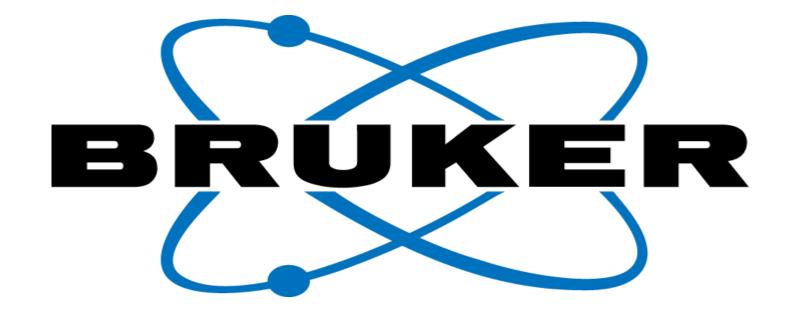
# **Advancing Blood Plasma Proteomics Using Enrich+ and timsTOF ULTRA**

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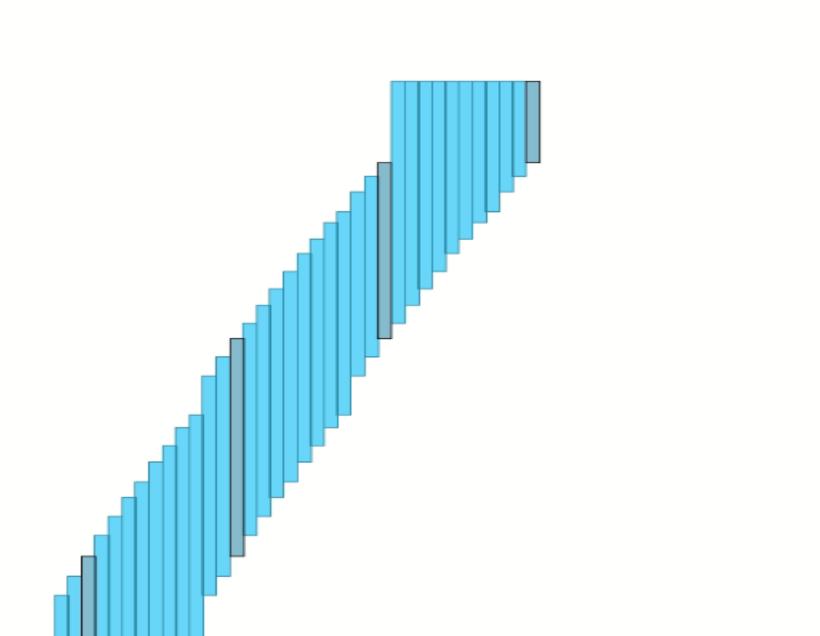
#### **USHUPO 2025**

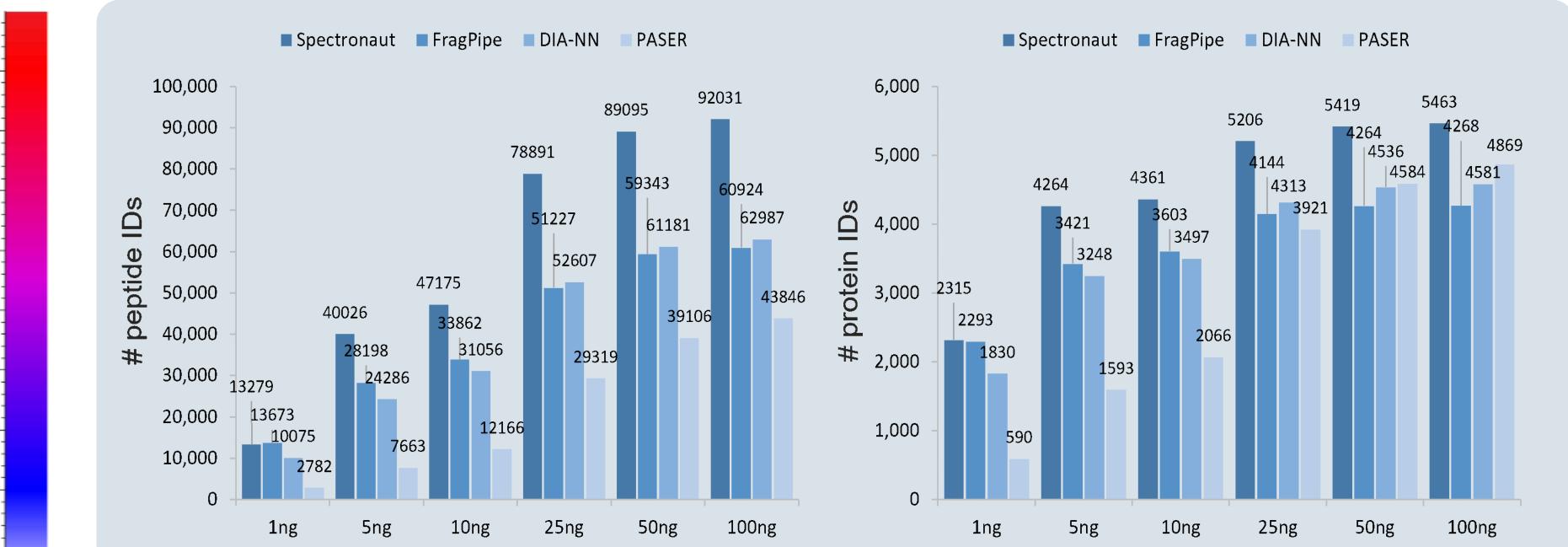
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# Introduction

