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Mass Spectrometry of Self-Assembled Monolayers: A New Tool for Molecular Surface Science

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Abstract

Most reactions can be performed in solution and on a surface. Yet the challenges faced in applying known reactions or in developing entirely new reactions for modifying surfaces remain formidable. The products of many reactions performed in solution can be characterized in minutes and even products having complex structures can be characterized in hours. When performed on surfaces, even the most basic reactions require a substantial effort—requiring several weeks—to characterize the yields and structures of the products. This contrast stems from the lack of convenient analytical tools that provide rapid information on the structures of molecules attached to a surface. This review describes recent work that has established mass spectrometry as a powerful method for developing and characterizing a broad range of chemical reactions of molecules attached to self-assembled monolayers of alkanethiolates on gold. The SAMDI-TOF mass spectrometry technique will enable a next generation of applications of molecularly defined surfaces to problems in chemistry and biology.

Keywords

biochip; interfacial reactions; label-free; self-assembled monolayer; SAMDI-TOF mass spectrometry

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The scientific and engineering disciplines have developed sophisticated toolboxes for making matter—including examples from organic chemistry to create small molecules with virtually unlimited complexity, molecular biology to prepare macromolecules with a precise arrangement of amino acids and a defined tertiary structure, and lithographic methods to fashion inorganic and metallic materials into integrated circuits and devices. Each of these approaches is based on distinct methods for assembling matter and each relies on analytical methods that can characterize the structures that they generate. In the bottom-up approaches common to synthetic chemistry, a range of methods including NMR and x-ray diffraction are used to delineate the connectivity of atoms within molecules. The biopolymers created using enzyme-mediated conversions are characterized using electrophoretic methods and high resolution mass spectrometry, while integrated circuits are characterized with electron microscopy, scanning probe tools, and sensitive spectroscopies. The tools remain fundamental to each of these areas, enabling further advances in the preparation of their respective targets.

The pursuit of structures with at least one dimension sized between 1–100 nm—which underlies the nanoscience and technology discipline that has grown over the past decade—is likewise dependent on analytical methods, and in several cases has been hampered by a lack of methods having appropriate performance.[1] For example, it remains difficult to characterize the products resulting from treatment of proteins or carbon nanotubes with organic reagents[2] and it is still difficult to characterize the dynamic structures of lipid rafts in bilayer membranes.[3] This review deals with the analogous challenge of characterizing the molecular structures of

organic surfaces. The development of self-assembled monolayers has in principle made it straightforward to functionalize surfaces with a broad range of molecular compositions to enable applications in biology and chemistry.[4] Yet, the common methods used in surface analysis do not have the resolution and sensitivity required to analyze complex molecular compositions and, in turn, have limited efforts to pursue the design and application of surfaces to these areas. In this review, I describe the recent development of mass spectrometric methods that are enabling applications of surface science in chemistry and biology. The review begins with an overview of the current methods that are broadly available, a description of recent work that expands the scope of surfaces that can be analyzed, and examples that illustrate the impact that these methods are having in the biological and chemical sciences.

Analytical Methods in Surface Science

Atomic Composition—The UHV Toolbox

Several surface analytical techniques have been developed and now provide a remarkable set of tools for the analysis of metallic and inorganic surfaces, and were recently highlighted with the award of the Nobel Prize in Chemistry to Gerhard Ertl. These methods offer exceptional sensitivity in characterizing the atomic composition and structure of clean substrates and in turn have been critical to developing materials and processes in semiconductor fabrication—in part for their high sensitivity in measuring the densities of impurities and dopants—and to understanding the mechanisms of heterogeneous catalytic processes.[5,6] An excellent survey of these methods is provided in the text by Adamson and Gast.[7] Included is x-ray photoelectron spectroscopy where a monoenergetic x-ray source is used to eject inner shell electrons that are then analyzed to quantitatively identify the composition of elements comprising a surface. Electron diffraction and ion scattering methods are powerful methods for characterizing the structures of ordered surfaces. A variety of optical methods—including ellipsometry, and surface plasmon resonance spectroscopy—measure changes in refractive index to provide information on the amount of molecules localized at the interface.

It is clear that these methods were and remain critical to the physics and fabrication of electronic structures and to surfaces used in catalysis—both which have relatively simple elemental structure and which often times rely on dopants present at low concentrations. Yet, they are less valuable for analyzing surfaces that are functionalized with complex chemical or biopolymeric structures. In these cases, it is the connectivity of the atoms into molecules, and changes in this connectivity following chemical or biological reactions, that are important, more so than the elemental composition.

Morphology, Shape and Forces—The Scanning Probe Toolbox

The characterization of the small feature sizes and out-of-plane structure of nanoscale assemblies has benefited enormously from the family of scanning probe techniques. Scanning tunneling microscopy has exquisite sensitivity and spatial resolution in characterizing the electronic properties of a surface.[8]. Among the most impressive applications to chemistry is a report by Allara, Tour and Weiss, who imaged the switching of individual conjugated molecules in a monolayer.[9]. Yet the skills and facilities required to perform these measurements limit their use to a relatively small number of aficionados. The introduction of atomic and lateral force microscopies that use a scanning probe to image the forces with a substrate are now widely available and provide straightforward characterization of the shapes, sizes and chemical properties of patterned nanostructures.[10] The extension of these methods with scanning probes that are functionalized with chemical or biological molecules are enabling unprecedented studies of the properties of individual proteins with new insights into the roles of force in biology.[11,12] Clearly, these methods have provided powerful tools for

characterizing the shapes, mechanics and properties of nanoscale structures, but they do not provide general routes for the molecular characterization of complex interfaces.

Molecular Structure—The Chemistry Toolbox

The introduction of self-assembled monolayers (SAMs)—including alkylsiloxanes on oxide substrates and alkanethiolates on the coinage metals—made it possible to display organic molecules and carry reactions of those molecules at surfaces.[13,14] These advances gave way to a substantial body of work that emphasized the need for surface analytical methods that could provide information on the molecular structure—in contrast to information on elemental composition described above—of monolayers. Several methods have been important to identifying molecular fragments, or functional groups, including grazing angle infrared spectroscopy and secondary-ion mass spectroscopy. The latter identifies masses of molecular fragments that derive from the high energy photodissociation of molecules at the surface. The data are can be analyzed to derive important information on the molecular surface structure, but are complicated and not routinely applied by the non-expert.[15]

Needs in Molecular Surface Science

The preceding discussion points to a clear disconnection between current surface analysis tools and applications in the chemical and biological sciences. Whereas the bulk characterization of organic and biological macromolecules is now routine, these methods cannot be applied to the small amounts of molecules that are present on surfaces (at densities of approximately 5×10^{14} molecules/cm^2). At the same time, the methods described above that have ultra sensitivity and can reveal elements present at low densities at surfaces do not offer information on the *molecular* structures of surfaces. In those cases where the methods do provide molecular information (for example, SIMS), they are not easily accessible or usable by the non-expert and therefore do not provide a routine tool, ultimately limiting their utility.

