Supporting information

Tuning oxidative modification by strong electric field using nanoESI of highly conductive solutions near minimum flow rate

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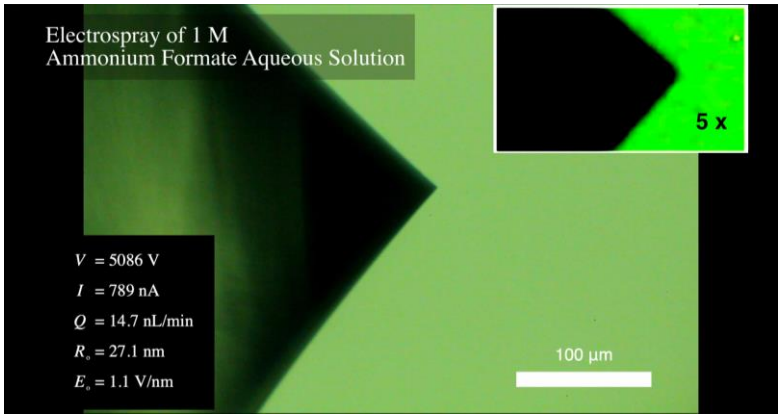
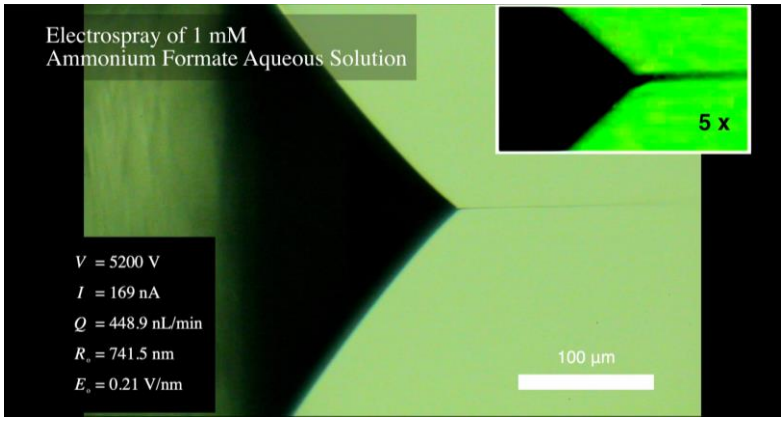
Screenshots and captions for movies



Movie 1. Electrospray of 1 M ammonium formate aqueous solution using micropipette tip (i.d. 0.4mm) under high-pressure (0.5 MPa gauge pressure) condition. The upper right inset shows the magnified (5×) image of the apex of the Taylor cone. The contrast and brightness are adjusted to increase the sharpness.
V: emitter voltage, I: measured spray current, Q: solution flow rate, R_o: droplet radius, E_o: electric field. Q, R_o, & E_o are calculated using Equations 1-3. Electric conductivity of the solution K = 8.67 S/m.



Movie 2. Electrospray of 1 mM ammonium formate aqueous solution. Electric conductivity of the solution K = 0.013 S/m.

