Compared dia-PASEF and prm-PASEF approaches for the absolute quantification of 500 human plasma proteins in colon cancer plasma samples.

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Introduction

Targeted proteomics methods are a traditional choice for absolute protein quantitation of proteins in biofluids. We recently evaluated the prm-PASEF acquisition strategy which, compared to traditional prm approaches, allows to further increase the number of addressable targets and the method's selectivity without compromising the sensitivity. In parallel, the even higher multiplexing potential of dia-PASEF approaches (now regularly used in discovery studies) also triggered our interest. We are now applying both approaches to the absolute quantitation of 500 blood proteins in colon cancer plasma samples.

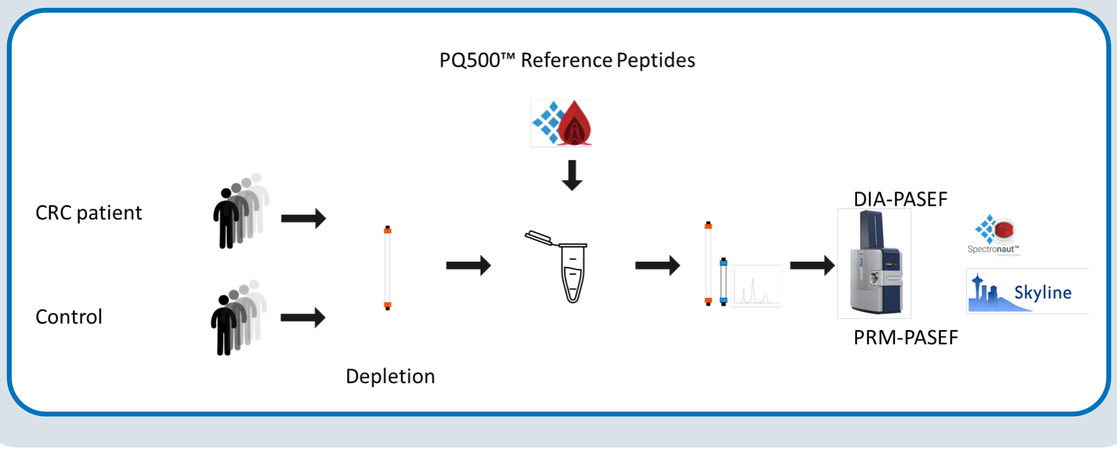


Fig. 1: Experimental setup.
Plasma from 10 CRC male patients and 10 age matched male control patients were spiked in with PQ500 after top 14 depletion, digested and analyzed by LC-dia-PASEF and LC-prm-PASEF.