The consequences of this lack of tools are striking. For example, there are far greater than 100,000 reports of homogeneous phase reactions,[16] yet of order 100 reports of reactions at self-assembled monolayers.[17] Applications in biology routinely use biochips, where arrays of peptides, proteins or other molecules are treated with samples to identify protein-protein interactions and enzyme activities.[18] These applications now rely on fluorescent or radioisotopic labels to identify activities, with the limitation that many biochemical activities are not easily labeled. Of greater concern, the need for labeling strategies requires that the activity to be measured is already defined, and therefore not open to the discovery of unanticipated activities.

Surface Chemistries for Biology

A host of interesting biological processes operate at surfaces and provide opportunities to develop and apply biologically active monolayers.[19] For example, molecular recognition and lateral organization of proteins at lipid bilayer membranes are important in signaling pathways and have prompted the development of model surfaces for studies of immune synapse function.[20] Most cells adhere to an insoluble protein matrix and studies of the ligand-receptor interactions that mediate adhesion have benefited from the development of monolayers that present peptide and carbohydrate ligands.[21] Immobilized molecules are now frequently used to perform high throughput assays of biochemical activities and benefit from the use of well-defined surface chemistries.[22] Self-assembled monolayers of alkanethiolates on gold have been the most important route to these biosurfaces, and are the focus of this review. Other important routes to biosurfaces have been reviewed elsewhere.[23]

SAMs combine several properties that make them the best available chemistries for modeling biological surfaces. First, monolayers that present short oligomers of the ethylene glycol group

are highly effective at preventing non-specific adsorption of proteins.[24] As a general rule, most proteins will adsorb non-specifically to most man-made surfaces. This unwanted adsorption can block the interaction of the immobilized ligands with soluble partners and can give rise to non-specific interactions. Hence, it is vital to have a non-interacting background for the presentation of ligands that can then interact with components of a proximal fluid. Second, these surfaces permit wide flexibility in tailoring the structure and properties, either by preparing monolayers from terminally-substituted alkanethiols or by carrying out reactions to elaborate the structure of a monolayer after it has assembled. This flexibility is important to implementing immobilization chemistries that give good control over the density and orientation—and therefore the activity—of attached biomolecules. Third, several methods are available that can pattern monolayers, to give control over the shapes of attached cells and to create arrays of distinct biomolecular content.[25–27] Fourth, because the underlying gold substrates are conductive, SAMs can be engineered to be redox-active and thereby allow applied potentials to be used to modulate the structures and properties of the monolayer.[28] Finally, the SAMs are compatible with several important analytical methods that are important in biology. SAMs supported on thin gold films (approximately 10 nm) are still optically transparent and permit optical and fluorescence microscopy. Monolayers on thicker gold films (40 nm) can be analyzed with surface plasmon resonance spectroscopy.[29]

My group's work in biosurfaces has revealed the many challenges associated with creating effective models of biological surfaces for studies of cell adhesion and in applications of biochips. These challenges do not stem from a shortage of reactions that can be employed to elaborate monolayers, but rather from the characterization of the products of chemical and biochemical reactions. In an early example, we demonstrated the use of a Diels-Alder reaction to immobilize biomolecules to a monolayer. The strategy relied on monolayers that presented a benzoquinone molecule—which reacts with cyclopentadiene to give the expected 4+2 cycloaddition product—at a low density against a background of tri(ethylene glycol) groups.[30] We used the benzoquinone in part because it is redox active and can be reduced to the corresponding hydroquinone by applying an electrical potential to the underlying gold (Figure 1). We could carry out cyclic voltammetry in the presence of a diene, and quantitate the amount of benzoquinone in each scan and therefore the rate of the Diels-Alder reaction. To gain evidence that the loss of redox activity of the quinone was indeed due to the anticipated reaction, we verified that a range of dienes reacted with the benzoquinone, and that analogous compounds that did not contain a conjugated diene unit had no affect on the quinone redox couple. Further, we could quantitatively derive second order rate constants for the reaction and found that the relative rate constants for a series of dienes agreed with the expected trend based on electronic effects. To gain structural evidence for the product, we used grazing angle infrared spectroscopy to observe the carbonyl functional group of the quinone and cycladdition product. We found that many of the established techniques—including XPS and ellipsometry—were less useful in identifying the products and yields of the reactions. The point of this example is that no single method—and certainly no method that is as convenient as is NMR in synthetic chemistry—could provide strong evidence for the product and yield of the reaction and therefore a combination of techniques had to be exploited to make the case for the product.

A related example comes from our development of a carbohydrate array to profile the binding specificities of proteins and the preferred substrates for enzymes.[31] We prepared a series of carbohydrate-diene conjugates and spotted these reagents onto a monolayer presenting the benzoquinone group to prepare an array of ten monosaccharides (Figure 2). We could easily characterize the binding specificities of lectins—proteins that bind to carbohydrates—by imaging monolayers that were treated with fluorescently-labeled proteins. Alternatively, surface plasmon resonance spectroscopy could be used to monitor the interactions of non-labeled proteins with immobilized carbohydrates, and recent work by Corn enables the application of this technique in an imaging format,[32] as do alternate configurations that are

based on plasmonic devices.[33] Yet, SPR does not have the sensitivity to monitor events that result in small changes to the mass of molecules at the surface, including those that result from enzymatic modification of the immobilized ligand. For example, the enzyme galactosyltransferase can append a galactose residue to terminal N-acetylglucosamine groups. To characterize the products that result from treatment of the array with the enzyme, we used a panel of lectins having known specificities to infer the pattern of reactivity of GalTase. By probing the array with fluorescently-labeled lectins before and after treatment with GalTase, we could determine those carbohydrates that were modified by the enzyme and could therefore identify preferred substrates for the enzyme.

This need for a direct labeling of the reaction product is common in all assays of biological activities and can be a challenge in implementing high throughput assays. This requirement is particularly limiting in experiments that seek to identify *unanticipated* biochemical activities, where a lack of knowledge of the modification prevents implementation of the appropriate labeling strategy. Outside of biology, there exists a similar need for analytical methods that can rapidly assess the products and yields of reactions performed on surfaces. We have found that matrix-assisted laser desorption-ionization time-of-flight mass spectrometry is well-suited to the characterization of self-assembled monolayers and below provide background to this technique and several examples of its application to chemical and biological applications of monolayers.

SAMDI-TOF Mass Spectrometry

Mass spectrometry methods, like the optical methods described above, are label-free in that they detect molecules according to their molecular weight and do not require modified forms of the analyte. But they carry significant advantages in providing molecular information that can discriminate the analytes. Because MS methods can identify species by their masses, they can be applied to the analysis of mixtures of activities provided that each activity can be identified by a peak at a unique m/z ratio. MS methods have the further advantage that they are not intrinsically limited in detecting small changes in a substrate. As described further below, whereas SPR methods are not able to identify a phosphorylation of a peptide substrate (a mass change of 80 Dalton), mass spectrometry can identify an exchange of hydrogen for deuterium in a molecule attached to a monolayer. The following section gives examples of SAMDI for characterizing biochemical activities and examples of this in chemistry and biology. These examples demonstrate the utility of SAMDI MS methods as a general tool for characterizing chemical and biochemical reactions at surfaces.

