Cleaning up MuDPIT: Triphasic Traps & Long Columns

Allis S. Chien, Andrew W. Guzzetta

Vincent Coates Foundation Mass Spectrometry Laboratory, Stanford University

Stanford University
MASS SPECTROMETRY

Overview

- Origin of the vented tetraphasic long column MuDPIT device
  - MuDPIT
  - Vented columns
- Triphasic traps
- Long columns
- Continuous & discontinuous configurations
  - HPLC valve diagrams
  - Results comparison
- Packing traps & columns

Parent Methodologies

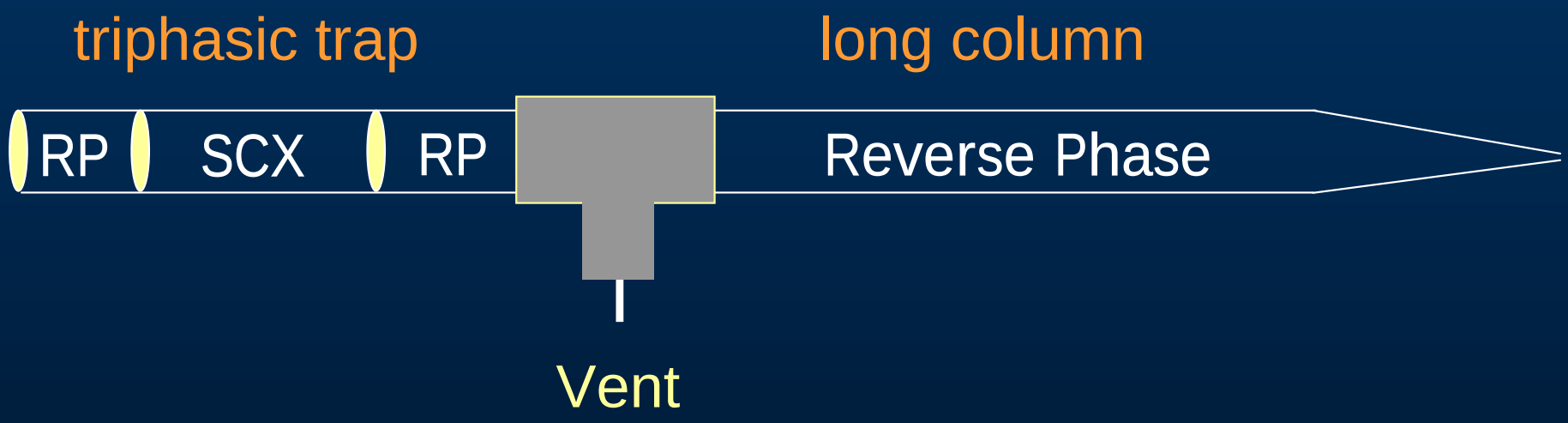
Triphasic MuDPIT

Vented Column

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

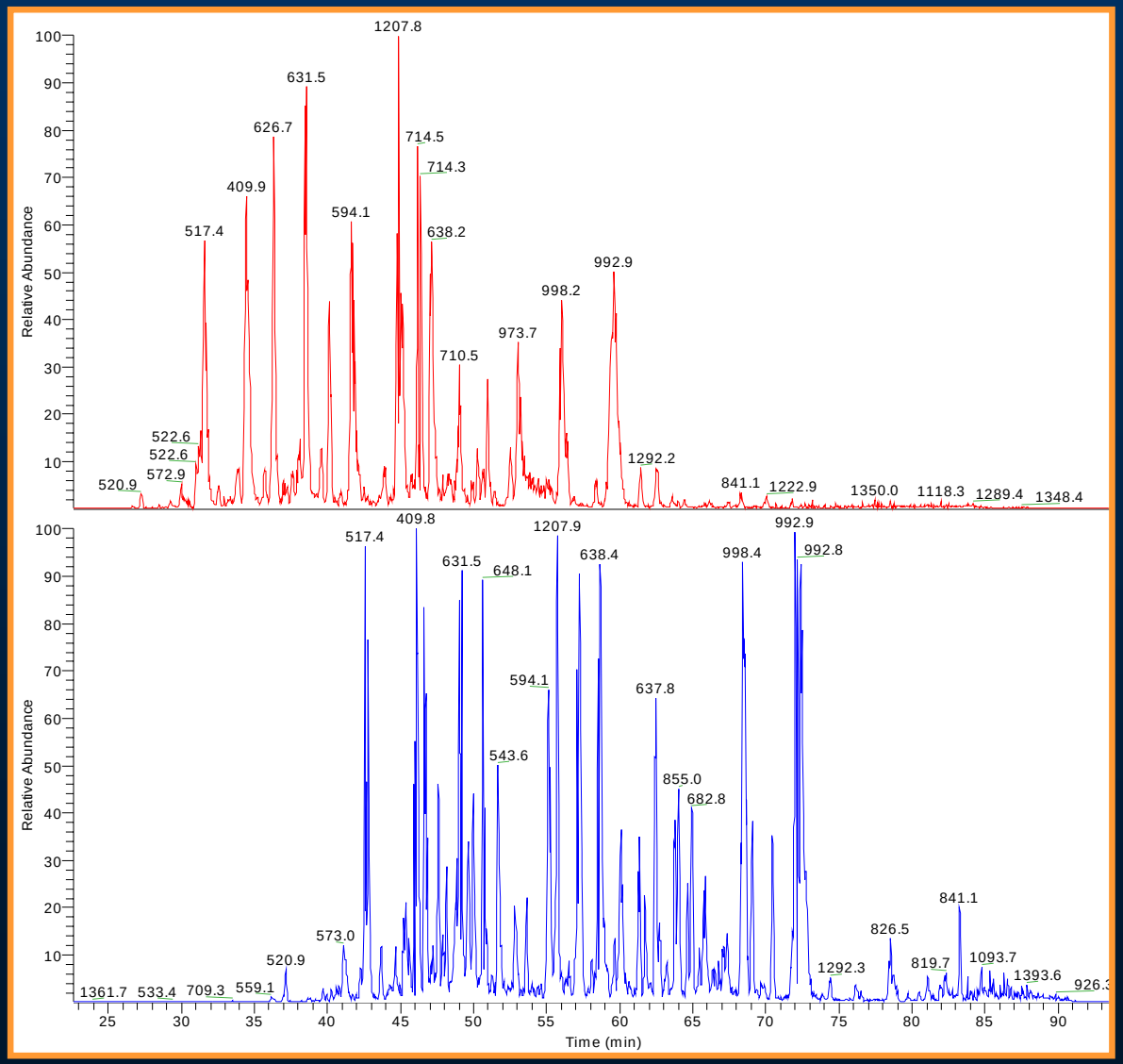
Vented Tetraphasic MuDPIT



- Online desalting
- 2-D separations
- Fast loading
- No salt on column

