Translational Research

In-depth plasma proteome profiling for high-throughput biomarker discovery using PreOmics® Kits and Thermo Scientific™ Orbitrap™ Astral™ Mass Spectrometer

Jared Deyarmin[1], Qingling Li[1], Jana Richter[2], Stephanie Samra[1]

[1]Thermo Fisher Scientific, San Jose, CA, USA, 95134; [2]Thermo Fisher Scientific (Bremen), GmbH, Bremen, Germany

Introduction

Plasma proteomics is key for biomarker discovery and clinical research due to its ease of collection and potential for new findings. Plasma contains signaling molecules and materials from diseased tissues, making it valuable for disease diagnosis, monitoring, and precision medicine. Mass spectrometry allows direct, unbiased analysis of thousands of plasma proteins. However, profiling is challenging due to the complexity and vast range of protein concentrations, spanning 12 orders of magnitude, where high-abundance proteins often mask low-abundance protein detection. To address this, sample preparation and mass spectrometry techniques have evolved.

To overcome scalability and increase depth of plasma proteome coverage needed for large clinical cohort biomarker discovery, PreOmics® GmbH has developed the in-StageTip (iST) and enrichment (ENRICH) technologies to offer reproducible, streamlined sample preparation for measuring the plasma proteome at scale using liquid chromatography mass spectrometry (LC/MS). In combination with the Thermo Scientific™ Vanquish™ Neo UHPLC system coupled to the Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer, we demonstrate the capabilities and power of coupling PreOmics and Orbitrap Astral mass spectrometer technologies for biomarker discovery workflows.

To further characterize the synergies of combining PreOmics sample preparation technologies with the Orbitrap Astral mass spectrometer in a biomarker discovery framework, we evaluated 3 different sample preparation workflows (PreOmics iST-BCT, PreOmics ENRICH-iST, and PreOmics ENRICHplus) using a small disease cohort with age, gender, and ethnicity-matched controls **(Figure 2).**

Experimental procedure

Sample preparation

Age, gender, and ethnicity matched biologically diverse samples (n=12) were obtained from BioIVT. Samples were thawed on wet ice, spun for plasma clarification, then processed using PreOmics iST-BCT, ENRICH-iST, and ENRICHplus sample preparation kits. Healthy donors were processed in triplicate to evaluate preparative technical reproducibility. Digested neat and enriched plasma samples were then evaporated using Thermo Savant™ speed vacuum concentrator, resuspended in 98% Optima grade Water, 2% Optima grade Acetonitrile in 0.1% Formic Acid. Peptides were quantitied using Peptide Fluorometric Quantitative Assay, then normalized to 75ng/uL prior to loading onto LC/MS.

Liquid chromatography-mass spectrometry parameters

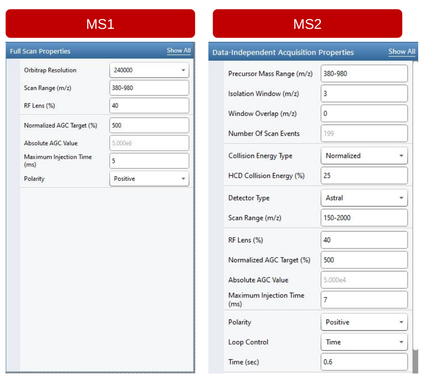
The Thermo Scientific™ Vanquish™ Neo™ UHPLC was used with the EASY-Spray™ HPLC ES906 column with a trap-and-elute injection configuration. Samples were separated using a 60 sample per day (SPD) chromatographic method with a 23-minute gradient **(Figure 1A)**. Column temperature was kept at 50˚C. Mobile phase A was 0.1% formic acid in H_2O and mobile phase B was 0.1% formic acid in 80% Acetonitrile. The critical narrow range data independent acquisition (DIA) MS1 and MS2 mass spectrometer parameters are shown in **Figure 1B**. Five hundred nanograms of eluted peptides were analyzed on an Orbitrap Astral mass spectrometer using all 3 sample preparation methods **(Figure 1C).** All data analysis was performed using Spectronaut® 19 software (Biognosys AG).

Figure 2. Liquid chromatography-mass spectrometry settings and TIC chromatogram for 60 SPD method deployed on 3 sample preparation strategies. (A). 60 SPD peptide separation column gradient using EasySpray ES906 column (150µm x 15cm, 2µm pore size). **(B).** Orbitrap Astral MS1 and MS2 scan parameters.**(C).** TIC of eluted peptides from 60 SPD triplicate matched healthy pool technical injections across 3 plasma sample preparation methods.

