Native LESA mass spectrometry: Direct analysis of proteins and their complexes

Rian L. Griffiths[1], Victor A. Mikhailov[2], Emma K. Sisley[1], Anna L. Simmonds[1], Iain B. Styles[1] and Helen J.Cooper[1]
[1]School of Biosciences, University of Birmingham, Birmingham, United Kingdom. [2]University of Oxford, Oxford, United Kingdom

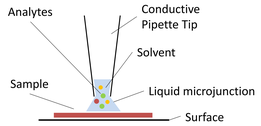
Overview

Liquid microjunction surface sampling using ammonium acetate based solvent systems is shown to be suitable for the analysis of a range of proteins in their native, folded, structure directly from complex sample substrates such as mouse liver and brain tissue.

Introduction

Liquid microjunction surface sampling via liquid extraction surface analysis (LESA) is an emerging tool for direct surface sampling of intact proteins and protein assemblies from biological substrates[1,2,3]. Recently, ammonium acetate based buffers have been described as suitable sampling solvents[4,5,6]. Here we show that native LESA MS can probe protein assemblies up to ~ 800 kDa and demonstrate improved methods for the analysis of folded intact protein species from thin tissue sections of bulk liver tissue and mouse brain for the first time. Furthermore, we show that chemical imaging of proteins and protein assemblies can be achived via native LESA MS. In addition, we explore native LESA MS for probing protein ligand-binding interactions from complex samples.

Experimental



Thin tissue sections (10 µm) thaw mounted onto glass slides were either analysed as is or after washing in 80 % ethanol. Human dried blood spots on filter paper were sampled a minimum of 24 hours after preparation. Protein standards were spotted onto glass slides. A Triversa Nanomate (Advion™) was coupled to either a Synapt G2S mass spectrometer (Waters, UK) or an Orbitrap Elite mass spectrometer (Thermo Scientific, UK). LESA extraction solvent comprised 200 mM ammonium acetate + 5 % methanol, or the same solvent system containing 1 mM biotin or bezabibrate.

Conclusions

- Native LESA MS is a suitable tool for direct analysis of intact proteins and protein assemblies from tissue substrates.
- Proteins can be spatially profiled using native solvent systems. Spatial distributions agree with previous reports and some proteins are described for the first time via LESA MS.
- Protein assemblies up to 800 kDa can be detected after extraction from a surface.
- High resolution native LESA data is shown on an Orbitrap Elite MS.

Native LESA MS: How big?

Protein Standards

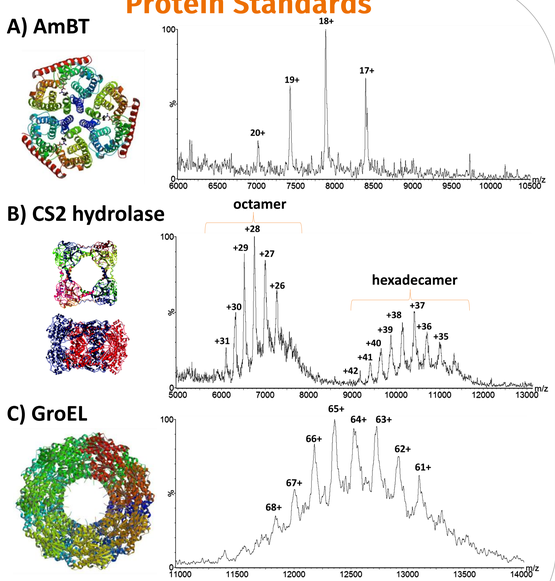


Figure 1 Native LESA mass spectra of protein complexes from glass substrate. A) Mass spectrum of avidin tetramer (64 kDa) B) CS_2 hydrolase: peaks correspond to ring octamer (189 kDa) and catenane hexadecamer (378 kDa). C) GroEL tetradecamer (803 kDa).

Native LESA MS: Thin Tissue Sections

Mouse Liver

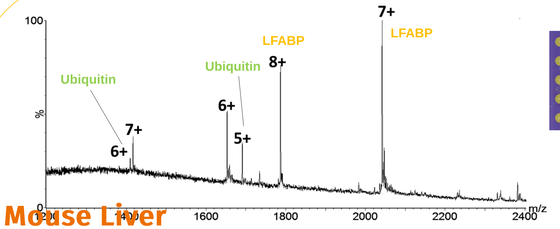


Figure 2 Native (contact) LESA MS of mouse liver tissue on a Synapt G2S mass spectrometer, led to the detection of a range of protein species in the range 8-14 kDa. Species such as liver fatty acid binding protein (LFABP) and ubiquitin were detected in low charge states, indicating that the protein remains folded.

Brain Mouse

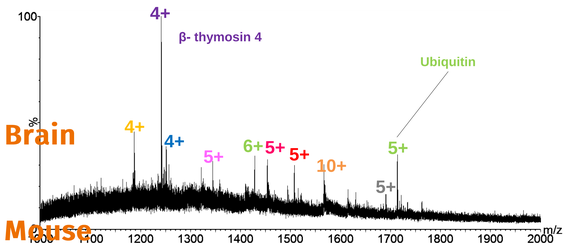


Figure 5 Native (contact) LESA MS of mouse liver tissue, led to the detection of a range of protein species in the range 4-15 kDa. Species such as β- thymosin 4 and ubiquitin were detected in low charge states, indicating that the protein remains folded.

