Supplemental Information

Transmission Geometry Laserspray Ionization *Vacuum*

Using an Atmospheric Pressure Inlet

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EXPERIMENTAL

Materials and Sample Preparation

Matrix compounds 3-nitrobenzonitrile (**3-NBN**), 2-nitrophloroglucinol (**2-NPG**), and 2,5-dihydroxybenzoic acid (**2,5-DHB**), proteins ubiquitin (bovine erythrocytes), insulin from bovine pancreas, α-chymotrypsinogen (bovine erythrocytes), and disialoganglioside isomers $GD1_a$ and $GD1_b$ were purchased from Sigma Aldrich (St. Louis, MO, USA). Matrix 2,5-dihydroxyacetophenone (**2,5-DHAP**) was purchased from Acros Organics (Fairlawn, NJ, USA). Angiotensin II was purchased from American Peptide (Sunnyvale, CA, USA). HPLC grade acetonitrile (**ACN**) and methanol (**MeOH**) were purchased from Fisher Scientific (Fairlawn, NJ, USA). HPLC grade water (**H_2O**) was obtained from EMD (Billerica, MA, USA) and glacial acetic acid from Mallinckrodt (St. Lois, MO, USA). A glass slide (Gold Seal, Portsmouth, NJ, USA) with sufficient laser transmission was used as the sample holder as previously employed for LSII.[1]

C57 Bl/6 mice were anesthetized at 20 weeks old by isoflurane and transcardially perfused with ice-cold 1x phosphate-buffered saline (PBS, 150 mM NaCl, 100 mM NaH2PO4, pH 7.4) for 5 minutes to remove trapped blood cells. Mouse brains were frozen at −22°C and sliced into 10 µm sections using a Leica CM1850 cryostat (Leica Microsystems Inc., Bannockburn, IL, USA) and mounted onto a glass microscopy slide that was pre-chilled and pre-coated with 3-NBN by gentle warming with a finger to allow sections to attach.[2] The tissue sections were transported on dry ice and stored at -80 °C before use. Directly before analysis, the tissue sections were thawed and placed into a dessicator to avoid water condensation. The 3-NBN matrix solution was prepared as 5 mg in 50 µL ACN and pipetted directly onto the mouse brain tissue section in 1 µL aliquots until the section was covered entirely by matrix and then allowed to dry. The glass slide was then placed onto the flat outer surface of the cone and held by the vacuum of the mass spectrometer.

Stock solutions of angiotensin I and II, ubiquitin, and α-chymotrypsinogen were prepared in pure water as 1 mg mL^{-1} concentration and bovine insulin in 50:50 MeOH:H_2O with 1% acetic acid as 1 mg mL^{-1} and further diluted to 5 or 10 pmol μL^{-1} concentrations accordingly. 2,5-DHAP matrix was used supersaturated (5 mg in 150 µL 50:50 ACN:H_2O) by gentle heating of the solution over a hot water bath and spotted (1:1 v/v) using the layer method.[3] 5 mg of 2-NPG matrix was dissolved in 100 µL 50:50 ACN:water, and spotted the layer method of 1 µL of analyte mixture to 2 µL of matrix solution on top and dried by heat gun. Matrix compound 3-NBN was prepared as 5 mg in 50 µL ACN and mixed with analyte and spotted in 1 µL aliquots onto glass slides. 5 mg of 2,5-DHB was dissolved in 50 µL 50:50 ACN:H_2O and mixed with analyte before being spotted onto the glass slide and allowed to dry. The mass spectral data from the SYNAPT G2 ion mobility spectrometry-mass spectrometry (**IMS-MS**) were analyzed by MassLynx v4.1 and DriftScope v2.1 (Waters Corporation) and Thermo Xcalibur2.1 software (Thermo) was used for data obtained on the LTQ Velos.

Methods and Instrumentation

The general set-up of a LSI source is displayed in **Scheme S1**. An inlet attachment (**Scheme S1A**, inset) was designed as an addition to an LTQ Velos Ion Max source that allowed conversion to an LSIV source. The extension, made of stainless steel was a cylinder 10 mm long with an inner diameter of 5 mm and attached to the inlet tube aperture. The extension fit over the inlet tube so that placing a glass slide over the opening (**Scheme S1A**) provided a backing pressure of 0.5 Torr. For LSII, the glass plate was held in front of but not touching the commercial inlet aperture (**Scheme S1B**), exposing the matrix/analyte sample to atmospheric pressure (760 Torr). The capillary temperature of the LTQ Velos was varied from 50 to 450 °C. The sample holder and modified atmospheric pressure Z-Spray (Waters) source is shown in **Scheme S2**. More details about LSIV on the Z-Spray source is provided in the main text.

RESULTS

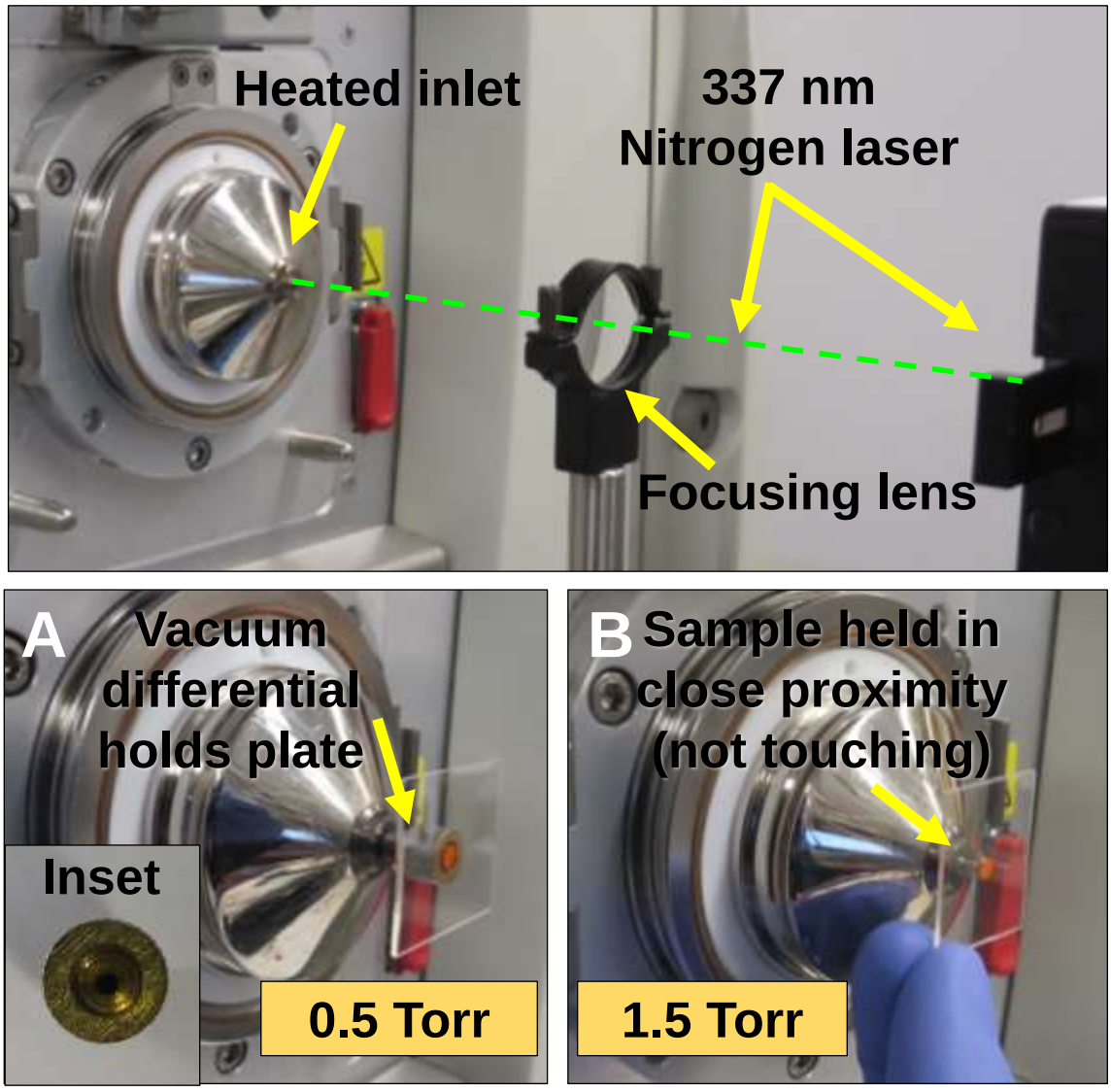
Details of Inlet Tube Measurements on an LTQ Velos

In both heated LSII and LSIV sources the matrix/analyte sample holder is handled at atmospheric pressure, however, the LSIV connecting ferrule allowed for the glass sample holder to adhere to the attachment, exposing the matrix/analyte sample to lower pressure and sealing off the entrance aperture while still allowing the source to maintain operation (**Scheme S1A**, inset). LSIV was operated with the first vacuum region gauge reading 0.5 Torr (**Scheme S1A**) and LSII with the vacuum gauge reading 1.5 Torr (**Scheme S1B**) using the LTQ Velos equipped with a heated inlet tube capable of being heated to 450 °C.[3] Using these two approaches, a temperature study in which the inlet tube was heated from 50 °C to 450 °C was performed. Initial temperature studies were performed using the LSIV extension to convert by sealing off the mass spectrometer (**Scheme S1A**) and also when using the commercial inlet aperture without the extension (**Scheme S1B**). Results are shown for bovine insulin (5.7 kDa) using 2,5-DHB and 2,5-DHAP, which are known to be the least volatile of the matrices tested (**Figure S1** and **Figure S2**). For LSIV, both matrices produced multiply charged analyte ions with the highest ion abundance at 350 °C (**Figure S1I.D** and **Figure S2I.D**). The same matrix compounds used with LSII produced the maximum ion abundance at 450 °C for 2,5-DHB and 350 °C for 2,5-DHAP (**Figure S1I.E** and **Figure S2I.D**). The observation of increasing ion abundance with increasing temperatures is in agreement with previous studies for MAII and LSII on the same instrument.[3] The introduction of increased vacuum with the extension allowed for lower inlet temperatures to be used for analyte ionization (**Figure S1** and **Figure S2**).

