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Exploring Spatial Top-Down Proteomics

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Understanding proteins within their functional contexts, whether in tissue units, cellular neighborhoods, small clusters of cells, or even at the single-cell level, remains a significant scientific challenge that pushes the boundaries of analytical methods. Traditional proteomic approaches largely rely on antibody-based techniques, which limit multiplexing and require prior knowledge of target proteins. While advancements like nanoPOTS-based bottom-up proteomics offer promising tools for analyzing small tissue sections, even single cells, these methods fall short in capturing proteoform-specific information. Proteoforms, representing distinct variations of proteins, are fundamental to cellular roles and functions. To address this gap, we have developed an approach that combines laser capture microdissection (LCM) nanoPOTS with mass spectrometry imaging (MSI), enabling both bottom-up and top-down proteomics. This integrated strategy has been applied across diverse systems, including human, murine, plant, and microbial tissues. For example, in studies of human pancreatic tissue, our methods provided an extensive proteoform landscape, identifying 500-1000 proteoforms and revealing unique variations of endocrine proteins that are often overlooked by conventional methods. MSI further enabled the spatial profiling of hundreds of islets, allowing clustering based on their proteoform signatures. LCM was instrumental for isolating both pooled and individual pancreatic islets, which were then analyzed using nanoPOTS for label-free quantitative top-down proteomics. Additionally, single islets and single cells were dissected to perform comprehensive bottom-up proteomic. These tools are now being applied to investigate chronic pancreatic diseases, T1D progression and therapeutic intervention.

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