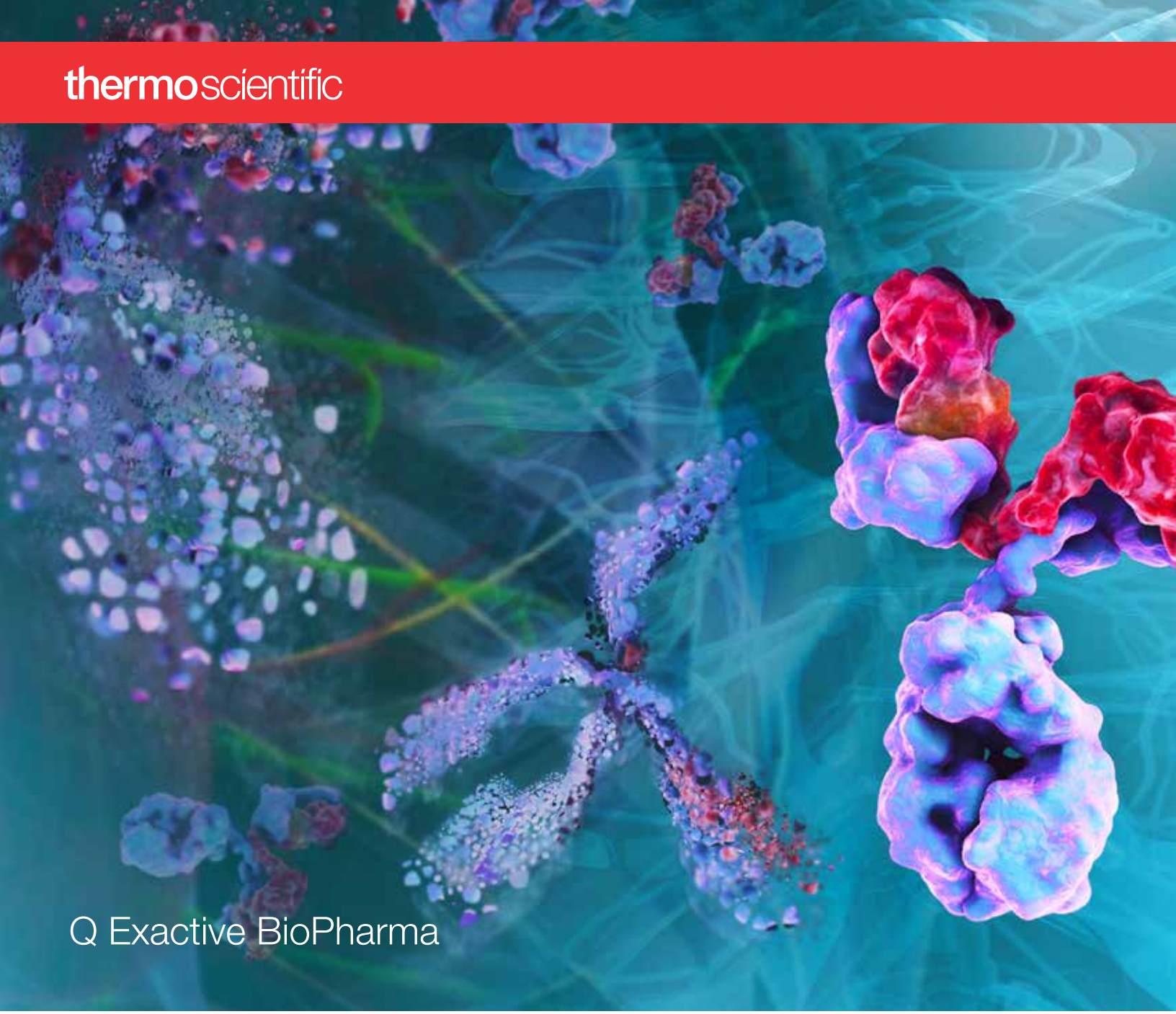
thermoscientific



Q Exactive BioPharma

The complete BioPharma characterization solution

ThermoFisher
SCIENTIFIC

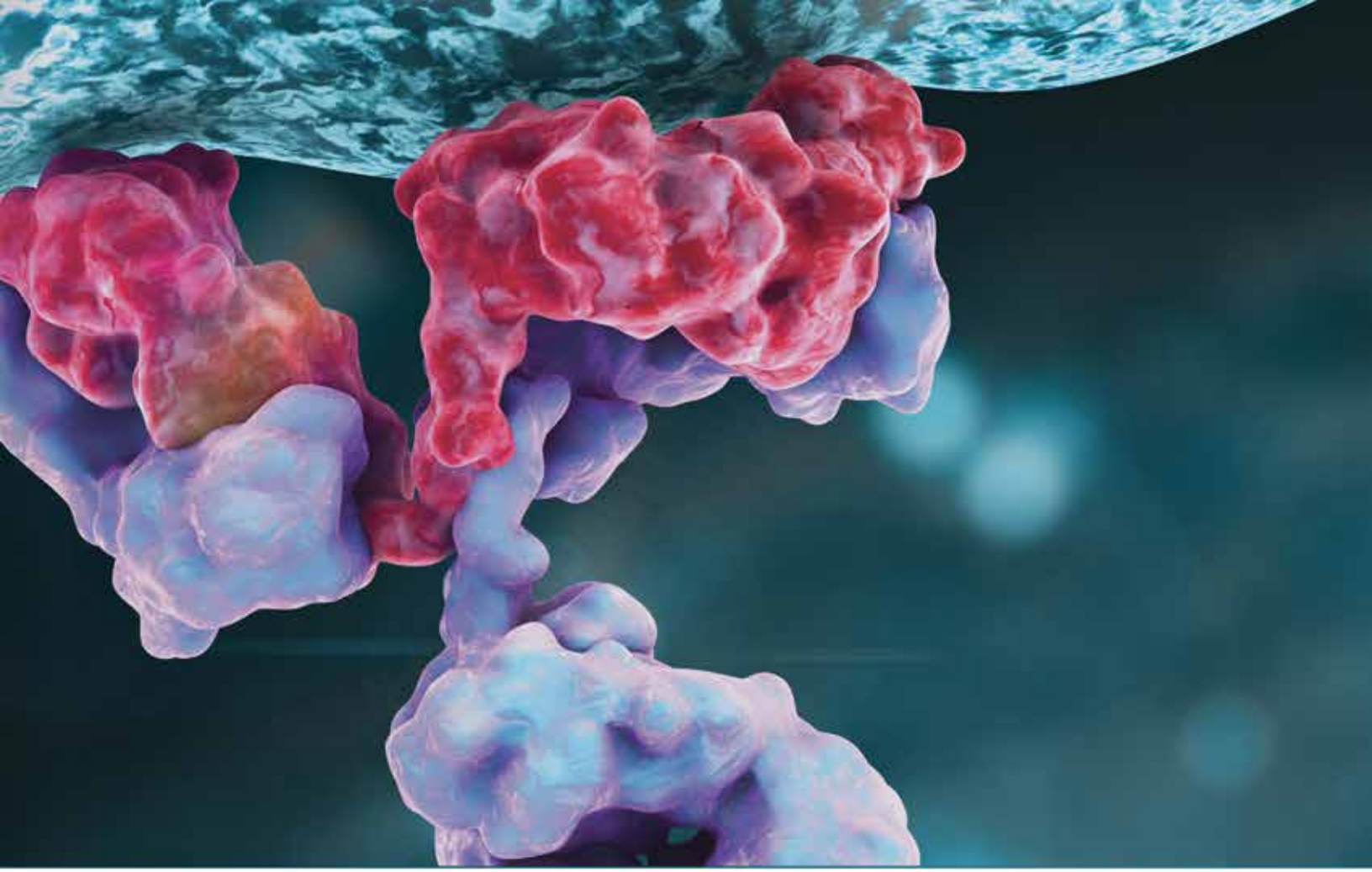
The most powerful system for every workflow

All in one package

"BioPharma Option" for the Thermo Scientific™ Q Exactive™ Plus and Q Exactive™ HF Hybrid Quadrupole-Orbitrap™ mass spectrometers adds **superior denatured and native MS intact analysis and subunit top/middle-down analysis capabilities to the most powerful benchtop peptide mapping instruments available**. When combined with Thermo Scientific™ BioPharma Finder™ software it provides a complete integrated hardware and software solution for BioPharma characterization.

