ThermoFisher SCIENTIFIC

Introducing the Thermo Scientific™ Q Exactive™ HF-X and ZipChip™ Technology for BioPharma Applications

Jonathan L. Josephs Ph.D.
Marketing Director
Life Sciences BU

Thermo Scientific Q Exactive BioPharma MS Offers a Complete Characterization Solution for BioPharma Customers

High Mass Range (HMR) Mode
Intact mAb and ADC Analysis

Optimized intact protein analysis under both native and denaturing conditions assures the highest quality and most informative spectra for the widest range of therapeutic proteins.

?

Protein Mode
Subunit Analysis Top/Middle-Down

Extreme resolving power of the Orbitrap™ mass analyzer ensures isotopic resolution of subunits and facilitates top/middle-down sequencing.

Standard Mode
Peptide Mapping

Perform peptide mapping with Orbitrap technology for unparalleled acquisition speed, mass accuracy, and spectral quality.

The most powerful system for every workflow

All in one package

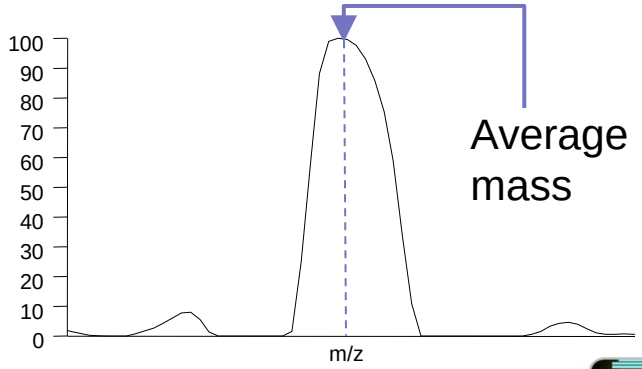
Two Types of Orbitrap Measurements for Intact Proteins

Average mass resolution

High Mass Range (HMR) mode

*Works for all proteins **(mAbs)***

Lowest resolving power

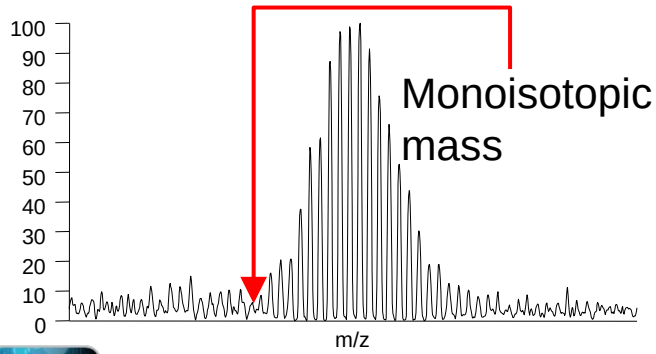


Isotopic resolution

Protein mode

Small / medium-sized proteins **(mAb subunits)**

Highest resolving power

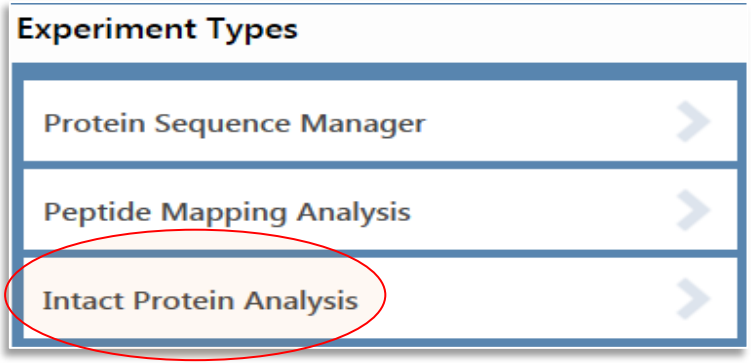


ReSpect deconcolution algorithm

Xtract deconvolution algorithm

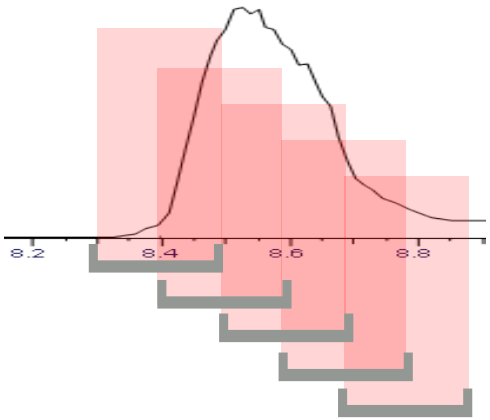
ThermoFisher SCIENTIFIC

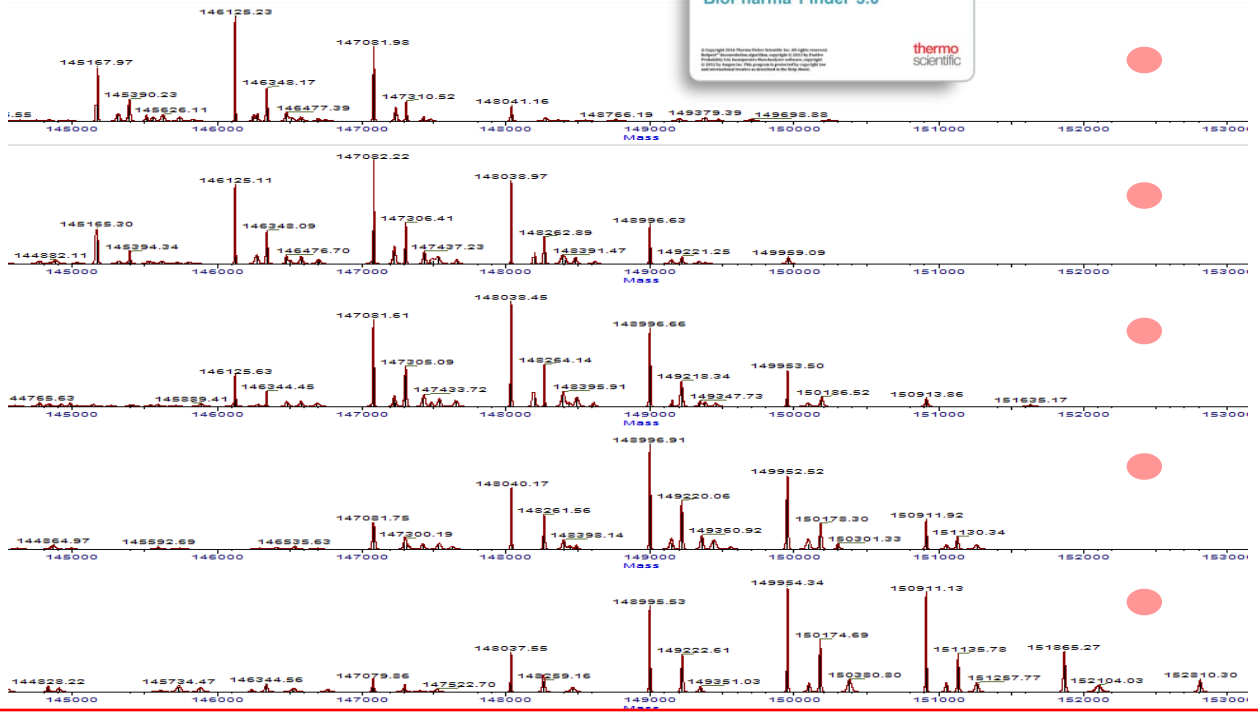
Intact Protein Analysis in Thermo Scientific BioPharma Finder

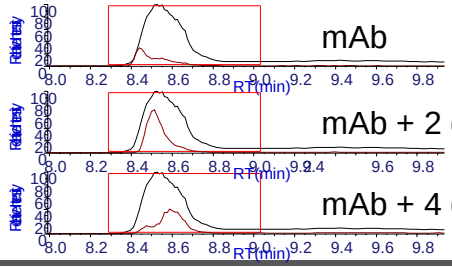


- Workflow software for intact protein mass determination
- Supports all **Orbitrap** mass spectrometers
- Includes 2 deconvolution algorithms:
  - **Xtract** for isotopically resolved proteins
  - **ReSpect™** for isotopically unresolved proteins (e.g. IgG)
- Batch processing/automation

Sliding Window Deconvolution is the Smart Choice for Chromatography

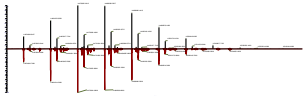




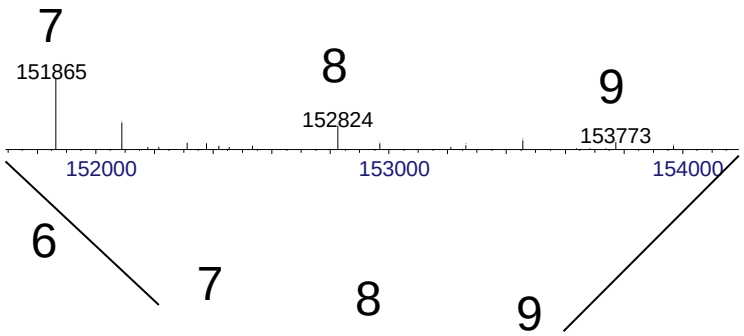


Components integrated

Comparison of Sliding Window and “Standard” Deconvolution



0 1 2 3 4 5



Sliding window detects the higher mass ADC components missed by “standard” deconvolution

Thermo Scientific Q Exactive BioPharma MS Offers a Complete Characterization Solution for BioPharma Customers

High Mass Range (HMR) Mode
Intact mAb and ADC Analysis

Optimized intact protein analysis under both native and denaturing conditions assures the highest quality and most informative spectra for the widest range of therapeutic proteins.

?

Protein Mode
Subunit Analysis Top/Middle-Down

Extreme resolving power of the Orbitrap™ mass analyzer ensures isotopic resolution of subunits and facilitates top/middle-down sequencing.

Standard Mode
Peptide Mapping

Perform peptide mapping with Orbitrap technology for unparalleled acquisition speed, mass accuracy, and spectral quality.

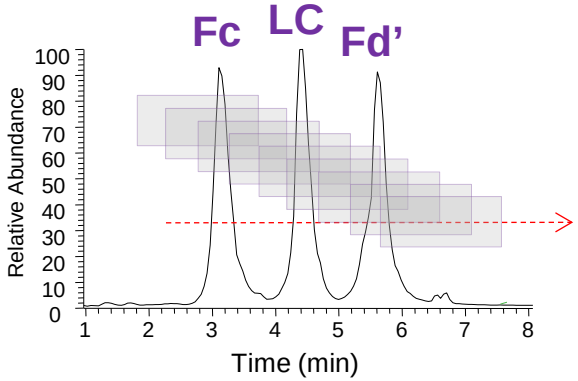
The most powerful system for every workflow

All in one package

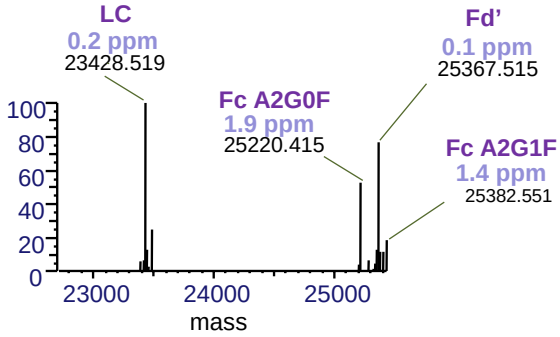
ThermoFisher SCIENTIFIC

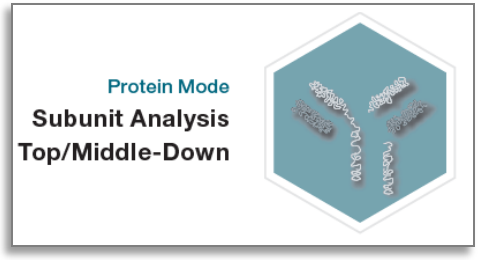
Subunit Analysis in Protein Mode on Thermo Scientific Q Exactive Plus MS

