



NRicher™ Beads for Targeted Serum Proteomic Biomarker Enrichment, Simplified and Diversified Workflows



BIOTECH SUPPORT GROUP
Sample Prep that Matters

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Introduction

Targeted proteomics is emerging as a foundation of next-generation clinical testing. As opposed to discovery proteomics, targeted proteomics focuses on pre-identified proteins —ones already implicated in disease — and seeks to quantify them precisely and reproducibly. However, biological fluids like serum/plasma present a technical hurdle due to the overwhelming influence of high abundance proteins and the changing sample landscape of proteins/peptides not associated with the selected targets. This results in ion suppression, spectral overlap, background noise and compromised clinical utility.

Methods

The NRicher™ platform chemistry addresses this challenge using proprietary bead chemistries derived from experience of over 15 years at the forefront of manufacturing beads (i.e., ionic, hydrophobic, hydrogen bonding, aromatic, polymeric) with differential proteome binding properties. NRicher™-based platform has derived commercial products including **AlbuVoid™**, **HemoVoid™** and related fit-for purpose kits. Over 30 journal articles using these products have been published. Now, NRicher™ based products provide an exceptional sample prep toolkit for targeted proteomics.

Proteoform Diversity: Human proteoform diversity is essential for normal biological processes and therefore proteoform measurements can be essential to understanding disease. Proteoforms are generated through three main processes—genetic variation, alternative splicing of RNA, and PTMs—to yield a variety of protein structures relative to the canonical protein structure (i.e., the “wild-type” protein).

As the characterization of different proteoforms on immuno- or ligand binding assays is challenging, alternate analytical approaches are available. Owing to its excellent molecular selectivity, mass spectrometry has become an attractive approach to measure protein biomarkers consisting of different proteoforms. While conventional ligand-binding assays measure a mixture of different proteoforms generating a weighted average concentration of the various proteoforms, LC-MS-based methods can distinguish different proteoforms and selectively quantify individual proteoform features.

Genetic variants over-expressed (i.e., Prothrombin G20210A), or under-expressed (i.e., alpha-1-antitrypsin deficiency) are examples of clinical consequence. Both genetic and other post-translational (PTM) variants in certain regions can impart function changes, even if expression levels are not necessarily affected. So, in the context of proteoform characterization, two key strengths of LC-MS, are the observation and quantification of specific functional regions (tryptic peptides), and its multiplexing capacity. As a result, LC-MS derived proteoform research can target select proteoforms or groups of proteoforms so as to generate precision medicine biomarkers. We advance strategies to utilize unique target enrichments for this purpose.

Typically, a heterozygous single-site variant results in the production of **two different protein isoforms**, the canonical functional sequence from the wild-type allele and a protein with a single amino acid substitution from the variant allele that may be **expressed in different amounts**. Genetic variants over-expressed (i.e., Prothrombin G20210A), or under-expressed (i.e., alpha-1-antitrypsin deficiency) are examples of clinical consequence. The resulting functional consequences may range from nothing significant to altered function, depending upon the specific type, expression level and location of the variant (i.e., ApoE4/E2). Targeted proteomic analysis can often distinguish these AA variant amounts and can thus correlate to disease.

Proteolytic cleavage can activate a protein through processing of a precursor protein (i.e., Prothrombin to Thrombin) or introduce a protein into a sample through proteolytic degradation (i.e., soluble membrane proteins). This is especially important for blood-based biomarkers as proteolytic truncations become the triggers for activation (i.e., coagulation and complement) and functional dysregulation (i.e., Serpins). Strict reliance on abundance-based measurements cannot account for these essentially important functional variants that greatly impact innate immunity, and chronic inflammation.

NRicher™ Serum/Plasma Knowledgebase for Targeted Proteomics

Purpose: Researchers can determine whether pre-identified protein targets of interest can be enriched by one or more NRicher™ beads; user defined targets may come from sources such as:

- Discovery proteomics
- Gene Expression
- Curated from public domain databases and publications

Function of the Knowledgebase

• Over 2000 proteins observed with corresponding relative MS signal intensities • Find your protein(s) of interest, and corresponding bead/method to best enrich for protein(s) of interest, free to review, accessible on a non-confidential basis • Annotation of 200 soluble membrane proteins, derived from ectodomain shedding, a common dysregulated disease mechanism • Annotation of 170+ prospective biofluid biomarkers associated with disease conditions, noting the optimal products for enrichment, and published references for the biomarker

Why Choose NRicher™

For over 15 years BSG has been at the forefront of developing synthetic beads (i.e., ionic, hydrophobic, hydrogen bonding, aromatic, polymeric) with differential proteome binding properties.

