Comparative MALDI MS analysis of human pancreatic islets – from tissues to individual cells

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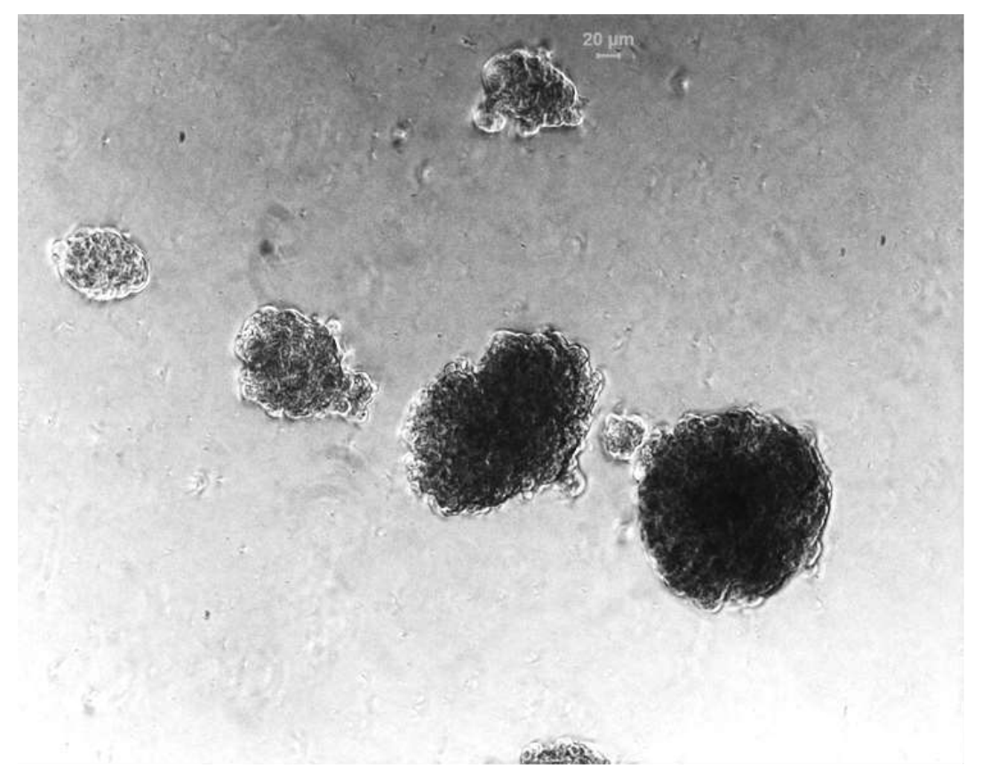
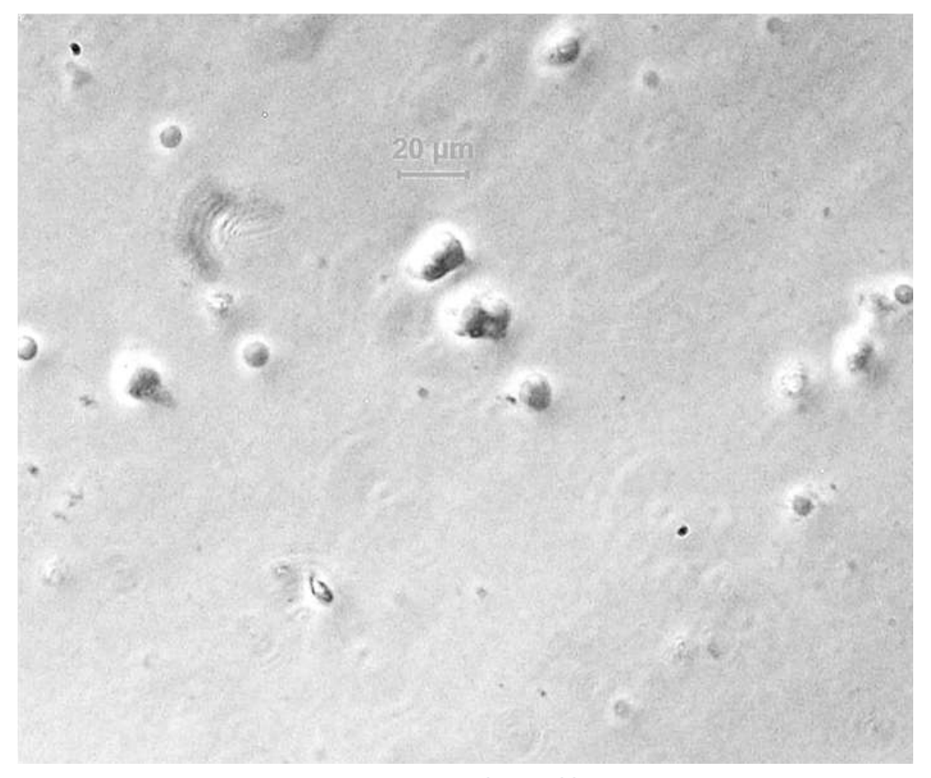
Introduction

Mass spectrometric (MS) investigation of chemical and structural complexities of the pancreatic islets (PI) allows the evaluation of their biochemical states including expression of hormones such as insulin, glucagon, and somatostatin as well as to estimate cellular composition in healthy and diseased conditions. Depending on the study goals and the specific instruments protocols, e.g. MS imaging of pancreatic tissue sections, whole islet MS analyte profiling, and single cell MS, distinct outcomes can be obtained. These approaches require different sample preparation steps and MS analysis parameters. We compare the outcomes of measurements of human islets using these three techniques with a focus on whole islet peptide profiling using MALDI MS.

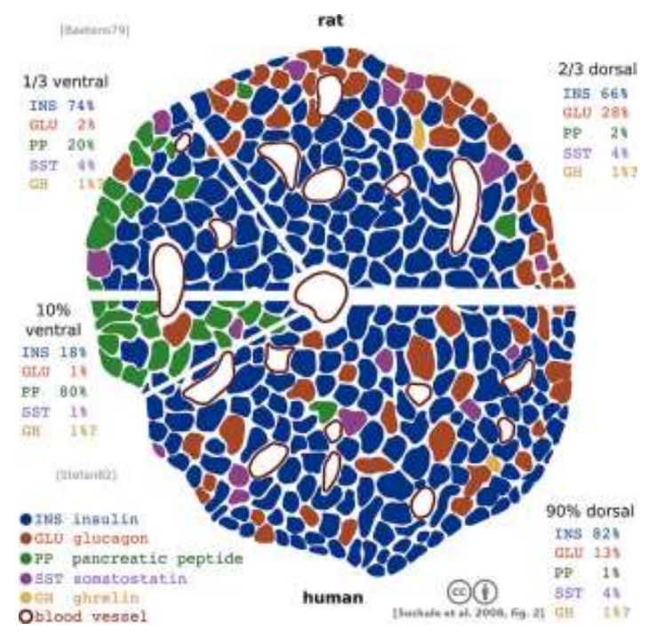
METHODS AND SAMPLE-RELATED INFORMATION

Isolated live pancreatic islets were obtained from the Human Pancreas Analysis Program (University of Pennsylvania). Human pancreatic tissue sections are produced by the Network for Pancreatic Organ Donors with Diabetes (University of Florida). The FT-ICR solariX and TOF-TOF ultrafleXtreme (Bruker, Billerica, MA) were used for MS imaging, high throughput single cell MS profiling, and single islet MS profiling. 2,5-Dihydroxybenzoic acid (DHB) matrix was deposited onto pancreatic tissue slices and populations of individual cells. Analytes from individual pancreatic islets were extracted in 30 mg/ml DHB aqueous solution. Islets were enzymatically treated and mechanically dissociated for single cell analysis. Analytes are detected in wide m/z 20-8000 range. Statistical evaluation of obtained data used principal component analysis and probabilistic latent semantic analysis.