Figure S1: Typical high-pressure nanoESI MS

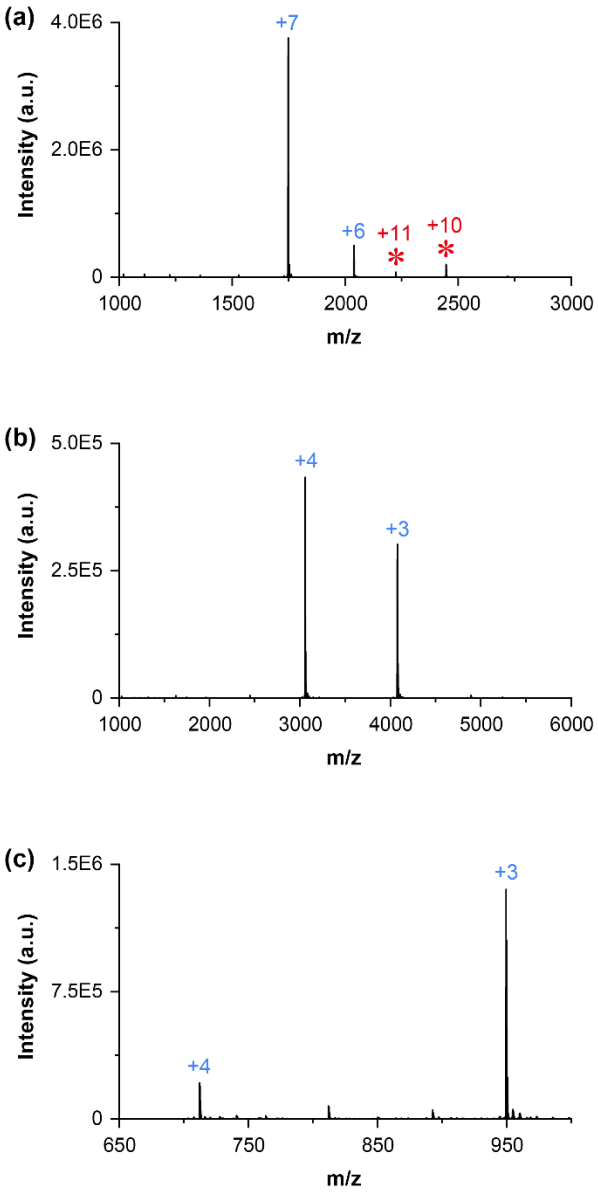


Figure S1. Typical high-pressure nanoESI mass spectra of protein and peptide acquired under nonoxidizing conditions. **a)** Cytochrome *c* (10 μM) in 100 mM ammonium formate. Asterisk * denotes the dimer. **b)** Cytochrome *c* in 100 mM tetraethylammonium bicarbonate. **c)** 10 μM melittin in 100 mM ammonium formate.

Figure S2: Cytochrome c in 1 M ammonium formate

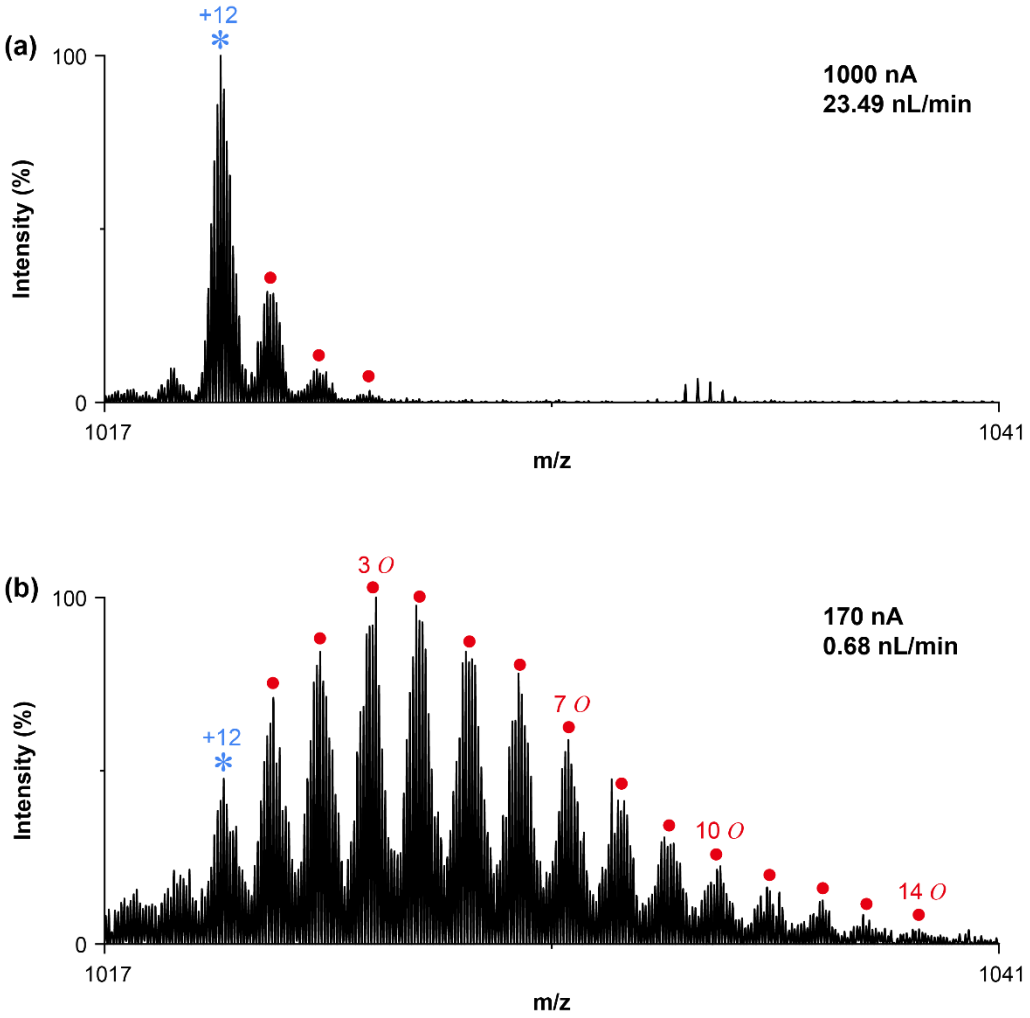


Figure S2. Mass spectra of cytochrome *c* in 1 M ammonium formate acquired at different spray currents and flow rates. **a)** Spray current: 1000 nA, flow rate: 23.5 nL/min. **b)** Spray current: 200 nA, flow rate: 0.9 nL/min. Red solid circle denotes the oxidized peak (M + n*O*). Asterisk denotes the unoxidized peak.

