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Proteomics

Unveil plasma proteomics with cutting-edge hybrid-DIA methods utilizing two strategies on the Orbitrap Astral Zoom MS

Authors

Qingling Li[1], Jared Deyarmin[1], Sophia Steigerwald[2], Stephanie Samra[1];

[1]Thermo Fisher Scientific, San Jose, CA, USA; [2]Thermo Fisher Scientific, GmbH, Bremen, Germany

Keywords

Hybrid-DIA, targeted peptide quantitation, data-independent acquisition, DIA, discovery proteomics, plasma proteomics, biomarker, PRM, SureQuant, Orbitrap Astral Zoom MS

Goal

Develop and evaluate hybrid-DIA methods utilizing two strategies to simultaneously perform targeted peptide quantitation and data-independent acquisition (DIA) discovery proteomics.

Introduction

Traditional proteomic approaches encompass two main segments: targeted quantitation and discovery proteomics. One of the most representative and widely used acquisition methods for discovery proteomics is DIA. DIA provides comprehensive proteome coverage, reduces missing values, and enables high-accuracy, large-scale studies by fragmenting all peptides in a sample simultaneously. However, DIA can sometimes lack the specificity needed for targeted studies. Targeted quantitation is better suited for achieving the highest sensitivity and accuracy for a specific list of low-abundance peptides but is limited in the number of peptides it can analyze. Deciding between comprehensive discovery proteomics profiling and sensitive targeted quantitation is often a predicament for scientists in translational research, especially when analyzing large sample cohorts. The hybrid-DIA method addresses these challenges by combining the strengths of both targeted and discovery proteomics.

Hybrid-DIA integrates targeted peptide quantitation with DIA discovery proteomics, enabling dynamic coordination of DIA scans and precise measurement of MS^2 scans for predefined peptide targets. This approach ensures accurate quantification of specific peptides of interest while maintaining the broad, unbiased nature of DIA, making it a powerful tool for translational research applications.

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One key application of hybrid-DIA in translational research is the study of plasma samples from patients with various diseases. Plasma is a readily accessible biological fluid rich in information about physiological states. Hybrid-DIA combines the specificity of targeted quantitation with the comprehensive coverage of DIA. This dual capability allows researchers to precisely quantify known low-abundant protein biomarkers while also exploring and identifying new biomarkers and protein signatures in plasma.

To leverage these advantages, we developed and evaluated hybrid-DIA methods using two strategies, targeted MS^2 (tMS^2) and the Thermo Scientific™ SureQuant™ Internal Standard Targeted Quantitation Workflow. The first approach combines a DIA scan with a targeted MS^2 experiment, involving an MS^1 scan, a targeted MS^2 acquisition with a mass list table of all peptides to be quantified, followed by a DIA experiment. The second approach integrates a DIA experiment with on-the-fly triggering scans. The results from the two hybrid-DIA approaches were compared with traditional acquisition strategies for standard DIA and targeted parallel reaction monitoring (PRM) analysis. Both hybrid-DIA approaches demonstrated promising results when compared to standard DIA and PRM methods. The established hybrid-DIA methods can be readily adapted to various sample matrices.

Materials and methods

Consumables and chemicals

- Fisher Scientific™ Optima™ LC-MS grade water with 0.1% formic acid (Cat. No. LS118-500)
- Fisher Scientific™ Optima™ LC-MS grade 80% acetonitrile with 0.1% formic acid (Cat. No. LS122500)
- Thermo Scientific™ SureSTART™ 9 mm screw caps
- Thermo Scientific™ SureSTART™ 0.2 mL TPX screw top microvial with glass insert
- Thermo Scientific™ EASY-Spray™ HPLC Column (Cat. No. ES906), 2 µm C18, 150 µm × 15 cm
- Thermo Scientific™ PepMap™ Neo Trap Cartridge, 5 µm C18 300 µm x 5 mm (Cat. No. 174500)
- PQ500™ Reference Peptides Kit (Biognosys, AG)
- Disease plasma from BioIVT

Instrumentation

- Thermo Scientific™ AccelerOme™ Automated Sample Preparation Platform
- Thermo Scientific™ Vanquish™ Neo UHPLC System
- Thermo Scientific™ Orbitrap™ Astral™ Zoom Mass Spectrometer
- Thermo Scientific™ Easy-Spray™ Source

Data analysis and visualization

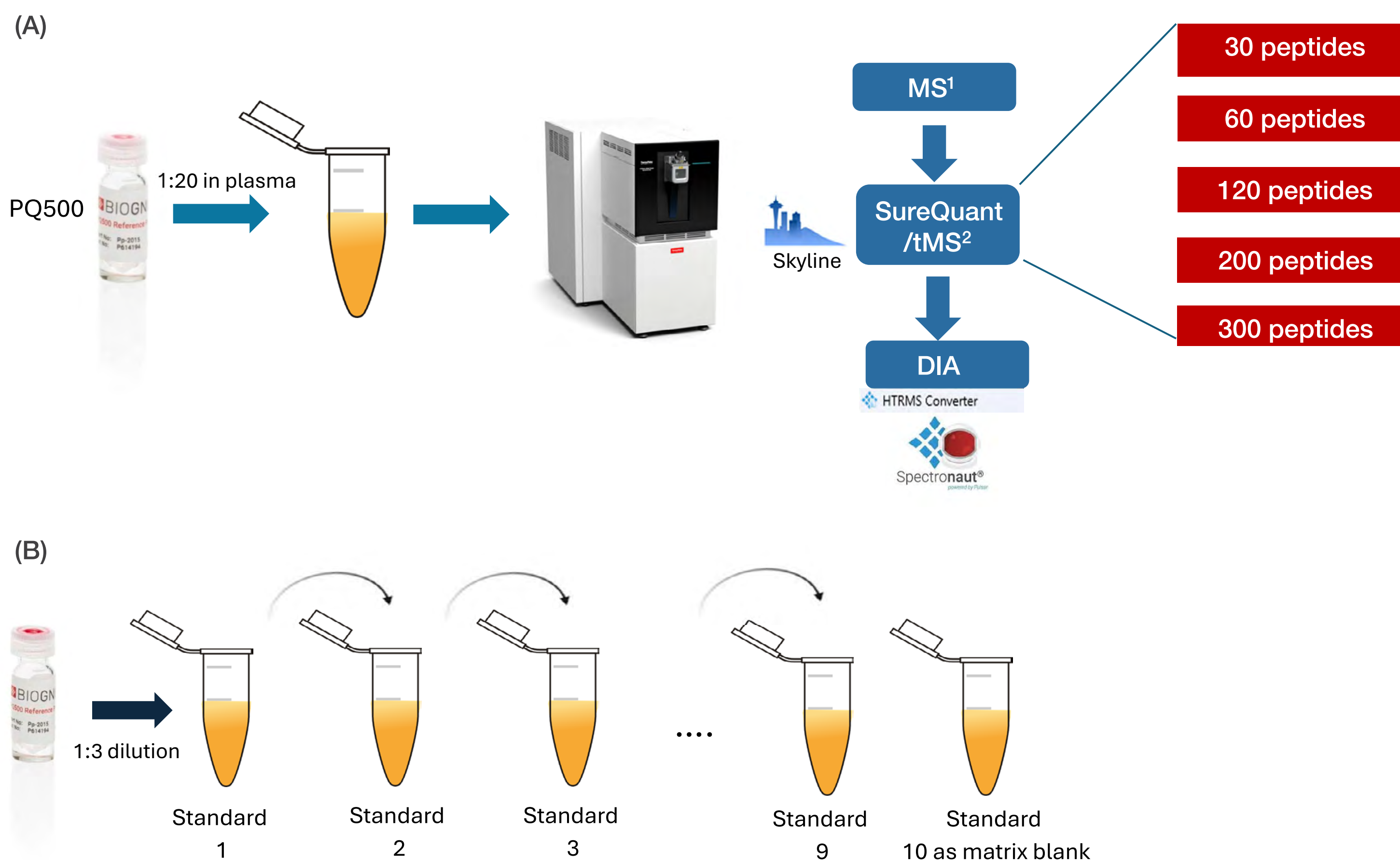
- University of Washington, MacCoss Lab. Skyline™ software (ver. 23.1.1.503)
- Spectronaut™ software (v20.1) (Biognosys, AG) with HTRMS converter
- Python™ software 3.0.1

Sample preparation and experiment design

PQ500 peptides were diluted according to the manufacturer's instructions. Neat human plasma disease samples were sourced from BioIVT and digested using the AccelerOme automated sample preparation platform. The digested disease plasma was then pooled together to form the sample matrix.