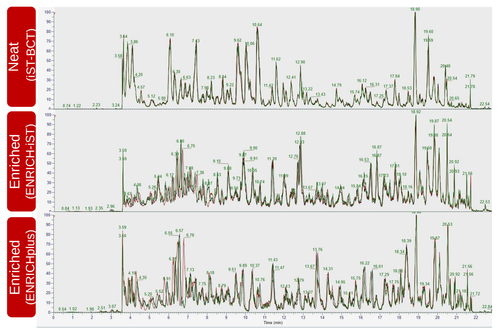
(A)

No	Time	Duration [min]	Flow [µl/min]	%B	Volume [µl]	No. of Column Volumes
1	0.000	Run				
2	0.000	0.000	2.000	10.0	0.00	0.00
3	0.300	0.300	2.000	10.0	0.60	0.34
4	0.600	0.300	0.800	10.0	0.42	0.24
5	13.600	13.000	0.800	22.5	10.40	5.86
6	20.500	6.900	0.800	35.0	5.52	3.11
7	20.900	0.400	2.000	55.0	0.56	0.32
8	20.900	Column Wash				
9	20.950	0.050	2.000	99.0	0.10	0.06
10	22.350	1.400	2.000	99.0	2.80	1.58
11	22.350	Stop Run				
12	22.350	Column Equilibration				

(B)



(C)



Experimental design

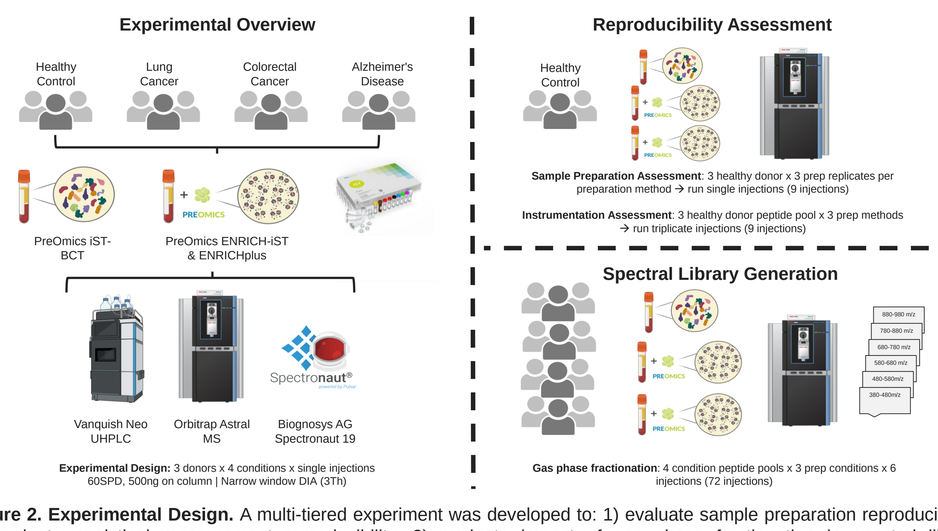


Figure 2. Experimental Design. A multi-tiered experiment was developed to: 1) evaluate sample preparation reproducibility, 2) evaluate analytical measurement reproducibility, 3) evaluate impact of gas phase fractionation in spectral library generation, and 4) evaluate capabilities of detecting differentiating biological signal in clinically relevant and representative plasma samples.

Sample preparation reproducibility

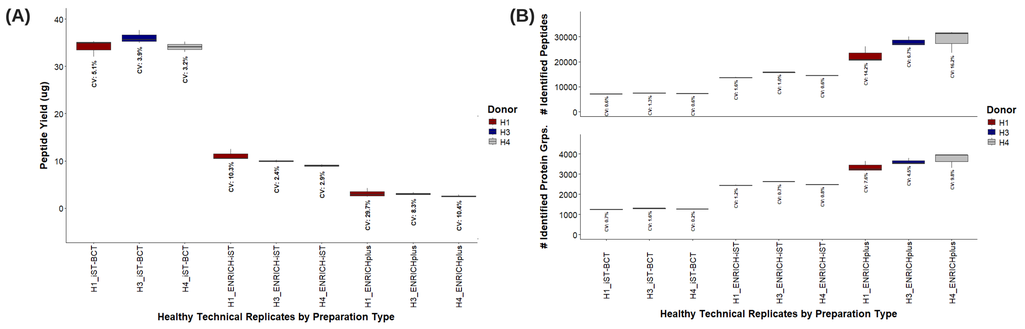


Figure 3. Evaluation of sample preparation reproducibility. (A). Sample preparation total yield across healthy donor technical triplicates across iST-BCT (neat plasma), ENRICH-iST, and ENRICHplus (enriched plasma) preparation methods. (B) Peptide and protein group identification reproducibility from 3 independent sample preparation technical triplicates.

