

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

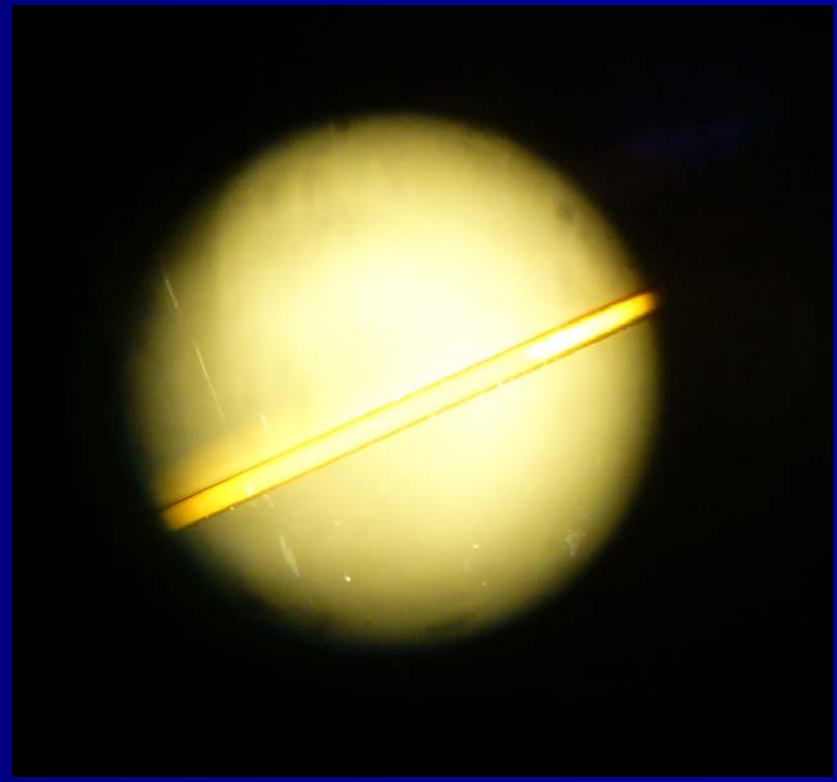
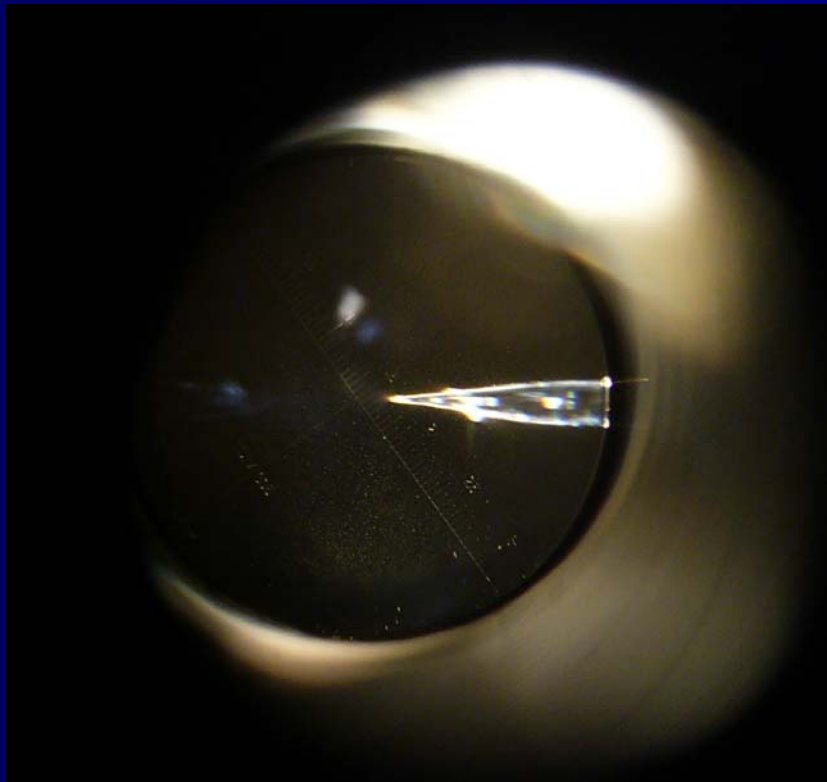
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Mass Spectrometry Laboratory  
Department of Chemistry  
Stanford University  
Stanford California

