Vented Column Technology Applied to Proteomic MudPIT Analysis on Long Capillary Columns

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Last year.... the long column and proteomics

What if?

Space
What if we could do MudPIT on a 60 cm column?

Available Column Technologies

Tri-Phasic MudPIT

- RP
- SCX
- Reverse Phase

Vented Technology

- RP
- Reverse Phase


MudPIT Meets Vented Column Technology Meets The Long Column.
Building the Double Vented Tetra Phasic MudPIT Device
Method

1. Place screen frit in the tee
2. Swage with short piece of PEEK
3. Pack Poros 10
4. Wash
5. Pack SCX (Whatman Partisil, 5um)
6. Wash
7. Pack final section of Poros 10
8. Unswage
9. Cut first segment of Poros 10 to length
10. Reswage with new PEEK
11. Cut off final length of fused silica capillary
Building The Triphasic Column
Continuous Vent
The Discontinuous Vent

Tri-Phasic On The Valve
## MudPIT Gradients

Optimized for speed to test different models, not optimized for proteomic performance

<table>
<thead>
<tr>
<th>Gradient 17</th>
<th>Gradient 18</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Load</strong></td>
<td><strong>Salt &amp;</strong></td>
</tr>
<tr>
<td><strong>Wash</strong></td>
<td><strong>Separate</strong></td>
</tr>
<tr>
<td>ACN bump</td>
<td></td>
</tr>
</tbody>
</table>

### Initial conditions
- 30ul/min 0%B
- 30ul/min 0%B

<table>
<thead>
<tr>
<th>T</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>12</td>
<td>80</td>
</tr>
<tr>
<td>12.1</td>
<td>5</td>
</tr>
</tbody>
</table>

**Purpose:** Load sample onto first RP and wash and elute onto SCX

<table>
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<tr>
<td>1</td>
<td>5</td>
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<tr>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>70</td>
<td>40</td>
</tr>
<tr>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>80.1</td>
<td>5</td>
</tr>
</tbody>
</table>

**Purpose:** Salt bump. Separate peptides on the analytical column.
10 cm Continuous Vent Human Serum MudPIT

RT: 0.00 - 116.03

0 mM

12.5 mM

25 mM

50 mM

250 mM

1000 mM

5000 mM
60 cm Continuous Vent Human Serum MudPIT

RT: 0.00 - 116.03

0 mM

12.5 mM

25 mM

50 mM

250 mM

1000 mM

5000 mM
Comparing 10 and 60 cm Columns at the 25 mM Salt Step Using the Continuous Vent Method

10 CM

RT: 22.58 - 95.12

Tim e (m in)

Relative Abundance

10 20 30 40 50 60 70 80 90 100

Relative Abundance

1207.8 631.5 626.7 714.5 714.3 409.9 594.1 638.2 992.9 998.2 973.7 522.6 522.6 572.9

NL: 4.03E9
Base Peak F: + c ESI Full m/z [400.00-1400.00] MS
25mm10cmc

60 CM

RT: 22.58 - 95.12

Tim e (m in)

Relative Abundance

10 20 30 40 50 60 70 80 90 100

Relative Abundance

1207.9 631.5 648.1 638.4 998.4 992.8 926.3 520.9 573.0 559.1

NL: 4.11E9
Base Peak F: + c ESI Full m/z [400.00-1400.00] MS
25mm60cmc
XIC Plots For 10 and 60 Columns At The 25 mM Salt Step With The Continuous Vent Method

10 cm

60 cm
Evaluating The CV and DCV Experiments By Counting Significant Peptides With Mascot

Formatting Options

<table>
<thead>
<tr>
<th>Format As</th>
<th>Select Summary (protein hits)</th>
<th>Help</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Significance threshold $p < 0.05$
- Max. number of hits $\text{AUTO}$
- Standard scoring ☑ MudPIT scoring ☐
- Ions score cut-off $20$
- Show sub-sets ☐
- Show pop-ups ☑ Suppress pop-ups ☐
- Sort unassigned $\text{Decreasing Score}$
- Require bold rel ☑
## CV vs. DCV

**Short vs. Long**

<table>
<thead>
<tr>
<th></th>
<th>10 cm</th>
<th>60 cm</th>
<th>% Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peptide Count</strong></td>
<td>537</td>
<td>635</td>
<td>18.3</td>
</tr>
<tr>
<td><strong>Peptide Count</strong></td>
<td>593</td>
<td>739</td>
<td>24.6</td>
</tr>
<tr>
<td><strong>% Gain</strong></td>
<td>10.4</td>
<td>16.4</td>
<td></td>
</tr>
</tbody>
</table>
## CV vs. DCV

**Short vs. Long**

<table>
<thead>
<tr>
<th>Peptide Count Category</th>
<th>10 cm</th>
<th>60 cm</th>
<th>% Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>6+</td>
<td><strong>447</strong></td>
<td><strong>498</strong></td>
<td><strong>11.4</strong></td>
</tr>
<tr>
<td>1-5</td>
<td><strong>90</strong></td>
<td><strong>137</strong></td>
<td><strong>52.2</strong></td>
</tr>
</tbody>
</table>

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<tr>
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<th>10 cm</th>
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<tbody>
<tr>
<td>6+</td>
<td><strong>486</strong></td>
<td><strong>589</strong></td>
<td><strong>21.2</strong></td>
</tr>
<tr>
<td>1-5</td>
<td><strong>107</strong></td>
<td><strong>150</strong></td>
<td><strong>40.2</strong></td>
</tr>
</tbody>
</table>
Conclusion

- Long columns are better at showing us a greater number of low level peptides.
- The CV method is superior to the DCV method.
- Manufacture of the CV device needs to be standardized and simplified.
Acknowledgements

- The Vincent & Stella Coates Foundation
- Mike MacCoss, University of Washington
- Andy Gieschen, Agilent
- ThermoFinnigan