Proteograph™ multi-nanoparticle proteins coronas enable deep plasma proteomics studies at scale with unmatched sensitivity in combination with trapped ion mobility

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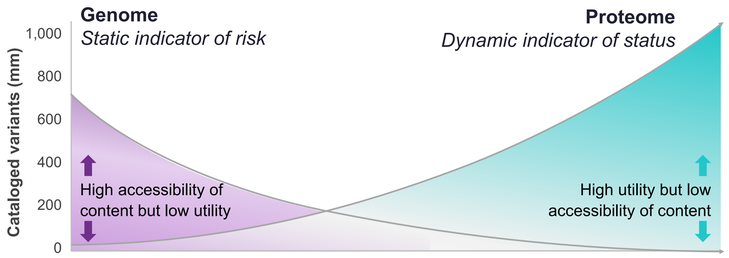
Proteograph Product Suite Delivers Unbiased, Deep and Rapid Proteomics at Scale

Blood plasma is a rich, readily available source of proteins that is commonly used in clinical profiling studies. However, plasma proteomics is inherently constrained by the large dynamic concentration range and complexity of the proteome. The ability to overcome these constraints while interrogating deeply and broadly into the plasma proteome has only been partially addressed by laborious, unscalable and low throughput workflows. To fully enable high-throughput plasma proteomics, we have developed a quantitative profiling solution, Proteograph Product Suite[1] that consists of a panel of 5 nanoparticles (NPs) with distinct physicochemical properties. This panel of NPs is used in parallel to enable high-performance plasma proteomics combining depth and breadth with precise and reproducible quantification.

Here we explore the synergy of the Proteograph assay with the Bruker timsTOF Pro mass spectrometer (MS), including; LC gradients making use of both data-dependent and data-independent acquisition (i.e., DDA and DIA) workflow coupled with our five nanoparticle Proteograph Assay using a control plasma sample.

Proteograph proteome profiling with the timsTOF Pro mass spectrometry was then evaluated in respect to depth of coverage and analysis throughput. We also investigated the linearity of response employing a multi-level proteome spike-in experiment.



