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Laser Desorption Postionization for Imaging MS of Biological Material

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Abstract

Vacuum ultraviolet single photon ionization (VUV SPI) is a soft ionization technique that has the potential to address many of the limitations of MALDI for imaging MS. Laser desorption postionization (LDPI) employs VUV SPI for postionization and is experimentally analogous to a MALDI instrument with the addition of a pulsed VUV light source. This review discusses progress in LDPI-MS over the last decade, with an emphasis on imaging MS of bacterial biofilms, analytes whose high salt environment make them particularly resistant to imaging by MALDI-MS. This review first considers fundamental aspects of VUV SPI including ionization mechanisms, cross sections, quantum yields of ionization, dissociation, and potential mass limits. The most common sources of pulsed VUV radiation are then described along with a newly constructed LDPI-MS instrument with imaging capabilities. Next, the detection and imaging of small molecules within intact biofilms is demonstrated by LDPI-MS using 7.87 eV (157.6 nm) VUV photons from a molecular fluorine excimer laser, followed by the use of aromatic tags for detection of selected species within the biofilm. The final section considers the future prospects for imaging intact biological samples by LDPI-MS.

Keywords

photoionization; vacuum ultraviolet; imaging; bacteria; laser desorption

1. Introduction

The goal of imaging mass spectrometry is to analyze known and unknown species within intact biological samples while maintaining information on their spatial distribution, allowing correlation of chemical ion signals to macroscopic biological structures.[1–4] One advantage of MALDI for imaging MS is the availability of sample preparation protocols for analysis of different analyte classes. Another is the availability of commercial instruments with high sensitivity, wide mass range, and high mass and spatial resolution. These advantages have lead to the widespread use of MALDI for imaging MS of peptides, proteins, drugs, and metabolites within intact tissue.[1–5]

Nevertheless, MALDI has several shortcomings associated with its use in imaging MS. Sample preparation typically requires multiple steps including tissue washings and matrix application. [4,6] The vast majority of molecules desorbed in MALDI are neutrals with an ion to neutral

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ratio estimated to range from $\sim 10^{-3}$ to $\sim 10^{-5}$. [7–9] MALDI can also display substance-specific ionization yields which can suppress signal for target analytes. Salts, tissue specific ion suppression, and interferences between multiple analytes lead to a dependence of ionization efficiency on the local environment. [10,11] Location-specific ion yield is a serious problem for imaging MS because it is difficult to determine if the absence of signal at a particular location is dependent on the actual absence of analyte or ion suppression, leading to the creation of images that may not be representative of true analyte distributions. [6] These issues argue for the use of an imaging MS technique that compliments the results obtained from MALDI.

Vacuum ultraviolet single photon ionization (VUV SPI) is a soft ionization technique that has the potential to address many of the aforementioned limitations of MALDI. The use of VUV SPI for a variety of MS applications beyond imaging MS of biological samples was recently reviewed. [12] Experimentally, the use of VUV SPI for postionization of laser desorbed neutrals is analogous to a MALDI instrument to which has been added a pulsed VUV light source. The method is termed laser desorption postionization (LDPI) or two laser mass spectrometry (L^2MS or $L2MS$), although postionization has also been performed via resonance enhanced multiphoton ionization [13] instead of VUV SPI. The separation of desorption and ionization steps as well as the similarity of VUV SPI cross sections for different analyte classes improves the possibility of quantification compared with multiphoton processes. Postionization also imparts some advantages to secondary neutral mass spectrometry over secondary ion mass spectrometry, as discussed elsewhere. [14,15]

A prior feature article in this journal reviewed the status of LDPI-MS as of a decade ago. [7] We focus in this paper on our LDPI-MS work over the last decade which has been directed towards imaging MS of bacterial biofilms, analytes whose high salt environment make them particularly resistant to imaging by MALDI-MS. We begin by reviewing fundamental aspects of VUV SPI including ionization mechanisms, cross sections, quantum yields of ionization, dissociation, and mass limits. We then describe the most common sources of pulsed VUV radiation and outline the features of our newly constructed LDPI-MS instrument with imaging capabilities. Next, we describe the detection and imaging of small molecules within intact biofilms by LDPI-MS and the use aromatic tags for detection of selected species within a bacterial biofilm. We finish with a brief overview of the future directions of imaging biological samples by LPDI-MS.

II. Fundamentals of VUV SPI

SPI is photon energy dependent and occurs for molecules whose ionization energies (IEs) are below the energy of irradiating photons. IEs for molecules below ~ 400 Da mostly fall between 6 and 12 eV, with the distribution peaking slightly below 9 eV, as indicated in Figure 1. [16] Figure 2 displays how IEs vary for several classes of compounds and how IEs drop with a given class as molecular weight increases. A VUV source generating 10.5 eV energy photons will ionize a large fraction of molecular analytes while excluding ionization of water, carbon dioxide, and other abundant species of little interest for imaging MS. On the other hand, use of lower photon energy provides a degree of selectivity in ionization which has the potential to greatly simplify the spectra collected from mixtures as a large number of species will not be detected.