Wilkins and Hanley have applied laser desorption mass spectrometry to the characterization of self-assembled monolayers and observed adducts corresponding to intact alkanethiols, including disulfides and their complexes with gold atoms.[34,35] The nature of the adducts, and the degree of fragmentation of the parent molecular ions were dependent on the laser fluence. This work used home-built mass spectrometers and did not investigate monolayers derived from alkanethiols that were substituted with functional groups or molecular fragments and did not characterize the products resulting from interfacial reactions. Based on these important examples, we reasoned that MALDI-TOF MS with a commercial instrument might be applicable to a far broader set of applications in surface chemistry (Figure 3). Indeed, we found this to be the case. Treatment of monolayers with the common energy adsorbing matrices used in MALDI MS resulted in spectra having major peaks corresponding to the masses of the alkanethiolates (and the respective disulfides).[36] Hence, this technique, which we have termed SAMDI-TOF MS owing to its combination of self-assembled monolayers and desorption-ionization mass spectrometry, provides information that can be interpreted in a straightforward manner. Indeed, in a first example, we characterized the products resulting from cycloaddition reactions to an immobilized maleimide group and of condensation reactions of a carboxylic

acid on the monolayer. These first examples reveal the information that can be collected on a monolayer in a rapid analysis and easily interpreted. Indeed, by comparing SAMDI spectra of a monolayer before and after a chemical treatment, it is possible to rapidly assess the number of and approximate yields of distinct products and, from a consideration of the mass of the new adducts, the nature of the product. We note earlier work that used SAMs as substrates for MALDI-TOF mass spectrometry, including the pioneering work of Nelson in the development of immunoassays and of the surface-enhanced laser desorption ionization (SELDI) methods that have been commercialized for identification of possible biomarkers.[37,38] These examples were not concerned with characterizing the alkanethiolates of the monolayer, as is SAMDI, but rather with characterizing proteins that interacted with the monolayer.

Several examples of the use of SAMDI to characterize chemical and biochemical reactions at surfaces follow, and serve to demonstrate the merits of this methods and the scope of problems for which it is suitable.

Electrochemical Interfacial Reactions

Electrochemical reactions of molecules attached to a monolayer are well known for several redox-active groups, and most extensively studied with the ferrocene redox couple.[39] The ability to perform oxidation or reduction reactions by applying electrical potentials to the gold film underlying the monolayer permits opportunities to create ‘dynamic substrates’ whose structures and properties can be switched. We have developed a family of strategies to electrochemically modulate the activities of biological ligands attached to a monolayer and have shown that these approaches could be employed to control the adhesion, migration and organization of mammalian cells.[40,41] Again, the development and optimization of these strategies requires a characterization of the products and yields of the electrochemical reactions. The SAMDI method has proven critical towards this activity. Figure 4 shows an example of an electrochemical protecting group for the aldehyde functionality. The protecting group is based on the redox-active hydroquinone unit and employs a pendant hydroxymethyl group to form an acetal from the aldehyde carbonyl group. This protected structure is stable under neutral conditions, but can be efficiently deprotected through oxidation of the hydroquinone ring. The SAMDI spectra show clearly that the deprotection proceeds in high yield and provides the aldehyde product, which can then be used to immobilize ligands.[42]

Immobilization of Ligands

A common step in the preparation of biologically active surfaces is the immobilization of molecules to a surface having appropriate chemistries, including activated esters, maleimides, epoxides and nucleophilic groups. It can be difficult to ensure that the immobilization reaction has proceeded to completion, or that other molecules in the sample have not been co-immobilized with the desired ligands. SAMDI provides a convenient and effective method for validating the preparation of these ‘biosurfaces’. In these examples a monolayer presenting a maleimide group at low density against a background of oligo(ethylene glycol) groups is treated with a thiol-tagged ligand.[43] The maleimide group selectively reacts with the thiol functionality of the ligand and the glycol groups are effective at preventing unwanted protein adsorption in subsequent assays. Characterization of the monolayer with mass spectrometry shows clear peaks corresponding to the maleimide-terminated alkanethiolate prior to the reaction and of the immobilized adducts following reaction.

A more complicated example is encountered in experiments that require the selective immobilization of proteins. For routes that rely on the selective reaction of a functional group on the protein with a capture group on the surface—for example, the reaction of a protein having a single cysteine residue with a maleimide group on the surface—it is necessary to first purify the recombinant protein to prevent competing reactions of other components in the

sample.[44] Because the purifications are often not complete, it can be important to verify that the protein of interest, and only this protein, was efficiently immobilized to the substrate. This problem is extremely challenging because the number of possible proteins in the sample that can compete for the surface is vast—making it impossible to explicitly check each other protein—and the common structures to all proteins make it difficult to apply spectroscopic methods to this problem. Here, too, the mass resolving ability of mass spectrometry methods makes it straightforward to verify the integrity of the immobilization process. We developed an active-site directed method whereby an irreversible inhibitor of an enzyme is presented on the monolayer surface and is used to covalently immobilize a fusion protein containing the target enzyme and the protein of interest to be displayed at the surface.[45] One example characterized the immobilization of a 40 kDa protein having cutinase linked to the cell adhesion domain from fibronectin to a monolayer presenting a phosphonate capture ligand.[46]

Kinase Activity Assays

The SAMDI assay is well-suited for performing a broad range of enzyme activity assays. In the 'solid-phase' format, the substrate is first attached to the monolayer and then treated with the enzyme to give the corresponding product, usually with a change in mass. Our first examples of this assay were directed towards measuring the activities of kinases, which represent a family of approximately 500 human enzymes that are responsible for the phosphorylation of proteins and play a role in essentially all cellular processes. Functional assays of these enzymes are important in biochemical research, drug discovery and diagnostics. Current solid phase assays use surfaces that present peptide substrates and monitor the phosphorylation of the peptide using either antibodies that recognize the modified peptide or ATP that is labeled at the terminal phosphate group with the ^{32}P isotope. The SAMDI format avoids the need for radioisotopic or antiobody reagents and can be applied to multiple assays in the same mixture. In one example, a monolayer presenting the RKRSRAE peptide was treated with the PKG kinase and ATP.[47] Spectra before and after treatment with kinase revealed that the mass of the peptide had increased by 80 Da, as expected for a single phosphorylation reaction (Figure 5). Further, the spectrum shows that all of the peptide was active towards the kinase. This ability of SAMDI to monitor both the substrate and the product of a biochemical reaction is an important benefit because it allows an estimate of the yield to be determined. It is also important because it serves as a quality control in the assay. With label-based assays, a lack of a signal is interpreted as a lack of activity but could be due to a failed immobilization of the peptide at the surface. Because SAMDI monitors all species on the monolayer, it can discriminate between true lack of activity and the false negatives that arise from defect substrates.

