

Observation of Zwitterion Formation in the Gas-Phase H/D-Exchange with CH₃OD: Solution-Phase Structures in the Gas Phase

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Infrared spectroscopy of gas-phase singly deuterated [Trp + K]⁺ (formed by H/D exchange with CH₃OD) shows that some (~20%) kinetically stable zwitterionic (ZW) conformer is formed, based on the diagnostic antisymmetric CO stretch of the deprotonated carboxylate moiety, $\nu_{\text{as}}(\text{CO}_2^-)$, at 1680 cm⁻¹. A majority of the deuterated [Trp + K]⁺ is found to be in the charge solvation (CS) conformation, with deuterium exchange occurring on both the acid and amino groups, which is consistent with H/D scrambling. Interestingly, H/D exchange with the more basic ND₃ reagent did not result in the stabilization of a kinetically stable zwitterion, although it is not clear yet what causes this observation. The result for CH₃OD shows that H/D exchange can in fact alter the structure of the analyte and, hence, care needs to be taken when interpreting gas-phase H/D exchange studies. Moreover, this result shows the possibility of forming solution-phase structures that are thermodynamically disfavored in the gas phase, thus opening a new area of study. (J Am Soc Mass Spectrom 2007, 18, 512–516) © 2007 American Society for Mass Spectrometry

H/D exchange (HDX) is one of the most popular techniques for the structural elucidation of biomolecules in mass spectrometry and has been widely implemented for both solution [1–4] and gas phase studies [5–18]. In solution, the extent of HDX can be directly related to the solvent accessibility of amino acid residues in proteins. However, in the gas phase there are many structural parameters apart from the surface availability that affect the HDX rates, as illustrated in a recent HDX study on ion-mobility-selected charge states of bovine ubiquitin [17]. Systematic studies on small peptide systems showed that the difference in proton affinity of the biomolecules and deuterated donor reagent plays a crucial role in the HDX mechanism [7–10, 19]. Beauchamp and coworkers [9] suggested that lower basicity reagents, such as D₂O and CH₃OD, proceed via a “relay” mechanism [20] for

protonated peptides, while the higher basicity ND₃ reagent gives rise to an “onium” mechanism [21]. In the case of amino acids cationized by an alkali metal ion rather than a proton, polar deuterating ligands such as D₂O can exchange via a similar relay mechanism involving charge solvation-zwitterion (CS-ZW) isomerization [18]. Modeling of observed HDX processes and quantum chemical calculations of the potential surfaces have made a strong circumstantial case that ZW structures are traversed in many cases during HDX with basic partners like D₂O, CH₃OD, and ND₃ [14, 16, 18]. Nevertheless, computations also generally indicate that the bare ZW is sufficiently disfavored energetically that its equilibrium presence in the M⁺/amino acid would be negligible [22, 23].

Here, we show unambiguous spectroscopic evidence that bare ZW can in fact be kinetically trapped as a result of the HDX of [Trp + K]⁺ with CH₃OD. Infrared multiple-photon dissociation (IR-MPD) spectroscopy of metal-tagged amino acids and peptides has already been shown to be a powerful probe for CS or ZW conformers in the gas phase [24–28]; the chemically

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different acid (COOH) or carboxylate (CO₂⁻) groups have characteristic CO stretch vibrations and thus IR spectroscopy is a direct probe of their presence.