Sensitivity in VUV SPI depends on the ionization yield which is determined by the photoionization cross section of the analyte. The yield of postionized neutrals by SPI is given by $Y = \sigma_{spi} I N_{gas}$ where σ_{spi} is the photoionization cross section, I is the intensity of VUV radiation, and N_{gas} is the density of gaseous neutrals. [12] SPI cross sections for molecules usually fall within one order of magnitude of each other, typically between 2 and 20 Mb (1 Mb = 10^{-18} cm²) for small molecules at 10.5 eV photon energy: alkanes have values of σ_{spi} near

3 Mb, aldehydes/ketones near 4 Mb, alkenes near 8 Mb, and dienes near 15 Mb. Within these narrow ranges, SPI cross sections differ between compound classes, but are quite similar within a given compound class at a fixed wavelength.[17] Thus, ratios of these cross sections are constant at a given wavelength and only one standard is required for quantification of multiple species of a given class.[17] Finally, it is unclear how σ_{spi} scales with molecular size: C_{60} displays a relatively large σ_{spi} of ~100 Mb at 10.5 eV photon energy,[18] but this may not be typical for large compounds due to the unusual photophysics of C_{60} .

Molecules can absorb VUV photons with energies below their IE, leading to photodissociation into neutral fragments rather than ionization. Figure 3 displays σ_{spi} and the VUV absorption cross section, σ_{a} , for anthracene as a function of photon energy. Anthracene absorbs strongly near 7.0 eV, below its 7.45 eV IE,[19] but no ions form. The quantum yield of ionization $\eta_{\text{i}} = \sigma_{\text{spi}}/\sigma_{\text{a}}$ [18] and η_{i} is also shown in Figure 3 for anthracene. As the energy of the absorbed photon increases, the quantum yield of ionization increases and approaches unity near 20 eV. This photon energy dependence in quantum yield of ionization has been observed for several small to moderate-sized compounds and is indicative of a direct ionization event.[18]

While consideration of η_{i} might argue for use of 20 eV photons for SPI of anthracene, increasing the photon energy can also increase fragmentation of the parent ion formed by VUV SPI. The large body of threshold photoelectron photoionization coincidence data generally supports the argument that increasing the photon energy up to ~10 eV above the IE increases fragmentation of the parent ion.[20] As a result, ionization as near as possible to the IE threshold has generally been preferred. However, this assumption has been called into question by the remarkably low fragmentation observed upon 26.5 eV soft X-ray photoionization of clusters of water, methanol, ammonia, carbon dioxide, sulfur dioxide, and metal oxides.[21,22] The role of fragmentation and internal energy is described further below.

Several other processes besides direct ionization may follow molecular absorption of a VUV photon whose energy exceeds its IE: direct dissociation, autoionization, predissociation, shape resonance and/or internal conversion. Both size and structure of the molecule and energy of the photon determine which of or whether those other processes will occur. Small molecules ionized with photons of energies within a few eV of their IE can undergo many of the above processes. However, for large molecules ionized with photon energies close to their IE, it has been argued that direct ionization and direct dissociation typically dominate over other ionization processes.[18] Vibrational relaxation may dominate autoionization in large molecules, allowing direct ionization to dominate over predissociation and direct dissociation.

The above arguments do not distinguish between vertical and adiabatic IEs. VUV SPI will create an ion on a femtosecond timescale, before geometric relaxation can occur. Such a vertical ionization can lead to immediate fragmentation if a dissociative ion state is populated or if Franck-Condon overlap between the ion and neutral state are poor. However, the rapid vibrational relaxation that dominates with increasing molecular mass can allow geometric rearrangement to a relaxed ion state. The difference in energy between the vertical and adiabatic IE that becomes available upon such relaxation can lead to dissociation and/or other processes. [21]

Early arguments that large molecules do not photoionize[23] were rebutted by later work. [24] While vibrational relaxation appears to prevent large molecules from undergoing autoionization, there appears to be consensus that large molecules can and do undergo efficient direct ionization.[18] This was supported by the observation of VUV SPI of a ~3,000 Da peptide covalently bound with anthracene,[25] gramicidin and clusters thereof, Ca-tryptophan complexes, and guanine clusters at masses up to 7,500 Da.[26,27] 7.87 eV SPI of gramicidin A was found to be 15× more efficient than multiphoton ionization at 266 nm.[27] However,

some of these studies utilized jet cooling of desorbed molecules,[26,27] a capability not yet implemented in LDPI-MS for imaging experiments and one generally considered incompatible with high detection efficiency.

Many of the above arguments are based upon traditional SPI mechanisms of isolated molecules in the gas phase, but cluster ionization should also be considered. Cluster formation occurs in MALDI[8,28] and delocalization of electron density by hydrogen bonding in clusters can lead to a decrease in IE by more than 1 eV.[29] Thus, clusters may undergo SPI at lower photon energies than their constituent isolated molecules.