1 cm of 150 mm ID = 4 cm of 75 mm ID

Long Column: Increased Resolution



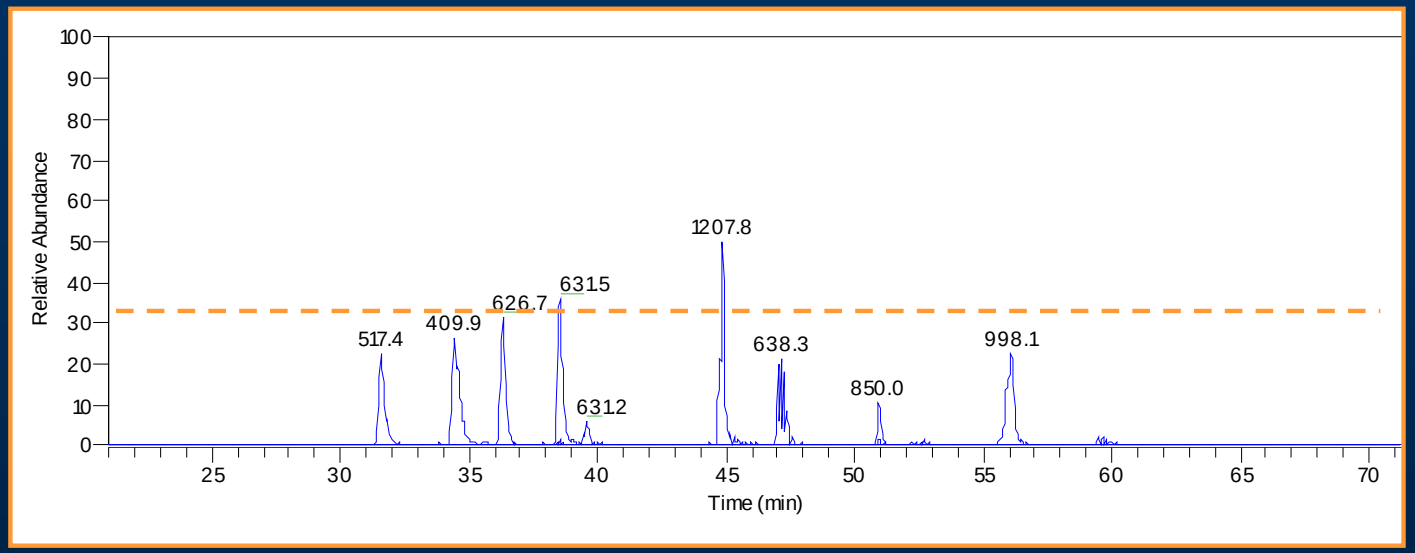
10 cm

60 cm

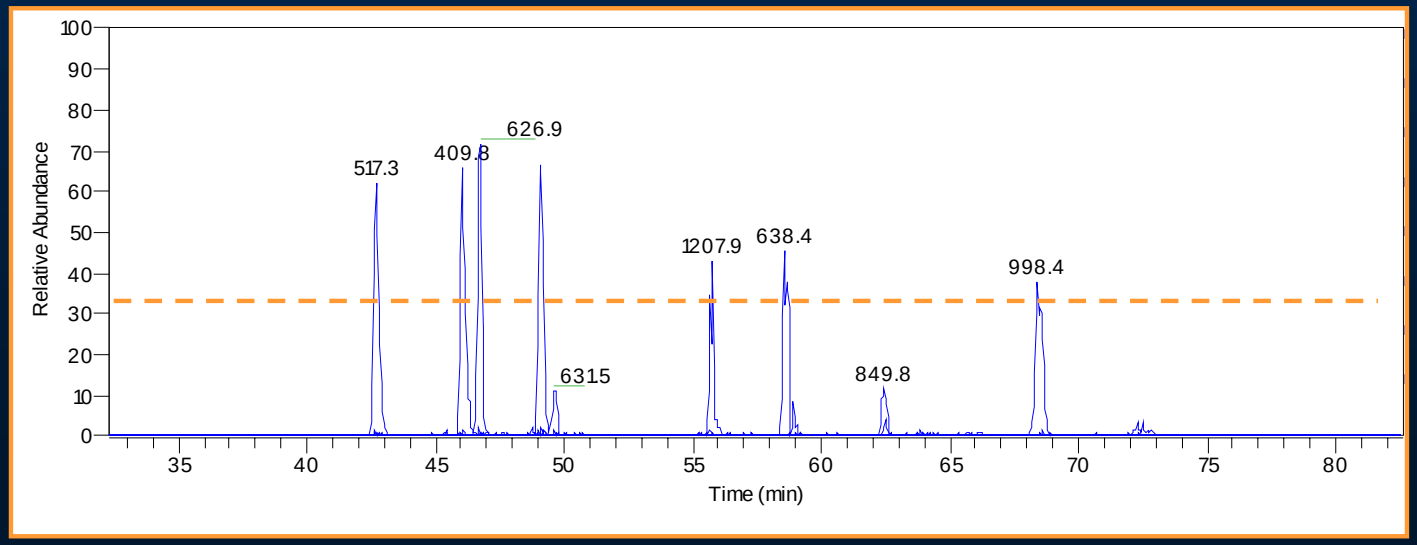
J. Proteome Res. 4(*6*), 2412 -2419, **2005**.

25 mM Salt Step

Extracted Ion Chromatograms



10 cm



60 cm

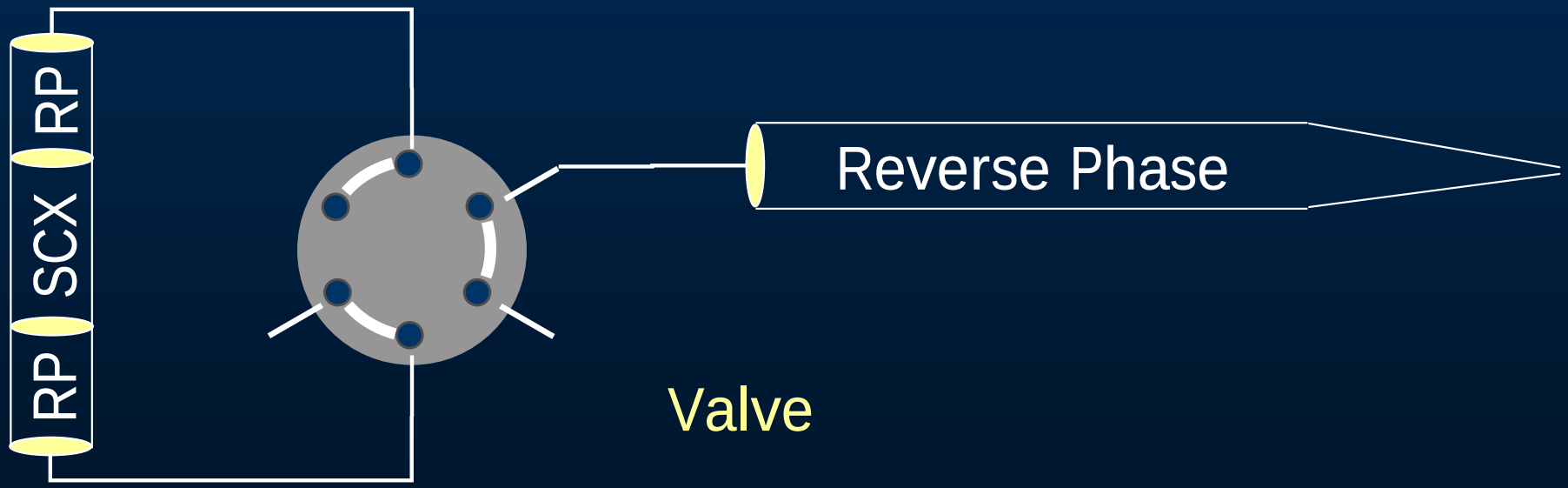
J. Proteome Res. 4(*6*), 2412 -2419, **2005**.

