https://doi.org/10.1016/j.aca.2017.11.079

Detection of Native Proteins using Solid-substrate Electrospray Ionization Mass Spectrometry with 0. Solvents

Bin Hu[a,b] and Zhong-Ping Yao[a,c,d*]

[a]State Key Laboratory for Chirosciences, Food Safety and Technology Research Centre and Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong SAR, China

[b]Institute of Mass Spectrometer and Atmospheric Environment, Jinan University, Guangzhou 510632, China

[c]State Key Laboratory of Chinese Medicine and Molecular Pharmacology (Incubation) and Shenzhen Key Laboratory of Food Biological Safety Control, Shenzhen Research Institute of The Hong Kong Polytechnic University, Shenzhen 518057, China

[d]Key Laboratory of Natural Resources of Changbai Mountain and Functional Molecules (Yanbian University), Ministry of Education, Yanji, Jilin 133002, China

* Corresponding author:

Dr. Zhong-Ping Yao

Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong Special Administrative Region, China

Tel: (852)3400-8792. Fax: (852)2364-9932.

E-mail: zhongping.yao@polyu.edu.hk

Abstract

Detection of native proteins, particularly directly from raw biological samples, has been a challenging task for mass spectrometry. In this study, we demonstrated that solid-substrate electrospray ionization mass spectrometry with nonpolar solvents such as n-hexane could allow detection of native proteins and protein complexes directly from raw biological samples. Mechanistic study revealed that the process involved rapid vaporization of the nonpolar solvent, temperature reduction of substrate surface, condensation of water from the ambient air, and spray ionization of the condensed water with analytes under the electric field. The fine spray with water at low temperature allowed the technique to detect native proteins, even directly from viscous samples (e.g., egg white) and solid samples (e.g., bone marrow). This study sheds new insight into the sampling and ionization process of mass spectrometry and provides a technique of great potential for characterization of proteins.

Key word: Native protein; Nonpolar solvent; Solid substrate; Electrospray ionization; Mass spectrometry.

1. Introduction

Characterization of proteins plays a critical role in fundamental and applied research in chemistry, biology and medicine. Techniques such as mass spectrometry (MS) [1, 2], nuclear magnetic resonance spectroscopy [3, 4] and X-ray crystallography [5, 6] are widely used for investigation of protein structures, conformations and interactions. Compared with other techniques, MS is advantageous in speed, sensitivity and specificity for characterization of proteins. Electrospray ionization (ESI) is a very gentle ionization technique in MS and has been commonly used to measure protein masses and investigate protein conformations and interactions [7-9]. Under ESI conditions, a protein is typically detected as a charge state distribution (CSD) that could be related to the protein conformation [7-9]. Native MS has been developed to enable detection of proteins in their native states [2, 10-15]. Such studies typically involved desalting and buffer exchange of the raw protein solutions into aqueous solutions with volatile buffers such as ammonia acetate, and analysis of the resulting solutions under very gentle ESI (or nanoESI) conditions. Direct detection of native proteins and protein complexes from real-life raw biological samples still poses a big challenge to MS, mainly due to the signal suppression of interferences such as salts and matrices present in the raw samples and the fact that conventional ESI cannot directly analyze some forms of samples such as viscous samples and solid samples.

In the last decade, ambient mass spectrometry was developed for direct analysis of raw samples under atmospheric conditions [16-20], and proteins have been the important analytes of these research efforts [18]. Various ambient ionization techniques, including desorption ESI (DESI) [20-23], nano-DESI [24], liquid-sample DESI [25, 26], electrospray laser desorption/ionization (ELDI) [27], extractive ESI [28, 29] and liquid

extraction surface analysis (LESA) [30] and others [18, 23] have been employed for analysis of proteins from various samples. However, very few successes have been achieved in direct detection of native proteins and protein complexes from real-life raw biological samples [18].

In recent years, much attention has been paid to electrospray ionization on solid substrates (e.g., copper coil, stainless steel needle, paper, wooden tip, aluminium foil and biological tissue) [31-38]. Unlike conventional ESI that uses capillaries that are relatively closed and with inert and smooth surfaces, solid-substrate ESI uses substrates with surfaces that can be porous and contain active functional groups for sample loading and ionization, and the samples on the substrates are exposed to the ambient air during the analysis [39]. Solid-substrate ESI thus can be used to directly analyze various forms of samples, and it has shown some features that are different from those of conventional capillary-based ESI. For example, it was found that ESI with stainless steel needles was more tolerant to salts and detergents for analysis of protein solutions [40, 41] and ESI with porous tips enabled separation and detection of proteins based on their sizes and shapes [38]. With simple sample preparation, ESI with paper tips (paper spray) could detect noncovalent protein complexes from human blood detritus without plasma [42]. Another interesting feature is that, unlike capillary-based ESI that typically uses relatively polar solvents such as water, methanol and acetonitrile as the ionization solvents and considers nonpolar solvents such as n-hexane as non-ESI-friendly solvents [43], ESI with paper [44] showed that nonpolar solvents such as n-hexane could allow ionization of small molecules including drugs, peptides, nucleotides and phospholipids. However, the mechanism for this is still obscure [44]. In this study, we demonstrated for the first time that solid-substrate ESI-MS with nonpolar solvents could allow

detection of native proteins, even from raw biological samples, and systematically investigated its underlying mechanism.

2. Experimental

2.1. Chemicals and materials

Proteins (lysozyme and myoglobin), chemicals (D_2O and reserpine) and organic solvents (n-alkanes) used in this study were purchased from Sigma (St. Louis, MO, USA). Water was Milli-Q water generated from a Synthesis A10 Milli-Q water generator (Millipore, Billerica, MA, USA). Wooden tips (wooden toothpicks, brand: Bestbuy), fresh eggs, and fresh porcine ribs were purchased from ParknShop (Hong Kong). The tip-end OD of the wooden tips was originally ~ 0.5 mm and further cut to ~ 0.2 mm before use [33]. The pore sizes of the wooden tips were at micrometer levels as measured using a scanning electron microscope (SEM) (Model: VEGA 3, TESCAN, Czech Republic) (see Figure S-1 for the SEM images), and the water content of the wooden tips was 9.34 ± 0.03 % (w/w) (n = 3) as measured by comparing the weights of the wooden tips before and after drying in an oven (Model: UM 400, Memmert-GmbH & Co., KG, Germany) at 105 ºC for 6 hours.

2.2. Mass spectrometric analysis of proteins

NanoESI-MS: NanoESI-MS experiments were carried out on a quadrupole time-of-flight mass spectrometer (Q-TOF2, Micromass, Manchester, UK). High voltage and cone voltage were set at 1.8 kV and 30 V, respectively. The ion source temperature was set at 20 ºC. The collision gas was set at 15 psi, and the collision energy was 10 eV.

