Construction of a Versatile High Precision Ambient Ionization Source for Direct Analysis and Imaging

Jason S. Sampson, Adam M. Hawkridge, and David C. Muddiman

W. M. Keck FT-ICR Mass Spectrometry Laboratory, Department of Chemistry, North Carolina State University, Raleigh, North Carolina, USA

The design and construction of a high precision ambient ionization source matrix-assisted laser desorption electrospray ionization (MALDESI) are described in full detail, including a complete parts list. The computer controlled high precision motion control system and high repetition rate Explorer laser are demonstrated during MALDESI-FT-ICR analysis of peptides and proteins ranging from 1 to 17 kDa. The high stability ionization source platform described herein demonstrates both the advantages of the new MALDESI source and versatility for application to numerous desorption and ionization techniques. (J Am Soc Mass Spectrom 2008, 19, 1527–1534) © 2008 American Society for Mass Spectrometry

The introduction and development of hybrid ambient ionization sources such as laser desorption atmospheric pressure ionization (LDAPI) [1, 2], fused droplet electrospray ionization/extraction electrospray (FD-ESI, EESI) [3, 4], direct analysis in real time (DART) [5], desorption electrospray ionization (DESI) [6], electrospray assisted laser desorption ionization (ELDI) [7–11], matrix-assisted laser desorption electrospray ionization (MALDESI) [12, 13], and infrared laser desorption electrospray ionization [14, 15] have advanced the capabilities of mass spectrometry. MALDESI, for example, is a pulsed ionization source that holds promise in areas ranging from top-down proteomics, tissue imaging, and ionization mechanism elucidation. The pulsed nature of MALDESI combined with its ability to generate multiply-charged ions are characteristics that are particularly well suited for Fourier transform ion cyclotron resonance (FT-ICR) and Orbitrap (LTQ-Orbi) mass spectrometry due to the inverse relationship of frequency to m/z and $\sqrt{(m/z)}$, respectively. The consequence of this relationship is high resolving power, <3 ppm mass measurement accuracy, and amenability to a multitude of tandem MS/MS techniques for bottom-up and top-down proteomics (e.g., CID, ETD, ECD, SORI, and IRMPD) [16–18]. Many of these MS/MS techniques in FT-ICR and Orbitrap instruments would benefit from a pulsed ionization source where intact proteins and polypeptides with complex post-translational modifications could be carefully interrogated rather than the typical "continuous" ionization encountered during a LC-MS/MS analysis where peak widths (i.e., analysis times) are about 10 to 30 s.

The advancement and acceptance of MALDESI and related hybrid ionization techniques are critically dependent on the widespread dissemination of detailed source designs such that results can be reproduced and improved in a variety of laboratories. Furthermore, there are certainly unanticipated potential applications that could benefit from novel or improved source designs. Herein, we provide a detailed description and characterization of the third version of the MALDESI source utilized in this laboratory. The further improvement to this design both within our laboratory as well as other laboratories should enable MALDESI and related hybrid ambient ionization sources to mature to a level of analytical robustness now widely enjoyed for ESI and MALDI.

Experimental

Materials

Bradykinin, angiotensin I, melittin, glucagon, bovine ubiquitin, lysozyme, myoglobin, and 2,5-dihydroxybenzoic acid were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. HPLC-grade acetonitrile and high purity water were purchased from Burdick and Jackson (Muskegon, MI). The electrospray solution was prepared by mixing acetonitrile and water 1:1 (vol:vol). The organic matrix solution was prepared by dissolving 150 mg DHB into 1 mL of the electrospray solution. All samples were prepared from 200 μM stock solutions and mixed 1:1 (vol:vol) with the matrix solution. Sample spots of 0.8 μL of the analyte matrix solution were deposited for each sample, equivalent to ~80 pmol analyte per spot.

Address reprint requests to Dr. D. C. Muddiman, W. M. Keck FT-ICR Mass Spectrometry Laboratory, Department of Chemistry, North Carolina State University, Raleigh, NC 27695, USA. E-mail: david_muddiman@ncsu.edu

1044-0305/08/$32.00
doi:10.1016/j.jasms.2008.06.013

Published online June 28, 2008
Received May 1, 2008
Revised June 19, 2008
Accepted June 20, 2008

Profilometery Measurements

A Tencor Alpha Step profilometer (5 nm resolution) was used to measure the diameter of the laser ablation crater in a gold coated QCM electrode. The distance was measured across the width of the laser ablation craters with three repeats at a scan speed of 50 μm/s. The QCM electrode (International Crystal Manufacturing, Oklahoma City, OK) consisted of 10 MHz A/T cut quartz crystal with a 1000 Å gold layer coated on a chromium base layer.

MALDESI-LTQ-FT Mass Spectrometry

MALDESI mass spectra were obtained using a hybrid LTQ-FT Ultra mass spectrometer (Thermo Electron Inc., San Jose, CA and Bremen, Germany) equipped with an actively-shielded 7 T superconducting magnet (Oxford Instruments, Concord, MA). The MALDESI source (Figure 1) was placed in front of the LTQ-FT fitted with a modified extended ion transfer capillary (part no. 54). Solvent was electrosprayed at 2.8 kV through a 75 μm i.d. fused silica capillary (part no. 40) with a 30 μm fused silica tapered PicoTip (part no. 44) using a Harvard PHD-2000 syringe pump (part no. 28) at a flow rate of 400 nL/min. The analyte was laser desorbed from each sample spot actively-dried [19] onto a stainless steel sample target (part no. 36) located directly below and between the ESI emitter and the ion transfer capillary. The stainless steel sample target was biased at 300 V. Each mass spectrum was single-acquisition with resolving power at 400 *m/z* set to $200{,}000_{FWHM}$ and the AGC is set to 1×10^6.

