TECHNICAL NOTE

Advancing Analytical Throughput and Depth in Plasma Proteomic Studies with the Proteograph® ONE Workflow

Introduction

Precision medicine requires a deeper understanding of the molecular underpinnings driving complicated phenotypes and disease heterogeneity. Though decades of genomic studies have provided important insights, they fall short of sufficiently capturing the complexity of biological states for medical applications.[1,2] That disconnect occurs because genomes and transcripts are well upstream of their gene products and the phenotypes they create.[3,4,5] Since proteins act as direct effectors of cellular function, proteomic profiling offers a more accurate view of molecular phenotypes and their clinical outcomes.

Deep unbiased mass-spectrometry (MS) proteomics provides researchers with the means to discover unknown protein associations and to uncover new functional insights on disease biology as well as individual response to stimuli like therapeutic treatments. Furthermore, MS proteomics provides peptide-level information, which facilitates the detection of more subtle protein variations with clinical relevance, like single amino acid substitutions, splice variants, and post-translational modifications.[3,6]

Though unbiased MS workflows have long been understood as the gold standard for proteomic studies, the challenging balance between depth, throughput, and reproducibility has historically hindered its broad adoption in large scale clinical biofluid studies.

Conventional approaches to MS proteomic analysis struggle to achieve deep protein detection in biofluid samples that exhibit a wide dynamic range of protein concentrations.[7] In these cases, highly abundant proteins can obscure the detection of less abundant yet relevant ones. For example, blood plasma, perhaps the most clinically relevant biofluid, exhibits a dynamic range greater than 12 orders of magnitude.[8] In fact, 22 proteins make up 99% of plasma protein by weight, and albumin alone accounts for 50%.[9]

Sample preparation methods such as depletion and fractionation that address the problems of dynamic

range have historically been manual and slow, drastically limiting their utility in large-scale studies. They also often introduce additional pre-analytical variability and decrease reproducibility.[10,11] Inconsistent sample prep has been shown to have a sizable impact on plasma proteomics study results.[12]

Seer's Proteograph® Product Suite addresses these challenges through its proprietary engineered nanoparticle (NP) technology, which enables rapid, deep characterization of biofluid proteins across wide dynamic ranges.[3,13,14] The Proteograph platform has consistently provided unmatched proteomic depth, excellent quantitative precision and linearity, and the most sensitive limits of quantification observed in comparative studies of academic and commercial proteomics approaches.[7,11,15,16] With the Proteograph Product Suite, researchers can perform deep, reproducible plasma proteomic studies at scale to detect and quantify clinically relevant protein biomarkers.

The Proteograph Product Suite, featuring the Proteograph ONE Assay, enables deep, reproducible, and unbiased proteomic analysis in just one MS injection. Its streamlined automated workflow enables processing up to 80 samples per run with sample to peptide preparation in less than 5 hours.

Here, we assess the performance of the Proteograph ONE workflow through comparison with manual direct digest workflows using two different MS systems: the Orbitrap™ Exploris™ 480 and Orbitrap Astral™ mass spectrometers

from Thermo Fisher Scientific. In addition, we investigate the cross-day, cross-plate, and cross-instrument reproducibility of the Proteograph ONE workflow to further demonstrate its utility for deep unbiased proteomics at scale.

Proteograph Product Suite

The Proteograph Product Suite (Figure 1) includes the Proteograph ONE Assay kit, the SP200 Automation Instrument, and the Proteograph Analysis Suite (PAS).

The Proteograph ONE Assay kit is a consumables package containing Seer proprietary engineered nanoparticles, reagents, plasticware, and controls for MS preparation of 40 or 80 samples at a time.

The SP200 Automation Instrument works seamlessly with the assay kit to perform automated plasma sample processing and peptide preparation for MS analysis, including optimized methods for desalting, quantification, and reconstitution. All told, the Proteograph ONE workflow on the SP200 Automation Instrument requires approximately 60 minutes of hands-on set-up time for the 80-sample configuration and and completes automated processing of samples to MS-ready peptides in less than 5 hours. The resulting peptides can be analyzed with most LC-MS instrument ecosystems, allowing for easy lab integration.

Following MS runs, researchers can directly upload their raw LC-MS data files to the Proteograph Analysis Suite, Seer's cloud-based analysis software, using its AutoUploader tool to process their MS results. PAS can handle large proteomics datasets much faster than other analysis approaches, leveraging popular open-source search and quantification tools. In turn, this allows novice and expert proteomics researchers alike to rapidly assess performance and perform interrogations of proteomics data to extract biological insights.

The Proteograph ONE Workflow

The Proteograph ONE workflow (Figure 2) consists of five steps. The first steps, nanoparticle-protein interaction and corona formation, protein corona wash, and peptide preparation, are automated within the SP200 Automation Instrument. The final steps, mass spectrometry (MS) and data analysis, occur outside the instrument.

Step 1: Protein corona formation

First, 120 µL of each plasma sample is aliquoted into a well of a 96-well plate. The SP200 instrument transfers 100 µL from each well into a second 96-well plate. The initial 120 µL volume allows for enough dead volume for the SP200 instrument to ensure consistent sample transfer from each well. Experimental controls are auto-loaded onto the second plate to monitor the performance of each stage of the sample processing (See *Proteograph Assay Controls*).

Each well is incubated with Proteograph ONE NPs for one hour to allow for corona formation. At first, the highest-abundance proteins bind to the NPs. Seer has tuned the physicochemical properties of the Proteograph ONE NPs to lower their affinity to high-abundance plasma proteins. Over time, the protein corona and plasma proteins reach an equilibrium , where higher affinity, lower abundance proteins and proteoforms displace high-abundance proteins in a reproducible manner.

