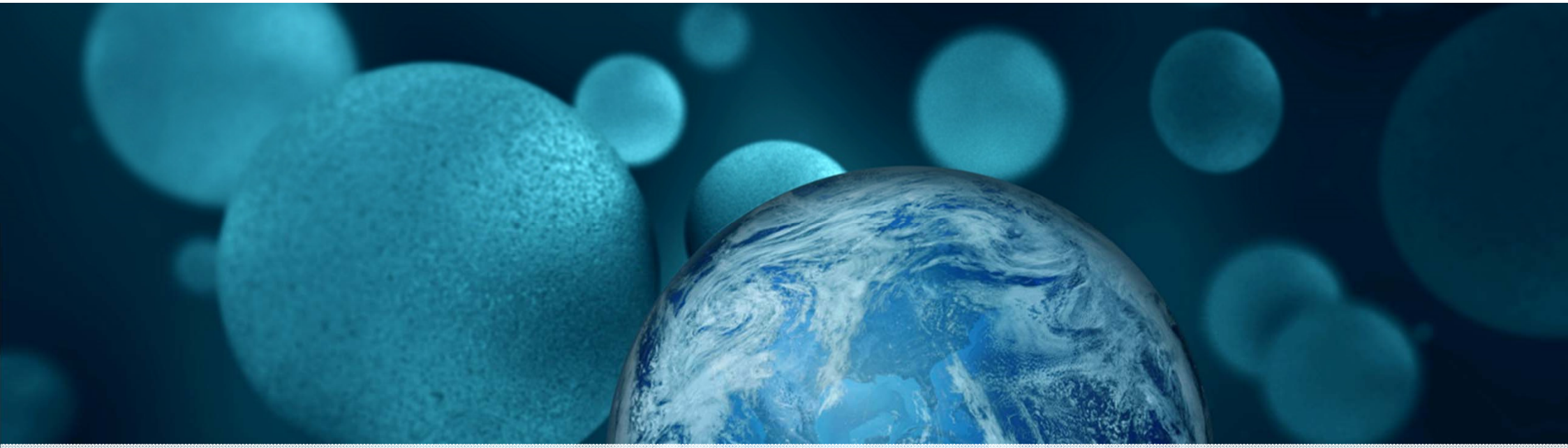

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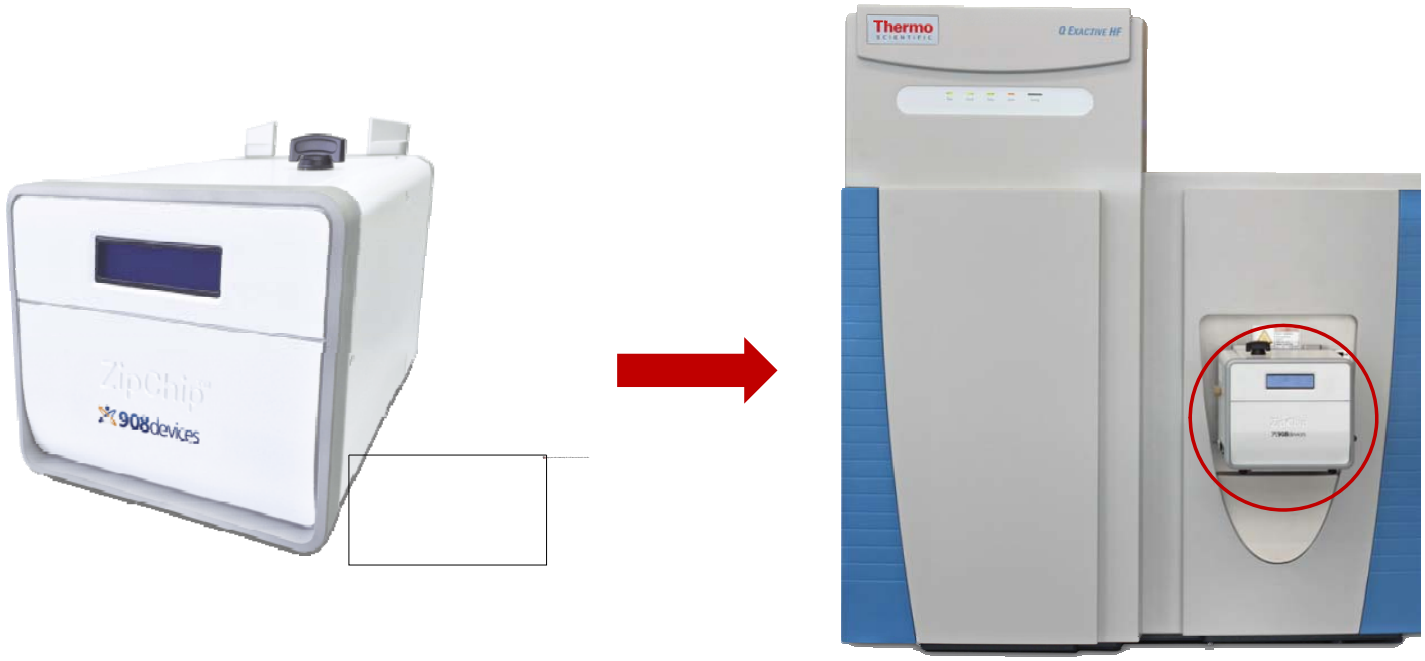
ZipChip™ and Thermo Scientific™ MS for CE/ESI-MS Analyses

Andrew Williamson

Applications specialist

What is the ZipChip System?

- The ZipChip system* uses integrated microfluidic technology to prepare, separate samples by capillary electrophoresis (CE), and then electrospray (ESI) analytes directly into a mass spectrometer (MS). Data collection, processing and reporting are through Xcalibur
- It is composed of the ZipChip interface and the microfluidic chip
- The CE separation and ESI occur on the microfluidic chip
- ZipChip Interface directly mounts onto the front end of a mass spectrometer
- ZipChip system is compatible with a broad range of biomatrices such as growth media, cell lysates, blood, plasma, and urine



* ZipChip system is sold exclusively by Thermo Fisher in Europe and APAC

Comprehensive Portfolio

ZipChip Interface

- Two versions: Autosampler operation version and manual operation version
- Compatible with all Thermo Scientific™ Exactive, Q Exactive Orbitrap MS, and LTQ Orbitrap MS instruments

ZipChip Autosampler

- Fully automated and controlled by the ZipChip software

ZipChips

- Disposable chips good for up to 125 injections per chip
- Two types: HR chip and HS chip

ZipChip Assay Kits

- 3 types of pre-made assay kits are designed for intact antibody, peptides, and metabolites analyses

Simple ZipChip-MS Analysis Workflow

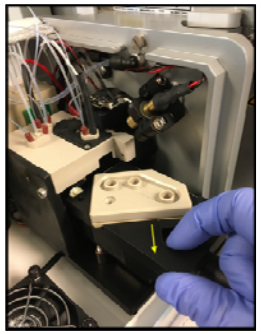
1

Select proper assay kit and ZipChip for your experiments

2

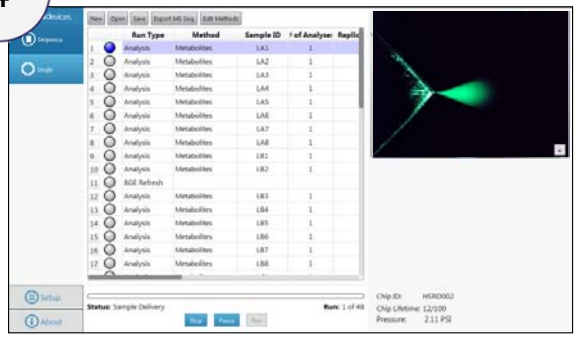
Simple Sample Prep

3



Place ZipChip and prepare the system

4



Set up sequence and collect CE-MS data

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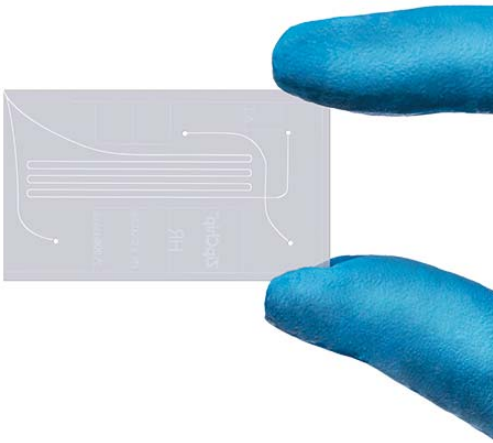
Why the ZipChip System?

- The **ONLY** commercially available integrated and portable CE/ESI interface for MS
- Offers extremely rapid CE separations, nano-spray level sensitivity, and HRAM mass spectrometry in one platform
- Requires minimal sample preparation with on-chip desalting capability
- Consumes only picograms to nanograms of sample per analysis



Fast CE separation • *Nano Spray Sensitivity* • *HRAM Mass Spectrometry*

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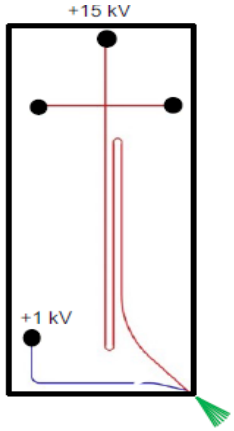
Common Applications Performed on ZipChip-MS Platform

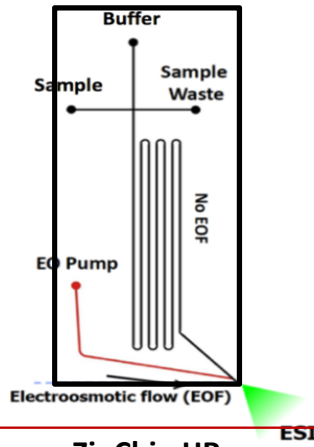
- Intact mAb/protein, and ADC characterization
- mAb subunit analyses
- Glycomics and glycoproteomics
- Peptide mapping
- Metabolomics

How ZipChip works

ZipChip™ System Introduction

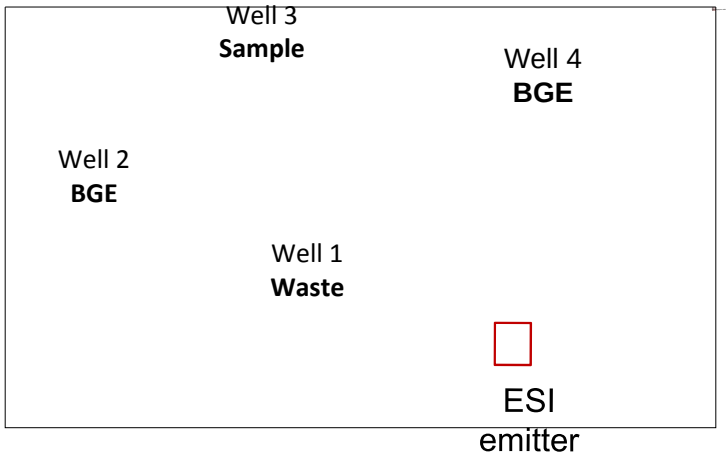
- The ZipChip™ system uses integrated microfluidic technology to prepare, separate samples by capillary electrophoresis (CE), and then electrospray (ESI) analytes directly into a mass spectrometer (MS). It is composed of the ZipChip interface and the chip
- ZipChip Interface directly mounts onto the front end of a mass spectrometer
- The CE separation and ESI occur on the microfluidic chip
- ZipChip system is compatible with a broad range of biomatrices such as growth media, cell lysates, blood, plasma, and urine
- Each analysis only consumes a few nano liters of sample containing pico grams to nano grams of analytes
- Only minimal sample preparation is needed



