Supporting Information

Electrostatic-Spray Ionization Mass Spectrometry

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SI-1: Chemicals and Microchip fabrication.

Chemical. Angiotensin I (trifluoroacetate salt, 98%) was obtained from Bachem. Myoglobin (horse heart, 90%), lactoferrin (bovine milk, 85%) and beta-lactoglobulin (bovine milk, 90%) were purchased from Sigma. Cytochrome C (horse heart, 95%), bovine serum albumin (98%) and ammonium bicarbonate (99.5%) were obtained from Fluka. Trypsin (bovine pancrease) and methanol (99.9% HPLC grade) were purchased from Applichem GmbH. Acetic acid (100%) was obtained from Merck. Acetonitrile (99.9% HPLC grade) was obtained from ABCR. 2,5-dihydroxybenzoic acid (98%) was purchased from Aldrich. All these reagents were used as received without further purification. Deionized water (18.2 MΩ cm) was obtained from an ultra-pure water system (Milli-Q 185 Plus, Millipore) and used for all experiments.

Microchip fabrication. A polyimide microchip was prepared by using laser ablation method with the design shown in scheme 1(a). The electrode was made by drilling an L-shape-like microchannel on a PolyImide (PI) substrate (125 μm thick, DuPont™ Kapton® polyimide film). The microchannel was then filled with carbon ink. In a second step, a microchannel was drilled on the same side of the polymer close to the electrode. At one end of the channel, a through hole was opened by laser drilling. Finally, the PI substrate was laminated with 25/10 μm PolyEthylene (PE)/ PolyEthylene Terephthalate (PET) composite sheets (Morane, Oxone, UK), where PE acted as the sealing agent when rolled at 130 °C and 3 bars for 3 s. To insure a good lamination, the entire structure was additionally cured for 1 hour at 80 °C. Before use, the tip of the microchannel was cut into a V-shape to facilitate the electrospray ionization.

SI-2: Mechanism of Electrostatic-Spray Ionization

- **Physical Parameters:**

Dielectric Constants (ε_r) of polyimide, silica, polypropylene and PMMA: 3.5, 3.9, 2.2-2.36 and 3.2 [1];

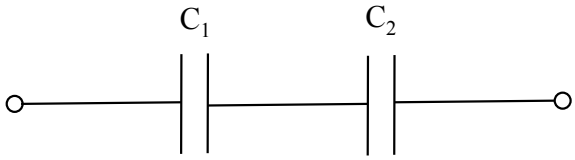
Surface Tension (γ) of 50%/50% (v/v) H_2O/MeOH and pure water: 0.03651-0.03286 N/m and 0.07201 N/m [2].

[1]: ε_r of polyimide is provided by DuPont for its product of Kapton® polyimide 125 μm thickness; ε_r of silica and polypropylene are obtained from Wikipedia http://en.wikipedia.org/wiki/Relative_permittivity; ε_r of PMMA is from Tesla Coil Mailing List http://www.pupman.com/listarchives/1998/April/msg00337.html. All these values correspond to a condition of room temperature and 1 kHz.

[2] *J. **Chem.** Eng. Data **1995,40,*** 611-614; values for 25 °C.

- **Physics Background**

Two capacitors in series:

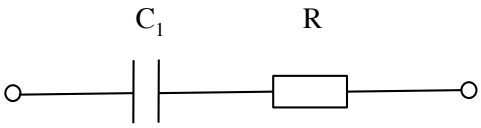


The equivalent capacitance for the whole system is given as:

$$C_{eq} = C_1C_2/(C_1 + C_2),$$

when $C_1 << C_2$, $C_{eq} = C_1$; when $C_2 << C_1$, $C_{eq} = C_2$.

RC circuit

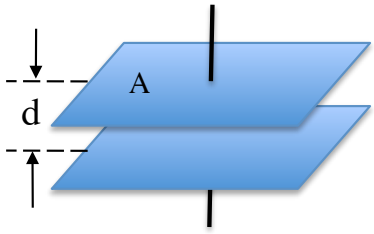


The current decrease with time is given as:

$$I(t) = I_0 e^{-t/RC}$$

where RC is the time constant (τ).

Capacitance for parallel-plate model:

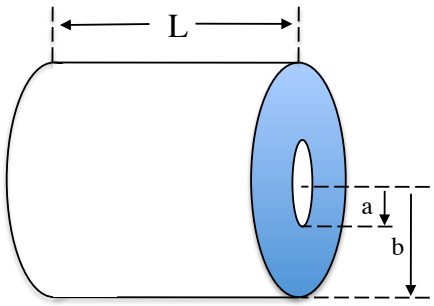


The capacitance for parallel-plate capacitor is given as:

$$C = \varepsilon A/d$$

ε is the permittivity of the insulator that can be calculated as $\varepsilon_r \times \varepsilon_0$, where ε_0 is the permittivity of vacuum. A is the area of the plate, and d is the distance between two plates.

Capacitance for Cylindrical Capacitor:



The capacitance for cylindrical capacitor is given as:

$$C = \frac{2\pi\varepsilon L}{ln\left[\frac{b}{a}\right]}$$

Electric field on the surface of a solid charged sphere

$$E = q/4\pi\varepsilon_0 r^2$$

where r is the radius of the sphere.

- **Mechanism of ESTASI**

ESTASI occurs when the electrostatic pressure at the tip of the emitter is larger than the Laplace pressure. The electrostatic pressure is a function of the surface charge accumulation

$$p_E = \sigma^2 / 2\varepsilon_0$$

where σ is the surface charge density. If the tip of the emitter, *e.g.* at the Taylor cone, is assumed to be hemispherical, the Laplace pressure is simply given by

$$p_L = 2\gamma / r$$

Therefore, small emitter microchannel radius and small liquid surface tension are preferred to realize ESTASI.