Figure S3: Ubiquitin in 1 M ammonium formate

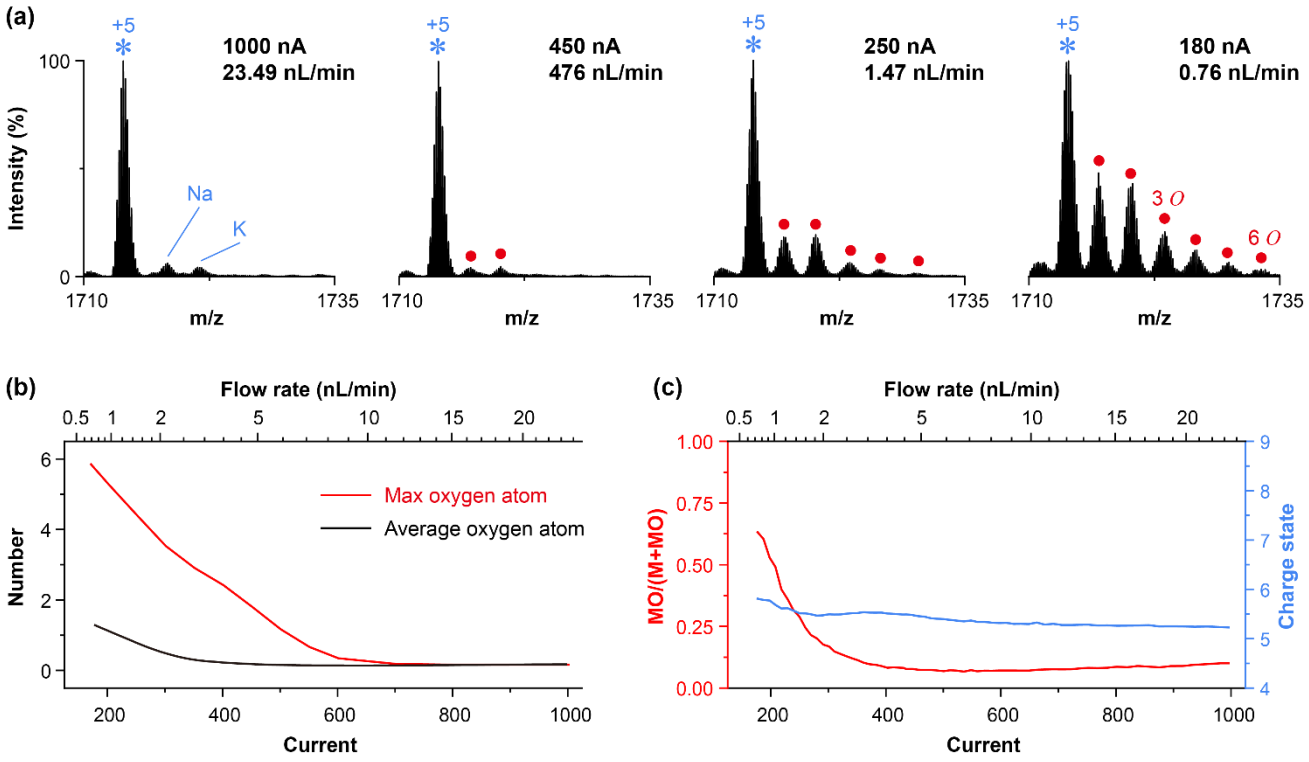


Figure S3. a) Mass spectra of ubiquitin (25 μM) in 1 M ammonium formate aqueous solution acquired at different spray currents. Red solid circle denotes the oxidized peak. Asterisk denotes the unoxidized peak. **b)** Maximum (red) and the calculated average (black) number of oxygen atoms versus spray current. **c)** Ratio of total oxidized ion species to the total ion signal (red) and the average charge state (blue) versus spray current.