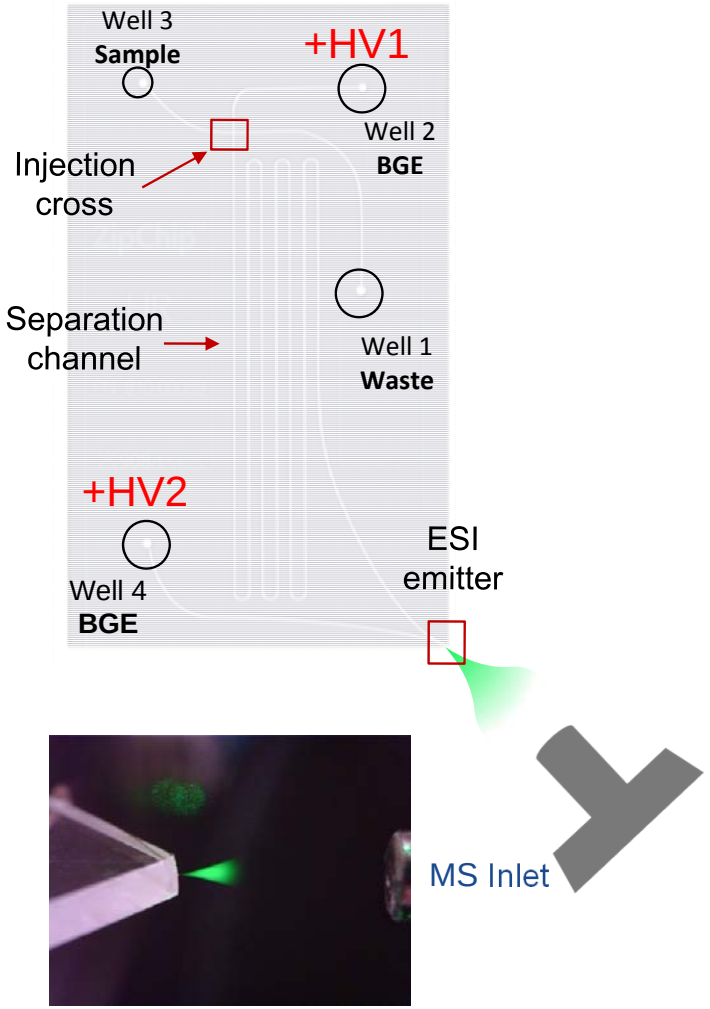


	ZipChip HS	ZipChip HR
Separation channel length (cm)	10	22
Flowrate (nL/min)	150	150
Maximum # of injections per chip	125	125
On Chip De-salting capability	Yes	Yes
Integrated ESI Emitter	Yes	Yes
EEPROMS (recognize chip type and track usage)	Yes	Yes
Recommended use	Small molecules or simple sample mixture	Big molecules or complex sample mixture
Typical analysis time	Up to 3 min	Up to 15 min

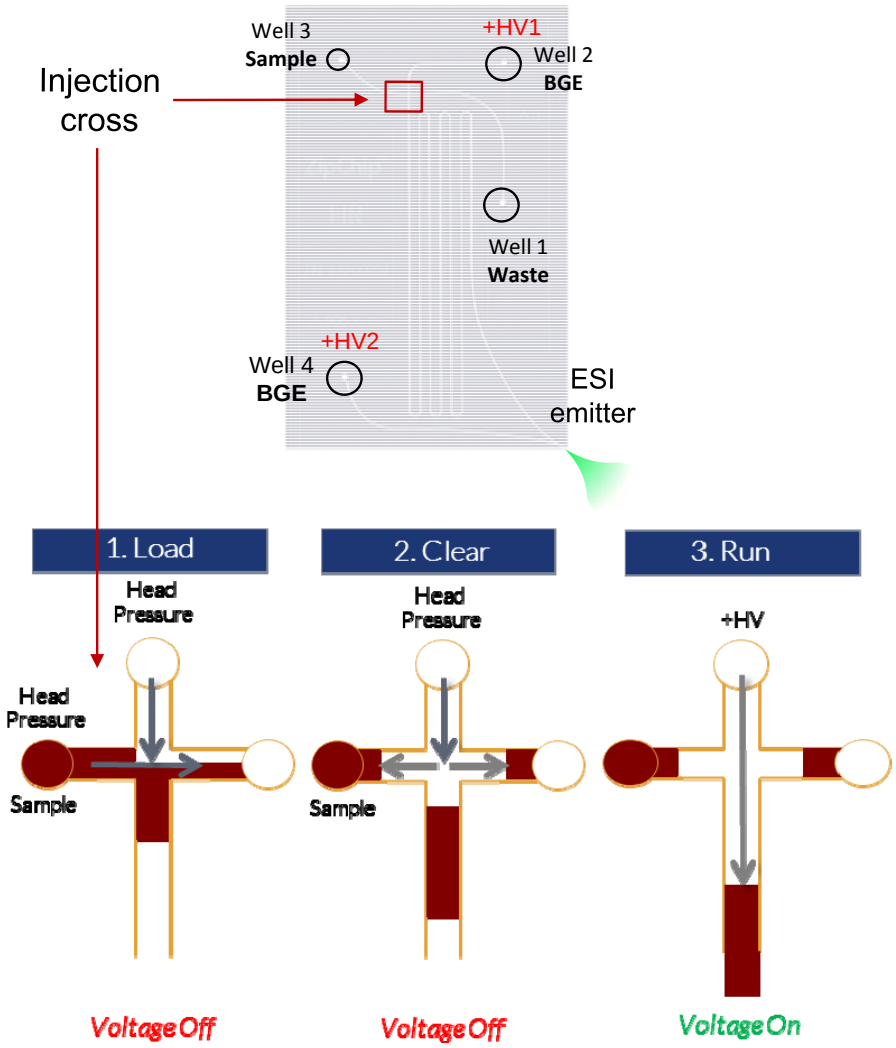
Anatomy of ZipChip



Glass microfluidic chip in PEEK housing



Sample Injection



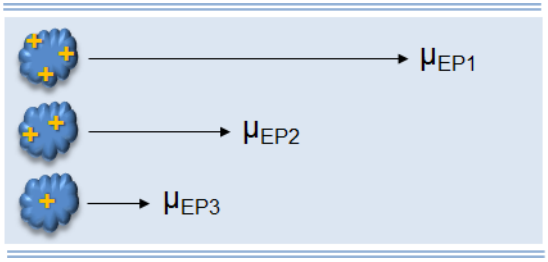
10 μM rhodamine-6G in BGE + 100 mM ammonium acetate

Sample Separation

- High voltage applied to Wells 2 and 4
- HV1 and HV2 determine field strength

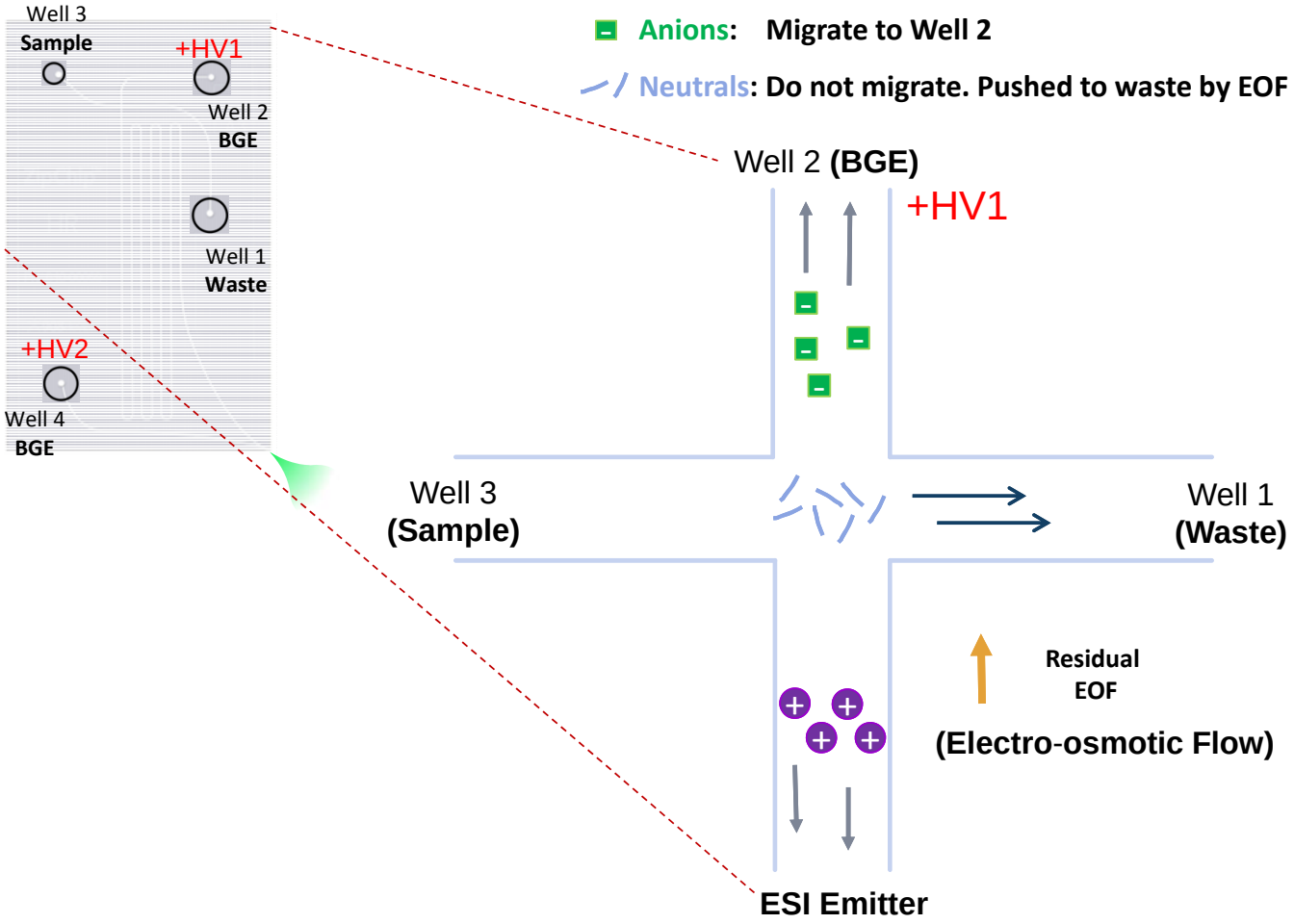
$$Field\ Strength = \frac{HV1 - HV2}{Channel\ Length}$$

