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MSiMass List: A Public Database of Identifications for Protein MALDI MS Imaging

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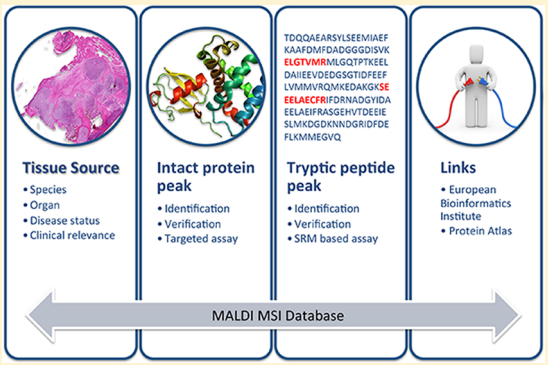
ABSTRACT: The clinical application of mass spectrometry imaging has developed into a sizable subdiscipline of proteomics and metabolomics because its seamless integration with pathology enables biomarkers and biomarker profiles to be determined that can aid patient and disease stratification (diagnosis, prognosis, and response to therapy). Confident identification of the discriminating peaks remains a challenge owing to the presence of nontryptic protein fragments, large mass-to-charge ratio ions that are not efficiently fragmented via tandem mass spectrometry or a high density of isobaric species. A public database of identifications has been initiated to aid the clinical development and implementation of mass spectrometry imaging. The MSiMass list database (www.maldi-msi.org/mass) enables users to assign identities to the peaks observed in their experiments and provides the methods by which the identifications were obtained. In contrast with existing protein databases, this list is designed as a community effort without a formal review panel. In this concept, authors can freely enter data and can comment on existing entries. In such, the database itself is an experiment on sharing knowledge, and its ability to rapidly provide quality data will be evaluated in the future.



KEYWORDS: *mass spectrometry imaging, MSI, protein identification, database*

INTRODUCTION

Mass spectrometry imaging (MSI) is a technique that uses spatially resolved proteomic and metabolomic MS methods to simultaneously reveal the distributions of hundreds of endogenous molecules directly from tissue samples.[1] Using essentially the same technology, peptides, proteins, pharmaceuticals, and metabolites can be analyzed, without the need for labeling and without prior knowledge of their presence or location within the tissue. One of the driving forces behind the increasing popularity of MSI is its ability to determine new molecular markers for patient diagnosis,[2−9] patient prognosis,[10,11] metastatic potential,[12] and response to molecular intervention.[13]

The clinical application of MSI has been largely based on protein imaging using MALDI MS for both intact proteins or via their tryptic peptides. Recent clinical examples of desorption electrospray[14] and MALDI imaging of lipids[15] indicate that this molecular class may also hold great potential. Nevertheless, the majority of current clinical MSI investigations have applied MALDI MSI for proteins, partially because of the ability to independently validate individual biomarkers using immunohistochemistry. Targeted assays based on selective reaction monitoring, a well-established technique for validating protein and metabolite biomarkers in tissue extracts and body fluids,[16] provide a more generally applicable method and have begun to be exploited for MALDI MSI.[17]

While several early examples of protein MALDI MSI included protein identities,[18−20] many others reported images of proteins but without their identifications. The identification of the protein is usually attempted through the analysis of tissue extracts but remains a challenge, and so many mass spectral peaks remain without annotation or experimental follow-up of any kind. An important hindrance to advancing this area has been the absence of a publicly accessible repository to store or retrieve MSI identifications. It is our opinion that such a database, however rudimentary, would be of great value for the field of MSI and allied technologies. In considering such a resource, it is instructive to first review the unique challenges facing the confident identification of the proteins detected in MSI experiments.