Assays of Multiple Activities

This feature of MS methods to identify multiple species on the surface can be exploited for performing multi-analyte assays. Current approaches to assaying several distinct kinase activities in a cell lysate, for example, would either separate the sample into several aliquots and perform individual assays on each or would pattern a set of peptides—where each was phosphorylated by only one kinase in the mixture—in an immobilized array. With mass spectrometry, however, the peptides could be immobilized as a mixture to a single monolayer and provided that the mass of each peptide is resolved from the others in the set, the phosphorylation of each peptide could be followed independently. Figure 5 shows a demonstration of this strategy using a monolayer to which four peptide substrates were immobilized. A SAMDI spectrum shows clear peaks for each of the peptide-alkanethiol conjugates. Treatment of the surface with a single kinase—here, CK1—results in a spectrum in which the peak for the selective peptide substrate has shifted by 80 Da and the peaks corresponding to substrates for other kinases remain unchanged.[47] Further, this concept can be applied to the analysis of unrelated activities—for example, those that report on protease,

kinase and glycosylation events—because the assay does not require processing steps that are often mutually exclusive for different classes of activity. With the sensitivities of modern MALDI-TOF instruments, we expect that more than a dozen assays can easily be accommodated in a common substrate.

Assays of Endogenous Cellular Activities

Characterizing the results of an immobilized format assay becomes more difficult with complex samples, including cell lysates, blood and other humoral fluids, since there are a nearly unlimited number of competing analytes that can interact with the surface or give cross-reactivities. One common example comes from the analysis of the family of caspase enzymes that underlie the apoptosis programs that cause cells to die. The proteases have distinct sequence specificities for the substrates that they cleave, but current assays based on fluorescence are not effective at resolving these activities. This limitation derives from the use of tetrapeptide substrates that have a fluorescent molecule conjugated to the amide targeted by the protease. The presence of this non-natural residue, together with the deletion of sequence that can be important for enzyme-substrate discrimination leads to substantial cross-reactivity of the peptides for the family of caspases. Label-free assays permit the use of a native peptide that more closely resembles the endogeneous substrates for the enzymes. We designed several peptides with this motivation in mind and performed assays of cell lysates on monolayers presenting the selective peptide substrates.[48] By analyzing the activities of lysates that were prepared at several times after induction of apoptosis, we found that the SAMDI assay provided a clearer resolution of specific caspase activities with comparable sensitivity to the fluorescence assays. Again, multiple caspase activities could be assays with a single monolayer by relying on the resolving ability of the mass spectra. We have also reported examples of the measurement of kinase activities in cellular lysates,[49] and of protein antigens in cerebral spinal fluid.[50]

Protein-Protein Interactions

Many applications in biointerfacial science rely on measuring the binding of proteins in a sample to ligands on a biochip surface. The current approaches rely almost exclusively on fluorescence measurement, either by directly labeling the target protein or by using labeled antibodies that are brought to the surface by way of the target protein. Here, too, the expense associated with preparing high affinity and selective antibodies for analytes has motivated the investigation of label-free methods for measuring protein binding. Surface plasmon resonance spectroscopy, along with analogous methods that measure changes in the refractive index of the medium near an interface, are the most important tools, but have the limitation that specific and non-specific binding are not resolved. We found that SAMDI is well-suited to analyzing proteins bound to the monolayer substrates and can identify proteins with masses up to 100,000 kD.[51,52] In one approach to characterizing protein-protein interactions with SAMDI, we immobilized his-tagged proteins to a monolayer presenting a Ni(II) chelate.[53] The mass spectrum of this surface revealed a sharp peak at m/z of 70 kD for the protein (Figure 6). When the surface was treated with a mixture of the his-tagged protein and a second protein that is known to interact with the first, we observed separate peaks for each protein. We did not observe the second protein when it was applied alone to the monolayer, demonstrating the effectiveness of the tri(ethylene glycol)-terminated monolayers for preventing non-specific adsorption of proteins. Further, we found that the same interaction could be observed in the other orientation, when the second protein was prepared in its his-tagged form. Because this assay uses microliter quantities of protein and does not require the protein to be rigorously purified, it could be well-suited to the global mapping of protein-protein interactions.

High Throughput Screening

Analytical techniques that can rapidly and quantitatively report on enzyme activities are valuable in screening programs that evaluate many thousands of reactions. In drug discovery, it is common to perform enzyme assays in the presence of small molecules from a library, and to identify those molecules that inhibit (or activate) the enzyme activity. We demonstrated that SAMDI is an effective approach to these screening applications. In one example, we developed an assay for the anthrax lethal factor toxin, which has proteolytic activity towards peptide substrates and we used this assay to identify inhibitors from a library containing 10,000 small molecules (Figure 7).[54] Droplets containing the enzyme and eight compounds were applied to a target plate having immobilized peptide and incubated for one hour. The plate was then rinsed and analyzed by mass spectrometry to identify those droplets that had no activity. Using this approach, we could evaluate several thousand compounds in a day and identified a small molecule that inhibited the lethal factor protease with a micromolar dissociation constant and that was active in cell culture models. Mass spectrometry had not previously been applied to screening applications, primarily because the effort required to prepare samples for analysis —which includes removing salts and enriching the desired analyte in the sample—are too slow and expensive. With the SAMDI method, a straightforward rinse of the surface accomplishes both objectives and is therefore compatible with large numbers of assays. The use of a label-free detection method also reduces the time to develop the assay and will be particularly important for assaying those activities that are difficult to label.

Chemical Reaction Discovery

These benefits of SAMDI also allow its application to the identification of new synthetic transformations in chemistry. The development of new reactions still employs a traditional route, which often begins with an unexpected observation and is followed with a linear sequence of optimizations. Recent work has employed parallel screening to identify reagents that effect known reactions in high yield[55] or with high enantioselectivity.[56] In both cases, the need for labeling strategies makes these strategies ill-suited for the discovery of unexpected, and potentially new, reactions. The SAMDI method, because it can identify any product that results from a reaction (provided that the mass has changed) can be applied to this latter goal. Indeed, SAMDI is well-suited to identifying the products that result from treatment of immobilized molecules with reagents. Figure 8 shows examples of the replacement of the hydrogen of a terminal alkyne with deuterium and of the palladium-catalyzed coupling of the alkyne with iodobenzne.[57] In both cases, SAMDI shows clear peaks corresponding to the mass of the expected product and has a mass resolution better than one Dalton. We recently reported fifteen reactions that were characterized in this way and extended that work to a screen to identify new reactions. In one example, we found an unexpected reaction of primary aldehydes with an immobilized amine to give N-alkylpyridinium products.

Perspectives

This last example illustrates the unique capabilities that the SAMDI MS method brings to molecular surface science. No other technique could be used to rapidly survey hundreds of reaction zones on a monolayer to identify those combinations of reactants and reagents that give products in high yield. The SAMDI method can do so at a rate exceeding 200 spots per hour and has a mass resolution that allows the identification of products that differ in mass from the substrate by a single Dalton. A further benefit of this method is that it can be performed using commercially available instruments. The examples described in this review used a Applied Biosystems Voyager DE-PRO instrument, and we have found that current MALDI-TOF mass spectrometers from other manufacturers are also effective for performing SAMDI. We note that the cost of the instruments is substantially lower than that of many UHV instruments used in surface science. This combination of cost, speed and commercial

instrumentation make SAMDI-TOF mass spectrometry a powerful tool—and for many programs, the enabling tool—for researchers that use self-assembled monolayers in studies of chemistry and biology.

