Supporting Information

Quantitative Imaging of Proteins in Tissue by Stable Isotope Labelled Mimetic Liquid Extraction Surface Analysis (SLiM LESA) Mass Spectrometry

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Materials and Methods

Materials

Water, methanol and formic acid were purchased from Fisher Scientific (Leicestershire, UK) and glass slides from Thermo Scientific. Fresh frozen mouse brain tissue (extraneous tissue from culled animals) was provided by Prof. Steve Watson at the University of Birmingham, fresh frozen control rat brain (extraneous tissue from culled animals) was provided by AstraZeneca.

Preparation of mimetic tissue models

Mimetic tissue models were prepared using the method described by Groseclose and Castellino.[1] Ten whole rat brains (thawed) were homogenized using a handheld homogenizer. Approximately 2.5 mL of homogenate was portioned into individual pre-weighed tubes and final weight homogenate per tube was calculated. C^{13},N^{15}-labelled ubiquitin (enrichment ≥98%, Sigma-Aldrich, Gillingham, UK) solutions were prepared in methanol and water (30:70), to final concentrations such that 1% final volume of tissue was added to each sample, this prevented large volumes of solvent diluting the homogenate. Magnetic stir bars (5 mm) were added to each tube and vortexed for 1 minute. Spiked homogenates were pipetted into plastic moulds before freezing for 1 hour at -80 °C. The final concentration of labelled ubiquitin in tissue was 0, 9.9, 19.9, 67.4, 68.6, 94.7, 122.6 and 163.0 nmol/g. Rat and mouse brains were dissected and flash-frozen in liquid nitrogen before storage at -80°C. Tissue mimetics, rat and mouse brain tissue were sectioned at -20°C (Leica CM 1850 Cryostat, Milton Keynes, UK) at 14 µm thickness, and thaw-mounted onto glass slides while placed adjacent to each other.

LESA MS method

Surface sampling was performed using a TriVersa Nanomate chip-based electrospray device (Advion, Ithaca, NY). The electrospray device was coupled to a Thermo Fisher Orbitrap Elite (Thermo Fisher Scientific, Bremen, Germany) instrument. The TriVersa Nanomate was controlled through LESA Points and ChipSoft 8.3.3 software (Advion, Ithaca, NY). The sample was mounted in the LESA universal adaptor plate and scanned using an Epson flatbed scanner. The scanned image was imported into LESA Points and the sampling locations were defined. Sampling locations were selected with an x, y spacing of 2 mm, covering the whole tissue section. The z coordinate was set to height 1.2 mm above the sample surface to allow the liquid microjunction to be formed. The solvent system for extraction and electrospray was methanol, water and formic acid (69.3:29.7:1). The robotic arm collected a conductive pipette tip and aspirated 2 µL of solvent from the reservoir. The arm relocated to the x, y

coordinates specified by LESA Points software. The pipette tip descended to the predetermined height (see above) and dispensed 1.3 µL solvent. The liquid microjunction was maintained for 10 seconds before re-aspiration. Each sample was delivered for 3 min *via* the Triversa Nanomate with a gas pressure of 0.3 psi and a tip voltage of 1.75 kV. Positive ion mass spectra were acquired in full scan mode (*m/z* 600 - 2000) at a resolution of 120 000 at *m/z* 400. The Automatic Gain Control was turned off and the injection time fixed at 1 ms.[2] Each scan was comprised of 5 co-added microscans.

Data analysis

RAW data files were analysed in Thermo Xcalibur Software (Thermo Fisher Scientific, Bremen, Germany) and further analysed in MATLAB (MathWorks, Massachusetts, US). RAW data imaging files were converted to mzML files using ProteoWizard's MSConvert software.[3] These files were converted into imzML files using imzML converter. ImzML files were analysed in MATLAB with SpectralAnalysis software.[4,5]

