iHUPO 2017

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Pushing the leading edge in protein quantitation: Integrated, precise, and reproducible proteomic workflows

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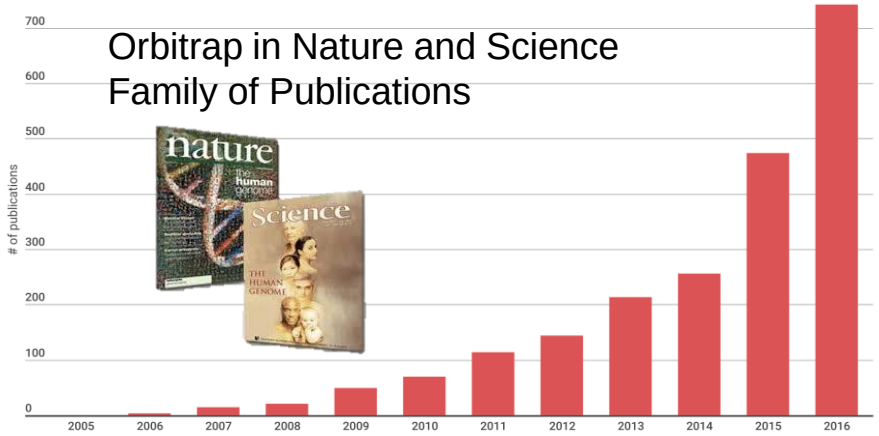
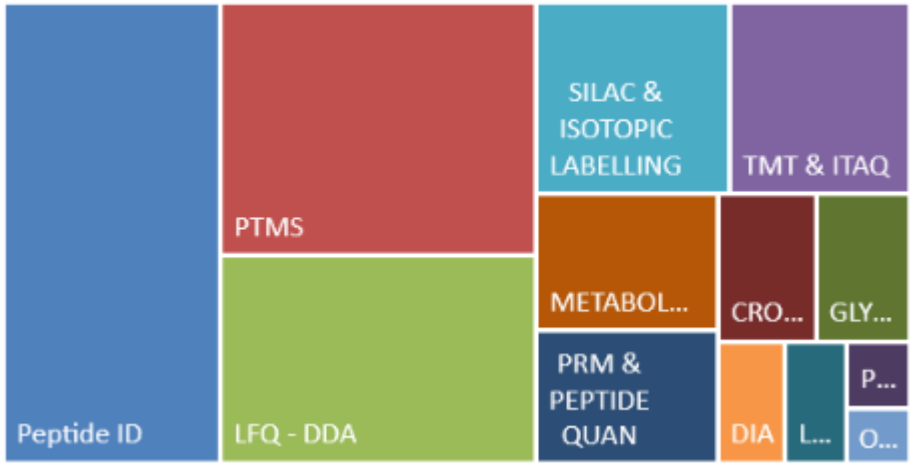
Strategic Marketing Specialist – Quantitative Proteomics
September 19, 2017

Pushing The Leading Edge in Protein Analysis





ASMS 2017

Q Exactive HF-X

ASMS 2014

Q Exactive HF

HUPO 2013

Q Exactive Plus

ASMS 2011

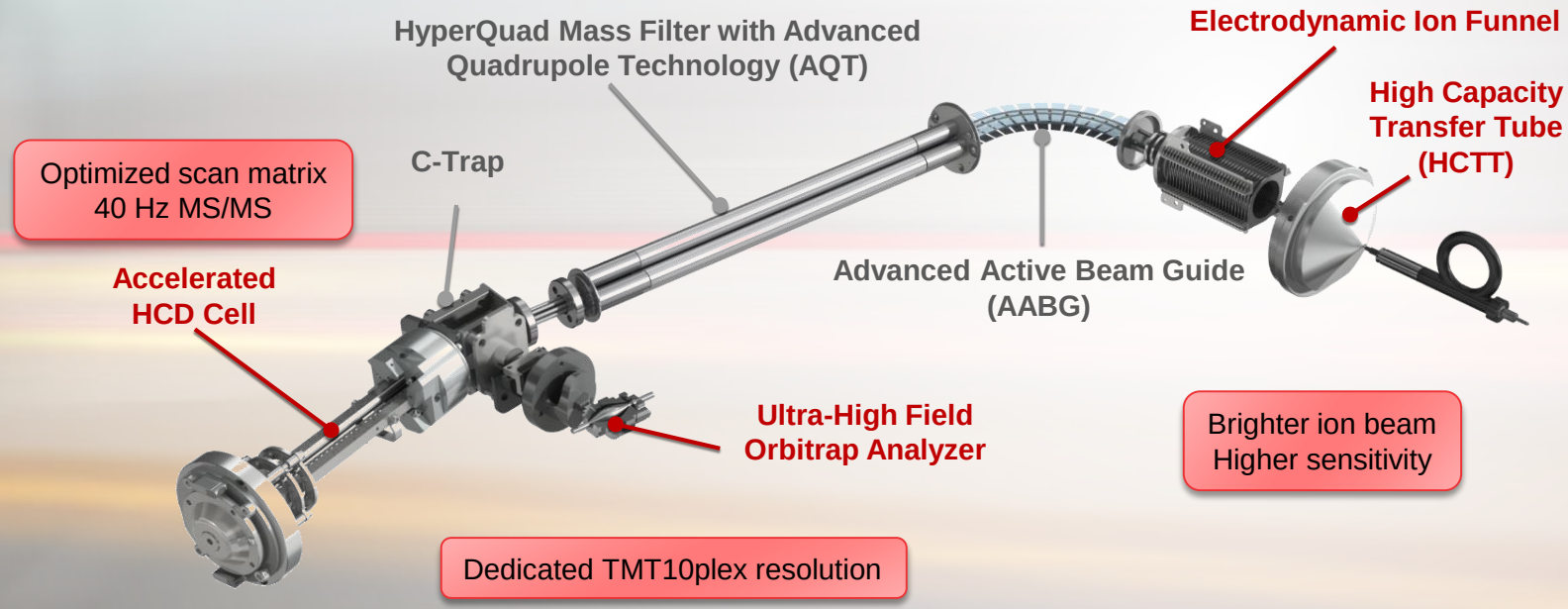
Q Exactive

Q Exactive Platform Family

Thermo Scientific™ Q Exactive™ HF-X Hybrid Quadrupole Orbitrap™ MS

new

- Novel architecture with a high capacity transfer tube (HCTT) and electrodynamic ion funnel that increases ion transmission and boosts sensitivity for all analytes.
- Increased acquisition speed (an optimized scan-matrix design now enables scan rates up to 40Hz) and advanced peak determination (APD) algorithm dramatically improves peptide sequencing depth at this speed.
- Empowering advances in TMT multiplexing, biomarker verification, and high-resolution PRM targeted analysis.



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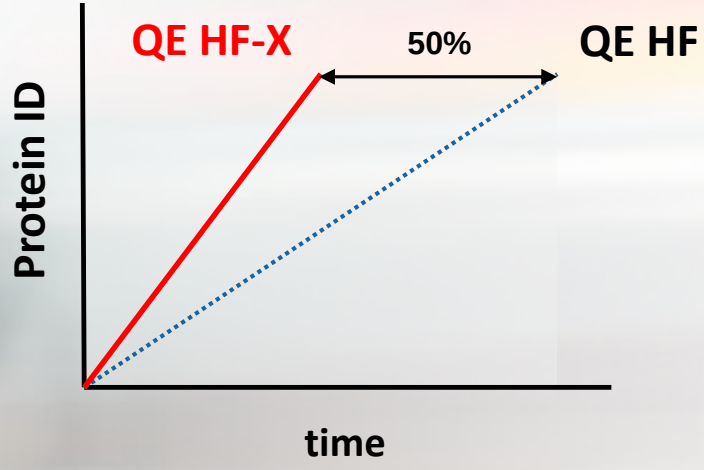
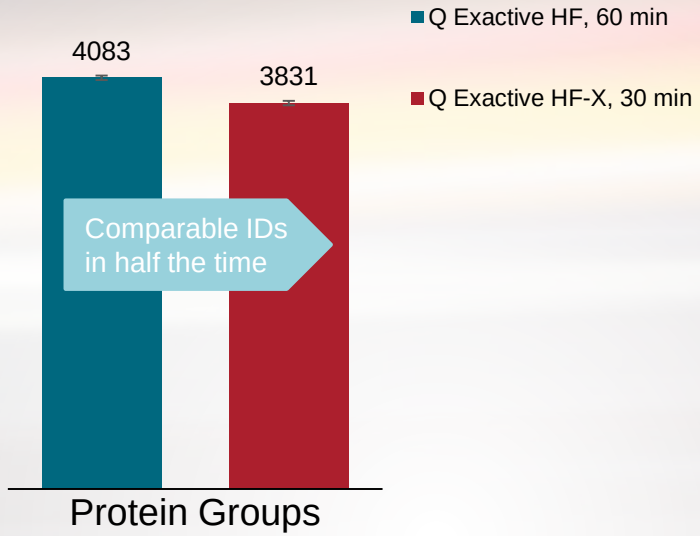
Q Exactive HF-X: Maximizing Efficiency For All Proteomics Applications

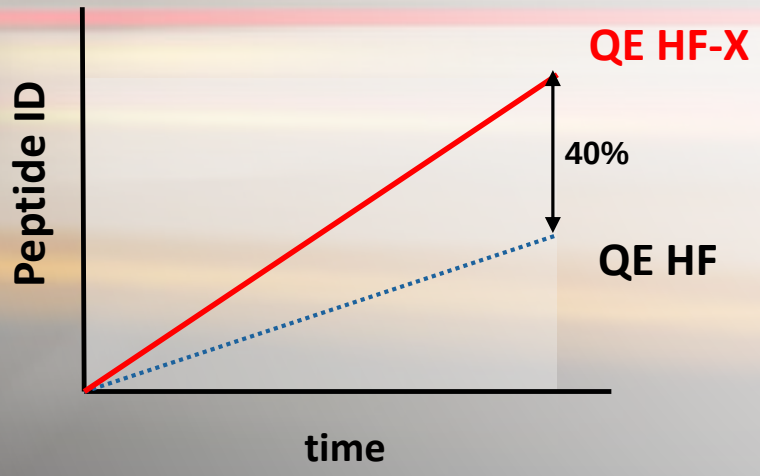
new

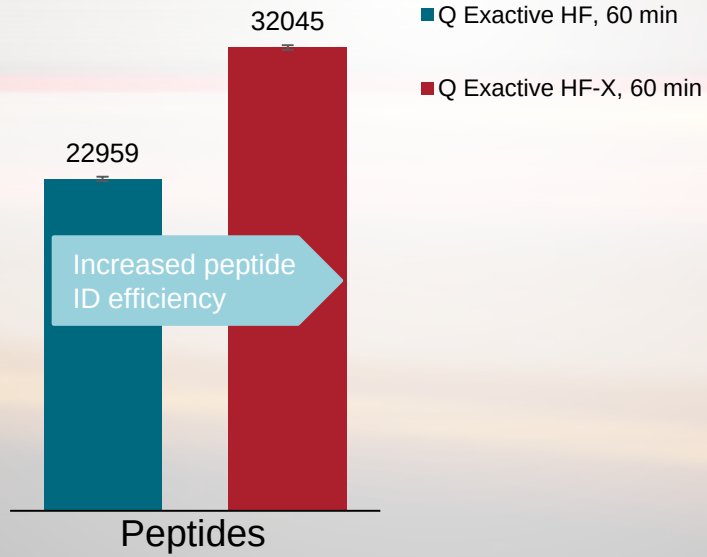




Maximizing protein identifications

- Quick screening of complex samples
- Quality control of complex samples
- Assessment of sample concentration





Maximizing peptide identifications

- Highest peptide coverage
- Deep proteome analysis
- Spectral library building

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The Goal: Quantitative, Standardized, High-throughput Proteomics

Needs

Analytical Workflows

TMT Multiplexing

Throughput and depth of analysis

- Large scale multiplexed quantitative proteomics
- Enables high throughput screening
- Most precise and reproducible quantitation

