Scale up

your biopharma experiments

Orbitrap Ascend
BioPharma Tribrid mass spectrometer

ThermoFisher SCIENTIFIC

thermo scientific

Scale up biopharma insight

Set new standards in biotherapeutic characterization

Built to meet the demands of biopharmaceutical experiments, the Thermo Scientific™ Orbitrap™ Ascend BioPharma Tribrid™ mass spectrometer adds innovations to scale up characterization of biotherapeutic products and native proteins. Achieve greater coverage using a revolutionary hardware design that features two ion routing multipoles. Characterize intact biopharmaceuticals with improved Native MS workflows. Perform sensitive and deep peptide-based analyses and enhance elucidation of even the most difficult-to-characterize molecules, such as oligonucleotides, using a combination of optional orthogonal fragmentation techniques. With these capabilities and more, access unprecedented experimental throughput, versatility and usability to meet tomorrow's challenges while achieving more insight today.

Native proteomics	Characterize ADCs and other biotherapeutics
Peptide mapping	Perform PTM and disulfide mapping
Top-down characterization	Completely characterize protein complexes and proteoforms
Oligonucleotides	Fully characterize oligonucleotides

"The Orbitrap Ascend BioPharma mass spectrometer combines two fundamental features that make it perfect for characterizing intact biopharmaceuticals. Multiple ion activation types are complemented by PTCR to simplify complex mass spectra and facilitate their interpretation. And the Native MS option increases the Orbitrap *m/z* range up to *m/z* 16,000. Together they enable researchers to perform sophisticated ion manipulations and analyze the most diverse molecules."

Luca Fornelli, PhD
Professor of Biology
University of Oklahoma

Orbitrap Ascend
BioPharma Tribrid mass spectrometer

Innovative technology addresses your biopharma needs

The Orbitrap Ascend BioPharma Tribrid mass spectrometer features innovations to scale up your biotherapeutic characterization with throughput, versatility and ease-of-use.

Real-time database/ library search
Database search/spectral-library-directed MS^n acquisition

Back ion routing multipole
Enables parallel analysis, performs HCD at MS^{3+} stage

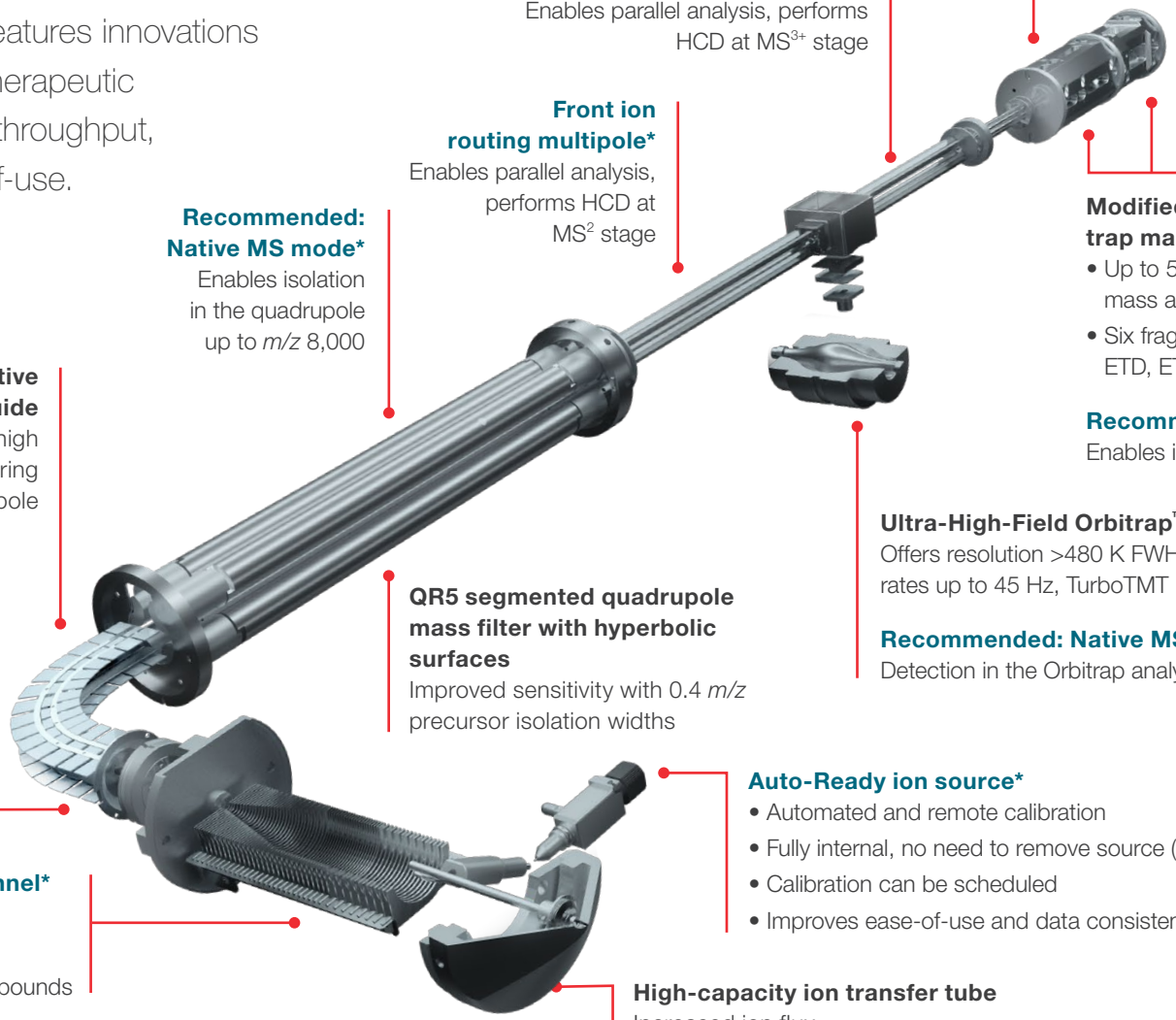
Front ion routing multipole*
Enables parallel analysis, performs HCD at MS^2 stage

