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Laser desorption from a room temperature ionic liquid

Peter Ronald Harris

*College of William & Mary - Arts & Sciences*

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Laser Desorption from a Room Temperature Ionic Liquid

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A Dissertation presented to the Graduate Faculty
of the College of William and Mary in Candidacy for the Degree of
Doctor of Philosophy

Department of Physics

The College of William and Mary
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Doctor of Philosophy

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We report laser desorption from a Room Temperature Ionic Liquid (RTIL) as a novel source for time of flight mass spectrometry. We use the 2nd harmonic of an Nd:YAG laser to deposit intensities of 1-50 MW/cm^2 via backside illumination onto our RTIL desorption sample. A microstructured metal grid situated on top of a glass microscope slide coated with RTIL serves as our desorption sample. The RTIL we use, 1-Butyl, 3-Methylimidazolium Hexafluorophosphate, remains liquid at pressures below 10^-8 torr. The use of liquid desorption sample allows for improved surface conditions, homogeneity and sample life as compared to Matrix Assisted Laser Desorption Ionization (MALDI) techniques. Our desorption technique is also unique as it allows the study of both multiphoton and acoustic desorption processes within the same time of flight spectra. Our technique yields intrinsically high resolution, low noise data. We observe differences between ion species in their preference for desorption by a particular desorption method. Specifically, we observe desorption solely by acoustic means of an entire RTIL molecule adducted with an RTIL cation. Finally, we report the applicability of this technique for the desorption of biomolecules.
For all my family and friends.
# Table of Contents

Acknowledgements ................................................................................................................... iv

List of Figures ............................................................................................................................. v

1 Introduction ............................................................................................................................... 2
   1.1 Motivation ............................................................................................................................. 3
   1.2 Review of Current Literature ............................................................................................... 7
   1.3 Dissertation Outline ............................................................................................................ 10

2 Theory of MALDI and Novel Desorption Techniques ......................................................... 12
   2.1 Conventional MALDI .......................................................................................................... 12
   2.2 Room Temperature Ionic Liquid ......................................................................................... 16

3 Experimental Apparatus ........................................................................................................... 20
   3.1 The Vacuum Chamber .......................................................................................................... 21
   3.2 Microchannel Plate Detector & Data Acquisition System .................................................. 24
      3.2.1 Microchannel Plate Detector ......................................................................................... 24
      3.2.2 Data Acquisition ........................................................................................................... 26
   3.3 Laser System ......................................................................................................................... 29
      3.3.1 Optics Layout ............................................................................................................... 31
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List of Figures

2.1 TOF-SIMS images of a SELDI sample.................................................. 15
2.2 Diagrams of 1-Butyl, 2-Methylimidazolium Hexafluorophosphate........ 18
3.1 Overall vacuum system schematic and photo.................................. 22
3.2 Schematic of microchannel plate detector and photomultiplier tube..... 25
3.3 Measured profile of Nd:YAG laser pulse and surface plot of CCD image of laser spot................................................................. 30
3.4 Schematic of optics table setup and orientation.............................. 32
3.5 Plot of neutral density filter calibration......................................... 34
3.6 Plot of ion count calibration......................................................... 34
3.7 Schematic of desorption sample holder assembly.......................... 35
3.8 Picture of copper grid used as part of desorption sample.................. 39
3.9 Schematic of grid holder sample assembly.................................... 39
3.10 TOF spectra of Titanium.......................................................... 42
3.11 TOF spectra of Copper............................................................ 42
4.1 Well resolved TOF spectra of Titanium........................................ 46
4.2 Well resolved TOF spectra of Copper........................................... 46
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3</td>
<td>FWHM measurement of PF$_6$ peak in time</td>
<td>47</td>
</tr>
<tr>
<td>4.4</td>
<td>FWHM measurement of PF$_6$ peak in mass</td>
<td>47</td>
</tr>
<tr>
<td>4.5</td>
<td>Conventional MALDI spectra low mass range peak</td>
<td>51</td>
</tr>
<tr>
<td>4.6</td>
<td>Desorption from RTIL spectra low mass range peak</td>
<td>51</td>
</tr>
<tr>
<td>4.7</td>
<td>Conventional MALDI spectra early arrival time peak resolution</td>
<td>52</td>
</tr>
<tr>
<td>4.8</td>
<td>Desorption from RTIL spectra early arrival time peak resolution</td>
<td>52</td>
</tr>
<tr>
<td>4.9</td>
<td>Delayed extraction schematic</td>
<td>54</td>
</tr>
<tr>
<td>4.10</td>
<td>Conventional MALDI spectra, high mass range</td>
<td>56</td>
</tr>
<tr>
<td>4.11</td>
<td>Conventional MALDI spectra, high mass range, single peak</td>
<td>56</td>
</tr>
<tr>
<td>4.12</td>
<td>Plot of PF$_6$ resolution versus acceleration voltage</td>
<td>62</td>
</tr>
<tr>
<td>4.13</td>
<td>Plot of PF$_6$ arrival time width versus acceleration voltage</td>
<td>62</td>
</tr>
<tr>
<td>4.14</td>
<td>Heat map of well resolved PF$_6$ peak</td>
<td>64</td>
</tr>
<tr>
<td>4.15</td>
<td>Heat map of PF$_6$ at low acceleration voltage</td>
<td>64</td>
</tr>
<tr>
<td>4.16</td>
<td>Plot of two PF$_6$ peaks acquired at different acceleration voltages</td>
<td>66</td>
</tr>
<tr>
<td>5.1</td>
<td>Positive TOF spectra, prompt desorption</td>
<td>69</td>
</tr>
<tr>
<td>5.2</td>
<td>Positive TOF spectra, prompt and acoustic desorption</td>
<td>69</td>
</tr>
<tr>
<td>5.3</td>
<td>TOF spectra of BMIM, prompt and acoustic</td>
<td>71</td>
</tr>
<tr>
<td>5.4</td>
<td>TOF spectra of titanium, only prompt desorption</td>
<td>71</td>
</tr>
<tr>
<td>5.5</td>
<td>TOF spectra of BMIM[RTIL], only acoustic desorption</td>
<td>73</td>
</tr>
<tr>
<td>5.6</td>
<td>Heat map taken near acoustic threshold of BMIM and BMIM[RTIL]</td>
<td>73</td>
</tr>
<tr>
<td>5.7</td>
<td>Single shots of positive TOF spectra, taken above and below the acoustic desorption threshold</td>
<td>75</td>
</tr>
</tbody>
</table>
5.8 Positive TOF spectra taken above the acoustic threshold showing delay in
calibration start time for acoustically desorbed ion peaks................. 76
5.9 Negative TOF spectra taken above the acoustic threshold.............. 78
5.10 Integrated ion count for BMIM and Imidazolium versus laser intensity, log
scale.................................................................................................................. 80
5.11 Integrated ion count for BMIM and Imidazolium versus laser intensity, linear
scale.................................................................................................................. 80
5.12 TOF spectra showing prompt and acoustic BMIM peak overlap......... 82
5.13 Integrated ion count for acoustic BMIM and acoustic [BMIM][RTIL] versus
laser intensity...................................................................................................... 82
5.14 Comparison of shot to shot laser power sensitivity for prompt versus
acoustically desorbed ions.................................................................................. 83
5.15 Integrated ion count of BMIM and Ti versus laser intensity, log scale..... 85
5.16 Integrated ion count of Ti and [BMIM][RTIL] versus laser intensity, log scale
............................................................................................................................. 85
5.17 Integrated ion count of Ti and acoustic BMIM versus laser intensity...... 86
5.18 Integrated ion count of Ti versus laser intensity, 1st fit........................ 86
5.19 Two BMIM spectra acquired with different laser spot sizes, showing variable
delay between acoustic and prompt BMIM..................................................... 88
6.1 Negative TOF desorption from a RTIL spectra of a biological sample...... 91
LASER DESORPTION FROM A ROOM TEMPERATURE IONIC LIQUID
CHAPTER 1

Introduction

Since the advent of pulsed lasers in the 1960’s, time of flight mass spectrometry has been one of their central applications. However, it was not until 1985 when Karas, Bachmann & Hillenkamp first introduced a matrix assisted laser desorption technique that their suitability to explore biophysics truly began [1]. Their technique, Matrix Assisted Laser Desorption Ionization (MALDI) pioneered the field of proteomics, allowing for the desorption and ionization of large biomolecules over a broad mass range in a survey technique. A new technique, presented in this dissertation, advances the field through the development of a cold ion source. This new technique incorporates the use of a Room Temperature Ionic Liquid (RTIL), allowing desorption below the MALDI power threshold, along with other advances.

The process of MALDI from a Room Temperature Ionic Liquid (RTIL) is a technique that is motivated by the underlying physical process as well as the potentially significant medical applications. I will attempt to limit the discussion of
the medical motivations to the following section. From a physical standpoint our desorption from RTIL technique has interesting characterizable behavior that is substantially different from that of conventional MALDI. Our technique distinguishes itself by avoiding the collisional MALDI plume used to achieve ionization of desorbed analyte species. Furthermore, our desorption technique is comprised of two processes, each with different behavior and mass spectroscopic advantages. The different features of the two processes potentially allow for the tuning of the primary desorption mechanism to match the characteristics of the analyte. Interestingly this tuning or desorption process selection can occur within a single spectra as these two processes are not mutually exclusive. The first process consists of a prompt desorption, while the second is a delayed acoustic desorption. First however, I describe the process of conventional Multiphoton Matrix Assisted Laser Desorption Ionization, which desorbs and ionizes analyte with a single laser pulse interaction, eliminating the need for a secondary ionization process.

1.1 Motivation

Over the past 30 years significant effort has gone into medical research seeking cures for various disease states afflicting humans. Many results from such research point to a need for early detection. Problematically, outward signs of disease often do not avail themselves until the disease has progressed significantly. In the case of many cancers, a biopsy is performed in which a tissue sample is taken from a patient and analyzed under a microscope by a clinician to determine if the sample is
cancerous. Such a technique is modestly effective at diagnosis, however biopsies occurs relatively late in a given disease state. Furthermore, the required removal of a tissue sample and individual analysis by a trained clinician as a method of diagnosis leaves obvious room for improvement.

Proteomics is a field of study that promises to improve the process of disease diagnosis. Proteomics is the study of all the proteins in a given sample. For the purpose of disease diagnosis, a sample often refers to blood serum which is significantly simpler to obtain than a tissue sample and is already regularly acquired during medical exams. One can envision the development of a blood test that could be administered at regular intervals, well before the known onset of a disease. This potentially allows for the extremely early diagnosis of disease from such blood serum samples. Proteomics relies on the knowledge that even in early stages of disease, significant changes arise in the type and quantity of proteins and peptides produced by the human body [2]. Distinguishing this approach from other blood tests is the survey nature of proteomics which allows the detection and potential identification of multiple peptides within a given sample. Rather than look at the abundance of a single protein (often done as a chemical test, in which a known reaction will occur), in proteomics, survey techniques allow us to look for proteomic patterns that essentially can act as “fingerprints” of a disease. To date this notion of proteomic patterns being more diagnostic than individual peptides has had limited success. However, advances in mass spectrometry may allow for future success. Regardless, survey techniques are currently useful during the discovery phase for a given disease,
when such techniques excel in the identification of biologically significant molecules (biomarkers) within samples. Once armed with the knowledge of such a disease fingerprint, the knowledge of disease biomarkers could be applied on a clinical level, where a blood serum sample can be scanned for matches to known disease fingerprints without a lengthy analysis by a clinician. Essentially, standard assays could by applied at the clinical level for detection of biomarkers that were identified during a successful discovery phase. Mass Spectrometry is a primary tool of proteomics for the detection and identification of peptides and proteins over a broad mass range.

Proteomics for disease diagnosis requires a successful discovery phase, where correlations between abundances of proteins and peptides with a given disease state are discovered. Time of flight mass spectrometry is well suited for this discovery phase with its ability to detect all the successfully ionized species for a sample. MALDI is a prominent mass spectrometry technique used in the discovery of biologically significant proteins. In MALDI, a sample is mixed with a chemical known as a matrix and allowed to dry into a crystalline form. This matrix chemical is selected for its ability to absorb laser light (sometimes referred to as an Energy Absorbing Matrix, EAM). When illuminated by a laser pulse, matrix material rapidly vaporizes and ionizes leading to a phase explosion from the sample surface. The resulting desorption plume contains ionized matrix molecules along with the original analyte material. Within this hot, dense plume analyte undergoes collisional ionization with matrix molecules. The resulting ionized plume then gets analyzed by
a time of flight mass spectrometer. The observed flight time of analyte yields their
mass to charge ratio \( m/z \). However, with many analytes being singly charged, as
protonation is the most common ionization pathway, often just the mass of the
observed ion is quoted with unit charge being assumed. MALDI is quite good at
detecting heavy proteins, with masses in the hundreds of kDa (Dalton, Da, is the
standard mass unit used within proteomics and is equivalent to an atomic mass unit,
amu).

MALDI has some drawbacks. MALDI matrices have an irregular crystal
structure that distorts the local electric field at the sample surface. MALDI struggles
as a quantitative tool for ion intensity measurements, partially from the sample
degradation that occurs with multiple laser shots. The hot collisional plume that is
required for ionization of analyte increases the initial velocity spread of ions,
degrading resolution. This is just a brief overview of MALDI drawbacks, only
intended as a part of the motivation for the multiphoton MALDI work presented in
this dissertation. A more thorough examination of MALDI drawbacks and their
consequences is presented in chapter 2.

