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NRicher™ Bead Platform Provides Unique Sub-Proteome Biases Creating Fit For Purpose Opportunities for Biomarker Discovery and Targeted LC-MS Quantification

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Introduction

Proteomic innovation continues to drive the need for new biomarkers to support personalized healthcare. For biofluid proteomics, a constant challenge is to observe and quantify low to mid-abundance sub-proteomes as the preponderance of high abundance proteins (>top 100) can obscure analysis. Similarly, for targeted proteomic workflows, efficiency and consistency in quantifying target peptides from different sample cohorts presents challenges due to the changing landscape of proteins/peptides not associated with the selected targets. A solution for both these challenges is now available through a platform of products, collectively called **NRicher™,** based on over 10 years experience at the forefront of derivatizing porous silica beads (i.e., ionic, hydrophobic, hydrogen bonding, aromatic, polymeric) with differential proteome binding properties[1,2]. Several commercial products based on singular bead surface features have been derived from the fundamental **NRicher™** bead chemistry platform, notably **AlbuVoid™** and **HemoVoid™**[3,4]. Following the BSG lead to circumvent immuno-affinity, corona-based separations adapted to magnetic nano-particles has been introduced. However, this necessitates a specialized instrument-based workflow, and associated capital and consumable expense. Consequently, **NRicher™**, offers a solution that stands out in its simplicity and versatility. Currently, the **NRicher™** platform consists of 10 different singular surfaces and a variety of bead cocktails, with others in development. These have been qualified to provide differential sub-proteome biases and can be adapted to meet specialized requirements. To further the utility of **NRicher™** derived products, a deeper LC-MS data characterization of the binding biases towards certain families, pathways or structurally related features of the sub-proteomes enriched is advantageous. We now report on four of Biotech Support Group's product derived sub-proteomes in such contextual variety as a first-pass characterization of their differential binding biases, and highlight the utilities that can be attained therefrom.

Methods Four products were evaluated using pooled human Platelet Rich Plasma (PRP).
AlbuVoid™ - initially developed for Albumin voidance, and enrichment of the low abundance proteome from serum/plasma
AlbuVoid™ PLUS - two products in series, an optimized Protein A for IgG depletion, followed by AlbuVoid™
HemoVoid™ - initially developed for Hemoglobin voidance, and enrichment of the low abundance proteome from whole blood and red cells
NRicher™ Mx – a cocktail of two other **NRicher™** bead chemistries, for general low abundance enrichment, notable for soluble membrane proteome enrichment.

Sample Prep

50 µL of neat PRP pool was processed in each replicate. Enrichment methods follow the protocol from product sheets[3,4,5,6] with one modification. Bound proteins were eluted with 300 µL Reagent 4 (7M urea, 2M thiourea, 4% CHAPS, 50 mM Tris pH 8, 0.25 M NaCl). Recovered proteome fractions (or in the case of Neat, 50 µL non-enriched PRP pool). Total protein material was normalized after the bead treatments. 40 µg of all samples was carried forward for reduction, alkylation, digestion. Alkylated samples were precipitated with 8 volumes cold acetone, 1 volume cold MeOH, 60 min at -80C prior to isolation of the pellet by centrifugation. Pellets were washed with three 250 µL aliquots cold MeOH and resuspended in 50 µL Digestion Buffer (50 mM Tris, 0.75 M urea, 0.1% NaDOC, 1.33 µg Trypsin/LysC per 100 µL). Predigestion proceeded for 2 h at 37C. A second 50 µL aliquot of Digestion Buffer was added to each sample and digestion proceeded overnight at 37C. Digested samples were quenched with 2.5% formic acid and NaDOC was removed by centrifugation. Acidified digests were cleaned by a reversed phase SPE using a Strata-X microelution 2 mg/well standard protocol. Samples were dried under liquid nitrogen and stored at -80C prior to resuspension for LC-MS. Immediately before LC-MS acquisition, samples were resuspended in 40 µL 3% DMSO, 0.2% formic acid, transferred to HPLC vials and centrifuged.

LC-MS Analysis

Peptides were injected on TripleTOF6600 LC-MS (Sciex), on a 45 minute gradient, in SWATH acquisition mode. Library was built with DIANN software using library-free fasta search mode at an FDR of 0.01 and "matching between runs" within each treatment group. Data integration was done on each group (kit/preparation) separately with DIANN using this generated library. Total protein and peptide identifications were extracted prior to imputation of missing values. Precursor signals were normalised using RT-LOESS and missing values were imputed using K- nearest neighbour. All peptides were retained.. Protein quantities used to calculate fold changes between groups represent the summed MS2 signal from the Top 5 associated precursors. Protein metadata was extracted from Uniprot based on the most likely protein accession from each identified protein group..Venn diagrams were generated using Venny Bioinfographics and Interactivenn. Protein abundances were mapped against the paxdb abundance database to estimate the relative coverage of low abundance proteins from each workflow. Gene ontology (GO) analysis was performed using Cytoscape on the genes whose proteins exhibited a significant positive fold change (p-value <0.05) between enriched and neat samples. Signal intensity clustering analysis was performed on protein z-scores using the Seaborn application within Allumiqs' in-house Python analysis pipelines.

