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dia-PASEF for targeted proteomics: development of large-scale assay for quantitation of more than 500 proteins in human plasma sample

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Introduction

Data-independent acquisition (DIA) has the advantage of reproducible and accurate protein identification and quantification across large sample cohorts. dia-PASEF (Meier et.al.,2020) merges the benefits of DIA with the advantages of ion mobility in proteomics experiments (Meier et al., 2018). The ion mobility dimension improves the alignment of precursor and fragment spectra and reduces interferences.

The benefits of dia-PASEF makes it an ideal method to be integrated in a platform for large-scale biomarker studies eliminating the need for tedious in-depth method optimization as is required for typical targeted approaches like PRM. The dia-PASEF approach has the additional advantage that both targeted and non-targeted extraction and quantitation can be performed on the same data set. Here, we use dia-PASEF in combination with the PQ500 kit (Biognosys) to develop a large-scale targeted quantitation assay for peptides in human plasma sample.

Methods

Individual plasma samples were digested using the iST kit from PreOmics. The PQ500™ kit (Biognosys) was prepared according to the manufacturer's instructions and spiked into the prepared digests. Tryptic peptides were separated on a 25cm C18 column (75µmx1.9µm, Aurora, IonOpticks) using a nanoElute coupled to a timsTOF HT mass spectrometer via a CaptiveSpray ionization source using a 30-min acetonitrile (ACN) gradient. For the dia-PASEF acquisition, a window placement scheme consisting of 6 TIMS ramps with 3 mass ranges per ramp spanning from 300–1200 m/z and from 0.6–1.40 1/K0 with a cycle time of 0.9 seconds, including one MS1 frame, was utilized. Data was processed in Spectronaut (v16, Biognosys) using an ion mobility annotated PQ500 library for targeted data extraction. The library-free directDIA workflow was used for discovery-based proteomics.

