Supplemental Section

Gas-Phase Ions Produced by Freezing Water or Methanol for Analysis using Mass Spectrometry

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Content

Additional mass spectra obtained on either a Thermo Scientific Orbitrap Exactive or LTQ Velos mass spectrometer using an inlet tube cooled with dry ice to initiate ionization are provided. **Figure S1** shows the increase in ion abundance for insulin that is obtained when the exterior inlet is cooled with dry ice relative to being at room temperature. **Figure S2** shows the mass spectra of myoglobin in an acidified methanol solution introduced into the exterior inlet that is cooled with dry ice with the interior inlet at 27 ºC and heated to 200 ºC. **Figure S3** shows ubiquitin obtained by placing a water solution onto dry ice and holding it close to a cooled mass spectrometer inlet with the interior inlet at 150 ºC. Very low ion abundance for ubiquitin is obtained introducing the sample directly to the inlet heated to 150 ºC. **Figure S4** shows the mass spectrum of two peptides in rainwater using ESI. **Figures S5 – S7** show ionization of benzyl pyridinium ions using cold ionization compared to ESI with and without 'in-source' fragmentation voltage. **Figure S8** is of bradykinin obtained by freezing (a) a rainwater solution and (b) freezing an HPLC solution. **Figure S9** is the negative ion mass spectrum of a mixture of angiotensin II and reserpine using freezing conditions. Only singly charged deprotonated ions are observed. Finally, a brief description is provided on how these results might relate to work by Thompson and Iribarne relative to lightning initiation.

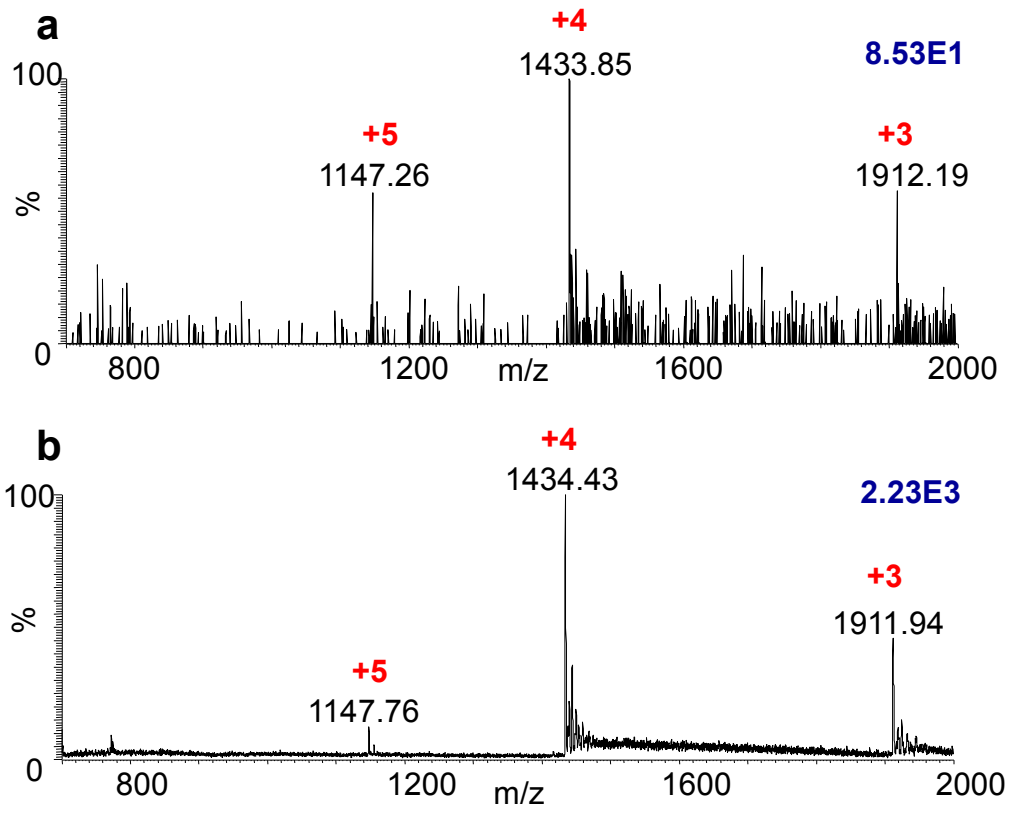

Figure S1: Summed mass spectra of a 3 µL injection of a 5 pmol μL^{-1} 1:1 water:methanol 0.1% formic acid solution of bovine insulin obtained on a Thermo LTQ Velos with the inner inlet at 75 ºC and (**a**) the exterior inlet at room temperature , (**b**) the exterior inlet cooled with dry ice.

