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Semi-automated liquid chromatography–mass spectrometric imaging platform for enhanced detection and improved data analysis of complex peptides

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Abstract

A semi-automated analytical platform featuring the coupling of monolithic reversed-phase liquid chromatography (RPLC) to matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI MSI) has been developed and evaluated. This is the first time that LC separation is readily coupled to MS imaging detection for the analysis of complex peptide mixtures both qualitatively and quantitatively. Methacrylate-based monolithic column with C₁₂ functional groups is fabricated for fast RPLC separation. The LC flow and matrix flow are collected on a commercially available MALDI plate which is mechanically controlled and analyzed with MALDI MSI subsequently. Both tryptic peptides digested from bovine serum albumin (BSA) and endogenous neuropeptides extracted from the blue crab *Callinectes sapidus* are analyzed with this novel LC–MSI platform. Compared with regular offline LC fractionation coupled with MALDI MS detection, LC–MSI exhibits significantly increased MS signal intensity due to retaining of temporal resolution from separation dimension via continuous sampling, which results in increased number of peptides detected and accurate quantitation. In addition, imaging signals enable improved data analysis based on either mass-to-charge ratio or retention time, which is extremely beneficial for the analysis of complex analytes. These findings have demonstrated the potential of employing LC–MSI platform for enhanced proteomics and peptidomics studies.

Keywords

Liquid chromatography; Mass spectrometric imaging; LC; MSI; Monolith; Neuropeptides; Quantitation

1. Introduction

The online coupling of liquid chromatography (LC) to mass spectrometry (MS) via electrospray ionization (ESI) source is recognized as one of the most predominating analytical platforms in the past decades, with numerous applications ranging from small

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Appendix A. Supplementary data: Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chroma.2013.03.042>.

molecules [1–3] to peptides and proteins [4–6]. As a complementary ionization method to ESI, matrix-assisted laser desorption/ionization (MALDI) generates mostly singly charged ions with higher tolerance to impurities and traces of additives [7,8]. However, unlike ESI, the inherent solid-phase nature of MALDI repels effective online coupling to separation dimensions, either LC or capillary electrophoresis (CE) [9]. As a result, most of the interfaces coupling separation with MALDI MS detection are designed to be offline via either direct or indirect fraction collection through home-built interfaces or commercially available spotters [10–13]. Compared with online coupling, offline LC–MALDI enables improved MS throughput by analyzing multiple fractionated samples at a time, more flexibility for re-analysis and on-target chemical reactions. Combined with the ionization efficiency complementary to ESI, these benefits attract researchers to take advantage of offline LC–MALDI based platform for various bio-analyses especially for complex sample analyses [10,14–17]. However, a great majority of the offline LC–MALDI coupling schemes suffer from lack of automation, labor-intensive data analysis and the loss of temporal/chromatography resolution from separation dimension due to discrete fraction collection. To date, there is still a lack of interface that can couple LC to MALDI in a similar degree of separation efficiency and automation compared with LC–ESI-MS.

Recently, the possibility of employing MALDI mass spectrometric imaging (MALDI MSI) for LC–MALDI and CE–MALDI coupling has been explored. Since its introduction in 1997 [18], MALDI MSI has almost been exclusively used for tissue imaging of various compounds [19–21], but the inherent characteristics of MSI also make it a great choice for coupling to a micro-scale separation method. In contrast to the regular offline LC/CE–MALDI coupling schemes as mentioned above, continuous collection of LC or CE flow can be easily achieved with MSI detection, which preserves the temporal resolution from separation dimension. In addition, the imaging software enables convenient data analysis based on either mass-to-charge (m/z) or retention time, indicating the potential which could be comparable to current LC–ESI-MS software. We have previously designed interfaces for CE–MALDI-MSI coupling [22,23], and further integrated CE–MALDI-MSI into multi-dimensional separations of neuropeptides from complex neural tissue extracts [24]. Significantly increased number of peptides has been detected when CE–MALDI-MSI is employed with accurate relative quantitation. Compared with CE, LC features a different separation mechanism (e.g., reversed phase LC is based on hydrophobicity) and is much more widely adopted; however, LC separation is impacted by Eddy diffusion and mass transfer kinetics within a column which requires additional consideration when coupled to MALDI MSI. To date, only one report was published on the coupling of LC and MSI by Weidner and Falkenhagen [25]. A T-connector was used to introduce matrix into LC column, and an electrospray deposition interface was built to spray the mixture of LC and matrix flow onto the MALDI target. However, due to the high LC flow rate, only a small portion of LC flow (0.5–2%) could be splitted, mixed with matrix and then sprayed to a relatively large area on MALDI target which could lead to reduced sensitivity and spatial resolution. With this interface, two polymers were separated with peak widths up to several minutes limiting its potential application to quantitation and complex samples.