Source Design

MALDESI Source Design and Construction

A new version of the MALDESI source was constructed based in part on the previous source design [12, 13]. A photo of the new source is shown in Figure 1 with major parts indicated. A complete parts list, grouped by manufacturer, is included in Table 1, with specifications for custom fabricated parts described in Table 2. A schematic of the ionization source labeled by part number is included in the Supplemental Material (Figure S1, which can be found in the electronic version of this article). The manual linear XYZ sample positioning stages were replaced with computer controlled motorized sample positioning stages (parts no. 2–5) and control unit (part no. 6) for precise sample positioning during analysis. The high speed long travel translational stages (parts no. 2, 3) allow for interrogation of samples across the full length and width of a standard MALDI target (part no. 36). Custom translation programming was accomplished using the supplied software (Newport, ESP); enabling one touch program execution for whole sample spot analysis, [a diagram of the ablation path is shown in the Supplemental Material (Figure S2)], as well as allowing the user to operate the source remotely. The position control can be actuated linearly without program execution for spot to spot analysis, as typically performed in MALDI. The high precision (0.035 μm resolution, 100 nm increments) of the positioning system enables accurate repeatable ($\pm$ 600 nm, bidirectional) positioning amenable to applications requiring a high degree of positioning control (e.g., tissue imaging).

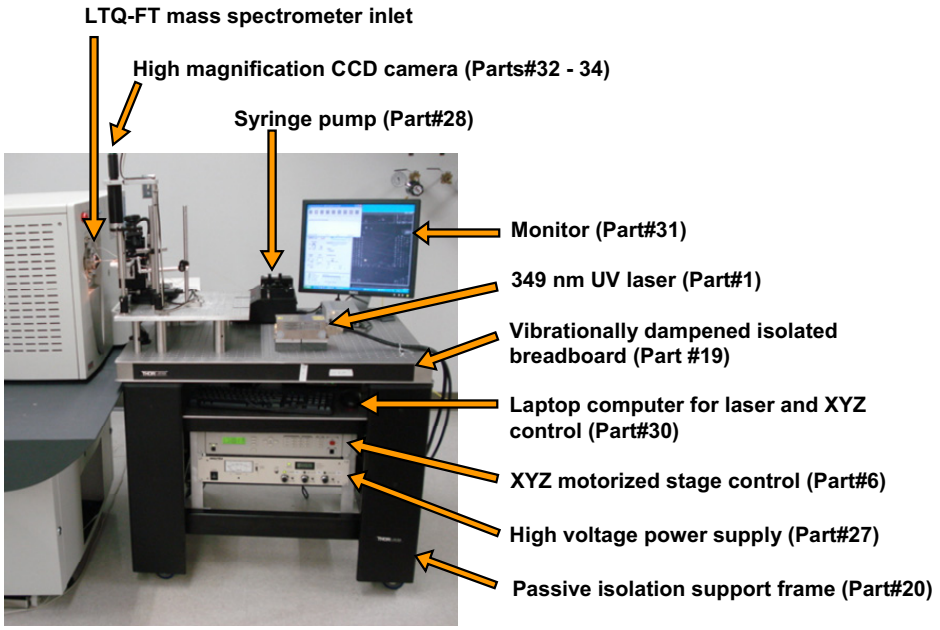

Figure 1. Photograph of the new MALDESI ionization source coupled to a LTQ-FT mass spectrometer with major parts indicated and part numbers that correspond to those listed in Table 1.