NanoESI emitters, i.e., nanoESI needles, were home-made with glass capillaries using a puller (Sutter Instrument, Novato, CA, USA) and a coater (Quorum Technologies, West Sussex, UK).

Wooden-tip ESI-MS: As shown in Figure 1a, similar to nanoESI needles, wooden tips (diameter: 2.0 mm, tip-end size: ~ 0.2 mm, length: 1.5 cm) were held by the Z-spray nanoESI ion source device (Micromass, Manchester, UK), as described in our previous work.[30] High voltage and cone voltage were set at 3.5 kV and 30 V, respectively. The ion source temperature was set at 20 ºC. The collision gas was set at 15 psi, and the collision energy was 10 eV. These wooden tips were perpendicularly placed to the mass spectrometer inlet with the tip end ~ 8.0 mm away from the MS inlet. To prepare a fixed amount of analytes (e.g. reserpine: 100 ng), 10 μL solution of analytes was loaded onto the wooden tips by pipette tips and then dried in the air for at least 20 minutes. Solid powders of protein standards (less than 1 mg) and fresh egg white (5 μL) were directly deposited onto the wooden tips for the analysis.

Solid-substrate ESI-MS of bone marrow: As shown in Figure 1b, bone marrow was cut into a triangle (sharp-end size: ~ 0.2 mm, bottom width: 2.0 mm, thickness: 1.0 mm, length: 4.0 mm, weight: 6.7 mg), pre-deposited with ammonium acetate powder (less than 0.1 mg), and was held with a metal clip to connect the high voltage supply of the mass spectrometer, as described in our previous work [45]. The biological tissue triangles were put parallel to the MS inlet with the sharp end ~ 8.0 mm away from the MS inlet.

Other MS settings: Various parameters, including the voltage of microchannel plate, MS profile and pumping pressure, were optimized to enhance the detection of native

proteins from the samples. The collision gas was set at 15 psi and the collision energy was set at 10 % - 30 % for the MS/MS experiments with the isolation width of 4 Da.

2.3. Others

Loading of non-polar solvents: Aliquots of non-polar solvents (e.g., 10 μL of n-alkanes) were rapidly loaded onto wooden tips and biological tissue using a micro-syringe (syringe code: 10F-7, syringe volume: 10 μL, SGE Analytical Science, Victoria, Australia) with a long (length: 70 mm) and small (OD: 0.47 mm) needle to eliminate evaporation of the nonpolar solvents before loaded onto the substrates.

Control of atmospheric conditions: The room temperature was at 20 ± 0.5 ºC and the relative humidity was at 60.0 ± 2.0 % in the laboratory. A nebulizer of water (Agilent Technologies, Santa Clara, CA, USA) was used to increase the humidity and gas flow of dry nitrogen was used to reduce the humidity. Relative humidity was monitored by a digital hygrothermograph (Tecpel Co., Ltd, Taipei, Taiwan).

Measurement of temperature: Temperature changes of wooden tips during evaporation of n-alkanes were measured using an Infrared Thermometer (model: ST663, Sentry Optronic Corp, Taipei, Taiwan), which shot a laser (diameter: 1-2 mm) onto the surface of the wooden tip to measure the local temperature of the surface.

Video recording: Growth and melting of ice dendrites as well as spray ionization on the wooden tips with pentane (or hexane) was visualized and recorded using a Sony T20 camera (Japan) with the assistance of a KL 1500 LCD halogen cold light lamp (Schott,

Mainz, Germany). Optical images of the ESI plume (Figure 4 and Figure S-3) were extracted from Video.

3. Results and discussion

3.1. Analysis of proteins

Powder of lysozyme, a typical protein sample, was attempted for analysis using this technique with a wooden tip and hexane as the added solvent. As shown in Figure 2a, very good signals of lysozyme were observed and the narrow charge state distribution (CSD) at high *m/z* range indicated that the protein was detected in its native state. To further confirm this, a myoglobin powder sample was analyzed (Figure 2c), and intact myoglobin without loss of the heme was observed at narrow charge states, demonstrating that the protein was detected at its native state. To our knowledge, this might be the first observation of directly using nonpolar solvents for ESI-MS analysis of proteins. Measurement using dried solution sample showed that 10 pmol lysozyme could gave S/N > 3, indicating the high sensitivity of the technique. Compared with the spectra obtained by nanoESI-MS analysis of aqueous solutions of lysozyme and myoglobin (Figures 2b and 2d), the spectra obtained by wooden-tip ESI-MS with n-hexane (Figures 2a and 2c) offered more narrow CSDs centred at higher *m/z* values. These observations were inconsistent with the previous assumptions that the ionization of analytes with nonpolar solvents might involve field desorption or spray ionization with nonpolar solvents [44], since native proteins normally are difficult to be detected by field desorption, which also typically does not generate a charge-state distribution of the analyte, and the proteins are not soluble in n-hexane [46, 47]. These results also indicated that n-hexane might not be the actual ionization solvent.

3.2. The role of moisture in the ambient air

To obtain insight into the ionization mechanism, we first investigated the source of protons for protonation observed in the spectra. Reserpine was analyzed using the technique with D_2O sprayed in the ambient air, in comparison with that obtained with H_2O at the same relative humidity (Rh), i.e., 70%. As shown in Figures S-2a and S-2b, addition of D_2O in the ambient air significantly increased abundance of *m/z* 610 that corresponded to $[reserpine+D]^+$. Furthermore, as showed in Figures S-2c and S-2d, the addition of D_2O in the ambient air for analysis of lysozyme caused increased *m/z* values of the protein ions, indicating that some mobile hydrogens of the protein exchanged with the deuteriums. This could be caused by the hydrogen/deuterium exchange occurred in the gas phase, or more likely that the moisture in the ambient air was condensed to serve as the solvent for the ionization.

To confirm the role of the moisture in the ambient air, the effect of the relative humidity of the ambient air on the detection of analytes was investigated. Wooden-tip ESI-MS was used to analyze reserpine pre-deposited on the tip end at a humidity range of 52% - 90% in the ambient air, with n-hexane as the added solvent. As shown in Figure 3a, the detected intensity of reserpine was found to increase linearly with the increasing humidity. This result was reasonable since at higher humidity more water could be condensed to dissolve more analyte to be detected, and further confirmed that the condensed water was responsible for ionization of the analytes. Effects of humidity on the ionization behavior of analytes had only been previously reported in plasma-based ionization methods [48-50]. This study showed its effect on ionization of ESI-based techniques and provides an additional pathway for protonation of analytes.

