CYCLIC ION MOBILITY ENABLED MASS SPECTROMETER AND APPLICATION TO HIGH THROUGHPUT PLASMA PROTEOMICS

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INTRODUCTION

Prostate cancer is a leading cause of cancer deaths for men in the U.S., with around 1 in 9 being diagnosed with the disease each year. Numerous OMIC-based studies into the disease have been conducted, proposing potential markers. However, in order to provide a comprehensive and statistically valid data set, samples from a large cohort of individuals are required. This ultimately provides an analytical challenge, particularly for proteomics research, where nanoscale chromatography is routinely adopted [1].

Here, we initially evaluate Cyclic™ IMS for the analysis of proteomic standards and then demonstrate high throughput strategies for proteomic profiling of plasma from prostate cancer individuals.

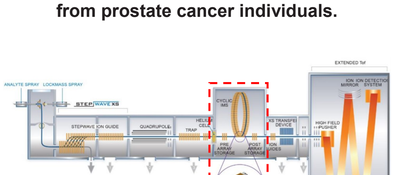


***Figure 1**. SELECT SERIES™ Cyclic IMS QToF Mass spectrometer.*

The highlighted IMS device allows multiple 1m IMS 'passes' to increase Ion Mobility resolution

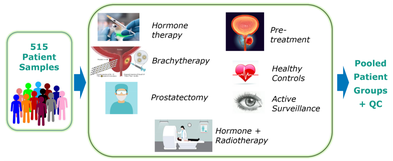
METHODS

SAMPLES

Discovery Proteomics:

Human K562 tryptic digest (Promega)

High Throughput Plasma Proteomics:



LIQUID CHROMATOGRAPHY

Nanoscale Discovery Proteomics:

Acquity™ M-Class
180 µm x 20mm trapping
75 µm x 25 cm HSS T3 analytical column
Solvent A: Aqueous 0.1% Formic Acid (FA)
Solvent B: 0.1% FA in acetonitrile
Trapping: 5 µL/min for 2 minutes
Analytical Flow Rate: 300 nL/min
Gradients: 5 to 35% B over 90 to 240 minutes

High Throughput Plasma Proteomics:

Acquity Premier I-Class
2.1 mm x 10 cm CSH column
Solvent A: Aqueous 0.1% FA
Solvent B: 0.1% FA in acetonitrile
Analytical Flow Rate: 150 µL/min
Gradients: 5 to 35% B over 15 minutes

MASS SPECTROMETRY

ESI +ve
50000 FWHM Mass Resolution
65 FWHM 'Single Pass' Ion Mobility Resolution
HDMS^E Acquisition Mode
50—2000 amu Mass Range
0.5 s (nano) and 0.15 s (HT) Integration Time

DATA PROCESSING

ProteinLynx Global Server and Progenesis™ QI for Proteomics
TIBCO Spotfire®
Metaboanalyst 5.0 [2]
Uniprot species specific protein databases
1% and 4% FDR

RESULTS—NANOSCALE

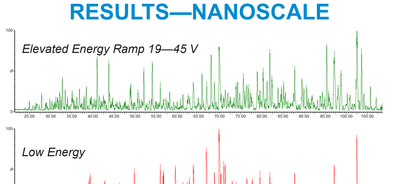


***Figure 2.** Nanoscale LC Separation of Human K562*

*During HDMS^E, the acquisition alternatively produces low energy and elevated energy chromatograms. The elevated energy fragmentation occurs in the transfer collision cell, which is located after the ion mobility separation indicated in **Figure 1**, hence precursors and fragments exhibit the same drift time information. During data processing, the fragment ions from the elevated scan are aligned with precursor information from function 1, based upon both retention and drift time information. The aligned data is then constructed into a binary file which can be readily database searched .*

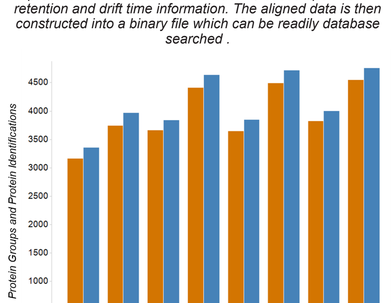


***Figure 3.** Database searches of Human K562 data*

*Protein identifications representing **protein groups**, whereby homology is collapsed and the **total number of proteins**, whereby homology is included, are shown. The corresponding peptide identifications follow a similar pattern, maximizing at approximately 60000 peptides. The data represents database searches consisting of an FDR of 1 and 4% with sample loadings from 25 to 100ng. Based on a 90 minute gradient, the data suggests that a loading of 50-75 ng is optimum. When increasing gradient length, peptide ions become more separated and data processing algorithms are more easily able to distinguish between ions. Identifications returned at 1% FDR increase from 3700 for 90min to 4500 for 240 minute. These data were derived from searching against a reviewed Uniprot database and, when searching the same data against a custom database containing homologs [3], the maximum identification reaches over 7200 proteins, 1% FDR.*

