Robust in-depth label-free plasma proteomics with engineered nanoparticle panels: An evaluation of micro-pillar array columns and FAIMS peptide separation

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Deeper dive into Plasma Proteome with Seer's Proteograph™ Platform

Introduction

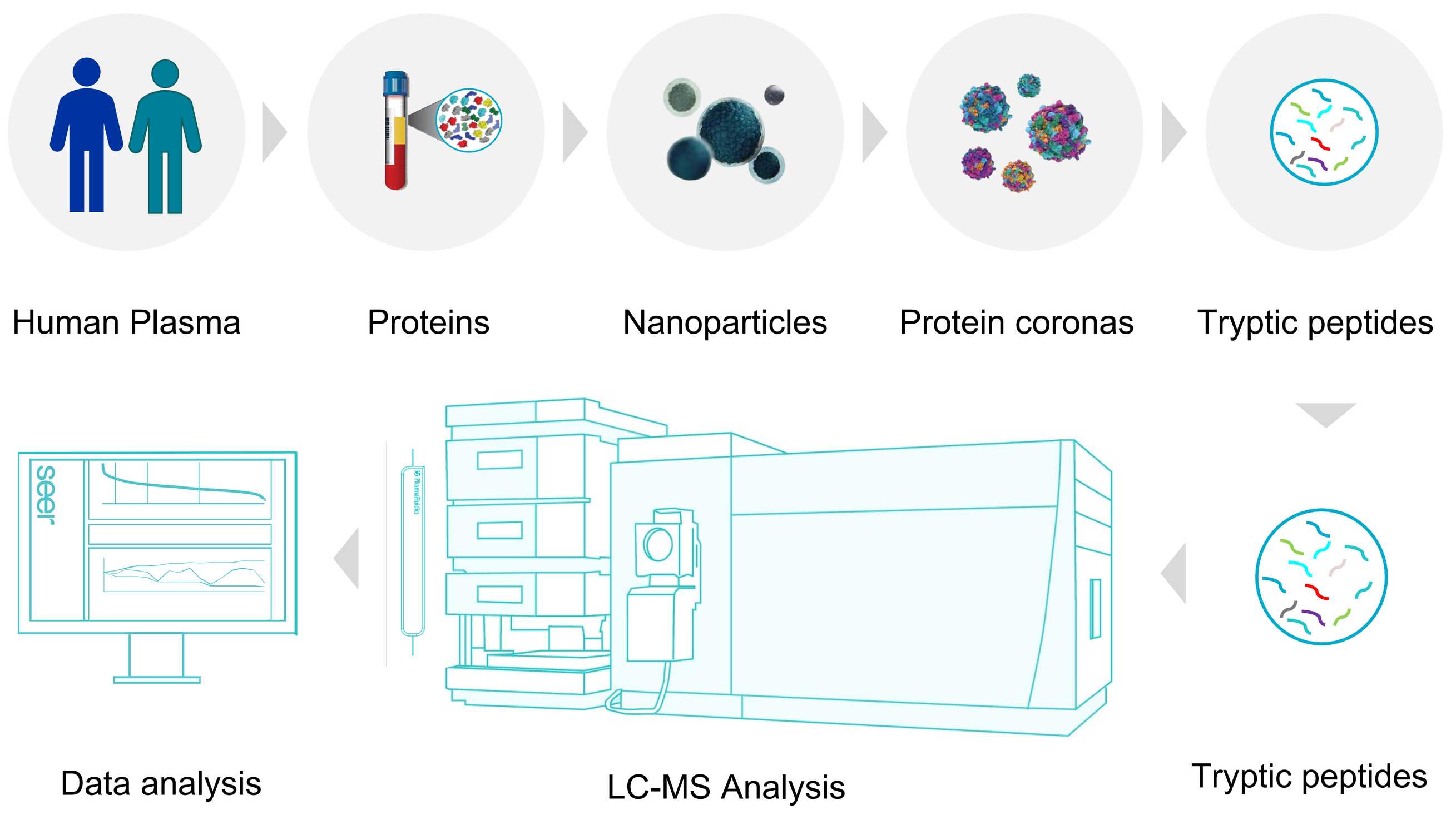
LC-MS-based proteomics analysis is a powerful analytical tool for identification and quantification of thousands of proteins in complex biological samples like human blood plasma. For large-scale proteomics analysis both LC and MS systems need to be robust, without compromising peptide and protein coverage and provide ease of use for users with any level of analytical expertise. Here we present a label-free proteomics workflow on a Orbitrap Fusion Lumos Tribrid mass spectrometer coupled to a High-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) interface and micro–Pillar Array Columns (μPAC™), as a robust analytical setup for in-depth proteomics analysis of plasma samples processed with Seer's Proteograph™ Product Suite.

