Proteograph: Efficient and Automated Multi-Nanoparticle Platform for Deep, Unbiased Plasma Protein Profiling and Protein-Protein Interaction Biological Insight

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Institute

REPRODUCIBLE AND **C**USTOM **M**ANUFACTURING



Different chemistries yield diverse but consistent NPs.

Specific protein corona formation is determined by unique chemistries on each NP surface (a, b, c). Scanning electron microscopy (d, e, f,) and dynamic light scattering (DLS, g, h, i) demonstrate that these NPs are highly reproducible in their physicochemical properties.

CONCLUSIONS

- 1. Proteograph enables deeper and higher throughput protein profiling in a simple and convenient format, enabling large-scale studies analogous to the genomics space.
- 2. Direct comparison to a typical proteomics profiling workflow highlights Proteograph's superior coverage, precision, and speed.

3. Exemplification demonstrates that high-throughput



PROTEOGRAPH

• ~7 hs turnaround time from sample to peptides • ~30m hands on time • To make high-throughput deep-proteomics practicable

PRECISION AND COVERAGE OF **NP**S

OHSU

Each NP can detect more proteins than neat plasma

Protein groups from protein corona of individual NPs measured in triplicate across a pooled sample (quantified by DDA LC-MS/MS, 1% protein and peptide FDR). Median count and standard deviations across triplicates are shown.

ACCURACY



Proteograph's NPs accurately measure differences across samples

Demonstrated with spike-recovery experiments and comparing measurements between MS signal intensities and ELISAs. Shown is the linearity of response for measurement for 4 peptides from C-reactive protein (CRP) across 2 orders of magnitude with an average slope of 0.9 (0.81-0.98, 95% CI). Similar results have been observed for 3 additional proteins.

DYNAMIC RANGE

Proteograph's NPs compress dynamic range of protein abundance enabling wide concentration range evaluation

Correlation of the maximum intensities of NP corona proteins vs. plasma proteins to the published concentration of the same proteins as determined by DDA LC-MS/MS in 3 representative NPs.

precision proteomics is both robust and efficient using Proteograph platform.



Proteograph offers significantly better protein coverage compared to alternative fractionation

method

4.1x more protein groups (1453 vs 351) were identified with Proteograph than the alternative high-pH fractionation method.

Proteograph allows better precision of measurements

CVs based on raw intensities were approximately 2x better than high-pH fractionation method.

Proteograph provides further insight into protein-protein interac-• 141 lung cancer and control tions (PPI) plasma samples 5NP panel A PPI map was constructed using the • Total time 2.5-weeks including STRING database and filtered for ~2 weeks LC-MS time (DIA) proteins previously observed in the blood proteome. Resulting map contained 21 clusters with >10 members identified. When mapping the protein groups, 15/21 and 1/21 interaction clusters were identified in which >10% of the members were covered.



Each NP retains comparable precision even with its increased coverage CV% distribution (precision) of 10 NPs and neat plasma (filtering for 3 out of 3

valid values across assay replicates).

Each NP's protein coverage is complementary between particles

1D annotation enrichment analysis comparing the protein intensity distribution of each NP against the average of all. 1D scores are plotted as a heat map for annotations.

EXEMPLIFICATION

any >=25%



Proteograph's NPs can interrogate a deeper proteome allowing more insights Ranked intensity plot matching a panel of 10 NPs and neat plasma to a plasma protein reference of MS intensities. Analysis shows that 5NPs achieve similar depth of coverage with 2x throughput.

~4x improvement in protein IDs vs depleted plasma Protein group counts by NP and depleted plasma. Each individual NP (green) yielded more IDs than depleted plasma

Detection Threshold (yellow) in the cohort of 141 subjects. 2499 protein IDs were found across the 5 NP panel and all 141 subjects (grey),

and 1992 protein IDs detected in at least 25% of all 141 subjects (blue).