LC-MS Analysis of IdeS-digested, Reduced Trastuzumab

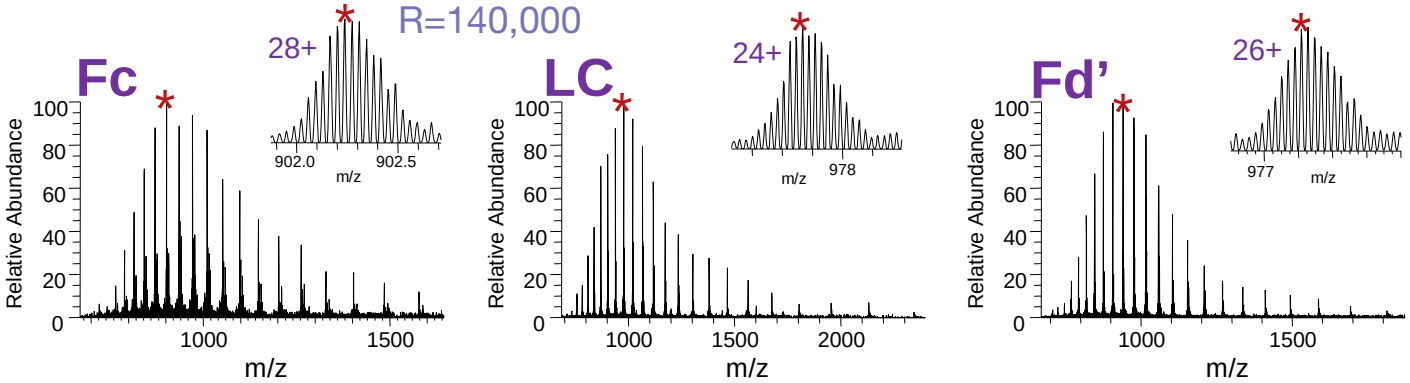


Sliding Window
Xtract™ deconvolution



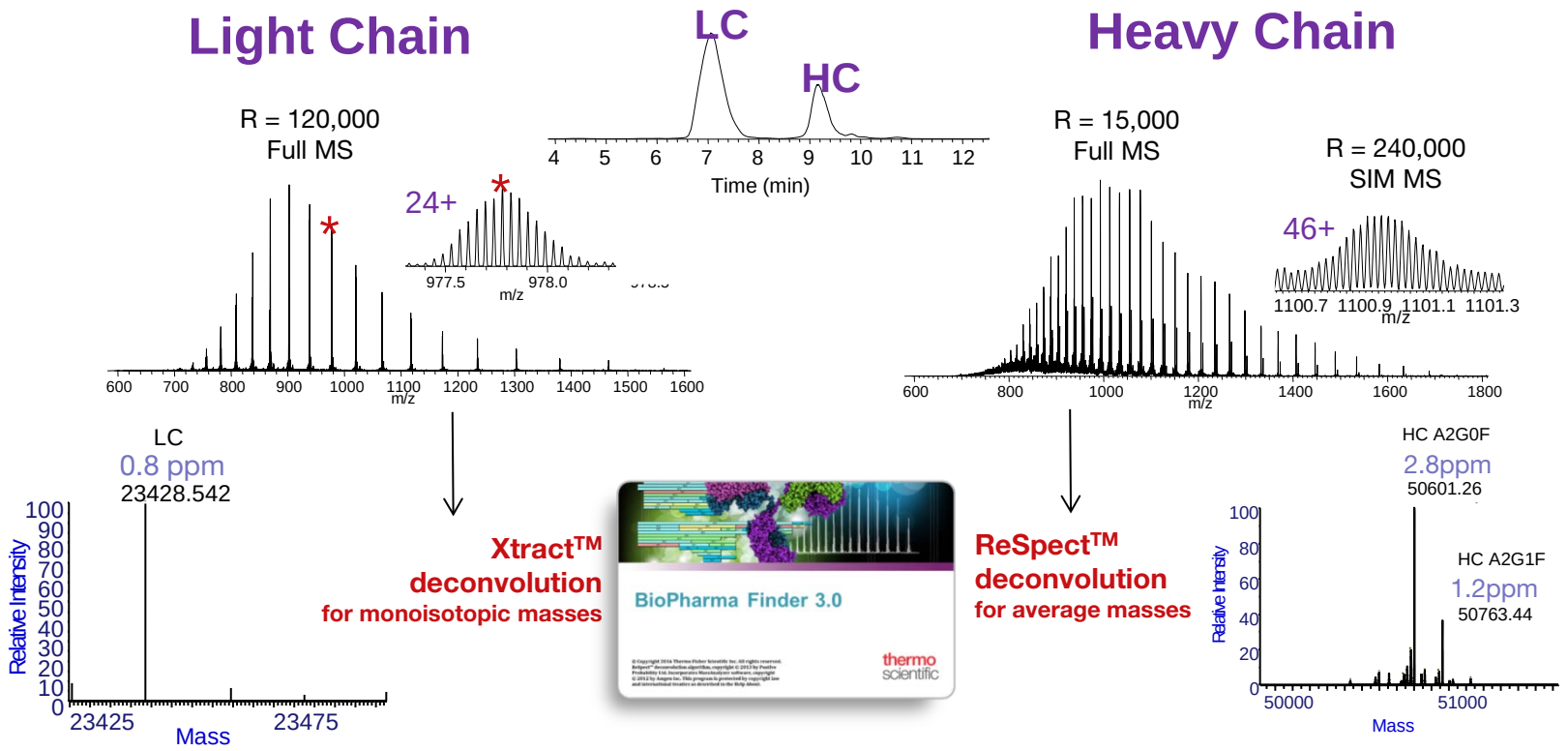


R=140,000



ThermoFisher SCIENTIFIC

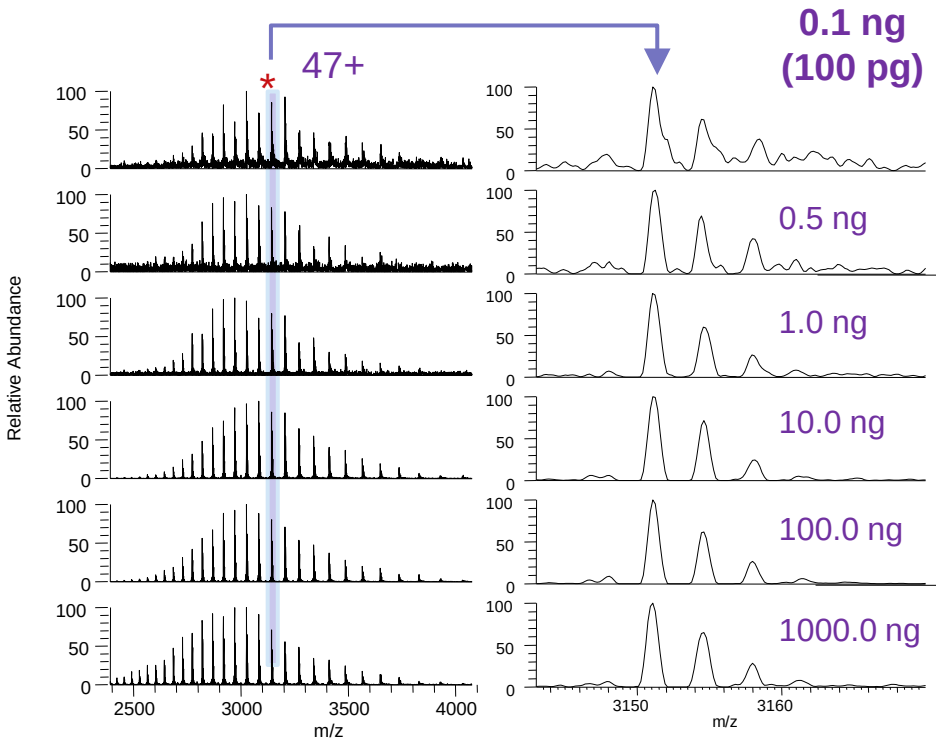
Subunit Analysis in Protein Mode on Thermo Scientific Q Exactive HF MS
LC-MS Analysis of Reduced Trastuzumab



ThermoFisher SCIENTIFIC

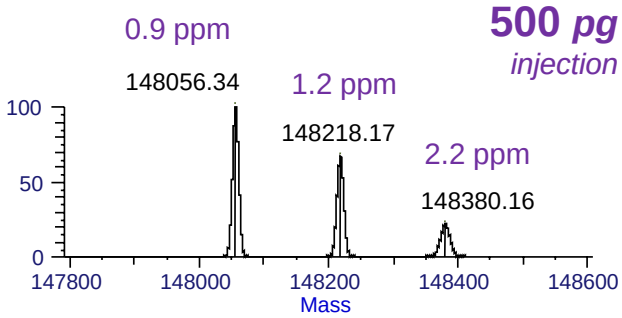
Denaturing LC-MS in HMR mode
Sensitive Intact Analysis of Trastuzumab Antibody

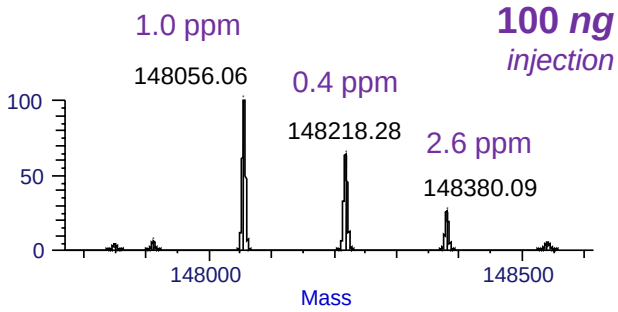
High Mass Range
(HMR) Mode
Intact mAb and
ADC Analysis



Q Exactive Plus in HMR Mode with MAbPac RP 2.1 x 50mm, 250 µl/min

ReSpect™ deconvolution

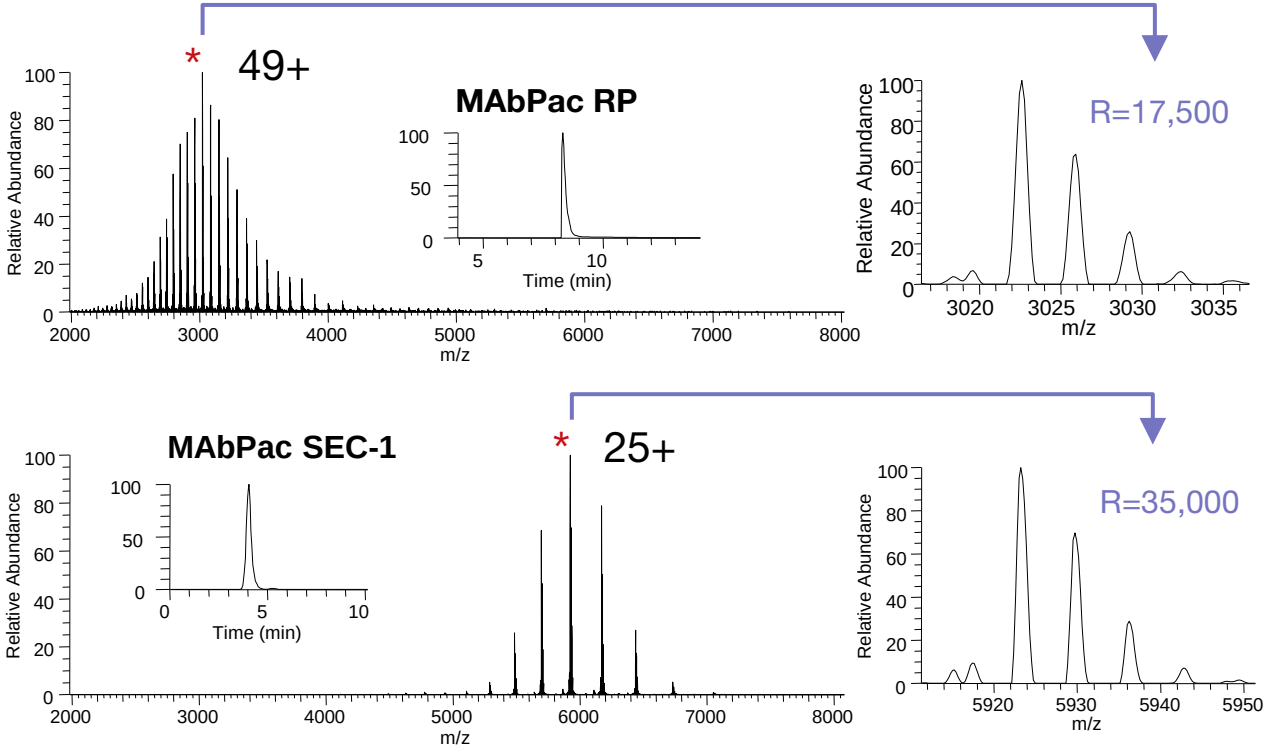




ThermoFisher SCIENTIFIC

Navigating Intact Protein Complexity with High Mass Range (HMR) Mode

High Mass Range (HMR) Mode
Intact mAb and ADC Analysis



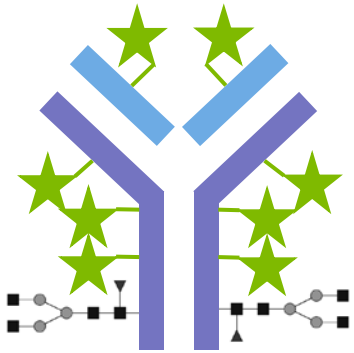
Denaturing MS is compatible with reverse phase HPLC separation.

Native MS is best for co-eluting high complexity samples like ADCs

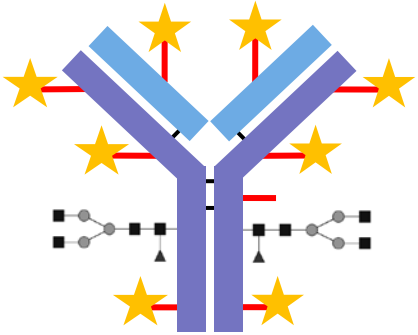
HMR mode intact analysis of Trastuzumab shows same answer in denaturing or native conditions

ThermoFisher SCIENTIFIC

Two Reasons Why Native MS Intact Analysis is a Powerful Addition to a Comprehensive ADC Characterization Strategy



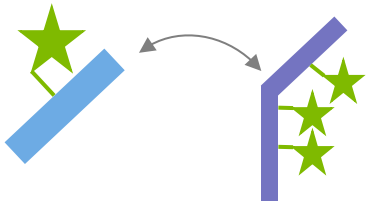
Cysteine-linked

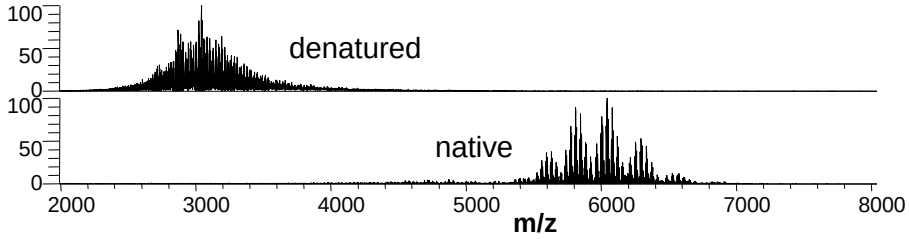


Lysine-linked

(1) Preservation of structural non-covalent interactions

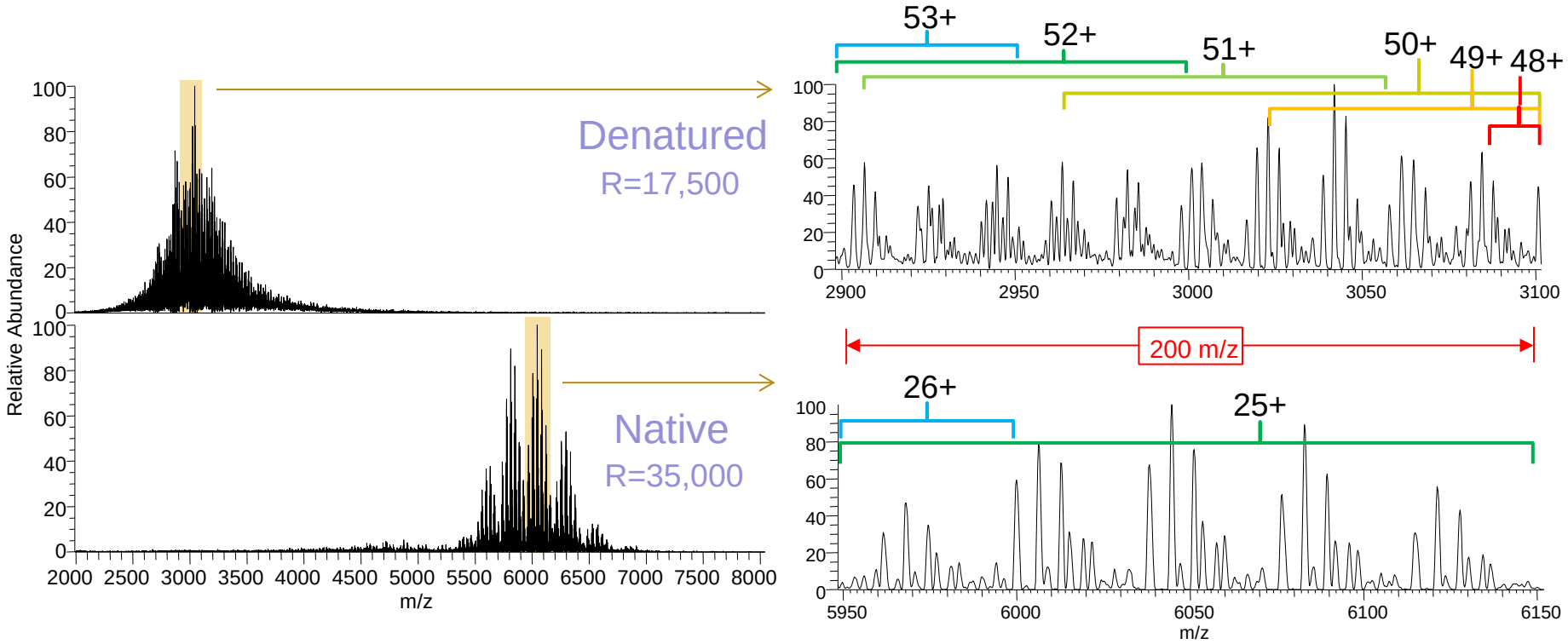
(2) Increased m/z separation in high mass range