Recommended: Native MS mode*
Enables isolation in the quadrupole up to *m/z* 8,000

Advanced active ion beam guide
Prevents neutrals and high velocity clusters from entering mass resolving quadrupole

Recommended: Thermo Scientific™ EASY-IC/ETD and PTCR ion source
Based on Townsend discharge, reliable and easy to use

Electrodynamic ion funnel*
- Efficient ion transfer
- Broad tuning curves
- Optimized for labile compounds



Recommended: UVPD
Unique fragmentation mode for analyte structure elucidation

Modified dual-pressure linear ion trap mass analyzer
- Up to 50 Hz MS^n and sensitive mass analysis
- Six fragmentation types: CID, HCD, ETD, EThcD, ETciD and UVPD

Recommended: Native MS mode*
Enables isolation up to *m/z* 8,000

Ultra-High-Field Orbitrap™ mass analyzer
Offers resolution >480 K FWHM and acquisition rates up to 45 Hz, TurboTMT

Recommended: Native MS mode*
Detection in the Orbitrap analyzer to *m/z* 16,000

QR5 segmented quadrupole mass filter with hyperbolic surfaces
Improved sensitivity with 0.4 *m/z* precursor isolation widths

Auto-Ready ion source*
- Automated and remote calibration
- Fully internal, no need to remove source (nESI, FAIMS)
- Calibration can be scheduled
- Improves ease-of-use and data consistency

High-capacity ion transfer tube
Increased ion flux

OPTIONS IC I ETD I PTCR I Native MS* I UVPD I FAIMS Pro Duo interface

*New on this platform

Peptides

Confidently map PTMs and characterize HCPs at analytical flow rates

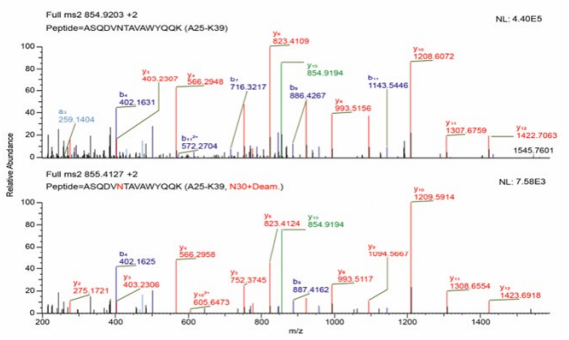
Scale up to quickly characterize low abundance modifications

High mass and structural complexity make biotherapeutics susceptible to post-translational modifications (PTM), some of which can be detrimental to their safety, stability and efficacy. A versatile approach to PTM characterization, peptide mapping often requires the analysis of numerous samples, which challenges the analytical method to generate high-confidence data over a wide dynamic range in short liquid chromatography (LC) runtimes. Identifying deamidation is particularly difficult due to the small mass shift and the overlapping isotopic envelopes of unmodified and deamidated peptides.

