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

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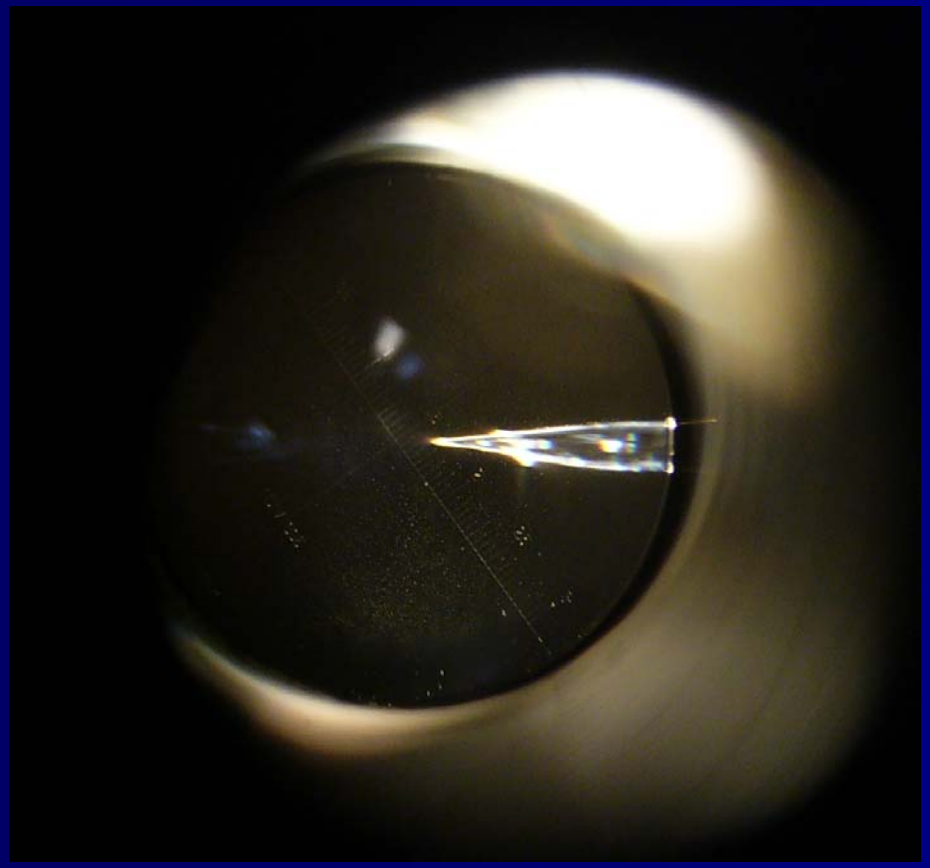
Last year…. the long column and proteomics

What if?

Space



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

Available Column Technologies

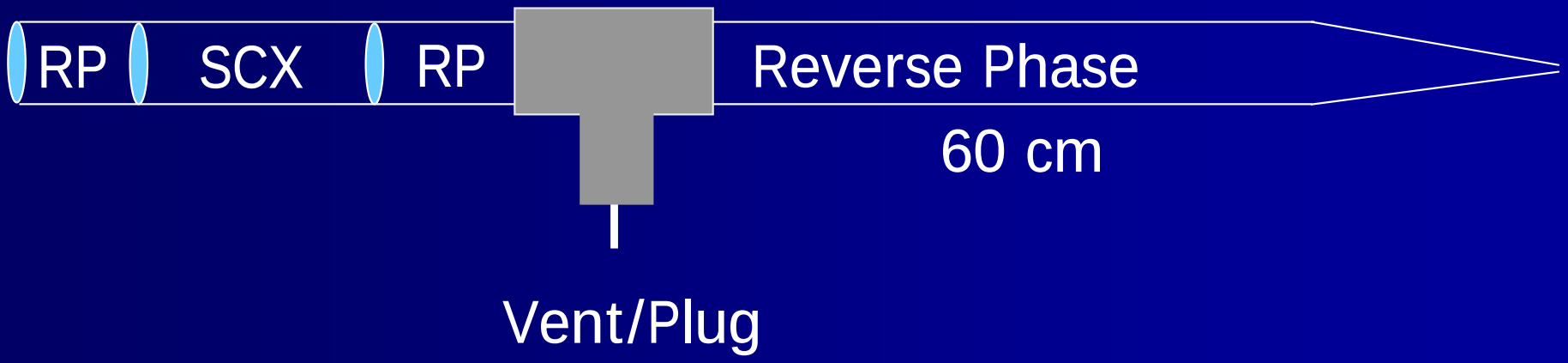
Tri-Phasic MudPIT

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. *Int J. Mass Spectrom.* **2002,** *219* (1), 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.

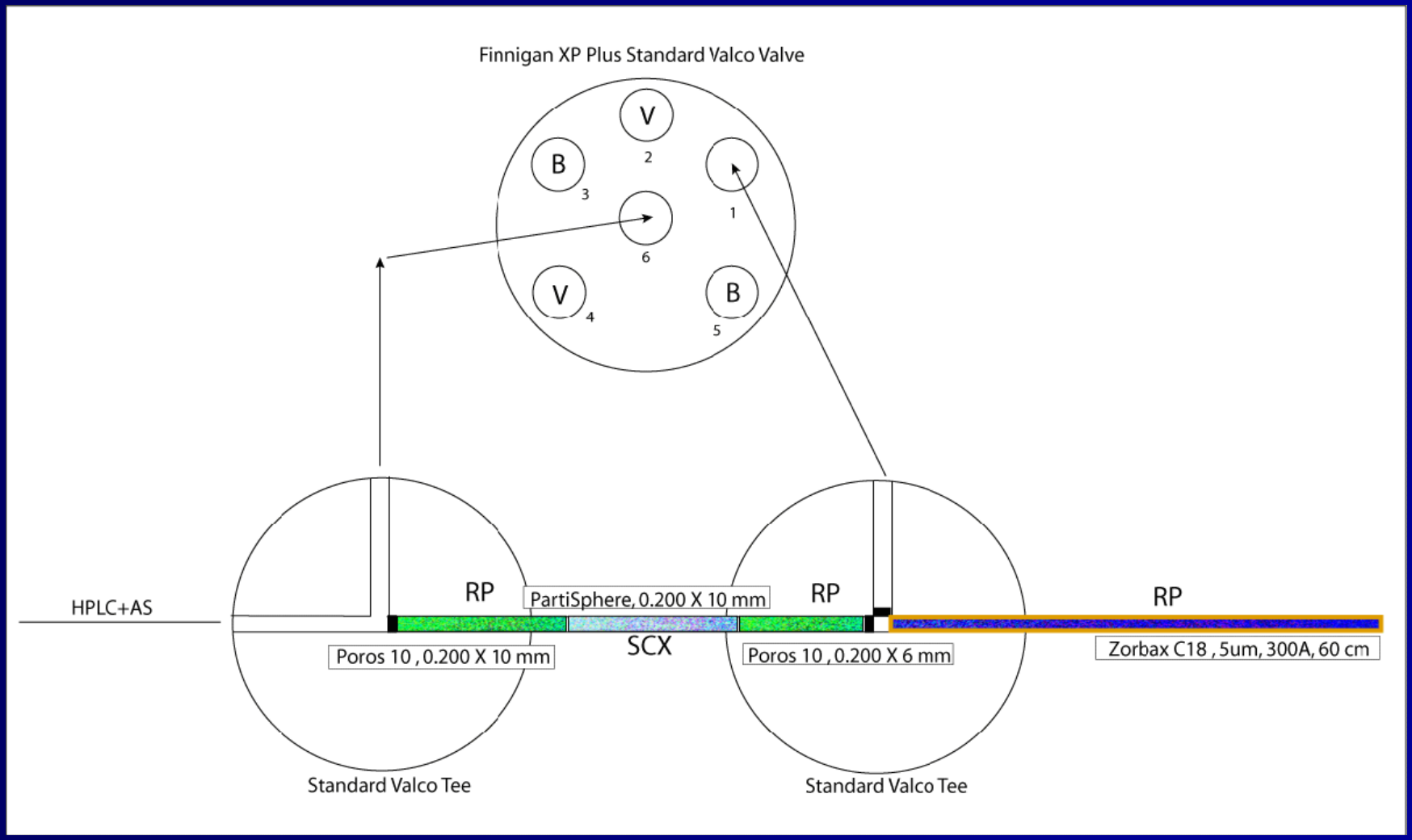
MudPIT Meets Vented Column Technology Meets the Long Column



