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An electrodynamic ion funnel interface for greater sensitivity and higher throughput with linear ion trap mass spectrometers

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Abstract

An electrospray ionization interface incorporating an electrodynamic ion funnel has been designed and implemented on a linear ion trap mass spectrometer (Thermo Electron, LTQ). We found ion transmission to be greatly improved by replacing the standard capillary–skimmer interface with the capillary–ion funnel interface. An infusion study using a serial dilution of a reserpine solution showed that ion injection (accumulation) times to fill the ion trap at a given automatic gain control (AGC) target value were reduced by ∼90% which resulted in an ∼10-fold increase in peak intensities. In liquid chromatography tandem MS (LC–MS/MS) experiments performed using a global protein digest sample from the bacterium, *Shewanella oneidensis*, more peptides and proteins were identified when the ion funnel interface was used in place of the standard interface. This improvement was most pronounced at lower sample concentrations, where extended ion accumulation times are required, resulting in an ∼2-fold increase in the number of protein identifications. Implementation of the ion funnel interface on a LTQ Fourier transform (FT) mass spectrometer showed a ∼25–50% reduction in spectrum acquisition time. The duty cycle improvement in this case was due to the ion accumulation event contributing a larger portion to the total spectrum acquisition time.

Keywords: Electrospray ionization; Electrodynamic ion funnel; Linear ion trap; Ion accumulation time; Sensitivity

1. Introduction

The linear ion trap mass spectrometer, a recent addition to the MS family, has increased ion capacity, improved ion trapping efficiency, and faster cycle times compared to the three-dimensional (3D) Paul trap mass spectrometer [1,2]. However, even with this increase in performance, major ion losses still occur in the electrospray ionization (ESI) interface. The benefits of reducing this ion loss is not as evident as it may seem due to the low ion population requirements of the linear ion trap (typically ∼30,000 ions) which can be easily delivered by most ion sources in a short period of time (∼1 ms) using a conventional interface (e.g. capillary–skimmer interface). The resulting short ion accumulation (ion injection) times to fill the ion trap in most cases only account for a small portion of the overall duty cycle. In addition, these instruments employ a technique called automatic gain control (AGC) which dynamically adjusts the ion injection time according to the intensity of the ion flux entering the ion trap [3,4]. This ensures that the optimal ion population is maintained regardless of the ion beam intensity which further obscures how much improvement can be realized by reducing ion loss in the ESI interface. We have addressed this question by designing an ESI interface that replaces the traditional skimmer with an electrodynamic ion funnel, and tested the effects of an increased ion beam on the linear ion trap mass spectrometer performance using both direct infusion and liquid chromatography (LC) MS experiments.

In ESI MS, ion losses can be substantial, with often only ∼1 out of every 10^2–10^4 ions generated by ESI at atmospheric pressure actually being detected using present instrument designs [5–7]. A large number of the ions are lost in the first vacuum stage of the mass spectrometer. Here, the gas stream exiting an inlet capillary or aperture rapidly expands into a supersonic jet followed by a so-called barrel shockwave and the formation of a mach disk several millimeters downstream of the capillary or aperture. Often, a conical metal skimmer is used to sample a portion of the ions in this expanding gas jet. The small size of the skimmer aperture (∼1 mm in diameter) limits both ion transmission efficiency and gas throughput preventing most of the neutral species (and entrained ions) from entering the lower pressure

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regions of the mass spectrometer. In order to reduce the ion losses associated with the skimmer, we have previously developed and reported on an electrodynamic ion funnel [8–12]. This device also has more recently been used and further explored by others on different mass spectrometers [13–15]. In contrast to the skimmer interface, the ion funnel can focus all of the ions in a broad *m*/*z* range exiting the inlet capillary and transmit them to the next lower pressure stage with a much lower degree of ion loss. Our present ion funnel design consists of a stack of ring electrodes with a front section of constant inner diameter (i.d.) creating a traditional stacked ring ion guide [16] and a back section that linearly decreases in i.d. creating an "ion funnel". A superimposed RF voltage and DC gradient is applied to the rings, which confines and transmits the ions through the device.

Several developments have further improved the performance of the ion funnel. For example, a disk electrode "jet disrupter" was placed in the entrance region of the ion funnel to disperse the gas jet exiting the inlet capillary, which reduced both the gas load to the following vacuum stage and the transmission of neutral species and charged residual clusters into the mass spectrometer [17]. The jet disrupter has also been used to regulate the ion beam intensity in order to improve mass measurement accuracy [18]. In addition, an adjustable *m*/*z* filter was created at the exit of the ion funnel to remove lower mass chemical background species in some applications [19]. Other ion funnels have been designed with duel electrospray inlets that allow combining ion streams from multiple ion sources [20]. Additionally, ion funnels have been incorporated in ion mobility spectrometers to serve as ion traps/gates and to eliminate ion diffusion loss in the drift tube, which greatly improves sensitivity [21]. The ion funnel has also been used to combine ESI and matrix-assisted laser desorption ionization (MALDI) into a single ion source [15].

