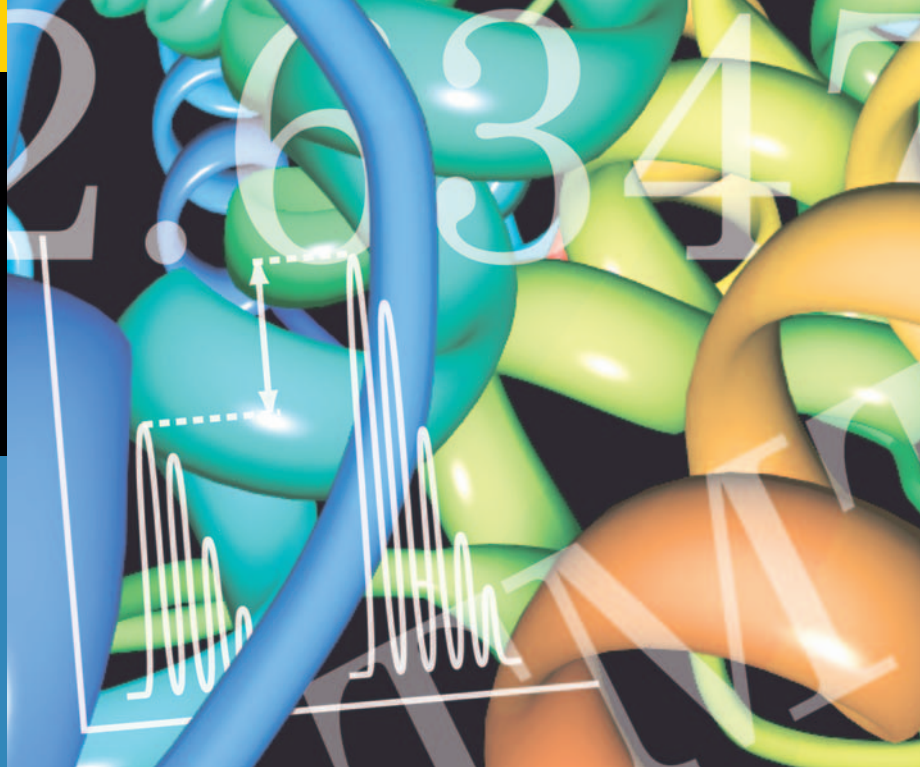


## Thermo Scientific Solutions for Quantitative Proteomics



*A leader in quantitative proteomics, we supply complete solutions that redefine the limits of quantitative proteomics and help you exceed your research goals.*

## Redefining Quantitative Proteomics

**TO UNDERSTAND** the functions of individual proteins and their place in complex biological systems, it is often necessary to measure changes in protein abundance relative to changes in the state of the system. As such, modern proteomics has evolved from an almost exclusively qualitative technique into one that spans a continuum of qualitative and quantitative approaches.

Traditionally, quantitative measurements of protein abundance were done by Western blotting. However, Western blotting almost always requires *a priori* knowledge of the system and the expected changes in order to obtain an appropriate target antibody. Antibodies are often not available, not specific, or very expensive. Antibodies for site-specific modifications can be even more difficult to obtain. In addition, Western blotting is sample intensive, has limited linear dynamic range, and typically only a single target is quantified in each Western blot.

Alternatively, liquid chromatography coupled to mass spectrometry (LC-MS) has emerged as a powerful technology for system-wide identification and quantitation of proteins. It can be used for both **discovery-based (untargeted)** and **targeted** determination of changes in protein abundance. It can also be used to measure changes in the abundance of protein-specific post-translational modifications (PTMs), facilitating location of the modified residue. Quantitative MS analyses require less sample than Western blotting and can detect and quantify multiple analytes in a single experiment.