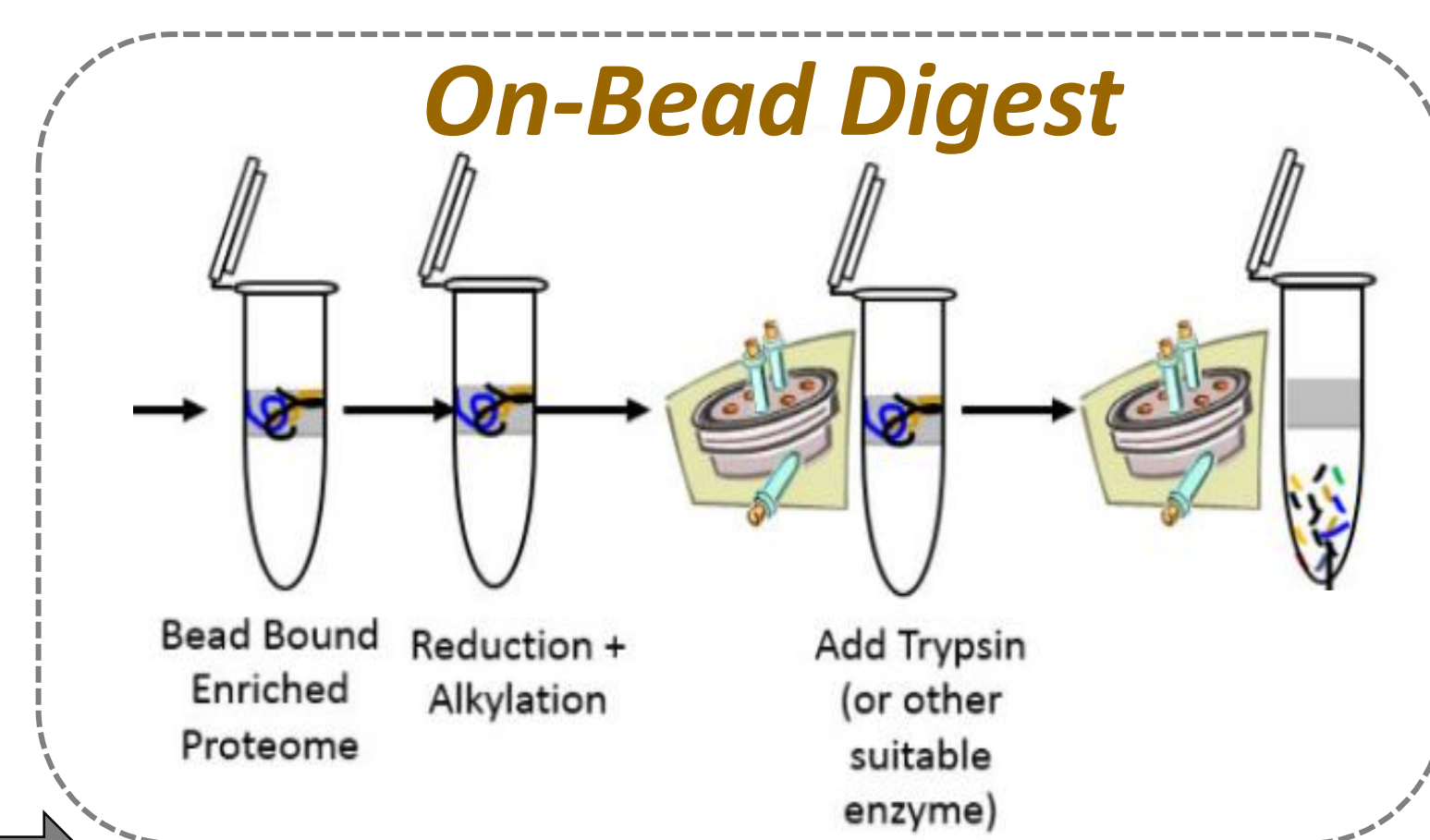
The NRicher™ Advantage

- Not derived from immuno-affinity: NRicher™ beads, are not species-specific. This allows wide applicability across various sample types.
- Cost-efficient: There's no need for an investment in high-end specialized equipment; a standard laboratory microfuge will suffice.
- Streamlined Analysis: Through a selected use of bead cocktails, NRicher™ products serve virtually all applications and analytical platforms, starting with sample volumes as low as 25 µl.
- On-bead digestion (optional): BSG pioneered Bead-Assisted Sample Prep (BASP™), offering workflow efficiencies (i.e., no added denaturants) for LC-MS proteomics of bead enriched sub-proteomes. •
- Format versatile: Beads can be purchased as part of catalog item kits, or in bulk; adaptable to 96-well and pipette tip formats

