RESEARCH ARTICLE

Structural and Energetic Effects in the Molecular Recognition of Acetylated Amino Acids by 18-Crown-6

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Abstract

Absolute 18-crown-6 (18C6) binding affinities of four protonated acetylated amino acids (AcAAs) are determined using guided ion beam tandem mass spectrometry techniques. The AcAAs examined in this work include: N-terminal acetylated lysine (N_α -AcLys), histidine (N_α -AcHis), and arginine (N_α -AcArg) as well as side chain acetylated lysine (N_ϵ -AcLys). The kinetic-energy-dependent cross sections for collision-induced dissociation (CID) of the $(\text{AcAA})\text{H}^+(18\text{C6})$ complexes are analyzed using an empirical threshold law to extract absolute 0 and 298 K $(\text{AcAA})\text{H}^+-18\text{C6}$ bond dissociation energies (BDEs) after accounting for the effects of multiple collisions, kinetic and internal energy distributions of the reactants, and unimolecular dissociation lifetimes. Theoretical electronic structure calculations are performed to determine stable geometries and energetics for neutral and protonated 18C6 and the AcAAs as well as the proton bound complexes of these species, $(\text{AcAA})\text{H}^+(18\text{C6})$, at the B3LYP/6-311+G(2d,2p)//B3LYP/6-31 G* and M06/6-311+G(2d,2p)//B3LYP/6-31 G* levels of theory. For all four $(\text{AcAA})\text{H}^+(18\text{C6})$ complexes, loss of neutral 18C6 corresponds to the most favorable dissociation pathway. At elevated energies, products arising from sequential dissociation of the primary CID product, $\text{H}^+(\text{AcAA})$, are also observed. Protonated N_α -AcLys exhibits a greater 18C6 binding affinity than other protonated N_α -AcAAs, suggesting that the side chains of Lys residues are the preferred binding sites for 18C6 complexation to peptides and proteins. N_α -AcLys exhibits a greater 18C6 binding affinity than N_ϵ -AcLys, suggesting that binding of 18C6 to the side chain of Lys residues is more favorable than to the N-terminal amino group of Lys.

Key words: Acetylated amino acids, Binding affinities, Collision-induced dissociation, 18-Crown-6, Hydrogen-bonding interactions, Molecular recognition

Introduction

Protein structures and protein–protein interactions play critical roles in all biological processes. As a result, studies aimed at the characterization and improved understanding of the three-dimensional structure of proteins and

the intra- and intermolecular interactions that stabilize their structures and complexes abound. These studies provide information key to understanding functional behavior in biological systems, and will therefore become increasingly pursued as the field of proteomics matures and evolves.

A variety of MS approaches have been used to characterize protein structure and intra- and intermolecular interactions that stabilize their structures such as H/D exchange [1–6], chemical cross-linking [7–15], and selective noncovalent adduct protein probing (SNAPP) [16–25]. SNAPP has been developed to exploit protein structure and folding states. The SNAPP method utilizes noncovalent

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recognition of amino acid residues, and in particular lysine (Lys) residues, to facilitate rapid identification and characterization of protein sequence, structure and conformational changes. In this approach, 18C6 was selected as the protein side chain tag because of its specificity for Lys side chains. The extent of 18C6 adduction to Lys side chains is determined by the number of accessible Lys side chains (i.e., those that are not involved in intramolecular interactions such as hydrogen bonds or salt bridges). Intramolecular interactions generally prevent the attachment of 18C6 and are directly correlated to the structure of the protein. Therefore, the number of 18C6 ligands that bind is also directly correlated to protein structure. Because the number of 18C6 ligands that bind to a protein can be easily determined by MS due to the large mass shift (264 Da per 18C6 ligand bound), protein structure and folding under varying solution conditions can be extrapolated.

The use of molecular recognition of crown ethers by various protein sequences and structures has also been pursued for a variety of other protein structure, function, and separation applications. Robinson and coworkers reported a novel charge reduction approach that is based on the collision-induced removal of noncovalently attached aza-18C6 from the charged side chain of tetrameric human transthyretin [26]. The highly selective binding of the crown ether to the protein contributes to the low quantity of aza-18C6 required, and reduces the chance of unintended side reactions in solution. Reduction of the charge state using molecular recognition of aza-18C6 does not cause dramatic structural change. Therefore, it significantly improves the stability of protein complexes, and protects the native state of proteins. Oshima and coworkers employed dicyclohexano-18C6 (DCH18C6) as an affinity ligand to extract the lysine-rich protein cytochrome *c* in Li_2SO_4 /polyethylene glycol (PEG) aqueous two-phase system [27]. Cytochrome *c* was quantitatively extracted into the PEG-rich phase in the presence of DCH18C6 within 5 minutes. Schneider and coworkers developed a strategy, using crown ethers as scaffolds for protein surface target recognition to explore protein folding and the mechanism of ligand binding. They designed a peptide receptor with 18C6 at one binding site for interaction with the peptide N-terminus and a peralkylammonium group as the other binding site for interaction with the C-terminus. In this work, the zwitterionic form of the unprotected tripeptide, Gly-Trp-Gly, was employed as a model peptide to develop a method for peptide differentiation based on length, amino acid composition, sequence, and the configuration of peptides and proteins [28, 29].

Although the protonated side chain of Lys has been shown to be the primary binding site for 18C6 complexation, the protonated side chains of His, Arg, and the N-terminal amino group may also compete for 18C6. Therefore, accurate thermochemical information regarding the binding between 18C6 and the basic amino acids may provide insight into the selectivity of the complexation process. However, very limited thermochemical data has

thus far been reported in the literature. In previous work, we examined the interactions between 18C6 and a series of protonated peptidomimetic bases that serve as mimics of the N-terminal amino group and the side chains of the basic amino acids in peptides and proteins [30]. The Lys mimic, *N*-butylamine (NBA), exhibits a higher 18C6 binding affinity than that of the His mimic, 4-methylimidazole (4MeIMID), and the Arg mimic, 1-methylguanidine (MGD), suggesting that Lys residues are the preferred binding sites for 18C6 complexation. The N-terminal amino group mimic, isopropylamine (IPA), exhibits a slightly higher binding affinity than that of the Lys mimic, NBA, suggesting that the N-terminal amino group can also serve as a competitive binding site for 18C6 complexation. In a follow-up investigation, we extended this work to include examination of the interactions between 18C6 and five protonated amino acids: glycine (Gly), Alanine (Ala), Lys, His, and Arg [31]. The measured 18C6 binding affinities of the protonated amino acids follow the order: Gly > Ala > Lys > His > Arg. Amongst the basic amino acids, Lys exhibits the highest binding affinity for 18C6, confirming that the side chains of Lys residues are the preferred binding site for 18C6. However, Gly and Ala exhibit greater binding affinities for 18C6, suggesting that the N-terminal amino group may indeed serve as a favorable binding site for 18C6.

