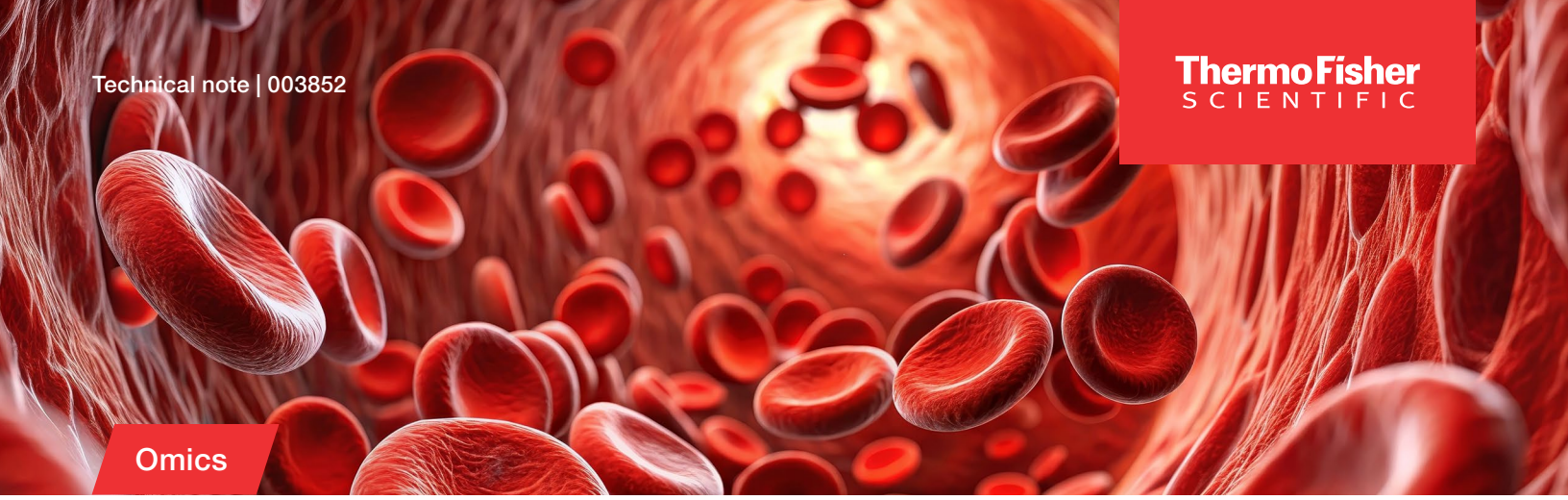

Technical note | 003852

Omics

Comprehensive and high-throughput plasma proteome profiling for biomarker discovery

Using the Orbitrap Astral Zoom Mass Spectrometer and the Seer Proteograph ONE Assay

Authors

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Keywords

Plasma, Proteograph ONE, Orbitrap Astral Zoom mass spectrometer, biomarker, nDIA, narrow-window, data-independent acquisition

Goal

Demonstrate the potential of plasma proteomics workflows with the Thermo Scientific™ Orbitrap™ Astral™ Zoom mass spectrometer and the Seer® Proteograph® ONE Assay to unlock unparalleled depth along with accurate and precise quantitation using narrow-window label-free data-independent acquisition (nDIA) methods. Combined with various plasma sample preparation strategies, including neat, immuno-depleted, and the Proteograph ONE workflow, the Orbitrap Astral Zoom MS empowers diverse throughput methods to maximize identification, quantitation, and high-throughput performance.

Introduction

Plasma proteomics, the study of proteins in blood plasma, has gained significant attention due to its accessibility and potential for novel biomarker discovery. By revealing complex biological processes, plasma proteomics plays a crucial role in understanding global signaling and immune responses,[1] making it a powerful tool for advancing disease diagnosis, monitoring, and personalized medicine.

However, profiling the plasma proteome is challenging due to its complex and dynamic composition. The wide range of protein concentrations—spanning up to 12 orders of magnitude[2]—can mask low-abundance proteins, impacting assay sensitivity and biomarker identification. In addition, preanalytical, analytical, and biological variables challenge the establishment of reliable biomarkers, necessitating optimized sample collection, sample preparation, and analytical techniques.[3]

Liquid chromatography-mass spectrometry (LC-MS)-based approaches excel in plasma proteomics due to their high analytical dynamic range and ability to directly measure peptides and characterize post-translational modifications (PTMs). Innovations in sample preparation, separation, mass spectrometry (MS), and data analysis are enhancing the sensitivity and robustness of these methods, allowing researchers to monitor changes and unravel disease mechanisms. Here, we demonstrate how the Orbitrap Astral Zoom MS enables high sensitivity and reproducibility for low-abundant proteins, facilitating comprehensive plasma proteome measurements and potential for biomarker discovery.

Plasma proteomics sample preparation methodology

Various sample preparation methods have been developed for plasma proteomics, each with its own advantages and challenges:

- **Neat plasma**
  - Simplifies sample preparation with minimal processing and sample manipulation.
  - Preserves the full spectrum of plasma proteins, maintaining biological representation and preservation.
  - Reduces the risk of sample loss and preparation-induced modifications.
  - Low-cost and highly reproducible sample preparation methods.
  - Low-abundant proteins may be masked by high-abundant proteins, limiting coverage.
- **Immunodepleted plasma**
  - Removes high-abundant proteins to enhance the detection and quantification of low-abundant proteins.
  - Improves sensitivity and dynamic range for biomarker discovery.
  - Potential for non-specific protein removal and the introduction of additional technical variation, which can offer opportunities for further optimization.
  - Compatible with automation or plate-based immunodepletion enables large studies to reduce technical variation and increase scalability.
- **Proteograph ONE processed plasma**
  - Uses Seer's proprietary engineered nanoparticles with physicochemical surface properties that increase low abundance proteins relative to high abundant proteins.[4]
  - Improves detection of low-abundant proteins and increases plasma proteome coverage, aiding in novel biomarker discovery.
  - End-to-end workflow automation reduces technical variation and increases scalability for large studies.
- **Fractionation schemes**
  - Separates digested peptides into less complex fractions, facilitating deeper protein identification.
  - Increases the time required for fractionation and analysis of each fraction.
  - Decreased scalability and reproducibility; not deployable for large scale clinical cohorts.

Optimized workflow

For optimal plasma proteomics analysis, a standardized high-throughput LC-MS workflow is essential. The Thermo Scientific™ Vanquish™ Neo UHPLC system, paired with the Orbitrap Astral Zoom MS, offers precise low flow rates and gradient formation, ensuring maximum sample recovery. The robustness of the analytical columns and UHPLC systems ensures reliable separation, minimizing variations in peak shape, retention time, and resolution across biological sample analyses. This consistency provides high-quality data acquisition in MS analyses, enabling precise detection and quantification of proteins across the concentration gradient, which is crucial given the complexity and wide dynamic range of plasma proteins.

In this technical note, we demonstrate the enhanced capabilities of the Orbitrap Astral Zoom MS in plasma proteomics, allowing researchers to gain deeper insights into the plasma proteome and drive innovations in disease diagnosis, monitoring, prognostics, patient stratification, and precision medicine. With the fine-tuned flexibility of methods tailored for high-throughput, quantitative, and maximum identification workflows, researchers are empowered to choose the best-suited methodology for plasma proteomic profiling based on their research requirements. This ranges from scalable and robust methods for population-scale studies to ultra-deep profiling for capturing the most extensive proteome depth of coverage.

