Matrix-Assisted Laser Desorption/Ionization Imaging Mass Spectrometry for the Investigation of Proteins and Peptides

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Annu. Rev. Anal. Chem. 2008. 1:689–705

First published online as a Review in Advance on March 18, 2008

The *Annual Review of Analytical Chemistry* is online at anchem.annualreviews.org

This article's doi:
10.1146/annurev.anchem.1.031207.112841

1936-1327/08/0719-0689$20.00

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Key Words

imaging, MALDI, mass spectrometry, proteins, peptides

Abstract

Mass spectrometry (MS) is an excellent technology for molecular imaging because of its high data dimensionality. MS can monitor thousands of individual molecular data channels measured as mass-to-charge (*m/z*). We describe the use of matrix-assisted laser desorption/ionization (MALDI) MS for the image analysis of proteins, peptides, lipids, drugs, and metabolites in tissues. We discuss the basic instrumentation and sample preparation methods needed to produce high-resolution images and high image reproducibility. Matrix-addition protocols are briefly discussed along with normal operating procedures, and selected biological and medical applications of MALDI imaging MS are described. We give examples of both two- and three-dimensional imaging, including normal mouse embryo implantation, sperm maturation in mouse epididymis, protein distributions in brain sections, protein alterations as a result of drug administration, and protein changes in brain due to neurodegeneration and tumor formation. Advantages of this technology and future challenges for its improvement are discussed.

1. INTRODUCTION

MS: mass spectrometry

MALDI: matrix-assisted laser desorption/ionization

IMS: imaging mass spectrometry

Over the past decade, proteomics has become a vital complement to genetic analysis in the investigation of nearly all aspects of the life sciences. These include the elucidation of cellular processes in both health and disease (1–3) and the discovery and evaluation of pharmaceutical compounds (4–9). Mass spectrometry (MS) has emerged as an essential analytical tool for the investigation of these molecular processes. Indeed, new advances in MS now provide the opportunity for investigative studies of molecular interactions in intact tissue. Unlike studies conducted with intact tissue decades ago, studies performed today can take advantage of the exquisite molecular specificity offered by MS. In particular, matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) allows investigators to analyze the spatial distribution of proteins directly in tissue specimens.

IMS can be used to localize specific molecules such as drugs, lipids, peptides, and proteins directly from fresh-frozen tissue sections with lateral image resolution of 30–50 μm. Thin frozen sections (10–15 μm thick) are cut and thaw-mounted on target plates; subsequently, an energy-absorbing matrix is applied. Areas with a typical target spot size of about 50 μm in diameter are ablated with a UV laser, thereby giving rise to ionic molecular species that are recorded according to their mass-to-charge (m/z) values. Thus, a single mass spectrum is acquired from each ablated spot in the array. Signal intensities at specific m/z values can be exported from this array to give a two-dimensional ion-density map, or image, constructed from the specific coordinate location of that signal and its corresponding relative abundance. For high-resolution images, matrix is deposited in a homogeneous manner to the surface of the tissue in such a way as to minimize the lateral dispersion of the molecules of interest. This can be achieved either by automatically printing arrays of small droplets or by robotically spraying a continuous coating. Each micro spot or pixel coordinate is then automatically analyzed by MALDI MS (**Figure 1**). From the analysis of a single section, images at virtually any molecular weight may be obtained, provided that there is sufficient signal intensity to record.

One of the most compelling aspects of IMS is that it provides the ability to simultaneously visualize the spatial arrangement of hundreds of analytes directly from tissue without any prior knowledge or need for target specific reagents such as antibodies. IMS enables the visualization of posttranslational modifications and proteolytic processing while retaining spatial localization. Other MS techniques, such as secondary ionization mass spectrometry (SIMS), have also been used for a variety of imaging applications. One of the major advantages of SIMS is that it is capable of high-resolution imaging (50–100 nm) for elements and small molecules (m/z <1000 Da). However, thus far it has not been shown to be effective for the analysis of proteins and large peptides.

This review focuses on MALDI IMS analysis of proteins and peptides in terms of basic instrumentation, sample preparation, and recent applications. MALDI MS is an effective technology for both qualitative and quantitative analysis of normal and diseased tissue and for assessing temporal changes in biological systems. In addition, this technology has been applied to the generation of three-dimensional protein