With enhanced speed and sensitivity, the Orbitrap Ascend BioPharma Tribrid mass spectrometer can achieve 100% sequence coverage with identification of low abundance modifications—including deamidation—at analytical flow rates, increasing both sample throughput and certainty in results.

Orbitrap Ascend BioPharma Tribrid mass spectrometer analysis of Trastuzumab peptides at a flow rate of 300 µL/min. A sequence coverage of 100% for the light and heavy chains of Trastuzumab at analytical flow rates was achieved. Low abundance modifications (<1% of the unmodified peptide's relative intensity) and the deamidation at 0.9% relative abundance were easily detected and characterized.

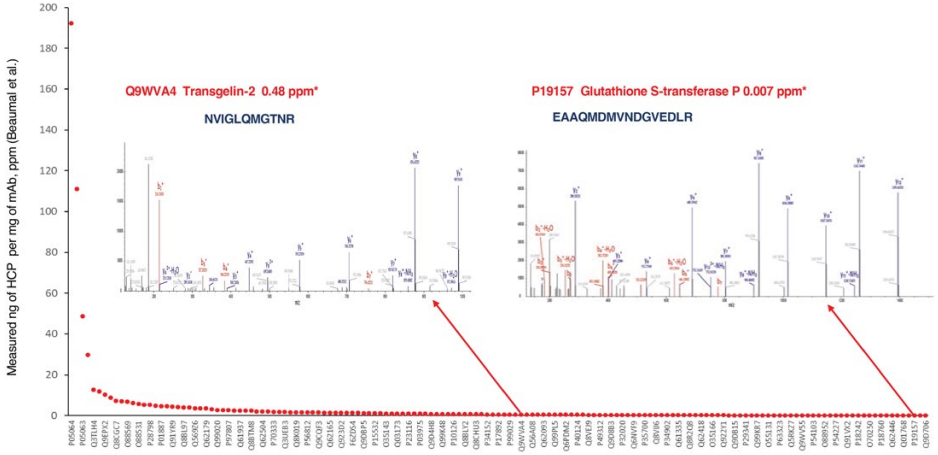
Proteins	Number of MS peaks	MS peak area	Sequence coverage
1:Heavy chain	2,420	46.3%	100.0%
2:Light chain	990	19.6%	100.0%



Scale up to confidently identify low-abundance HCPs

Host cell proteins (HCPs)—the biologic product impurities released during cell growth and processing—can detrimentally affect product safety and efficacy. LC with high-resolution accurate-mass (HRAM) MS analysis allows identification of multiple HCPs in a run and overcomes a typically huge intrasample dynamic range to detect very low abundance residual HCPs.

The sensitivity and dynamic range of the Orbitrap Ascend BioPharma Tribrid mass spectrometer provides rapid, high-confidence detection and identification of HCPs at less than 1 ppm concentration using analytical flow rates. Fragments generated via higher-energy collisional dissociation (HCD) enhance HCP identification.



The Orbitrap Ascend BioPharma Tribrid mass spectrometer identified over 260 HCP peptides down to 7 ppb concentrations at 300 µL/min flow rates.[1]

1. Beaumal C, Beck A, Hernandez-Alba O et al. (2023) Advanced mass spectrometry workflows for accurate quantification of trace-level host cell proteins in drug products: Benefits of FAIMS separation and gas-phase fractionation DIA. Proteomics and Systems Biology 23 (16). ***doi.org/10.1002/pmic.202300172****.*