VUV SPI can also induce dissociation of clusters and lead directly to the formation of protonated species. The observation of cluster SPI and proton transfer in supersonic jets[30, 31] indicates the feasibility of such processes. The excess energy between the vertical IE and either the adiabatic IE or the barrier to proton transfer for neutral clusters can drive their protonation/dissociation to form smaller protonated species.[21] Protonation mechanisms from atmospheric pressure photoionization (APPI) are also relevant to the discussion here.[32,33] APPI involves proton transfer or charge exchange from a secondary reagent ion species such as a purposely added dopant, solvent, or other component of the sample matrix. The process generally begins with formation of a radical cation from a reagent species by VUV SPI if the IE is below the photon energy. The reagent ion can then either proton transfer or charge exchange with the analyte, if either event is energetically favorable (i.e., if the proton affinity of the analyte is higher than that of the deprotonated reagent). Cluster dissociation/protonation and/or secondary APPI-like reactions in the desorption plume may also be important in LDPI, as evidenced in one case where an overabundance of protonated ions was observed with the addition of matrix.[34]

III. Vacuum Ultraviolet Sources

Several laboratory sources of pulsed VUV radiation are available for use in LDPI-MS. Nd:YAG lasers can be used to generate the ninth harmonic at 118 nm (10.5 eV) by passing their 355 nm third harmonic through a gas cell filled with xenon or a xenon/argon mixture. [35] Use of this source has been relatively widespread because it is easily coupled with time-of-flight instruments due to its ~5 ns pulse length and potentially high repetition rates. Furthermore, most analytes can be ionized by 10.5 eV radiation. However, the relatively low intensity of this source (~nJ/pulse) makes it difficult for use in a commercial instrument.

Another laboratory VUV source is the molecular fluorine excimer laser which has ~10 ns pulse length and emission wavelength of 157.6 nm, which corresponds to a photon energy of 7.87 eV. Fluorine lasers display repetition rates of up to 1000 Hz, sufficient for creating MS images from large samples. They are also commercially available with tens of millijoules of power, sufficiently intense to allow saturation of SPI. However, the 7.87 eV photon energy is below the IE of many species, allowing selective detection of only analytes with low IEs in mixtures and in other specific cases, as discussed further below.

Various other pulsed VUV sources are available that might be used in LDPI-MS.[12] There are several rare gas excimer sources that emit in the VUV and their broad emission bands are given in Figure 2.[12] They operate by electron beam pumping of a noble gas through a 300-nm silicon nitride foil leading to the formation of diatomic noble-gas excimers which emit in the VUV regime upon electronic relaxation and decay.[36] These sources can be operated in pulsed mode for coupling with pulsed laser desorption and may become commercially available in the near future.

Yet another useful source for SPI is the soft X-ray laser which produces ~ 10 μJ pulses of 26.5 eV photons at 12 Hz repetition rates.[37] The availability of pulsed soft X-ray or extreme UV sources has grown with their potential application to lithography.

Several new instruments have been recently developed that utilize tunable synchrotron radiation for VUV photoionization combined with either laser[38] or ion desorption.[14] Tunable VUV photoionization allows soft ionization just above the ionization threshold while maximizing sensitivity. Desorption by focused 1064 nm fundamental wavelength of a Nd:YAG laser was combined with synchrotron tunable VUV photoionization for study of petroleum saturates[38] and quinones[39] at the National Synchrotron Radiation Laboratory (Hefei, China). However, the use of this instrument for imaging MS has not yet been reported. Chemical imaging of surfaces at high resolutions can be achieved by photoionizing sputtered neutrals with tunable wavelength synchrotron VUV radiation. A modified commercial secondary ion mass spectrometer (TOF.SIMS⁵, ION-TOF USA, Chestnut Ridge, NY) has been coupled to the synchrotron light source on the Chemical Dynamics Beamline at the Advanced Light Source, Lawrence Berkeley National Laboratory (Berkeley, CA). This instrument was used to measure photoionization efficiency curves for As_m ($m = 1, 2$) and Au_n ($n = 1-4$) desorbed by a Bi_3^+ primary ion beam from GaAs and Au substrates, respectively.[14] Work is currently underway to incorporate pulsed laser desorption into this instrument.

One drawback of the use of VUV synchrotron radiation for SPI is that its quasi-continuous output cannot be fully utilized in conjunction with pulsed laser and ion desorption schemes predominant in imaging MS. However, a combination of Nd:YAG pump laser, dye lasers, and various harmonic generation strategies have been combined into a tunable, pulsed laboratory VUV source that operates from 7.4 to 10.2 eV photon energies.[40] It was argued that this laboratory source matches the VUV fluxes currently available at any synchrotron.

IV. New LDPI-MS Instrument for Imaging

Several different LDPI-MS instruments have been developed over the last decade. The Pellin group at Argonne National Laboratory (Argonne, IL) developed a series of LDPI-MS instruments that culminated in the SPIRIT machine[41,42] which was used for some of the previously published results reprinted here. Several versions of non-imaging LDPI-MS instruments were built in our laboratory at the University of Illinois at Chicago and used to collect some of the other results presented here.

A new instrument for LDPI-MS that allows imaging has been constructed in our laboratory, is indicated by Figure 4, and will be fully described in a separate publication. A 349 nm Nd:YLF laser (Explorer, Spectra-Physics, Irvine, CA) operated typically with a ~ 20 μm spot size at the sample and 100 Hz operational repetition rate is used for desorption (Figure 4, label f). The typical desorption energy is ~ 2 $\mu\text{J}/\text{pulse}$, but this can be increased to a maximum of 120 $\mu\text{J}/\text{pulse}$. Laser desorbed molecules are photoionized using a molecular fluorine excimer laser operating at 157 nm (7.87 eV, Optex Pro, Lambda Physik Inc., Ft. Lauderdale, FL) operated at 100 Hz repetition rate and 500 $\mu\text{J}/\text{pulse}$ laser power with a beam that is ~ 8 mm wide in the ionization region.