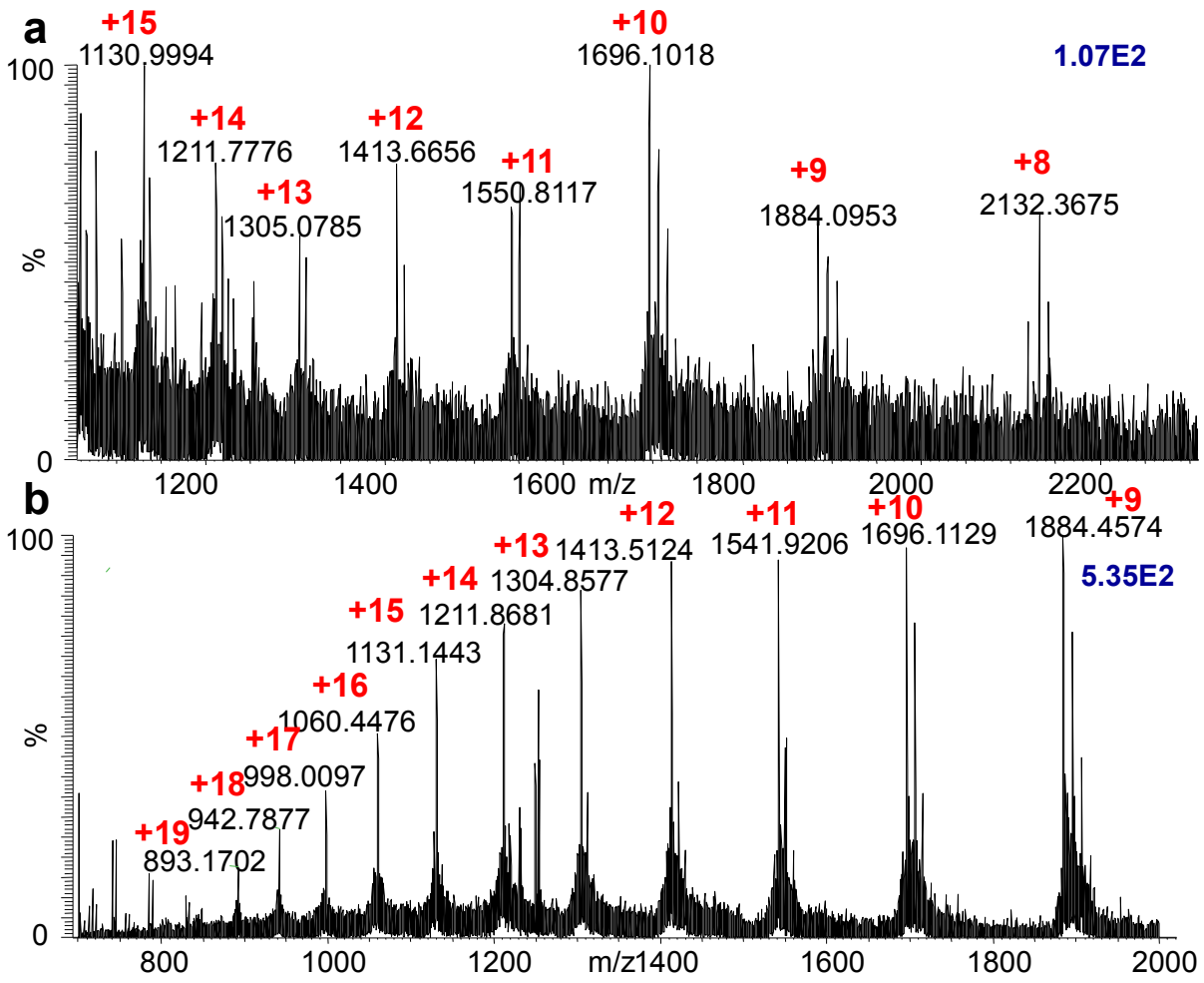

Figure S2: Mass spectra of horse heart myoglobin obtained on a Thermo Orbitrap Exactive summing 50 one second acquisitions of a 5 pmol μL^{-1} methanol solution infused into the exterior inlet cooled with dry ice with (**a**) the inner inlet at 27 ºC and (**b**) the inner inlet at 200 ºC.

