Title: Liquid Extraction Surface Analysis for Native Mass Spectrometry: Protein Complexes and Ligand Binding

Author: Victor A. Mikhailov Rian L. Griffiths Helen J. Cooper

PII: S1387-3806(16)30182-8
DOI: http://dx.doi.org/doi:10.1016/j.ijms.2016.09.011
Reference: MASPEC 15668

To appear in: *International Journal of Mass Spectrometry*

Received date: 26-5-2016
Revised date: 1-9-2016
Accepted date: 20-9-2016

Please cite this article as: Victor A.Mikhailov, Rian L.Griffiths, Helen J.Cooper, Liquid Extraction Surface Analysis for Native Mass Spectrometry: Protein Complexes and Ligand Binding, International Journal of Mass Spectrometry http://dx.doi.org/10.1016/j.ijms.2016.09.011

Liquid Extraction Surface Analysis for Native Mass Spectrometry: Protein Complexes and Ligand Binding

Victor A. Mikhailov[1#], Rian L. Griffiths[2#] and Helen J. Cooper[2*]

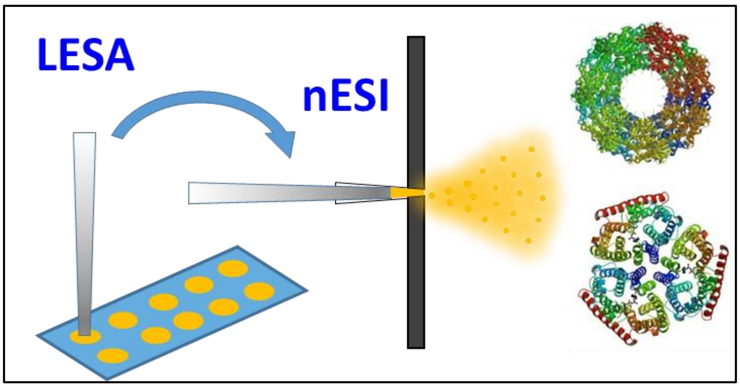
[1]Department of Chemistry, University of Oxford, Oxford, OX1 3TA, UK.

[2] School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.

*To whom correspondence should be addressed: h.j.cooper@bham.ac.uk

[#]These authors contributed equally to this work.

Graphical Abstract



Highlights

- Native LESA mass spectrometry is capable of the analysis of large (~800 kDa) non-covalent protein complexes.
- Both soluble and membrane protein complexes are amenable to LESA MS.
- Protein-ligand interactions can be interrogated by LESA mass spectrometry.

Abstract

Native liquid extraction surface analysis (LESA) mass spectrometry enables the direct sampling of protein complexes from a solid surface. We have previously demonstrated native LESA mass spectrometry of holomyoglobin (~17 kDa) from glass slides and tetrameric haemoglobin (~64 kDa) from dried blood spots and thin tissue sections. Here, we further explore the capabilities of this emerging technique by investigating a range of proteins which exist in various oligomeric states *in vivo.* Tetrameric avidin (~64 kDa), octameric (~190 kDa) and hexadecameric (~380 kDa) CS_2 hydrolase, and tetradecameric GroEL (~800 kDa) were all detected by native LESA mass spectrometry. Moreover, trimeric AmtB, a membrane protein, could also be observed by native LESA mass spectrometry. The suitability of LESA mass spectrometry for probing protein-ligand binding was also investigated. Non-covalent complexes of the ligand biotin with the proteins avidin, haemoglobin and bovine serum albumin were detected. The results indicate that non-specific binding is minimal and that native LESA mass spectrometry is a promising tool for the investigation of biologically significant ligand binding.

Keywords: liquid extraction surface analysis (LESA), native mass spectrometry, non-covalent protein complexes, protein-ligand interactions, drug binding.

Introduction

Knowledge of protein tertiary and quaternary structure is generally required for understanding protein function or malfunction. Experimental methods that provide this knowledge are capable of maintaining noncovalent bonds, such as hydrogen bonds and π-π interactions, thus preserving folded proteins in their native structure and the noncovalent bonding with those species with which they interact. Native electrospray mass spectrometry (MS) enables analysis of folded proteins and non-covalent protein complexes in the gas phase by choosing a non-denaturing electrospray buffer and careful optimisation of electric fields and pressures in the mass spectrometer [1-4]. In combination with other MS techniques, such as ion mobility measurements [5-7], gas-phase dissociation methods [8, 9], hydrogen-deuterium exchange [10-12] and non-covalent labelling [12-14] more specific information on the overall topology of non-covalent complexes, connectivity between different subunits in the complex and drug binding sites can be obtained. More recently, a native MS approach has been developed for membrane protein complexes [15, 16]. Stabilisation of these complexes in solution and the gas-phase requires that they are purified and electrosprayed in detergent micelles. The native MS approach also allows investigation of interactions of membrane protein complexes with lipids and drugs [17, 18].

Native mass spectrometry is characterised by ions in relatively low charge states – fewer protonation sites are available in folded proteins – and thus higher mass-to-charge ratios are recorded (for gas-phase protein complexes typical $m/z \geq 3000$) [1, 2]. Increasing the upper limit of the mass range to probe ever larger protein assemblies has been one of the major motives in development of the native MS approach. Q-TOF mass analysers have been the analysers of choice in native ESI, due to their high m/z range [2, 19]; however, more recently native MS has been successfully implemented on FT ICR and Orbitrap instruments [20, 21], which offer mass resolution superior to Q-TOF. To date, mass spectra of intact complexes as large as 18 MDa (an entire capsid assembly of bacteriophage HK97) have been reported [22], although the mass resolution of such large species is limited by incomplete desolvation of their gas-phase ions rather than by the inherent instrument resolution of the mass analysers employed [23].

