THE USE OF INFRARED MULTIPLE PHOTON DISSOCIATION AND FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY TO STUDY THE THERMOCHEMICAL DYNAMICS OF DEPHOSPHORYLATION AND THE SPECTRAL AND DENSITY FUNCTIONAL THEORY DETERMINED STRUCTURAL CHARACTERISTICS OF MONOSACCHARIDE ISOMERS

By

WRIGHT L. PEARSON III

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To God, my wife, family, colleagues, and friends who encouraged me to stay on the path
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Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

THE USE OF INFRARED MULTIPLE PHOTON DISSOCIATION AND FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY TO STUDY THE THERMOCHEMICAL DYNAMICS OF DEPHOSPHORYLATION AND THE SPECTRAL AND DENSITY FUNCTIONAL THEORY DETERMINED STRUCTURAL CHARACTERISTICS OF MONOSACCHARIDE ISOMERS

By

Wright Leroy Pearson III

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Chair: John R. Eyler
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In recent years, there has been an explosion of interest in the fields of saccharide and protein chemistry. Though much of the initial work has been conducted in solution, the desire to avoid complicated liquid-phase interactions has resulted in the promotion of gas-phase experiments. Capable of multiple stages of experimentation (MS^n) and long ion retention times while minimizing extraneous ion-ion/ion-molecule reactions, Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS) has proven to be a powerful instrument in these studies. Of particular importance is the addition of techniques, like infrared multiple photon dissociation (IRMPD), that promote ion dissociation/fragmentation for structural and thermochemical information. The current research employed two 4.7T FTICR mass spectrometers with different types of IR laser systems to examine the possibility of and the reasons for differentiation of saccharide isomers and the kinetic and energetic picture of dephosphorylation through multiple photon dissociation processes.

A LINOS OS-4000 continuous wave optical parametric oscillator (OPO) laser was purchased and the laser beam transfer optics were designed, positioned (within a purge box), and
focused on the ions in an ICR cell. Photon (vibrationally resonant) induced dissociation or action spectra, spanning the C-H-and O-H stretch regions, were then acquired for four rubidium cation-bound glycosides. Comparison of the experimental and Density Functional Theory calculated spectra revealed a network of intermolecular hydrogen bonding and rubidium attachments that provided the means to differentiate D-glucoside and D-galactoside anomers in the O-H stretching region of the infrared spectrum.

In the second project, two cw-CO₂ lasers were also set up for gas-phase experiments. Dephosphorylation rate constants (at varying laser powers) were obtained for both positively and negatively charged phosphopeptide ions at three different wavelengths. In these experiments, the dissociation rate constants favored the negatively charged species at all wavelengths (a result of lower activation energies) as the overall Arrhenius activation energies were independent of vibrational mode intensities.
CHAPTER 1
INTRODUCTION

Infrared multiple photon dissociation (IRMPD) of gas-phase ions has been of importance to mass spectrometrists for a number of years. A large part of this interest has been advanced through the use of Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS) in Penning traps and the development of tunable IR lasers. Penning traps make ideal mass spectrometers for these experiments due to two factors—low pressures and long ion storage times. Low pressure conditions (10^{-8} to 10^{-10} Torr) minimize ion-neutral interactions that lead to collisional energy transfer. Long ion retention times allow the ions to be exposed to IR photons for longer periods to facilitate multiple photon processes. In IRMPD, infrared photons arise from two sources, black body radiation from the trap’s walls and an external IR laser (CO_2, OPO, etc.) beam centered on the ions in the trap. Blackbody radiation can be adjusted by manipulating the trap’s temperature. Keeping the temperature relatively low decreases the intensity and frequencies of black body radiation, thus leaving most of the photon production to the focused, relatively high power monochromatic IR laser beam. Infrared light sources produce single or multiple (tunable) wavelength(s) with a continuous (continuous wave, cw) and/or a pulsed beam depending on the needs of the experiment. Multiple photon absorption is used to promote ion dissociation where the molecule’s resonant vibrational mode absorbs IR photons and undergoes excitation. The energy is then quickly distributed throughout the molecule’s vibrational modes raising the internal energy until the weakest bond(s) dissociates. Spectroscopy, kinetics, thermodynamics, structures, and energetics of many systems have been explored using IR laser beams directed onto ions contained in Penning traps.

In the present research, IRMPD has been applied to biological molecules, in particular saccharides and peptides. In conjunction with Dr. Cesar Contreras, vibrationally dependent
photodissociation spectra (action spectra) covering the C-H and O-H stretch region (2750 to 3750 cm\(^{-1}\)) of four cyclic D-aldohexose isomers have been measured with an FTICR mass spectrometer utilizing an optical parametric oscillator (OPO) laser light source. These spectra reveal areas of isomeric differentiation and help elucidate gas-phase structures with the aid of computational models. Kinetics and relative activation energies have also been determined for dephosphorylation of positively and negatively charged phosphopeptide ions, with a CO\(_2\) laser light source. These experiments have been repeated at three distinct widely spaced wavelengths to determine the effect of laser-vibrational resonance. Prior to describing the experiments and results, the basics of saccharides and proteins are presented.

**Saccharides**

Saccharides have been studied for many years and have very important biological and economic functions, from energy storehouses in the human body and sweeteners in our beverages and foods, to cellulose for plant structure, and potential biofuels. Emil Fischer (1852 - 1919), the father of saccharide chemistry, is responsible for carbohydrate classification and the initial studies of reaction mechanisms.\(^1\)\(^2\) His work has been carried forward through the tireless efforts of many in the scientific community, with experiments usually conducted in the solution phase. Given the complexity of solution phase chemistry, where carbohydrate interactions form derivatives and polymers that vary with temperature, solvent, molecular structure, etc., gas-phase chemistry is often utilized to help simplify the picture. Mass spectrometry is one of the more important instruments in these gas-phase studies. Saccharide ions are isolated in the gas phase to be examined through ion-molecule reactions, dissociation techniques (including IRMPD), and a host of other MS\(^n\) experiments to obtain information.\(^3\)\(^4\) Background information on saccharides is helpful for an appreciation of these complex interactions.
Monosaccharides

The basic units of saccharides are the monosaccharides that comprise one of two families: aldoses and ketoses. Each monosaccharide is made of 3 to 10 carbon atoms connected by C-C single bonds. When in the chain form, an alcohol is found on all but one carbon, which shares a double bond with an oxygen atom, resulting in the empirical formula \((C_nH_{2n}O_n)\). The most biologically significant saccharides, and the focus of this section, are the aldose sugars, which have the carbonyl on one end of the chain forming an aldehyde group. In ketoses, the carbonyl is found inside the chain resulting in a ketone group. Carbonyls of open monosaccharides are reducing agents, oxidized by mild oxidizing agents such as \(\text{Fe}^{+3}\) or \(\text{Cu}^{+2}\) (found in positive Tollens’ and Benedict’s tests). Tests to monitor the presence and the quantity of monosaccharides (reducing sugars) are common today, e.g., in glucose monitoring for diabetes patients.5-10

Emil Fischer developed a carbon numbering system and simple nomenclature to identify each monosaccharide (Fischer projections).11,12 Beginning with the carbonyl carbon designated as C-1 (in aldoses) or C-2 (in ketoses), each carbon is numbered sequentially ending with the \(\text{CH}_2\text{OH}\) group, which for D-glucose is C-6 (Figure 1-1). Monosaccharides with four, five, six, and seven carbon atoms are known as tetroses, pentoses, hexoses, and heptoses, respectively, and are given the title prefix keto- or aldo- depending on the family. The most naturally occurring are the hexose and pentose varieties, in particular the aldohexose D-glucose and ketohexose D-fructose.

Chiral centers. Most sugars have multiple chiral centers (carbons with four different functional groups). As a general rule, saccharides containing \(m\) chiral centers can have \(2^m\) stereoisomers. In the case of aldohexoses, which have 4 chiral centers, there are a total sixteen possible stereoisomers. These isomers are split into L and D categories (This is not to be
confused with the terms dextrorotatory (d or + for cw rotation) and levorotatory (l or – for ccw rotation), which specify the direction of rotation of plane polarized light when interacting with the electron clouds of chiral molecules. As shown in Figure 1-2, when the configuration of the chiral carbon farthest from the carbonyl is the same as the Fischer projection of D-glyceraldehyde (with the O-H to the right side of the chiral carbon), the sugar is designated as a D-enantiomer. With the L-enantiomer, the OH is pointing to the left (or S and R in the Cahn-Ingold-Prelog convention, respectively). For the aldohexoses, this results in two sets of eight uniquely named isomers, shown in Figure 1-3. The number of stereo isomers is further complicated by the formation of favored cyclic structures.

**Cyclic structures.** In solutions of monosaccharides containing five carbons or more, the combination of potentially reactive carbonyl and hydroxyl groups and a ring’s relative stability create conditions that promote cyclic structures in equilibrium with the sugar’s linear forms. This mechanism, known as mutarotation, is shown in Figure 1-4 for D-glucose. In D-glucose the carbonyl oxygen is protonated, opening the carbonyl C-1 to nucleophilic attack by the oxygen from the C-5 hydroxyl group to create a six-membered ring. The hydrogen atom from the former C-5 oxygen is subsequently lost, creating the hemiacetal structure with an anomeric carbon, C-1, at the point of further mutarotations. The ring also creates a new chiral center at C-1. However, the D designation is conserved as the C-6 hydroxyl group remains relatively unperturbed. The eight isomers of alpha D-aldohexose rings are illustrated in Figure 1-5.

Depending on the approach of the nucleophilic attack, above or below the plane of the carbonyl, the cyclic molecule will be in one of two anomeric forms, alpha (α) or beta (β). An alpha sugar is the result of a nucleophilic attack above the plane causing the newly formed C-1 hydroxyl group to be axial to the plane of the molecule. A beta sugar forms when the nucleophile
approaches from the opposite end (below) causing the C-1 hydroxyl group to be equatorial or in the plane of the ring. In nature, these two forms mutarotate resulting in an observed specific (optical) rotation $\left[\alpha\right]_D^{20}$, of +52.7 for D-glucose at 20°C. With the pure anomic forms having $\left[\alpha\right]_D^{20} = +112$ (α anomer) and $\left[\alpha\right]_D^{20} = +18.7$ (β anomer), at the D line of sodium, the equilibrium percent concentration is easily calculated to be 36% alpha and 64% beta in solution.\(^{15}\) Depending on the molecular structure, solvent, etc., these percentages vary for different sugars.

Mutarotation of the alpha and beta isomers can be halted through O-methylation of the anomic carbon, a process known as glycosylation. As shown in Figure 1-6, protonation of the C-1 hydroxyl group causes the release of water to create an oxonium ion intermediate. The methanol oxygen in turn attacks C-1 and, depending on the point of attack, forms an O-methylated alpha or beta isomer, which is a nonreducing sugar. Although the process is reversible in acidic media, the conformation is stable at moderate pH values.\(^{16}\)

**Cyclic conformations.** Influenced by the size of the ring, functional group interactions, solvent effects, etc., single-bonded rings can have many conformations. Five-membered rings are typically planar due to the steric conditions of the tightly grouped carbons. They are found in pentagon or envelope (E) shapes where some puckering and twisting does occur. The six-membered rings favored by D-aldohexoses can take on more forms. The most common are the boat and chair (Figure 1-7). Boat formations are designated as either $1,4^B$ or $B_{1,4}$ depending on whether both C-1 and C-4 carbons are pointed above or below the plane of the ring, respectively. The chair conformations are similarly designated as $4^C_1$ and $1^C_4$. With the $4^C_1$ structure, C-4 is above the plane of the ring and carbon C-1 is below.\(^{16}\) The $1^C_4$ chair is the opposite. In solution, the most abundant of these conformations is by far the $4^C_1$. However, in the gas phase, although
the $^4C_1$ chair is favored, a fair amount of the $^1C_4$ can exist.\textsuperscript{17} As with any flaccid ring, these structures can twist (i.e., skewed (S)) and bend (i.e., half chair (H)) into a number of variations.

**Disaccharides**

Most cyclic monosaccharides form larger molecules through glycosylation. The mechanism for O-glycoside linkage is similar to that described previously for O-methylation (Figure 1-6), except that the nucleophilic oxygen comes from a hydroxyl group located on one of the C-2 to C-6 carbons of a second cyclic monosaccharide (the glycosyl donor), not methanol. When a glycosidic bond is created between these two monomers the hemiacetal of the first cyclic monosaccharide, the glycosyl acceptor, is replaced by an acetal group. The acceptor’s acetal carbon C-1 is locked and unable to isomerize (nonreducing sugar), while the former glycosyl donor is now ready to act as acceptor on its reducing end. The exception to this is when the nucleophilic oxygen comes from the hydroxyl group on C-1, which produces only a nonreducing sugar.\textsuperscript{14}

Isomers of these molecules are numerous. If the acceptor is in the alpha/beta conformation the disaccharide is alpha/beta, respectively. Further, the structures multiply based on the nucleophilic oxygen’s position/orientation on the ring; e.g., one of the oxygen atoms of a pentose sugar (the glycosyl donor) can attack the C-1 carbon of another pentose sugar (the glycosyl acceptor) from different hydroxyl group positions ((C-2) OH → C-1, (C-3) OH → C-1, (C-4) OH → C1, or (C-6) OH → C-1) each with one of two orientations, axial or equatorial (Figure 1-8). Dissacharides are named in the following order: the configuration ($\alpha$ or $\beta$) of the nonreducing sugar, the name of that monomer, the numbered designation of the two carbons joined (C-1 → C-n), and the name of the second monomer. Furano and pyrano are added to the names of both
units depending on whether the ring consists of five or six carbons, respectively.\textsuperscript{18} Structures of larger polysaccharides are largely based on these linkages.

**Polysaccharides**

Polysaccharides (glycans) differ in the quantity and types of monosaccharide subunits, linkages (i.e., glycoproteins, S-glycoside linkages), and degree of branching. They are divided into two major groups: homopolysaccharides and heteropolysaccharides. Homopolysaccharides contain only one type of monosaccharide. Starch, glycogen in animals, and cellulose are all homopolysaccharides of D-glucose. Heteropolysaccharides consist of many different monomers and other subunits. They form the basis for proteoglycans, glycoproteins, glycolipids, etc., as well as many complex sugars. They are important in cell communication, structure, transport, and a host of other bodily functions.\textsuperscript{14} Carbohydrates form very complex structures and consist of many isomeric components, making saccharides challenging for gas-phase study.

**Saccharide Structure & Differentiation**

Early experiments with limited IR laser sources joined to mass spectrometers illustrated ion dissociation through the absorption of vibrationally resonant IR photons and set the stage for gas phase vibrational spectroscopy.\textsuperscript{19,20} By sequential scanning through the wavelengths of a tunable IR laser, instruments like the FTICR mass spectrometer can monitor the relative abundance of the fragments and precursor ions at each wavelength to create a dissociation or action spectrum.\textsuperscript{21,22} With new developments in laser technology and availability, the range of available wavelengths has expanded. Free electron laser facilities like the one at the FOM-Institute for Plasma Physics Rijnhuizen, in the Netherlands, make available IR wavelengths of 5-110\textmu m. Though impressive, the wavelength range does not include most of the near IR where the saccharide O-H and C-H stretches are found. The cw-OPO laser, however, covers a large part of the region, allowing the study of these important vibrational motions.
Infrared multiple photon dissociation in Penning traps has recently been applied to the problem of identification and structural elucidation of gas-phase di- and monosaccharides. In experiments at the FOM Institute with the free electron laser, Dr. Nick Polfer et al. showed that specific fragmentation patterns for eight isomers of lithium-tagged glucose-based disaccharide (1→2, 1→3, 1→4, 1→6) ions (scanned from 7.5 to 11.0 µm at a total irradiation energy of 340mJ) are capable of differentiating the isomers. Comparisons of the vibrational spectra of these isomers, however, prove less conclusive, due to the congested vibrational “fingerprint” region. The numerous saccharide hydroxyl groups led to the possibility that spectral differences may exist for the vibrationally isolated near IR O-H stretch region. In this research, four Rb\(^+\) tagged O-methylated monosaccharides were examined: Rb\(^+\) [O-methyl-\(\alpha\)-D-glucopyranoside (\(\alpha\)Glc)], Rb\(^+\) [O-methyl-\(\beta\)-D-glucopyranoside (\(\beta\)Glc)], Rb\(^+\) [O-methyl-\(\alpha\)-D-galactopyranoside (\(\alpha\)Gal)], and Rb\(^+\) [O-methyl-\(\beta\)-D-galactopyranoside] (\(\beta\)Gal)] (Figure 1-9). A cw-OPO laser provided the necessary wavelength range (1.38 - 2.0 and 2.28 - 4.67 µm) and power (50 - 150mW) in the O-H stretching region to dissociate the attached Rb\(^+\) ion. The results from these experiments, discussed in Chapter 5, give a promising means to differentiate each monomer complex. They also provide gas-phase structural information when the observed spectra are compared with those calculated theoretically.

**Peptides**

Proteins are the principle components of muscles and organs, and play crucial roles in chemical reactions (enzymes), communication (phosphorylation, etc.), transport (albumin, hemoglobin, etc.), and structure (organelles and cell membrane) within the body. Even though proteins are very important and have been subjected to intense experimental scrutiny, complex structures and their important domains have proven difficult to ascertain. With the development
of the polymerase chain reaction (PCR) the blueprint of proteins, deoxyribonucleic acid (DNA), became readily available and led to the mapping of the genetic component of protein production, promoting the study of proteins. Methods such as electrophoresis and electrospray ionization (ESI) mass spectrometry have made it easier to separate and analyze, respectively, a protein’s components, providing a wealth of new information.

The present work contributes to this body of knowledge by demonstrating the use of IRMPD for phosphorylation elucidation in Chapter 6. To gain a better understanding of these findings, a brief introduction to the structure and nature of peptides is presented.

Amino Acids

Of the numerous amino acids that exist in nature, only twenty are considered standard to protein composition. The others are produced after protein formation or are not involved. Figure 1-10 shows the 20 standard amino acids, all of which have a carboxyl group, amine group, hydrogen atom, and a side chain (R group) bonded to the same carbon, called the alpha carbon. In solution at physiological pH (~ neutral), amino acids form zwitterions by proton transfer from the COOH to the NH₂. Side chain groups determine the amino acid’s size, structure, overall charge (solubility), and role in the protein.

With the exception of glycine, whose R group is hydrogen, α carbons are chiral centers. Similar to the monosaccharides, if the chiral group is in the same position as L-alanine’s amine (on the left side of the α carbon) then it is a L isomer; when in the opposite configuration of D-alanine, a D isomer (Figure 1-11). Proteins are made up of L isomers, a result of asymmetric enzyme active sites in peptide formation. The carboxyl carbon is labeled 1, followed by the α carbon 2, and so forth continuing with the carbons of the R group.
The twenty R groups are subcategorized into five classes according to the relative polarity and charge (Figure 1-10). Nonpolar, aliphatic groups consist of alanine (Ala, A), glycine (Gly, G), isoleucine (Ile, I), valine (Val, V), leucine (Leu, L), proline (Pro, P), and methionine (Met, M). Hydrophobic by nature, these amino acids are often found in the interior of globular proteins and stabilize structure. Relatively nonpolar, aromatic R-groups include phenylalanine (Phe, F), tryptophan (Trp, W), and tyrosine (Tyr, Y). Aromatic side chains are important to enzyme formation and strongly absorb ultraviolet light at 280 nm, thus serving as important analytical tools for researchers. Polar neutral side chains are found in asparagine (Asn, N), cysteine (Cys, C), glutamine (Gln, Q), proline (Pro, P), serine (Ser, S), and threonine (Thr, T). The S-H groups of cysteine aid in the formation (through sulfide bonds) and maintenance of the tertiary structure of proteins. Serine and threonine (and tyrosine) are potential phosphorylation sites important to protein regulation and energy transport. Lysine (Lys, K), arginine (Arg, R), and histidine (His, H) are bases (positively charged at physiological pH). These hydrophilic molecules are often found on the surface of proteins. Histidine is a key component in hemoglobin, aiding the exchange of O₂ and CO₂, and is important in enzyme reactive sites. Lastly, aspartate (Asp, D) and glutamate (Glu, E) are acidic (negatively charged at physiological pH). These serve in cell transport and have important functions in neurotransmitters.

**Peptide bond.** The simplest peptide, a dipeptide, is produced by the condensation of the carboxyl group of one amino acid with the amino group of another, creating an amide linkage. Electron resonance stabilization of the amide linkage restricts its rotation (Figure 1-12) keeping the peptides in the predominant trans form in 99% of all linkages (Figure 1-13). The reaction, however, is thermodynamically unfavorable and requires more sophisticated reactions to explain peptide formation and orientation.
**Transcription and translation.** A peptide bond is created as part of a longer chain of reactions during the process of transcription and translation. Transcription begins in the cell’s nucleus with the opening of a deoxyribonucleic acid (DNA) section, from which a complementary messenger ribonucleic acid (mRNA) strand is created (with the help of RNA Polymerase). The transcribed mRNA strand moves out of the nucleus into the cell’s cytoplasm and attaches to the ribosome exposing two groups of three cyclic bases (a codon). Meanwhile, transfer RNA (tRNA), with the complementary three base sequence (anticodon) coded to a specific amino acid, binds to the amino acid and carries it to the ribosome. The tRNA anticodon forms H bonds with complementary mRNA on the first of two exposed codons. A second tRNA is then added to the other exposed codon, bringing the two amino acids in close proximity. The amino acids then form a peptide bond (with the help of peptidyl transferase). The ribosome moves down the mRNA exposing a new set of bases, a third tRNA comes into place, and the first tRNA leaves. The process of translation continues until the stop codon ceases the production and the peptide is released. This intricate process lowers the activation energy required for peptide bond formation and fixes L isomers in the trans state so that the bulky side chains are located in the energetically favored position on opposite sides of the C-N bond.

**Polypeptides**

Polypeptides consist of oligopeptides (~2-10 residues), polypeptides (~10-100 residues), and proteins (~more than 100 residues). Their structures are written with the free carboxyl group (-COO⁻), on the far right, the C-terminus, and the free amine group (-NH₂), N-terminus, located at the far left. Among their many roles, oligopeptides act as protease inhibitors, poisons, and means of drug transport, and they are often synthesized for peptide research. Polypeptides of 10-100 residues are primarily found in hormones and cell messengers particular to ribosomal activity as well as some enzymes. Proteins or large polypeptides, however, are described
according to a structural hierarchy. Primary structure specifies the amino acid sequence and the positions of S-S linkages. Alpha helixes and β-pleated sheets shape the primary strands into more elaborate secondary structures, as shown in Figure 1-14. The α helixes are typically right-handed (counterclockwise) coil-like spirals about ~5.4 Å wide with ~3.7 residues per turn (100° per amino acid) at a distance of ~1.5 Å along the axis. Built with a single primary strand, the spiral frame is supported by hydrogen bonds between the N-H of one residue and the C=O of another placed four groups before. Multiple amino acids strands are also joined to one another by hydrogen bonds from the N-H groups of one strand to the C=O’s of another. These β-pleated sheets get their name from the characteristic antiparallel or parallel zig zag pattern of the chain’s backbone, with alternating R groups protruding in and out of the sheet. Beta sheets are found as at turns and loops in many proteins with proline, glycine, threonine and/or serine residues at each turn and twist. Functional proteins are largely created from primary and secondary arrangements connected by hydrogen and disulfide bonds and hydrophobic and electrostatic interactions. These arrangements are known as tertiary (framing globular and other protein formations) structures. In some proteins, two or more tertiary structures group together and fashion even more complex quaternary structures. Supersecondary structures (motifs, folds, etc.) are found within these constructions, revealing specific protein functions in regions known as domains. From the quaternary structure of actin/tropomyosin/myosin/collagen in muscle tissue and hemoglobin for O2 transport to ribosomes and enzymes key to protein synthesis, proteins are among the most studied molecules in nature.

Many instruments and techniques are applied to illuminate peptide structure and function. X-ray crystallographic evidence for three dimensional arrangements is the basis for the knowledge about the aforementioned structural classes. Nuclear magnetic resonance (NMR)
provides information on the molecular make up as well as solution phase dynamics including conformational changes and protein folding, among other interactions. With the advance of ionization sources, mass spectrometry is currently another emerging tool for gas-phase experiments. Electrospray ionization (ESI), for instance, gently ionizes the protein(s) leaving multiple protonation (+ ions) or deprotonation (- ions) sites, depending on the conditions set by the experiment (discussed in Chapter 2). This multiply charged ionization capability allows large proteins to be mass detected within the limited mass-to-charge (m/q) range of most mass spectrometers. Study of these ions is carried out largely through fragmentation of the ions of interest, IRMPD providing a major means for dissociation. Fragmentation peaks (along with other tools) are used to determine peptide components and the kinetics of protein reactions under the proper conditions.  

**Unimolecular Dissociation of Phosphate Groups**

Dephosphorylation kinetics and relative activation energies have been determined for positively and negatively charged phosphopeptide ions trapped in a FTICR MS in work reported in Chapter 6. Phosphorylation and dephosphorylation regulate cellular processes that are important to metabolism, growth, and reproduction. The activation energies associated with dephosphorylation can give insight into the nature of phosphopeptide interactions and provide a clearer picture of the effects of phosphorylation.

One method of determining Arrhenius activation energies uses temperature controlled blackbody radiation as an energy source. Another approach is use of a low-intensity cw-CO₂ laser (in resonance with the P-O stretch) that models thermal properties of the blackbody radiator. In this picture, absorption and emission of the low-intensity infrared photons by the phosphopeptide molecule promotes thermal equilibrium with the surroundings, creating a
Boltzmann-like distribution of energy similar to a blackbody radiator with an effective temperature.

Dissociation of the lowest energy phosphate bond in the ion occurs when the internal energy of the ion exceeds a threshold energy ($E_t$), typically associated with the high energy tail of the Boltzmann distribution. The result is loss of a water and a neutral phosphate group, producing at a minimum two peptide fragment peaks ($P_{f,w/o\ H2O}$ & $P_{f,w/o\ H2O\ &\ H3PO4}$), and a phosphopeptide ($P_T$) peak of reduced intensity. Dissociation rate constants ($k_d$) are then obtained from the slopes of $\ln([P_T]/([P_T]+[P_{f,w/o}]))$ or $\ln$[relative ion abundance] vs. time plots.

When conditions for thermal equilibrium between the phosphopeptide ions and the environment exist, the rapid exchange limit (REX) is met. An Arrhenius plot of the natural log of the rate constants vs. natural log of the laser power (watts) associated with each $k_d$, can then be constructed. The slope of the plot gives the activation energy multiplied by a constant.

Overview

The objective of the research reported in this dissertation is the use of IRMPD-FTICR MS to give a picture of isomeric gas-phase saccharide structure (with density functional theory (DFT) calculations) and differentiation, as well as unimolecular dissociation from phosphopeptides. Chapter 2 will examine FTICR MS features, such as the ion trapping cell and ion sources that play key roles in the instrument’s operation. The theoretical framework of IRMPD will be the subject of Chapter 3, which will cover the nature of vibrational modes, the general IRMPD process, and related statistical/kinetic models. Carbon dioxide and OPO lasers are covered in Chapter 4. The laser systems’ theory, repair, and set up are discussed along with their relevance to the IRMPD experiments. Chapter 5 will present experimental and DFT-calculated IR spectra of four O-methylated D-glucopyranoside ions with a unique look into the gas-phase structures and the ability to differentiate between them. Unimolecular dissociation of
phosphate groups from both negative and positive phosphopeptide ions is the subject of Chapter 6. Vibrational, kinetic, and relative activation energy information and the relationship between photon absorption and the dissociation intensities are explored. Chapter 7 summarizes the research done to date and offers a look into future considerations for the continuation of these very promising experiments.

Figure 1-1. Emil Fischer’s numbering system applied to linear and cyclic D-glucose and alpha D-glucopyranose.

D-glucose

Figure 1-2. Glyceraldehyde L and D isomers.

Glyceraldehyde
Figure 1-3. The eight stereoisomers of D-aldohexose.
Figure 1-4. The mechanism for mutarotation of the alpha and beta cyclic structures and their corresponding specific rotations.