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

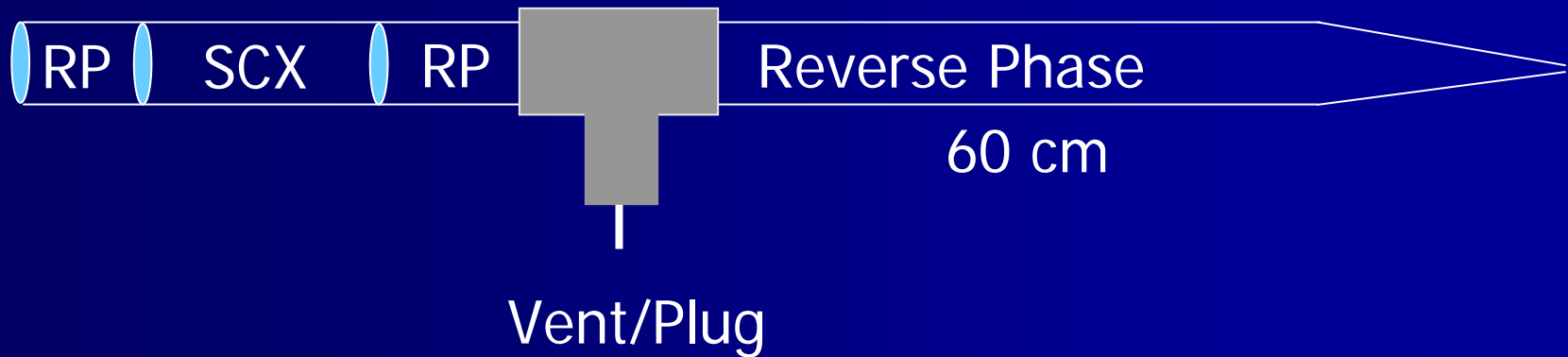


### Vented Technology

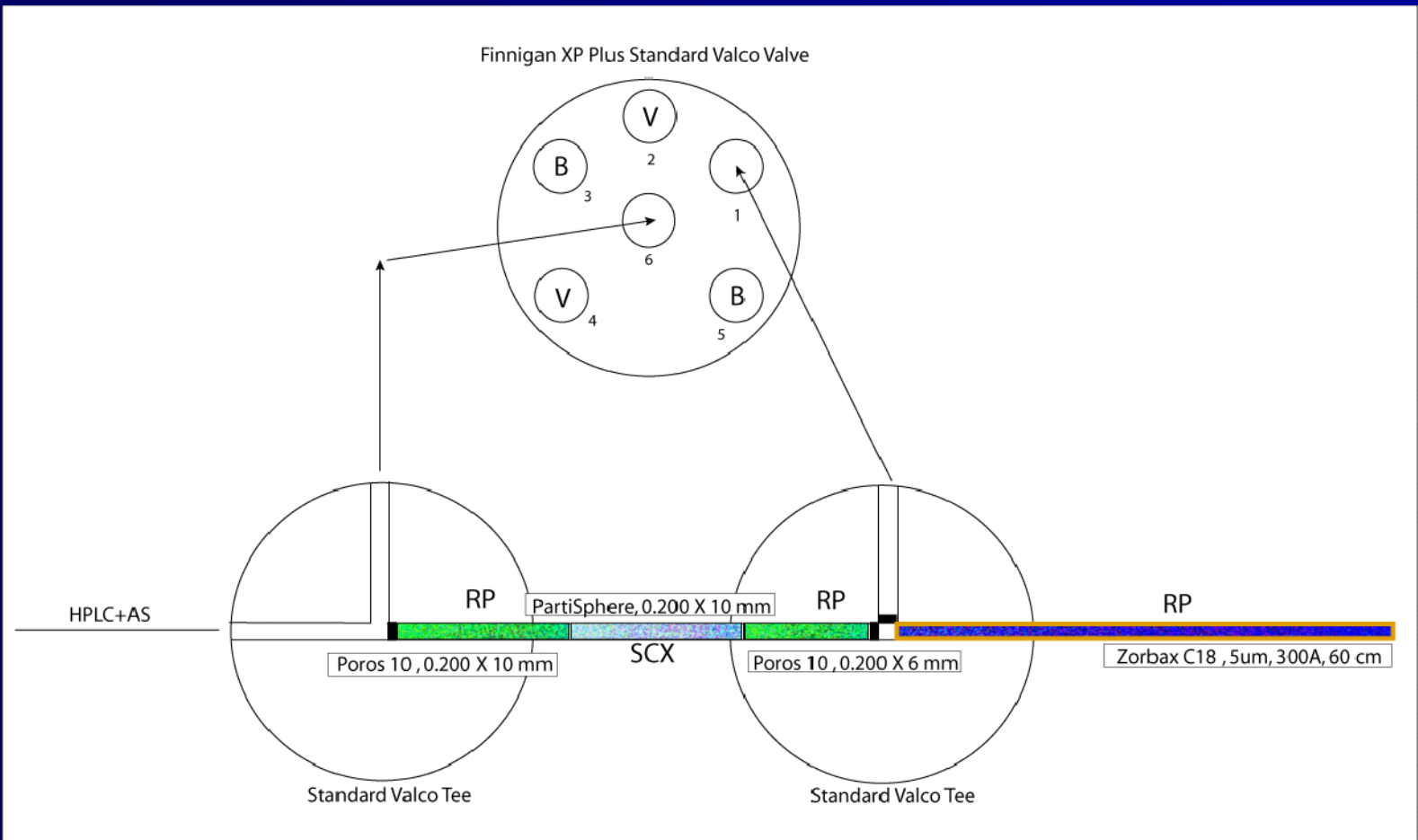


1. Comparison of three directly coupled HPLC MS/MS strategies for identification of proteins from complex mixtures: single-dimension LC-MS/MS, 2-phase MudPIT, and 3-phase MudPIT. W. Hayes McDonald, Ryoma Ohi, David T. Miyamoto, Timothy J. Mitchison, and John R. Yates, III *International Journal of Mass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

