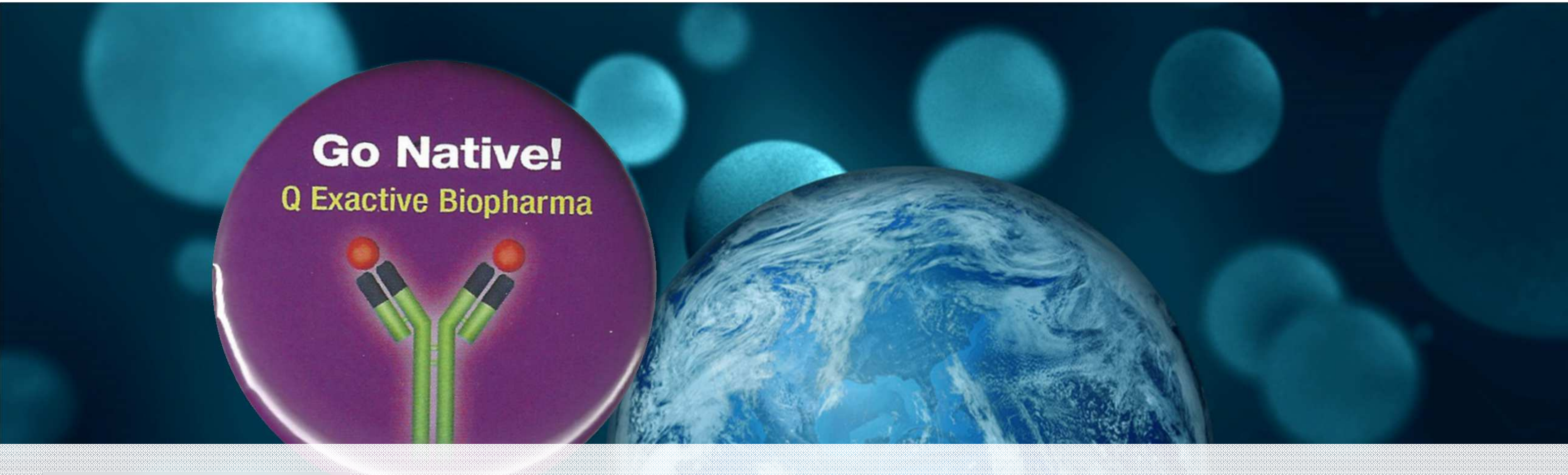


ThermoFisher SCIENTIFIC

The New Q Exactive^{TM} BioPharma Mass Spectrometer

Hermann Katzlinger

The world leader in serving science

PORTFOLIO so far

Exactive Series Portfolio

PERFORMANCE

Exactive Plus (EMR)

- Orbitrap analyzer
- Mass Range m/z 50 - 6000
- Mass Range EMR: 300 - 20,000
- Mass Accuracy: <1ppm
- Mass Resolution >140,000
- Scan Speed up to 12 Hz

Q Exactive & Q Exactive Plus

- Orbitrap analyzer
- Mass Range m/z 50 - 6000
- Mass Accuracy <1ppm
- Max. Mass Resolution >140,000
- Scan speed up to 12Hz
- Spectral Multiplexing
- AQT & AABG (QE Plus only)
- Optional Intact Protein Mode and Enhanced Resolution (280k) for QE Plus only

Q Exactive HF

- Ultra High Field Orbitrap analyzer
- Mass Range m/z 50 - 6000
- Mass Accuracy <1ppm
- Max. Mass Resolution >240,000
- Scan speed up to 18Hz
- Spectral Multiplexing
- AQT & AABG
- Optional Protein Mode

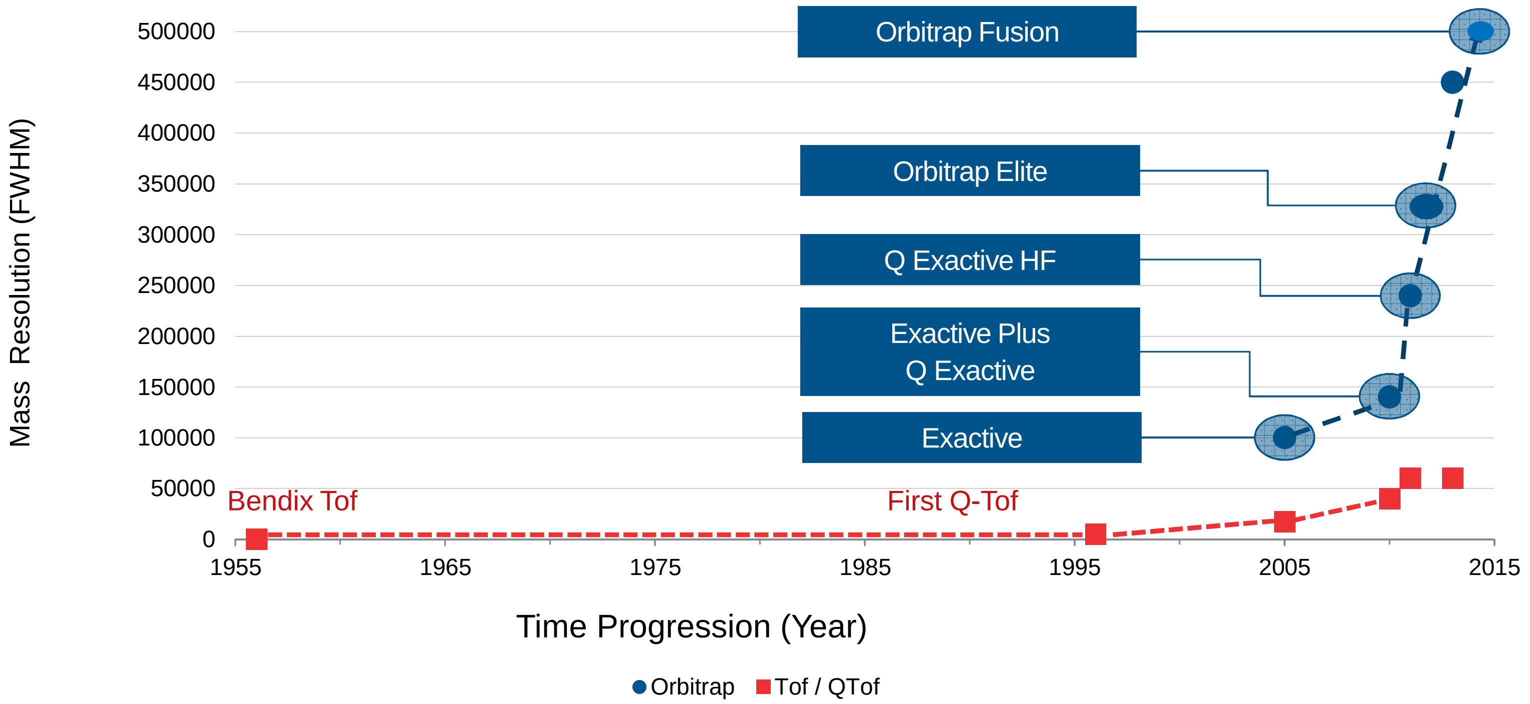
VALUE

Overview of Current Orbitrap MS Portfolio

Attributes	Exactive Plus (EMR)	Q Exactive (Plus)	Q Exactive HF	Orbitrap LTQ XL	Orbitrap Elite	Orbitrap Fusion
Max Resolution (FWHM) @ m/z 200	140k	140k (280k Enhanced Res)	240k	140k	340k	500k
Mass accuracy (ppm)	<1	<1	<1	<1	<1	<1
Scan rate (Hz)	12 Hz	12 Hz	18Hz	1 Hz	4 Hz	18 Hz
Polarity switching (s)	<1	<1	<1	Not during run	Not during run	1.1
Parallel Reaction Monitoring	NA	Yes	Yes	Yes	Yes	Yes
Multiplex (precursor/scan)	NA	10	10	NA	NA	Yes
DIA-MS / SWATH	NA	Yes	Yes	NA	NA	Yes
MS^n (n=10)	NA	NA	NA	Yes	Yes	Yes
ETD option	NA	NA	NA	Yes	Yes	Yes
Decision-tree (CID/HCD/ETD)	NA	NA	NA	NA	Yes	Yes
Synchronous MS^3	NA	NA	NA	NA	NA	Yes

Commercial High Resolution MS Technology Race

Orbitrap’s Spectacular Climb in Performance In 9-year Span!