ThermoFisher SCIENTIFIC

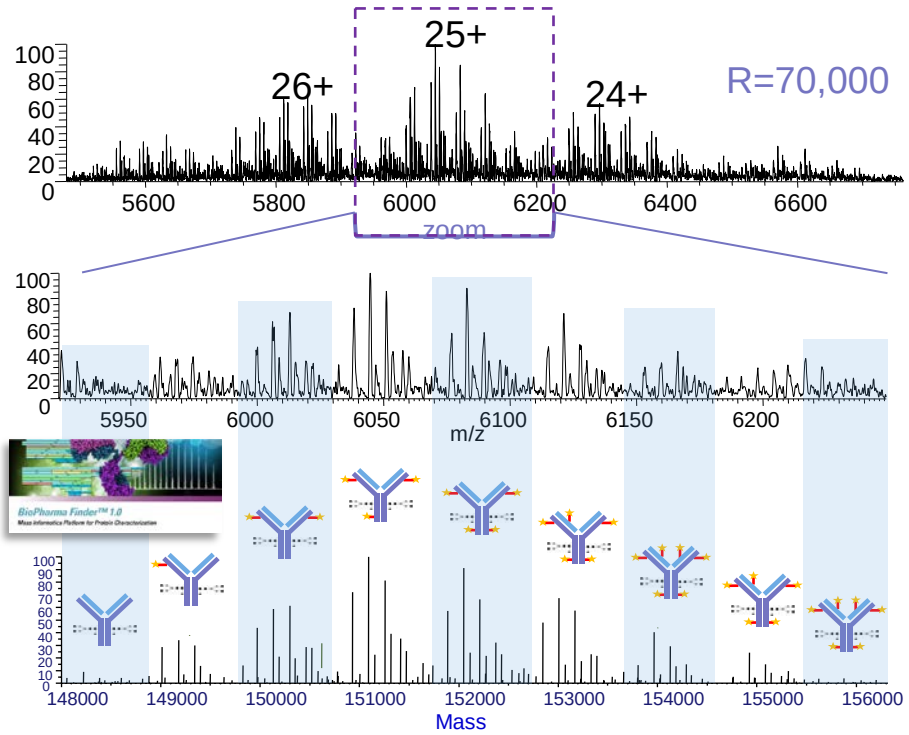
Intact Analysis of *Trastuzumab Emtansine* Lysine-linked ADC Denaturing vs. Native Conditions



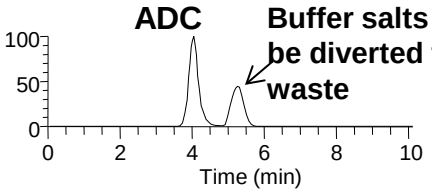
Native MS allows greater m/z separation of co-eluting species' charge states

ThermoFisher SCIENTIFIC

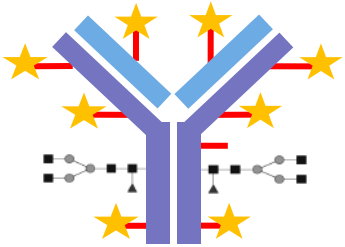
Native LC-MS in High Mass Range (HMR) Mode
Intact ADC Analysis of Trastuzumab Emtansine



Size Exclusion Chromatography



G0F/G1F DAR	Mass Accuracy (ppm)	Relative Abundance
D0	**6.49**	9.19
D1	21.69	34.26
D2	**0.05**	59.03
D3	**6.81**	100.00
D4	**5.17**	91.16
D5	**6.69**	67.42
D6	15.20	40.46
D7	**6.28**	24.28
D8	**3.78**	3.84

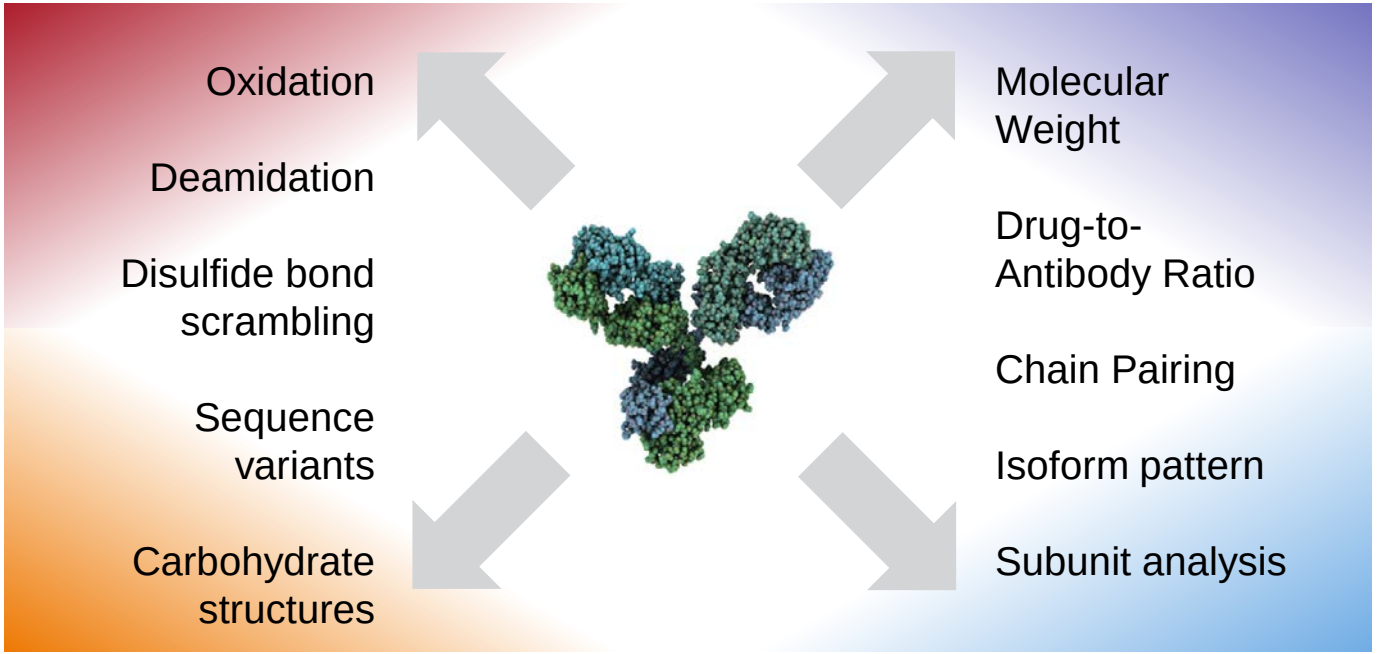


Trastuzumab Emtansine
Lysine-linked ADC

Average Drug-to-Antibody Ratio (DAR)
3.71

ThermoFisher SCIENTIFIC

Understanding Micro-heterogeneity and Critical Quality Attributes (CQAs)
Biologics are Always Complex Mixtures



Comprehensive characterization of therapeutic protein CQAs requires analysis from multiple perspectives.

Thermo Scientific UHPLC + Orbitrap MS + Data Analysis Software
A Powerful Solution to the Challenge of BioPharma Characterization

Thermo Scientific™
Vanquish™ Horizon
UHPLC system

Thermo Scientific™
Q Exactive™ Plus/HF/HF-X
BioPharma
Orbitrap™ Mass Spectrometer

Thermo Scientific™
BioPharma Finder™
Data Analysis Software Platform

ThermoFisher SCIENTIFIC

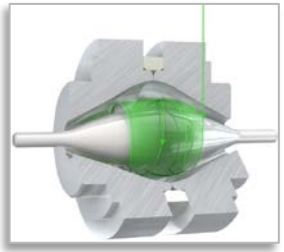
Thermo Scientific Q Exactive HF-X MS Specifications

Scan rate	Up to 40 Hz
Resolution	240,000 (FWHM) at m/z 200
Mass range	50 to 6,000 m/z Up to 8,000 m/z in BioPharma option
Mass Accuracy	3 ppm external, 1 ppm internal
Dissociation	Source CID, HCD
Multiplexing	Up to 10 precursor ions
Detectors	Orbitrap device
Polarity Switching	one full cycle in <1 sec (one full positive mode scan and one full negative mode scan at a resolution setting of 60,000)
Scan Functions	FS: Full Scan AIF: All Ion Fragmentation, SIM: Selected Ion Monitoring, PRM: Parallel Reaction Monitoring, DIA: Data Independent Acquisition, ddHCD: data dependent HCD
Options	BioPharma option

Thermo Scientific Q Exactive HF-X MS: Improved Architecture, Improved Performance

High Capacity Transfer Tube (HCTT)

Ultra-High Field Orbitrap Mass Analyzer



HCD Cell

C-Trap

Electrodynamic Ion Funnel

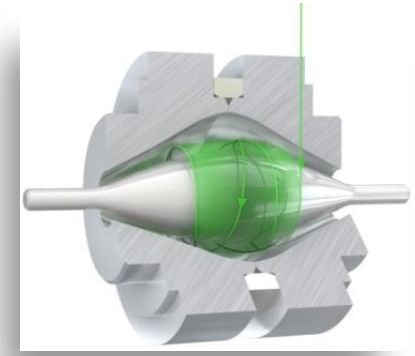
HyperQuad Mass Filter with Advanced Quadrupole Technology (AQT)

Advanced Active Beam Guide (AABG)

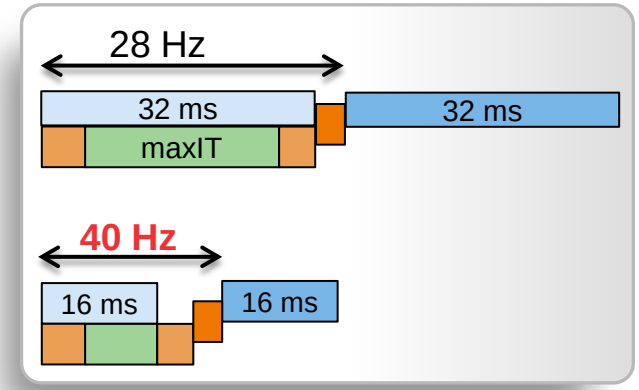
Key Technologies of Thermo Scientific Q Exactive HF-X MS

High Capacity Transfer Tube

Electrodynamic Ion Funnel

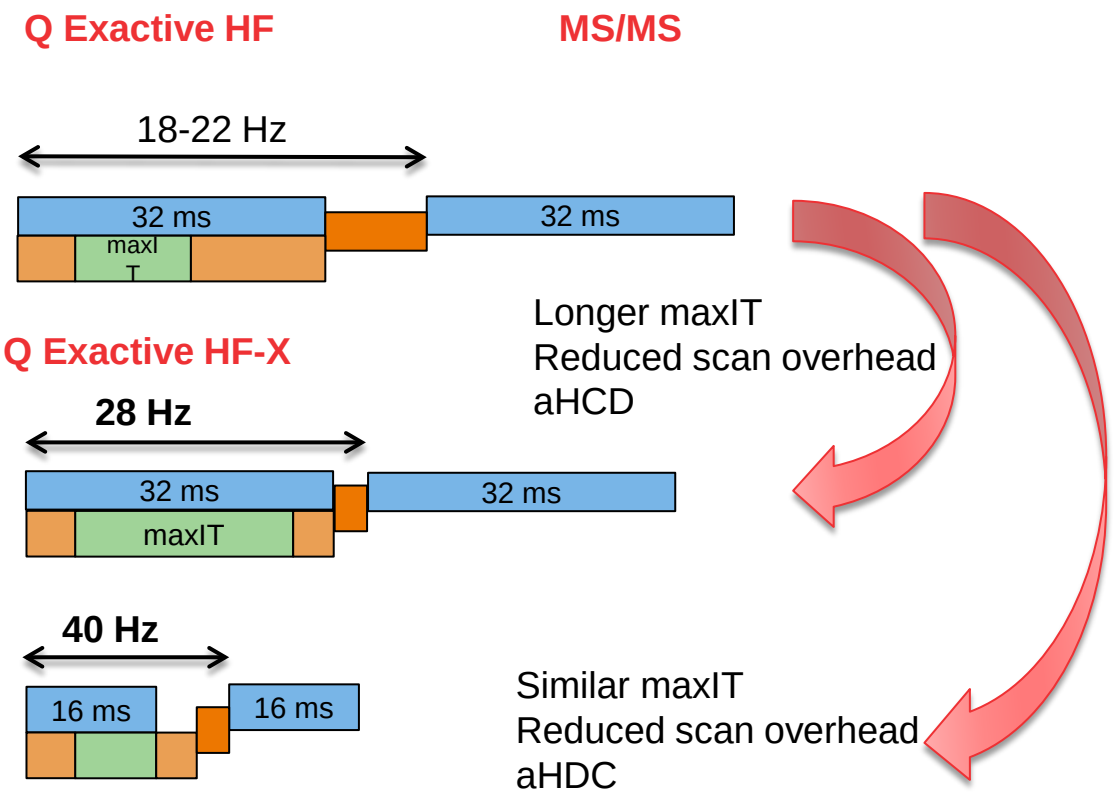


Ultra-High Field Orbitrap



Optimized Scan Matrix and new Transient lengths

Optimized Scan Matrix



- Brighter ion beam, reduced scan overhead, and accelerated HCD (aHCD) is boosting acquisition speed
- Advantage for both MS and MS/MS mode
- Fast and high quality MS/MS acquisition up the 40 Hz with new 16 msec transient (7,500 resolution setting)

Orbitrap detection

Maximum fill time with precursor ions

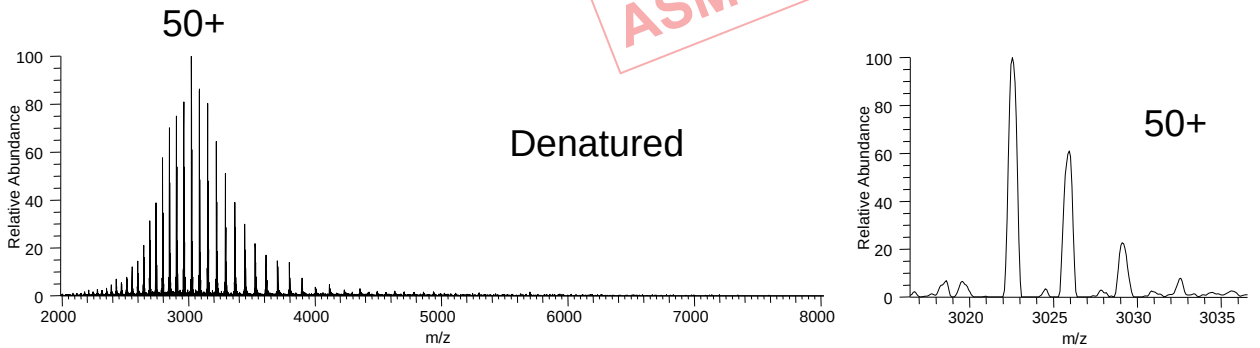
Inter and intra scan overheads

Intact Antibody Analysis with High Mass Range Mode
Thermo Scientific Q Exactive Plus MS with BioPharma Option