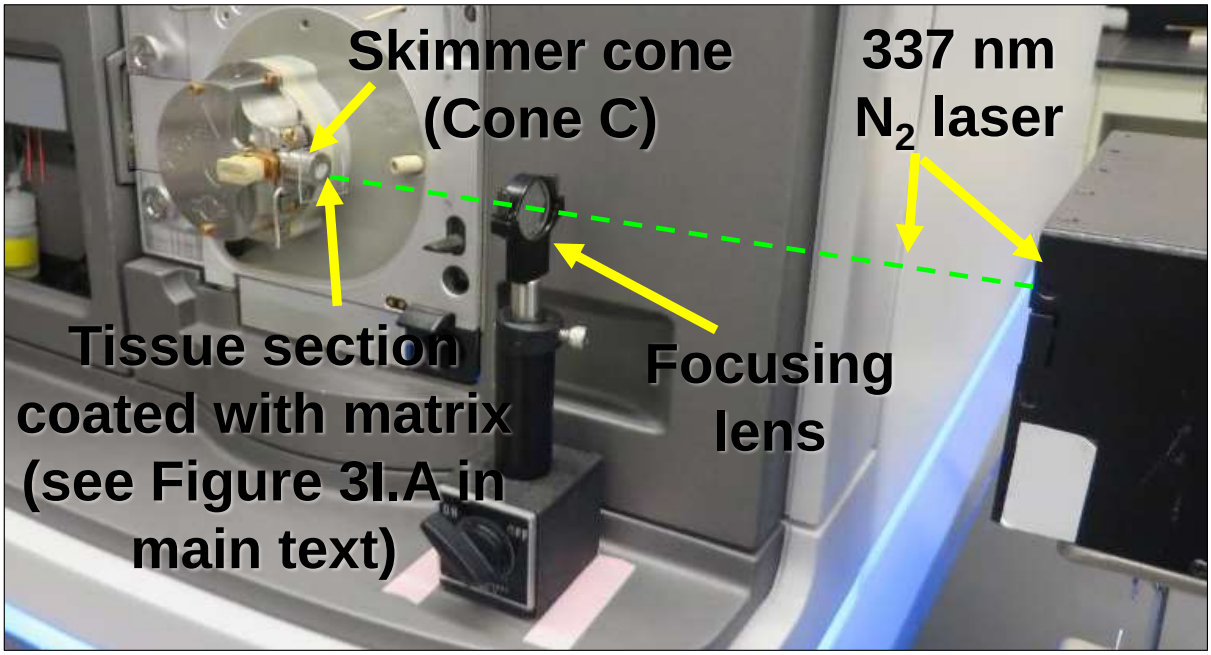
Although low analyte ion abundance and high chemical background in the low mass region was observed, the more volatile matrix compound 2,5-DHAP produced multiply charged ions at temperatures as low at 50 °C for both LSIV (**Figure S2I.A**) and LSII (**Figure S2II.A**); the ion

intensity using 2,5-DHB at this temperature is minute (**Figure S1I.A** and **Figure S1II.A**). Using matrix 2,5-DHAP, an inlet temperature of 350 °C produced the highest ion abundance for LSIV (**Figure S2I.D**) while a notable ion intensity drop is observed at a temperature of 450 °C (**Figure S2I.E**). LSII produced analyte ions of high intensity up to 450 °C, with only a small decrease in ion abundance between 350 °C and 450 °C (**Figure S2II.D-E**). Similarly, 2-NPG produced the highest ion abundance using LSIV at temperatures of 350 °C (**Figure S3I.D**), while LSII required a temperature of 450 °C to achieve high relative ion intensity (**Figure S3II.E**). Matrix 3-NBN produced the highest analyte ion abundance at the lowest inlet tube temperature of 50 °C for both LSIV and LSII (**Figure S4I.A** and **Figure S4II.A**).

Scheme S1. Experimental set-up on the LTQ Velos Ion Max source equipped with a heated inlet tube aperture design utilizing a 337 nm nitrogen laser and focusing lens in transmission geometry to ablate the sample on a UV transmitting substrate. (**A**) LSIV set-up with connecting extension (inset, bottom left) that seals off the vacuum of the inlet aperture with the glass sample holder, exposing the matrix/analyte to a pressure of 0.5 Torr and (**B**) LSII by holding the matrix/analyte in front of but not touching the inlet aperture for laser ablation in transmission geometry.



Scheme S2. Experimental set-up on the Z-Spray ion source of the Waters SYNAPT G2. Home-built LSIV source utilizing a 337 nm nitrogen laser and focusing lens in transmission geometry to ablate the sample on a UV transmitting substrate held by a cone (shown here, Cone C) and the vacuum inherent in the mass spectrometer. The sample holder (**Figure 2**, main text) or focusing lens (**Figure 3**, main text) can be moved by hand for laser ablation of different areas of the surface.