### Discovery-based analyses identify and quantify

With discovery-based quantitative analyses, the goal is to both identify proteins and measure their abundance changes across multiple sample sets. Several discovery-based techniques have been developed, including stable isotope labeling by amino acids in cell culture (SILAC), chemical labeling with isobaric mass tags, and label-free.<sup>1,2,3</sup>

### Targeted analyses are fast and sensitive

Targeted analyses can be performed once candidate proteins are identified by either discovery-based MS experiments or alternate, *a priori* information. Targeted analyses provide improved quantitation sensitivity (LOQ) and, combined with increased speed of analyses, enable analysis of expanded sample sets to assess the validity of the candidate proteins. Spiking biological samples with proteotypic, isotopically labeled peptide standards makes possible the absolute quantitation of each protein or post-translational modification of interest.

Choosing the most appropriate quantitative proteomics technique depends on experimental demands and instrumental capabilities. This brochure is intended to assist the decision-making process.

Technique	Type	Number Samples (per LC-MS run)	Precision (% CV)	Benefits	Drawbacks	Instruments
<b>Discovery-based (untargeted quantitative analysis coupling protein identification with quantitation)</b>						
Label-Free	Relative	1	< 30	<ul style="list-style-type: none"> <li>Applicable to any sample type</li> <li>Cost-efficient sample preparation</li> <li>Minimal sample handling</li> </ul>	<ul style="list-style-type: none"> <li>Each sample runs individually (low throughput)</li> <li>Requires extremely reproducible LC separations</li> <li>Requires multiple technical replicates</li> </ul>	Orbitrap
TMT	Relative	2 to 6	< 20	<ul style="list-style-type: none"> <li>Applicable to any sample type</li> <li>Multiplexing increases MS throughput</li> </ul>	<ul style="list-style-type: none"> <li>Requires extensive fractionation or long chromatographic gradients</li> </ul>	Orbitrap with HCD Ion Trap with PQD or Trap-HCD
SILAC	Relative	2 or 3	< 20	<ul style="list-style-type: none"> <li>Least susceptible to inter-sample variations in sample handling and preparation</li> <li>Multiplexing increases MS throughput</li> </ul>	<ul style="list-style-type: none"> <li>Only readily applicable to cell cultures</li> <li>Increases MS spectral complexity</li> </ul>	Orbitrap
<b>Targeted (analysis of predetermined peptides from discovery-based experiments or literature)</b>						
HR/AM-SIM	Relative or Absolute	1	< 20	<ul style="list-style-type: none"> <li>Uses the same MS system as discovery quantitation</li> <li>Easy method development using Pinpoint software</li> </ul>	<ul style="list-style-type: none"> <li>Requires reproducible LC separations</li> </ul>	Orbitrap
iSRM	Relative or Absolute	1	< 10	<ul style="list-style-type: none"> <li>Up to 15,000 SRM transitions per run</li> <li>Simultaneous protein quantitation and confirmation of identity</li> <li>Suitable for determining the absolute quantity of a protein in a complex biological matrix</li> <li>Easy method development using Pinpoint software</li> </ul>	<ul style="list-style-type: none"> <li>Requires reproducible LC separations</li> </ul>	TSQ triple quadrupole

Discovery-based and targeted quantitation techniques all have specific applications and advantages

# SILAC Improves the Throughput of Discovery-Based Quantitative MS Analyses and Increases Accuracy of Results

**STABLE-ISOTOPE LABELING BY AMINO ACIDS IN CELL CULTURE (SILAC)** is a powerful and widely used method of identifying and quantifying relative changes in complex protein samples. It can be applied to complex biomarker discovery and systems biology studies as well as to isolated proteins and protein complexes.

As its name implies, SILAC involves labeling protein samples *in vivo* by substituting an isotopically heavy form of an amino acid for the naturally occurring light

form. Because labeled and unlabeled samples are combined during the initial steps of sample preparation, SILAC minimizes the quantitative error inherent in handling separate samples in parallel. In addition, the mixing of samples permits a variety of enrichment techniques including immunoprecipitation. These can improve the detection of abundance changes for both low-abundance proteins and post-translational modifications such as phosphorylation or glycosylation.

