LESA FAIMS Mass Spectrometry for the Spatial Profiling of Proteins from Tissue

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ABSTRACT: We have shown previously that coupling of high field asymmetric waveform ion mobility spectrometry (FAIMS), also known as differential ion mobility, with liquid extraction surface analysis (LESA) mass spectrometry of tissue results in significant improvements in the resulting protein mass spectra. Here, we demonstrate LESA FAIMS mass spectrometry imaging of proteins in sections of mouse brain and liver tissue. The results are compared with LESA mass spectrometry images obtained in the absence of FAIMS. The results show that the number of different protein species detected can be significantly increased by incorporating FAIMS into the workflow. A total of 34 proteins were detected by LESA FAIMS mass spectrometry imaging of mouse brain, of which 26 were unique to FAIMS, compared with 15 proteins (7 unique) detected by LESA mass spectrometry imaging. A number of proteins were identified including α-globin, 6.8 kDa mitochondrial proteolipid, macrophage migration inhibitory factor, ubiquitin, β-thymosin 4, and calmodulin. A total of 40 species were detected by LESA FAIMS mass spectrometry imaging of mouse liver, of which 29 were unique to FAIMS, compared with 24 proteins (13 unique) detected by LESA mass spectrometry imaging. The spatial distributions of proteins identified in both LESA mass spectrometry imaging and LESA FAIMS mass spectrometry imaging were in good agreement indicating that FAIMS is a suitable tool for inclusion in mass spectrometry imaging workflows.

Spatial profiling of solid substrates via mass spectrometry imaging (MSI) can provide insight into biological systems. MSI techniques such as secondary ionization mass spectrometry (SIMS) can provide spatial resolution on the order of nm; however, only relatively low molecular weight species (below ~1000 Da) can be detected owing to the destructive nature of the technique. Matrix-assisted laser desorption/ionization (MALDI) provides softer ionization and the ability to ionize larger species such as proteins. Previous MALDI MSI reports have described the detection of numerous protein species from various tissues samples, such as liver and brain in mouse and rat models. The spatial distribution of protein species in bacterial colonies and plant matter have also been probed by MALDI MSI. The detection of high molecular mass protein species has also been reported: Franck et al. reported the detection of protein species up to 50 kDa from rat brain tissue sections and up to 70 kDa from ovarian cancer biopsy tissue sections. Nevertheless, MALDI typically leads to the formation of singly charged ions; hence the analysis of such large species is limited to time-of-flight mass analysers with the associated compromise in resolving power. Other limitations of MALDI MSI include lengthy sample preparation and matrix interference.

Electrospray-based MSI methods have been shown to provide complementary data to MALDI MSI. Desorption electrospray ionization (DESI), which involves the desorption of projectile droplets which have been electrosprayed onto a sample surface, was introduced by Cooks et al. in 2004. Eberlin et al. reported analysis of the same tissue section, first by desorption electrosprays ionization (DESI) imaging for lipid analysis followed by MALDI imaging for protein analysis, enabling correlation of lipid distributions with those of protein species. Although DESI has been shown to be suitable for the analysis of protein species up to ~17 kDa from (solid) solutions on a surface, the detection of such protein species directly from thin tissue sections has not been reported. A variant of DESI which involves liquid based samples (known as liquid DESI) has enabled the detection of protein species up to ~150 kDa.