Label-free Precursor Level Quantitation

New standard in quantitative sensitivity, accuracy and precision

- New DDA+ and HR-DIA workflow with precursor level quantitation methods
- Eliminates missing values
- Delivers reproducibility and accuracy for large sample cohorts

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

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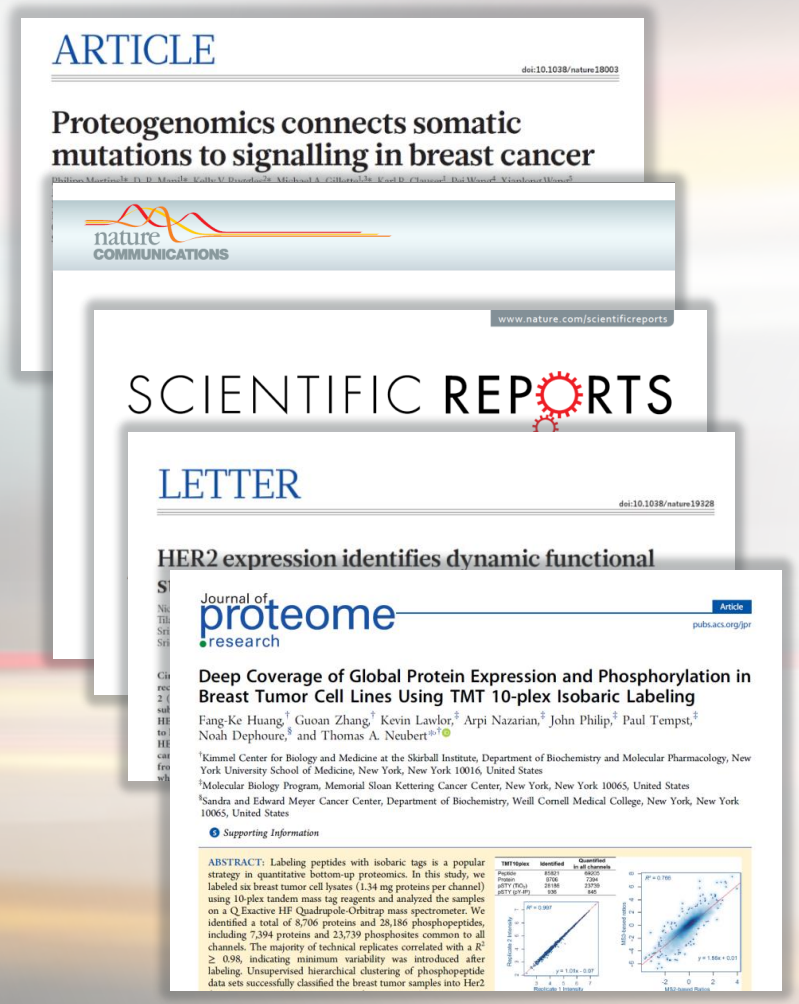
TMT Multiplexing Workflow - Proteogenomics Approaches in Cancer Research



Proteomics insights complement genomic results

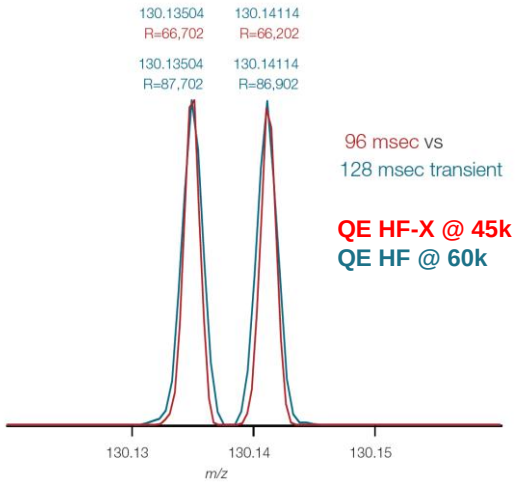
- Connects somatic mutations to **cancer signaling** and elucidates functional consequences
- **Proteogenomics signatures** have shown potential utility
- Creation of high-resolution proteome **maps of cancer histotypes** directly from clinical tissue.
- High Throughput Screening against drug panels to target **differentially regulated pathways** directly from clinical tissue.
- Phosphopeptide data sets to **classify breast** tumor samples into Her2 positive and negative groups

Major cancer centers adopting the TMT-based proteogenomic approach

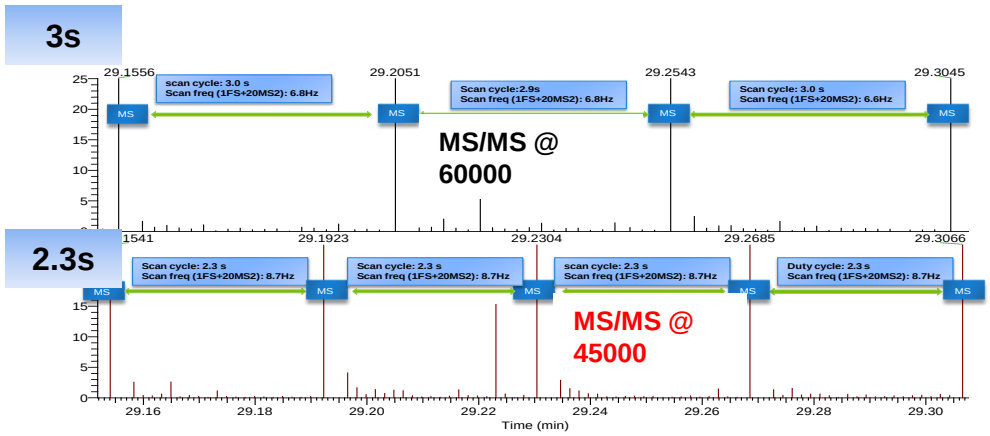
TMT Multiplexing Workflow for Precise Data in Less Time

Thermo Scientific™ TMT™ 11-Plex Reagents

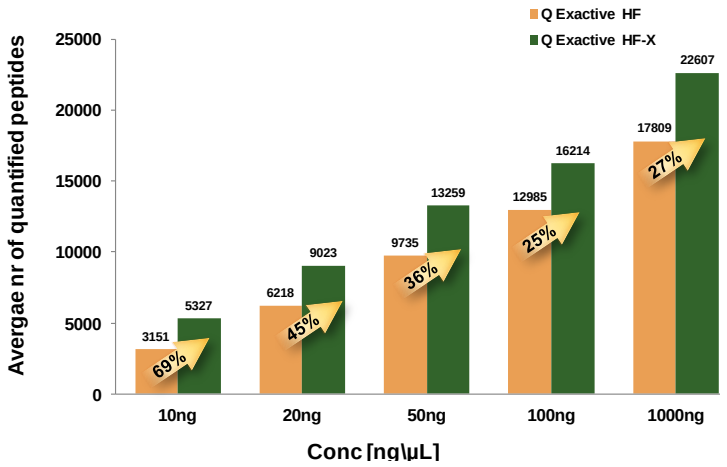
- TMT11-131C can be used in combination with TMT10plex reagents to multiplex 11 different samples for MS analysis
- Increased throughput with concurrent MS analysis of multiple samples
- Sample origin flexibility
- Fewer missing values
- 11-plex data analysis is supported by Proteome Discoverer 2.2

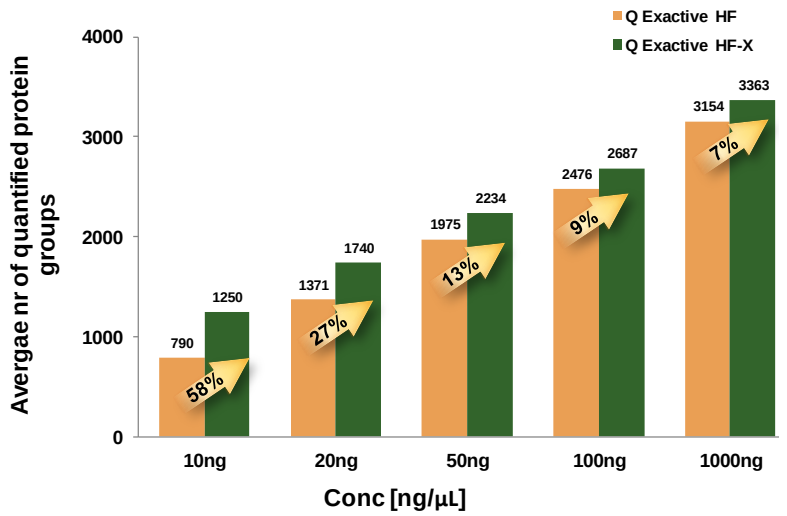


Dedicated TMT resolution



~25% improvement in scan speed





Pain points: From Discovery to High Throughput Clinical Proteomics

Proteome coverage

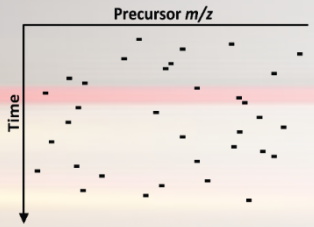
Discovery

Evaluation

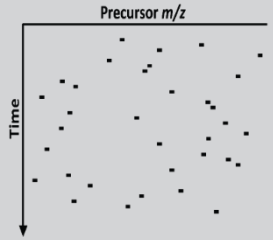
Clinical

Identification

Label-free precursor quantitation workflows



Data Dependent Acquisition (DDA)



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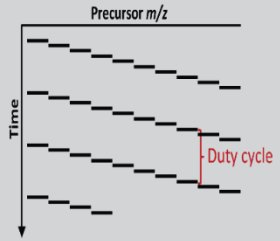
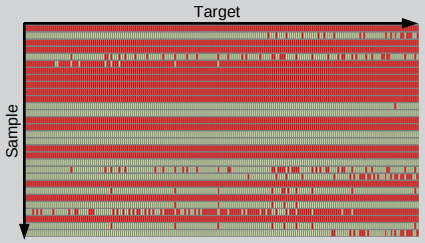


Data Independent Acquisition (DIA)



Parallel Reaction Monitoring (PRM)

Pain points

Not optimized for quantitation

Selectivity and dynamic range

Run-to-run reproducibility

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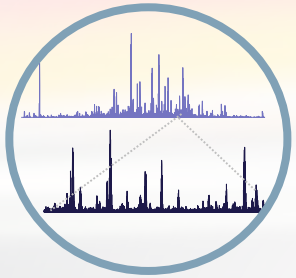
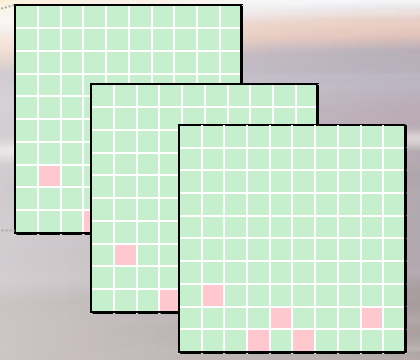
Workflow Solutions for Quantitative, Standardized, High-Throughput Proteomics

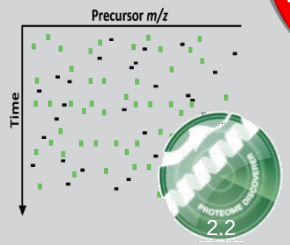
Sample Preparation → **Separation** → **Data Acquisition** → **Data Analysis**

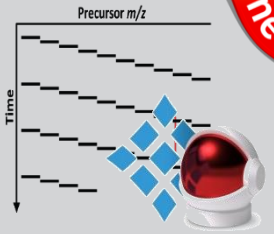
QUANTITATION | **PRECISION** | **REPRODUCIBILITY** | **STANDARDIZATION**

new



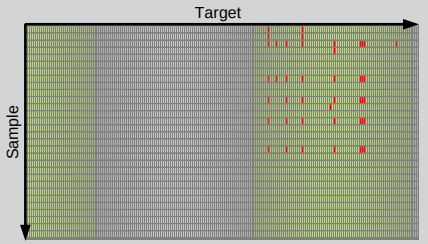
Data Dependent Acquisition Plus (DDA+)

new



High Resolution MS1 DIA (HR-DIA)

new



Dynamic Retention Time PRM (dRT-PRM)