Most native MS experiments are carried out by direct electrospray infusion of the purified sample into the mass spectrometer. Analytical applications of the native MS approach can be expanded considerably with sampling of non-covalent protein-protein and protein-ligand complexes directly from surfaces: the avenues for future explorations include probing diverse biological samples, such as dried blood spots and thin tissue sections, protein/ligand assays and biomimicking membranes. Surface sampling can be coupled to electrospray ionisation (essential for native MS) by desorption electrospray ionisation (DESI) or liquid microjunction based technologies such as liquid extraction surface analysis (LESA) [24, 25]. In DESI, a jet of electrosprayed solvent is directed at a surface containing analyte molecules in order to desorb and deliver them into the mass spectrometer [26-28]. This technique produces multiply charged ions similar to direct ESI infusion [29, 30]. DESI has been successfully applied to the surface analysis of small molecules, peptides and proteins up to ~ 25 kDa [27, 31, 32] , however direct analysis of intact proteins from biologically relevant samples has not been reported to date. A variant of DESI in which liquid samples are probed (liquid-DESI) has demonstrated that intact proteins and protein complexes up to ~ 150 kDa can be detected [29].

In another approach, DESI can be used to probe non-covalent interactions between immobilised proteins and ligands. In this method proteins are immobilised on a surface and treated with a mixture of ligands. The non-binding ligands are then washed away, and the assay is subjected to DESI analysis with denaturing solvents leading to the detachment of the bound ligand molecules from the protein and their delivery to the mass spectrometer [33]. This approach can be used for high-throughput screening for binding ligands, but the indirect assessment of non-covalent interactions between the protein and the ligands *via* detection of the ligand molecules by DESI is not entirely conclusive, because the presence of the immobilising agent can produce false positive results [34]. Furthermore, these studies cannot provide information on the stoichiometry of binding, and are not applicable to samples with heterogeneous protein targets, such as blood spots and tissue sections.

Alternative surface sampling techniques that are potentially capable of extracting a diverse range of non-covalent complexes for MS analysis are liquid microjunction based methods such as liquid extraction surface analysis (LESA) [24, 25] or continuous flow liquid microjunction sampling [35]. LESA enables sampling of discrete locations on a surface by placing a small droplet of the solvent on a spot which is then re-aspired with a conductive pipette tip prior to ESI MS. LESA MS analysis of intact proteins has been achieved from a variety of substrates: dried blood spots on filter paper [36-39], thin tissue sections thaw-mounted onto glass slides [40-43] and *E.coli* colonies grown on agar gel [44]. Recently, protein species up to ~15 kDa were reported by ‘nanoDESI’ imaging of tissue sections [45]. Despite its name, nanoDESI is actually based on continuous flow liquid microjunction surface sampling [46].

We recently reported the use of LESA with non-denaturing ammonium acetate based solvent for the MS analysis of noncovalent protein complexes from glass and polyvinylidene fluoride (PVDF) substrates [47]. Using this ‘native’ LESA MS method, intact ~17.5 kDa holomyoglobin, a non-covalent myoglobin-heme complex, and a range of non-covalently bound species from a haemoglobin sample: α^H and β^H-globin subunits associated with heme,~16 kDa each; the $(\alpha\beta)^{2H}$ heterodimers (~32 kDa) and the intact $(\alpha\beta)_2{}^{4H}$ tetrameric complexes, ~64 kDa, were detected. Furthermore, the intact haemoglobin tetramer could be detected from dried blood spots on filter paper. Griffiths *et al* subsequently demonstrated direct detection of the tetrameric haemoglobin complex from vasculature in thin tissue sections of mouse liver thaw-mounted onto glass slides [48].

In the work presented here, we sought to further characterise the capabilities of native LESA MS. We demonstrate a significant extension of the mass range of our method, and show that it is possible to detect intact protein assemblies as large as ~ 800 kDa. We also demonstrate that membrane protein complexes, that are prepared in micelle-containing solutions, are amenable to native LESA MS and can be probed directly from dried spots on a surface. The mass spectra produced by native LESA MS are similar to those obtained from 'standard' native mass spectrometry. Furthermore, protein-ligand interactions have been probed by LESA MS, and the detection of biotin noncovalently bound to avidin, haemoglobin and BSA is presented.

Materials and Methods

Materials: Protein standards (avidin from chicken egg white, bovine serum albumin (BSA), human haemoglobin, Chaperonin 60 from *Escherichia coli* (GroEL)), biotin and ammonium acetate were purchased from Sigma-Aldrich (Gillingham, UK). GroEL was further purified as described elsewhere [1]. CS_2 hydrolase from *Acidianus A1-3* was a gift from Jasmin Mecinovic, Radboud University Nijmegen, Netherlands [49]. Ammonium channel (AmtB) was expressed and purified in-house at the University of Oxford as described elsewhere [50]. Octyltetraglycol (C8E4) was purchased from Avanti (Alabaster, AL, USA).

Sample Preparation: Proteins were prepared in 200 mM ammonium acetate (pH=7.5) resulting in a final protein concentration 1-10 µM. GroEL and BSA proteins were further desalted using Micro-BioSpin columns (BioRad, Hercules, CA, USA). AmtB was prepared in ammonium acetate solution containing C8E4 at twice the critical micelle concentration. 1 µl of protein solution was spotted onto a cleaned glass slide for LESA. The sample spots were air-dried at room temperature and probed using LESA with a delay of 20-90 min between the deposition of the sample and its extraction for MS analysis.

