Integrated Plasma Multi-Omics Using Nanoparticle Technology and Single Shot Capillary MS

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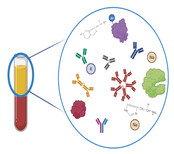
Introduction

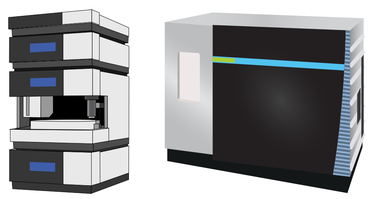
Plasma Proteomics with Seer

- Plasma is one of the most biomolecule-rich sample types
- Plasma proteins exist at concentrations that vary by 10 orders of magnitude, creating a huge dynamic range problem
- Seer uses **Nanoparticle (NP)** technology to differentially select proteins from human plasma
- Proteins differentially aggregate to each of the 5 NP's based on the physiochemical properties of the beads
- Plasma samples can be processed in an automated workflow using the Seer Proteograph Suite



Multi-omics using MOST

- Simultaneous analysis of proteins, lipids, and metabolites would provide and exceptionally powerful and efficient means of biomolecule analysis
- Mass Spectrometry is well suited to analyze many classes of molecules
- Potential applications include biomarker analysis, precision medicine, diagnostics, metabolomic and proteomic studies
- **Multi-Omic Single Shot Technology (MOST)** enables proteomic and lipidomic analysis from a singular MS run using a modified LC gradient
- Recently, MOST has been adapted for nano-flow LC-MS
- MOST technology is compatible with proteomic samples from many cell types and species



Combining -omic Strategies

- Expansion and optimization of plasma biomolecule analysis necessitates a depletion of high abundance proteins
- Plasma samples processed in the Seer Proteograph Suite result in peptide samples for a broad selection of plasma proteins
- NP peptide samples can be analyzed in tandem with lipid samples using MOST technology

- Peptide separations can be enhanced using a **high-field asymmetric waveform ion mobility spectrometry (FAIMS)** device which separates ions before transfer into the Mass Spec based on their mobility in various electric fields

Conclusions & Future Work

- Combining protein enrichment from the Seer Proteograph Suite and the multi-omic analysis strategy of MOST can identify over 2000 biomolecules in two hours
- The addition of FAIMS to compliment LC separations consistently increased peptide sampling and protein identifications from plasma peptide samples
- More work is needed to optimize use of FAIMS with proteins and lipids
- Improved Method development is needed to extract lipids prior to Proteograph analysis

References & Acknowledgments

We thank Seer, National Institutes of Health grant P41 GM108538 awarded to JJC, and National Institutes of health grant T32 HL07899 for providing financial support of this work.

He, Y., et. al. (2021). Multi-Omic Single-Shot Technology for Integrated Proteome and Lipidome Analysis. Analytical Chemistry, 93(9), 4217–4222. https://doi.org/10.1021/acs.analchem.0c04764

Hebert et al., (2018) . Comprehensive Single-Shot Proteomics with FAIMS on a Hybrid Orbitrap Mass Spectrometer. Analytical Chemistry, 95(15) 9529-9537.https://doi.org/10.1021/acs.analchem.8b02233

Method Validation

Figure 1: The MOST gradient enables effective separations of proteins and lipids in a single LC-MS experiment. Lipid samples are initially loaded onto the column, followed by peptide samples. For the first 80 minutes, the gradient is predominantly mobile phase A (white) which elutes peptides off of the C18 capillary column using water and formic acid. For the following 40 minutes, mobile phase B (green) is increased significantly and then more gradually to elute lipids. **A.** A full MOST experiment where a lipid sample was not added to the column. **B.** A full MOST experiment with both peptides from Seer NP 4 and lipids from human plasma.

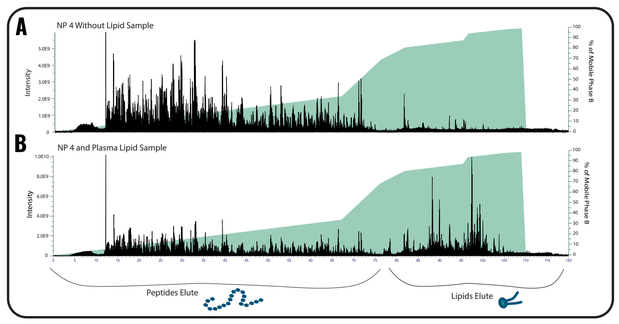


Figure 3: (Right) Wilcoxon Rank Sum analysis indicates that there is no significant difference between the amount of protein groups that can be identified when NP samples are run with and without lipids (p=0.1041).