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DDA+ Workflow: Standardized, Precursor-based Quantitation Solution

new

Robust LC Separation

UltiMate 3000 RSLCnano

- *Nano, cap, micro-flow rate range*
- *Direct inject or pre-concentration mode*
- *Easy connections with nanoViper fittings*

150 µm EASY-Spray Columns

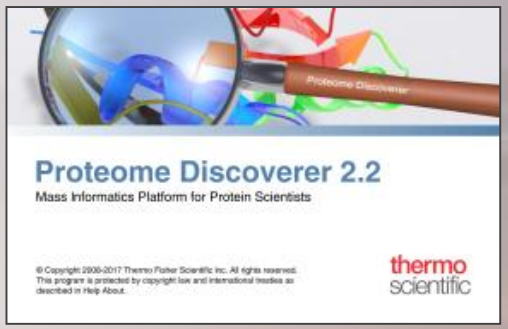
- *2 µm Acclaim PepMap*
- *Sensitivity and robustness*
- *RT stability <1% CV observed for 350 injections*

Optimized MS Acquisition

Q Exactive HF-X

- *Improved ion transmission*
- *Increased acquisition speed*
- *Advanced peak determination (APD)*
- *Same # of protein IDs half the time*

Comprehensive Data Informatics



Proteome Discoverer™ 2.2

- *No more 'missing values'*

Designed for quantitative precision and reproducibility

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Separations Designed for Routine Analysis of Large Sample Cohorts

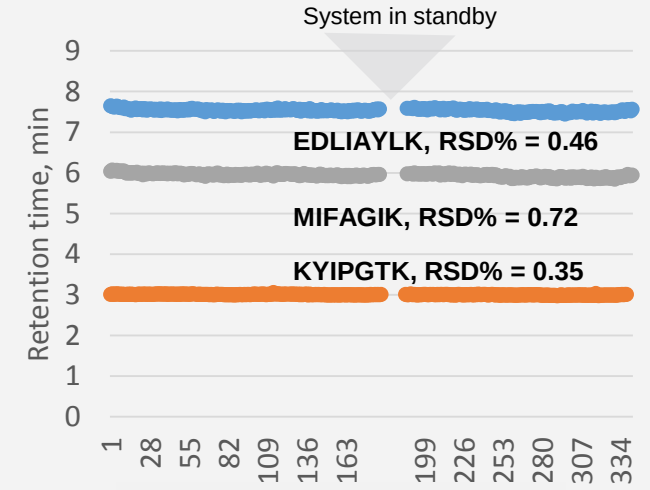
new

- New 150 µm ID EASY-Spray columns with PWHM as low as 3 s at 3 µL/min flow rate
- Provide excellent chromatographic performance, ease-of-use and robustness
- Retention time stability (RSD < 1%) was observed for 350 injections
- Peak area stability for 150 consecutive injections over 8 days with peak area RSD of less than 10%

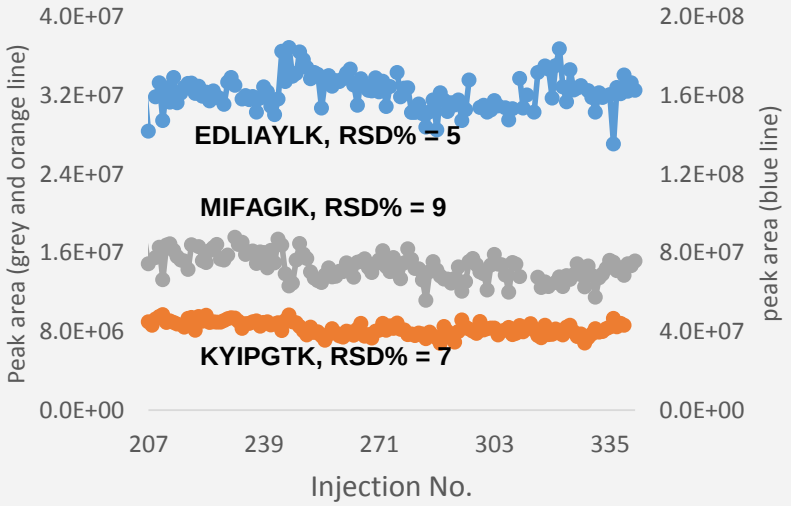
B-017*: Novel capillary flow LC HRAM MS platform for fast targeted analysis and robust profiling of complex samples.*

E-018*: Revolutionary Proteome Profiling and Quanitation without Compromising Speed, Sensitivity, and Selectivity.*

EASY-Spray column
150 µm ID x 150 mm,
2 µm Acclaim PepMap
15 µm ID emitter



Retention Time Stability



Peak Area Stability

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Dependable, Robust LC Performance for Demanding Applications

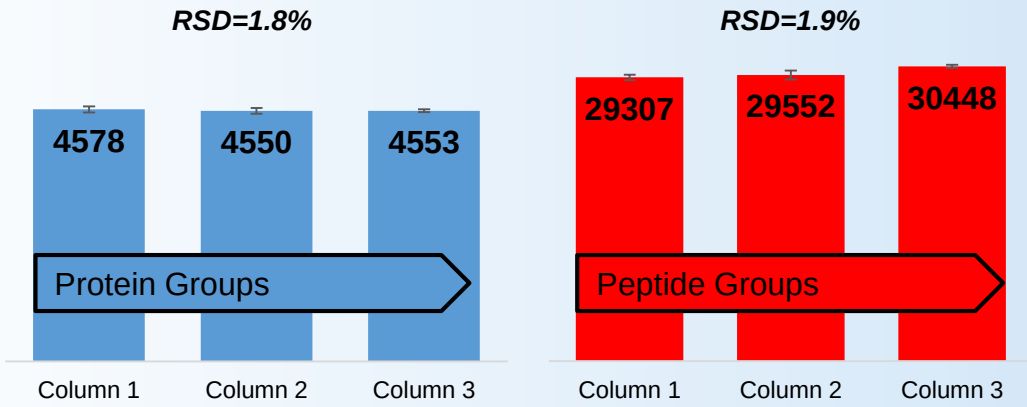
new

EASY-Spray column
150 µm ID x 150 mm

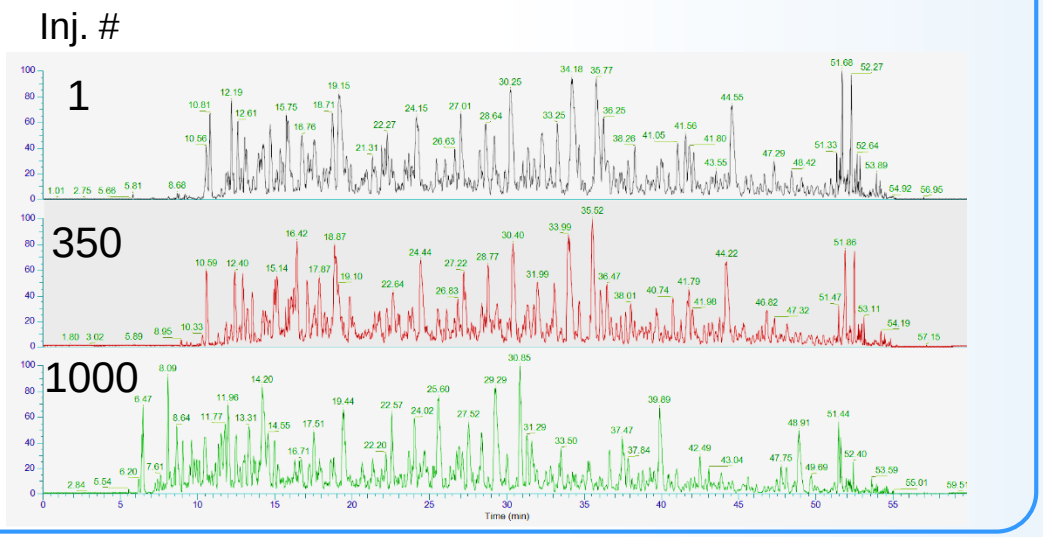
UltiMate 3000, 1.2 µL/min
4 µg HeLa, 60 min method

QE HF-X, 120K/7.5K, Top40

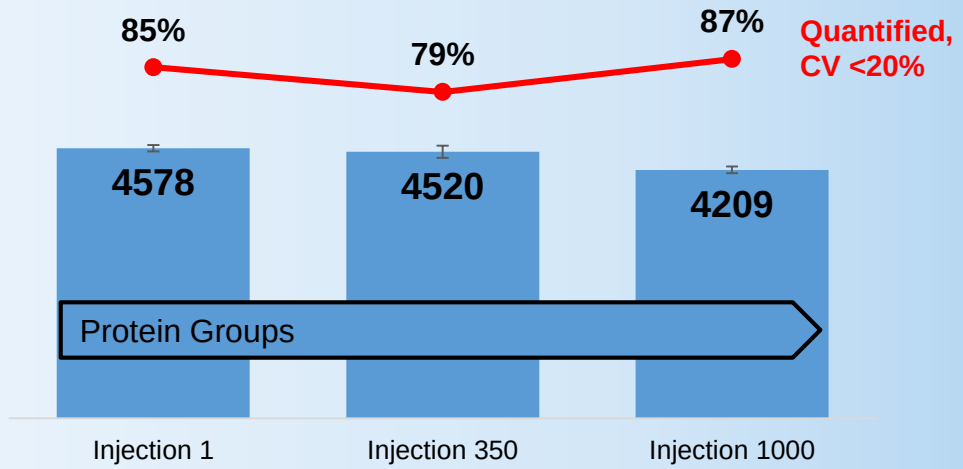
Column-to-column consistency



Durable response factor



Long-term quantitative performance



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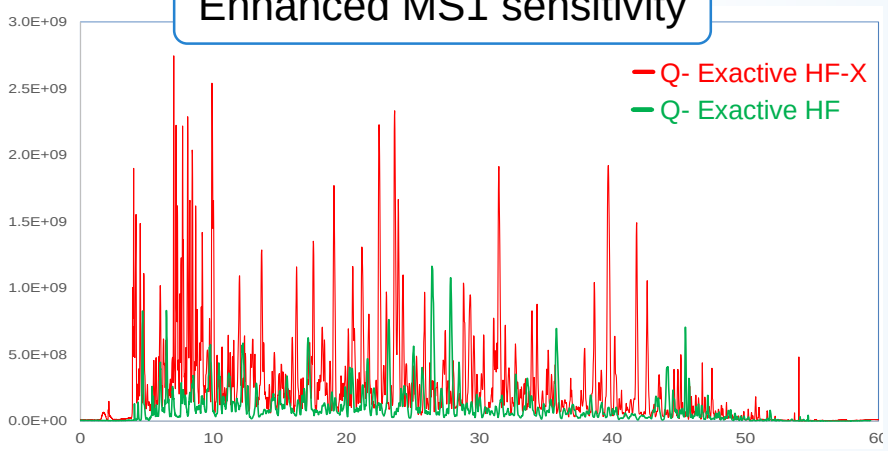
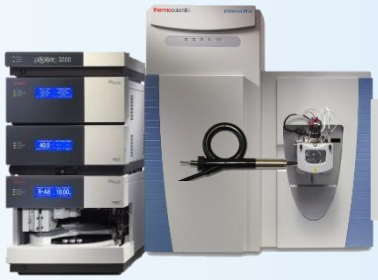
Maintaining Sensitivity with Increased Robustness: Cap-flow LC with QE HF-X