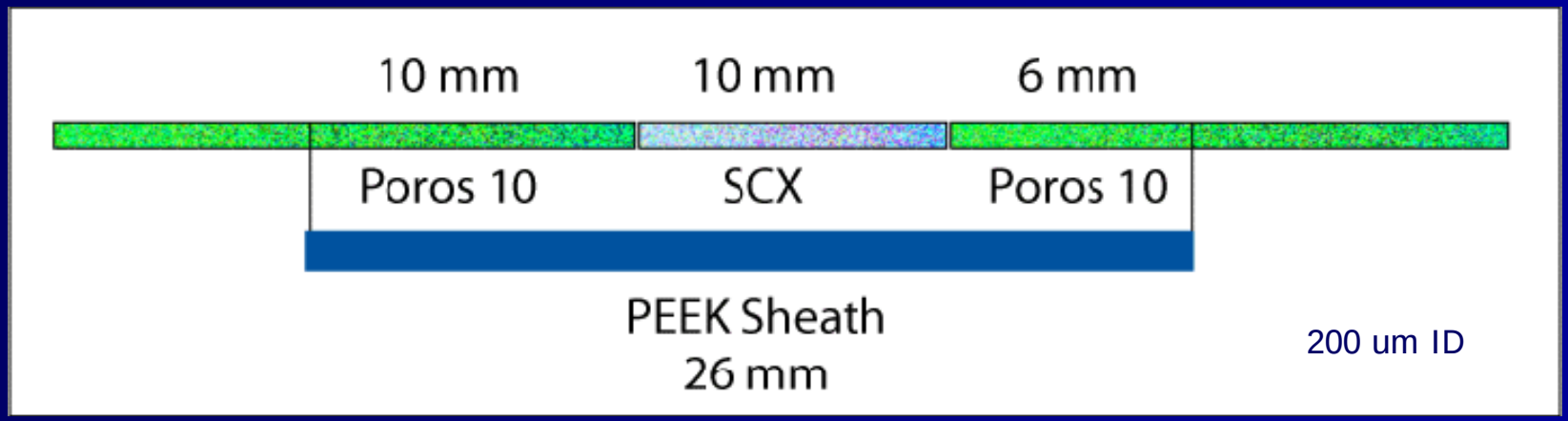
HPLC: Standard Flow Shimadzu 10ADVP with homemade splitter
MS: XP Plus Ion Trap mass spectrometer

Guzzetta AW, Chien AS. Related Article: A double-vented tetraphasic continuous column approach to MudPIT analysis on long capillary columns demonstrates superior proteomic coverage.
J. Proteome Res. **2005** Nov-Dec, *4* (6), 2412-9.

