Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/jprot

Performance evaluation of a dual linear ion trap-Fourier transform ion cyclotron resonance mass spectrometer for proteomics research☆

Chad R. Weisbrod[a], Michael R. Hoopmann[a], Michael W. Senko[b], James E. Bruce[a,*]

[a]Department of Genome Sciences, University of Washington, Seattle, WA, United States
[b]Thermo Scientific, San Jose, CA, United States

ARTICLE INFO

Available online 13 April 2013

Keywords:
Velos-FT
Dual cell fragmentation
Instrumentation
Dynamic range
Yeast
Duty cycle

ABSTRACT

A novel dual cell linear ion trap Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) and its performance characteristics are reported. A linear ion trap-Fourier transform ion cyclotron resonance mass spectrometer has been modified to incorporate a LTQ-Velos mass spectrometer. This modified instrument features efficient ion accumulation and fast MS/MS acquisition capabilities of dual cell linear RF ion trap instruments coupled to the high mass accuracy, resolution, and dynamic range of a FT-ICR for improved proteomic coverage. The ion accumulation efficiency is demonstrated to be an order of magnitude greater than that observed with LTQ-FT Ultra instrumentation. The proteome coverage with yeast was shown to increase over the previous instrument generation by 50% (100% increase on the peptide level). In addition, many lower abundance level yeast proteins were only detected with this modified instrument. This novel configuration also enables beam type CID fragmentation using a dual cell RF ion trap mass spectrometer. This technique involves accelerating ions between traps while applying an elevated DC offset to one of the traps to accelerate ions and induce fragmentation. This instrument design may serve as a useful option for labs currently considering purchasing new instrumentation or upgrading existing instruments.

Biological significance
A novel hybrid mass spectrometer that allows increased MS/MS acquisition rates with high mass measurement accuracy and new ion fragmentation methods greatly improves the number of proteins, posttranslational modifications and protein–protein interactions that can be identified from cells.
This article is part of a Special Issue entitled: New Horizons and Applications for Proteomics [EuPA 2012].

1. Introduction

The era of modern mass spectrometry has largely been dominated by advancements in instrumentation [1–6], sample handling [7–10], and database searching technologies [11–13]. Innovations made in these areas have made possible incredible improvement in the analysis of complex samples. The drive to decipher information within the proteome [8,10] has been a

☆ This article is part of a Special Issue entitled: New Horizons and Applications for Proteomics [EuPA 2012].

* *Corresponding author at:* Department of Genome Sciences, University of Washington, 815 Mercer St., Seattle, WA 98109, United States. Tel.: +1 206 543 0220; fax: +1 206 616 0008.
E-mail address: jimbruce@uw.edu (J.E. Bruce).

1874-3919/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jprot.2013.04.009

major force influencing technological development of mass spectrometry methods for biological applications. The current paradigm in proteome research involves shotgun [9] or bottom-up experiments in which protein samples (pure protein, cell lysates, tissue lysates, etc.) are enzymatically digested into peptide mixtures. The resulting peptide mixtures can have wide dynamic range in peptide concentration [14] and heterogeneity [15]. In fact, the overall complexity of these peptide mixtures exceeds the sensitivity and efficiency in detection of all available modern instruments [4]. The primary impediments to routine whole organism proteome measurements by mass spectrometry are acquisition speed, sensitivity, and dynamic range. Further development of instrumentation technology is required for routine high coverage, in-depth proteome analyses.

Mass spectrometry instrumentation [16] has evolved dramatically over the last twenty years. A current configuration for proteomics research today is a hybrid type and consists of two coupled mass analyzers, each capable of independent data acquisition. A conventional example combines a linear ion trap (LTQ) with a Fourier transform mass spectrometer (FTMS). In this case, the FTMS is either an ion cyclotron resonance mass spectrometer (LTQ-FT) or Orbitrap mass spectrometer. In general, hybrid mass analyzers benefit proteomic research by incorporating desired characteristics from each analyzer to yield unique capabilities.

