Human Plasma Sample Preparation Workflow Using the Seer Proteograph™ Product Suite

Introduction

Proteins are functional drivers of biology and key indicators of homeostasis in living organisms. While proteomics provides more mechanistic insights into the state of an organism than genomics, proteomic studies have been limited in scale due to challenges with accessibility, reproducibility, and workflow complexities. Blood plasma, an easily accessible biofluid that interacts with many tissues across the human body, is an attractive target for proteomics analysis. However, due to the large dynamic range of protein concentrations, interrogating the plasma proteome in depth has only been addressed by laborious, low throughput, and non-scalable workflows.[1]

The Proteograph Product Suite was developed to address these limitations by enabling high-throughput, in-depth plasma proteome identification and quantification. A panel of five proprietary engineered nanoparticles (NPs) with distinct physicochemical properties allows sampling of plasma proteins across the wide dynamic range of the proteome without compromising relative quantitation, enabling unbiased biomarker discovery. Seer's NP-based approach with the Proteograph Product Suite enables unbiased, deep, rapid proteomics at scale with higher reproducibility than conventional, manual deep proteomics methods.

Here, we describe the Proteograph Product Suite and workflow for complete plasma proteomics sample preparation using the SP100 automation instrument. We demonstrate the baseline comparison to neat plasma proteomics analysis using a standardized liquid chromatography–mass spectrometry (LC–MS) method on two MS instrument platforms for discovery proteomics: the timsTOF Pro (Bruker) and the ORBITRAP EXPLORIS 480 (Thermo Fisher Scientific). Finally, we will describe the cross-plate and cross-site reproducibility of the workflow, demonstrating the platform's power for unbiased, deep, and rapid proteomics at scale.

Previous studies have shown that NPs provide access to low abundance proteins, enabling identification of new biomarkers from samples such as blood plasma.[2,3] With the Proteograph technology, labs will be able to perform deep proteomics at scale, gaining more insights to biology than ever before.

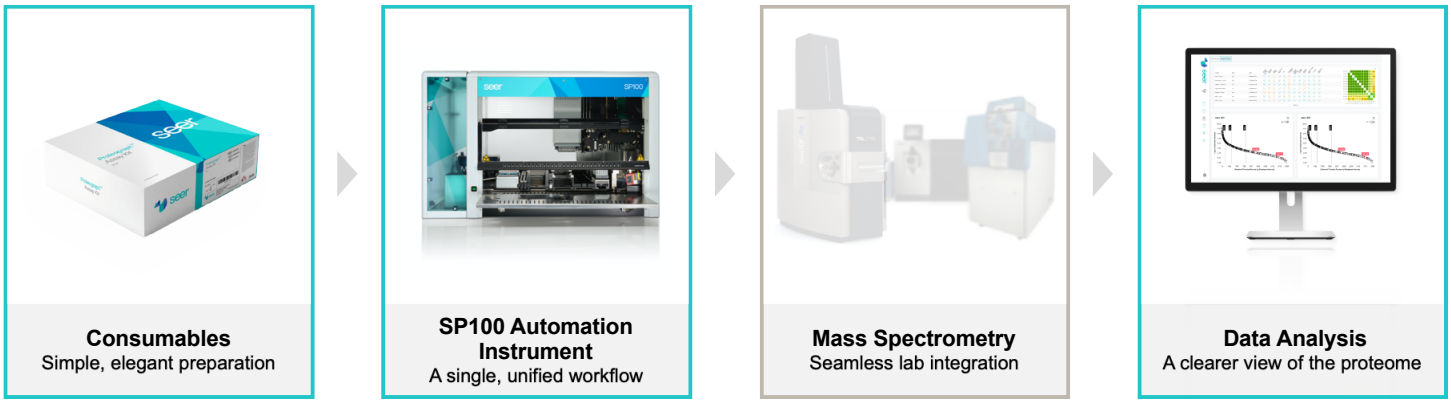

Figure 1. The Proteograph Product Suite. Consumable reagents and nanoparticles are supplied for sample preparation, carried out on the SP100 automation instrument. The final peptide product is compatible with any proteomics-capable LC–MS system, allowing for seamless lab integration. The Proteograph™ Analysis Software allows for downstream analysis of samples, group analysis for biological insight and visualization of assay controls and results files compatible with existing advanced informatics toolkits.