Figure S4: Heatmaps of cytochrome c and ubiquitin

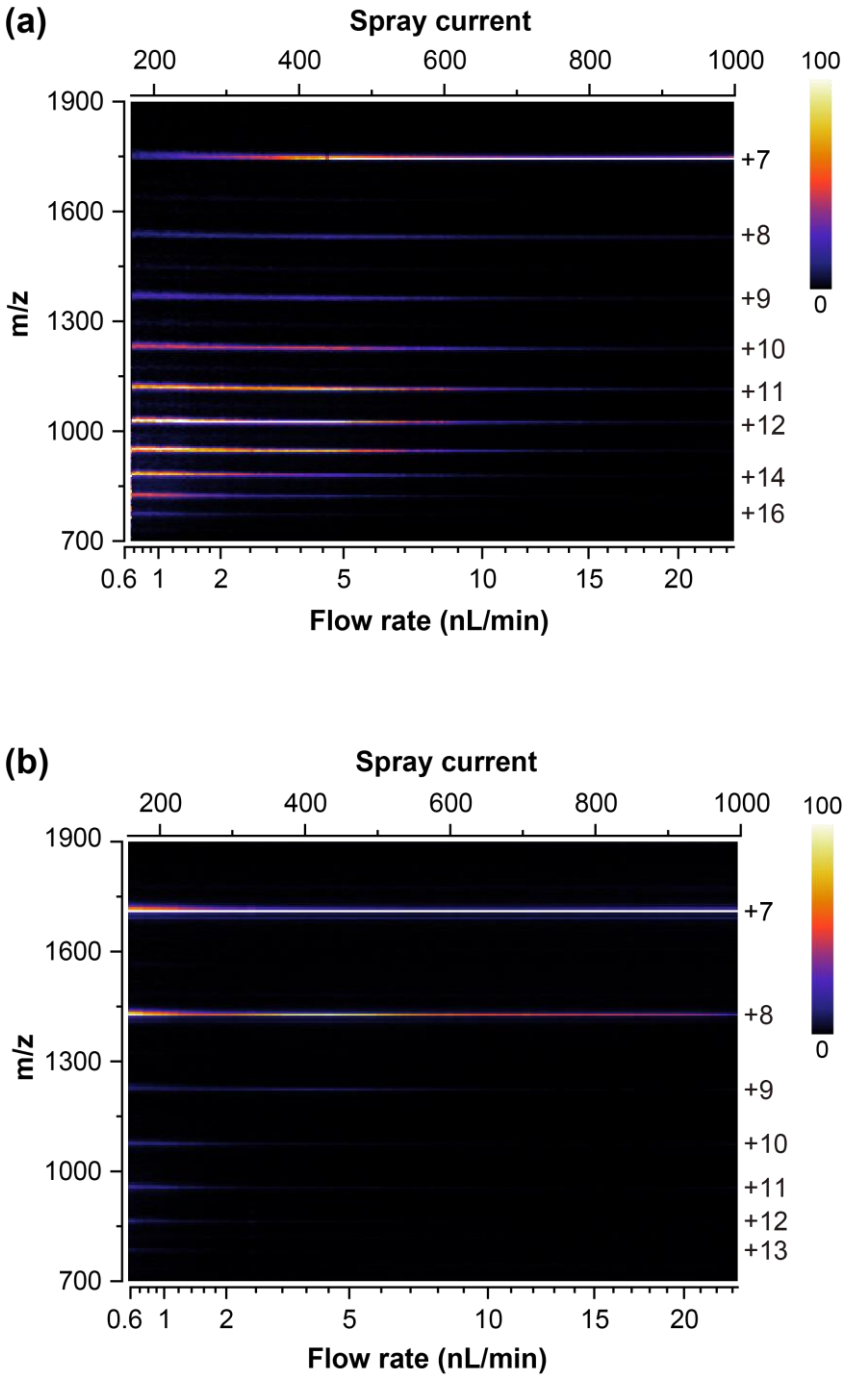


Figure S4. Heatmaps showing the effect of flow rate on the charged state of a) cytochrome *c* (25 μM) and b) ubiquitin (25 μM) in 1 M ammonium formate aqueous solution.

Table S1: Amino acid sequence

Apo-cytochrome *c* (CC) 11572.35

GDVEKGKKIFVQKCAQCHTVEKGGKHKTGPNLHGLFGRKTGQAPGFSYTDANKNKGITW
GEETLMEYLENPKKYIPGTKMIFAGIKKKGEREDLIAYLKKATNE

Ubiquitin (UB) 8564.84
MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKE
STLHLVLRLRGG

Melittin (MLT) 2847.5
GIGAVLKVLTTGLPALISWIKRKRQQ

Angiotensin (AG) 1296.49
DRVYIHPFHL

	Cys (C)	Met (M)	Trp (W)	Tyr (Y)	His (H)	Phe (F)	Total
CC	2	2	1	4	3	4	16
UB	0	1	0	1	1	2	5
MLT	0	0	1	0	0	0	1
AG	0	0	0	1	2	1	4

Table S1. Amino acid sequences of apo-cytochrome c, ubiquitin, melittin, and angiotensin. The amino acids whose R group is easily oxidized are marked in red.