Most data acquisition and sample movement functions of the instrument are under computer control (LabView v.8.2, National Instruments, Austin, TX). A 12 bit, 125 MS/s data acquisition card with 128 MS memory (CompuScope 8229, DynamicSignals LLC, Lockport, IL) is used to acquire signal from the ion detectors.

Label a in Figure 4 (expanded inset) shows the ion optics for the ionization/extraction region which incorporate a custom pseudo-orthogonal delayed pulsed extraction and acceleration to 6.1 keV. This extraction scheme allows for separation of direct ions and postionized molecules,

optimizing detection of the latter. The instrument is capable of operating in both linear and reflectron modes, with an ion mirror in the reflectron consisting of 40 plates connected by 1 M3 resistors (ITT Power Solutions, West Springfield, MA) of demonstrated 1200 mass resolution at m/z 397. The detector indicated by label c (Advanced Performance Detector, Burle, Lancaster, PA) is biased at -2.4 keV and used for reflectron detection while a dual microchannel plate detector (18 mm dual multichannel plate, R.M. Jordan Co., Grass Valley, CA) is used for linear detection.

An ultrahigh vacuum compatible translation stage (Micos LS-120, Micos USA, Irvine, CA) indicated by label d is used for the manipulation of the sample with spatial resolution limited by the laser spot size. The stage has maximum travel length of 40 mm, integrated limit switches and unidirectional repeatability of ± 0.2 μm . A new load lock for quick sample entry was also constructed and is indicated by label e. Base pressure in the load lock is 5×10^{-6} Torr and base pressure inside the main chamber is 3×10^{-8} Torr. A digital single lens reflex camera (D300 with Telephoto AF Micro Nikkor 200 mm f/4.0D lens, Nikon, Tokyo, Japan) is used for sample viewing on a high definition television and correlation of visual and mass spectrometric images.

V. 7.87 eV LDPI-MS of Small Molecule Analytes in Bacterial Biofilms

Bacterial biofilms are communities of microorganisms which grow on surfaces and undergo communal behavior controlled by chemical signaling known as quorum sensing.[43] Bacterial biofilms play a central role in many microbial processes including serving as a persistent source of many hospital infections.[44] The study and improved treatment of bacterial biofilm infections would be facilitated by an improved understanding of spatial chemical distributions within intact biofilms. Imaging MS is one strategy for probing these chemical distributions, as discussed in prior work.[45,46]

A popular dental composite used for filling cavities and other dental restorations is made from a polymerizable resin matrix bound to glass filler particles using silane coupling agents.[47] Dental plaque is a multispecies bacterial biofilm which grows on the surfaces of human teeth and dental composites alike, leading to degradation of both these natural and synthetic materials.[48] Dental composites are chemically complex, so a model dental composite consisting of a covalently bound overlayer system was previously developed to study their degradation.[49] Prior work demonstrated the ability of LDPI-MS to detect a major component of the resin phase of a dental composite, bisphenol A diglycidyl methacrylate (BisGMA), when it was covalently bound via a silane coupling agent to a porous silicon oxide surface.[49,50] Both 10.5 and 7.87 eV LDPI-MS could detect BisGMA in this model overlayer system, but the 7.87 eV laser showed a much higher sensitivity, presumably due to its higher VUV intensity.

A model BisGMA overlayer was prepared and used as a substrate to culture a *Staphylococcus sobrinus* biofilm for three days. Figure 5 shows the analysis of these intact biofilms by 7.87 eV LDPI-MS and MALDI-MS (the latter using α -cyano-4-hydroxycinnamic acid matrix). LDPI-MS (Figure 5A) shows a definitive BisGMA parent peak at m/z 512 with 7% normalized intensity. Control studies determined that the BisGMA detected by LDPI-MS was freed from the porous silicon oxide surface via degradation by the bacterial biofilm. MALDI-MS in Figure 5B shows only a very low intensity ($\sim 0.4\%$ normalized) peak of the parent BisGMA. No matrix addition or other sample preparation was utilized for LDPI-MS. BisGMA degradation peaks were also identified by both MALDI-MS and LDPI-MS, (labeled *) but LDPI-MS was able to detect more degradation or fragment ion peaks of BisGMA compared to MALDI-MS: degradation products at m/z 427, 359, 341 and 283 were observed by LDPI-MS while MALDI-MS only observed a single degradation product at m/z 277. Some of the peaks observed in MALDI-MS are due to the matrix while others have not been identified. Finally, the intense

signal below m/z 250 is typical of 7.87 eV LDPI-MS and may represent considerable analytic opportunity for an instrument with higher mass resolution and/or tandem capabilities.

Some caveats should be considered in this comparison. The new imaging LDPI-MS instrument depicted in Figure 4 was used to collect the LDPI-MS in Figure 5, while a commercial MALDI-MS was used to collect the MALDI-MS (4700, Applied Biosystems, Foster City, CA). Similar regions of the biofilm were analyzed by both methods and multiple analyses confirmed the different sensitivities. In principle, the LDPI-MS instrument could have been used to collect the MALDI-MS data. In practice, the LDPI-MS ion optics are optimized to preferentially collect postionized neutrals while rejecting direct ions, reducing its MALDI-MS sensitivity.

