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SureQuant intelligence-driven MS: a new paradigm for targeted quantitation

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Introduction to traditional, targeted LC-MS approaches

While mass spectrometry (MS) proteomics is often envisioned as an unbiased discovery platform for protein quantitation, many users utilize the technology for directed monitoring of relevant predefined targets. This is becoming especially true with the rise of proteomics research in clinical validation, translational medicine, and biological studies that seek to precisely quantify known pathways and biomarkers of health and disease. The requirement for reliable and sensitive quantitation of a defined set of analytes has spawned new advances in liquid chromatography, mass spectrometry (LC-MS) approaches. Historically, the gold standard for LC-MS targeted quantitation has been selected reaction monitoring (SRM) workflows using a triple quadrupole mass spectrometer.[1] These workflows differ from discovery-based analyses since the goal is not to comprehensively survey and identify all the components of a sample but rather to monitor and quantify a selected panel of targets with the highest sensitivity, specificity, accuracy, precision and reproducibility. For this purpose, triple quadrupole mass spectrometers are typically programmed to monitor specific unique ions derived from the target of interest and to devote the entire analysis time towards measurement of those surrogate ions [Figure 1a].

For example, peptides representing proteins of interest can be selected to generate an ‘assay’ for their routine detection and targeted quantification. In this case, the first and third quadrupoles (Q1 and Q3) act as dual mass filters allowing selected peptide ions (i.e., precursor ions) to be isolated, fragmented, and their resulting specific fragment ions (i.e., product ions) to be isolated and detected. The recorded trace of signal intensity versus retention time (RT) for precursor-product ion pairs (i.e., transition) can be used to quantify the target peptide, and by extension, the target protein. The addition of a reference synthetic isotope-labeled peptide, to be used as an internal standard (IS), into the sample mixture allows ‘absolute’ quantification of the endogenous peptide to be derived from the ratio of unlabeled to labeled peak area. The use of SRM has been widely adopted for a variety of targeted quantification applications owing to its relative simplicity, speed and relatively straightforward data analysis. Targeted SRM assays do not require time-consuming and expensive antibody development and selection, so this approach

inherently offers significant time and cost savings over antibody-based assays. However, SRM has several limitations including low mass resolution of the quadrupole filters. In complex mixtures such as biological matrices, this can limit the selectivity of measurements since near isobaric interferences cannot be sufficiently discriminated from true analyte signal.[2] This selectivity issue can manifest as reduced signal-to-noise ratio (S/N) and reduced limits of quantitation (LOQ) for target signals, especially in complex matrices, thereby limiting the measurement sensitivity.

Many of the limitations associated with SRM assays can be addressed using high-resolution, accurate-mass (HRAM) mass spectrometers. Orbitrap-based mass spectrometers with quadrupole mass filters are an ideal platform for these types of experiments significantly improving the overall selectivity and sensitivity of targeted experiments. The approach taken on these instruments is referred to as parallel-reaction monitoring (PRM). PRM methodology uses the quadrupole of the mass spectrometer to isolate a target precursor ion, fragment it in the collision cell, and then detect all the resulting product ions simultaneously in the mass analyzer. Quantification is carried out by extracting and integrating one or more fragment ions traces with 5–10 ppm mass windows and comparing this information across multiple sample sets. PRM experiments on HRAM systems differs by the ability to monitor all peptide fragment ions in parallel in a single MS^2 spectrum at high-resolution using the Orbitrap mass analyzer [Figure 1b].

The acquisition of HRAM MS^2 spectra on quadrupole-Orbitrap systems provides several benefits. First, the distinction between fragment ion signals and interferences can be readily achieved, which allows for greater measurement selectively, lower limits of detection, and in turn better quantification sensitivity.[3] Second, the instrument trapping capabilities allow enhanced accumulation of low abundance analytes, which can increase the S/N of these targets in complex matrices and their overall detectability.[2] Lastly, the PRM approach simplifies assay development time since time-consuming iterative selection of transitions associated with SRM and instrument parameters can be eliminated through the acquisition of a single, data-rich MS^2 spectra containing all potential fragment ions. This allows for post-acquisition selection of optimal fragment ions for target quantification without the need to preselect target transitions, and significantly reduces the need for iterative peptide-level parameter optimization steps and separate data acquisitions. However, there are still considerable limitations with PRM experiments. The first is related to the fundamentally slower acquisition of the mass analyzer than that is used in SRM which can reduce the overall duty

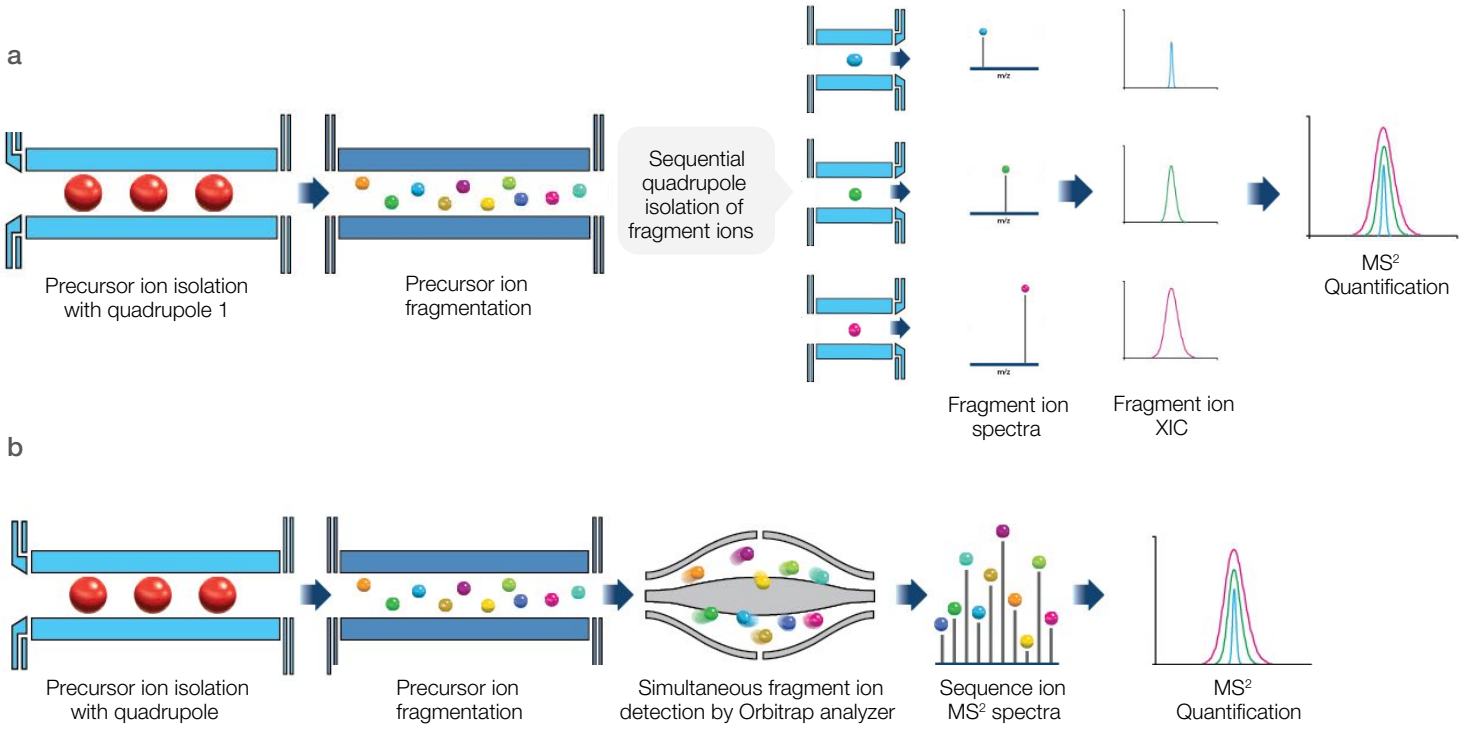

Figure 1. SRM and PRM are conventional targeted LC-MS approaches. (a) In SRM assays the mass spectrometer is programmed to monitor for the presence of one or more precursor ions, collisionally fragment these ions, isolate, and detect the resulting fragment ions in sequential steps. The integration of extracted ion chromatograms (XIC) from the diagnostic fragment ions allows quantitation of the target. (b) PRM shares similarities with SRM, however, fragment ions are isolated and detected in parallel using high-resolution, accurate-mass detectors such as Thermo Scientific™ Orbitrap™ mass analyzers. This enables post-acquisition determination of the optimal fragment ions for quantification as well as higher measurement selectivity.