Liquid extraction surface analysis (LESA), which enables the extraction of analytes from solid substrates via the formation of a liquid microjunction between a pipet tip and the sample surface, has been shown to be suitable for the analysis of intact proteins from a variety of sample substrates. Previously, we have shown that intact protein species can be extracted from substrates including dried blood spots on filter paper, thin tissue sections thaw-mounted onto glass and Escherichia coli colonies growing on agar. Moreover, intact protein complexes can be sampled by LESA. LESA MSI has been used as a high sensitivity profiling technique for the determination of...
drug distributions, as a precursor tool to high spatial resolution MALDI MSI experiments of smaller tissue areas, and in the analysis of various drug dosed animal studies. \(^{25,24}\) Randall et al. applied LESA MSI to the simultaneous analysis of lipids, proteins and a drug compound in mouse brain tissue sections. \(^{27}\) In addition, Wiszorksi et al. have recently described the use of LESA as an extraction protocol to provide spatially resolved proteomics data. \(^{28}\) LESA MSI suffers from low spatial resolution, typically 500 μm to 1 mm, but has the advantages of sensitivity, ability to be coupled with high resolution mass analysers and potential for native MSI. It can therefore be considered to be complementary to high spatial resolution imaging techniques. Recent work by Hsu et al. showed imaging of proteins up to 15 kDa in brain tissue by nanospray desorption electrospray ionization (nanoDESI) at approximately 200 μm lateral resolution. \(^{30}\) NanoDESI is somewhat of a misnomer as it is a variant of liquid microjunction surface sampling in which a continuous solvent stream replaces the discrete liquid microjunction, similar to the technology developed by Van Berkel et al. \(^{30}\) and now commercialized as the flowprobe system by Prosolia.

Previously, we have shown that coupling of LESA to a high-field asymmetric ion mobility spectrometry (FAIMS, also known as differential ion mobility spectrometry) device, \(^{14}\) after extraction/ionization and before mass spectrometric analysis, provides significant improvements in the analysis of complex biological samples. \(^{32,33}\) The inclusion of FAIMS results in improved signal-to-noise ratios, shorter acquisition times, and separation of molecular classes, that is, proteins and lipids. FAIMS enables gas phase separation of ions at atmospheric pressure based on differences in their mobility in high and low electric fields. Ions are passed between two electrodes by a carrier gas (air in this case) and an asymmetric waveform is applied to the electrodes such that the ions are exposed to alternating high and low electric fields perpendicular to their trajectory. Ions diverge from the original trajectory due to their differential mobility, and would discharge on the electrode if a dc compensation field (CF) were not superimposed. It is possible to selectively transmit different ions, or subsets of ions, by careful selection of CF. The benefits afforded by FAIMS suggest that the technique could find applications in LESA MSI protocols.

The inclusion of ion mobility spectrometry has previously been shown to offer advantages for MSI. McLean et al. demonstrated that inclusion of drift tube ion mobility spectrometry in MALDI MSI on a time-of-flight mass spectrometer \(^{34}\) enabled imaging of isobaric lipid and peptide species from mouse liver tissue sections. Bennett et al. coupled FAIMS to DESI for mass spectrometry imaging of lipids in sections of rat brain and 4-hydroxybenzoic acid in sea algae. \(^{35}\) Inclusion of the ion mobility device in the MSI workflow was shown to improve signal-to-noise ratios of lipid analytes in rat brain, providing greater insight as to the true spatial distributions of these molecular species in the tissue sections and improving image contrast.

Here, we demonstrate LESA FAIMS mass spectrometry imaging of proteins in tissue sections of mouse brain and liver and compare the results with LESA MSI in the absence of FAIMS. FAIMS was operated in static mode, i.e., the dispersion field and compensation field were kept constant throughout the analysis. The DF and CF values applied are optimum for transmission of proteins with molecular weight >30 kDa. \(^{32}\) The results show an increase in number of protein species detected:

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**METHODS**

**Materials.** Thin Tissue Sections. Brain and liver from wild-type mice (extraneous tissue from culled animals) were the gift of Prof Steve Watson (University of Birmingham). Organs were frozen on dry ice prior to storage at −80 °C. Sections of murine liver tissue and brain tissue of area ~1.5 cm² were obtained at a thickness of 10 μm using a CM1810 Cryostat (Leica Microsystems, Wetzlar, Germany) and thaw mounted onto glass slides.

**Solvents.** The following solvents were used: acetonitrile (J. T. Baker, The Netherlands), ethanol (Fisher Scientific, Loughborough, UK), HPLC grade water (J. T. Baker, The Netherlands), and formic acid (Sigma-Aldrich Company Ltd., Dorset, U.K.).

