Multi-nanoparticle Workflow Enables Deep Plasma Proteomics at Scale, with Enhanced Precision, and Depths of Coverage

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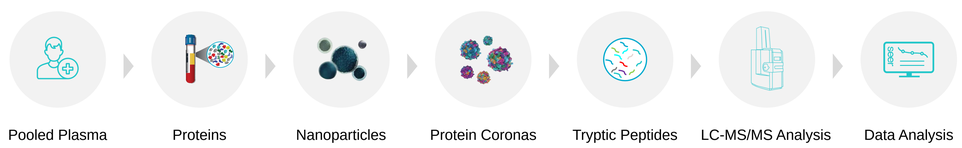
Proteograph™ Product Suite Delivers Unbiased, Deep and Rapid Plasma Proteomics

Blood plasma is a rich source of protein biomarkers for early detection of diseases. To overcome limitation of deep plasma proteomics in large cohorts, we have developed a fast and scalable technology that employs intricate protein-nano interactions. Introducing a nanoparticle (NP) into a biofluid such as blood plasma leads to the formation of a selective, specific, and reproducible protein corona at the nano-bio interface, driven by the relationship between protein-NP affinity, protein abundance and protein-protein interactions. We previously demonstrated that this process, incorporated within the Seer Proteograph™ Product Suite, offers superior performance in terms of depth, breadth, precision, and throughput compared to conventional deep plasma proteomics workflows[1]. The ratio of plasma-to-nanoparticles plays an important role in protein corona composition and can be optimized to enhance and differentiate protein selectivity. Here we investigate effects of different conditions on protein corona composition enabling enhanced proteome depth performance of Proteograph.

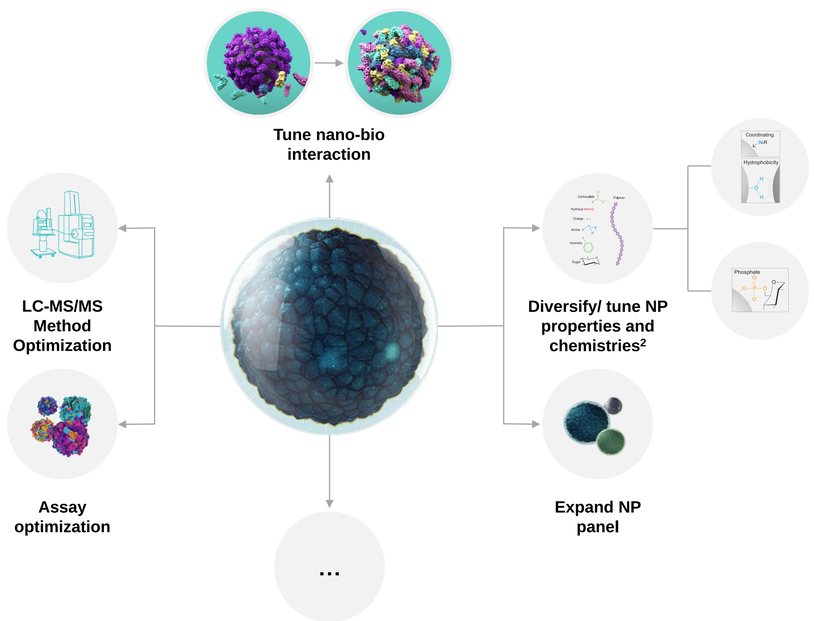
Here we have investigated compositional changes of protein coronas from 5 NPs with blood plasma at different ratios. Samples were analyzed with timsTOF Pro mass spectrometers with UltiMate 3000 HPLC system using a 30min dia-PASEF LC-MS/MS method. We evaluated depth, dynamic range, coverage, and precision of quantification at a wide range of concentrations for each NP. Strategies including limitation of the available binding surface of NPs and increasing the binding competition, improved depth of coverage in plasma proteome by increasing the competition, enabling reproducible protein identification and quantification across assay replicates. In addition, protein selectivity was enhanced, leading to improved coverage of plasma proteome when using multiple physicochemically distinct NPs.

Proteograph Product Suite

From Sample to Peptides, Ready for Analysis on Most LC-MS/MS Instruments

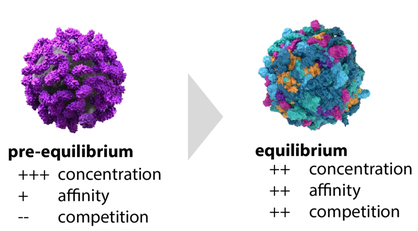


Strategies to Enhance NP-based Deep Plasma Proteomics



Proprietary Engineered Nanoparticles Enables Enhanced Coverage of Hormone/Cytokine Signaling Proteins

