Enhanced Competition at the Nano–Bio Interface Enables Comprehensive Characterization of Protein Corona Dynamics and Deep Coverage of Proteomes

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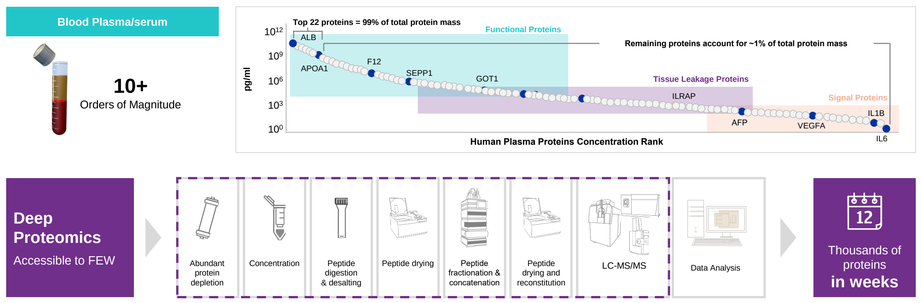
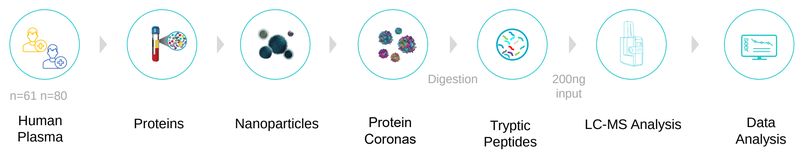
Experimental and Computational Dissection of Nano-Bio Interactions and Protein Corona Dynamics

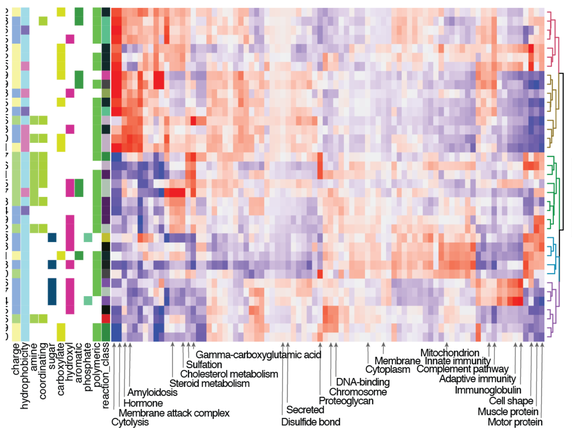
Panels of proprietary engineered nanoparticles (NPs) can overcome the high dynamic range of proteomes in samples like blood plasma at a scale to discover new low abundance biomarkers in a fully automated workflow. This is enabled by a reproducible and quantitative dynamic range compression at the nano-bio interface (protein coronas). Moreover, any nanomaterial used in biomedical applications (e.g., for drug delivery) will form molecular coronas as function of the subject's proteome and the physicochemical properties of nanomaterial, putatively affecting pharmacokinetics and dynamics. We demonstrate a combination of nanoengineering, state-of-the-art DIA mass spectrometry workflows, and machine learning that enhances our fundamental understanding of nano-bio interactions resulting in deeper coverage of proteomes and improved precision of the workflow.

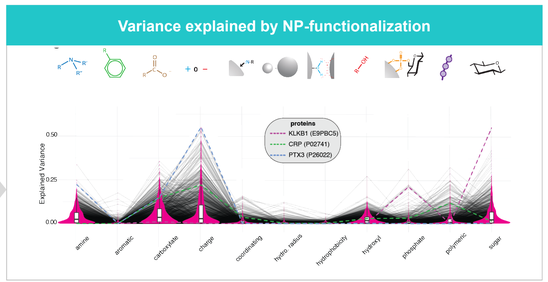


The Proteograph™ Product Suite enables deep, unbiased, rapid, and scalable access to the plasma proteome



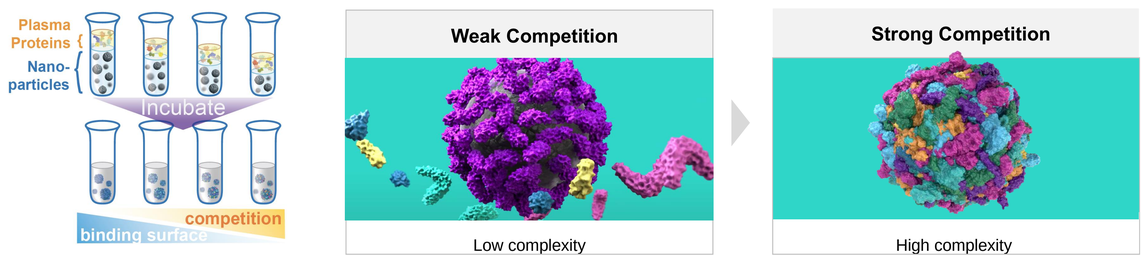
Multiple avenues to explore and tune nano-bio interactions and NP-based deep proteomics





