POSTER APP NOTE

# Multi-omic Analyses Enabled by Microchip Capillary Electrophoresis and Trapped Ion Mobility Mass Spectrometry

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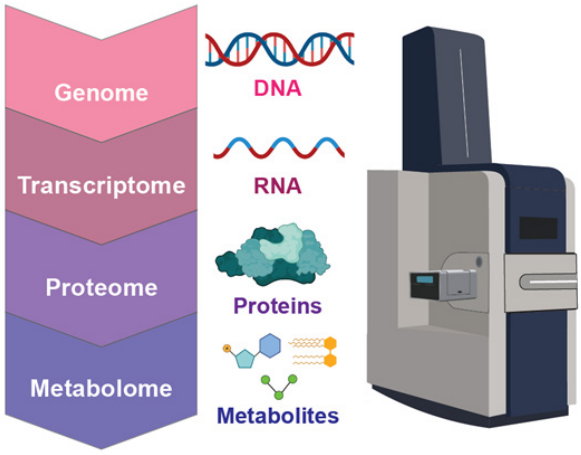
## Overview

- Use of a microchip capillary electrophoresis device coupled to a trapped ion mobility mass spectrometry for rapid multiomic analyses.
- A broad range of polar metabolite classes identified including good coverage of amino acids and acylcarnitines.
- Over 4500 peptides corresponding to 1300 proteins were quantified with total of 7.5-minute replicate runs.
- Initial development of a top-down proteomics method for the analysis of human heart protein extract.

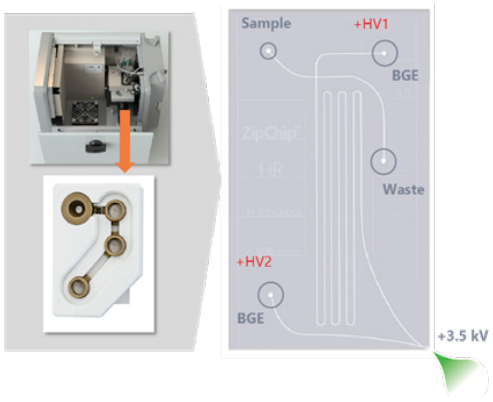
## Introduction

The quantitation of biomolecules has traditionally been accomplished through liquid chromatography-mass spectrometry (LC-MS). Comprehensive LC-MS analysis of biological samples typically involves long chromatography methods to resolve complex analytes prior to MS analysis.[1] In recent years, capillary electrophoresis (CE) has been explored as a rapid alternative to LC. ZipChip is a microfluidic CE device that can be directly coupled to multiple MS interfaces, providing both high resolution separation and high sensitivity detection due to the fact that it is a nanoflow separation that enables nano-electrospray, which has high ionization efficiency. Here we provide a survey of applications including metabolomics, bottom-up, and top-down proteomics and demonstrate the potential of these methods for rapid multiomic analyses.




## ZipChip Device

ZipChip is designed to perform fast separations of biological samples. A sample plug is introduced via a pressure-based injection. Only positive analytes migrate down the separation channel where they are separated based on their electrophoretic mobility and electrosprayed onto the mass spectrometer source.



# Methods

## Metabolomics

Metabolites were extracted from human heart tissue by homogenization in methanol and dried in vacuo. Metabolites were resolved with a field strength of 1000 V/cm over 5 minutes. MS analysis was performed using data dependent Parallel Accumulation Serial Fragmentation (ddaPASEF). Data analysis was performed using MetaboScape 2022b (Bruker) and Skyline.

## Top-Down Proteomics

Pierce Intact Protein Mix (Thermo Fisher) was injected (0.2 ng) and separated with a field strength of 500 V/cm over 7 minutes. Yeast extract was obtained from Promega. Protein was extracted from human heart tissue by homogenization in HEPES buffer, pH 7.4.

## Bottom-Up Proteomics

K562 standard (Promega) was injected (5 ng) and separated with a field strength of 300 V/cm over 7.5 minutes. MS analysis was performed via diaPASEF. Data analysis was performed using DIA-NN.

# Metabolomics

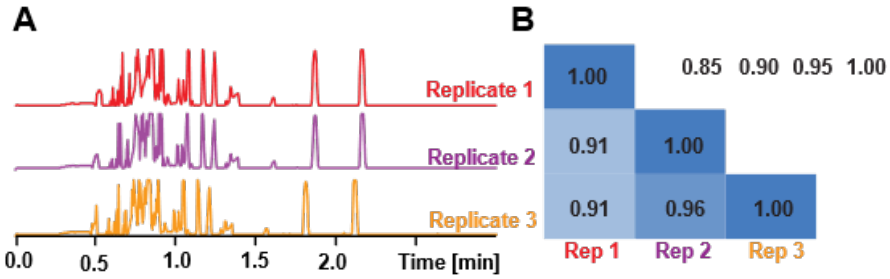



*Figure M1. Reproducibility: analysis of polar metabolites extracted from human heart tissue. A) Base peak electropherograms (BPE) showing the technical replicate analysis of polar metabolites extracted from human heart tissue. B) Pearson correlation analysis of the 3 replicates.*