[Chemical structures and reactions diagram]

the alpha anomer $[\alpha]_D = +112$

the beta anomer $[\alpha]_D = +18.7$
Figure 1-5. The eight stereoisomers of alpha-D-aldohexose rings.
Figure 1-6. The mechanism for O-methylation of the alpha and beta cyclic structures to prevent mutarotation is also known as glycosylation. Glycosylation is responsible for attaching two monosaccharides together to form larger molecules, where the ROH group belongs to another monosaccharide.
Figure 1-7. The chair and boat conformations of cyclic D-glucose.

Figure 1-8. Examples of disaccharides: (a) Glcβ1-2Glc, (b) Glcβ1-3Glc, (c) Glcβ1-4Glc, and (d) Glcβ1-6Glc.
Figure 1-9. Structures of the four methylglycosides examined in the work reported in Chapter 5: Rb\[O\text{-methyl-}\alpha\text{-D-glucopyranoside (}\alpha\text{Glc})], Rb\[O\text{-methyl-}\beta\text{-D-glucopyranoside (}\beta\text{Glc})], Rb\[O\text{-methyl-}\alpha\text{-D-galactopyranoside (}\alpha\text{Gal})], and Rb\[O\text{-methyl-}\beta\text{-D-galactopyranoside (}\beta\text{Gal})].
Figure 1-10. The amino acid groups separated by polarity and charge in their zwitterion forms.
Figure 1-11. Alanine L and D isomers as zwitterions.

Figure 1-12. Electron resonance stabilization of the amide linkage
Figure 1-13. The trans and cis form of the amide linkages, where 99% of all peptide linkages are in the trans configuration keeping R groups and alpha carbons on either side.

Figure 1-14. Three dimensional and general structure of α–helix and β–pleated sheets, respectively; the alpha helix illustration gives the agreed upon secondary structure as pictured in the original paper by Pauling, Cory, and Branson.27 [Reprinted with permission from the National Academy of Sciences. Pauling, L.; Corey, R.B.; Branson, H.R. 1951. The structure of proteins: Two hydrogen-bonded helical configurations of the polypeptide chain.28 Proc. N.A.S. (Volume 37, Page 207, Figure 2) and Elsevier. Nesloney, C.L.; Kelly, J.W. 1996. Progress Towards Understanding β-sheet Structure. Bioorganic & Medicinal Chemistry. (Volume 4, Page 740, Figure 1).]
During the last 50 years, ion cyclotron resonance mass spectrometry (ICR MS), and later Fourier transform ICR MS (FTICR MS), have evolved into one of the more powerful tools in mass spectrometry. Beginning with the discovery of ion cyclotron motion, the first ICR mass spectrometers were single frequency scanning instruments where slow measurements and simple ion sources (chemical and electron impact ionization) limited their potential. With the advance of computers, a fast Fourier transform algorithm was developed and put to use in mass spectrometry. Marshall and Comisarow’s application of the technology with a new cell design led to the creation of the FTICR MS. The total ion signal, taken virtually at once, provides relatively quick \( m/q \) determination of all trapped ions and has promoted real-time gas-phase experiments. Development of external ion sources like electrospray ionization (ESI), a nondestructive multiple-charge state platform, has allowed the study of very large molecules, greatly expanding the instrument’s applications. Because of these and other advances and long ion retention times FTICR MS has been very useful for the study of gas-phase dissociation processes.

History and Fundamentals

**Ion Cyclotron Radius**

Lawrence and Livingston in 1932 discovered that, as an ion moves through a uniform magnetic field, it encounters a force that propels the ion in a circular motion perpendicular (x,y plane) to the magnetic field (z axis). This inward directed force is known as the Lorentz force and is given by

\[
Force = m \frac{d\nu}{dt} = q\nu \times B
\]  

(2-1)
where \( m \) is the mass of the ion, \( \mathbf{v} \) is the velocity vector of the ion, \( q \) is the ion charge, and \( \mathbf{B} \) is the magnetic field vector. The Lorentz force acting as a centripetal force is countered by an equivalent outward directed centrifugal force \( (m \frac{d\mathbf{v}}{dt} = m \frac{\mathbf{v}^2}{r}) \), which creates a stable orbit for the ion(s) such that

\[
\frac{m\mathbf{v}_{x,y}^2}{r} = (m\omega^2 r) = q\mathbf{v}_{x,y} B_0
\]

where \( B_0 \) is the magnetic field strength along the \( z \) axis and the \( x,y \) components of \( \mathbf{v} \) are defined by \( \mathbf{v}_{x,y} = \sqrt{\mathbf{v}_x^2 + \mathbf{v}_y^2} \). Ion cyclotron motion is a circular orbit with a frequency obtained from a simple rearrangement of Equation 2-2, where the cyclotron angular frequency \( \omega = \frac{\mathbf{v}_{x,y}}{r} \) is determined by the \( m/q \) of the ion(s) and the magnetic field strength,

\[
\omega = 2\pi f_{freq} = \frac{qB_0}{m}.
\]

The implications of this result for mass spectrometry are clear. An ensemble of ions of varying \( m/q \) have different frequencies in the presence of a uniform magnetic field, making it possible to discriminate ions with equal \( m/q \) without concern as to differences in velocity or translational energy (kinetic energy focusing is unnecessary). A spectrum of these frequencies is easily converted into a mass spectrum by the implementation of Equation 2-3. Lawrence further revealed that the ion’s cyclotron motion is excited to a larger coherent radius by applying a relatively low-energy radio frequency (rf) voltage resonant with the angular frequency. Thus, the ions of interest can be discriminated by either changing the magnetic field with a static applied resonant frequency or by having a static magnetic field with changing or concurrent resonant rf.
**Ion Cyclotron Resonance Mass Spectrometer**

The first ICR mass spectrometer determined the mass of the ions by sequentially changing the magnetic field while using an applied static rf voltage to excite the ions as they came into resonance. Ions were then detected by electrometer plates placed in the magnetic field or by energy loss due to absorption by resonant frequencies.\(^{41,42}\) These operating features are found in the Omegatron and Llewelyn’s ICR spectrometers, respectively. The process of sequential scanning made the task of taking a spectrum long and tedious, and severely limited the resolution. Double resonance techniques (the ability to excite/isolate one \(m/q\) ion separately from another), however, made the ICR MS an instrument of choice for the study of gas-phase ion molecule reactions.

**Fourier Transform Ion Cyclotron Resonance Mass Spectrometer**

In 1965 with the advance of computing, Cooley and Tukey developed a fast Fourier transform algorithm.\(^{43}\) The ability to convert large quantities of data into discrete frequency components in a short period of time opened up new possibilities. In NMR, discrete signals from an ensemble of varying frequencies of nuclear moment precession could be detected and used to acquire data in fractions of the time. Marshall had seen the development of FT-NMR a number of years earlier and in the early 1970’s approached Comisarow, who had recently built an ICR mass spectrometer, with a proposition to create a Fourier transform version of the instrument. Two main obstacles stood in the way: how to excite and detect the ions.

The excitation of the ions is difficult due to the large frequency bandwidth associated with the \(m/q\) range in ICR MS. To test the project’s feasibility, an rf pulse of a narrow frequency range (typically used in FT-NMR) was applied to a makeshift cell within the bore of the ICR’s magnet. The resonant ions responded to give a detectable signal. Comisarow and Marshall reasoned that a shorter timed pulse could potentially increase the bandwidth for ICR excitation.
However, as the frequency amplitude is proportional to the area of the time of the pulse, shortening the pulse duration calls for a much higher voltage, high enough to make the project unfeasible. It had been long understood that a slow frequency sweep of around 20 minutes at rf amplitude in millivolts could create a spectrum. So, Comisarow and Marshall decided to try a 1 ms rf sweep of ~10Volts for broadband detection, and they met success.\(^{35}\)

Detection of the ions required a different vision. Ion motion had been simulated as a rotating monopole mimicking the cyclotron motion. The model predicted that by exciting the ICR motion to a sufficient radius, an image current would be produced on recording metal plates. This current is stated as

\[
I(t) = \frac{Nq^2r}{md} \sin \omega t
\]  

(2-4)

where \(I\) is the image current, \(N\) is the number of ions in the cell, \(r\) is the radius of motion, and \(d\) is the width of the ICR cell.\(^{36}\) Detection of the signal was achieved by converting the image current to voltage through a broadband RC circuit. However, the key to making all this work lay in the basic design of the cell.

**Infinity Cell**

The ICR cell takes on many shapes and sizes (Figure 2-1), but a common configuration is a cylinder in a quadrupolar linear configuration with the excitation and detection plates on the left/right and top/bottom, respectively, forming the four quarters of the cylinder and two trapping plates on the front and back (Figure 2-2).\(^{44}\) Centered inside the bore of a superconducting electromagnet, the middle of the cell is aligned with the homogeneous region of the magnetic field. The ions created by either an external or internal ion source enter through a small opening on one side of the trapping plates (along the z-coordinate) parallel to the direction of the magnetic field. As mentioned, the Lorentz force causes the ions to move perpendicular (in the
x,y plane) to the axis of the magnetic field at a velocity $u_{x,y}$ establishing a stable cyclic motion with a radius $r$. Positive ions move in the counterclockwise direction; negative ions move in a clockwise direction (Figure 2-3) according to Equation 2-1.\textsuperscript{45} In order to prohibit ejection along the z axis, a small equivalent voltage is applied to each of the trapping plates, causing the ions to oscillate harmonically within the cell’s small central region. A rf voltage applied to the excitation plates then provides the resonant electric field necessary for the ions to spiral outward to a detectable orbit, where an induced ion image current is recorded through the detection plates. This configuration allows stable ion positioning as well as discrete excitation and detection at broad bandwidths. A closer look into this operation might prove helpful in understanding the FTICR mass spectrometer.

**Oscillation and Magnetron Motion and Trapping**

When a positive (or negative) ion passes to the back of the cell it is repelled by a positive (or negative) voltage applied to the rear plate and travels in the opposite direction encountering an equal voltage now applied to the front entrance plate. Oscillating back and forth within the saddle of the electric field’s potential surface, the ion is trapped. This 3-D quadrupolar trapping potential ($\Phi$) is defined as,

$$\Phi(r,z) = V_{\text{trap}}[\gamma + \frac{\alpha}{2a^2} (2z^2 - r^2)] ,$$

where $V_{\text{trap}}$ is the trap voltage, $a$ is a measure of trap dimensions, $\gamma$ and $\alpha$ are constants based on the trap’s shape, and $r = \sqrt{x^2 + y^2}$.\textsuperscript{46}

The electric field and force components of the surface help shed light onto the oscillating and magnetron motions. By taking the negative derivative of the 3-D quadrupolar trapping potential with respect to the direction of the ion, the equation for the electric field is
\[ E(z) = -\frac{\partial \Phi}{\partial z} = -\frac{2V_{\text{trap}} \alpha}{a^2} z \]  \hspace{1cm} (2-6)

corresponding to an axial directed force of

\[ F_{\text{radial}} = qE(z) = -\frac{2qV_{\text{trap}} \alpha}{a^2} z. \]  \hspace{1cm} (2-7)

This produces an oscillating frequency on the z axis similar to simple harmonic motion and the frequency is given by,

\[ \omega_z = 2\pi v_z = \sqrt{\frac{2qV_{\text{trap}} \alpha}{ma^2}}. \]  \hspace{1cm} (2-8)

Isolated, the axial force moves the ions in harmonic motion along the z axis but has no net effect on their x-y motion. Radial forces are another matter. The radial force \( F_{\text{radial}} = qE(r) \) component acts in the x,y plane and is calculated as

\[ F_{\text{radial}} = qE(r) = -q \frac{\partial \Phi}{\partial z} = \frac{qV_{\text{trap}} \alpha}{a^2} r. \]  \hspace{1cm} (2-9)

The outward radial force operates against the Lorentz force and adds another wrinkle to the ions’ motion and a term to Equation 2-2, such that

\[ m\omega^2 r = q \nu_{x,y} B_0 - \frac{qV_{\text{trap}} \alpha}{a^2} r. \]  \hspace{1cm} (2-10)

Simplification and rearrangement yield the quadratic relation,

\[ \omega^2 - \frac{qB_0}{m} \omega + \frac{qV_{\text{trap}} \alpha}{ma^2} = 0. \]  \hspace{1cm} (2-11)

Equation 2-11 indicates that \( \omega \) is independent of the radius, meaning that the ion’s position has no effect on these complex motions.\(^{47}\) The cyclotron motion is redefined by a solution to Equation 2-11, which gives two equations of motion: the reduced cyclotron (\( \omega_c \)) and magnetron (\( \omega_m \)) frequencies. These are
\[ \omega_+ = \frac{\omega_c}{2} + \sqrt{\left(\frac{\omega_c}{2}\right)^2 - \frac{\omega_z^2}{2}} \]  

and

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

respectively, where \( \omega_c \) is the unperturbed cyclotron frequency and \( \omega_z \) is the oscillation frequency. The trapping oscillation, magnetron, and cyclotron rotations are illustrated in Figure 2-4. Cyclotron motion interacting with the trapping fields results in the additional magnetron motion. Both of these motions move the ion in the x, y plane as they simultaneously travel back and forth in the z direction in the trap’s potential well. Detection is hardly affected by the magnetron and oscillating trapping movements due to their insignificant role in defining the orbital radius after compensation for the reduced cyclotron motion. Only when the cell is misaligned and/or the radius of the ions approaches the limits of the trap are these effects even noticed.

**Cyclotron Motion and Excitation**

The moment the ions enter the trap, the phase of their cyclotron motion is incoherent. Detection is impossible, as any induced charge on one plate is canceled by the 180° out-of-phase signal of another ion on the opposite plate. The radius is also too small for any notable detection. A simple rearrangement of Equation 2-2 gives the simplified (not taking into account magnetron motion, etc.) radius as,

\[ r = \frac{mv_{x,y}}{qB_0} \]  

Even considering the molecule’s equilibrium thermal energy,
\[ \frac{mv_{x,y}^2}{2} = k_B T \tag{2-15} \]

with the radius redefined as,

\[ r = \frac{1}{q B_0} \sqrt{2k_B T m} \tag{2-16} \]

in which \( r \) is the ion’s cyclotron radius, \( k_B \) is the Boltzmann constant, and \( T \) is the temperature, excitation is necessary to bring all but the largest \( m/q \) ions into larger orbits.

This is accomplished by applying an oscillating electric field of sufficient amplitude between the excitation plates perpendicular to \( B_0 \). Ions in resonance with the electric field absorb energy causing the ions to spiral outward in the plane. The off-axis movement of the spiraling motion also brings the ions into spatial coherence, as shown in Figure 2-5.\textsuperscript{50} The final orbit radius is determined by the amplitude of the frequency.

Instantaneous amplitude is given as

\[ A(t) = q^2 \frac{E_{0t}^2 t_{\text{excite}}}{4m} \tag{2-17} \]

By integrating over time the absorbed energy is

\[ E_{\text{abs}} = \int_0^t A(t) dt = q^2 \frac{E_{0t}^2 t_{\text{excite}}^2}{8m} \tag{2-18} \]

As the absorbed energy of the ion is equal to its kinetic energy, setting terms in Equation 2-2 equal to Equation 2-18 and rearranging for \( r \) gives

\[ \frac{mv^2}{2} = \frac{m \omega^2 r^2}{2} = \frac{q^2 E_{0t}^2 t_{\text{excite}}^2}{8m} \rightarrow r = \frac{E_{0t} t_{\text{excite}}}{2B_0} \tag{2-19} \]

Approximating the electric field as produced by the voltage placed on two infinitely extended plates,
\[ E_0 = \frac{V_{p-p}}{d} \]  \hspace{1cm} (2-20)

with the radius is redefined as
\[ r = \frac{V_{p-p} t_{\text{excite}}}{2dB_0} \approx \frac{\beta_{\text{dipolar}} V_{p-p} t_{\text{excite}}}{2dB_0} \]  \hspace{1cm} (2-21)

where \( A(t) \) is the amplitude, \( E_0 \) is the electric field, \( t_{\text{excite}} \) is the excitation period, \( E_{\text{abs}} \) is the absorbed energy, \( \beta_{\text{dipolar}} \) is the scaling factor for finite trap, and \( V_{p-p} \) is the peak to peak voltage, a detectable ion radius is reached with the appropriate excitation period and voltage.\(^{45,52}\) This post-excite radius is independent of the ion’s mass. Thus, all masses of the same charge generate an equivalent image current and resulting signal. In other words, as long as an equivalent voltage and pulse duration are applied, the mass spectrum of similarly charged species should reflect the relative abundances of these ions. Increasing the excitation energy much further can cause ion dissociation (here absorbed resonant frequency energy (translational energy) is converted into internal energy through collisions with background gas resulting in ion fragmentation, i.e., sustained off-resonance irradiation collisional induced dissociation, SORI–CID), ion-molecule reactions, or ion ejection from the trap \[ \left( r \geq \frac{d}{2} \right) . \] \(^{44}\)

As previously mentioned, a \( t_{\text{excite}} \) pulse can excite ions at broader bandwidths than its specified frequency (\( \omega_0 \)) according to the relation,
\[ \frac{1}{t_{\text{excite}}} \geq 2\pi | \omega - \omega_0 | \]  \hspace{1cm} (2-22)

However, increasing the bandwidth by decreasing the excitation time requires an increase in the plate voltages to maintain the same amplitude and radius (Equations 2-4 through 2-7). Using this method, the ICR bandwidth (kHz to MHz) necessitates a pulse so short that the compensating
high voltage produces other intrusions. In order to minimize these problems, a modulated radio frequency (chirp) is applied to the plates. This covers the entire bandwidth while maintaining the amplitude at minimal voltages. In equation 2-21 $t_{\text{excite}}$ converts to $\frac{1}{\sqrt{\text{Sweep rate}}}$ set at the desired frequency range.

Though idealized, these equations are very similar to the conditions in the Bruker Infinity cell.\(^{44,51}\) As mentioned, the cell is placed where the magnetic field is most homogeneous with only small perturbations affecting $B_0$, and to the small ion the cell excitation plates appear as infinitely long. The applied electric field is also engineered to provide as consistent a field as possible, not withstanding trapping voltage interference. Even so, any necessary corrections are connected by a constant multiplied factor easily applied to these equations. For cylindrical traps this factor is $\beta_{\text{dipolar}} = 0.8018$.\(^{52}\)

**Detection**

Once the ions are excited and achieve a detectable coherent orbit, an image current is produced by a change in the image charge at both plates such that

$$I_s = \frac{\partial Q}{\partial t} = \frac{\partial}{\partial t} \left( -\frac{Nqr \cos \omega t}{d} \right) = \frac{Nqr \omega}{d} \sin \omega t .$$  \hspace{1cm} (2-23)

The alternating current is then converted into voltage through an RC circuit where the solution to the RC circuit differential equation,

$$I_s = \frac{V_s(t)}{R} + C \frac{\partial V_s}{\partial t} = 0$$  \hspace{1cm} (2-24)

gives

$$V_s(t) = \frac{Nqr \omega}{d} \sqrt{\frac{1}{R^2 + \omega^2 C^2}} \sin(\omega t + \phi) .$$  \hspace{1cm} (2-25)
In Equations 2-24 and 2-25, the resistance in the circuit is denoted by $R$, the capacitance by $C$, the instantaneous ICR voltage by $V_s$, and the phase difference between $I_c$ and $I_R$ by $\phi$. The ion signal reflects not only the relative number of diverse $m/q$ ions in the trap, but also the multiple of each ion’s revolutions in the cell according to the superposition principle. This multichannel advantage allows the FTICR MS to gather $N$ data points simultaneously in $\frac{1}{N}$ amount of time of the older scanning ICR methods.\textsuperscript{44}

Signal-to-noise and resolution are improved as well. The signal-to-noise ratio is is described by the relationship $\frac{V_s}{V_n}$. The noise in the voltage ($V_n$) is understood to be the product of the noise in the current (cell) and the impedance in the RC circuit such that

$$V_n(rms) = \sqrt{\frac{4k_B T_R \Delta f \frac{1}{R}}{\frac{1}{R^2} + \omega_c^2 C^2}}$$

where the temperature of the resistor is $T_R$ and $\Delta f$ is the detection bandwidth. By taking Equation 2-26 and dividing into $V_s(rms) = \frac{N q \omega R}{\sqrt{2d} \sqrt{\frac{1}{R^2} + \omega^2 C^2}}$ from Equation 2-25, the S/N is

$$\frac{S}{N} = \frac{V_s}{V_n} = \frac{N q \omega R^{1/2}}{\sqrt{2d} \sqrt{4k_B T_R \Delta f R}}.$$  \hspace{1cm} (2-27)

Superposition multiplies the effect of $N$ ions many times over causing the signal to increase over the noise.\textsuperscript{53,54} Signal is also improved by an increase the ion’s radius, charge, and frequency (smaller $m/q$), as noted before, and the resistance between the detection plates (Ohm’s law $IR=V$). Resolution is defined as the full width at half the height of the peak (FWHM) $\Delta m_{1/2}$ and
the resolving power given by $\frac{m}{\Delta m_{1/2}}$. In terms of the corresponding angular frequency, the derivative of $\omega_c$ with respect to mass yields,

$$\frac{\partial \omega_c}{\partial m} = \frac{\partial}{\partial m} \frac{qB_0}{m} = -\frac{qB_0}{m^2} = -\frac{\omega_c}{m} \quad (2-28)$$

where

$$\frac{m}{\Delta m_{1/2}} = -\frac{\omega}{\Delta \omega_{1/2}} = -\frac{B_0}{\frac{m}{q} \Delta \omega_{1/2}} \quad (2-29)$$

The increased number of data points generates better frequency discrimination, which decreases $\Delta \omega_{1/2}$ and improves the mass resolving power and resolution. Resolving power also increases inversely with $m/q$ and is enhanced by the magnetic field strength.  

The signal is recorded in the time domain as the free induction decay (FID) or transient response. This signal is often damped and broadened over time due to ion collisional interactions associated with cell pressure conditions as well as radial ejection from the trap (Figure 2-6). But the remaining signal is converted through the use of a fast Fourier transform algorithm into a corresponding spectral plot of the amplitude vs. frequency. Amplitude is equivalent to the number of ions of the same $m/q$ and the frequencies are directly related to their corresponding mass to charge. Prior to FT, the FID is apodized to correct broadened profiles of the peak bases. The cell is the heart of the mass spectrometer; however, the heart cannot beat without ions.

**Ion Sources**

Ion sources for the FTICR MS create gas-phase ions for detection. This is accomplished with a number of devices that are categorized in two groups: internal and external. When the molecules are introduced directly into the trap by a leak valve or probe, internal ion sources, such
as electron impact, chemical ionization, and photo ionization, are often employed to ionize neutral species. External ionization sources like matrix assisted laser desorption ionization (MALDI) and electrospray ionization (ESI) form ions outside cell. The generated ions are injected through regions of differential pumping, where a series of ion optics and/or quadru/hexapole ion guides steer the charged molecules to the lower pressure region of the cell.

**Internal Ion Sources**

Volatile molecules are introduced by means of inserting a container into a sealed port from which a leak valve allows the neutrals to enter. Nonvolatile components are often inserted on heated probes to be vaporized. In both cases, the internal ion source interacts with the molecule in the cell to produce ions. Electron impact (EI), one of the earliest ionization methods, uses a high energy electron beam (~70eV) centered on the molecules to remove a valence electron (positive charge) or a low energy electron beam that results in the capture of an electron (negative charge).\(^{55-57}\) Multiple ion fragmentation often occurs due to the excess energy absorbed by the molecule and can promote chemical ionization of uncharged species and ion molecule reactions. Chemical ionization (CI) is a softer technique, which ionizes the analyte through charge, proton, or larger group transfer from a reagent gas in excess (methane, ammonia, etc.) or via another radical/ionized molecule.\(^{58-60}\) Photons (visible light, ultraviolet lasers, etc.) with sufficient energy can cause the ejection of an electron in the process known as photoionization.\(^{61,62,63}\) A type of photoelectric effect, the probability of photoionization depends primarily on the threshold energy and the cross section of the interaction.\(^{64}\)

**External Ion Sources**

The two most widely used external ion sources today are MALDI and ESI. Matrix assisted laser desorption ionization (MALDI) is a technique that marries the features of fast atom bombardment (FAB) and laser desorption ionization (LDI). Like FAB, the analyte is supported
in a matrix. This design enhances the transfer of energy from a laser beam aimed at the matrix surface (LDI) to the analyte, thus promoting desorption and ionization. Electrospray ionization (ESI) applies electrochemical and thermospray principles. A solution (sometimes acid or base modified) is passed through a charged capillary/needle (the working electrode) and exits as a nebulized spray (thermospray) into an electric field between the needle and a plate mounted on the opposite side (counter electrode). In the electric field, the spray breaks down into very small droplets. Some of these remaining droplets (and already ionized components) then pass through a heated metal capillary (and/or heated drying gas) to emerge as gaseous ions.

**Matrix-assisted laser desorption/ionization.** Three crucial areas are necessary for an understanding of Matrix-assisted laser desorption/ionization (MALDI): the makeup/preparation of the matrix, the function of the laser, and their interaction. As previously mentioned, matrices are designed to facilitate desorption and ionization. The substrate is an analyte-specific nonvolatile chromophore that absorbs the laser’s light to readily energize the matrix. Gentisic acid (2,5-dihydroxybenzoic acid, DHB), which is mixed with other components to form “super DHB,” and 2,6-dihydroxyacetophenone (DHAP) are two common matrix substrates for saccharides and peptides, respectively. These ingredients are mixed with the analyte in experimentally determined ratios (typically ~ 500 to 5000:1, substrate:analyte) and are often spiked with Na⁺, K⁺, Ag⁺, Cu⁺ or other monovalent cations to optimize and control ionization characteristics. Relatively volatile solvents like acetone are used to dissolve the matrix, which is then spread out evenly in target spots on polished semiconducting plates. Small, even-layered crystals, ideal for light absorption and matrix interactions, are produced through gentle heating of the plates.
The plate is then mounted on a stage and a laser is focused on it to a 100 - 200µm spot size (Figure 2-7). Pulsed at 3-10ns, the UV Nitrogen (UV MALDI) and, at 6-200ns, the IR YAG (IR MALDI) lasers are common and provide the energy absorbed by the sample. This energy is distributed from substrate to analyte causing desorption from the surface. Ionization occurs either before and/or after the molecules’ removal from the surface. Internal ionization is promoted through analyte derivatives, matrix interactions (energy resonance transfer, metal additions, etc.), and changes in pH. Post-laser gas-phase photoionization, electron capture, and charge exchange are additional means of external charging. Power attenuated, the laser is tuned to supply an optimal amount of energy for desorption and ionization above the threshold laser irradiance of \( \sim 10^6 \frac{W}{cm^2} \). Each pulse is repeated until enough ions are collected for a clean, low S/N spectrum.

By raising the laser irradiance, examining the fragmentation yields, and piecing together the fragment puzzle, MALDI has been be used for structural characterization of \( N \)-linked glycan (Man)\(_6\)(GlcNAc)\(_2\) in DHB among other saccharide complexes. Matrix assisted laser desorption ionization, though useful, does present problems, including preparation of the matrix, laser alignment and the inclination to produce only single-charge states. These are among the reasons why ESI has gained a more prominent role as an external ion source.