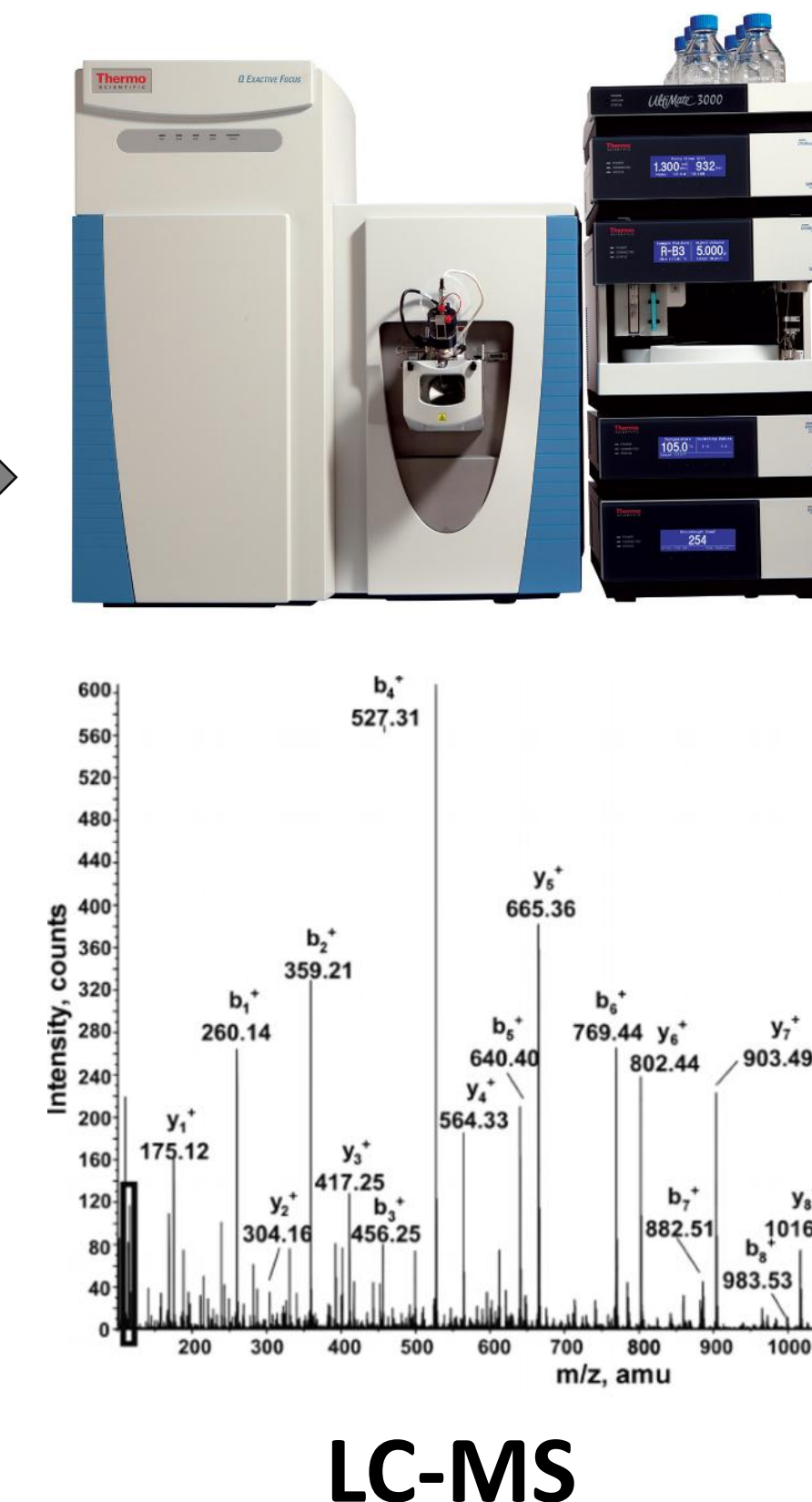
NRicher™ workflow
High abundance (i.e., Albumin) proteins selectively pass