25 mM Salt Step

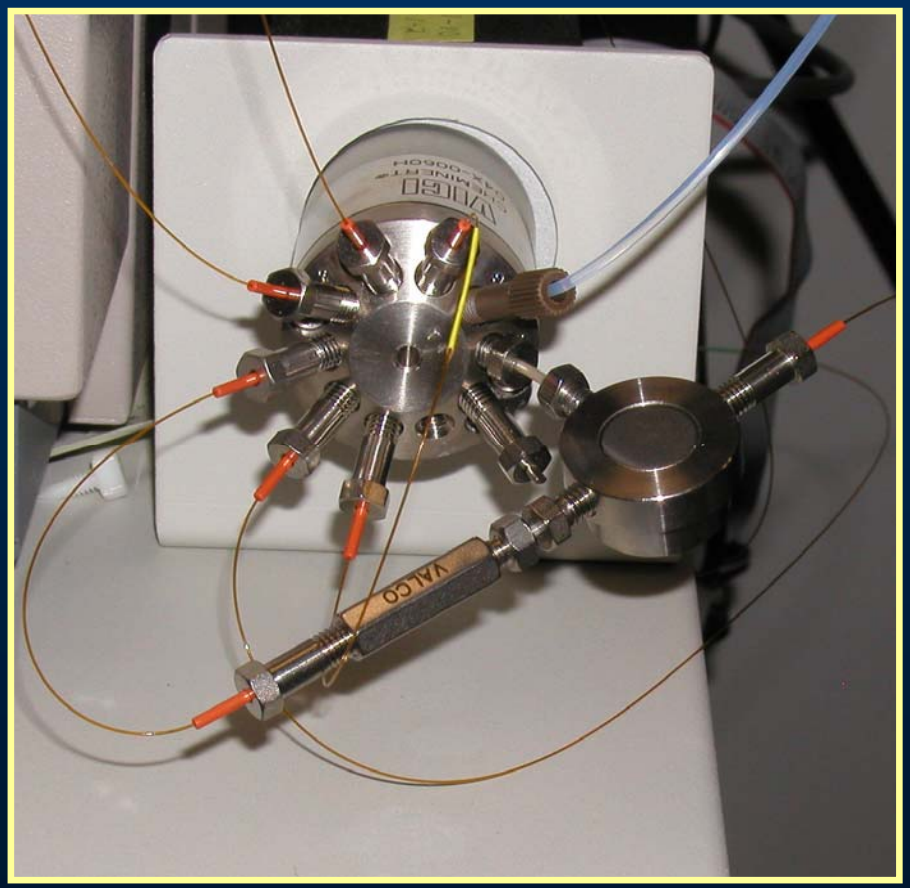
Alternate Configurations

Continuous

Discontinuous

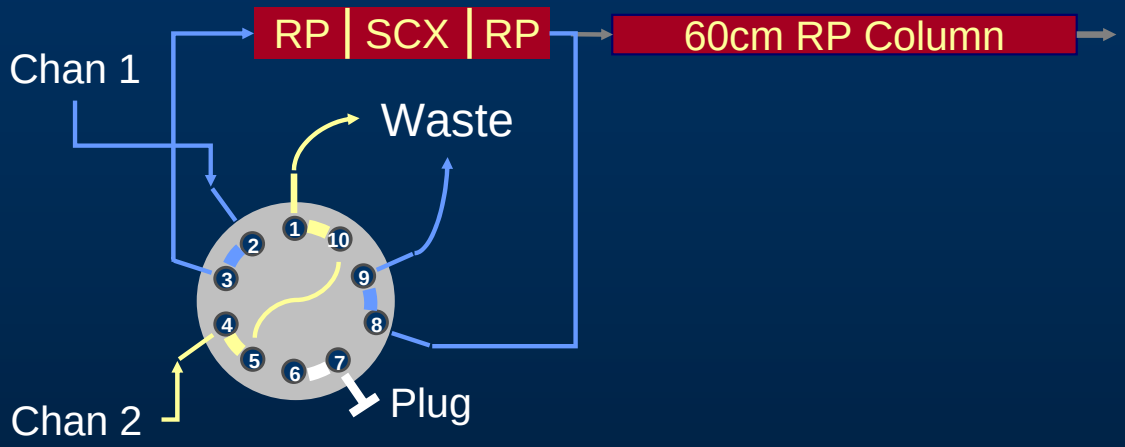


Continuous - Triphasic Vent

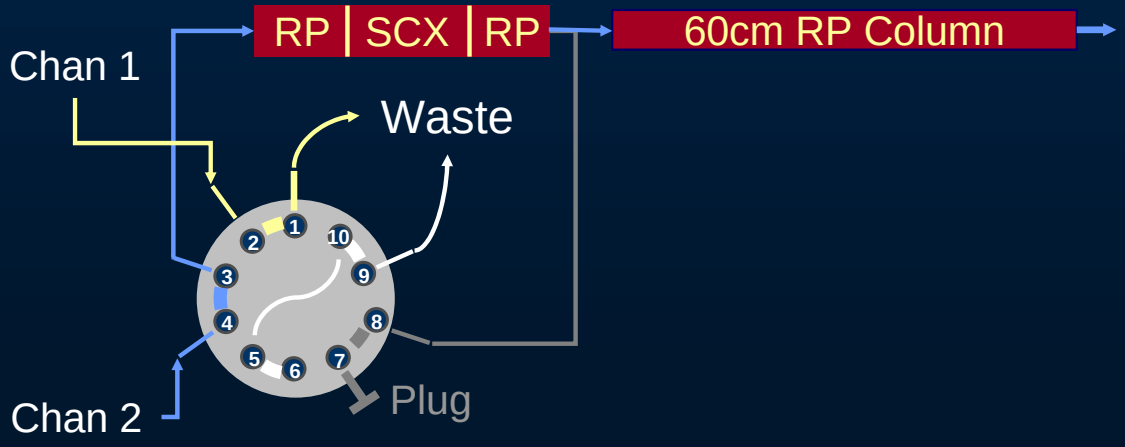


Vent 10-1, Plug 1-2

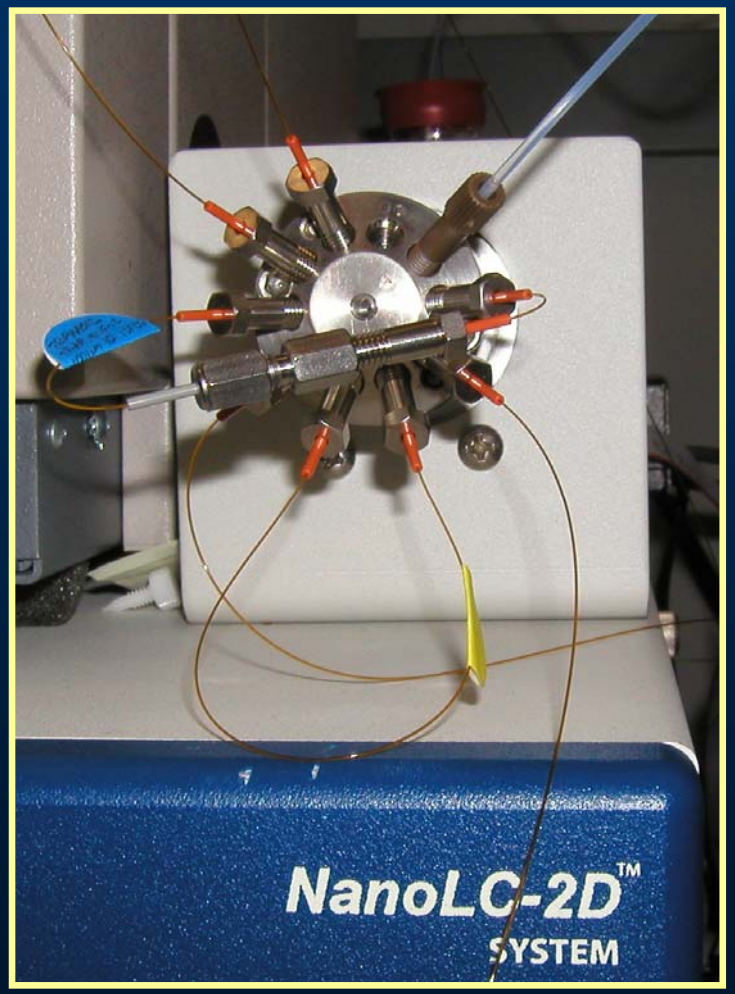
Load sample, organic bump, SCX



Reverse phase gradient

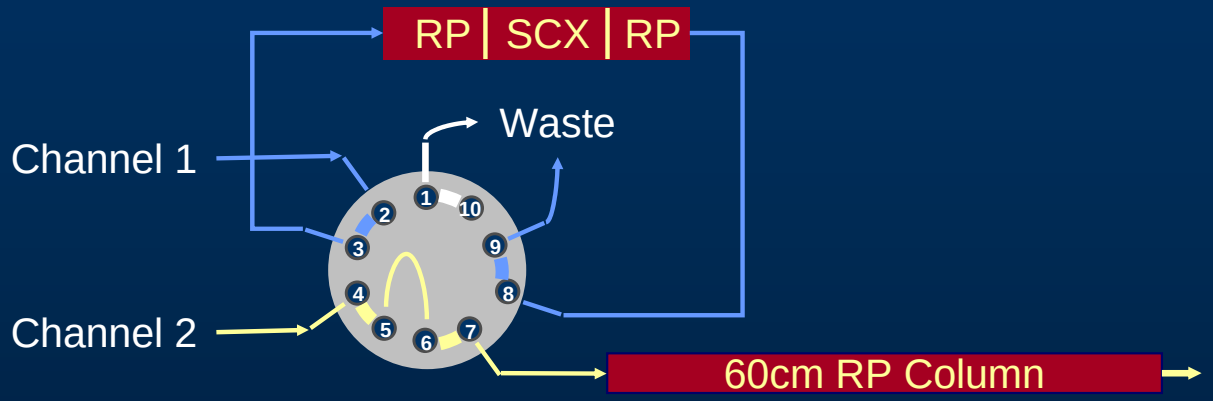


Discontinuous - Triphasic Valve

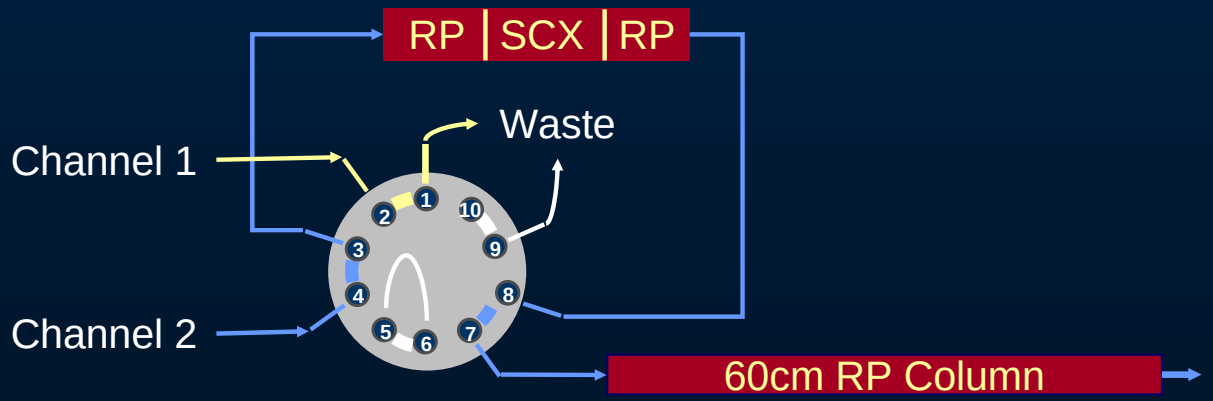


Load 10-1, Inject 1-2

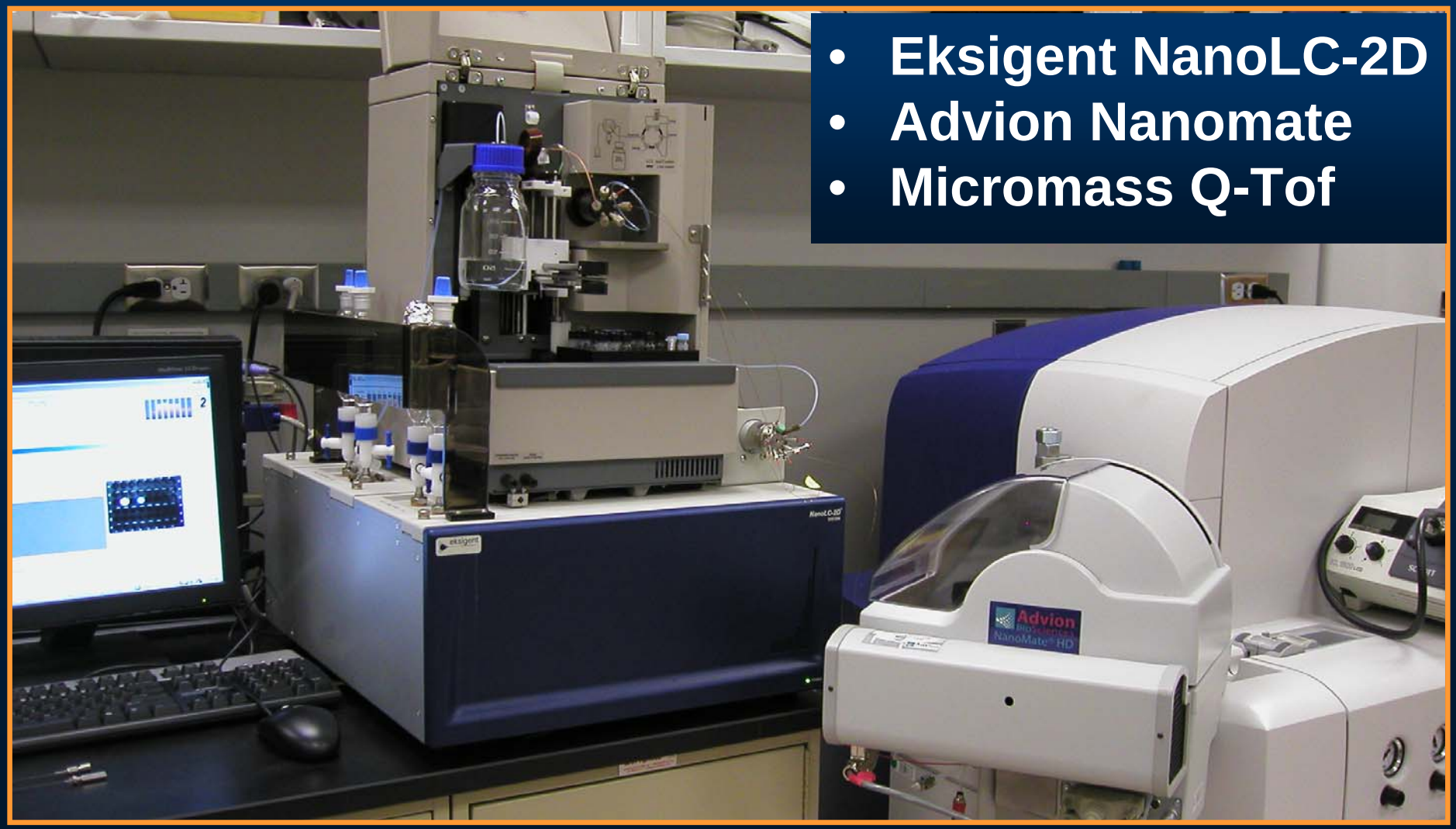
Load sample, organic bump, SCX



Reverse phase gradient