Experimental approach

Common consumables

- Water with 0.1% formic acid (FA) (v/v), Optima™ LC-MS grade, Fisher Chemical™ (P/N LS118-500)
- 80% Acetonitrile (ACN), 20% water with 0.1% formic acid, Optima™ LC-MS, Fisher Chemical™ (P/N LS122500)
- Formic acid, 99.0+%, Optima™ LC-MS grade, Fisher Chemical™ (P/N A117-50)
- Thermo Scientific™ EASY-Spray™ HPLC Column, 2 µm, C18, 150 µm × 15 cm (P/N ES906)
- Thermo Scientific™ EASY-Spray™ PepMap™ Neo UHPLC Column, 2 µm, C18, 75 µm × 50 cm (P/N ES75500PN)
- Thermo Scientific™ PepMap™ Neo Trap Cartridge, 5 µm, C18, 300 µm × 5 mm (P/N 174500)
- Thermo Scientific™ High Select™ Depletion Spin Columns (P/N A36370)
- Thermo Scientific™ AccelerOme™ Label-Free MS Sample Prep Kit (P/N A50945)
- Thermo Scientific™ Pierce™ Quantitative Fluorometric Peptide Assay Kit (P/N 23290)
- Seer® Proteograph® ONE Assay Kit
- Thermo Scientific™ SureSTART™ 9 mm screw caps (P/N 6PSC9STB1)
- Thermo Scientific™ SureSTART™ 0.2 mL TPX screw top microvial with glass insert (P/N 60180-1655)

Instrumentation

- Thermo Scientific™ AccelerOme™ Automated Sample Preparation Platform
- Thermo Scientific™ Savant™ SpeedVac™ Concentrator
- Vanquish Neo UHPLC system
- Orbitrap Astral Zoom MS
- Seer® SP200 Automation Instrument

Sample preparation

Plasma peptides—neat, immunodepleted, and those generated by the Seer Proteograph ONE workflow—were prepared from a matched K_2EDTA pooled human plasma sample across all sample preparation methodologies.

Neat plasma peptides were prepared using the AccelerOme Label-Free MS Sample Prep Kit on the AccelerOme Automated Sample Preparation Platform. Specifically, 1.5 µL of plasma was mixed with 48.5 µL of LYSE solution and samples were loaded onto the AccelerOme platform for protein preparation, digestion with Trypsin/Lys-C, and peptide clean-up.

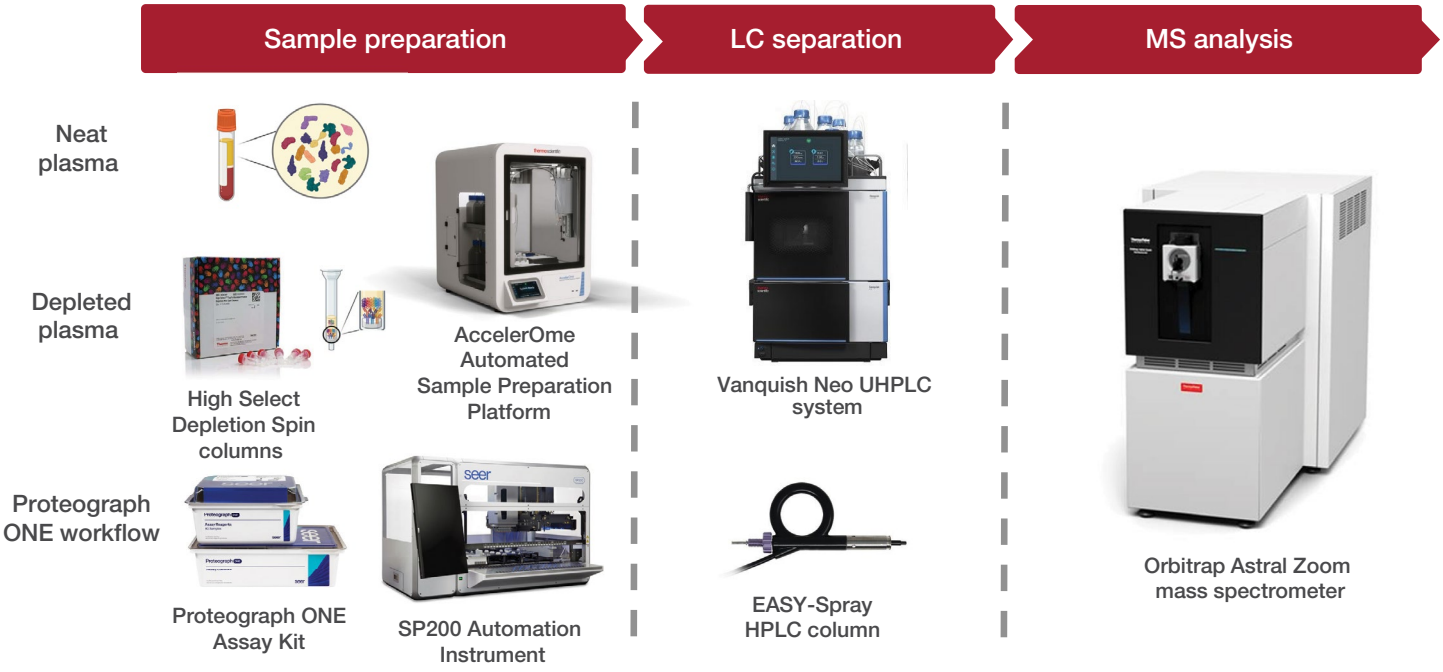

Figure 1. High-throughput plasma proteomics workflow using the Orbitrap Astral Zoom MS. This end-to-end solution, from sample handling to data acquisition, offers exceptional flexibility for translational research, enabling rapid analysis for large cohorts and deeper insights for advanced proteomics.

Peptides were then dried down with a Savant SpeedVac concentrator and reconstituted in water with 0.1% formic acid and 2% acetonitrile to a final concentration of 100 ng/µL. Peptide concentrations were quantified using the Pierce Quantitative Fluorometric Peptide Assay Kit. Depending on the LC-MS throughput, 200 ng, 500 ng, or 1,250 ng of peptide mass was loaded on the column for LC-MS analysis.

Immunodepleted plasma peptides were prepared using the High Select Depletion Spin columns. Specifically, 10 µL of plasma was used per depletion using the columns and the associated protocol was followed with no deviations. Depleted protein samples were concentrated using the Savant SpeedVac concentrator and reconstituted in 50 µL of LYSE solution from the AccelerOme Label-Free MS Sample Prep Kit. Samples were loaded directly into the AccelerOme Automated Sample Preparation Platform for protein preparation, digestion with Trypsin/Lys-C, and peptide clean-up. Peptides were then dried down with a Savant SpeedVac concentrator and reconstituted in water with 0.1% formic acid and 2% acetonitrile to a final concentration of 100 ng/µL. Peptide concentrations were quantified using the Pierce Quantitative Fluorometric Peptide Assay Kit. Depending on the LC-MS throughput, 200 ng, 500 ng, or 1,250 ng of peptide mass was loaded on the column for LC-MS analysis.

Plasma peptides were prepared using the Seer Proteograph ONE workflow. Specifically, 120 µL of plasma sample was loaded onto the Seer SP200 Automation Instrument and 100 µL was automatically mixed with the nanoparticles included in the Proteograph ONE Assay Kit. Sample-nanoparticle mixtures were incubated for one hour (37 °C) for protein corona formation based on physicochemical properties of the particles. A series of washes were performed to remove non-specific and weakly bound proteins. Plasma proteins bound to nanoparticles were then reduced, alkylated, and digested with Trypsin/Lys-C. Digested peptides underwent cleanup and desalting using a particle-based system. Eluted peptides were quantified using the Pierce Quantitative Fluorometric Peptide Assay Kit. Peptides were then dried down with a Savant SpeedVac concentrator and reconstituted in water with 0.1% formic acid and 3% acetonitrile to a final concentration of 50 ng/µL. Depending on the LC-MS throughput, 200 ng, 500 ng, or 1,250 ng of peptide mass was loaded on the column for LC-MS analysis.

