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Innovation in Mass Spectrometry Instrumentation for Applications in Cryogenic Ion Vibrational Spectroscopy

By

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Chapter 8: The need for instrument innovation: why advances in software and hardware pave the way for the study of new and more complex chemical environments

Section 8.1: Preface

One of the most important things we, as scientists and as people in general, can do is explain the motivation behind our work to those around us. Whether our audience be our families, our friends or our funding agencies, writing or speaking about our projects helps us better understand our own work. Even more so, it often helps us identify the parts that we ourselves don't understand as well as we should. In a way, it keeps us accountable and keeps us on track. Communication is a way to include and involve the world around us in our lives, especially those who may not be in the field.

Inspired by this idea, I present a final chapter explaining my graduate research to a non-scientific audience. While I have had many avenues to practice science communication during my education, this has been the most fruitful since my graduate research has been so niche, so specialized. I am grateful to the Wisconsin Institute for Science Literacy (WISL) for sponsoring, supporting and, most importantly, encouraging students to practice science communication in a tangible manner. I am especially grateful to Prof. Bassam Shakhashiri, Cayce Osbourne and Elizabeth Reynolds for their help in making this chapter possible. By reading this chapter and delving into the mind of a half-engineer-half-chemist, I hope the reader is able to understand my love for and desire to create new and improved chemical instruments to help advance the field that has grown so much over the past five years.

Section 8.2: Introduction

I was always told that a task is easier when you use the right tool. Don't believe me? Let me convince you. Have you ever tried to eat soup with a fork? Have you ever tried to open a door with your foot instead of your hand? These examples might seem absurd and off-kilter but there are less egregious examples too. For example, have you ever tried to make coffee without a filter? It doesn't work very well because the grounds are left floating in the coffee and you can't avoid them while trying to drink your coffee. Have you ever tried to use a screwdriver that was too small for the screw you were trying to remove? This is a particularly good example because even with the correct tool, details matter. If you use a screwdriver with too small of a head, you are likely to strip the screw and then it's even harder to get it out. Much like the tasks we face in our daily

lives, scientists are faced with similar challenges when approaching experiments and new challenges in their fields. In order to help facilitate scientific discovery, I've spent my graduate research developing new and improved tools so that scientists' jobs are easier.

I work in a fundamental physical chemistry lab that studies different chemical systems on the atomic scale. As a group, we are interested in understanding how the chemical structure of a system affects its function. Performing these studies requires very specific instrumentation that cannot be purchased commercially. Therefore, a huge part of the work in our group is to continue to develop the instruments and methods by which we study atomic systems. The method by which we study these systems is a tandem approach that combines mass spectrometry and infrared spectroscopy. Mass spectrometry is a technique that identifies the mass per unit charge of a system or species you are studying. For my applications in mass spectrometry, this is just the mass because I make singly charged species and anything divided by one is itself. Mass spectrometry is widely regarded as one of the most useful techniques for identification of mass but it does lack structural information, i.e. how the individual parts of a molecule are connected to one another. That is where infrared spectroscopy comes in. Infrared spectroscopy is a technique that connects a molecule's characteristic absorption of light to its structure. Using mass spectrometry and infrared spectroscopy together provide us with both the mass of a molecule as well as its connectivity. This is extremely useful because we get two pieces of information from one test.

During my time in the Garand Group, I focused on developing the current tandem technique in three main areas. The first deals with the tool/instrument that we use. I developed a prototype instrument that decreased the cost and complexity of the current instrumentation. The second two advances center around data acquisition. The prototype I built and the new approach utilizing alternate waveforms to control the ions addresses the lack of selectivity, selectivity being the precision with which you can make and keep only the molecule you are interested in. Finally, a modular housing approach and simplified ion trap design has resulted in the formation of the first peptide with a multi-solvent system surrounding it. A peptide is a small chain of amino acids linked together. Amino acids are the building blocks of proteins and most of human life, so these are important species to study in both micro- and macro-scale experiments. Here, a multi-solvent system around a peptide means that there is more than one type of molecule that can be placed around the molecule of interest to understand how it would react in an environment more closely

adjacent to the natural world, one where there is not just water in a lake but likely pollutants or pesticides. The advances in instrumentation open the door for the study of more complex and, in some cases, more realistic chemical environments. This permits us to study and begin to understand systems as they exist in nature.

Section 8.3: The Current Approach: Cryogenic Ion Vibrational Spectroscopy (CIVS)

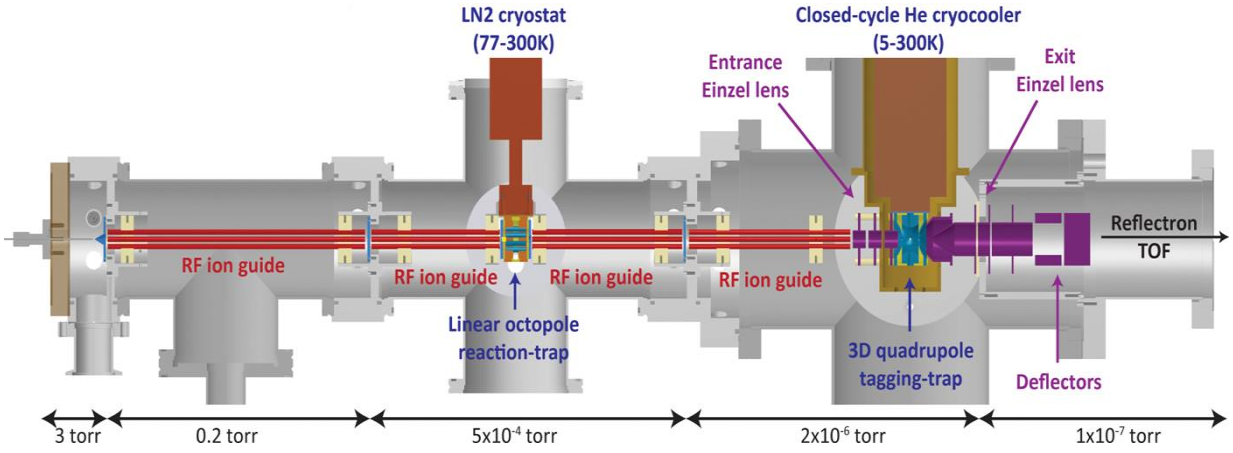

Figure 8.1. CAD schematic of the cryogenic spectrometer currently used in the Garand Group.

The first step in identifying ways to improve an instrument is to understand how it works. Figure 8.1 shows a schematic of the current instrument. Starting at the left side of Figure 8.1, we begin with an electrospray interface. Electrospray is a technique that uses high voltages applied across the output of a very small glass needle as a solution is flowed through it and out of the pointed tip. The solution contains the molecules that we are interested in studying and as they encounter the high voltage applied across the small tip, the solution is aerosolized (transformed from the liquid phase to the gas phase) and ionized (made to have an electric charge). The ions, what we call the resultant charged particles created in the electrospray interface, are pulled into the instrument via a pressure differential. The pressure differential is a result of two mechanical pumps pulling out the air from the front stages of the instrument. The ions are then guided through a series of ion guides composed of long stainless-steel rods with specific voltages applied to them. These devices are called ion guides and the voltages applied to the rods create an electric field that balances the ion within them. Given the correct parameters, the ion guides can be set to transfer ions from one location to the next.