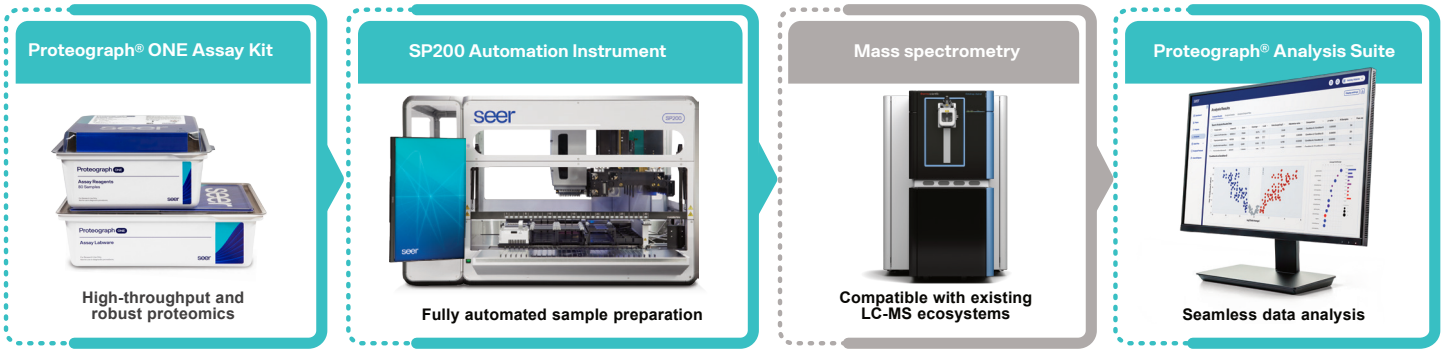

Figure 1. The Proteograph ONE Workflow. The Proteograph ONE Assay kit includes reagents and labware for either 40-sample or 80-sample processing, which is carried out on the SP200 Automation Instrument. The final desalted peptides are compatible with analysis with any bottom-up proteomics method on a variety of LC-MS systems, allowing for seamless lab integration. The Proteograph Analysis Software allows for downstream peptide and protein identification, group analysis for biological insight, visualization of assay controls, results files compatible with existing advanced informatics toolkits, and proteogenomics analysis. The components provided by Seer are highlighted in teal.

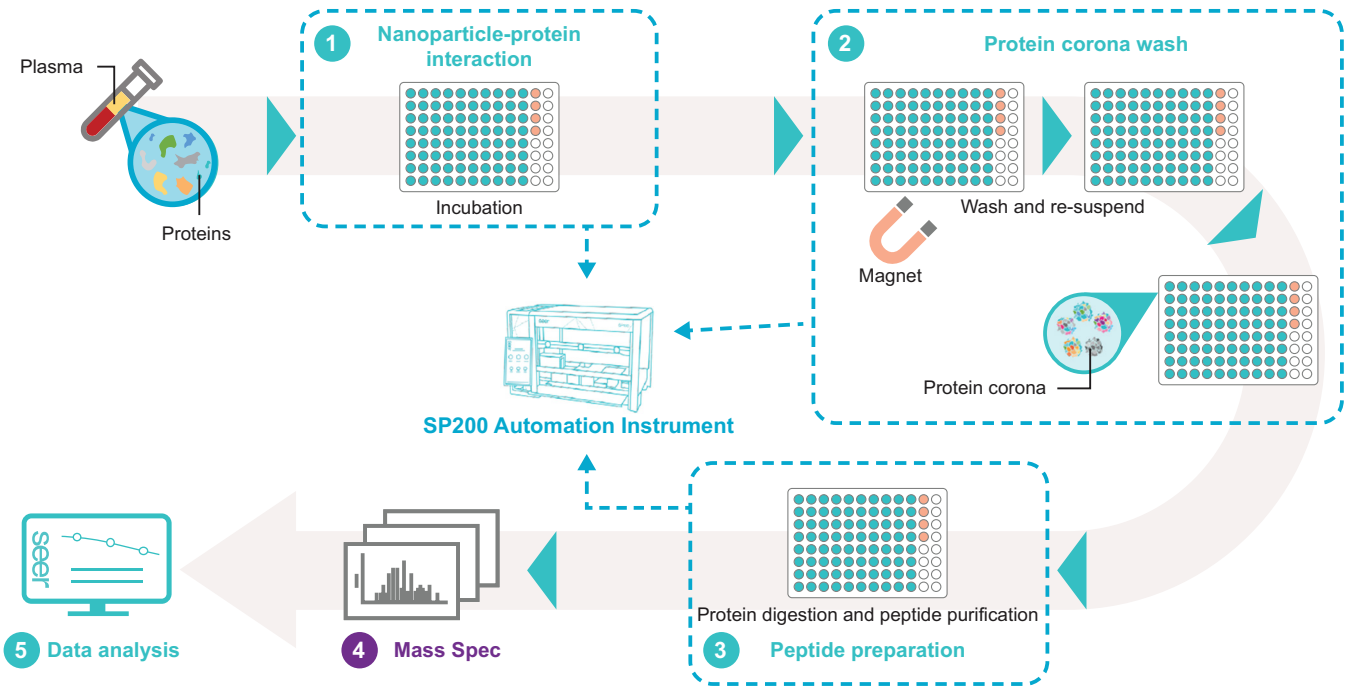

Figure 2: Proteograph ONE Workflow Schematic. **(1)** Upon addition of biofluid to Seer's nanoparticles, a stable and reproducible protein corona is formed based on the particle's physicochemical properties. **(2)** Corona-containing NPs are pulled down and washed, taking advantage of the superparamagnetic core. **(3)** Proteins are then denatured, reduced, alkylated, and digested directly on the particles using a standard one-pot sample preparation workflow, resulting in tryptic peptides released into the supernatant. The resulting peptide mixture is then cleaned up using solid phase extraction – all above steps taking place on the SP200 Automation Instrument. Peptides are then quantified using a fluorescence spectrometer and dried. When ready for MS analysis, the peptides are resuspended on the SP200 Automation Instrument before injection onto a **(4)** LC-MS system. **(5)** LC-MS data can be transferred directly to the Proteograph Analysis Suite for peptide and protein identification, quantification, and other biological insights.

Step 2: Protein corona wash

Once the incubation ends, a magnetic field is used to accumulate the superparamagnetic NPs to allow for a series of gentle washes that remove non-specific and weakly bound proteins as well as other contaminants. The resulting specific protein corona ultimately enables deep coverage of the plasma proteome.

Step 3: Automated Peptide Preparation

In a single reaction, NP-bound proteins are reduced, alkylated, and digested with Trypsin/Lys-C directly on the Proteograph ONE NPs to generate tryptic peptides for downstream LC-MS analysis. A second proprietary engineered particle solution aggregates the peptides to clean up and desalt the digestion mixture. Seer designed this particle-based step to explicitly improve peptide clean-up.

The resulting peptides are eluted from the particles into a collection plate using an organic solvent/water mixture.

