High Pressure Ion-Neutral Clustering and Hydrogen/Deuterium Exchange to Rationalize Gas Phase Ion Conformations

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INTRODUCTION

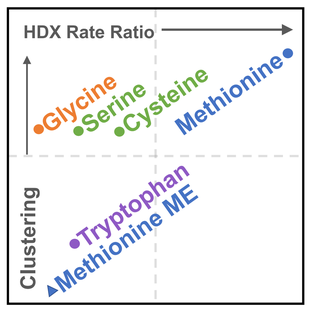
Ion-neutral Clustering

- The traditional ion mobility experiment focuses explicitly on the collision behavior of ions and neutrals.
- Introducing reactive vapor dopants promotes ion-neutral clustering and alters arrival time distributions.
- Select vapor modifiers exhibit site-specific uptake mechanisms the prompt questions regarding the mechanism of interaction.

Hydrogen Deuterium Exchange (HDX)

- Recent efforts in low-pressure gas-phase HDX studies allowed for many deuterium sources and faster reaction times
- Experimental limitations to low-pressure techniques restricted deuterium incorporation and quantitation

Quantifying the degree of vapor association and HDX showed clustering affects exchange reaction kinetics for a series of amino acids.



AIMS

- Utilize site-specific ion-neutral clustering with simultaneous gas-phase HDX for model peptides
- Combine experimental data with theoretical calculations for vapor modifier binding times and binding locations
- Rationalize possible gas-phase ion conformations by comparing experiment and theory

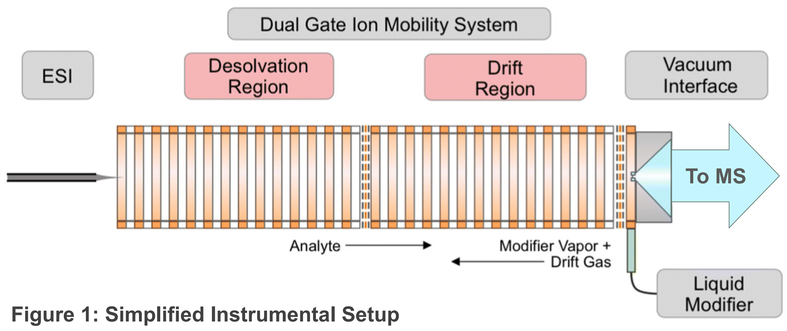


Figure 1: Simplified Instrumental Setup
The atmospheric pressure, dual gate ion mobility spectrometer is coupled to an LTQ mass spectrometer. The sample solution is introduced via electrospray into the drift tube. Ion packets are pulsed into the drift region through the first gate and are separated by mobility. Mobility spectra are obtained by encoding the data in the frequency domain. The frequency-encoded mobility spectra may be used to recover drift time spectra for m/z selected ions using fast Fourier transform. Changes in isotope distribution are monitored by the LTQ as a function of the modifier flow rate. The modifier is introduced through the primary drift gas inlet through a GC injection liner at variable flow rates using a syringe pump.

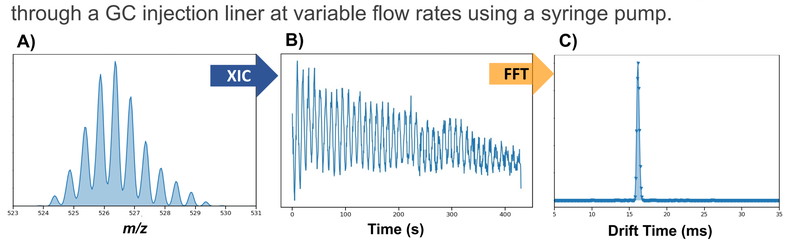


Figure 2: Sample Data Processing Scheme
A) A sample mass spectrum where methanol-OD was introduced at 80 µL/hr. The *m/z* 530.7 is the monoisotopic mass of the double-charged peptide. B) The mass-selected ion chromatogram from the highlighted isotopic window. This mass range is used for all data points. C) The recovered mobility peak. The frequency is converted to an arrival time using the sweep rate of the two synchronized gates.