Herein, we describe the new ion funnel/linear ion trap MS interface design and evaluate its performance compared with the standard ESI interface using direct infusion, LC–MS, and LC tandem MS (MS/MS) analyses of a MS calibrant and enzymatically digested protein samples. We observed an ∼90% reduction in ion trap injection times, which produced an ∼10-fold increase in the signal intensity reported by the instrument (due to the correction for the shorter ion accumulation times). Additionally, LC–MS/MS of protein tryptic digest samples that covered a range of concentrations resulted in the identification of more peptides and proteins, with increased performance at lower concentrations (∼2× the number of proteins were identified with the ion funnel interface at the lowest concentration) as a direct consequence of faster MS/MS acquisition times. Finally, the ion funnel produced a larger improvement to the duty cycle of a LTQ Fourier transform (FT) mass spectrometer which requires greater ion populations than the LTQ.

2. Experimental

2.1. Sample preparations

A 0.1 mM stock solution of reserpine (Sigma-Aldrich, St. Louis, MO, USA) was prepared by dissolving it in a 50:50 solution of n-propanol (Fisher Scientific, Pittsburgh, PA, USA) and water (Nanopure Infinity purification system, Barnstead, Dubuque, IA, USA). From this stock solution, a serial dilution was performed using a 50:50 solution of methanol (Fisher Scientific) and water (Nanopure) and 1% acetic acid (Sigma-Aldrich) to provide a range of molar concentrations from 10–0.001 μM.

Proteomic samples from the bacterium *Shewanella oneidensis* were prepared by isolating and digesting the cellular proteins, as previously described [22]. Briefly, the cells were lysed by bead beating, centrifuged for 5 min at 14,000 rpm and 4 °C, and digested for 3 h with Trypsin (Promega, Madison, WI, USA) in a 1:50 enzyme to protein ratio at 37 °C. The digested sample was cleaned using a C18 SPE column (Supelco, Bellefonte, PA, USA) and then concentrated in a speed-vac to a volume of 50 μL. A BCA protein assay was then performed to determine the final concentration. From this stock solution, a range of concentrations were made by diluting with pure water.

2.2. Ion funnel

The ion funnel consisted of 100 ring electrodes made from 0.5 mm brass plates and separated by 0.5 mm thick Teflon spacers. The tapered section of the ion funnel included 42 electrodes that decreased linearly in i.d., starting at 25.4 mm and ending at 2.5 mm. These electrodes were followed by a DC-only electrode (conductance limit) with an i.d. of 2.0 mm. The 6.5 mm diameter jet disrupter electrode was located ∼2 cm from the entrance of the ion funnel (approximately at Plate 20 of the 100 plate stack). The electrical circuitry was mounted onto two circuit boards and consisted of a chain of 500 kΩ resistors for the DC gradient and 10 nF capacitors for the RF voltage. The boards were attached to the ion funnel with two custom zero insertion force (ZIF) connectors (Tactic Electronics, Plano, TX, USA). An RF of 615 kHz and 60 $V_{p\text{-}p}$ (peak-to-peak) was applied to the ion funnel by a custom high-Q head with a built in waveform generator and RF power amplifier. The DC voltages were supplied by a 9 output DC power supply (Model TD-9500, Spectrum Solutions, Russellton, PA, USA). The DC gradient of ∼20 V/cm on the ion funnel was generated by applying 200 V on the first funnel RF/DC electrode and 3 V on the last RF/DC electrode. The conductance limit electrode was biased at 1 V, and the jet disrupter voltage was adjusted for maximum ion transmission, which resulted in a voltage range of 15–25 V less than the first funnel electrode voltage. More in depth descriptions and characterizations of ion funnels can be found elsewhere [8,20].

The ion funnel was interfaced to the linear ion trap mass spectrometer (Thermo Electron, LTQ, San Jose, CA) by a special holder that mounted the ion funnel on one side and housed the LTQ collisional quadrupole (Q00) assembly on the other side. Fig. 1 shows the placement of the Q00 in the standard LTQ interface (Fig. 1a) and the ion funnel interface (Fig. 1c). The backside of the ion funnel holder was modeled after the Q00 holder in the LTQ. The lens (L0) and the Q00 were removed from the standard LTQ interface and attached to the ion funnel holder. The ion funnel was then attached to the holder by using four threaded rods inside ceramic tubes that ran the length of