Surface Sampling: LESA was carried out using the Triversa Nanomate chip-based ESI source (Advion Biosciences, Ithaca, USA) coupled with the Synapt G2S mass spectrometer (Waters, Manchester, UK). The sample was mounted onto the LESA universal adaptor plate and an image was acquired using an Epson Perfection V300 photo scanner. The exact location to be sampled was selected using the LESA Points software (Advion). The extraction/ionisation solvent was 200 mM ammonium acetate in water (HPLC grade, JT Baker, Deventer, The Netherlands). For biotin binding studies, the ammonium acetate solution was used to spot the protein samples on the slide, the extraction solution contained either 1.0 or 0.1 mM biotin in 200 mM ammonium acetate. In the extraction process, the solvent was aspirated from the solvent well, before the robotic arm relocated to a position above the sample slide. Some of the solvent was then dispensed and a liquid microjunction was maintained between the pipette tip and the sample surface before aspirating the solvent and delivering it to the electrospray chip. The distance between the nanospray chip and the entrance to the sample cone was 3-6 mm. The details of the LESA extraction procedure and ion source (Nanomate) parameters for each protein are given in **Table 1.**

Mass Spectrometry: A speedivalve (BOC Edwards, Crawley, Sussex, UK) was installed between the rotary and turbo pumps that pump the ion source region in Synapt G2S (Waters, Wilmslow, UK). The pressure in the ion source can be controlled by careful throttling of the pumping by the speedivalve with the aim of improving the transmission of ions with high *m/z* that are characteristic for intact protein complexes. The backing pressure was altered from 3 to 9 mbar for these experiments. Mass spectra were acquired in the full scan TOF mode on the Synapt G2S. The scan time was set at 2-5 s, the *m/z* range was altered depending on the species investigated (usually ≥ 10,000). The capillary temperature was set to 30°C and the cone voltage was set at 45 V. In some experiments (see text) collision induced dissociation (CID) was carried out on the ions in the trap region of the mass spectrometer. Collision voltage was varied from 50 to 200 V. All data were analysed by Mass Lynx software (version 4.1, Waters). The raw mass spectra have been averaged for 20-100 scans and presented here with minimal smoothing and background subtraction. The native LESA MS spectra have been compared against the mass spectra of the same proteins using direct infusion electrospray with Nanomate on the same mass spectrometer and the mass spectra from the previous work. For selected examples (*e.g.* avidin-biotin binding), LESA MS spectra were compared with high-resolution native MS spectra obtained on Orbitrap Q-Exactive instrument (Thermo, Bremen, Germany) in the University of Oxford modified for high *m/z* range, similar to the modifications previously reported by Rose *et al* [21].

Results and Discussion

Protein-protein Complexes

A range of proteins which exist in oligomeric states *in vivo* and in non-denaturing solutions were investigated. Their non-covalent complexes vary in mass from ~60 kDa up to ~800 kDa and exist in different oligomeric states, from trimers (AmtB) to hexadecamers (CS_2 hydrolase). **Figure 1A** shows the results obtained for avidin, a soluble tetrameric glycoprotein, which has been long-used as a standard for native MS [51]. The mass of the avidin tetramer is similar to that of the haemoglobin tetramer described in LESA MS studies in our previous works [48, 52]. Sampling the dried protein on glass with 200 mM ammonium acetate solution resulted in the detection of the tetrameric species (~64 kDa) in charge states ranging from 12+ to 16+ at *m/z* ~ 5300, 4900, 4600, 4300 and 4000 respectively, and are in accordance with the charge state distribution usually observed in the mass spectra of avidin from non-denaturing electrospray [53]. The tetramer peaks shown in the spectrum are relatively broad (~200 Th). Usually peak broadening in native MS is caused by incomplete desolvation of the gas-phase molecular ions and the presence of salts [47], but can also be a sign of protein degradation. A high-resolution mass spectrum of avidin, produced by direct nano-electrospray on an Orbitrap Q-Exactive mass spectrometer that was modified for high *m/z* range, shows the presence of peaks corresponding to these modifications within the envelop of each charge state of the tetramer (**Figure S1A,** Supporting Information). Mass spectra of the charge states (14+ to 18+) of the avidin tetramer obtained on the Nanomate/Synapt G2S apparatus by direct infusion demonstrate peak broadening similar to LESA MS (**Figure S1B** *versus* **Figure 1A**). Furthermore, collision induced dissociation (CID) of the avidin tetramer reveals multiple peaks for each charge state of the fragment monomer **(Figure S1B)**, indicating the presence of multiple modifications on the protein backbone. These results suggest the avidin tetramer ions observed in LESA MS have the same structure as those observed in 'standard' native MS. Further evidence that avidin retained its biologically relevant state in the LESA MS approach came from our results for its binding to biotin (see below).

Carbon disulphide hydrolase (CS_2 hydrolase) is an enzyme that converts carbon disulphide to carbon dioxide and hydrogen sulphide. It exists as a 190 kDa octameric ring and a 380 kDa hexadecameric catenane (locked-ring structure). The catenane formation was reported as biologically relevant in 2013 by Eldijk *et al* [54]. Surface sampling of dried solutions of this protein led to the detection of both of these oligomeric forms. The octameric ring structure was detected in charge states ranging from +26 to +31 between *m/z* 6000 to 8000 as shown in **Figure 1B**. In the same mass spectrum, the hexadecameric catenane is recorded in charge states between 35+ to 42+ (*m/z* range 9000-12000). Similar mass spectra of the CS_2 hydrolase were reported in earlier direct infusion native MS [49], and were obtained in this work: direct infusion nanoelectrospray of the same protein solution used for LESA MS resulted in the mass spectrum shown in **Figure S1C.** The fact that both biologically relevant oligomeric forms of CS_2 hydrolase survived the drying stage of the procedure and could then be detected by LESA MS emphasises the biological relevance of this method.

The largest soluble complex analysed here by LESA MS was GroEL, a chaperone protein which forms a ~800 kDa tetradecameric complex consisting of two ring heptamers. GroEL tetradecamer is a soluble protein complex which has often been used to test the upper mass range in native MS applications [21, 55-57]. GroEL was detected by LESA MS from dried protein samples in charge states ranging from 61+ to 68+ at *m/z* ~ 13100, 12900, 12750, 12500, 12350, 12200, 12000 and 11850 respectively (**Figure 1C)**. Previous native electrospray ionisation studies in ammonium acetate based solvent systems have reported the detection of the GroEL complex in the similar *m/z* range with slight variations in the charge state distribution that was apparently dependent on the type of mass spectrometer used [21, 56, 57]. We also detected unfolded monomeric subunits of GroEL at m/z < 4000 (~58 kDa, data not shown), which might be an indication of either partial degradation of the complex in the dry spots before LESA extraction, or collision induced dissociation in the ion source. Nevertheless, most of the GroEL tetradecamer was retained during the LESA procedure and produced mass spectra similar to native MS directly from solution, indicative of the similarity of the gas-phase GroEL ions formed in these two methods.