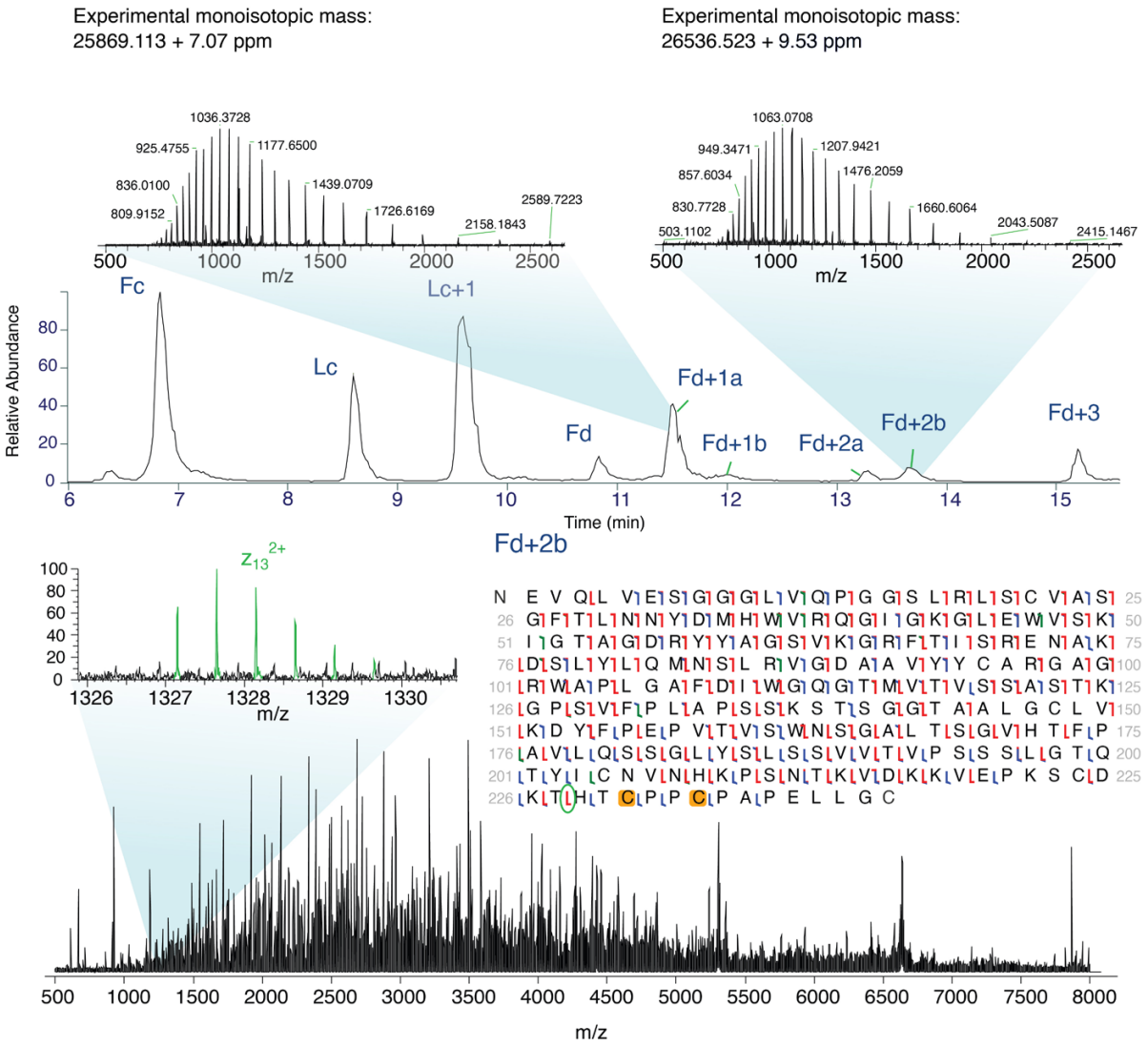
Fully characterize ADC with middle-down analysis and versatile orthogonal fragmentation

ADC

Scale up ADC characterization

Antibody drug conjugation (ADC) plays a crucial role in improving the selectivity, efficacy and safety of therapeutic interventions. Determination of the drug-to-antibody ratio and the location of the drug conjugate is essential for the characterization of ADC-based therapeutics. Use of a combination of orthogonal MS fragmentation techniques can be necessary to characterize these therapeutics with certainty.

The versatility of the Orbitrap Ascend BioPharma Tribrid mass spectrometer enables scientists to perform a simple, middle-down ADC digestion while still getting accurate intact results for the number and types of drugs bound to the target protein. The nearly intact antibody chains can be fragmented using the instrument's various orthogonal fragmentation techniques—including HCD, recommended electron transfer dissociation (ETD) and ultraviolet photodissociation (UVPD)—to obtain nearly complete sequence coverage of the protein and the exact location of the conjugation. Analytical throughput is increased by combining intact antibody and fragmentation analyses into a single experimental workflow.



