Ion Structure and Energetics in the Gas Phase Characterized
Using Fourier Transform Ion Cyclotron Resonance Mass
Spectrometry

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Ion Structure and Energetics in the Gas Phase Characterized Using
Fourier Transform Ion Cyclotron Resonance
Mass Spectrometry

Chad A. Jones

A dissertation submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

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ABSTRACT

Ion Structure and Energetics in the Gas Phase Characterized Using Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

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In this dissertation, I use Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) to study the structure and energetics of gas phase ions.

Infrared multiphoton dissociation spectroscopy (IRMPD) is a technique for measuring the IR spectrum of gas phase ions in a Penning trap. I use this technique to investigate the conformation of cucurbituril complexes, terminal diamines, and protonated amino acids.

Cross sectional areas by Fourier transform ion cyclotron resonance mass spectrometry (CRAFTI) is a technique developed by the Dearden lab to measure the cross section of gas phase ions. In this work, I further develop a fundamental understanding of this technique. I investigate the role of dissociation in this and other FTICR-MS techniques. I also show that the principles of the CRAFTI technique can be used to measure the pressure inside the cell of an FTICR-MS. This technique, linewidth pressure measurement (LIPS), allows for a quantitative measurement and comparison of CRAFTI cross sections. To demonstrate the improvements to the technique, I measure the CRAFTI cross sections for the 20 standard amino acids and compare these to literature values measured by ion mobility measurements.

Keywords: FTICR-MS, CRAFTI, IRMPD, LIPS
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1. Introduction to FTICR-MS

An important part of my education in graduate school has been learning about and growing to appreciate the contributions of scientists that preceded me. I have therefore chosen to include a brief section on the history of mass spectrometry and the work that has directly influenced my research.

1.1. Mass Spectrometry: A History

Measuring an atom or molecule's mass has been an important consideration since the early days of chemistry. Both William Prout\(^1\) and J.L.G. Meinecke observed in 1815 that several elements were composed of integer multiples of the mass of hydrogen (H\(_2\)).\(^2\) An accurate mass measurement is therefore key to many experiments. Interestingly, the development of the mass spectrometer did not necessarily come about from any experimental need. Instead, the development of mass spectrometry came about from observations from physicists in the late 19th and early 20th century.

In 1896, Eugen Goldstein was conducting experiments in the von Helmholtz lab on cathode ray tubes. He observed that when a high electrical potential is applied between the cathode and the anode, luminous rays can be seen traveling along the distance of the tube in the opposite direction of the cathode rays. Goldstein called these rays "canal rays", or \textit{Kanalstrahlen}.\(^3\) Later, it was discovered that while cathode rays are electrons moving towards the positively charged anode, canal rays are positively charged ions moving towards the negatively charged cathode.

Two years later, while working in the same lab, Wilhelm Wien showed that canal rays can be deflected into a parabola by strong magnetic fields — an experiment that could be
considered the first experiment towards modern mass spectrometry techniques. Wien later quantified the ions by their charge-to-mass ratio \((q/m)\), the inverse of the currently used mass-to-charge ratio \((m/q)\), and was the first to measure the charge of a proton. The parabolas in Wien's spectra were poorly resolved and unclear. The low resolution of the \(q/m\) values was attributed to either variability in the electric charge or mass of the ions. Although both of these options are obviously untrue when taken in context of modern atomic theory, it should be noted that important discoveries about the nature of the atom were not available to Wien.

J.J. Thomson continued to investigate Goldstein's canal rays, seeking to refine the resolution of Wien's parabolas. Thomson hypothesized that the variability in the \(q/m\) parabolas was due to collisions with background neutrals, rather than variability in the charge or mass of the ion. In 1905, Thomson made low pressure measurements that resolve the fine structure of these parabolas. These experiments by Thomson were the first observed ion/molecule reactions and could easily be considered the earliest work in my specific field of study. It is interesting to see the parallels between his work and much of the work in this dissertation (Chapters 4-6). Specifically, the behavior of ions in a magnetic field and the effect that pressure has on these measurements are key aspects in both experiments. As vacuum technology improved, so did the resolution of these experiments. Work by Arthur Dempster in 1918 and Francis Aston in 1919 lead to the development of a mass spectrograph (the first instrument with the now familiar name) capable of defining isotope structure.

Work in the field during the late 1920s through 1950s was more focused on understanding the reaction dynamics of ion/molecule reactions. Work done by Oscar Rice and Herman Ramsperger, and L.S. Kassel in 1927 and 1928 was generalized by Rudolph Marcus in 1952 into RRKM theory, an important theory that allows for the calculation of unimolecular rate
constants. The 1950s brought another important innovation: Hans Georg Dehmelt and Wolfgang Paul developed the first ion traps.\textsuperscript{8} Development of the first Fourier transform ion cyclotron resonance mass spectrometer followed in 1974.

1.2. FTICR-MS

The primary technique used in this work is Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS), a mass spectrometry technique developed in 1974 by Comisarow and Marshall.\textsuperscript{9} The technique relies on measurement of the cyclotron motion — a fundamental property of an ion in a magnetic field. FTICR-MS utilizes this cyclotron motion to experimentally determine the mass-to-charge ratio of gas phase ions. Ultrahigh resolution of an ion's mass-to-charge ratio is possible using this technique. For example, a typical high resolution mass spectrometer has a mass resolution ($m/\Delta m$) of ~ 50,000. FTICR-MS, on the other hand, is capable of a mass resolution greater than 1,000,000. This ultrahigh resolution can also be coupled with ion activation techniques that can be used to probe an ion's gas phase structure and energetics. These ion activation techniques will be the focus of Chapter 2. In this chapter, I will focus on ion creation, isolation, and detection in FTICR-MS.
1.2.1. Ion creation

The first step in any FTICR-MS experiment is an ionization event, as the mass-to-charge ratio cannot exist without a charged species. There are many techniques for ion creation and detailing all of them is well beyond the scope of this work. However, it will be of some use to describe the two primary ionization techniques used in the Dearden lab: electrospray ionization (ESI) and electron ionization (EI).

ESI is the primary ionization technique in the Dearden lab. It is a very soft ionization method; very little ion fragmentation occurs during the ionization event. ESI was developed in the late 1980s as a way to ionize nonvolatile chemicals and put them into the gas phase. It is done by applying high voltage (in the Dearden lab typically ~6 kV) to a capillary tube. Solutions sprayed through this capillary tube will form a "Taylor cone" as seen in Figure 1 - 2. Charged
droplets formed at atmospheric pressures will vaporize. As the solvent evaporates, it leaves behind charged nonvolatile compounds that can be transported through a differentially pumped vacuum to the low pressure region where ions are trapped in the cell. Figure 1 - 1 shows the FTICR-MS setup used in the Dearden lab.

Electron ionization is another possible ionization technique. It is a much harder ionization source than ESI. For this reason it is rarely used in the Dearden lab, as our research interests are focused on weakly bound complexes. However, EI is a useful ionization method for volatile organic compounds or gaseous mixtures — both of which can be leaked into the low pressure region and ionized inside the trapping cell. EI is based on the early work of Walker Bleakney, who studied the ionization of mercury by single electron impact.\textsuperscript{12} EI is primarily used in this work to ionize neutral Ar in the trapping cell.

1.2.2. Cyclotron motion

In a magnetic field, an ion will be acted upon by the Lorentz force as described in Equation 1 - 1:

\[ \mathbf{F} = m \frac{d\mathbf{v}}{dt} = q (\mathbf{E} + \mathbf{v} \times \mathbf{B}) \]  

(1 - 1)
where \( F \) is the Lorentz force vector, \( m \) is the mass, \( \mathbf{v} \) is the velocity vector, \( \mathbf{E} \) is the vector of the electrical component of the electromagnetic field, and \( \mathbf{B} \) is the strength of the magnetic field.

Considering only the magnetic field, this equation simplifies to Equation 1 - 2.

\[
\mathbf{F} = m \frac{d\mathbf{v}}{dt} = q (\mathbf{v} \times \mathbf{B}) \tag{1 - 2}
\]

This Lorentz force bends the ion into a circular path. This bending motion, called cyclotron motion, is at the core of FTICR-MS theory. The Lorentz force can also be written in terms of velocity in the plane perpendicular to the magnetic field using Equation 1 - 3.

\[
\mathbf{F} = \frac{mv^2}{r} = qv_B B \tag{1 - 3}
\]

The angular frequency, \( \omega_c \), of this cyclotron motion can be obtained by combining (1 - 1 with Equation 1 - 3 to yield Equation 1 - 4.

\[
\omega_c = \frac{qB}{m} \tag{1 - 4}
\]

\( \omega_c \) in this equation is the "unperturbed" cyclotron frequency.\(^{13}\) Besides this unperturbed cyclotron frequency, ions in an FTICR-MS trap will also experience trapping oscillations and magnetron motion, both of which will be discussed later. The application of Equation 1 - 4 to mass spectrometry will be readily apparent to anyone acquainted with the field; the unperturbed cyclotron frequency is inversely proportional to the mass-to-charge ratio that all types of mass spectrometers are designed to measure. Thus, by experimentally determining an ion's cyclotron frequency, the mass-to-charge ratio has also been determined. Experimental determination of an ion's cyclotron frequency is much more simple than a direct mass measurement using the Lorentz force because this frequency is an inherent property of the ion that does not depend on an ion's velocity. Measuring the Lorentz force directly to determine the mass, however, would be
a velocity dependent measurement. Furthermore, the typical cyclotron frequency of an ion ranges from 10 kHz to 200 kHz, a value that is within an electrically accessible region. These points make cyclotron motion an excellent physical property to exploit for molecular mass information. The Dearden lab has an interest in using FTICR-MS to obtain molecular reaction and structural information of macrocyclic compounds. Much of the work in this dissertation is on method development to improve these studies.

1.2.3. Trapping oscillations

Once ions have been created, they can be effectively trapped in the \( x-y \) plane of a properly centered trapping cell by the strength of the magnetic field alone. Ions may still escape the trapping cell in the \( z \)-axis. To trap ions in the \( z \)-axis a small voltage (~1-3 volts) is applied along the \( z \)-axis, creating a potential well that traps the ions. For ions formed outside the cell, a pulse of neutral background gas at the time of ion injection can increase the collision frequency and sufficiently cool ions that would otherwise have enough energy to escape the trapping cell.

These trapping voltages add trapping oscillations, another form of ion motion orthogonal to the cyclotron motion. The frequency of these oscillations can be calculated using Equation 1-5 below.

\[
\omega_z = \sqrt{\frac{2qV\alpha}{ma^2}} \tag{1 - 2}
\]

Here \( \alpha \) is a geometric factor of the cell and \( a \) is the \( z \)-axis length of the cell.

1.2.4. Magnetron motion

Magnetron motion is an ion motion introduced through the electric field. The trapping potential described in 1.2.3 above creates a radial force perpendicular to the Lorentz force in the \( z \)-axis. The total force on the ion is then:
\[ \mathbf{F}_{\text{tot}} = \mathbf{F}_{\text{cyc}} - \mathbf{F}_{\text{rad}} = m\omega^2 r = q\mathbf{B}\alpha r - \frac{qV\alpha}{a^2} r \]  

(1 - 6)

where \( F_{\text{cyc}} \) is the reduced Lorentz force from Equation 1 - 2 (which we derived earlier to yield the first term), \( F_{\text{rad}} \) is this new radial force just described, \( \alpha \) is a constant that depends on the geometry of the trapping cell, \( a \) is the length of the trapping cell, and \( V \) is the voltage applied to the cell. It can be noted that this equation is a quadratic equation in \( \omega \):

\[ m\omega^2 - q\mathbf{B}\alpha + \frac{qV\alpha}{a^2} = 0 \]

The solutions for which are:

\[ \omega_+ = \frac{\omega_0}{2} + \sqrt{\left(\frac{\omega_0}{2}\right)^2 - \frac{\omega_z^2}{2}} \]  

(1 - 7)

and

\[ \omega_- = \frac{\omega_0}{2} - \sqrt{\left(\frac{\omega_0}{2}\right)^2 - \frac{\omega_z^2}{2}} \]  

(1 - 8)

Equation 1 - 7 is the reduced cyclotron frequency, or the ion's actual cyclotron frequency in the presence of the trapping electric field. Equation 1 - 8 is the magnetron frequency where \( \omega_z \) is the trapping oscillation from Equation 1 - 5.

Magnetron motion can be excited in FTICR-MS, though in all cases in this work magnetron excitation is unintentional. The excitation is usually minimal and can be safely ignored.

1.2.5. Excitation/Detection

Ions are detected in FTICR-MS as an image current on a pair of conducting plates. Every FTICR-MS experiment employs at least one RF excitation event. The purpose of this excitation event is to induce spatial coherence of the ion packet and translationally excite the packet to a
large enough radius that an image charge can be created on the detection plates. The cyclotron
radius will decay (through, for example, collisions with background neutrals or coupling to the
excitation/detection electronics). A Fourier transform of the time domain decay transient gives
the cyclotron frequency of ions present in the trapping cell. Figure 5-1 is shows the ion motion as
a result of RF excitation, and Chapter 5 of this work details experiments that have been done to
help uncover the role that dissociation specifically plays in dephasing an ion from the coherent
packet.

Several types of excitation events are possible. The most simple excitation event is a
single frequency excite, which utilizes an RF frequency resonant with a trapped ion. When an
external RF voltage is applied, the radius expands according to Equation 1 - 9.

\[ r = \frac{\beta V_{p-p} T_{excite}}{2dB} \]  

(1 - 9)

Here \( \beta \) is a geometry factor of the trapping cell (0.897 for the cell used in this work), \( V_{p-p} \)
is the peak-to-peak amplitude of the applied voltage, \( T_{excite} \) is the length of the RF pulse, \( d \) is the
diameter of the cell, and \( B \) is the strength of the magnetic field. Typically an excitation radius
\(~75\%\) of the trapping cell radius will give optimal signal. The kinetic energy of an ion excited by
a single frequency excitation event can be calculated with Equation 1 - 10 below.

\[ KE_{(post excitation)} \times 10^7 = \frac{1.20607 \beta^2 z^2 V_{p-p} T_{excite}}{d^2 m} \]  

(1 - 10)

Notice that the while both the cyclotron frequency and the ion's kinetic energy depends
on its mass and charge, the cyclotron radius is independent of both. A single frequency excitation
can also be used to perform a simultaneous excite/detect event (SED) in which excitation and
detection electronics are simultaneously active. SED events allow for real-time monitoring of the
ion packet, and are also used in CRAFTI, a new analytical technique that will be discussed in
detail in Chapters 2, 4, 5, and 6. SED events are also diagnostically useful, as the RF from the excite plates can be detected using the detection plates. A "received during transmit" or RDT event can help diagnose electronic problems between the cell and acquisition computer.

Chirp excitation is a commonly used broadband excitation method. A "sweep" of the desired frequencies is performed such that the RF applied will be resonant with the applied frequency for a short amount of time.

Figure 1 - 3 Simulated time domain of a chirp excitation.

Figure 1 - 3 shows the time domain of a simulated chirp excitation. The name "chirp" comes from audible corollary to the RF waveform, which sounds like a bird chirping. The Dearden lab uses a linear chirp waveform, described by Equation 1 - 11:

$$f(t) = f(0) + kt$$

(1 - 11)

Where \( f(t) \) is the frequency at time \( t \), \( f(0) \) is the frequency at time 0, and \( k \) is the chirp rate, a constant that describes how quickly the frequency sweeps through the desired range of frequencies. The frequency sweep can be done from low-to-high frequency or from high-to-low
frequency. The effects of this choice will be discussed more in Chapter 5. A chirp waveform is simple to construct (compared to SWIFT waveforms, which will be discussed later) but have several downsides; the power over a broadband excitation will vary, chirp waveforms are a non-selective waveform meaning all frequencies in a given range will be equally excited (not considering the natural power variation already mentioned). The cyclotron radius and kinetic energy post-chirp excitation will be discussed more in Chapter 5; however, the general equation is similar to those of Equation 1 - 9 and Equation 1 - 10, respectively.

It is often the case that a non-uniform excitation waveform is desired. Stored waveform inverse Fourier transform (SWIFT) waveforms are broadband excitation waveforms capable of producing non-uniform excitation. For example, an ion or group of ions can be preferentially excited to expand different ions to different cyclotron radii, or ions can selectively ejected from the cell with a high amplitude while leaving nearby ions unperturbed. Single isotope isolations are possible for complexes that aren't highly charged (high charge states will coalesce the isotope peaks, making isolation more difficult).

1.3. Conclusions

Mass spectrometry is a vital component of many fields of chemical research. Early discoveries in the late 19th and early 20th century made this technique possible. Later innovations made it more powerful and widespread. FTICR-MS is an ultrahigh resolution mass spectrometry technique. In the following Chapter I will discuss the three ion activation techniques used in my research to study the structure and energetics of ions in the gas phase using FTICR-MS. These three techniques are: 1) Sustained off-resonance irradiation collision-induced dissociation (SORI-CID) 2) Infrared multiphoton dissociation (IRMPD), and 3) Cross sectional areas by Fourier transform ion cyclotron resonance mass spectrometry (CRAFTI). Each
of these techniques utilize the excitation techniques discussed in this chapter in one way or another.
1.4. References

2. Ion activation in FTICR-MS

FTICR-MS is a powerful mass spectrometry technique with high resolution capabilities. This fact alone makes it a valuable tool in chemical analysis. However, unlike most other mass spectrometry techniques, detection in FTICR-MS is a non-destructive process. This means that ions can be trapped for long periods of time (seconds to minutes). Trapped ions can be further manipulated by adding energy either by collisional excitation, irradiation, applying resonant RF frequencies, in situ reactions, or any combination of these.

All FTICR experiments employ at least one excitation event at or near the resonant frequency to expand the radius of ion motion and impose phase coherence, enabling detection. Many experiments employ additional excitation events to eject ions from the trap or to translationally excite the ions and facilitate subsequent collisional activation. Exciting at the resonant frequency causes the ion orbit radius to expand rapidly and imparts a large amount of kinetic energy. While this allows the kinetic energy to be precisely controlled, on resonance excitation has several drawbacks for tandem mass spectrometry experiments, including short transients, often severe space charge effects, and other issues. In addition, collisions that result from resonant excitation may not transfer sufficient kinetic energy to induce rapid dissociation, and the collision dampens the translational energy stopping any further ion activation. This is especially true for large ions since the maximum center-of-mass kinetic energy available for activation decreases as mass increases.