Advances in proteomics are essential for the identification of biomarkers and elucidation of molecular mechanisms of health and disease. This

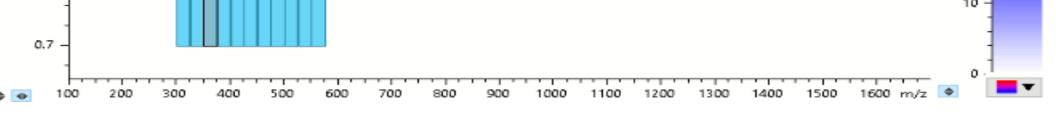




study presents an optimized workflow leveraging the PreOmics Enrich+ kit in conjunction with the timsTOF ULTRA mass spectrometer and the nanoElute 2 ultrahigh-performance liquid chromatography (UHPLC) system. PreOmics Enrich+ kit is designed for robust blood plasma protein enrichment to enhance the detection of a diverse proteins, including low-abundance targets that are difficult to detect using conventional proteomic approaches.

# **Methods**

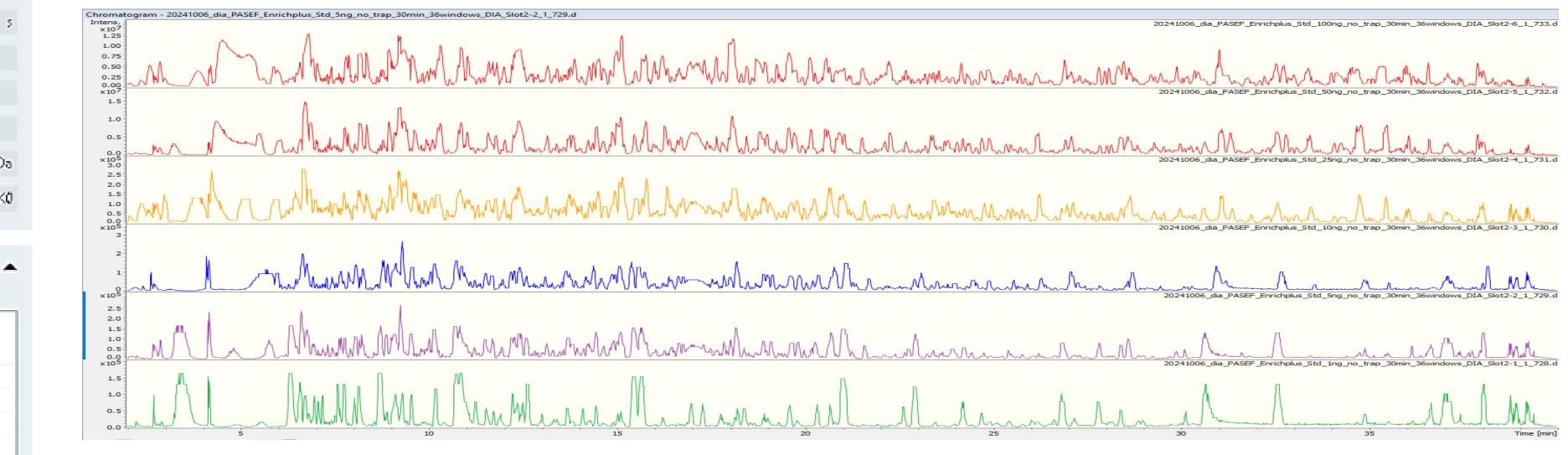
Ten ug of lyophilized commercial human plasma digest standard was provided by PreOmics. A dilution series was prepared ranging from 100ng to 1ng. Proteomic analysis (performed in triplicated) was performed using the timsTOF ULTRA mass spectrometer coupled with a nanoElute 2 liquid