Middle-down analysis of a digested ADC mimic and sequencing of the protein Fd+2b using EThcD and UVPD orthogonal fragmentation methods.

Data courtesy of Professor Luca Fornelli, University of Oklahoma.

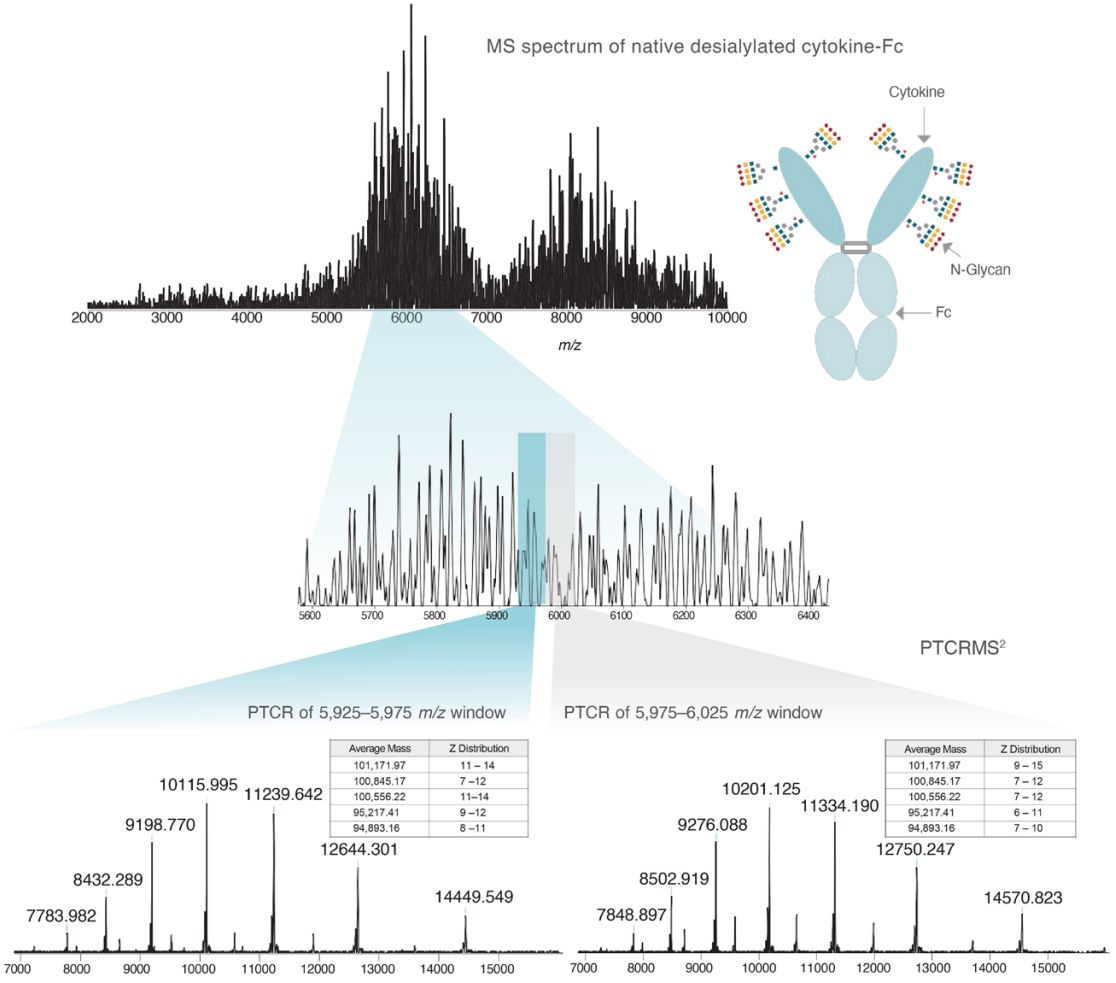
Native-DIA-PTCR

Comprehensively analyze therapeutic proteins with Native MS and PTCR

Scale up native protein characterization

Protein therapeutics often generate undecipherable spectra due to their high levels of intrinsic heterogeneity. By using Orbitrap Ascend BioPharma Tribrid mass spectrometer's recommended Native MS and proton transfer charge reduction (PTCR) options, interpretable spectra can be generated from heterogeneous protein samples. The Native MS option extends the quadrupole isolation range to *m/z* 2,000–8,000, which can be used in combination with narrow data-independent acquisition (DIA) windows to simplify the ion population in each scan. The Native MS option also extends Orbitrap analyzer detection to *m/z* 16,000. These capabilities enable unambiguous identification of previously indiscernible or low-abundance proteoforms. PTCR generates perfluoroperhydrophenanthrene (PFPP) ions for subsequent gas-phase, ion-ion reactions that produce lower charge state distributions to enhance protein characterization.