LC-MS analysis

All LC-MS runs for neat, depleted, and Proteograph ONE processed peptides were analyzed using a Vanquish Neo UHPLC system in trap and elute configuration, paired with an Orbitrap Astral Zoom MS. Peptides were separated on the Vanquish Neo UHPLC system using EASY-Spray analytical columns, and chromatographic gradients were formed using 0.1% formic acid in water for mobile phase A and 0.1% formic acid in 80% acetonitrile for mobile phase B. Liquid chromatography parameters and gradient settings can be found in Tables 1A, 1B, and 1C. Mass spectrometer source parameters and scan parameters can be found in Table 2.

Table 1A. High performance liquid chromatography parameters for 100 SPD gradient throughput

Gradient		
Time (min)	*% Mobile phase B*	*Flow (µL/min)*
0	4.0	2.5
0.2	4	2.5
0.3	8	2.0
7.9	22.5	2.0
11.5	35.0	2.0
11.55	55.0	2.5
12.6	99.0	2.5
LC parameters		
LC configuration	Trap and Elute	
Fast loading/equilibration mode	Pressure Control	
Loading/equilibration/wash pressure	Max Pressure	
Equilibration factor	3	
Sampler temperature (°C)	7	
Mobile phase A / weak wash	0.1% Formic acid in water	
Mobile phase B / strong wash	0.1% Formic acid in 80% acetonitrile	
Zebra wash	Enabled	
Zebra wash cycles	4	
Analytical column temperature (°C)	50	
Column specifications		
Analytical column	EASY-Spray HPLC column, 2 µm C18, 150 µm × 15 cm (P/N ES906)	
Trap column	Thermo Scientific™ PepMap™ Neo Trap Cartridge, 5 µm C18, 300 µm × 5 mm, (P/N 174500)	

Table 1B. High performance liquid chromatography parameters for 60 SPD gradient throughput

Gradient		
Time (min)	*% Mobile phase B*	*Flow (µL/min)*
0	10	2.0
0.3	10	2.0
0.6	10	0.8
13.6	22.5	0.8
20.5	35.0	0.8
20.9	55.0	2.0
20.95	99.0	2.0
22.35	99.0	2.0

LC parameters	
LC configuration	Trap and Elute
Fast loading/equilibration mode	Pressure Control
Loading/equilibration/wash pressure	Max Pressure
Equilibration factor	3
Sampler temperature (°C)	7
Mobile phase A / weak wash	0.1% Formic acid in water
Mobile phase B / strong wash	0.1% Formic acid in 80% acetonitrile
Zebra wash	Enabled
Zebra wash cycles	4
Analytical column temperature (°C)	50

Column specifications	
Analytical column	EASY-Spray HPLC Column, 2 µm C18, 150 µm × 15 cm (P/N ES906)
Trap column	PepMap Neo Trap Cartridge, 5 µm C18, 300 µm × 5 mm, (P/N 174500)

Table 1C. High performance liquid chromatography parameters for 24 SPD gradient throughput

Gradient		
Time (min)	*% Mobile phase B*	*Flow (µL/min)*
0	8	0.5
2.5	8	0.5
3.0	8	0.25
37.0	28	0.25
48.5	35	0.25
48.9	98	0.25
49.0	98	0.5
54.0	98	0.5

LC parameters	
LC configuration	Trap and Elute
Fast loading/equilibration mode	Pressure Control
Loading/equilibration/wash pressure	Max Pressure
Equilibration factor	3
Sampler temperature (°C)	7
Mobile phase A / weak wash	0.1% Formic acid in water
Mobile phase B / strong wash	0.1% Formic acid in 80% acetonitrile
Zebra wash	Enabled
Zebra wash cycles	4
Analytical column temperature (°C)	50

Column specifications	
Analytical column	EASY-Spray PepMap Neo UHPLC Column, 2 µm, C18, 75 µm × 50 cm (P/N ES75500PN)
Trap column	PepMap Neo Trap Cartridge, 5 µm C18, 300 µm × 5 mm, (P/N 174500)

Table 2. Orbitrap Astral Zoom MS parameters: (A) global source and mass spectrometer parameters, (B) MS1 full scan experiment parameters, and (C) MS2 DIA scan experiment parameters

(A) Global parameters (Source and MS)	
Positive ion voltage (V)	2,100
Ion transfer tube temperature (°C)	290
Expected peak width (s)	10
Default charge state	2
Lock mass correction	Off

(B) MS1 full scan experiment parameters	
Orbitrap resolution	240K
Scan range (*m/z*)	380–980
RF lens (%)	40
Normalized AGC target (%) / Absolute AGC value	500% / 5.00e6
Maximum injection time	5 milliseconds (24-60 SPD) or 3 milliseconds (100 SPD)
Microscans	1

(C) MS2 DIA scan experiment parameters	
Precursor mass range (*m/z*)	380–980
Isolation window (*m/z*)	2.5 (24 SPD) or 3 (60-100 SPD)
Window placement optimization	On
AGC target	Custom
Normalized AGC target (%) / Absolute AGC value	500% / 5.00e4
Maximum injection time (ms)	7
DIA scan range (*m/z*)	150–2,000
HCD collision energy (%)	25
RF lens (%)	40
Pre-accumulation	On
Loop control	Time
Time (s)	0.6

LC-MS data processing and analysis

All acquired LC-MS data was processed using library-free analysis approaches with the Proteograph® Analysis Suite (PAS) (Seer Inc). All results were processed and filtered with a 1% precursor and 1% protein group false discovery rate (FDR). Exported tables were imported to Rstudio™ (2023.09.0 Build 463) with R (v4.3.1) for downstream data analysis and visualization.

Results and discussion

Neat plasma

Across various neat plasma throughputs and injection amounts, the median peptide and protein identifications with respective technical replicate injection median CVs were the following:

- For 100 samples per day (SPD), a median of 5,492 peptides and 682 protein groups were identified, with quantitative precision of 7.6% peptide CV and 5.6% protein group CV (Figures 2–5).
- For 60 SPD, a median of 6,343 peptides and 868 protein groups were identified, with quantitative precision of 7.4% peptide CV and 5.4% protein group CV (Figures 2–5).
- For 24 SPD, a median of 8,999 peptides and 1,165 protein groups were identified, with quantitative precision of 7.1% peptide CV and 4.8% protein group CV (Figures 2–5).

Among all protein groups identified at least once in neat plasma, 185 (14.7%) are unique to neat plasma and not detected in other workflows (Figure 6). Enriched Gene Ontology (GO) categories show blood related and immune related categories enriched and KEGG pathway enrichment analyses show enrichment in coagulation, adhesion, and other metabolic pathways (Figure 7). Due to the wide dynamic range of protein concentration in neat plasma, the ability to detect mid and low abundant protein groups were compromised relative to other workflows (Figure 8).

Top 14 protein depletion

To achieve greater plasma proteomic depth of coverage, High Select Depletion Spin columns were used to immunocapture and remove the 14 most abundant proteins. Across various depleted plasma throughputs and injection amounts, the median peptide and protein identifications with respective technical replicate injection median CVs were the following:

- For 100 SPD, a median of 10,887 peptides and 1,410 protein groups were identified, with quantitative precision of 7.8% peptide CV and 5.9% protein group CV (Figures 2–5).
- For 60 SPD, a median of 12,124 peptides and 1,667 protein groups were identified, with quantitative precision of 6.7% peptide CV and 4.9% protein group CV (Figures 2–5).
- For 24 SPD, a median of 18,273 peptides and 2,403 protein groups were identified, with quantitative precision of 7.1% peptide CV and 4.8% protein group CV (Figures 2–5).

