

Conversion of ProteomeX to Nanospray

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The ProteomeX system can easily be converted from micro-spray mode to nano-spray mode. The use of smaller diameter reversed-phase separation columns 75 μm ID results in increase sensitivity of about a factor of 10 compared to the standard setup. The two dimensional separation methods combines a high flow separation of peptides in a first dimension with high sensitivity and nanoflow detection of peptide fragments using 75 μm ID packed tip columns in a second dimension.

Installation

1. Nanospray Source: Follow the instruction for installation of the nanospray source. Note: the system need to be tuned specifically for nanoflow.
2. Exchange the two reversed-phase columns with two peptide traps and wash the traps with 50% ACN 0.1% formic acid for 20 minutes using the sample pump. See Image 1. Adjust the flow rate after the peptide traps to 10 $\mu\text{L}/\text{min}$. This will be the flow rate of the sample pump for later two-dimensional methods. Switch the valve manually to wash both peptide traps and make sure that the flow rate after both peptide traps is the same.

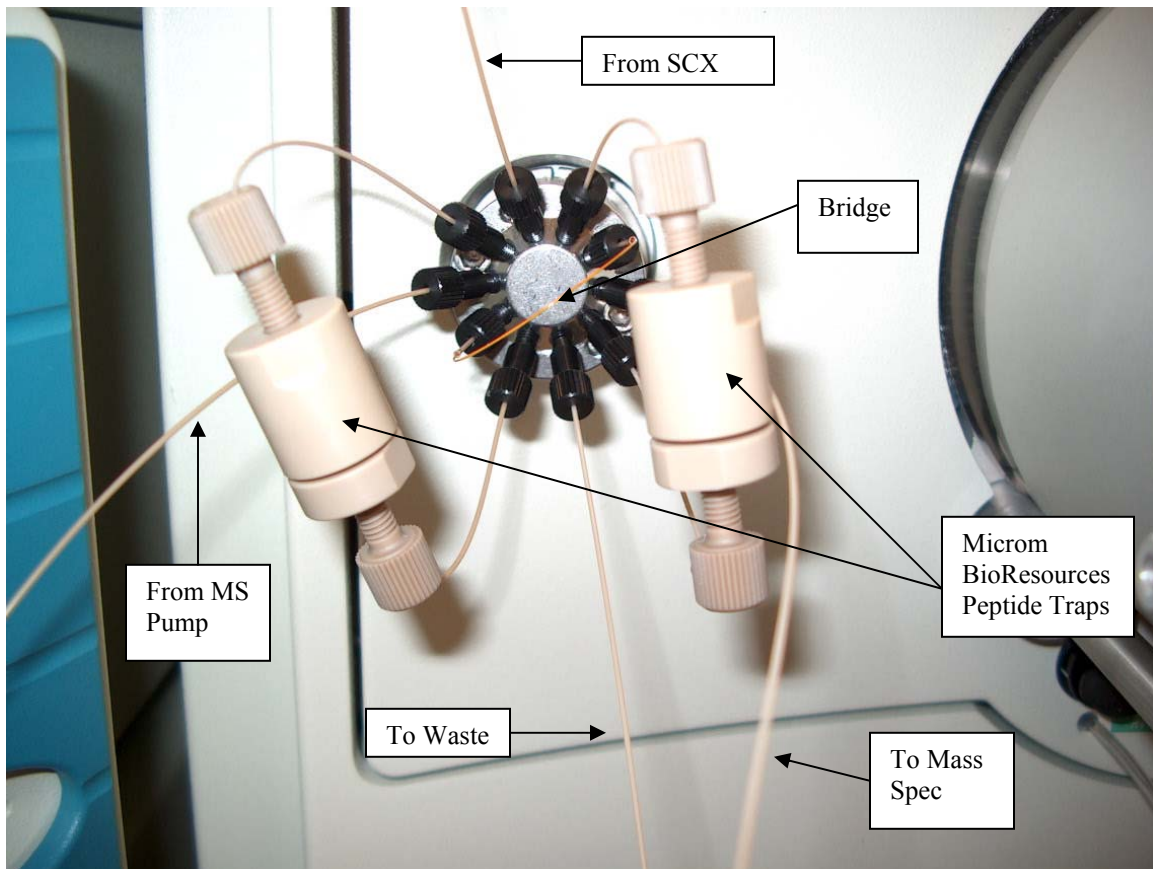


Image 1. 10-port valve plumbing for the peptide traps. All other tubing is the same as those used with the ProteomeX system. Notice the orientation of the peptide traps.

3. Install the PicoFrit column in the Nanospray source and connect to the 10 port valve. Do not attach the outer ring of the Nanospray source to the nanospray extension yet. Use the MS pump to wash the PicoFrit for 20 minutes with 50% ACN 0.1% formic acid. Measure the flow rate at the tip using a glass capillary and adjust the flow rate at the pump to achieve a flow rate of 200nL/min. See Image 2. Attach the source to the extension and move the PicoFrit close (~3 mm) to the entrance of the mass spectrometer (ion transfer tube). The spray can be optimized by adjusting the PicoTip position, the spray voltage (typically 1.8 kV - 2.0 kV), and the flow rate (100 - 200nL/min). See Image 3.

