Supplemental Information

Laserspray Ionization Imaging of Multiply Charged Ions using a Commercial Vacuum MALDI Ion Source

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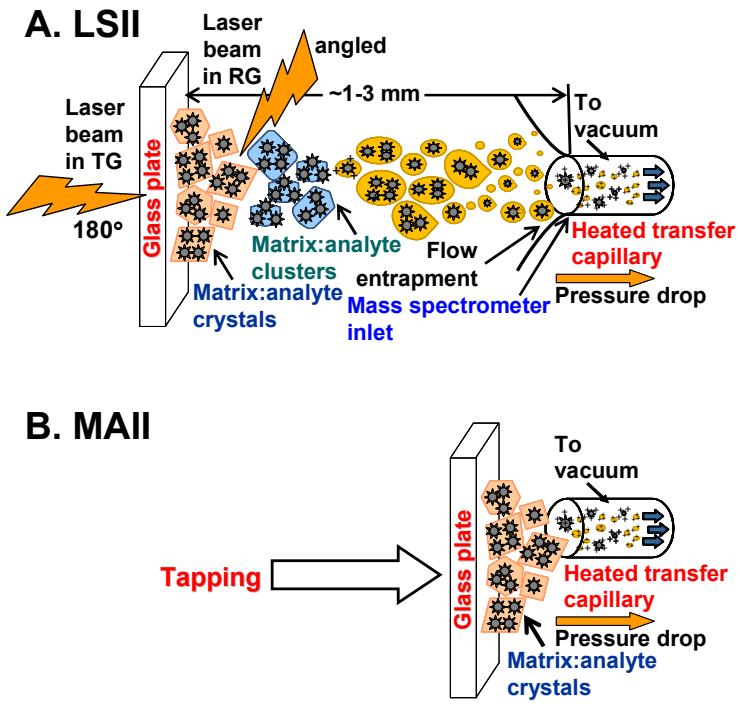

Scheme S1. Cluster model representation at atmospheric pressure of (**A**) LSII in transmission geometry and reflection geometry laser ablation and (**B**) MAII with no laser of the matrix/analyte crystals forming charged matrix/analyte clusters. Multiply charged ions are produced in the presence of a pressure drop region from atmospheric pressure to vacuum and desolvation of the matrix from the clusters in the heated transfer capillary.