The native LESA MS approach was also applied to a different class of protein complexes: membrane proteins. The encapsulation of hydrophobic membrane complexes in detergent micelles is required to retain their stability in solution and the gas phase. Native ESI mass spectra of membrane protein complexes in micelles result in unstructured features due to the heterogeneity of the micelles. Collision activation is used to liberate the membrane complex from the gas-phase micelles and resolve its charge states [16]. We used a similar approach in our LESA MS experiment. AmtB is a membrane transporter protein which forms a trimeric complex with a mass of approximately 140 kDa. AmtB in C8E4 micelles in ammonium acetate solution was spotted on the glass slides and, after the spots were dried, was extracted and analysed by LESA MS using pure ammonium acetate solution. To resolve the charge states of this trimer complex we used an elevated collision voltage of 200 V in the trap part of the Synapt G2S. We detected charge states of the intact trimer ranging from +17 to +20 at *m/z* ~ 8400, 7900, 7400 and 7000 respectively. These peaks were relatively narrow, ≤ 50 Th wide (**Figure 1D**), in comparison with the width of the peaks of the soluble complexes (**Figure 1A-C**)**.** This effect is probably due to the fact that the membrane protein is not likely to retain many solvent molecules because of its limited hydrophilic surface and the large activation energy that had to be applied to its gas-phase ions in order to remove the detergent molecules and reveal the charge states. A similar mass spectrum of AmtB trimer was produced by direct infusion nanoelectrospray (**Figure S1D**).

Protein-Ligand Interactions

Biotin (244 Da) binds to avidin specifically and with very high affinity (one biotin molecule per each avidin monomer). This property is exploited in biotechnology to isolate biotinylated molecules in affinity assays or chromatography, where immobilised avidin or homologous proteins are used to anchor the molecules of interest [58]. As biotin binding is fast, a solution of biotin in ammonium acetate was used to sample dried spots of avidin by LESA in order to test whether the binding to avidin can take place during this procedure. Sampling with 200 mM ammonium acetate solution containing 1 mM biotin led to a mass shift for the major peaks of the charge of avidin tetramer, as shown in **Figure 1A inset**. This mass shift, averaged for 14+ to 16+ charge state, relates to an addition of ~ 960 Da, indicating that as many as four biotin molecules (244 Da × 4 = 976 Da) are bound to the avidin tetramer. At present we are limited by the resolving power of the Synapt mass spectrometer and the relatively small *m/z* range of the Orbitrap coupled to LESA in Birmingham (*m/z* < 4000) to demonstrate specific binding of biotin to avidin tetramer with higher resolution by this method. We have also tested biotin binding to avidin on the Orbitrap Q-Exactive (upper *m/z* limit 30000 Th, University of Oxford) by the direct infusion of avidin/biotin solution using a nanospray capillary**, Figure S2A, B**. As the fine structure of the charge states of avidin can be resolved on the Orbitrap, the mass shift of the peaks corresponding to biotin binding was calculated to be exactly 976 Da (four biotin molecules bound). Moreover, CID of the avidin/biotin non-covalent conjugate at 100 V collision voltage resulted in the production of apo-avidin tetramer (-976 Da mass shift, **Figure S2C**). Therefore, we conclude that implementing LESA MS on a higher-resolution/higher mass range instrument in future should be able to resolve the fine structure of multiply modified proteins and protein complexes and the exact stoichiometry of the drug binding to them.

Determination of the overall pharmacokinetic profile of a drug requires measurements of its affinity toward plasma proteins [59]. Native LESA MS was applied to the investigation of binding of biotin to two blood proteins, BSA and human haemoglobin, in a fashion similar to the avidin experiments. The degree of modification of wild type BSA is smaller than that of multiply glycosylated avidin. It was therefore possible to resolve peaks corresponding to the binding of individual biotin molecules (at least up to four) to BSA (charge states 13+ to 17+) on the Synapt G2S coupled to LESA, **Figure 2,** although the resolution was not high enough to estimate the maximum number of bound molecules. Biotin binding to haemoglobin dimers, $(\alpha\beta)^{2H}$ was also detected, with better mass resolution and relatively high signal intensity, in charge states ranging from +12 to +8 between m/z 2500 to 4250, **Figure 3**. At least seven molecules of biotin bound to the haemoglobin dimer were detected at zero collision energy in the trap region of Synapt G2S. The tetrameric haemoglobin $((\alpha\beta_2)^{4H})$ bound to biotin was also detected with a lower mass resolution in the charge states from 14+ to 16+, and with a positive mass shift in respect to the peaks of the charge states of unbound haemoglobin tetramers (data not shown)**,** but the exact number of the bound biotin molecules was impossible to estimate with this level of mass resolution. Peaks corresponding to α- and β- globin monomer and monomer-heme species in charge states ranging from 8+ to 16+ were also observed, but it is not apparent in the mass spectra whether any biotin binding took place for the monomers **(Figure 3B)**: it is, however, possible to conclude that apo-protein monomeric ions are much more abundant than the ions which might be corresponding to protein/biotin complexes, so the binding of biotin to the monomeric haemoglobin cannot be as strong as to the dimers. These results are in accord with a previously reported study on biotin binding to human blood plasma proteins [60]. Although the authors did not investigate the biotin binding to specific proteins, they found out that 12 % of biotin in plasma is covalently bound and 7 % is bound reversibly.