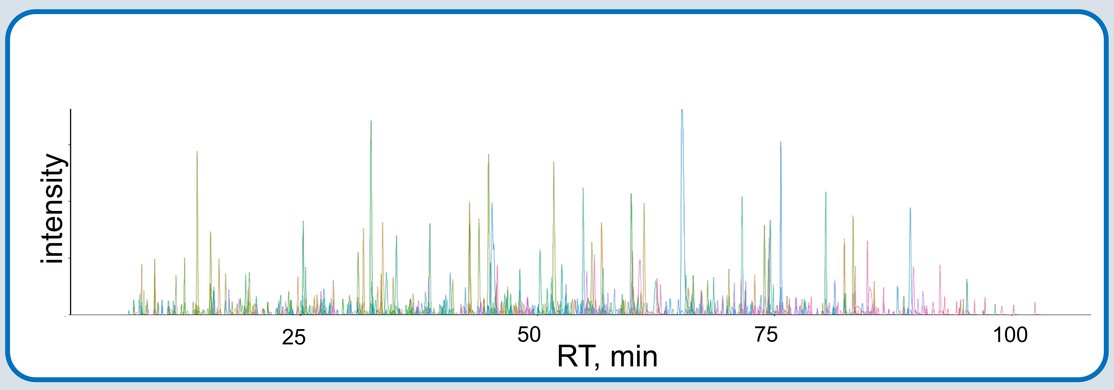
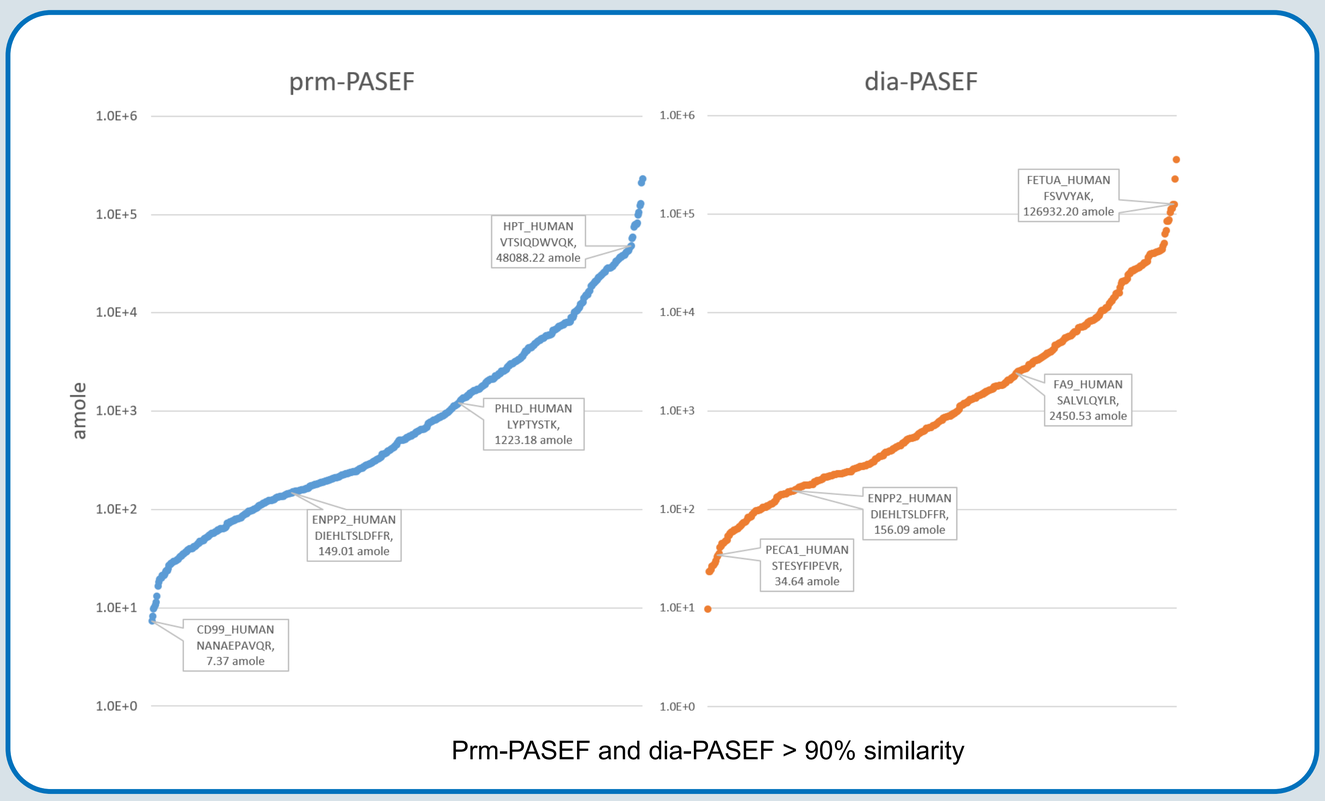


Fig. 2: prm-PASEF data extraction.
1564 Extracted ion chromatograms (XICs) are generated from the signal of fragments ions. The quantification is based on the area under the peak (Skyline).