Experimental and Calculations

The experiments were carried out on a laboratory-built 4.7 Tesla actively shielded Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer, described in detail elsewhere [29]. This mass spectrometer is coupled to the beam line of the free electron laser for infrared experiments (FELIX) [30], thus enabling IR spectroscopy experiments on ions in the gas phase. [Trp + K]⁺ was generated in a commercial electrospray ionization (ESI) source (Z-spray, Micromass, Manchester, UK) and were accumulated in a storage hexapole (500 ms). As the ions exit the hexapole, they pass through a quadrupole deflector/bender (ABB Extrel) into an octopole rf ion guide. Upon entering the octopole, the kinetic energy of the ions is typically about ~35 eV. By pulsing the octopole DC bias from ground to about -35 V while the ions are traveling through, the potential energy of the ions can be rebiasd without affecting their kinetic energy [26]. As the ions enter the ICR cell, they have to overcome the potential energy difference from -35 V to ground, thereby reducing their kinetic energy. It is then possible to trap the ions in the ICR cell using moderate trapping voltages (i.e., <10 V), without the need of employing a gas pulse. This approach minimizes the heating of the ions due to collisional activation, which is an advantage in terms of obtaining higher-quality IR spectra.

The trapped [Trp + K]⁺ ions were exposed to CH₃OD, which was pulsed in up to a pressure of ~1 × 10⁻⁵ mbar and then pumped away (delay 8 s). The singly deuterated [Trp + K]⁺ (*m/z* 244) was mass-isolated and then irradiated with 10 pulses from FELIX; FELIX delivers high-energy pulses (typically 60 mJ) over a duration of 5 μs of widely tunable IR light (3 to 250 μm) at a repetition rate of 5 Hz. The ion photo-dissociates when the laser wavelength is resonant with a vibrational mode of the molecule and, thus, the IR spectrum is recorded by monitoring the yield of photo-dissociation (here appearance of K⁺) as a function of wavelength. Note that the ion has to absorb multiple photons (i.e., tens to hundreds) to reach the dissociation threshold. The mechanism of infrared multiple-photon dissociation (IR-MPD) has been described in detail previously [31, 32]. In a separate experiment, [Trp + K]⁺ was deuterated using a constant background pressure of ND₃ (8 × 10⁻⁷ mbar) for 6 s. The singly deuterated ion (at *m/z* 244) was again mass isolated and probed by IR spectroscopy, as described above.

The calculations were carried out at the DFT level (B3LYP/6-311 + G(d,p)) using the Gaussian software package [33], and were zero-point energy corrected [27]. The frequencies were scaled (0.987) and a Gaussian convolution was applied (FWHM = 30 cm⁻¹).

