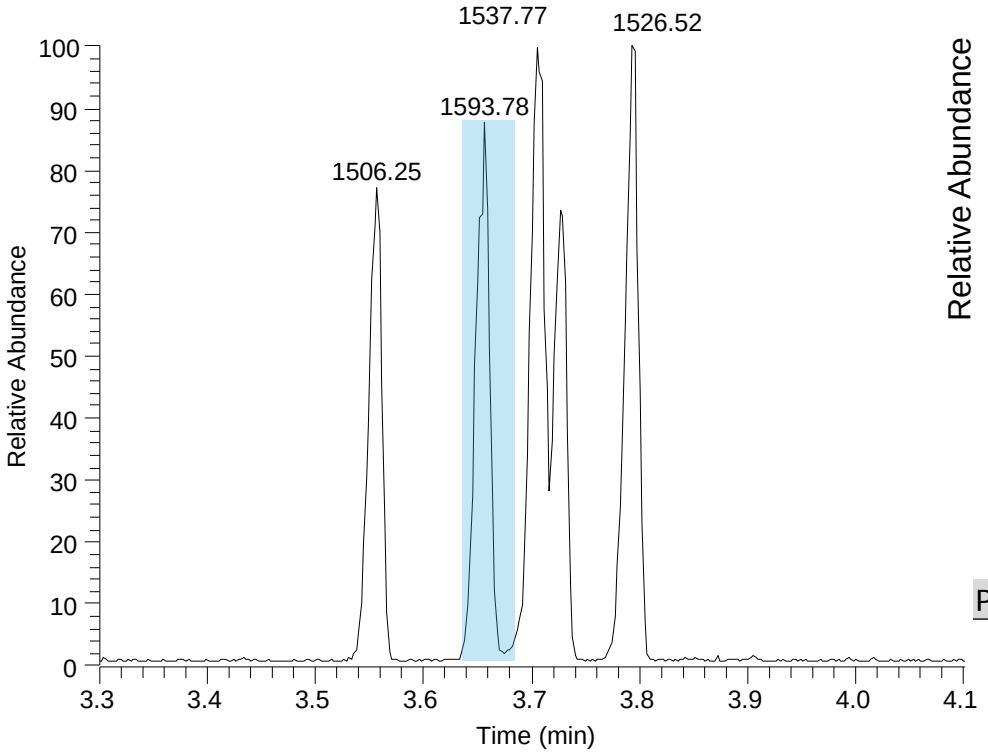
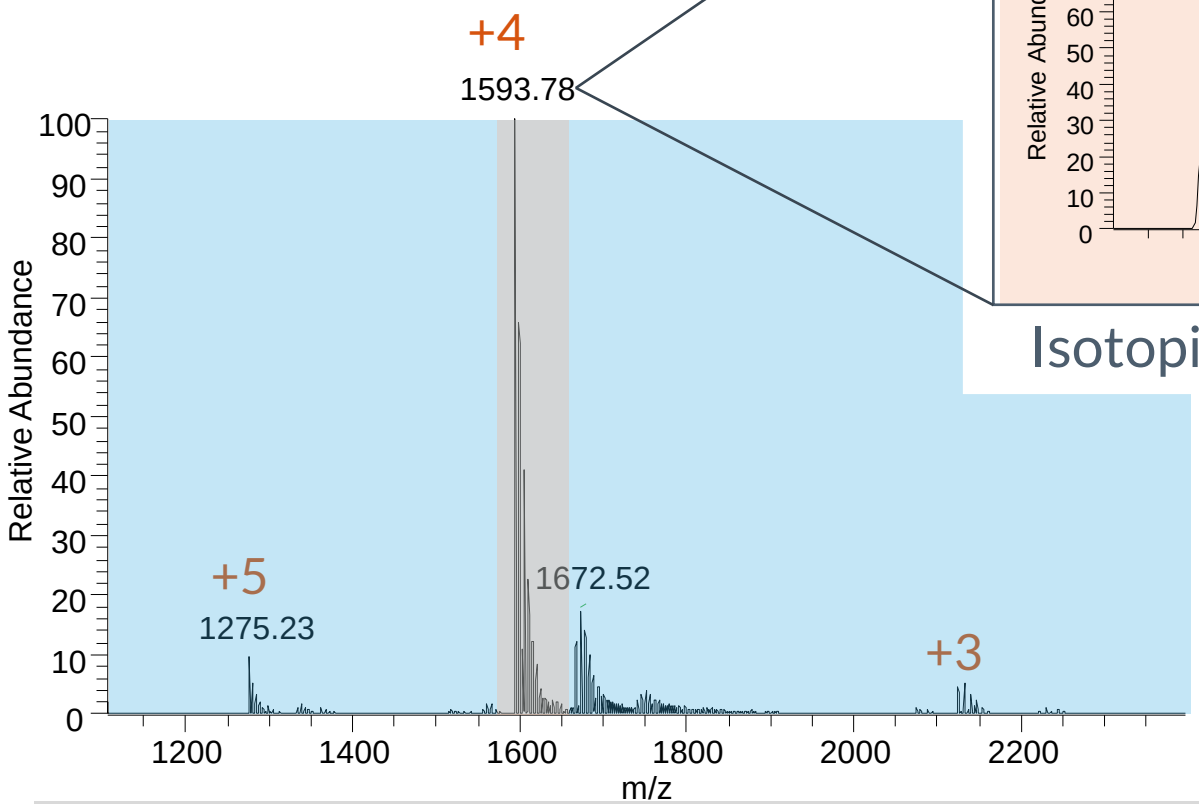
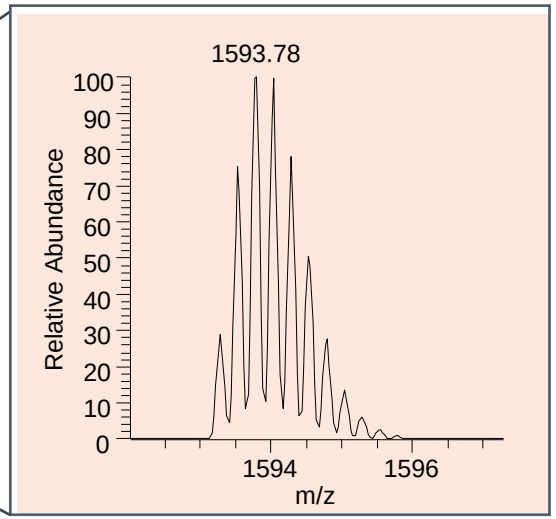
Oligonucleotide Analysis

Mixture of 5 primers (3 uM)
HRB Chip
Prototype BGE (pH 8.5)
Exactive Plus EMR
35,000 Resolution







Isotopic resolution

Peak #	Sequence	Monoisotopic Mass (Da)	Theoretical Mass (Da)	Mass Error (ppm)
1	TTT TTT TTT TTT TTT TTT TT	6018.979	6018.96503	2.3
2	ACG GCT ACC TTG TTA CGA CTT	6369.100	6369.08531	2.3
3	AGA GTT TGA TCC TGG CTC AG	6145.056	6145.05147	0.74
4	/5Phos/TTT TTT TTT TTT TTT TTT TT	6098.949	6098.93136	2.9
5	GGC CAC GCG TCG ACT AGT AC	6100.062	6100.05255	1.5

