

# Unfolding of Hydrated Alkyl Diammonium Cations Revealed by Cryogenic Ion Mobility-Mass Spectrometry

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## S Supporting Information

**ABSTRACT:** Hydration of the ammonium ion plays a key role in determining the biomolecular structure as well as local structure of water in aqueous environments. Experimental data obtained by cryogenic ion mobility-mass spectrometry (cryo-IM-MS) show that dehydration of alkyl diammonium cations induces a distinct unfolding transition at a critical number of water molecules,  $n = 21$  to  $23$ ,  $n = 24$  to  $26$ , and  $n = 27$  to  $29$ , for 1,7-diaminoheptane, 1,8-diaminooctane, and 1,10-diaminododecane, respectively. Results are also presented that reveal compelling evidence for unique structural transitions of hydrated ammonium ions associated with the development of the hydrogen-bond network around individual charged groups. The ability to track the evolution of structure upon stepwise dehydration provides direct insight into the intricate interplay between solvent–molecule interactions that are responsible for defining conformations. Such insights are potentially valuable in understanding how ammonium ion solvation influences conformation(s) of larger biomolecules.

A number of competing effects dictate conformational preferences of biomolecules as they transition from solution to a solvent-free environment, including solute–solvent interactions,<sup>1–6</sup> Coulombic repulsion of like charges,<sup>7–10</sup> and/or intramolecular charge solvation.<sup>11–19</sup> These interactions have been studied by theoretical and experimental approaches that probe specific effects of solvation on peptide and protein structure, and it has been established that, for most biological molecules, the preferred water binding sites are the accessible hydrogen bond donors or acceptors and the charged side chains.<sup>1,20</sup> Sequential hydration studies of ammonium, guanidinium, and carboxylate ion groups reveal the size of the first solvation shell(s),<sup>21–26</sup> preferences for self-solvation, and salt-bridge formation,<sup>27–31</sup> and in a number of cases, binding energies of the first few water molecules have been determined.<sup>20,32–38</sup> For multiply charged ions, the interplay between Coulombic repulsion and water–ion interactions has been shown to induce conformational changes during the desolvation processes of electrospray ionization (ESI).<sup>19</sup> Tracking the structure(s) and dynamics of small ions at intermediate extents of hydration in an effort to deconvolute these effects will improve our understanding of ion hydration and the structural preferences of biomolecules.

Here, cryogenic ion mobility-mass spectrometry (cryo-IM-MS) is employed to measure changes in the structures of a series of hydrated doubly charged ions including 1,7-diaminoheptane (DAH), 1,8-diaminooctane (DAO), and 1,10-diaminododecane (DAD) as a function of the numbers ( $n$ ) of attached water molecules. The cryo-IM-MS instrument has been described in detail previously.<sup>6</sup> Ions are generated via ESI and guided into a variable temperature drift tube, which is maintained at  $80 \pm 2$  K via liquid nitrogen cooling. This approach is used to capture hydrated ions formed by ESI that are separated on the basis of size-to-charge (IM) and mass-to-charge ( $m/z$ ) by time-of-flight MS. Cryogenic cooling of the drift tube is required for the preservation of these weakly bound cluster ions that have been shown to evaporate under ambient conditions.<sup>6</sup> Here, we show that hydrated alkyl diammonium cations undergo an abrupt unfolding transition upon stepwise desolvation over a narrow range of water molecules:  $n = 21$  to  $23$  for 1,7-DAH,  $n = 24$  to  $26$  for 1,8-DAO, and  $n = 27$  to  $29$  for 1,10-DAD.