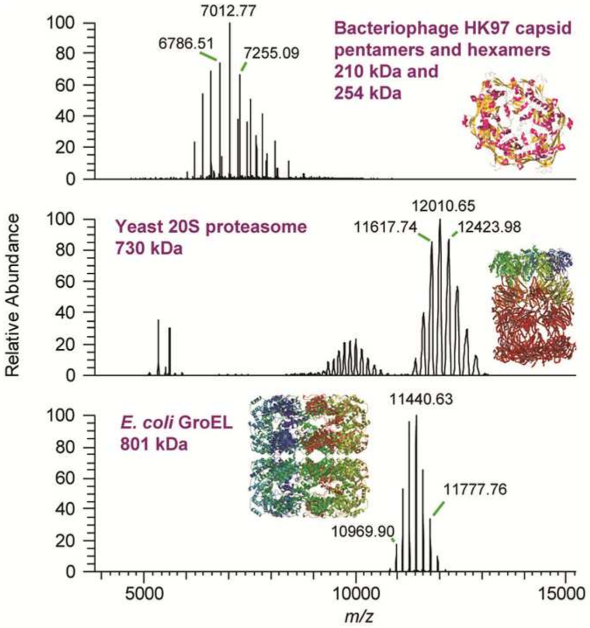


High Resolution only with Orbitrap technology

Exactive Plus EMR

Analyze protein complexes in native-like states

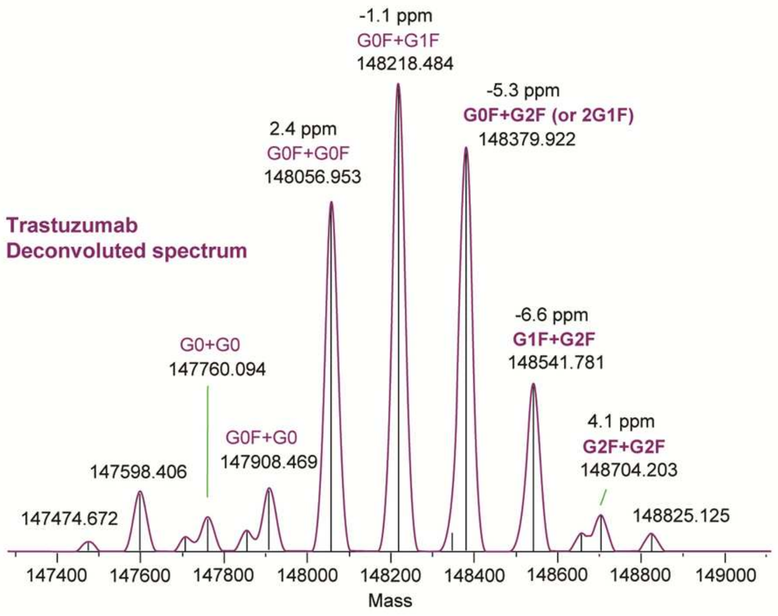
- Detect intact protein assemblies up to 1 MDa
- Study quaternary structures
- Study protein-substrate interactions



Exactive Plus EMR

Identify and characterize antibodies and other intact proteins

- Separate isoforms of denatured and native proteins
- Determine glycosylation states with excellent mass accuracy
- Investigate protein-drug conjugates

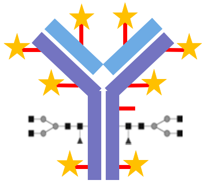


Q Exactive BioPharma Offers a Complete Characterization Solution for BioPharma

Q Exactive BioPharma Is Optimized For Top 3 Protein Characterization Workflows

High Mass Range (HMR) Mode

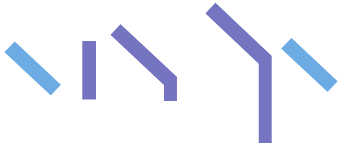
Intact mAb and
ADC Analysis



Optimized intact protein analysis under both native and denaturing conditions. Highest quality 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 Q Exactive with the NEW BioPharma Option

- The BioPharma Option is available for the Thermo Scientific™ Q Exactive™ Plus and Q Exactive™ HF Hybrid Quadrupole Orbitrap™ mass spectrometers.

- The BioPharma Option adds superior denatured and native MS intact analysis and subunit top/middle-down analysis capabilities to the most powerful benchtop peptide mapping instruments available.

- The BioPharma Option offers distinct operational modes that have been optimized for the top 3 protein characterization workflows:
  - Denatured and native MS intact analysis with the new High Mass Range (HMR) mode
  - Subunit and top/middle-down analysis with Protein mode
  - Peptide mapping with Standard mode

The Q Exactive with the NEW BioPharma Option

- For the Q Exactive Plus mass spectrometer, the BioPharma Option includes
  - Standard Mode
  - Protein Mode
  - Enhanced Resolution Mode with resolution up to 280,000 @ m/z 200
  - High Mass Range Mode with extended mass range up to m/z 8000

- For the Q Exactive HF mass spectrometer the BioPharma Option includes
  - Standard Mode
  - Protein Mode
  - High Mass Range Mode with extended mass range up to m/z 8000

Unique Features of the BioPharma Option

- **High Mass Range mode with mass detection up to m/z 8000**
- Robust mass accuracy and efficient desolvation have become hallmarks of Orbitrap technology for intact protein analysis. Based on the engineering behind the Thermo Scientific™ Exactive Plus™ EMR Orbitrap™ mass spectrometer we have developed High Mass Range (HMR) mode for intact protein analysis on the BioPharma-ready Q Exactive Plus and Q Exactive HF mass spectrometers.

- HMR with mass detection up to m/z 8000 mode was designed specifically for intact therapeutic protein workflows with the aim of providing ease of use and flexibility as needed.

- We have optimized Orbitrap performance for improved transmission of high m/z ions in HMR mode resulting in increased sensitivity for all types of large molecules.

- With the introduction of HMR mode we present a platform that is optimized for both conventional intact monoclonal antibody analysis performed in denaturing conditions, as well as native MS analysis which is ideal for antibody drug conjugates (ADCs) and other heterogeneous antibody samples.

Any Disadvantage of Having BioPharma Option Installed on my Instrument?

- **NO!**
- BioPharma Option **does not compromise** the performance of the Q Exactive Plus and Q Exactive HF instruments in Standard Mode
- The instruments with BioPharma Option cover all Pharma and BioPharma applications

