Optimizing LC-MS/MS settings for plasma proteomics analysis with cap-flow LC separation and dia-PASEF

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Introduction

Plasma is a widely-available biopsy containing information on health state and proteomics data from blood plasma can significantly facilitate clinical research. The high dynamic range of protein abundances is a challenging aspect that impairs deep protein coverage. Recently, the introduction of bead-based depletion/enrichment technologies, that significantly increase the plasma proteome coverage. Statistically reliable data rely on huge cohorts of donors, demanding rapid and robust data acquisition of hundreds of samples/day. We optimized reversed-phase LC separation using capillary flow rates for rapid sample loading and separation within 5-10 minutes. MS data acquisition was performed by a dia-PASEF program with short acquisition cycles. Thereby, we increased the rate from 40 SPD for nanoLC up to 220 SPD, enabling data acquisition of larger cohorts. Up to 6100 protein groups were reported for cell lysates and plasma samples achieved 450-550 protein IDs for neat samples and up to 1200 protein groups for ENRICHed plasma.

Plasma preparation

Plasma samples from 20 healthy donors were obtained with EDTA as anti-coagulant from BioMex prepared with one centrifugation step at 1200 xg (1-spun) and two subsequent centrifugations at 1200 xg (2-spun) to efficiently remove platelet residues.

All plasma samples were prepared with the iST-BCT kit to obtain neat plasma digests and with the ENRICH-iST kit for ENRICH plasma digests (both from PreOmics). After sample cleanup, peptide solutions were dluted to ~0.5 ug/ul.

High-turnover cap-LC method with dia-PASEF acquisition

- Fast gradients were established on a Waters M-Class HPLC operated in HyStar via a novel driver pack.
- Samples were separated on Bruker-Max (RP-C18, 10cmx150µmx1.5 µm) operated at 60°C in the column toaster (Bruker) directly connected to the ion source of the TimsTOF HT.
- Chromatographic gradients were optimized for direct injection, short separation with consistent peak width and minimal carry-over and equilibration time. (fig. 1 B)
- dia-PASEF-MS acquisition was optimized to yield an average of 5 datapoints over the chromatographic signal. (fig 1 C+E)

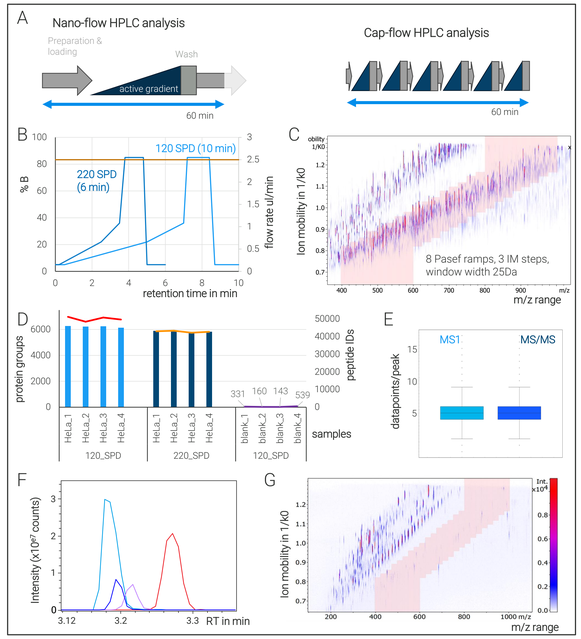

Fig.1: A) Enhancing large sample cohorts by rapid-sample turnover at higher flow rates. B) HPLC gradients for 120 SPD (10 min) and 220 SPD (6min) using the M-Class HPLC system, C) dia-PASEF acquisition cycle and conditions for cap-LC, D) protein and peptide IDs for capLC gradients, peptide IDs for blanks are labelled, all runs resulted in 0 PG, E) distribution of datapoints/chromatographic peak for >60k tryptic peptides from HeLa, F) chromatographic peak shape for 4 precursors, C histogram plot for blank run