3.3. Effects of non-polar solvents

Different nonpolar solvents, e.g., n-alkanes with carbon number from 5 to 10, have different evaporation rates [51] and thus different efficiency for water condensation. In this study, reserpine deposited on wooden tip was analyzed with various n-alkanes, in order to investigate their effects on the analysis. As shown in Figure 3b, the signal intensity of reserpine was found to be well correlated with the carbon number and evaporation rate of the solvent. The strongest intensity of reserpine was obtained with n-pentane, which has the fastest evaporation rate [51] and could lead to the lowest surface temperature (-14.3 ± 0.4 ºC) and largest amount of water condensed to dissolve more analytes on the surface of wooden tip. Solvent evaporation and water condensation was also remarkable for n-hexane, but was significantly reduced for the higher homologues (C7 - C10) with lower evaporation rates [51], correspondingly leading to higher temperatures on the substrate surface (0.1 ± 0.4, 13.4 ± 0.5, 14.7 ± 0.4, 17.8 ± 0.3 and 18.2 ± 0.3 ºC for n-hexane, n-heptane, n-octane, n-nonane and n-decane, respectively, as measured), less water condensed for analyte dissolution, and shorter signal duration (Figure S-3) and weaker accumulated signals of the analyte.

As shown in Figure S-3, n-pentane offered the longest but fluctuant signals. This was believed to be related to formation of ice dendrites that were only available with n-pentane. We propose that when n-pentane was used, the analyte on the substrate surface was initially dissolved in the condensed water which was immediately frozen and grown into ice dendrites. The ice dendrites were quickly melted and electrospray was induced from their tips. Some tips of the ice dendrites might not contain any analyte, but the nearby ice that contained the analyte were melted at the same time and moved to the spraying tip end to produce signals. This process, together with the multiple sprayings

from different dendrites (see Figure 4a), caused the signal fluctuation. n-hexane offered stable and relatively strong signals of the sample. A photo of wooden-tip ESI with n-hexane as the added solvent is shown in Figure S-4. A spray plume much smaller than that of normal wooden-tip ESI [37] could be observed.

Compared with other n-alkanes used for the investigation, n-pentane has a much higher evaporation rate, and formation of ice dendrites was observed when n-pentane was used as the added solvent (Figure 4a), a result that was consistent with the previous observation [44]. In this study, the growth of ice dendrites and their roles in the ionization process were investigated. The applied electric field was found to have significant effect on the growth of the ice dendrites. As shown in Figure 4b, without electric field, only ice was observed on the tip surface; under lower electric fields (i.e., 0.5 - 2.0 kV), ice dendrites were formed but only at the tip end, presumably due to intensification of the electric field at the tip end, and the length of ice dendrites increased with the increased voltage; under higher electric fields (i.e., 2.5 - 4.5 kV), ice dendrites grew more and more in a wider range of the tip surface with the increased voltage, but the length of the ice dendrites no longer increased. This observed effect of electric field on the growth of ice dendrites was believed to be caused by the electric field accelerating the flux of water and the substrate (i.e., the wooden tip) providing a suitable electro-nucleation for formation of ice dendrites [52-54]. The ice was found to melt almost immediately, and under the applied electric field, spraying was observed from the ice dendrites and wooden tip (see the video in Supporting Information), generating the mass spectra. The applied electric-field induced formation of ice dendrites as well as spray ionization with the melting of ice.

3.4. Mechanism of solid-substrate ESI-MS with nonpolar solvents

According to the above observations, as shown in Figure 1c, the mechanism of solid-substrate ESI-MS with nonpolar solvents was proposed as below: 1) The added low-boiling-point nonpolar solvent is rapidly evaporated, causing absorption of heat, rapid reduction of ambient temperature, and subsequently rapid condensation of water in the ambient air onto the substrate surface. 2) Under the applied electric field, spray ionization is induced from the sharp end of the solid substrate and ice dendrites with the condensed or melted water as the solvent. Nonpolar solvent is evaporated mainly before or during the spray process, and the observed spectra are about the analytes in the water. Non-polar solvents enable condensation of water from the ambient air and allow the spray ionization to occur at lower temperatures and with fine plume. These are believed to lead to detection of proteins in their native states.

3.5. Detection of native proteins from egg white

The technique was then explored for detection of native proteins from raw biological samples. n-Hexane was chosen as the non-polar solvent for the analysis due to its stable and reasonably high signals. In this study, the technique was attempted to detect proteins from raw egg white, which contains proteins such as lysozyme and ovalbumin that are of importance to biological properties and nutritional values [55-57]. Egg white is highly viscous and contains complex biological matrices, and direct detection of native proteins from egg white has represented an analytical challenge. Although some MS techniques have been recently developed for direct analysis of viscous samples, they are limited to analysis of small molecules [58-60], and successful detection of intact proteins from egg white typically requires extensive sample preparation prior to the MS analysis [61]. In this study, ~ 5 μL of fresh egg white was deposited onto a wooden tip and analyzed with n-hexane as the added solvent. As shown in Figure 5a, lysozyme and ovalbumin were

predominately observed in the spectrum. Ovalbumin was observed with a narrow CSD at +10, +11 and +12, an observation that was in agreement with the results obtained for detection of native ovalbumin using a modified equipment for analysis of ovalbumin standard in buffer solution [61]. For lysozyme, the observed narrow CSD at +7, +8 and +9 was in good agreement with that previously obtained using the present technique for analysis of the lysozyme standard (Figure 2a), confirming the observation of native lysozyme from the raw sample. According to the concentration of lysozyme in fresh egg white (~ 0.34 % (w/w)) [55, 62], the analyzed egg white sample contained ~ 1.2 nmol lysozyme. The unambiguous detection of native lysozyme from egg white indicated the good sensitivity of the technique for detection of native proteins from raw samples. It was noted that the lysozyme peaks obtained from the egg white (Figure 5) had slightly larger *m/z* values than those from the standard (Figure 2a), i.e., *m/z* 1599.0, 1800.0 and 2062.9 vs.1590.4, 1789.1 and 2044.6 for +9, +8 and +7 ions, respectively, corresponding to a mass increase of 90 Da. Ion at *m/z* 1800 (+8) was selected for collision-induced dissociation and fragment ions corresponding to loss of 1, 2, 3, 4 and 5 water molecules were observed in the MS/MS spectrum (Figure S-5), suggesting that the detected lysozyme from egg white non-covalently bound five water molecules (~ 90 Da). This might be resulted from the incomplete desolvation, but it was more likely that lysozyme in fresh egg white originally bound five water molecules, since such an addition of 5 water molecules was not observed for the lysozyme standard under similar conditions. It has been reported that some proteins bind small molecules such as water to modulate their structures and functions in biosystems [63, 64]. This study provides new insight into the lysozyme structure and interactions in fresh egg white.