Q Exactive BioPharma - Specifications

Q Exactive Plus with the BioPharma Option

Including Enhanced Resolution, Protein mode, and HMR mode

Max Resolving Power

280,000 @ m/z 200

Max Scan Rate

Up to 12 Hz at resolution setting of 17,500 @ m/z 200

Q Exactive HF with the BioPharma Option

Including Protein mode and HMR mode

Max Resolving Power

240,000 @ m/z 200

Max Scan Rate

Up to 18 Hz at resolution setting of 15,000 @ m/z 200

Mass Range

m/z 50-8,000

Mass Accuracy

Internal: < 1 ppm RMS
External: < 3 ppm RMS under defined conditions

Dissociation

In-Source CID, HCD

Scan Functions

FS, AIF, SIM, PRM, DIA, ddHCD

Quad Isolation

Step-less from full mass range down to 0.4 amu

Polarity Switching

One full cycle in < 1 sec

Multiplexing

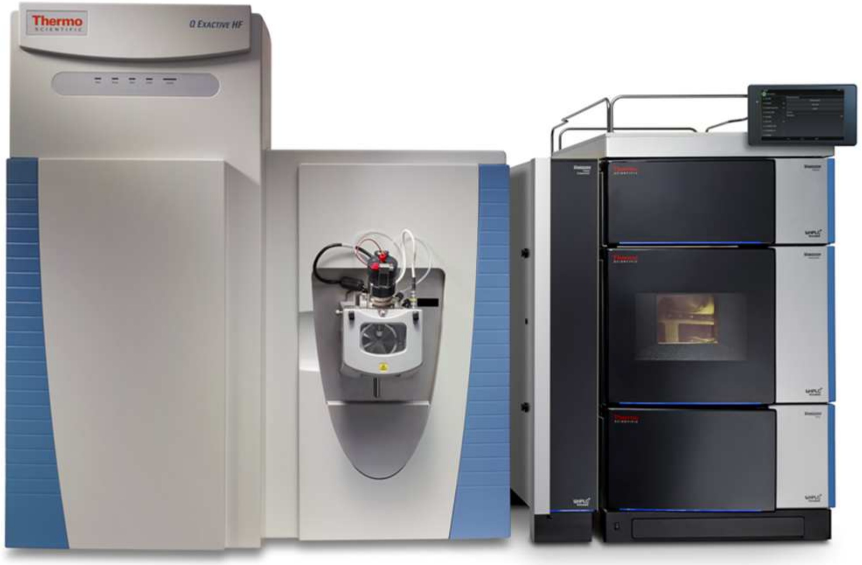
Up to 10 precursors/scan

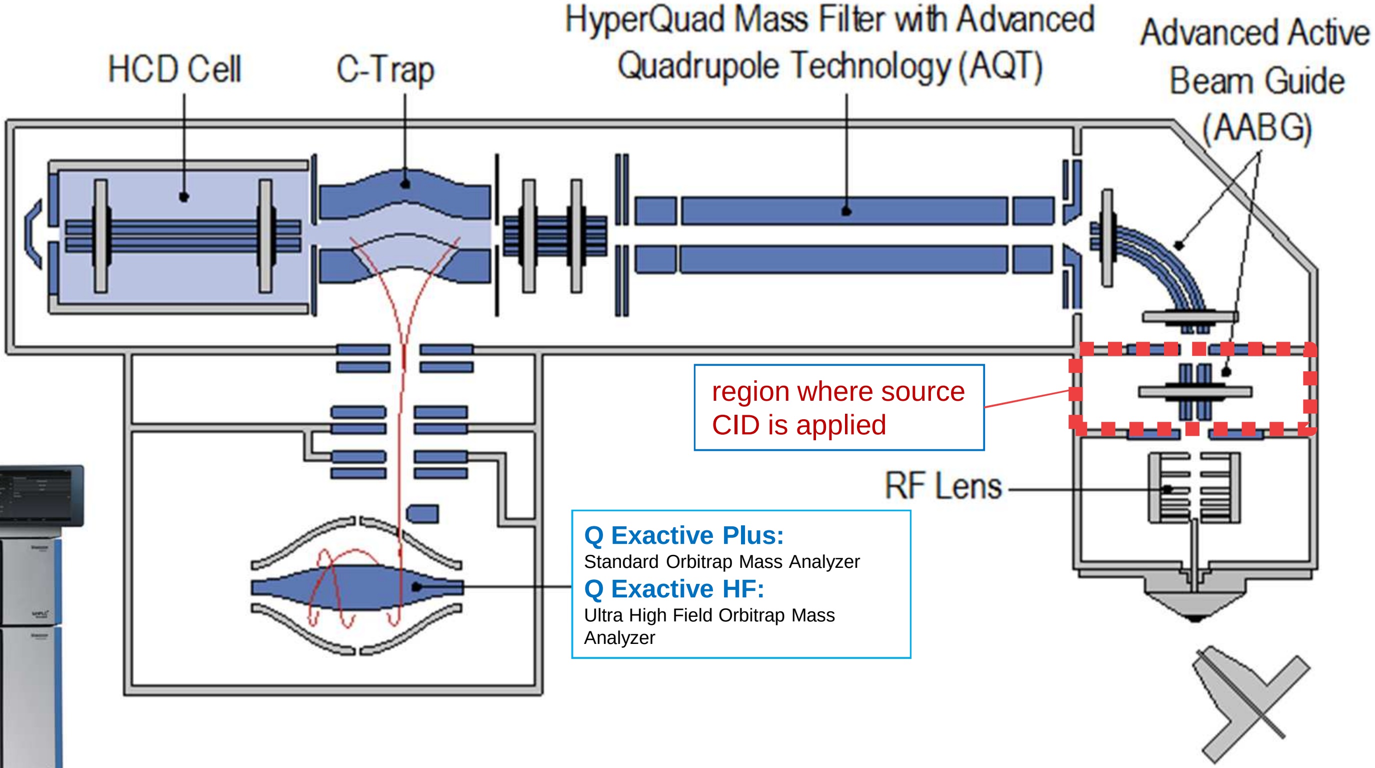
Analog Inputs

One (1) analog input (0 - 1 V)
One (1) analog (0 - 10 V)

Quad isolation up to m/z 2500

Q Exactive BioPharma - Hardware Overview

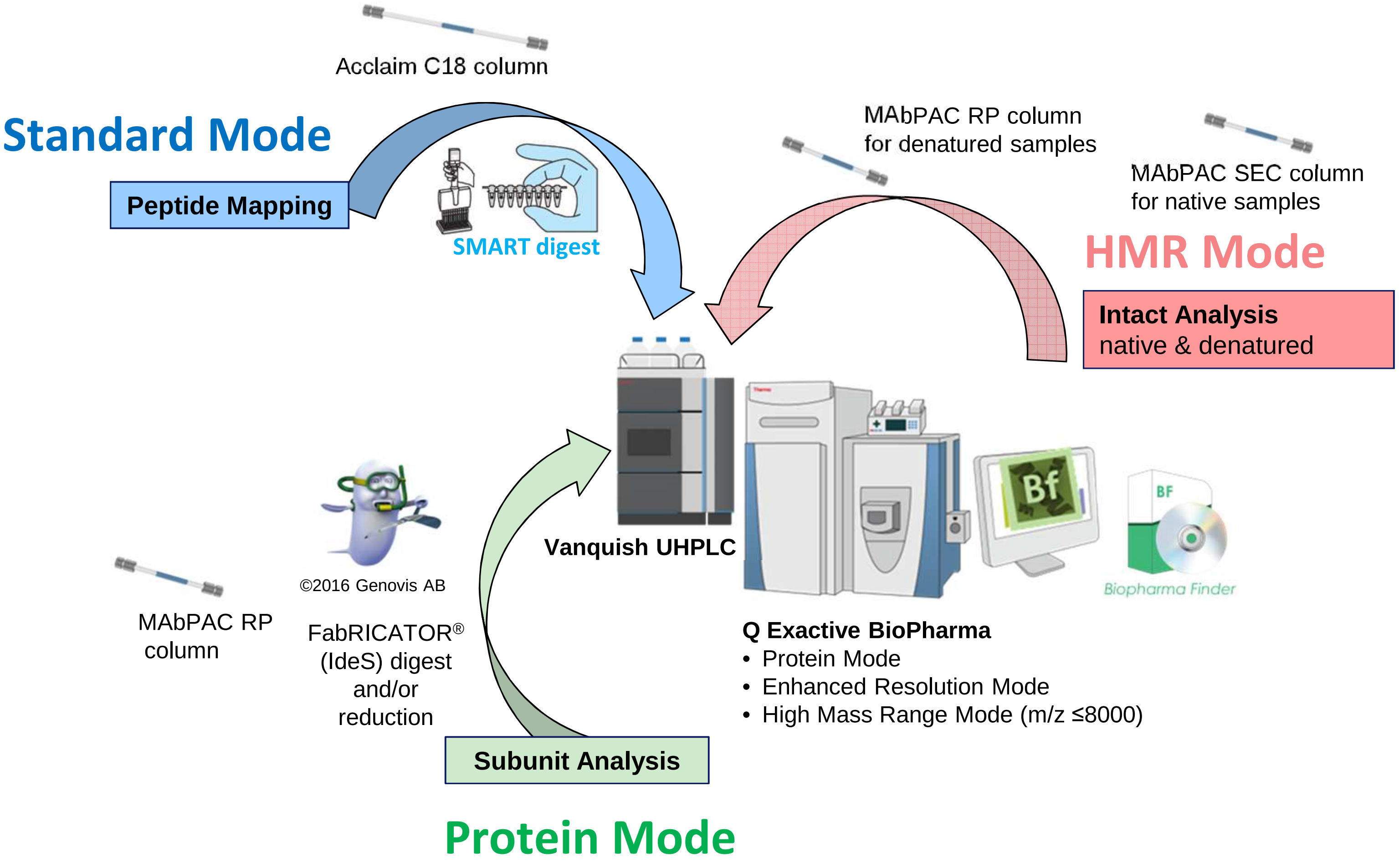




region where source CID is applied

Q Exactive Plus:
Standard Orbitrap Mass Analyzer
Q Exactive HF:
Ultra High Field Orbitrap Mass Analyzer

Q Exactive BioPharma Operating Modes for the 3 Major BioPharma Workflows



Acclaim C18 column

Standard Mode

Peptide Mapping

SMART digest

MAbPAC RP column
for denatured samples

MAbPAC SEC column
for native samples

HMR Mode

Intact Analysis
native & denatured

Vanquish UHPLC

©2016 Genovis AB

MAbPAC RP
column

FabRICATOR®
(IdeS) digest
and/or
reduction

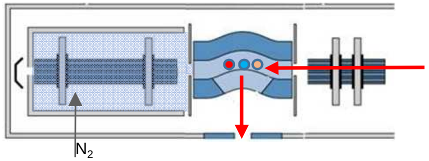
Subunit Analysis

Protein Mode

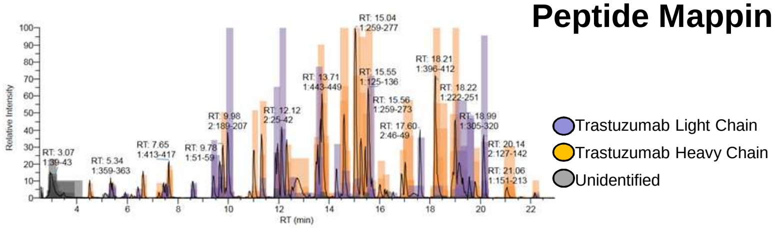
Q Exactive BioPharma
- Protein Mode
- Enhanced Resolution Mode
- High Mass Range Mode (m/z ≤8000)

Differences in the Trapping Path and Pressure Settings in the Three Different Operating Modes Available

Standard Mode

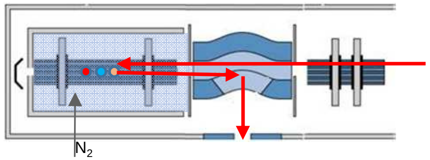


Trapping in Standard Mode: trapping gas pressure setting 1 (fixed on setting 1)

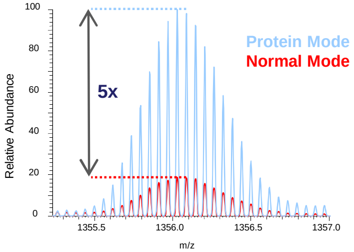


Peptide Mapping

Protein Mode

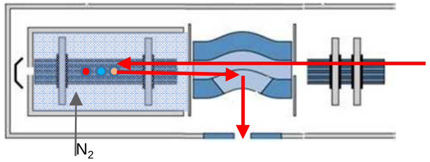


Trapping in Protein Mode: trapping gas pressure default setting 0.2 (range 0.2-1)