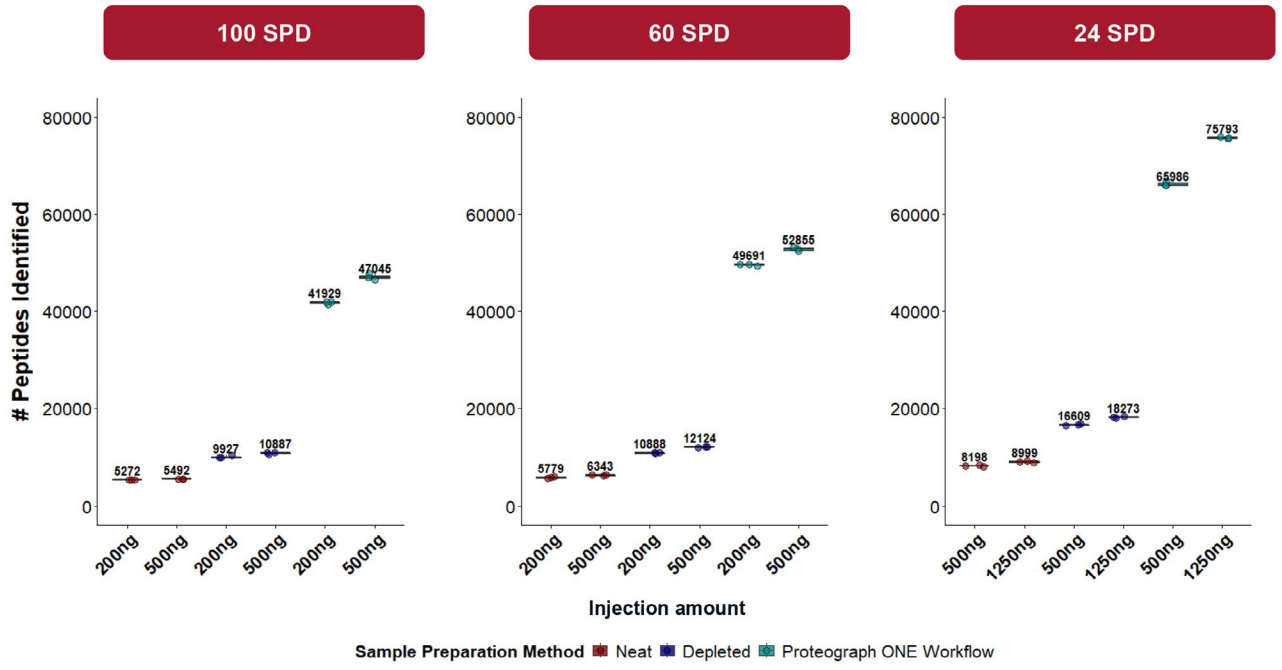

Figure 2. Orbitrap Astral Zoom MS peptide identifications from neat, depleted, and plasma processed using the Proteograph ONE workflow. Box plots display the peptide identifications for each sample preparation strategy: neat (dark red), depleted (navy), and the Proteograph ONE workflow (cyan). The box plots are ordered from highest throughput to maximum identification throughput from left to right. Each combination of sample preparation strategy, throughput, and load on column was analyzed in triplicate, with individual run identification numbers represented by circles. The median number of peptides is shown above each box plot.

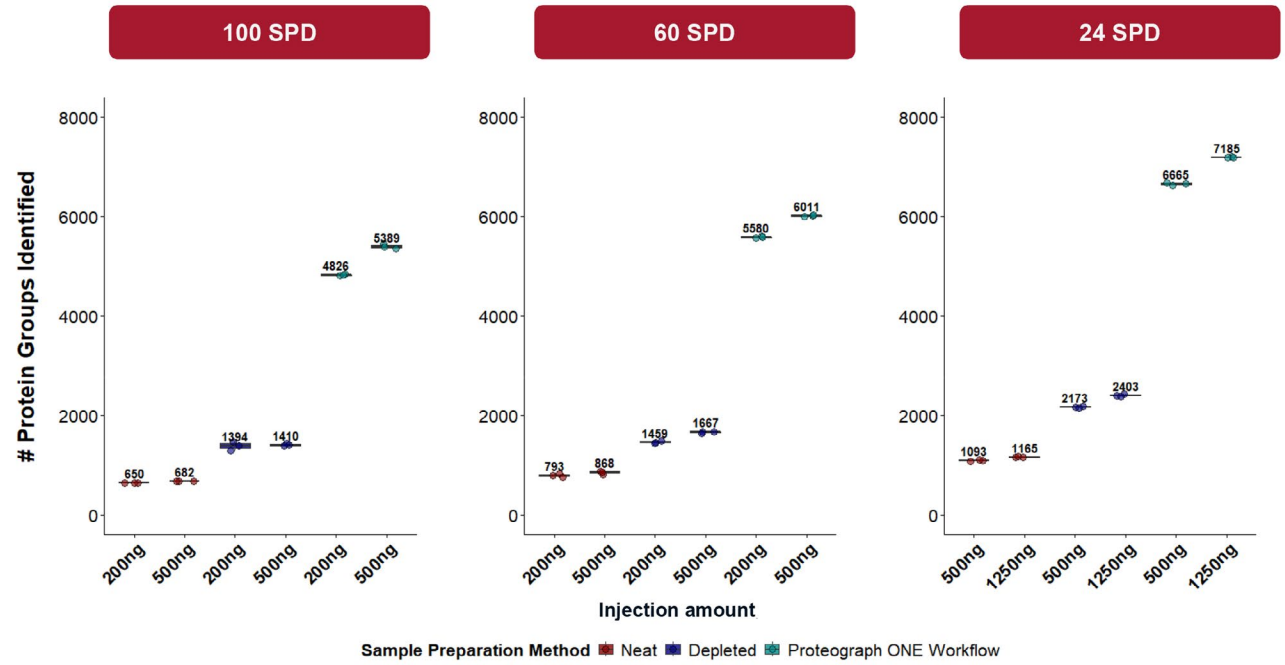

Figure 3. Orbitrap Astral Zoom MS protein group identifications from neat, depleted, and plasma processed using the Proteograph ONE workflow. Box plots display the protein group identifications for each sample preparation strategy: neat (dark red), depleted (navy), and Proteograph ONE workflow (cyan). The box plots are ordered from highest throughput to maximum identification throughput from left to right. Each combination of sample preparation strategy, throughput, and load on column was analyzed in triplicate, with individual run identification numbers represented by circles. The median number of protein groups is shown above each box plot.