What are the current limitations of the SAMDI method? The primary variable in this method concerns the choice and application of matrix. We have used the standard matrices developed for MALDI-TOF MS and find that the optimal matrix can vary with the structures of monolayer and analyte that are being characterized. We also find that the deposition of matrix can result in patches of the surface giving good or poor signal. Current efforts to develop strategies to uniformly apply matrix may improve this step. MALDI can be used to provide a spatial mapping of the chemistry of the monolayer. Modern instruments have a one micron scanning capability and sufficient resolution to provide images of products with better than ten micron resolution. We also note that SAMDI does not have the quantitative character that other surface analytical methods do. For a given analyte on the monolayer, the intensity of the m/z peak is generally related to the density of that species, but different molecules ionize with varying efficiencies, making it difficult to directly compare the relative amounts of distinct molecules. Peaks corresponding to the unfunctionalized alkanethiolate in the monolayer can be used to calibrate the intensities of peaks corresponding to different molecules, but doing so requires authentic standards and reduces the throughput of the method. Finally, the monolayers do not have the stability required in some applications. Temperatures in excess of 70 C, ultraviolet light in the presence of oxygen, and chemical reagents that are strong oxidants, bases or acids, cause damage to the monolayer. For biological assays, which require moderate temperatures and neutral aqueous environments, stability of the monolayers is not limiting.

Self-assembled monolayers are now a basic component of the toolbox for nanoscience. At the most basic level, these nanoscale two-dimensional structures are important for modifying the physical properties of a surface. In current applications, the monolayers provide a route to preparing surfaces that are decorated with a vast array of molecular content and that in turn enable applications in chemistry and biology. These applications often require a sequence of reactions to assemble the final monolayer and to analyze it after its application. The SAMDI method brings an unprecedented ability to identify the alkanethiolates and their reaction products, to identify proteins, polymers or other molecules that are associated with the monolayer, and to verify the integrity of the monolayer in experiments. Several researchers have recently adopted this method and we expect that it will become a basic component of the characterization toolbox for nanoscience.

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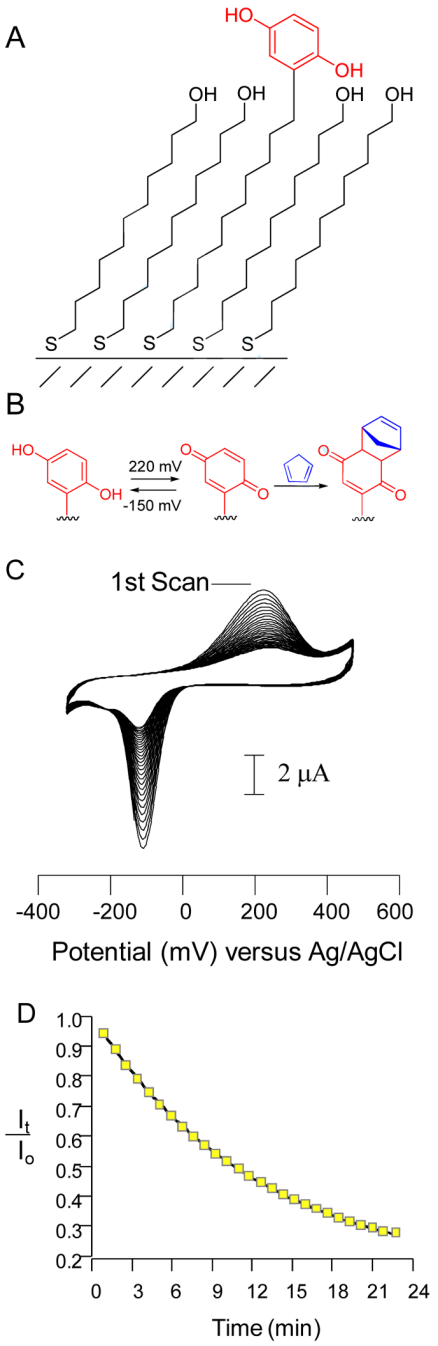

Figure 1.
Interfacial reactions can be characterized with electrochemical methods. In this example, a hydroquinone group attached to a monolayer (A) undergoes oxidation to give the corresponding benzoquinone group, which selectively reacts with cyclopentadiene to produce the cycloadduct (B). Cyclic voltammetry reveals waves for the oxidation and reduction reactions of the hydrquinone, and shows a decreasing intensity of those waves as the reaction proceeds (C). Integration of the area under the waves provides kinetic information and shows that the reaction is first order in benzoquinone (D).