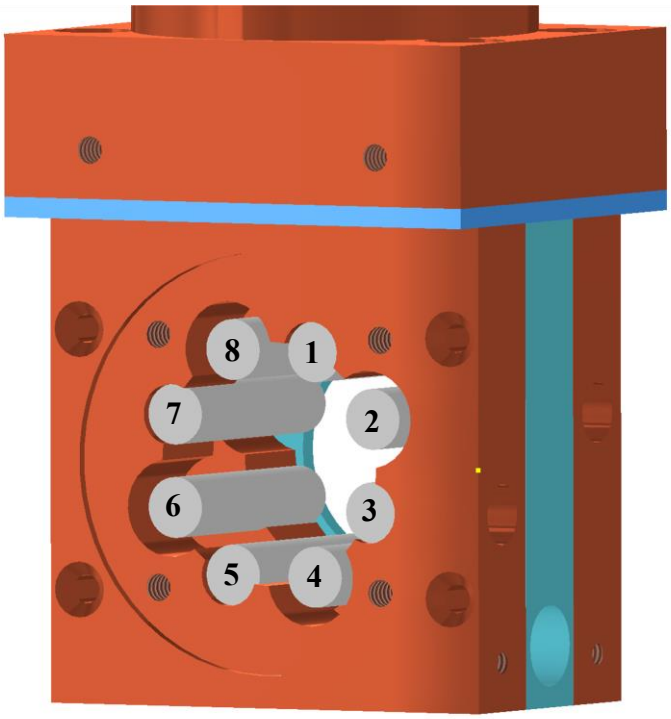

Figure 8.2. A schematic of the linear octupole ion trap equipped with 8 rods that create the trapping field to hold ions for a user-defined amount of time.

After passing through two ion guides separated by a small spacing plate that helps decrease the pressure by three orders of magnitude, the ions encounter a shortened version of an ion guide that has two end caps. The end caps act as voltage-regulated walls that prevent the ions from being guided in or out thus creating an ion trap. The ion trap in Figure 8.2 is a linear octupole trap meaning that it has eight stainless steel rods creating the trapping field that holds the ions within the end cap boundaries. The ions are trapped by introducing a small pulse of inert gas such as Helium into the trap prior to the ions entering. An inert gas is one that is non-reactive and, therefore, ideal for collisional cooling of the ions since it simply helps to stop them by giving them something to crash into as they enter the trap. The presence of the buffer gas cools down the fast moving and energetic ion packet, the term used to describe the group of ions that travel in one trapping cycle, as it enters the region where we would like it to remain for a user-determined period of time.

In addition to the cooling effects of the gas, the octupole ion trap is attached to a liquid nitrogen reservoir which cools the trap to 80K (approx. -310 °F). The cooling is an essential component to the approach since it allows for ions to be in a less energetic state. The lower energy ions permit us to perform reaction chemistry within the traps. One specific example of reaction

chemistry that is of particular relevance in the work presented in this thesis is solvation of ions with a variety of solvents including water, H_2O, and methanol, CH_3OH. Solvation is a specific type of chemical reaction that is meant to mirror an environment that a molecule exists in in nature. An example would be a protein with a shell around it that is composed of the different components of blood. This example is something all of us can relate to because our bodies are filled with blood and we all have proteins that we get from eating certain foods. In this context, we define an ion of interest surrounded by a solvent molecule as a solvent cluster. Namely, the protein surrounded by the components of blood would be the solvent cluster. In my experiments, the solvent clusters are formed by seeding the non-reactive gas with a small amount of the solvent such that each ion packet is able to react with a small amount of the solvent that then chemically reacts and adheres to the outside of the molecule. This is often referred to as the solvent shell or solvent complex. Figure 8.3 shows an example of a cartoon peptide (green) and the peptide solvated with water

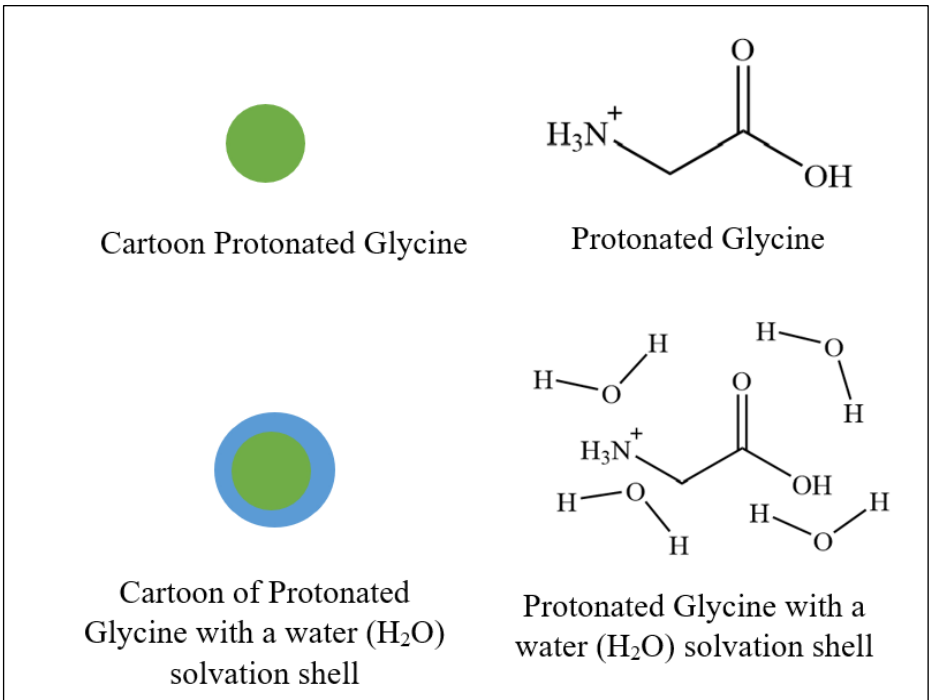

Figure 8.3. Cartoon depiction of the protonated amino acid glycine (top left) and chemical structure (top right) followed by a cartoon depiction of protonated glycine solvated with water (bottom left) and a potential chemical configuration of the waters around protonated glycine (bottom right).

(green and blue) on the left. The right side of the figure shows the chemical representation of the amino acid glycine and what it looks like when it is solvated with water. Once the solvent complexes form, the ions are pulsed out of the trap by dropping the voltage on the end cap about 5-10V below the trapping voltage. The solvated ions are guided by another set of ion guides and an additional spacing plate to another type of ion trap. As opposed to the first geometry made of a series of stainless-steel rods, the second ion trap in the instrument is a three-dimensional trap (Figure 8.4). Its geometry consists of two hyperbolic end caps and a circular, donut-like ring. This ion trap is connected to an even colder freezer that reaches temperatures down to 4K (approx. -450 °F). This temperature facilitates the infrared spectroscopy approach used.

The spectroscopy used in this particular type of ion trap is called action spectroscopy and requires the condensation or attachment of a non-perturbative tag to the ion packet. Unlike the solvent molecules which form a chemical bond to the species of interest, the tag molecule is non-perturbative because it does not disturb and chemically change the molecule. It is meant to act like a price tag on an item of clothing that can be removed without changing the composition of the shirt, pair of pants, etc. The tag is loosely-bound to the solvated molecule via the cryogenic or ultra-cold cooling of the freezer attached to the 3D Paul trap. The geometry of the three-dimensional ion trap is critical to the next stage where mass analysis occurs.

