High-Performing PepSep Advanced Columns Enable Deep Profiling of P2-Enriched Plasma Samples on the Orbitrap Astral

Bettina Streckenbach[1], Christopher Below[1], Sandra Schaer[1], Vanessa Buehlmann[1], Sebastian Mueller[1], Leander Runtsch[2], Martin Schirmer[3], Nils A. Kulak[2], Roland Bruderer[1]

[1]Biognosys AG, Schlieren, Switzerland; [2]PreOmics GmbH, Martinsried, Germany; [3]Bruker Daltonics GmbH & Co. KG, Bremen, Germany

Poster Number: MP 382

Introduction

Blood plasma is a crucial biofluid for studying health and disease in humans as it provides an accessible biomarker reservoir. The study of the human plasma proteome, however, has been hampered by the large dynamic range of protein concentrations in plasma. Recently proposed technologies enable deep profiling of the plasma proteome through particle-based enrichment, resulting in rich samples with a dynamic range optimal for potent liquid-phase separation in modern analytical columns. To maximize the information obtained from plasma samples, we benchmark a novel *PepSep Advanced* separation column on a fast-cycling *Orbitrap Astral* mass spectrometer and report their performance by combining them for the first time with the *P2-iST* enrichment technology.

Materials and Methods

Enriched Plasma Samples

For testing of the instrumental setup, a human blood plasma pool was prepared using the *P2-iST Plasma* workflow (*Biognosys Group*). Additional evaluation was performed on a sample cohort of *P2-iST* enriched plasma samples from patients with pancreatic cancer (n=23), lung cancer (n=29) and from cancer-free participants (healthy controls, n=34). The *P2-iST* enriched plasma pool sample served as enriched plasma quality control.



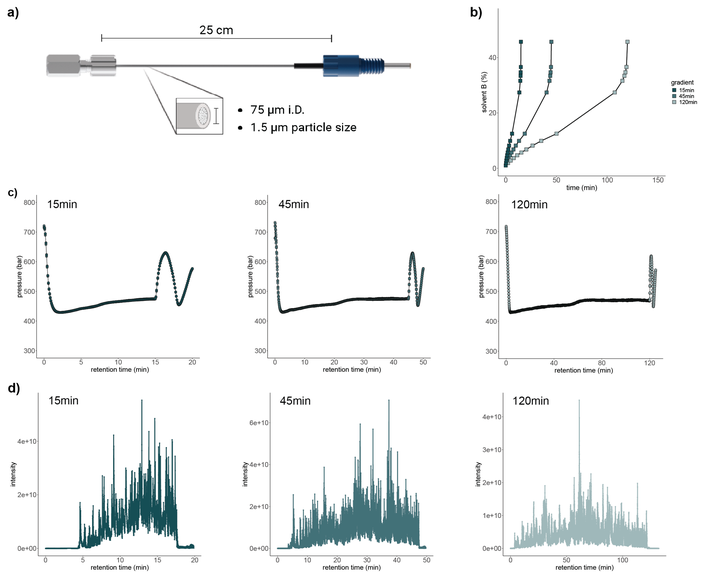
Figure 1: Workflow for *P2-iST* enriched plasma sample analyzed on a FAIMS - *Orbitrap Astral* platform with the *PepSep Advanced* column. For setup-testing, a pool of human blood plasma samples enriched with the *P2-iST* technology was used.

LC-MS Setup

In this study, 800 ng of enriched plasma samples were infused on a *PepSep Advanced* 25 cm separation column (*Bruker*) coupled to a 10 µm inner diameter nano-flow bullet type emitter and connected to a *Vanquish Neo UHPLC* (both *Thermo Fisher Scientific*). For the setup-testing, tryptic peptides were separated in 15-, 45- and 120-minute optimized non-linear gradients at 60 °C on a *Montara BioLabs* column heater and recorded on an *Orbitrap Astral* with a *FAIMS Pro* interface (*Thermo Fisher Scientific*). For the biological sample cohort, chromatographic separation was performed in a 45- and 80-minute optimized non-linear gradient on the same LC-MS platform. All data was searched with *Spectronaut 21* using *directDIA*.

Results

Platform Evaluation



In this study, the *PepSep Advanced* 25 cm column was benchmarked on the *Orbitrap Astral* connected to a *Vanquish Neo* using a *P2-iST* enriched human plasma pool. The column demonstrated good compatibility with the *Orbitrap Astral* by showing stable performances in terms of column parameters, peptide distributions and identifications across different gradients (**Figure 2, Figure 3**).

Figure 2: Column specifications and overview of the platform evaluation. (**A**) Representative illustration of the *PepSep Advanced* column specifications. (**B**) Applied non-linear gradients, solvent B: 80% ACN, 0.1 % FA in water. (**C**) Stacked representation of column back pressure profiles and (**D**) TICs for each gradient.