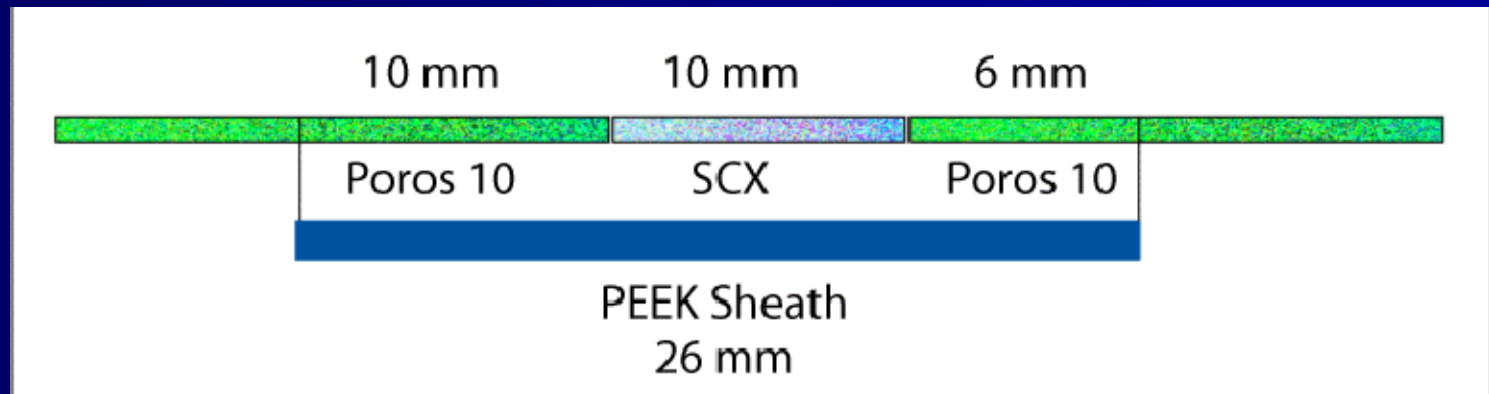


# Building the Double Vented Tetra Phasic MudPIT Device



# Building The Triphasic MudPITter

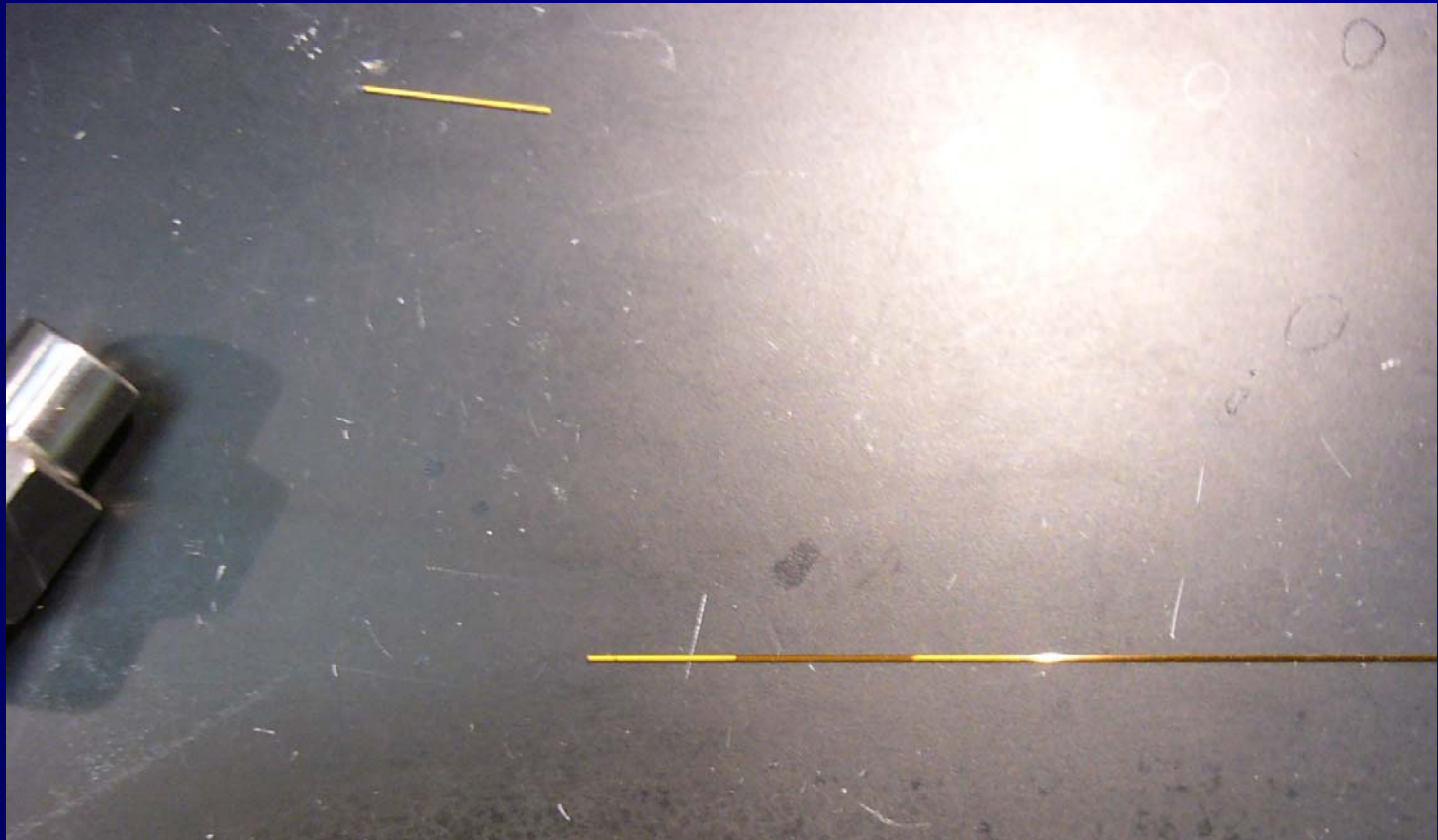