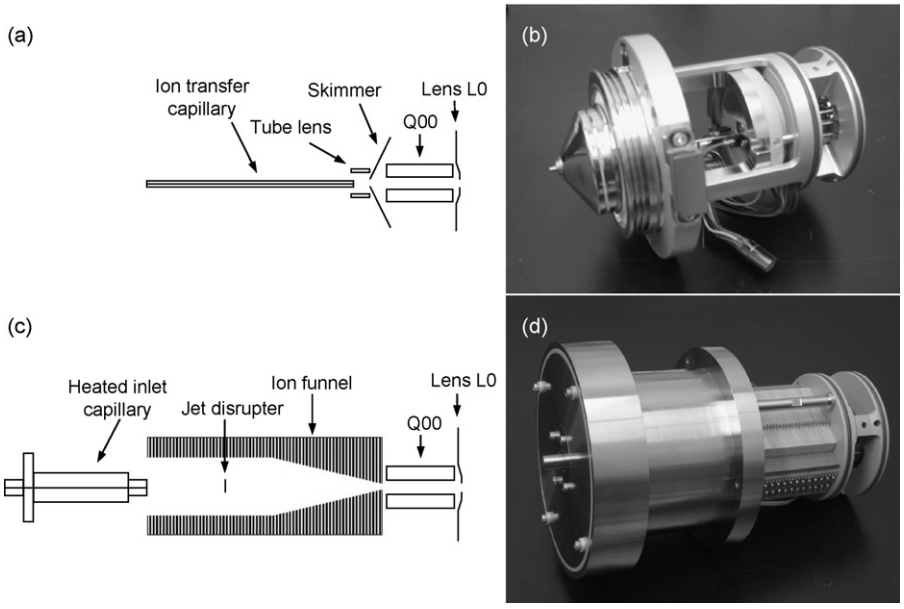

Fig. 1. Diagram and photo of the standard linear ion trap ESI-MS interface (a and b) and the ion funnel interface (c and d) showing how the ion funnel replaces the ion transfer capillary, tube lens, and skimmer and utilizes the same collisional quadrupole (Q00) and lens as the standard interface.

the ion funnel in holes machined in the electrode plates. Once mounted, there was a 2.0 mm gap between the conductance limit of the ion funnel and the Q00. The vacuum can for the ion funnel was then attached to the ion funnel holder making the ion funnel interface a single device that could be easily inserted into the LTQ instrument manifold (Fig. 1d) similar to the standard LTQ interface (Fig. 1b). A 580-μm i.d., 6.4-cm long inlet capillary was used for introduction of ions into the ion funnel and heated to 140 °C by using two 100 W cartridge heaters (Watlow, St. Louis, MO, USA). The heated capillary was also biased to 10 V higher than the DC voltage on the first ion funnel electrode. The pressure in the ion funnel chamber was 1.3 Torr, and it was pumped by the rough pump on the LTQ. The instrument was retuned after switching ion interfaces, using the auto-tune feature of the instrument control software.

2.3. Infusion experiments

ES emitters were prepared by pulling sections of a 75-μm-i.d./200-μm-o.d. fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA) by hand with a butane torch. The ES emitter was then connected to a transfer capillary and a 25-μL syringe (Hamilton, Las Vegas, NV, USA) by a stainless steel union that also served as the connection point for the ES voltage. All solutions were infused from the lowest concentration to the highest concentration at a flow rate of 0.3 μL/min by a syringe pump attached to the LTQ instrument. Voltages from 1.9–2.0 kV were applied to the ES emitter via the high voltage source from the LTQ. The ES emitter "tip" was positioned to an optimal electrospray distance of ∼2 mm from the mass spectrometer inlet by a Newport *x-y-z* stage (Newport Corporation, Irvine, CA, USA). The *m/z* range of the MS spectra was 50–1000. Peak intensities were obtained by using the Xcaliber data analysis software on the Finnigan LTQ after averaging 10 spectra to reduce intensity fluctuations from the ES. Injection times were obtained by averaging the injection times reported in the header file of 30 consecutive spectra. The header file is an automatically generated list that records the settings and times for each acquisition.

2.4. LC–MS and LC–MS/MS experiments

The LC system was constructed in house and is described previously [22]. Briefly, a pair of 100-mL Isco Model 100 DM syringe pumps and a Series D controller (Isco Inc., Lincoln, NE, USA) were used in conjunction with Valco valves (Valco Instruments Co., Houston, TX, USA). A 10-μL sample loop was used for manual sample loading and injection. Reversed-phase capillary LC columns were manufactured in-house by slurry packing 5-μm Jupiter C_{18} stationary phase (Phenomenex, Torrence, CA, USA) into a 60-cm length of 360-μm-o.d. × 150-μm-i.d. fused silica capillary tubing (Polymicro Technologies). Mobile phase A consisted of 0.2% acetic acid and 0.05% TFA in water, and mobile phase B consisted of 0.1% TFA in 90% acetonitrile/10% water. The mobile phase selection valve was switched from position A to B 20 min after sample injection to create an exponential gradient as mobile phase B displaced A in the mixer. Flow through the capillary LC column was ∼1.8 μL/min when equilibrated to 100% mobile phase A.

