Description of a Novel Multi-Column/Multi-Dimensional LC-MS/MS Platform for Automated Proteome Analysis

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Objective

We demonstrate a unique multi-dimensional nano-LC/MS platform system for the analysis of complex proteomes.

Background

Multi-dimensional LC-MS/MS for the analysis of complex biological samples has been utilized extensively. On-line techniques, like MUDPIT[1], use capillary column and nano-flow chromatography in two dimensions but are limited to single-use columns. Off-line techniques can fractionate larger quantities of material repeatedly on the same column but require analytic scale columns and flow rates, and fractions require concentration and analysis on a nano-column. Our goal is to design a system that combines the larger sample capacity of offline systems to nano-flow rates seen in on-line methods without sample loss or changing columns.

System Characteristics

- Two separation dimensions (SCX and RP) provide comprehensive analysis of complex mixtures.
- Analytical SCX column (1x50mm) enables high sample loading capacities (20-100ug) coupled to three modular nanospray RP columns (0.075x100 mm) that provide sensitive MS detection.
- At any point in time, peptides are being loaded on one column, eluting from another column, while the third column is being washed and re-equilibrated.

Novel Aspects

- On-line analysis reduces sample losses associated with off-line fraction collection, drying, and reconstitution.
- Increased throughput is achieved with three columns that afford continuous loading, elution, and washing of the reverse phase dimension and continuous acquisition of MS data.
- Increased dynamic range by enabling the use of larger quantities of starting material.
- Chromatographic reproducibility is achieved with modular manufactured columns, three parallel pumps with isolated flow paths, and simplified pumping and low volume connections.
- A three column nanospray source with translational slide, and control software provides full automation.
- Simplified linear flow paths reduce system complexity and eliminates channel-to-channel variability.

Methods / Instrumentation

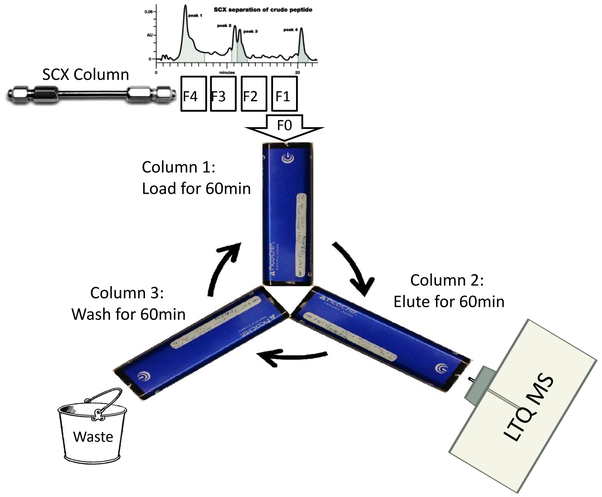

Fig 1. Diagram showing the simultaneous on-line loading of SCX separated peptides, elution via reverse phase, and washing/re-equilibrating of three PicoCHIP nanospray columns.

Lysis/Digestion: Yeast (strain YJM789) was prepared according to published protocol[2]

Hardware: Dionex Ultimate 3000 RSLC system containing a total of 4 gradient pumps, Thermo LTQ MS, New Objective PicoSLIDE column switching system and Valco linear port valve using chemistry as follows:

- SCX mobile phase A is water/0.1% formic acid
- SCX mobile phase B is 500mM ammonium acetate pH 2.9.
- RP buffer A is water/0.1% formic acid
- RP buffer B is acetonitrile/0.1% formic acid

nLC-MS² analysis: Using a Dionex U3000 RSLC system containing three sets of binary pumps, 18ug of digested yeast (strain YJM789) peptide was loaded onto a 5x1mm Sigma BioBasic SCX column with SCX-A at 300nL/min for 60min. During a ten hour gradient from 0mM to 400mM SCX-B, **three phases run simultaneously** in 60min segments. Valve and column changes occur at the beginning of every 60min segment in the order Load->Elute->Wash. Reverse phase electrospray columns used are New Objective 0.075x100mm 5µm Reprosil R0 modular columns.