Table 1. MALDESI parts list

Part no.	Description	Distributor	Stock no.	Quantity
1	Explorer Q-Switched DPSS Laser	Newport (Irvine, CA)	EXPL-349-120-1KE	1
2	High performance low profile linear stage	Newport (Irvine, CA)	436	3
3	Motorized actuator (x and y axes)	Newport (Irvine, CA)	LTA-HS	2
4	Vernier Micrometer (z axis)	Newport (Irvine, CA)	SM-50	1
5	Angle bracket (90°)	Newport (Irvine, CA)	360-90	1
6	2 Axis motion controller/driver	Newport (Irvine, CA)	ESP300-11N1N1	1
7	19 in. rack mount brackets (ESP300)	Newport (Irvine, CA)	ESP300-R	1
8	Slotted base	Newport (Irvine, CA)	B-05A	3
9	2 in. post holder	Newport (Irvine, CA)	VPH-2	3
10	2 in. ss post	Newport (Irvine, CA)	SP-2	1
11	4 in. ss post	Newport (Irvine, CA)	SP-4	1
12	6 in. ss post	Newport (Irvine, CA)	SP-6	2
13	12 in. ss post	Newport (Irvine, CA)	SP-12	2
14	Right angle post connector	Newport (Irvine, CA)	CA-1	1
15	Adjustable angle post connector	Newport (Irvine, CA)	CA-2	1
16	1 in. lens mount	Newport (Irvine, CA)	LH-1	1
17	UV fused silica plano convex lens AR10	Newport (Irvine, CA)	SPX017 + AR.10	1
18	UV enhanced aluminum mirror	Newport (Irvine, CA)	10D20AL.2	2
19	Performance plus breadboard	Thorlabs (Newton, NJ)	PBI11111	1
20	Passive support frame (27.5″H × 36″L × 30″W)	Thorlabs (Newton, NJ)	PFP51505	1
21	Periscope assembly	Thorlabs (Newton, NJ)	RS99	1
22	4 in. stainless steel post	Thorlabs (Newton, NJ)	RS4	1
23	4 in. mounting post	Thorlabs (Newton, NJ)	P4	4
24	Swivel casters (3 in.)	Grainger	1G196	4
25	Caster brake kit	Grainger	4X698	4
26	Power strip (6 plug)	Grainger	6X953	2
27	DC power supply	Analytica of Branford	103510	1
28	PHD 2000 syringe pump	Harvard Apparatus	70-2000	1
29	Fiber-Lite Light source	Dolan Jenner	MI-150	1
30	Dell laptop computer (Pentium M, 1.4 GHz)	Dell	N/A	1
31	Dell 19 in. monitor	Dell	N/A	1
32	CCD camera	Hitachi	KP-M1AN	1
33	Leica optical amplifier	Vashaw Scientific	312996	1
34	Leica monozoom 7	N/A	N/A	1
35	Happauge Win-TV USB-2	Circuit City	N/A	1
36	SS 192 well MALDI plate	Applied Biosystems	4333375	1
37	RS232 to USB converter	Tiger Direct	N/A	1
38	High Voltage wire (5kV, 2 pcs. 12 in., 24 in.)	N/A	N/A	1
39	LTQ high voltage plug	Connectronics Corp.	10334-02	1
40	24 in. fused silica capillary (75 um i.d.)	Polymicro Technologies	2000019	1
41	Stainless steel union	Valco Instrument Co.	ZUIXC	1
42	Syringe adapter	Valco Instrument Co.	VISF-2	1
43	PEEK tubing sleeve orange 0.062 × 0.016	Upchurch Scientific	F-230	2
44	Silica tip (30 um i.d. tapered)	New Objective Inc.	FS360-75-30-N-20	1
45	Hamilton gas tight syringe 100 uL	Fisher Scientific	14-813-138	1
46	Breadboard	Fabricated in house	N/A	1
47	Camera support bracket	Fabricated in house	N/A	1
48	Teflon ESI holder	Fabricated in house	N/A	1
49	Sample target high voltage clip	Fabricated in house	N/A	1
50	19 in. rack support bracket	Fabricated in house	N/A	2
51	Sample target right angle bracket	Fabricated in house	N/A	1
52	Sample plate Teflon insulator	Fabricated in house	N/A	1
53	Computer shelf	Fabricated in house	N/A	1
54	Extended capillary	Fabricated in house	N/A	1
55	Laser spacing block	Fabricated in house	N/A	1

The manually actuated pulsed nitrogen laser (337 nm) was replaced by the Explorer laser system (part no. 1), a Q-switched diode pumped solid-state ultraviolet laser (349 nm) with first pulse suppression, precise internally measured laser power (0–120 μJ) and repetition rate control (0–5000 Hz) using the supplied software (Spectra-Physics, L-Win). The laser power, repetition rate and translation stage velocity control (0–5 mm/s) are invaluable for applications to various samples, surfaces and desorption conditions; preventing damage to solid substrate and depletion of analyte during analysis. The high-resolution CCD camera

Table 2. Description of parts fabricated in-house

46	16 in. × 16 in. × 3/8 in. thickness aluminum (thickness may be reduced to 1/4 in.), drilled and tapped 1/4 × 20 (1″ on center)
47	8 in. × 1/2 in. thickness aluminum, two piece clamp for Monozoom lens support
48	2 in. × 1/2 in. × 1/4 in. thickness Teflon; cut-out to receive a stainless steel union, threaded tube inserted across width to attach aluminum retainer and electrical contact for ESI voltage
49	1¼ in. × 1/8 in. × 1/32 in. thickness stainless steel with custom bend for electrical contact with sample target
50	16 in. × 2½ in. (1½ in. along component attachment region) × 1/4 in. thickness aluminum, slots at each end for attachment to support frame (part no. 20)
51	3 in. length × 2½ in. height, 1/4 in. thickness aluminum, (2) vertical slots cut in short side for attachment to XYZ stage, (2) clear holes (no. 8) in long side for Teflon target insulator attachment
52	2½ in. × 2½ in. × 1/4 in. thickness Teflon with (2) small stainless steel posts to accommodate the sample target and (2) holes drilled and tapped 8/32 in. from bottom to attach to sample target bracket (part no. 51)
53	1/4 in. thickness aluminum, cut to fit across upper cross members of support frame (part no. 20)
54	2 in. extended stainless steel capillary with lug to fit LTQ atmospheric pressure interface
55	7½ in. × 3¾ in. × 1½ in. aluminum block including ½ in. slots on each end for ¼ × 20 screw attachment to the breadboard (part no. 19)

(parts no. 32–34) was connected using the WinTV PCI tuner (part no. 35) to the laptop computer and monitor (parts no. 30, 31) enabling real time on screen visualization and recording of the sample spot and tracking of the laser spot on target during laser ablation, which is particularly useful for analysis of small sample spots as well as tissue sections for imaging.

