Precision Mass Spectrometry: Advances and Applications

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Vice President, Omics Marketing
Life Sciences Business Unit

October 2017

The world leader in serving science

50 Years of “Firsts” in MS - Thermo Fisher Scientific’s Legacy of Innovation

1967

First commercial triple quad MS

First commercial GC ion trap MS

First benchtop MALDI-ToF MS

First LC ion trap MS

First high-res triple quadrupole MS

First radial ejection linear ion trap

First commercial ion trap FT-ICR MS

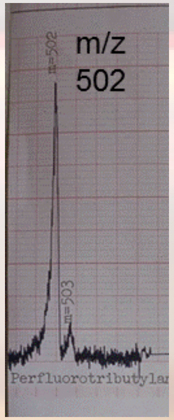
First commercial Orbitrap MS

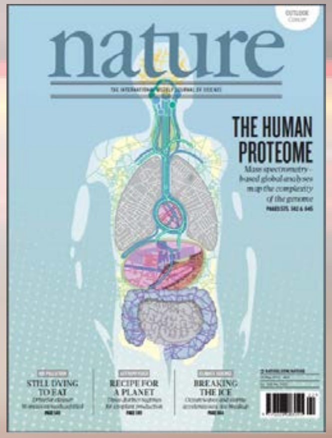
First commercial Linear Trap ETD

First benchtop quadrupole-Orbitrap MS

First tribrid MS

Continuing the Legacy of MS Innovation in 2017…





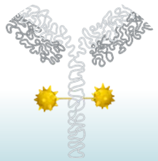
1980 1982 1991 1996 2001 2004 2005 2006 2011 2013

Major Product Introductions at ASMS 2017

new

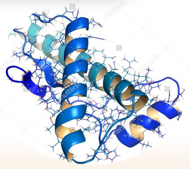
Food and Beverage

Environmental



Biopharmaceuticals

Translational Research/ Precision Medicine



Life Science Research “omics”

Thermo Scientific™ Instruments

Orbitrap HRAM MS

Orbitrap Fusion™ Lumos™ MS Options

- UVPD
- 1M
- APD

Q Exactive™ HF-X LC-MS

Exactive™ GC Orbitrap™ GC-MS

Triple Quadrupole LC-MS

TSQ Altis™ MS

TSQ Quantis™ MS

Thermo Scientific™ Software

- Small molecule analysis
- Protein analysis
- Cloud Applications

Compound Discoverer™ Software

Orbitrap™ GC-MS HRAM Metabolomics Library

Proteome Discoverer™ Software

Sample Prep, Kits and Consumables

- MS Crosslinking reagents
- Capillary flow HPLC columns
- Metabolomics Standards Kits

Biocrates AbsoluteIDQ p400HR Kit

DSSO and DSBU MS-Cleavable Linkers

Easy-Spray PepMap 150µm Column

Thermo Scientific TSQ Triple Quadrupole Mass Spectrometers

Sensitivity and robustness without compromise

TSQ Altis Triple Quad LC-MS

Sensitivity and robustness without compromise

- **Ultimate sensitivity and speed**
- **Increased selectivity with H-SRM**
- **Unmatched analytical flexibility and reproducibility**
- **For the most demanding applications**

AIM+ TECHNOLOGY

Active Ion Management+

Optimize ion management precision, reliability, speed, and reproducibility.

TSQ Quantis Triple Quad LC-MS

True quantitative workhorse

- **Ultimate robustness**
- **Best in class sensitivity**
- **Increased selectivity with H-SRM**
- **Ease of use and maintenance**

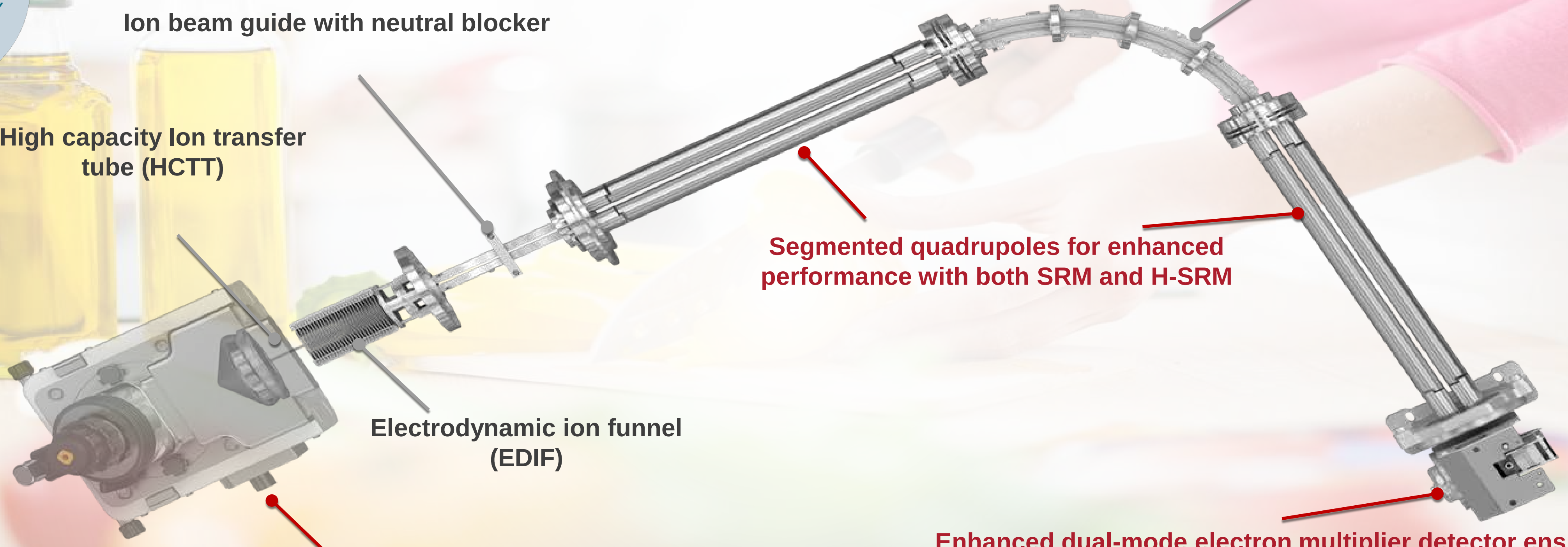
"………"With the **new innovations** in the **Thermo Scientific TSQ Altis MS**, my lab can **develop quantitative methods** for biotherapeutic proteins and target receptors with **extreme sensitivity**, **selectivity**, **accuracy** and **precision**. This is very exciting for our research since this capacity is very unique"

Jun Qu,
University of Buffalo

NEW Thermo Scientific TSQ Altis Triple Quadrupole Mass Spectrometers

Innovations that deliver new quantitative performance

AIM+ TECHNOLOGY



Active collision cell facilitating more SRMs/sec

Ion beam guide with neutral blocker

High capacity Ion transfer tube (HCTT)

Segmented quadrupoles for enhanced performance with both SRM and H-SRM

Electrodynamic ion funnel (EDIF)

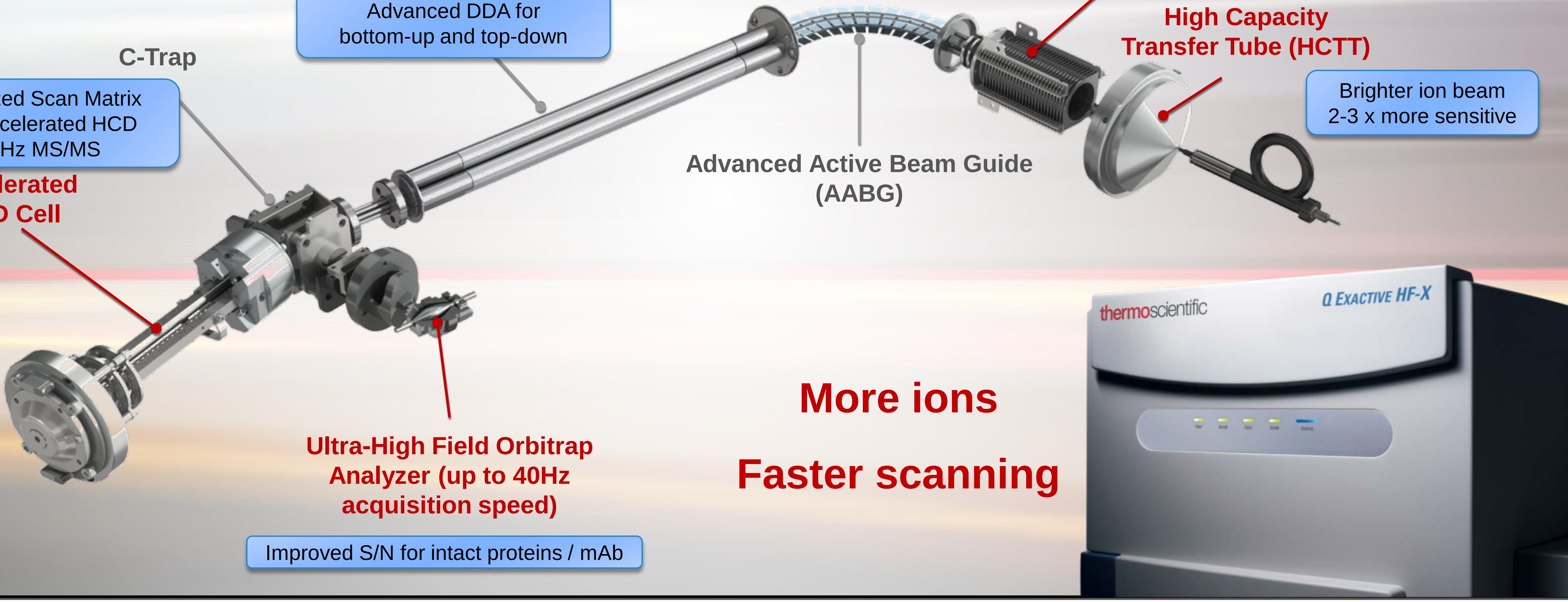
Enhanced dual-mode electron multiplier detector ensuring excellent linearity and dynamic range

OptaMax^{TM} NG ion source with integrated APCI

Thermo Scientific Q Exactive HF-X MS – New Innovations

new

Fastest, most powerful Q Exactive MS



HyperQuad Mass Filter with Advanced Quadrupole Technology (AQT)

Advanced DDA for bottom-up and top-down

Electrodynamic Ion Funnel

High Capacity Transfer Tube (HCTT)

Brighter ion beam
2-3 x more sensitive

C-Trap

Optimized Scan Matrix with accelerated HCD
40 Hz MS/MS

Accelerated HCD Cell

Advanced Active Beam Guide (AABG)

Ultra-High Field Orbitrap Analyzer (up to 40Hz acquisition speed)