**LESA.** Thin tissue section samples of mouse brain were washed in 80% ethanol for 10 s (to remove abundant lipid species from the sample) and then left to air-dry. Thin tissue sections of mouse liver were not washed. Samples were loaded onto a universal LESA adapter plate. The plate was placed into the TriVersa NanoMate chip-based electrospray device (Advion, Ithaca, NY) coupled to the Thermo Fisher Scientific Orbitrap Elite (Thermo Fisher Scientific, Bremen, Germany). The solvent system used for LESA extraction/electrospray was 40:60 acetonitrile:water with the addition of 1% formic acid.

**LESA and LESA FAIMS MSI and Optimization Experiments.** 1.5 μL samples were aspirated from the solvent well. The robotic arm relocated to a position above the tissue and descended to a height 0.8 mm above the surface of the sample. 1.0 μL of the solution was dispensed onto the sample surface to form a liquid microjunction. The liquid microjunction was maintained for 10 s; then 1.1 μL were reaspirated into the pipet tip. The sample solvent was mixed once for mouse liver experiments. All MSI experiments were acquired at 2 mm × 2 mm spacing. The LESA Points software in which sampling locations are selected is limited to 1 mm spacing increments; 2 mm spacing was chosen to prevent overlapping of sampled areas from location to location.

**LESA and LESA FAIMS MS/MS Experiments.** Four micro-liters were aspirated from the solvent well before the robotic arm relocated to a position 0.8 mm above the surface of the tissue sample. 1.0 μL of the solution was dispensed onto the sample surface, forming a liquid microjunction that was maintained for 10 s, before 1.5 μL was reaspirated. The sample was mixed twice (redispensed and held for a further 10 s). Samples were introduced into the mass spectrometer via the TriVersa NanoMate, with gas pressure 0.3 psi, a tip voltage of 1.70 kV, and a capillary temperature of 250 °C for experiments conducted without the FAIMS device coupled to the mass spectrometer and 350 °C when the FAIMS device was coupled.

**FAIMS.** The TriVersa NanoMate was coupled to a miniaturized ultra-FAIMS device (Owlstone, Cambridge, UK) which was coupled to an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, Bremen Germany). FAIMS separation was carried out in positive ion mode using an NC chip (Owlstone) with trench length 78.1 mm, gap width 101.3 μm and total thickness 700 μm. The FAIMS device was operated either in static or 1D mode. In static mode, the dispersion field (DF) and compensation field (CF) were 270...
and 2.6 Td, respectively (brain), and 270 and 2.68 Td, respectively (liver). In 1D mode, the dispersion field was fixed at 270 Td and the compensation field was varied from −1 to 4 Td over 180 s.

Mass Spectrometry. Experiments were performed on a Thermo Fisher Orbitrap Elite mass spectrometer.

MSI. Mass spectra were recorded in full scan mode (m/z 600–2000) at a resolution of 120 000 at m/z 200. Automatic gain control (AGC) was turned off for spatial profiling experiments. The fill time was optimized prior to analysis (by sampling serial tissue sections at a central location) by acquiring data with the AGC on with extended maximum injection times. Subsequent interrogation of those data revealed the actual approximate fill times required to accumulate 1 × 10^6 charges. The following fill times were optimal: 2 or 5 ms (mouse brain and liver respectively) for LESA experiments with no FAIMS device attached to the mass spectrometer, 20 ms for LESA experiments with the FAIMS device attached (no field applied), and 150 or 100 ms (mouse brain and liver respectively) for LESA (static) FAIMS experiments. Data were acquired for 2 min at each location.

MS/MS. LESA FAIMS (DF = 270 Td, CF = 2.6 Td) tandem mass spectrometry experiments were conducted via collision induced dissociation (CID). For these experiments, AGC was used with a target of 1 × 10^6 charges and a maximum injection time of 1000 ms. CID was performed in the ion trap at a normalized collision energy between 20% and 23% and fragments were detected in the orbitrap. The isolation width was between 3.0 and 5.0 Th. Each scan comprised of 1 microscan. Data were recorded for between 2 and 7 min (~245–475 scans).

Data Analysis. Protein Identification. Data were analyzed using Xcalibur version 3.0.63 software. All mass spectra were deconvoluted using the Xtract function in Xcalibur to obtain monoisotopic masses of species detected in each experiment. Mass spectra were processed with a signal-to-noise ratio of 2. Identity was confirmed by manual analysis using Protein Prospector (http://prospector.ucsf.edu/prospector/mshome.htm).

