Robust, high throughput and deep plasma proteomics workflow with engineered nanoparticle panels

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The Proteograph Product Suite enables rapid sample preparation for reproducible, deep and unbiased plasma proteomic analysis

Data Independent Acquisition (DIA) proteomics methods are powerful methods for cataloging thousands of proteins in complex biological samples like human blood plasma in a high throughput LC-MS proteomics approach. However, for any large-scale proteomics studies, both LC and MS systems need to be robust, without compromising on peptide and protein coverage and provide ease of use for users with any level of analytical expertise. Here we present a high throughput label-free plasma proteomics workflow on a Orbitrap Exploris™ 480 mass spectrometer coupled to an UltiMate™ 3000 HPLC system and PharmaFluidics™ micro-pillar Array Columns (µPAC™), as a robust, high throughput analytical setup for in-depth proteomics analysis of plasma samples processed with Seer's Proteograph™ Product Suite[1].

Methods

- Neat PC5 control pooled plasma (a low complex pooled healthy control plasma sample) and Seer's five-nanoparticle (5NPs) enriched PC5 plasma proteins were digested on the Proteograph SP100 automated sample preparation platform.
- Desalted, tryptic peptides processed by SP100 were analyzed with label-free MS data acquisition in DIA, top speed mode. LC-MS analysis was performed with a flow rate of 1µL/min using a gen1, 50 cm, C18 µPAC columns coupled to an Orbitrap Exploris 480 mass spectrometer (Thermo Fisher Scientific).
- Performance of different workflows were evaluated with a 30,120 and 150 min single injection method or five separate injections with a 30 min DIA method for each of the 5NPs and neat plasma digest.
- The DIA data is analyzed with DIA-NN in Seer's Proteograph™ Analysis Suite (PAS) using a spectral library free DIA-NN approach[2], for label-free data analysis providing improved peptide and protein coverage with a 1% FDR rate.

Label-Free DIA Workflow

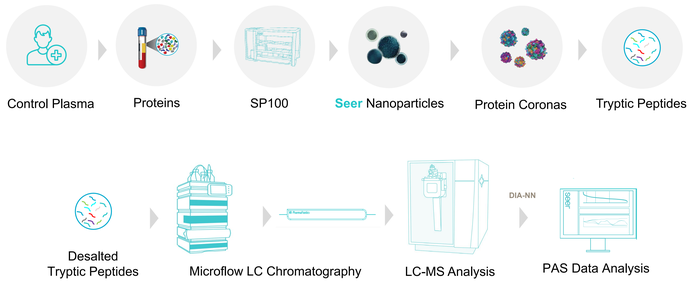

Figure 1. Architecture diagram of VAE neural network.

- Plasma samples were processed using Seer's Proteograph™ product suite. Samples were analyzed on an Ultimate3000 nanoLC with a 50 cm, C18 µPAC column (PharmaFluidics) coupled to an Orbitrap Exploris 480 mass spectrometer using a 33min total run time, 120min total run time method, and a 150min total run time DIA method. With data processed in Proteograph™ Analysis Suite (PAS) using DIA-NN with fully tryptic peptide search.

Deep and unbiased plasma proteomics performance with robust, reproducible and high-throughput DIA workflow

Results

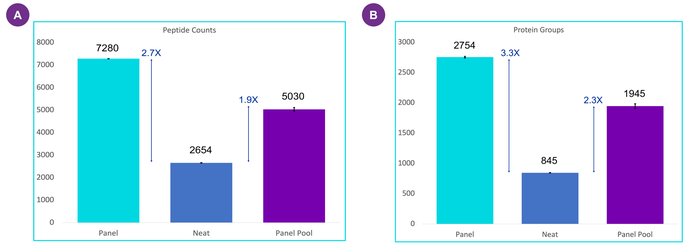

Figure 2. Peptide and protein groups identification. Data set comparison of neat plasma, Seer's 5NP panel enriched plasma pooled into a single injection and 5 individual injections for each NP analyzed with a 33min DIA method. **(a)** Seer's 5NP enriched plasma panel show a significant increase in peptide identification compared to neat plasma digest and pooled 5NPs peptide analyzed in single injection. **(b)** Seer's 5NP enriched plasma panel shows significant increase in the number of protein groups using Seer's Proteograph Product Suite compared to neat plasma digest.