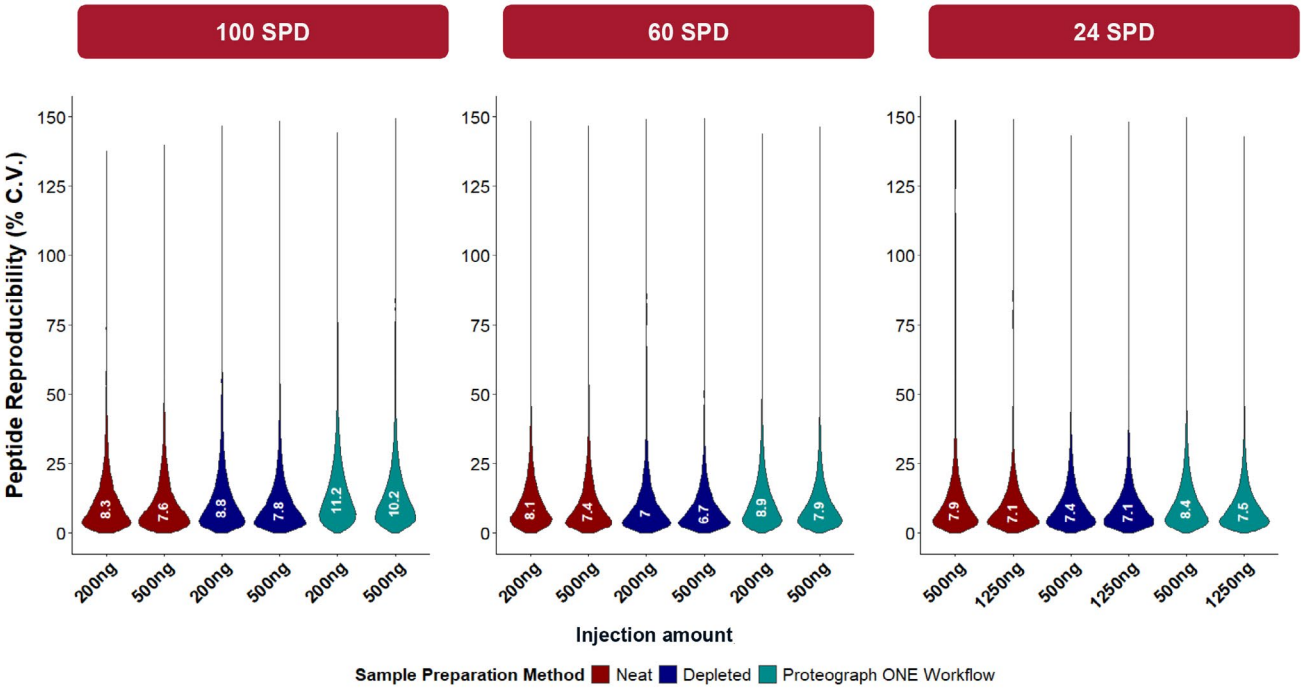

Figure 4. Orbitrap Astral Zoom MS peptide measurement quantitative precision from neat, depleted, and plasma processed using the Proteograph ONE workflow. Violin plots display the peptide coefficient of variation (% CV) for each sample preparation strategy: neat (dark red), depleted (navy), and Proteograph ONE workflow (cyan) calculated from triplicate LC-MS injections of each sample preparation strategy, throughput, and load on column combination. The violin plots are ordered from highest throughput (100 SPD) to maximum identification throughput (24 SPD) from left to right. The median peptide % CV per combination is shown vertically in each violin plot.

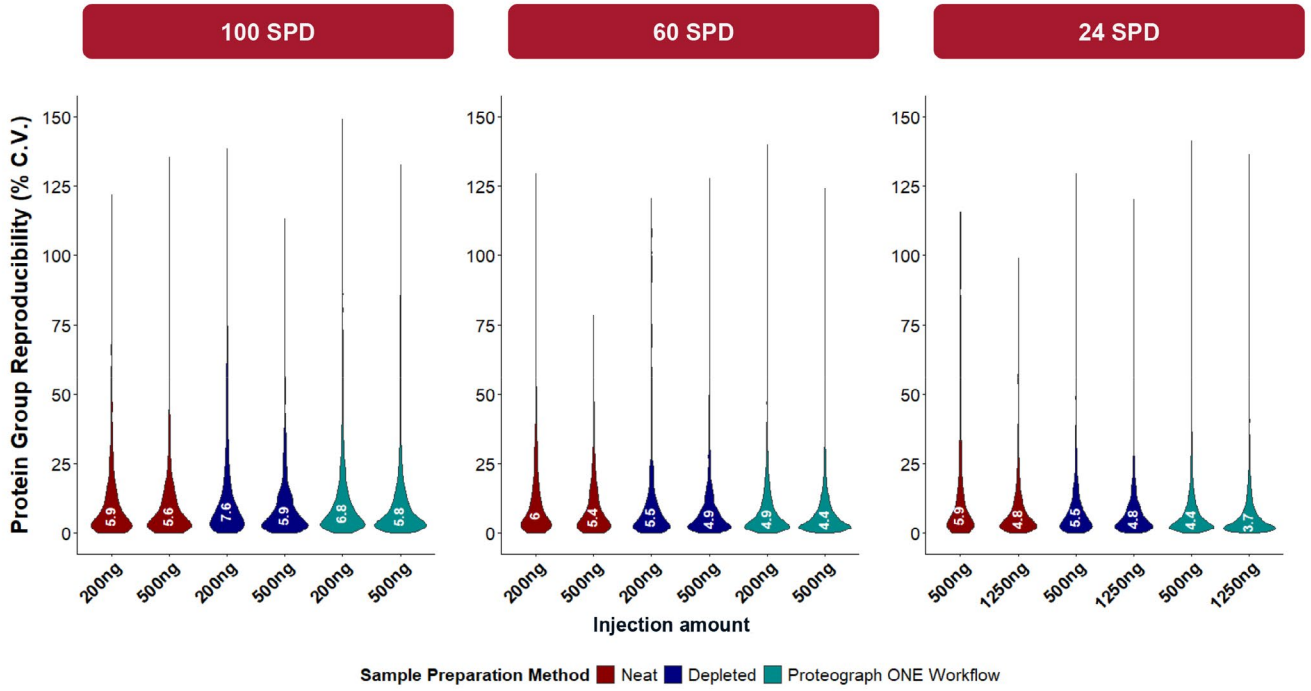

Figure 5. Orbitrap Astral Zoom MS protein group quantitative precision from neat, depleted, and plasma processed using the Proteograph ONE workflow. Violin plots display the protein group coefficient of variation (% CV) for each sample preparation strategy: neat (dark red), depleted (navy), and Proteograph ONE workflow (cyan) calculated from triplicate LC-MS injections of each sample preparation strategy, throughput, and load on column combination. The violin plots are ordered from highest throughput (100 SPD) to maximum identification throughput (24 SPD) from left to right. The median protein group % CV per combination is shown vertically in each violin plot.

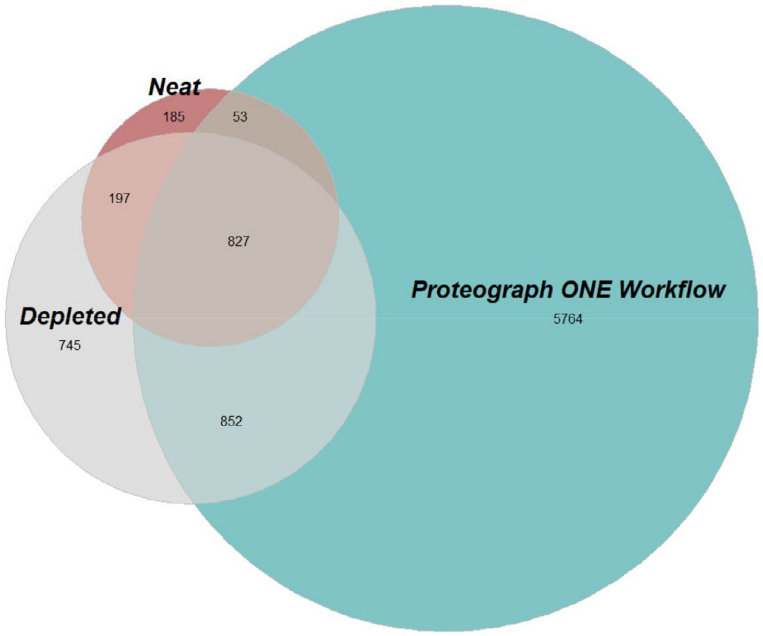

Figure 6. Protein group intersection from neat, depleted, and plasma processed using the Proteograph ONE workflow in combination with the Orbitrap Astral Zoom MS. The Euler plot displays the intersection and unique protein groups identified at least once in the 24 SPD, 1,250 ng throughput/load on column combination. Sample preparation methods are separated by color: neat (dark red), depleted (gray), and the Proteograph ONE workflow (cyan).