After completing peptide elution on the SP200 Automation Instrument, a Pierce™ Fluorescent Assay kit (Thermo Fisher Scientific, 23290) is used to determine the peptide yield from each well. The peptides are then dried down in a SpeedVac (3 hours to overnight). At this point, the resulting dried peptides can be moved to the next step or can be stored at -80 °C for later analysis.

Step 4: Mass Spectrometry

Using the peptide quantification assay results, the SP200 Automation Instrument reconstitutes the peptides to their final desired concentration. The samples are now ready for analysis and compatible with most LC-MS instrument systems, allowing for seamless lab integration. On average, the assay kit yields ~1200 ng per sample of tryptic peptides, supplying enough material for at least two injections from each well. The second injection volume serves as a backup in case any sample must be reinjected due to MS sampling issues.

Step 5: Data Analysis

Once LC-MS data collection is complete, researchers can use the Proteograph Analysis Suite (PAS) to process raw data files and perform bioinformatic analysis. This cloud-scalable solution can handle a large number and size of data files with only minor increases in computation time, which greatly accelerates access to biological insights. The PAS software includes:

- Tools to organize your experiments and data files
- Intuitive step-by-step data analysis setup wizards
- Analysis protocols for both data-independent acquisition (DIA) and data-dependent acquisition (DDA) modes using industry standard search engines
- Pre-configured visualizations for rapid reporting.

The latest PAS version also includes DIA-NN protocols with improvements for data processing.

Proteograph Assay Controls

In addition to one user-provided control, the fully automated Proteograph ONE workflow includes three Proteograph assay controls in each plate to assess the performance of each sample preparation step. Those internal controls are:

1. **Process Control**: Lyophilized undigested plasma proteins that are reconstituted and run through the full assay.
2. **Digestion Control**: Lyophilized undigested plasma proteins that are reconstituted and run from digestion onward.
3. **Cleanup Particles Control**: Lyophilized bulk digested plasma proteins that are run through the cleanup steps only.

Measuring the control peptide yields allows users to actively monitor performance over time, allowing for rapid issue identification and intervention as needed. If the process control peptide yield falls within its acceptable variation range,* users know the workflow was executed correctly. If not, users can compare the variations of the three controls to determine where non-optimal performance occurred.† Closely tracking control yield variation across plates helps ensure that large-scale plasma proteomics studies generate consistent analytical performance across days, users, instruments, and sites.

Methods

Plasma Samples

Sixteen individual human plasma samples, each comprised of plasma pooled from three to five genetically distinct individuals, were processed on a Proteograph ONE Assay plate to assess the depth of protein coverage compared to samples prepared using a direct plasma digestion workflow.

Four different control plasmas consisting of K_2EDTA plasma from BioIVT (HUMANPLK2-0101355 and HUMANPLK2-0101354) were used to evaluate the performance of the Proteograph ONE workflow.

For plasma spike-in studies, K_2EDTA bovine plasma was sourced from Innovative Research (IGBOPLAK2E500ML).

Sample Prep

Peptides generated from four 40-sample and four 80-sample Proteograph ONE Assay kits were prepared on two separate SP200 Automation Instruments. This evaluation was conducted over four days to evaluate Proteograph ONE Assay reproducibility across plates, days, and SP200 Automation Instruments.

For the manual direct digest workflow, 10 µL of each plasma sample was diluted from the corresponding enriched sample into 240 µL of TE buffer (25X dilution). The diluted samples were then added to the preparation plate directly after corona washing prior to digestion.

Liquid Chromatography & Mass Spectrometry

When comparing the Proteograph ONE and direct digest workflows, two different LC-MS instrument setups were used:

1. Thermo Fisher Scientific™ Orbitrap™ Exploris™ 480 Mass Spectrometer coupled with an Ultimate™ 3000 nanoLC system.
2. Thermo Fisher Scientific Orbitrap™ Astral™ Mass Spectrometer coupled with a Vanquish™ Neo UHPLC system.

For analysis on the Orbitrap Exploris, 8 µL of 0.06 µg/µL peptides were loaded on an Acclaim PepMap 100 C18 (0.3 mm ID x 5 mm) trap column and then separated on an Ultimate 3000 HPLC System and a 50 cm µPAC HPLC column (Thermo Fisher Scientific) at a flow rate of 1 µL/min using a gradient of 5 to 25% solvent B (0.1% FA, 100 % ACN)

* Please note that this range can vary depending on the type of plate reader and lot used.
† Seer Field Application Scientist team will also help first-time users interpret the control results.

in solvent A (0.1% FA, 100% water) over 22 minutes. This resulted in a 33-minute total run time on the Thermo Fisher Orbitrap Exploris™ 480 mass spectrometer. LC-MS analysis was done in DIA mode using 10 m/z isolation windows from 380-1000 m/z. MS1 and MS2 scans were acquired at 60K and 30K resolution, respectively. The DIA data were analyzed with DIA-NN (v1.8.1) using standard settings with a spectral library-free approach based on the Uniprot Human FASTA database.

For analysis on the Orbitrap Astral, identical trap column, column, and flow rates were used. For sample pickup, 8 µL of 0.05 µg/µL peptides (400 ng) were loaded. Total gradient from 0 to 35% solvent B (0.1% FA, 100% ACN) in solvent

