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

and

Practical Aspects of Long Column Chromatography

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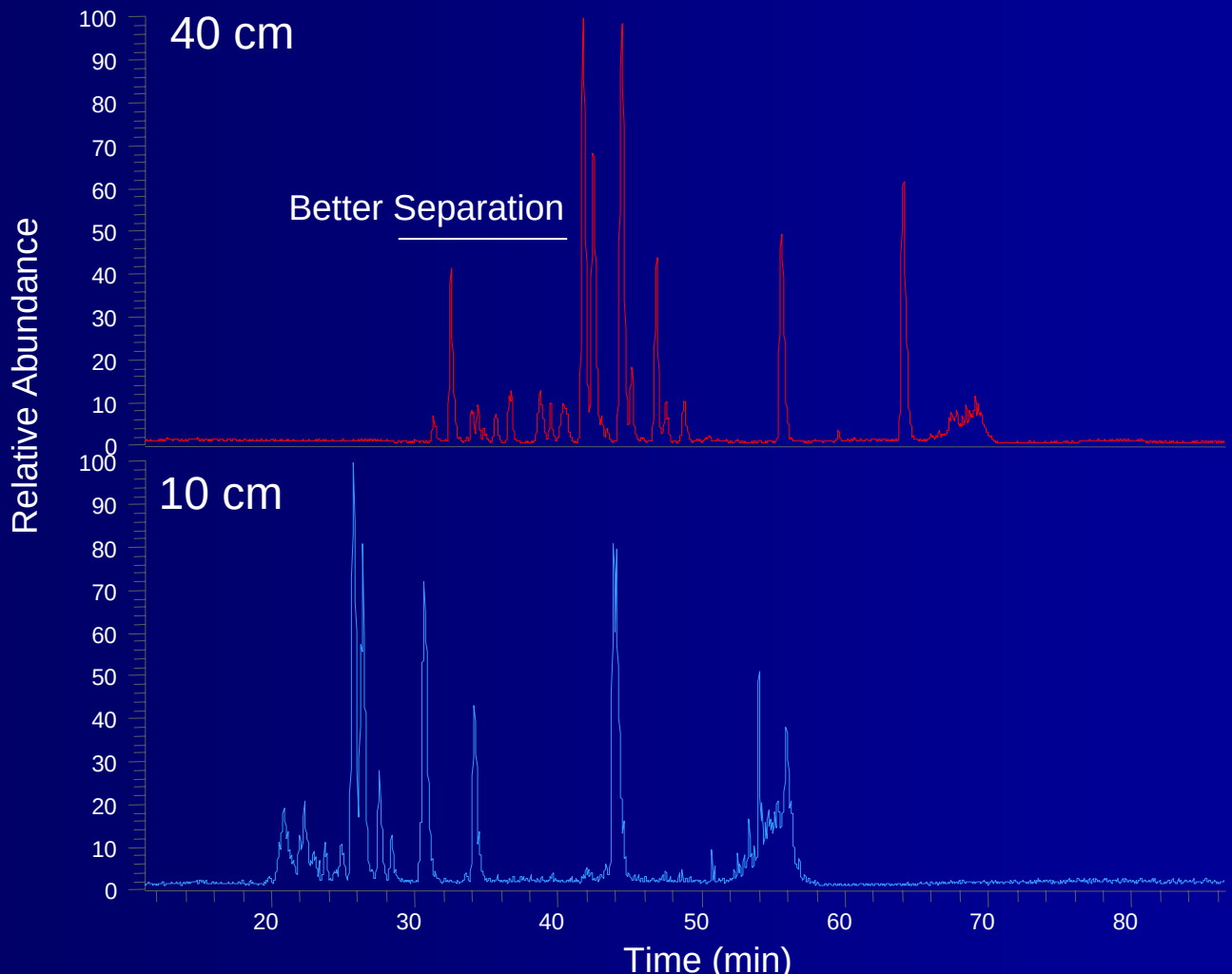
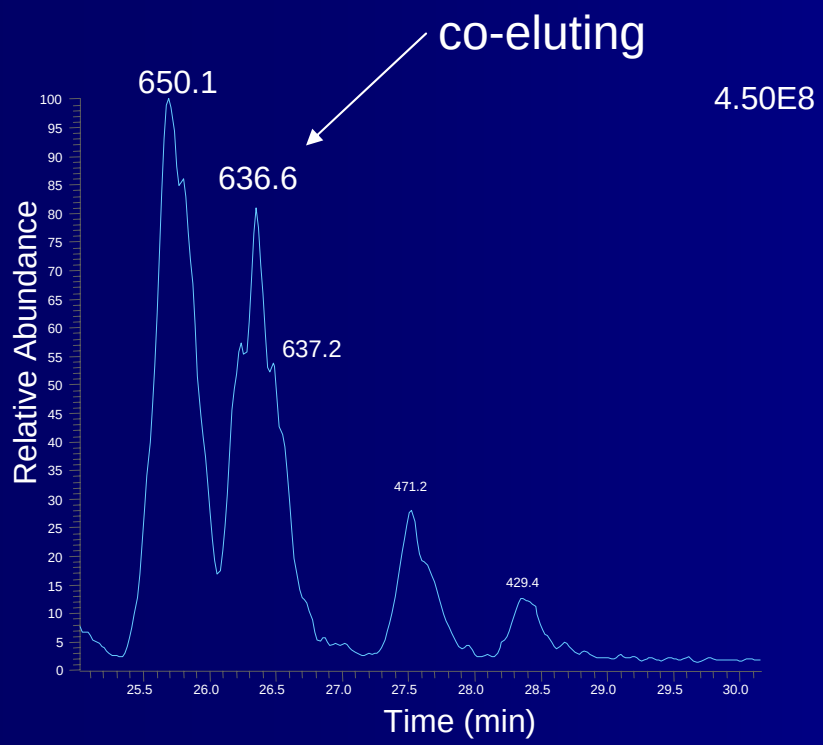
Recent Configuration