3.6. Detection of noncovalent protein complexes from bone marrow

The technique was also attempted for detection of noncovalent protein complexes from raw solid biological samples. Bone marrow can produce red blood cells via hematopoiesis and is associated with many diseases such as multiple myeloma and myelodysplastic syndrome [65, 66]. In this study, a small piece of porcine bone marrow was held with a metal clip connected to the high voltage, and directly analyzed with n-hexane as the added solvent. As shown in Figure 5b, intact hemoglobin, which consists of four subunits (i.e., 2α and 2β) and one heme group in each subunit, was detected in the spectrum. Dimers (α_2 and β_2) and monomers (α and β) of the subunits and the heme were also observed, presumably because that hemoglobin had been partially denatured in the sample when analyzed. Lipids, another important composition of bone marrow, were also detected, but were much less and at much lower abundances, compared with the spectrum previously obtained using methanol containing 0.1 % formic acid as the added solvent [37], in which only denatured individual proteins were observed for proteins. In addition to the functions as discussed above, n-hexane was believed to play a role in removing lipids and enhancing detection of proteins and protein complexes in the sample.

4. Conclusions

In conclusion, this study revealed the mechanism of ESI on solid substrates with nonpolar solvents and explored application of the technique for analysis of proteins. The rapid and almost concurrent evaporation of non-polar solvents, temperature reduction, water condensation and spray process allowed spray ionization to occur with fine plume of water and under low temperatures. These enabled the technique to detect proteins and protein complexes in their native states, including directly from raw viscous and solid biological samples. In addition to wooden tips, other substrates and biological tissues

[39, 67] can be used for the technique. This study sheds new insights into the sampling and ionization process of mass spectrometry, and demonstrates a highly potential technique for rapid characterization of native proteins.

Acknowledgements

This work was supported by Natural Science Foundation of China (Grants No. 81373369 and No. 21405127), Hong Kong Research Grants Council (CRF Grant No. C5031-14E and GRF Grant No. 5029/13P), and Science and Technology Planning Project of Guangdong Province (Grant No.2014A020221106).

References

[1] R. Aebersold, M. Mann, Mass spectrometry-based proteomics, Nature, 422 (2003) 198-207.

[2] J.L.P. Benesch, B.T. Ruotolo, D.A. Simmons, C.V. Robinson, Protein complexes in the gas phase: Technology for structural genomics and proteomics, Chem Rev, 107 (2007) 3544-3567.

[3] L.A. Baker, M. Baldus, Characterization of membrane protein function by solid-state NMR spectroscopy, Curr Opin Struc Biol, 27 (2014) 48-55.

[4] J.H. Ardenkjaer-Larsen, G.S. Boebinger, A. Comment, S. Duckett, A.S. Edison, F. Engelke, C. Griesinger, R.G. Griffin, C. Hilty, H. Maeda, G. Parigi, T. Prisner, E. Ravera, J. van Bentum, S. Vega, A. Webb, C. Luchinat, H. Schwalbe, L. Frydman, Facing and Overcoming Sensitivity Challenges in Biomolecular NMR Spectroscopy, Angew Chem Int Ed, 54 (2015) 9162-9185.

[5] M. Kornreich, R. Avinery, R. Beck, Modern X-ray scattering studies of complex biological systems, Curr Opin Biotech, 24 (2013) 716-723.

[6] E.F. Garman, Developments in X-ray Crystallographic Structure Determination of Biological Macromolecules, Science, 343 (2014) 1102-1108.

[7] J.F. Delamora, Principles and Practice of Electrospray Ionization Mass-Spectrometry for Large Polypeptides and Proteins, Mass Spectrom Rev, 11 (1992) 431-434.

[8] A. Laganowsky, E. Reading, J.T.S. Hopper, C.V. Robinson, Mass spectrometry of intact membrane protein complexes, Nat Protoc, 8 (2013) 639-651.

[9] J.B. Fenn, M. Mann, C.K. Meng, S.F. Wong, C.M. Whitehouse, Electrospray Ionization for Mass-Spectrometry of Large Biomolecules, Science, 246 (1989) 64-71.

[10] B.C. Bohrer, S.I. Mererbloom, S.L. Koeniger, A.E. Hilderbrand, D.E. Clemmer, Biomolecule Analysis by Ion Mobility Spectrometry, Annu Rev Anal Chem, 1 (2008) 293-327.

[11] B.T. Ruotolo, C.V. Robinson, Aspects of native proteins are retained in vacuum, Curr Opin Chem Biol, 10 (2006) 402-408.

[12] J.A. Loo, Studying noncovalent protein complexes by electrospray ionization mass spectrometry, Mass Spectrom Rev, 16 (1997) 1-23.

[13] J.A. Loo, Electrospray ionization mass spectrometry: a technology for studying noncovalent macromolecular complexes, Int J Mass Spectrom, 200 (2000) 175-186.

[14] A.J.R. Heck, Native mass spectrometry: a bridge between interactomics and structural biology, Nat Methods, 5 (2008) 927-933.

[15] Z.P. Yao, M. Zhou, S.E. Kelly, M.A. Seeliger, C.V. Robinson, L.S. Itzhaki, Activation of ubiquitin ligase SCF(Skp2) by Cks1: insights from hydrogen exchange mass spectrometry, J Mol Biol, 363 (2006) 673-686.

[16] M.Z. Huang, C.H. Yuan, S.C. Cheng, Y.T. Cho, J. Shiea, Ambient Ionization Mass Spectrometry, Annu Rev Anal Chem, 3 (2010) 43-65.

[17] R.G. Cooks, Z. Ouyang, Z. Takats, J.M. Wiseman, Ambient mass spectrometry, Science, 311 (2006) 1566-1570.

[18] Z.P. Yao, Characterization of proteins by ambient mass spectrometry, Mass Spectrom Rev, 31 (2012) 437-447.

[19] R.B. Cody, J.A. Laramee, H.D. Durst, Versatile new ion source for the analysis of materials in open air under ambient conditions, Anal Chem, 77 (2005) 2297-2302.

[20] Z. Takats, J.M. Wiseman, B. Gologan, R.G. Cooks, Mass spectrometry sampling under ambient conditions with desorption electrospray ionization, Science, 306 (2004) 471-473.

[21] S. Myung, J.M. Wiseman, S.J. Valentine, Z. Takats, R.G. Cooks, D.E. Clemmer, Coupling desorption electrospray ionization with ion mobility/mass spectrometry for analysis of protein structure: Evidence for desorption of folded and denatured states, J Phys Chem B, 110 (2006) 5045-5051.

[22] Y.S. Shin, B. Drolet, R. Mayer, K. Dolence, F. Basile, Desorption electrospray ionization-mass spectrometry of proteins, Anal Chem, 79 (2007) 3514-3518.

[23] K.A. Douglass, A.R. Venter, Protein analysis by desorption electrospray ionization mass spectrometry and related methods, J Mass Spectrom, 48 (2013) 553-560.

[24] P.J. Roach, J. Laskin, A. Laskin, Nanospray desorption electrospray ionization: an ambient method for liquid-extraction surface sampling in mass spectrometry, Analyst, 135 (2010) 2233-2236.