Similar as native ESI, there is also an onset voltage for ESTASI. The value of this onset voltage is influenced by the capacitances of the two capacitors illustrated in scheme 2. Before the ESTASI happens, the system is two capacitors in series. The first capacitor is with a constant capacitance (C_1) during experiments when a specific

emitter is selected. The capacitance of the second capacitor (C_2) varies with the distance between emitter tip and MS inlet. The equivalent whole capacitance (C_{eq}) of the system is: $C_{eq} = C_1C_2/(C_1 + C_2)$. Upon application of a high voltage U, the maximum charge on the second capacitor representing the tip of the emitter is UC_{eq}. By placing the emitter far from the MS inlet, C_2 becomes very small. C_{eq} is then equal to C_2, and the maximum charge at the tip of the emitter is UC_2. Since C_2 is very small, this charge is too small to induce electrospray. By placing the emitter close enough to MS inlet, C_2 cannot be neglected and the charge at the tip is UC_{eq}, which must be large enough to onset the spray if the ESTASI can happen.

In the case of parallel plate capacitor,

$$C_1 = \varepsilon A/d$$

Therefore, small distance between plates, large plate area, and large dielectric constant for insulator will result in large capacitance of the first capacitor, and are preferred for the ESTASI.

In the case of cylindrical capacitor,

$$C_1 = \frac{2\pi\varepsilon L}{ln\left[\frac{b}{a}\right]}$$

Therefore, large length, small b/a ratio, and large dielectric constant for insulator are preferred for the ESTASI.

When this condition is realized, ESTASI happens and the second capacitor can be considered as a leaky capacitor with a diode in parallel, therefore is a resistance (R). The system is then a RC circuit, with the time constant (τ) of:

i) in the case of parallel plate capacitor:

$$\tau = \frac{R\varepsilon_r\varepsilon_0 A}{d}$$

ii) in the case of cylindrical capacitor

$$\tau = \frac{2\pi\varepsilon_r\varepsilon_0 LR}{ln\left[\frac{b}{a}\right]}$$

The current of this RC circuit decrease with time is then expressed as:

$$I(t) = I_0 e^{-t/\tau}$$

- **Case studies**

i) ESTASI with polyimide microchip

In the case of microchip ESTASI, the microchannel for electrospray is with a cross section dimension of 50 μm × 100 μm. The first capacitor is formed by two plates and

an insulator: the first plate is a band carbon electrode with the surface area of 50 μm × 5 mm; the insulator is polyimide with the thickness of 2 mm; and the second plate is electrolyte solution in microchannel, which is larger than the first plate (scheme 1(a)). The solvent for spray is 50%water/50%methanol

For an approximate calculation, the parallel-plate model is used, and the capacitance of the first capacitor C_1 is calculated as ~4 fF. Under a high voltage of 6 kV, UC_1 is ~20 pC. This charge is the maximum theoretical charge on the second capacitor, when C_2 is much larger than C_1.

If a hemispherical droplet is formed at the tip of emitter with a radius of 50 μm, the maximum surface charge density (σ) on the droplet can reach 1.3 mC/m^2, resulting in an electrostatic pressure of 100 kPa. The Laplace pressure is 1.4 kPa for a droplet with the radius of 50 μm and surface tension of 0.035 N/m (50% water 50% methanol), which is much smaller than the electrostatic pressure. Therefore, the ESTASI can happen under this condition. However, a larger carbon band electrode and a smaller distance between the electrode and the microchannel will benefit the ESTASI.

Scheme SI-2.1. Microchip with electrode integrated in the microchannel.

The resistance of electrospray during ESTASI should be same as that of native ESI if the emitter geometry and the distance between emitter and the MS inlet keep constant. We have fabricated a microchip with a microchannel for ESI integrated with a carbon electrode (scheme SI-2.1) holding similar geometry as the microchip used for ESTASI in scheme 1(a). During experiments, the voltage applied for native ESI was 4 kV, and the current measured by an amperometer was around 0.1 μA. Therefore, the resistance of electrospray during ESTASI from microchip can be estimated with a value around 40 GΩ.

The time constant (τ) is then ~0.16 ms for microchip ESTASI. The value is very small and did not limit our experiments. The experimental time constant is in the order of 0.1 s and stems mainly from the rise time of the switch box system. Indeed, the maximum operation frequency of ESTASI in our lab was 5 Hz limited by the switch box.

ii) ESTASI with silica capillary

In the case of capillary ESTASI, silica capillary with inner diameter of 50 μm and outside diameter of 360 μm was used, and the solvent for spray was 50%water/50%methanol. The C_1 is then calculated as ~200 fF with a cylindrical capacitor model where the length is 2 mm and the insulator is silica. Under a high voltage of 6 kV, UC_1 is ~1 nC.

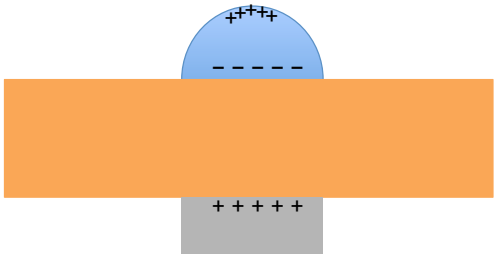
Assuming that a hemispherical droplet is generated with the radius (r) of 25 μm at the end of the channel, the maximum surface charge density (σ) on the droplet can reach 0.25 C/m^2, resulting in an electrostatic pressure of 3 GPa, which is very large. Therefore, the ESTASI can happen under this condition.

By experiments, we also estimated the resistance of electrospray performed with silica capillary as ~70 GΩ. And then the RC time constant is ~14 ms, still very small and not limiting the practice.

iii) ESTASI with micropipette tip

In case of ESTASI from micropipette tip, the capacitance of the first capacitor can still be calculated with the cylindrical capacitor model as ~600 fF, where L is 2 mm, a is 200 μm, b is 300 μm and ε_r is 2.3. Therefore, UC_1 is ~4 nC. The hemispherical droplet formed at the tip of emitter was large with a radius of ~100 μm. The maximum surface charge density (σ) on the droplet can reach 0.06 C/m^2, resulting in an electrostatic pressure of 200 MPa, large enough to induce ESTASI.