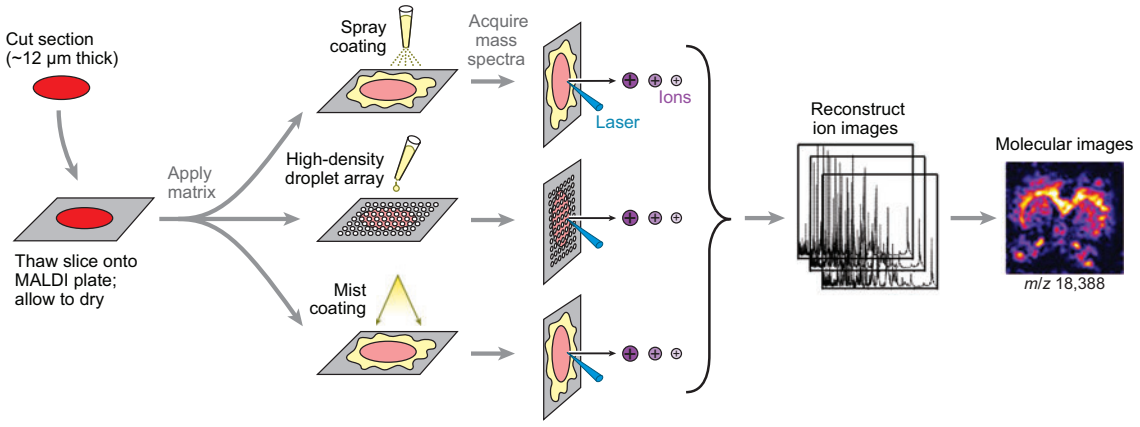

Figure 1

Scheme of a typical direct tissue imaging experiment. Three types of matrix application are shown: spray coating, high-density droplet array, and mist coating. Subsequent image acquisition is shown for each technique. Abbreviation: MALDI, matrix-assisted laser desorption/ionization.

images of brain, whole-body reconstructions, and the measurement of protein changes in specific tissues after systemic drug administration.

2. THE TECHNOLOGY

The MS instrumentation best suited for the analysis of peptides and proteins directly from tissues is MALDI time-of-flight (TOF) technology. The ablation process directed by the focused laser beam, together with the high frequency of the laser pulse, has rendered TOF the most widely used ionization method for imaging. The duty cycle of a modern TOF analyzer is an ideal match for the pulsed laser process and also has the advantages of a theoretically unlimited mass range, high ion-transmission efficiency, multiplex detection capability, and simplicity in instrument design and maintenance (10).

A brief description of a MALDI TOF MS experiment follows for those who are not currently practitioners and who seek introductory information; more detailed treatments can be found in other works (11, 13). A typical analysis of proteins directly from tissue is described for illustrative purposes. There are two main experimental approaches: profiling and imaging. Profiling involves analyzing discrete areas of the tissue sections and subjecting the resulting protein profiles to computational analysis. Typically, this uses 5–20 spots, each measuring approximately 0.2–1 mm in diameter. These experiments are designed to make comparisons between representative areas on pieces of tissue, such as a normal healthy area versus a diseased area, or between two different specimens. Thus, in the profiling mode, fine spatial resolution is not required. A sufficient number of areas must be sampled to gain statistical confidence in the results; the number varies depending on the specific experiment. On the other hand, high-resolution imaging of a tissue requires that the entire tissue section be

TOF: time of flight

SA: 3,5-dimethoxy-4-hydroxy-cinnamic acid

analyzed through an ordered array of spots, or raster, in which spectra are acquired at intervals that define the image resolution (e.g., every 50 μm in both the *x* and the *y* directions). Two-dimensional ion-intensity maps, or images, can then be created by plotting the intensity of any signal obtained as a function of its *xy* coordinates. The resulting images allow rapid assessment of protein localization differences between and among samples.

Tissues used for analysis should be frozen in liquid nitrogen immediately after resection to preserve morphology and minimize protein degradation through proteolysis. The tissue is usually sectioned in a cryostat to give 10- to 12-μm-thick sections and is then thaw-mounted onto an electrically conductive sample plate (11). Sample plates include gold-coated or stainless steel metal plates and glass slides that have a conductive coating. The tissue may be gently rinsed with ethanol acting as a fixative and a wash to remove lipids and salts. Alternatively, IMS-compatible tissue-staining protocols can be used in conjunction with the optically transparent glass slides, allowing correlation of IMS data with histological features of the same section by optical microscopy (14).

MALDI IMS requires the application of energy-absorbing matrix. The matrix is typically a small organic molecule that cocrystallizes with the analytes of interest on the tissue surface. The matrix is capable of absorbing laser energy, thereby causing the analyte to desorb from the sample surface and ionize. The most commonly used matrices include 3,5-dimethoxy-4-hydroxy-cinnamic acid (SA), 2,5-dihydroxybenzoic acid (DHB), and α-cyano-4-hydroxy-cinnamic acid (HCCA). Solvent combinations and the matrix used vary depending on the type of molecule and tissue being investigated (11). A 50:50 (v/v) acetonitrile/water or ethanol/water mixture is generally recommended for use in tissue analysis (12). SA is an excellent matrix for the analysis of protein (11), and DHB and HCCA are primarily used for the analysis of peptides and lower-molecular-weight analytes.