Figure S5: Oxidation using ESI plus corona discharge under atmospheric pressure

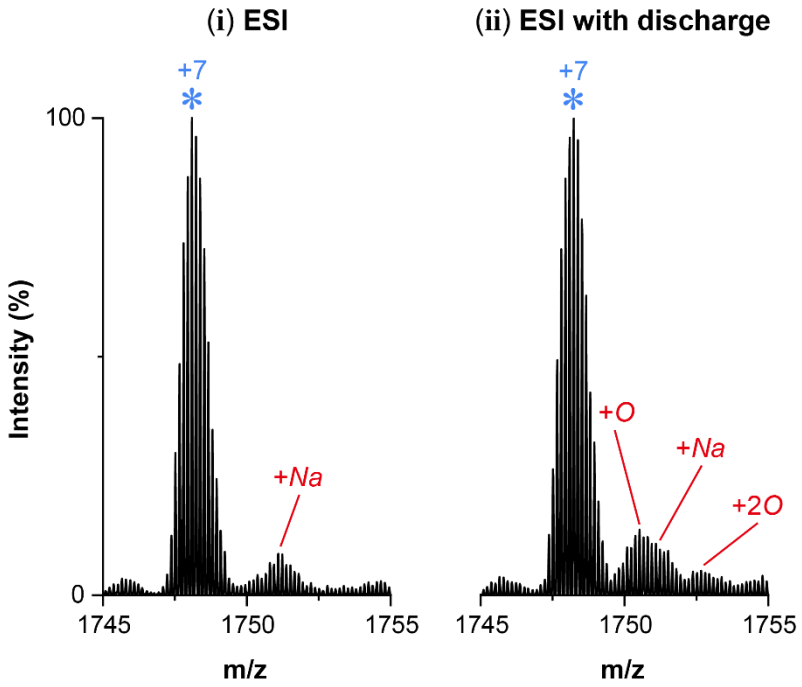


Figure S5. Mass spectra of cytochrome *c* (10 μM) in 100 mM ammonium formate in 50% ethanol. **(i)** Standard atmospheric pressure ESI. **(ii)** Atmospheric pressure ESI with a higher voltage to induce an electric discharge with a current of 8 μA. The solution flow rate is 0.5 μL/min.