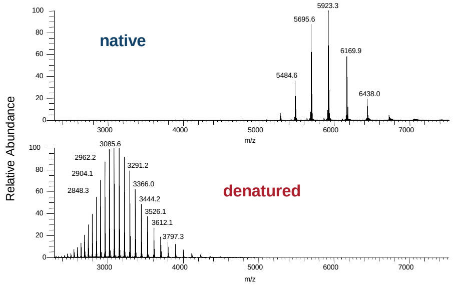


Subunit Analysis

HMR Mode



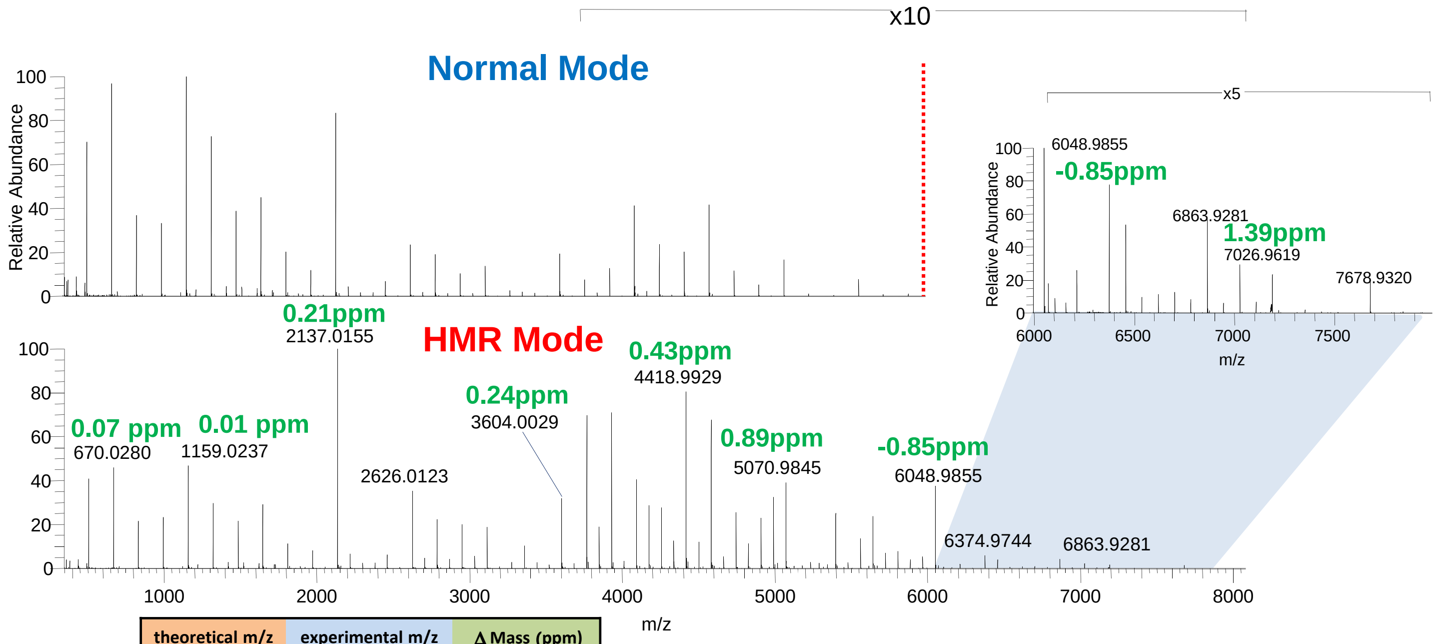
Trapping in HMR Mode: trapping gas pressure default setting 1 (range 1.0-1.5)



Intact mAb Analysis

Calibration of HMR Mode with Ammonium Hexafluorophosphate

Full MS spectra of ammonium hexafluorophosphate direct infusion experiment in Normal and HMR modes



theoretical m/z	experimental m/z	Δ Mass (ppm)
670.02805	670.0280	0.07
1159.02371	1159.0237	0.01
2137.01504	2137.0155	-0.21
3604.00204	3604.0029	-0.24
4418.99481	4418.9929	0.43
5070.98903	5070.9845	0.89
6048.98036	6048.9855	-0.85
7026.97170	7026.9619	1.39

ACROS ORGANICS
A Thermo Fisher Scientific Brand

Chemical Identifiers

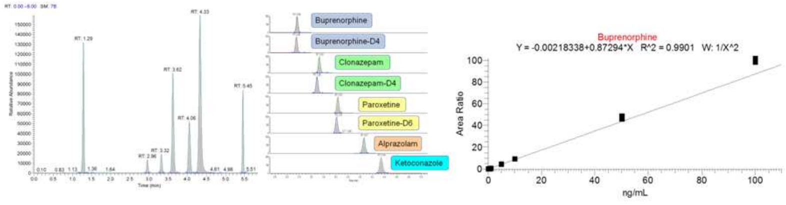
CAS	16941-11-0
Assay Percent Range	99%
Molecular Formula	H_4F_6NP
Linear Formula	NH_4PF_6
MFCD Number	MFCD00064642
Chemical Name or Material	Ammonium hexafluorophosphate, 99.5%

part number: **AC202320050**

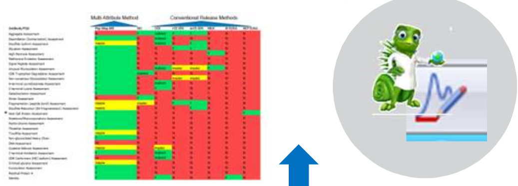
GSA/VA

Q Exactive for Pharma and BioPharma Complete Characterization

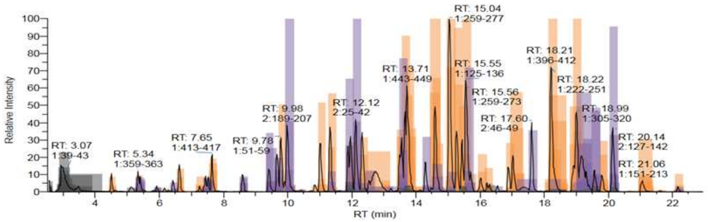
Pharma Qual/Quan



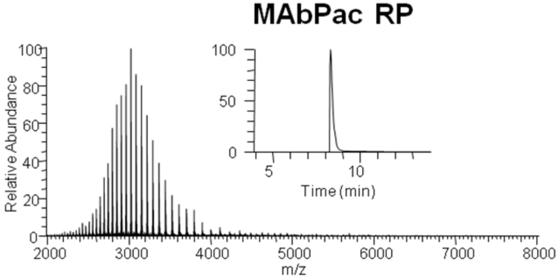
MS in QC



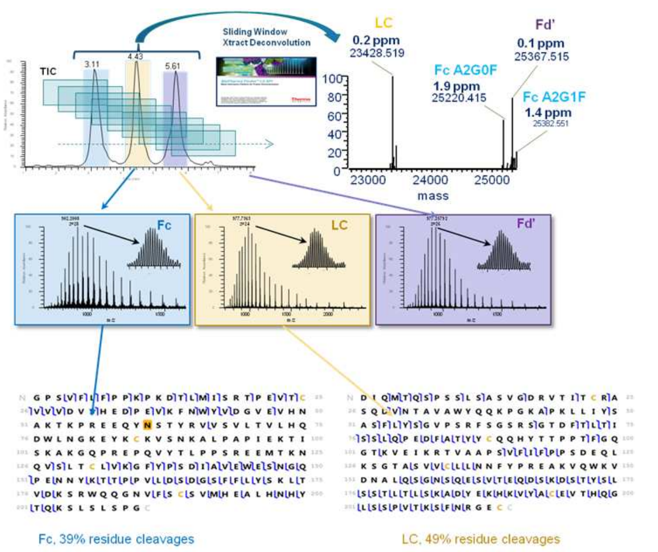
Peptide Mapping



Intact: Denatured



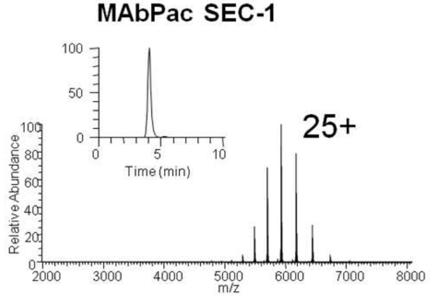
Subunit Top/Middle Down



Fc, 39% residue cleavages

LC, 49% residue cleavages

Intact: Native



HDX

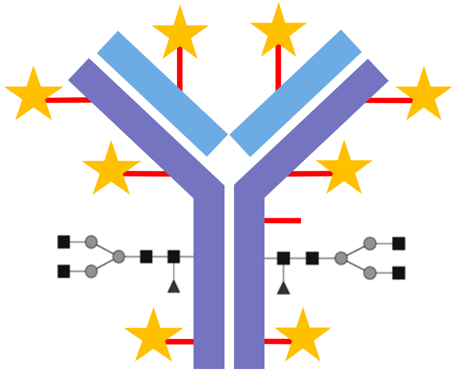
Native MS in HMR Mode: Unraveling Antibody Drug Conjugates (ADC) Complexity