1 cm = 7 cm



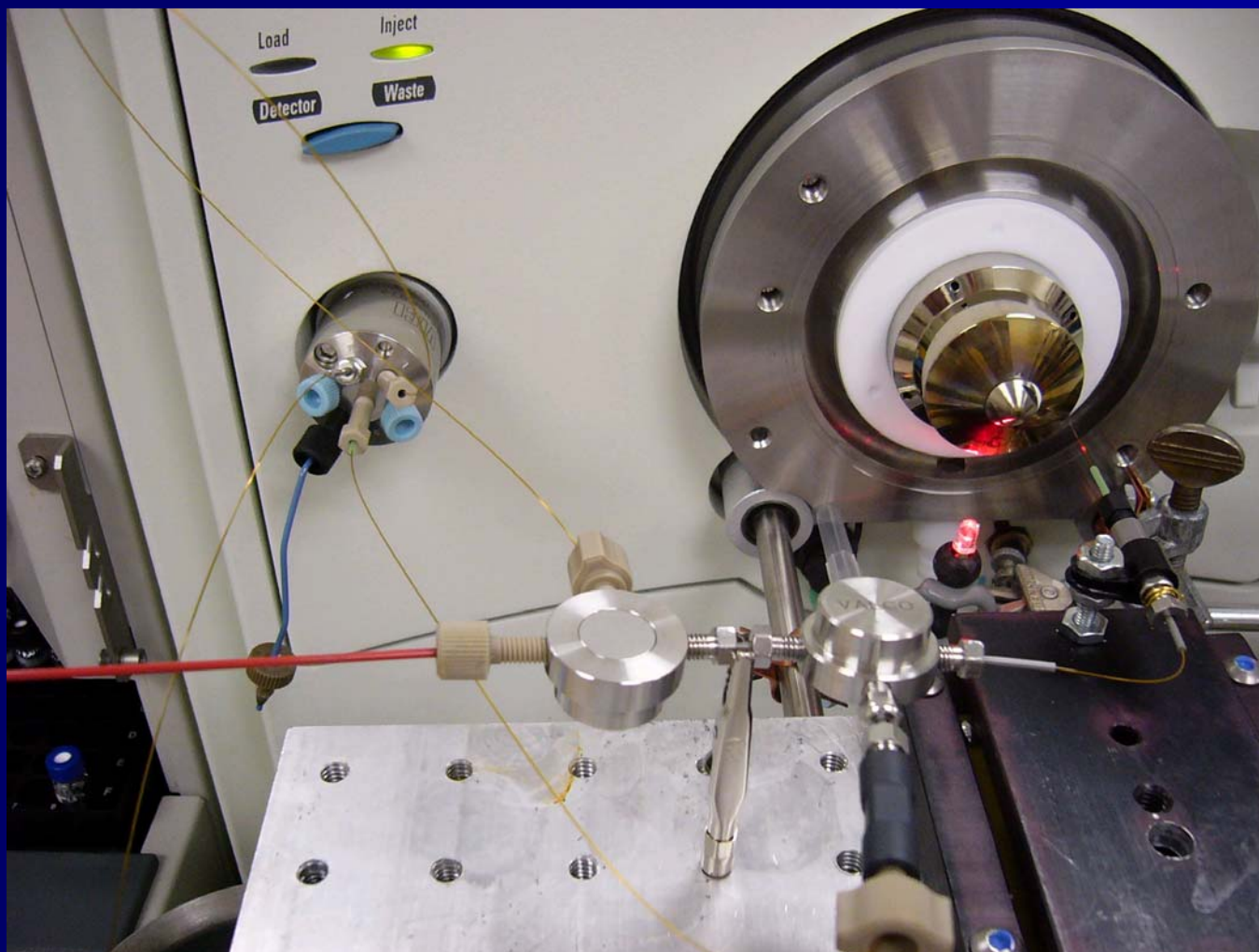
## Method

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



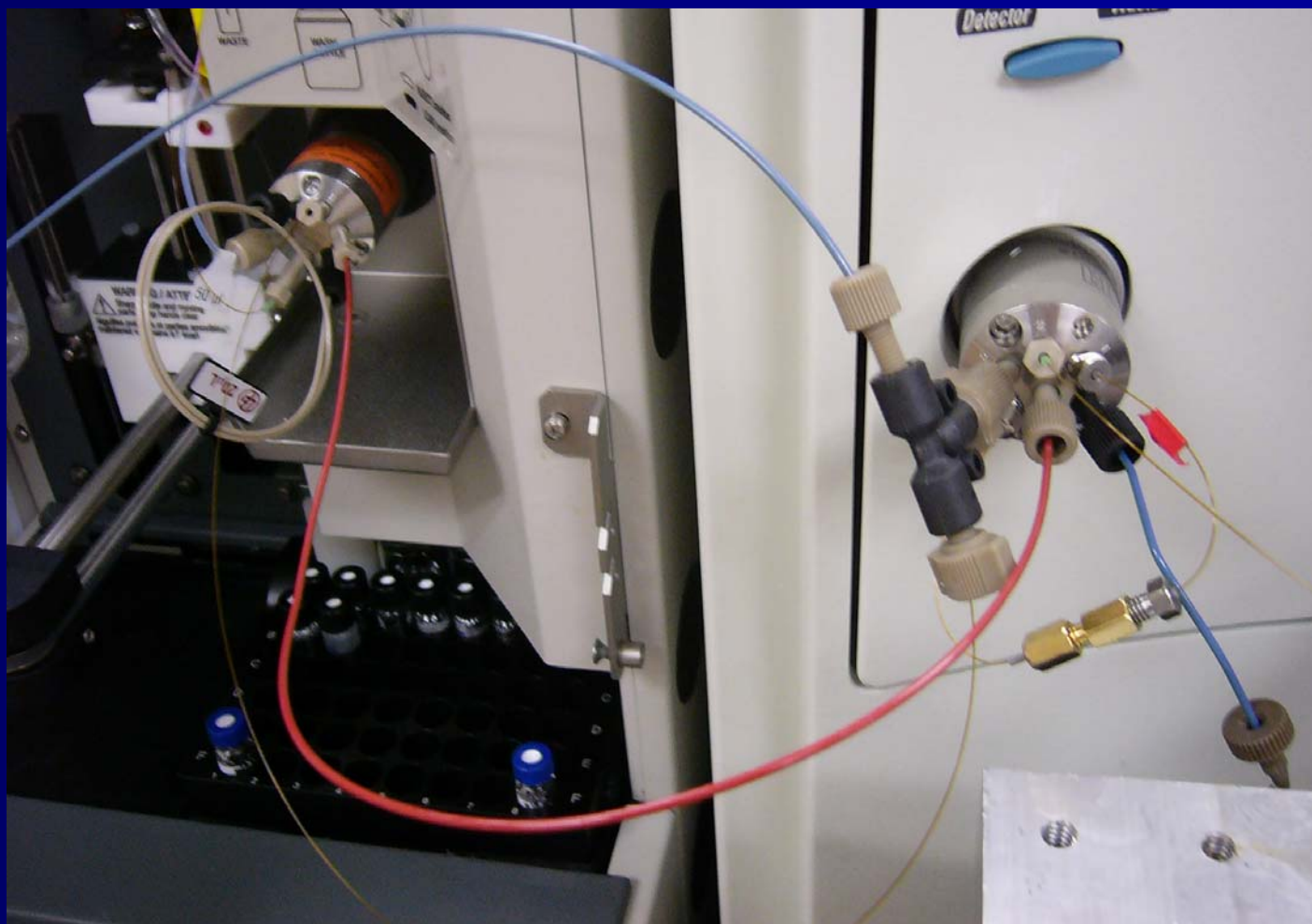
