Multi-Segment Direct Inject nano-ESI-LTQ-FT-ICR-MS/MS For Protein Identification

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Abstract
Reversed phase high performance liquid chromatography (HPLC) interfaced to electrospray tandem mass spectrometry (MS/MS) is commonly used for the identification of peptides from proteolytically cleaved proteins embedded in a polyacrylamide gel matrix as well as for metabolomics screening. HPLC separations are time consuming (30-60 min average), costly (columns and mobile phase reagents), and carry the risk of column carry over between samples. The use of a chip-based nano-ESI platform (Advion NanoMate) based on replaceable nano-tips for sample introduction eliminates sample cross-contamination, provides unchanging sample matrix, and enhances spray stability with attendant increases in reproducibility. Recent papers have established direct infusion nano-ESI-MS/MS utilizing the NanoMate for protein identification of gel spots based on full range MS scans with data dependent MS/MS. In a full range scan, discontinuous ion suppression due to sample matrix can impair identification of putative mass features of interest in both the proteomic and metabolomic workflows. In the current study, an extension of an established direct inject nano-ESI-MS/MS method is described that utilizes the mass filtering capability of an ion-trap for ion packet separation into four narrow mass ranges (50 amu overlap) with segment specific dynamic data dependent peak inclusion for MS/MS fragmentation (total acquisition time of 3 minutes). Comparison of this method with a more traditional nanoLC-MS/MS based protocol utilizing solvent/sample stream splitting to achieve nanoflow demonstrated comparable results for protein identification from polyacrylamide gel matrices. The advantages of this method include full automation, lack of cross-contamination, low cost, and high throughput.

Keywords: Direct inject, Nano-ESI, LTQ-FT-ICR-MS/MS, Protein identification

Background
One of the major goals of proteomics is to be able to identify proteins of interest [1]. For protein spots on 1D- or 2D-SDS-PAGE gels this is a challenge due to the large diversity of protein spot abundances and the complexity of the matrix. The great number of protein spots or gel slices which can approach several hundred per experiment demands automated, high throughput, cost-effective analytical methods with high sensitivity [2].

For the proteomics workflow, high performance liquid chromatography (HPLC) is typically coupled via an electrospray source (nanoESI or ESI) to either an ion trap or time-of-flight (TOF) mass analyzer with ion packet selection via mass filter (quadrupole or ion trap) [2-9]. Following parent mass feature selection, tandem mass spectrometry (MS/MS) based on collision induced dissociation (CID) or electron capture dissociation (ECD) of peptide parent mass/charge features are used to generate mixed ion populations of peptide fragments which are then utilized for protein identification. The acidified HPLC eluent serves two purposes: a) to separate peptide mixtures thus simplifying the parent mass composition of ion packet; and b) to competitively eliminate salts. However this workflow has several drawbacks including potential sample cross-contamination due to column carryover and the lengthy sample run time [2,8]. The cost of instrument time, columns, and solvents drives the total cost per sample.

To address the problem of lengthy sample run time and expensive instrument time usage, direct infusion sample introduction methods without HPLC separation prior to the electrospray source has been developed [2,8,10-16]. The advantages of the chip-based ESI method include fully automated, high throughput, a lack...
of cross-contamination, enhanced spray stability and reproducibility, and constant sample matrix [2,8,13-16]. In each case, protein spots were analyzed with one broad mass range MS scan followed by MS/MS scans.

In the current study, a method of direct inject LTQ-FT-ICR-MS/MS combined with automated chip-based nanoESI is introduced which is broadly applicable to a variety of mass spectrometry types. Proteolytic peptides were subjected to a MS prescan (00–2000 m/z) followed by four narrow mass range scans with 50 amu overlap and including iterative MS/MS of mass features in the selected window with total acquisition time of 3 min per sample. Dynamic data dependent exclusion and charge state screen were enabled in these ranges to ensure that many +2 and +3 charged peaks were selected based on ion intensity. This new multi-segment direct inject nano-ESI method was compared to a traditional RPLC-nanoESI-LTQ-FT-ICR-MS/MS method with an approximate run time of ~30 minutes. The ability to identify proteins from rat liver 2D-SDS-PAGE gel spots was compared between methods.

