# Deep and quantitative succinylation profiling from dietary treatment in liver

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## Introduction

Protein succinylation is critical in cellular physiology and pathology. Moreover, it is an unexplored PTM and thought to have dramatic consequences on protein structure and thereby function given the large size of the succinyl moiety (100 Da) and amino acid site selectivity (K) changing charge from +1 to -1. Mass spectrometry (MS)-based proteomics is a powerful technology for the identification and characterization of succinylated peptides and its positional isomers. In this study, we combine succinylated peptide-level enrichment with timsTOF technology (DDA[1] and DIA[2]) to both identify sites of lysine succinylation and measure changes in succinylation upon dietary treatment in mouse liver tissues. Here, we show highly reproducible and accurate quantification using this instrument with the PASEF acquisition method and the software packages Spectronaut and PaSER which have optimized several parameters of their algorithms for the processing of 4-dimensional PASEF data.

## Methods

Control diet and treated (NS diet) mouse livers (N=8) were homogenized, digested and enriched for succinyl modifications using the anti-succinyl (Succ-K) affinity motif from Cell Signaling Technologies. A nanoElute (Bruker Daltonics) nano-flow LC was coupled to a high-resolution TIMS-QTOF (timsTOF Pro, Bruker Daltonics) with a CaptiveSpray ion source (Bruker Daltonics). The peptide mixtures were loaded onto a 250 mm pulled emitter column (IonOpticks). Chromatographic separation was carried out using a linear gradient of 2-30% buffer B (100% ACN and 0.1% FA) at a flow rate of 250 nl/min over 100 min. Data were acquired both in DDA[1] and DIA[2] mode using variable CE's and mobility windows. Data was analyzed by PaSER (DDA) and Spectronaut v. 15 in direct DIA mode (Library free approach).

1 Meier et al., J Proteome Res., 2015; 14(12); 5378-87
2 Meier et al., Nature Methods, 2020; 17; 1229-1236

## Results

The DDA search results produced in real time by PaSER identified more than 6300 peptides, in single shot analysis, of which greater than 3400 were succinylated. DDA-PASEF sample was prepared by pooling all 8 samples and injecting an amount equal to 2.5 uL on column. Within biological replicates DIA-PASEF identified on average 8200 peptide groups of which on average 4600 were succinylated. Moreover, a total of approx 1600 protein groups (PGs) was identified. An amount equal to 4 uL was injected on column for each DIA-PASEF run. Comparison between the NS diet *vs.* Control diet shows 389 significantly altered proteins (with a q-value less than 0.01 and an absolute fold change (AFC) greater than 0.58).