The HPLC column was coupled to the mass spectrometer using an in-house manufactured interface. No sheath gas or make-up liquid were used. The heated capillary temperature and spray voltage were 200 °C and 2.2 kV, respectively, for the standard interface and 140 °C and 2.2 kV, respectively, for the ion funnel interface. Eluent from the HPLC was scanned using an *m/z* range of 400–2000. The LC–MS measurements used a series of MS scans (max ion injection time: 10 ms), and the LC–MS/MS measurements used a repeating sequence of 1 MS

scan (max ion injection time: 10 ms) followed by 10 MS/MS scans (max ion injection time: 100 ms) of the 10 most abundant ions from the MS scan, using a collision energy setting of 35%. The target ion population was set to 30,000 ions. Dynamic exclusion software settings allowed for data dependent discrimination against previously analyzed ions by excluding ions that fell within −0.5 to +1.5 *m/z* units of the analyzed ion for 1 min.

S. oneidensis samples were analyzed by LC–MS/MS using a LTQ FT by injecting 10 μL of a 0.1 μg/μL sample to the LC. The separation, ion funnel, and linear ion trap method and settings were the same as described above. Ions were detected by the FT ion cyclotron resonance (ICR) mass analyzer for both the MS and MS/MS spectra. The target ion population was 500,000 ions and the maximum injection times were 1000 ms for the MS analysis and 2000 ms for the MS/MS analysis. The resolution of the FTICR was set at 100,000.

3. Results and discussion

3.1. Reserpine infusion experiment

We determined the level of increase in the intensity of the ion beam, produced by the implementation of an ion funnel on the linear ion trap mass spectrometer, by infusing a broad range of concentrations of a calibrant analyte to the ESI source. Sample solutions ranging from 10–0.001 μM of reserpine (MW = 608.7) were infused separately via a syringe pump and electrosprayed with the standard or the ion funnel interface. The same ES emitter, transfer line, syringe, and flow rate were used for all experiments, and the emitter was positioned to an optimal ∼2 mm from the instrument inlet for each interface.

Fig. 2 shows the intensity versus the reserpine concentration in log–log scale using both the standard and ion funnel ESI interfaces. The ion funnel consistently provided a ∼10-fold increase in reserpine signal intensity, indicating a large increase in ion flux through the interface and into the ion optics of the mass spectrometer compared to the skimmer interface. Interestingly, this increase in intensity does not provide a similar increase in signal-to-noise (*S/N*). This can be explained by understanding

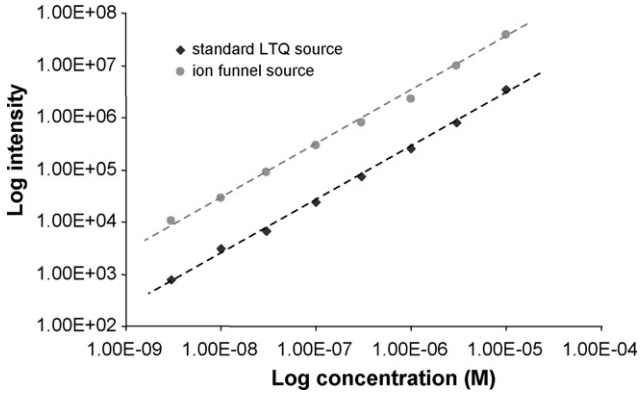

Fig. 2. Intensity versus concentration plot for the infusion of a series of reserpine solution concentrations for comparing the standard and the ion funnel interfaces. The same ES emitter, flow rate, and voltage were used for both interfaces along with placement of the emitter at ∼2 mm from the inlet.

how AGC aims to "fill" the ion trap to the targeted level. The target ion population (30,000 ions for these experiments) is chosen for optimal performance (e.g. resolution) of the ion trap mass analyzer. Before ion accumulation, the instrument detects the ion flux in a "pre-scan" and calculates the accumulation time (injection time) needed to reach the target ion population; a larger ion flux will result in a shorter time to "fill" the trap. In contrast, a lower efficiency ion interface producing a weaker ion flux will require a longer injection time. If a sufficient maximum fill time is allowed, the different injection times all result in a trap "filled" to the same ion population, and result in similar spectrum *S/N*. If there was no correction in ion intensity, the detected mass spectra from these two different ion fluxes would have similar peak intensities. To recapture the quantitative information, the ion intensities shown in the mass spectra are all normalized by dividing the ion signal from the detector by the ion injection time. In summary, the largest impact to instrument performance provided by the ion funnel interface in the infusion study results from a decrease in the ion injection times required to fill the linear ion trap.

A comparison of the two interfaces using identical solution concentrations showed that the use of the ion funnel decreased the injection times by ∼90%, which corresponds to an ion flux increase of ∼10-fold. For instance, the 1.0 μM concentration produced ion injection times of 1.27 ± 0.08 ms and 0.13 ± 0.02 ms for the standard and ion funnel interfaces, respectively. We found that as the concentration of reserpine decreased, the ion injection times increased, but ultimately leveled off due to the ion population in the ion trap being increasingly dominated by "chemical noise" (e.g. solvent and solution contaminant related species and solvent clusters associated with ESI) instead of reserpine ions. In other words, if the ion beam consisted of pure analyte ions then the magnitude of ion injection times should reflect the magnitude of analyte concentration. However in ESI, other ions (e.g. "background", solvent related, or contaminant) are always present that partially fill the ion trap, and become more significant for longer accumulation times. We found that for the infusion experiments, the chemical background started to dominate the ion population around the 1 μM concentration, causing the injection times to level off for the more dilute reserpine solutions. As noted earlier, the mass spectrum ion intensities reflect the true ion beam intensity after being normalized by dividing the ion signal from the detector by the ion injection time. In the case of the ion funnel, the ∼10-fold increase in peak intensity shown in Fig. 2 is a direct consequence of the 90% reduction in ion injection times.

