Quantifying 500+ Human Plasma Proteins in a Single Run with SureQuant

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INTRODUCTION

Plasma proteome is one of the most challenging biological matrices because of its large dynamic range. A classical untargeted proteomics method suffers from this due to oversampling of abundant proteins. Targeted workflows have a higher dynamic range but are difficult to setup and are not as comprehensive as discovery methods.

We evaluated the use of a plasma protein assay panel containing SIS peptides measured with a new targeted acquisition method called SureQuant. This method uses the spiked-in SIS peptides to trigger the acquisition of the endogenous peptides. This greatly simplifies the classical targeted acquisition workflows by not needing to schedule peptide acquisition by their retention times. However, analyzing data acquired from such a pipeline poses new challenges. Towards this end, we adapted our analysis pipeline in SpectroDive software to optimally analyze SureQuant data.

To validate our pipeline, we measured a dataset consisting plasma from 30 patients (15 healthy, and 15 non-small-cell lung carcinoma (NSCLC)) using SureQuant acquisition and compared it with our previous analysis of these samples using MRM acquisition.

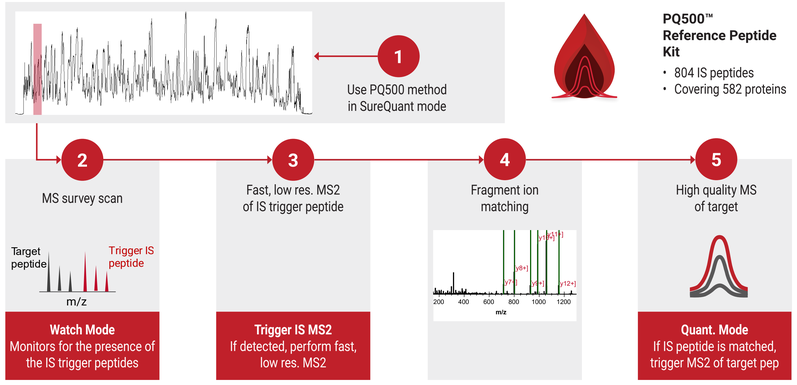


Figure 1. SureQuant workflow with PQ500 standard is an out-of-the-box solution for quantifying plasma proteins

1) Spike-in PQ500 reference peptides in the plasma sample and select PQ500 method in the SureQuant mode of Exploris 2) SureQuant mode works by first watching for the IS trigger peptides in the MS survey scans based on a PPM tolerance. 3) When an IS peptide candidate is detected in the survey scan, it triggers a low resolution MS2 scan of that peptide. 4) The acquired low resolution MS2 spectra is matched with the corresponding transitions in the method, again with a PPM tolerance. 5) If the IS peptide is matched based on a minimum number of transitions, the instrument triggers a high-resolution MS2 for the target peptide.

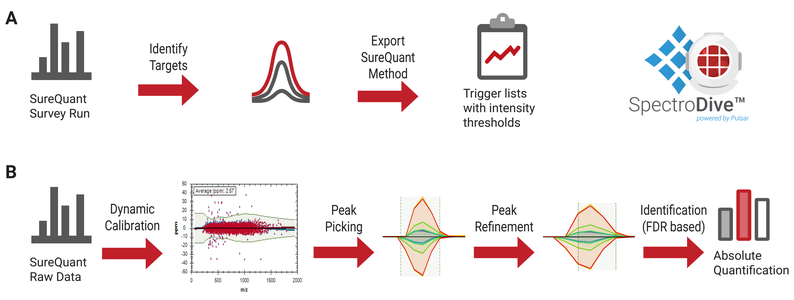


Figure 2. SpectroDive is a complete solution for analyzing SureQuant data

(**A**) For an optimal SureQuant acquisition, one needs to create a transition list with intensity thresholds for the trigger peptides based on MS1 quantification. SpectroDive 10 will make this a one-click process to easily set up a SureQuant method. (**B**) Once the SureQuant raw data is acquired for your sample, it can be analyzed using SpectroDive with its standard targeted analysis pipeline featuring automatic parameter optimization, powerful peak detection algorithms, and target-decoy based false discovery rate analysis (FDR). SpectroDive 10 has new SureQuant-specific processes to optimize the data analysis. For example, it refines the peaks to account for the fact that the target is triggered after the reference peptide is detected.

METHODS

Non-depleted plasma samples from 30 patients (15 healthy and 15 NSCLC) were spiked with the PQ500 kit (Biognosys) containing 804 SIS peptides for 582 proteins. The samples were acquired with a 60-minute gradient using a Orbitrap Exploris 480 in SureQuant acquisition mode. The data was analyzed with SpectroDive 10 software with 1% peptide FDR. Previously, we had measured the same sample in MRM mode. This data was reanalyzed using the same SpectroDive version and used as a gold standard to validate SureQuant analysis.