## SILAC WORKFLOW

### Cell Cultures

2-3 samples grown in light- and heavy-labeled amino acid media



Combine cells

Extract proteins

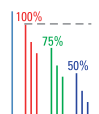
Denature, reduce, alkylate, and digest

LC-MS/MS

EASY-nLC

Orbitrap technology

MS



Automated data analysis using Proteome Discoverer software

Quantitation is performed at the MS-level by comparing the intensities of the light- and heavy-labeled precursor ions

MS/MS



Proteins are identified using the accurate-mass precursor information and the CID, HCD or ETD fragmentation data



Thermo Scientific Pierce SILAC protein quantitation kits contain all of the reagents needed for comparing two or three sample types in a wide variety of mammalian cell lines. They are compatible with many Thermo Scientific protein/peptide enrichment technologies.

## KEY POINTS

### Advantages:

- 2-3 samples
- Reduced sample handling decreases variability
- Data analysis using Proteome Discoverer software

### Disadvantages:

- Cell cultures only
- High-resolution MS (FT or Orbitrap technology) required



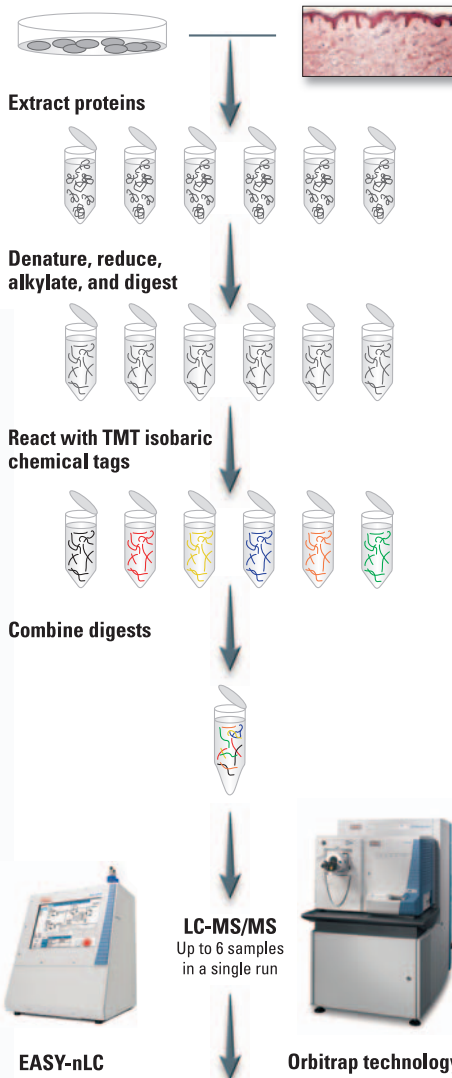
# TMT Labeling Increases the Number of Samples Analyzed, and Peptides Identified and Quantified, in a Single Analysis

**ISOBARIC CHEMICAL TAGS** are a more universal alternative to SILAC for simultaneous identification and quantitation of proteins in multi-sample sets. They can facilitate relative quantitation of a wide variety of samples including cells, tissues, and biological fluids.

Thermo Scientific Tandem Mass Tag (TMT) reagents are isobaric mass tags consisting of an MS/MS reporter group, a spacer arm, and an amine-reactive group. Amine-reactive groups covalently bind to peptide N-termini or to lysine residues. Each tag fragments during MS/MS, producing unique reporter ions. Protein quantitation is accomplished by comparing the intensities of the reporter ions.

## TMT WORKFLOW

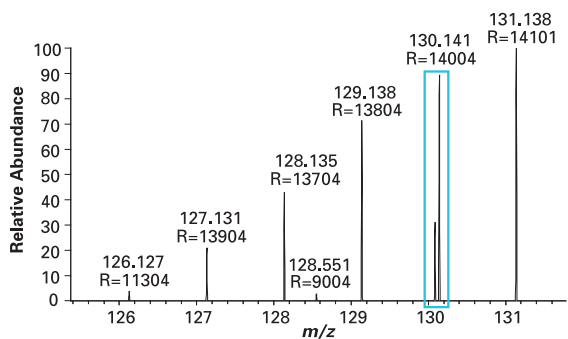
Cell Culture or Tissue  
2-6 samples



### KEY POINTS

**Advantages:**

- 2-6 samples
- Any sample type
- Ion trap- or Orbitrap-based instruments
- Data analysis using Proteome Discoverer software



