Experimental & Computational Approach to Profile Nanoparticle–Protein Interactions for Deep Plasma Proteomics

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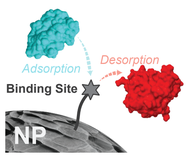
Nanoparticle–protein interactions are at the core of the Seer plasma proteomics platform

Introduction

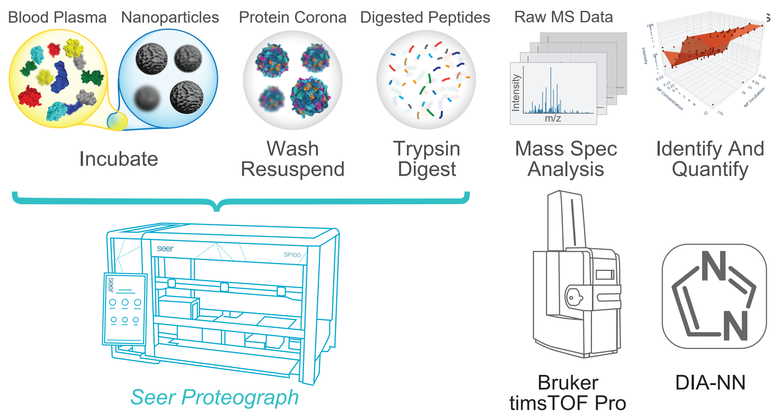


Seer's nanoparticles combined with mass spectrometry enable unbiased analysis of the blood plasma proteome at scale and depth[1,2,3].

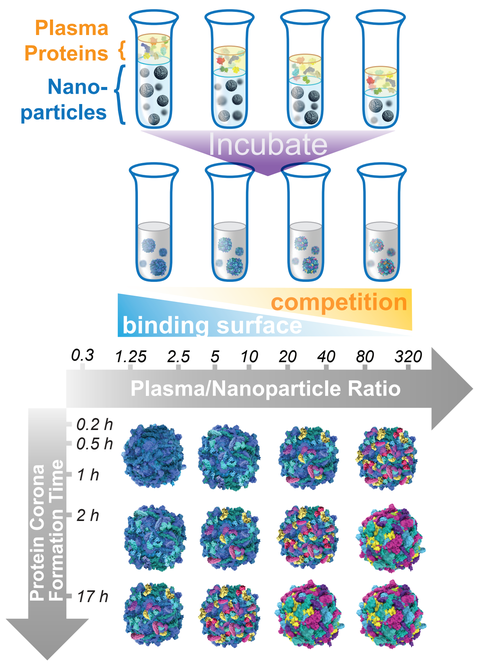
This is facilitated through the adsorption of proteins onto the nanoparticle surface, which boosts the signal of high-affinity low-abundance proteins and suppresses the low-affinity high-abundance ones, a process known as *Vroman effect*[4].

High diversity of structural and chemical properties across plasma proteome as well as the wide dynamic range of protein concentrations determine a nanoparticle adsorption-desorption dynamics in complex protein mixtures.

Seer Proteograph™ Product Suite enables reproducible, deep and unbiased plasma proteomics



Vroman Effect Profiling: Experimental Design



To profile the dynamics of NP–protein interactions, we measured *protein coronas* of NPs across a wide range of conditions[1]:

- ***3*** distinctly functionalized Seer nanoparticles
- ***9*** plasma/nanoparticle ratios
- ***5*** nanoparticle incubation times

By modulating conditions, we shift the dynamic equilibrium of NP–proteins system, which is reflected in protein intensities measured by LC-MS/MS.