High abundance (i.e., Albumin) proteins selectively pass or void through

the beads, concentrating and enriching sub-proteomes on the beads

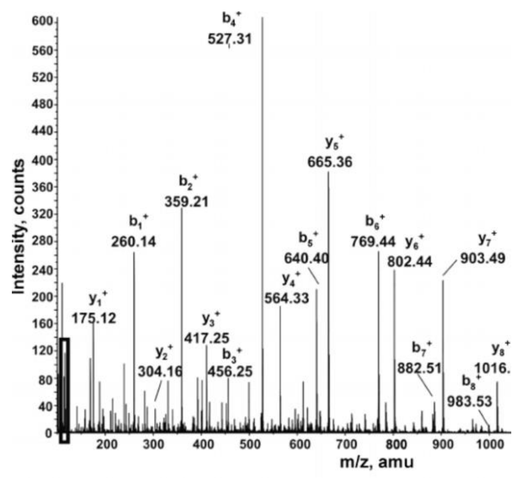
NRicher™ workflow

Features simple, fast bind/wash/elute protocol
Standard Laboratory Equipment
No Specialized Instruments

Other Options (not evaluated here)

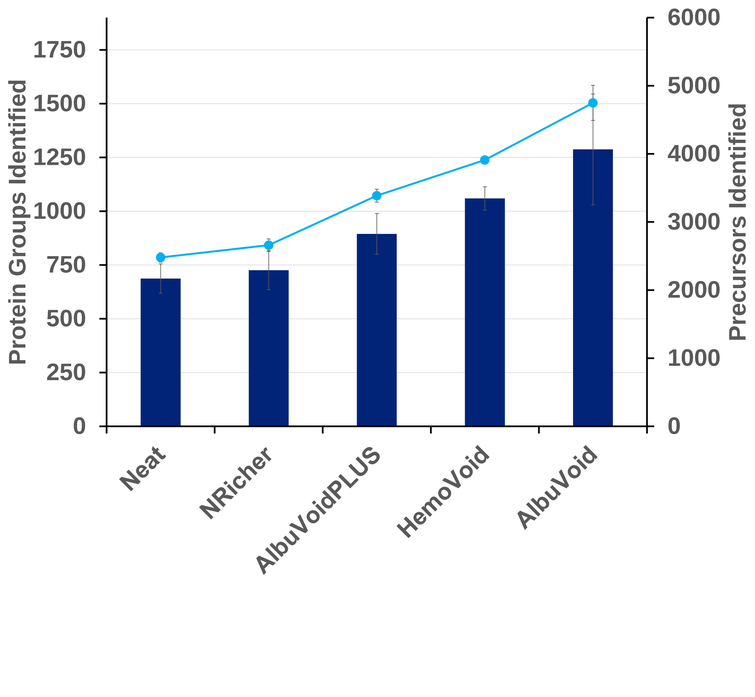
>On-bead digestion

>Eluates applied to other proteomic analyses:
•Enzymatic/Functional assays
•2DE
•ELISA/immunoassay