Two experiments were conducted to evaluate the performance of hybrid-DIA by two different approaches. The first experiment was investigating the effects of targeted peptide numbers using both hybrid-DIA approaches (Scheme 1A). PQ500 heavy peptides were spiked into pooled neat plasma digest at 1:20 dilution. Five hybrid-DIA methods were established with 30, 60, 120, 200, and 300 targeted peptides in the PRM panel. These peptides were evenly distributed across the 32-minute gradient. For SureQuant-based hybrid-DIA, both heavy and light peptides were included in the targeted peptide panel. For tMS^2 hybrid-DIA, only light peptides were included in the mass list table.

The second experiment was to evaluate the linearity, limit of detection (LOD), and limit of quantification (LOQ) of targeted peptides using hybrid-DIA analysis on the Orbitrap Astral Zoom MS (Scheme 1B). Peptides were diluted into a 250 ng/µL pooled plasma digest with a 3-times serial dilution. The dilution curve spanned a wide dynamic range for different heavy peptides, from standard level 1 (100%) to standard level 9 (0.005%), with a 100% plasma blank for the final level (standard level 10). A 1 µL standard solution containing 250 ng plasma digest was loaded onto the column.



Scheme 1. Experimental scheme to evaluate the performance of the two hybrid-DIA approaches. (A) Experiment to investigate protein group (PG) and peptide ID numbers in five hybrid-DIA methods with 30–300 targeted peptides. (B) Serial dilution experiment to evaluate the linearity, precision, accuracy, LOD, and LOQ of the targeted peptides in the hybrid-DIA method.

LC-MS analysis

The Vanquish Neo UHPLC system was used with the EASY-Spray HPLC column with a trap-and-elute injection scheme. HPLC gradients and parameters were optimized and are shown in Table 1. The column temperature was maintained at 50 °C. Mobile phase A consisted of 0.1% formic acid in water, and mobile phase B consisted of 0.1% formic acid in 80% acetonitrile. Samples were analyzed by the Orbitrap Astral Zoom mass spectrometer. The tMS^2 hybrid DIA method included three experiments (Figure 1A): an MS^1 experiment, a DIA experiment, and a tMS^2 experiment, which included the PRM mass list table. The MS^1, DIA, and tMS^2 mass spectrometer experiment parameters are shown in Table 2. The SureQuant-based hybrid-DIA method included two experiments (Figure 1B): a SureQuant PRM and a DIA experiment. Hybrid-DIA method parameters are shown in Table 3. The eluted peptides were analyzed on the Orbitrap Astral Zoom mass spectrometer using both methods.

Hybrid-DIA method set up

1. tMS^2 hybrid-DIA method setup

The method includes three experiments (Figure 1A): a full MS^1, a tMS^2, and a DIA experiment. The parameters for all three experiments are shown in Table 2. To set up the PRM method, a DIA run was first performed using the PQ500 pure standards solution to determine the charge state and retention times. A minimum of three most abundant transitions were selected in Skyline software using the PRM Conductor. The mass list table with scheduled retention times for the tMS^2 experiment was generated from Skyline software.

Table 1. HPLC conditions for hybrid-DIA methods.

LC parameters	Column	EASY-Spray HPLC column, 2 µm C18, 150 µm × 15 cm (Cat. No. ES906)	
	Column temperature	50 °C	
	Fast loading/ equilibration	Pressure Control	
	Pressure for loading/ equilibration/ wash	Max Pressure	
	Equilibration factor	2	
	Sampler temperature	7 °C	
	Mobile phase A	0.1% FA in water	
	Mobile phase B	0.1% FA in 80% ACN	
Gradient	Time	%B	Flow (µL/min)
	0	3	0.8
	0.5	3	0.8
	22.5	30	0.8
	30	45	0.8
	30.2	98	0.8
	32.5	98	0.8

2. SureQuant hybrid-DIA method setup

The setup for the SureQuant-based hybrid-DIA method is shown in Figure 1B. The SureQuant experiment was established in two steps.[2] In the first step, a Survey Run was conducted to characterize the heavy peptide standards for the target panel. This involved a data-dependent acquisition (DDA) analysis with an inclusion list of precursor ions of the heavy peptides in multiple charge states. It used MS conditions identical to the subsequent SureQuant analysis, establishing optimal precursors and fragment ions for the heavy peptides. The Survey Run provided empirical data on signal intensity response for each heavy peptide and determined a triggering intensity threshold. In the second step, the heavy peptide information from the Survey Run was used to program the SureQuant experiment. The SureQuant experiment was followed by a DIA experiment, in which the method parameters were the same as those used in standard DIA and tMS^2 hybrid-DIA methods.

Table 2. Mass spectrometer parameters in tMS^2 hybrid-DIA. (A) Mass spectrometer parameters in DIA experiment. (B) Mass spectrometer parameters in MS^1 experiment. (C) Mass spectrometer parameters in tMS^2 experiment.

(A) DIA experiment parameters	
Precursor mass range (*m/z*)	350–980
Isolation window (*m/z*)	5
Scan range (*m/z*)	150–2,000
HCD collision energy (%)	25
RF lens (%)	40
AGC target (%)	500
Maximum injection time (ms)	7
Window placement optimization	On
Loop control	Time
Time (s)	0.6

(B) MS^1 experiment parameters	
Resolution	240,000
Scan range (*m/z*)	350–980
RF lens (%)	40
AGC target (%)	500
Maximum injection time (ms)	5

(C) tMS^2 experiment parameters	
Isolation window (*m/z*)	2
Activation type	HCD
HCD collision energy (%)	25
AGC target (%)	500
Maximum injection time (ms)	5
Polarity	Positive
Loop control	All
Retention time window (min)	2
Source fragmentation	Disabled
Detector type	Astral
RF lens (%)	40
Scan range	200–1,500

Table 3. Mass spectrometer parameters in SureQuant hybrid-DIA. (A) Mass spectrometer parameters in DIA experiment. (B) Mass spectrometer parameters in MS^1 experiment. (C) Mass spectrometer parameters in tMS^2 (SureQuant PRM) experiment.

(A) DIA experiment parameters	
Precursor mass range (*m/z*)	350–980
Isolation window (*m/z*)	5
Scan range (*m/z*)	150–2,000
HCD collision energy (%)	25
RF lens (%)	40
AGC target (%)	500
Maximum injection time (ms)	7
Window placement optimization	On
Loop control	Time
Time (s)	0.6

(B) MS^1 experiment parameters	
Resolution	120,000
Scan range (*m/z*)	300–1,200
RF lens (%)	40
AGC target (%)	500
Maximum injection time (ms)	10

(C) tMS^2 experiment parameters		
	Heavy	**Light**
Isolation window (*m/z*)	1.6	2
Activation type	HCD	HCD
HCD collision energy (%)	25	25
AGC target	100 (Standard)	300
Maximum injection time (ms)	3	5
Polarity	Positive	Positive
Mass tolerance (ppm)	Low: 10 High: 10	Low: 20 High: 20
Retention time window (min)	2	2
Detector type	Astral	Astral
RF lens (%)	40	40
Scan range	150–1,800	150–1,800
Isolation offset (*m/z*)	N/A	-5.004 (R +2) -4.007 (K +2) -3.336 (R +3) -2.671 (K +3)