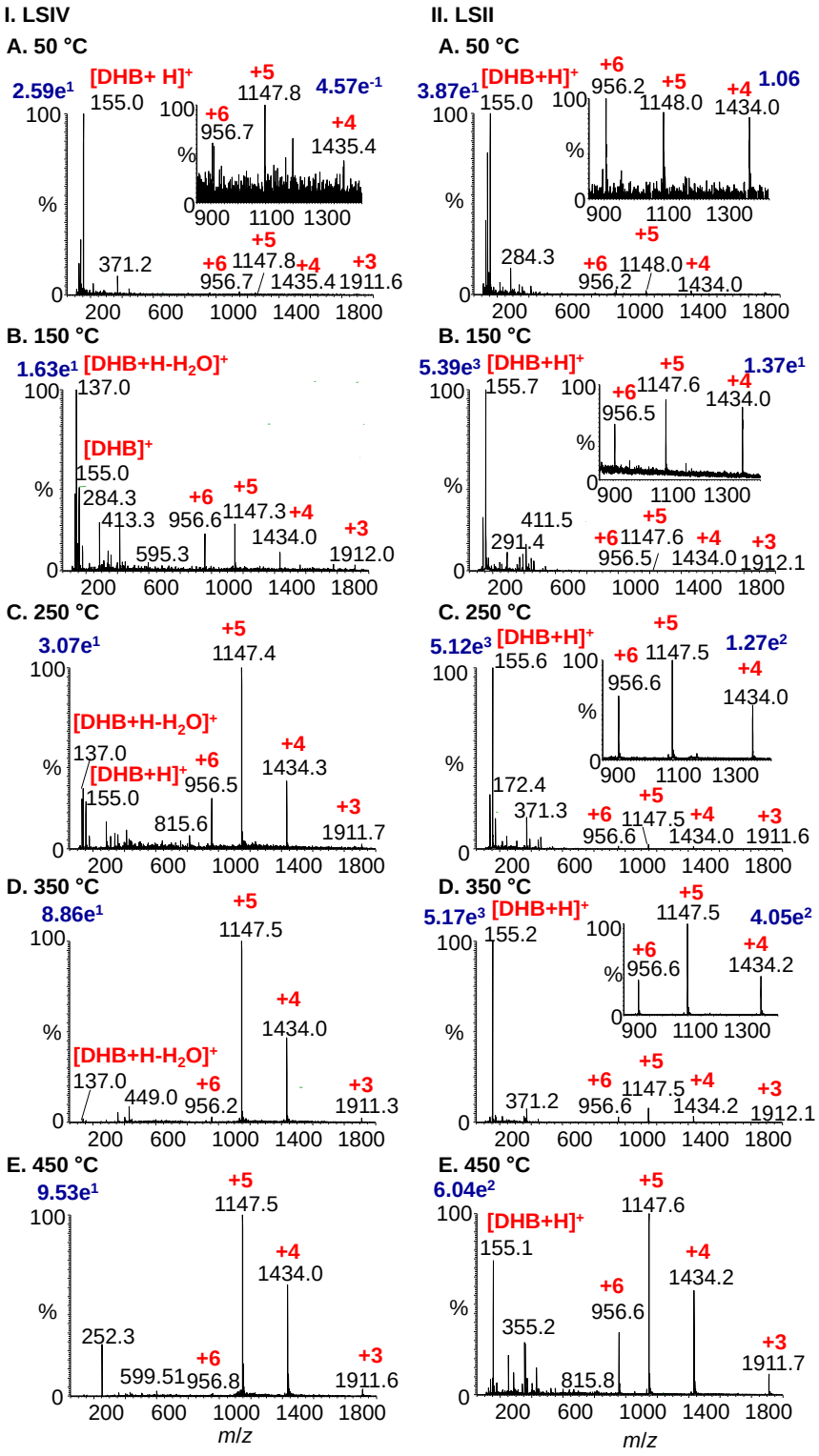


Figure S1. Mass spectra of bovine insulin (MW 5,730) using 2,5-DHB matrix on an LTQ Velos by (**I**) LSIV at ~0.5 Torr backing pressure, (**II**) LSII from atmospheric pressure at increasing inlet temperatures of (**A**) 50 °C, (**B**) 150 °C, (**C**) 250 °C, (**D**) 350 °C, and (**E**) 450 °C. Insets show charge state distribution from *m/z* 800 to 1400. Ion intensity is indicated in blue in top left corner.

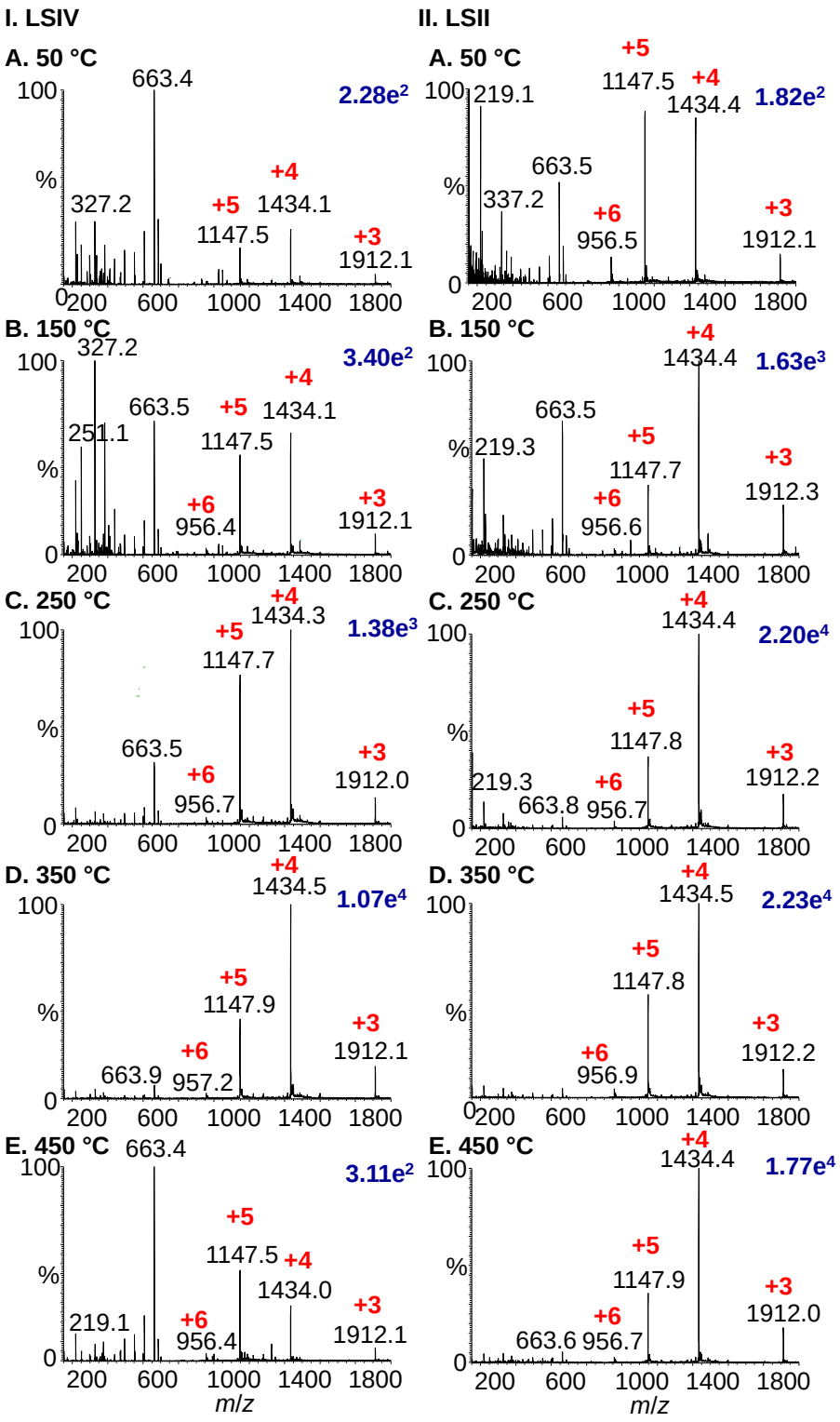


Figure S2. Mass spectra of bovine insulin (MW 5,730) using 2,5-DHAP matrix on an LTQ Velos by (**I**) LSIV at ~0.5 Torr backing pressure, (**II**) LSII from atmospheric pressure at increasing inlet temperatures of (**A**) 50 °C, (**B**) 150 °C, (**C**) 250 °C, (**D**) 350 °C, and (**E**) 450 °C. Ion intensity is indicated in blue in top right corner.

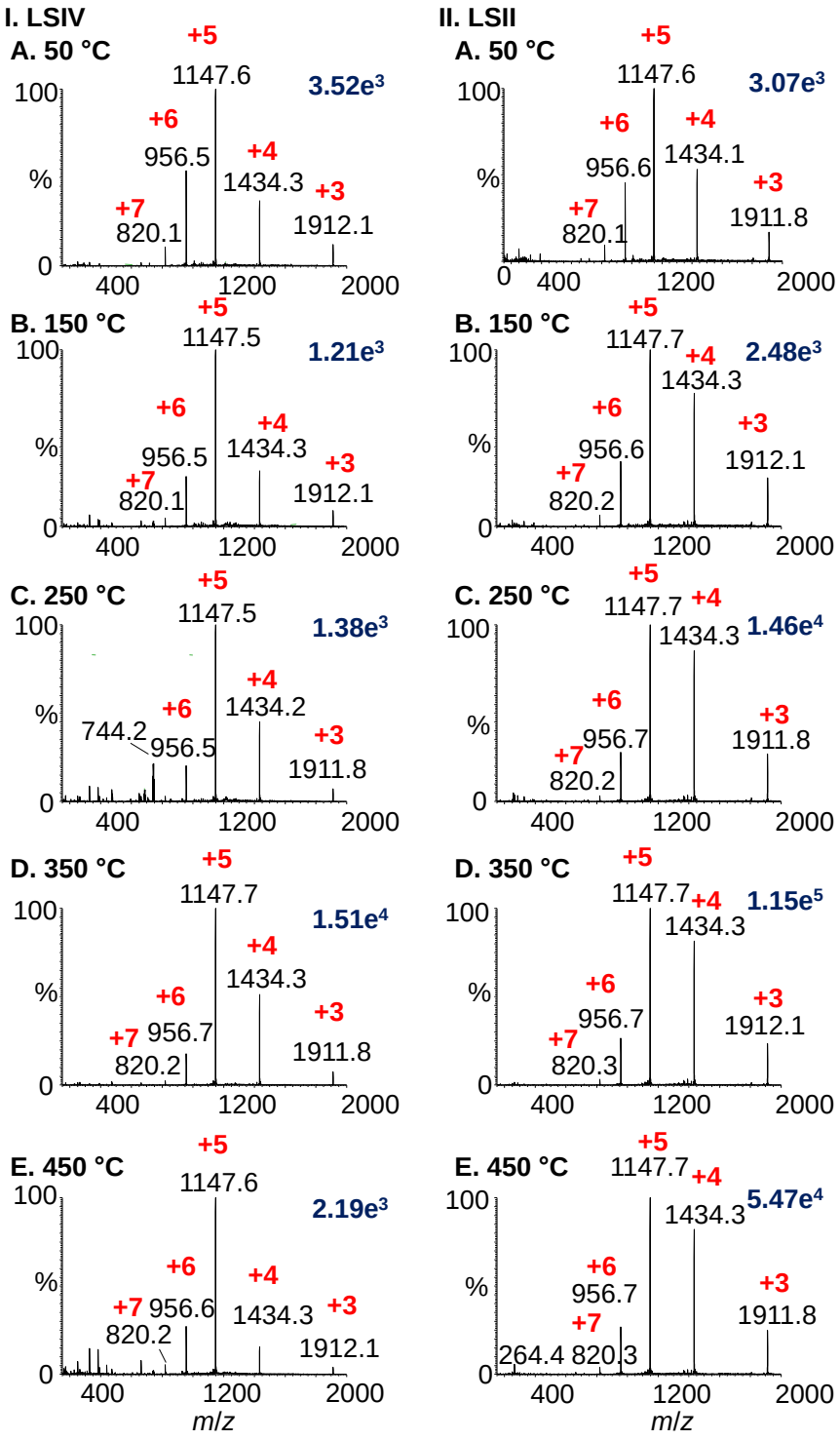


Figure S3. Mass spectra of bovine insulin (MW 5,730) using 2-NPG matrix on an LTQ Velos by (**I**) LSIV at ~0.5 Torr backing pressure, (**II**) LSII from atmospheric pressure at increasing inlet temperatures of (**A**) 50 °C, (**B**) 150 °C, (**C**) 250 °C, (**D**) 350 °C, and (**E**) 450 °C. Ion intensity is indicated in blue in top right corner.