MALDI MSI of Intact Proteins

MALDI MS of larger intact proteins (above 5 kDa) generates singly charged ions that are too large for collision-activated dissociation to be effective. The development of laser spray ionization, capable of generating multiply charged protein ions

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Measured m/z	Species	Tissue	DOI	Protein Name	UniProt ID	Ion Type	Ident. Type
2790.00	mouse	pancreas	10.1371/journal.pone.0039424.t002	ALB1	P07724	intact	pcr, sequence, PMF
2812.00	mouse	pancreas	10.1371/journal.pone.0039424.t002	TMSB4X	Q0P5T0	intact	pcr, sequence, PMF
2829.00	mouse	pancreas	10.1371/journal.pone.0039424.t002	TMSB4X	Q0P5T0	intact	pcr, sequence, PMF
8404.00	human	lung	10.1021/pr901008d	CRIP1_Human	P50238	intact	mass
3371.00	human	lung	10.1158/1078-0432.CCR-09-1091	DEFA1	P59665	intact	pcr
3442.00	human	lung	10.1158/1078-0432.CCR-09-1091	DEFA2	P59665	intact	pcr
3485.00	human	lung	10.1158/1078-0432.CCR-09-1091	DEFA3	P59666	intact	pcr
4355.00	human	prostate	10.1158/1078-0432.CCR-08-2892	MEKK2	Q9Y2U5	intact	mass
4073.00	mouse	brain	10.1016/S0003-2697(02)00386-X	Beta-amyloid protein	Q7M088	intact	mass
4130.00	mouse	brain	10.1016/S0003-2697(02)00386-X	Beta-amyloid protein	Q7M088	intact	mass
4229.10	mouse	brain	10.1016/S0003-2697(02)00386-X	Beta-amyloid protein	Q7M088	intact	mass
4328.20	mouse	brain	10.1016/S0003-2697(02)00386-X	Beta-amyloid protein	Q7M088	intact	mass
4512.30	mouse	brain	10.1016/S0003-2697(02)00386-X	Beta-amyloid protein	Q7M088	intact	mass
3744.00	human	lymphnodes	10.1016/j.jprot.2011.04.013	DEFA 1	P59665	intact	mass
4748.00	human	lymphnodes	10.1016/j.jprot.2011.04.013	thymosin β-4	O14604	intact	mass
4737.00	human	lymphnodes	10.1016/j.jprot.2011.04.013	thymosin β-10	P63313	intact	mass
10091.00	human	lymphnodes	10.1016/j.jprot.2011.04.013	S100A6, Calcyclin	Q9HB71	intact	
10625.00	human	lymphnodes	10.1016/j.jprot.2011.04.013	S100B	P04271	intact	
11653.00	human	lymphnodes	10.1016/j.jprot.2011.04.013	S100A11, Calgizzarin	P31949	intact	
16791.00	human	lymphnodes	10.1016/j.jprot.2011.04.013	Calmodulin	P62158	intact	
3700.00	human	head and neck squamous cell	10.1038/sj.onc.1209770	Hemoglobin subunit beta	P68871	intact	mass
9973.00	human	head and neck squamous cell	10.1038/sj.onc.1209770	Acyl-CoA-binding	P07108	intact	mass
11043.00	human	squamous cell	10.1038/sj.onc.1209770	Cystatin A	P01040	intact	mass
11312.00	human	head and neck squamous cell	10.1038/sj.onc.1209770	Histone H4	P62805	intact	mass
15150.00	human	head and neck squamous cell	10.1038/sj.onc.1209770	Hemoglobin a	P69905	intact	mass
15885.00	human	head and neck squamous cell	10.1038/sj.onc.1209770	Hemoglobin b	P68871	intact	mass
27805.00	human	head and neck squamous cell	10.1038/sj.onc.1209770	Stratifin	P31947	intact	mass

Figure 1. Screenshot of the MSiMass list available on www.maldi-msi.org/information/MSiMassList.

from MALDI MSI preparations,[21] offers a potential solution by enabling electron-transfer dissociation-based tandem mass spectrometry (MS/MS)[22] but will require high resolving power ion-isolation capabilities to isolate specific protein ions from the highly complex mass spectra obtained from tissues. Liquid extraction surface analysis,[23] the use of immobilized proteases,[24,25] or taking protein extracts from microdissected samples, followed by LC−MS/MS characterization, are methods to identify proteins from localized regions of tissue.

