Ultra-high sensitivity nanoflow lipidomics with Trapped Ion Mobility Spectrometry (TIMS) and Parallel Accumulation SErial Fragmentation (PASEF)

Catherine G. Vasilopoulou[1], Karolina Sulek[2], Andreas-David Brunner[1], Sven W. Meyer[3], Ulrike Schweiger-Hufnagel[3], Ningombam Sanjib Metei[4], Matthias Mann[1,2], Florian Meier[1]

[1]Max-Planck Institute of Biochemistry, Martinsried, Germany; [2]NNF Center for Protein Research, Copenhagen, Denmark; [3]Bruker Daltonik GmbH, Bremen, Germany, [4]PREMIER Biosoft, Indore, India

max planck institute of biochemistry

1. Introduction

Despite consisting of few building blocks, lipids form a highly diverse group of biomolecules with important biological function. Established liquid chromatography – mass spectrometry (LC-MS) workflows sample the lipidome with high throughput, but limited selectivity and high starting amounts. We present a high-sensitivity workflow based on nanoflow separation and trapped ion mobility spectrometry (TIMS) [1]. By synchronizing TIMS separation with precursor selection (PASEF), we have recently demonstrated an over 10-fold increase in MS/MS acquisition rates without any loss in sensitivity [2, 3]. Here, we explore and establish a rapid and sensitive PASEF lipidomics workflow capable of comprehensively analyzing low sample amounts. To investigate the potential of the additional TIMS dimension, we set out to compile a high-precision lipid CCS library from body fluids, tissue samples and human cell lines.

2. timsTOF Pro

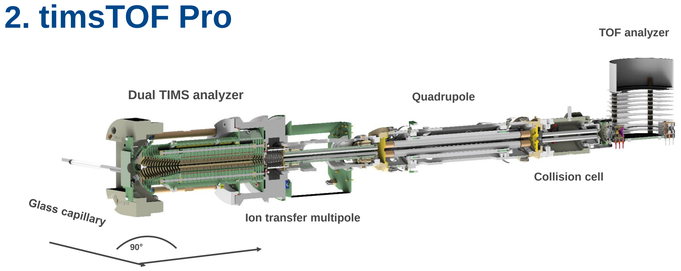


Figure 1. Instrument schematic of the timsTOF Pro. Ions are generated in an electrospray source, transferred into the vacuum system through a glass capillary, deflected by 90˚ , and focused into the dual TIMS analyzer. In the first TIMS analyzer ions are accumulated while another batch of ions is separated by ion mobility in the second TIMS analyzer. Ions transferred through a multipole and can be isolated by the analytical quadrupole mass filter for optional subsequent fragmentation in the collision cell. Afterwards, narrow ion packages are accelerated into a field free drift region by the orthogonal deflection unit for high-resolution time-of-flight mass analysis.