Figure S6: Measurement using in-emitter electrochemical reactor

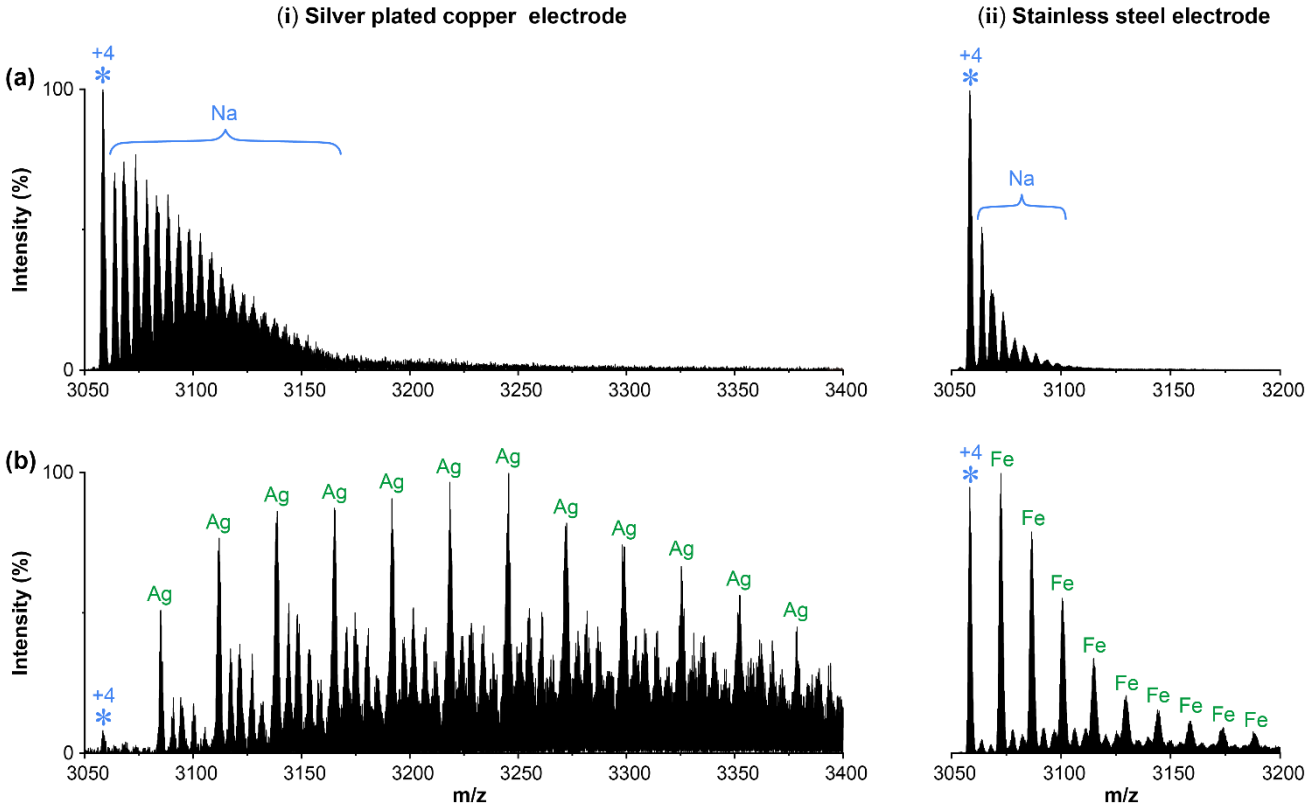


Figure S6. Mass spectra of cytochrome *c* in 100 mM tetraethylammonium bicarbonate acquired by nanoESI with an in-emitter electrochemical (EC) reactor. The oxidation electrodes are (i) Silver-plated copper and (ii) stainless steel. (a) and (b) are the mass spectra acquired at 0 min and 20 min after the turn ON of the EC reactor.

Figure S7: Oxidation of melittin in $H_2{}^{18}O$

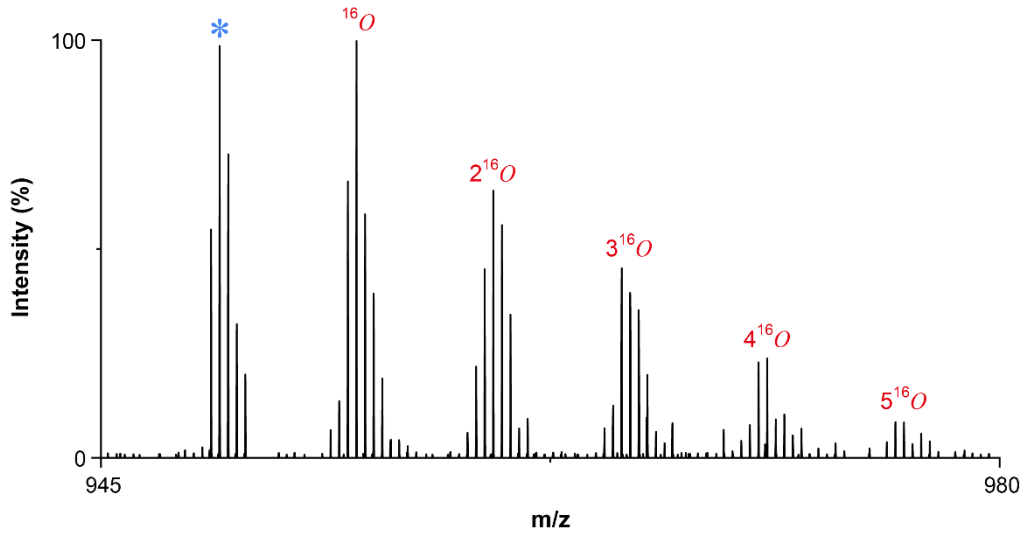


Figure S7. Mass spectrum of melittin oxidized by strong electric field-induced oxidation. The sample solution is prepared using ammonium acetate (~1.5 M) in water-^{18}O. Asterisk denotes the unoxidized peak.

Figure S8: Detection of H_2O_2 using ADHP

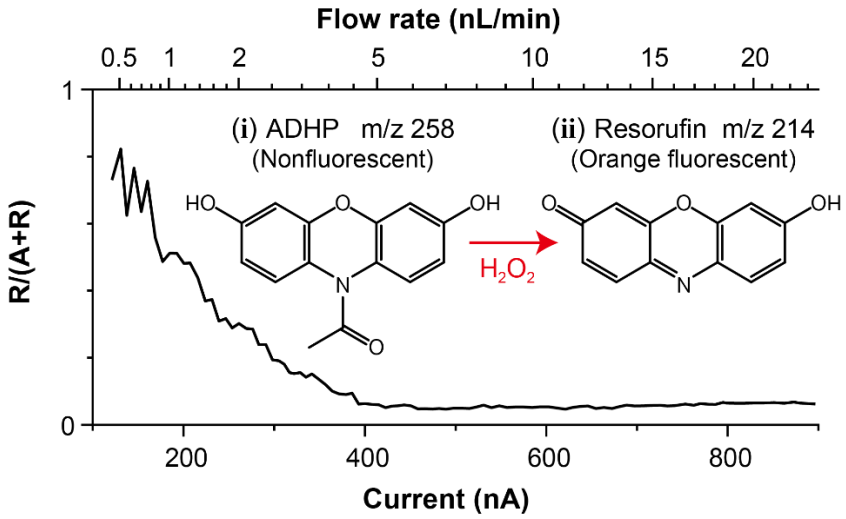


Figure S8. Detection of hydrogen peroxide H_2O_2 using ADHP.