- Field strength drives the ZipChip separation



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

q - *charge*
η - *viscosity*
a - *hydrodynamic radius*

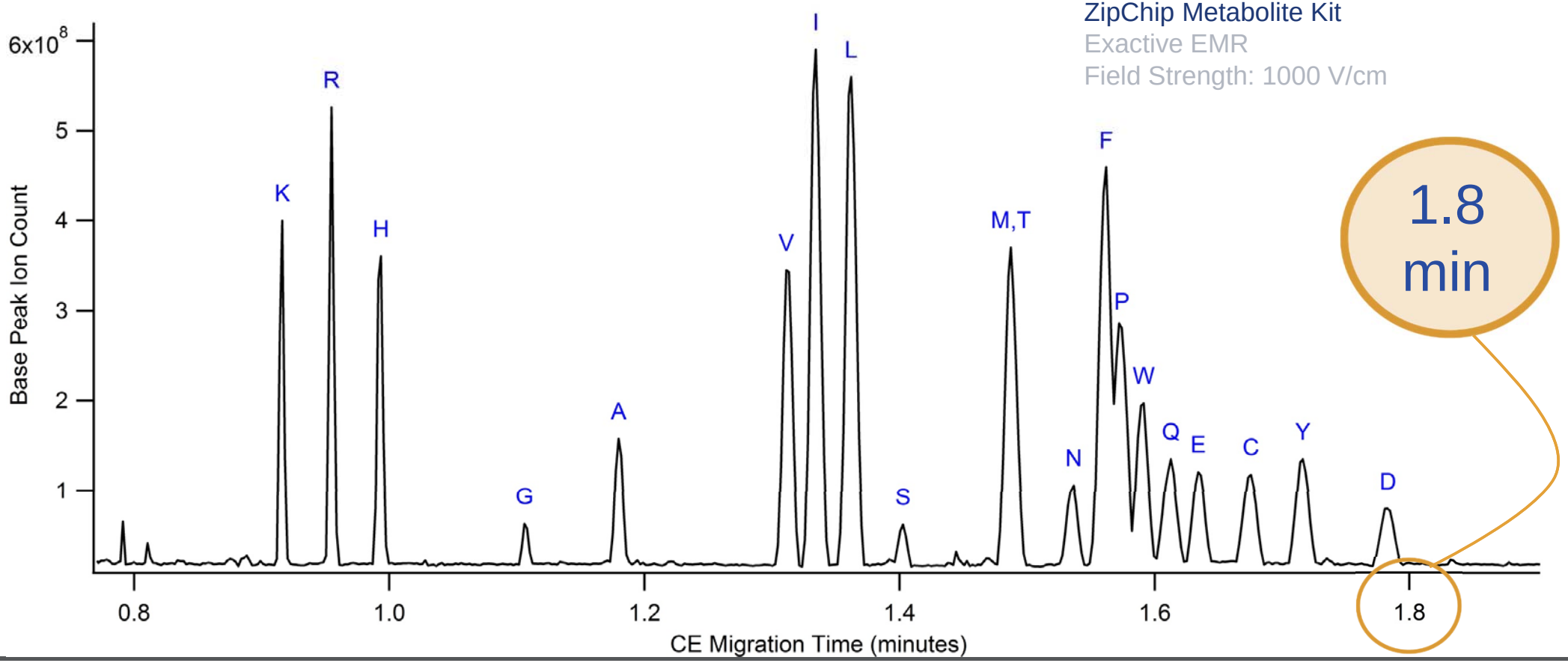


For ZipChip analysis analytes must be positively charged in solution

Small Molecule Analysis/ Metabolomics

ZipChip Separation of Amino Acids

2.5 µM Promega Complete AA Mix
ZipChip HS
ZipChip Metabolite Kit
Exactive EMR
Field Strength: 1000 V/cm



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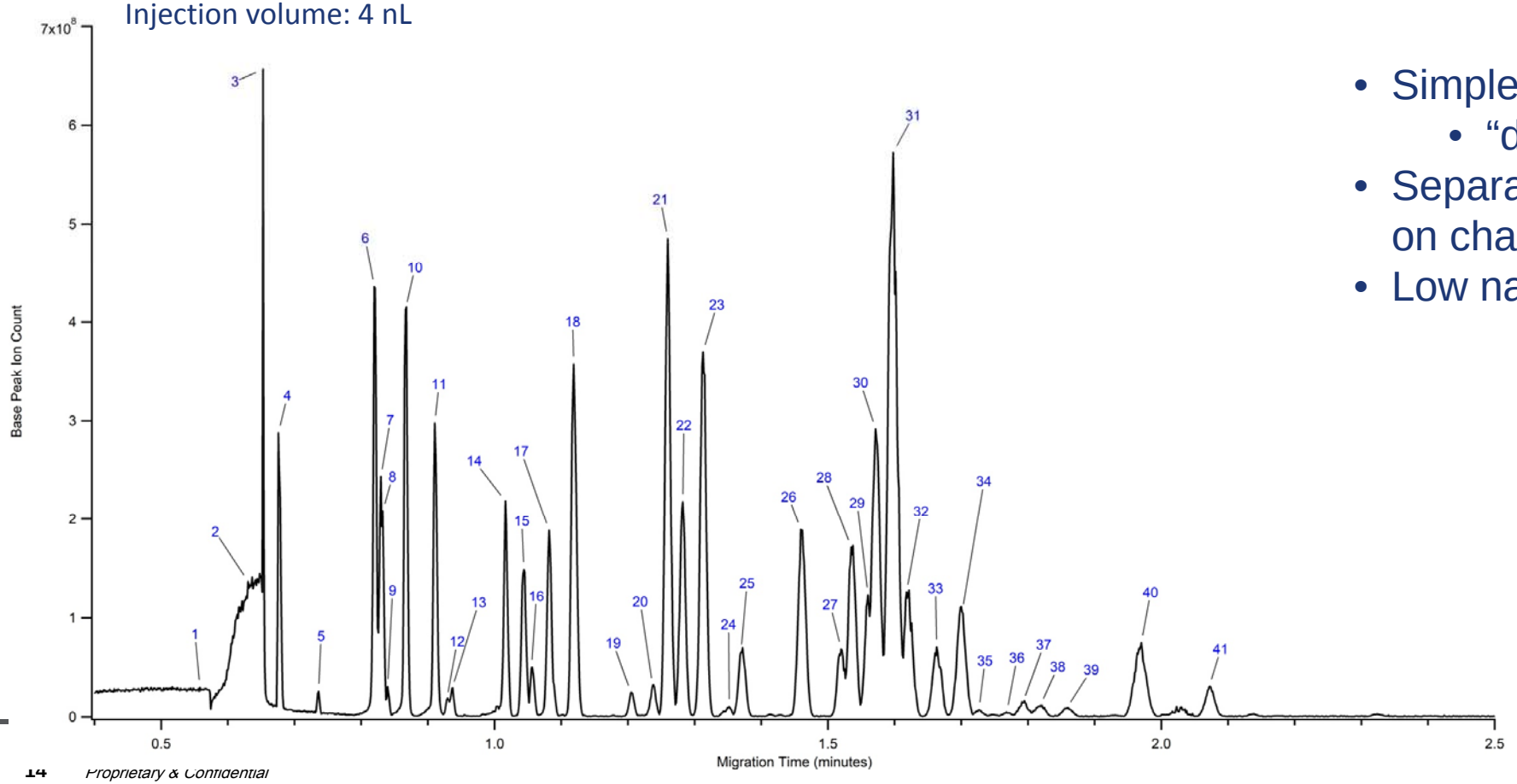
ZipChip Analysis of Human Plasma

Chip type: ZipChip HS
BGE: Metabolite kit (methanol/water/formic acid)
Field Strength: 1000 V/cm
Injection volume: 4 nL



- Simple sample prep
  - "dilute and shoot"
- Separation based only on charge and size
- Low nanomolar LODs

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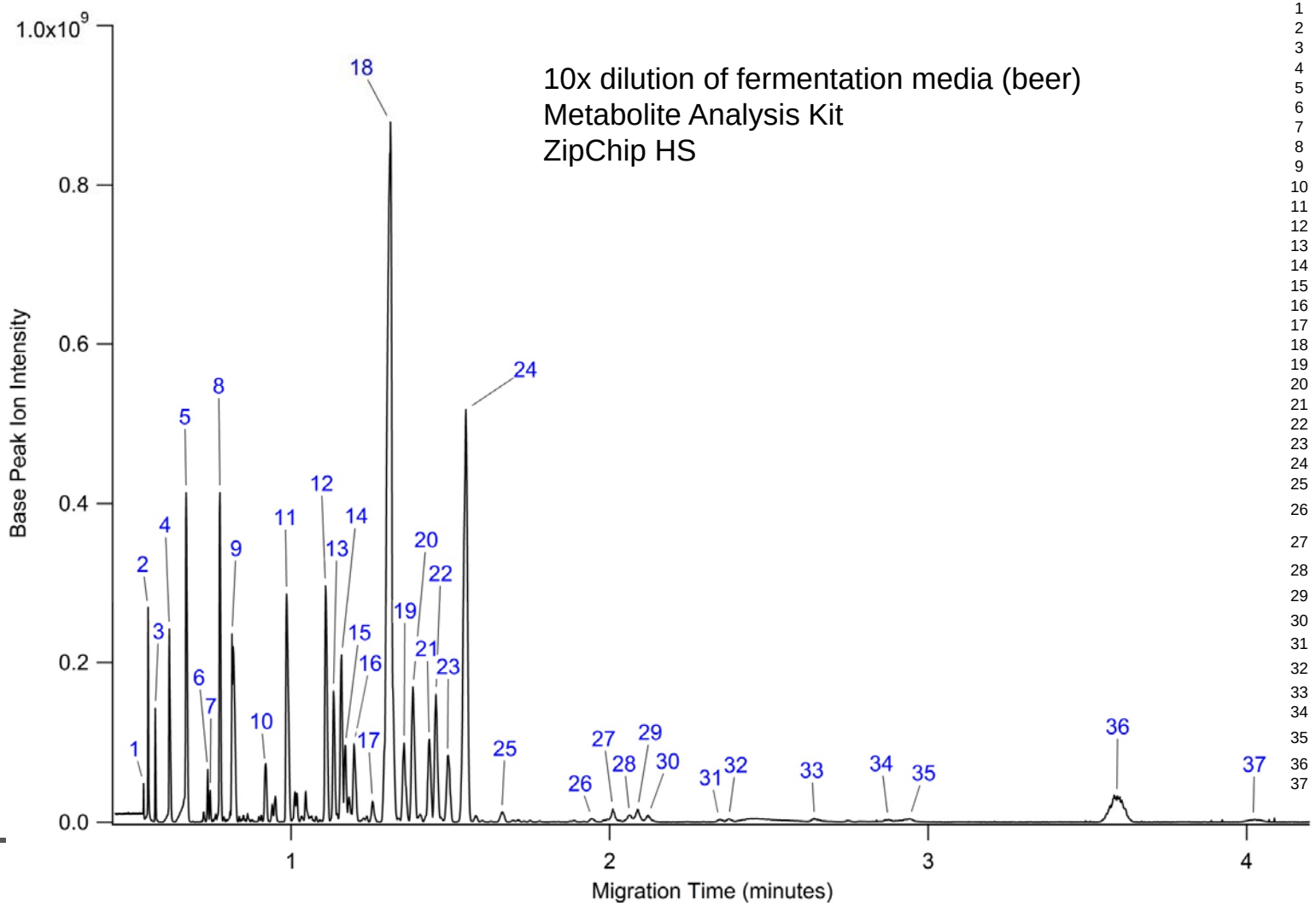
Metabolite Analysis with ZipChip