The Thermo Scientific™ Q Exactive™ BioPharma platform 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



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.

Introducing:

High Mass Range mode

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 mode was designed specifically for intact therapeutic protein workflows with the aim of providing ease of use and flexibility as needed.

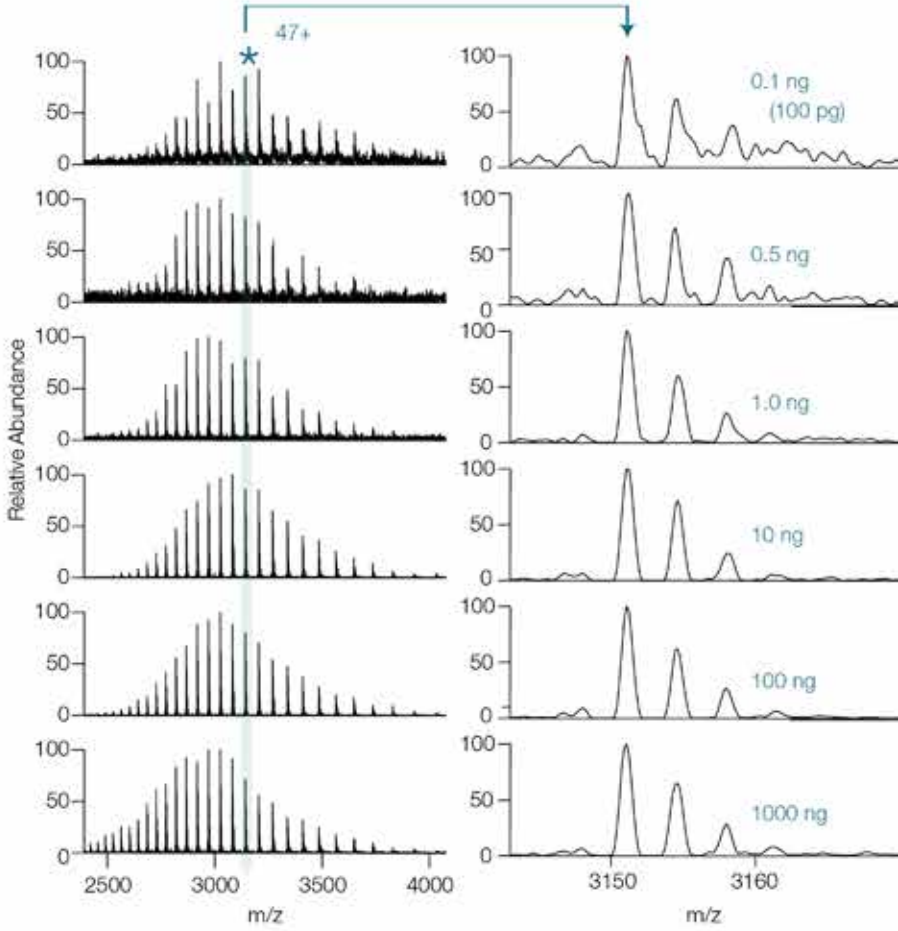
We have improved transmission and scanning up to *m/z* 8000 resulting in increased sensitivity for all types of large molecules.

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.

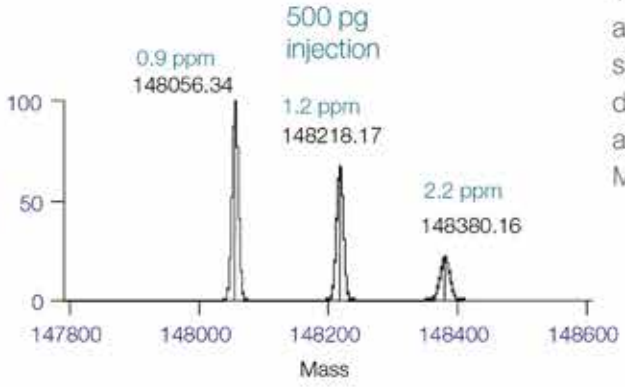
High sensitivity denaturing analysis

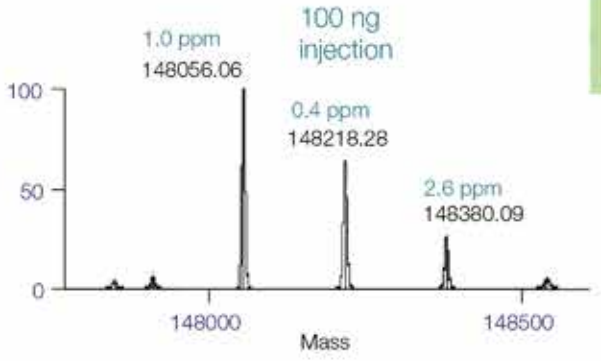
Reverse phase chromatography with online desalting is the conventional method for intact analysis of monoclonal antibodies under denaturing conditions.

Serial dilution of Trastuzumab, shows a consistent charge envelope over a range of 4 orders of magnitude. High mass accuracy can be achieved regardless of the concentration of the sample used.



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





State-of-the-art intact protein analysis

The introduction of HMR mode brings the highest quality and sensitivity for intact protein analysis to the Q Exactive series instruments. The 500 pg on-column injection demonstrates the superior sensitivity and mass accuracy achieved with the Q Exactive Plus running in the new High Mass Range mode.

ReSpect™ Deconvolution
for average masses

Intact antibody analysis
with High Mass Range mode

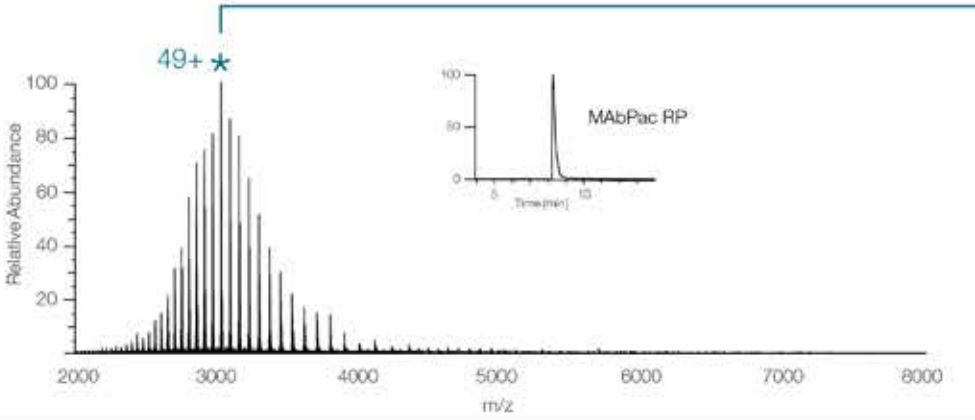
High Mass Range (HMR) mode enables increased scanning range up to 8,000 *m/z*, providing the flexibility to analyze your intact proteins in either denaturing or native MS conditions.

Denaturing MS is compatible with reverse phase HPLC separation.