**Results**

As shown in Figure 1, scanning across different mass ranges impacts the number and intensity of the parent ion LTQ-FT-ICR-MS spectra generated from tryptic digests of protein spots. In Figure 1A, a selected segment from the multi-step method (400-750 m/z) is shown. In Figure 1B, a scan of the full experimental mass range was collected (400-2000 m/z) with a zoom to the mass region comparable to that shown in Figure 1A (400-750 m/z). Clear differences in the number and intensity of ions present in the spectra are found with the sole difference being mass range selection of ion packet—this highlights the impact of discontinuous ion suppression and ion population characteristics on spectral features. Specifically, the loss of numerous moderate to low abundance ion features is evident in Figure 1B (mass range scanned 400-2000 m/z). Figure 1C & 1D are identical spectra from Figure 1A and 1B but zoomed to the range of 400-500, respectively, for enhanced comparison with the identical outcome of a greater number of ion features present in the narrow scan range (400-750 m/z; Figure 1C) versus the broad ion scan (400-2000 m/z; Figure 1D). A greater number of +2 and +3 charged ions were assigned (not identified for simplicity) when the smaller range was scanned further highlighting the utility of this method for iterative data dependent MS/MS analysis.

![Figure 1](http://www.proteomesci.com/content/9/1/38)
In Table 1 (Figure 2 liver gel image with spot numbering), the ability to identify proteins from 16 protein containing gel spots by these two methods was compared. A total of 41 proteins were identified by HPLC-MS/MS method while 38 proteins were identified by chip-based nanoESI/MS/MS method. This reflects the known outcome of multiple proteins present in protein spots from 2D gels. A total of 33 common proteins were identified with both methods. Twenty out of the 33 common proteins identified by both methods possess higher MOWSE scores with the multistage direct inject nanoESI/MS/MS method. For the remaining 13 common proteins, the MOWSE scores are comparable between methods. Interestingly a lack of putative identity hits from decoy database searching (reverse amino acid sequence for proteins used to assess false positive identifications) was observed for the multistep nanoESI/MS/MS method. However, when utilizing the LC-based separation method, spurious putative identifications were present. A larger study will be necessary to confirm that the multistage method yields higher identification certainty with lower false-positive rate. Of note, the multistage direct inject method yielded a greater number of peptides matched per protein and a higher percentage coverage for the majority of the common proteins identified by both methods (22 out of 33). For the remainder, 6 possess equal scores and 5 are of comparable ranking.

Discussion
A comparison of data-dependent MS/MS fragmentation within the proposed direct inject multi-step small range parent ion MS scan and a direct inject single step full range parent ion MS scan were conducted on selected 2D gel protein spots and the results were compared with the more traditional HPLC MS/MS method. The only difference between the two direct inject methods were the scan range before the data-dependent MS/MS fragmentation; the former one (multi-step) utilized 4 segments of overlapping narrow range parent ion MS scans and a direct inject single step full range parent ion MS scan were conducted on selected 2D gel protein spots and the results were compared with the more traditional HPLC MS/MS method. The only difference between the two direct inject methods were the scan range before the data-dependent MS/MS fragmentation; the former one (multi-step) utilized 4 segments of overlapping narrow range parent ion MS scans followed by daughter ion MS/MS scans, while the latter one (full range) utilized a full range parent ion MS scan (400-2000 m/z) followed by daughter ion MS/MS scans. The MOWSE scores of the multi-step small range scan were greater than those of full range scan method due to discontinuous ion suppression leading to fewer candidate parent ions. The results of the multi-step small range scan was comparable with the results of the HPLC method (data not shown). In the HPLC method, due to the variance of peak width for the eluting peptides, it is not feasible to adjust the time used for each segment in the MS scan of each parent ion. Some peaks lose a great degree of information when the multistage approach is employed, particularly the moderate to low abundance peaks. The use of direct injection with continuous, stable parent ion composition makes the multistage parent ion scanning feasible.