Our desorption from a Room Temperature Ionic Liquid (RTIL) technique
addresses the drawbacks of conventional MALDI. We replace the crystalline
MALDI matrix with an RTIL, removing the crystalline surface irregularities as well
as extending a sample’s useful lifetime. More importantly our process does not
require the creation of a MALDI plume, meaning we have a colder process enabling
increased resolution. The bulk of the dissertation is centered around studies of the
behavior and physical mechanisms that govern our unique desorption process. A thorough understanding of dual desorption process is crucial if we seek to extend its use to these significant medical motivations.

1.2 Review of Current Literature

Proteomics has advanced towards medical significance with the implementation of successful biomarker discovery techniques. Techniques such as MALDI and TOF-SIMS have had a good degree of success in desorbing and detecting biological samples. This dissertation describes novel desorption processes that differ from these more standard techniques. Early pioneers in novel desorption techniques were Lindner and Seydel, who first reported Laser Induced Acoustic Desorption (LIAD), which was accomplished by the back irradiation of a thin solid sample as a means of desorbing ions from the front surface [3]. Initially unsure of the acoustic nature of the process, Lindner and Seydel chose target desorption molecules that were susceptible to thermal breakdown in order to eliminate the possibility of a thermal, rather than acoustic effect driving the observed desorption process. While they concluded an acoustic process was responsible for desorption, they had not measured or characterized the desorbing shockwaves, nor did they develop a coherent model describing physically how such a process would work. Furthermore, it was concluded that LIAD suffered from a low ionization rate of desorbed species, generally requiring post ionization by other means [4, 5, 6]. This requirement of an additional post ionization step led to the cessation of further LIAD development.
The development of matrix-free laser desorption/ionization has been actively pursued beyond acoustic desorption. One such method is that of Desorption Ionization On Silicon (DIOS), presented by Wei, Buriak & Siuzdak in 1999 [7]. This technique relies on nano-structured surfaces to desorb low mass molecules. Such structures are formed by the etching of porous silicon or by the creation of thin films via deposition. Typical structured components have sizes in the tens of nanometers. Interestingly, it was found that the thickness of thin films for decent desorption spectra needed to be at least 50nm thick, while thicknesses greater than 100nm offered no further advantages [8]. Unlike MALDI, with its strong wavelength dependence, desorption via DIOS is found to be relatively independent of laser wavelength. This wavelength independence is due to the band gap of crystalline silicon (1.1eV), which is exceeded by photons with wavelength less than 1100nm, associated with the range of light from ultraviolet to the near-infrared [8].

As much of the work presented here is being compared to that of conventional MALDI, it is worthwhile to review the history of this technique. Pulsed lasers have been used in time of flight mass spectrometry since the 1960’s in a number of desorption studies. However, it wasn’t until 1985 when Karas, Bachmann & Hillenkamp first introduced a matrix assisted technique that laser desorption’s suitability to biophysics truly began [1]. The first MALDI type samples consisted of a laser-absorbing molecule mixed with alanine, dried onto an aluminum plate, with a sample thickness of 5-10µm. Here they showed desorption of alanine molecules in the presence of an absorbing matrix material at irradiance a factor of ten below that of
the alanine molecules alone [1]. This work stood in contrast to previous laser desorption work where little was found regarding the influence of the laser wavelength on desorption efficiency. From this, Karas, et al. proposed the use of a fixed laser wavelength matched to a strongly absorbing matrix as a method of controlling the energy deposition for a large variety of organic samples, regardless of their preferred absorption ranges.

In 1998, Handschuh, Nettesheim and Zenobi, reported the study of desorbate from multiple desorption methods by collecting desorbate on a trapping plate and subsequently studying images of these plates. Desorbate from MALDI, laser induced thermal desorption, IR ablation and substrate heating were compared. Handschuh et al. concluded MALDI excelled at the desorption of intact individual molecules, while other techniques tended to yield larger particle sizes that suffered from reduced ionization probability and detection efficiency [6].

The incorporation of RTIL into desorption sources has recently taken shape, however it has been limited to the development of new MALDI matrices. First attempts by Armstrong, et al., yielded little success, having involved the simple replacement of MALDI matrices with standard RTILs. Zabet-Moghaddam, et al., found improvements when classical MALDI matrices were mixed with RTILs. The final step in the evolution of RTILs for use in MALDI matrices was carried out by Armstrong et al., having designed novel ionic liquids matrices that incorporate the base from ionic liquids with acidic MALDI matrices to form what is now known as Ionic Liquid Matrices (ILMs) [9, 10, 11, 12, 13]. The primary advance achieved with
the incorporation of RTIL into the design of MALDI matrices has been a more homogeneous matrix/analyte mixture [14]. Inhomogeneous sample surfaces are known to contribute to significant shot to shot variability within MALDI spectra [15, 16]. This variability has hampered efforts to use MALDI spectra for quantitative measurements of peptide abundances from analyte samples. Thoely, et al. reported the incorporation of ILMs allowed for the quantitative analysis of low molecular weight compounds, reducing shot to shot peak height variability from 60% to 10%. The reduction of this variability, while notable, does not change the fundamental mechanisms or problems associated with conventional MALDI. Thoely, et al. also report MALDI spectra incorporating ILMs still suffer from low mass resolution, high background and poor ionization efficiency [8].

1.3 Dissertation Outline

This dissertation is divided into six chapters. Chapter one gives an overview of the work, along with motivation and an introduction to proteomics. The literature review in chapter one covers three main topics: conventional MALDI, novel desorption techniques and methods that blend both. Acoustic desorption, conventional MALDI, MALDI incorporating ionic liquid matrices and “matrix free MALDI” are reviewed. Chapter two describes the theory beyond the desorption techniques discussed in this dissertation. Specifically, Room Temperature Ionic Liquids and MALDI matrix crystals are discussed in detail. Chapter three focuses on the experimental apparatus and procedures used for data acquisition. The vacuum
chamber and laser system are described. Procedures for sample exchange and operational techniques are reviewed. Finally chapter three reveals some example data our time of flight system is capable of producing. Chapter four solely discusses resolution. The resolution limitations found in conventional MALDI are compared to those found use the desorption techniques in this work. Finally, implications of our observed resolution are discussed. Chapter five reveals the bulk of the data presented in this work. The focus of chapter five is split between the two processes that occur within our desorption technique. The behavior and characteristics are presented and discussed. Conclusions and outlook are in chapter six, with the biological implications first mentioned in chapter one again discussed in light of this work.
CHAPTER 2

Theory of MALDI and Novel Desorption Techniques

The desorption work presented in this thesis shares similarities to conventional MALDI, the theory of which will be discussed in this section. Furthermore, an introduction to room temperature ionic liquids is presented. Finally a theoretical justification for the desorption techniques presented in this work is described.

2.1 Conventional MALDI

Here I present a description of conventional MALDI. I seek to provide a detailed overview of the topic that should be sufficient for comparison and consideration with the techniques presented in my work. Matrix Assisted Laser Desorption Ionization (MALDI) exploits the known absorption bands of known matrix molecules. Matrix molecules are not the primary analyte of a sample, rather matrix molecules are mixed with analyte to
form crystalline sample surfaces. These matrix molecules are extremely efficient
absorbers at selected wavelengths, as such, analyte molecules are expected to be
significantly less efficient by comparison, thereby protecting them from direct ionization
and associated fragmentation. This description of MALDI is essentially what gives rise
to its claims to be a ‘soft’ ionization method, as matrix molecules absorb the bulk of
deposited laser energy [17, 18].

Multiple models have been proposed for the ionization of matrix molecules via
the MALDI process, including multiphoton, energy pooling, excited-state proton transfer
and thermal ionization processes. Generally, multiphoton ionization is believed to be the
most reasonable pathway for matrix ionization, especially in the case of UV MALDI
[17]. In this model, matrix molecules are directly ionized via multiphoton ionization,
where an electron is liberated by n photons from a neutral matrix molecule as in equation
2.1.

\[ \text{Matrix} + n(h\nu) \Rightarrow \text{Matrix}^+ + e^- \]  

2.1

After the creation of matrix ions, matrix-matrix reactions occur that create protonated
matrix ions, as in equation 2.2, where an excited matrix ions interacts with a neutral
matrix molecule.

\[ \text{Matrix}^+ + \text{Matrix} \Rightarrow \text{MH}^+ + (M - H) \]  

2.2

The protonation of matrix ions in equation 2.2 is significant in MALDI because most
observed analyte ions are protonated, with the protonated matrix ion serving as an H+
donor, as seen in the proton transfer reaction in equation 2.3, where ‘A’ stand for an
analyte molecule.
\[ MH^+ + A \Rightarrow M + AH^+ \]  

A similar model dictates the formation of negative analyze ions. The deprotonated matrix molecule from equation 2.2 is excited and can capture a free electron forming a deprotonated matrix ion in equation 2.4.

\[(M - H) + e^- \Rightarrow (M - H)^-\]  

The deprotonated matrix ion formed in equation 2.4 can in turn deprotonate anaylte, as seen in equation 2.5.

\[(M - H)^- + A \Rightarrow M + (A - H)^-\]  

Aside from the role of the deprotonated matrix ion, dissociative electron capture reactions are also believed to be a primary mechanism for the formation of analyze anions (equation 2.6) [17,18].

\[ A + e^- \Rightarrow (A - H)^- + H\]  

The MALDI reactions described in equations 2.1 through 2.6 are believed to occur within the MALDI plume that forms rapidly after the UV laser energy deposition. The high number density (remaining at a few percent of the solid density for the first few ns) of the plume allows for frequent collisions that initiate such reactions. MALDI ions formed within the plume can obtain high velocities (1000 m/s), and exhibit a range of post desorption speeds. The UV MALDI plume is the result of an explosive phase transition from the solid to gas phase. The control and characterization of the MALDI plume is complex and varies with choice of matrix, surface characteristics, etc.

Both MALDI and Surface Enhance Laser Desorption Ionization (SELDI) have complex surface structures that influence conditions within the desorption plume.
Figure 2.1: Time of Flight Secondary Ion Mass Spectrometry (TOF-SIMS) image of a prepared SELDI IMAC-Cu sample chip. The crystalline sample surface is rough and irregular. The sample pictured consists of pooled blood serum and a MALDI matrix. Both SELDI and MALDI samples have similar surface structures after matrix application. Such structures and features hinder resolution and reproducibility of TOF spectra.
SELDI is a technique that is quite similar to MALDI, but takes extra care in sample surface preparation by chemical means for the creation of desired surface affinities for peptides. SELDI uses prepared surfaces that will bind to specific amino acids and peptides of interest, a process known as Immobilized by Metal Affinity Capture (IMAC). After deposition of peptides, SELDI samples are then washed before the application of a matrix solution. SELDI allows undesired (and unbound) proteins to be washed away from the analyte of interest before final application of the matrix compound. Both MALDI and SELDI have complex resultant surface structures [16]. Figure 2.1 is an ion image (acquired by a PHI Thrift III, Time-Of-Flight Secondary Ion Mass Spectrometry, TOF-SIMS, instrument, courtesy of Dasha Malyarenko) of a prepared SELDI IMAC-Cu chip sample containing pooled blood serum and matrix material that has been prepared for use on a Ciphergen PBS-2 SELDI-TOF instrument [19]. Such complex surface structures play a significant role in reproducibility issues experienced by MALDI, as the surface morphology changes with increasing laser shots and associated damage.

2.2 Room Temperature Ionic Liquid

A central difference of our laser desorption from a RTIL as compared to MALDI is the use of a room temperature ionic liquid (RTIL) in place of a conventional crystalline MALDI matrix. Room temperature ionic liquids are comprised of positive and negative ions, similar to conventional salts, however, as the name suggests, RTILs are liquid at room temperature. Beyond this, RTILs have the unusual, yet well documented, property of being liquids with extraordinarily low vapor pressures. In an attempt to measure a
vapor pressure for [BMIM][PF₆], the RTIL studied in this work, we looked for evidence of RTIL evaporation from a series of microdroplets (r = 20-100 μm) situated on a stainless steel plate and kept under a vacuum of 10⁻⁹ torr for over a week. Comparing optical pictures of our RTIL microdroplets taken before and after vacuum exposure, we found no evidence of evaporation. This unusually low vapor pressure drove early interest in RTILs by chemists as a new class of "green" solvents [20]. The continued classification of RTILs as 'green' solvents is uncertain; while RTILs are non-volatile, many are toxic. Regardless of their role in chemistry, in physics the extremely low vapor pressure of RTIL makes them suitable for use under high vacuum, a domain typically dominated only by solids or rarified gases [21, 22, 23, 24]. We use RTIL as an integral part of our desorption process for three primary reasons. First, RTIL's composition of pre-charged species allows for potential ionization pathways of analyte via gentle attachment of RTIL primary cations and anions to analyte. This is in contrast to the conventional MALDI ionization mechanism which relies on the collisional ionization of analyte with excited ions & molecules within the MALDI plume. Second, the liquid surface of RTIL gives us a good smooth, repeatable surface for desorption. Conventional MALDI surfaces (fig. 2.1) are jagged crystalline insulators, having with poor initial electric field properties that only get further degraded with increasing numbers of laser shots. The inhomogeneous electric field along with the vertical position spread for desorption both degrade the temporal resolution of MALDI. Finally, as a liquid, RTIL has a self healing property in the sense that material removed by laser desorption can be replenished, unlike that of a solid, crystalline surface.
Figure 2.2: Diagram showing structure of (a) BMIM$^+$, (b) PF$_6^-$, and (c) Imidazolium$^+$, the primary fragment of BMIM$^+$. These three ions are the most abundant species present in our desorption spectra, in addition to any metal ions. Aside from Imidazolium, there is another remnant BMIM fragment (56 Da) present in desorption spectra, though it is less stable, and frequently fragments further.
While RTILs comprise an entire class of liquids, in our studies we focus on one of the most common: 1-butyl, 3-methylimidazolium hexafluorophosphate, BMIM\(^+\) PF\(_6^-\), whose structure and mass are shown in figure 2.2. We chose BMIM\(^+\) PF\(_6^-\) because it is one of the better studied RTILs and is commercially available from major chemical companies (Sigma-Aldrich, Fluka #70956, Switzerland). In addition, BMIM\(^+\) PF\(_6^-\) has all the properties just mentioned, namely, a low vapor pressure, liquid at room temperature and an ionic composition. Like most RTILs, it has one notable drawback as it tends to breakdown at high temperatures. For [BMIM\(^+\)][PF\(_6^-\)] this is known to begin above temperatures of 100°C [25]. In our work, we observe PF\(_6^-\) to be extremely stable, while BMIM\(^+\) tends to additionally produce a large primary fragment, Imidazolium\(^+\), at higher laser powers.

