

6.3: Electrospray Ionization (ESI) Mass Spectrometry

Electrospray Ionization Mass Spectrometry is a technique used to determine molecular weights for proteins, peptides, and other biological macromolecules such as oligosaccharides¹. Originally described by Canadian-American Physicist Sir Arthur J. Dempster in an article titled "A new method of positive ray analysis."⁷ His work was the first modern mass spectrometer, which used positive rays to determine the mass-to-charge ratio of various isotopes of lithium and magnesium. Dempster showed that it was possible to determine the isotopes' relative proportions and atomic weights using this method⁷. Mass spectrometry in general is useful for structure elucidation (when combined with chromatography separation techniques), peptide sequencing (when combined with ion traps), and quantification (when combined with a triple-quadrupole mass analyzer) for example, and is mainly limited by the ability of the ions generated to remain stable until their arrival at the detector. Electrospray Ionization describes the method by which the macromolecules are ionized via a "soft" ionization, which does not fragment nor harshly degrade the macromolecules and ionizes by multiple charging¹. Rather, the macromolecules are ionized into small droplets². The droplets are then desolvated further, effectively decreasing the droplet size into molecules with protons¹. The protonated/desolvated molecules enter the mass analyzer and subsequently the detector to determine the mass/charge ratio¹. The main advantage is that the samples can easily be introduced to the instrument in solution with the ability to detect very small masses (atomic masses) to very large molecules (MDaltons) with detection limits down to the pico-, femto-, and attomole levels⁵. Furthermore, this analytical technique has become of great importance within the last decade or so due to advancements enabling even higher sensitivity and its ability to be combined with high-throughput automation for "-omics" studies with high applicability in drug discovery.

Sample Preparation

Samples for ESI-MS are typically purified, which is ideal for the instrument because mixtures of components with different physicochemical properties are not good analytes for the technique (e.g. glycans vs. peptides)¹. Methods such as high-performance liquid chromatography, capillary electrophoresis, and liquid-solid column chromatography are commonly employed for the purification step, and subsequently injected into the ESI-MS¹. Usually, the purification method is attached directly to the capillary needle¹.

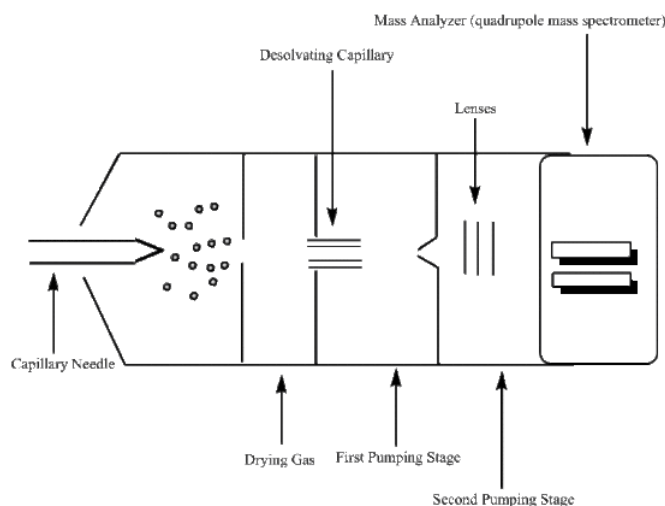


Figure 6.3.1: Diagram of Electrospray Ionization-Mass Spectrometry (Murphy, 2016)

Electrospray Ionization (ESI)

A mechanical syringe pump injects the liquid sample (usually less than one mM in a polar volatile solvent) into the capillary needle, and thereby nebulizing it into a fine mist¹. Typically an analyte will undergo three major processes after injection and transfers into the gas phase: it will produce charged droplets from the high-voltage capillary tip, repeatedly evaporate solvent from the charged droplet followed by droplet disintegration into much smaller droplets, and lastly transfer of the ion into the gas-phase⁵. The capillary needle typically has an inner diameter of ~0.1mm and outer diameter of ~0.2mm, and a low flow rate around 1 to 20 $\mu\text{L}/\text{min}$ ¹. A voltage is applied to the tip of the capillary of around 2-6 kV, where the surrounding source-sampling cone is located around 1-3 cm from the spray needle tip (see Figure 6.3.1)².

