Achieving Multiple Hydrogen/Deuterium Exchange Timepoints of Carbohydrate Hydroxyls Using Theta-Electrospray Emitters

H. Jamie Kim, Elyssia S. Gallagher*

Department of Chemistry and Biochemistry, Baylor University, One Bear Place #97348, Waco, TX, 76798

* Corresponding author:

Elyssia S. Gallagher

Email: elyssia_gallagher@baylor.edu

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Figure S1. Schematic drawing of a theta capillary cross-section. The two channels are divided by a glass septum. The height of each channel, measured perpendicular to the middle of the septum, is measured as the "outer diameter" because two separate droplets form from the two channels. The average of the diameters are calculated as the tip opening size.

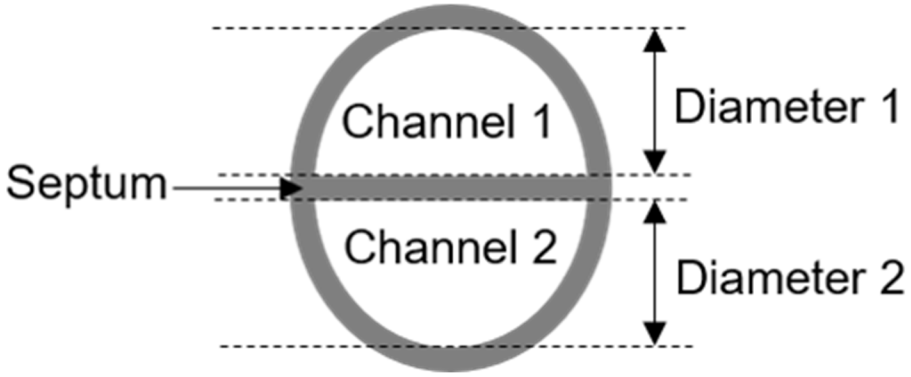


Figure S2. **(A)** Diagram of the open-source setup used for theta-ESI experiments. Platinum wires are inserted into each barrel of the pulled theta tips and voltage is applied using alligator clips connecting the instrument power supply to the platinum wires. Due to the shape of the open instrument source and our XYZ setup, the theta tip was positioned so that the direction of the opening of the tip was perpendicular to the opening of the cone. **(B)** Pictures of the open-source setup used for theta-tip HDX experiments. A theta capillary holder (Warner Instruments, Hamden, CT) secures the pulled theta tips in place with the platinum wires inserted inside each barrel of the tip. The capillary holder system is mounted on an XYZ stage (Thorlabs, Newton, NJ) with a custom-built setup to secure the capillary holder.