[BMIM\(^+\)][PF\(_6^-\)] is a transparent liquid and is non absorbent at 532nm, the wavelength of our laser light. Unlike conventional MALDI, desorption from RTIL does not rely on a wavelength tuned matrix. At sufficient intensities we force absorption of laser light by RTIL and subsequently produce desorption spectra. Metal ions, sourced from a component of the sample assembly are observed as products of direct multiphoton ionization. Similar to MALDI, ionization via attachment can occur in our desorption process. Rather than matrix molecules that have been protonated or deprotonated in the dense, violent MALDI plume serving as adducts for analyte, we propose acoustic attachment of BMIM\(^+\) and PF\(_6^-\) to analyte as an ionization pathway.
CHAPTER 3

Experimental Apparatus

This dissertation is centered on time of flight (TOF) experiments conducted in a series of custom vacuum chambers. In this chapter I describe the multiple components that comprise the final version of the experimental apparatus. All experimental interactions occur within the vacuum chamber; it serves as the housing for the microchannel plate detector, time of flight drift tube as well as the laser desorption/ionization sample assembly. Beyond the vacuum chamber the laser system generates pulses that initiate ion desorption events, while the data acquisition system stores and analyzes resulting experimental data. The following sections describe each of these components in detail, followed by an example of a quality TOF spectra this apparatus has generated.
3.1 The Vacuum Chamber

The vacuum chamber is constructed from standard stainless steel 6” Conflat hardware: a Conflat 6-way cross, an 8.5” long Conflat full nipple and a spherical octagon chamber consisting of eight 2 ¾” Conflat ports along the edge of a ring with 6” Conflat ports situated above and below. The layout of these components is shown in figure 3.1, where the chamber is oriented vertically with the sample region at the bottom, allowing easy underside laser access to the desorption sample. The 6-way cross is a portion of the field free flight region as well the central connector for the remainder of the chamber. The remainder of the field free flight region is comprised of the full nipple flange, greatly extending the total flight path. The spherical octagon at the base of the chamber houses the sample assembly, along with the window and high voltage feedthrough ports.

The entire assembly is mounted in a custom Unistrut structure, secured on top of an 8’ x 4’ optics table. Vacuum pumps are mounted off an elbow flange from the central six-way cross. Pressures below 10⁻⁸ torr are maintained with a Leybold Turbovac 152 turbomolecular pump (145 l/s) backed by a Varian SD-200 Rotary-Vane roughing pump (~50 l/s 180 l/min). The roughing pump provides the turbomolecular pump with a fore pressure in the range of 10⁻³ torr. The final pressure is measured using a Varian 0564-K2500-303 Bayard-Alpert style ionization gauge coupled with a Varian Multi-Gauge Controller (Part No. L8350301). Pressures in the range of 10⁻⁸ torr are suitable for our time of flight experiments as this equates to a
Figure 3.1: Vacuum chamber assembly overview. Turbomolecular pump mounted off side of six-way cross. Detector mounted at top of six-way cross. Incident laser beam enters chamber from bottom via 6” CF window port at base of Spherical Octagon Chamber. Straight pipe serves as an extension of flight tube. Focusing lens and mirror mounted on independent 3-D translation stages outside vacuum.
mean free path of 44,000km for PF₆ which is much longer than the 51cm flight tube.

We calculate the mean free path (\( \lambda \)) in equation 3.1 [26]:

\[
l = 3.107 \times 10^{-20} \left( \frac{\frac{T}{d_m^2 P}}{(4 \times 10^{-10})^2 (1.3 \times 10^{-6})} \right) \approx 4.4 \times 10^7 \text{cm}
\]

where we have used SI units and assumed a pressure (\( P \)) of 1 \times 10⁻⁸ torr and a molecular diameter (\( d_m \)) of 4Å [27]. The extended field free flight tube yields a decent temporal resolution as noted in the final section of this chapter.

Sample exchange is accomplished by first bringing the entire chamber up to atmosphere and removing the spherical octagon chamber from the bottom of the assembly. The detachable bottom portion of the chamber houses the entire sample assembly including ion focusing lens and all required electrical feedthroughs. This arrangement allows for easy testing of a sample installation before final vacuum pump down. Once the spherical octagon is removed, the new sample can be installed, as described in detail in section 3.5, followed by the reassembly of the vacuum chamber. All vacuum seals are made using fresh copper gaskets to ensure a high quality vacuum. A typical sample exchange as measured from venting the chamber to restoring 10⁻⁸ torr takes less than six hours.
3.2 Microchannel Plate Detector & Data Acquisition System

3.2.1 Microchannel Plate Detector

At the top of the vertically oriented chamber is a matched chevron pair microchannel plate (MCP) detector coupled to a fast phosphor screen. This detection system, shown in figure 3.2, has the ability to provide excellent gain, as well as excellent spatial & temporal resolution. The use of a specialized E36 phosphor manufactured by El-Mul Technologies (designed to yield 2.4 ns rise times with peak fluorescence at ~395 nm), preserves the time & gain characteristics of the MCP while allowing for ion imaging. The microchannel plates are made of specialized glass that has been optimized for its secondary electron emission properties. These plates have channels (10 µm diameter holes) passing through them at 12° with respect to the plate surface, with center to center channel separations of 12.5 µm as shown in figure 3.2. Gain in a microchannel plate detector is achieved via secondary electron emission that is initiated by a sufficiently energetic charged particle striking the interior of a channel wall. Emitted secondary electrons are then accelerated by the bias voltage down a channel, through the plates, resulting in a cascade process in which more collisions occur (enhanced by the channel angling), yielding more secondary electrons. Between the first and second plate, the electrons emitted from one channel...
Figure 3.2: Chevron microchannel plate detector coupled to a fast E36 phosphor screen. The plates have a diameter of 25mm, while the channels are at a 12° bias angle with a 10μm diameter and a center to center channel separation of 12.5μm. The chevron assembly shows the 180° rotation of the top plate with respect to the bottom. This arrangement helps preserve time resolution by reducing the likelihood of back streaming ions from starting another electron cascade event. This entire assembly is under vacuum & mounted to a CF window flange. Outside the vacuum lies the photomultiplier tube, preceded by a glass filter, BG-3, to avoid saturation by laser light.
in the first plate spread, entering multiple localized channels in the second plate. While this somewhat reduces the spatial resolution of the detector, it strongly enhances the gain. With the first plate grounded and the second plate biased at +1800V, we can achieve a final gain of $\sim 10^6$. The front microchannel plate is kept at ground in order to define the end of the field free flight region for our time of flight apparatus. The microchannel plates are in a chevron configuration, meaning the two plates are rotated 180 degrees with respect to one another, such that the channels align in a "V" type configuration. This arrangement helps limit the contribution of false event counts coming from back-streaming positive ions that have starting an electron cascade from a sufficiently energetic channel wall collision. The chevron assembly effectively reduces the distance before a channel wall collision thereby reducing the chance it will have the necessary kinetic energy to induce secondary electron emission, which would reduce the detector’s temporal resolution [28].

### 3.2.2 Data Acquisition

Secondary electrons exiting the second microchannel plate are accelerated towards the fast phosphor screen which is biased at +4250V, with an efficiency of $\sim 3$ photons per incident electron. The photons exit the vacuum chamber via the window flange to which the detector assembly is mounted. Emitted light from the phosphor screen is collected into a 1P28 photomultiplier tube (PMT), which is outside vacuum, directly above the chamber. We enhance our collection efficiency by a factor of two by placing a weak convex lens on top of the vacuum window before the PMT.
housings. The photomultiplier also operates via an electron cascade process, converting the TOF phosphor signal into an electrical signal suitable for storage by our digitizer. We choose not to directly collect the TOF signal from the second microchannel plate for two reasons. First, we can avoid the difficulties associated with separating our signal from the high bias voltage of the second microchannel plate. Second, we know from our preliminary work the setup as is allows for the imaging of the phosphor screen under the same MCP conditions, simply by removing the PMT. The photomultiplier tube is operated with its cathode between -500 and -675 Volts. Operating at higher voltages, while increasing gain, also has the unwanted effect of increasing the likelihood of producing a ring following large signals, which typically appears at a characteristic delay of 110ns. To avoid saturation of our PMT from the scattered incident laser light we use a thick (3mm) glass filter, BG-3 (Schott Glass), which effectively blocks 532nm light (transmittance <0.1%), while passing 390nm (transmittance >90%). The filter is so effective at blocking 532nm light, we intentionally position it between the MCP and PMT such that a very small amount of light bypasses the filter and still reaches the PMT, suitable for timing and monitoring of laser power on a TOF data record. The sensitivity of the 1P28 is reasonable over a range from ~200nm-600nm, making it capable of detecting both phosphor and laser light.

The 50 ohm output of the 1P28 photomultiplier tube is collected and recorded onto a CPU via a DP211 Acqiris Technologies 8-bit digitizer board (Acqiris Technologies was acquired by Agilent Technologies in 2007). The Acqiris board can
be run through Matlab or through the Acqiris data acquisition program. For large
data runs, acquisition through Matlab is advantageous for incorporating simple data
processing before storage. For more typical smaller data runs, the Acqiris data
acquisition program is useful as all trigger and acquisition setting can be simply
stored for consistent future use. For data acquired through either method, Matlab is
employed for post acquisition data analysis. To acquire data the output of the PMT is
sent directly into channel one of the DP211. The data acquisition board has an
external trigger input where we input a photodiode signal from laser light scattered
from a prism two meters before the vacuum chamber entry window. This photodiode
signal is only used for triggering an acquisition, the absolute timing for a TOF record
is accomplished through leaked laser light to the PMT or the electron peaks that are
present in negative ion spectra. Typical data runs consist of the acquisition of 100
shots in succession, stored individually, displaying waveforms during an acquisition.
Post analysis data displayed in this dissertation is often the summed 100 shot data in
an effort to avoid inconsistencies introduced by laser fluctuations that occur on a shot
to shot basis. For live monitoring during data acquisition, the Acqiris data acquisition
program is equipped with both display and zoom windows. Often the display
window is used to give an overview of all ions being desorbed, while the zoom
window allows for monitoring of transitions between desorption processes for an
indicative ion peak, such as BMIM\(^+\). Considering the quantity of data being
recorded, the live monitoring window is useful for identifying unusual shots that
might be missed if looking only at summed 100 shot data.
3.3 Laser System

We use the second harmonic (532nm) of a Quanta-Ray DCR-2 Nd:YAG pulsed laser to generate 2ns rise time pulses at a repetition rate of 10Hz. Figure 3.3 is a measurement of the beam's temporal profile using a photodiode. The pulse clearly has temporal structure, the laser Q-switch yields a central pulse that is surrounded by two side bands of reduced energy. From this profile and knowledge of the average laser power we can deduce the peak power of the laser pulse. The average power of the laser pulse train is measured using a thermal power meter, typically 0.19mW before attenuation. The average power per pulse is known as our pulses run at 10Hz, yielding 19µJ per pulse. The energy in a single laser pulse (19µJ) is simply the integral of our measured pulse shape (power vs. time) as shown in figure 3.3a. We then can determine the peak power per pulse using the extent of the pulse (12.9ns), yielding a peak power per pulse of ~ 1.5kW. Solving for the peak power, using the measured average energy of 19µJ, we find:

\[
\Rightarrow \text{PeakPower} = \frac{\text{TotalEnergy}}{550 \cdot \text{SampleTime}} = \frac{19\mu J}{(550)(2 \times 10^{-2} \text{ ns})} \sim 2kW
\]

Figure 3.3c is a surface plot captured by a CCD microscope image of our laser spot in air. While the spot is not completely uniform, there is a clearly defined central region, beyond which intensity drops off rapidly. Before focusing our spot we have used an iris to block to more inhomogeneous portion of the input beam, as seen in figure 3.4a.
Figure 3.3: a) Measured profile of the Nd:YAG laser pulse. The width of the central peak is less than 5ns with a peak power of 3kW. The Q-switch also passes two shoulder peaks of lesser intensity. b) The integral of the pulse shape, representing the energy as a function of time within the pulse. The final value of this integral is equivalent to the total pulse energy of 19µJ.

c) 3D surface plot of our laser spot intensity, imaged by a CCD camera, notice the central intense region is well defined.
3.3.1 Optics Layout

We use the 2nd harmonic of the Nd:YAG laser, which exits the frequency doubling crystal with a spot diameter of ~ 1 cm. The optical path of the beam from the doubling crystal onwards is shown in figure 3.4. For stable operation of our Nd:YAG laser system we always run the oscillator at full power. We require only a small fraction of this output for our desorption experiments, as such we only use the light that has reflected from the front face of a prism situated in our beam path. From this reduced power level we carry out laser desorption studies as a function of laser power by further attenuating our beam through the use of ND filters. After attenuation, a mirror at 45° with respect to the optical table surface reflects the beam upward towards the vacuum chamber window. This mirror is mounted on a 3-D translation stage allowing for easy alignment before insertion of the focusing lens. A second translation stage adjusts our convex 10 cm lens (1” diameter), which focuses our beam down to a sub 50 μm spot on the desorption sample. Generally, when we need to move the laser spot small amounts (less than 2 mm), we simply translate the focusing lens.

