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**BAMS: A Robust Analytical Platform for Quantifying Histone Modifications and Various Proteoforms by IP MALDI MS**

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The identification of proteoforms is a challenging element of proteomics research due to the vast level of heterogeneity and dynamic range needed to span the full proteome. Proteoforms—which result from a variety of genetic and post-translational modifications (PTMs) dictate a protein’s functional state. This extends out to histones proteins which are heavily modified with post-translation modifications. We configured an immunoaffinity platform to independently screen multiple post-translational modifications within a single region of a protein using BAMS. In this poster, we assessed the analytical merits of BAMS for quantification of histone post-translation modifications.

Synthetic peptides were purchased from Thermofisher Scientific. Cells were cultured in SILAC DMEM media and exposed to DMSO or SAHA (5uM). Cells were collected at 0, 10, 30min and 4, 8 and 24hrs following SAHA treatment. Nuclei were isolated and lysed in the presence of detergents. 100ug of protein was digested with LysC in 0.5% SDC & 12mM SLS. Digestion was terminated by heating to 90 °C for 5 min. Affinity capture was performed using site-specific antibodies coupled to magnetic agarose beads. Assays were performed in 0.17% SDC & 3mM SLS and washed in ACN, PBS and water. Target peptides were eluted prior to MALDI MS analysis using a timsTOF Flex instrument (Bruker Daltonics). A panel of BAMS affinity-capture reagents, targeting site-specific epigenetic post-translational modifications on histones were used. The panel was generated by coupling magnetic agarose beads (375 μm, +/- 25 μm) to site-specific antibodies to monitor the following histone PTMs: H4 (K5ac/K8ac/K12ac/K16ac), H3 (K9ac) and H3 (K9me). Reproducible micro-arrays were generated using a MALDI matrix sprayer, efficiently eluting captured peptide analytes from the antibody-coupled beads. The BAMS microarray slide was analyzed using the timsTOF Flex instrument. High correlation and reproducibility were observed among replicate high density BAMS microarray slides. High degree of linearity (R2 ~0.98, precision (C.V. <5%), and dynamic range spanning 3 orders of magnitude was observed utilizing synthetic peptides as internal standards. Multiple H3 histone post-translational modifications were quantified over a wide time course (0, 10, 30 240, 480, 1440 min) using SILAC based quantification to assess kinetics of an HDAC inhibitor.

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**Highly multiplexed label-free imaging of tissue proteoforms using individual ion mass spectrometry**


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Proteoforms are the key effectors in human health and disease. Mapping proteoform localizations in tissues is critical in characterizing the human proteome. Imaging mass spectrometry (MS) is a powerful technique for label-free imaging of proteins in tissues. Despite the advances in spatial resolution, imaging MS is limited by proteome coverage and the molecular specificity to characterize proteoforms. Herein, we introduce proteoform imaging mass spectrometry (PiMS) as a highly multiplexed technique to image intact tissue proteoforms of molecular weight up to 80 kDa. PiMS utilizes nanospray desorption electrospray ionization (nano-DESI) MS as a sampling and ionization probe, and charge detection via Orbitrap-based individual ion mass spectrometry (I2MS) is employed in data acquisition. In a PiMS experiment, proteoforms from different locations on tissue are sampled and detected as multiply-charged individual ions. By means of I2MS processing, molecular masses of proteoforms are directly obtained, and putative proteoform IDs are assigned using intact mass tag (IMT) approach. For each assigned proteoform, an ion image is constructed by plotting the absolute individual ion count against the spatial coordinates on tissue. A first demonstration of PiMS in human kidney tissue yields the identification of 169 of the ~400 detected proteoforms, including key enzymes in primary cell metabolisms, blood proteoforms, structural proteoforms, etc. The identified proteoforms show distinct localizations to the cortex, the medulla, and the vasculature in kidney. To further identify the detected tissue proteoforms, we developed a multiplexed top-down PiMS2 workflow to maximize proteoform identification. This workflow utilizes PiMS on tissue as a survey of the intact masses, charge states, and spatial distributions of the proteoforms. Data-dependent top-down MS/MS experiments were performed on an adjacent region. MS/MS data was collected in either ensemble or I2MS2 mode, which enables efficient fragment detection in a broad mass range. In a proof-of-concept experiment performed on a human ovarian cancer tissue, 63 out of 84 proteoforms in the 3-50 kDa range at >5% relative abundance were identified in a single experiment. PiMS is poised to serve as a powerful imaging tool for biomarker discovery and disease diagnostics in various human tissues.

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