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Published in:
Journal of Proteome Research

DOI:
[10.1021/acs.jproteome.9b00009](https://doi.org/10.1021/acs.jproteome.9b00009)

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Krutilin, A., Maier, S., Schuster, R., Kruber, S., Kwiatkowski, M., Robertson, W. D., Hansen, N-O., Miller, R. J. D., & Schlueter, H. (2019). Sampling of Tissues with Laser Ablation for Proteomics: Comparison of Picosecond Infrared Laser and Microsecond Infrared Laser. *Journal of Proteome Research*, 18(3), 1451-1457. <https://doi.org/10.1021/acs.jproteome.9b00009>

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Sampling of Tissues with Laser Ablation for Proteomics: Comparison of Picosecond Infrared Laser and Microsecond Infrared Laser

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Supporting Information

ABSTRACT: It was recently shown that sampling of tissues with a picosecond infrared laser (PIRL) for analysis with bottom-up proteomics is advantageous compared to mechanical homogenization. Because the cold ablation of tissues with PIRL irradiation is soft, proteins remain intact and even enzymatic activities are detectable in PIRL homogenates. In contrast, it was observed that irradiation of tissues with a microsecond infrared laser (MIRL) heats the tissue, thereby causing significant damage. In this study, we investigated the question if sampling of tissues with a MIRL for analysis of their proteomes via bottom-up proteomics is possible and how the results are different from sampling of tissues with a PIRL. Comparison of the proteomes of the MIRL and PIRL tissue homogenates showed that the yield of proteins identified by bottom-up proteomics was larger in PIRL homogenates of liver tissue, whereas the yield was higher in MIRL homogenates of muscle tissue, which has a significantly higher content of connective tissue than liver tissue. In the PIRL homogenate of renal tissue, enzymatic activities were detectable, whereas in the corresponding MIRL homogenate, enzymatic activities were absent. In conclusion, MIRL and PIRL pulses are suited for sampling tissues for bottom-up proteomics. If it is important for bottom-up proteomic investigations to inactivate enzymatic activities already in the tissue before its ablation, MIRL tissue sampling is an option, because the proteins in the tissues are denatured and inactivated by the heating of the tissue during irradiation with MIRL irradiation prior to the ablation of the tissue. This heating effect is absent during irradiation of tissue with a PIRL; therefore, sampling of tissues with a PIRL is a choice for purifying enzymes, because their activities are maintained.

KEYWORDS: laser ablation, tissue sampling and homogenization, proteomics, mass spectrometry

INTRODUCTION

A critical first step in the analysis of proteomes of tissues is the extraction of proteins from their native environment. Depending on the nature of the tissue, and especially the content of connective tissue, different disruption methods are required. Mortar and pestle, Dounce and Potter-Elvehjem grinder, TissueLyser, bead mill, barocycler, cryogrinding, and sonification are frequently applied tools for mechanical homogenization of tissues.^{1–5} However, these techniques suffer from requiring relatively large tissue specimens to generate sufficient amounts of proteins.⁶

One emerging sampling technique is laser capture microdissection (LCM).⁷ Here, a tissue specimen is sliced in thin sections and placed into a microscope. A laser then is used to separate the area of interest from the residual tissue for subsequent homogenization and analysis. This procedure preserves spatial information on the selected cells and enables even single cell analysis, but its downsides are the time required for sampling and the problem that fresh frozen tissue is difficult to process. LCM is a two-dimensional approach, and

Received: January 6, 2019

Published: January 23, 2019

an additional homogenization step is required for solubilizing the biomolecules of interest. To overcome these drawbacks and to minimize time-consuming sampling and mechanical homogenization procedures, a tissue sampling method was developed utilizing a picosecond infrared laser (PIRL). By irradiation of the tissue with the PIRL beam, the tissue is converted into an aerosol, thereby solubilizing molecules of the tissue, as was recently demonstrated by Kwiatkowski et al.^{8,9} and Ren et al.¹⁰ This process reduces the sampling and homogenization of the tissue to a single step.