For high-resolution imaging, the matrix solution should be homogenously deposited across the tissue section in such a manner as to avoid significant lateral migration of analytes. Currently, this is achieved by applying matrix solution to the tissue in either a spotted array or a homogenous spray coating (11). A continuous and homogenous spray coating allows the highest spatial resolution, but densely spotted arrays yield higher reproducibility and generally better spectra quality. Heterogenous matrix coating gives rise to hot and cold spots for ablation due to random crystal formation, therefore producing poor, highly pixilated images. Numerous robotic spotting devices are commercially available and utilize acoustic (15), piezoelectric (16), ink-jet printer (17), and capillary deposition techniques (18). Several robotic spray coating devices are also commercially available and utilize a mist-nebulizing method (19) or a thermally assisted spray method (20).

Protein analysis is usually performed on a linear TOF instrument to achieve the highest possible sensitivity. Ions formed and desorbed during the laser pulse are extracted and accelerated into the field-free region of the TOF analyzer. Ions are usually detected by a multichannel plate detector and the TOF of the various ions is inversely proportional to their *m/z* values. This time measurement is then converted to *m/z* through appropriate calibration procedures. For the analysis of

low-molecular-weight species, an ion mirror or reflectron can be used in the ion flight path to compensate for the initial velocity/energy distribution and can thus improve resolution (21).

Other analyzer combinations that have been used with MALDI IMS include TOF-TOF (16), orthogonal TOF and orthogonal quadrupole-TOF (22, 23), ion mobility (24), Fourier transform ion cyclotron resonance (25, 26) and ion trap technologies (27, 28). These additional tools have provided capabilities for protein identification, high mass resolution acquisition, and detection of small molecules such as drugs and metabolites. Although it is beyond the scope of this article to describe these techniques in more detail, the reader is referred to other works for more thorough descriptions (16, 22–25, 27).

3. QUANTITATION

To assess changes in protein concentrations in a given tissue, pixel-to-pixel reproducibility must be high; that is, two pixels close together having the same protein concentrations should give the same spectra within acceptable standards. Although these standards vary from experiment to experiment, typically variations of $\pm 15\%$ (or less) are deemed acceptable. Factors bearing directly on this aspect of the analysis include ionization efficiency of a given molecule, ion-suppression effects, extraction efficiency of the matrix-deposition process, and the robustness and effectiveness of postacquisition processing. High pixel-to-pixel reproducibility can be achieved if careful attention is paid to sample preparation and matrix application. In addition, instrument parameters such as voltage settings and laser power must be kept constant within a given experiment. A robotic matrix application device is invaluable in removing operator-to-operator variations. In a recent study of protein distribution in brain, relative standard errors of 3.7–9.6% were obtained between striatal regions in multiple animals (22). However, one must keep in mind that because ionization processes can be affected by the physical and chemical processes of molecules, it is difficult to estimate relative concentrations of two completely different proteins by comparing their peak heights alone.

4. DATA PROCESSING AND ANALYSIS

It is important to assess the reproducibility of the mass spectra so that variations in peak intensities can be correlated to biological endpoints. To do so, one must perform the two general types of data analysis: preprocessing and statistical analysis. The preprocessing step reduces the experimental variance between spectra through the removal of background, normalization of the peak intensity to the total ion current, and peak alignment algorithms. Normalization of the spectra minimizes variation arising from day-to-day instrument fluctuations, differences in matrix crystallization across tissue sections, and changes in sample preparation and the chemical properties of the underlying tissue (29). Various algorithms are employed for all of the spectra processing steps: baseline subtraction, peak alignment, normalization and peak picking. Ion images are generated directly from these processed data sets.

The next phase of data processing involves downstream events such as calculation of average spectra for each specimen type or targeted area in the tissue (as determined by the investigator) and subsequent statistical analysis procedures. Principal components analysis can be performed to reduce the dimensionality of a given data set using processes to extract, display, and rank the variance within the data set. By identifying patterns in data, the investigator can more easily determine similarities and differences (30). Significance analysis of microarrays (SAM) can be performed to assign a score to each feature on the basis of change in its spectral intensity relative to the standard deviation of repeated measurements. For features with a score greater than a predetermined threshold, SAM uses permutations of the repeated measurements to estimate the percentage of features identified by chance (the false discovery rate) (31). This algorithm is used to elucidate features that change significantly between two groups of specimens. A hierarchical clustering analysis (HCA) may also be applied to the data set, enabling samples to be grouped blindly according to their expression profiles. HCA functions by calculating the dissimilarity between the individual analyses (32).