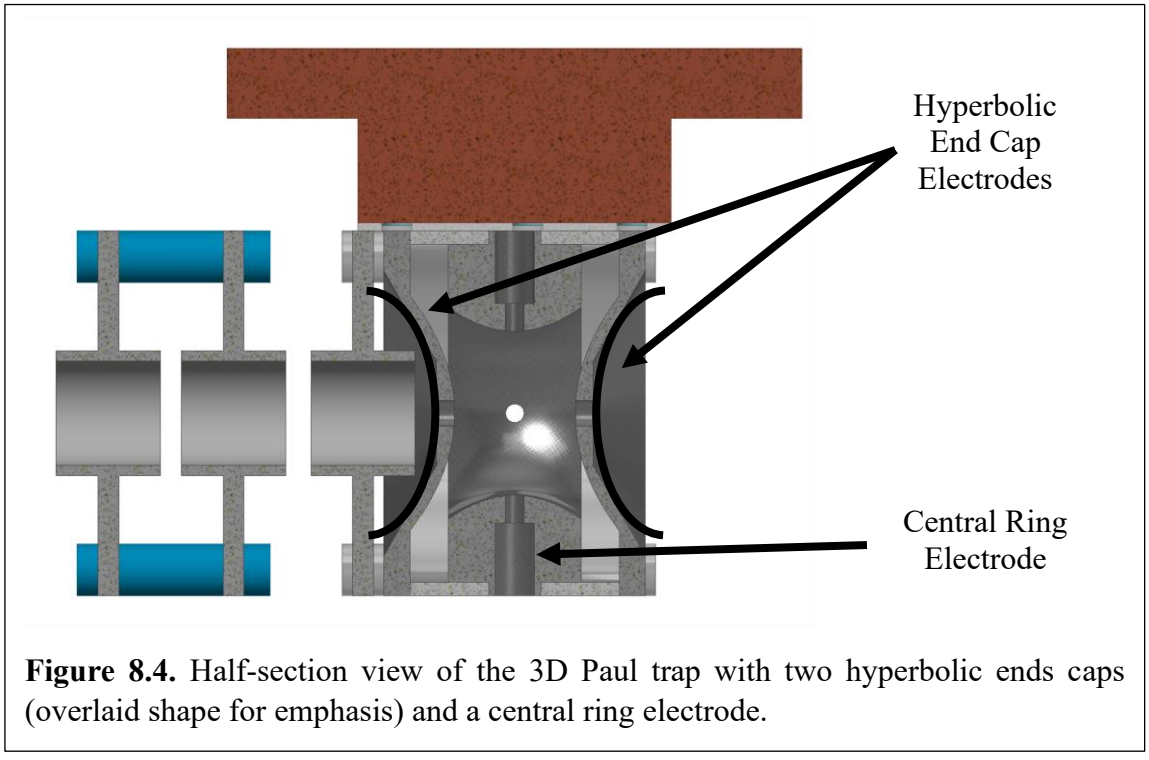

Figure 8.4. Half-section view of the 3D Paul trap with two hyperbolic ends caps (overlaid shape for emphasis) and a central ring electrode.

The ion packet with the condensed tag is pulsed out of the second trap into a series of two to three plates that are floated at a voltage near that of the end cap of the 3D Paul Trap. The last of the plates, however many there are, is pulsed much like the end cap of an ion trap is. The tight ion packet is introduced into a time of fight (TOF) tube that separates the ions by measuring how fast they travel down the tube. The principle of TOF is much like a 400-meter dash. Each ion within a packet starts at the same place (the start line) and is given the chance to travel (run) the straight path down the tube to the detector. The only difference is that ions can't start themselves down the path and so we must start them, which we do by giving all the ions a voltage pulse that gives each of them the same kinetic energy. Since all ions start with the same kinetic energy, the lighter ions travel faster since they are most affected by the pulse and easier to move. The faster ions reach the detector first and thus the ion packet is separated and detected based on the mass-to-charge spread of the ions.

In summary, mass-to-charge is calibrated based on the length of time it takes ions of different size to travel the same distance and hit the detector. To detect the ions that are separated

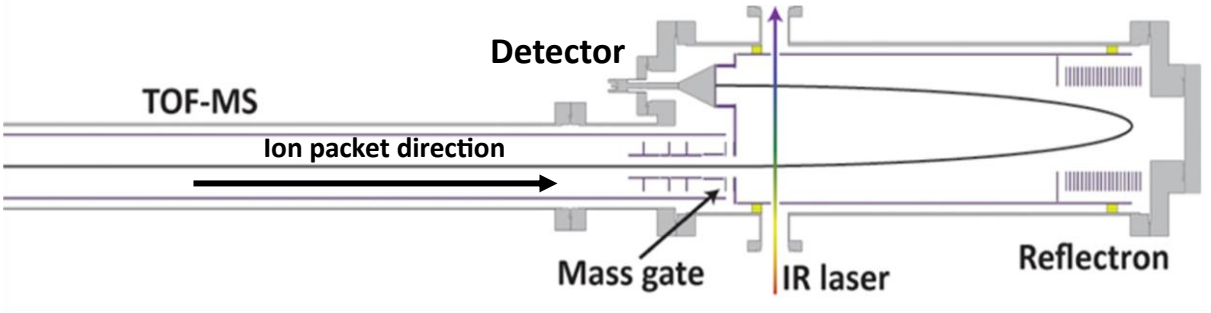

Figure 8.5. Schematic of a time of flight (TOF) region equipped with a reflectron. The ions move down the TOF (left) and then are turned by the reflectron (right) and projected onto the detector (center top).

in the TOF tube, we use a multi-channel plate (MCP) detector. A MCP detector is a flat plate with many micron-sized channels that are coated in a material that allows them to amplify ion current. So, when ions of any mass-to-charge hit the detector, they are recorded as an ion current. Ion current is amplified and is taken to be directly proportional to output signal (i.e. the number of ions of a specific mass-to-charge that hit the detector).

In order to perform spectroscopy, the tagged ion packet is perpendicularly intersected by a laser beam of user-defined wavelength in the second stage of the TOF region (Figure 8.5). As mentioned before, spectroscopy is a technique that utilizes light to characterize the bonds within a molecule to reveal the connectivity of atoms that create the molecule we are studying. In this technique, when the laser wavelength set by the user is resonant with a molecular bond energy, the bond absorbs the energy and begins to move. The movement associated with similar energies of light and matter is called resonance. Only when the energy of the input light is matched to the bond energy does the tag fall off. A good analogy for this process is scratching an itch on your arm. You could scratch your leg or have someone scratch your back, but it doesn't meet the necessary conditions to solve the problem. You have to identify the right place to scratch and then scratch in the right manner. You wouldn't want to scratch so hard that you made yourself bleed but just simply touching your arm also won't fix your itch. Much like scratching an itch, the laser has to intersect the ion packet in the right spot and it has to scratch it in the right manner which means it has to be at a resonant wavelength. In the technique of action spectroscopy, the laser is scanned over a variety of input energies (wavelengths) so that all the different bonds in the molecule can be characteristically identified and pieced back together. This is how the Garand Group uses the tandem mass spectrometry infrared spectroscopy technique to study small molecular species.

Section 8.4: What can't we do with the current hardware?

Now that we know how the current instrument works, it is important to identify where we can improve our technique. This approach to instrumentation ties back to some of the original analogies I presented you with but I'd mostly like to focus on the screwdriver analogy. We have a screwdriver and it works but we can pick a different size head on our screwdriver which might work better and/or make our job a little easier to execute. This is the approach that my graduate work has taken to the tandem technique used to study small molecules.

First and foremost, the linear octupole shown in Figure 8.2, which we use to produce chemically-manipulated molecules, results in a large distribution of solvated clusters. Figure 8.6 shows a tripeptide (three amino acids linked together) with an increasing number of water molecules bonded around it. Panel 1 shows the tripeptide (Ala_3H^+) with the one, two and three water clusters attached (the following three peaks). Panel 4, which had to be zoomed by five times

on the y-axis to be seen at this intensity, shows the tripeptide with waters four up to fifteen in the solvation shell. Note that each peak represents a tripeptide with a different number of waters.