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

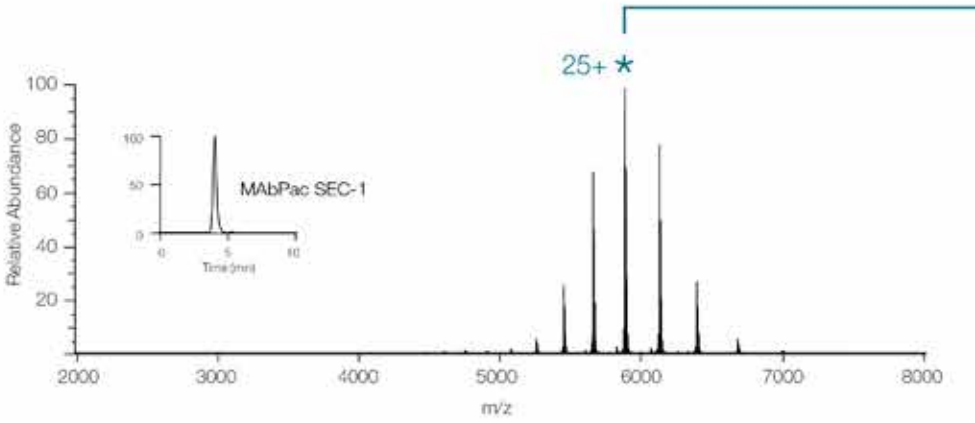
Denaturing

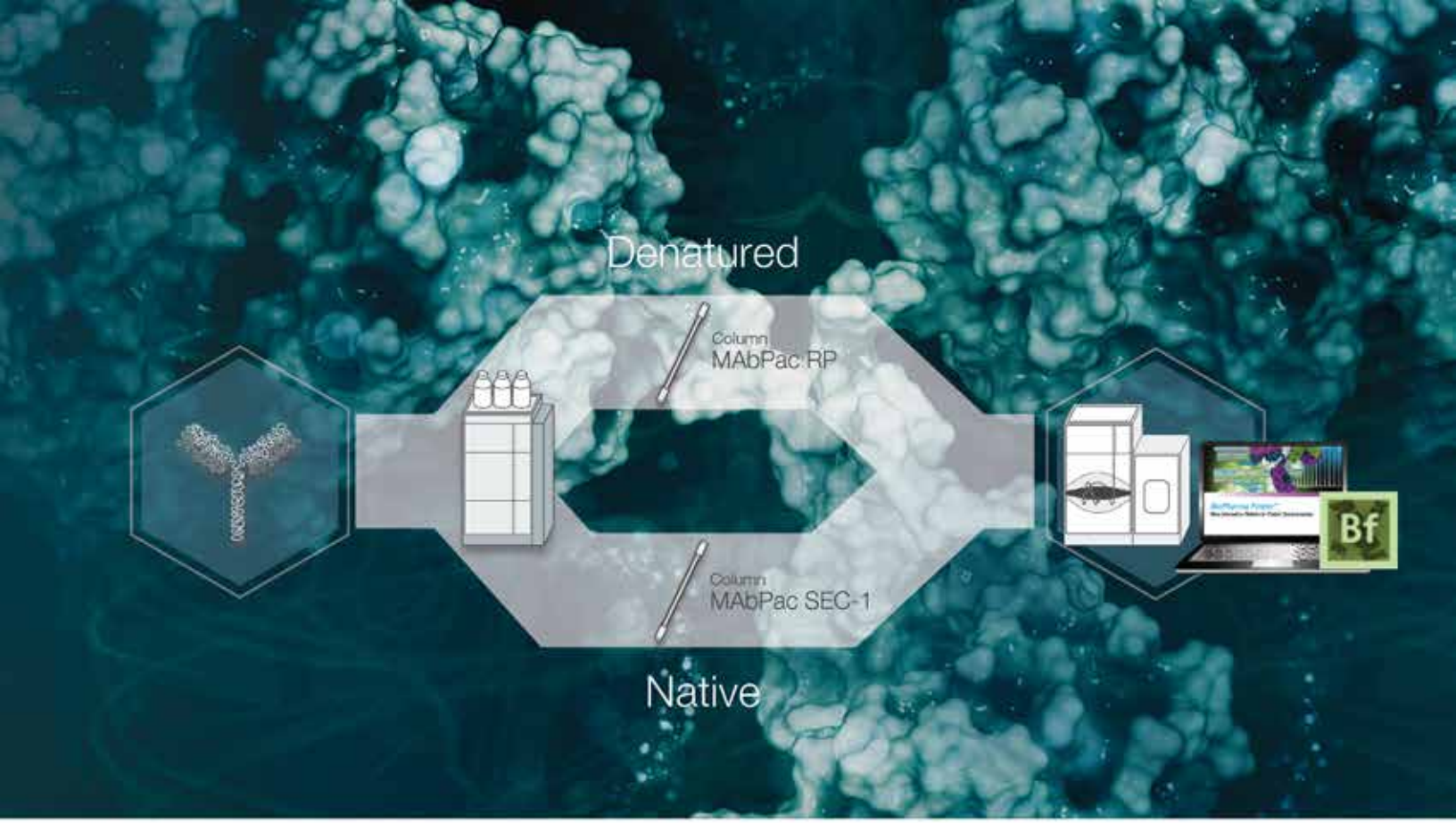
Conventional denaturing conditions, including mobile phase containing organic and acidic pH, result in protein unfolding and increased protonation. Denaturing MS is compatible with reverse phase chromatography, which allows sensitive, salt-free conditions for identifying intact proteins at lower *m/z* ranges.



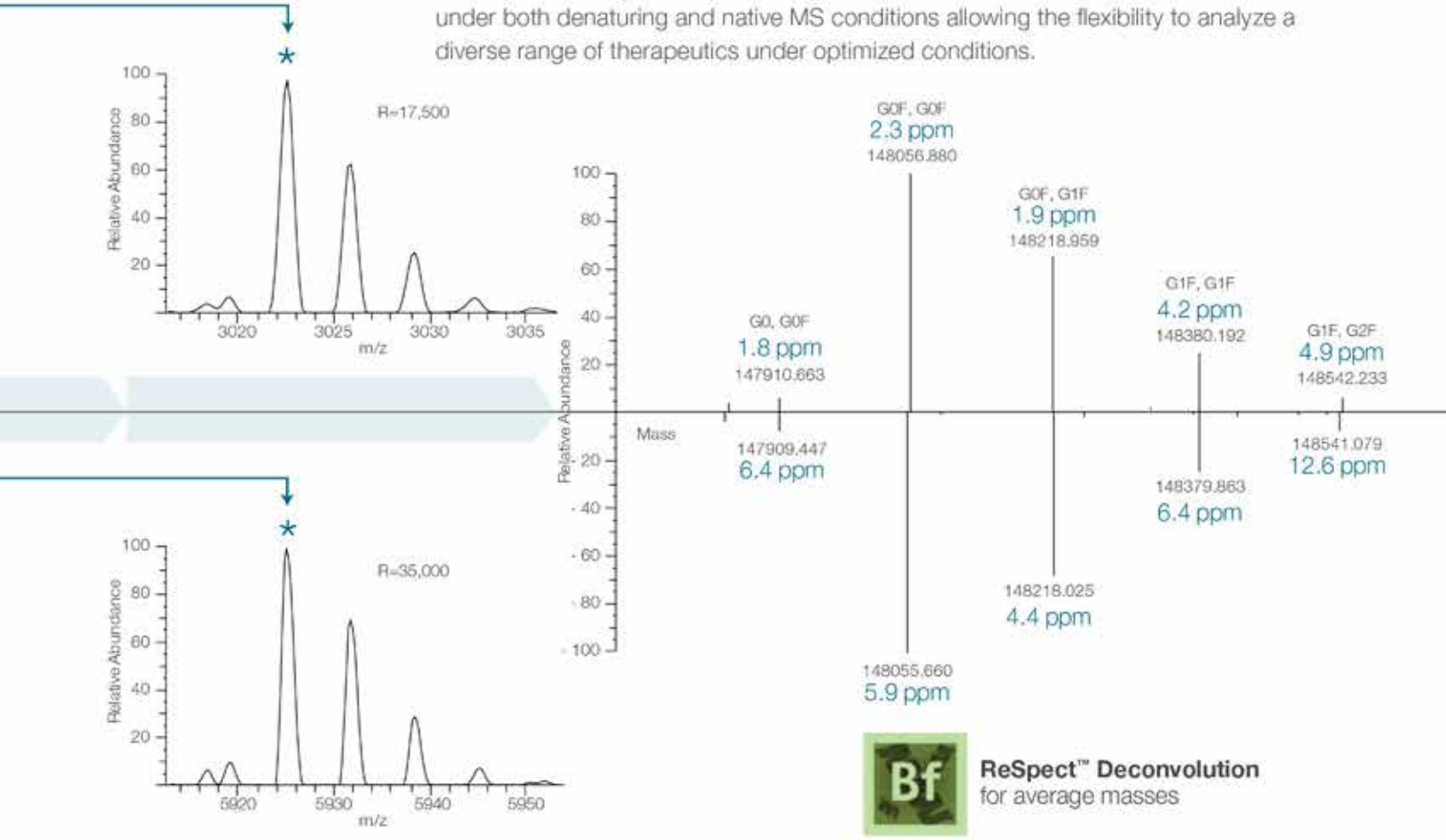
Native

Native MS conditions using volatile salt-based buffers at physiological pH retain compact or native conformations resulting in lower charge states. Native MS is compatible with size exclusion chromatography for observation of intact proteins at high *m/z* ranges. This strategy reduces mass interferences in complex protein spectra by increasing peak capacity in *m/z* space.





Multiple Analysis Options. HMR mode provides accurate intact mAb analysis under both denaturing and native MS conditions allowing the flexibility to analyze a diverse range of therapeutics under optimized conditions.