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

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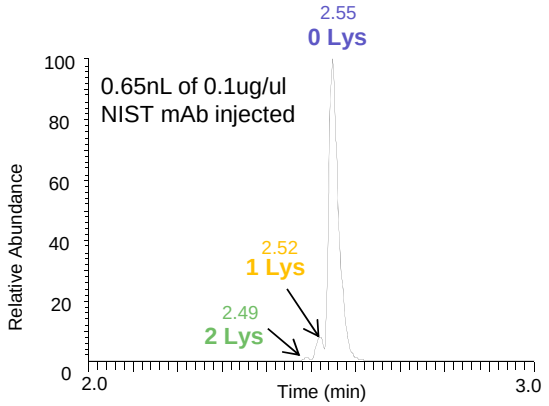
Biopharmaceuticals

Intact NIST mAb Analysis in HMR Mode

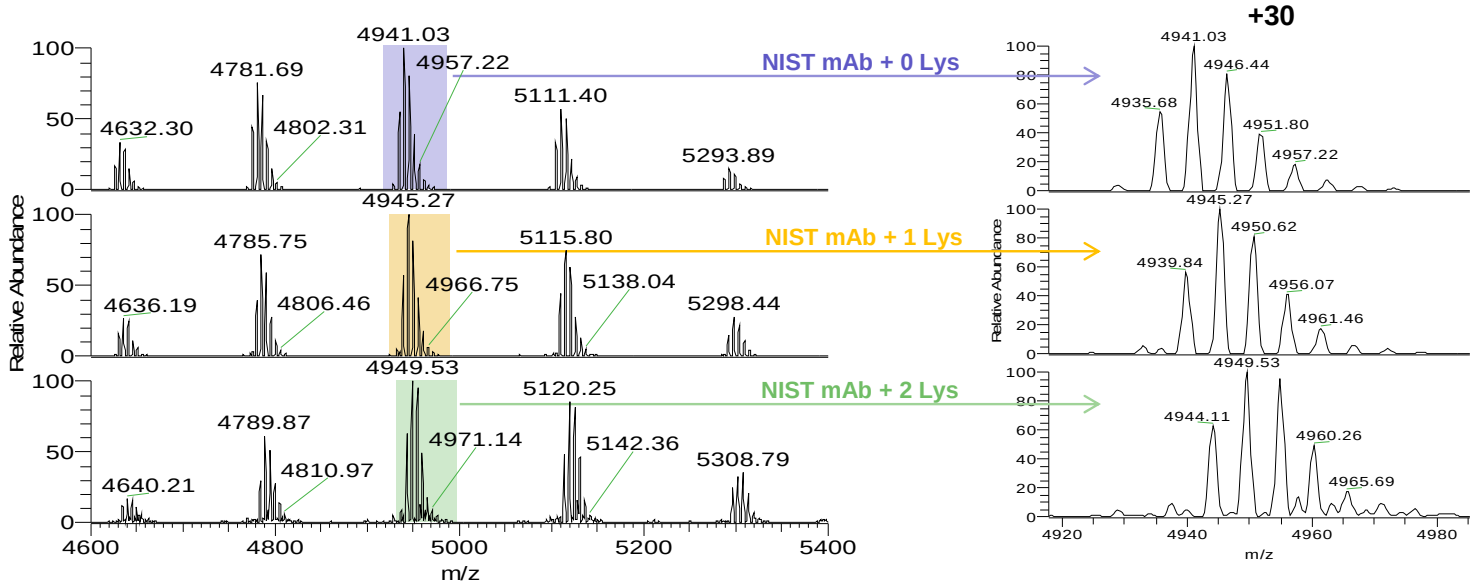
The rapid separation and accurate identification of highly differently abundant charge variants can also be consistently achieved by the ZipChip™ system and Q Exactive™ platform

- Near baseline separation of intact NIST mAb charge variants with abundance ranging over 2 orders of magnitudes can be achieved by the ZipChip system
- High resolution accurate mass data of each Lysine variant is confidently obtained on Q Exactive™ Plus/HF/HF-X
- Glycoform with abundance as low as 0.16% of the base peak can be detected and identified
- All 5 major glycoforms from each of the three different Lysine variants are identified by BioPharma Finder

MS data was acquired on a QE HF with BioPharma Option
CE separation was achieved on ZipChip HR



Protein Name	Modification	Average Mass (Da)	Theoretical Mass (Da)	Matched Mass Error (ppm)	Intensity	Relative Abundance	RT (min)
NIST	1xG0F_G0F	148038.8	148037.1	11.5	1.28E+09	58.82	2.55
NIST	1xG0F_G1F	148200.0	148199.3	5.0	2.18E+09	100.00	
NIST	1xG1F_G1F	148362.1	148361.2	6.0	1.82E+09	83.26	
NIST	1xG1F_G2F	148522.7	148523.5	5.8	1.00E+09	46.00	
NIST	1xG2F_G2F	148684.6	148685.7	7.2	4.84E+08	22.21	
NIST_plus1K	1xG0F_G0F	148165.6	148165.3	1.9	7.66E+07	3.51	2.49
NIST_plus1K	1xG0F_G1F	148327.4	148327.4	0.1	1.39E+08	6.36	
NIST_plus1K	1xG1F_G1F	148489.7	148489.4	2.5	1.18E+08	5.40	
NIST_plus1K	1xG1F_G2F	148652.3	148651.7	3.6	5.97E+07	2.74	
NIST_plus1K	1xG2F_G2F	148811.9	148813.9	13.4	2.90E+07	1.33	
NIST_plus2K	1xG0F_G0F	148295.4	148293.5	13.1	1.04E+07	0.48	2.42
NIST_plus2K	1xG0F_G1F	148456.9	148455.6	9.0	1.56E+07	0.72	
NIST_plus2K	1xG1F_G1F	148618.4	148617.5	5.6	1.51E+07	0.69	
NIST_plus2K	1xG1F_G2F	148779.4	148779.9	3.1	8.07E+06	0.37	
NIST_plus2K	1xG2F_G2F	148942.0	148942.0	0.4	3.49E+06	0.16	



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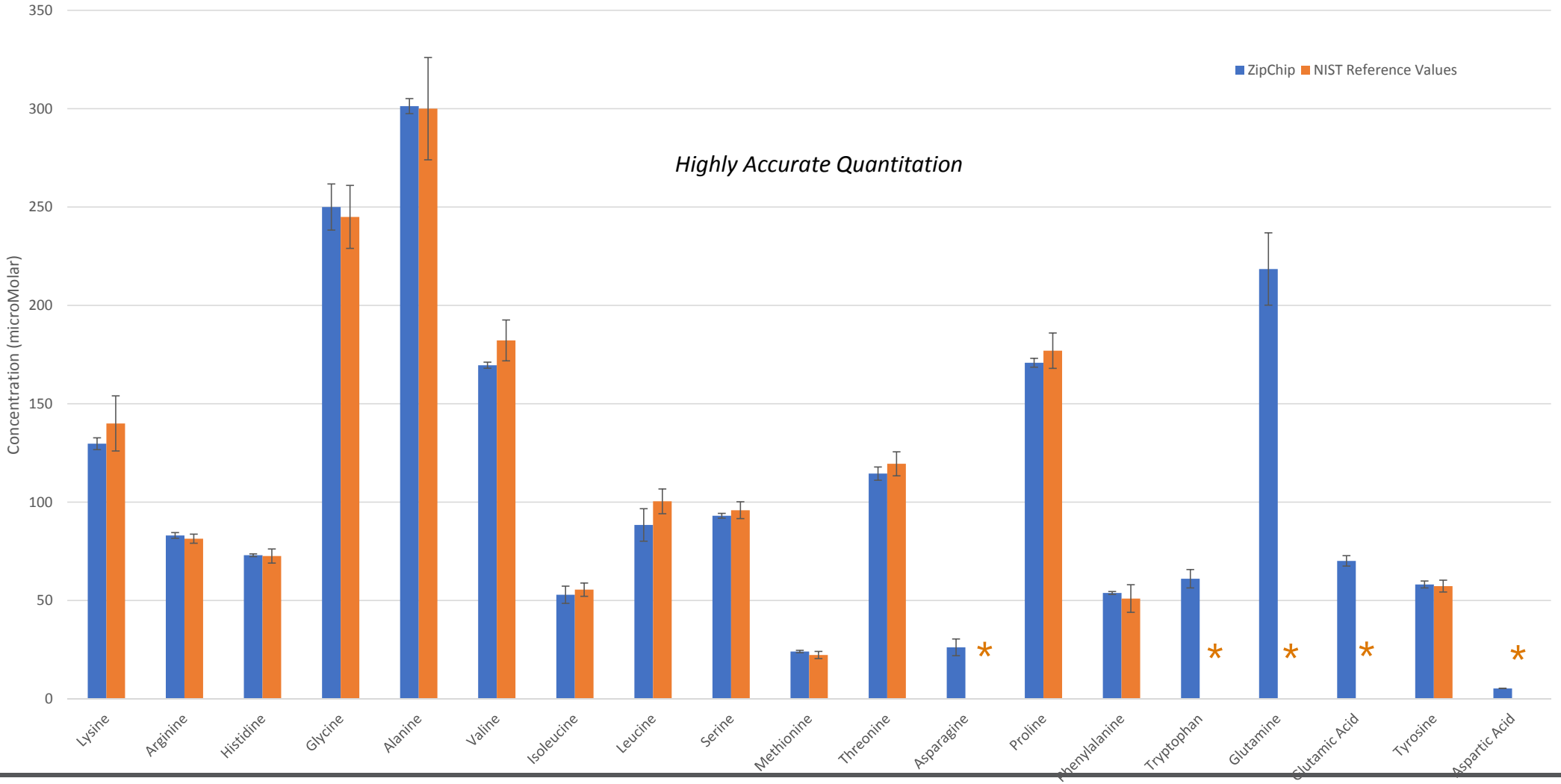
ZipChip Analysis of NIST Human Plasma, SRM 1950