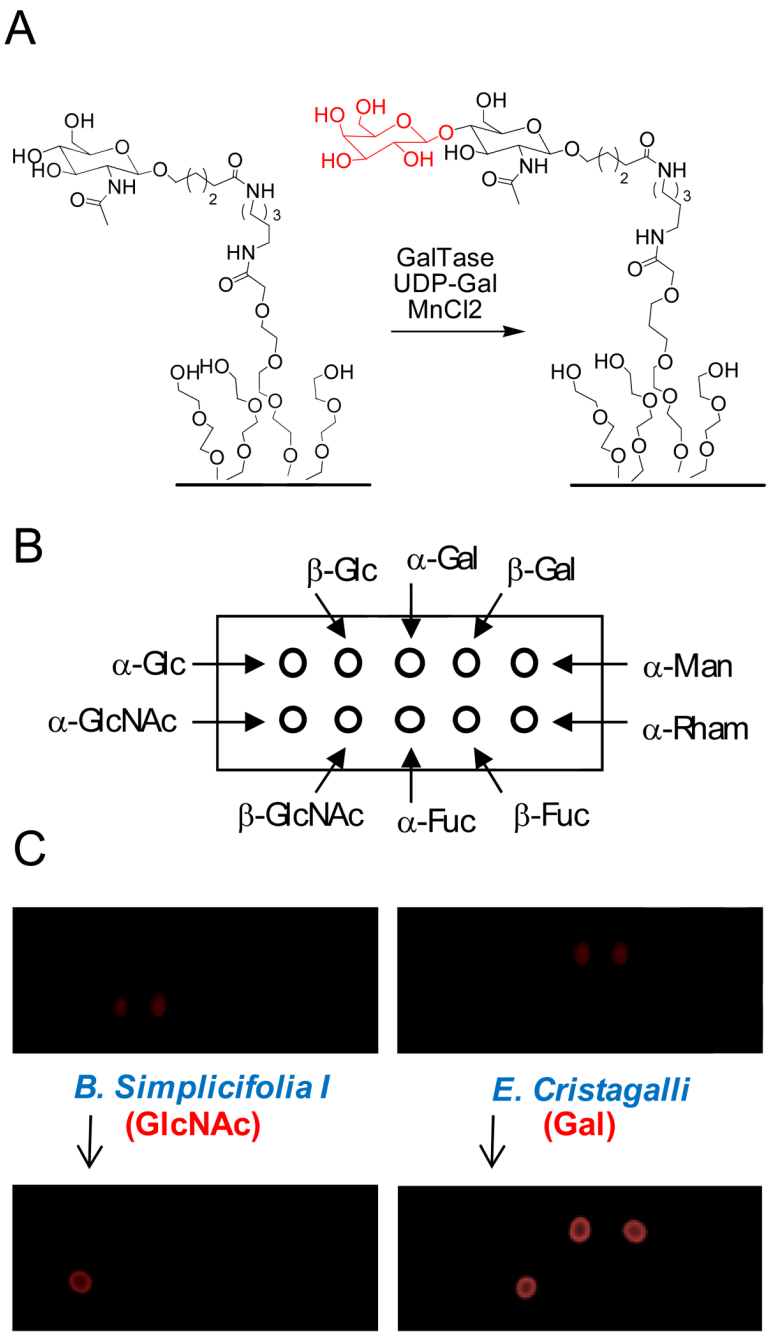

Figure 2.
Biochemical interactions can be assayed using biochips and fluorescence detection. In this example, an array of ten carbohydrates is prepared and treated with a galactosyltransferase enzyme (A). Identical arrays (B) are treated with carbohydrate-binding proteins before and after the enzyme reaction to reveal changes in the structures of the immobilized sugars, and therefore the sugars that are substrates for the enzyme (C).

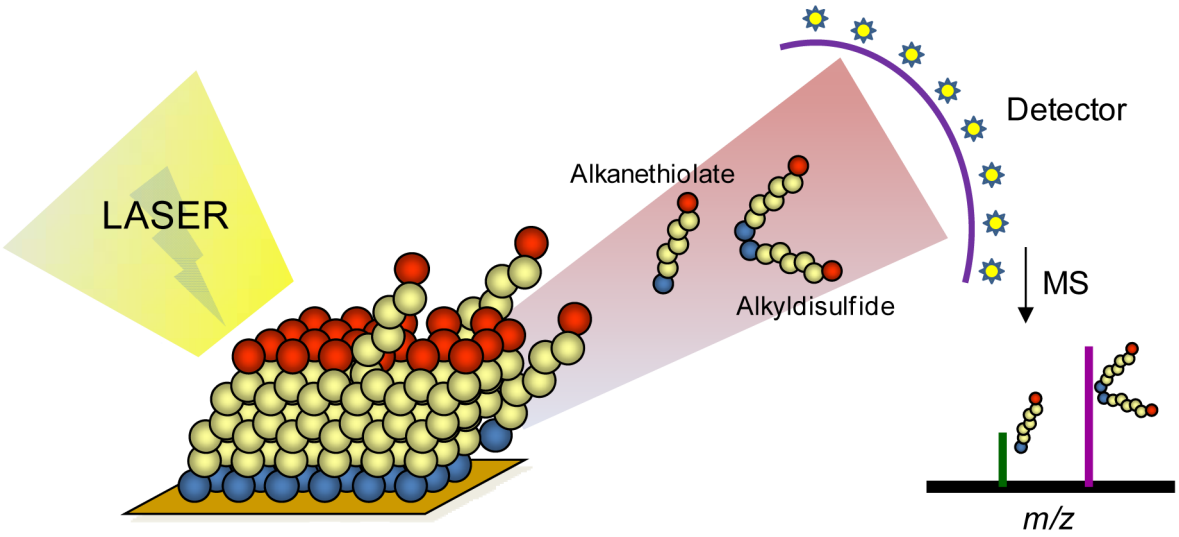

Figure 3.
The combination of self-assembled monolayers and matrix assisted laser desorption-ionization mass spectrometry—in a technique termed SAMDI MS—permits rapid characterization of the masses of alkanethiolates in the monolayer. Both alkanethiolate and dialkyldisulfide forms of the molecules are observed. This method enables applications of model surfaces to a broad range of problems in chemistry and biology.

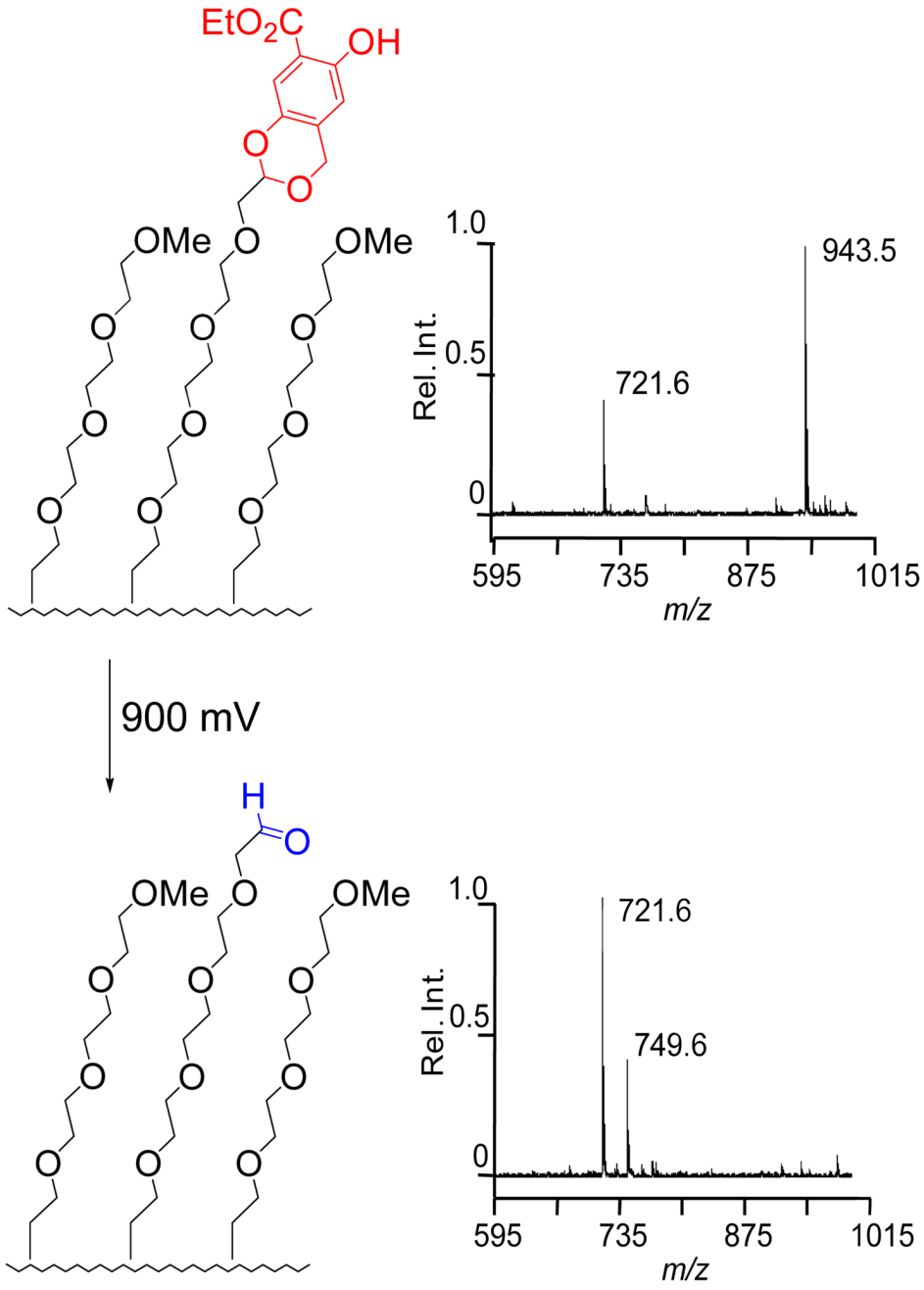

Figure 4.
SAMDI MS can be applied to characterizing the products of electrochemical reactions of monolayers. In this example, a monolayer is functionalized with an acetal derived from hydroquinone. Application of a 900 mV potential to the underlying gold film results in oxidative hydrolysis of the acetal to reveal the aldehyde group. SAMDI spectra before and after the reaction show clear peaks for the disulfide containing the functionalized monolayer (944 and 750 Da, respectively) and for the disulfide derived from the background alkanethiolates (721 Da).