Kebarle and co-workers previously reported data for the sequential attachment of the first few water molecules to alkyl diammonium cations.<sup>39,40</sup> Based on the measured binding energies, they proposed that water molecules bind to both charged groups in an alternating fashion. Studies of larger hydrated clusters reported formation of magic number clusters for hydrated ammonium ions,  $\text{NH}_4^+(\text{H}_2\text{O})_n$ , as well as primary amines; specifically,  $n = 20$  has been attributed to the formation of a clathrate cage structure similar to the compact structure of  $\text{H}^+(\text{H}_2\text{O})_n$  ( $n = 21$ ).<sup>22,41–45</sup> However, the magic number cluster  $n = 40$  has been absent from the spectra of many doubly protonated diammonium ions such as 1,12-diaminododecane and 1,7-DAH, suggesting that both charged groups may favor solvation by a single water droplet as opposed to separate solvation shells.<sup>22,46</sup> Williams and co-workers used IRPD spectroscopy to analyze the sequential hydration of 1,7-DAH with up to 30 associated water molecules and, through a comparison with the singly protonated heptylamine ion, determined that, for clusters with  $>14$  water molecules, the linear molecule begins to fold and form a single droplet that bridges both charged groups;<sup>46</sup> the suberate dianion exhibits similar behavior.<sup>47</sup> Computational studies confirmed that such a folding transition exists for alkyl diammonium cations, but

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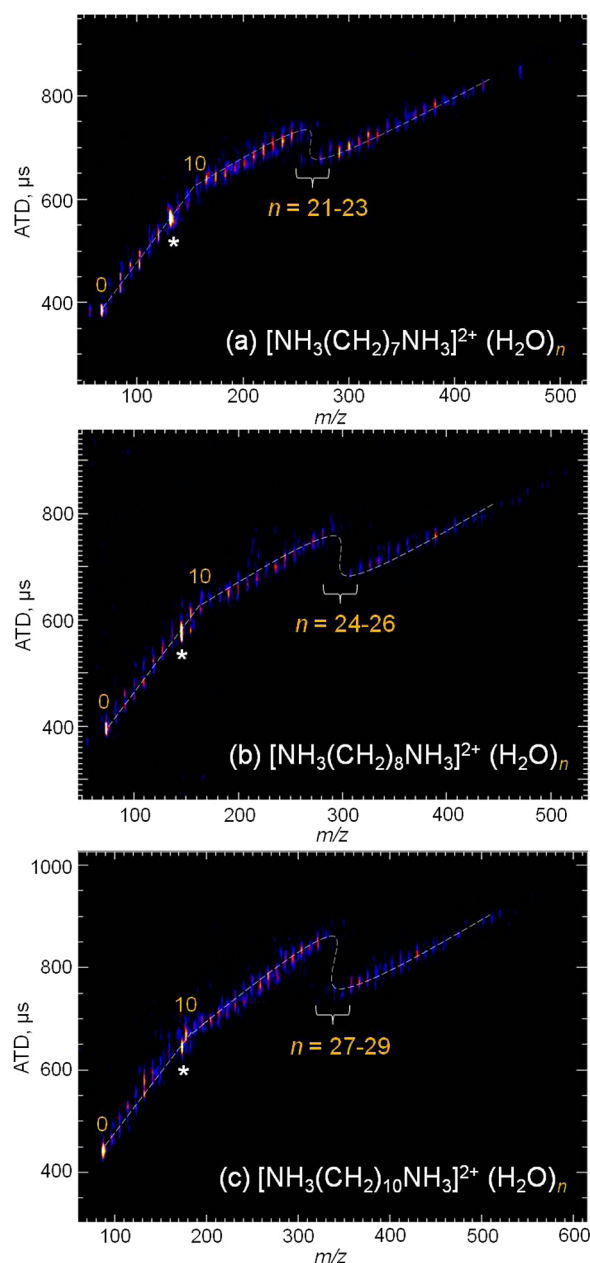


reports on the exact size of the droplet required to bridge the two charged groups have varied. Janhangiri et al. reported the water-induced folding transition using molecular dynamics (MD) simulations, but found evidence for this transition occurring at 22 water molecules for 1,7-DAH and varying as a function of alkyl chain length.<sup>48</sup>

