Ion mobility (IM) based data independent acquisition has recently become available on timsTOF Pro. Due to its parallel accumulation-serial fragmentation (PASEF) strategy, it achieves a high duty cycle and has shown promise in biological applications. We have previously shown that high-precision retention time calibration benefits DIA analysis (Bruderer, 2016). Upon applying this concept to ion mobility for analyzing dia-PASEF data, we could identify a high number of proteins, competitive to state-of-the-art DIA methods. Figure 1: High-precision IM in Spectronaut Ion mobility calibration ensures optimal IM extraction width (red line, right panel) is automatically used for data analysis as shown here with a 400 ng Hela sample.

Figure 2: Triplicate Hela lysate dia-PASEF runs with varying sample amounts (10ng, 50ng, 400ng) on a 100 min gradient

Top Panel: Average number of proteins identified per injected sample amount with 1% peptide and protein FDR. The library consisting of over 10,000 protein groups was created from 15 fractions using Pulsar search engine with 1% FDR (PI2A, precursor and protein)

Bottom Panel: The analysis time per run (in minutes) for the library generation (PASEF) and subsequent, targeted analysis of the 400 ng dia-PASEF runs is significantly lower than the acquisition time (100 min gradient + 20 min overhead)

Figure 3: UPS2 proteins spiked in 50 ng mouse cerebellum background matrix in 5 different steps, 2 replicates each

Top Panel: Average number of proteins identified by different Spectronaut versions. The red line indicates the library size in proteins.

Bottom Panel: Regulation analysis was performed using an unpaired t-test of protein quantities calculated from both MS1 and MS2 (Huang, 2019) in SN 14. For SN 13, the t-test was based on precursor quantities calculated from MS2. Upon sorting all the regulated candidate pairs by p-value, we found 34 UPS2 candidate pairs in the top 100. This is comparable to what we have previously observed in this sample (Muntel, 2019).