Enhanced MS1 sensitivity





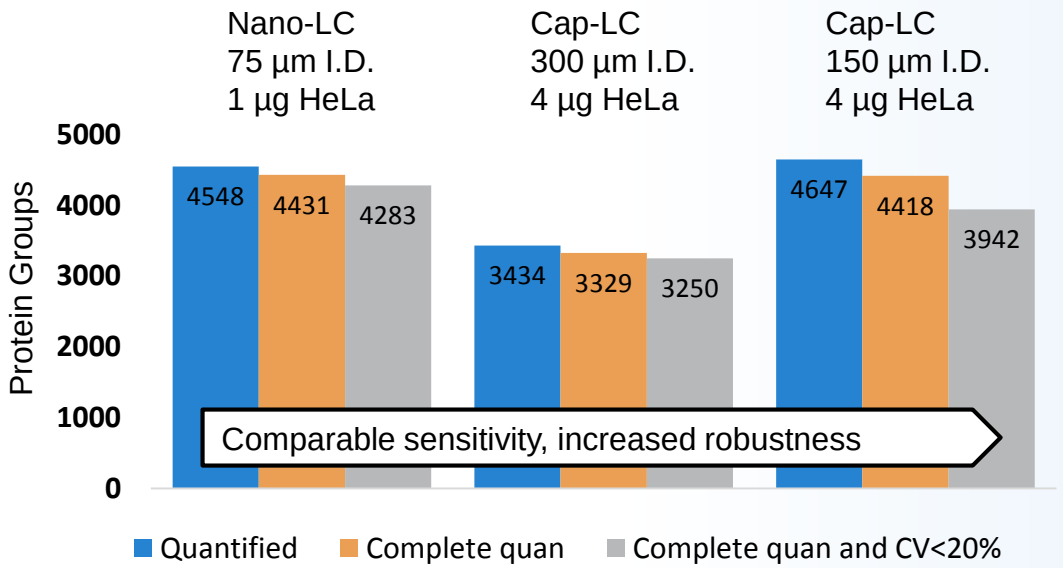
DDA+ Workflow

Analytical Advantages

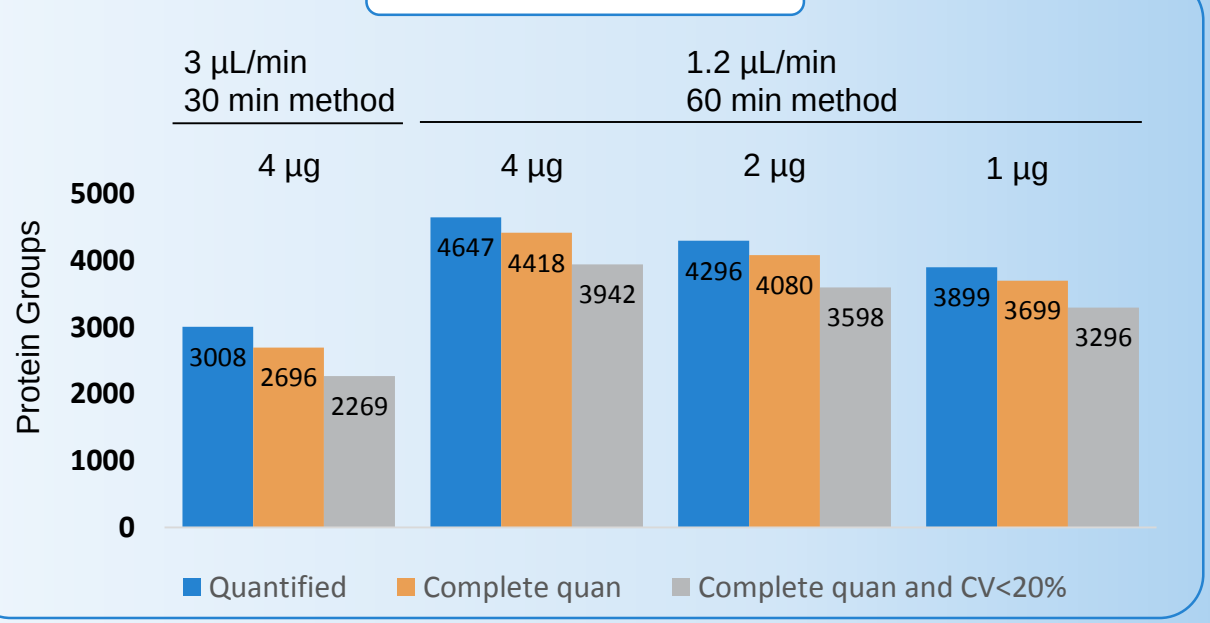
Reproducible, high-throughput cap-flow

Ease-of-use and robust chromatography

Brighter source improves MS sensitivity



EASY-Spray 150 µm



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DDA+ Empowered by Proteome Discoverer 2.2

Key Benefits

- Automates data analysis, and simplifies MS^n data analysis of large-scale, complex proteomic studies through customizable workflows
- Improved Label-Free Quantitation
  - Minora Feature Detector node
    - Retention time alignment node
    - Feature Mapper and linking across files
- **Minimizes 'missing data points' and maximizes quantitative insights**
- Improved statistics and enhanced results views
  - p-values for peptides/protein groups
  - Principal components analysis, volcano plots, trend charts, chromatographic trace charts, etc.

Comprehensive data analysis platform for qualitative and quantitative proteomics research

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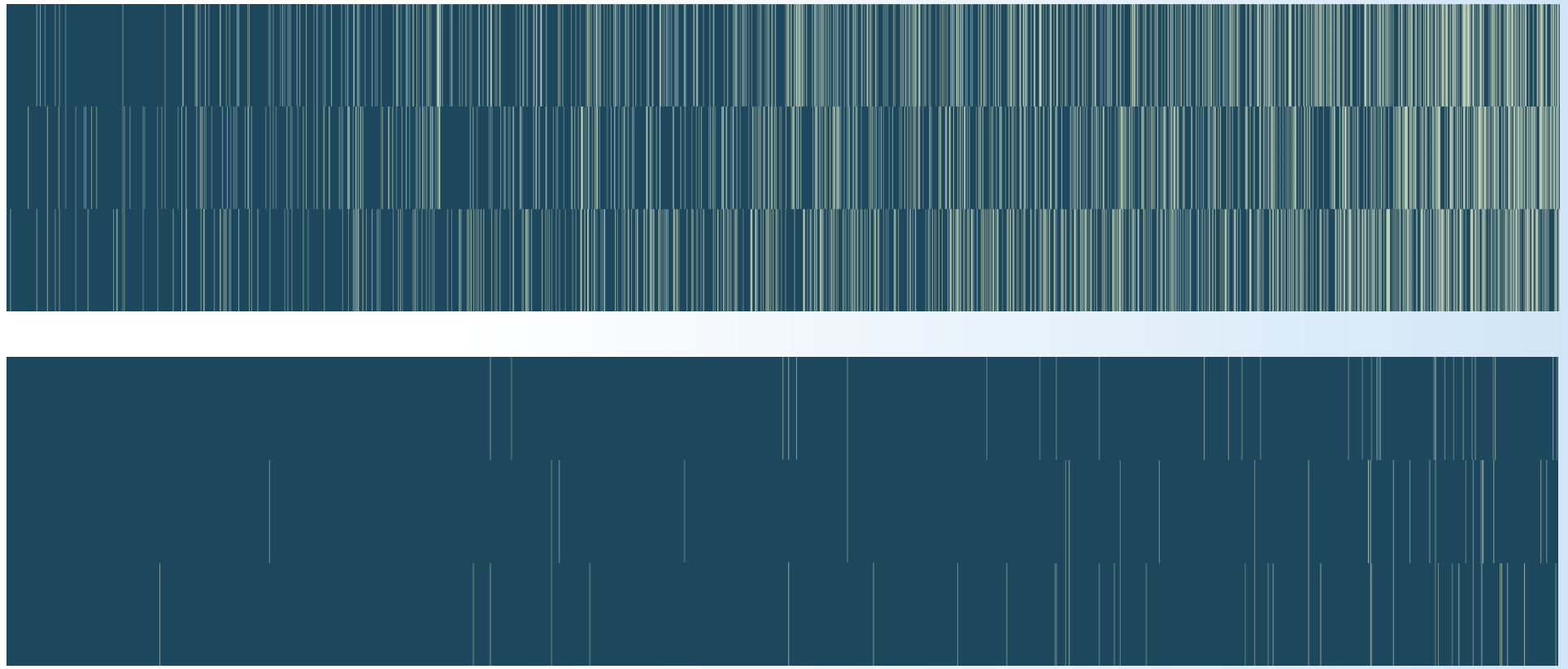
DDA+ : Maximize Quantitative Reproducibility and Reduce Missing Data Points

new

DDA+, 4ug HeLa, 60min, 120K/7.5K, Top 40

Completely quantified peptides



Missing data = sparse quantitation

Complete quantitation

50% quantified during MS

97% quantified from MS and PD 2.2

Quantitation → Precision → Reproducibility → Standardization

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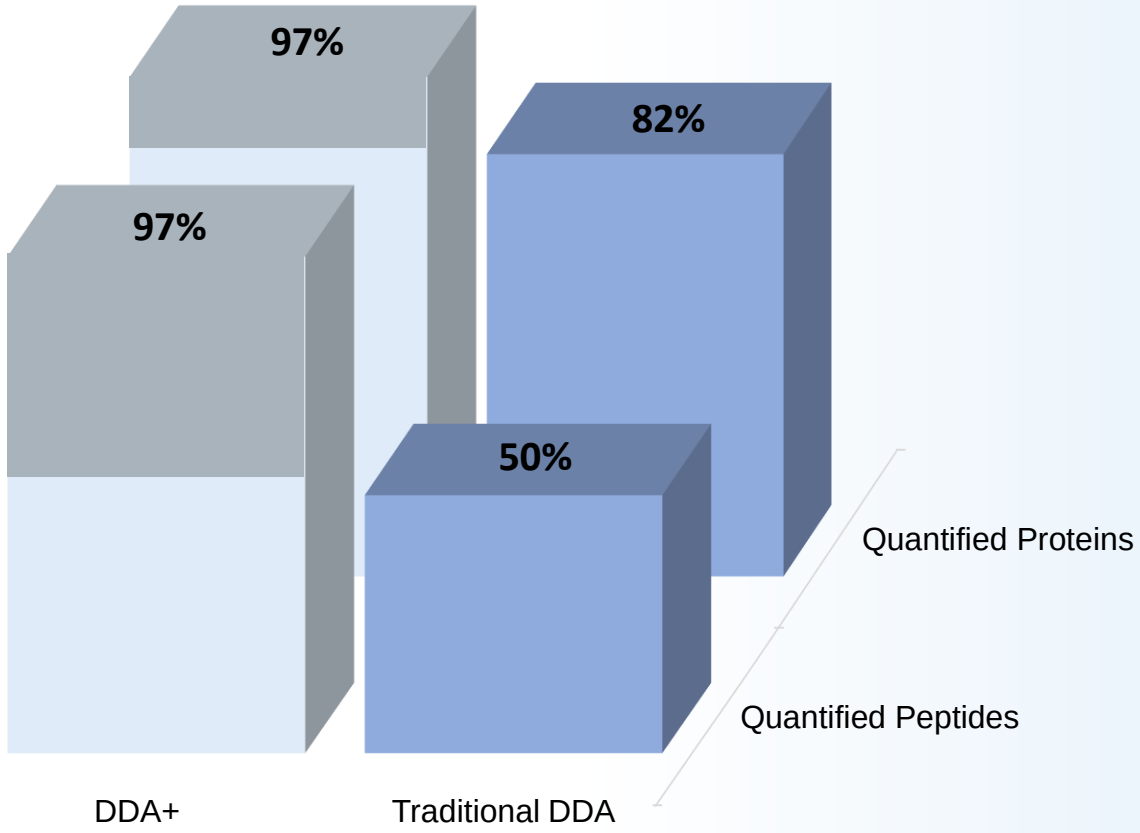
DDA+ Workflow Maximizes Quantitative Reproducibility Across Samples