An important factor to be considered is that post-mortem degradation means many clinical tissue samples contain endogenously generated protein fragments.[26] For example, a top-down LC−MS/MS analysis of (nontryptic) peptides extracted from human chondrosarcoma tissue reported 39 fragments of vimentin, an abundant protein indicative of mesenchymal tissue.[27] A recent LC−MS/MS analysis of the proteins contained in the extracted matrix following MALDI MSI sample preparation found 124 N- and C-terminal protein fragments.[28] While bottom-up LC−MS/MS approaches would still assign such fragments to the full proteins, on the basis of the detected tryptic peptides, *intact* MALDI MSI detects the intact masses. Accordingly, if the protein fragment has not been previously reported, a positive identification remains difficult. Several of the biomarkers reported by MALDI MSI have been such protein fragments.[3,5,27,29] Even though it has been demonstrated that post-mortem degradation can lead to the generation of protein fragments,[26] these previous results indicate that the protein fragments may be used as discriminative surrogates of biological processes.

It is currently normal practice to extract the proteins from adjacent tissue sections. Peptides, small proteins, and protein fragments are best identified by top-down LC−MS/MS to provide an unequivocal link between the identification and the intact mass of the ion. Larger proteins, or more complex extracts, may rely on enzymatic digestion, followed by LC−MS/MS, but are first fractionated using one of an array of technologies, including 1-D SDS-PAGE, size-exclusion chromatography, or other forms of liquid chromatography.

MALDI MSI of Tryptic Peptides

MALDI MSI of tryptic peptides lacks the peptide separation step of conventional bottom-up LC−MS/MS proteomics approaches, and consequently, the spectra display an abundance of peptide masses spanning the entire mass range from 800 to 4000 *m/z*, including many isobaric ions. The tandem mass spectra obtained via on-tissue MS/MS will include fragment ions from multiple precursors because of the large number of isobaric ions. It has been previously demonstrated that the presence of so-called chimera spectra adversely affects the ability to identify a protein.[30]

It is now common practice to compare the peaks detected in MALDI-MSI with the results obtained by LC−MALDI or LC−MS/MS analysis of adjacent tissue sections, assigning identities on the basis of mass accuracy.[31] The moderate overlap between ions detected by MALDI with those detected by ESI,[32] the ionization method of LC−MS/MS experiments, means any assignments based on LC−MS/MS generated peptide lists need to be verified. The same reasons that make the on-tissue identification of intact proteins and tryptic peptides challenging also complicate MS-based verification of the identities. Accordingly, the majority of studies perform MALDI MSI in a strict "lead discovery" mode, in which final validation is performed using orthogonal technologies, for example, immunohistological staining of tissue microarrays.

Depth of Coverage

The success of protein identification in MALDI MSI can be attributed to the shallower depth of coverage of a MALDI MSI experiment. A significant fraction of the total protein content in mammalian cells is constituted by a small number of abundant proteins. For example, it was recently reported that the "first 184 proteins [of 8000 proteins detected from cell lysates] already accounted for half the proteome mass, while the last 5600 [detected proteins] added up to less than 5%".[33] In MALDI MSI, because all proteins are subject to the same extraction, desorption, ionization, and mass analysis process, the highly abundant, soluble proteins are preferentially detected. A recent analysis of the proteins contained in the matrix after MALDI MSI sample preparation supports this conclusion.[28] LC−MS/MS analysis of an acetonitrile extract of the MALDI matrix, following MALDI MSI sample preparation of colon adenoma tissue, led to the identification of 809 proteins. LC−MS/MS of the corresponding tissue extract identified more than 2800 proteins. These results demonstrate that only a fraction of the proteome is effectively sampled during MALDI MSI sample preparation.

MALDI MSI Database of Identified Peptides and Proteins

The characteristics of the MALDI MSI experiment and the challenges associated with the unequivocal identification of the detected peaks warrant the generation of a publicly accessible database of protein identifications. Accordingly, we have initiated a database, the initial aim of which is to provide the information necessary to assign the protein peaks detected in clinical MALDI MSI research and to provide methods to confirm the identification. This first step, while limited in scope, provides essential information for the entire MSI community. We have begun to collect the masses of peptides and proteins observed in MSI experiments as well as their identities and to list the corresponding reference. This simple process has rapidly generated a valuable resource by which researchers can assign identities. This work builds on the recently reported work by Maier et al., who reported the bottom-up LC−MS/MS characterization of the proteins in MALDI MSI sample preparations.[28] However, in this report the vast majority of the proteins are larger than those normally detected in MALDI MSI, and the employed bottom-up identification strategies are insensitive to the protein fragments commonly detected in MALDI MSI. A further important difference concerns accessibility: instead of a closed journal manuscript and its associated supporting information, the MSiMass list has been created to be an open community identification resource that is MSI-centric in nature and that will be continually updated and revised by community participation.