3.2. LC–MS and LC–MS/MS of a bacterial global tryptic protein digest

Does an order of magnitude increase in ion beam intensity (and therefore an order of magnitude decrease in ion accumulation time) affect the performance of a linear ion trap mass spectrometer? In the case of a single MS scan analysis the improvement in duty cycle is minimal as a typical scan time for the instrument in MS mode is only 150 ms which results in a duty cycle improvement of ∼1%. However, the main analytical

power of the linear ion trap is not the ability to acquire single mass spectra but it is the selection, isolation, and subsequent fragmentation of analytes to perform MS/MS experiments. This process typically requires much longer ion injection times and a large reduction in accumulation time here should improve instrument performance. To test this hypothesis, we analyzed a complex mixture of peptides obtained by enzymatically digesting a global proteome sample from the bacterium, *S. oneidensis*. The sample was analyzed by both LC–MS and LC–MS/MS.

The linear ion trap mass spectrometer was fitted with either the standard interface or the ion funnel interface, and the same LC system and conditions (same LC column, ES emitter, solvents, etc.) were employed for all experiments. Fig. 3 shows the resulting base peak chromatograms from LC–MS analyses of the bacteria sample, obtained using Xcaliber software with seven point Gaussian smoothing, for the standard LTQ interface (Fig. 3a) and the ion funnel interface (Fig. 3b). It is important to note that both chromatograms exhibit similar separation profiles indicating good separation reproducibility and a minimal *m/z* bias between the two different interfaces. The largest impact

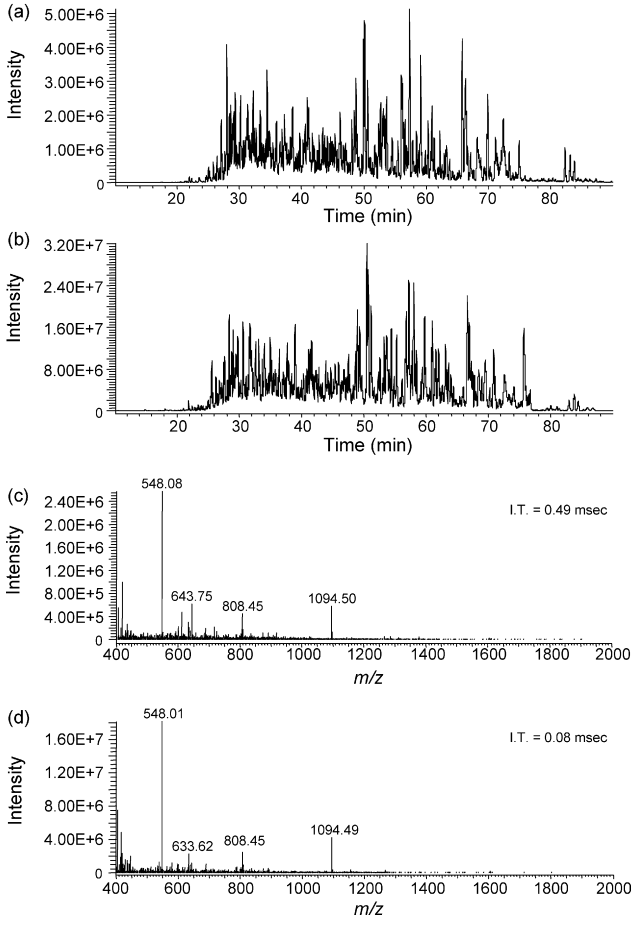

Fig. 3. Base peak total ion chromatograms from the LC–MS analysis of a global tryptic digest from *S. oneidensis* with the standard LTQ interface (a) and the ion funnel interface (b). A representative mass spectrum from (a) and (b) are shown in the lower portion. The *m/z* 548 peak was chosen randomly and the spectrum from the highest point in the elution profile is shown from the standard LTQ interface (c) and the ion funnel interface (d). IT: Injection times to fill the ion trap.

was the increase in sensitivity when the ion funnel was used. A representative mass spectrum from each analysis is shown in the lower portion of this figure. The *m/z* 548 peak was chosen at random and the spectrum from the maximum of the elution profile is shown (standard LTQ interface in Fig. 3c and the ion funnel interface in Fig. 3d). Here, an ~7-fold increase in peak intensity was obtained by using the ion funnel interface. It is also important to note that the other peaks in the spectra are also similar (within normal variance for different LC runs), indicating similar ion transmission profiles between the two interfaces across the *m/z* range. In order to obtain a statistical number for the decrease in ion injection times by using the ion funnel interface, we randomly selected 30 additional peaks that were observed in the two analyses and compared their injection times at the maximum of their elution profile. We found the average injection time for the standard LTQ interface was 1.26 ms, the average injection time for the ion funnel interface was 0.18 ms, and an overall reduction in injection time of $84 \pm 3\%$ between each of the 30 peaks when the ion funnel LTQ interface was used.