3.3.2 Laser Intensity Settings

Desorption studies at various laser powers are common and we accomplish this by changing the neutral density filters in the beam path. After data acquisition the same set of neutral density filters is calibrated using an optical power meter and a
Figure 3.4: a) Top view schematic of optical table setup. b) Side view schematic of optics, beyond the neutral density filter set.
CW green laser pointer. We use the CW green laser pointer instead of our pulsed laser system when calibrating to avoid saturation of our Newport model 835 optical power meter. Figure 3.5 is a plot of magnitude of the pre-calibration error versus the neutral density filter combination. For a typical focused spot size area of $1400\mu m^2$ ($r \sim 20\mu m$), a laser energy of $10\mu J$ per pulse is well above the threshold required to desorb ions from the metal grid/RTIL interface, such energies typically yield good sized ion signals, while avoiding saturation and temporal broadening effects.

Considering our typical spot sizes, and a laser energy of $10 \mu J$, our laser power is $0.77kW$, while our laser intensity is $55MW/cm^2$ for clean, beyond threshold spectra. Regarding the ion production threshold, dropping the laser energy down to $\sim 1-2\mu J (<10MW/cm^2)$ per pulse quickly ceases all ion production, reflecting the observed strong threshold behavior. Chapter 5 examines the laser dependence of ion production in detail.

We normalize our ion count by looking at multiple single shot spectra taken near production thresholds and integrate the signal area under a single representative peak at a known mass. We do this at a known mass to avoid dark current. Figure 3.6 shows five shots taken near threshold, typical of those used for ion count calibration.

### 3.4 Laser Desorption Sample Assembly

Figure 3.7 presents a layout of our multiphoton MALDI sample holder which consists primarily of a stack of six identical $1.4'' \times 1.4'' (3.56cm \times 3.56cm)$ stainless steel square plates (eV parts series C, Kimball Physics Inc., Wilton, NH), each with a
Figure 3.5: Plot of the difference between the pre and post calibrated laser intensity as a function of the neutral density filter combination. These neutral density combinations yield a laser intensity range from $0 - 60$ MW/cm$^2$.

Figure 3.6: Plot of five individual spectra, taken near threshold, one of which registers a BMIM$^+$ ion peak. We calibrate the ion count in our spectra by setting the integrated area of a near threshold single shot peak to be equivalent to the detection of a single ion. From this value we renormalize the integral of our summed data peaks from voltage to ion counts.
Figure 3.7: Sample holder assembly, including Einsel lens stack. Stainless steel plates are electrically isolated from each other with the use of alumina rods and spacers. The plates are 1.4\" x 1.4\" square, 0.026\" thick and have a 0.187\" diameter hole in the center. The inter-plate spacing is 0.300\", with the exception of the bottom two plates, whose spacing is 0.038\", the thickness of the glass microscope slide they sandwich. The right half of the figure shows a blowup of the microscope slide supporting the metal grid and RTIL. The laser light enters this assembly from the bottom, being focused and steered by a lens outside the vacuum chamber.
0.187” (0.475cm) diameter hole in the center. All plates are electrically isolated from one another and ground, by alumina tubes and spacers. The spacing between the plates is 0.300” (0.762cm), accomplished by using standard identical spacers. The exception to this spacing is at the holder base, where laser light enters the sample holder, here the two plates secure a standard glass microscope slide, which determines their spacing of 0.038” (0.097cm). The microscope slide serves as a transparent platform for our various metal grid/RTIL samples. Electrical contact between the metal grid and 2\textsuperscript{nd} plate is direct, though in some trials we instead rely on the DC conductivity of RTIL, which is similar to that of sea water. Generally, the base of this assembly (1\textsuperscript{st} and 2\textsuperscript{nd} plates) is biased at a high voltage of +/- 3kV, serving as the acceleration voltage for our time of flight measurements, where the next (3\textsuperscript{rd}) plate is grounded, defining our acceleration region. Switching the polarity of this base plate allows for the collection of either positive or negative ion spectra. In some studies we do not ground the 3\textsuperscript{rd} plate, allowing us to examine acceleration field versus extraction field effects on desorption, in such a case the 4\textsuperscript{th} plate then defines the end of the acceleration region. The subsequent three plates act as an Einzel lens, where the 4\textsuperscript{th} and 6\textsuperscript{th} plates are grounded, while the 5\textsuperscript{th} plate is at a variable voltage to provide ion focusing. Ion collection was generally enhanced with the focus plate set to ~ 2/3 of the acceleration voltage, with the same polarity. Kapton wiring is bolted to all sample assembly elements, and is then linked to our high voltage feedthrough flange, which has four individual isolated outputs, and is mounted to one of the 2 ¾” CF side ports of the spherical octagon chamber. Kapton
is an excellent insulating material that is coated on the high voltage wires, its extremely low outgassing rate makes it suitable for ultrahigh vacuum (UHV) work. The laser desorption sample holder from figure 3.7 is mounted inside an 8 Multi-CF Spherical Octagon Chamber (Kimball Physics, Wilton NH), using the Groove Grabber system on the bottom 6” Conflat port. The Groove Grabber system consists of Grabber Grooves which are present in our chamber and Groove Grabbers which are mounted to our multi-plate sample stack and clamp down onto Grabber Grooves. Underneath the mounted assembly is the large 6” CF window (from figure 3.4b) allowing for laser access to the desorption sample, as well as access for imaging the laser spot position on the sample grid with a CCD camera. The remainder of the spherical octagon chamber has eight 2 ¾” CF ports, each closed with either a blank, a window or a high voltage feedthrough flange. Four independent high voltage power supplies are used for the MCP, PMT and various elements in the desorption sample holder assembly: two Hewlett Packard model 6516A serving as variable +/-3kV supplies and two variable +/-5kV supplies by Matsusada Precision. The specifics on the preparation of the desorption sample assembly follow in section 3.5.

3.5 Grid / RTIL Sample Preparation

The careful preparation of desorption samples is crucial, especially when dealing with liquids in vacuum. RTIL has a tendency to wick along any metal surfaces, so care must be taken when securing a sample with metal shims or plates. From start to finish the sample preparation proceeds as follows. The desorption
techniques in this dissertation rely on back illumination, therefore the use of a fresh
glass microscope slide for each sample preparation, ensuring it is free of laser
damage, is worthwhile. A metal grid, shown in figure 3.8, is centered on the
microscope slide to allow for easier clamping of the retaining plate onto the retaining
shim, which holds the edge of the grid in place. This arrangement is shown in figure
3.9. The choice of metal grid can vary in both composition and cell shape and size.
We have used square, hexagonal and rectangular shaped grid cells with sizes ranging
from 40-200 μm and compositions of gold, copper or titanium. Figure 3.8 is a picture
of one of our grids, in this case, copper rectangular. Once a sample grid is firmly
secured within the laser desorption sample assembly, the RTIL can be applied. It is
crucial that the droplet of RTIL not touch the metal retaining shim during the
application. This droplet should be small, ~1 μL, as to avoid spreading upon
deposition to the grid edges, again to ensure the retaining shim does not come in
contact with the RTIL. If the droplet does touch the metal retaining shim, it is almost
a certainty that the majority of the RTIL will wick away from the grid, into the space
between the microscope slide and retaining shim. Assuming the RTIL droplet has
been placed appropriately, it will, upon pump down, spread thinly into the grid cells,
with a thickness limited by that of the grid. If the majority of RTIL has wicked away,
it becomes obvious at data acquisition as no ion signal will be present in the central
grid cells (including metal ions). It is possible to reapply a droplet to the grid once
more in an effort to repair a poor sample preparation caused by RTIL wicking. The
side ports of the spherical octagon chamber allow for access to add more RTIL.
Figure 3.8 Picture of a rectangular copper grid cells, used as a sample in RTIL desorption experiments. Open cells are 200µm x 40µm. The entire grid is circular, with a diameter of 3mm.

Figure 3.9: Grid holder sample assembly diagram. The grid is positioned on the microscope slide such the retaining shim only covers the outer edges of the grid, ensuring good electrical contact. The clamping plate holds the other three components together by clamping them to the fixed sample assembly apparatus shown in figure 3.4.
without changing the alignment of the sample. If this second droplet application fails, it is essentially required that one begins with a fresh sample. Repeated application of RTIL droplets eventually saturates the sample, completely covering the grid, with RTIL thickness greater than 100\(\mu\)m, shutting down ion production completely.

### 3.6 Time to Mass Conversion

As described, the experimental apparatus is well suited for high precision time of flight measurements. Fortunately, the time to mass conversion is easily derived from first principles and is quite accurate. Beyond calculating this conversion, we can rely on known calibrants within our sample. The following is a brief description of this time to mass conversion. Once desorbed from the sample surface, ions travel through the acceleration/extraction region. The energy gained traversing this region gives the ion’s final kinetic energy (and final velocity):

\[
qV = \frac{1}{2}mv_f^2 \Rightarrow v_f = \sqrt{\frac{2qV}{m}} \tag{3.4}
\]

However, the average velocity through the acceleration region is determined by the ion’s average kinetic energy. Here we assume the initial kinetic energy of the ion is close to zero, this approximation is reasonably valid, as any initial kinetic energy is certainly small compared to that gained by a 2kV acceleration field. As the electric potential increases linearly between the source and following plate, we can compute
the average velocity of an ion by first computing its average kinetic energy in the field:

$$\overline{KE} = \frac{0 + qV}{2} = \frac{1}{2} mv^2 \Rightarrow v = \sqrt{\frac{qV}{m}}$$ \hspace{1cm} (3.5)

An ion’s velocity in the drift region is determined by the kinetic energy imparted by the electric field:

$$\overline{KE} = qV = \frac{1}{2} mv^2 \Rightarrow v = \sqrt{\frac{2qV}{m}}$$ \hspace{1cm} (3.6)

The total flight time of an ion is simply the sum of the time spent in the acceleration (t_a) and in the drift regions (t_d):

$$t_{total} = t_a + t_d = \frac{d_a}{v_a} + \frac{d}{v_d} = d_a \sqrt{\frac{m}{qV}} + d \sqrt{\frac{m}{2qV}} = \left(\frac{d_a + \frac{d}{\sqrt{2}}}{\sqrt{qV}}\right) m$$ \hspace{1cm} (3.7)

Allowing a to be the travel length term in parenthesis above, we can solve for m/q:

$$\frac{m}{q} = \frac{V(t_{total})^2}{\alpha^2}$$ \hspace{1cm} (3.8)

Substituting typical values found in our system for a PF_6^- ion (10.05µs flight time for a -2kV acceleration field across the 0.76cm acceleration region, then drifting for 50.5cm in the field-free flight tube):

$$m = \frac{(t_{total})^2 qV}{\alpha^2} \Rightarrow \frac{(10.05e-6)^2 (1.602e-19)(2000)}{(0.365)^2} = 145\text{amu}$$ \hspace{1cm} (3.9)

We correctly find the mass of our PF_6^- ion to be 145amu.

Generally, to reduce experimental uncertainties, we rely on known mass calibrants rather than measured flight tube distances and voltages to determine the
Figure 3.10: Multiphoton MALDI, summed 100 shot spectra, displaying well resolved Titanium isotope peaks.