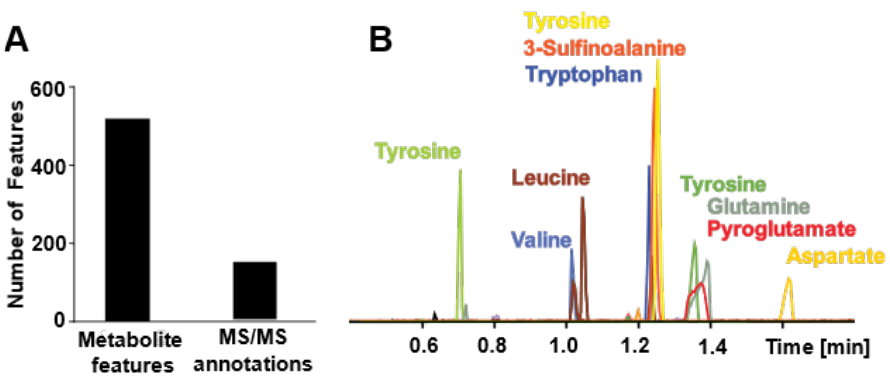



*Figure M2. Amino acids and metabolite features. A) Bar graph showing the number of metabolic features detected including those identified by fragmentation matching against publicly available libraries. Multiple metabolites were identified using the ZipChip for analysis of polar metabolite extracts including acylcarnitines, amino acids, bigenic amines, creatine, dipeptides, neurotransmitter/modulators, polyamines, purines, and vitamins. B) Representative extracted ion electropherograms for amino acids.*

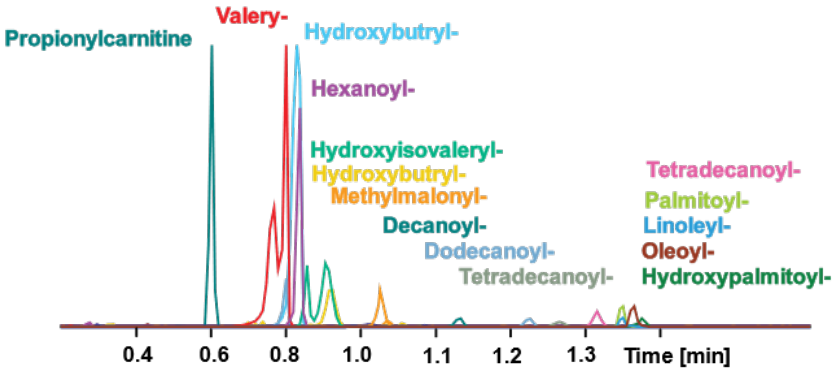



*Figure M3. Acylcarnitines. Representative extracted ion electropherograms of acylcarnitines. Coverage spanned from propionylcarnitine (C3) to oleoylcarnitine (C18:1).*

# Proteomics

## Top-Down Proteomics

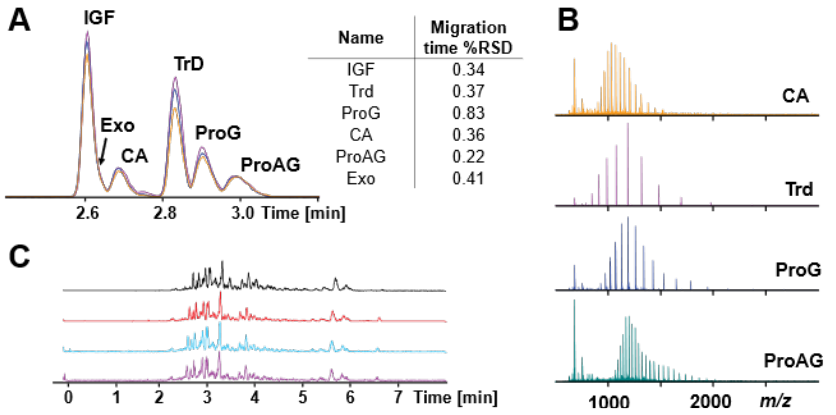



*Figure P1. Reproducibility of standard proteins and yeast extract. A) Overlaid BPEs and table showing that the reproducibility of all migration times was below 1% RSD. B) Mass spectra of select protein standards. C) Reproducible separation of a yeast extract.*