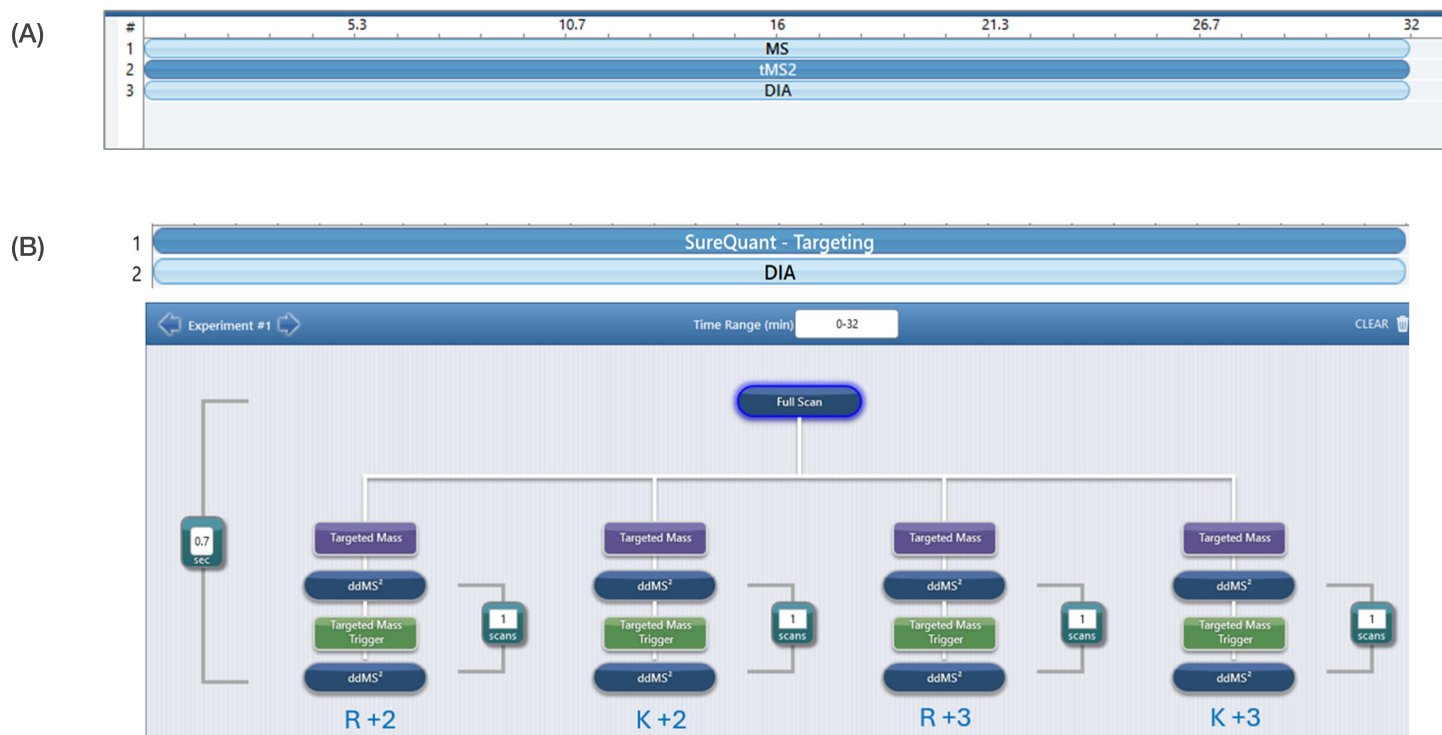


Figure 1. tMS^2 hybrid-DIA and SureQuant hybrid-DIA methods setup. (A) Method setup for tMS^2 hybrid-DIA. (B) Method setup for SureQuant hybrid-DIA.

Data analysis

For both tMS² and SureQuant-based hybrid-DIA analyses, DIA scans were extracted using the HTRMS converter tool from Spectronaut software (v20.1). The converted DIA files were then used for directDIA™ searches in Spectronaut software. MS files from both standard DIA and hybrid-DIA conversions were searched using Spectronaut software's library-free approach with factory settings and the human database (UniProt reference proteome 2025 release, 20,420 entries) FASTA file. Carbamidomethylation of cysteine was set as a fixed modification, while oxidation of methionine was set as a variable modification. Targeted peptides from the hybrid-DIA raw files were processed using Skyline software. LOD and LOQ were determined using a three-times signal-to-noise ratio. The concentration of each peptide was calculated based on the manufacturer's supplied amount and the volume of diluent used for resuspension. All the statistical analyses were graphed and plotted using Python software 3.0.1.

Results and discussion

tMS² hybrid-DIA results and discussions

Protein group and peptide ID numbers were obtained by searching the extracted DIA files in Spectronaut software. As shown in Figure 2, the number of identified protein groups was 692 in the standard DIA method, which decreased in the tMS² hybrid-DIA methods, ranging from 593 to 645. There was no decrease in protein group IDs as the number of targeted peptides increased in the hybrid-DIA methods. On average, the protein group IDs decreased by 9.5% in the five hybrid-DIA methods compared to the standard DIA method. The coefficient of variation (CV%) for protein group IDs was 3.2% across the five different tMS² hybrid-DIA methods with 30–300 targeted peptides. The average decrease in the number of identified peptides was 4.2% compared to the standard DIA method. The CV% for the number of peptide IDs was 1.5% across the different hybrid-DIA methods. The observed loss of protein IDs in the hybrid-DIA methods was predominantly among low-abundance proteins, which can be attributed to the high dynamic range of protein levels in neat plasma. This was confirmed by the protein ranking plot shown in Figure 3, which indicates that the dynamic range of detected protein intensities exceeded six orders of magnitude.

The mean values of data points per peak in the DIA experiment were 5.0–5.3 for panel sizes ranging from 30 to 120 targeted peptides, decreasing to 4.5–4.7 when the method included 200 and 300 targeted peptides (Figure 4). The data shows that with the current data points per peak, the CV values for protein quantities were comparable to those of the standard DIA method,

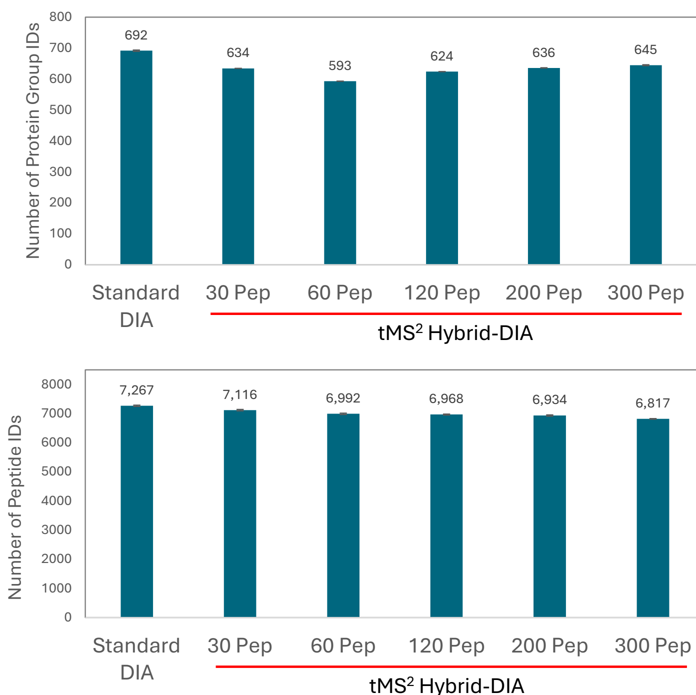


Figure 2. Hybrid-DIA had an average of 9.5% fewer protein groups and 4.2% fewer peptide IDs compared to standard DIA but still maintained a good protein depth in plasma. Bar charts show the number of protein groups and peptides identified.

ranging from 6% to 8% (Figure 5A), while the CV values for peptide intensities were approximately 11% to 13% (Figure 5B). Thus, the data points per peak in hybrid-DIA methods, even with 200 and 300 targeted peptides, guaranteed robust protein and peptide quantification.