In this chapter I will describe several ion activation techniques used in FTICR-MS to overcome these difficulties. So called "slow heating" methods deposit small amounts of energy to incrementally heat the ion. Instead of dissociating as the result of a single, high energy
collision, the ions dissociate as the result of many low energy collisions. There are a number of slow heating methods available in FTICR-MS. In this chapter I will look specifically at infrared multiphoton dissociation (IRMPD) and sustained off-resonance irradiation collision induced dissociation (SORI-CID). I will also describe cross sectional areas by Fourier transform ion cyclotron resonance mass spectrometry (CRAFTI), a technique that can be used to measure cross sections of gas phase ions inside the cell of an FTICR-MS. These three techniques are the fundamental ion activation techniques that will be discussed in later chapters.

2.1 Sustained Off-Resonance Irradiation Collision Induced Dissociation (SORI-CID)

Sustained off-resonance irradiation collision induced dissociation (SORI-CID) is a widely used FTICR-MS ion activation technique developed in the early 1990s by Gauthier et al. In SORI-CID, an RF voltage is applied with a frequency that is slightly off-resonance from the ion's cyclotron frequency (in the Dearden lab this off-resonance is typically about 1 kHz). This off resonant excitation creates a beat pattern, with the ion's cyclotron radius expanding and contracting as the kinetic energy increases and decreases over time.

Ion collisions with a background neutral can happen at any point during this excitation process. This means that the kinetic energy of the ion at the time of the collision is unknown. Also, the fraction of translational energy that is deposited into internal modes is also unknown. For these two reasons, SORI-CID experiments, as currently performed, do not yield any quantitative information about the bond dissociation energy. However, SORI-CID can be used qualitatively to compare the relative bond strengths. In this section I will describe the kinetic energy of an ion during a SORI-CID experiment as well as what happens when non-optimal conditions are chosen. This work is partially from a paper co-authored with Daniel Mortensen.
Laskin et al. have shown that the kinetic energy of an ion excited by off-resonance excitation can be described by Equation 2 - 1 below.\(^4\)

\[
KE_{\text{lab}} = \frac{q^2 \beta^2 V_{p-p}^2}{16md^2 \Delta f^2} \left[1 - \cos(\Delta f) t\right]
\]

(2 - 1)

Here, \(q\) is the elementary charge, \(\beta\) is a geometry factor for the trapping cell (0.897 for the work presented here)\(^7\), \(V_{p-p}\) is the amplitude of the applied voltage, \(m\) is the mass of the ion, \(d\) is the diameter of the cell, \(\Delta f\) is the frequency offset, and \(t\) is the length of the applied RF pulse. It can be seen from this equation that the kinetic energy of an ion increases and decreases in a sinusoidal manner over the course of a SORI-CID experiment. The maximum kinetic energy occurring when \(\cos(\Delta f) t\) is equal to -1, and Equation 2 - 1 becomes:

\[
KE_{\text{lab}} = \frac{q^2 \beta^2 V_{p-p}^2}{8md^2 \Delta f^2}
\]

(2 - 2)

Of course, the collision partner will greatly affect the center-of-mass collision energy.

The center-of-mass collision energy can be calculated from the lab frame using (2 - 3 below).

\[
KE_{\text{cm}} = \frac{M}{m+M} KE_{\text{lab}}
\]

(2 - 3)

Equation 2 - 2 is a description of the maximum kinetic energy of the excited ion. However, experimentally the quantity \(E_{\text{SORI}}\) is used. This quantity is the relative energy deposited during multiple collisions during the SORI-CID process. An equation for \(E_{\text{SORI}}\) has been derived by Zhang et al.\(^8\)

\[
E_{\text{SORI}} = n_o \sigma K_s f_{\text{coll}}^t \left(\frac{\beta^3 q^3 V_{p-p}^3}{128 \pi^3 d^3 (\Delta f)^3} \left(\frac{M}{M+m}\right)^{\frac{3}{2}} \left(\frac{M}{m}\right)^{\frac{3}{2}} \right)
\]

(2 - 4)
Here, \( n_n \) is the number density, \( \sigma \) is the collision cross section, \( K_v \) is the proportionality constant relating the ion's velocity to the maximum velocity of the activated ion, \( f_E \) is the fraction of energy deposited during a collision (assumed to be a constant), and \( t_{\text{coll}} \) is the excitation event length. It has been shown\(^9\) that for optimal detection of fragment ions \( t_{\text{coll}} \) should be chosen as an integer multiple of \( 1/\Delta f \). Additionally, we observed enhanced fragmentation for SORI events that use non-integer numbers of cycles.\(^6\) While studying the fragmentation of \([\text{mc5+Li+Cs}]^{2+}\), Daniel Moretensen observed that the relative abundance of the parent ion varied greatly. Upon further analysis, he recognized that increased dissociation occurs when the total number of SORI cycles is a non-integer multiple of the frequency offset.

![Figure 2 - 1 Relative abundance of \([\text{mc5+Cs+Li}]^{2+}\) as a function of SORI time (ms).\(^6\)](image-url)

Figure 2 - 1 Relative abundance of \([\text{mc5+Cs+Li}]^{2+}\) as a function of SORI time (ms).\(^6\)
Figure 2 - Dissociation curves for 18-crown-6·Cs+ with varying excitation frequency offsets, 0.5% (0.95 kHz), 1.0% (1.81 kHz), and 1.5% (2.73 kHz) of the resonant frequency (181.6 kHz). The continuous curves are sinusoidal fits to the data. (a) When plotted vs. the length of the SORI event, the variation in parent ion abundance depends on the frequency offset. (b) When plotted vs. the number of SORI cycles, all three frequency offsets give similar results.

These results can be explained by considering the mechanism of SORI-CID activation in the absence of collisions. As ions are excited off resonance, after a few SORI cycles they are in phase with the driving RF at the beginning of each cycle. The ions are excited to higher kinetic energies by the driving field, but as the cycle proceeds the phase mismatch between the ion orbits and the driving RF increases. Halfway through the cycle the ions have been accelerated to maximum kinetic energy but are 180° out of phase with the driving RF, so no further kinetic energy is added. In real SORI experiments the ions undergo collisions randomly during the cycle, but the average behavior of the ions should be quite similar to what we have described. If the SORI activation does not last for an integer number of SORI cycles, the ions are left
translationally excited, just as if they had been excited by a short on-resonant excitation; subsequent collisions convert translational energy to internal energy, and the ions then dissociate. Hence, the process observed here might better be termed “activated ion CID:” the ions are slowly activated via SORI, then may undergo harder collision-induced dissociation due to the residual kinetic energy, with all of this occurring in a single event in the experimental sequence.

Overall the pattern in dissociation with excitation time is sinusoidal with nearly complete dissociation occurring halfway through a SORI cycle. Experiments were also performed at 0.5%, 1.0%, and 1.5% off-resonance. When these dissociation curves are plotted against time (Figure 2 - 2 Dissociation curves for 18-crown-6·Cs+ with varying excitation frequency offsets, 0.5% (0.95 kHz), 1.0% (1.81 kHz), and 1.5% (2.73 kHz) of the resonant frequency (181.6 kHz). The continuous curves are sinusoidal fits to the data. (a) When plotted vs. the length of the SORI event, the variation in parent ion abundance depends on the frequency offset. (b) When plotted vs. the number of SORI cycles, all three frequency offsets give similar results. Figure 2 - 2a) the sinusoidal trend is evident at each frequency offset, but the time scales vary as expected because the SORI period varies with the frequency offset. In fact, once the dissociation curves are normalized by $1/\Delta f$ and plotted against SORI cycles (Figure 2 - 2b) they show good agreement with each other and are comparable with the results from the decamethyl-cucurbit[5]uril complex. This demonstrates that activated ion dissociation due to residual kinetic energy occurs over a range of frequency offsets and is compelling evidence for the dissociation mechanism described above. The chemical systems we examined for this study are weakly bound. However, we expect the effects demonstrated here to be general, especially when the residual kinetic
energies at non-integer numbers of SORI cycles are greater than the binding energy for the ion under study.

In this work it was discovered that if comparison of dissociation energies is the goal, care should be taken when choosing the lengths of SORI events so that they are integer multiples of the SORI cycle time; otherwise, large errors are likely. As with any phase error, the effects are cumulative so may be more apparent at longer times. Conversely, deliberately setting the length of the SORI excite to a non-integer multiple of the SORI cycle is a means for imparting additional energy to the ions, combining slow heating via multiple collisions during SORI with a harder collision from residual kinetic energy at the end of the event. It is likely these effects will show some molecular size dependence. Larger molecules will be slower to activate due to RRKM effects, and radiative cooling could also come into play. As is well known, if the molecule radiatively cools more quickly than the collisional activation rate, it may not be possible to deposit sufficient energy in a large ion to cause dissociation. In such cases, it is desirable to carry out the activation quickly, and the “activated ion collision-induced dissociation” demonstrated here may help.

2.2 Infrared multiphoton dissociation (IRMPD)

Infrared vibrational spectra of gas phase ions can yield important structural information. For example, the IR spectrum can reveal which functional groups are present, the location of the functional groups, or reveal any intermolecular interactions such as hydrogen bonding. Beauchamp and co-workers first reported laser induced dissociation of ions in a Penning trap of an FTICR-MS.10 Ion densities in FTICR-MS are very low, making standard absorption measurements difficult as measurements of light attenuation are not sufficiently sensitive to yield any relevant information. To circumvent this problem an action spectrum, rather than an
absorption spectrum, is obtained. As the experiment takes place inside a high resolution mass spectrometer the ion to be analyzed can be mass selected prior to obtaining a spectrum. Mass selected ions of interest are trapped in the cell of an FTICR-MS and irradiated with IR photons from a tuneable laser at a particular frequency. The photons will be absorbed if the ion of interest has a vibrational frequency resonant with the frequency of the IR photons. The ratio of fragment ions to total ion population is proportional to the absorption efficiency at that frequency. Plotting this ratio as a function of laser frequency yields an action spectrum that is equivalent to a gas phase absorption spectrum.

In IRMPD, multiple photon absorption events occur before dissociation occurs. The energy absorbed by the ions is quickly distributed through intramolecular vibrational relaxation between all available degrees of freedom. This eventually leads to the dissociation of the ion by the lowest energy pathway. Figure 2 - 3 is a schematic of this mechanism.

![Figure 2 - 3 Dissociation mechanism in IRMPD. From Polfer review on IRMPD.](image-url)
The ion absorbs a photon at frequency $v_0 \rightarrow v_1$, and the energy from this absorbed photon is distributed into the available degrees of freedom, thereby vibrationally relaxing $v_1$. The process continues as additional photons are absorbed at $v_0 \rightarrow v_1$ until the dissociation threshold is reached. The dissociation pathways in IRMPD are comparable to those in SORI. In both techniques the ions are slowly heated. Energy gained by either collisions (SORI-CID) or IR absorption (IRMPD) causes the ion to dissociate by the lowest energy pathway. In an IRMPD experiment, however, high resolution mass measurements can be made immediately following the dissociation event. For SORI-CID experiments the gas pulsed into the system must first be evacuated.

IMPRD action spectra can be compared to frequency spectra obtained using \textit{ab initio} calculations. This allows for distinctions to be made regarding the ion's gas phase structure. IRMPD experiments performed in the Dearden lab will be the subject of Chapter 3 of this work.

2.3 Cross sectional areas by Fourier transform ion cyclotron resonance mass spectrometry (CRAFTI)

A molecule's chemistry is largely determined by its conformation. For example, amino acids — the building blocks of life — polymerize into larger proteins. These proteins are the key component to most cellular activity, and their conformation determines how they will interact with other proteins.\textsuperscript{13} Indeed, the misfolding of these proteins can cause a wide range of biological diseases.\textsuperscript{14} The binding of drug molecules to their targets,\textsuperscript{15} the physical properties of polymers,\textsuperscript{16} or non-covalent interactions with macrocycles are all affected by the conformation of the molecules involved.

The gold standard for measuring a molecule's collision cross section - its molecular size - is ion-mobility mass spectrometry (IMS-MS).\textsuperscript{17} IMS-MS is a mass spectrometry technique
capable of separating and characterizing gaseous ions by their mass-to-charge ratio as well as by their size and shape. The extra dimension of analysis makes IMS-MS an ideal technique for analyzing proteins, lipids, glycans, and other metabolites. IMS-MS measures the time it takes for a gaseous ion to travel the distance of a drift tube. The drift region of the instrument is filled with a buffer gas, typically helium or nitrogen, and a low level electric field is applied. The ion velocity is proportional to the strength of the electric field, which is maintained at a low enough level that the energy from the electric field is less than the energy of thermal ion-neutral collisions. The proportionality constant that connects ion velocity to electric field strength is given in Equation 2 - 5:

$$K = \left( \frac{3q}{16N} \right) \left( \frac{2\pi}{k_bT} \right)^{\frac{1}{2}} \left( \frac{m + M}{mM} \right)^{\frac{1}{2}} \left( \frac{1}{\Omega} \right)$$

(2 - 5)

where \(q\) is the charge of the ion, \(k_b\) is Boltzmann's constant, \(N\) is the number density of the buffer gas, \(m\) is the mass of the neutral buffer gas, \(M\) is the mass of the ion, and \(\Omega\) is the collision cross section of the ions. If the cross section of the ion is desired it can be derived from Equation 2 - 5 to yield Equation 2 - 6.

$$\Omega = \left( \frac{18\pi}{16} \right)^{\frac{1}{2}} \frac{ze}{(k_bT)^{\frac{1}{2}}} \left[ \frac{1}{M} + \frac{1}{m} \right] t_D E \frac{760}{T} \frac{1}{P} \frac{273.2}{N}$$

(2 - 6)

where \(t_D\) is the ion's drift time, \(E\) is the applied electric field, \(L\) is the drift length, and \(P\) is the pressure of the buffer gas.

Recently, the Dearden lab has introduced a new method for measuring the cross section of gas phase ions using an FTICR-MS called cross sectional areas by Fourier transform ion cyclotron resonance mass spectrometry (CRAFTI). The ion motion in FTICR-MS experiment are described in detail in Chapter 5 of this work. Figure 5 - 2 shows the time domain decay
transient of a typical FTICR-MS. Parisod et al. observed that by analyzing either this decay transient or the linewidth in the frequency domain the nonreactive ion-molecule collision frequencies could be determined. Early work from the Beauchamp group also showed that the number density of the neutral gas correlated linearly with the linewidth of the frequency domain spectrum.

Yang and Dearden developed these early observations into CRAFTI by assuming that the rate of the time domain transient decay is limited by collisions between ions and background neutrals. The signal strength is proportional to the total amount of charge in the coherent packet. Once an ion is scattered from the packet it no longer contributes to the total signal. In a coherently orbiting packet of $N$ ions, the number of ions lost from the coherent packet, $dN$, in a given time will be proportional to the number of ions and the collision frequency. This collision frequency is dependent on the ions mass, $m$, the mass of the neutral, $M$, the collision cross section, $\sigma$, the neutral number density, $n_n$, and the relative velocity of the ion and neutral. (2 - 7, therefore, can be used to describe the ions lost during time $t$.

$$dN = -\frac{M}{m+M} \sigma n_n v dt$$

(2 - 7)

We assume that during time $t$ the ion velocity, $v$, is constant. This assumption is valid if ions orbit in a perfectly smooth magnetic field, all the ions are equally excited by an RF waveform with no deviations, and ions are dephased by a single collision. Under these conditions, the ions remaining in the packet can be determined by Equation 2 - 8.

$$N(t) = N_0 \exp\left(-\frac{M}{m+M} \sigma n_n v t\right)$$

(2 - 8)

The Fourier transform of this time domain equation gives Equation 2 - 9, the frequency equation.
\[ \text{Mag}(\omega) = \frac{\text{Mag}_0}{1 + \left( \frac{\omega - \omega_0}{m + M} \frac{1}{\sigma n_n \nu} \right)^2} \]  
\( (2 - 9) \)

Here \( \text{Mag}_0 \) is the magnitude mode peak amplitude and \( \omega_0 \) is the unperturbed cyclotron frequency. Under pressure limited conditions, the linewidth is dominated by ion-neutral collisions, creating Lorentzian peaks with linewidths that can be described by Equation 2 - 10 below.

\[ \text{FWHM} = 2 \frac{M}{m + M} \sigma n_n \nu \]  
\( (2 - 10) \)

The ion velocity can be described by Equation 2 - 11 below:

\[ v = \frac{\beta V_{p-p_t \text{exc}}}{2d} \frac{q}{m} \]  
\( (2 - 11) \)

Combining Equation 2 - 10 and 2 - 11 and rearranging to solve for \( \sigma \) gives Equation 2 - 12, shown below.

\[ \sigma = \frac{\text{FWHM} (m + M) m}{n_n} \frac{d}{q \beta V_{p-p_t \text{exc}}} \]  
\( (2 - 12) \)

This equation can be used to experimentally determine the CRAFTI cross section for an ion in an FTICR-MS cell. This equation will be discussed at length in this work. In Chapter 4 the equation will be used to determine the pressure in the FTICR-MS, Chapter 5 will discuss the effects that dissociation plays on the dephasing cross section, and in Chapter 6 I present results for the CRAFTI cross sections of the 20 standard, naturally occurring amino acids.

There are several important differences between CRAFTI and IMS-MS. CRAFTI utilizes a single, high energy collision to dephase an ion from the coherent ion packet that produces signal. The kinetic energies of these collisions range between several hundred to several thousand eV, a much higher energies than those used in IMS-MS. In a typical IMS-MS...
experiment, the energy added to the ion by the applied electric field is \( \leq 1\% \) of the thermal collision energy.\(^{17}\) Thus, the collision energy of a CRAFTI experiment is 30,000-500,000 times more energetic than in IMS-MS. As a result, bond dissociation plays a much larger role in a CRAFTI experiment than in IMS-MS.