Preparation of mimetic tissue models

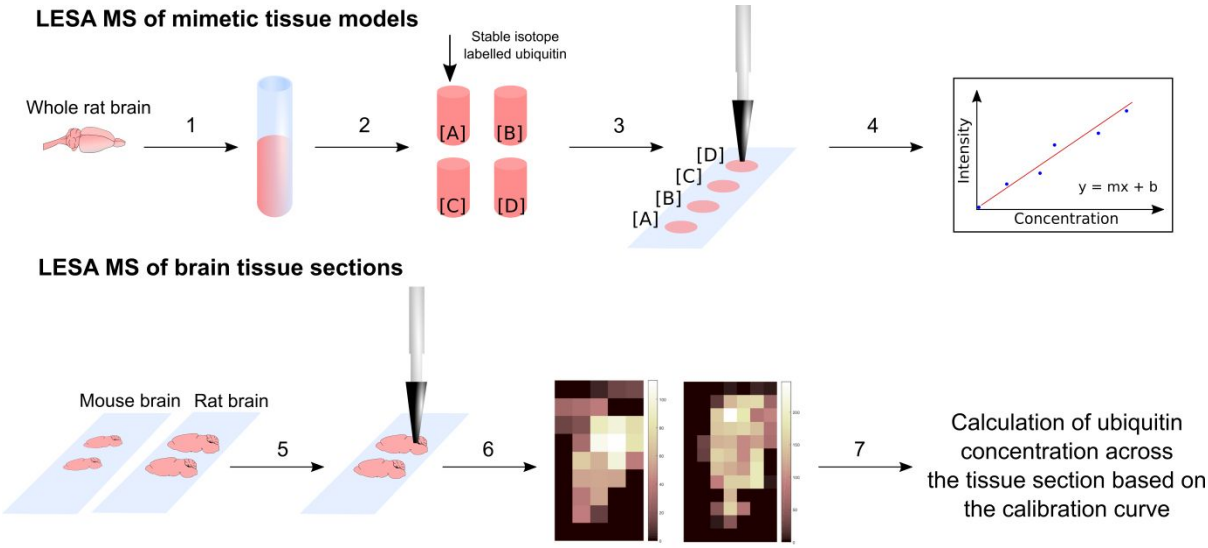

Figure S1. Workflow describing the preparation of mimetic tissue models. (1) Whole rat brains are homogenized and (2) the homogenate is divided equally into pre-weighed tubes. The labelled protein of interest (ubiquitin) is spiked into homogenates with stirring and allowed to freeze at -80 °C. (3) The labelled mimetics with different concentrations of the labelled protein (A, B, C, D) are cryosectioned and thaw-mounted onto glass slides. (4) LESA MS analysis of the tissue mimetics is performed and a calibration curve is plotted. (5, 6) Subsequently, LESA MS imaging of rat and mouse brain tissue sections preserves the spatial distribution of the protein of interest. (7) The calibration curve is used to quantify the amount of protein across the tissue section or in the individual areas of brain.

Exclusion of three data points

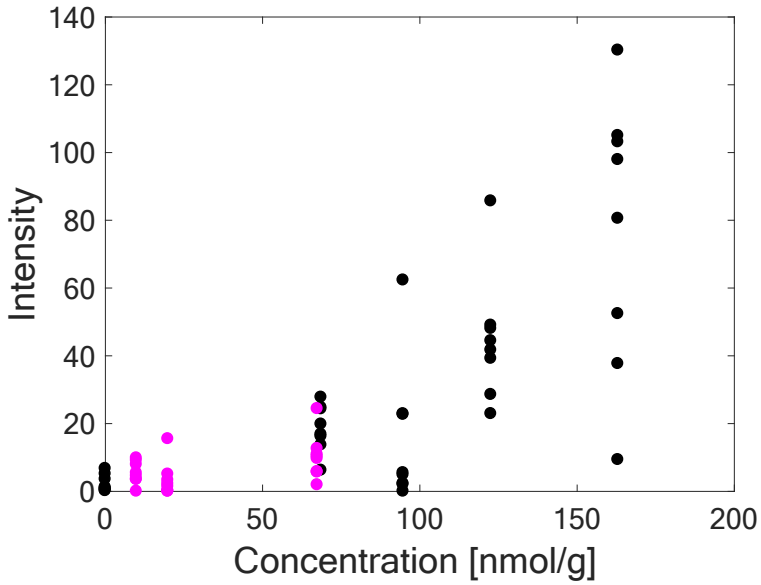

Figure S2. Data obtained from LESA MS of mimetic tissue, charge 10+, m/z 904.7624. Excluded datapoints which did not meet the requirements for LLOQ are shown in magenta.

Assessment of homogenate mixing

Two sections were taken from the top and one from the bottom of the mimetic tissue model (see *Figure S3*) and all three were subject to LESA MS under the same sampling conditions. Ion images of endogenous and labelled ubiquitin were produced which reveal that a similar level of variation is seen for both species. This suggests that the labelled ubiquitin was assumed to be distributed uniformly throughout the homogenate prior to freezing. It also suggests that the variation in intensity is due to variation in LESA sampling and not due to inhomogeneity in the sample.