5. MOLECULAR IMAGES OF DISEASE

To date, considerable effort has been focused on finding molecular markers that represent early indicators of disease. MALDI IMS provides a means to visualize molecularly specific information while maintaining spatial integrity. For example, cancer progression depends on essential characteristics such as the presence of growth factors, insensitivity to growth-inhibiting signals, evasion of apoptosis, high replicate potential, sustained angiogenesis, and tissue invasion and metastasis (33). Alterations in protein expression, proteolytic processing, and posttranslational modifications all contribute to cellular transformation. IMS analyses of tissue sections reflect the overall status of the tissue; therefore, analyses of tissues in various states can reveal differences in the expression of proteins that otherwise cannot be predicted. IMS has been used to image protein distributions in multiple types of cancer. Imaging analysis has been used to probe proteome changes in mouse breast and brain tumor (34, 35), glioblastoma biopsies (36), and human lung metastasis to the brain (12) and prostate (34, 37). Identifying features that reveal differential expression patterns between cancerous and normal tissue can provide valuable insight into the molecular mechanisms of cancer, provide molecular diagnostic and prognostic signatures, and identify possible drug targets in implicated pathways.

Ion images obtained from a mouse brain with a developed glioblastoma tumor in the right hemisphere after injection with GL26 glioma cells (S.L. Frappier, unpublished data) are shown in **Figure 2*a***. Histone H4 localizes in the tumor tissue in contrast to guanine nucleotide-binding protein $\gamma 7$ and cytochrome c oxidase polypeptide VIIc, which localize in the surrounding normal brain tissue. Hundreds of ion images were produced from a single data-acquisition process.

Three-dimensional MALDI IMS was first reported in 2005 for the depiction of myelin basic protein isoform 8 in the corpus callosum of a mouse brain (38); more recently, it was used to explore a three-dimensional protein volume in the

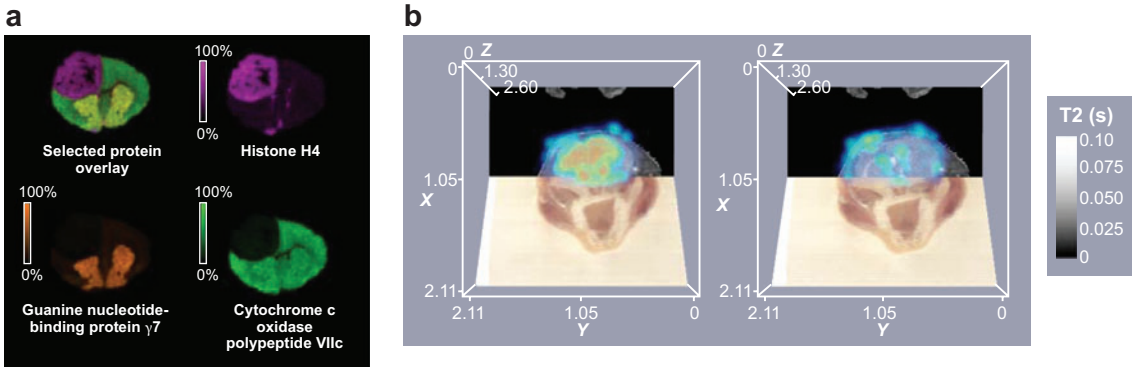

Figure 2

Imaging mass spectrometry (IMS) analysis of a glioma tumor mouse brain. (*a*) Ion images from a two-dimensional IMS experiment of a mouse brain with a glioma tumor located in the right hemisphere. (*b*) Spatially resolved three-dimensional IMS volumes coregistered to in vivo magnetic resonance imaging data and the optical volume of a glioma tumor mouse brain.

substantia nigra in a mouse model of Parkinson's disease (39). The acquisition of three-dimensional molecular images also raises the exciting possibility of combining these IMS images with magnetic resonance imaging (MRI) and perhaps positron emission tomography (PET) imaging, so that the molecularly specific MS data can be coregistered with the MRI data. The latter technique relies on the relaxation properties of excited hydrogen nuclei in water and lipids after exposure to a powerful, uniform magnetic field (40).

Figure 2*b* shows the results of experiments wherein MS and MRI were combined to analyze a mouse brain injected with glioma cells and to visualize the data in three dimensions (41). The entire mouse head was sectioned from the olfactory bulb to the beginning of the spinal cord. Coronal sections of the mouse brain were collected at 20-μm increments as a high-resolution camera acquired blockface images every 40 μm. Tissue sections for IMS analysis were collected every 160 μm, coated with SA, and imaged at 150-μm lateral resolution. In the left panel of **Figure 2*b***, histone H2B is associated with the tumor as it presents in the contralateral ventricle. Conversely, the right panel of **Figure 2*b***, showing myelin basic protein isoform 4, demonstrates that corpus callosum can be accurately spatially correlated in a three-dimensional sample-specific context. A good correlation of the tumor between MALDI ion intensities, contrast variation in the magnetic resonance parameters, and the optical volume was observed.