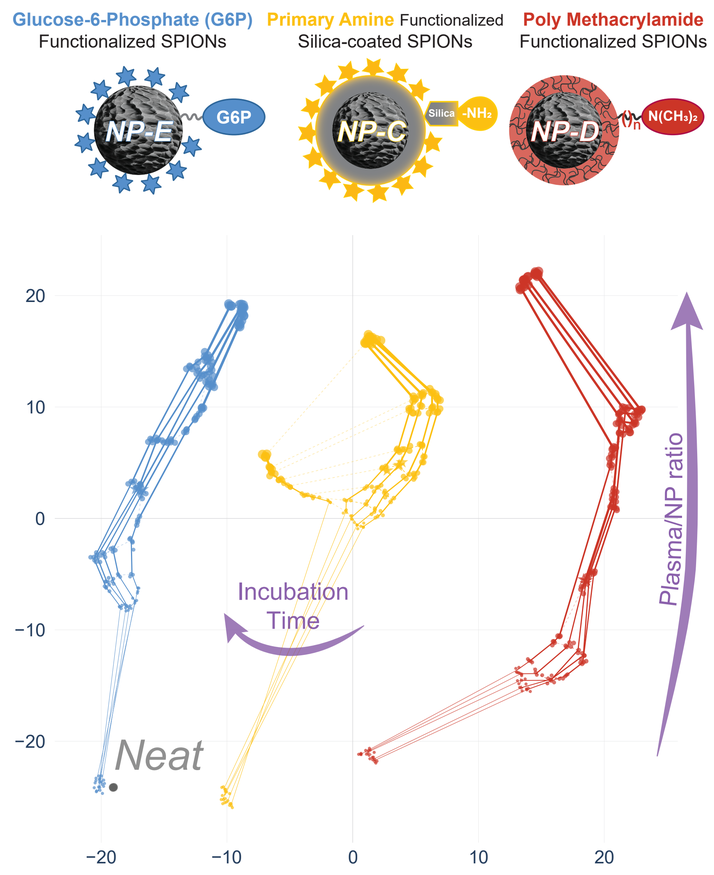
In total we have measured ***534*** protein corona samples using a *Bruker timsTOF Pro* in *diaPASEF* mode. The MS data were analyzed with *DIA-NN* yielding intensity profiles for ***3184*** protein groups.

Vroman Effect Landscape of 3 Seer NPs: Nanoparticle Corona-Centric View

UMAP plot of protein intensity profiles for each of 534 measured protein coronas.

Each dot represents one protein corona experiment. Experiments with the same plasma/NP ratio or incubation time are connected.

The distance between the dots reflects the similarity of protein coronas. The plot confirms high reproducibility and consistency of the Seer NP workflow.

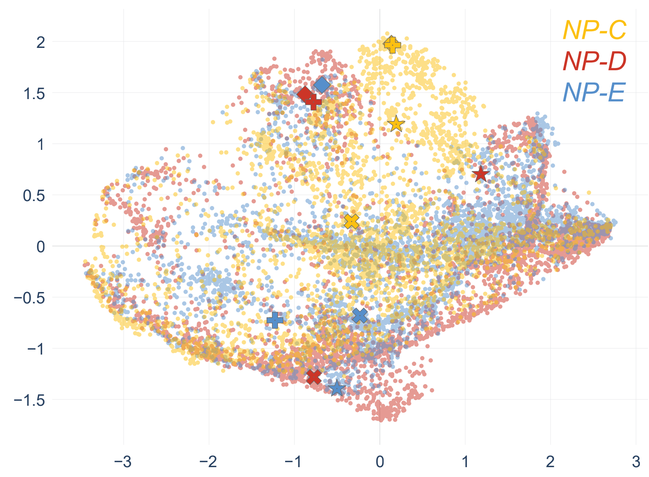


Vroman effect profiling enables neat protein abundance reconstruction

Vroman Effect Landscape of 3 Seer NPs: Protein-Centric View

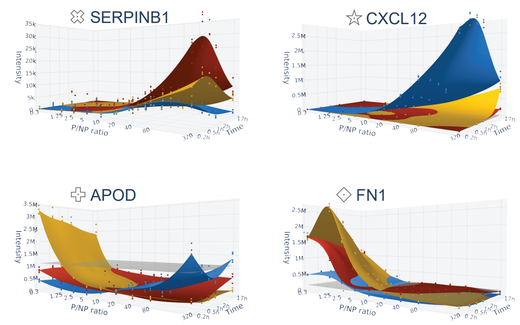
UMAP plot of protein–nanoparticle intensity profiles for each of 3184 identified protein groups across all tested conditions. One dot corresponds to an intensity profile of a specific protein group in a corona of a specific NP (indicated by the dot color).

The clouds of protein profiles for individual NPs largerly overlap, supporting the notion that, while NPs have distinct specificities, the process of protein corona formation is governed by the same biophysical principles.



