SPATIAL DISTRIBUTION AND IDENTIFICATION OF PROTEIN IN TUBERCULOSIS GRANULOMAS USING DESI AND CYCLIC ION MOBILITY

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OVERVIEW

- **DESI analysis of tissues was acquired using the SELECT SERIES™ Cyclic™ IMS System.**
- **Intact peptides and small proteins were detected and separated by ion mobility in different trends.**
- **Identification of some of these peptides is under way.**

INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), is the leading cause of death by a single infectious agent and remains a serious global health threat. The TB granuloma is a dynamic three-dimensional structure that contains a highly complex mix of innate and adaptive immune cells that have diverse phenotypes. Imaging of proteins in TB granulomas has the potential to inform on host-responses that lead to granuloma formation to enable the identification of novel signaling pathways and new therapeutic interventions. DESI cIMS was utilized to determine the spatial distribution of proteins in TB granulomas. We applied tissue washing protocols to improve the detection sensitivity and biomolecular coverage.

EXPERIMENTAL

Rabbits were infected with *Mtb strain HN878 for 16-20 weeks. Lesions were resected, sterilized, sectioned at 10 µm thickness and transferred to glass slides for histology and mass spectrometry imaging.*

For protein analysis, the tissues were treated to remove the lipids and small molecule metabolites. The tissue samples were washed using the following protocol:

- 1 minute with 70% Ethanol/30% Water
- 1 minute with 100% Ethanol
- 30 seconds with 100% Dichloromethane
- 4 minutes under vacuum desiccator

For the DESI solvent, a solution of Acetonitrile/Water (80:20) with 0.1% formic acid and Leucine Enkephalin (200 ng/mL) was infused with a flow rate of 3 µL/min. The transfer line was heated at 450 °C. The sprayer voltage was set to 0.85 kV in positive ionization. Initial experiments were performed using one pass along the Ion Mobility cell using a Traveling Wave Height of 26 V at 2 pushes per bin. MS/MS of the most abundant peptide have been performed to elucidate structural information of the peptides and proteins. The data was acquired in MassLynx™ version 4.2. The images were visualized using the High Definition Imaging (HDI™) version 1.6.

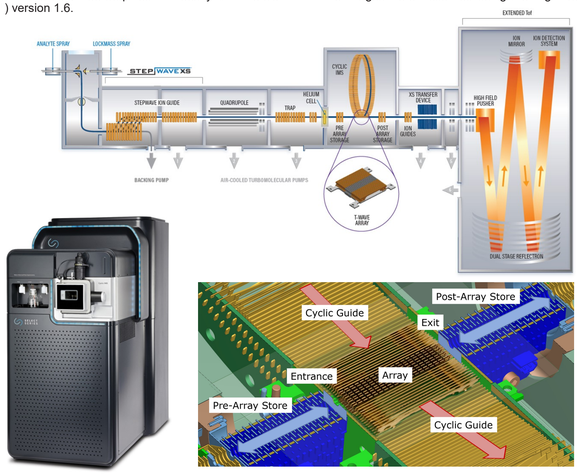
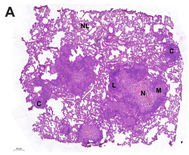


Figure 1. *(Top) Schematic of the Cyclic IMS QTOF Instrumentation. It contains three main regions: the trap region, the cyclic ion mobility device, and the transfer region. (Bottom) Zoom in of the cyclic ion mobility device.*

RESULTS & DISCUSSION

The TB Granuloma, Drug Penetration and Host-Directed Therapy



B

HDT Action	Activation of autophagy	Breakdown of granuloma structure	Increase anti-inflammatory response
Drug compounds	Rapamycin, Metformin, Valproic acid, Satin, Vitamin D, Phenylbutyrate	Etanercept, Bevacizumab	Ibuprofen, Diclofenac, Acetylsalicylic acid, Vitamin D
Response	Enhanced intracellular Mtb killing	Increased drug penetration – higher sterilizing capability	Reduced inflammation and tissue damage

Figure 2. A) Hematoxylin and eosin stained section of a region of infected lung tissue containing a caseous necrotic granuloma (N) that is surrounded by macrophages (M) and lymphocytes (L), several smaller necrotic granulomas and cellular granulomas (C) are also present. **B)** Summary of current host-directed therapeutic compounds that have demonstrated efficacy in treating *Mtb* infection.

