Proteome characterization of tunneling nanotubes with dda-PASEF acquisition mode

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Introduction

Cell-to-cell communication is a crucial prerequisite for the development and maintenance of multicellular organisms. Ultrafine intercellular structures often referred as tunneling nanotubes (TNTs) are involved in crucial cell to cell communication and the spread of pathogens or toxins[1]. Their proteome characterization is central to understand which proteins are involved in these processes and their regulations. Bottom-up proteomics characterization of small amounts of TNTs is challenging due to the low amounts of material. To overcome this challenge, use of PASEF (Parallel Accumulation and Serial Fragmentation) technology on tims-TOF Pro and 4D-Proteomics enables the generation of high-quality peptide spectra with unsurpassed sequencing speed and sensitivity.

Methods

Whole cells and cellular protrusions like filopodia and TNTs were isolated using Laser Capture Microdissection[3]. Proteins were extracted and digested with the PreOmics kit[4]. Peptides were directly loaded on a IonOpticksAurora 25 cm column using nanoELute coupled to a timsTOF Pro instrument via a Captive Spray ionization source. Data were acquired using DDA-PASEF© in low sensitivity mode method. Data were directly streamed to PaSER box for all DDA data unless otherwise specified. Data were filtered at a FDR of 1% for peptide and protein groups.
Analyses were performed by injecting 4 uL of sample (upon reconstitution in 7 uL of initial chromatographic mobile phase) and measured over a 60-minute gradient. Samples prepared from 1000 or 500 whole cells treated with H_2O_2 generated around 1300 protein groups (PG) and approximately 5000 peptides. Meanwhile samples prepared from 3000 filopodia cuts or 3000 TNT cuts from cells treated with H_2O_2 resulted in 150 PGs on average and about 500 peptide IDs.

Results

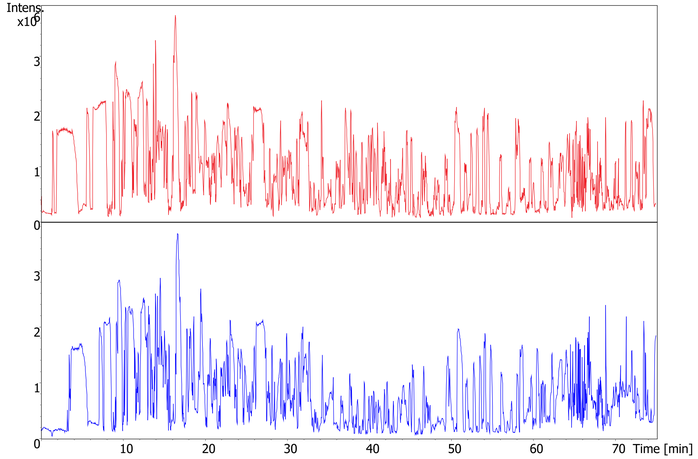

Fig. 1. Representatives BPCs for digest from (a) 1000 whole cells cut or (b) 500 whole cells cut from cells treated with H_2O_2

LC-MS-Orbitrap-Fusion Tribrid		
Sample types	*# of LCM sample isolated*	*# Protein IDs*
Whole cells	3000 cuts	1139
Whole cells	3000 cuts	851
Whole cells	3000 cuts	862
Average		**951**
Filopodia	6000 cuts	72
Filopodia	6000 cuts	86
Filopodia	6000 cuts	143
Filopodia	6000 cuts	36
Average		**84.25**
TNTs	12000*	103
TNTs	6000 cuts	71
Average		**87**

timsTOF Pro		
Sample types	*# of LCM sample isolated*	*# Protein IDs*
Whole cells	1000 cells	1331
Whole cells	1000 cells	1426
Whole cells	1000 cells	1468
Average		**1408**
Whole cells	500 cells	1259
Average		**1259**
Filopodia	3000 cuts	105
Average		**105**
TNTs	3000 cuts	203
TNTs	3000 cuts	119
Average		**161**

Summary tables of the number of protein IDs using LC-MS-Orbitrap-Fusion Tribrid *vs* timsTOF Pro. Increase sensitivity of timsTOF Pro allows for a reduction in required LCM samples. Interestingly, cutting the number of fixed cells down to 500 gave results similar to those obtained with 1000 cells, thus lowering by half the samples required for MS analysis compared to our previous published results[5]. The first TNT sample using the Fusion Tribrid required 12,000 cuts because the cells were fixed using glutaraldehyde and the protein were not extracted as published in Gordon et al., 2018. All the other samples followed our optimized fixation and protein extraction protocols[3,4,5].

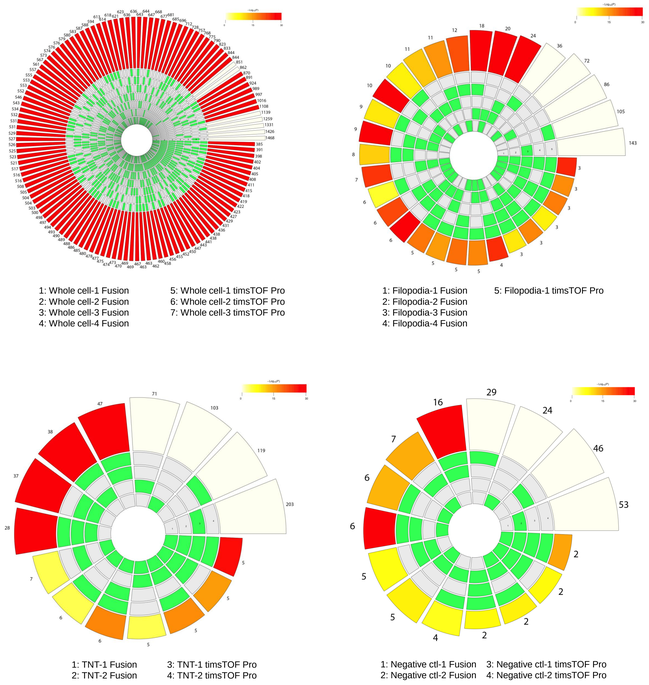

Fig. 2. The overlap per sample from the two mass spectrometers (a Thermo Orbitrap Fusion Tribrid and a Bruker timsTOF Pro) used for shotgun protein identification are highly significant.
Using the R package SuperExactTest[6] the four TNT samples have an overlap of 5 proteins (Fold Enrichment over expected overlap (FE): 329,533; P-Val.: 4.60e-27); the five filopodia samples have an overlap of 3 proteins (FE: 236,529,527; P-Val.: 2.75e-25); and the seven whole cell samples have an overlap of 385 proteins (FE: 17,806,770; P-Val: 0. These overlaps makes it clear that the machines are catching the same type of proteins per sample, therefore the LCM cutting, the extraction method, and the shotgun protein identification is extremely accurate from machine to machine.

Conclusion

- timsTOF Pro has a greater depth and sensitivity compared to Orbitrap-Fusion Tribrid mass spectrometer:
  - With a third less sample, we obtained an approx. 1.5-fold increase in the number of protein IDs for whole cells
  - With a quarter to a half less sample with TNTs, we obtained a 1.85 fold increase in the number of protein IDs
- timsTOF Pro allows for less starting material, in addition to enabling acquisition with shorter chromatographic gradients
- Our LC-MS approach enables sensitive and reproducible results that can be used for the proteome characterization of Filopodia and TNTs

timsTOF Pro

References

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