**Electrospray ionization.** Electrospray ionization (ESI) occurs in three stages: the creation of charged ion droplets, the reduction of the droplets’ sizes and the production of desolvated ions. A pump-assisted syringe, or liquid chromatography (LC) instrument, delivers a solution from the transfer line to the high-voltage capillary. At the capillary ±3-5 kV is applied (working
electrode) creating an electric field between the capillary tip and a parallel plate (counter electrode) placed 0.5 to 2 cm away. At a strength of \( \sim 10^6 \frac{V}{m} \), the electric field is given by

\[
E_e = \frac{2V_c}{r_c \ln \frac{4d}{r_c}}
\]

(2-30)

where \( V_c \) is the applied potential, \( r_c \) is the outer radius of the capillary, and \( d \) is the distance between the working and counter electrodes.\(^{76}\) In the positive ion mode, the capillary voltage is positive; the anions move toward the inner surface of the capillary surface as cations continue to the tip. The voltage and electrophoretic movement are opposite for negative ions. Cations at the needle’s end interact with the electric field and draw the solution from the surface to form a Taylor cone (Figure 2-8), where further oxidation occurs (reduction for negative ions).\(^{77}\) Once the tip of the cone builds up enough charge density, the force of the electric field overcomes the opposing solution surface tension and a charged droplet leaves in the direction of the electric field. Consistent spray comes from the controlled rate of charged droplet formation. This in turn is a product of proper solution chemistry, reasonable solution flow rates, and the appropriate voltage to oxidize/ionize the electroactive species. The rate is best characterized by the electrospray current given as

\[
i_{ESI} = Hv_f \sigma_s E_c \, ,
\]

(2-31)

where the surface tension and dielectric constant are given a value \( H \), the solution’s flow rate is \( v_f \) and specific conductivity is \( \sigma_s \).\(^{77,78}\) As the droplet travels in the field, the solvent begins to evaporate. The electrostatic repulsions grow until they almost equal the force of the droplet’s surface tension or the Raleigh limit, specified as

\[
q_R = 8\pi \sqrt{e_o \gamma R^3} \, ,
\]

(2-32)
where the excess charge is $q_R$, $\varepsilon_0$ is the vacuum permittivity, $\gamma$ is the surface tension, and $R$ is the radius.\textsuperscript{79} Coulomb fission then occurs, resulting in two droplets. According to droplet jet fission, this happens at the elongated end of the comet shaped droplet as it slowly breaks down the volume into smaller components (Figure 2-9).\textsuperscript{77} A heated gas and/or metal capillary helps the process, although two competing processes explain the final ion construction. The charge residue model (CRM) states that the desolvation continues until the solvent disappears.\textsuperscript{80,81} The ion evaporation model (IEM) maintains that the electric field provides enough energy for the ion to escape the confines of the droplet.\textsuperscript{82,83} Many of these ions hit the opposing plate (counter electrode) and are reduced (or oxidized if they are negative ions) completing the electrochemical circuit maintaining the current. However, a significant number enter the mass spectrometer to be analyzed in the ICR cell.

By adjusting the applied voltages, the pH (protonating or deprotonating agent), the solvent polarity, and/or adding a cation(s) or anion(s), the charge state of the analyte can vary greatly. This helps place large $m/q$ ions in the mass spectrometer’s frame of reference and allows for interesting multiple-charge-state experiments. For example, electrospray ionization has been used to study molecules the size of myoglobin, a mass of 16,951 Daltons, with charge states of +8 to +23, to give a complete, although at times overwrought, spectrum.\textsuperscript{84} The addition of microspray, varying gas assisted systems, and other designs further helps in spray efficiency and control.

**Ion Accumulation and Ion Optics**

Often beginning at atmospheric pressure, ions from an external ion sources are subject to various stages of pumping as they are guided through each region to enter the cell. Ions exiting the heated metal capillary pass through a set of skimmers that help concentrate them by removing excess neutrals. These ions, all with different kinetic energies, are collected in a
hexapole where they are held by a trapping voltage for 500 to 2000ms. This allows the ions to assume uniform energy and to accumulate, maximizing ion concentration. Afterwards the ions are ejected from the hexapole trap as a group toward the cell. Ions are guided to the analyzer trapping cell in the Bruker FTICR mass spectrometer through cylindrically shaped electrostatic plates or einzel lenses. As seen in Figure 2-10, the ion optics designated PL-1, FOCL1, PL9, and FOCL2 are hollow cylinders whose primary purpose is to keep the electrostatically repulsive ions focused by application of like potential. The split cylinders DPL2, DPL4, XDFL, and YDFL, steer the ions into place through differentially controlled potentials with HVO. Octapole ion guides serve the same purpose in other FTICR MS instruments. The principles of IRMPD in an FTICR MS cell are explored in Chapter 3.

Figure 2-1. Ion trap configurations are: (a) cubic, (b) cylindrical, (c) cylindrical w/segmented endcaps, d&e-opened ended with change in capacitance coupling, f-dual, and g-matrix where excitation is abbreviated E, detection D, and trapping T. [Reprinted with permission from John Wiley & Sons, Ltd. Marshall, A.G.; Hendrickson, C.L.; Jackson, G.S. 1998. Fourier Transform Ion Cyclotron Resonance Mass Spectrometry: A Primer. Mass Spectrom. Rev. (Volume 17, Page 12, Figure 12).]
Figure 2-2. A quadrupolar cylindrical trap consisting of excitation and detection plates.

Figure 2-3. The cyclotron motion of positive and negative ions. [Reprinted with permission from Elsevier. Marshall, A.G.; Hendrickson, C.L. 2002. Fourier transform ion cyclotron resonance detection: principles and experimental configurations. Int. J. Mass Spectrom. (Volume 215, Page 60, Figure 1).]
Figure 2-4. The pictures define the outside ion orbit where the ion cyclotron motion $v_c$ is coupled with the magnetron motion $v_m$ providing the variations in the motion as the ions oscillate $v_T$ between the two trapping plates. [Reprinted with permission from John Wiley & Sons, Ltd. Marshall, A.G.; Hendrickson, C.L.; Jackson, G.S. 1998. Fourier Transform Ion Cyclotron Resonance Mass Spectrometry: A Primer. Mass Spectrom. Rev. (Volume 17, Page 14, Figure 13).]

Figure 2-5. Ion cyclotron incoherent motion (left center) is excited coherently exciting the ions out to a higher radius (left) with the application of a resonant rf electric field. The final radius (right) coupled with coherent motion leads to detection through an image current induced on the detection plates, which is then converted to voltage through an RC circuit. [Reprinted with permission from John Wiley & Sons, Ltd. Comisarow, M.B.; Marshall, A.G. 1996. The Early Development of Fourier Transform Ion Cyclotron Resonance (FTICR) Spectroscopy. J. Mass Spectrom. (Volume 31, Page 583, Figure 1).]
Figure 2-6. Complete picture of the FTICR signal production, from detection, free induction decay (FID) of the time domain signal, Fourier transform of the signal (frequency spectrum not pictured), to m/q spectrum. [Reprinted with permission from the author. Hixson, K. K. 2000. FTICR Theory. Environmental Molecular Sciences Laboratory. http://www.emsl.pnl.gov/docs/msd/mass_spec/home/fticrtut.html, Volume 2008 (July 2009).]

Figure 2-7. The UV MALDI source depicting the power attenuated laser source, plate/matrix sample and plasma. [Reprinted with permission from Elsevier. Mamyrin, B.A. 1994. Laser assisted reflection time-of-flight mass spectrometry. Int. J. Mass Spectrom. (Volume 131, Page 5, Figure 3).]
Figure 2-8. The picture of electrospray as an electrochemical cell with the Taylor cone formed due to the competing cohesive forces of the droplet and the applied electric field. When charged droplets come close to the Raleigh limit they are separated from the Taylor cone and begin to break down further as they move toward the counter-electrode and mass spectrometer entrance. [Reprinted with permission from the American Chemical Society. Kebarle, P.; Tang, L. 1993. From ions in solution to ions in the gas phase—the mechanism of electrospray mass spectrometry. Anal. Chem. (Volume 63, Page 974A, Figure 1).]

Figure 2-9. The droplet jet fusion model depicts the comet-shaped droplet as more likely to undergo coulomb fission at the tail end. [Reprinted with permission from the American Chemical Society. Kebarle, P.; Tang, L. 1993. From ions in solution to ions in the gas phase—the mechanism of electrospray mass spectrometry. Anal. Chem. (Volume 63, Page 977A, Figure 3).]
Figure 2-10. Focusing and guiding ion lenses in the Bruker FTICR mass spectrometer. [Reprinted with permission from Contreras, C.S. 2008. Carbohydrates and Amino Acids: Infrared Multiple Photon Dissociation Spectroscopy and Density Functional Theory Calculations. Ph.D. dissertation (Page 66, Figure 2-8) University of Florida, Gainesville, Florida.]
CHAPTER 3
INFRARED MULTIPLE PHOTON DISSOCIATION THEORY

Fifty years ago the scientific community believed infrared radiation from a blackbody or light source was incapable of causing molecular dissociation. The vibrational picture had been that of isolated diatomic bonds oscillating with minimal interactions within a larger molecular framework. From this perspective, it follows that even though a photon in resonance with a bond’s vibrational frequency is initially absorbed, as the excited molecule climbs up the anharmonic vibrational energy ladder, it moves out of resonance and energy is no longer transferred. Under this paradigm, the only way a bond would break is through the application of consecutive frequencies resonant with the anharmonic vibrational levels until critical bond energy is reached, causing dissociation. This proved impossible for the instruments of the day. However, with the development of high-power IR light sources, such as the CO₂ laser, infrared radiation at a fixed frequency proved sufficient to fragment molecules. The “anharmonic bottleneck” problem proved surmountable, which led to the theory of multiple photon dissociation. In order to understand these processes, the theories of vibrational motion, light-matter interactions, and IRMPD spectroscopy/quasi BIRD will be reviewed.

**Vibrational Motion**

Molecular motion is expressed by three interacting components: translation, rotation, and vibration. Translation describes the molecule’s movement through space. Rotation depicts motion around the molecule’s central axes. Vibration concerns changes in the positions of atoms within the molecule. Vibrational movement builds from diatomic motion, to linear combinations of diatomic vibrations (modes), to an interconnected collection of modes (that describes most polyatomic molecules). In this section, each of these concepts is considered as well as their harmonic and anharmonic behavior.
**Diatomic Vibrational Motion**

Diatomic molecular vibrations are the building blocks for the complex motions of bonds. Starting with the application of the classic harmonic picture to quantum and anharmonicity treatments, an understanding of intramolecular molecular motion is possible.

**Classical mechanics picture.** Molecular vibrational motion is often pictured as two atoms attached by a spring that oscillates harmonically from an equilibrium position. This classical viewpoint gives the diatomic oscillation frequency \( v_{freq} \) as

\[
v_{freq} = \frac{1}{2\pi} \sqrt{\frac{k_f}{\mu}}
\]  

(3-1)

where \( k_f \) is the bond force constant (a measurement of the bond strength), and \( \mu \) is the reduced mass of two atoms: one with the mass \( m_1 \), the other with mass \( m_2 \) such that

\[
\mu = \frac{m_1 m_2}{m_1 + m_2}.
\]  

(3-2)

The energy of a bond’s harmonic motion is broken down into potential and kinetic energy parts. Potential energy reaches a maximum at the two extreme ranges of motion. Kinetic energy reaches its maximum at the equilibrium point. In between, the energies are characterized by a potential loss and kinetic gain of energies as the bond moves from the pure potential energy to kinetic energy point and vice versa. Continuation of this action is due to the elastic restoring force between the two atoms. The restoring force \( (F_{restore}) \) is equal to the product of the spring’s force constant \( k_f \) and the displacement \( (R) \) from equilibrium \( (R_e) \) such that

\[
F_{restore} = -k_f x
\]  

(3-3)

where \( x = R - R_e \). The vibrational (harmonic) potential energy \( (U) \) is given by

\[
\int dU = \int F_{restore} dx \rightarrow U = \frac{1}{2}kx^2
\]  

(3-4)
and describes the motion of a parabola, which is the characteristic movement of a harmonic oscillator (Figure 3-1). To better describe this picture, quantum considerations are necessary.

**Quantum considerations.** A solution to the quantum picture begins with the time-dependent Schrödinger equation,

\[ i\hbar \frac{\partial \psi(e,N,t)}{\partial t} = H \psi(e,N,t) . \]  

(3-5)

In Equation 3-5, \( \psi \) is the wavefunction described by the electronic \( e \) and nuclear coordinates \( N \), time is \( t \), and \( H \) is the Hamiltonian. The Hamiltonian is described by potential and kinetic terms such that

\[ H= K_{\text{Tot}} + U_{\text{tot}} = K_e + K_N + U_{ee} + U_{NN} + U_{Ne} . \]  

(3-6)

The total potential energy \( U_{\text{tot}} \) consists of energy contributions from the repulsions between the electrons \( (U_{ee}) \), the nuclei \( (U_{NN}) \), and the attraction between nuclei and electrons \( (U_{Ne}) \). Kinetic energy \( K_{\text{Tot}} \) contributions are the motions of the electrons \( (K_e) \) and nuclei \( (K_N) \).

If the potential energy is considered independent of time, Equation 3-5 is simplified by the separation of the time and spatial variables to give

\[ \psi(N,e,t) = \psi(N,e)\varphi(t) . \]  

(3-7)

The general form of the time-independent Schrödinger equation becomes

\[ H\psi(N,e) = E\psi(N,e) \]  

(3-8)

where the operator \( H \) acts on the wave function \( \psi(N,e) \) to give the eigenvalue \( E \) and the original function \( \psi(N,e) \).

Further simplification is found through the Born-Oppenheimer approximation, which states that the large, slower-moving nuclei are considered fixed with respect to the fast moving electrons, in order to separate and solve the electronic and nuclear components separately. The first part of the solution involves the use of the electron Hamiltonian \( (H_e) \).
\( H_e = K_e + U_{ee} + U_{Ne} \). (3-9)

Solving the electronic time-independent Schrödinger equation produces the electronic potential \( E_e \) for the arrangement of the positioned nuclei. The solutions for a number of possible nuclei arrangements help define a potential energy surface whose minimum identifies the molecule’s ground state equilibrium conformation. The effective potential is then incorporated into nuclear Hamiltonian

\[
H_N = K_N + U_{NN}
\]

(3-10)

to give the nuclear component of the time-independent Schrödinger equation

\[
(H_N + E_e(N)) \psi(N) = E_N \psi(N).
\]

(3-11)

The solutions describe the translational, rotational, and vibrational molecular motions.

In terms of the simple harmonic oscillator, the time-dependent Schrödinger equation is given as

\[
i \hbar \frac{d\psi}{dt} = -\frac{\hbar}{2\mu} \frac{d^2\psi}{dx^2} + \frac{1}{2} k_f x^2 \psi
\]

(3-12)

where \( \hbar = \frac{\hbar}{2\pi} \) and \( \hbar \) is Planck’s constant. Separating the time \((\varphi)\) and spatial \((\psi)\) components results in

\[
-\frac{\hbar}{2\mu} \frac{d^2\psi}{dx^2} + \frac{1}{2} k_f x^2 \psi \varphi = i\hbar \psi \frac{d\varphi}{dt}.
\]

(3-13)

Dividing Equation 3-13 by \( \psi \varphi \) gives

\[
-\frac{\hbar}{2\mu} \frac{1}{\psi} \frac{d^2\psi}{dx^2} + \frac{1}{2} k_f x^2 = i\hbar \frac{1}{\varphi} \frac{d\varphi}{dt}
\]

(3-14)

and the time and spatial terms are isolated. For a time-independent function, the right side of Equation 3-14 is constant, meaning the left side is as well. Furthermore, as the left side of the
equation is an expression of the Hamiltonian operator with the dimensions of kinetic and potential energy, it follows that their product, the eigenvalue, is the total energy such that

\[- \frac{\hbar}{2\mu} \frac{d^2\psi}{dx^2} + \frac{1}{2} k_j x^2 \psi = E \psi \quad (3-15)\]

and

\[i\hbar \frac{d\varphi}{dt} = E \varphi . \quad (3-16)\]

Solving the differential Equation 3-16 gives the time-dependent wavefunction as

\[\varphi = e^{-\frac{iEt}{\hbar}} \quad (3-17)\]

and a solution to differential Equation 3-15 gives the energy values

\[E_{vib} = \hbar \omega \left( \nu_n + \frac{1}{2} \right) \quad \nu_n = 0,1,2,3,4,... \quad (3-18)\]

Equation 3-18 indicates that the energy values are quantized (\(\nu_n\)) and evenly spaced along the harmonic vibrational ladder (Figure 3-1). The ladder begins with the lowest possible energy or the zero point energy \(E_{vib} = \frac{1}{2} \hbar \omega\) and moves from this position to the higher energy levels, whose difference is defined by \(\Delta E_{vib} = E_{n+1} - E_n = \hbar \omega = \hbar \nu \quad \text{88,89}\)

**Anharmonicity.** The harmonic picture adequately describes the behavior near the bottom of the potential energy well. However, as the vibrational motion is excited and the system moves up the energy ladder, the restoring force and the displacement are no longer proportional. The result is that the displacement from equilibrium becomes greater, and energy levels become more closely spaced, redefining the harmonic potential energy curve as anharmonic (Figure 3-1). At longer internuclear distances (i.e., high vibrational levels), the restoring force eventually becomes zero and the bond dissociates with a dissociation energy defined by the potential energy...
from the ground state. A means of describing the anharmonic potential energy curve is through the Morse potential,

\[ U = \hbar c D_e (1 - e^{-a\nu})^2 \]  

(3-19)

where \( D_e \) marks the curve minimum, \( c \) is the speed of light, and \( a \) is the potential energy curve width, defined by

\[ a = \sqrt{\frac{\mu \omega_v^2}{2\hbar c D_e}}. \]  

(3-20)

The potential energy function can be placed into Equation 3-8, the time-independent Schrödinger equation, and solved to produce an equation for vibrational energy corrected by an anharmonic factor such that,

\[ E_{\nu} = \hbar c G_{\nu} = \left( \nu_n + \frac{1}{2} \right) \hbar \omega_v - \left( \nu_n + \frac{1}{2} \right)^2 \hbar \omega_v \chi_e. \]  

(3-21)

In Equation 3-21, the anharmoncity constant \( \chi_e \) is given as

\[ \chi_e = \frac{a^2 \hbar}{2 \mu \omega_v} = \frac{\omega_v}{8\pi D_e} = \frac{\nu_v}{4D_e}. \]  

(3-22)

Equations 3-21 and 3-22 illustrate that the anharmonic correction is subtracted from the harmonic term such that as \( \nu_n \) gets larger (overall energy increase), \( \Delta E_{\nu} \) becomes smaller, decreasing the gap between each successive vibrational energy level. Although the Morse potential is an approximation (additional corrective terms to Equation 3-21 are required when a better fit is necessary), it does provide a fairly good match for simpler systems. It predicts the maximum vibrational level for dissociation to be

\[ \nu_{n,\text{max}} < \frac{\hbar c D_e}{\frac{1}{2} \hbar \omega_v} - \frac{1}{2} \]  

(3-23)
and the dissociation energy \(D_0\) as

\[
D_0 = D_e - \frac{1}{2} \frac{\hbar \omega_0 (1 - \chi_e)}{\hbar c} = D_e - \frac{E_0}{\hbar c}
\]  

(3-24)

where \(E_0\) is the zero point energy.\(^{90,91}\)

**Polyatomic Vibrations**

Diatomic molecules have only one stretching motion. Larger molecules contain multiple vibrational motions with bond lengths and angles that change with respect to other vibrations. To represent each of these movements and their interactions precisely requires complicated kinetic and potential energy treatments. In order to simplify the problem, linear combinations \(Q_v\) of vibrational movements are employed. For example, the linear combinations for carbon dioxide’s two C-O stretches (Figure 3-2) are

\[
Q_1 = \frac{1}{\sqrt{2m}} (m_c^{1/2} q_1 - 2m_o^{1/2} q_2 + m_c^{1/2} q_3) \quad \kappa_1 = \frac{k_f m_c}{m_c m_o}
\]  

(3-27)

and

\[
Q_2 = \frac{1}{\sqrt{2}} (q_1 - q_3) \quad \kappa_2 = \frac{k_f}{m_n}
\]  

(3-26)

where \(m_c\) or \(m_o\) is the mass of the carbon or oxygen, \(m\) is the total mass (=\(m_c + 2m_o\)), \(q_n\) are the weighted coordinates, \(q_n = \sqrt{m_n x_n}\) (\(x_n\) is the displacement of atom \(n\)), \(n\) is equal to 1 (oxygen atom), 2 (carbon atom), and 3 (oxygen atom), and \(\kappa\) is the effective force constant. The dynamics of the antisymmetric \((Q_1)\) and symmetric \((Q_2)\) stretches (Figure 3-2) are seen as the sum and difference of mutual and opposing directions of the C-O stretching motions, respectively. A new effective force constant corrects for the dynamic combinations of these motions. Linear molecules like CO\(_2\) have 3N-5 (N is equal to the number of atoms, i.e., 3(3)-5=4) degrees of freedom (DOF) and nonlinear molecules have 3N-6 normal vibrational DOF, each corresponding
to a linear combination of vibrational motions. Normal coordinates for the translational $Q_T$ and rotational $Q_R$ motions are subtracted from the total (3N) DOF. Linear and nonlinear molecules both have 3 translational DOF and their rotational DOF are 2 and 3, totaling 5 and 6 nonvibrational DOF, respectively. Besides the symmetric and asymmetric stretches, other molecular normal vibrational modes include rocking, wagging, twisting, and scissoring motions or varying combinations of the six movements.

The Hamiltonian for the harmonic normal modes is represented as a sum of terms,

$$H = \sum_i -\frac{1}{2} \hbar^2 \frac{\partial^2}{\partial Q_i^2} + \frac{1}{2} \kappa_i Q_i^2 .$$

(3-27)

The molecule’s vibrational wavefunction is a product of each mode’s wavefunctions,

$$\psi = \prod_i \psi_{\nu_i} (Q_i) ,$$

giving the Schrödinger equation

$$\frac{1}{2} \hbar^2 \frac{\partial^2 \psi(Q_i)}{\partial Q_i^2} + \frac{1}{2} \kappa_i Q_i^2 \psi(Q_i) = E \psi(Q_i) .$$

(3-28)

The solution of Equation 3-28 gives total harmonic vibrational energy of the molecule as

$$E_{\nu_i} = \sum_i (\nu_i + \frac{1}{2}) \hbar \omega_i .$$

(3-29)

Similar to the diatomic picture, an anharmonic correction to the Schrödinger equation can be performed

$$-\sum_i \left( \nu_i + \frac{1}{2} \right)^2 \hbar \omega_i \chi + \ldots .$$

However, further corrections are more involved, because they account for complications, such as electrical anharmonicity from the interplay of dipole moments contributing to the corrective terms.$^{88,92,93}$

**Interaction of Light and Matter**

Electromagnetic (e/m) resonance occurs when the oscillating electromagnetic (e/m) radiation of light of a given frequency comes into contact with a vibrational mode that has the
same frequency as the oscillating e/m radiation. The mode’s interaction with the oscillating e/m radiation results from a change in its electric dipole moment ($\mu$) induced and/or amplified by the vibration. Once the light energy is absorbed, this energy results in a higher vibrational energy level. Normal modes that have the ability to absorb energy are infrared active. Other modes that do not affect a change in dipole moment are infrared inactive.

**Diatomic Electric Dipole Moment**

In a one-dimensional oscillator, the electric dipole moment depends on the positions of the electrons and nuclei. An approximate relationship considers the dipole moment as a product of the two charges $\pm dq$ and their displacement from the equilibrium position as, $R = R_e + x$, such that

$$\mu = Rdq - R_e dq + xdq = \mu_o + xdq$$

(3-30)

where $\mu_o$ is the electric dipole moment at the equilibrium distance. From Equation 3-30 it follows that,

$$\langle \nu_x | \mu | \nu_i \rangle = \mu_o \langle \nu_x | \nu_i \rangle + dq \langle \nu_x | x | \nu_i \rangle ,$$

(3-31)

where $\langle \nu_x | \mu | \nu_i \rangle = \int \psi_{\nu_x} \mu \psi_{\nu_i} d\tau$ is the expression for the transition dipole moment. The equilibrium dipole moment $\mu_o \langle \nu_x | \nu_i \rangle$ cancels, because $\nu_x, \nu_i$ are orthogonal ($\nu_x \neq \nu_i$ then $\int = 0$) when the nuclei are at equilibrium. The addition of the relationship $dq = \frac{du}{dx}$ allows Equation 3-31 to be rewritten as

$$\langle \nu_x | \mu | \nu_i \rangle = \frac{du}{dx} \langle \nu_x | x | \nu_i \rangle .$$

(3-32)

Equation 3-32 illustrates that, if there is no change in the dipole with a change in displacement $\left( \frac{du}{dx} = 0 \right)$, then the transition dipole moment is zero. Following from $\langle \nu_x | x | \nu_i \rangle$, utilizing the
appropriate Hermite polynomial wavefunctions, the selection rules for harmonic vibrational transitions are $\Delta \nu = \pm 1$. An expansion of Equation 3-32 leads to an expression more appropriate for the anharmonic case,

$$\langle \nu_f | \mu | \nu_i \rangle = \left( \frac{d\mu}{dx} \right) \langle \nu_f | x | \nu_i \rangle + \frac{1}{2} \left( \frac{d^2\mu}{dx^2} \right) \langle \nu_f | x^2 | \nu_i \rangle + \ldots$$

(3-33)

As the vibrational energy is increased, the displacement $x$ becomes greater and is no longer proportional to the dipole moment. When this occurs the second term in Equation 3-33 becomes more important and the associated electrical anharmonicities allow transitions of $\Delta \nu = \pm 2$ and so on.\textsuperscript{90-94} These transitions contribute to the first overtones or second harmonics in vibrational spectra.

**Polyatomic Electric Dipole Moment**

In a similar fashion, the normal modes depend upon the displacement of charge over the linear combinations of vibrational modes such that,

$$\mu = \sum_i \left( \frac{\partial \mu}{\partial Q_i} \right) Q_i + \ldots$$

(3-34)

The transition dipole moment for each normal mode is

$$\langle 00...\nu_f ...0 | \mu | 00...\nu_j ...0 \rangle = \left( \frac{d\mu}{dQ_j} \right) \langle \nu_f | Q_j | \nu_i \rangle$$

(3-35)

In terms of the CO\textsubscript{2} molecule’s normal modes $Q_1$ and $Q_2$, the changes in $x$ along the normal coordinate (the direction of motion) are active if they will produce a change in $\mu$. For $Q_1$, the asymmetric stretch, opposing and attracting motions causes a net change in the charge distribution, which in turn indicates a change in the dipole moment ($dx dq = d\mu$) of the molecule. The symmetric stretch $Q_2$, however, does not produce a net change in charge distribution and, therefore, is inactive.\textsuperscript{88,92-94}
Similar to the diatomic case, anharmonicities are also taken into account by additional terms in Equation 3-35. Along with overtones and harmonics, combination bands ($\nu_i \pm \nu_j$) and Fermi resonances have to be taken into consideration. Combination bands arise due to the mixing of similar normal modes and produce peaks of lower intensity than fundamental transitions ($\nu_i$ to $\nu_j$). The exceptions are Fermi resonances that occur due to interactions between two combination normal modes (i.e., fundamental and an overtone) of the same symmetry and similar energies. This interaction results in the “mixing” of some of the fundamental’s intensity into the overtone, producing overtone peaks with higher intensity than the fundamental and complicating the assignment of peaks for vibrational spectra.\textsuperscript{92,94}

**Infrared Photodissocation Spectroscopy**

Absorption spectroscopy measures the energy of electromagnetic radiation absorbed or transmitted by a sample. Electromagnetic radiation in resonance with active vibrational modes of strong/weak transition moments is absorbed strongly/weakly resulting in an absorbance ($A$) of

$$A = \ln \frac{I_o}{I} = \varepsilon [C] L_{cell}$$

(3-36)

where $I_o$ is the incident light intensity, $I$ is the transmitted light intensity, $\varepsilon$ is the molar absorptivity (molecular extinction coefficient), $[C]$ is the concentration, and $L_{cell}$ is the e/m radiations path length through the sample. Equation 3-36 is commonly known as the Beer-Lambert law. A vibrational spectrum consists of peaks (absorbance) or valleys (%T) corresponding to the vibrations’ resonant modes, with intensities varying according to the strength of the change in dipole moment.

Absorption spectroscopy requires a minimum concentration of $10^{10}$ molecules per cm$^3$ for proper detection. In the cell of the FTICR mass spectrometer, ion concentrations are
approximately $10^6$ ions per cm$^3$ (due in part to space charge effects, size of the trap, etc.) producing ($[\sim 10^6 \text{ ions per cm}^3]$, $\varepsilon \sim 1 \times 10^4 \text{ M}^{-1}\text{cm}^1$, $L_{\text{cell}} \sim 12$ cm) an absorbance of $2 \times 10^{-10}$, a value far below the limits of detection. Under these conditions, probing the vibrational modes requires a different approach. By monitoring the dissociation products at varying infrared wavelengths, vibrational spectra are acquired, although the intensities are now products of light-dipole interactions and the complex IRMPD mechanisms.

