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An in-depth and high throughput plasma proteomics workflow powered by Orbitrap Exploris 480 mass spectrometer using multi nanoparticle-based workflow

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Introduction

Plasma proteomics using mass spectrometry (MS) remains a promising method to discover disease biomarkers. However, for large-scale plasma proteomics studies, a robust liquid chromatography-mass spectrometry (LC-MS) setup that does not compromise on protein identification, sequence coverage, dynamic range and analysis precision is required. Here we present a high-throughput and a maximum identification (Max-ID) workflow on an Thermo Scientific™ Orbitrap™ Exploris 480 mass spectrometer for in-depth analysis of plasma (Figure 1). The plasma samples were processed with Seer's Proteograph™ XT Assay utilizing multi-nanoparticles (NPs) for an unbiased and deep proteomics enrichment and analysis at scale (Figure 2).

Materials & methods

Proteograph XT assay

Samples are processed using 100 µL aliquots of each plasma sample, mixed with each of two NP wells included in the Proteograph XT Assay Kit. A one-hour incubation allows high-affinity proteins to displace high-abundance proteins, resulting in a reproducible protein corona on each NP surface that probes the depth of the plasma proteome.

A series of gentle washes remove non-specific and weakly bound proteins. The paramagnetic property of the NPs allows for accumulation of NPs with protein corona after each wash step. This results in a highly specific and reproducible protein corona that contains the high-affinity protein binding partners selected by the NPs. Protein coronas are reduced, alkylated, and digested with Trypsin/Lys-C. All steps are performed in a one-pot reaction directly on the NPs. The in-solution digestion mixture is then desalted using a mixed-media filter plate and positive pressure (MPE) system.

LC method

High-throughput method:

- Thermo Scientific™ EASY-Spray™ PepMap™ Column, 2 µm C18, 150 µm x 15 cm
- Thermo Scientific™ PepMap™ Neo Trap Cartridge, 5 µm 300 µm x 5 mm
- Flow rate: 1.5 µL/min on a Trap & Elute workflow

Max-ID method:

- Thermo Scientific™ EASY-Spray PepMap™ Neo Column, 2 µm C18, 75 µm x 75 cm
- Flow rate: 0.25 µL/min on a Direct Injection workflow

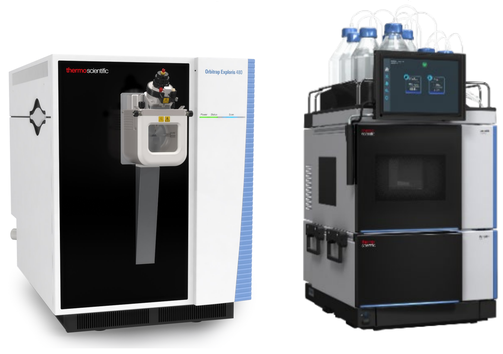
MS method

The Proteograph XT Assay Kit generates two sets of enriched peptides at the end of analysis. For the high throughput method, 2 individual injections of 24 minutes each were used for a total instrument time of 48 minutes per sample. For the Max ID method, the two peptide wells were pooled, and LC-MS analysis was done in single injection using a 102 minutes total gradient (Table 1 and 2)

Data analysis

Proteograph™ Analysis Suite (PAS) with match between run (MBR) was used for data analysis of the resulting LC-MS files unless stated otherwise. Data was exported and processed by Pandas and Seaborn packages in Python for data visualization. Spectral library free search was done on Spectronaut 18 software, DIA-NN (v1.8.1) or Thermo Scientific™ Proteome Discoverer™ 3.1 software using CHIMERYS™ intelligent search algorithm by MSAID. In all software packages, 1% FDR cut off was applied at both protein and peptide levels.

Figure 1. Orbitrap Exploris 480 mass spectrometer and Thermo Scientific™ Vanquish™ Neo UHPLC system.



Results

The optimized DIA method enabled the identification of > 3,000 protein groups, > 20,000 peptides from the high-throughput method (Figure 3A and B) and > 3,800 protein groups, > 27,000 peptides in the Max-ID method (Figure 3C and D) using the Proteograph Analysis Suite with match between run (MBR).

Table 1. LC gradients for high-throughput and max-ID methods.