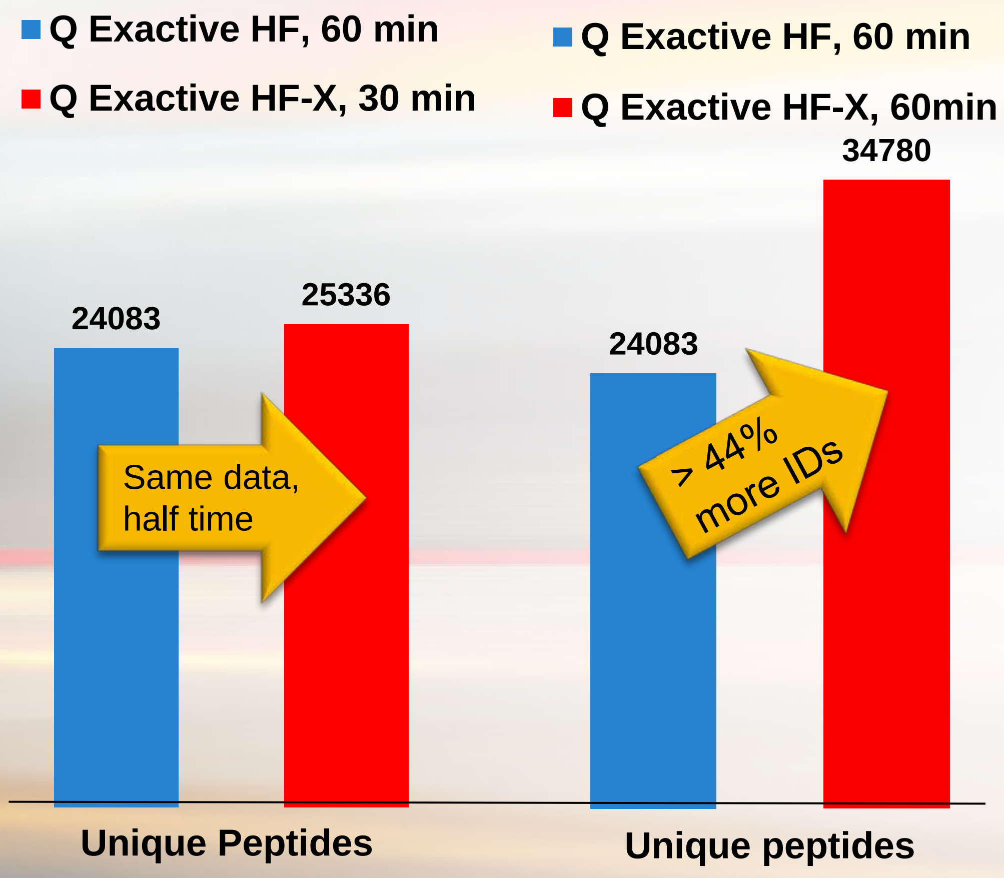
Improved S/N for intact proteins / mAb

More ions

Faster scanning

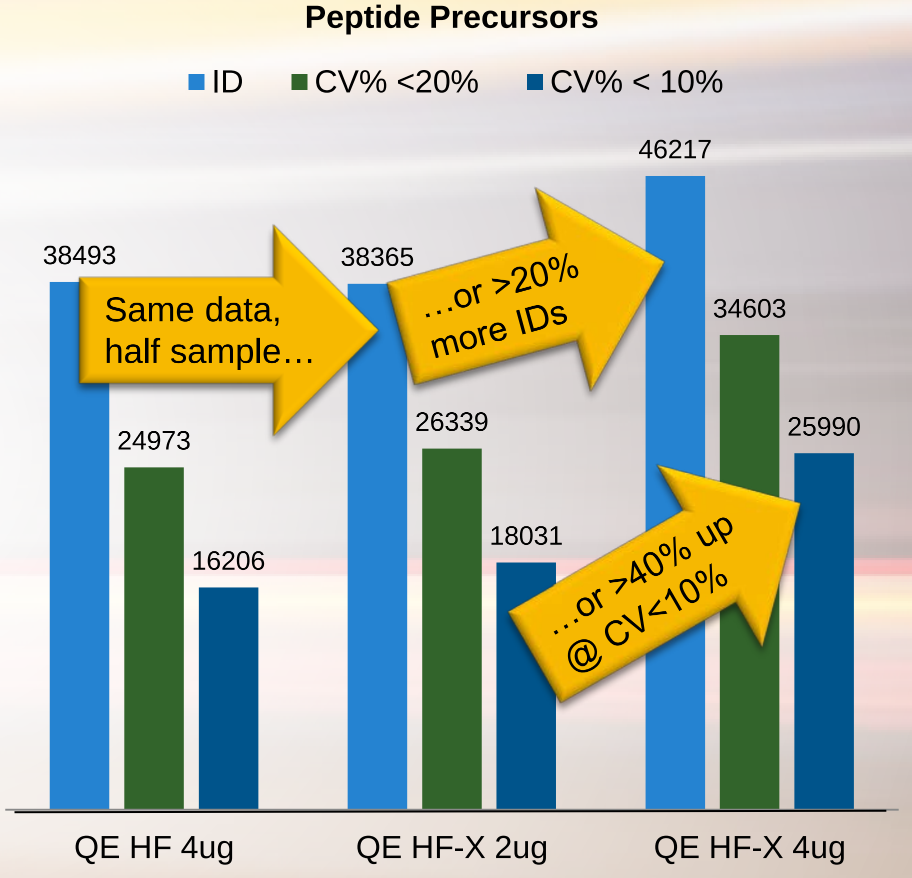
Thermo Scientific Q Exactive HF-X MS — Faster, More Sensitive

Productivity increase in DDA



Sample: 1ug Pierce Hela digest

Peptide quantitation in DIA



Sample: Pierce Hela digest, 60 min CapLC

ASMS 2017: TP 389, T.N. Arrey et al.

ASMS 2017: ThP237, Y. Xuan et al.

Thermo Scientific Orbitrap Fusion Lumos Tribrid Mass Spectrometer

new

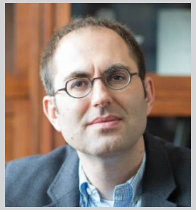
Delivering even more power - UVPD, 1M and APD Options

"The **1 million resolution Orbitrap** is a unique tool to allow fluxomics analysis of lipids with sensitivity **comparable to radioactivity tracing**."

Matthew Mitsche,
UT Southwestern Medical Center

"…….**UVPD-MS/MS and MS^n** promises an unprecedented level of structural information for the improved ID and characterization of lipids, **not possible to attain on any other commercial MS system**"

Gavin Reid,
University of Melbourne



"The **new APD algorithm** provides new peptide precursors to go after and amounts to a 25–35% identification bonus. This will be a key technology in achieving **deeper coverage and higher throughput for proteome analyses**."

Josh Coon,
University of Wisconsin-Madison

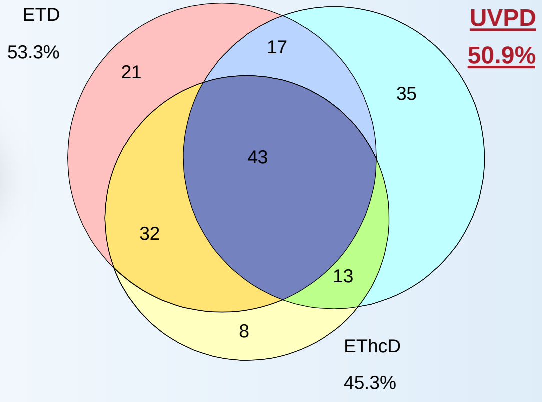
Thermo Scientific Orbitrap Fusion Lumos Tribrid Mass Spectrometer

new

Delivering even more power – UVPD Option

Integrated UVPD

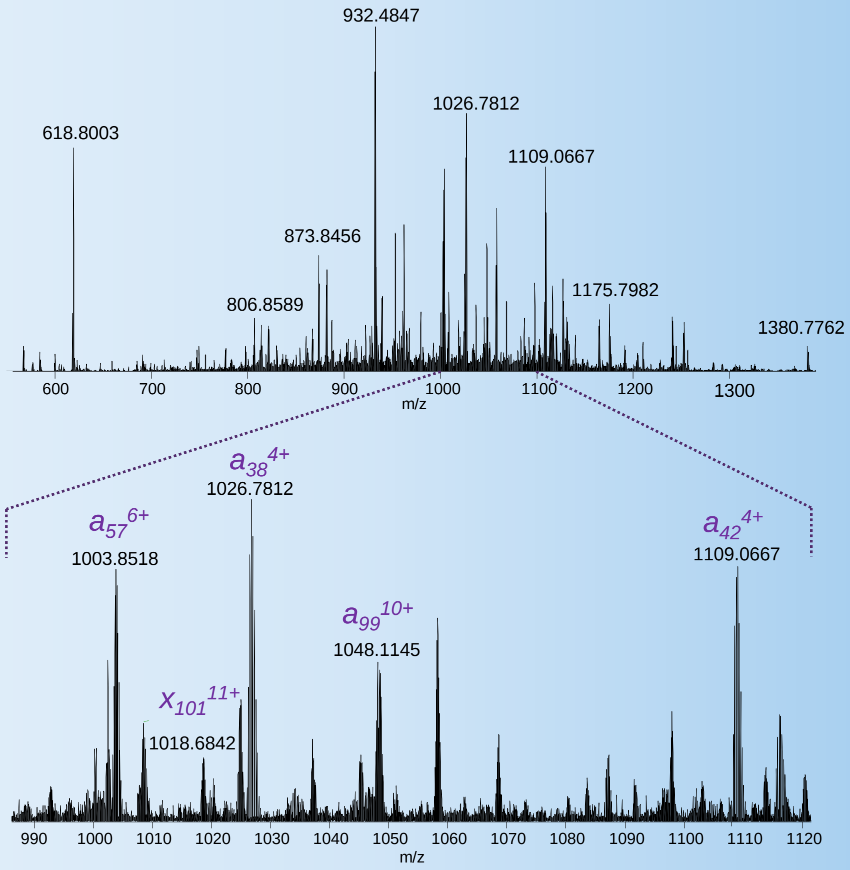
Improves sequence coverage with new fragmentation technique. Over 20% unique bond cleavages. Lipid double bond localization.



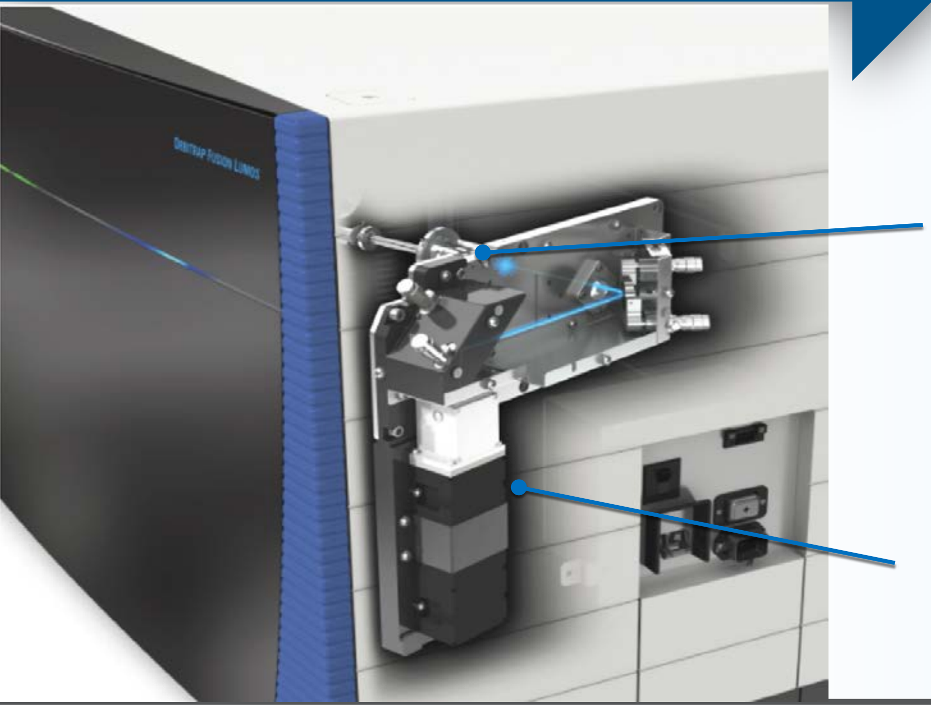
21% unique coverage

79.7% total coverage

Glycosylation site locations



Light Chain UVPD MS^2 scan was measured at 120,000 FWHM resolution after a UVPD activation time of 8 ms



DUAL PRESSURE LINEAR ION TRAP
MS^n and sensitive mass analysis of fragments resulting from CID, HCD, ETD, EThcD and UVPD