The XYZ stage (parts no. 2–5, 38, 49, 51, 52), second stage laser beam positioning mirror (part no. 18, mounted in the upper portion of the periscope assembly parts no. 21, 11, 8), focusing lens assembly (parts no. 8, 9, 11, 13, 14, 16, 17), ESI emitter assembly (parts no. 8, 9, 12, 12, 15, 40–44, 48), and CCD camera assembly (parts no. 8, 9, 10, 13, 32–34, 47) were all mounted onto the custom fabricated breadboard (part no. 46). Schematics of the desorption and ionization region with a description of each subassembly are shown in Figures 2 and 3. A clear hole drilled in the breadboard (part no. 46) allows the passage of the laser beam from the first stage directional mirror (parts no. 18, 21, 22) mounted on the main breadboard work surface (part no. 19) to the second stage directional mirror (parts no. 8, 11, 18, 21) mounted on the custom breadboard (part no. 46). The breadboard (part no. 46) with all mounted equipment was then mounted onto the vibrationally isolated main breadboard (part no. 19) using 4 in. offset posts (part no. 23). The laser (part no. 1) mounted to a custom fabricated spacing block (part no. 55), first stage laser beam positioning mirror (part no. 18 mounted in lower portion of the periscope assembly, part no. 21, attached to a 4 inch post, part no. 22), syringe pump (part no. 28), light source (part no. 29) and monitor (part no. 31) were mounted on the main breadboard (part no. 19). The field of the main breadboard (part no. 19) and custom breadboard (part no. 46) were fabricated with tapped holes (1/4 × 20) on one inch center; a supply of appropriate fasteners is required to attach various components to the breadboards.

The high voltage power supply (part no. 27) and motion controller (part no. 6) were mounted to the support frame (part no. 20) using custom fabricated rack mounting brackets (part no. 50). The high voltage power supply (part no. 27) was attached to the sample target high voltage clip (part no. 49) using 5 kV insulated wire (part no. 38) for contact with the stainless steel sample target (part no. 36) mounted on the Teflon insulator (part no. 52). The keyboard, mouse, and

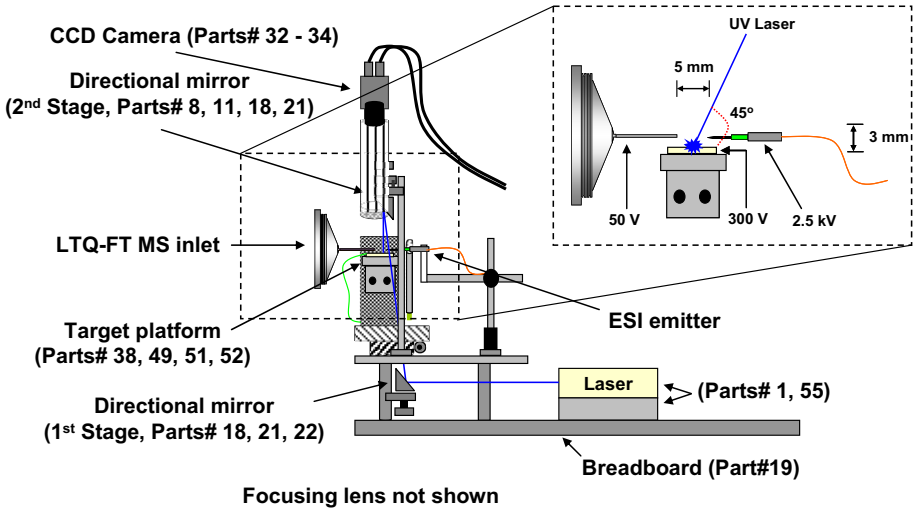

Figure 2. Side view schematic of the ionization region of the MALDESI source with the numbers corresponding to the parts listed in Table 1.