Instrumentation



- Eksigent NanoLC-2D
- Advion Nanomate
- Micromass Q-Tof

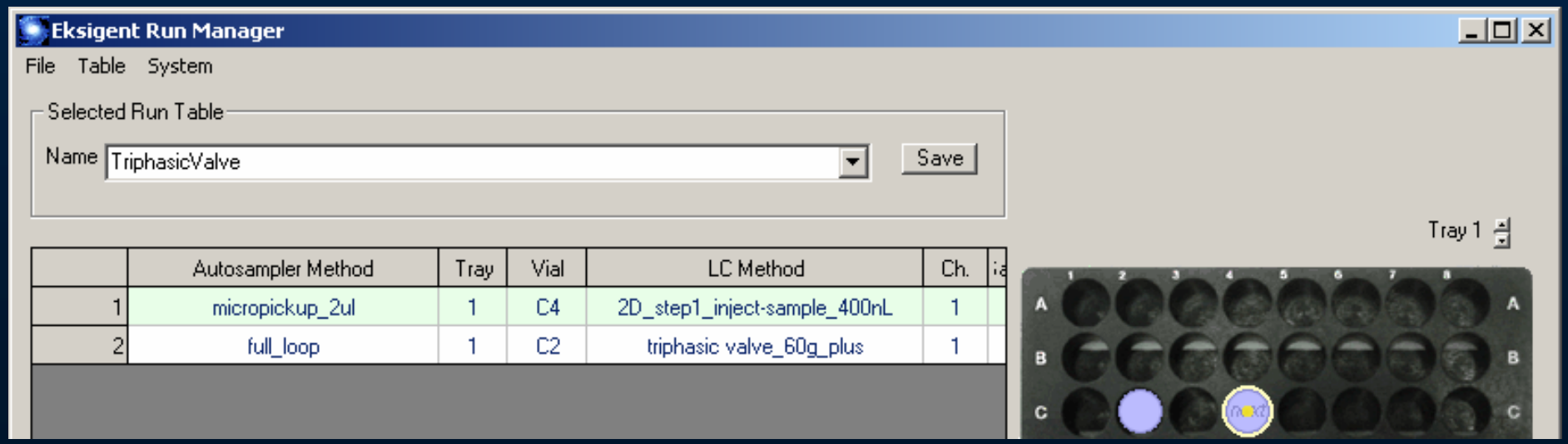
Eksigent Sequence

Procedure:

- Load and wash peptides on 1st RP segment of trap
- Bump peptides onto SCX with injection of high organic
- Run series of salt steps and RP gradient separations

Translation into instrument methods:

1. Injection - sample load & wash
2. 2D-LC - high organic injection, salt steps & RP gradients



Experimental

- Trap: 100 um ID x 35 mm (12/15/8 mm RP/SCX/RP)
  - Poros 10 um RP
  - Partisphere 5 um SCX
  - alternate: 150 um ID x 16mm (6/6/4 mm RP/SCX/RP)