Isolated human pancreatic islets

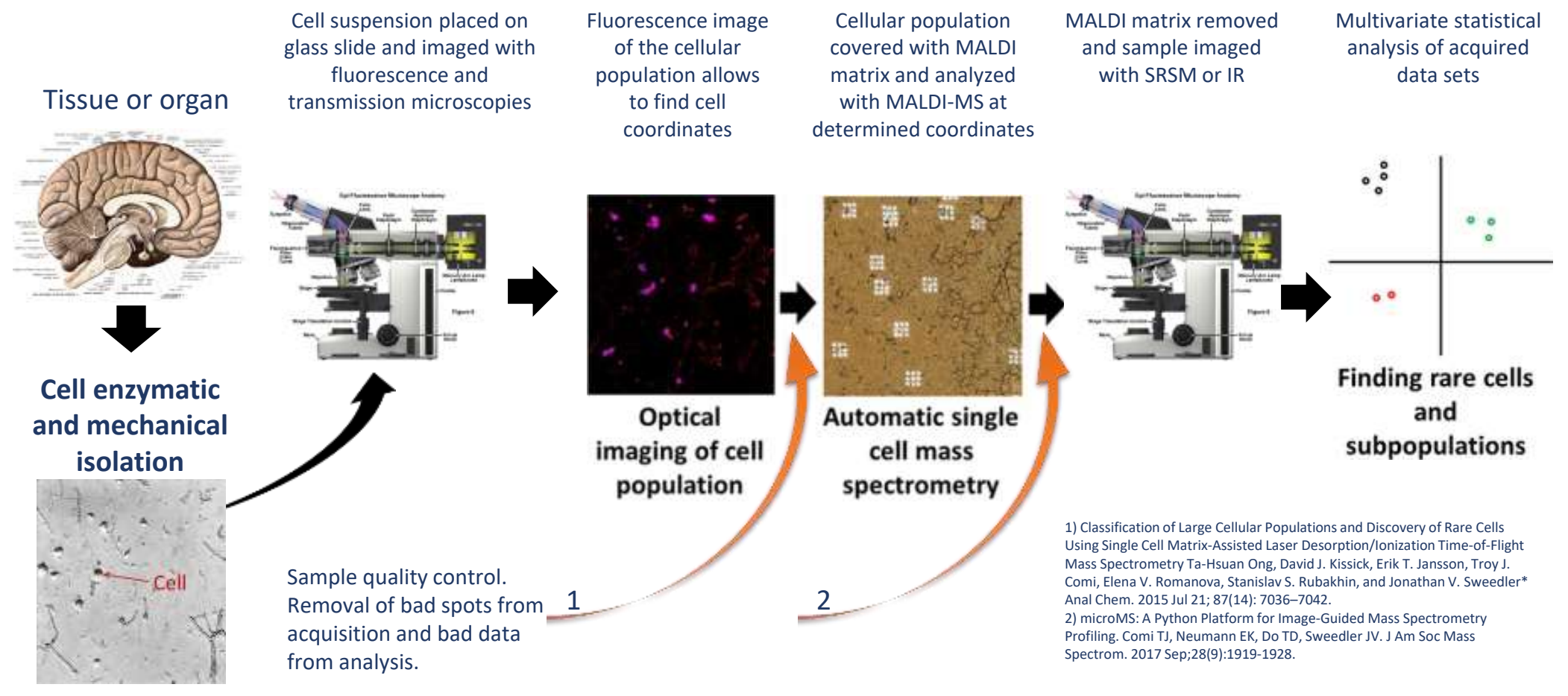

Individual cells

Cellular organization of the endocrine pancreatic islets



Cell type	Main prohormone	% of total
Alpha cells	glucagon	20
Beta cells	insulin and amylin	70
Delta cells	somatostatin	<10
Epsilon cells	ghrelin	<1
PP cells (gamma cells or F cells)	Pancreatic prohormone (or pancreatic	<5

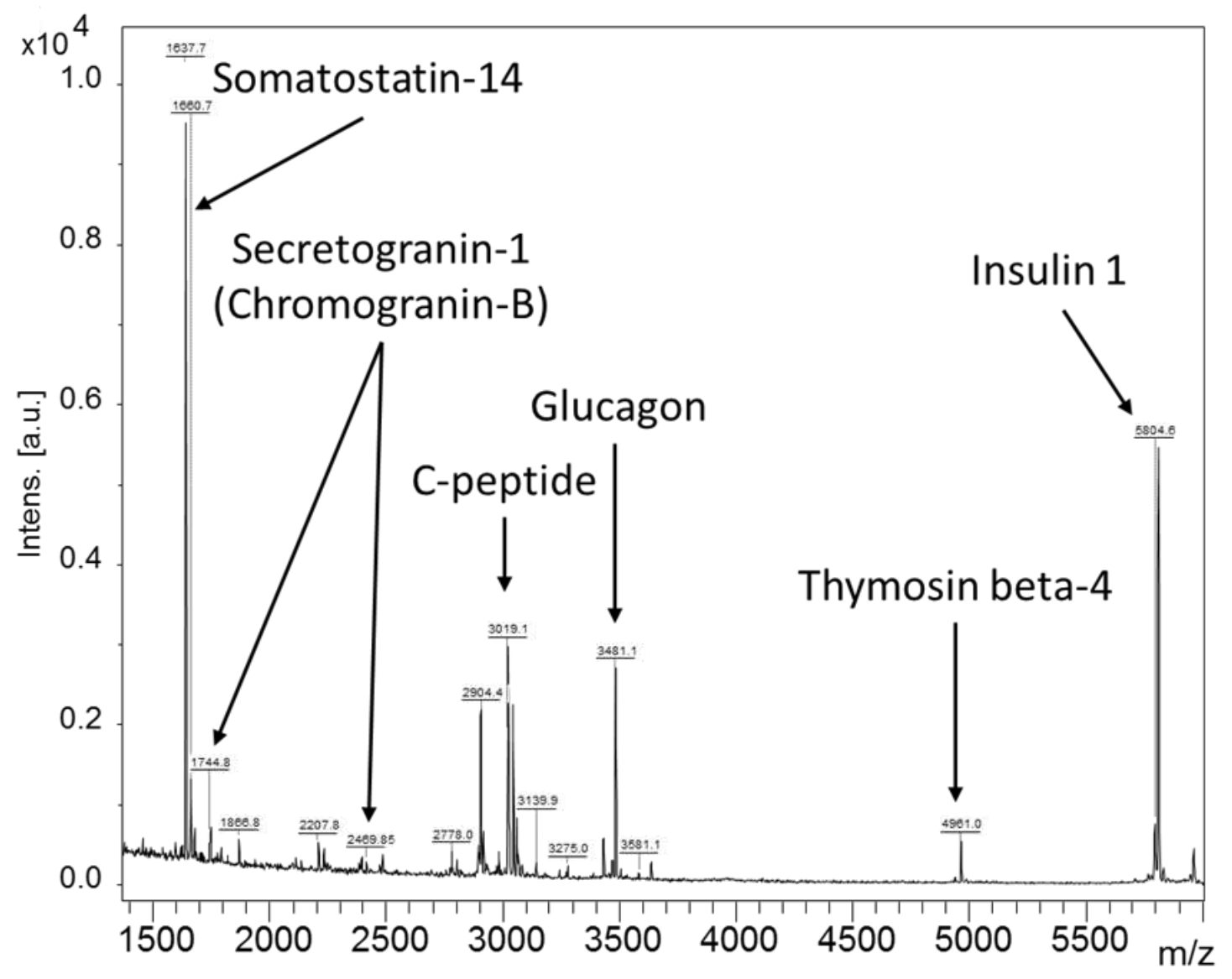
Approach for high-throughput single cell preparation and analysis using MicroMS guided SRSM and MALDI-MS



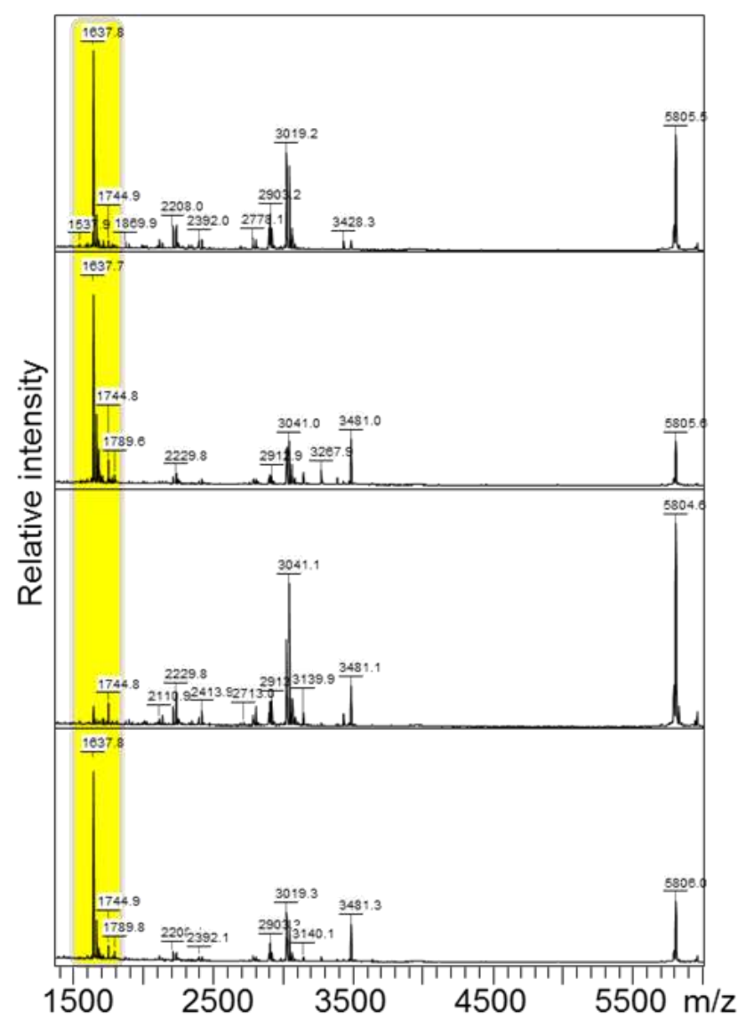
1) Classification of Large Cellular Populations and Discovery of Rare Cells Using Single Cell Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry Ta-Hsuan Ong, David J. Kissick, Erik T. Jansson, Troy J. Comi, Elena V. Romanova, Stanislav S. Rubakhin, and Jonathan V. Sweedler* Anal Chem. 2015 Jul 21; 87(14): 7036–7042.
2) microMS: A Python Platform for Image-Guided Mass Spectrometry Profiling. Comi TJ, Neumann EK, Do TD, Sweedler JV. J Am Soc Mass Spectrom. 2017 Sep;28(9):1919-1928.