Utility of Information

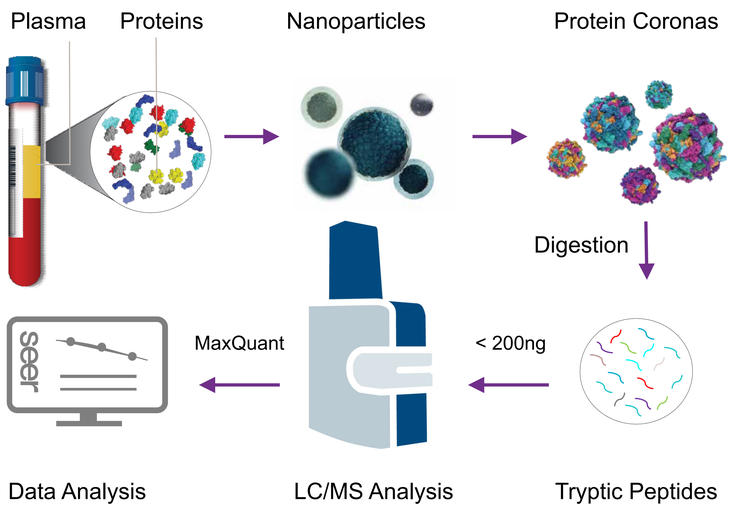
10M+ human exomes
1M+ genomes

~695M genetic variants catalogued

< 0.2% of genetic variants fully characterized

Core Technology

Proprietary Engineered Nanoparticles



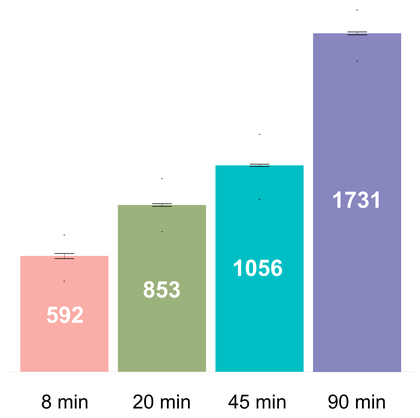
Proteograph Product Suite

Sample is ready to be analyzed on most LC/MS instruments

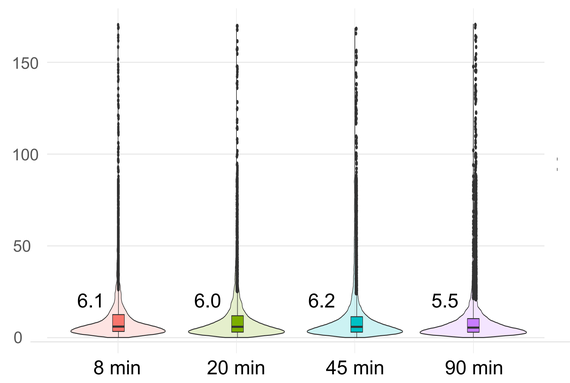
Unbiased, Deep and Rapid Method for Plasma Proteomic Analysis at Scale with the Proteograph Product Suite and the Bruker timsTOF Pro Mass Spectrometer

Deep and Rapid LC/MS Method for Plasma Proteomics

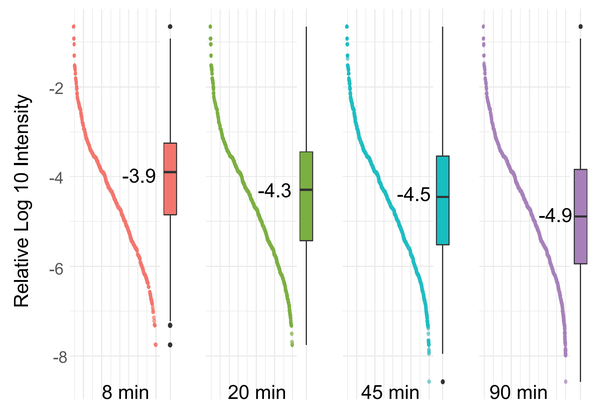
1A Data Dependent Acquisition (DDA)



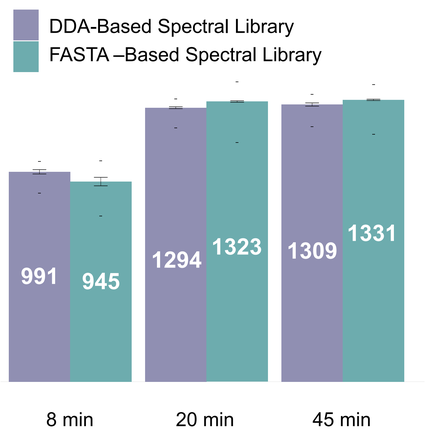
2A Precision of DDA Method (Peptide CVs)



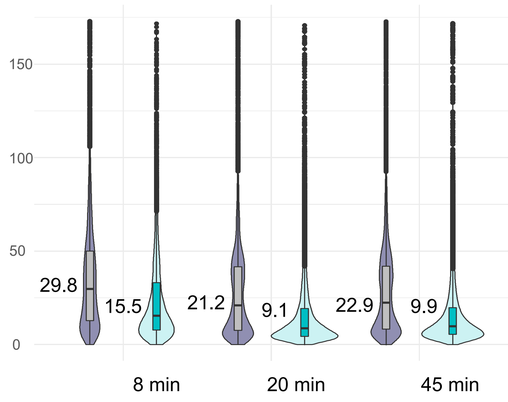
3A Protein Abundance (High to Low)



1B Data Independent Acquisition (DIA)



2B Precision of DIA Method (Peptide CVs)



3B Protein Abundance (High to Low)