**Figure 1:** 26 Da window (36 total windows) dia-PASEF window placement scheme. The cycle time was 0.85s

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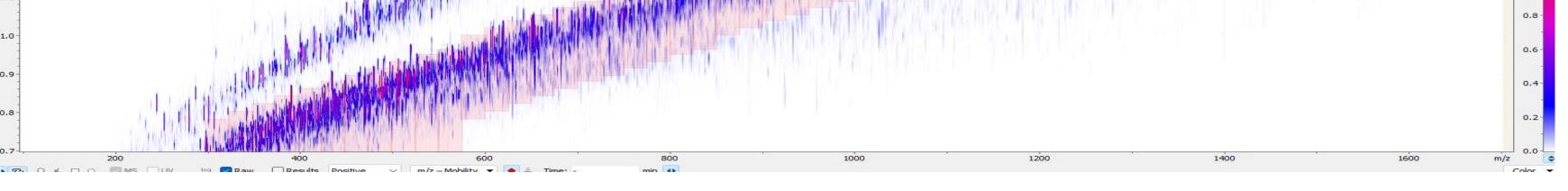
**Figure 3:** Analysis of commercial plasma digest standard analyzed using a PepSep Ultra (25 cm x75  $\mu$ m x1.5  $\mu$ m) column, coupled to the nanoElute2<sup>®</sup> and timsTOF ULTRA. A dia-PASEF method with 36 total windows, using 3 steps was used. Forty samples per day throughput was achieved with a 30-minute gradient. Ion mobility (IM) was set at 0.7 (1/k0 start) to 1.3 (1/k0 end). Data analysis was conducted using several data independent acquisition software Proteoscape (no MBR), DIA-NN, Spectronaut, and Fragpipe DIA Tracer.



**Figure 4:** Base peak chromatogram comparison between commercial plasma standard. Plasma peptides were separated on a 30 min gradient run on a PepSep Ultra column coupled to nanoElute2 and timsTOF Ultra mass spectrometer

chromatography system and PepSep Ultra column for high-resolution peptide separation. Dataindependent acquisition (dia-PASEF - figures 1 & 2) was employed to achieve highly sensitive detection from a single-shot 30-minute analysis of 50  $\mu$ L of human blood plasma. This workflow enhances the efficiency of large-scale sample processing, making it well-suited for biomarker discovery and clinical research applications.

For dia-PASEF acquisition, a windowing schema utilizing 26 Da windows (36 windows total) was implemented, covering an ion mobility range of 0.7–1.3 and a mass-to-charge (m/z) range of 300– 1200. Ion accumulation and ramp times were set to 65 milliseconds, yielding a total cycle time of 0.85 seconds. Tryptic peptides from a human lysate digest were analyzed, and data processing was conducted using DIA-NN 1.9.2, Fragpipe/DIA Tracer + DIA-NN, Spectronaut 19 and Bruker Proteoscape (tims DIA-NN). The FASTA database used was from uniport.org (human). The Spectral library used either generated from Spectronaut (.d import + FASTA), DIA Tracer + MSFragger, DIA-NN generated, Bruker provided spectral library.



**Figure 5:** timsTOF Ultra heat map with overlaying dia-PASEF windows of Enrich+ plasma standard at 100ng. Peptides were separated on a nanoelute2 with pepsep Ultra column.

## Results

We achieved sensitive detection of 5,000 protein groups and 92,000 peptides in a single shot 30-minute analysis of 50 uL of human blood plasma (Figure 3). This workflow offers potential improvements for the efficiency of processing large sample cohorts, rendering it advantageous for large-scale biomarker discovery and clinical research applications. The synergistic combination of Proteomics Enrich+ method with the timsTOF ULTRA and nanoElute 2 establishes a powerful platform for advancing proteomic analysis in the context of personalized medicine with improved proteomic depth and scalability, thereby facilitating insights into potential disease mechanisms and identification of potential therapeutic targets

## Conclusions

High Sensitivity & Speed: Detects 5,000 protein groups and 92,000 peptides in a



**Figure 2:** TimsControl 6.0 instrument parameters for the timsTOF Ultra mass spectrometer. Ion mobility range of 0.7–1.3 and a mass-to-charge (m/z) range of 300–1200. Ion accumulation and ramp times were set to 65 milliseconds, yielding a total cycle time of 0.85 seconds. High sensitivity detection was enabled. Denoising was disabled.

30-minute plasma analysis.

Scalable for Large Studies: Optimized for high-throughput biomarker discovery and clinical research.

Deeper Proteomic Insights: Enhances scalability and disease mechanism discovery for personalized medicine



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