Building the Double Vented Tetra Phasic MudPIT Device



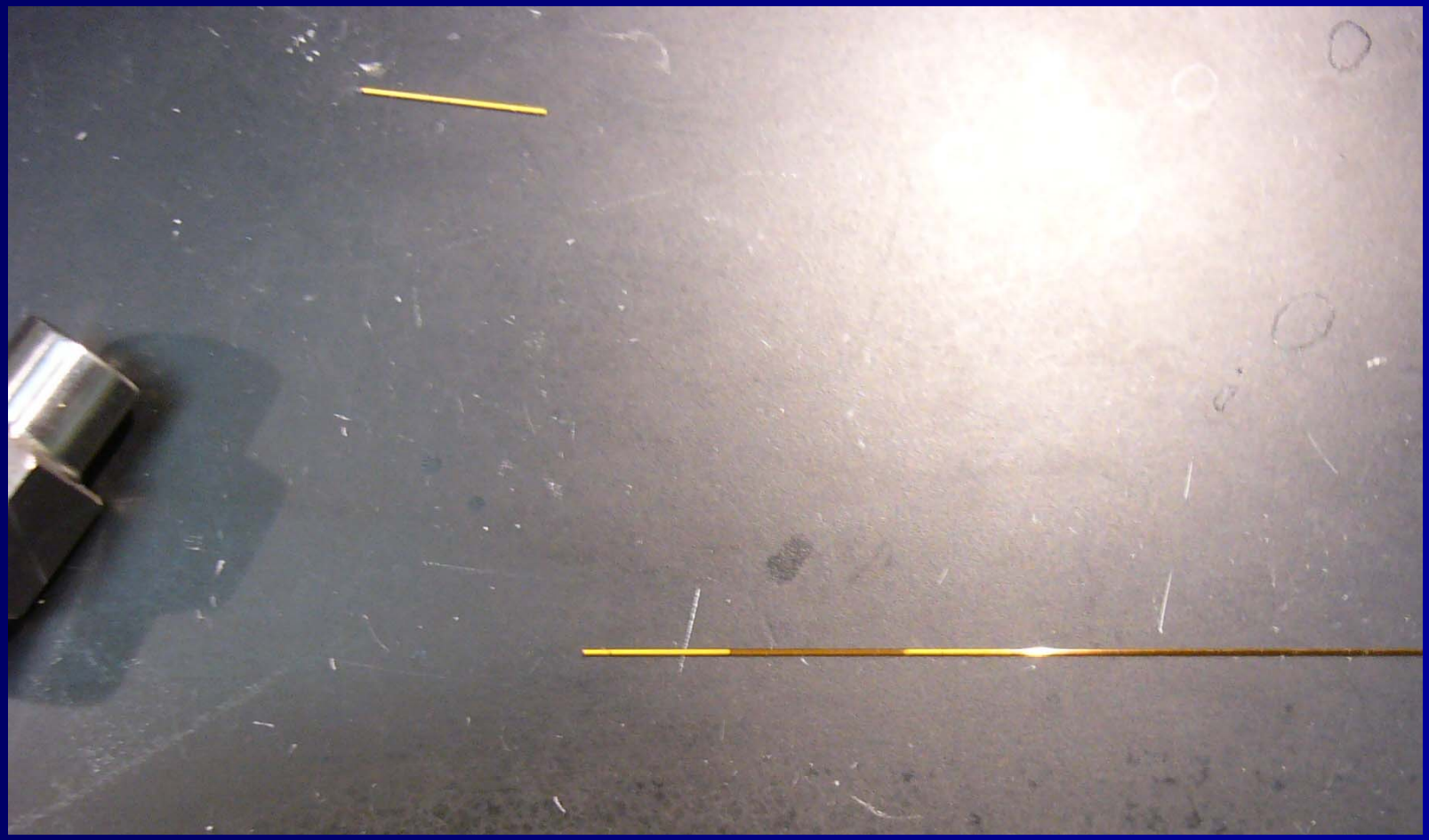
Building the Triphasic MudPITter



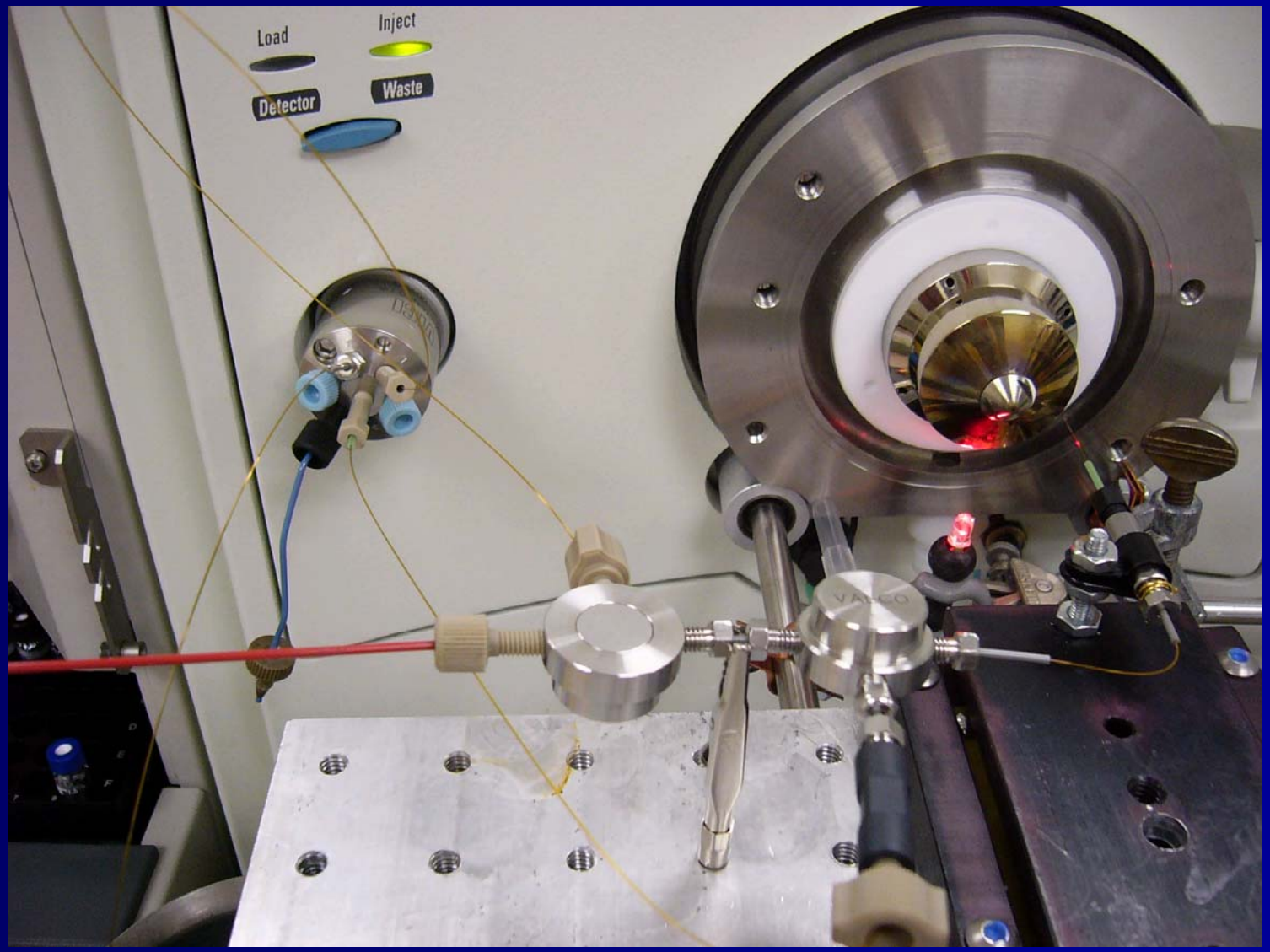
Method

1. Place screen frit in the tee
2. Swage with short piece of PEEK
3. Pack Poros 10
4. Wash
5. Pack SCX (Whatman Partisphere, 5μm)
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 MudPIT Column

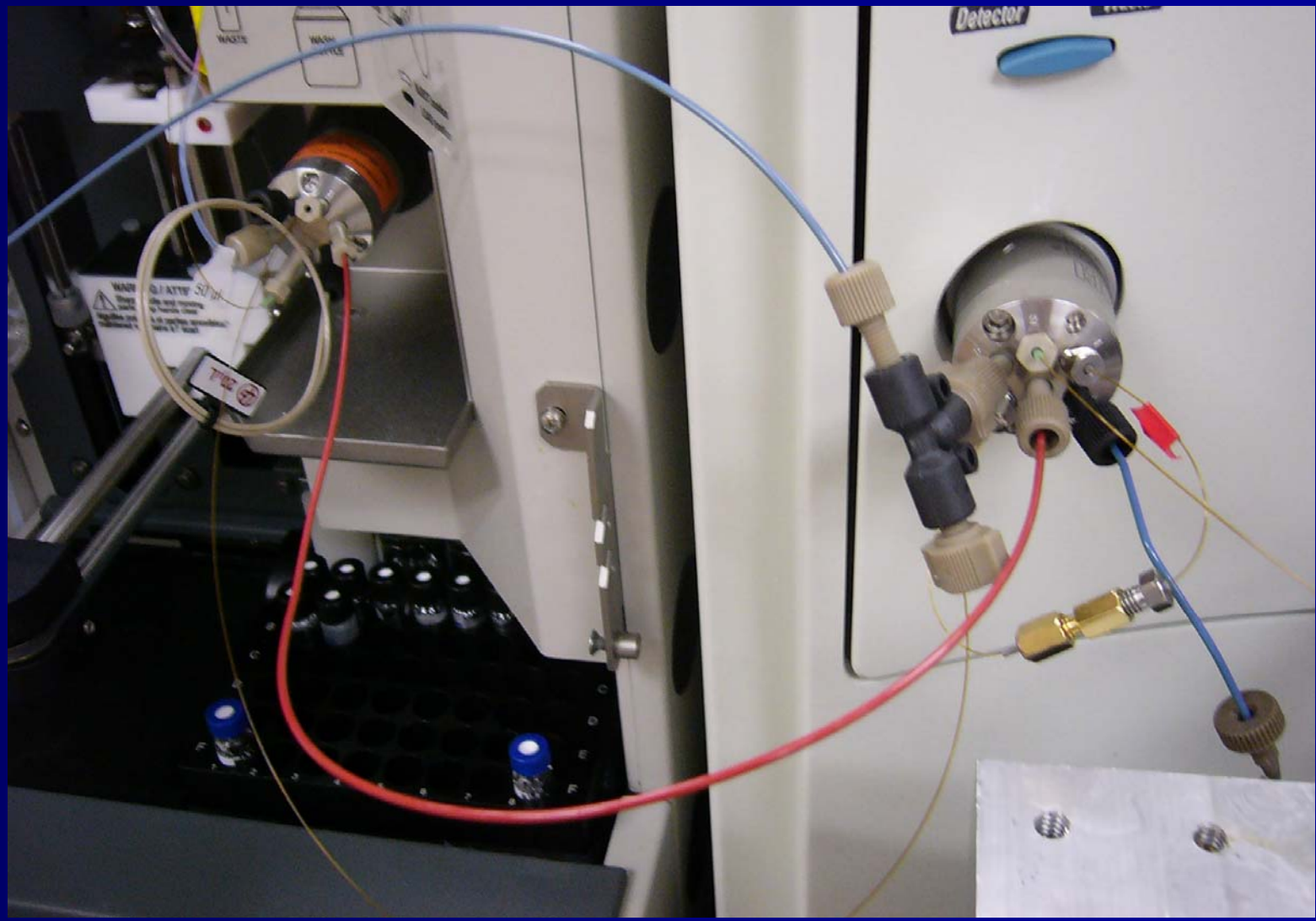


Continuous Vent



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
30µl/min 0%B

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

Purpose: Load sample onto first RP. Wash and elute onto SCX.