The hydroxyl radical can form hydrogen peroxide H_2O_2 in solution. The detection of H_2O_2 in the solution is performed using 10-Acetyl-3,7-dihydroxyphenoxazine (ADHP, Amplex Red) which is a widely used fluorescent probe molecule for H_2O_2. ADHP converts to resorufin by H_2O_2. Because the electric field-induced oxidation takes took place only within a small volume of the order of $(10\ nm)^3$, the direct fluorescent measurement at the Taylor cone tip is difficult. Nevertheless, resorufin and the ADHP are readily detected as protonated species by the present nanoESI-MS. Figure S8 shows the plot of the ratio of resorufin intensity to the intensity of (ADHP + resorufin) versus the spray current. The abundance of H_2O_2 is the highest at the strongest electric field at the lowest spray current and flow rate.

Figure S9: Oxidation of ubiquitin in oxygen

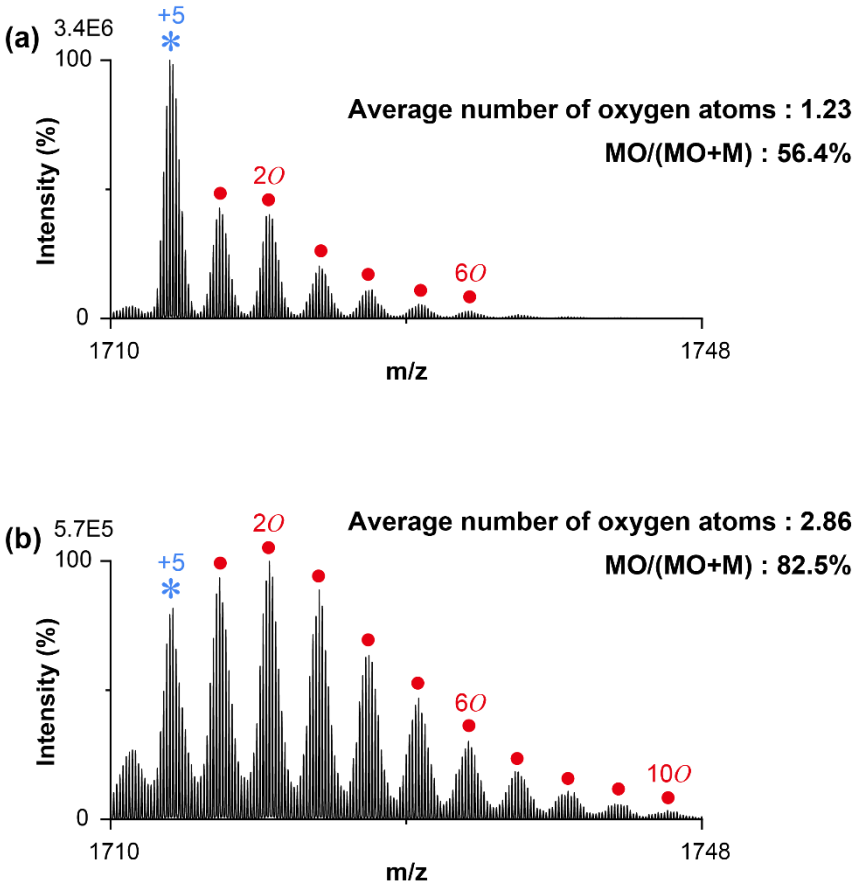


Figure S9: Oxidation of ubiquitin using air and oxygen as operating gases.

a) 0.5 MPa dry air (default in this study).

b) 99.5 % oxygen at 0.3 MPa.

The values show the average number of the incorporated oxygen atoms and the percentage of oxidation for charge state +5. The spray current is 200 nA. Ubiquitin concentration: 20 μM. The solvent is 1 M ammonium acetate aqueous solution. Red solid circle denotes the oxidized peak. Asterisk denotes the unoxidized peak.