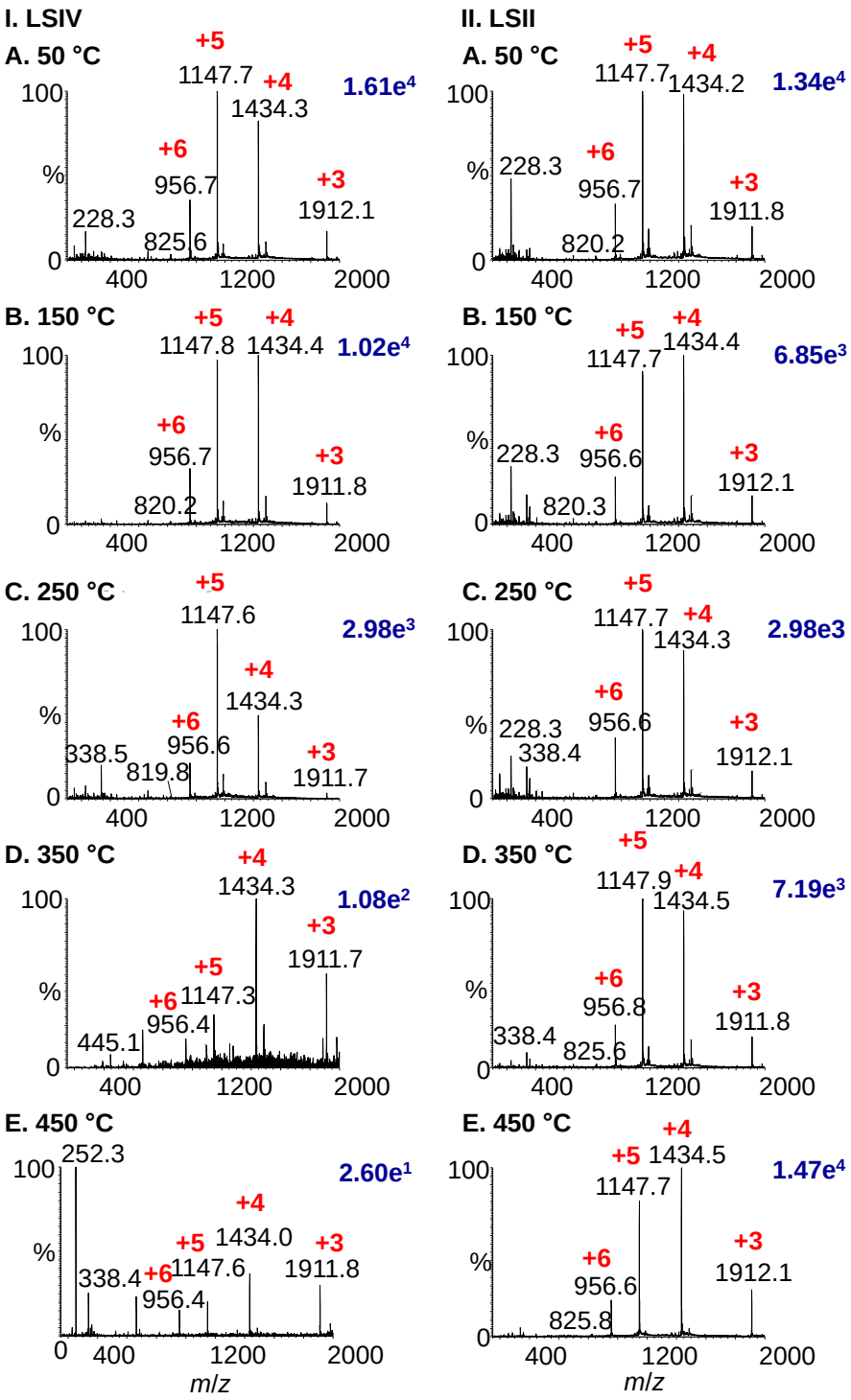


Figure S4. Mass spectra of bovine insulin (MW 5,730) using 3-NBN matrix on an LTQ Velos by (**I**) LSIV at ~0.5 Torr backing pressure, (**II**) LSII from atmospheric pressure at increasing inlet temperatures of (**A**) 50 °C, (**B**) 150 °C, (**C**) 250 °C, (**D**) 350 °C, and (**E**) 450 °C. Ion intensity is indicated in blue in top right corner.

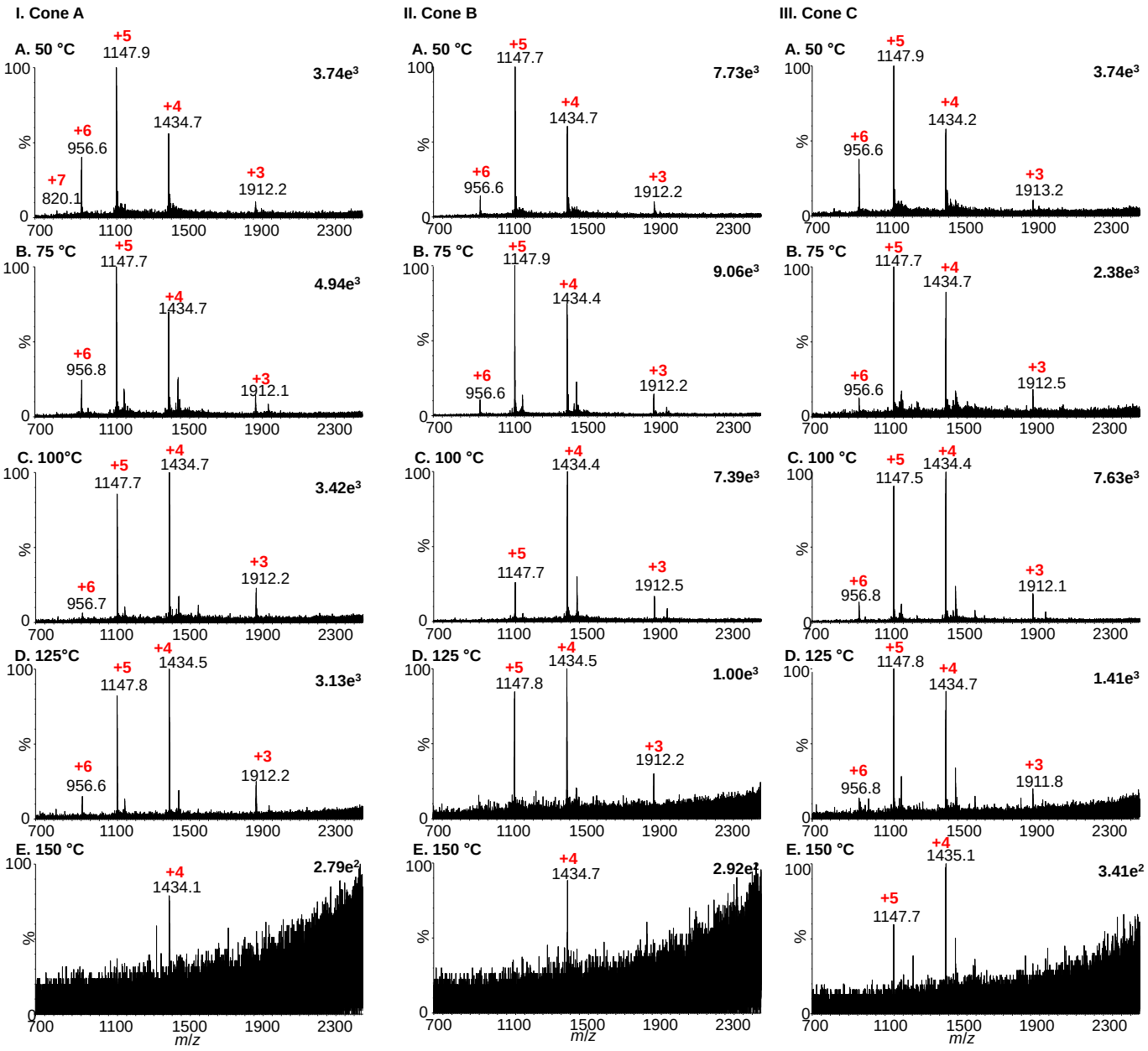


Figure S5. Matrix 3-NBN and mass spectra of bovine insulin (MW 5,730) by LSIV using (**I**) Cone A, (**II**) Cone B, and (**III**) Cone C at source temperatures of (**A**) 50 °C, (**B**) 75 °C, (**C**) 100 °C, (**D**) 125 °C, and (**E**) 150 °C on a Waters SYNAPT G2. Ion intensity is indicated in top right corner.