Imaging. Single location data were saved as a single mass spectrum using the write to raw file function in the Xcalibur software. These new single mass spectrum.raw files were converted to the mzML format using msconvert as part of ProteoWizard and then to the imzML format using imzMLConverter. Data in imzML format were then loaded into MATLAB (version 2013a, The MathWorks Inc., Natick, Massachusetts) using imzMLConverter and in house software.

Staining. Serial tissue sections were stained with H&E as follows: tissue was twice washed in water for 2 min before immersing in Harris’s hematoxylin for 4 min. The tissue was then washed in water (2 min), Scott’s Tap Water Substitute (0.5 min) and water (2 min) before immersing in Eosin for 1 min. Finally, the tissue was washed in water (2 × 2 min), alcohol (4 × 2 min), and xylene (3 × 2 min). Anatomical features were manually assigned by visual inspection and correlation to the sagittal Allen Mouse Brain Atlas.
RESULTS AND DISCUSSION

Mouse Brain Tissue Sections. Serial sections of mouse brain tissue were sampled in a sequential fashion by LESA or LESA coupled to (static) FAIMS, allowing the spatial mapping of detected species, as shown in the photographs inset in Figure 1. Previously we reported that protein species in mouse brain were optimally transmitted through the chip-based FAIMS device at a dispersion field of 270 Td and a compensation field 2.6 Td,32 hence those conditions were employed here for data acquisition via LESA (static) FAIMS. Data were acquired at 2 mm spacing in order to prevent any oversampling between sampling locations. Oversampling is often used in MSI to improve spatial resolution; however, that approach is only applicable for methods in which the surface is fully ablated. LESA does not result in complete ablation and previous work in our laboratory39 has shown that resampling a tissue location alters the subsequent mass spectrum, possibly due to washing effects or degradation of the wetted surface. For the sampling volumes used here, the sampling area has diameter ∼1.8 mm. (The LESA points software allows sampling locations to be spaced at increments of 1 mm only). Data were acquired with the automatic gain control (AGC) switched off, using a defined fill time which had been optimized prior to full tissue profiling experiments, see above. Automatic gain control governs the number of charges accumulated in the linear ion trap prior to transmission to the orbitrap. To allow comparisons of the relative abundances of species at different sampling locations, AGC was not used and instead the fill time was given a defined value.

Profiling of mouse brain tissue by LESA and LESA FAIMS led to the detection of a number of multiply charged protein species. Figure 1A shows the summed mass spectrum from a specific location (location 4 on the inset photograph) sampled by LESA (no FAIMS device coupled). A variety of multiply charged protein species were detected, corresponding to several

Figure 2. LESA FAIMS MSI of mouse brain tissue at DF = 270 Td, CF = 2.6 Td. (A) LESA sampling positions. LESA FAIMS MS ion images of (B) m/z 1315 (MW 6568 Da; 5+), (C) m/z 957 (MW 6694 Da; 7+, 6.8 kDa proteolipid), (D) m/z 1299 (MW 7789 Da; 6+), (E) m/z 1185 (MW 8289 Da; 7+), (F) m/z 1069 (MW 9614 Da; 9+), (G) m/z 1166 (MW 9321 Da; 8+), (H) m/z 1237 (MW 12365 Da; 10+, macrophage migration inhibitory factor), (I) m/z 1382 (MW 12428 Da; 9+), and (J) m/z 789 (MW 14792 Da; 19+, α-globin).
different protein species; however, a greater number of protein species were detected from the equivalent location on the serial section when LESA was coupled to FAIMS, as shown in Figure 1B. Deconvolution of the mass spectra, using the Xtract function in Xcalibur, shows that 11 separate protein species (4−17 kDa) were detected in the LESA experiment compared to 14 protein species in the LESA (static) FAIMS experiment. A similar analysis of the mass spectra obtained at a different location (location 9, Figure 1 inset photographs) reveals detection of 10 separate protein species in the LESA experiment and 19 separate species between 4−17 kDa in the LESA (static) FAIMS experiment. The spatial distributions of a selection of the species unique to the LESA (static) FAIMS experiment are shown in Figure 2. (Figure 1 inset shows an H&E stained serial section for comparison). As described above, the spatial resolution afforded by the LESA sampling regime is low; nevertheless it is possible to profile multiple protein species by LESA FAIMS.