LC-MS analysis was performed with 50cm and 200cm C18 μPAC columns (PharmaFluidics, Belgium) coupled to an Orbitrap Fusion Lumos Tribrid mass spectrometer and FAIMS Pro Interface (Thermo Fisher Scientific). Neat plasma, Seer's five-nanoparticle (5NPs) enriched plasma proteins digested on Proteograph Product Suite, and standard HeLa digest (Pierce) were analyzed with label-free MS data acquisition in DDA, top speed mode with multi-CV FAIMS peptide fractionation based on peptide mass and charge states. Performance were evaluated with a 300 min single injection method. MSFragger software were used for label-free data analysis, additionally Proteome Discoverer™ 3.0 software was evaluated with further improved peptide and protein coverage with a 1% FDR rate.

Method

- Human Plasma sample (PC5, 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.
- Peptide samples were analyzed with a reversed phase C18 μPAC columns run on the Orbitrap Fusion Lumos Tribrid MS with and without FAIMS with microflow and nanoflow LC set up on Ultimate U3000 LC system.
- Label-free data analysis performed with MSFragger and Proteome Discoverer 3.0 with CHIMERYS + MSPepSearch + Sequest HT + INFERYS Rescoring.

Proteograph Product Suite



From Sample to Peptides, Ready for Analysis on Most LC-MS/MS Instruments

Evaluation of DDA Workflows for Deep Plasma Protein Coverage

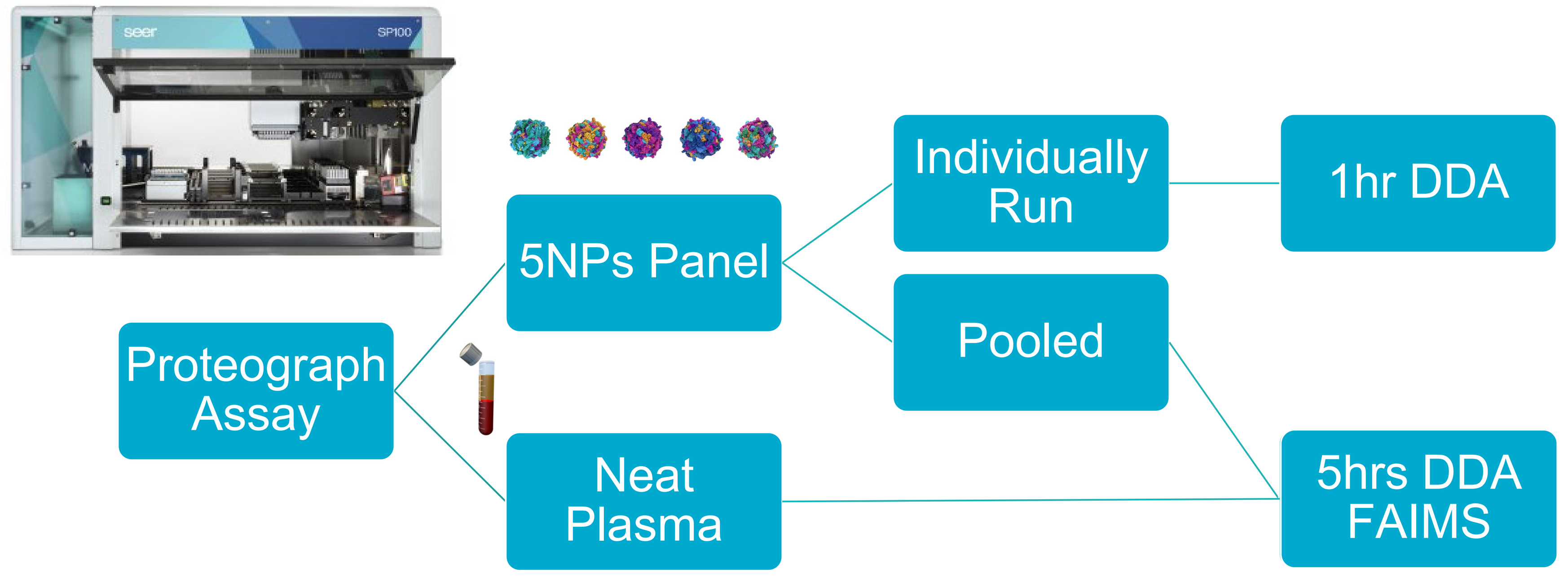


Figure 1. Experimental Workflow. Plasma samples enriched with Seer's 5NPs panel are individually analyzed with our 1hr microflow DDA method (total time: 5hrs per panel) or with a pooled 5NP peptides in single injection nanoflow, 5hrs DDA FAIMS method. Neat Plasma digest is also analyzed with 5hrs DDA FAIMS method with same injection mass for direct comparison to 5NP enriched peptides..