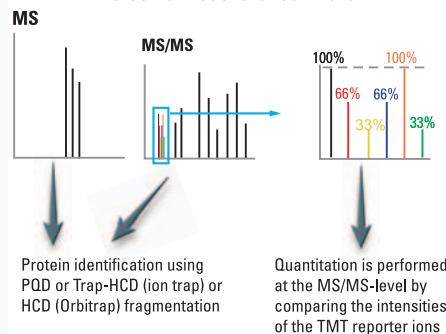
### High-resolution HCD-MS/MS improves quantitative precision in TMT® analyses

The ability to generate low-*m/z* reporter ions and to distinguish them from isobaric interferences is essential for consistent, precise TMT quantitation. This is best accomplished using HCD fragmentation combined with the high-resolution-at-low-*m/z* detection that is available on Orbitrap™-based systems.



TMT kits are available in 2-plex for comparing two samples in small profiling studies. They are also available in 6-plex for comparing up to six samples in complex analyses with multiple conditions (e.g. time courses, dose responses, replicates, and multiple-sample comparisons). TMT kits can be combined with many Thermo Scientific peptide enrichment technologies.

### Automated data analysis using Proteome Discoverer software



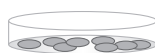


# Label-Free Quantitation Enables Exploration of the Proteome in Greater Depth

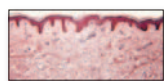
**LABEL-FREE DIFFERENTIAL ANALYSIS** has gained popularity for discovery-based quantitative proteomics. It requires no specific sample preparation and accommodates large numbers of diverse samples. Label-free quantitation involves extracting peptide chromatograms from LC-MS/MS runs and integrating peak areas over the chromatographic time scale. Typically favored for shotgun proteomics, the label-free approach has been incorporated into large-scale biomarker discovery studies measuring disease-related changes. It has demonstrated high reproducibility and linearity at both peptide and protein levels.<sup>3</sup>

## LABEL-FREE WORKFLOW

**Cell Culture or Tissue**  
Unlimited number of samples



**Extract proteins**



**Denature, reduce, alkylate, and digest**



**LC-MS/MS**

Each sample run individually  
Reproducible chromatography and high-resolution MS are required



**EASY-nLC**



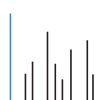
**Orbitrap technology**

**Automated data analysis using SIEVE software**



Quantitation is performed at the MS-level by comparing chromatographic peak areas for precursor ions between individual raw files

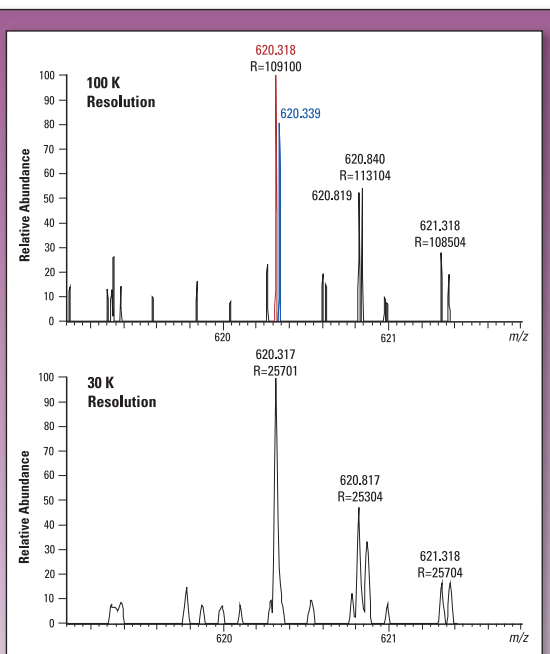
**MS/MS**



Protein identification using the accurate-mass precursor information and the CID, HCD or ETD fragmentation data

Each of the highly complex samples found in a typical label-free differential analysis study is run individually. Therefore, meticulous sample handling, sample preparation, reproducible chromatography between technical and biological replicates, and sensitive, high-resolution, accurate-mass MS are all essential. Thermo Scientific solutions for label-free differential analysis are complete and integrated. Thermo Scientific EASY-nLC systems use a split-free design to achieve exceptional stability and reproducibility. They easily couple to all Thermo Scientific mass spectrometers, including the Orbitrap-based systems that have the high resolution necessary for successful label-free quantitation.

