Supporting Information

High-Precision, Gas-Phase Hydrogen/ Deuterium Exchange Kinetics by Mass Spectrometry Enabled by Exchange Standards

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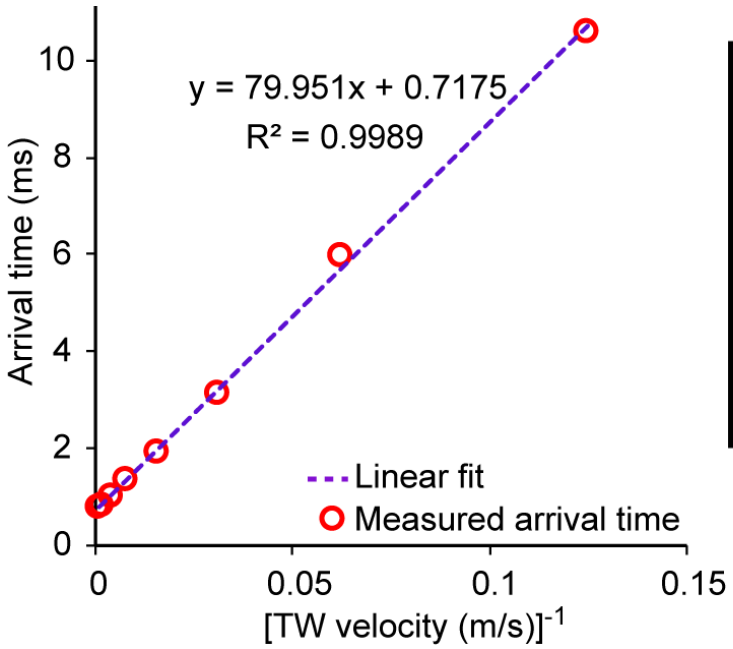
Contents:



TW velocity (m/s)	Transfer TWIG residence time (ms)
512	0.12
256	0.31
128	0.63
64	1.21
32	2.43
16	5.26
8	9.88

Figure S1. Calculation of the exact gHDX times in the TW transfer TWIG. The plot shows measured the arrival time of standard compounds as a function of the inverse transfer TW velocity ranging from 512 to 8 m/s (red circles). A linear fit was used for assessing the residence time in the other sections of the mass spectrometer including the IMS cell and the downstream optics in the time of flight region (y-intercept). Subtraction of this time from the measured arrival time was used to calculate the residence time solely in the transfer TWIG (shown on the table on the right).