Salt &
Separate

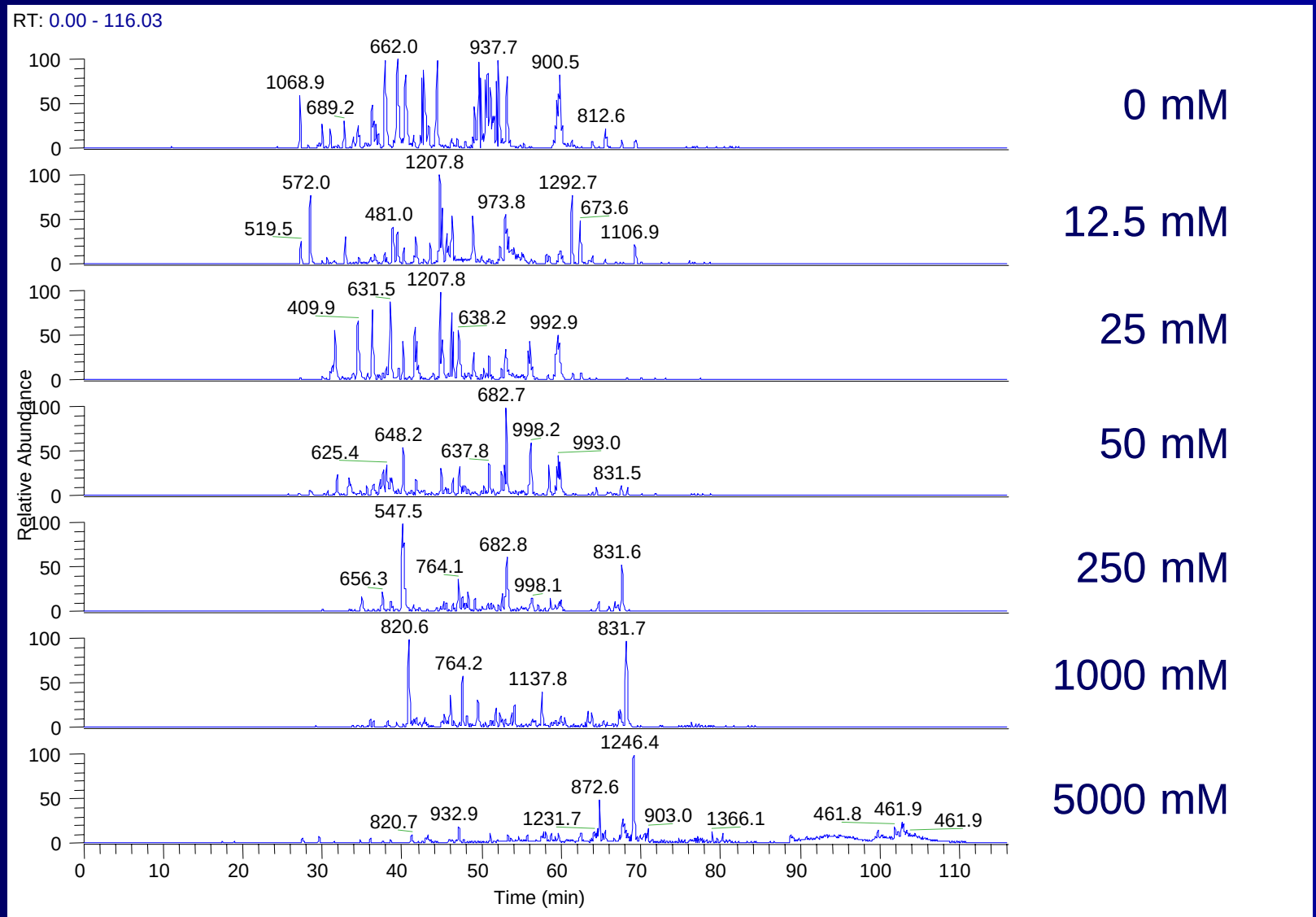
Gradient 18

Initial conditions
30µl/min 0%B

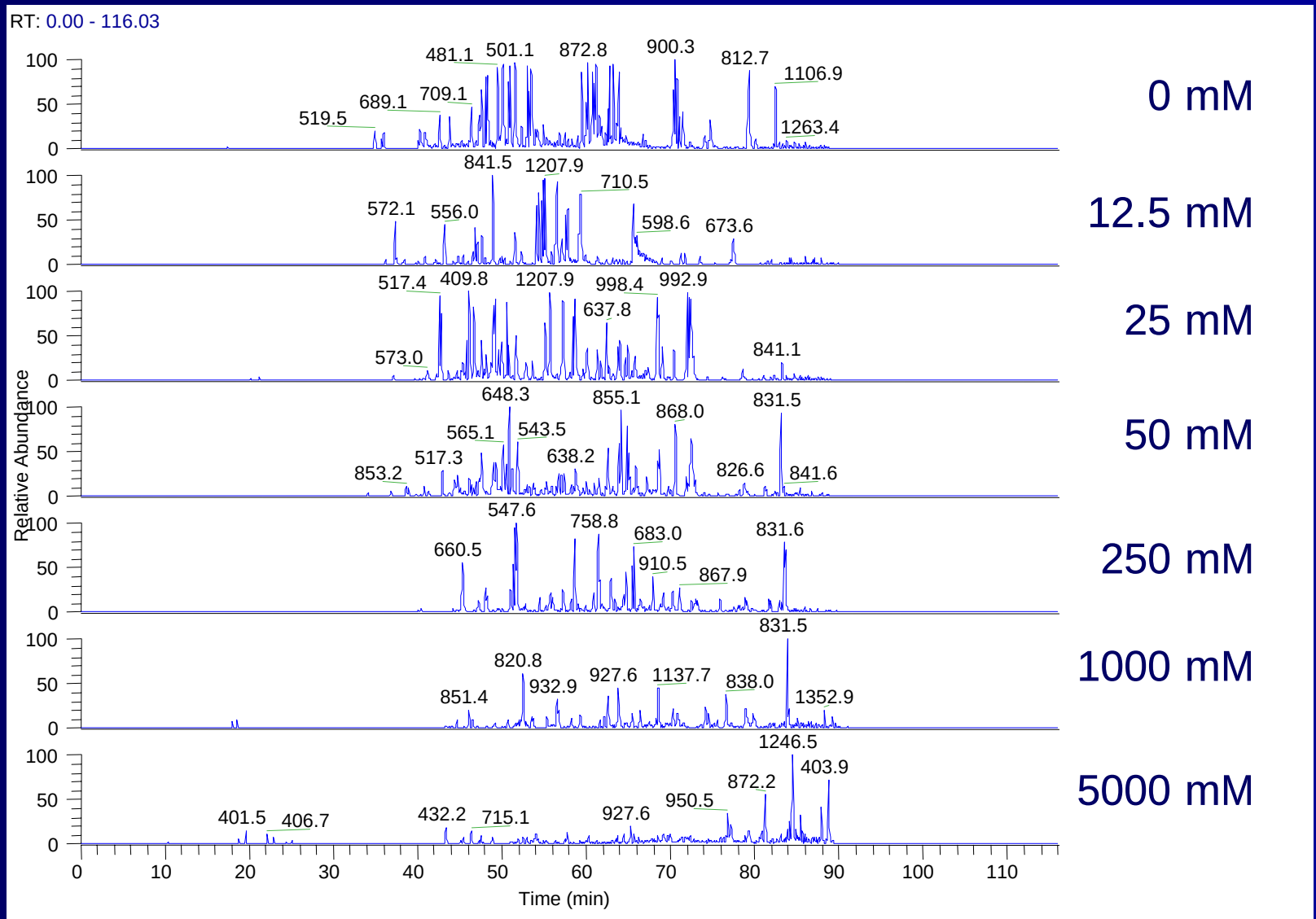
T	%B
1	5
10	5
70	40
80	80
80.1	5
120	Stop

