# Deep Plasma Proteomics at Scale with Proteograph™ Product Suite: A Performance Evaluation with Label-free and TMT Multiplexing Methods

**Ryan W. Benz**[1], Xiaoju Zhang[1], Tianyu Wang[1], Mathew Ellenberger[1], Gabriel Castro[1], Ray Schmidt[1], Veder Garcia[1], Qiu Yang[1], Chenyi Zhao[1], Purvi Tandel[1], Michelle Dubuke[1], Ramón Díaz Peña[2], Alex Campos[2], and Khatereh Motamedchaboki[1]

[1]Seer, Inc., Redwood City, CA 94065, USA, [2]Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA 92037, USA

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

## Proteograph Product Suite enables high levels of plasma sample processing reproducibility for both label-free and TMT, LC-MS proteomics workflows

## Introduction

Human blood plasma is a widely accessible sample for assessing individual health status. However, the large dynamic range of circulating proteins combined with the diversity of proteoforms present in plasma have limited the comprehensive characterization of the plasma proteome in a high throughput manner. To address such challenges, current plasma proteomics workflows combine immunodepletion of high abundance proteins, peptide fractionation and sample multiplexing approaches such as tandem mass tags (TMT). Recent advancement in sample preparation (Seer's Proteograph™ Product Suite), coupled with improved mass spectrometry instrument sensitivity and speed, enable the quantification of thousands of proteins from plasma without compromising throughput or reproducibility, creating a unique opportunity to detect robust protein biomarkers for complex diseases[1]. Here we evaluate the performance of label-free Data Dependent Acquisition (DDA) and TMT multiplexing methods with a set of control plasma samples processed with Proteograph Product Suite for deep plasma proteomic analysis.

### Proteograph Product Suite

Seer core technology provides unbiased, deep, and rapid proteomics at scale

**From sample to peptides, ready for analysis on most LC-MS instruments with a variety of proteomics methods including; label-free and TMT workflows**

## Methods

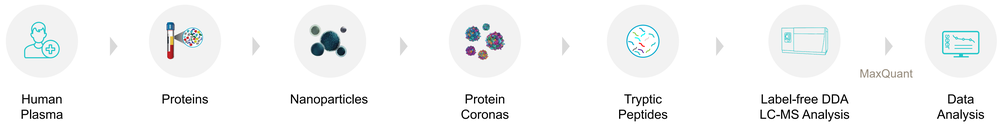
### Label-Free DDA Analysis

A control pooled human plasma sample was processed with two different Proteograph automation instruments SP100, on two separate days each, resulting in a total of 4 batches (plates). Each batch contained 16 replicates of the control pooled plasma proteins enriched with 5 nanoparticles to produce 80 total wells of tryptic digested and desalted peptides for downstream LC-MS analysis. Tryptic peptides from 6 of the 16 total replicates were analyzed using a Thermo Fisher Scientific Orbitrap Exploris 480 Mass Spectrometer in DDA mode with a 30-minute LC gradient (15 hours per batch of 6 replicates). LC-MS data files were processed using MaxQuant, with 1% FDR at the protein and peptide levels.

### TMT Analysis

Two different control pooled human plasma samples were processed with Proteograph automation instruments, SP100 in 4 batches prepared on 4 different days. Tryptic peptides enriched with 5 nanoparticles were pooled together in one fraction and labeled with one of the TMTpro™ 16plex reagents followed by peptide fractionation (24 high pH RP fractions) and LC-MS analysis on a Thermo Fisher Scientific Orbitrap Fusion Lumos Tribrid Mass Spectrometer and a FAIMS Pro Interface. LC-MS analysis were performed with a 48-hour workflow for 16 samples analysis, with 2-hr LC separation and 3 CV (compensation voltage) FAIMS peptide separation.