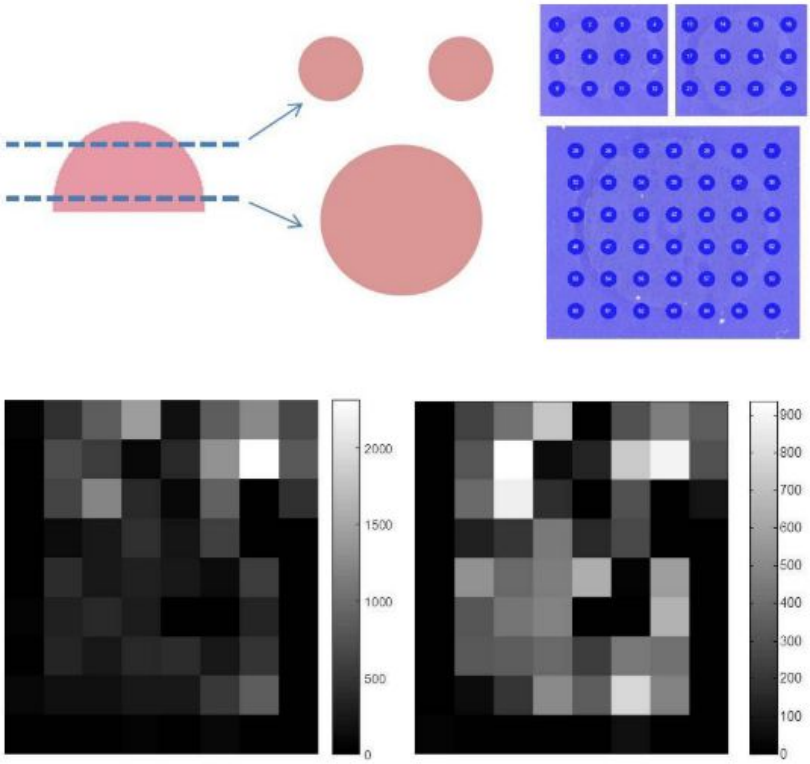

Figure S3. Clockwise from top left: schematic describing section locations within mimetic, locations selected for LESA sampling, LESA MS image of ions detected at m/z 904.6543 (isotopically labelled ubiquitin) and LESA MS image of ions detected at m/z 857.3632 (endogenous ubiquitin).