# Continuous Vent





# The Discontinuous Vent

Tri-Phasic On The Valve



# MudPIT Gradients

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

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

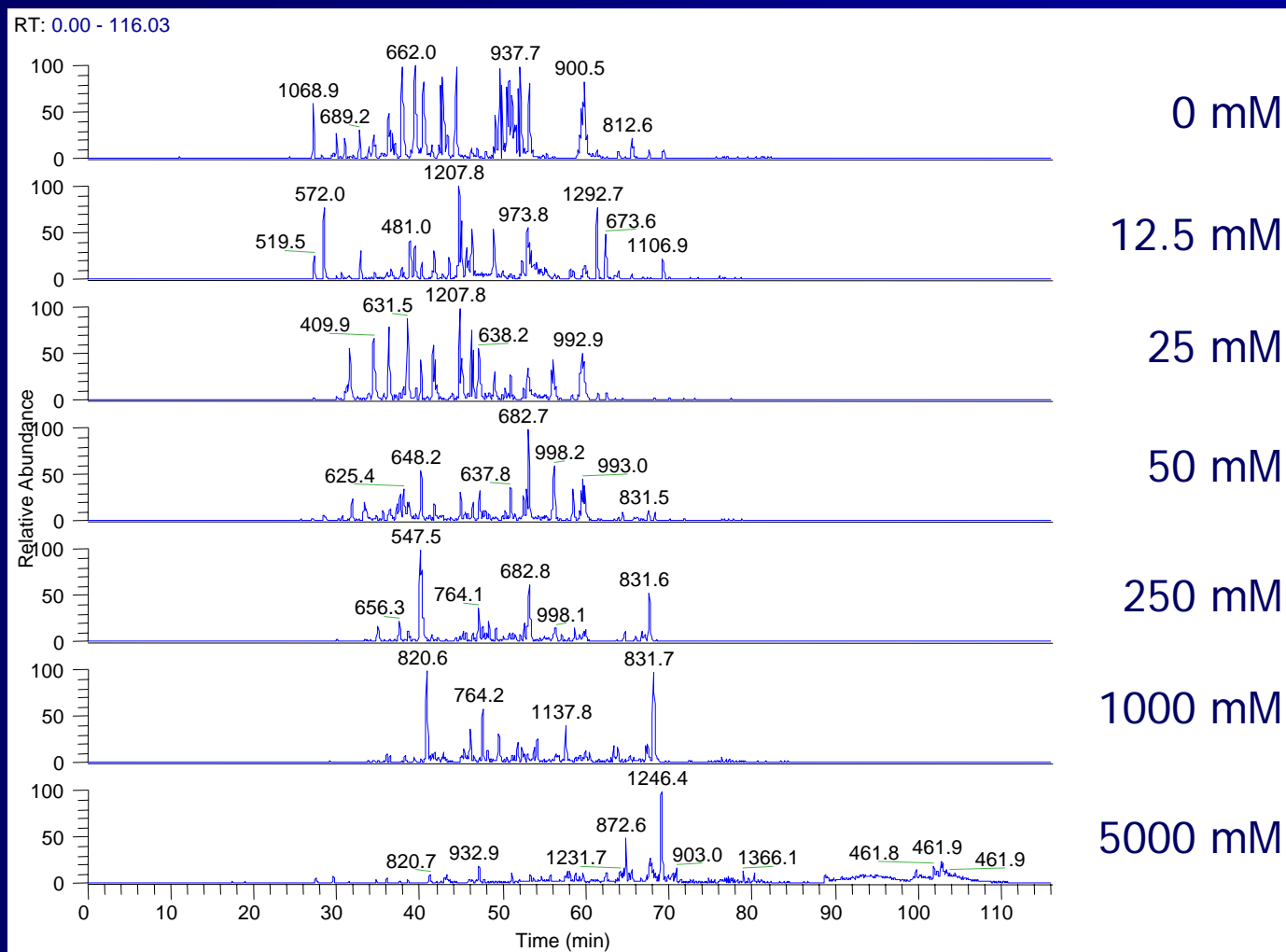
Gradient 18

Initial conditions  
30ul/min 0%B

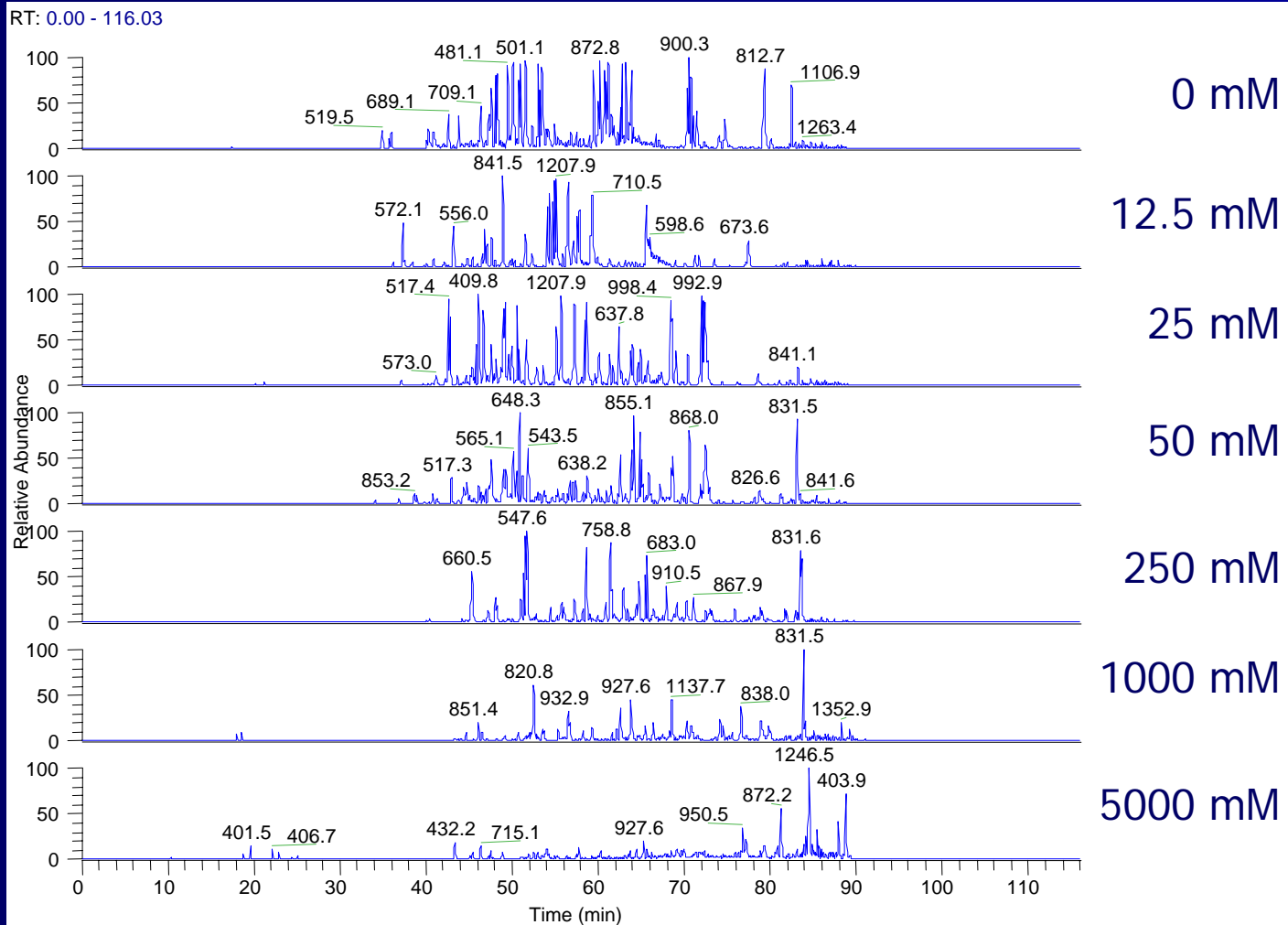
T	%B
1	5
10	5
70	40
80	80
80.1	5
120	Stop

