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Proteomics

A high-throughput plasma proteomics workflow on a Thermo Scientific Orbitrap Exploris 480 mass spectrometer

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Keywords

Plasma, Orbitrap Exploris 480 mass spectrometer, data-independent acquisition, DIA, Proteograph XT Assay, Vanquish Neo UHPLC, CHIMERYS, Spectronaut software, Proteome Discoverer software

Goal

To develop a high-throughput plasma proteomics workflow on a Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer

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 is required that does not compromise on protein identification, protein sequence coverage, dynamic range, and analysis precision. Here, a high-throughput workflow and a maximum identification (Max-ID) workflow on an Orbitrap Exploris 480 mass spectrometer for in-depth analysis of plasma are presented. The plasma samples were processed with the Seer Proteograph™ XT workflow utilizing proprietary engineered nanoparticles for an unbiased and deep proteomics protein sampling and analysis at scale. See Figure 1.

Vanquish Neo UHPLC system and Orbitrap Exploris 480 mass spectrometer

Materials and methods

General consumables

- Fisher Scientific™ LC-MS grade water with 0.1% formic acid (P/N LS118-500)
- Fisher Scientific™ Optima™ LC-MS grade 80% acetonitrile with 0.1% formic acid (P/N LS122-500)
- Fisher Scientific™ Optima™ LC-MS grade 100% acetonitrile with 0.1% formic acid (P/N LS120-212)
- Thermo Scientific™ SureSTART™ 9 mm Screw Caps, Level 3 High Performance Applications (P/N 6PSC9STB1)
- Thermo Scientific™ SureSTART™ 0.2 mL TPX Screw Top Microvial with Glass Insert for <2 mL Samples, Level 3 High Performance Applications (P/N 60180-1655)
- Seer Proteograph™ XT Assay Kit 40 Samples (P/N S55R4004)

Method-specific consumables

High-throughput method

- Thermo Scientific™ EASY-Spray™ PepMap™ Column, 2 µm C18, 150 µm × 15 cm (P/N ES906)
- Thermo Scientific™ PepMap™ Neo Trap Cartridge, 5 µm, 300 µm × 5 mm (P/N 174500)

Max-ID method

- Thermo Scientific™ EASY-Spray™ PepMap™ Neo Column, 2 µm C18, 75 µm × 75 cm (P/N ES75750)

Sample preparation

Human plasma sample (a pooled healthy control plasma) was prepared using the automated, scalable, and robust Seer Proteograph™ Product Suite, SP100 automation system with Proteograph XT Assay kit. A 0.5 µg sample of tryptic peptides was analyzed on a reversed phase C18 PepMap column coupled to an Orbitrap Exploris 480 MS with both nanoflow and capillary flow LC set up on a Vanquish Neo UHPLC system utilizing “Fast Loading” and “Fast Equilibration” features.

Instrumentation

Table 1. List of workflow components with part numbers

Workflow components	Description
Liquid chromatography	Thermo Scientific™ Vanquish™ Neo UHPLC System (P/N VN-S10-A-01): • Binary Pump N • Split Sampler NT • Solvent Rack, Vanquish System Controller • System Base with Drawer • Vanquish Display (P/N 6036.1180) • Vanquish Split Sampler Sample Loop, 100 µL (P/N 6252.1950) • Vanquish Column Compartment N (P/N VN-C10-A-01)
Analytical columns	• EASY-Spray PepMap Column, 2 µm C18, 150 µm × 15 cm (P/N ES906) • EASY-Spray PepMap Neo Column, 2 µm C18, 75 µm × 75 cm (P/N ES75750)
Trap column	Thermo Scientific™ PepMap™ Neo Trap Cartridge, 5 µm, 300 µm × 5 mm (P/N 174500)
Emitter	Thermo Scientific™ EASY-Spray™ Nano & Capillary adapter (P/N ES993)
Source	Thermo Scientific™ EASY-Spray™ ion source (P/N ES082)
Automated sample preparation platform	Seer SP100 Automation Instrument (P/N S55R4008)
Mass spectrometer	Orbitrap Exploris 480 Mass Spectrometerr
Data analysis software	• Seer Proteograph Analysis Suite (PAS) • Thermo Scientific™ Proteome Discoverer™ 3.1 Software with CHIMERYS™ Intelligent Search Algorithm by MSAID • Spectronaut™ 18 Software (Biognosys) • DIA-NN Software (v1.8.1)