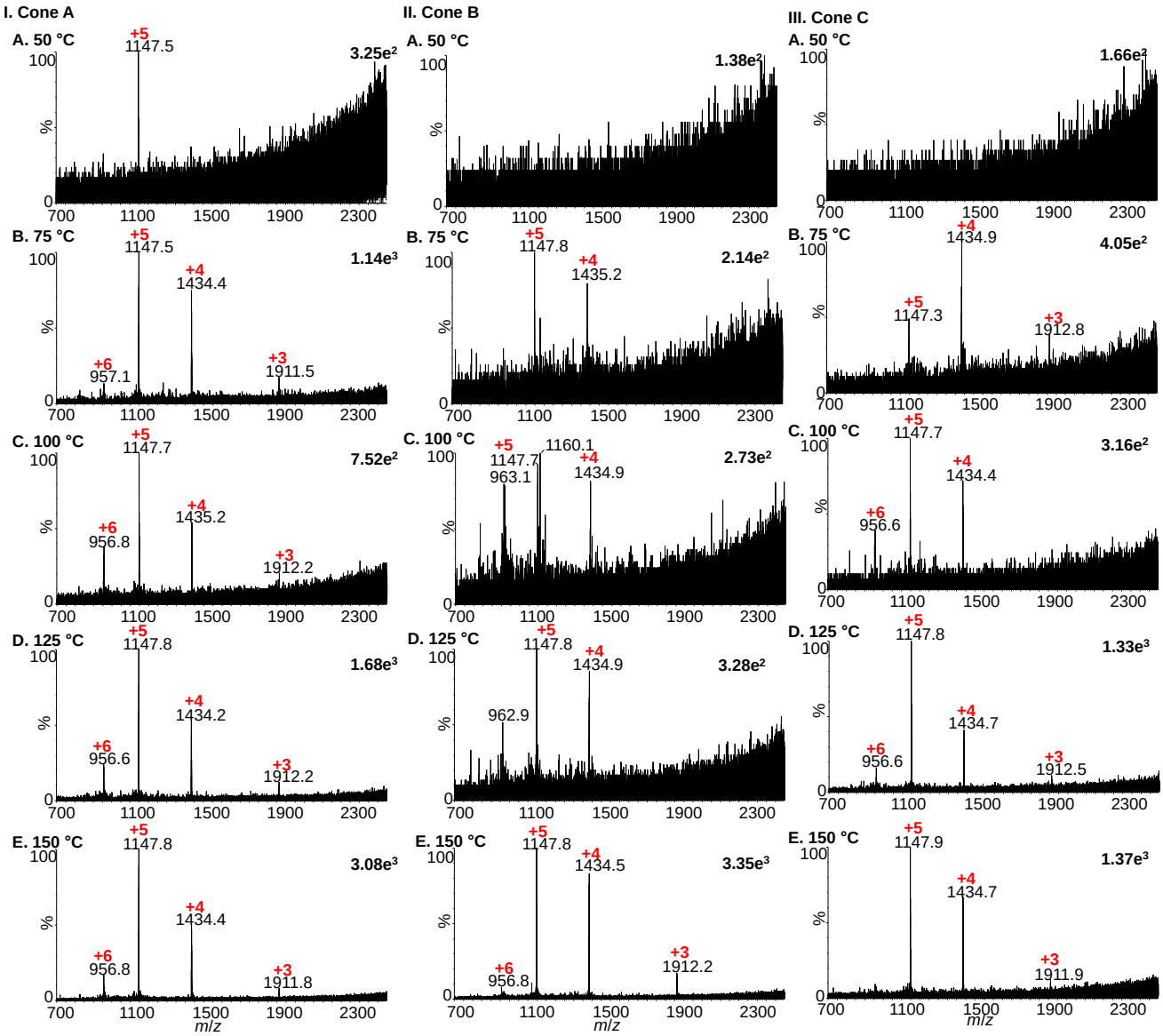


Figure S6. Matrix 2-NPG and mass spectra of bovine insulin (MW 5,730) by LSIV using (**I**) Cone A, (**II**) Cone B, and (**III**) Cone C at source temperatures of (**A**) 50 °C, (**B**) 75 °C, (**C**) 100 °C, (**D**) 125 °C, and (**E**) 150 °C on a Waters SYNAPT G2. Ion intensity is indicated in top right corner.

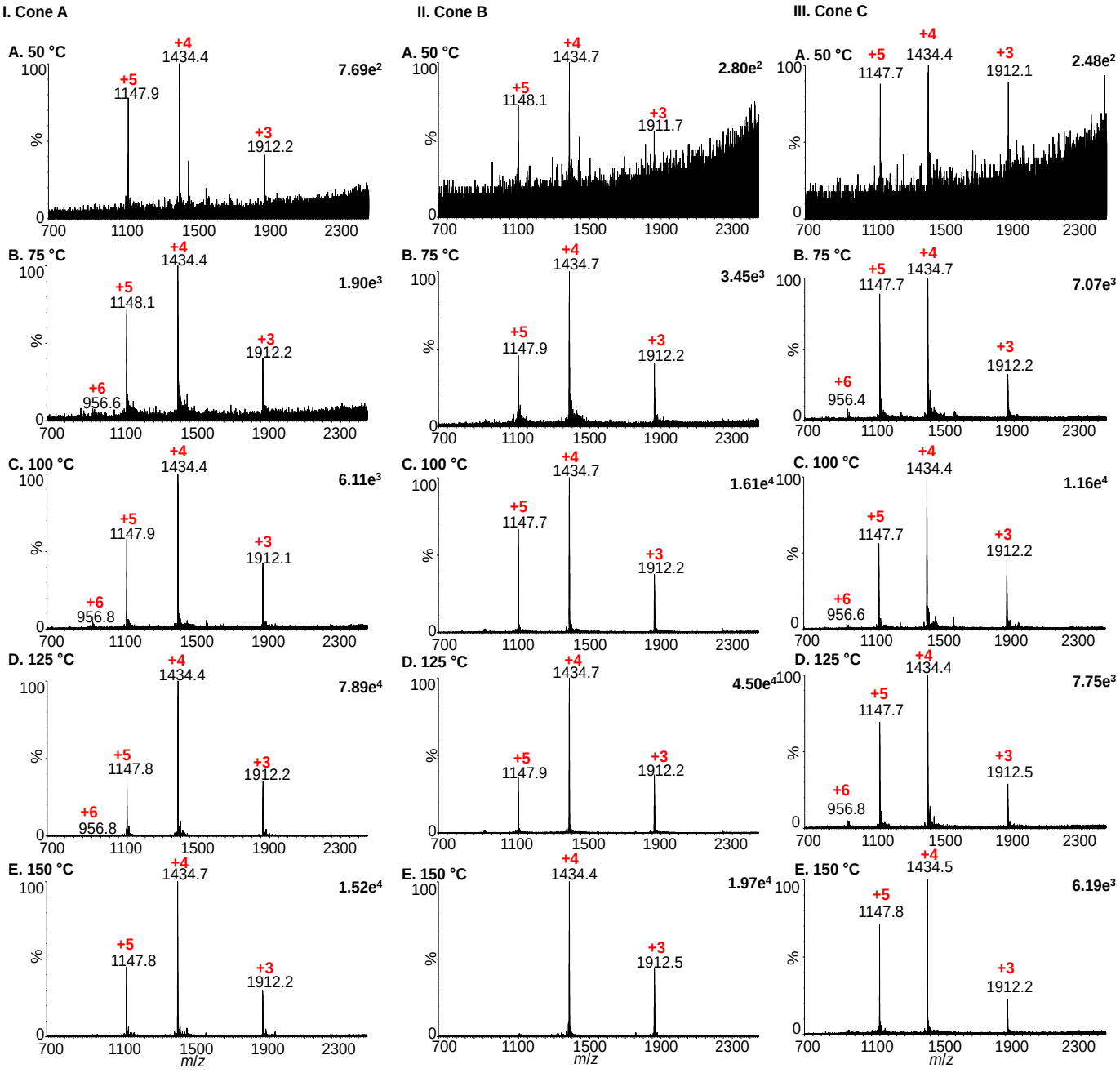


Figure S7. Matrix 2,5-DHAP and mass spectra of bovine insulin (MW 5,730) by LSIV using (**I**) Cone A, (**II**) Cone B, and (**III**) Cone C at source temperatures of (**A**) 50 °C, (**B**) 75 °C, (**C**) 100 °C, (**D**) 125 °C, and (**E**) 150 °C on a Waters SYNAPT G2. Ion intensity is indicated in top right corner.