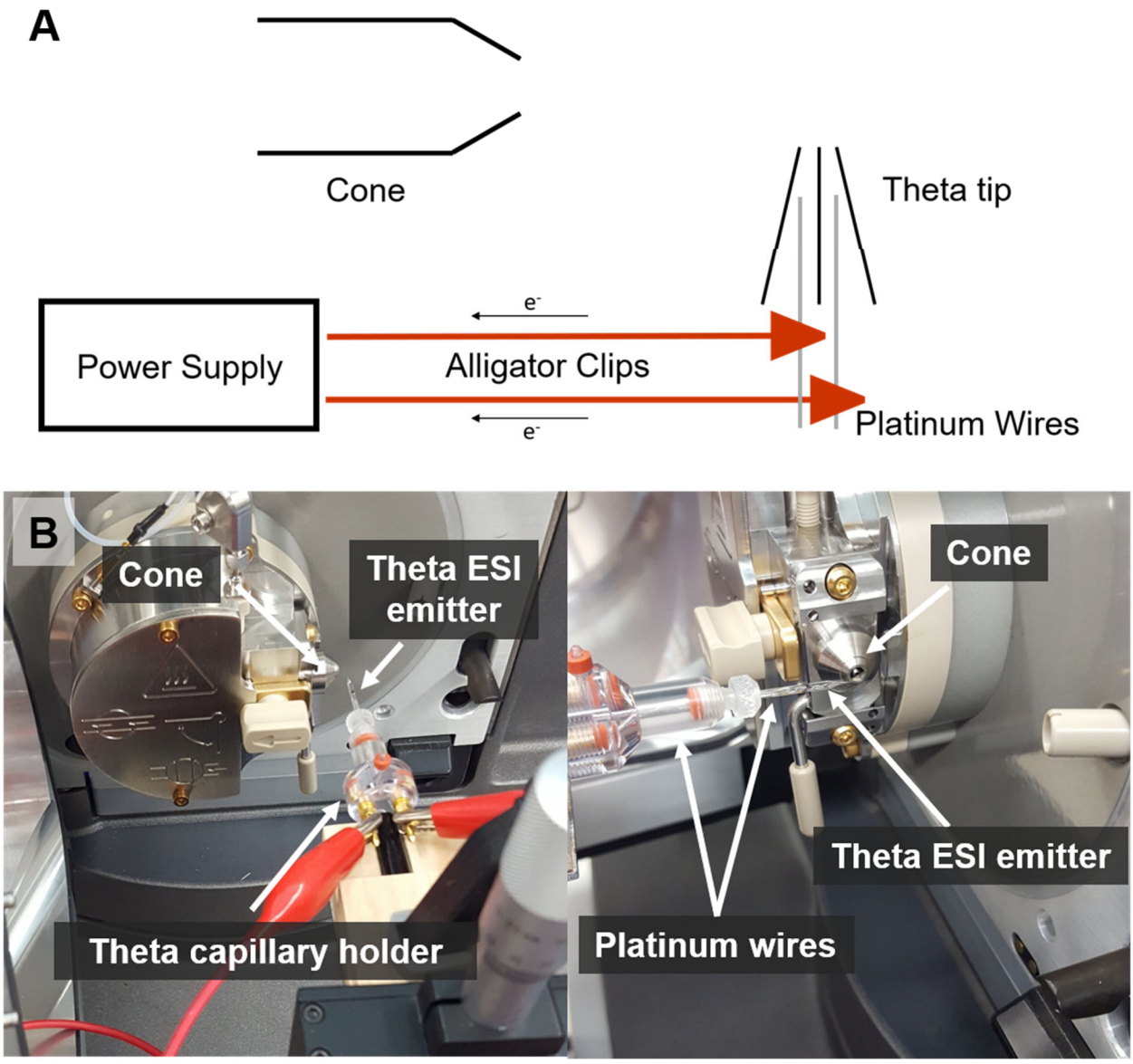
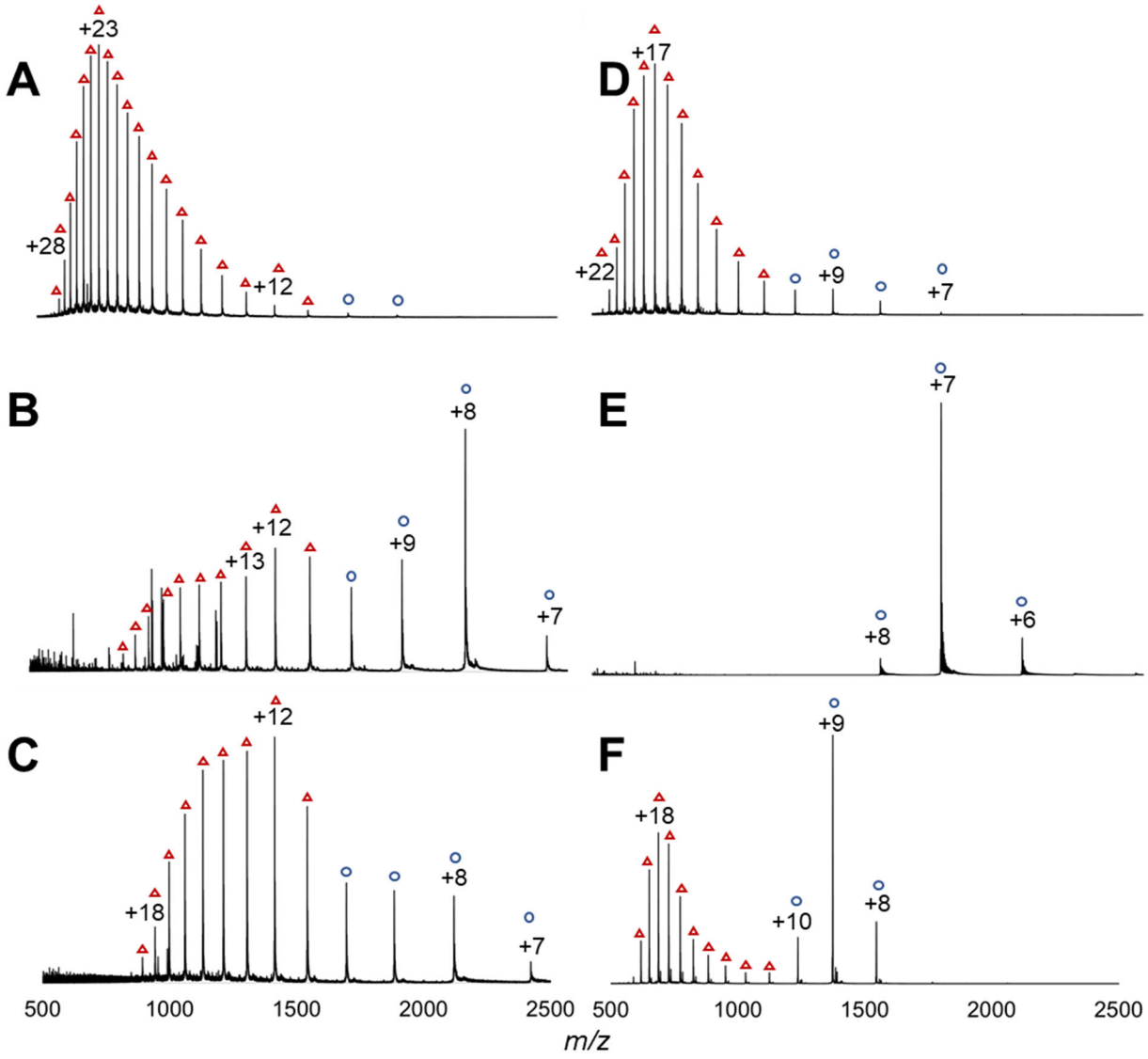