The PIRL emits light with a wavelength of around 2.94 μm , which corresponds to the peak absorption in the IR spectrum of water. The energy of light at this wavelength induces the intramolecular stretching vibrations in the oxygen–hydrogen bonds of the water molecules. The vibrational modes cause a translational motion, which drives the ablation. The very short picosecond pulse of PIRL results in an ultrafast transition of water molecules from liquid to gaseous phase, which is much faster than the thermal and acoustic energy transfer to the surrounding tissue.^{11–13} The applied optical energy moves out of the irradiated volume within the emerging ablation aerosol.¹² The adiabatic expansion of the aerosol plume into the gas phase has a significant cooling effect. Therefore, irradiation with PIRL converts tissue via cold vaporization into an aerosol. Thus, the time period during which the biomolecules are exposed to high temperatures is considerably shorter, explaining why in the aerosols produced by PIRL proteins remain intact and even enzymatic activities are detectable (Kwiatkowski et al.^{8,9}).

Microsecond infrared laser (MIRL) pulses consist of pulses that are 6 orders of magnitude longer than those of PIRL. Therefore, irradiation of tissues with MIRL causes a significant degree of thermal damage¹⁴ due to the unconfined energy deposition into the tissue.¹² Though severe thermal damage to the adjacent tissue utilizing the MIRL is observed,¹⁴ the ablation process produces an aerosol. For this study, it was hypothesized that in tissue aerosols produced by MIRL also proteins are present but enzymatic activities are not detectable because of the heating of the tissue during irradiation with MIRL causes denaturation of proteins and thereby inactivation of enzymes in the tissue. Thus, in this study, we compared sampling of tissues with MIRL and PIRL as sample preparation step subsequent to bottom-up proteomics, and we investigated if enzymatic activities are detectable in the aerosol produced by irradiation of renal tissue with MIRL pulses.

MATERIALS AND METHODS

Chemicals, Solvents, and Reagents

All chemicals were purchased from Sigma-Aldrich (Steinheim, Germany) or Fisher Scientific (Bremen, Germany) and used without further purification. Sequence-grade trypsin was purchased from Promega (Mannheim, Germany). Coomassie Brilliant Blue G 250 was purchased from Carl Roth (Karlsruhe, Germany). Unstained Precision protein standards broad range was purchased from Bio-Rad (Munich, Germany).

Sample Preparation

Rat tissue samples from Wister rats were received from the research animal facilities of the Medical Center Hamburg-Eppendorf (UKE). The experiments were performed following the principles of the 3Rs, namely reduction; only tissue of rats that had been sacrificed for other projects was used. Fresh muscle, liver, and kidney tissues were cut in four pieces,

immediately frozen in liquid nitrogen, and stored prior to ablation at $-80\text{ }^{\circ}\text{C}$.

Tissue Homogenization

Figure S1 of the Supporting Information shows an illustration of the experimental setup. For the laser system, a PIRL-HP3-1064 OPA-3000 (Attodyne Inc., Toronto, Canada) and a commercial MIRL (Er:YAG laser, MEY-1-A EX-2, J Morita Mfg Corp., Kyoto, Japan) was used for the experiments. Both lasers emitted a Gaussian shape beam with a wavelength of $3000 \pm 90\text{ nm}$ for PIRL and $2.94\text{ }\mu\text{m}$ for MIRL. The durations of the PIRL and MIRL pulses were 400 ps and 300 μs , respectively. With respect to the different characteristics of the laser systems employed, the laser parameters were carefully considered to result in a comparable ablation speed (tissue volume per time) and focus spot size with the least possible thermal collateral damage. Additional laser parameters are described in the Supporting Information (Table S1). These lasers were connected to a home-built scanning system with an autofocus function. The ablation area was adjusted to $4 \times 4\text{ mm}^2$, and the ablation time for each specimen was 260 s. The ablated volume depends not only on the applied fluence but also on the tissue type. It can be estimated that the ablated volume in the case of PIRL irradiation is around 13 mm^3 in liver tissue. For MIRL irradiation, the ablated volume in the tissue can be evaluated to range from around 10 to 20 mm^3 depending on the fluence.¹⁵ A home-built sample stage was used and adjusted to $-10\text{ }^{\circ}\text{C}$. During the ablation process, the generated plume was transferred to a cryo-trap via low pressure created by the membrane pump (MZ 2C VARIO, VACUUBRAND GmbH & CO KG, Wertheim, Germany).