A summary of all protein species detected in the LESA and LESA FAIMS MSI experiments according to location, as indicated in the H&E stained section Figure 1 inset, is given in Supporting Information Tables 1 and 2. A total of 34 separate protein species were detected in the mass range 4 to 17 kDa across the tissue section profiled by LESA (static) FAIMS, 26 of which were not detected in the LESA analysis. Note that we previously reported that surface sampling of mouse brain tissue at a specific discrete location via LESA (static) FAIMS experiments (at DF = 270 Td and CF = 2.6 Td) led to the detection of 29 different protein species in the mass range ~5 to ~37 kDa.32 Clearly different species were detected in the current experiment. This observation is not unexpected: There will be differences in the anatomical regions being sampled, which naturally vary in protein composition. In addition, the smaller extraction volumes used for here for optimized spatial resolution may result in reduced extraction efficiency.

To identify some of the protein species unique to the FAIMS MSI experiment, top-down collision induced dissociation (CID) analysis was performed in a further experiment. LESA (static) FAIMS CID MS/MS of the 20+ ions centered at m/z 749.99 (MW_{meas} 14970.71 Da, MW_{calc} 14971.82, ∆ 5.6 ppm) resulted in identification of α-globin with 22% sequence coverage, see Figure 3A. This protein was detected in most of the on-tissue locations (9/21, see Supporting Information Table 2). The ion distribution of the 19+ charge state of the same protein (detected at m/z 789) is shown in Figure 2 Panel J. The 7+ precursor ion at m/z 957.80 (MW_{meas} 6693.58 Da, MW_{calc} 6693.58) was identified as 6.8 kDa mitochondrial proteolipid with a sequence coverage of 24%, see Figure 3B. This is the first reported identification of this protein from brain tissue by LESA. Finally, the 9+ precursor ion m/z 1375.69 (MW_{meas} 13265.18 Da, MW_{calc} 13265.14, ∆ 3.2 ppm) was identified as macrophage migration inhibitory factor, see Figure 3C. Although the sequence coverage achieved was low (7%), this protein has previously been reported by surface sampling (coupled to LC-MS) of mouse brain tissue sections by Schey et al.40

A number of species (MW = 2105.07, 2401.19, 2404.18, 2475.27, 3215.59, 3343.65, 4744.39, 4979.27, 5368.70, 5455.79, 5703.08, 8976.23, and 10069.75 Da) were detected in the LESA profiling experiment that were not detected in the LESA (static) FAIMS experiment. Six of these species were in the mass range 2−4 kDa whereas the remainder fell within the mass range detected in the FAIMS experiment (4−17 kDa).

The charge states varied from 3+ to 10+, see Supporting Information Table 3. The absence of these species in the FAIMS data set may be the result of low abundance (FAIMS is associated with a reduction in absolute sensitivity despite the improvements in signal-to-noise32) or that the static FAIMS conditions used here are not suitable for their transmission. Similarly, the species with MW 4960 Da (assigned as β-thymosin 4, see below) was detected in most (10/12) on-tissue locations in which protein signals were detected in the LESA experiment, but in only two locations in the LESA (static) FAIMS experiment. Supporting Information Figure 1C shows the spatial mapping (ion image) of m/z 828 (6+ charge state) together with the corresponding LESA sampling method (Supporting Information Figure 1B). To determine whether the static FAIMS conditions were limiting transmission of these ions, a LESA 1D FAIMS MSI experiment was performed in which the dispersion field was fixed at 270 Td and the compensation field was varied from −1 to 4 Td. (Note that this experiment was performed on a nonserial section of mouse brain tissue). β-Thymosin 4 was detected at each tissue location; however, the optimum CF field for its transmission was 3.6 Td. That observation explains the inconsistent results from the static FAIMS analysis, that is, the static FAIMS...
conditions (DF = 270 Td, CF = 2.6 Td) were not optimum for transmission of $\beta$-thymosin 4 ions.

