High-Throughput Robotic Nanoliter Sample Manipulation with SLIM IM-MS Analysis

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Overview

- First online coupling of nanoPOTS (**nano**droplet **P**rocessing in **O**ne pot for **T**race **S**amples) robotic system with Structures for Lossless Ion Manipulations (SLIM)
- nanoPOTS provides a platform for high-throughput small sample manipulations (<25 nL)
- High sensitivity SLIM IM-MS platform allows for rapid (~sec) analysis of small sample volumes with long-path (>6 m) traveling wave ion mobility (TW IM) separations for improved peak capacity

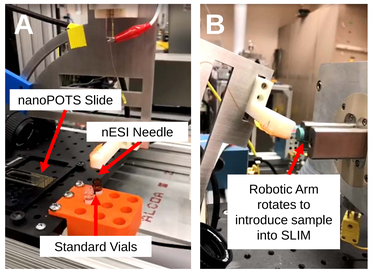
Introduction

- SLIM has been used for improved proteomics analysis by direct analysis of complex samples without online separations[1]
- SLIM provides ultrahigh resolution TW IM separations for separation of peptide isomers that are otherwise difficult to separate[2]
- Accumulation of large ion populations within the SLIM IM module provides increased sensitivity[3]
- The nanoPOTS platform allows for sampling and manipulation of very small volumes[4]
- In this work, the nanoPOTS robotic system is coupled with SLIM IM-MS for rapid, small volume sampling of various proteomics samples including BSA digest; LC-fractionated HeLa cell extracts collected and reconstituted on a nanoPOTS plate; and heavy-labeled phosphopeptides with several challenging isomers

Methods

- **Traveling wave (TW) SLIM IM-MS module coupled to Agilent 6538 QTOF**
- **SLIM Module:** 12.3 m path length; 5,4 electrode arrangement; Operated at 2.5 Torr nitrogen gas[5]
- **Onboard Accumulation:** 6 m ion accumulation region; achieved by blocking ions at interface between two major TW regions
- **TW Parameters:** TW Amplitude 25 V_{p-p}; TW speed of 142 m/s; Guard electrode +15 V
- **Software:** Custom developed LabView-based software for control of nanoPOTS robot system. Custom software for SLIM IM-MS instrument control and data acquisition. Data processing performed using custom software tools[6] and Agilent IM-MS Browser B.08.00 and Mass Profiler

Figure 1. A) The nanoPOTS robot system utilizes a 20 µm ID nESI tip for manipulations (sample reconstitution, withdrawal, and infusion). **B)** The robotic arm then rotates the nESI tip to the capillary inlet of the SLIM IM-MS. The process is repeated for high-throughput sampling.



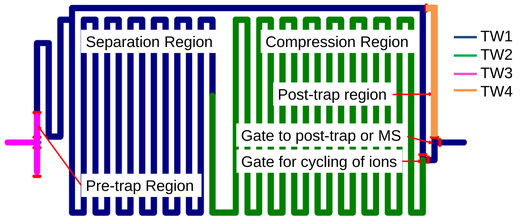


Figure 2. Layout of SLIM module indicating the various trapping and TW regions. During on-board accumulation, ions are accumulated in the first 6 m separation region before undergoing separation in the second 6 m region.

Results

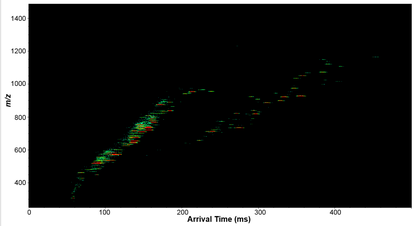


Figure 3. SLIM IM-MS analysis of BSA digest demonstrates the ability to use the nanoPOTS robot for analysis of small volumes (<25 nL) for rapid (~seconds) high-throughput sampling of complex mixtures

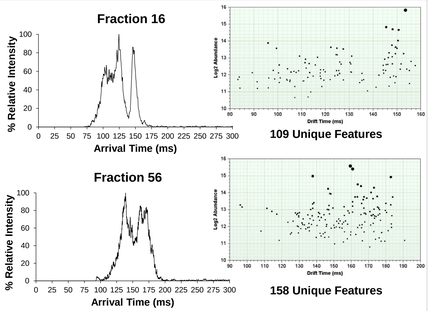


Figure 4. Rapid SLIM IM-MS analysis of 60 LC-fractionated HeLa cell extracts collected on nanoPOTS plate allows improved peak capacity (>60) and rapid identification of unique peptides (~72 features/fraction)

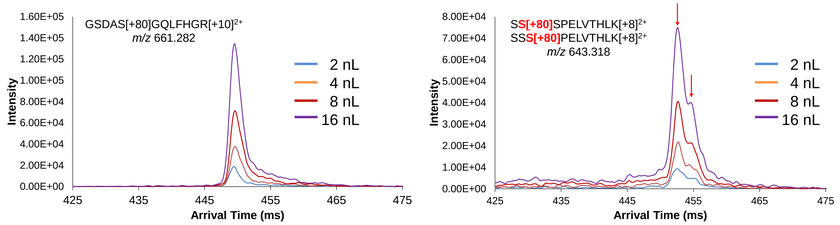


Figure 5. Analysis of 2-16 nL of phosphoproteomics samples with 18 m separation, demonstrating potential quantitative capabilities of nanoPOTS-SLIM IM-MS : A) phosphopeptide from human PLCG1 protein; and B) isomers from human RHG01 protein, with partial resolution of adjacent serine phosphorylation sites

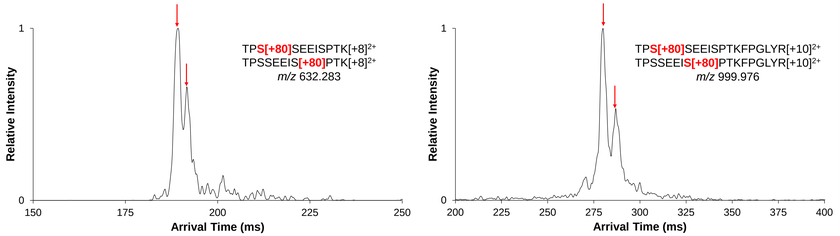


Figure 6. SLIM IM-MS separation of phosphopeptide isomers from human RBP1 protein, demonstrating the ability to separate peptides with different serine phosphorylation sites with a 6 m SLIM IM separation

Conclusions

- A nanoPOTS sample manipulation platform successfully coupled with the SLIM IM-MS platform
- Several sample types were analyzed including a BSA digest; LC-fractionated HeLa cell extracts collected and reconstituted on a nanoPOTS plate; and heavy-labeled phosphopeptides with several challenging isomers
- Very small sample volumes (<25 nL) and a SLIM IM module using on-board ion accumulation were utilized for rapid, high sensitivity measurements
- This combination provides for analysis of complex mixtures (BSA digest, HeLa cell extracts) with improved peak capacity for identification of unique peptides
- Ultrahigh resolution SLIM IM-MS platform allows for separation of peptide phosphorylation site isomers; much greater resolution feasible
- Current studies underway to further investigate the quantitative capabilities of the nanoPOTS-SLIM IM-MS system

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