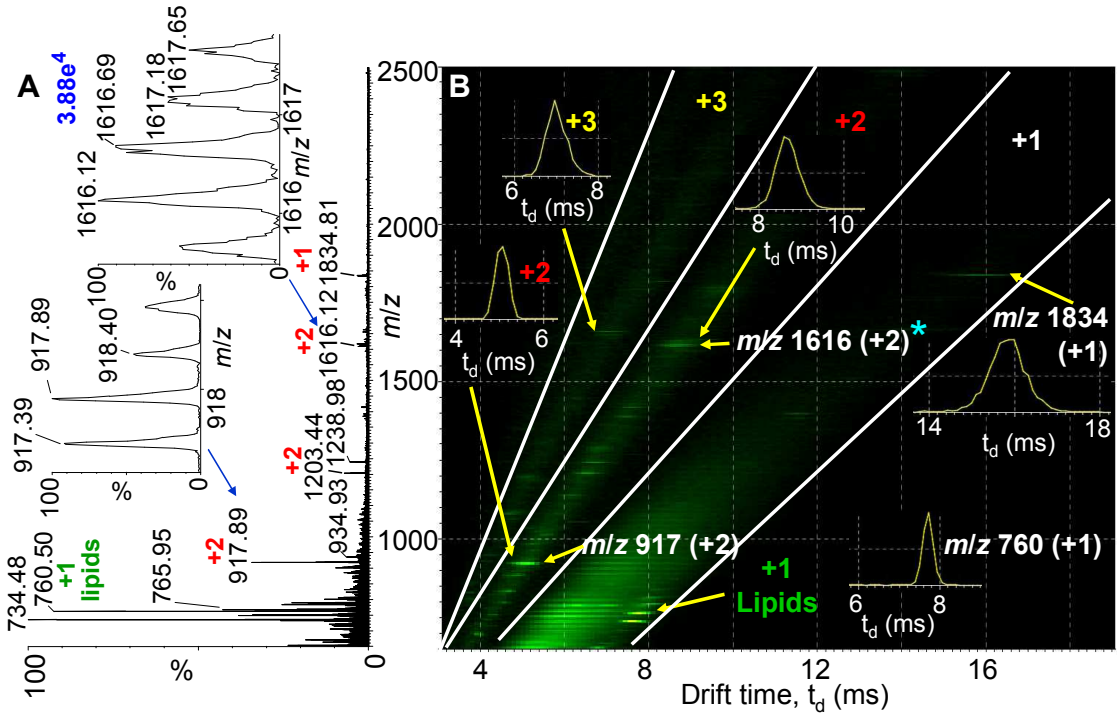

Figure S1. LSIV-IMS-MS **(A)** total mass spectrum and (**B**) two-dimensional plot of drift time vs. *m*/*z* of delipified mouse brain tissue spotted with 2,5-DHAP matrix and acquired using 'LSI settings'[39,42] on a commercial intermediate pressure MALDI source of the SYNAPT G2 mass spectrometer. Insets show (**A**) isotopic distribution of +2 ions of the identified *N*-acetylated MBP peptide (*m*/z 917.39) and the highest MW detected, ~3.2 kDa (*m*/*z* 1616.12) (see *), and (**B**) drift times of +3, +2, and +1 ions.

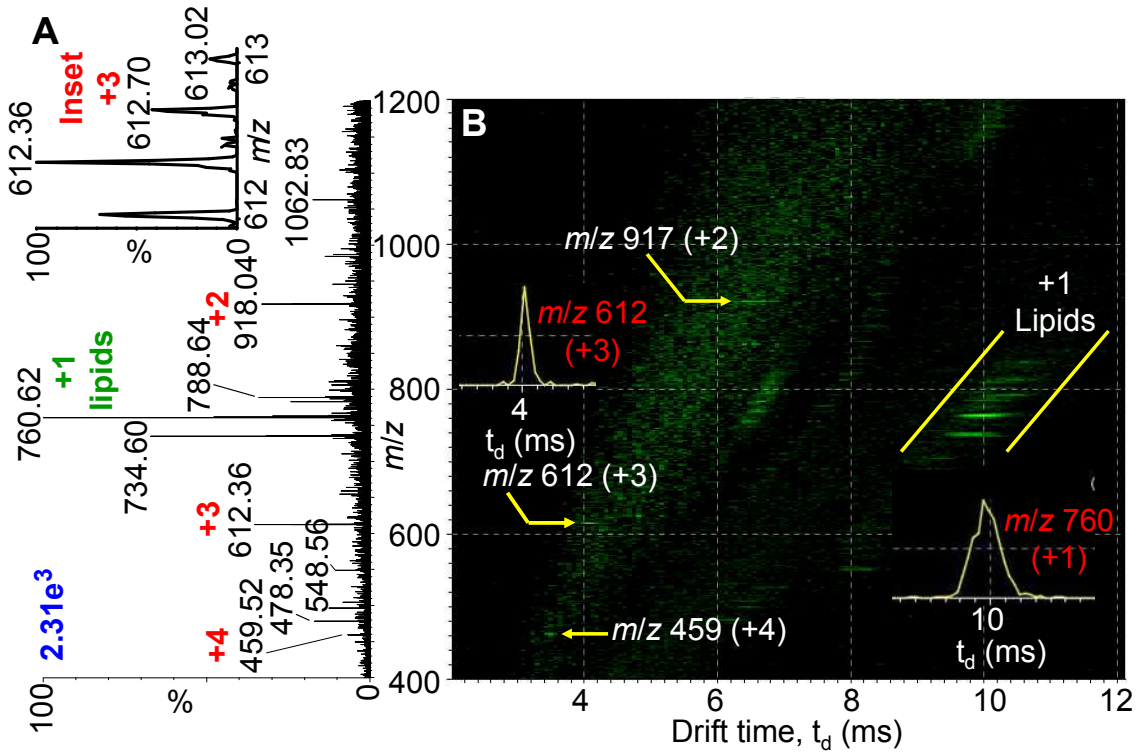

Figure S2. LSII-IMS-MS **(A)** total mass spectrum and (**B**) two-dimensional plot of drift time vs. *m*/*z* of non delipified mouse brain tissue spotted with 2,5-DHAP matrix and acquired using the nanoESI source of the SYNAPT G2 mass spectrometer with a homebuilt skimmer cone to perform LSII in transmission geometry. Insets show in (**A**) isotopic distribution and (**B**) drift times of +3 charge state ion (*m*/*z* 612.36) of the identified *N*-acetylated MBP peptide and +1 drift time of a lipid (*m*/*z* 760.62).