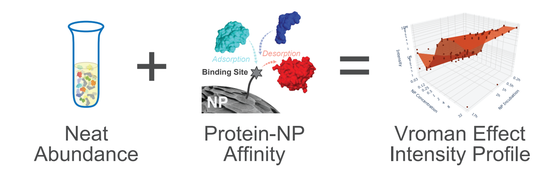
Individual NP–Protein Profiles

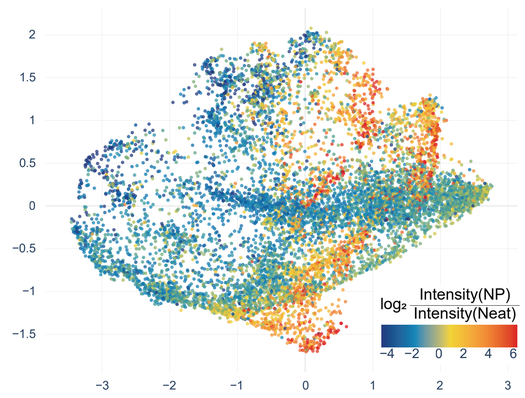
The symbol specifies the position of the profile on the UMAP.



Vroman Effect Profiles Correlate with Neat Protein Abundance

Projecting "neat" (NP-less) plasma protein intensity information onto UMAP reveals strong correlation between the NP-protein intensity profile and its neat abundance.





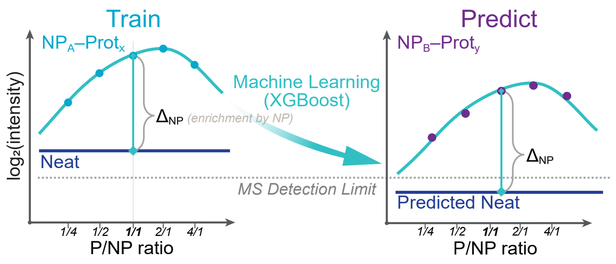
Reconstructing Absolute Neat Protein Abundance Using Vroman Effect Profiles

By compressing the dynamic range, Seer Proteograph workflow allows robust and accurate measurement of protein abundance variation *between the samples*, even for the very low abundant proteins.

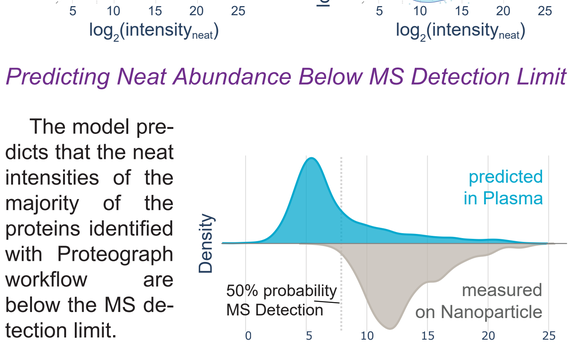
We have applied machine learning to Vroman effect profiling data to *decompress* the dynamic range and reconstruct the *absolute neat plasma abundance* of proteins, even when it is *below the detection limit* of the conventional plasma workflow.

Using ML to Predict Protein Enrichment in NP

Similar Vroman effect profiles indicate similar NP enrichment.



ML Enhances Absolute Neat Protein Intensity Estimates



Predicting Neat Abundance Below MS Detection Limit

The model predicts that the neat intensities of the majority of the proteins identified with Proteograph workflow are below the MS detection limit.

Conclusions

Understanding the nanoparticle–protein interactions on the quantitative level opens new possibilities for the nanoparticle-enabled LC-MS/MS analysis. Vroman effect profiling allows to streamline the design of nanoparticles interrogating specific physicochemical fraction of the proteome occupied by hundreds to thousands of proteoforms and to improve the processing of the retrieved data, including large cohort studies.

References

1. Ferdosi, Stukalov et al, *Enhanced Competition at the Nano–Bio Interface Enables Comprehensive Characterization of Protein Corona Dynamics and Deep Coverage of Proteomes*, **Advanced Materials** (2022)
2. Blume et al, *Rapid, deep and precise profiling of the plasma proteome with multi-nanoparticle protein corona*, **Nat Comm** (2020)
3. Ferdosi et al, *Engineered nanoparticles enable deep proteomics studies at scale by leveraging tunable nano–bio interactions*, **PNAS** (2022)
4. Vroman et al, *Interaction of high molecular weight kininogen, factor XII, and fibrinogen in plasma at interfaces*, **Blood** (1980)

Publications

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