Distinguishing transthyretin marker peptides from amyloidosis model tissue using MALDI

Jennifer Aguilan[1,2*], Joshua Fischer[4], Cristina Silvescu[4], Katherine Stumpo[4], Yan Sun[3], Simone Sidoli[3] and Shannon Cornett[4]

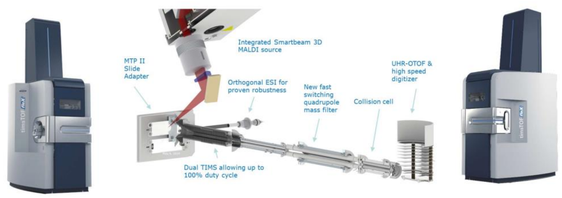
[1] *Laboratory for Macromolecular Analysis and Proteomics, Albert Einstein College of Medicine, New York, NY, USA*
[2] *Department of Pathology, Albert Einstein College of Medicine, New York, NY, USA*
[3] *Department of Biochemistry, Albert Einstein College of Medicine, New York, NY, USA*
[4] *Bruker Daltonics Inc., Billerica, MA, USA*

Introduction

Amyloidosis is a rare disease characterized by extracellular deposition of protein fibrils that can lead to organ failure. Diagnosis is challenging due to the ~30 different amyloid protein subtypes. Immunohistochemistry and Laser capture microdissection (LCM) with LC-MS/MS are two widely used but time intensive clinical assays. Using a transthyretin model tissue, the goals are to test if timsTOF fleX can directly map transthyretin peptide markers on tissue (for faster turnaround time) and, use the trapped ion mobility (TIMS) and ultrahigh resolution to resolve isobaric peptides. For proof-of-concept, initial results demonstrate that the timsTOF fleX can map and resolve isobaric peptides observed from transthyretin amyloidosis (ATTR) negative and positive tissues which suggests that this could be a potential alternative amyloidosis assay to develop.

On-tissue peptide imaging analysis by timsTOF fleX

- **Fully integrated with high throughput, high spatial resolution MALDI source**
- **Proven dual-source geometry from scimaX**
- **Proven SmartBeam 3D MALDI laser from rapiflex**



Conclusions

1. Sensitivity and specificity were improved when isobaric transthyretin peptides from Congo red positive and Congo red negative tissue samples were resolved by the timsTOF fleX using combined ultra-high TOF resolution and TIMS.
2. The trapped ion mobility added another dimension for separation of isobaric peptides based on collision cross section (CCS) and resolved transthyretin peptides from the interfering background peptides present in the complex tissue environment.
3. The results demonstrated from this proof-of-concept work is promising and encouraging to further evaluate the potential applications of timsTOF fleX for peptide marker tissue imaging for amyloid protein identification and subtyping.

*Manuscript in preparation.

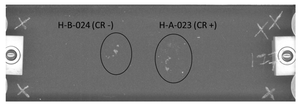
Methods

1. On-tissue trypsin digestion was performed on formalin-fixed paraffin embedded (FFPE) heart biopsy and autopsy tissue sections.
2. Tissues were either coated with HCCA matrix using the TM sprayer for imaging or peptides were extracted with 10% and 40% acetonitrile/0.1% trifluoroacetic acid and pooled.
3. Digested peptides from pure transthyretin standard were used as positive control.
4. Peptide digests mixed with HCCA were analyzed by MALDI-TOF using the timsTOF fleX.
5. Pure transthyretin peptide standard was spiked in the peptide extracts to test the capability to differentiate isobaric peptides.

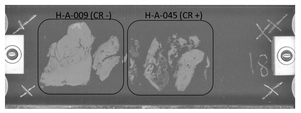
Results

On-tissue peptide extracts analysis by timsTOF fleX

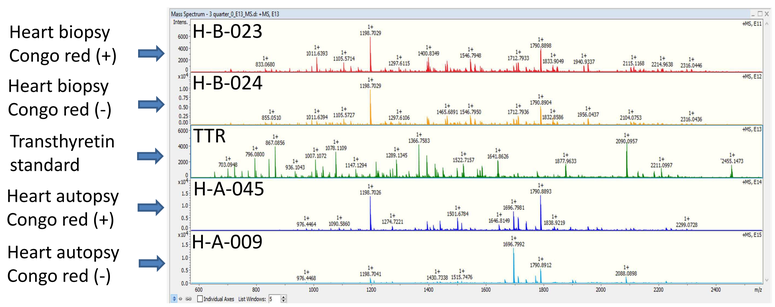
Heart Biopsy



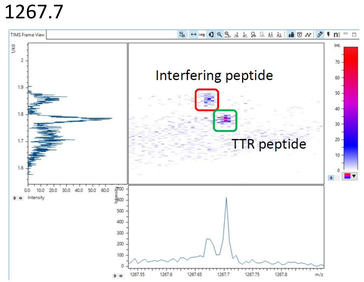
Heart Autopsy



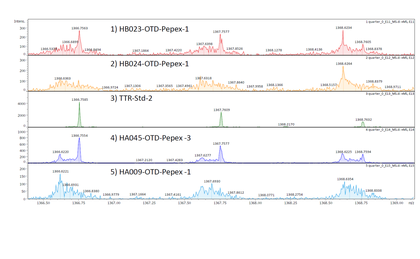
Transthyretin (TTR) peptide digest profile on the timsTOF fleX