**Multiple Photon Dissociation**

As mentioned previously, in the early years of spectroscopy it was thought that all resonant vibrational absorption occurred as part of a stepwise coherent mechanism. Under this scheme, a vibrational mode in resonance with an IR laser of a given frequency undergoes excitation but as the excited molecule climbs the vibrational energy ladder, the frequency of the IR laser becomes out of resonance and energy can no longer be absorbed (Figure 3-3). This process is still true for some molecules (with high dissociation energies and limited DOF), but it no longer applies to the extent once thought.

Multiple photon dissociation is based on the generally incoherent mechanism that takes place in three stages, characterized by vibrationally coherent excitation, internal vibrational redistribution into the quasicontinuum, and photodissociation, as seen in Figure 3-4. At lower vibrational levels, the relatively harmonic behavior and rotational-vibrational coupling allow for the resonant transfer of energy from the IR laser to the molecule at the fundamental frequency. As the excited molecule climbs the ladder, internal vibrational redistribution (IVR) of the energy occurs through anharmonic coupling of the vibrational mode with a number of background modes that in turn distribute the energy to the other states, etc. This process continues with an increasing number of interacting states which shifts and broadens resonant absorption levels until a quasicontinuum of vibrational states is reached. In the quasicontinuum,
photons of all IR wavelengths are able to be absorbed as the mutually interacting high density of states makes the molecule resonant. During this time, the IR laser, still focused at the fundamental vibrational mode, is continually producing photons that are being absorbed and redistributed until the molecule reaches a critical energy for the dissociation of the weakest bond. This is physically allowed because the time frame for IVR (~ ps) is much shorter than the time required for absorption of a photon at the fundamental, and the energy builds up at a faster rate than stimulated or collisionally induced emissions (~ms) are able to counter.

At room temperature, ions in equilibrium with their environment have multiple populations of differing energy, governed by their Boltzmann thermal distribution. Thus, the energy absorbed by the ions $E_{\text{abs}}$ ($\approx$ product of the laser’s fluence and the ion’s absorption cross section) does not have the same effect on every ion in the distribution. Add this to the coupling of the vibrational-rotational modes and the increasing anharmonicity of excited ions, and the spectrum becomes broader and shifts to the red (i.e., to lower energies). This effect is decreased at lower fluences, which tend to perturb the system less and also reduce complicating issues, such as Rabi broadening.*

Because the efficiency of the multiple photon dissociation depends on the closely spaced (high density) states, larger molecules are generally better candidates for this process. In fact, at room temperature some large molecules are already in the quasicontinuum and dissociate readily. Conversely, small molecules with low density of vibrational states are more prone to stop absorbing laser energy at the anharmonic bottleneck. However, with higher laser fluence, even some of these reluctant molecules are capable of IRMPD.

*The interaction of a vibrational level with high energy laser e/m field causes oscillation in the fixed vibrational motion, bringing other frequencies into resonance.
**Action Spectroscopy**

In order to obtain an action spectrum in FTICR MS instruments, the laser beam is first aligned with the ion cloud in the ICR cell. The ion signal is optimized, isolated, and the laser beam triggered to coincide with the ion’s retention time. Mass spectra, at a series of consecutive wavelengths (at given powers and set times), are acquired and any IRMPD products are noted. The action spectrum is then created from a plot of the natural log of the power-corrected relative abundances of the total ion population divided by the relative abundance of the parent ion population versus wavelength. Positions of the spectral peaks are primarily a result of resonant laser/vibrational mode interactions for a given wavelength. Peak intensities are based on ion dissociation, which depends on the ion’s initial internal energy, the interplay of the resonant frequencies and the laser fluence with the vibrational transition dipole moment, and the IRMPD process.

Among the first to implement these techniques in Fourier transform ion cyclotron resonance mass spectrometry were Dunbar, Eyler, and Beauchamp.\textsuperscript{106-110} Of particular interest was the use of a two-laser CO\textsubscript{2} system, which allowed Watson and Eyler to overcome the lack of power (causing low or lack of ion dissociation) in the tunable laser, by following the tunable laser with a high-powered pulsed laser. The first laser excites on-resonance vibrational modes to quasicontinuum states. Now resonant with all infrared frequencies within the continuum, the ions absorb photons from the fixed off-resonance pulsed laser and dissociate.\textsuperscript{111} The spectra from these experiments are a bit broader than spectra obtained without the assistance of a second laser but illustrate clearly defined peaks.

**Multiple fragmentations.** It is not uncommon for an ion to have a number of competing channels for dissociation, with multiple fragments created by similar dissociation energy profiles. However, most multiple fragmentation pathways are the result of excess internal energy
carried over from IRMPD. Here, new low energy pathways are made available with each fragment, often creating a congested spectrum, especially at high laser fluencies. Products from larger molecules are excellent candidates for these processes. Multiple fragments can still be utilized to form an action spectrum, although sensitivity and peak resolution suffer due to the uncertainty in the final ion populations and the complicated energy picture.

**Messenger technique.** In order to correct for the above, lower laser fluences are used to probe the resonant vibrational frequencies of weakly bound ligand-molecule complexes in what is known as the messenger technique. Designed to lower the number of photons required to dissociate the ligand without perturbing the vibrations of the molecule, the messenger ligand increases the density of states to favor dissociation. The spectra also produce very sharp features, a result of increased sensitivity (a one- or two-photon dissociation process) due to the relatively low coupling of the van der Waals forces in the ligand attachment.\(^{95,96}\) Of interest in these processes are the kinetic and energetic pictures for ion dissociation, which are specific to the phosphopeptide dissociation experiments discussed later in Chapter 6.

**Unimolecular Dissociation Kinetics**

In the ICR cell, when the ions’ absorption of IR photons (from a low-intensity CO\(_2\) laser energy source) is equivalent to the stimulated and spontaneous emission, a thermal equilibrium with the surroundings is reached. This creates a Boltzmann-like distribution of energy with an effective temperature. Dissociation of an ion’s lowest energy bond occurs when the ion’s energy exceeds a threshold energy (\(E_t\)) typically associated with the high energy tail of the Boltzmann distribution. The result is loss of a neutral, producing a daughter (D) peak (parent – neutral) and a parent (P) peak of reduced intensity. The effect is carried out over a wide range of wavelengths to produce action spectra. Alternatively, if the laser is kept at a given wavelength and power while the irradiation times are varied, the outcome is used to obtain dissociation rate constants
(k_d) from the slopes of ln{[P]/([P]+[D])} or ln[Relative Ion Abundance] versus time plots. Activation energies E_a are then determined by the slope of the line from a modified Arrhenius plot of the natural log of the rate constant (k_d) versus the natural log of the power associated with each k_d, such that

$$\frac{d \ln k_d}{d \ln P} C = E_a .$$  \hspace{1cm} (3-38)

The constant (C) represents the relationship between laser power and temperature and is determined using two different approaches, which will now be discussed.

**Laser Intensity and Temperature**

A CO_2 laser of sufficient fluence with an IR wavelength in resonance with an absorbing vibrational mode can imitate a blackbody radiator with an effective temperature. However, determining the exact relationship between the laser’s intensity or power and the complementary temperature of a blackbody radiator is difficult. In order to accomplish this, two different models have been proposed independently by Robert Dunbar and by Kolja Paech and Evan Williams.

**The Dunbar model.** In the early 1990s, Dunbar suggested that an FTICR mass spectrometer, with long, relatively collision-free retention times, provides an excellent means to study slow kinetic information that evolves from ions irradiated in the cell. \(^{112-114}\) Carbon dioxide lasers, with wavelengths in the congested vibrational region, served as Dunbar’s laser source of choice, as many molecules are already in resonance. Within this setup, an IR laser can be viewed as a thermal source leading to a thermal analysis (i.e., Arrhenius) of the affected systems, provided that certain conditions are met. The continuous wave (cw) laser power must be kept relatively low and serve as the principal energy source in the thermal-equilibrium-like processes (equivalent photon absorption and emission), with the kinetics governed accordingly. And the Einstein coefficients define the system, where spontaneous relaxation is more important than the
corresponding collisional processes. \textsuperscript{113} Dunbar derived Equation 3-38 by approximating the relationship of the laser’s intensity and the blackbody temperature through Planck’s equation,

\[
I(h\nu) = I_o(h\nu) \left[ \exp\left( \frac{h\nu}{k_BT} \right) - 1 \right]^{-1} \tag{3-39}
\]

where \(I(h\nu)\) is the intensity of the laser (power) at a given frequency, \(I_o\) is the absolute light intensity (constant set by the data), and \(T\) is the effective temperature. The derivative of the natural log of Equation 3-39 with respect to \(\frac{1}{k_BT}\) results in the relation,

\[
\frac{d \ln I}{d \frac{1}{k_BT}} = -h\nu \frac{1}{\exp\left( \frac{h\nu}{k_BT} \right) - 1} = -qh\nu . \tag{3-40}
\]

In comparison, the derivative of the natural log of the Arrhenius equation with respect to \(\frac{1}{k_BT}\) gives

\[
\frac{d \ln k_d}{d \frac{1}{k_BT}} = \frac{d \ln \left[ Ae^{\frac{-E_a}{k_BT}} \right]}{d \frac{1}{k_BT}} = \frac{d \ln A - \frac{E_a}{k_BT}}{d \frac{1}{k_BT}} = -E_a . \tag{3-41}
\]

Solving Equations 3-40 and 3-41 for \(\frac{d \frac{1}{k_BT}}{d \ln P}\) and equating these terms (with Intensity=Power) provides the activation energy as

\[
\frac{d \ln k_d}{d \ln P} qh\nu = E_a \tag{3-42}
\]

where \(q\) is estimated by the partition function at frequency \(\nu\) and is often a value between 1.00-1.10 for temperature ranges associated with these laser dissociation experiments (often taken as
the average, 1.05). The constant expressed in Equation 3-38 is simply a multiple of $g$, $h$, and $v$ (i.e., at a wavelength of 943 cm$^{-1}$, the constant is 8.14). Dunbar expressed two potential issues with this treatment. The first involved the interaction of strong radiative intensities near but not on resonance with the CO$_2$ laser. Dunbar dismissed this issue as, at most, a scalar multiplier to blackbody results. Thus, as long as equilibrium conditions are met, the activation energy is not affected. The second problem involved the possibility of extensive unaccounted-for IR radiative (spontaneous) emissions from vibrational intensities away from the laser’s frequency. The higher the number of nondegenerate vibrational DOF, the greater the possibility of misrepresenting the distant modes and the number of emitted photons, resulting in low activation energies. A second treatment by Paech and Williams compensates for these emissions by using a steady state approximation, and they obtained a new constant that applies to peptides and proteins.

**Paech and Williams.** The Paech and Williams model$^{33}$ begins with the assumption that the system is in thermal equilibrium ($k_i \approx k_{ji}$). Here, the dissociation rate ($k_d$) is small enough to assume that a relative steady state exists, according to the equilibrium condition, $k_i \approx k_{ji} \gg k_d$ (the rapid exchange limit, REX), such that

$$
\sum_v \beta(v) \rho(v) P(v) h v = \sum_v \left( A(v) + \beta(v) \right) \rho(v) P''(v) h v
$$

(3-43)

where $\beta$ and $A$ are the Einstein coefficients, $\rho(v)$ is the energy density, and $P(v)$ is the vibrational level occupation probability. The Einstein $\beta$ and $A$ coefficients are given by

$$
\beta = \frac{c^3 A}{8 \pi \hbar v^3}
$$

(3-44)

and

$$
A = 2.88 \times 10^{-9} v^2 I_{abs}
$$

(3-45)
where \( I_{\text{abs}} \) is the absorption intensity. The left side of Equation 3-43 is related to stimulated absorption (\( \beta \)) and the right side is related to both stimulated (\( \beta \)) and spontaneous (\( A \)) emission, as they govern the movement of energy up and down multiple vibrational energy levels according to their respective probabilities, \( P(v,T) \) and \( P''(v,T) \). Further, the occupation probabilities of an ion population with a Boltzmann distribution at an effective temperature \( T \) are characterized by

\[
P(v,T) = \sum_{n=0}^{\infty} e^{-\frac{nhv}{k_B T}} \left( 1 - e^{-\frac{nhv}{k_B T}} \right) (n+1) \tag{3-46}
\]

and

\[
P''(v,T) = \sum_{n=0}^{\infty} e^{-\frac{nhv}{k_B T}} \left( 1 - e^{-\frac{nhv}{k_B T}} \right) n \tag{3-47}
\]

In Equations 3-46 and 3-47, \( n + 1 \) and \( n \) are quantum numbers that represent squares of the creation and annihilation operators. These terms correspond to the higher probability of absorption and emission at higher vibrational occupation levels. Setting Equation 3-43 to zero gives

\[
0 = \sum_{v'} [vA(v)P''(v,T) + v\beta(v)\rho(v)(P''(v,T) - P(v,T))] . \tag{3-48}
\]

From Equations 3-46 and 3-47, \([ P(v,T) - P''(v,T) ] = 1 \). Using this simplification and removing the resonant laser frequency from the summation gives

\[
0 = v'A(v_{\text{laser}})P(v_{\text{laser}}) - v'\beta(v_{\text{laser}})\rho(v_{\text{laser}}) + \sum_{v'} [v'A(v')P''(v',T) - v'\beta(v')\rho(v')]. \tag{3-49}
\]

Equation 3-49 is then rearranged to isolate \( \rho(v_{\text{laser}}) \) (= \( I_{\text{laser}} \) measured as the laser’s power)

\[
\rho_{\text{rel.}}(v_{\text{laser}}) = \frac{1}{v'\beta(v_{\text{laser}})} \left( \sum_{v} [v'\beta(v')\rho(v') - v'A(v')P''(v',T)] - v'A(v_{\text{laser}})P''(v_{\text{laser}},T) \right). \tag{3-50}
\]

Equation 3-50 was then computationally evaluated for four peptides (leucine enkephalin,
gramicidin S, bradykinin, and melittin) at different temperatures, giving their related laser intensities. In this procedure, the frequencies and transition dipole moments for each peptide were computed (AM 1) and the dipole moments (x1.8) were corrected. The corresponding wavelengths and the calculated transition probabilities, within a 100cm⁻¹ region, were added and averaged and the model based on Equation 3-50 executed. A plot of the natural log of the laser powers versus reciprocal of the temperatures was taken and the relationship established through the slope(s) such that,

\[
\frac{d \ln \rho(v_{\text{laser}})}{d \frac{1}{T}} = \frac{d \ln I(v_{\text{laser}})}{d \frac{1}{T}} = \frac{d \ln P(v_{\text{laser}})}{d \frac{1}{T}} = \text{slope} = s .
\] (3-51)

Figure 3-5 illustrates that the slopes of all four peptides are very similar, deviating less than 9%, over a wide range of temperatures and laser powers. Equation 3-51 is an expression of the relative power density (i.e., relative laser power). The relative power density removes the scalar multipliers, i.e., \( \frac{1}{v' \beta(v_{\text{laser}})} \), from Equation 3-50, as seen in the derivative of the natural log of the laser power (= Equation 3-50) with respect to \( \frac{1}{T} \) such that,

\[
\frac{d \ln \rho_{\text{rel.}}(v_{\text{laser}})}{d \frac{1}{T}} = \frac{d}{d \frac{1}{T}} \ln \left( \sum_v \left[ v' \beta(v') \rho(v') - v' A(v') P^v(v', T) \right] - v' A(v_{\text{laser}}) P^v(v_{\text{laser}}, T) \right) .
\] (3-52)

Paech et al. believe this relationship makes the proportionality constant \( s \) applicable to many different peptides, above the REX limit. For instance, decreasing/increasing the peptide’s size, though decreasing/increasing the degrees of freedom, does not significantly alter the relative intensities of absorption (peptides ~biological polymers), and since scalar multipliers are not a factor, a similar vibrational profile should be observed. Similarly, uncertainty between the
measured and actual laser power the peptides experience has no appreciable effect on the slope (s). Thus, the proportionality factor $s$ should be in close agreement with other similar peptides, if not peptides as a whole (within the REX), and applicable to a range of power conditions. Solving Equations 3-41 (except $\frac{d \ln k_d}{d \frac{1}{T}} = \frac{E_a}{k_B}$) and 3-51 for $d \frac{1}{T}$ and equating these terms, the value for the Arrhenius activation energy is

$$\frac{d \ln k_d}{d \rho(v_{laser})} k_B s = \frac{d \ln k_d}{d P(v_{laser})} k_B s = 4.34E_a.$$ \hspace{1cm} (3-53)

Paech et al. compared Arrhenius activation energies from IRMPD simulated blackbody radiation utilizing Dunbar’s and Paech and Williams’ constants with actual blackbody data (i.e., blackbody infrared radiative dissociation (BIRD) experiments, in which the ICR cell is physically heated to a given temperature, producing black body photons for ion dissociation). The results are presented in Table 3-1. Activation energies, found through IRMPD experiments utilizing Paech and Williams’ treatment and the BIRD experiments are in general agreement for all four peptides (at different charge states) with one exception (the authors were unsure of the reason, and cite another identical experiment that illustrates agreement). Not counting the exception, Dunbar’s constant underestimated the activation energies by about ~40%. Paech and Williams effectively account for spontaneous emission as part of the radiative equilibrium of peptide molecules at the REX. However, Dunbar’s model is relevant for smaller molecules with lower density of states producing far fewer modes that spontaneously emit (i.e., stimulated emission $>>$ spontaneous emission). By examining key differences between the molecules’ dissociation kinetics, DOF, and transition states, molecular categories are developed that aid the experimenter in determining the best course of action.
Molecular Categories

Boltzmann distributions differ according to the size of the molecules (density of states) and the energy of those states. Not surprisingly, the molecular dynamics and DOF greatly affect the nature of the distribution. In general, the smaller the molecule, the more likely that the distribution is severely truncated. However, with molecules of increasing size, the distribution begins to appear normal until an equilibrium state is reached. As a result, the assigned categories are somewhat fluid, as shown in Figure 3-6 for hydrocarbons, and should be approached carefully. To better understand the differences in the distributions, three molecular categories are described in terms of their kinetics, degrees of freedom (DOF), transition states, and the models used to correct any deficiencies.\textsuperscript{31,32,115}

**Small molecule kinetics.** The overall kinetic picture of unimolecular dissociation is given by,

\[ P \xrightarrow{k_1} P \xrightarrow{k_{uni}} D_{(p-frag)} + P_{depleted} \quad (3-54) \]

where \( k_1 \), \( k_{uni} \), and \( k_{uni} (=k_d) \) are the governing rate constants of absorption, emission, and dissociation, respectively. Each category is related directly to the kinetic picture in Equation 3-54 and its effect on the Boltzmann distribution. In the small molecule category, thermal equilibrium is not maintained as \( k_{uni} \gg k_{uni} \).\textsuperscript{116-119} Molecules of this size (no greater than 100 DOF) do not have the ability to reach a steady state with their surroundings, due to the low and/or degenerate density of states. Thus, the energy builds up quickly and the lowest energy bond dissociates at a faster rate than relaxation. If the transition state is entropically favored or loose (i.e., direct bond cleavage reaction), dissociation is further promoted. The transition state is measured by the Arrhenius coefficient, where the entropy of activation (\( \Delta S^+ \)) is given by a simple rearrangement of
\[ A^\infty = \left( \frac{e^{k_T}}{h} \right) e^{\frac{\Delta S^\infty}{R}}. \]  

(3-55)

In the case mentioned, \( A^\infty \approx 10^{14.5} \) and greater are common.\(^{120}\) If these assumptions are reasonably true, the Boltzmann distribution is considered truncated and Equation 3-38 yields low activation energies, which are corrected by the modified Tolman approach.\(^{113}\)

Tolman’s theory states that the activation energy is equal to the average energy of the molecules that are reacting minus the average energy of the nonreacting reactant molecules.\(^{121,122}\) Dunbar’s new approach rearranged Tolman’s equation in terms of the threshold energy, adding a few corrective terms to give

\[ E_t = E_a + \langle E' \rangle - (\Delta E_{\text{rad}} + \Delta E_{\text{depl}}) \]  

(3-54)

where \( E_t \) is the threshold energy (molecules that absorbed energy at or above the \( E_t \) limit are removed), \( E' \) is the average energy of the population remaining (calculated from a Boltzmann distribution), \( \Delta E_{\text{rad}} \) is a correction factor for the radiation field’s temperature dependence, and \( \Delta E_{\text{depl}} \) is the depletion of reactant molecules within the high energy population, given by \( E_t h \nu_{\text{avg}} \).

The corrective terms \((\Delta E_{\text{rad}} + \Delta E_{\text{depl}})\) partially cancel each other and can be represented by 300 cm\(^{-1}\) or just around 0.9 Kcal/mole.\(^{123}\) The Arrhenius activation energy is taken from the plot based on Equation 3-38. Average energy, \( E' \), is found by considering an estimated value for \( E_t \) and using it to truncate the calculated Boltzmann distribution of energies (Figure 3-7), and \( E' \) is obtained from the remaining energies. The distribution is computed from the molecule’s vibrational frequencies (through computational means) and the effective temperature of the laser pulse through Planck’s equation. Then, these values are applied to the Boltzmann equation, given by
\[
P(E)dE = \frac{W(E)e^{\frac{-E}{kT}}dE}{\int W(\varepsilon)e^{\frac{-\varepsilon}{kT}}d\varepsilon} \tag{3-55}
\]

where \( P \) represents the probability that the molecule has internal energy \( E \), and \( W(E) \) is the density of states found through the direct count method of Beyer-Swinehart. The average energy of the truncated Boltzmann distribution is then determined and the value of \( E_t \) is calculated using Equation 3-54 and compared to the estimated value. Then \( E_t \) is approximated again and \( E' \) is calculated until the estimated and calculated \( E_t \) energies match. The value for \( E_t \) thus obtained is taken to be the true dissociation energy.

**Medium-sized molecule kinetics.** The medium category finds the Boltzmann distribution as reduced, though not truncated, as the rate of dissociation is competitive with the rate of the spontaneous and stimulated emission of photons, \( k_{uni} \approx k_{ij} \).\textsuperscript{124-126} In this case, the density of states (~intermediate DOF) is greater than the value for an average small molecule, but it is not large enough to result in relative steady state equilibrium. Ions are removed at a fast enough rate that the Boltzmann distribution is perturbed (Figure 3-8). If the transition state is relatively tight with \( A^\infty \approx 10^{12.4} \), where simple but entropically unfavorable dissociation reactions occur (e.g., the McLafferty rearrangement), the rates of reaction begin to disfavor dissociation.\textsuperscript{120} This can move some of these molecules into the large molecule kinetic limit. Assuming this is not the case, master equation modeling is necessary to account for the distribution’s defects.

Master equation modeling separates the overall molecular kinetics into two categories, the rate of dissociation \( k_d N_i(0) \) and the rate of internal vibrational transitions \( \sum k_{i,j} N_i \), such that

\[
dN_i(t) = [k_d N_i(0) + \sum k_{i,j} N_i(0)]dt \tag{3-56}
\]
where \( N \) represents the initial population fraction (Boltzmann distribution) that corresponds to the energy level \( i \) for \( N_i \), \( j \) for \( N_J \), and \( dN \) is the change in the time dependent population.\(^{124,127}\)

The rate of dissociation is modeled by Rice, Ramsperger, Kassel, Marcus (RRKM) theory illustrated by

\[
 k(E) = \frac{\sigma N^\tau (E - E_0)}{h\rho(E)}
\]

where \( \sigma N^\rho (E-E_0) \) is the sum of states, \( h\rho(E) \) is the density of states, and \( E_0 \) is the RRKM threshold energy. Similar to the Boltzmann calculation, the density of states can be considered through a direct counting method with the frequency values taken from computational calculations.\(^{128}\) The value of \( E_0 \) can be treated by the sudden death approach, where any population above \( E_0 \) is considered to be dissociated. This is confirmed when dissociation above \( E_0 \) accelerates rapidly, thereby establishing the dissociation point.

The state transition rate constant \( (k_{i,j}) \) measures the transfer of the ion population into and out of the energy levels and is equal to the rates of absorption \( (k_{abs}) \) and emission \( (k_{emis}) \) of the IR radiation. The value for \( k_{abs} \) is

\[
 Rate \ absorption(i \rightarrow j) = \sum_{E(i,j)=h\nu(m)} \rho(h\nu)\beta P_m^n.
\]

In Equation 3-58, \( P_m^n \) is the probability of energy occupation for the \( n^{th} \) vibrational level in the \( m^{th} \) mode. This probability is statistically similar to that given by Equation 3-55.

The emission rate constant is

\[
 Rate \ emission(j \rightarrow i) = \sum_{E(i,j)=h\nu(m)} A P_m^{n+1}.
\]

Summation terms in Equation 3-58 and 3-59 consider all the modes of all the states with the condition that the \( i \) and \( j \) energy level difference be equal to the mode frequency. To clarify
the dissociation, absorption and emission rates are added for each population within a 100 cm\(^{-1}\) frequency range and summed across all ranges. Using the frequency and intensities from computational calculations and substituting these values into the master equation, at a given effective temperature \(T\), dissociation of the ensemble is simulated at different times. Rate constants are then determined for the given temperature and the process is repeated for different temperatures to later form a theoretical Arrhenius plot.

The master equation is then adjusted to better mimic the behavior of the experimental line. This is done by altering the variables of the master equation until a fit is found. Parameters include: threshold energy \(E_t\), radiation absorption \((I_{\text{abs}})\), transition dipoles \((\mu)\), and specific transition frequencies, the most critical being \(E_0\) from RRKM theory. The value for \(E_0\) that satisfies the experiment is taken as the true activation energy. Accordingly, the Arrhenius constant is modified through transition dipole moment scaling factors and by altering the transition frequencies associated with the entropy of activation, until the intercepts are equivalent. Master equation modeling and RRKM theory are used for all categories including the large molecular category discussed in the next section, which details one statistical aspect of the IRMPD theory.

**Large molecule kinetics.** Kinetics in the large molecule category are governed by the principle \(k_{-1} \gg > k_{\text{uni}} \text{ or } k_f > > k_{d}.\) A large density of states (~high DOF) allows for the distribution of energy throughout many vibrational modes through intramolecular vibrational relaxation (IVR). The molecules accumulate energy more slowly and dissociate only at the high energy tail of the distribution, thus maintaining an equilibrium state with the environment. If the transition state is very tight \((A^\infty \approx 10^{9.9})\), intricate reaction mechanism(s) create conditions that are very entropically unfavorable, further deterring dissociation while promoting equilibrium.\(^{120}\)
Distribution of the Boltzmann energy is in a relatively steady state and the Arrhenius energies are reasonable in the condition otherwise known as the rapid exchange limits (REX). However, because the relationship between the temperature and the laser is modeled, the activation energy is considered relative.

The type of laser involved in the above processes is of some importance. Ideally, infrared lasers should be precise, coherent, durable instruments, capable of high enough fluence to promote dissociation and the appropriate wavelengths to get the job done. As two types of lasers were used in the research reported in this thesis, an OPO laser for the action spectroscopy studies utilizing the messenger technique (with CO$_2$ laser reinforcement for the CH stretches) and a CO$_2$ laser for the kinetics analysis, a review of their operations is presented in Chapter 4.

Figure 3-2. Representations of the four vibrational modes of CO$_2$ molecule: asymmetric stretch, the doubly degenerate bending modes, and the symmetric stretch.

Figure 3-3. The coherent stepwise absorption results in the discontinuation of absorption of once resonant photons, due to the anharmonic bottleneck effect.
Figure 3-4. The three stages of multiple photon dissociation. [Reprinted with permission from Los Alamos Science, Los Alamos National Laboratory. Lyman, J.L.; Galbraith, H.W.; Ackerhalt, J.R. 1982. Multiple Photon Excitation. Los Alamos Science. (Volume 3, Page 78, Figure 11).].
Figure 3-5. The plot of the relative intensity vs. the reciprocal of the temperature through the use of steady state approximations for four peptides; leucine enkephalin (triangles), gramicidin s (squares), bradykinin (diamonds) and melittin (circles). [Reprinted with permission from the American Chemical Society. Paech, K.; Jockusch, R.A.; Williams, E. R. 2002. Slow Infrared Laser Dissociation of Molecules in the Rapid Energy Exchange Limit. J. Phys. Chem. A. (Volume 106, Page 9764, Figure 3).]