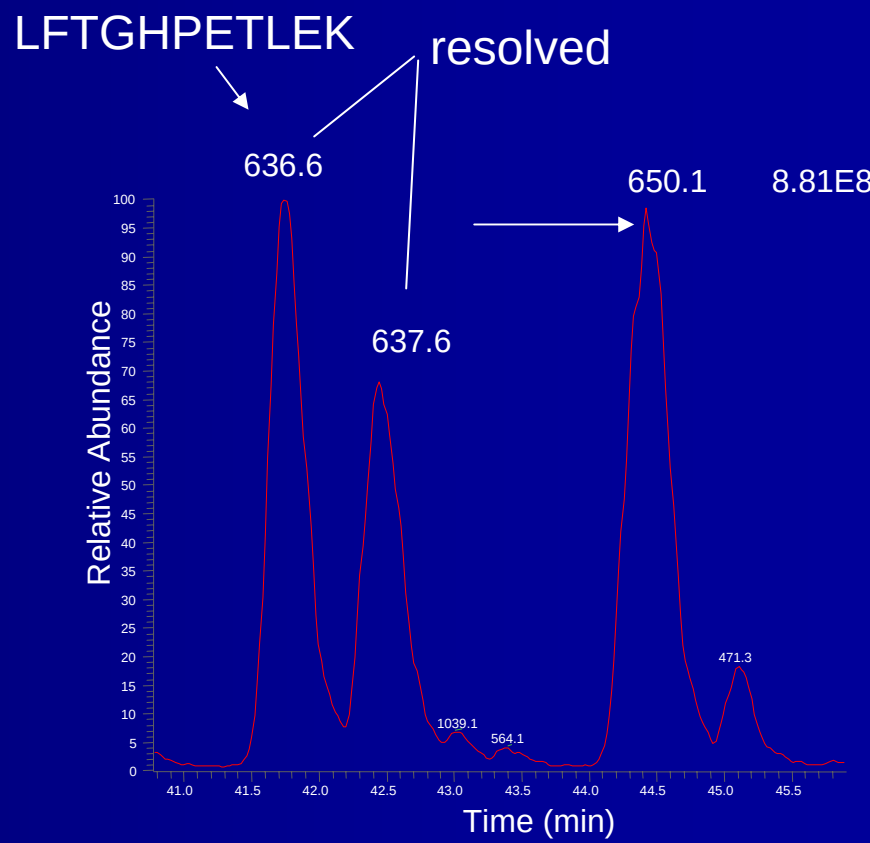
Myoglobin Tryptic Digest
400 mm vs. 100mm



Separation of Near Isobars and Added Selectivity



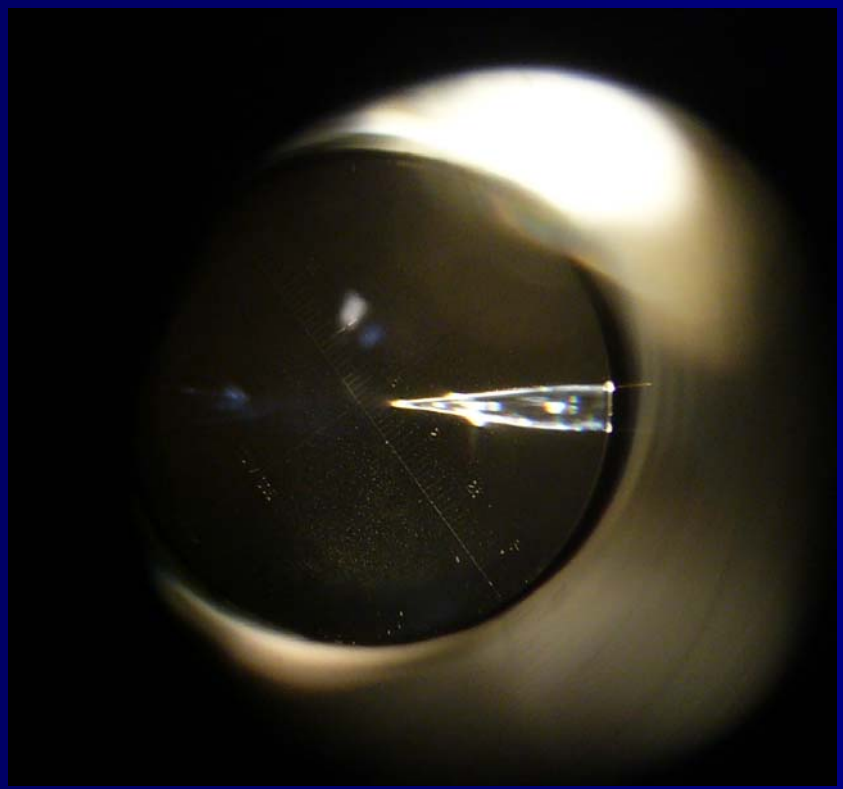
100 mm



400 mm

What if?

Space



Could we do MudPIT on a 60 cm column?