Non-specific binding of ligands to proteins or non-specific formation of non-covalent protein complexes is sometimes observed in native mass spectrometry [1, 2]. In order to exclude non-specific binding from the mass spectra, they are often collected either in a series with diminishing ligand or protein concentrations in the electrospray solution in order to decrease the chances of non-specific association in the electrospray microdroplets, or with extra ion activation (*e.g.,* carefully elevated sampling cone or collision voltage) to dissociate non-specific complexes in the gas-phase. LESA mass spectra of haemoglobin and haemoglobin / biotin with a collision voltage of 100 V in the trap region of Synapt G2S are shown in **Figure 3C and D.** These mass spectra demonstrate that some of the biotin molecules remain bound to haemoglobin dimers even under the harsh activation conditions. This result indicates that at least some of the biotin binding to haemoglobin heterodimers observed by LESA MS is specific and therefore biologically significant. Assuming that biotin does not bind specifically to unfolded haemoglobin monomers, the absence of strong MS signals corresponding to biotin binding to the monomers haemoglobin **(Figure 3B, D**) also indicates that non-specific binding in our LESA experiments was minimal.

Conclusions

Liquid extraction surface analysis (LESA) of protein species on glass substrates has been shown to be successful for protein complexes up to ~800 kDa (GroEL). This mass is significantly greater than previously reported (~64 kDa, haemoglobin). Remarkably, the investigated complexes could survive a relatively long time, from 20 min to over an hour, on the glass substrate before their extraction by LESA. The results also show that native LESA MS has potential for applications in membrane protein studies that involve the use of stabilising detergent micelles, as demonstrated by the successful LESA MS detection of the AmtB membrane complex. Furthermore, the potential of native LESA MS to probe protein-ligand interactions has been shown for the first time with the binding of biotin to avidin, BSA and haemoglobin. The results obtained for biotin binding to avidin confirmed that avidin remained in the biologically active state during LESA. As our previous results for haemoglobin demonstrated [47], improved mass resolution can be achieved by interfacing this method with mass spectrometers of higher resolving power, e.g. Orbitrap.

In summary, native LESA mass spectrometry is (1) suitable for the analysis of high mass protein complexes; (2) interrogating complexes of both soluble and membrane proteins; and (3) probing protein-ligand interactions. The results are highly encouraging for future attempts to extend the use of the native LESA MS method to the analysis of protein/ligand binding assays, the analysis of membrane protein complexes from biomimicking membranes, and *in situ* analysis of heterogeneous biological surfaces, such as dry blood spots and tissue sections.

Acknowledgments

RLG and HJC are funded by EPSRC (EP/L023490/1). Waters Corporation is acknowledged for a Fellowship to VAM. The Waters Synapt G2S mass spectrometer was funded by EPSRC (EP/K039245/1). Supplementary data supporting this research is openly available from the University of Birmingham data archive at http://findit.bham.ac.uk/. Joseph Gault and Denis Shutin (Carol Robinson's group) from the University of Oxford are acknowledged for providing a sample of AmtB and their help with collecting MS data on the Orbitrap Q-Exactive.

References

1. Hernandez, H. and C.V. Robinson, *Determining the stoichiometry and interactions of macromolecular assemblies from mass spectrometry.* Nature Protocols, 2007. **2**(3): p. 715-726.
2. Heck, A.J. and R.H. van den Heuvel, *Investigation of intact protein complexes by mass spectrometry.* Mass spectrometry reviews, 2004. **23**(5): p. 368-389.
3. Sharon, M., *Structural MS Pulls Its Weight.* Science, 2013. **340**(6136): p. 1059-1060.
4. Marcoux, J. and C.V. Robinson, *Twenty years of gas phase structural biology.* Structure (London, England : 1993), 2013. **21**(9): p. 1541-50.
5. Hall, Z., A. Politis, and C.V. Robinson, *Structural Modeling of Heteromeric Protein Complexes from Disassembly Pathways and Ion Mobility-Mass Spectrometry.* Structure, 2012. **20**(9): p. 1596-1609.
6. Politis, A., et al., *Integrating Ion Mobility Mass Spectrometry with Molecular Modelling to Determine the Architecture of Multiprotein Complexes.* Plos One, 2010. **5**(8).
7. van Duijn, E., et al., *Chaperonin Complexes Monitored by Ion Mobility Mass Spectrometry.* Journal of the American Chemical Society, 2009. **131**(4): p. 1452-1459.
8. Benesch, J.L.P., *Collisional Activation of Protein Complexes: Picking Up the Pieces.* Journal of the American Society for Mass Spectrometry, 2009. **20**(3): p. 341-348.