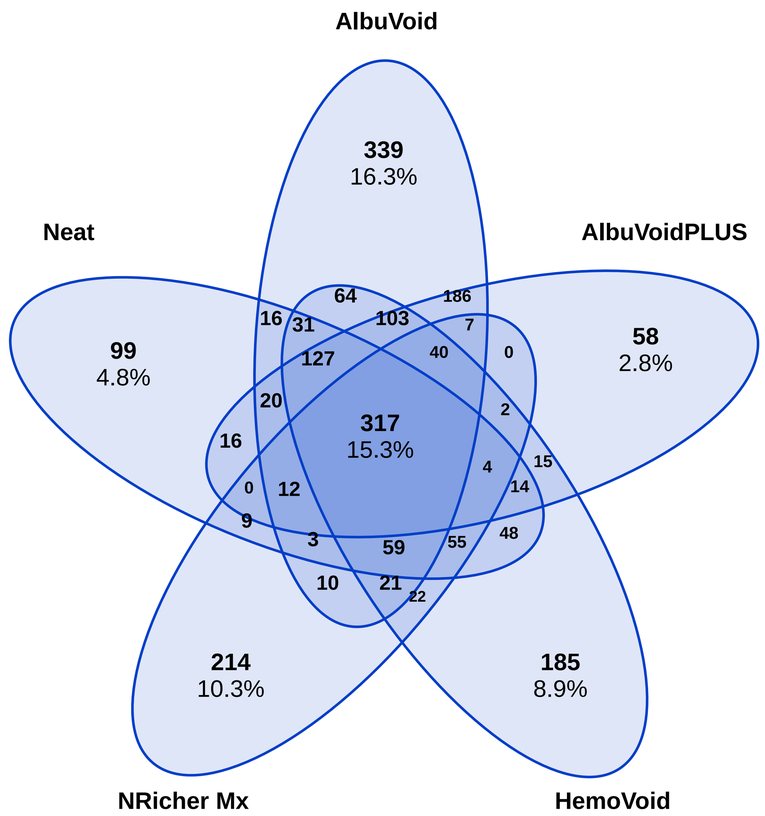


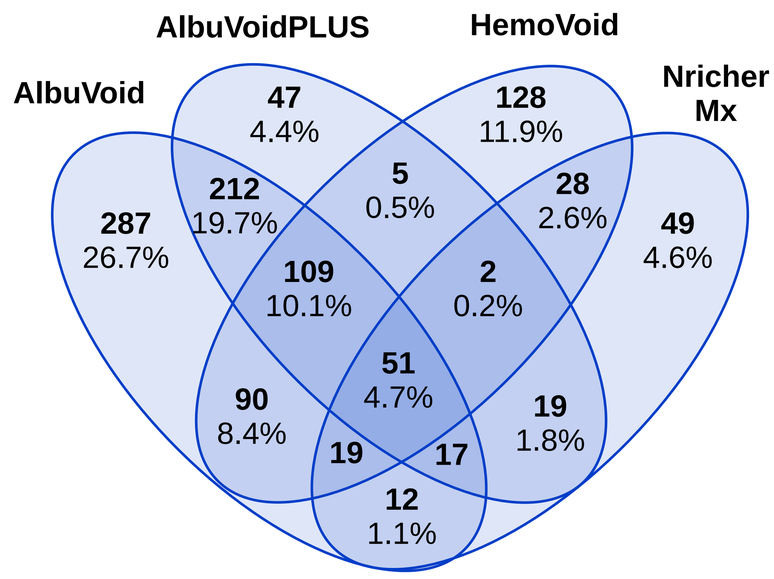
LC-MS

Total identifications from each sample prep and neat PRP (no enrichment)