Theoretical calculations indicate that in the complexes to His and Arg, the preferred binding site for 18C6 is to the protonated backbone amino group, rather than the side chain. Therefore, the trends in the 18C6 binding affinities of the protonated amino acids determined in that work [31] do not represent the trends in the binding affinities of the side chains of Lys, His, and Arg. In addition, intermolecular and intramolecular interactions can prevent complexation of 18C6 to the side chains of amino acid AA residues in peptides and proteins. Due to the conformational flexibility of peptides and proteins, the backbone may also influence the complexation of 18C6 with the protonated AA residue side chains. Therefore, we extend this work to include acetylated AAs as additional models for noncovalent interactions between 18C6 and peptides or proteins. N_α -acetylation ensures side chain binding, while side chain acetylation results in preferred binding of 18C6 to the N-terminal amino group. In order to investigate backbone effects on the molecular recognition of the basic AAs by 18C6, absolute 18C6 affinities of four acetylated AAs are determined here using guided ion beam tandem mass spectrometry techniques. The acetylated AAs examined here include: N_α -AcLys, N_ϵ -AcLys, N_α -AcHis, and N_α -AcArg, as shown schematically in the model peptide of Figure 1. The energy-dependent cross sections for collision-induced dissociation (CID) of the $(\text{AcAA})\text{H}^+(18\text{C6})$ complexes are analyzed using methods previously developed that explicitly include the effects of the kinetic and internal energy distributions of the reactants, multiple ion-neutral collisions, and the kinetics of unimolecular dissociation. Absolute $(\text{AcAA})\text{H}^+ - 18\text{C6}$ bond dissociation energies (BDEs) for four $(\text{AcAA})\text{H}^+(18\text{C6})$ complexes are

Figure 1. Model peptide showing the structures of the acetylated amino acids examined here including: N_ε-AcLys, N_α-AcLys, N_α-AcHis, and N_α-AcArg

derived and compared to theoretical estimates for these BDEs computed here. The effects of acetylation on the 18C6 binding affinities of the AAs are assessed by comparing present results to those for the AAs previously investigated [31].

Experimental

General Procedures

Cross sections for CID of four protonated acetylated amino acid-18C6 complexes, (AcAA)H⁺(18C6) with Xe, where AcAA = N_α-AcLys, N_ε-AcLys, N_α-AcHis, and N_α-AcArg are measured using a guided ion beam tandem mass spectrometer that has been described in detail previously [32]. The (AcAA)H⁺(18C6) complexes are generated by electrospray ionization (ESI) under conditions similar to those described previously [30, 31]. The ions are effusively sampled from the source region, focused, accelerated, and focused into a magnetic sector momentum analyzer for mass analysis. Mass-selected ions are decelerated to a well-defined kinetic energy and focused into an rf octopole ion guide that traps the ions in the radial direction. The octopole passes through a static gas cell containing Xe at low pressure (~0.05–0.20 mTorr) to ensure that multiple ion-neutral collisions are improbable. After collision, products and remaining reactant ions drift to the end of the octopole, are focused into a quadrupole mass filter for mass analysis, and subsequently detected with a secondary electron scintillation detector and standard pulse counting techniques.

Data Handling

Ion intensities, measured as a function of collision energy, are converted to absolute CID cross sections as described previously [33]. Uncertainties in the absolute and relative cross sections are approximately ±20% and ±5%, respectively. The absolute zero and distribution of the ion kinetic energies are determined using an rf octopole ion guide as a retarding potential analyzer as previously described [33]. The ion kinetic energy distributions are found to be Gaussian with a full width at half maximum (FWHM)

between 0.2 and 0.5 eV (lab) for these experiments. The uncertainty in the absolute energy scale is ±0.05 eV (lab). Ion kinetic energies in the laboratory frame, E_{lab} , are converted to energies in the center-of-mass frame, E_{CM} , using the formula $E_{\text{CM}} = E_{\text{lab}}m/(m + M)$, where M and m are the masses of the ionic and neutral reactants, respectively. All energies reported below are in the center-of-mass (CM) frame unless otherwise noted.

Thermochemical Analysis

The threshold regions of the CID cross sections are modeled using procedures developed elsewhere [33–35], as described previously for similar systems [30, 31]. Details of the analysis procedures, which include explicitly accounting for the kinetic and internal energy distributions of the reactants, the effects of multiple ion-neutral collisions, and the lifetime of the dissociation ions using a loose phase space limit (PSL) transition state (TS) are provided in the [Supplemental Information](#).

Theoretical Calculations

To obtain stable geometries, vibrational frequencies, and energetics for neutral and protonated 18C6 and the AcAAs, as well as the proton bound (AcAA)H⁺(18C6) complexes, theoretical calculations were performed using HyperChem [36] and the Gaussian 09 [37] suites of programs. Neutral and protonated 18C6 and the AcAAs as well as the (AcAA)H⁺(18C6) complexes exhibit many stable low-energy structures. Therefore, potential low-energy candidate structures were obtained via a simulated annealing procedure employing the Amber force field. The most stable conformers accessed at the end of each annealing cycle were subjected to additional analysis. All structures within 30 kJ/mol of the lowest-energy structure found via the simulated annealing procedure, as well as others representative and encompassing the entire range of structures found were further optimized using density function theory. Additional details regarding the simulated annealing procedure can be found in the [Supplemental Information](#).

Geometry optimizations for neutral and protonated 18C6 and the AcAAs as well as the proton bound (AcAA)H⁺(18C6) complexes were performed using density functional theory at the B3LYP/6-31G* level of theory [38, 39]. Vibrational analyses of the geometry-optimized structures were performed to determine the vibrational frequencies of the optimized species for use in modeling of the CID data and to allow zero-point energy (ZPE) corrections to be determined. The frequencies calculated were scaled by a factor of 0.9804 [40]. The scaled vibrational frequencies and rotational constants are listed in Tables S1 and S2 of the [Supplemental Information](#). Single-point energy calculations were performed at the B3LYP/6-311+G(2d,2p) and M06/6-311+G(2d,2p) levels of theory using the B3LYP/6-31G* optimized geometries. To obtain accurate energetics, ZPE and basis set super position

error (BSSE) corrections using the counterpoise approach [41, 42] are included in the computed (AcAA)H⁺–18C6 BDEs and proton affinities (PAs) of the AcAAs.

Polarizability is one of the key factors that contribute to the strength of noncovalent interactions. Thus, the isotropic molecular polarizabilities of the ground-state conformations of the neutral and protonated AcAAs and 18C6 are calculated using PBE0 hybrid functional and the 6-311+G(2d,2p) basis set using the B3LYP/6-31G* optimized geometries. This level of theory was chosen because polarizabilities determined using the PBE0 functional [43] exhibit much better agreement with experimentally determined polarizabilities than the B3LYP and M06 functionals employed for energetics here [44].

Results

Cross Sections for Collision-Induced Dissociation

Experimental cross sections were obtained for the interaction of Xe with four (AcAA)H⁺(18C6) complexes, where AcAA = N_α-AcLys, N_ε-AcLys, N_α-AcHis, and N_α-AcArg. Figure 2 shows representative data for the (N_α-AcLys)H⁺(18C6) complex. Experimental cross sections for the other (AcAA)H⁺(18C6) complexes are shown in Figure S1 of the [Supplemental Information](#). The most favorable process for all complexes is loss of an intact 18C6 ligand in the CID reactions 1.

At elevated energies, products arising from the sequential dissociation of the primary H⁺(AcAA) CID product were also

Figure 2. Cross sections for collision-induced dissociation of the (N_α-AcLys)H⁺(18C6) complex with Xe as a function of kinetic energy in the center-of-mass frame (lower *x*-axis) and laboratory frame (upper *x*-axis). Data are shown for a Xe pressure of 0.2 mTorr

observed for all complexes as shown in Figure 2 and Figure S1 of the [Supplemental Information](#). Because the fragmentation of H⁺(AcAA) is not of specific interest here, these minor sequential fragmentation pathways will not be discussed further.

