Ultra-high coverage of the serum proteome using a multi-nanoparticle based workflow

Carleen M Kluger[1]; Elena Kunold[1]; Florian Flenkenthaler[1]; Philipp Skroblin[3]; Till Kindel[1]; Thomas Wild[1]; Lucy Williamson[2]; Purvi Tandel[2]; Daniel Hornburg[2] and Andreas Tebbe[1]

[1]Evotec (München) GmbH, Neuried, Germany; [2]Seer, Inc., Redwood City, CA; [3]Evotec International GmbH, Göttingen, Germany

Introduction

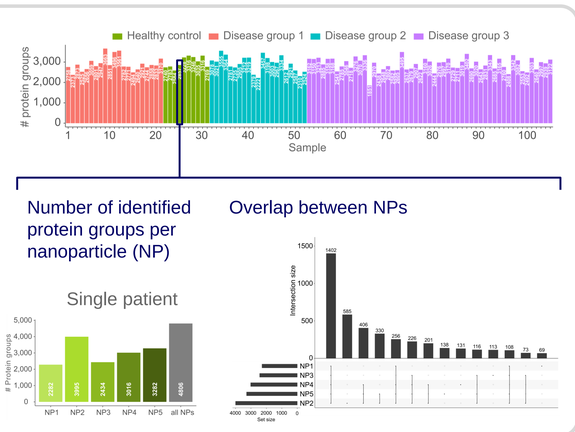
For years, the ultra-wide abundance range of proteins in plasma and serum has limited clinical applications by unbiased mass spectrometry based proteomics to just a few hundred high-abundance proteins. We show that by using the proprietary multi-nanoparticle based Proteograph™ technology to enrich low abundant proteins from serum, these challenges can now be efficiently addressed allowing detection of more than 5,000 proteins in a patient cohort with more than 3,000 proteins in individual patient serum samples.

Methods

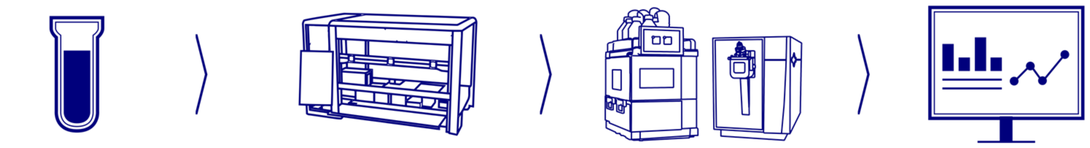
105 human serum samples from NURTuRE (nurturebiobank.org), a cohort of patients with chronic kidney disease or idiopathic nephrotic syndrome and healthy control participants, were incubated with a set of proprietary nano-particles followed by tryptic digestion and sample clean-up. All steps were performed using the fully automated Proteograph Product Suite (Seer Inc.). Peptides were combined and analyzed by 2h single-shot data independent acquisition (DIA) LC-MS/MS on a Q-Exactive HF-X instrument. MS raw data were analyzed using the DIA-NN software package.

Analysis of patient cohort

Identification of more than 5,000 protein groups and around 3,200 protein groups per individual sample in single-shot DIA measurements of pooled nanoparticles.



In non-pooled measurements of single nanoparticle samples from a single patient, more than 2,200 protein groups could be identified with each nanoparticle type alone and around 4,800 protein groups across all nanoparticles.

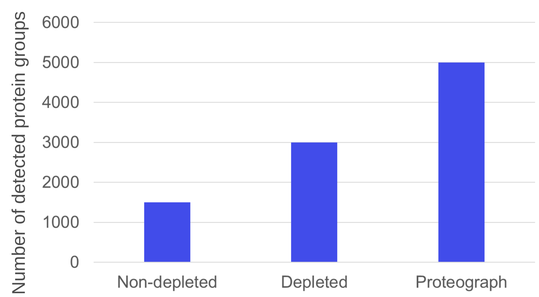


Fully integrated multi-nanoparticle based high performance proteomics workflow. Workflows are established for plasma and serum and will be further extended to various sample types (e.g. cerebrospinal fluid, urine, conditioned media) and other applications.