As described above, the multistage direct inject nanoESI/MS/MS method yielded at a minimum comparable, and in most cases greater, confidence in the correct identification of proteins from a polyacrylamide gel matrix. The 16 spots analyzed covered a wide mass range, from 14,200 to 164,000 amu including the low, medium, and high abundance levels indicating that the multistage direct inject method is robust for protein identification. The advantages of this chip-based nanoESI/MS/MS method include fully automated, lack of cross-contamination, and high throughput with sensitivity sufficient to identify multiple proteins per sample. The number of segments, mass range and running time for each segment may be optimized to accommodate the complexity of different matrices.

Mass spectrometry methods have been recognized as a general strategy for protein characterization. Although HPLC-MS/MS is commonly utilized for protein identification [8] it has the disadvantage of a lengthy run time, high cost per sample due to instrument time, and possible sample cross-contamination due to column carryover. Here we introduce a novel 4-segment multistage direct inject nanoESI LTQ-FT-ICR-MS/MS method that can be used to analyze a proteolytic digest within approximately 3 minutes. Multiple overlapping narrow range parent ion mass scans followed by iterative MS/MS can effectively minimize the discontinuous ion suppression observed with broad range mass spectral windows (400-2500 m/z) common in other direct inject nanoESI-MS/MS methods for protein identification. The current method is robust with wide mass range coverage and multiple protein identification in samples. While an LTQ-FT-ICR-MS was utilized for the current comparison, high mass accuracy and resolution is not a requirement of the method. Indeed, as MS/MS agreement with fragmentation patterns is the basis of peptide identification, the method is amenable to use on a variety of mass spectrometry platforms which provide sufficient parent ion resolution for selection of multiple peptide features for fragmentation—ie all commercially available mass spectrometry platforms currently used for proteomics workflows. As this method can substitute for the traditional HPLC-MS/MS method for the protein analysis of 2D gel spots with a much lower cost, we propose that the current practice of identification of protein spots of interest should change to an analysis of the entire population of proteins present on 2D gels to enhance the understanding of the population represented within an experiment.

Conclusion
A multi-step, chip-based nanoESI-MS/MS method is proposed for protein identification. This method consists of 4 segments with overlapping narrow range scans
Table 1 Comparison of identification of 2D gel separated spots of rat liver protein by chip-based nanoESI/MS/MS and nanoHPLC-MS/MS