Available Column Technologies

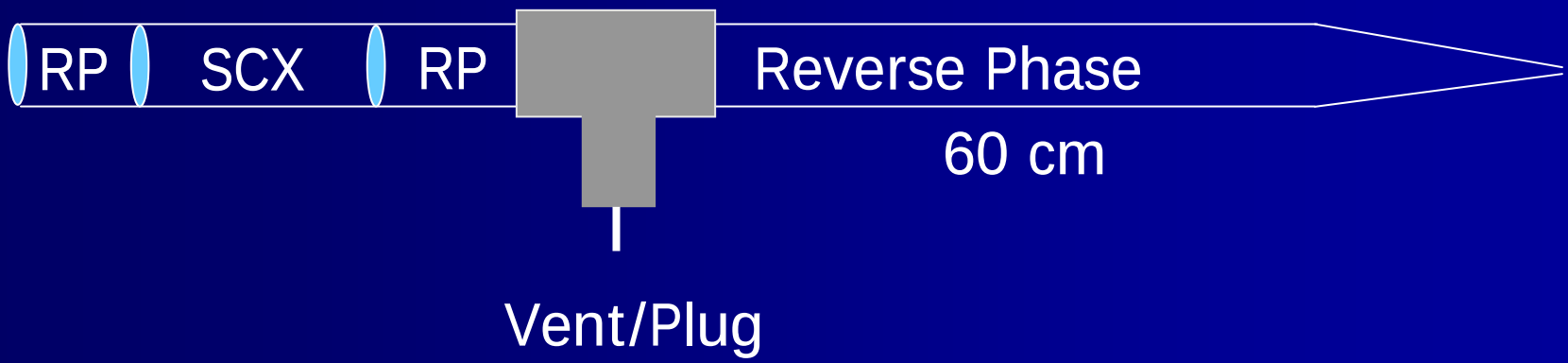
Tri-Phasic MudPIT

Vented Technology

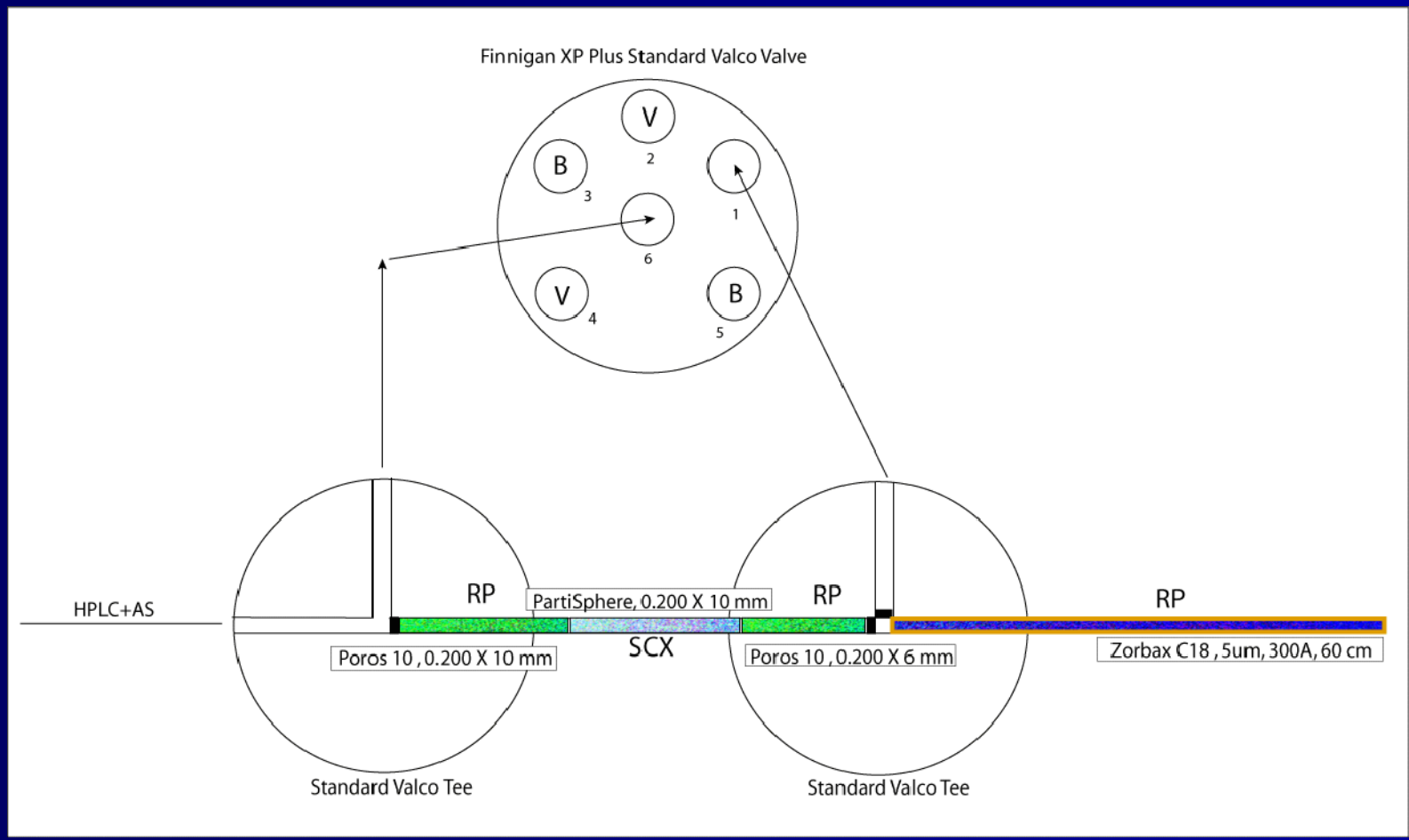
1. Comparison of three directly coupled HPLC MS/MS strategies for identification of proteins from complex mixtures: single-dimension LC-MS/MS, 2-phase MudPIT, and 3-phase MudPIT. W. Hayes McDonald, Ryoma Ohi, David T. Miyamoto, Timothy J. Mitchison, and John R. Yates, III International Journal of Mass Spectrometry, Volume 219, Issue 1 , 1 **August 2002**, Pages 245-251

2. Automation of Nanoscale Microcapillary Liquid Chromatography-Tandem Mass Spectrometry a Vented Column. Lawrence J. Licklider, Carson C. Thoreen, Junmin Peng and Steven P. Gygi* Anal. Chem. **2002**, 74,3076-3083

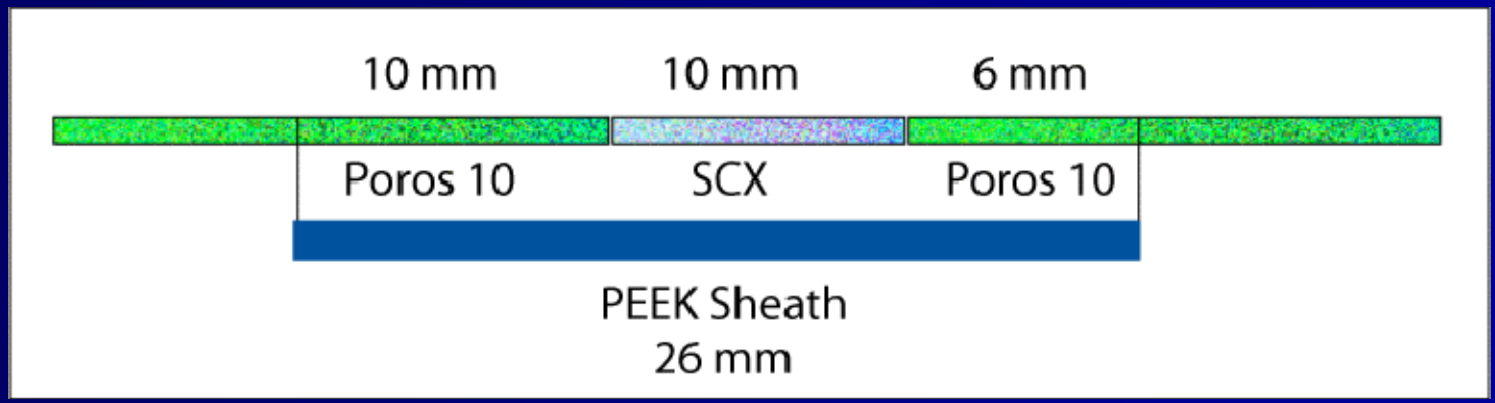
MudPIT Meets Vented Column Technology Meets The Long Column.



