Increasing Molecular Coverage in Complex Biological and Environmental Samples by Using IMS-MS

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Introduction

Why are we interested in using IMS-MS platforms?

1. IMS adds complementary information to MS measurements which helps lower false discovery rates, separates isomers and allows faster LC separations

2. IMS-TOF MS provides greater dynamic range of detection relative to trapping (e.g. Orbitrap) instruments

3. Detection of structural changes in peptides/proteins can help characterize specific disease states (structural biomarkers)
IMS-MS instrumentation

**Features:**

- NanoESI ion source with 2 inlets for on-the-fly calibration
- Off-axis hourglass ion funnel/accumulation trap before IMS
- Rear ion funnel after IMS
- Segmented quadrupole for CID
- High dynamic range Agilent TOF or Q-TOF MS
Multiplexed IMS-MS

Multiplexing utilizes more drift time space and increases signal

Multiplexed LC-IMS-MS

De-multiplexed IMS-MS spectra

Multiplexed IMS-MS spectra

Multiplexing utilizes more drift time space and increases signal

8 peptides spiked in human serum

<table>
<thead>
<tr>
<th>Spiking Level</th>
<th>Non-Serum Peptide</th>
<th>60-min LC-IMS-TOF MS</th>
<th>60-min LC-TOF MS</th>
<th>100-min LC-Velos-Orbitrap</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 pg/mL</td>
<td>Melittin</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>100 pg/mL</td>
<td>Dynorphin A Porcine Fragment 1-13</td>
<td>✓</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1 ng/mL</td>
<td>Des Pro Ala Bradykinin</td>
<td>✓</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1 ng/mL</td>
<td>Leucine Enkephalin</td>
<td>✓</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10 ng/mL</td>
<td>3X FLAG Peptide</td>
<td>✓</td>
<td>✓</td>
<td>ND</td>
</tr>
<tr>
<td>10 ng/mL</td>
<td>Substance P</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>100 ng/mL</td>
<td>Methionine Enkephalin</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>100 ng/mL</td>
<td>[Ala92]-Peptide 6</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

Sample analyzed using Velos-Orbitrap, TOF MS and IMS-TOF MS instruments
Benefits of IMS drift time separation

1. Improved Sensitivity & Increase Feature Detection & Confidence

QTOF MS of Bradykinin (100 pM)  
IMS-QTOF MS of Bradykinin (100 pM)
Benefits of IMS drift time separation

2. Separates by Shape and Charge State

BSA tryptic digest (25 µg/mL) (5 sec acquisition)

Human Plasma tryptic digest (0.5 mg/mL) (summed LC run for 50 minutes)
Benefits of IMS drift time separation

3. Distinguish different classes of compounds

Peptides and lipids are easily distinguished
Benefits of IMS drift time separation

4. Distinguish sequence isomers

NW Chem used to model 3-D conformations
Benefits of IMS drift time separation

5. Characterize Aggregation Levels & Analyze Interactions

Peptide mixture from C18 column
Benefits of IMS drift time separation

5. Characterize Aggregation Levels & Analyze Interactions

Transthyretin (TTR) Tetramer
Both compact and extended conformers

Drug stabilizes compact (solution phase) structure

Samples from Catherine Costello
Biological diversity studies

- Thousands of samples need to be analyzed to understand the diversity in a population
- IMS-MS allows for faster analysis of many samples with high sensitivity
Chronic liver disease

- **Multiple Factors**
  - **Hepatitis (A,B,C)**
  - Alcohol (ALD)
  - Diabetes
  - Various autoimmune and recessive conditions

- Estimated 130 million people world-wide have HCV
- Blood borne pathogen with no vaccine
Liver fibrosis study

- **Discovery Phase:** 60 matched (age, sex, fibrosis stage) patients correlated by biostatistician

E. S. Baker, et al. “Advancing the High Throughput Identification of Liver Fibrosis Protein Signatures Using Multiplexed Ion Mobility Spectrometry” accepted in MCP.
Liver fibrosis study

Discovery Phase

- Analyzed 60 post-liver transplant patients with LC-IMS-MS
- At least 2 unique peptides were required to identify a protein; significant peptides have p and q values <0.05
- Statistical analysis identified 136 proteins that distinguish between conditions
Liver fibrosis study

Non-transplant Comparison

- Analyzed 60 non-transplant patients with Ishak score 0-1 versus 4-6
- At least 2 unique peptides were required to identify a protein; significant peptides have p and q values < 0.05
- 63 statistically significant proteins between conditions

![Venn diagram showing significant proteins in transplant and non-transplant conditions, with 63 proteins overlapping.](image)
# Liver fibrosis study

## 19 Example Proteins
- Classified into 4 groups: liver metabolism, immune response, oxidative stress and liver architecture
- Overall trends with increasing fibrosis
  - Liver metabolism decreases
  - Oxidative stress increases
  - Extracellular matrix proteins (within liver architecture) increase in fast progressors
  - Differences between slow and fast progressor liver architecture proteins observed