Acquisition optimization

- coverage of more than 6000 protein groups in 120 SPD separations from ~400ng of cell lysate with excellent reproducibility of protein/peptide identification and quantitation (figure 1 D-F)
- high reproducibility of retention times and narrow chromatographic peaks
- Low sample carry over even after multiple injections of high sample amounts, as demonstrated by absence of signal intensity and peptide identifications (fig. 1 G)

Plasma proteomics at high turnover

- Cap-flow chromatography separation at 120 SPD identified 450-550 protein groups in neat plasma and 670-1150 protein groups in ENRICH plasma samples (fig. 2A) in 7 min active gradient time covering ~5 orders of magnitude (fig. 2 B)
- Overlap of protein groups is ~80% for single-spun and double -spun plasma for neat and ENRICH plasma, respectively and ~85% for neat and ENRICH plasma (fig. 2 C+D)
- High-abundant proteins display high reproducibility over all 20 donors with higher abundance and less variation in ENRICH-iST plasma compared to neat plasma (fig. 2 E)

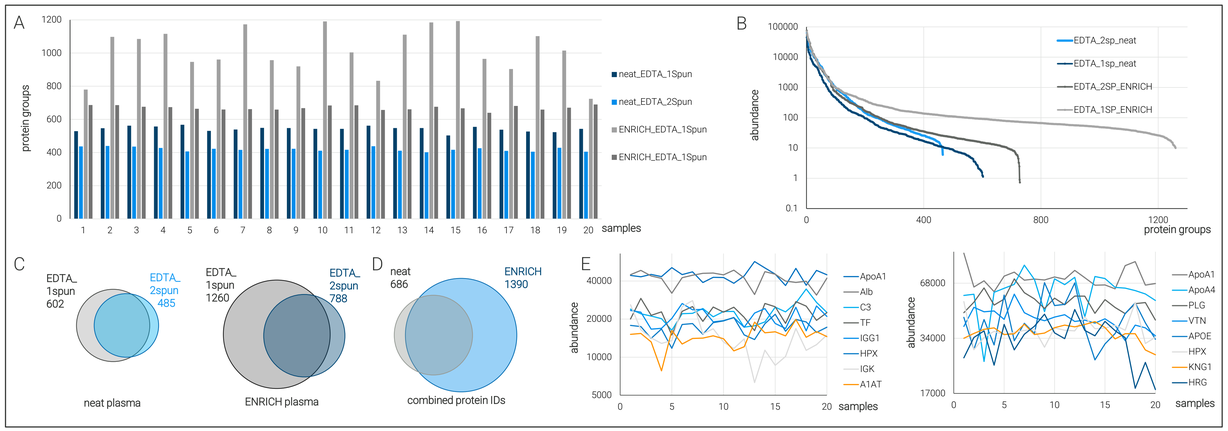

Fig. 2 A) protein identifications by capLC-MS for neat and ENRICH plasma preparations from single-spun and double-spun EDTA plasma from 20 donors, B) average abundance of protein groups in neat and ENRICH plasma samples, C) overlap of protein IDs for neat and ENRICH samples from single-spun and double-spun plasma, D) overlap of protein IDs in neat and ENRICH plasma, E) abundance of the 8 most abundant proteins in neat and ENRICH plasma over 20 donors

Summary

Capillary flow LC provides fast and reproducible alternative to acquire proteomics data for large sample cohorts. Fast separation gradients are combined with rapid dia-PASEF programs with <0.5 sec acquisition cycles to provide sufficient quantitative data. Plasma sample preparation with Enrich-iST yielded up to 1000 protein IDs in single spun plasma and up to 700 IDs in double-spun plasma, but reveals more variability in protein IDs.

Images were generated with Instant Clue

Nolte, H., MacVicar, T.D., Tellkamp, F. et al. Instant Clue: A Software Suite for Interactive Data Visualization and Analysis. Sci Rep 8, 12648 (2018). https://doi.org/10.1038/s41598-018-31154-6

Conclusion

- Cap-flow HPLC applications increase sample throughput to >100 – 200 sample/day enabling plasma proteomics for clinical studies
- Increased flow rates significantly reduce overhead times such as column washing, equilibration and sample loading
- Dia-PASEF acquisition provides robust acquisition cycles to fit narrow chromatographic peaks

Technology