| Spot | Method  | Protein                                                      | Score | Peptidesmatched | % Cover | ProteinMW (× 10^{-3}) | Decoy Discovery Rate | False Discovery Rate | Accession     |
|------|---------|--------------------------------------------------------------|-------|----------------|---------|-----------------------|---------------------|---------------------|---------------|
| 1    | HPLC    | Protein disulfide-isomerase                                  | 211   | 7              | 12      | 57.1                  | 0                   | 0                   | gi129729      |
|      | Multistep|                                                              | 280   | 15             | 26      | 0                     | 0                   | 0                   | gi202549      |
| 2    | HPLC    | Lodothyronine 5' monodeiodinase                              | 211   | 7              | 13      | 54.0                  | 0                   | 0                   | gi148702818   |
|      | Multistep|                                                              | 280   | 15             | 25      | 0                     | 0                   | 0                   | gi148702819   |
| 3    | HPLC    | Prolyl 4-hydroxylase, beta polypeptide, isoform CRA_a        | 211   | 7              | 11      | 59.0                  | 0                   | 0                   | gi148702818   |
|      | Multistep|                                                              | 280   | 15             | 25      | 0                     | 0                   | 0                   | gi148702819   |
| 4    | HPLC    | Methionine adenosyltransferase I, alpha                      | 304   | 7              | 21      | 43.5                  | 0                   | 0                   | gi19526790    |
|      | Multistep|                                                              | 378   | 6              | 28      | 0                     | 0                   | 0                   | gi6677739     |
| 5    | HPLC    | Regucalcin                                                   | 230   | 5              | 18      | 33.4                  | 0                   | 0                   | gi6677739     |
|      | Multistep|                                                              | 211   | 4              | 14      | 0                     | 0                   | 0                   | gi6677739     |
| 6    | HPLC    | Carbamoyl-phosphate synthetase 1                            | 1286  | 28             | 18      | 164.5                 | 4                   | 17.39              | gi124248512   |
|      | Multistep|                                                              | 697   | 23             | 14      | 0                     | 0                   | 0                   | gi124248512   |
| 7    | HPLC    | Carbamoyl-phosphate synthetase 1                            | 1221  | 27             | 18      | 164.5                 | 3                   | 17.65              | gi124248512   |
|      | Multistep|                                                              | 1112  | 31             | 20      | 0                     | 0                   | 0                   | gi124248512   |
| 8    | HPLC    | Glutamate de-hydrogenase 1                                  | 416   | 9              | 17      | 61.3                  | 1                   | 9.09               | gi6680027     |
|      | Multistep|                                                              | 364   | 10             | 17      | 0                     | 0                   | 0                   | gi7106255     |
| 9    | HPLC    | Arginase 1                                                   | 284   | 5              | 16      | 34.8                  | 1                   | 12.5               | gi7106255     |
|      | Multistep|                                                              | 290   | 8              | 26      | 0                     | 0                   | 0                   | gi7106255     |
|      | HPLC    | Short-chain specific acyl-CoA dehydrogenase, mitochondrial   | 134   | 4              | 10      | 44.9                  | 0                   | 0                   | gi584714      |
|      | Multistep|                                                              | 192   | 6              | 19      | 0                     | 0                   | 0                   | gi1009706     |
| 10   | HPLC    | Sorbitol dehydrogenase precursor                            | 176   | 4              | 11      | 40.7                  | 0                   | 0                   | gi22128627    |
|      | Multistep|                                                              | 207   | 6              | 17      | 0                     | 0                   | 0                   | gi22128627    |
| 11   | HPLC    | Sorbitol dehydrogenase                                       | 176   | 4              | 12      | 38.2                  | 0                   | 0                   | gi22128627    |
|      | Multistep|                                                              | 207   | 6              | 18      | 0                     | 0                   | 0                   | gi22128627    |
| 12   | HPLC    | L-iditol 2-dehydrogenase                                     | 176   | 4              | 11      | 42.8                  | 0                   | 0                   | gi397357      |
|      | Multistep| Sorbitol dehydrogenase, isoform CRA_a                       | 176   | 4              | 14      | 32.0                  | 0                   | 0                   | gi149023127   |
| 13   | HPLC    | Fumarate-acetoacetase                                        | 191   | 4              | 10      | 46.2                  | 0                   | 0                   | gi50973       |
|      | Multistep|                                                              | 290   | 6              | 15      | 0                     | 0                   | 0                   | gi50973       |
| 14   | HPLC    | 4-hydroxy-phenyl-pyruvate dioxygenase                        | 197   | 4              | 10      | 45.1                  | 0                   | 0                   | gi849053      |
|      | Multistep| F1 protein                                                   | 197   | 4              | 10      | 43.6                  | 0                   | 0                   | gi849053      |
| 15   | HPLC    | Chain A, Crystal Structure of Fumarylacetoacetate Hidrolase Complexed With (Hydroxymethylphosphinoyl)-3-Oxo-Butanoic Acid | 191 | 4 | 10 | 46.2 | 0 | 0 | gi13399972 |
|      | Multistep| Chain A, Crystal Structure of Fumarylacetoacetate Hidrolase Complexed With Fumarate And Acetoacetate | 191 | 4 | 10 | 46.2 | 0 | 0 | gi8569272 |
| 16   | HPLC    | Chain A, Crystal Structure of Fumarylacetoacetate Hidrolase Complexed With Fumarate And Acetoacetate | 191 | 4 | 10 | 46.2 | 0 | 0 | gi8569274 |
|      | Multistep| Glutamate-ammonia ligase                                    | 187   | 5              | 15      | 42.0                  | 0                   | 0                   | gi2144563     |
|      | HPLC    | Glutamine synthetase                                         | 187   | 5              | 15      | 42.1                  | 0                   | 0                   | gi31982332    |