Here we report an improved LC–MSI device for quantitative analysis of complex peptides. Lauryl methacrylate-co-ethylene dimethacrylate (LMA-EDMA) monolithic column is fabricated for RPLC separation. The LC flow and matrix flow are independently controlled and mixed on a commercially available ground stainless steel MALDI plate. With MALDI plate moving along x -axis, a straight, continuous and homogenous LC trace is formed on the surface of the MALDI plate directly. Combined with the micro-meter scale spatial resolution from MSI acquisitions, this novel interface design retains temporal resolution for the separation down to sub-second time scale, resulting in enhanced mass profiling and accurate quantitation. This system is highly robust and is semi-automated highlighting its great

potential as an attractive alternative to the widespread use of LC–ESI-MS for comparative proteomics and peptidomics studies due to its complementary feature and unique capabilities.

2. Materials and methods

2.1. Chemical and materials

Acetic acid, hydrochloric acid, sodium hydroxide, acetone, acetonitrile, urea and ammonium bicarbonate were obtained from Fisher Scientific (Pittsburgh, PA, USA). Lauryl methacrylate (LMA, 96%), ethylene dimethacrylate (EDMA, 98%), butandiol (99%), 1-propanol (99.5%), 3-(trimethoxysilyl) propyl ethacrylate (98%), 2,2 -Azobis(2-methylpropionitrile) (AIBN, 98%), trifluoroacetic acid (TFA), -Cyano-4-hydroxycinnamic acid (CHCA, 99%), iodoacetamide (IAA) and bovine serum albumin (BSA) were purchased from Sigma–Aldrich (St. Louis, MO, USA). D/L-dithiothreitol (DTT) and sequencing grade modified trypsin were from Promega (Madison, WI, USA). Ethanol (200 proof) was purchased from Decon Laboratories (King of Prussia, PA, USA). C18 Ziptip column from Millipore was used for sample cleaning, and all water used was doubly distilled on a Millipore filtration system (Bedford, MA, USA). The physiological saline consisted of 440 mM NaCl, 11 mM KCl, 26 mM MgCl₂, 13 mM CaCl₂, 11 mM Trizma base, and 5 mM maleic acid in pH 7.45.

2.2. Sample preparation

The tryptic peptides were digested from BSA. Briefly, 30 µg of BSA was reconstituted in 20 µL of 8 M urea followed by adding 1 µL of 1 M DTT. The tube was gently vortexed and incubated in 37 °C for 30 min. 20 µL of 200 mM IAA was added to the tube after reducing, and incubated for another 30 min at room temperature in dark. 4 µL of DTT was then added to consume residual alkylating reagent. 120 µL of 25 mM ammonium bicarbonate solution was then added to dilute urea before adding 1 µg of trypsin. The digestion was kept at 37 °C overnight, and quenched by adding 1 µL of formic acid with gentle vortex. The BSA tryptic peptides was stored at –80 °C and desalted with Ziptip C18 column before usage. DTT, IAA and urea were dissolved in 25 mM ammonium bicarbonate solution.

Neuropeptides were extracted from the pericardial organ (PO) of blue crabs (*Callinectes sapidus*). The crabs were purchased from local grocery store and kept in an artificial seawater tank at 10–12 °C without food. During dissection, the crabs were first anesthetized for 15–30 min in ice, and POs were dissected in physiological saline. Three POs were combined, homogenized in 50 µL acidified methanol (1% acetic acid in 90% methanol) in ice and centrifuged at 16,100 rcf for 8 min before harvesting the supernatants. The extraction was repeated for 3 times and the supernatants were combined and dried. The residue was reconstituted in 10 µL of 0.1% TFA and desalted with Ziptip C18 column.