$\beta$-Thymosin 4 was identified following LESA CID MS/MS of the 5+ ions at $m/z$ 993.70 (MW$_{\text{meas}}$ 4960.47 Da, MW$_{\text{calc}}$ 4960.49 Da, $\Delta$ 2.7 ppm, 61% sequence coverage), see Supporting Information Figure 2A. In addition, the species with MW 8559 Da, which was detected in both the LESA and the LESA (static) FAIMS experiment, was identified as ubiquitin (MW$_{\text{meas}}$ 8559.59, MW$_{\text{calc}}$ 8559.62, $\Delta$ 3.0 ppm, 27% coverage) following LESA CID MS/MS of the 7+ ions at $m/z$ 1224.50, see Supporting Information Figure 2B. The 12+ ions observed at $m/z$ 1400.33 were identified as calmodulin acetylated at position 95 (MW$_{\text{meas}}$ 16779.80, MW$_{\text{calc}}$ 16779.81, $\Delta$ 0.7 ppm, 13% coverage), see Supporting Information Figure 2C.

Recently, Hsu et al. reported nanoDESI mass spectrometry imaging of proteins from coronal sections of mouse brain. In their study, ubiquitin was described to be homogeneously distributed across the tissue whereas $\beta$-thymosin 4 was detected in greater intensity in the hippocampus. We detected $\beta$-thymosin 4 and ubiquitin across the tissue section (Supporting Information Figure 1C and D); however, both proteins were detected in greater intensity in the cerebral nuclei. The hippocampus was not sampled in the tissue sections analyzed here; the hippocampus is a small anatomical feature which is not consistently present in sagittally sectioned brain. Hsu et al. also reported that $\alpha$-globin was present in high abundance in certain locations, possibly corresponding to vasculature features. In this work, $\alpha$-globin was only detected when FAIMS was incorporated in the MSI workflow, see Figure 2J.

The signal intensity is particularly high in location 3 which could be due to similar vasculature features, although no such features were visible to the naked eye.

The protein calmodulin (MW 16780 Da) was detected in both the LESA experiment and the LESA (static) FAIMS experiments. Monitoring of the various charge states of this species shows that the spatial distributions and relative abundances of the 9+ through 12+ charge states within each experiment are in good agreement with one another, see Supporting Information Figure 3; however, comparison of the spatial distributions of ions detected between each experiment is hindered by the fact that the LESA sampling locations are not identical in the two serial sections. This variation is a consequence of the LESA software which requires manual positioning of the sampling locations. For example, in the photographs inset in Figure 1, the bottom row of the LESA image has sampled the edge of the tissue whereas the equivalent row in the LESA (static) FAIMS image has sampled on tissue. In order to ensure that the presence of the FAIMS field does not affect the spatial distribution of detected analytes, an imaging experiment was performed on a single tissue section in which each individual LESA sample was analyzed both with the FAIMS field off and on. As the LESA extraction was performed as a single step in each experiment, any differences in location and extraction efficiency are controlled for and the spatial distributions in LESA MSI and LESA (static) FAIMS MSI can be directly compared. Figure 4 shows the spatial distributions of the 9+ ($m/z$ 1866), 10+ ($m/z$ 1679), 11+ ($m/z$ 1527), and 12+ ($m/z$ 1399) charge states of calmodulin with and without the FAIMS field applied. The spatial distributions...
observed in the LESA MSI and LESA FAIMS MSI are in agreement. The differences in absolute intensity between the two ion maps are the result of improved S/N observed with FAIMS. Similar data are shown for the 6+ (m/z 1409), 7+ (m/z 1208), and 8+ (m/z 1057) charge states of the protein with MW 8446 Da in Supporting Information Figure 4. Again, the spatial distributions of these ions are in agreement.