Building the Double Vented Tetra Phasic MudPIT Device



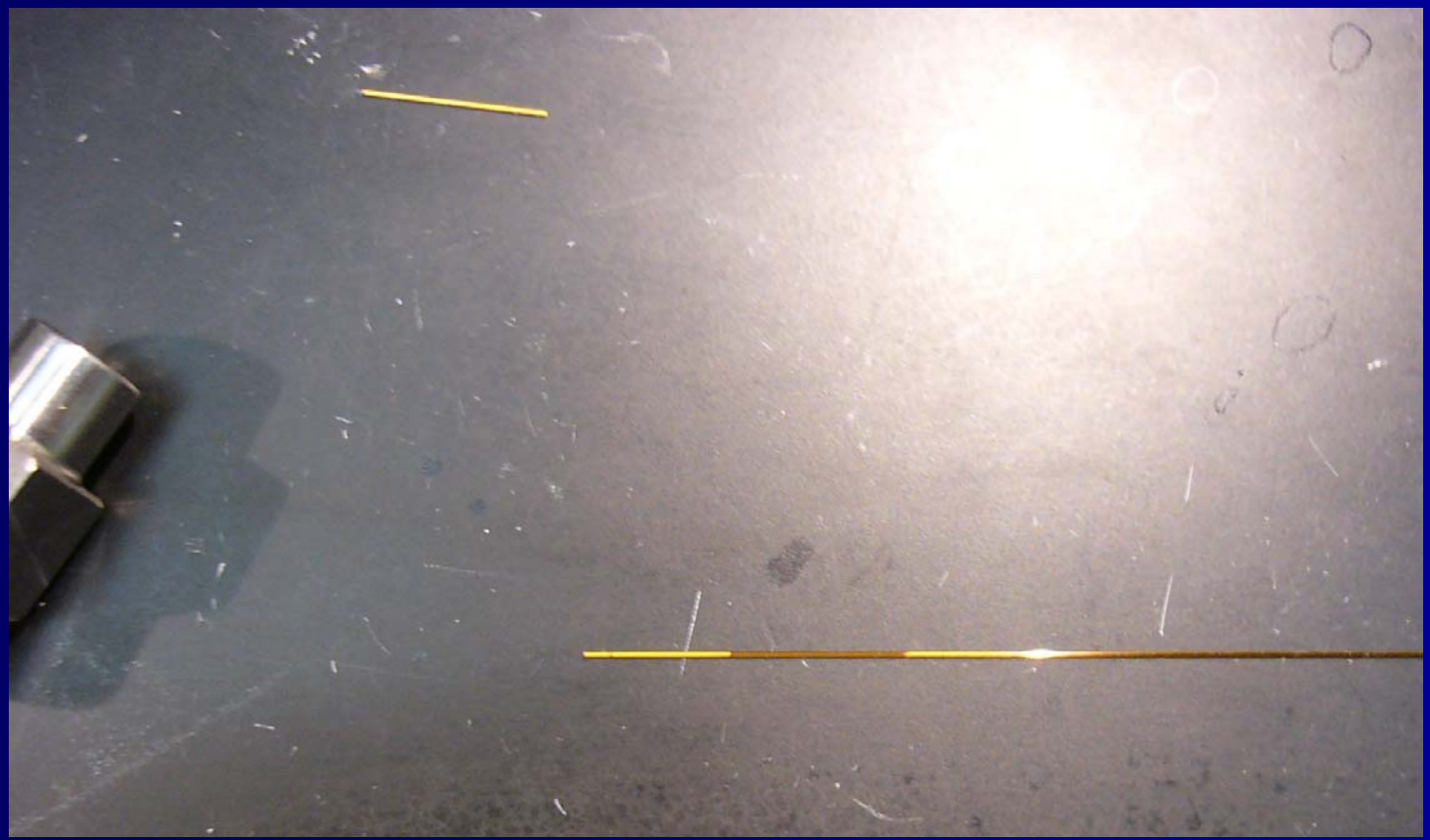
Building The Triphasic MudPITter



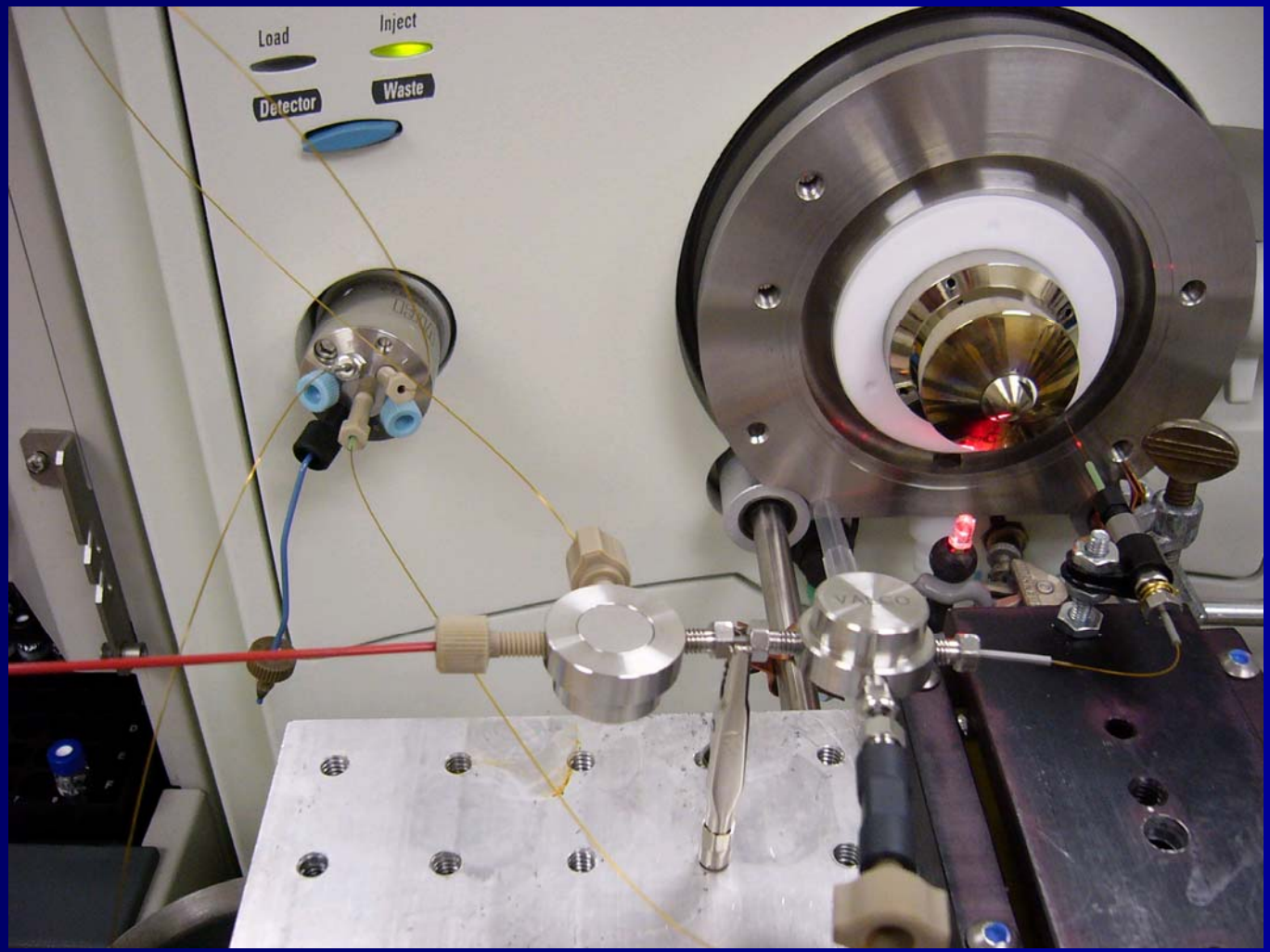
Method

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

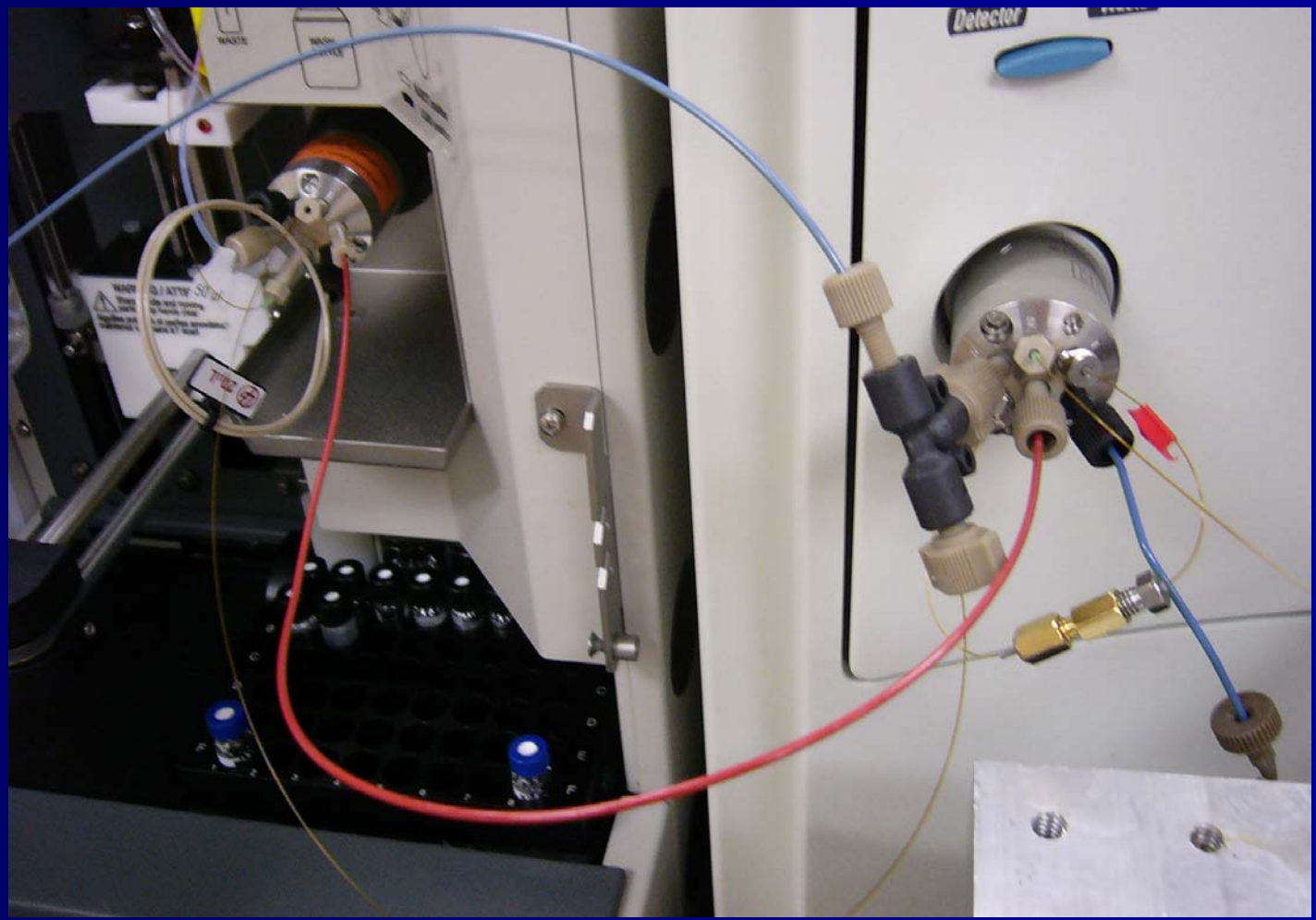