Condensates of the aerosols of renal tissue induced by irradiation with PIRL or MIRL pulses were used without further preparation, screening for enzymatic activities via MALDI analysis (see paragraph below). Muscle and liver homogenates were prepared as follows: After thawing, the samples were quickly aliquoted into two parts. One part was mixed 1:1 (v:v) with 6 M urea and centrifuged at $15\text{ }000g$ for 3 min. The supernatants were transferred into a 2 mL reaction vial and stored at $4\text{ }^{\circ}\text{C}$ until the proteomic experiment (for more details, see section 4 in the Supporting Information). The second part was mixed with 100 μL of 5 $\times$ Laemmli buffer (225 mM Tris-HCl, pH 6.8, 50% glycerol, 5% sodium dodecyl sulfate; 0.05% bromophenol blue; 250 mM dithiothreitol) and immediately transferred into a boiling water bath for 5 min. The homogenates were centrifuged at $15\text{ }000g$ for 3 min. The supernatants were transferred into a 2 mL reaction vial and stored at $-20\text{ }^{\circ}\text{C}$. The protein amounts obtained were measured with a 2-D Quant Kit following the manufacture's protocol.

SDS-PAGE

For SDS-PAGE, 30 μg of homogenate was loaded on a 10% Bis-Tris gel (Criterion XT Bis-Tris gel, Bio-Rad, Munich, Germany). Electrophoresis was performed at a constant voltage of 120 V for 60 min. The gel was stained (40% MeOH, 10% acetic acid, 0.025% Coomassie Brilliant Blue G 250, dissolved in ultrapure water) overnight and destained with 40% MeOH.

Mass Spectrometry

Tryptic in-solution digestion of tissue homogenates was performed in accordance with Wiśniewski et al.,¹⁶ and the experiment was carried out in centrifuge filter with a 10 kDa

cutoff. LC–MS/MS analysis was performed using reversed-phase ultra high-performance liquid chromatography (Dionex UltiMate 3000 RSLCnano, Thermo Scientific, Bremen, Germany) coupled via electrospray ionization (ESI) to a quadrupole orbitrap mass spectrometer (Orbitrap Fusion, Thermo Scientific, Bremen, Germany). For the LC–MS/MS analysis, 5 μ L of the original homogenates from MIRL and PIRL irradiation were used for the acquisition of LC–MS/MS data (Xcalibur Software Version 3.1). The samples were loaded on a trapping column (nanoACQUITY UPLC Symmetry C18 trap column, 180 μ m $\times$ 20 mm, 5 μ m, 100 Å; buffer A, 0.1% formic acid in ultrapure water; buffer B, 0.1% formic acid in acetonitrile, Waters, Manchester, U.K.) at a flow rate of 5 μ L $\cdot$ min^{−1} with a 2% buffer B for 5 min. In the next step, the analytes were transported into the separation column (nanoACQUITY UPLC column, BEH 130 C18, 75 μ m $\times$ 250 mm, 1.7 μ m, 100 Å, Waters, Manchester, U.K.) and passed through with a flow rate of 200 nL $\cdot$ min^{−1}. A 35 min separation gradient from 3 to 28% B, followed by a 5 min separation gradient from 28 to 35% B was used to elute the peptides. For spray generation, a fused-silica emitter (i.d. 10 μ m, New Objective, Woburn, U.S.) at a capillary voltage of 1650 V was used. For data acquisition, the mass spectrometer was performed in positive ion mode.