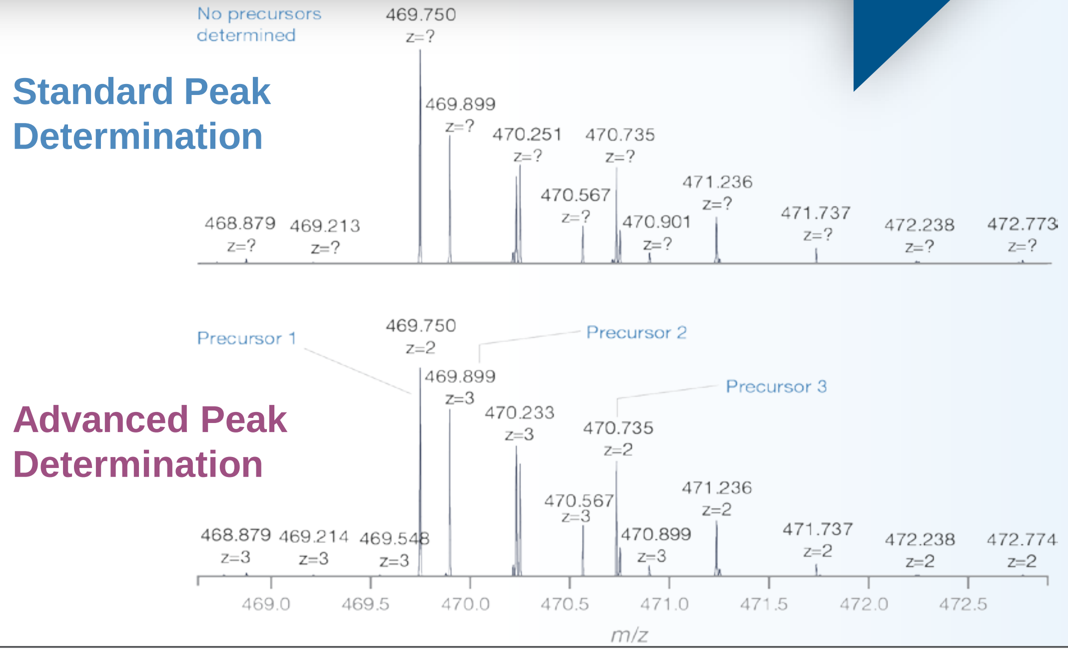
UVPD SOURCE
Embedded inside the mass spectrometer for optimal performance and reliability

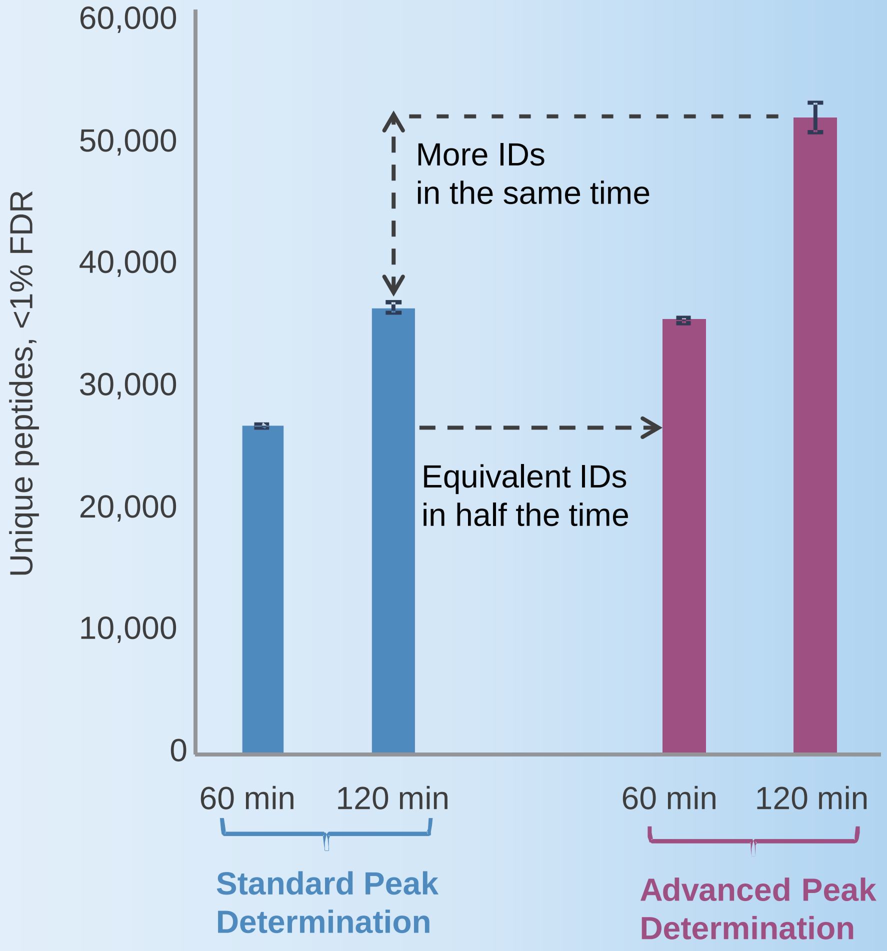
new

Delivering even more Identication power - APD

Advanced Peak Determination

Assigns more monoisotopic peaks and charge states in complex spectra which significantly increases the population of precursors available for data-dependent analysis to deliver more unique peptide identifications.





new

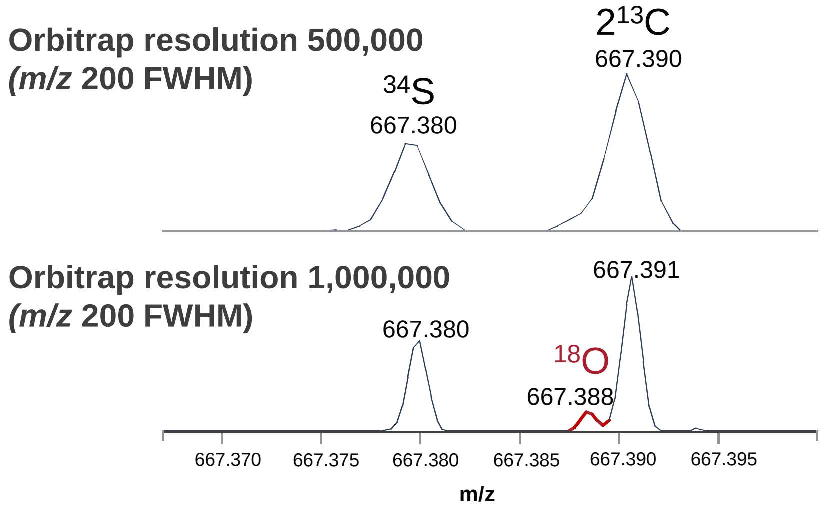
Delivering even more power – 1M Option

1M Resolution

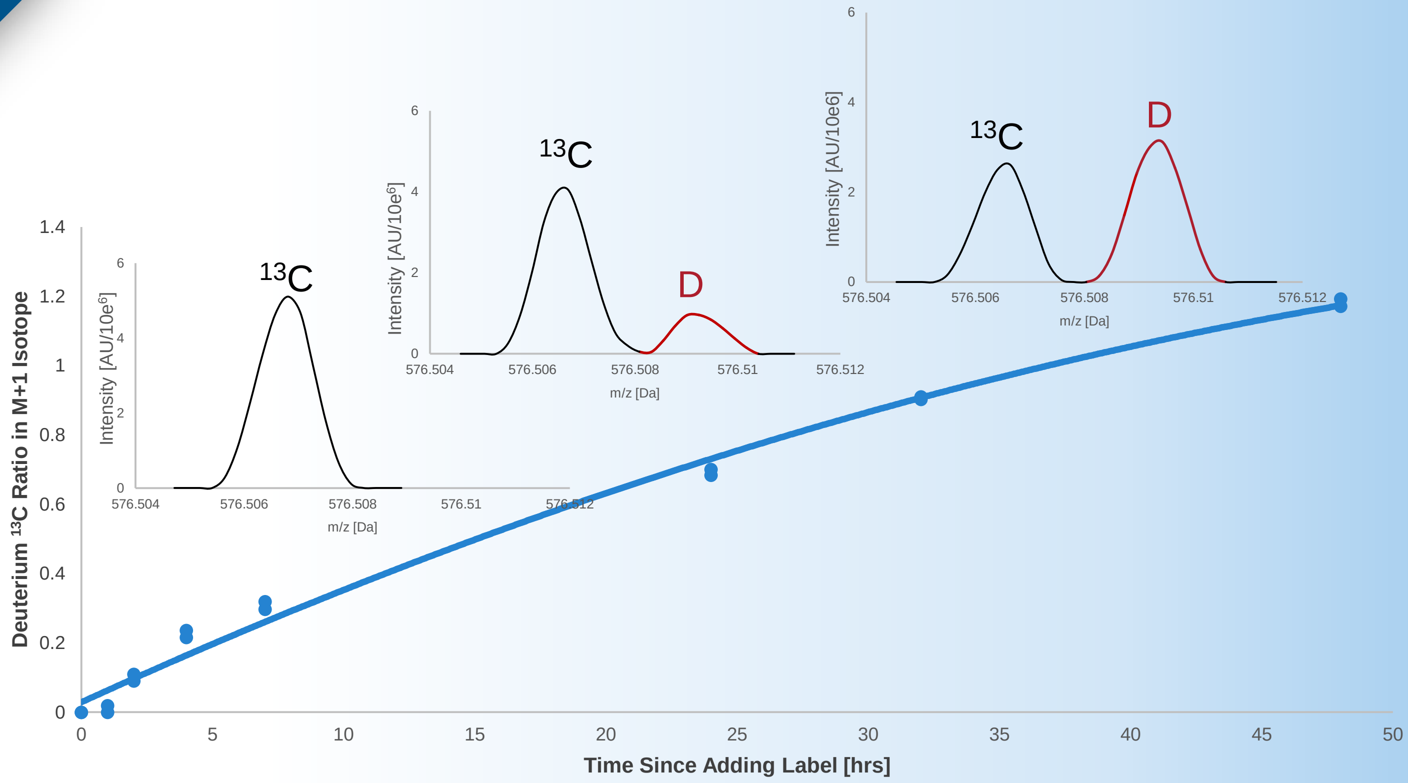
Resolves fine isotope structure of small molecules and enables lipid metabolic flux analysis

Fine Isotope Structure of Irganox:

^{18}O and 2^{13}C isotopes resolved at 1M



Labeled deuterium and natural ^{13}C isotopes resolved on LC timescale



Major Software Releases

new

Unleashing the potential of MS data

Proteomics and Protein Analysis

Thermo Scientific™ Proteome Discoverer™ 2.2 Software

Thermo Scientific™ ProteinCenter™ 4.0 Software **

Thermo Scientific™ ProSightPC™ 4.0 Software

Instrument Control and Qualitative Analysis

Thermo Scientific™ FreeStyle™ 1.3 Software

Thermo Scientific™ Xcalibur™ 4.1 Software

Quantitative Analysis

Thermo Scientific™ TraceFinder™ 5.0 Software **

- Maximizing investment in instrumentation
- Rigorous data processing and results

Small Molecule Analysis

Thermo Scientific™ Compound Discoverer™ 2.1 Software

Biopharmaceutical Analysis

Thermo Scientific™ Biopharma Finder™ 3.0 Software **

** preview

Cloud Applications

new

MS

MS Instrument Connect
Remote monitoring dashboard for multiple instruments

SP

Sample Profiler
Profiling of authentic products, add/compare new samples and determine trends and differences