Figure S10: Oxidation of PC in oxygen

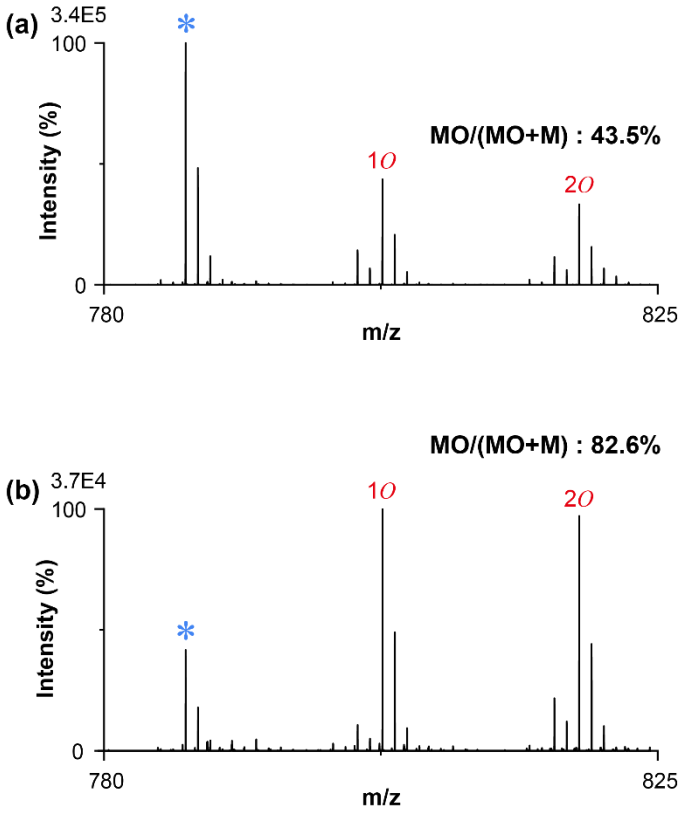


Figure S10. Oxidation of L-phosphatidylcholine (PC 18:1/18:1) using air and oxygen as operating gases.

a) 0.5 MPa dry air (default in this study). Percentage of oxidation = 43.5 %

b) 99.5 % oxygen at 0.3 MPa. Percentage of oxidation = 82.6 %

Asterisk denotes the unoxidized peak. MO is the total intensity of all oxidized species. M is the intensity of the unoxidized species. The spray current is ~250 nA in both cases. PC concentration: 5 μM. The solvent is 500 mM HCl in water:acetonitrile 9:1. K = 14.7 S/m.

Figure S11: Oxidation using glass capillary with 5 μm i.d.

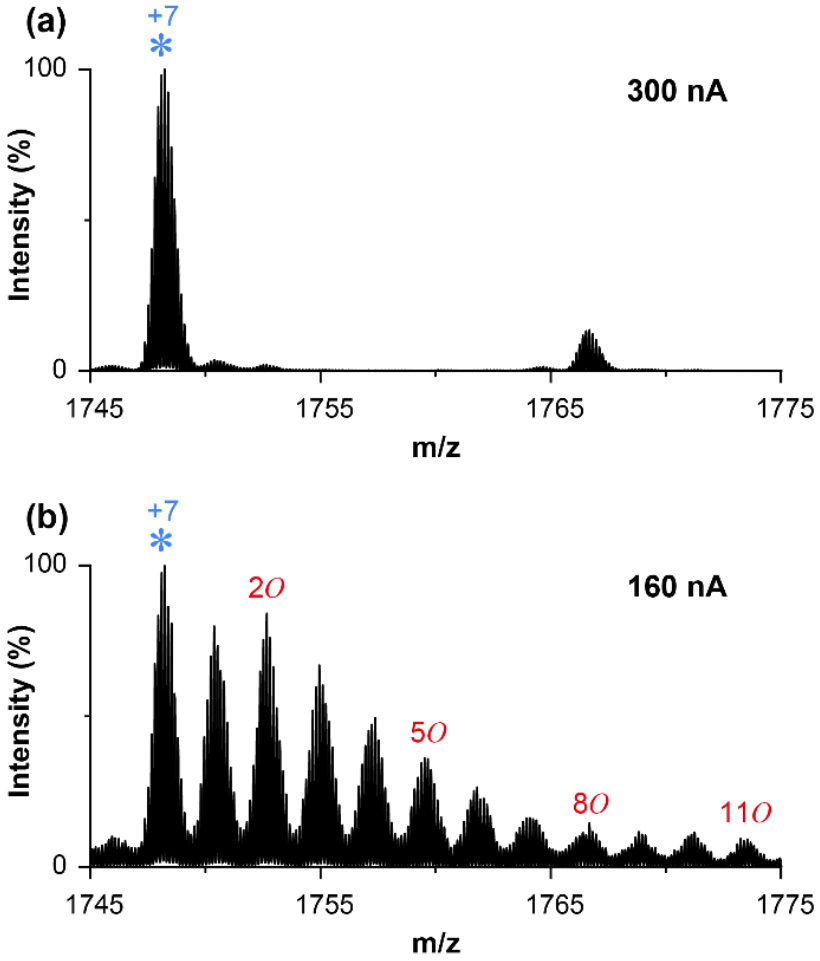


Figure S11: Oxidation of cytochrome *c* using pulled-glass capillary with i.d. of 5 μm.

a) Mass spectrum acquired at 300 nA spray current. Non-oxidizing condition.

b) Mass spectrum acquired at 160 nA. Oxidizing condition.

Sample: 20 μM cytochrome c in 1 M ammonium formate aqueous solution.

Operating gas: air at 0.5 MPa. Asterisk denotes the unoxidized peak.