Theoretical Results

Theoretical structures for neutral and protonated 18C6 and the AcAAs as well as the (AcAA)H⁺(18C6) complexes were calculated as described in the [Theoretical Calculations](#) Section. The B3LYP/6-31G* ground-state structures of the (AcAA)H⁺(18C6) complexes are shown in Figure 3. Structures and M06/6-311+G(2d,2p) relative energies of several representative low-energy conformations of the (AcAA)H⁺(18C6) complexes computed here are shown in Figure S2 of the [Supplemental Information](#). Results for select stable low-energy conformations of neutral and protonated 18C6 and the AcAAs are shown in Figure S3 of the [Supplemental Information](#). The (AcAA)H⁺–18C6 BDEs at 0 K calculated at the M06/6-311+G(2d,2p)//B3LYP/6-31G* and B3LYP/6-311+G(2d,2p)//B3LYP/6-31G* levels of theory including ZPE and BSSE corrections, are listed in Table 1. Comparison of the measured and calculated values suggests that the M06 results are most reliable (see [Comparison of Theory and Experiment](#) Section). Therefore, the following discussion will focus on energetics calculated at the M06/6-311+G(2d,2p) level of theory using the B3LYP/6-31G* optimized structures unless otherwise specified.

18C6

The present work is a follow-up to earlier studies where the interactions between a series of protonated peptidomimetic bases and several protonated amino acids and 18C6 were

Figure 3. B3LYP/6-31G* optimized geometries of the ground-state conformers of the (AcAA)H⁺(18C6) complexes

Table 1. (AcAA)H⁺–18C6 Bond Dissociation Enthalpies at 0 K in kJ/mol^a

AcAA	TCID	M06 ^b			B3LYP ^c		
		<i>D_e</i>	<i>D₀</i> ^d	<i>D_{0,BSSE}</i> ^{d,e}	<i>D_e</i>	<i>D₀</i> ^d	<i>D_{0,BSSE}</i> ^{d,e}
N _α -AcLys	179.9 (7.7)	217.3	205.6	192.8	161.5	149.8	139.2
N _ε -AcLys	172.4 (5.8)	204.0	192.0	177.5	144.1	132.1	119.4
N _α -AcArg	137.3 (5.2)	167.3	157.9	146.9	127.5	115.1	106.1
N _α -AcHis	129.9 (5.3)	154.7	148.4	137.8	116.5	110.2	101.3
AEU/MAD	6.0 (1.2)		21.1 (3.2)	8.9 (3.3)		28.3 (9.4)	38.4 (11.1)

^a Present results, uncertainties are listed in parentheses.^b Calculated at M06/6-311+G(2d,2p)//B3LYP/6-31G* level of theory.^c Calculated at B3LYP/6-311+G(2d,2p)//B3LYP/6-31G* level of theory.^d Including ZPE corrections with B3LYP/6-31G* frequencies scaled 0.9804.^e Also includes BSSE corrections.

examined [30, 31]. Because the neutral and protonated forms of 18C6 were examined in detail in that work, only a brief summary of the structures of 18C6 most relevant to the current work is given here. The ground-state conformation of neutral 18C6 is of *C_i* symmetry; four of its six ether oxygen atoms are directed inward from the ether backbone, while the other two are directed outward. A weak intramolecular C–H···O interaction helps stabilize the ground-state conformer (Figure S3). A stable conformer with *D_{3d}* symmetry was also found that lies 9.3 kJ/mol higher in energy than the ground-state structure. In this conformation, each of the oxygen atoms are directed inward from the ether backbone, forming a nucleophilic cavity for very favorable interaction with guest cations.

Acetylated Amino Acids

The B3LYP/6-31G* optimized geometries and the M06/6-311+G(2d,2p) relative stabilities of the ground-state and stable low-energy conformations of the neutral and protonated AcAAs are provided in Figure S3 of the Supplemental Information. The preferred site of protonation for N_α-AcLys, N_α-AcArg, and N_α-AcHis is at the side chain substituent. In contrast, protonation of the N-terminal amino group along the backbone is preferred for N_ε-AcLys. The ground-state and low-energy structures of the neutral and protonated AcAAs are stabilized by intramolecular hydrogen bonds between the backbone amino, carboxyl, and acetyl moieties and the side chain substituent. Protonation is found to significantly alter the preferred conformations of N_α-AcLys and N_ε-AcLys. In both cases, protonation occurs at the free amino group altering it from a hydrogen bond acceptor to a hydrogen bond donor. As a result, the hydrogen bonding interactions that stabilize the protonated forms differ from those that stabilize the neutral species. In contrast, the preferred conformations of N_α-AcArg and N_α-AcHis are virtually unaffected by protonation. In both cases, the proton binds to a side chain nitrogen atom that does not participate in the hydrogen bonding interactions that stabilize the neutral, and thus the hydrogen bonding interactions are retained in the protonated species. A brief description of the ground-state

conformations of the neutral and protonated AcAAs is provided below, while details for select excited low-energy conformers are provided in the Supplemental Information.

N_α-AcLys

The ground-state structure of N_α-AcLys is stabilized by two intramolecular hydrogen bonds, one between the backbone carboxyl hydrogen and the side chain amino nitrogen atoms, the other between the backbone carbonyl oxygen and amino hydrogen atoms (Figure S3). The ground-state structure of H⁺(N_α-AcLys) is also stabilized by two intramolecular hydrogen bonds, one between the backbone carbonyl oxygen and amino hydrogen atoms, and the other between the acetyl oxygen and one of the protonated side chain amino hydrogen atoms (Figure S3).

N_ε-AcLys

The ground-state structure of N_ε-AcLys is stabilized by an intramolecular hydrogen bond between the amino hydrogen atom of the acetylated side chain and the backbone amino nitrogen atom (Figure S3). The ground-state structure of H⁺(N_ε-AcLys) is also stabilized by two intramolecular hydrogen bonds between the backbone carbonyl and side chain acetyl oxygen atoms and two of the amino hydrogen atoms of the protonated backbone N-terminal amino group (Figure S3).

N_α-AcHis

The ground-state structure of N_α-AcHis is stabilized by two intramolecular hydrogen bonds, one between the side chain amino hydrogen and the acetyl oxygen atoms, and the other between the backbone amino hydrogen and carbonyl oxygen atoms (Figure S3). The ground-state structure of H⁺(N_α-AcHis) exhibits a very similar conformation to that of neutral N_α-AcHis, except the side chain is now protonated, that is also stabilized by the same two hydrogen bonding interactions (see Figure S3).

N_{α} -AcArg

The ground-state structure of N_{α} -AcArg is stabilized by two intramolecular hydrogen bonds, one between the acetyl oxygen and side chain amino hydrogen atoms, and the other between the backbone carbonyl oxygen and amino hydrogen atoms (Figure S3). The ground-state structure of $H^+(N_{\alpha}$ -AcArg) exhibits a very similar conformation to that of neutral N_{α} -AcArg, except that the side chain is now protonated, that is also stabilized by the same two intramolecular hydrogen bonds (see Figure S3).