RESULTS

Comparing HDX and Vapor Induced Mobility Shifts

As seen previously with select amino acids, the shifts in mobility induced by transient ion-neutral clustering correlate surprisingly well with the pseudo-first-order semilogarithmic kinetics plots.

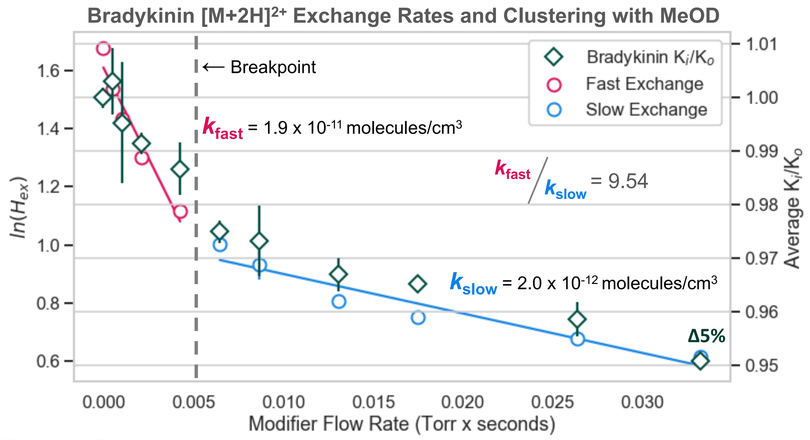


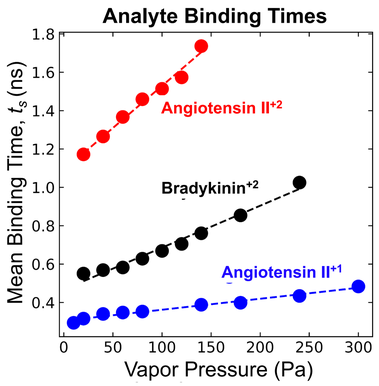
Figure 3: HDX kinetics and clustering behavior for bradykinin
At least two rates are observed for $[M+2H]^{2+}$ ions of bradykinin with methanol-OD: a fast rate (k_{fast}) and slow rate (k_{slow}) coefficient calculated from the slope of the linear fits. A total of 6 exchangeable hydrogens are observed after only 40 ms of exposure to the vapor through the drift cell. Following similar trends, the mobility reaches a 5% change (**green diamonds**) with 300 µL/hr of the methanol-OD.

Analyte	Fast rate (x10^{-12})	Slow rate (x10^{-12})	R^2	Relative Rate	% K_0 change
Bradykinin^{+2}	18.8	2.0	0.99	9.54	5%
Angiotensin II^{+2}	60.7	17.7	0.99	3.43	5.5%
Angiotensin II^{+1}	17.5	8.6	0.99	2.04	3%

Table 1: Experimental Summary for HDX and Vapor Association of Select Model Peptides. The observed rates of exchange are analyte dependent. Consistent with the literature, angiotensin II is a particularly fast exchanger. The greater shifts in mobility for the doubly charged species highlight the role of charge in vapor association.

Bradykinin RPPGFSPFR **Angiotensin II** DRVYIHPF

Molecular Dynamics Simulations



While the experimental results highlight differences in reactivity due to structural differences, modeling the vapor association events elucidates specifically how many vapors bind, where they bind, and for how long.

Figure 4: Analyte Binding Times
Consistent with previous work, the greater shifts in mobility correlate to a longer time a vapor spends bound. However, showing the time bound as a function of modifier vapor pressure, it is shown that the increase in vapor pressure influences the time-bound. This means the modifier vapors influence one another in the ion-neutral complex.