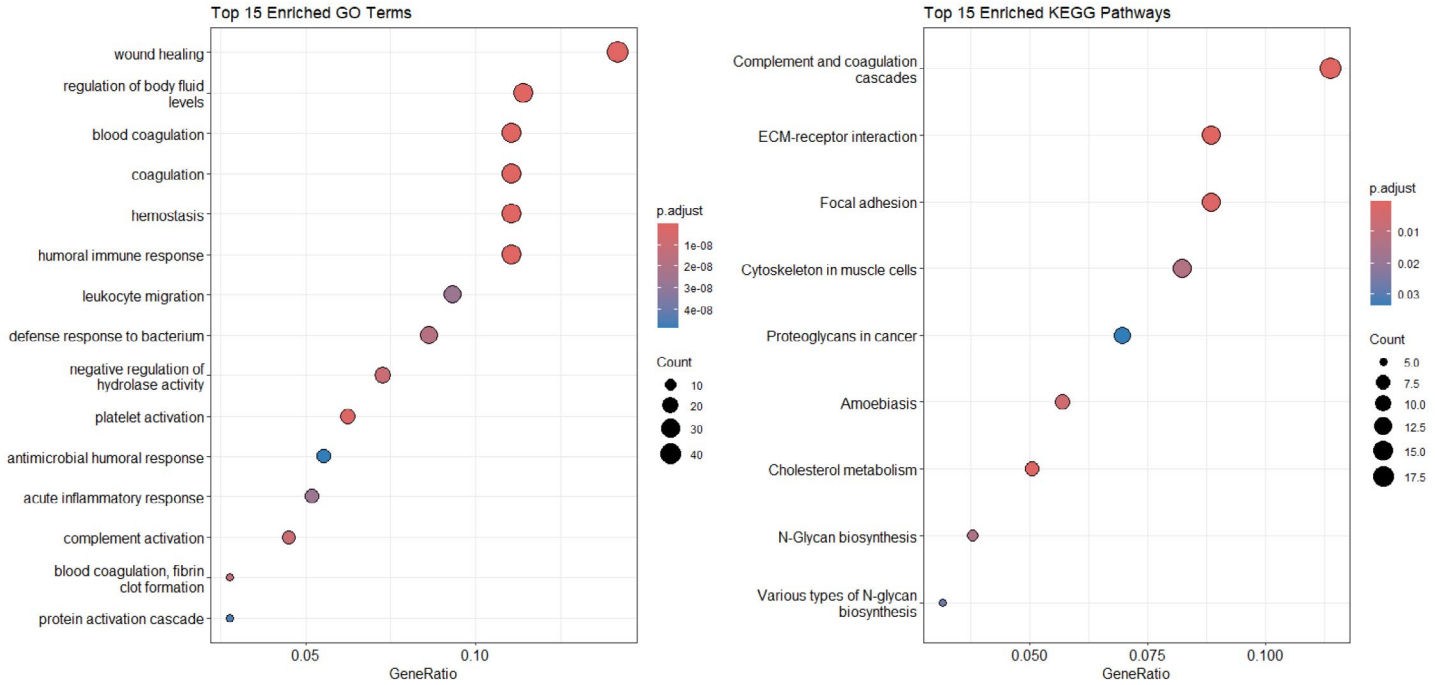

Figure 7. Neat plasma gene ontology and KEGG pathway enrichment. For GO and KEGG pathway enrichment analyses, p-value and q-values were cut off at 0.05 and p-values were adjusted using the Benjamini-Hochberg procedure to control FDR. Fisher's exact test was used to compare the proportion of proteins associated with GO terms or KEGG pathways in identified protein lists from neat plasma against the background list proportions. The top 15 GO biological processes are represented in the dot plot on the left. The top 15 enriched KEGG pathways are represented in the dot plot on the right. Statistical significance is noted by color scale and measured proteins by size.

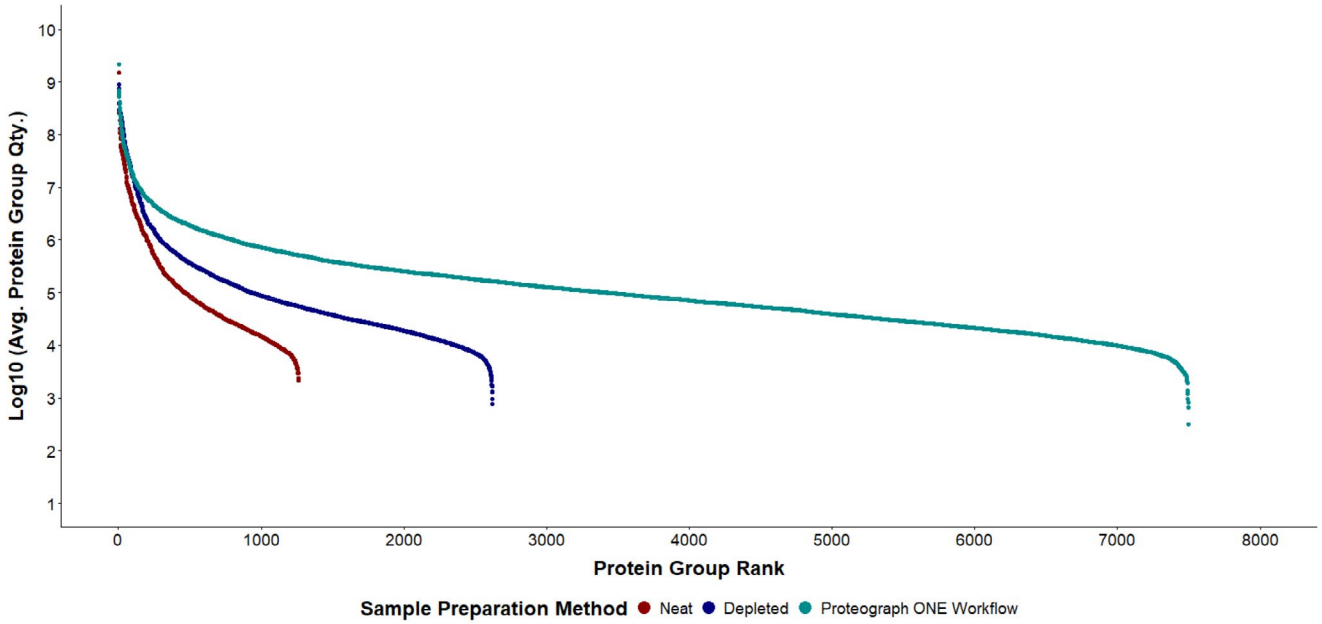

Figure 8. Protein group ranking from neat, depleted, and plasma processed using the Proteograph ONE workflow in combination with the Orbitrap Astral Zoom MS. The rank plot displays protein groups identified at least once in the 24 SPD, 1,250 ng throughput/load on column combination relative to the measured $\log_{10}$ (average protein group quantity) to represent abundance. Sample preparation methods are separated by color: neat (dark red), depleted (navy), and the Proteograph ONE worklfow (cyan).

Out of all protein groups identified at least once in depleted plasma, 745 (28.4%) of those protein groups are unique to depleted plasma that are not identified in other workflows (Figure 6). Enriched GO categories show blood related and immune related categories enriched and KEGG pathway enrichment analyses show enrichment in coagulation, intracellular signaling, adhesion, and other metabolic pathways (Figure 9). Due to the wide dynamic range of protein concentration in plasma, more comprehensive proteome depth is achieved by abundant protein depletion using immunocapture combined with LC-MS analysis on the Orbitrap Astral Zoom MS, allowing for additional mid and low abundant protein groups to be detected (Figure 8).

Seer Proteograph ONE Workflow processed plasma

To achieve maximum plasma proteomic depth of coverage, the Seer Proteograph ONE Workflow was used to compress the dynamic range of plasma proteome. Across various Proteograph ONE processed plasma throughputs and injection amount combinations, the median peptide and protein identifications with respective technical replicate injection median CVs were the following:

- For 100 SPD, a median of 47,045 peptides and 5,389 protein groups were identified, with quantitative precision of 10.2% peptide CV and 5.8% protein group CV (Figures 2–5).
- For 60 SPD, a median of 52,855 peptides and 6,011 protein groups were identified, with quantitative precision of 7.9% peptide CV and 4.4% protein group CV (Figures 2–5).
- For 24 SPD, a median of 75,793 peptides and 7,185 protein groups were identified, with quantitative precision of 7.5% peptide CV and 3.7% protein group CV (Figures 2–5).

Out of all protein groups identified at least once in Proteograph ONE processed plasma, 5764 (76.9%) protein groups are unique to this method and are not detected in other workflows (Figure 6). Enriched GO categories show blood-related, immune-related, and multiple metabolic categories enriched, and KEGG pathway enrichment analyses show enrichment in neurodegenerative diseases, metabolic pathways, immune signaling, and other metabolic pathways (Figure 10). Furthermore, when comparing protein groups identified at least once in Seer Proteograph ONE processed plasma to the latest version of the Human Plasma Peptide Atlas (v 2023-04) containing 4,608 canonical proteins,[5] 54.9% of proteins were identified using the Seer Proteograph ONE workflow, in addition to 4,964 protein groups that have not been routinely detected in plasma (Figure 11).