ReSpect™ Deconvolution
for average masses

Native MS ADC analysis in HMR mode
with Size Exclusion Chromatography

Native MS is now as easy as LC-MS

Intact protein analysis under native MS conditions can now be performed routinely using size exclusion chromatography (SEC) coupled to the Q Exactive Plus or Q Exactive HF mass spectrometers.

The Thermo Scientific™ Vanquish™ UHPLC system combined with the Thermo Scientific™ MAbPac™ SEC-1 Size Exclusion Column allows automatic buffer exchange to be performed in-line with the mass spectrometer.

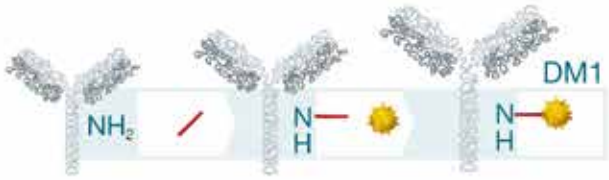
High throughput native LC-MS intact protein analysis with the MAbPac SEC-1 column enables greater-than-baseline separation of antibody and formulation buffer salts, which cause ion suppression. As a result, therapeutic antibody samples in formulation buffer may be injected directly without sample clean-up.

Coupling SEC to MS can produce clean intact protein spectra with resolution settings even as high as 70,000, allowing clear distinction of peak shoulders and low-level species.

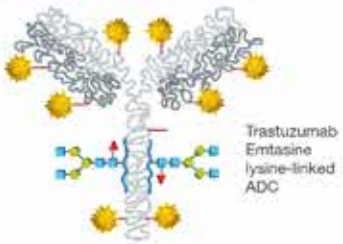
Native

Analysis of Trastuzumab Emtasine, a lysine-linked Antibody Drug Conjugate (ADC) based on Trastuzumab

Step-wise conjugation of antibody lysines with a bi-functional linker and payload can create moderate to very high sample heterogeneity comprised of varying degrees of glycosylation, linker-drug (DM1) conjugation, and linker-only conjugation.



Trastuzumab Emtasine

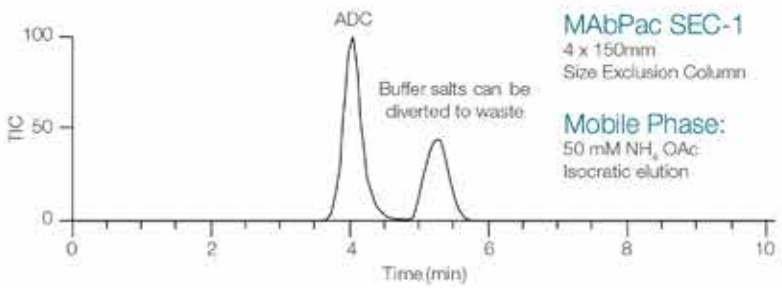


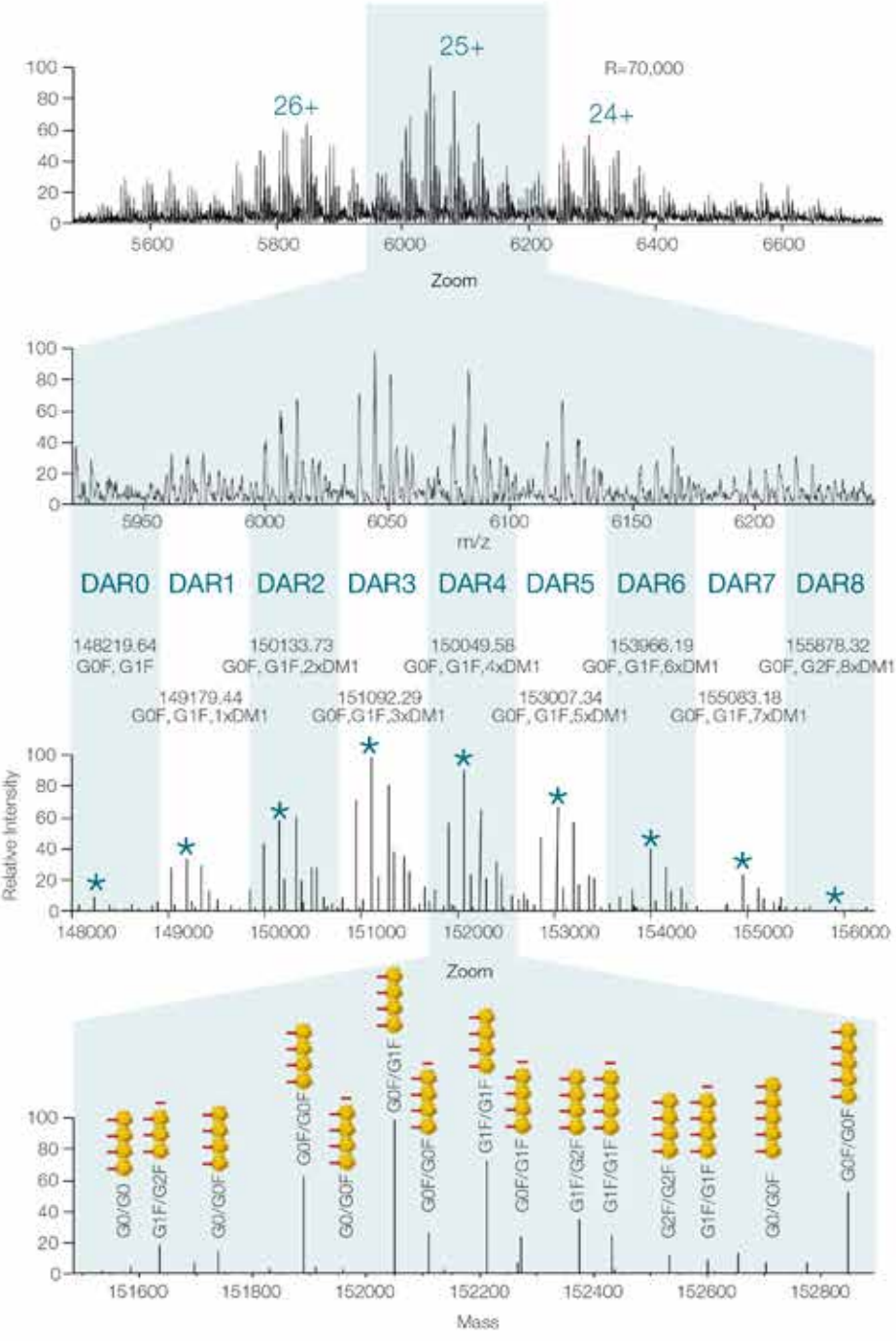
Average Drug-to-Antibody (DAR) Ratio

3.71

* G0F/G1F DAR form	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

Native





Measuring DAR using high resolution native MS.

The next generation of high resolution native MS has arrived.

Performing intact protein analysis in native MS allows greater separation between sequential charge states. For ADC samples, native MS allows the low and high abundance DAR forms to be separated and thus accurately measured. Native MS DAR analysis of complex ADC samples is now clear enough to confirm by eye.