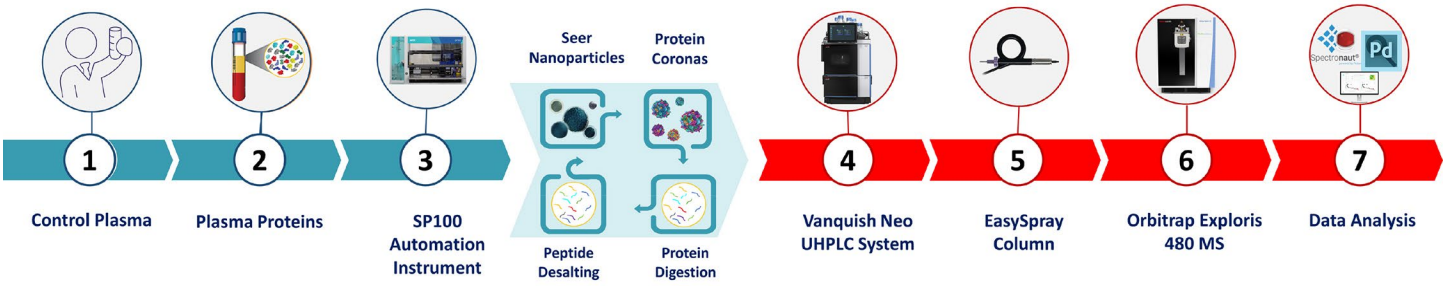

Figure 1. High-throughput plasma proteomics workflow

LC-MS analysis

The Proteograph XT assay kit creates two peptide sets for each sample at the end of sample processing. For the high-throughput method, two individual injections per sample were analyzed with the 24-minute LC-MS method with trap-and-elute sample loading, a total instrument time of 48 minutes per sample. For the Max-ID method, the two fractions were pooled and samples were analyzed in a single run using a 102-minute total gradient time coupled with direct injection to minimize sample loss. Upon the completion of each injection, result files were automatically uploaded to Thermo Scientific™ Ardia™ platform by Thermo Scientific™ Xcalibur™ acquisition software.

Source parameters, including spray voltage and ion transfer tube temperature, are tunable parameters and must be optimized for the individual setup. The details of the LC gradient, LC parameters, and MS method are reported in Table 2.

Data analysis

Seer Proteograph Analysis Suite (PAS) was used for data analysis of the resulting LC-MS files. 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 software (v1.8.1), or Proteome Discoverer 3.1 software using CHIMERYS intelligent search algorithm. In all software packages, 1% FDR cut off was applied at both protein and peptide levels.

Table 2. Summary of all LC and MS method parameters. Parameters not mentioned in the table are set to default values.

Separation column specifications for ES906 column (Vanquish Neo system)	
Inner diameter	150 µm
Length	15 cm
Maximum pressure	1,000 bar
Maximum flow	4 µL/min
Maximum temperature	60 °C

Separation column specifications for ES75750 column (Vanquish Neo system)	
Inner diameter	75 µm
Length	75 cm
Maximum pressure	1,500 bar
Maximum flow	1 µL/min
Maximum temperature	60 °C

Separation column specifications for ES75750 column (Vanquish Neo system)	
Inner diameter	300 µm
Length	0.5 cm
Maximum pressure	1,500 bar
Maximum flow	200 µL/min
Maximum temperature	60 °C

LC method (High-throughput)			
Column temperature	50 °C		
Fast loading/equilibration	PressureControl		
Pressure for loading/ equilibration/wash	Max Pressure		
Equilibration factor	2		
Sampler temperature	7 °C		
Gradient	Time	%B	Flow (µL/min)
	0	4	2
	0.2	8	2
	14.6	20	1.5
	21.5	35	1.5
	21.9	99	2
	22.6	99	2

LC method (Max-ID)			
Column temperature	50 °C		
Fast loading/equilibration	PressureControl		
Pressure for loading/ equilibration/wash	1,500 bar		
Equilibration factor	2		
Sampler temperature	7 °C		
Gradient	Time	%B	Flow (µL/min)
	0	1	0.25
	0.1	6	0.25
	60.1	20	0.25
	90.1	35	0.25
	91.1	99	0.25
	102	99	0.25

MS parameter	Max ID method	High-throughput method
MS1		
Resolution	60,000	60,000
Scan range (*m/z*)	380–750	380–780
AGC	300%	300%
Max-IT	Auto	Auto
MS2		
Resolution	15,000	15,000
Scan range (*m/z*)	145–1,450	145–2,000
Isolation window (*m/z*)	4	4
Window placement optimization	On	On
AGC	1,000%	800%
Max-IT	Auto	28 ms

Results and discussion

The optimized DIA method enabled the identification of >3,000 protein groups, >20,000 peptides from the high-throughput method (Figures 2A and 2B) and >3,800 protein groups, >27,000 peptides in the Max-ID method (Figures 2C and 2D) using PAS.

