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

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Introduction

As plasma's availability, accessibility and physiological role make it an attractive support to perform diagnosis, prognosis or theragnosis assays, Label-Free Quantification Proteomics (LFQ-Proteomics) now appears to be a method of choice to search for candidate biomarkers. Despite formidable progresses made at the sample preparation, data acquisition and data processing levels, the number of replicates that are required to compensate for the biological background variability does still constitute a formidable challenge: it does not only call for a sample preparation reproducibility, but for a perfect stability of the measuring conditions. If some MS architectures like the TIMS-Q-TOF have proven their robustness to this regard, the nano-LC separation devices traditionally used in such approaches are still constituting the main source of variability and time lag. In this communication, we are reporting the results obtained using a high-Throughput (Fig.1) and reproducible cap-flow separation setup, coupled to a last generation timsTOF system.

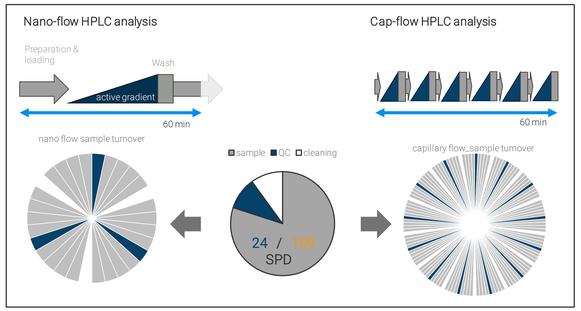

Fig.1: Comparison of HPLC set-up with respect to HPLC overhead time a resulting sample throughput assuming a ratio of 80:10:10 of samples to quality control run and system cleaning.

Nano-LC MS of the plasma proteome with dia-PASEF

Nano-LC-MS/MS is currently the gold standard for deep plasma proteomics analysis. A plasma sample was prepared with iST-BCT and ENRICH plus kits and analyzed using nano-LC combined with dia-PASEF data acquisition covering precursors from 400-100m/z with 25 Da windows in 8 dia-PASEF ramps. 200 ng of obtained peptides were separated on a 30 min gradient on a Ionopticks Aurora column (250x0.075mm, 1.7 µm) and peptides were detected in a modified TimsTOF HT mass spectrometer. All data were analyzed in Spectronaut 19 with directDIA+ against a canonical human fasta database.

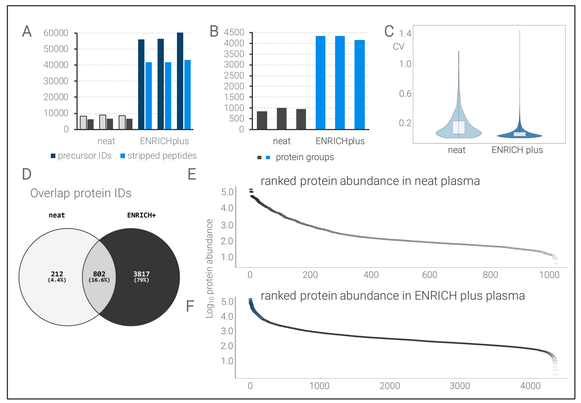

Fig. 2 Plasma proteomics prepared with iST-BCT and ENRICH plus protocol and analyzed with nanoLC using dia-PASEF. A) Precursor and peptide IDs in 3 injection replicates; B) Identified in protein groups (3 injection replicates); C) CVs of all protein groups; D) Overlap of protein IDs between neat and ENRICH plus samples; E) ranked protein hits in neat plasma; F) ranked protein groups in ENRICH plus

Results I

- Almost 1000 protein groups were detected in neat (iST-BCT) plasma samples and ~4500 protein groups with more than 40000 peptide sequences from ENRICH plus treated plasma.
- Both datasets report good reproducibility over 3 injection replicates (median CV < 20%)
- Protein enrichment successfully reduces the dynamic range of plasma proteins thereby covering a wider range of plasma proteins

Cap-LC MS of the plasma proteome with diaPASEF

For capLC separation, the nanoElute was equipped with a PepSep HPLC column (80x0.150 mm, 1.5 µm) operated at 2 ul/min and coupled to a TimsTOF HT mass spectrometer. A 7 min separation gradient was optimized on K562 injections, the dia-PASEF isolation scheme and IM range was identical to nanoLC data, however the ramp time was reduced to generate sufficient datapoints/peak for reproducible quantitation. 200ng of the prepared plasma proteome samples were injected in triplicates and all data were analyzed in SN19 using directDIA+.