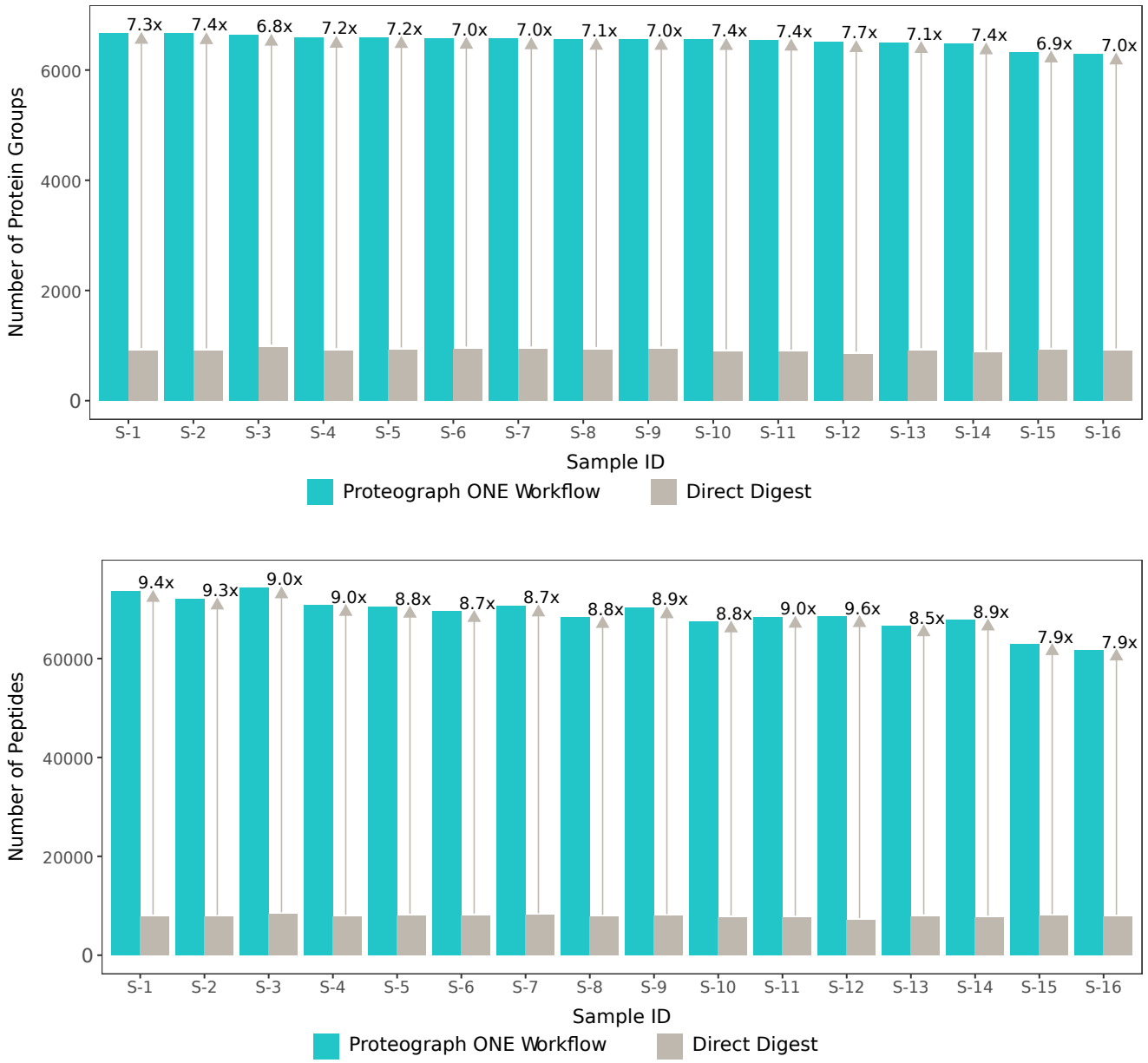

Figure 3: Proteomic Depth by Plasma Sample for Both the Proteograph ONE and Direct Digest Workflows.
Evaluation of protein and peptide identification rates and discovery depth was conducted by processing 16 plasma samples through Proteograph ONE and direct digestion workflows, respectively. The number of protein group (**top**) and peptide IDs (**bottom**) are shown, with fold-improvement between Proteograph ONE and direct digestion workflows plotted directly. The Proteograph ONE workflow detects between 6.8X – 7.7X more protein groups, and 7.9 X – 9.6X more peptides compared to the direct digestion workflow for this set of samples.

A (0.1% FA, 100% water) over 20.8 minutes, resulting in a 24-minute total run time on the Astral™ mass spectrometer. LC-MS analysis was done similarly to the Exploris™ 480 methods, with the following exceptions: Data was acquired in DIA mode, with a 3 m/z isolation windows from 380–980 m/z. MS1 scans were acquired at 240K resolution with an AGC target set at custom at 500%, RF lens set at 40%, and collision energy set at 25%. The DIA data were analyzed with DIA-NN (v1.8.1) using the same search parameters described in the Exploris 480 methods.

ELISA

All ELISAs were run using the Quantikine™ kit (R&D Systems) following the manufacturer's protocol. A seven-point standard curve was generated between 0.78 ng/mL and 50 ng/mL. Plasma samples were diluted 50X and run at 450 nm on a Molecular Devices SpectraMax M2 microplate reader.

The initial ELISA tested seven human plasma samples, four pools, and three individuals for selection for the spike-in assay. The plasma sample with the lowest endogenous CRP concentration, IPS4-1 at 1.23 µg/mL, was chosen as the spike-in candidate. A 4-PL model was used on the prepared CRP standard curve comparing absorbance to concentration to determine sample CRP concentration.

A 9.3 mg/mL CRP (Millipore Sigma) stock solution was diluted 5X and 10X for use as the spike-in to IPS4-1 plasma to generate 2X, 5X, 10X, and 100X CRP-spiked plasma samples. The spiked-in plasma samples were then run on MS with eight reps each from both Proteograph ONE and direct digest samples. Four reps of the assay plate were run on the Astral and Exploris MS instruments following methods laid out in earlier method sections.

A confirmatory ELISA was run on spike-in samples with two reps of each. For each spike-in level, two dilutions were made with two reps for each dilution. These were averaged to determine CRP concentration. Dilutions of up to 1:10000 were made to bring spike-in levels within the linear range of the ELISA assay.

Using the dilution ratios and a 4-PL model based on the standard curve, CRP concentrations were back-calculated for the spike-in samples. ELISA CRP concentrations and MS abundance of select CRP protein peptides were translated to $\log_2$. Log_2 MS abundance was plotted against the $\log_2$ CRP protein concentration as determined by ELISA.