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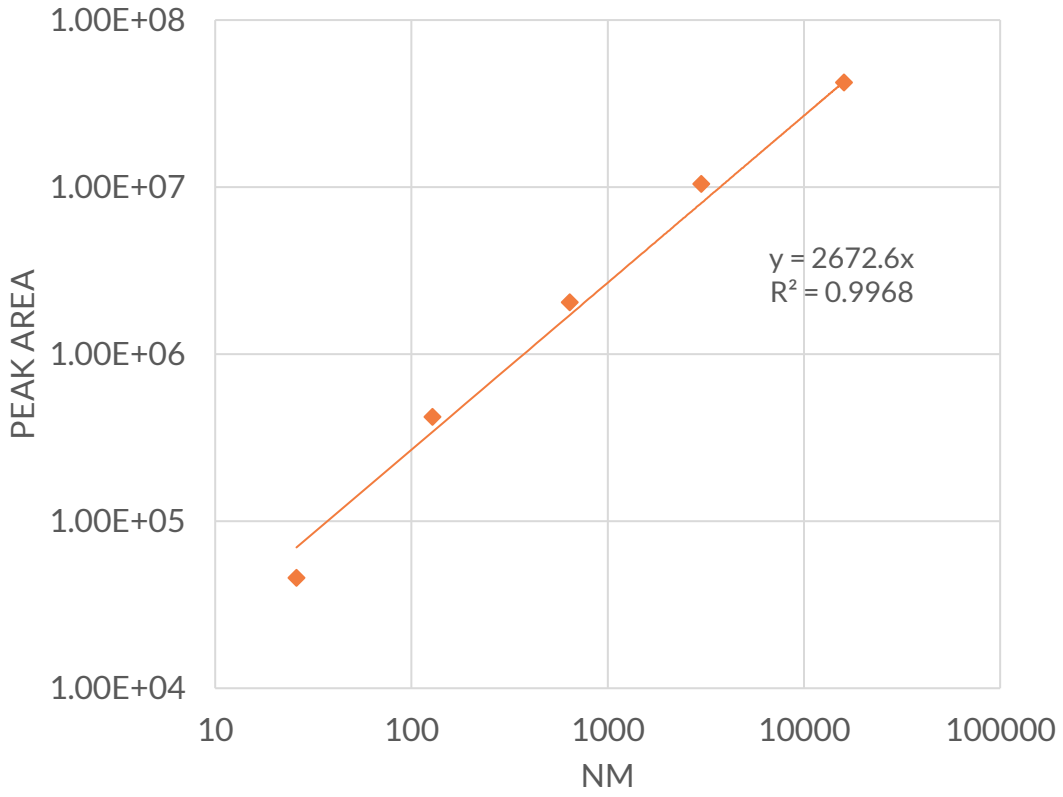
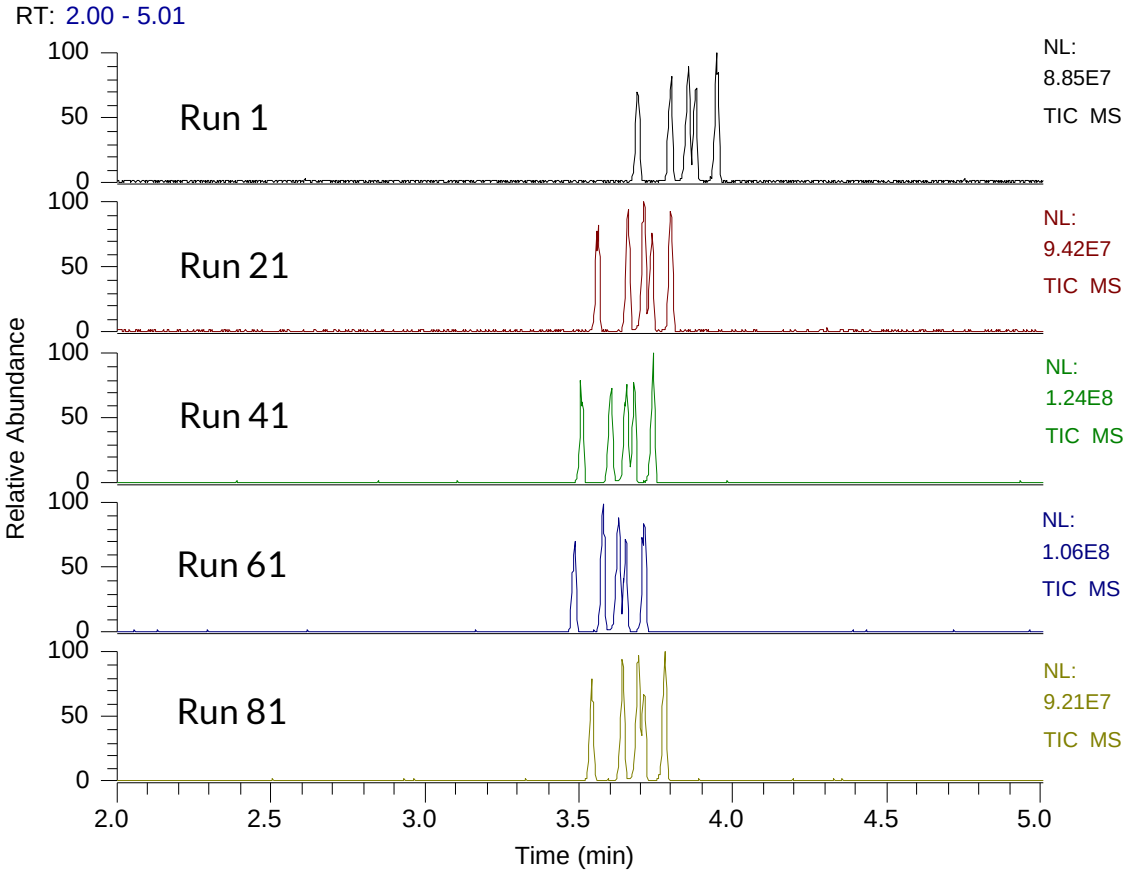
Confident identification of oligonucleotides

Oligonucleotide Analysis



Linear response with estimated LODs at 10 nM



Migration time RSDs 1.6%
Peak area RSDs 13%

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Example Applications

- Single method to determine charge heterogeneity, mass information & glycoform characterization
- Resolve basic and acidic variants in fully native mAb characterization
- Resolve charge variants at the intact & subunit level
- Resolve glycoform variants
- Resolve lower abundance basic variants from the high abundance main variant
- Determine different drug-to-antibody (DAR) ratios
- Direct sampling from bio reactors with fewer cleanup steps
- Intact mass, reduced, subunit, peptide mapping, or metabolite ID (cell culture/spent media) all possible in the same conditions

Single Platform for Multi-Characterization of Proteins

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Summary

ZipChip allows a direct microfluidic CZE nano-ESI interface to traditional MS with:

- Minimal sample preparation
- No de-salting requirements
- Fast analysis times
- Low (nL) sample volume injections.

ZipChip provides an orthogonal, high-throughput solution to traditional LC combining CZE with high resolution mass data for intact, reduced, subunit, peptide, and metabolite biotherapeutic profiling.

Customer Support Portal

Visit my.908devices.com for:

- Latest software release
- Ordering information
- 908 recommended protocols with example data
- Sample guide
- Chip care guide
- System shutdown guide

For any questions please email help@908devices.com or call +1.857.254.1500

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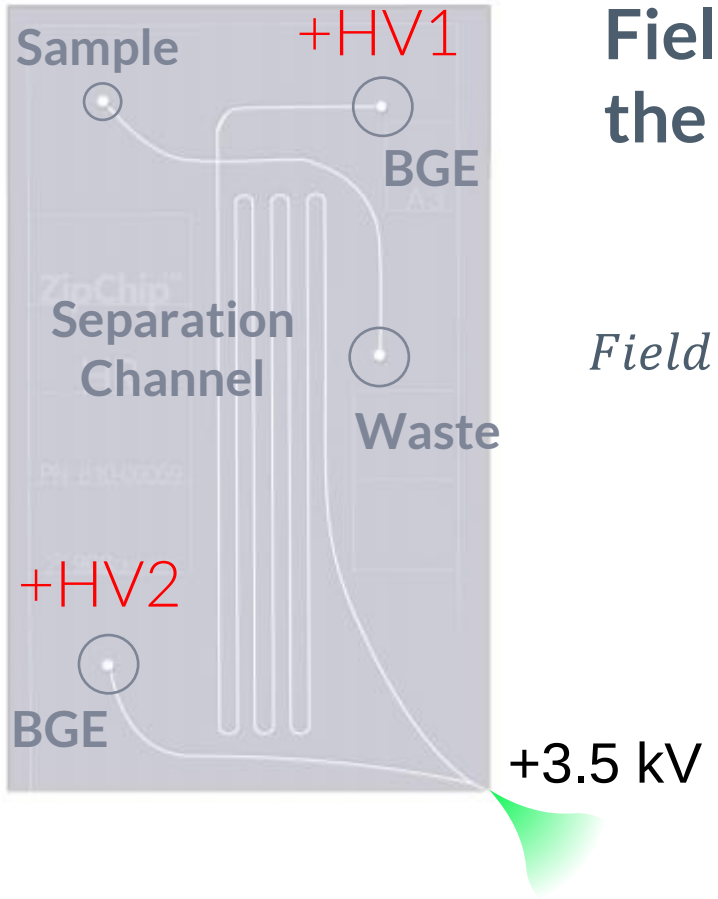
Thank You

https://908devices.com/products/zipchip

ZipChip – How it Works

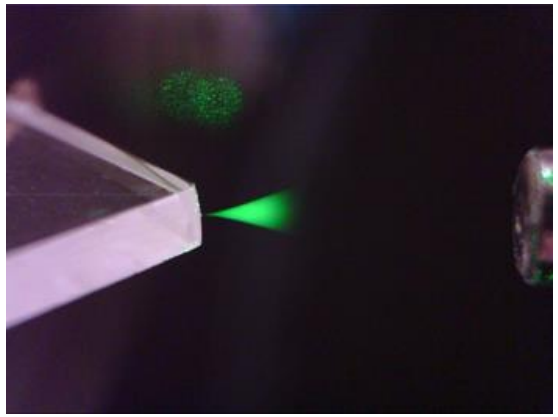
Chip Anatomy – Fast, High Resolution Separations