Vanquish UHPLC

Sliding Window ReSpect™ Deconvolution for highly accurate average masses

Subunit and middle-down analysis with Protein mode

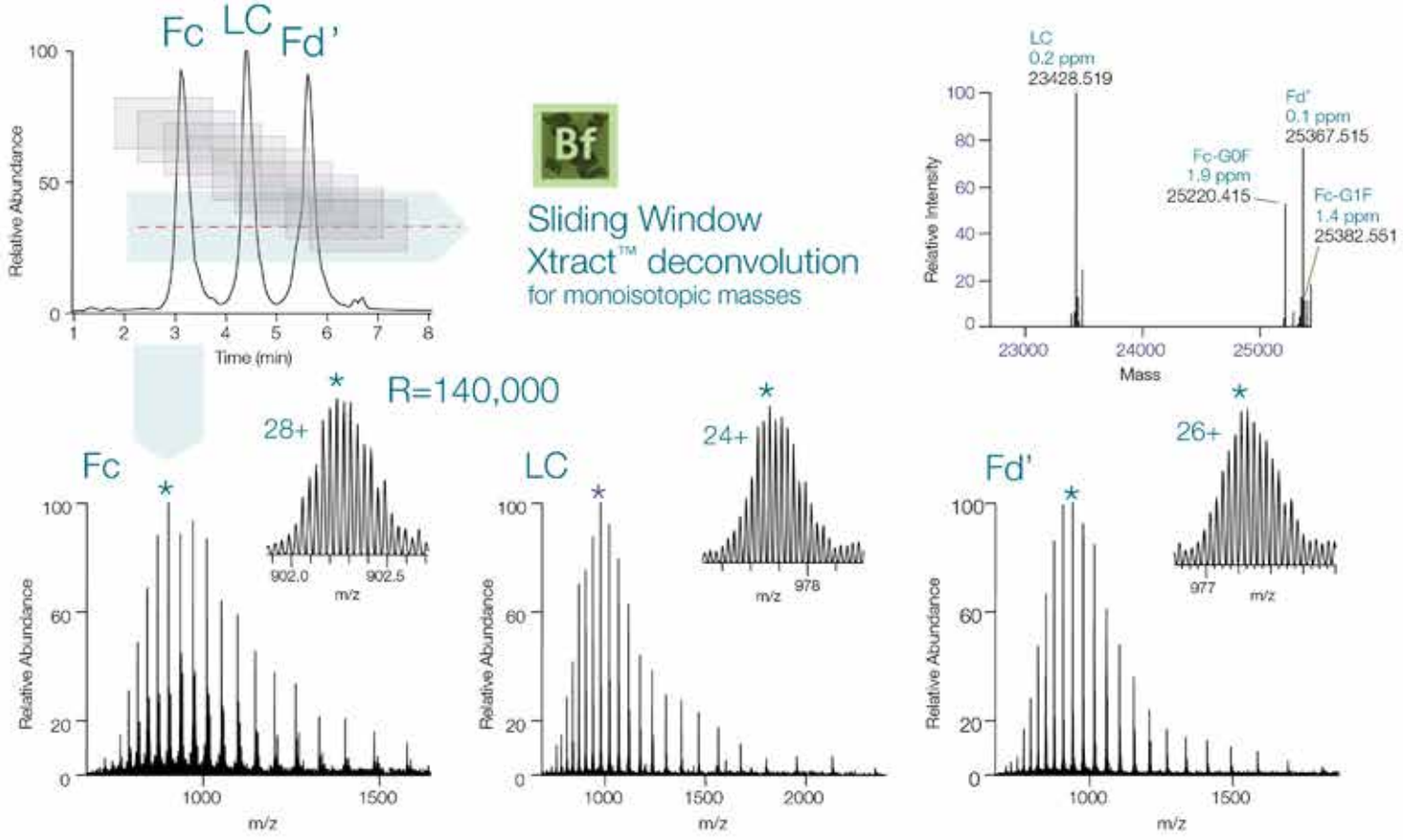
Q Exactive Plus with BioPharma Finder software

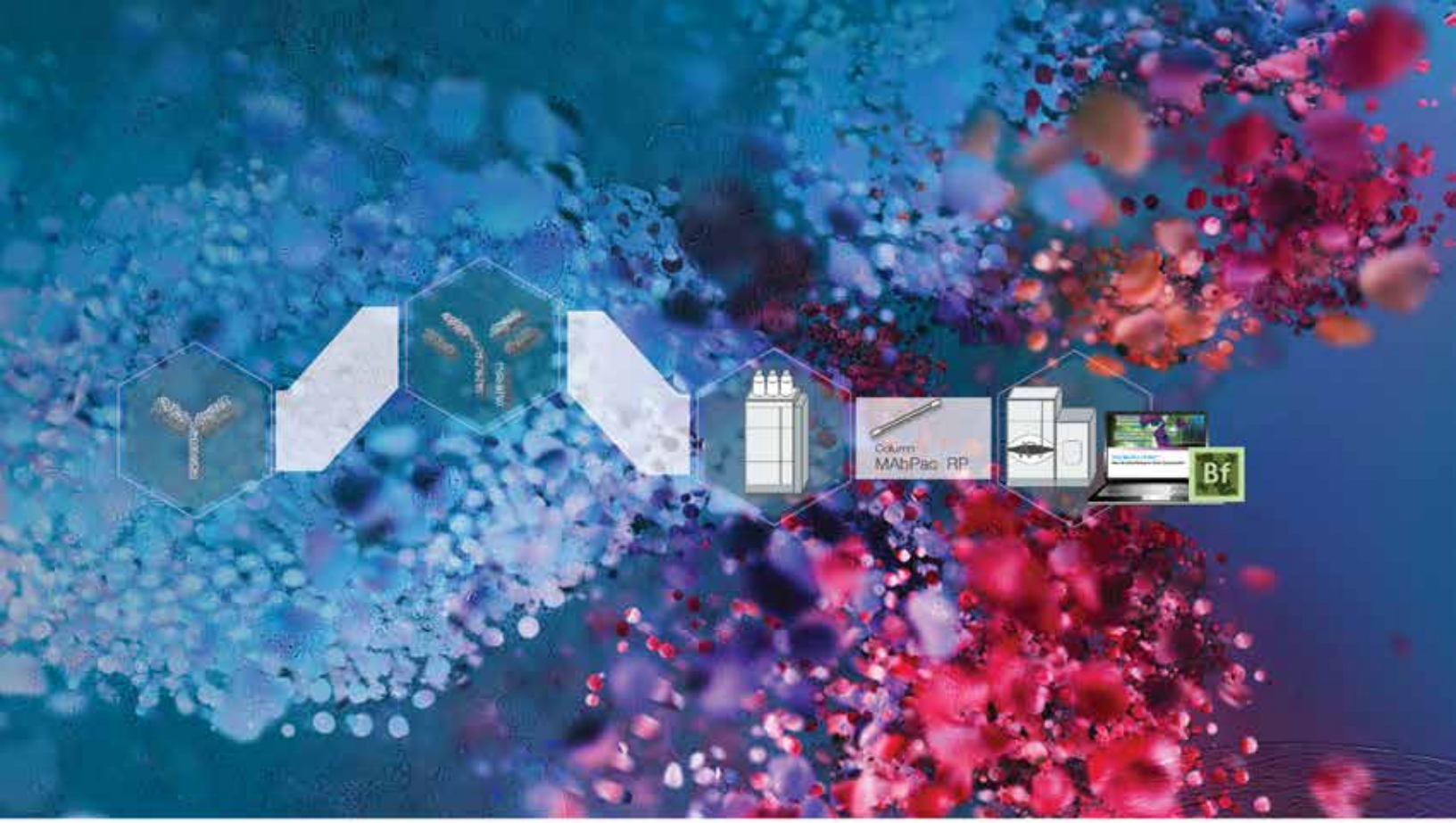
Baseline isotopic resolution of mAb subunits is routinely achievable in Protein mode. The cysteine protease produced by S. pyogenes, known as IdeS, is highly specific for IgG antibody molecules. IdeS digestion followed by reduction will generate 3 subunits which are readily separated by reverse phase HPLC using the Thermo Scientific™ MAbPac™ RP. Deconvolution of subunit MS spectra is easy and powerful with Thermo Scientific™ BioPharma Finder™ software.

The Sliding Window method and the Thermo Scientific™ Xtract™ deconvolution algorithm in BioPharma Finder software enables monoisotopic mass determination of the mAb subunits.

Middle-down analysis of mAb subunits is easily performed as a targeted LC-MS/MS experiment using HCD fragmentation and ultra high resolution scanning. BioPharma Finder accurately deconvolves ultra high resolution MS/MS spectra and middle-down fragment maps can be generated using ProSight Lite software.