Results

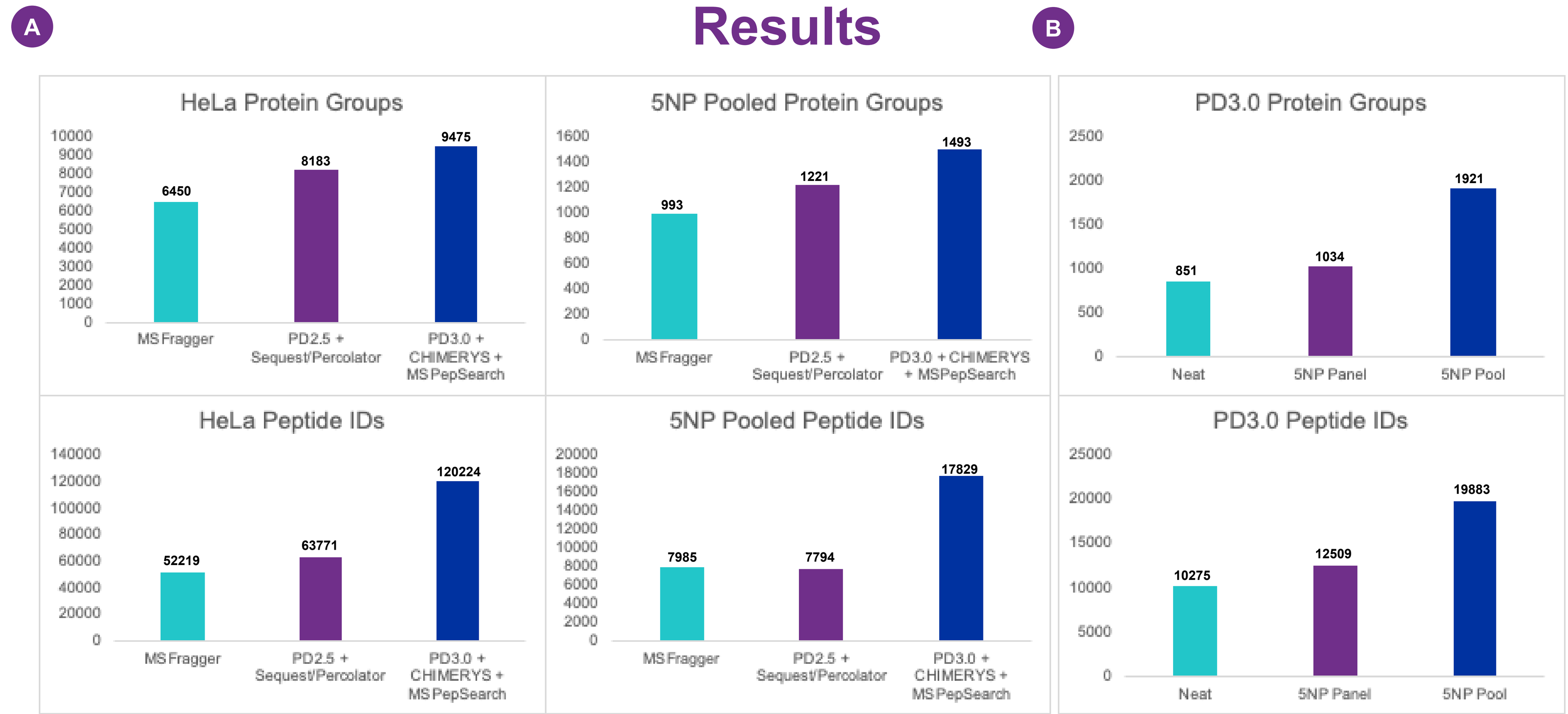


Figure 2. Identification performance with different search engines. A) HeLa standard digest and 5NP pooled plasma peptides were analyzed with 5hrs DDA FAIMS method. Data was analyzed with SFragger, Proteome Discoverer 2.5 with Sequest HT, and Proteome Discoverer 3.0 with CHIMERYS + MSPepSearch. ~9.5k protein groups and 120K peptide were identified with 4ug HeLa digest in a single injection. **B)** the optimized single injection DDA FAIMS method was then used for Neat, 5NP pooled plasma peptides; resulting in ~2K, 20K protein groups/peptide IDs with Proteome Discoverer 3.0.