- **Integrated sample handling**
- **Uniform and stable surface coating**
- **Zone electrophoretic separation**
- **Direct Nano-electrospray ionization (ESI)**



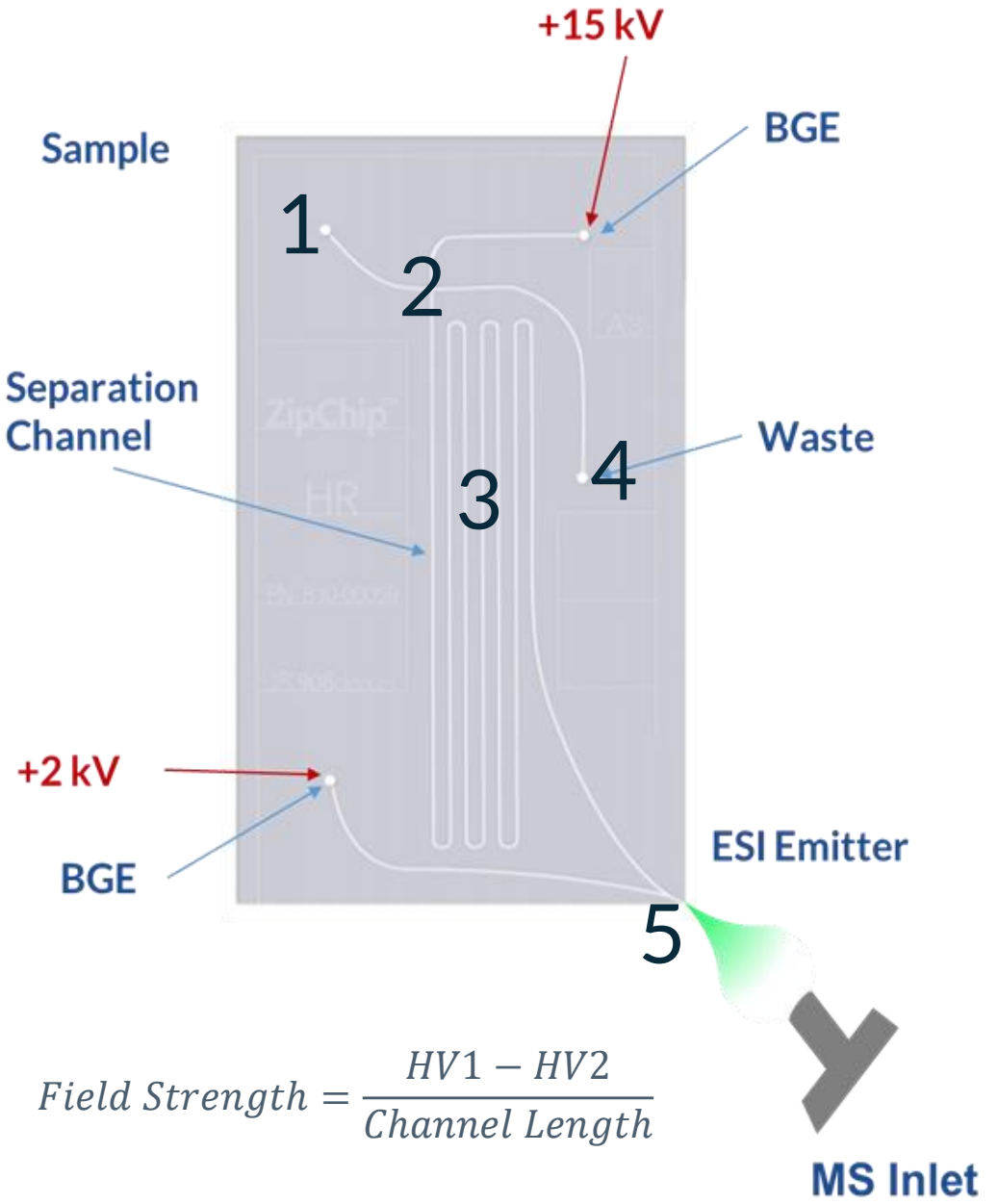
Field strength drives the ZipChip separation

$$Field\ Strength = \frac{HV1 - HV2}{Channel\ Length}$$



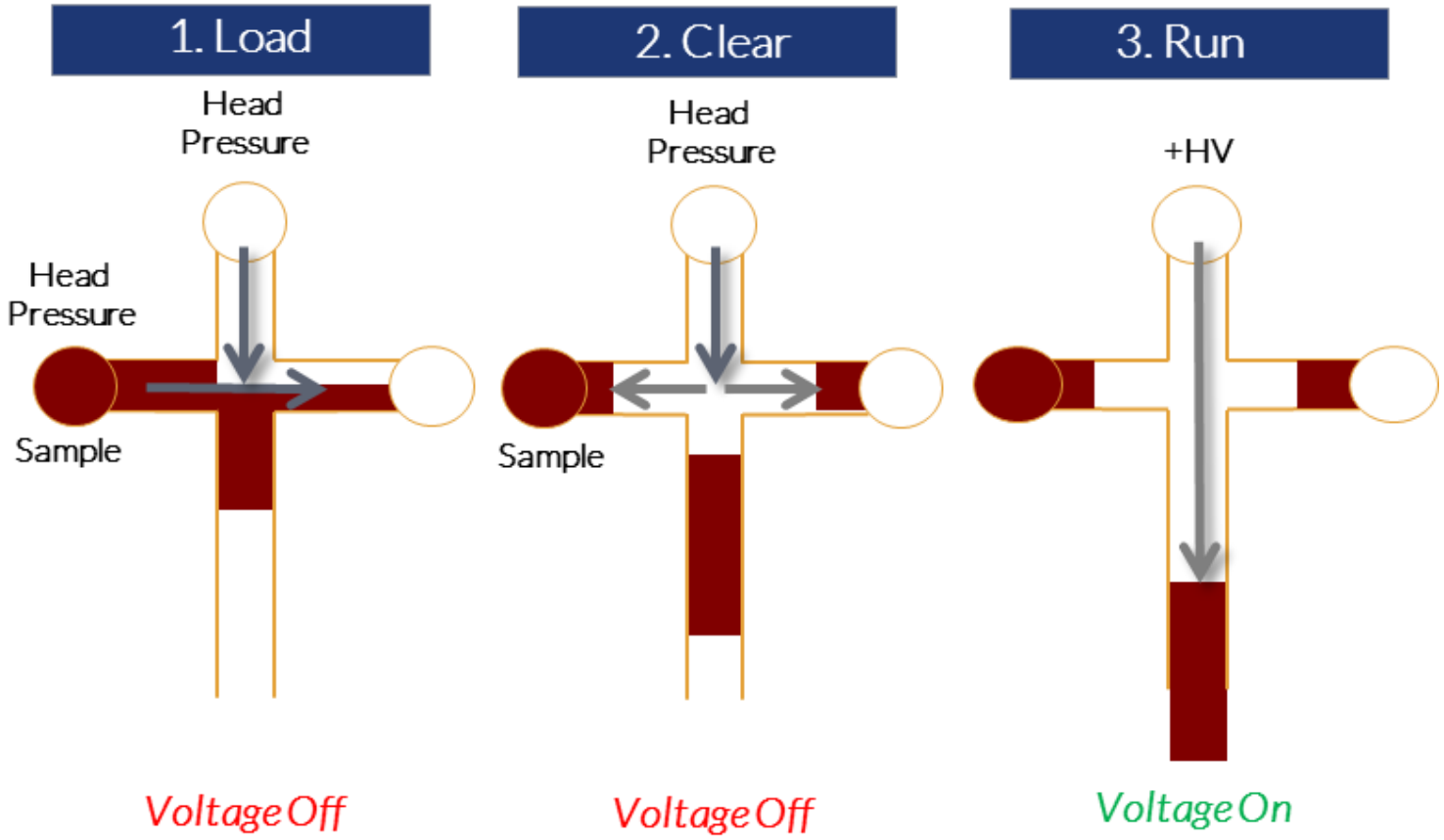
Sequence of Events

1. A small sample plug is pressure-injected into the ZipChip.
2. Voltage is applied across the separation channel
3. Sample migrates through BGE and separates based on charge and size
4. Negatives and neutrals to waste
5. Positive analytes are separated and electrospray into the MS.



$$Field\ Strength = \frac{HV1 - HV2}{Channel\ Length}$$

A Closer Look at Sample Handling



- Injections performed by applying pressure to wells
- Voltages are not applied until after injection is complete