Mouse Liver Tissue Sections. In further experiments, serial sections of mouse liver were sampled by LESA and LESA (static) FAIMS (DF = 270 Td; CF = 2.68 Td). A summary of all protein species detected in the LESA and LESA FAIMS experiments according to location, as indicated in Figure 5A and Supporting Information Figure 5A, is given in Supporting Information Tables 4 and 5. Again, a greater number of protein species were detected from an equivalent location on the serial section when LESA was coupled with (static) FAIMS. A total of 40 separate protein species in the mass range 4−16 kDa were detected across the tissue section profiled by LESA (static) FAIMS, 29 of which were not detected in the LESA analysis. Tissue profiling by LESA revealed 24 protein species in that mass range, 13 of which were unique to that data set.

Figures 5B−F show the spatial distributions of a selection of protein species detected in the LESA (static) FAIMS experiment. A vascular feature can be seen in the tissue sections, see Figure 5A, and the location of α- and β-globin clearly correspond with that feature (Figure 5B and 5C, respectively). Interestingly, the protein species unique to the LESA (static) FAIMS experiment were predominantly detected in pixel locations corresponding to the vascular feature. For example, see Figure 5G, SH, and 5I. Figure 5F shows the spatial distribution of liver fatty acid binding protein FABP1 within the section. FABP1 appears to be predominantly located toward the outer of the section. For comparison, the spatial distributions of α-globin, β-globin and FABP1 detected in the LESA experiment are shown in Supporting Information Figure 5. Again, the globin chains correlate with the position of the vascular feature and the FABP1 is distributed toward the outer of the section.

■ CONCLUSIONS

The results show that inclusion of FAIMS in the LESA MSI workflow increases the number of protein species detected. For the mouse brain sample studied here, 15 proteins (in mass range 4−17 kDa) were detected by LESA MSI compared with 34 proteins detected by LESA (static) FAIMS MSI. In total, 41 protein species were detected in the range 4−17 kDa. Twenty-six of those proteins were unique to LESA (static) FAIMS MSI. Seven proteins in that mass range were unique to LESA MSI. A further six species in the mass range 2−4 kDa were observed in LESA MSI only. For the mouse liver sample studied, 24 species (in the mass range 4−16 kDa) were detected by LESA MSI compared with 40 species detected by LESA (static) FAIMS MSI. In total, 53 protein species were detected in the range 4−16 kDa. Twenty-nine of those proteins were unique to LESA (static) FAIMS MSI. Thirteen proteins in that mass range were unique to LESA MSI. The static FAIMS conditions used here were optimized for higher molecular weight species thus preventing transmission of the lower molecular weight species (<4 kDa). In addition, although the static FAIMS conditions (DF = 270 Td, CF = 2.6 Td) were broadly optimized for
proteins of MW > 4 kDa, there are some outliers such as β-thymosin 4 (CF = 3.6 Td), or species of very low abundance, which were not transmitted by FAIMS.

A number of proteins in mouse brain were identified by collision-induced dissociation: LESA (static) FAIMS CID MS/MS resulted in the identification of α-globin (MW 14972 Da), 6.8 kDa mitochondrial proteolipid (MW 6693 Da) and macrophage migration inhibitory factor (MW 12665 Da). LESA CID MS/MS resulted in identification of ubiquitin (MW 8560 Da), β-thymosin 4 (MW 4960 Da), and calmodulin (MW 16780 Da), all of which were also observed in the FAIMS data set.

Eight protein species were detected in both the LESA MSI and LESA (static) FAIMS MSI analysis of mouse brain. The presence of these allowed comparisons of the spatial distributions measured with and without FAIMS. The good agreement between the two distributions confirms the suitability of FAIMS as a useful tool in mass spectrometry imaging.

**ASSOCIATED CONTENT**

Supporting Information

Tables of protein masses detected in LESA MSI and LESA FAIMS MSI of mouse brain, unique species detected in LESA MSI of mouse brain, and protein masses detected in LESA MSI and LESA FAIMS MSI of mouse liver (**PDF**)

Figures showing ion images obtained following LESA MSI of mouse brain, LESA CID MS/MS spectra of proteins detected following LESA of mouse brain, spatial distribution of a protein in various charge states in the presence and absence of FAIMS field, and ion images obtained following LESA MSI of mouse liver (**PDF**)

**AUTHOR INFORMATION**

Notes

The authors declare no competing financial interest.

Supplementary data supporting this research is openly available from the University of Birmingham data archive at http://findit.bham.ac.uk/.

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