iv) ESTASI from microdroplet on the PMMA plate

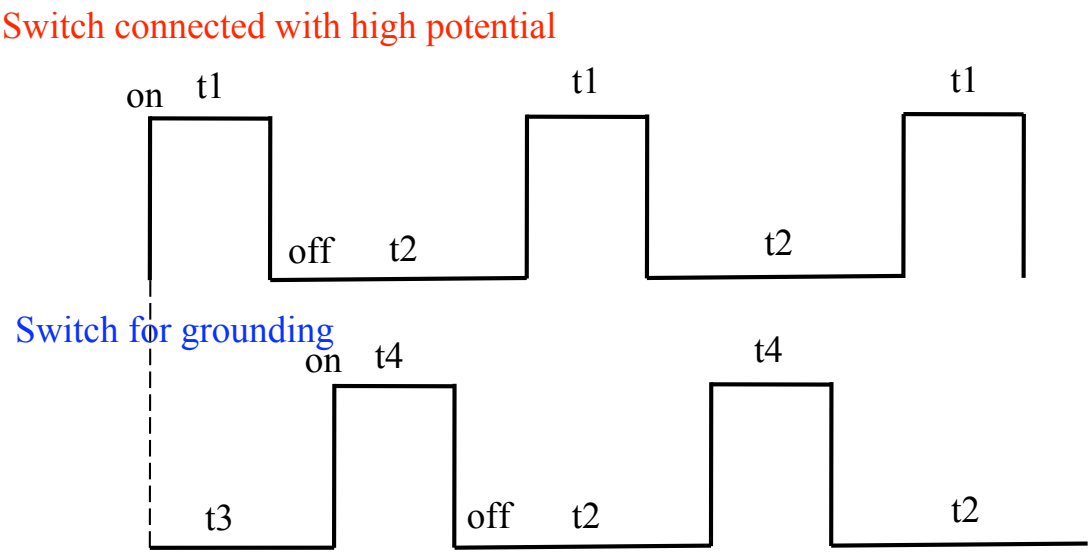


Scheme SI-2.2. Schematic presentation of the spray from droplet on PMMA plate.

In the case of ESTASI from microdroplet deposited on a PMMA plate, the capacitance of the first capacitor can be easily calculated by the parallel plate mode as ~10 fF, where the surface area for electrode is π/4 mm^2, the thickness of PMMA plate is 2 mm. The charges are separated in the droplet with negative charge at the bottom and positive charge on the top, scheme SI-2.2. It is hard to know the radius of the positively charged part on the droplet, which should be very small in principle to keep a largest distance from the electrode and a smallest distance to the MS inlet. Since the UC_1 is ~60 pC, the positively charged droplet should be with a radius smaller than 340 μm to be sprayed, where the surface tension is 0.07201 N/m for pure water.

Of course, decreasing the thickness of the PMMA plate can benefit the ESTASI. Indeed, we have also tried PMMA plate with thickness of 1 mm and 0.5 mm, where ESTASI was easily realized.

SI-3: LabView programme for controlling the performance of switches



Scheme SI-3. Schematic representation of the performance of the two switches controlled by a LabView programme.

SI-4: Limit of detection of native ESI with a microchip emitter or with the commercially equipped ESI source

The limit of detection (LOD) was characterized by looking for a certain amount of sample that could generate a peak on the mass spectrum with signal-to-noise ratio (S/N) of 3. Since there is normally a sudden MS signal drop during the decrease of sample concentrations, it is hard to obtain an exact value for the LOD. We decreased or increased the sample concentration by 5 nM each time during the experiments. Therefore, we could determine the LOD in a range of X nM to X+5 nM, when X nM of sample generated a peak on MS but with a S/N smaller than 3 and when X+5nM of sample generated a peak on MS with a S/N larger than 3. It was also possible to obtain the LOD in a range of X−5 nM to X nM, when X nM of sample generated a peak on MS with S/N larger than 3 and when X−5 nM of sample did not generate a peak on MS.

The microchip shown in scheme SI-2.1 was used as emitter for native ESI, where the cross-section of the microchannel is 100 µm × 50 µm. 4kV high voltage was applied on the electrode to induce ESI. A solution of angiotensin I (20 nM in 50% MeOH/49% H_2O/1% acetic acid) or cytochrome C (50 nM in 50% MeOH/49% H_2O/1% acetic acid) was infused into the microchip by a syringe pump at a flow rate of 20 µl/h for ESI MS.

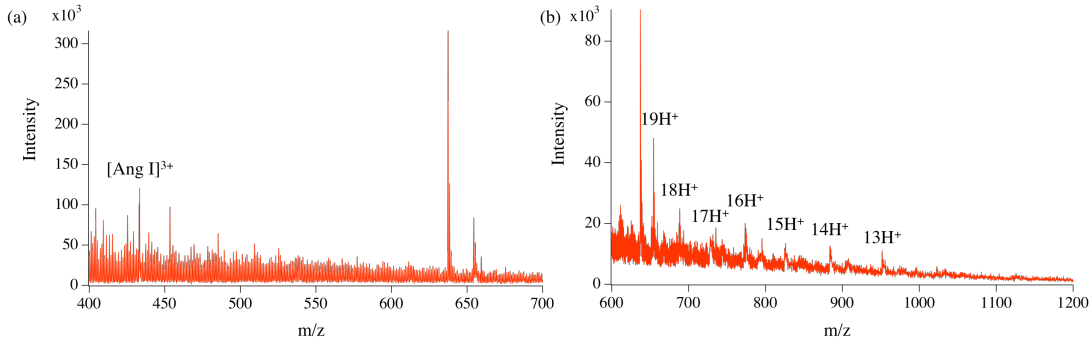


Figure SI-4.1 The mass spectra of angiotensin I ((a) 20 nM) and cytochrome C ((b) 50 nM) under positive MS mode. Samples were all prepared in a buffer of 50% MeOH/49% H_2O/1% acetic acid. The ions were generated by native ESI with a microfluidic chip as emitter.