Figure 3-6. The generalized effects of DOF, rate constants, and transition states on small, medium, and large molecular kinetics for hydrocarbons. [Reprinted with permission from John Wiley & Sons, Ltd. Dunbar, R.C. 2004. BIRD, Evolution, Principles, and applications. Mass Spectrom. Rev. (Volume 23, Page 137, Figure 4).]
Figure 3-7. The Boltzmann distribution truncated at the threshold energy (E_t) (where ΔE_{depl} = E_t - hν_{avg}) according to the sudden death approximation for modified Tolman modeling for small molecule kinetics. [Reprinted with permission from the American Institutes of Physics. Dunbar, R.C. 1991. Kinetics of low-intensity infrared laser photodissociation. The thermal model and application of the Tolman theorem. J. Chem. Phys. (Volume 95, Page 2542, Figure 3).]

Figure 3-8. The equilibrium and nonequilibrium (depleted) Boltzmann distribution for medium molecule kinetics. [Reprinted with permission from American Chemical Society. Dunbar, R.C. 1994. Kinetics of Thermal Unimolecular Dissociation by Ambient Infrared Radiation. J. Phys. Chem. (Volume 98, Page 8708, Figure 5).]
Table 3-1  Comparison of Arrhenius activation energies from IRMPD experiments utilizing the Dunbar and Paech & Williams corrections, and values determined using blackbody infrared radiative dissociation (BIRD) experiments for four peptides: leucine enkephalin, gramicidin S, bradykinin, and melittin. The experiments are taken from the papers of Schnier et al. \textsuperscript{b,132} \textsuperscript{d,133} Busman et al. \textsuperscript{g,134} Jockusch et al. \textsuperscript{c,136} h;\textsuperscript{135} Freitas et al. \textsuperscript{e,138} Paech et al. \textsuperscript{f,33}

<table>
<thead>
<tr>
<th>compound</th>
<th>charge state</th>
<th>mass (Da)</th>
<th>$E_a$ (BIRD)</th>
<th>modeled $E_a$ (eV)</th>
<th>error (%)</th>
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<tr>
<td>leucine enkephalin</td>
<td>1+</td>
<td>555</td>
<td>1.1\textsuperscript{b}</td>
<td>0.66</td>
<td>1.09\textsuperscript{c}</td>
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<tr>
<td>bradykinin</td>
<td>1+</td>
<td>1061</td>
<td>1.3\textsuperscript{d}</td>
<td>0.63</td>
<td>1.16\textsuperscript{e}</td>
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<tr>
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<td>1061</td>
<td>1.3\textsuperscript{d}</td>
<td>1.17</td>
<td>1.94\textsuperscript{e}</td>
</tr>
<tr>
<td>bradykinin</td>
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<td>0.8\textsuperscript{d}</td>
<td>0.50</td>
<td>0.90\textsuperscript{e}</td>
</tr>
<tr>
<td>melittin</td>
<td>2+</td>
<td>2845</td>
<td>-</td>
<td>0.77</td>
<td>1.3\textsuperscript{f}</td>
</tr>
<tr>
<td>melittin</td>
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<td>0.77</td>
<td>1.3\textsuperscript{f}</td>
</tr>
<tr>
<td>ubiquitin</td>
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<td>8565</td>
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<td>0.7</td>
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CHAPTER 4
CARBON DIOXIDE AND OPTICAL PARAMETRIC OSCILLATOR LASERS

An infrared multiple photon dissociation experiment requires a monochromatic infrared light source with a relatively high fluence in order to promote dissociation. These sources come in a variety of types, from carbon dioxide and nitrogen gas lasers to more sophisticated optical parametric oscillator and free electron lasers. Regardless of complexity, light amplification by stimulated emission of radiation (LASER) consists of three basic components: a lasing medium, a source to “pump” energy into the medium, and a resonant cavity. Each laser type employs different media to promote the emission of coherent radiation of the desired wavelengths. This in turn requires a specific means of exciting the medium and a suitable resonant cavity to enhance the power. Copious laser systems of varying complexities result. The focus of this chapter is the CO$_2$ and OPO lasers, both of which were used in this work.

**Carbon Dioxide Laser**

In the early 1960s, Patel produced the first CO$_2$ laser at Bell Laboratories. Building on the earlier development of the helium-neon laser and Barker and Adels’ studies on CO$_2$ vibrational rotation transitions, Patel explored the use of the CO$_2$ molecule as a potential gas lasing medium. The first laser consisted of two tubes connected in a rectangular formation, similar to that shown in Figure 4-1 (initially without the nitrogen gas). The top small tubing served as an inlet and excitation vessel (electrical discharge) for the CO$_2$ gas and was connected on the far ends to a larger tube, the resonance cavity. The resonance cavity, set in a solid frame, had fixed mirrors on both ends, one fully reflective and the other with a nonreflecting center of a radius ~ 5mm (a partially reflective mirror was also used for comparison). The excited carbon dioxide molecules entered the second chamber and emitted photons as the molecules relaxed to their ground state. The resonance chamber, designed to allow specific wavelengths of light to
undergo constructive interference, amplified these emissions via a successive buildup of emitted photons reflected between the two mirrors. Lasing was achieved once the coherent light of a specific wavelength passed the threshold energy of the cavity. With his initial experiments, Patel attained powers of 1mW in continuous-wave mode, with somewhat higher pulsed powers. Laser frequencies covered the P branches of two vibrational-rotational transitions. Later, Patel excited N$_2$ gas via an electrical discharge (Figure 4-1) and mixed the excited gas with the CO$_2$ gas to promote resonant energy transfer between the two molecules. This initially improved the laser’s efficiency by 100% where later even higher powers were achieved.

Today, CO$_2$ lasers come in a variety of sizes. Though the basic construction is similar, major differences include improvements to the resonance chamber, closed laser systems, and tunable CO$_2$ systems for expanded wavelength range and power. With modern carbon dioxide lasers, all processes take place in a single liquid-jacketed sealed resonance chamber, where electrical discharge results from a potential difference between anode and cathode posts or rings placed inside. Within the jacket, cold water is continuously circulated from a constant temperature bath (~20°C) dissipating heat. The removal of the excess energy increases laser efficiency and allows higher potentials to be used for increased energy output. Many of these lasers are also sealed, with H$_2$O (~0.2 torr) added to the mixture to ensure the continual reprocessing of carbon monoxide to carbon dioxide (i.e., CO + H$_2$O $\rightleftharpoons$ CO$_2$ + H$_2$) for longer operation without recharging.

With tunable lasers, the totally reflecting mirror is replaced with a transparent window (i.e., zinc selenide, (ZnSe)) set at the Brewster angle. The Brewster angle allows for the complete transmission of light where the reflected (at angle $\theta_b$) and refracted (at angle $\theta_r$) light rays are
perpendicular to one another, such that \( \theta_B + \theta_r = 90^\circ \). Incorporating these conditions into Snell’s law gives

\[
n_1 \sin \theta_B = n_2 \sin (90^\circ - \theta_B) = n_2 \cos \theta_B
\]

where \( n_1, n_2 \) are the refractive indices of the gas and the window, respectively. Equation 4-1 is rearranged to find the Brewster angle \( (\theta_B) \) as

\[
\theta_B = \arctan \left( \frac{n_2}{n_1} \right).
\]

Light traveling through the Brewster window encounters a wavelength tunable diffraction grating and reflects back to the resonance chamber. By changing the grating angle, wavelengths in the R or P branches of two vibrational-rotational transitions are tuned into resonance with the optical cavity. The two vibrational-rotational transitions are part of a four level system that expands available wavelengths and improves the efficiency of the CO\(_2\) laser.

**Four Level System**

At the heart of a CO\(_2\) laser is the vibrational structure of the carbon dioxide molecule. As mentioned above, the linear molecule has 4 vibrational modes (3N-5), the asymmetric and symmetric stretches and two degenerate bending modes. The lower level vibrational/rotational energy order of these modes establishes a four-stage system (Figure 4-2).\(^{147,149}\) Resonance energy transfer from N\(_2\) to the CO\(_2\) molecules occurs at the first energy level of the asymmetric stretch, 00\(^0\)1 (\(\Sigma_u^+\)). These molecules relax (stimulated and spontaneous emission) to two lower vibrational-rotational levels (\(\Sigma_g^+\)): the first level of the symmetric stretch 10\(^0\)0 and the second level of the bending motions 02\(^0\)0. The \(\Sigma_g^+\) levels relax (helium gas aids these processes, in particular the 10\(^0\)0 (an IR inactive mode) that undergoes thermal relaxation)\(^{150,151}\) to the third
stage, the first vibrational level of the bending motions $01^00 (\Pi_u)$, and finally, to the ground state $(\Sigma_g^+)$.

The ability of the medium to reach the $00^01$-excited state rapidly and to dissipate the energy from stage two quickly creates a population inversion that promotes the net production of emitted photons. This is aided by the addition of nitrogen and helium. Electrically pumped nitrogen gas provides the necessary excitation energy through collisional (near) resonant energy transfer. Nitrogen’s fairly large cross-section (the lowest excited energy level 2330.7 cm$^{-1}$ of N$_2$ is very nearly resonant with $00^01$ at 2349.16 cm$^{-1}$) and slow deactivation rate (~8.8 sec @ 2torr) make N$_2$ ideal for the transport and transfer of energy.$^{152,\dagger}$ Helium aids in the depopulation of the laser’s second stage through collisional contact. Also, the high thermal conductivity of He helps dissipate heat.

**Vibrational-Rotational Picture**

The laser’s capabilities are further enhanced by the many rotational levels associated with each vibrational level. The rotational levels are a product of the molecules’ axial rotational motion (x, y, z coordinates), which spatially displace the atoms from a fixed position. As the CO$_2$ molecule is linear, only two assigned coordinates (by convention y and z) result in a change in the molecule’s position.

Simple rotational motion can be modeled by a linear rigid rotor with rotational energies of

\[
E(J) = \frac{\hbar^2}{8\pi^2 I} J(J + 1).
\]

Here, $J$ is the rotational quantum number with values of 0, 1, 2, 3…, and $I$ is the moment of

$\dagger$ Nitrogen (g) is a homonuclear diatomic molecule that does not lose vibrational energy through electric dipole radiation but relaxes by interacting with other molecules.
inertia \((I=2m_{\text{oxygen}} R^2)\). From Equation 4-3, the difference in the rotational energy levels is given by

\[ \Delta E(J) = 2\tilde{B}J_{J+1} \]  

where \(J_{J+1}\) is the higher rotational energy level and the rotational constant \(\tilde{B}\) is defined by

\[ \tilde{B} = \frac{\hbar}{8\pi^2I} \text{ (in Hz)} \quad \text{or} \quad \tilde{B} = \frac{B_c}{c} \text{, in cm}^{-1}. \]

Equation 4-3 indicates that the rotational energy levels are not equally spaced but increase with increasing values of \(J\). Rotational levels are also more closely spaced than their vibrational counterparts \((\nu\text{\textsubscript{vib}} > \tilde{B})\), with each vibrational level corresponding to a set of associated rotational levels. Figure 4-3 illustrates how P \((\Delta J=-1)\) and R \((\Delta J=+1)\) branches arise when transitions between these vibrational-rotational levels occur. Each line within the branch corresponds to a different wavelength of light, increasing in energy from the P to the R branch, according to the selection rule \(\Delta J = \pm 1\). The central Q branch, however, is forbidden \((\Delta J \neq 0)\) for the CO\(_2\) molecule, leaving a gap between the P and R branches. In the harmonic case, the energy (in cm\(^{-1}\)) of a particular vibrational-rotational energy level is

\[ \tilde{T}(\nu, J) = \frac{E(\nu, J)}{\hbar c} = \tilde{\nu} \left( \nu + \frac{1}{2} \right) + \tilde{B}_c J(J + 1). \]

To better approximate the energy of a vibrational-rotational level, anharmonicity and bond distortion are taken into account.\(^{88}\) Because an excited molecule’s bond length increases with increasing vibrational levels, the moment of inertia increases and the rotational constant \(\tilde{B}\) decreases, thus decreasing the distance between each rotational level. The result is a coupling of both vibrational and rotational states through anharmonicity. The correction for the coupling of the rotational component is given by
\[ E_{\text{vib-rot coupling}} = \alpha \left( \nu + \frac{1}{2} \right) J (J+1) \]  

where \( \alpha \) is the vibrational-rotational coupling constant. Also, molecular bonds are not rigid as assumed by the rigid rotor model. Distortion occurs with the rotational motion and causes a second corrective term given as

\[ E_{\text{centrifugal distortion}} = D_e J^2 (J+1)^2 \]  

where \( D_e \) is the centrifugal distortion constant. Equations 4-3, 4-4, 4-5 and Equation 3-29 (limited to one vibrational mode) combine to yield,

\[
\tilde{T}(\nu, J) = \frac{E(\nu, J)}{hc} = \tilde{v}_v \left( \nu + \frac{1}{2} \right) - \tilde{v}_v \tilde{\chi}_e \left( \nu + \frac{1}{2} \right)^2 + \tilde{B}_e J(J+1) - D_e J^2 (J+1)^2 - \alpha_e \left( \nu + \frac{1}{2} \right) J(J+1).
\]

The transition intensities from 00\(^0\) to 10\(^0\) and 02\(^0\) are related to Boltzmann statistics\(^{94}\) given by

\[ N(E) = (2J + 1) e^{-\tilde{\nu} J(J+1)/k_B T}. \]  

Rotational levels of each vibrational level are populated according to the distribution. Laser transitions are most probable from rotational states that have maximum population at 00\(^0\). This corresponds to the intensity of the laser’s output at that given transition frequency.

Figure 4-4 gives the distribution of the corresponding P and R branches for both transitions. Photon emissions from the first excited state of the asymmetric stretch (00\(^0\)) to the second excited state of the bending motion (02\(^0\)) and the first excited state of the symmetric stretch (10\(^0\)) give wavelengths of 9.174 – 9.354 (P)/9.443 – 9.773 (R) and 10.105 - 10.365 (P) and 10.441 - 10.885 (R) \(\mu\)m, respectively. The gaps (/) between the R and P branch are due to the
forbidden Q branch. This phenomenon creates multiple energy transitions covering an important area of the congested fingerprint in IR spectra.

**Experimental Carbon Dioxide Lasers**

The experiments discussed in this work were performed on two separate laser setups: the optical parametric oscillator (OPO) laser/CO$_2$ apparatus and two tunable CO$_2$ lasers. These were each aligned with two different FTICR mass spectrometers in the Mass Spectrometry Services lab and in the Eyler lab at the University of Florida, respectively. The OPO-CO$_2$ beams were aligned with the ions in the cell of the FTICR mass spectrometer in order to perform a two-laser experiment. Here, an off-resonant CO$_2$ laser was added to aid the dissociation of ions that were excited into the quasicontinuum by vibrationally resonant wavelengths from the OPO laser. This system is discussed in some detail in the next section of this chapter. The second system was developed after blackbody radiation photons (through heating the ICR cell) failed to dissociate phosphate groups from phosphopeptide ions. A tunable Apollo CO$_2$ laser in disrepair was placed into service to provide a more direct and powerful (high fluence) source of IR photons. This arrangement included the complete set up of transfer mirrors, a laser gate, and other devices to finish the experiments. Later, an additional tunable CO$_2$ laser was purchased and integrated into the system.

**Apollo Carbon Dioxide Laser**

A tunable Apollo CO$_2$ laser, used in past experiments, was available but in need of repair. In order to facilitate these changes, the CO$_2$ laser was transported to the physical chemistry teaching lab with permission of Dr. Kathryn Williams and repaired with the aid of Lawrence (Larry) Hartley. Initially, the laser was checked to be sure the glass resonance cavity, hoses, etc., were in good shape. To this end, a rebuilt water chiller and connections were added, some of the old tubing replaced and secured, and the visible high-voltage connections and probes checked,
with no notable problems associated with the cavity itself. As the existing schematics were incomplete, the electronic circuits were traced and schematics drawn. From these developed sources, the problems were isolated to the preamp control board, relays, and interlocks. After the replacement of a number of diodes and relays, the creation of a piggyback board that replaced worn circuits, and the reconfiguration of an interlock, the laser appeared operational. In other words, laser plasma was evident, though the laser was not lasing due to a severely misaligned cavity. With Prof. John Eyler’s assistance, the alignment of the laser was completed within a short period. The laser was moved back to the Eyler lab for integration with the FTICR mass spectrometer.

Figure 4-5 illustrates the original CO$_2$ laser set up. With the aid of Cesar Contreras, the laser was placed within the northwest quadrant of a 12’x 4’ platform supported by two tables. All the connections were completed and the laser tested. Platforms were made to lift the CO$_2$ laser beam to the approximate center of the ICR cell window. According to plan, the focusing mirror was mounted, placed onto a sliding bracket, and secured to a long guiding rod perpendicular to the laser beam on the east side of the table. This platform was designed to allow 360° adjustment and movement along the rail. The focusing mirror was in line with a second “back” mirror at the FTICR cell’s entrance, as seen in Figure 4-6. An NaCl window was added central to the output end of the CO$_2$ laser (west) and the focusing mirror (east). On the other sides of the salt window, a wavemeter (south) and helium-neon laser mirror (north) were placed. Parallel and north of the CO$_2$ laser, the mutually aligned helium-neon laser allowed for further tuning of the CO$_2$ laser and provided a visible and safe beam for the positioning of the system. Once the pieces were in place, the system was first adjusted using the helium-neon laser followed by optimization with the CO$_2$ laser. Subsequently, the power meter was placed in between the helium-neon laser
mirror and the salt window, and the readout was placed above the table. *Predator*, the mass spectrometer’s acquisition program, turned on the laser during the reaction delay sequence in the program, while the beam-cell alignment was maximized by monitoring the absolute abundances of precursor and product ions in the mass spectra.

A few early problems led to a number of additions to the system. Initially, feedback from the triggered CO$_2$ laser’s control circuitry froze the *Predator* program. The problem was solved by the introduction of an opto-isolator box into the circuit. As the triggered beam power readings were later found to be inconsistent (high initial output prior to settling at a given power), the trigger was later abandoned for the laser gate system (the laser continuously running) as seen in Figure 4-7.

With the laser gate, a high or low voltage from *Predator* is sent to a control box that is connected to a power amplifier and a rotary solenoid attached to a thin, centered aluminum rod with a mirror and counter weight on each end (gate). When the voltage is high or the experiment is not running, power from the amplifier is relayed to the rotary solenoid raising the gate and closing off the laser from the cell. The power meter, now placed at the same height and in position to receive the reflected beam, measures the full power of the laser at that point. Conversely, if the voltage is low, the gate drops during the sequence chosen (typically the reaction delay) allowing the beam to pass into the cell. By raising the voltage at the next event in the sequence, the beam is again blocked, regulating the reaction time to a single event window.

Additional features included the installation of a new antireflective-coated zinc selenide Brewster and FTICR cell window to improve power transmission.

A second repair was accomplished after a coolant water connection leak damaged the laser. Tracing the circuits, it was discovered that the problem was related to the high voltage supply
circuitry, in particular a faulty power tube. The power tube was replaced and the high voltage restored, but still the laser did not work. Upon close inspection, it was found that the anode inside the glass resonance chamber was damaged. Joe Caruso at the University of Florida chemistry glass shop was unable to repair the damage. However, he produced a second glass cavity, whose repairs were not as extensive, that fit the Apollo laser. After installing the second chamber, the cavity was realigned and did work but at a fraction of the original power. At that point, Dr. Eyler decided to purchase a second CO$_2$ laser, which was integrated into the existing system.

**Lasy Carbon Dioxide Laser**

The Lasy-20G-AT tunable laser was purchased and installed as shown in Figure 4-8. The only modification to the existing set-up included the addition of two other mirrors, both with the same degree of motion as previously mentioned; the first guided the laser beam from the Lasy CO$_2$ laser toward the beam of the Apollo laser, and the second mirror was set at the exact intersection of the two beams to guide the new laser along the same path. Both lasers were in turn aligned to the equipment and the cell. A second gate was built for the proposed two laser experiments, utilizing the CO$_2$ lasers in the same manner as the double laser OPO/CO$_2$ experiments discussed later in this chapter and in Chapter 5.

Unlike the Apollo, the Lasy CO$_2$ laser is sealed and does not require a constant supply of gas. The laser also is capable of remote control, with on/off and cw/pulsed switches, power potentiometer, and wavelength control. Wavelength and power control units are wired and placed near the FTICR mass spectrometer’s computer workstation. These lasers aided in many experiments in the Eyler lab and encouraged a number of dissociation and action spectroscopy experiments, including those in described in Chapter 6.
Optical Parametric Oscillator Laser

In 1961, Franken et. al. discovered that second harmonic generation of light is produced when a relatively high-powered laser is passed through a material with nonlinear optical properties. Optical parametric amplification and oscillation soon followed and in 1965 Giordmaine and Miller used this information to build the first tunable OPO laser. Applications of these and other advances led to stimulated Raman scattering, coherent antistokes (CARS), and inverse (IRS) and gain (SRGS) spectroscopy, in addition to a host of other applications. Since then, better cavity designs and the improvement of nonlinear materials have resulted in more efficient and powerful tunable lasers, providing the spectroscopist with very precise broadband energies. The OS 4000 OPO from LINOS is a continuous wave IR-tunable laser, whose wavelength ranges from 1.38 - 2.0 and 2.28 - 4.67 µm for the signal and idler beams, respectively, at thousandths of a nm accuracy, while producing 25 - 75mW per beam. This section will focus on the basic theory of the OPO laser, setup, and operation.

Optical Parametric Oscillator Theory

Referring to Figure 4-9, when a relatively high intensity pump laser beam of a set frequency (ω_p) is focused into any period of a periodically poled lithium niobate crystal, signal (ω_s) and idler (ω_i) frequencies are created, according to the relationship ω_p = ω_s + ω_i (keeping with the law of conservation of energy). In the crystal, the signal and idler beams are amplified and the pump depleted as phase matching conditions are met. The two beams are then amplified further in the OPO resonance cavity until their power surpasses a threshold and the lasing action begins. In order to understand the workings of the OPO, the theory and experimental set up will now be discussed.
Nonlinear Optics

Linear optics covers the typical behavior of light (i.e., reflection, refraction, transmission, etc.) as it interacts with different media. As such, linear optics can be compared to the sound from speakers in an idealized stereo system driven at appropriate powers with no distortion. Here, the frequencies, though reflected and absorbed by the surrounding walls, etc., are still clear and linear. Once the amplifier is turned up, however, the now overpowered speakers produce harmonic and anharmonic distortions of fundamental frequencies, and the music, once clearly heard, becomes less discernable. These effects are analogous to what occurs in nonlinear optics. In nonlinear optics, relatively powerful coherent lasers focused on a material induce nonlinear effects from distortion of the medium. These effects include generation of sum-frequency (different multiple frequencies producing one frequency), difference-frequency (a single frequency forming multiple frequencies), and harmonic-frequency (multiples of the same frequency producing harmonic frequencies) from the fundamental (pump) frequency(s).\(^{170}\)

Both linear and nonlinear interactions are expressed in terms of the induced polarization of light, given as

\[
P(\omega) = \varepsilon_0 [\chi^{(1)} E(\omega) + \chi^{(2)} E^2(\omega) + \chi^{(3)} E^3(\omega) + ...]
\]

where \(P(\omega)\) is induced polarization, \(\varepsilon_0\) free space permittivity, \(\chi^n\) is the nth order susceptibility, and \(E(\omega)\) is the electric field strength. The first order of susceptibility \(\chi^{(1)}\) covers linear optics. Second-order susceptibility \(\chi^{(2)}\) corresponds to the beginning of nonlinear behavior, where second harmonic generation (e.g. wavelength doubling from 1064 nm to 532 nm) and optical parametric oscillation (pump wavelength producing signal and idler wavelengths) occur. Second-order susceptibility is a third-degree tensor having 27 elements.\(^{170}\) These nonlinear coefficients describe the off/near-resonant interactions between the applied electromagnetic fields and the
outer electron clouds of an atom or molecule in a medium. Here, a field-stimulated change in the
electron cloud excites the entire molecule to an intermediate (virtual) state, with the annihilation
of a pump photon, whereupon immediate relaxation (back to original state) creates the signal and
subsequent idler photons \((h\nu_p = h\nu_s + h\nu_i)\).\textsuperscript{171,172} With the lithium niobate crystal, these
frequencies are adjustable, as the nonlinear refractive index (important to the interactions of light
with the medium) varies in a controllable manner with the crystal’s temperature.\textsuperscript{168,173-175} If the
temperature is raised or lowered (within the operational window of 50 - 170°C for the Linos
OPO laser), different signal and idler frequencies are produced. The summation of these events
gives an overall statistical measurement of the induced polarization. However, unlike odd-
ordered susceptibilities, even-ordered susceptibility tensors disappear, along with their nonlinear
properties, with the inverse symmetry operations of centrosymmetric materials. Thus, production
and optimization of noncentrosymmetric (no inversion symmetry) crystals (e.g., LiNbO\(_3\),
K\(_2\)NbO\(_3\), and Ba\(_2\)NaNb\(_3\)O\(_{13}\)) with high transmissivity and optical damage thresholds is necessary
to create efficient second-order nonlinear effects.\textsuperscript{176-178}

Isolating the second-order nonlinear terms and noting that the electric and magnetic fields
are propagated along the z axis in a direction resonant with the cavity, Equation 4-10 simplifies
to

\[
P_c = \sum_x \sum_y \chi_{x,y,z} E_x E_y.
\]

Further expansion of the terms in Equation 4-11 reveals the interaction of the different electric
fields coupling the pump, signal, and idler beams,

\[
P(\omega_p) = \varepsilon \chi^2(\omega_s, \omega_i) E(\omega_s) E(\omega_i)
\]

\[
P(\omega_s) = \varepsilon \chi^2(\omega_p, -\omega_i) E(\omega_p) E^* (\omega_s)
\]

\[
P(\omega_i) = \varepsilon \chi^2(\omega_p, \omega_s) E(\omega_p) E^* (\omega_s)
\]
From these equations, interacting phase conditions are made apparent through the slow amplitude approximation.\textsuperscript{173,179,180}

The slow amplitude approximation treats the nonlinear wave equations above as monochromatic plane waves propagated in the z-direction.\textsuperscript{181-183} Each function is then transformed into an expression for its amplitude ($A_n(z)$) change with respect to the z direction or $\frac{\partial A_n(z)}{\partial z}$. In the first stage, the electric fields’ components are expanded according to their unit vectors ($a_m$), scalar amplitude functions ($A_n$), and wave vectors ($k_n$) in the z direction,

\begin{align}
E(\omega_p, z) &= a_p A_p(z) e^{ik_p z} \\
E(\omega_s, z) &= a_s A_s(z) e^{ik_s z} \\
E(\omega_i, z) &= a_i A_i(z) e^{ik_i z}.
\end{align}

The general equation for the amplitude scalar to the wave vector is

\[ k = \frac{2\pi}{\lambda} n_o(\omega) \]

where $n_o$ is the nonlinear refractive index. Substituting Equations 4-15, 4-16, 4-17 into Equations 4-12, 4-13, 4-14 gives the generated polarizability in terms of the amplitude and wave vector functions as

\begin{align}
P(\omega_p, z) &= \varepsilon_o \chi^2(\omega_p, \omega_s, \omega_i) a_p a_s A_p(z) A_i(z) e^{i(k_p-k_s)z} \\
P(\omega_s, z) &= \varepsilon_o \chi^2(\omega_p, \omega_s, \omega_i) a_p a_i A_p(z) A_i(z) e^{i(k_p-k_s)z} \\
P(\omega_i, z) &= \varepsilon_o \chi^2(\omega_p, \omega_s, \omega_i) a_p a_s A_p(z) A_s(z) e^{i(k_p-k_s)z}.
\end{align}
In the second approximation, the general electric field component \( E(\omega, z) = a_o A(z)e^{ikz} \) is placed in the modified general nonlinear wave equation (from Maxwell’s equations) given as,

\[
\frac{d}{dz} \times \frac{d}{dz} E(\omega, r) + \mu_o \omega^2 \varepsilon(\omega) E(\omega, r) = \mu_o \omega^2 \varepsilon(\omega) P(\omega, r)
\]

where the linear dielectric constant is \( \varepsilon(\omega) \) and is simplified to give the equation of the slow amplitude approximation (derivation noted in references), \(^{170,175}\)

\[
\frac{\partial A(\omega, z)}{\partial z} = \frac{ik}{2\varepsilon(\omega)} \left[ a_o P'(\omega, z) \right] e^{ikz}.
\]

Substituting Equations 4-19, 4-20, 4-21 into Equation 4-23 leads to,

\[
\frac{\partial A_p(z)}{\partial z} = \frac{ik_p}{2n^2(\omega_p)} a_p \chi^2(\omega_p, \omega_i) a_s a_s(z) A_i(z) e^{-i\Delta k z}
\]

\[
\frac{\partial A_s(z)}{\partial z} = \frac{ik_s}{2n^2(\omega_s)} a_s \chi^2(\omega_p, \omega_i) a_p a_p(z) A_i(z) e^{-i\Delta k z}
\]

\[
\frac{\partial A_i(z)}{\partial z} = \frac{ik_i}{2n^2(\omega_i)} a_i \chi^2(\omega_p, \omega_s) a_p a_p(z) A_i(z) e^{-i\Delta k z}
\]

where the change in the wave vector, \( \Delta k = k_p - k_i - k_s = n_p \omega_p - n_i \omega_i - n_s \omega_s \approx \left( \frac{n_p}{\lambda_p} - \frac{n_s}{\lambda_s} - \frac{n_i}{\lambda_i} \right) \), is a measure of the coupled wave equations. Here, three-way mixing takes place between the coupled waves of the applied electric fields, resulting in the parametric interaction of pump, signal, and idler. This provides a mechanism for the exchange of energy among the fields. For instance, an amplitude gain for signal and idler waves (parametric amplification) and a corresponding decrease in the pump energy occurs so long as phase matching conditions are met (i.e., \( \Delta k \rightarrow 0 \) or ideally \( \Delta k = 0 \)).
Quasi-Phase Matching

Phase velocity mismatch (between pump, idler, and signal) occurs as velocity synchronism breaks down due to dispersion and other effects. There are two general methods for correcting these problems.\textsuperscript{184,185} Traditional phase matching involves utilizing the birefringence effect of the refractive index to reduce dispersion. The other complementary technique uses periodically poled regions, spaced between points of phase divergence, to bring the fields back into phase.\textsuperscript{176,186} This process is known as quasi-phase matching. As previously stated, interacting fields step out of phase as they travel through the crystal, thus affecting the gain. The maximum interaction length over which amplification of the signal and idler fields is sustained is known as the phase coherence length. Phase coherence lengths are determined at the point where the phase of the pump and the sum of the idler and signal frequencies are 180° out-of-phase. Rotation of alternating coherent length segments (periodically poled elements in one period) in the crystal assures that the pump and the idler and signal frequencies are back in phase. These modifications are accomplished through crystal alteration or electric field poling.\textsuperscript{187,188} Adaptation of the crystal is commonly achieved by growing the crystal with periodic elements in place or by invasive alteration to an existing crystal. Electric field poling applies to ferroelectric crystals (i.e., lithium niobate), where long metal electrode strips are applied in a periodic fashion to the top of the crystal with the bottom uniformly covered and grounded.\textsuperscript{189,190} When a static electric field of sufficient strength is applied, the periods under the field are reversed. The periodically poled lithium niobate crystal in the LINOS OS 4000 OPO laser operates in the latter fashion, where the depleted pump and surging signal and idler beams leave the crystal in phase to resonate in the cavity.
Pound, Drever and Hall Technique

Small frequency fluctuations in lasers are common due to variations in temperature, current, voltage, etc. Thus, a means to keep the pump laser in resonance with the cavity is critical to achieve wavelength precision and constant power. The LINOS OPO does this by considering the laser resonance cavity as a Fabry-Perot interferometer and implementing the Pound, Drever, and Hall (PDH) technique\textsuperscript{164,165} to lock the laser in place. With the LINOS OPO, the Nd:YAG laser is modulated to produce two 12 MHz side bands on either side of the principal beam.\textsuperscript{166} Assuming the cavity and pump are generally aligned with the system, these side bands enter the resonance cavity, resonate, and escape from the cavity as shown in Figure 4-10.