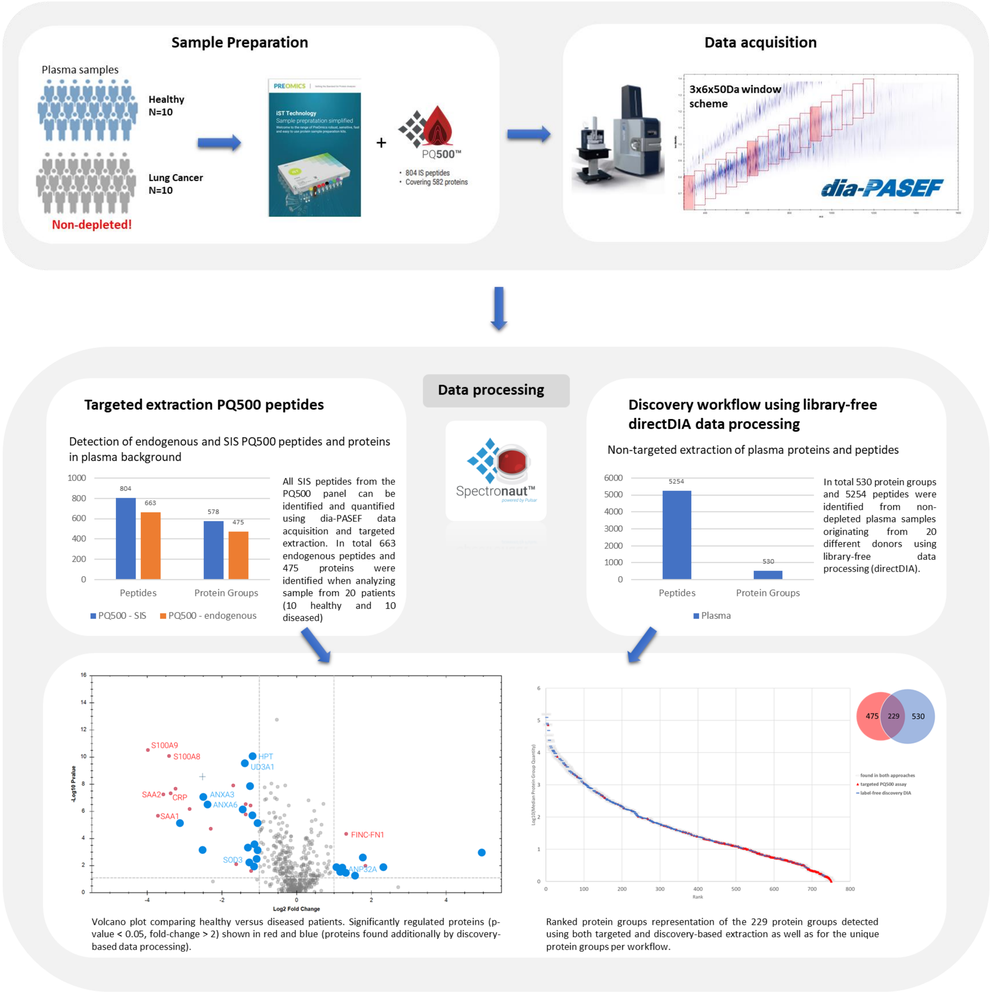

Fig. 1: Workflow for large-scale quantitation of more than 500 proteins in human plasma

Results

We developed a scalable assay consisting of plasma sample preparation using PreOmics' iST kit, addition of the PQ500™ reference kit for absolute quantitation of target peptides, combined with dia-PASEF data acquisition on the timsTOF HT and processing using Spectronaut software. The PQ500™ kit covers more than 500 target proteins amongst them 130 FDA approved clinical markers. We used a 30-min gradient to enable high sample throughput without sacrificing analytical depth of the assay. By covering a mobility range from 0.6 to 1.4 1/K0 and an m/z range from 300 to 1200, the complete PQ500 panel was included in the dia-PASEF method. Application of 6 TIMS ramps using 3 mass ranges per ramp resulted in a total cycle time of 0.9s (including one MS1 frame per cycle) ensuring good coverage of the chromatographic peak.

The assay was applied to a proof-of-concept study of non-depleted plasma samples from patients diagnosed with lung cancer. All 804 SIS peptides and 578 protein groups from the PQ500 panel could be detected. In total, 663 peptides and 463 protein groups were identified, covering around 80% of the PQ500 panel. Of those, 55 proteins were found to be significantly regulated (p-value < 0.05, fold change > 2). Three of the proteins (Fibronectin, Immunoglobulin lambda-like polypeptide 1, Immunoglobulin lambda-like 1 light chain) were detected to be higher abundant in healthy donors, whereas the remaining proteins showed significant upregulation in donors diagnosed with lung cancer. For example, elevated plasma levels of serum amyloid A (SAA) proteins (SAA1 and SAA2) have been detected in patients diagnosed with lung cancer.

With dia-PASEF not only targeted peptides can be monitored, but quantitation information of all detectable peptides is preserved. Data processing using directDIA™ allows identification and quantitation without the need for library generation. In total 530 protein groups and 5254 peptides were identified during the experiment. Additional 26 protein groups were found to be significantly regulated, which were not part of the targeted quantitation assay. Among those, extracellular superoxide dismutase (SOD3) was found, which is known to be more highly expressed in tumor cells than in normal cells.

Summary

Our results show that the applied multiplexed approach has the potential to identify disease biomarkers in non-depleted plasma samples without in-depth expert knowledge by using a standard proteomics workflow supported on the timsTOF platform.

A high correlation of the targeted dia-PASEF method with an advanced prm approach (prm-PASEF), which fully exploits the multiplexing capabilities of the timsTOF mass spectrometer, was already shown before (Lesur et al., 2022). In the referenced study, the PQ500 kit was spiked in depleted human plasma samples originating from 20 patient plasma samples from a colorectal cancer cohort and analyzed using a 100-min gradient. The results clearly showed that both techniques could be used as diagnostic tool for different diseases in future.

In our study we further evaluated and optimized the dia-PASEF approach as it is less complex to set up and provides both targeted and non-targeted data extraction capabilities. Our results obtained from a 30-min gradient using non-depleted plasma show that an increase in sample throughput is possible without sacrificing analytical depth further extending the diagnostical potential of the developed assay.

Conclusion

A workflow for targeted quantitation in non-depleted human plasma using dia-PASEF was evaluated. The PQ500 kit enables reliable targeted quantitation of several hundreds of proteins in plasma.

- The **workflow eliminates tedious and time-consuming method development** required for standard targeted workflows and still yields excellent targeted quantitation results.
- The complete PQ500 panel can be covered in just 30-min run time using dia-PASEF approach.
- By using a data-independent approach additional proteins not included in the target panel are measured and can be quantified **resulting in a combination of targeted and discovery proteomics**.
- Application to a biologically relevant lung cancer study shows regulation of peptides known to be associated with cancer. This confirms the potential of applying the approach for clinical research studies.

timsTOF HT