Figure S3. Representative mass spectra of apomyoglobin (A-C, 4.5 ± 0.3 μm channels) and cytochrome c (D-F, 20. ± 9 μm channels) in protein folding experiments via theta tips. The top panels (A, D) represent denatured proteins in 0.1% formic acid, the middle panels (B, E) represent folded proteins at equilibrium in a premixed sample of denatured protein and 100 mM ammonium acetate at 1:1 (v/v), and the bottom panels (C, F) represent proteins folded during ESI after sprayed from theta tips. Protein folding via theta spray (C, F) show intermediate charge states between unfolded (A, D) and equilibrium (B, E) states. The peaks are labeled with blue circle and red triangle to show the charge states that have been used to calculate folded and unfolded populations, respectively, following the guidelines provided by Mortensen *et al.*[1, 2]



Apomyoglobin has two folding states, one at a 7 μs time constant[3] and one that occurs with a time constant greater than 1 ms,[4] to which we attribute the multi-modal distribution observed for charge states of apomyoglobin in equilibrium. The presence of both folded and unfolded charge states for apomyoglobin and primarily folded states for cytochrome *c* at equilibrium (B and E) are consistent with the previous reporting of Mortensen *et al.*[1, 2]

References

1. D. N. Mortensen and E. R. Williams, Investigating Protein Folding and Unfolding in Electrospray Nanodrops Upon Rapid Mixing Using Theta-Glass Emitters, *Anal. Chem.*, 2015, **87**, 1281-1287.
2. D. N. Mortensen and E. R. Williams, Ultrafast (1 μs) Mixing and Fast Protein Folding in Nanodrops Monitored by Mass Spectrometry, *J. Am. Chem. Soc.*, 2016, **138**, 3453-3460.
3. R. M. Ballew, J. Sabelko and M. Gruebele, Direct Observation of Fast Protein Folding: The Initial Collapse of Apomyoglobin, *P. Natl. Acad. Sci.*, 1996, **93**, 5759-5764.
4. Robert H. Callender, R. Brian Dyer, Rudolf Gilmanshin and W. H. Woodruff, FAST EVENTS IN PROTEIN FOLDING: The Time Evolution of Primary Processes, *Annu. Rev. Phys. Chem.*, 1998, **49**, 173-202.