$(AcAA)H^+(18C6)$ Complexes

The B3LYP/6-31G* optimized geometries of the ground-state conformations of the $(AcAA)H^+(18C6)$ complexes are shown in Figure 3. 18C6 binds to the protonated side chain substituent in the complexes to N_{α} -AcLys, N_{α} -AcArg, and N_{α} -AcHis, and to the protonated backbone amino group in the complex to N_{ϵ} -AcLys. Thus, complexation to 18C6 does not alter the preferred site of protonation to these AcAAs. In all cases, binding of 18C6 occurs via N-H $\cdots$ O hydrogen bonds. The conformation of 18C6 in all of these complexes bears great similarity to the D_{3d} excited conformer of the neutral crown with a nucleophilic cavity in the center for interaction with the protonated AcAA (see Figure S3 of the [Supplemental Information](#)). A brief description of the ground-state conformations of these complexes is provided below, while details for select excited low-energy conformers are provided in the [Supplemental Information](#).

$(N_{\alpha}$ -AcLys) $H^+(18C6)$

Binding of 18C6 to $H^+(N_{\alpha}$ -AcLys) results in cleavage of the N-H $\cdots$ O hydrogen bonding interaction between the protonated side chain amino hydrogen and acetyl oxygen atoms, such that $H^+(N_{\alpha}$ -AcLys) exhibits an extended conformation in the complex. Cleavage of this hydrogen bonding interaction allows the protonated side chain amino group to interact with 18C6 via three nearly ideal (i.e., nearly linear) N-H $\cdots$ O hydrogen bonds. The other hydrogen bonding interaction that stabilizes $H^+(N_{\alpha}$ -AcLys), between the backbone car-

bonyl and amino hydrogen atoms, is preserved upon binding of 18C6 (compare Figure 3 and Figure S3 of the [Supplemental Information](#)).

$(N_{\epsilon}$ -AcLys) $H^+(18C6)$

Binding of 18C6 to $H^+(N_{\epsilon}$ -AcLys) results in cleavage of both N-H $\cdots$ O hydrogen bonding interactions between the protonated side chain amino hydrogen atoms and the carbonyl and acetyl oxygen atoms, such that $H^+(N_{\epsilon}$ -AcLys) exhibits an extended conformation in the complex. Cleavage of these hydrogen bonding interactions allows the protonated backbone amino group to interact with 18C6 via three nearly ideal N-H $\cdots$ O hydrogen bonds (compare Figure 3 and Figure S3 of the [Supplemental Information](#)).

$(N_{\alpha}$ -AcHis) $H^+(18C6)$

In the ground-state structure of the $(N_{\alpha}$ -AcHis) $H^+(18C6)$ complex (Figure 3), the conformation of $H^+(N_{\alpha}$ -AcHis) is remarkably similar to the conformation of the isolated ground-state species (Figure S3 of the [Supplemental Information](#)), in which the protonated side chain amino hydrogen atom forms a hydrogen bond with the backbone acetyl oxygen atom. In this conformer, $H^+(N_{\alpha}$ -AcHis) binds to the O1 and O4 atoms of a distorted D_{3d} conformer of 18C6 via two N-H $\cdots$ O hydrogen bonds.

$(N_{\alpha}$ -AcArg) $H^+(18C6)$

In the ground-state conformation of the $(N_{\alpha}$ -AcArg) $H^+(18C6)$ complex (Figure 3), the conformation of $H^+(N_{\alpha}$ -AcArg) is remarkably similar to the conformation of the isolated ground-state species, in which the acetyl oxygen atom forms an intramolecular hydrogen bond with one of the protonated side chain amino hydrogen atoms. The protonated side chain interacts with the O1, O2, O4, and O5 atoms of 18C6 via four N-H $\cdots$ O hydrogen bonds.

Threshold Analysis

The model of equation (S1) of the [Supplemental Information](#) was used to analyze the thresholds for reactions 1 in four $(AcAA)H^+(18C6)$ complexes. The results of these analyses are provided in Table 2. Representative results are shown in

Table 2. Threshold Dissociation Energies at 0 K and Entropies of Activation at 1000 K for Loss of 18C6 from $(AcAA)H^+(18C6)$ Complexes^a

AcAA	σ_o^b	n^b	E_0 (eV) ^c	E_0 (PSL) (eV) ^b	kinetic shift (eV)	$\Delta S^\ddagger$ (PSL) (J mol ⁻¹ K ⁻¹)
N_{α} -AcLys	81(11)	1.4 (0.1)	3.71 (0.13)	1.87 (0.08)	1.84	112 (4)
N_{ϵ} -AcLys	100 (8)	1.2 (0.1)	3.40 (0.10)	1.79 (0.06)	1.61	120 (4)
N_{α} -AcArg	47 (2)	1.3 (0.1)	2.61 (0.10)	1.42 (0.05)	1.19	114 (4)
N_{α} -AcHis	80 (4)	1.3 (0.1)	2.49 (0.09)	1.35 (0.06)	1.14	78 (4)

^a Present results, uncertainties are listed in parentheses.

^b Average values for loose PSL transition state.

^c No RRKM analysis.

Figure 4. Zero-pressure-extrapolated $\text{H}^+(\text{N}_\alpha\text{-AcLys})$ CID product cross section of the $(\text{N}_\alpha\text{-AcLys})\text{H}^+(18\text{C}6)$ complex in the threshold region. The solid lines show the best fits to the data using equation (1) convoluted over the ion and neutral kinetic energy distributions. The dotted lines show the model cross section in the absence of experimental kinetic energy broadening for reactants with an internal energy corresponding to 0 K

Figure 4 for the $(\text{N}_\alpha\text{-AcLys})\text{H}^+(18\text{C}6)$ complex. The analyses for the other three $(\text{AcAA})\text{H}^+(18\text{C}6)$ complexes are shown in Figure S4 of the [Supplemental Information](#). In all cases, the experimental cross sections for reactions 1 are accurately reproduced using a loose PSL TS model [35]. Previous work has shown that this model provides the most accurate assessment of the kinetics shifts for CID processes for electrostatically bound ion-molecule complexes [45–53]. Good reproduction of the data is obtained over energy ranges exceeding 3.0 eV and cross section magnitudes of at least a factor of 100. Table 2 also lists E_0 values obtained without including the RRKM lifetime analysis. Comparison of these values with the $E_0(\text{PSL})$ values shows that the kinetic shifts are the largest for the most strongly bound systems, and decrease in the order $\text{N}_\alpha\text{-AcLys} > \text{N}_\epsilon\text{-AcLys} > \text{N}_\alpha\text{-AcArg} > \text{N}_\alpha\text{-AcHis}$. This trend in the magnitudes of kinetic shifts is consistent with expectations that the observed kinetic shifts should directly correlate with the density of states of the activated complex at threshold, which increases with energy.

The entropy of activation, $\Delta S^\ddagger$, is a measure of the looseness of the TS and the complexity of the system. It is determined from the molecular parameters used to model the energized molecule and TS for dissociation as listed in Table S1 and S2. The $\Delta S^\ddagger(\text{PSL})$ values at 1000 K are listed in Table 2 and vary between 78 to $120 \text{ J mol}^{-1} \text{ K}^{-1}$ across these systems. These values are consistent with the noncovalent nature of the binding in these systems. The $\Delta S^\ddagger(\text{PSL})$ values are the smallest for the complex to $\text{N}_\alpha\text{-AcHis}$, $78 \text{ J mol}^{-1} \text{ K}^{-1}$, where only two hydrogen bonds are cleaved in the CID process, and larger

for the remaining complexes 112 to $120 \text{ J mol}^{-1} \text{ K}^{-1}$, where three or four hydrogen bonds are broken.