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

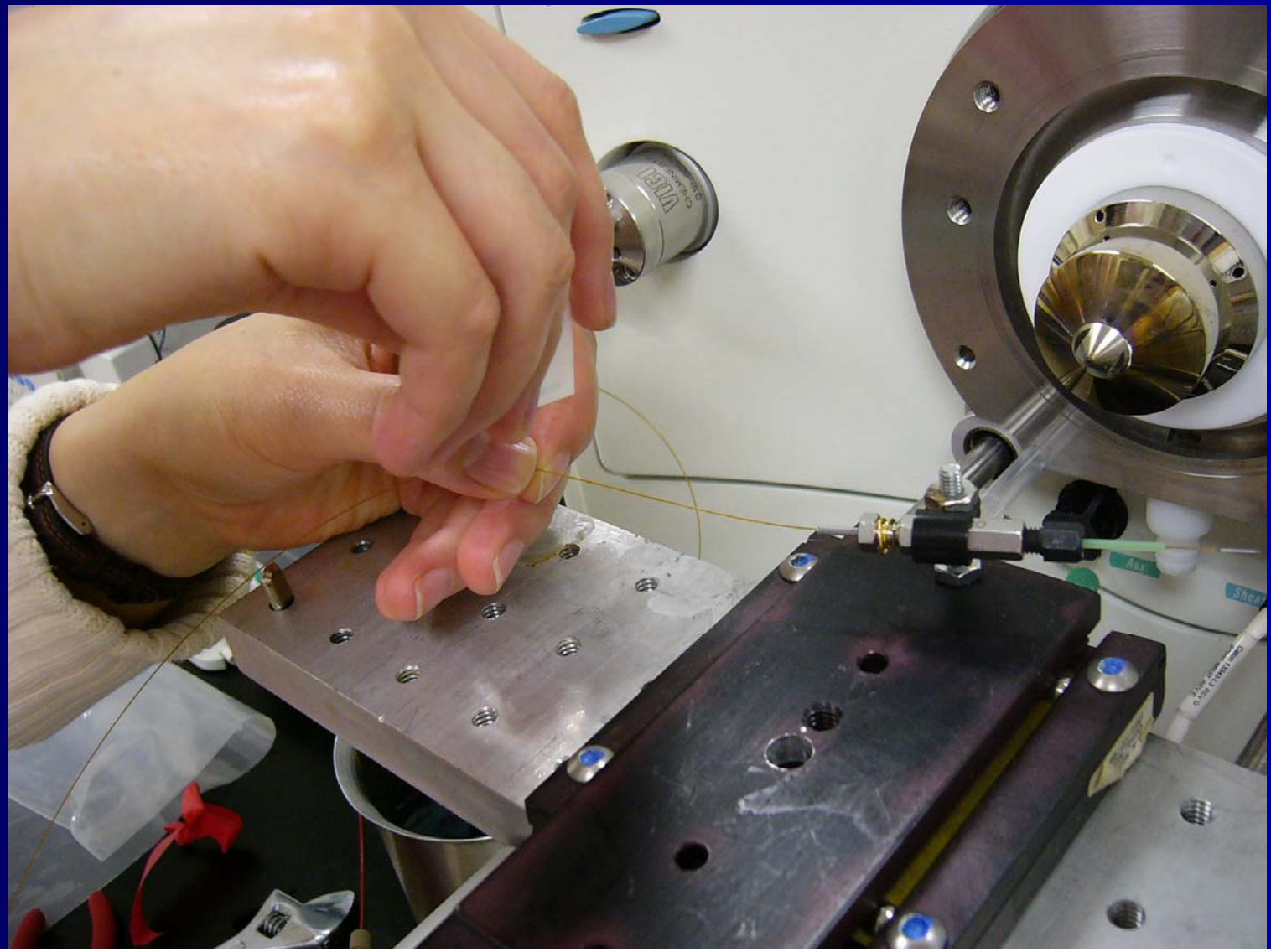
10 cm Continuous Vent Human Serum MudPIT



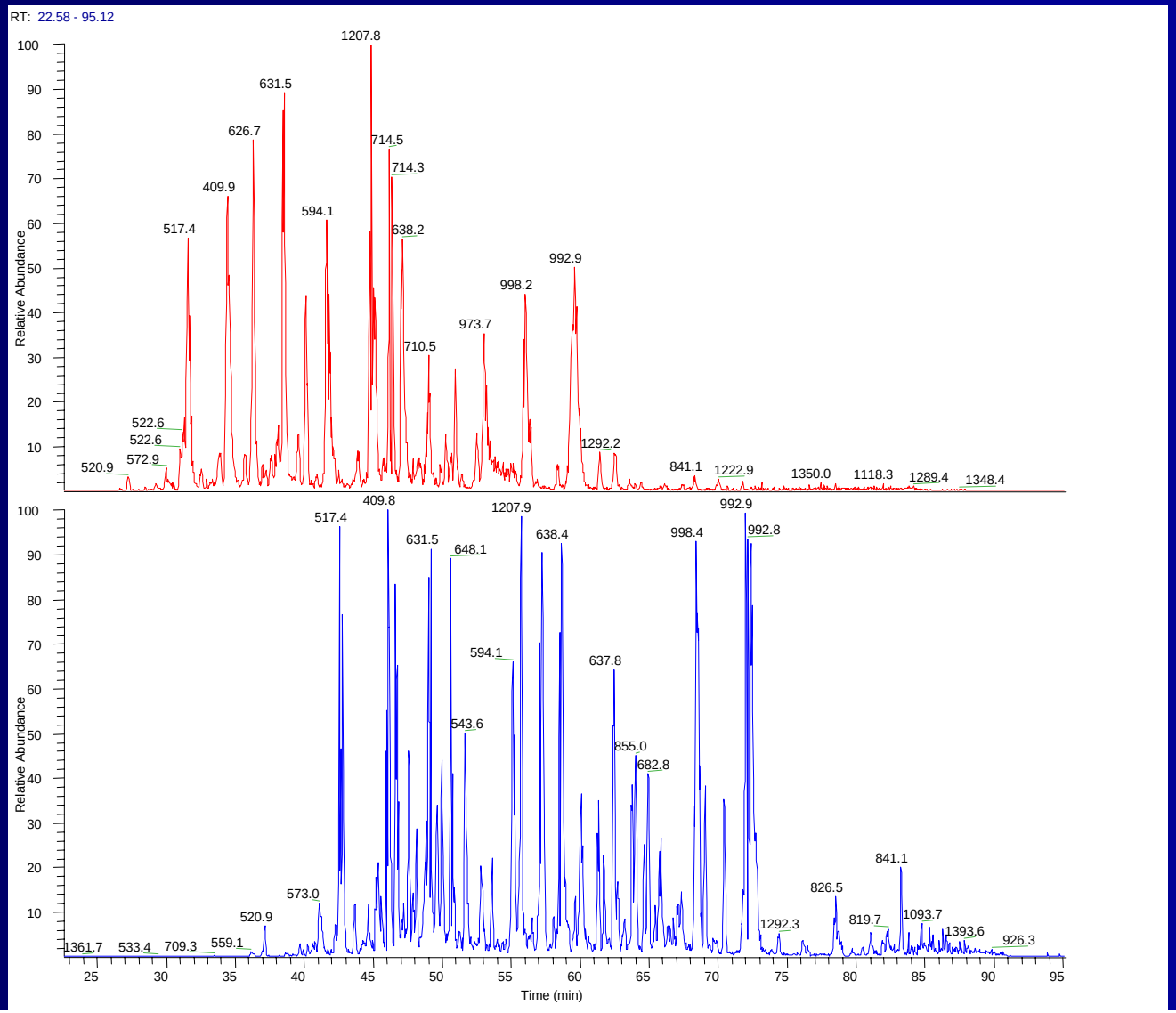
60 cm Continuous Vent Human Serum MudPIT



Making the Cut



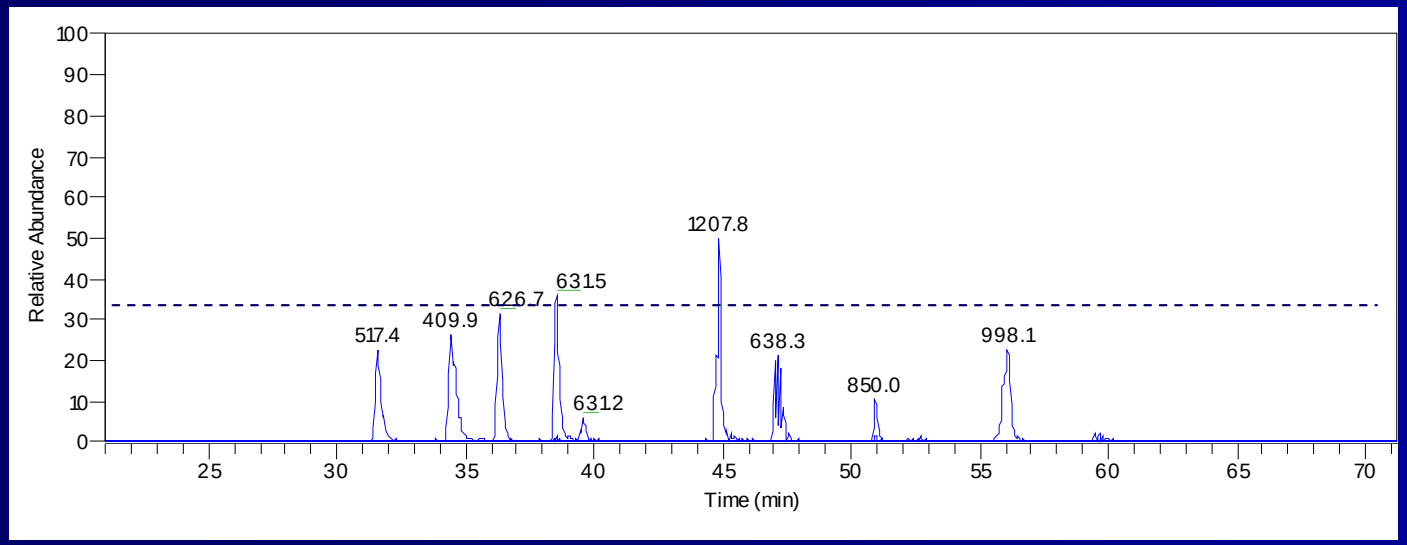
Comparing 10 and 60 cm Columns at the 25 mM Salt Step using the Continuous Vent Method