The flexibility of the Orbitrap Ascend BioPharma Tribrid mass spectrometer enables scientists to carry out unique experiments that combine the recommended Native MS option with PTCR, as well as other dissociation techniques, making it a powerful instrument for comprehensive characterization of therapeutic proteins in their native state.



Orbitrap Ascend BioPharma Tribrid mass spectrometer analysis of native desialylated cytokine-Fc analyzed with Native MS option. Two DIA windows were fragmented for comprehensive characterization.

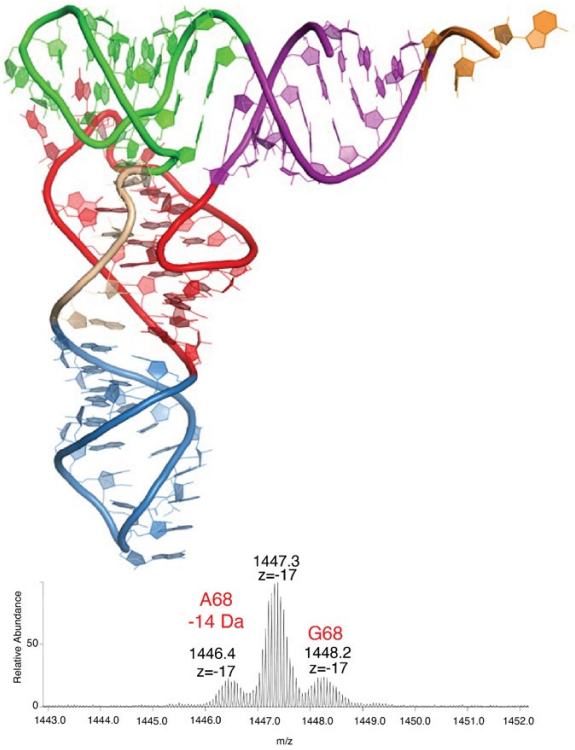
Data courtesy of Wendy Sandoval.