2.3. In solution formaldehyde labeling

Two aliquots of sample (BSA tryptic peptides or PO extraction) were each dissolved in 5 µL of 50% acetonitrile in 0.1% TFA after desalting, and added with 1 µL of borane pyridine (C₅H₈BN, 120 mM in 10% methanol). The samples were then pairwise labeled with formaldehyde-H₂ (4% in water, v/v) and formaldehyde-D₂ (4% in water, v/v) in 1:1 concentration ratio, respectively, followed by 37 °C incubation for 15 min. 1 µL of 0.2 M ammonium bicarbonate was then added to quench the reaction, and the two labeled aliquots were mixed and dried. After desalting, the combined sample was reconstituted in 10 µL of 0.1% TFA and stored at –80 °C before analysis.

2.4. LC column and condition

LMA-EDMA monolithic column was fabricated based on a modified procedure from our previous report [26]. Fused-silica capillary with 40 μm i.d. and 360 μm o.d. (Polymicro Technologies, Phoenix, AZ, USA) was quickly rinsed with acetone and water, and then flushed with 0.2 M NaOH for 30 min. After rinsed with water, the capillary was pumped with 0.2 M HCl for another 30 min, followed by water and ethanol. Silanization was performed by flushing 3-(trimethoxysilyl) propyl methacrylate (in 95% ethanol) at a flow rate of 0.5 $\mu\text{L}/\text{min}$ for 90 min, and followed by acetone and a stream of nitrogen overnight. The polymerization mixture consists of 24% LMA, 16% EDMA, 40% 1-propanol and 20% 1,4-butanediol (w/w, with 1% AIBN as initiator). The mixture was degassed with nitrogen and filled into silanized capillary which was then sealed at both ends. Thermal-initiated polymerization was carried out in a water bath at 70 $^{\circ}\text{C}$ for 24 h. After polymerization, the capillary was washed with acetonitrile and water, and cut into 12 cm long as LC column. LC fractionation was conducted with a Waters nanoACQUITY UPLC system. The mobile phases contained solution A (0.1% formic acid in deionized water) and solution B (0.1% formic acid in acetonitrile). LC separation started at 95% of solution A, dropped to 80% at 10 min and 30% at 30 min. The flow rate was set at 0.5 $\mu\text{L}/\text{min}$ with 1 μL sample loading amount.

2.5. LC–MSI interface

LC–MSI interface consists of a monolithic LC column, a matrix-delivery capillary as well as a commercially available ground stainless steel MALDI plate from Bruker Daltonics (Bremen, German). As shown in Fig. 1, matrix flow (saturated CHCA in 50% ACN/0.1% TFA) was delivered via a capillary at 0.7 $\mu\text{L}/\text{min}$ by a syringe pump (Pump 11 Elite, Harvard Apparatus, Holliston, MA, USA). The matrix flow and LC flow were mixed at the capillary tips and collected on the surface of MALDI plate which is mechanically controlled. The MALDI plate moved along x -axis at the speed of 4.5 mm/min, equaling to the distance between two spots on the MALDI plate. A lamp was used during fraction collection to dry the solvents quickly so that approximately 0.8 mm wide, straight and homogenous LC traces can be deposited on the plate surface with minimal diffusion. LC eluent from 2 min to 25 min was collected which contained majority of separated analytes.

2.6. MALDI MS imaging

The MS images of collected LC traces were acquired with Autoflex III MALDI-TOF/TOF (Bruker Daltonics, Bremen, Germany) equipped with 200 Hz Smartbeam II laser. Positive reflectron mode was adopted with the following parameters: ion source 1 voltage 18.93 kV, ion source 2 voltage 16.49 kV, reflector 1 voltage 20.90 kV, reflector 2 voltage 9.59 kV and lens voltage 8.65 kV. For each pixel, the step size was set at 100 $\mu\text{m} \times 100 \mu\text{m}$ (on the x/y axis), and 200 shots at 60% laser power were accumulated in a random mode for each pixel. The imaging data was processed with FlexImaging 2.0 (Bruker Daltonics, Bremen, Germany) and Quantinetix (ImaBiotech, Loos, France).