Figure 8.6 demonstrates one of the main challenges associated with this technique. Namely, it is challenging because we are not forming just the tripeptide - water cluster we want to investigate but a whole suite of them that make the output cluttered and detract from the formation of the one we are interested in studying. Another difficult aspect of this technique regarding a

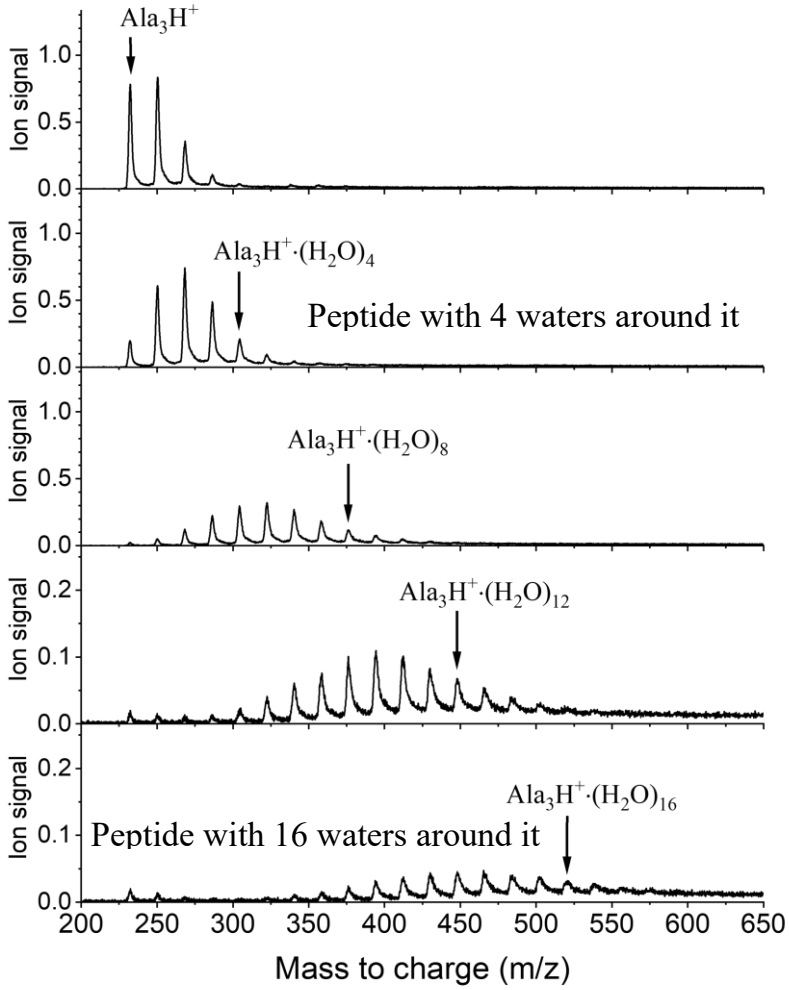

Figure 8.6. A small chain peptide (Ala_3H^+) with an increasing number of solvent water molecules in each panel.

large distribution is that we only have the capability of studying one cluster size at a time which is increasingly difficult when the signal intensity is low as is seen by the zooming necessary to see the peaks in panels 3, 4 and 5 of Figure 8.6. Signal intensity is essentially the amount of a species

that is detected. This means we produced seven-times as little signal of the largest clustered species compared to the original. This makes the experiments harder to do because there is less material to work with.

In addition to the lack of selectivity for which cluster forms, the linear octupole ion trap is the sole location for chemical reactions or solvation experiments. The second trap, the three-dimensional trap, is dedicated to tag condensation which requires a vastly different set of experimental parameters than those required for solvation with water. With only one location to perform a chemical reaction, our experiments are limited to one chemical change per system. A specific example of a two-step reaction that we are interested in studying is the formation and then solvation of a catalytic intermediate. A catalytic intermediate is a species that must be formed *in-situ* meaning that it cannot be formed beforehand or bought ready to be studied. It has to be made in the experimental setup and, being an intermediate, it is usually short lived because it is not energetically stable.

In the past, we have used our linear octupole trap to form the intermediate species and then studied it using spectroscopy[1,2]. We were required to use two separate traps because of the different set of experimental parameters that forming the intermediate requires when compared to solvation with water. Different experimental settings for different processes is analogous to putting on your shoes compared to putting on your shirt. They are both involved in the process of getting dressed, but come at different times and require different approaches. The formation of the chemical intermediate requires using ozone as the buffer gas. Ozone is a reactive gas that changes the charge state of the intermediate. Solvation, on the other hand, requires the use of a buffer gas that will not change the charge state of the molecule. Because the conditions required to perform these experiments are so different, they cannot be performed in the same ion trap. It requires two separate environments. Many studies have been performed to try to understand the mechanism by which the intermediate is formed and reacts in the cycle, but none have been able to directly probe the solvated intermediate because of the experimental limitations discussed[3–5]. Here I present the work I have done to create a new type of instrument that can study the two-step reaction.

A slightly less complicated example of a two-step reaction is one that simply involves putting two different solvents species with different chemical properties on the outside of a molecule of interest. This is like mixing two paint colors in a painting to more accurately depict

the scene. In our ion traps, we need more than one solvent because the natural world is complex and made up of many different solvents and surroundings. In order to better replicate nature to understand why things work the way they do, we need to put more than one thing around our molecule of interest. And it really does help make the picture more realistic just like when you mix dark and light blue to make a more beautiful Lake Mendota!! The two solvents added are water (H_2O) and methanol (CH_3OH). Water and methanol have vastly different properties that cause one to exist as a liquid for a long period of time (think of water in a lake that doesn't just magically disappear overnight) and one to rapidly move from the liquid to the gas-phase at room temperature. Therefore, these two solvents require starkly different experimental settings to form and maintain clusters so they act as a good model study for isolating a species' chemical environment, making a chemical change and then passing it on to perform another step in a reaction process.

Section 8.5: Instrument Innovation - Hardware

In order to address the challenges presented in the previous section, we decided to attack both the hardware and software sides of the issue. To address the hardware components and the physical need for more environments for chemical reactions or manipulation, we designed a

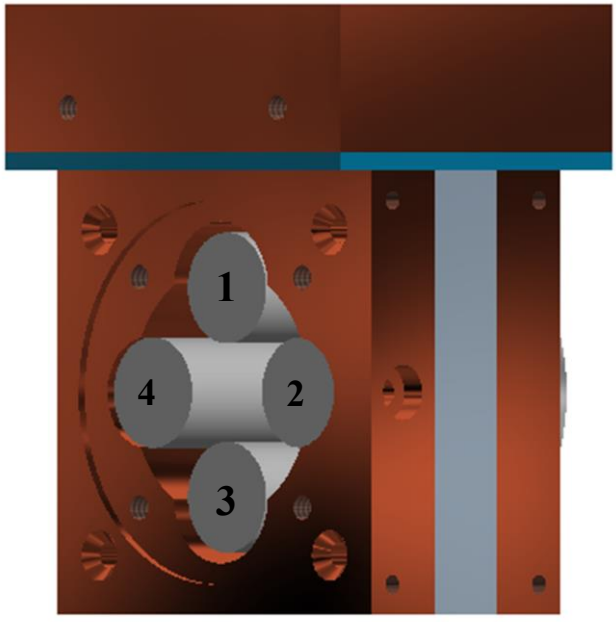

Figure 8.7. Schematic of the simplified quadrupole ion trap geometry used in the digital ion trap prototype instrument.

molecular housing and simplified linear trap design for plug-and-play or LEGO-like ion trapping. Figure 8.8 shows the prototype developed for proof-of-concept experiments. The software portion of the project addressed the lack of selectivity within ion traps. The approach I chose to pursue was to convert the ion traps from analog to digital traps. All the data discussed in this chapter was taken on a digital ion reaction trap prototype. To be clear, the prototype is only capable of mass spectrometry. The motivation for the work will eventually be applied to the current tandem cryogenic spectrometer instrument, but the advancements here are only on the side of mass spectrometry. No infrared work will be shown and no lasers were used to collect this data set.