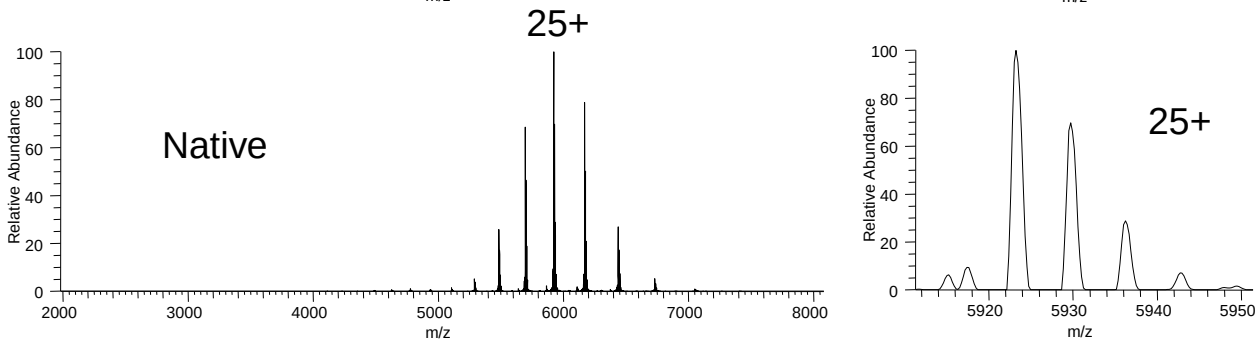
- Orbitrap scanning up to 8000 m/z
- Flexibility for denaturing conditions or Native MS

Last year at ASMS 2016

Column: MAbPac RP
(reversed phase LC)



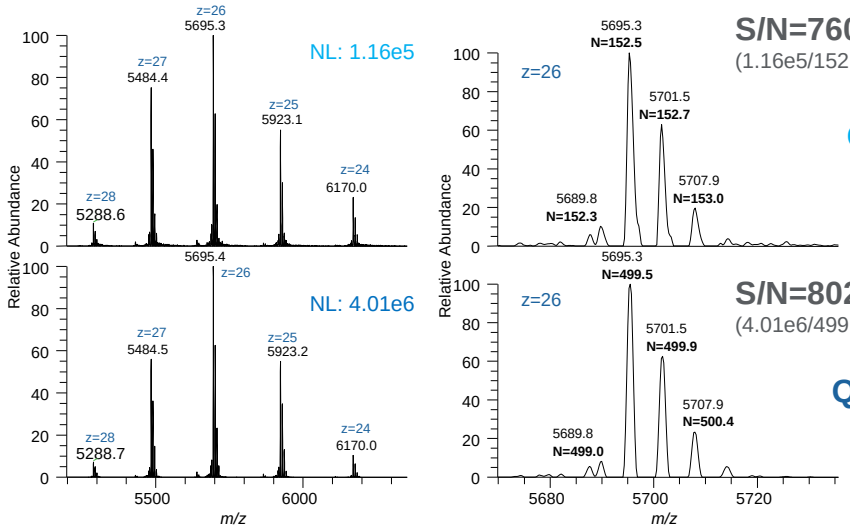
Column: MAbPac SEC-1
(native size exclusion LC)



ThermoFisher SCIENTIFIC

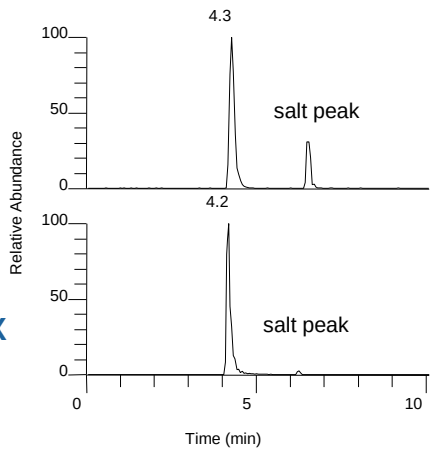
Analysis of Intact Trastuzumab under Native Conditions in HMR Mode

Improved S/N ratio on the Q Exactive HF-X by a factor of ~5-10.



Q Exactive HF

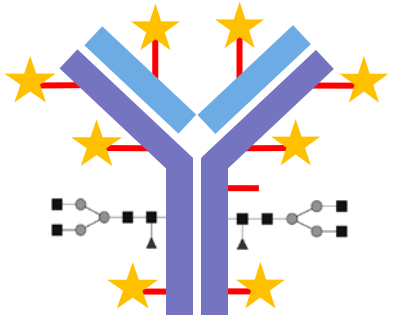
Q Exactive HF-X

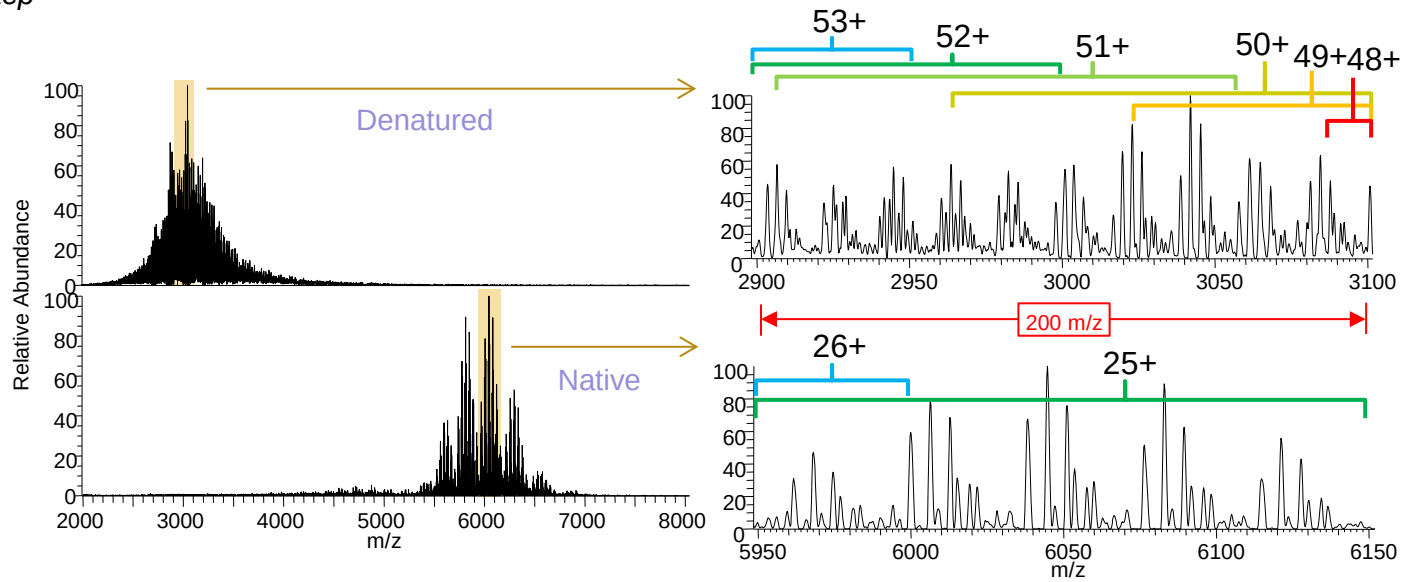


SEC LC-MS analysis of intact Trastuzumab monoclonal antibody using Acclaim SEC column, 4.6 x 300 mm, 300 µl/min flow rate, 50 mM ammonium acetate. Full MS, HMR mode, m/z 2500–8000, resolution setting 30k, 10 µscans. Spectra show an average of 3 scans (10 µscans each).

Intact Analysis of *Trastuzumab Emtansine* Lysine-linked ADC Denaturing vs. Native Conditions

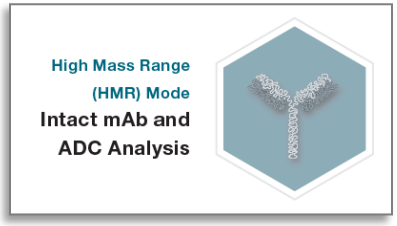
- *ADC construction can create **layers** of sample **heterogeneity***
- *Native MS allows greater inter-charge state separation*
- *Native MS allows "True Intact" analysis of heterogeneous samples*
  - *No deglycosylation step*

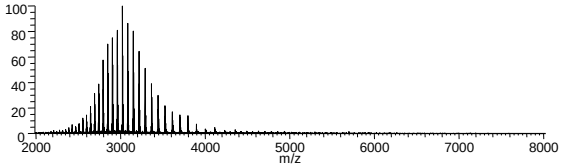


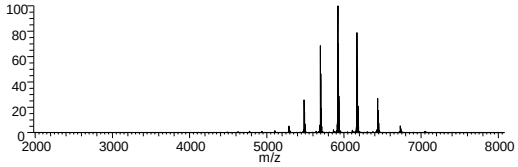


ThermoFisher SCIENTIFIC

Choosing the Right Strategy for Intact Protein Analysis

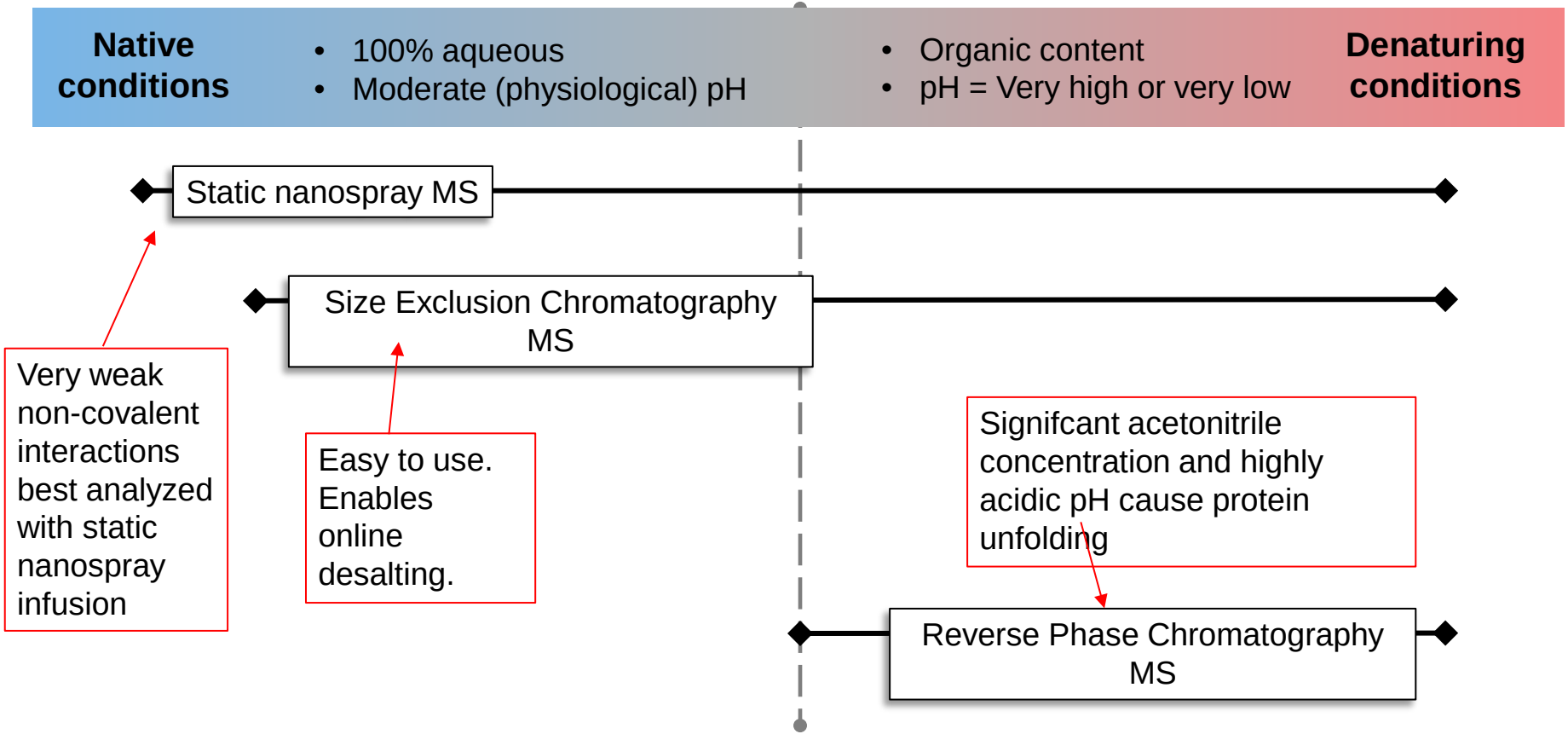




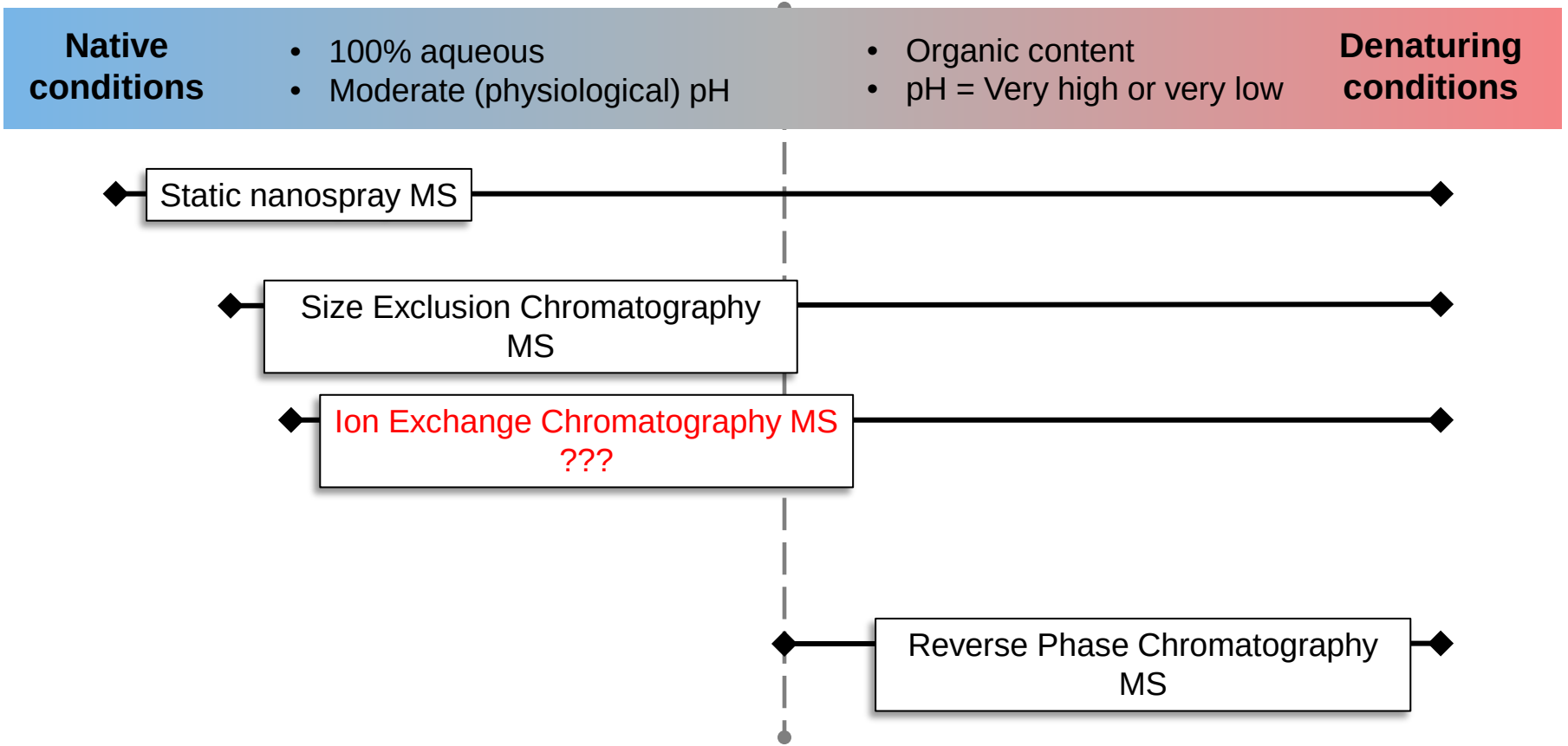


	Denaturing MS	Native MS
***Most sensitive* detection** of simple mAbs and subunits, low abundance protein isoforms	★	
High resolution separations Reverse Phase (RP)	★	
***Most confident* analysis** of heterogeneous mixtures, ADCs, glycoproteins, PEGylation		★
On-line desalting Size Exclusion (SEC)		★

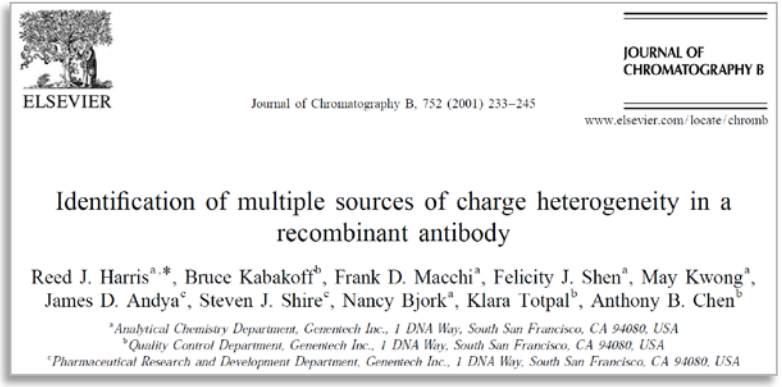
Intact Protein MS: What is “Native”? What is “Denatured”?