PO

Pathway Overrepresentation
Find significant pathways from gene or protein lists

OC

Omics Comparator
See overlap and uniqueness comparing protein or gene lists

Thermo Fisher Cloud

Addressing evolving needs to connect scientists, instruments, software and data

Supporting Segment Evolution

Enabling breakthrough research to fight cancer

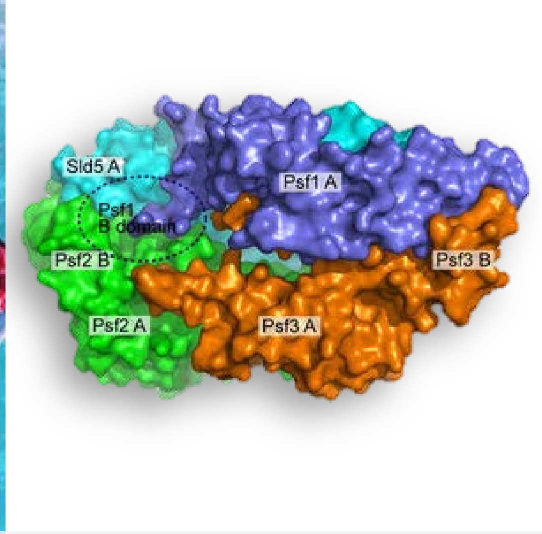
Translational Research and Personalized Medicine

Standardized metabolic phenotyping

Metabolomics

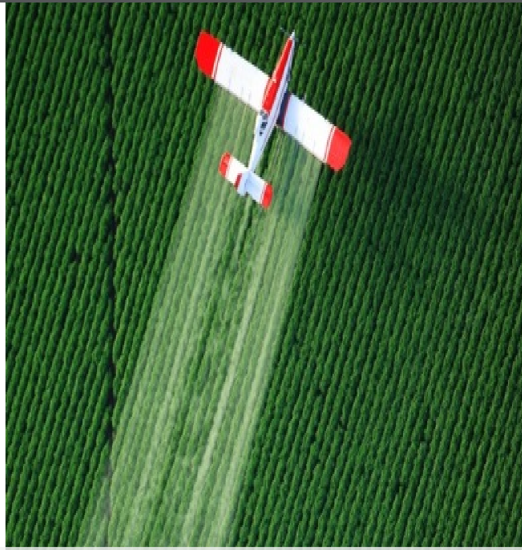
Confident characterization of biologics

Biopharmaceutical Analysis and QA/QC



Next-generation structure analysis

Integrative Structural Biology



Tomorrow’s quantitation today

Food/Env
Tox
Clinical apps
pK

Proteome Profiling for Precision Medicine

From Sample to Read-Out In a Day

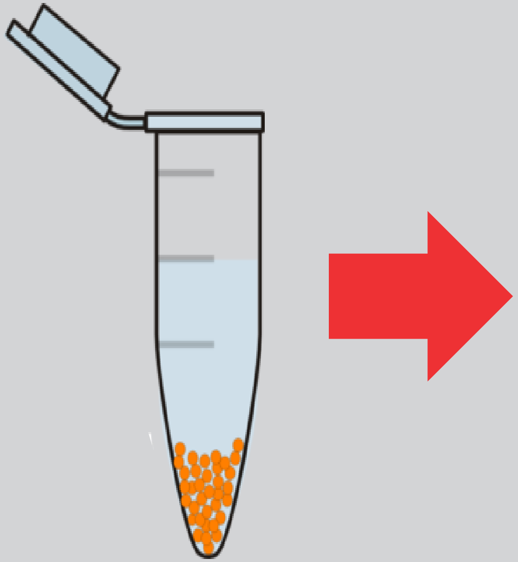
FFPE archive

SkBr3
(EGFR+)

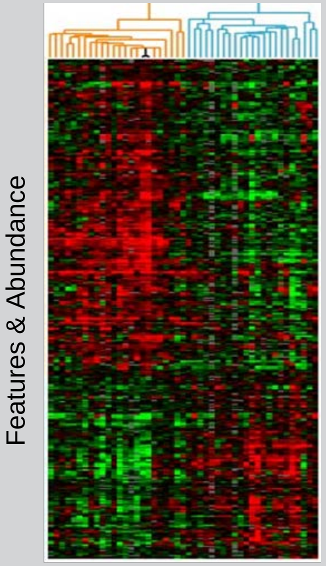
U251
(EGFR++)

A431
(EGFR+++)

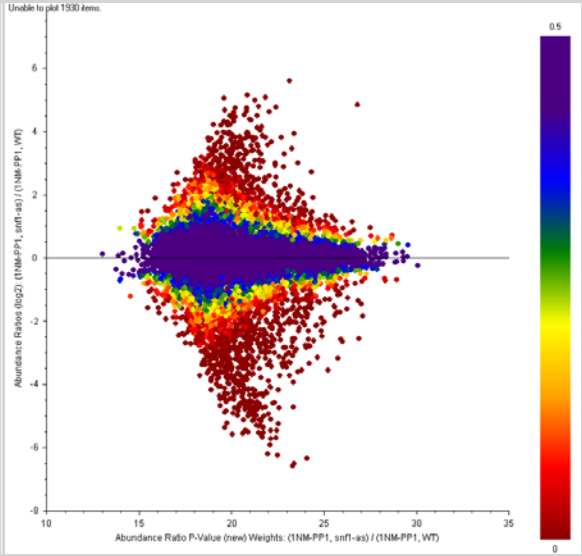
Fresh/frozen tissue



Proteome Signature
Classified histotypes



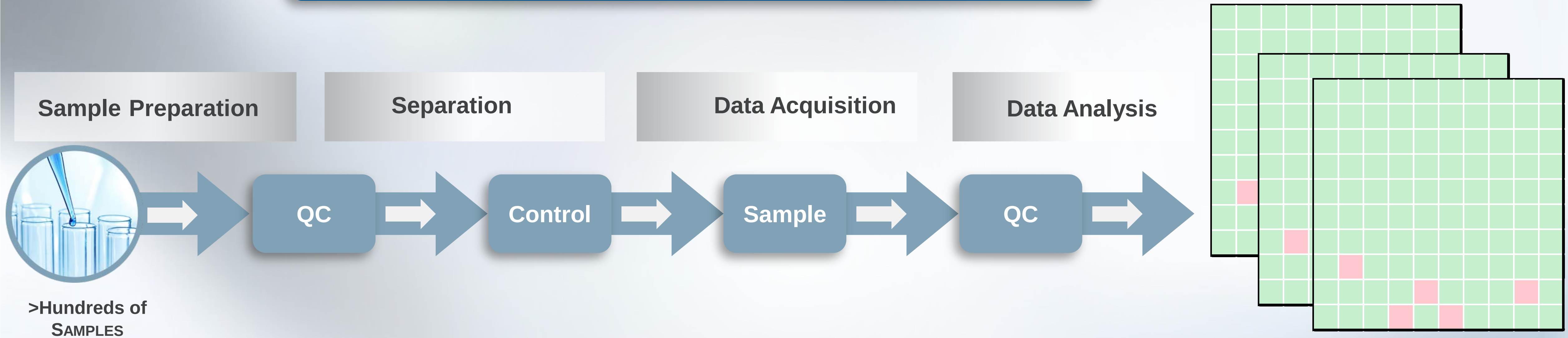
Biomarkers
Differentially regulated pathways
Dysregulated functional modules



QUANTITATIVE **REPRODUCIBLE** **STANDARDIZED** **SCALABLE**

The Goal: Standardized, High Throughput Proteomics

Large Scale Proteomics



>Hundreds of SAMPLES

Standardization Method

- Defined QC, optimized separation, efficient data acquisition
- Defined analysis criteria (# replicates, <1%FDR, < 20%CV)

Reproducible Results

- Highest specificity & confidence
- Optimal sensitivity & depth of coverage

NOTE:

- **COMPLETE quantitation** Peptides reproducibly quantified across all repeats
- **SPARSE quantitation** Peptides identified, but not quantified across all repeats

Delivering comprehensive proteome profiles with reproducibility and precision

Precursor Label Free Quantitation Workflows Built On HRAM Orbitrap MS

Data Dependent Acquisition
DDA+


Proteome Discoverer 2.2
Mass Informatics Platform for Protein Scientists
thermo scientific

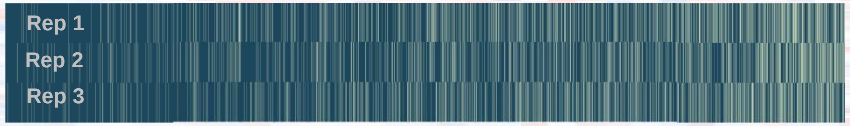
Thermo Scientific™ Proteome Discoverer™ Software

Thermo Scientific™
Q Exactive ™ HF-X MS

Data independent Acquisition
HR-DIA


spectronaut
STAR-QUALITY ANALYSIS SOFTWARE
A new era in proteomics.
BIOGNOSYS

Biognosys Spectronaut Software



Missing data/sparse quantitation

Complete quantitation

Standardization

- Complete quantification
- Designed for large sample cohorts
- Flexible workflows serving the community

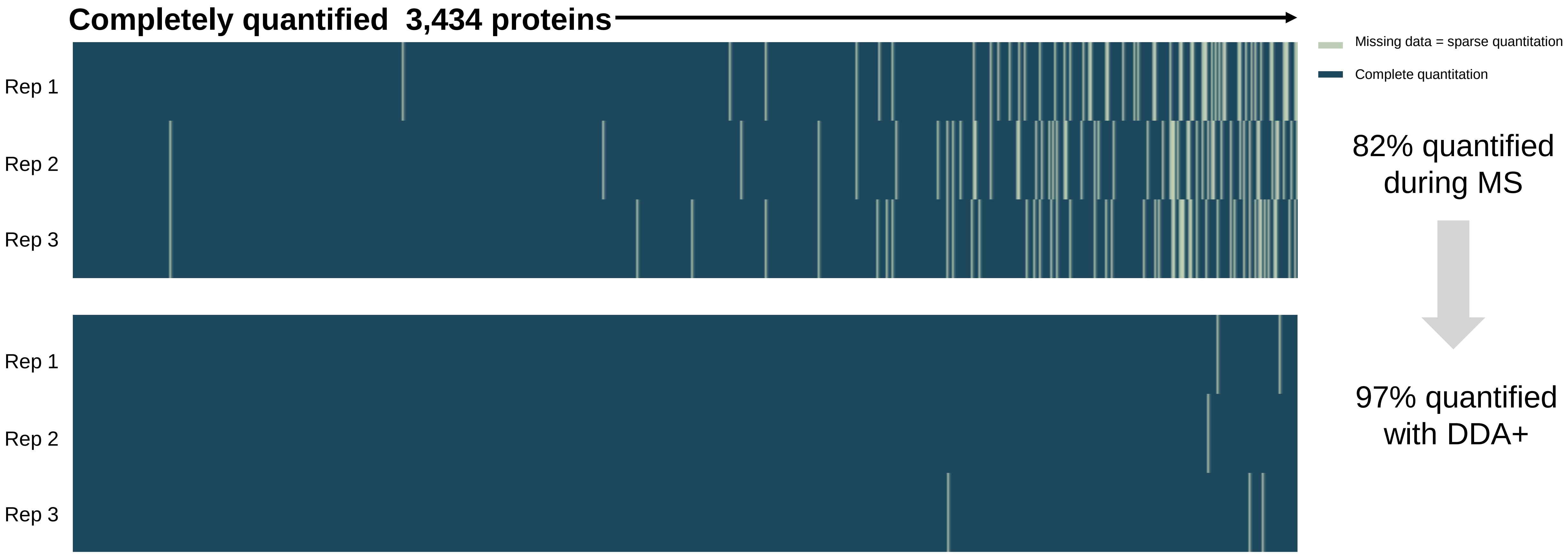
Supports both

- Biospecimen profiling/digital archiving
- Mechanism of action studies high quantitative
- precision

Driving quantitative reproducibility, precision and standardized workflow solutions