Figure S1 contains ESI mass spectra of 1,7-DAH, 1,8-DAO, and 1,10-DAD  $[\text{NH}_3(\text{CH}_2)_x\text{NH}_3]^{2+}(\text{H}_2\text{O})_n$  ions, where  $x = 7, 8, \text{ or } 10$ , respectively. The relative abundances of the hydrated ions in the mass spectra reflect the relative stabilities of specific cluster ions. Note that magic number clusters are not detected for either dication because both charged groups prefer to be solvated by a single droplet at large numbers of  $n$ .<sup>22,46</sup> The peaks labeled with asterisks (see Figures 1 and S1) fall at  $m/z$  values that correspond to either the dehydrated singly charged  $[\text{M} + \text{H}]^+$  ion or a proton-bound dimer ion,  $[2\text{M} + 2\text{H}]^{2+}$ . Regardless, these ions appear to be formed by a different mechanism because hydrated forms of the ions are not observed. A plausible mechanism is by a charge reduction reaction to form  $[\text{M} + \text{H}]^+$  ions, as was reported previously,<sup>19</sup> i.e., loss of  $\text{H}^+(\text{H}_2\text{O})_n$  ions via proton transfer from  $[\text{M} + 2\text{H}]^{2+}(\text{H}_2\text{O})_n$  ions.

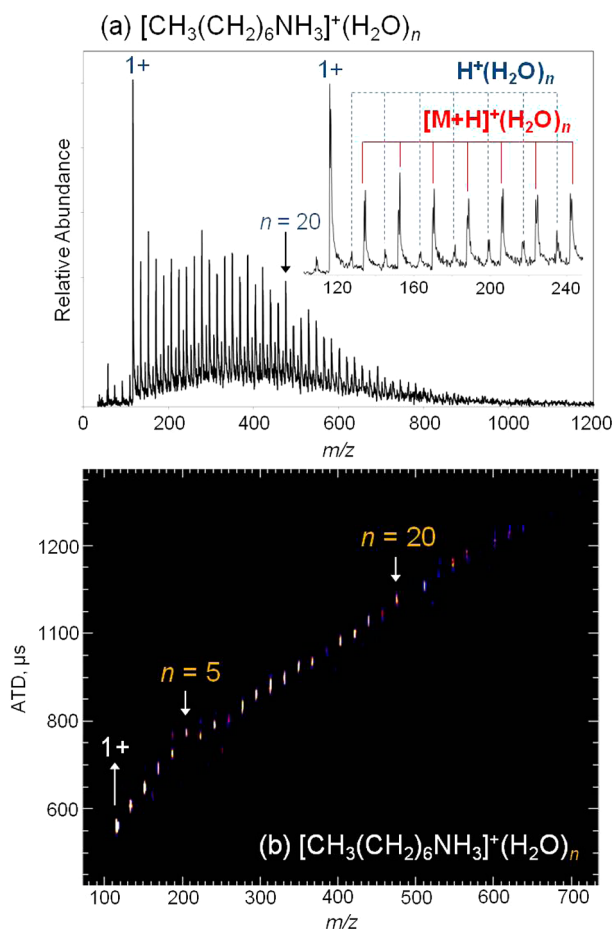
In order to investigate the conformational preferences of the doubly charged ions as a function of the number of attached water molecules, two-dimensional contour plots of ion mobility arrival time distribution (ATD) versus  $m/z$  were generated (Figure 1). The plots reveal a rather obvious discontinuity in the  $[\text{M} + 2\text{H}]^{2+}(\text{H}_2\text{O})_n$  ATD trendlines over a narrow range of water molecules for each dication. This deviation is consistent with an unfolding event upon stepwise dehydration for the  $[\text{M} + 2\text{H}]^{2+}(\text{H}_2\text{O})_n$  ions of each diammonium cation. For 1,7-DAH (Figure 1a), the ATDs for clusters  $n > 23$  fall along a uniform trendline and the structures of these ions are attributed to a folded conformation in which both ammonium ions are solvated by a single droplet. The folded structure is stabilized on the surface of the droplet because energetically favorable solute–solvent interactions compensate for the Coulombic repulsion between the two charged groups and the water molecules effectively bridge the ammonium ions.<sup>46</sup> In this region, loss of sequential water monomers to evaporation results in minor changes to the overall structure of the molecule. As the droplet continues to evaporate, it shrinks in size and repulsive Coulombic interactions between the charge sites force the solvated ion to elongate, splitting the water droplet into two clusters which solvate each ammonium ion separately. The resulting elongated ion conformation is accompanied by an increase in arrival time for the “unfolded” hydrated clusters, clearly evident in the mobility trendline shown in Figure 1a. For 1,7-DAH, this transition occurs over the range  $n = 21$  to 23, consistent with computational studies reported by Peshlherbe and co-workers.<sup>48</sup> The longer alkyl chain lengths of 1,8-DAO and 1,10-DAD result in a shift of the unfolding transition to larger cluster ions owing to the increased separation of charged groups in the molecule and subsequent increase in water molecules required to bridge between the ammonium ions. The 2-D plots shown in Figure 1b and 1c confirm that this is the case for 1,8-DAO and 1,10-DAD, as an identical transition is observed over the range  $n = 24$  to 26 and  $n = 27$  to 29, respectively.