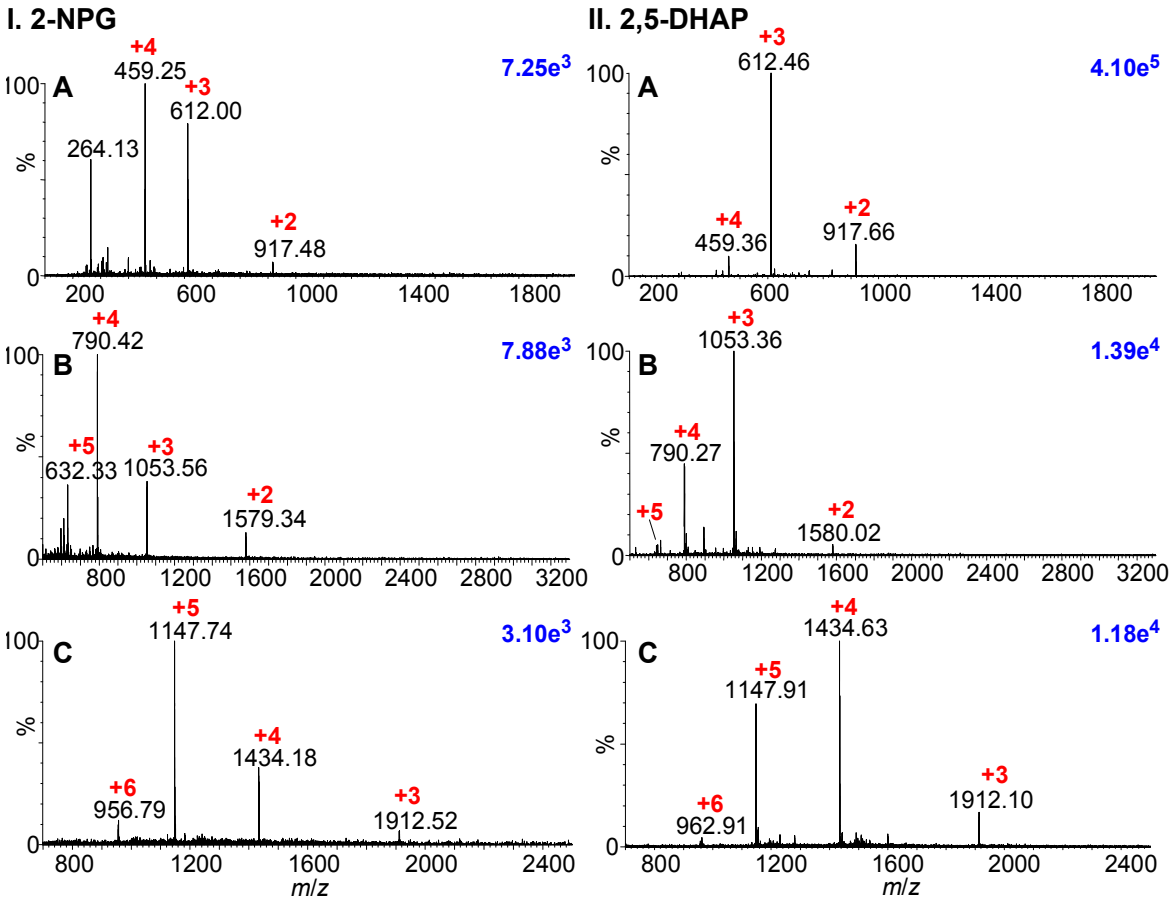

Figure S3. LSIV-MS using (**I**) 2-NPG matrix at low laser power (4.2 J cm^{-2}) and (**II**) 2,5-DHAP matrix at higher laser power (7.3 J cm^{-2}) of (**A**) *N*-acetylated MBP peptide (MW 1833 Da), (**B**) galanin (MW 3150 Da), and (**C**) bovine insulin (MW 5731 Da) acquired using 'LSI settings'[39,42] on a commercial intermediate pressure MALDI source of the SYNAPT G2 mass spectrometer.