Figure 3.11: Multiphoton MALDI, summed 100 shot spectra, displaying well resolved Copper isotope peaks.
value of $qV/a^2$ (from equation 3.9). Aside from the primary ions of our RTIL, the metal ions from the desorption sample grids serve as excellent mass calibrants. Furthermore, the detection of clearly resolved isotopic structure, appearing with their naturally occurring abundances serves as a nice confirmation of the experimental apparatus. The five isotopes of titanium are clearly resolved in figure 3.10. Similarly, the two isotopes of copper are clearly resolved in figure 3.11. Integrating under the curve in figure 3.11 we find abundance of 67% and 33% for the two copper isotopes. This agrees nicely with NIST accepted values of 69.17% and 30.83% [29]. The distinctive isotopic structure of titanium also serves as a nice landmark for real time monitoring of spectra displayed in the acquisition time domain window. Ultimate resolution and its implication for ion desorption is examined in detail in the following chapter.
CHAPTER 4

Resolution

In general, the merit of time of flight mass spectrometry data is judged by its ultimate resolution. In the case of proteomics work, this is especially true as protein and peptide identification rely on the ability to resolve individual ion species and even isotopic patterns from a vast assembly of ion species. Conventional MALDI excels at resolving heavy mass ions, however, this is only after the incorporation of more advanced techniques such as delayed extraction and high acceleration voltages. This is in contrast to the work presented in this dissertation where laser desorption from a room temperature ionic liquid is shown to produce well resolved spectra before the implementation of any such additional resolution enhancing techniques. This difference is especially clear during the investigation of relatively low mass range spectra.
4.1 Defining Resolution

Principally, resolution in time of flight mass spectrometry is the ability to identify ions of different species within a given single spectra. Laser desorption from a room temperature ionic liquid produces prompt ions that meet this definition very well, figures 4.1 & 4.2 illustrate well resolved ion peaks, displaying the isotopic structure of titanium and copper.

Beyond a qualitative definition, resolution is generally discussed as a ratio in terms of mass or time. In MALDI, the mass resolution of an instrument is often quoted as a measure of quality. However, in this dissertation we will focus on the closely related temporal resolution as this relates more clearly to the physical processes being studied. Fortunately mass and time resolutions are simply related. The temporal resolution of a peak (equation 4.1) is defined as the ratio of its central arrival time \( t \), to its arrival time spread \( \Delta t \), measured as the full width at half maximum.

\[
\text{resolution} = \frac{t}{\Delta t}
\]

The full width at half maximum and the central arrival time are obtained directly from time of flight spectra, as shown for a PF\(_6\) peak in figure 4.3. Similarly, the mass resolution can be measured directly from spectra displayed in the mass domain, as demonstrated in figure 4.4 for the same PF\(_6\) peak. These figures illustrate that these two measurements of resolution differ, yet are simply related as:
Figure 4.1  Multiphoton MALDI, summed 100 shot spectra, displaying well resolved Titanium isotope peaks.

Figure 4.2  Multiphoton MALDI, summed 100 shot spectra, displaying well resolved Copper isotope peaks.
**Figure 4.3** PF$_6$ peak displaying the central arrival time ($t$) and the peak width ($\Delta t$), measured at the full width at half maximum. The temporal resolution for this peak is $\sim 1:370$. Negative 3kV data.

**Figure 4.4** PF$_6$ peak displaying the central arrival mass ($m$) and the peak width ($\Delta m$), measured at the full width at half maximum. The mass resolution for this peak is $\sim 1:190$. Negative 3kV data.
\[
\frac{m}{\Delta m} = \frac{t}{2\Delta t}
\]

Equation 4.2

The relation between mass and time resolution, as stated in equation 4.2, is straightforward to derive [30]. For a given ion peak a spread in arrival times exists, where this spread, \(\Delta t\), can be described as the difference between \(t_1\) and \(t_2\), the arrival times at the peak’s full width at half maximum. The central peak arrival time, \(t\), is equivalent to the average of \(t_1\) and \(t_2\) for a symmetric peak shape. These times, \(t_1\) and \(t_2\), translate to distinct mass values, \(m_1\) and \(m_2\), yielding a mass spread, \(\Delta m\). Equation 4.3, using conservation of energy, equates the energy gain due to the acceleration field, to the ions final kinetic energy, thereby expressing \(m_1\) and \(m_2\) in terms of \(t\) and \(\Delta t\).

\[
qV = \frac{1}{2}mv^2 \Rightarrow \begin{cases} 
\frac{2Vq}{L^2}\left( t - \frac{\Delta t}{2} \right)^2 &= m_1 \\
\frac{2Vq}{L^2}\left( t + \frac{\Delta t}{2} \right)^2 &= m_2
\end{cases}
\]

Equation 4.3

Computing the mass resolution, incorporating the definitions in equation 4.3 we find

\[
\frac{m}{\Delta m} = \frac{\frac{2Vq}{L^2}t^2}{\frac{2Vq}{L^2}\left( t + \frac{\Delta t}{2} \right)^2 - \frac{2Vq}{L^2}\left( t - \frac{\Delta t}{2} \right)^2} = \frac{t^2}{2t\Delta t} = \frac{1}{2} \frac{t}{\Delta t}
\]

Equation 4.4

the mass and time resolutions are related as previously stated in equation 4.2.
Resolution can also be expressed in terms of the central final velocity and the final velocity spread. Relating the ratio of the final velocity and the final velocity spread back to temporal resolution, equation 4.5 expresses the final velocities in terms of arrival times and arrival time spreads.

$$\frac{v_f}{\Delta v_f} = \frac{1}{\frac{1}{t} - \frac{1}{t+\Delta t}} = \frac{1}{\left(1 - \frac{\Delta t}{2t}\right)^{-1} - \left(1 + \frac{\Delta t}{2t}\right)^{-1}} \approx \frac{1}{\left(1 + \frac{\Delta t}{2t} + ... \right) - \left(1 - \frac{\Delta t}{2t} - ...ight)}$$ 4.5

Performing a binomial expansion of equation 4.5 yields the result found in equation 4.6, where temporal resolution is simply related to the final velocity and final velocity spread.

$$\frac{v_f}{\Delta v_f} \approx \frac{t}{\Delta t}$$ 4.6

Good resolution, whether in time or mass, is crucial for the generation of useful time of flight spectra especially when handling multiple ion species of a limited range. Ideally, resolution would not be a consideration at all, as each ion species would be uniformly desorbed and would arrive at precisely the same time. Clearly this is not a realistic expectation, as physical processes give rise to a spread in ion birth conditions, in turn, yielding our observed final time spreads. Looking ahead: in section 4.3, the causes and implications of our observed time spreads are investigated and discussed. Presently, in section 4.2, resolution issues regarding conventional MALDI systems are discussed as are the methods employed to improve them.
4.2 Conventional MALDI Resolution

Conventional MALDI intrinsically does not have excellent resolution. Its desorption and ionization process, consisting of a hot expanding collisional plume, creates a range of initial energies for a given ion species. This degrades resolution; figure 4.5, a low mass range spectra produced by conventional MALDI (20kV) displays this result [31]. For comparison, figure 4.6 shows prompt ion peaks, within the same mass range as figure 4.5, that was desorbed using our laser desorption from RTIL technique. Figures 4.5 & 4.6 are both plotted over a 10 Da window for ease of comparison. Figure 4.6 is an overlay of two sets of laser desorption from RTIL spectra, one positive, one negative, both from the same mass range. Aside from the improved resolution between figures 4.5 and 4.6, our desorption technique also offers a much more uniform, clean peak shape. While figures 4.5 & 4.6 show a comparison of ions of a similar mass, this is not a completely fair comparison as the peaks compared have different arrival times, as conventional MALDI uses significantly higher acceleration voltages than our technique. This improved resolution is not an artifact of the acceleration voltages differences. Figures 4.7 & 4.8 display two low mass ion spectra over the same arrival time window. Clearly, even comparing peaks of similar arrival times, conventional MALDI does not match the resolution of laser desorption from RTIL for low masses. Conventional MALDI does produce decent resolution at higher mass ranges, to do so it employs a few techniques that greatly enhance resolution, including the use of high acceleration fields, delayed extraction techniques and operation in a reflectron mode.
Figure 4.5 Conventional MALDI, quality control spectra, taken at EVMS on a Ciphergen PBS2 instrument, displaying one of the better resolved peaks within the low mass range. This peak suffers from poor resolution and a distorted peak shape.

Figure 4.6 RTIL desorption, summed 100 shot spectra, displaying both positive (blue) and negative (red) spectra over the same mass range. BMIM+ (139.2 Da) and PF$_6^-$ (145.0 Da) peaks are clearly present with a mass resolution of ~300. The shoulder on the right side of the BMIM+ peak is indicative of its isotopic structure. PF$_6^-$ is without isotopes.
Figure 4.7 Conventional MALDI, quality control spectra, plotted in the time domain over a 1\(\mu\)s range. The two early time, low mass ion peaks are not well resolved when compared to the ion peak of similar arrival time shown in figure 4.8. This species has a mass just over 200 Da and likely arises from matrix compounds present in the MALDI sample.

Figure 4.8 RTIL desorption, summed 100 shot spectra, displayed in the time domain over the same 1\(\mu\)s range as figure 4.7. This 29 Da peak is better resolved than its conventional MALDI counterparts of similar arrival times, despite a lower acceleration voltage (20kV vs. 3kV).
The use of high acceleration fields improves resolution for high mass peaks taken with a conventional MALDI system. Such large fields overcome resolution degradation effects associated with a large initial energy spreads. Considering a collection of desorbed ions with an initial energy spread of $E_0 \pm \Delta E$, equation 4.7 derives the expected mass resolution, incorporating the knowledge that an initial energy spread will be preserved while traversing conservative electric fields.

$$\frac{m}{\Delta m} = \frac{E_f}{\Delta E_f} = \frac{qV + E_0}{\Delta E} \approx \frac{qV}{\Delta E}$$ \hspace{1cm} 4.7

Equation 4.7 illustrates that increasing the final energy of desorbed ions by increasing the acceleration field is an effective way to increase mass resolution. In fact, if we ignore $E_0$ as it is quite small compared to the energy gain from the electric field, we find the resolution is roughly linear with the acceleration voltage. This is true as long as there is no field dependence in the initial energy spread, which is reasonable as this spread is largely due to thermal processes. MALDI typically employs acceleration voltages in the 10-20kV range, compared to the 2-3kV acceleration fields used in our desorption technique.

Delayed extraction is a clever technique that takes a group of ions of the same mass but with different initial velocities and focuses them back together for simultaneous arrival at the detector [32]. By delaying the start of the application of the extraction field, after the desorption process, same mass ions are allowed to separate based on their initial velocities, at which point the application of the field
a. Energy gain due to field

\[ \text{Energy gain due to field} = a^*qV_0 \]

b. Applied voltage \( = V_0 \)

\[ a = \frac{x}{L} \]

Energy gain due to field

\[ = a^*qV_0 \]

c. Figure 4.9  Delayed Extraction Schematic.  

a. Same mass ions with different initial velocities are allowed to separate spatially, before the application of an extraction field.  

b. Extraction field applied such that each ion experiences a different position based acceleration.  

c. Same mass ions, each with different final velocities, arrive simultaneously at detector.
gives a different kick to each ion, based on their current position within the extraction region, as shown in figure 4.9. Selecting the appropriate amount of delay is done by monitoring the width of an ion peak upon arrival and tuning the delay to maximize resolution. Delayed extraction does not match the final velocity of same mass ions, instead it yields whatever velocity is required to match the ions arrival time. This technique works remarkably well, but can only function over a limited mass range. A sufficiently small delay would align low mass ions, while that same delay would not allow heavy ions to separate enough for the differential acceleration to compensate for their initial velocity spread. Similarly, during a relatively long delay suited for high mass ions, the electric field would turn on after light ions have either left the extraction region or separated too far so that the field overcompensates for their initial velocity distribution.

MALDI time of flight is often operated in a reflectron mode, further enhancing resolution. A reflectron is an electrostatic mirror that can further correct a kinetic energy spread (in the direction of travel) within a given ion species [33]. This electrostatic mirror allows more energetic ions to travel further before reflection, where fast ions will have a longer path length than slow ions, thus regrouping them. The detector is then placed at the focal point of the electrostatic mirror, the point at which the arrival times will match for a single ion species. An additional benefit from a reflectron instrument is the effective doubling of the field free flight tube length without the need for a physically longer, more cumbersome apparatus.
Figure 4.10 Conventional MALDI quality control spectra, displaying multiple well resolved peaks across a high mass range, using delayed extraction, high acceleration voltage and a reflectron.

Figure 4.11 Conventional MALDI quality control spectra, in the high mass range, using delayed extraction, high acceleration voltage and reflectron. Peptide of mass 7777 Da with a good mass resolution of ~600.
The incorporation of the above mentioned techniques in MALDI produces high quality time of flight spectra. Specifically at the higher mass ranges, mass resolutions greater than 500 can be obtained. Figure 4.10 shows multiple well resolved peaks within the high mass range of a conventional MALDI, quality control spectra [31]. Figure 4.11 highlights the excellent resolution MALDI can obtain within the high mass range, after the incorporation of the techniques discussed in this section. The blood serum peptide displayed in figure 4.11 is of mass 7777 Da [31]. For reference, Albumin, the most abundant peptide in human blood serum is mass 67 kDa and is within the detection range of MALDI techniques. Our laser desorption from a room temperature ionic liquid technique holds promise to be competitive with resolutions achieved by conventional MALDI. Our technique has the added advantage of not yet having incorporated the resolution enhancing techniques discussed above.

