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NEXT GENERATION PROTEOMICS

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Single Shot Quantification of 4000 Proteins in Blood Plasma Using Data Independent Acquisition Mass Spectrometry

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Introduction

Human blood is a readily available, highly relevant sample type carrying information from virtually all parts of the human body. Until recently, single shot proteome coverage in human blood using mass spectrometry was rather limited due to the large dynamic range of protein abundance, spanning more than ten orders of magnitude. Improvements at every step of the workflow have led to huge increases in proteome coverage in the last year. We show evidence that this is due to the particular shape of the protein abundance distribution of blood. Finally, we show some data how modern workflows can quantify 4000 plasma proteins using single shot data independent acquisition mass spectrometry.

Methods

Human plasma samples were measured using Biognosys' TrueDiscovery platform. In short, samples were depleted and downstream sample prep was performed in 96-well format. Samples were analyzed on a Thermo Scientific EASY-nLC 1200 coupled to a Thermo Scientific Orbitrap Exploris 480. A FAIMS Pro was connected to the Exploris 480. LC-MS DIA methods were optimized for the respective gradient lengths. DIA data was either directly searched using directDIA or analyzed with a library using Spectronaut (Biognosys).

Results

We optimized every step of a single shot data independent acquisition mass spectrometry workflow including sample preparation, liquid chromatography separation, ion mobility separation (FAIMS), mass spectrometry acquisition and data analysis.

We show that with this optimized workflow we can quantify 4000 plasma proteins in a human cancer plasma study. This corresponds to a 800% improvement compared to the roughly 500 proteins which were achieved few years ago using single shot acquisition workflows.

We show evidence that the massive gains in proteome coverage with this optimized workflow are caused by the inherent protein abundance distribution of plasma. The data indicate that current workflows reached a dynamic range such that the lower end is at the point of highest

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protein density in plasma and hence any improvements in dynamic range lead to significant increases in proteome coverage.

Going forward we see possibilities for improvements on every step in the workflow cumulatively pushing the boundaries of unbiased, ultra-deep plasma profiling in the coming years. Proteomics in plasma will become an indispensable tool for precision medicine and will deliver biomarkers used as surrogate endpoints in clinical trials.

Mass spectrometry related innovations

A high throughput sample preparation and LC-MS setup is estimated to profile up to 4000 proteins in human plasma studies