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). This makes dia-PASEF 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.

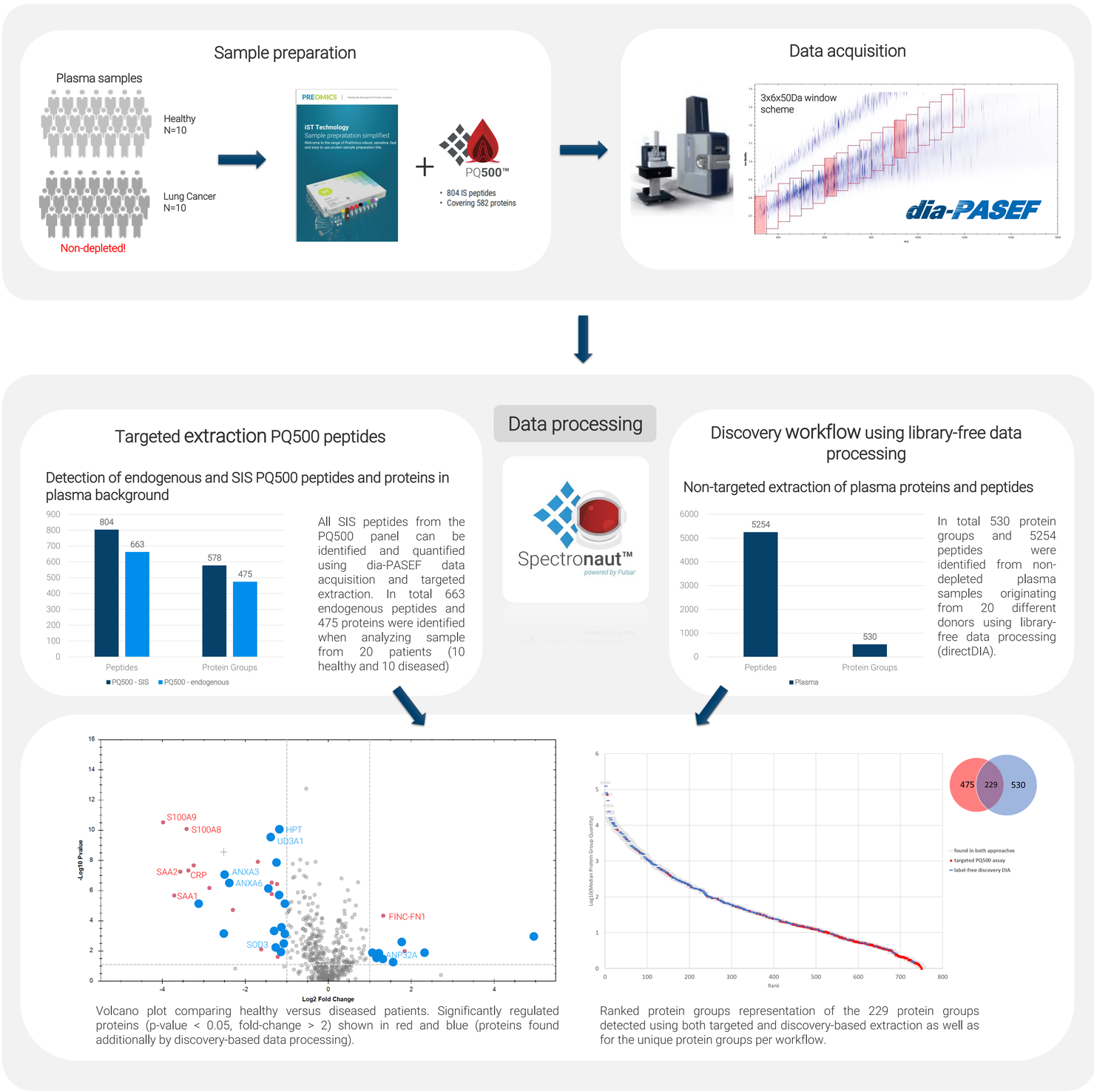
Methods

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 column (75µm x 1.9µm, Aurora, IonOpticks) using a nanoElute coupled to a timsTOF HT via a CaptiveSpray ionization source using a 30-min 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 0.7 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.

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 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).

With dia-PASEF not only targeted peptides can be monitored, but quantitation information of all detectable peptides is preserved. 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.



Volcano plot comparing healthy versus diseased patients. Significantly regulated proteins (p-value < 0.05, fold-change > 2) shown in red and blue (proteins found additionally by discovery-based data processing).

Ranked protein groups representation of the 229 protein groups detected using both targeted and discovery-based extraction as well as for the unique protein groups per workflow.

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

Summary

In our study we evaluated the application of the dia-PASEF approach for a large-scale quantitation assay of peptides in human plasma samples. The dia-PASEF approach is less complex to set up compared to other targeted approaches and provides both targeted and non-targeted data extraction capabilities.

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.

Conclusion

- Targeted quantitation using dia-PASEF **eliminates tedious and time-consuming method development** required for standard targeted workflows.
- By using a DIA approach additional proteins not included in the target panel are measured and can be quantified **resulting in a combination of targeted and discovery proteomics**.

timsTOF HT