- LOAD: Peptides eluting off the SCX column are loaded onto one RP nanospray column at 300nL/min for up 60min in 100% RP buffer A.
- ELUTE: A reverse phase gradient from 0% RP buffer B to 35% elutes loaded SCX peptides through the second nanospray column into a Thermo LTQ MS.
- WASH: After eluting the column is desalted for 15min with 100% RP buffer A to prevent salt precipitating in high organic, washed for 25min with 70% RP buffer B, then equilibrated with 100% RP buffer A for 20min.

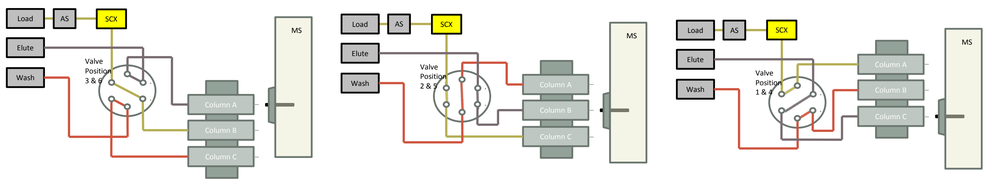

Fig 3. Schematic depicting linear isolated Load/Elute/Wash pump flow paths and valve switching mechanism when each column is in position and eluting peptides.

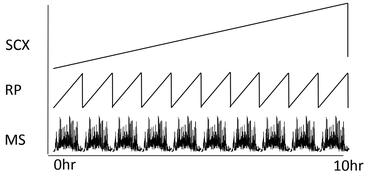

Fig 4. Diagram detailing the timing of the salt gradient, reverse phase gradients and the acquisition of the MS data.

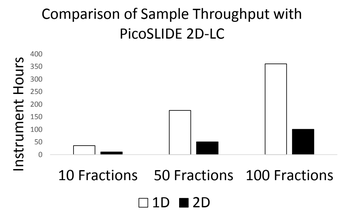

Fig 5. Instrument time needed to run a multi-dimensional fractionation method can be reduced by 70% or more depending on gradient lengths, data based on 1hr load/elute cycles.

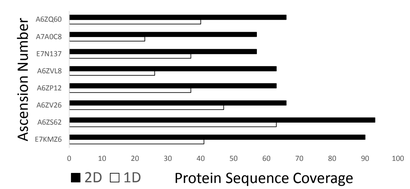

Fig 6. Example proteins eluting in both 1D and 2D were selected at random for coverage assessment. Initial experiments show at least 2-3x improvement during a 60 minute run.

Results

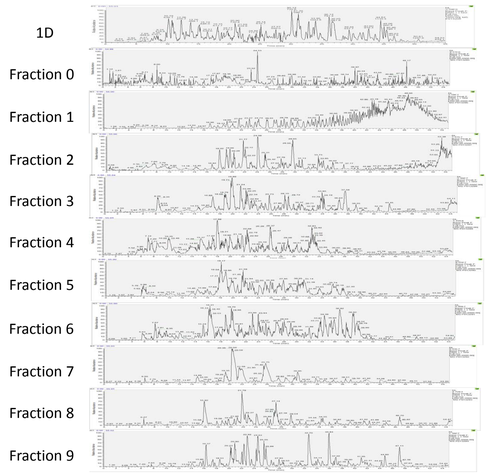

Fig 2. Representative 1D chromatogram compared to ten 2D salt fractions from the same sample, showing separation of distinct populations of peptides in each 60min fraction segment. Initial experiments show a 3-fold increase in protein identification, a 2-3x increase in sequence coverage, and a quantitative increase in the integrated peak areas that was proportional to the total amount of protein loaded on the SCX column. Future work will aim to optimize the analysis time, loading capacity, separation dimensions and overall performance of the system.

Acknowledgments

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References:

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2. Hebert AS, Richards AL, Bailey DJ, Ulbrich A, Coughlin EE, Westphall MS, Coon JJ. Mol Cell Proteomics. 2014 Jan;13(1):339-47.