Single-shot quantitative plasma proteomics using the PASEF method

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Introduction

The plasma proteome is one of the most accessible sources for studies both in biological and clinical settings. However, plasma presents a huge dynamic range in protein concentration that precludes in-depth analysis in a high-throughput manner. Direct analysis of un-depleted samples without fractionation is a very attractive workflow for large cohort studies but such a strategy requires a very fast mass spectrometer to achieve reasonable depth in the proteome. Parallel acquisition and serial fragmentation strategy (PASEF) on the timsTOF Pro platform enables very fast sequencing, efficient utilization of ions, and boosts the sensitivity by time focusing the ions in the TIMS front-end. Here we investigate the performance of PASEF method for single-shot plasma proteome analysis using label free quantification.

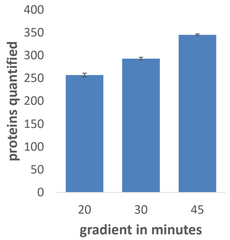
Methods

Pooled plasma samples were purchased from Sigma and digested with trypsin and cleaned up using Preomics iST sample preparation kits. For depletion, plasma proteins were loaded serially through a Agilent Human-6 and Sigma IgY14 depletion columns. Depleted samples were also digested using iST sample preparation kits. About 200 ng of peptides were loaded on a Aurora 25 cm column using the nanoElute HPLC system and analyzed using PASEF acquisition strategy in the timsTOF pro system. For short 20-minute gradients, Bruker TEN column was used with 200 ng peptide load. All samples were analyzed using "Short gradient protein identification" – one of the default methods available in oTOF control software. Raw data were processed in MaxQuant computational software suite for DDA analysis and Spectronaut from Biognosys was used for dia-PASEF analysis. Protein groups and peptides were filtered at 1% FDR.

Results

Single-shot analysis of neat plasma is a enables high-throughput analysis of few hundreds to thousands of samples in a cohort. Depletion of so many samples would be both expensive and the process might also lead to quantitative distortion of the proteome. We initially checked different method conditions and found that PASEF spanning a mobility range of 0.85 - 1.35 provides the best results in terms of number of proteins quantified. We performed multiple runs in 20, 30 and 45-minute gradients resulting in a maximum of 345 protein groups quantified on average in a 45-minute gradient. Matching the 30-minute gradient to a library from depleted plasma sample yielded more than 550 proteins quantified using default settings in MaxQuant. When we reduced this to very stringent matching windows (retention time of 6 secs, mobility window of 0.01) we could still quantify more than 460 proteins on average. Our initial experiments with 25-minute dia-PASEF runs matched to library yielded more than 410 proteins quantified in undepleted plasma.

Fig 1: Proteins identified in different gradient lengths from undepleted plasma. Matching the single-shot runs to depleted plasma runs increased the number of quantified proteins significantly despite using very tight matching windows for retention and mobility values



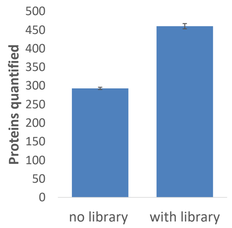


Fig 2: DDA runs with matching enabled has reasonable data completeness

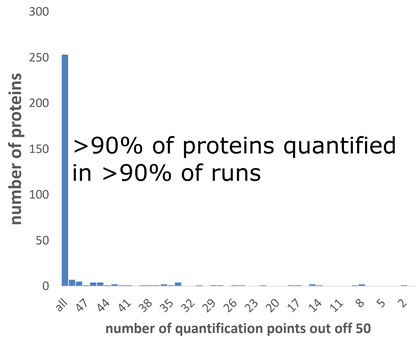
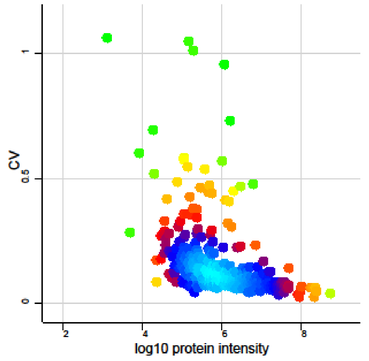


Fig 3: Density plot of protein LFQ CV versus protein log 10 abundance



Conclusion and Outlook

- Single-shot DDA PASEF measurement leads to deep coverage of plasma proteome
- Matching to depletion library significantly improves the depth of coverage in DDA runs
- Initial results from dia-PASEF runs indicate that more than 400 proteins could be quantified in 20 minutes.
- Relatively long gradients would be interesting to explore for quantification of close to 1000 proteins
- Generating a library would be beneficial for DDA runs
- Library free DIA runs in longer gradients might also be a promising approach

Technology