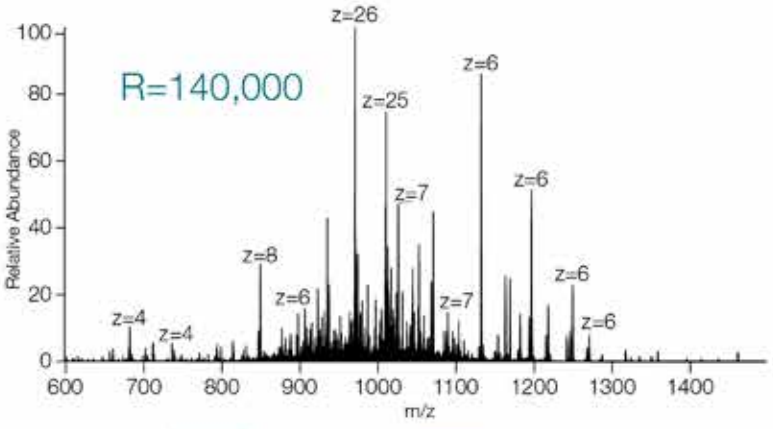
LC-MS analysis of Ides-digested, reduced Trastuzumab



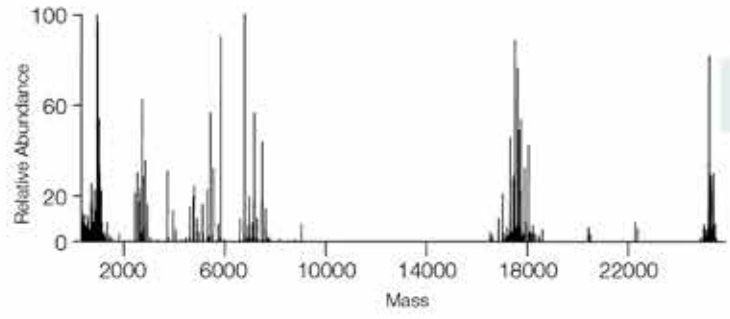


Middle-down analysis of IdeS-digested Trastuzumab subunits

HCD Fragmentation



Bf Xtract™ deconvolution



Light Chain

49% residue cleavages

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
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
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
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
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
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
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
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
L S S P V T K S F N R G E C

Fd'

38% residue cleavages

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
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
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
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
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
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
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
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
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
T H T C P P C P A P E L L G C

Fc

39% residue cleavages

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
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
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
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
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
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
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
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
T Q K S L S L S P G C

Reduced antibody and top-down analysis with Protein mode

Q Exactive HF with BioPharma Finder software

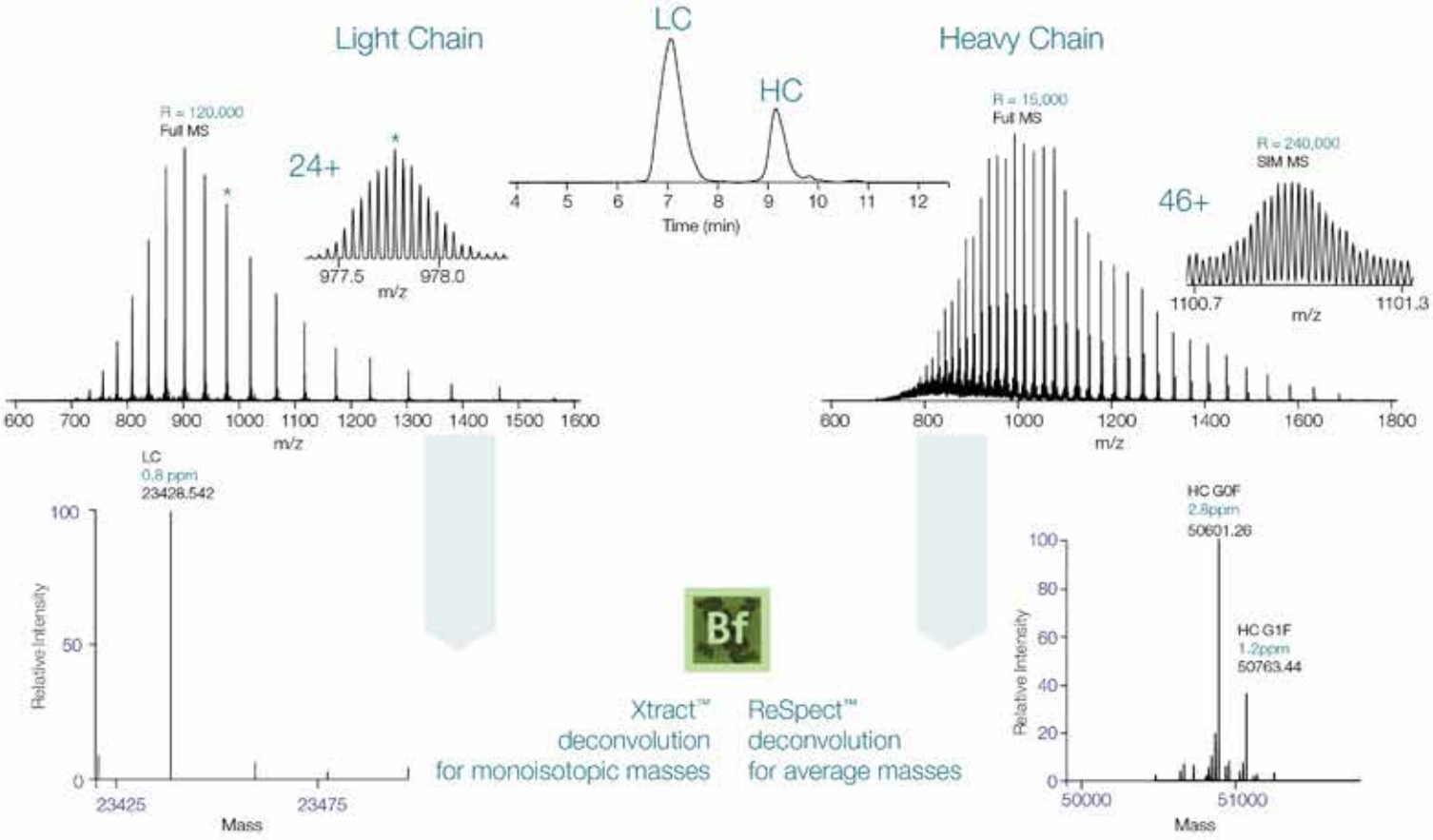
Protein mode on the Q Exactive HF is a powerful combination that has set the standard for the highest quality isotopically resolved data achievable on a benchtop mass spectrometer.

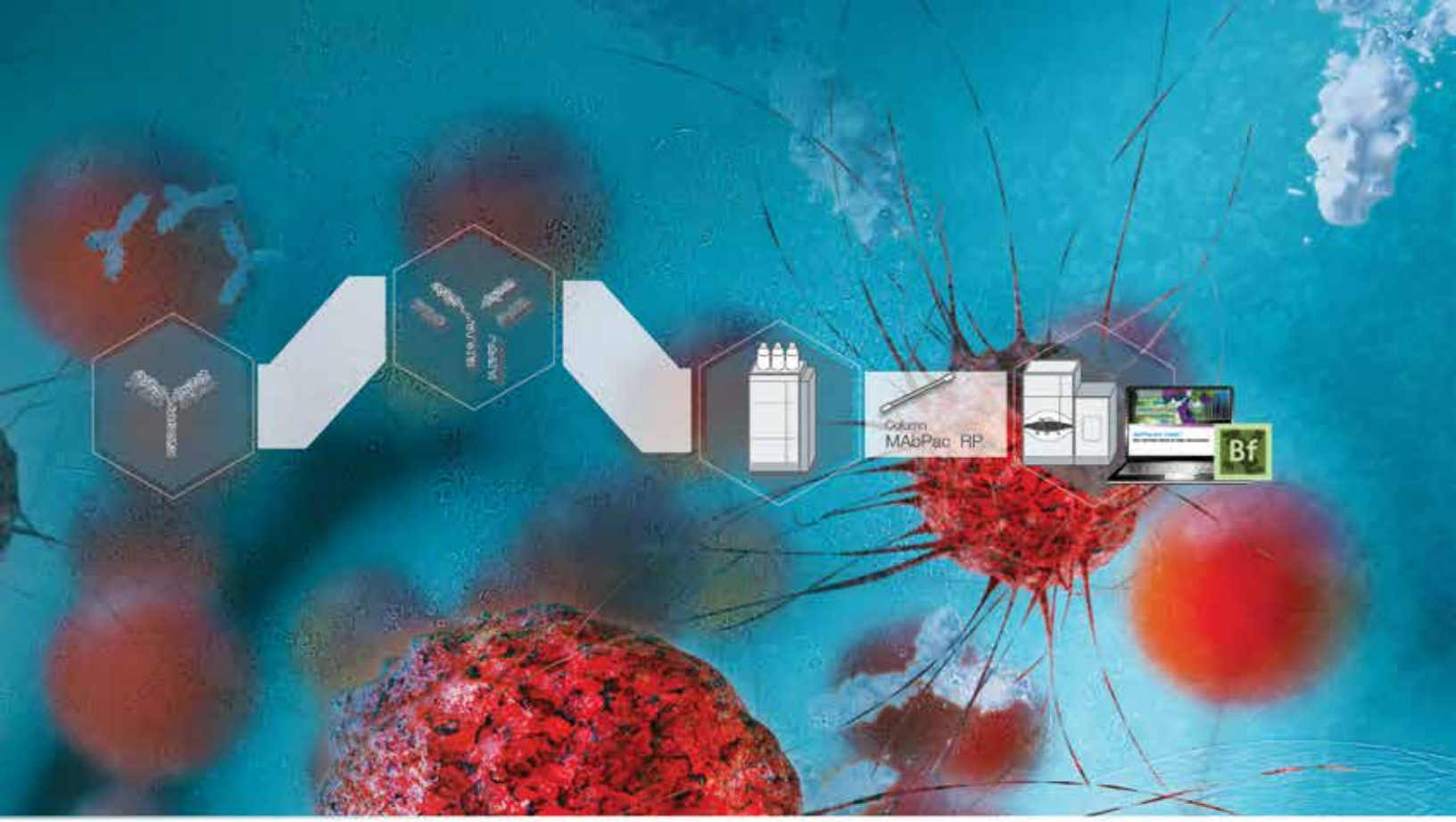
The MAbPac RP provides complete separation of reduced mAb light and heavy chains. Full MS scans using high (120,000) and low (15,000) resolution allow mass determination of the light and heavy chains, respectively.