Enhanced Protein Groups and Peptide Detection Across Plasma Proteome



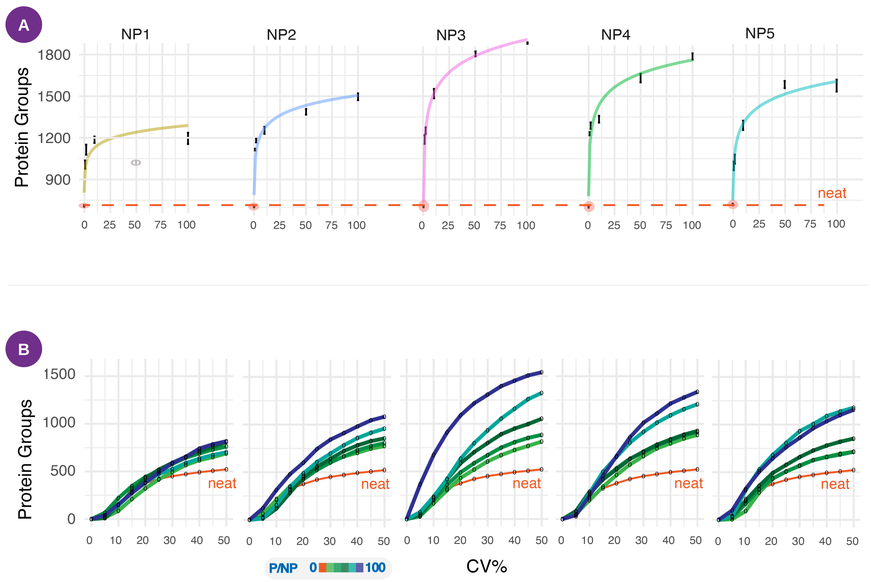


Figure 1. Tuning nano-bio interaction to diversify protein corona composition.

At equilibrium, strong competition for binding increases selective sampling of biomolecules by specific binding affinities, leading to effective ratio compression.

Figure 2. Number of identified protein groups as a function of enhanced nano-bio interaction.

A) By limiting the available binding surface of NPs and increasing the binding competition, we can quantify 20 – 60% more proteins with better reproducibility on the surface of each NP at 1% peptide and protein level FDR. **B)** CV/ ID accumulation analysis shows that enhanced nano-bio interaction yields more protein group identifications at high precision.

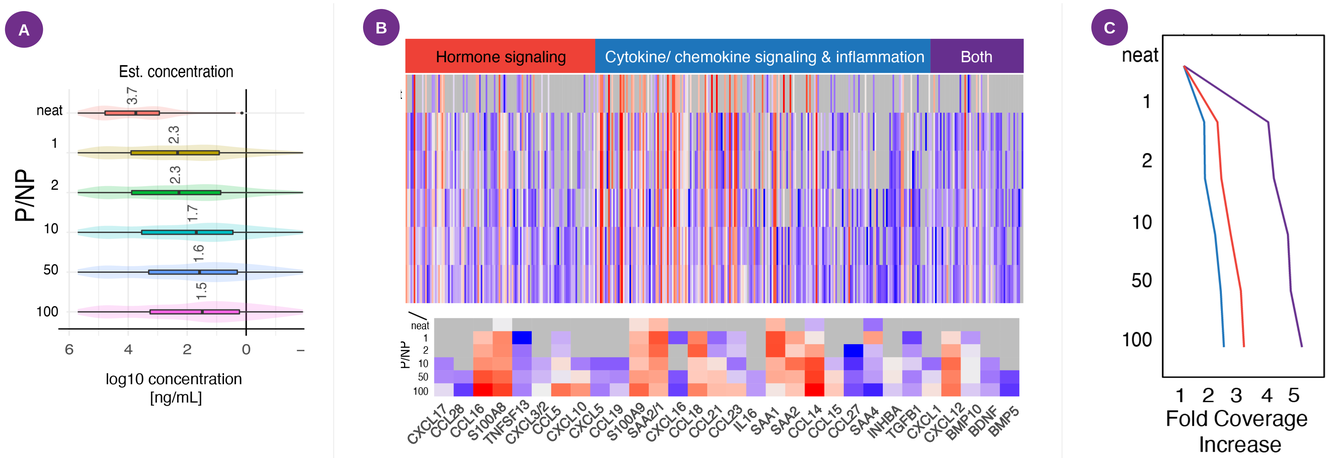


Figure 3. Identification of proteins annotated with hormone/cytokine signaling.

A) Distribution of the concentration of hormone/cytokine signaling proteins shows that by increasing the Protein:NP ratio we can identify more proteins in this group at a lower concentration level. **B)** By increasing the Protein:NP, the coverage of proteins with these annotations increases. Some of these proteins are highlighted in the figure. **C)** Compared to neat plasma as the baseline the coverage of these groups of proteins increases by 2 – 5-fold as we limit the binding surface.

Conclusion

- Proteograph enables deep, rapid, and scalable plasma proteomics exploiting complex nano-bio interactions superior to conventional plasma workflows.
- By increasing the binding competition, we identify 20 – 60% more proteins on the surface of each NP with higher reproducibility in quantification.
- Nanoparticles with optimized workflow, capture a large and diverse set of proteins and biological pathways based on their specific physicochemical makeup.

References

[1] Blume et al. *Nat. Comm.* (2020)
[2] Ferdosi et al. 2021, PNAS in press
[3] Hornburg et al. bioRxiv (2022)

Publications

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