Protein	Isoelectric point	Charge	Domains
PTX3	4.8	-23	
CRP	5.7	-7	Beta-sheet rich
KLKB1	7.3	-2	PAN

NP engineering: Changing physical characteristics including surface chemistries, porosity, curvatures can change the 'appearance' of NPs and hence the subset of molecules attracted to it. To efficiently sample the universe of opportunity, design decisions are guided by a comprehensive understanding of what drives biomolecules to associate with surfaces.



Assay tuning: Conditions including buffers, temperature, incubation times, and ratio of binding sites to competing binders (Vroman effect) will affect nano-bio interactions and protein corona formation which could be utilized for optimizing the assay performance.

Nanoparticle Coronas Enable Deep Coverage of Biological Content From Proteins to Peptide to Posttranslational Modifications

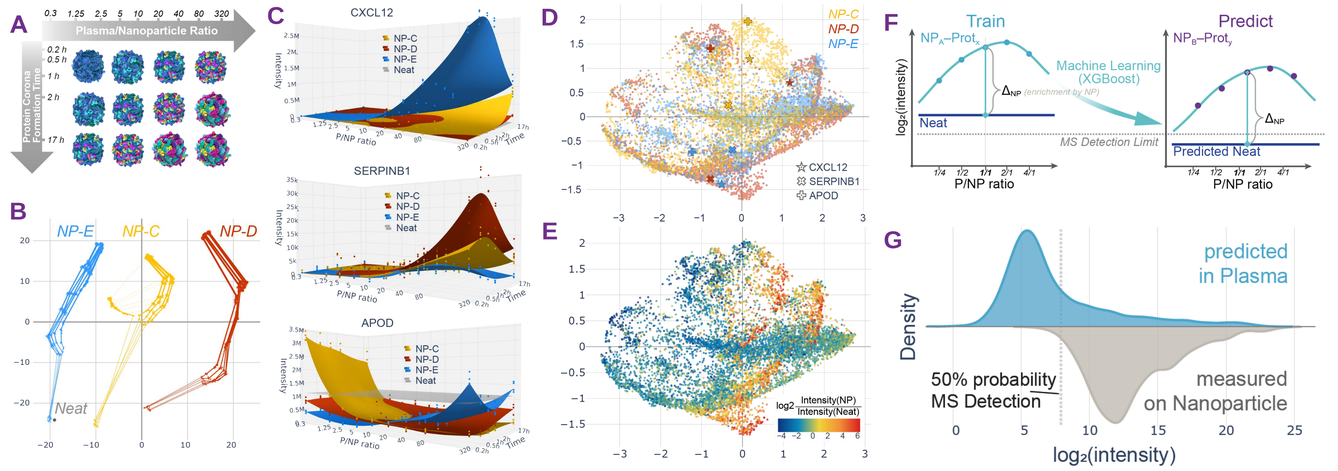


Figure 1. Modeling Protein Corona Dynamics. (A) Protein corona composition at different NP incubation times and concentrations. The plasma proteome was sampled by NP-C, NP-D and NP-E using all combinations of incubation times and concentrations. **(B)** 2D UMAP. Each dot represents 1 MS run and is colored by NP. Experiments with the same incubation time or NP concentration are connected by the solid or dashed lines, respectively. The distance between the MS runs reflects the similarity of protein intensities measured in these experiments. **(C)** Protein intensities of Stromal cell-derived factor 1 (CXCL12), Leukocyte elastase inhibitor (SERPINB1), and Apolipoprotein D (APOD); points represent intensity measurements in individual experiments, surfaces are the thin plate splines-based interpolation of the measured intensities across different P/NP ratios and corona formation times; the point and surface colors denote the NP of the corresponding experiments. **(D-E)** 2D UMAPs of the protein-NP dynamic profiles. Each dot represents the intensities of a given protein group across all NP corona formation times and P/NP ratios. The distance between the points reflects the similarity of protein-NP dynamics. The color of the dot denotes the NP of the corresponding dynamic profile **(D)**, or the log2-fold change of a given protein intensity at the reference NP condition (P/NP=10, 1h NP corona incubation time) in comparison to the neat plasma **(E)**. **(F)** Using ML to predict protein enrichment in NPs. **(G)** The model predicts that the neat intensities of most proteins identified with Proteograph™ workflow are below the MS detection limit.