Thermo Scientific SIEVE software enables researchers to analyze label-free data according to common, predefined experimental designs such as two-group randomized controlled studies or single-group longitudinal studies. Trend analysis can be performed to detect changes associated with dosage effects or time points for the comparison of multiple classes of samples.



### High-resolution MS is required to distinguish isobaric co-eluting peptides

The simultaneous high resolution (>100,000) and spectral dynamic range afforded by Orbitrap technology enable label-free quantitation by providing essential discrimination between isobaric co-eluting ions and separating analyte signal from noise. With 30K resolution, it is impossible to resolve the co-eluting isobaric peaks at  $m/z$  620.317. However, 100K resolution reveals two distinct peaks at  $m/z$  620.318 and  $m/z$  620.339.

## KEY POINTS

### Advantages:

- Unlimited number of samples
- Any sample type
- Data analysis using SIEVE software

### Disadvantages:

- Multiple technical replicates
- Reproducible chromatography is essential
- High-resolution MS (Orbitrap technology) required

## Targeted Protein Quantitation: Improving Sensitivity, Accuracy, and Throughput

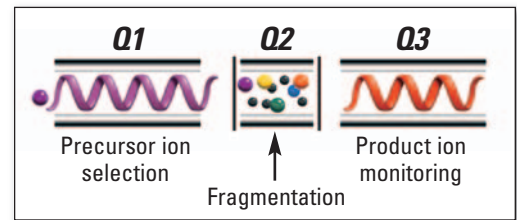
**MASS SPECTROMETRY-BASED TARGETED QUANTITATION** is widely used to determine relative or absolute abundances of peptides of interest. It provides a high degree of accuracy and sensitivity, and allows the profiling of hundreds of targets in a single experiment. It is a powerful, more-flexible alternative to time- and resource-intensive, enzyme-linked immunosorbent assays (ELISAs).

Targeted quantitation is frequently applied to large sample sets to validate putative biomarkers identified in earlier discovery experiments or to analyze wide-scale changes in biological systems. While target peptides are often selected through analysis of data from previous MS/MS discovery experiments, hypotheses and *a priori* knowledge of targeted systems can also be used to select proteotypic peptide targets.

Targeted quantitation is most frequently associated with triple quadrupole mass spectrometers such as the Thermo Scientific TSQ family, but can also be accomplished using the high resolution, mass accuracy, and MS/MS capabilities of Orbitrap-based hybrid mass spectrometers.<sup>4</sup>

### Triple quadrupoles and iSRM – simultaneous quantitation and identity confirmation with high sensitivity and throughput

Selected reaction monitoring (SRM) on a triple quadrupole mass spectrometer provides an outstanding combination of specificity, sensitivity, accuracy, precision, and reliability. Up to 250 peptide-specific pairs of precursors and product ions can be monitored per second. Thermo Scientific TSQ Quantum Ultra and Vantage mass spectrometers are the only triple quadrupoles capable of using 0.2-Dalton resolution for precursor ion selection. This dramatically reduces chemical interferences while maintaining high transmission efficiency.<sup>5</sup>



Selected reaction monitoring on a triple quadrupole mass spectrometer provides an outstanding combination of specificity, sensitivity, accuracy, and reliability.