Robust, Reproducible Proteome Profiling

capLC DDA, 4ug HeLa, 60min, 120K/7.5K, 19ms, Top 40 -> PD 2.2 Label free quant



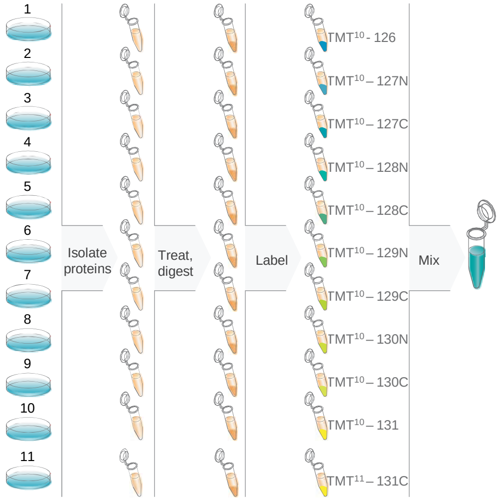
82% quantified during MS

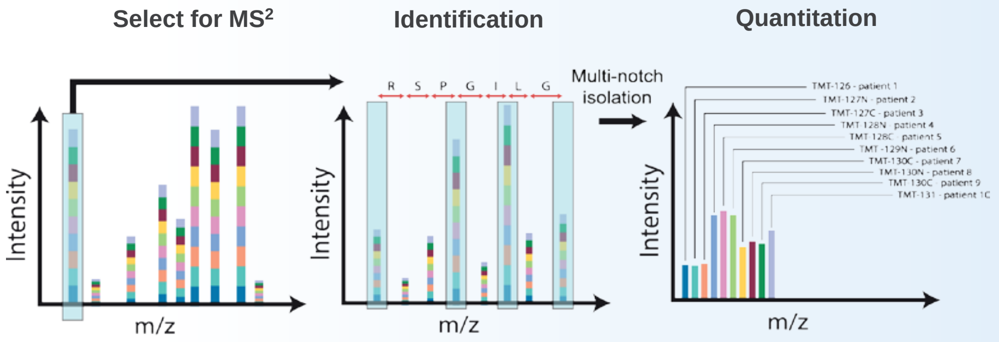
97% quantified with DDA+

Of 3434 proteins (1%FDR) 3329 (97%) are quantified across all replicates with using PD 2.2 MS1 peak detection/alignment and label free quant capabilities

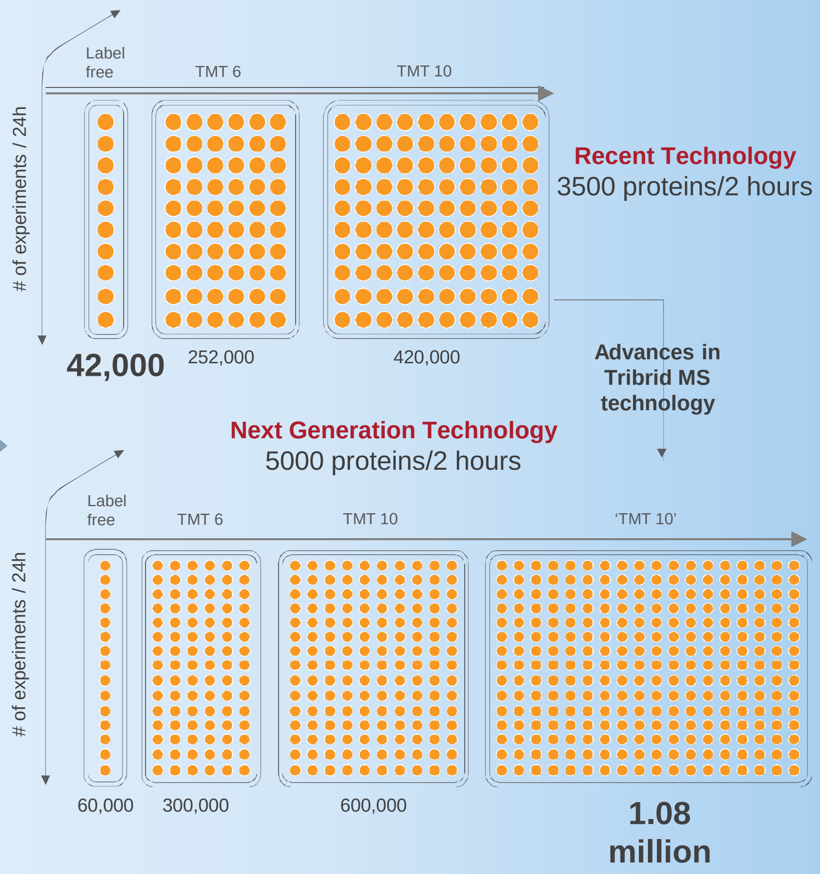
Proteomics can deliver very deep levels of analysis with complete quantitation

TMT Multiplexing Workflow: 11 Sample Results in a Single LC/MS Analysis





Number of protein quantifications per day



Recent Technology
3500 proteins/2 hours

42,000

Advances in Tribrid MS technology

Next Generation Technology
5000 proteins/2 hours

1.08 million

Sample Labeling and Preparation → **LC-MS/MS (SPS MS3) Analysis** → **Data Analysis**

Thermo Scientific™ TMT™ 11-Plex Reagents

Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ MS with Method Templates


Proteome Discoverer 2.2
Mass Informatics Platform for Protein Scientists

ProteinCenter
Professional Edition

Thermo Scientific™ Proteome Discoverer™ Software and ProteinCenter™ Software

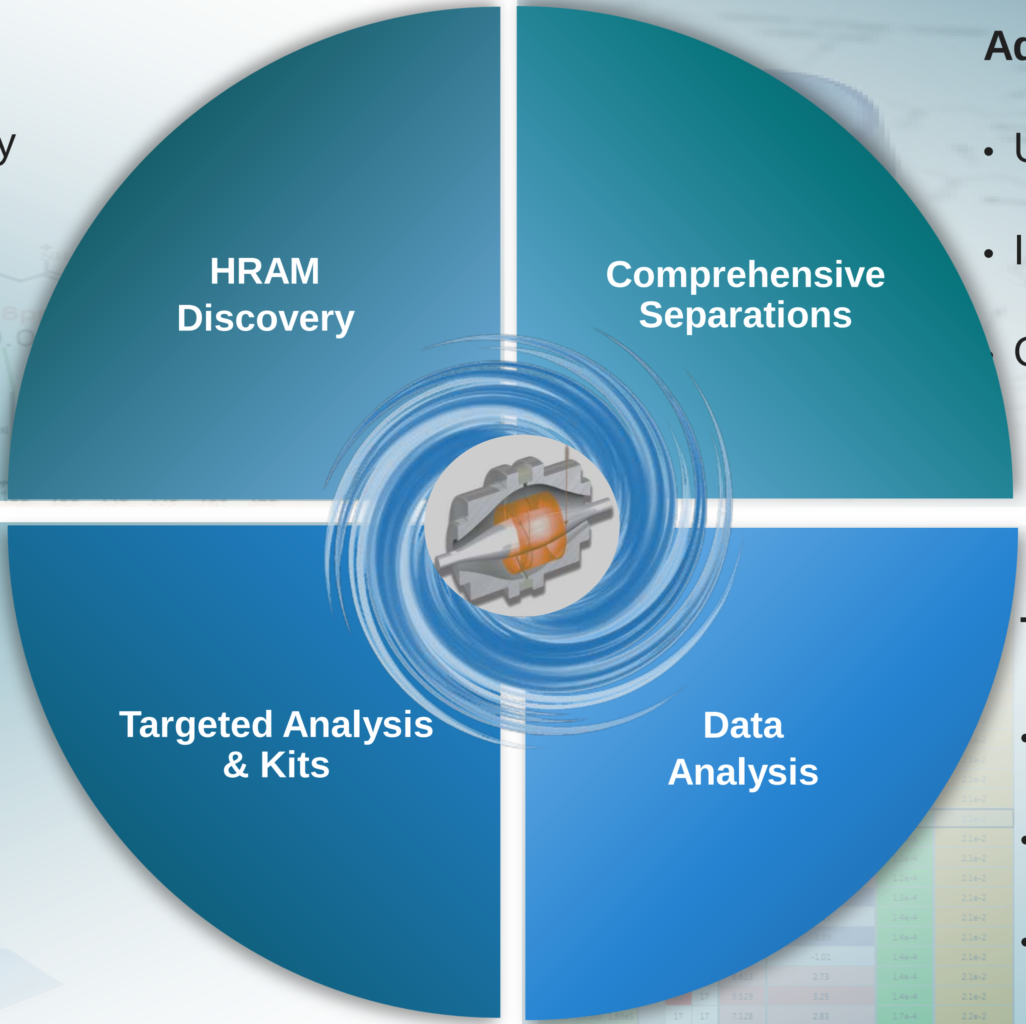
Unique Thermo Fisher Scientific workflow with potential for massive throughput

Driving Rigor and Standardization

A Comprehensive Solution for Metabolomics Analysis

Highest Quality Data

- Comprehensive untargeted discovery
- Unambiguous Orbitrap HRAM accuracy
- Robust for large cohort studies



HRAM Discovery

Comprehensive Separations

Targeted Analysis & Kits

Data Analysis

Addressing Chemical Diversity

- UHPLC for broad coverage
- IC for polar compounds
- GC for volatiles, complements UHPLC

Target Metabolites of Interest

- Full scan HRAM quantitation
- Biocrates Absolute*IDQ* p400 HR Kit
- Downstream targeted SRM triple quadrupole quantitation

Turn complex data to knowledge

- Robust, rigorous statistical analysis
- Curated libraries for confident ID
- Interactive visualization of results

Core of successful metabolomics powered by Orbitrap technology

Complex Data into Knowledge – Software for the Total Solution

Customers Demanding Insights

- Go from “features” to confident identifications
- Reduce the risk of false positives
- Designed to mine rich Orbitrap data

Thermo Scientific™ Compound Discoverer™ 2.1 Software

Complete ID and Characterization Solution

- Differential analysis
- Rigorous statistical analysis
- Confident identification
- Structure elucidation
- Pathway mapping

Database and Spectral Libraries

High Quality Curation

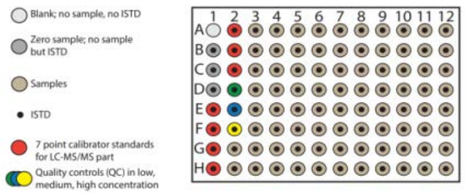
- mzCloud* online fragmentation library
  - ~7,000 compounds
  - > 2 million spectra
- New Thermo Scientific™ Orbitrap™ GC-MS Metabolomics Library

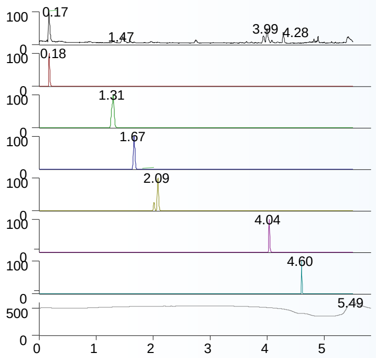
* HighChem LLC

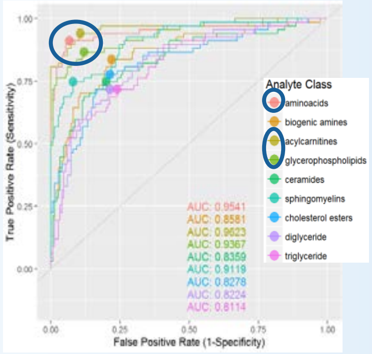
Significant investment to deliver robust and rigorous results that matter