The prototype mass spectrometer has two major advances that distinguish it from the current model in our lab. First, the ion traps are linear quadrupoles instead of octupoles. That means the traps only have four rods total which makes them easier to construct. The quadrupoles operate on the same principles and with the same electric field as the octupole traps. The second advancement is the modular housing design used to hold the ion traps and other parts/guides/etc. The design consists of three parts: connecting blocks (front stages and ion guides), liquid nitrogen reservoir holders and the cube that houses the traps. The cube creates a plug-and-play design for ion trapping as it is self-containing of the trap which allows for minimal disruption of standard parts when modifications or cleaning is necessary for the traps. It also promotes experimental flexibility as the linker or connector blocks can connect as many cubes as the user would like. Hence, it is possible to have as many ion traps stacked in a row as the user desires since each is self-contained.

The prototype itself is very similar to the mass spectrometry setup described for the tandem mass spectrometry infrared spectroscopy instrument. In brief, the same electrospray interface takes a solution phase system and makes a charged particle in the gas-phase (an ion) that moves into a differentially pumped region. The ions pass through a skimmer and then two hexapole ion guides separated by a spacer plate that helps decrease the pressure. The ions then enter the linear digital quadrupole ion trap where they are collisionally cooled by a pulsed, inert gas. The ion trap is connected to a liquid nitrogen reservoir so that the trap temperature is 80K (approx. -310 °F). The ions are manipulated, a chemical reaction is performed or other changes are made to the ions. The ions are then pulsed out of the trap using the same voltage gate setup described previously.

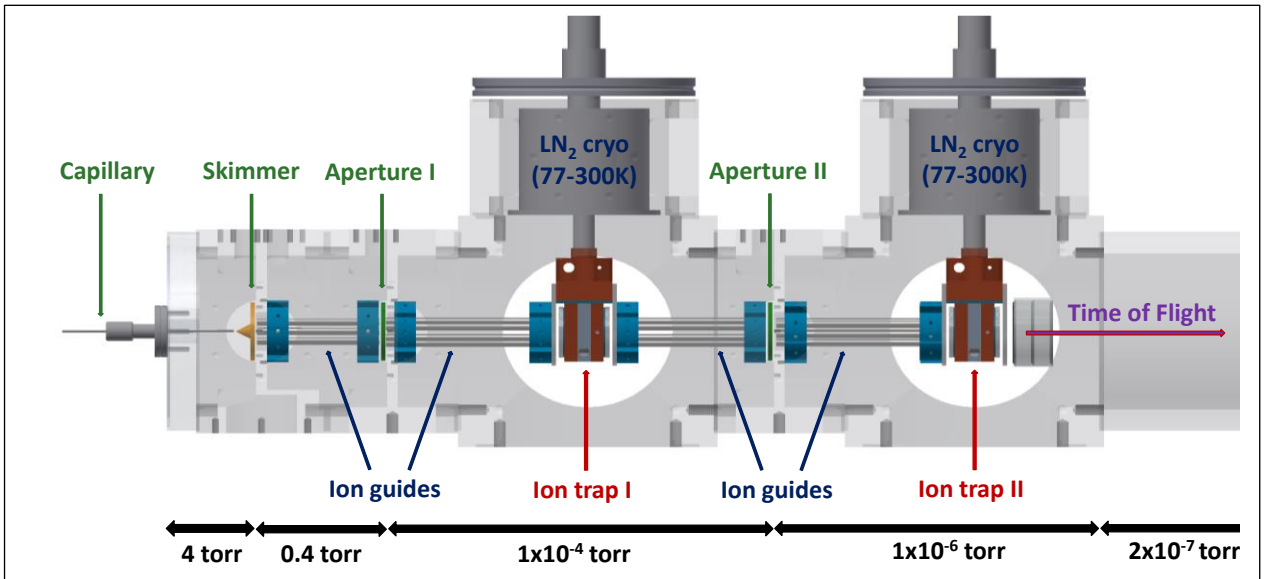

Figure 8.8. Schematic of the tandem mass-selective cryogenic digital ion trap prototype instrument.

create an even lower pressure in the second, identical linear digital ion trap. The beauty of the design of the modular, LEGO-like prototype is that the same easy to construct design works over and over again. And the work demonstrated here proves that even though the design is simplified, it works just as well as the octupole trap. After being pulsed out of the second trap, the ions are sent through some focusing plates and then enter a TOF region for final analysis using a MCP detector.

Section 8.6: Digital Ion Traps: What are they and why are they useful?

What does it mean to have a digital ion trap and how does it address the lack of selectivity in current approaches? First off, a digital ion trap is an ion trap whose electric field for trapping ions is created by a different type of waveform. Specifically, a digital waveform that has an on and an off state much like the 1's and 0's that you might see in scenes from the Matrix. Another way to think about digital waveforms are as electrical switches or square waveforms that have discrete high and low states. Conventionally, ion traps are run on continuous waveforms known as sine waves (Figure 8.9, left).

Labs across the world have invested in developing ion traps that run on the discrete, switching waveforms[6–12]. As is expected, when you change one parameter (the type of waveform that is used to trap the ions), other parameters change in suit. The adjustable parameters available in digital or square waveforms are the frequency and duty cycle, both of which are user defined

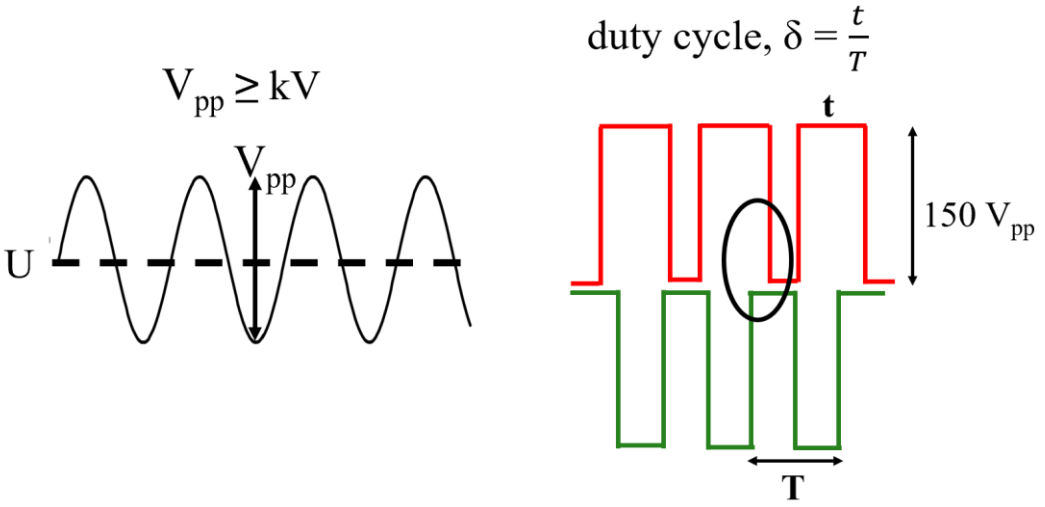

Figure 8.9. Representation of a sine wave used to drive quadrupole filters and/or ion traps with amplitude, V_{pp}, on the order of kV, DC offset U and an adjustable frequency (left); representation of two square waves with an adjustable frequency, amplitude, V_{pp} of 150V and a non-standard duty cycle creating a potential offset, U.

variables that can be changed mid-experiment. The frequency of a waveform can be summarized as the rate at which the waveform switches from the high state to the low state. In terms of switching the waveform, high frequency waveforms switch from the high state to the low state very quickly, even up to the order of 10^6 per second! Low frequency waveforms switch slowly, on the order of seconds. The other adjustable parameter is called the duty cycle. The duty cycle is defined as the amount of time that the waveform spends in the high state of the switch relative to one full period of the waveform. One period of the waveform is the amount of time it takes the wave to complete one oscillation from low state, switching to the high state, sitting in the high state and finally switching back to the low state. The right side of Figure 8.9 shows a non-zero or adjusted duty cycle on the red wave. The red wave spends more time in the high state relative to the amount of time it spends in the low state. This parameter is adjustable for the low and high side of the waveform.