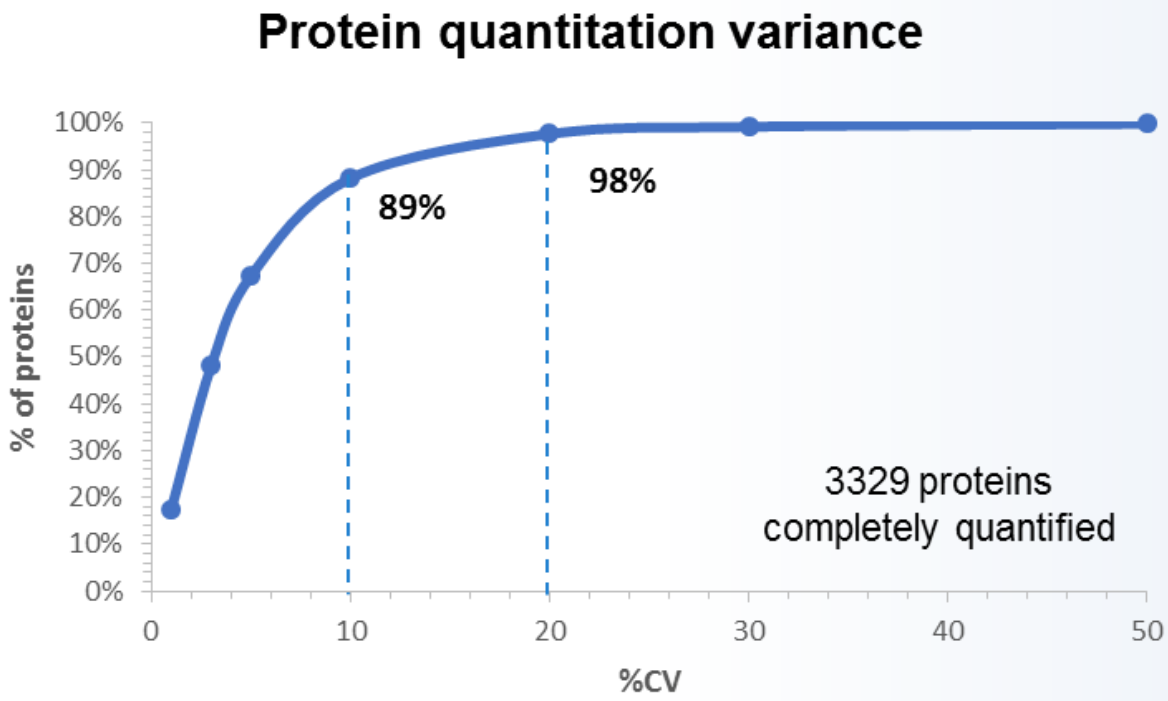
DDA+, 4ug HeLa, 60min, 120K/7.5K, Top 40

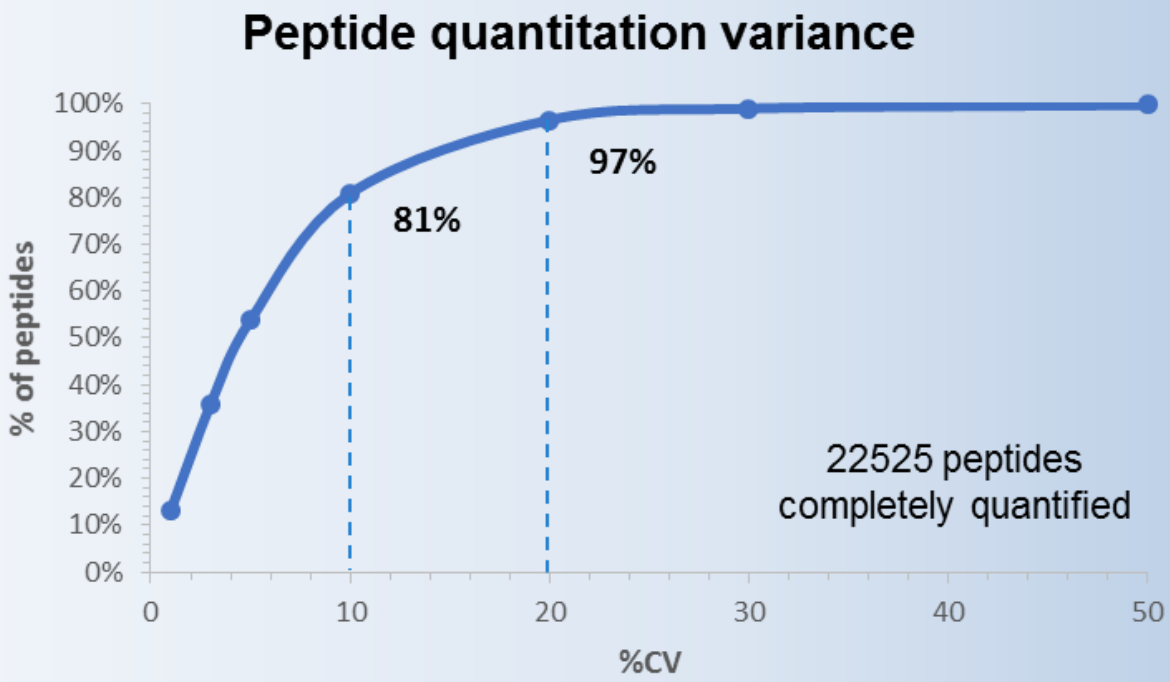
DDA+ workflow compared to DDA:

- 15% gain in completely quantified proteins
- 47% gain in completely quantified peptides
- Boosts quantitative information and enables comprehensive analysis
- **Minimizes 'missing data points' and maximizes quantitative insights**

DDA+ Enables Quantitative Confidence Through High Precision

DDA+, 4ug HeLa, 60min, 120K/7.5K, Top 40





Quantitation | **Precision** | Reproducibility | Standardization

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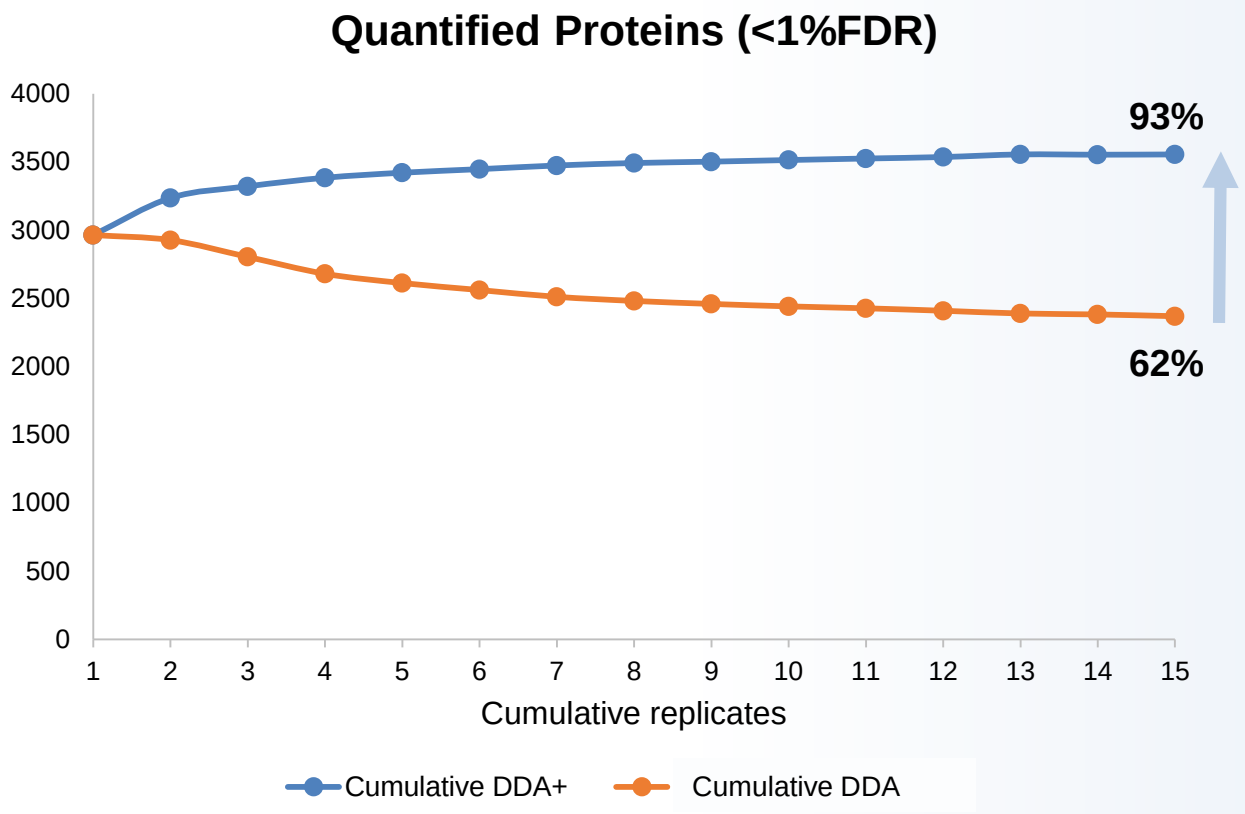
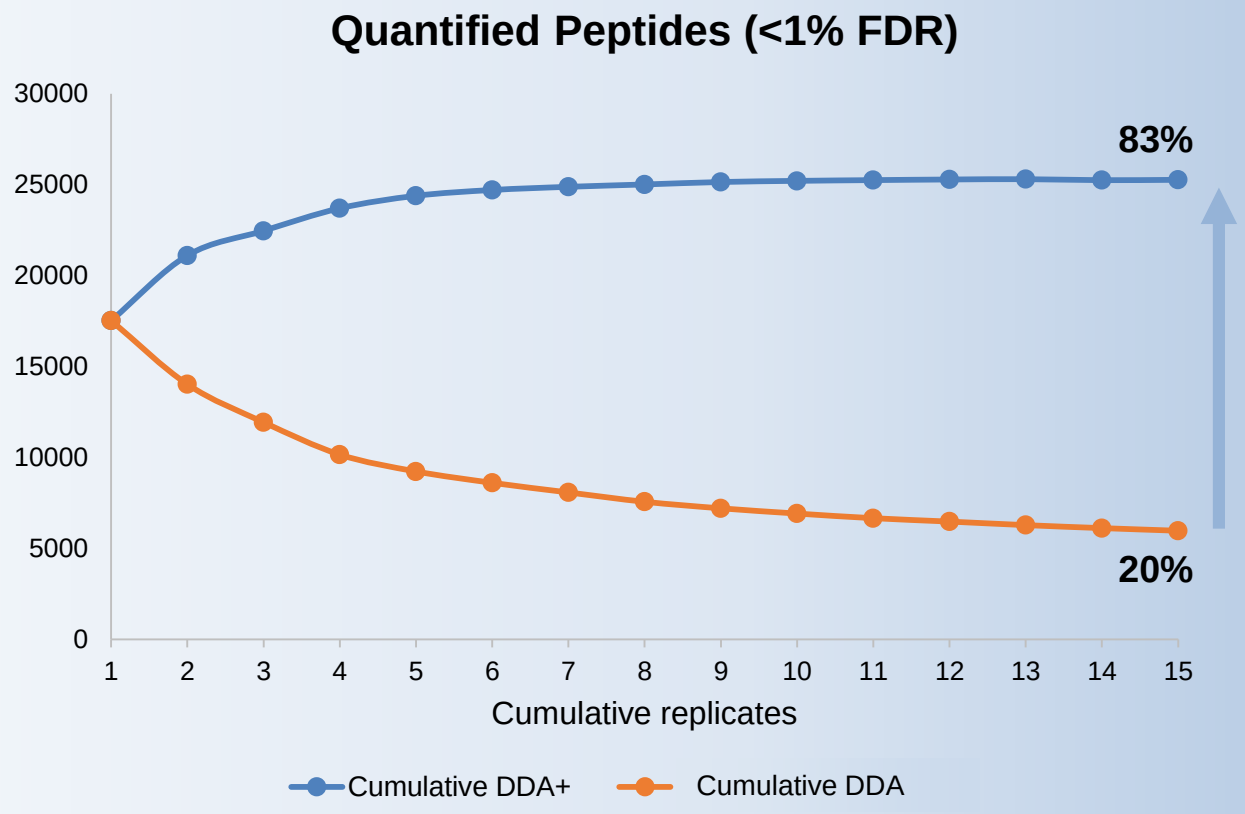
DDA+ Workflow Maximizes Quantitative Reproducibility Across Samples