Data Processing

Proteins were identified from the LC–MS/MS raw data with the MaxQuant Software (Version 1.5.2.8, Max Planck Society, Munich, Germany) using the Andromeda search engine against the *Rattus norvegicus* Uniprot-Swiss-Prot database (www.uniprot.org, downloaded January 29, 2016, 7934 sequence entries). The search parameters were defined to allow a precursor tolerance of 10 ppm and MS/MS tolerance of 0.5 Da, carbamido-methylation for cysteine residues as a fixed modification, and two missed cleavages were allowed. Only proteins identified with at least two unique peptides were taken into account.

For the analysis of the proteins in the SDS-PAGE in the range of 3–6 kDa, we used the same MaxQuant version as described above with the same parameters. In addition, we used the Perseus software (version 1.5.1.6, Max Planck Society, Munich, Germany) to identify the number of proteins in each gel band. Proteins with at least two unique peptides were taken into account.

Data Availability

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE^{17–19} partner repository with the data set identifier PXD012208, PXD012440, and PXD012209.

Screening Enzymatic Activities by MALDI Analysis

The MALDI analysis was used to determine the enzyme activities by measuring the products of proteolysis of angiotensin I (Ang 1–10). Prior to MALDI analysis, the condensates of the aerosols of renal tissue were incubated with angiotensin I ($c_{\text{Ang1–10}} = 10^{-5}$ M; 1:9, v:v; homogenate: Ang 1–10) for 0, 3, 6, and 24 h. For each time point, the mixture was mixed with DHB (2,5-dihydroxybenzoic acid 20 mg $\cdot$ mL^{−1} in 50% ACN, 0.1% TFA) in a volume ratio of 1:2 (v:v), respectively. The samples were stored at 4 °C for further measurement. The MALDI spectra were recorded via a time-of-flight (TOF) mass spectrometer (Bruker UltrafleXtreme Smartbeam II Laser, Bremen, Germany), and each spectrum

was generated through 2000 shots in partial sample mode. The instrument was operated in positive reflector ion mode with a repetition rate of 20 Hz, 40% laser intensity, 20 kV first acceleration voltage, 18.5 kV second acceleration voltage, and 250 ns extraction delay. The recorded m/z range was from 500 to 5040.

RESULTS AND DISCUSSION

Total Protein Amounts in the Condensates of the Tissue Aerosols

Rat liver ($n = 9$) and muscle tissue ($n = 9$) was ablated with each laser system, and the total amount of protein contained within the collected condensate was measured. For rat liver tissue, 470 ± 188 μ g and 216 ± 131 μ g of protein was measured for ablation by PIRL or MIRL irradiation, respectively, in the condensates (Table S2). The condensates of rat muscle tissue obtained with PIRL and MIRL yielded 342 ± 142 and 479 ± 208 μ g of proteins, respectively.

Surface of Ablated Area

Figure 1 shows the ablated areas of kidney tissue after the exposure time of 260 s with PIRL and MIRL. The ablated

Figure 1. Ablated kidney tissues after 260 s of irradiation with PIRL (A) and MIRL (B). The 4 $\times$ 4 mm² areas were ablated in both cases. Burnt residue is observed after MIRL irradiation. The adjacent tissue after PIRL irradiation indicates no significant alteration to the naked eye and appears red and moist.

volume can be seen as a rectangular pattern. The remaining tissue after ablation with PIRL exhibited no visible changes and has a planar surface. In contrast, the MIRL ablated tissue shows a burnt residue around the irradiation area. The ablation surface is formed by laser pulse impact. This observation and those made in Figure 1 cover the theoretical concepts of material ablation for subnanosecond pulses like PIRL pulses. The energy transferred to water molecules in the excited volume by a picosecond pulse causes a rapid phase transition from the liquid phase into the gas phase. Because of the speed of this transition, the energy cannot dissipate into the adjacent tissue; therefore, nearly no residual heat accumulates and thus no burnt residue is observed after picosecond IR irradiation.¹⁰ In contrast, during a microsecond pulse, in the excited volume the critical temperature for a phase transition takes much more time²⁰ so that a significant part of the supplied energy dissipates into adjacent tissue, cooking the tissue in the excited volume as well as in adjacent cells, which are not ablated by MIRL irradiation.