Results

Depth of Proteome Coverage of the Proteograph ONE Workflow

To assess the depth of proteome coverage, we compared the number of detectable unique plasma protein groups and peptides found using the Proteograph ONE and direct digest workflows across a set of sixteen different pooled plasma samples. The Proteograph ONE workflow detected significantly more proteins and peptides than direct digest for all sixteen pooled plasma samples (Figure 3). The

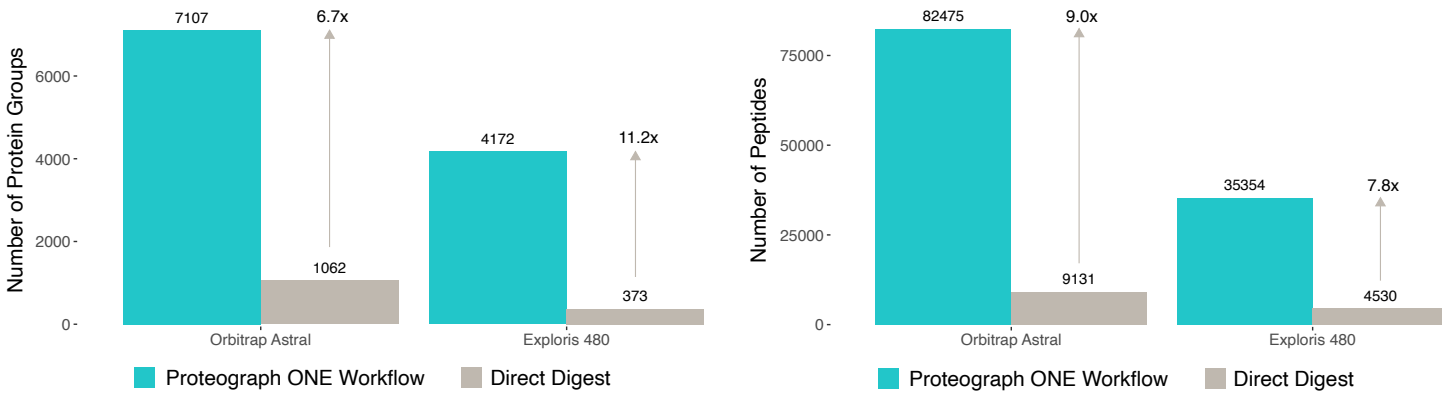

Figure 4: Proteomic Depth by MS Instrument for Both the Proteograph ONE and Direct Digest Workflows.
Evaluation of protein and peptide identification rates and discovery depth was conducted by processing 16 plasma samples through Proteograph ONE and direct digestion workflow, respectively. The total number of Protein groups (**left**), and Peptide IDs (**right**) were collected and reported, with fold-improvement between Proteograph ONE and direct digestion workflows plotted directly with regards to two different LC-MS instrument setups used — Thermo Fisher Scientific Orbitrap Astral, and Thermo Fisher Scientific Orbitrap Exploris 480. The Proteograph ONE workflow provides a significant boost toward protein identification depth regardless of MS of choice.

Proteograph ONE workflow resulted in a ~7.2-fold average increase in unique protein groups detected compared to direct digest, measuring >7000 unique human plasma proteins across samples, (Figure 3, top). Even more starkly, the Proteograph ONE workflow detected ~8.8-fold more peptides (Figure 3, bottom).

Notably, large improvements were measured on both LC-MS setups (Figure 4). The Proteograph ONE workflow detected far more total protein groups (Figure 4, left) and peptides (Figure 4, right) across the set of sixteen pooled plasma samples than the direct digest using the Astral and Exploris 480. Its depth advantage was particularly pronounced on the Exploris 480, yielding 11X more protein groups than direct digest, compared to 6.7X using the Astral. However, on the Astral—the newer, more sensitive MS designed for deep proteomics—the Proteograph ONE workflow detected nearly 3000 more protein groups (7107) compared to those detected with the Exploris (4172). The Astral system also improved the detection of low-abundance proteins in the direct digest workflow (1062 vs 373), explaining why the fold change between the workflows is smaller compared to Exploris (Figure 4, left).

Proteograph ONE workflows also detected >4-fold more proteins cataloged in the Human Plasma Proteome Project (HPPP) with both LC-MS systems (Figure 5). Arranging HPPP proteins from most to least abundant revealed that Proteograph ONE workflow also detected HPPP proteins across a wider distribution of abundance, whereas direct digest analysis predominately detected high-abundance proteins (Figure 5, top). In fact, the direct digest detected very few proteins in the lowest quartile (Figure 5, bottom), indicating that direct digest struggles to detect important, yet

