Robust and Deep Plasma Proteomics using a Multi Nanoparticle-based Workflow coupled with an Orbitrap Exploris 480 Mass Spectrometry and FAIMS Pro Interface

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Abstract

Introduction

Data-independent acquisition (DIA) is a powerful method for cataloging thousands of proteins in complex biological samples like human plasma. However, for any large-scale proteomics studies, both LC and MS systems need to be robust, without compromising on peptide and protein coverage and analysis precision. Here we present a high-throughput single injection, label-free plasma proteomics workflow with a Thermo Scientific™ Orbitrap Exploris™ 480 Mass Spectrometer coupled to a Thermo Scientific™ FAIMS Pro™ Interface as a robust analytical setup for in-depth analysis of plasma samples processed with Seer's Proteograph™ Product Suite utilizing a multi-nanoparticle-based approach for an unbiased and deep plasma proteomics analysis at scale.

Method

Neat plasma and Seer's enriched plasma proteins digested on Proteograph Product Suite, were analyzed using a 75cm, Thermo Scientific™ EASY-Spray™ PepMap™ Neo UHPLC Column on a Thermo Scientific™ Vanquish™ Neo UHPLC System at 250nl/min, coupled to an Orbitrap Exploris 480 mass spectrometer with and without FAIMS Pro interface. Performance of the whole workflow was evaluated with a single injection using a 90 min effective gradient that allowed for 160 min total analysis time sample to sample. The data were analyzed with DIA-NN in Seer's Proteograph™ Analysis Suite providing enhanced peptide and protein coverage with a 1% FDR rate.

Results

Proteograph™ with label-free DIA method resulted in identification of 3423 protein groups. The addition of FAIMS improved the identifications by 10%. The single injection of 2ug pooled 5NPs plasma digest was used for improvement in throughput without compromise on protein and peptide coverage with nanoflow sensitivity.

This optimized workflow provides an easy-to-use, robust workflow for high throughput, deep plasma proteomics analysis at scale. In addition, we foresee opportunities to further increase the throughput by further optimizing loading, equilibration and washing steps by up to 30% improvement.

Methods

Human plasma control sample (PC6, a low complex pooled healthy control plasma) were enriched on Seer's multi-nanoparticles technology via an automated, scalable, and robust Proteograph™ Product Suite, SP100 automation system with Proteograph Assay Kit. 2µg of tryptic peptides were analyzed on a reversed phase C18 PepMap column coupled to an Orbitrap Exploris 480 MS with and without FAIMS Pro Interface with nanoflow LC set up on Vanquish Neo UHPLC system utilizing "Fast Loading" and "Fast Equilibration" features.

LC Method

- Analytical Column: EASY-Spray PepMap Neo UHPLC Column, 2µm C18, 75µmx75cm
- Mobile phases: [A] 0.1% FA in H_2O, [B] 0.1% FA in 80% ACN
- Flow rate: 250 nL/min, direct injection

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				

Results

The optimized DIA method enables identification of over 3000 protein groups and approximately 15500 peptides in each individual replicate. When the 4 replicates were searched together 3423 protein groups and close to 19000 peptides were identified with high reproducibility (Standard Deviation = 10.4)

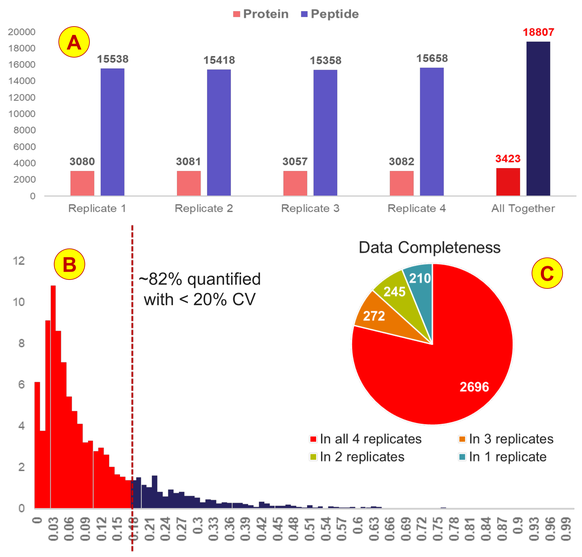

Figure 1. A: total number of protein groups and peptides identified. **B:** Superior quantitative reproducibility was observed among 4 replicate injections. **C:** 87.6% of the proteins were identified in all 4 replicate injections.

Results (cont.)

