B.1 Automated workflows for DIA data using DIA-NN on the PaSER platform

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Data independent acquisition (DIA) has become the go to method for deep and quantitative proteomic analysis given the ability to sample large m/z windows in a reproducible and non-stochastic manner. Using a method termed dia-PASEF on a TIMS enabled Q-TOF lends additional advantages in both duty cycle and selectivity using the ion mobility space. DIA-PASEF allows for deep proteomes in short gradient times (<20 min.) and even deeper proteomes with longer greater times. Despite the rapid advancements in hardware and applications of proteomics, which has directly lead to the generation of thousands of proteomic data sets, the proteomic bottleneck remains data analysis. The PaSER platform provides a solution through its GPU-powered ability to perform CCS-enabled DDA and DIA analysis in real time. With the release of PaSER 2022c, we introduced the first vendor integrated version of DIA-NN, namely TIMS DIA-NN: a CCS-enabled analysis tool for the identification and quantification of dia-PASEF data. TIMS-DIA-NN takes advantage of the TIMScore model that greatly increases peptide identifications to build more robust libraries. Here we present PaSER 2023 which includes significant improvements to the peak picking and match between runs algorithms. To show the capability of this platform, we used Human, Yeast and Ecoli (HYE) mixtures at different but known ratios to identify over 140000 precursors and over 12500 proteins excellent with quantitative accuracy. Total number of unique peptides across all analyses was 15,000 while number of identified protein groups were 500, 800 and 1,600 (control, recovered and death groups, respectively). Among several down and up-regulated proteins, this study could quantify significant proteins related to bleeding episodes and liver damage, which are common symptoms in severe conditions and in accordance with clinical condition of those patients. The high relevance of this study is the quantification of blood proteins as potential biomarkers of yellow fever for a precise, sensitive and fast methodology by proteomics approach.

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B.2 Yellow Fever Blood Proteomics Study by Trapped Ion Mobility Mass Spectrometry

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Yellow fever is a mosquito-borne viral illness where 15% of cases present severe symptoms and the mortality can approach 50% because of renal, hepatic, neurological impairment and hemorrhage. As diagnosis still requires information from patient and laboratory findings such as PCR and immunomeasays, here we have performed proteomics analysis on serum samples from different patients who either recovered or died from yellow fever using trapped ion mobility spectrometry coupled to quadrupole-time of flight mass spectrometry to evaluate and understand this disease progression. Pooled serum samples from a control group, a group recovered from infection and group from patients who died were analyzed on timsTOF Pro 2 instrument combined to a nanoElute (Bruker Daltonics) operating with an Aurora nano column (25 cm x 75 µm ID, C18 - IonOpticks, Australia) on DDA and dia-PASEF acquisition modes. Data were processed using MSFragger or DIA-NN 1.8. Non-depleted samples showed about 876 and 1,400 protein groups by DDA and dia-PASEF respectively. Total number of unique peptides across all analyses was 15,000 while number of identified protein groups were 500, 800 and 1,600 (control, recovered and death groups, respectively). Among several down and up-regulated proteins, this study could quantify significant proteins related to bleeding episodes and liver damage, which are common symptoms in severe conditions and in accordance with clinical condition of those patients. The high relevance of this study is the quantification of blood proteins as potential biomarkers of yellow fever for a precise, sensitive and fast methodology by proteomics approach.

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