[25] Z.X. Miao, S.Y. Wu, H. Chen, The Study of Protein Conformation in Solution Via Direct Sampling by Desorption Electrospray Ionization Mass Spectrometry, J Am Soc Mass Spectrom, 21 (2010) 1730-1736.

[26] C. N. Ferguson, S. A. Benchaar, Z. X. Miao, J. A. Loo, H. Chen, Direct Ionization of Large Proteins and Protein Complexes by Desorption Electrospray Ionization-Mass Spectrometry, Anal Chem, 83 (2011) 6468-6473.

[27] J. Shiea, C.H. Yuan, M.Z. Huang, S.C. Cheng, Y.L. Ma, W.L. Tseng, H.C. Chang, W.C. Hung, Detection of native protein ions in aqueous solution under ambient conditions by electrospray laser desorption/ionization mass spectrometry, Anal Chem, 80 (2008) 4845-4852.

[28] H.W. Chen, S.P. Yang, M. Li, B. Hu, J.Q. Li, J. Wang, Sensitive Detection of Native Proteins Using Extractive Electrospray Ionization Mass Spectrometry, Angew Chem Int Ed, 49 (2010) 3053-3056.

[29] B. Hu, S.P. Yang, M. Li, H.W. Gu, H.W. Chen, Direct detection of native proteins in biological matrices using extractive electrospray ionization mass spectrometry, Analyst, 136 (2011) 3599-3601.

[30] N.J. Martin, R.L. Griffiths, R.L. Edwards, H.J. Cooper, Native Liquid Extraction Surface Analysis Mass Spectrometry: Analysis of Noncovalent Protein Complexes Directly from Dried Substrates, J Am Soc Mass Spectrom, 26 (2015) 1320-1327.

[31] C.M. Hong, C.T. Lee, Y.M. Lee, C.P. Kuo, C.H. Yuan, J. Shiea, Generating electrospray from solutions predeposited on a copper wire, Rapid Commun Mass Spectrom, 13 (1999) 21-25.

[32] K. Hiraoka, K. Nishidate, K. Mori, D. Asakawa, S. Suzuki, Development of probe electrospray using a solid needle, Rapid Commun Mass Spectrom, 21 (2007) 3139-3144.

[33] B. Hu, P.K. So, H.W. Chen, Z.P. Yao, Electrospray Ionization Using Wooden Tips, Anal Chem, 83 (2011) 8201-8207.

[34] B. Hu, L. Wang, W.C. Ye, Z.P. Yao, In Vivo and Real-time Monitoring of Secondary Metabolites of Living Organisms by Mass Spectrometry, Sci Rep, 3 (2013) 2104.

[35] H. Wang, J.J. Liu, R.G. Cooks, Z. Ouyang, Paper Spray for Direct Analysis of Complex Mixtures Using Mass Spectrometry, Angew Chem Int Ed, 49 (2010) 877-880.

[36] B. Hu, P.K. So, Z.P. Yao, Electrospray ionization with aluminum foil: A versatile mass spectrometric technique, Anal Chim Acta, 817 (2014) 1-8.

[37] B. Hu, P.K. So, Z.P. Yao, Analytical Properties of Solid-substrate Electrospray Ionization Mass Spectrometry, J Am Soc Mass Spectrom, 24 (2013) 57-65.

[38] B. Hu, Z.P. Yao, Mobility of Proteins in Porous Substrates under Electrospray Ionization Conditions, Anal Chem, 88 (2016) 5585-5589.

[39] P.K. So, B. Hu, Z.P. Yao, Electrospray ionization on solid substrates, Mass Spectrom, 3 (2014) S0028.

[40] M.K. Mandal, L.C. Chen, K. Hiraoka, Sequential and Exhaustive Ionization of Analytes with Different Surface Activity by Probe Electrospray Ionization, J Am Soc Mass Spectrom, 22 (2011) 1493-1500.

[41] M.K. Mandal, L.C. Chen, Z. Yu, H. Nonami, R. Erra-Balsells, K. Hiraoka, Detection of protein from detergent solutions by probe electrospray ionization mass spectrometry (PESI-MS), J Mass Spectrom, 46 (2011) 967-975.

[42] Y. Zhang, Y. Ju, C.S. Huang, V.H. Wysocki, Paper Spray Ionization of Noncovalent Protein Complexes, Anal Chem, 86 (2014) 1342-1346.

[43] P. Jiang, C.A. Lucy, Enhancement of ionization efficiency of mass spectrometric analysis from non-electrospray ionization friendly solvents with conventional and novel ionization techniques, Anal Chim Acta, 897 (2015) 45-52.

[44] A.Y. Li, H. Wang, Z. Ouyang, R.G. Cooks, Paper spray ionization of polar analytes using non-polar solvents, Chem Commun, 47 (2011) 2811-2813.

[45] B. Hu, Y.H. Lai, P.K. So, H.W. Chen, Z.P. Yao, Direct ionization of biological tissue for mass spectrometric analysis, Analyst, 137 (2012) 3613-3619.

[46] L.E. Bromberg, A.M. Klibanov, Transport of Proteins Dissolved in Organic-Solvents across Biomimetic Membranes, Proc Natl Acad Sci USA, 92 (1995) 1262-1266.

[47] K.C.S. Figueiredo, H.C. Ferraz, C.P. Borges, T.L.M. Alves, Structural Stability of Myoglobin in Organic Media, Protein J, 28 (2009) 224-232.

[48] G.A. Newsome, L.K. Ackerman, K.J. Johnson, Humidity Affects Relative Ion Abundance in Direct Analysis in Real Time Mass Spectrometry of Hexamethylene Triperoxide Diamine, Anal Chem, 86 (2014) 11977-11980.

[49] G.A. Newsome, L.K. Ackerman, K.J. Johnson, Humidity Effects on Fragmentation in Plasma-Based Ambient Ionization Sources, J Am Soc Mass Spectrom, 27 (2016) 135-143.

[50] J.-C. Wolf, L. Gyr, M.F. Mirabelli, M. Schaer, P. Siegenthaler, R. Zenobi, A Radical-Mediated Pathway for the Formation of [M + H]+ in Dielectric Barrier Discharge Ionization, J Am Soc Mass Spectrom, 27 (2016) 1468-1475.

[51] R. Tuckermann, S. Bauerecker, B. Neidhart, Evaporation rates of alkanes and alkanols from acoustically levitated drops, Anal Bioanal Chem, 372 (2002) 122-127.

[52] J. Maybank, N.N. Barthakur, Growth and Destruction of Ice Filaments in an Electric Field, Nature, 216 (1967) 50-52.

[53] K.G. Libbrecht, V.M. Tanusheva, Electrically induced morphological instabilities in free dendrite growth, Phys Rev Lett, 81 (1998) 176-179.