Using the adjustable frequency and duty cycle parameters, digital ion technology (DIT) allows me to perform mass-selection *within* my ion traps instead of adding an additional piece of hardware to an already complicated instrument. This is a great advantage in the tandem mass spectrometry infrared spectroscopy technique that the Garand Group uses since adding additional

hardware components that mass-select ions, done by some groups already, usually results in more ion loss than it does selection of the species of interest[13–19]. To motivate instrument innovation and improvement in design, I again mention that having the right tool for the job makes a task easier. Digital waveforms that allow in-trap mass-selection is a better tool to approach the tandem mass spectrometry and infrared spectroscopy technique because it makes acquiring data and interpreting spectra much easier.

In order to use square waveforms to perform in-trap mass-selection, I adjusted the square wave parameters of frequency and duty cycle to selectively remove all mass below or above a user-defined mass-to-charge. Figure 8.10 and Figure 8.11 show what happens when the frequency and duty cycle are adjusted, respectively. The top panel of each figure shows a distribution of water clustered around a molecule of interest, in this case it was a small protein but it could be anything that we are interested in studying. In panel 3 of Figure 8.10, a lower frequency was applied for a short period of time, which removed the peptide and the first and second water cluster while still preserving the larger clusters. Remember, a cluster is just a molecule of interest with a

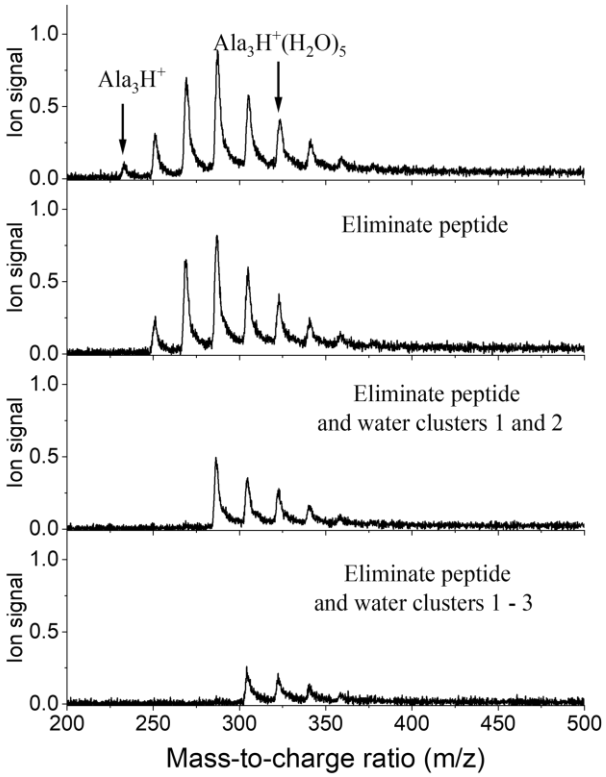

Figure 8.10. Formation of a $Ala_3H^+\cdot(H_2O)_n$ cluster distribution followed by a high pass m/z filter by changing the frequency.

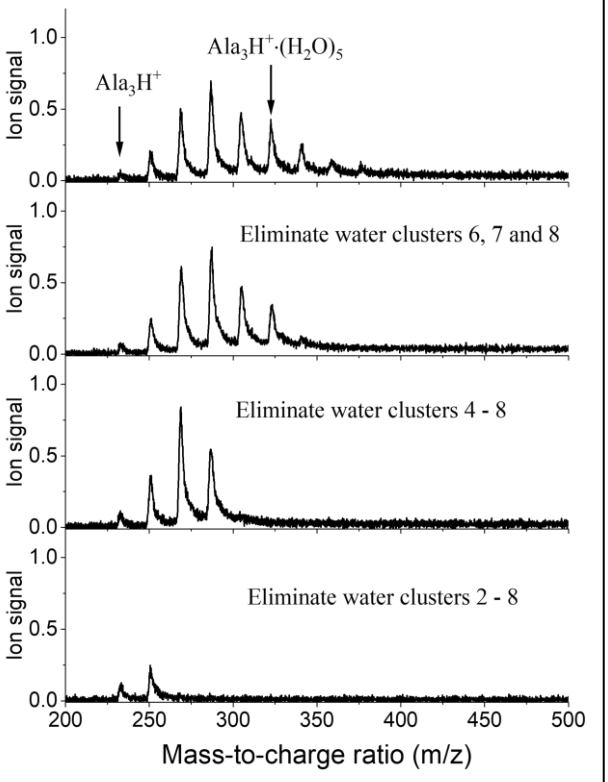

Figure 8.11. Formation of a $Ala_3H^+\cdot(H_2O)_n$ cluster distribution followed by a low pass m/z filter by changing the duty cycle.

set specific number of solvent molecules added. In this case, the solvent is water so the larger the cluster, the more water is present around the small protein. Panel 4 shows what happens when I change the frequency I apply for a short period of time. Namely, it removes the next cluster one at a time. The other parameter, the duty cycle, eliminates water clusters starting with heavier (higher mass-to-charge) species. The third panel of Figure 8.11 shows how I adjusted the duty cycle of the square wave to eliminate water clusters four through eight.

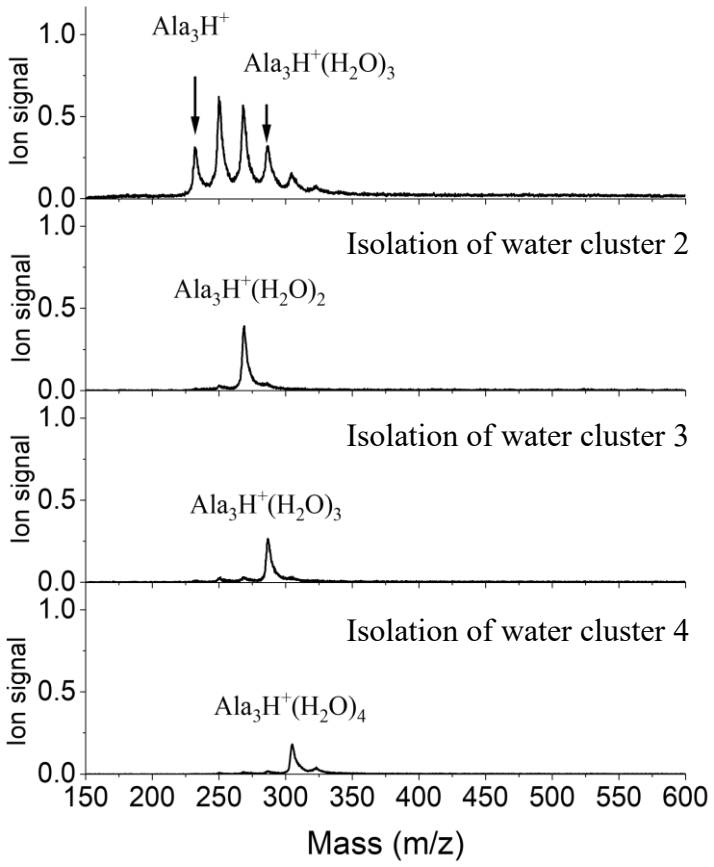

Figure 8.12. Formation of a $Ala_3H^+ \cdot (H_2O)_n$ cluster distribution followed by mass isolation of selected water clusters using sequential low pass and high pass filters.