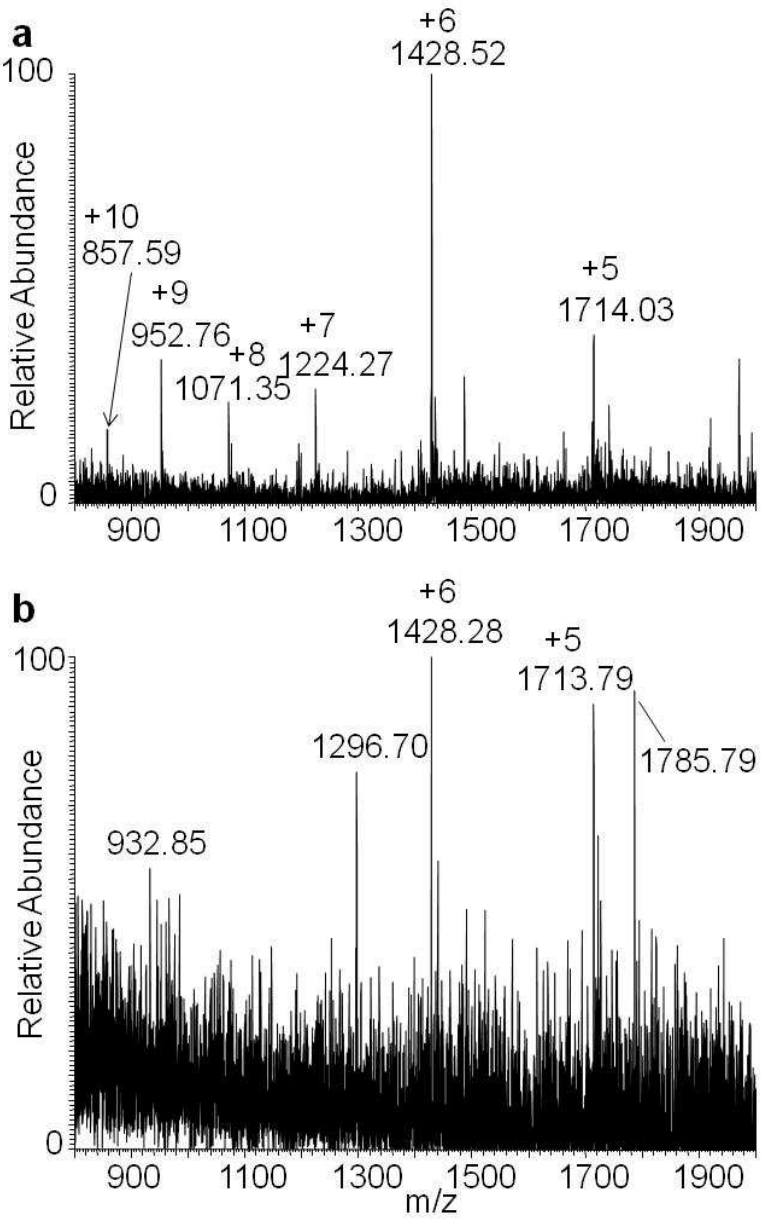

Figure S3: Mass spectra of an aqueous solution of the protein ubiquitin (**a**) obtained using an LTQ Velos mass spectrometer with the outer inlet cooled with water ice, the analyte introduced from the surface of dry ice but not touching the inlet aperture, and the interior section of the inlet tube heated to 150 °C, and (**b**) the same aqueous solution of ubiquitin introduced in solution directly into the interior inlet tube heated to 150 °C.