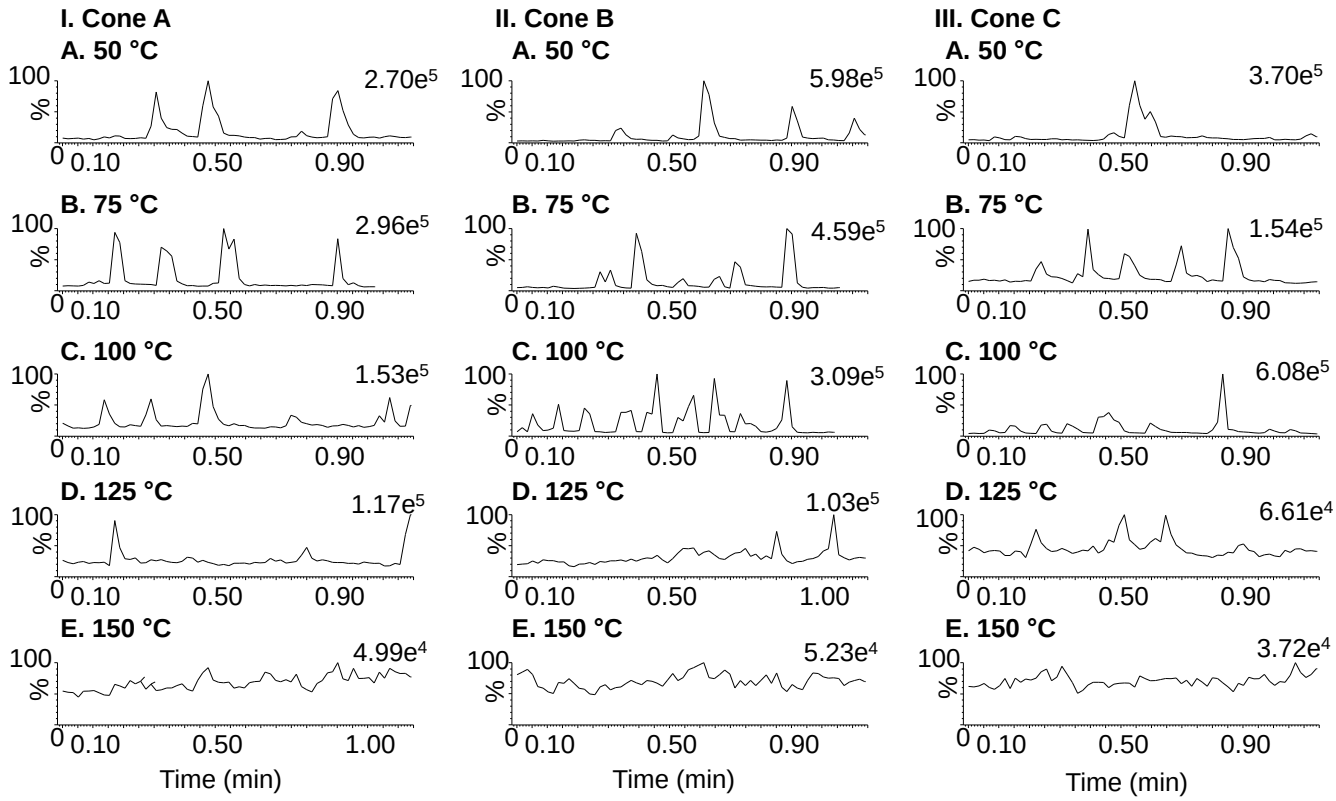


Figure S8. Matrix 3-NBN and total ion chronogram (TIC): (**I**) Cone A, (**II**) Cone B, and (**III**) Cone C, at source temperatures of (**A**) 50 °C, (**B**) 75 °C, (**C**) 100 °C, (**D**) 125 °C, and (**E**) 150 °C on a Waters SYNAPT G2. A more detailed description of the cone designs can be found in the main text. Ion intensity is indicated in top right corner.

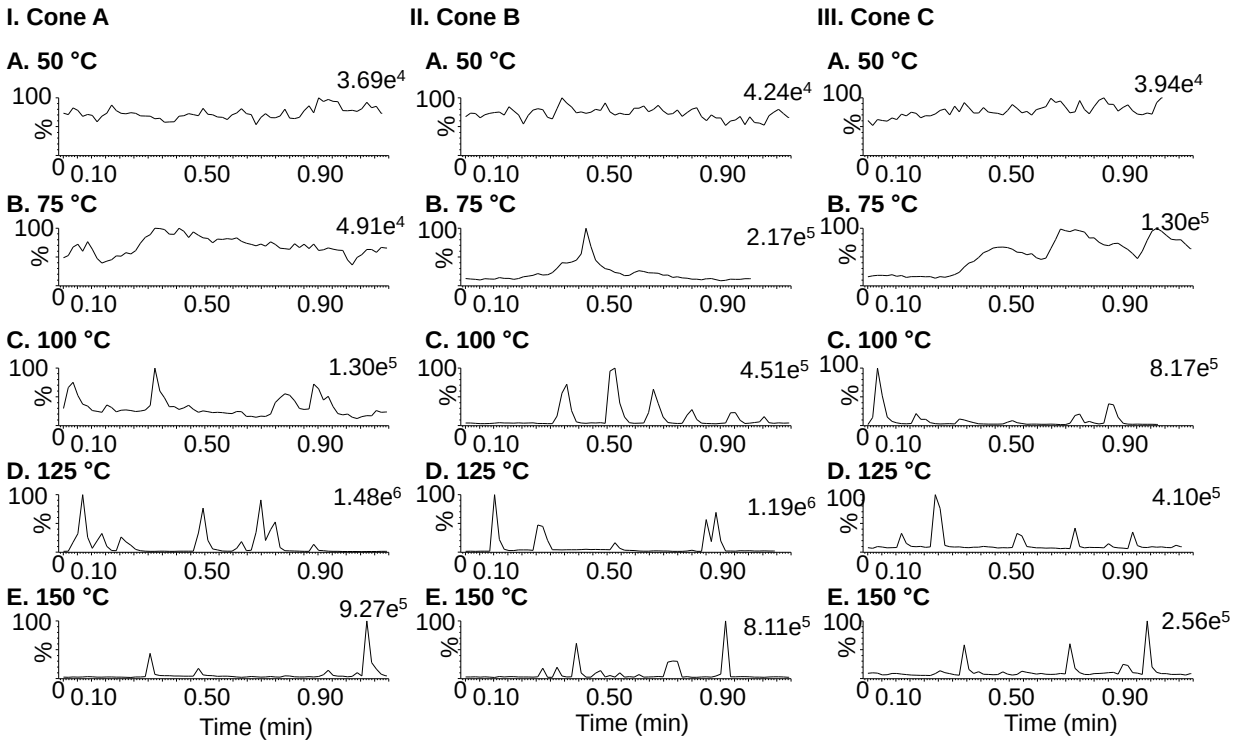


Figure S9. Matrix 2,5-DHAP and total ion chronogram (TIC): (**I**) Cone A, (**II**) Cone B, and (**III**) Cone C, at source temperatures of (**A**) 50 °C, (**B**) 75 °C, (**C**) 100 °C, (**D**) 125 °C, and (**E**) 150 °C on a Waters SYNAPT G2. A more detailed description of the cone designs can be found in the main text. Ion intensity is indicated in top right corner