Intact ADC analysis:
Goal is to look at untouched molecule

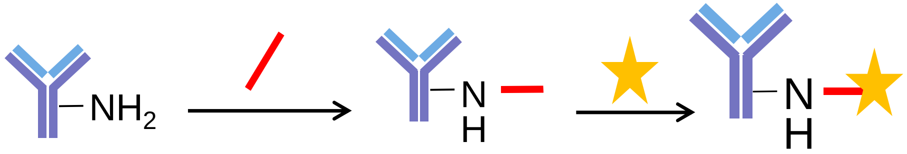
ADC construction can create
layers** of sample **heterogeneity

Many diverse ADC forms possible

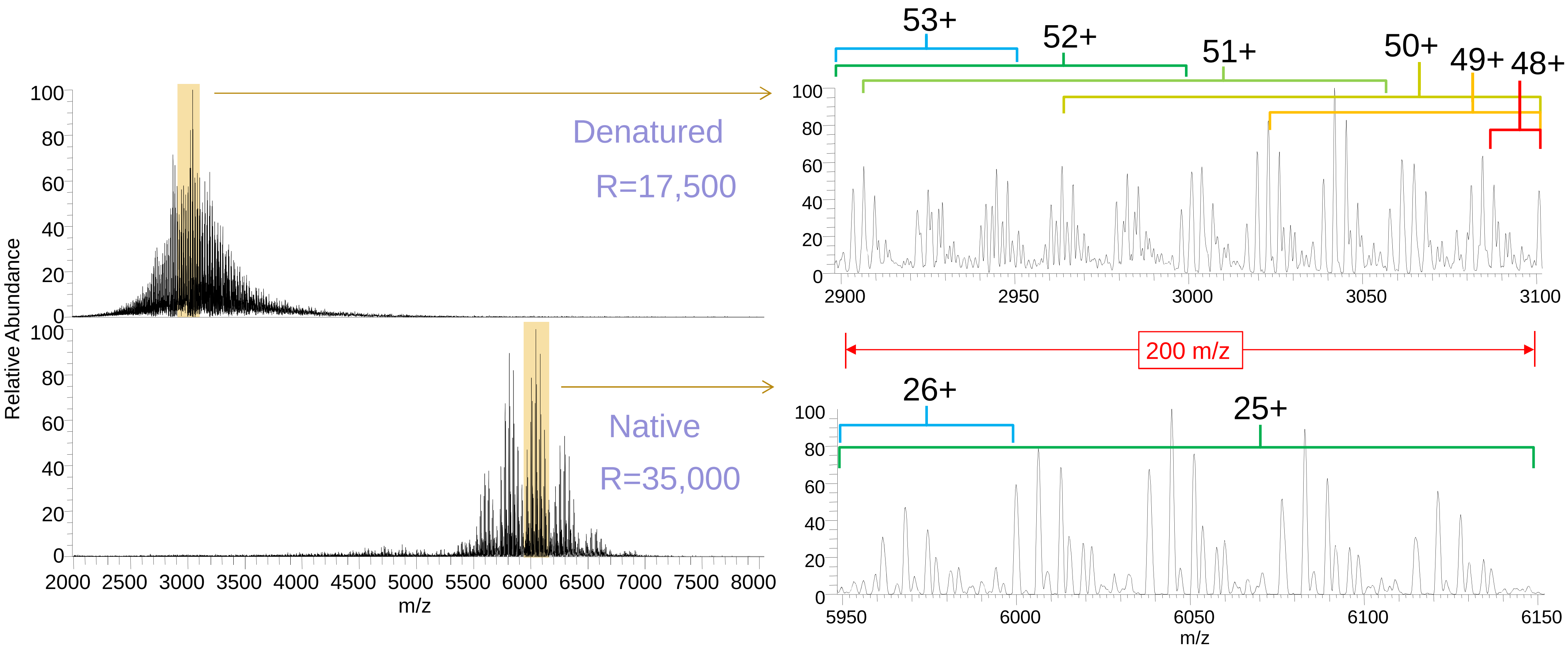
- Antibody
    - Glycan variants
    - Minor PTM variants
    - Sequence/clipping variants
- Conjugation
    - Linker-payload forms
    - Free-linker forms



Trastuzumab Emtansine
Lysine-linked ADC

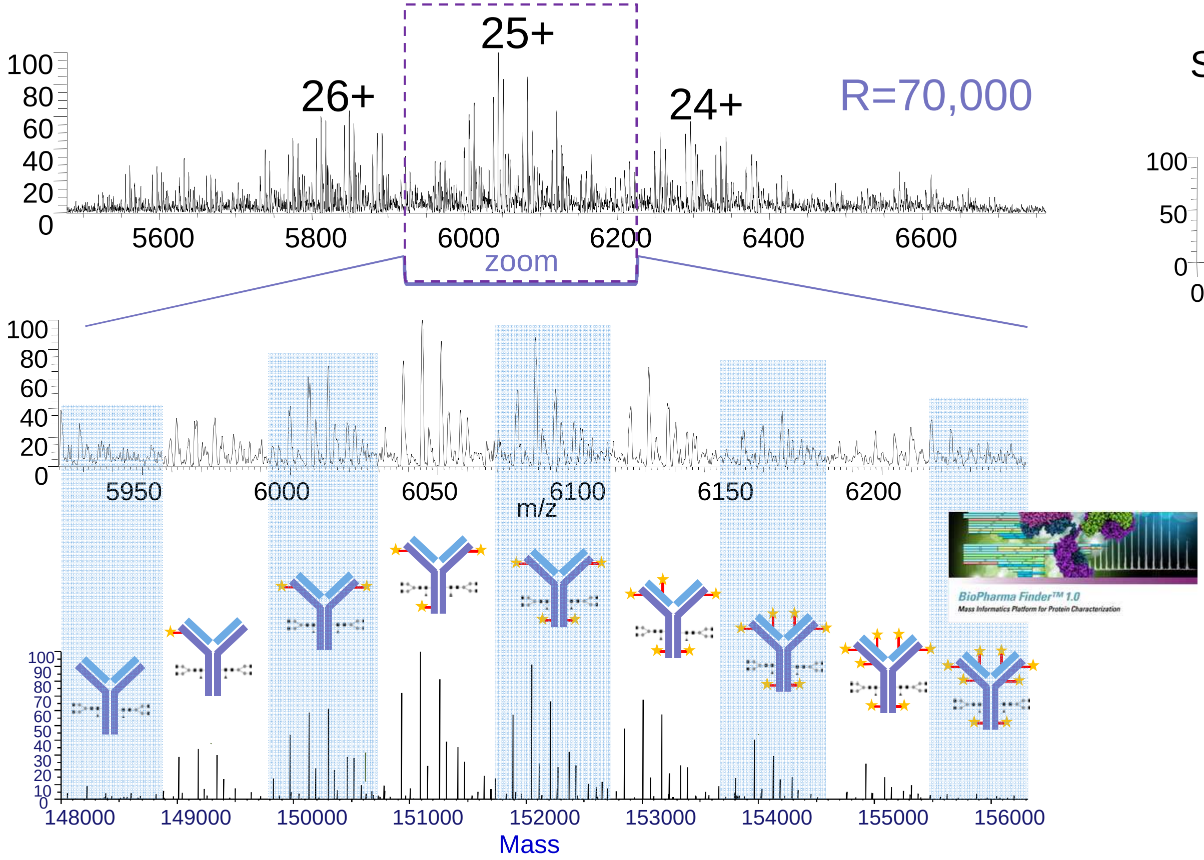


Denaturing vs. Native Conditions: Intact Analysis of Trastuzumab Emtansine Lys-linked ADC

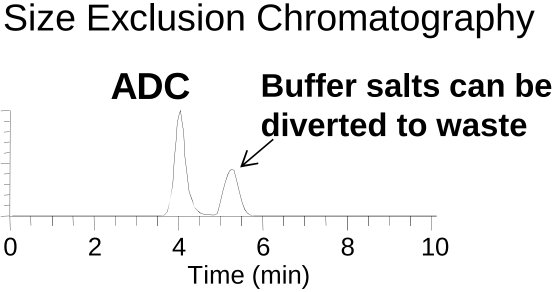


Native MS allows greater m/z separation of co-eluting species' charge states

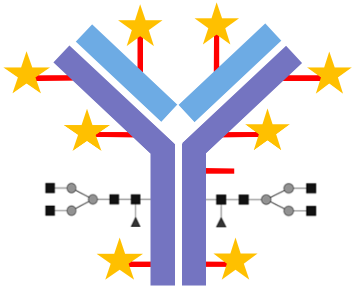
Native LC-MS in HMR Mode: Analysis of Intact Trastuzumab Emtansine Lys-linked ADC