cycle and impact the practical number of monitorable targets in a given analysis. Second, the faster duty cycle and greater sampling rate of SRM in principle may allow increased sampling of the peptide elution profile and better overall representation of the peak area compared to PRM.

Fundamental limitations of traditional targeted methodologies

One major constraint of both SRM and PRM targeted approaches is the relationship between target multiplexing (i.e., the number of analytes that can be reliably measured) within the desired cycle time, and the amount of measurement time devoted to each of these analytes within that fixed period of time [Figure 2].

The (maximum) intended cycle time is directly constrained by the chromatographic properties of the liquid chromatography setup (i.e., peak width). It needs to be calculated to ensure sufficient collection of data points as the target elutes over time (typically 6–10 points/elution peak) thus providing an accurate description of the target elution profile, which is critical for quantification precision. For example, with a 20 s peak width and 10 points of measurement, a 2 s max cycle time is required. Once this cycle time is established, a finite amount of acquisition time is available to be allocated for the measurement of each analyte. On HRAM systems where PRM is performed, the goal is to parallelize the operation of the C-trap/HCD cell and the Orbitrap mass analyzer so that overhead time is minimized. In other words, the allowable ion trapping/fragmentation time should not exceed the Fourier transform transient time that is occurring in parallel. In this way, the selectivity (based on resolution) and sensitivity (a function of maximum fill time) are adjusted in unison to maintain near equivalent timing [Figure 2]. Thus, there is always a tradeoff between highest quantitative performance, in terms of sensitivity/selectivity, and the number of targets per analysis. For high sensitivity, longer acquisition time is devoted to measurement of each target at the expense of the total number of targets. Conversely, for a large number of targets, the effective acquisition time for each target needs to be reduced, compromising the data quality. This highlights the challenge of current targeted workflows which seek to achieve the highest quantitative performance (in terms of sensitivity, selectivity, and precision) while still quantifying hundreds to thousands of targets in a single analysis.

The second major limitation of SRM and PRM targeted approaches is the overall sub-optimal efficiency of the targeted acquisition methodology itself. In a typical LC-MS targeted analysis, the analytes will elute from the chromatographic interface at a predictable time during the analysis, called elution time, or RT. Time-scheduled targeted analysis, where RT monitoring windows are specified in the instrument method, offers one way to minimize the number of unproductive scans that would occur in unscheduled acquisition during portions of the analysis where the intended target is not expected to be observed [Figure 3a].

Time-scheduled targeted analysis reduces the overall cycle time needed to collect a set of measurements since the number of peptides per cycle is managed more effectively. However, RT remains a loose analyte property, often impacted by the chromatographic setup variability (column performance, solvent composition, laboratory temperature, etc.). In practice, this property can be especially troublesome for configurations using nano- or capillary-flow rates which improve the sensitivity considerably but result in RTs that

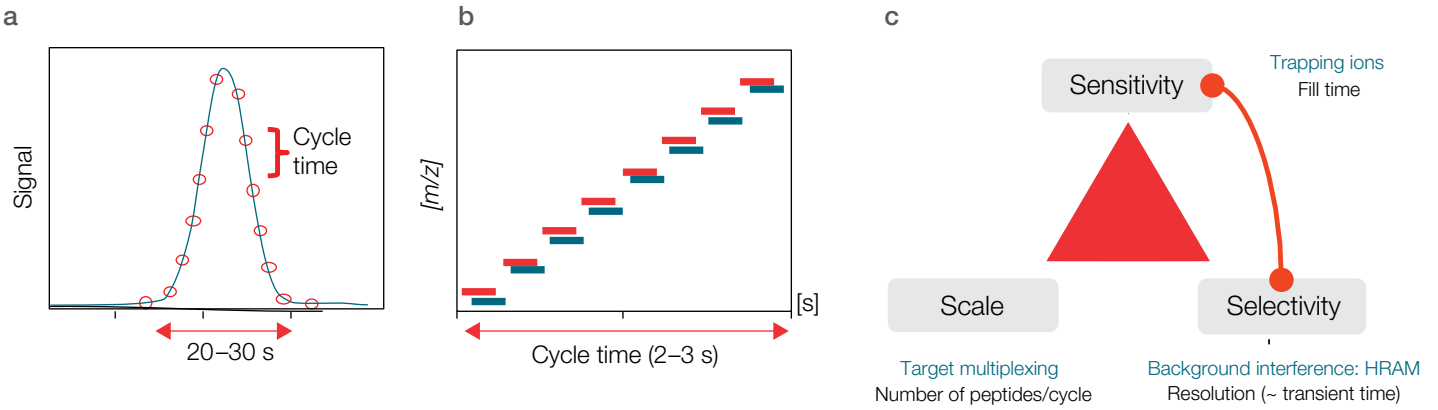

Figure 2. Inter-dependencies between experiment scale, sensitivity, and selectivity. (a) Chromatographic elution properties of the analyte and the desired sample rate (b) will ultimately determine the amount of time the mass spectrometer can spend taking measurements. (c) Within this fixed time, the instrument can be used to collect fewer measurements with high selectivity and sensitivity or alternatively, take more measurements but at a reduced sensitivity.