Another target for study by imaging MS within intact bacterial biofilms are antibiotics. Various arguments have been made regarding the mechanism of biofilm antibiotic resistance.[51] One question is whether antibiotics can fully penetrate the biofilm. Fluorescence microscopy can be used to probe antibiotic penetration through a biofilm,[52] but it requires fluorescent labeling of the antibiotic and it cannot detect chemical modification of the antibiotic within the biofilm. A complementary strategy is to use imaging MS to locate antibiotics within an intact biofilm.

We previously demonstrated that low IE antibiotics can be detected by 7.87 eV LDPI-MS, [46] and ongoing work is expanding this list of detectable antibiotics. Figure 6 shows a mass spectrum and MS image of the intensity distribution of the parent ion peak of the antibiotic rifampicin at m/z 822 within a *Staphylococcus epidermidis* biofilm, also measured with the new machine shown in Figure 4. The biofilm was grown on polycarbonate membrane for several days,[46] then cut into a wedge. Rifampicin was then added to one side of the biofilm and allowed to diffuse across it for five minutes. Figure 6 (top) shows a typical peak distribution of rifampicin using 7.87eV LDPI MS: the parent ion peak appears at m/z 822 along with four characteristic fragments at m/z 795, 398, 299, and 99. The image in Figure 6 (bottom) shows the partial diffusion of antibiotic through the biofilm, with higher concentration at the origin of the antibiotic on the left side of the image. The best image spatial resolution currently possible with this instrument is $\sim 20\text{ }\mu\text{m}$ and is limited by desorption laser spot size and instrumental vibrations.

The data in Figure 6 was acquired by continuously moving the sample stage at a horizontal velocity of $250\text{ }\mu\text{m/sec}$ with respect to the desorption laser running at 100 Hz collecting the spectrum corresponding to each position. The stage moves in a back and forth raster pattern, shifting vertically at the end of each row. The total mass range collected was m/z 2 to 1450, and each saved spectrum was the average of 20 spectra. About ten minutes was required to collect an $8 \times 8\text{ mm}$ image, depending upon the velocity of the translation stage. Mass spectral signal was integrated from m/z 821 to 823 for each of position of the desorption laser, then used to create the contour plot in Figure 6 of this integrated intensity at each sample stage position. Data analysis software (Origin 7.5, Origin Lab, North Hampton, MA) was used to process and merge the data from multiple files.

VI. Derivatization for Detection of High IE and Selected Chemical Species in a Biofilm

One of the primary goals in imaging MS is detection of intact peptides and proteins. However, peptides either have a higher IE than the photon energy available or if they contain tryptophan, tend to undergo fragmentation upon 7.87 eV VUV SPI. One solution that has previously been shown is labeling peptides and amino acids with a chromophore to lower their IE sufficiently to allow SPI, analogous to a strategy initially reported for multiphoton ionization.[53] Fmoc, dimethyl amino benzoic acid, tryptophan, fluorescein, naphthalene, and anthracene were all

shown to act as low IE chromophores when covalently bound to analytes for 7.87 eV SPI. [34,54,55]

The concept behind tagging with a chromophore is analogous to their use in fluorescence microscopy: the chromophore maintains its low IE which allows it to be detected by VUV SPI even after it is coupled to the analyte. An *ab initio* calculation of the ground state relaxed geometry of a hexapeptide GAPKSC tagged with anthracene demonstrated this phenomenon, as shown in Figure 7.[34] The calculated IE for the anthracene-GAPKSC compound was 7.5 eV, near unbound anthracene's IE of 7.45 eV and sufficient for ionization by 7.87 eV photons. The highest occupied molecular orbital for this compound is shown in Figure 7 to be localized on the anthracene group, indicating that the tag serves as the moiety which allows threshold SPI. Furthermore, the calculations indicated that the positive charge was delocalized across the anthracene tag after ionization, reducing the possibility of charge-initiated fragmentation.

7.87 eV LDPI-MS of anthracene-GAPKSC supported the calculations.[34] The anthracene tag acted as a chromophore so the parent ion of the anthracene-GAPKSC peptide could be detected without any addition of matrix, although matrix addition did enhance neutral desorption efficiency (data not shown). The anthracene tag decreased the IE of GAPKSC just below the 7.87 eV threshold, so the compound was ionized with little excess energy, minimizing fragmentation. Additionally, anthracene appears to have stabilized the positive charge of the radical cation, further minimizing its fragmentation.

Another example of a VUV tag is tryptophan, whose obvious advantage would be that it occurs naturally in or can be genetically engineered into a target peptide. Tryptophan has been studied thoroughly by tunable VUV SPI and has been shown to undergo fragmentation when it absorbs a photon with energy of 7.87 eV, only ~0.6 eV above its IE.[56] The m/z 204/130 parent/fragment ratio from photoionized tryptophan can also be used as a thermometer of the laser desorption process. Figure 8 shows 7.87 eV LDPI-MS of neat tryptophan and the anthracene-tryptophan compound.[34] Neat tryptophan underwent fragmentation resulting in a low intensity molecular ion with the m/z 130 fragment ion dominating the spectrum, consistent with laser-desorbed tryptophan having an internal temperature in excess of 600K. Tagging tryptophan with anthracene suppressed fragmentation and showed a strong parent ion which dominated the mass spectrum. The relatively large size of anthracene-tryptophan is also expected to have induced a kinetic shift in dissociation compared to neat tryptophan.

