Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supplementary Information

A Multichannel Rotating Electrospray Ionization Mass Spectrometry (MRESI): Instrumentation and Plume Interactions

Contents

Solvent delivery method in MRESI setup

Unlike normal ESI-based ionization techniques, spray solvent needs to be infused into rotating spray emitters in MRESI, and the method of solvent delivery in the MRESI setup is shown in Figure S1. The external power source, syringe pump and stepper motor, are placed directly below the MRESI setup. Three parts of MRESI setup are colorized, in which part 1 and 2 are transmission parts whereas part 3 is rotating part, and their motion mode are also shown in the schematic diagram. In particular, through the gear drive, stepper motor rotate part 3 and all other parts connect to it, such as syringes, spray emitters, and part 2. The thrust force of syringe pump is transmitted to syringe through part 1 and part 2. Part 1 moves along the rotation axis, while part 2 not only move forward with part 1, but also rotate with part 3. A plane bearing is sandwiched between part 1 and 2 to reduce the friction. Then, the spray solvent in syringe is infused into spray emitter to produce electrospray through a short PTFE tube. In this version of MRESI, one syringe pump could drive 1-3 syringe(s), and the flow rate is easy to control. Normally, different channels share the same flow rate unless using syringes of different capacities (such as 100 µL, 250 µL, 500 µL and so on).

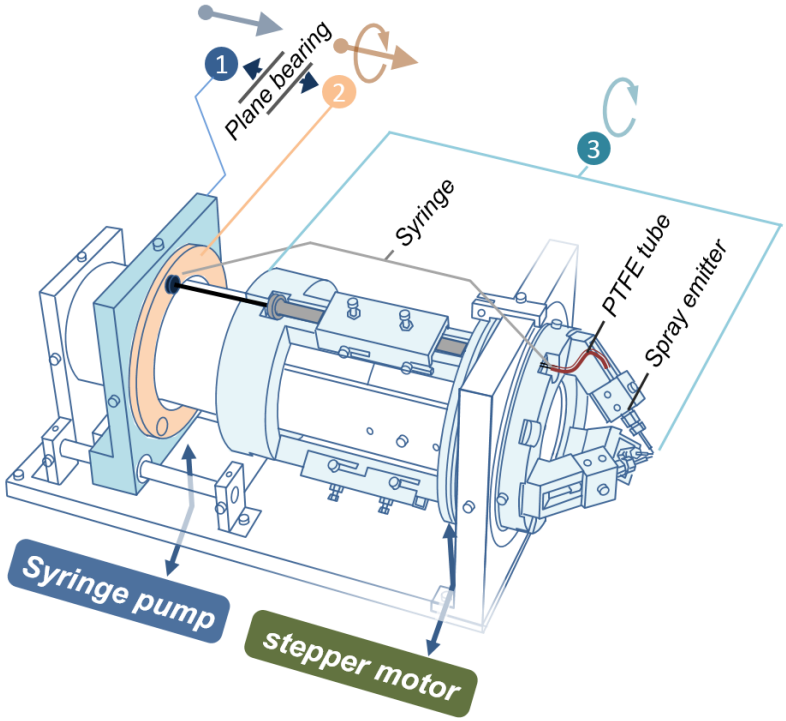

Figure S1. Schematic illustration of the MRESI setup. Three parts related to solvent delivery are colorized and their motion mode are shown above.

Electrical contact method of MRESI setup

In the MRESI setup, voltage is applied to spray emitters through the contact of electric brush, metal ring, and electric wire, as shown in Figure S2. Metal ring is surrounded on rim of the rotating part and rotates with it. For each spray emitter, there is an electric wire by which connect it to the metal ring. So all spray emitters share the same voltage.