3. Results and discussions

3.1. Optimization of monolithic LC column

The LC flow rate and matrix flow rate must be compatible to be combined for LC–MSI coupling. Meanwhile, fast separation is desired so that LC eluent with separated analytes can be collected on a MALDI plate with limited length. As a routine method, when particle-packed nano-RPLC column was employed for complex peptide characterization as we previously reported, low flow rate and high pressure drop were observed which resulted in long separation time up to 120 min that cannot be collected in full duration with MALDI plate [27]. As a dilemma, when a regular 4.5 mm i.d. RPLC column was used as reported by

Weidner and Falkenhagen [25], the mL/min level of LC flow rate was not compatible with $\mu\text{L}/\text{min}$ level of matrix flow rate. Thus, a very limited portion of the LC flow was splitted and collected for MALDI MS detection which caused significant loss of sensitivity.

Compared with regular particle-packed column, monolithic column's wide through-pores and small skeletons enable similar separation efficiency at a significantly lower pressure drop [28,29]. At a flow rate similar to matrix flow, fast separation can be achieved with LC flow fully collected on the MALDI plate. In addition, the pore size and channel depth of a monolithic column could be individually adjusted, which allows us to optimize separations based on different sample requirements [30,31]. LMA-EDMA monolith with C_{12} functional groups was adopted here for the separation of trace-level peptide mixtures. The composition and ratios of monomers and porogens were individually evaluated. As tested under nano-LC system, a pressure drop of 1100 psi was observed under $0.5 \mu\text{L}/\text{min}$ flow rate (5% ACN: 95% 0.1% TFA), with a separation window from 9 min to 19 min for both tryptic peptides and neuropeptides. A scanning electron microscope (SEM) was employed (Hitachi S570, Pleasanton, CA, USA) to capture the internal structure of the home-made LMA-EDMA monolithic column (Fig. 2).

3.2. LC–MSI interface

The collection of LC trace with minimal loss in sensitivity and resolution is critical for LC–MSI coupling. Therefore, instead of using a spray device which could cause loss of resolution due to diffusion, we modified our CE–MSI interface for LC–MSI coupling [23]. Briefly, a commercially available ground stainless steel MALDI plate was used, with two capillaries delivering LC flow and matrix flow separately. Compared with other reported separation–MALDI coupling interfaces, our device is economical and much simpler without the need of vacuum [32] or spray [25]. With an effort for automation, both LC flow and matrix flow were individually controlled with pumps, while the movement of MALDI plate was controlled mechanically (Fig. 1A). As a result, narrow, continuous and homogenous LC traces could be collected directly on the MALDI plate and were quickly dried to minimize diffusion. Fig. 1B shows a LC trace, which was co-crystallized from LC eluent and CHCA matrix, collected on the MALDI plate surface. With the $100 \mu\text{m} \times 100 \mu\text{m}$ pixel size from MSI detection, each pixel covers approximately 1.2 s of LC separation, however, the pixel size could be further reduced to $50 \mu\text{m} \times 50 \mu\text{m}$ with prolonged acquisition time to bring the step size down to 0.6 s of LC separation per pixel, further improving chromatographical resolution achievable with our LC–MSI platform.

The stability of the LC–MSI platform was tested with BSA tryptic peptides. Three LC runs under the same condition were performed with LC traces subjected to MSI analysis on different days. Fig. 3 shows the MS full scan of three runs. Very similar spectra were observed with comparable numbers of peptides detected. We further selected six tryptic peptides with representative m/z values and retention times, and calculated the relative standard deviation (RSD) of their migration times among three runs. Results have shown that the RSDs ranged from 0.4% to 1.4%, indicating excellent stability and reproducibility of the LC–MSI system.

3.3. Image-based data analysis and peptide characterization

Routinely, collecting LC fractions on a MALDI plate spot-by-spot and acquiring mass spectrum from each time point are extremely labor intensive. The data analysis requires a manual search of the mass spectra generated from each spot. Compared with that, a unique feature of LC–MSI data analysis is the ability to analyze data based on either mass-to-charge ratio or retention time from the constructed image. With the imaging software, a “mass filter” can be specified on the mass spectrum and the image for the selected mass range will