Pearson correlation was calculated to compare protein quantities between hybrid-DIA and standard DIA methods. Figure 6A illustrates an example correlation between hybrid-DIA with 30 targeted peptides and standard DIA, with a coefficient (r) value of 0.98. Figure 6B presents a Pearson correlation heatmap showing the correlation between each method. The r values between the data from each method exceeded 0.97, indicating very strong correlations between standard DIA and tMS² hybrid-DIA, as well as strong correlations between different tMS² hybrid-DIA methods.

The effects of DIA scans on PRM acquisition were also investigated in hybrid-DIA analysis. Figure 7 illustrates the normalized intensity of all 200 peptides. Quantitatively, the data demonstrated a median intensity ratio between standard PRM and hybrid-DIA of 1.11 and an average ratio of 1.08. This indicates an approximate 10% decrease in targeted peptide intensities when using the hybrid-DIA method. However, this slight decrease was not shown to affect the LOD and LOQ for

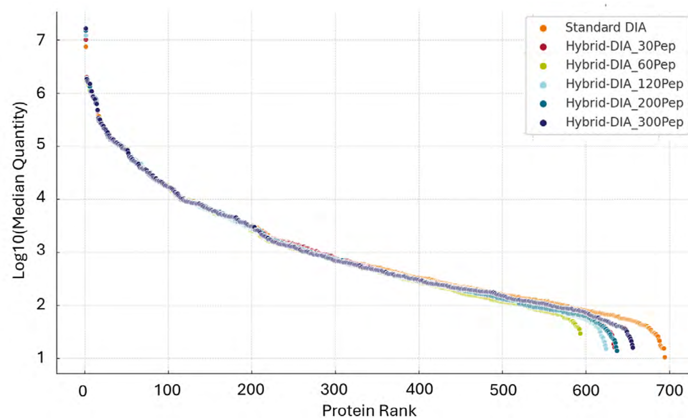


Figure 3. The dynamic range of detected protein intensities is more than 6 orders of magnitude. Protein group ranking from standard and tMS² hybrid-DIA methods on the Orbitrap Astral Zoom MS.

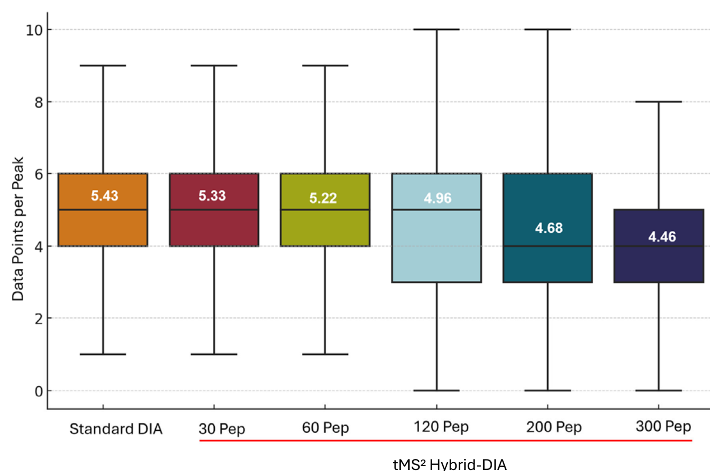


Figure 4. Data points per peak from standard and tMS² hybrid-DIA on the Orbitrap Astral Zoom MS.

Table 4. Summary of the two hybrid-DIA approaches.

	tMS² hybrid-DIA	SureQuant hybrid-DIA
Need heavy standard	No	Yes
Method set up	Straightforward	More complex
Peak completeness	Yes	No
Transition list	Not required	Required
Included targeted peptides	Less	More
Data points per peak	Less	More
Peptide identification	Potential interference if no heavy peptide confirmation	Improved confidence and accuracy/ robustness for peptide ID
Adaptive RT	Yes	No

the quantified targeted peptides. Furthermore, a very strong correlation was observed between peptide intensities in standard PRM and tMS² hybrid-DIA with r=0.994.

Calibration curves for 300 targeted peptides were calculated using Skyline software. For linearity, 89% of peptides had R^2 values greater than 0.99, and 99% of peptides had R^2 values exceeding 0.90 (Figure 8B). Using the peptide LEYLLLSR as an example, the linearity and accuracy (100 ± 20%) of the calibration curves were maintained across a concentration range of 6 orders of magnitude from level 1 to level 9 (Figure 8A and 8C). Notably, at a quantity of approximately 6 amol on column, a good signal-to-noise ratio was still observed for this peptide (Figure 8D). Additionally, more than 92% of peptides had an LOD below 25 amol, over 97% had an LOD below 50 amol, and more than 82% of peptides had an LOQ below 50 amol (Figure 9). These results showed that the Orbitrap Astral Zoom MS has exceptional sensitivity for peptide quantitation using hybrid-DIA methods.

SureQuant hybrid-DIA results and discussions

The SureQuant-based hybrid-DIA method was evaluated similarly to the tMS² hybrid-DIA, including assessments of protein group and peptide identifications, CV%, protein and peptide intensity correlations, etc.

The number of identified protein groups in SureQuant-based hybrid-DIA ranged from 596 to 614 (Figure 10). There was an average decrease of 12.8% in the number of protein group IDs compared to the standard DIA method. The coefficient of variation (CV%) for protein group IDs was 1.2% across different hybrid-DIA methods. The number of identified peptides ranged from 6,745 to 6,932, which is slightly lower compared to the standard DIA method with 7,272 peptides identified. The average decrease in the number of peptide IDs was 6.7% compared to standard DIA, with a CV% of 1.3% across SureQuant-based hybrid-DIA methods with 30–300 targeted peptides.

The CV% values for protein quantities ranged from 5% to 7% in standard DIA and hybrid-DIA with 30–300 targeted peptides, while the CV values for peptide intensities ranged from 8% to 12.2% (Figure 11). This demonstrates good reproducibility of protein and peptide quantification from DIA acquisition in hybrid-DIA analysis. Similar to tMS² hybrid-DIA, the dynamic range of detected protein intensities exceeded six orders of magnitude (Figure 12).

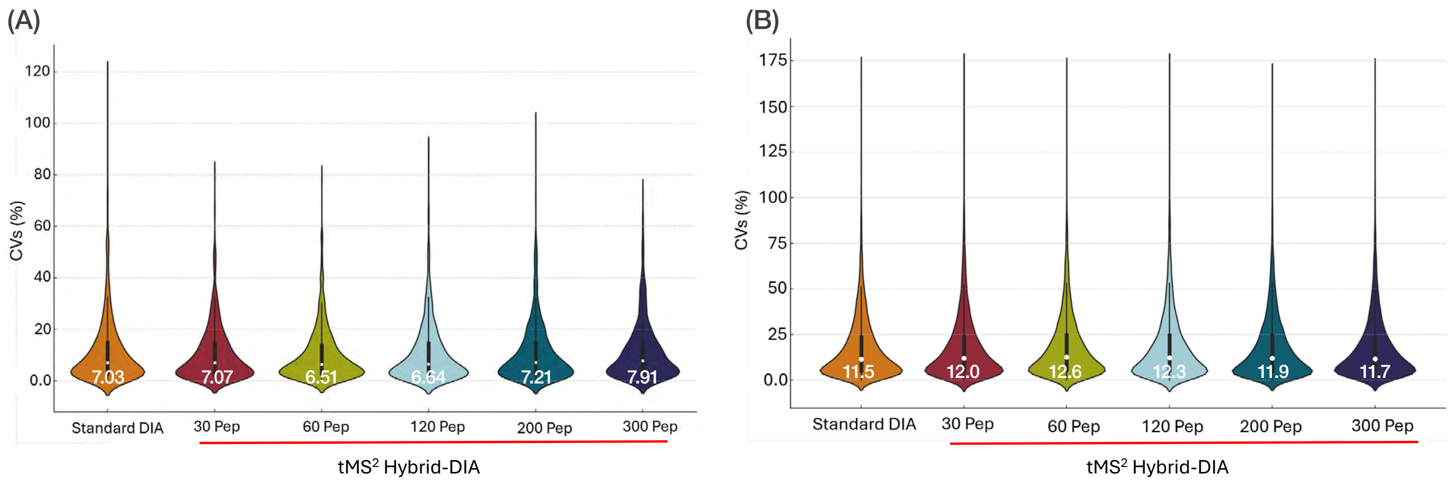


Figure 5. The Orbitrap Astral Zoom MS quantitation has good precision. Protein quantity and peptide intensity CV% from tMS^2 hybrid-DIA methods with different numbers of targeted peptides.