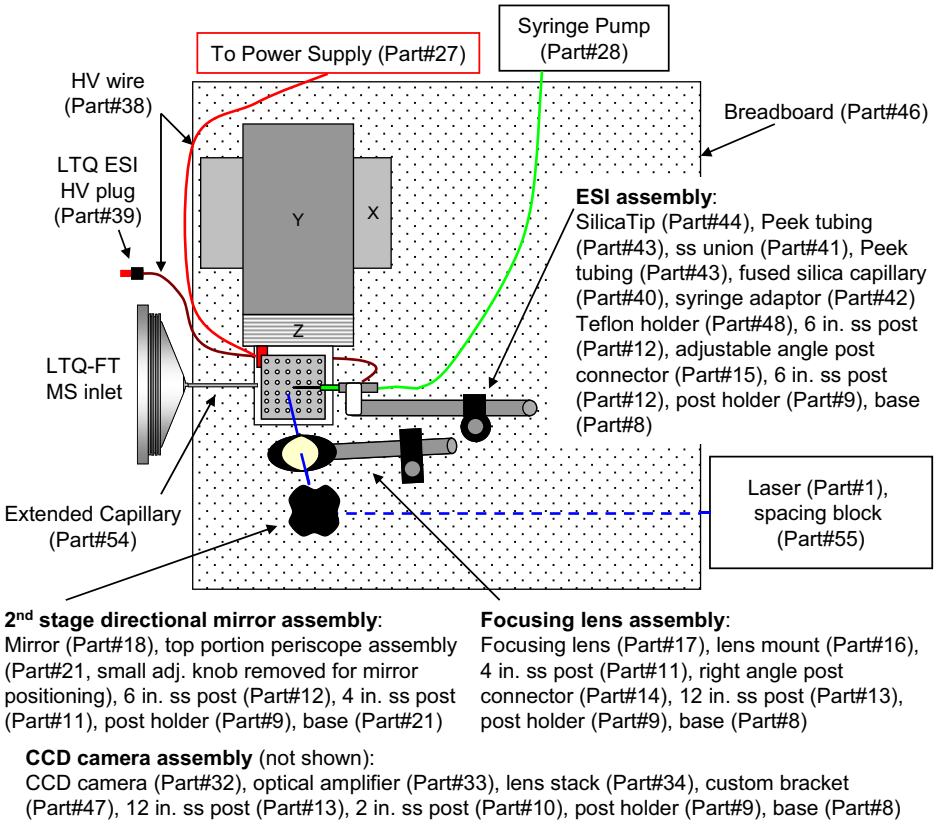

Figure 3. Top view schematic of the ionization region with a listing of the parts for each sub-assembly.

laptop computer (part no. 30) were placed onto a custom fabricated shelf (part no. 53) situated on the upper cross beams of the support frame (part no. 20). Power management was provided using two 6-plug power strips (part no. 26) installed on the inside of the vertical supports on the rear of the support frame (part no. 20). Before assembly, casters (parts no. 24, 25) were installed to replace the existing adjustable feet on the support frame for mobility. The versatility of this ionization source platform allows for interchangeability of the source between instruments as well as installation of new components onto the existing platform.

Desorption and Ionization Source Versatility

The high stability ionization source platform may be implemented using various desorption and ionization techniques for application specific analysis. Existing ionization sources may be installed onto the platform due to the inherent versatility of the breadboard for roll-up accessibility. Analyte desorption may be induced by one of a number of regimes, including laser desorption as in MALDESI, laser-induced acoustic desorption (LIAD) [20], heated nitrogen gas amplification, sequencing, and annotation of plastomes (ASAP) [21], and impact of high velocity charged droplets as in DESI [6], followed by a number of post-desorption ionization methods including atmospheric pressure photo ionization (APPI) [22], atmospheric pressure chemical ionization (APCI) [23], and electrospray ionization (ESI) [24]. In addition to the hybrid ionization sources listed above, this platform is amenable to basic electrospray ionization [24] and atmospheric pressure matrix-assisted laser desorption ionization [25].

Results and Discussion

Explorer Laser Beam Characterization

The laser beam spot size was measured at a laser power of 112 μJ (measured internally) using a gold coated quartz crystal microbalance electrode as the laser target. A photo of the QCM electrode following laser ablation is shown in Figure 4b, with an expanded view of a single ablation crater shown in Figure 4c. In these experiments, the thin gold top layer (1000 Å) was removed exposing the chromium base layer with some re-deposition along the perimeter of the ablated crater. The elliptical laser beam spot size (60 μm $\times$ 80 μm) was determined by measuring the actual diameter of the ablation craters using a Tencor Alpha Step profilometer. A representative scan plot is shown in Figure 4a.

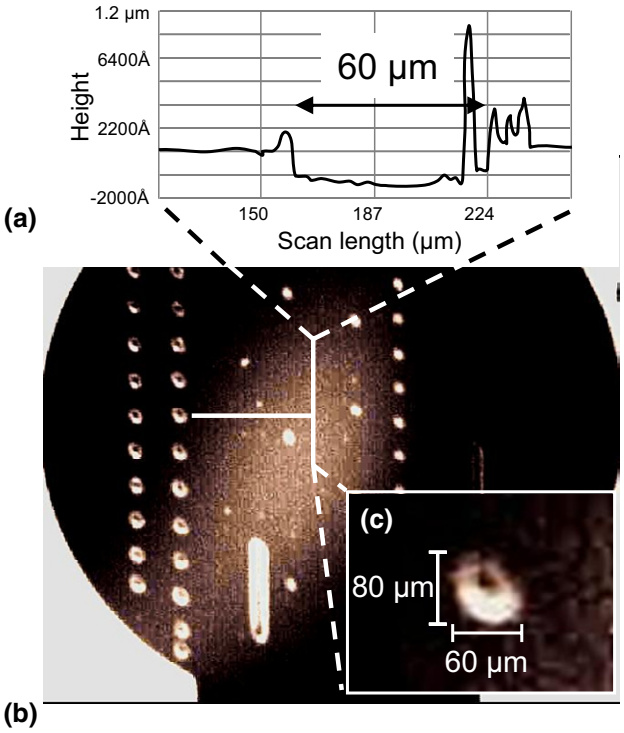

Figure 4. (**a**) Profilometer scan plot across the width (60 μm) of the laser ablation crater in the gold QCM electrode. (**b**) Photograph of the QCM electrode after laser desorption. (**c**) Expanded view of an elliptical ablation crater, the diameters (60 μm × 80 μm) were measured for multiple ablation craters across the QCM electrode.