Because an increased temperature can increase the number of oxidized methionines within a proteome,²¹ a comparison was performed regarding the amount of oxidized methionine. There was no significant difference of methionine oxidation

between the samples obtained by tissue sampling with PIRL and MIRL irradiation. Obviously, the oxidation of methionine during sampling of tissues with both lasers does not play a role. This phenomenon can be explained by the fast ablation process. The time course from the release of proteins from tissues upon irradiation with the lasers until the condensation of the tissue aerosols is estimated to be less than 1 s. In addition within both sampling methods the small water droplets containing the proteins in the aerosol are cooled by the adiabatic expansion of the plume.

The postablation results of the surface for muscle and liver tissue are shown in the [Supporting Information, Figures S2 and S3](#).

In this study we did not perform a histological analysis of the residues of the tissues irradiated with PIRL and MIRL pulses, because histological investigations comparing the action of PIRL and MIRL pulses on tissues were already performed in detail in *ex vivo* studies by Boettcher et al.¹⁴ using human vocal folds, Hess et al.²² investigating human vocal folds, Jowett et al.²³ ablating chicken bones, and Boettcher et al.²⁴ irradiating human laryngeal tissue.

SDS-PAGE for Muscle and Liver Tissue

In [Figure 2](#), two technical replicates are presented for liver (A) and muscle (B) homogenate using PIRL pulses with $1 \text{ J}\cdot\text{cm}^{-2}$

Figure 2. SDS-PAGE of the condensates of the tissue aerosols produced by irradiation of liver (A) and muscle (B) with PIRL and MIRL. The PIRL samples (L1, L2, M1, and M2) were obtained with an average fluence of $1 \text{ J}\cdot\text{cm}^{-2}$. Ablation with MIRL was performed with $40 \text{ J}\cdot\text{cm}^{-2}$ (samples L3 and M3) and $60 \text{ J}\cdot\text{cm}^{-2}$ (samples L4 and M4). PS: protein standard.

and MIRL pulses with 40 and $60 \text{ J}\cdot\text{cm}^{-2}$, respectively. A general comparison of the lanes from PIRL (L1 and L2) and MIRL (L3 and L4) in the condensates of the aerosols of liver (A) showed a similar pattern; however, a different distribution of the ratio of intensities of the protein bands was observed.

The protein patterns in the condensates of the aerosols of rat muscle tissue (B) obtained by PIRL (M1 and M2) and MIRL (M3 and M4) are similar as well. Nearly all protein bands are present in both cases; however, again there are significant differences in the ratio of the intensities of the bands, which appear to be protein weight-dependent. In the region of 3–6 kDa of all SDS-PAGE gels of condensates of the tissue aerosols obtained with MIRL pulses, a gradient band is shown.

This band is not present in the SDS-PAGE gels of the condensates of the tissue aerosols obtained with PIRL pulses.

Because the increased intensity of the 3–6 kDa band in the samples obtained with MIRL irradiation might have been formed by staining of fragments from proteins being much larger than 3–6 kDa, these bands were cut and subjected to tryptic in gel digestion in accordance with Shevchenko et al.²⁵ ([Figure S4 and Table S2](#)), followed by LC–MS/MS analysis. The results showed a similar number of proteins for both lasers in this region. Identified proteins with molecular masses higher than the protein standard suggests were considered as fragmented. We found a comparable number of proteins which exceed the molecular mass of the protein standard for both MIRL and PIRL homogenate. Based on this result it can be excluded that the increased staining for MIRL homogenate of the 3–6 kDa area was due to the binding of Coomassie to a higher number of small proteins or protein fragments. Furthermore, we concluded that the extraction methods here play a minor role in the fragmentation of proteins observed in the SDS-PAGE bands. Protein degradation, which was observed here, may occur *in vivo* by proteolytic processes.²⁶ Additional impact on the protein degradation is the removal of a tissue from a living organism. After excursion (*ex vivo*), proteases can rapidly change the proteome composition.²⁷