Standardized Workflow for Targeted Metabolomics with Biocrates Kit

new

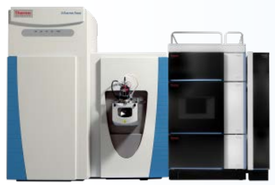






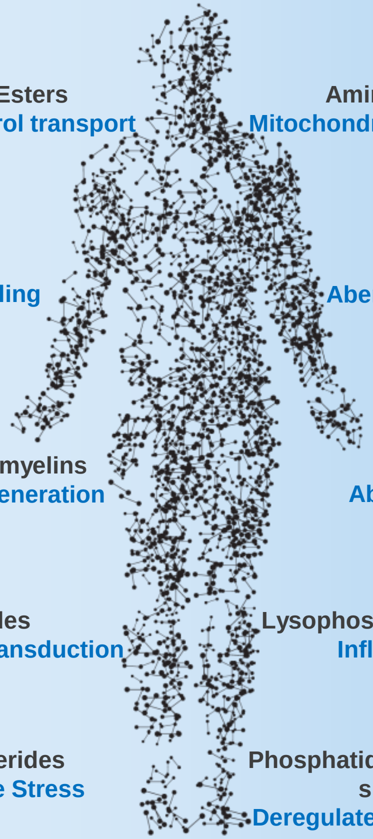
Sample Labeling and Preparation → **LC-MS/MS and FIA-MS/MS Analysis** → **Quantitation and Kit Validation Analysis**

Biocrates AbsoluteIDQ p400 HR Kit verified for Thermo Scientific™ Q Exactive™ MS



Thermo Scientific™ Q Exactive™ MS Family

Biocrates MetIDQ Software



Hexoses
Insulin resistance

Cholesterol Esters
Aberrant cholesterol transport

Amino Acids
Mitochondrial dysfunction

Ceramides
Aberrant cell signaling

Biogenic Amines
Aberrant cell cycle control

Sphingomyelins
Neurodegeneration

Acylcarnitines
Aberrant fatty acid oxidation

Diglycerides
Aberrant signal transduction

Lysophosphatidylcholines
Inflammation

Triglycerides
Oxidative Stress

Phosphatidylcholines
Deregulated growth

10 µL

Delivering standards, throughput and reproducibility across studies

Thermo Fisher Scientific : A Global Partner in Precision Medicine

The Boston Globe

Thermo Fisher targets a growing niche: precision medicine

Multi-Omic profiling and data integration in practice

- Targeted NGS Cancer Panels
- Proteome & Metabolome profiling
- BRIMS Center: working lab

The Economist Events

HEALTH CARE FORUM

WAR ON CANCER

"We have the possibility to collaborate with academia, pharma, the industry and patients at large to make better outcomes"

Corina Shtir
Head of Precision Medicine
Thermo Fisher Scientific

waroncancer.economist.com

#WarOnCancer

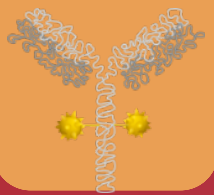
Pillars for Success | QUANTITATIVE | REPRODUCIBLE | STANDARDIZED | SCALABLE

Thermo Fisher Scientific — Perfect Partner to the Biopharmaceutical Industry

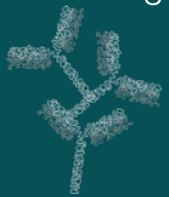
Disciplines for full biotherapeutic characterization

Higher Order Analysis

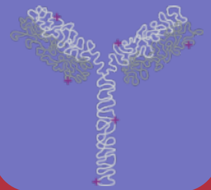
Intact Analysis



Aggregate Screening

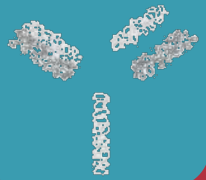


Charge Variant Screening

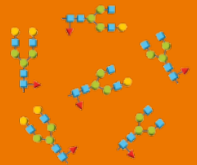


Glycan Analysis

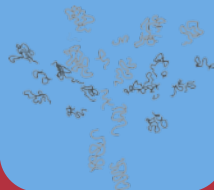
Sub-Unit Analysis



Released Glycan



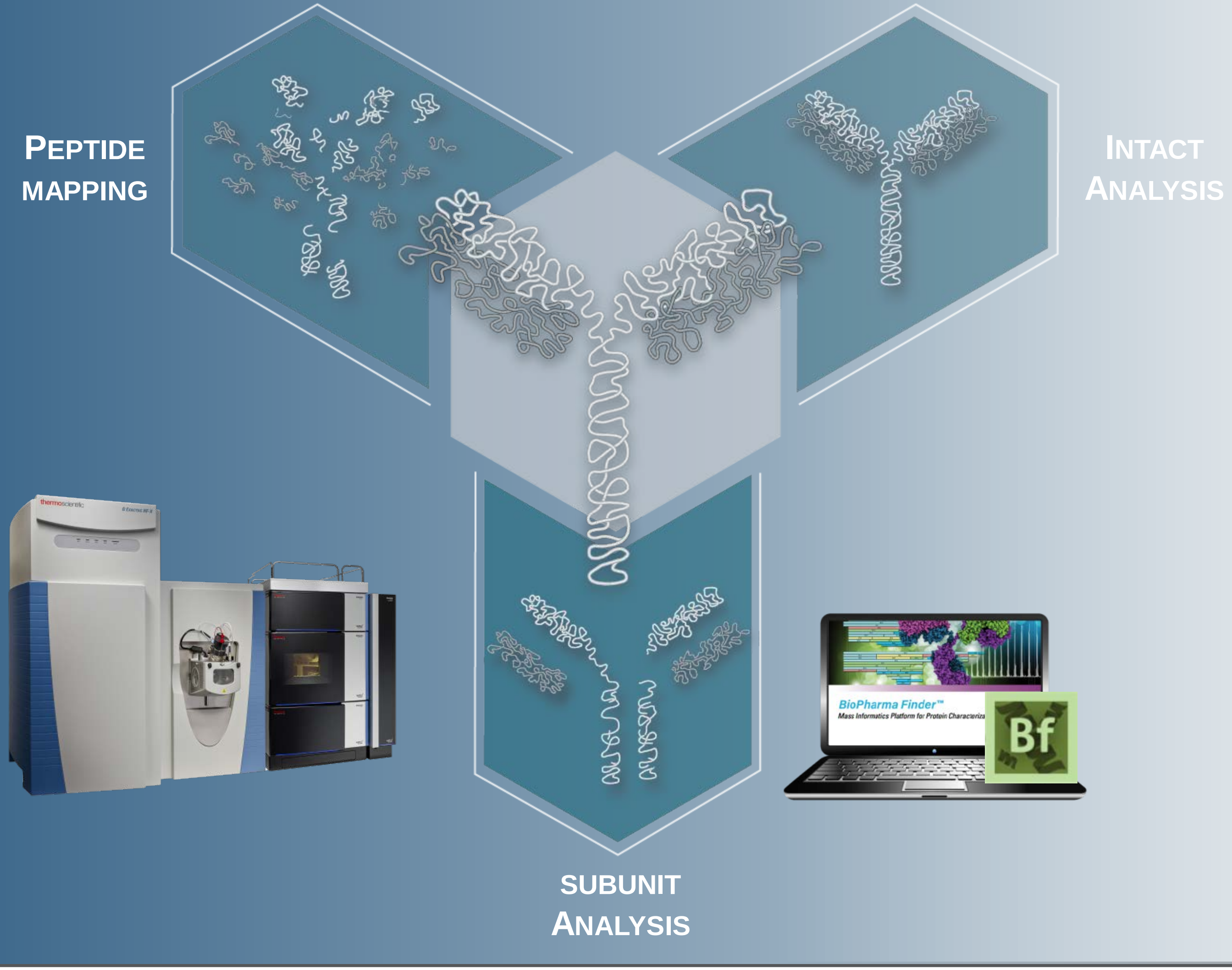
Peptide Mapping



Thermo Scientific Q Exactive HF-X with Biopharma Option

new

Expanded functionality for complete characterization



High Mass Range mode for intact native proteins and ADCs

Protein Mode with high sensitivity and speed reveals mAb subunits even without LC separation 5-10X S/N improvements

Brighter ion source and enhanced speed ensures comprehensive coverage for peptide mapping

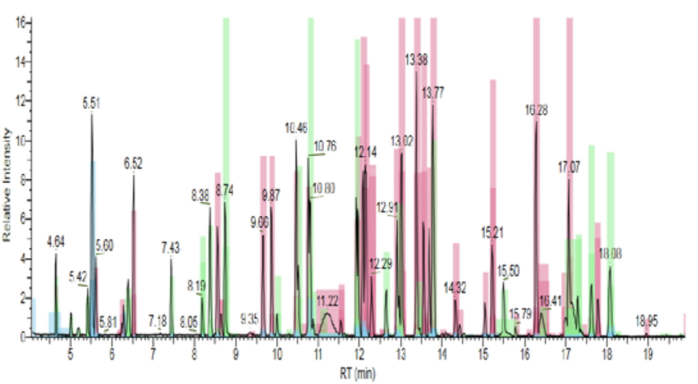
- Advancing MS technology for multi attribute measurements in QC

Thermo Scientific Q Exactive HF-X MS with Biopharma Option

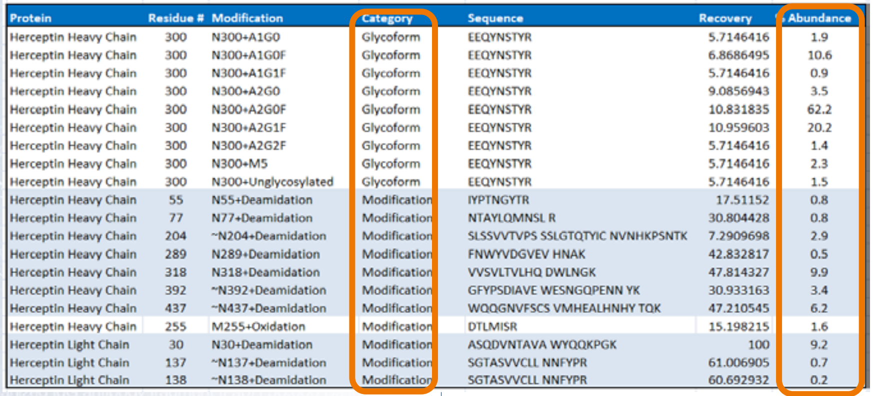
Peptide mapping of trastuzumab

Digestion → **LC-MS/MS Analysis** → **Data Analysis**

100% Sequence coverage Identifcation and quantitation of modifications

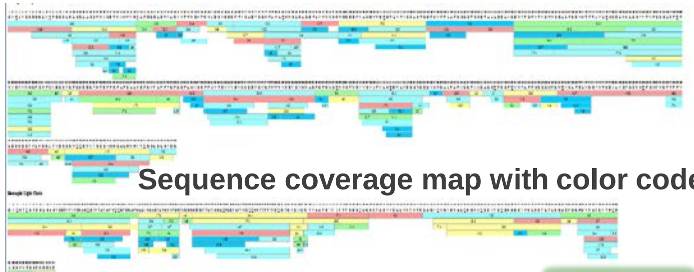


Identified peptides highlighted by color coded peak shading in chromatogram

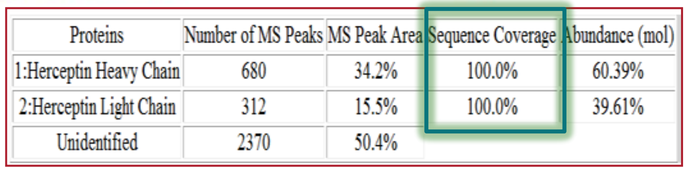


Protein	Residue #	Modification	Category	Sequence	Recovery	Abundance
Herceptin Heavy Chain	300	N300+A1G0	Glycoform	EEQYNSTYR	5.7146416	1.9
Herceptin Heavy Chain	300	N300+A1G0F	Glycoform	EEQYNSTYR	6.8686495	10.6
Herceptin Heavy Chain	300	N300+A1G1F	Glycoform	EEQYNSTYR	5.7146416	0.9
Herceptin Heavy Chain	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