Continuous Vent



The Discontinuous Vent

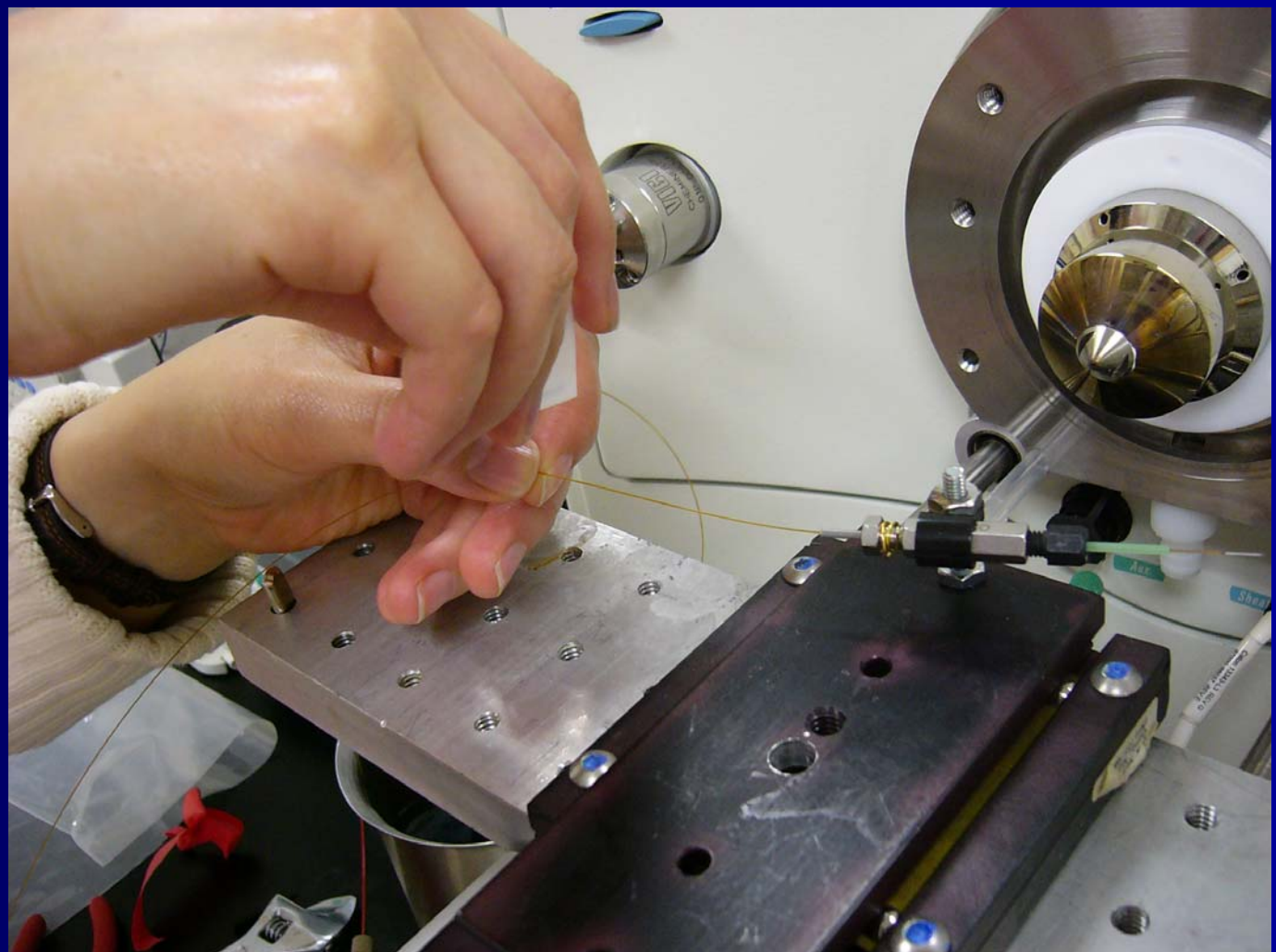
Tri-Phasic On The Valve



The Matrix

10 cm DCV (trap on the valve)	60 cm DCV (trap on the valve)
10 cm CV	60 cm CV

Making the Cut



MudPIT Gradients