### 4.3 Limitations on Resolution

The resolution of MALDI type instruments is limited by two primary contributions, a spread in birth velocities and a finite birth time associated with the laser desorption process. The same species ions desorbed with a spread in birth velocities, as would be expected from a high temperature source such as MALDI, would yield a reduced resolution. This initial velocity spread at the source would persist upon entry into the free flight region, although it will narrow somewhat after passing through the acceleration region. Besides an initial energy/velocity spread, the
primary desorption event has an associated birth time, which in turn provides a
characteristic time over which ions are desorbed from the surface. Section 4.3
reviews the consequences of these two effects and incorporates physical data to
indicate their magnitude.

The existence of an initial time spread, essentially the birth time of the ions, is
typically dictated by the laser pulse duration. For our laser desorption process, this
birth time, is the Nd:YAG laser pulse length which is approximately 6 ns. This birth
time serves, at best, as a minimum arrival time spread. If birth time effects were the
ultimate limit on our resolution, assuming there is no initial velocity distribution
within a given species of ion, our resolution would be as shown in equation 4.8:

\[
\frac{t_{\text{arrival}}}{\Delta t} = \frac{t_{\text{arrival}}}{t_{\text{birth}}}
\]

with the birth time setting \( \Delta t \), leaving \( t_{\text{arrival}} \) to govern the resolution. Therefore, in
such a scenario, with \( t_{\text{arrival}} \) determining the resolution one should expect improved
resolution with lower acceleration fields or a longer flight tube, as both would drive
up the value of \( t_{\text{arrival}} \). Therefore a birth time of 6 ns limits a PF\(_6\) peak, from our laser
desorption from RTIL -3kV spectra, to a temporal resolution of < 1300. It should be
pointed out that our MCP and PMT combination currently limit our observed width to
10ns, which exceeds that of our laser pulse time.

Birth time effects are not the limiting factor in our observed resolution, initial
velocity distributions play a significant role in conventional MALDI and less of a role in
our laser desorption from RTIL technique. Conventional MALDI is a highly thermal
process, with initial temperatures during the creation of the MALDI plume upwards of 1000K. MALDI is known as a fluence (J/cm$^2$) dependent rather than irradiance (W/cm$^2$) dependent process, where the birth time associated with the laser plays a minimal role in determining ion packet characteristics [17]. MALDI’s thermal characteristics impart a significant thermal energy spread to desorbed ions. We observe that laser desorption from RTIL suffers from initial velocity spread based degradation to a much lesser extent than that of MALDI.

Initial velocity distributions are a manifestation of the energy spread deposited during the desorption process. Therefore for a given ion species, we express its energy spread in terms of its resulting initial velocity spread. Resolution can then be expressed in terms of the initial velocity, $v_0$, and the initial velocity spread, $\Delta v$, as shown in equation 4.9.

$$\frac{t}{\Delta t} = \frac{v_f}{\Delta v_f} = \frac{\sqrt{v_0^2 + \frac{2qV}{m}}}{\sqrt{\left(v_0 + \frac{\Delta v_0}{2}\right)^2 + \frac{2qV}{m}} - \sqrt{\left(v_0 - \frac{\Delta v_0}{2}\right)^2 + \frac{2qV}{m}}}$$ 4.9

Factoring out the kinetic energy gain due to the acceleration field, we can Taylor expand the expression in equation 4.10:

$$\frac{t}{\Delta t} = \frac{mv_0^2}{\sqrt{2qV}} + 1 \approx \frac{1 + \frac{1}{2}}{\frac{mv_0^2}{2qV} + 1}$$ 4.10

59
where the first term in each of the square roots is less than one. This first term is essentially the ratio of the ion’s initial energy to that of the kinetic energy gain due to the acceleration field. Expressing this ratio in terms of experimental data from our laser desorption from RTIL technique with typical values for a PF₆ molecule with an arrival time of 9μs:

\[
\frac{m v_0^2}{2qV} = \frac{v_0^2}{v_{field}^2} = v_0^2 \left( \frac{9 \times 10^{-6}}{0.42} \right)^2 = v_0^2 \left( 2 \times 10^{-11} \right) \leq 1
\]

Equation 4.11

The energy ratio calculated in equation 4.11 is less than one, considering typical values for \( v_0 \) from conventional MALDI are between \( 10^2 \) and \( 10^3 \) m/s [34].

Incorporating the value of the ratio from equation 4.11 into equation 4.10 yields a simple result in equation 4.12 that summarizes the expected resolution for a desorbed ion with an initial velocity and initial velocity spread:

\[
\frac{t}{\Delta t} \cong \frac{2qV}{mv_0 \Delta v_0}
\]

Equation 4.12

For MALDI type processes, we expect resolution to be linear with increasing acceleration field and inversely proportional to the product of the initial velocity and velocity spread. Equation 4.12 summarizes the need for MALDI to use large acceleration voltages to overcome resolution loss associated with a largely thermal desorption process.
4.4 Implications of Resolution

Having reviewed a few potential limits of resolution, section 4.4 presents data relating to its dependence on the acceleration field. In principle, a higher acceleration field should yield a better resolution. MALDI relies on this to combat a relatively large initial velocity spread, typically employing voltages in the tens of kilovolts. As discussed in the previous section, a higher acceleration field will narrow a given initial velocity spread for an ion species, thereby increasing resolution. Interestingly, in laser desorption from RTIL, we do not always see this effect; here we will discuss the implications.

Figure 4.12 shows that the temporal resolution remains relatively constant despite a factor of two change in the acceleration field. This is in clear contrast to the resolution’s expected (equation 4.12) linear dependence on the acceleration field. To better examine this behavior, figure 4.13 displays the arrival time spread for PF₆, Δt, as a function of the acceleration voltage over an expanded range. The arrival time spread drops quickly with increasing acceleration voltage then levels off to a minimum temporal width. The arrival time spread can be described as the sum of the preserved initial time spread (birth time) and a portion due to initial velocity effects,

\[ \Delta t_{\text{arrival}} = t_{\text{birth}} + \Delta t_{\text{velocity}} \]  

4.13

examining the 2

\[ \frac{2\Delta t}{t} = \frac{\Delta E}{E} \]  

4.14
Figure 4.12 PF$_6$ peak resolution plotted as a function of the applied acceleration voltage. The temporal resolution is remarkably constant, not displaying the expected linear dependence on the acceleration voltage.

Figure 4.13 PF$_6$ peak arrival time width plotted as a function of the applied acceleration voltage. The time width is unusual, as it is remarkably constant at higher acceleration voltages. At acceleration voltages below 1500V, collection efficiency begins to suffer and width measurements become less reliable.
allowing the arrival time spread from equation 4.13 to be reexpressed:

\[
\Delta t_{\text{arrival}} = t_{\text{birth}} + \frac{\Delta E \ t}{E \ 2}
\]

Equation 4.15 relates the initial energy spread of desorbed ions to the observed arrival time spread. By expressing \(E\) and \(t\) in terms of known physical system parameters and the acceleration voltage, for PF\(_6\) we find:

\[
\Delta t = \Delta t_{\text{birth}} + \Delta E \sqrt{\frac{m L^2}{2q V}} \frac{1}{2qV} = \Delta t_{\text{birth}} + \frac{\Delta E}{q} \cdot \left(10^9\right) \cdot V^{-\frac{3}{2}}
\]

where \(\Delta t\) is expressed as the sum of the birth time spread and the initial energy spread in eV. According to this model, we would expect an increase in peak width at low acceleration voltages, with the initial energy spread governing the turn-on point for the width increase. Qualitatively, the model in equation 4.16 matches the behavior shown in figure 4.13. Recall figure 4.13 is a plot of the measured arrival time width as a function of acceleration voltage for 100 shot summed data for a PF\(_6\) peak. The increasing width at the low acceleration voltages would seem to be an indicator of a measureable initial energy spread as predicted by equation 4.16. However this isn’t the case because in addition to not following the functional form consistent with that of an energy spread, it is clear that the increase in arrival time width is primarily due to increased shot to shot variation as apparent in the heat maps that follow.

The resolution of our prompt peak is dominated by the birth time associated with the desorption process. A closer examination of the composition of this data reveals this low acceleration voltage data is less reliable. Our collection efficiency suffers at low acceleration voltages, with significant temporal width variation.
Figure 4.14  Heat map displaying a well resolved PF₆ peak over the course of a 100 shot data run, taken at -1500V. The color bar represents the raw ion intensity recorded in volts. Only a few shots, numbers 91-93, appear to be significantly broader than the rest.

Figure 4.15  Heat map displaying a PF₆ peak acquired at an acceleration voltage of -1300V where a dual behavior is observed. The color bar represents the raw ion intensity recorded in volts. Compared to figure 4.12, this spectra is not as well resolved, due to the roughly 20 broader shots.
between shots. Figure 4.14 is a heat map of a well-resolved PF$_6$ peak, taken at -1500V, with almost all shots quite narrow. Figure 4.15 is a heat map of PF$_6$ taken at -1300V, otherwise at the same conditions as figure 4.14. Two changes are apparent. First, the collection efficiency has fallen off considerably at the lower acceleration voltage (-1300V, figure 4.15). Second, the ion peaks acquired at the lower acceleration voltage are significantly broader, as shown in figure 4.16, a plot of two PF$_6$ peaks, each a single representative shot from the -1300V and -1500V data sets. The apparent width change of the PF$_6$ peak between the -1300V and -1500V spectra can not be attributed to the presence of an initial velocity spread due to the desorption process for two reasons. First, this observed width change is occurring over the voltage range where the collection efficiency of the TOF system is quickly deteriorating. Second, all changes in PF$_6$ peak width cease as the acceleration voltage is increased beyond -1500V, the same point where the collection efficiency stabilizes.

Our process, laser desorption from a room temperature ionic liquid, produces ions with an arrival time width that is governed by the desorption birth time, as evidenced by the arrival time width’s independence on the acceleration field. At extremely low acceleration voltages (a factor of two below typical operation values), peak widths increase, however this is due to collection and detection degradation effects, not fundamental desorption processes. The stability of ion peak width is encouraging for future improvements to instrument resolution. In particular, we could strongly enhance resolution by increasing flight times, through the
Figure 4.16  PF$_6$ peak from two single shot spectra taken at different acceleration voltages. The spectra taken at a -1300V is broader than that of the -1500V spectra. This change is not indicative of the presence of any initial velocity spreads arising from the desorption process. This width change only appear at low acceleration voltages, over the range where the TOF system’s collection efficiency is dropping to zero.
incorporation of a reflectron. Additionally, we stand to benefit from a post-acceleration region in order to enhance the detection efficiency of slow, late arriving ions. Unlike MALDI, we don’t see improved resolution at increased acceleration voltages, which aside from avoiding the need for improved high voltage feedthroughs in our system, implies our initial energy spread is significantly narrower and therefore colder than that of MALDI.
CHAPTER 5

Ion Production Mechanisms

Paramount to any time of flight mass spectrometry technique is the method of desorption and ionization of analyte. This chapter describes experiments incorporating two different methods of desorption/ionization initiated by the same single laser pulse. Both methods differ from conventional MALDI, with one not reliant on collective effects while both are not reliant on complex plume dynamics. Each offer advantages when compared to MALDI, in essence yielding two interesting and complementary tools for desorption. Moreover, the ability to study two processes within a single spectra is a unique and promising opportunity.

5.1 Two Processes

The generation of ion spectra through the incorporation of techniques presented in this dissertation can yield the occurrence of two processes within a single spectra. The first is a multiphoton process, in the sense that RTIL is transparent at 532nm, yet
Figure 5.1: Time of flight spectra, 100 shot summed data, taken at a source voltage of +3kV. Typical well resolved prompt desorption ion spectra, with minimal background and clearly identifiable ion peaks. The primary RTIL cation, BMIM, its primary fragment, Imidazolium, and metal ions from the sample grid, Titanium, are all labeled.

Figure 5.2: Time of flight spectra, 100 shot summed data, taken at the same laser spot position, laser spot size, source voltage and focus voltage, but at 25% higher laser power. Spectra containing peaks produced by two mechanisms of ionization/desorption. Higher laser power yields higher intensity ion peaks from the prompt desorption process, in addition to acoustically generated ion peaks, that appear delayed and more broad than typical multiphoton MALDI peaks.
we can force absorption at this wavelength with a sufficiently high intensity. This multiphoton process desorbs/ionizes analyte at the arrival time of the laser pulse, yielding narrow ion peaks of high resolution, with the transit through RTIL serving to cool desorbed ions. The second process is acoustic, occurring at a delay (~100ns) with respect to the laser pulse, occurring at the surface of the RTIL with the delay tied to the laser spot size. This acoustic process is not as well resolved as the multiphoton process, but excels at the production of heavier ion species and is more gentle at desorption.

A fresh titanium grid (300 mesh) sample was prepared with 1 μL of [BMIM][PF₆] as described in Chapter 3. Our prompt desorption process routinely generates summed 100 shot time of flight data as seen in figure 5.1, acquired at a source voltage of +3kV. Spectra such as those in figure 5.1 contain readily identifiable peaks that are easily calibrated from the laser shot time and system parameters. Figure 5.2 is a positive time of flight spectra taken from the same sample, at the same acceleration voltage, laser spot position and focus voltage as that of figure 5.1, except at a 25% higher laser power. Under these conditions an additional process occurs, this acoustic process appears at a delay with respect to the prompt desorption process start time (essentially the laser time). Interestingly, for the primary RTIL cation, BMIM⁺, ions peaks associated with both processes are readily identifiable. Figure 5.3 features an overlay of figures 5.1 and 5.2 in the window around the BMIM⁺ peak arrival time. This overlay allows the clear identification of the first peak in the higher laser power spectra as the prompt desorption peak, as it
Figure 5.3: BMIM$^+$ peak from figures 5.1 and 5.2. The first data set, shown in red, is a purely prompt desorption spectra. The second data set, shown in blue, has two BMIM$^+$ peaks, the first clearly aligning with the prompt desorption peak from the red spectra, while the second arises from an acoustic desorption process. The acoustic peak is delayed by 100ns and is broad compared to the prompt desorption peak.