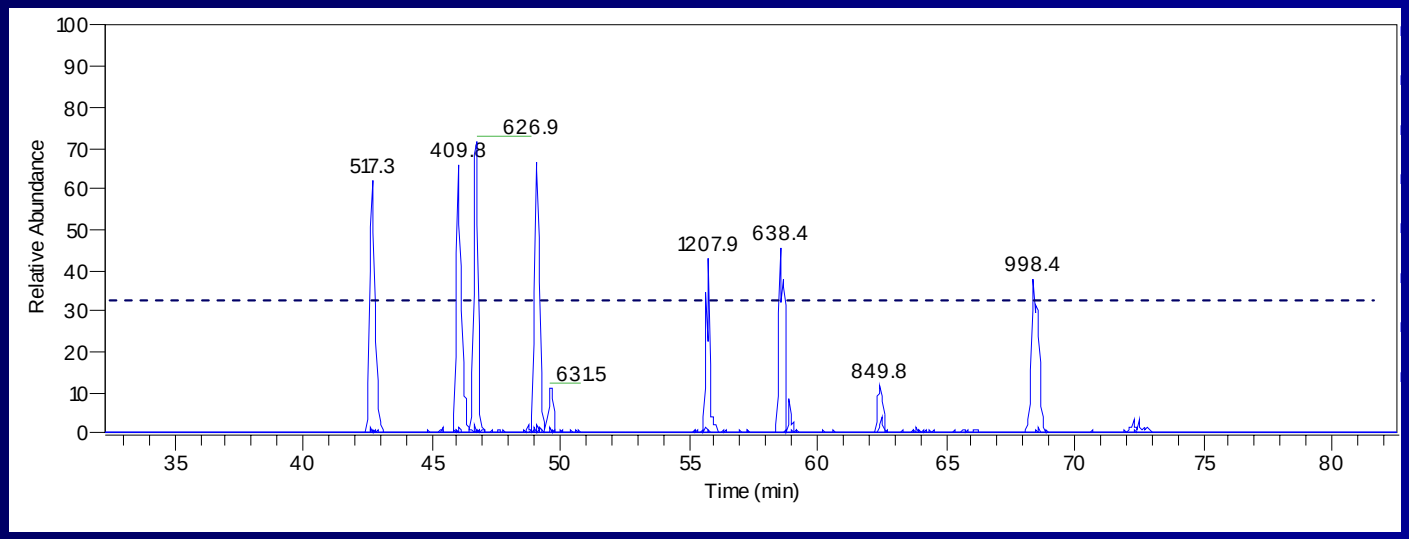
10 cm

60 cm

XIC Plots for 10 and 60 Columns at the 25 mM Salt Step using 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	

Our First Proteomics Evaluation Method:

Counting Unique Peptides on the Mascot Significant Hits List

10cmDCV			60cmDCV			10cmCV			60cmCV	
10cmDCV			60cmDCV			10cmCV			60cmCV	
43232						47319			44212	
P01024	53		P01024	61		P01024	65		P01024	75
P01023	50		P01023	50		P02768	60		P02768	63
P02768	46		P02768	45		P01023	51		P01023	53
P02787	25		P04114	34		P02787	35		P02787	41
P01028	23		P01028	31		P04114	32		P01028	33
P00450	23		P02787	23		P01028	24		P04114	29
P01857	18		P00450	19		P08603	17		P08603	21
P04114	18		P00747	17		P01857	16		P00450	20
P00747	15		Q14624	17		Q14624	15		P01871	18
P08603	14		P01857	15		P00450	15		P02647	18
P01871	13		P01871	15		P01009	14		P01009	16
P02774	12		P01009	15		P02647	14		P01857	15
P02647	11		P02647	15		P00738	13		P00738	14
P01009	10		P02774	14		P00747	11		P19823	14
P19827	10		P19827	14		P19827	10		P02774	14
P02790	10		P08603	13		P04217	10		P01876	13
P01859	9		P19823	12		P01876	9		P19827	13
P01876	9		P01859	9		P19823	9		P00747	13
P00738	9		P01876	9		P02774	9		P04217	10
Q14624	9		P04217	8		P01042	9		P04003	10
P04003	9		P02790	8		P20742	8		P01859	9
P19823	8		P02751	8		P01859	7		P01042	9
P01042	7		P04003	8		P01834	7		P01011	8
P01834	6		P01834	7		P01842	7		P01834	7
P01842	6		Q95LB0	7		P02765	7		P01842	7
P01011	6		P01842	6		P00734	6		P02765	7
P04217	6		P01011	6		P06727	6		Q14624	7
P02749	6		P01042	6		P01860	5		P00734	7
P02751	6		P00738	6		P01011	5		P06727	7
P02765	5		P02765	5		P01620	4		P10909	6
P00734	5		P20742	5		P01019	4		P02751	6
P01860	4		P02760	5		P04004	4		P04004	6
P06396	4		P06396	5		O43866	4		P01860	5
O43866	4		P04004	4		P06396	4		P02790	5
P01861	3		P00734	4		P02652	3		P02749	5
P80748	3		P09871	4		P02766	3		P00739	4
P02652	3		P00739	4		P00760	3		P02649	4
P04004	3		O43866	4		P02790	3		P01019	4
P00760	2		P02748	4		P02751	3		P02763	4
P01625	2		P00751	4		P02753	3		P02760	4
P01597	2		P01861	3		P04003	3		P00760	3
P01620	2		P01860	3		P02649	3		P01787	3
P01019	2		P01597	3		P80748	3		P02656	3
P00739	2		P02652	3		P03952	3		P01620	3
P46782	2		P02753	3		P02749	3		P02652	3
P01714	2		P01620	3		P01861	2		P02766	3
P01616	2		P03952	3		P00739	2		P01606	3
Q9UGM5	2		P06727	3		P01597	2		P80748	3
P01764	2		P80748	3		P01625	2		P00751	3
P08649	2		P10909	3		P02760	2		P02747	3
P10909	2		P01031	3		P01764	2		P05546	3
P27169	2		P05546	3		P02748	2		O43866	3
P02766	2		P00760	2		P02763	2		P02753	3
P04220	1		P01786	2		P01617	2		P02748	3
P20742	1		O60841	2		P10643	2		P03952	3
P01766	1		P02766	2		Q28522	1		P01861	2
P01619	1		P04430	2		P01766	1		Q28372	2