Statistical Analysis of the LC–MS/MS Data of the Condensates of the PIRL- and MIRL-Induced Aerosols of Muscle and Liver Tissue

The efficiency of the extraction of the proteins from tissue induced by laser ablation, for both the PIRL and MIRL, was investigated by subjecting the condensates of the aerosols of muscle and liver tissue to statistical analysis of the LC–MS/MS data. [Figure 3](#) summarizes the results.

Figure 3. Venn diagram of identified proteins from rat liver (A) and rat muscle (B) sampled with MIRL and PIRL in a LC–MS/MS analysis. Proteins were counted if 2 or more of their unique peptides have been identified and are detected in two technical replicates of rat liver and muscle, respectively.

A higher number of proteins were identified with MIRL ablation on the rat muscle tissue. Because of the higher fluence in the case of MIRL irradiation, the ablation depth per pulse is considerably higher, on the order of several tens of micrometers, compared to PIRL irradiation.²⁸ Therefore, a precise layer-by-layer ablation with an ablation depth per laser pulse of a few micrometers as in the case of PIRL was not achieved in this experiment. The difference in pulse energy requirements is due to the differences in efficiencies for ablation in the two different pulse regimes. More energy is lost by acoustic transport and thermal diffusion for MIRL relative

Figure 4. MALDI-TOF mass spectra of angiotensin 1–10 ($c = 10^{-5}$ M) incubated with the condensates of the aerosols obtained from renal tissue by (A) PIRL and (B) MIRL. The reaction mixtures were analyzed after 0, 3, 6, and 24 h of incubation time. Signals of the generated angiotensin peptides and angiotensin 1–10 are marked by arrows. Matrix: 2,5-dihydroxybenzoic acid (2,5-DHB).

to PIRL irradiation, as evident in Figure 1 by the burning for similar ablation rates with respect to material removal.

The total number of proteins identified from liver tissue by bottom-up proteomics following MIRL extraction was 581 proteins and with PIRL, 926. From that, 326 were identified in both MIRL and PIRL samples. In contrast, the total number of identified proteins in muscle tissue with MIRL irradiation was 883 proteins, and with PIRL, 642. From that, 406 were identified in both MIRL and PIRL samples. The comparatively low number of identified proteins can be explained by using a 35 min gradient to separate the peptides and by using at least two unique peptides per protein for protein identification.

There is a correlation between the tissue with a high (muscle) and with a low content of connective tissue (liver) and the yield of proteins, both the total amount and the number of individual proteins. It can be assumed that the significant higher fluence of MIRL pulses in the range of 40–60 J cm⁻² in comparison with PIRL pulses at 1 J cm⁻² is responsible for a more effective extraction of the muscle tissue, which is much more robust because of its larger content of connective tissue compared to liver tissue and the actin and myosin filaments within the myocytes. Further studies utilizing higher-fluence PIRL should be performed to investigate the effect. The present system is limited in pulse energy for the needed focusing conditions to compare the two pulse regime. In comparison to muscle tissue, liver tissue is lower in collagen content.²⁹ As a result, the fluence used here with the PIRL is high enough for an efficient ablation and homogenization of the liver tissue. In consequence, a significantly higher yield of the total protein amount and of the number of individual

proteins was achieved by the ablation of liver tissue with PIRL in comparison to MIRL pulses.

Enzyme Activity

The enzymatic activity retained in the condensates of tissue aerosols obtained with MIRL and PIRL was investigated using angiotensin I (amino acid sequence: DRVYIHPFHL), because in renal tissue³⁰ and in blood,³¹ which is still present in the tissue, several proteases are present, which can convert angiotensin I into smaller peptides. MALDI-TOF spectra of the reaction products of the incubation of angiotensin I with condensates (see protein concentration measurement Figure S4) of the aerosols of renal tissue after defined incubation times are shown in Figure 4.