* No NIST Reference Value

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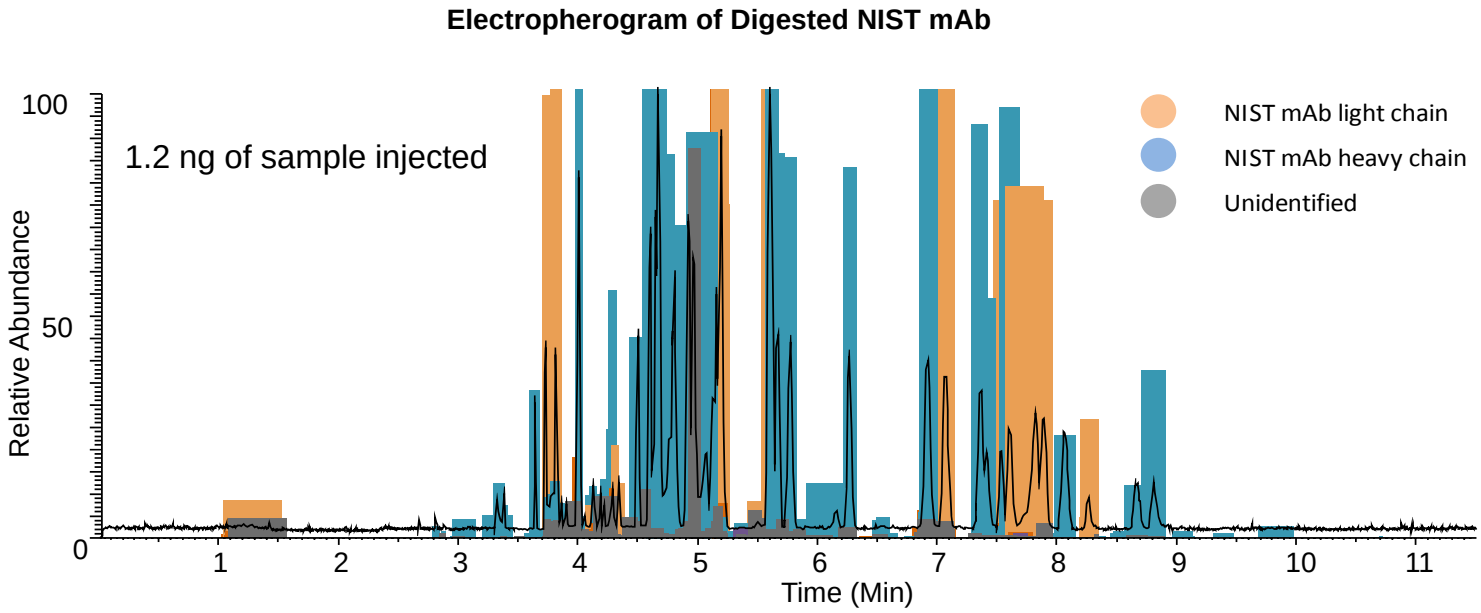
Peptide Mapping Analysis in Standard Mode

The combination of ZipChip™ sample separation, Q Exactive™ Plus/HF/HF-X produced HRAM MS and MS/MS spectra, and BioPharma Finder™ software enables fast and accurate peptide identification

- Plug and play ZipChip™ delivers stable nano spray and nano spray level sensitivity
- CE-MS/MS analysis can be completed in 10 minutes
- Only a few nanograms of sample are sufficient for the analysis
- 98% sequence coverage based on MS/MS data for the light chain and heavy chain is confidently achieved

MS data was acquired on a QE HF
with BioPharma Option
CE separation was achieved on ZipChip HR



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

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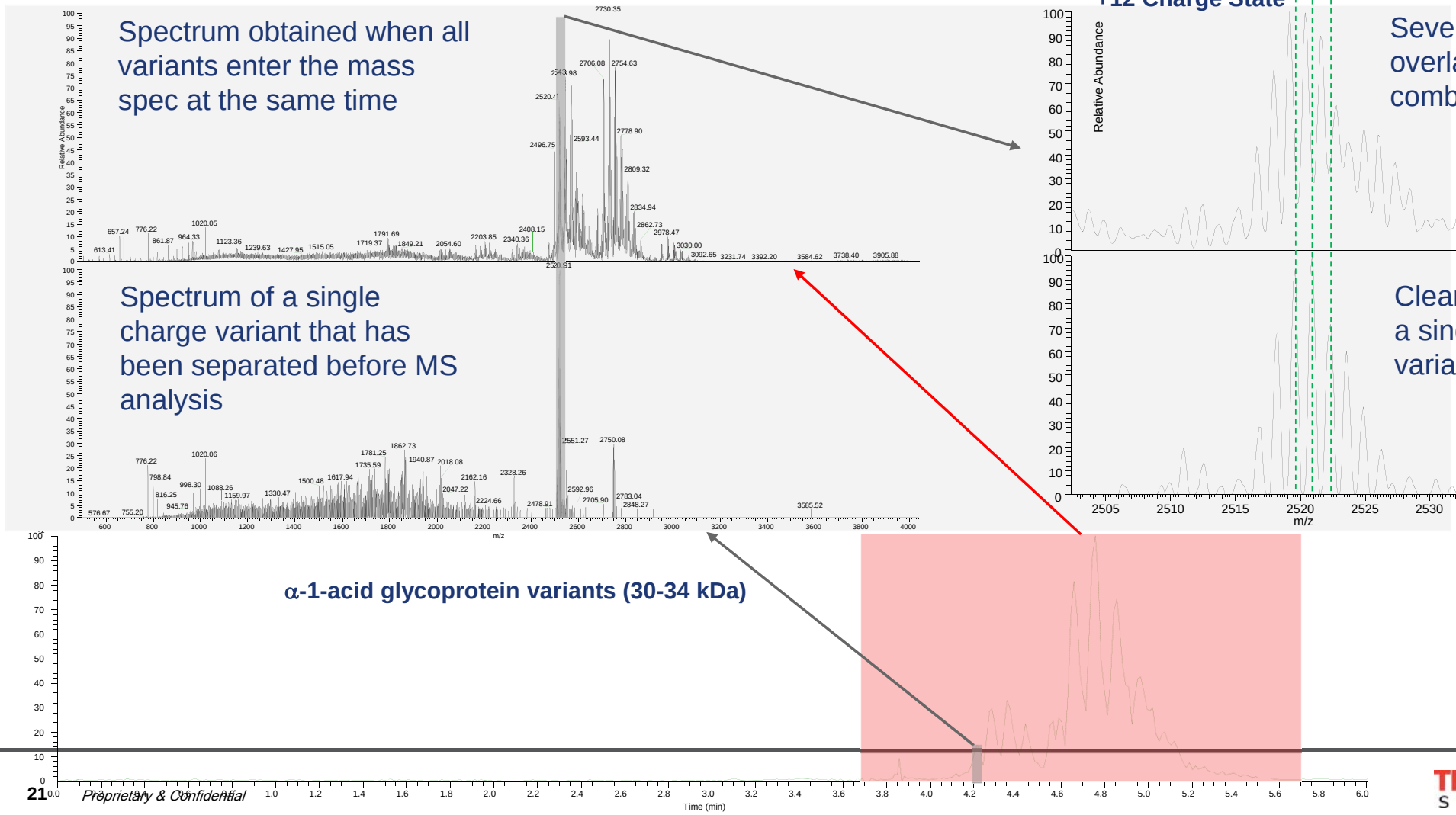
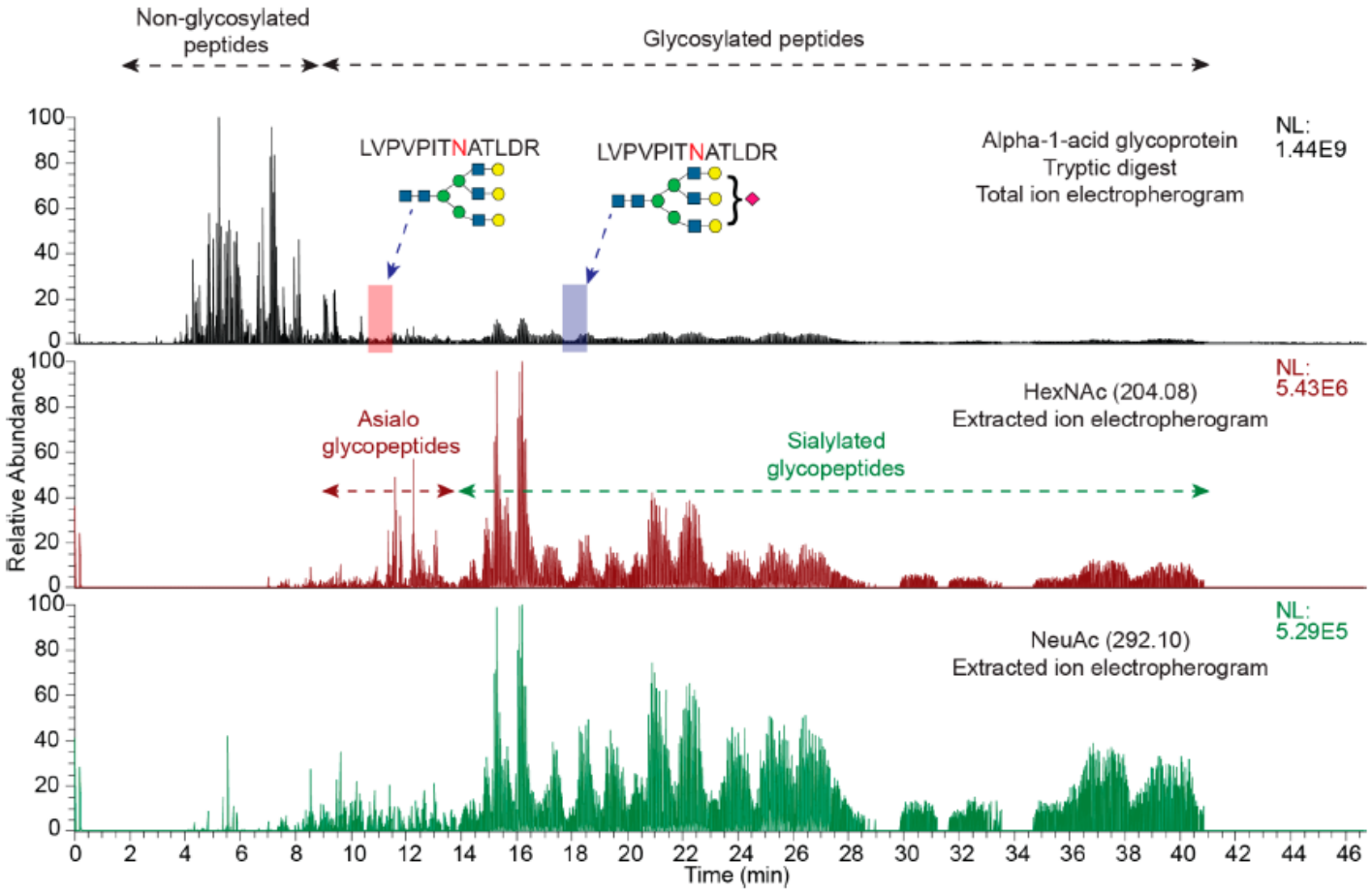
Glycoproteomics

Intact Analysis of Complex Glycoproteins



Glycoproteomics with ZipChip

Alpha-1-acid glycoprotein tryptic digest
ZipChip HR
Thermo Q Exactive Plus



- Glycopeptides naturally separate away from aglyco peptides
- In depth characterization of protein glycopeptides
- Improved separation resolution between glycopeptides compared to LC
- Reduced analysis times compared to LC

Khatri, K. et al; **Microfluidic Capillary Electrophoresis-Mass Spectrometry for Analysis of Monosaccharides, Oligosaccharides, and Glycopeptides**. *Anal. Chem*. 10.1021/acs.analchem.7b00875

