Investigation of a prototype 150 um microfluidic device and IMS-ToF mass spectrometry for high-throughput multi-omic profiling

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Duke Proteomics Core Facility

Collaborative creation of the Duke School of Medicine, the Institute for Genome Sciences & Policy - established 2007, fully on-line 2008

Provides support for basic and clinical research scientists
- Support for >600 projects for >150 Principle Investigators
- Just ran our 10,000th sample

Name Changing to “Duke Proteomics and Biological Mass Spectrometry Facility” in 2013
- Addition of support for small molecule analysis, including DMPK, metabolomics, and lipidomics

www.genome.duke.edu/cores/proteomics
A Case for 150 um Separations

Assumptions:  UPLC conditions of 0.45 cm/sec linear velocity
Aiming for fast, efficient separation (10-15 min per sample or fraction)
Signal is proportional to analyte concentration
Swept volume is 3 ul
So, while there is nothing ‘magical’ about 150 um separations...

- It offers a nice blend of:
  - Sensitivity of capillary scale
  - Solvent and sample use of capillary scale
  - Throughput of analytical (UPLC) scale

- But, there have been ‘historical’ hangups:
  - Spray stability and robustness at 2-4 ul/min
  - Columns that are easy to install, with UPLC performance
### Benefits and Compromises of Changing Column Diameters for Various Applications

<table>
<thead>
<tr>
<th>Diameter</th>
<th>Benefits</th>
<th>Compromises</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.075 mm</td>
<td>2-4x Speed &amp; Efficiency/time</td>
<td>4-5x sample required</td>
</tr>
<tr>
<td>0.150 mm</td>
<td>1/20 Solvent/Sample Consumption</td>
<td>10-20% increase in time</td>
</tr>
<tr>
<td>2.1 mm</td>
<td></td>
<td>Source/ionization flexibility</td>
</tr>
</tbody>
</table>

*Must be weighed for each individual application*
Common and Emerging Workflows in our Lab
- where would decreasing analysis time add value?

Qualitative Proteomics Experiments
- Protein ID, confirmation
- Immunoprecipitation, Protein/Protein Interaction

PTM Characterization
- Phosphorylation (TiO₂ Enrichments), S-Nitrosylation (RAC enrichments)
- Acetylation (Antibody Based Enrichment)
- Qualitative or Quantitative Analysis (Label-Free or various SILAC/covalent labeling strategies)

Differential Expression and/or Targeted Quantitation
- Global or targeted quantitation of individual protein expression as a function of disease, treatment, etc.

Metabolite Quantitation, Pharmacokinetic Analysis
- Non-targeted analysis of polar or nonpolar metabolites
- Targeted quantitation of metabolites
- Drug metabolism and Pharmacokinetic Analysis

Vs.
Tile Design and Flow Diagram

- ESI Emitter Assembly
- Incoming flow
- Analytical Column
- Trap Column
- Electrical Connections (EEPROM, Heater)
Evaluation Areas for Prototype 150 um Tile

Label-Free Quantitation, Proteomics

Targeted Peptide Quant, Method Development and Deployment

Metabolomics (RPLC and HILIC)

Lipid Profiling (Flow Injection)
Summary of Multi-Omics Sample Preparation Strategy

Cell Disruption
(Sonication in AmBic pH8)

Bradford Assay, 1.8mg/sample
(normalize by total lysate)

- **Polar Metabolites**
  - ~48%
  - 80/20 MeOH/water
  - 1 hr extraction, N₂ dry
  - Resuspend
  - 2/1/0.2 MeCN/Formic Acid/HFBA
  - Inject 1% for LC-MS/MS (30 min/sample)

- **Lipids**
  - ~48%
  - 80/20 MTBE/MeOH
  - 1 hr extraction, N₂ dry
  - Resuspend
  - 4/2/1 IPA/MeOH/CHCl₃
  - Inject 4% for FIA (10 min/sample)

- **Proteins**
  - ~4%
  - 0.25% w/v Rapigest
  - DTT/IAA/trypsin overnight
  - Acidify
  - 1/2/97 TFA/MeCN/water
  - Inject 20% for 2DLC-MS/MS (3 hr/sample)
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150 um Prototype Tile
Direct Inject/Flow Injection Fluidic Diagram