High-throughput

No	Time	Duration [min]	Flow [µl/min]	%B	Volume [µl]	No. of Column Volumes
1	0.000	Run				
2	0.000	0.000	2.000	4.0	0.00	0.00
3	0.200	0.200	2.000	8.0	0.40	0.23
4	14.600	14.400	1.500	20.0	25.20	14.19
5	21.500	6.900	1.500	35.0	10.35	5.83
6	21.500	Column Wash				
7	21.900	0.400	2.000	99.0	0.70	0.39
8	22.600	0.700	2.000	99.0	1.40	0.79
9	22.600	Stop Run				
10	22.600	Column Equilibration				

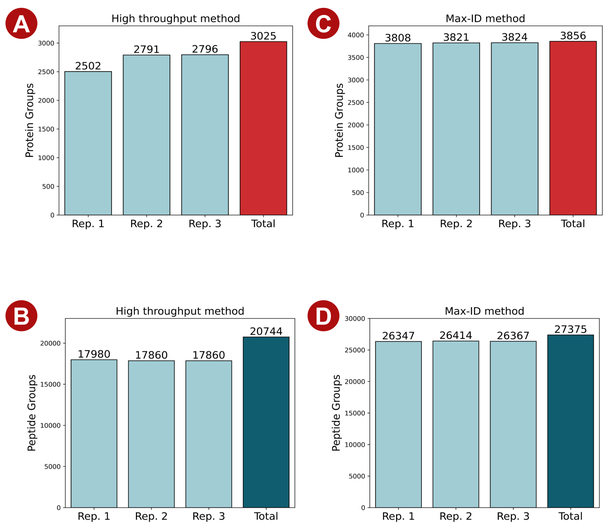
Max-ID

No	Time	Duration [min]	Flow [µl/min]	%B	Volume [µl]	No. of Column Volumes
1	0.000	Run				
2	0.000	0.000	0.250	1.0	0.00	0.00
3	0.100	0.100	0.250	6.0	0.03	0.01
4	60.100	60.000	0.250	20.0	15.00	6.76
5	90.100	30.000	0.250	35.0	7.50	3.38
6	90.100	Column Wash				
7	91.100	1.000	0.250	99.0	0.25	0.11
8	102.000	10.900	0.250	99.0	2.73	1.23
9	102.000	Stop Run				
10	102.000	Column Equilibration				

Table 2. MS parameters for high-throughput and max-ID methods.

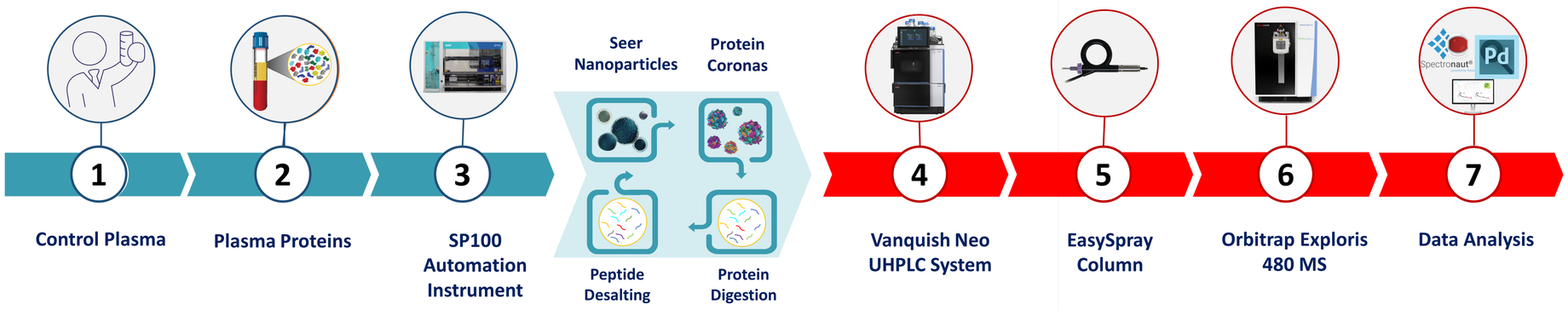
	MS Parameter	Max ID	High Throughput
MS1	Resolution	60K	60K
	AGC	300%	300%
	Max-IT	Auto	Auto
MS2	Resolution	15K	15K
	AGC	800%	1000%
	Max-IT	Auto	28ms
	Isolation Window	4Da	4Da

Figure 3. Orbitrap Exploris 480 MS enables high-throughput and in-depth plasma proteome coverage.