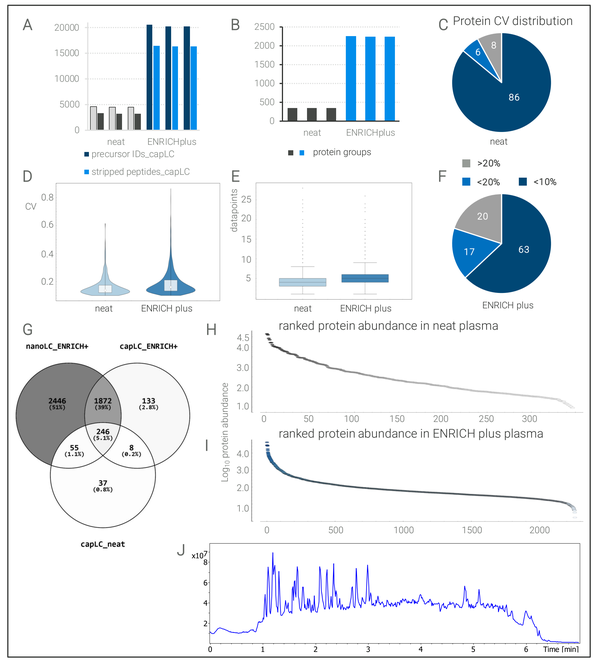

Fig. 3 capLC-MS/MS analysis of plasma proteomics samples prepared with iST-BCT and ENRICH plus protocols. A) Precursor and peptide IDs from 3 injection replicates; B) Identified in protein groups; C) CV distribution in neat samples; D) CVs of all protein groups; E) distribution of datapoints for peak determination; F) CV distribution in ENRICH plus samples; G) overlap of proteins groups in capLC data with ENRICH plus data from nanoLC; H) ranked protein hits in neat plasma; I) ranked protein groups in ENRICH plus; J) TIC of capLC separation of ENRICH plus prepared plasma proteins.

Results II

- Cap-flow chromatography separation increased the sample throughput by factor 4, identifying 350 protein groups in neat plasma and more than 2200 protein groups in ENRICH plus samples in 7 min active gradient time
- Cap-flow LC data report excellent reproducibility with median CVs of 7.5% and 11% in neat and enriched plasma samples, respectively.
- The overlap of protein groups is more than 90% with nanoLC data.

Summary

Nano-flow LC-MS/MS represents the gold standard to generate deep plasma proteome datasets for low sample numbers and identifies more than 4500 protein groups in enriched plasma samples in 30 min gradient time.

Capillary-flow LC-MS/MS reduced the gradient length and overhead time to increase the sample throughput by 4-6fold with a reduced coverage of plasma proteome. More than 2200 protein profiles were observed in 7 min gradient time for enriched plasma samples.

Dia-PASEF technology on TimsTOF mass spectrometers can be adapted to provide sufficient coverage of chromatographic peaks (5 datapoints) resulting in excellent reproducibility (CV ~10%) for short gradients.

Images were generated with Venny 2.1.0 and Instant Clue

Oliveros, J.C. (2007-2015) Venny. An interactive tool for comparing lists with Venn's diagrams. https://bioinfogp.cnb.csic.es/tools/venny/index.html

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 larger clinical cohorts
- Quantitative reproducibility of dia-PASEF acquisition is retained for short separation gradients
- LC-MS/MS analysis with nano-flow provides the deepest proteome coverage and can be utilized to generate cohort specific spectral libraries

Technology