Multiphase trap chips for improved protein identification and quantitation in discovery and targeted proteomics

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ABSTRACT

Over the past decade, proteomics has become one of the dominant applications in understanding complex biological and disease derived processes. Typically, numerous sample preparation and processing steps are required, prior to mass spectrometric (MS) analysis, to reduce the sample complexity for improved identification and quantification. One of the commonly employed strategies to reduce sample matrix is through offline fractionation; however, it can be time consuming and may involve multiple manual sample handling steps that are prone to significant sample losses. Here we report the development of an online, multiphase trap chip for efficient trapping and fractionation of peptides that is suitable for proteomics applications.

INTRODUCTION

Unlike genomics, advancement in proteomic analysis has been impeded in the past decade due to the complex nature of the protein matrix which spans a dynamic range up to 12 orders of magnitude in plasma,[1] and the lack of PCR-equivalent technique to amplify the low-abundant species. Numerous offline sample fractionation strategies had been proposed to reduce sample matrix; however, lack of automation and significant sample losses during transferring steps had limited their use. Perhaps the most promising techniques to reduce sample complexity are online 2D-LC and long column approaches. Both the orthogonal natural of the 2nd dimension, and longer column length greatly improve the separation capacity, but the performance of 2D-LC methods are often offset by poor resolution of the 1st dimension with the same peptides elute in multiple fractions,[2] while long column approaches are offset by high back pressure and long gradient time with limited gain in peak capacity.[3] Hoping to address these issues, we developed a multiphase trap chip which allows efficient trapping and separation of peptide fractions for improved protein identification and quantitation.

With this new multiphase trap chip, we have developed a 5-step fractionation scheme in a trap-elute type configuration to effectively separate digested peptides and demonstrated utility with both non-depleted human plasma and cancer cell line lysates. This workflow shows exceptional peptide retention reproducibility, and highly distinct peptide elution profile with about 90% of all identified peptides found exclusively in only one fraction. Although the total analysis time for the multiphase trap workflow is ~8hr, we anticipate the improved identification and quantification of targeted peptides with minimal peptide overlap throughout fractions outweigh the longer analysis time, and the simplicity of this automated multiphase trap chip workflow makes online fractionation more accessible and useful to discovery and targeted proteomics applications.

MATERIALS AND METHODS

Chip Design and Fabrication
Multiphase trap chips were fabricated using standard multilayer photolithography. Multiphase chip is composed of 3 segments (1x1x1cm, 200µm ID), and each segment was packed in order of reverse phase-strong cationic exchange-reverse phase (RP-SCX-RP) respectively. Multiphase trap chips work with the standard cHiPLC® system (Eksigent, part of AB SCIEX) configuration (Figure 1a).

Materials
2 types of SCX phases were used, 3µm and 5µm PolySULFOETHYL A (PolyLC Inc.). 5µm C18-300Å was used as RP phases. Non-depleted human plasma and cell lysate digests from SW-1736 thyroid cancer cell line were used to evaluate the performance of this new multiphase trap chip LC-MS workflow.

NanoLC Conditions:
NanoLC Ultra 2D system (Eksigent, part of AB SCIEX) coupled with standard cHiPLC® system was used for online fractionation and separation of digested peptides. In a typical workflow (Figure 1b), peptides were loaded onto the multiphase trap, and separated using either 4 or 5 salt steps (0 - 1500mM ammonium acetate) followed by 30min acetonitrile gradients using a 15cm, 75µm or 200 µm ID RP chip analytical column.

MS/MS Conditions:
Eluted peptides were either analysed by SRM-MS on a 4000 QTRAP® system (AB SCIEX) or MS/MS on a TripleTOF® 5600 system (AB SCIEX). Peptides were searched against the human SwissProt database using MASCOT with a precursor mass tolerance of 50 ppm and fragment mass tolerance of 0.5 Da.

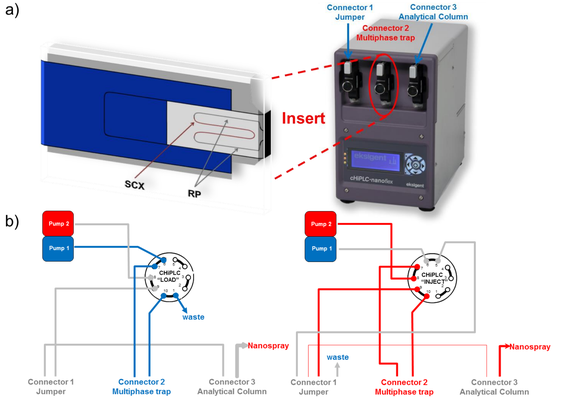
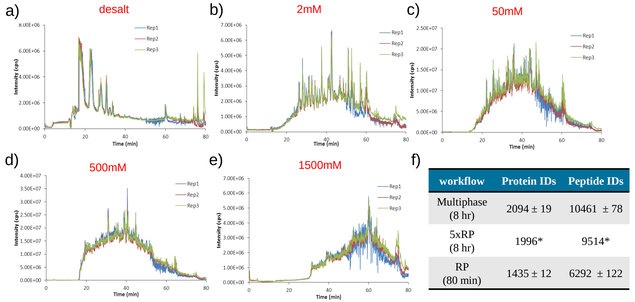


Figure 1. Multiphase trap chips. a) Chip design. b) Multiphase trap chip for online SCX fractionation followed by RP separation.