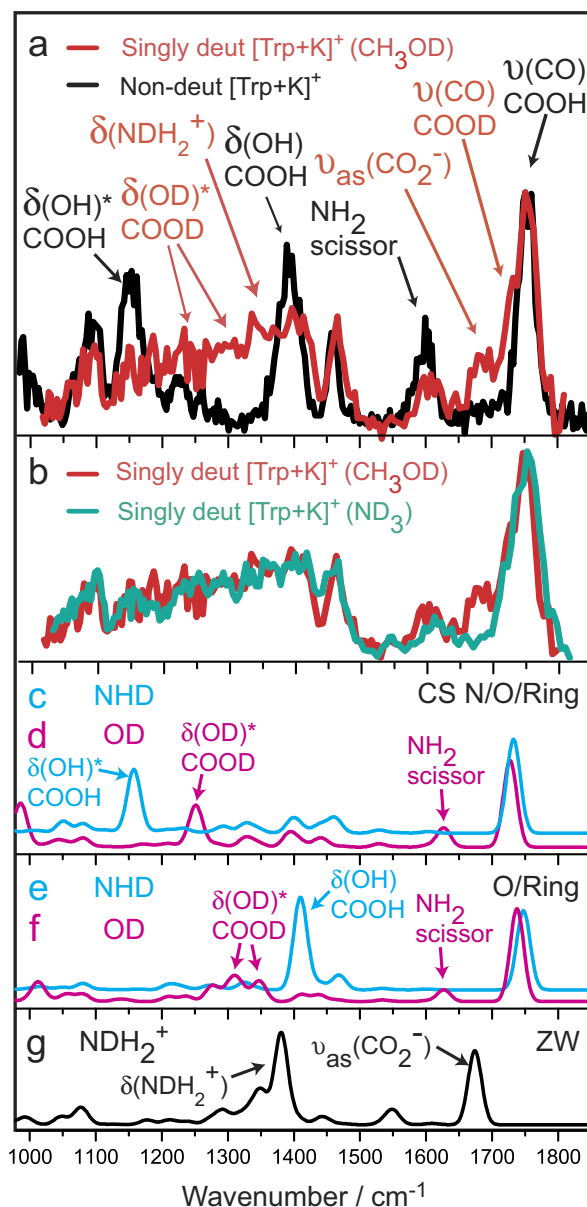
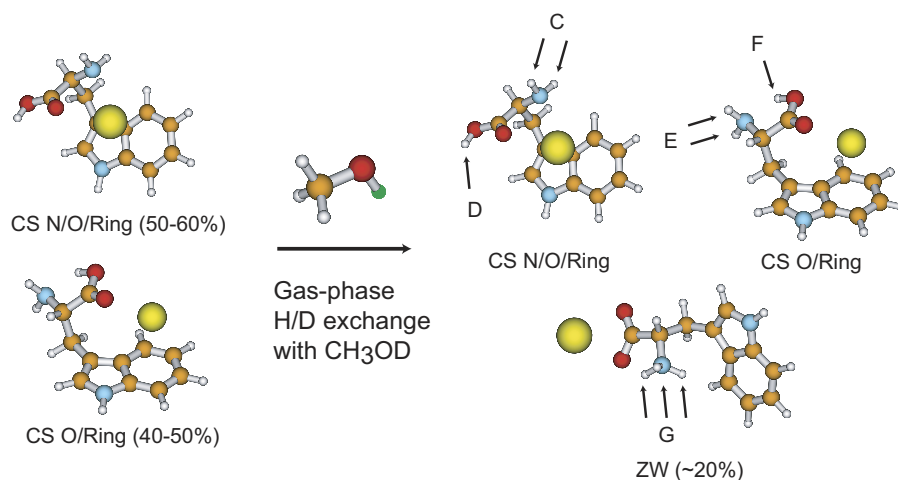


Figure 1. (a) Overlapped IR spectra of singly deuterated (using CH₃OD) (red) and nondeuterated (black) [Trp + K]⁺. Annotation is color-coded to show peaks that appear in both spectra (black) and peaks that only appear in the singly deuterated spectrum (red). (b) Overlapped IR spectra of singly deuterated [Trp + K]⁺ (CH₃OD, red; ND₃, cyan). Calculated IR spectra for singly deuterated CS N/O/Ring conformer: (c) deuterated NHD group; (d) deuterated acid OD group; CS O/Ring conformer: (e) deuterated NHD group; (f) deuterated acid OD group; and (g) ZW deuterated NDH₂⁺ group. The character of the modes marked with an asterisk is heavily mixed with other molecular motions.

Results and Discussion

In a previous study [27], it was shown from the IR spectrum of nondeuterated [Trp + K]⁺ (Figure 1a, black trace) that the population consists of two CS conformers, tridentate N/O/Ring (50 – 60%) and bidentate O/Ring (40 – 50%), and that there is no observable presence of ZW (for structures see Scheme 1). This is not



Scheme 1. Schematic drawing of $[\text{Trp} + \text{K}]^+$ gas-phase deuteration using CH_3OD , showing the approximate amounts in parentheses. The sites of deuteration are highlighted by arrows and the corresponding theoretical spectra in Figure 1 are indicated by upper case letters.

surprising, as N/O/Ring (+1 kJ mol⁻¹) and O/Ring (0 kJ mol⁻¹) are almost isoenergetic, while ZW is much less favorable energetically (+15 kJ mol⁻¹).