be shown. Alternatively, since the position of image reflects retention time, by selecting a pixel or a region of interest (ROI) on the image of the LC trace, the mass spectrum can be retrieved and displayed for the selected retention time. Fig. 4 shows an example of how to analyze a mass spectrum with complex and low abundance peaks using image-based data analysis. A BSA tryptic peptide EYEATLEECCAK (m/z 1502.6) is observed as shown in Fig. 4A; however, there are a few lower-abundance peaks in the mass range around m/z 1500 which are suppressed by the peak at m/z 1502.6. With imaging software, we set up a 1 Da wide mass filter at m/z 1500 (the pink stripe region in Fig. 4), and observed its image distribution. There are two distinguishable colored regions, indicating peptides with different retention times separated by LC. By clicking on the colored regions, the mass spectrum can be displayed for each region, and mass spectral peaks at m/z 1500.8 (as in Fig. 4B) and m/z 1498.7 (which was co-eluted with m/z 1502.6, as in Fig. 4C) are observed with significantly enhanced sensitivity and resolution.

The easy three-step “select mass range (filter)->obtain image->extract ions from pixel” as summarized in Fig. 4 can also be used for searching of complex unknown analytes by screening the mass filter across the spectrum. As an example shown in Fig. S1, when the spectrum was screened with a 1-Da wide mass filter, two colored image regions around m/z 2061 were observed, indicating overlapped peaks due to the low S/N and limited instrument resolution (Fig. S1A). With MSI we can extract the ions from each region from Fig. S1B by a simple click and get the spectrum for each separated peaks at m/z 2060.9 and m/z 2061.1 (Fig. S1C and D). Tandem MS can also be performed by gas-phase fragmentation of selected ions from the colored regions for further structural elucidation.

The improved data analysis combined with the high separation resolution retained from LC provides greater capability in peptide detection and characterization. By loading 1 μ L of 1.8 μ M tryptic peptides into the LC-MSI system, a total of 69 tryptic peptides (or 78 including variable modifications) were identified by Mascot search, representing 81% of protein sequence coverage. With diluted tryptic peptide mixture, we were able to detect most of the major peptides with 20 fmol loading amount. Considering each peak was covered by more than 20 pixels in most cases, the limit of detection for each pixel was below 1 fmol as measured here. The number of peptides detected by LC-MSI is significantly higher than regular LC-MALDI with discrete fraction collection every 30 s where a total of 47 tryptic peptides were detected (representing 66% of protein sequence coverage). We also compared the analysis of the same type of tryptic peptides by LC-MSI with the CE-MSI platform as we recently developed [23]. In our opinion, these two techniques are complementary to each other in that they accommodate different sample loading amounts (CE-MSI in the nanoliter regime and LC-MSI allows microliter volume injection) and rely on different separation mechanisms, providing researchers alternative method based on specific experimental needs. Similar to the situation when comparing regular LC-MS and CE-MS, each method has its own advantages and shortcomings. However, with higher loading capacity of LC as presented in this study (1 μ L for LC vs. less than 100 nL for CE), we have observed higher peptide coverage (69 tryptic peptides) than previous CE-MSI based analysis where a total of 46 tryptic peptides were detected. A Venn diagram for the comparison of LC-MALDI, LC-MSI and CE-MSI is shown in Fig. S2.

The newly developed LC-MSI platform was also applied to the separation and detection of complex neuropeptides extracted from the blue crab *C. sapidus*. The analysis of neuropeptides is challenging due to their extremely low concentration and diverse chemical and physical properties in a complex biological background. As we recently reported that by employing CE-MSI, a total of 150 putative neuropeptides had been detected, representing a significantly enhanced coverage than using other analytical platforms. Here with a higher loading capacity, 262 putative neuropeptides were detected with LC-MSI, including 65

previously identified from our home-built neuropeptide database, which is higher than the number of peptides detected with CE–MSI. All of the tryptic peptides and putative neuropeptides detected with LC–MSI are listed in Tables S1 and S2 as supporting material.

