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PRESENTATION TITLE: Multimodal Mass Spectrometry Imaging Approaches for Probing Complex Biological Systems

ABSTRACT: The human kidney is composed of over 26 cell types that actively coordinate to form higher order structures, such as glomeruli and tubules. While the scientific community generally understand the roles of these cell types and structures, it is not known how these cells vary molecularly and numerically throughout a single organ or between organs within the human population, particularly as a function of demographic. Understanding both the underlying similarities and differences between these demographics has major ramifications for functional efficiency, transition to disease, and disease severity. We developed and employed a multimodal approach consisting of imaging mass spectrometry, multiplexed immunofluorescence, autofluorescence microscopy, and histopathology to explore the molecular and cellular differences between human kidneys as a function of sex and BMI. Human kidney tissue was flash frozen, embedded in carboxymethylcellulose, and cryosectioned to a 10 µm thickness. Autofluorescence microscopy was acquired. Samples for MALDI IMS were coated with DAN using an HTX TM Sprayer. MALDI IMS was performed on a timsTOF fleX MS system in positive and negative ion modes at a 10 µm pixel size. For multiplexed immunofluorescence, tissues underwent antigen retrieval, rehydration, fixation, primary antibody incubation, and further fixation. The CODEX system automatically dispensed complementary, fluorescent barcodes. Microscopy was performed using EGFP, DAPI, Cy7 and DsRed filters. Data were processed using a combination of commercial and inhouse software. Here, we developed a workflow consisting of sample preparation, pathological assessment, multiplexed immunofluorescence, MALDI imaging mass spectrometry, and histological staining to obtain rich molecular and cellular profiles of 17 human kidneys. In total, we sampled from both men and women between the ages of 21 to 77 years old. The size and depth of coverage enabled creation of the most extensive atlas of the human kidney to date, including >200 identified lipids and 23 antibody labels. We then used supervised and unsupervised approaches to create molecular and cellular profiles of glomeruli and proximal tubules. By correlating our suite of analytical approaches with MALDI IMS, we have hypothesized the physiological roles of cell type defining lipid features. For example, phosphatidylinositol lipids were globally detected with lower abundance in female glomeruli with the largest differences observed in PI(34:2), PI(36:4), and PI(32:0). Based on multimodal data, we hypothesize that these molecules are critical substrates for prostaglandin synthesis in glomerular mesangial cells. The function of most lipids remains unknown because they are difficult to assess, demonstrating the importance and power of the approach for understanding the role of discrete lipids. Moreover, the established methodology is demonstrated for the human kidney but can be expanded and validated for use in other organ systems, such as the heart or spleen, or animal models, such as the mouse. Because the workflow is flexible, a variety of samples can be accommodated for high content atlas creation and exploration of biological systems.

BIOGRAPHY: Elizabeth Neumann is an assistant professor in the chemistry department that joined in July. Her research focuses on understanding the molecular and cellular architecture behind neurological diseases. This highly interdisciplinary research involves developing analytical tools and multimodal imaging methods for understanding complex biological phenomena. Before joining UC Davis, she was a National

Institutes of Health postdoctoral fellow at Vanderbilt University in Nashville, Tennessee, developing an open, global atlas of the human body at the cellular level. Additionally, she earned her doctorate in analytical chemistry at The University of Illinois at Urbana-Champaign, Urbana, Illinois, where, among other honors, she received a graduate student leadership award and a National Science Foundation-Graduate Research Fellowship.