Evaluation of engineered multi-nanoparticle-based proteomics analysis for unbiased, deep, and rapid analysis of fetal bovine serum derived cell culture media

Bryn Levitan*; Xiaoyan Zhao; Heather Ha; Margaret Donovan; Ryan Benz; Mathew Ellenberger; Khatereh Motamedchaboki; Asim Siddiqui; Marty Goldberg and Omid Farokhzad

Proteograph™ Product Suite Delivers Untargeted, Deep and Rapid Proteomics at Scale

Proprietary Engineered Nanoparticles Enables Deep and Rapid Proteomics Analysis of Cell Culture, Conditioned Media

Introduction

The conditioned media of different cell cultures are widely used for a variety of biological applications in-vitro; including characterization of secreted proteins from different cell types into the media under different conditions or treatments to understand underlying observed biological functions. However, to keep cells viable, a set of abundant proteins in fetal bovine serum (FBS) is included in the media for mammalian cell culturing, which creates an extra level of complexity by affecting the dynamic range of the protein concentration. This complexity and wide dynamic range is similar to challenges researchers have in plasma proteomics for in-depth proteomics analysis by liquid chromatography–tandem mass spectrometry (LC-MS/MS) which necessitates complex workflow and trade-offs between throughput, scalability, coverage, and precision. To solve this challenge, we applied a deep and scalable proteome profiling platform[1] to analyze FBS based HeLa cell media directly on the automated Proteograph™ Product Suite. This approach leverages multiple nanoparticles (NPs) engineered with distinct physicochemical properties to provide broad coverage of the complex proteomes at scale.

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

In this study, we first compared Proteograph™ workflow with conventional proteomic workflows for plasma proteomics analysis to demonstrate the advantage of Proteograph™ with its proprietary nanoparticles. We processed plasma samples with four methods: Proteograph™ workflow, high-pH fractionation, plasma depletion and direct digestion of neat plasma. For secreted media, we used 250 uL of media harvested from HeLa cells. We processed the conditioned media directly with Proteograph™ automation instrument and tryptic peptides generated, were analyzed using a Thermo Fisher Scientific Orbitrap Fusion Lumos Tribrid Mass Spectrometer in a DDA mode with a 2hrs LC gradient per NP per sample. LC-MS/MS raw data files were processed using Proteograph™ Analysis Suite with MaxQuant, applying a 1% FDR cutoff at the protein and peptide levels.

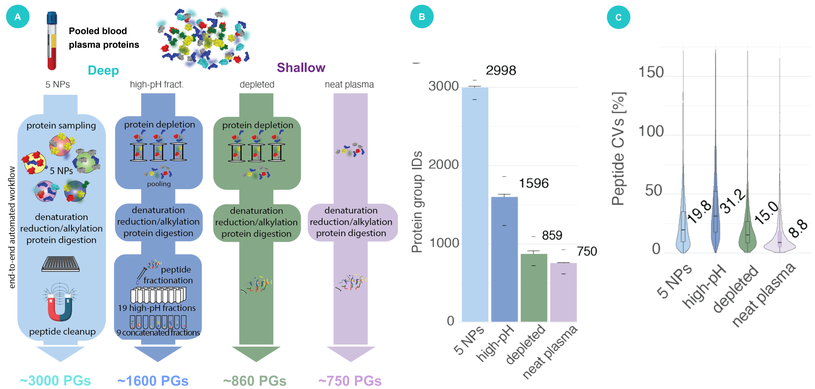


Figure 1. Depth of coverage and analysis precision achieved with different label-free plasma proteomics workflows. **(A)** Conventional label-free plasma proteomics workflows compared to Proteograph Product Suite with a 120 minute DDA analysis for each of the five nanoparticles in the Proteograph™ Assay and total analysis time of 10 hrs per sample. **(B)** Proteograph data resulted in ~3000 protein groups identification (1% FDR at protein and peptide level) across a dynamic range of 7 orders of magnitude with MaxQuant. **(C)** Proteograph assay precision showed improved replicate CV compared to fractionation methods[2] with ~2X more protein groups identified.

Label-Free DDA Workflow

Figure 2. Label-free DDA workflow. In the label-free DDA workflow, conditioned media samples were processed through the automated Proteograph sample preparation workflow using a panel of five nanoparticles. After processing, a 96-well plate with digested peptides is dried and reconstituted, ready for LCMS analysis.