MALDI MS profiling of analyte extracts collected from individual pancreatic islets

MALDI MS detects a variety of bioactive molecules including hormones in the human pancreatic islets

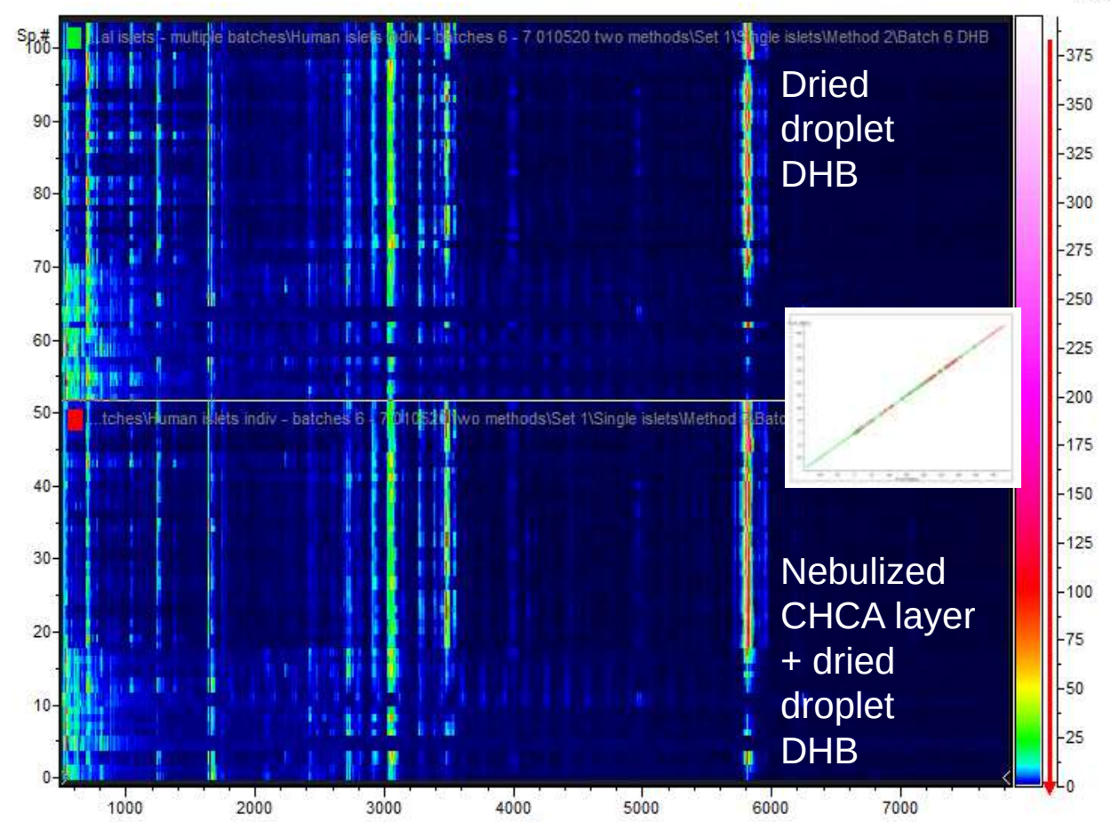


Representative mass spectrum acquired from extract of an individual human islet

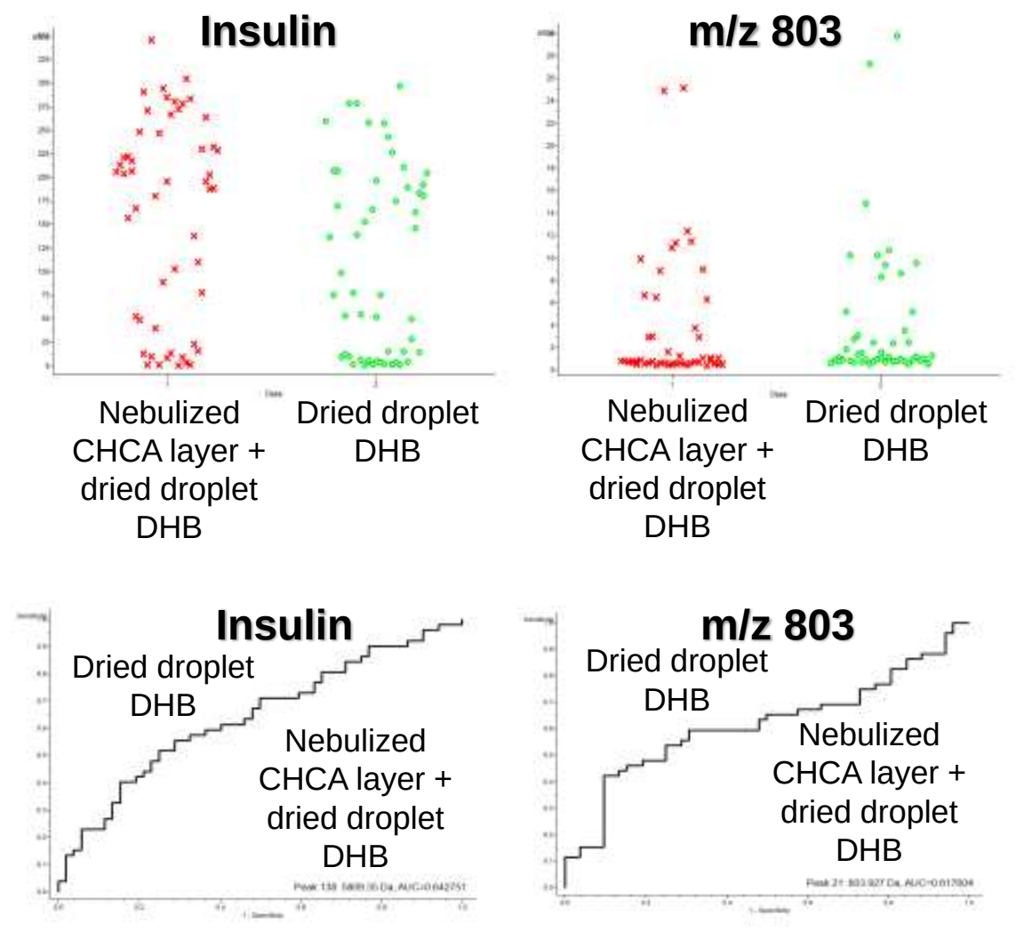


Differences in relative intensities of signals in mass spectra of analyte extracts are indicative of pancreatic islet chemical and cellular heterogeneities

MALDI MS analysis of analyte extracts of different pancreatic islets demonstrated high repeatability of measurements for many of detected compounds