The kinetic energies of an ion in a magnetic field excited by applying a single frequency RF pulse resonant with the ion's cyclotron frequency can be calculated by Equation 2 - 13.

\[
KE_{\text{post-excitation}} = \frac{1.20607 \times 10^7 \beta^2 z^2 V_{p-p} T_{\text{excite}}^2}{d^2 m}
\] (2 - 13)

Here, \( \beta \) is a geometric factor for the trapping cell, \( z \) is the charge of the ion in multiples of elementary charge, \( V_{p-p} \) is the amplitude of the applied RF, \( T_{\text{excite}} \) is the length of the applied RF, \( d \) is the cell diameter, and \( m \) is the mass of the ion in Daltons. The excitation radius of this ion can be calculated using Equation 2 - 14 below.

\[
r = \frac{\beta V_{p-p} T_{\text{excite}}}{2dB}
\] (2 - 14)

Here, \( B \) is the strength of the magnetic field in Tesla. Interestingly, while the kinetic energy of an ion depends on both \( m \) and \( z \), the excitation radius is independent of both of these variables. Thus, an ion of any given mass or charge will have the same cyclotron radius within the trapping cell of an FTICR-MS. Optimal signal can be achieved at \( \sim 75\% \) of the trapping cell diameter.

As CRAFTI measurements depend on a single collision to dephase an ions there is a mass limit to measurements that can be made using this technique. This upper mass limit occurs when the center-of-mass kinetic energy for an ion/neutral collision is less than the energy required to dephase that ion from the coherent ion packet. As there are competing mechanisms for ion dephasing (either by scattering or dissociation), a simple calculation of this upper mass
limit is not currently possible. However, a rough estimation can be made by assuming that a collision of at least 10 eV is necessary to dephase an ion. This estimation follows from the assumption that 10% of the energy in a collision is transferred into internal modes and a typical non-covalent interaction will have a bond dissociation energy of about 1 eV. Implied in this assumption is that scattering collisions will require much more energy for large ions than the energy required to dephase by dissociation. Given this assumption we can use Equation 2 - 13 and Equation 2 - 14 to estimate the upper mass limit possible in any given magnetic field. For magnetic field strengths of 3 T, 4.7 T, 9.4 T, 14.5 T, and 22 T the upper mass limit for a singly charged ion is 726 Da, 1148 Da, 2316 Da, 3584 Da, and 5447 Da respectively. As the kinetic energy increases with the square of the charge on an ion this upper mass limit will quickly increase for multiply charged species. Figure 2 - 4 is a plot of how the center-of-mass kinetic decreases as mass increases for various magnetic field strengths.

![Figure 2 - 4](image.png)

Figure 2 - 4 Center-of-mass kinetic energy as a function of mass. Energy represents an ion excited to a cyclotron radius equal to 75% of the trapping cell radius.

The weakness of this analysis is in the fact that dephasing can occur either by dissociation or by scattering collisions. Thus, the upper mass limit for CRAFTI is likely higher
than this estimation. Scattering an ion from the coherent ion packet without dissociation will still result in the dampening of the time domain signal and therefore will still contribute to the line broadening necessary in CRAFTI. Further work in the Dearden lab will probe the upper mass limit of CRAFTI (see Chapter 7 for more discussion).
2.4 References


3. Development of an IRMPD experiment and preliminary results

My initial work in the Dearden lab focused around the development of an experimental set-up for infrared multiphoton dissociation (IRMPD). The details of IRMPD theory were discussed in Chapter 2. This chapter will discuss the experimental design of the IRMPD set-up in the Dearden lab that comprised the first 2-3 years of my graduate career.

3.1 Experimental

3.1.1 Materials

Samples of L-tryptophan and L-phenylalanine were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. Electrospray samples were prepared by first dissolving in 88% formic acid (Mallinckrodt Baker Inc., Phillipsburg, NJ) and then diluting with methanol/water (50:50) to a final concentration of 100-200 µM.

3.1.2 Instrumentation

All IRPMDP experiments described in this chapter were performed on a Bruker model APEX 47e Fourier transform ion cyclotron resonance mass spectrometer. The instrument is equipped with an infinity cell and a 4.7 T superconducting magnet. Ions are generated in a microelectrospray source modified from an Analytica (Branford, MA) design. The MIDAS Predator data system (National High Magnetic Field Laboratory; Tallahassee, FL) is employed to control the FTMS instrument as well as the laser shutter.

Mid-IR light was generated by a LaserVision OPO, pumped by a Continuum YAG laser, average power of signal+idler was 300 mJ, had a bandwidth of 1 cm\(^{-1}\). Output from the OPO/OPA was measured using an Ocean optics USB4000 spectrometer. IR light was focused
into the trapping cell of a Bruker FT-ICR 4.7 T magnet. Ions of each species were photolyzed for about 300 laser pulses, and then mass analyzed using FT-ICR. An action spectra was created using Equation 3 - 1 below.

$$PDY = \frac{\sum A_{\text{frag}}(t_{\text{irr}}, \hbar \omega)}{A_{\text{prec}} + \sum A_{\text{frag}}(t_{\text{irr}}, \hbar \omega)}$$

Here PDY is the photodissociation yield, which will be equal to the sum of all fragments seen in the mass spectrum divided by the sum of all ion peaks in the mass spectrum (both product and parent ion peaks). When no dissociation occurs the PDY is therefore equal to zero while complete dissociation corresponds to a PDY equal to one.

3.1.3 Computational details

Computations of structure and normal mode frequencies were performed using RDFT, using NWChem 4.7 with an M06-2x hybrid functional, and a 6-31+G* basis set. Computed frequencies were firsts scaled. As recommended, different scaling factors for OH (0.976) and NH (0.959) were used to separate and correspond more closely to experimental values. Computational spectra were then line broadened to ~5 cm\(^{-1}\) to accurately reflect the experimental spectra.

3.2 Experimental considerations

Much of the work completed on this particular project is not publishable in a peer-reviewed journal. Replication of known data played an important role in my early work. This type of replication, while necessary to validate the experimental set-up, would not contribute any new or significant data to the field and would therefore not warrant publication. During this process, however, a number of important experimental considerations were discovered. While not fit for publication this information will likely prove useful to further work in the Dearden lab.
In this section I will be addressing those experimental considerations as well as giving my thoughts on how future experiments can run more smoothly.

3.2.1 Nitrogen purging

Most IRMPD experiments utilize a nitrogen purging system to ensure optimal beam power enters the FTICR-MS trapping cell. In the Dearden lab this proved to be somewhat difficult and, in the end, unnecessary. The beam path was purged from the exit of the OPO/OPA to about 25 cm before the entrance to the vacuum. A simple PVC pathway was originally built and later an acrylic purge box was designed and built to simplify the alignment process. I noticed, however, that nitrogen purging was decreasing the laser's power over the pathway that was purged. This was likely due to either a turbulent air flow or water vapor that had contaminated the nitrogen gas. In any case, I discovered that because of Utah's dry climate the total laser power in an unpurged pathway was sufficient for the IRMPD experiment. The work presented here was done without nitrogen purging.

3.2.2 Alignment

Laser alignment is, of course, a key consideration for these experiments. Photons are created in the Nd:YAG laser and wavelength selection and idler amplification is done in the OPO/OPA. IR photons are guided into the FTICR-MS using the steering optics. A 100 cm focusing lens is placed ~120 cm from the center of the FTICR-MS trapping cell. This allows for the beam that is well aligned to be expanding inside the cell, irradiating a larger ion population.

There is some difficulty in aligning a beam of photons into the center of cell that is under vacuum. As the ion steering optics and source drying tube prevent photons from exiting the front of the instrument, it cannot be verified that the beam is in fact passing through the center of the cell. Any off center irradiation causes signal problems described in 3.2.3. There are several ways
that I found to verify that the beam path is in fact through the center of the cell. First, an acrylic cover can be placed on the front of the instrument in lieu of the standard ion creation source (including the drying tube, skimmer, and hexapole). This acrylic cover allows for the vacuum in the cell to be retained while still visualizing the cell. A visible light laser can be aimed through the FTICR-MS from the front to the back. This beam can then serve as a reference point for the z-axis beam path that will pass directly through the center of the cell. Two irises on the back of the instrument can be aligned with this visible light laser beam as a reference point for future IR alignments once the acrylic plate has been removed and the ion creation source has been replaced. A second, and much less accurate, option is to shine a bright flashlight down the bore of the magnet until the IR window is seen. Two irises are then placed such that the IR window can still be seen while looking through the irises. This second option is much faster, but also less accurate. However, this method has proved accurate enough to see small amounts of IRMPD fragmentation that can be used to tune the beam to a true center. This second method also does not require breaking the source vacuum, thus saving a substantial amount of time.

3.2.3 Gold and nickel in signal

A consequence of the difficult and inefficient alignment techniques described above was the appearance of gold and nickel appearing frequently in the mass spectra. When the beam is not properly aligned with the center of the cell a significant portion of the laser's power is focused on the cell itself, which is made of gold and nickel. I noticed very early that gold and nickel would consistently show up in the mass spectrum during an IRMPD experiment — both while doing the initial laser alignment and after the experiment itself had been running. In both cases gold and nickel in the mass spectrum was evidence of laser misalignment; either it had not been correctly aligned to begin with or the beam path had drifted.
3.3 Initial attempts using β-glucopyranoside

To ensure that the alignment was correct and that dissociation would occur as expected, the first step in this project was the replication of a published IRMPD experiment. The initial suggestion was to use a rubidiated β-glucopyranoside published by Eyler. For this complex, several intense peaks are expected between 3350 cm$^{-1}$ and 3750 cm$^{-1}$. Dissociation of this complex was never seen upon irradiation in our trapping cell. Later discussions with Dr. Nicolas Polfer suggested that β-glucopyranoside would be a poor choice for an initial survey of the experimental set-up as he had seen poor absorption (and therefore dissociation) in sugars as compared to other compounds. This poor absorption makes accurate laser alignment more critical. Instead it was suggested that protonated tryptophan would be an easier compound to see dissociation even if the laser was slightly misaligned. Then, to improve the signal the alignment can be tuned until maximum dissociation is seen.

3.4 IMRPD spectrum of protonated amino acids

3.4.1 IRMPD spectrum of protonated tryptophan

The gas phase IRMPD action spectrum has been previously reported by Polfer et al. In this study they found a strong absorption band at 3550 cm$^{-1}$ corresponding to the carboxylic acid -OH stretching mode, a doublet just below 3500 cm$^{-1}$ corresponding to the indole stretch and a NH$_3^+$ stretching modes that begin at 3340 cm$^{-1}$ with the asymmetric stretching. They found that protonated tryptophan (m/z 205) fragments by loss of neutral ammonia. The fragmentation peak (m/z 188) can further fragment by loss of ethenone (CH$_2$CO). No further fragmentation is seen from this peak (m/z 146) as none of the initial IR absorbing moieties remain. The IRMPD action spectrum for these fragments (m/z 188 and m/z 146) were also obtained by creating the
fragmentation products using nozzle-skimmer CID and isolating the products in the cell. Figure 3 - 1 shows the IRMPD action spectra reported by Polfer et al.

Figure 3 - 1 IRMPS action spectra for protonated tryptophan and related fragmentation products as reported by Polfer et al.\textsuperscript{1a}

I chose protonated tryptophan as the system that would be used to verify the IRMPD experimental set up because the ion signal was easily obtained (electrospraying tryptophan from an acidic solution required no major tuning of the ion optics to see a significant signal for TrpH\textsuperscript{+}), protonated tryptophan is also well studied system, and complete fragmentation is seen in as little as 1.5 seconds of irradiation. This last point means that if the laser is slightly misaligned fragmentation will still be seen at the strongest band (3550 cm\textsuperscript{-1}). The laser alignment can then
be tuned until this peak is fully fragmented. In our system full fragmentation is seen after 3-5 seconds of irradiation from a well aligned laser. The full IRMPD action spectrum obtained from the set-up in the Dearden lab is seen in Figure 3 - 2 below.

![Gas phase IRMPD action spectrum of protonated tryptophan](image)

As expected, there is a strong absorption band at 3550 cm$^{-1}$, corresponding to the carboxylic acid -OH stretching mode, a doublet just below 3500 cm$^{-1}$ corresponding to the indole stretch and the asymmetric -NH$_3^+$ stretching mode at 3340 cm$^{-1}$. While the -NH$_3^+$ stretching modes are expected to continue after the asymmetric mode at 3340 cm$^{-1}$, this region of the IR spectrum was not probed, as this was only a preliminary study to verify the laser alignment. Fragmentation patterns also occur as expected, with protonated tryptophan (m/z 205) fragmenting by loss of neutral ammonia (yielding m/z 188). This product further fragments by loss of ethenone (yielding m/z 146). Although Polfer et al. report the IRMPD action spectra of these two fragmentation peaks, I did not replicate these results since the purpose of the initial replication was to verify correct alignment of the laser.
3.4.2 IRMPD spectra of protonated phenylalanine

After successfully verifying the laser alignment using TrpH⁺, the IRMPD action spectrum of protonated phenylalanine (PheH⁺) was obtained. PheH⁺ is structurally similar to TrpH⁺, so little difference was expected between the two IRMPD action spectra. The IRMPD action spectrum of PheH⁺, which has not been reported in the literature, is also a good stepping stone towards obtaining an IRMPD action spectrum of PheH⁺ bound to cucurbit[7]uril (discussed in more detail later in this chapter). The fragmentation pattern of PheH⁺ is more simple than that of TrpH⁺, as shown in Figure 3 - 3. While TrpH⁺ fragments by loss of neutral ammonia followed by a secondary loss of ethenone, PheH⁺ fragments only once by loss of neutral CO and H₂O. This removes the resonant carboxylic acid stretch and prevents further absorption and fragmentation.

![Figure 3 - 3 Dissociation of protonated phenylalanine. Mass spectrum obtained after 5 seconds of irradiation at 3545 cm⁻¹.](image-url)

The full IRMPD action spectrum of PheH⁺ can be seen in Figure 3 - 4 below.
Figure 3 - 4 IRMPD action spectrum of protonated phenylalanine.

Missing in the IRMPD action spectrum for PheH$^+$ is a doublet just below 3500 cm$^{-1}$ corresponding to the indole stretch and the asymmetric -NH$_3^+$ stretching mode at 3340 cm$^{-1}$. As an indole moiety is not present in PheH$^+$ the lack of a corresponding stretch is expected. However, as an -NH$_3^+$ moiety is present the lack of this absorption band in the IRMPD action spectrum is unexpected. Also, the carboxylic acid stretch at 3550 cm$^{-1}$ has been red-shifted by roughly 15 cm$^{-1}$. Both of these differences between TrpH$^+$ and PheH$^+$ can be explained as red-shifts due to coupling with the phenyl group. As the indole group is not present in PheH$^+$, the -NH$_3^+$ moieties interact more with the phenyl pi system. This red-shift is seen in the carboxylic acid stretch and may explain why the -NH$_3^+$ stretch is not seen. It may be that the stretch is below the range probed in this work. Indeed, the computational spectra of PheH$^+$ show the -NH$_3$ stretching modes beginning at 3300 cm$^{-1}$, just to the red of the experimental range (see Figure 3 - 13 later in this Chapter for the computational IR spectra). Figure 3 - 5 is a comparison between the experimental TrpH$^+$ and PheH$^+$ action spectra.
3.5 IRMPD spectra of protonated $\alpha,\omega$ diamines

Further IRMPD work was done to determine how hydrogen bonding can affect an IRMPD action spectrum. For this work, protonated $\alpha,\omega$ diamines were used as a model system. In the gas phase, singly protonated $\alpha,\omega$ diamines will bend so that the terminal amines can form a hydrogen bonding system with one bridging proton between two -$\text{NH}_2$ moieties. The lowest energy conformers are pictured in Figure 3 - 6.
As seen in this figure, this hydrogen bonding system makes very subtle changes as carbons are added and the length of the hydrocarbon chain increases. Probing the effect that these subtle changes will have is the purpose of using protonated α,ω diamines as a model.

3.5.1 Computational data

Computational results for the IR frequencies of H_{2}N(CH_{2})_{n}NH_{3} for n = 3-6 can be seen in Figure 3 - 7. The computational IR spectrum for protonated α,ω propane diamine reveals symmetric and antisymmetric -NH_{3} stretches at 3310 cm\(^{-1}\) and 3370 cm\(^{-1}\) as well as symmetric and antisymmetric -NH_{2} stretches at 3340 cm\(^{-1}\) and 3410 cm\(^{-1}\), and similar features exist in the IR spectra for butane, pentane, and hexane diamine, though as the carbon chain length increases the features are red-shifted. An increasing carbon chain allows the -NH_{2} moiety to interact more with the protonated -NH_{3}, resulting in a red-shift of the frequencies.
Along with this red-shift, the symmetric and antisymmetric stretching frequencies are closer together for even numbered carbon chains than for odd numbered carbon chains. A possible explanation of this behavior is that for odd numbered carbon chains the -NH₂ hydrogens are forced into a gauche conformation, while for even numbered carbon chains the -NH₃ hydrogens are in an anti conformation.

Although the H₂N - - - HNH₂ system has been modeled here as having one hydrogen bound primarily to one nitrogen and hydrogen bound to the second, it would be more correct to
model the hydrogen as a bridging hydrogen. To get an idea for which chain length allows for the optimal amount of sharing of this proton, I have measured the inner atomic distance between the short N-H bond and the longer, hydrogen bond. The ratio of these bonds gives an idea of how well the bridging hydrogen is shared between the two nitrogen atoms. A ratio equal to 1 means an equal sharing. Figure 3 - 8 shows a plot of this bond ratio as carbon chain length increases.

![Bond ratio plot](image)

Figure 3 - 8 Bond ratio of the long and short N-H bonds in cyclized diamines. A bond ratio equal to 1 would indicate an equal sharing of the bridging hydrogen.

This figure suggests that the optimal chain length for a cyclized diamine is 4 carbons, or a cyclical molecule formed by 7 atoms.

3.5.2 Experimental data

IRMPD action spectra for protonated propane, butane, and hexane diamine were experimentally observed. The results can be seen in Figure 3 - 9 below.
During the course of these experiments the laser path within the OPO/OPA had some alignment issues, therefore the complete spectra for these diamines were not obtained. The features that were observed were peaks at 3278 cm$^{-1}$ and 3335 cm$^{-1}$ for propane diamine, a peak with a slight shoulder at 3325 cm$^{-1}$ for butane diamine, and a peak at 3355 cm$^{-1}$ for hexane diamine. Other smaller features are possibly present, but replicate measurements would show if these peaks are above the noise level.

