HIGHLY REPRODUCIBLE, ABSOLUTE QUANTIFICATION OF >500 PLASMA PROTEINS WITH PQ500 AND SUREQUANT

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BIOGNOSYS
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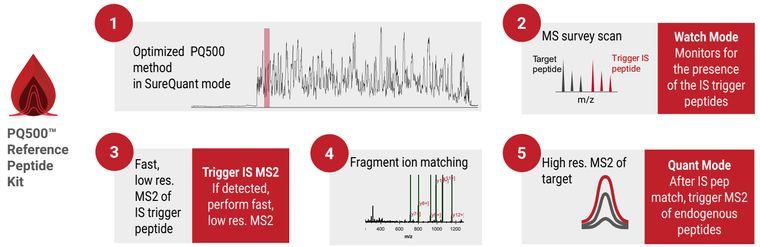
INTRODUCTION

The application of proteomics in clinical studies requires robust and highly reproducible methods with a high throughput.
Here, we elucidated the use of PQ500 in combination with the SureQuant acquisition method in a heart failure study. The PQ500 kit consists of stable isotope labeled (IS) peptides covering 578 human plasma proteins. Within the SureQuant method, the MS monitors for the presence of the spiked IS peptides and only triggers a scan on the endogenous peptide, when the labeled peptide was found. Compared to classical MRM or PRM methods, SureQuant requires only a minimum of method development and is robust against variations in the chromatography.



CONCLUSIONS

- **PQ500-SureQuant method allowed the quantification of 568 plasma proteins in 1h per sample.**
- **The method provided low technical CVs (sample preparation and acquisition) across the protein abundance range.**
- **Absolute quantification covered 6 to 7 orders of magnitude and including several heart failure markers.**
- **Optimization of PQ500 panel and intensity thresholds resulted in only minor improvements (+3% full profiles and CVs < 20%).**

RESULTS

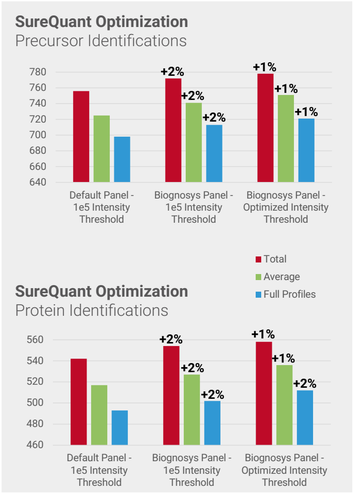
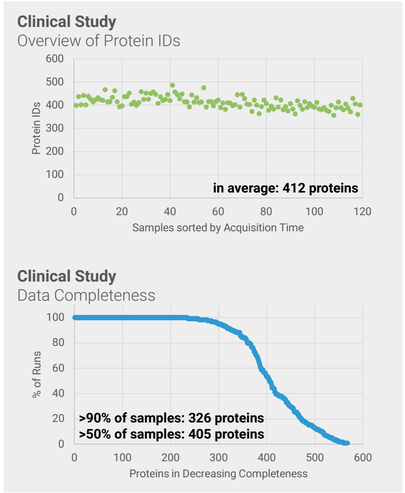
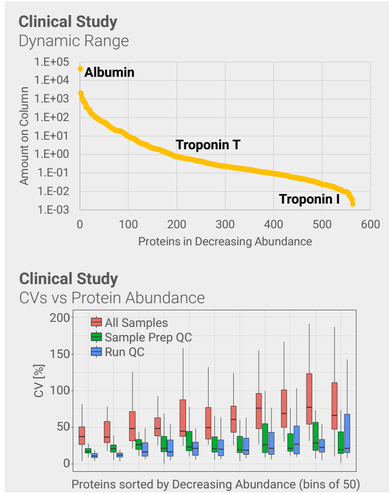


Figure 1: Optimization of SureQuant
The optimization of the PQ500 panel and intensity thresholds for the trigger of the IS peptides resulted in only minor improvements (triplicate injections of a pooled sample).
Determination of intensity thresholds and data analysis was performed with SpectroDive (Biognosys).

Figure 2: IDs and data completeness
120 plasma samples from a heart failure clinical study were prepared using a published protocol (Bruderer, et al., 2019). PQ500 (Biognosys) was spiked into the samples. Samples were acquired by a 40min gradient using an Easy nLC1200 LC coupled online to an Orbitrap Exploris 480 MS (both Thermo Fisher Scientific).





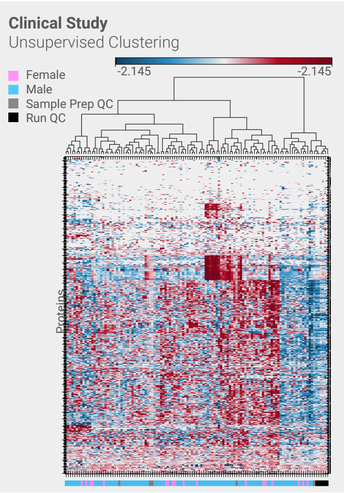


Figure 3: Overview of dataset
The PQ500-SureQuant methods enabled the absolute quantification of in average 412 proteins per sample including two heart failure markers: Troponin T and I.
The dynamic range covered 6 to 7 orders of magnitude, which is comparable to state-of-the-art MRM methods.
We noticed an increasing CV with decreasing protein abundance and only a low CV of the LC-MS (Run QC) and workflow (Sample Prep QC). This indicated that the main variance was related to biological differences.
An supervised clustering (z-score transformed values) revealed clustering of the data. We mapped the samples to the gender of the study subjects and could exclude a gender bias.