A comparison of the IR spectra of singly deuterated (using CH_3OD) to nondeuterated $[\text{Trp} + \text{K}]^+$ in Figure 1a shows new bands that are only observed in the singly deuterated IR spectrum (labeled in red), while other bands appear in both (labeled in black). Most intriguing is the new band at 1680 cm⁻¹, which gives unequivocal evidence for the presence of the ZW structure. This peak can only be assigned to the diagnostic antisymmetric CO stretch of the deprotonated carboxylate moiety, $\nu_{\text{as}}(\text{CO}_2^-)$, of the ZW (Figure 1g). Conversely, bands which are unaffected by the HDX experiment, such as bands associated with aromatic CH bending (1080 and 1450 cm⁻¹), are essentially identical in both spectra. Additional evidence for the assignment of the 1680 cm⁻¹ band is provided by a comparison to the singly deuterated spectrum of $[\text{Trp} + \text{K}]^+$ generated in a different way, using ND_3 as the HDX exchange reagent (Figure 1b), where this band is clearly missing, while the remainder of the spectrum is nearly identical. This shows that the ZW is formed in the HDX process with CH_3OD , but is not formed under all HDX conditions. As the ZW structure is calculated to be energetically much less favorable, the observed ZW (~20%) is merely kinetically trapped.

The majority (~80%) of singly deuterated $[\text{Trp} + \text{K}]^+$ (using CH_3OD) is in the CS form, as seen by the intense carboxylic acid C=O stretch mode, $\nu(\text{CO})$ COOH. The interesting question then arises as to where these CS structures are deuterated, and a number of possibilities exist (Scheme 1). The changes in the spectrum shown in Figure 1a, including the appearance of new features between 1100 to 1400 cm⁻¹, can be rationalized by a mixture of structures (i.e., N/O/Ring and O/Ring) and sites of deuteration. Partial deuteration of the acid group is indicated by the appearance of the O—D

bending modes, $\delta(\text{OD})$ COOD (1250 to 1300 cm⁻¹), and the C=O stretching mode of COOD (1730 cm⁻¹), as well as by the substantial decrease of the COOH bending modes $\delta(\text{OH})$ COOH at 1400 cm⁻¹ and 1160 cm⁻¹. Partial deuteration of the amino group is supported by a reduction in the intensity of the NH_2 scissoring band (1600 cm⁻¹). Deuteration at the indole NH site is unlikely, as this site is known to exchange more slowly in $[\text{Trp} + \text{H}]^+$ [34], and given that HDX with CH_3OD only resulted in a maximum of three deuterium exchanges out of a possible four sites. While a mixture of structures and sites of deuteration is present, it is difficult to establish the relative contribution of each sub-group.

The observation that both NHD and COOD structures are formed shows that no site is preferentially deuterated and is consistent with H/D scrambling. It is not clear yet whether HDX of $[\text{Trp} + \text{K}]^+$ with CH_3OD always proceeds via the ZW, nor whether the kinetic trapping captures all, or only a fraction, of the transiently-formed ZW structures.

A number of complementary experiments were carried out to determine what other factors contribute to ZW formation. The relative amounts of CS/ZW are hardly affected by the trapping time in the ICR cell (in the range from 4 to 8 s), showing that the kinetically trapped bare ZW are stable on the time-scale of seconds. Moreover, the CH_3OD pressure (in the range of 5×10^{-6} to 2×10^{-5} mbar) did not appear to have an impact on the amount of ZW that was generated, although it did affect the extent of HDX. However, the CH_3OD pressure range that could be explored was limited by the constraint that the ion signal of the singly deuterated species could not be allowed to fall too low. It is therefore difficult to know whether the ratio of CS and ZW would be very different outside of this relatively narrow experimental window.

In order for “bare” ZW to be observed, an energy

gap of 15 kJ mol⁻¹ has to be overcome. Clearly, the thermal energy is insufficient, as the ZW is not observed for [Trp + K]⁺ before HDX. Nonetheless, the internal energy of a floppy molecule such as tryptophan at room temperature is already relatively high (~1500 cm⁻¹) and the kinetic energy of the ions in these experiments was up to 6 eV, given that this was the trapping voltage used. Due to collisions with CH₃OD, some of this kinetic energy would be transferred into internal energy, thus accounting for the energy of conversion from bare CS to bare ZW.