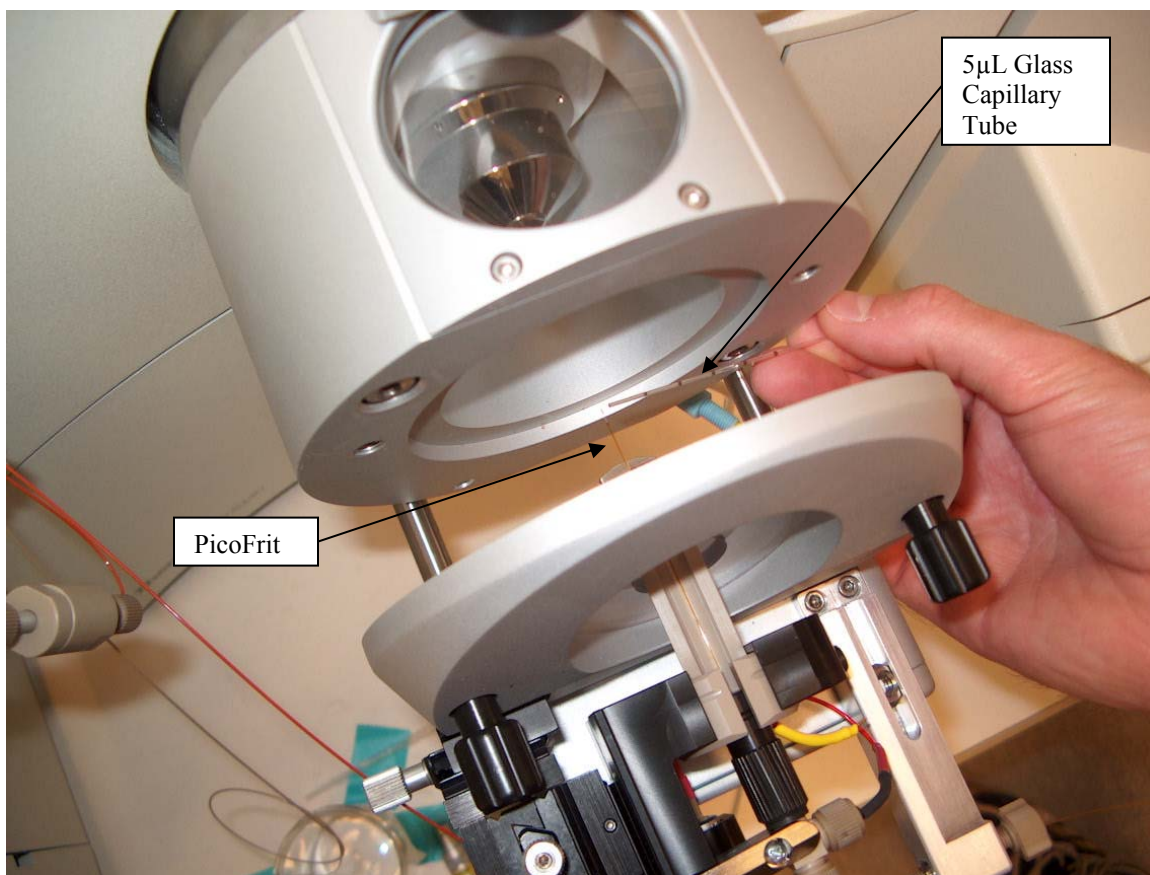


Image 2. Use a 5µL capillary tube to measure the flow rate. Allow buffer to flow for 5 minutes then collect the drop. Its volume should be approximately 1µL.