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

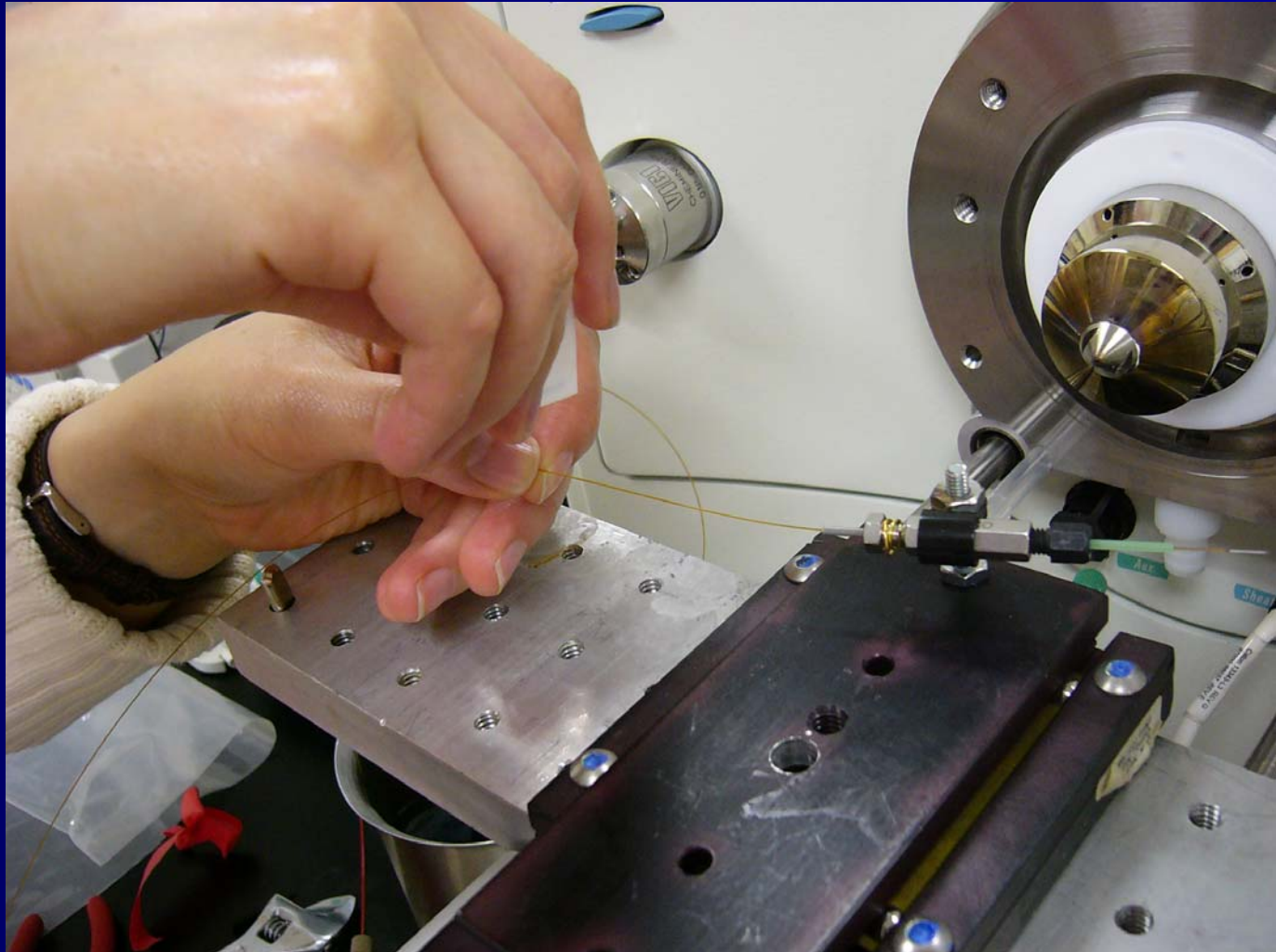
# 10 cm Continuous Vent Human Serum MudPIT



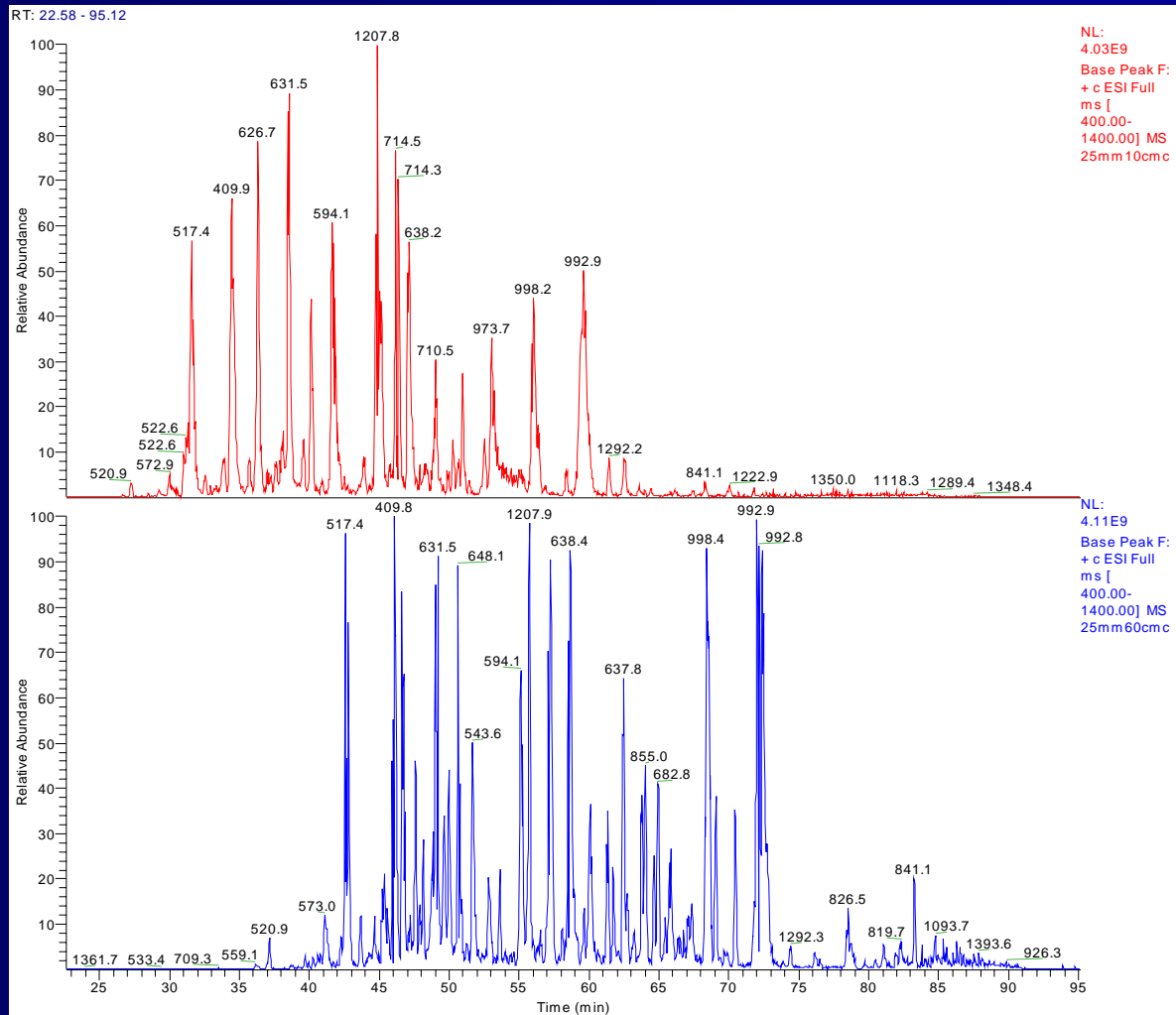
# 60 cm Continuous Vent Human Serum MudPIT