9. Zhou, M., C. Huang, and V.H. Wysocki, *Surface-Induced Dissociation of Ion Mobility-Separated Noncovalent Complexes in a Quadrupole/Time-of-Flight Mass Spectrometer.* Analytical Chemistry, 2012. **84**(14): p. 6016-6023.
10. Wales, T.E. and J.R. Engen, *Hydrogen exchange mass spectrometry for the analysis of protein dynamics.* Mass Spectrometry Reviews, 2006. **25**(1): p. 158-170.
11. Engen, J.R., *Analysis of Protein Conformation and Dynamics by Hydrogen/Deuterium Exchange MS.* Analytical Chemistry, 2009. **81**(19): p. 7870-7875.
12. Konermann, L., X. Tong, and Y. Pan, *Protein structure and dynamics studied by mass spectrometry: H/D exchange, hydroxyl radical labeling, and related approaches.* Journal of Mass Spectrometry, 2008. **43**(8): p. 1021-1036.
13. Takamoto, K. and M.R. Chance, *Radiolytic protein footprinting with mass Spectrometry to probe the structure of macromolecular complexes.* Annual Review of Biophysics and Biomolecular Structure, 2006. **35**: p. 251-276.
14. Pan, Y., et al., *Structural Characterization of an Integral Membrane Protein in Its Natural Lipid Environment by Oxidative Methionine Labeling and Mass Spectrometry.* Analytical Chemistry, 2009. **81**(1): p. 28-35.
15. Barrera, N.P., et al., *Micelles protect membrane complexes from solution to vacuum.* Science, 2008. **321**(5886): p. 243-246.
16. Laganowsky, A., et al., *Mass spectrometry of intact membrane protein complexes.* Nature Protocols, 2013. **8**(4): p. 639-651.
17. Laganowsky, A., et al., *Membrane proteins bind lipids selectively to modulate their structure and function.* Nature, 2014. **510**(7503): p. 172-+.
18. Marcoux, J., et al., *Mass spectrometry reveals synergistic effects of nucleotides, lipids, and drugs binding to a multidrug resistance efflux pump.* Proceedings of the National Academy of Sciences of the United States of America, 2013. **110**(24): p. 9704-9709.
19. Sobott, F., et al., *A tandem mass spectrometer for improved transmission and analysis of large macromolecular assemblies.* Analytical Chemistry, 2002. **74**(6): p. 1402-1407.
20. Li, H., et al., *Native Top-Down Electrospray Ionization-Mass Spectrometry of 158 kDa Protein Complex by High-Resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometry.* Analytical Chemistry, 2013. **86**(1): p. 317-320.
21. Rose, R.J., et al., *High-sensitivity Orbitrap mass analysis of intact macromolecular assemblies.* Nature Methods, 2012. **9**(11): p. 1084-+.
22. Snijder, J., et al., *Studying 18 MDa Virus Assemblies with Native Mass Spectrometry.* Angewandte Chemie International Edition, 2013. **52**(14): p. 4020-4023.
23. Lossl, P., J. Snijder, and A.J.R. Heck, *Boundaries of Mass Resolution in Native Mass Spectrometry.* Journal of the American Society for Mass Spectrometry, 2014. **25**(6): p. 906-917.
24. Eikel, D., et al., *Liquid extraction surface analysis mass spectrometry (LESA-MS) as a novel profiling tool for drug distribution and metabolism analysis: the terfenadine example.* Rapid Communications in Mass Spectrometry, 2011. **25**(23): p. 3587-3596.
25. Rao, W., et al., *Ambient DESI and LESA-MS Analysis of Proteins Adsorbed to a Biomaterial Surface Using In-Situ Surface Tryptic Digestion.* Journal of the American Society for Mass Spectrometry, 2013. **24**(12): p. 1927-1936.
26. Takáts, Z., et al., *Mass Spectrometry Sampling Under Ambient Conditions with Desorption Electrospray Ionization.* Science, 2004. **306**(5695): p. 471-473.
27. Shin, Y.-S., et al., *Desorption Electrospray Ionization-Mass Spectrometry of Proteins.* Analytical Chemistry, 2007. **79**(9): p. 3514-3518.
28. Stokes, A.A., et al., *Top-down protein sequencing by CID and ECD using desorption electrospray ionisation (DESI) and high-field FTICR mass spectrometry.* International Journal of Mass Spectrometry, 2010. **289**(1): p. 54-57.

29. Ferguson, C.N., et al., *Direct Ionization of Large Proteins and Protein Complexes by Desorption Electrospray Ionization-Mass Spectrometry.* Analytical Chemistry, 2011. **83**(17): p. 6468-6473.
30. Liu, P., et al., *Measuring Protein–Ligand Interactions Using Liquid Sample Desorption Electrospray Ionization Mass Spectrometry.* Analytical Chemistry, 2013. **85**(24): p. 11966-11972.
31. Douglass, K.A. and A.R. Venter, *Protein analysis by desorption electrospray ionization mass spectrometry and related methods.* Journal of Mass Spectrometry, 2013. **48**(5): p. 553-560.
32. Wiseman, J.M., et al., *Desorption electrospray ionization mass spectrometry: Imaging drugs and metabolites in tissues.* Proceedings of the National Academy of Sciences, 2008. **105**(47): p. 18120-18125.
33. Yao, C., et al., *Screening of the Binding of Small Molecules to Proteins by Desorption Electrospray Ionization Mass Spectrometry Combined with Protein Microarray.* Journal of The American Society for Mass Spectrometry, 2015. **26**(11): p. 1950-1958.
34. Annis, D.A., et al., *Affinity selection-mass spectrometry screening techniques for small molecule drug discovery.* Current Opinion in Chemical Biology, 2007. **11**(5): p. 518-526.
35. Van Berkel, G.J., V. Kertesz, and R.C. King, *High-Throughput Mode Liquid Microjunction Surface Sampling Probe.* Analytical Chemistry, 2009. **81**(16): p. 7096-7101.
36. Edwards, R.L., et al., *Hemoglobin Variant Analysis via Direct Surface Sampling of Dried Blood Spots Coupled with High-Resolution Mass Spectrometry.* Analytical Chemistry, 2011. **83**(6): p. 2265-2270.
37. Edwards, R., et al., *Top-Down Proteomics and Direct Surface Sampling of Neonatal Dried Blood Spots: Diagnosis of Unknown Hemoglobin Variants.* Journal of The American Society for Mass Spectrometry, 2012. **23**(11): p. 1921-1930.
38. Edwards, R.L., et al., *Compound heterozygotes and beta-thalassemia: Top-down mass spectrometry for detection of hemoglobinopathies.* Proteomics, 2014. **14**(10): p. 1232-1238.
39. Griffiths, R.L., et al., *Liquid extraction surface analysis field asymmetric waveform ion mobility spectrometry mass spectrometry for the analysis of dried blood spots.* Analyst, 2015. **140**(20): p. 6879-6885.
40. Sarsby, J., et al., *Top-Down and Bottom-Up Identification of Proteins by Liquid Extraction Surface Analysis Mass Spectrometry of Healthy and Diseased Human Liver Tissue.* Journal of The American Society for Mass Spectrometry, 2014. **25**(11): p. 1953-1961.
41. Sarsby, J., et al., *Liquid extraction surface analysis mass spectrometry coupled with field asymmetric waveform ion mobility spectrometry for analysis of intact proteins from biological substrates.* Analytical Chemistry, 2015. **87**(13): p. 6794-6800.
42. Rian L. Griffiths, et al., *LESA FAIMS mass spectrometry for the spatial profiling of proteins from tissue.* Analytical Chemistry Under Revision, 2016.
43. Griffiths, R.L., et al., *Formal Lithium Fixation Improves Direct Analysis of Lipids in Tissue by Mass Spectrometry.* Analytical Chemistry, 2013. **85**(15): p. 7146-7153.
44. Randall, E.C., J. Bunch, and H.J. Cooper, *Direct Analysis of Intact Proteins from Escherichia coli Colonies by Liquid Extraction Surface Analysis Mass Spectrometry.* Analytical Chemistry, 2014. **86**(21): p. 10504-10510.
45. Hsu, C.-C., P.-T. Chou, and R.N. Zare, *Imaging of Proteins in Tissue Samples Using Nanospray Desorption Electrospray Ionization Mass Spectrometry.* Analytical Chemistry, 2015. **87**(22): p. 11171-11175.
46. Roach, P.J., J. Laskin, and A. Laskin, *Nanospray desorption electrospray ionization: an ambient method for liquid-extraction surface sampling in mass spectrometry.* Analyst, 2010. **135**(9): p. 2233-2236.
47. Martin, N., et al., *Native Liquid Extraction Surface Analysis Mass Spectrometry: Analysis of Noncovalent Protein Complexes Directly from Dried Substrates.* Journal of The American Society for Mass Spectrometry, 2015: p. 1-8.