Tile options tested:
- 5, 10, 20 cm
- BEH C18
- HSS T3 C18
- CSH C18
- BEH C4
- BEH Amide HILIC
Infusion Tile
RPLC Metabolomics Method

Analysis used 1% of isolate:
150 um x 10 cm 1.7 um BEH C18 tile, F = 2.0 ul/min at 45°C
Mobile phase A: 0.1% Formic acid, 0.02% HFBA, in water
Mobile Phase B: 0.1% Formic acid in 10/90 IPA/MeCN
Mass Spectrometry: Synapt G2 HDMS, Resolution mode (25,000 Rs) @ 5Hz
Retention Time Shift and Principal Components Analysis for RPLC Metabolomics Method
Peak Width Distribution for 12 min RPLC Metabolite Separation

Chromatographic Efficiency, Width at Base (n=4,737 CS=1 metabolites)
‘Direct’ HILIC Method Translation from 2.1mm to 0.15 mm

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2.1 mm UPLC (Column)</th>
<th>0.15 mm UPLC (Tile)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column Length</td>
<td>150 mm</td>
<td>100 mm</td>
</tr>
<tr>
<td>Mobile phase A/B</td>
<td>0.1% FA in H$_2$O/MeCN</td>
<td>0.1% FA in H$_2$O/MeCN</td>
</tr>
<tr>
<td>Flow Rate (Velocity)</td>
<td>0.4 ml/min (0.45 cm/sec)</td>
<td>0.002 ml/min (0.44 cm/sec)</td>
</tr>
<tr>
<td>Loop offline*</td>
<td>0.1 min</td>
<td>2.5 min</td>
</tr>
<tr>
<td>Gradient</td>
<td>99% to 30% B in 5.9 min</td>
<td>99% to 30% B in 7 min</td>
</tr>
<tr>
<td>Sample reconstitution*</td>
<td>50/50 MeCN/H$_2$O</td>
<td>99/1 MeCN/H$_2$O</td>
</tr>
</tbody>
</table>

**Diagram:**
- Methionine
- Xanthene
- Lactose
- Arginine
The Beauty of the Tile Interface...

seamless column installation for UPLC

Pressure

Solvent A Flow

Solvent B Flow

Injections w 20 cm column

TILE CHANGE

Injections with 10 cm column
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  Acidify
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  Inject 20% for 2DLC-MS/MS
  (3 hr/sample)
Lipid Profiling using Flow Injection Analysis and an Infusion Tile

Approximately 600 unique lipid species quantified in a 4 minute run (5 min cycle)

Analysis of the Lipid Isolate from MCF7 cells (prepared using MTBE/MeOH extraction).
- Ion-Mobility Data-Independent Analysis
- Synapt G2, 0.6 sec scans (6V or 15-45V)
- 3 ul/min flow rate
- Mobile phase was 10/90 IPA/MeCN with 0.1% formic acid
Lipid Quantitation (AUC)
Sum Lipid Composition using Accurate Mass

"-M vs. +M"

P <= 0.01

TG (52:3)
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1/2/97 TFA/MeCN/water
Inject 20% for 2DLC-MS/MS
(3 hr/sample)
150 um Prototype Offers Improvements in Time Required for Method Development in Biomarker Verification Studies

- Data Analysis and Generation of Next Method
- LC-MS/MS Analysis Time

**Nanoscale LC (0.4ul/min)**
- Total Met. Dev. Time: 230 minutes

**150 um tile (3 ul/min)**
- Total Met. Dev. Time: 100 minutes

**150 um tile (3 ul/min)**
- Total Met. Dev. Time: 50 minutes
Chromatographic Performance and Raw Signal Intensity, Nano (75 um) versus 150 um

Peptide K.TFAEALR.I from Enolase

Peptide R.EALDFFAR.G from ADH

Nano LC conditions

F = 0.4 ul/min  
W₁/₂ = 12 sec

T150 Conditions

F = 3 ul/min  
W₁/₂ = 2.4 sec
150 um Prototype Tile
2D with Dilution Fluidics

RP1 - Xbridge-BEH130 C18 NanoEase Column, 5μm, 300 μm x 50 mm
Trap - UPLC Symmetry C18 Trap, 5 μm, 180 μm x 20 mm
MS/MS - Synapt G2 – hdDIA (hdMS\textsuperscript{E})
## Goals for High-Throughput Proteomics Analysis Using 2DLC and TRIZAIC