Detect and identify unexpected low-abundance tRNA modifications

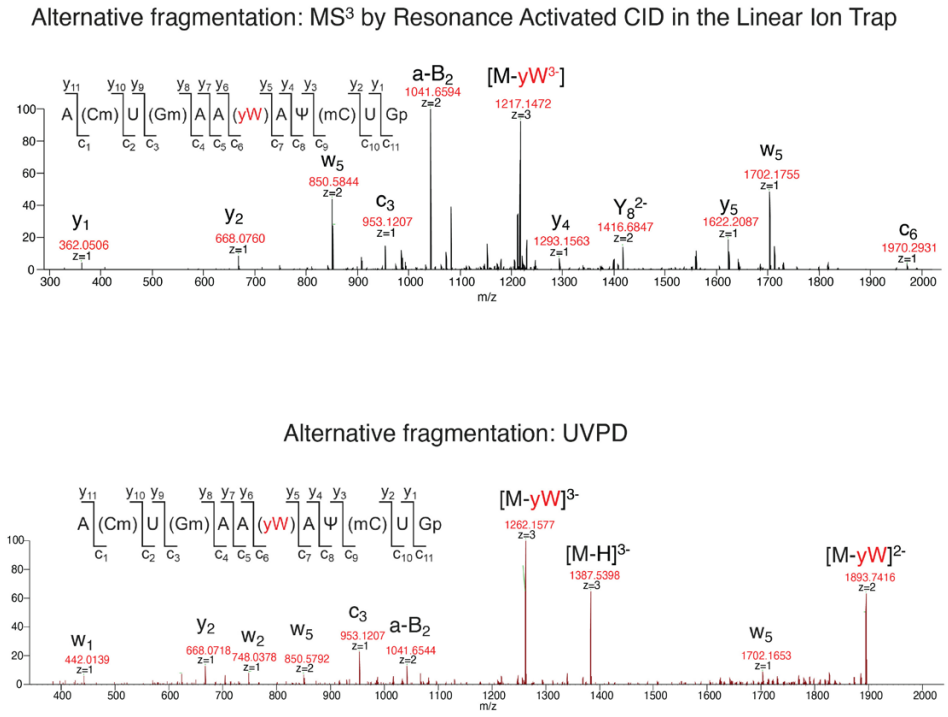
Oligos

Scale up oligonucleotide characterization

Therapeutic oligonucleotides produced by chemical synthesis can carry various types of impurities, some of which are present at very low levels. One method to achieve good separation and identification of modified intact tRNA is ion-pairing reversed-phase chromatography (IP-RPLC) coupled to HRAM MS analysis using the Orbitrap Ascend BioPharma Tribrid mass spectrometer. Intact detection of low-abundance oligonucleotide modifications can be paired with well-established digestion techniques for in-depth characterization using ion trap MS^3-based collision-induced dissociation (CID) and the added versatility provided by recommended UVPD.



IP-RP coupled to Orbitrap Ascend BioPharma Tribrid mass spectrometer analysis enables separation of the modified tRNA species and identification of the known isodecoder and an under-modified variant.



After digestion, the tRNA modification at yW-14 was located and well characterized using MS^3-based CID and recommended UVPD.