LC–MS/MS experiments were then conducted using the linear ion trap mass spectrometer with either the standard or the ion funnel interface, using the *S. oneidensis* digest sample. Fig. 4 compares LC–MS/MS spectra for a randomly chosen peptide with the two arrangements. The upper portion of this figure displays the MS spectrum for the peptide (*m/z* of 881) obtained with the standard LTQ interface (Fig. 4a) and the resulting MS/MS spectrum (Fig. 4b). The lower portion shows the results obtained for the same peptide (MS in Fig. 4c and MS/MS in Fig. 4d), but obtained using the ion funnel interface. Unlike the MS scans, the MS/MS scans require longer injection times (actual inject times are shown in the upper right of each spectrum) due to a larger AGC target value for MS/MS analysis. The amount of time in the MS/MS scan not associated with ion injection is ~200 ms. This results in a 17% improvement in duty cycle for this particular MS/MS acquisition when the ion funnel interface was used. Since the injection time is directly related to the sample concentration, the impact of increasing the ion flux and reducing the injection times by an ion funnel should become more prominent with more dilute samples.

We then analyzed a series of *S. oneidensis* sample dilutions (1.0, 0.3, 0.1, and 0.03 μg/μL) with LC–MS/MS analyses using the standard interface and ion funnel interface. The resulting MS/MS spectra were analyzed using SEQUEST and filtered using criteria previously shown to provide >95% confidence in peptide identification [23]. The resulting number of identified peptides, unique peptides (following removal of multiple peptide identifications), and unique proteins are given in Table 1. For the highest concentration tested (1.0 μg/μL), the ion funnel afforded only a small increase in the number of identifications. We found that as the concentration was reduced, the effect of the ion funnel became more pronounced, and at the lowest concentration tested (0.03 μg/μL), the number of identified proteins was doubled by using the ion funnel interface. Although there is a level of false positives, the filtering used strict criteria that provide a similarly high level of confidence in the added identifications. We also analyzed the 1.0 μg/μL sample in triplicate using both interfaces which showed acceptable reproducibil-

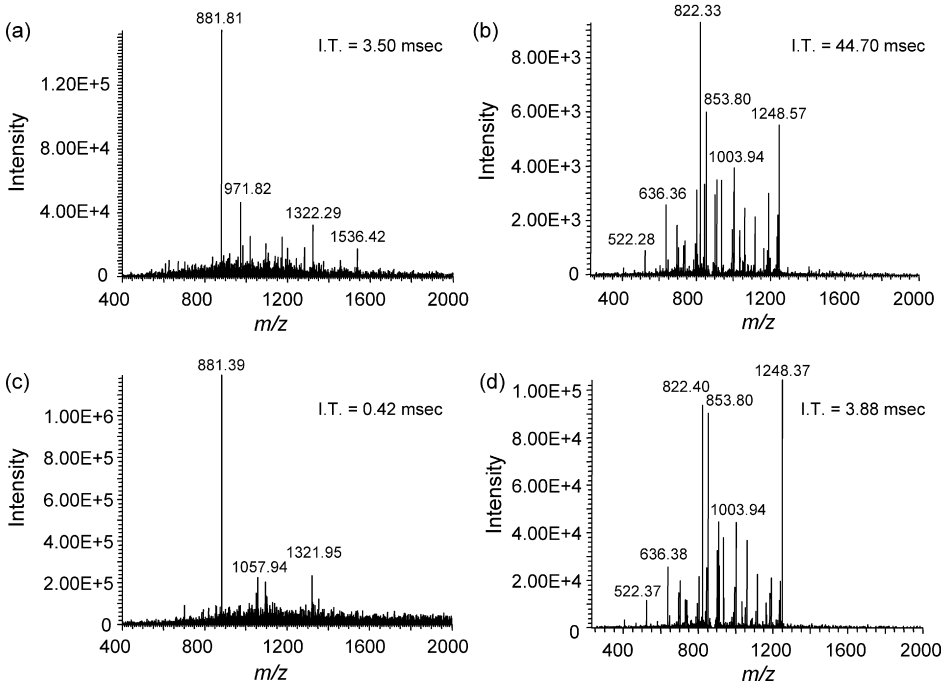

Fig. 4. Mass spectra for the randomly chosen peptide, *m/z* 881 (identified as ANSGHPGAPMGMADIAEVLWNDFLK) in the LC–MS/MS analysis of a global tryptic digest from *S. oneidensis* with the standard linear ion trap interface ((a) and (b)) and the ion funnel interface ((c) and (d)). The MS-only spectra are shown in (a) and (c), and the resulting MS/MS spectra are shown in (b) and (d). The injection time (IT) to fill the ion trap is given for each spectrum.

ity of the peptide identification process. The standard interface resulted in 4978 ± 180 peptide identifications, 3216 ± 95 unique peptides, and 966 ± 22 unique proteins. The ion funnel interface resulted in 5342 ± 134 peptide identifications, 3356 ± 199 unique peptides, and 980 ± 28 unique proteins.

