Multiomic Analyses of Pancreatic Islets via Laser Capture Microdissection-Mass Spectrometry and timsTOF fleX MALDI-2 Imaging

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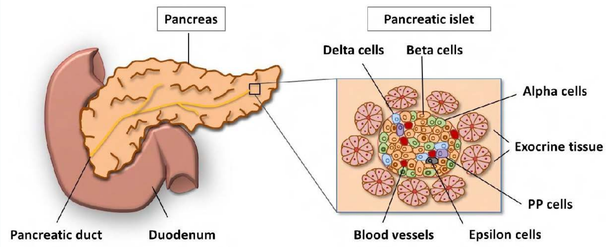
Objectives

- To develop methods for high-spatial resolution, multiomic mass spectrometry (MS) analyses of pancreatic islets of Langerhans.

- To use laser capture microdissection (LCM) and MS imaging to analyze the metabolome, peptidome, glycome, and proteome of pancreatic islets in healthy and diabetic states.

Introduction

Pancreatic islets of Langerhans perform the endocrine functions (peptide hormone synthesis) of the pancreas. Islets only compose approximately 3% of organ mass. The most abundant islet cell types are α and β-cells, which synthesize glucagon and insulin, respectively. Other less common cell types include δ and ε-cells, which synthesize somatostatin and ghrelin respectively.[1-2]



Pancreatic disease, such as cancer and diabetes, often impairs islet function, potentially leading to fatal complications. Understanding disease-induced biomolecular changes in islets at the single-islet and single-cell level using LCM-LC-MS/MS[3-4] and MALDI-MS imaging[5-6] can help improve our understanding of pancreatic disease pathology and identify molecules of interest for biomarker discovery or therapeutic targeting. Here, we seek to develop these methods and apply them to cadaveric pancreas tissues.

Experimental Methods

Figure 1: Workflow for LCM-LC-MS/MS. Formalin-fixed, paraffin-embedded (FFPE) pancreas sections are stained to visualize islets, which are collected on a tube cap, and lysed via sonication in SDS or acidified MeOH. Extracted analytes are analyzed intact or are digested with trypsin using an S-Trap.[7] Peptides are analyzed using reversed-phase nanoflow LC-MS/MS. Database searching is done using MaxQuant.

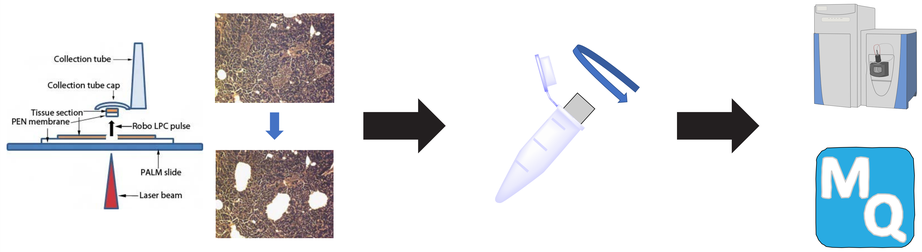
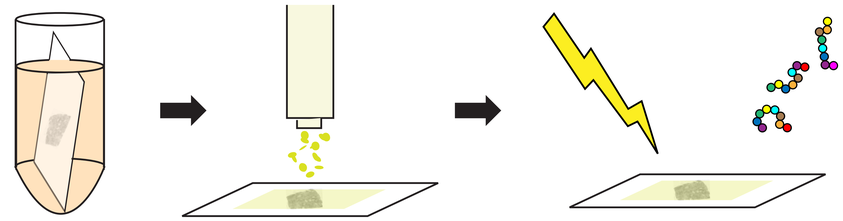


Figure 2: Workflow for MALDI-MS imaging. Pancreas tissues used are either flash frozen and embedded in 3% carboxymethylcellulose or FFPE. Based on the biomolecular class of interest, sections are pre-treated with a washing step before matrix application. Tissues are imaged using either a timsTOF fleX MALDI-2 or a rapifleX Tissuetyper TOF/TOF and analyzed using SCiLS Lab.