	10cmDCV			60cmDCV			10cmCV			60cmCV	
P08649	2		P10909	3		P02760	2		P02747	3	
P10909	2		P01031	3		P01764	2		P05546	3	
P27169	2		P05546	3		P02748	2		O43866	3	
P02766	2		P00760	2		P02763	2		P02753	3	
P04220	1		P01786	2		P01617	2		P02748	3	
P20742	1		O60841	2		P10643	2		P03952	3	
P01766	1		P02766	2		Q28522	1		P01861	2	
P01619	1		P04430	2		P01766	1		Q28372	2	
P02743	1		P41339	2		P01786	1		P01625	2	
P01612	1		P19652	2		P49065	1		O14791	2	
Q03591	1		P01019	2		P01742	1		P01768	2	
P04433	1		P01605	2		P01765	1		P25311	2	
P01742	1		P05155	2		Q59072	1		P01614	2	
P18902	1		P02763	2		P01781	1		P01031	2	
P01631	1		P01625	2		P01700	1		P05155	2	
P13671	1		P04196	2		P05546	1		P01598	2	
P11426	1		Q28522	1		P01593	1		P01611	2	
P01765	1		P04220	1		Q8RE44	1		P02743	2	
O18500	1		P35568	1		P01621	1		P13671	2	
P01825	1		P97279	1		Q61838	1		P02775	2	
P05156	1		P01596	1		P01784	1		P10643	2	
O60841	1		P02749	1		P08649	1		Q28522	1	
P04196	1		P46782	1		P01785	1		P02769	1	
P01591	1		P01598	1		O83270	1		P04220	1	
P01611	1		P01593	1		Q9S7P9	1		P01877	1	
P01617	1		P01619	1		P01029	1		P49065	1	
P01700	1		P08649	1		P18527	1		P01766	1	
P35827	1		P01717	1		P01973	1		P01762	1	
P01784	1		P14227	1		Q9UGM5	1		P08649	1	
P01763	1		P01700	1		P01714	1		P01770	1	
			P27169	1					P01742	1	
			Q9UGM5	1					P07360	1	
			P22752	1					P01779	1	
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			P98137	1					Q03591	1	
			P02745	1					P01700	1	
			P37093	1					P07225	1	
			Q03591	1					P01743	1	
			Q62910	1					P05090	1	
			P02746	1					P18982	1	
									Q864W0	1	
	537			635			593		P80108	1	
									O84877	1	
									Q59072	1	
									P01806	1	
									P50042	1	
									P00736	1	
									P01825	1	
									P01935	1	
									Q97RH2	1	
									P97313	1	
									P01717	1	
									Q09687	1	
									P04937	1	
									P01618	1	
									P01767	1	
									P33449	1	
									Q13619	1	
											739

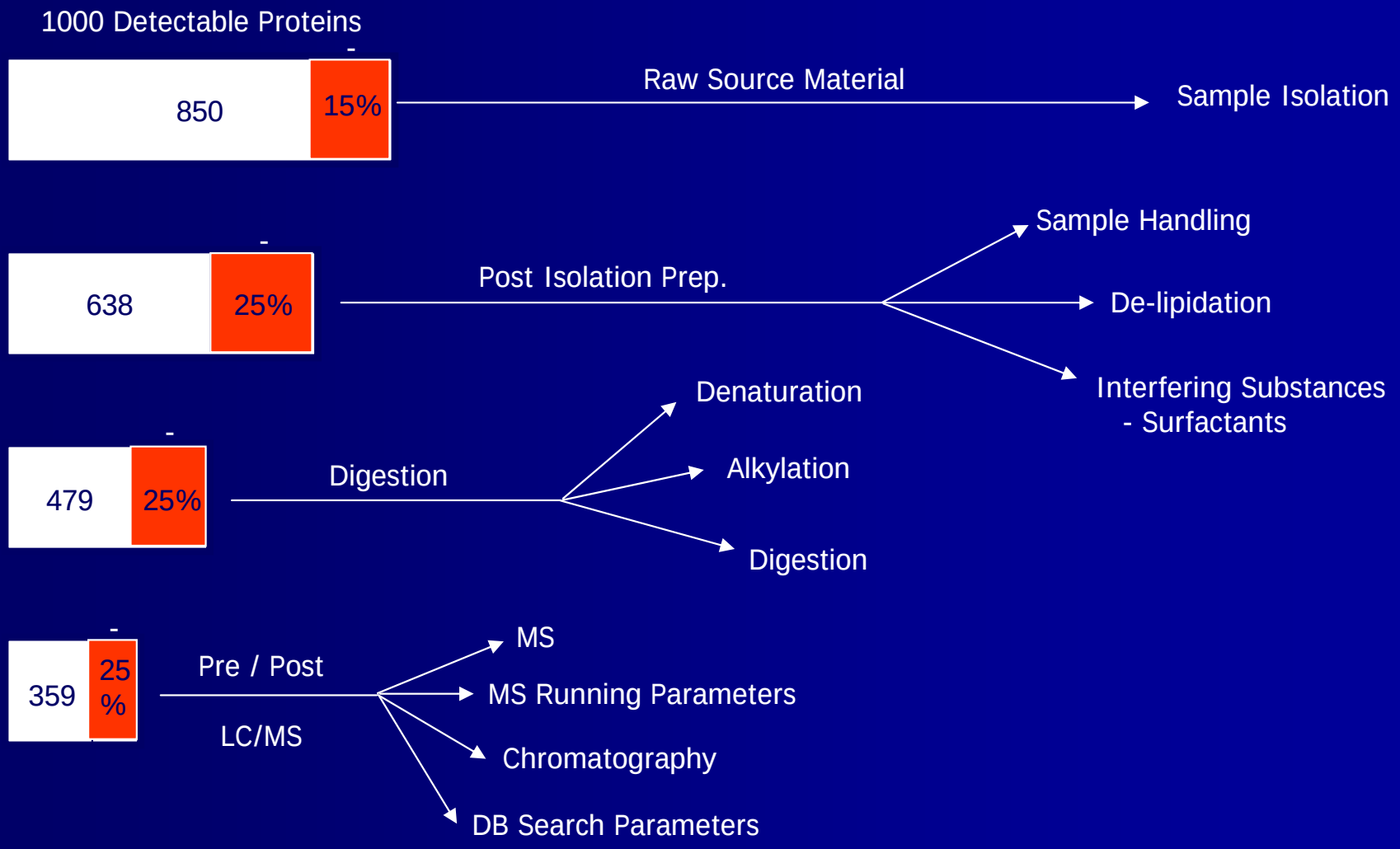
CV vs. DCV

Short vs. Long

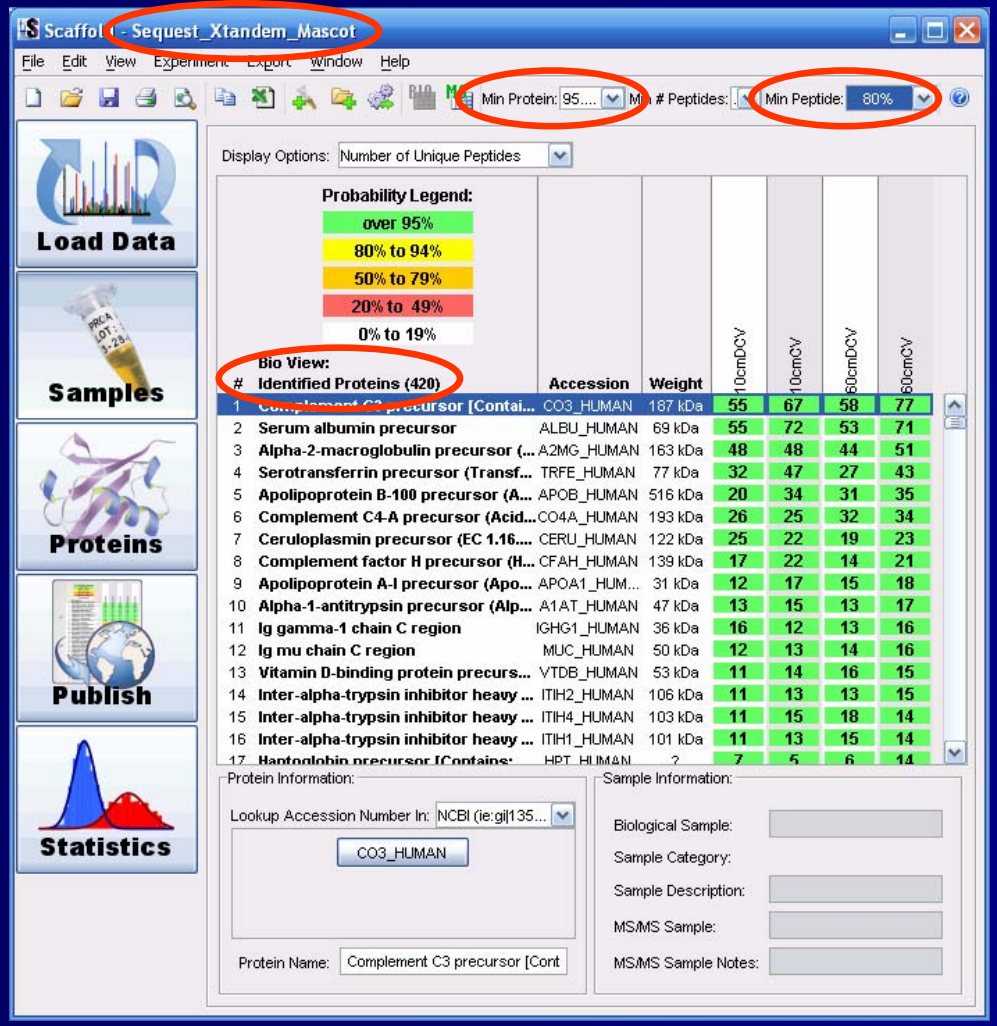
Peptide Count Category (protein with n peptides)	10 cm DCV	60 cm DCV	% Gain
6+	447	498	11.4
1-5	90	137	52.2
	10 cm CV	60 cm CV	
6+	486	589	21.2
1-5	107	150	40.2