TIMS separation adds another dimension of identification when combined with ultra-high TOF resolution. TTR standard mixed with H-B-023 peptide extract. Mass and mobility resolved for peptide m/z 1267.7



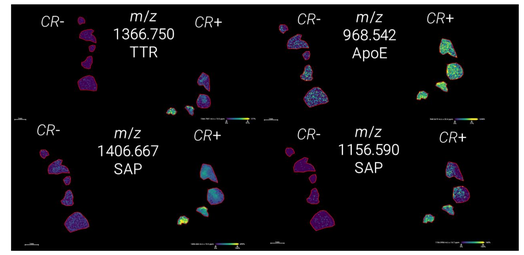
The timsTOF fleX's enhanced sensitivity enabled the detection of the TTR standard peptide GSPAINVAVHVFR m/z 1366.8 found to be present only in Congo red (+) samples: H-B-023 and H-A-045

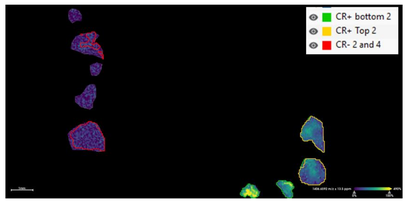


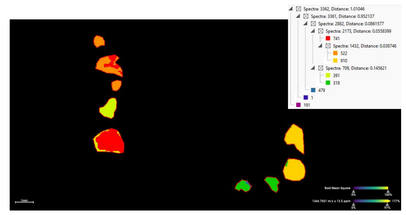
Results

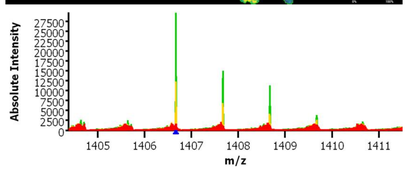
On-tissue peptide imaging by timsTOF fleX

Potential peptide markers observed in CR+ tissue for transthyretin (TTR) amyloid protein. Serum amyloid P component (SAP) and Apolipoprotein E (ApoE) are associated universal markers that co-localize with amyloid proteins.



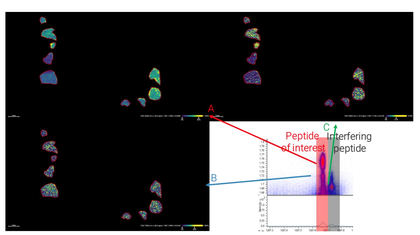




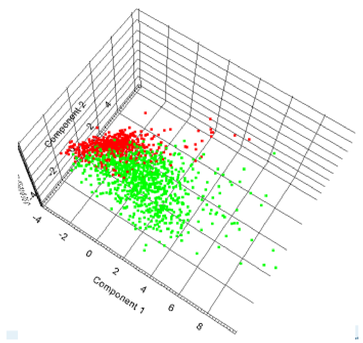


Extracted average mass spectra from segmentation analysis used to distinguish between CR- and CR+ tissue biopsies

Segmentation analysis used to cluster statistically similar regions into extractable regions



TIMS can resolve overlapping peptide peaks from complex tissue samples which is useful for screening peptide markers



PCA analysis using SCiLS showed clustering of CR(+) and CR(-) tissue biopsies

Isobaric peptides observed from CR (+) and CR (-) tissues resolved by MALDI-ultrahigh resolution and collision cross section (CCS) values using TIMS

Protein name/ Gene Name	m/z meas.	CCS (Å²)	Mob. 1/K0	Protein name/ Gene Name	m/z meas.	CCS (Å²)	Mob. 1/K0
Transthyretin	1267.673	356.5	1.761	Serum Amyloid P	1156.594	328.6	1.621
TTR	1267.675	361.5	1.785	SAP	1156.606	328.6	1.622
	1268.662	342.5	1.692		1156.632	316.8	1.563
	1281.647	341.9	1.689		1406.667	352	1.741
	1281.683	342.6	1.692		1811.865	367.5	1.821
	1281.695	340.9	1.684		1811.891	368.3	1.825
	1396.717	342	1.691				
	1396.719	347.3	1.717	Apolipoprotein E	948.539	293.4	1.444
	1396.728	341.4	1.688	ApoE	1497.772	371.9	1.84
	1396.753	341.8	1.69		1497.827	363	1.796
	1494.851	378.4	1.872				