<table>
<thead>
<tr>
<th>Function</th>
<th>Gene</th>
<th>SP_Descriptions</th>
<th>Change (SP/ NP)</th>
<th>Change (FP/NP)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liver Metabolism</strong></td>
<td>F2</td>
<td>Prothrombin</td>
<td>Decrease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BCHE</td>
<td>Cholinesterase</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RBP4</td>
<td>Retinol-binding protein 4</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>TTHY</td>
<td>Transthyretin</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>IGFALS</td>
<td>Insulin-like growth factor-binding protein complex acid labile subunit</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>IGFBP3</td>
<td>Insulin-like growth factor-binding protein 3</td>
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<tr>
<td><strong>Immune Response</strong></td>
<td>C4A</td>
<td>Complement C4-A</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>CD14</td>
<td>Monocyte differentiation antigen CD14</td>
<td>Increase</td>
<td></td>
</tr>
<tr>
<td><strong>Oxidative Stress</strong></td>
<td>QSOX1</td>
<td>Sulfhydryl oxidase 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GPX3</td>
<td>Glutathione peroxidase 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Liver Architecture</strong></td>
<td>ECM1</td>
<td>Extracellular matrix protein 1</td>
<td>Increase</td>
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<tr>
<td></td>
<td>LGALS3BP</td>
<td>Galectin-3-binding protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACTB</td>
<td>Actin, cytoplasmic 1</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>FA12</td>
<td>Coagulation factor XII</td>
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<td></td>
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<tr>
<td></td>
<td>TGFBI</td>
<td>Transforming growth factor-beta-induced protein ig-h3</td>
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<td></td>
<td>FA10</td>
<td>Coagulation factor X</td>
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<td>Decrease</td>
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<tr>
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<td>CO5</td>
<td>Complement C5</td>
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</tr>
<tr>
<td></td>
<td>VTN</td>
<td>Vitronectin</td>
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</tr>
<tr>
<td></td>
<td>LUM</td>
<td>Lumican</td>
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</tr>
</tbody>
</table>

**Decrease** = Green  
**Increase** = Red
Liver fibrosis study

Western Blots

**Liver Metabolism**

- **Patient Pair X**: F2
  - Liver Metabolism
  - PP22 vs PP16

**Log2 Abundance Values from LC-IMS-MS**

- **Patient Pair X**: 18.6
  - Stage 4 vs Stage 1
  - PP22

- **Patient Pair Y**: 18.6
  - Stage 3 vs Stage 1
  - PP16

**Innate Immune Response**

- **C4β for C4A**: PP21 vs PP29

- **Patient Pair X**: 19.6
  - Stage 3 vs Stage 1
  - PP21

- **Patient Pair Y**: 19.6
  - Stage 3 vs Stage 1
  - PP29

**Oxidative Stress**

- **QSOX1**: PP24 vs PP22

- **Patient Pair X**: 16.6
  - Stage 3 vs Stage 1
  - PP24

- **Patient Pair Y**: 16.6
  - Stage 1
  - PP22

**Liver Fibrosis**

- **ECM1**: PP21 vs PP23

- **Patient Pair X**: 15.6
  - Stage 3 vs Stage 1
  - PP21

- **Patient Pair Y**: 15.6
  - Stage 3 vs Stage 0
  - PP23

- **LGALS3BP**: PP24 vs PP21

- **Patient Pair X**: 19
  - Stage 3 vs Stage 1
  - PP24

- **Patient Pair Y**: 19
  - Stage 3 vs Stage 1
  - PP21
Future Projects & Directions
Fast lipidomic/metabolomic analyses

<table>
<thead>
<tr>
<th></th>
<th>IMS-MS</th>
<th>QTOF-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direct Infusion</td>
<td>LC</td>
</tr>
<tr>
<td>Lipid Extract from Plasma</td>
<td>463</td>
<td>736</td>
</tr>
</tbody>
</table>

50-min LC Gradient

IMS cannot completely counter the ion suppression from direct infusion, but more features are observed with LC-IMS-MS than LC-QTOF MS alone

3 peaks observed for m/z = 637.31 all with the same elution time
Na\(^+\) Isomer separations with IMS

Normalized Intensity (AU)

Drift Time (ms)

MW = 150.05
Isomer separations difficult with HILIC

\[ \alpha\text{-D-Ribose 5-phosphate (r5p)} \]
\[ \text{D-Ribulose 5-phosphate (ru5p)} \]

\[ \text{D-Fructose-6-phosphate (f6p)} \]
\[ \text{D-Glucose-6-phosphate (g6p)} \]
Preliminary IMS-MS analysis of urine

Positive ESI, 5 µL of pooled urine prepared

Positive ESI, 5 µL of pooled urine + 15 mM sugars (glucose, xylose, mannose and galactose) prepared
Conclusions

IMS-MS:

• Increase the throughput of sample analysis, while still detecting lower level species
• Analyze difficult samples and obtain information that was previously not detected
• Start running biological diversity studies to evaluate peptide/protein markers in hundreds to thousands of patients

Future directions:

• Perform fast lipidomic and metabolomic analyses
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