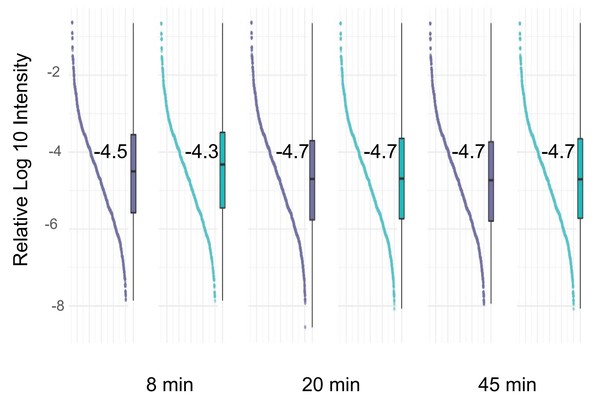


Figure 1. Comparison of protein identification across different gradients. A) In DDA mode, protein identification is improved by increasing the length of the gradient. B) In DIA mode, protein identification was similar using 20 min and 45 min gradients and significantly higher than the 8 min gradient. A library-free approach resulted in similar identifications when compared to a DDA-based spectral library approach.

Figure 2. Replicate CV for median-normalized peptide intensities. A) In DDA mode, the median CV of ≤ 6.2 was observed across different gradient lengths. B) In DIA mode, significantly better CV distribution was observed than in the library-free approach. The 20 min gradient had the lowest median CV when compared to the other gradient lengths.

Figure 3. Dynamic range of identified proteins matched with normalized protein intensities from the previously published data[2]. A) In DDA mode, the dynamic range coverage is improved by increasing the gradient length, up to one order of magnitude compared to the shortest gradient. B) In DIA mode, the dynamic range coverage is similar using both experimental library-based and library-free approaches. A slight improvement was observed when increasing the gradient to 20 min or longer.

Quantitation Accuracy with Optimized DDA Method

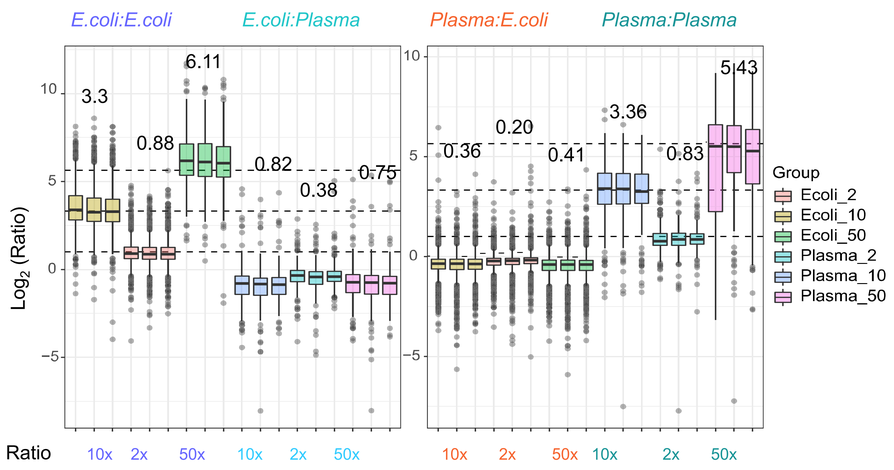


Figure 4. Evaluation of accuracy and precision. In a spike-in E.coli:Control Plasma experiment, a 2, 10 and 50-fold range of experimental ratio was analyzed with a good accuracy across a wide dilution range in a 90 min DDA run.

Conclusions

- For TIMS-TOF DDA analysis, protein coverage improves with increasing gradient length, with a median peptide CV ≤ 6.2
- In DDA the median dynamic range of a 90 min method improved by an order of magnitude when compared to the 8-min analysis
- TIMS-TOF Pro DIA data vs. DDA showed improved protein coverage when comparing same gradient lengths
- DIA data analysis using a library-free approach (MaxQuant ref) potentially omits the need for generation of experimental spectral libraries by maintaining the protein identification information and improving the quantification precision. It also highlights the utility of this approach for multi-species proteomics such as microbiome where an unknown proteome may be present in the samples without prior knowledge
- Proteome spike-in experiments showed a good quantitation accuracy across 2–50 fold changes
- Proteograph Product Suite in combination with the timsTOF Pro provides a high-performance combination for rapid deep, precise, and accurate proteome profiling for biomarker discovery and biomedical research

References

1. Blume et al. Nat. Comm. (2020)
2. Keshishian et al. Nature Protocols (2017)

Publications

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