- Column : 75 um ID x 60 cm, Vydac 5 um C18
- Flow rates:
  - Channel 1, 8 uL/min
  - Channel 2, 400 nL/min
  - Column equilibration: 500 nL/min, 40 min
- Backpressure: 3500-4000 psi; max observed 4500 psi
  - Use PEEK sleeves; don't use teflon sleeves or 1-piece fittings
- SCX steps: 0, 10, 25, 50, 250, 1000, 5000 mM ammonium acetate
- RP gradient: 5-40% B over 60 min (short test method)
- Eluents:
  - Channel 1A, 5M ammonium acetate, 0.1% formic acid
  - Channel 1B, 0.1% formic acid in water
  - Channel 2A, 0.1% formic acid in water
  - Channel 2B, 0.1% formic acid in acetonitrile

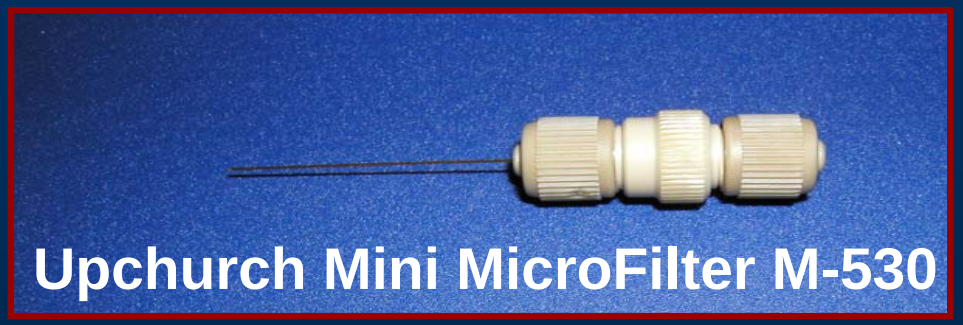
Advantages of Vent vs. Valve

Vent	Valve
Minimal dead volume	Identical plumbing to standard trap and column setup
No delay between trap & column	Continuous flow through column: constant pressurization, enables shorter methods: • column equilibrates during salt steps & washes • elevated flow rates during re-equilibration
Better peak shape	Better retention time reproducibility
10% more peptides identified	**2-3x lower RT RSDs** (average 0.13% vs. 0.36%)
Maximum peptide ID	**Differential expression, fraction collecting**