Native LESA MS: Imaging

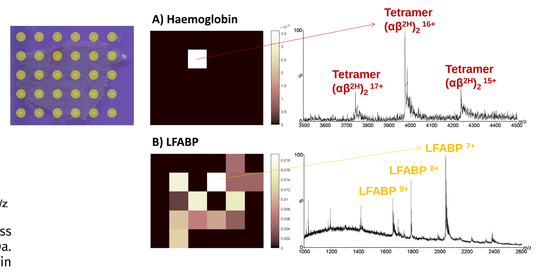


Figure 3 Native LESA MS imaging of mouse liver tissue. Sampling in sequential locations, led to the detection of predominantly LFABP in the bulk tissue and haemoglobin in a visible vasculature feature.

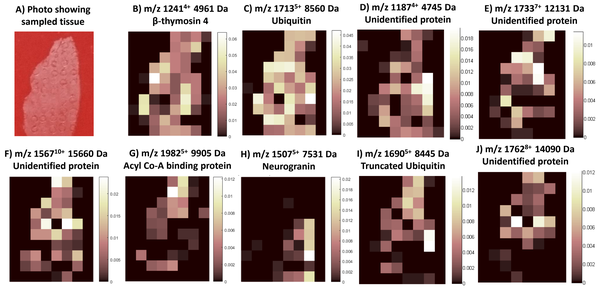


Figure 6 Native LESA MS imaging of mouse brain tissue. Sampling in sequential locations, led to the detection of various proteins in the range 4-14 kDa. Spatial distributions of native proteins are consistent with previous reports.

HRMS native LESA

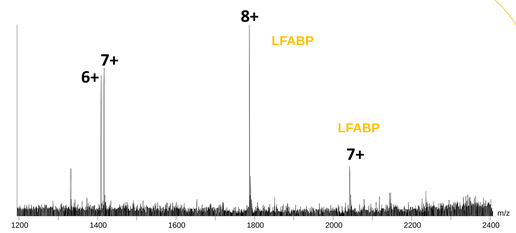


Figure 4 Native (contact) LESA MS of mouse liver tissue on an Orbitrap mass spectrometer, led to the detection of abundant protein species such as liver fatty acid binding protein (LFABP) and ubiquitin in low charge states.

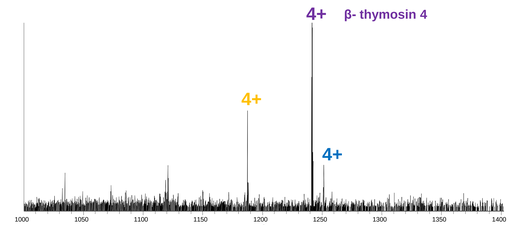


Figure 7 Native (contact) LESA MS of mouse brain tissue on an Orbitrap mass spectrometer, led to the detection of abundant protein species such as β- thymosin 4 in low charge states. Fewer protein species were detected in comparison to similar experiments on a Synapt G2S mass spectrometer as fewer gas pressures can be tuned.

Native LESA MS: Protein-Ligand binding

Protein Standards

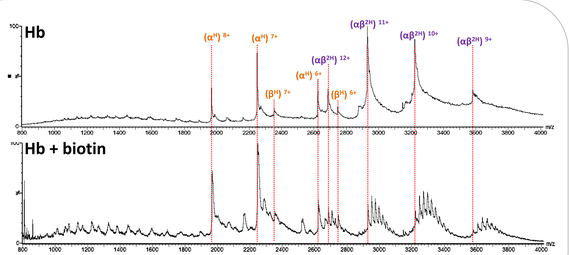


Figure 8 Native LESA mass spectra of haemoglobin from glass substrate sampled with either 200 mM ammonium acetate or 200 mM ammonium acetate including 1 mM biotin.

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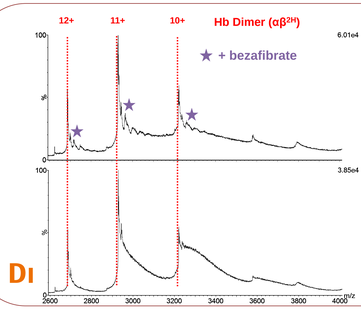

Figure 9 Native LESA MS of human dried blood spots. Sampling with ammonium acetate buffer led to the detection of the haemoglobin dimer. Re-sampling of the same tissue location with a sampling buffer containing 1 mM bezafibrate drug led to the detection of additional peaks corresponding to binding of the drug to each protein species.

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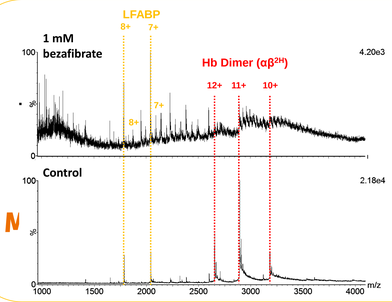

Figure 10 Native LESA MS of mouse liver tissue. Sampling with ammonium acetate buffer led to the detection of LFABP and haemoglobin dimer species. Re-sampling of the same tissue location with a sampling buffer containing 1 mM bezafibrate drug led to the detection of additional peaks corresponding to binding of the drug to each protein species.

References

[1]Edwards, R., et al. , J. Am. Soc. Mass Spectrom., 2012. **23**(11): p. 1921-1930.
[2]Sarsby, J., et al. , J. Am. Soc. Mass Spectrom., 2014. **25**(11): p. 1953-1961.
[3]Randall, E.C., et al. , Anal. Chem., 2014. **86**(21): p. 10504-10510.
[4]Martin, N.J., et al. , J. Am. Soc. Mass Spectrom., 2015. **26**(8): p. 1-8.
[5]Griffiths, R.L. and H.J. Cooper, Anal. Chem., 2016. **88**(1): p. 606-609.
[6]Mikhailov, V.M., et al. , Int. J. Mass Spectrom., 2016. DOI: 10.1016/j.ijms.2016.09.01

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