Analytes must be *positively charged* in solution

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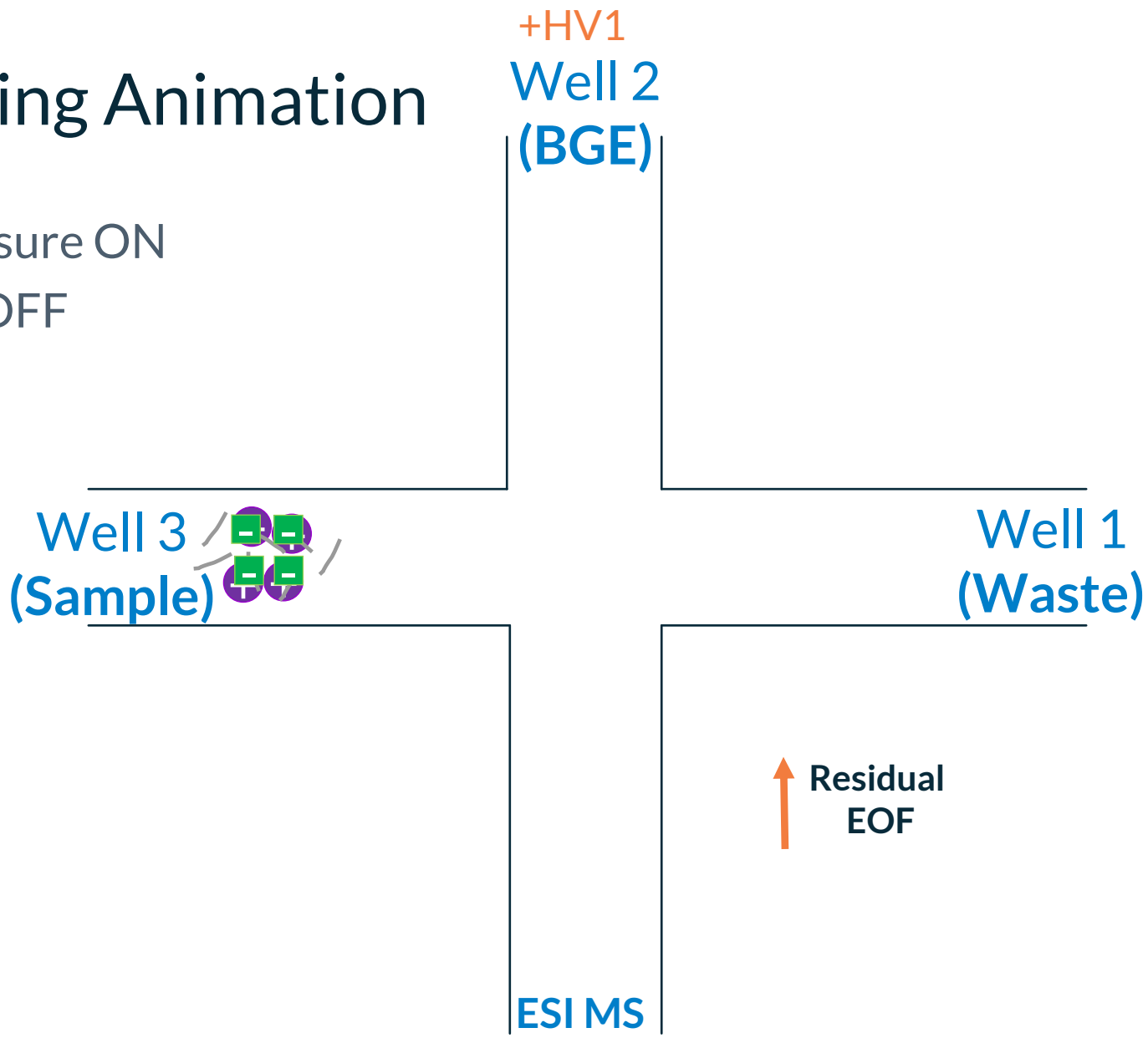
Sample Handling Animation

- Pressure ON
- HV OFF



Analytes must be *positively charged* in solution

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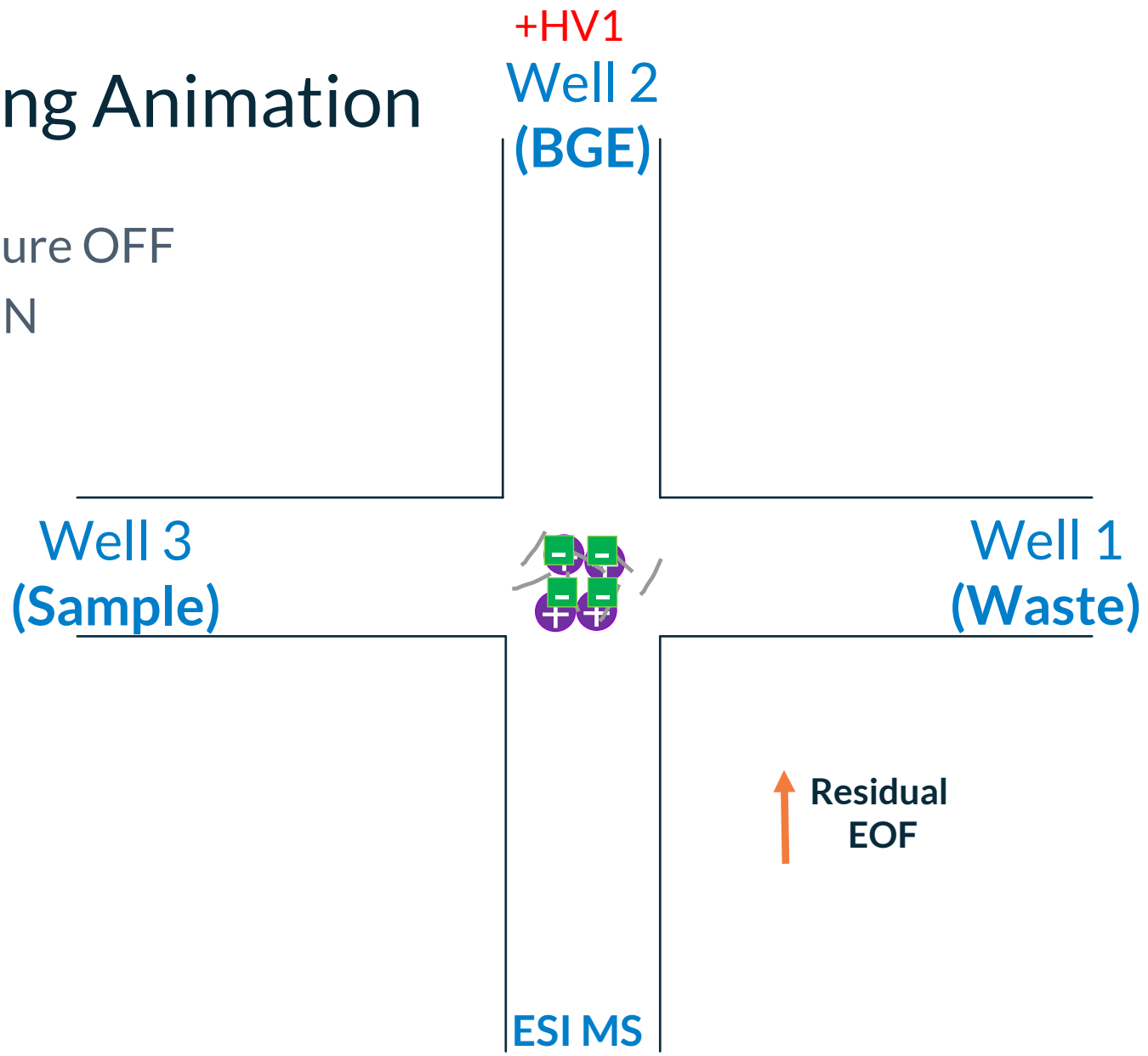
Sample Handling Animation

