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Unveiling hidden protein depths: a high-throughput plasma proteomics workflow for enhanced biomarker discovery on Orbitrap Astral MS

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Abstract

Purpose: The aim is to create effective plasma proteomics workflows using diverse sample preparation techniques and multiple throughput methods. This will enable comprehensive plasma proteomics analysis and accurate quantitation using a label-free data-independent acquisition (DIA) strategy on the advanced Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer.

Methods: Plasma samples were enriched by Seer Proteograph XT kit or depleted by Thermo Scientific™ High Select™ Depletion Spin Columns. All samples were digested by trypsin/Lys-C and the peptides were loaded onto a 15-cm or 50-cm Thermo Scientific™ EASY-Spray™ PepMap™ column for a throughput of 60 and 100 sample per day (SPD) or 24 SPD, respectively. To further improve proteome coverage, a 16 SPD method was separated with the 60-cm IonOpticks TS UHPLC column. The peptides were resolved by using a Thermo Scientific™ Vanquish™ Neo UHPLC system. The eluted peptides were analyzed on an Orbitrap Astral MS operated in DIA mode (Figure 1).

Results: Our innovative sample processing, in combination with the advanced Orbitrap Astral mass spectrometer, results in the most extensive plasma proteome coverage to date. We were able to identify over 4000, 5000, 6000 and 8000 protein groups with the 100, 60, 24 and 16 SPD workflow, respectively. Furthermore, the coefficient of variance (CV) were approximately 4-6% for each method, highlighting the unmatched precision of the workflow. Collectively, we have developed a range of sample preparation methods, each paired with varieties of LC-MS method with a different and flexible sample analysis throughput. This advancement will significantly contribute to translational and biomarker discovery research providing the depth of proteome coverage necessary for early biomarker detection through comprehensive plasma proteome analysis.

Introduction

The analysis of blood plasma for biomarker discovery research presents a promising avenue for early disease detection, but the complexity of the associated workflows has hindered progress in this area. One of the significant advantages of plasma proteomics is the ease of sample collection through routine blood draws.

Mass spectrometery-based proteomic analysis, in conjunction with advanced separation technology such as Liquid Chromatography (LC), LC-MS analysis is a leading method for studying low-abundant proteins due to its sensitivity, unambiguous peptide identification, and accurate and precise quantitation. In addition, the new cutting-edge Orbitrap Astral MS offers unprecedented sensitivity, which allows for the most comprehensive plasma proteome coverage to facilitate the discovery of potential biomarkers across wide dynamic range of plasma proteome, crucial for reproducible investigation of low-abundant plasma proteins from precious samples.

Materials and methods

Sample preparation

The neat plasma sample used in this experiment was from a pooled sample collected from multiple healthy donors. The sample was prepared using the Thermo Scientific™ EasyPep ™Mini MS Sample Prep Kit (PN A40006). For plasma protein enrichment, 240 µL of plasma sample was mixed with each of the two nanoparticles suspension (NPs) with the Proteograph XT Assay Kit (Seer Inc.) according to manufacturer's instruction. The top 14 abundant proteins in human plasma samples were depleted using depletion mini spin columns from the High Select™ Depletion Spin Columns (PN A36370). Depleted protein samples were dried in a Speedvac, followed by processing with EasyPep Mini MS Sample Prep Kit

Data analysis

The LC-MS data has been processed by Spectronaut™ software (Biognoys, v18) using a directDIA approach, DIA-NN (v1.8.1) or Thermo Scientific™ Proteome Discoverer™ software (v3.1.0.618) with CHIMERYS™ intelligent search algorithm by MSAID. The ensuing tables were imported to Python for downstream data analysis and visualization.

Results

Neat plasma

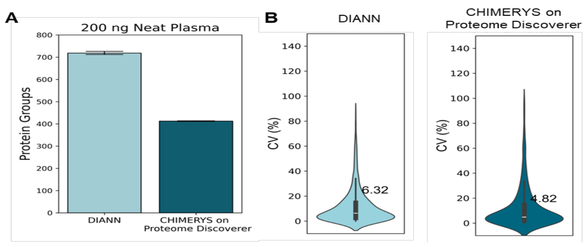
The 60 SPD workflow offers a good balance of throughput and proteome coverage, given that no protein depletion or enrichment is required. With the sensitivity and higher throughput of the Orbitrap Astral MS, the coverage of neat plasma for short LC gradients surpasses that of the current data to date. With the 60 samples per day method, we identified approximately 720 protein groups along with 7700 peptide groups and a CV of approximately 6% which suggests excellent precision and is crucial for accurate quantitation (Figure 2).