IMS has also been utilized to study neurodegenerative diseases such as Alzheimer's and Parkinson's. Neurodegeneration is widespread in Alzheimer's disease, affecting several brain regions. β-Amyloid plaques accumulate causing plaque formation and subsequent neurodegeneration (42, 43). A mutation of the amyloid precursor protein gene causes higher production of β-amyloid and β-amyloid peptides in the cortical and hippocampal structures of the brain. MALDI images, prepared by coating tissue with SA and imaging at a resolution of 100 μm, show that the β-amyloid peptides

MRI: magnetic resonance imaging

PET: positron emission tomography

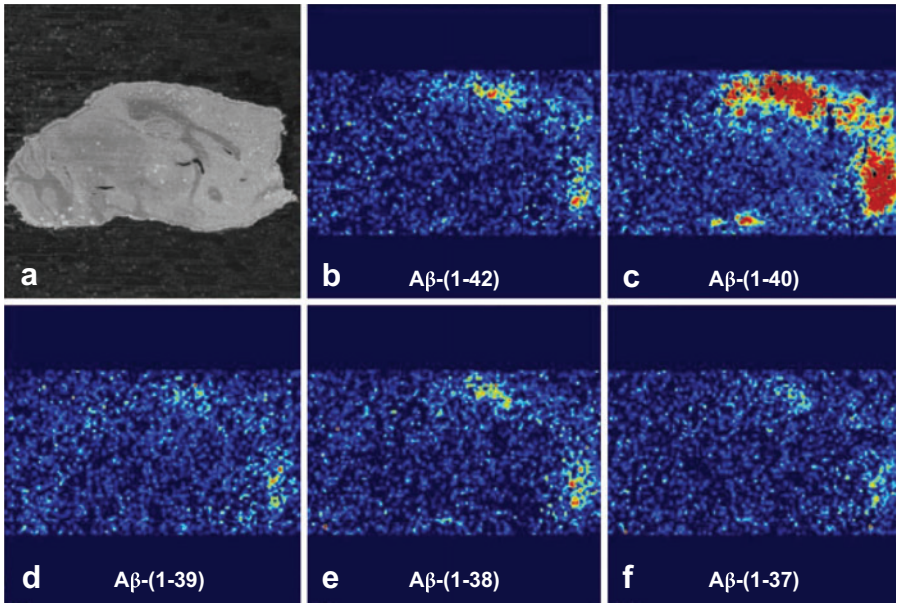

Figure 3

Imaging mass spectrometry images from a diseased brain section from an Alzheimer's patient. (*a*) Optical image of the sagittal diseased brain section. (*b*) Aβ-(1-42) molecular image (*m/z* 4515.1). (*c*) Aβ-(1-40) molecular image (*m/z* 4330.9). (*d*) Aβ-(1-39) molecular image (*m/z* 4231.7). (*e*) Aβ-(1-38) molecular image (*m/z* 4132.6). (*f*) Aβ-(1-37) molecular image (*m/z* 4075.5). Reprinted with permission from Reference 44.

(I-40) and (I-42) are the most abundant amyloid peptides in those brain regions in an Alzheimer's disease mouse model (44) (**Figure 3**). The extensive deposits of the Aβ (I-40) are seen localized in the cortex of the sagittal section.

From previous studies of Parkinsonism, a condition that affects regions of the brain that control motor function (such as the substantia nigra and the striatum), it was found that dopamine neurons in the nigrostriatal pathway are gradually decreased in the striatum. IMS technology has been applied to the 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP) animal model of Parkinsonism (45); the investigators found that the protein PEP-19, a neuronal calmodulin-binding protein, was significantly reduced (>30%) in the striatum compared to control regions in normal brain. Further study is needed to determine PEP-19's specific involvement in neurodegeneration.

6. NEW INSIGHTS INTO BIOLOGY

MALDI IMS of tissue sections can provide information on the spatial and temporal action of proteins involved in processes that take place in organs or tissue substructures containing heterogeneous cell types. A major advantage of MALDI IMS is that it allows the investigator to visualize molecular events while retaining spatial

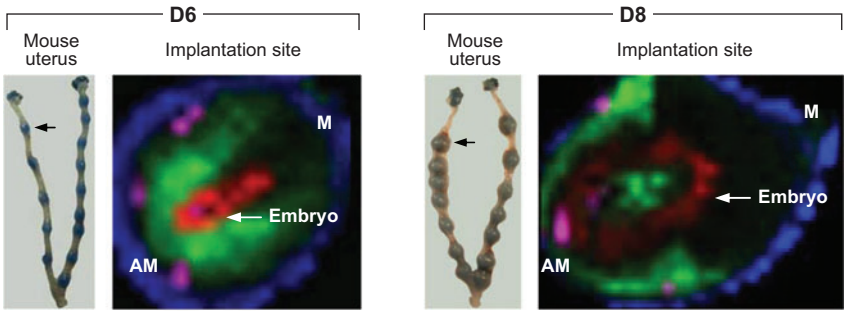

Figure 4

Optical images of a day 6 (D6) pregnant mouse uterus and day 8 (D8) pregnant mouse uterus along with the corresponding ion images (arrows indicate the implantation site). D6 implantation site and D8 implantation site ion-density maps obtained for: *m/z* 5923 (*purple*), *m/z* 4039 (*green*), *m/z* 6717 (*blue*), *m/z* 8193 (*red*). See text for details. Abbreviations: AM, antimesometrial; M, mesometrial.

information that would be lost using techniques that require tissue homogenization and extraction.