Fittings & Hardware for Packing

Valco
Tee MT1XCS6

Union ZU1XC



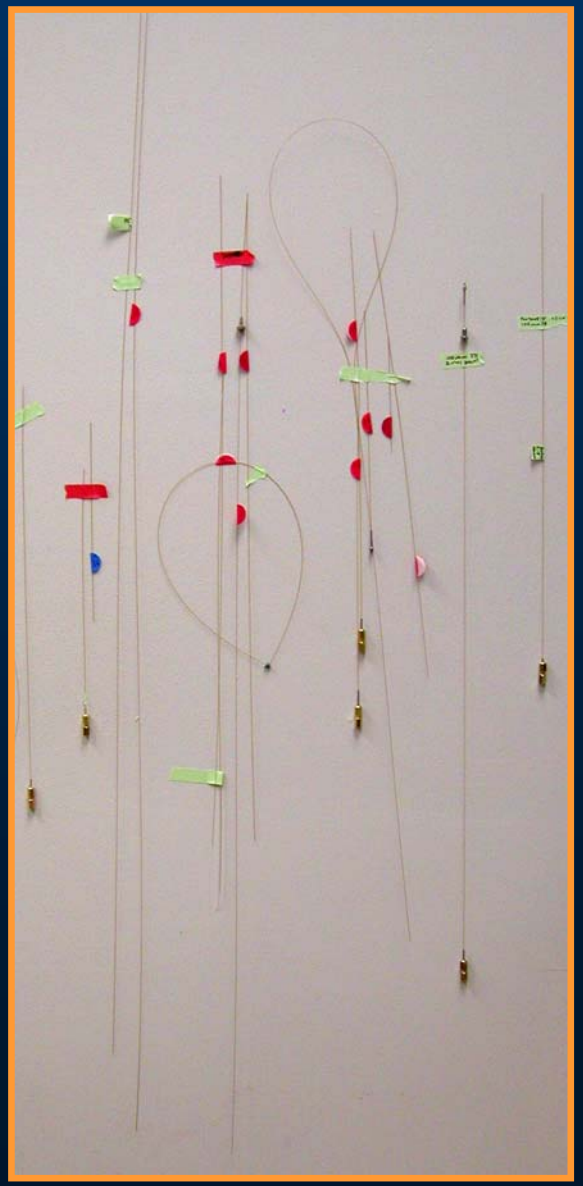
Upchurch Mini MicroFilter M-530

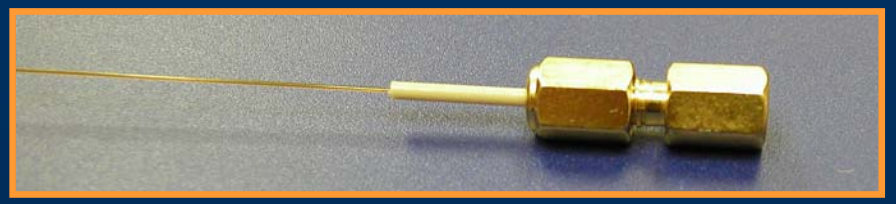
Upchurch MicroTight adaptor P-770

Valco union E2UIC
Frit 2SR1-10

Grey PEEK
Upchurch 1565

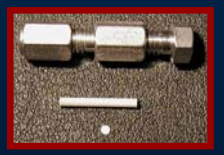
Packing the long column





1. Start with 150 um ID tubing, 5 um particles
2. Cut a length of fused silica 2-3x longer than final column length

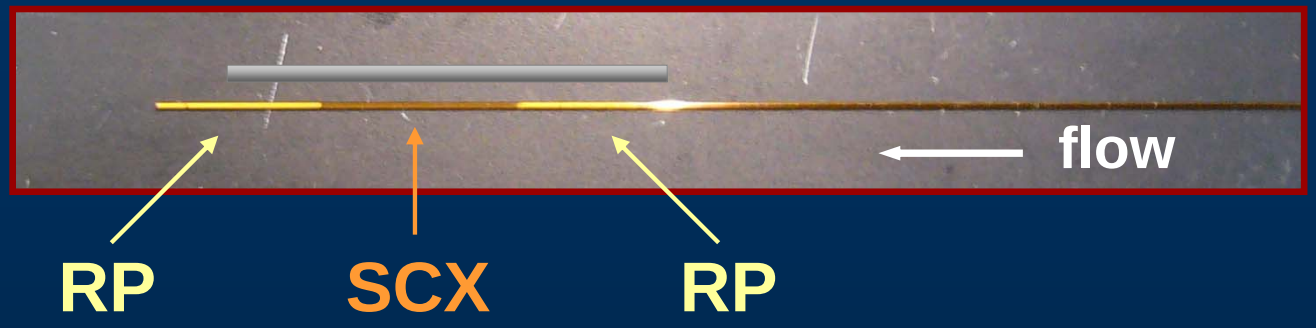
3. Push a thick but free flowing slurry of stationary phase in isopropanol into the tubing using a syringe
4. When tubing is full, swage into a fritted union



5. Use an HPLC to consolidate the column bed

- If column isn't long enough:
  - Allow column to dry out, then reverse direction and try again
  - Place a low-resistance frit on "empty" end of tubing, re-pack from "full" end
- Smaller ID tubing & smaller particles are more difficult to pack

Packing the triphasic trap



Method:

1. Fit ~10-15 cm fused silica with temporary frit
2. Over pack RP
3. Wash, mark lengths
4. Pack SCX to exact length
5. Wash
6. Pack final section of RP
7. Wash
8. Remove temporary frit
9. Cut first RP segment to length
10. Reswage with PEEK sheath
11. Cut fused silica capillary to final length

Conclusions

- 2D-LC with triphasic traps & long columns
  - Traps: no salt on RP column; fast loading, salt steps, washing
  - Long columns: improved resolution, peak capacity, peak shape
  - No extraordinary equipment or efforts required
- Choose configuration based on purpose:
  - Vent for protein ID
  - Valve for retention time reproducibility
- Pack your own traps & columns