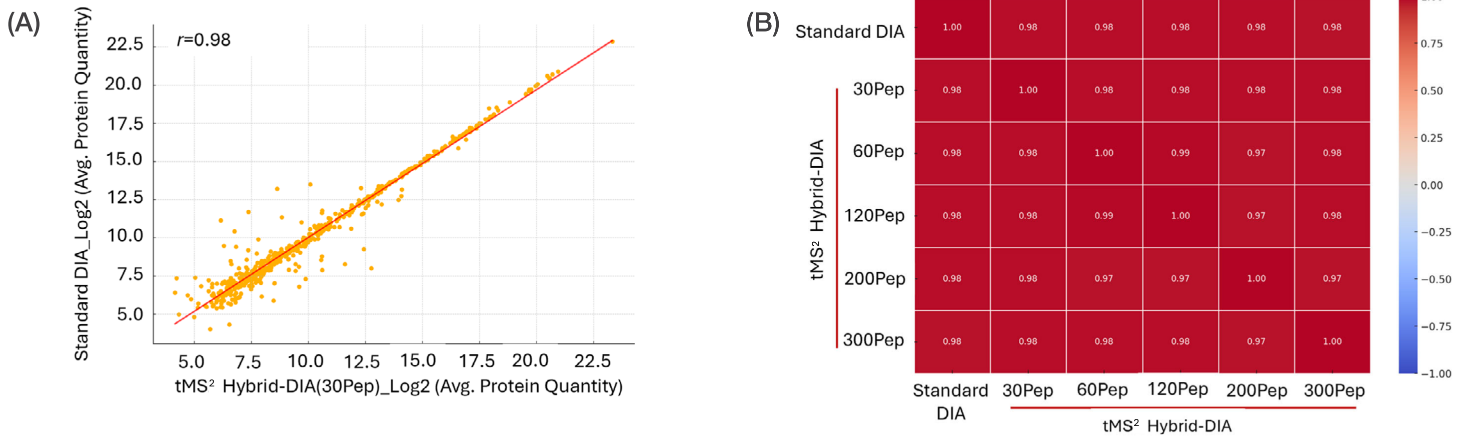


Figure 6. Protein quantity correlations between standard DIA and different tMS^2 hybrid-DIA methods.

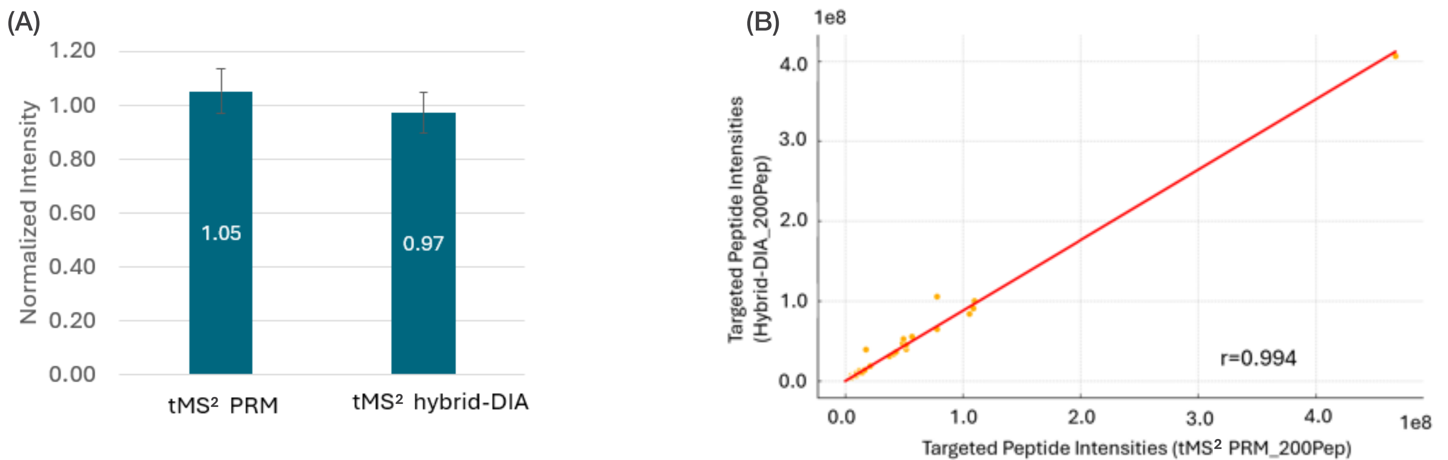
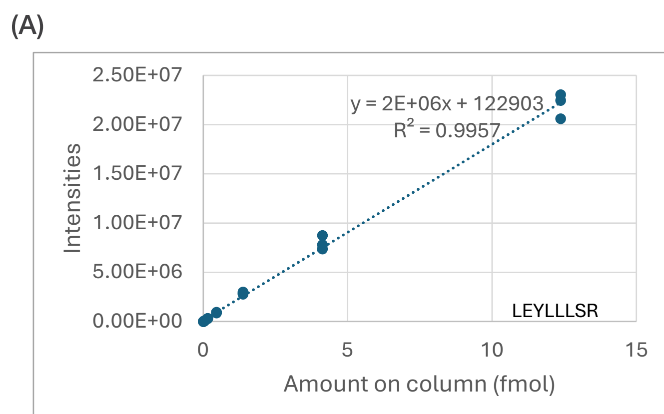
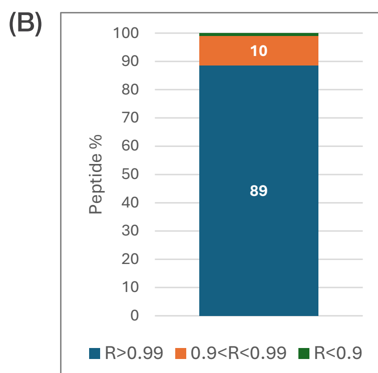


Figure 7. Targeted peptide intensities were not significantly affected by DIA acquisition in the tMS^2 hybrid-DIA method. (A) Normalized peptide intensities from standard tMS^2 PRM and tMS^2 hybrid-DIA methods. (B) Pearson correlation of targeted peptide intensities between tMS^2 hybrid-DIA and standard PRM methods.

(A)



(B)



(C)

level	fmol on column	Accuracy(%)	CV(%)
1	12.4	99.6	5.8
2	4.1	108.1	8.9
3	1.4	117.3	3.9
4	0.46	111.7	4.2
5	0.15	115.6	4.9
6	0.05	106.9	9.6
7	0.02	99.6	12.5
8	0.0057	87.1	15.2
9	0.0019	100.7	12.2

(D)

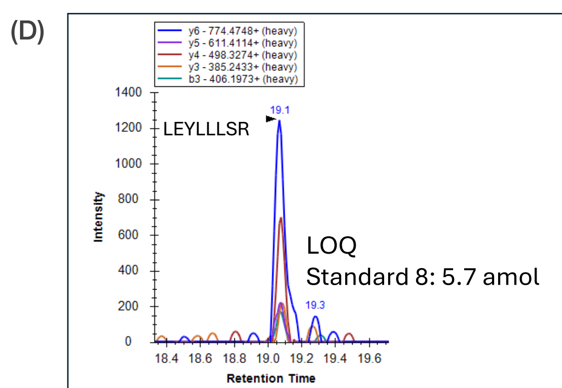


Figure 8. Linearity, accuracy and precision of targeted peptides using the tMS² hybrid-DIA method. (A) Calibration curve of the example peptide LEYLLLSR. (B) Calibration curve linearity coefficients R values for all 300 peptides. (C) Accuracy and precision (n=3) of the calibration curve. (D) The peptide peak of LEYLLLSR at LOQ level (5.7 amol).