Modification Summary: Quantification of glycoforms, deamidation, oxidation



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

Thermo Scientific Q Exactive HF-X MS with Biopharma Option

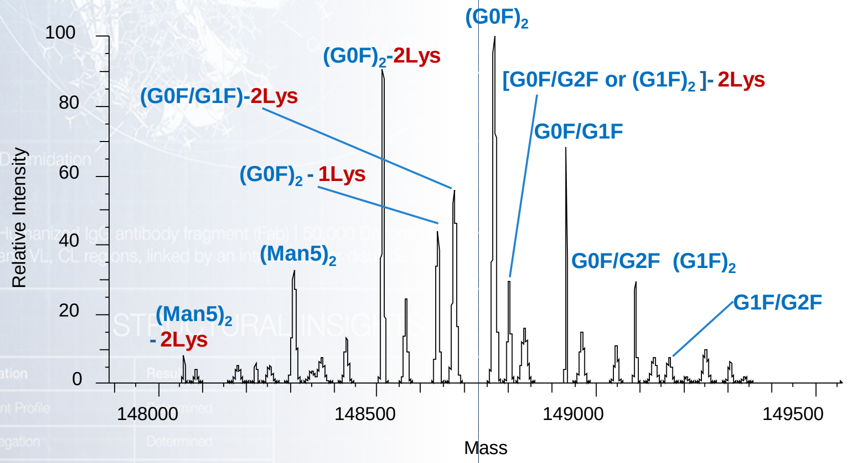
new

Intact mass analysis – Native and denatured proteins

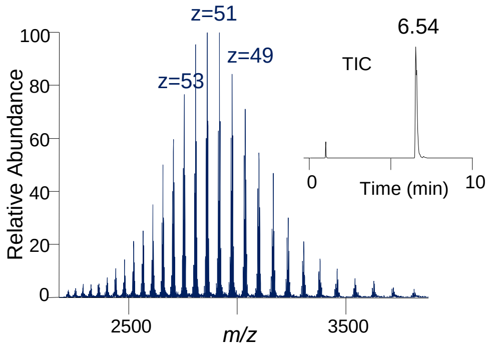
Superior analysis of intact proteins under native and denaturing conditions

Resolves protein modifications such as glycosylation and adducts

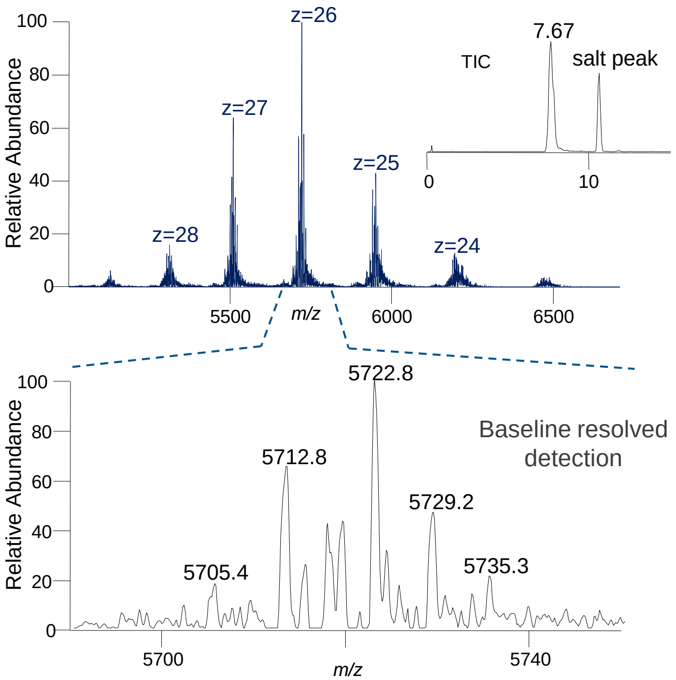
Example : Intact infliximab



Denatured Conditions



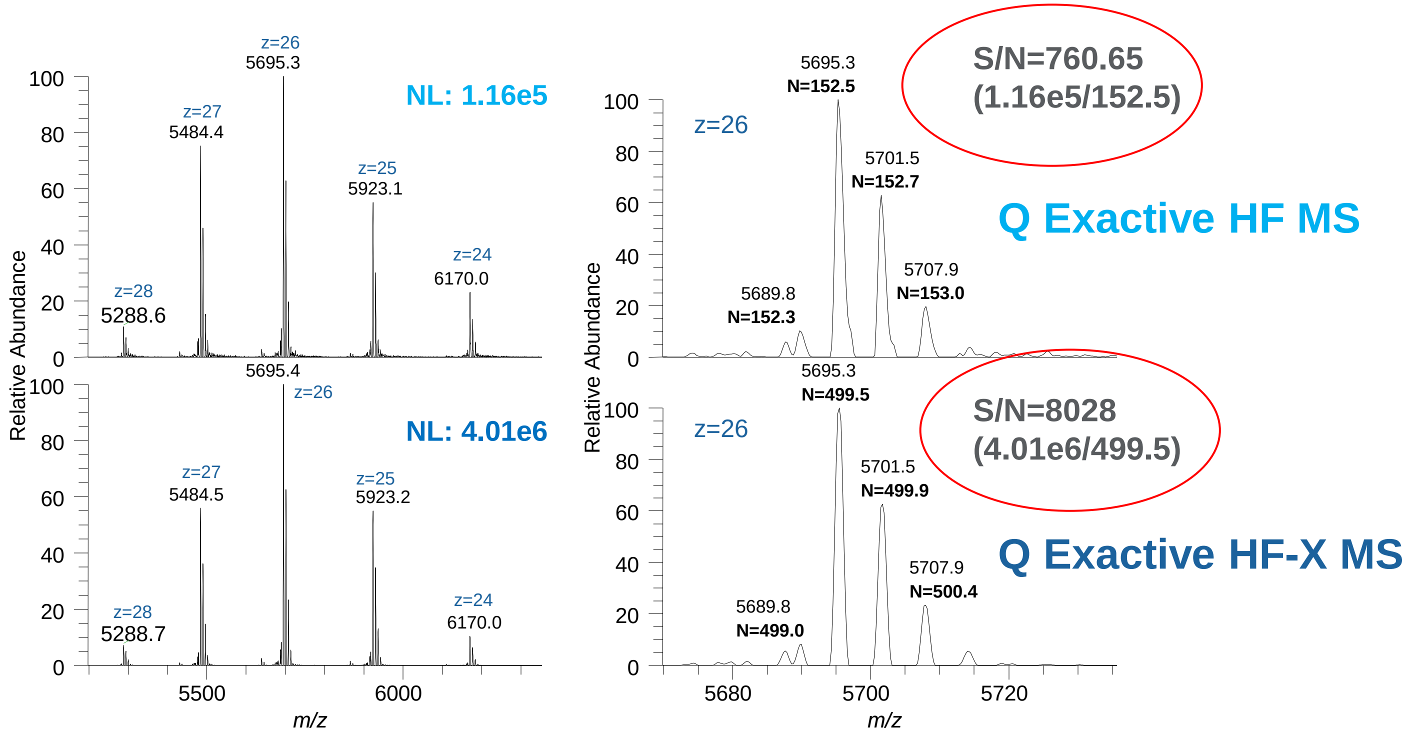
Native Conditions



Deconvoluted spectrum of the complex glycoforms under native & denaturing conditions

Analysis of Intact Trastuzumab under Native Conditions in HMR Mode

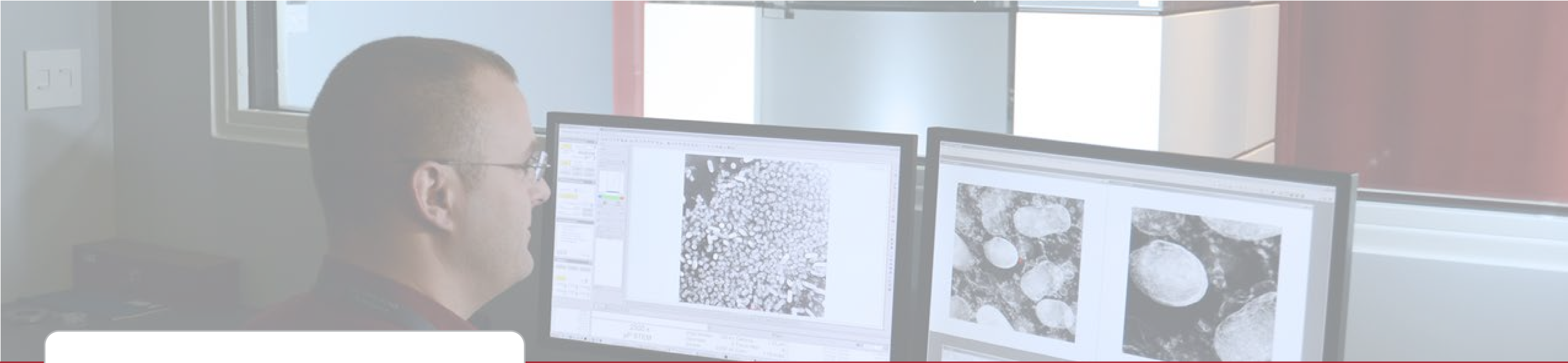
S/N ratio improved by ~5-10 on the Q Exactive HF-X MS



SEC-LCMS analysis of intact trastuzumab monoclonal antibody using Acclaim SEC column, 4.6 x 300 mm, 300 µl flow, 50mM ammonium acetate.

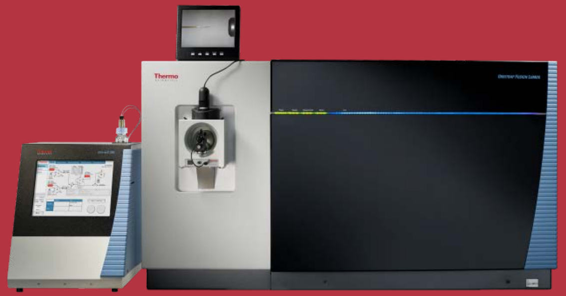
Full MS, HMR mode, m/z 2500–8000, resolution setting 30k, 10 µscans. Spectra show an average of 3 µscans each.

Thermo Fisher Scientific — Clear Leadership in Structural Biology



FEI

part of **Thermo Fisher Scientific**



Complimentary Leadership in Cryo-EM and Orbitrap MS

Publications in Nature and Science

The Nobel Prize in Chemistry 2017

The Royal Swedish Academy of Sciences has decided to award the Nobel Prize in Chemistry 2017 to