Figure 5.4: Ti$^+$ peaks for two sets of summed 100 shot spectra are plotted as a function of arrival time. Both data sets contain only Ti$^+$ peaks produced by the prompt desorption process. The Ti$^+$ spectra in blue is the same data set shown in blue in figure 5.3, displayed in the inset. The blue spectra is above threshold for the production of both acoustic and prompt desorption BMIM$^+$ peaks. Despite this, Ti$^+$ only has a prompt desorption peak. Metal ions are not created acoustically.
aligns with the prompt desorption peak from figure 5.1. The second peak is acoustic and arrives 100ns later than the multiphoton peak and is notably broader.

Further investigation of these two spectra at arrival times other than that of BMIM\textsuperscript{+}, reveal all ion species are not treated equally by the two desorption/ionization processes. Take for example, the Titanium ions shown in figure 5.4, in which both spectra contain narrow Titanium peaks generated by the prompt desorption process, while neither has delayed acoustically generated counterparts. Titanium\textsuperscript{+}, unlike BMIM\textsuperscript{+}, is not pre-ionized, and is therefore not found acoustically which is primarily a desorption, and not an ionization, mechanism. In addition, the acoustic process desorbs from the surface of the RTIL, not from the grid. While this reveals a limitation for acoustic desorption, it is potentially overcome for some analyte via attachment to BMIM\textsuperscript{+} ions. This holds promise for acoustic desorption of amino acids via RTIL attachment.

We observe BMIM\textsuperscript{+} attachment within these same spectra at later arrival times as shown in figure 5.5, where there is an acoustically desorbed [BMIM\textsubscript{2}]PF\textsubscript{6}\textsuperscript{+} ion peak in the higher power spectra. Furthermore, this heavy ion is unique in that it does not have an associated prompt desorption peak. This behavior highlights the unique potential for the acoustic desorption of molecules where our prompt desorption process fails. Careful examination of the lower power spectra in figure 5.5 does reveal an extremely small [BMIM\textsubscript{2}]PF\textsubscript{6}\textsuperscript{+} peak. We can refer to the heat map used to generate the summed data, revealing this small peak is comprised of only 5
Figure 5.5: BMIM[RTIL]^+ peak, mass 425 Da, acoustically desorbed. Neither the high power (blue) or low power (red) spectra yield a BMIM[RTIL]^+ peak via the prompt desorption process. This highlights the potential for desorbing heavy molecules via acoustic desorption process in a region where a more typical desorption process fails.

Figure 5.6: Heat map showing only five shots contain [BMIM]_2[PF_6]^+ acoustic peaks from the low laser power 100 shot spectra. These five shots also contain acoustically generated BMIM^+ peaks.
out of 100 shots, as seen in figure 5.6. Our high and low power spectra are chosen near, but on opposite sides of the threshold for the acoustic process. The heat map in figure 5.6 highlights the sharpness of this threshold which yields a sensitivity to shot to shot laser fluctuations. Figure 5.7 displays individual spectra from two consecutive laser shots from the heat map data (figure 5.6) that display this sharp threshold behavior. These two shots were taken at the same laser power setting, having only small (+/- 10%) shot to shot variations inherent in our laser setup.

The laser power dependence for our two processes clearly differs. While the laser power fluctuations between 5.7a and 5.7b are enough to turn on our acoustic desorption process, the same laser power fluctuations hardly affect the amplitude of the prompt ion peaks of BMIM$^+$ and its primary fragment, Imidazolium$^+$.

To properly identify the process giving rise to ion peaks found in time of flight spectra, we needn’t solely rely on the characteristic widths or shapes. Figure 5.8a displays a mass calibration set to the time offset to the laser firing time which we associate with the prompt desorption process. This calibration equation is:

$$\frac{m}{z} = 2.12 \left( \frac{t - 531}{500} \right)^2$$

$$t_{laser} = t - 531$$

where $t$, the time index (2ns per time point), refers to the start of a time of flight record and $t_{laser}$ refers to the time at which the laser strikes the desorption sample. Equation 5.1 correctly assigns mass to the prompt BMIM$^+$, Imidazolium$^+$ and Ti$^+$ peaks. It fails for the acoustic desorption ion peaks. Assuming this failure arises
Figure 5.7: Single shot time of flight positive spectra obtained from two consecutive laser shots near the acoustic desorption threshold. These are shots 59 (a) and 60 (b) from the heat map in figure 5.6. Nominally, all 100 shots where taken at the same laser power, shot to shot variation results in the occasional above threshold shots in this data run. BMIM$^+$ and its primary fragment (imidazolium) are present in both spectra and are of similar amplitude. A Ti$^+$ peak is clearly present only in spectra b, though it is not the result of acoustic desorption. Acoustic desorption occurs in spectra b with the generation of a [BMIM]$_2$[PF$_6$]$^+$ peak and the additional BMIM$^+$ peak.
**Table 5.8:**

<table>
<thead>
<tr>
<th>Ion</th>
<th>Ti</th>
<th>Imidazolium</th>
<th>BMIM</th>
<th>[BMIM]₂[PF₆]</th>
</tr>
</thead>
<tbody>
<tr>
<td>m/z</td>
<td>47.9</td>
<td>83</td>
<td>139</td>
<td>425</td>
</tr>
</tbody>
</table>

**Table 5.9:**

<table>
<thead>
<tr>
<th>Ion</th>
<th></th>
<th>BMIM, prompt</th>
<th>BMIM, acoustic</th>
<th>BMIM Δₜarrival</th>
</tr>
</thead>
<tbody>
<tr>
<td>t_arrival</td>
<td>8096 ns</td>
<td>8226 ns</td>
<td>130 ns</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 5.8:** Single shot time of flight positive spectra transformed into m/z domain using two different start time within the calibration equation. **5.8a** sets t₀ equal to the laser time and yields correct mass assignments for the prompt desorption peaks. **5.8a** fails to assign correct masses to the acoustic peaks. **5.8b** accounts for the time delay (130ns) associated with the acoustic desorption process. This time delay is measured as the difference in the arrival times of the BMIM⁺ peaks.
from the time delay associated with an acoustic process, it's reasonable to attempt a simple shift in the desorption start time within the same calibration equation.

\[
m/z = 2.12 \left( \frac{t - 596}{500} \right)^2
\]

5.2

In fact, equation 5.2 assigns the proper mass for the acoustic desorption ion peaks of BMIM\(^+\) and [BMIM\(_2\)][PF\(_6\)]\(^+\) as shown in figure 5.8b. We set the change in the time offset between equations 5.1 and 5.2 at 130ns, measured as the difference between the arrival time of the two BMIM\(^+\) peaks (prompt and acoustic) in figure 5.8. The ability of a simple shift in the start time to properly align the masses of two ion peaks, BMIM\(^+\) and [BMIM\(_2\)][PF\(_6\)]\(^+\), that have arrival times differing by almost a factor of two is good evidence the acoustic process indeed occurs at a fixed delay with respect to the laser time.

Negative ion spectra exhibit similar behavior as do their positive counterparts. Here the dominant ion species is PF\(_6^-\), the anion of our RTIL, along with fast electrons as shown in figure 5.9a. Further investigation of the PF\(_6^-\) peak, in figure 5.9b, reveals there are both acoustic and prompt PF\(_6^-\) peaks, along with a prompt PF\(_5\)OH\(^-\) ion peak. Similar to the positive ion spectra where we observed acoustically desorbed [BMIM\(_2\)][PF\(_6\)]\(^+\), figure 5.9b & 5.9c reveal its negative analog, [PF\(_6\)]\(_2\)[BMIM\(^-\)], is found acoustically desorbed, except via PF\(_6^-\), rather than BMIM\(^+\) attachment. In addition, [PF\(_6\)]\(_2\)[BMIM\(^-\)] is not produced by the prompt desorption process, as was found for [BMIM\(_2\)][PF\(_6\)]\(^+\) in the positive spectra.
Figure 5.9: A representative single shot time of flight negative ion spectra obtained above the acoustic desorption threshold. Spectra a displays all ion peaks, including the electrons. Plot b highlights the region around PF$_6^-$ . Plot c displays the required calibration correction for an acoustic peak is simply the delay time for the acoustic process.
5.2 Laser Power Dependence

The existence of two distinct processes is clear from the arrival timing of same species ion peaks as well as ion peak shape and width. Further bolstering this argument is the distinct laser power dependence for the prompt versus acoustic processes. Both processes exhibit distinct laser power dependences, while Titanium stands in a class of its own.

The prompt BMIM$^+$ peak and its primary fragment Imidazolium$^+$ are plotted on a logarithmic scale as a function of laser intensity in figure 5.10. The individual data points represent the summed ion event count over a time window around the selected ion species during a 100 spectra acquisition with the background subtracted. The subtracted background is calculated as the integral of the same length time window at a slightly later (or earlier) arrival time in which there are no ion peaks. In figure 5.10 it clear both prompt peaks, BMIM$^+$ and Imidazolium$^+$ quickly rise from 0-10 MW/cm$^2$, then grow much more slowly from 10 MW/cm$^2$ onward. Both prompt BMIM$^+$ and Imidazolium$^+$ have similar laser power dependences, except for the appearance of a deviation at very high laser powers as evident by figure 5.11, essentially figure 5.10 plotted linearly. This apparent deviation should not been trusted as a difficulty arises when calculating the integrated intensity for the prompt BMIM$^+$ peak at high laser powers. At higher intensities the acoustic BMIM$^+$ peak overlaps with the prompt BMIM$^+$ peak, resulting in an overstatement of the prompt BMIM$^+$ if straightforward time summations are being used. Fortunately, Imidazolium$^+$ serves as a good proxy for BMIM$^+$, as they grow together with laser
Figure 5.10: BMIM\(^+\) and Imidazolium\(^+\) summed 100 shot ion counts plotted as a function of laser intensity, on a logarithmic scale. Both prompt peaks experience a rapid rise from 5-10 MW/cm\(^2\), leveling off to slower linear growth (on log scale) at around 10 MW/cm\(^2\).

Figure 5.11: Integrated Imidazolium\(^+\) and BMIM\(^+\) ion signals from 100 shot spectra plotted as a function of laser power. The plotted prompt BMIM\(^+\) peak integrated intensity grows with laser power in a similar fashion to the Imidazolium\(^+\). The noticeable deviation above 30 MW/cm\(^2\) is exaggerated due to overlap of the acoustic BMIM\(^+\) peak with the prompt BMIM\(^+\) peak, thereby inflating its integrated intensity. In reality, BMIM\(^+\) and Imidazolium\(^+\) behave quite similarly with increasing laser intensity.
power, only deviating as the acoustic BMIM$^+$ overlap begins to occur. Figure 5.12 gives an example of the overlap between prompt and acoustic BMIM$^+$ at higher laser powers. This overlap is primarily due to the significant broadening of the acoustic BMIM$^+$ peak in summed data taken at high laser power. The acoustic process yields naturally broad ion peaks and is made to look even broader in the summed data as shot to shot variations in acoustically desorbed ion peak arrival times are significant. After recognizing the contributions from the acoustic BMIM$^+$ signal to the prompt BMIM$^+$, it is clear both primary prompt desorption peaks behave remarkably similar with respect to their dependence on laser intensity.

Acoustically generated ion peaks exhibit significantly different laser power dependence. Compared to the prompt ion peaks, these acoustic peaks have a strong threshold behavior, not turning on until a higher laser power. Acoustic desorption has a clear on/off behavior, with limited variation in desorbed ion intensity. Figure 5.13 displays this behavior with both acoustically generated peaks, BMIM$^+$ and [BMIM$_2$][PF$_6$]$^+$ remain essentially at zero until reaching a laser power significantly past the point at which prompt ion peaks are generated. Furthermore, once the acoustic process turns on, it does not grow exponentially with laser power. In figure 5.13 there is growth in the acoustic desorption signal just past threshold, however this quickly levels out at higher laser power. It is important to recall that acoustic desorption is especially sensitive to shot to shot variation in laser power. It is for this reason it appears as if acoustic production occurs before 40 MW/cm$^2$, however this is
Figure 5.12: Two sets of 100 shot summed data taken at high laser power displaying the BMIM+ ion peaks. At high laser powers significant overlap occurs between the prompt and acoustic ion peaks, complicating efforts to accurately measure integrated ion signal from each process.