Figure 3 - 9 Experimental and Theoretical IRMPD action spectra for NH$_2$(CH$_2$)$_n$NH$_3$ for n = 3, 4, and 6.
For propane diamine the experimental peaks are red-shifted from the computational peaks by roughly 35 cm\(^{-1}\). The hexane diamine experimental peaks, however, are blue-shifted by about 15 cm\(^{-1}\). Only one feature is seen in the butane diamine spectra and therefore it cannot be attributed as either red or blue shifted. This shift could be explained as a systematic error, a red-shift in one spectrum and a blue-shift in another. The red-shift/blue-shift may be because of the way the systems were modeled. If the energy to transfer the proton from one nitrogen to the other is low enough then a mobile proton would affect the vibrational frequencies that are seen experimentally. Unfortunately, without a full spectrum to analyze any it would not be possible to make any definitive conclusions about these systems. Future work in the Dearden lab should focus on fixing the alignment within the OPO/OPA system so that these experiments can continue.

3.5.3 Dissociation mechanism for protonated diamines vs. protonated monoamines

Figure 3 - 10 is my proposed dissociation mechanism for protonated gas phase diamines. In every case for the protonated \(\alpha,\omega\) diamines the dissociation product is loss of neutral ammonia. This dissociation product is the only observed product in both IRMPD and SORI-CID experiments. Although the lowest energy conformation is the formation of intermolecular hydrogen bonds between the protonated nitrogen and the non-protonated nitrogen, the protonated nitrogen can rotate away from the non-protonated nitrogen. This places the -NH\(_3\) in an ideal position to act as a leaving group for an intermolecular \(S\text{\textsubscript{N}2}\) cyclization reaction, forming the product seen in Figure 3 - 10.
This mechanism has a conformational requirement that neither tryptophan nor phenylalanine has, which explains the low dissociation yields seen for protonated diamines (~20% after 30 seconds of irradiation as compared to ~100% after 5 seconds for protonated tryptophan and phenylalanine). This proposed mechanism is supported further by the dissociation products of protonated monamines. IRMPD was attempted on protonated monoamines, but when no dissociation products were seen I attempted SORI-CID. Instead of loss of ammonia, the SORI-CID products for the protonated monoamines were different for each of the monoamines. In each case the fragmentation involved breaking a carbon-carbon bond instead of losing neutral ammonia. These fragmentation patterns has been documented for monoamines\(^3\) but not for the diamines. Further studies examining this fragmentation pattern is warranted to verify the proposed fragmentation mechanism.
3.6 IRMPD spectra of cucurbituril complexes

Cucurbiturils are macromolecular structures originally synthesized by Robert Behrend in 1905 by the condensation of glycoluril with formaldehyde. Although Behrend found that the newly synthesized substance had a high affinity for alkali metals and organic dyes, the structure of this interesting compound was not discovered for another 75 years. In 1981 Mock et al. used x-ray crystallography to show that cucurbit[n]urils, CB[n]s, are macrocyclic molecules with n repeating glycoluril monomers. These macrocycles have a distinct resemblance to a pumpkin, a member of the plant family cucurbitaceae, from which their name is derived. Cucurbiturils are highly symmetric, having two carbonyl portals with a high binding affinity for cationic guests. This binding is due to a combination of ion-dipole interaction involving the carbonyl groups and hydrophobic forces of the inner cavity. The binding of cucurbiturils to amino acids, peptides, and proteins has also been studied. CB[6], as a host molecule, is too small to encapsulate amino acids. However, Buschmann et al. have shown using isothermal titration calorimetry (ITC) that not only do amino acids have a high binding affinity (K_a ~1x10^3 M^-1), but that binding affinity has little variability as the size of the amino acid is increased. From this data, the Buschmann group determined that the amino acids have very little interaction with the inner cavity and instead form externally bound complexes. Tao and coworkers later showed that the cavity of CB[7] was large enough to encapsulate two equivalents of phenylalanine, leucine, or tyrosine inside the cavity.

An interesting application to cucurbiturils as synthetic receptors was demonstrated by Urbach et al. Capitalizing on the strong binding affinity of CB[7] to phenylalanine, a direct assay for insulin is reported. A fluorescent dye is first complexed with CB[7], which acts as a fluorescent quencher. When aliquots of insulin are added an increase in fluorescence is seen as
the phenylalanine residue binds within CB[7], displacing the fluorescent dye. The effect is quantitative, and is seen even in a mixture of common blood proteins. These insulin@CB[7] and amino acids@CB[n] studies were the motivation for the study of amino acids@CB[7] described below. The hope was to use IRMPD to probe the conformation of amino acids bound to CB[7] in the gas phase. These conformations were first studied computationally and then attempts were made to obtain IRMPD spectra for the gas phase protonated complexes.

3.6.1 Computational IRMPD spectra for cucurbituril complexes

3.6.1.1 [Phenylalanine@CB[7]]^+

Monte Carlo conformational searches found two families of conformations for cucurbit[7]uril bound to protonated phenylalanine in the gas phase, as shown in Figure 3 - 11 below:

![Low energy conformations of PheH^+@CB[7].](image)

External bound  
~ 64 kJ/mol

Internally bound  
0 kJ/mol

Figure 3 - 11 Low energy conformations of PheH^+@CB[7].
The internally bound family of conformations were the lowest energy conformations found by ab initio calculations (M06-2x; 6-31+G*). There is also an externally bound family of complexes whose energy is roughly 64 kJ/mol higher than the internal structures. Verifying the binding site of host:guest interactions is a key aspect of macromolecular chemistry. IRMPD provides a convenient way to determine the binding site, as the vibrational frequencies of an internally bound complex should be distinguishable from the vibrational frequencies of an externally bound complex. This can be seen in the computational IR spectra of PheH⁺@CB[7] Figure 3 - 12 below:

Figure 3 - 12 Computational IR spectra of PheH⁺@CB[7].
In Figure 3 - 12 the computational IR spectra for two possible experimental set-ups is seen. The top spectrum is the computational IR spectrum for the frequency range that could be obtained using a free-electron laser. These experiments use a high energy beam to probe the 600-1800 cm\(^{-1}\) range\(^{10}\). This range includes most C-C vibrational\(^{11}\) frequencies as well as many of the vibrational frequencies found in metal-organic compounds, which are often studied using this technique\(^{12}\). However, the IRMPD spectrum of PheH\(^+\) at these wavelengths suggest that a free electron laser would be a poor choice to distinguish the internal from the external complexes. The two spectra are very similar due to the largely unchanged conformation of the CB[7] host. Even with some changes that occur as PheH\(^+\) binds externally vs. internally, these similarities in the host lead to a largely unchanged IR spectra in the C-C range. However, in the -NH and -OH stretching region the spectrum is much more simplified, and unique features could be probed using a tabletop OPO/OPA like the one available in the Dearden lab.

Figure 3 - 13 shows the computational IR spectra for the internally bound PheH\(^+\), the externally bound PheH\(^+\), and the unbound PheH\(^+\). As reported earlier, the IRMPD action spectra of PheH\(^+\) has been observed to have a carboxylic acid stretch just above 3500 cm\(^{-1}\), appearing experimentally at about 3535 cm\(^{-1}\). This is slightly blue-shifted from the computational predictions. The carboxylic acid -OH stretch shifts ~120 cm\(^{-1}\) from the unbound phenylalanine to the internally bound complex. From the internally bound to the externally bound complex another red-shift of ~150 cm\(^{-1}\). These red-shifts are the primary features that could be used to distinguish the internally bound complexes from the externally bound complexes.
3.6.1.2 Tryptophan@CB[7]

I also attempted to obtain an IRMPD spectrum of protonated tryptophan@CB[7]. The computational results were similar to that of protonated phenylalanine@CB[7]. A low energy conformational family exists for the internally bound TrpH⁺ as well as an externally bound conformational family that is about 53 kJ/mol higher than the internally bound complex. The range accessible using a free electron laser is also nearly indistinguishable while the range of 2800-3400 cm⁻¹ seems like an excellent range to determine if the complex is internally or externally bound. The -OH stretch in TrpH⁺@CB[7] is red-shifted by about 150 cm⁻¹ if the complex is bound externally. For both of these complexes — PheH⁺@CB[7] and TrpH⁺@CB[7] — no IR induced dissociation was ever seen. Possible reasons for this are discussed later in this Chapter.
3.6.1.3 Computational IR spectrum for MeOH and [MeOH@CB[5]NaK]^{2+}

The important features in the IR spectra that have been previously studied have been the -OH stretches of the guest molecule. A third interesting system is that of neutral methanol trapped inside CB[5]. Two alkali metals, Na^+ and K^+, act as caps to the CB[5] cage. This system could be used as a more simple model to understand the red-shifting of an -OH moiety that is bound in the cavity of a cucurbituril. Computational results, seen in Figure 3 - 15 suggest that, once again, a red-shift of ~100 cm\(^{-1}\) is seen for a bound -OH moiety as compared to an unbound -OH moiety.

Figure 3 - 14 Computational IR spectra for TrpH+@CB[7].
The low energy dissociation pathway of the \([\text{MeOH@CB}[5]+\text{NaK}]^{2+}\) complex is by loss of the internal MeOH. This in spite of the metals that appear to be "blocking" its exit through the carbonyl portals.
This dissociation can occur because the activated complex has a "plunger" vibrational mode in which the host molecule moves in the y-direction opposite to the three guest molecules. When sufficient energy is added to this vibrational mode the MeOH has a low energy pathway to dissociation. This low energy dissociation pathway should make the \([\text{MeOH@CB}[5]+\text{NaK}]^{2+}\) an ideal candidate for IRMPD experiments, though all attempts to date have not yielded any experimental results.

3.6.2 Experimental IRMPD spectra of cucurbituril complexes

It is possible that the dissociation of cucurbituril complexes has not been seen because of dye tuning effects that are commonly seen in these systems. The polarizability of cucurbiturils has been studied using solvatochromic probes, such as 2,3-diazabicyclo[2.2.2]oct-2-ene (DBO). The inverse oscillator strength of the near-UV absorption band of DBO has a linear correlation to the polarizability of the solvent environment. Results showed that the polarizability of the inner cavity is extremely low (0.12), below that of even perfluorohexane. As a comparison, most other macrocycles have a cavity whose polarizability is similar to that of alkanes or water. The interior of CB[7] to date has the lowest measured polarizability.\textsuperscript{13}

An interesting consequence of such low polarizability is a decrease in the radiative decay rate, \(k_r\), of fluorescent states.\textsuperscript{14} This decay rate is determined by the Stickler-Berg equation:

\[
k_r = \frac{\phi_f}{\tau_f} \propto n^2
\]

Where \(\Phi_f\) is the fluorescence quantum yield, \(\tau_f\) is the fluorescence lifetime, and \(n\) is the refractive index of the environment. Some fluorescent dyes, when bound to cucurbiturils, display their longest recorded fluorescent lifetimes. This effect is seen even when the fluorescent dye is
too large to fit completely inside the cucurbituril, as is the case with DBO. These results further support the idea that carbonyl binding as well as inner-cavity binding are important.

What is even more surprising, given that complexation leads to decreased radiative decay, is that the quantum yield (defined as the ratio of the radiative decay rate to the sum of all decay rates including non-radiative decay) of a dye when complexed with with CB[7] increases. The significance of this statement is that cucurbituril complexation both decreases the radiative decay rate and increases the fluorescence quantum yield, a scenario that the Stickler-Berg equation seems to prohibit. This paradoxical result seems to suggest that cucurbituril complexation protects the dye from non-radiative relaxation pathways. One of these non-radiative relaxation pathways is, of course, dissociation. Thus, dissociation would not be expected if the rate of dissociation is affected such that IR emission becomes a competitive relaxation process.

Another possible (and if fact, more likely in my opinion) explanation is RRKM effects. Dissociation in IRMPD occurs when energy from the infrared excites vibrational modes in the complex. This initial absorption rarely leads directly to dissociation. Instead, the energy moves throughout all vibrational modes until it reaches the mode associated with the lowest energy dissociation pathway and the complex dissociates. As the number of vibrational modes increases, the rate at which a complex will dissociate decreases due to the time it takes for the energy to move throughout all the available modes. For a molecule with a high number of vibrational modes this timescale may be long enough that IR emission will be more statistically likely than dissociation, and IRMPD fragmentation will not be seen.
If RRKM effects are responsible for the lack of dissociation a simple solution is to simply add more energy to the system. The higher energy will increase the dissociation rate and dissociation will become more statistically likely than IR emission. This has been done by other groups by exciting first with a tuneable OPO/OPA laser similar to that used used in this work followed by irradiation with a CO₂ lasers tuned to a resonant frequency. The strength of the secondary laser pulse (CO₂) is tuned such that dissociation is only seen when energy is added by absorption of the photons from the OPO/OPA. Another option is to seek out a more powerful laser system that is tuneable along the desired range, such as the tunable infrared light produced by the Free-Electron Laser for IntraCavity Experiments FELICE.

3.7 Conclusions

IRMPD is a powerful analytical tool that allows structural determination of gas phase molecules. To date the Dearden lab has successfully begun experimental work on several interesting systems. While in the Dearden lab I began by designing the alignment scheme for later experiments to be done. The IRMPD action spectrum of protonated tryptophan was the first recorded spectrum in the lab followed soon thereafter by protonated phenylalanine. These protonated amino acids fragment very easily making them ideal candidates to verify the alignment of any new or recently misaligned experimental set-up. Next I began collecting IRMPD spectra for protonated α,ω diamines. Though problems with the OPO/OPA interrupted my work, these simple systems may hold interesting clues about the effects that hydrogen bonds have on IRMPD spectra.

IRMPD action spectra for cucurbituril complexes have not to date been recorded. There are several possible explanations for why this may be the case. A more powerful laser system, or a dual laser system will likely result in fragmentation being seen. There are a number of
interesting questions waiting to be answered. For example, what effect on -OH moieties is seen when an ion is bound inside the cavity of a cucurbituril? Can an IRMPD action spectrum be used to determine if a complex is internally or externally bound?

One may ask why IRMPD should be used to determine the conformation of gas phase CB[n] complexes. This may be especially true for the Dearden lab as we already have a proven technique for determining molecular size - CRAFTI (see Chapters 4-6 of this work). However, CRAFTI is only able to determine the ensemble average properties of a coherent ion packet. If multiple conformations of a system are present - which may be the case with these CB[n] complexes where conformational families exist in very close energies - then IRMPD could possibly be used to determine the relative abundances of each conformation. For example, if an obvious IRMPD feature is shifted a known amount the relative intensities of those peaks could contain information about the relative conformation abundances in the total ion population. This relative abundance information could then be used to determine the cross sections of two conformations present in a single coherent packet using CRAFTI.
3.8 References

4. Absolute pressure measurement in FTICR

Gas pressure plays a fundamental role in many important chemical processes, including the establishment of physical and chemical equilibria and the determination of rates of chemical reactions. Accurate measurement of pressure is essential for characterizing such processes, and pressure measurement is often the limiting factor in many quantitative experiments.¹

Specifically, in Fourier transform ion cyclotron resonance (FTICR) mass spectrometric measurements of reaction rates, collisional dissociation thresholds, or collision cross sections, accurate pressure determinations in the ion trapping cell are essential. In the relevant pressure range (typically $10^{-10}$—$10^{-5}$ mbar), pressures are almost always measured using ionization-based techniques.² Most commonly, these involve either a Bayard-Alpert style "hot cathode" type ionization gauge³ or a Penning-style cold cathode gauge.⁴ All ionization-based methods, including these, fundamentally involve ionization of the neutral gas and measurement of the resulting ion current.² The ion current is proportional to the neutral gas pressure and also depends on the ionization cross section of the gas.

However, the high magnetic field required for FTICR experiments presents some special challenges, because ion motion and the resulting ion current measurement are strongly affected by the magnetic field. Most commonly, ionization gauge transducers on FTICR instruments are placed outside the high-field region of the magnet, sometimes as much as a meter or more away from the ion trapping cell. Gas conductance between the transducer and the trapping cell can result in pressure differences between the transducer and trapping cell of as much as an order of magnitude.⁵ When pressure measurements are critical, corrections for this difference must be made, typically by measuring the rate of a well-characterized ion-molecule reaction in the
trapping cell and using the known rate constant to determine the actual pressure in the trapping cell.

In addition to the problems inherent to operating in a high magnetic field, ionization-based methods suffer other problems. They act as low-speed ion pumps, so their operation may perturb the measurement. Ionization techniques are influenced by deposition of material on the surfaces of the transducer, which may change with time. Hot cathode devices require bakeout and thermal equilibration, and outgassing from the heated filament may additionally perturb the pressure. In addition, in cold cathode devices, it can be difficult at low pressures to initiate the plasma that is required to ionize the neutral gas, and small non-linear discontinuities between pressure and ion current have been reported. As a result, the sensitivity of an ionization-based measurement may vary by as much as a factor of 2-3 over a period of several days.

We recently introduced a new method of measuring collision cross sections using an FTICR instrument (collision cross sectional areas from analysis of Fourier transform ion cyclotron resonance, or "CRAFTI"). CRAFTI measurements are based on the dependence of FTICR linewidths (FWHM) on collisions that dephase the coherently-orbiting ions (under pressure-limited conditions), and depend inversely on the neutral collision gas number density \( n_n \), as shown in.

\[
\sigma = \frac{\text{FWHM}}{n_n} \frac{(m_{\text{ion}} + M_{\text{neutral}})}{M_{\text{neutral}}} \frac{m_{\text{ion}}}{q} \beta V_{pp} t_{exc} \quad (4 - 1)
\]

Here, \( \sigma \) is the dephasing cross section, \( m_{\text{ion}} \) and \( M_{\text{neutral}} \) are the masses of the ion and neutral, respectively, \( q \) is the ion charge, \( d \) is the cell diameter, \( \beta \) is the cell geometry factor, \( V_{pp} \) is the peak-to-peak excitation voltage, and \( t_{exc} \) is the duration of the excitation event. It is easy to see that Equation 4 - 1 can be solved for neutral number density, and that (all other things being
equal) number density is directly proportional to the FWHM linewidth. All the terms in the resulting equation except \( \sigma \) are either known or easily measured. This suggests that for systems where \( \sigma \) is known and the linewidth is pressure-limited, FTICR linewidth measurements can be used to determine absolute pressures. This is an attractive idea because it might enable measurement of pressures directly in the FTICR cell, without depending on ionization potential or requiring an ion current measurement.