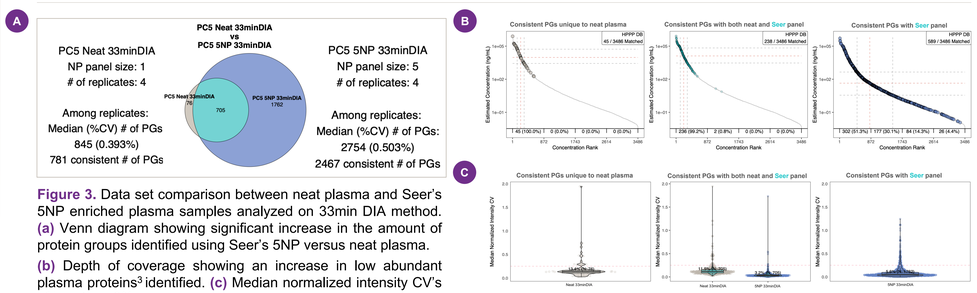

Figure 3. Data set comparison between neat plasma and Seer's 5NP enriched plasma samples analyzed on 33min DIA method. **(a)** Venn diagram showing significant increase in the amount of protein groups identified using Seer's 5NP versus neat plasma. **(b)** Depth of coverage showing an increase in low abundant plasma proteins[3] identified. **(c)** Median normalized intensity CV's of less than 6%.

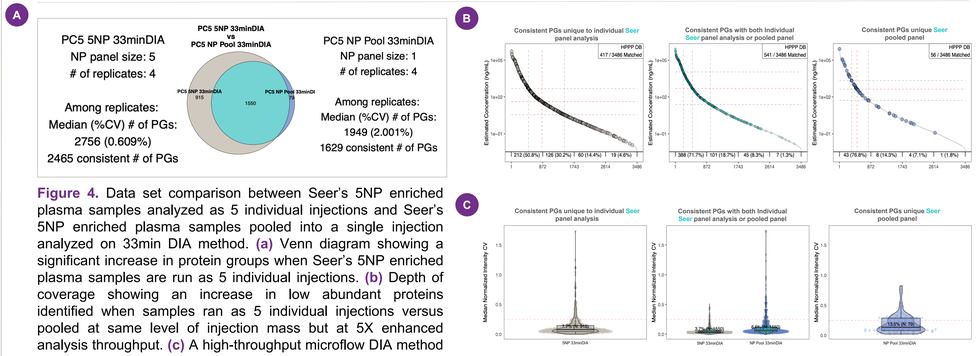

Figure 4. Data set comparison between Seer's 5NP enriched plasma samples analyzed as 5 individual injections and Seer's 5NP enriched plasma samples pooled into a single injection analyzed on 33min DIA method. **(a)** Venn diagram showing a significant increase in protein groups when Seer's 5NP enriched plasma samples are run as 5 individual injections. **(b)** Depth of coverage showing an increase in low abundant proteins identified when samples ran as 5 individual injections versus pooled at same level of injection mass but at 5X enhanced analysis throughput. **(c)** A high-throughput microflow DIA method with median normalized intensity CV's of less than 4%.

Figure 5. Performance evaluation of high-throughput DIA workflow for plasma proteomics. Data set comparison of 3 different DIA methods using neat plasma and Seer's 5NP enriched plasma peptides pooled into a single injection. **(a)** Peptide counts for neat plasma show an increase as gradient length is extended, but when using 5NP pooled sample more peptide counts are seen using a 33minDIA method. **(b)** Protein groups also show an increasing trend in neat plasma with longer gradient DIA methods; however, a shorter 33min DIA method is sufficient for 5NP pooled sample type providing more protein groups identification on gen1, 50 cm, C18 µPAC columns using microflow LC-MS analysis. Further method optimization is required for longer gradient DIA method for pooled 5NP peptide analysis in single injection DIA method.

Conclusions

- Seer' Proteograph product Suite provides significant increase in the peptide and protein groups counts versus neat plasma using a robust high throughput 33min microflow DIA method.
- Significant enhancement in peptide and protein groups identification when Seer's 5NP plasma samples are run as 5 individual injections versus a pooled sample.
- The 33minDIA method has demonstrated to be a robust high throughput workflow with high reproducibility on gen1, 50 cm, C18 µPAC columns using microflow LC-MS analysis.
- Further method optimization is required for longer gradient microflow DIA method for a single injection pooled 5NPs enriched plasma peptide LC-MS analysis.

References

[1] Blume et al. Nat. Comm. (2020)
[2] Demichev et al. Nat Comm. (2020)
[3] Keshishian, et al. Nature Protocols (2017)