DDA+, 4ug HeLa, 60min, 120K/7.5K, Top 40





Quantitation → Precision → **Reproducibility** → Standardization

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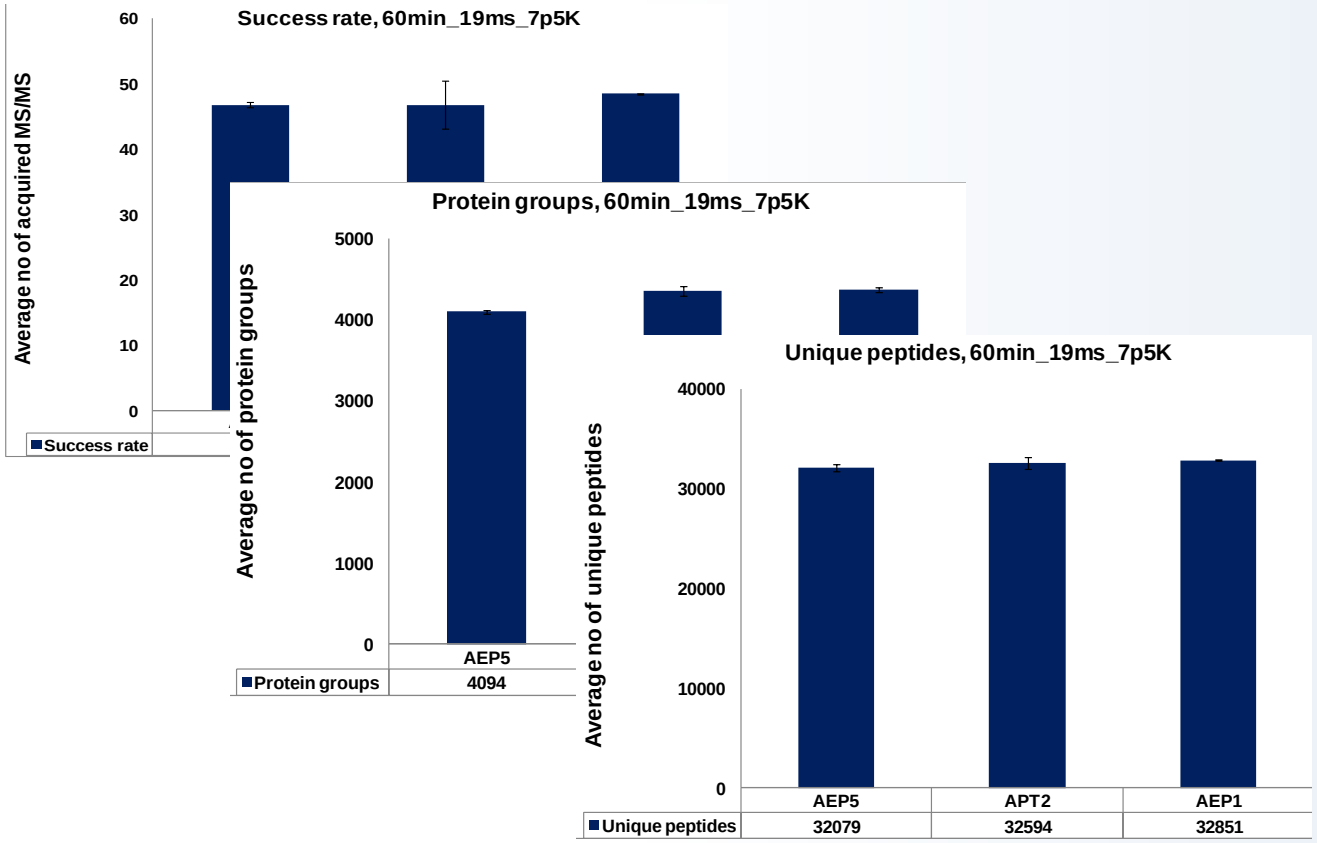
QE HF-X: Standardization With Reproducible Results Across Different Instruments



- Large-scale initiatives require standardization across many instruments and laboratories
- Transfer of methods and SOPs requires reliability and consistency
- Q Exactive HF-X demonstrated strong concordance of measurements across instruments and locations
  - Peptide identification efficiency of ~48% (PSM/total # of scans)
  - Protein group coverage of cell digest: 4271, 4% CV
  - Unique peptide groups: 32508, 1% CV

Quantitation → Precision → Reproducibility → **Standardization**

HR-DIA: Unparalleled Proteome Coverage and Reproducibility

new

Robust LC Separation

UltiMate 3000 RSLCnano

- *Nano, cap, micro-flow rate range*
- *Direct inject or pre-concentration mode*
- *Easy connections with nanoViper fittings*

150 µm EASY-Spray Columns

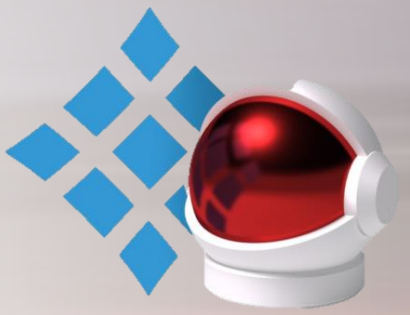
- *2 µm Acclaim PepMap*
- *Sensitivity and robustness*
- *RT stability <1% CV observed for 350 injections*

Optimized MS Acquisition

Q Exactive HF-X

- *Improved ion transmission*
- *Increased acquisition speed*
- *Advanced precursor determination*
- *Same # of protein IDs half the time*

Comprehensive Data Informatics



Spectronaut™

BIOGNOSYS

- *High-resolution MS1 quantitation*

Designed for comprehensiveness and reproducibility

ThermoFisher SCIENTIFIC

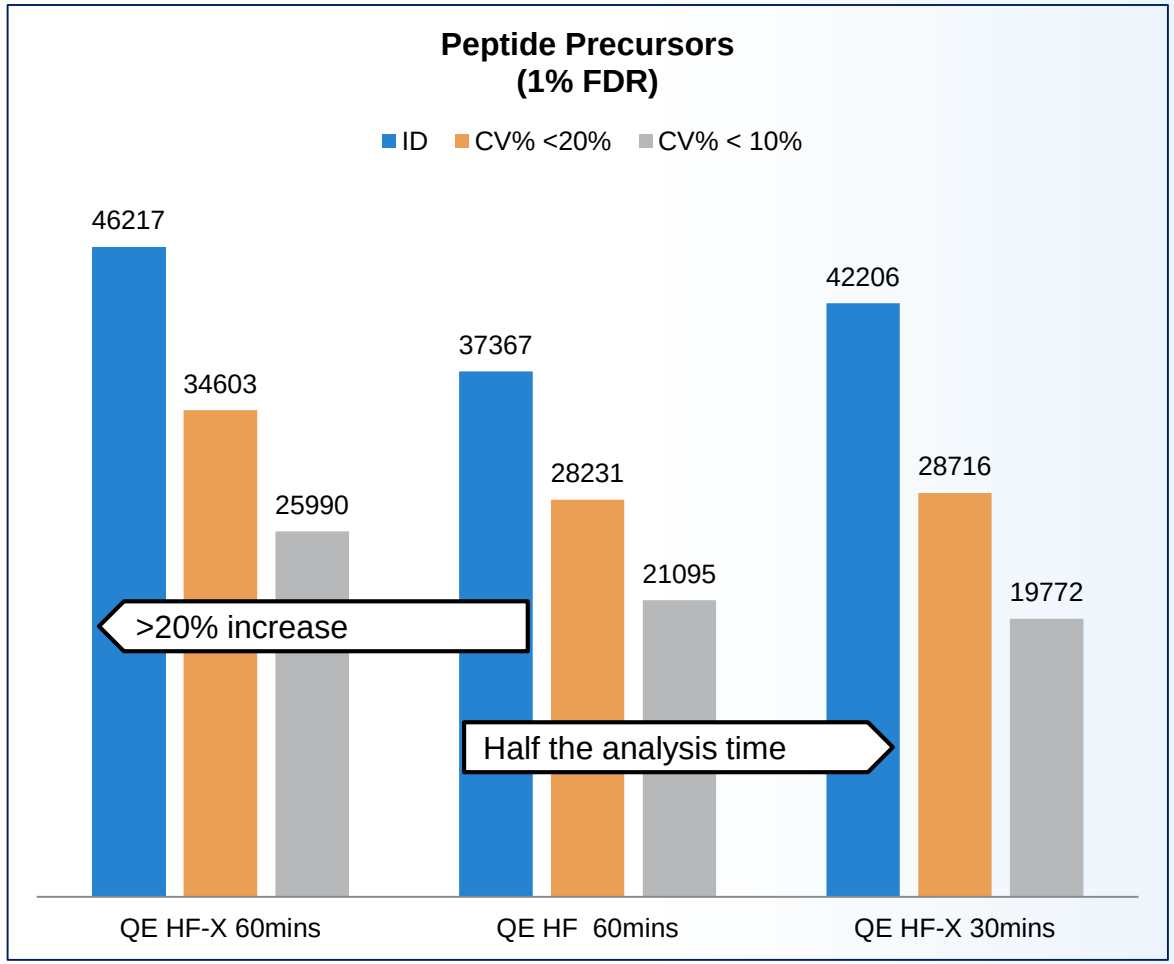
Spectronaut by Biognosys

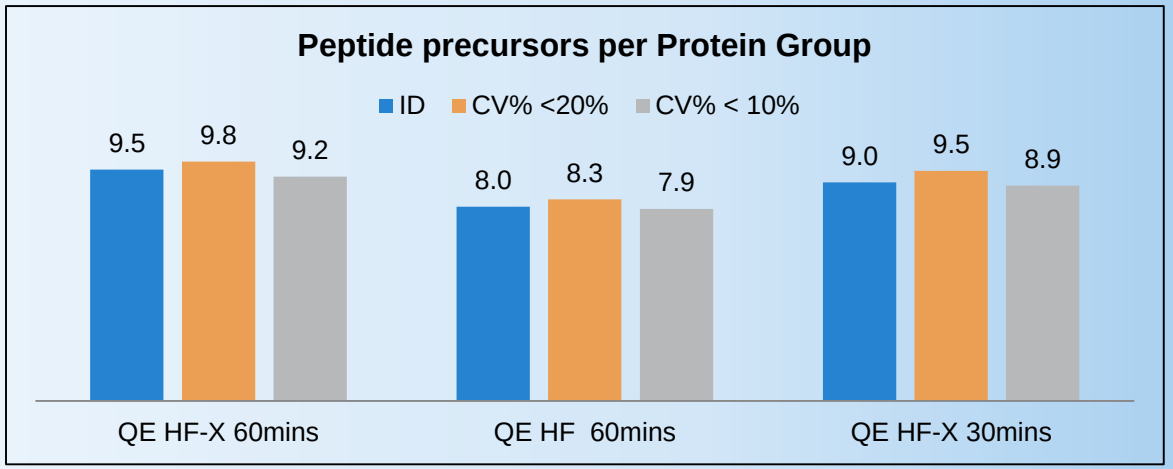
Key Benefits

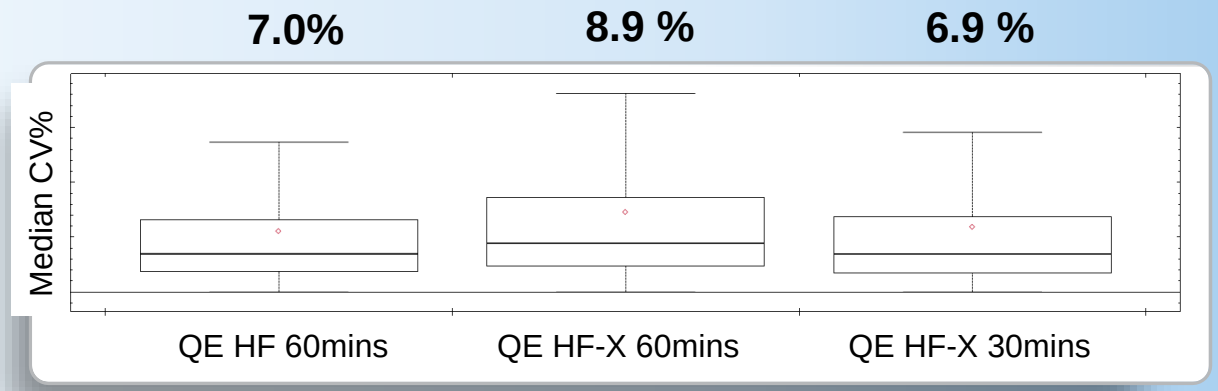
- Spectronaut software is specifically developed for the analysis of DIA data sets
- Data analysis with retention time correction based on spiked reference peptides - HRM calibration kit or iRT Kit
- Spectral library generation from Proteome Discoverer search results
- Powerful peak picking
- Direct visualization of qualitative and quantitative results on protein level
- Fast data analysis speed in **less than 2 min per run**