For such an approach, well-characterized collision cross sections are needed. Both ion mobility spectrometry (IMS)\(^8\) and traveling-wave ion mobility spectrometry (TWIMS)\(^9\) have generated large databases of measured collision cross sections. However, identification of suitable ions with known cross sections is complicated by the fact that ion-neutral cross sections are dependent on the kinetic energy of the collision and on the neutral collision gas. Therefore, directly comparing cross sections to IMS-MS or TWIMS is not straightforward because the experimental conditions for these techniques are quite different from those that pertain in the FTICR cell when using CRAFTI. For example, while both IMS and TWIMS involve many low energy (thermal) collisions (usually with He), CRAFTI measurements involve a single high energy (~1-10 keV) collision (usually with Ar or some other collision partner heavier than He). This problem is further complicated by the fact that all dephasing collisions, including collisions that lead to dissociation, play a role in determining CRAFTI cross sections, whereas ion mobility measurements are dominated by momentum transfer collisions. As a result, CRAFTI cross sections are typically larger than measured IMS, TWIMS, or computed cross sections that are designed to model IMS or TWIMS. This makes cross sections measured using mobility methods difficult to use for our purposes.
Fortunately, a suitable set of kinetic energy-resolved cross sections is available for collisions of Ar⁺ with neutral Ar.¹⁰ This is convenient because our primary application is pressure measurements in support of CRAFTI cross section determinations, and Ar is our collision gas of choice. The purpose of this paper is to demonstrate this new method of measuring pressures without relying on ion current measurements, specifically to show that FTICR linewidth measurements can be used to accurately measure absolute pressures in the trapping cell of FTICR instruments. We term the new method linewidth pressure measurement, and refer to it herein by the acronym "LIPS".

4.1 Experimental

4.1.1 Materials

Argon gas (99.95%) was purchased from Airgas.

4.1.2 Instrumentation

All experiments were performed using a Bruker APEX 47e Fourier transform ion cyclotron resonance mass spectrometer with an Infinity trapping cell.¹¹ The instrument was controlled using a MIDAS Predator data system (National High Magnetic Field Laboratory; Tallahassee, FL).¹² RF excitation amplitudes were measured using an oscilloscope at the output of the final excitation amplifier. Pressures within the trapping cell were varied using a Freiser-type⁵ pulsed leak valve consisting of a 0.004" orifice solenoid pressurization valve backed by a 28 psig Ar supply line and a 0.039" orifice solenoid evacuation valve connected to a mechanical vacuum pump (both valves from General Valve Corp.; Fairfield, NJ). Both solenoid valves were connected to the high-pressure side of a precision variable leak valve (Varian; Palo Alto, CA).
Steady-state pressures obtained with the pulsed leak system were varied by varying the length of
time the pressurization solenoid was left open.

4.1.3 Procedures

Conventional pressure measurements were performed using a cold cathode gauge
(Balzers; Fürstentum, Lichtenstein) mounted outside the high field region of the instrument,
about 1 m from the trapping cell. All pressures were adjusted for the ionization potential of
neutral Ar.¹

Ar⁺ ions were created by first pulsing neutral Ar into the trapping cell to about \(5 \times 10^{-7}\)
mbar and ionizing with a 50 ms, 100 eV pulse from an electron gun mounted on the trapping cell
axis. Following ionization, the pulsed leak was evacuated and the system was pumped for 500
ms to remove neutral Ar and return the system to baseline pressure.

For experiments measuring total ion intensity, a 5 ms, 100 eV pulse from the electron gun
was used to produce ions. Because the total FTICR experimental sequence was long enough to
allow reactive collisions (mainly charge transfer to form H₂O⁺) at the pressures employed, the
total ion intensity of all species was measured as a function of the steady-state Ar pressure
achieved at various pulsed valve pressurization durations. A calibration curve was generated to
relate total ion intensity to steady-state Ar pressure as determined using the cold cathode tube.

A typical LIPS experiment was done by first creating Ar⁺ as described above. Next,
neutral Ar was leaked into the system at various pulsed valve pressurization durations and the
Ar⁺ linewidth was calculated using the Igor Pro software package (version 6, Wavemetrics; Lake
Oswego, OR).⁷, using the appropriate cross section for the experimental Ar⁺ kinetic energy,
yields a pressure corresponding to that particular pulsed leak pressurization duration. Because
the pressures created by pulsed leak valve were very reproducible,\textsuperscript{13} in practice the pulsed leak duration could be used thereafter to generate known steady-state pressures.

4.2 Ar\textsuperscript{+}/Ar momentum transfer cross sections as a calibrant

As seen in Equation 4 - 1 and described above, the absolute value of a CRAFTI cross section depends on the number density of the neutral collision gas. If an accurate cross section is known the pressure in the cell can be directly measured using the linewidth in the mass spectrum. Finding a suitable system with a known value of \( \sigma \) is more difficult than it might seem at first glance. Both IMS-MS and TWIMS have generated large databases of measured collision cross sections. Unfortunately, directly comparing cross sections to IMS-MS or TWIMS is not straightforward because the experimental conditions for these techniques are quite different from those of CRAFTI. For example, while IMS-MS utilizes many low energy (thermal) collisions (usually with He), CRAFTI measurements involve a single high energy (~1-10 keV) collision (usually with Ar or some other collision partner heavier than He).

This makes the absolute cross section values very different, though qualitative comparisons between IMS-MS and CRAFTI have been found to be very similar (see Chapter 2 of this work). This problem is further complicated by the fact that all dephasing collisions, including collisions that lead to dissociation, play a role in measuring collision cross section by the CRAFTI technique, whereas IMS-MS measurements are dominated by momentum transfer collisions. As a result, CRAFTI cross sections are typically larger than measured IMS-MS or TWIMS cross sections or computed cross sections that are designed to model IMS-MS or TWIMS. The effect that dissociation plays in FTICR-MS is discussed in greater detail in Chapter 5 of this work.
Momentum transfer cross sections have been measured for Ar$^+$ colliding with Ar.$^{14}$ These data are plotted in Figure 4 - 1.

![Graph showing collision cross sections of Ar$^+$ in Ar over a wide range of kinetic energies.](image)

**Figure 4 - 1.** Collision cross sections of Ar$^+$ in Ar over a wide range of kinetic energies.

These literature data make an excellent comparison to CRAFTI measured cross sections for two reasons: First, the cross sections decrease as kinetic energy in the lab frame increases in a predictable way (logarithmically), making it possible to determine the cross section at any kinetic energy in this range. Second, the range of these cross sections is within the energy range convenient for use in CRAFTI.

To compare these literature data to CRAFTI cross sections the Lorentzian linewidth of Ar$^+$ in Ar was measured in the FTICR over a wide range of kinetic energies (2-14 keV). These linewidths were then used to minimize the error between the literature value cross sections and the CRAFTI cross sections, using $n_n$ as the only free parameter. The results of this least squares analysis are seen in Figure 4 - 2 below.
The absolute values of the CRAFTI collision cross sections in this figure are not of any particular merit, as they were determined using a fitting routine, but of particular interest is that all of the data points in the figure were well fit using only Ar neutral number density as a free parameter. This means that the collision cross sections for Ar⁺ in Ar measured by the CRAFTI technique behave similarly to the cross sections previously reported in the literature. The fitting procedure yields a number density (pressure) for the Ar collision gas in the FTICR cell, and the pressure obtained in this way is typically within a factor of 3 of the pressure obtained using the cold cathode tube (vide infra); we take this as strong evidence that the pressures obtained from CRAFTI are reasonable.

Therefore, Ar pressures in the FTICR cell can be measured using a LIPS technique. This technique is very similar to the CRAFTI experiment described above, though the pulse sequence is slightly different. In LIPS pressure calibration an initial pulse of argon gas fills the cell. After allowing the cell to reach a steady state (~750 ms) an electron gun ionizes the argon. Thus after creating the Ar⁺ needed for the pressure measurements, the pulsed leak is evacuated to return the
trapping cell to baseline pressure and avoid interference with subsequent pressure measurements. Next a second pulse of gas leaks neutral Ar back into the cell. A single frequency, simultaneous excite-detect event is then used to determine the Ar$^+$ linewidth. Literature data is then used to determine the Ar number density in the cell (using the literature value for the momentum transfer cross section). This same process is repeated for each pulsed leak valve time that will be used in an experiment. This calibration process is fast enough that it can be performed both before and after any experiment and averaged to yield an accurate pressure measurement that is representative of the conditions that were in the cell during the actual experiment.

It is noteworthy that for the Ar$^+$ in Ar system, cross sections decrease with increasing kinetic energy as expected from collision theory. This is in contrast to the CRAFTI cross sections for larger molecular ions colliding with Xe we reported earlier,\textsuperscript{7} which generally increase with increasing kinetic energy. We believe this occurs because of increasing contributions to the CRAFTI cross section from collision-induced dissociation as kinetic energy increases; this phenomenon will be the subject of Chapter 5.

4.3 Comparison of LIPS linewidth measured pressure with cold cathode gauge measured pressures

To check the accuracy of these pressure measurements, we compared the pressures measured by the cold cathode gauge with those measured using this new LIPS technique. Results of this comparison can be seen in Figure 4 - 3.
In this figure, the pressure measured using the LIPS technique (plotted as a red x) is compared with the pressure measured by a cold cathode gauge (plotted as a blue circle). The error bars in both blue and red represent one standard deviation in the measured values (N=20 over several days). Since these measurements were all taken within a short amount of time, the true variation in the cold cathode gauge is not measured by this standard deviation. The black error bars are added to represent this error, and are an arbitrary value of 1/2 the measured value. This value is consistent with the variability that we see due to maintenance of the gauge and daily drift of the measured pressure.

Two things are immediately apparent from Figure 4 - 3. First, the amount of variability in pressure seen in the LIPS measured pressures (red error bars) is smaller than those seen in the cold cathode gauge measured pressures (blue error bars). This improved precision is an important benefit of the measuring in-cell pressures using the LIPS technique. Second, the pressures measured at the gauge agree very well with the linewidth measured pressures at low pressures,
but as pressure increases the cold cathode gauge measured pressures increase much more quickly than the pressures measured by LIPS linewidth. This can be explained by realizing that the two methods are probing the pressure at different locations. The cold cathode gauge is measuring pressure that is very close to the pulsed leak valve while the LIPS linewidth method is measuring pressures that are inside of the FTICR-MS trapping cell. At longer valve times (higher pressures) the molecular flow will be different at the vacuum entrance where the cold cathode gauge is than inside the trapping cell. At very short valve times (low pressures), however, the vacuum is affected very little by the pulsed leak valve activity and therefore the two pressure measurements agree very well with each other.

A second way of visualizing these pressure measurements is a calibration plot with the cold cathode gauge measured pressures on the y-axis and the LIPS measured pressures on the x-axis, as seen in Figure 4 - 4.
There is a linear correlation between the pressure measured by the cold cathode gauge and the pressure measured using the LIPS technique. Error bars in this figure represent one standard deviation in the measured values (N=20 over several days). The pressures measured by the cold cathode gauge correlate well with the pressures measured using the LIPS method, with a correlation coefficient of 0.995. However, such a calibration plot should not be used to determine absolute pressures inside the cell based on the cold cathode gauge reading because of the inherent gauge variability as well as molecular flow effects that were discussed previously.

4.4 Comparison of LIPS with total ion intensities.

One of the difficulties in attempting to compare LIPS pressures with pressures measured using other techniques is that most other techniques (such as the cold cathode tube used here) do not directly measure the pressure in the trapping cell, but measure it at some remote location in the vacuum chamber. We therefore sought another method for comparing pressures more directly in the ion trapping region.

Signal intensity in FTICR is proportional to the number of charges held in the ion trapping cell.15 Further, as long as the space charge limit is not approached, the number of ions produced by an electron beam pulse through the trapping cell should be proportional to the number of neutral molecules irradiated by the electron beam, which is in turn proportional to the neutral pressure. This suggests that total FTICR signal intensity following a fixed-length, constant energy pulse of electrons through the trapping cell should be a viable means of measuring the neutral pressure in the trapping cell. In fact, this approach was taken by Jiao et al.13 to characterize the rise times for different pulsed leak valve configurations.
We used total FTICR signal intensity following a 5 ms electron beam event as a means of probing the total neutral pressure in our trapping cell, for comparison with pressures measured using LIPS. Steady-state pressure measurements using this method following pulsed-leak pressurization of the instrument showed that total signal intensity increased linearly with pulsed leak pressurization duration, indicating these experiments were conducted below the space charge limit. This linearity also suggests that the initial assumption that pressure increases linearly with pulsed leak valve time is valid. Steady-state pressures read from the cold cathode tube were measured and used to generate a calibration curve relating total FTICR signal to cold cathode pressures. Thus, measurement of total FTICR signal intensity yields another method for determining absolute pressures, recognizing that the absolute pressures obtained from this calibration contain systematic error due to calibration with the cold cathode tube and its location remote from the trapping cell. Unsurprisingly, pressures measured in this way agree within about a factor of two with LIPS pressures.

However, independent verification of the LIPS technique can be obtained from these experiments, by measurement of the pulsed leak valve rise time at the ion trapping cell. Because the total ion signal is produced only during the 5 ms electron beam pulse, measurement of total FTICR signal offers a means of measuring pressures with higher time resolution (down to the duration of the electron beam pulse, in principle) than is possible with the cold cathode tube and its associated electronics. Similarly, LIPS measurements can be made rapidly (as long as the signal transient is comparable to the desired time resolution). Both types of measurements reflect pressure changes within the ion trapping cell, and should show changes occurring at the same rate.
A comparison of the two techniques, measuring pressures at various time delays following 100 ms activation of the pulsed leak, is shown in Figure 4 - 5. Within experimental error, both FTICR signal intensity and LIPS give the same rise time constant (about 160 ms in this case), suggesting both methods measure the same pressure time profile in the trapping cell.

4.5 Comparison of LIPS with conventional high vacuum pressure measurement.

LIPS offers several advantages in comparison with traditional methods of measuring high vacuum. For our applications, a chief advantage is that because the ion trapping cell is the transducer, LIPS measures pressure in the cell, exactly where we want to make the measurement. Thus, LIPS techniques avoid the need to measure the pressure at some remote point and then correct back to cell pressure, potentially yielding more accurate results for experiments where pressure plays a crucial role.
Other advantages derive from the fact the LIPS measurements do not depend on ionization cross sections. Therefore, LIPS determinations are independent of the ionization potential of the neutral gas. Although LIPS does require ions, the ions need not be made by electron impact, so perturbation of the pressure arising from the presence of a heated filament is avoided. Therefore, there is no need for bakeout of a gauge tube or for thermal equilibration of the system, there are no ion pumping effects, and there is no outgassing from a hot filament. Similarly, there is no need to ignite a plasma.

Because electrons are not required, the production of soft X-rays that occurs when electrons collide with surfaces, and the corresponding residual current, is not a limiting factor for LIPS measurements as it is for ionization-based pressure measurement. Finally, relative to the cold cathode technique we use extensively, LIPS measurements have better reproducibility, and less day-to-day drift.

In comparison with conventional techniques, the greatest current weakness of LIPS is its limited applicability; the technique is only appropriate for instruments that generate a transient signal (from coherently-moving ions) that has a pressure-limited lifetime. Thus, LIPS is demonstrated to work for FTICR instruments and will likely work in Orbitrap mass spectrometers, but currently is not applicable elsewhere.

LIPS measurements depend on the collision cross section of ion-neutral collisions, which are kinetic energy-dependent. Because few kinetic energy-resolved collision cross sections in the energy range appropriate for FTICR measurements are available, there are currently few options for calibrating LIPS. However, CRAFTI techniques offer a means of measuring collision cross
sections and their kinetic energy dependence in the appropriate range, so that more options should soon become available.

The time resolution of LIPS measurements is limited by the length of the required excite/detect event, which is in turn limited by the requirement that the decaying transient signal not be transform-length-limited. Because the transient decay rate is pressure-dependent, the time required to adequately characterize the linewidth is also pressure-dependent, so the time resolution of LIPS will decrease at lower pressures.

Finally, factors other than pressure certainly limit FTICR linewidths at low pressures and will likely determine the low-pressure limit of the LIPS technique, which is currently not known. Because pressure and cross section are inversely proportional, the use of larger ions for LIPS may offset these problems and improve the low-pressure limit of the technique.

4.6 Applications: Absolute values in CRAFTI cross sections

By calibrating pressure measurements using this LIPS technique, I have been able to improve both the precision and accuracy of our pressure measurements. This is an important step in the improvement of CRAFTI because cross sections measured in this way depend critically on the neutral gas number density.

In the past, the Dearden lab has used cold cathode gauges to measure the pressure inside the trapping cell during a CRAFTI experiment. This proved useful enough to make comparative measurements between macromolecular complexes. Our group also has unpublished work for CRAFTI cross sections of crown ether/amine complexes in which the cold cathode gauge was used to measure cell pressures. The results from those experiments show that the cold cathode gauge is sufficient to get cross sections that can be compared for qualitative purposes. However,
if these experiments are done on different days, drift in the gauge can be significant enough to make even qualitative comparisons difficult. Our group has seen in the past that pressure measurements using a cold cathode gauge can vary by a factor of 2-3 from day to day. This corresponds to a change in the measured CRAFTI cross sections on the same order of magnitude. Thus, until now CRAFTI measurements for molecules to be compared had to be done on the same day, preferably back-to-back.