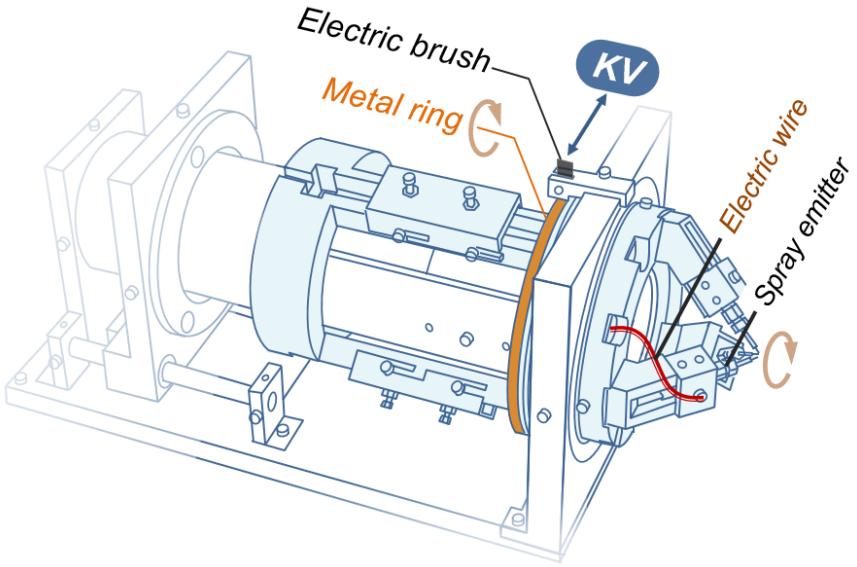

Figure S2. Schematic illustration of the MRESI setup. Voltage is applied to spray emitters through the contact of electric brush, metal ring, and electric wire.

The denaturation phenomenon of insulin in MRESI analysis

An interesting protein denaturation phenomenon was observed in MRESI when insulin solution and formic acid solution were sprayed from two separate ESI emitters simultaneously. Compared to the MRESI spectrum obtained by concurrently spraying insulin solution and methanol/water (50/50, v/v) in two channels (Figure S3a), the MRESI mass spectrum after adding 1% formic acid in methanol/water (50/50, v/v) while keeping other else constant (Figure S3b) exhibits a relatively wide distribution of charge states of insulin with 5+ being the base peak, indicating that the protein is partially denatured.