Knowing that I could eliminate high and low mass-to-charge species, I decided to write a code that would allow me to perform both types of filters in one trapping cycle. Therefore, I was able to select one single mass-to-charge. Selecting one mass-to-charge is called mass-selection and the code I wrote allows me to perform mass-selection all within one ion trap. Mass-selection allows me to create an initial distribution and then keep only the one I want. If I go back to the

getting dressed analogy I used earlier, in-trap mass-selection is like trying on six shirts in your closet and then selecting one to wear out. Not being able to do this in your closet – say having to go to the store, find six new shirts, try them on in the dressing room, etc. - would require a lot of extra work and time. This is exactly what we are trying to avoid by providing a mechanism for in-trap mass-selection. Figure 8.12 shows how tuning, or adjusting slightly, the two adjustable parameters allows me to select any species within a distribution that I want without significant loss.

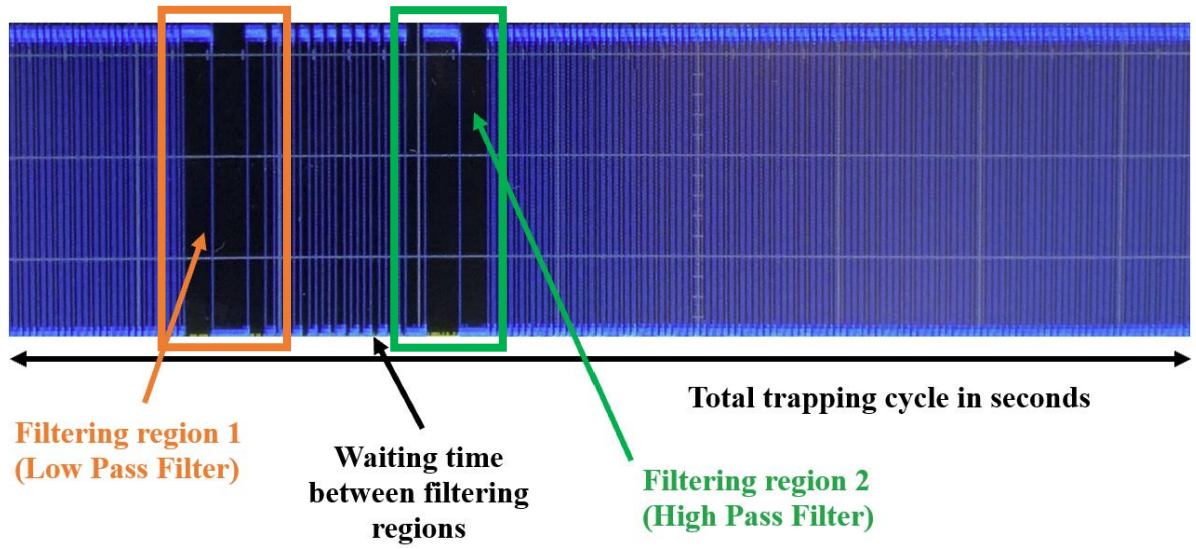

Figure 8.13. Scope trace showing square waveforms (blue) applied to the rods of the quadrupole rods to create the electric field used to trap ions. The square waveforms are shown under their normal conditions (blue, left), adjusted in a first region (orange) as a sample of low pass filter settings, returned to the normal settings (blue, "Waiting Time"), adjusted for a second region (green) showing a sample of high pass filtering and finally returned to their normal conditions (remaining blue portion).

One of the biggest challenges was finding the correct combination of frequency and duty cycle to perform mass isolation because of the mathematical relationship that relates them. They are dependent variables in the context of an ion's mass-to-charge which means that when you change one of them, the other changes slightly as well. In order to circumvent this issue, I rewrote a portion of the code that controls the trapping waveforms so that it could perform the low pass filtering first and then, after a set amount of time, perform a high pass filtering step. This is demonstrated in Figure 8.13. As a reminder, low pass filtering is when only ions of lower mass-to-charge than the user-defined mass-to-charge can pass. And a high pass filter is the opposite. A

high pass filter is like the signs at an amusement park that say "You must be at least this tall to ride". Based on a user-defined mass-to-charge, the applied frequency essentially tells the ions

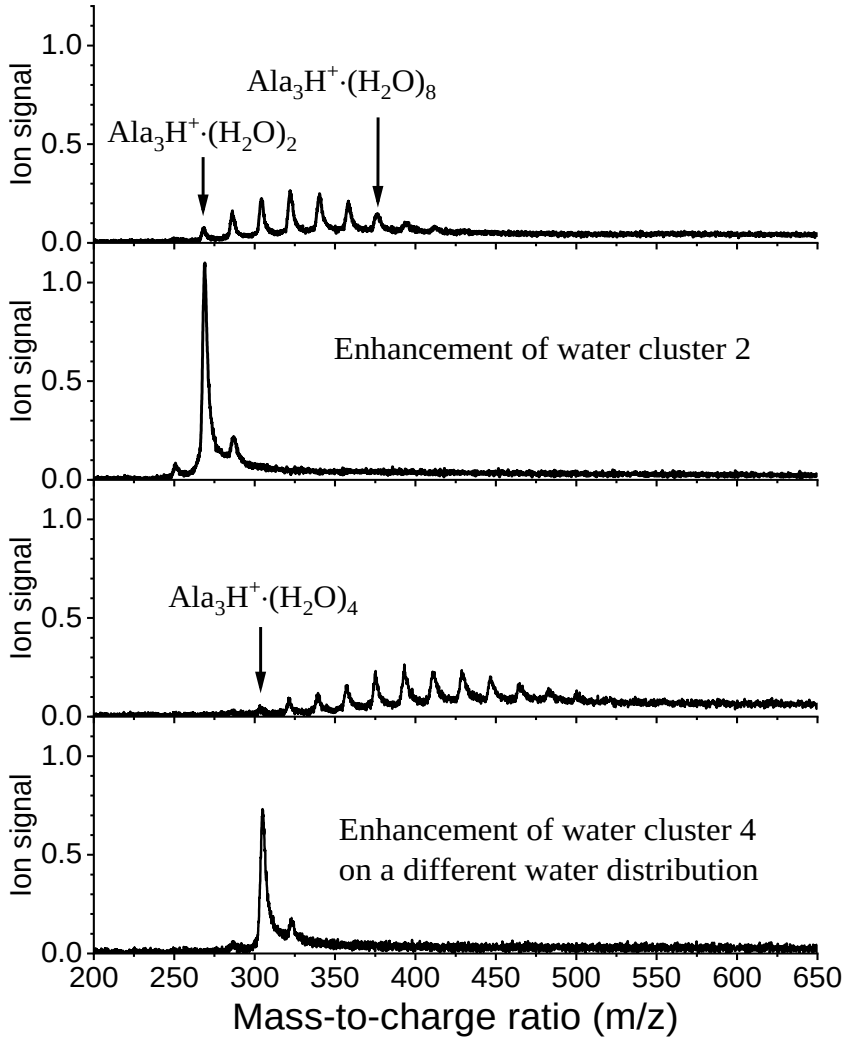

Figure 8.14.. Enhanced formation and isolation of specific $Ala_3H^+\cdot(H_2O)_n$ cluster.

"You must be at least this heavy to pass". My adjusted code decoupled the frequency and duty cycle variables, which allowed them to operate as if they were not tied to one another. Not only did this work beautifully for mass-selection, but I discovered that by changing the timing of when each filter is applied, I could selectively enhance the mass-to-charge that I was selecting.

Figure 8.14 shows two examples of cluster enhancement and selection from a distribution of water clusters on the same parent tripeptide (a small chain of three amino acids linked together).

The top panel of Figure 8.14 shows a distribution of water clusters. The second panel shows selection and enhancement of the second water cluster. We saw enhancement of nearly 25 times the selected water cluster, which was really exciting! In order to get this much enhancement, you must apply the low pass filter while clusters are forming. When I apply the filter while the clusters are forming in the trap, the process is gentler and allows the water molecules to remain intact. Thus, the water (the solvent in this case) funnels down to the clusters that survived the filtering process.