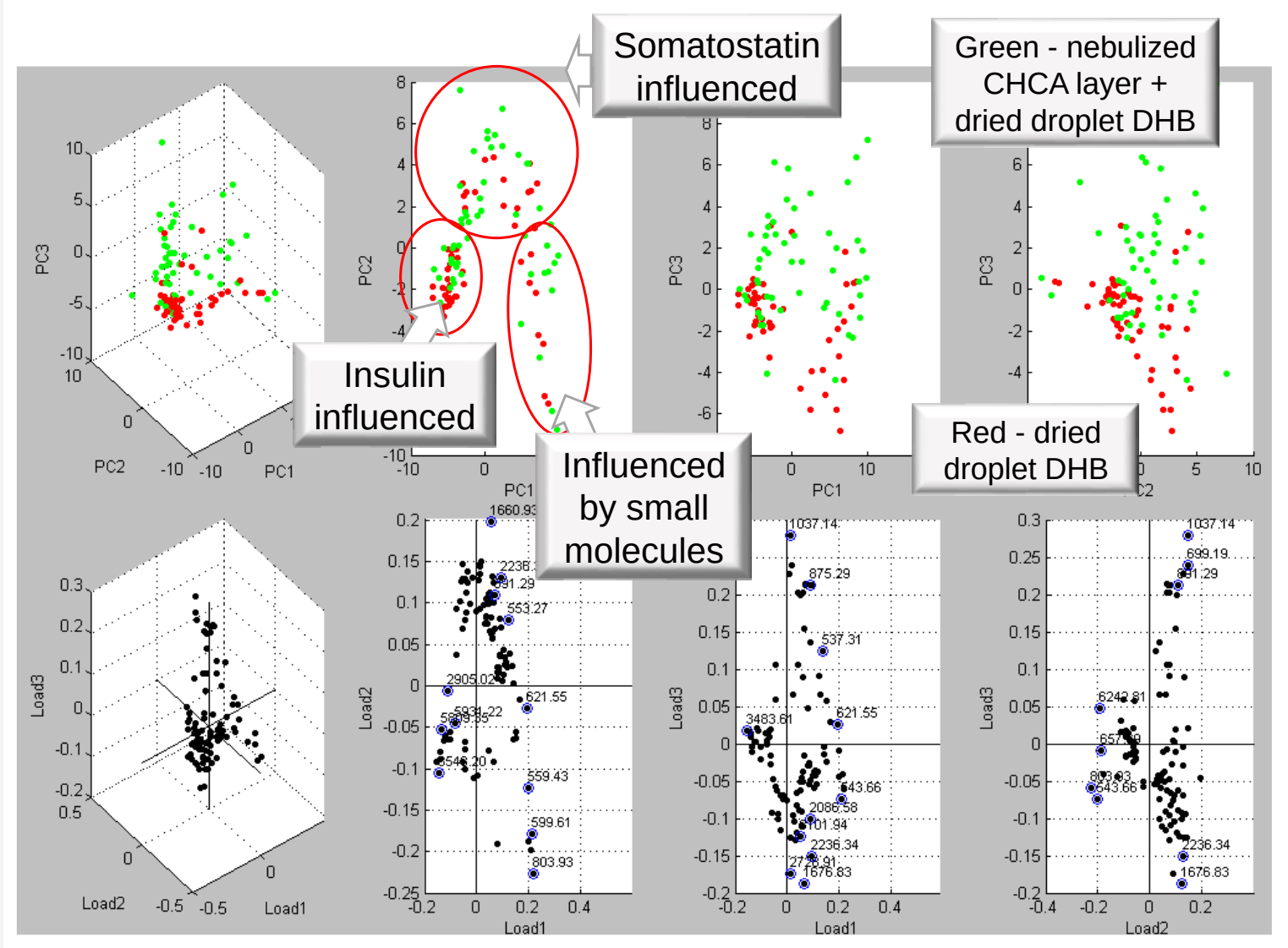


Gel view of mass spectra acquired from the same set of individual islet extracts aliquoted processed at different sample preparation conditions, and analyzed in duplicates: 1) DHB and analyte-containing droplet dried on metal surface; 2) DHB and analyte-containing droplet dried on metal surface coated with a layer of dry CHCA. Analyte extracts of islets from one donor are analyzed.

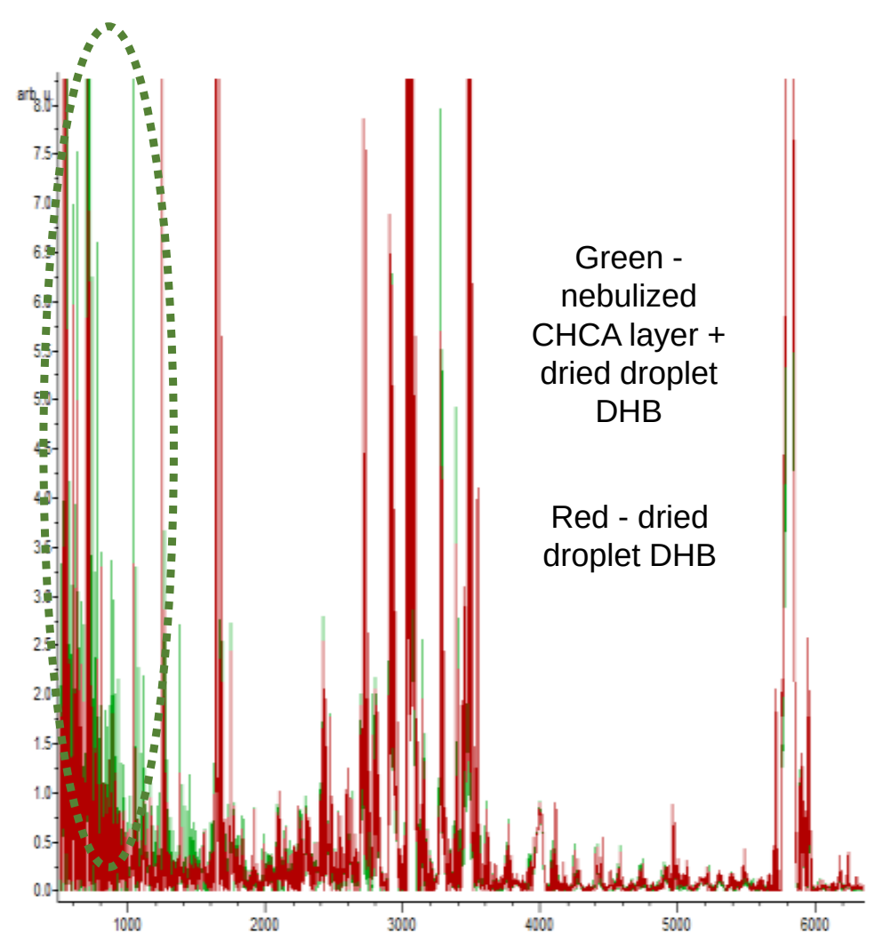


Histograms and ROCs illustrating similarity in results of MS measurements of samples prepared using two approaches

Analysis of cumulative influences of sample preparation conditions allows distinguishing of different sample types