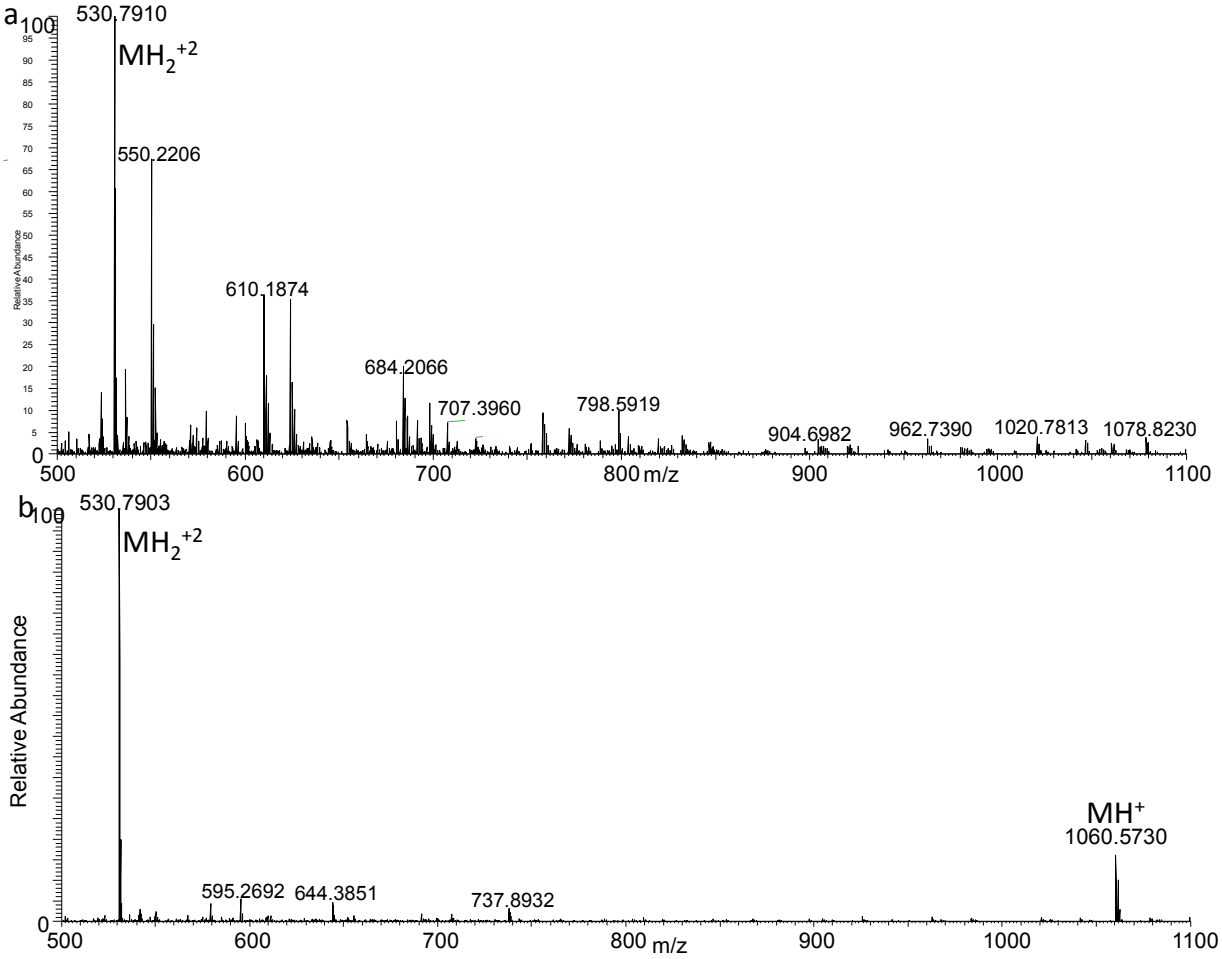

Figure S4. Positive ion cold ionization (exterior inlet cooled with dry ice and internal inlet at 35 °C on Orbitrap Exactive) of 1 pmol μL^{-1} bradykinin. **a**: rainwater collected during a thunderstorm in Philadelphia, PA (abundance MH_2^{+2} = 1.3e5), **b**: HPLC water (abundance MH_2^{+2} = 2.9e5). The peptide ions are labeled.