Practical issues in pressure measurement make the problem even more difficult. For example, changing the backing pressure to the pulsed leak valve, changing the leak settings for the leak valve, or cleaning the cathode itself all cause non-negligible changes to the pressure readings without necessarily changing the absolute pressure itself. If CRAFTI measurements are to be compared between labs the problem is intensified, as the gauge placement, model, and other factors change significantly between labs. Using the LIPS calibration method will mean that pressures measured in any lab under any reasonable experimental conditions should give a precise and accurate measurement of the pressure. This means that LIPS calibration makes comparing CRAFTI results that weren't collected at the same time — or even on the same instrument — a much easier process. Furthermore, any FTICR-MS experiments with inherent pressure dependences can now be quantitatively compared, even if the work was done in different labs with different cell conditions. Further work (and collaboration with a second FTICR-MS lab) will need to be done to verify this last claim, but we have already seen evidence of this measurement stability in some preliminary, unpublished results. While CRAFTI results calculated using pressures from a cold cathode gauge can vary by a factor of 2-3 if not collected within a day or two, CRAFTI results calculated using the LIPS method for pressure
measurement remain precise even when instrument cleaning, cathode cleaning, and long time periods (> 1 week) separate the measurements.

4.7 Applications: Measuring pulsed leak valve performance

Freiser et al. first reported a pulsed leak valve for use with FTICR-MS in 1996. Pulsed leak valves are useful experimentally because the pressure of a background neutral can be quickly increased, held constant, and decreased in a controlled way. In the 1996 paper, Freiser et al measured the rise time of the pressure leaking into the FTICR-MS cell by measuring the signal intensity of Ar\(^+\) ions produced via electron impact ionization. An electron gun was used to ionize the neutral argon, with electrons gated through the FTICR trapping cell for a constant amount of time. As long as the trapping cell is not near the space charge limited trapping capacity, the signal intensity increases linearly with the neutral argon pressure. Freiser et al. reported a rise time for their pulsed leak system that was roughly 500 ms. After this short rise time the pulsed leak valve is able to maintain a constant increased pressure for a long period of time (seconds to minutes).
Pressure measurements made in the original Freiser paper during the "steady state" from 1-10 seconds still had a noticeable amount of variation. Freiser et al. observed this variation in their measurements and claimed that it was a fluctuation in the stability of the electron gun and that the pressure was in fact more steady than the graph seemed to show.

The LIPS pressure measurement technique described above was used to recreate the data in the original Freiser pulsed leak paper. Experimentally, this was done using the same sequence as described in 4.2, but instead of allowing a long delay time (up to steady state values) after the second pulsed leak event the delay was varied between 100 ms and 1 second to probe the linewidth (and therefore the pressure) during the valve rise time. Figure 4 - 7 shows the result of this experiment.
It can be seen that the variation present in the original Freiser pulsed leak valve paper is not present in our data. This supports the conclusion by Freiser et al. that the variation in pressure that they saw was more a function of electron gun behavior than it was of actual pressure measurements. These results also show a simple method for determining the time dependence in variable-pressure experiments. Using LIPS pressure measurements can streamline the efficiency of an experiment; knowing the rise time of a pulsed leak valve in any given system means less experimental time "wasted" waiting for a steady pressure to be reached.

4.8 Conclusions

Measurement of FTICR linewidth for species with known collision cross sections yields absolute pressure measurements in the FTICR trapping cell that are in reasonable agreement with conventional ionization-based techniques but avoid problems arising from thermionic production of ions and a remotely-located pressure transducer. LIPS therefore represents a fundamentally new method for measuring low pressures, which should enable more accurate results for other ion chemistry experiments that are pressure-dependent. In particular, pressures determined from
the LIPS technique can be used to make absolute CRAFTI cross section measurements, which in turn will make available more possibilities for ions that can be used in LIPS.
4.9 References


5. Role of Dissociation in FTICR

In Chapter 1 of this dissertation I discussed the detection method used in FTICR-MS. In this chapter I will show that, in general, the role that dissociation plays in FTICR-MS is underappreciated and that this dissociation, especially under pressure-limited conditions, can be quite large. This dissociation can affect the linewidth as well as the relative abundance of the parent ion. Dissociation during the excite-for-detect event should be carefully considered when measuring either dissociation yield or linewidth as a function of pressure, \( V_{p-p} \), or excite time. This is especially true when detecting small, non-covalent complexes. Additionally, when performing ion isolation events the direction of the frequency sweep should be carefully considered.

Every experimental sequence in FTICR-MS uses at least one RF excite event at or near the resonant frequency of an ion that is to be detected. The purpose of this excite event is to bring the cyclotron motion of the ions into phase coherence and to increase the cyclotron radius. The resulting "ion packet" can then be detected via the image current it induces as it passes near the detection plate (see Figure 5 - 1).
Figure 5 - 1 The effects of RF excitation on ions in an FTICR-MS trapping cell. The RF excitation brings the cyclotron motion of the ions into phase coherence and increases the radius of this motion. It should be noted that the position of the ions is not to scale, as detection is best achieved from ions in the center of the trapping cell.

Before excitation ions trapped in the cell produce no net image current on the detection plates. After excitation the ions are moving coherently and produce an image current in the form of a decaying transient. A Fourier transform of this data yields the cyclotron frequency, which is inversely proportional to the ion's mass-to-charge ratio (see Figure 5 - 2).

Figure 5 - 2 FTICR-MS signal intensity as a function of time (left) and m/z, converted from frequency (right) for Ar⁺.
Of note for this discussion is the decaying transient seen in the left of Figure 5 - 2. This decay occurs as ions are removed from the coherent packet by either dissociation, scattering collisions, or collisional cooling with background neutrals. Ions can also be removed through mechanisms that do not involve collisions such as coupling with the trapping and detection electronics as well as irregularities in the magnetic field. At high pressures (roughly $10^{-8}$-$10^{-5}$ mbar) collisional mechanisms will dominate while at low pressures non-collisional mechanisms will dominate. Under optimal conditions a minimum of roughly 10 charges are necessary for detection in FTICR-MS. To improve the detection limit in FTICR-MS, multiple remeasurements can be employed. As FTICR-MS employs a non-destructive measurement (that is, ions do not strike a surface to be detected as in other mass spectrometry methods) the image current of an ion packet can be measured several times in succession. This is done by performing the standard excitation/detection event followed by a cooling period as the ions relax back to the center of the cell through collisions with the background neutrals. Then, the same packet of ions is again excited and detected. Although ion loss is seen after each excitation/detection event, Williams et al. reported that 92-98% of the ion population is retained for the next measurement. This ion loss was mainly attributed to scattering collisions and z-axis ejection. Later experiments by Guan et al. showed that remeasurements could be performed for hundreds of cycles over time frames up to at least one hour.

5.1 Experimental

5.1.1 Materials

Ar gas (99.95%) was purchased from Airgas. Samples of cesium chloride, rubidium chloride, barium chloride, and 18-crown-6 were obtained from Sigma-Aldrich (St.
Louis, MO) and used without further purification.

5.1.2 Instrumentation

All experiments were performed using a Bruker APEX 47e Fourier transform ion cyclotron resonance mass spectrometer with an Infinity trapping cell. The instrument was controlled using a MIDAS Predator data system (National High Magnetic Field Laboratory; Tallahassee, FL). Pressures within the trapping cell were varied using a Freiser-type pulsed leak valve consisting of a 0.004" orifice solenoid pressurization valve backed by a 28 psig Ar supply line and a 0.039" orifice solenoid evacuation valve connected to a mechanical vacuum pump (both valves from General Valve Corp.; Fairfield, NJ). Both solenoid valves were connected to the high-pressure side of a precision variable leak valve (Varian; Palo Alto, CA). Pressure measurements were performed using a cold cathode gauge (Balzers; Fürstentum, Lichtenstein) mounted outside the high field region of the instrument, about 1 m from the trapping cell. All pressures were adjusted for the ionization potential of neutral Ar.

Linear chirp excitation waveforms of frequencies from 75-1500 kHz (corresponding to roughly 1000-50 m/z) were created with a chirp rate of 10-1000 Hz/μsec using the MIDAS Predator data system. Waveform outputs were amplified using an ENI 2100L RF power amplifier to a range of 20-60 V_{pp} (lower amplitudes were not sufficient to produce signal and higher amplitudes ejected ions from the trapping cell).

5.2 Results

5.2.1 Dissociation of non-covalent complexes as a function of sweep direction

My earliest work in the Dearden lab focused around the development of an experimental set-up for infrared multiphoton dissociation (IRMPD, see Chapters 2 and 3 for more details). The
The first step in an IRMPD experiment is isolation of the complex to be studied. This ensures that any dissociated products seen in the mass spectra are produced by the infrared irradiation instead of by source conditions during the injection or by being collisionally activated before the irradiation. While trying to isolate the rubidiated β-glucopyranoside complex, I noticed that rubidium (two isotopes, m/z 85, 87) was consistently present in the mass spectrum, even when strong SWIFT, CHIRP, and single frequency isolation events were used to eject ions from the cell. This suggested to me that the dissociation seen was occurring after the isolation events but before the detection event. One possible explanation for the appearance of these ions is a meta-stable parent complex dissociating after the isolation event had occurred. To ensure this wasn't happening I added a 1 second wait time after ions were injected into the cell both before and after an isolation event was used, but the signal was unaffected. Thus, the dissociation seen cannot be explained by decay of meta-stable ions created during ion injection.

To investigate the nature of the dissociation observed during the excite-for-detect event I first changed the sweep direction. When the sweep was performed from high to low frequency, only the rubidiated complex was seen. However, when sweeping in the low to high frequency direction (i. e. high mass to low mass, so that resonant excitation of the complex occurs before the resonant frequency of the fragment ion is reached) the rubidium ion was seen in the mass spectrum. This suggests that the non-covalent complex was being dissociated during the excite-for-detect event. We then varied the excite amplitude within a range suitable to obtain signal. The relative abundance of the rubidium ion to rubidiated complex increased as the amplitude increased, showing a nearly linear correlation between excite amplitude and complex dissociation. When the sweep direction was in the opposite direction (from high frequency to
low frequency), the rubidium fragment ion was not observed. Figure 5 - 3 shows the dissociation of Rb$^+$ from β-glucopyranoside as a function applied RF voltage.

This dissociation is also dependent on the chirp rate. Figure 5 - 4 shows that the amount of dissociation decreases as the chirp rate is increased. For this work [Cs+18-crown-6]$^+$ was used.
Finally, the amount of dissociation of \([\text{Cs}^{+18}\text{-crown-6}]^+\) was studied as a function of pressure. Results for this experiment are seen in Figure 5 - 5.
The error bars in this figure represent the standard deviation in repeated measurements. From this figure it can be seen that the dissociation of [Cs+18-crown-6]+ increases as pressure increases.

5.2.2 The effect of dissociation on CRAFTI cross section

The effect that dissociation plays in FTICR-MS has also been apparent to us while measuring collision cross sections using the CRAFTI technique. In this technique, the Lorentzian linewidth (FWHM) of an ion is plotted as a function of neutral number density. The linewidth broadening increases linearly with neutral number density. At a molecular level this is due to ions dephasing from the coherent ion packet, seen as a decrease in the image current in the time domain signal. As ions dephase more quickly at higher pressures this shortens the lifetime of the time domain signal, which in turn broadens the linewidth in the frequency domain after the Fourier transform.

A simple model of this time domain dampening is to consider the ions as being dephased only by scattering collisions. Ions dephased in this manner are spatially removed and no longer contribute to the coherent ion motion that creates the image current, thereby dampening the time domain signal. For polyatomic ions it is also possible to dephase the ions by dissociation. This means that collisions that may not be energetic enough to scatter an ion from the coherent packet may still be energetic enough to induce dissociation. This dissociation may not spatially remove the ions from the coherent ion packet, but the change in cyclotron frequency has the same effect; ions are no longer part of the coherent packet, the time domain signal is dampened, and the frequency domain linewidth is broadened. Because of this added broadening due to dissociation care should be taken when interpreting CRAFTI results, as dissociation can increase the absolute value of the CRAFTI cross section.
As discussed in Chapter 4 of this dissertation, the cross section of Ar\(^+\) in Ar neutral background gas decreases as kinetic energy increases. This inverse correlation between kinetic energy and cross section is what is expected and what is seen in cross section measurements performed by other methods. Figure 5 - 6 shows the cross section of Ar\(^+\) in Ar neutral background gas at various kinetic energies.

Figure 5 - 6 Collision cross section of Ar\(^+\) with Ar neutral as a function of kinetic energy (measured with CRAFTI and compared with published literature values)

This behavior is what should be expected while measuring collision cross section. Collision theory describes the impact parameter, b, the minimum non-collision distance between two collision partners. In other words, b is the closest that two collision partners can approach without a collision occurring. For a spherical molecule the cross section is \(\pi b^2\). As kinetic energy increases the impact parameter, and therefore the cross section, also decreases.

Interestingly, though, the opposite behavior is seen when larger complexes are analyzed using CRAFTI. Figure 5 - 7 shows the collision cross section of various macrocyclic/alkali metal complexes.

Figure 5 - 7 Collision cross section of various macrocyclic/alkali metal complexes with Ar neutral as a function of kinetic energy measured with CRAFTI
The CRAFTI cross sections of Cs+, Rb+, BaCl+, and Cs2Cl+ with Ar neutral were also measured as a function of kinetic energy. Although an absolute value for the collision cross section of Cs+ in Ar at varying kinetic energies is not available in the literature, it is possible to get a rough estimation of the hard sphere radius from the ionic radius of Cs+ from literature crystallography data and the van der Waals radius of Argon. This back of the envelope calculation gives a hard sphere cross section for the Cs+/Ar system of 23 Å². This value can then be used to calibrate CRAFTI measurements. A linear fit of the Cs+ data was obtained in such a way that the y-intercept is 23 Å². The reason for this fit is the assumption that the crystallography/van der Waals data corresponds closest to a gas phase system with zero energy.

For experiments measuring the CRAFTI cross sections of Rb+, BaCl+, and Cs2Cl+ the pressures inside the cell were first calibrated using these Cs+ fits in the same way that Ar+/Ar values were used as described in Chapter 4. CRAFTI cross sections of Cs+ were calculated using pressures measured using a cold cathode gauge. Subsequent alkali metal/halogen complexes were measured using pressures defined by Cs+ cross sections in a method similar to the LIPS pressure measurement method discussed in Chapter 4. Thus, a quantitative measurement between Cs+ and

Figure 5 - 7 Kinetic energy dependence of CRAFTI cross sections determined using SF₆ collision gas. Dotted lines are exponential fits to the data; error bars represent ± 1 standard deviation for repeated determinations.
the other alkali metal complexes presented here is not possible. It should be noted that Cs\(^+\) cross sections reported here are significantly smaller than Rb\(^+\) cross sections. As a reliable value for Cs\(^+\) cross sections is not known, accurate pressures from inside the trapping cells will not be possible using Cs\(^+\) cross sections. The absolute values of alkali metal cross sections reported here should not be considered. Instead, they are reported to compare the relative cross section trends as kinetic energy increases. Furthermore, a comparison - even qualitative - between the measured cross sections of alkali metals is not possible from this data as pressure was measured only once per day using the Cs\(^+\) data and assumed to remain constant throughout all other experiments. In subsequent work (see Chapter 6) a LIPS measurement was obtained before and after each experiment to reduce error from pressure changes inside the cell. LIPS is a technique that gives an absolute pressure measurement from an external calibrant of known cross section (Ar\(^+\) in Ar). Using this technique to measure CRAFTI cross sections of alkali metals yields both quantitative and qualitative data. This work is currently being performed in the Dearden lab by Anupriya.

Figure 5 - 8 CRAFTI cross sections of Cs\(^+\) with Ar neutral. Dotted lines are logarithmic fits to the data; error bars represent ± 1 standard deviation for repeated determinations.
The CRAFTI cross sections of Rb+, BaCl+, and Cs2Cl+ in Ar neutral can be seen in Figure 5 - 9, Figure 5 - 10, and Figure 5 - 11 respectively. Pressures were measured as described above, with a measurement of the Cs+/Ar cross section being taken once per day. CRAFTI cross sections of all alkali metals and alkali metal/halogen complexes that have been measured decrease logarithmically as kinetic energy increases.

Figure 5 - 9 CRAFTI cross sections of Rb+ in Ar neutral. Dotted lines are logarithmic fits to the data; error bars represent ± 1 standard deviation for repeated determinations.
Figure 5 - 10 CRAFTI cross sections of BaCl⁺ in Ar neutral. Dotted lines are logarithmic fits to the data; error bars represent ± 1 standard deviation for repeated determinations.

Figure 5 - 11 CRAFTI cross sections of Cs₂Cl⁺ in Ar neutral. Dotted lines are logarithmic fits to the data; error bars represent ± 1 standard deviation for repeated determinations.
5.3 Discussion

The dissociation that is seen as a function of pressure, $V_{p-p}$, and chirp rate can be explained as follows: the excitation begins at a low frequency and initially excites the low frequency (high $m/z$) complex. Although much of the energy is deposited into translational modes, increasing the orbit radius and phase-grouping the ions to allow detection, a small amount of energy is deposited internally into vibrational modes (probably via collisions with background gas). For some of the complexes, this is sufficient to induce dissociation. The dissociated ions are then detected as the sweep continues to higher frequencies and the fragment ions come into resonance. This same vibrational excitation and dissociation still occurs when the sweep direction is reversed, but the dissociated ions are not observed because the excite sweep has already passed the higher resonant frequencies needed to excite the dissociated ions.

Using RRKM theory it is possible to calculate how much energy would be necessary in excess of the dissociation threshold for the dissociated product to be seen in the mass spectrum. Dissociation must occur on the timescale of the frequency sweep of the excite-for-detect chirp waveform. For the [Cs+18-crown-6]$^+$ complex with a Cs$^+$ dissociation product this is roughly 2 milliseconds. Any dissociation that occurs after 2 milliseconds will not be present in the mass spectrum. Equation 5 - 1 is a simplified version of the equation used to determine the RRKM rate constant.

$$k(E) = \nu \left(1 - \frac{E_0}{E}\right)^{s-1} \quad (5 - 1)$$

Here $\nu$ is the vibrational frequencies of the activated complex divided by the vibrational frequencies of the transition state complex. For our purposes we can simplify this to mean the vibrational frequency of the complex that is associated with unimolecular dissociation. $E_0$ is the
barrier to activation, $E$ is the energy in the activated complex, and $s$ is the degrees of freedom. Using this equation we can see that 10% dissociation of the $[\text{Cs}+18\text{-crown-6}]^+$ complex occurs at an energy of about 8.95 eV. We can also make the reasonable assumption that 10% of the energy from a collision is deposited into internal vibrational modes. Therefore, the center-of-mass kinetic energy necessary to see dissociation at that time scale is roughly 90 eV, which fits well with the actual kinetic energy of 123 eV (see calculation of this energy below).

