Multiplexed, scalable, and functionality compatible platforms for 3D spatially resolved proteomic profiling

Yuxuan Tian

Technical summary:

In order to systematically study complex biological systems, it's essential to examine the cellular and molecular information from the perspective of multiplexed molecular networks in spatially contextual microenvironment. Due to the highly dynamic, deeply interconnected network of various biomolecules, it is necessary to characterize the spatial distribution and molecular functionality of biomolecules within the context of their interactive role in the whole biomolecular network. Spatial proteomics serves as an indispensable tool for extracting and deciphering cellular and molecular information of proteins, one of the important category of functional biomolecules, within biological systems. However, compared with high throughput technologies such as single cell sequencing or spatial transcriptomics, its widespread application is often hindered by the constrained volumetric scalability and multiplexing capability inherent in current approaches.

Additionally, existing spatial proteomics focus more on capturing the information of molecular identity and their spatial locations, often neglecting the information of functionality. Since proteins, together with other functional biomolecules (glycans, neurotransmitters, lipids, etc.), are major participants of biological processes, their functionality profile can provide valuable insights into molecular recognition, signal transduction, and functional abnormality directly related to disease diagnostics and therapeutics. However, the widely used chemical fixation protocols don't support functional detection of proteins, triggering the development of new tissue preservation technology for the unfulfilled aim of spatially resolved functional detection.

To address these challenges, two projects were implemented during my PhD to improve the volumetric scalability, multiplexing capacity, and the possibility of spatially resolved functional detection of spatial proteomics. Here we firstly present a robust, easy-to-implement 3D proteomic mapping platform with both volumetric and multiplexing scalability. With this platform, multiround, multichannel protein detection can be achieved on millimeter scale tissues in a unique buffer environment. We showcased two modalities for 3D proteomic analysis: dense labeling, better at morphological characterization of cytoarchitectures, and stochastic sparse labeling, compatible to combinatorial barcoding strategy for enhanced multiplexing scalability. Using our platform, we performed comparative analysis of the pathology and cytoarchitecture of human brain diagnosed with Alzheimer's Disease (AD) and healthy control. We evaluated the region-specific features of AD pathological hallmarks, as well as their correlations to neuronal compositions, innate immune systems, and axonal connectivity properties. Additionally, in the second project, we developed a fixative-free technology enabling 3D in situ functional detection of proteins. As a demonstration, we confirmed the mechanical

interlocking effect on various types of biological tissues, and verified the entanglement between dense polymer chains and biomolecules. As we predicted, signals of enzymatic activity from endogenous proteases in mouse colon tissues were successfully captured in 3D, spatially resolved way. Together, we envision that our results will serve as pioneering technologies to enable spatial proteomic profiling with better volumetric scalability, multiplexing capacity, and possibility of spatially resolved functional detection.

Thesis supervisor: Kwanghun Chung

Title: Associate Professor of Chemical Engineering