Platform Enables Deep Plasma Proteome Profiling

This novel *PepSep Advanced* setup achieved maximal identifications of 6'834 protein groups (PGs) with the 120min gradient (**Figure 3b**). Furthermore, 83% of all PGs were quantified with a coefficient of variation (CV) below 20% (**Figure 4**). While shorter gradients resulted in less IDs (15min: 5'742 PGs), the high robustness in quantified peptide and PGs as well as the median CVs in PG signals < 9 % across all tested gradients (**Figure 4b**) underlined the strong quantitative performance of this platform.

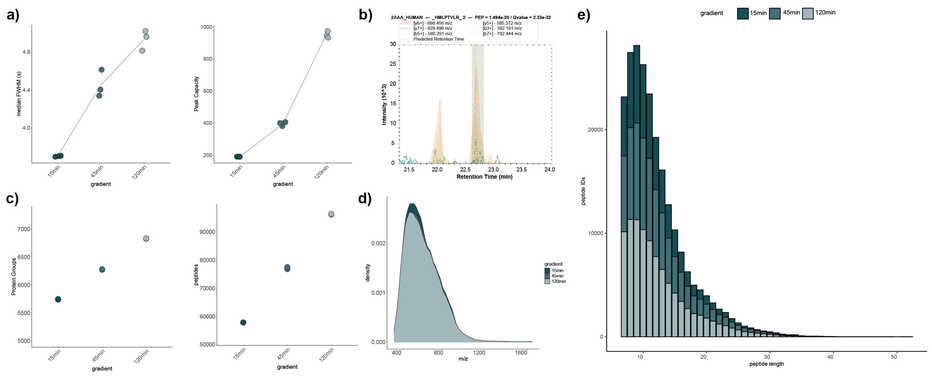


Figure 3: Performance of the *PepSep Advanced* column on the *Astral* platform. (**A**) Median peak width in FWHM (in s) and peak capacity for each tested gradient. (**B**) Exemplary Peak shape: extracted ion chromatogram (XIC) of an MS2 signal in the 45min gradient. (**C**) Identifications on protein group and peptide level. (**D**) Mass range distribution and (**D**) length distribution of detected peptides for each gradient.

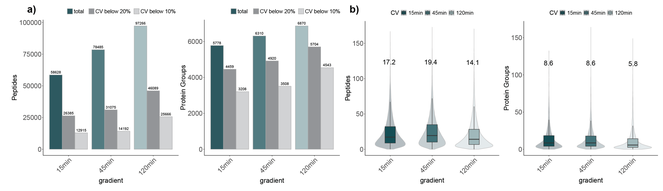


Figure 4: Quantitative performance of the platform. a) Precision of quantified peptides and protein groups, and b) median CVs of peptide and protein group intensities across the tested gradients (n=3).

Benchmarking on a Biological Cohort

The *PepSep Advanced* setup was tested on a *P2-iST* enriched biological sample cohort including patients with lung cancer, pancreatic cancer and healthy participants (total: 82 samples). With optimized methods, high plasma coverage was achieved with 7'710 and 8'177 sparse PG identifications for the 45min and 80min gradients, respectively, which revealed cohort-specific differences and allowed to reproduce previously reported biological differences[1].

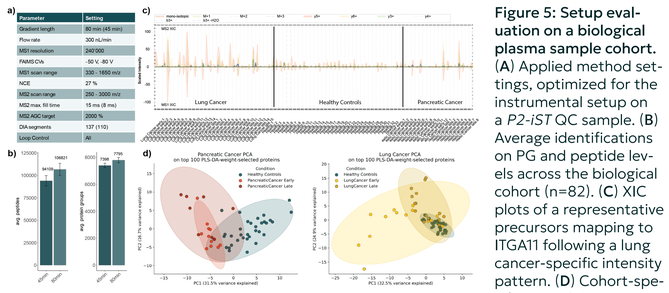


Figure 5: Setup evaluation on a biological plasma sample cohort. (**A**) Applied method settings, optimized for the instrumental setup on a *P2-iST* QC sample. (**B**) Average identifications on PG and peptide levels across the biological cohort (n=82). (**C**) XIC plots of a representative precursors mapping to ITGA11 following a lung cancer-specific intensity pattern. (**D**) Cohort-specific PCA plots.

Conclusion

Benchmarking of the *PepSep Advanced* column on the *Orbitrap Astral* setup demonstrated high performance by achieving deep plasma proteome profiling while retaining quantitative precision. Applied on a biological sample cohort, the setup showcased its promising applicability for discovery workflows.

Reference

- Tognetti et al., J Proteome Res. 2022 May 23;21(7):1718-1735.

Contact

For further information about this poster, please contact:

Bettina Streckenbach, PhD

Senior Scientist, R&D

Email: bettina.streckenbach@biognosys.com

www.biognosysgroup.com

Conflict of Interest Disclosure: Bettina Streckenbach, Christopher Below, Sandra Schaer, Vanessa Buehlmann, Sebastian Mueller and Roland Bruderer are employed by Biognosys AG, Leander Runtsch and Nils A. Kulak are employed by PreOmics GmbH, and Martin Schirmer is employed by Bruker Daltonics GmbH & Co. KG.