Sweep direction is therefore an important consideration when working with weakly bound complexes. To illustrate this importance we can imagine the collision of neutral Ar with $[\text{Cs}+18\text{-crown-6}]^+$ during the excite-for-detect event. Equation 5 - 2 can be used to calculate the excitation radius of an ion excited with a chirp excite.

\[
r = \frac{\beta V_{pp}}{2dB} \sqrt{\frac{1}{\text{Sweep Rate}}} \quad (5 - 1)
\]

Here $\beta$ is a geometric factor for the trapping cell, $V_{pp}$ is the peak-to-peak amplitude of the applied RF, $d$ is the cell diameter, and $B$ is the strength of the magnetic field. It is interesting to note that the excitation radius does not depend on the ion's mass or charge. All ions in the cell will be excited to the same cyclotron radius, though their cyclotron frequencies will differ. The kinetic energy of an ion that has been excited using a chirp excitation can be calculated using (5 - 2):

\[
KE_{(\text{post excitation})} = \frac{1.20607 \times 10^7 \beta^2 z^2 V_{pp}^2}{d^2 m} \frac{1}{\text{Sweep Rate}} \quad (5 - 2)
\]

Sweep rate here is in Hz/sec. Note that although the excitation radius of a given ion does not depend on its mass or charge, the post excitation kinetic energy will depend on the square of the charge ($z$) and the inverse of the mass ($m$).
In FTICR-MS, a strong signal can be obtained for ions excited to ~75% of the cell diameter. For the collision of neutral Ar with [Cs+18-crown-6]+ using a 4.7 T magnet this corresponds to $V_{pp}$ of 14.25 V and a sweep rate of 1000 Hz/µsec. With these applied parameters, the center-of-mass kinetic energy for Ar neutral colliding with [Cs+18-crown-6]+ is 123 eV. This energy is more than enough to induce dissociation of Cs+ from 18-crown-6, which has a bond dissociation energy of 1.31 ± 0.09 eV. In contrast, the center-of-mass kinetic energy for a small protein (ubiquitin, 8.5 kDa) under these same conditions is only 0.29 eV, which would not be sufficient energy to induce dissociation. From these calculations we can conclude that small, non-covalent complexes will be most affected by collisions during the excite-for-detect event. Non-covalent complexes have low energy dissociation pathways that can be accessed through collision with background neutrals. Larger complexes that also have low energy dissociation pathways should not be expected to dissociate, however, because the center-of-mass collision energies of these complexes with a small background neutral is much smaller. For monoatomic ions, the effect is obviously not an issue, as dissociation is not possible.

Williams et al. reported a 92-98% efficiency in retaining ions in the cell for multiple remeasurements. In that work it is noted that the 2-6% loss per remeasurement cycle is due to scattering collisions and z-axis ejection. Dissociation induced during the excite-for-detect event is another important factor to consider when performing multiple remeasurements of a single ion population of low m/z ions. This factor was not discussed by Williams et al., but is not likely a large contributor to ion loss in that system as gramicidin D (the ion used in that study) is a large enough ion that the center-of-mass kinetic energy of collisions during the excite-for-detect event will be much lower than the amount of energy required to induce dissociation. For Cs+ (the other ion used in that study) dissociation is obviously not a possibility as it is monoatomic.
smaller, non-covalently bound complexes, the effects from dissociation during the excite-for-detect event may be significant enough to make multiple remeasurements of the same ion population difficult. As dissociation increases with an increase in $V_{p-p}$, a smaller applied RF voltage may reduce the ion loss due to dissociation during the excite-for-detect event. Alternatively, higher RF amplitudes combined with faster chirp rates could also be used. Although the frequency resolution decreases as the chirp rate increases, a sufficiently fast chirp rate would give less time for the ions to dissociate during the frequency sweep. Reduced ion loss by one of these techniques may make more remeasurements possible with smaller, non-covalently bound complexes. As complexes become larger, and the center-of-mass kinetic energy of collisions with background neutrals decreases this mode of ion loss will approach zero. However, as larger magnetic fields become accessible for use with FTICR-MS, the kinetic energy of ions being detected also increases (see Chapter 2 of this work). Thus, it is possible that with higher magnetic fields being used in FTICR-MS that multiple remeasurements may not be possible due to dissociation during the excite-for-detect event.

Similar to this remeasurement technique, Guan et al. used the non-destructive nature of the FTICR-MS detection scheme to monitor the real-time reaction of horse heart myoglobin with diethylamine. The same discussion above applies to experiments of this type if performed with low mass complexes: collisions with center-of-mass kinetic energies sufficiently high to induce dissociation should be avoided or the excite-for-detect event will affect the results of the reaction measurement.

The dissociation that occurs during and excite-for-detect also plays an important role in the measurement of CRAFTI cross sections. The CRAFTI cross sections for all small molecules ($\text{Ar}^+, \text{Cs}^+, \text{Rb}^+, \text{BaCl}^+, \text{Cs}_2\text{Cl}^+$) that have been measured decrease as kinetic energy increases (as
expected). However, the CRAFTI cross sections for all macrocyclic compounds measured so far increase as kinetic energy increases. We can explain this discrepancy with theory by realizing that the dephasing collisions are high energy collisions. The center-of-mass kinetic energy is well above the amount of energy necessary to induce dissociations in these complexes. Figure 5 - 12 shows an example reaction coordinate for a unimolecular reaction. As the energy of the collision increases and becomes much larger than the barrier to dissociation the rate of dissociation will continue to increase. This increased rate of dissociation can be explained in three different, yet not mutually exclusive, ways. First, head-on collisions convert kinetic energy to internal energy more efficiently than glancing collisions. At higher energies a larger fraction of collisions deposit enough internal energy to cause dissociation because more glancing collisions will do so. A second explanation is that as the kinetic energy increases, collision rates increase because the ion velocities are higher, and higher number of collisions means more opportunities for scattering or dissociation. Finally, increased kinetic energy results in a faster dampening of the time domain transient as RRKM effects remove the ions from the coherent ion packet by faster dissociation at higher energies, which in turn will increase the linewidth upon Fourier transform. It is this linewidth increase that results in a higher collision cross section. Therefore, collisions occurring during the excite-for-detect event (which in CRAFTI is a simultaneous excite/detect event) induce dissociation and affect the measured value.
Figure 5 - 12 Example reaction coordinate with the energies of a CRAFTI experiment and a IMS-MS experiment.

This explanation of fits well with the observed CRAFTI cross section trends for the macrocyclic compounds as well as Ar⁺. It does not, however, explain why the CRAFTI cross sections of alkali metal/halogen complexes decrease as kinetic energy increases. As these complexes do have accessible dissociation pathways it might be expected that CRAFTI cross sections would increase as kinetic energy increases. The important difference (and possible explanation for these observations) is that all of the studied macrocyclic compounds have a low energy dissociation pathway while the alkali metal/halogen complexes do not. Although the center-of-mass kinetic energies used for the alkali metal/halogen complexes were higher than the bond dissociation energies of those complexes it may be that the effect that dissociation plays in increasing the CRAFTI cross section is not apparent until the kinetic energy is sufficiently higher.
than the bond dissociation energy. Until a sufficiently high energy is reached the rate of
dissociation is not increased enough to affect the decay transient and resulting linewidth in the
frequency domain.

The implication of these results to CRAFTI measurements are that care should be taken
when comparing CRAFTI cross sections. CRAFTI cross sections are generally larger than cross
sections obtained through computational or more traditional cross section measurements
(TWIMS/IMS-MS). Although quantitative CRAFTI cross sections are now possible (see
Chapters 4 and 6 of this work), the absolute values of CRAFTI cross sections will be influenced
by bond dissociation energies. Thus, a comparison of complexes with similar bond dissociation
energies is possible but as the difference between bond dissociation energies becomes larger the
strength of that comparison weakens. To illustrate this problem we can imagine two complexes
of similar size, complex A and complex B. If complex A has a low energy dissociation pathway
that is not accessible to complex B the resulting CRAFTI cross sections will be larger for
complex A than for complex B, notwithstanding their similar molecular size.

The effect that kinetic energy has on CRAFTI cross section has also been seen in the
Dearden lab while studying crown ether/amine complexes. Complexes of 12-crown-4, 15-crown-
5, 18-crown-6, and dibenzo 18-crown-6 bound to terminal monoamine complexes were studied.
Kinetic energy was held constant in the lab reference frame and measured CRAFTI cross
sections were compared with computational cross sections. CRAFTI cross sections should be
expected to correlate linearly with computational cross sections (as seen in Chapter 6). In these
results we see a second order polynomial fit with computational cross sections increasing faster
than CRAFTI cross sections. This deviation is most likely due to the measurements being
performed at constant energy in the laboratory reference frame. This means that higher mass
complexes will have softer collisions with the background neutral and the CRAFTI cross section will be smaller. These results will be discussed in detail more in the Anupriya's dissertation.

A final problem that arises while measuring CRAFTI cross sections of alkali metal/halogen complexes is the formation of multiply-charged multimers with the same mass-to-charge ratio as singly-charged monomers. For example, it would be convenient to preferentially "create" Cs₂Cl⁺ in the trapping cell of an FTICR-MS. However, multimers of the type Cs₂nClₙⁿ⁺ will also be formed during the electrospray process. These n-charged n-mers have the same mass-to-charge ratio as the singly charged monomers but distinct isotope patterns. If present, these multimers will contribute to the total measured CRAFTI cross section. I suspect that the measured CRAFTI cross section will be a weighted average of the CRAFTI cross section for complexes present in the ion packet population (current work in the Dearden lab is being done to verify this). However, it has been observed that CRAFTI cross section increases when multiply charged multimers are present in higher abundance. The abundance of these multimers in the ion population can be controlled by adjusting conditions at the electrospray source and in the ion transfer optics. Softer conditions lead to higher multimer abundances. While performing the work reported in Figure 5 - 11, large abundances of multimers were observed. Results from those measurements can be seen in Figure 5 - 13.
As seen in Figure 5 - 13, the CRAFTI cross section as well as the error associated with those cross sections is significantly increased when multiply-charged multimers are present in the ion population. When signal becomes weak it can be difficult to recognize the appearance of these multimers. I therefore suggest that when multiply-charged multimers are a possibility the CRAFTI cross section of the M+1 or M+2 peak be measured. The contribution from the multimers will be minimized for sufficiently small complexes. For larger complexes (proteins, for example) this approach would not be successful and other methods of minimizing the population size of multimers, such as carefully chosen source parameters, should be considered.

5.4 Conclusion

The role that dissociation plays in FTICR-MS has been underestimated in the time since cyclotron motion was first used to measure the mass-to-charge ratio of an ion. While scattering collisions and z-axis ejection as a result of the excite-for-detect event have been discussed in the
literature as the primary mechanism for ion loss,\textsuperscript{3} the effect that dissociation plays has not yet been mentioned. This dissociation during the excite-for-detect event is most evident in small, non-covalently bound complexes. Care should be taken when performing any experiments where this dissociation may affect the resulting mass spectra. Comparing spectra obtained with a frequency sweep in both directions will ensure that the excite-for-detect event is not affecting the acquired mass spectra.

Dissociation also affects the absolute value of measured CRAFTI cross sections. It is the most probable reason that CRAFTI cross sections are larger than computationally measured cross sections or cross sections measured using other experimental methods. Care should be taken when comparing CRAFTI cross sections of complexes with large differences in their bond dissociation energies.
5.5 References

6. CRAFTI on Protonated Amino Acids

In this chapter the CRAFTI cross sections of the 20 naturally occurring amino acids are reported. To better understand these cross sections, comparisons between two previously reported ion mobility spectrometry - mass spectrometry (IMS-MS) experiments of the same amino acids are also reported as a benchmark for correlation between cross section measurements. This benchmark is found to have a correlation coefficient of 0.9866, while CRAFTI compares to these measurements with a correlation coefficient of 0.9492. Correlation between these IMS-MS measurements and exact hard sphere (EHS) computational results are also discussed. Correlation between EHS and CRAFTI measurements agree very well ($R^2 = 0.9775$), and this correlation can be used to provide important structural information about gas phase amino acids.

6.1 Experimental

6.1.1 Materials

Amino acids were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. Electrospray samples were prepared by first dissolving in 88% formic acid (Mallinckrodt Baker Inc., Phillipsburg, NJ) and then diluting with methanol/water (50:50) to a final concentration of 100-200 µM. Argon gas was purchased from Airgas (Radnor, PA) at a purity of 99.995%.

6.1.2 Instrumentation

All experiments were conducted using a Bruker model APEX 47e Fourier transform ion cyclotron resonance mass spectrometer controlled by a MIDAS data system and equipped with an infinity cell and a 4.7 T superconducting magnet. Ions are generated in a microelectrospray
source modified from an Analytica (Branford, MA) design, with a heated metal capillary drying tube based on the design of Eyler.

RF excitation amplitudes, $V_{p-p}$, were measured directly with an oscilloscope each time an ion was excited. The amplifier used in this work amplifies RF voltage with a slight frequency dependence. This change in the actual $V_{p-p}$ output is small enough that in most cases it is unnoticeable. In a typical CRAFTI experiment, though, even small changes in $V_{p-p}$ can have a large effect on the final measured CRAFTI cross section. By directly measuring the output from the amplifier I was able to better control constant kinetic energy conditions.

Pressure measurements were completed using the Ar$^+$/Ar method described in Chapter 4 of this work. Briefly, the linewidth of Ar$^+$ in Ar neutral was measured for each of the pulsed leak valve times used in the CRAFTI experiment. These linewidths were used to calculate the number density expected in the cell at the given valve time for the pulsed leak valve. Ar$^+$/Ar pressure measurements were performed both before and after each amino acid CRAFTI experiment and averaged. CRAFTI cross sections were calculated using this average number density. No major shifts or drift were seen between the averaged Ar$^+$/Ar measurements. Full details of a typical CRAFTI experiment have been described in detail elsewhere in the literature$^1$ as well as in Chapter 2 of this work.

6.1.3 Computational modeling

Molecular structures were obtained using the Spartan '08 package (Wavefunction, Inc.; Irvine, CA) for conformational searching using the MMFF force field provided in the package, requesting 10,000 starting conformers (systematic searches sometimes completed after examining fewer than 10,000 structures). In each case, the amino acid was protonated on the
amino group. CPK surface areas for each conformer were also computed using Spartan ’08. For each protonated amino acid, the five conformers with the lowest MMFF energies were analyzed using the MOBCAL package and their momentum transfer collision cross sections were computed using the projection approximation, using the exact hard sphere scattering method, and using the trajectory method as implemented in MOBCAL. The resulting cross sections for these lowest-energy conformers were then weighted using a 300 K Boltzmann distribution function and averaged. We justify this averaging by noting that none of the techniques used for experimentally measuring the cross sections have sufficient resolution to resolve the different conformers; in particular, the experimental arrival time distributions in the literature show single peaks for each amino acid. A similar averaging approach has been used previously.

6.1.4 Data analysis

The CRAFTI experimental results were processed using the Igor Pro software package (version 6, Wavemetrics; Lake Oswego, OR). Details for the analysis process are reported elsewhere. Briefly, the analysis involved calculation of the power spectrum and measurement of the full width at half maximum linewidth of the frequency domain peak as a function of collision number density (which had been previously determined as described in 6.1.2). Plots of linewidths vs. collision gas number density are generally linear. The slope was calculated from a linear least-squares fit and used in the determination of the CRAFTI cross section. The complete set of CRAFTI amino acid cross sections was measured and calculated before making any comparisons to IMS-MS data.

6.1.5 Ion energetics

CRAFTI experiments were performed at constant lab frame kinetic energy of 1.9 keV. This amount of excitation corresponds to an excitation radius of 1.60 cm to 2.13 cm in the
trapping cell before collisions occur (collisions that may occur before the excitation event is completed do not affect the linewidth as discussed in Chapter 2).

6.1.6 Computational modeling

Molecular structures were obtained using the Spartan '08 package (Wavefunction, Inc.; Irvine, CA) for conformational searching using the MMFF force field provided in the package, requesting 10,000 starting conformers (but systematic searches sometimes completed after examining fewer than 10,000 structures). In each case, the amino acid was protonated on the amino group. CPK surface areas for each conformer were also computed using Spartan '08. For each protonated amino acid, the five conformers with the lowest MMFF energies were analyzed using the MOBCAL package \(^2\) and their momentum transfer collision cross sections were computed using the projection approximation, using the exact hard sphere scattering method, and using the trajectory method as implemented in MOBCAL. The resulting cross sections for these lowest-energy conformers were then weighted using a 300 K Boltzmann distribution function and averaged. We justify this averaging by noting that none of the techniques used for experimentally measuring the cross sections have sufficient resolution to resolve the different conformers of a given amino acid; in particular, the experimental arrival time distributions in the literature \(^3\) show single peaks for each amino acid. A similar averaging approach has been used previously. \(^4\)

6.2 Results

6.2.1 CRAFTI cross sections of amino acids

Figure 6 - 1 shows a plot of the CRAFTI cross section in Å\(^2\) as a function of molecular mass, and is a tabulated version. As should be expected, the cross section is positively correlated
with molecular mass, though the cross sections do not increase monotonically, as our lab has previously seen is the case with crown-ether/ammonium complexes. It is this lack of monotonic increase, however, that gives more credibility to the idea that CRAFTI cross sections yield structurally relevant data. For example, it could be possible that the measured CRAFTI cross sections are simply a function of molecular mass. If this were the case, then no structural information could be obtained from CRAFTI as the linewidth/pressure correlation would only be mass dependent. The correlation seen in Figure 6 - 1, however points to a more nuanced correlation that does yield structural information. This information is discussed in more detail later as a comparison to computational data.