48. Griffiths, R.L. and H.J. Cooper, *Direct Tissue Profiling of Protein Complexes: Toward Native Mass Spectrometry Imaging.* Analytical Chemistry, 2016. **88**(1): p. 606-609.
49. van Eldijk, M.B., et al., *Evidence that the catenane form of CS2 hydrolase is not an artefact.* Chemical Communications, 2013. **49**(71): p. 7770-7772.
50. Reading, E., et al., *The Role of the Detergent Micelle in Preserving the Structure of Membrane Proteins in the Gas Phase.* Angewandte Chemie-International Edition, 2015. **54**(15): p. 4577-4581.
51. Schwartz, B.L., K.J. Light-Wahl, and R.D. Smith, *Observation of noncovalent complexes to the avidin tetramer by electrospray ionization mass spectrometry.* Journal of the American Society for Mass Spectrometry, 1994. **5**(3): p. 201-204.
52. Martin, N.J., et al., *Native Liquid Extraction Surface Analysis Mass Spectrometry: Analysis of Noncovalent Protein Complexes Directly from Dried Substrates.* Journal of The American Society for Mass Spectrometry, 2015: p. 1-8.
53. Bush, M.F., et al., *Collision Cross Sections of Proteins and Their Complexes: A Calibration Framework and Database for Gas-Phase Structural Biology.* Analytical Chemistry, 2010. **82**(22): p. 9557-9565.
54. van Eldijk, M.B., et al., *Evidence that the catenane form of CS 2 hydrolase is not an artefact.* Chemical Communications, 2013. **49**(71): p. 7770-7772.
55. Benesch, J.L.P., et al., *Protein complexes in the gas phase: Technology for structural genomics and proteomics.* Chemical Reviews, 2007. **107**(8): p. 3544-3567.
56. Mikhailov, V.A., et al., *Mass-Selective Soft-Landing of Protein Assemblies with Controlled Landing Energies.* Analytical Chemistry, 2014. **86**(16): p. 8321-8328.
57. Dyachenko, A., et al., *Allosteric mechanisms can be distinguished using structural mass spectrometry.* Proceedings of the National Academy of Sciences, 2013. **110**(18): p. 7235-7239.
58. Morag, E., E.A. Bayer, and M. Wilchek, *Immobilized nitro-avidin and nitro-streptavidin as reusable affinity matrices for application in avidin-biotin technology.* Analytical Biochemistry, 1996. **243**(2): p. 257-263.
59. Brauckmann, C., et al., *The interaction of platinum-based drugs with native biologically relevant proteins.* Analytical and Bioanalytical Chemistry, 2013. **405**(6): p. 1855-1864.
60. Mock, D.M. and M.I. Malik, *Distribution of biotin in human plasma: most of the biotin in plasma is not bound to protein.* Am. J. Clin. Nutr., 1992. **56**: p. 427-432.

Figure 1 Native LESA mass spectra of protein complexes from glass substrate. A) Mass spectrum of avidin tetramer (64 kDa) after collision activation of avidin ions in the trap region (100 V collision voltage). Peaks of fragment monomers (*m/z* $\leq$ 2000) show fine structure corresponding to multiple modifications. Inset: peaks of avidin sampled with 1 mM biotin solution (bottom) are mass-shifted in respect to avidin sampled with pure ammonium acetate (top) in accordance with binding four biotin molecules (no collisional activation used). B) CS_2 hydrolase: peaks correspond to ring octamer (189 kDa) and catenane hexadecamer (378 kDa). C) GroEL tetradecamer (803 kDa). D) AmtB trimer (140 kDa): a membrane protein complex in C8E4 micelles. Collision activation (200 V) was used in the trap region to strip the complex off the detergent molecules.