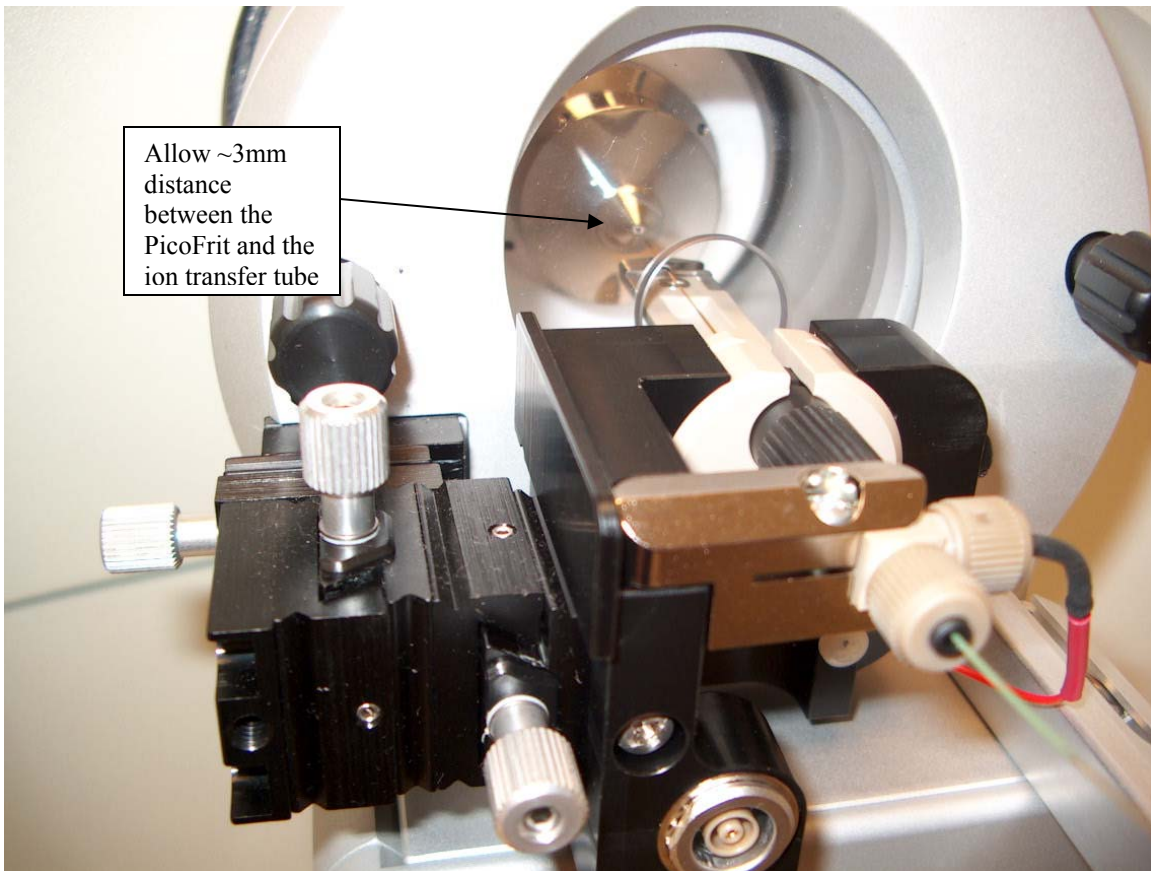


Image 3. Move the PicoFrit to approximately 3mm from the opening of the ion transfer tube. Adjust this position, along with the spray voltage and flow rate, to optimize the spray.