While various tryptophan-containing peptides have been found to undergo 7.87 eV SPI, their extensive fragmentation has so far limited this as an analytical strategy in LDPI-MS.[55] However, jet-cooled peptides with tryptophan-containing complexes such as gramicidin, clusters thereof, and Ca-tryptophan complexes up to 7,500 Da showed formation of intact parent ions during 7.87 eV SPI.[26,27] It appears that one limitation of LDPI-MS is the lack of cooling following the laser desorption step.

The strategy of analyte tagging to allow detection by 7.87 eV LDPI-MS was used for detection of peptides within intact biofilms.[45] ERGMT, a quorum sensing peptide in *Bacillus subtilis* that regulates sporulation and competence, was tagged with both anthracene and a commercial quinoline-based compound, then detected by 7.87 eV LDPI-MS. Furthermore, the tags were applied to the intact biofilm, allowing the tagged peptide to be spatially imaged. By contrast, MALDI-MS could only detect this peptide within intact biofilms when the microbes were electrophoretically lysed.

Both tags described above bind to primary and secondary amines, which make them non-specific for any particular type of peptide or amino acid. N-(1-pyrene)maleimide (NPM) is a cysteine-specific tag that is commonly used in fluorescence detection.[57] NPM acts as a chromophore for 7.87 eV photons and binds selectively to cysteine via a stable sulfur bond.

Cysteine, glutathione as well as AIVCF and AIVCFW were tagged with NPM and detected neat using 7.87 eV LDPI-MS. Figure 9 shows the LDPI-MS of glutathione (top trace labeled LDPI) and AIVCFW (third trace from top labeled LDPI) spiked into an intact *S. epidermidis* biofilm at ~90 μ M concentration, then tagged with NPM. The NPM-glutathione tagged parent ion was detected at m/z 604 (label a in Figure 9) along with the glutathione parent ion without NPM at m/z 308 (label c), NPM itself at m/z 297 (label d), and the NPM dimer at m/z 594 (label b). A parent ion was also detected for AIVCFW-NPM at m/z 1034 (label e). AIVCFW-NPM also showed neutral losses of tryptophan (m/z 904 labeled f), NPM tag (m/z 738 labeled g), and both (m/z 607 labeled h). The two LD traces in Figure 9 show data collected with the fluorine laser turned off and in neither of these cases were ions were detected in the absence of postionization. The neat biofilm without any NPM tagging showed no peaks in LDPI-MS.

Various attempts to detect these peptides within the biofilms by MALDI-MS were unsuccessful. For example, after spiking peptides into the biofilm, matrix was airbrushed on top of the biofilm and they were analyzed with a commercial MALDI-MS (4700 MALDI-TOF, Applied Biosystems, Foster City, CA). However, no peptide signal was observed by MALDI-MS (data not shown). This failure is consistent with the dearth of MALDI-MS of intact biofilms reported in the literature.

VII. Conclusions and Future Directions

The prospects are bright for the use of laser desorption vacuum ultraviolet postionization in imaging MS. The work here has shown that VUV SPI can detect analytes in bacterial biofilms where MALDI-MS produces little or no signal. However, further investigation of a wider array of biological sample classes is required to determine when LDPI-MS should be considered in lieu of MALDI-MS or secondary ion mass spectrometry, currently the predominant methods of imaging MS. LDPI-MS will also be greatly expanded when introduced into instruments which incorporate other instrumental advances such as higher mass resolution and/or tandem MS capabilities.

Most of the work discussed here has focused on use of the 7.87 eV fluorine laser for postionization, but other higher photon energy VUV sources also show great potential. Use of the 10.5 eV source greatly expands the analytes that can be targeted,[12,31,49,58] although its low intensity make it unlikely to appear in a commercial instrument anytime soon. However, other more robust VUV[12] and higher photon energy sources[37] are becoming available and promise to dramatically expand the capabilities and ease of the technique.

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References

1. Rubakhin SS, Jurchen JC, Monroe EB, Sweedler JV. Drug Discov Today 2005;10:823. [PubMed: 15970265]
2. Reyzer ML, Caprioli RM. Curr Opin Chem Biol 2007;11:29. [PubMed: 17185024]
3. McDonnell LA, Heeren RMA. Mass Spectrom Rev 2007;26:606. [PubMed: 17471576]
4. Seeley EH, Caprioli RM. Proc Nat Acad Sci USA 2008;105:18126. [PubMed: 18776051]