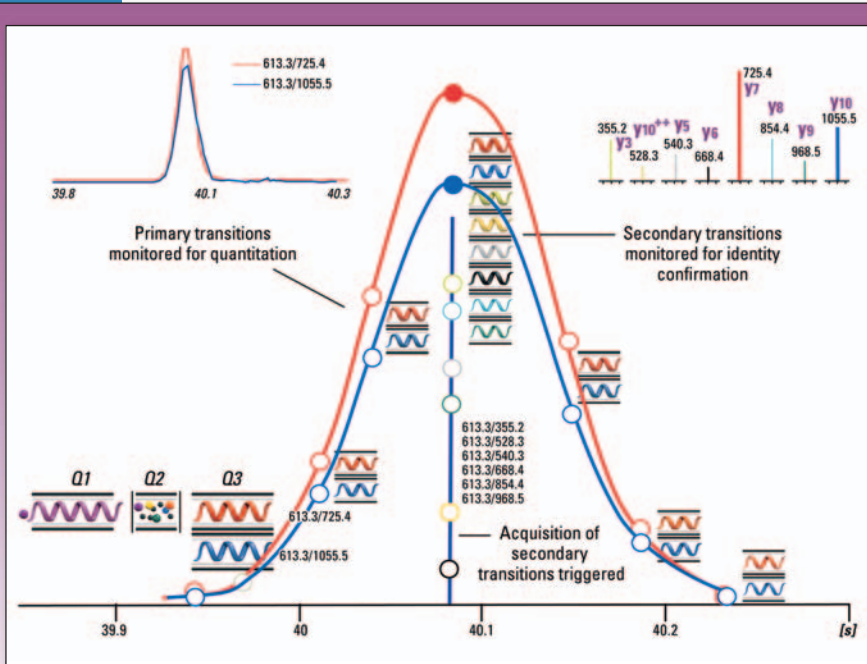
### KEY POINTS - SRM

#### Advantages:

- Higher throughput
- Higher MS dynamic range
- Synthetic peptides available for spectral library building

#### Disadvantages:

- Larger peptides fragment poorly
- Internal His, Arg, and/or Lys can divide precursor signal
- May not have spectral libraries for ion ratio determination



Intelligent prioritization of SRM increases sensitivity, throughput, and precision by monitoring a few primary SRM transitions for quantitation. The measured intensity of the primary SRM transitions are used to trigger acquisition on an expanded set of secondary SRM transitions for identity confirmation.

Thermo Scientific quadrupole mass spectrometers incorporate exclusive *intelligent* SRM (iSRM) technology. iSRM achieves selectivity and specificity using two interdependent steps.<sup>6</sup> The first step provides compound-specific quantitation by using time-based SRM (t-SRM) to monitor several primary transitions for each peptide. Once all primary transitions reach a user-specified threshold, the second step is initiated. Additional (secondary) transitions are monitored to create a composite MS/MS spectrum for peptide sequence confirmation. Through this intelligent use of data acquisition cycles, iSRM uniquely enables confirmation of peptide identity at SRM-level sensitivity without sacrificing throughput. Simultaneous quantitation and identity confirmation of as many as 1,000 peptides can be accomplished in a single LC-MS/MS run.

## Easier transition to targeted quantitation with high resolution and accurate mass

Proteomic discovery experiments are most often performed on high-resolution, accurate-mass (HR/AM) Orbitrap mass spectrometers. Using the same instrument for targeted quantitation can simplify the transition to targeted quantitation methods.

Thermo Scientific Orbitrap-based hybrid mass spectrometers can simultaneously quantify and confirm identification of multiple targeted proteins/peptides even in complex biological matrices. High-resolution, accurate-mass selected ion monitoring (HR/AM SIM) in the Orbitrap mass analyzer provides accurate quantitation. Fast, sensitive MS/MS analysis of targeted peptides is used to confirm identities. The high resolution, mass accuracy, and MS/MS mass range up to 4000  $m/z$  enable targeting larger, non-tryptic and heavily modified peptides using the same acquisition methods applied to smaller, unmodified tryptic peptides.



Targeted quantitation can also provide absolute quantitation of specific proteins by incorporating appropriate stable-isotope-labeled peptides as internal standards.<sup>5,7</sup> Custom-made Thermo Scientific HeavyPeptide AQUA Ultimate, QuantPro and Basic peptides offer solutions for both absolute and relative quantitation of proteins by MS, while tailored crude peptide libraries like Thermo Scientific PEPotec SRM support the development of SRM assays in MS workflows.