Multi-nanoparticle based Proteograph technology allowed detection of more than **5,000 proteins in patient-derived plasma/serum samples**

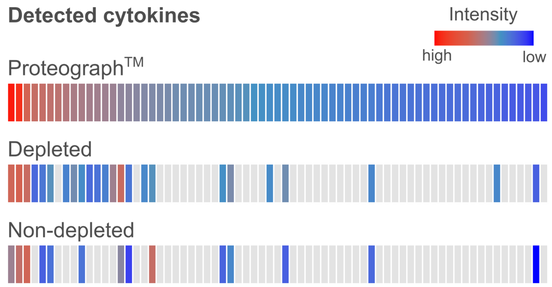
Unparalleled Proteome Coverage

Proteome depth applying **Evotec's plasma proteomics workflows**



Evotec's plasma proteomics workflows with or without antibody based depletion of high abundant proteins already deliver outstanding proteome coverage (about 1,500 to 3,000 proteins, respectively). Application of Proteograph technology boosts proteome coverage to up to 5,000 proteins in patient cohort studies (>3,000 proteins per individual sample).

Unprecedented Proteome Depth



Application of Proteograph technology significantly expands the dynamic range of quantified proteins compared to workflows with and without antibody-based depletion. Identification of 30% more cytokines compared to large scale plasma proteome study relying on extensive depletion and fractionation.

Possibilities

- Automated high-throughput analysis of large cohorts of challenging clinical samples, such as plasma, urine and cerebrospinal fluid
- Unbiased proteome-wide discovery of novel biomarkers
- Patient stratification

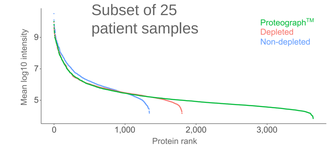
#RESEARCHNEVERSTOPS

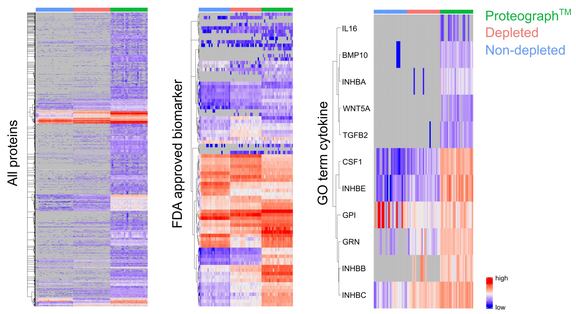
Your contact: Dr. Carleen Kluger
Group Leader Clinical Proteomics
Carleen.Kluger@evotec.com

Proteograph data in comparison to other sample preparation workflows

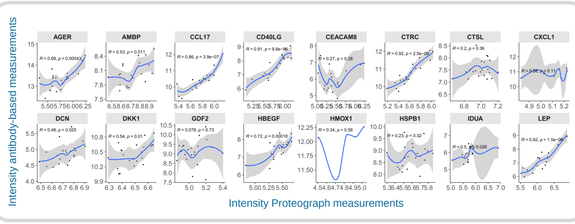
Data from a kidney cohort was compared to results obtained from non-depleted and antibody-depleted serum from the same study.

Significantly increased dynamic range of detected proteins. Identification of additional low-abundant proteins.





Identification completeness depends on protein abundance. Very similar behavior for high abundant proteins using different preparation workflows. Increased coverage of cytokines using the multi-nanoparticle based Proteograph technology.



Comparison of unbiased, multi-nanoparticle based Proteograph data with an antibody-based inflammation marker panel. High correlation for many proteins across multiple orders of magnitude.

Conclusions

We present here a comprehensive serum proteomics workflow that can be used for patient stratification as well as potential identification of novel biomarkers which are not accessible through other strategies. Ultra-high coverage serum proteomics increases number of quantified proteins by 3-5 fold compared to depleted and un-depleted serum proteomics.