- Pressure OFF
- HV ON



Analytes must be *positively charged* in solution

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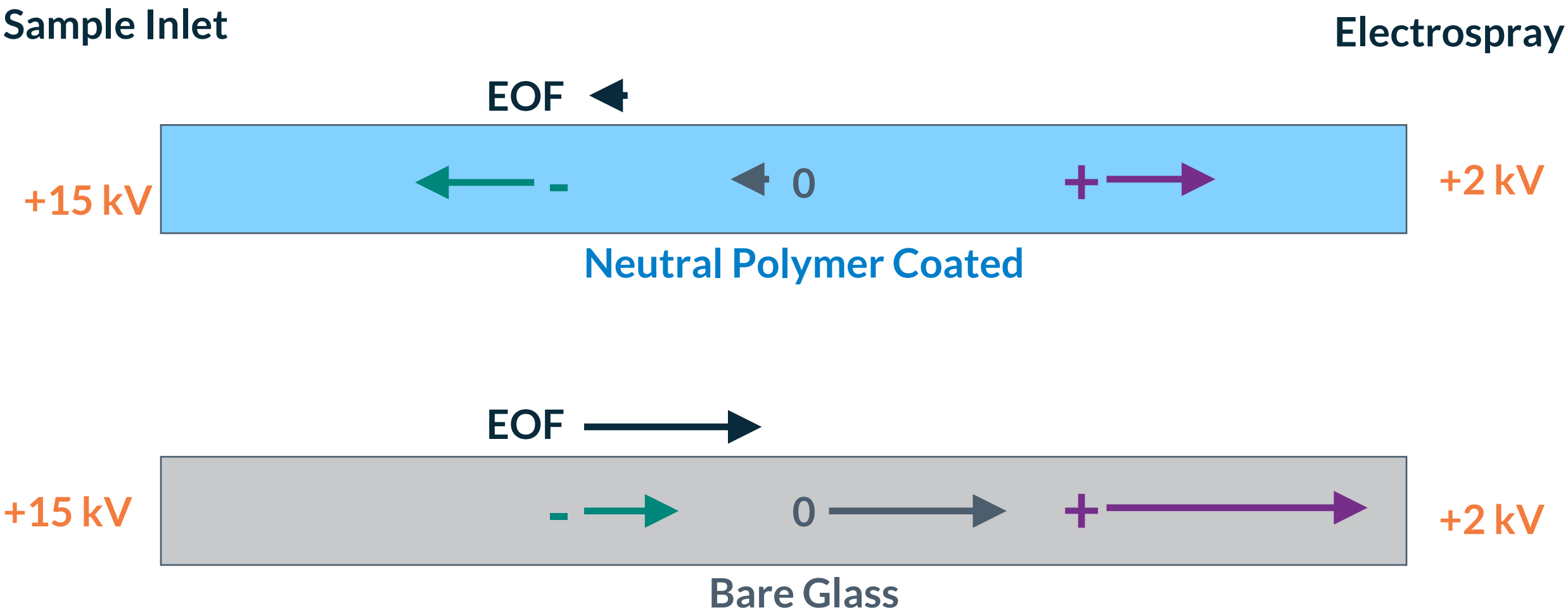
Impact of Chip Type - Analyte Migration



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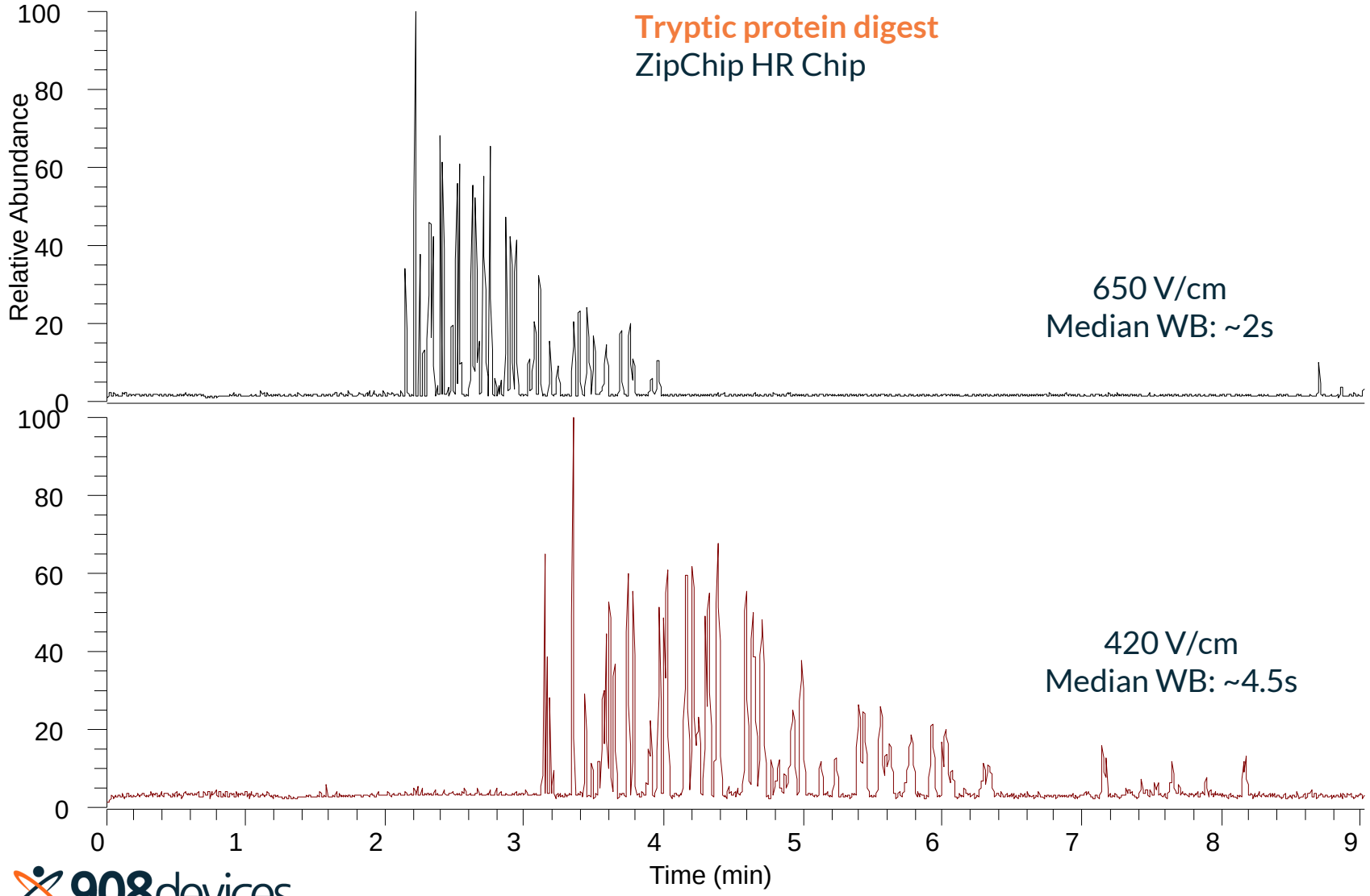
Impact of Field Strength – Migration Time



Changing the field strength will speed up or slow down the ZipChip migration

$$Field\ Strength = \frac{HV1 - HV2}{Channel\ Length}$$

Reducing the field strength may be important for MS/MS data collection

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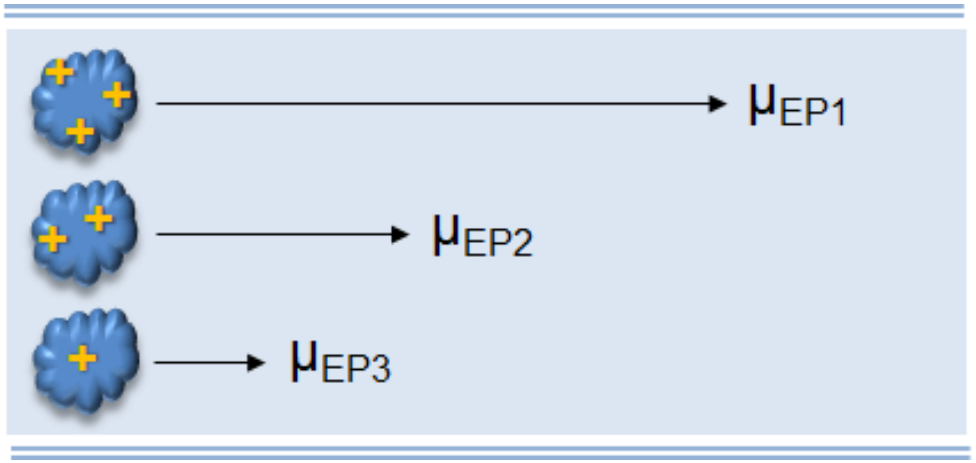
Impact of BGE - Separation

ZipChip performs capillary zone electrophoresis (CZE) separations, a mechanism based on electrophoretic mobility (μEP)

$$\mu_{EP} = \frac{q}{6\pi\eta a}$$

q - charge
η - viscosity
a - hydrodynamic radius



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Minor Separation Impact – Hydrodynamic Radius and Viscosity