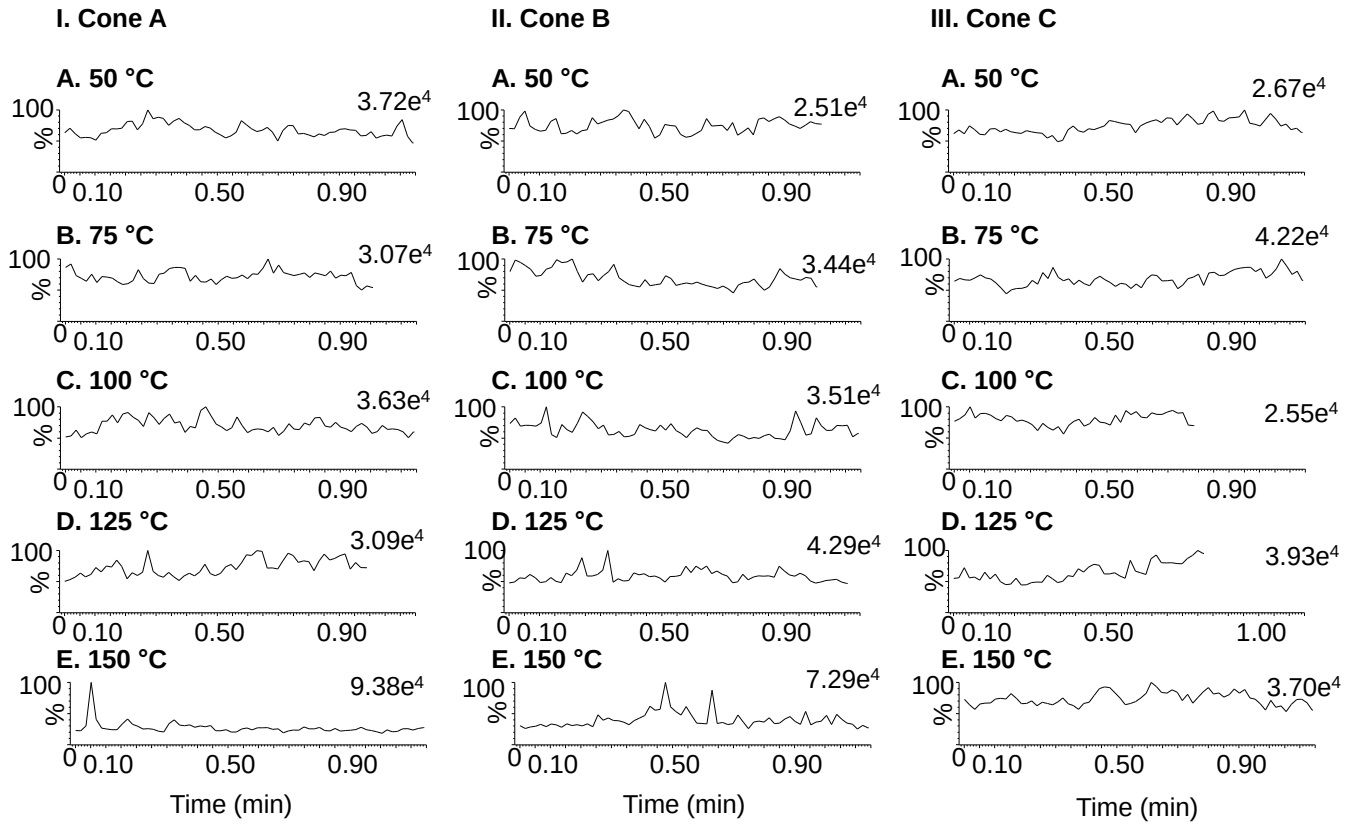


Figure S10. Matrix 2-NPG and total ion chronogram (TIC): (**I**) Cone A, (**II**) Cone B, and (**III**) Cone C, at source temperatures of (**A**) 50 °C, (**B**) 75 °C, (**C**) 100 °C, (**D**) 125 °C, and (**E**) 150 °C on a Waters SYNAPT G2. A more detailed description of the cone designs can be found in the main text. Ion intensity is indicated in top right corner

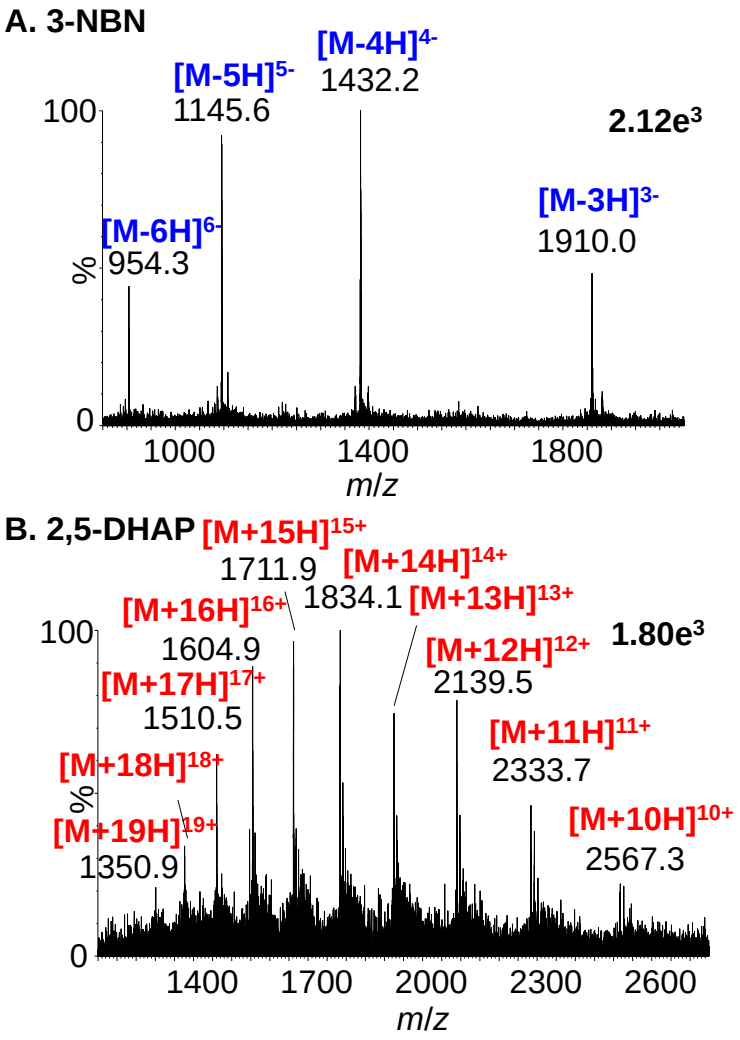


Figure S11. Transmission geometry LSIV mass spectra of (**A**) bovine insulin using matrix 3-NBN in the negative mode at a source temperature of 50 °C (Cone C) summed over 60 scans and (**B**) α-chymotrypsinogen (MW 25,656) using matrix 2,5-DHAP in positive mode at a source temperature of 125 °C (Cone C) summed over 120 scans. Data was obtained on the Z-Spray ion source of the Waters SYNAPT G2. Ion intensity is indicated in top right corner. A more detailed description of Cone C can be found in the main text.

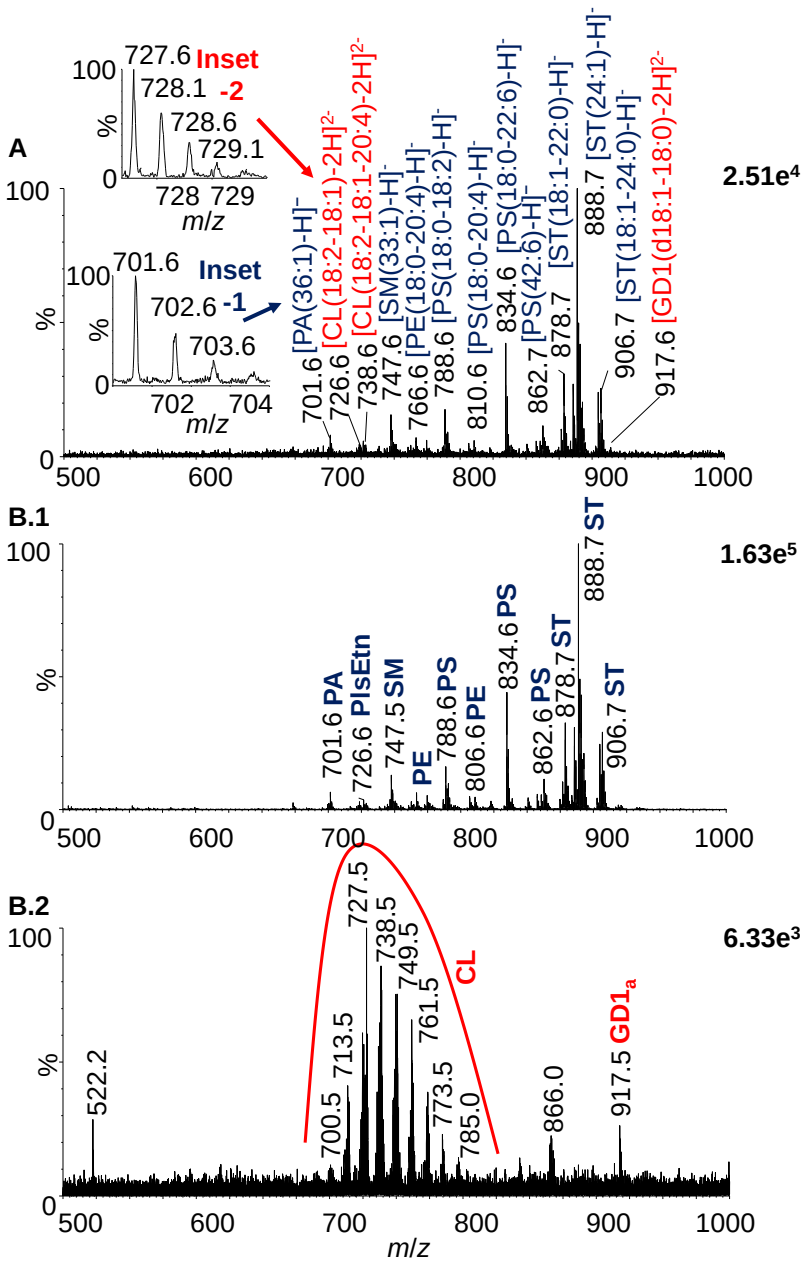


Figure S12. (**A**) Total mass spectrum obtained in the negative mode and extracted mass spectra from the 2-D plot in main text **Figure 3I.B** of the (**B.1**) singly charged family containing sulfatides (ST), phosphatidylserines (PS), phosphatidylethanolamines (PE), phosphatidic acids (PA), and sphingomyelin (SM) as well as the (**B.2**) the doubly charged family containing cardiolipins (CL) and ganglioside ($GD1_a$). Data obtained from laser ablation of a 10 µm mouse brain tissue section spotted with matrix 3-NBN using Cone C at a source temperature of 80 °C on a Waters SYNAPT G2. Lipids were tentatively assigned according to literature.[4-10] Insets in (**A**) show $[M-2H]^{2-}$ and $[M-H]^-$ isotopic distributions of CL and PA lipids, respectively. Ion intensity is indicated in top right corner. A more detailed description of Cone C can be found in the main text.