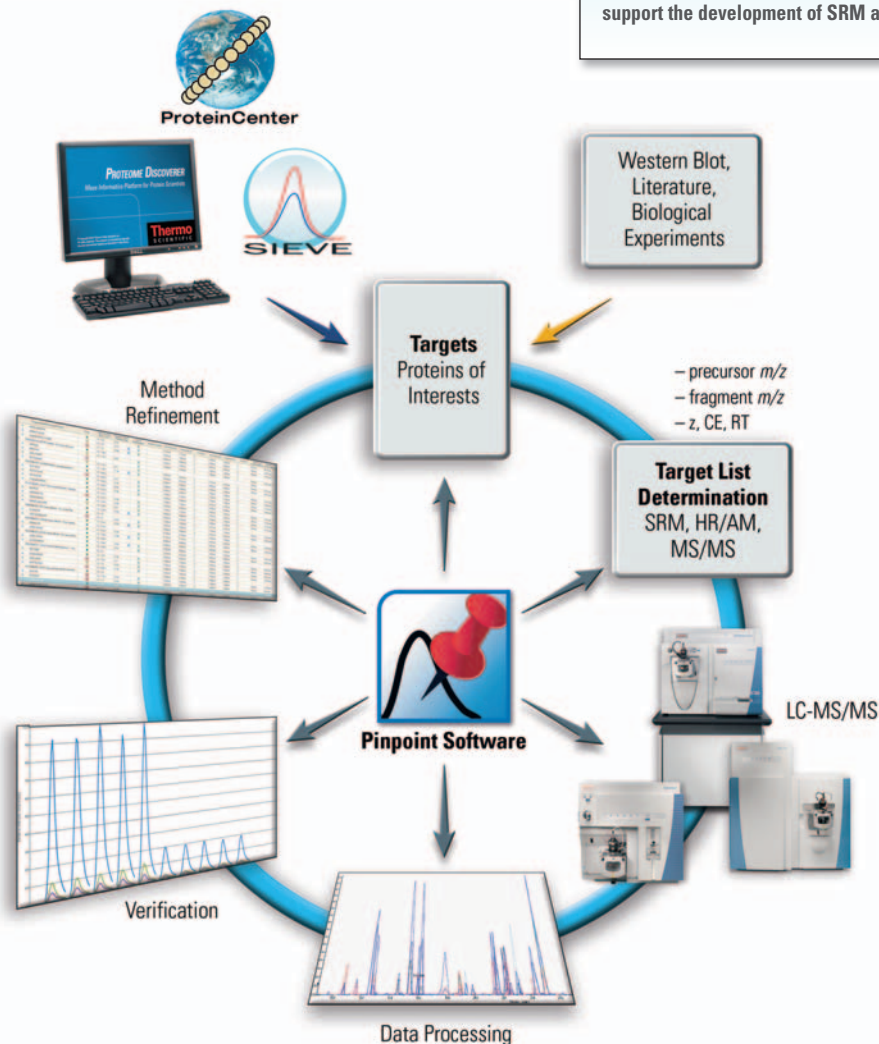
### KEY POINTS - HR/AM SIM

#### Advantages:

- Leverages multiple charge states
- Can monitor larger peptides
- Simultaneous qual/quant

#### Disadvantages:

- Peak capacity considerations are essential
- Less efficient for smaller peptides/low  $m/z$  ranges
- Lower throughput



## Pinpoint software facilitates targeted quantitation

Thermo Scientific Pinpoint software simplifies the creation of targeted assays and facilitates the transition from discovery to targeted quantitation. For triple quadrupoles, it can identify peptide targets, determine the best SRM transitions, and build the instrument method using data from discovery experiments or its own internal prediction engine. For Orbitrap-based instruments, it can identify the peptide targets and build the instrument method with scheduled MS/MS for identity confirmation. Pinpoint software includes tools for data analysis and iterative method refinement, allowing target peptides with the best responses to be identified and retained in a final method. This ensures that instrument resources are used to analyze only peptides that provide high-quality quantitation. Pinpoint software interfaces seamlessly with Thermo Scientific ProteinCenter software.

Pinpoint software facilitates many tasks within quantitative proteomics workflows



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