## 1. Overview/Abstract

Similar to all other organs, kidney is composed of many functionally different cell types that would need single-cell level tools to investigate the disease mechanisms and identify targets for clinical treatment. Recent sequencing-based transcriptomic technologies have revolutionized kidney research by their capability of thorough classification of cell subtypes and their varied gene expression. However, genome-wide protein information that bridges the gap between gene expression and clinical diagnosis has been lacking particularly at the single-cell resolution. Functional proteins (hundreds as known so far) have been well known for representing phenotypes, physiological activities, drug targets, signaling pathways and regulations for cells. The current functional proteome tools either only analyze dozens of proteins in single cells or lack the sufficient sensitivity. In this project, we will optimize a multiplex in situ tagging (MIST) technology and apply it to isolated single cells for functional proteome studies, and we will also develop a spatial functional proteome technology to study kidney biopsy samples. Single-cell MIST has been demonstrated to measures >450 proteins for T cells and ~200 proteins for solid tissue samples in our preliminary study. With the high capacity of multiplexity, we will compose a large kidney-specific panel of proteins that include biomarkers, important signaling proteins and transcription factors. Two aims we propose are to (1) Optimize scMIST to analyze blood and urine cells from patients with kidney disease; and (2) Develop single-cell spatial MIST for analysis of human kidney biopsy specimens. The completion of this project will generate enabling technologies and methods widely accessible in the kidney research community, and will produce the critical preliminary data for proposals targeting large external grants.