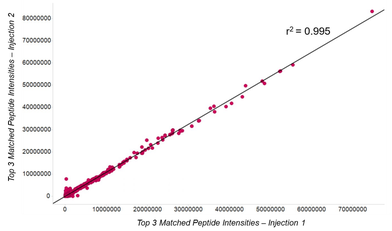


***Figure 4.** Nanoscale LC Reproducibility*

An important aspect for discovery experiments is injection to injection reproducibility, both in terms of signal intensity for quantitation purposes and quality of spectra for identification rates. These metrics are also an indication of the robustness of measurements achievable with the LC-MS system where some sample data acquisitions with tens or hundreds of samples can last several weeks. Shown is a comparison between protein signal intensities of two replicate injections, specifically using the top3 most intense peptides per protein from the search results. Translating this reproducibility into the identification rates obtained in 2 out of three injections 3602 proteins were identified with a 1% FDR, which equates to 78% of the total identifications

HIGH THROUGHPUT PLASMA PROTEOMICS

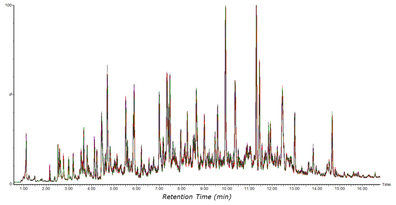


***Figure 5.** Analytical Scale LC Robustness*

Each sample was acquired in triplicate and each set of 24 injections run in a randomized manner. 500 ng of the plasma tryptic digest was injected on-column for each MS acquisition.

Shown is the typical chromatography obtained from the plasma digest samples and is an overlay of 10 chromatograms taken randomly throughout the analysis. Key to successful analysis of clinical proteomic samples, is the reproducibility and robustness of the LC-MS system.

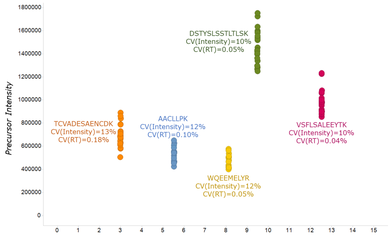


***Figure 6.** Retention Time and Protein Identification Reproducibility*

Each raw datafile was processed using Progenesis QI for Proteomics and extracting processed data for five different peptides from three different proteins over the whole elution time, excellent reproducibility for retention time and signal intensity is shown. A maximum coefficient of variance (CV) of 13% was measured for signal intensity, whilst a maximum of 0.18% CV was calculated for retention times.

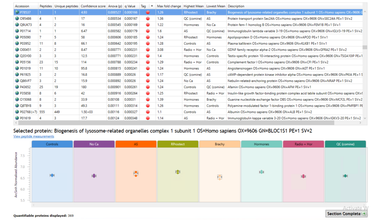
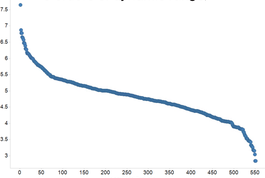


***Figure 7.** Protein Quantitation and Dynamic Range*

TOP: The 8 samples analysed, comprising 24 runs in total, were specified in the PQIP experimental set up and it is found that 369 proteins can be reproducibly quantified at an ANOVA p value of <0.05. These searches were performed using the reviewed uniprot human database.
BOTTOM: In addition, 551 proteins were identified in at least one injection and matched peptide intensities covered nearly 5 orders of dynamic range,



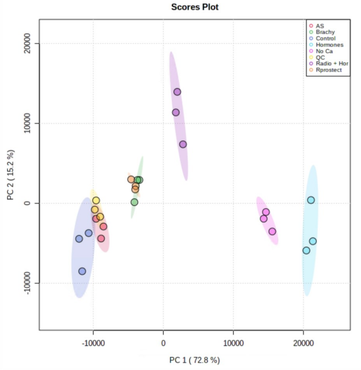


***Figure 8.** PCA Separation of sample groups*

The data were exported from the PQIP into Metabonanalyst for additional statistical analysis. Unsupervised principal component analysis (PCA) showed clear separation between the different sample treatments or disease states in the experiment .

CONCLUSIONS

- **The performance characteristics of Cyclic IMS in qualitative discovery and quantitative plasma proteomic experiments have been described.**
- **Cyclic IMS is found to exhibit excellent performance for the analysis of complex peptide digest mixtures.**
- **Clear separation observed using PCA between different groups of disease state or treatment has been demonstrated.**
- **Excellent reproducibility and robustness has been demonstrated, suggesting that analysis of a large sample cohort would be possible.**

References

1. Lennon et al., High-Throughput Microbore Ultrahigh-Performance Liquid Chromatography-Ion Mobility-Enabled-Mass Spectrometry-Based Proteomics Methodology for the Exploratory Analysis of Serum Samples from Large Cohort Studies, J Proteome Res. 2021; 20 (3):1705-1715.
2. Pang et al., (2021) MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights Nucl. Acids Res. (doi: 10.1093/nar/gkab382)
3. Meier et al., 2018, Molecular & Cellular Proteomics, *17*, 2534–2545

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