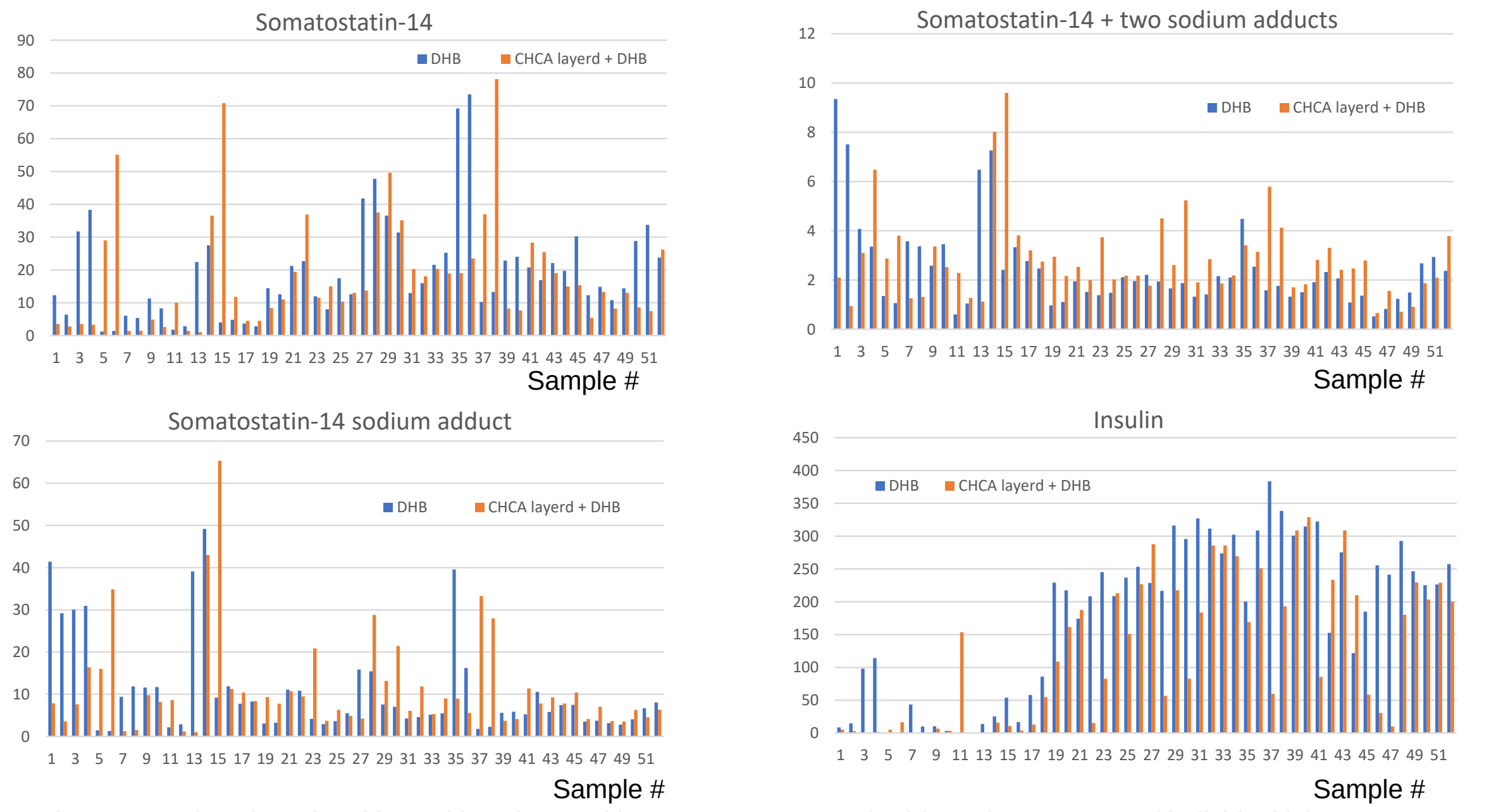


PCA of data acquired of mass spectra acquired from the same set of individual islet extracts aliquoted, processed at different sample preparation conditions, and analyzed in duplicates: 1) DHB and analyte-containing droplet dried on metal surface; 2) DHB and analyte-containing droplet dried on metal surface coated with a layer of dry CHCA. Analyte extracts of islets from one donor are analyzed.



Overlay of average mass spectra of the same samples prepared at different conditions – difference in small molecule region is visible

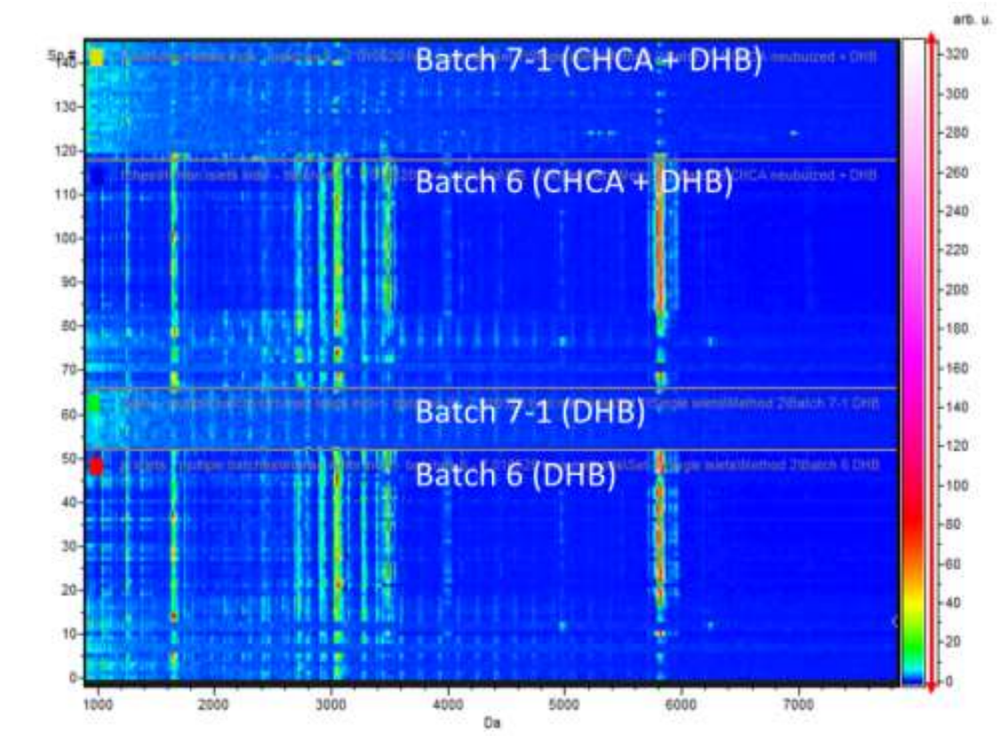
Measurements of multiple samples of the same extracts reduces but does not eliminate influences of chemical matrix and MALDI matrix-analyte cocrystallization effects on repeatability of measurements



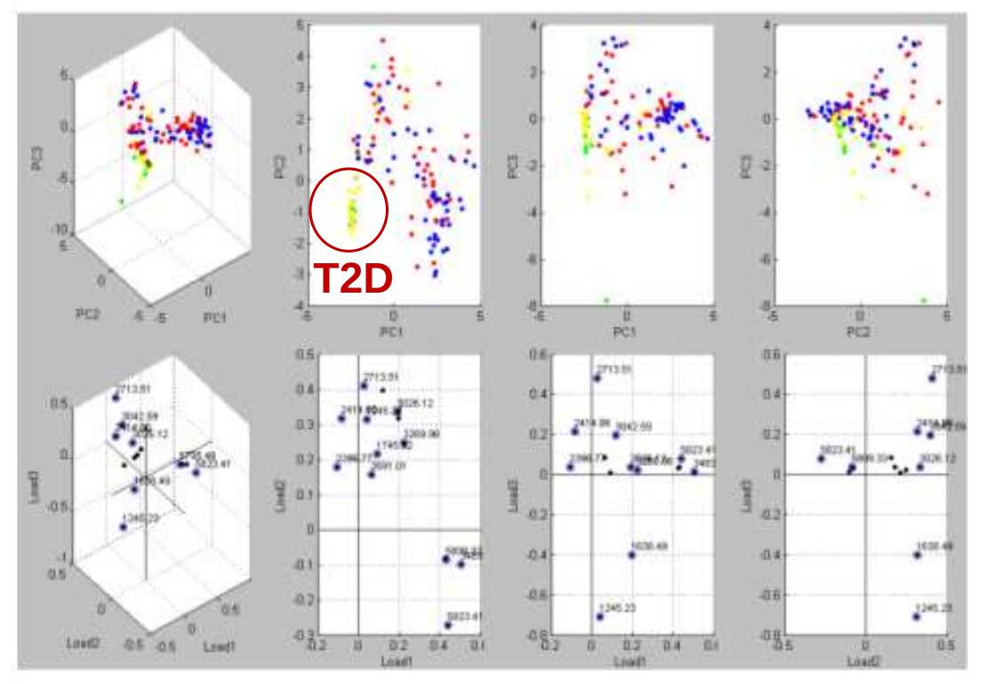
Histograms of analyte signal intensities observed in mass spectra acquired from the same set of individual islet extracts aliquoted, processed at different sample preparation conditions, and analyzed in duplicates

Biochemical differences between affected by type two diabetes and healthy donors pancreatic islets

Large difference in analyte profiles of the pancreatic islets obtained from healthy donor and donor with type two diabetes is observed