3. Nanoflow PASEF lipidomics workflow

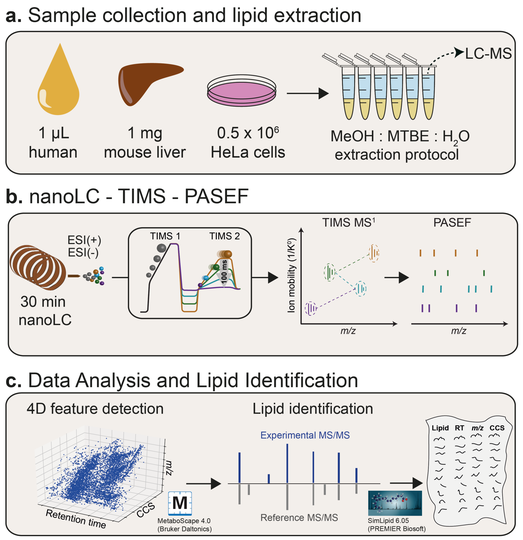


Figure 2. Nanoflow lipidomics with trapped ion mobility mass spectrometry. a. Lipids from various biological sources, such as body fluids, tissues and cells, are analyzed using a single MeOH:MTBE extraction. **b.** The crude extract is injected into a nanoflow liquid chromatography (LC) system coupled online to a high-resolution TIMS quadrupole time-of-flight mass spectrometer (timsTOF Pro, Bruker Daltonics). In the dualTIMS analyzer, ions are accumulated in the front part (TIMS 1) while another batch of ions is mobility separated in the rear part (TIMS 2). PASEF synchronizes precursor selection and ion mobility separation, which allows to fragment multiple precursors in a single TIMS scan at full sensitivity. **c.** Features are extracted from the four-dimensional (retention time, *m/z*, ion mobility, intensity) space by T-REX 4D in MetaboScape and assigned to PASEF MS/MS spectra for automated lipid identification and compilation of comprehensive lipid CCS libraries. Lipids were identified using SimLipid. *MeOH* = methanol, *MTBE* = methyl-tert.-butyl ether, *CCS* = collisional cross section.

4. PASEF in Lipidomics

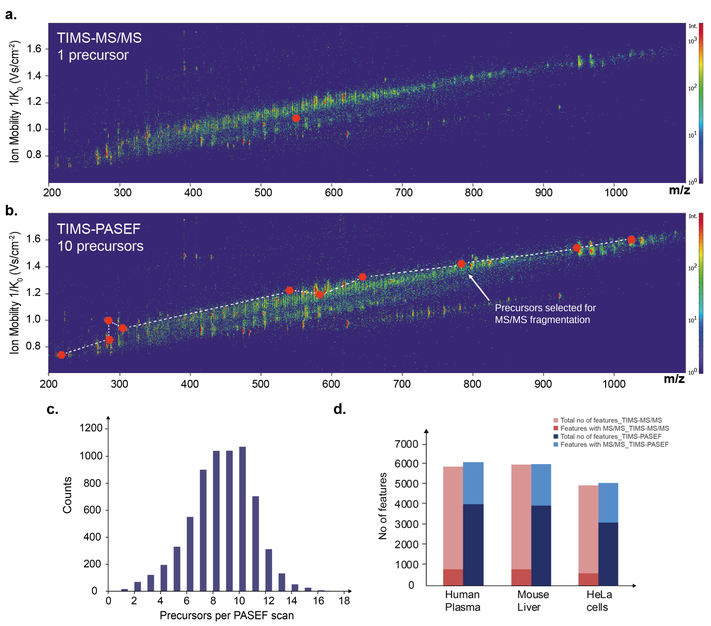
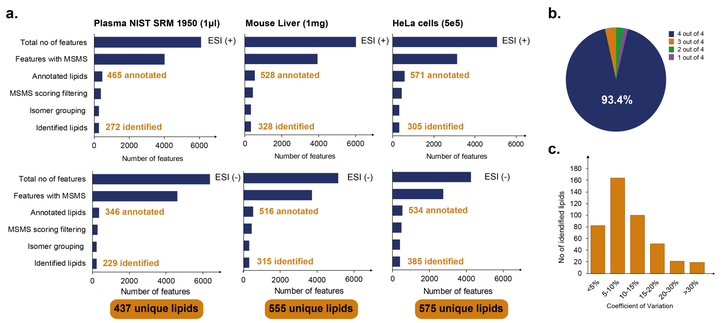


Figure 3. Evaluation of PASEF in lipidomics. a.b. Heat-map visualization of a representative trapped ion mobility resolved mass spectrum of human plasma. Red dots indicate precursors selected for MS/MS fragmentation in the subsequent 100 ms PASEF scan. **c.** Distribution of the number of precursors per PASEF scan analyzing human plasma lipid extract. **d.** Total number of 4D features extracted from 30 min runs of human plasma (n=4), mouse liver (n=5) and human cancer cells (n=5) in positive ion mode without (TIMS-MS/MS, red) and with PASEF (PASEF, blue). The fraction of features assigned to MS/MS spectra is indicated by a darker color.

5. Accurate identification and label-free quantification



MS/MS- and accurate mass-based identification on head group and fatty acyl level.

Figure 4. Lipid identification and label-free quantification. a. Sequential data analysis steps from the total number of detected 4D features to unique lipids for human plasma, mouse liver and human cancer cells in both ionization modes. **b.** Fraction of lipids quantified in *N* out of four replicate injections of plasma. **c.** Coefficients of variation for 437 lipids label-free quantified in at least two out of four replicate injections of plasma.