Intriguingly, HDX of [Trp + K]⁺ using ND₃ did not give rise to the stabilization of the ZW (see Figure 1b). The ND₃ experiment shown in Figure 1b was carried out at a constant background pressure of 8 × 10⁻⁷ mbar, under which conditions collisional cooling would be less effective than in the CH₃OD experiments. The ND₃ experiment was therefore repeated at the higher constant pressure of 5 × 10⁻⁶ mbar (not shown). However, no ZW was observed under these conditions either. In spite of numerous attempts, it has so far not been possible to generate the ZW in the HDX experiments with ND₃. This failure could in principle arise due to a number of possibilities: (1) HDX using more basic reagents such as ND₃ may have a very different energy landscape compared with low-basicity reagents such as CH₃OD, thus making stabilization of the thermodynamically less favorable ZW structure inherently more difficult. (2) ZW structures may not be formed in the HDX mechanism with ND₃, not even as reaction intermediates. The latter explanation would contradict the current understanding of HDX with ND₃ [9, 16, 21], and it therefore requires more controlled experiments to verify this hypothesis. In particular the temperature of ions cannot be accurately controlled in the current experiments, despite the fact that it is probably the most important parameter in terms of stabilizing the ZW. In the current experiment, the kinetically trapped ZW structures are probably stabilized by collisions with CH₃OD molecules, which reduce their internal energy sufficiently so that they are stable on the timescale of seconds. By conducting the HDX experiments at a lower temperature (i.e., lower than room temperature), it would be possible to see if the yield of kinetically trapped ZW in the CH₃OD HDX experiments can be increased, thus showing if ZW structures are always formed.

Conclusions

Although HDX using ND₃ and CH₃OD both give rise to a mixture of deuterated CS structures, only CH₃OD yielded a kinetically stable ZW. The mechanistic elucidation of this unexpected finding requires considerable future work. In this light, careful temperature control of HDX experiments could give a quantitative understanding of the underlying mechanism(s). Importantly, these results show that HDX can be an invasive technique, causing a change in the structure of the analyte

and, hence, care needs to be taken when inferring structural information based on HDX studies. Moreover, this finding shows that *solution-phase* structures can be generated in the gas-phase environment by interaction with polar solvent molecules, thereby enabling controlled gas-phase experiments on solution-phase structures; thus, an interesting approach for future study is that the unfavorable ZW conformer could be enriched by selectively dissociating the CS conformer with IR-MPD (at e.g., 1750 cm⁻¹), and studied further under gas-phase conditions.