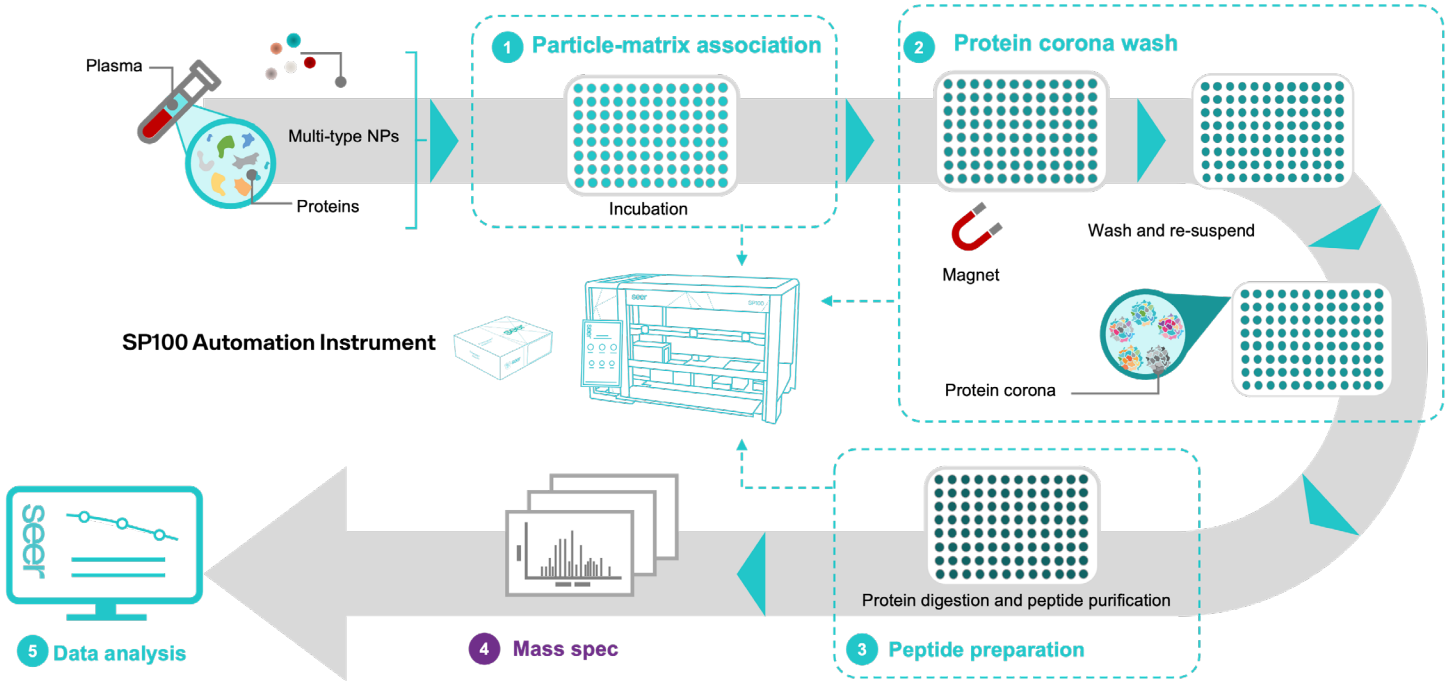

Figure 2. The Proteograph workflow. Upon addition of plasma to Seer's nanoparticles (NPs), a stable and reproducible protein corona is formed based on the particle physicochemical properties (1). Corona-containing NPs are pulled down and washed, taking advantage of the paramagnetic core (2). Proteins are denatured, reduced, alkylated, and digested directly on the particles using a standard one-pot preparation, resulting in tryptic peptides released into the supernatant. The resulting peptide mixture is then desalted using solid-phase extraction on the SP100 (3). Peptides are quantified, and then dried and resuspended before injection onto an LC-MS system (4). LC-MS data can be transferred directly to the Proteograph Analysis Suite for protein identification, quantification, and other biological insights (5).