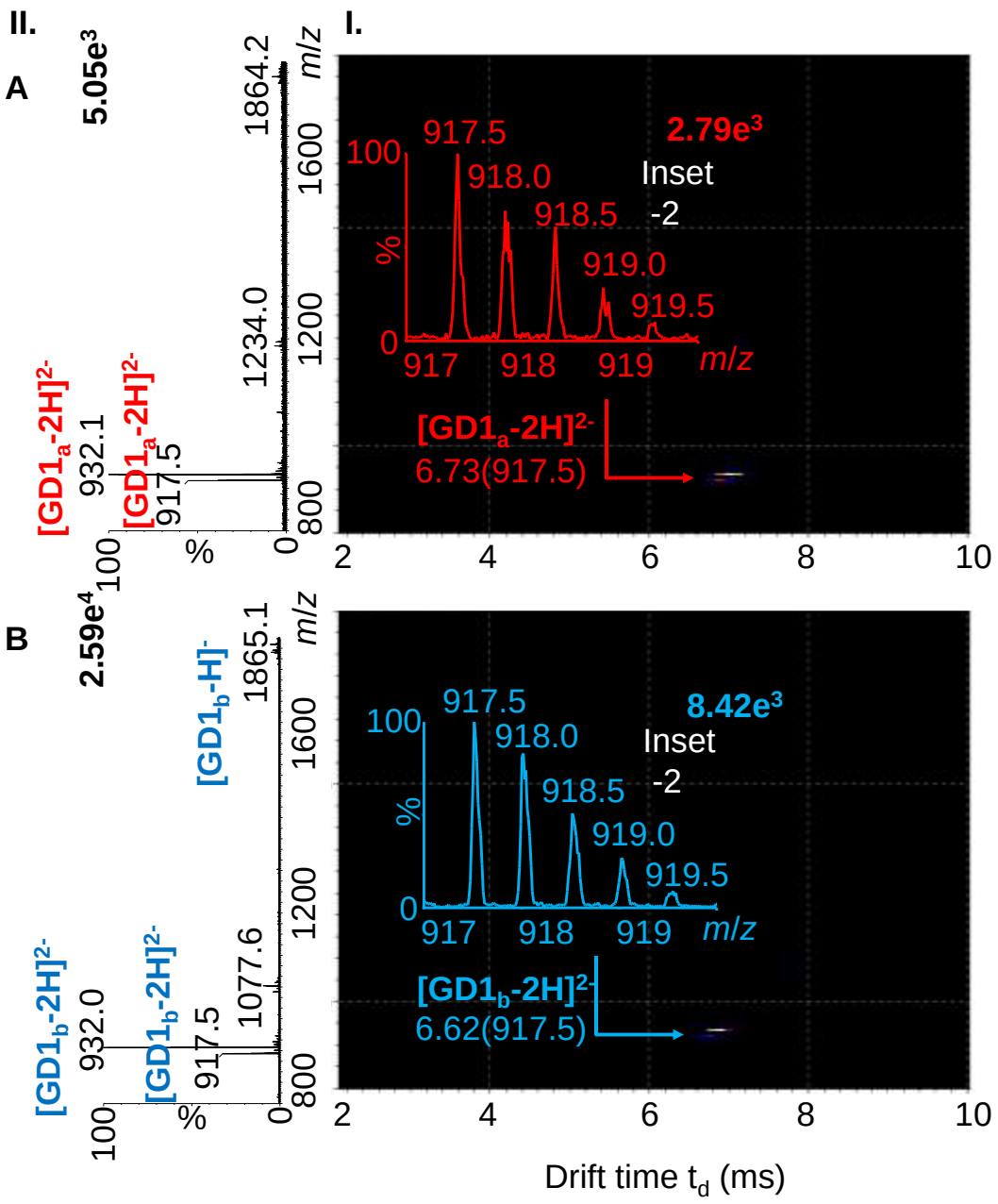


Figure S13. LSIV-IMS-MS of ganglioside standards in the negative ion detection mode: (**I**) 2-Dimensional plot and (**II**) mass spectra of (**A**) $GD1_a$ and (**B**) $GD1_b$ using Cone C and matrix 3-NBN at 80 °C on the SYNAPT G2. Inset in (**II**): isotopic distribution of $[M-2H]^{2-}$ at *m/z* 917.5 and drift times of 6.73 and 6.62 ms for (**A**) $GD1_a$ and (**B**) $GD1_b$, respectively. Ion intensity is indicated in top right corner of the mass spectra. A more detailed description of Cone C can be found in the main text.

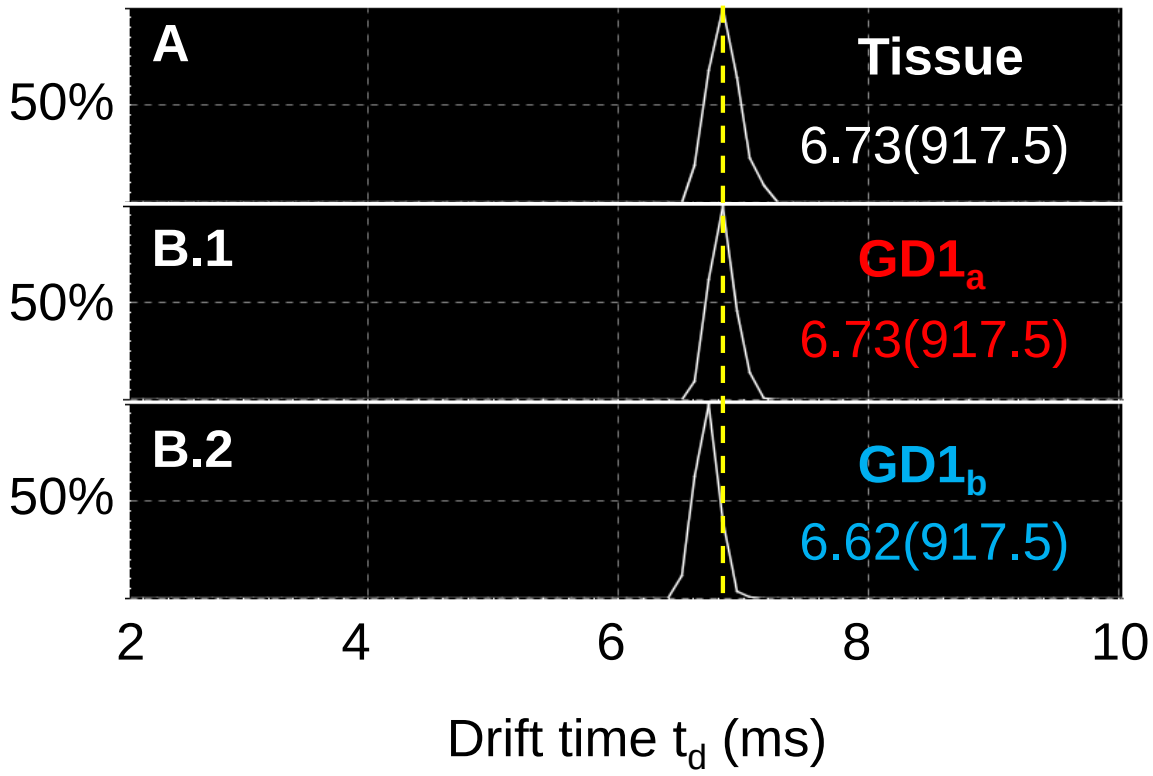


Figure S14. Drift times and nested data set displayed as drift time in milliseconds by *m*/*z* of (**A**) GD1 ganglioside detected in mouse brain tissue section (for details see **Figure 3** in main text), standards (**B.1**) $GD1_a$ and (**B.2**) $GD1_b$. The drift time obtained from *m*/*z* 917.5 in the tissue section is identical to that of the drift time for the $GD1_a$ standard at 6.73 ms, allowing for the tentative assignment of $GD1_a$ detected in the brain tissue section. A more detailed description of Cone C can be found in the main text.

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