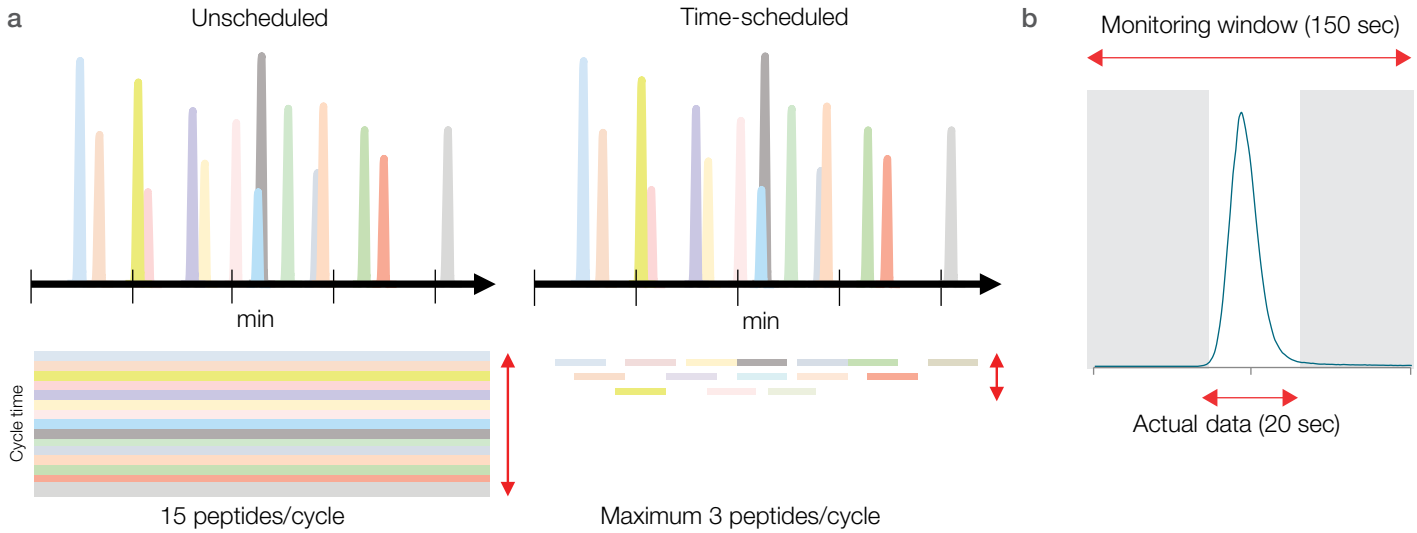

Figure 3. Targeting efficiency can be improvement with RT scheduling. (a) The simplest and least efficient targeted approach continuously collects measurements for all analytes in the experiment, even at periods in the analysis when the targets are not likely to have chromatographically eluted (left). Time-scheduled targeted approaches are more efficient and seek to measure the analyte only during expected elution time ranges (right). This can dramatically improve the cycle time and allow higher sampling rates. (b) Time-scheduled approaches are still prone to some inefficiency as the monitoring window is usually programmed to be longer than the actual analyte elution to safeguard against RT drift.

can be erratic and unpredictable. This RT variance can be problematic and result in missing analyte measurements in cases where the analyte RT has drifted outside of the programmed RT window due to severe fluctuations or in cases where the RT window itself is too narrow to accommodate accepted technical variance. This latter situation leads to the next issue of inefficiency where the RT window is generally established to be longer than the actual elution profile itself to buffer against RT drift. However, for a typical 150 s RT window monitoring a 20 s elution peak, this ultimately means that >85% of acquisition scans are not productive and thus an inefficient use of instrument resources [Figure 3b].

The next paradigm for turnkey targeted quantitation

In order to overcome some of the limitations of PRM, internal standard-triggered PRM (IS-PRM) was developed. This approach leveraged spiked-in internal standard reference probes to dynamically guide the targeted analysis in real-time. The concept of IS-PRM has been described previously, and in its original implementation significantly improved the inefficiencies associated with time-scheduled targeted methods by actually measuring analytes only while they are eluting. This allowed even larger numbers of targets to be reliably detected without sacrificing data quality and measurement sensitivity.[4] However, IS-PRM showed limited adoption in the proteomics community mainly due to its requirement for sophisticated application-programing interfaces and the need for specific informatics tools to support assay development and method preparation. The Thermo Scientific™ SureQuant™ IS Targeted Quantitation workflow is an evolution of the original IS-PRM approach, but with refinements to enhance the implementation, usability, and robustness of the method while still maintaining the highest level of quantitative performance.

The SureQuant method also relies on synthetic internal standards, spiked into a sample, to guide the instrument in real-time during the experiment. More specifically, the mass spectrometer alternates between two acquisition modes, i.e., Watch mode and Quant mode, in which acquisition parameters, such as fill time and resolution, are adjusted on-the-fly to maximize sensitivity and selectivity at the precise time-point in the analysis when the target of interest is eluting. Importantly, in the SureQuant method implementation the acquisition no longer requires time scheduling of RT windows and therefore removes the associated inefficiencies mentioned for traditional targeted methods. The schematic in Figure 4 illustrates the real-time process for SureQuant method peptide quantitation.

First, synthetic IS corresponding to the targets of interest are spiked into the sample at easily detectable amounts, and then subjected to an LC-MS SureQuant method. In 'Watch Mode' the instrument is programmed to continuously monitor for the presence of the reference IS using a combination of MS^1 and MS^2 scans, but using parameters favoring acquisition speed. A high-resolution full scan is first performed to evaluate whether a precursor *m/z*

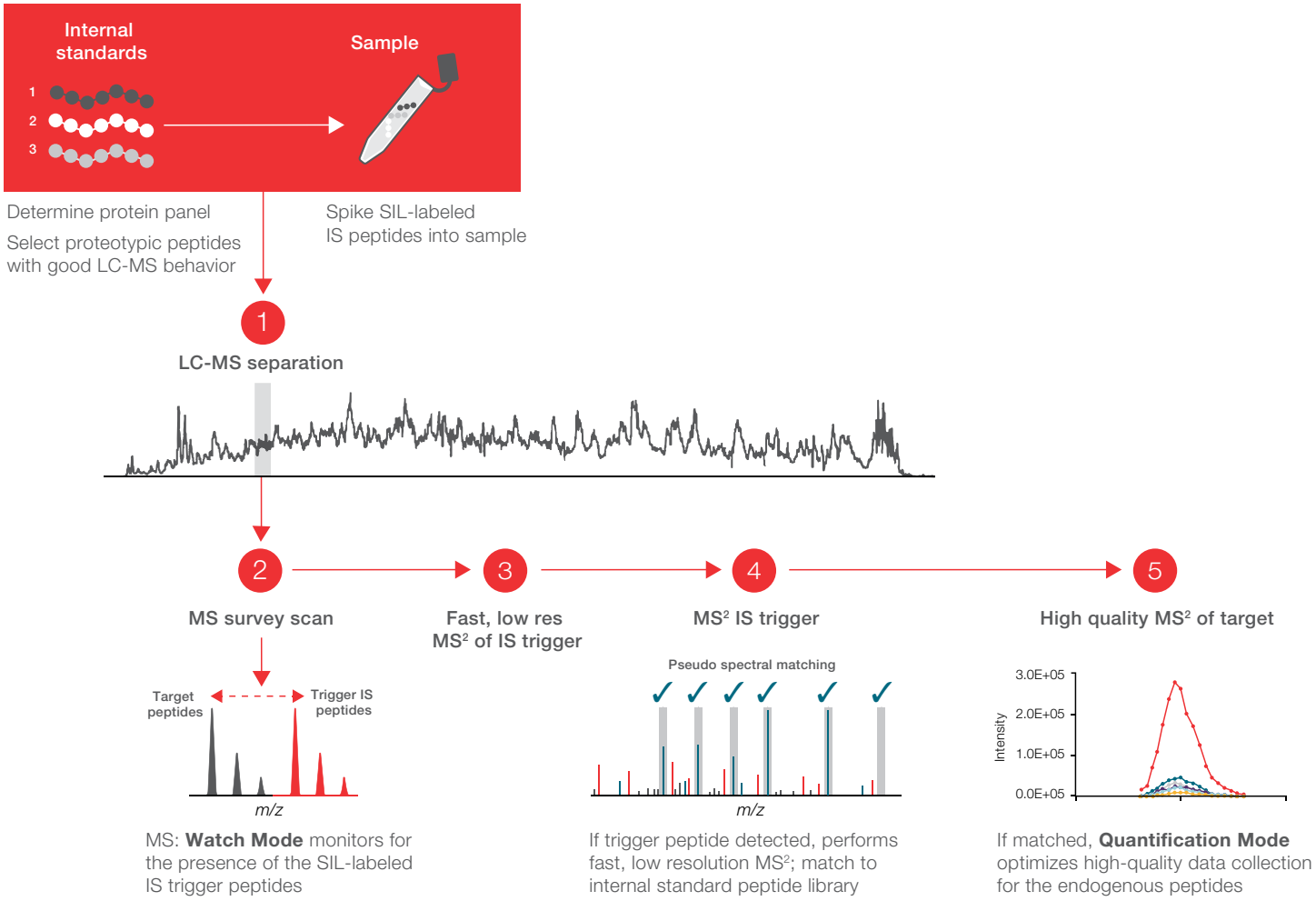

Figure 4. SureQuant Internal Standard Targeted Quantitation method for robust, high-performance quantitation.