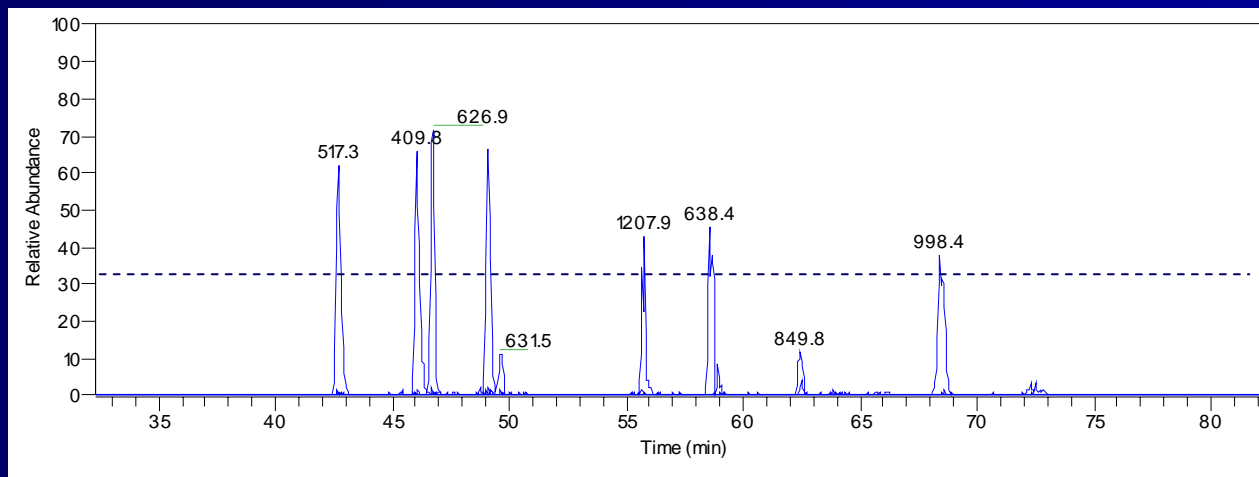
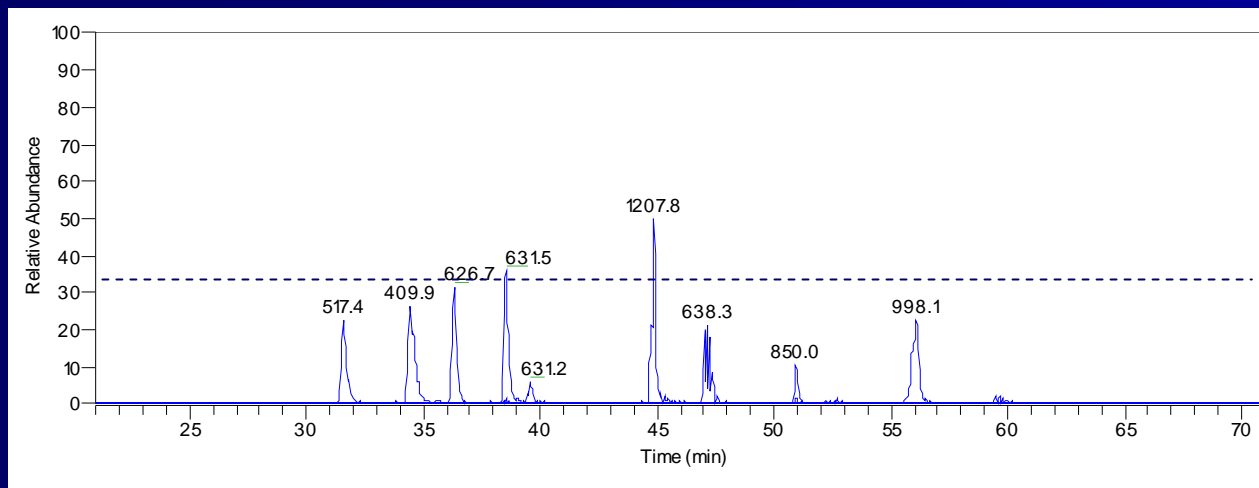
# Making the Cut



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



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



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

## Formatting Options

Format As	Select Summary (protein hits) ▾		<a href="#">Help</a>
	Significance threshold p < 0.05	Max. number of hits AUTO	
	Standard scoring <input type="radio"/> MudPIT scoring <input checked="" type="radio"/>	Ions score cut-off 20	Show sub-sets <input type="checkbox"/>
	Show pop-ups <input type="radio"/> Suppress pop-ups <input checked="" type="radio"/>	Sort unassigned Decreasing Score ▾	Require bold red <input checked="" type="checkbox"/>



# 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	

# 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

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- Mike MacCoss, University of Washington
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- ThermoFinnigan