Figure 1 shows a screenshot of the MSiMass list. The database contains a number of fields that can be used as search criteria or to order the results. These are:

Measured m/z − reports the measured mass as reported in the original research article. Note: Users should bear in mind the expected mass accuracy of the mass analyzer when comparing their MALDI MSI data sets with those previously reported.

Species − refers to the organism from which the reference MALDI MSI identity was obtained. The Uniprot ID, which is also a link to its entry in the Uniprot database, provides a rapid method by which the reader can ascertain to what degree the protein's primary structure is conserved in different species.

Tissue − refers to the tissue analyzed in the indicated organism. For human proteins the Uniprot ID can be used within the Human Protein Atlas to access immunohistochemical expression patterns for a range of tissues and diseases to assess if the MALDI MSI measurements are consistent with that protein's known expression.

DOI − provides a direct link to the original research article and thus the appropriate identification method as well as maintains the primacy of original peer-reviewed research.

Protein name − lists some commonly used names for the identified protein.

UniProt ID − provides a link to the protein information contained in the UniProt protein knowledge base.

Ion Type − the ion type refers to the MALDI MSI experimental method. 'Intact' means the ions were detected from the tissue without the addition of exogenous enzymes. Accordingly all neuropeptides and the nontryptic protein fragments commonly detected in clinical tissues (as a result of post-mortem degradation or other biological process) are included as 'intact' proteins. 'Digest' refers to ions that were detected following the addition of a protease to the tissue section. The vast majority of on-tissue digestion work has used trypsin as the protease. Accordingly, unless otherwise stated, the proteolytic peptides are tryptic peptides.

Identification Type − Several methods have been used to assign protein identities in MALDI MSI. Intense peptide ions have been identified by performing MALDI MS/MS directly from the tissue; these are termed 'AA sequence'. Owing to the difficulties associated with direct identification of peptides/proteins directly from tissue (see discussion at beginning of paper), most intact proteins and tryptic peptides have been assigned on the basis of the agreement between the measured mass and proteins/peptides identified by orthogonal LC−MS/MS analysis of tissue extracts. These are termed "mass"-based identifications. Alternatively, identification has been performed on tissue extracts using a peptide mass fingerprint protocol, as widely used in proteomics or confirmation by PCR. The purpose of this data field is to give the user an indication of the confidence of the assignment. Obviously, a solely mass-based identification should be treated as tentative assignment, whereas a sequence confirmation is stronger data to support the protein's identity.

Addition-to and Revision-of MSiMass List

The reporting of errors and the addition of new identifications to the mass list have been designed to be a simple process to foster contributions. Errors and additions can be reported on a dedicated forum of the www.MALDI-MSI.org website after creating an account and logging-in. The database is thus open to the entire MSI community without formal review but will

then be assessed and corrected by the MSI community via a simple feedback mechanism. Importantly, this will allow the database to grow rapidly and foster participation. The launch of this initiative follows from a Nordforsk funded initiative on signaling in tissues and a meeting of the European network COST Action BM1104 on MALDI MSI and represents the recognition that its further clinical development is best achieved as a community. By providing assignments for each mass and the methods by which they may be confirmed, it will enable MALDI MSI investigators to rapidly assign an observed signal and to directly access the validation method, thus enabling research efforts that focus on the biomedical potential of MSI.

■ FUTURE DEVELOPMENTS

This database itself is an experiment in knowledge sharing. It offers a platform for the MSI community to share and consume data, and future use will show if this is a concept that can be further expanded. The goal is create a database containing information about tissue origin, disease status, clinical impact, detected masses, protein identifications, and validation methods for the identified peaks (if available). On the basis of community feedback the database could be further expanded, for example, to enable the development of tissue- and disease-specific libraries of MS-assays. Links with other databases, for example, the human protein atlas,[34] will integrate MSI investigations with the wider and extensive bioanalytical infrastructures.

The MALDI MSI database will benefit the entire MSI community and could be subsequently expanded to include different molecular classes (lipids, metabolites) as well as different ionization methods (DESI, SIMS, NIMS).

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Notes

The authors declare no competing financial interest.

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