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Alexey Stukalov
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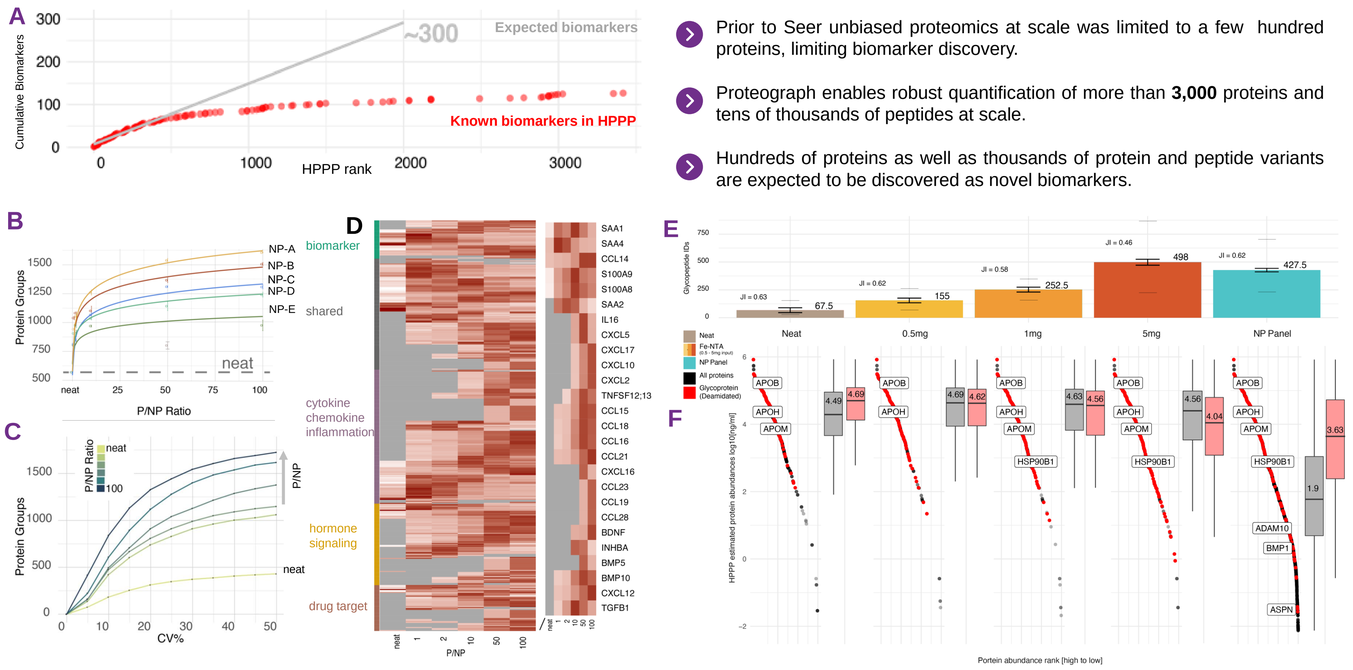

Figure 2. Enhanced Nano Enable Quantification of Low Abundance Proteins and Peptides. (A) Accumulation of biomarkers across the dynamic range. Grey line extrapolates biomarker accumulation from the first 500 proteins to the next 1500 proteins suggesting hundreds of biomarkers among the top 2000 plasma proteins. **(B)** Number of identified proteins at different P/NP ratios and in neat plasma. **(C)** Number of protein groups measured at 5 NP panel level below given coefficient of variation (CV) for different P/NP. **(D)** z-score protein intensities for biomarker, drug target, hormone signaling, cytokine/chemokine signaling, and inflammation annotations identified across the panel of 5 NPs at different NP dilution ratios. **(E)** Number of glycopeptides identified in a single pooled plasma: Neat, Fe-NTA enrichment using digested plasma, and Proteograph (equivalent to 2 mg plasma protein per NP). **(F)** Depth of coverage in each workflow is shown based on the estimated protein abundance in the HPP.

- Prior to Seer unbiased proteomics at scale was limited to a few hundred proteins, limiting biomarker discovery.
- Proteograph enables robust quantification of more than **3,000** proteins and tens of thousands of peptides at scale.
- Hundreds of proteins as well as thousands of protein and peptide variants are expected to be discovered as novel biomarkers.

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Conclusion

- Understanding the nanoparticle–protein interactions on the quantitative level enables the design of NP-based assays to interrogate specific physicochemical fraction of the proteome occupied by hundreds to thousands of proteoforms.
- NPs can identify significantly more proteins and peptides at high precision. Including many low abundant cytokines and chemokines as well as post translationally modified proteins and peptides.

References

Blume et al. (2020). Nature Comm
Ferdosi et al. (2022) PNAS
Ferdosi et al. (2022) Adv Mat
Schwenk, et al. (2017) Journal of Proteome Research

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