Designed for high throughput DIA data analysis

Double Your Throughput: Capillary LC DIA – QE HF-X vs QE HF







HR-DIA: 4ug HeLa, 120k MS1, 30K DIA,10 m/z (80 windows)

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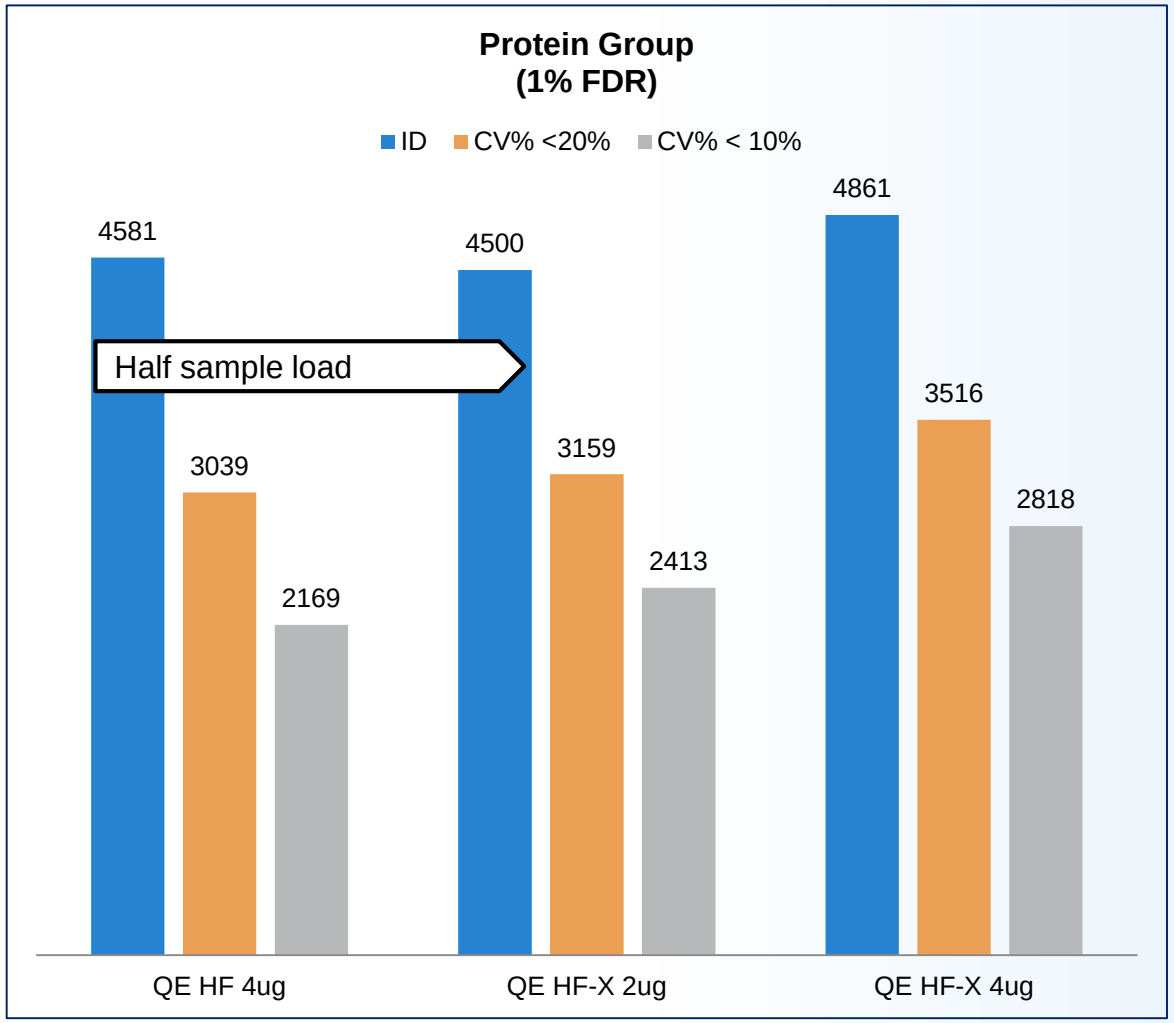
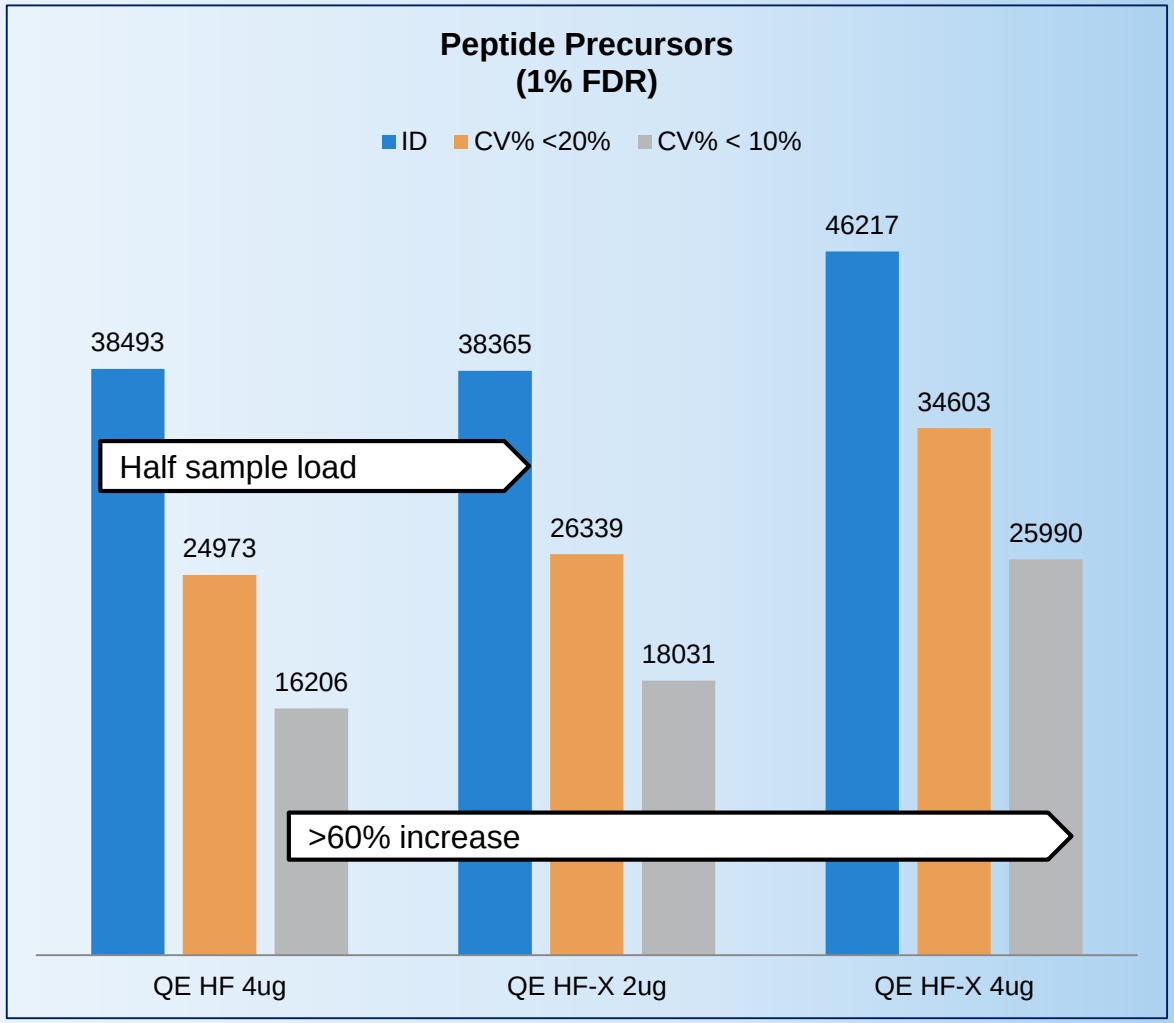
Double Your Sensitivity: Capillary LC DIA – Q Exactive HF-X vs HF





HR-DIA: 4ug HeLa, 120k MS1, 30K DIA,10 m/z (80 windows)

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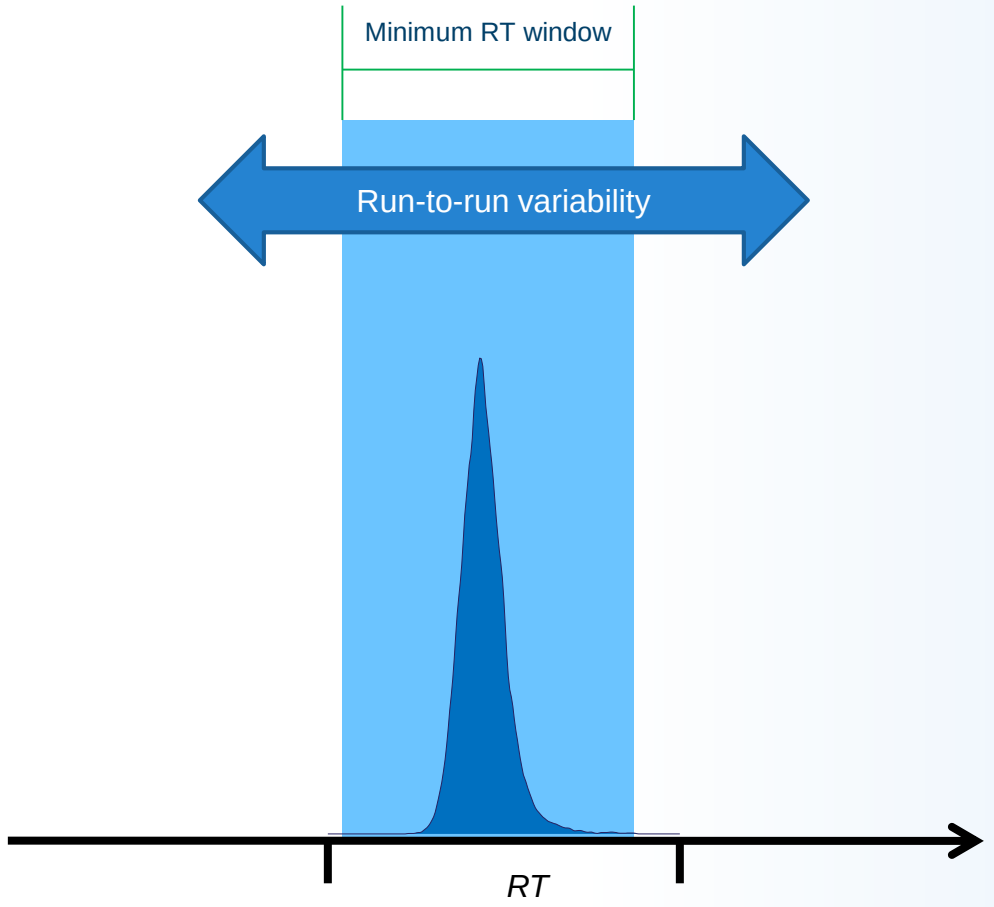
Dynamic Retention Time PRM (dRT- PRM)

