Mouse fatty liver proteomes in dda-PASEF and dia-PASEF from low input using the timsTOF SCP

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Introduction

Liver diseases are a global health issue and especially pronounced with a western diet, including a continually increasing disease burden from non-alcoholic fatty liver disease. There is both a need for biomarkers and ability to test potential drugs for this condition and thus a need for translational mouse studies. The mice in this study were fed a Western diet for 16 weeks and their livers were collected and flash frozen. The liver samples were from mice over-expressing the protein of interest and on the high fat diet or a control group of matched wild type were used for proteomic analysis with liquid chromatography and tandem mass spectrometry analysis.

Methods

Mouse livers were harvested and frozen with liquid nitrogen. Frozen cells were thawed, reduced, and alkylated. 10ng of the protein was digested with trypsin and cleaned up with STRAP purification. Chromatography was performed on a nanoElute (Bruker Daltonics) using an Aurora nano column (25 cm x 75 µm ID, C18 - IonOpticks, Australia) at 400 nl/min with a 90 min gradient. LC-TIMS MS/MS data were acquired on a timsTOF SCP instrument operated in dda-PASEF and dia-PASEF modes. DDA data were analyzed using PEAKS OnLine (Bioinformatics Solutions). and dia-PASEF and raw data were analyzed with Spectronaut 17 (Biognosys) at a 1% FDR.

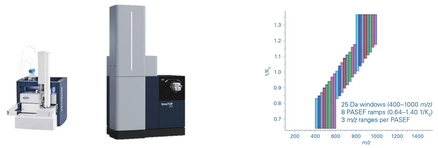

Fig. 1. A] Bruker NanoElute and timsTOF SCP B] dia-PASEF windows

Results

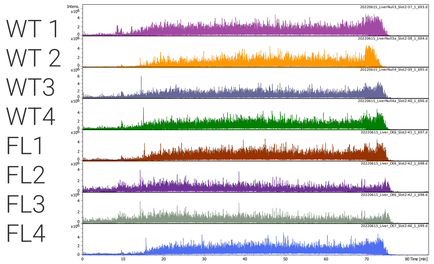

Fig. 2 Total Ion Chromatogram of the MS2 of replicate runs

Sample Name	# Precursor	#PSM	#Peptides	# Protein Groups	# PSM / # Precursor
All (8 samples)	1,871,262	718,290	92,442	5,704	38%
WT 1	218,285	84,319	42,505	3,779	39%
WT2	221,475	84,998	42,856	3,804	38%
WT 3	230,860	86,599	43,620	3,859	38%
WT4	185,550	69,441	36,430	3,189	37%
Average	214,043	81,339	41,353	3,658	38.00%
FL1	241,723	98,997	49,228	4,113	41%
FL2	265,269	94,947	48,143	4,148	36%
FL3	239,567	98,859	49,118	4,047	41%
FL4	268,533	100,130	50,561	4,271	37%
Average	253,773	98,233	49,263	4,145	38.75%

Table 2: PEAKS Results from 10ng of sample on column

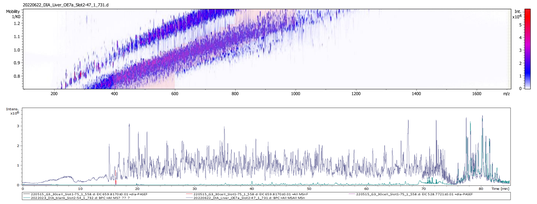

Fig. 3 dia-PASEF A] Ion cloud coverage B] base peak chromatogram in dark blue over the blank run in green.

Parameter	Value
Precursors	75,303 of 75,313
Modified Peptides	59,014 of 59,017
Peptides	55,113 of 55,115
Proteotypic Peptides	11,766
Protein Groups	5,996 (5,996)
Proteins	12,586 (12,543)
Single Hits	1,042 (1,159)
Fragments	450,309 of 1,701,688
Library Source	Spectronaut
Software Version	17.4.230317.55965
Merged	False
Search Engine Platform	Pulsar
Digest Type	Specific
Missed Cleavage	3
Min Peptide Length	7
Max Peptide Length	52
Toggle N-terminal M	True
Digest Rule	Trypsin/P
Protein Databases	

Average XIC Width	1.47 min
Median Absolute Delta iRT	0.19
Median Absolute Delta RT	0.08
MS1 Average Delta (ppm)	2.92
MS2 Average Delta (ppm)	2.04
MS1 Average Tolerance (ppm)	6.5
MS2 Average Tolerance (ppm)	6.5
Average IM Width	0.05
Peak Capacity	544.72 (NaN)
Median FWHM	0.092026 (NaN)
Median Peak Width	0.155975 (NaN)
Data Points per Peak (MS1)	10 (NaN)
Data Points per Peak (MS2)	10 (NaN)
Gradient Length	85

Table 3: Spectral library and characteristics

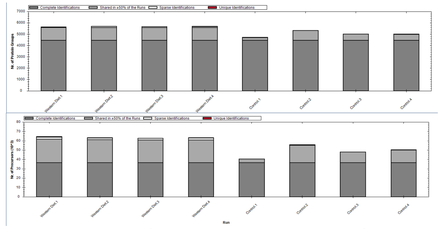

Fig. 4. dia-PASEF A] protein groups per condition B] precursors per condition

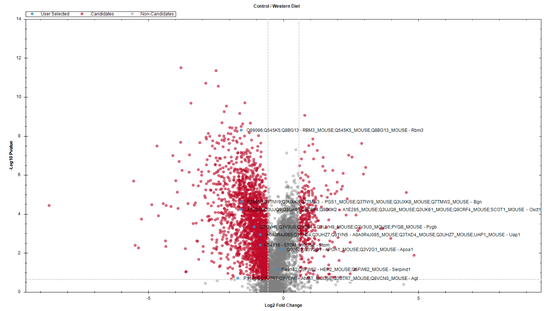

Fig. 5. dia-PASEF volcano plot highlighting genes that are known in fatty liver early disease

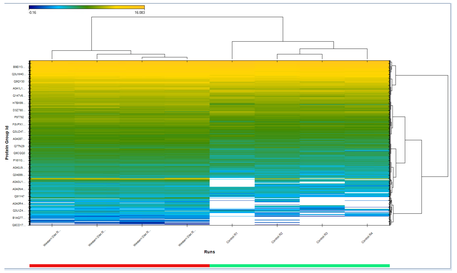

Fig. 6. DIA heat map with independent clustering separates high fat diet wild type control from wild type control

Summary

dda-PASEF Results

Control samples had good technical reproducibility as well as consistency of detected protein groups between biological replicates.

Wild Type directly loaded at 10ng on column gave an average of 3,365 protein groups and 41k peptides. The fatty liver that was 16 weeks on a western diet 10ng averaged 4,415 protein groups at an FDR of 1% and 49k peptides.

dia-PASEF Library

From a small sample size [n=4] biological with 2 technical replicates a spectral library representing 5,996 Protein groups and 55k peptides and 75 k precursors was generated.

dia-PASEF Results

Results from just 1ng on column averaged 4,500 protein groups in the wild type samples , with 40k peptides, 45k sequences, and over 5,500 protein groups in the fatty liver with western diet, with 50k peptides, and 60k sequences.

Conclusion

TIMS-PASEF in DDA mode was able to identify over five thousand protein groups from 10ng of mouse liver and in DIA over four thousand protein groups were identified from just 1ng of protein. This methodology allows deep proteome coverage from very small amounts of tissue and enables isolation of proteomes from specific regions of tissue vs whole tissue.

timsTOF SCP Technology