**A Label-Free DDA Workflow**




**B TMTpro 16plex Workflow**

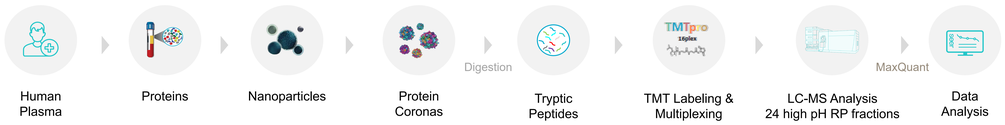



**Figure 1. Label-free DDA and TMTpro 16plex plasma proteomics workflows with Proteograph Product Suite.**

A) In the label-free DDA workflow, 16 replicates of a pooled human plasma sample (PC5) were processed through the Proteograph sample preparation workflow on SP100 instrument, using a panel of five nanoparticles. After processing, a 96-well plate with digested peptides is ready for LC-MS analysis. B) In the TMTpro 16-plex workflow, 4 aliquots of pooled human plasma sample (PC3) and two aliquots of pooled human plasma sample (PC5) were processed on 4 and 2 plates respectively on 4 different days on same Proteograph automation system , SP100. Each of 4 PC3 processed peptides were aliquoted in 3 vials (total 12 samples) and each of 2 PC5 processed peptides were aliquoted in 2 vials (total 4 samples), resulting in 16 samples for peptide labeling with Thermo Scientific TMTpro 16plex reagents. Labeled peptides were pooled into a single multiplexed pooled sample for LC-MS analysis. Peptides were fractionated by high pH RP chromatographic separation into 24 fractions and each fraction was analyzed with a 3 CV FAIMS, MS2 method for TMT LC-MS analysis.

## Results

### Label-Free DDA Data & Results

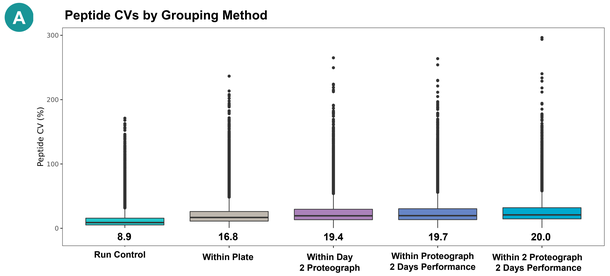



**Figure 2. Peptide and protein groups intensity CVs across two SP100 instruments and days.**

Peptide and protein groups intensity CVs, with four different grouping methods: within plate (batch), within day across SP100 instruments, within SP100 across days, and between days and SP100 instruments. For reference, LC-MS run controls intensity CVs are plotted at the left most box which represent the variability in LC-MS performance during the batch runs. The CV values show the overall distribution of CVs computed within each of the nanoparticles. A) The median peptide intensity CVs are at or below 21% across the grouping methods. B) The median protein groups intensity CVs are all at or below 20% across the grouping methods.

### TMTpro 16plex Data & Results

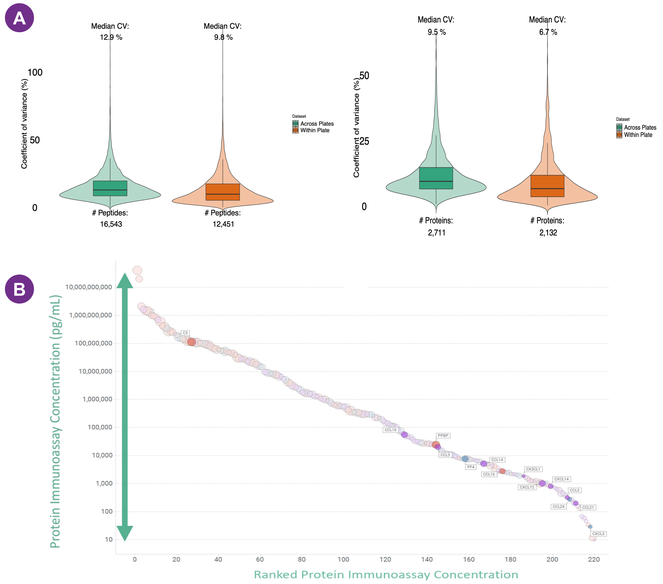



**Figure 3. Peptide and protein groups identifications and intensity CVs with SP100 across plate and days.**

A) Peptide and protein groups intensity CVs computed for two different control pooled plasma samples within and between batches (plates) for the TMTpro 16plex runs. Peptide and protein groups CVs are below 13% and 10%, respectively, across plates and a few points lower within plates showing an overall high degree of reproducibility across ~16,000 peptides and ~2700 proteins. B) Proteins from the TMT experiments were mapped to the Human Plasma Proteome Project (HPPP) protein database[2] showing detection across the entire concentration range of the database spanning 9 orders of magnitude.

## Conclusions

- The median CV (%) of the entire workflow including SP100 sample preparation and LC-MS analysis is ~20% within & across 4 batches for reported label-free DDA workflow and < 10% for TMT workflow within & across batches.
- We have detected plasma proteins covering 9 orders of magnitude dynamic range reported in HPPP.
- In summary, the Proteograph Product Suite enables rapid, automated and reproducible sample preparation for deep plasma proteomic analysis for large cohort plasma proteomics studies.

## References

[1] Blume et al. *Nat. Comm.* (2020)
[2] Schwenk, et al. *Journal of Proteome Research.* (2017)

Publications

Seer, Inc., Redwood City, CA - *rbenz@seer.bio