After an incubation time of 3 h, an intense signal of m/z 1181.7 was present, which corresponds to angiotensin 1–9 (DRVYIHPFH) and likely to have been produced by a carboxy-peptidase like the angiotensin-converting-enzyme-2. In addition, signals with smaller intensities are visible with m/z = 1046.5 and m/z = 899.5, corresponding to angiotensin II (angiotensin 1–8, DRVYIHPF) and angiotensin 1–7 (m/z = 899.5, DRVYIHP). These signals indicate enzyme activities of several peptidases like the angiotensin-converting enzymes (ACE1 and ACE2), chymase, and/or neprilysin. The increase of the signal intensities of the enzymatic degradation products of angiotensin I represents the increasing enzymatic conversion.

In contrast, the MALDI spectra of the incubation of angiotensin I with the condensate of the aerosol of renal tissue, obtained with MIRL pulses, showed no signals even after an incubation time of 24 h, indicating that no significant enzymatic activities were present.

It can be assumed that this result is related to the heating of the adjacent tissue, which occurs during irradiation of tissue with MIRL pulses as well as degradation of the species during ablation. The heating effect of MIRL irradiation is already very obvious on the surface of the ablation zone (Figure 1). The “whitening” of this area can be termed “cooked” tissue, causing protein denaturation which occurs for most proteins above 45 °C and thereby inactivating enzymes. By the heating of tissue, enzymes like proteases can be inactivated, as demonstrated, for example, by Stingl et al.³² In the case of the sampling of renal tissue with PIRL pulses during ablation, the tissue is not heated; therefore, the peptidases in the tissue are not inactivated by heat denaturation and thus are able to degrade the model substrate peptide, angiotensin I, as shown in Figure 4A. Furthermore, these results also demonstrate that the temperature in the plume of the PIRL tissue aerosol are below the temperature responsible for heat inactivation of enzymes.

CONCLUSION

In this study, we utilized a commercial MIRL, which is typically used in surgery, for sampling of liver tissue and muscle tissue for bottom-up proteomics and compared the results with respect to the total protein amount, number of identified individual proteins, and enzymatic activities with those obtained with a PIRL. MIRL in comparison to PIRL sampling yielded more identifiable proteins from tissue with a high content of connective tissue. Vice versa, PIRL in comparison to MIRL sampling yielded more identified proteins in tissue with a low content of connective tissue. In contrast to MIRL, PIRL sampling did not induce residual tissue heating or damage and maintained enzymatic activities. Thus, PIRL irradiation is the first choice for tissue sampling, if minimal disruption of proteins preserving enzymatic activities is the goal and if an intact tissue remaining after ablation is needed. The application of MIRL pulses is suited for tissue types with a larger amount of connective tissue and/or if enzymatic activities must be suppressed within the intact tissue already.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jproteome.9b00009.

Additional figures, tables, and discussion as described in the text (PDF)

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Notes

The authors declare the following competing financial interest(s): R.J.D.M. is the cofounder of AttoDyne Inc. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE^{17–19} partner repository with the data set identifier PXD012208, PXD012440, and PXD012209.

ACKNOWLEDGMENTS

We acknowledge funding through the Max Planck Society in Cooperation Frauenhofer-Gesellschaft MPG-FhG “DIVE SPOT” for financial support; the research animal facilities of the Medical Center Hamburg-Eppendorf (UKE) for sample support; and Djordje Gitaric and Khashayar Shojaei-Asanjan for the design of the ablation box.

ABBREVIATIONS

LCM, laser capture microdissection; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; LC-MS/MS, liquid chromatography tandem-mass spectrometry; MIRL, microsecond infrared laser; PIRL, picosecond infrared laser; TFA, trifluoroacetic acid; ACN, acetonitrile; ESI, electrospray ionization

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