3.4. LC–MSI quantitation

Relative quantitation has been evaluated by isotopic formaldehyde labeling. BSA tryptic peptides or extracted neuropeptides were labeled with light formaldehyde (FH₂, +28.03 Da for each incorporated label) and heavy formaldehyde (FD₂, +32.05 Da for each incorporated label) in 1:1 concentration ratio, generating a 4.02 Da (or multiple of 4.02 Da) mass difference between each peak pair. Although there was a concern that isotopic labeling might change migration behaviors between light and heavy labeled analytes, we did not observe such phenomenon and all peak pairs exhibited similar retention times and signal intensities. The instrument-specific software FlexImaging does not have the function to display peak intensities; however, we have employed Quantinetix, a software package capable of dealing with quantitative MS imaging data, to calculate the intensities from selected regions of interest (ROI) from the peak images. 5–15% errors for most of the peak pairs were observed. An example is shown in Fig. 5. A peak pair at *m/z* 1333.6/1345.6 is from BSA tryptic peptides after formaldehyde labeling in 1:1 concentration ratio (FH₂:FD₂). Based on MS full scan (Fig. 5A), both peaks exhibit similar peak intensities as well as highly identical image distribution (Fig. 5B and C). With Quantinetix, we calculated the peak intensities for each of the peak and obtained a peak ratio of 1.04:1 (Fig. 5D and E), indicating accurate relative quantitation with LC–MSI.

4. Conclusions

We have developed a monolithic LC–MSI system for complex peptide mixture analysis. This semi-automated platform retains the resolution from separation dimension and enables improved data analysis with imaging software. We performed the first LC–MSI analysis of complex peptide mixtures, as well as the first LC–MSI based quantitation. Fast separation and enhanced MS sensitivity have been observed resulting in significantly increased number of peptides being detected than other techniques, including regular LC–MALDI and our recently reported CE–MSI platforms. Accurate relative quantitation was demonstrated with isotopic formaldehyde labeling. The LC–MSI system is simple and robust with highly reproducible results, providing a new strategy of LC–MALDI coupling with great potential in proteomics and peptidomics studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Jemal M. Biomed Chromatogr. 2000; 14:422–429. [PubMed: 11002279]
2. Lee MS, Kerns EH. Mass Spectrom Rev. 1999; 18:187–279. [PubMed: 10568041]

3. Petrovic M, Hernando MD, Diaz-Cruz MS, Barcelo D. *J Chromatogr A*. 2005; 1067:1–14. [PubMed: 15844508]
4. Aebersold R, Mann M. *Nature*. 2003; 422:198–207. [PubMed: 12634793]
5. Anderson L, Hunter CL. *Mol Cell Proteomics*. 2006; 5:573–588. [PubMed: 16332733]
6. Ning ZB, Zhou H, Wang FJ, Abu-Farha M, Figeys D. *Anal Chem*. 2011; 83:4407–4426. [PubMed: 21491952]
7. Marshall AG, Hendrickson CL. *Annual Review of Analytical Chemistry (Palo Alto)*. 2008; 1:579–599.
8. Bodnar WM, Blackburn RK, Krise JM, Moseley MA. *J Am Soc Mass Spectrom*. 2003; 14:971–979. [PubMed: 12954165]
9. Pes O, Preisler J. *J Chromatogr A*. 2010; 1217:3966–3977. [PubMed: 20304406]
10. Williams BJ, Russell WK, Russell DH. *Anal Chem*. 2007; 79:3850–3855. [PubMed: 17411015]
11. Preisler J, Hu P, Rejtar T, Karger BL. *Anal Chem*. 2000; 72:4785–4795. [PubMed: 11055691]
12. Ericson C, Phung QT, Horn DM, Peters EC, Fitchett JR, Ficarro SB, Salomon AR, Brill LM, Brock A. *Anal Chem*. 2003; 75:2309–2315. [PubMed: 12918971]
13. Zhang BY, McDonald C, Li L. *Anal Chem*. 2004; 76:992–1001. [PubMed: 14961730]
14. Hattan SJ, Vestal ML. *Anal Chem*. 2008; 80:9115–9123. [PubMed: 19551981]
15. Mateos J, Lourido L, Fernandez-Puente P, Calamia V, Fernandez-Lopez C, Oreiro N, Ruiz-Romero C, Blanco FJ. *J Proteomics*. 2012; 75:2869–2878. [PubMed: 22245418]
16. Funke S, Azimi D, Wolters D, Grus FH, Pfeiffer N. *J Proteomics*. 2012; 75:3177–3190. [PubMed: 22480906]
17. Soderberg CAG, Lambert W, Kjellstrom S, Wiegandt A, Wulff RP, Mansson C, Rutsdottir G, Emanuelsson C. *PLoS ONE*. 2012; 7:e38927. [PubMed: 22723907]
18. Caprioli RM, Farmer TB, Gile J. *Anal Chem*. 1997; 69:4751–4760. [PubMed: 9406525]
19. Heeren RMA, Smith DF, Stauber J, Kukrer-Kaletas B, MacAleese L. *J Am Soc Mass Spectrom*. 2009; 20:1006–1014. [PubMed: 19318278]
20. Greer T, Sturm R, Li LJ. *J Proteomics*. 2011; 74:2617–2631. [PubMed: 21515430]
21. Amstalden van Hove ER, Smith DF, Heeren RMA. *J Chromatogr A*. 2010; 1217:3946–3954. [PubMed: 20223463]
22. Wang JH, Ye H, Zhang ZC, Xiang F, Girdaukas G, Li LJ. *Anal Chem*. 2011; 83:3462–3469. [PubMed: 21417482]
23. Zhang Z, Ye H, Wang J, Hui L, Li L. *Anal Chem*. 2012; 84:7684–7691. [PubMed: 22891936]
24. Zhang ZC, Jia CX, Li LJ. *J Sep Sci*. 2012; 35:1779–1784. [PubMed: 22807360]
25. Weidner SM, Falkenhagen J. *Anal Chem*. 2011; 83:9153–9158. [PubMed: 22017593]
26. Zhang ZC, Wang JH, Hui LM, Li LJ. *Electrophoresis*. 2012; 33:661–665. [PubMed: 22451059]
27. Ma MM, Wang JH, Chen RB, Li LJ. *J Proteome Res*. 2009; 8:2426–2437. [PubMed: 19222238]
28. Svec F, Huber CG. *Anal Chem*. 2006; 78:2100–2107.
29. Zhao L, Qin H, Wu RA, Zou H. *J Chromatogr A*. 2012; 1228:193–204. [PubMed: 21993515]
30. Svec F. *J Chromatogr A*. 2010; 1217:902–924. [PubMed: 19828151]
31. Jiang T, Mallik R, Hage DS. *Anal Chem*. 2005; 77:2362–2372. [PubMed: 15828768]
32. Rejtar T, Hu P, Juhasz P, Campbell JM, Vestal ML, Preisler J, Karger BL. *J Proteome Res*. 2002; 1:171–179. [PubMed: 12643537]