In the DIA acquisition from the SureQuant hybrid-DIA method, the mean points per peak decreased from 5.80 to 5.56, showing a smaller decrease compared to tMS² hybrid-DIA (Figure 13). This indicated that an increased size of the targeted panel had very little effect on the data points per peak for DIA acquisition in the SureQuant hybrid-DIA assay. With a larger number of targeted panels, SureQuant hybrid-DIA could have more data points per peak in DIA acquisition compared to tMS² hybrid-DIA. This may be due to the fact that in the SureQuant hybrid-DIA method, MS² spectra are acquired for the heavy/light target peptides only when there is spectral evidence for their presence. Conversely, the PRM assay acquires MS² spectra throughout the active target retention time window.

Pearson correlation analysis revealed strong positive correlations ($r > 0.97$) between protein intensities obtained from standard DIA and SureQuant hybrid-DIA, as well as between different hybrid-DIA methods (Figure 14). Similarly, the targeted peptide intensities also demonstrated a strong correlation between hybrid-DIA and SureQuant PRM assay (Figure 15). The standard SureQuant method exhibited slightly higher intensities compared to the SureQuant hybrid-DIA method, with a median intensity ratio of 1.13 between SureQuant and hybrid-DIA analysis, indicating approximately a 13% decrease in hybrid-DIA SureQuant acquisition versus standard SureQuant analysis. Again, this small decrease is not expected to affect the LOD and LOQ levels of targeted peptides when running SureQuant-based hybrid-DIA analysis.

In the end, the results from the two different hybrid-DIA approaches were cross-validated by Pearson correlation (Figure 16). There was a strong correlation of targeted peptide intensities between SureQuant acquisition in the SureQuant hybrid-DIA and tMS² acquisition in the tMS² hybrid-DIA assays. For protein intensities from DIA acquisition using the two hybrid-DIA approaches, the correlation coefficients were all greater than 0.97, showing consistent protein and peptide results using both hybrid-DIA approaches.

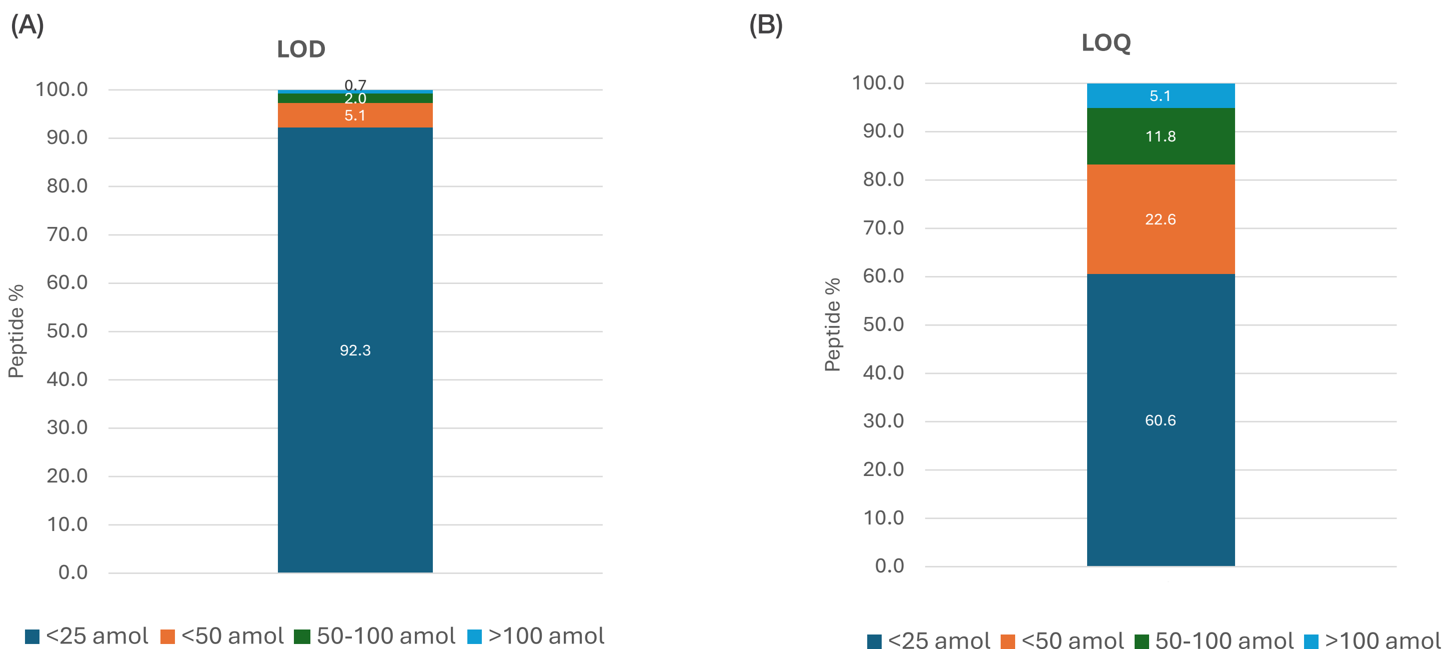


Figure 9. The Orbitrap Astral Zoom MS has exceptional sensitivity for peptide quantitation. Summary of LOD and LOQ of 300 targeted peptides using the tMS[2] hybrid-DIA method.

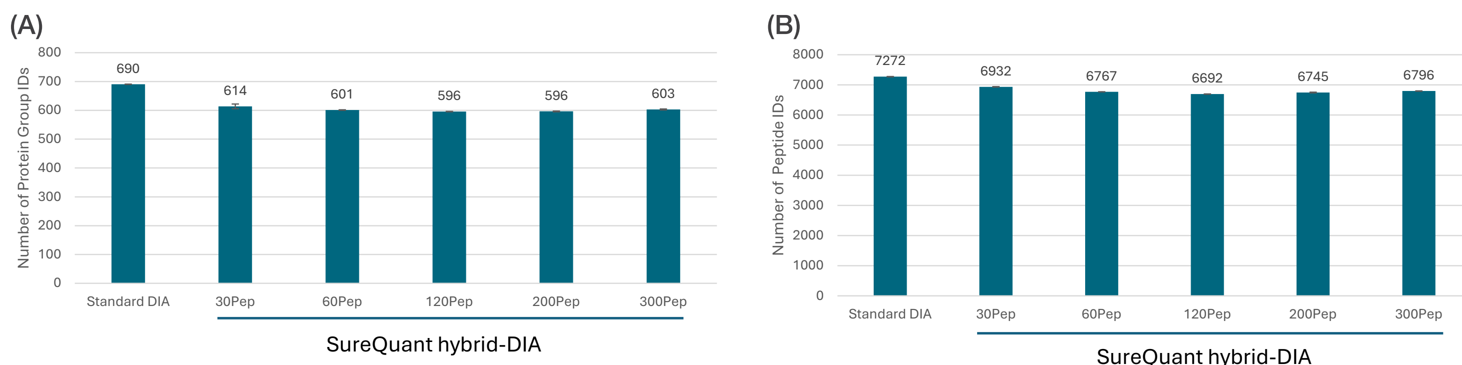


Figure 10. Hybrid-DIA had an average of 12.8% fewer protein groups and 6.7% fewer peptide numbers compared to standard DIA but still maintain a good protein depth in plasma. Bar charts show the number of protein groups and peptides identified.