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References

1. Verma, S.; Pomerantz, S. C.; Sethi, S. K.; McCloskey, J. A. Fast atom bombardment mass spectrometry following hydrogen-deuterium exchange. *Anal. Chem.* **1986**, *58*, 2898.
2. Katta, V.; Chait, B. T.; Carr, S. Conformational changes in proteins probed by hydrogen-exchange electrospray-ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **1991**, *5*, 214.
3. Konermann, L.; Simmons, D. A. Protein-folding kinetics and mechanisms studied by pulse-labeling and mass spectrometry. *Mass Spectrom. Rev.* **2003**, *22*, 1.
4. Hydrogen exchange and covalent modification: focus on biomolecular structure, dynamics and function. 18th Sanibel Conference on Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **2006**, *17*, 1481.
5. Winger, B. E.; Light-Wahl, K. J.; Rockwood, A. L.; Smith, R. D. Probing qualitative conformation differences of multiply protonated gas-phase proteins via hydrogen/deuterium isotopic exchange with water-d2. *J. Am. Chem. Soc.* **1992**, *114*, 5897.
6. Suckau, D.; Shi, Y.; Beu, S. C.; Senko, M. W.; Quinn, J. P.; Wampler, F. M.; McLafferty, F. W. Coexisting stable conformations of gaseous protein ions. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 790.
7. Campbell, S.; Rodgers, M. T.; Marzluff, E. M.; Beauchamp, J. L. Structural and energetic constraints on gas phase hydrogen/deuterium exchange reactions of protonated peptides with D₂O, CD₃OD, CD₃CO₂D, and ND₃. *J. Am. Chem. Soc.* **1994**, *116*, 9765.
8. Gard, E.; Green, M. K.; Bregar, J.; Lebrilla, C. B. Gas-phase hydrogen/deuterium exchange as a molecular probe for the interaction of methanol and protonated peptides. *J. Am. Soc. Mass Spectrom.* **1994**, *5*, 623.
9. Campbell, S.; Rodgers, M. T.; Marzluff, E. M.; Beauchamp, J. L. Deuterium exchange reactions as probe of biomolecule structure. Fundamental studies of gas phase reactions of protonated glycine oligomers with D₂O, CD₃OD, CD₃CO₂D, and ND₃. *J. Am. Chem. Soc.* **1995**, *117*, 12840.
10. Green, M. K.; Lebrilla, C. B. Ion-molecule reactions as probes of gas-phase structures of peptides and proteins. *Mass Spectrom. Rev.* **1997**, *16*, 53.
11. Valentine, S. J.; Clemmer, D. E. H/D exchange levels of shape-resolved cytochrome *c* conformers in the gas phase. *J. Am. Chem. Soc.* **1997**, *119*, 3558.
12. McLafferty, F. W.; Guan, Z.; Haupts, U.; Wood, T. D.; Kelleher, N. L. Gaseous conformational structures of cytochrome *c*. *J. Am. Chem. Soc.* **1998**, *120*, 4732.
13. Freitas, M. A.; Marshall, A. G. Rate and extent of gas-phase hydrogen/deuterium exchange of bradykinins: Evidence for peptide zwitterions in the gas phase. *Int. J. Mass Spectrom.* **1999**, *182*, 221.
14. Wyttenbach, T.; Paizs, B.; Barran, P.; Brecci, L. A.; Liu, D.; Suhai, S.; Wysocki, V. H.; Bowers, M. T. The effect of the initial water of hydration on the energetics, structures and H/D exchange mechanism of a family of pentapeptides: An experimental and theoretical study. *J. Am. Chem. Soc.* **2003**, *123*, 13768.
15. Jurchen, J. C.; Cooper, R. E.; Williams, E. R. The role of acidic residues and of sodium ion adduction of the gas-phase H/D exchange of peptides and peptide dimers. *J. Am. Soc. Mass Spectrom.* **2003**, *14*, 1477.
16. Cox, H. A.; Julian, R. R.; Lee, S.-W.; Beauchamp, J. L. Gas-phase H/D exchange of sodiated glycine oligomers with ND₃: Exchange kinetics do not reflect parent ion structures. *J. Am. Chem. Soc.* **2004**, *126*, 6485.