[54] L.F. Evans, The Growth and Fragmentation of Ice Crystals in an Electric Field, J Atmos Sci, 30 (1973) 1657-1664.

[55] Y. Mine, Recent Advances in the Understanding of Egg-White Protein Functionality, Trends Food Sci Tech, 6 (1995) 225-232.

[56] A.J. Harvey, G. Speksnijder, L.R. Baugh, J.A. Morris, R. Ivarie, Expression of exogenous protein in the egg white of transgenic chickens, Nat Biotechnol, 20 (2002) 396-399.

[57] E.D.N.S. Abeyrathne, H.Y. Lee, D.U. Ahn, Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents-A review, Poultry Sci, 92 (2013) 3292-3299.

[58] R.C. Simas, R.R. Catharino, I.B.S. Cunha, E.C. Cabral, D. Barrera-Arellano, M.N. Eberlin, R.M. Alberici, Instantaneous characterization of vegetable oils via TAG and FFA profiles by easy ambient sonic-spray ionization mass spectrometry, Analyst, 135 (2010) 738-744.

[59] W.S. Law, H.W. Chen, J.H. Ding, S.P. Yang, L. Zhu, G. Gamez, K. Chingin, Y.L. Ren, R. Zenobi, Rapid Characterization of Complex Viscous Liquids at the Molecular Level, Angew Chem Int Edit, 48 (2009) 8277-8280.

[60] X. Li, B. Hu, J.H. Ding, H.W. Chen, Rapid characterization of complex viscous samples at molecular levels by neutral desorption extractive electrospray ionization mass spectrometry, Nat Protoc, 6 (2011) 1010-1025.

[61] Y. Yang, A. Barendregt, J.P. Kamerling, A.J.R. Heck, Analyzing Protein Micro-Heterogeneity in Chicken Ovalbumin by High-Resolution Native Mass Spectrometry Exposes Qualitatively and Semi-Quantitatively 59 Proteoforms, Anal Chem, 85 (2013) 12037-12045.

[62] H. McGee, On Food and Cooking: The Science and Lore of the Kitchen, Scribner 2007.

[63] E. Reading, A. Laganowsky, T.M. Allison, C.V. Robinson, Membrane Proteins Bind Lipids Selectively To Modulate Their Structure And Function, Protein Sci, 23 (2014) 231-231.

[64] A. Laganowsky, E. Reading, T.M. Allison, M.B. Ulmschneider, M.T. Degiacomi, A.J. Baldwin, C.V. Robinson, Membrane proteins bind lipids selectively to modulate their structure and function, Nature, 510 (2014) 172-175.

[65] B. Bjorkstrand, P. Ljungman, H. Svensson, J. Hermans, A. Alegre, J. Apperley, J. Blade, K. Carlson, M. Cavo, A. Ferrant, A.H. Goldstone, A. deLaurenzi, I. Majolino, R. Marcus, H.G. Prentice, K. Remes, D. Samson, A. Sureda, L.F. Verdonck, L. Volin, G. Gahrton, Allogeneic bone marrow transplantation versus autologous stem cell transplantation in multiple myeloma: A retrospective case-matched study from the European group for blood and marrow transplantation, Blood, 88 (1996) 4711-4718.

[66] A. Koc, R. Oner, C. Oner, D. Aktas, M. Sozen, E. Tuncbilek, C. Altay, Myelodysplastic syndrome (MDS) associated with increased hemoglobin F and trisomy 8: presentation of a patient, Hematol Cell Ther, 41 (1999) 187-189.

[67] P.K. So, B. Hu, Z.P. Yao, Mass spectrometry: towards in vivo analysis of biological systems, Mol Biosyst, 9 (2013) 915-929.

Figure Captions

Figure 1. a-b) Illustration of experimental setup of wooden-tip ESI-MS (a) and solid-substrate ESI-MS of biological tissue (b). c) Process of solid-substrate ESI-MS with non-polar solvents. G: gas, L: liquid, S: solid.

Figure 2. Mass spectra obtained using wooden-tip ESI-MS with hexane as the added solvent for analyzing powders of lysozyme (a) and myoglobin (c), and using nanoESI-MS for analyzing 100 μM aqueous solutions of lysozyme (b) and myoglobin (d).

Figure 3. a) The relationship between the observed intensity of reserpine (100 ng) and relative humidity of the ambient air (52% - 90%), obtained with hexane as the added solvent; b) Intensity of reserpine (100 ng) and evaporation rate of n-alkanes in ambient air, plotted as a function of carbon number of n-alkanes (inset shows the relationship between the observed intensity and the evaporation rate). Data of evaporation rate of n-alkanes were from the literature [51].

Figure 4. a) Ice dendrites formed on wooden tip surface and spray plume from ice dendrites with n-pentane as the added solvent (the photo was extracted from the video in the supporting information); b) ice dendrites formed on the wooden tip under different electric fields (0 - 4.5 kV).

Figure 5. Spectra obtained using wooden-tip ESI-MS with hexane as the added solvent for analysis of hen egg white (a) and porcine bone marrow (b).