Jacques Dubochet
University of Lausanne, Switzerland

Joachim Frank
Columbia University, New York, USA

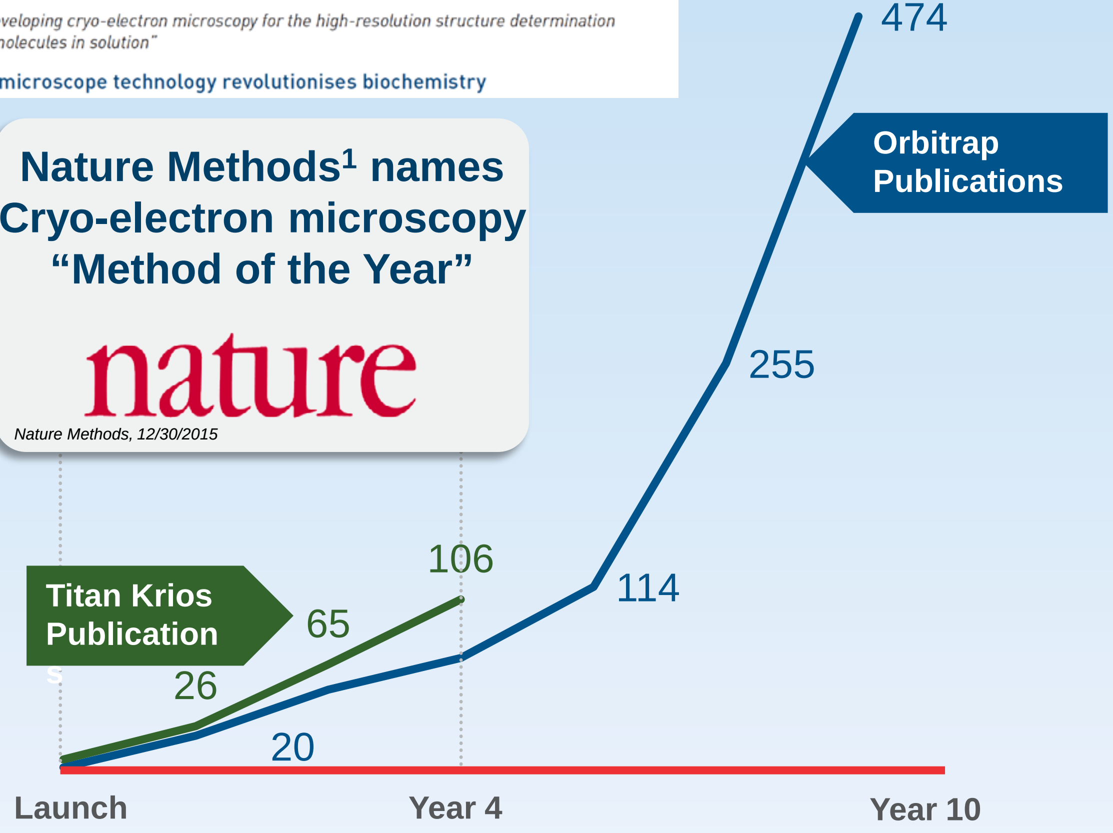
Richard Henderson
MRC Laboratory of Molecular Biology, Cambridge, UK

"for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution"

Cool microscope technology revolutionises biochemistry

Nature Methods[1] names Cryo-electron microscopy "Method of the Year"

Nature Methods, 12/30/2015



"This represents a **new era in imaging of proteins** in humans with immense implications for drug design"

Francis Collins, M.D.

Integrative structural biology answers :

- 3D Structure
- Stoichiometry
- Composition
- Topology (binding partners, cofactors, messengers, etc.)
- Binding affinity
- Dynamics
- Aggregates
- Biological function

Thermo Fisher Scientific Integrative Structural Biology Solutions

Structure-function information based upon chemical information


A Sponsored Supplement to *Science*

Accelerating the path from structure to function through integrative structural biology solutions

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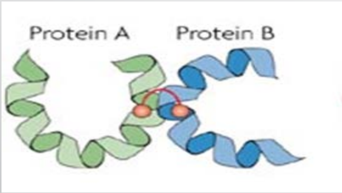
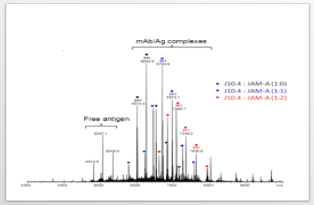
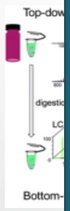
3D structure-function information from single protein imaging

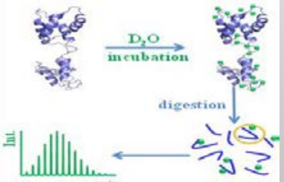


Crosslinking MS

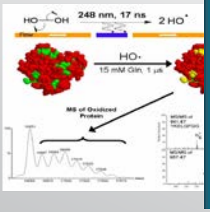


Native MS

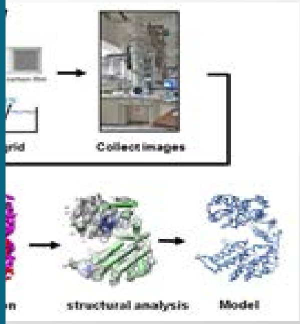




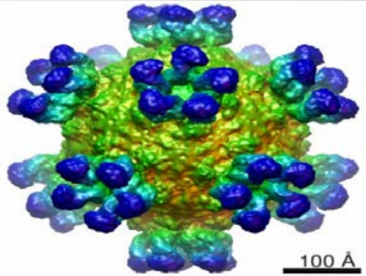
HDX



FPOP



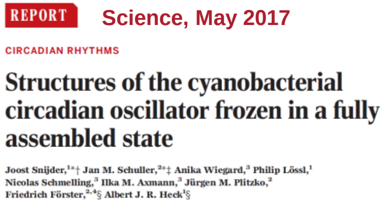
M



Understanding the structure-function context has game changing potential

Integrative Structural Biology

Albert Heck
Utrecht University



REPORT Science, May 2017

CIRCADIAN RHYTHMS

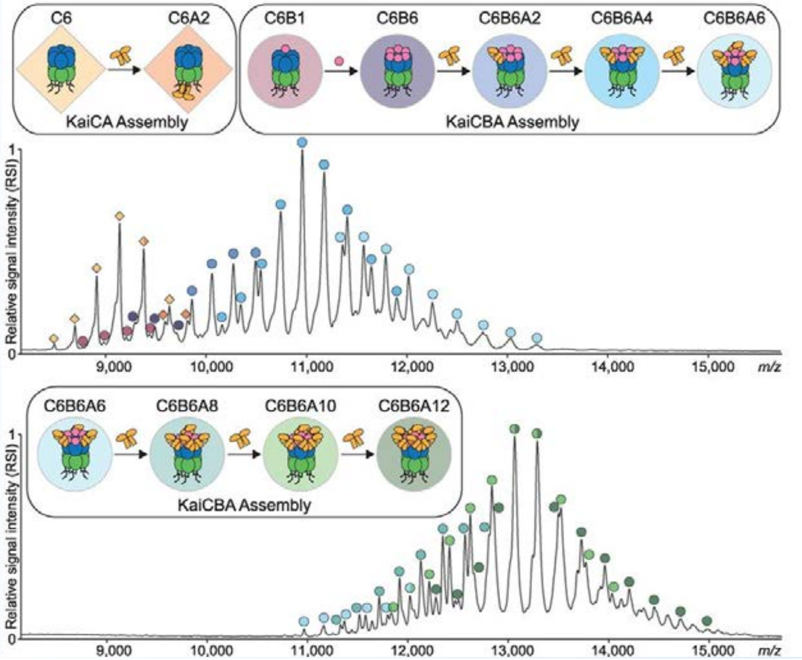
Structures of the cyanobacterial circadian oscillator frozen in a fully assembled state

Joost Snijder,[1*†] Jan M. Schuller,[2*‡] Anika Wiegard,[3] Philip Lössl,[1]
Nicolas Schmelling,[3] Ilka M. Axmann,[3] Jürgen M. Plitzko,[2]
Friedrich Förster,[2,4§] Albert J. R. Heck[1§]

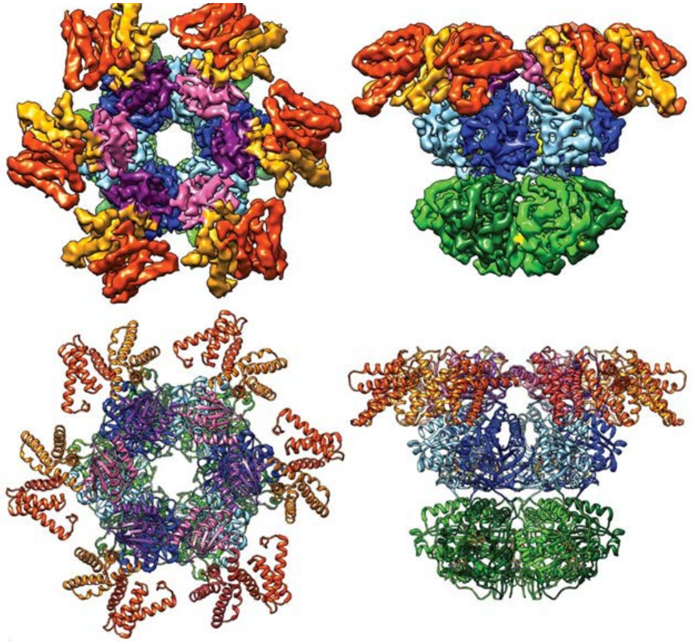
Sunday User Meeting Plenary

“There are Moments When Time does Stop”: Characterizing a Circadian Oscillator with Hybrid MS Methods and Cryo-EM

Native MS for monitoring assembly dynamics

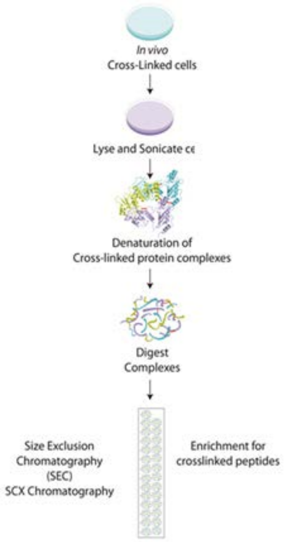


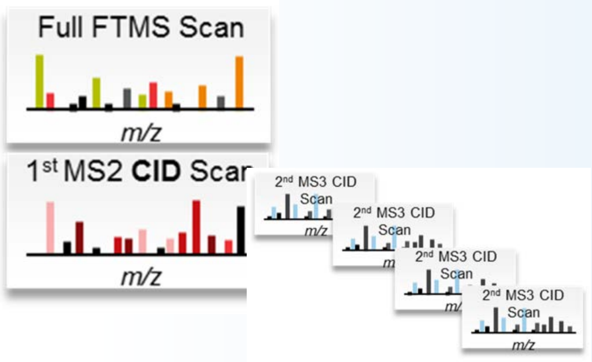
Cryo-EM map and model

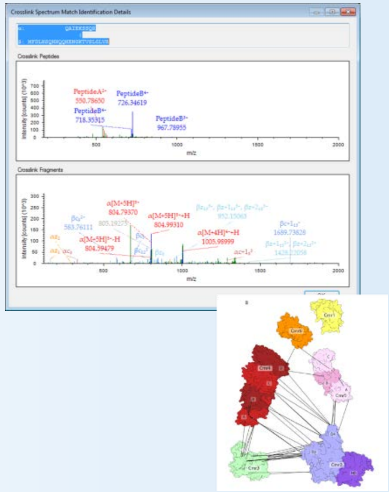


Combined methods provide structural basis to understand protein assembly

Crosslinking Mass Spectrometry Workflow

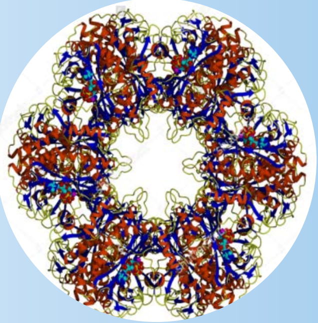






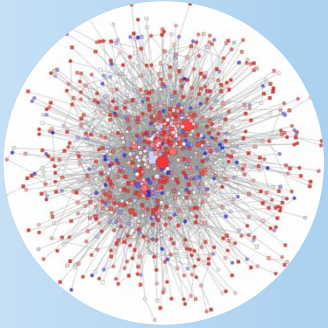
Protein Structure

Protein Complexes



Sample Labeling and Enrichment → **MSⁿ and Multiple Fragmentation Analysis** → **Data Analysis**

Interactome



Thermo Scientific™ DSSO and DBSU MS Cleavable Linkers

Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ MS with Method Templates

Thermo Scientific™ Proteome Discoverer™ Software with XlinkX Node

Unique workflow that is complete , easy to use and sensitive

Summary

Precision Mass Spectrometry

New Product Introductions for Precision Mass Spectrometry – Continuing the legacy of innovation

- Extending leadership in HRAM Orbitrap MS
- Driving quantitation solutions for complex analyses with new QQQ’s and Orbitraps
- Major software releases that unleashing the potential of MS data

Leading the Way with Scientific Rigor and Precision in Key Application Areas

- Delivering standardized solutions for proteomics and metabolomics based translational research/precision medicine
- Game-changing structure function studies by integrative structural biology solutions
- Comprehensive and pioneering workflows for biopharma characterization and QA/QC