Figure 5.13: Integrated acoustic BMIM and [BMIM]_2[PF_6] ion signals from 100 shot spectra plotted as a function of laser power. A sharp threshold exists between 30-40 MW/cm^2. The apparent rise before this point is due to shot to shot fluctuations that exceed the acoustic production threshold.
Figure 5.14: Three individual consecutive shots taken with the laser intensity set to 29.8 MW/cm², near the observed acoustic production threshold. The effect of shot to shot fluctuation is apparent for acoustic production. **a)** Arrival time window centered around the BMIM⁺ peak. All three shots yield small amplitude variations in the prompt BMIM⁺ peak, while shot 28 clearly shows its acoustic counterpart. **b)** Arrival time window centered around the \([\text{BMIM}]_2[\text{PF}_6]^+\) (acoustic) peak. The same laser fluctuations present in figure 5.14a have a dramatic impact on the appearance of consecutive \([\text{BMIM}]_2[\text{PF}_6]^+\) acoustic peaks.
due to a limited number of above threshold shots. Figure 5.14b displays [BMIM]₂[PF₆]⁺ for 3 consecutive shots taken at a laser intensity of 29.8 MW/cm².

Figure 5.14a displays Imidazolium⁺ for the same three consecutive shots. Comparing these two figures it is apparent shot to shot variation do affect the Imidazolium⁺ peak amplitude, however, this in no way compares to the threshold style behavior seen in the three acoustic [BMIM]₂[PF₆]⁺ peaks. Clearly, acoustically desorbed ions differ in their laser intensity dependence compared to prompt ions with their sharper threshold at 40 MW/cm².

Interestingly, Ti⁺, while having a peak shape and timing of the prompt desorption process, seems to differ not only from acoustic ions, but other prompt ions as well. Figure 5.15 compares Ti⁺ production to prompt BMIM⁺ production as a function of laser intensity. Ti⁺ grows as intensity to the n while BMIM⁺ rises more quickly until 10 MW/cm², then essentially levels out or grows slowly & linearly.

Figure 5.16 compares Ti⁺ production to acoustic [BMIM]₂[PF₆]⁺ production. Here again, Ti⁺ goes as lⁿ, while [BMIM]₂[PF₆]⁺ remains low with minimal ion production. Subsequently [BMIM]₂[PF₆]⁺ roughly matches the Ti⁺ desorbed ion count at higher laser intensities, where acoustic production begins beyond 30 MW/cm². Furthermore, the acoustic production of [BMIM]₂[PF₆]⁺ quickly levels off at high intensities, exhibiting almost an bimodal on/off style behavior. The same hold true for acoustic BMIM⁺ at high intensities as shown in figure 5.17. A closer examination of the laser
Figure 5.15: BMIM$^+$ and Ti$^+$ summed ion counts plotted as a function of laser intensity, on a logarithmic scale. The prompt desorption BMIM$^+$ count rises more quickly than Ti$^+$ initially, however it almost completely levels off starting at 10 MW/cm$^2$, where Ti$^+$ continues to increase.

Figure 5.16: Prompt Ti$^+$ and acoustic [BMIM]$_2$[PF$_6$]$^+$ integrated ion count plotted on a logarithmic scale as a function of laser intensity. Ti$^+$ follows an intensity raised the n dependence, while the acoustic ions are more difficult to characterize due to the severity of their laser intensity threshold.
Figure 5.17: Integrated Ti (prompt) and BMIM (acoustic) ion signals from 100 shot spectra plotted as a function of laser power. Interestingly, Ti⁺, while not timed to align with the acoustic process, exhibits a similar laser power dependence as the acoustically generated ion series. The total desorbed ion counts for the two species are quite similar.

Figure 5.18: Ti⁺ integrated ion count plotted on a logarithmic scale as a function of laser intensity. Ti⁺ follows intensity raised the n dependence, as shown by the two fits with n set to 3 and 5.
intensity dependence of Ti⁺ itself in figure 5.18 reveals a clear 1ⁿ behavior with limits of n between 3 and 5. Such behavior is indicative of a multiphoton desorption process.

We have the ability to alter the laser spot size incident on the desorption sample by changing the size of an iris located 2 meters before our sample assembly (iris location, optical table layout described in chapter 3). Laser spot size variation allows for the investigation of collective effects, such as our acoustic pulse. Interestingly, the variation of our spot size dictates the delay in our acoustic desorption pulse. In figure 5.19a, we’ve induced a rather dramatic change in spot size which yields a difference of 82 ns between the two acoustic BMIM⁺ arrival times. Images of the laser spot imaged onto a CCD camera are shown in figure 5.19b. While the laser spot is not symmetric, it is clear the size of the ‘hot’ region within each spot is significantly different. Interestingly, the ratio of the delay of the acoustic pulse is roughly equivalent to the ratio of the area or laser energy (as the energy scales with area):

\[
\frac{126 \text{ns}}{44 \text{ns}} = 2.86 \approx 2.78 = \frac{490 \mu m^2}{176 \mu m^2}
\]

Future work calls for the use of a more uniform spot shape and readily controllable spot size so the dependence on area and laser energy could be well mapped out and further explored [35, 36].
Figure 5.19:  

a. BMIM$^+$ 100 shot summed data, two spectra. The two spectra were taken with different laser spot sizes. Changing the laser spot size changes the arrival time delay of the acoustic BMIM$^+$ peak. The time delay for the acoustic pulse scales roughly with spot size area. 

b. Images of the laser spots, imaged on a CCD camera, after spectra acquisition.
CHAPTER 6

Conclusions

Desorption from a room temperature ionic liquid opens new opportunities to study two complementary processes in tandem. The primary desorption process is the multiphoton desorption of metal ions from the sample grid. The secondary desorption process is acoustic and combined with the use of room temperature ionic liquids is not solely limited to the desorption of neutral species. Combined, these processes present a novel method of desorbing and ionizing biomolecules. Final results further highlighting this potential are discussed in section 6.1. The technique presented in this work opens future avenues for continued study of multiple desorption techniques within a single spectra. This is possible due to the flexibility of our apparatus; the tuning of the laser spot location, laser power, and laser spot size allows for the quasi independent control of two distinct processes within the same time of flight record. Improvements to the layering of RTIL onto desorption surfaces will likely yield improved stability, control and the ability to tailor samples for studies.
over broader laser intensity ranges. Furthermore, the addition of a post acceleration stage to the time of flight apparatus should improve the ability to detect larger biomolecules, as increasing the speed at which slow ions strike the MCP enhances the probability of detection.

6.1 Detection of Biomolecules

Part of the early motivation presented for this work was its potential suitability for the detection of biomolecules. Angiotensin II (MW=1046.2 Da) is a peptide found in blood that is linked to constriction of blood vessels. We attempt to detect Angiotensin II using our desorption from a RTIL technique. We choose Angiotensin II as it is commercially available, biologically significant and falls within a mass range we can currently handle. Angiotensin II is comprised of the amino acid chain: Asp-Arg-Val-Tyr-Ile-His-Pro-Phe, which is convenient as it allows us to watch for the presence of these amino acids as signs of peptide fragmentation.

We prepare a titanium grid sample coated with RTIL as discussed in detail in chapter three. After pumping down the sample we return it to atmosphere to add our Angiotensin II droplet. We deposit a 5μL droplet of Angiotensin II 984μM solution onto the RTIL coated sample grid and allow it to dry. Upon deposition the droplet spreads over the grid and partially onto the stainless steel support plate with ~5 nanomoles of Angiotensin II covering an approximate area of 30mm². Considering the area of our laser spot is approximately 1400μm², we have roughly 0.25 picomoles of Angiotensin II within our desorption region (as defined by our laser spot).
Figure 6.1  Negative ion data (-3kV) taken from [BMIM][PF₆], Titanium grid prepared desorption sample coated with 5µL of Angiotensin II (MW=1046.2 Da). a) Three spectra showing an ion peak in m/z range of Angiotensin II, most likely a negative ion adduct of Angiotensin II. b) Closer view of Angiotensin II associated ion peaks.
Figure 6.1 displays three single shot negative (3kV) ion spectra taken at 30MW/cm² from this desorption sample. PF₆⁻, [PF₆]₂[BMIM]⁻ and e⁻ peaks are clearly present, with the former two both showing an acoustic desorption peak, indicating we are above threshold for acoustic desorption in these shots. In addition there is a peak at an m/z of ~1150 Da, in the range of Angiotensin II (MW =1046.2 Da). This peak appears unique to this sample, and is likely Angiotensin II with a negative ion adduct. Adduction is common as we have seen in our work with [PF₆]₂[BMIM]⁻ and [BMIM]₂[PF₆]⁺, and is also well documented in the literature, especially in the case of Na⁺ adducts [38, 39]. Some studies in MALDI using ionic liquid matrices (ILMs) have also reported the presence of adduction mechanisms [40].

These results are encouraging and should be improved upon. The minimal background around the Angiotensin II peak in figure 6.1b is excellent for future work, as noise and dark current counts clearly do not mask the small observed peak. In addition, the pure RTIL & Ti desorption sample contributes little in the way of ion peaks beyond 500 Da, leaving a clear window for investigation of peptides beyond this range. Even at masses below 500 Da, recurrent RTIL and Ti related peaks are readily identifiable, leaving open the possibility for studying lighter amino acids. As mentioned, our ability to successfully detect heavy ions is partially limited by the lack of a post flight tube acceleration region before our microchannel plate detector. The addition of such a region would improve the detection efficiency for late arriving ions, such as most biomolecules of interest. Furthermore, optimization of the
application of biological samples to the desorption surface can be improved. The dried droplet method we have used tends to accumulate the majority of solute around the edges of the deposited droplet, which is large compared to our desorption (laser spot) size. A simple improvement such as the application of microdroplets for the deposition of peptides has the advantage of necessarily containing solute, even after drying, within desorption grid cells. In addition, microdroplets would have the advantage of minimizing solution wicking along the sample surface.

6.2 Acoustic Process Characterization

In addition to multiphoton desorption we observe acoustically desorbed ion peaks. Such ion peaks require no post ionization step as room temperature ionic liquids are essentially a pre-ionized species. This process would benefit from further characterization regarding control of the delay between the prompt and acoustic desorption time. We observe an acoustic desorption threshold of $\sim 30\text{MW/cm}^2$, for our typical laser spot area of $1400\mu\text{m}^2$. From this we can rule out a shock wave generated via laser induced cavitation, as such cavitation events have a much higher threshold of $\sim 1\text{GW/cm}^2$. It has been observed by L. Berthe, A. Sollier, et al, that the duration of laser induced acoustic pressure pulses decreases with increasing laser intensity on an aluminum target in water [37]. Improvements to our laser spot control should result in further improvements to our acoustic pulse duration, and in turn, our acoustic ion peak resolution.
6.3 Prompt Desorption

The presence of prompt desorption ion peaks amongst the acoustically desorbed peaks represents an advance towards a more accurate calibration of the acoustically desorbed process. The timing of the prompt process is simple and tied directly to the laser pulse time. The power dependence of titanium is clearly a multiphoton desorption process. The power dependence of BMIM$^+$ and Imidazolium$^+$ exhibits a threshold behavior with a sharp turn on at ~ 5MW/cm$^2$, growing roughly as $t^2$ thereafter. Furthermore, the prompt desorption process yields excellent resolution, even in the low mass range, at low acceleration voltages, a regime where MALDI falters. Both processes yield especially low background TOF spectra when compared to MALDI, which will aid in the detection of species with unknown ion signatures.

6.4 Outlook

Future studies should focus on the turn on transition for acoustic spectra on a shot by shot basis. Shot to shot fluctuations in laser power are significant enough near the acoustic threshold that the laser power should be monitored and recorded on a shot by shot basis. The improved knowledge of laser power on a single shot basis along with an improved laser spot shape should allow for the control or accounting of much of the instabilities seen in the acoustic ion production process.
These improvements that benefit the acoustic desorption process should greatly increase prospects for the detection of biomolecules. Furthermore, improvements to the sample exchange process will allow a higher throughput of desorption samples. Specifically, the decoupling of the sample assembly source from the entire assembly (ion optics stack) should be accomplished. Aside from increased ease of sample exchange, the current procedure is not well suited for future novel deposition methods of biomolecules (such as microdroplets) onto sample surfaces. Also, other room temperature ionic liquids should be explored in an effort to find others suited to simple adduction to peptides.

Lastly, the independence of the two desorption processes discussed in this work should be further explored. Recall, within single spectra, we observed BMIM\(^+\) can be either promptly desorbed, or acoustically desorbed, or both within the same trace. Interestingly, acoustic desorption was limited to only a few ion species, while prompt desorption was limited to mostly lighter ion species. We also observed both processes had at least one ion species unique to each desorption process. Investigations into the limitations of each process can be carried out by the addition of other low mass molecules to the desorption sample to test limits of acoustic desorption. New samples incorporating other heavier peptides and amino acids will reveal further limitations of the prompt process and further reinforce the usefulness and complementary nature of having both processes present within single spectra.
BIBLIOGRAPHY


VITA

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Peter Ronald Harris was born on June 15, 1980, in Rockville Centre, New York. He grew up in Wantagh, New York, on the south shore of Long Island. After graduating from Wantagh High School in 1998, he enrolled at the College of the Holy Cross in Worcester, Massachusetts. He graduated in 2002 with a BA in Physics. In the fall of 2002 he enrolled in the graduate program at the College of William and Mary in Williamsburg, Virginia. After receiving his MS in 2003, he joined the group of Dr. William Cooke to study novel desorption methods for use in time of flight mass spectrometry. This thesis was defended on January 20th, 2009 at the College of William & Mary in Williamsburg, Virginia. After successfully defending his dissertation he accepted a postdoctoral position within the Physics Division of the Oak Ridge National Laboratory in Oak Ridge, Tennessee.