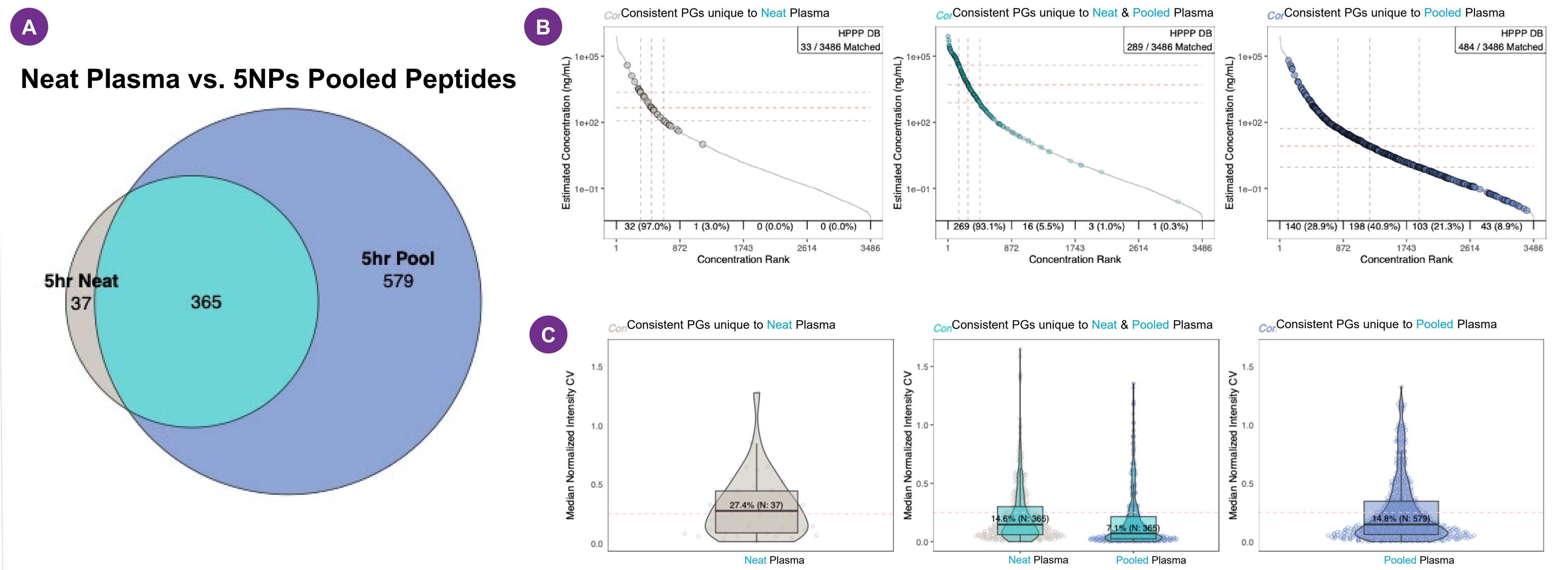


Figure 3. DDA OT/IT FAIMS method performance with neat plasma digest vs. pooled 5NPs peptide.
A) Venn diagram comparison of Protein Group identifications where Seer's pooled 5NPs identified significantly more protein groups than neat digested plasma. **B)** Depth of plasma proteomics coverage where we were able to identify more lower abundant proteins with pooled sample. **C)** Medium Normalized Intensity CVs below 15% with Proteograph platform.