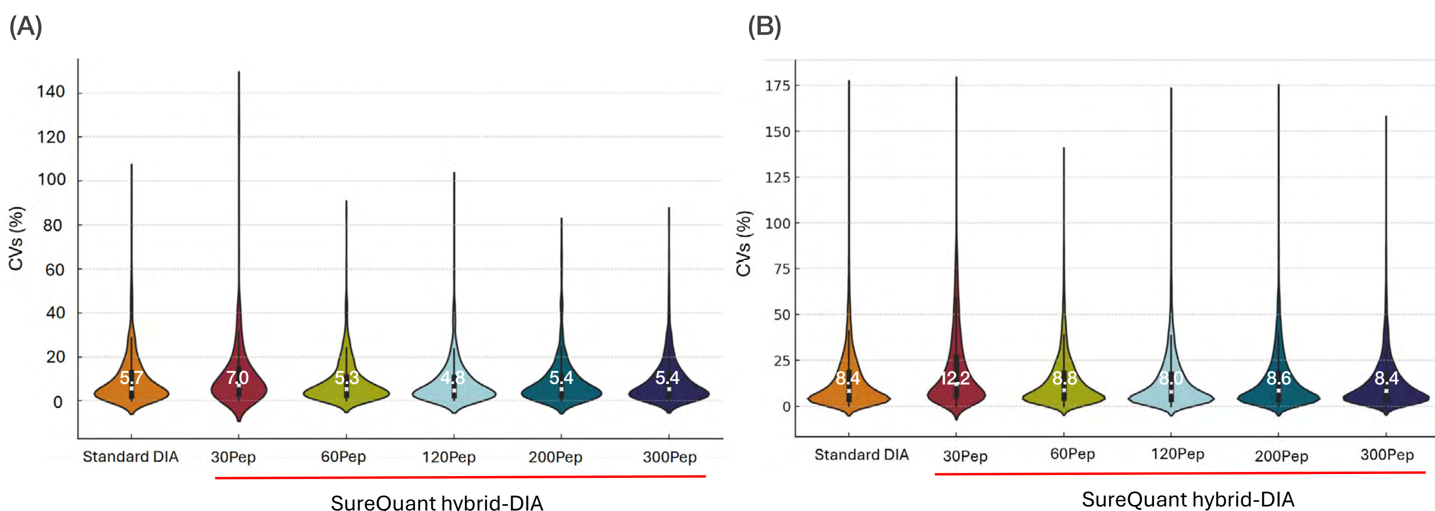
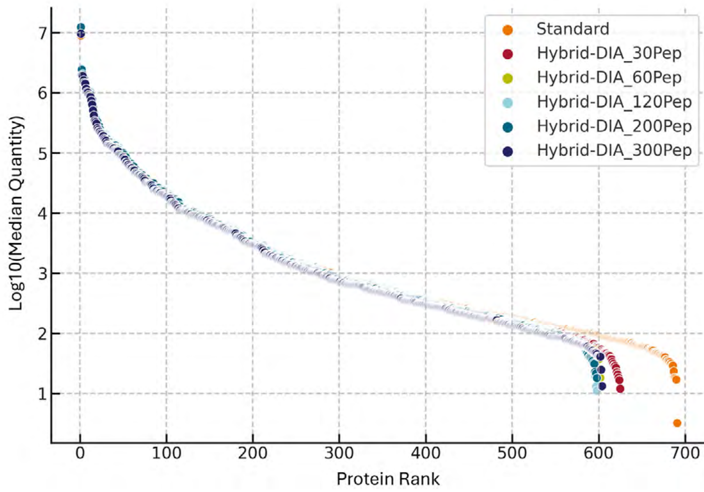


Figure 11. The Orbitrap Astral Zoom MS quantitation showed good precision. Protein quantity and peptide intensity CV% from three injection replicates using SureQuant hybrid-DIA methods with 30–300 targeted peptides.



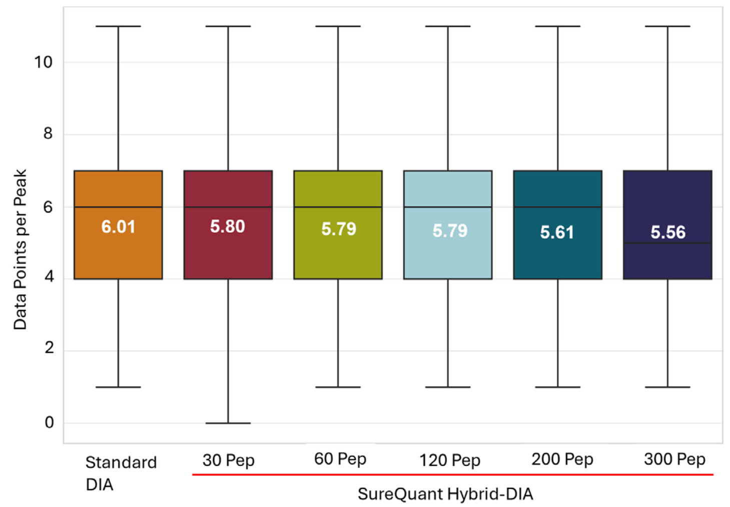
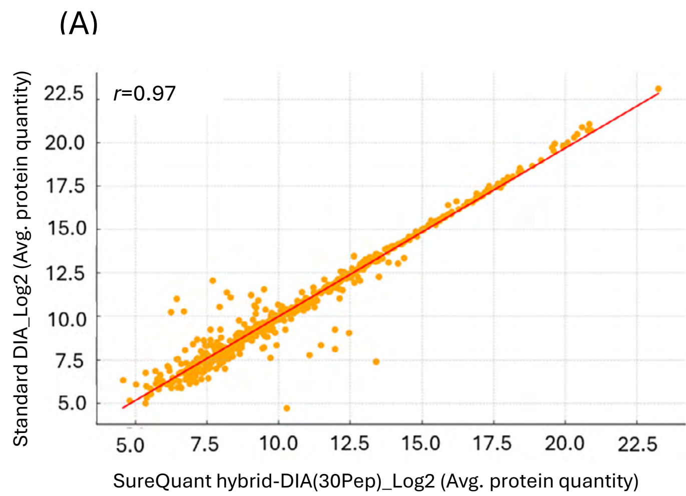


Figure 12. The dynamic range of detected protein intensities is more than 6 orders of magnitude. Protein group ranking using standard and SureQuant hybrid-DIA methods on the Orbitrap Astral Zoom MS.

Figure 13. Data points per peak from standard and SureQuant hybrid-DIA on the Orbitrap Astral Zoom MS.



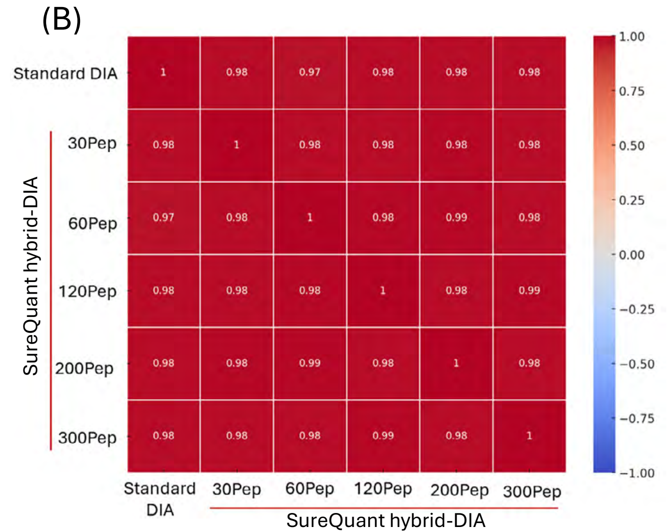
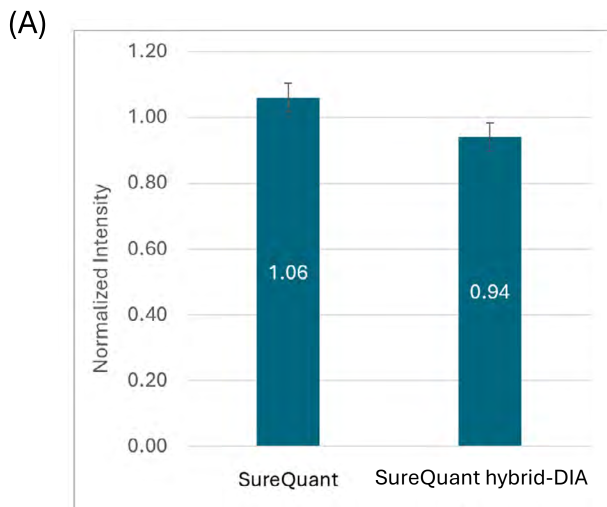


Figure 14. Protein quantity correlations between standard DIA and different SureQuant hybrid-DIA methods.