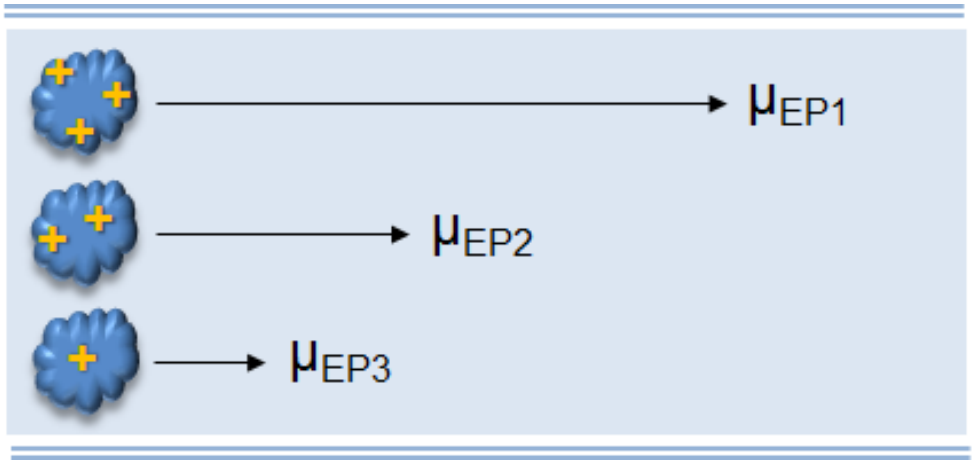
Separation is driven by electrophoretic mobility which is inversely proportional to a product of the BGE viscosity and molecule hydrodynamic radius

$$\mu_{EP} = \frac{q}{6\pi\eta a}$$

q - charge

η - viscosity

a - hydrodynamic radius



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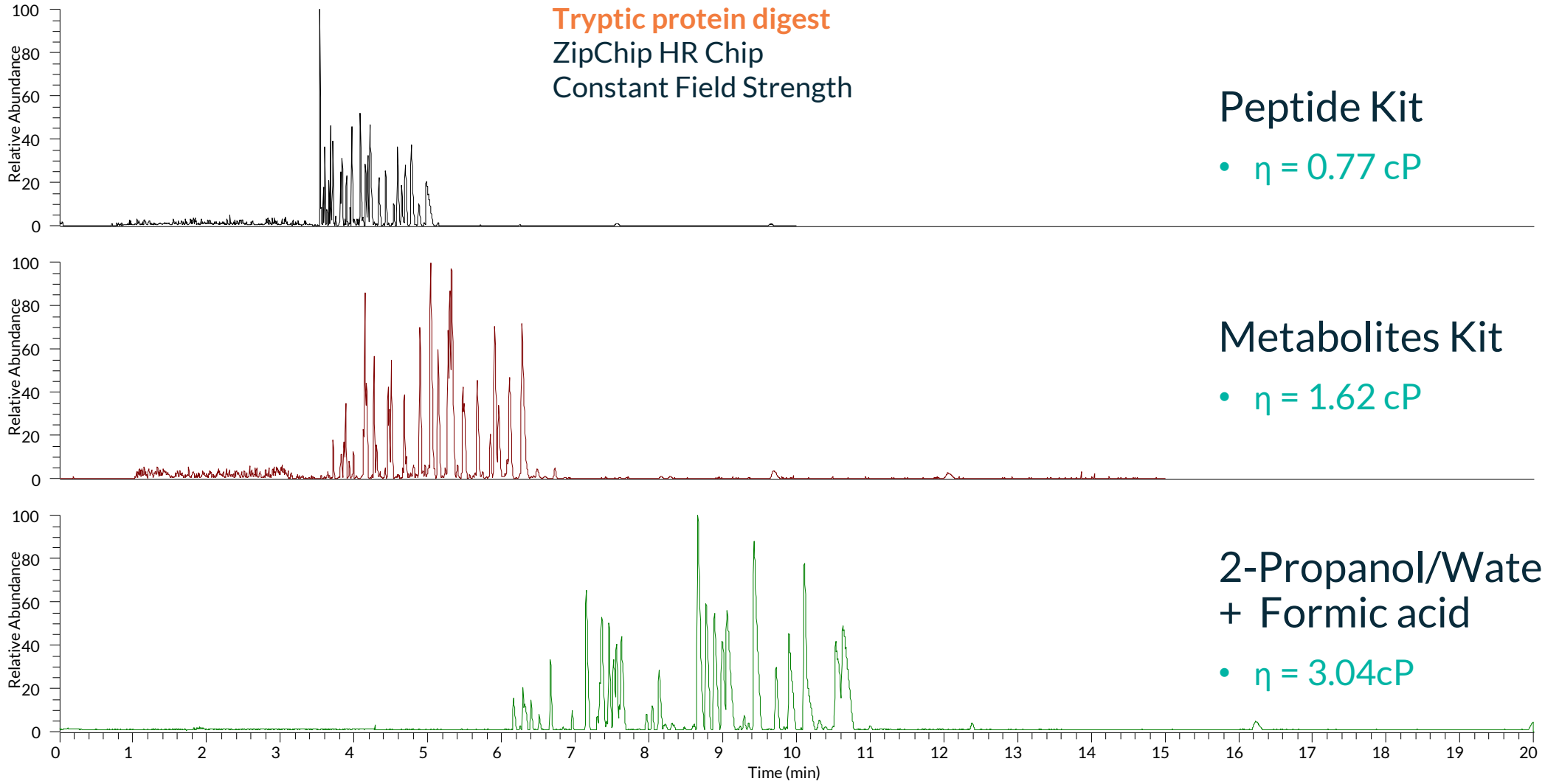
Impact of Viscosity - Separation



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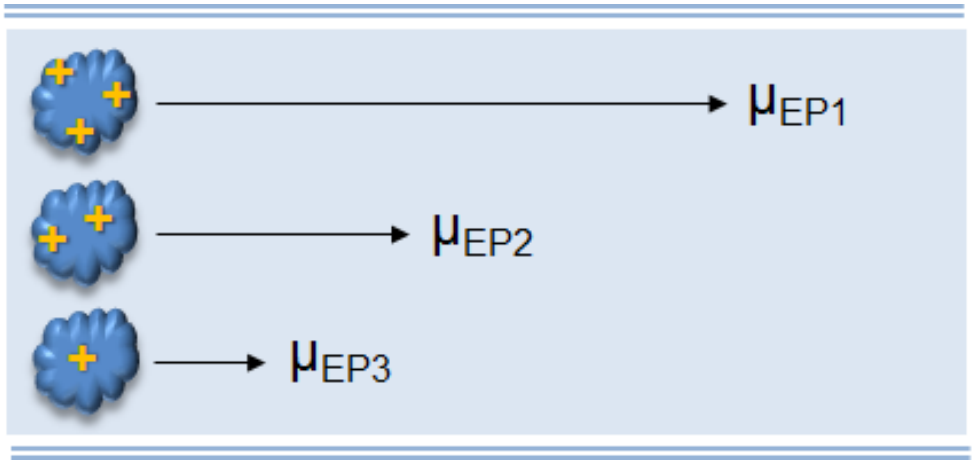
Higher viscosity produces longer migration times

Major Separation Impact - Charge

Separation is driven by electrophoretic mobility which is directly proportional to charge

$$\mu_{EP} = \frac{q}{6\pi\eta a}$$

q - charge
η - viscosity
a - hydrodynamic radius



Analytes must be *positively charged* in solution

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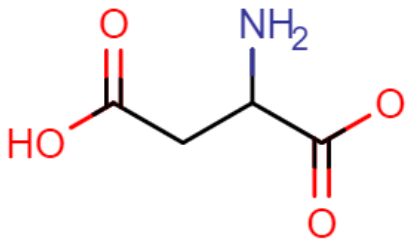
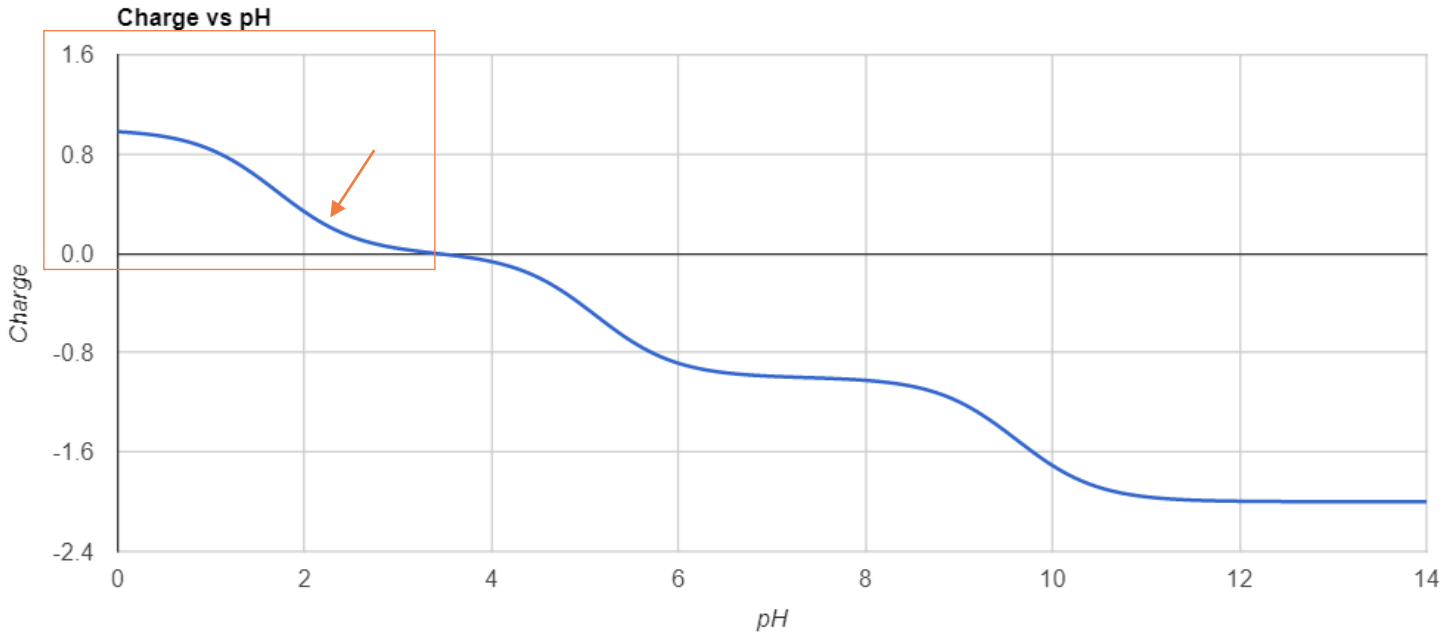
Consider the pH of the BGE vs. the Isoelectric Point