The proposed transition, from a folded structure in which both ammonium ions are solvated by a single water cluster at larger numbers of  $n$  to an elongated structure in which the ammonium ions are solvated separately, was tested by



**Figure 1.** Two-dimensional contour plots of ATD versus  $m/z$  for  $[\text{M} + 2\text{H}]^{2+}(\text{H}_2\text{O})_n$  ions of (a) 1,7-diaminoheptane (DAH), (b) 1,8-diaminooctane (DAO), and (c) 1,10-diaminododecane (DAD). All solutions were prepared at a concentration of 500  $\mu\text{M}$  with pure water (18 M $\Omega$ ) containing 0.1% formic acid, and the heated capillary ion inlet temperature was maintained between 338 and 349 K in order to control the extent of desolvation. The dashed lines are included to guide the eye along the  $[\text{M} + 2\text{H}]^{2+}(\text{H}_2\text{O})_n$  trendlines. The peaks labeled with an asterisk correspond to either the  $[\text{M} + \text{H}]^+$  ion or a proton-bound dimer,  $[2\text{M} + 2\text{H}]^{2+}$ .

comparing the hydration of heptylamine. The heptylammonium ion,  $[\text{CH}_3(\text{CH}_2)_6\text{NH}_3]^+$ , has a single ammonium group that will serve as the primary site of hydration; consequently, this ion should not adopt a folded hydrated structure.<sup>46</sup> The mass spectrum of heptylamine is shown in Figure 2, and both the  $[\text{CH}_3(\text{CH}_2)_6\text{NH}_3]^+(\text{H}_2\text{O})_n$  and  $\text{H}^+(\text{H}_2\text{O})_n$  series of hydrated clusters are observed. The 2-D mobility plot of  $[\text{CH}_3(\text{CH}_2)_6\text{NH}_3]^+(\text{H}_2\text{O})_n$  ions (Figure 2b) reveals a single hydration trendline which appears uniform in the region  $n = 20$



**Figure 2.** (A) ESI mass spectrum and (B) 2-D contour plot of ATD versus  $m/z$  for heptylammonium  $[\text{CH}_3(\text{CH}_2)_6\text{NH}_3]^+(\text{H}_2\text{O})_n$  ions. The inset in panel (A) contains an expanded view of the region surrounding  $[\text{CH}_3(\text{CH}_2)_6\text{NH}_3]^+(\text{H}_2\text{O})_n$  ( $n = 0$  to  $7$ ). In addition to hydrated heptylammonium ions, protonated water clusters are formed from ESI in small abundance.

to 30, indicating similarly structured conformer populations in this range that are consistent with elongated structures hydrated by a single solvation droplet around the ammonium ion.

The 2-D plot of the heptylammonium ion reveals a more subtle deviation in the trendline at  $n = 5$  to  $6$  that may shed light on a similar deviation observed in the low-mass region of the spectra for the diammonium cations shown in Figure 1. There, a second structural transition is observed at  $n = 10$  to  $11$ , evident from the abrupt change in slope observed for ATDs falling on the  $[\text{M} + 2\text{H}]^{2+}(\text{H}_2\text{O})_n$  trendlines. Previous cryo-IM-MS studies of protonated water clusters,  $\text{H}^+(\text{H}_2\text{O})_n$ , elucidated structural transitions for small ions corresponding to changes associated with the evolution of the hydrogen-bond network around the excess proton.<sup>49</sup> Upon desolvation, clusters transitioned from 2-D net-type structures into elongated chain structures over the range  $n = 8$  to  $11$ . This transition was characterized by the change in slope observed for ATDs along the hydration trendline indicating structural differences in the two types of clusters. Such a transition is predictable from spectroscopic results,<sup>50–52</sup> but the structural implications are directly revealed in the cryo-IM-MS hydration trendlines. The transition observed at  $n = 10$  for 1,7-DAH, 1,8-DAO, and 1,10-

DAD may result from a similar development of the H-bond network around the ammonium ion.