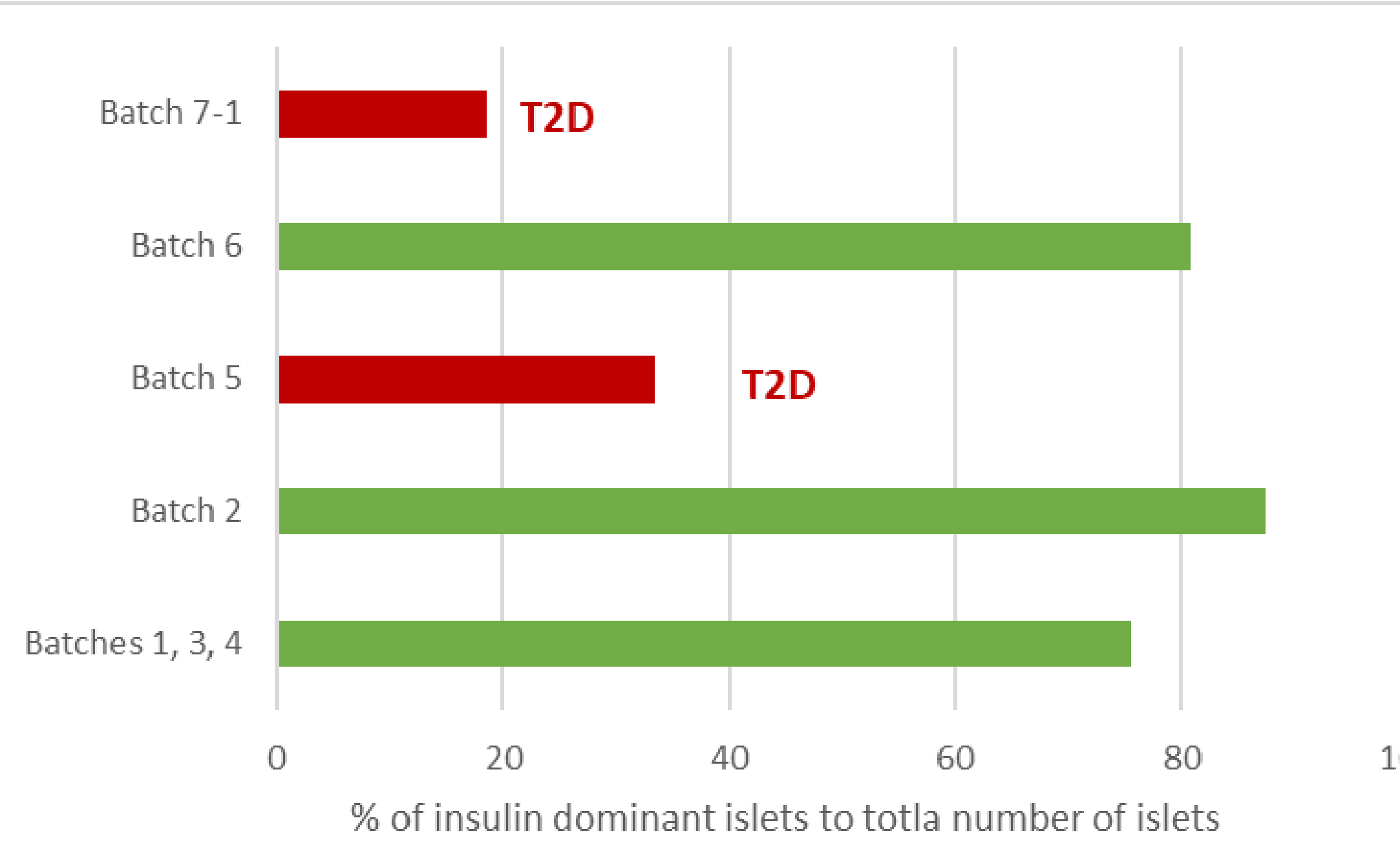
Gel view of mass spectra acquired from 65 individual islet extracts aliquoted processed at different sample preparation conditions, and analyzed in duplicates: 1) DHB and analyte-containing droplet dried on metal surface; 2) DHB and analyte-containing droplet dried on metal surface coated with a layer of dry CHCA. Batch 6 islets are collected from healthy donor and batch 7-1 islets are collected from donor with type 2 diabetes.



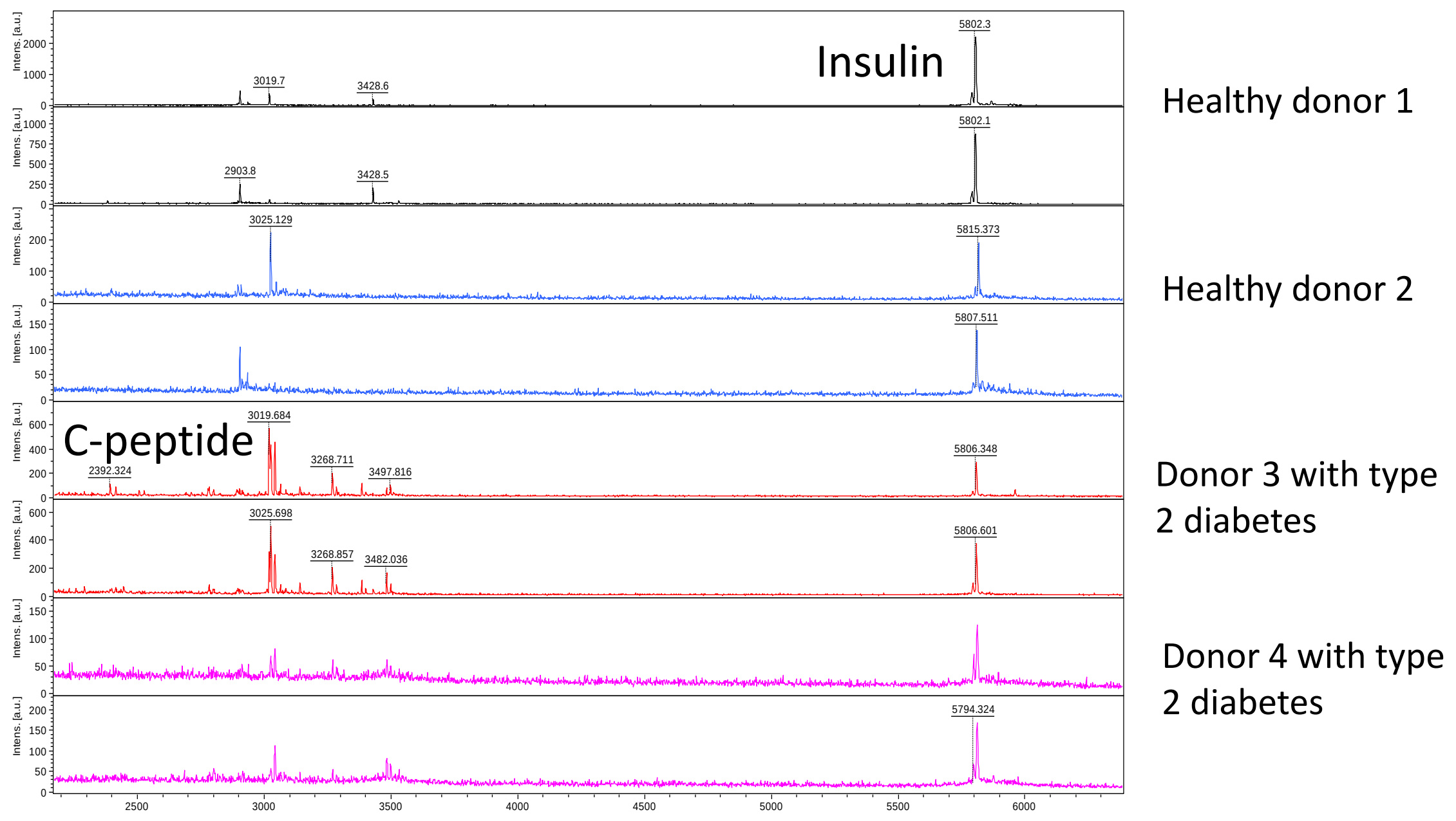
PCA of data acquired from extracts of 65 islets: red - healthy donor, green – donor with T2D. Both sets of samples prepared by deposition of DHB and analyte-containing droplet onto metal surface; blue - healthy donor, yellow – donor with T2D. Both sets of samples prepared by deposition of DHB and analyte-containing droplet onto metal surface coated with a layer of dry CHCA.

Different batches of islets obtained from healthy donors and donors with type 2 diabetes (T2D) demonstrated a variety of dominant chemical profiles

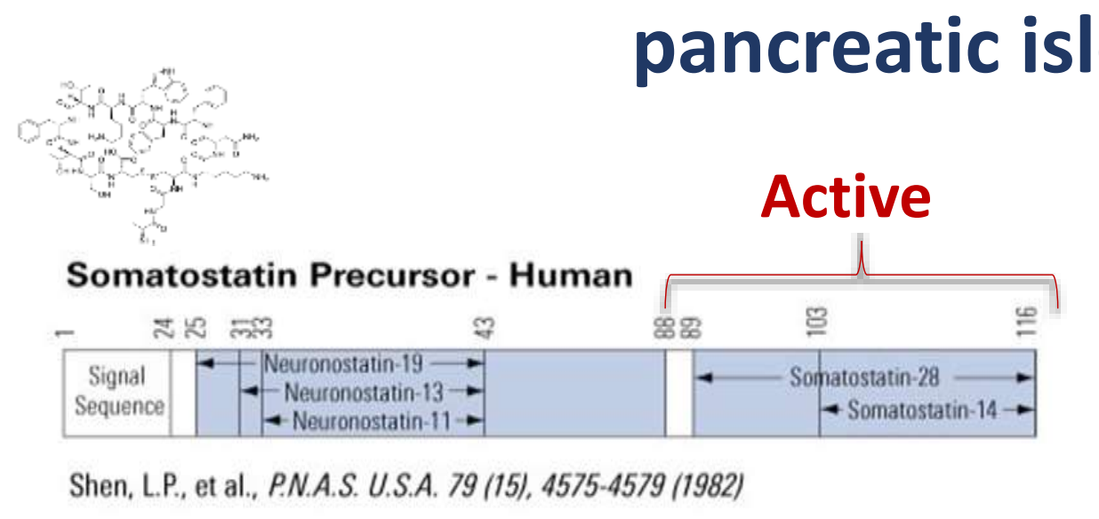
Mass spectra acquired from extracts of pancreatic islets obtained from donors with type two diabetes (T2D) exhibit less frequently dominant insulin signal



