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.