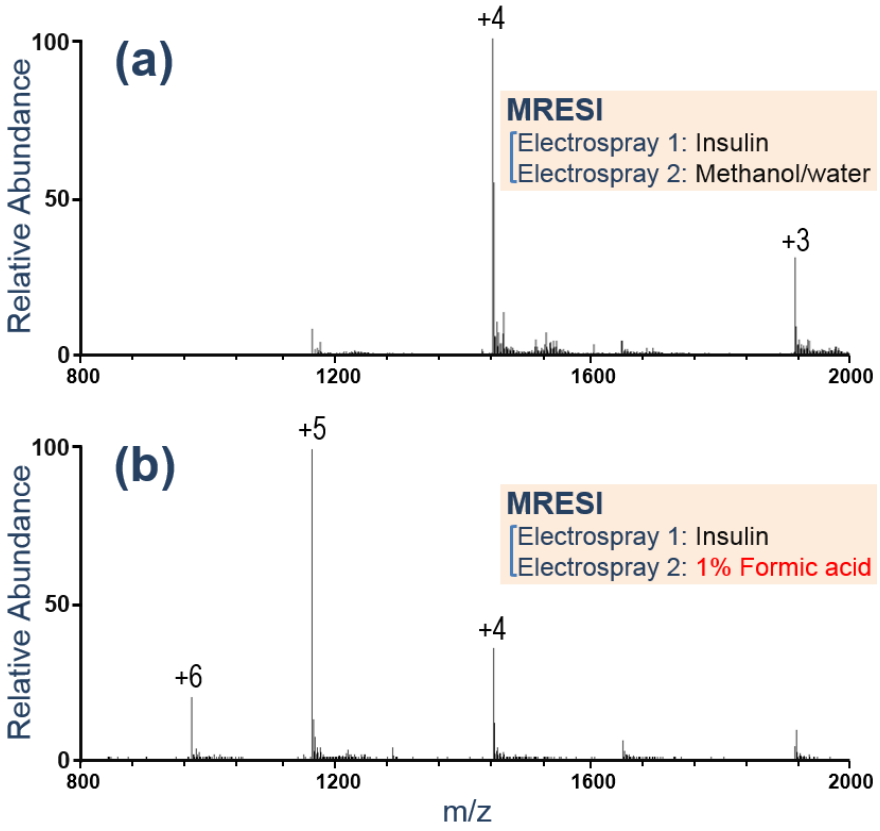

Figure S3. MRESI mass spectra obtained by concurrently spraying 10 μM insulin solution in one channel and methanol/water (50/50, v/v) (a) without adding formic acid and (b) adding 1% formic acid in another channel.

The shift of protein CSD caused by introducing gas phase formic acid molecules to a MRESI spray which contains cytochrome *c*

In order to verify that gas phase formic acid could react with the electrospray contains protein and result in an observed shift of protein charge state distributions (CSD) in the mass spectra, we design a simple experiment to introduce gas phase formic acid molecules to the spray by loading 2 μL formic acid on the extension capillary 2 mm away from the inlet. As shown in Figure S4, before loading acid, a narrow distribution of charge states of cytochrome *c* with +8 being the base peak was obtained. Once the volatile denaturing reagent formic acid was loaded, the gas phase formic acid molecules vaporized form droplets and diffused to spray area, then reacted with cytochrome *c* in spray droplets and resulted in a dramatic shift of protein CSD. As shown in the average mass spectrum of the first 2 min, a relatively wide distribution from +8 to +17 of charge states of cytochrome c with +10 being the base peak were obtained. However, as the concentration of gas phase formic acid decreases over time, the average mass spectrum of 3-4 min shows a drop of the average protein charge state and the average mass spectrum of 5-6 min is similar to that before loading formic acid. These results have proved that gas phase formic acid could induce the shift of protein CSD.