Analytical measurement reproducibility

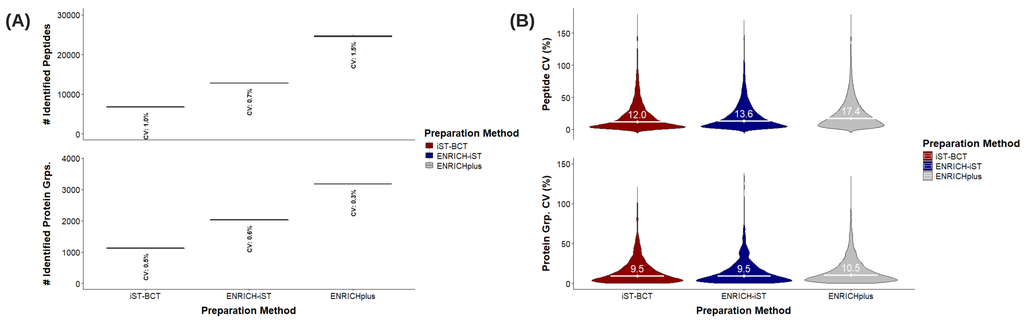


Figure 4. Evaluation of analytical measurement reproducibility. (A). Triplicate technical injections of healthy donor peptide pool peptide and protein group identification reproducibility across sample preparation methods. **(B).** Peptide and protein group coefficients of variation (%CV) across sample preparation workflows.

Spectral library assembly

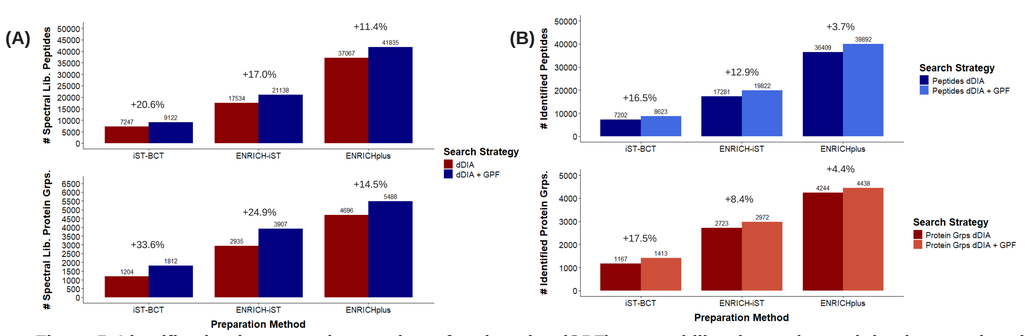


Figure 5. Identification increases in gas phase fractionation (GPF) spectral libraries and search implementation. (A). Peptides and protein groups in spectral library using library free and GPF assisted libraries across sample preparation methods. **(B).** Peptide and protein group identifications across experiment including biological samples using library free and GPF assisted libraries across sample preparation methods.

Assay identification performance

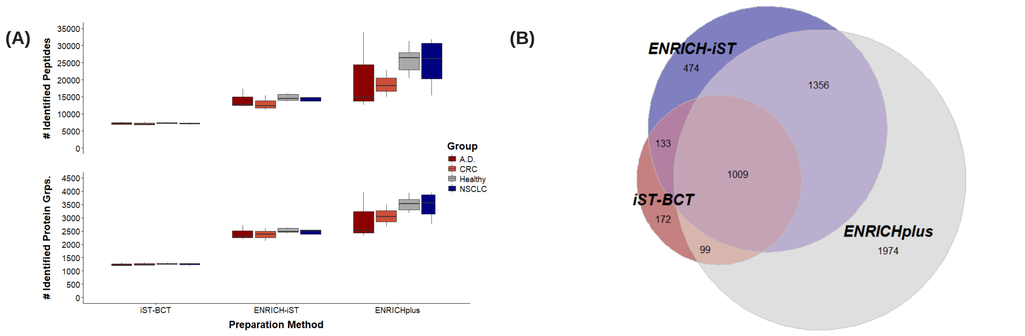


Figure 6. Identifications across experiment using biological samples and complementarity of sample preparation methods. (A). Peptides and protein group identifications across various biological samples and sample preparation methods. **(B).** Protein group overlaps and complementarity across all identified protein groups identified using each sample preparation method.