Is Ion Exchange Chromatography Compatible with LC-MS?



Highly Cited Article on Trastuzumab Charge Variant Analysis (Salt-based Elution)



ELSEVIER

Journal of Chromatography B, 752 (2001) 233–245

JOURNAL OF CHROMATOGRAPHY B

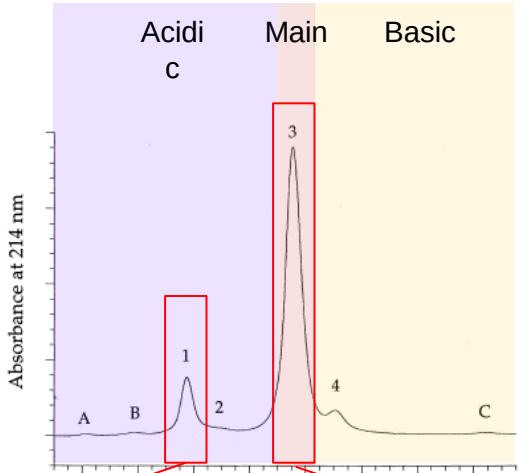
www.elsevier.com/locate/chromb

Identification of multiple sources of charge heterogeneity in a recombinant antibody

Reed J. Harris[a,*], Bruce Kabakoff[b], Frank D. Macchi[a], Felicity J. Shen[a], May Kwong[a], James D. Andya[c], Steven J. Shire[c], Nancy Bjork[a], Klara Totpal[b], Anthony B. Chen[b]

[a]Analytical Chemistry Department, Genentech Inc., 1 DNA Way, South San Francisco, CA 94080, USA
[b]Quality Control Department, Genentech Inc., 1 DNA Way, South San Francisco, CA 94080, USA
[c]Pharmaceutical Research and Development Department, Genentech Inc., 1 DNA Way, South San Francisco, CA 94080, USA

Harris et al., 2001



Fractionation + peptide mapping

1 x deamidation on light chain residue D30

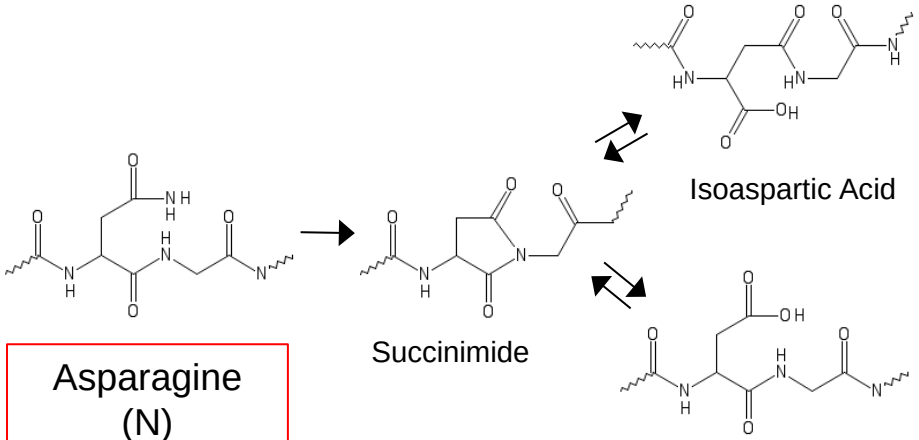
LC-UV
0 - 200 mM NaCl gradient in 20 mM $NaPO_4$

Fractionation + peptide mapping

Unmodified mAb

ThermoFisher SCIENTIFIC

Asparagine Deamidation Results in Very Small Mass Increase, Very Small pI Decrease



Asparagine
(N)
(pK_a = 8.80)

Aspartic Acid
(D)
(pK_a = 3.9)

N→D Δ mass =	Monoisotopic	Average
	0.984016 Da	0.9848 Da

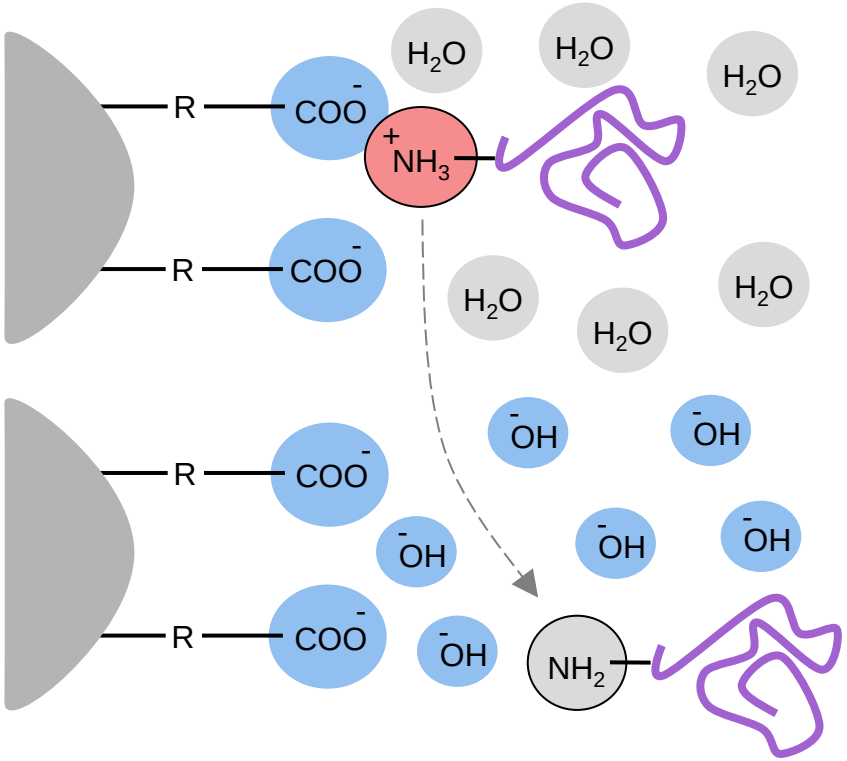
Calculated isoelectric point (pI) change upon Trastuzumab deamidation

Unmodified	1 x Deamidation
pI = 8.648	pI = 8.576

Δ pI = - 0.07 units

Data source for pKa values: ExPasy
https://www.protpi.ch/Calculator/ProteinTool

Elution by pH Gradient, Most Sensitive ESI in Native Cation Exchange LC-MS



Binding conditions

pH = ~ neutral

Protein pI = basic

Eluting conditions

pH = basic

constant
50 mM
ammonium acetate
buffer

Method for Native Weak Cation Exchange (WCX) LC-MS

Thermo Scientific™
ProPac™ WCX-10
Weak cation exchange column

- Buffer A = 50 mM AmAce, pH 7
- Buffer B = 50 mM AmAce, pH 10

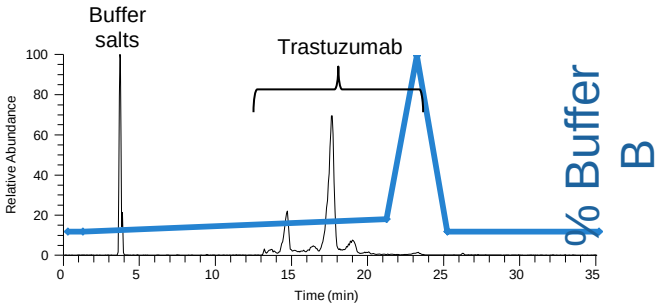
- Flow rate = 0.3 mL/min

Thermo Scientific™
Vanquish™ Horizon
UHPLC system

Time (min)	% Buffer B
0	1
1	1
21	8
23	100
25	1
35	1

Thermo Scientific™
Q Exactive™ HF-X
Orbitrap MS
with BioPharma Option

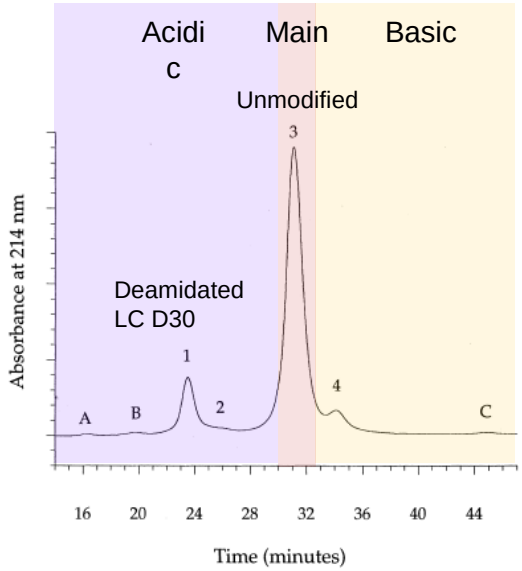
- 60,000 resolution setting
- 10 microscans



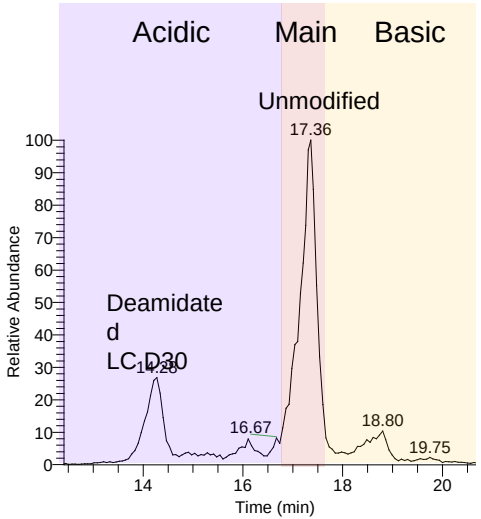
<u>On-column sample concentration</u> and <u>online desalting</u> are key features of native cation exchange LC-MS

ThermoFisher SCIENTIFIC

Native WCX-MS Method is Comparable to Separation in Literature



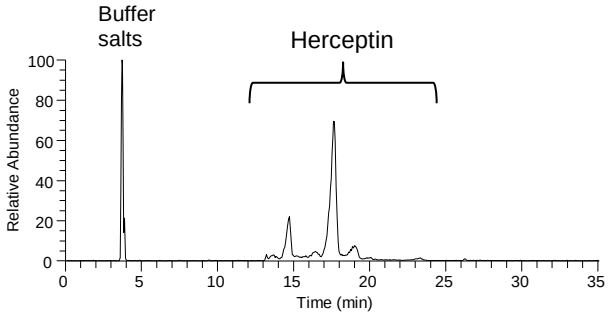
Harris et al., 2001
LC-UV
0 - 200 mM NaCl gradient
in 20 mM $NaPO_4$
Salt elution

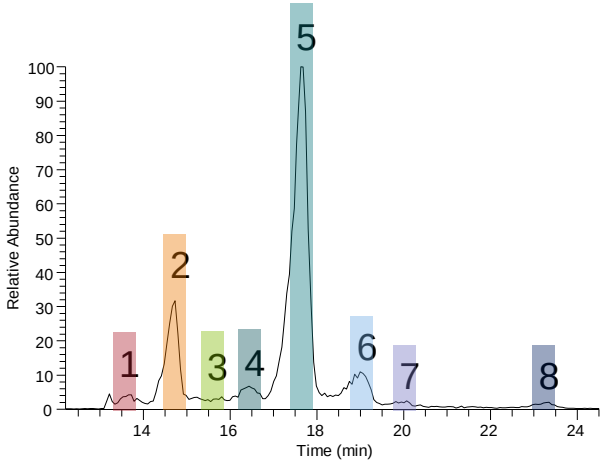


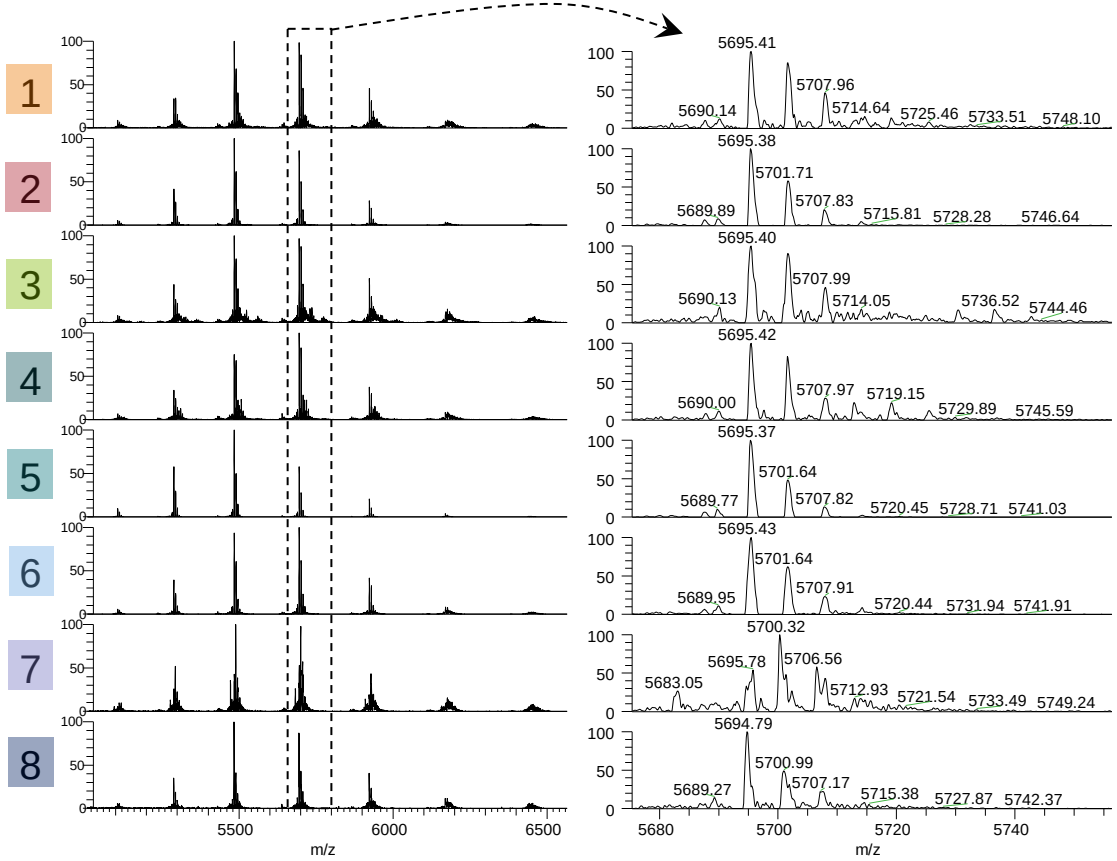
Thermo Scientific™
Q Exactive™ HF-X BioPharma
LC-MS
pH 7-10 gradient (1-8%)
in 50 mM $NH_4CH_3CO_2$
pH elution