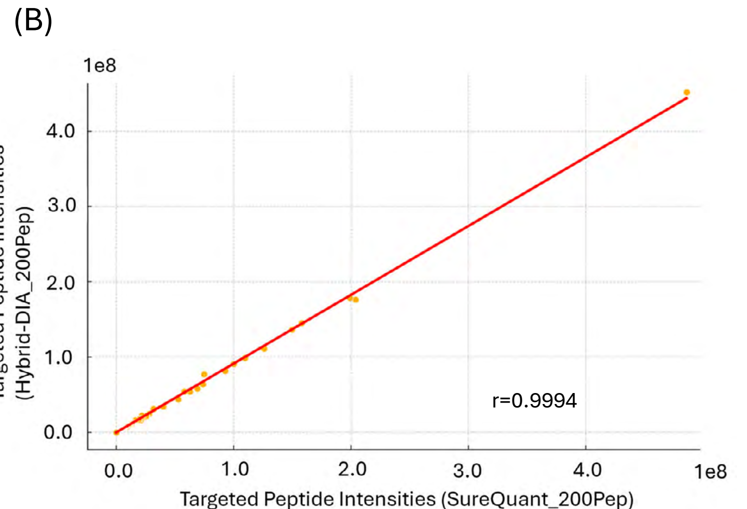


Figure 15. Targeted peptide intensities decreased by about 13% in the SureQuant hybrid-DIA compared to the SureQuant PRM method. (A) Normalized peptide intensities from SureQuant PRM and SureQuant hybrid-DIA methods. (B) Pearson correlation of targeted peptide intensities between SureQuant hybrid-DIA and SureQuant PRM methods.

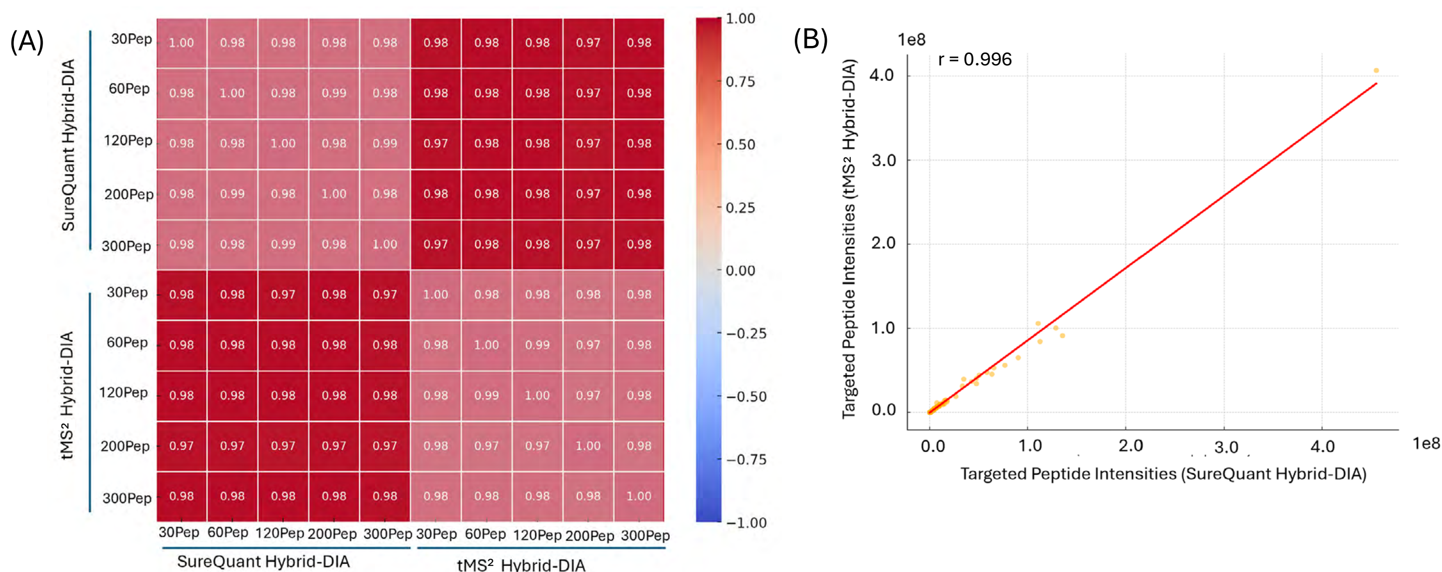


Figure 16. Cross-validation between SureQuant hybrid-DIA and tMS² hybrid-DIA results. (A) Correlation of protein quantities from DIA acquisition between SureQuant hybrid-DIA and tMS² hybrid-DIA methods. (B) Correlation of targeted peptide intensities between SureQuant hybrid-DIA and tMS² hybrid-DIA methods.

Conclusions

Overall, the data demonstrated that both the SureQuant hybrid-DIA and tMS² hybrid-DIA methods are effective for simultaneously achieving targeted peptide quantitation and DIA discovery scans. Table 4 highlights the pros and cons of tMS² hybrid-DIA and SureQuant hybrid-DIA approaches.

The tMS² hybrid-DIA method does not require heavy peptide standards, has a straightforward setup, ensures peak completeness, and supports adaptive retention time (adaptive RT). The data showed that there are sufficient data points per peak, even with 300 targeted peptides in the PRM panel. However, users should be cautious about the number of data points per peak when using excessively large, targeted peptide panels. Additionally, users may experience potential interference in peptide identification without heavy-labeled peptide confirmation.

SureQuant hybrid-DIA includes more targeted peptides, provides more data points per peak, and offers improved confidence and accuracy in peptide identification. On the downside, it requires heavy peptide standards, has a more complex setup, does not ensure peak completeness, and does not need adaptive RT.

Each method has its own advantages and features, making it suitable for different experimental needs.

Highlights of hybrid-DIA methods:

- **Hybrid-DIA still maintains a good depth of protein group and peptide numbers compared to the standard DIA method**. The increased number of targeted peptides did not impact the protein group number in the DIA acquisition of the hybrid-DIA method.
- **The Orbitrap Astral Zoom MS demonstrated exceptional sensitivity for targeted peptides in hybrid-DIA analysis.** The DIA acquisition in the hybrid-DIA method did not significantly affect the sensitivity of targeted peptide scans compared to the standard PRM method. More than 92% of peptides had a LOD below 25 amol, and more than 82% of peptides had a LOQ below 50 amol.
- **Data from the hybrid-DIA method showed a very strong positive correlation with data from both the standard DIA and standard PRM methods.** Protein intensities measured using the hybrid-DIA method exhibited a strong correlation with those obtained from the standard DIA method. Similarly, targeted peptide intensities from the hybrid-DIA method showed a strong correlation with the standard PRM method.
- **The two hybrid-DIA approaches, SureQuant hybrid-DIA and tMS² hybrid-DIA, demonstrated consistent results.**

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References

1. Martínez-Val, A. et al. Hybrid-DIA: intelligent data acquisition integrates targeted and discovery proteomics to analyze phospho-signaling in single spheroid. *Nature Communications* **2023**, *14*, 3599.
2. Gajadhar, A. Thermo Fisher Scientific Technical Note 65873 - SureQuant intelligence-driven MS: a new paradigm for targeted quantitation. https://documents.thermofisher.com/TFS-Assets/CMD/Technical-Notes/tn-65873-ms-surequant-targeted-quantitation-tn65873-en.pdf

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