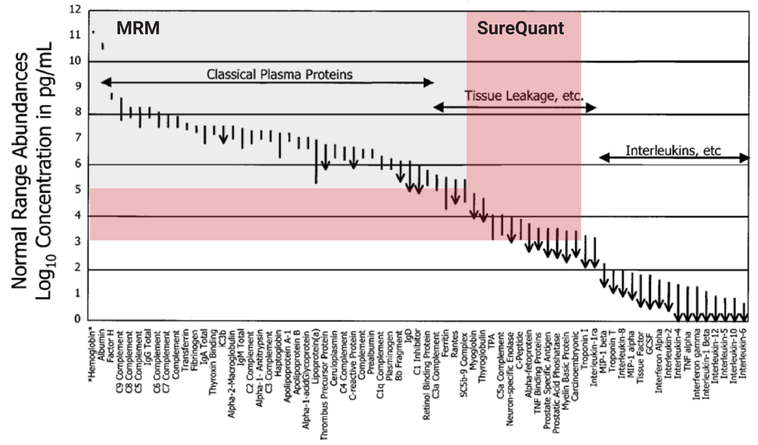


Figure 3. SureQuant has a higher dynamic range than MRM

Mapping proteins that were identified in at least 30% of the samples to proteins with known concentration in plasma (adapted from [Anderson, 2002]) shows that we can measure more than 8 orders of magnitude in dynamic range of plasma protein concentration using SureQuant as opposed to 7 orders of magnitude with MRM. This corresponds to an extension of measuring classical plasma proteins towards measuring potentially interesting tissue leakage proteins.

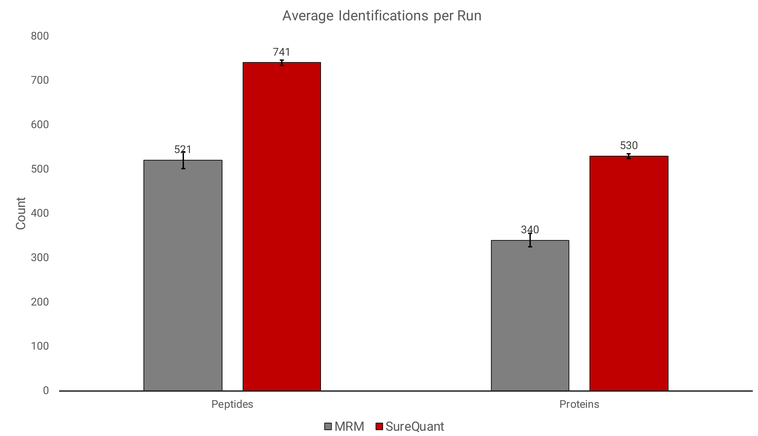
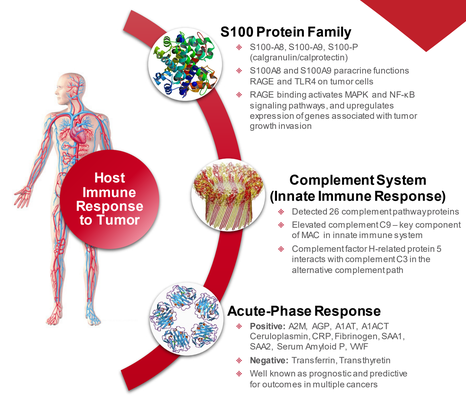


Figure 4. Comparison of identifications between SureQuant and MRM

On average, SpectroDive identified 42% more peptides and 55% more proteins with SureQuant acquisition mode compared to MRM with a 1% FDR (false discovery rate). Analyzing a triplicate of the pooled samples gave a median CV of 6.5% at the peptide level, indicating high reproducibility of the method.

A



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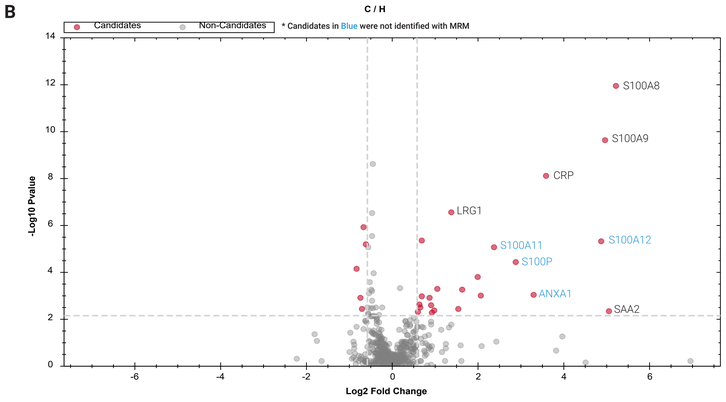

Figure 5. Pathway analysis and biomarker mechanistic origins from SureQuant analysis

(**A**) Biomarker analysis summary highlights that the circulating proteome of subjects with NSCLC is comprised of a larger fraction of proteins associated with the host immune response to the tumor. (**B**) Differentially regulated candidates show that acute phase signaling, complement system as well as the S100 protein family were significantly enriched in subjects with NSCLC, with respect to the normal donors. While the top candidates remained same between the two analysis (black font), new candidates from the S100 family along with ANXA1 which plays a role in the innate immune response were identified with SureQuant analysis (blue font).

CONCLUSIONS

- By removing the major hurdle of scheduling, SureQuant acquisition, in combination with PQ500 reference assay kit, is an out-of-the-box solution for targeted analysis of plasma proteome (Figure 1)
- We created an optimized SureQuant analysis pipeline within SpectroDive software which can identify more than 500 plasma proteins per sample (Figure 2, Figure 4)
- SureQuant extends the dynamic range by > 1 order of magnitude compared to MRM well into the interesting range of tissue leakage proteins (Figure 3)

CONTACT INFORMATION

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