Translational Research

Comprehensive and high-throughput plasma proteome profiling for biomarker discovery using the Orbitrap Astral Zoom Mass Spectrometer and the Seer Proteograph ONE workflow

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Introduction

Plasma proteomics, the study of proteins in blood plasma, is crucial for biomarker discovery and understanding global signaling and immune responses[1], aiding disease diagnosis, monitoring, and personalized medicine. However, the plasma proteome's complexity and dynamic range—spanning up to 12 orders of magnitude[2]—pose challenges, especially for detecting low-abundance proteins. Additionally, preanalytical, analytical, and biological variables complicate reliable biomarker identification, necessitating optimized techniques[3].
Liquid chromatography-mass spectrometry (LC-MS) excels in plasma proteomics due to its high analytical dynamic range and capability to measure peptides and characterize post-translational modifications (PTMs). Advances in sample preparation, separation, MS, and data analysis are improving method sensitivity and robustness. We demonstrate the Thermo Scientific™ Orbitrap™ Astral™ Zoom Mass Spectrometer's high sensitivity and reproducibility in detecting a comprehensive plasma proteome across neat, depleted, and plasma processed with the Seer® Proteograph® ONE workflow, facilitating potential biomarker discovery[4].

Experimental Procedure

Sample preparation

Neat Plasma Peptides: 1.5 µL of human plasma was mixed with 48.5 µL of LYSE solution from the Thermo Scientific™ AccelerOme™ Label-Free MS Sample Prep kit. The mixture was processed on the AccelerOme automated platform for protein preparation, Trypsin/Lys-C digestion, and peptide clean-up. Peptides were dried using a Savant™ SpeedVac concentrator and reconstituted in water with 0.1% formic acid and 2% acetonitrile to 100 ng/µL, then pooled. Peptide concentrations were quantified with the Thermo Scientific™ Pierce™ Quantitative Fluorometric Peptide Assay Kit.
Immunodepleted Plasma Peptides: 10 µL of human plasma was depleted using High Select Depletion Spin Columns as per the protocol. Depleted samples were concentrated, reconstituted in 50 µL of LYSE solution, and processed on the AccelerOme automated platform for protein preparation, Trypsin/Lys-C digestion, and peptide clean-up. Peptides were dried, reconstituted in water with 0.1% formic acid and 2% acetonitrile to 100 ng/µL, quantified, normalized, and pooled.
Seer® Proteograph ONE Workflow Peptides: 120 µL of human plasma was loaded onto the Seer SP200 instrument, with 100 µL mixed with nanoparticles from the Proteograph ONE Assay Kit. Samples were incubated at 37° C for 1 hour for protein corona formation. Non-specific proteins were washed off, and bound proteins were reduced, alkylated, and digested with Trypsin/Lys-C. Peptides were cleaned, desalted, quantified, dried, and reconstituted in water with 0.1% formic acid and 3% acetonitrile to 50 ng/µL, then pooled.

Liquid chromatography-mass spectrometry & data analysis

All LC-MS runs for neat, depleted, and Proteograph ONE processed peptides were separated and analyzed using a Thermo Scientific™ Vanquish™ Neo UHPLC System in trap and elute configuration, paired with an Orbitrap Astral Zoom Mass Spectrometer. Peptides were separated on the Vanquish Neo UHPLC System using Thermo Scientific™ EASY-Spray™ PepMap™ analytical columns (ES906, 2µm C18, 150µm × 15 cm for 100 and 60 SPD and ES75500PN 2µm C18, 75µm × 50 cm for 24SPD) and 12.6-, 22.35-, and 54-minute chromatographic gradients, respectively, 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. Samples were injected with 2 loading mass amounts (200/500 ng for 100/60 SPD; 500/1250 ng for 24 SPD) in triplicate. Liquid chromatography parameters are shown below in Table 1A. Mass spectrometer scan parameters can be found below in Table 1B. All data was processed using the Proteograph® Analysis Suite (PAS) (Seer). Output files were imported to Rstudio (2023.09.0 Build 463) with R (v4.3.1) for downstream data analysis and visualization.