Conversion from 0 to 298 K

To allow comparison to commonly employed experimental conditions, we convert the 0 K bond energies determined here to 298 K bond enthalpies and free energies. Table S3 lists 0 and 298 K enthalpy, free energy, and enthalpic and entropic corrections for all systems experimentally determined. Details of the enthalpy and entropy conversions are provided in the [Supplemental Information](#).

Discussion

Comparison of Theory and Experiment

The measured and calculated 18C6 binding affinities of $\text{N}_\alpha\text{-AcLys}$, $\text{N}_\epsilon\text{-AcLys}$, $\text{N}_\alpha\text{-AcHis}$, and $\text{N}_\alpha\text{-AcArg}$ at 0 K are summarized in Table 1. The agreement between M06/6-311+G(2d,2p)//B3LYP/6-31G* theory and experiments is illustrated in Figure 5. For all systems, M06 theory systematically overestimates the measured $(\text{AcAA})\text{H}^+ - 18\text{C}6$ BDEs with a mean absolute deviation (MAD) of $8.9 \pm 3.3 \text{ kJ/mol}$. The agreement between B3LYP theory and the measured BDEs is less satisfactory. B3LYP theory systematically underestimates the measured $(\text{AcAA})\text{H}^+ - 18\text{C}6$ BDEs by $38.4 \pm 11.1 \text{ kJ/mol}$. The average experimental uncertainty (AEU) in the measured $(\text{AcAA})\text{H}^+ - 18\text{C}6$ BDEs is $6.0 \pm 1.2 \text{ kJ/mol}$, slightly smaller than the MAD for M06 theory, and significantly smaller than the MAD for B3LYP theory. Clearly, M06 theory does a much better job of describing the binding in these systems.

Figure 5. M06/6-311+G(2d,2p) theoretical versus experimental $(\text{AcAA})\text{H}^+ - 18\text{C}6$ 0 K BDEs. Values are taken from Table 1. Theoretical values include ZPE and BSSE corrections. The black line shows perfect agreement, while the red line is shifted up by 8.9 kJ/mol, the MAD

Trends in the 18C6 Binding Affinities

The measured $(\text{AcAA})\text{H}^+$ –18C6 BDEs determined here follow the order: $\text{N}_\alpha\text{–AcLys} > \text{N}_\epsilon\text{–AcLys} > \text{N}_\alpha\text{–AcArg} > \text{N}_\alpha\text{–AcHis}$. The interactions of 18C6 with protonated $\text{N}_\alpha\text{–AcLys}$ and $\text{N}_\epsilon\text{–AcLys}$ involve three nearly ideal linear $\text{N–H}\cdots\text{O}$ hydrogen bonds, which results in the strongest noncovalent interactions between 18C6 and the AcAAs investigated here. 18C6 interacts with protonated $\text{N}_\alpha\text{–AcArg}$ via four less than ideal hydrogen bonds with four oxygen atoms of the crown (O1, O2, O4, and O5) to form a somewhat less strongly bound complex. Protonated $\text{N}_\alpha\text{–AcHis}$ interacts with 18C6 via two non-ideal hydrogen bonds to alternate oxygen atoms (O1 and O4) to form a low symmetry conformer, and exhibits the weakest binding to 18C6. These trends in the $(\text{AcAA})\text{H}^+$ –18C6 BDEs confirm that the geometry, even more importantly than the number of hydrogen bonding interactions, is critical to the strong binding necessary for molecular recognition.

The analogous trend was also observed in our previous study of protonated peptidomimetic base–18C6 complexes [30]. The peptidomimetic bases (B) that bind to 18C6 via three $\text{N–H}\cdots\text{O}$ hydrogen bonds exhibit the greatest binding affinities for 18C6. The Lys mimic, NBA, exhibits a higher 18C6 binding affinity than the His mimics, imidazole (IMID) and 4-MeIMID, and the Arg mimic, 1-methylguanidine (MGD). The trend in the 18C6 binding affinity between His and Arg is not readily predictable from the peptidomimetic base study because the bases examined did not mimic the side chain substituents of Lys, His, and Arg in a completely systematic fashion, and the 18C6 binding affinity of the Arg mimic, MGD, is 0.2 kJ/mol lower than the His mimic, IMID, but is 8.2 kJ/mol higher than the other His mimic, 4MeIMID.

The 18C6 binding affinities of Lys, Arg, and His were examined in a follow-up study and found to follow the order, $\text{Lys} > \text{His} > \text{Arg}$ [31]. Theoretical calculations suggest that the protonated side chain of Lys is the preferred binding site for 18C6 complexation. In contrast, the protonated backbone amino group is the preferred site of binding for 18C6 to protonated His and Arg. Therefore, these results confirm that the Lys side chains are the preferred 18C6 binding sites to peptides and proteins. However, the trends in the 18C6 binding affinities determined in that work for His and Arg do not represent the trends in the binding affinities of their side chains. Based on the relative stabilities computed for the most stable conformers of the $(\text{His})\text{H}^+(18\text{C6})$ and $(\text{Arg})\text{H}^+(18\text{C6})$ complexes where 18C6 binds to the protonated backbone amino group (ground-state) and protonated side chain (excited conformers), theory suggests that the trend in the binding affinities will be preserved and His side chains are preferred over Arg side chains.

Binding Sites of Amino Acid Side Chains

The 18C6 binding affinity of protonated $\text{N}_\alpha\text{–AcLys}$ is 7.5 kJ/mol higher than that of protonated $\text{N}_\epsilon\text{–AcLys}$,

42.6 kJ/mol higher than that of protonated $\text{N}_\alpha\text{–AcArg}$, and 50.0 kJ/mol higher than that of $\text{N}_\alpha\text{–AcHis}$, indicating that the Lys side chain is the preferred binding site for 18C6 complexation amongst the basic AAs in proteins or peptides. Similar results were also found in our previous study of protonated peptidomimetic bases with 18C6 complexes [30]. The 18C6 binding affinity of the protonated form of the Lys mimic, NBA, is 48.8 kJ/mol higher than that of the His mimic, IMID, and 49.0 kJ/mol higher than that of the Arg mimic, MGD. The same trend was also observed in a follow-up study where the 18C6 binding affinity of several amino acids including all of the basic amino acids were examined. The 18C6 binding affinities of the protonated form of Lys is 11.4 kJ/mol higher than that of His, and 26.6 kJ/mol higher than that of Arg [31]. The same trend was also reported by Julian and Beauchamp [16] when a 1:1:1 mixture of NBA, guanidine (GD), and IMID was sprayed with 18C6. They found that the $(\text{NBA})\text{H}^+(18\text{C6})$ complex dominates the spectrum, and is the base peak (100 % relative abundance), while the relative intensity of the $(\text{GD})\text{H}^+(18\text{C6})$ and $(\text{IMID})\text{H}^+(18\text{C6})$ complexes is much smaller, 3.5 % and 1 %, respectively. These results suggest that the Lys side chains should remain the preferred binding sites for 18C6 complexation to peptides and proteins.