Figure 2: Comparison of peptide peak quantification for individually analyzed nanoparticle samples analyzed with and without a lipid sample on the MOST gradient. Label Free Quantification (LFQ) of each peak indicates that incorporation of lipids to the gradient has no effect on peak quantification.

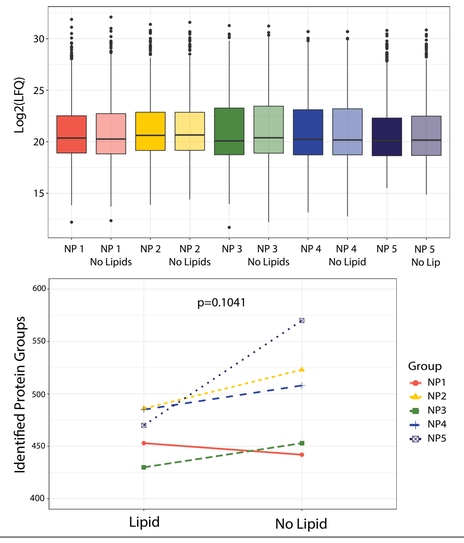
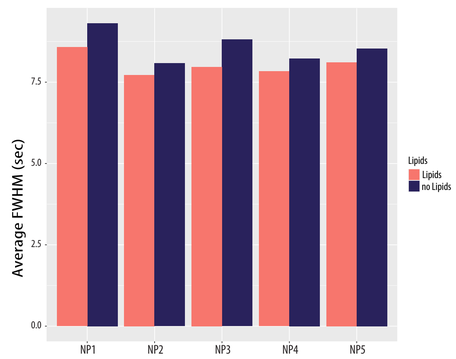


Figure 4: The average Full Width at Half Maximum (FWHM) of NP peptide samples processed with and without lipids remains consistent. Consistency in FWHM indicates that having both lipids and peptides does not have a detrimental impact on the chromatographic method. Narrower and consistent peaks enable better MS resolution and better peptide identification.



Optimization & Results

Figure 5: An overall comparison of protein identifications from each nanoparticle. NP samples were run separately and then their uniquely identified proteins were compared in silico (blue). From the individual experiments, the top two and three NP samples that yielded the most protein group identifications were combined into a single sample and reanalyzed alongside a pooled sample with all 5 NPs in it. All samples were compared to a neat plasma digest (no NPs used), which consistently yields fewer protein groups.

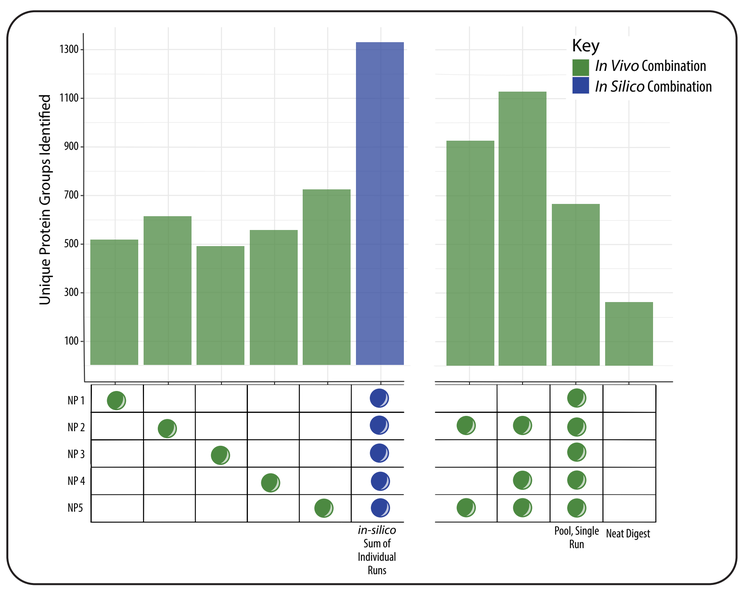


Figure 6: Venn Diagram analysis illustrates the overlap between several sample sets. **A.** Of the 272 identified protein groups in neat plasma, 60% of them are also identified in a pooled sample of all 5 NP samples. **B.** Though each NP individually identifies between 500-700 proteins, there is a significant amount of overlap between each one. An analysis of the top 3 NPs indicates that each one isolates around 200 unique proteins. This overlap in proteins contributes to the difference between the in-silico sum of proteins and observed results from pooled samples, as seen in Fig. 5.