matching the IS target list is detected (within tight tolerance, typically set at ±3–10 ppm around theoretical *m/z*) and can be observed at a specified triggering intensity threshold. If the criteria are satisfied, a fast MS² scan of the IS occurs and real-time fragment ion matching against predefined associated reference fragment ion confirms the identity of the IS (typically using at least 5 of 6 reference fragments detected with low-ppm tolerance). Since the IS will co-elute with the endogenous target, it acts as a landmark instructing the instrument to switch to acquisition parameters favoring data quality at the appropriate time. At this point 'Quant Mode' is enabled and high-fill time, high-resolution MS² scans are collected for the endogenous target, allowing high-quality measurement of the elution peak and quantification of the target.

An example of the triggered acquisition process is depicted in Figure 5 along with the critical method parameters optimized for the highest data quality and sensitivity of triggering as a first priority but also to retain sufficient triggering specificity. For example, the IS precursor ion is measured with high-resolution in the Orbitrap mass analyzer to facilitate its detection in complex matrices, and with low-ppm measurement error to enable selective confirmation of its actual elution. Additionally, an IS triggering intensity threshold corresponding to ~1% of the apex chromatographic intensity is a further constraint to ensure sensitivity and selectivity of triggering.

The SureQuant method has been implemented in the instrument control software of HRAM Orbitrap systems (Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer, Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ mass spectrometer, and legacy Orbitrap MS systems compatible with Tune v3.3 or higher). An example of the method structure is shown in Figure 6.

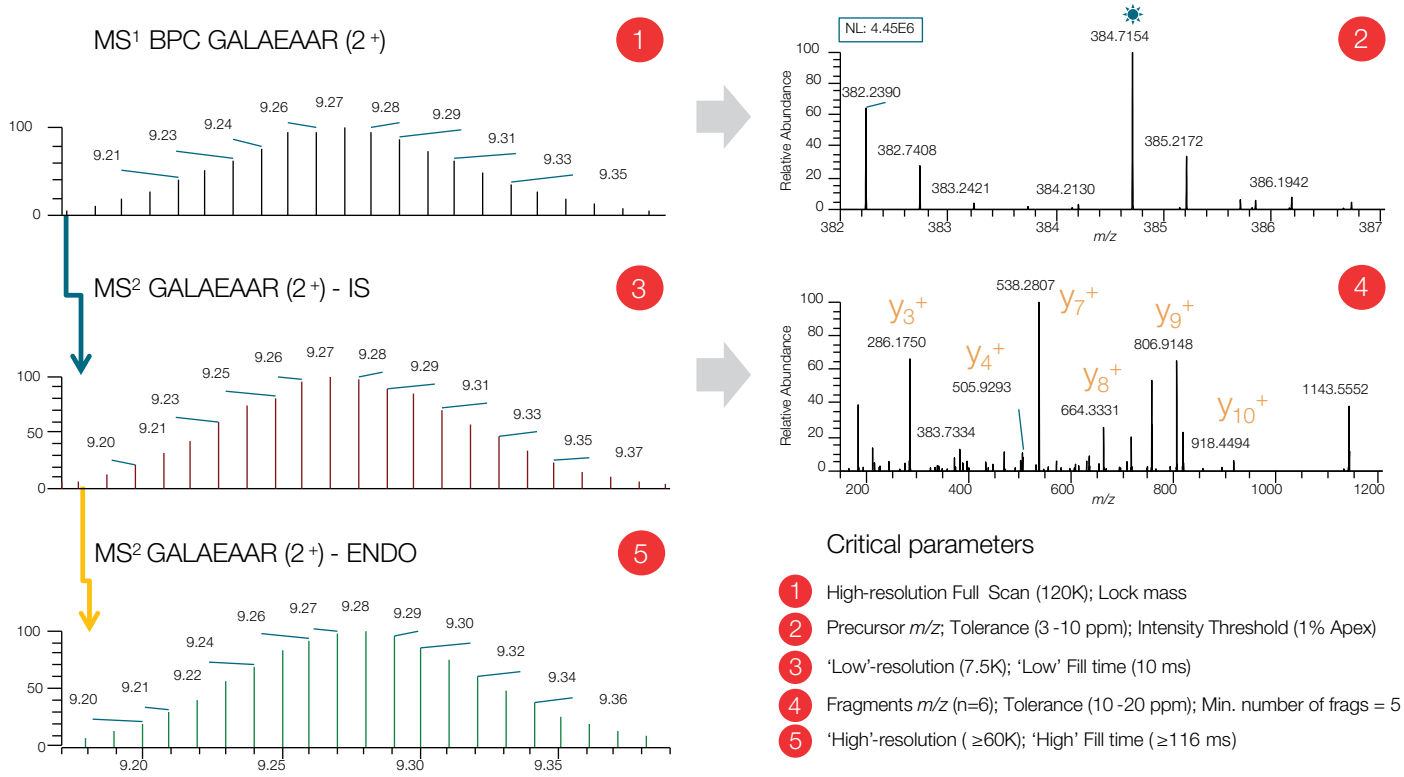

Figure 5. Example of SureQuant acquisition sequence and critical parameters for each of stage of detection and quantification. For each cycle, high-resolution full scans monitor for the presence of putative internal standard precursor ions (1) and assess whether signal abundance is within the expected intensity range (2). Candidate ions are isolated, fragmented and undergo fast MS² analysis (3) for on-the-fly confirmation of the target (4). After reference standard confirmation, high-sensitivity isolation, fragmentation and MS² analysis of endogenous target is conducted (5).

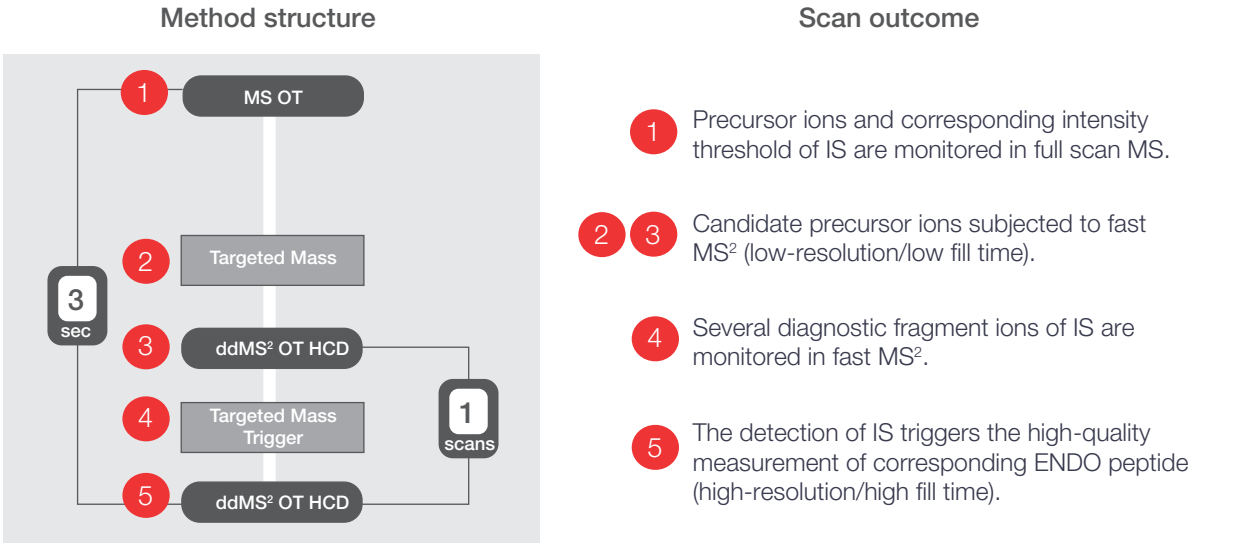

Figure 6. SureQuant method scan structure on Orbitrap mass spectrometers. Internal standard-triggered acquisition schemes can be created natively on the instrument control software of HRAM Orbitrap systems. This allows intuitive, decision-tree based SureQuant methods to be created without the need for instrument application programming interface (iAPI) knowledge.