Size Exclusion Chromatography

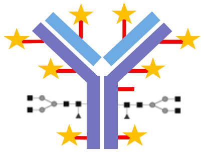


Buffer salts can be diverted to waste



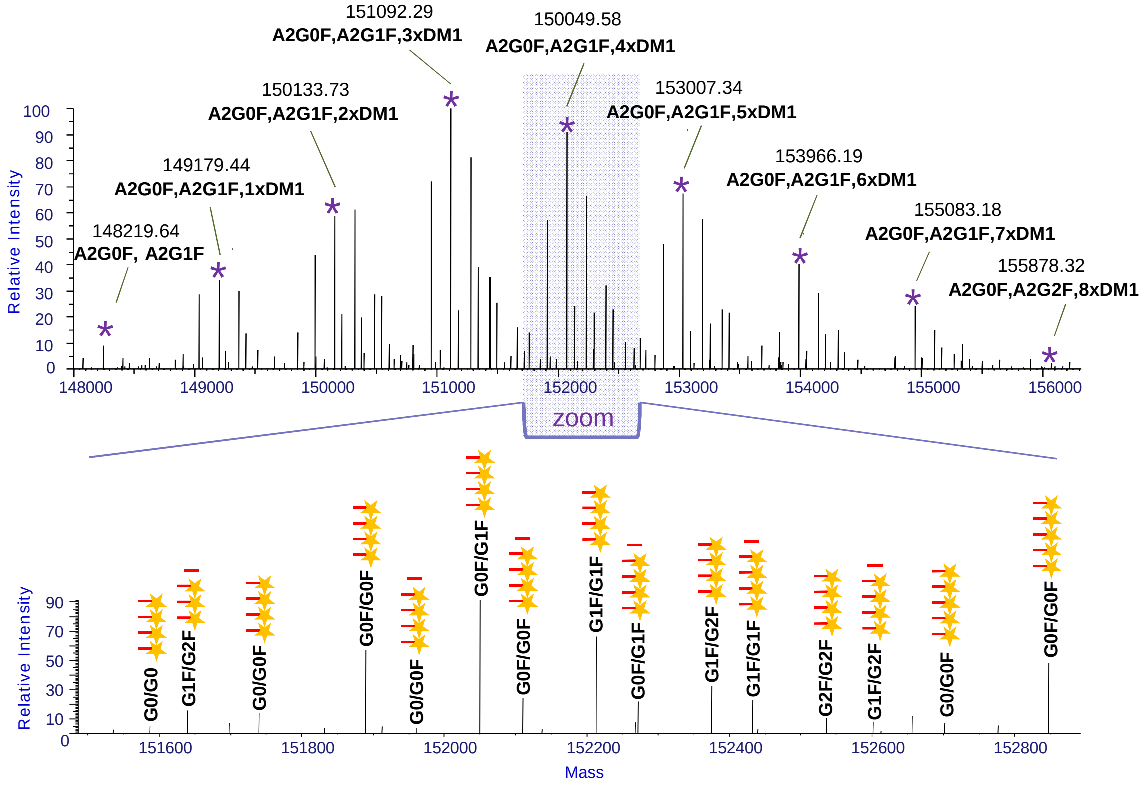
Trastuzumab Emtansine
Lysine-linked ADC

Native LC-MS in HMR Mode: Analysis of Intact Trastuzumab Emtansine Lys-linked ADC



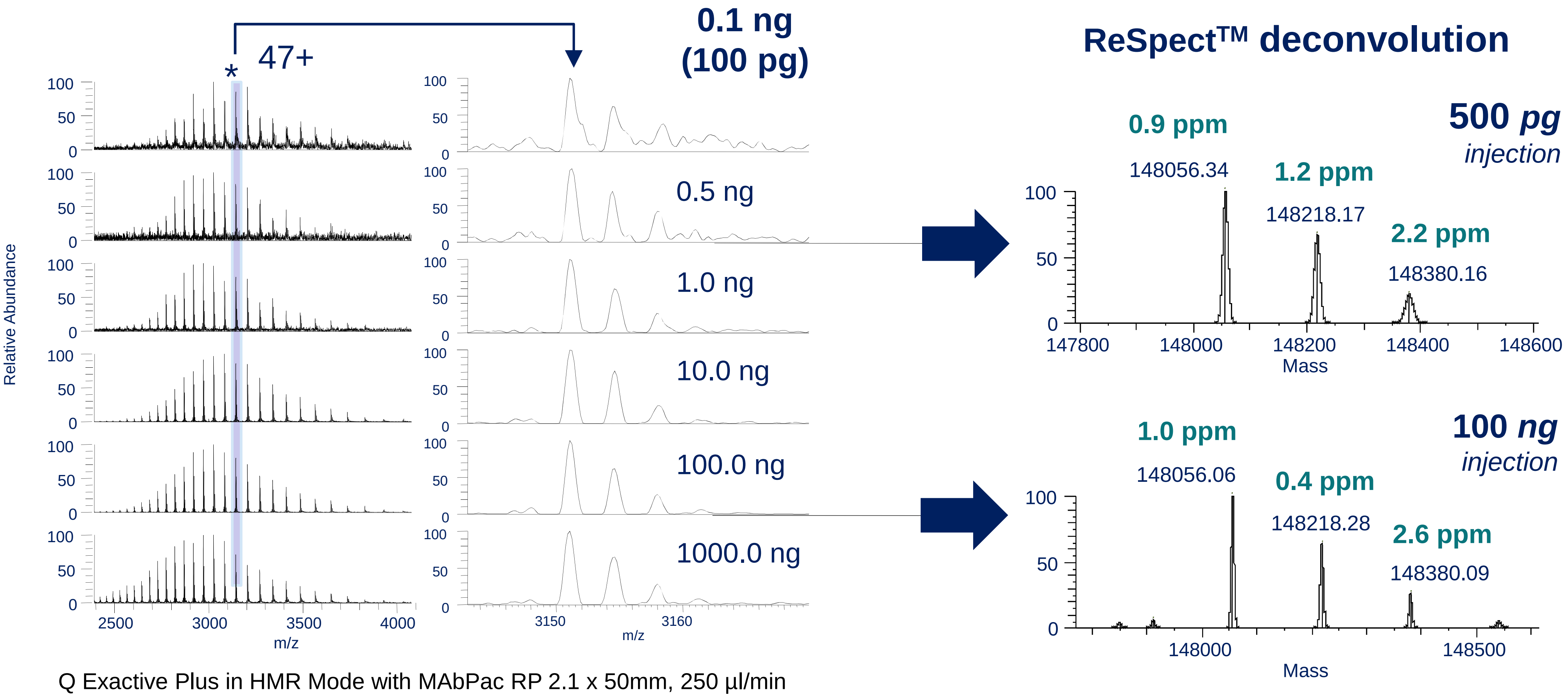
Average
Drug-to-Antibody
Ratio (DAR)
3.71

* G0F/G1F DAR	Mass Accuracy (ppm)	Relative Abundance
DAR0	6.49	9.19
DAR1	21.69	34.26
DAR2	0.05	59.03
DAR3	6.81	100.00
DAR4	5.17	91.16
DAR5	6.69	67.42
DAR6	15.20	40.46
DAR7	6.28	24.28
DAR8	3.78	3.84



Achieving unprecedented detail in intact ADC analysis with high resolution Native MS

Dilution Series Analysis of Intact mAb Trastuzumab - Denaturing LC-MS in HMR Mode

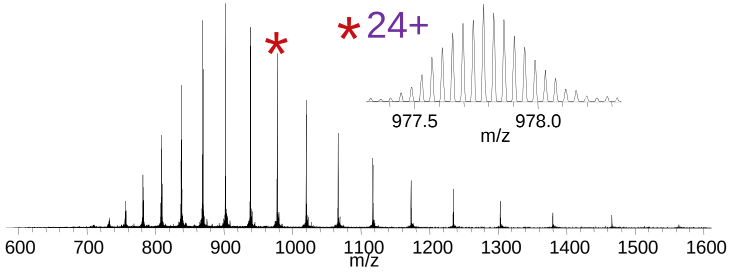


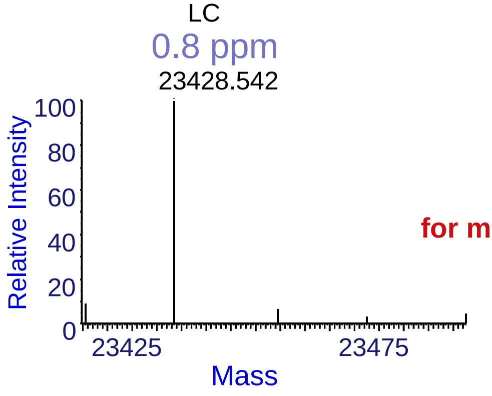
Q Exactive Plus in HMR Mode with MAbPac RP 2.1 x 50mm, 250 µl/min