The ultra-high-field Thermo Scientific™ D20 Orbitrap™ mass analyzer allows baseline isotopic resolution for each species. Using a narrow isolation window in a selected ion monitoring (SIM) scan allows isotopic resolution of the heavy chain on the chromatographic time scale.

Top-down MS/MS spectra are recorded at ultra high resolution of 240,000 to limit mass interferences of highly charged fragment ions. Reliable deconvolution in BioPharma Finder and HCD fragment matching in ProSight Lite provide high quality top-down coverage for structural confirmation.

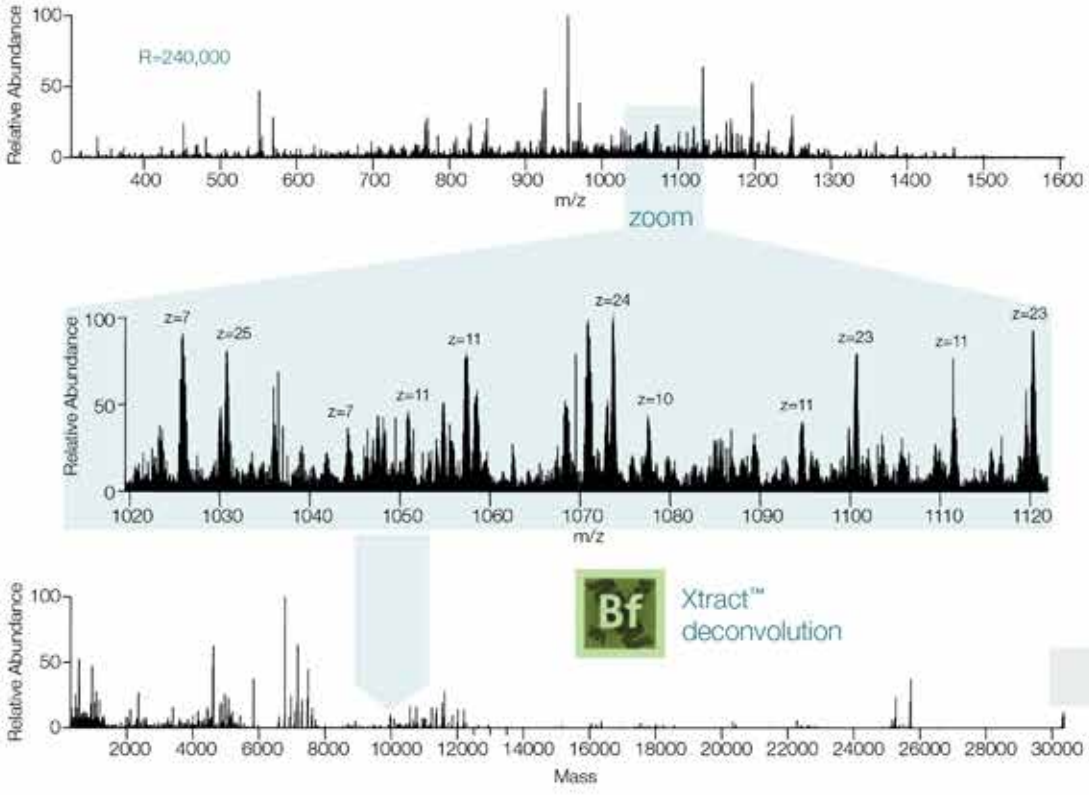
LC-MS analysis of reduced Trastuzumab light and heavy chains





Top-Down analysis of reduced Trastuzumab light and heavy chains

HCD Fragmentation



Light Chain

43% residue cleavages

Heavy Chain

24% residue cleavages

Peptide mapping
with Standard mode

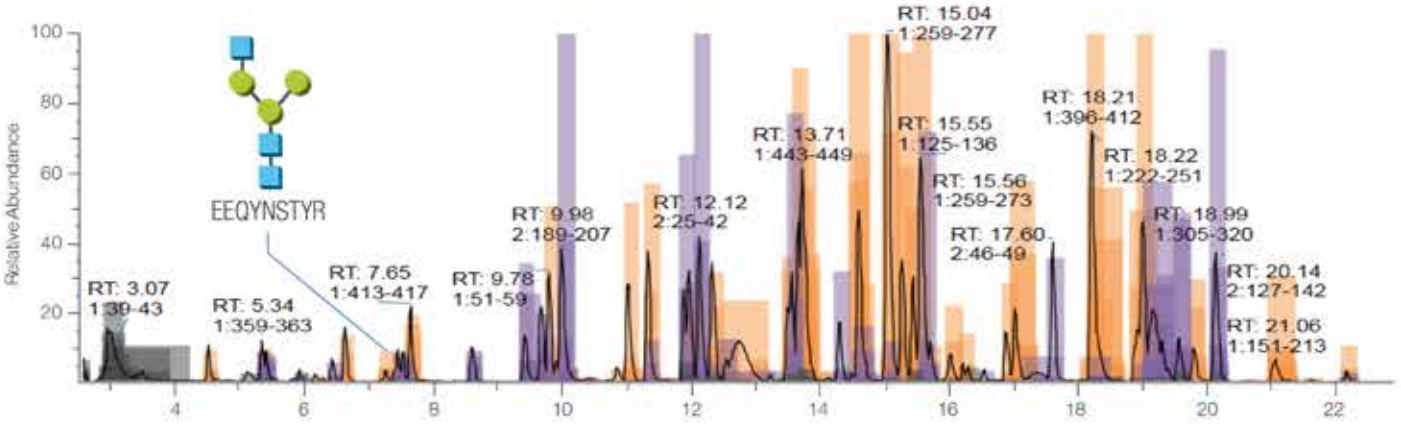
The state-of-the-art peptide mapping data possible with the Q Exactive BioPharma platforms are a culmination of decades of leadership in the development of data dependent acquisition (DDA) combined with the unmatched mass accuracy and resolution achieved by the Orbitrap mass analyzer.

Peptide separation using the Thermo Scientific™ Acclaim™ RSLC 120 C18 column allows 100% sequence coverage in a 20 minute LC-MS/MS experiment. Low abundance peptides resulting from sequence variants and other important modifications are detected at levels as low as 0.001% base peak abundance. High quality HCD fragmentation spectra combined with the predictive algorithms in BioPharma Finder software ensures high mAb sequence coverage with confident peptide IDs even for low abundance glycopeptides.

The highest confidence in peptide identifications is possible using the industry-leading algorithms in BioPharma Finder software combined with the high mass accuracy, high resolution, and the reliably detailed MS/MS spectra of the Orbitrap.