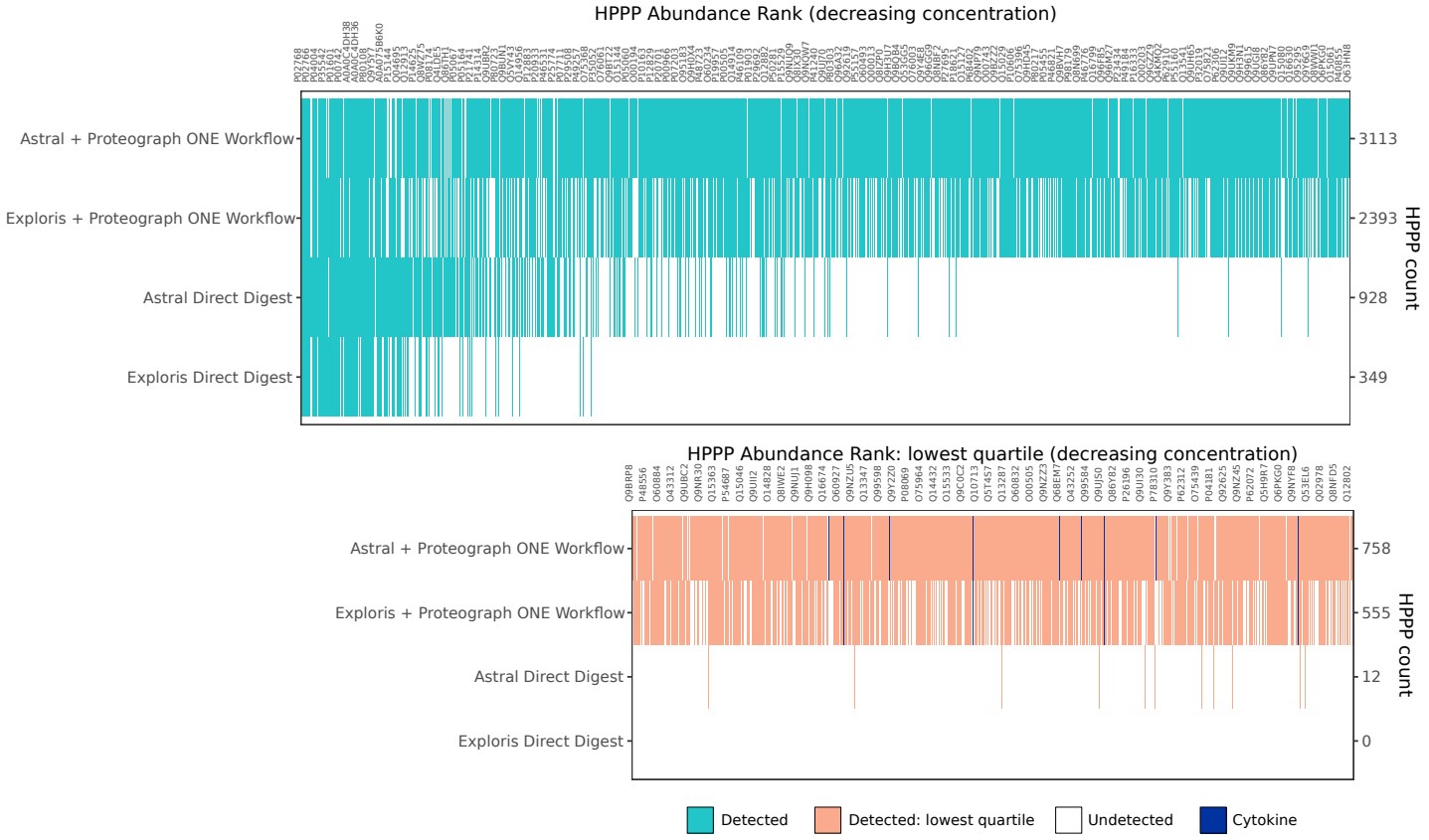

Figure 5: Evaluation of Proteomic Depth by Referencing HPPP database. **(Top)** Protein identifications from identical samples processed with (1) Proteograph ONE Workflow paired with Astral MS, (2) Proteograph ONE Workflow paired with Exploris MS, (3) Direct Digestion paired with Astral MS, (4) Direct Digestion paired with Exploris MS were mapped toward the HPPP database. The protein estimated concentrations were taken from HPPP data central (https://peptideatlas.org/hupo/hppp/) and protein concentration are rank ordered in decreasing abundance from left to right. **(Bottom)** The protein identifications are plotted for the bottom 25% concentrated proteins according to HPPP, and cytokines are plotted in blue, on the plot. Improvement in protein identifications for the least 25% abundant proteins are more pronounced compared to the entire HPPP concentration range.

low abundance proteins like cytokines (**Figure 5, bottom, dark blue**). This observation again held true regardless of the mass spectrometer used.

These results indicate that the Proteograph ONE workflow provides significantly more proteomic depth across a wide plasma abundance range than direct digests. By achieving considerably more depth, the Proteograph ONE workflow allows users to perform deep, unbiased proteomics studies that generate more complete plasma protein sequence coverage, better capturing the roles of low-abundance proteins, proteoforms, and post-translational modifications (PTMs).

Reproducibility of the Proteograph ONE Assay

To begin assessing the reproducibility of the Proteograph ONE workflow, we first measured peptide yields from identical Proteograph ONE control samples across 17 plates on multiple separate days to evaluate inter-day process consistency (**Figure 6**). Peptide yields were consistent across all measurement days within three standard deviations from the mean (**Figure 6**). These data show that the Proteograph ONE workflow provides the consistent sample processing necessary for highly reproducible data.