Traveling back toward the pump laser, the beams are isolated from the incident-modulated beam via a Faraday isolator (#4) and directed to a photodiode (#5). The signal is then compared to the modulated beam through a mixer to create an error signal. If the compared side bands are the same (error signal is zero), then the cavity is in resonance. If the error signal is not zero, then the control electronics send the appropriate signal to the piezoelectric crystal attached to the back-reflecting mirror (#16) for a zero error adjustment. In the lock mode, a feedback loop keeps the pump laser in resonance, which in turn allows the pump resonant signal and idler beams to stay in resonance.

An off-resonance intensity appears the same from either side of the resonant intensity (Gaussian-like beam profile). Thus, the direction of the corrective change is difficult to determine. This is corrected by taking the derivative of the intensity with respect to its frequency, where the on-resonance condition is achieved when \( \frac{dI}{d\omega} = 0 \). Off-resonance positions are detected as either being in-phase with the modulation (above resonance, positive derivative) or 180° out-of-phase (below resonance, negative derivative), as shown in Figure 4-11. The
The aforementioned mixer reflects this by a positive or negative output, respectively. This in turn is dependent on the resonance conditions inside the cavity. To better understand these conditions, a general characterization of the interaction of the laser beam components is necessary.

The electric field \( E_{\text{inc}} \) incident to the cavity is

\[
E_{\text{inc}} = E_o e^{i(\omega t + \beta \sin \Omega t)}
\]

where \( E_o \) is the initial electric field, \( \omega \) is the primary frequency, \( \Omega \) is the phase modulation of \( \omega \) (phase dithering), and \( \beta \) is the modulation index \( \left( \beta = \frac{\Delta \omega}{\Omega} \right) \). Equation 4-27 is expanded through Bessel functions to give

\[
E_{\text{inc}} \approx E_o [J_0(\beta) + 2iJ_1(\beta) \sin \Omega t] e^{i\omega t} = E_o [J_0 e^{i\omega t} + J_1 e^{i(\omega + \Omega) t} - J_1 e^{-i(\omega - \Omega) t}]
\]

where \( J_0 \) and \( J_1 \) are zero and first order Bessel functions. The final term in Equation 4-28 illustrates the inclusion of the primary frequency \( (J_o) \) and the two side bands \( (J_1) \). The total power \( (P_o) \) associated with the incident beam is

\[
P_o = |E_o|^2
\]

such that \( P_o \approx P_c + 2P_s \), where \( P_c \) is the power in the carrier beam \( (P_c = J_o^2 \times P_0) \) and \( P_s \) is the power in the side bands \( (P_s = J_1^2 \times P_o) \). These beams are reflected in the Fabry-Perot cavity (OPO resonance chamber) where the transfer function is given by

\[
F(w) = \frac{E_{\text{ref}}}{E_{\text{inc}}} = \frac{r \left( e^{i \frac{\omega}{\Delta \nu_{FSR}}} - 1 \right)}{1 - r^2 \left( e^{i \frac{\omega}{\Delta \nu_{FSR}}} \right)}.
\]

In Equation 4-30, the reflected electric field is \( E_{\text{ref}} \), \( r \) is the amplitude reflection coefficient for the two mirrors, and \( \Delta \nu_{FSR} \) is the free spectral range (FSR) of the chamber. The free spectral
range is defined by the difference between the resonant (constructively interfering) modes in the cavity such that

$$\Delta v_{fr} = \frac{c}{2L}$$

where $c$ is the speed of light and $L$ the length of the resonance cavity. Equation 4-30, also known as the reflection coefficient $\left(\frac{E_{ref.}}{E_{inc.}}\right)$, is rearranged to solve for the reflected electric field $E_{ref}$. The incident electric field equation (Equation 4-28) is then converted to

$$E_{ref.} = E_o \{ F(\omega)J_o(\beta)e^{i\omega t} + F(\omega + \Omega)J_1(\beta)e^{i(\omega + \Omega)t} - F(\omega - \Omega)J_1(\beta)e^{-i(\omega - \Omega)t} \}$$

As mentioned, the reflective field is detected by a photodetector, which measures power.\textsuperscript{191}

Thus, the reflective power ($P_{ref}$) is found by taking the square of the absolute value of $E_{ref}$ (Equation 4-29), which when expanded is

$$P_{ref.} = P_o |F(\omega)|^2 + P_l \{ [F(\omega + \Omega)]^2 + [F(\omega - \Omega)]^2 \}
+ 2 \sqrt{P_o P_l} \{ R_e [F(\omega)F(\omega + \Omega) - F(\omega)F(\omega - \Omega)] \cos \Omega t + I_m [F(\omega)F(\omega + \Omega) - F(\omega)F(\omega - \Omega)] \sin \Omega t \}
+ (2\Omega terms)$$

where $R_e$ and $I_m$ are the real and imaginary parts of the equation, respectively. The first line of Equation 4-33 represents the unperturbed power contributions from the fundamental and sidebands. Line two gives the power addition from the interference between the waves of the carrier and sidebands. Line three is the input of the sidebands’ interference with one another. Of importance is the interference or beat patterns of line 2 that provide an indirect measurement of the primary wave in terms of power. This line is isolated from the others by the use of a mixer and low-pass filter, where the sine and cosine parts are effectively separated. The real or cosine terms are used to measure low frequency modulation, where the cavity response is quicker than
the phase modulation. The mixer compares the original modulated signal (Ω') from the laser with the signal from the reflecting beam (Ω) according to

$$\cos(\Omega't) \cos(\Omega t) = \frac{1}{2} \cos(\Omega' - \Omega)t + \cos(\Omega' + \Omega)t$$ 4-34

where the original (incident) signal sine (Ω') is shifted 90° with a phase shifter. The mixer sends out a dc signal which is passed through a low-pass filter to isolate the error signal, given by

$$e = 2\sqrt{P_c P_s}\{R_c[F(\omega + \Omega) - F(\omega)F(\omega - \Omega)]\}$$ 4-35

which is approximated by

$$e \approx 2\sqrt{P_c P_s}\left[2 \text{Re}\left(F(\omega) \frac{d}{d\omega} F(\omega)\right)\right] \approx 2\sqrt{P_c P_s} \frac{d|F|^2}{d\omega} \Omega$$ 4-36

when Ω is small, as is the case here. Given $\sqrt{P_c P_s} \approx P_o \frac{\beta}{2}$, the Pound, Drever, and Hall error is

$$e \approx P_o \frac{d|F|^2}{d\omega} \Omega \beta \approx 2\sqrt{P_c P_s} \frac{d|F|^2}{d\omega} \Omega$$ 4-37

with the resonance state represented by the zero point in the error signal or $\frac{d|F|^2}{d\omega} = 0$.

In the scanning mode of the Linos OPO laser, as implemented in our laboratories, an oscilloscope monitors two channels of the lock control continuously: the sinusoidal-like error signals and the corresponding pump modes in the cavity. When switched to lock, two horizontal lines appear, one at the point of the zeroed signal and the other at the top of the pump mode, indicating that the cavity is in resonance and ready for operation.

**Optical Parametric Oscillator Operation and Set Up**

The Linos OS 4000 optical parametric oscillator laser is installed in the Mass Spectrometry Services laboratory at the University of Florida and aligned with the cell of a 4.7T FTICR mass
spectrometer. The laser is placed directly behind the mass spectrometer in an adjacent room (due to lack of space), where a rectangular hole in the wall allows for the laser beam’s passage.

Installation involved the placement of two laser tables for the OPO and related electronics, the connection of the OPO laser with the lock/temperature and YAG control modules, the calculated arrangement and careful placement of guiding mirrors for signal and idler beams, the integration of power meter, wavemeter, iris, and oscilloscope, as well as the design and execution of nitrogen purge box. An overview of the OPO operations, setup, and tuning is given below.

**Optical Parametric Oscillator Operation**

As previously mentioned, there are three qualities that make up a laser: the photon producing medium, the medium’s energy source, and resonance chamber. The OS 4000 OPO laser operates under the nonlinear principles of second-order optics. The medium is a periodically poled lithium niobate crystal consisting of eighteen different poling periods, each of which corresponds to consecutive wavelength ranges accessed by changing the crystal’s position. A continuous wave Neodymium Yttrium Aluminum Garnet (Nd:YAG) laser (operating at 2 watts) acts as the pump, whose beam is guided through a series of polarizers, mirrors, and focusing optics (Figure 4-12) to bisect one of the periodically poled periods in the crystal. For each pump photon \( E_p \) lost, two photons of lower energy, signal \( E_s \) and idler \( E_i \), are created, such that \( E_p = E_s + E_i \). These signal and idler photons continue to gain energy from the pump beam as they travel through the crystal, where periodically poled regions maintain quasiphase matching. After leaving the crystal, they resonate in a chamber defined by a piezo-mounted reflecting mirror in the back (#16) and a partially reflective surface on the front of the crystal (#13) where the incident pump beam enters. The energy of the idler and signal beams increases with the constant input of pump energy and multiple passes inside the cavity until the threshold energy of around 0.3W is met and laser action begins. Two pairs of signal and idler beams are
directed from the front and back of the cavity. Exiting the front of the cavity, idler 1 and signal go through the pump-reflecting steering mirror (#10) and are separated by the signal-reflecting mirror (#18) just behind it. Here, idler 1 passes through and is directed to the outside as the signal is reflected by a third mirror to also exit the laser housing (SM1). The other pair, idler 2 and signal, is taken from the cavity where a selective filter allows only idler 2 to pass, which then exits from the back of the laser housing (SM2).

**Tuning.** Before examining the laser set up, a few words about the laser’s tuning are in order. Tuning the laser occurs in three basic stages. The first two are noted in Figure 4-13. Stage 1 involves the adjustment of the crystal to one of the eighteen poling periods, based on the required wavelength range. The periodically poled crystal sits on a stage where north-south adjustments are made by the turn of a knob aligning the period with the perpendicular static pump laser beam. Within that period there are strong and weak regions that are noted by strong and weak power output, respectively, at a set wavelength. By monitoring the wavelength’s power while changing the crystal, a strong region is found (adjustment of the etalon (stage 2) is often required to acquire a stable wavelength) and the period is set. The free spectral range (FSR) of the cavity is further sampled by the FSR of the etalon in Stage 2. By adjusting the micrometer attached to the etalon’s platform, discrimination of wavelengths in the etalon’s FSR is possible. For fine differences in wavelength, the adjustment of the etalon(s) is sufficient to cover wavelength differences of ~thousandths of a nanometer. Coarse adjustment or wavelength sampling for action spectroscopy is based on finding a wavelength within a broader range (i.e., one nanometer) that produces the highest most stable power for optimal dissociation. Stage 3 is temperature tuning within the poling period. Figure 4-14 is a plot of the temperatures versus the wavelengths of each poling period for the signal (top row) and idler (bottom row). A temperature
increase results in a decrease in the idler wavelength and an increase in the signal wavelength, although the total energy remains the same. Temperature tuning from 50 - 170°C covers all the wavelength ranges between the poling periods with an additional 20% overlap, and is controlled by a separate module (in the same housing as the lock electronics) with fine and coarse adjustments carefully monitored on the display.

**Optical Parametric Oscillator Set Up**

Two laser tables were set up in the OPO laser room, and their heights adjusted to the corresponding height of a previously installed CO₂ laser system in the back of the FTICR. As shown in Figure 4-15, the laser housing was placed on the first table with the front of the laser pointing toward the mass spectrometer. A separate stage was created and set on the second table to lift the temperature/lock electronics housing and oscilloscope out of the beam’s path. The YAG controller was placed on a shelf under the laser table. After all the main components were assembled, previously configured mirrors were set into place. These stages of mirrors guided three beams of the OPO to their prospective locations. The signal beam coming out the front of the laser housing (SM1) was directed into the wavemeter placed in the far front right corner of the second table. The signal wavelength was then converted to the idler wavelength by the relationship

\[
\left( \frac{1}{\lambda_p} - \frac{1}{\lambda_s} = \frac{1}{\lambda_i} \right) = \left( \frac{1}{1064_{nm}} - \frac{1}{\text{wavemeter}_{nm}} = \frac{1}{\lambda_i} \right). 
\]

Idler 1 was steered around the perimeter of the table to the back far right side of the laser table. Idler 2 from the back of the OPO was guided to a position side-by-side to idler 1 and equidistant from a path traced from the centerline of the ICR cell. Here, the beams acted as two sides of an isosceles triangle on a horizontal plane raised and guided to the center of the cell. They were precisely configured based on a program that considered geometric and trigometric principles.
Precise alignment was achieved by monitoring dissociation products from the rubidium tagged D-glucosides (discussed further in the Chapter 5), where the right mirror was adjusted until maximum dissociation was reached, and then the process repeated with the left mirror. The CO$_2$ laser was then integrated into the system by careful adjustment of its mirror aligning the CO$_2$ beam central to and at an equivalent height with the OPO beams without interfering with their paths. As mentioned earlier, the additional laser provided a means to incorporate a two-laser experiment. This was put into use with the C-H stretches of the rubidium-tagged D-glucoside ions, which did not readily dissociate with the OPO laser alone as will be discussed in Chapter 5.

Along the path of these beams toward the ICR cell, the irises were placed to regulate the time of irradiation and measure the power. Power was taken when the irises were closed, and the beams were reflected into a power meter. A nitrogen purge box was also designed and built in four sections to cover the OPO laser table, the travel through the wall, the CO$_2$ laser, and the entrance into the electronic region of the cell. The system was assembled with the assistance of Cesar Contreras.

The CO$_2$ and OPO laser systems were designed and assembled to provide a means to complete the sugar and phosphopeptide experiments. As such, their use spread beyond these goals to aid many others in the Eyler and Polfer labs as tools of action spectroscopy, as well as a means of fragmentation. The analysis of the action spectra for the four D-glucoside isomers using the OPO/CO$_2$ system forms the subject of Chapter 5.
Figure 4-1. A picture of the CO₂ laser’s resonance chamber from Patel’s original paper. Note the reflective mirror to the right and the transmitting window to the left, micrometers for alignment, and inlets for both CO₂ and N₂ gas. [Reprinted with permission from the American Physical Society. Patel, C.K.N. 1964. Selective Excitation through Vibrational Energy Transfer and Optical Maser Action in N₂-CO₂. Physical Review Letters (Volume 13, Page 618, Figure 2).]

Figure 4-2. Patel’s original description of the four-stage CO₂ laser system illustrates the laser’s chemical basis for operation. Excitation of N₂ gas and subsequent resonance transfer of energy to CO₂ molecules initiates the reaction. This energy is released from the first excited vibration level of the asymmetric stretch to the first and second levels of the symmetric stretch and bending motions, respectively. These vibrational-rotational transitions produce photons of multiple wavelengths consistent with a set of P and R branches for each vibrational level transition. Energies from these last levels further decay to the bending motion’s first vibrational energy level and then back down to the ground state. [Reprinted with permission from the American Physical Society. Patel, C.K.N. 1964. Selective Excitation through Vibrational Energy Transfer and Optical Maser Action in N₂-CO₂. Physical Review Letters (Volume 13, Page 617, Figure 1).]
Figure 4-3. An illustration of rotational-vibrational transitions where P and R branches are created from the spacing between the two rotational-vibrational levels in accordance with the selection rules.

Figure 4-4. The P and R branches from Barker and Adels’ studies on CO$_2$ vibrational-rotational transitions that inspired the work for Patel’s creation of the CO$_2$ laser. In this molecule, the central “Q” branch is forbidden. [Reprinted with permission from the American Physical Society. Patel, C.K.N. 1964. Continuous-Wave Laser Action on Vibrational-Rotational Transitions of CO$_2$. Physical Review (Volume 136, Page A1189, Figure 1).]
Figure 4-5. The initial setup of the Apollo CO\textsubscript{2} laser consisting of the controller, chiller, gas mixture, optics, power meter, wavemeter, and helium-neon laser.

Figure 4-6. Final stage of the CO\textsubscript{2} laser beam alignment with the cell of the FTICR mass spectrometer. The iris serves as a guide, and the guide rod protects cell control electronics.
Figure 4-7. Addition of the laser gate and associated control electronics to the Apollo CO₂ laser to promote consistent laser power for the length of the experiment.
Figure 4-8. The addition of the sealed tube tunable Lasy CO\textsubscript{2} laser, power amplifier, control box, and chiller with additional optics.

Figure 4-9. General description of the OPO laser’s resonance chamber and nonlinear optical behavior induced by focusing a YAG pump laser (yellow) into a poling period of a periodically poled lithium niobate crystal to produce idler (blue) and signal (red) wavelengths from two sides of the cavity according to \( \frac{1}{\lambda_p} = \frac{1}{\lambda_s} + \frac{1}{\lambda_i} \).
Figure 4-10. A picture of LINOS OPO laser with appropriate features labeled corresponding to the Pound, Drever, and Hall method for locking in the resonance cavity. (for complete assignments see Figure 4-12).

Figure 4-11. Derivative of the intensity with respect to frequency of a Gaussian-like beam profile, where the on resonance condition is achieved at the origin. [Reprinted with permission from the American Association of Physics Teachers. Black, E. D. 2001. An Introduction to Pound-Drever-Hall Laser Frequency Stabilization. American Journal of Physics (Volume 69, Page 83, Figure 6).]
Figure 4-12. The LINOS OPO laser with appropriate labels including the housing (1) YAG (pump) laser (2), quarter- / half- waveplate and half -wave plate polarizers (3,6), Faraday isolator (4), photodiode unit (5), telescope (7), pump beam steering mirror (8), iris (9), beam splitting (signal/idler from pump) and pump steering mirror (10), three-stage focusing lens (11), cover for crystal/oven (not shown (12)), oven with PPLN crystal (13), folding mirrors (14), etalon housings (15), piezo-mounted back or end mirror (16), first and second beam separation units (17, 18).

Figure 4-13. Wavelength tuning of the OPO begins with the adjustment of the PPLN crystal or stage one. Based on the wavelength range desired, the PPLN stage is moved to a place within the corresponding period that maximizes power at a set wavelength. Stage two adjusts the etalon to discriminate between wavelengths in its FSR.
Figure 4-14. Temperature vs. wavelength plots for each poling period provide a means of tracking the third stage of tuning through temperature. Here, a controlled change in the PPLN crystal temperature produces a different range of wavelengths in succession. The signal and idler changes are seen in the top and bottom rows, respectively.
Figure 4-15. General set up of the OPO laser, including placement of the OPO laser and the electronics, idler 1 & 2 and CO₂ alignment with the cell, signal and wavemeter placement, and the iris and power meter arrangement (only one iris is shown).
CHAPTER 5
SPECTRAL AND MOLECULAR DIFFERENCES IN O-METHYLATED GLYCOSIDE ISOMERS

Increasingly, FTICRMS is being employed to study carbohydrate composition in the gas phase, eliminating solution phase carbohydrate interactions that can complicate the structural picture. With mass spectrometry, a difficulty lies in differentiating between the many possible biological saccharide isomers of the same m/q, 80-90% of which are chiral. This is further complicated by a saccharide’s varying linkage positions, anomericity (α, β), etc. The common approach of differentiation employs tandem mass spectrometry to isolate and fragment ions of known isomers (often derivatized to lock the structure), through dissociation techniques like collision induced dissociation (CID). Here, key spectral differences particular to each isomer’s fragmentation pattern and relative abundances provide a template for differentiation of unknown saccharides. Significant fragmentation differences are not common to all isomers, however, making some saccharides impossible to differentiate within the usual uncertainties associated with these experiments. The use of IRMPD to probe wavelength dependent fragmentation is one possible means to overcome the aforementioned problem.

Another possibility is IRMPD-promoted action spectroscopy. Comparison of the isomers’ vibrational “action” spectra not only provides a means of differentiation, but with the appropriate computational work, it also gives an intimate study of the responsible gas-phase structures and their intramolecular interactions. Because the ion concentration in a FTICR cell is very low, an absorption spectrum is impossible to detect. But action spectroscopy measures the vibrational character indirectly by monitoring the ion’s dissociation while tuning across a given wavelength range (usually reported as wavenumbers (υ or cm⁻¹)). As described in Chapter 3, the process begins when the laser’s wavelength is in resonance with a vibrational energy level, causing the transfer of multiple infrared photons. The energy associated with each absorbed
photon spreads throughout the ion via intramolecular vibrational relaxation (IVR), increasing the ion’s internal energy until the weakest bond dissociates.\textsuperscript{208}

To improve sensitivity and resolution, the “messenger technique” is employed,\textsuperscript{209,210} whereby a weakly bound metal cation-saccharide complex is formed to promote the cation removal through a one- or two-photon absorption process. Common cations are taken from the alkali metals whose binding strengths increase with decreasing size.\textsuperscript{211,212} Here, simple protonation results in glycosidic bond cleavage.\textsuperscript{213} Strongly bound lithium-sugar complexes, which were used by Polfer et al. in IRMPD wavelength dependent fragmentation, cleave across saccharide rings and at the glycosidic bond.\textsuperscript{22} Potassium, rubidium, and cesium ions, however, all dissociate readily without fragmenting the saccharide. Of these, rubidium promotes clear spectra and was the choice for the experiments reported in this chapter.

Earlier IRMPD spectra obtained by Valle et al. explored the infrared range from 600 - 1700 cm\textsuperscript{-1} to examine vibrational characteristics of rubidium-glycoside isomers O-methyl-\(\alpha\)-D-glucoside (\(\alpha\)Glc), O-methyl-\(\beta\)-D-glucoside (\(\beta\)Glc), O-methyl-\(\alpha\)-D-galactoside (\(\alpha\)Gal), and O-methyl-\(\beta\)-D-galactoside (\(\beta\)Gal) (Figure 5-1) at the Free Electron Laser for Infrared eXperiments (FELIX) facility at the FOM-Institute for Plasma Physics Rijnhuizen in the Netherlands.\textsuperscript{214-216} In these experiments, only a few questionably differentiable characteristics were found between the isomers (Figure 5-2). After some consideration and computational verification, the four hydroxyl groups in the relatively isolated O-H stretch region (~ 3200 - 3650 cm\textsuperscript{-1}) were thought to have a greater potential for differentiation of the monosaccharide structures.

An optical parametric oscillator (OPO) laser aligned with the ions in the cell of a Fourier transform ion cyclotron resonance (FTICR) mass spectrometer provided the wavelength range (2,270 - 4,670 nm/4405 - 2141 cm\textsuperscript{-1}) and power (50 - 150 mW) necessary to obtain the spectra of
the C-H and O-H stretches for the four complexes. A comparison of the spectra revealed differentiable features. Computational studies were carried out utilizing density functional theory (DFT) with the B3LYP hybrid functional and 6-31+G (d) and LANLD2DZ basis (for Rb), from which zero point energies and vibrational frequencies for a number of conformers were obtained. The adjusted theoretical spectra of the lowest energy conformers were compared to the experimental spectra, revealing candidate gas-phase structures responsible for the vibrational bands observed. This included the discovery that multiple conformers, differing largely in their hydrogen bond interactions, were responsible for the variation of O-H stretches in the two glucoside spectra.

Experimental Methods

Materials

The four monosaccharide isomers of O-methyl D-glycoside were obtained from Dr. Brad Bendiak of the Department of Cellular and Structural Biology at the University of Colorado Health Sciences Center. Methanol and rubidium chloride were purchased from Sigma-Aldrich and milliQ water was acquired from the physical chemistry teaching laboratory at the University of Florida. Stock solutions were prepared by dissolving 10mg of each monosaccharide in four separate 10 ml solutions of 80:20 MeOH:H₂O. Samples were taken as needed and diluted to 1x10⁻⁴M at the same solvent ratio. Rubidium chloride (cationizing agent) was then added in an equimolar amount.

Instrumentation

A tunable continuous-wave optical parametric oscillator (OPO) laser aligned to the cell of a 4.7T Bruker Apex II FTICR mass spectrometer (Bruker, Billerica, MA) with an Analytica electrospray (ESI) source (Analytica of Branford, Inc., Branford, CT), was employed for these experiments (Mass Spectrometry Services Laboratory at the University of Florida). Electrospray
ionization at a flow rate of 2μL per min was assisted by nebulizer and desolvation gas (N₂) flows at a rate of 35 and 155 L/hr, respectively. A 3.6kV potential difference was applied between the ESI needle and capillary. Parent ions were isolated using swept frequency ejection pulses and irradiated. The products (depleted parent and rubidium ions) were detected in a 70 - 500 m/q window by broadband detection. Bruker Xmass data acquisition system 7.0 was responsible for setting instrument parameters and data collection. The LINOS OS 4000 OPO laser was purchased from LINOS Photonics (Munich, Germany).