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

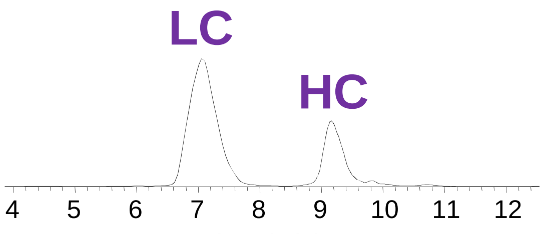
Light Chain

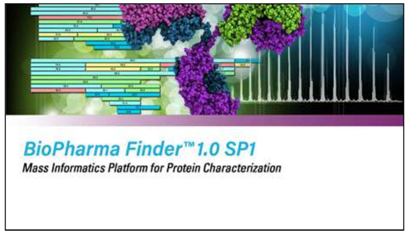
R = 120,000
Full MS





Xtract™
deconvolution
for monoisotopic masses





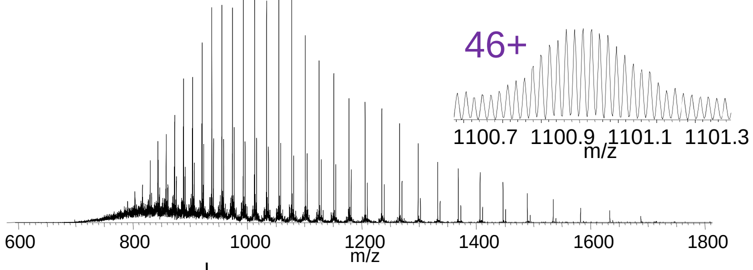
BioPharma Finder™ 1.0 SP1
Mass Informatics Platform for Protein Characterization

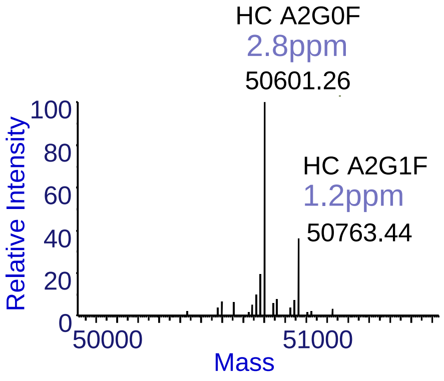
ReSpect™
deconvolution
for average masses

Heavy Chain

R = 15,000
Full MS

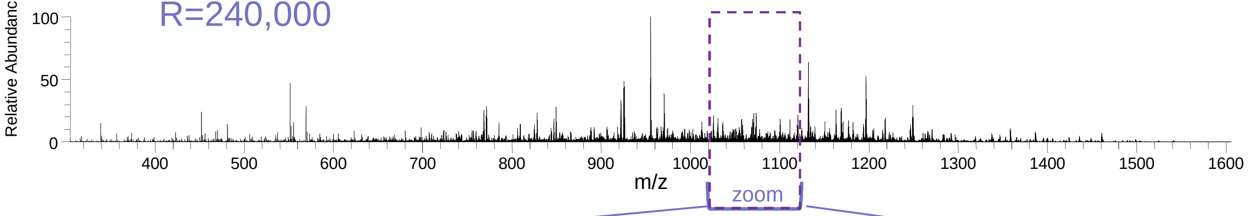
R = 240,000
SIM MS

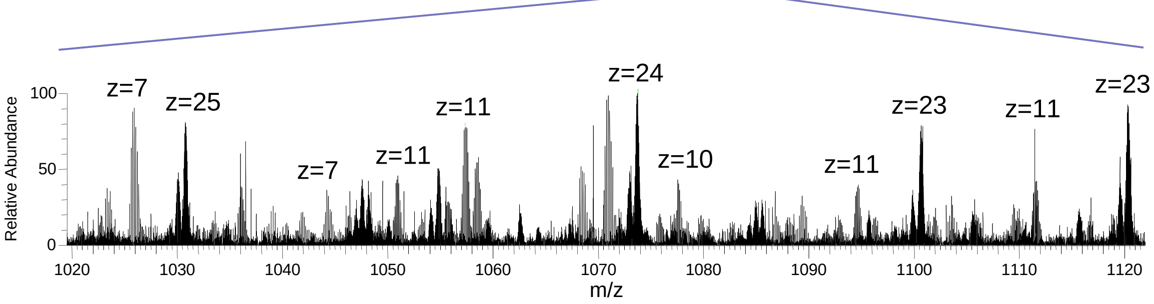




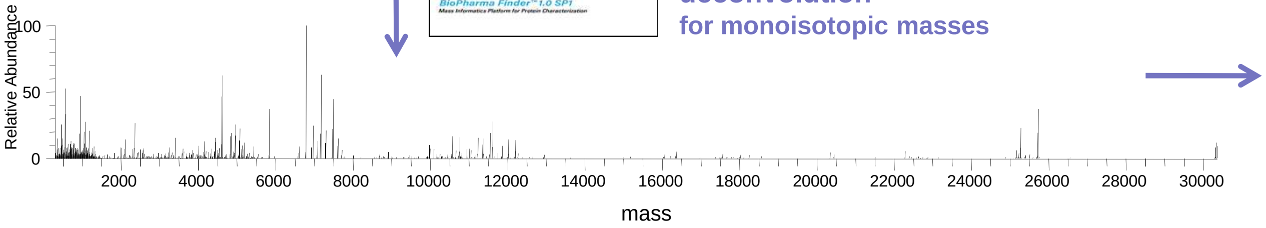
Top-Down Analysis in Protein Mode: HCD Fragmentation of Reduced Trastuzumab LC and HC