Fig. 1. LC-MSI interface. (A) The scheme of the interface consisting of a monolithic LC column, a capillary for matrix flow delivery and a ground stainless steel MALDI plate. (B) A uniform LC trace deposited on the surface of a MALDI plate with approximately 0.8 mm width. Distance between two spots on the plate is 0.45 mm.

Fig. 2.
SEM micrograph showing the internal structure of LMA-EDMA monolith fabricated within 40 μm i.d. capillary. Scale bar is 5 μm.

Fig. 3. Stability test of monolithic LC-MSI platform. Upper: the MS full scan of three runs using BSA tryptic peptides. Bottom: the average retention times and RSDs of six representative tryptic peptides detected.

Fig. 4. LC-MSI data analysis. (A) MS full scan showing the mass range from m/z 1490 to m/z 1520. A 1-Da wide mass filter was selected and shown in pink strip, with the imaging signal being enlarged and highlighted in two white boxes. (B) and (C) Mass spectrum for each colored region from the selected 1-Da mass range. (For interpretation of the references to color in figure legend, the reader is referred to the web version of the article.)

Fig. 5.

Relative quantitation with LC-MSI for peak pair m/z 1333.6/1345.6. (A) The MS full scan of peak pair at m/z 1333.6/1345.6. The sample was labeled with formaldehyde in 1:1 concentration ratio (FH2:FD2). (B) Image for the peak at m/z 1333.6. (C) Image for the peak at m/z 1345.6. Both (B) and (C) display images with similar intensities and distribution patterns, indicating roughly 1:1 ratio in relative abundances of the peptide pair. (D) and (E) The extracted ion intensities of m/z 1333.6/1345.6 using Quantinetix software. With a region of interest (ROI) selected from image, peak intensities were obtained and averaged by pixel, which were listed in figure as “average calculated intensity”. A ratio of 1.04:1 was observed for this peak pair.