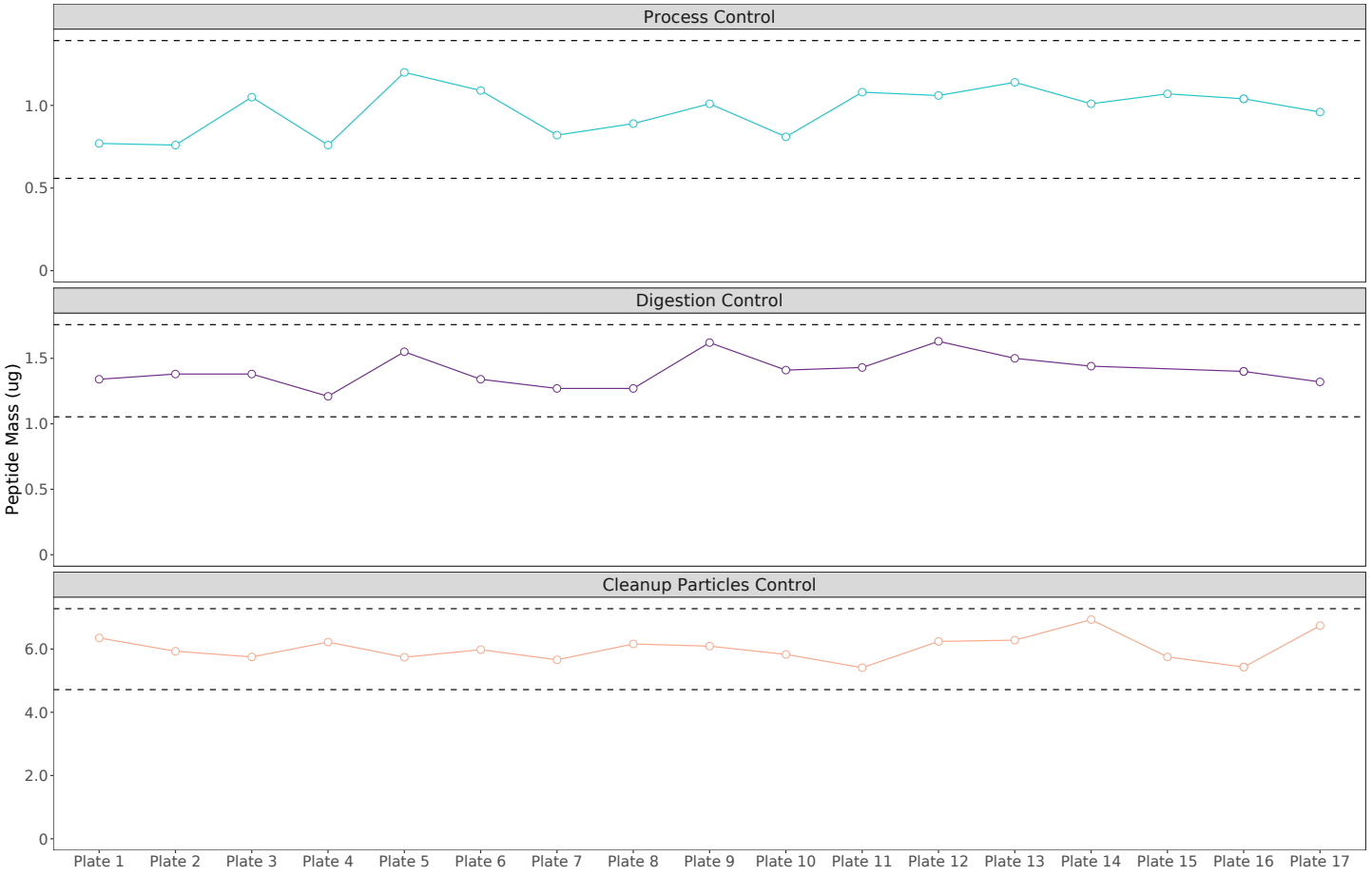

Figure 6: Built-in Controls to Monitor Instrument Performance and Demonstrate Processing Consistency Across Days. The Proteograph ONE Assay has multiple controls built in to offer user traceability to monitor SP200 Automation Instrument performance to ensure day-to-day reproducibility across months of sample processing. The peptide yield was plotted for **(top)** Process control, **(middle)** Digestion Control, and **(bottom)** Cleanup Particles Control. Controls are strategically placed during different stages of proteomic sample preparation to set up multiple checkpoints, hence, when a particular plate or sample performed under or over our specifications, the combinations of controls would allow users to better pinpoint the root cause (i.e., enzyme digestion efficiency, peptide clean-up performance, or nanoparticle corona formation quality).

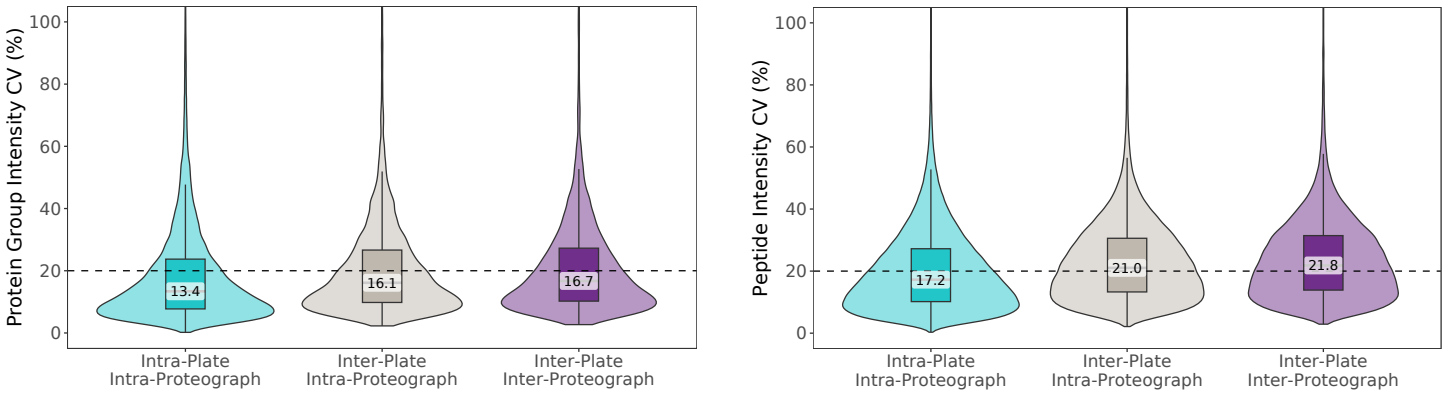

Figure 7: Proteograph ONE Reproducibility Assessment. Control pooled plasma was run in replicates on three different experimental settings: **(teal)** intra-plate, intra-Proteograph (comparison was made within the same plate and same SP200 Automation Instrument), **(gray)** inter-plate, intra-Proteograph (comparison was made with the same SP200 Automation Instrument, but different processing plates), and **(purple)** inter-plate, inter-Proteograph (comparison was made with different SP200 Automation instruments and different processing plates). The label-free intensity coefficient of variation (CV %) was plotted for (**left**) Protein groups, and (**right**) Peptides, respectively. Median CV % was plotted and annotated directly on the plot, with dotted line denoting 20% CV.

Performance was also assessed through CVs for protein groups intensity and peptide intensity. The intra-plate and inter-plate CVs for protein group intensity were 13.4% and 16.1%, respectively (**Figure 7, left**). These CVs are similar to or better than those collected with other methods and commercial platforms.[7,11,17,18] With the intra-plate CV at 17.2% and inter-plate CV at 21%, peptide data showed a similar albeit slightly elevated trend (**Figure 7, right**). This increase in CV is consistent with expectations for median peptide CV compared to median protein group CV.

To further pressure test CV results, plates were also processed by two different SP200 Automation Instruments. When combining inter-instrument analysis with inter-plate comparisons, protein group (16.7%) and peptide (21.8%) CVs remained low, only increasing slightly (**Figure 7**).

Collectively, median protein group and peptide CVs within and between plates and SP200 instruments indicate the Proteograph ONE workflow provides excellent precision and experimental reproducibility, even when varying days, plates, and instruments. These results are especially impactful because the Proteograph ONE workflow captures many more low-abundance proteins.

Quantification Accuracy of the Proteograph ONE Workflow

To evaluate the quantification performance of the Proteograph ONE workflow, we used a mixed proteome spike-in experiment using K_2 EDTA bovine plasma to assess relative fold-change accuracy.[19] Bovine plasma was spiked into human plasma to create samples with different bovine to human ratios (1:11, 1:5, and 1:3). From there, fold-changes between spiked sample pairs were measured and compared against the known values (1.5X, 2X, and 3X). The Proteograph ONE workflow accurately measured the spike-in samples, collecting observed fold-changes close in value to the actual changes (1.56X, 1.95X, and 3.09X) and those collected by direct digest (**Figure 8**).

To further vet the suite's quantification accuracy, we used ELISA as an orthogonal analytical method to examine the quantitative linearity using a spike-recovery study with C-Reactive-Protein (CRP). CRP was added to pooled human plasma samples at 2X, 5X, 10X, and 100X the endogenous level and measured by ELISA. Plotting ELISA vs Theoretical values resulted in a linear R^2 value of 0.989 (data not shown).

A portion of these same spiked samples were then processed with the Proteograph ONE workflow and analyzed by MS

to assess the correlation between MS and ELISA data. The peptide intensity data for Proteograph ONE workflow samples strongly correlated with ELISA protein concentration for four CRP peptides, with R^2 values between 0.97 and 0.98. (Figure 9, left). The Proteograph ONE workflow samples also provided comparable quantification accuracy as the direct digest workflow (Figure 9, right), given similar correlation values (R^2 between 0.97 and 0.98).