Aspartic Acid

Isoelectric point



Isoelectric point:	3.41

pH	Charge
1.7	0.50
4.6	-0.23
6.5	-0.96
7.4	-1.00
8.0	-1.02

Aspartic Acid at pH 2.2 net charge is ~+0.24

Plots generated using chemicalize.org

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Analyte Banding - Transient isotachophoresis (tITP)

What is Transient isotachophoresis?

- When using a stationary phase (LC), loading can be increased due to analyte capture.
- In CZE, tITP is a concentration enhancement technique which allows for larger sample injection volumes without losing separation performance.
- tITP utilizes a leading electrolyte!

tITP Recommended	tITP Generally Not Necessary
Small Molecules & Metabolites, Amino Acids, Peptides	Intact, Reduced, or Subunits

For effective use of tITP, always use Kit Diluent

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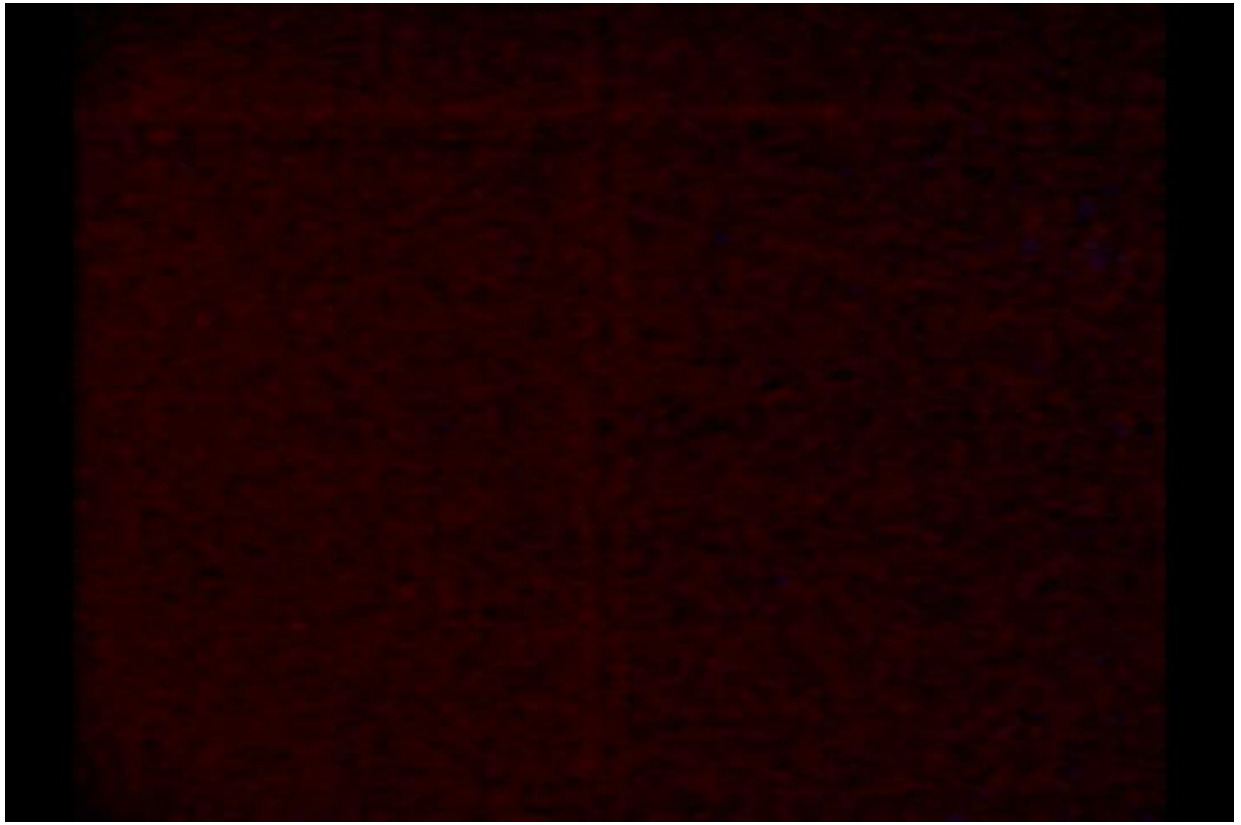
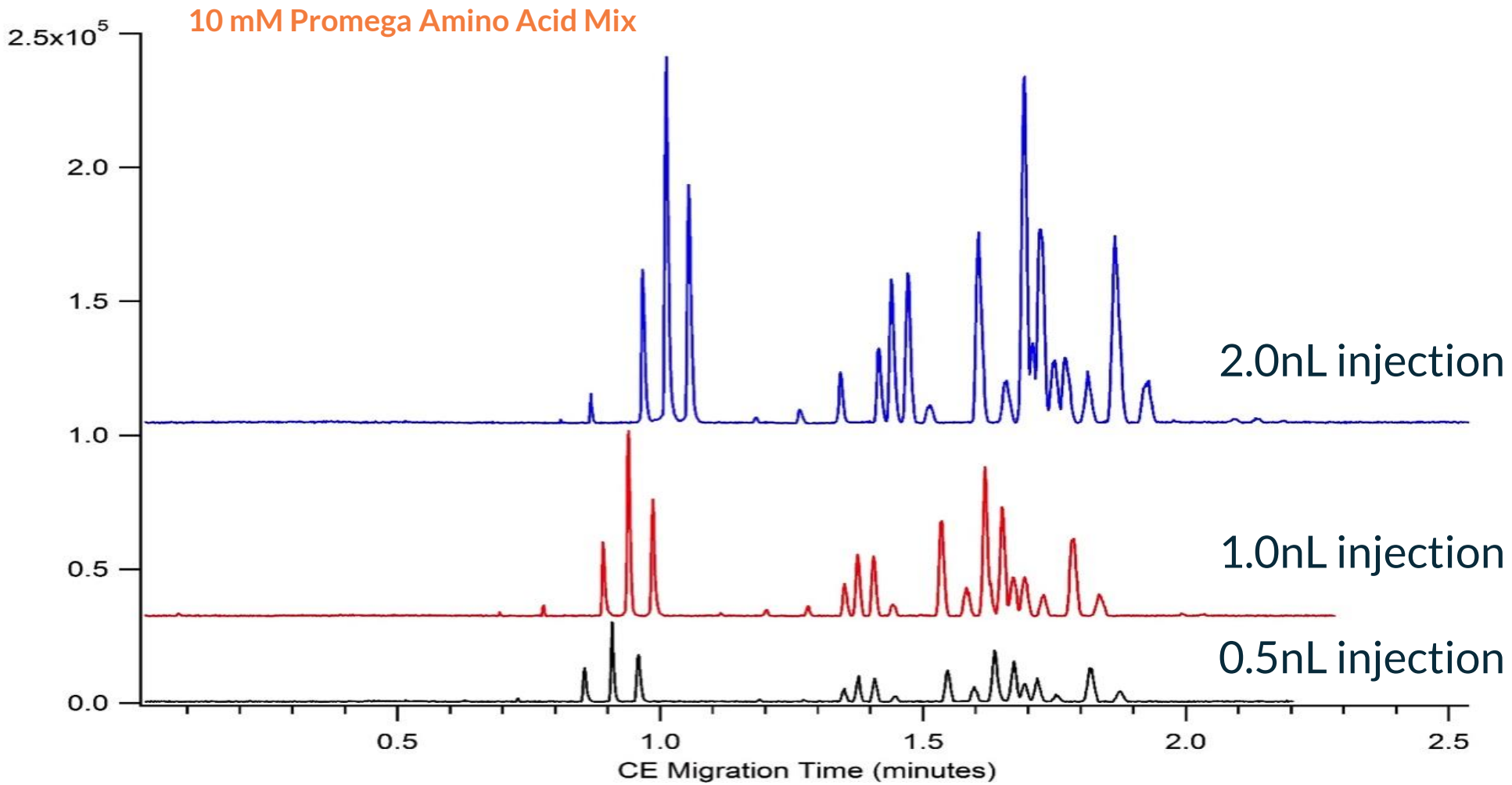
tITP Animation