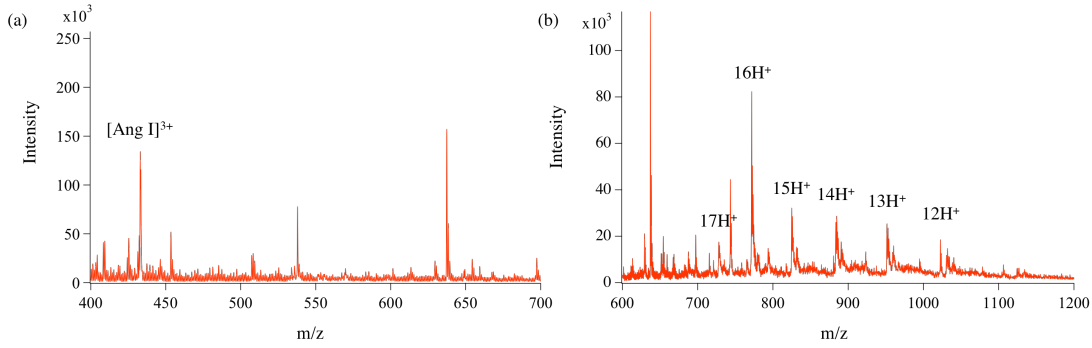


Figure SI-4.2 The mass spectra of angiotensin I ((a) 50 nM) and cytochrome C ((b) 70 nM) under positive MS mode. Samples were all prepared in a buffer of 50% MeOH/49% H_2O/1% acetic acid. The ions were generated by native ESI with the standard commercial ionization source from Thermo Scientific. The ionization was performed under optimized condition: 3 µl/min sample infusing flow rate, 3.7 kV for ESI and 5 psi of nitrogen sheath gas pressure.

SI-5: ESTASI MS and native ESI MS analysis of large proteins.

Large proteins of bovine serum albumin (BSA) and bovine lactoferrin were analyzed by both ESTASI MS and native ESI MS. The ESTASI MS was performed with the microchip in scheme 1(a) under a high voltage of 6 kV. Pulse sequence parameters were set to: t_1=t_4=0.05 s, t_2=0.25 s and t_3=0.15 s. A solution of BSA (15 µM in 50% MeOH/49% H_2O/1% Acetic acid) or bovine lactoferrin (13 µM in 50% MeOH/49% H_2O/1% Acetic acid) was infused into the microchip by a syringe pump at a flow rate of 20 µl/h for ESTASI MS

The native ESI MS was performed with the microchip in scheme SI-2.1 under a high voltage of 4 kV. Proteins with same concentrations were infused into the microchip under a same flow rate for native ESI MS.

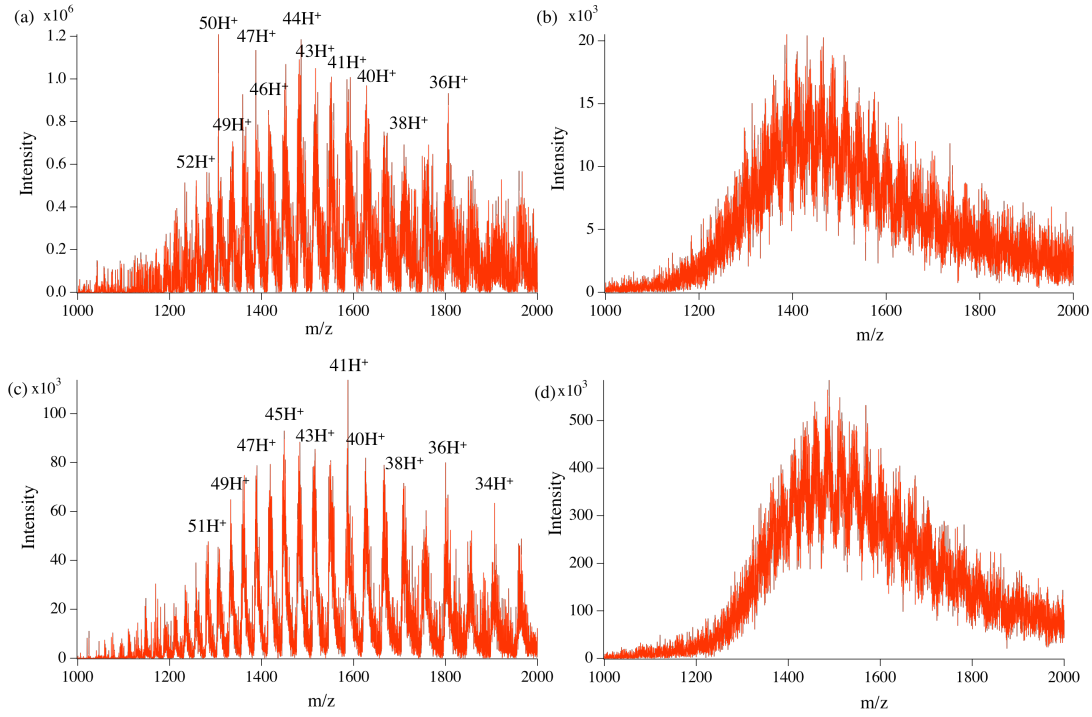


Figure SI-5. (a) BSA and (b) bovine lactoferrin identified by ESTASI MS. (c) BSA and (d) bovine lactoferrin identified by native ESI MS.