ThermoFisher SCIENTIFIC

Native WCX-MS: 1 uL Injection of Formulation Trastuzumab (21 ug/uL)



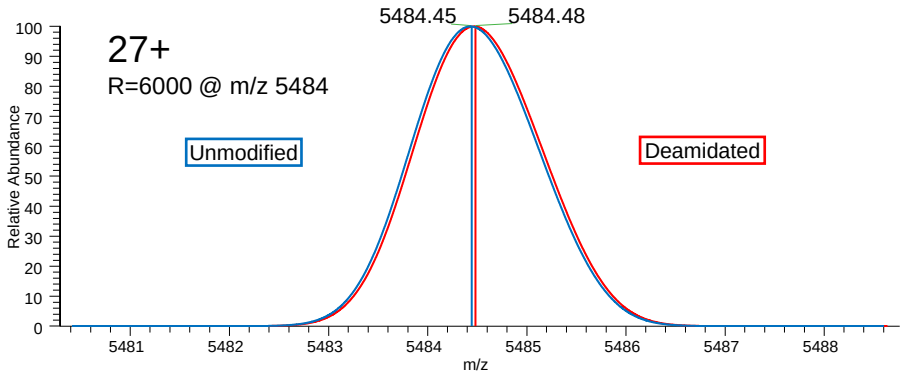


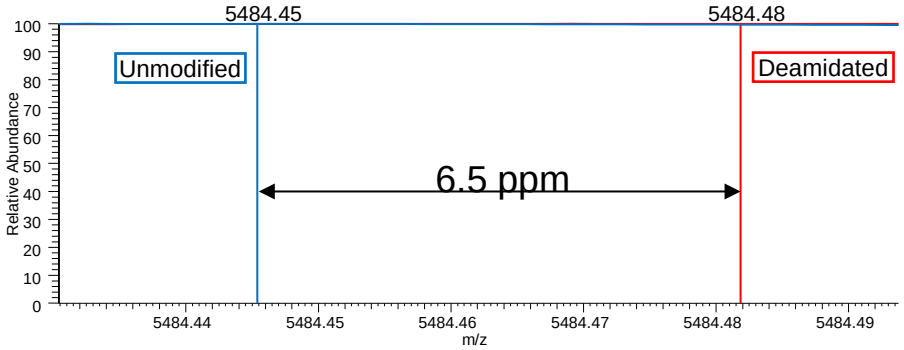


ThermoFisher SCIENTIFIC

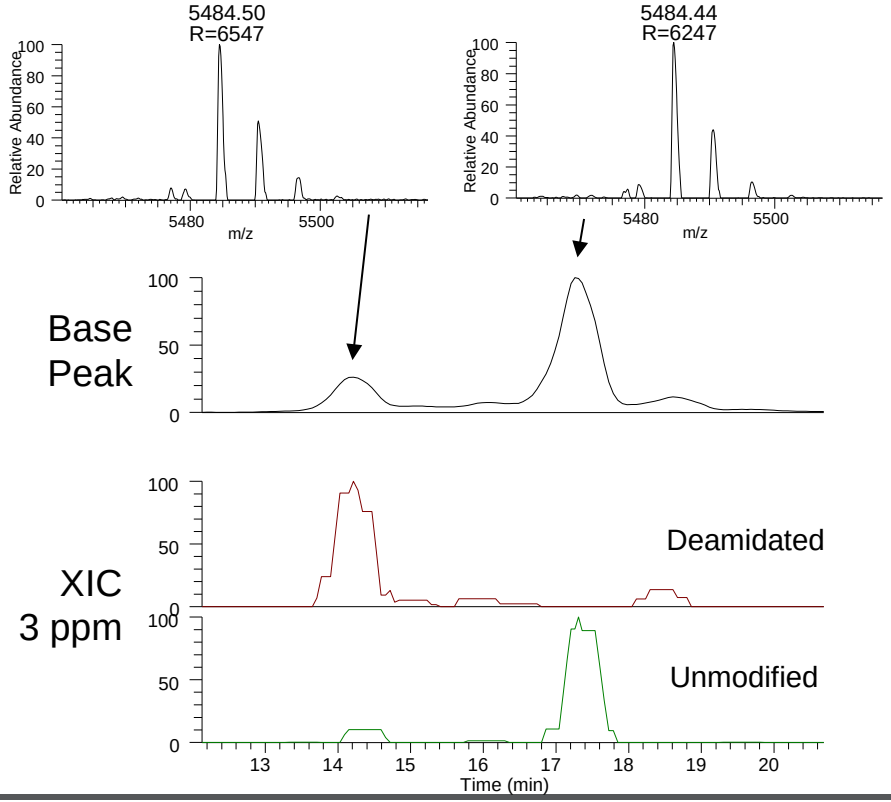
Raw Data Reflect Mass Accuracy of Unmodified and Deamidated Trastuzumab

Simulated Spectrum





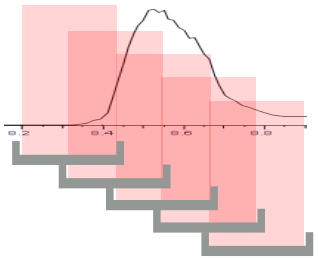
Experimental Data

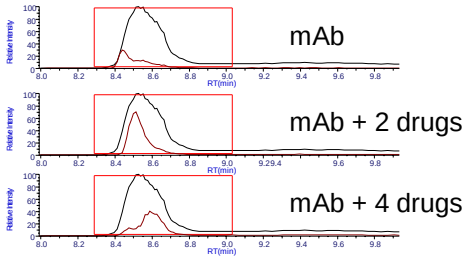


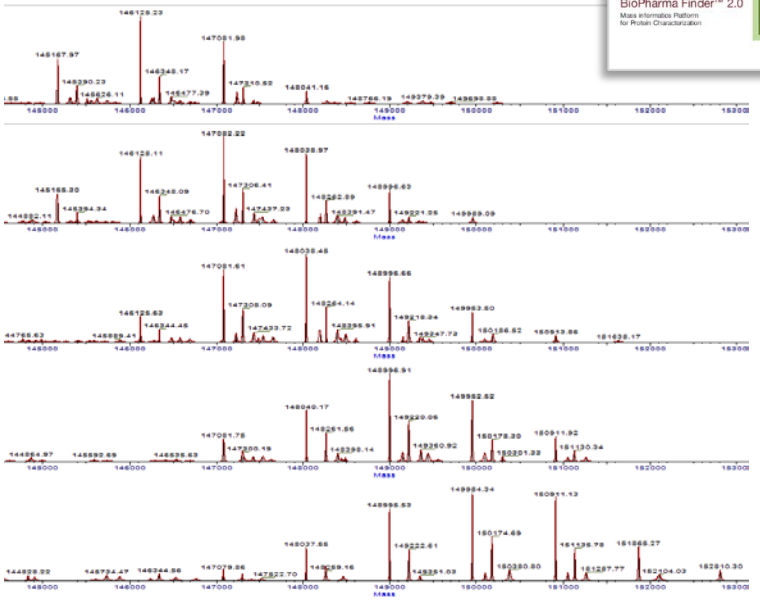
ThermoFisher SCIENTIFIC

Sliding Window Deconvolution is the Smart Choice for Chromatography

- **Automatically** performs Xtract or ReSpect deconvolution along separations timescale
- **Removes user bias** from deconvolution analysis
- Improves quality of **batch analyses**
- Sensitive and confident identification + relative quan
- **All biologics benefitted** - simple mAbs, complex ADCs



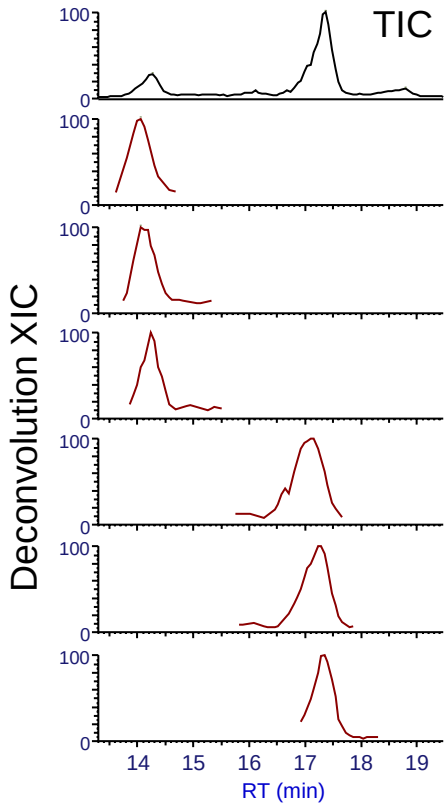




LC peak area automatically integrated

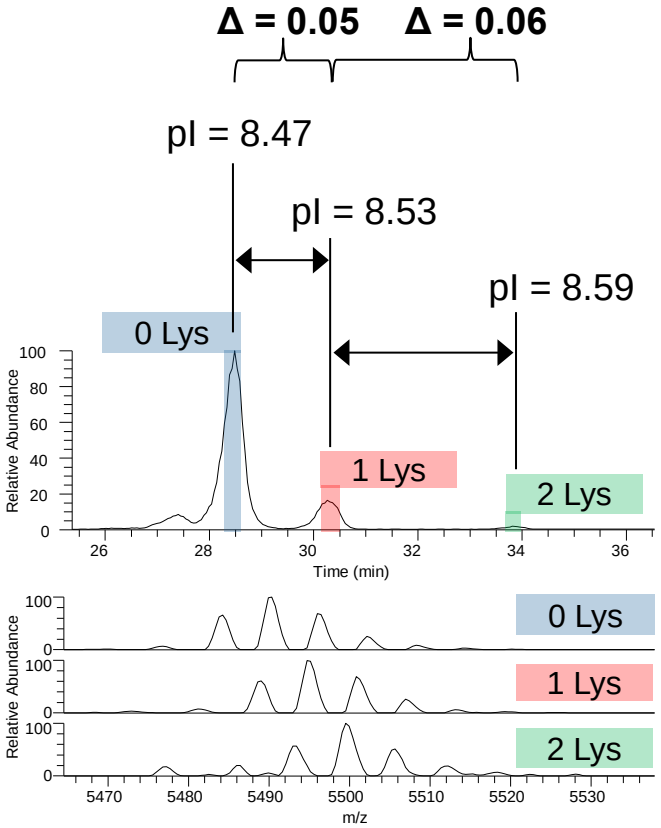
ThermoFisher SCIENTIFIC

ReSpect Sliding Window Results for Native WCX-MS with Automatic Annotation



Modification	Average Mass (Da)	Theoretical Mass (Da)	Matched Mass Error (ppm)	Relative Abundance	RT Range
2xA2G1F,1xDeamidation	148381.53	148381.80	1.8	6.81	13.521 - 14.782
1xA2G0F,1xA2G1F,1xDeamidation	148219.47	148219.66	1.3	19.60	13.648 - 15.412
2xA2G0F,1xDeamidation	148057.69	148057.52	1.2	30.75	13.774 - 15.602
2xA2G1F	148380.81	148380.82	0.1	27.11	15.665 - 17.790
1xA2G0F,1xA2G1F	148219.00	148218.68	2.2	73.91	15.728 - 17.979
2xA2G0F	148056.95	148056.54	2.8	100.00	16.800 - 18.421

NIST mAb Lys-variants are Readily Separated and Detected by Native WCX-MS

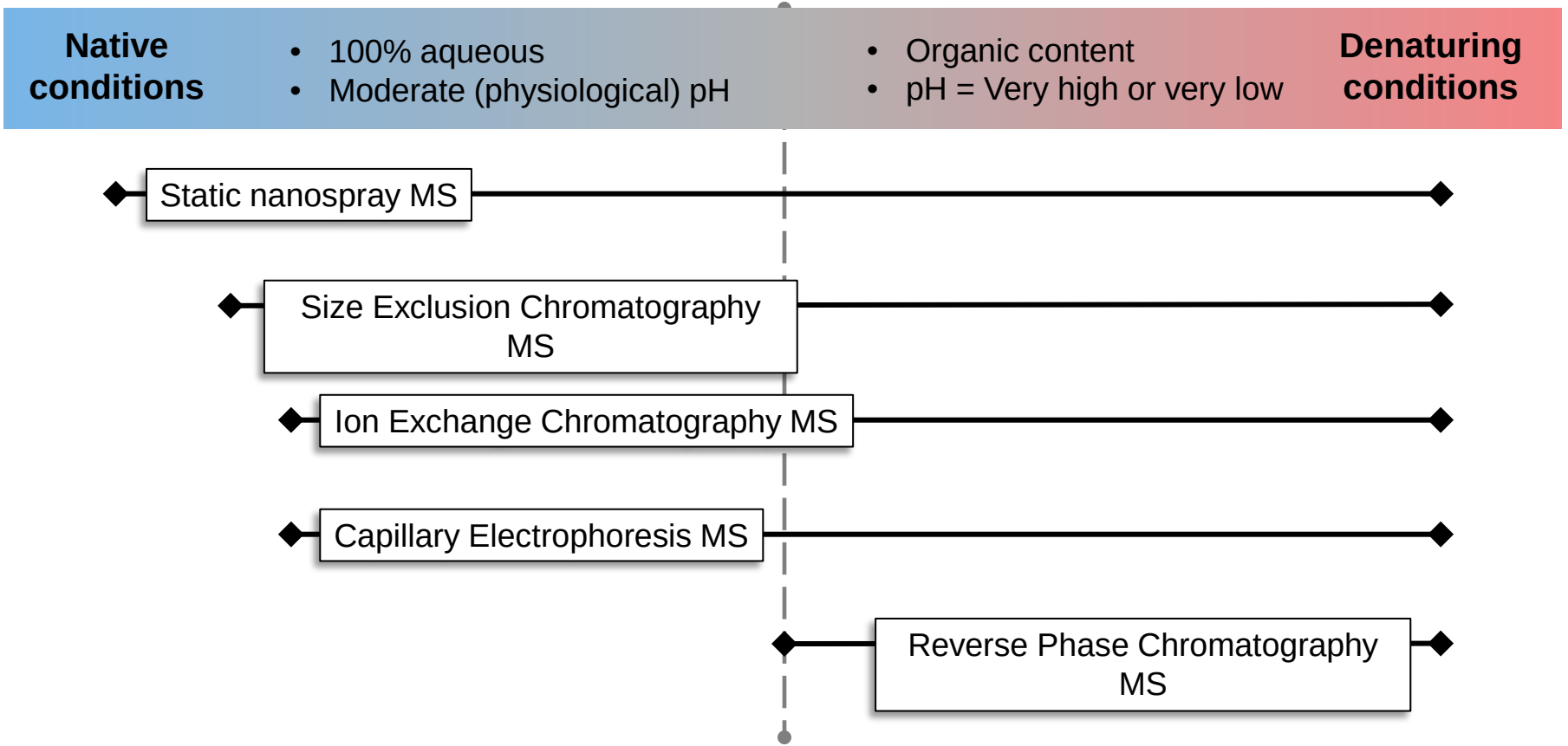


- Native WCX-MS method baseline resolves pI differences of < 0.05 units
- Without separation true impurities will cause mass interferences