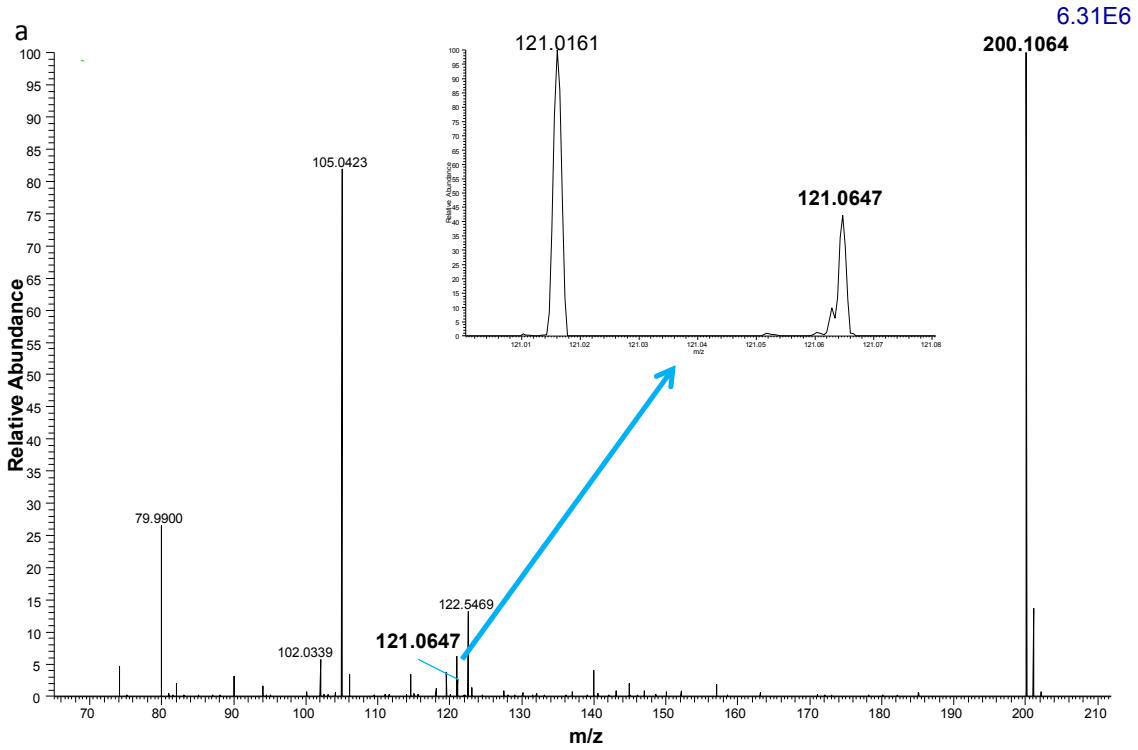

Figure S5. Cold ionization of 1-(4-methoxybenzyl)pyridinium chloride in acetonitrile:water with the exterior inlet cooled with dry ice, the internal inlet at 40 ºC, capillary 50 V, tube lens 90 V, and skimmer 30 V on an Orbitrap Exactive.

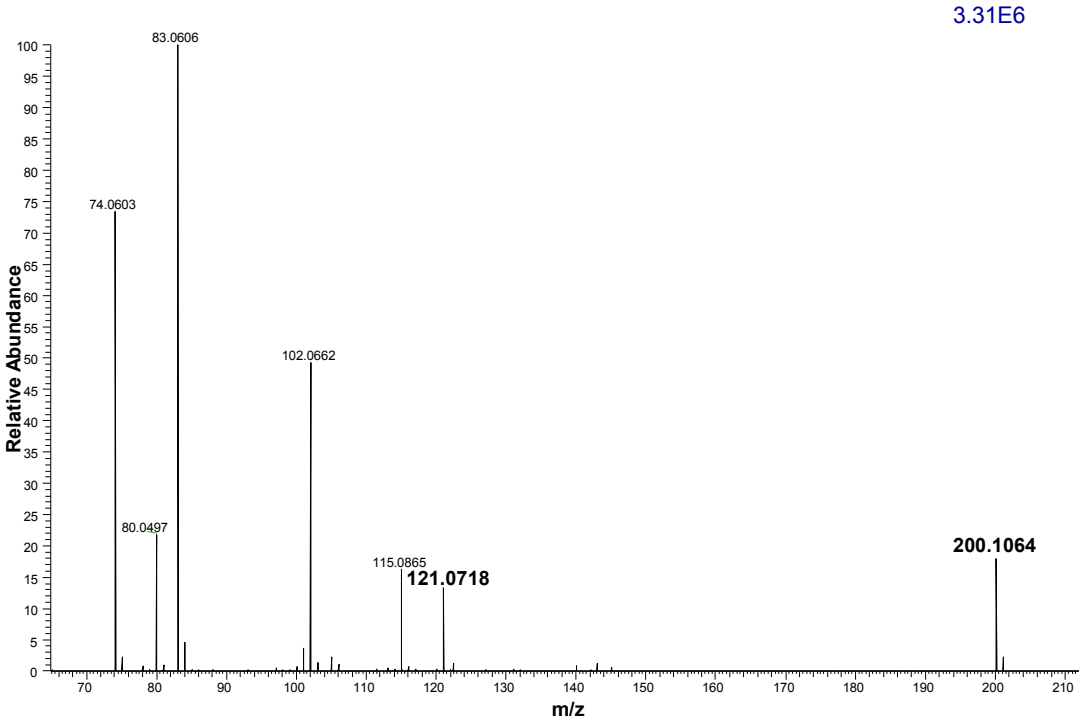

Figure S6. ESI of 1-(4-methoxybenzyl)pyridinium chloride in acetonitrile:water with the exterior inlet cooled with dry ice, the internal inlet at 40 ºC, capillary 50 V, tube lens 90 V, and skimmer 30 V on an Orbitrap Exactive.