The deviation in the trendline of hydrated clusters is observed at  $n = 10$  to  $11$  for the diammonium cations whereas a similar deviation is observed at  $n = 5$  to  $6$  for the heptylammonium ion, a difference that is consistent with symmetric solvation of the two charged groups. This supports the assignment of these deviations to structural changes in the H-bond network surrounding the ammonium ion, consistent with interpretation of spectroscopic data. Williams and co-workers probed the free-OH stretch spectral regions of 1,7-DAH hydrated clusters and showed that bands associated with one-coordinated water molecules, which are present in chain-like structures, dropped off sharply at  $n = 10$ , whereas the band attributed to three-coordinated water molecules, present in larger 2-D net structures, began to grow in simultaneously at  $n = 9$ .<sup>46</sup> This suggests that, for 1,7-DAH, the transition from chain-like structures into net-like structures should occur around  $n = 10$ , with five water molecules surrounding each charged group separately. IRPD spectra of hydrated heptylammonium clusters revealed a similar decrease in intensity of the free-OH stretches corresponding to one-coordinated water molecules at  $\sim n = 5$  to  $6$ .<sup>46</sup> Again, this is consistent with the transitions observed in the 2-D plots for the diammonium cations shown in Figure 1, as well as the deviation observed at  $n = 5$  for the heptylammonium ion (Figure 2), which possesses only a single charged group.

Collectively, results indicate that the structural evolution of 1,7-DAH upon desolvation is characterized by multiple transitions. While the water-induced unfolding transition was predicted based on spectroscopic studies, results presented here definitively establish that the transition occurs at a specific, narrow range of water molecules. When greater than 23 water molecules are bound to the ion, it adopts a folded conformation in which both charged groups are brought into close proximity by solvation from a single droplet of water. Loss of water monomers through evaporation coupled with Coulombic repulsion then promotes the elongation and separation of the ammonium ions located on opposite ends of the molecule. This unfolding occurs abruptly over the range  $n = 21$  to  $23$  for 1,7-DAH and varies as a function of alkyl chain length. Clusters smaller than  $[\text{M} + 2\text{H}]^{2+}(\text{H}_2\text{O})_n$  ( $n = 21$ ) exist as extended structures in which both charged groups are solvated by separate droplets. In this range, the water molecules solvating the ammonium ions form an interconnected network of H-bonds made up of two- and three-coordinated water molecules. Beginning at  $n = 10$ , loss of water monomers results in expansion of the net-type hydrated ions into chain-like structures possessing one-coordinated water molecules. This transition has been previously observed for protonated water clusters,  $\text{H}^+(\text{H}_2\text{O})_n$ , but is shown to occur over a different range indicating subtle differences between hydration of the ammonium and hydronium ions. The ammonium ion is present in a wide range of peptides and proteins and plays an essential role in the structural preferences of these molecules, especially in the presence of water. Understanding the hydration behavior and conformational changes of small systems as a function of the number of waters attached provides insight into the interactions, hydrations, and structural transitions that would be expected in more complex biological molecules.

## ■ ASSOCIATED CONTENT

## ● Supporting Information

ESI mass spectra of diammonium cations shown in Figure 1. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b05448.

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## Notes

The authors declare no competing financial interest.