5. Stoeckli M, Staab D, Schweitzer A. *Inter J Mass Spectrom* 2007;260:195.
6. Heeren RMA, Smith DF, Stauber J, Kükrer-Kaletas B, MacAleese L. *J Amer Soc Mass Spectrom* 2009;20:1006. [PubMed: 19318278]
7. Hanley L, Kornienko O, Ada ET, Fuoco E, Trevor JL. *J Mass Spectrom* 1999;34:705. [PubMed: 10407355]
8. Dreisewerd K. *Chem Rev* 2003;103:395. [PubMed: 12580636]
9. Tsai ST, Chen CH, Lee YT, Wang YS. *Mol Phys* 2008;106:239.
10. Walker AK, Wu Y, Timmons RB, Kinsel GR, Nelson KD. *Anal Chem* 1999;71:268. [PubMed: 9921134]
11. Luxembourg SL, McDonnell LA, Duursma MC, Guo X, Heeren RMA. *Anal Chem* 2003;75:2333–2341. [PubMed: 12918974]
12. Hanley L, Zimmermann R. *Anal Chem* 2009;81:4174. [PubMed: 19476385]
13. Pomerantz AE, Hammond MR, Morrow AL, Mullins OC, Zare RN. *J Am Chem Soc* 2008;130:7216. [PubMed: 18484722]
14. Takahashi LK, Zhou J, Wilson KR, Leone SR, Ahmed M. *J Phys Chem A* 2009;113:4035. [PubMed: 19371112]
15. Kriesekotte C, Cantz T, Haberland J, Zibert A, Haier J, Köhler G, Schöler HR, Schmidt HHJ, Arlinghaus HF. *J Mass Spectrom* 2009;44:1417. [PubMed: 19753579]
16. King BV, Pellin MJ, Moore JF, Veryovkin IV, Tripa CE. *Appl Surf Sci* 2003;203/204:244.
17. Adam T, Zimmermann R. *Anal Bioanal Chem* 2007;389:1941. [PubMed: 17874081]
18. Berkowitz, J. *Atomic and Molecular Photoabsorption: Absolute Total Cross Sections*. Academic Press; London: 2002.
19. Lias SG, Bartmess JE, Liebman JF, Holmes JL, Levin RD, Mallard WG. *J Phys Chem Ref Data* 1988;17:18.
20. Ng CY. *Inter J Mass Spectrom* 2000;200:357.
21. Heinbuch S, Dong F, Rocca JJ, Bernstein ER. *J Chem Phys* 2007;126:244301. [PubMed: 17614543]
22. Dong F, Heinbuch S, Xie Y, Bernstein ER, Rocca JJ, Wang ZC, Ding XL, He SG. *J Amer Chem Soc* 2009;131:1057. [PubMed: 19119810]
23. Schlag EW, Levine RD. *J Phys Chem* 1992;96:10608.
24. Becker CH, Wu KJ. *J Amer Soc Mass Spectrom* 1995;6:883.
25. Edirisinghe, PD. PhD thesis. Department of Chemistry. University of Illinois; Chicago: 2006. Derivatization 7.87 eV postionization mass spectrometry and its application to biofilm characterization.
26. Marksteiner M, Haslinger P, Ulbricht H, Sclafani M, Oberhofer H, Dellago C, Arndt M. *J Amer Soc Mass Spectrom* 2008;19:1021. [PubMed: 18499471]
27. Marksteiner M, Haslinger P, Sclafani M, Ulbricht H, Arndt M. *J Phys Chem A* 2009;113:9952. [PubMed: 19705815]
28. Knochenmuss R, Zenobi R. *Chem Rev* 2003;103:441. [PubMed: 12580638]
29. Kinsel GR, Knochenmuss R, Setz P, Land CM, Goh SK, Archibong EF, Hardesty JH, Marynick DS. *J Mass Spectrom* 2002;37:1131. [PubMed: 12447889]
30. Kostko O, Belau L, Wilson KR, Ahmed M. *J Phys Chem A* 2008;112:9555. [PubMed: 18578483]
31. Hu Y, Bernstein ER. *J Phys Chem A* 2009;113:639. [PubMed: 19123776]
32. Syage JA. *J Amer Soc Mass Spectrom* 2004;15:1521. [PubMed: 15519219]
33. Marchi I, Rudaz S, Veuthey J. *Talanta* 2009;78:1–18. [PubMed: 19174196]
34. Edirisinghe PD, Moore JF, Calaway WF, Veryovkin IV, Pellin MJ, Hanley L. *Anal Chem* 2006;78:5876. [PubMed: 16906735]
35. Van Bramer SE, Johnston MV. *J Am Soc Mass Spectrom* 1990;1:419.
36. Mühlberger F, Streibel T, Wieser J, Ulrich A, Zimmermann R. *Anal Chem* 2005;77:7408. [PubMed: 16285693]
37. Heinbuch S, Grisham M, Martz D, Rocca JJ. *Opt Express* 2005;13:4050. [PubMed: 19495315]
38. Guo W, Bi Y, Guo H, Pan Y, Qi F, Deng W, Shan H. *Rap Comm Mass Spectrom* 2008;22:4025.