Driftscope™ , Spectra and MS Images

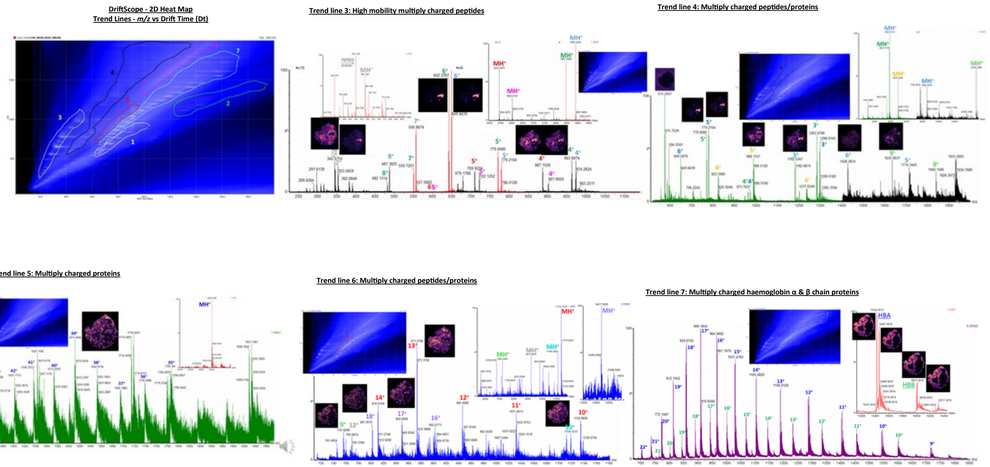


Figure 3. Driftscope view (Drift time vs. m/z) and corresponding spectra taken from trend lines 3-7. Multiple charged ions and the deconvoluted spectra are shown for each trend line, along with representative peptide and protein ion images.

Peptide /Protein MS Images

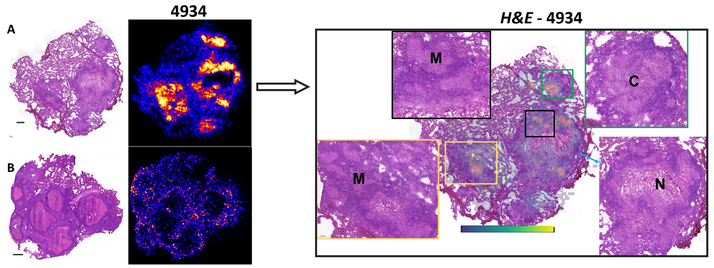


Figure 4. Left panel - Hematoxylin and eosin stained sections showing several necrotic and cellular granulomas (A) and necrotic granulomas (B), along with corresponding MS images of a macrophage-specific peptide/protein. **Right panel -** Merged H&E-MS image demonstrating the peptide/protein at 4934 Da is mapped to regions of macrophage (M) accumulation surrounding necrotic granulomas (N) and in the center of the cellular granulomas (C_).

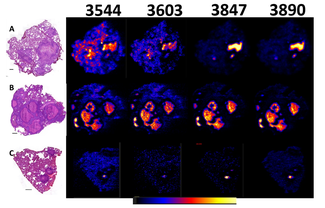


Figure 5. MS Images of peptides/proteins detected with highest signal intensity in the necrotic regions of TB granulomas

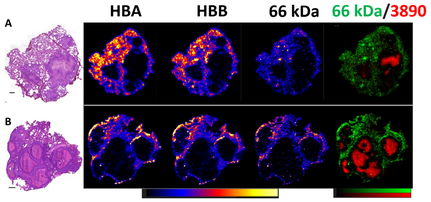


Figure 6. MS Images of the hemoglobin alpha and beta chains and a protein detected at 66 kDa that demonstrates similar spatial distribution. Right panel are merged ion images of the high molecular weight protein and a peptide/protein mapped to the necrotic core of TB granulomas.

CONCLUSIONS

- **DESI XS and Cyclic Ion Mobility enable the detection of a wide range of peptides and proteins directly from tissue.**
- **Tissue washing and heated transfer line (450 C) are also essential for the transmission and detection of peptides/proteins.**
- **Ion mobility separation improves the signal-to-noise of the multiply charged peptides/proteins.**
- **Small peptides and proteins detected within distinct macrophages populations and lesion regions may have significant functional impact.**
  - **Therapeutic targets**
- **Evaluation of both large and small proteins is essential when studying clinical and preclinical samples (*)**

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