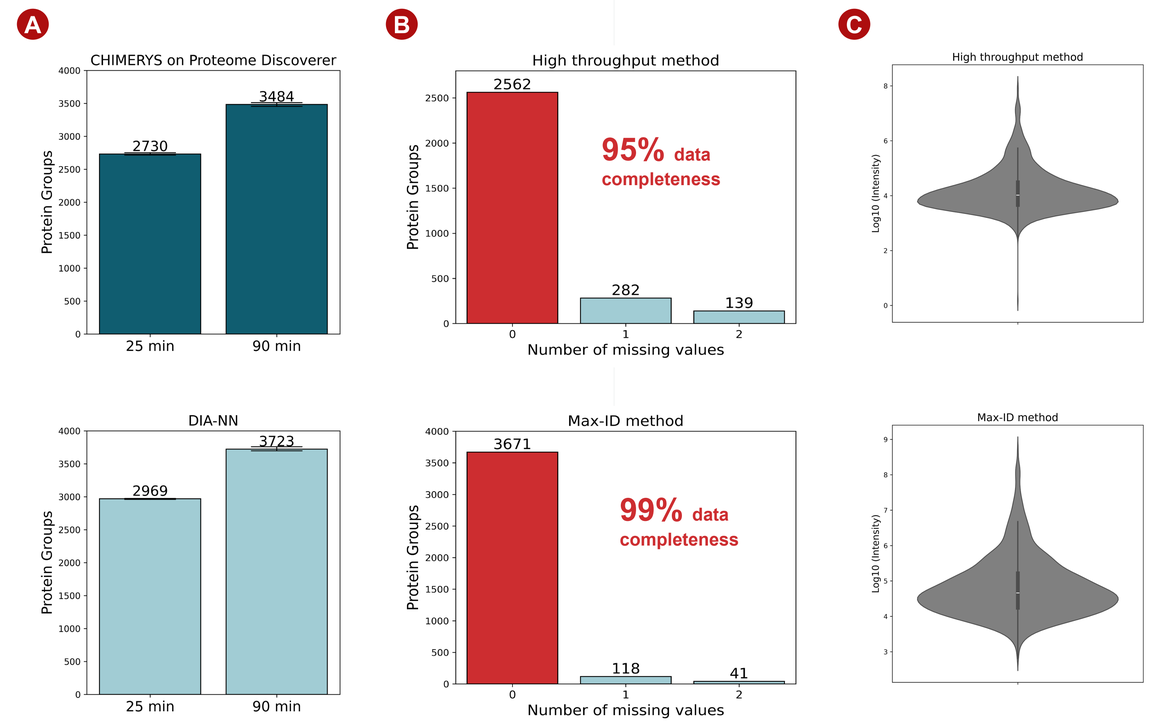
Number of protein groups in the (A) high-throughput (>3,000) and (C) Max-ID (>3,800) method. Number of peptide groups in the (B) high-throughput (>20,000) and (D) Max-ID (>27,000) method

Figure 2. High-throughput plasma proteomics workflow on Orbitrap Exploris 480 MS.