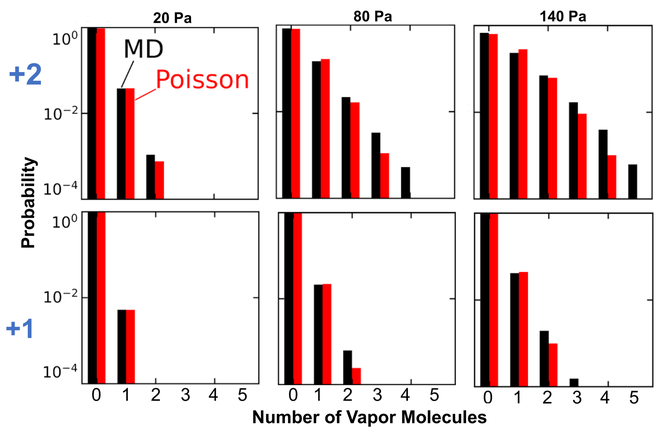


Figure 5: Number of Vapor Molecules Bound for Angiotensin II +1 and +2 Ions
The probability of having *n* vapors bound at 20, 80, and 140 Pa for $[M+2H]^{2+}$ ions (top row) and $[M+H]^{+}$ ions (bottom row). This clearly shows the role of charge in forming ion-neutral complexes.

Predicted Per Residue Association Probabilities

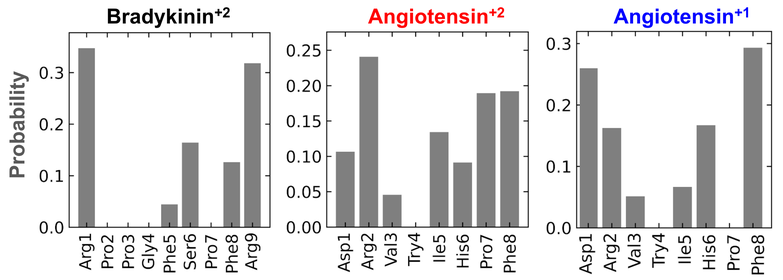
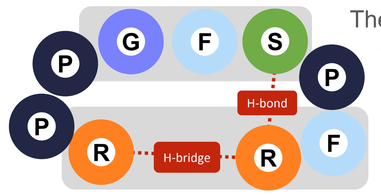


Figure 6: Probability of Vapor Association on Per Reside Basis for bradykinin $[M+2H]^{2+}$ (left), angiotensin II $[M+2H]^{2+}$ (middle), and $[M+H]^{+}$ (right) at 140 Pa. All species show substantial probabilities for phenylalanine interactions. For bradykinin, the vapor interacts mostly with the arginine residues at the termini of the peptide. Nearly all residues in angiotensin II have a measurable probability to host a modifier. Interestingly, interactions at Pro7 are starkly different between the two charge states.

CONCLUSIONS

Bradykinin Structural Constraints

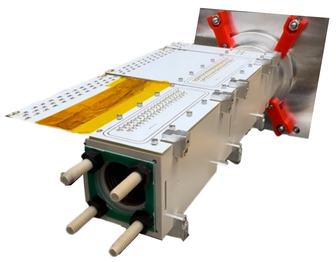


The previously proposed structural motif of doubly charged bradykinin includes a proton bridge between the arginine residues and a possible H-bond between the side chains of Ser6 and Arg9. This structure is consistent with the regions calculated by the per residue association studies highlighted in grey. We predict that the exchanged hydrogens would be included in these regions as well.

Angiotensin II Structural Constraints

Both the singly and doubly charged angiotensin II ions are reported in the literature to be linear. Since all but one of the residues is shown to be interacting with the modifier and reacts very fast with methanol-OD, our data is consistent with this structure. The difference in binding between the +1 and +2 at Pro7 indicates a change in adding a second proton onto the peptide.

FUTURE WORK



Coupling this work with dissociation experiments is necessary to examine HDX of peptide fragments and expand to larger model protein systems. In addition, a new heated drift cell is operated to capture the impact of temperature on these conclusions. Switching from a drift tube, SLIM-HDX experiments are planned to enable time-varying experiments (See Clowers' Poster).

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