MALDESI-LTQ-FT-ICR of Peptides and Proteins

MALDESI-FT-ICR mass spectra of peptides and proteins (1–8.6 kDa) including bradykinin, angiotensin I, melittin, glucagon, and ubiquitin each mixed with organic matrix have been shown previously [12, 13]. Comparable data were obtained using the new source; representative MALDESI-FT-ICR mass spectra of bradykinin and melittin both mixed with DHB are included in Supplemental Material (Figure S3). Lysozyme and myoglobin (not shown previously) were each mixed 1:1 (vol:vol) with DHB, 0.8 μL was deposited onto the sample target and actively dried [19]. The ESI solution flow-rate was set to 400 nL/min and stable electrospray was obtained. The motion controller was preprogrammed to raster the sample surface under the laser beam as illustrated in the Supplemental Material (Figure S2). The laser was actuated using computer control at a laser power of 50 μJ (measured internally) and repetition rate of 10 Hz and the motion program initiated. Multiply-charged ions were generated and detected for each peptide and protein; demonstrating a molecular weight range from 1 to 17 kDa using this source. Representative MALDESI-FT-ICR mass spectra of myoglobin and lysozyme from this experiment are shown in Figure 5, with an observed resolving power of ~40,000.

The amount of material ablated during analysis was calculated using the amount of material spotted (~80 pmol), collection time (200 ms), laser repetition rate (10 Hz, 2 shots per collection), area of the laser spot (3846 μm^2), and the area of the dried spot (A = 3.14 mm^2); 196 fmol were removed per spectrum, assuming uniform distribution of analyte and complete removal at each laser shot. The development and implementation of molecular transport devices such as the air amplifier [26, 27], air ejector [28], and remote analyte sampling, transport, and ionization relay (RASTIR) [29], which enable the efficient transport of ions into the mass spectrometer have been investigated and may prove important to increasing sensitivity.

Liquid Drop Sample Analysis

Liquid drop analysis of 0.8 μL droplets of 200 μM ubiquitin and myoglobin mixed 1:1 (vol:vol) with DHB (150 mg/mL) and deposited onto the sample target, biased at 300 V, for immediate analysis is demonstrated using MALDESI with ESI post-ionization. Liquid drop laser desorption analysis with ESI post-ionization has been demonstrated previously [11, 30]. The ESI emitter was biased at 2.8 kV to electrospray 50% acetonitrile in water at a flow rate of 400 nL/min. The liquid sample was continuously irradiated using the Explorer laser without moving the sample target, yielding relatively constant ion abundance over the lifetime of the droplet (~30 s). liq-MALDESI-FT-ICR mass spectra of myoglobin and ubiquitin with ESI post-ionization are shown in Supplemental Material (Figure S4A and B), respectively.

Conclusions

The MALDESI ionization source described herein couples high stability with precision motion and laser control. This design enables high mass resolving power analysis of biological molecules including intact and top-down characterization in addition to facilitating potential imaging applications.

The mobile design provides a stable analytical platform which may be used to implement a number of desorption regimes including laser desorption (e.g., MALDESI), high velocity solvent droplets (e.g., DESI), a stream of heated nitrogen gas (e.g., ASAP), and post-desorption ionization using electrospray ionization (ESI), chemical (e.g., APCI), and photon ionization (e.g., APPI) for analysis of various types and classes of molecules on multiple MS platforms. The modular configuration of the MALDESI ionization source can facilitate the substitution of parts as necessary for specific applications or budgeting constraints. Furthermore, an IR laser could be mounted to the laser table breadboard for infrared laser MALDESI applications (IR-LDESI) [14, 15].