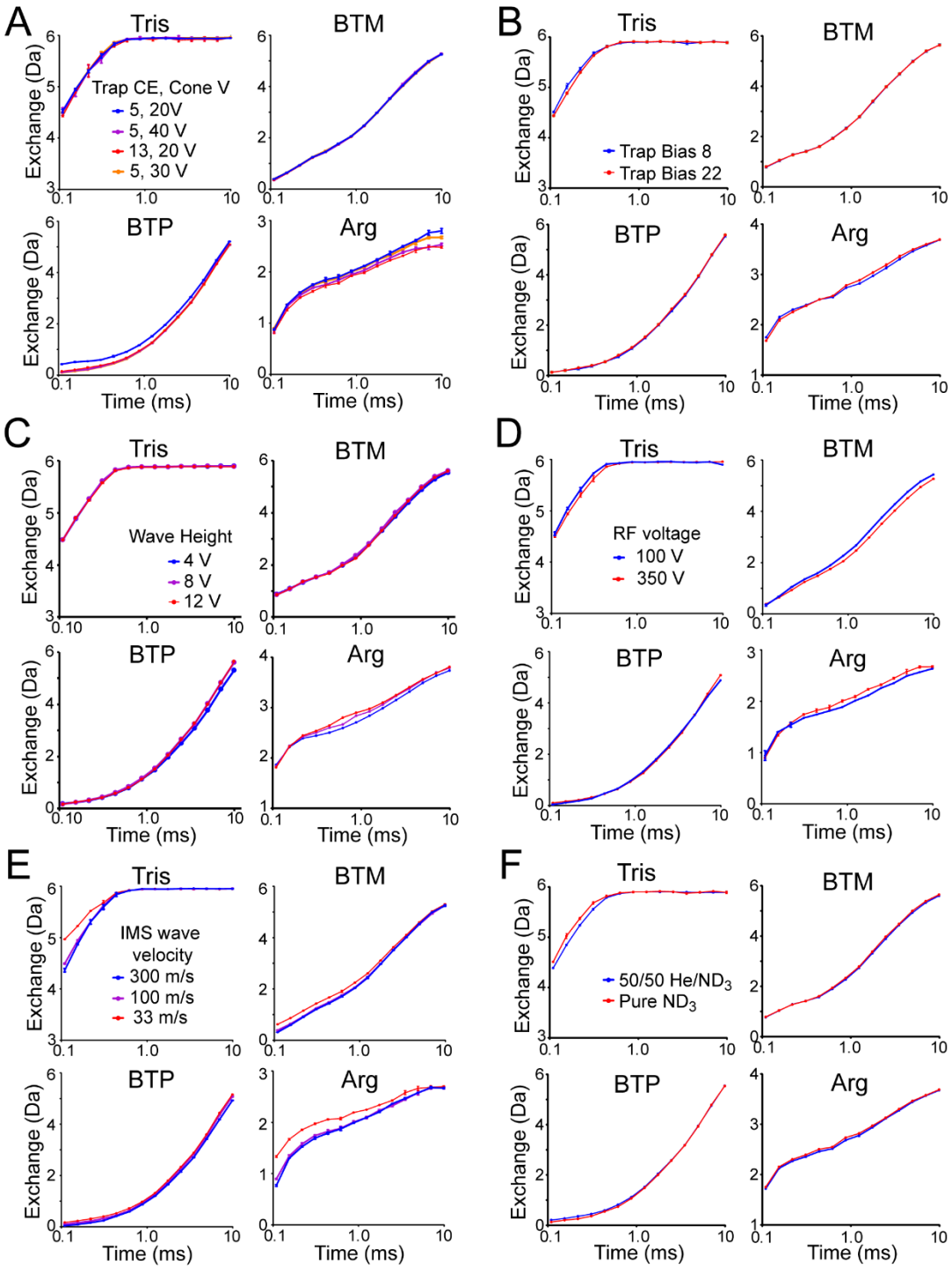


Figure S2. Effect of MS settings on gHDX for tris compounds and arginine. Various conditions for cone voltage and trap CE **(A)**, Trap bias **(B)**, transfer TW height **(C)**, transfer RF confining voltage **(D)**, IMS wave velocity **(E)** and comparison of pure ND_3 vs. a 1:1 mixture of ND_3/He **(F)**. Specific conditions are listed in the inset, and all other conditions are default as specified in the methods.