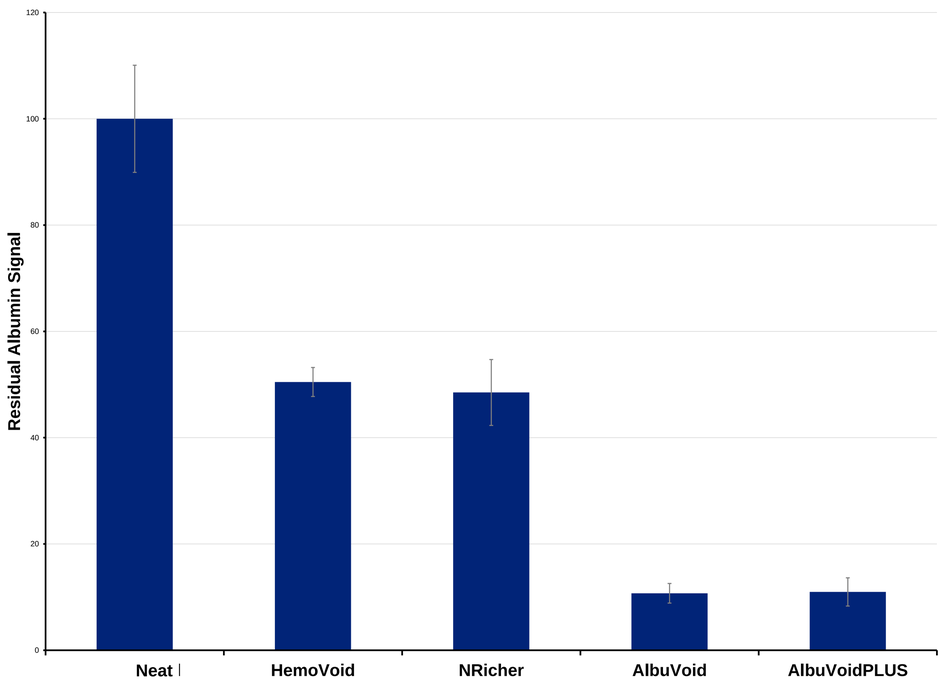


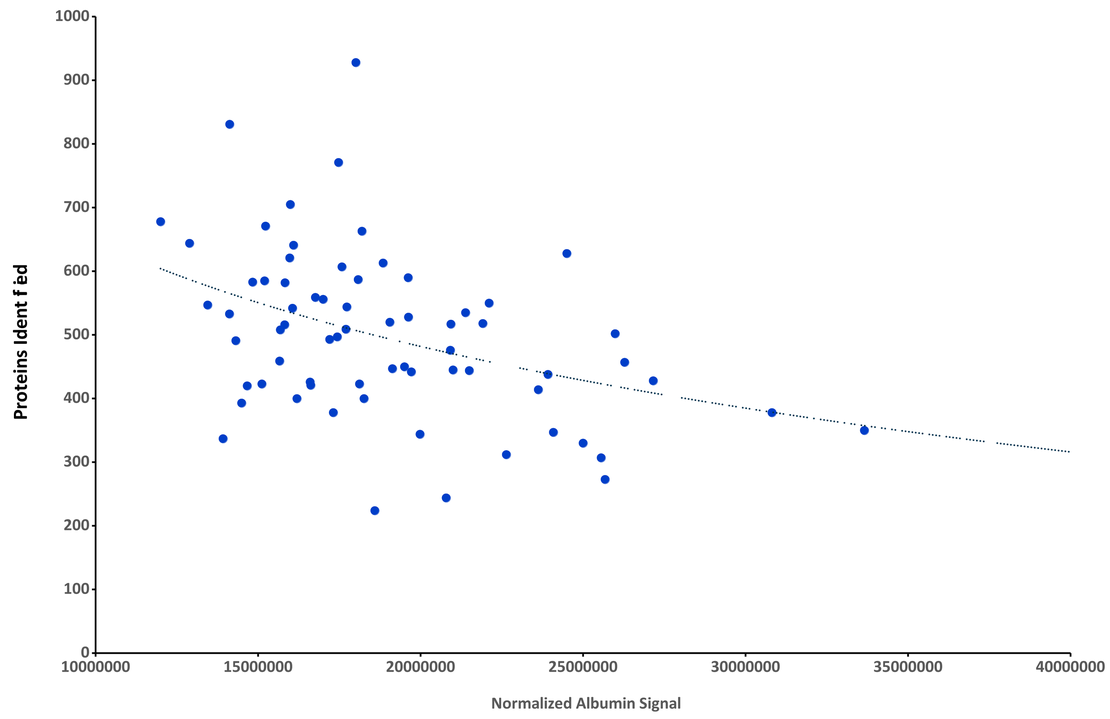
Of the 4 enrichment strategies, AlbuVoid & AlbuVoidPLUS enhance proteome coverage the most relative to neat PRP. Each prep provides differential coverage.