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 (Spectronaut 18 software and Proteome Discoverer software with CHIMERYS) and academic software (DIA-NN) packages employed for library-free analysis of the plasma data (Figure 3A). Both the high-throughput and Max-ID methods showed high data completeness (Figure 3B) with wide dynamic range of analysis (Figure 3C).

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 4A). 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 4B.

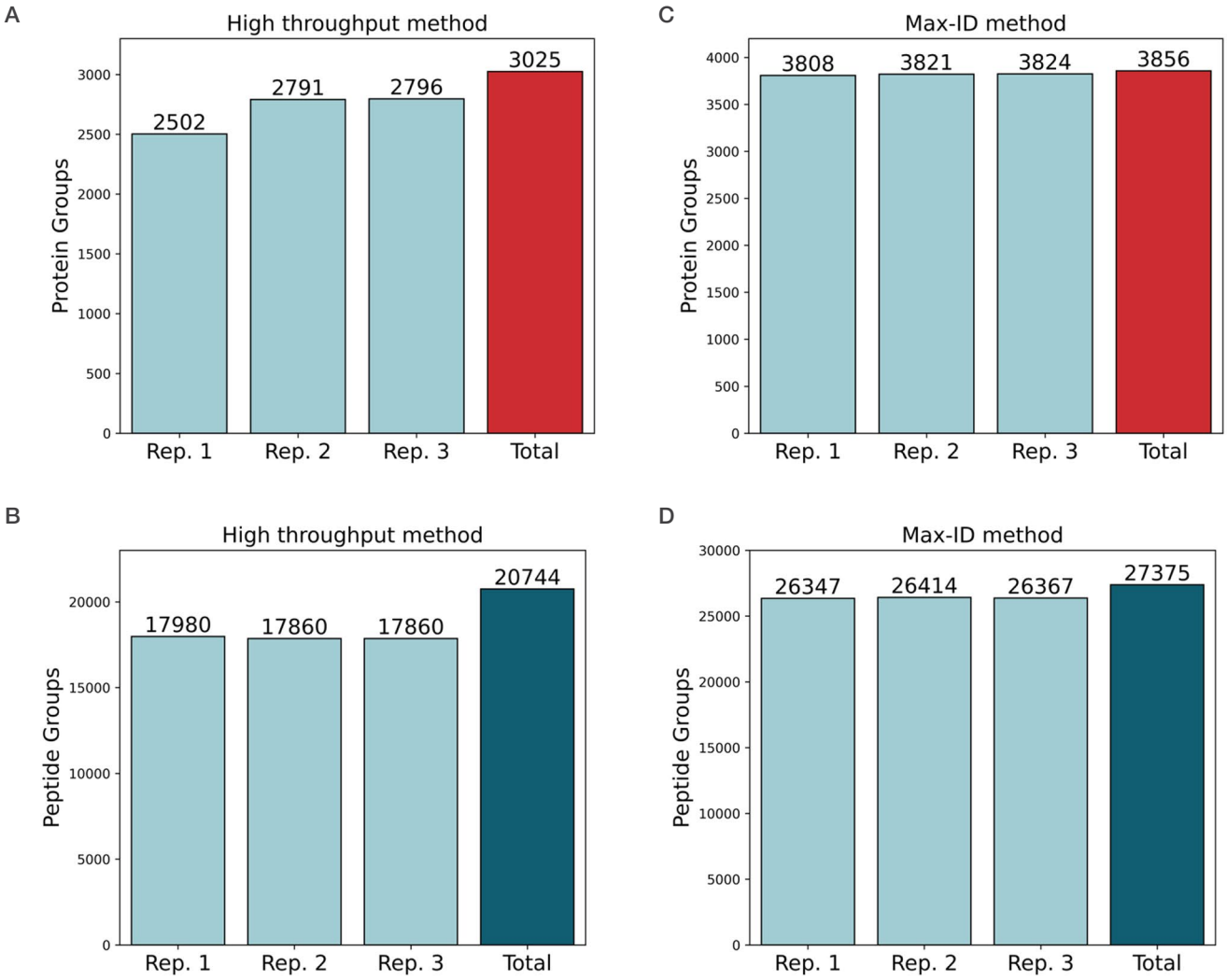

Figure 2. Number of protein groups in the (A) high-throughput (>3,000) and (C) Max-ID (>3,800) method; number of peptide groups in the (B) high-throughput (>20,000) and (D) Max-ID (>27,000) method