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

Load
Wash
ACN bump

Gradient 17

Initial conditions
30ul/min 0%B

T	%B
1	5
4	5
10	80
12	80
12.1	5
20	Stop

Purpose load sample onto first RP and wash and elute onto SCX

Salt &
Separate

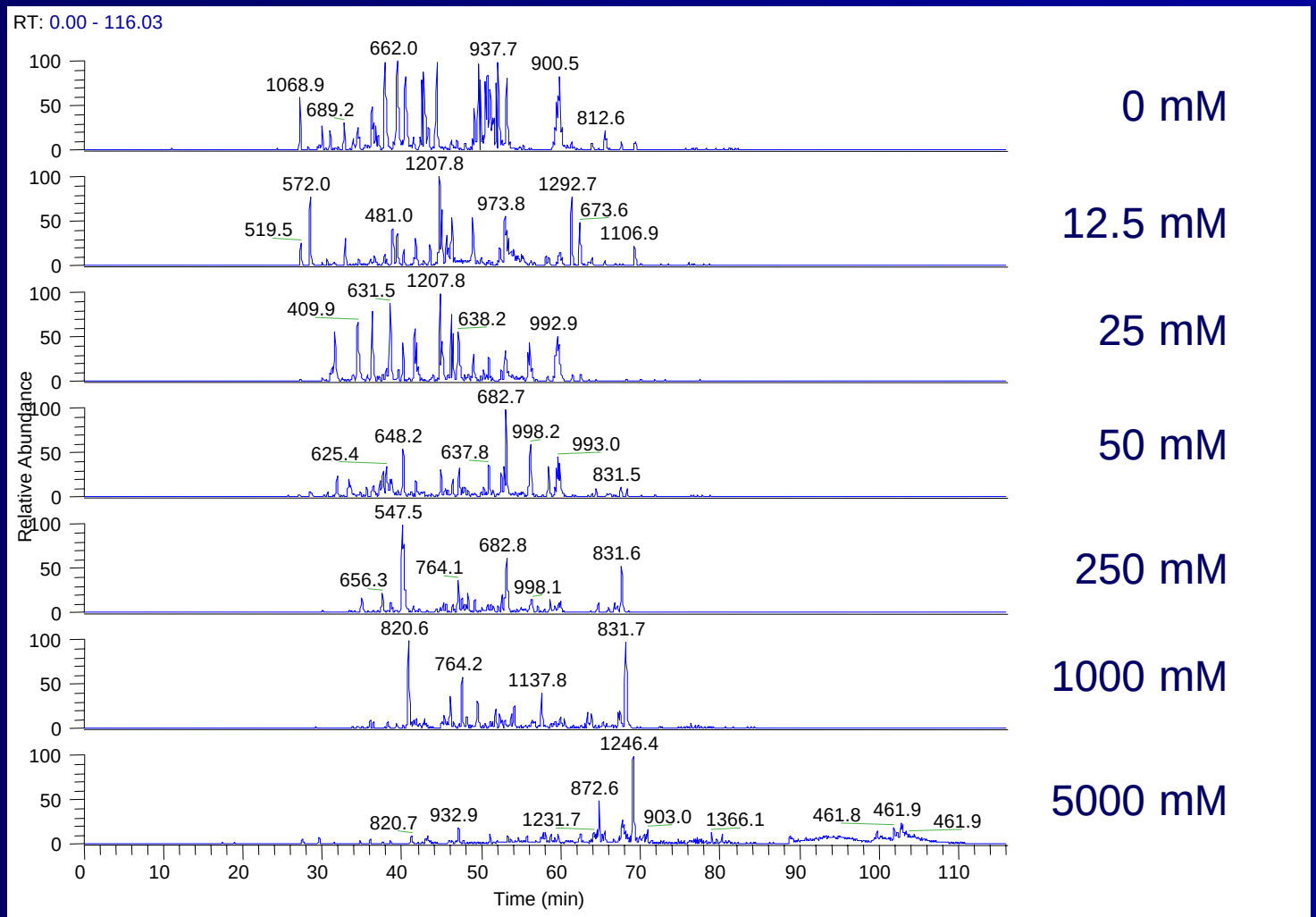
Gradient 18