Figure 6 - 1 CRAFTI cross section measurements as a function of molecular mass.
<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Molecular weight (Da)</th>
<th>Measured cross section (Å²)</th>
<th>Boltzmann-weighted average computed cross section (Å²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IMS³</td>
<td>IMS⁵</td>
<td>TWIMS⁴</td>
</tr>
<tr>
<td>Gly</td>
<td>76.0</td>
<td>48.4 ± 0.8</td>
<td>111.0</td>
</tr>
<tr>
<td>Ala</td>
<td>90.1</td>
<td>52.8 ± 4.3</td>
<td>110.4</td>
</tr>
<tr>
<td>Ser</td>
<td>106.1</td>
<td>52.4 ± 0.2</td>
<td>111.4</td>
</tr>
<tr>
<td>Pro</td>
<td>116.1</td>
<td>54.1 ± 0.4</td>
<td>110.8 62.4</td>
</tr>
<tr>
<td>Val</td>
<td>118.1</td>
<td>58.5 ± 0.2</td>
<td>115.2 64.8</td>
</tr>
<tr>
<td>Thr</td>
<td>120.1</td>
<td>55.1 ± 0.4</td>
<td>113.3</td>
</tr>
<tr>
<td>Cys</td>
<td>122.0</td>
<td>57.6 ± 1.5</td>
<td>114.1</td>
</tr>
<tr>
<td>Ile</td>
<td>132.1</td>
<td>63.0 ± 1.3</td>
<td>69.0 133.1 ± 1.2</td>
</tr>
<tr>
<td>Leu</td>
<td>132.1</td>
<td>67.0 ± 1.1</td>
<td>120.6 70.5</td>
</tr>
<tr>
<td>Asn</td>
<td>133.1</td>
<td>57.7 ± 0.4</td>
<td>114.9</td>
</tr>
<tr>
<td>Asp</td>
<td>134.0</td>
<td>58.8 ± 0.4</td>
<td>116.8 62.9</td>
</tr>
<tr>
<td>Gln</td>
<td>147.1</td>
<td>61.1 ± 1.4</td>
<td>116.6 62.9</td>
</tr>
<tr>
<td>Lys</td>
<td>147.1</td>
<td>65.7 ± 0.1</td>
<td>116.7</td>
</tr>
<tr>
<td>Glu</td>
<td>148.1</td>
<td>62.1 ± 0.4</td>
<td>117.7 63.4</td>
</tr>
<tr>
<td>Met</td>
<td>150.1</td>
<td>64.8 ± 1.2</td>
<td>118.9</td>
</tr>
<tr>
<td>His</td>
<td>156.1</td>
<td>64.2 ± 0.6</td>
<td>117.4</td>
</tr>
<tr>
<td>Phe</td>
<td>166.1</td>
<td>73.6 ± 1.0</td>
<td>125.6</td>
</tr>
<tr>
<td>Arg</td>
<td>175.1</td>
<td>73.1 ± 0.9</td>
<td>122.0</td>
</tr>
<tr>
<td>Tyr</td>
<td>182.1</td>
<td>80.8 ± 1.1</td>
<td>131.6</td>
</tr>
<tr>
<td>Trp</td>
<td>205.1</td>
<td>87.6 ± 1.2</td>
<td>134.0</td>
</tr>
</tbody>
</table>

Table 6 - 1 Amino acid cross sections from several different methods

6.2.2 Comparison to DRIFT data

Ion-mobility spectrometry–mass spectrometry (IMS-MS) is considered to be the gold standard for gas phase collision cross section measurements. IMS-MS measures the time required for a gaseous ion to travel the length of a drift tube. The drift region of the instrument is filled with a buffer gas, typically helium, and a low level electric field is applied. The ion velocity is proportional to the strength of the electric field, which is maintained at a low enough
level that the energy from the electric field is less than the energy of ion-neutral collisions.\textsuperscript{6} The collision cross section is measured in IMS-MS using Equation 6 - 1 below.

\[
\Omega = \frac{(18\pi)^{1/2} e}{16 \left( k_b T \right)^{1/2}} \left[ \frac{1}{m_I} + \frac{1}{m_B} \right]^{1/2} \frac{1}{N_0} \frac{1}{K_0}
\]  

(6 - 1)

Here $\Omega$ is the collision cross-section of the ions, $ze$ is the charge of the ion, $k_b$ is Boltzmann's constant, $T$ is the temperature, $m_B$ is the mass of the neutral buffer gas, $m_I$ is the mass of the ion, $N_0$ is the number density of an ideal gas at STP, and $K_0$ is the reduced mobility for the ion. Ions that are more compact will have a higher mobility through the drift tube and therefore a smaller cross section than larger ions.

The 20 naturally occurring amino acids have been measured at least twice in the past.\textsuperscript{7} This makes amino acids an excellent system to study using CRAFTI, as we can compare how well one IMS-MS measurement compares to another as well as how well CRAFTI correlates to IMS-MS. Figure 6 - 2 shows the correlation between two reported sets of amino acid cross section measurements. For each of these data sets the reduced mobility ($K_0$) was reported and the cross section ($\Omega$) was calculated using Equation 6 - 1.
The linear correlation between these two data sets has an $R^2$ value of 0.9866. I consider this to be a fairly good correlation, given that the measurements were carried out on different IMS-MS instruments at different times under slightly different conditions. Although this is the only correlation study between IMS-MS instruments that I have done, I propose that this correlation coefficient be considered an acceptable correlation between methods.

Figure 6 - 3 shows the correlation between CRAFTI results reported in Figure 6 - 1 and the IMS-MS measurements reported by Hill et al.
The correlation coefficient for these data sets is 0.9442. Although lower than the correlation between the two IMS-MS data sets, I still consider it to be a fairly good correlation, given the differences in the two techniques. The correlation between CRAFTI measurements and IMS-MS measurements is just as good as the correlation between IMS-MS measurements. One obvious difference, however, is in the absolute values of the measurements. The CRAFTI measurements span a much wider range of values than the IMS-MS measurements. This is to be expected, though, because the conditions between IMS-MS and CRAFTI experiments are quite different. For example, CRAFTI cross sections are measured using a single, high energy (7.4 eV - 11.7 eV in this work) collision with Ar while IMS-MS cross sections are measured using multiple, low energy (0.025 eV) collisions (typically with He or N2). For CRAFTI cross sections this means that both dissociation and scattering collisions contribute to the damping of the time domain signal (and therefore the broadening in frequency domain that is used to calculate cross sections), whereas for IMS-MS the primary mechanism is believed to be momentum transfer collisions.
6.2.3 Comparison to computational data

The ultimate goal in cross section experiments is to obtain structural information about the ion. For this work that structural information is gained by comparing computed collision cross sections for candidate conformers to the experimentally measured CRAFTI cross sections. In this work exact hard sphere (EHS) values are presented, although other calculated values (obtained using the trajectory method or using the projection approximation) were also obtained, yielding similar results. Figure 6 - 4 shows the correlation between EHS cross sections and the measured CRAFTI cross sections presented in this work.

![Graph showing correlation between EHS and CRAFTI cross sections](image)

Figure 6 - 4 Correlation of the computationally modeled EHS cross sections with CRAFTI cross sections

With a correlation of 0.9775 it can be easily stated that CRAFTI cross sections agree well with the EHS cross sections. Error bars in this figure are on the order of the size of the data points themselves, and for clarity were not included. Again, the same observation can be made that the CRAFTI cross sections are much higher than the EHS computed cross sections, but this
is once again expected as the computational data are obtained by modeling IMS-MS experimental conditions of multiple low energy, non-dissociative collisions with N₂ while CRAFTI relies on a single, high energy collision with Ar to dephase the ion from the coherent ion packet.

Figure 6 - 5 is a two-axis plot of the computational EHS cross sections and the experimental CRAFTI cross sections. As mentioned briefly in 6.2.1, these data are evidence that CRAFTI cross sections reflect changes in molecular structure and not just increasing molecular weight.

![Figure 6 - 5 Comparison of computational EHS cross sections with experimental CRAFTI cross sections](image)

This figure shows that CRAFTI cross sections correlate well with amino acid molecular structure. Both the computational and CRAFTI results follow a general upward trend in cross section as mass increases. This is expected, of course, as an increased molecular size should correlate with an increased molecular mass. Important deviations in this general trend are present in both the computational and experimental results. Figure 6 - 6 shows these deviations in greater detail.
In this figure it can be seen that valine, threonine, and cysteine in both the computational and experimental cross sections decrease in collision cross section as molecular mass increases; the exact opposite of the general expected trend. This deviation is easily explained, however, when the chemical structures are considered.

The side chains of valine and threonine have the same length. They differ in that valine has a -CH$_3$ substituent on the side chain while threonine has a -OH group, making threonine the more massive compound. However, the -OH group also allows for intramolecular hydrogen bonding in threonine, which makes its gas phase structure more compact. In both the glutamine/glutamic acid pair and the asparagine/aspartic acid pair a -NH$_2$ moiety is replaced by a -OH moiety. The computational results for both of these pairs predicts the carboxylic acid to be more compact, a prediction that is verified experimentally. The gas phase structure of the carboxylic acid is more compact because of hydrogen bonding effects. Cysteine's cross section,
which is smaller than that of either valine or threonine, can be explained by the presence of sulfur, which adds mass to the amino acid but takes up less volume than a methyl group.

For the collision cross sections of isoleucine and leucine the computational results predict a cross section for leucine that is larger than isoleucine. This small cross section difference is created by moving one of the -CH₃ substituent by one position in the side chain. The leucine/isoleucine system is an interesting system that allows us to determine the resolving power of CRAFTI. Experimentally, the leucine cross section was found to be significantly larger than that of isoleucine, as predicted by the computational results.

Next, these same computational EHS values were compared to the IMS-MS measurements discussed previously. This was done to get an idea of how well CRAFTI was correlating with EHS as compared to IMS-MS. These results are seen in Figure 6 - 7 below:
It can be noted once more that CRAFTI cross sections correlate just as well, if not better, than the reported IMS-MS experiments, which have a correlation coefficient of 0.9204 for the data of Hill et al.\textsuperscript{7b} and 0.8384 for the data of Dennis et al.\textsuperscript{7a} In the case of Dennis et al., the poorest linear correlations are for the amino acids with cross sections around 110 Å\textsuperscript{2}. This is likely the largest contributor to the relatively low correlation coefficient. These data points are for glycine, alanine, proline, and serine (the 4 lightest naturally occurring amino acids). The experimental conditions under which these values were obtained did not include any mass
selection upstream of the drift tube, leaving the possibility that solvent attachment may have occurred, increasing the experimental cross sections for these 4 amino acids.

6.3 Conclusion

Most of the work on the CRAFTI technique, until now, has been in the initial development and improvement of the technique (see also Chapter 4 of this work). This work is one of the first attempts to apply the technique to gain structural information. I have shown here that CRAFTI results for the 20 common amino acids compare well to both IMS-MS results and EHS computational results. Both CRAFTI and IMS-MS results compare to computational results much better than cross sectional results obtained using TWIMS. By comparing CRAFTI results to EHS computational results I have shown the general trend that collision cross section generally increases as molecular mass increases. However, there are several important deviations from this trend that are apparent in both EHS and CRAFTI results. This study yields important information about the structures of gas phase protonated amino acids as well as demonstrates the structural resolution possible using CRAFTI.

It should be noted that I am not attempting to claim that CRAFTI is a superior method to IMS-MS. In fact, I don't believe that the differences in correlation coefficient provide evidence for that at all. While the correlation coefficients for CRAFTI in each of the correlations discussed until this point have been higher it should be remembered that this is probably not an accurate representation of how all IMS-MS measurements would correlate (if more data were available to make a complete comparison). One important way that IMS-MS is superior to CRAFTI is in its ability to separate conformational isomers. CRAFTI measurements analyze cross section by exciting on a single RF frequency. For any conformational isomers in the cell this frequency will be the same and the FTICR-MS signal and resulting CRAFTI cross section will be a weighted
average of these cross section for the isomers present (although this has yet to be shown by experimentation).
6.4 Reference


7. Summary and Perspective

Mass spectrometry is a useful tool for measuring molecular mass. The techniques discussed in this dissertation, however, go well beyond a simple mass analysis. Fourier transform ion cyclotron mass spectrometry (FTICR-MS) can be used to investigate fundamental properties of gas phase ions. In this dissertation I have shown several methods for measuring these properties.

Infrared multiphoton dissociation spectroscopy (IRMPD) probes the IR spectrum of gas phase ions. In this dissertation I used IRMPD to investigate the gas phase conformations of protonated amino acids, cucurbituril complexes, and terminal diamines. Several important observations came from IRMPD experiments. First, if the IRMPD action spectrum of protonated phenylalanine is compared to the previously measured action spectrum of protonated tryptophan the carboxylic acid stretch at 3550 cm\(^{-1}\) has been red-shifted by roughly 15 cm\(^{-1}\). I attribute this red shift to the carboxylic acid moiety coupling with the pi system of the phenyl group.

The gas phase spectrum of protonated phenylalanine was measured in preparation for investigating protonated phenylalanine bound to cucurbit[7]uril. The computational IR spectra for protonated phenylalanine and protonated tryptophan reveal two conformational families - one internally bound and one externally bound. The -OH band in the externally bound complexes are red shifted by \(~150\) cm\(^{-1}\) from the free complexes. This band is further red shifted by \(~150\) cm\(^{-1}\) when the protonated amino acid is bound internally. With red shifts this large, IRMPD is an excellent choice for determining whether the amino acid binds internally or externally. However, during the course of my research I was unable to see any signs of dissociation of these
complexes by the IRMPD mechanism. I discussed in Chapter 3 several possible ways to overcome this problem.

I investigated the effect that hydrogen bonding has on the gas phase spectrum using terminal diamines as a model system. Computational modeling shows that these compounds are cyclical, with the protonated amine wrapping around towards the unprotonated end. The IRMPD action spectra for these complexes were only partially obtained before problems with the OPO/OPA system began. However, computationally I observed a red shift as the chain length increases.

The dissociation of terminal diamines is an interesting observation found in this dissertation. Previous work by McLafferty described the mechanism of dissociation for primary monoamines, which fragment by α or β cleavage. On the subject of diamines, however, McLafferty only mentions that they fragment by loss of neutral ammonia. The mechanism of this dissociation is not discussed, nor has it been discussed in any later work. The work presented here suggests that terminal diamines fragment through an internal $S_N2$ reaction. This mechanism should be further investigated as described below.

Cross sectional areas by Fourier transform ion cyclotron resonance mass spectrometry (CRAFTI) has been shown to be a valuable technique for measuring the collision cross sections for gas phase ions. In this work I investigated the fundamental properties of gas phase ions in a FTICR-MS Penning trap that affect this measurement. Dissociation of the ion, for example, plays an important role in dephasing an ion from the coherent packet. This increases the measured cross section. This effect is discussed in Chapter 5.
A correct pressure measurement is critical for the CRAFTI technique. For some time now, it has been obvious that current methods of measuring pressure inside the cell of an FTICR-MS has been lacking. A quantitative measurement of the CRAFTI cross sections is not possible without an accurate measurement of the cell pressure. However, the CRAFTI technique itself can be used to measure that pressure. By measuring the linewidth of an ion with a known collisions cross section over a wide range of kinetic energies I was able to develop an accurate and reproducible method for measuring the pressure. The linewidth pressure measurement technique (LIPS) has been used to drastically improve the quantitative aspects of the CRAFTI technique. LIPS combined with CRAFTI was used to measure the cross sections of the 20 standard protonated amino acids. The current outlook for the technique is very promising. Other groups have already begun investigating this technique. The key in the Dearden lab will be to quickly explore new systems and gain important structural insight using CRAFTI.

To further investigate the role that dissociation plays in FTICR-MS, I suggest that the CRAFTI cross sections for alkali metal complexes be measured once again using the LIPS pressure measurement technique to calibrate. Monoatomic ions cannot dissociate and non-covalent ions dissociate readily. These two extremes have already been probed, and CRAFTI cross sections of monoatomic ions decrease with increasing kinetic energy while non-covalent complexes' cross sections increase with increasing kinetic energy. Alkali metal/halogen complexes provide a way to investigate the behavior of tightly bound complexes. However, as discussed in Chapter 5, care should be taken when investigating these complexes as the presence of multiply charged multimers can affect the results. I suggest that the M+1 or M+2 peaks of these complexes be analyzed, depending on the isotope pattern for the complex being investigated. Analyzing these peaks will diminish the presence of multiply charged multimers.
CRAFTI could also be used to further investigate the dissociation mechanisms of terminal mono- and diamines. I suggest measuring the CRAFTI cross sections of the monoamine series CH₃(CH₂)ₙNH₃⁺ and comparing them to the CRAFTI cross sections for the similar diamine series NH₂(CH₂)ₙNH₃⁺. The diamine cross sections should be smaller due to the cyclization that occurs in the gas phase. Next, the CRAFTI cross sections for the SORI-CID fragments of the diamine series should also be measured. These products contain only one amine group and n-1 heavy atoms. Thus, if the diamines do in fact dissociate by the internal SN₂ mechanism proposed in this work, the CRAFTI cross sections of these dissociated complexes will be significantly smaller because the ionic products of the dissociation will be cyclic. However, if the proposed mechanism is not correct, the cross sections for the NH₂(CH₂)ₙNH₃ fragments should be the same as those of the CH₃(CH₂)ₙ₋₁NH₃ monoamines.

The upper mass limit of CRAFTI should be determined. The simplest way to do this is to incrementally increase the mass of ions being analyzed by CRAFTI. It will be important to the technique to know whether the upper limit will be able to accommodate peptides and small proteins. I expect that as this limit is reached the linewidths plotted as a function of pressure will become non-linear. This will happen because single collisions will no longer be energetic enough to dephase the ions. The upper mass limit of CRAFTI will be reached when single collisions cannot sufficiently dephase the ions. The limit could be increased by increasing the magnetic field strength or by using heavier neutral collision gases.

Lastly, ion trajectories should be modeled to investigate ion behavior during a CRAFTI experiment. This would be a useful endeavor to see and better understand ion behavior. Moreover, CRAFTI cross sections are currently compared to computational methods that model ion mobility experiments. CRAFTI cross sections compare fairly well to these computational
results, but development of a computational model based on CRAFTI techniques will improve the structural comparisons that can be made.

The work I have done in the Dearden lab will leave a lasting impression. LIPS in particular makes quantitative measurements of CRAFTI cross sections possible. This makes CRAFTI a much more attractive technique for other groups to begin using. Without a calibrated pressure measurement technique, comparison of values obtained by groups in different labs would not be possible. Now, with more quantitative CRAFTI measurements possible comparisons between labs also becomes possible. The comparison between CRAFTI cross sections and computational results are also strengthened by a quantitative CRAFTI cross section.
7.1 References