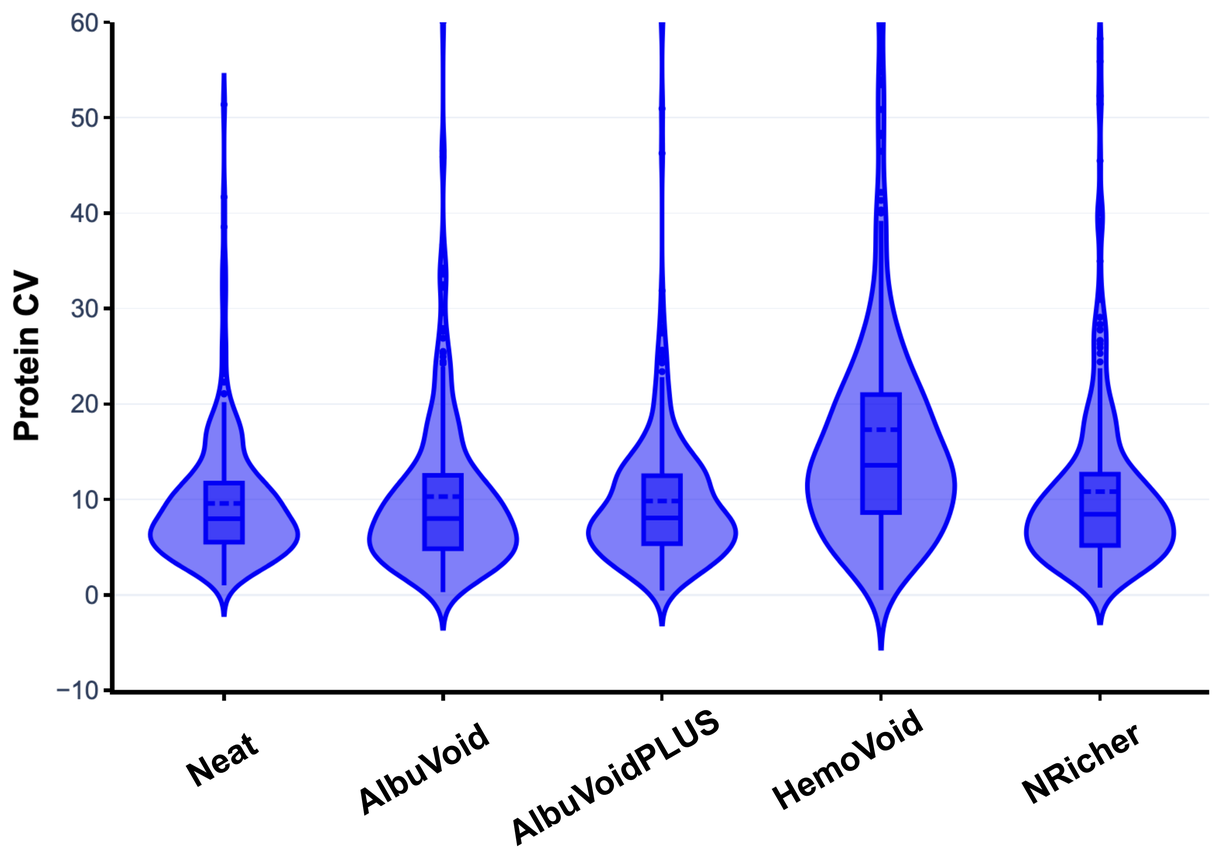


Lower albumin signal correlates with greater protein identifications





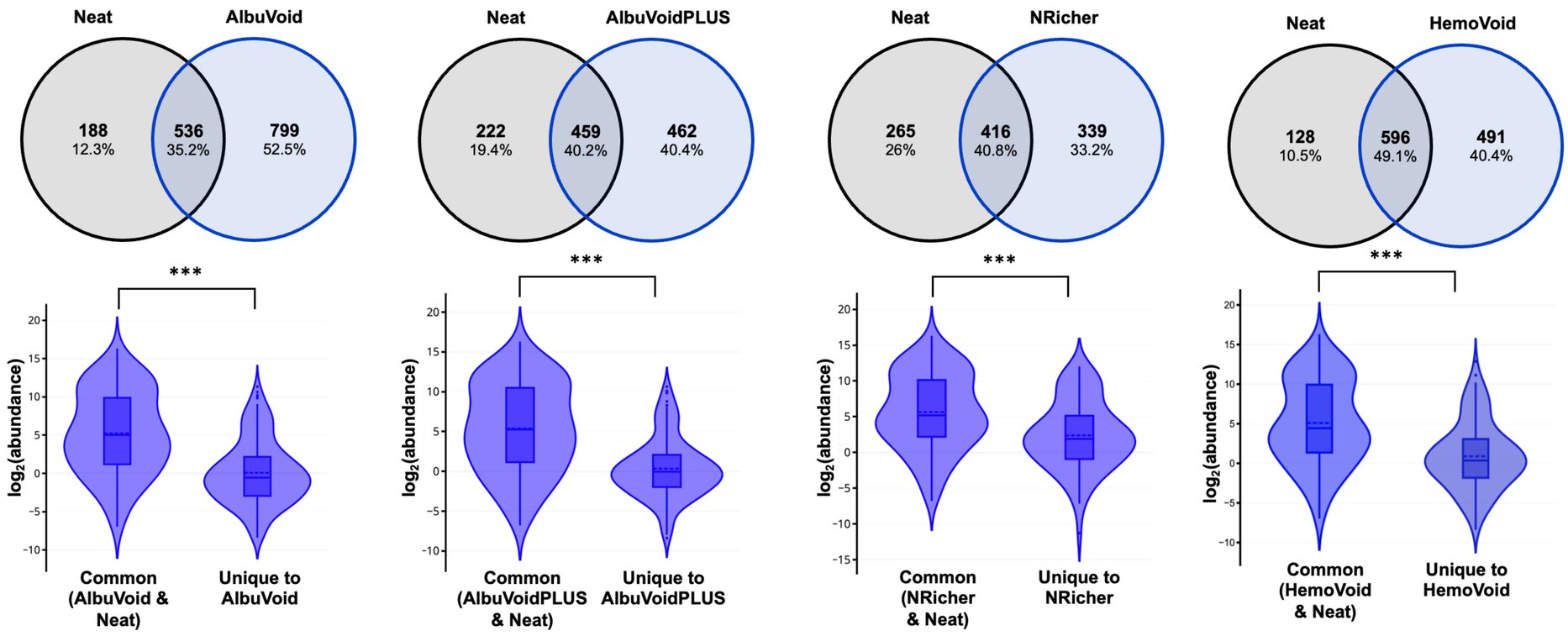
CV distributions of commonly identified proteins, excluding the Top 100 ranked by mean Neat intensity