Figures

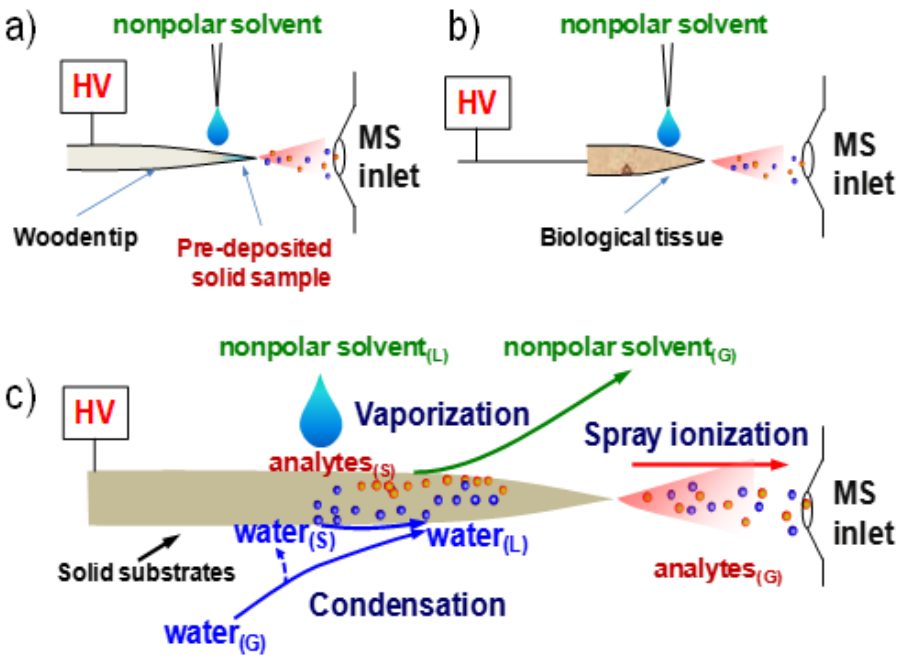

Figure 1

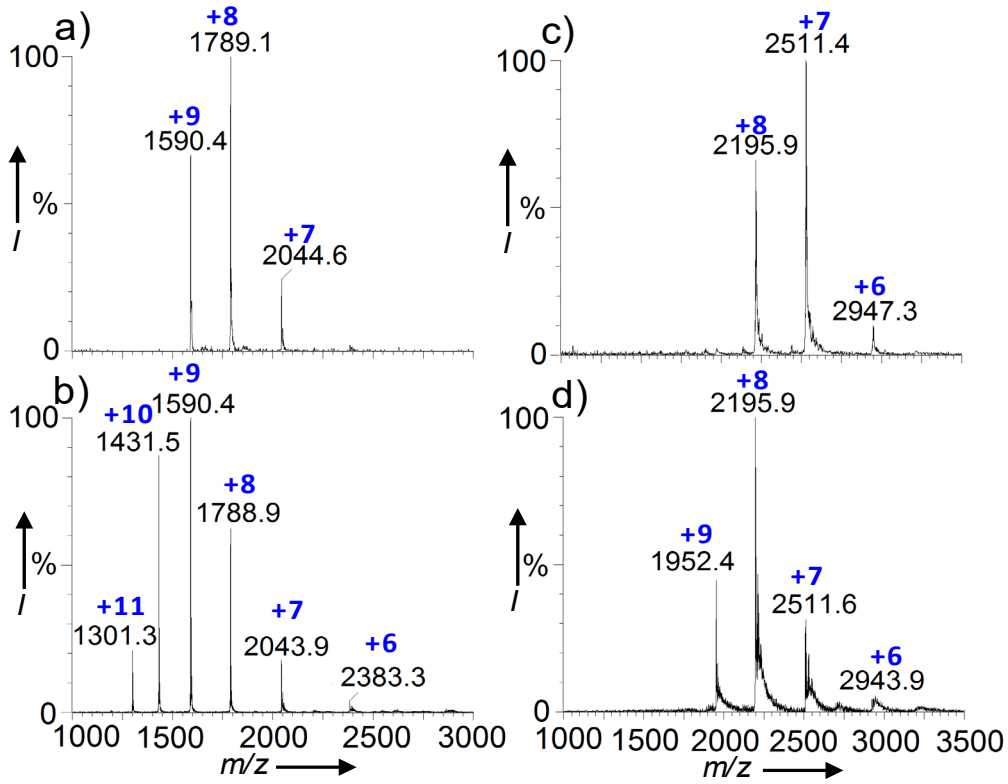
a)
+8
1789.1
+9
1590.4
+7
2044.6
100
0
%
I
b)
+9
1590.4
+10
1431.5
+8
1788.9
+11
1301.3
+7
2043.9
+6
2383.3
1000 1500 2000 2500 3000
m/z
c)
+7
2511.4
+8
2195.9
+6
2947.3
d)
+8
2195.9
+9
1952.4
+7
2511.6
+6
2943.9
1000 1500 2000 2500 3000 3500

Figure 2

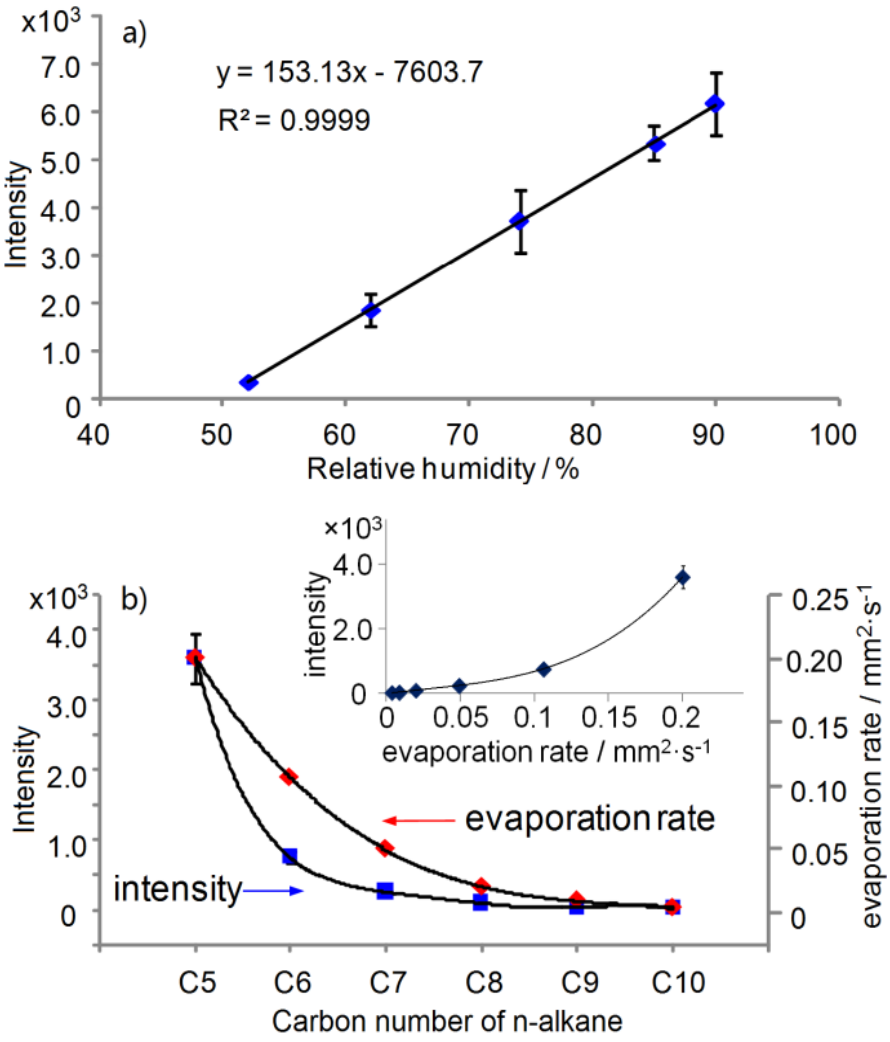
a)
y = 153.13x - 7603.7
R² = 0.9999
x10³
Intensity
Relative humidity / %
b)
intensity
evaporation rate / mm²·s⁻¹
evaporation rate
intensity
evaporation rate / mm²·s⁻¹
C5
C6
C7
C8
C9
C10
Carbon number of n-alkane