ThermoFisher SCIENTIFIC

Top down and bottom up proteomics

Combined Top-Down and Bottom-Up Analysis in One Platform

thermoscientific

Combined Top-down and Bottom-up Proteomics using Capillary Electrophoresis-Mass Spectrometry

Chien-Hsun Alex Chen, Aaron Gajadhar, Ioanna Ntai, Andreas Huhmer

Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, CA 95131

- **CE-MS Workflow: Using one CE chip in a sequential run, instead of two LC columns in separate setups**

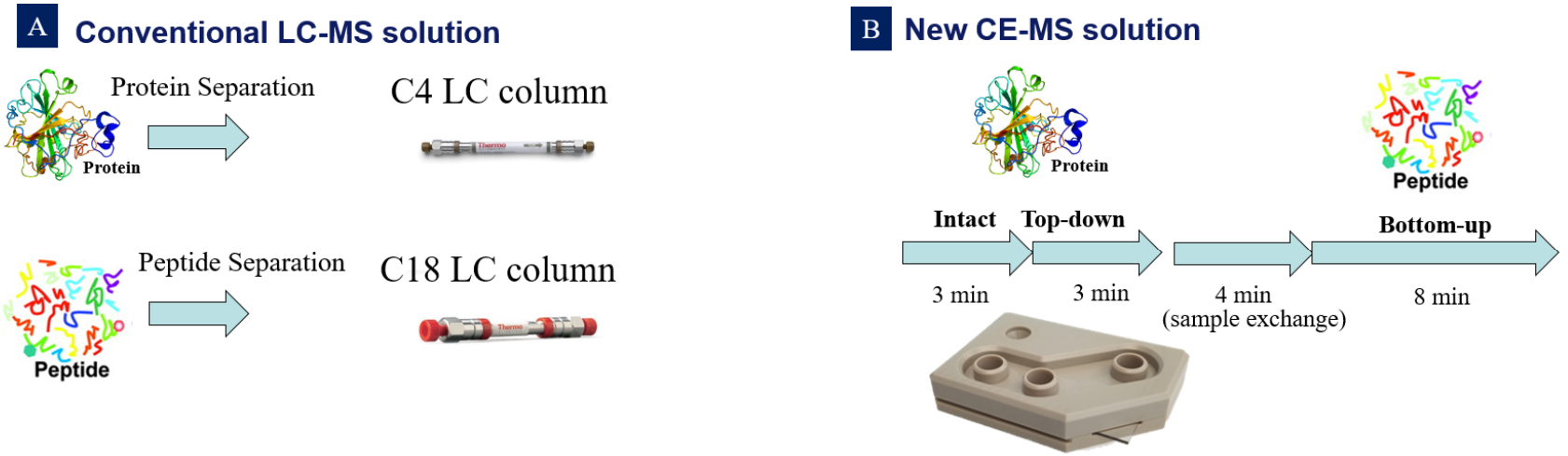
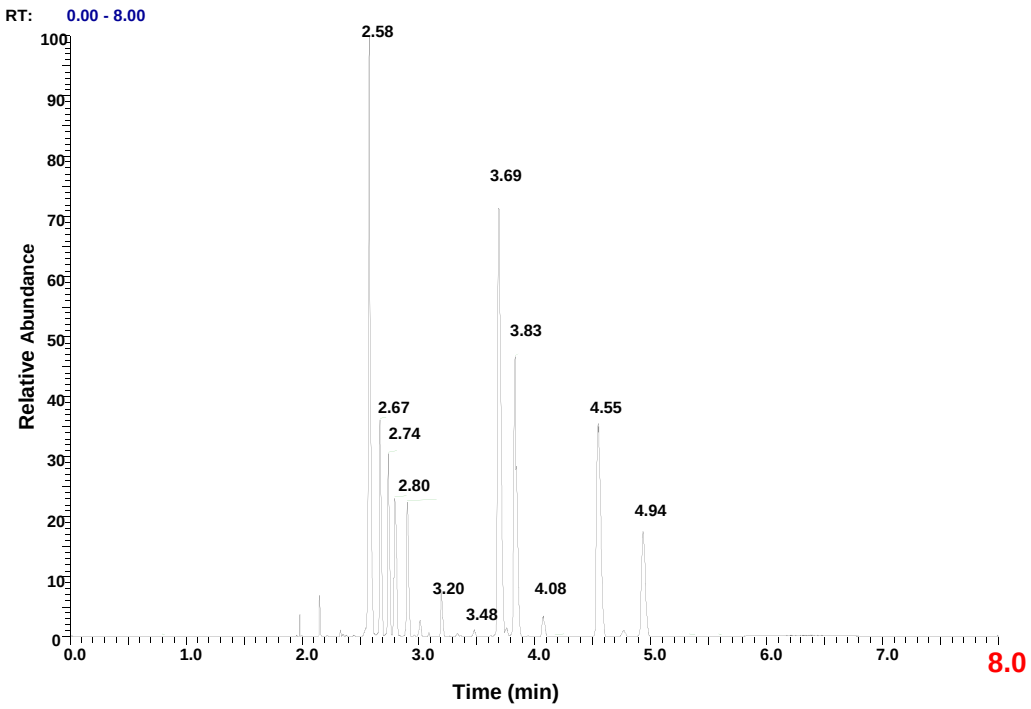


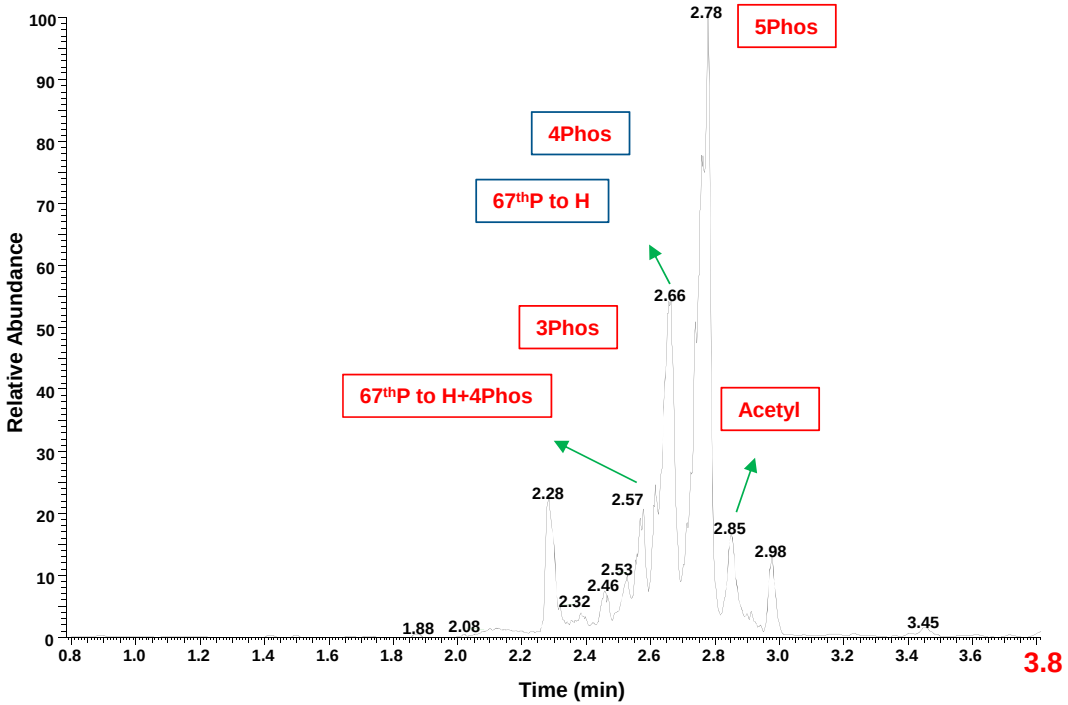
Figure 1. Schematics of combined top-down and bottom-up workflow. A) Conventional LC-MS workflow requires two LC platforms. One C4 column for protein separation, and one C18 column for peptide separation. B) New CE-MS workflow only requires one CE chip. Intact, top-down, and bottom-up analysis can be integrated into 18-min analysis.

Top-down + Bottom-up

Bottom Up Analysis of Cytochrome C



Top Down Analysis of Beta Casein



	Intact Protein	Bottom-up	Top-down	
	Proteoforms	Sequence coverage	Residue Cleavage	Modifications
Cytochrome C	1	97%	50%	2
Carbonic anhydrase	1	97%	19%	1
KRAS	10	89%	15%	4
Beta-casein	11	86%	16%	7

- Fast runs, efficient separations, and rapid method switching enable multi-level characterization with no wasted time

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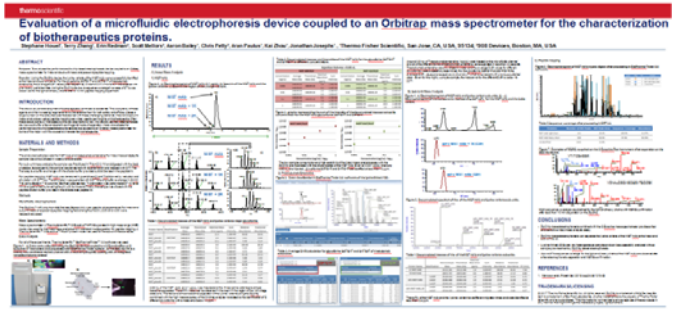
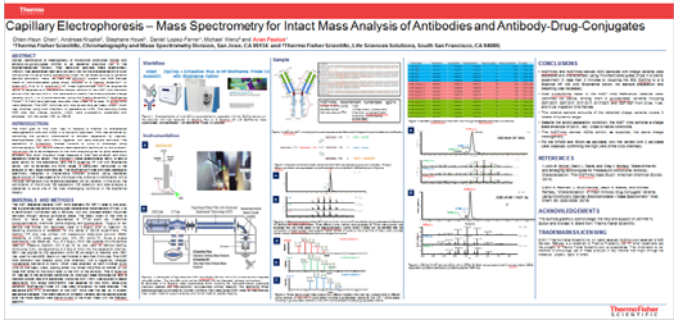
The ZipChip Advantage

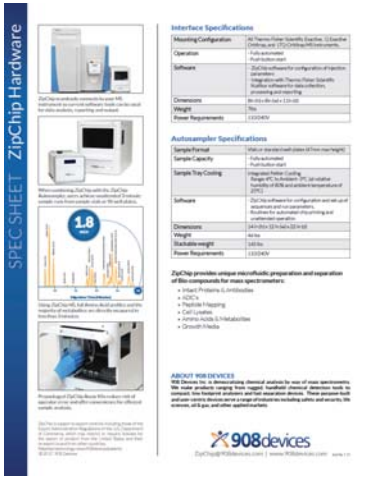
- Efficient separations regardless of size
- Small molecules to large intact proteins
- Fast separations
- Sensitive and stable nano-ESI
- Minimal sample prep
- No analyte labeling
- Separations based only on charge and size
- Fast and easy switching between analyte classes