Top14 protein depletion

With a daily throughput of 100 samples, we were able to identify approximately 1100 protein groups using a 500 ng sample load. The observed CV was around 10%, indicating extensive plasma proteome coverage and effective quantitation from the depletion column. We further improved the plasma proteome coverage to 1380 protein groups using the 60 samples per day method (Figure 3).

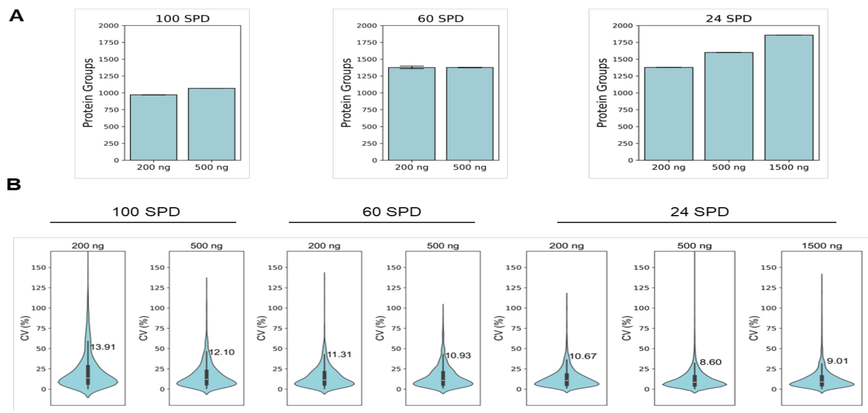
In an effort to improve plasma proteome coverage, we analyzed the depleted plasma samples using a method that allowed us to process 24 samples per day. This strategy led to the identification of 1600 and 1850 protein groups from the 500 ng and 1500 ng sample loads, respectively (Figure 3).

Figure 2. Orbitrap Astral MS improves proteome coverage from neat plasma digest.



(A) The number of protein groups identified from 200 ng of neat plasma digest. Samples were analyzed at a throughput of 60 samples per day, and the ensuing raw files were processed by DIA-NN or CHIMERYS on Proteome Discoverer. **(B)** Coefficient variance percentage of protein groups from three individual runs.

Figure 3. Top 14 protein depletion improves plasma proteome coverage.



(A) The number of protein groups (upper panel) and peptide groups (lower panel) from the top 14 mini column-depleted plasma samples at a throughput of 100, 60, or 24 samples per day on Orbitrap Astral mass spectrometer. **(B)** Violin plots showing the percentage of the coefficient of variance from three replicates of sample preparation.

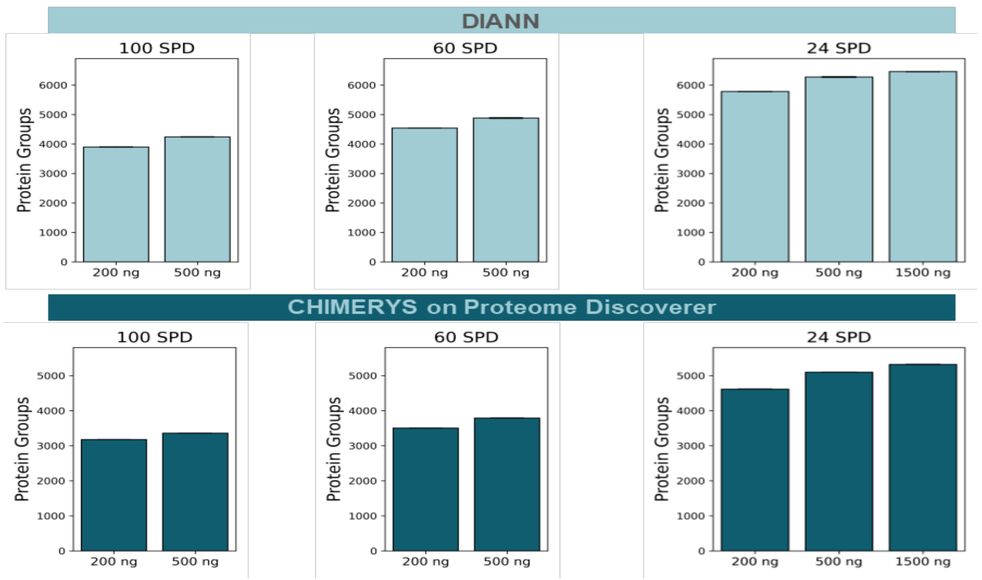
Proteograph XT processed plasma

The Proteograph XT Assay (Seer Inc.) generated two NP fractions were pooled into one sample in equal quantity and analyzed on Orbitrap Astral MS. At a throughput of 100 samples per day, we identified approximately 3900 or 4300 protein groups from 200 ng and 500 ng enriched plasma samples, respectively (Figure 4). Moreover, the quantitation precision was evidenced by a CV % of 6.