Initial conditions
30ul/min 0%B

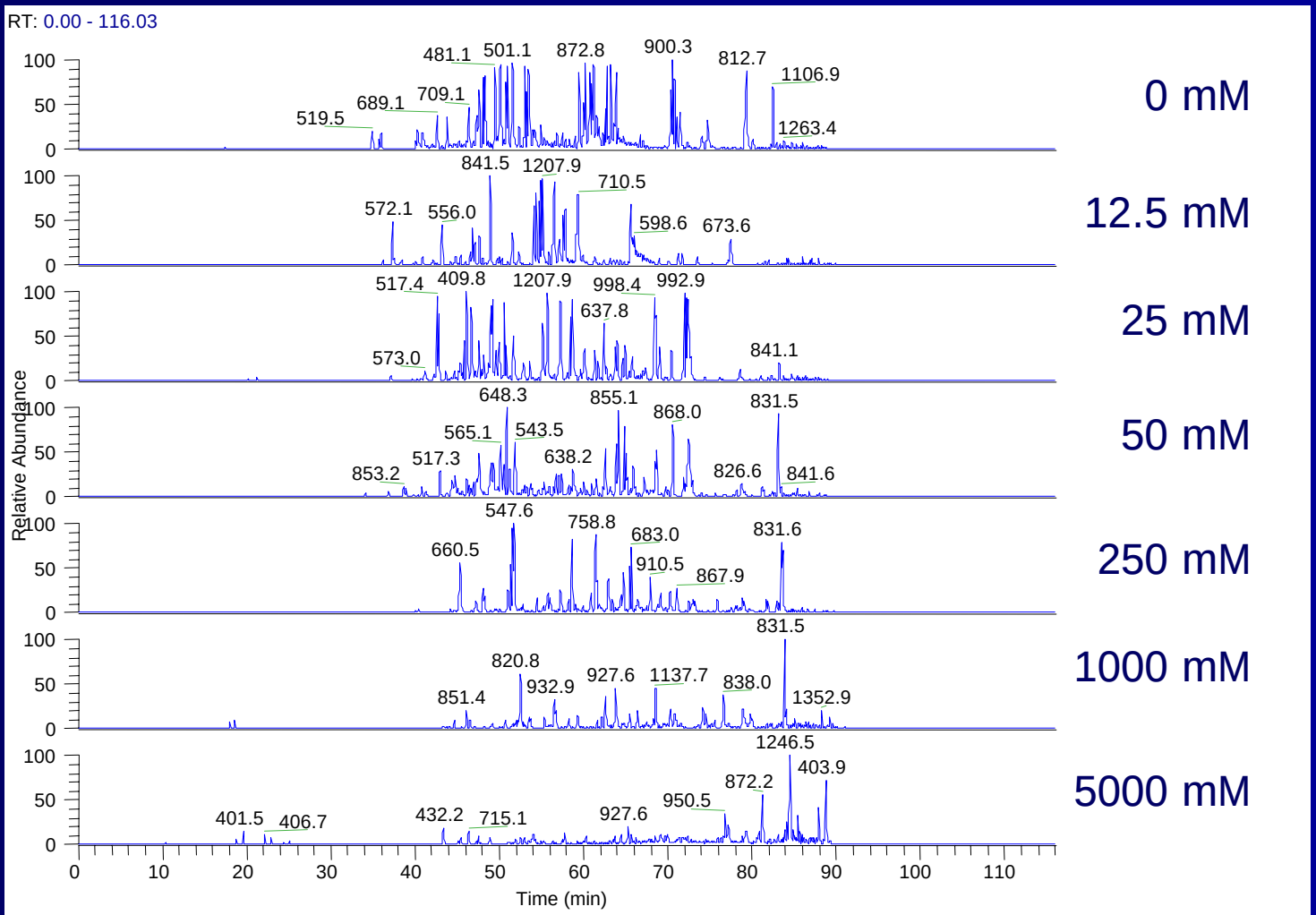
T	%B
1	5
10	5
70	40
80	80
80.1	5
120	Stop

Purpose: Salt bump. Separate peptides on the analytical column.

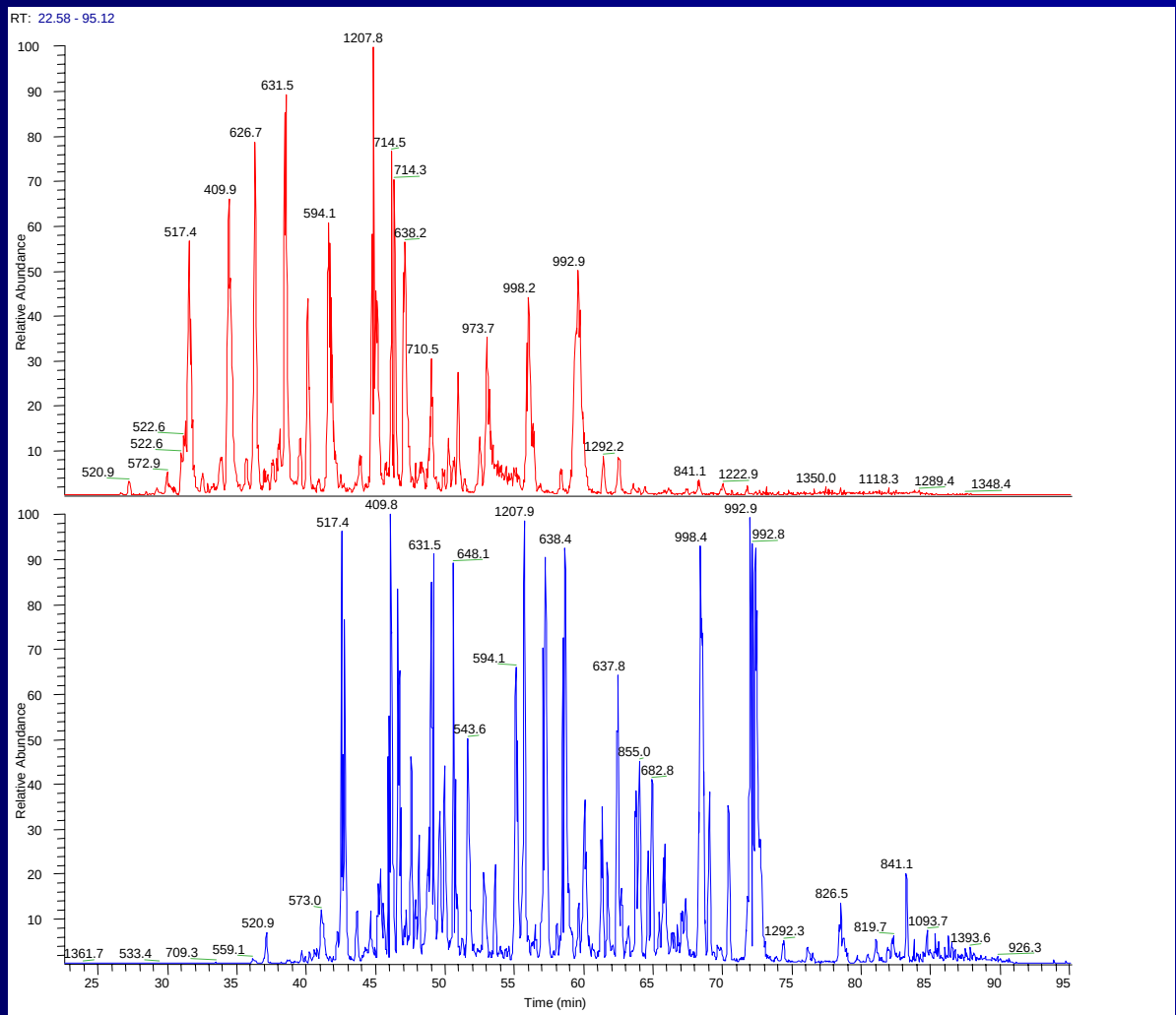
10 cm Continuous Vent Human Serum MudPIT