Your system is now ready to go. We recommend testing the system in a one dimensional mode first. You'll need to remove the SCX column from the system to do this. Inject 500 fmol of a protein digest (BSA or myoglobin) directly to the peptide trap using the sample pump for 10 minutes. Switch the valve and elute the peptides with a short gradient. Make sure that both peptide traps are working. Check your plumbing if you have problems in the one dimensional mode. You are now ready for the two-dimensional separation. Re-install the SCX column and check for leaks. Use the ProteomeX software to generate the method.

2D-LC/MS/MS Method

The samples were analyzed using a modified version of the ProteomeX workstation. The two reversed phase columns were replaced by two poly (styrene-divinylbenzene) peptide traps (Michrom Bioresources, Auburn, CA USA) and the ESI source with the 30 μm ID metal spray needle was replaced by the nanospray ionization source including a reversed-phase PicoFritTM column (5 μm BioBasic C18, 300 \AA pore size, 75 μm x 10cm, tip 15m, New Objective, Woburn, MA, USA). The PicoFritTM column is placed directly in front of the mass spectrometer, limiting the peak dispersion after the column. The peptides were loaded onto the strong cation ion exchange column. The peptides were eluted from

the SCX column by injection of increasing salt concentrations (40, 100, and 400 mM for BSA) and loaded onto one of the peptide traps, while the other peptide trap and the PicoFrit™ column run the 0% to 65% mobile phase B gradient in 90 minutes. The reversed phase solvents used were 0.1% formic acid in water (A) and 0.1% formic acid acetonitrile (B) solutions. The solvents for sample pump were 0.1% formic acid in water (C). A flow rate of 75µL/min before the split and 200 nl/min after the split was used for MS pump, and a flow rate of 150µL/min before splitting and 2 µl/min after the splitting was used for sample pump. The eluted peptides were analyzed by an LCQ Deca XP Plus ion trap mass spectrometer. The spray voltage was 1.8 KV and the capillary temperature was 160 °C and 35 units of collision energy were used to get fragment spectra. Two MS/MS spectra of the most intense peaks were obtained followed by each full scan mass spectrum. The dynamic exclusion feature was enabled to obtain MS/MS spectra on co-eluting peptides.

2D Nanospray for ProteomeX Conversion Parts List

Upchurch:

PeekSil Tubing (4 needed) 1/32 OD 25um ID 10 cm # 32510

PeekSil Tubing (1 needed) 1/32 OD 25 um ID 30 cm # S-32530 (only ThermoFinnigan can order this item)

Fitting (4 needed) 1/32 OD tubing for Valco Valve # M-645

Ferrule (5 needed) 1/32 OD tubing for peptide traps and connection to nanospray # F-113

Nut (5 needed) 1/32 OD tubing for peptide traps and connection to nanospray # F-300

Peptide trap holder (2 needed) # A-356

Microm Bioresources:

Peptide Traps (2 needed) # 004/25109/32

New Objective:

PicoFrit columns # PFC7515-BI-10-3PK

Valco:

Fittings (4 needed) 1/32 OD tubing for Valco Valve # C-NNFLFPIC

Spare Parts: (recommended)

All PeekSil Tubing 1 extra

Fittings 4 extra

Ferrules, Nuts 4 extra

Peptide traps 2 extra

Option:

Agilent peptide trap and holder

5065-9915 holder

#5065-9913 trap