**Optical Parametric Oscillator Laser**

The tunable cw-OPO laser operates according to the nonlinear optic principles of second order susceptibility as seen in Chapter 4. Here, electromagnetic radiation from the continuous-wave pump laser (Nd:YAG), operating at 2W and a fixed wavelength of 1064 nm, interacts with a periodically poled lithium niobate crystal in one of eighteen poling periods, to produce two different frequencies

\[ \omega_p = \omega_s + \omega_i \]  

(5-1)

where \( \omega_p, \omega_s \) and \( \omega_i \) are the frequencies of the pump, signal, and idler, respectively. A gradual increase in temperature from 50 to 150 °C increases the signal wavelengths and decreases the idler wavelengths within the capacity of the period and the temperature range. This process brings consecutive groups of wavelengths into resonance as the previous wavelengths disappear. Wavelength discrimination is further enhanced by the inclusion of an etalon in the resonance cavity. After acquisition of data for a given wavelength range, the temperature was reduced and the crystal’s position changed to an adjacent poling period to introduce the next range of wavelengths. The 18 poling periods available with the LINOS laser cover wavelength ranges of
1.38 - 2.0 and 2.28 - 4.67 µm or 7246 - 5000 and 4405 - 2141 cm⁻¹ for signal and idler, respectively.

The wavelength used for dissociation (from the idler beams) is calculated from the wavemeter’s output through a rearrangement of the conservation of energy relationship (based on Equation 5-1) between the wavelengths of the pump (νₚ =1064nm), signal (νₛ = wavemeter), and idler (νᵢ) given as

\[
\frac{1}{\nu_i} = \frac{1}{\nu_p} - \frac{1}{\nu_s}.
\]  

(5-2)

For the C-H stretch region, a monochromatic (10.6 µm) cw Synrad CO₂ laser (Mukilteo, WA) beam was directed central to and on-axis with the idler beams for off-resonant dissociation of OPO excited ions in the quasicontinuum.

**Experimental Procedure**

After preparing a solution of one of the four Rb⁺ [O-methylated-glycoside] complexes, the ions were introduced to the FTICR mass spectrometer via electrospray ionization, accumulated in a hexapole ion trapping region for 1s, and transferred to the cell through a series of ion optics. Once trapped in the cell, the Rb⁺ [O-methylated-glycoside] precursor ions were isolated and irradiated by the OPO idler beams at a set wavelength for 10s (for O-H stretches). Mass spectra of the resulting ions were then acquired. Ten scans were accumulated to improve signal to noise and the relative abundances of the precursor and product (⁸⁵Rb⁺ and ⁸⁷Rb⁺) ions were recorded. Subsequently, the OPO laser was adjusted for the next wavelength and the process was repeated. Mass spectra consisted of either the Rb⁺ [O-methylated-glycoside] precursor ions with no dissociation products (nonresonant wavenumbers) or the depleated precursor ions with the Rb⁺ dissociation products (resonant wavenumbers). The action spectrum was then plotted as the
power-corrected natural logarithm of the $\frac{P + F}{P}$ ratio versus $\bar{\nu}$, where $F$ corresponds to the combined relative abundance of $^{85}\text{Rb}^+$ and $^{87}\text{Rb}^+$ ions and $P$ the relative abundance of the depleted precursor ions. When only the precursor ions exist, the value for $\ln \frac{P + F}{P}$ reduces to zero.

Rubidium ions were readily dissociated by resonant absorption of infrared photons in the OH stretch region. However, the C-H stretches’ relatively weak absorption generated little or no rubidium peaks under the same conditions (Figure 5-4A). Fluence assistance to the OPO photon resonant excited ions (in the quasicontinuum) was provided by off-resonant photons from the CO$_2$ laser. The off-resonance condition was assured by past action spectra (Figure 5-2), which illustrated a relatively featureless absorption region at the CO$_2$ laser’s wavelength of 10.6μm. Also, any blackbody and/or off-resonant effects were avoided by changing the CO$_2$ laser’s power and irradiation time until a product-free spectrum was achieved while operating only the CO$_2$ laser over the total irradiation period. Following the aforementioned protocol, the trapped and isolated ions were irradiated for ten seconds with the OPO laser, with the additional fluence of the CO$_2$ laser during the last three seconds. This resulted in little dissociation. A few more trials were completed until a total OPO irradiation time of 15 seconds, with the CO$_2$ laser operating concurrently for the last 7 seconds, produced sufficient fragmentation, as shown in Figure 5-4B. All other conditions were the same for both O-H and C-H stretches. Two to three spectra were obtained and averaged (bin size 2cm$^{-1}$) for each of the four Rb$^+$ [O-methylated-glycoside] complexes.
**Computational Methods**

General structures of the four isomers of O-methylated D-glycosides were drawn in Hyperchem\textsuperscript{222} according to the stable gas-phase conformations found in the literature: the \( ^4 \text{C}_1 \) conformation with the OH groups involved in a hydrogen-bonding network oriented in a clockwise arrangement.\textsuperscript{220,221,223,224} A rubidium cation was artificially attached to the O-1 oxygen and placed above and central to the glycosidic ring system (at a distance of \( \sim 3 \text{Å} \)). Ring dihedral angles and H-C-O-H torsional angles, promoting the rotation of the hydroxyl groups around the C-O bonds, were defined such that the angles changed independently of one another. During the conformational search, the dihedral angles were modified through Hyperchem’s flex and torsion algorithm while the torsional angles were randomly varied, for a total of 1000 structures. Subsequently, the structures were geometrically optimized with AMBER force field (AM 1) and comparisons were made to eliminate duplicate and higher energy structures. Duplicate criteria were set to disregard conformers whose threshold energies, dihedral angles and atomic positions were within 0.05Kcal/mol, 10° and 0.25Å of another, respectively. The higher energy threshold was set 15Kcal/mole above the lowest energy conformer to contain the range of possible conformers. Geometric optimization of these remaining conformers was carried out using B3LYP/6-31+G(d) in addition to LANDL2DZ basis\textsuperscript{228,229} for the sugar and the rubidium ion, respectively, followed by the vibrational calculations, with *Gaussian 03*.\textsuperscript{217} When these structures proved inadequate to explain the existing glucoside anomeric spectra, other ring structures were created and analyzed. Scaling factors of 0.97 and 0.96 were used for the frequencies associated with the O-H and C-H stretches, respectively, within the error of the corresponding level of theory and the results for similar saccharides.\textsuperscript{220,221,224,230-234}
Experimental Results and Discussion

The vibrational spectra of the lowest energy conformations for these species, convoluted with a Gaussian band profile of 20 cm\(^{-1}\), were compared in the Ph.D. thesis of Cesar S. Contreras to those obtained in the aforementioned experiments by Valle et al. in the 900 - 1400 cm\(^{-1}\) range. Of these, the lowest energy conformer’s spectrum agreed with experiment as shown in the Figure 5-5. However, the experimental bands were too broad and similar to reveal any major differentiable features. For this reason, the O-H stretches were used in the present work.

Glucosides

O-methyl-\(\beta\)-D-glucoside. The action spectrum of O-methyl-\(\beta\)-D-glucoside ranges from 2750 to 3750 cm\(^{-1}\) and includes both O-H and C-H stretches, which consist of four and two bands, respectively. These bands are labeled I - VI proceeding from the higher to lower wavenumbers, as seen in Figure 5-6. Even though C-H stretches from 2800 to 3050 cm\(^{-1}\) are in general agreement with the calculated lowest energy structure, the theoretical spectrum is unable to account for all bands in the experimental O-H stretch region from 3378 to 3700 cm\(^{-1}\). In order to complete the coverage of these O-H bands, the four lowest energy conformers (all within the error associated with the level of theory) are considered and differ in their intramolecular hydrogen bond(s) and/or Rb\(^+\) [O-methyl-\(\beta\)-D-glucoside] interactions.

The two lowest energy conformers, 1\(\beta\)Glc and 2\(\beta\)Glc, at 0.00 and 0.8 kcal per mole, respectively, have similar structures and vibrational spectra, as seen in Figure 5-7. Here the Rb\(^+\) cation is found above the ring near the pocket created by the C-6 hydroxyl group, O-1, and the C-1 O-methyl group. Conformers 1\(\beta\)Glc, 2\(\beta\)Glc, and 3\(\beta\)Glc exist in the \(^4C_1\) ring conformation. The next highest in energy at 5.18 and 6.49 kcal per mole are conformers 3\(\beta\)Glc and 4\(\beta\)Glc, respectively. The Rb\(^+\) attached to conformer 3\(\beta\)Glc exists outside the ring between hydroxyl groups on C-4 and
C-6. In conformer $4_{\beta\text{Glc}}$, the Rb$^+$ ion is under the ring (with respect to the C-6 carbon above) between the C-2 and C-4 hydroxyl groups and O-1. This position is made favorable by the $^1\text{C}_4$ half-chair ring structure of $4_{\beta\text{Glc}}$.

The assignments of the bands are as follows. Conformers $1_{\beta\text{Glc}}$, $2_{\beta\text{Glc}}$, and $4_{\beta\text{Glc}}$ in Figure 5-7 account for band I, which is centered at 3677 with 21 cm$^{-1}$ full width at half maximum (fwhm). This band relates to the O-H stretch modes that are relatively unencumbered by inter- or intramolecular bond interactions. Free O-H stretches are associated with the hydroxyl group on C-2 for $1_{\beta\text{Glc}}$ and $2_{\beta\text{Glc}}$ and the hydroxyl groups on C-2 and C-4 in structure $4_{\beta\text{Glc}}$.

Band II at 3637 cm$^{-1}$ with a fwhm of 9 cm$^{-1}$ is the result of either a hydrogen bond or Rb$^+$ [O-methyl-\(\beta\)-D-glcoside] interactions. A hydrogen bond between the C-4 hydroxyl hydrogen and the C-3 oxygen occurs in $1_{\beta\text{Glc}}$, $2_{\beta\text{Glc}}$, and $3_{\beta\text{Glc}}$. Structure $3_{\beta\text{Glc}}$ introduces a second weaker interaction between the C-3 hydroxyl hydrogen and the C-2 oxygen. There is also rubidium interplay with the hydroxyl oxygen on C-6 for conformers $1_{\beta\text{Glc}}$ and $2_{\beta\text{Glc}}$. These motifs weaken and shift the O-H stretches from band I to band II.

Band III at 3560 cm$^{-1}$ with a fwhm of 20 cm$^{-1}$ is accounted for by the $3_{\beta\text{Glc}}$ and $4_{\beta\text{Glc}}$ conformers. In structure $3_{\beta\text{Glc}}$, the rubidium cation interacts with the C-4 hydroxyl oxygen to further weaken the hydrogen bonded O-H stretch mentioned above, and this interaction corresponds to the larger peak (3597 cm$^{-1}$) in band III. The interrelation between the hydroxyl of C-6 and O-methyl oxygen of C-1 creates one of two interconnected hydrogen bonds in the $4_{\beta\text{Glc}}$ structure, which in this case moves the O-H vibrational mode to the smaller peak (3540 cm$^{-1}$) in band III.

Band IV is centered at 3450 cm$^{-1}$ with a fwhm of 32 cm$^{-1}$. With the $4_{\beta\text{Glc}}$ conformer’s $^1\text{C}_4$ structure (which tucks the C-4 hydroxyl group under the ring), the hydroxyl hydrogen from C-3
is attracted to the oxygen on C-6 forming a second hydrogen bond across the ring. The network of hydrogen bonds helps shift this frequency to band IV.

The C-H region consists of two bands V, VI, that are made more evident by the two-laser experiment considered earlier. The C-H stretches correspond to the symmetric (VI) and antisymmetric stretches (V). Lowest in energy, the symmetric modes are related combinations of degenerate C-H motions from the ring, the ethyl group on C-6, and methyl group on C-1. Antisymmetric stretches are related to the ethyl group on C-6 and the methyl group on C-1 (from lowest to highest energies of vibration, respectively). These have higher predicted energies than the values in the experimental spectra. The predicted spectra of all four conformers, shown with the experimental spectrum in Figure 5-8, illustrate good overlap with all bands.

**O-methyl-α-D-glucoside.** The O-H stretch region of O-methyl-α-D-glucoside correlates well with the βGlc spectrum bands with the notable exception of a nonexistent band II (Figure 5-9). Bands I, III, and IV have bandwidths of 21, 54, and 45 cm\(^{-1}\), respectively. The C-H stretches are in similar positions as well, although the intensities are reversed and the overall wavenumber range is decreased to 2900 - 3050 cm\(^{-1}\). Comparable to the βGlc, the O-H stretching region of O-methyl-α-D-glucoside’s lowest energy conformer (Figure 5-10) contains bands I and II, but the O-H stretches in bands III and IV are not accounted for. As band II does not exist in the experimental spectrum, two other calculated conformers are considered, 1\(_{αGlc}\) (\(^1\)C\(_4\)) and 2\(_{αGlc}\) (\(^O,3\)S), as seen in Figure 5-11.

Unperturbed hydroxyl groups on C-2 and C-4 for 1\(_{αGlc}\), as well as on C-3 for 2\(_{αGlc}\), correspond to band I. Conformer 1\(_{αGlc}\) has only one hydrogen bond, the hydroxyl hydrogen on C-3 to the oxygen on C-6, which accounts for band III. Band IV is formed by structure 2\(_{αGlc}\), the result of hydrogen bonding from the hydroxyl hydrogen of C-6 to the oxygen on C-4 and
interaction of the Rb$^+$ with oxygen on C-6. Due to the lack of precise overlap between the computational conformers and the experimental bands III and IV, further computational work is necessary. Although the peak heights are reversed, the C-H stretches agree in general with the assignments of βGlc, following the pattern of symmetric (VI) to antisymmetric (V) stretches.

**Galactosides**

**O-methyl-β-D-galactoside.** For βGal, four bands (II, III, V, and VI) appear at 3650, 3580, 2959, 2915 cm$^{-1}$, with bandwidths of 30, 17, 17, 29 cm$^{-1}$, respectively. Experimental and computational spectra are illustrated in Figure 5-12 with the corresponding lowest energy structure. The general composition of the conformer is $^4$C$_1$ with the rubidium cation above and central to the C-6 oxygen, O-1, and C-1 O-methyl group.

The O-H stretch region spans 3550 to 3680 cm$^{-1}$ covering bands II and III. Relatively weak interaction of the C-2 and C-4 hydroxyl hydrogens and the oxygen of C-3 places the C-3 and C-2 O-H stretches at the blue shifted band II (band II’s energy is slightly higher in βGal than in βGlc and includes some overlap within the region of band I). A hydrogen bond between the hydroxyl hydrogen of C-6 to the oxygen on C-4 and the C-6 oxygen connection with the rubidium cation further shifts these modes to band III. Although the overall coverage is good, the theoretical peaks are a bit red shifted with slightly better vibrational mode resolution in comparison to those in the experimental spectrum.

The C-H stretch assignments of βGal follow the pattern of βGlc. Comparison of computational (2855 to 3062 cm$^{-1}$) and experimental (2855 to 3000 cm$^{-1}$) regions indicates common spectral features with a higher energy spread for the theoretical antisymmetric stretches.

**O-methyl-α-D-galactoside.** The experimental spectrum of the αGal isomer consists of five bands centered at 3660, 3600, 3550, 2960, 2915 cm$^{-1}$, with respective broad bandwidths of 30, 27, 38, 27 and 30 cm$^{-1}$. Figure 5-13 includes the αGal experimental and computational spectra of
the lowest energy conformer (including structure) relating to bands II, III, V, and VI. Similar to βGal, αGal’s band II is blue shifted from the αGlc position. The best agreement of theoretical and experimental spectra for alpha galactose, as for βGal is the $^4C_1$ structure with the rubidium cation between O-1 and the oxygen of C-6.

The lowest energy structures and computational spectra of αGal and βGal are similar, with two notable differences: the computed αGal spectrum shows better resolution of the vibrational modes and predicts a red shift in the features of band III. When compared to the experimental spectrum in Figure 5-13, these distinctions become apparent. The experimental spectrum of αGal has two clear peaks within the blue shifted band II (compared to only a slight splitting for βGal), where the vibration of relatively free hydrogen from the C-3 hydroxyl group is of slightly higher energy than the C-2 O-H stretch, which weakly interacts with the C-3 oxygen. Two distinct areas of lower and higher energy also exist for the C-4 and C-6 O-H stretches (band III), respectively. It appears that the closer rubidium interaction (due to the displaced oxygen in the axial position of the alpha sugar) with the C-6 O-H stretch shifts the mode to the lower energy region of band III, while the C-4 O-H stretch experiences a corresponding slight increase in energy. Overall, the computationally determined spectra of the O-H stretches overlap with the experiment, although a red shift is noted. The long shoulder that tails into band IV is yet to be accounted for (experiment pending).

Alpha-galactoside has a C-H stretch pattern comparable to that of αGlc. The computational and experimental spectra of the C-H stretches (Figure 5-13) illustrate some differences in bands V and VI. However, symmetric stretches (VI) agree in position but are not of the same intensity. In the predicted spectrum, asymmetric stretches of greater energies also form distinct peaks above 2980cm$^{-1}$. 
Isomeric differentiation. There are two general methods of differentiating isomers from their corresponding spectra. The first is noting the wavelength(s) where the presence or absence of isomer band(s) takes place with respect to the other isomer band(s). The second is contrasting overlapping spectral features and looking for defining ratio differences.

Figure 5-14 groups together all four isomers’ O-H stretch spectra where isomeric differences are found for each band. Band I is comprised of the free O-H stretches of αGlc and βGlc anomers. Even though the tail ends of αGal and βGal are present, the large intensity difference or a careful choice of wavenumbers (in a range >3689cm⁻¹) allows the differentiation between the two sets of D-glycosides. Band II indicates strong peak intensities for βGal and βGlc, with smaller peaks for αGal, and absence of a peak for αGlc. Due to the blue shifted βGal peak, if the ratio of the peaks of βGlc/βGal = 3637cm⁻¹/3649cm⁻¹ is >1, <1, ≈1 then the presence of βGlc and/or βGal, βGal and/or αGal (no βGlc), and αGlc (no other isomers), respectively, is likely.

Further comparison between the ratios βGlc and βGal for the >1, <1 conditions

\[
\left( \frac{\beta\text{Glc}}{\beta\text{Gal}} \right)_{3637\text{cm}^{-1} \text{ to } 3649\text{cm}^{-1}} \quad \text{and} \quad \left( \frac{\beta\text{Gal}}{\beta\text{Glc}} \right)_{3637\text{cm}^{-1} \text{ to } 3649\text{cm}^{-1}}
\]

provides another means of differentiation, where differences in the size of the ratios offer the potential for determining the presence of isomers indicated by the subscripts (=,≠). This technique is easily extended to and between other bands in the spectra. All isomers are accounted for in band III. The shoulder at ~3597cm⁻¹ signifies αGlc and/or αGal and at 3609.5cm⁻¹ αGal
existence. A lack of dissociation in the region $3544 \text{ cm}^{-1}$ and $3572 \text{ cm}^{-1}$ points toward the nonexistence of all isomers, with the possible exception of the respective $\beta$Gal and $\alpha$Gal ions. There are also relative intensity differences between the D-glycosides and each anomer. Band IV is dominated by the $\alpha$Glc and $\beta$Glc peaks where, in their absence, the $\alpha$Gal is barely present and $\beta$Gal is completely missing. If dissociation is there, the difference in the intensities of the peaks $\alpha$Glc, $\beta$Glc, and $\alpha$Gal and/or comparison to other spectral features (bands) can offer a means of differentiation. With the C-H stretches (Figure 5-15), the intensities of band V and VI for alpha and beta anomers are inverse of each other, where a simple ratio should indicate the anomeric form. The beta anomers also possess a low energy tail where alpha anomers do not.

**Conclusion**

Frequencies ($\text{cm}^{-1}$) of the O-H and C-H stretches for the ions of rubidium-D-glycoside isomers, $\text{Rb}^+[\text{O-methyl-}\alpha\text{-D-glucoside}]$, $\text{Rb}^+[\text{O-methyl-}\beta\text{-D-glucoside}]$, $\text{Rb}^+[\text{O-methyl-}\alpha\text{-D-galactoside}]$, and $\text{Rb}^+[\text{O-methyl-}\beta\text{-D-galactoside}]$ are capable of being characterized through IRMPD in a FTICR mass spectrometer. Early attempts to find a means of differentiating four D-glycoside isomers in the mid-IR range found little discernable difference. However, in examining the less congested O-H and C-H stretch regions, distinctions are found. These differences follow patterns of H-bonding and $\text{Rb}^+$ ion interactions. The highest energy band I is the result of the unperturbed motion of the O-H stretch. From here, weaker hydrogen bonding along the ring and independent $\text{Rb}^+$ interaction shifts the vibrations to band II. In band III, the rubidium interaction with oxygen involved with ring hydrogen bonds further weakens the interrelated O-H stretch. In addition, other combinations of hydrogen bonds and single cross ring hydrogen bonds in the $^1\text{C}_4$ conformers appear to also shift the frequencies to band III. The cross ring hydrogen bonding, when combined with another interconnected hydrogen bond, shifts the former into band IV. As expected, the general trend appears to indicate that the more involved
the rubidium ion and the stronger the interaction(s) of the hydrogen bond(s), the weaker the O-H stretch. The differentiation of all four isomers is the result.

Figure 5-1. The basic structure of O-methyl-α-D-glucopyranoside (αGlc), O-methyl-β-D-glucopyranoside (βGlc), O-methyl-α-D-galactopyranoside (αGal), and O-methyl-β-D-galactopyranoside (βGal) isomers (all with a molecular weight of 194) with the C-1 O-methyl and C-5 hydroxyl groups in the axial/equatorial positions for alpha/beta and the glucoside/galactoside conformations respectively.
Figure 5-2. Action spectra of the four glycoside isomers αGlc, βGlc, αGal, and βGal from 600-1700 cm⁻¹ obtained by Valle et al. at FELIX [Adapted with permission from Valle, J.J. 2005. Differentiation of Carbohydrate Stereoisomers by Infrared Multiple Photon Dissociation Using a Free Electron Laser and a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer. Ph.D. dissertation (Page 115, Figure 5-5) University of Florida, Gainesville, Florida.]
Figure 5-3. The OPO laser general set up with the idler 1/signal and idler 2 beams coming from the front and back of the laser box (corresponding to the resonance cavity inside of the OPO 4000), respectively. The signal beam travels to the wavemeter as both idler 1 & 2 are optically guided to the center of the FTICR cell. An off-resonance CO\textsubscript{2} laser is used in the double laser experiments. Two iris shutters (one illustrated here), regulate the beams’ entry and, when closed, guide the beams into the power meter.

Figure 5-4. Differences in the C-H stretch in action spectra of βGlc. A) resonant OPO laser only; B) resonant OPO laser coupled with an off-resonant CO\textsubscript{2} laser. The CO\textsubscript{2} laser provides additional energy to the already excited ions by the OPO laser in the quasicontinuum.
Figure 5-5. Theoretical (color) and experimental (grey) spectra of αGlc(A), βGlc(B), αGal(C) and βGal (D), indicating the similarities between each isomer in the 900-1400 cm\(^{-1}\) region. [Adapted with permission from Contreras, C.S. 2008. Carbohydrates and Amino Acids: Infrared Multiple Photon Dissociation Spectroscopy and Density Functional Theory Calculations. Ph.D. dissertation (Page 134-135, Figures 6-7, 8, 9) University of Florida, Gainesville, Florida.]

Figure 5-6. Action spectrum of O-methyl-β-D-glucoside (βGlc) indicating the general ranges and specific bands of C-H (2 bands) and O-H (4 bands) stretching motions.
Figure 5-7. Comparison of the experimental (red) and theoretical spectra (with structures) of the four conformers ($1_{\beta\text{Glc}}$, $2_{\beta\text{Glc}}$, $3_{\beta\text{Glc}}$, $4_{\beta\text{Glc}}$) of O-methyl-$\beta$-D-glucoside.
Figure 5-8. Overview of the theoretical and experimental spectra for rubidium tagged βGlc, with conformers $1_{\beta\text{Glc}}$ (dk blue), $2_{\beta\text{Glc}}$ (green), $3_{\beta\text{Glc}}$ (aqua), and $4_{\beta\text{Glc}}$ (dk grey) compared to the experimental spectrum (red). All six bands are accounted for.

Figure 5-9. The IRMPD spectra of αGlc (blue) and βGlc (red) illustrates similar overlap with the noted absence of band II.
Figure 5-10. Action spectra of O-H stretching region for αGlc (blue) and the calculated lowest energy conformer. [Reprinted with permission from Contreras, C.S. 2008. Carbohydrates and Amino Acids: Infrared Multiple Photon Dissociation Spectroscopy and Density Functional Theory Calculations. Ph.D. dissertation (Page 141, Figure 6-19) University of Florida, Gainesville, Florida.]
Figure 5-11. The IRMPD spectra and structures for conformers $1_{\alpha}\text{Glc}$ (black and left) and $2_{\alpha}\text{Glc}$ (blue-green and right) and the experimental spectrum (blue) [Adapted from Contreras, C.S. 2008. Carbohydrates and Amino Acids: Infrared Multiple Photon Dissociation Spectroscopy and Density Functional Theory Calculations. Ph.D. dissertation (Page 142, Figure 6-20, 21) University of Florida, Gainesville, Florida.]

Figure 5-12. Experimental and theoretical spectra for $\beta\text{Gal}$ (exp.: neon green, theory: black) and lowest energy conformer structure.
Figure 5-13. Experimental and theoretical spectra for αGal (exp.: brick red, theory: black) and lowest energy conformer structure.

Figure 5-14. Spectra of the O-H stretch region for all four isomers with spectral band labels.
Figure 5-15. Spectra of the C-H stretch region for all four isomers with spectral band labels.
CHAPTER 6
THERMOCHEMICAL DYNAMICS OF DEPHOSPHORYLATION

In recent years, mass spectrometry has evolved into one of the most important tools in protein chemistry. Significant to proteomics is the identification and characterization of posttranslational modifications; principally phosphorylation of the amino acids serine, threonine, and tyrosine. Catalyzed by kinases, phosphorylation is an exothermic process that occurs through the transfer of a phosphate group from adenosine triphosphate (ATP) to the alcohol side chain of the amino acid. Since it is reversible, phosphorylation controls many cellular functions, like metabolism, protein synthesis, growth, and reproduction. This is largely accomplished through peptide conformational changes brought about by the insertion/interaction and removal/noninteraction of the phosphate group and its negatively charged oxygens (under physiological conditions) with the protein. Understanding the kinetics and the activation energies associated with dephosphorylation gives insight into the nature of the phosphate-peptide relationship.

Traditionally, tandem mass spectrometry is used to identifying the presence and/or sequence of amino acids bearing phosphate groups through their fragmentation products. In order to accomplish this collision-induced dissociation (CID), electron-capture dissociation (ECD), infrared multiple photon dissociation (IRMPD), etc., are employed as sole and combined fragmentation techniques within the field of mass spectrometry. Of the approaches used, Fourier transform ion cyclotron resonance (FTICR) mass spectrometry is one of the most capable with MS^n capability, high resolution, and mass accuracy. Here, precursor ions are isolated, activated, and then dissociated. The presence of phosphopeptides is noted by the neutral phosphate loss from their precursor ions (typically HPO_3 and H_3PO_4, which measure 80 and 98 Daltons, respectively). A difficulty in these techniques is the fragile posttranslational...
modification that readily dissociates requiring careful determination of dissociation conditions. This restriction, however, allows for the relative isolation of the dephosphorylation reaction and simplifies the study of the phosphopeptides’ mechanisms through the use of kinetics, energetics, and IRMPD spectroscopy.  