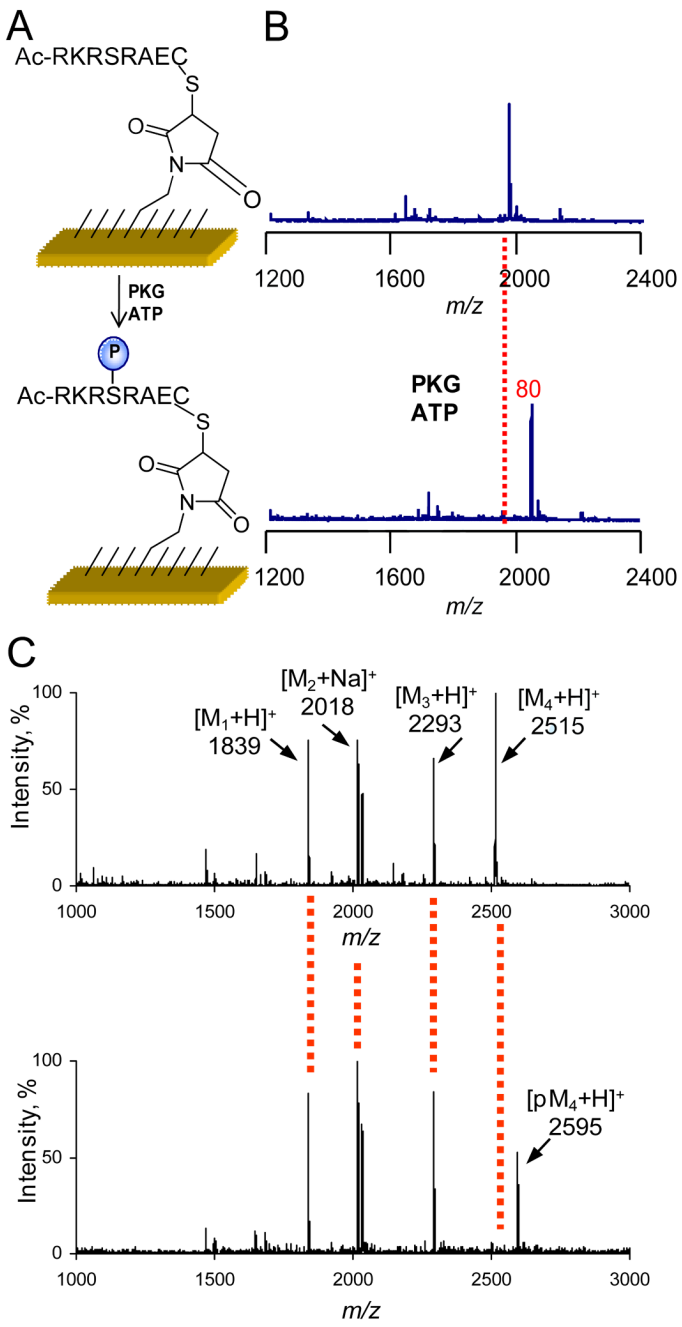

Figure 5.

SAMDI MS can be applied to assays of enzyme activities. In this example, a peptide substrate for the PKG kinase was immobilized to a monolayer presenting a maleimide group (A). SAMDI spectra of the monolayer before and after the enzyme reaction reveal that the mass of the peptide-terminated alkanethiol increased by 80 Dalton, corresponding to the expected phosphorylation. A multi-analyte assay was performed by immobilizing a mixture of four peptide substrates. Treatment of the monolayer with the CK1 kinase resulted in phosphorylation of only a single peptide, which was easily detected in the SAMDI spectra.

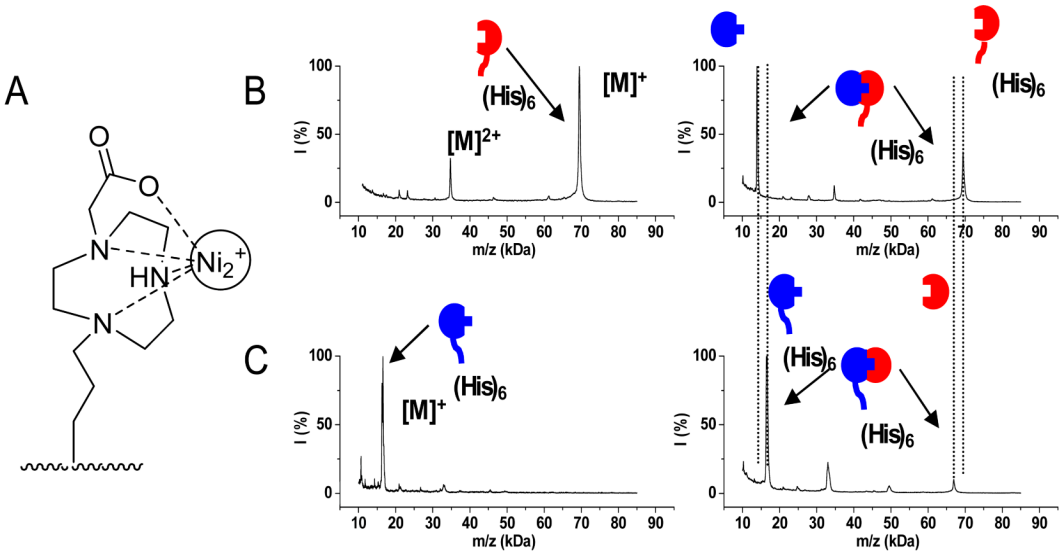

Figure 6.

Protein interactions can be monitored with the SAMDI method. A monolayer presenting a chelate of the Ni(II) ion was used to immobilize his-tagged proteins (A). SAMDI MS shows clear peaks for a 70 kD protein (B). The monolayer was treated with a mixture of this protein and a 14 kD protein known to interact with the first and then analyzed by SAMDI to reveal the presence of both proteins. The same interaction could be identified in the opposite orientation, by using a his-tagged version of the smaller protein (C).