SI-6: Detection of anions by MS during ESTASI induced by a positive voltage

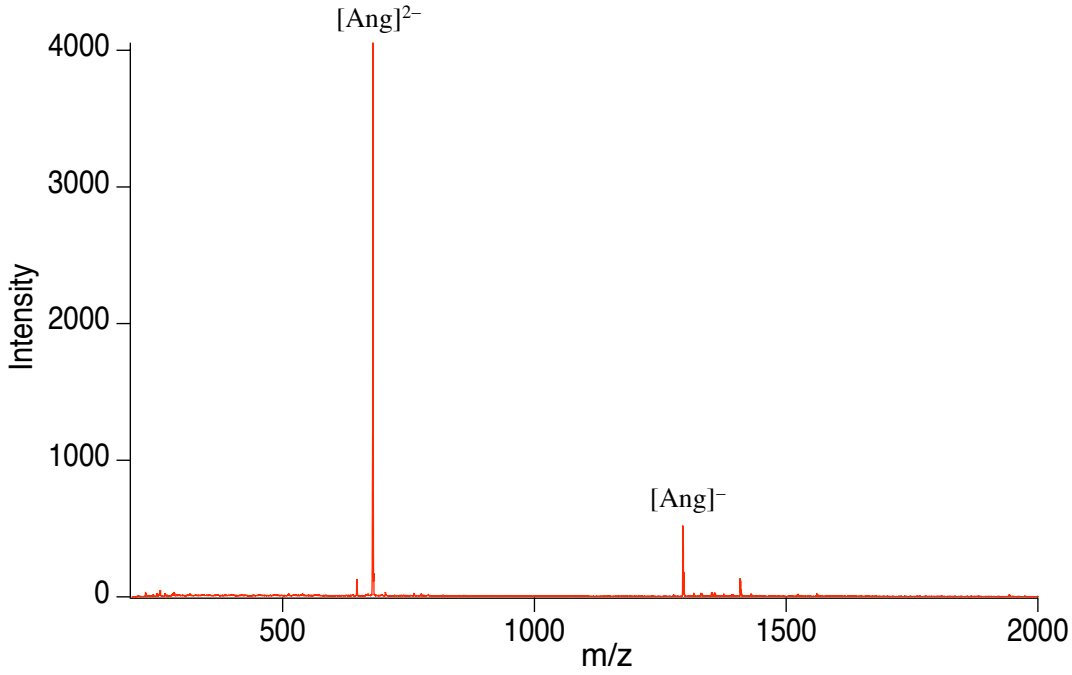


Figure SI-6. The mass spectrum of deprotonated angiotensin I (0.1 mM in 20 mM NH_4HCO_3 50% water 50% methanol) under negative MS mode. The ions were generated by ESTASI with a positive potential (6 kV) applied to the electrode. A silica capillary (50 μm inner diameter) was used as the emitter. The sample solutions were infused into the capillary by a syringe pump at a flow rate of 20 μl/h.

SI-7: Analysis of proteins by ESTASI MS using commercial capillary as emitter

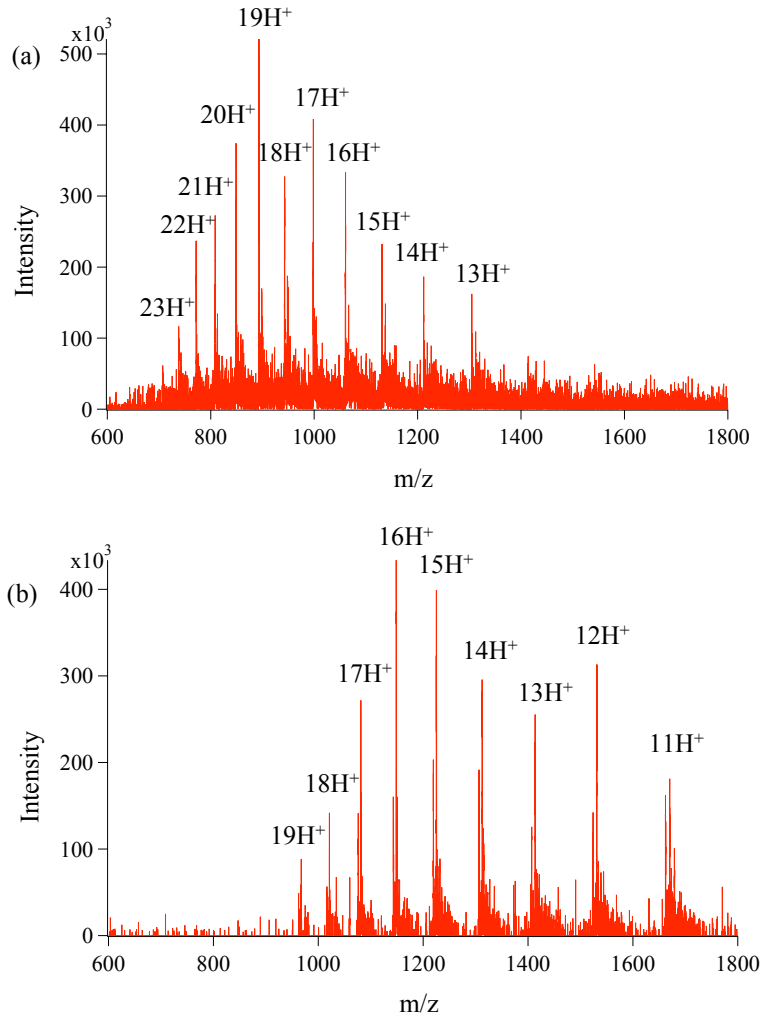


Figure SI-7.1. Mass spectra of (a) myoglobin and (b) beta-lactoglobulin (5 μM in 50% MeOH/49% H_2O/1% acetic acid, respectively) under positive MS mode. The ions were generated by ESTASI with a positive potential (6 kV) applied to the electrode. A silica capillary (50 μm inner diameter) was used as the emitter. The sample solutions were infused into the capillary by a syringe pump at a flow rate of 20 μl/h. Pulse sequence parameters were set to: t_1=t_4=0.05 s, t_2=0.25 s and t_3=0.15 s.