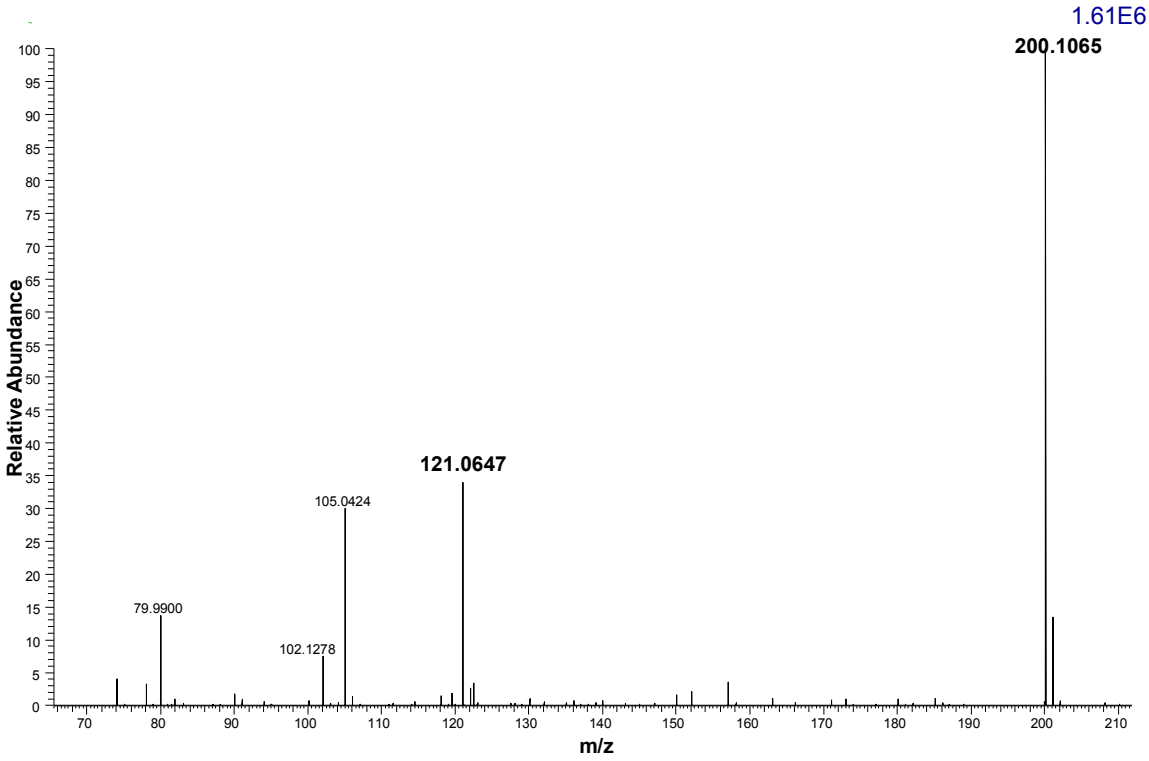

Figure S7. Cold ionization of 1-(4-methoxybenzyl)pyridinium chloride in acetonitrile:water with the exterior inlet cooled with dry ice, the internal inlet at 40 ºC, capillary 50 V, tube lens 90 V, skimmer 30 V, and 100 V applied to 'in-source' fragmentation on an Orbitrap Exactive.