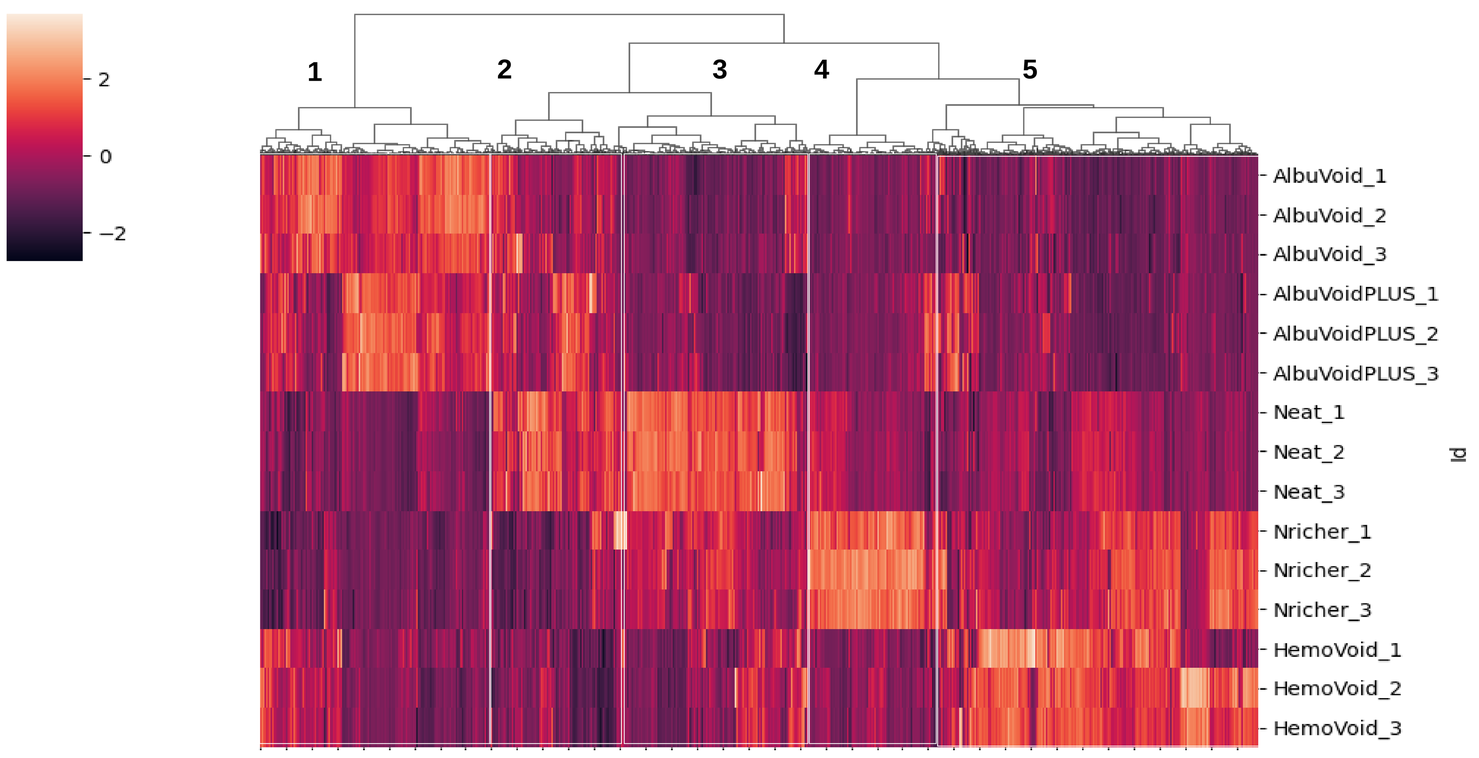
	Mean CV	Median CV	Low Abundance Coverage*
Neat	9.6	8.0	-
HemoVoid	17.3	13.6	+24%
NRicher Mx	10.8	8.4	+10%
AlbuVoidPLUS	9.8	8.0	+20%
AlbuVoid	10.3	8.0	+30%

Proteins with low abundance <10 ppm (800 ng/mL, assuming 80 mg/mL plasma protein)