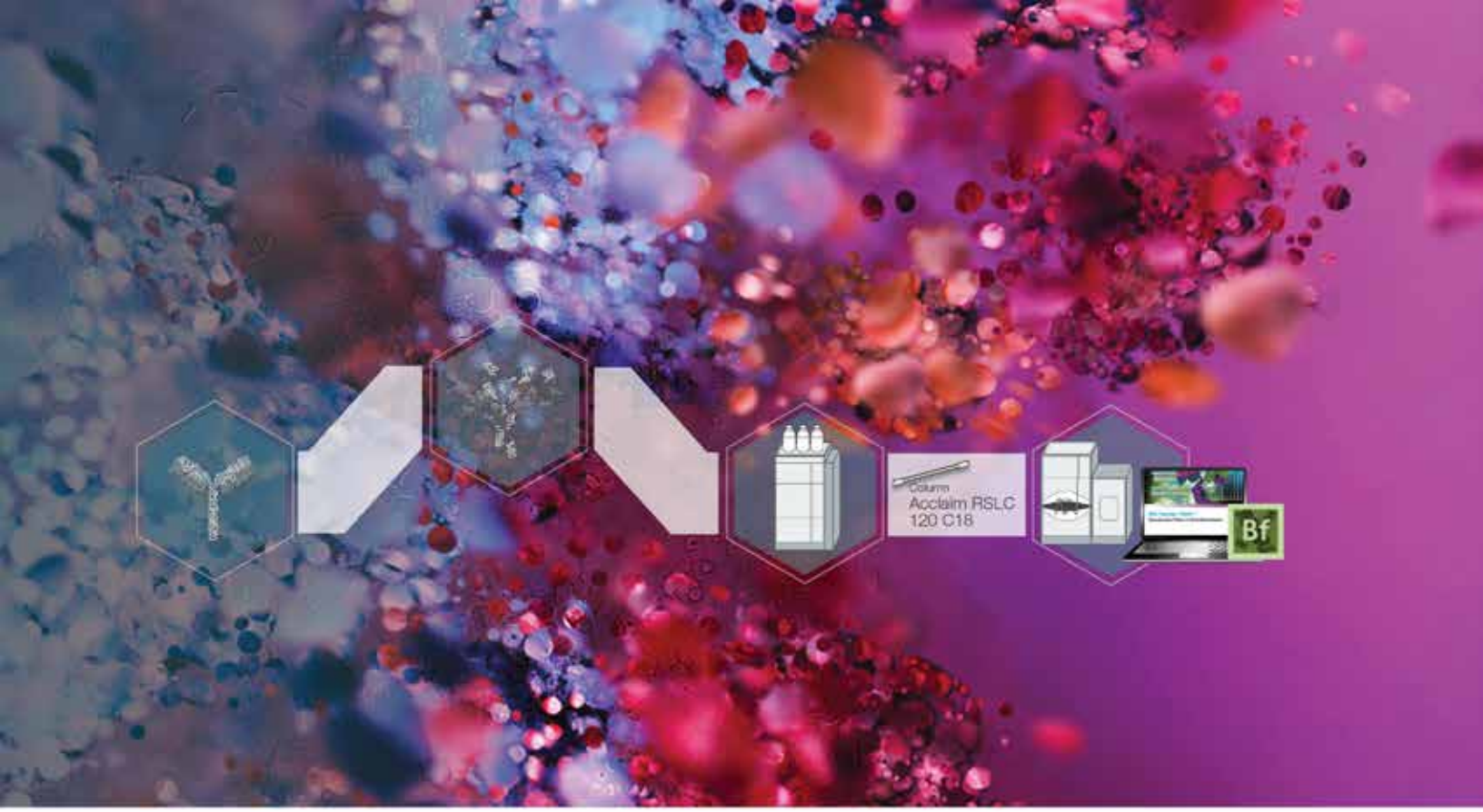
Sensitivity driven by the excellent absolute response and the rapid high resolution scanning of the Orbitrap allows discrimination between components which are near-isobaric or co-eluting.

Proteins	Number of MS Peaks	MS Peak Area	Sequence Coverage	Abundance (mol)
1:Trastuzumab Heavy Chain	1474	60.70%	100.00%	60.79%
2:Trastuzumab Light Chain	726	27.80%	100.00%	39.21%
Unidentified	3819	11.50%		



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

Accurate peptide IDs are based on high fidelity, true MS/MS spectra analyzed with predictive spectral algorithms and visualized in a color coded display by BioPharma Finder software.



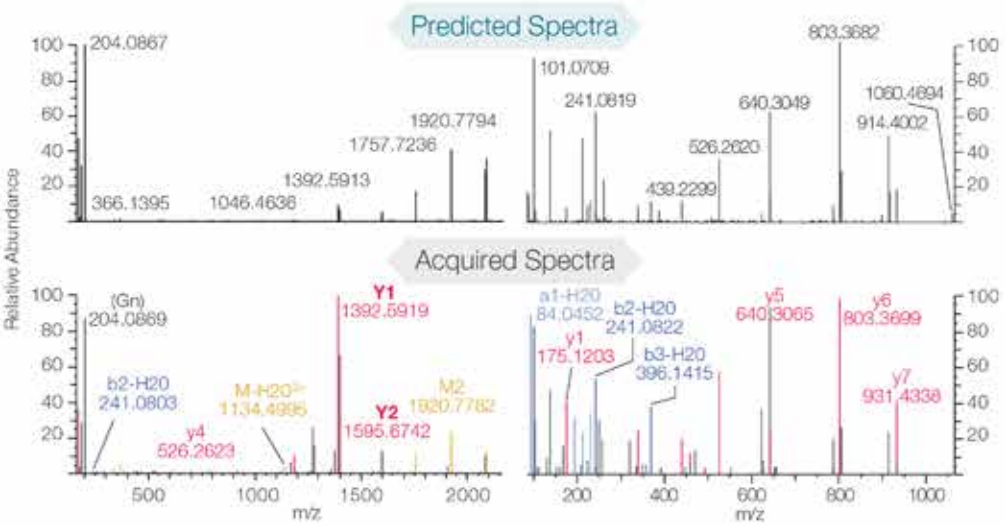
Protein: Herceptin Heavy Chain
Residue #: 300
Category: Glycoform
Sequence: EEQYNSTYR

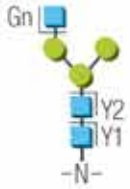
Mod.	Peptides
N300+A1G0	1:E296-R304=1188.50473m(N300+A1G0)
N300+A1G0F	1:E296-R304=1188.50473m(N300+A1G0F)
N300+A1G1F	1:E296-R304=1188.50473m(N300+A1G1F)
N300+A2G0	1:E296-R304=1188.50473m(N300+A2G0)
N300+A2G0F	1:E296-R304=1188.50473m(N300+A2G0F)
N300+A2G1F	1:E296-R304=1188.50473m(N300+A2G1F)

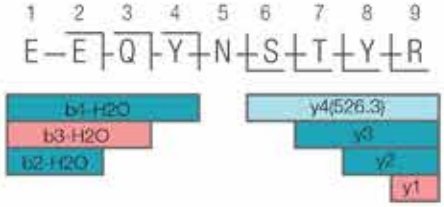
Glycosylation site occupancy can be measured accurately at the peptide level and signature HCD fragment ions for glycans (Gn) and glycopeptides (Y1, Y2) are identified in BioPharma Finder software.

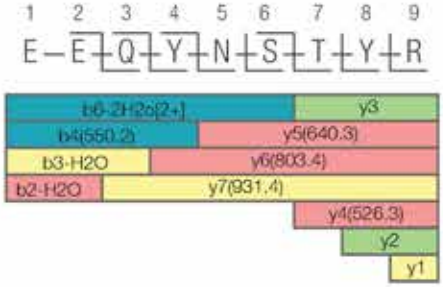
Trastuzumab glycopeptide analysis

HCD Fragmentation









Thermo SCIENTIFIC

Q Exactive BioPharma

Q Exactive Plus with BioPharma Option

Including Enhanced Resolution, Protein mode, and HMR mode

Max Resolving Power

280,000 at *m/z* 200

Max Scan Rate

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

Q Exactive HF with BioPharma Option

Including Protein mode and HMR mode

Max Resolving Power

240,000 at *m/z* 200

Max Scan Rate

Up to 18 Hz at resolution setting of 15,000 resolution at *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)

Ordering information

Product	Cat. No.
BioPharma	
Q-Exactive Plus System	0726030
BioPharma Option for Q Exactive Plus	0726055
Q Exactive HF System	0726041
BioPharma Option for Q Exactive HF	0726060
Vanquish Flex Binary UHPLC	5400.0225
Vanquish Binary Pump H UHPLC	5400.0105
MAbPac SEC-1 (4.0 x 150 mm)	075592
MAbPac RP (2.1 x 100 mm)	088647
Acclaim RSLC 120 C18 (2.1 x 250 mm)	074812
BioPharma Finder software	OPTON-30592

Find out more at **thermofisher.com /qebp**

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