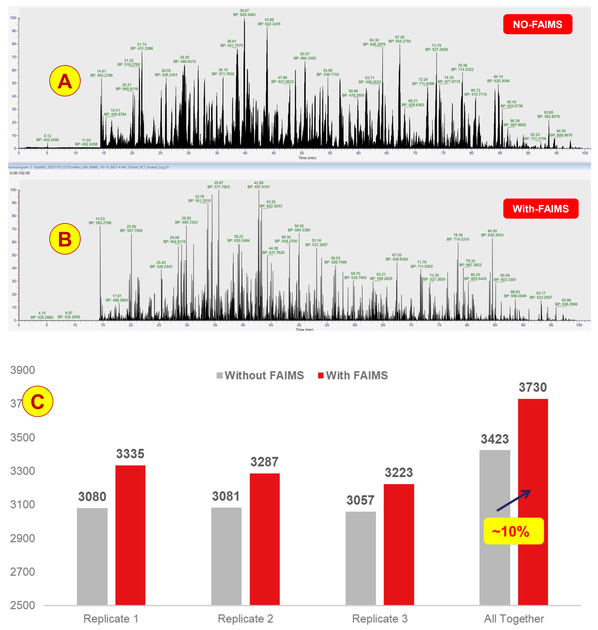

Figure 3. A: Base Peak chromatogram of one of the replicate injections without FAIMS and **B:** with FAIMS. FAIMS clearly improves the signal by removing unwanted charge states. **C:** Improvement in the number of protein group identifications when FAIMS is used. Approximately 10% increase in the number of protein group identification was observed in each individual replicate and when all 4 replicates were searched together .

Proteograph Assay

Samples (16 samples/plate) are processed using 40 µL aliquots of each plasma sample, mixed with each of five NPs aliquots included in the Proteograph 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 to generate tryptic peptides for LC-MS analysis. All steps are performed in a one-pot reaction directly on the NPs. The in-solution digestion mixture is then desalted, and all detergents are removed using a mixed-media filter plate and positive pressure (MPE) system. Clean peptides are eluted in a high-organic buffer into a deep-well collection plate.

MS Method

A 90 minutes (active gradient, 102 minutes total run time) data independent acquisition method was used with MS1 resolution set to 60K, AGC target of 3e6 and automatic MaxIT injection. The DIA experiment was set to have 4 m/z isolation window with 1 m/z overlap making approximately 100 DIA events across the mass range. Orbitrap resolution was set to 15K and a high value of 1e6 was used for AGC to be able to better control the ion population using MaxIT that was set to 28 ms. Normalized collision energy of 28 was used with a scan range of 145-1450 m/z.

For the method where FAIMS Pro Interface was used, two compensation voltages of -45 V and -75 V were used in 2 different experiments.

Data Analysis

The Proteograph Analysis Suite (PAS) is used for analysis of the resulting LC-MS data files using DIA-NN v1.8, applying a 1% FDR cutoff at the protein and peptide levels.

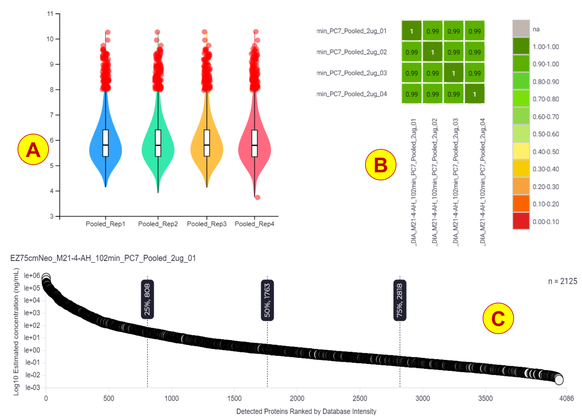

Figure 2. A: Protein group intensities with distribution of protein sequence coverage for each replicate. **B:** Statistical correlation of protein group intensities between samples based on the Pearson correlation coefficient. **C:** Dynamic range of proteins identified in one run compared to a deep plasma proteome coverage reported in human plasma proteome (HPPP) index.

CONCLUSIONS

- Enrichment of plasma proteins using Seer's multi-nanoparticles technology, Proteograph Product Suite and Proteograph Assay Kit helps dig deeper into the plasma proteome dynamic range.
- Using a Vanquish Neo UHPLC system with a PepMap Easy-Spray column coupled to an Orbitrap Exploris 480 MS provides a robust and reproducible setup for identification and quantification of plasma proteins.
- Addition of FAIMS Pro Interface to the proposed workflow improves the number of identification by 10% resulting in a total of 3423 protein groups identified from 2ug of enriched sample.

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