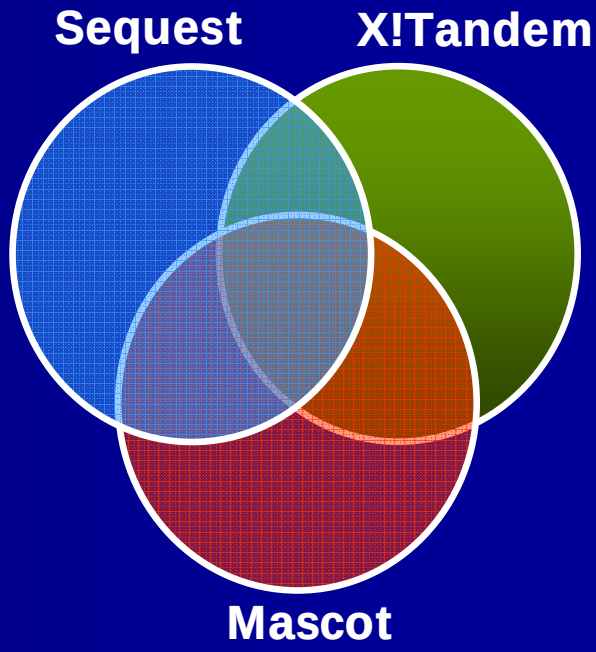
Improving the Efficiency of the Process

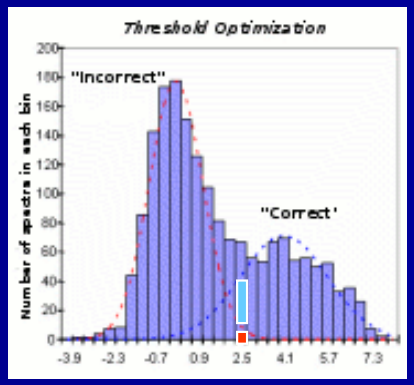
Worshipping at the altar of incrementalism



Scaffold Proteomics Evaluation Method





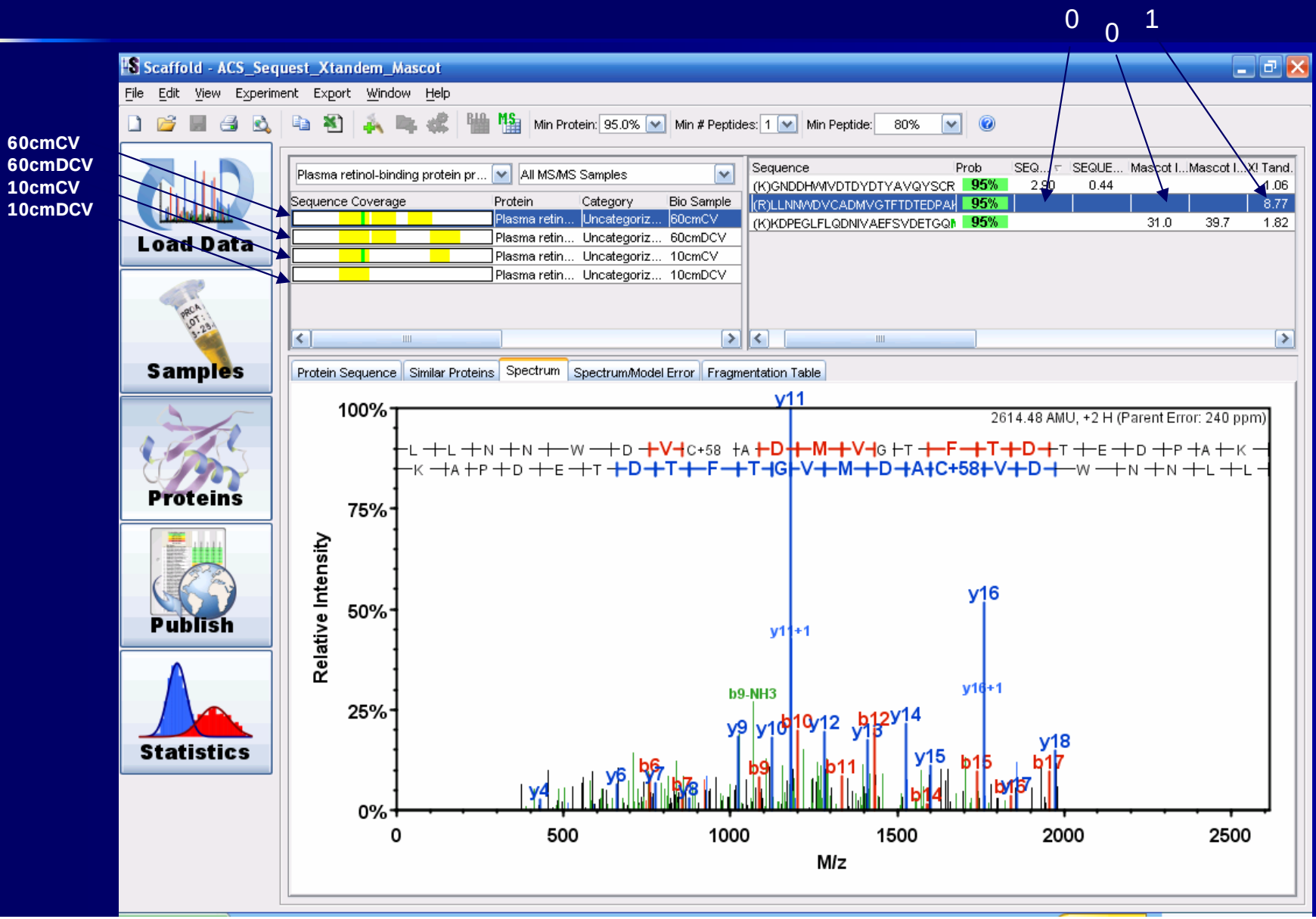


www.proteomesoftware.com

Keller, A. et al *Anal. Chem.* **74,** 5383-5392

Example

50th on the list



Reworking the Data With Scaffold

CV vs. DCV

Short vs. Long

	10 cm DCV	60 cm DCV	% Gain
Peptide Count	728	829	14 %
original Mascot numbers ->	537 (36%)	635 (31%)	
	10 cm CV	60 cm CV	
Peptide Count	787	894	14 %
original Mascot numbers ->	593 (33%)	739 (21%)	
% Gain	8 %	8 %	166 / 23%

Conclusion

- Long columns are better at showing us a greater number of low level peptides.
- The CV method is superior to the DCV method.
- Be an incrementalist.

Acknowledgements

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