Supporting Information

Ultrafast (1 μs) Mixing and Fast Protein Folding in Nanodrops Monitored by Mass Spectrometry

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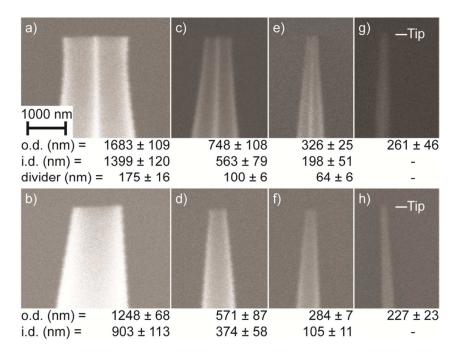
a)

1000 nm

c)

e)

g) —Tip

o.d. (nm) =	1683 ± 109	748 ± 108	326 ± 25	261 ± 46
i.d. (nm) =	1399 ± 120	563 ± 79	198 ± 51	-
divider (nm) =	175 ± 16	100 ± 6	64 ± 6	-

b)

d)

f)

h) —Tip

| o.d. (nm) = | 1248 ± 68 | 571 ± 87 | 284 ± 7 | 227 ± 23 |
| i.d. (nm) = | 903 ± 113 | 374 ± 58 | 105 ± 11 | - |

Fig. S1. Electron micrographs of the tips of the theta-glass emitters with average o.d.s of (a,b) 1465 ± 134, (c,d) 659 ± 135, (e,f) 305 ± 32, and (g,h) 244 ± 61 nm. The inner divider is perpendicular to and parallel to the sample stand in the upper and lower panels, respectively. White lines were added to (g) and (h) to indicate where the tips end. For the ~244 nm o.d. tips, the i.d. and divider width are not resolved.

Mixing Efficiencies

To determine if complete mixing occurs between solutions loaded into the different barrels of the theta-glass emitters, a rapid equilibration complexation reaction is performed. If incomplete mixing occurs between the solutions loaded into either barrel of the theta-glass emitters, only a portion of the reagents in either solution will interact and the product to reagent ratio in the resulting mass spectra will be less than in that of a premixed solution that is in equilibrium. 18-crown-6 (18C6) and K^+ form the complex $[18C6 + K]^+$ with a forward rate constant of 2.45 × 10^9 mol s^{-1} (average of values measured by others).[1] Protonated 18C6 is not observed in the mass spectra, so the product to reagent ratio cannot be directly observed. Therefore, NaCl is added to the 18C6 solution to form the complex $[18C6 + Na]^+$.

Aqueous solutions containing (A) 50 µM 18C6 and 50 µM NaCl and (B) 5 µM KCl are mixed both at a 1:1 ratio prior to nanoESI (equilibrium) and using the theta-glass emitters with flow rates between 48 and 2878 pL/s (rapid mixing). These flow rates are obtained using various tip sizes and backing pressures. A mass spectrum of these solutions mixed prior to nanoESI, acquired at 913 pL/s, is shown in Figure S2a. $[18C6 + Na]^+$ and $[18C6 + K]^+$ are both present at a ratio of 1 to 1.6 ± 0.1, respectively. The ratio, $[18C6 + K]^+/[18C6 + Na]^+$, for both the equilibrium and rapid mixing experiment are shown in Figure S2b as a function of flow rate. These data were corrected for the relative abundance of $[18C6 + K]^+$ in the mass spectra of solution A at each flow rate. The ratios of these complexes are the same to within error between the equilibrium and rapid mixing experiments at each flow rate. This result indicates that this reaction reaches equilibrium during nanoESI and that complete mixing occurs between the solutions. The ratio of the complexes in these experiments has a relatively constant value of ~1.3 at each flow rate obtained using the ~1465 nm o.d. tips, a slightly higher value of ~1.6 when the ~659 nm o.d. tips are used, and significantly higher values of ~2.1 and ~1.9 when the ~305 and

~244 nm o.d. tips are used, respectively. The diminished tip diameter of the smaller tips results in an increased tip surface area relative to the solution volume in these experiments, and this could result in more K^+ being released from the glass, resulting in an increased ratio of $[18C6 + K]^+$ to $[18C6 + Na]^+$ in the resulting mass spectra. The change in tip geometry may also result in an increased ionization efficiency of $[18C6 + K]^+$ relative to $[18C6 + Na]^+$ for the smaller tip sizes, resulting in the increased relative abundance of $[18C6 + K]^+$ in the resulting mass spectra.
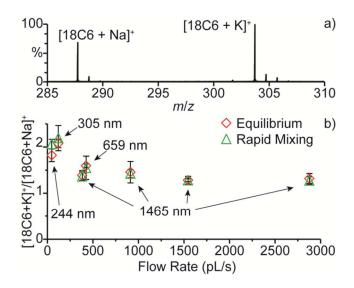

Fig. S2. (a) Mass spectrum of a solution containing Na^+, 18C6, and K^+ at a ratio of 10:10:1. (b) The ratio, $[18C6 + K]^+/[18C6 + Na]^+$, in the mass spectra obtained from the equilibrium (diamonds) and rapid mixing (triangles) experiments as a function of the solution flow rate. The average o.d. of the tips used in these experiments are indicated.

Reference

(1) Izatt, R. M.; Bradshaw, J. S.; Nielsen, S. A.; Lamb, J. D.; Christensen, J. J.; Sen, D. *Chem. Rev.* **1985**, *85*, 271-339.
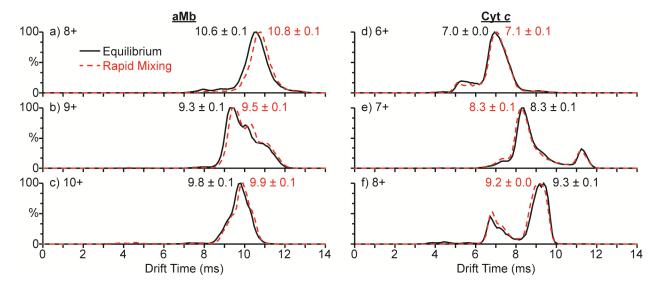
Fig. S3. TWIMS drift profiles of the (a) 8+, (b) 9+, and (c) 10+ charge states resulting from mixing an acidified aqueous aMb solution (pH = 2.9) with a 100 mM aqueous ammonium acetate solution at a 1:1 ratio prior to nanoESI (equilibrium; pH = 4.7) (black lines) and using a theta-glass emitter (dashed red lines). TWIMS drift profiles of the (d) 6+, (e) 7+, and (f) 8+ ions resulting from mixing an acidified aqueous cyt *c* solution (pH = 2.8) with a 100 mM aqueous ammonium acetate solution at a 1:1 ratio prior to nanoESI (equilibrium; pH = 4.4) (black lines) and using a theta-glass emitter (dashed red lines).