Proteograph Product Suite

The Proteograph Product Suite (Figure 1) consists of four components, three of which are provided by Seer. The Proteograph™ Assay kit is a consumables package containing plastics and reagents, including controls, for each step of sample preparation and analysis. The steps needed to go from plasma to MS-ready peptides are automated and take about 30 minutes of hands-on time, followed by 6.5 hours of hands-off assay time. The resulting peptides are compatible with most modern proteomics LC-MS instrument setups, allowing for seamless lab integration. Raw LC-MS data files can be uploaded directly off the instrument to the Proteograph Analysis Suite—a cloud-based, scalable data analysis platform—using an optional automatic upload to allow even novice proteomics scientists to rapidly assess assay performance and interrogate their proteomics data for new biological insights.

Proteograph Assay

The full workflow consists of five steps (Figure 2), and the main assay is completed in the first three steps.

Step 1: Particle-Matrix Association

40 µL aliquots of each plasma sample are mixed with each of five NPs aliquots included in the Proteograph Assay Kit. Samples (16 samples/plate) are plated across rows and NPs are plated down columns (five NPs) for a total of 80 reaction wells in an intuitive plate layout, including controls for each stage of the process (Figure 3). 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.

Step 2: Protein Corona Wash

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.

Step 3: Peptide Preparation

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. Immediately after peptide elution, an optional peptide quantitation assay fully automated on the SP100 (using the Pierce Fluorescent Assay Kit, p/n 23290) can be used to determine the peptide yield for each well. The plate is then dried down in a SpeedVac (3 hours – overnight), and the resulting dried peptides can be stored at -80 °C or directly analyzed by LC-MS.

Step 4: LC-MS Analysis

Using the results from the peptide quantitation assay, peptides are reconstituted to their final desired concentration. Up to 1000 ng of tryptic peptides are available for each LC-MS injection, with at least 2 injections of 500 ng from each assay well.

Step 5: Data Analysis with PAS

The Proteograph Analysis Suite (PAS) can be used for analysis of the resulting LC-MS data files. The software package includes an experiment data management system, analysis protocols for both data-independent acquisition (DIA) and data-dependent acquisition (DDA) modes with industry standard algorithms, an analysis setup wizard, and tools for reviewing and visualizing results. This cloud-scalable solution can handle a large number and size of data files, reducing the time needed to go from data acquisition to biological insights.

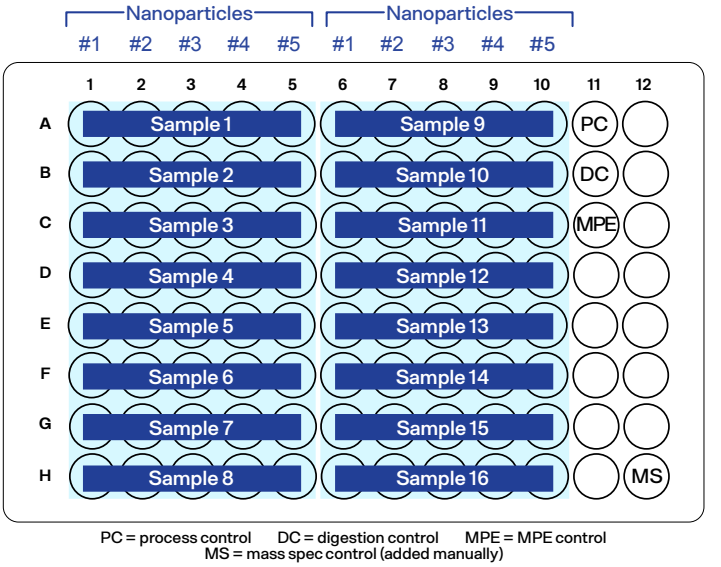

Figure 3. Proteograph Assay Plate layout. PC5 plasma peptides prepared with the SP100, and neat plasma digests, were analyzed using 30-minute DIA methods on both the ORBITRAP EXPLORIS 480 MS and timsTOF Pro mass spectrometers.