17. Robinson, E. W.; Williams, E. R. Multidimensional separations of ubiquitin conformers in the gas phase: Relating ion cross sections to H/D exchange measurements. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 1427.
18. Rožman, M.; Bertoša, B.; Klasinc, L.; Sric, D. Gas phase H/D exchange of sodiated amino acids: Why do we see zwitterions? *J. Am. Soc. Mass Spectrom.* **2006**, *17*, 29.
19. Gard, E.; Willard, D.; Bregar, J.; Green, M. K.; Lebrilla, C. B. The site-specificity in the H-D exchange reactions of gas-phase protonated amino-acids with CH₃OD. *Org. Mass Spectrom.* **1993**, *28*, 1632.
20. Wyttenbach, T.; Bowers, M. T. Gas phase conformations of biological molecules: The hydrogen/deuterium exchange mechanism. *J. Am. Soc. Mass Spectrom.* **1999**, *10*, 9.
21. Zhu, C.; Balta, B.; Aviyente, V.; Lifshitz, C. The interaction of protonated diglycine with ammonia: A density functional theory model study. *J. Phys. Chem. A* **2000**, *104*, 7061.
22. Hoyau, S.; Ohanessian, G. Interaction of alkali metal cations (Li⁺–Cs⁺) with glycine in the gas phase. *Chem. Eur. J.* **1998**, *4*, 1561.
23. Hoyau, S.; Pelicier, J.-P.; Rogalewicz, F.; Hoppilliard, Y.; Ohanessian, G. Complexation of lycine by atomic metal cations in the gas phase. *Eur. J. Mass Spectrom.* **2001**, *7*, 303.
24. Kapota, C.; Lemaire, J.; Maître, P.; Ohanessian, G. Vibrational signature of charge solvation and salt bridge isomers of sodiated amino acids in the gas phase. *J. Am. Chem. Soc.* **2004**, *126*, 1836.
25. Polfer, N. C.; Paizs, B.; Snoek, I.; Compagnon, I.; Suhai, S.; von Helden, G.; Meijer, G.; Oomens, J. Infrared fingerprint spectroscopy and theoretical studies of potassium ion tagged amino acids and peptides in the gas phase. *J. Am. Chem. Soc.* **2005**, *127*, 8571.
26. Polfer, N. C.; Oomens, J.; Moore, D. T.; von Helden, G.; Meijer, G.; Dunbar, R. C. Infrared spectroscopy of phenylalanine Ag(I) and Zn(II) complexes in the gas phase. *J. Am. Chem. Soc.* **2006**, *128*, 517.
27. Polfer, N. C.; Oomens, J.; Dunbar, R. C. IRMPD spectroscopy of metal-ion/tryptophan complexes. *Phys. Chem. Chem. Phys.* **2006**, *8*, 2744.
28. Kamariotis, A.; Boyarkin, O. V.; Mercier, S. R.; Beck, R. D.; Bush, M. F.; Williams, E. R.; Rizzo, T. R. Infrared spectroscopy of hydrated amino acids in the gas phase: protonated and lithiated valine. *J. Am. Chem. Soc.* **2006**, *128*, 905.
29. Valle, J. J.; Eyler, J. R.; Oomens, J.; Moore, D. T.; van der Meer, A. F. G.; von Helden, G.; Meijer, G.; Hendrickson, C. L.; Marshall, A. G.; Blakney, G. T. A free electron Fourier-transform ion cyclotron resonance mass spectrometry facility for obtaining infrared multiphoton dissociation spectra of gaseous ions. *Rev. Sci. Instrum.* **2005**, *76*, 23103.
30. Oepts, D.; van der Meer, A. F. G.; van Amersfoort, P. W. The free-electron-laser user facility FELIX. *Infrared Phys. Technol.* **1995**, *36*, 297.
31. Bagratashvili, V. N.; Letokov, V. S.; Makarov, A. A.; Ryabov, E. A. Multiple Photon Infrared Laser Photophysics and Photochemistry; Harwood: Chur, Switzerland, 1985.
32. Oomens, J.; Sartakov, B.; Meijer, G.; von Helden, G. Gas-phase infrared multiple photon dissociation spectroscopy of mass-selected molecular ions. *Int. J. Mass Spectrom.* **2006**, *254*, 1.
33. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A. J.; Stratman, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, A. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gombertz, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanyakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S. and Pople, J. A. *GAUSSIAN 03*; Gaussian Inc.: Pittsburgh, PA, 2003.
34. Rožman, M.; Kazazic, S.; Klasinc, L.; Sric, D. Kinetics of gas-phase hydrogen/deuterium exchange and gas-phase structure of protonated phenylalanine, proline, tyrosine, and tryptophan. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 2769.