“Why, then, the world's mine oyster, which I with sword will open”

William Shakespeare
'The Merry Wives of Windsor’, 1600
While many practical limitations to charged particle analysis……
NanoESI current loss and transmission to MS

0.5 μL/min flow rate to electrospray; 490 μm i.d. single inlet capillary (120°C) to MS

Charges/s to MS can approach $10^{11}$
More than $10^4$-fold (potentially $>10^6$) miss match with ion utilization rate!

Page et al., *JASMS*, 18, 1582-1590 (2007)
Orbitrap MS maximum peak intensities during LC separation

- Maximum ion utilization rate by FTICR or Orbitrap MS is $\sim 10^6$/s
- Significant variation in detection levels due to variation in ion trap accumulation times (reported levels corrected for differences in AGC set accumulation times)
- Maximum dynamic range in a single spectrum $\sim 10^3$
Drift tube ion mobility separations

Ions move (and can separate) much faster in gases than liquids.

Commonly achieved separation speeds (e.g., in proteomics applications):

**LC**
- Total separation time ~ 3000 s
- Minimum peak width ~ 10 s
- Effective peak capacity ~200-300
- Peak generation rate*: ~15 s/peak

**IMS**
- Total separation time ~ 50 ms
- Minimum peak width ~ 0.6 ms
- Effective peak capacity (peptides) ~10-20
- Peak generation rate*: ~5 ms/peak
IMS stage added to MS without significant ion loss


Insulin spectrum (200 fM/µL) before and after addition of drift tube

528 c/s  
ESI/MS

536 c/s  
ESI/IMS/MS
• Dual nanoESI source
• Off-axis ‘hourglass’ ion funnel/accumulation trap region before IMS
• Ion funnel after IMS for lossless transmission
• Segmented quadrupole for CID between IMS and MS
• High dynamic range (ADC-based) Agilent TOF or Q-TOF MS
Continuous ion transmission (lossless) mode

ESI Source

High Pressure Ion Funnel

Hourglass Ion Funnel

Drift Cell

Rear Ion Funnel

Q1

Q2

MS

Ion Gate

Low Voltage (Continuous Beam)

4-12 Torr

281.1618

395.2411

480.6085

547.3141

653.3598

740.4036
Pulsed IMS mode for separations

ESI Source

High Pressure Ion Funnel

Hourglass Ion Funnel

Drift Cell

Rear Ion Funnel

Q1

Q2

MS

Ion Gate

High Voltage

(Pulsed Beam)

mass

m/e

Mobility - Scans
Multiplexed ion fragmentation

Collisional activation after IMS stage

Parent IMS-MS Spectrum

Fragmentation IMS-MS Spectrum
Multiplexing utilizes more drift time space; increases signal and extends LOD

Increased ion utilization efficiency and saturated peaks

- TOF with ADC detector recovers very quickly from saturation
- Allows minor peaks in ‘isotopic envelope’ to be used for correction
Base peak chromatogram from LC-IMS-MS of human plasma sample

Original

Saturation-corrected
LC-IMS-MS and proteome coverage

100-min LC-MS Data from LTQ Orbitrap Velos
17,778 LC-MS Features Found

60-min LC-IMS-QTOF MS Data
23,115 LC-MS Features Found
Comparing data for one time-point during LC separation

Velos – 6 ions deisotoped in this region

Zoomed Region Velos

Zoomed Region IMS-QTOF

Multiplexed IMS-QTOF MS – 17 ions deisotoped in this region
Detection of peptides spiked into 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></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 MS and TOF MS with and without IMS stage

Baker et al., *J. Proteome Res.* **2010**, **9**, 997-1006
LC-IMS-MS 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

Baker et al., Molecular and Cellular Proteomics, 13, 1119-1127 (2014)
LC-IMS-MS 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 studies
Larger scale LC-multiplexed IMS-MS application

Analyzed 3518 serum samples depleted of major proteins from study of sarcopenia in men*

In total >6000 analyses (including replicates) over 9+ months

Collaboration with Eric Orwoll and coworkers (OHSU)
Sources of measurement variation

- 3518 serum samples; immunoaffinity depletion of 12 most abundant proteins
- Sources of variation determined* using 990 higher intensity peptides from ~200 control samples interspersed with clinical serum samples

* Shannon McWeeney, OHSU
\textbf{(Sugar+Na)^+ Isomer separations with IMS}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{chart.png}
\caption{Normalized intensity (AU) vs. drift time (ms) for Xylose and Arabinose.}
\end{figure}

MW = 150.05

D-Arabinose  \quad D-Xylose
IMS-MS of protein complexes

Transthyretin (TTR) Tetramer
Both compact and extended conformers

TTR Tetramer with 2 (Diflunisal; MW=250)
No extended conformers

Drug stabilizes compact (solution phase) structure

Samples from Catherine Costello
The Classical MS Paradigm

Ionization / transport to MS $\rightarrow$ $m/z$ analysis / detection
The Modern MS Paradigm

Ionization etc.  →  Ion Manipulations  →  m/z analysis etc.