HCD MS/MS
R=240,000





Xtract^{TM}
deconvolution
for monoisotopic masses



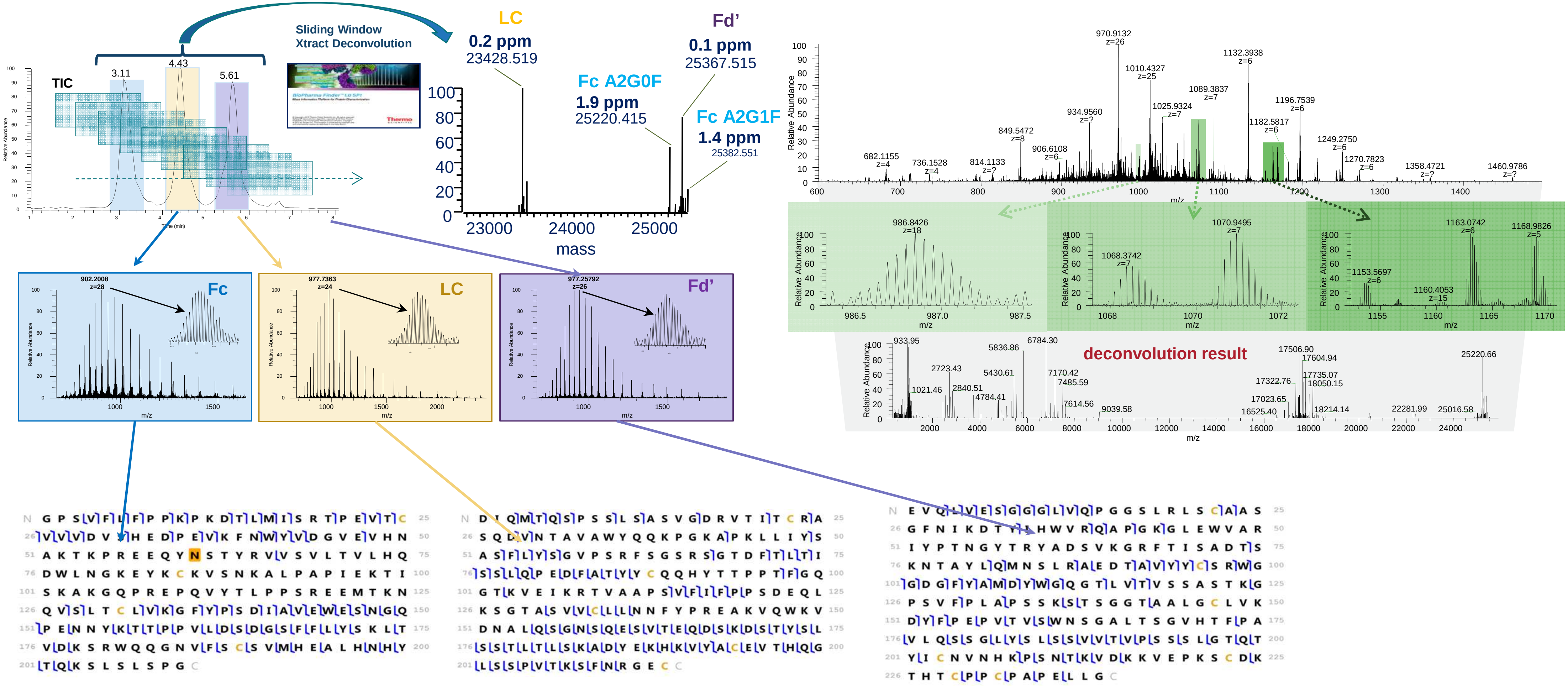
Light Chain

43% residue cleavages

Heavy Chain

24% residue cleavages

Intact and Top-Down Subunit Analysis after IdeS Digest and Reduction

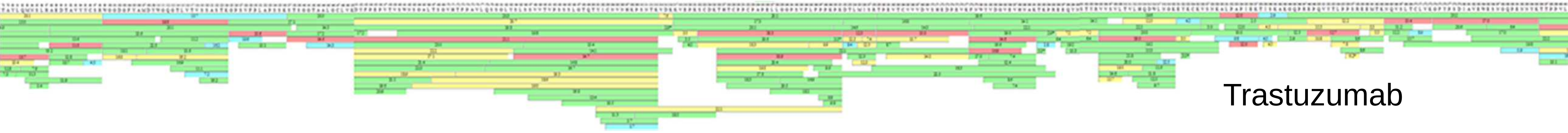


Fc, 39% residue cleavages

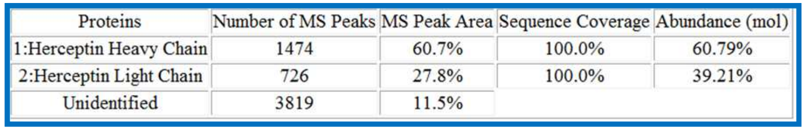
LC, 49% residue cleavages

Fd’, 38% residue cleavages

Antibody Peptide Mapping

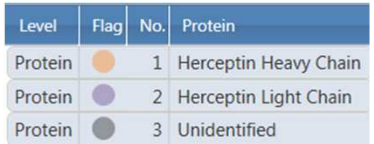


Trastuzumab

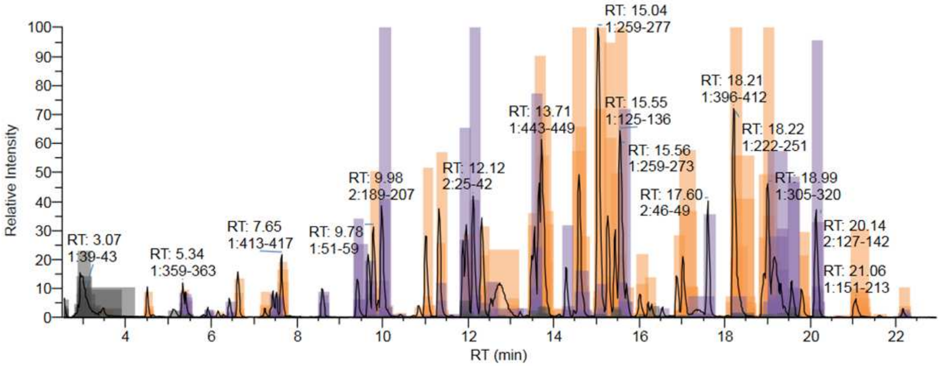


Proteins	Number of MS Peaks	MS Peak Area	Sequence Coverage	Abundance (mol)
1:Herceptin Heavy Chain	1474	60.7%	100.0%	60.79%
2:Herceptin Light Chain	726	27.8%	100.0%	39.21%
Unidentified	3819	11.5%		

🟥 >1.4e+007 🟨 >7.5e+005 🟩 >4.0e+004 >2.2e+003 >1.2e+002



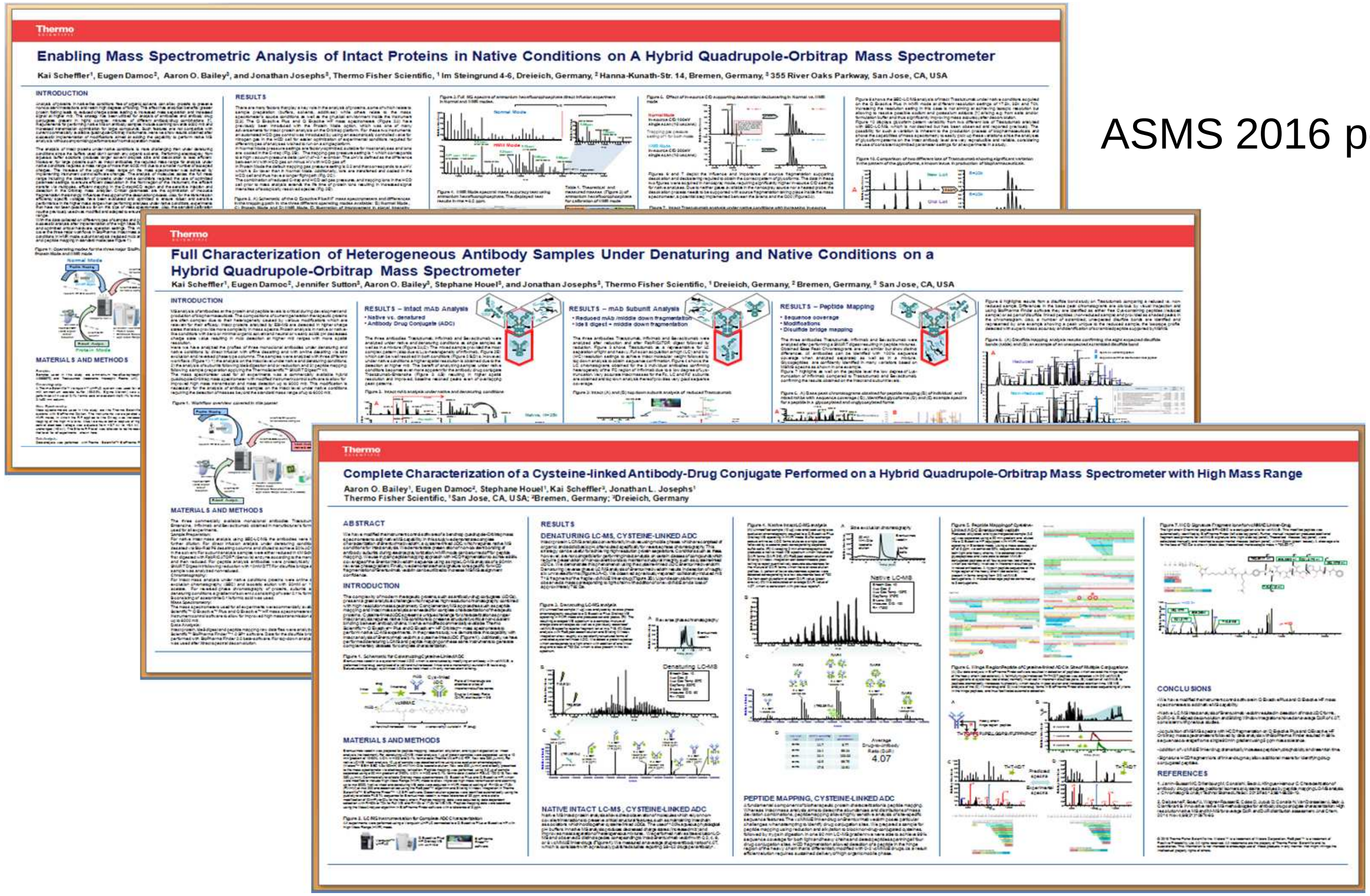
Level	Flag	No.	Protein
Protein	●	1	Herceptin Heavy Chain
Protein	●	2	Herceptin Light Chain
Protein	●	3	Unidentified



Sensitivity / Accuracy

Rapid, high resolution scanning of the Orbitrap mass analyzer allows discrimination between components which are near-isobaric or co-eluting. Identifies sequence variants, modifications, glycosylation, impurities.

Q Exactive BioPharma - Available Collateral



Enabling Mass Spectrometric Analysis of Intact Proteins in Native Conditions on A Hybrid Quadrupole-Orbitrap Mass Spectrometer

Kai Scheffler[1], Eugen Damoc[2], Aaron O. Bailey[3], and Jonathan Josephs[3], Thermo Fisher Scientific, [1] Im Steingrund 4-6, Dreieich, Germany, [2] Hanna-Kunath-Str. 14, Bremen, Germany, [3] 355 River Oaks Parkway, San Jose, CA, USA

Full Characterization of Heterogeneous Antibody Samples Under Denaturing and Native Conditions on a Hybrid Quadrupole-Orbitrap Mass Spectrometer

Kai Scheffler[1], Eugen Damoc[2], Jennifer Sutton[3], Aaron O. Bailey[3], Stephane Houel[3], and Jonathan Josephs[3], Thermo Fisher Scientific, [1] Dreieich, Germany, [2] Bremen, Germany, [3] San Jose, CA, USA

Complete Characterization of a Cysteine-linked Antibody-Drug Conjugate Performed on a Hybrid Quadrupole-Orbitrap Mass Spectrometer with High Mass Range

Aaron O. Bailey[1], Eugen Damoc[2], Stephane Houel[1], Kai Scheffler[3], Jonathan L. Josephs[1]
Thermo Fisher Scientific, [1] San Jose, CA, USA; [2] Bremen, Germany; [3] Dreieich, Germany

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BioPharma Option* for Q Exactive Plus	726055
Q Exactive HF MS System	726041
BioPharma Option* for Q Exactive HF	726060
Vanquish Flex Binary UHPLC	5400.023
Vanquish Binary Pump H UHPLC	5400.011
MAbPac SEC-1 (4.0 x 150 mm)	75592
MAbPac RP (2.1 x 100 mm)	88647
Acclaim RSLC 120 C18 (2.1 x 250 mm)	74812
BioPharma Finder Software	OPTON-30592

* For details on the BioPharma Option please refer to slide 5

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