Comprehensive Profiling of the Proteome, Lipidome, and Metabolome Enabled Using a Prototype UPLC-Compatible Microfluidic Device

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Benefits and Compromises of Changing Column Diameters for Various Applications

0.075 mm

Benefits
- 2-4x Speed & Efficiency/time

Compromises
- 4-5x sample required

0.150 mm

Benefits
- 1/20 Solvent/Sample Consumption
- 20-40x Sensitivity

Compromises
- 10-20% increase in time
- Source/ionization flexibility

2.1 mm

Must be weighed for each individual application
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)
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

>17,000 metabolite Features in 12 minutes
Lipid Profiling using Flow Injection Analysis and an Infusion Tile

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

Approximately 600 unique lipid species quantified in a 4 minute run (5 min cycle)
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_<sub>E</sub>)
## Goals for High-Throughput Proteomics Analysis Using 2DLC and TRIZAIC

### Initial Trapping Step
- **1D**
  - Nano*
  - 90 min gradient @ 0.4 ul/min

- **2D**
  - Nano* TriZAIC
  - 37 min gradient @ 0.4 ul/min (nano) or 3 uL/min (Tile)

- **2D**
  - TriZAIC
  - 18.5 min gradient @ 3 uL/min (Tile)

- **2D**
  - TriZAIC
  - 18.5 min gradient @ 3 uL/min (Tile)

### Fraction Elution to 2\textsuperscript{nd} Dimension
- **1D**
  - Nano*

- **2D**
  - Nano* TriZAIC

- **2D**
  - TriZAIC

### Analytical Separation

### Time per sample (hr)

<table>
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<tr>
<th>Type</th>
<th>Column</th>
<th>$\Phi$</th>
<th>$\Phi$/min</th>
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<td>Nano*</td>
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<td>0.8</td>
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<td>Nano* TriZAIC</td>
<td>295*</td>
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<td></td>
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<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)
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 18.5 min, 3.0 ul/min
TRIZAIC 150 2DLC Configuration
Chromatographic Evaluation versus 75 um Capillary Column technology
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