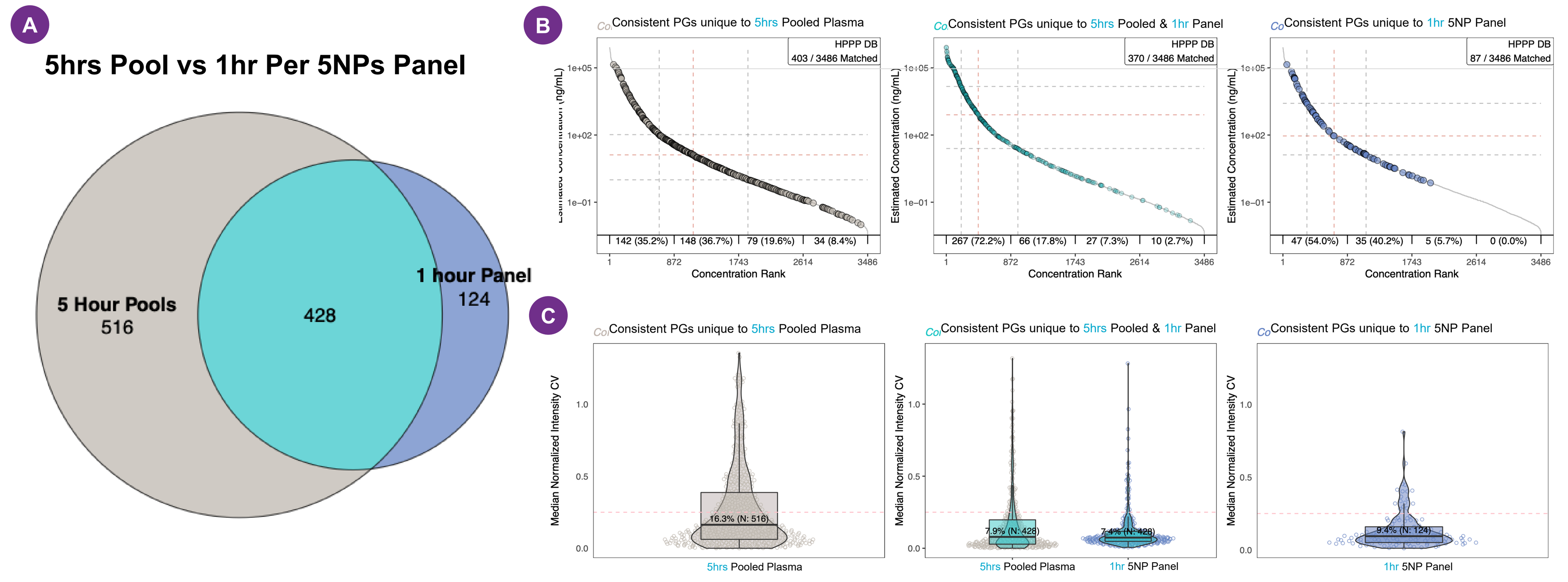


Figure 4..Method performance with single injection DDA OT/IT FAIMS vs. individual 5NP panel analysis with 1hr DDA OT/OT method. A) Venn diagram comparison of Protein Group identifications where Seer's pooled 5NP sample outperforms the 1hr 5NP panel (5hrs total time) with FAIMS OT/IT method. **B)** Depth of plasma proteomics coverage where we were able to identify more unique lower abundant proteins with FAIMS Pro Interface. **C)** Medium Normalized Intensity CVs below 17%.

Conclusion

- Seer's Poteograph Product Suite facilitates access to lower abundant proteins in the plasma proteome compared to neat plasma digestion for high-throughput unbiased plasma proteomics analysis.
- Utilizing long DDA gradients with FAIMS Pro Interface allows for deeper dive and greater number of unique proteins identified in a single injection LC-MS analysis with future plan to have a shorter gradient method on gen2- 110 cm column to enhance the DDA plasma proteomics analysis workflow.
- Proteome Discoverer 3.0 with CHIMERYS providing deeper protein coverage with FAIMS data with multiple CVs.

References

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

Publications