We have used IMS to study the mammalian reproductive process involving implantation and early development of the embryo. The heterogeneous cell types of the uterus, each of which has a unique function, pose a challenge for studying events during early pregnancy. In rodents and humans, one limiting factor is the availability of adequate amounts of tissue for biochemical and molecular biology experiments. Studying the uterine molecular landscape using conventional approaches presents an arduous task as some proteins are localized to particular cell types on a specific day of pregnancy and do not reappear later in the process. We studied implantation sites from mice on day 6 (D6) and day 8 (D8) of pregnancy and obtained protein distributions that were unique to embryo, surrounding muscle, and uterine stroma within an area measuring 2 mm^2 (K. Burnum, unpublished data). The ion-intensity maps presented in **Figure 4** depict how protein location and expression change during early pregnancy. The ions depicted in red and green are expressed in the presence of the embryo only on D6 through D8 of pregnancy. The ion shown in red is expressed at the site of the embryo and increases by D8. The uterus has two poles; the mesometrial pole (M), where the blood supply enters the uterus, and the antimesometrial pole (AM), where the embryo implants. The ion depicted in purple remains at the AM pole in these images, whereas the ion depicted in green shifts solely to the AM pole by D8. The ion shown in blue represents the muscle surrounding the uterus. Disregulation of events prior to, during, or immediately after implantation contributes to poor pregnancy rates. Therefore, understanding protein localization during implantation is essential to providing potential targets for treating infertility and developing novel contraceptive approaches.

In another project involving mammalian reproduction, researchers studied the spatial distribution of proteins in the sexually mature mouse epididymis (46), a structure that contains a coiled tube attached to the back of the testis. Immature sperm

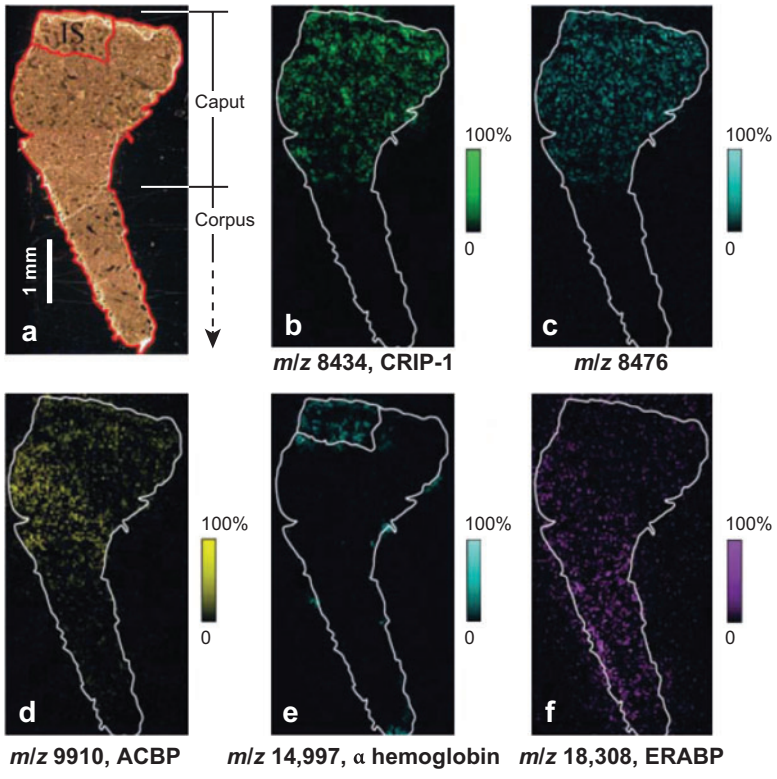

Figure 5

High-resolution imaging mass spectrometry of caput epididymis. (*a*) Optical image of a 12-μm section mounted on a sample plate prior to matrix application. (*b–f*) Ion-density maps obtained for *m/z* 8434 (CRIP-1), *m/z* 8476, *m/z* 9910 (ACBP), *m/z* 14,997 (α hemoglobin), and *m/z* 18,308 (ERABP). Reprinted with permission from Reference 46.

becomes competent for fertilization as it travels through the epididymis and interacts with proteins located along this tubule. The epididymis consists of a head (caput), body (corpus), and tail (cauda). Molecular images were generated for the whole epididymis and specific proteins were mapped over this structure. Evidence for the regional localization of some of the major epididymal proteins in the mouse as observed by IMS are shown in **Figure 5**. From these and additional images, 30 protein signals that showed regionalized patterns along the epididymis were targeted and identified.