ZipChip injection **with** tITP

ZipChip injection **without** tITP



Loading Impact - Transient isotachophoresis (tITP)



tITP mediates separation at higher injection volumes

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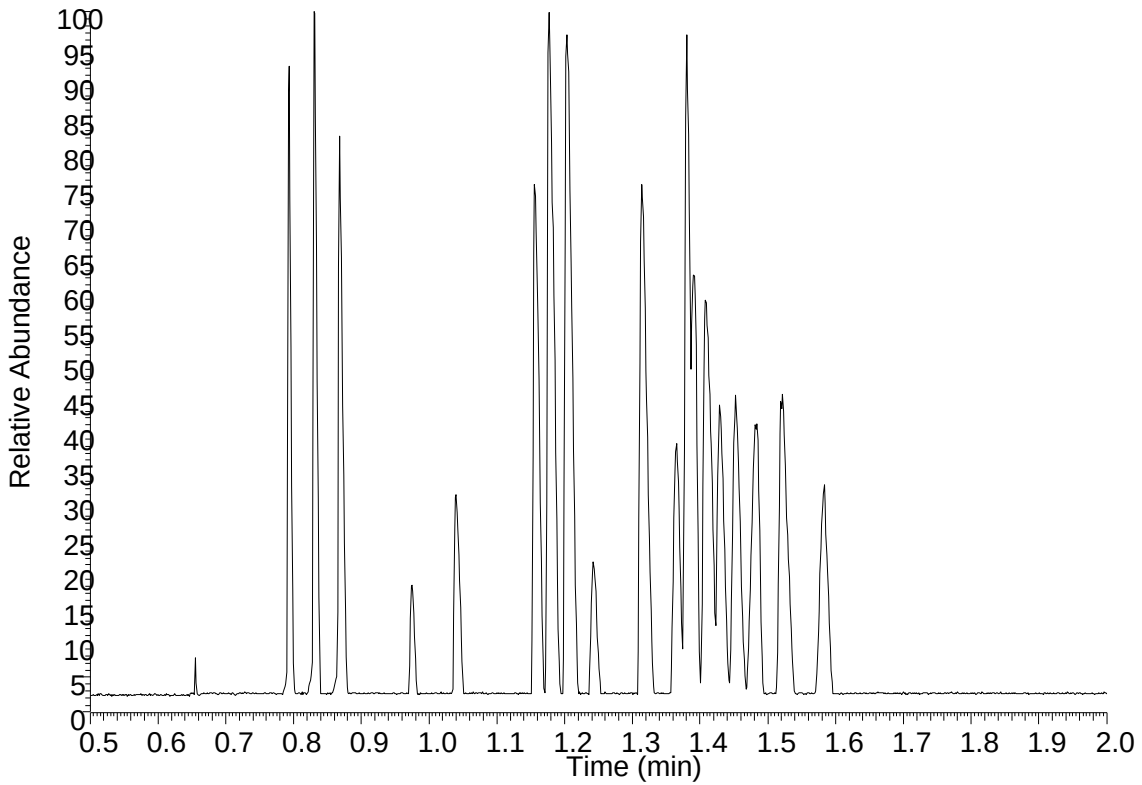
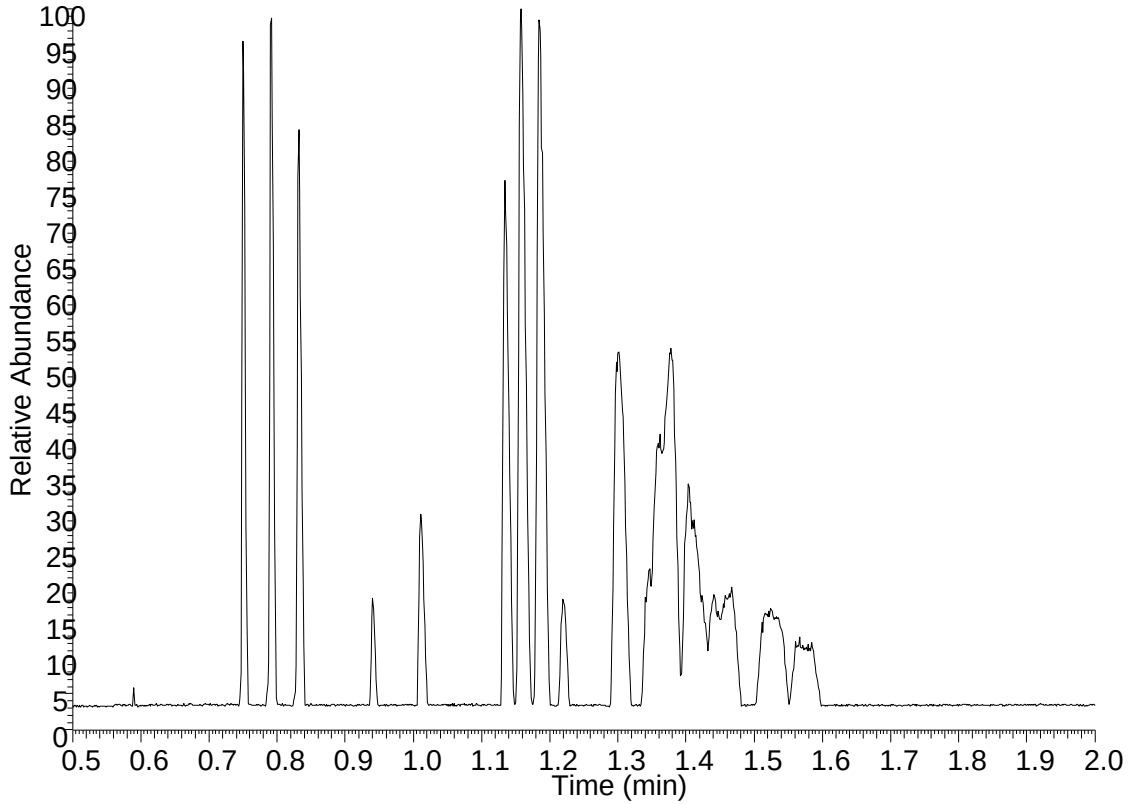
Peak Shape Impact - Transient isotachophoresis (tITP)

Proper level of leading electrolyte



- Sharp peaks
- Consistent shape across separation window

Too little leading electrolyte



- Jagged and misshapen peaks, especially later in migration times

Fix by diluting more with sample diluent or adding more leading electrolyte

Sample Matrix Considerations

Reference and consult the *Sample Guide* at my.908devices.com

In General:

- No de-salting is typically required – samples can be diluted directly from formulation buffer
- Anionic or neutral components are OK.
  - SDS, Urea, Ammonium salts, PBS, Phosphates. . .
- Avoid slow-moving cations
  - TRIS, Guanadine HCl, DMF, TCEP

For any questions please email help@908devices.com or call +1.857.254.1500

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Sample Loading Considerations

Type of BGE	Applications	Limit of Detection	Target Concentration	Onset of Overloading
Metabolite	Cell Culture Monitoring, Reduced & Subunit, Intact Mass, Peptide Mapping	1-10 nM	10 µM	50 µM
Peptide	Peptide Mapping, N-Glycans	1-10 nM	0.25 -0.5 mg/mL	50 µM
Intact Antibody / Native / CVTOF	Intact mAb or other proteins	0.001 mg/mL	0.5 mg/mL	1-5 mg/mL

Sample injections are very low volume (1-10 nL), however, the required minimum transfer volume for the sample well on the chip is 10 µL

Additional Best-Practices, Tips, and Tricks

- BGE & Transfer lines are HIGHLY susceptible to over-tightening (Autosampler version only)
- Less than ideal chip spray can often be improved by lightly wiping the corner with a kimwipe – to be demonstrated in the lab.
- When in doubt, second guess the BGE – Is it expired? If required, does it have acid in it? Was it prepared recently?
- If still in doubt, second guess the sample matrix – Solubilized with TRIS? Reduced with TCEP? Diluted with sample diluent?

Questions?