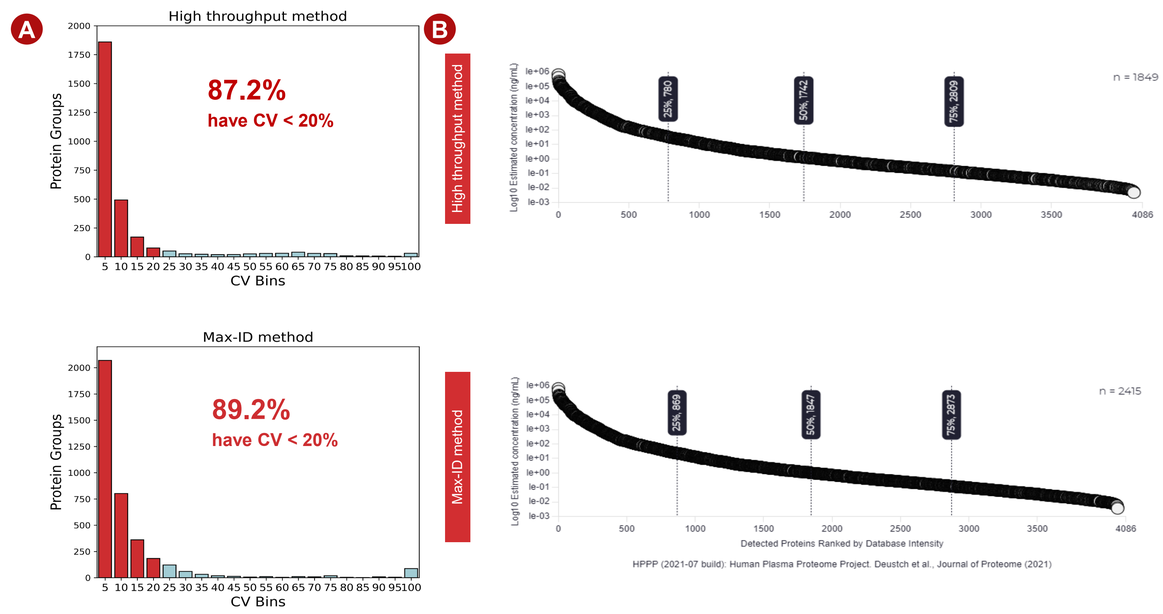
Results

Figure 4. Orbitrap Exploris 480 MS enables highly reproducible plasma proteomics analysis.



(A) Number of protein groups obtained from library-free searches on CHIMERYS with Proteome Discoverer software and DIA-NN; (B) Data completeness of high-throughput (95%) and Max-ID (99%) method; (C) Wide dynamic range afforded by the Orbitrap Exploris 480 mass spectrometer in both the high-throughput and Max-ID method to facilitate reproducible and accurate quantitation.

Figure 5. Excellent quantitative performance achieved by Orbitrap Exploris 480 MS



(A) Protein group coefficient of variance (CV) distribution of the high-throughput and Max-ID methods demonstrate the excellent quantitation and precision from the Orbitrap Exploris 480 mass spectrometer; (B) Dynamic range of proteins identified in one run compared to a deep plasma proteome coverage reported in human plasma proteome (HPPP) index.

PAS offers pre-configured DIA-NN workflows with the options to run with or without a spectral library. Recent developments in data analysis software (e.g., using machine-learning approaches for in silico prediction of high-quality spectral libraries) have made library-free approaches a valid time- and cost-effective alternative. Commercially available software (CHIMERYS with Proteome Discoverer) and academic software (DIA-NN) packages employed for library-free analysis of the plasma data (Figure 4A). Both the high-throughput and Max-ID methods showed high data completeness (Figure 4B) with wide dynamic range of analysis (Figure 4C).

In addition to protein identification, precise measurement is necessary to identify potential biomarkers of biological insights. The data must be highly precise and accurate to reflect subtle changes in biological systems. Inaccurate quantitation leads to lack of statistical power that is both a waste of time and resources but can also require years of repeating experiments in the biomarker discovery pipeline. This is counter to the end goal of improving patient care by negatively impacting treatment decisions and outcomes. The results for both the high-throughput and Max-ID methods showed excellent quantitation precision with 87.2% and 89.2% of the proteins having a CV of <20%, respectively (Figure 5A).

The dynamic range of proteins identified in one run compared to a deep plasma proteome coverage reported in the human plasma proteome project (HPPP) index is plotted in Figure 5B.

Conclusions

- Enrichment of plasma proteins using Seer's Proteograph XT technology helps dig deeper into the plasma proteome dynamic range.
- LC-MS workflow with Vanquish Neo UHPLC system with a PepMap Easy-Spray column coupled to a Thermo Scientific™ Orbitrap Exploris™ 480 MS provides a robust and reproducible setup for identification and quantification of plasma proteins.
- High throughput capillary flow method enables identification of over 3,000 protein groups, of which 87.2% have a CV below 20%.
- Max-ID method allows for deeper proteome coverage along with excellent quantitation performance on Orbitrap Exploris 480 MS.

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