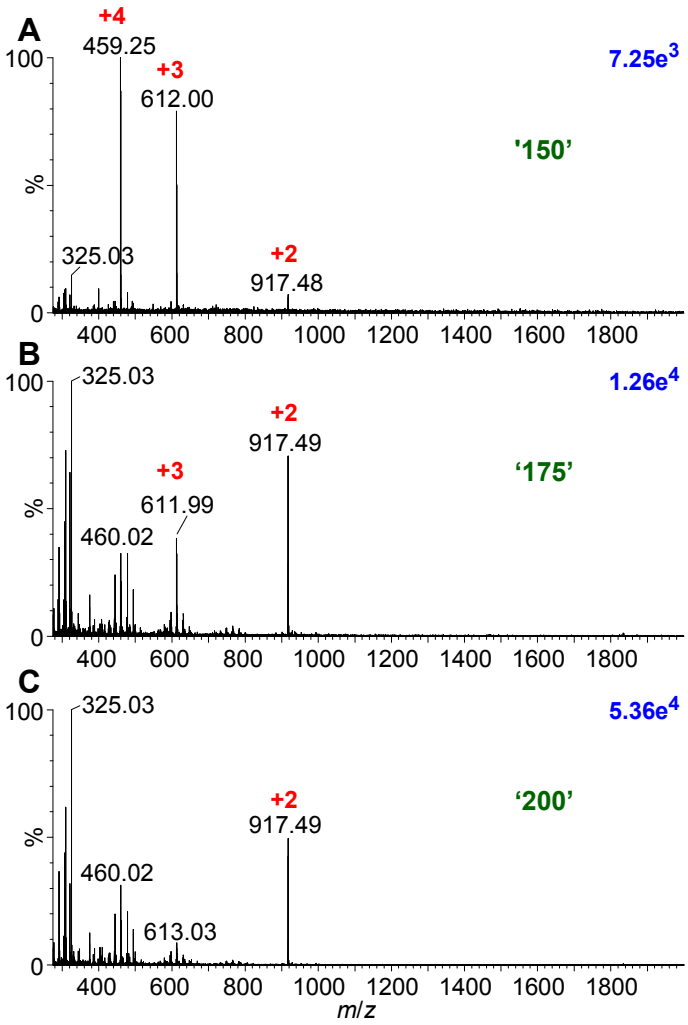

Figure S4. LSIV-MS of *N*-acetylated MBP peptide (MW 1833 Da) with 2-NPG matrix using different laser power: (**A**) 4.2, (**B**) 5.7, and (**C**) 7.3 J cm^{-2} acquired using 'LSI settings'[39,42] on a commercial intermediate pressure MALDI source of the SYNAPT G2 mass spectrometer.