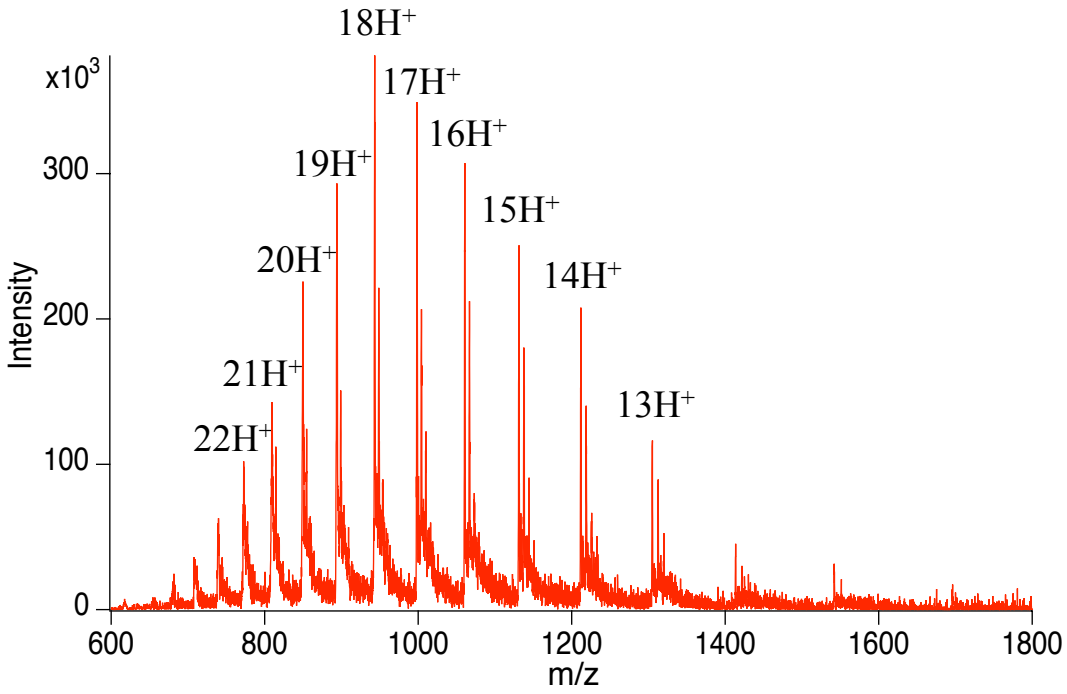


Figure SI-7.2. The mass spectrum of myoglobin (5 μM in 50% MeOH/49% H_2O/1% acetic acid) under positive MS mode. The ions were generated by ESTASI with a negative potential (−6 kV) applied to the electrode. A silica capillary (50 μm inner diameter) was used as the emitter. The sample solutions were infused into the capillary by a syringe pump at a flow rate of 20 μl/h. Pulse sequence parameters were set to: t_1=t_4=0.05 s, t_2=0.25 s and t_3=0.15 s.

SI-8: PMMA substrate patterned with wells as an insulating plate for the ESTASI

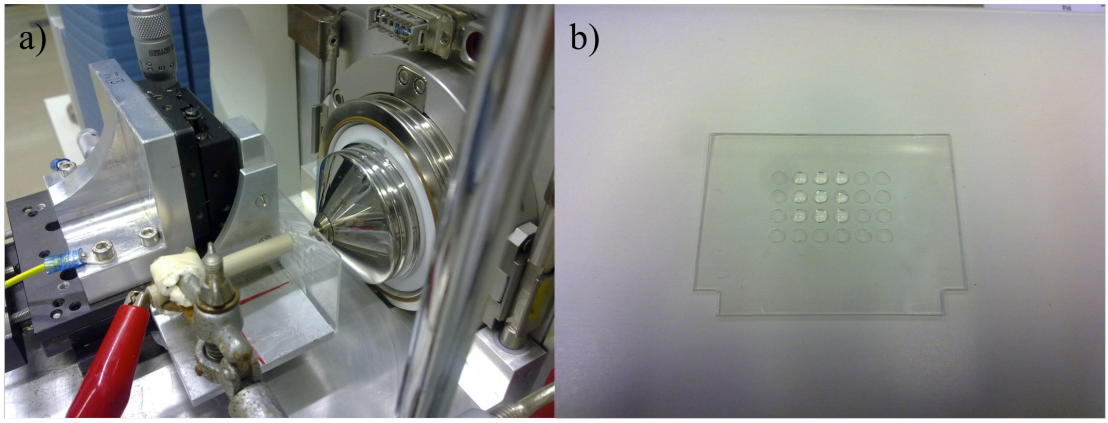


Figure SI-8. a) Droplet spray by ESTASI to produce ions for MS analysis; b) the PMMA substrate patterned with wells and deposited with droplets.

SI-9: ESTASI of myoglobin digest deposited as droplets on the polymer plate

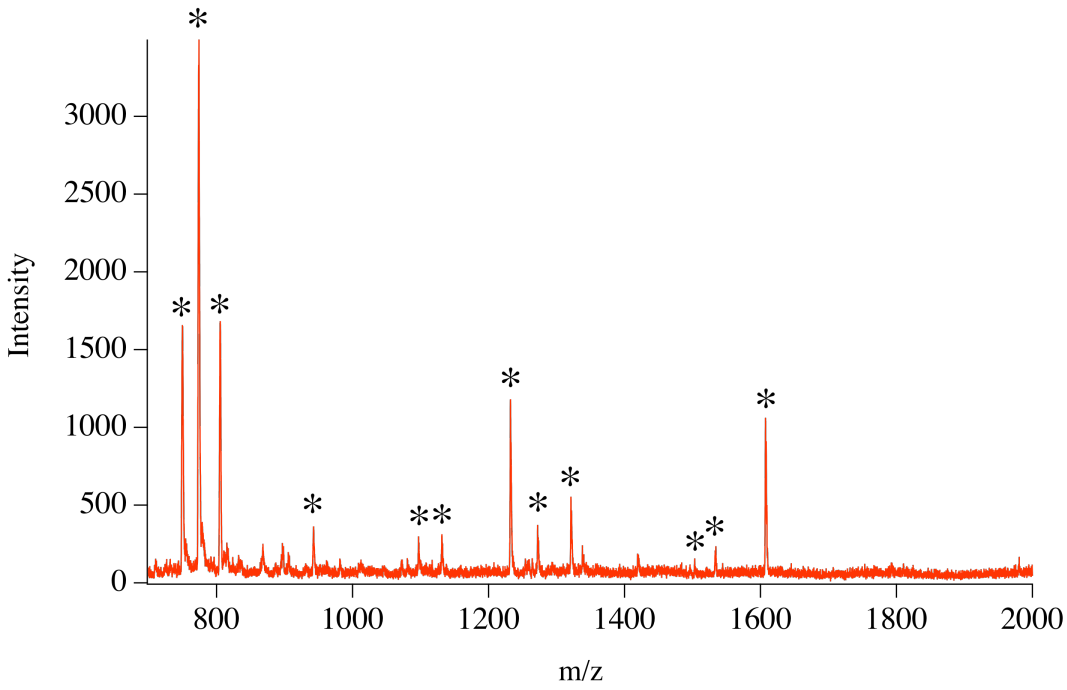


Figure SI-9. Mass spectrum of myoglobin tryptic digest (30 µM in 50% MeOH/49% H_2O/1% acetic acid) under positive MS mode. The ions were generated by ESTASI when a pulsed positive high potential (6 kV) was applied to the electrode and when 1 µl of the sample solution was deposited on an insulating plate. The LabView time sequence parameters were set to: t_1=t_4=0.05 s, t_2=0.25 s and t_3=0.15 s. *: identified peptides from myoglobin digest.