60 cm Continuous Vent Human Serum MudPIT



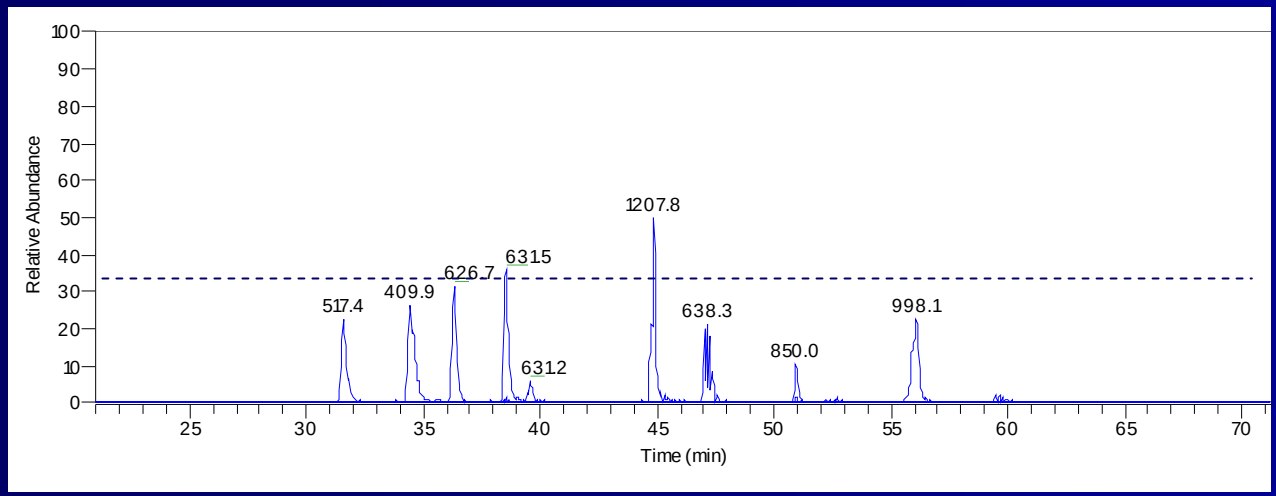
Comparing 10 and 60 cm Columns at the 25 mM Salt Step Using the Continuous Vent Method



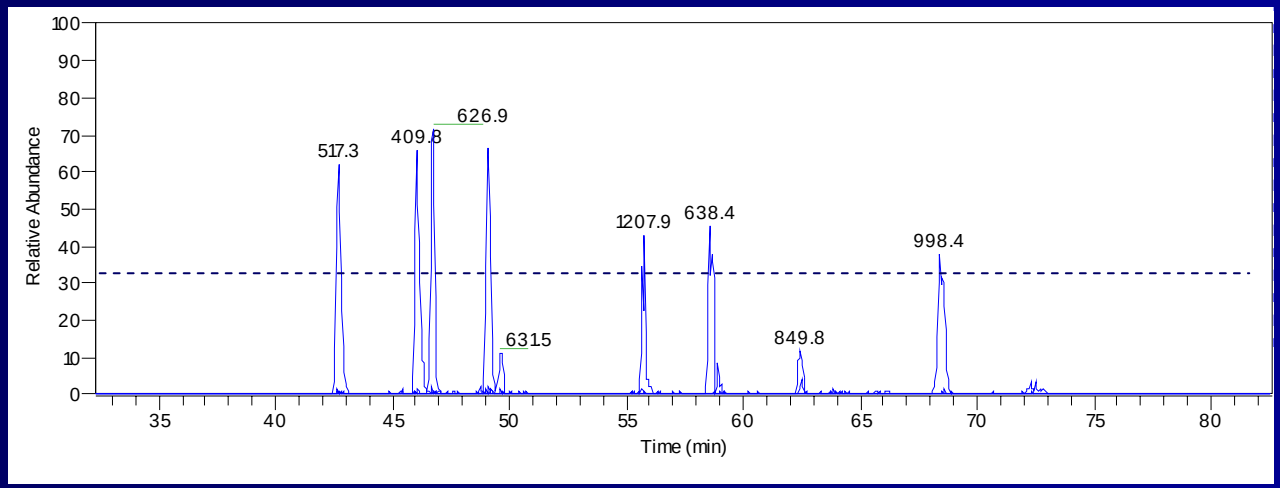
10 CM

60 CM

XIC Plots For 10 and 60 Columns At The 25 mM Salt Step With The Continuous Vent Method



10 cm



60 cm

CV vs. DCV

Short vs. Long

	10 cm DCV	60 cm DCV	% Gain
Peptide Count	537	635	18.3
	10 cm CV	60 cm CV	
Peptide Count	593	739	24.6
% Gain	10.4	16.4	37.6

CV vs. DCV

Short vs. Long

Peptide Count Category (protein with n peptides)	10 cm DCV	60 cm DCV	% Gain
6+	447	498	11.4
1-5	90	137	52.2
	10 cm CV	60 cm CV	
6+	486	589	21.2
1-5	107	150	40.2

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

10 cm Segregation

60 cm Segregation