Data source for pKa values: ExPasy

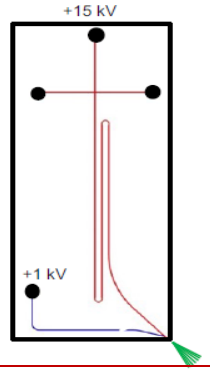
https://www.protpi.ch/Calculator/ProteinTool

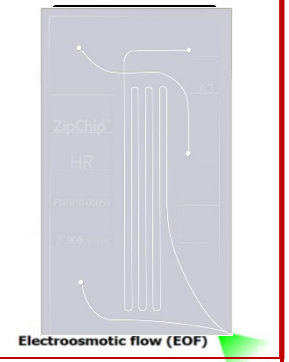
Intact Protein MS Separation Solutions also Include Capillary Electrophoresis (CE)



908 Devices ZipChip System

- The 908 Devices ZipChip™ system interface directly mounts onto the front end of a compatible mass spectrometer
- The ZipChip system uses integrated microfluidic technology to prepare, separate samples by capillary electrophoresis (CE), and then electrospray (ESI) analytes directly into mass spectrometers (MS)
- Each analysis only consumes a few nanoliters of sample containing pico grams to nano grams of analytes
- Most analyses can be completed in a few minutes





	Zip Chip HS	Zip Chip HR
Separation channel length (cm)	10	22
On Chip De-salting capability	Yes	Yes
Integrated ESI Emitter	Yes	Yes
EEPROMS	Yes	Yes
Max chip life (# of injections)	125	125
Recommended use	Small molecules or simple sample mixture	Big molecules or complex sample mixture
Typical anlaysis time	Up to 3 min	Up to 12 min

ThermoFisher SCIENTIFIC

908 Devices ZipChip System Hardware and Consumables

ZipChip Interface

- Compatible with all Thermo Scientific™ Exactive™ and Q Exactive™ Hybrid Quadrupole-Orbitrap™ MS, as well as the Thermo Scientific™ LTQ-Orbitrap XL™ Hybrid Ion Trap-Orbitrap series MS instruments
- Also compatible with Thermo Scientific™ ion trap MS and select triple-stage quadrupole MS instruments
- Data collection, processing and reporting through Thermo Scientific ™ Xcalibur™ software

Autosampler

- Fully automated and integrated into the ZipChip software
- Compatible with both 48-vial plate and 96-well plate

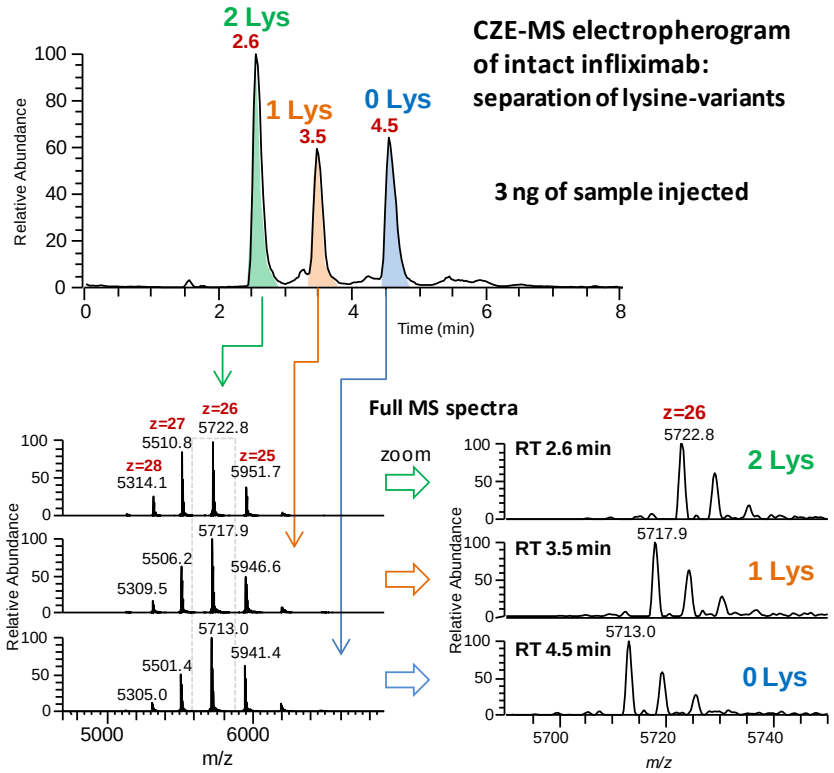
Zip Chips

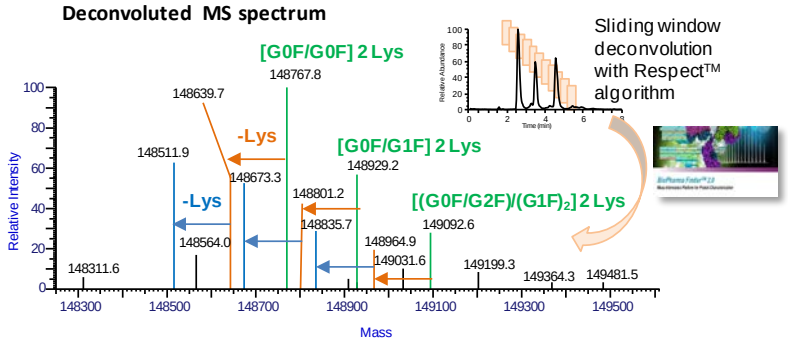
- Disposable chips
- Two types: HR chip and HS chip
- Each chip is a single piece of glass, about the same size and shape as a microscope slide
- Microfluidic channel and electrospray is integrated into both types of chips
- 5 chips per pack

ZipChip Assay

- Three types of pre-packed assay kits are designed for intact protein, peptides, and metabolites analyses, respectively
- Individual bottles can be directly loaded into the 908 autosampler
- Packaged in individual bottles to match to 96 well-plate solvent usage
- Good for 500 injections per box of assay kit

ZipChip - Thermo Scientific Q Exactive HF-X MS Analysis of Intact Infliximab Antibody

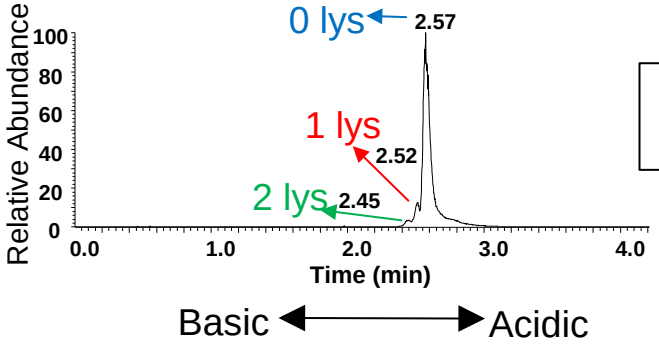




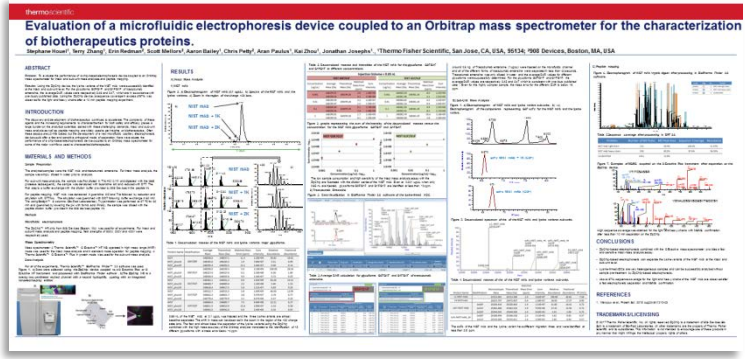
Theoretical and experimental masses of infliximab glycoforms of lysine variants

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

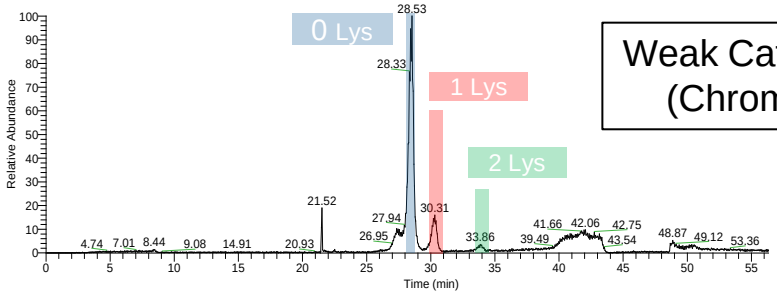
WCX and CE Show Complementarity, Reverse Elution Order of NIST mAB Lys-variants



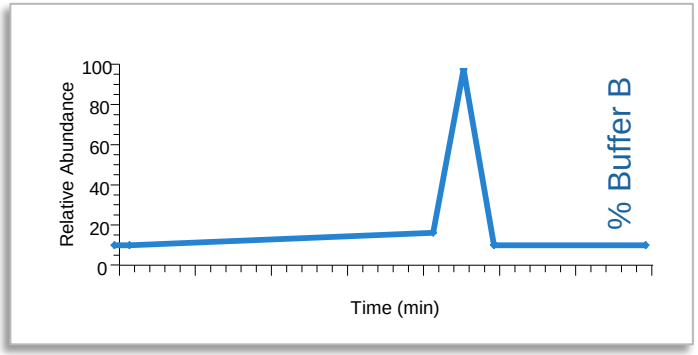
ZipChip
(Electrophoresis)


Evaluation of a microfluidic electrophoresis device coupled to an Orbitrap mass spectrometer for the characterization of biotherapeutics proteins.

Acidic ⟷ Basic



Weak Cation Exchange
(Chromatography)



ThermoFisher SCIENTIFIC

Conclusions

- Native LC-MS is an easy to use solution for analyzing micro-heterogeneous samples
- Thermo Scientific™ Q Exactive™ HF-X MS with BioPharma Option offers increased sensitivity for native mAb analysis
- Native WCX-MS is a powerful method which expands offerings for native LC-MS intact protein analysis
  - Online desalting
  - On-column sample concentration
  - Baseline resolves pI differences of < 0.05 units
- Without separation true micro-heterogeneous impurities can cause interferences and mass errors

Thank you!

Genentech

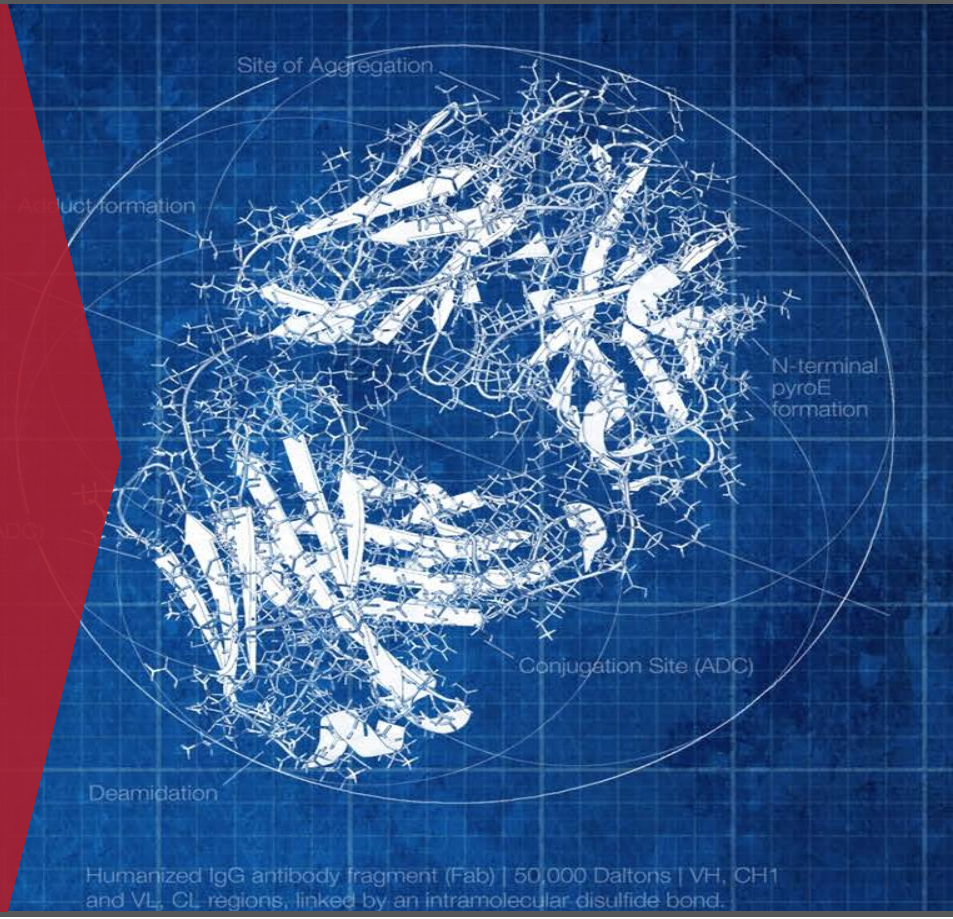
- Wendy Sandoval
- Guanghui Han
- Wilson Phung
- Chengfeng Ren

BioPharma Finder Team

- Paul Gazis
- Jennifer Sutton

Pharma/BioPharma Team

- Michael Blank
- David Brant
- Kelly Broster
- Kate Comstock
- Simon Cubbon
- Stephane Houel
- Rowan Moore
- Keeley Murphy
- Kyle D'Silva
- Terry Zhang
- John Rontree

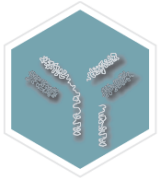


Thermo Scientific Q Exactive BioPharma MS Offers a Complete Characterization Solution for BioPharma Customers

High Mass Range (HMR) Mode
Intact mAb and ADC Analysis

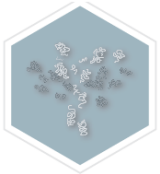
Optimized intact protein analysis under both native and denaturing conditions assures the highest quality and most informative spectra for the widest range of therapeutic proteins.

Protein Mode
Subunit Analysis Top/Middle-Down



Extreme resolving power of the Orbitrap™ mass analyzer ensures isotopic resolution of subunits and facilitates top/middle-down sequencing.

Standard Mode
Peptide Mapping



Perform peptide mapping with Orbitrap technology for unparalleled acquisition speed, mass accuracy, and spectral quality.