Figure 3

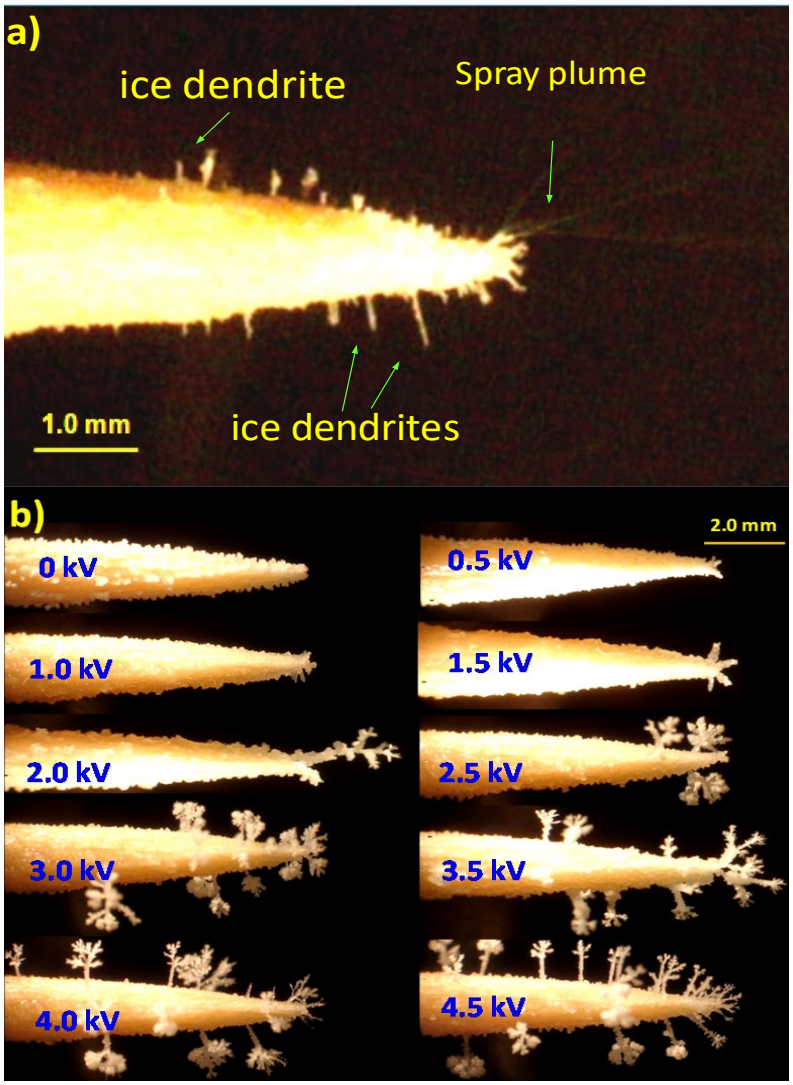
a)
ice dendrite
Spray plume
1.0 mm
ice dendrites
b)
2.0 mm
0 kV
0.5 kV
1.0 kV
1.5 kV
2.0 kV
2.5 kV
3.0 kV
3.5 kV
4.0 kV
4.5 kV

Figure 4

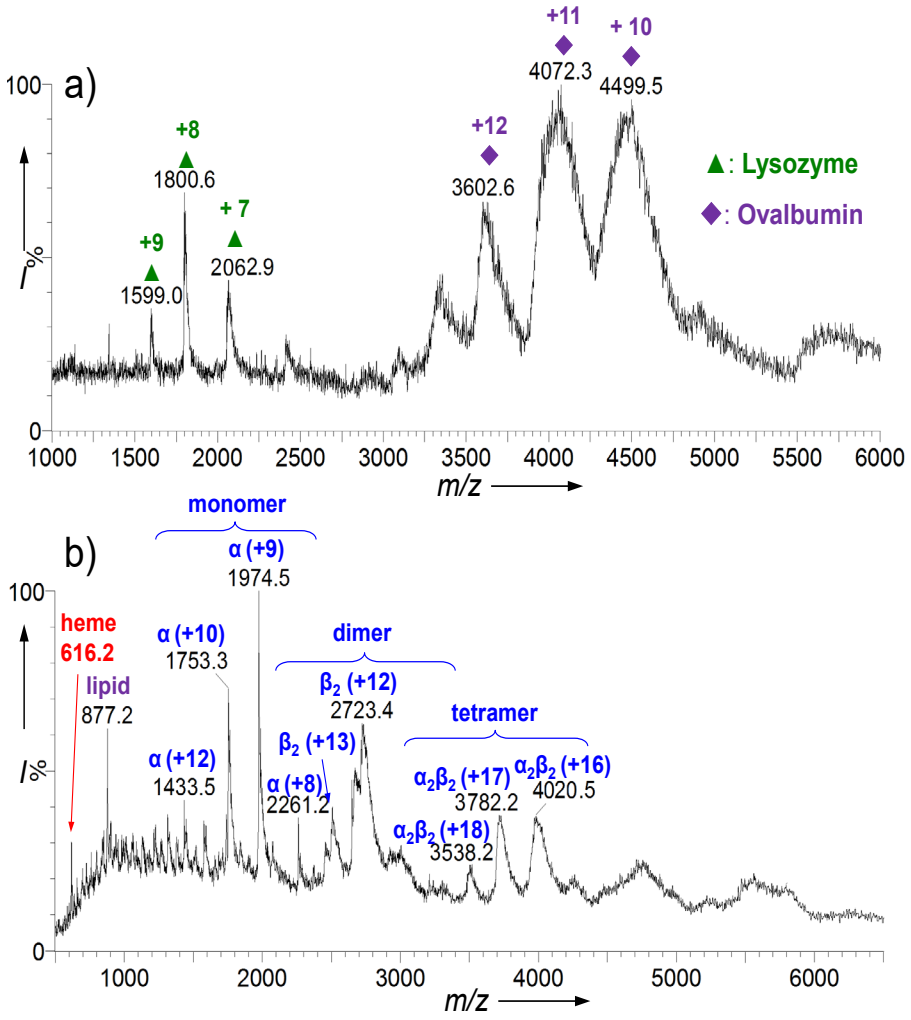
a)
100
0
I%
1000 1500 2000 2500 3000 3500 4000 4500 5000 5500 6000
m/z
+8
1800.6
+ 7
2062.9
+9
1599.0
+12
3602.6
+11
4072.3
+ 10
4499.5
▲: Lysozyme
◆: Ovalbumin
b)
100
0
I%
1000 2000 3000 4000 5000 6000
m/z
monomer
α (+9)
1974.5
heme
616.2
lipid
877.2
α (+10)
1753.3
α (+12)
1433.5
dimer
β2 (+12)
2723.4
β2 (+13)
α (+8)
2261.2
tetramer
α2β2 (+17)
3782.2
α2β2 (+16)
4020.5
α2β2 (+18)
3538.2

Figure 5