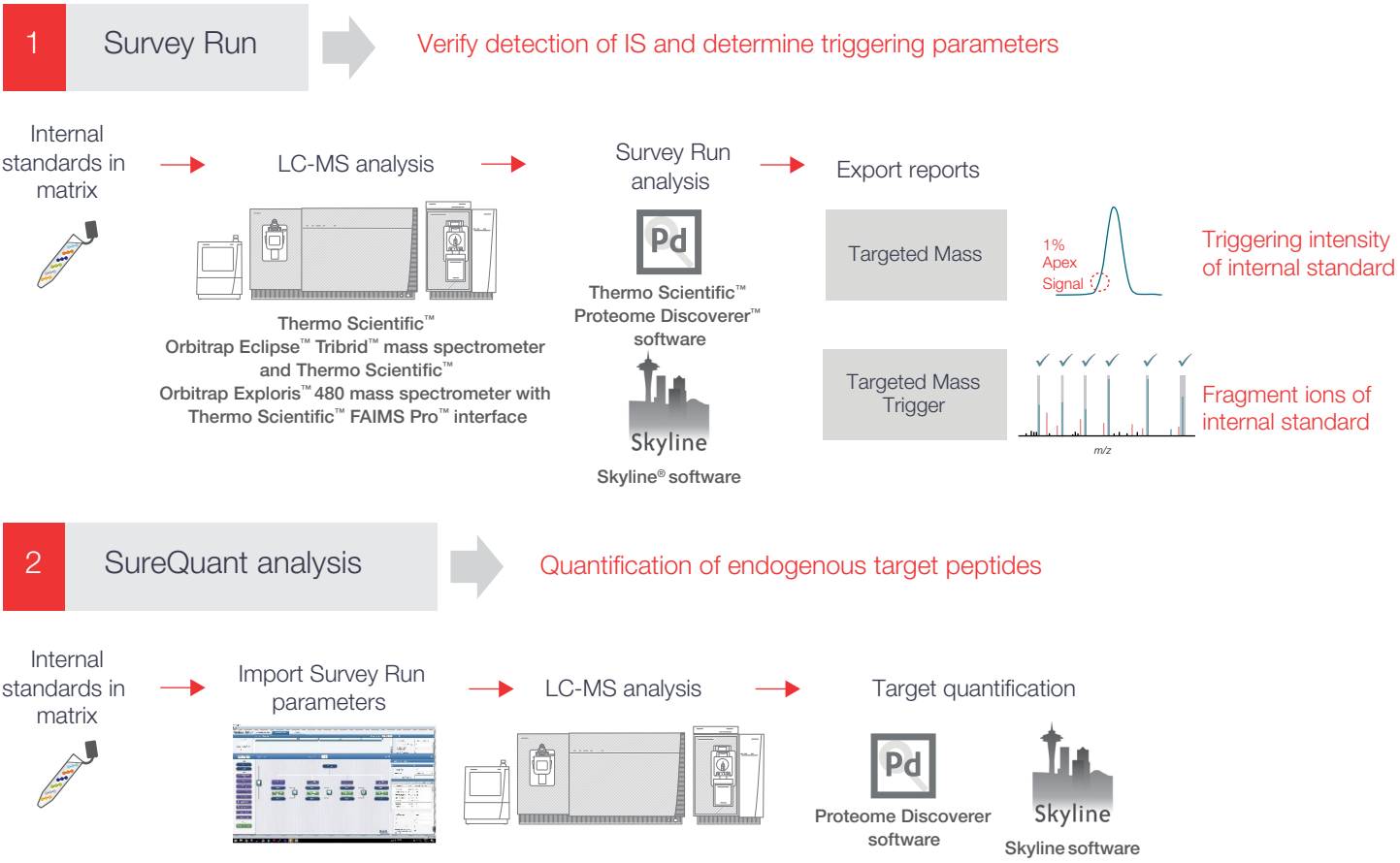

Figure 7. Overall methodology and SureQuant workflow. To prepare the SureQuant method for routine quantification, a scouting or 'Survey Run' is first performed to ensure detectability of the IS mixture with the user's analytical setup, and to characterize the triggering parameters such as the 1% triggering intensity and optimal reference fragment ions of each IS. In the second stage, this information is exported from the Survey Run analysis and transferred into the SureQuant method analysis template. Using this updated method, routine SureQuant analysis of samples spiked with the IS mixture can be performed without additional adjustments or time-scheduling.

In principle, some analyte properties need to be collected prior to conducting the actual SureQuant method analyses. Establishing a SureQuant targeted quantitation assay can be streamlined into two general stages as shown in Figure 7.

In the first stage a 'Survey Run' is performed to characterize the IS corresponding to the target panel, spiked into a representative matrix. The acquisition itself is a directed data-dependent acquisition (DDA) analysis containing an inclusion list composed of the precursor ions of the IS in the mixture (under multiple theoretical charge states). The Survey Run achieves several purposes, including detectability assessment of the IS using the user's specific LC-MS configuration and to ensure system suitability (MS sensitivity, chromatography, etc.). This preliminary analysis is performed using MS conditions identical to those to be used in 'Watch Mode' of the subsequent targeted SureQuant analysis and therefore establishes the optimal precursor ions and associated optimal fragment ions for the IS. Of key importance, the Survey Run also provides empirical data on the signal intensity response for each IS and allows determination of a specific triggering intensity threshold for each individual IS. A value corresponding to ~1% of the apex IS signal is recommended for triggering intensity thresholds in the SureQuant method to ensure selective and sensitive monitoring of the IS.

In the second stage, the pertinent IS information from the Survey Run (i.e., precursor *m/z*, intensity threshold, fragment ion *m/z*) is then used to program the SureQuant instrument method. After this one-time adjustment, IS can be spiked into samples and the targeted method can be performed routinely without any further intervention.

Embedded, pre-set instrument method templates support the use of Thermo Scientific™ SureQuant™ Targeted MS Assay Kits and third party targeted panels, while generic SureQuant method templates are also available to simplify the development of custom targeted panel assays.

Benefits of the SureQuant IS Targeted Quantitation Workflow

Highest efficiency acquisition

Compared to conventional targeted approaches, SureQuant methodology offers superior acquisition efficiency, translating into several benefits. As mentioned earlier, a typical time-scheduled experiment is prone to many unproductive scans which are collected within the RT scheduled window because the window is generally far wider than the target elution peak [Figure 3b]. Thus, the number of productive MS^2 scans, or scans providing meaningful data on the target, can be quite low, in this case ranging from 10–15% of scans in the RT window. In contrast, SureQuant method acquisitions are considerably more productive providing 80–90% efficiency under the same scenarios [Figure 8].