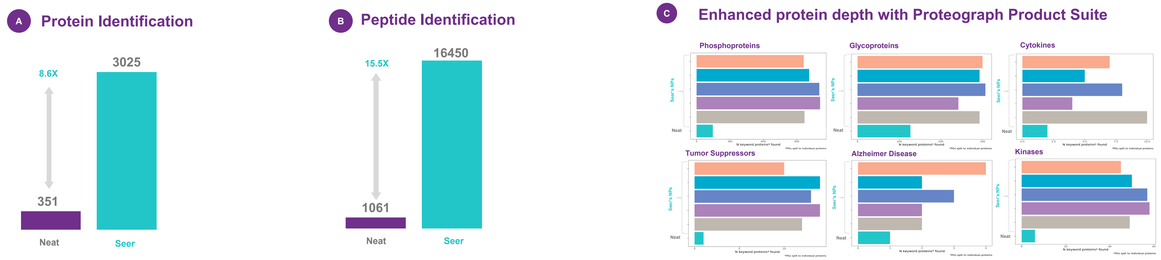
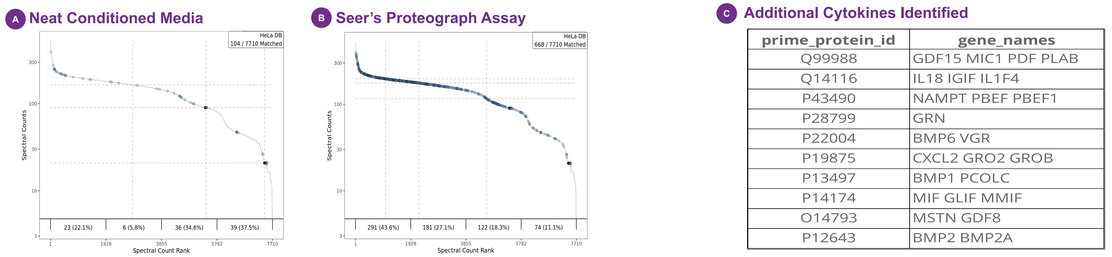


Figure 3. Proteograph provides enhanced proteins, peptides detection across diverse ranges of proteins in cell culture media. Our results demonstrate detection of more than **(A)** 3000 cell-derived proteins and **(B)** over 16000 peptides in the HeLa cell culture supernatant containing FBS. The Proteograph™ platform offered ~10X improvement in depth of proteome coverage compared to results derived from direct digestion of same conditioned media material, enabling identification of **(C)** lower abundant cytokines in the culture media, which were not robustly detected by conventional proteome approaches and only achievable with complex proteomics workflows including concentration and fractionation.

.



C Additional Cytokines Identified

prime_protein_id	gene_names
Q99988	GDF15 MIC1 PDF PLAB
Q14116	IL18 IGIF IL1F4
P43490	NAMPT PBEF PBEF1
P28799	GRN
P22004	BMP6 VGR
P19875	CXCL2 GRO2 GROB
P13497	BMP1 PCOLC
P14174	MIF GLIF MMIF
O14793	MSTN GDF8
P12643	BMP2 BMP2A

Figure 4. Identification of low abundant cytokines and other low abundance proteins with Proteograph Product Suite. Using Proteograph we are able to detect more lower abundance proteins. In **(A)** neat media analyzed with direct digestion workflow and **(B)** media analyzed with Proteograph workflow (showing results derived from one of the nanoparticles), relative abundance value of proteins was plotted against abundance rank based on HeLa database. These demonstrate protein detection across the entire concentration range of the HeLa proteome database[3] spanning 7 orders of magnitude in Proteograph derived analysis vs neat media digestion. **(C)** This table shows examples of low abundance cytokines detected by Proteograph.

Conclusion

- Proteomics provides invaluable insights into biology, but it is challenging to collect deep proteome data at scale
- Proteograph provides deep and untargeted proteomics workflow for conditioned media with less sample volume required
- The Proteograph Product Suite enables rapid and automated sample preparation for large scale conditioned media proteomics

References

[1] Blume et al. Nat. Comm. (2020)
[2] Ferdosi et al, in press (2022)
[3] Mann et al. Mol Cell Proteomics. (2012)

Seer, Inc., Redwood City, CA 94065, USA | *blevitan@seer.bio

Publications