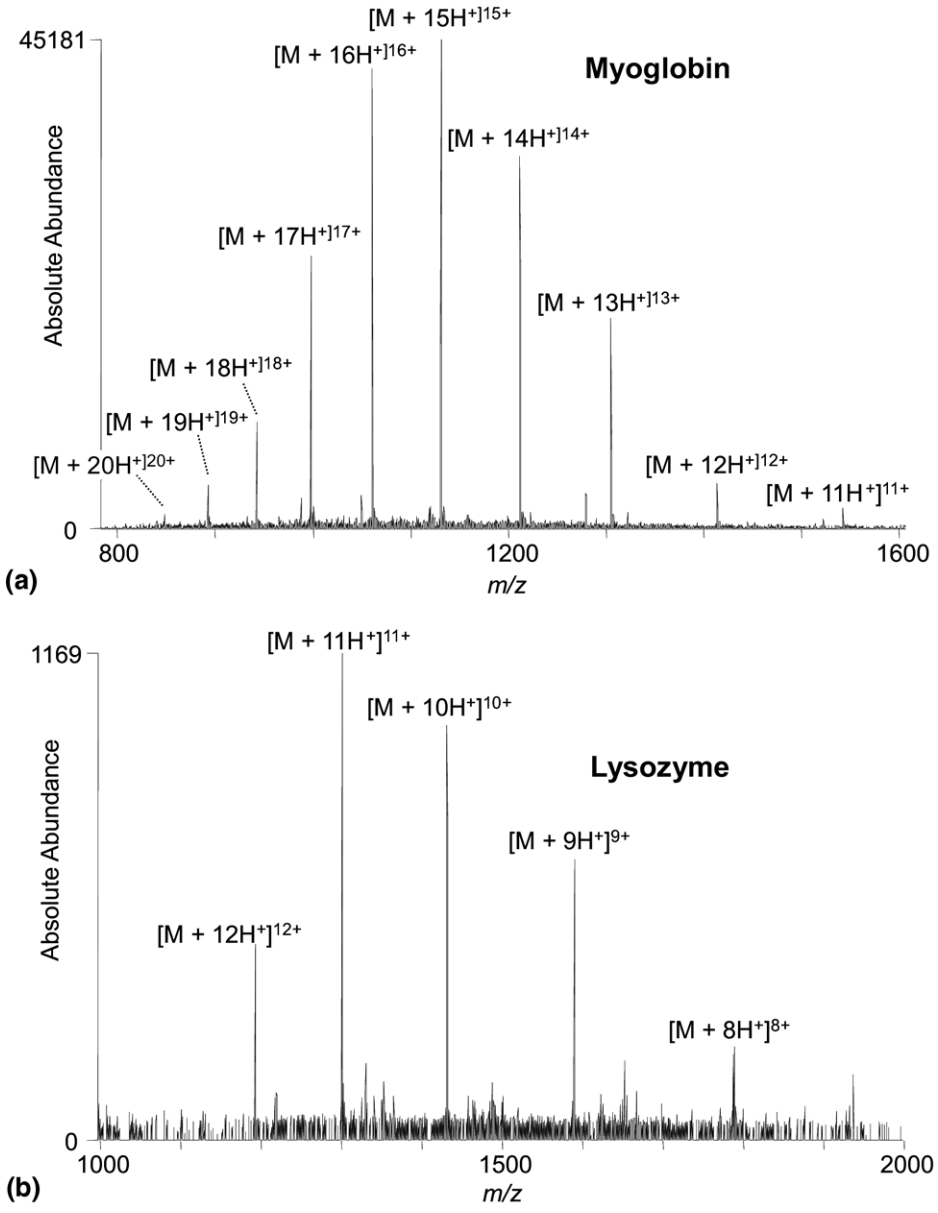

Figure 5. MALDESI-FT-ICR mass spectra of 0.8 μL actively dried spot of 200 μM (**a**) myoglobin (16.9 kDa) and (**b**) lysozyme C (14.3 kDa) mixed 1:1 (vol:vol) with 150 mg/mL DHB.

Acknowledgments

The authors thank Dr. John A. Mclean of Vanderbilt University for assistance in acquiring the Explorer laser system, Dr. Fred Stevie in the Analytical Instrumentation Facility at North Carolina State University for assistance with the profilometer measurements, and the machine shop personnel in the College of Physical and Mathematical Sciences at North Carolina State University for their assistance with the MALDESI source construction. The authors gratefully acknowledge financial support received from the National Cancer Institute, National Institutes of Health (R33 CA105295), the W. M. Keck Foundation, the William R. Kenan Jr. Fund for Engineering, Technology and Science, and North Carolina State University.

References

1. Kolaitis, L.; Lubman, D. M. Detection of Nonvolatile Species by Laser Desorption Atmospheric-Pressure Mass-Spectrometry. *Anal. Chem.* **1986,** *58*(11), 2137–2142.
2. Coon, J. J.; McHale, K. J.; Harrison, W. W. Atmospheric Pressure Laser Desorption/Chemical Ionization Mass Spectrometry: A New Ionization Method Based on Existing Themes. *Rapid Commun. Mass Spectrom.* **2002,** *16*(7), 681–685.
3. Chang, D. Y.; Lee, C. C.; Shiea, J. Detecting Large Biomolecules from High-Salt Solutions by Fused-Droplet Electrospray Ionization Mass Spectrometry. *Anal. Chem.* **2002,** *74*(11), 2465–2469.
4. Chen, H. W.; Venter, A.; Cooks, R. G. Extractive Electrospray Ionization for Direct Analysis of Undiluted Urine, Milk. and Other Complex Mixtures Without Sample Preparation. *Chem. Commun.* **2006,** *19,* 2042–2044.
5. Cody, R. B.; Laramee, J. A.; Durst, H. D. Versatile New Ion Source for the Analysis of Materials in Open Air Under Ambient Conditions. *Anal. Chem.* **2005,** *77*(8), 2297–2302.
6. Takats, Z.; Wiseman, J. M.; Gologan, B.; Cooks, R. G. Mass Spectrometry Sampling Under Ambient Conditions with Desorption Electrospray Ionization. *Science* **2004,** *306*(5695), 471–473.
7. Shiea, J.; Huang, M. Z.; Hsu, H. J.; Lee, C. Y.; Yuan, C. H.; Beech, I.; Sunner, J. Electrospray-Assisted Laser Desorption/Ionization Mass Spectrometry for Direct Ambient Analysis of Solids. *Rapid Commun. Mass Spectrom.* **2005,** *19*(24), 3701–3704.
8. Huang, M. Z.; Hsu, H. J.; Lee, J. Y.; Jeng, J.; Shiea, J. Direct Protein Detection from Biological Media through Electrospray-Assisted Laser