39. Pan Y, Zhang L, Zhang T, Guo H, Hong X, Qi F. *J Mass Spectrom* 2008;43:1701. [PubMed: 18636469]
40. Hanna SJ, Campuzano-Jost P, Simpson EA, Robb DB, Burak I, Blades MW, Hepburn JW, Bertram AK. *Inter J Mass Spectrom* 2009;279:134.
41. Veryovkin IV, Calaway WF, Moore JF, Pellin MJ, Lewellen JW, Li Y, Milton SV, King BV, Petravic M. *Nucl Instr Meth Phys Res B* 2004;219–220:473.
42. Veryovkin IV, Calaway WF, Pellin MJ. *Nucl Instr Meth Phys Res A* 2004;519:353.
43. Ghannoum, M. *Microbial Biofilms*. O'Toole, GA., editor. Washington, D. C: ASM Press; 2004.
44. Parsek MR, Singh PK. *Annu Rev Microbiol* 2003;57:677. [PubMed: 14527295]
45. Edirisinghe PD, Moore JF, Skinner-Nemec KA, Lindberg C, Giometti CS, Veryovkin IV, Hunt JE, Pellin MJ, Hanley L. *Anal Chem* 2007;79:508. [PubMed: 17222014]
46. Gasper GL, Carlson R, Akhmetov A, Moore JF, Hanley L. *Proteom* 2008;8:3816.
47. Ferracane JL. *Crit Rev Oral Biol Med* 1995;6:302. [PubMed: 8664421]
48. Marsh PD. *J Clin Periodontol* 2005;32(Suppl 6):7. [PubMed: 16128825]
49. Zhou M, Wu C, Edirisinghe PD, Drummond JL, Hanley L. *J Biomed Mater Res* 2006;77A:1.
50. Zhou M, Wu C, Akhmetov A, Edirisinghe PD, Drummond JL, Hanley L. *J Amer Soc Mass Spectrom* 2007;18:1097. [PubMed: 17449273]
51. Musk DJJ, Hergenrother PJ. *Curr Medic Chem* 2006;13:2163.
52. Rani SA, Pitts B, Stewart PS. *Antimicrob Agents Chemother* 2005;49:728. [PubMed: 15673757]
53. Houston CT, Reilly JP. *J Phys Chem A* 2000;104:10383.
54. Edirisinghe PD, Lateef SS, Crot CA, Hanley L, Pellin MJ, Calaway WF, Moore JF. *Anal Chem* 2004;76:4267. [PubMed: 15283559]
55. Hanley L, Edirisinghe PD, Calaway WF, Veryovkin IV, Pellin MJ, Moore JF. *Appl Surf Sci* 2006;252:6723.
56. Wilson KR, Jimenez-Cruz M, Nicolas C, Belau L, Leone SR, Ahmed M. *J Phys Chem A* 2006;110:2106. [PubMed: 16466244]
57. Ogony J, Mare S, Wu W, Ercal N. *J Chromat B* 2006;843:57.
58. Chen YF, Sullards MC, Hoang TT, May SW, Orlando TM. *Anal Chem* 2006;78:8386. [PubMed: 17165831]
59. Jochims HW, Baumgartel H, Leach S. *Astron Astrophys* 1996;314:1003.
60. Mallocci G, Mulas G, Joblin C. *Astron Astrophys* 2004;426:105.

Figure 1. Atoms and molecules that can be ionized by a single photon of a given energy, compiled previously[16] from gas phase ion thermochemical data.

Figure 2.

Ionization energies of molecules at a given weight and class as well as photon energies of several available pulsed and continuous vacuum ultraviolet (VUV) light sources. Reprinted with permission from *Anal. Chem.* 2009, 81, 4174–4182. 2009 American Chemical Society.

Figure 3.

Quantum yield of ionization, η_i ; absorption cross section, σ_a ; and single photon ionization (SPI) cross section, σ_{spi} as a function of photon energy for anthracene. η_i and σ_a replotted from literature[59,60] and used to calculate σ_{spi} . Adiabatic ionization energy of anthracene is 7.45 ± 0.03 eV.[19]

Figure 4.

Laser desorption postionization mass spectrometer (LDPI-MS) instrument schematic with inset showing expanded view of (a) ionization/extraction region ion optics. (b) is reflectron, (c) is reflectron detector, (d) is translation stage, (e) is load lock, (f) is 349 nm Nd:YLF desorption laser, and (g) is 157 nm fluorine laser for VUV postionization.

Figure 5. (A) LDPI-MS and (B) MALDI-MS of bisphenol A diglycidyl methacrylate (BisGMA) detected within intact *Staphylococcus sobrinus* biofilm cultured for three days on BisGMA covalently bound overlayer prepared on porous silicon oxide surface.

Figure 6.

Top shows LDPI-MS of rifampicin detected within intact *Staphylococcus epidermidis* biofilms grown on polycarbonate membrane. Bottom image depicts spatial distribution of rifampicin parent ion m/z 822 $\pm$ 1 within biofilm cut into shape of wedge. Linear dependence of ion intensity in image indicated by color: Pink > red > yellow > green > aqua > blue.

Figure 7. *Ab initio* ground state geometry and highest occupied molecular orbital of anthracene-tagged GAPKSC hexapeptide. Reprinted with permission from *Anal. Chem.*, 2006, 78, 5876–5883. 2006 American Chemical Society.

Figure 8. Tryptophan (W) and anthracene tagged tryptophan (Anth-W) detected using 7.87 eV LDPI-MS. Reprinted with permission from *Anal. Chem.*, 2006, 78, 5876–5883. 2006 American Chemical Society.

Figure 9.

7.87 eV LDPI-MS of glutathione (top traces) and AIVCFW hexapeptide (third trace from top) spiked into a *S. epidermidis* biofilm which was subsequently treated with NPM to tag these two peptides. Laser desorption (LD) MS is shown for glutathione in biofilm (second trace from top). Bottom two traces are LD and LDPI-MS of biofilm without any peptide spikes or NPM treatment.