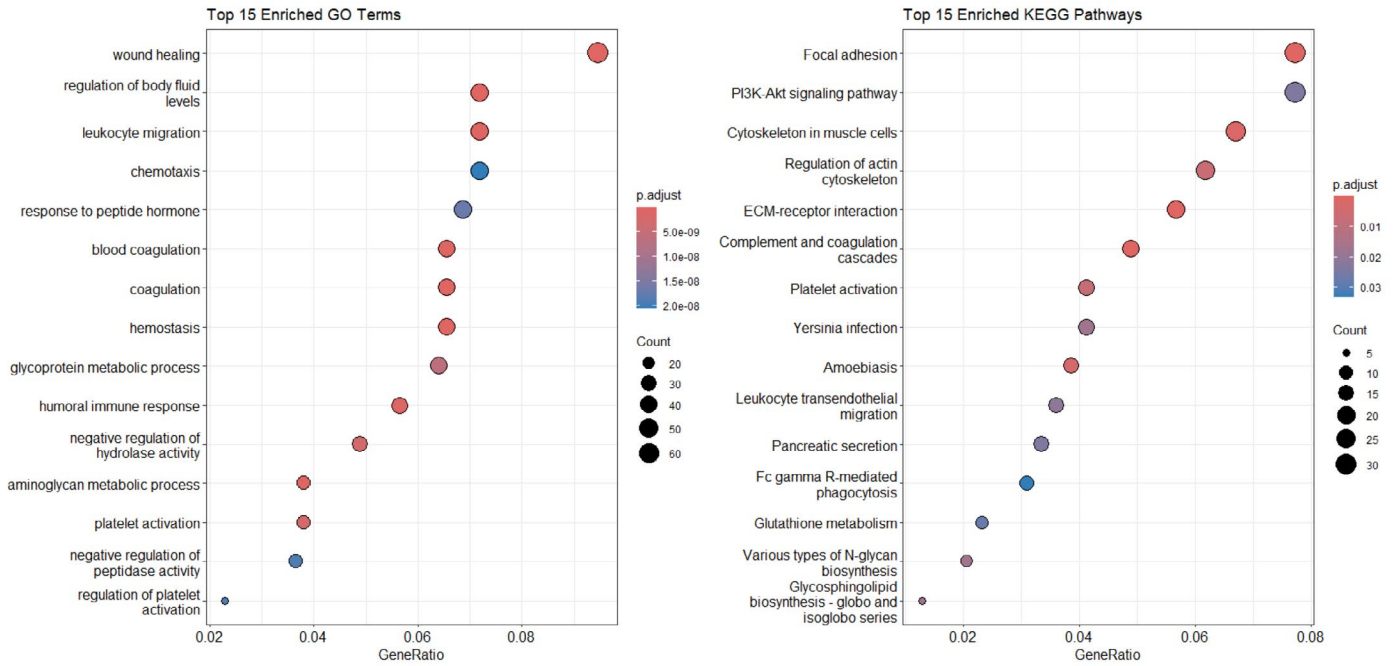

Figure 9. Depleted plasma gene ontology and KEGG pathway enrichment. For GO and KEGG pathway enrichment analyses, p-value and q-values were cutoff at 0.05 and p-values were adjusted using the Benjamini-Hochberg procedure to control FDR. Fisher's exact test was used to compare the proportion of proteins associated with GO terms or KEGG pathways in identified protein lists from depleted plasma against the background list proportions. The top 15 GO biological processes are represented in the dot plot on the left. The top 15 enriched KEGG pathways are represented in the dot plot on the right. Statistical significance is noted by color scale and measured proteins by size.

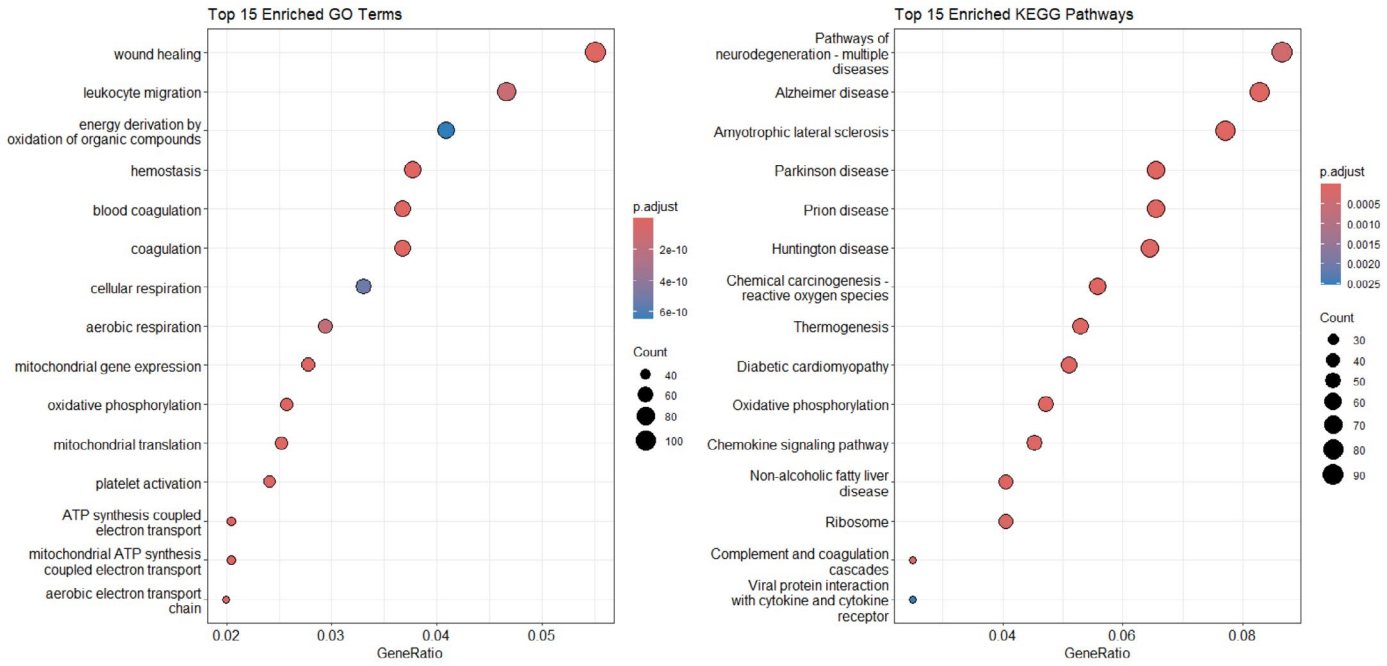

Figure 10. Plasma processed using the Proteograph ONE workflow gene ontology and KEGG pathway enrichment. For GO and KEGG pathway enrichment analyses, p-value and q-values were cutoff at 0.05 and p-values were adjusted using the Benjamini-Hochberg procedure to control FDR. Fisher's exact test was used to compare the proportion of proteins associated with GO terms or KEGG pathways in identified protein lists from Proteograph ONE processed plasma against the background list proportions. The top 15 GO biological processes are represented in the dot plot on the left. The top 15 enriched KEGG pathways are represented in the dot plot on the right. Statistical significance is noted by color scale and measured proteins by size.