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Table 1 Comparison of identification of 2D gel separated spots of rat liver protein by chip-based nanoESI/MS/MS and nanoHPLC-MS/MS (Continued)

| Multistep | Long-chain acyl-CoA dehydrogenase | 122 | 3 | 6 | 48.0 | gi726095 |
|-----------|-----------------------------------|-----|---|---|------|---------|
| 10 HPLC   | Arginase 1                         | 232 | 4 | 13 | 34.8 | 0       | 0       | gi7106255 |
| Multistep | Aldo-keto reductase family 1, member D1 | 120 | 3 | 7 | 37.4 | 0       | 0       | gi20302063 |
| 11 HPLC   | Arginase 1                         | 232 | 4 | 13 | 34.8 | 0       | 0       | gi7106255 |
| Multistep | Ornithine transcarbamylase, isoform CRA_f | 298 | 5 | 14 | 42.0 | 1       | 14.29   | gi148703731 |
| 12 HPLC   | Alcohol dehydrogenase 1 (class I) | 156 | 3 | 9 | 39.7 | 0       | 0       | gi6724311 |
| Multistep | Alcohol dehydrogenase 1 (class I), isoform CRA_b | 156 | 3 | 11 | 35.3 | 0       | 0       | gi148680154 |
| 13 HPLC   | Electron transferring flavoprotein, alpha polypeptide | 155 | 3 | 7 | 35.0 | 0       | 0       | gi13097375 |
| 14 HPLC   | Glutathione S-transferase mu 1     | 193 | 5 | 19 | 26.0 | 1       | 25      | gi6754084 |
| 15 HPLC   | Hemoglobin beta                    | 378 | 6 | 47 | 15.7 | 0       | 0       | gi229301 |
| 16 HPLC   | Fatty acid binding protein 1, liver | 149 | 3 | 22 | 14.2 | 0       | 0       | gi8393343 |

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followed by data-dependent MS/MS fragmentation. With a narrow range scan, a greater number of moderate to low abundance ion features are found than when a full experimental range parent ion scan is utilized. The advantages of this method include full automation, lack of cross-contamination, low cost, and high throughput. It should be noted that the cost of the direct inject platform (Advion Nanomate) and associated disposables (tips, chip) is analogous to a moderate cost HPLC system.

**Materials and methods**

All reagents and solvents for mass spectrometry of LC/MS grade were obtained from Fisher Scientific (Waltham, MA, USA). Modified trypsin was purchased from Promega (Madison, WI, USA) or Sigma-Aldrich (St. Louis, MO, USA). ZipTipsC18 was purchased from Millipore (Bedford, MA, USA).

**2D-SDS-PAGE of Liver Proteins**

Adult C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Animals were housed by the University of Louisville in a dedicated room at 22°C, with a 12 hour alternating light/dark cycle, and were maintained on Purina LabDiet #5015 and water *ad libitum*. The University of Louisville is an ‘Association for Assessment and Accreditation of Laboratory Animal Care’ (AAALAC)-approved facility. Rat liver was homogenized on ice in 7 M urea, 2 M thiourea with 4% CHAPS detergent. 500 μg liver protein (measured by the Bradford method) was separated by isoelectric focusing with non-linear, pH 3-10, 180 mm × 3 mm × 0.5 mm Immobiline DryStrips (GE Healthcare, Piscataway, NJ) according to the manufacturer’s instructions. Passive rehybridization was accomplished in a 7 M urea, 2 M thiourea, and 4% CHAPS solution containing 2.8 mg/ml DTT and 2% pH 3-10 IPG buffer. The proteins were focused for 28,000 Vhrs. Before transfer of proteins for second dimension separation according to molecular weight, the strips were equilibrated first with 3.5 mg/mL DTT contained in equilibration buffer (1.5 M Tris pH 8.8 buffer containing 6 M urea, 34.5% glycerol, and 2% SDS) for 30 minutes and second with 45 mg/ml iodoacetamide in equilibration buffer for an additional 30 minutes. The strips were then loaded onto 12% acrylamide gels and separated at 70 volts for 24 hours at room temperature. Gels were stained with colloidal Coomassie Blue G-250 and washed in deionized water for at least 48 hours. Gel images were collected using an Epson Expression 1680 desktop scanner with transparency adapter at 266 dpi.