Results and Discussion

Figure 3: Results from LCM-LC-MS/MS of pancreatic islets. Acidified methanol was used to extract peptide hormones, while SDS was used to extract proteins, including those from the extracellular matrix (ECM). **A)** Pie graph of hormones identified by peptide family. Many families were identified, unsurprisingly including insulin and glucagon-related peptides. **B)** Comparison of ECM proteins identified using urea-only extraction, in-solution digestion followed by C18 cleanup versus SDS extraction and on-trap cleanup and digestion. Use of the S-Trap for sample preparation identified a larger number of ECM proteins.

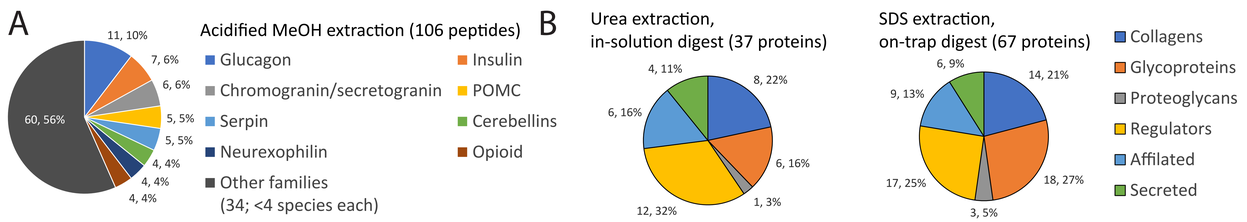
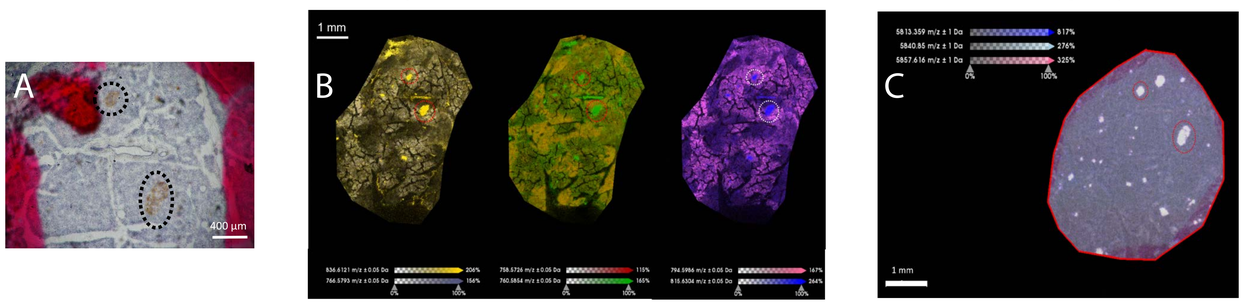


Figure 4: Results from MALDI-MS imaging of pancreatic tissue sections at 10 µm step size. Lipids were analyzed after washing tissue sections in ammonium acetate and application of CHCA matrix using a timsTOF fleX MALDI-2 in reflectron mode. Hormones were analyzed after washing in ethanol solutions and application of CHCA matrix using a rapifleX Tissuetyper TOF/TOF in linear mode. **A)** Optical image of pancreas tissue stained to visualize islets (in brown). These two reference islets are circled in both the optical and MALDI images. **B)** Overlaid images of phosphocholine (PC) lipids distinguish islets from exocrine cells. Islets are distinguished using PC(40:5), PC(34:1), and PC(38:2) , while exocrine cells are distinguished by PC(P-36:4)/(O-36:5), PC(34:2), and PC(P-38:4)/(O-38:5) (L-R). C) Overlaid hormone images of three adducts of insulin (protonated, sodiated, and potassiated) reveal heterogeneity in islet size.



Conclusions and Future Work

- SDS lysis and on-trap digestion using the S-Trap enables higher coverage of islet ECM proteins compared to in-solution digestion with urea lysis.
- MALDI-MS enables multiomic high-spatial resolution imaging of individual pancreatic islets.
- The methods developed here will be applied to diabetes and pancreatic cancer tissue for multiomic single-islet and single-cell analyses.

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