Desorption Ionization/Mass Spectrometry. *J. Proteome Res.* **2006,** *5*(5), 1107–1116.
9. Huang, M. Z.; Hsu, H. J.; Wu, C. I.; Lin, S. Y.; Ma, Y. L.; Cheng, T. L.; Shiea, J. Characterization of the Chemical Components on the Surface of Different Solids with Electrospray-Assisted Laser Desorption Ionization Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **2007,** *21*(11), 1767–1775.
10. Lin, S. Y.; Huang, M. Z.; Chang, H. C.; Shiea, J. Using Electrospray-Assisted Laser Desorption/Ionization Mass Spectrometry to Characterize Organic Compounds Separated on Thin-Layer Chromatography Plates. *Anal. Chem.* **2007,** *79*(22), 8789–8795.
11. Peng, I. X.; Shiea, J.; Loo, R. R. O.; Loo, J. A. Electrospray-Assisted Laser Desorption/Ionization and Tandem Mass Spectrometry of Peptides and Proteins. *Rapid Commun. Mass Spectrom.* **2007,** *21*(16), 2541–2546.
12. Sampson, J. S.; Hawkridge, A. M.; Muddiman, D. C. Generation and Detection of Multiply-Charged Peptides and Proteins by Matrix-Assisted Laser Desorption Electrospray Ionization (MALDESI) Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **2006,** *17*(12), 1712–1716.
13. Sampson, J. S.; Hawkridge, A. M.; Muddiman, D. C. Direct Characterization of Intact Polypeptides by Matrix-Assisted Laser Desorption Electrospray Ionization Quadrupole Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **2007,** *21*(7), 1150–1154.
14. Nemes, P.; Vertes, A. Laser Ablation Electrospray Ionization for Atmospheric Pressure in Vivo and Imaging Mass Spectrometry. *Anal. Chem.* **2007,** *79*(21), 8098–8106.
15. Rezenom, Y. H.; Dong, J.; Murray, K. K. Infrared Laser-Assisted Desorption Electrospray Ionization Mass Spectrometry. *Analyst* **2008,** *133*(2), 226–232.
16. Marshall, A. G.; Guan, S. H. Advantages of High Magnetic Field for Fourier Transform Ion Cyclotron Resonance Mass Dpectrometry. *Rapid Commun. Mass Spectrom.* **1996,** *10*(14), 1819–1823.
17. Kelleher, N. L.; Lin, H. Y.; Valaskovic, G. A.; Aaserud, D. J.; Fridriksson, E. K.; McLafferty, F. W. Top-Down Versus Bottom-Up Protein Characterization by Tandem High-Resolution Mass Spectrometry. *J. Am. Chem. Soc.* **1999,** *121*(4), 806–812.
18. Kelleher, N. L. Top-Down Proteomics. *Anal. Chem.* **2004,** *76*(11), 196A–203A.
19. Williams, T. I.; Saggese, D. A.; Wilcox, R. J.; Martin, J. D.; Muddiman, D. C. Effect of Matrix Crystal Structure on Ion Abundance of Carbohydrates by Matrix-Assisted Laser Desorption/Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **2007,** *21*(5), 807–811.
20. Golovlev, V. V.; Allman, S. L.; Garrett, W. R.; Taranenko, N. I.; Chen, C. H. Laser-Induced Acoustic Desorption. *Int. J. Mass Spectrom.* **1997,** *169,* 69–78.
21. McEwen, C. N.; McKay, R. G.; Larsen, B. S. Analysis of Solids, Liquids, and Biological Tissues Using Solids Probe Introduction at Atmospheric Pressure on Commercial LC/MS Instruments. *Anal. Chem.* **2005,** *77*(23), 7826–7831.
22. Robb, D. B.; Covey, T. R.; Bruins, A. P. Atmospheric Pressure Photoionization: An Ionization Method for Liquid Chromatography-Mass Spectrometry. *Anal. Chem.* **2000,** *72*(15), 3653–3659.
23. Shahin, M. M. Mass-Spectrometric Studies of Corona Discharges in Air at Atmospheric Pressures. *J. Chem. Physics* **1966,** *45*(7), 2600.
24. Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. Electrospray Ionization for Mass-Spectrometry of Large Biomolecules. *Science* **1989,** *246*(4926), 64–71.
25. Laiko, V. V.; Baldwin, M. A.; Burlingame, A. L. Atmospheric Pressure Matrix Assisted Laser Desorption/Ionization Mass Spectrometry. *Anal. Chem.* **2000,** *72*(4), 652–657.
26. Zhou, L.; Yue, B. F.; Dearden, D. V.; Lee, E. D.; Rockwood, A. L.; Lee, M. L. Incorporation of a Venturi Device in Electrospray Ionization. *Anal. Chem.* **2003,** *75*(21), 5978–5983.
27. Dixon, R. B.; Muddiman, D. C.; Hawkridge, A. M.; Fedorov, A. G. Probing the Mechanisms of an Air Amplifier Using a LTQ-FT-ICR-MS and Fluorescence Spectroscopy. *J. Am. Soc. Mass Spectrom.* **2007,** *18*(11), 1909–1913.
28. Dixon, R. B.; Bereman, M. S.; Muddiman, D. C.; Hawkridge, A. M. Remote Mass Spectrometric Sampling of Electrospray- and Desorption Electrospray-Generated Ions Using an Air Ejector. *J. Am. Soc. Mass Spectrom.* **2007,** *18*(10), 1844–1847.
29. Dixon, R. B.; Sampson, J. S.; Hawkridge, A. M.; Muddiman, D. C. Novel Ambient Aerodynamic Ionization Source for Remote Analyte Sampling and Mass Spectrometric Analysis. *Anal. Chem.* **2008,** *80*(13), 5266–5271.
30. Sampson, J. S.; Hawkridge, A. M.; Muddiman, D. C. Development and Characterization of a New Ionization Technique for Analysis of Biological Macromolecules: Liquid Matrix-Assisted Laser Desorption Electrospray Ionization. *Anal. Chem.* **2008,** in press.