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## ■ REFERENCES

- Wytenbach, T.; Liu, D.; Bowers, M. T. *Int. J. Mass Spectrom.* **2005**, *240*, 221–232.
- Barran, P. E.; Polfer, N. C.; Campopiano, D. J.; Clarke, D. J.; Langridge-Smith, P. R. R.; Langley, R. J.; Govan, J. R. W.; Maxwell, A.; Dorin, J. R.; Millar, R. P.; Bowers, M. T. *Int. J. Mass Spectrom.* **2005**, *240*, 273–284.
- Wytenbach, T.; Bowers, M. T. *Annu. Rev. Phys. Chem.* **2007**, *58*, 511–533.
- Wytenbach, T.; Bowers, M. T. *Chem. Phys. Lett.* **2009**, *480*, 1–16.
- Pierson, N. A.; Chen, L. X.; Valentine, S. J.; Russell, D. H.; Clemmer, D. E. *J. Am. Chem. Soc.* **2011**, *133*, 13810–13813.
- Silveira, J. A.; Servage, K. A.; Gamage, C. M.; Russell, D. H. *J. Phys. Chem. A* **2013**, *117*, 953–961.
- Gross, D. S.; Williams, E. R. *J. Am. Chem. Soc.* **1995**, *117*, 883–890.
- Serner, J. L.; Johnston, M. V.; Nicol, G. R.; Ridge, D. P. *J. Am. Soc. Mass Spectrom.* **1999**, *10*, 483–491.
- Chang, T. M.; Prell, J. S.; Warrick, E. R.; Williams, E. R. *J. Am. Chem. Soc.* **2012**, *134*, 15805–15813.
- Skinner, O. S.; McLafferty, F. W.; Breuker, K. *J. Am. Soc. Mass Spectrom.* **2012**, *23*, 1011–1014.
- Loo, J. A. *Mass Spectrom. Rev.* **1997**, *16*, 1–23.
- Rodriguez-Cruz, S. E.; Klassen, J. S.; Williams, E. R. *J. Am. Soc. Mass Spectrom.* **1997**, *8*, 565–568.
- Breuker, K.; McLafferty, F. W. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 18145–18152.
- Breuker, K.; Brueschweiler, S.; Tollinger, M. *Angew. Chem., Int. Ed.* **2011**, *50*, 873–877.
- Wytenbach, T.; Bowers, M. T. *J. Phys. Chem. B* **2011**, *115*, 12266–12275.
- Nagornova, N. S.; Rizzo, T. R.; Boyarkin, O. V. *Science* **2012**, *336*, 320–323.
- Silveira, J. A.; Fort, K. L.; Kim, D.; Servage, K. A.; Pierson, N. A.; Clemmer, D. E.; Russell, D. H. *J. Am. Chem. Soc.* **2013**, *135*, 19147–19153.
- Fort, K. L.; Silveira, J. A.; Pierson, N. A.; Servage, K. A.; Clemmer, D. E.; Russell, D. H. *J. Phys. Chem. B* **2014**, *118*, 14336–14344.
- Servage, K. A.; Silveira, J. A.; Fort, K. L.; Russell, D. H. *J. Phys. Chem. B* **2015**, *119*, 4693–4698.
- Liu, D.; Wytenbach, T.; Barran, P. E.; Bowers, M. T. *J. Am. Chem. Soc.* **2003**, *125*, 8458–8464.
- Misaizu, F.; Sanekata, M.; Fuke, K.; Iwata, S. *J. Chem. Phys.* **1994**, *100*, 1161–1170.
- Lee, S. W.; Freivogel, P.; Schindler, T.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1998**, *120*, 11758–11765.
- Robertson, W. H.; Diken, E. G.; Price, E. A.; Shin, J. W.; Johnson, M. A. *Science* **2003**, *299*, 1367–1372.
- Walters, R. S.; Pillai, E. D.; Duncan, M. A. *J. Am. Chem. Soc.* **2005**, *127*, 16599–16610.
- Cooper, T. E.; O'Brien, J. T.; Williams, E. R.; Armentrout, P. B. *J. Phys. Chem. A* **2010**, *114*, 12646–12655.
- O'Brien, J. T.; Williams, E. R. *J. Phys. Chem. A* **2011**, *115*, 14612–14619.