The Proteograph ONE workflow provides excellent quantification performance, supporting its capacity to detect true clinically relevant expression differences between sample groups in biomarker discovery programs.

Conclusions

The Proteograph ONE workflow enables faster, larger-scale deep proteomic studies, expanding the scope of biological discovery.

- The Proteograph ONE Assay and SP200 Automation Instrument combine to offer a new, fully automated, deep, unbiased workflow that enables the processing of up to 80 samples per shift.
- Streamlined to a single MS injection, the Proteograph ONE workflow reduces total sample-to-peptide time to less than 7 hours per batch, while lowering expenses associated with MS time and consumables.

Figure 8: Mixed Proteome Spike-in Evaluation of Quantification Accuracy. **(Top)** Three representative pairs of spiked-in samples and the expected fold-changes of bovine proteins concentration in these pairs. **(Bottom)** Distribution of observed fold-changes of bovine proteins for 3 selected comparisons of spiked-in samples. The color indicates the data source: protein identifications unique to direct digestion **(gray)**, protein identifications shared between the Proteograph ONE workflow and direct digestion **(purple)**, or protein identifications unique to the Proteograph ONE workflow **(teal)**. The horizontal dashed lines indicate the expected fold-changes.

- The automated workflow supports consistent peptide processing to minimize pre-analytical variability, which users can monitor using built-in controls and PAS.
- Harnessing Seer's proprietary engineered nanoparticle technology, the Proteograph ONE workflow captures deep proteomic information from human plasma for biomarker discovery, profiling proteins largely invisible to traditional deep plasma proteomics approaches.
- The Proteograph ONE workflow facilitates excellent experimental reproducibility within and between plates across multiple days, using different SP200 Automation Instruments.
- Confirmed by both mixed proteome and spike-in assays, the Proteograph ONE workflow accurately quantifies protein levels to allow for tracking of expression changes with clinical relevance.
- Taken together, the Proteograph ONE workflow allows researchers to measure thousands of proteins in thousands of samples in just a few weeks for comprehensive, unbiased proteomic analysis.

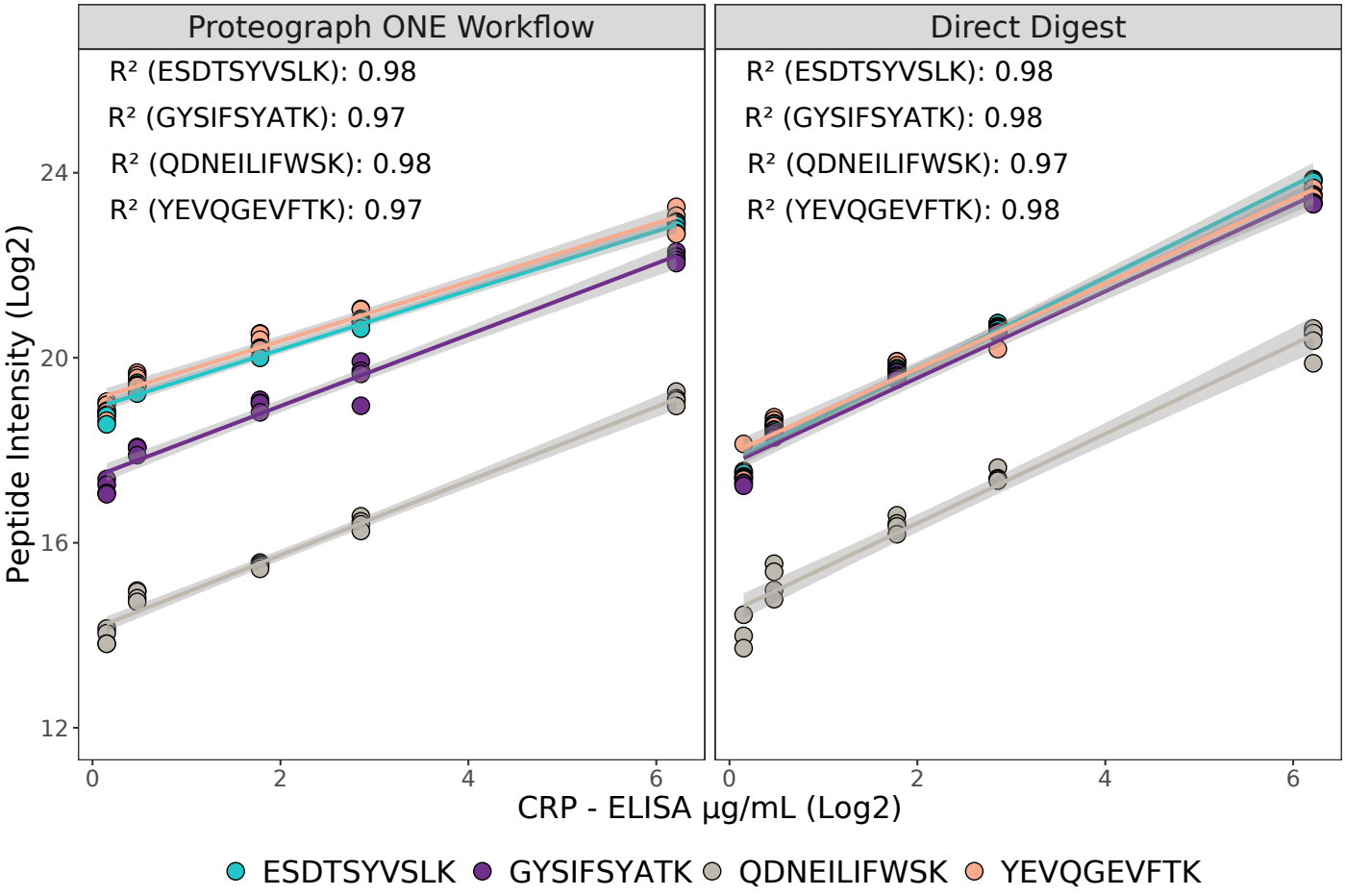

Figure 9: ELISA Detection of CRP Protein for Orthogonal Validation of Quantification Linearity. Pooled plasma samples were spiked with CRP at different dilutions to investigate quantification linearity for both the Proteograph ONE **(left)** and direct digest **(right)** workflows against results from an ELISA assay using same samples. The Proteograph ONE workflow data from four different peptides correlated closely with ELISA data (R^2 = 0.97-0.98), showing nearly identical quantification linearity.

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