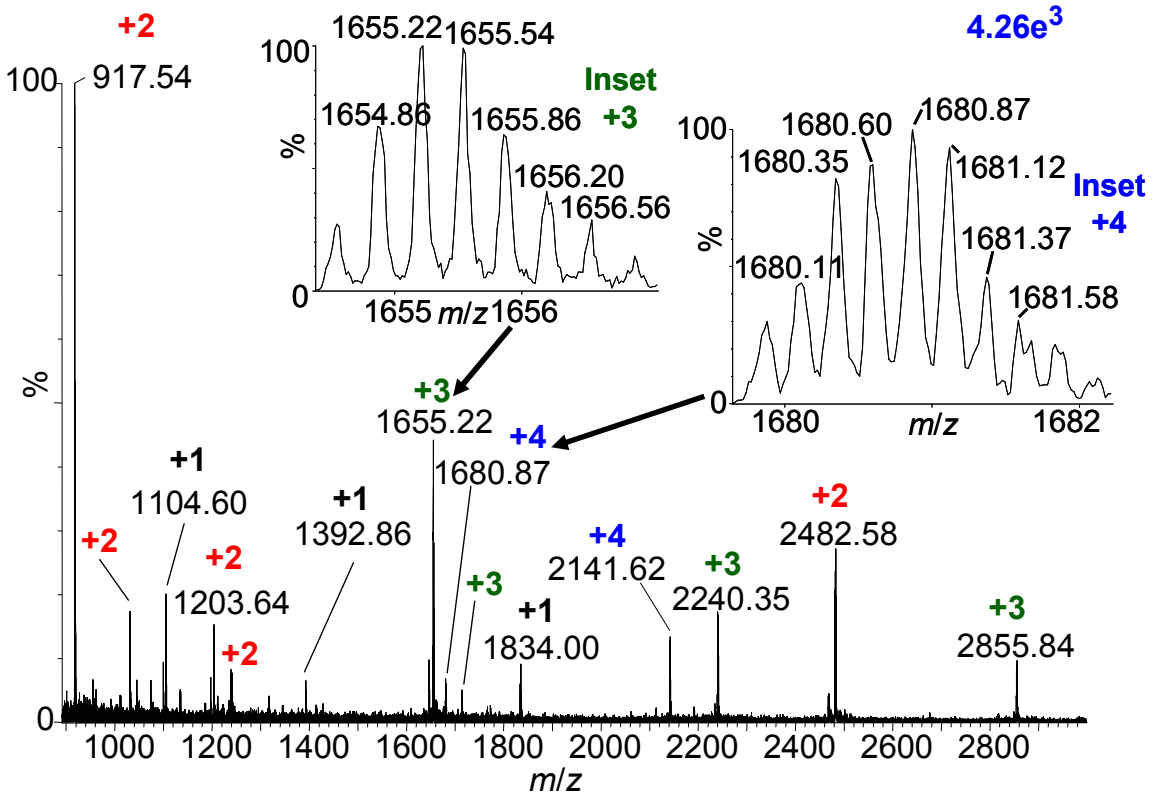

Figure S5. LSIV-IMS-MS of delipified mouse brain tissue spotted with 2-NPG matrix shown in **Figure 2A**. Shown here is the total mass spectrum of the multiply charged peptide and protein ions with the large molecular weight detected, an ~8.6 kDa protein (*m*/*z* 2855.84) with insets of the isotopic distributions of higher charge states: +3 (*m*/*z* 1655.22) and +4 (*m*/*z* 1680.87) ions. Data acquired using 'LSI settings'[39,42] on a commercial intermediate pressure MALDI source of the SYNAPT G2 mass spectrometer.

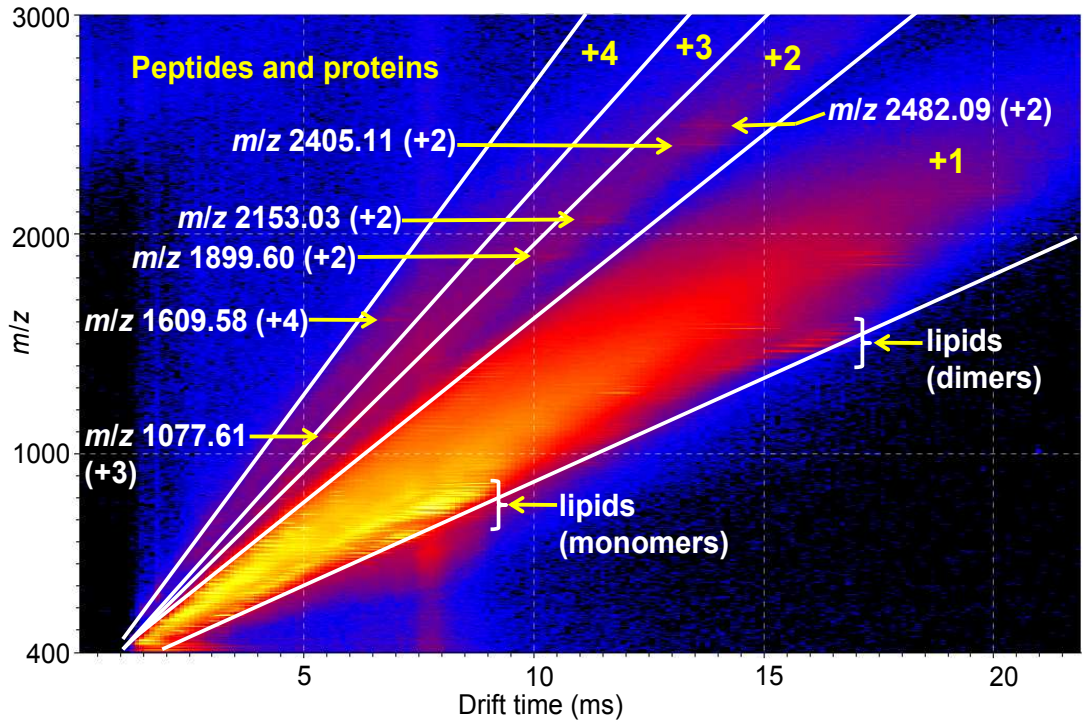

Figure S6. LSIV-IMS-MS two-dimensional plot of drift time vs. *m*/*z* of delipified mouse brain tissue mounted on CHCA precoated glass plate spray coated with binary matrix mixture of 90% 2,5-DHAP and 10% 2-NPG and acquired using 'LSI settings'[39,42] on a commercial intermediate pressure MALDI source of SYNAPT G2 mass spectrometer.