through the beads, enriching sub-proteomes on the beads



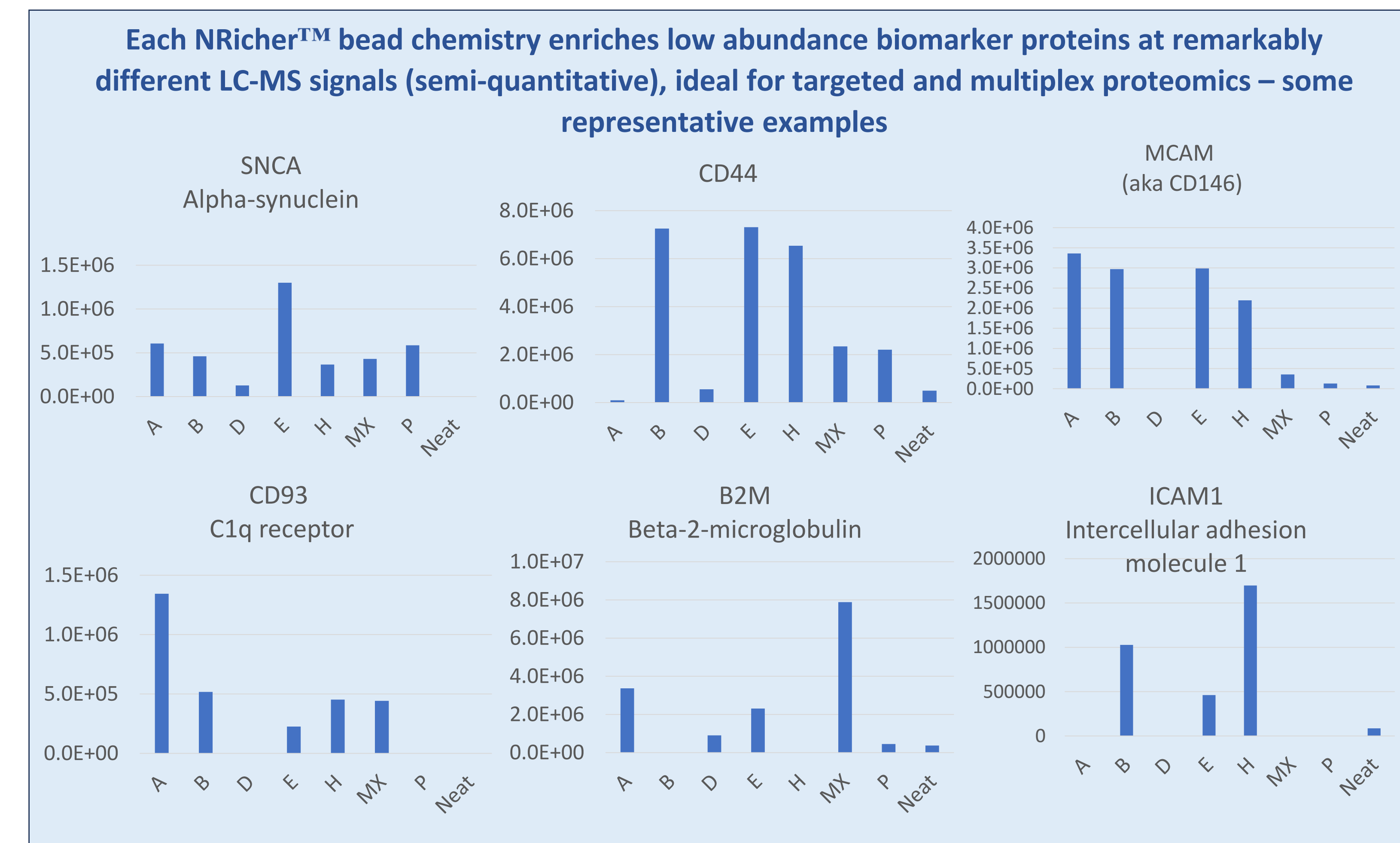
Proteomic Analytical Options
Eluates from NRicher™ beads can be applied to other common analyses:
• Enzymatic/Functional assays
• 2DE
• ELISA/immunoassay



Results

LC-MS analyses were conducted at the Rutgers Center for Integrative Proteomics. We report on the efficiency and selection bias properties of 7 different NRicher™ bead chemistries. The accumulation of the identifications and relative signal intensities are reported within an open access Knowledgebase of >2000 serum proteins, most of which are not observable without enrichment.