The increase in the number of identifications for the lower sample concentrations shown in Table 1 was contributed, in large part, by the greater speed of ion accumulation. These MS/MS analyses were ran using parameters from our proteomics facility which were optimized for complex biological samples and to minimize under-sampling. For example, a MS scan is first acquired. From this scan, the top 10 peaks are automatically selected for MS/MS analysis. The 10 MS/MS scans are then acquired in a determined order. It is easy to understand the necessity of speed in this situation because, if the initial MS/MS scans take too long, the later MS/MS scans have the potential to be acquiring after the chosen analyte has fully eluted from the LC column and those peptides will not be identified. In order to minimize this, time limits are applied to the injection time; therefore, it is imperative that the ion trap have a significant number of analyte ions if/when this limit is reached. The ion funnel interface increased the intensity of the ion beam which allowed the ion trap to obtain larger ion populations when lower concentrated samples were analyzed. The larger ion populations resulted in MS/MS spectra with improved quality, and in-turn, increased the number of peptide and protein identifications.

3.3. LC–MS/MS analysis using a LTQ FT mass spectrometer

The results from the use of an ion funnel with a linear ion trap mass spectrometer indicate that the greatest advantage due to the ion funnel is reduced ion injection times. The level of improvement to MS performance is thus correlated to how large of a role the ion accumulation event has in the acquisition sequence. In other words, instruments that require longer injection times should show increased improvement when an ion funnel interface is used. Fourier transform mass analyzers, such as an ICR cell or Orbitrap, fundamentally require larger ion populations than the linear ion trap which consequently increases the required injection times to fill the ion trap. The longer ion injection times contribute a larger portion to the duty cycle, and the use of an ion funnel should have a greater impact. To evaluate this, we installed the ion funnel interface on a LTQ FT mass spec-

Table 1
Comparison of LC–MS/MS results with the standard interface and the ion funnel interface, using a range of *S. oneidensis* tryptic digest concentrations and 10 μL injections on the same LC column

	Standard interface				Ion funnel interface			
Concentration (μg/μL)	1.0	0.3	0.1	0.03	1.0	0.3	0.1	0.03
Peptide identifications	4770	3681	2548	1415	5187	4712	4106	3851
Unique peptide identifications	3107	2405	1726	1012	3126	2835	2302	1992
Unique protein identifications	940	780	587	357	953	895	757	699

Table 2
Comparison of MS/MS injection times with a hybrid linear ion trap-FTICR mass spectrometer using both the standard and the ion funnel interfaces

Peptide (*m/z*)	Standard LTQ interface (ms)	Ion funnel interface (ms)	Percent reduction (%)
476.31	539.4	40.0	92.6
520.33	495.3	89.7	81.9
797.45	394.2	83.0	78.9
831.41	474.3	69.9	85.3
863.45	331.6	68.5	79.3
900.46	384.2	20.7	94.6
949.45	612.5	42.3	93.1
998.88	1050.0	67.1	93.6
1057.15	745.9	110.7	85.2
1103.25	920.9	81.9	91.1

The *m/z* of 10 randomly chosen peaks are shown that were selected and identified as peptides by MS/MS in both runs from the analysis of 1 μg of a *S. oneidensis* proteome tryptic digest sample with the corresponding injection times and percent reduction.

trometer. A 1 μg sample of the *S. oneidensis* tryptic digest was analyzed with both the standard and the ion funnel interfaces. Table 2 shows the injection times of the MS/MS analysis of 10 randomly chosen peptides from the two runs. The standard interface resulted in injection times ranging from ∼300 to 1000 ms. The time interval of the scan not consisting of the injection time was ∼820 ms, which includes the AGC prescan, ion selection/fragmentation, ion transfer, and ICR detection (obtained from the information in the header file of the mass spectra). Thus, the injection times range from ∼25 to 55% of the duty cycle during peptide elution. Replacing the standard interface with the ion funnel lowered the injection times to ∼20–100 ms for the same 10 peptides. This reduced the overall spectrum acquisition times by ∼25–50% for MS/MS analyses, thus allowing for more MS/MS analyses during an LC separation.