**Proteolytic Digestion of Selected Protein Spots**

Selected spots representing high and low molecular weight, intense and faint protein expression were excised from 2D-SDS-PAGE gels and destained with 50% ethanol (EtOH) and 50 mM NH₄HCO₃ at room temperature with a minimum of 5 washes. The gel pieces were dehydrated in ethanol and subjected to in-gel protease digestion with modified trypsin (10 mg/μL, Promega) in 50 mM NH₄HCO₃ for 18 hours. Tryptic peptides were extracted via sequential steps of 50% EtOH in 0.1% formic acid followed by 95% EtOH in 0.1% formic acid. The extracted peptides were split into 2 aliquots, desalted with C18 zip-tips (Millipore, Bedford, MA, USA), lyophilized, and stored at -80°C until analysis.

**LC-nano-ESI-MS/MS**

The extracted peptides were dried and resolubilized in 30 μL of 3% acetonitrile (ACN), 97% water, 0.1% formic acid and transferred to auto-sampler vials for LC-MS/MS analysis. The binary gradient elution mobile phase initially consisted of 90% A: 10% B. A was 3% acetonitrile (ACN) and 0.1% formic acid in water, and B was 97% ACN and 0.1% formic acid in water. Mobile phase A at 90% was maintained for the first 6 min and decreased linearly to 45% over 10 min, maintained for 0.1 min and then changed to 10% and maintained for 2 min. Finally, the mobile phase A was increased to 90% and the column was re-equilibrated for a further 8 min. Sample temperature was

![Figure 2](http://www.proteomesci.com/content/9/1/38)
maintained at 10°C in the autosampler prior to analysis. The flow-rate was 10 μL/min and split at approximately a 1:10 ratio before introduction to the mass spectrometry system.

An LTQ-FT-ICR MS system (Thermo-Electron, Waltham, MA, USA) was utilized for data acquisition. The mass analysis method consisted of one segment with three scan events with an initial delay of 2 min following injection and sample spray initiation. The 1st scan event was a broad range FT-ICR-MS scan (400-1200 m/z) with 100,000 resolution for parent ion selection followed by 2 other scan events with data dependent MS/MS for fragmentation of the 2 most intense ions with +2 or +3 charges. Unassigned, +1, and +4 charges were rejected. The ion fragmentation process was conducted in the ion trap (35 eV CID energy) with a parent mass feature isolation width of 2 m/z. Dynamic exclusion to prevent multiple fragmentation events with same parent mass feature utilized a m/z window of 0.5-1.5 m/z; repeat and exclusion windows of 15 s and 30 s and an exclusion list size of 48. The total acquisition time for the HPLC-ESI-MS/MS method was 26 min.

Multi-step direct inject nano-ESI-FT-ICR-MS/MS
An aliquot (7 μL) of the liver protein spot tryptic digest was introduced via chip-based nanoelectrospray with an Advion TriVersa Nanomate interfaced to the LTQ-FT-ICR-MS. This chip-based nanoESI-MS/MS method consisted of a prescan (400-2000 m/z) followed by 4 segments overlapping narrow range scans (400-600, 550-750, 700-950, and 900-1200 amu; 50 amu overlap) with data-dependent MS/MS fragmentation of selected parent ions of the top most intense +2 or +3 peaks in each segment. The top 10 most intense +2 or +3 peaks were subjected to CID fragmentation from each segment. For each segment, a total of 11 scan events were included: an initial FT-ICR parent mass scan with 100,000 resolution followed by 10 other scan events with data dependent MS/MS of +2 or +3 charged ions. These 11 scan events were cycled until the segment time was exceeded. Unassigned, +1, and +4 charges were rejected. Parent mass fragmentation was initiated in the ion trap (35 eV CID energy) with an ion isolation width of 2 m/z. Dynamic exclusion was employed with a mass width 0.5-1.5 m/z, repeat and exclusion duration are 180.00 s with exclusion list size of 500. Scan times for the full range and the narrow mass ranges are both 500 microseconds while the scan time for MS/MS is 100 microseconds. The sample spray characteristics were stable (greater than 10 minutes) with ion current between 10-90 nA (1.5 kV spray voltage and 0.10 psi head pressure). The duration for the 4 segments were 1.4, 1.0, 0.4, and 0.2 minutes, respectively, with a total acquisition time of 3 minutes.