Bead-based enrichment improves coverage of low-abundance proteome

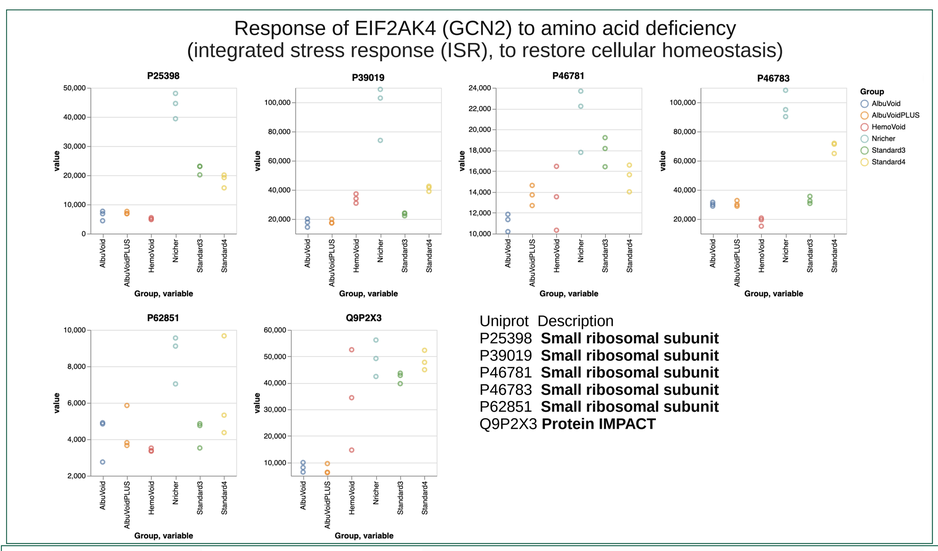


Bead-based enrichment shows signal intensity cluster biases, indicating quantitative differences between enrichments

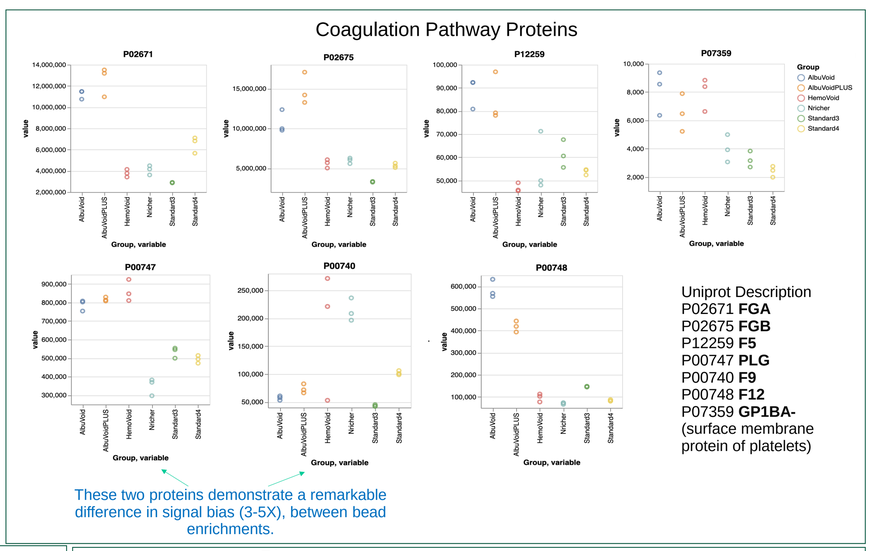


All observed proteins

Enrichment of Pathways Associated with Complement & Coagulation Cascades, Lipoproteins, Stress Response



Uniprot Description
P25398 **Small ribosomal subunit**
P39019 **Small ribosomal subunit**
P46781 **Small ribosomal subunit**
P46783 **Small ribosomal subunit**
P62851 **Small ribosomal subunit**
Q9P2X3 **Protein IMPACT**



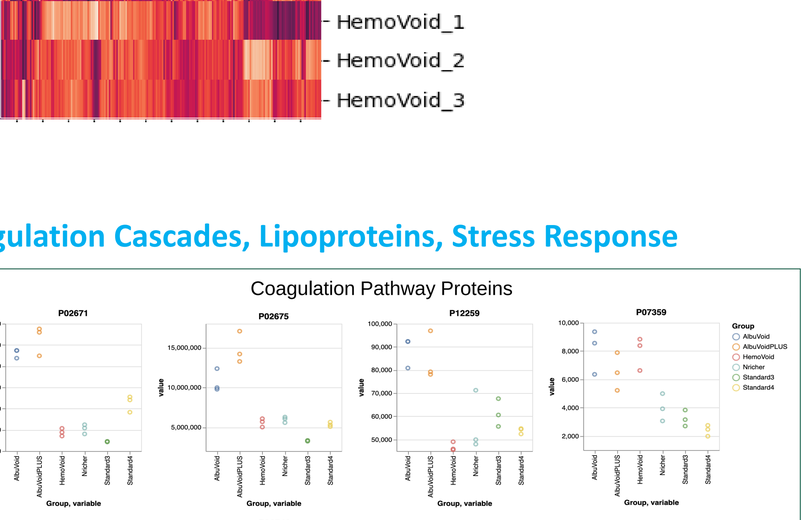
These two proteins demonstrate a remarkable difference in signal bias (3-5X), between bead enrichments.