RESULTS



workflow	Protein IDs	Peptide IDs
Multiphase (8 hr)	2094 ± 19	10461 ± 78
5xRP (8 hr)	1996*	9514*
RP (80 min)	1435 ± 12	6292 ± 122

Figure 2. Reproducibility of online fractionation workflow using the multiphase trap chip. 5 fractionation steps separation of Thyroid cancer cell line SW1736. The steps consists of a) desalt, b) 2mM ammonium acetate, c) 50mM ammonium acetate, d) 500mM ammonium acetate, and e) 1500mM ammonium acetate. f) protein and peptide identification of cell lysate analyzed by 5 step multiphase (5 µg), 1 hr. RP (5 µg) and 5x 80 min RP (5x5 µg), * sum of 5 RP runs, not performed in triplicates, thus SD calculation not applicable.

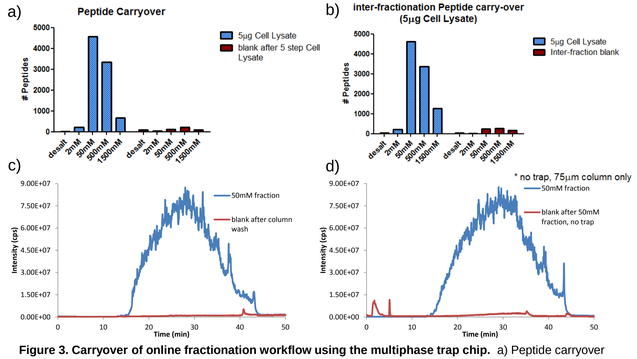


Figure 3. Carryover of online fractionation workflow using the multiphase trap chip. a) Peptide carryover in a blank after the analysis of 5µg cell lysate indicates minimal carryover from multiphase chip. b) Peptide carryover in a blank after each fractionation step, no trapping of the blank injection shows most carryover is contributed by the analytical column. c) Overlaid TIC traces of 50mM fraction of the analysis of 5µg cell lysate and its blank after column wash. d) Overlaid TIC traces of 50mM fraction of the analysis of 5µg cell lysate and its blank after the fractionation step, no trapping of the blank injection.

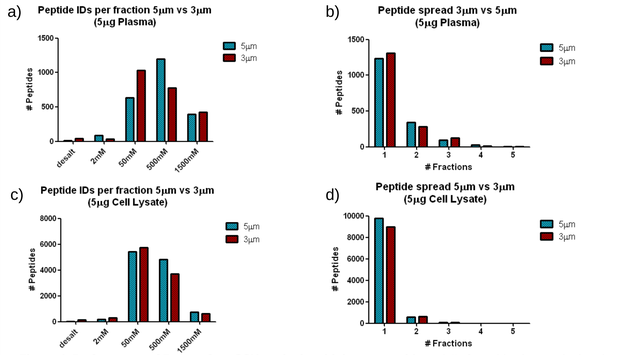


Figure 4. Performance of 5µm vs 3µm SCX packed multiphase trap chips. a) # of peptides in non-depleted plasma, and c) cell lysate peptide identification across all fractions. b) # of proteins in non-depleted plasma, and d) cell lysate peptide spread across all fractions. The results indicated comparable performance of the 5µm against 3µm SCX phase. 5µm SCX phase showed slightly greater performance.

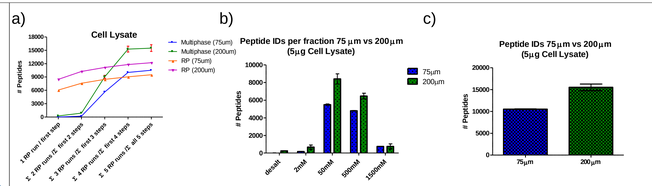


Figure 5. Performance of 5 µm multiphase trap chip follow by 75 µm or 200 µm ID RP analytical column. a) Cumulative unique peptide IDs of 5 step Multiphase (5µg) or 5 x 1 hr RP (5x5 µg = 25 µg) analysis of cell lysate. b) Peptide identification across fractions of cell lysate. c) Total unique peptide identification in cell lysate.

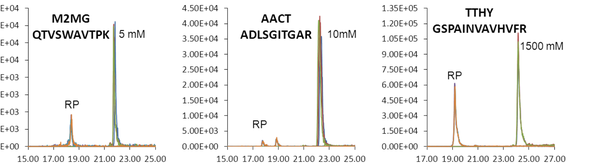


Figure 6. Multiphase chip separation vs RP chip separation applied to selected reaction monitoring (SRM) for quantitation. Extracted Ion chromatograms of selected SRM peptides in nondepleted human plasma demonstrate increased signal following multiphase fractionation.

CONCLUSIONS

This new multiphase trap cHiPLC-MS workflow demonstrated exceptional separation and reproducibility of peptides from cell lysates and human plasma samples. Key to the performance is the excellent precision of the salt-based fractionation (Fig. 4b & 4d), increased depth of coverage (Fig. 5) , compatibility with small sample loads and complete hands-free operation.

The excellent reproducibility is a key feature that enables ready integration with SWATH-MS workflows (see Krisp et al., ThOE 2.50pm)

REFERENCES

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