Table SI-9. Peaks for peptides observed on figure SI-9.

m/z	z	Sequences of Peptides
748.4	1	(K)ALELFR(N) [135-140][1, 2]
772.5	1	(L)TALGGILK(K) [71-78][3]
804.0	2	(K)VEADIAGHGQEVLIR(L) [18-32]
941.5	1	(K)YKELGFQG(-) [147-154][4]
1096.1	2	(L)NVWGKVEADIAGHGQEVLIR(L) [13-32]
1130.6	1	(K)GHHEAELKPL(A) [81-90]
1231.6	1	GLSDGEWQQVL(N) [2-12]
1271.7	1	(R)LFTGHPETLEK(F) [33-43]
1320.7	1	(K)YLEFISDAIIH(V) [104-114]
1502.7	1	(K)HPGDFGADAQGAMTK(A) [120-134]
1532.8	1	(K)YLEFISDAIIHVL(H) [104-116]
1606.9	1	(K)VEADIAGHGQEVLIR(L) [18-32]

1. The sequence information includes the amino acid sequences of identified peptides, the amino acids before and after the sequences in parentheses, and the positions of the peptides in myoglobin in square brackets.

2. The peptides in black color are resulted from specific cleavage of myoglobin by trypsin, and are identified by comparing the observed molecular weights with the theoretical molecular weights with the help of FindPept tool on the server of ExPASy (http://web.expasy.org/findpept/).

3. The peptides in red color are generated by semi-specific cleavage of myoglobin by trypsin, where only one terminal is cleaved besides lysine or arginine. These sequences were identified by ESI tandem MS using collision induced dissociation with the help of FindPept tool on the server of ExPASy (http://web.expasy.org/findpept/).

4. (-) means the peptides is generated from a terminal of the protein.

SI-10: CE ESTASI MS and CE MALDI MS

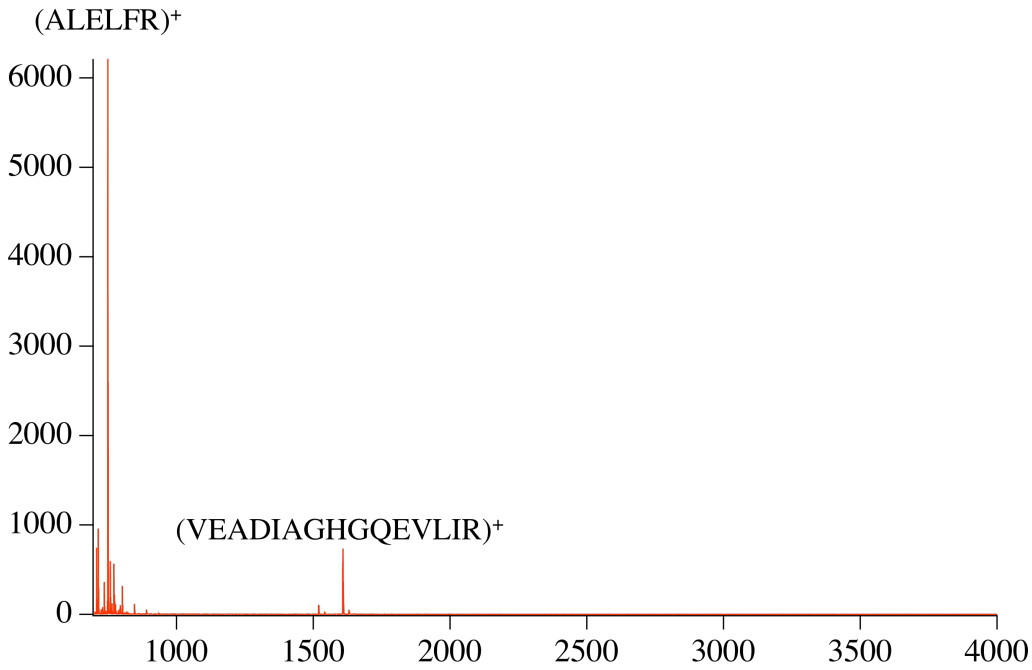


Figure SI-10.1. MALDI MS of fraction 9.

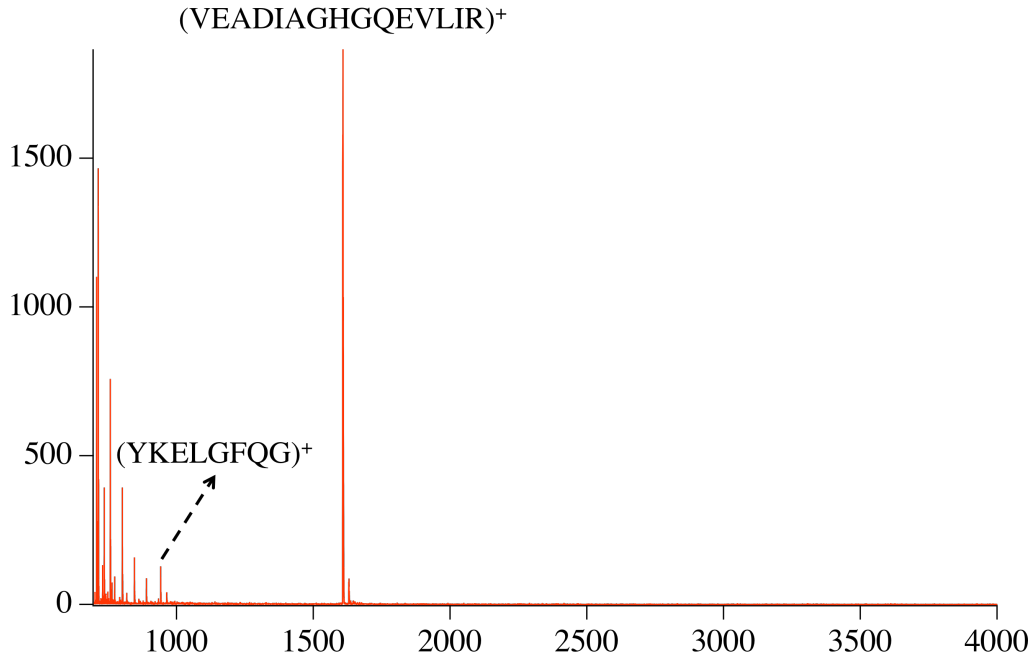


Figure SI-10.2. MALDI MS of fraction 10.

Table SI-10. Comparison of peptides observed by ESTASI MS and MALDI MS from the CE fractions.