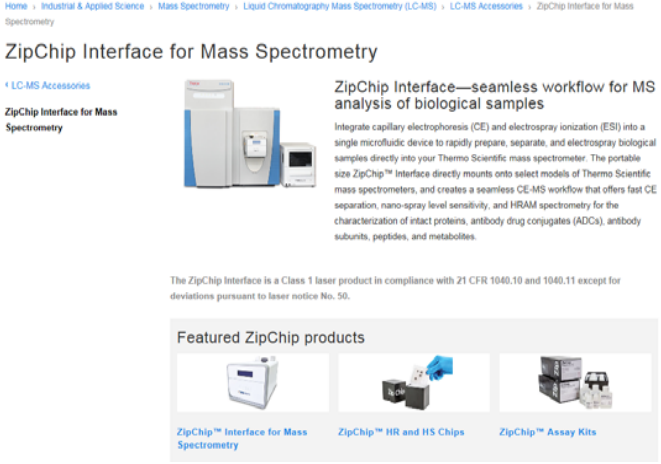
Available Resources

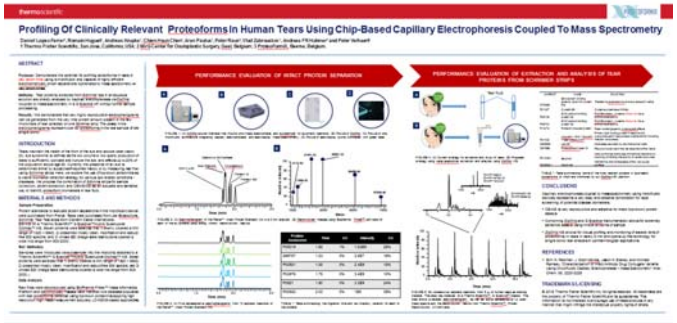
- Brochure
- Spec sheets
- Posters
- TF.com page
- Appl. Note











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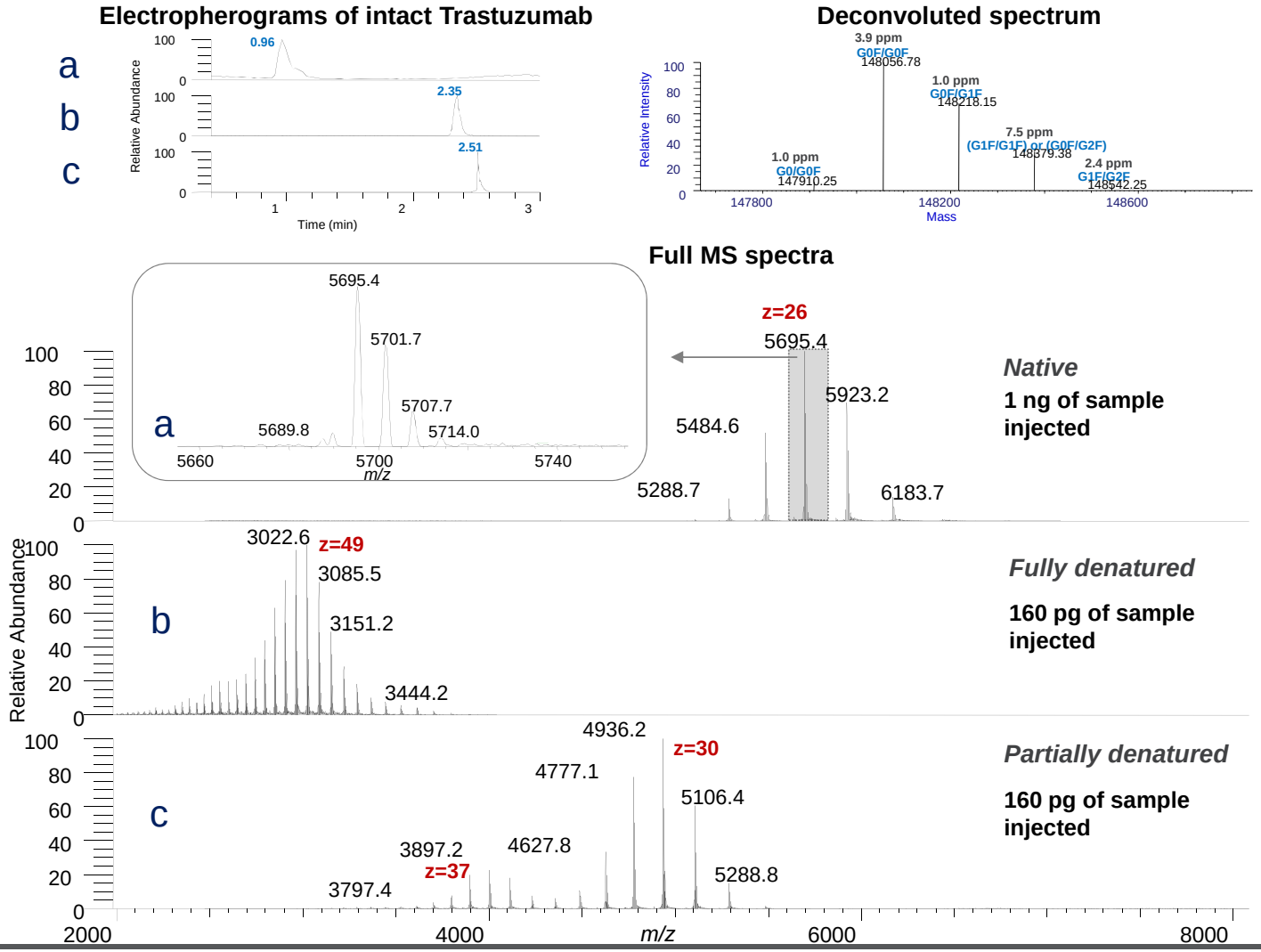
Intact mAb-- Trastuzumab Analysis in HMR mode

The ZipChip™ system coupled with the Q Exactive™ platform can quickly analyze intact mAbs in native, partially denatured, and fully denatured conditions to support biotherapeutics characterizations under a diverse range of conditions

- CE/ESI-MS analysis can be completed within 3 minutes
- High resolution accurate mass spectra in intact native, partially denatured, and fully denatured states on the Q Exactive™ Plus/HF/HF-X with BioPharma option are confidently achieved
- Sample consumption can be as low as pico grams to nano grams
- Major glycoforms are identified by BioPharma Finder

MS data was acquired on a QE HF-X with BioPharma Option
CE separation was achieved on ZipChip HR

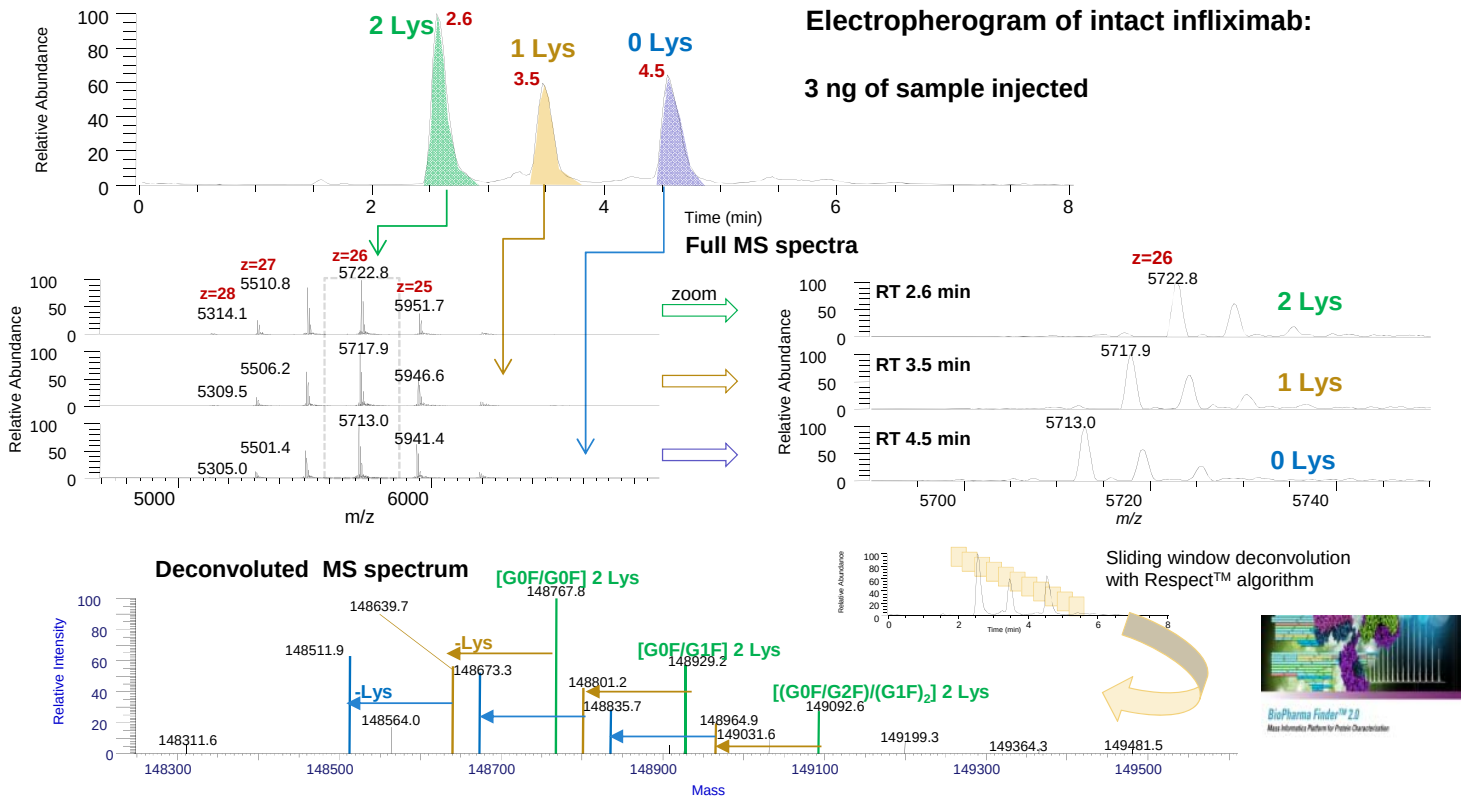


Intact mAb– Infliximab Analysis in HMR mode

The ZipChip™ system coupled with the Q Exactive™ platform is unique and powerful to separate and identify different intact antibody charge variants in native, partially denatured, and denatured conditions

- Baseline separation of intact mAb charge variants resulting from different levels of Lys-clipping can be achieved within three minutes by ZipChip™ system
- High resolution accurate mass spectra of all lysine variants are confidently detected on Q Exactive Plus/HF/HF-X with BioPharma option
- Three major glycoforms from each of the three lysine variants are identified by BioPharma Finder with mass accuracies better than 10 ppm

MS data was acquired on a QE HF-X with BioPharma Option
CE separation was achieved on ZipChip HR



Theoretical and experimental masses of Infliximab glycoforms of Lysine variants

Lys-Variant	Glycoform	Theor. average mass	Exp. average mass	Δ Mass (ppm)
2 Lys	G0F/G1F	148930.7	148929.2	9.8
2 Lys	G0F/G0F	148768.5	148767.8	4.8
2 Lys	(G1F)2 or (G0F/G2F)	149092.8	149092.6	1.3
1 Lys	G0F/G0F	148640.3	148639.7	4.3
1 Lys	G0F/G1F	148802.5	148801.2	8.6
1 Lys	(G1F)2 or (G0F/G2F)	148964.6	148964.9	-1.8
0 Lys	G0F/G0F	148512.2	148511.9	1.6
0 Lys	G0F/G1F	148674.3	148673.3	6.8
0 Lys	(G1F)2 or (G0F/G2F)	148836.5	148835.7	5.1