7. PROTEOME RESPONSE TO DRUG ADMINISTRATION

In addition to assisting in disease differentiation and diagnosis, the proteome signature of a tissue can also be used to determine the effects of drug/small-molecule administration to an animal model or patient. Over the past decade, proteins have

become principal targets for drug discovery, and proteomics-oriented drug research has come to the forefront of activity in this area. Proteomics play a major role in drug development, specifically in target identification, target validation, drug design, lead optimization, and preclinical and clinical development (47).

Pharmaceutical drug discovery is a laborious and tedious process: It can take 8–10 years, at great expense, to bring a drug candidate into the market (4). Obviously, it is essential to find ways to expedite this process. With early assessment of the distribution of a drug candidate in targeted tissues, IMS can greatly assist in the discovery and validation of processes related to drug administration, distribution, metabolism, and excretion (48, 49). For example, IMS can detect the presence and location of an individual drug and its metabolites in a label-free protocol, a significant advantage over other small-molecule imaging techniques that typically require the addition of a radioactive tag to the molecule of interest, such as in autoradiography. Another advantage of IMS is that it is capable of providing information on both the pharmacological and biological effects of a drug in that it can detect molecular features that may be markers of drug efficacy or toxicity. Other imaging techniques provide little information on the molecular identity of these biological endpoints. Thus, IMS can monitor the analyte of interest and also the corresponding proteome response. For example, investigators have discovered transthyretin as a marker for gentamicin-induced nephrotoxicity in rat (50). Gentamicin-induced nephrotoxicity is seldom fatal and is usually reversible but often results in long hospital stays. Thus, there is great interest in finding potential markers of early toxicity and also in helping to elucidate the molecular mechanism. Investigators utilized MALDI IMS to determine differential protein expression within the rat kidney (cortex, medulla, and papilla), identified features of interest between dosed and control rat, and then applied downstream protein identification procedures. Transthyretin was significantly increased in the treated mouse kidney over control; these findings were validated with Western blot and immunohistochemistry.

To study the relationship between drug distribution in tumors and the resulting protein alterations, mass spectral images were obtained from MMTV/HER2 tumors excised from mice (35). Investigators were able to identify markers that indicate a response of the tumor to administration of erbB receptor inhibitors OSI-774 and Herceptin®. Inhibition of tumor cell proliferation and induction of apoptosis and tumor reduction were predicted by a >80% reduction in thymosin β4 and ubiquitin levels that were detectable after 16 h of a single drug dose before any evidence of in situ cellular activity.

The same procedures can also be applied to whole-body animal tissue sections for a system-wide analysis in a single experiment (22). For whole-body sagittal tissue sections, using the same sample preparation and analysis conditions described for IMS experiments of tissues, signals unique to individual organs were detected and used to produce a two-dimensional protein map of a control rat (**Figure 6**). By expanding the capabilities of MALDI IMS to investigate multiple tissue types simultaneously across a whole-body tissue section, distinct protein patterns can be identified and used to monitor whole-body system dynamics.

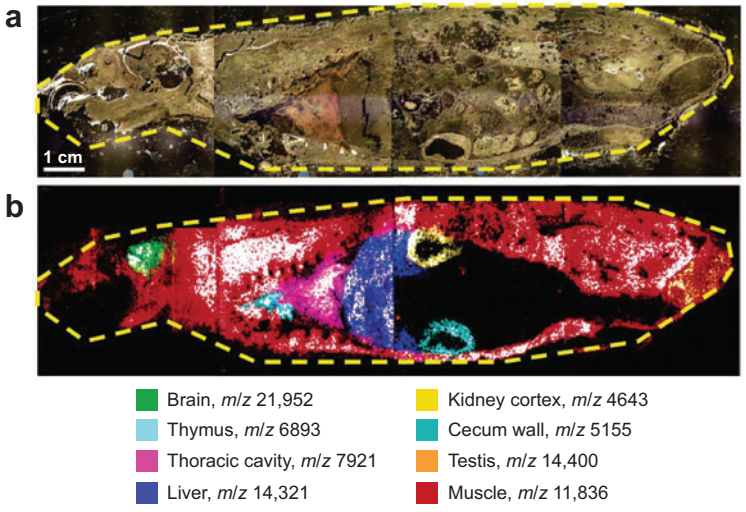

Figure 6

Whole-body protein analysis of rat sagittal tissue section by imaging mass spectrometry. (*a*) Optical image of rat sagittal tissue section across four gold matrix-assisted laser desorption/ionization target plates. (*b*) Ion image overlay of unique organ signals. Reprinted with permission from Reference 22.