Representative mass spectra acquired from eight beta cells isolated from four individual pancreatic islets



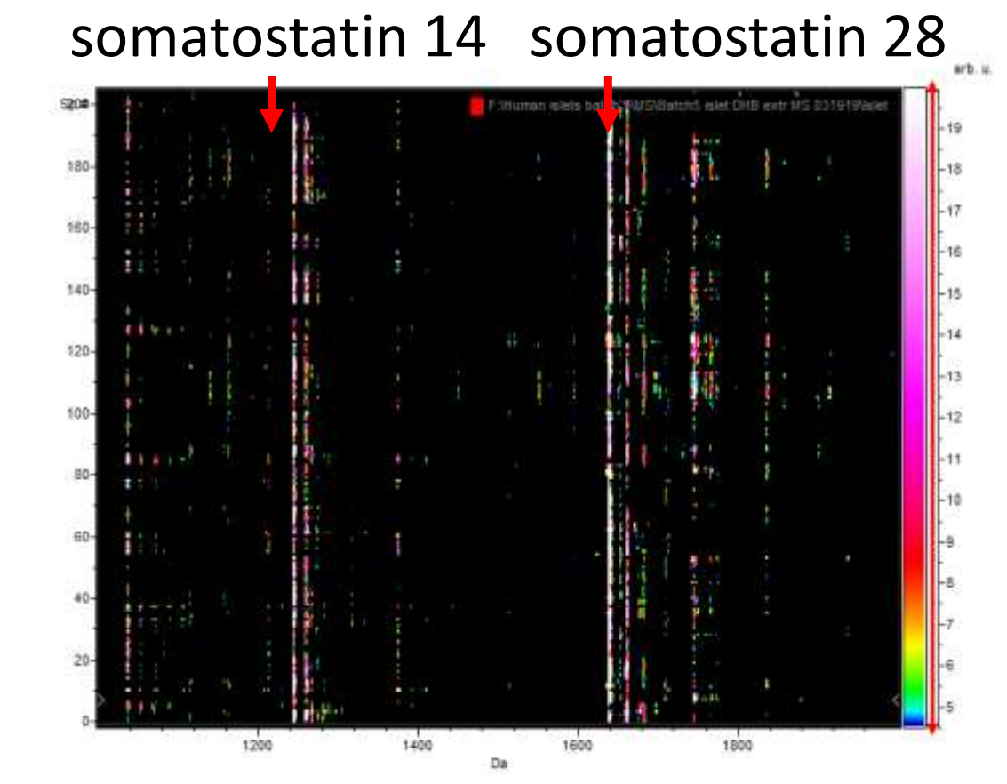
Somatostatin prohormone processing in individual pancreatic islets and cells



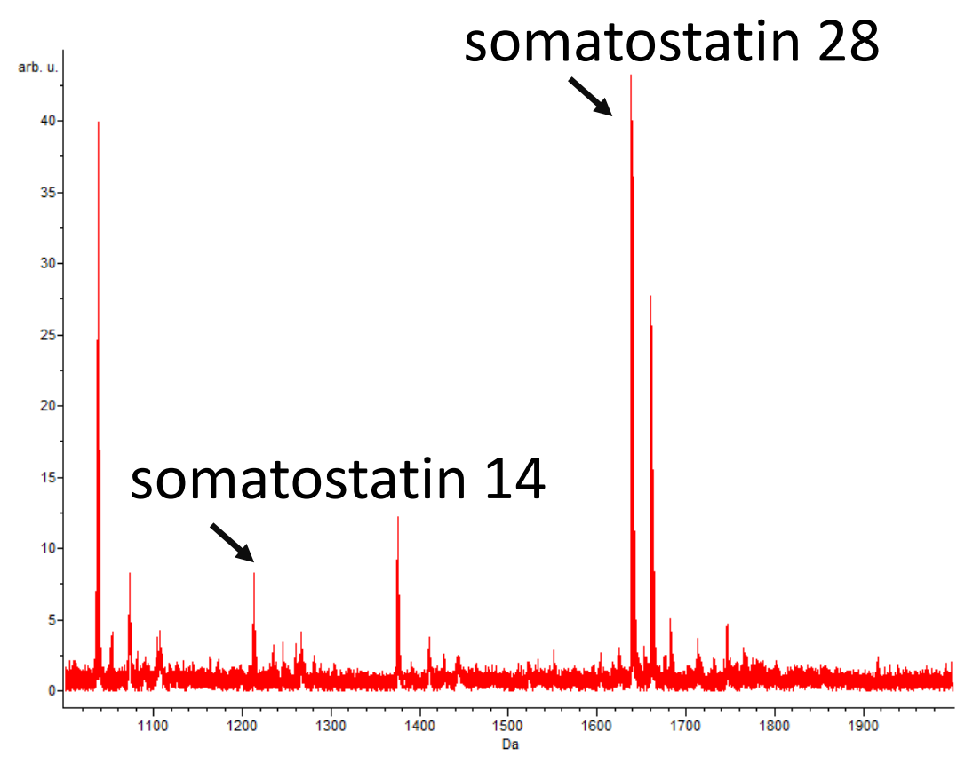
Shen, L.P., et al., *P.N.A.S. U.S.A. 79 (15), 4575-4579 (1982)*

Somatostatin - peptide hormone regulating the endocrine system and affecting neurotransmission and cell proliferation via interaction with G-protein-coupled somatostatin receptors leading to inhibition of the release of numerous secondary hormones.

Different somatostatins are detected in analyte extracts from individual islets of Langerhans

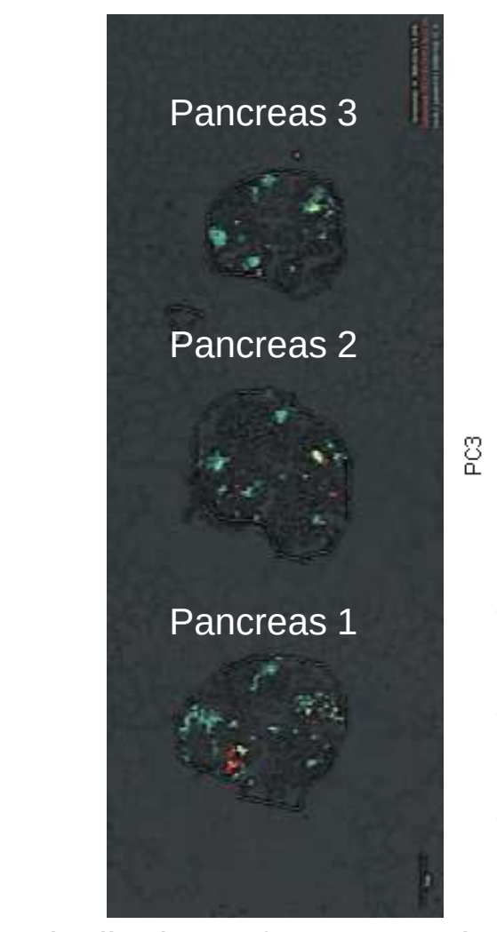


Gel view of mass spectra acquired from extracts of multiple individual pancreatic islets