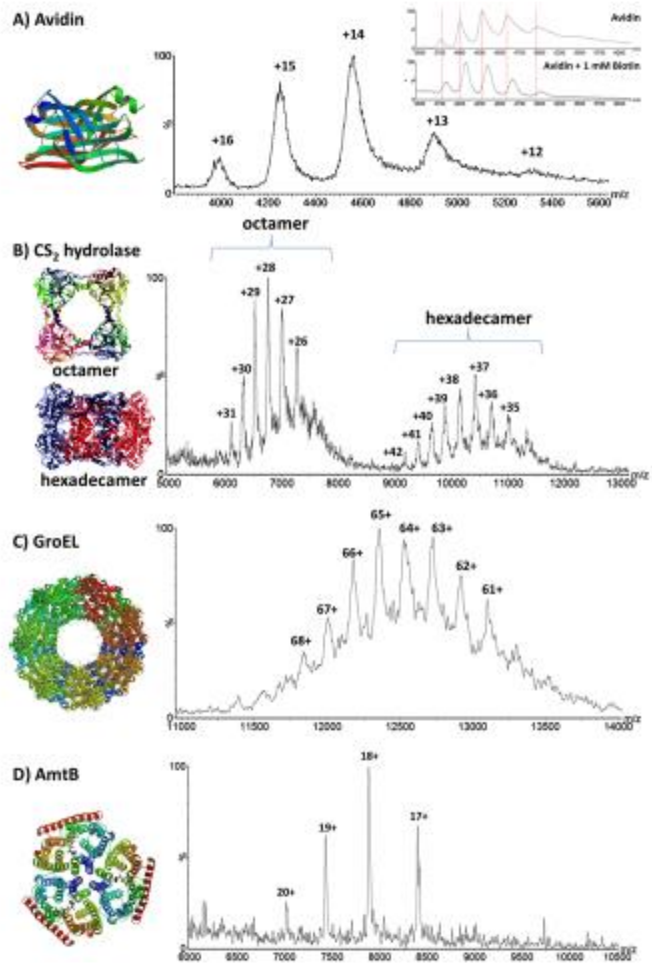


Figure 2 Native LESA mass spectra of BSA from glass substrate. A) BSA spots sampled with 200 mM ammonium acetate. B) BSA spots sampled with 200 mM ammonium acetate / 1 mM biotin.

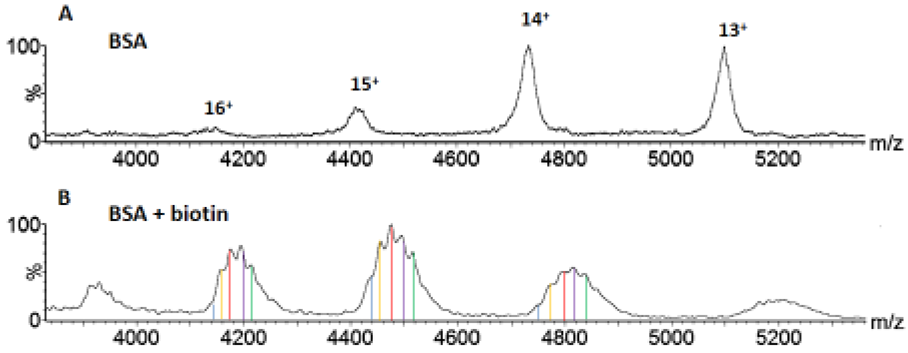


Figure 3 Native LESA mass spectra of haemoglobin from glass substrate. A) and C) Haemoglobin spots sampled with 200 mM ammonium acetate. B) and D) Haemoglobin spots sampled with 200 mM ammonium acetate/ 1 mM biotin. C) and D) 100 V trap collision voltage was used.

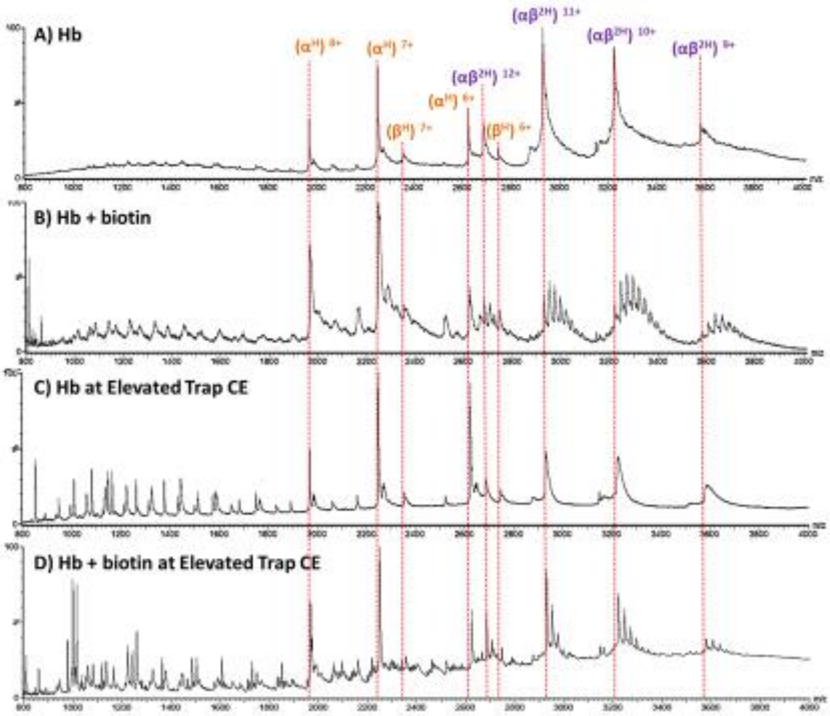


Table 1. Parameters of LESA extraction procedure and ion source conditions.

Protein	Volume aspirated from solvent well (µl)	Height above the surface (mm)	Volume dispensed onto the surface (µl)	Delay time (secs)	Volume reaspirated (µl)	Gas pressure (psi)	Cone Voltage (kV)
AmtB	2.0	0.8	1.5	60	2.0	0.3	1.9
CS_2 hydrolase	2.0	0.8	1.5	60	2.0	0.3	1.9
GroEL	4.0	0.6	2.0	30	2.5	0.15	1.8
Haemoglobin	4.0	0.6	2.0	30	2.5	0.15	1.8
Avidin	4.0	0.6	2.0	30	2.5	0.15	1.8
BSA	4.0	0.6	2.0	30	2.5	0.15	1.8