8. IN SITU PROTEOMICS

Because the simple linear MALDI IMS analysis only provides a measurement of the molecular weight of a given protein, off-line techniques involving extraction, LC separation, digestion, and LC/MS/MS analyses with database searches are performed to unequivocally identify a protein. These approaches are effective, but are very time consuming and costly. Direct in situ protein identification would provide a significant advantage in such cases. We have developed a protocol that allows digestion of proteins and peptides directly on thin tissue sections using well-defined microspotted arrays of trypsin that can subsequently be spotted with matrix for IMS and MALDI MS/MS analyses (16). In one such study (**Figure 7**), we applied trypsin in 150-μm diameters in an array with spots located 200 μm apart on a section of mouse brain. For two specific proteins, PEP19 and neurogranin, four tryptic peptides from each were identified and sequenced by MALDI MS/MS directly from the tissue digest spots. The image of each peptide was then constructed (**Figure 7**).

The application of IMS to the analysis of formalin-fixed, paraffin-embedded (FFPE) tissue sections has also been undertaken. FFPE is the most convenient method to preserve samples in hospital tissue banks; this method is of great interest as millions of tissue samples are stored worldwide, many of which are linked to detailed patient histories and outcomes. However, analysis of the proteins in formalin-fixed tissues is a major challenge: The fixation process alters the structures of the proteins by cross-linking, thereby changing the molecular weight, often in unpredictable ways. Recent studies have focused on protocols to analyze such samples and thus far have

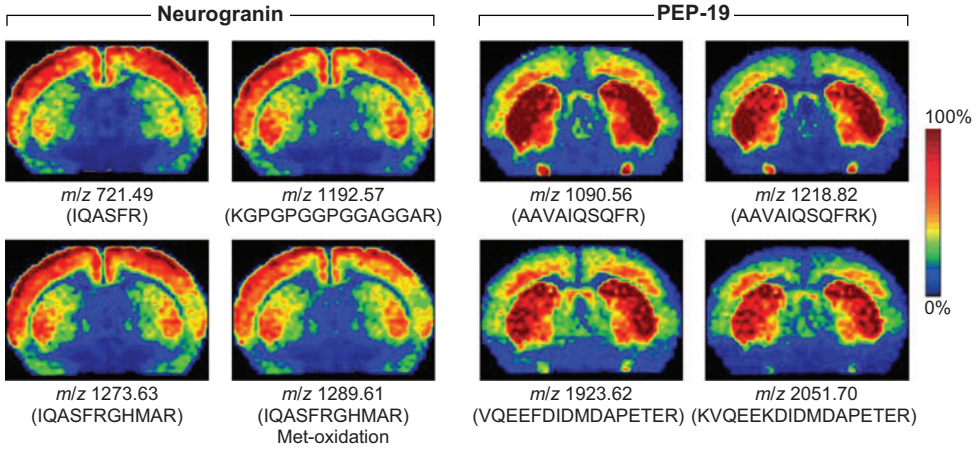

Figure 7

Examples of on-tissue tryptic digestion and subsequent matrix-assisted laser desorption/ionization imaging mass spectrometry analysis. *Left*: Tryptic peptides generated from the digestion of the 7.5-kDa protein, neurogranin. *Right*: Tryptic peptides generated from the digestion of the 6.7-kDa protein, PEP-19. Reprinted with permission from Reference 16.

shown promise. For example, IMS was used to analyze two-year-old archived FFPE rat brain tissues and the results showed the same localization of various proteins as that of the proteins within frozen tissue sections (51).

9. PERSPECTIVES AND CONCLUSIONS

The molecular complexity of biological systems obviates the need for molecularly specific tools to elucidate proteomic events in both spatial and temporal distributions. One of the most effective ways to present such information is in the form of images, as demonstrated through MRI, PET, and confocal microscopy. Similarly, images of the molecular composition of living systems will allow us to gain a more comprehensive view of biological processes and reveal complex molecular interactions. This approach will be vital in elucidating molecular aspects of disease and also drug effectiveness by providing spatial and relevant time-based information.

MALDI IMS provides unique information on peptides, proteins, lipids, metabolites, xenobiotics, and other endogenous compounds directly from complex tissue samples, bringing powerful new capabilities to biological research. Sample preparation is relatively easy and is amenable to robotics for high-throughput analysis. Most importantly, it is an excellent discovery tool, as the identity of the analytes do not need to be known prior to analysis. Many hundreds of signals can be recorded from a single experiment with high mass measurement accuracy. Because the spatial integrity of the tissue is maintained, molecular images and patterns can be correlated with histopathological analyses and other molecular imaging modalities.

Given the current rapid advances in both physics and chemistry, the prospects for MALDI IMS are bright. Such advances are sure to bring higher spatial resolution,

increased dynamic range of detection, and improved visualization of the molecular complexities within living systems. This emerging technology, and enabling variations of it, will continue to be an essential way to present complex data. Moreover, this technology appeals to the human intellect and to our ability to understand complexity in a visual format.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This work was supported by RMC grants NIH/NIGMS 5R01 GM58008-09 and DOD W81XWH-05-1-0179. K.B. is supported by NICHD training grant no. HD007043-31A2. The authors would like to thank Dr. Pierre Chaurand and Dr. Shannon Cornett for providing insights throughout this work.

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