Section 8.7: Moving towards the study of more complex chemical systems

With this new tool set, I set out to prove that it indeed expanded the number and types of systems that the tandem technique could be used to investigate. Figure 8.15 demonstrates the use of the modular ion trap design (hardware) and in-trap mass selection (software) for the study of multi-solvent clusters. Remember, multi-solvent clusters are any molecules that have more than

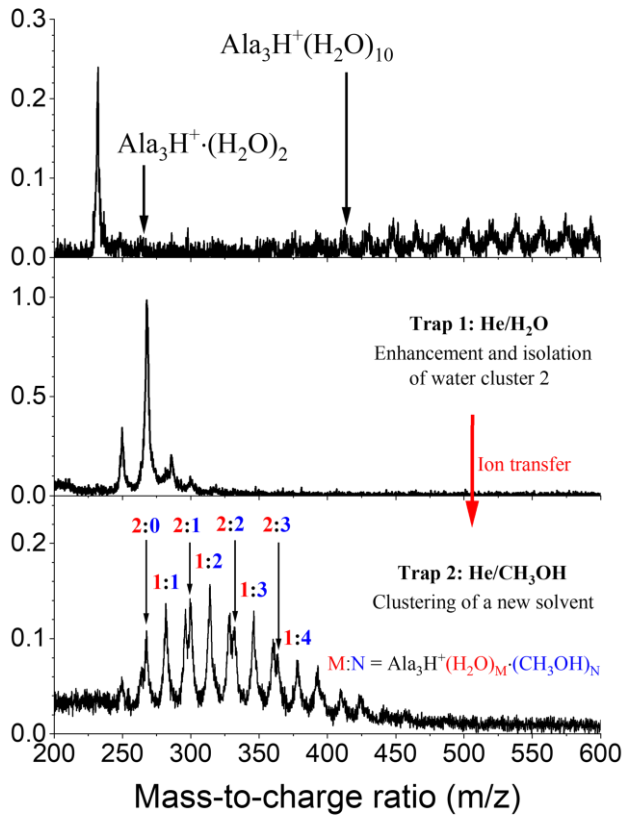

Figure 8.15. Two trap experiment where $Aa_3H^+\cdot(H_2O)_2$ is formed and enhanced in the first ion trap. Methanol is then clustered on top of the selected cluster in the second trap.

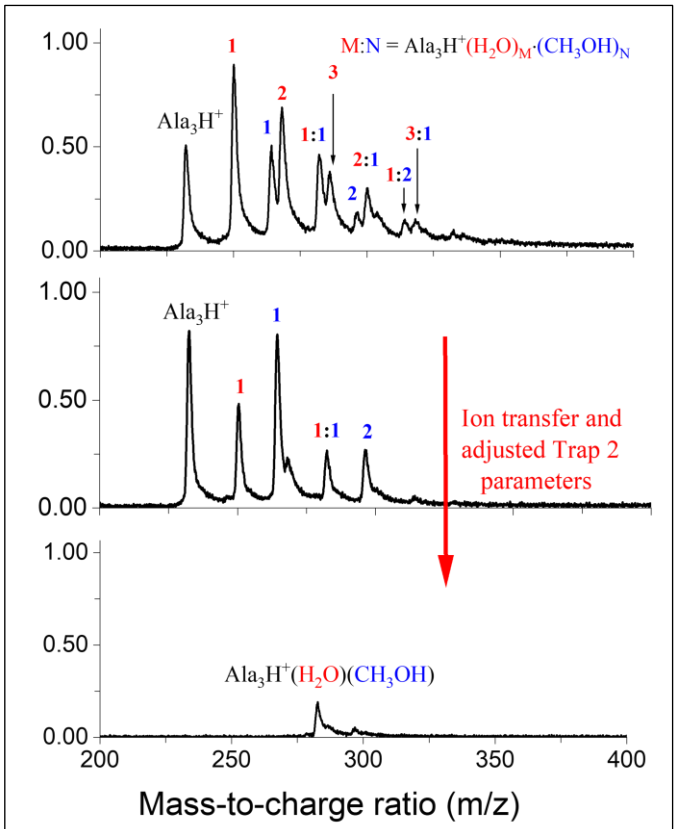

Figure 8.16. Two trap experiment where enhancement occurs in the first trap followed by methanol clustering and final selection of the multi-solvent cluster.

one thing around them. This is very important because the natural world is complex and often involves more than one component for any process.

The top panel of Figure 8.15 shows a distribution of water clusters on a small chain of peptides that I was studying at the time. The second panel shows selection and enhancement of the second water cluster in the first digital, quadrupolar ion trap using the same process described earlier. The ions are transferred from the first trap to the second, identical trap where instead of using water as the solvent, I used methanol (CH_3OH). I chose methanol because it has a much lower vapor pressure and therefore requires significantly different trapping and clustering settings. Vapor pressure is essentially a measure of how quickly a liquid evaporates or moves from the liquid to the gas phase.

The lower the vapor pressure, the quicker the liquid becomes a gas and so methanol required me to work a lot faster since it disappeared faster. The third panel of Figure 8.15 shows that despite the different requirements for clustering and the delicate nature of the mass-selected clusters, indeed appropriate settings and completely separate conditions can be created and maintained such that complex, multi-solvent clusters can be formed.

While it took a slightly different approach based on software limitations, Figure 8.16 shows the fruition of the ultimate goal of the new instrument that I built: mass selection of multi-solvent, complex clusters created in two separate ion traps with two separate solvents. The top panel of Figure 8.16 shows a distribution of complex clusters created using both quadrupole ion traps. Similar to Figure 8.15, the first trap was used to cluster water and the second trap was used to cluster methanol on top of the pre-existing and transferred H_2O clusters. The second panel shows both traps being used to enhance the cluster I wanted to study. Enhancement involves the application of low pass filtering during cluster formation in each trap. Thus, the second panel shows the effect that enhancement settings had on multi-solvent cluster formation. Most notably, the removal of higher order clusters to prevent the spread of second solvent cluster formation. The final panel of Figure 8.16 combines the approaches demonstrated in the first two panels with an added high pass filter at the end of the second trapping cycle to remove lower mass-to-charge species. The combination of enhancement and mass-selection within each trap permits optimizing parameters for each different cluster formation. Tying this back to our screwdriver analogy, it would be like using a Phillips head screwdriver to remove the screws that cover the battery

compartment of a toy and a flathead screwdriver to help you pry the batteries out to replace them. Each screwdriver serves a separate purpose and the distinct heads are important to apply at different times in the process of replacing batteries for the toy.

Section 8.8: Conclusions and Future Work

I hope that this chapter has convinced you that having the right tool for the job really does make it easier to do the job and to do it well. For the Garand Group, the new tools that we have – digital ion technology, simplified geometry of ion traps and a modular housing design – make the job - tandem mass spectrometry infrared spectroscopy – easier to perform and open the door for the study of new, more complex systems. Specifically, the advances in in-trap mass-selection and mass-selection coupled with cluster enhancement have decreased the complexity of the instrumentation and is expected to decrease the amount of time it takes to record data. Moreover, my work has opened the door to create complex, multi-solvent clusters with a variety of different solvents to better understand more realistic chemical environments for things like catalysts, environmental contaminants and biological species. We will know this after we install the digital prototype on the front of the current tandem instrument. This is expected to happen this summer!

Furthermore, in a world that is going digital, my work has provided another application and avenue for the digitization of a technique. Going from an instrument that takes up nearly an entire room and requires multiple recording systems to a condensed prototype run off of my personal laptop is the first step in lowering the barrier to entry for this powerful yet difficult technique.

So next time you approach a problem, I encourage you to take a few moments to consider the best tool for the job. The extra few moments can make a huge difference whether it be a better cup of coffee in the morning or a screw that isn't stripped next time you have to take it out, it's worth the time to consider the tool.

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