The strong electric field causes the nebulized particles to carry a charge, thus, becoming electrospray droplets where a drying or sheath gas flows around the capillary improving nebulization¹.

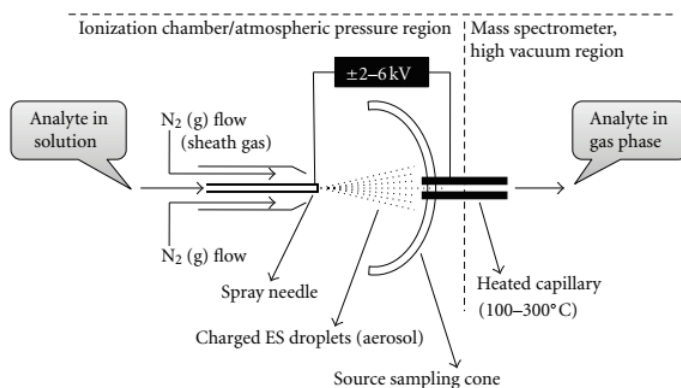


Figure 6.3.2: Diagram of Electrospray Ionization (Banerjee & Mazumdar, 2012)

The electric field also directs the spray toward the mass spectrometer while droplets diminish in size (solvent evaporation)¹. This process is described pictorially in Figure 6.3.3 below. Once the electrospray droplets pass through the heating capillary (Figure 6.3.2, labeled as desolvating capillary in Figure 6.3.1), the ions completely desolvate¹. The heating capillary is typically around 0.2mm inner diameter, 60mm in length, at a temperature controlled in a range from 100-300°C for desolvation and continual droplet shrinkage¹. At this point two forces become dominant: surface tension in the droplets acting to retain the shape of the droplet, and Coulomb force of repulsion between like charges on the surface which act to break down the shape of the droplet².

As the droplets travel through the heating capillary, they have a high enough electric field density that causes like charges to repel one another, increasing surface tension (first to second step in Figure 6.3.3)². The droplets then reach the Rayleigh limit, which describes the limit of the number of charges that can be present on a charged droplet before fission occurs and is broken down (third step in Figure 6.3.3)¹. It is at this point the surface tension can no longer sustain the Coulomb force of repulsion, and a “Coulomb explosion” or “Coulomb fission” occurs (fourth step in Figure 6.3.3)¹. The parent droplets disintegrate into much smaller droplets of positive or negative charge, with a much higher mass-to-charge ratio¹. These small droplets have high mass-to-charge ratios because smaller portions of parent charges are carried off and distributed in many offspring droplets compared to the mass carried off from parent droplets. An example time history is shown below of methanol droplets produced by electrospray in Figure 6.3.4.

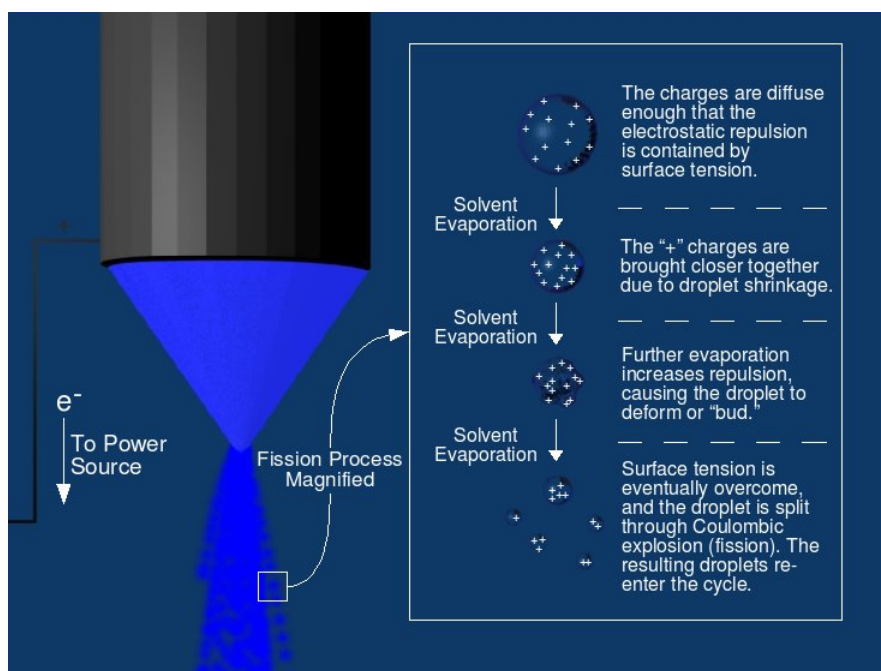


Figure 6.3.3: Fission Process Diagram (McFarland, 2008)