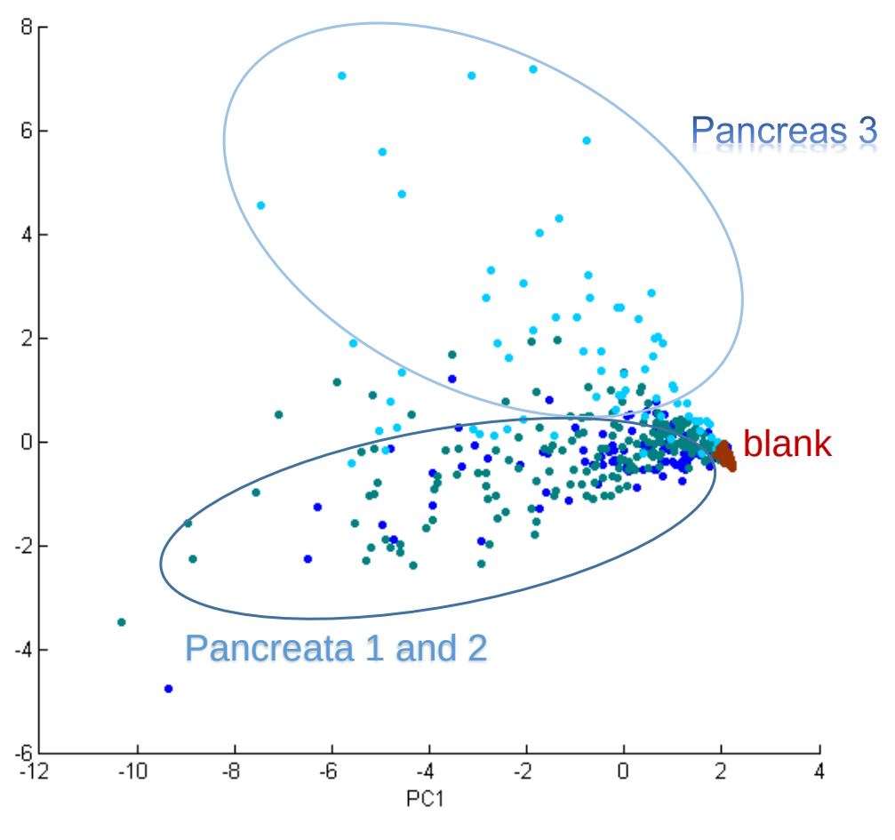


Somatostatin peptides detected in a single isolated pancreatic islet

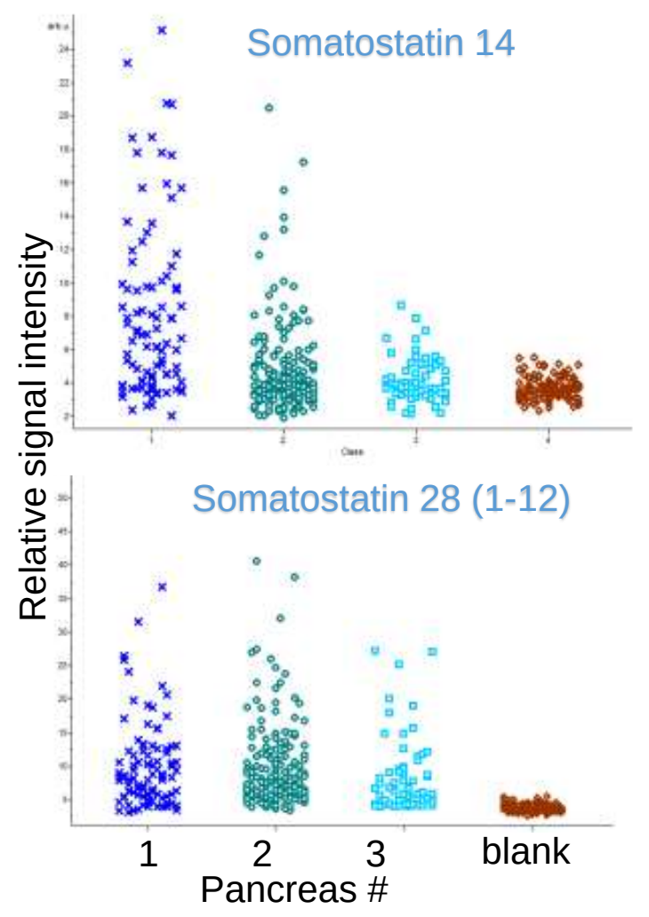
Mass spectrometry imaging detection of somatostatin peptides in human pancreatic tissue sections



Distributions of somatostatin 14 (yellow), somatostatin 28 (1-12) (red), and insulin C peptide (green) in 3 pancreatic sections

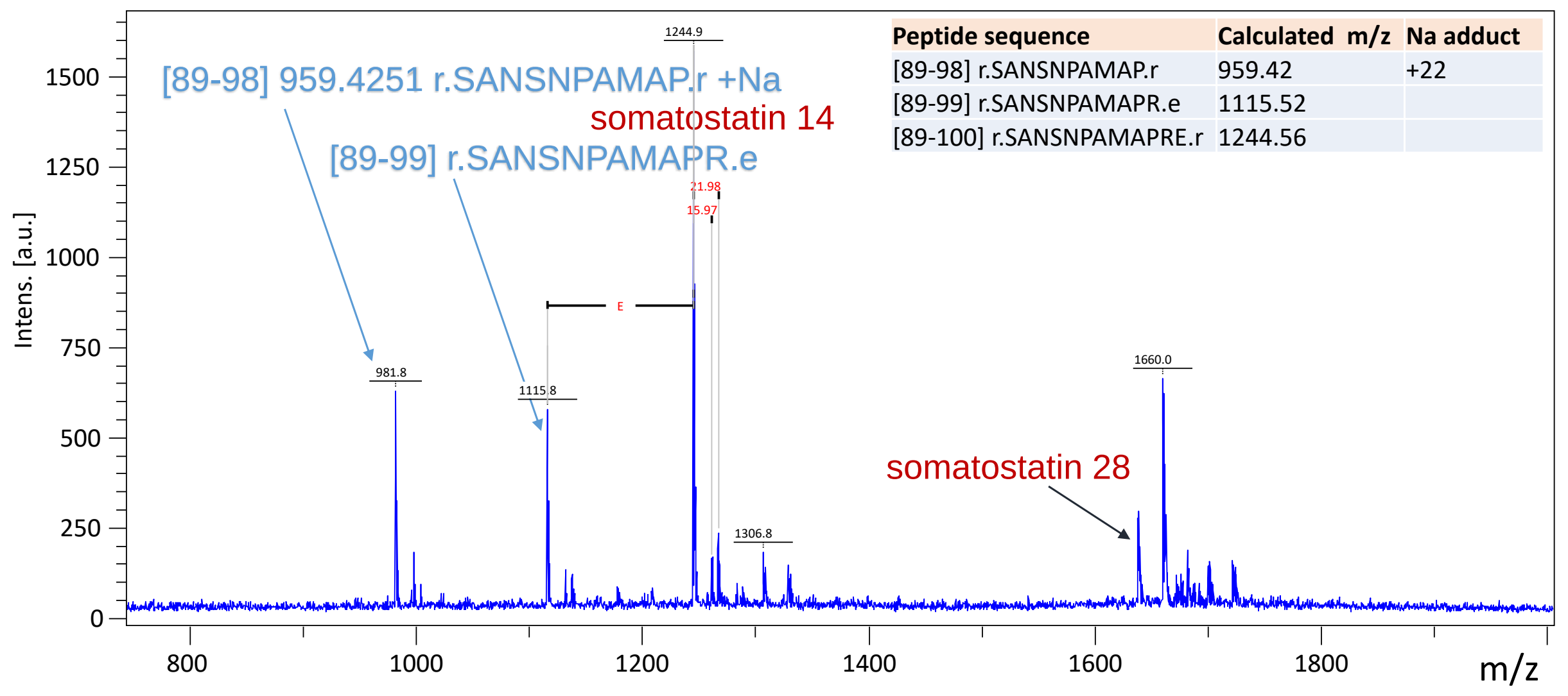


Results of PCA of data acquired using mass spectrometry imaging in islet-occupied areas of 3 pancreatic tissue sections



Histograms of relative signal intensity distributions for somatostatin 14 somatostatin and 28 (1-12) peaks

Somatostatin peptides detected in a single pancreatic islet cell



Peptide sequence	Calculated m/z	Na adduct
[89-98] r.SANSNPAMAP.r	959.42	+22
[89-99] r.SANSNPAMAPR.e	1115.52	
[89-100] r.SANSNPAMAPRE.r	1244.56	

CONCLUSIONS

1) All major peptides related to insulin, somatostatin, and glucagon along with less common peptides were detected using MALDI MS in analyzed human pancreatic islets.
2) High level of heterogeneity of individual islet profiles were observed with 11% not producing signals in the peptide molecular mass region. These structures may represent small pieces of the acinar tissue collected during islet isolation.
3) Among the analyzed islet-like structures, 60% have peptide profiles dominating by insulin signal and just 4% by glucagon signal.
4) Somatostatin 14 and somatostatin-28 (1-12) dominated profiles are detected in 16% and 10% in correspondence. Importantly, ratio of islets with dominating somatostatin prohormone-related signals is highest in the T2D samples.
5) High throughput single cell MS analysis uncovered cells expressing somatostatin-28 (1-12) along somatostatin 14 and several other smaller peptides likely derived from the somatostatin prohormone.
6) In mass spectrometry imaging experiments, only one islet among twenty detected in tissue sections of additional three healthy donors expressed somatostatin-28 (1-12).
7) The results of these work demonstrated comparable and complimentary nature of the outputs from the three MS methods.

ACKNOWLEDGEMENTS

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(http://www.diabetes.org/)