Figure 4. Simplified QC Assessment and Performance Monitoring. PAS provides automatic quality control evaluation of LC-MS data. Available QC metrics include protein and peptide groups counts and intensities, peak width, retention time, TIC, sequence coverage for FASTA-based searches, missed cleavage rate, peptide quant, and identification rates.

Proteograph Assay Controls

The fully automated Proteograph assay provides four internal controls, three of which are built-in on the assay plate, to assess the performance of each step of sample preparation and LC-MS analysis (Figures 3 and 4). QC performances can be monitored in PAS with actionable measures. This guarantees successful completion of large-scale plasma proteomics studies with consistent analytical performance across instruments and sites.

1. Process Control (PC): Seer control plasma is mixed with NP1 in position A11. This serves as a control for the entire assay workflow.
2. Digestion Control (DG): Diluted neat Seer control plasma is digested in well B11. This is a control for the protein digestion process through peptide cleanup.
3. MPE Control (MPE): A purified, neat peptide mixture from the Seer control plasma is added to well C11 and mixed with the digestion reagents. This is the control for the peptide cleanup process.
4. Mass Spec Control (MS): A separately provided aliquot of digested plasma peptides, same as the MPE control, can be added to the plate immediately prior to LC-MS analysis. This control adds a layer of confidence to the LC-MS setup's performance prior to analyzing precious samples.

Methods

PC5 pooled peptide control material consists of pre-pooled K_2EDTA plasma from ProMedDx (Norton, MA) derived from healthy subjects.

For LC-MS analysis, peptides were loaded on an Acclaim PepMap 100 C18 (0.3 mm ID x 5 mm) trap column and then separated on a 50 cm µPAC analytical column (PharmaFluidics, Belgium) at a flow rate of 1 µL/minute using a gradient of 5 – 25% solvent B (0.1% FA, 100 % ACN) in solvent A (0.1% FA, 100% water) over 22 minutes, resulting in a 33 minute total run time. For analysis on the ORBITRAP EXPLORIS 480 mass spectrometer, 150-400 ng of material per NP was analyzed in DIA mode using 10 m/z isolation windows from 380-1000 m/z. MS1 scans were acquired at 60k resolution and MS2 at 30k resolution. For the timsTOF Pro, 200 ng of material per NP was analyzed in diaPASEF mode using ion mobility range of 0.57 – 1.47 V.s/cm^2 with 100 ms accumulation time.

The DIA data from both instruments were analyzed with DIA-NN using the default settings in PAS, with a spectral library-free approach based on the Uniprot Human FASTA database.[4]