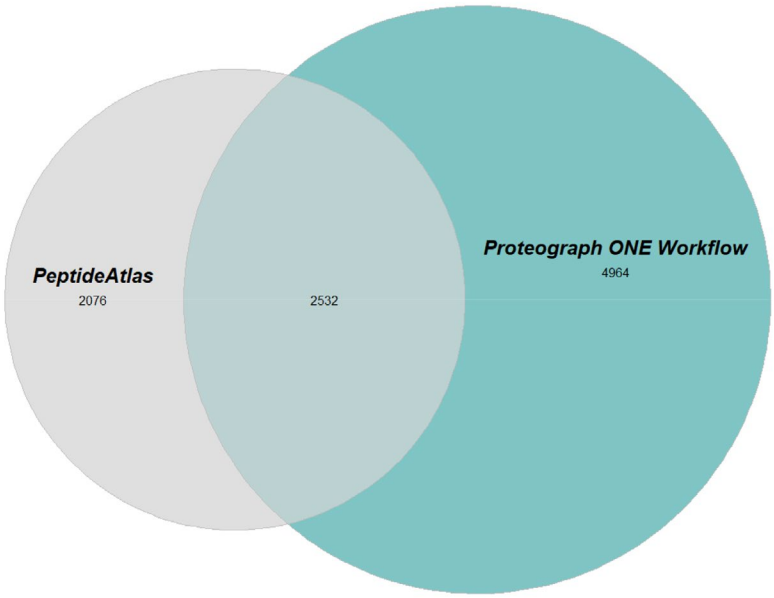

Figure 11. Plasma processed using the Proteograph ONE workflow protein group identifications compared to Human Plasma Peptide Atlas. The Euler plot displays protein groups identified at least once in the 24 SPD, 1,250 ng throughput/load on column combination relative to the canonical protein groups in the Human Plasma Peptide Atlas repository. Protein groups in each category are represented by different colors: protein groups identified using the Proteograph ONE workflow are represented in cyan and protein groups represented in Human Plasma Peptide Atlas in gray.

The extended plasma proteome depth is due to identifying and quantifying more proteins from the mid to low abundance range, which have biological interest as mid to low abundant tissue leakage and signaling proteins are now measurable from unique physicochemical properties of protein/nanoparticle interactions fine-tuned for dynamic range compression and capture of lower abundant proteins (Figure 8). With increased depth of the plasma proteome using the Proteograph ONE workflow, clinically relevant FDA biomarkers are also detected, highlighting the capabilities of capturing established relevant biomarkers in addition to enhanced depth (Figure 12). In combination with the speed and sensitivity of the Orbitrap Astral Zoom MS, extended plasma proteome depth is achieved with superior analytical precision.

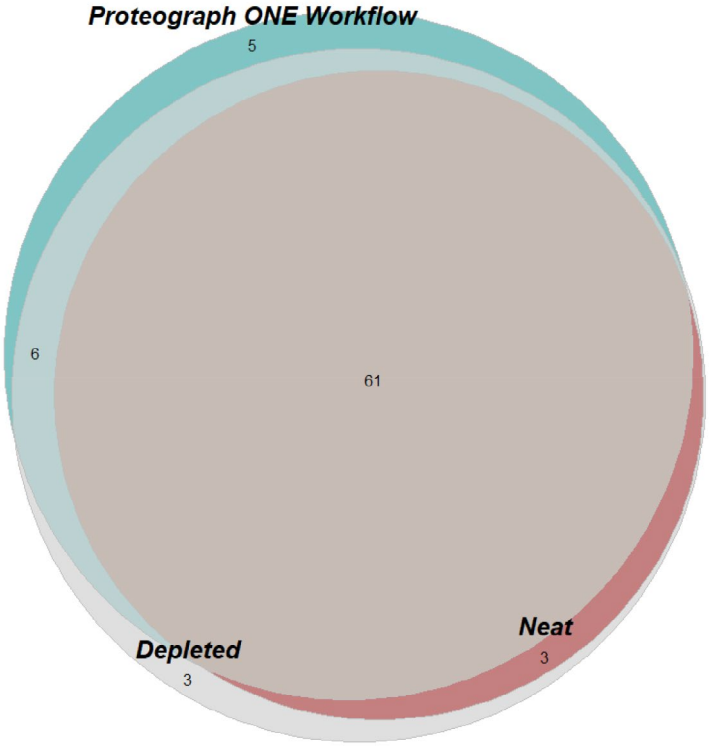

Figure 12. FDA approved protein biomarker overlap from neat, depleted, and plasma processed using the Proteograph ONE workflow in combination with the Orbitrap Astral Zoom MS. The Euler plot displays protein groups identified at least once in the 24 SPD, 1,250 ng throughput/mass on column compared to the 109 plasma and/or serum clinical assays approved by the FDA[6]. Sample preparation methods are separated by color: neat (dark red), depleted (gray), and the Proteograph ONE worklfow (cyan).

Conclusion

Neat plasma

- Across various depleted plasma throughputs and injection amounts, the median peptide and protein identifications, along with their respective median CVs, were as follows:
  - For 100 SPD: 5,492 and 682 median peptide and protein groups were identified, with respective 7.6% and 5.6% median peptide and protein group CV.
  - For 60 SPD: 6,343 and 868 median peptide and protein groups were identified, with respective 7.4% and 5.4% median peptide and protein group CV.
  - For 24 SPD: 8,999 and 1,165 median peptide and protein groups were identified, with respective 7.1% and 4.8% median peptide and protein group CV.
- Using the Orbitrap Astral Zoom MS, neat plasma proteome coverage exceeds 1,000 protein groups in a single pooled sample, providing exceptional quantitative precision without additional sample handling steps.

Depleted plasma

- Across various depleted plasma throughputs and injection amounts, the median peptide and protein identifications, along with their respective median CVs, were as follows:
  - For 100 SPD: 10,887 and 1,410 median peptide and protein groups were identified, with respective 7.8% and 5.9% median peptide and protein group CV.
  - For 60 SPD: 12,124 and 1,667 median peptide and protein groups were identified, with respective 6.7% and 4.9% median peptide and protein group CV.
  - For 24 SPD: 18,273 and 2,403 median peptide and protein groups were identified, with respective 7.1% and 4.8% median peptide and protein group CV.
- Using High Select Depletion Spin columns to remove abundant proteins increased coverage by 1.92x (60 SPD) and 2.08x (24 SPD) compared to neat plasma.

Seer Proteograph ONE processed plasma

- Across various Proteograph ONE processed plasma throughputs and injection amounts, the median peptide and protein identifications, along with their respective median CVs, were as follows:
  - For 100 SPD: 47,045 and 5,389 median peptide and protein groups were identified, with respective 10.2% and 5.8% median peptide and protein group CV.
  - For 60 SPD: 52,855 and 6,011 median peptide and protein groups were identified, with respective 7.9% and 4.4% median peptide and protein group CV.
  - For 24 SPD: 75,793 and 7,185 median peptide and protein groups were identified, with respective 7.5% and 3.7% median peptide and protein group CV.
- The Proteograph ONE workflow further increased coverage by 6.63x (60 SPD) and 5.94x (24 SPD) compared to neat plasma, offering flexibility for different cohort sizes and biomarker needs.
- Using this workflow with the Orbitrap Astral Zoom MS, 41,929–75,793 median peptides and 4,826–7,185 median protein groups were identified with high precision (CV of 3.7–6.8% for protein groups).
- The combination of the Proteograph ONE workflow and the Orbitrap Astral Zoom MS extends the measurable dynamic range of the plasma proteome. This method detects 5,764 unique protein groups not seen with other workflows and captures more mid to low abundant proteins that may be of biological interest.

Due to the speed, sensitivity, and robustness of the Orbitrap Astral Zoom MS, large-scale cohorts for biomarker discovery in translational research are now more feasible than ever. With throughputs of ≥60 SPD, balancing depth and throughput, over 18,000 samples can be measured per year on a single mass spectrometer, breaking barriers in population-scale studies. From neat sample preparation methods covering ≥64 FDA biomarkers[6] to profiling ≥7,000 protein groups per plasma sample with the Proteograph ONE workflow, the high-throughput sample preparation methods in combination with the Orbitrap Astral Zoom MS unlock vast opportunities for groundbreaking discoveries.

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