new

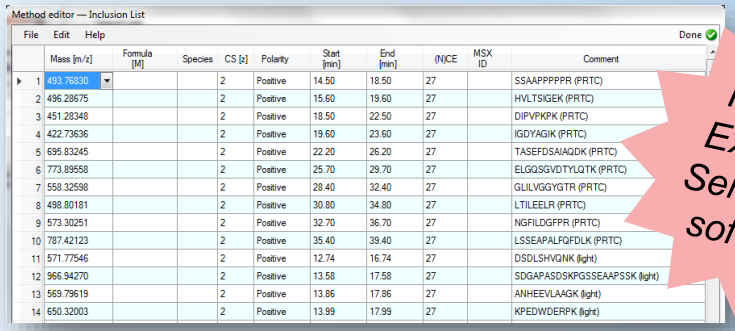
Dynamic retention time adjustment



dRT-PRM Workflow

Skyline

Pierce™ Peptide Retention Time Calibration Mixture as Landmark Peptides



	Mass [m/z]	Formula [M]	Species	CS [z]	Polarity	Start [min]	End [min]	(N)CE	MSX ID	Comment
1	493.76830			2	Positive	14.50	18.50	27		SSAAPPPPPR (PRTC)
2	496.28675			2	Positive	15.60	19.60	27		HVLTSIGEK (PRTC)
3	451.28348			2	Positive	18.50	22.50	27		DIPVPKPK (PRTC)
4	422.73636			2	Positive	19.60	23.60	27		IGDYAGIK (PRTC)
5	695.83245			2	Positive	22.20	26.20	27		TASEFDSAIAQDK (PRTC)
6	773.89558			2	Positive	25.70	29.70	27		ELGQSGVDTYLQTK (PRTC)
7	558.32598			2	Positive	28.40	32.40	27		GLILVGGYGTR (PRTC)
8	498.80181			2	Positive	30.80	34.80	27		LTILEELR (PRTC)
9	573.30251			2	Positive	32.70	36.70	27		NGFILDGFPR (PRTC)
10	787.42123			2	Positive	35.40	39.40	27		LSSEAPALFQFDLK (PRTC)
11	571.77546			2	Positive	12.74	16.74	27		DSDLSHVQNK (light)
12	966.94270			2	Positive	13.58	17.58	27		SDGAPASDSKPGSSEAAPSSK (light)
13	569.79619			2	Positive	13.86	17.86	27		ANHEEVLAAGK (light)
14	650.32003			2	Positive	13.99	17.99	27		KPEDWDERPK (light)

New in Exactive Series 2.9 software

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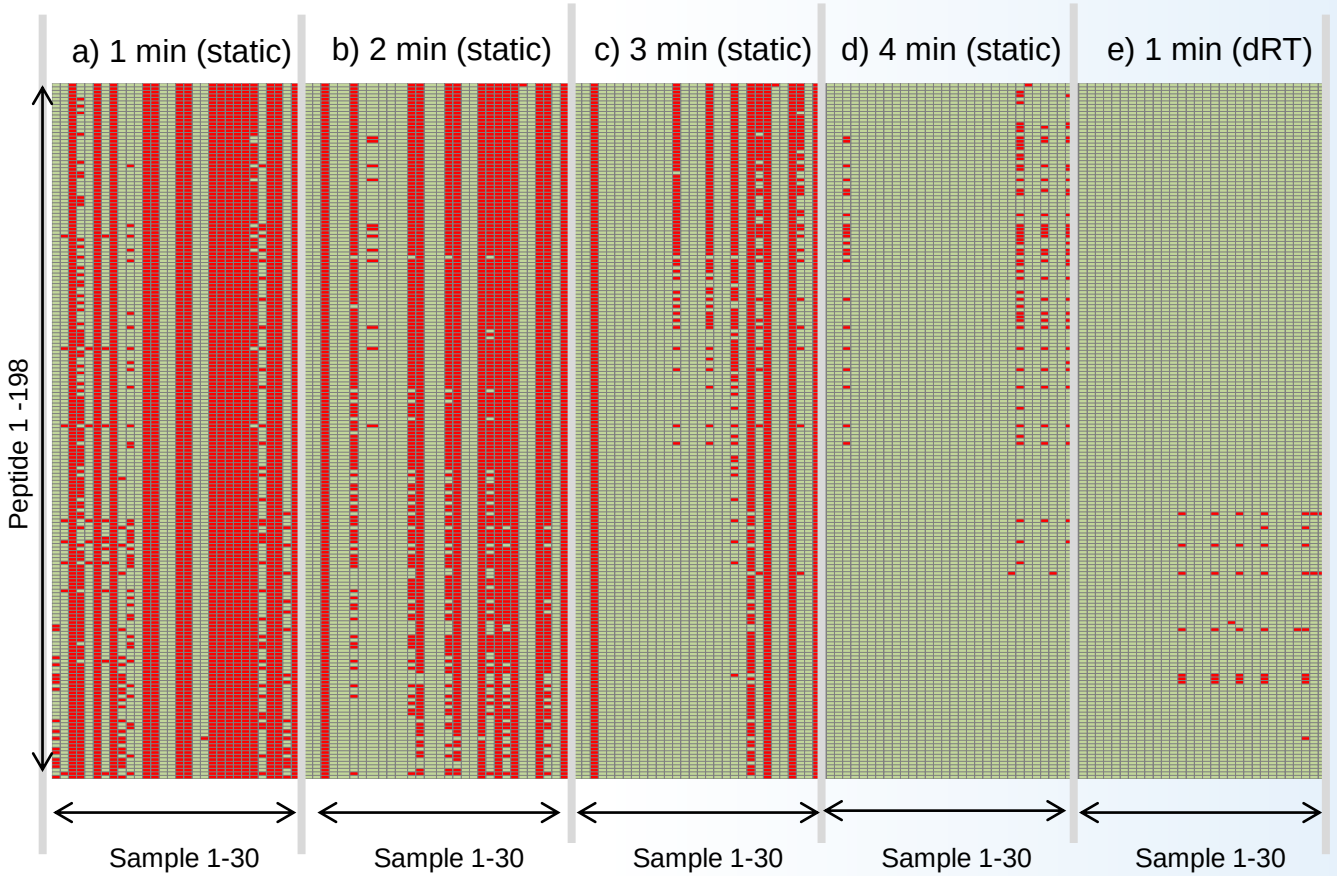
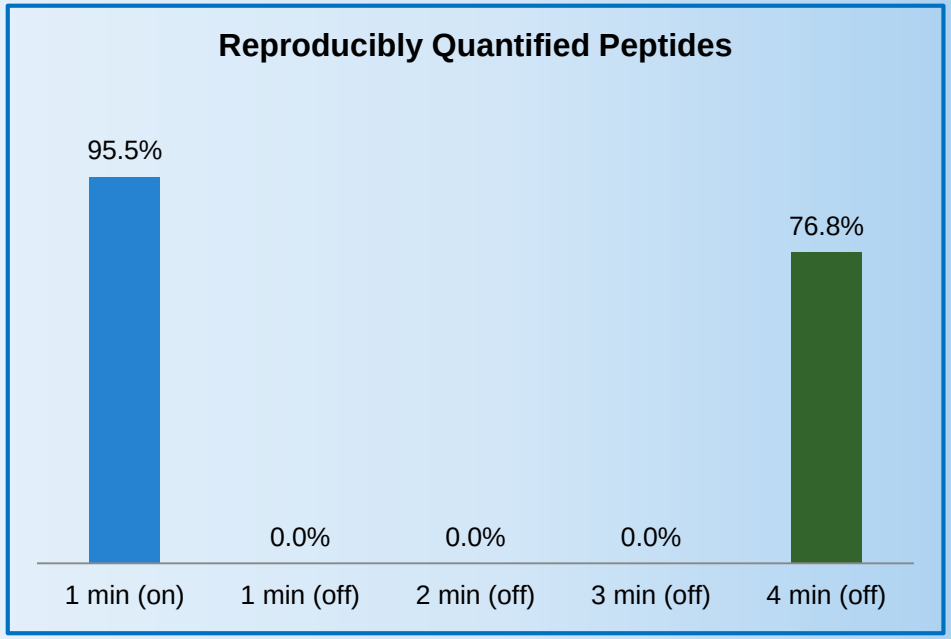
Robust and Reproducible Targeted PRM Quantitation



Peak within the RTW

Peak (part.) outside of the RTW

HeLa (500 ng/uL) was spiked with 50 fmol/uL PRTC-Kit peptides



- 198 peptides
- 5 different LC gradients
- Randomized technical replicates
- 30 technical runs each LC gradient

New in Exactive Series 2.9 software

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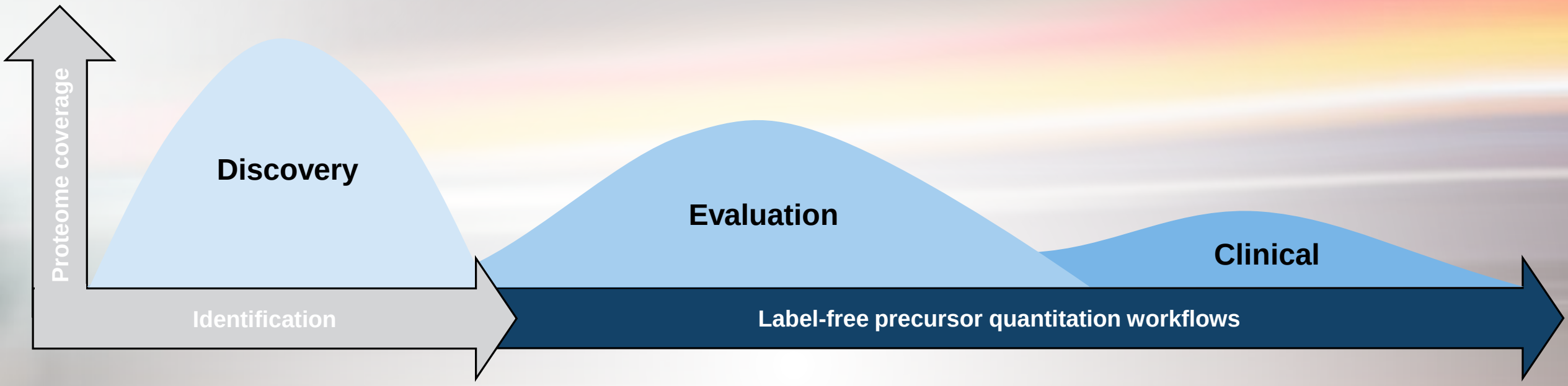
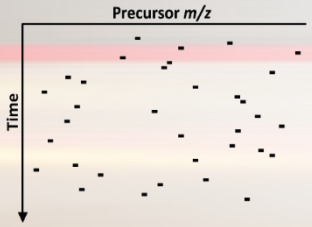
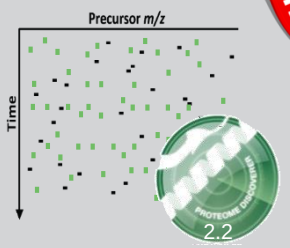
Workflow Solutions for Quantitative, Standardized, High-Throughput Proteomics

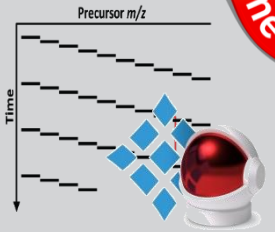




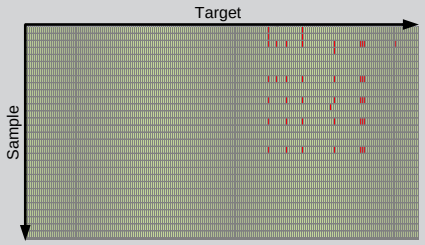
Data Dependent Acquisition (DDA)



Data Dependent Acquisition Plus (DDA+)



High Resolution MS1 DIA (HR-DIA)



Dynamic Retention Time PRM (dRT-PRM)

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Questions?

B-017: Novel capillary flow LC HRAM MS platform for fast targeted analysis and robust profiling of complex samples.

E-018: Revolutionary Proteome Profiling and Quantitation without Compromising Speed, Sensitivity, and Selectivity.

E-006: Towards comprehensive signaling pathway monitoring using advanced PRM methods

D-087: The Power of Multiplexing- Combining TMT discovery and targeted label free quantitation for biomarker analysis

A-077: Quantitative Analysis of Signaling Pathways Using 11plex TMT Reagents and Comprehensive Phosphopeptide Enrichment Strategies

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