- Wytenbach, T.; Paizs, B.; Barran, P.; Breci, L.; Liu, D.; Suhai, S.; Wysocki, V. H.; Bowers, M. T. *J. Am. Chem. Soc.* **2003**, *125*, 13768–13775.
- Lemoff, A. S.; Bush, M. F.; Wu, C. C.; Williams, E. R. *J. Am. Chem. Soc.* **2005**, *127*, 10276–10286.
- Bush, M. F.; Prell, J. S.; Saykally, R. J.; Williams, E. R. *J. Am. Chem. Soc.* **2007**, *129*, 13544–13553.
- Drayß, M. K.; Blunk, D.; Oomens, J.; Polfer, N.; Schmuck, C.; Gao, B.; Wytenbach, T.; Bowers, M. T.; Schäfer, M. *Int. J. Mass Spectrom.* **2009**, *281*, 97–100.
- Gao, B.; Wytenbach, T.; Bowers, M. T. *J. Phys. Chem. B* **2009**, *113*, 9995–10000.
- Meot-Ner, M.; Field, F. H. *J. Am. Chem. Soc.* **1974**, *96*, 3168–3171.
- Klassen, J. S.; Blades, A. T.; Kebarle, P. J. *J. Phys. Chem.* **1995**, *99*, 15509–15517.
- Lemoff, A. S.; Bush, M. F.; Williams, E. R. *J. Am. Chem. Soc.* **2003**, *125*, 13576–13584.
- Ye, S. J.; Moision, R. M.; Armentrout, P. B. *Int. J. Mass Spectrom.* **2005**, *240*, 233–248.
- Wincel, H. *J. Phys. Chem. A* **2007**, *111*, 5784–5791.
- Gao, B.; Wytenbach, T.; Bowers, M. T. *J. Am. Chem. Soc.* **2009**, *131*, 4695–4701.
- Donald, W. A.; Leib, R. D.; Demireva, M.; Williams, E. R. *J. Am. Chem. Soc.* **2011**, *133*, 18940–18949.
- Klassen, J. S.; Blades, A. T.; Kebarle, P. J. *J. Am. Chem. Soc.* **1994**, *116*, 12075–12076.
- Blades, A. T.; Klassen, J. S.; Kebarle, P. J. *J. Am. Chem. Soc.* **1996**, *118*, 12437–12442.
- Khan, A. *Chem. Phys. Lett.* **2001**, *338*, 201–207.
- Diken, E. G.; Hammer, N. I.; Johnson, M. A.; Christie, R. A.; Jordan, K. D. *J. Chem. Phys.* **2005**, *123*, 164309.
- Douady, J.; Calvo, F.; Spiegelman, F. *J. Chem. Phys.* **2008**, *129*, 154305.
- Willow, S. Y.; Singh, N. J.; Kim, K. S. *J. Chem. Theory Comput.* **2011**, *7*, 3461–3465.
- Chang, T. M.; Cooper, R. J.; Williams, E. R. *J. Am. Chem. Soc.* **2013**, *135*, 14821–14830.
- Demireva, M.; O'Brien, J. T.; Williams, E. R. *J. Am. Chem. Soc.* **2012**, *134*, 11216–11224.
- Yang, X.; Fu, Y.-J.; Wang, X.-B.; Slavíček, P.; Mucha, M.; Jungwirth, P.; Wang, L. S. *J. Am. Chem. Soc.* **2004**, *126*, 876–883.
- Jahangiri, S.; Legris-Falardeau, V.; Peshherbe, G. H. *Chem. Phys. Lett.* **2015**, *621*, 85–90.
- Servage, K. A.; Silveira, J. A.; Fort, K. L.; Russell, D. H. *J. Phys. Chem. Lett.* **2014**, *5*, 1825–1830.
- Jiang, J.-C.; Wang, Y.-S.; Chang, H.-C.; Lin, S. H.; Lee, Y. T.; Niedner-Schatteburg, G.; Chang, H.-C. *J. Am. Chem. Soc.* **2000**, *122*, 1398–1410.
- Shin, J. W.; Hammer, N. I.; Diken, E. G.; Johnson, M. A.; Walters, R. S.; Jaeger, T. D.; Duncan, M. A.; Christie, R. A.; Jordan, K. D. *Science* **2004**, *304*, 1137–1140.
- Headrick, J. M.; Diken, E. G.; Walters, R. S.; Hammer, N. I.; Christie, R. A.; Cui, J.; Myshakin, E. M.; Duncan, M. A.; Johnson, M. A.; Jordan, K. D. *Science* **2005**, *308*, 1765–1769.