In the early 1990s, Dunbar proposed that the long, relatively collision-free retention times of an FTICR mass spectrometer offers a means to study slow kinetic processes that develop from ions irradiated in the cell. He theorized that a continuous wave infrared laser, at low power, is capable of acting as a primary thermal source in equilibrium with the spontaneous and stimulated emission of photons from the ions in an ICR cell. Under these conditions, a Boltzmann-like distribution of energy with an effective temperature is established. Dissociation of an ion’s lowest energy bond occurs when the ion’s energy exceeds a threshold energy ($E_t$) typically associated with the high energy tail of the distribution. By plotting the natural log of the dissociated parent ion fraction versus time, dissociation rate constants ($k_d$) are calculated. And through the application of a modified form of the Arrhenius equation, relative activation energies are determined according to

$$\frac{d \ln k_d}{d \ln P} C = E_a$$  

where $k_d$ is the unimolecular rate constant, $P$ the laser power, $C$ a proportionality factor, and $E_a$ the relative activation energy as seen in Chapter 3. However, the exact relationship between the laser power and temperature is difficult to quantify. The association was made clearer after McMahon and Dunbar discovered that blackbody infrared dissociation (BIRD) is possible through the heating of the ICR cell. Through master equation modeling, the discovery of the rapid exchange limit and comparative experimental results between the BIRD and IRMPD data, Paech and Williams established a means to correlate power to temperature for a series of
varying sized peptides, where the constant C in Equation 6-1 is given a value of \( \frac{1}{4.34} \) (Dunbar C= \( q\hbar \) as defined in Chapter 3)\(^{33,120,123-135,138,139}\)

Two tunable cw-CO\(_2\) lasers (the Apollo and Lasy-20G-AT) were used in the course of these experiments at wavelengths of 9.294, 9.588, and 10.632 \( \mu \)m. Each wavelength was used in turn to examine the kinetic and activation energy similarities and differences for both +2 and -2 charge states of a monophosphorylated peptide ion consisting of 16 residues and a mass of 2061.8291 Da.

Action spectra were also taken for both charge states. These experiments involved the Lasy-20G-AT tunable cw-CO\(_2\) laser beam focused on the phosphopeptide ions in the cell of a 4.7T FTICR mass spectrometer. The dissociation process begins when the IR photon’s wavelength is in resonance with an ion(s) vibrational mode, resulting in energy absorption. The energy is then redistributed to background vibrational states through internal vibrational relaxation (IVR), building up to eventually dissociate the weakest bond(s).\(^{99}\) By measuring the fraction of the precursor ion remaining in the mass spectrum particular to a specific wavelength, for a series of mass spectra/wavelengths, an IRMPD spectrum is formed.\(^{95}\) These results are used to help determine the effect, if any, of wavelength choice and the ion’s charge on the kinetic/energetic picture.

**Experimental Methods**

**Materials**

The monophosphopeptide was purchased from Sigma-Aldrich as part of the phosphopeptide positive control set (product number P9615) and consisted of Phe-Gln-pSer-Glu-Glu-Gln-Gln-Thr-Glu-Asp-Glu-Leu-Gln-Asp-Lys residues taken from an HPLC purified tryptic digest of B-Casein. Methanol and formic acid were purchased from Sigma-Aldrich and
Fischer-Scientific, respectively, and the milliQ water was obtained from the physical chemistry teaching lab at the University of Florida. Stock solutions were prepared by dissolving 0.1mg of the monophosphopeptide in 1000 µl of milliQ water. From this supply 100 µl was taken and diluted to 1000 µl comprising a 50:50 MeOH:H2O solution. Protonation was accomplished through the addition of 1% formic acid and controlled deprotonation of the acidic peptide through the addition of ~1.18x 10⁻⁶ M formic acid.

Instrumentation

The experiments were performed on two instruments. Positively charged ion experiments were executed in the Infinity cell of a passively shielded 4.7T (Magnex Scientific Ltd; Abington, U.K.) Bruker (Bruker-Daltronics; Billerica, MA) FTICR mass spectrometer (BioApex 47e) united to an Analytica (Branford, CT) electrospray ionization (ESI) source and a modified heated metal capillary (120°C) with a conical inlet. Phosphopeptide solutions of 4.847 x10⁻⁶ M were introduced into the ESI source, via a fused silica transfer line, at a flow rate of 15µL per hr. and a 2.3 kV potential was applied between the ESI needle and capillary. Predator (an extension of the modular ICR data and acquisition and analysis system (MIDAS)²⁵³ and the external source control (XSRC) programs designed by Dr. David Dearden’s lab at Brigham Young University were used to control the functions of the infinity cell/data acquisition and the ion optics, respectively. Apollo and Lasy-20G-AT tunable cw-CO₂ lasers were used as the source of IR photons.

Negatively charged ion experiments were performed in the Infinity cell of an actively shielded 4.7T Bruker Apex II FTICR mass spectrometer coupled to an Analytica ESI source (Mass Spectrometry Services Laboratory at the University of Florida). Solutions of 4.847 x10⁻⁶ M were used at a flow rate of 2µL per min. The ESI source was assisted by a nebulizer and
desolvation gas (N₂) at a rate of 35 and 155 L/hr, respectively. A 2.9kV potential was applied between the ESI needle and capillary. Instrument parameters and data collection were performed by the Bruker Xmass data acquisition system 7.0. A Lasy-20 G-AT tunable cw-CO₂ laser was the sole source of IR photons.

Initial positive ion experiments were begun with the repaired Apollo CO₂ laser. The Lasy-20G-AT CO₂ laser was later integrated into the system, as seen in Figure 4-8, and became the laser of choice due to automated wavelength control and superior power stability. The laser beams were reflected from a series of gold mirrors (the second to last a focusing mirror) to pass through a ZnSe window into the cell located directly opposite the ions’ entrance. A laboratory-constructed laser gate was installed to regulate the irradiation time. In the upright position, a mirror mounted on the gate redirected the beam to the power meter. When activated from Predator’s reaction delay event, the gate lowered and allowed the beam to pass.

**Experimental Procedure**

After preparing the appropriate solutions and injecting the sample into the source, ions were accumulated for 2s in a hexapole storage region and transferred via ion optics to the cell of the FTICR mass spectrometer. The phosphopeptide ion signal was found, optimized, and isolated. Positive ion isolation took place in two stages utilizing stored waveform inverse Fourier transform (SWIFT) and chirp ejections. Negative ions were isolated with swept frequency ejection pulses. A 0.5sec delay time was introduced after each isolation step to cool the ions. Once trapped and isolated the ions were irradiated by the CO₂ laser at one of three wavelengths (9.294, 9.588, and 10.632 µm) at 1W of power for an irradiation time of 1s. Thirty scans were taken to improve s/n and the relative abundances of precursor and product ions. The procedure was repeated for 2s, 3s, etc., the power adjusted, and the process continued for each successive increase in power until the data for the kinetic and Arrhenius plots were complete. The
wavelength was then changed and the process repeated until all the wavelengths were finished. Mass spectra of the precursor phosphopetide (P) and product (F) ions were analyzed and their relative abundances noted in an Excel spreadsheet, as seen in Table 6-1. The natural log of \([P]/([P]+[F])\) versus irradiation time plots were created and the dissociation rate constants \((k_d)\) obtained from the absolute value of the slopes. Activation energies \((E_a)\) were then determined from the modified Arrhenius plot of the \(\ln [k_d]\) versus \(\ln [\text{laser Power}]\) associated with each \(k_d\), as seen in Equation 6-1.

Ions for the action spectra were introduced and isolated in the cell by the methods previously mentioned. The irradiation time, however, was limited to 1s at 5W for positively charged ions and 2.5 watts for the negatively charged ions. A mass spectrum was taken for each wavelength in the CO\(_2\) spectrum from 9.174 – 9.354/9.443 – 9.773 and 10.105 - 10.365/10.441 - 10.885 \(\mu\)m omitting wavelengths that lacked appropriate powers (at the far ends of the P and R branches). From the mass spectrum the precursor (P) and dissociation product ions (F) were noted and plotted as the \(\ln \left(\frac{P+F}{P}\right)\) versus \(\tilde{\nu}\).

**Experimental Results and Discussion**

**Phosphopeptide Mass Spectra**

Infrared multiple photon dissociation mass spectra for positively and negatively charged phosphopeptide ions are seen in Figure 6-1 and 6-2. Irradiated at 10.6\(\mu\)m at 6W for 4sec and at 2.5W for 3.5sec, the respective positive and negative ions mass spectra illustrate the same pattern of phosphate loss. The dissociation pathway is made clearer through the knowledge that \(\beta\) elimination is the phosphate dissociation mechanism for phosphoserine (Figure 6-3), where the \(\text{H}_3\text{PO}_4\) loss creates the serine replacement dehydroalanine and corresponding product\(^{255,256}\).

Thus, the phosphate dissociation products are seen as originating with the precursor ion
[P1±2H+]±2 and precursor ions minus one/two water molecules ([P1±2H+]±2 - H2O) and ([P1±2H+]±2 - 2H2O).

Differences between the spectra include evidence of phosphopeptide backbone fragmentation and dissociation products. The excess internal energy in the irradiated ion cleaves the positively charged phosphopeptide ion between y7 (m/q of 977.5 (TENELQNK)) and b9 (m/q of 1088.5 (FQpSEEQQQ)) with further dissociation at [b9] –H2O. Here, the dephosphorylation and the backbone cleavage appear to be competing low energy pathways. The negatively charged phosphopeptide ion spectra do not contain evidence of competing pathways, though excess internal energy creates further fragmentation (m/q 835, 826, and 819) from dephosphorylated products (m/q 981,972, and 965, respectively), each consistent with a loss of 146 m/q (q=-2). The relatively small mass difference, intact peptide, and the low internal energy indicate a small peptide loss, probably located at one of the termini, cleaved at the amide bond. The dipeptide consisting of phenylalanine (F) and glutamine (Q) at m/q of 292(y2) is consistent with the loss of the y fragment (neutral oxazolone or +1 charge).

Phosphopeptide Kinetics

Once the phosphopeptide IRMPD spectra were acquired, a graph comparing the fraction of the parent ion depleted over time, at different powers, was created for each wavelength. These pseudo-first order kinetic plots for both the positive (at 9.294, 9.588, 10.632 µm) and negative (at 9.294 and 10.632 µm) ions are seen in Figure 6-4 and 6-5, respectively. Readily apparent (Table 6-2) are the differences between the dissociation rate constants of the negative and positive ion modes. These differences favor dephosphorylation of the negatively charged phosphopeptide ions by as much as 30 to 50 times that of their positively charged counterparts. Given the similar pattern of phosphate loss and common β elimination mechanism, it would
appear that conformational differences and the corresponding phosphate interactions within these structures account for the kinetic disparity between the two charge states.

The work of Yan-Ting Guo *et. al.* demonstrates one possible explanation for kinetic differences in the charge dependent ratio of trans and cis conformers, based on the orientation of the alpha carbons of proline and serine (phosphorylated and nonphosphorylated) residues in the smaller peptides, Ac-ApSPK-NH$_2$ and Ac-ASPK-NH$_2$, respectively.$^{257}$ Utilizing nuclear magnetic resonance (NMR) to determine equilibrium constants (\( K_{\text{trans}} = \frac{[\text{trans}]}{[\text{cis}]})\), the authors calculated the cis ratio (%) for the nonphosphorylated and the -1 and -2 charge states of the phosphorylated peptides resulting in values of 8.4, 10.0, and 18.1, respectively. The increased cis ratio is the product of increased isomer stability due to the H-bonding between the negatively charged oxygen(s) on the phosphate ion(s) and nearby amide protons (Figure 6-6). Even so, the energy of the cis structure is still higher than the trans counterpart. This is communicated by \( K_{\text{trans}} >1\) and the cis isomer’s mass spectra, where the fragmentation is much greater than for the trans ion under the same conditions. Cis energy is also implied by the very nature of peptides, where the trans isomer is favored placing bulky side chain (-R) groups (attached to the alpha carbons) on opposite sides of the C-N bond. The implication is that positively charged phosphopeptide ions with neutral phosphate groups favor the trans form more than their negative complements. Therefore, positive ions do not dissociate as rapidly. The principle might help explain part of the kinetic picture in the current experiment; however, with larger systems many interactions are possible, as a pseudo first order reaction would suggest. Computational studies isolating the environment of the phosphate group could be useful in unraveling these mysteries.
Relative Activation Energies and Infrared Multiple Photon Dissociation Spectra

Kinetic differences can also be a reflection of different activation energies. Examination of the Arrhenius plots’ slopes in Figures 6-7 and 6-8 indicates that the corresponding activation energies for the positive ions are greater than for the negative ions. This is confirmed in Table 6-3 where the relative activation energies are given along with their 95% confidence limits (CI) indicating that the lower the energy required for dissociation, the faster the dissociation rate. In other words, the threshold energy associated with the high-energy tail of the Boltzmann distribution is lower for negative ions resulting in more ions leaving the distribution compared to the positive ions under the same conditions. The large error associated with the relative activation energies might throw this idea into question. Besides the nature of the experimental conditions and the error calculations, which are believed to account for a large part of the error,‡ the kinetic picture and mutual agreement among both positive and negative relative activation energies appear to lend credence to the above interpretation.

Further comparison between the relative activation energies of positive and negative ions and the IRMPD spectra (Figure 6-9) reveals that differences in the IRMPD spectral intensities do not fundamentally affect the relative activation energies. The agreement follows from the idea that the laser acts as a thermal source, exciting ions through laser wavelength-ion vibrational resonant absorption creating a Boltzmann distribution of energies. As such, so long as the rapid exchange limit (REX: where the exchange of photons emitted and absorbed by the ensemble is much greater than dissociation) is met and holds true, the number of photons absorbed by changing the power, time, or the efficiency of absorption (i.e., by choosing a vibrational mode

‡ It is believed that the large error associated with the relative activation energies of the positively charged phosphopeptide ions is in part due the effect of the relatively small product yield/peak differences. However, both charge states suffer from the relationship between the number points that define the line of the plots and their 95% CIs. These issues can be remedied by larger parent signal and the acquisition of more rate constants.
with a stronger transition dipole moment), etc., only changes the number of ions crossing the energy barrier but does not mischaracterize the activation energy. However, because the exact relationship between temperature and laser power is unknown, the laser power dependent Boltzmann distribution finds correlation to the temperature distribution through different approaches, like the Dunbar and Paech and Williams models.\(^{31,33,113,139}\)

Of these two approaches, the Paech Williams model is considered the more accurate for the current monophosphorylated peptide, which corresponds to the same class (peptides and proteins) of ions, is within the REX limit, and has many DOF (where spontaneous emission events are far greater than stimulated emission(s) at the resonant vibrational mode(s)).\(^{33}\) Relative activation energies determined with the Dunbar approach are placed in Table 2-2 for comparative purposes.

Flora and Muddiman’s experiments on synthesized\(^{34}\) and harvested phosphopeptides,\(^{137}\) [GAG-pS-GAG]\(^{-1}\) and [TRDIYETDpYYRK]\(^{-2}\) modeled by Dunbar’s relationship, gave dephosphorylation relative activation energies of 0.48 eV and 0.43 eV respectively. These values are 0.17 eV and 0.12 eV above the highest value obtained at 9.294 µm, mainly 0.31 ± 0.09, though they do fall within the error noted by both experiments. Comparisons, however, are made difficult due to the difference in the charge state of the first relationship, where higher charge states typically correspond to greater dissociation yields and lower activation energies\(^{135}\) and the different (nonserine), amino acid tyrosine in the second case. Differences in the size and structure of these molecules may also play a crucial role.

The higher relative activation energy value for the positive ion taken at 9.294 µm is a bit of an anomaly. It is clearly greater than the other positive ion values, though it does agree within
error. Some of the discrepancy may be accounted for in the last line of the kinetics plot that, once corrected through further experiments, should bring the energy into line with the current trend.

Conclusion

Dephosphorylation of a monophosphorylated peptide resulted in the loss of an $\text{H}_3\text{PO}_4$ neutral from the precursor ion as well as subsequent water loss product ions. Illustrating competing low energy pathways (positive mode) and excess internal energy fragmentation (positive and negative modes), the phosphate dissociation was still unencumbered by interferences. This made the analysis of the unimolecular dissociation kinetics and the relative activation energies easier. Negative ion dephosphorylation at all wavelengths provided evidence of higher rate constants than positive ion motifs. This in turn corresponded to lower negative ion relative activation energies believed to be due to possible conformational interaction(s) between the negatively charged oxygen(s) of the phosphate group and its surroundings. Relative activation energies were also found to be independent of vibrational mode intensities (at least as measured by the dissociation action spectra). Overall, the activation energy for dephosphorylation of the -2 charge state was found to be $0.51 \pm 0.11 \text{eV}$ and the +2 charge state $0.82 \pm 0.25 \text{eV}$. 
Figure 6-1. An example of an IRMPD phosphopeptide mass spectrum of the +2 charge state after CO₂ laser irradiation time of 4.0s at 10.632 µm and 6W. Loss of the phosphopeptide ion occurs through β elimination from the precursor ion and its water elimination products. Fragmentation of the phosphopeptide backbone is also noted by the \( y_7 \) and \( b_9 \) fragments.
Figure 6-2. An example of an IRMPD phosphopeptide mass spectrum of the -2 charge state after CO₂ laser irradiation time of 3.5s at 10.632 µm and 2.5W. Loss of the phosphopeptide ion occurs through β elimination from the precursor ion and its water elimination products. Excess internal energy results in the loss of y₂ fragment from the dephosphorylated peptide.

\[
\text{pSer}
\]
\[
(185-\text{H}_2\text{O}) = 167 \text{ Da}
\]
\[
(\text{Ser}+80) = 185 \text{ Da}
\]

Figure 6-3. Diagram illustrating the β elimination product for dephosphorylation of phosphoserine.
\[
\ln P_f(3.15 \text{ Watts}) = -(9 \pm 4) \times 10^{-3}t - 0.019 \pm 0.011
\]
\[
\ln P_f(3.65 \text{ Watts}) = -(0.013 \pm 0.005)t - 0.029 \pm 0.010
\]
\[
\ln P_f(4.15 \text{ Watts}) = -(0.026 \pm 0.009)t - 0.03 \pm 0.02
\]
\[
\ln P_f(4.70 \text{ Watts}) = -0.05 \pm 0.03t - 0.005 \pm 0.067
\]
Figure 6-4. Pseudo-first order kinetic plots (w/ 95% confidence limits) measuring the dephosphorylation of positively charged (+2) phosphopeptide ions over time at the given powers for A) 9.294 μm (w/ selected error bars depicting general range of error in these experiments), B) 9.588 μm, and C) 10.632 μm wavelengths. The absolute value of each slope gives the corresponding unimolecular dissociation rate constant for the given CO₂ laser power.
Figure 6-5. Pseudo-first order kinetic plots (w/ 95% confidence limits) measuring the dephosphorylation of negatively charged (-2) phosphopeptide ions over time at the given powers for A) 9.294 µm and B) 10.632 µm wavelengths. The absolute value of each slope gives the corresponding unimolecular dissociation rate constant for the given CO$_2$ laser power.
Figure 6-6: Cis trans conformers from phosphopeptide study illustrating a possible reason for the differences in kinetics and relative Arrhenius activation energies observed in the results reported in this dissertation. [Reprinted with permission from Springer. Guo, Y.; Li, Y.; Zhu, Z.; Zhao, Y. 2005. Effect of the Phosphate Group with Different Negative Charges on the Conformation of Phosphorylated Ser/Thr-Pro Motif. Int. J. Pept. Res. Ther. (Volume 11, Page 160, Figure 1).]

\[
\ln k_{\text{obs}} (s^{-1}) = (4.2 \pm 1.7) \ln P_{\text{Watts}} - 9.6 \pm 2.3
\]
Figure 6-7. Arrhenius relative activation energy plots (w/ 95% confidence limits) for unimolecular dissociation of phosphate neutrals from positively charged (+2) phosphopetide ions at A) 9.294 μm, B) 9.588 μm, and C) 10.632 μm wavelengths.
Figure 6-8. Arrhenius relative activation energy plots (w/ 95% confidence limits) for unimolecular dissociation of phosphate neutrals from negatively charged (-2) phosphopetide ions at A) 9.294 μm and B) 10.632 μm wavelengths.
Figure 6-9. The IRMPD (Action) spectra (with selected error bars depicting general range of error) for both positively (A) and negatively (B) charged phosphopeptides covering the majority of the tunable CO$_2$ wavelength range from 9.193 to 9.354 (Blue), 9.458 to 9.714 (Red), 10.125 to 10.365 (Green) and 10.476 to 10.787 (Purple) μm.
Table 6-1. Example of the conversion of the product and fragment ions’ relative abundances from a mass spectrum to a point on a kinetic plot of \( \ln([P]/([P]+[F])) \) versus time. This point represents the dephosphorylation of a positively charged (+2) monophosphopeptide ion irradiated for 4.0s at 6W and 10.632 \( \mu \)m.

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<th>6.408</th>
<th>4.539</th>
<th>8.087</th>
<th>1.416</th>
</tr>
</thead>
<tbody>
<tr>
<td>ln(( \frac{[P]}{[P]+[F]} )) vs. 10.632( \mu )m</td>
<td></td>
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<tr>
<td>0.766</td>
<td>-0.267</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Table 6-2. Unimolecular rate constants for dephosphorylation of both negatively and positively charged phosphopeptide ions at the given wavelengths and corresponding powers.

<table>
<thead>
<tr>
<th>Positive Ions</th>
<th>Unimolecular dissociation rate constants, ( k_d ) (s(^{-1})) / Power (W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.294( \mu )m</td>
<td>0.009/2.15 0.013/3.15 0.026/4.15 0.048/4.70</td>
</tr>
<tr>
<td>9.588( \mu )m</td>
<td>0.002/1.92 0.007/3.64 0.021/4.00 0.035/4.60</td>
</tr>
<tr>
<td>10.632( \mu )m</td>
<td>0.019/4.00 0.035/4.50 0.049/5.00 0.070/6.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Negative Ions</th>
<th>Unimolecular dissociation rate constants, ( k_d ) (s(^{-1})) / Power (W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.294( \mu )m</td>
<td>0.077/0.78 0.160/1.03 0.287/1.50 0.513/1.74</td>
</tr>
<tr>
<td>10.632( \mu )m</td>
<td>0.058/1.50 0.102/2.00 0.20/2.5 0.372/3.50</td>
</tr>
</tbody>
</table>
Table 6-3. Relative Arrhenius activation energies with 95% confidence limits utilizing both Dunbar’s and Paech and William’s models.

<table>
<thead>
<tr>
<th></th>
<th>Dunbar’s Model</th>
<th>Williams’ Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rel. $E_a$ (eV)</td>
<td>Average</td>
</tr>
<tr>
<td>Positive Ion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.294μm</td>
<td>0.59±0.24</td>
<td>Average</td>
</tr>
<tr>
<td>9.588μm</td>
<td>0.42 ± 0.28</td>
<td>0.46±0.14</td>
</tr>
<tr>
<td>10.632μm</td>
<td>0.39±0.21</td>
<td>0.74±0.4</td>
</tr>
<tr>
<td>Negative Ion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.294μm</td>
<td>0.31±0.09</td>
<td>Average</td>
</tr>
<tr>
<td>10.632μm</td>
<td>0.28±0.08</td>
<td>0.29±0.06</td>
</tr>
</tbody>
</table>


CHAPTER 7
CONCLUSIONS AND FUTURE WORK

Infrared multiple photon dissociation of ions in the cell of a Fourier transform ion cyclotron resonance mass spectrometer has led to the creation of action spectra for four rubidium-D-glycoside isomer complexes and subsequent structural elucidation based on frequency agreement with theoretical structures as well as the determination of thermochemical properties for positively and negatively charged monophosphorylated peptide ions. These experiments also introduced three laser systems into the Eyler and Polfer labs: two carbon dioxide gas lasers and an optical parametric oscillator laser that are still being utilized for a myriad of experiments.

The OPO laser takes advantage of the nonlinear properties of a periodically poled lithium niobate crystal that exhibits second order susceptibility characteristics. Key among these properties is the ability to convert the pump laser beam photon (Nd:YAG laser) into two separate photons (signal and idler) of differing wavelengths, the sum of whose energy equals that of the original photon. The laser, covering a range of 1.38 - 2.0 and 2.28 - 4.67 µm for the respective signal and idler beams, was acquired in order to explore the vibrationally uncongested O-H, N-H, and C-H stretch regions with IRMPD spectroscopy. The hope was to find a means to differentiate saccharide isomers, D-glycosides in particular, through differences in their O-H stretch modes.

Initial experiments began with the setup of the OPO, which included the design and alignment of external transfer optics, Uniblitz shutters, power meter, gas purge box, etc. Once established, with the aid of Cesar Contreras, action spectra of four rubidium tagged D-glycoside isomers were obtained and revealed differentiable bands. Upon comparison of the action spectra to vibrationally consistent computational structures, the assigned vibrational motions appeared to
correspond to an extensive hydrogen bonding network made more complex through rubidium interactions and ring structure fluctuations. This culminated in the determination that up to four conformers were responsible for the action spectra of the Rb\(^+\)[O-methyl-\(\beta\)-D-glucoside] complex.

Accepting such a wide range of conformers for one isomer and the general difficulty in finding adequate matches between the IRMPD spectra and the DFT determined frequencies has led to the implementation of different strategies. One inherent weakness in the past was the conformational search, where the applied semi-empirical AM 1 force field did not work well with the rubidium tagged glucoside conformers. To correct for this shortcoming, a template of each geometrically optimized isomer was created from which a rubidium cation was added to one of many predetermined zones. Using the Gausview 3.0 program, this process was repeated for different quadrants until the coverage was complete. The resulting geometries from the many newly created conformers were then saved as Cartesian coordinates where the Gaussian job files were created and executed. Another potential problem was finding an effective core potential that could properly model the unique interaction of the rubidium ion with isomeric states. Here, the LANDLDZ basis was replaced with SDD (Stuttgart/Dresden) ECP/ basis as the result of SDD’s recent success with similar sugars. These calculations are currently in progress and hopefully will yield results that are more amicable.

As a long-term project, the expanded study of the D-hexose ions is in order with a nod to the study of di- and tri- saccharide isomers of these components. Development of a library of sugar action spectra and schemes for differentiation can then be started and added to with each successive experiment, perhaps resulting in the development of diagnostic software. The additional examination of structure through vibrational spectra comparison could expand into
other arenas to help unlock sugars’ complex role with proteins, lipids and other biologically relevant molecules.

After failed attempts at acquiring unimolecular dissociation rate constant information from black body infrared radiation dissociation (BIRD) procedures, a tunable Apollo CO$_2$ laser was repaired and pressed into service. The repair and the laser set up (table, optics, windows wave meter etc.) were accomplished with the help of Lawerence Hartley and Cesar Contreras, respectively. Later, a Lasy tunable CO$_2$ laser was purchased and incorporated into the design. Both infrared lasers serve as the source of IR photons for a number of experiments. Of particular interest was the determination of the unimolecular kinetics and relative activation energies of positively (+2) and negatively (-2) charged monophosphorylated peptide ions. Comparison of the slopes of the kinetic plots revealed that the positive ions dissociated at a much slower rate than the negative ions, a pattern that held up for all positive/negative ions recorded at different wavelengths. Arrhenius activation energies appeared to confirm that the higher dissociation yields of the negative ions were directly related to their lower activation energies. Kinetics determined at other wavelengths confirmed this result. The 95 % confidence limits suggest that further expansion of the kinetics for each wavelength would result in more points for their Arrhenius plot line and serve as a means to make the data clearer.

The interesting question is why this disparity exists in the first place and how can it best be modeled? Along these lines further research exploring the interactions of phosphopeptide ions with their surroundings is warranted. Taking a cue from studies of larger proteins, regions most likely to interact with phosphate group (domains) can be extracted and the relationship studied to later incorporate each local difference into the larger picture. Here, additional exploration of the
effects of the possible cis isomer should also be examined and kinetic modeling implemented to further break down the constituents of the pseudo first order rate constants.
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BIOGRAPHICAL SKETCH

Wright (Lee) Pearson III was born in Coral Gables, Florida, to Wright L. Pearson, Jr. and Betty Pearson. The oldest of two boys, he grew up primarily in Miami, Florida, with his family. After graduating from Miami Lakes High School, he attended Miami Christian College and was consistently on the dean’s list throughout his college career. Lee graduated from Miami Christian College with dual bachelor’s degrees in psychology and theology. Following his schooling, he gained considerable work experience in a variety of industries.

In June of 1995, Lee resolved to continue his education by pursuing a Doctorate of Philosophy in physical chemistry. He briefly attended Miami Dade College, where he earned an award for Chemistry Student of the Year in 1999. Following his acceptance to the University of Florida, he continued to earn other awards—the Hypercube Scholar award in 2000 for post baccalaureate research in computational chemistry and Chemistry Teaching awards in 2002 and 2004. Upon completion of his Ph.D. program in the summer of 2009, Lee pursued postdoctoral research in spectroscopy.