Assay Characterization

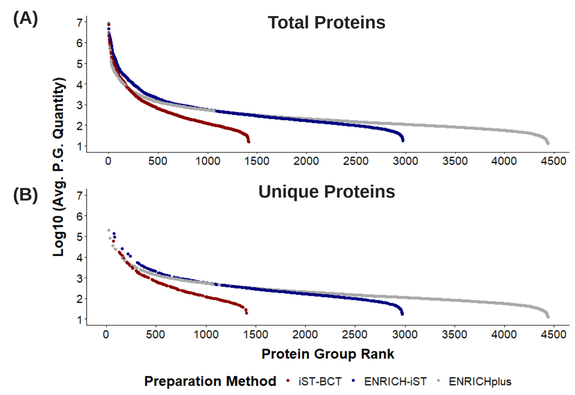


Figure 7. Protein Group Rank plots across sample preparation methods (A). Protein group rank plot across each sample preparation method. **(B).** A protein group rank plot with all overlapping protein groups identified across multiple methods removed, demonstrating unique protein groups for each sample preparation method.

Biological Differentiation

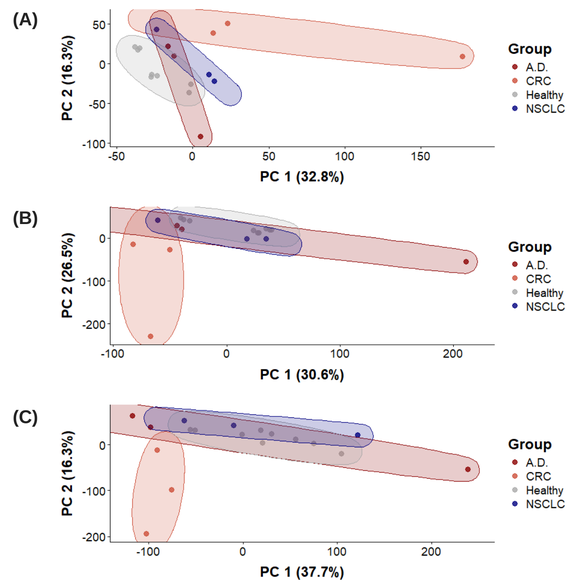


Figure 8. Principle Components Analysis (PCA) across sample preparation methods. (A). iST-BCT. **(B).** ENRICH-iST. **(C).** ENRICHplus.

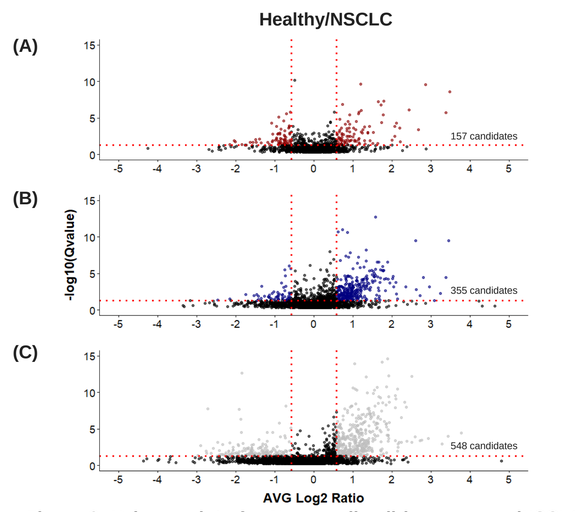


Figure 9. Volcano plots for Non small cell lung cancer (NSCLC) across sample preparation methods. (A). iST-BCT. **(B).** ENRICH-iST. **(C).** ENRICHplus.

Conclusions

- PreOmics kits combined with the Orbitrap Astral mass spectrometer enable a scalable, reproducible plasma proteomics workflow without compromising proteome depth.
- ENRICH-IST and ENRICHplus enhance proteome depth by over 2x and 3.14x, respectively, compared to neat plasma in biologically diverse double-spun K2EDTA plasma.
- Gas phase fractionation improves spectral library comprehensiveness and boosts peptide and protein group identifications significantly.
- ENRICHplus identifies more low abundant proteins, spanning the entire concentration range.
- With PreOmics Enrichment technology, the number of differentially abundant candidate proteins in biological comparisons between healthy and disease groups increases, demonstrating increased potential of finding novel biomarker candidates.
- The Orbitrap Astral mass spectrometer enables in-depth biological discovery, paving the way for novel findings and advancements in clinical cohort and population-scale translational research.

Trademarks/licensing

Learn more at https://www.thermofisher.com/astral