This enhanced efficiency can be leveraged to gain several analytical advantages, including:

1. Enhanced data quality—settings favoring higher sensitivity and selectivity (higher resolution/fill time) can be used to improve limits of quantitation.
2. Increased target scale—a higher number of targets can be monitored and quantified in the same amount of total analysis time as PRM without sacrificing the duty cycle.
3. Increased throughput—higher productivity can be achieved by reliably quantifying targets in less total instrument time, while still achieving acceptable data quality.
4. Enhanced detection success rate—more reliable and consistent target measurement can be achieved since the IS guides the measurement of the target of interest at precisely the right time. This leads to less intra-run and less inter-run missing values.

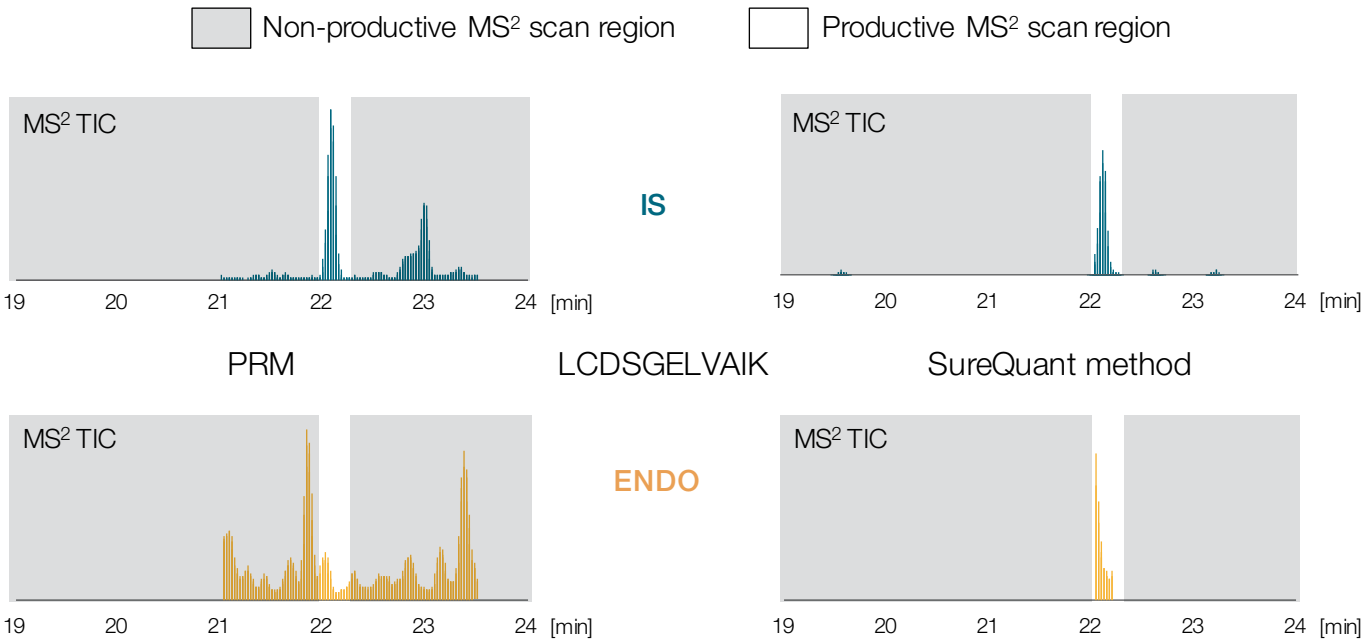

Figure 8. SureQuant method intelligent detection of targets maximizes instrument efficiency and productivity. The IS and endogenous detection of a representative peptide, LCDSGELVAIK, is shown from PRM and SureQuant acquisition. In the PRM experiment, many uninformative MS^2 scans are captured for the IS and endogenous target (gray region) during the 2.5 min monitoring window, and a smaller proportion of MS^2 scans are captured during the actual target elution time (white region). The dynamic nature of SureQuant acquisition minimizes unproductive scans allowing shorter duty cycles and higher productivity. Experiment details: 50 fmol IS spiked into 250 ng Thermo Scientific™ Pierce™ HeLa Protein Digest Standard. PRM MS^2 settings: 2.5 min RT window, 15,000 resolution, 20 ms IT. SureQuant method MS^2 settings: Watch mode 7500 resolution, 10 ms IT; Quant mode 60,000 resolution, 116 ms IT.

Independence from time-scheduling

With SureQuant method acquisition, the mass spectrometer is continuously monitoring the reference IS to determine the optimal time to record measurements of the desired target. Therefore, the actual elution time of the target of interest is not used as a constraining acquisition parameter and does not need *a priori* determination for SureQuant analysis as in a conventional LC-MS targeted analysis. This independence from RT scheduling provides several advantages in analytical performance. First, less time and resources are required for method development/optimization to establish appropriate RT windows. Second, the risk of missing a target measurement during an analysis is greatly minimized even in the case of unpredictable target elution times. Overall, this allows for more consistent, reliable, and robust measurement and quantification of targets even under experimental conditions of inconsistent chromatographic behavior [Figure 9].

Minimize duty cycle times for higher sampling rates, improved sensitivity and superior data quality

The selection of instrument parameters such as resolution and ion injection fill time will influence the overall time required for a complete cycle of MS^2 measurements to be collected for co-eluting targets. This duty cycle time is proportional to the acquisition time used for the MS^2 scans. As mentioned earlier, the fill time for a particular experiment is determined once the Orbitrap mass analyzer resolution is selected in an effort to ensure parallelization of the C-trap/HCD cell and the Orbitrap mass analyzer. Therefore, as the resolution/fill time are increased within an experiment, the duty cycle will also increase proportionally [Figure 10a]. Extending the cycle time will have a negative impact on the overall sampling rate and measurement precision.