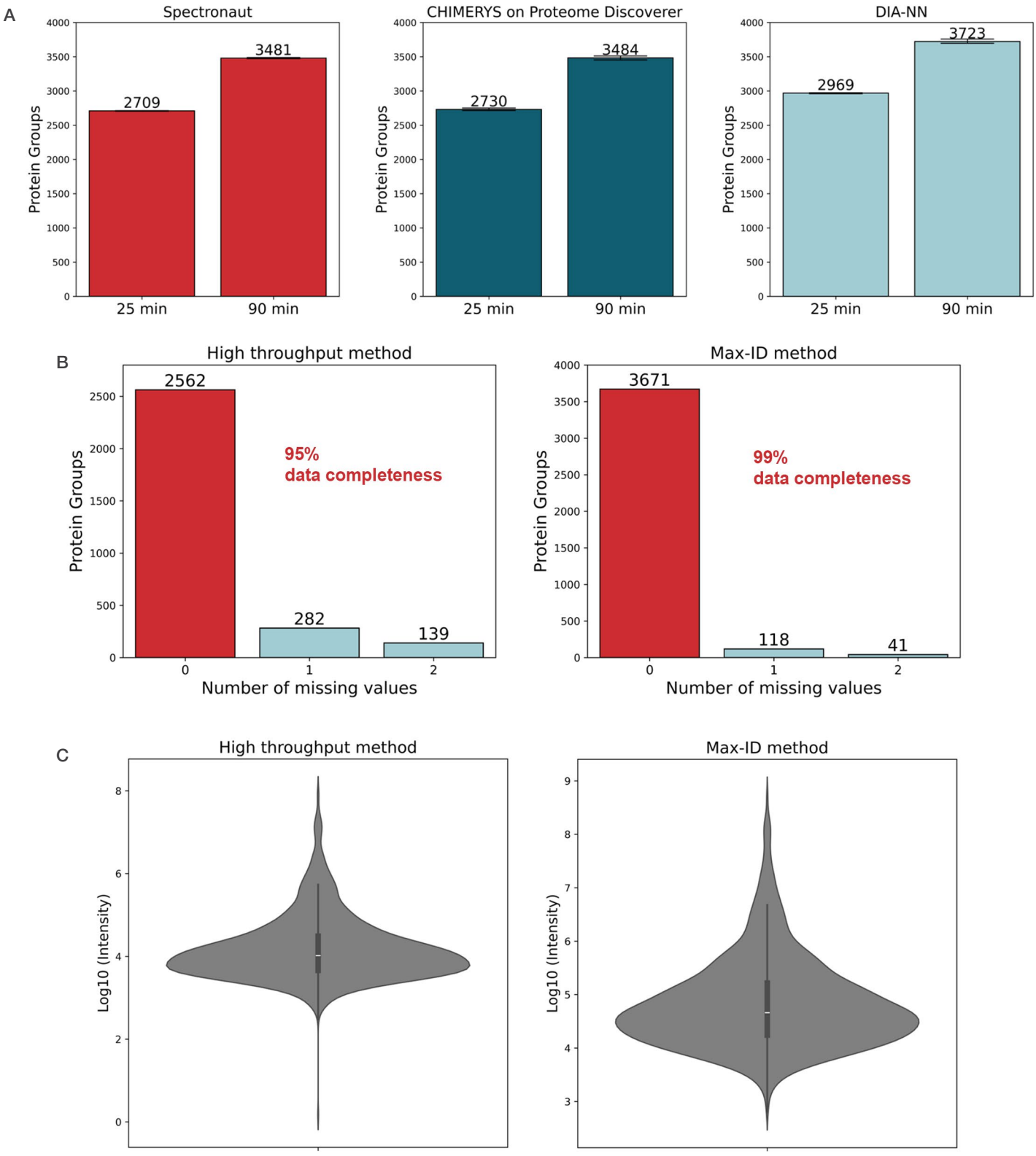

Figure 3. (A) Number of protein groups obtained from library-free searches on Spectronaut software, CHIMERYS on Proteome Discoverer software and DIA-NN software; (B) data completeness of high-throughput (95%) and Max-ID (99%) method; (C) wide dynamic range afforded by the Orbitrap Exploris 480 mass spectrometer in both the high-throughput and Max-ID method