Each NRicher™ bead notated as A,B,D,E,H,MX,P has a different surface chemistry and sub-proteome selectivity profile; with a substantial depletion of Albumin. Note that NRicher™ “A” is the BSG product called AlbuVoid™. A selection of one or more NRicher™ beads can be customized to meet the application requirements. Beyond the selection of bead chemistries, further optimization can be achieved through process protocols, such as load and bind/wash buffer adjustments, to which we can provide further guidance.



After NRicher™, target peptides have enhanced spectral signal, even as gradient times are reduced. NRicher™ sub-proteome enrichment can minimize acquisition time, collectively improving overall throughput, cost, and outstanding gains in productivity. Specific target peptides that report functional (PTM) or gene variant regions promise actionable insights and potential multiplex biomarkers for disease. NRicher™ methods offer scalability, automation compatibility, and cost-effectiveness. If you do not see your protein(s) of interest, please contact us. We also supply a trial screening kit that contains 4 different bead chemistries, each with substantial Albumin removal.

Representative Reporting Signals from the NRicher™ Knowledgebase

Uniprot Identifier	Gene Name	Relative Total Signal Intensities corresponding to each NRicher Beads, LC-MS DIA acquisition						
		A	B	D	E	H	MX	P
P13473	LAMP2			1.3E+05		1.1E+05	2.3E+05	1.5E+05
P24593	IGFBP5	5.5E+05	7.6E+05	1.4E+05	4.7E+05	5.8E+05	2.3E+06	5.3E+05
P98160	HSPG2	6.0E+06	7.9E+06	3.1E+06	9.4E+06	1.1E+07	2.0E+06	8.6E+06
O94919	ENDOD1				1.1E+05	1.0E+05		
P58546	MTPN		1.8E+05	1.3E+05				2.4E+05
P07900	HSP90AA1	1.8E+06	2.3E+06	4.2E+06	2.5E+06	1.7E+06	6.4E+06	7.2E+06
P40925	MDH1	1.3E+05		8.6E+05	1.1E+06	1.7E+05	4.2E+05	1.7E+06
Q14766	LTBP1	1.1E+06	4.0E+05	5.8E+05	5.6E+05	5.9E+05	1.8E+05	1.1E+06
P23470	PTPRG				4.3E+05			
P31946	YWHAB	6.7E+04	1.6E+05		2.9E+05			
P01344	IGF2	3.3E+06	5.9E+06	3.0E+06	4.8E+06	7.9E+06	5.5E+06	1.1E+06
P13224	GP1BB			3.1E+05		1.6E+05	8.9E+05	3.4E+05
P05362	ICAM1		1.0E+06		4.6E+05	1.7E+06		

Conclusions

The NRicher™ Bead Chemistry Platform, is a unique proteome subfractionation technology, not based on immuno-affinity, achieving simple, cost-effective workflows, without the need for HPLC or additional capital expenditures.

To enhance efficiency and productivity in targeted proteomic workflows through precise sub-proteome enrichment, researchers can leverage specialized bead chemistries tailored to specific applications, while further process refinements will streamline workflows, reduce acquisition time, and improve cost-effectiveness. Our open access NRicher™ Knowledgebase supports this opportunity. Each NRicher™ bead chemistry enriches low abundance biomarker proteins at remarkably different LC-MS signals, ideal for targeted and multiplex proteomics.

This addresses a key challenge in proteomics: the inability of current ‘omic’ platforms to distinguish functional proteoforms and pathway outcomes. There remains a conventional proteomic assumption that all protein functions correlate to relative abundances, a carry-over viewpoint from gene expression. However, this assumption can be egregiously misleading regarding functional outcomes. The NRicher™ technology is designed to encourage the investigation of differentiated functional post-translational modifications (PTMs), and non-canonical variant regions, as well as imbalances due to activating and regulating sub-proteomes of innate immunity and chronic inflammation.

Let us assist in new research avenues for biomarker discovery, and personalized medical decisions, overcoming the limitations of strictly counting proteins as a benchmark. For more information and to download the Knowledgebase, visit <https://www.biotechsupportgroup.com/category-s/340.htm>