Uniprot Description
P02671 **FGA**
P02675 **FGB**
P12259 **F5**
P00747 **PLG**
P00740 **F9**
P00748 **F12**
P07359 **GP1BA-** (surface membrane protein of platelets)

Complement Cascade

P00736 **C1R**
P02745**C1QA**
P02746**C1QB**
P02748 **C9**
P06681 **C2**
P07357 **C8A**
P07358 **C8B**
P07360 **C8G**
P04004 **Vitronectin**
P09871 **C1S**
P10643 **C7**
P13671 **C6**

Plasma lipoprotein assembly, remodeling and clearance



P02647 **APOA1**
P02649 **APOE**
P02656 **APOC3**
P06727 **APOA4**
Q13790 **APOF**

Several proteins are signal depleted by AlbuVoid and enriched in HemoVoid & NRicher, demonstrating the quantitative biases between bead chemistries

Results and Discussion Signal intensity cluster analysis demonstrates low abundance enrichment of many proteins, quantifiably differentiated by both signal and bead chemistry.

Pathway analysis demonstrates the binding biases of different beads towards different pathway sub-proteomes, relative to each other and to untreated (neat).

The Applied Utility for Cluster/Pathway Analysis

For known pathways of interest, drug development biomarkers for example, the knowledgebase of enriched pathway sub-proteomes can be utilized to acquire quantitative data surrounding pathway regulation. For serum and plasma, biological pathway regulation is often achieved through proteolytic processing of parent proteins; Complement and Coagulation being good examples of this phenomena. To measure these changes in disease, peptide level monitoring of the split regions is a viable strategy. Through proper selection of **NRicher™ beads,** multiplex peptide targets from specific functional regions, such as spanning regions of proteolysis, truncations, amino acid variants or other PTMs can be explored in depth. This extends research beyond strict abundance level quantitation, to more discretionary functional levels. Protein level enrichment can serve to enhance signal to noise of the functionalities associated with post-translational modifications (PTMs).

For discovery proteomics, bead-based enrichment casts a wider net of pathway observations, to compare samples, normal vs. disease, disease vs. disease, etc.

Bead-based enrichment of the low abundance sub-proteome (<top 100) produces equivalent CV to neat, while concurrently expanding coverage; beneficial when comparing relative signal intensities between samples. For this, **NRicher™- derived sub-proteomes** ultimately generate better peptide signal to noise levels, with reduced acquisition time and enhanced productivity, compared to neat (not enriched) serum or plasma. This is achieved with simple methods and at economy for high throughput investigations.

Highlights

The **NRicher™** bead chemistry platform provides a new tool kit for proteomic enrichments. Currently, 10 bead chemistries are available, another 10 are being pre-qualified and are expected to be added by year end.

Each of the individual beads has differentiated sub-proteome binding biases, applicable towards any discovery or targeted goal; for example, proteins within functional pathways or multiplex biomarkers.

The beads can be supplied separately or in cocktail combinations, to support specialized sample prep goals in simple, cost-efficient workflows.

References

1. Wan, C., Borgeson, B., Phanse, S. *et al.* Panorama of ancient metazoan macromolecular complexes. *Nature* 525, 339–344 (2015). https://doi.org/10.1038/nature14877
2. Oka, Amita R., et al. "Functional proteomic profiling of phosphodiesterases using SeraFILE separations platform." *International Journal of Proteomics* 2012 (2012).
3. https://www.biotechsupportgroup.com/AlbuVoid-Albumin-Depletion-and-Low-Abundance-p/avk.htm
4. https://www.biotechsupportgroup.com/HemoVoid-Hemoglobin-Depletion-From-Erythrocytes-p/hvk.htm
5. https://www.biotechsupportgroup.com/v/vspfiles/templates/257/pdf/NRicherMxProductSheet091823.pdf
6. https://www.biotechsupportgroup.com/AlbuVoid-PLUS-p/np-avk.htm