Here we present results of our efforts to modify the LTQ-FT hybrid by combining a dual cell linear RF ion trap and FT-ICR mass spectrometer, referred to as a Velos-FT mass spectrometer. This instrument has many unique attributes such as the ability to rapidly accumulate ions, MS^n analysis at a higher repetition rate, and the ability to perform beam-type CID fragmentation between the dual cell linear RF ion traps. The Velos-FT has improved ability to accumulate ions, with observed ion accumulation time reduction of an order of magnitude or more (~3 ms vs. 40 ms) compared with conventional LTQ-FT instrumentation. When performing MS^n, selected ion monitoring (SIM), or data independent acquisition (DIA), this speed improvement can be very significant. Related to this, we show that with the Velos-FT, the number of peptide identifications per run is increased by 100% when compared with the LTQ-FT Ultra (50% on the protein group level). Top down proteomics [17] requires isolation and fragmentation prior to analysis. For top-down experiments, in many cases it is difficult to accumulate enough ions to achieve coherent cyclotron motion for the duration required to resolve high mass ions [18,19]. Here we show that the ability to accumulate large biomolecules with the custom Velos-FT is greatly improved over those with the LTQ-FT Ultra. The dual cell linear RF ion trap instrument configuration enables operation of a unique fragmentation method, which we call dual cell fragmentation (DCF), performed by transferring ions between the two cells through the background gas using elevated DC potentials to accelerate and induce dissociation. This technique generates fragmentation patterns which share some similarities with spectra acquired on QTOF and triple quadrupole instruments. The so called "1/3 rule" which limits the lower mass limit for product ions generated by resonance excitation in RF based ion traps was shown to be reduced with DCF. The overall peptide identification rate was comparable to that obtained with resonance excitation CID, and many peptides were identified in both methods demonstrating the utility of DCF for peptide identification with spectra that also contain lower m/z ions. Furthermore, a distinct subset of peptides was identified with each method that may be a result of repeated analyses or subtle differences between the fragments observed in DCF and CID. Finally, with the cost of high performance instrumentation currently inflating much faster than available funds from most granting agencies that support mass spectrometry, the upgrade design of the Velos-FT presented here may present a useful option for other labs to consider.

2. Experimental

2.1. *Modification of the LTQ-FT Ultra*

An LTQ-FT Ultra mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) was modified to include an LTQ Velos mass spectrometer. The linear ion trap (LTQ) was removed and replaced with a dual cell linear radio frequency ion trap mass spectrometer[5] (Velos, Thermo Fisher Scientific, San Jose, CA). The two linear radio frequency ion traps will be referred to throughout the manuscript as the high pressure cell (HPC) and low pressure cell (LPC). No ion optic modifications were necessary for the coupling process as the flanges designed to mate the LTQ and Velos to the Orbitrap or the FT mass spectrometer are identical. However, ion trap control software modifications were required for operation of the custom FT-ICR. Software in control of the LPC was modified so that it acts as a simple RF multipole during the transmission of ions from the HPC to the ICR cell for FT-MS acquisition mode. The standard FT ion transmission calibration script was modified to include the center lens, front, center and back sections of the LPC, along with the back lens of the dual cell trap assembly. Optimized DC voltages for each of these elements are applied during the transmission event of an FTMS acquisition. The ion transfer efficiency between the linear ion trap and ICR cell was determined to be similar for both the LTQ-FT Ultra and custom FT-ICR MS based upon unscaled total ion current measurements conducted using the same ion target value for both instruments (see Supplemental data for details).

2.2. *Ion-Trap Control Language (ITCL) DCF program*

Direct modification of factory installed ITCL code was performed in-house for the implementation of DCF. These modifications operate within the framework of the factory installed code for the LTQ-FT Ultra mass spectrometer. In short, CID collision energy setting of 1.0 in the user interface was used to enable DCF. This allows for the execution of DCF both directly from Tune as well as during method based operation. When DCF is enabled, ion accumulation and isolation proceed in the HPC without alteration of factory installed ITCL code. However, no resonance excitation is applied before initial transfer to the LPC. Ions are then transferred immediately back to the HPC. The HPC center section potential is adjusted to induce fragmentation through application of a large negative DC bias. This DC potential bias is determined as a function of precursor ion m/z, in a similar fashion as with beam-type CID collision cell experiments [20].