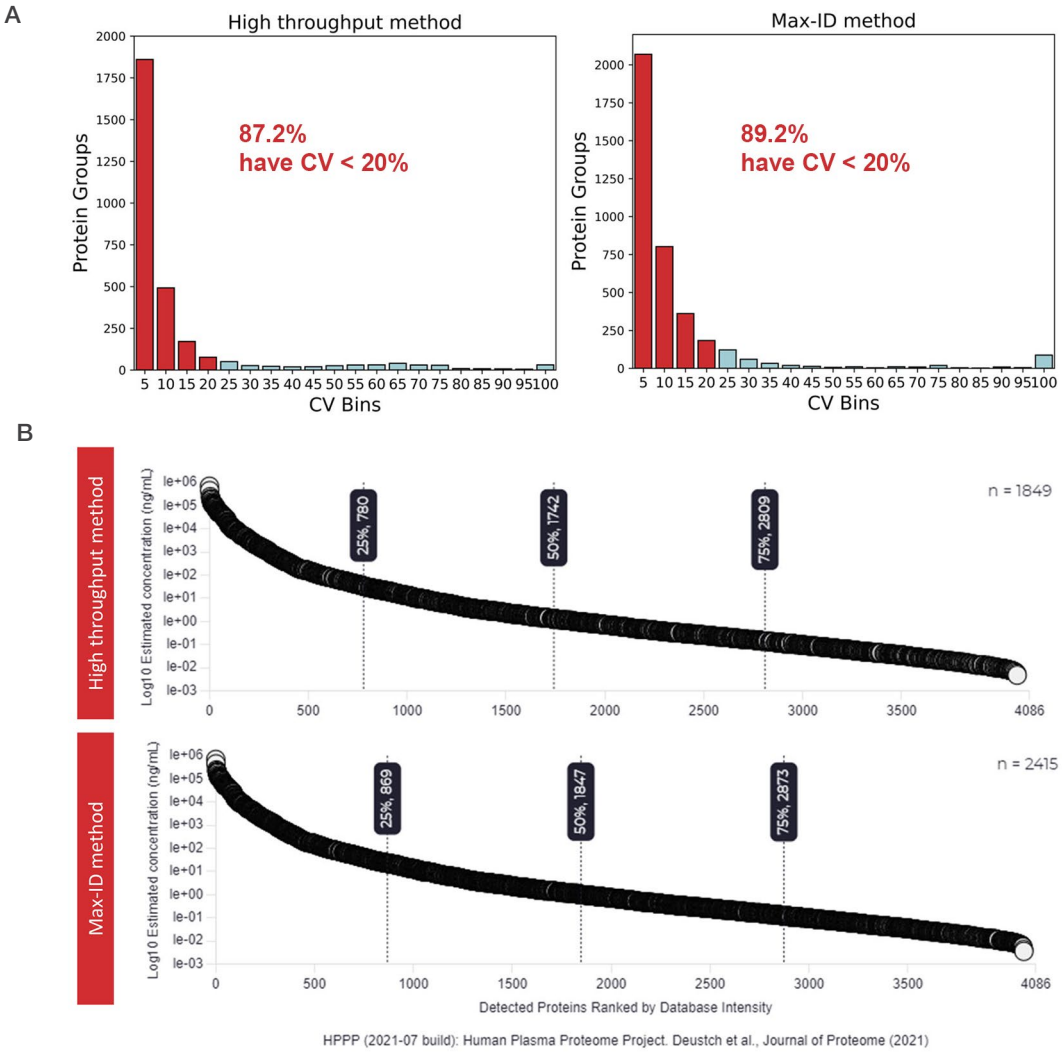

Figure 4. (A) Protein group coefficient of variance (CV) distribution of the high-throughput and Max-ID methods demonstrate the excellent quantitation and precision from the Orbitrap Exploris 480 mass spectrometer; (B) dynamic range of proteins identified in one run compared to a deep plasma proteome coverage reported in human plasma proteome project (HPPP) index

Conclusion

- Proteograph XT workflow enables deeper protein coverage across a wide dynamic range of plasma proteome.
- Using a Vanquish Neo UHPLC system with a PepMap Easy-Spray column coupled to an Orbitrap Exploris 480 mass spectrometer provides a robust and reproducible setup for identification and quantification of plasma proteins.
- The high-throughput capillary flow method enables identification of over 3,000 protein groups, of which 87.2% have a CV below 20%.
- The Max-ID method allows for deep proteome coverage along with excellent quantitation performance on an Orbitrap Exploris 480 mass spectrometer.
- In summary, this robust LC-MS method enables researchers to do large plasma cohort studies at a new scale and depth.

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