(A)

Vanquish Neo UHPLC Parametersa	
LC Configuration	Trap and Elute
Fast Loading/Equilibration Mode	Pressure Control
Loading/Equilibration/Wash Pressure	Max Pressure
Equilibration Factor	3
Sampler Temperature	7° C
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

(B)

MS1 & DIA Scan Experimental Parameters	
Orbitrap Resolution	240K
Precursor Scan Range (*m/z*)	380-980
RF Lens (%)	40
Normalized AGC Target (%) / Absolute AGC Value (MS1/ MS2)	500% / 5.00e6 500% / 5.00e4
Maximum Injection Time (MS1/MS2)	3 ms (100SPD & 60SPD); 5 ms (24SPD) / 7 ms
DIA Isolation Window Width (*m/z*)	3 (60,100 SPD) / 2.5 (24 SPD)
DIA AGC Target	Custom
DIA Scan Range (*m/z*)	150-2000
HCD Collision Energy (%)	25

Table 1. Liquid chromatography-mass spectrometry settings. (A). Vanquish Neo UHPLC parameters **(B).** Orbitrap Astral Zoom MS1 and MS2 scan parameters.

Experimental Workflow & Design

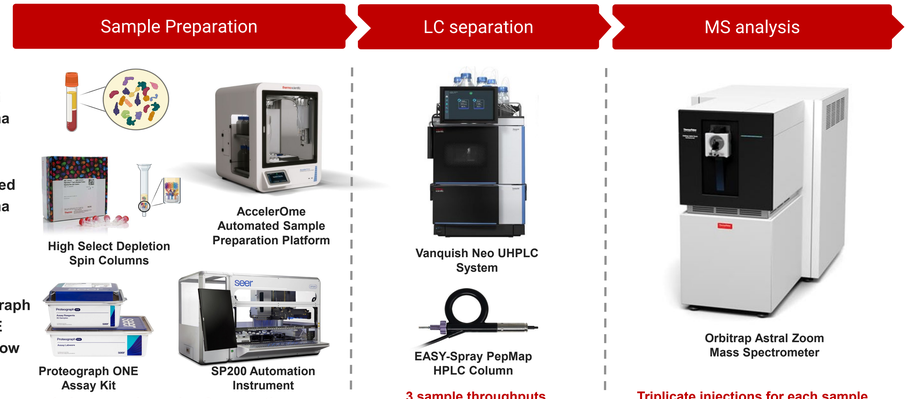

Figure 1. High-throughput plasma proteomics workflow using the Orbitrap Astral Zoom Mass Spectrometer. This end-to-end solution, from sample handling to data acquisition, offers exceptional flexibility for translational research, enabling rapid analysis for large cohorts and deeper insights for advanced proteomics. With this workflow, a multi-tiered experiment was designed to assess proteomic depth of coverage, instrument analytical precision, and biological representation across the 3 sample preparation workflows.

Assay Characterization

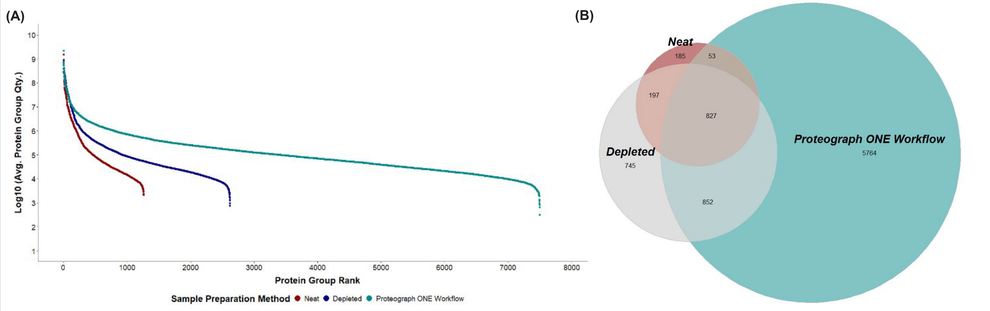

Figure 2. Protein group ranking (A) and intersection (B) from neat, depleted, and plasma processed using the Proteograph ONE workflow in combination with the Orbitrap Astral Zoom mass spectrometer. The rank plot displays protein groups identified at least once in the 24 SPD, 1250ng throughput/ mass on column combination relative to the measured $\log_{10}$(average protein group quantity) to represent abundance **(A)**. The Euler plot displays the intersection and unique protein groups identified at least once in the 24 SPD, 1250 ng throughput/ mass on column combination **(B)**. Sample preparation methods are separated by color: neat (dark red), depleted (grey), and the Proteograph ONE workflow (cyan).