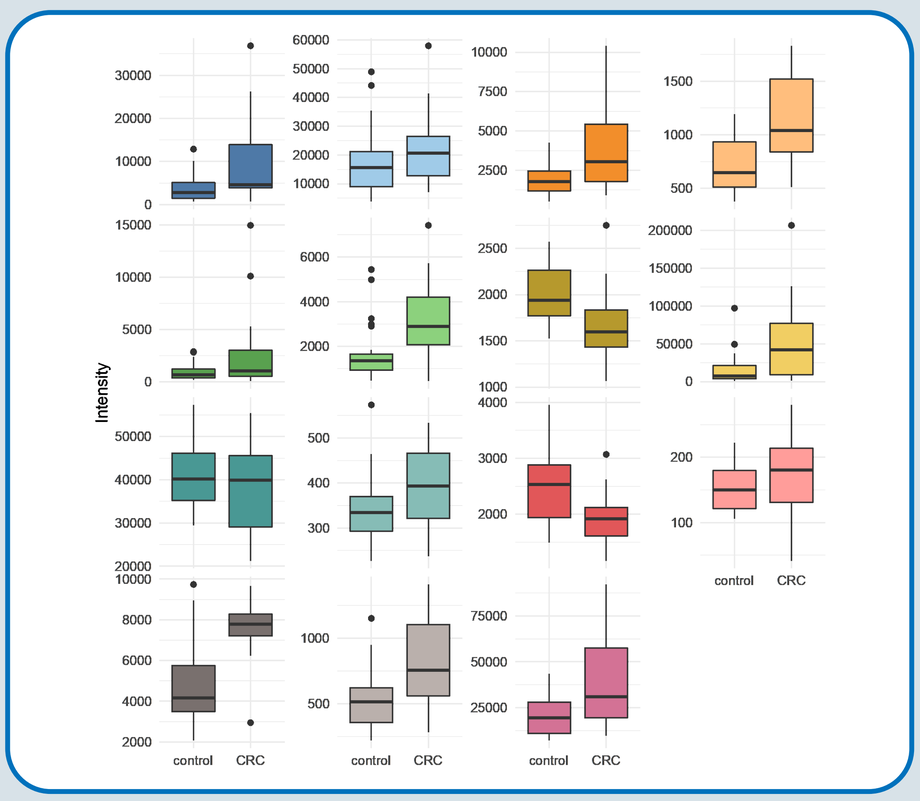


Fig. 3: compared prm-PASEF and dia-PASEF quantitation results
Abundance of the targeted proteins in a control sample. Abundances are calculated from each PQ500 peptide's calibration curve using its heavy form as an internal standard.

Fig. 4: Proteins significantly regulated in CRC patient cohorts
Compared protein expression levels measured in the control (left) and CRC (right).

Methods

The plasma sample cohort consisted in 10 patients affected by a colon cancer (adeno carcinoma, CRC) and 10 controls. Plasma samples were depleted with a Mars 14 depletion column (Agilent), digested with a trypsin protease and spiked the PQ500 (Biognosys) synthetic peptides mixture. Samples and controls were separated by nano-HPLC (nanoElute, Bruker Daltonics) on a 25cm pulled emitter column (IonOpticks) using a 100 min gradient. Peptides were analyzed on a timsTOF Pro2 instrument (Bruker Daltonics) operated in prm-PASEF and dia-PASEF modes. Data processing has been done with Spectronaut (Biognosys), MaxQuant and Skyline-daily **(Fig.1)**.

Results

We monitored1564 precursor-ions corresponding to 782 peptides from 565 proteins while using a 2 min retention time window **(Fig.2)**. Some peptides could be quantified down to 7 amol with prm-PASEF and 20 amol with dia-PASEF **(Fig.3)**. The median relative standard deviation of the signal of the peptides was of 3%. 98% of the 574 quantified peptide pairs could be quantified from the prm-PASEF experiment, while 96% could be quantified using dia-PASEF. The results obtained with both approaches were highly correlated. The application of the method to the CRC sample cohort revealed 15 significantly regulated proteins which had formerly been identified in more invasive cancer tissue studies **(Fig.4)**.

Conclusions

- Both prm-PASEF and dia-PASEF approaches allowed to quantify a large proportion of the targeted peptide pairs with > 90% similarity
- The proteins exclusively quantified with prm-PASEF were the ones with the lowest expression level
- The proteins highlighted by the approach had formerly been revealed by cancer tissue studies. We have been able to track those with a much less invasive plasma-based study, hence opening interesting clinical research perspectives.

timsTOF PRO2