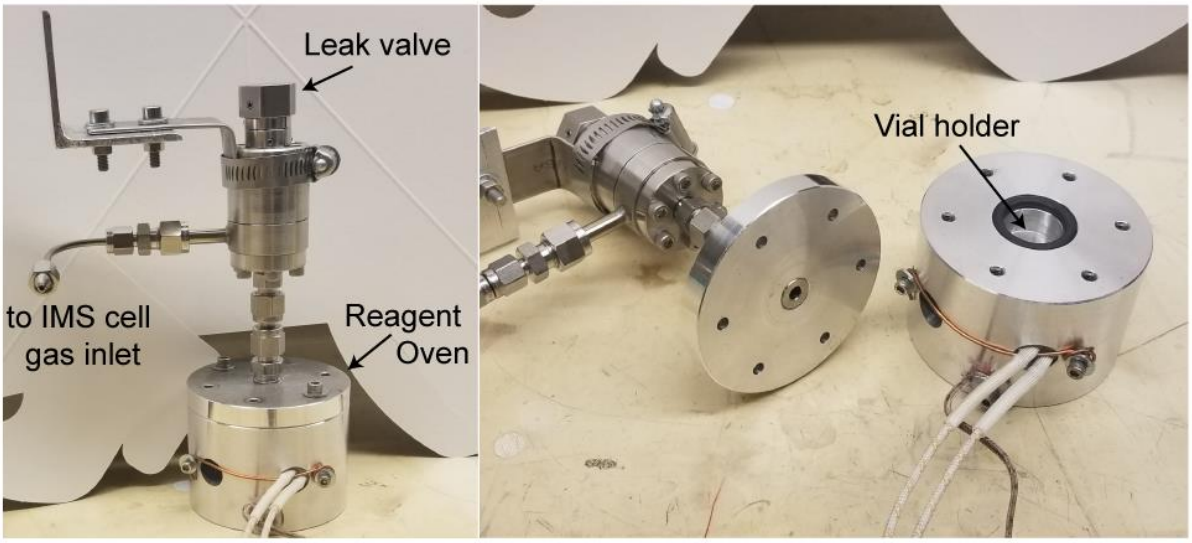


Figure S3. The heated leak valve assembly to control the flow of solvent vapor (methanol or water) into the mass spectrometer. A vial of reagent is placed into the oven assembly. The reagent oven is sealed and heated to a specific temperature and the leak valve is adjusted to achieve a specific pressure of the reagent within the IMS cell.