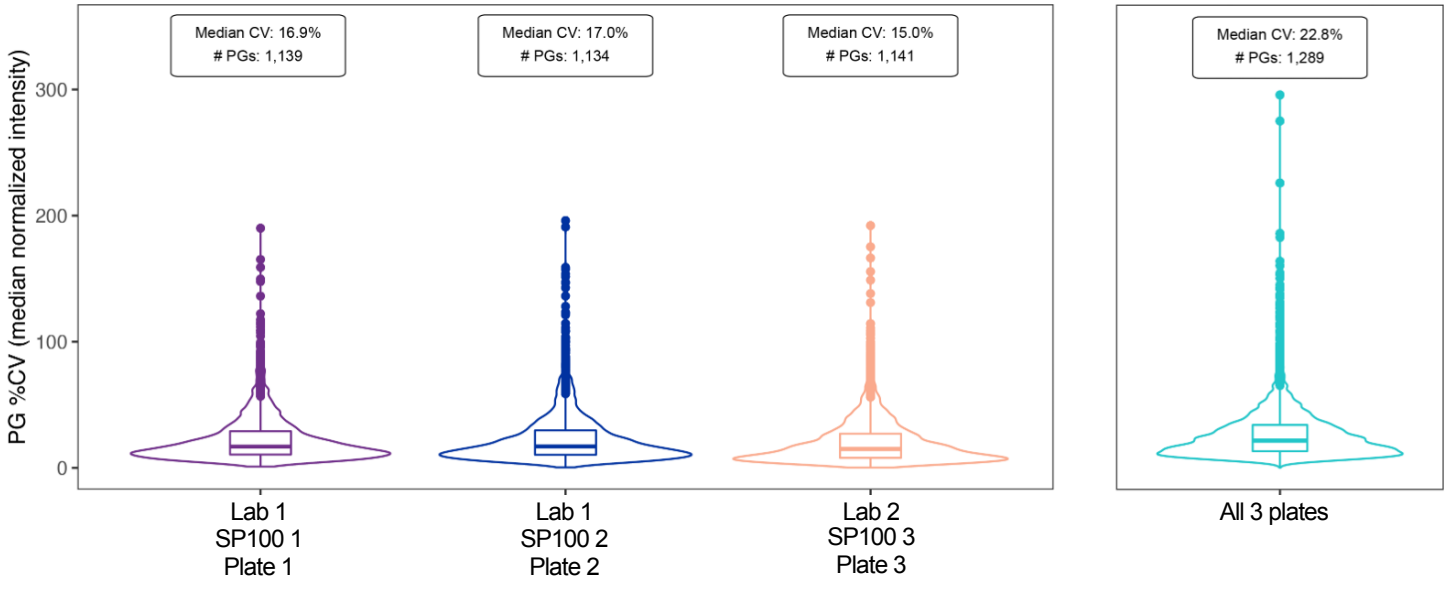

Figure 5. Quantitative reproducibility of samples. Biosample PC5 was run in four preparative replicates on three different plates and SP100 instruments in two different labs in the United States. The samples were analyzed on the same timsTOF Pro LC-MS setup at Seer. The number of PGs (protein groups) represent the total number of unique PGs withinn each plate. For each plate, protein group analysis showed quantitative median CVs of ≤17%. When analyzed together, the total multi-site experimental CV showed a median %CV of <25% within the required range for large-scale proteomics studies.

Instrument	Sample	Peptides	Protein Groups
ORBITRAP EXPLORIS 480 30-min. DIA	PC5 (Neat)	2643 ± 28	498 ± 4
	PC5 (5NP)	7751 ± 12	1600 ± 7
timsTOF Pro 30-min. DIA	PC5 (Neat)	2408 ± 36	524 ± 8
	PC5 (5NP)	6845 ± 24	1588 ± 4

Table 1. In a separate experiment, PC5 plasma peptides prepared with the SP100 and neat plasma digests were analyzed using 30-minute DIA methods on both the ORBITRAP EXPLORIS 480 MS and timsTOF Pro mass spectrometers. Values are shown as peptides and protein groups, +/- standard deviation of 4 preparative replicates.

Results

Unbiased, deep, and rapid proteomics at scale with Proteograph workflow

The Proteograph workflow was performed at two different labs across the United States, with each lab running either one or two SP100 instruments. On each instrument, four preparative replicates of Seer control plasma (PC5, a low complexity plasma sample) were prepared, as well as a neat PC5 plasma digest. These peptides were returned to Seer and analyzed using a 30-minute DIA method on either the ORBITRAP EXPLORIS 480 or timsTOF Pro mass spectrometer. Exemplification data demonstrating the depth of coverage on both instruments is shown in Table 1, with both instruments showing similar results on the same control plasma sample. Additionally, cross-site and cross-SP100 samples were analyzed on the timsTOF Pro only (Figure 5), demonstrating intra-plate quantitative reproducibility with CVs ≤17% and inter-plate reproducibility with CVs <25%. Together, these results show that the Proteograph workflow enables large-scale studies across multiple instruments and sites, regardless of the MS instrumentation used for data acquisition.

Conclusions

- The SP100 instrument offers a fully automated workflow for processing up to 16 biofluid samples in one day.
- Controls are included for each key step of the assay and automatically handled in the downstream analysis software.
- Peptides are ready for quantification and reconstitution with automated workflows on the SP100.
- Resulting peptides are compatible with any modern LC-MS proteomics setup, and cross-plate and cross-site data show workflow reproducibility that enables unbiased, deep, and rapid proteomics at scale.

Product Information

The Proteograph Product Suite includes the SP100 Automation Instrument, a five-nanoparticle panel with associated consumables, and the Proteograph Analysis Suite of software that easily integrates with existing LC-MS instruments from most spectrometry vendors.

Learn more at

seer.bio/product/proteograph-product-suite

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References

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