6. Accurate and precise ^{TIMS}CCS measurement

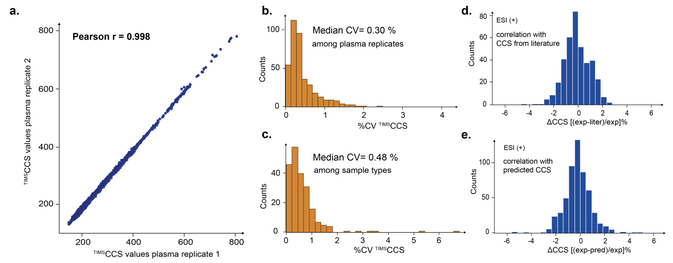


Figure 5. Precise and accurate determination of lipid ^{TIMS}CCS values. a, Pearson correlation of ^{TIMS}CCS values of 6,100 4D features detected in two replicate injections of a human plasma lipid extract. **b,** Coefficients of variation (CV) of ^{TIMS}CCS values for lipids commonly identified in replicate injections of the same sample (plasma) and **c,** three different samples (plasma, liver, HeLa). **d,** Relative deviation of experimental ^{TIMS}CCS values in this study from literature reports [4, 5] and **e,** machine learning predictions [5].

7. The TIMS lipidomics landscape

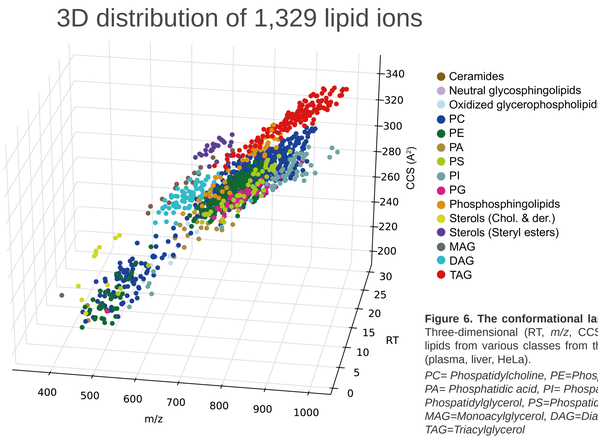


Figure 6. The conformational landscape of lipid ions. Three-dimensional (RT, *m/z*, CCS) distribution of 1,329 lipids from various classes from three biological samples (plasma, liver, HeLa).
PC= Phospatidylcholine, PE=Phospatidylethanolamine, PA= Phosphatidic acid, PI= Phospatidylinositol, PG= Phospatidylglycerol, PS=Phospatidylserine, MAG=Monoacylglycerol, DAG=Diacylglycerol, TAG=Triacylglycerol

8. Conclusions

- Trapped ion mobility separation enabled us to fragment on average nine precursors per 100ms PASEF scan by rapidly switching the mass position of the quadrupole and acquire MS/MS spectra for almost all detectable isotope patterns in short nanoLC runs (30 min).
- Our nanoflow PASEF lipidomics workflow is readily applicable to a broad range of biological samples and scales well from minimal amounts of body fluids (1 µL blood plasma) to larger tissue samples (1mg mouse liver) and cell cultures (0.5 million cells).
- TIMS-PASEF positions each lipid in a four-dimensional space with a precision <2 ppm for masses, <0.2% for CCS and about 1-5% for retention times.
- We compiled a library of over 1,300 high-precision lipid CCS values directly from unfractionated biological samples.

We conclude that TIMS and PASEF enable highly sensitive and accurate 4D lipidomics, and generate comprehensive digital archives of all detectable species along with very precise ion mobility measurements – a wealth of information which awaits full exploration and application.

[1] Ridgeway,...., Park. Int. J. MS, 2018; 425:22-35.
[2] Meier,...., Mann. J Proteome Res. 2015; 14:5378-87.
[3] Meier,...., Mann. Mol. Cell. Prot. 2018.; 17: 2534-45.
[4] Leaptrot,....,McLean. Nat. Commun. 2019; 10.
[5] Zhou, …, Zhu. Anal Chem. 2017; 89:9559-66.

e-mail: vasilopoulou@biochem.mpg.de