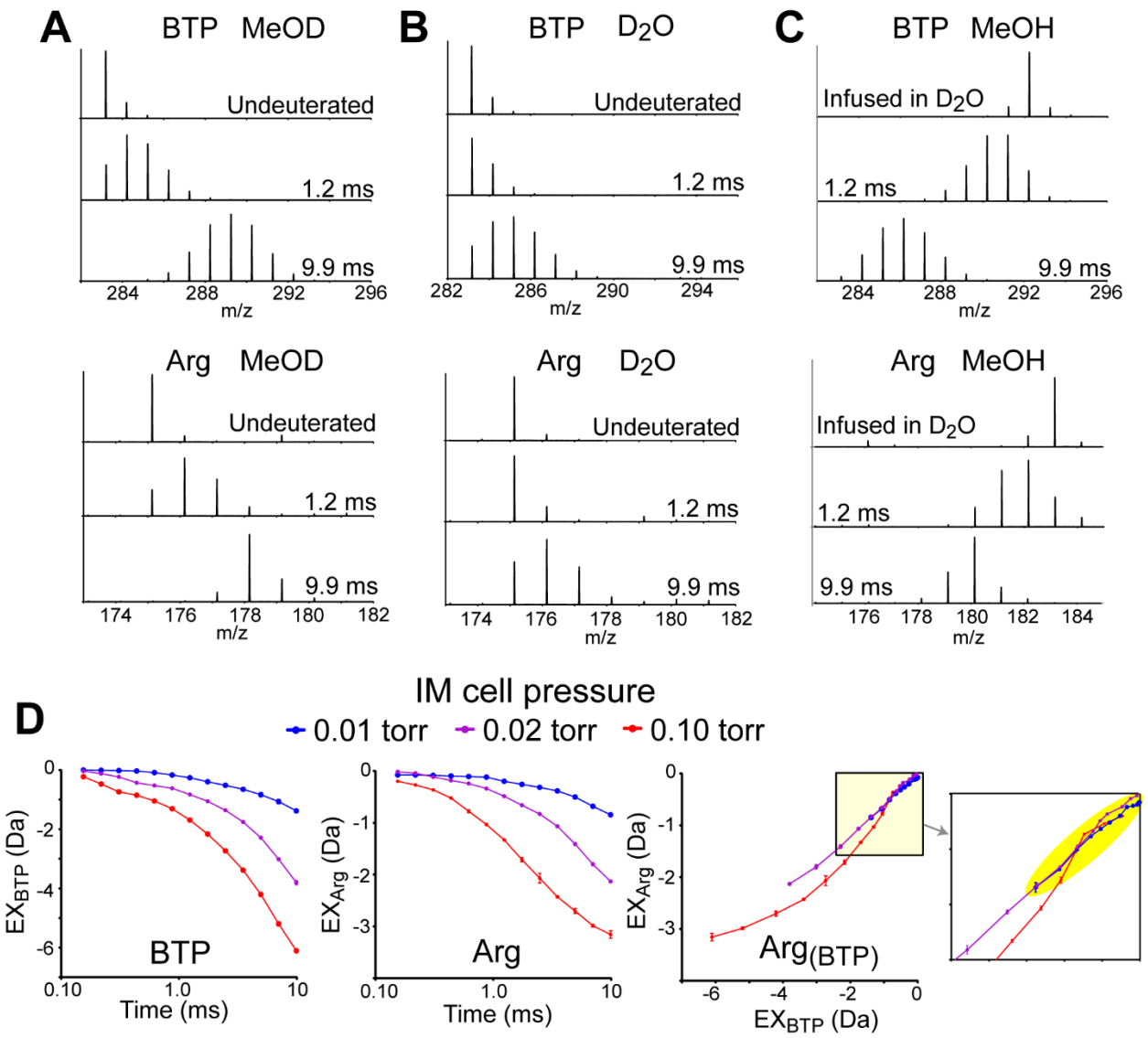


Figure S4. Mass spectra for BTP (top) and arginine (bottom) using either MeOD **(A)** or D_2O **(B)** as the exchange reagent. The spectra for the reverse exchange ("off-exchange") with fully deuterated BTP and arginine using undeuterated methanol is shown in **(C)**. **D)** Off exchange for BTP and arginine are shown in the left and middle plots at three different pressures of methanol from 0.01 (blue) to 0.1 (red) torr. The exchange of arginine referenced to BTP ($Arg_{(BTP)}$) is shown on the right. The boxed region is zoomed to indicate the region of the exchange that is effectively corrected by referencing (highlighted in yellow).

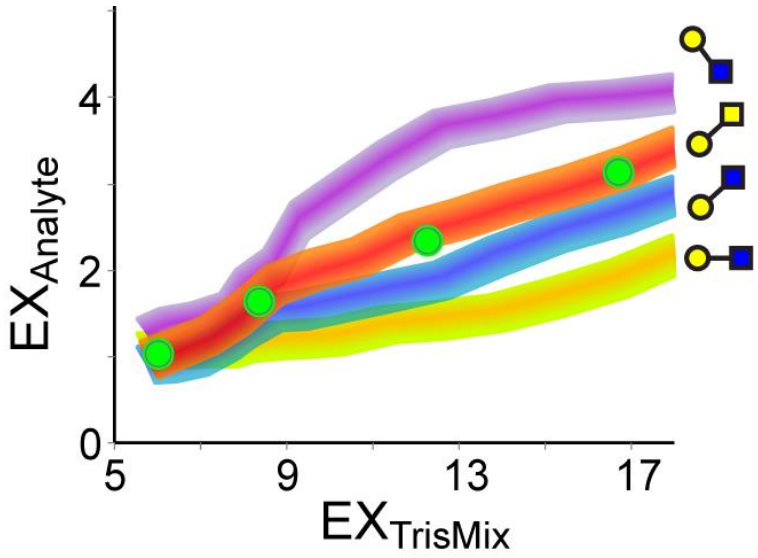


Figure S5. Example of how standardized gHDX data can be used for identification of isomeric analytes. The four highlighted bands represent exhaustive gHDX collected for four isomeric glycans relative to the TrisMix: Gal(β1-6)GlcNAc (purple); Gal(β1-3)GalNAc (red); Gal(β1-3)GlcNAc (blue); Gal(β1-4)GlcNAc (yellow); as described previously [20]. The green circles are 4 time points that were collected for an unknown analyte also relative to the TrisMix. Based on this comparison against the known gHDX profiles of each disaccharide, the unknown analyte can be identified based on its consistency with the exchange profile of the Gal(β1-3)GalNAc species (red band).