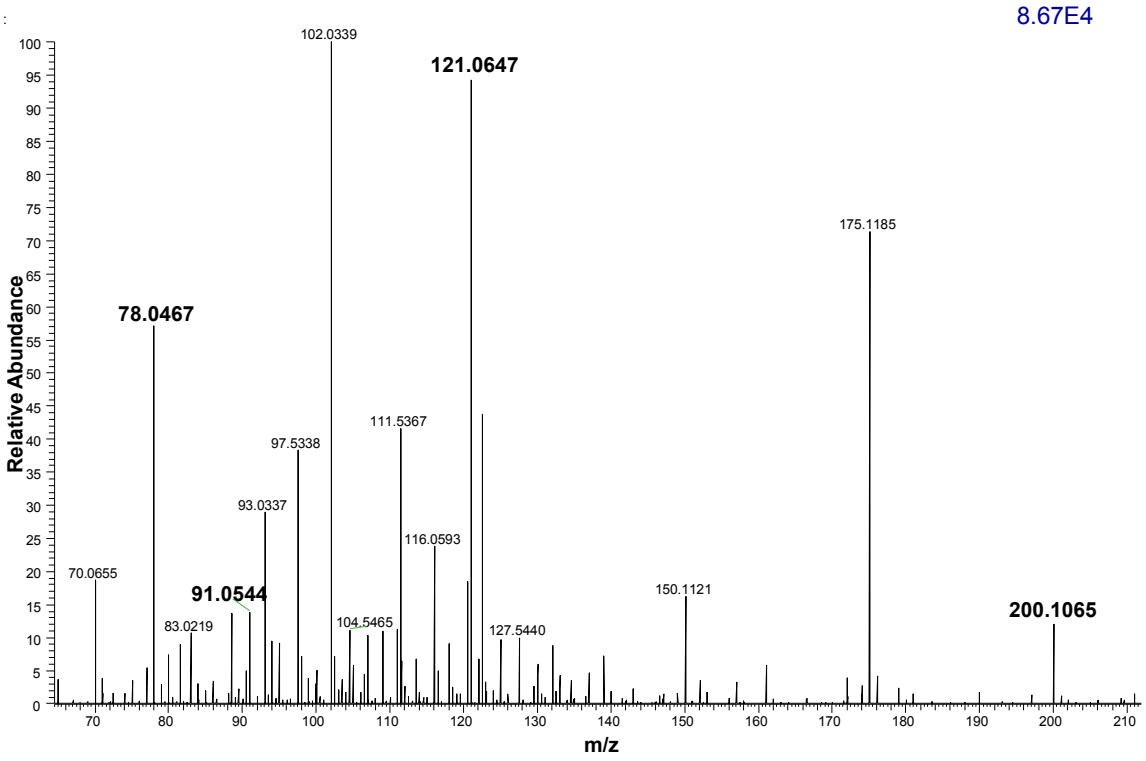

Figure S8. ESI of 1-(4-methoxybenzyl)pyridinium chloride in acetonitrile:water with the exterior inlet cooled with dry ice, the internal inlet at 40 ºC, capillary 50 V, tube lens 90 V, skimmer 30 V, and 100 V applied to 'in-source' fragmentation on an Orbitrap Exactive.

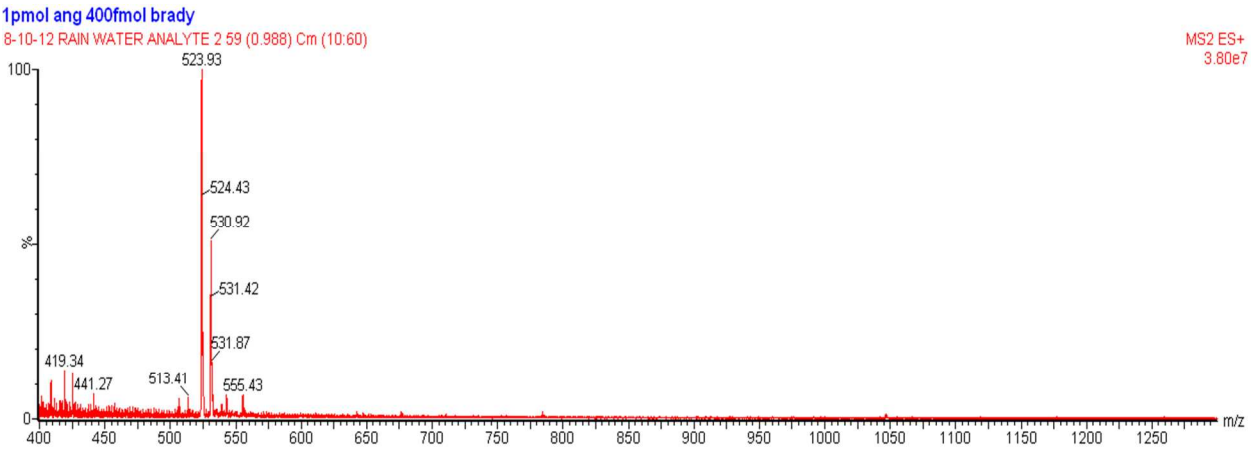

Figure S8. ESI-MS of 1 pmolμL^{-1} of angiotensin II and 0.4 pmolμL^{-1} of bradykinin in rainwater collected during a thunderstorm in Scranton, PA. The peak observed at m/z 523.33 is the MH_2^+ ion of angiotensin II and the peak at 530.92 is the MH_2^+ ion of bradykinin. The identical concentration sample in HPLC water gave an ion abundance of 8.9e7 for the same scan range or 2.3X more abundance than with rainwater.