Identification & Analytical Measurement Precision

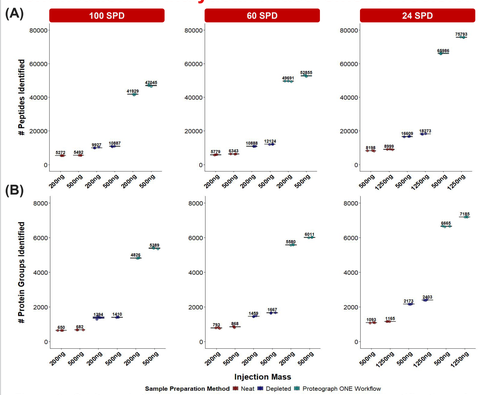

Figure 3. Orbitrap Astral Zoom MS peptide & protein group identifications from neat, depleted, and plasma processed using the Proteograph ONE Workflow. Box plots display the peptide **(A)** and protein group **(B)** identifications for each sample preparation strategy. Each combination of sample preparation strategy, throughput, and mass on column was analyzed in triplicate, with individual run identification numbers represented by circles. The median number of peptides **(A)** or protein groups **(B)** is shown above each box plot.

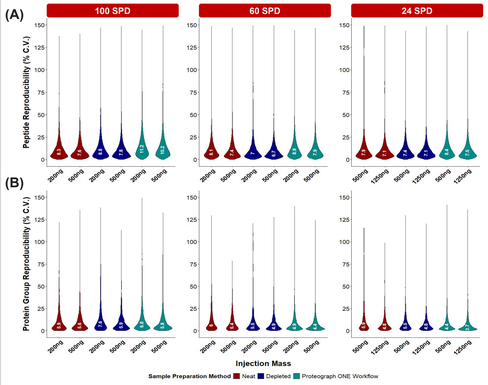

Figure 4. Orbitrap Astral Zoom MS peptide measurement quantitative precision from neat, depleted, and plasma processed using the Proteograph ONE Workflow. Violin plots display the peptide coefficient of variation (% CV) for each sample preparation strategy calculated from triplicate LC-MS injections of each sample preparation strategy, throughput, and mass on column combination. The median peptide **(A)** or protein group **(B)** % CV per combination is shown vertically in each violin plot.

Plasma Proteome Relevance

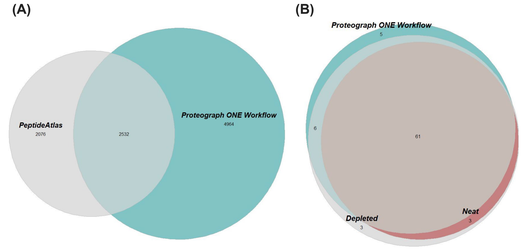

Figure 5. Plasma processed using the Proteograph ONE Workflow protein group identifications compared to Human Plasma PeptideAtlas (A) and FDA approved protein biomarker overlap (B). The Euler plot displays protein groups identified at least once in the 24 SPD, 1250ng throughput/ mass on column combination relative to the canonical protein groups in the Human Plasma PeptideAtlas repository (Build 2023-04)[4] **(A)**. The Euler plot displays protein groups identified at least once in the 24 SPD, 1250ng throughput/ mass on column compared to the 109 plasma and/or serum clinical assays approved by the FDA[5] **(B)**.

Conclusions

- With the Orbitrap Astral Zoom Mass Spectrometer, neat plasma proteome coverage exceeds 1,000 protein groups in a single pooled sample, providing exceptional quantitative precision without additional sample handling steps.
- High Select Top 14 abundant protein depletion enhances plasma proteome depth by ~2x across 100, 60, and 24 SPD throughputs with comparable or superior analytical measurement precision.
- Seer Proteograph ONE enhances plasma proteome depth by 7.9x (100SPD), 6.9x (60SPD), and 6.2x (24SPD) with comparable or superior analytical measurement precision and no compromise on sample preparation throughput.
- The combination of the Orbitrap Astral Zoom Mass Spectrometer and the Proteograph ONE Workflow allows for over 7,000 protein groups to be identified from a single injection.
- The speed, sensitivity, and robustness of the Orbitrap Astral Zoom Mass Spectrometer enables in-depth biological discovery, paving the way for novel findings and advancements in clinical cohort and population-scale translational research.

References

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