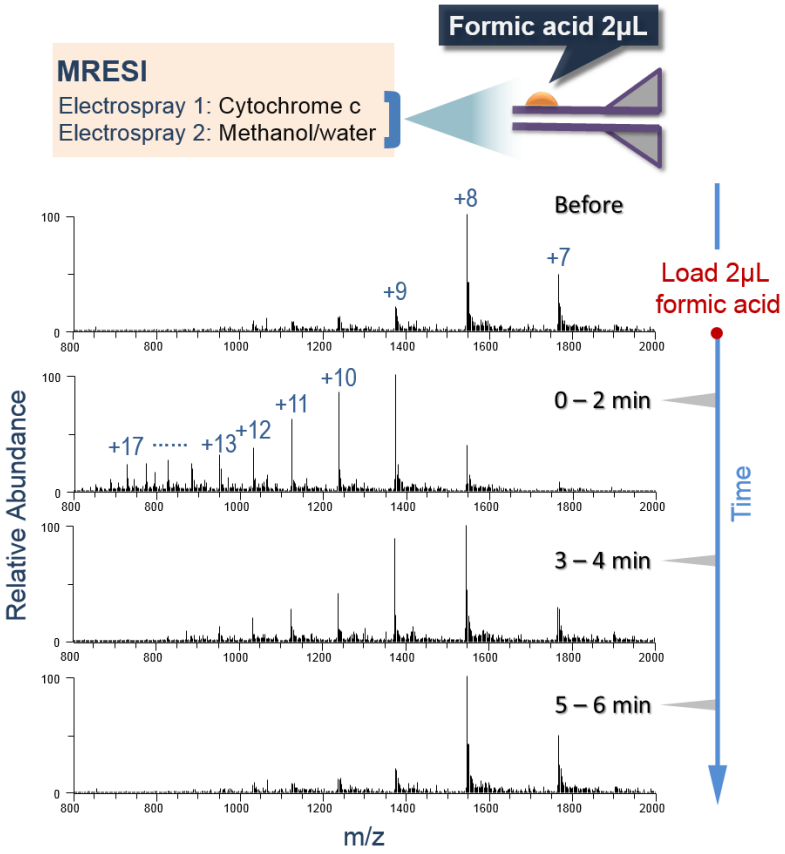

Figure S4. MRESI mass spectra obtained by concurrently spraying 20 µM cytochrome *c* and methanol/water (50/50, v/v) in two separate channels before and after loading 2 µL formic acid on the extension capillary 2 mm away from the inlet.

Supercharging Experiment in MRESI

When proteins hold more charges induced by "supercharging reagent(s)" (Ref. 43-46 in the main text), it is easier for commercial mass analyzers to measure its molecular weight due to the obtained lower mass to charge ratios. Moreover, in tandem MS mode, the supercharged ions are often more fragile, which would generate more structurally informative fragments. So the studies on supercharging effect are important in proteomics. We tried to *in situ* induce supercharging of denatured cytochrome *c via* our MRESI instrument. As shown in Fig S5, two supercharging reagent, glycerol and *m*-nitrobenzyl alcohol, respectively, were used to try to induce supercharging effect. The supercharging reagent was sprayed from one emitter, while the denatured protein solution was sprayed from another emitter. However, the obtained mass spectra were similar to the blank control without higher charged ions. The results again confirm that in MRESI, when non-volatile reagents were applied, cross talks between spray emitters can be neglected.

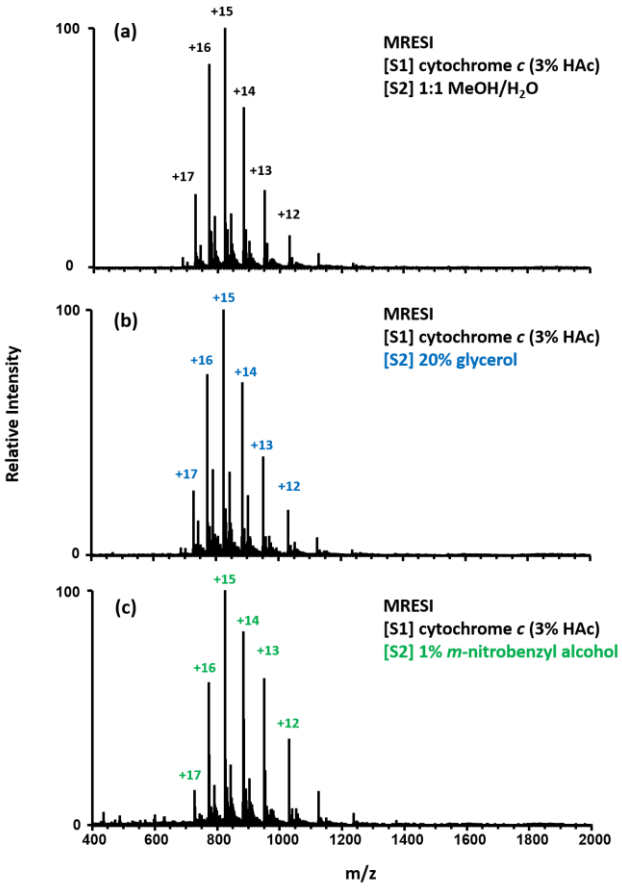

Fig S5. (a) MRESI experiment when one spray [S1] is cytochrome *c* solution with 3% HAc and another spray [S2] is 1:1 MeOH/H_2O, this experiment is conducted as blank control. (b) Similar to (a), but [S2] is changed to 20% glycerol solution. (c) Similar to (a), but [S2] is changed to 1% *m*-nitrobenzyl alcohol solution.