- Storage (ion traps)
- Separations:
  - Low field mobility
  - High field differential mobility
  - Mobility in interacting gases and vapors
- Reactions:
  - Collisionally activated ion dissociation; heating
  - Other ion-molecule (e.g. charge reduction, H/D exchange)
  - Ion-surface interactions (e.g. surface induced dissociation)
  - Ion-Ion (e.g. ETD, chemistry for crosslinking, derivatization)
Structures for Lossless Ion Manipulations (SLIM)
Confining ions using inhomogeneous electric fields

Quadrupole ion trap

D. Gerlich, Advances in Chemical Physics, VLXXXII, 1992
Structures for Lossless Ion Manipulations (SLIM)

Assembling complex devices from SLIM components to provide new capabilities

- Ions effectively repulsed from surfaces by pseudo-potential due to RF of alternating phase applied to central electrodes
- Ions confined by DC of guard electrodes
- DC potentials to central ‘rung’ electrodes drive ion motion

DC to Guard electrodes for ion confinement
RF to central ‘rung’ electrodes for ion repulsion from surfaces
Central electrode DC steps to e.g. drive ion drift separations
Effective potential due to RF applied to SLIM central rung electrodes

\[ f = 800 \text{ KHz}; \ V_{p-p} = 130 \]

Tolmachev et al., Anal. Chem., in press
In combination RF and DC potentials create ion conduits

Typical RF+/- electrode dimensions: ~6 mm x 0.75 mm
Typical board (Y-dimension) spacing: ~5 mm
Initial PCB based SLIM implementation

- Guard electrodes
- Rung electrodes
- SLIM component
Assembly of simple SLIM modules
Arrangement for initial SLIM module evaluation
New ion funnel developed for ion transfer to SLIM

- DC + RF
- Rung electrodes
- Guard electrodes
- SLIM
Current probe inserted between SLIM surfaces

Distance from point of ion injection to 45 cm linear SLIM module

![Graph showing ion current (pA) vs. distance (cm)]
DC gradient in Z-direction for ion mobility separations in SLIM

Similar gradient in side guard electrodes also required.
SLIM IMS resolving power

Drift Time (ms)

$m/z$ 622: $R = 39$

$m/z$ 922: $R = 44$

$m/z$ 1222: $R = 60$
Effect of RF amplitude on SLIM IMS performance

No significant effect of RF amplitude on observed IMS drift times or resolving power.
SLIM IMS resolving power

- SLIM linear arrangement
- Theory

Drift field intensity (V/cm) vs. Resolving Power
Forming ion accumulation / trapping regions in SLIM

Ion trapping using 15 cm of a 30 cm linear SLIM module

From ion funnel
Extended trapping times show lossless ion storage

Signal for ions from ESI, stored for different times in a 15 cm SLIM trap, and then sent to MS

![Graph showing integrated signal for major ions trapped (AU) against trapping time (seconds).]
Extended trapping times show lossless ion storage
The SLIM switch

Switch on: ions turned and transmitted to MS

Switch off: ions continue on linear path

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Switch On</th>
<th>Switch Off</th>
</tr>
</thead>
<tbody>
<tr>
<td>Switching Guard</td>
<td>643 V</td>
<td>543 V</td>
</tr>
<tr>
<td>Switching Rung</td>
<td>528 V</td>
<td>533 V</td>
</tr>
</tbody>
</table>

All other voltages remain unchanged when switching
Resolving Power

SLIM IMS turn vs. straight resolving power

- **SLIM linear arrangement**
- **Theory**
- **SLIM with 90° turn**

Graph showing the resolving power for different electric field intensities (E [V/cm]). The data points are plotted for SLIM linear arrangement, theory, and SLIM with 90° turn. The x-axis represents E [V/cm], and the y-axis represents the resolving power.
SLIM path switching

Ions turned and transmitted to MS

14V/cm through Turn

Intensity (a.u.)
m/z

Switching guard biased +100 V above “normal”, first electrode of turn path lowered 11 V

Ions switched to linear path

14V/cm Switching Off

Intensity (a.u.)
m/z

Switching guard biased +100 V above “normal”, electrode of trace of turn path raised 11 V

Webb et al., Anal. Chem., submitted
Fast switching for mobility selection
Fast switching for mobility selection

Drift Time (ms)
Fast switching for mobility selection
Fast switching for mobility selection

\( m/z \ 1222 \)
Fast switching for mobility selection

Nine peptide mixture
Assembling complex devices from simple components to provide new capabilities
Support

NIH National Institute of General Medical Sciences
P41 Biomedical Technology Research Resource

DOE Office of Biological and Environmental Research
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