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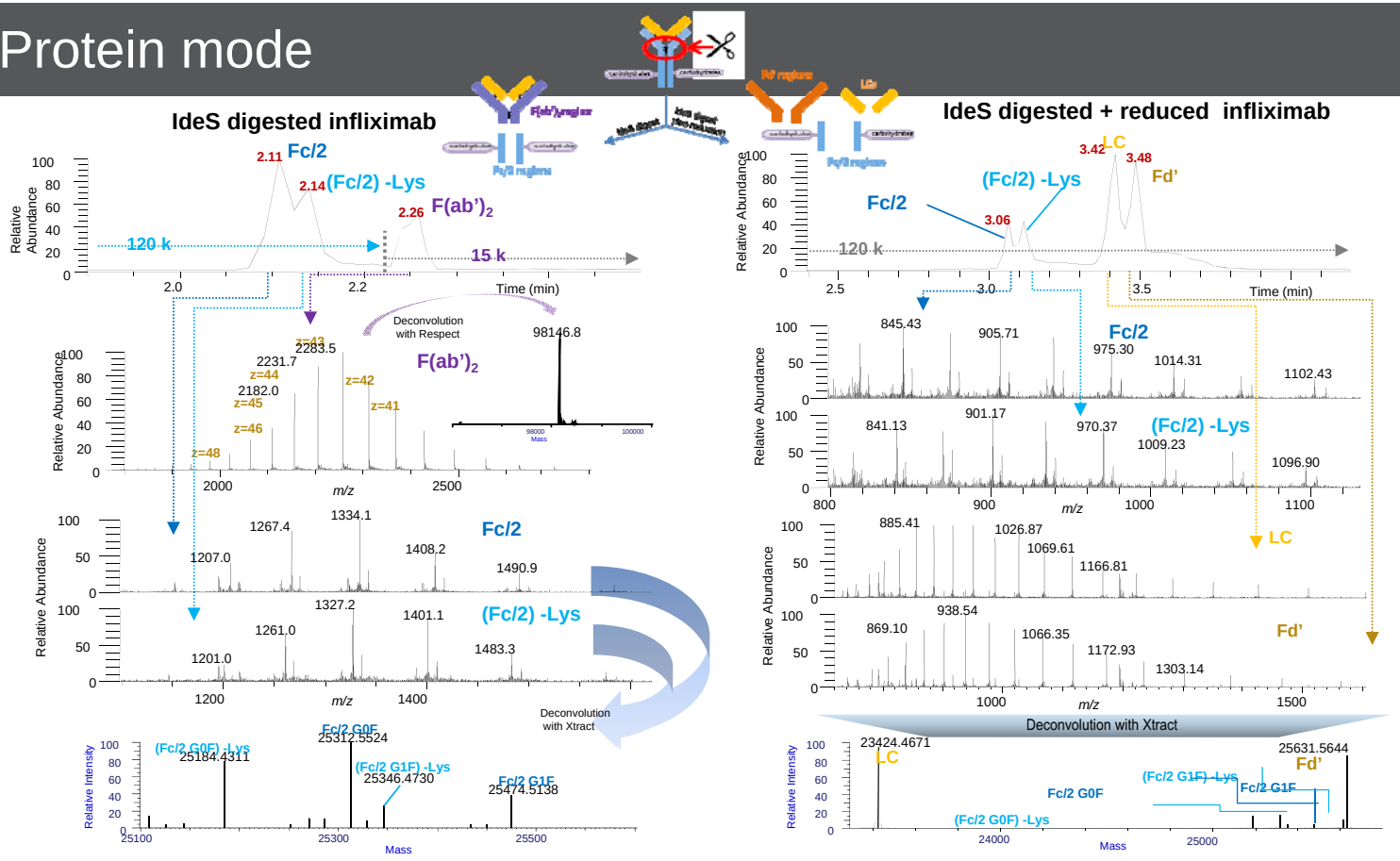
mAb Subunits Analysis in Protein mode

Fast, sensitive, and accurate antibody subunit analysis can be accomplished by the ZipChip™ system and Thermo Scientific™ Q Exactive™ Plus or HF/HF-X platform

- Separation of infliximab mAb subunits can be achieved in 3 minutes by ZipChip™ system
- The sliding window method combined with Xtract™ deconvolution algorithm in BioPharma Finder ™ software enables monoisotopic mass determination of each subunit
- Lysine variants and their major glycoforms of the subunits can be identified by BioPharma Finder software

MS data was acquired on a QE HF-X with BioPharma Option
CE separation was achieved on ZipChip HR



Subunit	Monoisotopic theo. mass	Experimental mass	Δ Mass (ppm)
IdeS digest plus reduction			
Light chain	23424.3946	23424.4671	3.1
Fd'	25631.5325	25631.5644	1.2
Fc/2 G0F	25316.5863	25316.6345	1.9
Fc/2 G1F	25478.6391	25478.5753	2.5
(Fc/2 G0F) -Lys	25188.49131	25188.5763	3.3
(Fc/2 G1F) -Lys	25350.54413	25350.5246	0.8
IdeS digest			
F(ab')2	98146.3 (av.)	98146.8	-4.8
Fc/2 G0F (2 SS bonds)	25312.5550	25312.5524	0.1
Fc/2 G1F (2 SS bonds)	25474.6078	25474.5138	3.7
(Fc/2 G0F) -Lys (2 SS bonds)	25184.4600	25184.4311	1.1
(Fc/2 G1F)- Lys (2 SS bonds)	25346.5128	25346.4730	1.6

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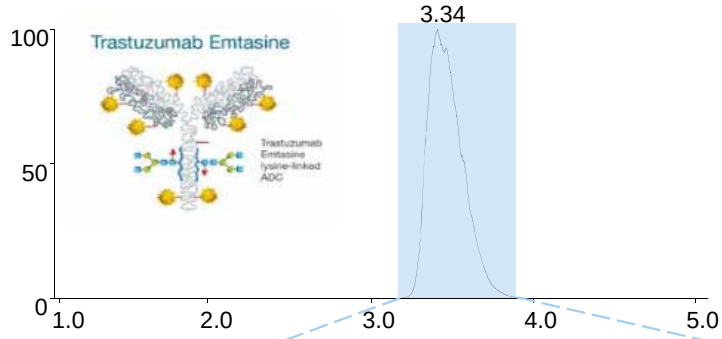
Antibody-Drug Conjugate (ADC) Analysis in HMR Mode

Heterogenetic ADCs can be successfully characterized within 1 minute without sample pre-treatment by ZipChip™system and Q Exactive™ Plus/HF/HF-X, and the powerful BioPharma Finder™ software

- Powerful sliding window capability enabled by BioPharma Finder
- All of the different forms of Trastuzumab Emtansine can be analyzed in less than 40 seconds with only 3 nanograms of sample injected
- No sample pre-treatment required
- The calculated average DAR values are consistent with previous published data

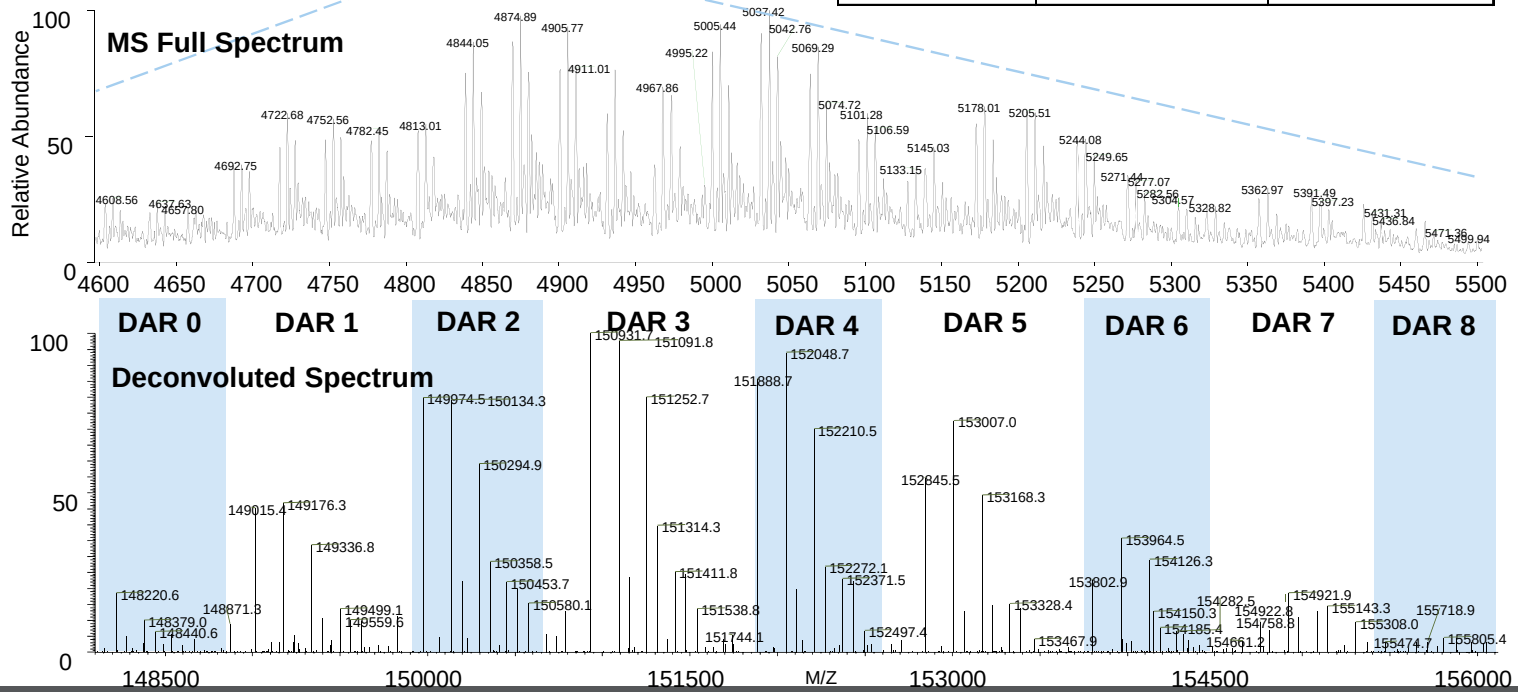
MS data was acquired on a QE HF with BioPharma Option
CE separation was achieved on ZipChip HR

Electropherogram of Trastuzumab Emtansine



Average Drug-to-Antibody Ratio (DAR) is 3.47

G0F/G1F DAR Form	Mass Accurancy (ppm)	Relative abundance
DAR0	10.72	9.95
DAR1	7.45	33.47
DAR2	1.23	58.97
DAR3	2.37	79.90
DAR4	5.63	69.92
DAR5	9.08	49.14
DAR6	13.89	28.97
DAR7	9.93	12.64
DAR8	8.41	2.83



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