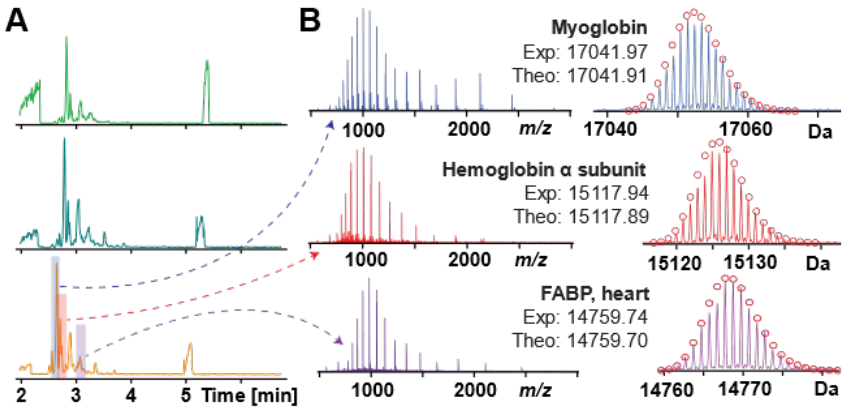



*Figure P2. Human heart tissue extract. A) BPEs displaying three replciate separations of a HEPES protein extract from human heart tissue. B) Example mass spectra including myoglobin, hemoglobin α subunit, and heart fatty acid-binding protein with high mass accuracy.*

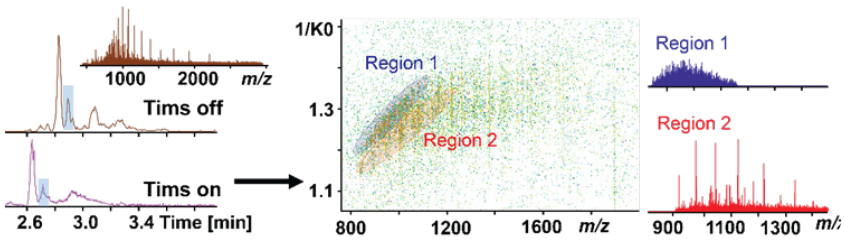



*Figure P3. Trapped ion mobility. Additional separation from ion mobility allows the distinction of overlapping proteoforms in a human heart extract.*

## Bottom-Up Proteomics

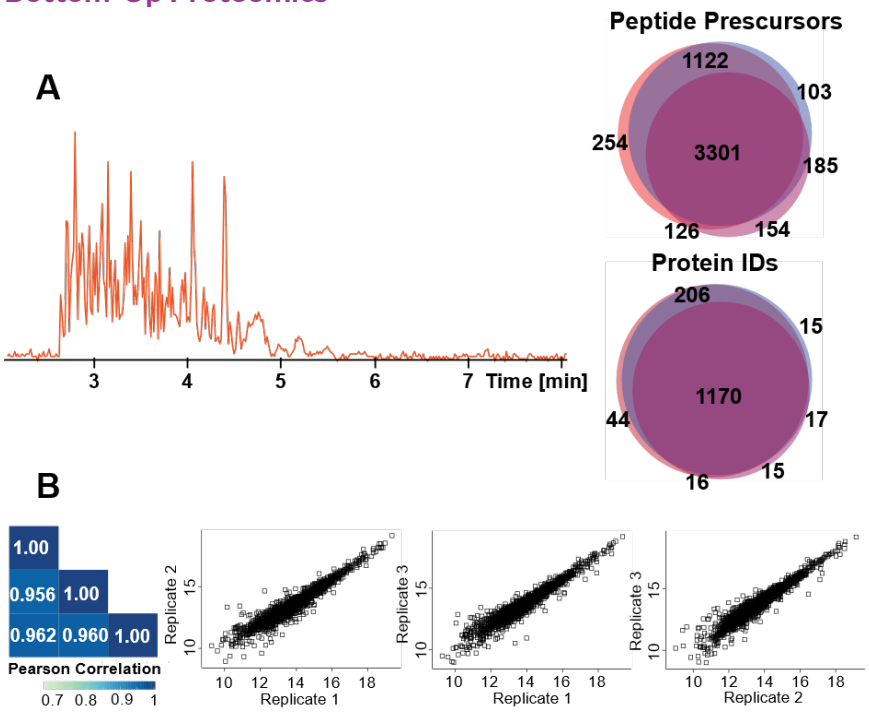



*Figure P4. Bottom-up proteomics. A) Example BPE showing rapid separation and Venn diagrams representing peptide precursors and identified proteins over three injection replicates. Over 1300 total proteins were identified from 4500 peptide precursors at 1% FDR. B) Pearson correlation score and individual plots corresponding to each set of replicates.*

## Conclusions

- Microchip capillary electrophoresis coupled to a trapped ion mobility mass spectrometry promises high "omic" coverage in impressively short time and with minimal sample preparation.
- We have shown applications to metabolomics and proteomics in both bottom-up and top-down format.

## References

1. Hasin, Y.; Seldin, M.; Lusis, A. Multi-omics approaches to disease. Genome Biol 18, 83 (2017).
2. Carillo, S.; Kulkarni, A.; Bones, J.; & Redman, E. Direct Microchip CE/MS Peptide Mapping Workflow for the Analysis of mAb CQAs. LCGC Webinar (2023).

Generic molecules in introductory figure are from Biorender.

## Acknowledgements

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