Peptide identification
Common to the two workflows above, peptides were identified by NCBIr database searching with an in-house Mascot Server (Matrix Scientific). Parameters for the searches were: Mammalian proteins, ± 0.8 daltons for the parent peptide, ± 0.4 daltons for fragmentation masses, 2 missed trypsin cleavage sites allowed, and carbamidomethylation of cysteins as a variable modification. Acceptable protein identifications required a Mascot MOWSE score greater than 65 and a minimum of 2 peptides.

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Authors’ contributions
JC and REN planned the experiments; LC was responsible for the preparation of 2D gel spot samples; JC carried out the experiments, performed data analysis and wrote the first draft of the manuscript; LC and REN critically reviewed the content of the manuscript before submission. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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References
1. Elliott MH, Smith DS, Parker CE, Borchers C: Current trends in quantitative proteomics. J Mass Spectrom 2009, 44:1637-1660.
2. Pereira-Medrano AG, Sterling A, Snijders AP, Reardon KF, Wright PC: A systematic evaluation of chip-based nanoelectrospray parameters for rapid identification of proteins from a complex mixture. J Am Soc Mass Spectrom 2007, 18:1714-1725.
3. Lafrentz BR, LaPana SE, Call DR, Wiens GD, Cain KD: Proteomic analysis of Flavobacterium psychrophilum cultured in vivo and in iron-limited media, Dis Aquat Organ 2009, 87:171-182.
4. Gromov P, Gromova I, Bunkenberg J, Cabozon T, Moreira JM, Timmermans-Wielenga V, Roepstorff P, Rank F, Celis JE: Up-regulated proteins in the fluid bathing the tumour cell microenvironment as potential serological markers for early detection of cancer of the breast. Mol Oncol 2010, 4:65-89.
5. Ouyang H, Luo Y, Zhang L, Li Y, Jin C: Proteome Analysis of Aspergillus fumigatus Total Membrane Proteins Identifies Proteins Associated with the Glycoconjugates and Cell Wall Biosynthesis Using 2D LC-MS/MS. Mol Biotechnol 2010, 44:177-189.
6. Urbanovicius S, Wiggers H, Parker CE, Borchers C. Current trends in quantitative proteomics. J Mass Spectrom 2009, 44:1637-1660.
7. Lindholt JS, Yorston H, Honoré B: Proteomic analysis identifies mitochondrial metabolic enzymes as major discriminators between different stages of the failing human myocardium. Acta Cardiol 2009, 64:511-522.
8. Abildsø J, Mann M: Mass spectrometry-based proteomics. Nature 2003, 422:198-207.
9. Zhang S, Van Pelt CK, Henion JD: Automated chip-based nanoelectrospray-mass spectrometry for rapid identification of proteins separated by two-dimensional gel electrophoresis. Electrophoresis 2003, 24:3620-3632.
10. Wilm M, Mann M: Analytical properties of the nanoelectrospray ion source. Anal Chem 1996, 68:1-8.
11. Chen S: Rapid protein identification using direct infusion nanoelectrospray ionization mass spectrometry. Proteomics 2006, 6:16-25.
12. Gabelica V, Vreuls C, Flée P, Duval V, Joris B, Pauw ED: Advantages and drawbacks of nanospray for studying noncovalent protein-DNA complexes by mass spectrometry. Rapid Commun Mass Spectrom 2002, 16:1723-1728.
13. Zhang B, Liu H, Karger BL, Foret F: Microfabricated Devices for Capillary Electrophoresis-Electrospray Mass Spectrometry. Anal Chem 1999, 71:3258-3264.
14. Deng Y, Zhang H, Henion J: Chip-Based Quantitative Capillary Electrophoresis/Mass Spectrometry Determination of Drugs in Human Plasma. Anal Chem 2001, 73:1432-1439.
15. Li J, Leftiche T, Tremblay TL, Wang C, Bonneil E, Harrison DJ, Thibault P: Application of Microfluidic Devices to Proteomics Research: Identification of Trace-level Protein Digests and Affinity Capture of Target Peptides. Mol Cell Proteom 2002, 1:157-168.
16. Tan A, Benetton S, Henion JD: Chip-Based Solid-Phase Extraction Pretreatment for Direct Electrospray Mass Spectrometry Analysis Using an Array of Monolithic Columns in a Polymeric Substrate. Anal Chem 2003, 75:5504-5511.