Binding Affinities of AcAAs versus AAs

The 18C6 binding affinity of protonated Lys increases by 12.2 kJ/mol upon N_α -acetylation. This is understood by the electron withdrawing effect of the acetyl group, which increases the charge retained by the side chain primary amino hydrogen atoms. The increased charge on the side chain primary amino hydrogen atoms induces higher charge on the oxygen atoms of 18C6 that results in slightly stronger electrostatic interactions between the primary amino hydrogen and ether oxygen atoms. In contrast, N_α -acetylation on His decreases the 18C6 binding affinity by 26.4 kJ/mol. This decrease in binding affinity occurs because 18C6 binds to the protonated side chain in the $(\text{N}_\alpha\text{–AcHis})\text{H}^+(18\text{C6})$ complex instead of the protonated backbone amino group as in the $(\text{His})\text{H}^+(18\text{C6})$ complex. In addition, the acetyl carbonyl oxygen atom forms a hydrogen bonding interaction with the protonated amino group of the side chain, providing additional stabilization to the protonated amino acid. However, this interaction stabilizes the isolated AA more than its complexes to 18C6. Thus, the charge on the protonated side chain hydrogen atoms decreases, and consequently decreases the induced charges on the ether oxygen atoms of 18C6. As a result, the electrostatic interaction between the protonated side chain and 18C6 becomes weaker.

In the ground-state structure of the $(\text{N}_\alpha\text{–AcArg})\text{H}^+(18\text{C6})$ complex, 18C6 binds to the protonated side chain of Arg. In contrast, 18C6 binds to the protonated backbone amino group in the ground-state conformer of the $(\text{Arg})\text{H}^+(18\text{C6})$ complex. As a result, the 18C6 binding affinity of protonated Arg

decreases by 3.8 kJ/mol upon N_α -acetylation. The protonated backbone amino group of Arg interacts with 18C6 via three ideal $N-H\cdots O$ hydrogen bonds, which results in a stronger binding interaction as compared to that between 18C6 and the protonated side chain of Arg where binding to 18C6 involves four non-ideal $N-H\cdots O$ hydrogen bonds. In the ground-state structure of the $(N_\alpha\text{-AcArg})H^+(18C6)$ complex, the acetyl oxygen atom is hydrogen bonded to an amino hydrogen atom of the protonated side chain. This hydrogen bonding interaction decreases the charge on the hydrogen atoms of the protonated side chain. However, unlike His this hydrogen bond stabilization does not directly involve any of the atoms involved in the hydrogen bonding interactions with 18C6. Therefore, its effect on the binding of 18C6 is not significant, and thus alters the binding interactions very little.

Side Chain versus N-terminal Binding to Lys

Protonated N_α -AcLys exhibits an 18C6 binding affinity that is 7.5 kJ/mol higher than that of protonated N_ϵ -AcLys, suggesting that the side chain of Lys residues are the preferred binding sites for 18C6 complexation in peptides or proteins. Theoretical calculations also suggest that binding of 18C6 to the protonated side chain of Lys is 4.2 kJ/mol more favorable than binding to the protonated backbone amino group [31]. The Lys side chain exhibiting a higher 18C6 binding affinity than the N-terminal amino group can be understood based on differences in the steric interactions of the carboxyl group and side chain in the complexes to N_ϵ -AcLys versus N_α -AcLys that constrain their complexation to 18C6. The X-ray study of Krestov and coworkers suggests that the steric interaction with the N-terminal amino acid side chain could constrain its complexation to 18C6 [54]. They found that the “depth of penetration” of the ammonium group into the 18C6 cavity for complexation exhibits a significant difference between diglycine and dialanine. The ammonium group in diglycine is much closer than that of dialanine during complexation. Steric interactions with the methyl side chain in proximity to the amino group in dialanine do not allow 18C6 to approach as closely and therefore bind as strongly. Thus, the 18C6 binding affinity of the N-terminal amino group should depend on the nature of the side chain. As a result, the Lys side chain constrains the complexation of the N-terminal amino group to a slightly greater extent than the backbone constrains complexation of the side chain amino group and leads to the 18C6 binding affinity of protonated N_α -AcLys being 7.5 kJ/mol greater than that of protonated N_ϵ -AcLys.

Measured BDEs versus PA of AcAAs

In our previous study of the binding in protonated peptidomimetic base-18C6 complexes, $(B)H^+(18C6)$, an inverse correlation between the 18C6 binding affinity and the PA of the peptidomimetic base is found as a result of the shorter N-H bonds and the decreased charge retained on the

amino protons. In a follow-up study of the binding in protonated amino acid-18C6 complexes, $(AA)H^+(18C6)$, an inverse correlation between the 18C6 binding affinity and the PA of the amino acid is also found. As for these other studies, the AcAAs investigated in this study involve different types and numbers of hydrogen bonding interactions with 18C6, thus correlations between the PA of the AcAA and the measured BDEs differ depending on the nature of the binding geometries to 18C6. Roughly inverse correlations between the measured 18C6 binding affinity and the PA of the AcAAs are also observed in the systems examined here. The PA of N_α -AcLys is 984.8 kJ/mol and increases to 987.0 kJ/mol for N_ϵ -AcLys, 996.0 kJ/mol for Lys, 1051.0 kJ/mol for Arg [55, 56], and 1061.0 kJ/mol for N_α -AcArg. Accordingly, the measured $(AcAA)H^+-18C6$ BDEs decrease from 179.9 kJ/mol for N_α -AcLys, to 172.4 kJ/mol for N_ϵ -AcLys, 167.7 kJ/mol for Lys, 137.3 kJ/mol for N_α -AcArg, and 129.9 kJ/mol for Arg [31].

The inverse correlation between the measured BDEs and PAs still loosely holds for the N_α -AcLys, N_ϵ -AcLys, His, and N_α -AcHis complexes, although they exhibit different binding interactions with 18C6. The PA of N_α -AcHis is 988.2 kJ/mol, 0.2 kJ/mol higher than that of His [57], 3.4 kJ/mol higher than that of N_α -AcLys, and 1.2 kJ/mol higher than that of N_ϵ -AcLys. Accordingly, the measured $(N_\alpha\text{-AcHis})H^+-18C6$ BDE is 129.9 kJ/mol, 26.4 kJ/mol lower than that of His [31], 42.5 kJ/mol lower than that of N_ϵ -AcLys, and 50.0 kJ/mol lower than that of N_α -AcLys. This inverse correlation was explained based on the N-H bond lengths and the charge retained on the amino protons. The AcAAs with higher PAs bind the proton tighter and lead to weaker interactions with 18C6, resulting in lower dissociation thresholds.

Conclusions

The kinetic energy dependence for CID of four $(AcAA)H^+(18C6)$ complexes, where $AcAA = N_\alpha\text{-AcLys}$, $N_\epsilon\text{-AcLys}$, $N_\alpha\text{-AcArg}$, and $N_\alpha\text{-AcHis}$ with Xe is examined by guided ion beam tandem mass spectrometry techniques. For all four systems, the primary dissociation pathway observed for these noncovalently bound complexes is loss of neutral 18C6. Thresholds for these CID processes are determined after consideration of the effects of the kinetic and internal energy distributions of the reactants, multiple collisions with Xe, and the lifetimes for unimolecular dissociation. The ground-state structures and theoretical estimates for the CID thresholds are determined from density functional theory calculations performed at the B3LYP/6-311+G(2d,2p)//B3LYP/6-31G* and M06/6-311+G(2d,2p)//B3LYP/6-31G* levels of theory. The agreement between M06 theory and experiment is reasonably good with a MAD of 8.9 ± 3.3 kJ/mol. The agreement between B3LYP theory and the measured BDEs is much less satisfactory. B3LYP theory systematically underestimates the measured $(AcAA)H^+-18C6$ BDEs by 38.4 ± 11.1 kJ/mol. Thus, it is clear that M06 theory describes the noncovalent

interactions responsible for the binding in these complexes much more effectively than B3LYP theory.