### Time per sample (hr)

<table>
<thead>
<tr>
<th>Type</th>
<th>Column</th>
<th>Φ (min)</th>
<th>Φ (μL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1D</td>
<td>Nano*</td>
<td>112</td>
<td>0.8</td>
</tr>
<tr>
<td>2D</td>
<td>Nano* TriZAIC</td>
<td>295*</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>405</td>
<td>1.35</td>
</tr>
<tr>
<td>2D</td>
<td>TriZAIC</td>
<td>350</td>
<td>2.0</td>
</tr>
<tr>
<td>2D**</td>
<td>TriZAIC</td>
<td>350</td>
<td>2.6**</td>
</tr>
</tbody>
</table>

* Current “standard” configurations

**Potential elimination of between-fraction trapping time with dual-trap 2DLC prototype (K. Fadgen and M. Staples)

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Initial Trapping Step

- Fraction Elution to 2\(^{nd}\) Dimension
- Analytical Separation

- 90 min gradient @ 0.4 μL/min
- 37 min gradient @ 0.4 μL/min (nano) or 3 μL/min (Tile)
- 18.5 min gradient @ 3 μL/min (Tile)
- 18.5 min gradient @ 3 μL/min (Tile)

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150 um Tile Loading Test
q1D configuration, 150 um x 100 mm

Loading test performed with E. Coli lysate
Method: 5 to 40% MeCN in 37.1 min, 3 ul/min, 35C
Only very minor effects on chromatographic efficiency at 4 ug load
2D LC/MS/MS on Synapt G2
nanoLC vs 150 um Tile

75 um x 150 mm BEH C18 column
7 to 35% MeCN in 37 min, 0.5 ul/min

150 um x 100 mm BEH C18 nanoTile
7 to 35% MeCN in 37 min, 3.0 ul/min
Cutting Run Time in Half while Maintaining the Separation Efficiency

150 um x 100 mm BEH C18 nanoTile
7 to 35% MeCN in 37 min, 3.0 ul/min

150 um x 100 mm BEH C18 nanoTile
7 to 35% MeCN in 18.5 min, 3.0 ul/min

3 ug EColi, 5 fxn, 37 min grad, HDMSE, Fxn 5
3 ug EColi, 5 fxn, 18p5 min grad, HDMSE, Fxn 1
TRIZAIC 150 2DLC Configuration

Chromatographic Evaluation versus 75 um Capillary Column technology

Analysis Method

<table>
<thead>
<tr>
<th>Analysis Method</th>
<th>Mean Width at Base (min)</th>
<th>Separation Space (min)</th>
<th>Peak Capacity (min/mb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 min x 3ug</td>
<td>0.2</td>
<td>40</td>
<td>200</td>
</tr>
<tr>
<td>18 min x 9ug</td>
<td>0.4</td>
<td>80</td>
<td>400</td>
</tr>
<tr>
<td>37 min x 3ug</td>
<td>0.8</td>
<td>120</td>
<td>600</td>
</tr>
<tr>
<td>37 min x 9ug</td>
<td>1.0</td>
<td>160</td>
<td>800</td>
</tr>
<tr>
<td>37 min Cap</td>
<td>0.4</td>
<td>80</td>
<td>400</td>
</tr>
</tbody>
</table>
Preliminary Data on Performance for Peptide Identifications

• Take home messages:
  – Even with 4.5x loading and higher chromatographic efficiency, max peptide IDs with 150 um x 100 mm Tile is ~25% less than 75 um x 150 mm, however throughput is 2x.
  – Performance of 18.5 min separation with correct loading rivals 37 min separation
  – Higher throughput should allow 5-fraction 2DLC in time of “traditional” nanoscale 1D LC
Acknowledgments

Duke University Proteomics Core Facility
http://www.genome.duke.edu/cores/proteomics/

Funding
NIH S10 grant
Duke School of Medicine
CTSA grant UL1RR024128