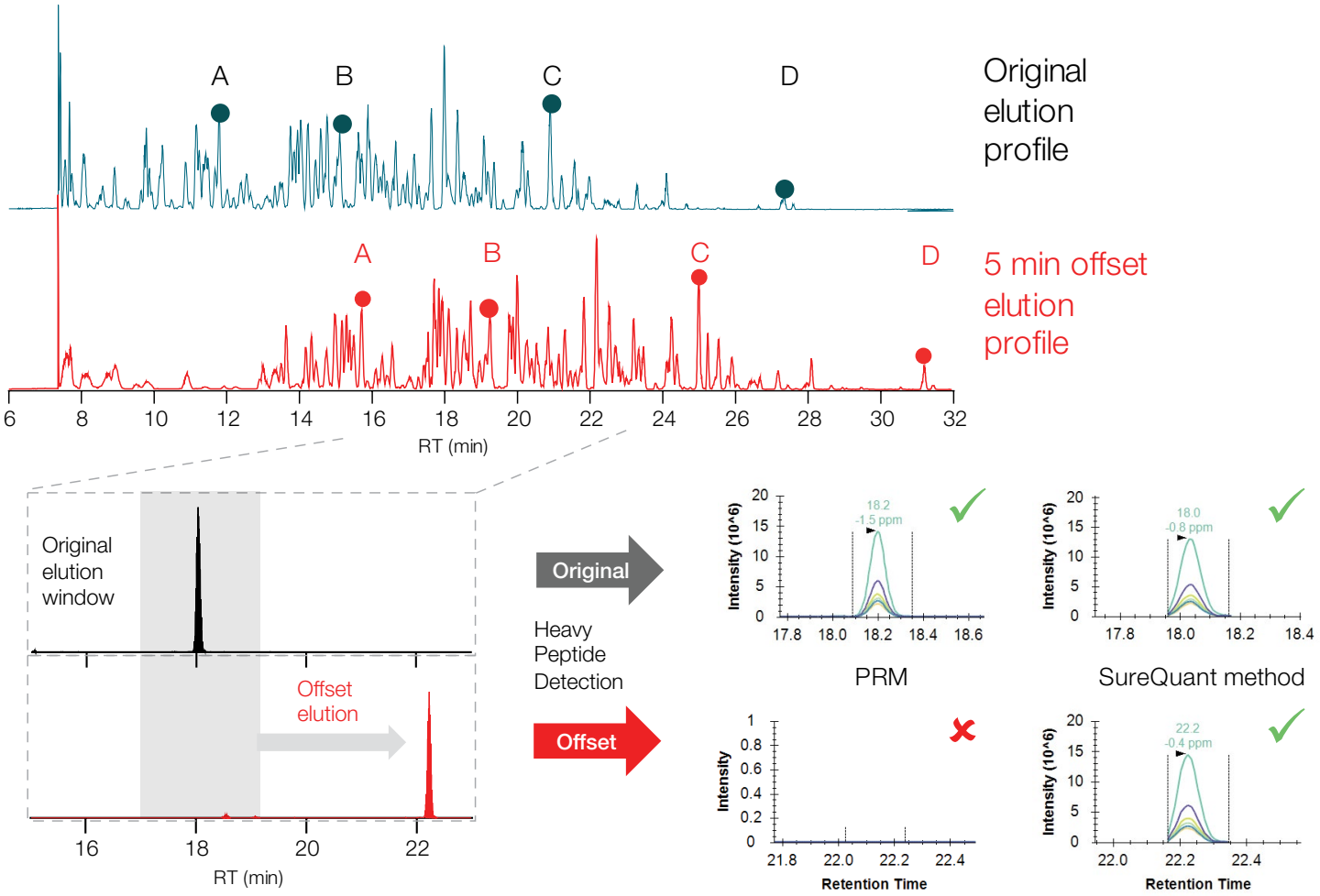

Figure 9. SureQuant acquisition robustness overcomes chromatographic fluctuations. Targeted analysis of AKT-mTOR pathway proteins was performed by PRM and SureQuant acquisition using a standard gradient and an offset gradient which introduced a 5-minute artificial time delay to simulate LC retention time variations that can commonly occur (landmark peptides A-D are indicated for comparison). As an example, the observed heavy peptide LFDAPEAPLPSR (*m/z* 611.8526 ++) elution is shown at the original and offset retention times (bottom left). Notably, while PRM acquisition failed to capture the signal of the peptides with delayed elution, SureQuant acquisition maintained reliable measurement under these conditions.

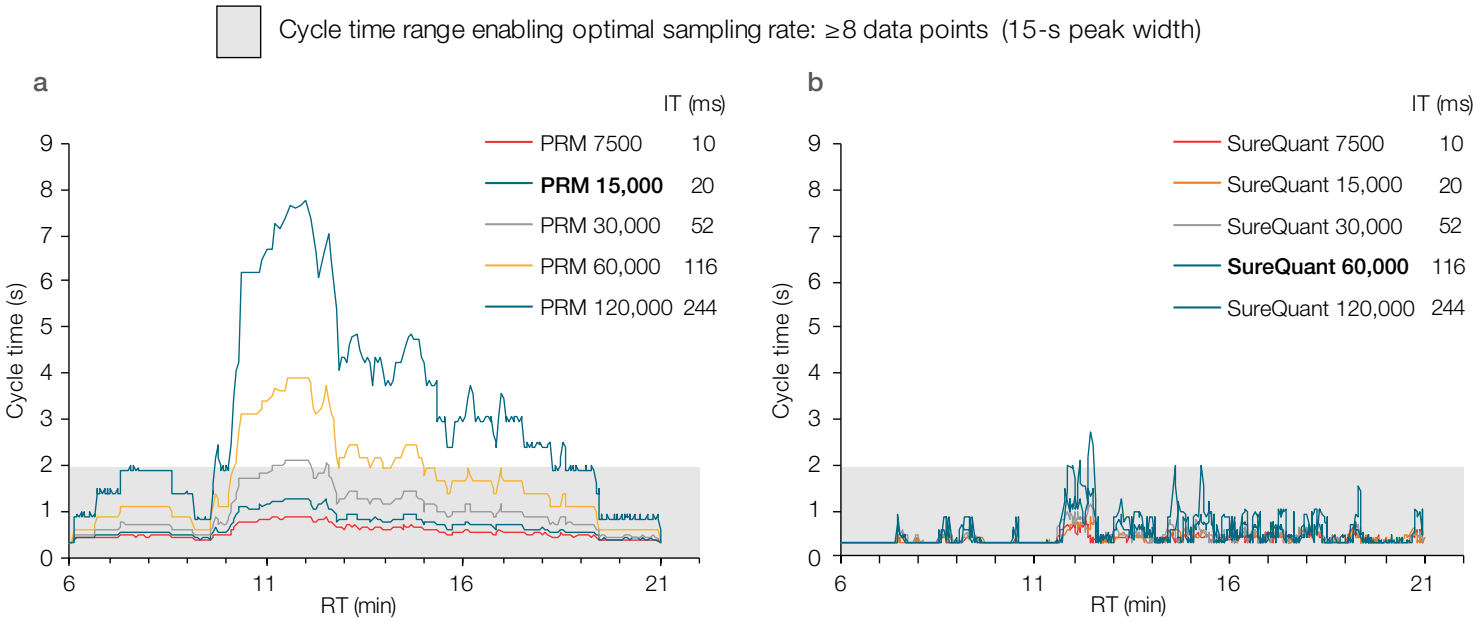

Figure 10. SureQuant IS targeted quantitation method reduces acquisition inefficiencies allowing more target measurement time without compromising cycle times. Comparison of cycle times observed using various MS^2 scan parameters for analysis of 30 AKT-mTOR IS and endogenous peptides using PRM or SureQuant acquisition. Shaded area represents maximum allowable cycle time of 1.875 sec to ensure sampling of ≥8 data points per peak. Green trace represents MS^2 settings allowing optimal sampling for both PRM and SureQuant methods. Experiment details: 20 fmol IS Thermo Scientific™ Pierce™ SureQuant™ AKT Pathway Multiplex Panel spiked into 1000 fmol 6 protein digest mixture (Pierce HeLa Protein Digest Standard). Thermo Scientific™ EASY-Spray™ PepMap RSLC C18 15 cm × 150 µm ES806 column, 1.2 µL/min flow rate, 30 min gradient. PRM setting: 2.5 min RT window. SureQuant method settings: Watch mode 7500 resolution, 10 ms IT.

For illustration, a comparison of various ion injection fill time/MS^2 resolution settings was performed for PRM analysis of 30 AKT-mTOR pathway targets. This comparison established maximum parameters of 20 ms/15,000 resolution to achieve sufficient sampling rates [Figure 10a, teal trace]. However, due to the more specific and efficient SureQuant acquisition, settings of 116 ms/60,000 resolution can be used for the analysis of this panel without exceeding the desired duty cycle [Figure 10b, teal trace]. This represents an effective gain of 6-fold in fill time, providing a concomitant boost in measurement sensitivity.

