A.33 Evaluating timsTOF Pro 2 for the analysis of histone PTMs measured in Global Chromatin Profiling using dia-PASEF and prm-PASEF
Sebastian Vaca¹, Malvina Papanastasiou¹, William R. Hawkins¹, Andrew H. Reiter², Simone Sidoli², Steven A. Carr¹
¹Broad Institute of MIT & Harvard, ²Albert Einstein College of Medicine

The functional roles of chromatin post-translational modifications (PTMs), including phosphorylation, methylation and acetylation, in transcriptional regulation and disease are only beginning to be elucidated, limited in part by our ability to characterize and quantify the modifications present. We previously developed and applied a high-throughput, quantitative MS-based assay termed Global Chromatin Profiling (GCP) that enables the study of 80 combinatorial PTMs on histone tails in cells and tissues primarily on histones H3 and H4. While this set is of high biological interest, it does not represent the full spectrum of modifications and does not fully cover other histones like H2A/H2B. In the current study, we evaluated the use of timsTOF Pro 2 for the analysis of histone PTMs using both dia-PASEF and prm-PASEF acquisition modes in an effort to improve the overall coverage of modifications as well as to increase the speed and sensitivity of the analyses.

We investigated the linearity range, limits of detection and optimum collision energies for fragment ion intensities. To accelerate the development of prm-PASEF methods, we implemented a workflow based on dia-PASEF. Individual values for retention time, optimal collision energy and ion mobility for all histone PTMs were extracted from dia-PASEF runs in Skyline. We further investigated the effect of accumulation time in the TIMS cell on peptide intensities and selected optimum values to prevent signal saturation. Optimized dia-PASEF and prm-PASEF methods were employed for the study of cancer cells perturbed with epigenetic drugs, known to modulate histone PTMs. Results from both acquisition methods were compared to previous results obtained employing our conventional workflow using a Thermo Orbitrap QE +. The application of ion mobility and recent developments in acquisition techniques allowed us to better discriminate between isobaric PTMs, obtain more accurate measurements and improve PTM coverage. Further, acquisition using dia-PASEF also allows for future mining of additional, low abundance lysine PTMs chemically related to acetylation (i.e. crotonylation), thereby providing a more comprehensive view of the histone PTM landscape. Methodological improvements, including advanced sample preparation protocols, sensitivity and throughput, as well as current limitations in the field towards using low numbers of cells will be presented.

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A.34 Proteome imaging of human pancreas with a novel microPOTS platform
Marija Velickovic¹, Le Z. Day², Sarah M. Williams¹, Sara J. Gosline², James C. Pino², Vincent Danna², Thomas L. Fillmore³, Camillo Posso², Isaac K. Attah², Julia Laskin², Kristin E. Burnum-Johnson¹, Ying Zhu¹, Paul D. Piehowski¹
¹Environmental and Molecular Sciences Division, Pacific Northwest National Laboratory, Richland, WA, ²Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA, ³Department of Chemistry, Purdue University, West Lafayette, IN, 47907, USA

At PNNL, significant emphasis has been placed on improving the sensitivity of global proteomics. These efforts have resulted in the development of the nanoPOTS platform (nanodroplet processing in one pot for trace samples). This approach greatly enhances the efficiency of sample digestion and protein recovery. Another advantage of this approach is facile coupling to laser capture microdissection (LCM) for tissue analysis. In this work, we designed a new, larger chip that increases the processing volume up to 2 μL, which allows for simple manual operation while maintaining much of the critical advantages of the nanoPOTS approach. Further, we combined this approach with isobaric labeling and our in-house built nanofractionation system to create a robust platform for spatial proteomics capable of quantifying >5000 proteins from human tissues with ~200 μm spatial resolution.

To demonstrate the utility of this approach, we applied it to pancreas tissue from human and rat models. In our first experiment, we collected 5 samples containing microdissected islets and 5 samples containing acinar tissue, followed by a larger “boost” sample containing roughly equal amounts of both tissue structures. The samples were digested on-chip and incorporated into a single TMT-11plex for fractionation into 12 fractions for LC-MS/MS analysis. Differential analysis using linear mixed model for microarray data (LIMMA) R package found, 2,300 proteins differentially expressed (adjusted p-value <0.05). Functional enrichment results revealed significant enrichment of secretion and exocytosis related terms in islets, and metabolic processes in the acinar tissue.

For proteome imaging of human pancreas, we created a grid pattern using the LCM software that centered a single voxel on a pancreatic islet, with the remainder of the imaging voxels covering the surrounding microenvironment. A total of seven independent images were collected from a single tissue section to capture a variety of islets and environmental contexts. Mapping insulin and glucagon, markers of the healthy islet show localization predominantly to the islet voxel. In contrast, AMY2B and LIPPP are preferentially abundant in the acinar tissue. Further analysis and tool development is currently underway to more efficiently leverage the spatial information contained in this dataset.

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