4. Conclusion

The use of an electrodynamic ion funnel interface with a linear ion trap mass spectrometer greatly reduced ion losses in the ESI-MS interface. This improvement was most evident in the reduced (∼90%) ion trap injection times. The *S/N* remained unchanged between the two different ion interfaces due to the "filled" ion trap having a similar population of ions. The improvements to instrument performance were dependant on the type of MS experiment and the proportion of the total scan time associated with the ion injection event. The ability to fill an ion trap faster provided more improvement for MS/MS analyses than MS. This was mainly due to the higher AGC level in the MS/MS analyses which requires longer ion injection times. The enhancement was more pronounced at more dilute sample concentrations and provided higher quality MS/MS spectra which increased peptide and protein identifications. The increase in duty cycle was also more pronounced when a LTQ FT instrument was used due the larger ion population requirement that necessitates longer injection times.

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References

[1] D.J. Douglas, A.J. Frank, D. Mao, Mass Spectrom. Rev. 24 (2005) 1.
[2] J.C. Schwartz, M.W. Senko, J.E.P. Syka, J. Am. Soc. Mass Spectrom. 13 (2002) 659.
[3] J.C. Schwartz, X.-G. Zhou, M.E. Bier. US Patent (1996) 5,572,022.
[4] M.E. Belov, V.S. Rakov, E.N. Nikolaev, M.B. Goshe, G.A. Anderson, R.D. Smith, Rapid Comm. Mass Spectrom. 17 (2003) 627.
[5] N.B. Cech, C.G. Enke, Mass Spectrom. Rev. 20 (2001) 362.
[6] P. Kebarle, L. Tang, Anal. Chem. 65 (1993) 972A.
[7] R.D. Smith, L.A. Loo, C.G. Edmonds, C.J. Barinaga, H.R. Udseth, Anal. Chem. 62 (1990) 882.
[8] K. Taeman, A.V. Tolmachev, R. Harkewicz, D.C. Prior, G. Anderson, H.R. Udseth, R.D. Smith, T.H. Bailey, S. Rakov, J.H. Futrell, Anal. Chem. 72 (2000) 2247.
[9] S.A. Shaffer, K. Tang, G.A. Anderson, D.C. Prior, H.R. Udseth, R.D. Smith, Rapid Commun. Mass Spectrom. 11 (1997) 1813.
[10] S.A. Shaffer, D.C. Prior, G.A. Anderson, H.R. Udseth, R.D. Smith, Anal. Chem. 70 (1998) 4111.
[11] S.A. Shaffer, A.V. Tolmachev, D.C. Prior, G.A. Anderson, H.R. Udseth, R.D. Smith, Anal. Chem. 71 (1999) 2957.
[12] J.S. Page, A.V. Tolmachev, K. Tang, R.D. Smith, J. Am. Soc. Mass Spectrom. 17 (2006) 586.
[13] R.R. Julian, S.R. Mabbett, M.F. Jarrold, J. Am. Soc. Mass Spectrom. 16 (2005) 1708.
[14] E.C. Lynn, M.C. Chung, C.C. Han, Rapid Commun. Mass Spectrom. 14 (2000) 2129.
[15] C. Stacey, T. Kim, Z. Zhang, T. Knudsen, M. Park, Proceedings of the 53rd ASMS Conference on Mass Spectrometry, San Antonio, TX, June 5–9, 2005, p. MP245.
[16] D. Gerlich, State-Selected and State-to-State Ion Molecule Reaction Dynamics, Wiley, New York, 1992.
[17] T. Kim, K. Tang, H.R. Udseth, R.D. SmithRD, Anal. Chem. 73 (2001) 4162.
[18] J.S. Page, B. Bogdanov, A.N. Vilkov, D.C. Prior, M.A. Buschbach, K. Tang, R.D. Smith, J. Am. Soc. Mass Spectrom. 76 (2005) 244.
[19] J.S. Page, A.V. Tolmachev, K. Tang, R.D. Smith, J. Mass Spectrom. 40 (2005) 1215.
[20] K. Tang, A.V. Tolmachev, E. Nikolaev, R. Zhang, M.E. Belov, H.R. Udseth, R.D. Smith, Anal. Chem. 74 (2002) 5431.
[21] K. Tang, A.A. Shvartsburg, H.N. Lee, D.C. Prior, M.A. Buschbach, F.M. Li, A.V. Tolmachev, G.A. Anderson, R.D. Smith, Anal. Chem. 77 (2005) 3330.
[22] M.S. Lipton, L. Pasa-Tolic, G.A. Anderson, D.J. Anderson, D.L. Auberry, J.R. Battista, M.J. Daly, J. Fredrickson, K.K. Hixson, H. Kostandarithes, C. Masselon, L.M. Markillie, R.J. Moore, M.F. Romine, Y. Shen, E. Strittmatter, N. Tolic, H.R. Udseth, A. Venkateswaran, K.-K. Wong, R. Zhao, R.D. Smith, Proc. Natl. Acad. Sci. 99 (2002) 11049.
[23] W.J. Qian, T. Liu, M.E. Monroe, E.F. Strittmatter, J.M. Jacobs, L.J. Kangas, K. Petritis, D.G. Camp, R.D. Smith, J. Proteome Res. 4 (2005) 53.