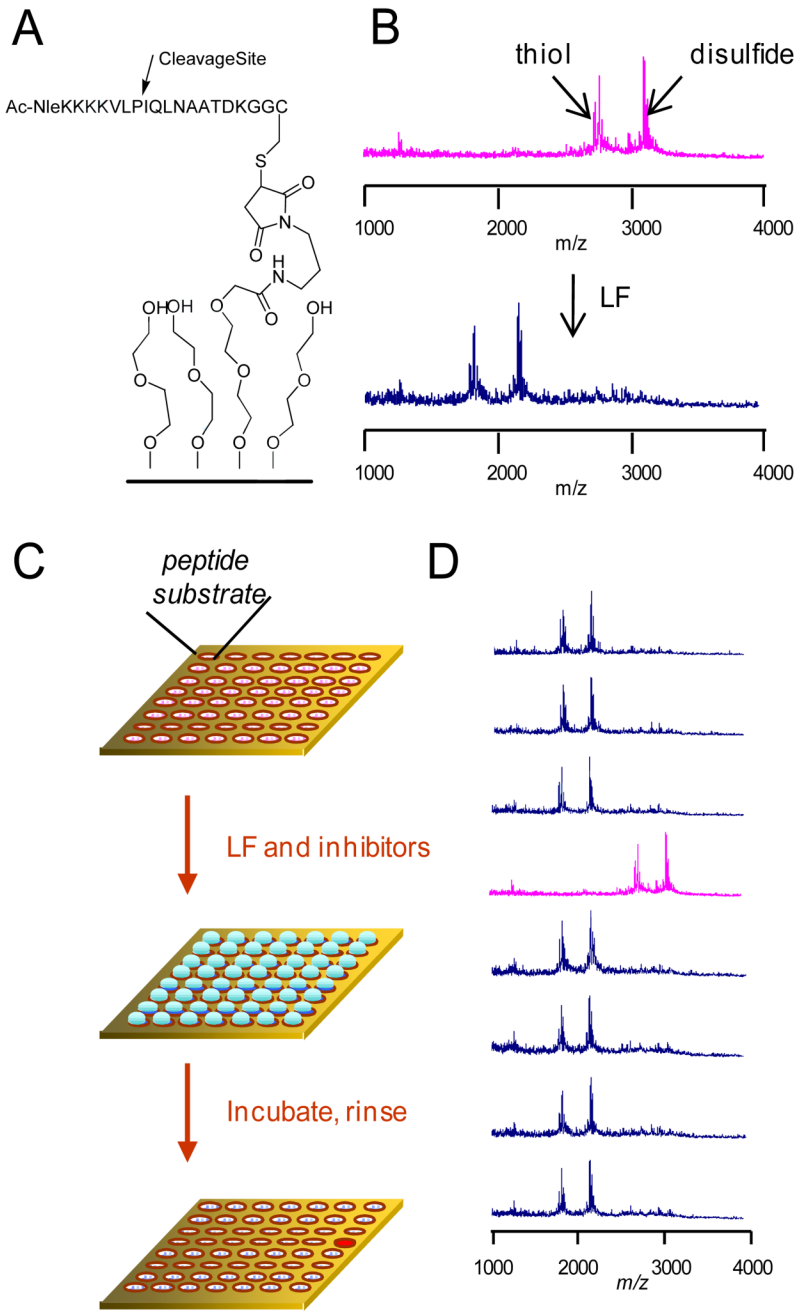

Figure 7.

SAMDI was used to perform a screen of 10,000 small molecules to identify inhibitors of the anthrax lethal factor protease. (A) A peptide substrate for lethal factor was immobilized to a monolayer presenting maleimide groups. (B) Treatment of the monolayer with recombinant protease resulted in cleavage of the peptide, which could be analyzed by SAMDI mass spectrometry. (C) Chemical screens were performed by arraying one hundred droplets that contained the protease and eight compounds from the library, followed by analysis of the spots with mass spectrometry, which clearly identified those spots having an inhibitor in the reaction mixture (D, pink spectrum).

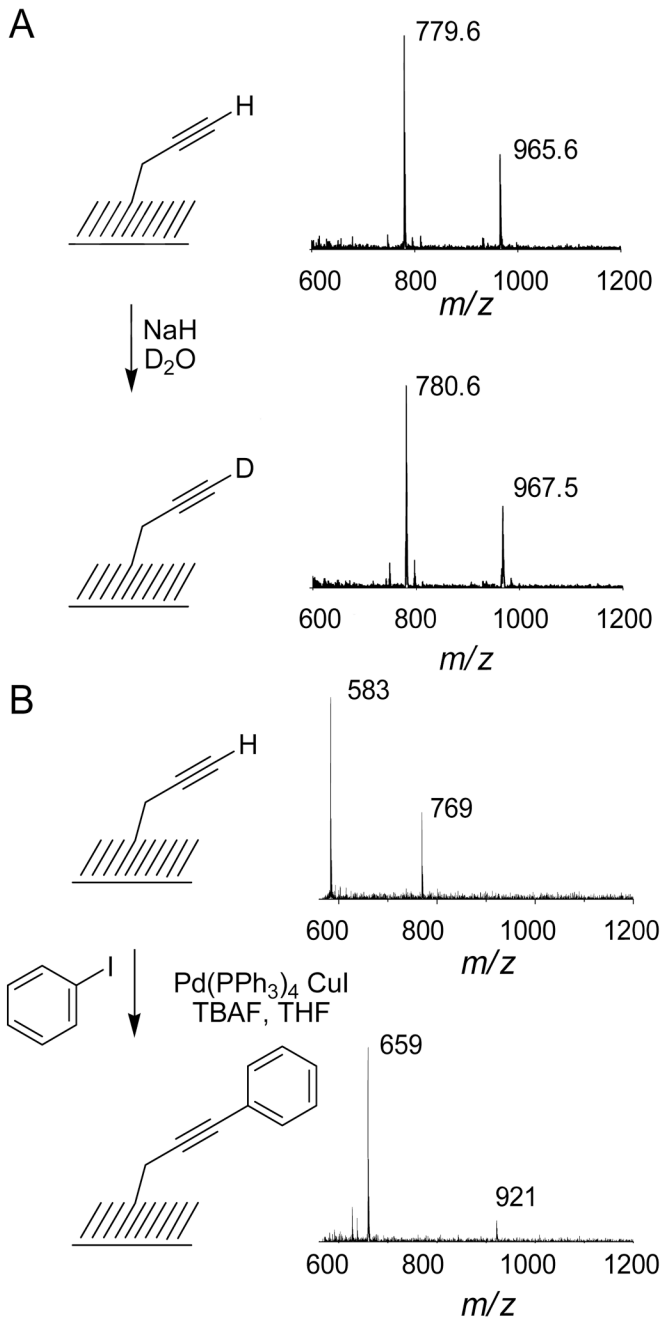

Figure 8.

Chemical reactions of molecules attached to a monolayer are rapidly characterized with SAMDI MS. Treatment of a monolayer presenting a terminal alkyne group with sodium hydride and deuterated water results in the exchange of the terminal hydrogen for a deuterium atom (A). SAMDI MS identifies the product with a mass change of one Dalton (at 780.6) and the associated disulfide product (967.5). A Sonagashira coupling of iodobenzene to the alkyne also proceeds in high yield to produce the phenylalkyne adduct (B).