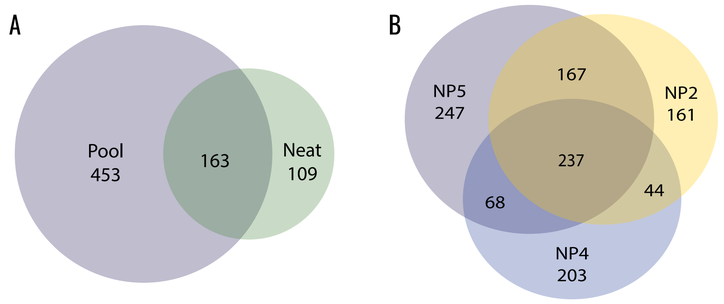


Figure 8: Comparison of a set of short (**A**) and long (**B**) gradients found that a 60 minute gradient consistently resulted in the most protein identifications. This corresponded to the lowest Full Width at Half Maximum (FWHM) values (**C, D**) which indicates high resolving power and enables better identifications. Especially notable in the long gradient set (**D**) is the doubling of FWHM as the active gradient length increases. Following analysis were done in 60 minutes.

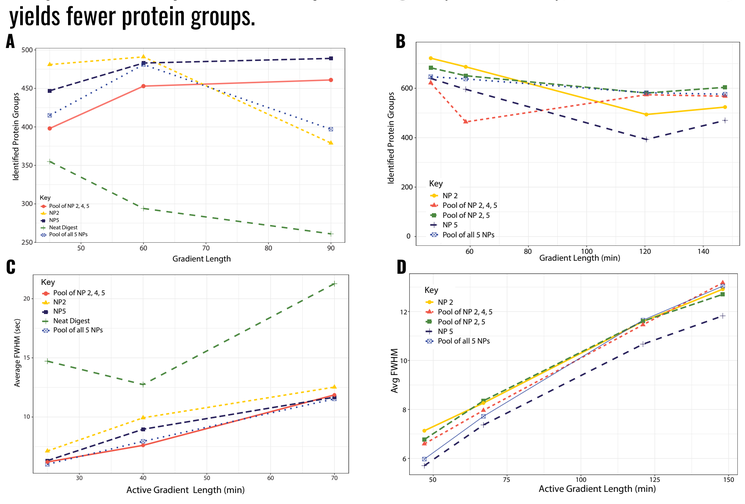


Figure 7: A ranked analysis of all proteins identified from a pool of all 5 NPs reveals a large increase in identified low-abundance proteins. **A.** The neat digest (orange) favors high abundance proteins, while the pooled NP (purple) can identify a larger amount of proteins over a wider range. **B.** The sequence coverage of proteins identified from the pooled NP sample decreases for lower abundance proteins, relative to high abundance proteins. The distribution of protein group density also indicates that the majority of identified proteins have somewhat low sequence coverage, with a median of 29% coverage.

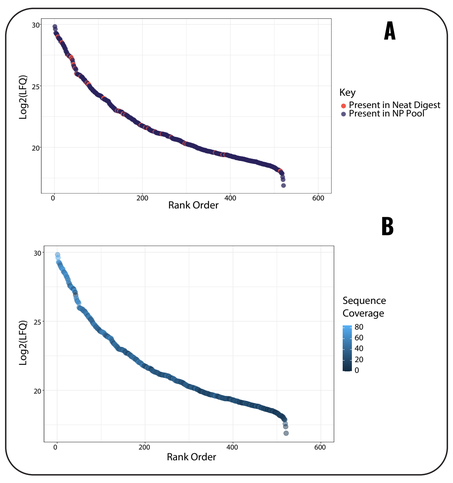


Figure 9: Incorporation of FAIMS technology using two switching compensation voltages for ion mobility separations increases protein identifications in neat and pooled samples of the top tow and all 5 NPs. For the paired NP sample, there was a statistically significant increase. In each sample FAIMS separations enabled better sampling and lead to more identifications.