Higher acquisition efficiency allows longer acquisition time to be devoted to the measurement of targets without exceeding the duty cycle constraints. This directly translates into higher levels of sensitivity. This is reflected by the comparison of PRM and SureQuant analyses of the 30 AKT-mTOR pathway targets directly from a HeLa cancer cell line sample using parameters required for comparable duty cycle between the respective approaches [Figure 11]. The SureQuant acquisition detected and quantified 26 out of 30 targets even without immunoprecipitation enrichment that is typically needed for reliable detection by conventional PRM analysis. This enhanced and comprehensive detectability is a direct consequence of higher sensitivity measurements which can be achieved with longer fill time MS^2 scans using the SureQuant method. For all targets, the number and quality of MS^2 spectra are improved and this ultimately allows confident quantitation of more targets from the panel, including several targets that would have otherwise been undetectable.

Conclusion

Historically, discovery and targeted LC-MS experiments were employed for diametrically opposed objectives. For example, LC-MS proteomics approaches can be neatly divided into discovery workflows favoring high-scale coverage with sub-optimal quantitative performance, or conversely, targeted workflows with a stronger focus on highest quantitative performance at the expense of low-scale coverage.

The SureQuant IS Targeted Quantitation workflow represents a truly novel paradigm in the LC-MS toolbox, filling the sweet spot on the continuum for large-scale target profiling while still maintaining superior quantitative performance. In principle, this approach also opens the door to new possibilities outside of proteomics, including metabolomics or other small-molecule quantification which rely on labeled standards as well. Together with the

analytical improvements benefiting acquisition efficiency, productivity, reliability and robustness of quantification, and measurement sensitivity, the practical challenges of method development and deployment can also be reduced by the SureQuant method [Table 1]. Intelligent acquisition methods like the SureQuant IS Targeted Quantitation workflow, along with advances in data processing and automation, are moving us closer towards a reality of truly turnkey targeted solutions.

References

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Protein	Peptide	PRM 15K-20ms	SQ 60K-116ms
GSK3β	TPPEAIALC[+57]SR	1	20
AKT2	LPFYNQDHER	0	17
IGF1R	AENGPGPGVLVLR	1	15
PTEN	IYNLC[+57]AER	0	15
GSK3β	LC[+57]DSGELVAIK	5	15
GSK3β	LLEYTPTAR	0	15
GSK3α	VTTVVATLGQGPER	6	15
AKTS1	GALAEAAR	5	13
AKT1	NDGTFIGYK	0	13
AKT2	SDGSFIGYK	0	13
IGF1R	IDIHSC[+57]NHEAEK	1	12
PTEN	AQEALDFYGEVR	10	11
MTOR	LFDAPEAPLPSR	11	11
MTOR	TLDQSPELR	2	10
AKT1	YSFQTHDR	0	10
KS6B1	FEISETSVNR	0	9
TSC2	SNPTDIYPSK	0	9
GSK3α	SQEVAYTDIK	1	9
TSC2	GQPEGPLPSSSPR	0	8
AKTS1	SSDEENGPPSSPDLDR	0	8
IGF1R	TTINNEYNYR	0	7
MTOR	ETSFNQAYGR	2	6
MTOR	GNNLQDTLR	0	6
TSC2	GYTISDSAPSR	0	5
PTEN	NNIDDVVR	0	4
KS6B1	DGFYPAPDFR	0	1
AKT3	LVPPFKPQVTSETDTR	0	0
IRS1	SVSAPQQIINPIR	0	0
AKT3	TFHVDTPEER	0	0
IRS1	TGIAAEEVSLPR	0	0
Number of peptides detected:		11	26

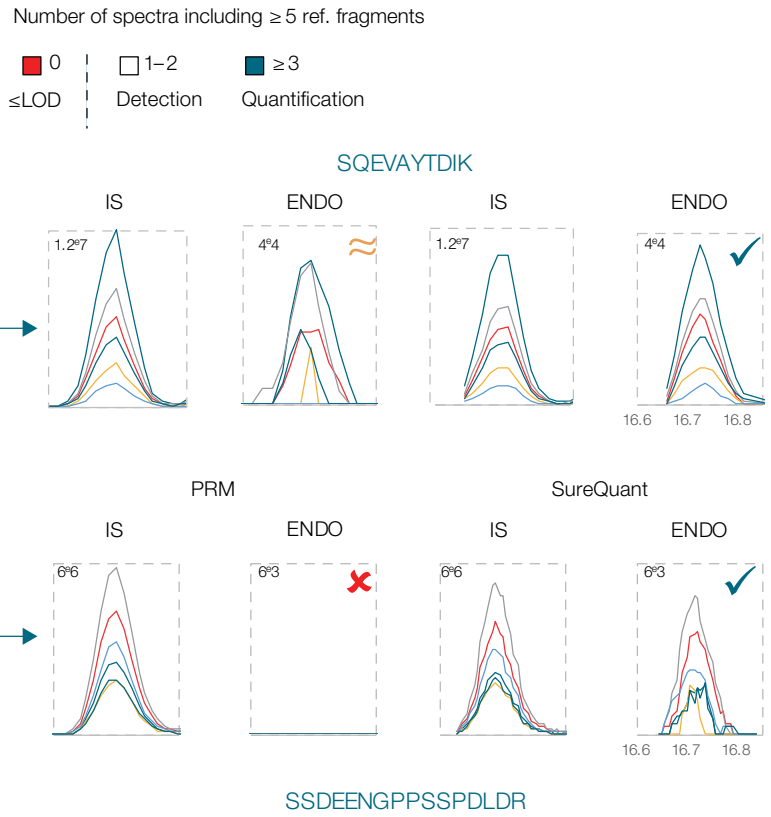

Figure 11. Longer fill time MS² scans using the SureQuant method translates into higher sensitivity measurements and higher detection rate. Comparison of results for analysis of 30 AKT-mTOR IS and endogenous peptides using PRM or SureQuant acquisition. MS settings allowing equivalent cycle times were used. MS² chromatograms are shown for peptides from GSK3α and AKTS1 proteins to illustrate higher detectability of targets. Experiment details: 50 fmol Pierce SureQuant AKT Pathway Multiplex Panel mixture spiked into 250 ng Pierce HeLa Protein Digest Standard. EASY-Spray PepMap RSLC C18 15 cm × 150 µm ES806 column, 1.2 µL/min flow rate, 30 min gradient. PRM MS² settings: 2.5 min RT window, 15,000 resolution, 20 ms IT. SureQuant MS² settings: Watch mode 7,500 resolution, 10 ms IT; Quant mode 60,000 resolution, 116 ms IT.

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Table 1. Summary of PRM/SRM and SureQuant method capabilities. Traditional targeted approaches require tradeoffs between high-density targeted panels or high-sensitivity measurements, while the SureQuant IS Targeted Quantitation workflow offers both large-scale profiling while still maintaining the highest quantification performance.

	SureQuant	PRM/SRM	PRM/SRM
Value proposition	Up to 500 pairs of H/L targets/30 min *Highest targeting efficiency *Complete quan profile every analysis *Maintain sensitivity and target scale	Up to 100 pairs of H/L targets in 30 min (60,000–116 ms) *Favoring sensitivity	Up to 500 pairs of H/L targets in 30 min (7500–10 ms) *Favoring target number
Quantification performance (Precision/accuracy)	● ● ● ●	● ● ● ●	● ● ● ●
Sensitivity (LLOQ)	● ● ● ●	● ● ● ●	● ● ● ●
Scale (# Targets)	● ● ● ●	● ● ● ●	● ● ● ●
Efficiency (Productive Scans)	● ● ● ●	● ● ● ●	● ● ● ●
Load-and-play (Minimal adjustment)	● ● ● ●	● ● ● ●	● ● ● ●

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

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