The 18C6 binding affinities determined here combined with structural information obtained from theoretical calculations provides useful insight into the processes that occur in the molecular recognition of peptides and proteins by 18C6 for protein structure and sequence investigation. N_α -AcLys exhibits the highest binding affinity for 18C6, suggesting that the side chains of Lys residues are the preferred binding sites for 18C6 complexation. N_α -AcLys exhibits a higher binding affinity for 18C6 than N_ϵ -AcLys, again suggesting that the side chain of Lys residues are the preferred binding site for 18C6 as compared to the N-terminal amino group of Lys. N_α -acetylation increases the 18C6 binding affinity for Lys. In contrast, N_α -acetylation decreases the 18C6 binding affinity of His and Arg, again confirming that Lys residues are the preferred binding sites for 18C6 complexation, and that competition by His and Arg residues for 18C6 complexation is not significant.

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References

- Smith, D.L., Deng, Y., Zhang, Z.: Probing the non-covalent structure of proteins by amide hydrogen exchange and mass spectrometry. *J. Mass Spectrom.* **32**, 135–146 (1997)
- Engen, J.R., Smith, D.L.: A powerful new approach that goes beyond deciphering protein structures. *Anal. Chem.* **73**, 256A–265A (2001)
- Kaltashov, I.A., Eyles, S.: Studies of biomolecular conformations and conformational dynamics by mass spectrometry. *Mass Spectrom. Rev.* **21**, 37–71 (2002)
- Hoofnagle, A.N., Resing, K.A., Ahn, N.G.: Protein analysis by hydrogen exchange mass spectrometry. *Annu. Rev. Biophys. Biomol. Struct.* **32**, 1–25 (2003)
- Eyles, S.J., Kaltashov, I.A.: Methods to study protein dynamics and folding by mass spectrometry. *Methods* **34**, 88–99 (2004)
- Garcia, R.A., Pantazatos, D., Villarreal, F.J.: Hydrogen/deuterium exchange mass spectrometry for investigating protein–ligand interactions. *Assay Drug Dev. Technol.* **2**, 81–91 (2004)
- Sinz, A.: Chemical cross-linking and mass spectrometry for mapping three-dimensional structures of proteins and protein complexes. *J. Mass Spectrom.* **38**, 1225–1237 (2003)
- Brunner, J.: New photolabeling and crosslinking methods. *Annu. Rev. Biochem.* **62**, 483–514 (1993)
- Kluger, R., Alagic, A.: Chemical cross-linking and protein–protein interactions: a review with illustrative protocols. *Bioorg. Chem.* **32**, 451–472 (2004)
- Melcher, K.: New chemical crosslinking methods for the identification of transient protein–protein interactions with multiprotein complexes. *Curr. Prot. Pept. Sci.* **5**, 287–296 (2004)
- Kodadek, T., Duroux-Richard, I., Bonnafous, J.C.: Techniques: oxidative cross-linking as an emergent tool for the analysis of receptor-mediated signaling events. *Trends Pharmacol. Sci.* **26**, 210–217 (2005)
- Back, J.W., de Jong, L., Muijsers, A.O., de Koster, C.G.: Chemical cross-linking and mass spectrometry for protein structural modeling. *J. Mol. Biol.* **331**, 303–313 (2003)
- Friedhoff, P.: Mapping protein–protein interactions by bioinformatics and cross-linking. *Anal. Bioanal. Chem.* **381**, 78–80 (2005)
- Trakselis, M.A., Alley, S.C., Ishmael, F.T.: Identification and mapping of protein–protein interactions by a combination of cross-linking, cleavage, and proteomics. *Bioconj. Chem.* **16**, 741–750 (2005)
- Petrochenko, E.V., Pedersen, L.C., Borchers, C.H., Tomer, K.B., Negishi, M.: The dimerization motif of cytosolic sulfotransferases. *FEBS Lett.* **490**, 39–43 (2001)
- Julian, R.R., Beauchamp, J.L.: Site specific sequestering and stabilization of charge in peptides by supramolecular adduct formation with 18-crown-6 ether by way of electrospray ionization. *Int. J. Mass Spectrom.* **210/211**, 613–623 (2001)
- Julian, R.R., Beauchamp, J.L.: The unusually high proton affinity of Aza-18-crown-6 ether: Implications for the molecular recognition of lysine in peptides by lariat crown ethers. *J. Am. Soc. Mass Spectrom.* **13**, 493–498 (2002)
- Julian, R.R., Beauchamp, J.L.: Selective molecular recognition of arginine by anionic salt bridge formation with Bis-phosphate crown ethers: Implications for Gas phase peptide acidity from adduct dissociation. *J. Am. Soc. Mass Spectrom.* **15**, 616–624 (2004)
- Julian, R.R., Akin, M., May, J.A., Stoltz, B.M., Beauchamp, J.L.: Molecular recognition of arginine in small peptides by supramolecular complexation with dibenzo-30-crown-10 ether. *Int. J. Mass Spectrom.* **220**, 87–96 (2002)
- Julian, R.R., May, J.A., Stoltz, B.M., Beauchamp, J.L.: Biomimetic approaches to gas phase peptide chemistry: Combining selective binding motifs with reactive carbene precursors to form molecular mousetraps. *Int. J. Mass Spectrom.* **228**, 851–864 (2003)
- Ly, T., Julian, R.R.: Using ESI-MS to probe protein structure by site-specific noncovalent attachment of 18-crown-6. *J. Am. Soc. Mass Spectrom.* **17**, 1209–1215 (2006)
- Ly, T., Julian, R.R.: Protein–metal interactions of calmodulin and α -synuclein monitored by selective noncovalent adduct protein probing mass spectrometry. *J. Am. Soc. Mass Spectrom.* **19**, 1663–1672 (2008)
- Liu, Z., Cheng, S., Gallie, D.R., Julian, R.R.: Exploring the mechanism of selective noncovalent adduct protein probing mass spectrometry utilizing site-directed mutagenesis to examine ubiquitin. *Anal. Chem.* **80**, 3846–3852 (2008)
- Ly, T., Liu, Z., Pujanauski, B.G., Sarpong, R., Julian, R.R.: Surveying ubiquitin structure by noncovalent attachment of distance constrained Bis(crown) ethers. *Anal. Chem.* **80**, 5059–5064 (2008)
- Yeh, G.K., Sun, Q., Meneses, C., Julian, R.R.: Rapid peptide fragmentation without electrons, collisions, infrared radiation, or native chromophores. *J. Am. Soc. Mass Spectrom.* **20**, 385–393 (2009)
- Pagel, K., Hyung, S.J., Ruotolo, B.T., Robinson, C.V.: Alternate dissociation pathways identified in charge-reduced protein complexes. *Anal. Chem.* **82**, 5363–5372 (2010)
- Oshima, T., Suetsugu, A., Baba, Y.: Extraction and separation of a lysine-rich protein by formation of supramolecule between crown ether and protein in aqueous two-phase system. *Anal. Chim. Acta* **674**, 211–219 (2010)
- Hossain, M.A., Schneider, H.J.: Sequence-selective evaluation of peptide side-chain interaction. New artificial receptors for selective recognition in water. *J. Am. Chem. Soc.* **120**, 11208–11209 (1998)
- Peczu, M.W., Hamilton, A.D.: Peptide and protein recognition by designed molecules. *Chem. Rev.* **100**, 2479–2494 (2000)
- Chen, Y., Rodgers, M.T.: Structural and energetic effects in the molecular recognition of protonated peptidomimetic bases by 18-crown-6. *J. Am. Chem. Soc.* **134**, 2313–2324 (2012)
- Chen, Y., Rodgers, M. T.: Structural and energetic effects in the molecular recognition of amino acids by 18-Crown-6. *J. Am. Chem. Soc.* **134**, 5863–5875 (2012).
- Rodgers, M.T.: Substituent effects in the binding of alkali metal ions to pyridines studied by threshold collision-induced dissociation and Ab initio theory: The methylpyridines. *J. Phys. Chem. A* **105**, 2374–2383 (2001)
- Ervin, K.M., Armentrout, P.B.: Translational energy dependence of $\text{Ar}^+ + \text{XY} \rightarrow \text{ArX}^+ + \text{Y}$ ($\text{XY} = \text{H}_2, \text{D}_2, \text{HD}$) from thermal to 30 eV cm. *J. Chem. Phys.* **83**, 166–189 (1985)
- Khan, F.A., Clemmer, D.E., Schultz, R.H., Armentrout, P.B.: Sequential bond energies of chromium carbonyls $\text{Cr}(\text{CO})_x^+$, $x = 1-6$. *J. Phys. Chem.* **97**, 7978–7987 (1993)
- Rodgers, M.T., Ervin, K.M., Armentrout, P.B.: Statistical modeling of collision-induced dissociation thresholds. *J. Chem. Phys.* **106**, 4499–4508 (1997)
- HyperChem Computational Chemistry Software Package, Ver. 5.0; Hypercube Inc: Gainesville, FL (1997)

37. Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R., Scalmani, G., Barone, V., Mennucci, B., Petersson, G.A., Nakatsuji, H., Caricato, M., Li, X., Hratchian, H.P., Izmaylov, A.F., Bloino, J., Zheng, G., Sonnenberg, J.L., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Vreven, T., Montgomery Jr., J.A., Peralta, J.E., Ogliaro, F., Bearpark, M., Heyd, J.J., Brothers, E., Kudin, K.N., Staroverov, V.N., Kobayashi, R., Normand, J., Raghavachari, K., Rendell, A., Burant, J.C., Iyengar, S.S., Tomasi, J., Cossi, M., Rega, N., Millam, J.M., Klene, M., Knox, J.E., Cross, J.B., Bakken, V., Adamo, C., Jaramillo, J., Gomperts, R., Stratmann, R.E., Yazyev, O., Austin, A.J., Cammi, R., Pomelli, C., Ochterski, J.W., Martin, R.L., Morokuma, K., Zakrzewski, V.G., Voth, G.A., Salvador, P., Dannenberg, J.J., Dapprich, S., Daniels, A.D., Farkas, Ö., Foresman, J.B., Ortiz, J.V., Cioslowski, J., Fox, D.J.: Gaussian 09, Revision A.1. Gaussian, Inc, Wallingford (2009)
38. Becke, A.D.: Density-functional thermochemistry. III. The role of exact exchange. *J. Chem. Phys.* **98**, 5648–5652 (1993)
39. Lee, C., Yang, W., Parr, R.G.: Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Phys. Rev. B* **37**, 785–789 (1988)
40. Foresman, J.B., Frisch, A.E.: Exploring chemistry with electronic structure methods, 2nd edn, p. 64. Gaussian, Pittsburgh (1996)
41. Boys, S.F., Bernardi, R.: The calculation of small molecular interactions by the differences of separate total energies. Some procedures with reduced errors. *Mol. Phys.* **19**, 553–566 (1970)
42. van Duijneveldt, F.B., van Duijneveldt-van de Rijdt, J.G.C.M., van Lenthe, J.H.: State of the art in counterpoise theory. *Chem. Rev.* **94**, 1873–1885 (1994)
43. Adamo, C., Barone, V.: Toward reliable density functional methods without adjustable parameters: The PBE0 model. *J. Chem. Phys.* **110**, 6158–6170 (1999)
44. Smith, S.M., Markevitch, A.N., Romanov, D.A., Li, X., Levis, R.J., Schlegel, H.B.: Static and dynamic polarizabilities of conjugated molecules and their cations. *J. Phys. Chem. A* **108**, 11063–11072 (2004)
45. Rodgers, M.T., Armentrout, P.B.: Collision-induced dissociation measurements on $\text{Li}^+(\text{H}_2\text{O})_n$, $n=1-6$: The first direct measurement of the Li^+-OH_2 bond energy. *J. Phys. Chem. A* **101**, 1238–1249 (1997)
46. Rodgers, M.T., Armentrout, P.B.: Absolute binding energies of lithium ions to short chain alcohols, $\text{C}_n\text{H}_{2n+2}\text{O}$, $n=1-4$. Determined by threshold collision-induced dissociation. *J. Phys. Chem. A* **101**, 2614–2625 (1997)
47. Rodgers, M.T., Armentrout, P.B.: Absolute alkali metal ion binding affinities of several azoles determined by threshold collision-induced dissociation. *Int. J. Mass Spectrom.* **185/186/187**, 359–380 (1999)
48. Rodgers, M.T., Armentrout, P.B.: Absolute binding energies of sodium ions to short chain alcohols, $\text{C}_n\text{H}_{2n+2}\text{O}$, $n=1-4$, determined by threshold collision-induced dissociation experiments and Ab initio theory. *J. Phys. Chem. A* **103**, 4955–4963 (1999)
49. Armentrout, P.B., Rodgers, M.T.: An absolute sodium cation affinity scale: Threshold collision-induced dissociation experiments and ab initio theory. *J. Phys. Chem. A* **104**, 2238–2247 (1999)
50. Amunugama, R., Rodgers, M.T.: Absolute alkali metal ion binding affinities of several azines determined by threshold collision-induced dissociation and ab initio theory. *Int. J. Mass Spectrom.* **195/196**, 439–457 (2000)
51. Rodgers, M.T., Armentrout, P.B.: Noncovalent interactions of nucleic acid bases (Uracil, Thymine, and Adenine) with alkali metal ions. Threshold collision-induced dissociation and theoretical studies. *J. Am. Chem. Soc.* **122**, 8548–8558 (2000)
52. Rodgers, M.T., Armentrout, P.B.: Statistical modeling of competitive threshold collision-induced dissociation. *J. Chem. Phys.* **109**, 1787–1800 (1998)
53. Rodgers, M.T.: Substituent effects in the binding of alkali metal ions to pyridines studied by threshold collision-induced dissociation and ab initio theory: The aminopyridines. *J. Phys. Chem. A* **105**, 8145–8153 (2001)
54. Kulikov, O.V., Krestov, G.A.: Thermodynamics and mechanism of complexation of peptides with 18-crown-6 in water. *Pure Appl. Chem.* **67**, 1103–1108 (1995)
55. Hunter, E.P., Lias, S.G.: Evaluated gas phase basicities and proton affinities of molecules: An update. *J. Phys. Chem. Ref. Data* **27**, 413–656 (1998)
56. Cannington, P.H., Ham, N.S.: He(I) and He(II) photoelectron spectra of glycine and related molecules. *J. Electron Spectrosc. Relat. Phenom.* **32**, 139–151 (1983)
57. Bouchoux, G., Buisson, D.A., Colas, C., Sablier, M.: Protonation thermochemistry of alpha-aminoacids bearing a basic residue. *Eur. J. Mass Spectrom.* **10**, 977–992 (2004)