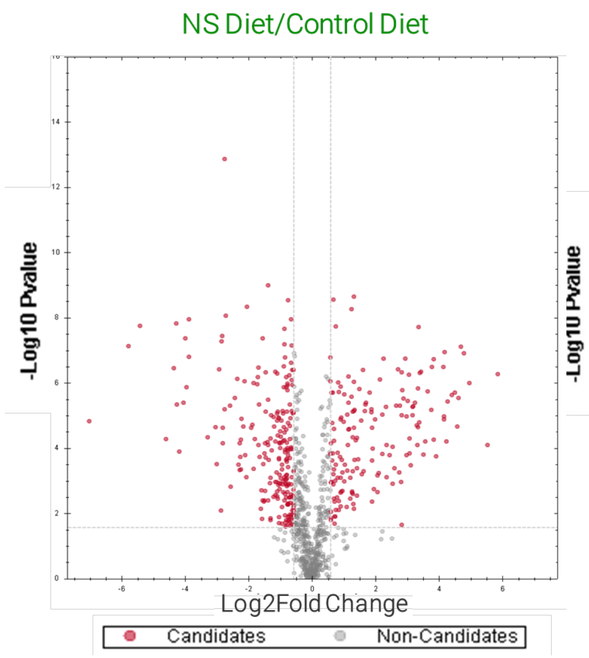


**Figure 1: volcano plot shows 166 upregulated proteins and 223 downregulated proteins**

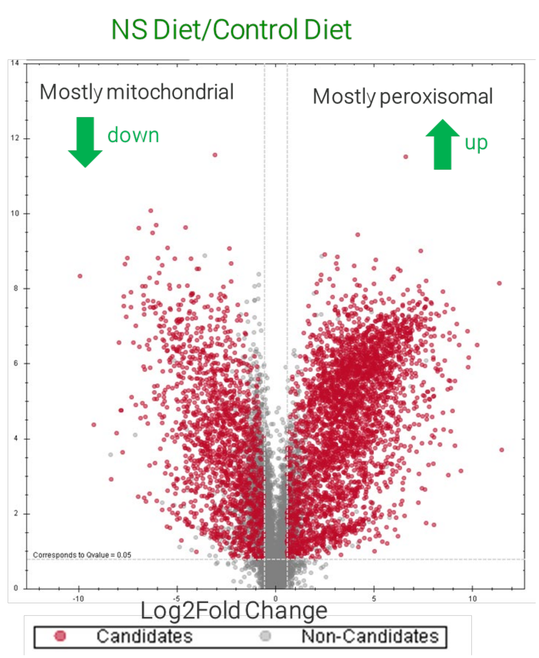


**Figure 2: volcano plot shows 3152 upregulated peptides and 1543 downregulated peptides**

timsTOF and CCS-enabled analysis

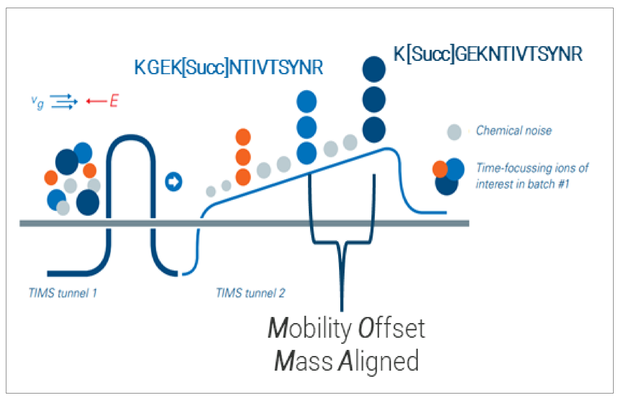


**Figure 3: separation in TIMS device of the 2 co-eluting peptides at RT = 37.04 minutes**

Meanwhile, comparison between the NS diet *vs.* Control diet, at the peptide level, shows 4695 significantly altered peptides (with a q-value less than 0.01 and an AFC greater than 0.58). Collectively, the data show a high number of peptides detected and a deep remodeling of the liver succinylome. The very high number of detected peptides is a direct result of the DIA-PASEF technology enabling a deep coverage and unambiguous differentiation of succinylated isomeric peptides separated in the ion mobility space (PASEF principle shown in figure 3 and representative example shown in figure 4 below).

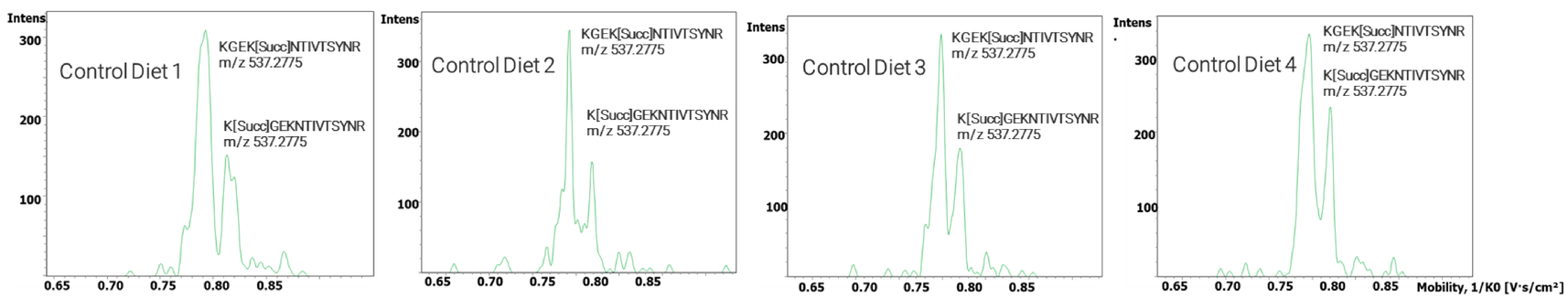


**Figure 4: extracted ion mobilograms (EIMs) of m/z 537.2766 eluting at 37.04 min showing 2 mobility peaks corresponding to KGEK[Succinyl]NTIVTSYNR and K[Succinyl]GEKNTIVTSYNR peptides, with a mobility value of 0.794 and 0.805 1/K0, respectively. Ion mobility values are repeatable across biological quadruplicates.**

## Conclusions

- High numbers of succinylated peptides enable a better understanding of the dynamic changes of the succinylome
- Ion mobility separation of succinylated isomeric peptides is reproducible and quantitative across runs

timsTOF Pro