

Spatial and Single Cell Proteoform Analysis Using Direct Sampling Single Molecule Mass Spectrometry

“Proteoform” is a term describing the actual molecular forms of proteins including sequence variations and posttranslational modifications, bringing us one step closer to phenotypes and dynamic states. In addition, direct proteoform measurement bypasses steps in conventional proteomics experiment (e.g., proteolytic digestion, chromatographic separation), providing a path to proteomics in spatial tissue compartments and single cells at unprecedented sensitivity and throughput.

Mass spectrometry (MS) is superior for proteoform measurement by accurate determination of molecular masses and connectivity of amino acid sequences and modifications. To achieve sensitive MS detection of proteoforms, we employed individual ion mass spectrometry (I2MS), an Orbitrap-based charge detection MS technique in conjunction with direct sampling MS approaches. We demonstrate proteoform imaging mass spectrometry (PiMS) using nanospray desorption electrospray ionization (nano-DESI) has enabled highly-multiplexed imaging and identification of tissue proteoforms up to 70 kDa. These proteoforms not only help discern tumor margins in ovarian cancer tissues at ~20 μm spatial resolution, but also report anatomical regions and cellular neighborhood of human kidney, contributing to our knowledge in reference tissue atlases. Such approach has been extended to high-throughput single cell profiling at a speed of >1000 cells per day, enabling proteoform-based cell typing in complex cell mixture from rat brain hippocampus. We discovered small populations of glial cells and resident immune cells using novel proteoform signatures. Finally, nanosecond infrared laser ablation was coupled to Orbitrap MS that allowed for molecular analysis of intact proteins and their complexes in their native physiological environment. Together these tools unveiled previously unknown molecular channels that may be employed for future fundamental biological research, biomarker discovery and disease diagnostics.

User consent

yes

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