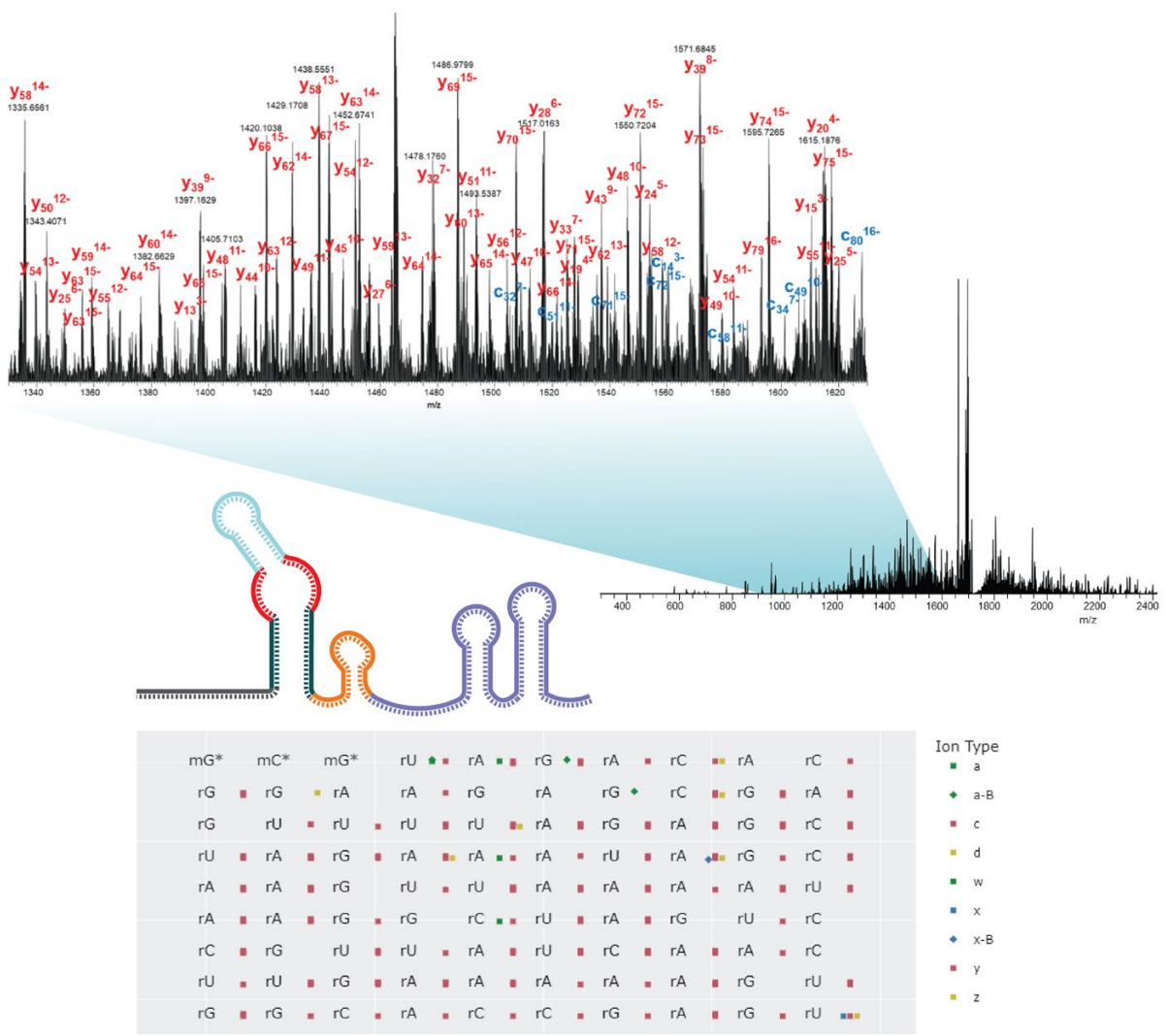
Top-down

Achieve nearly 100% base-pair coverage for 100-mer sgRNA using low-q CID

Scale up characterization of sgRNA

Single guide RNA (sgRNA) plays a crucial role in the CRISPR-Cas9 gene editing system by guiding the Cas9 enzyme to specific genomic locations, enabling precise and targeted modifications of the DNA sequence. Characterization of sgRNA is essential to ensure precision from initial experiments to future therapies. Using the low frequency (q) CID capability of the Orbitrap Ascend BioPharma Tribrid mass spectrometer combined with intact and top-down methods, *de novo*-level fragmentation patterns of intact sgRNA can be obtained, providing nearly 100% sequence coverage for a 100-base-pair structure.

The low-q CID technique adds experimental flexibility by reducing precursor ion activation; the resulting product ion spectra are predominantly composed of sequence ions and base losses from the precursor. The reduction in product ion spectral complexity in favor of sequence-informative species simplifies and increases the confidence of *m/z* peak annotation and therefore confirmation or elucidation of the primary RNA sequence.



Orbitrap Ascend BioPharma Tribrid mass spectrometer analysis of a 100-mer sgRNA using low-q CID provided almost 100% sequence coverage.

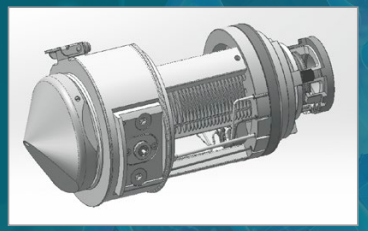
Experience more high-quality results with less hassle using automated, remote and schedulable system checks and calibrations

Scale up convenience and ease-of-use

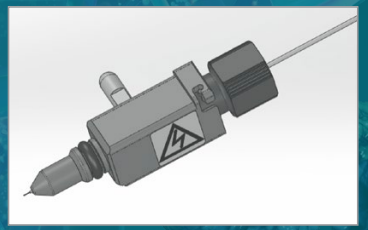
The Auto-Ready ion source is a fully integrated, standard, easy-to-use feature that increases laboratory productivity with automated, remote and schedulable system checks and internal calibrations. Because there is no need to remove the source (HESI, nESI or high-field asymmetric waveform ion mobility spectrometry [FAIMS]), there are no experimental setup interruptions required to perform internal calibrations. The user can automate the calibration to start at a scheduled time—for example, every week—when there are no experiments planned to run on the instrument. The calibration can run completely remotely, regardless of the nature of the last experiment. Because the calibration can be scheduled to occur regularly and automatically without interrupting vital work, users can expect to maintain mass spectrometer performance, improve data consistency and achieve more accurate and precise quantitation.

Auto-Ready ion source

Separate ion transfer tube

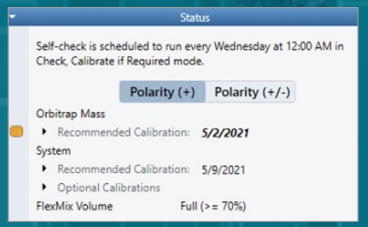


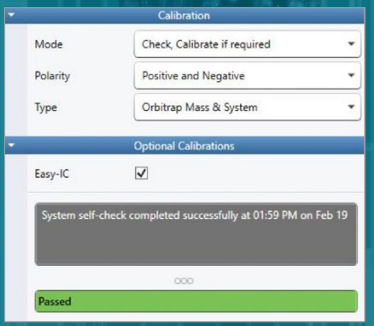
Dedicated emitter



Robust delivery system

Automated weekly calibrations





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