Supplemental Information for:

Effect of Droplet Lifetime on Where Ions are Formed in Electrospray Ionization

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Phone: (510) 643-7161 e-mail: <u>erw@berkeley.edu</u> **Table S-1.** Summary of droplet lifetimes obtained from acidified water containing cytochrome c and aqueous buffered solutions with theta emitters that have tip diameters ranging in size from 0.24 µm to 4.4 µm using cytochrome c folding constant.^{1,2} Nitrogen gas backing pressure used to change the solution flow rate and resulting droplet lifetime is either 10 psi or 40 psi.

tip size (µm)	Backing pressure (psi)	Droplet lifetime (µs)	Solution
0.24	10	1 ± 0	100 mM AA
0.3	10	2.8 +/- 0.6	100 mM AA
1.5	10	9 +/- 2	100 mM AA
1.5	40	20 +/-3	100 mM AA
1.4	10	25 +/- 7	water
1.4	10	7 +/- 2	500 mM AA
1.7	40	38 +/- 2	water
4.4	10	99 +/-14	water



Figure S-1. Electrospray ionization mass spectra obtained from solutions consisting of 10 μM βlactoglobulin in 100 mM ABC (a-c) and in 100 mM AA (d-f) using 1.7 μm theta tip emitters at source temperatures of 160 °C (a, d), 230 °C (b, e), and 300 °C (c, f). Peaks at slightly higher m/zcorresponds to lactosyl covalently bound to β-lactoglobulin.³



Figure S-2. Electrospray ionization mass spectra obtained from solutions consisting of 10 μM βlactoglobulin in 100 mM ABC with 3% ammonium hydroxide using 300 nm and 4.4 μm theta tip emitters at source temperatures of 160 °C (a, c) and 300 °C (b, d). The lifetime of the droplets formed from 317 nm and 4.4 μm theta emitter is approximately 1 μs and 50 μs, respectively. Peaks at slightly higher *m/z* corresponds to lactosyl covalently bound to β-lactoglobulin.³



Figure S-3. Electrospray ionization mass spectra obtained from solutions consisting of 10 μ M β lactoglobulin in 10 mM AA using 300 nm (a-c), 1.7 μ m (d-f), and 4.4 μ m (g-i) theta tip emitters at source temperatures of 160 °C (a, d, g), 230 °C (b, e, h), and 300 °C (c, f, i). The lifetime of the droplets formed from 317 nm, 1.7 μ m, and 4.4 μ m theta emitter is approximately 1 μ s, 10 μ s and 50 μ s, respectively. Peaks at slightly higher *m/z* corresponds to lactosyl covalently bound to β -lactoglobulin.³



Figure S-4. Example tryptophan fluorescent emission results of β -lactoglobulin measured from 200 nm to 390 nm wavelength at temperatures between 25 °C and 87.5 °C measured in 2.5 °C increments.



Figure S-5. Electrospray ionization mass spectra obtained with electrothermal supercharging of 10 μ M β -lactoglobulin in 100 mM ABC (a-c) and 100 mM AA (d-f) at a spray voltage of 0.8 kV using 1.7 μ m single barrel emitters at source temperature 160 °C (a, d), 230 °C (b, e), and 300 °C (c, f). Peaks at slightly higher *m*/*z* corresponds to lactosyl covalently bound to β -lactoglobulin.³ * indicates polydimethylsiloxane clusters that are present as an impurity.



Figure S-6. Electrospray ionization mass spectra obtained from solutions consisting of 10 μ M β lactoglobulin in 100 mM ABC using 1.7 μ m theta tip emitters at source temperatures of 300 °C with spray voltage of 0.8 kV (a) and 1.3 kV (b). Peaks at slightly higher *m/z* corresponds to lactosyl covalently bound to β -lactoglobulin.³ * indicates polydimethylsiloxane clusters that are present as an impurity.

Reference

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