LESA MS imaging of rat and mouse brain tissue sections

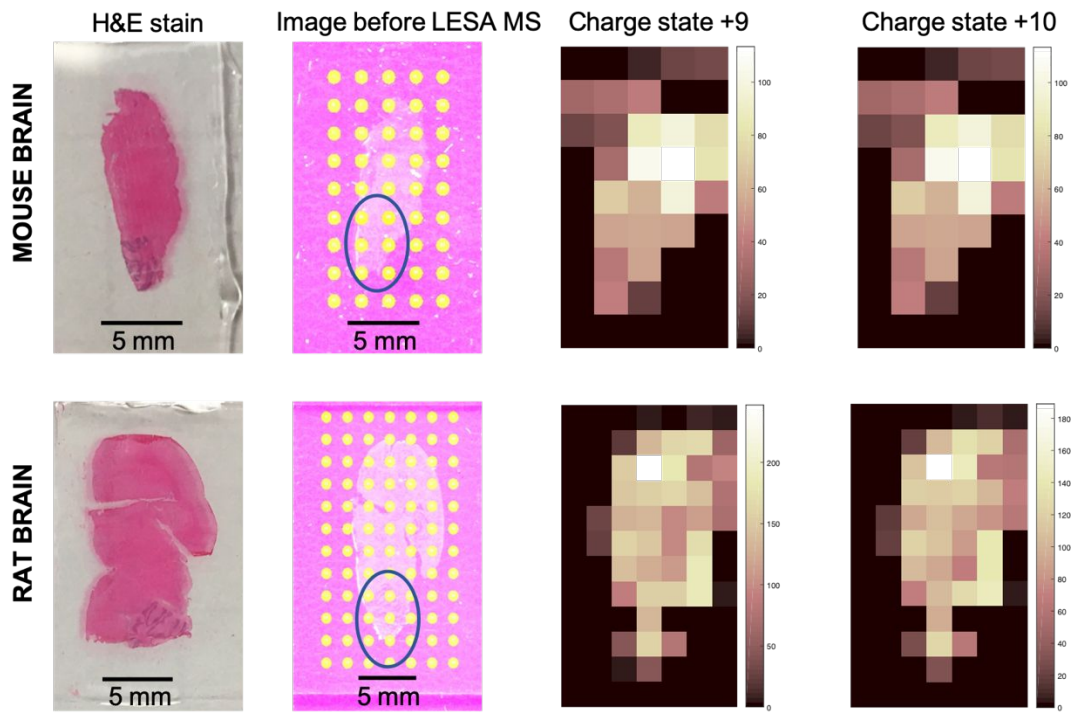

Figure S4. LESA MS imaging of brain tissue sections from mouse (top) and rat (bottom). Left: Tissue sections stained with hematoxylin and eosin (H&E). Middle: Photograph of tissue section prior to LESA MS imaging with sampling location array (2 mm spacing) shown. Blue ovals indicate position of cerebellum which aids orientation of the tissue section. Right: Ion intensity images of 9+ and 10+ charge states of ubiquitin (*m/z* 952.0732 and *m/z* 856.9863 respectively).

The initial tissue mimetics experiment

The whole experiment was performed and completed 1 year after the preliminary results. The *Figure S5* summarizes the LESA MS preliminary experiment.

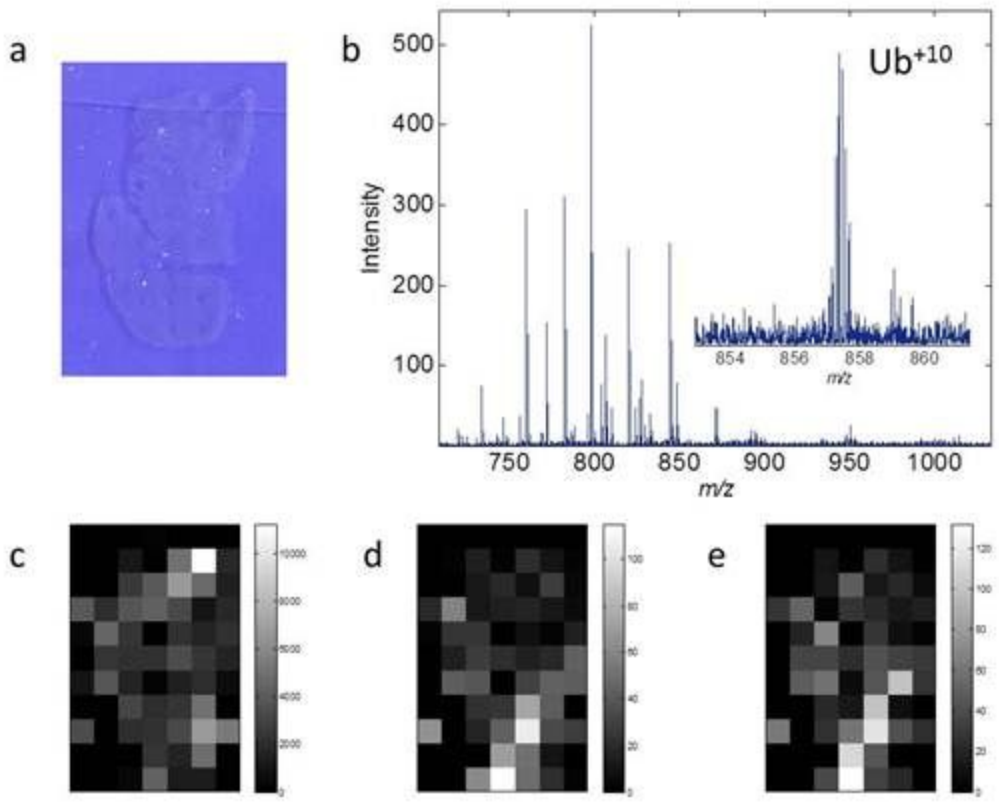

Figure S5. LESA MS imaging of rat brain. (a) An optical image of rat brain tissue after sampling. (b) An example of mass spectrum with endogenous ubiquitin in charge state 10+. (c), (d), (e) A representation of ion images of heme (616.1717 *m/z*), endogenous ubiquitin in charge state 10+ (857.3632 *m/z*) and endogenous ubiquitin in charge state 9+ (952.6230 *m/z*).

References

(1) Groseclose, M. R.; Castellino, S. A mimetic tissue model for the quantification of drug distributions by MALDI imaging mass spectrometry. *Anal. Chem.* **2013**, *85*, 10099-10106.
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(4) Race, A. M.; Styles, I. B.; Bunch, J. Inclusive sharing of mass spectrometry imaging data requires a converter for all. *J. Proteomics* **2012**, *75*, 5111-5112.
(5) Race, A. M.; Palmer, A. D.; Dexter, A.; Steven, R. T.; Styles, I. B.; Bunch, J. SpectralAnalysis: Software for the Masses. *Anal. Chem.* **2016**, *88*, 9451-9458.