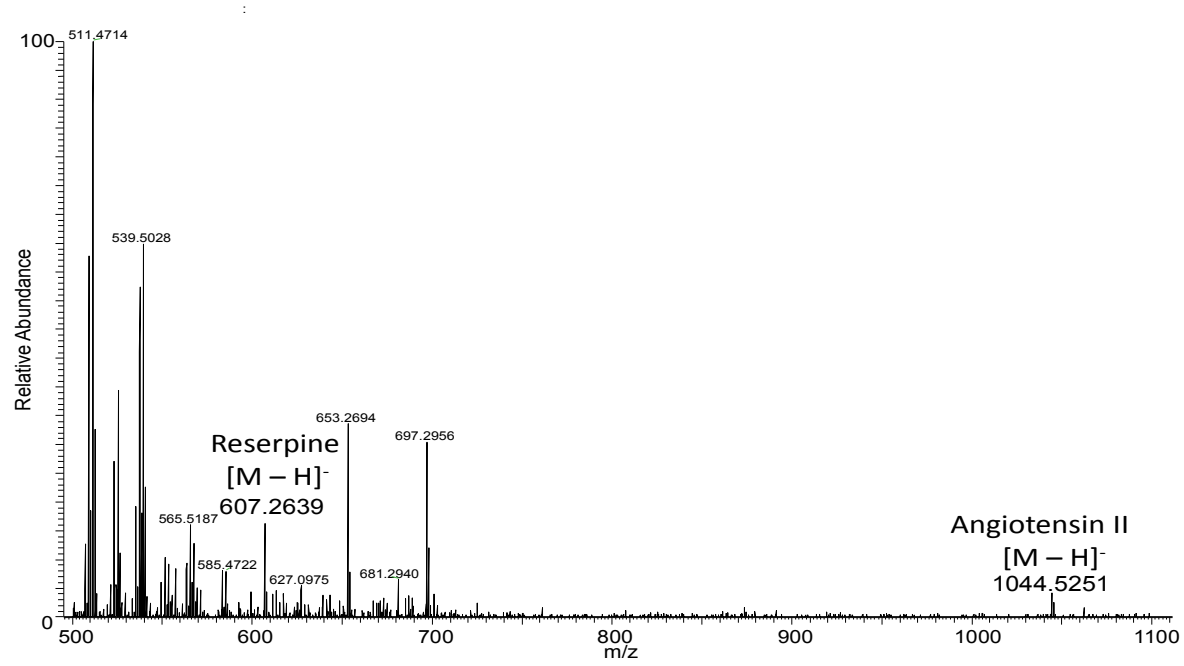

Figure S9. Negative ion mass spectrum from freezing rainwater (exterior inlet cooled with dry ice and interior inlet at 35 °C on the Orbitrap Exactive) containing 2 pmol μL^{-1} of reserpine and 1 pmol μL^{-1} angiotensin II. The singly charged deprotonated peptide ions are labeled. All other ions are from impurities in the solution.

Comments on Coulomb Fission Events in Thunderclouds and the Relationship to Lightning Initiation

In Thompson and Iribarne's paper, "The Fate of Electrical Charges in Evaporating Cloud Droplets", they concluded that coulomb fission events could occur in charged cloud droplets, but that salts and other impurities in the rainwater would prevent ion evaporation releasing bare ions.[1] To determine if their conclusion that particulates and salts in cloud droplets would prevent formation of bare ions under freezing conditions,[1] we collected rainwater from thunderstorms for analysis by MS using a hot and cold inlet, as well as with ESI. The peptide angiotensin II was added to rainwater collected during thunderstorms occurring near Scranton and in Philadelphia, Pennsylvania, and to HPLC water to make equal concentrations (1 μM) in each solution. Ionization of angiotensin II was compared from the two solutions by ESIand under freezing conditions using mass spectrometry. The doubly charged ions from angiotensin II were observed by both methods and from all solutions. Analyte ion abundance in the rainwater was reduced ca. 50% relative to HPLC water for all methods, suggesting that impurities in the collected rainwater had only a moderate influence on coulomb fission events that produce the bare analyte ions. The similarity of results except for ion abundance which was lower with cold ionization is consistent with the methods producing bare ions by similar processes. These results also suggest that at least in some thunderstorms, the contaminants are not sufficient to halt the process whereby bare ions are produced.

Thus, Thompson and Iribarne's hypothesis that under subliming/evaporating conditions, which occurs near the leading edge of a cloud or in up or down drafts,[2] columbic fissure can result in large numbers of smaller highly charged droplets/particles typically carrying several times more charge-to-mass than the parent droplet. It is now known that the water droplets and ice particles in a thundercloud can carry hundreds, even thousands, of charges.[3,4] Even though the total charge would not change, the higher charge mobility would increase the conductivity of

the air. High local conductivity and low fields have been measured in thunderclouds.[5] The breakdown potential of air has also been shown to decrease during up and downdraft events,[6,7] and corona emission from charged hydrometeors,[7-10] has been suggested as a means of initiating lightning in a high electric field. Coulomb fission processes occurring under high field conditions in thunderclouds could also have implications for lightning initiation by creating a localized high conductivity region promoting electric breakdown.[8] The cold ionization experiments which are carried out in a field-free environment concentrating charge onto very small particles demonstrates these processes occur without an external electric field. Thus, higher air conductivity following coulomb fission events could be a source of lightning initiation irrespective of local electric fields.

References

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