The most powerful system for every workflow

All in one package

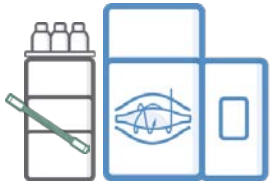
ThermoFisher SCIENTIFIC

Peptide Mapping of Trastuzumab With Digestion Using SMART Digest^{T} Trypsin Kit

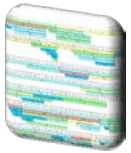
Digestion

SMART Digest Kit

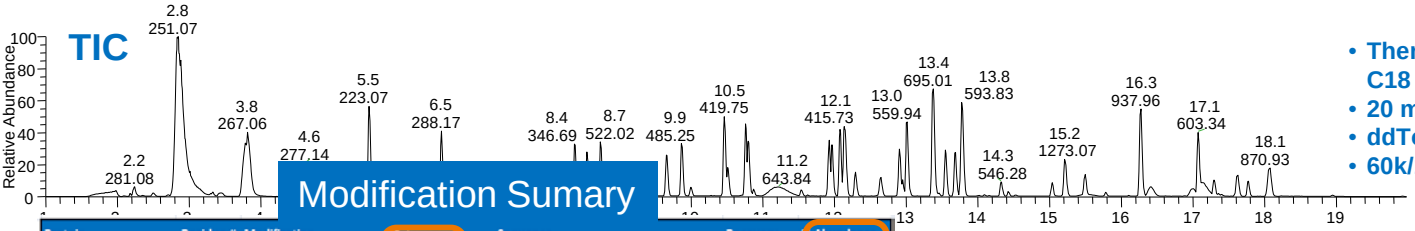
LC-MS



Data Analysis



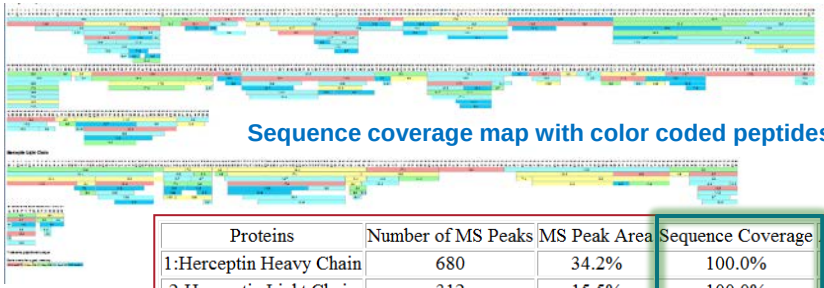
- Sequence coverage
- Sequence variants
- ID of unexpected mods
- Modifications ID +quan



- Thermo Scientific™ Acclaim™ VANQUISH™ C18 UHPLC column
- 20 min gradient
- ddTop5 Method
- 60k/15k resolution settings (MS/MS^{2})

Modification Sumary

Protein	Residue #	Modification	Category	Sequence	Recovery	Abundance
Herceptin Heavy Chain	300	N300+A1G0	Glycoform	EEQYNSTYR	5.7146416	1.9
Herceptin Heavy Chain	300	N300+A1G0F	Glycoform	EEQYNSTYR	6.8686495	10.6
Herceptin Heavy Chain	300	N300+A1G1F	Glycoform	EEQYNSTYR	5.7146416	0.9
Herceptin Heavy Chain	300	N300+A2G0	Glycoform	EEQYNSTYR	9.0856943	3.5
Herceptin Heavy Chain	300	N300+A2G0F	Glycoform	EEQYNSTYR	10.831835	62.2
Herceptin Heavy Chain	300	N300+A2G1F	Glycoform	EEQYNSTYR	10.959603	20.2
Herceptin Heavy Chain	300	N300+A2G2F	Glycoform	EEQYNSTYR	5.7146416	1.4
Herceptin Heavy Chain	300	N300+M5	Glycoform	EEQYNSTYR	5.7146416	2.3
Herceptin Heavy Chain	300	N300+Unglycosylated	Glycoform	EEQYNSTYR	5.7146416	1.5
Herceptin Heavy Chain	55	N55+Deamidation	Modification	IYPTNGYTR	17.51152	0.8
Herceptin Heavy Chain	77	N77+Deamidation	Modification	NTAYLQMNSL R	30.804428	0.8
Herceptin Heavy Chain	204	~N204+Deamidation	Modification	SLSSVVTVPS SSLGTQTYIC NVNHKPSNTK	7.2909698	2.9
Herceptin Heavy Chain	289	N289+Deamidation	Modification	FNWYVDGVEV HNAK	42.832817	0.5
Herceptin Heavy Chain	318	N318+Deamidation	Modification	VVSVLTVLHQ DWLNGK	47.814327	9.9
Herceptin Heavy Chain	392	~N392+Deamidation	Modification	GFYPSDIAVE WESNGQPENN YK	30.933163	3.4
Herceptin Heavy Chain	437	~N437+Deamidation	Modification	WQQGNVFSCS VMHEALHNHY TQK	47.210545	6.2
Herceptin Heavy Chain	255	M255+Oxidation	Modification	DTLMISR	15.198215	1.6
Herceptin Light Chain	30	N30+Deamidation	Modification	ASQDVNTAVA WYQQKPGK	100	9.2
Herceptin Light Chain	137	~N137+Deamidation	Modification	SGTASVVCLL NNFYPR	61.006905	0.7
Herceptin Light Chain	138	~N138+Deamidation	Modification	SGTASVVCLL NNFYPR	60.692932	0.2



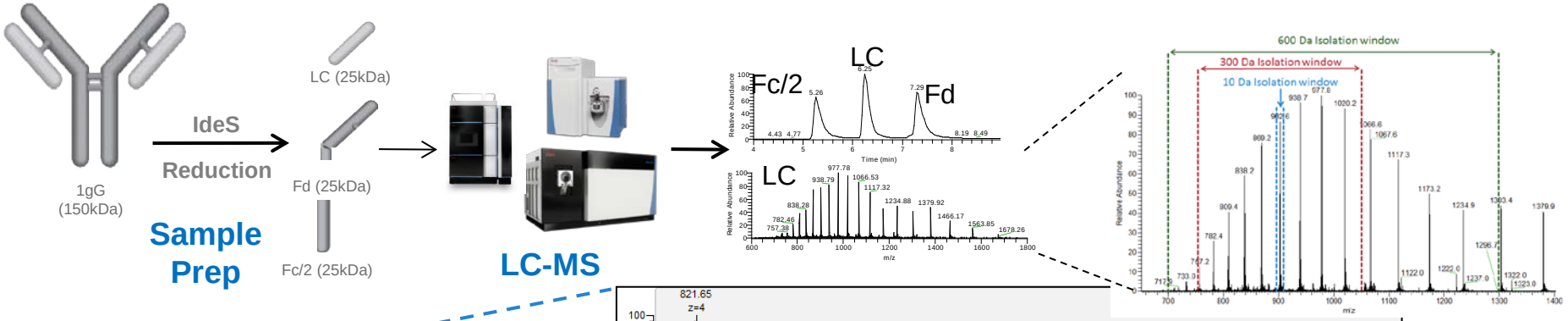
Sequence coverage map with color coded peptides

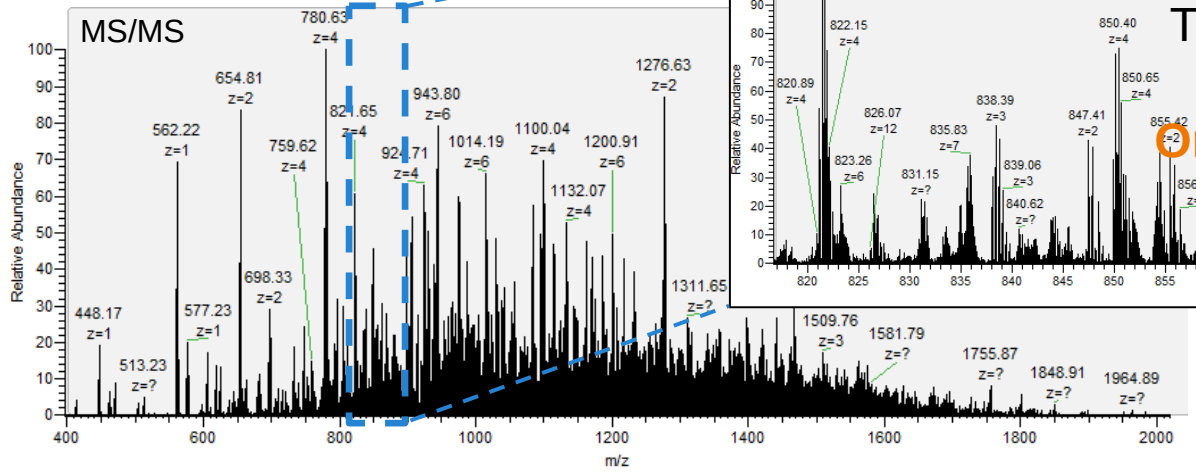
Proteins	Number of MS Peaks	MS Peak Area	Sequence Coverage	Abundance (mol)
1:Herceptin Heavy Chain	680	34.2%	100.0%	60.39%
2:Herceptin Light Chain	312	15.5%	100.0%	39.61%
Unidentified	2370	50.4%		

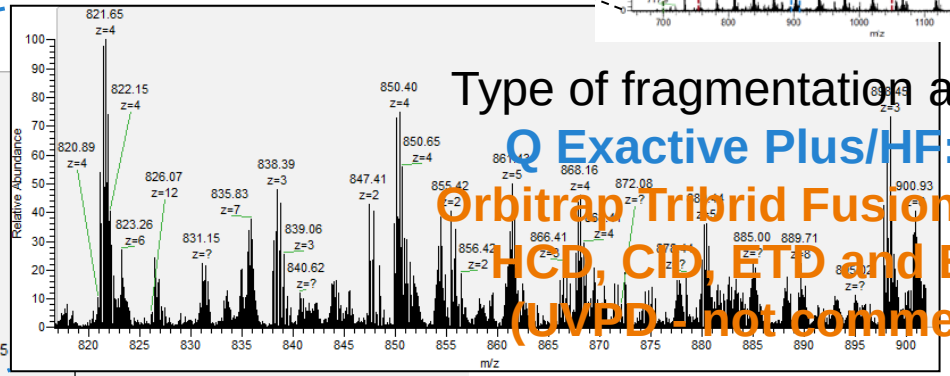
100% sequence coverage based on MS/MS spectra

ThermoFisher SCIENTIFIC

Workflow for a Middle Down Experiment







Type of fragmentation available:

Q Exactive Plus/HF: HCD

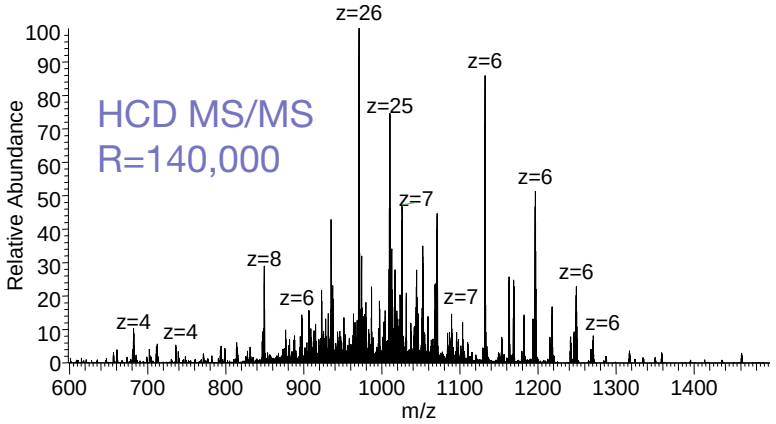
Orbitrap Tribrid Fusion/Lumos: HCD, CID, ETD and EThcD

(UVPD – not commercial)

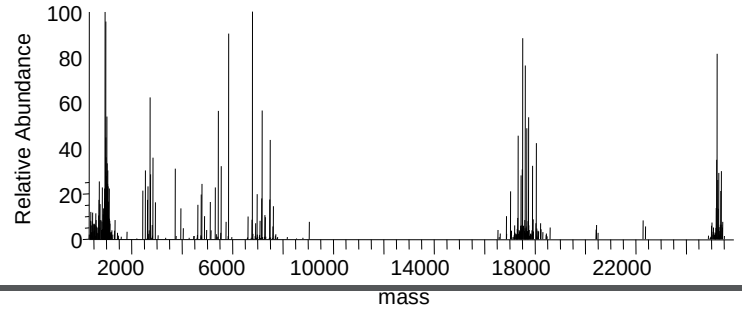
Due to the complexity of the spectra in top-down analysis, high resolution is required

ThermoFisher SCIENTIFIC

Sequence Coverage with HCD Fragmentation on a Thermo Scientific Q Exactive Plus MS (Trastuzumab)



Xtract™ deconvolution



ProSight Lite v1.3
Simple, Targeted, Top-Down Proteomics
NORTHWESTERN UNIVERSITY

Light Chain
49% residue cleavages

N D I Q M T Q S P S S L S A S V G D R V T I T C R A 25
26 S Q D V N T A V A W Y Q Q K P G K A P K L L I Y S 50
51 A S F L Y S G V P S R F S G S R S G T D F T L T I 75
76 S S L Q P E D F A T Y Y C Q Q H Y T T P P T F G Q 100
101 G T K V E I K R T V A A P S V F I F P P S D E Q L 125
126 K S G T A S V V C L L N N F Y P R E A K V Q W K V 150
151 D N A L Q S G N S Q E S V T E Q D S K D S T Y S L 175
176 S S T L T L S K A D Y E K H K V Y A C E V T H Q G 200
201 L S S P V T K S F N R G E C 214

Fd’
38% residue cleavages

N E V Q L V E S G G G L V Q P G G S L R L S C A A S 25
26 G F N I K D T Y I H W V R Q A P G K G L E W V A R 50
51 I Y P T N G Y T R Y A D S V K G R F T I S A D T S 75
76 K N T A Y L Q M N S L R A E D T A V Y Y C S R W G 100
101 G D G F Y A M D Y W G Q G T L V T V S S A S T K G 125
126 P S V F P L A P S S K S T S G G T A A L G C L V K 150
151 D Y F P E P V T V S W N S G A L T S G V H T F P A 175
176 V L Q S S G L Y S L S S V V T V P S S S L G T Q T 200
201 Y I C N V N H K P S N T K V D K K V E P K S C D K 225
226 T H T C P P C P A P E L L G C 239

Fc
39% residue cleavages

N G P S V F L F P P K P K D T L M I S R T P E V T C 25
26 V V V D V S H E D P E V K F N W Y V D G V E V H N 50
51 A K T K P R E E Q Y N S T Y R V V S V L T V L H Q 75
76 D W L N G K E Y K C K V S N K A L P A P I E K T I 100
101 S K A K G Q P R E P Q V Y T L P P S R E E M T K N 125
126 Q V S L T C L V K G F Y P S D I A V E W E S N G Q 150
151 P E N N Y K T T P P V L D S D G S F F L Y S K L T 175
176 V D K S R W Q Q G N V F S C S V M H E A L H N H Y 200
201 T Q K S L S L S P G 214

ThermoFisher SCIENTIFIC

Thank you!

Aaron Bailey
Eugen Damoc
Kai Scheffler
Stephane Houel
Alexander Harder
Thomas Moehring
Alexander Makarov
Jessica Wang
Jennifer Sutton

Michael Blank
Terry Zhang
Kate Comstock
Keeley Murphy
David Brant
Tina Settineri
Ken Miller
Paul Gazis

Shanhua Lin
Shane Bechler
Seema Sharma
David Horn
Fred Zinnel
Mark Sanders
Keith Waddell
Brenda Kesler