The throughput of 60 samples per day resulted in the quantification of approximately 4900 protein groups. It is noteworthy that the CV % for these quantifications was 6%, indicating a relatively low level of variability.

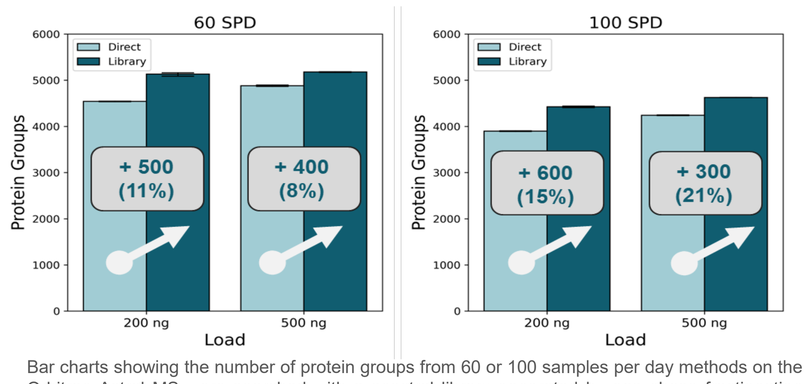
Incorporating a peptide spectral library into the search frequently enhances proteome coverage and data completeness, particularly when it comes to short gradient methods. To comprehend the advantages of spectral library-based search in our high-throughput workflow, we created a spectral library using a MS-based gas phase fractionation paired with the 60 samples per day method. This combination resulted in the incorporation of spectra from 7300 protein groups into our library, which further improved the identification by an additional 300 to 600 protein groups (Figure 5).

Figure 4. Deep plasma proteomics workflow with the Orbitrap Astral mass spectrometer and Proteograph XT Assay



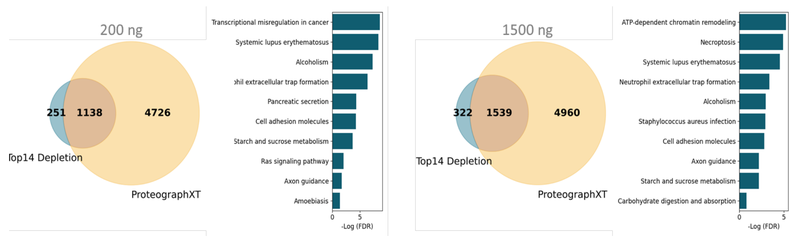
The number of protein groups **(A)** and peptide groups **(B)** identified from different loads of Proteograph XT Assay enriched plasma. Samples were analyzed on the Orbitrap Astral MS with high-throughput methods, including 100 and 60 samples per day (SPD) or a 24 SPD method to maximize identification.

Figure 5. Utilizing spectral library to improve proteome coverage for high-throughput workflows.



Bar charts showing the number of protein groups from 60 or 100 samples per day methods on the Orbitrap Astral MS were searched with a spectral library generated by gas phase fractionation (Library) or through a library-free data analysis approach. .

Figure 6. Differential plasma protein identification with Seer Proteograph XT and the top 14 depletion workflow.



Venn diagrams showing the overlap between Proteograph XT and the top 14 depletion workflow from the 24 samples per day method. Bar charts showing the KEGG pathway analysis from protein groups exclusively identified from the top 14 depletion method.

Enhance plasma proteome coverage with various sample preparation approaches

It's noteworthy that about 20-25% of proteins identified through the top 14 depletion workflow were not detected by the Proteograph XT enrichment method, indicating this to be a cost-effective supplementary method to further enhance plasma proteome coverage if those proteins are biologically relevant in the study (Figure 6).

Conclusions

- Neat plasma workflows, while easy to handle due to minimal sample preparation steps, suffer from plasma wide dynamic ranges of protein concentrations, which result in shallow coverage of the plasma proteome compared to enriched or depleted plasma workflows.
- The use of high select depletion spin columns for plasma top 14 high abundant protein depletion has proven to significantly increase plasma proteome coverage. Compared to neat plasma, it enhances proteome coverage by 2- to 3-fold. This method provides an economical workflow and is a viable alternative to other depletion strategies used in plasma proteomics research.
- The Proteograph XT workflow coupled with the Orbitrap Astral mass spectrometer offers the most comprehensive depth of plasma proteome analysis, effectively balancing the need for high throughput automated plasma proteomics workflows. This makes it a more technically efficient method for large cohort plasma studies.

Trademarks/licensing

Learn more about plasma proteomics workflow on Orbitrap Astral MS