	Peptides observed	
Spot N°	**by MALDI-MS**	**by ESTASI MS**
1	×	×
2	(K)KGHHEAELKPL(A) [80-90][1, 3]; (K)HKIPIK(Y) [98-103][2]	(K)KGHHEAELKPL(A) [80-90]; (K)HKIPIK(Y) [98-103]
3	(K)GHHEAELKPL(A) [81-90]; (K)TEAEMK(A) [52-57]	(K)GHHEAELKPL(A) [81-90]; (K)TEAEMK(A) [52-57]
4	(K)GHHEAELKPL(A) [81-90];	(R)NDIAAKYKELGFQG(-) [141-154][4]
5	(K)ASEDLKK(H) [58-64]	(K)ASEDLKK(H) [58-64]
6	×	×
7	(R)LFTGHPETLEK(F) [33-43]	(R)LFTGHPETLEK(F) [33-43]
8	(R)NDIAAK(Y) [141-146]	×
9	(K)ALELFR(N) [135-140]; (K)VEADIAGHGQEVLIR(L) [18-32]	(K)ALELFR(N) [135-140]
10	(K)VEADIAGHGQEVLIR(L) [18-32]; (K)YKELGFQG(-) [147-154]	(K)VEADIAGHGQEVLIR(L) [18-32]
11	(K)YKELGFQG(-) [147-154]	(K)YKELGFQG(-) [147-154]
12	×	(K)ASEDLK(K) [58-63]; (K)FDKFK(H) [44-48]
13	(K)ASEDLK(K) [58-63]; (K)FDKFK(H) [44-48]	(K)ALELFRNDIAAK(Y) [135-146]
14	(K)YLEFISDAIIH(V) [104-114] (K)ALELFRNDIAAK(Y) [135-146]	×
15	(K)YLEFISDAIIHVL(H) [104-116]	(K)YLEFISDAIIHVL(H) [104-116]
16	×	×
17	×	(K)TEAEMKASEDLKK(H) [52-64]
18	×	×

1. The sequence information includes the amino acid sequences of identified peptides, the amino acids before and after the sequences in parentheses, and the positions of the peptides in myoglobin in square brackets.

2. The peptides in black color are resulted from specific cleavage of myoglobin by trypsin, and are identified by comparing the observed molecular weights with the theoretical molecular weights with the help of FindPept tool on the server of ExPASy (http://web.expasy.org/findpept/).

3. The peptides in red color are generated by semi-specific cleavage of myoglobin by trypsin, where only one terminal is cleaved besides lysine or arginine. These

sequences were identified by ESI tandem MS using collision induced dissociation with the help of FindPept tool on the server of ExPASy (http://web.expasy.org/findpept/).

4. (-) means the peptides is generated from a terminal of the protein.

SI-11: Native ESI MS

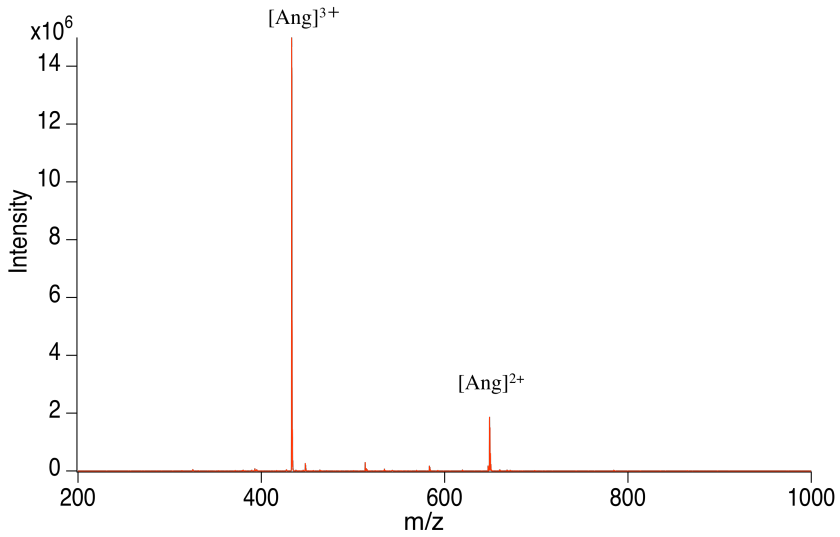


Figure SI-11 (a). Mass spectrum of Angiotensin I (0.1 mM in 49% water 50% methanol and 1% acetic acid) detected by microchip ESI MS. The microchip shown in scheme SI-2.1 was used as emitter. The flow rate was 20 µl/h. A high voltage of 4 kV was used to induce the ESI.

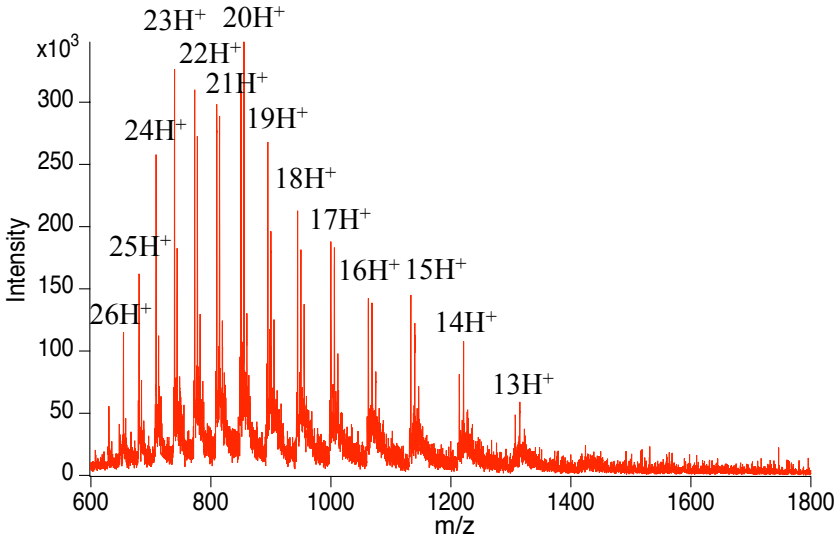


Figure SI-11 (b). Mass spectrum of myoglobin (5 µM in 49% water 50% methanol and 1% acetic acid) detected by capillary ESI MS. A silica capillary with 50 µm inner diameter and 360 µm outside diameter coated with a thin layer of silver ink at the tip was used as the emitter. The flow rate was 20 µl/h. A high voltage of 4 kV was used to induce the ESI.