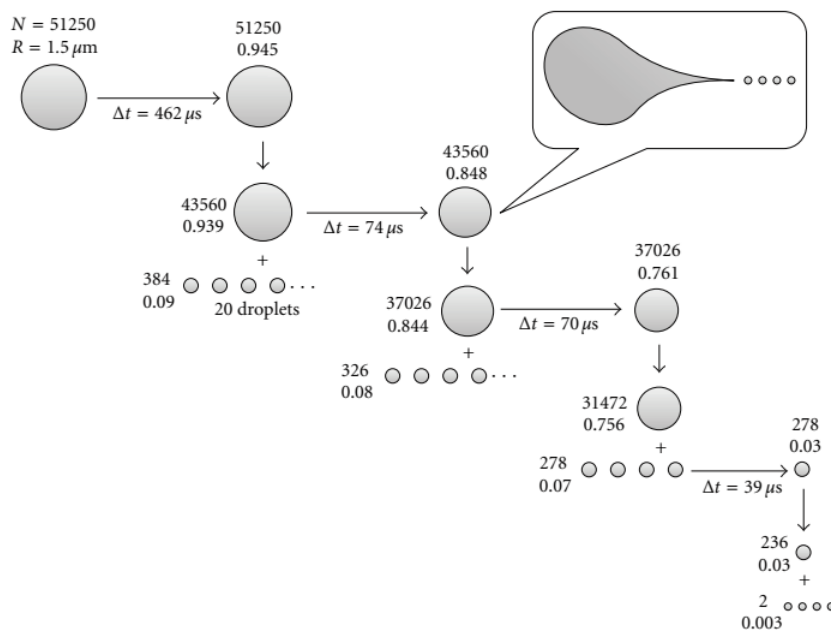


Figure 6.3.4: Methanol Droplets produced by microelectrospray process. The upper left droplet is a parent droplet created at the electrospray capillary tip. N is the number of elementary charges, R is the radius of the droplet in micrometers, Δt is the time in microseconds for solvent evaporation to shrink the droplet (right arrows) to the point where Coulomb fission occurs (down arrows). Only the first three successive solvent evaporation/Coulomb fission steps are shown. The inset is a drawing of a droplet undergoing jet fission (Banerjee & Mazumdar, 2012).

The decomposition of a droplet from ESI occurs in manner similar to the inset of Figure 6.3.4, and is dictated by the Rayleigh equation:

$$q^2 = 8\pi^2\epsilon_o\gamma D^3 \quad (6.3.1)$$

Where q is charge, epsilon 'naught' is the permmissivity of the medium, γ is the surface tension of the droplet, and D is the droplet diameter.

Mass Analyzers

From the ion beam, the mass analyzer takes different types of ions and separates them based on their mass-to-charge ratio. Afterwards, the ions are passed onto the detector. There are many types such as magnetic (B)/electric (E) sector mass analyzers, linear quadrupole ion trap (LIT), three-dimensional qudrupole ion trap (QIT), orbitrap (Mass Analyzer Orbitrap), time-of-flight mass analyzer (TOF, Mass Analyzer Time of Flight), and ion cyclotron resonance mass analyzer (ICR)¹. They all take advantage of dynamic of static magnetic/electric fields based on Lorentz force law (Equation (1), a charge experiences electric and magnetic forces when traveling through magnetic/electric fields)³ and Newton's second law of motion (Equation (2), objects accelerate based on their mass and net forces acting on the object)⁴.

$$F = qE + qvxB \quad (6.3.2)$$

$$F = mxa \quad (6.3.3)$$

Where in Equation (2), F is the Lorentz force, q is the charge of the ion, E is the electric field, v is the velocity of the ion, and B is the magnetic field. In Equation (3), F is Force, m is mass, and a is acceleration.

Table 1 below briefly summarizes comparisons between different mass analyzers. The mass analyzer uses electrostatic lenses (see Figure 6.3.1) to direct the beam into the analyzer, and is kept at a high vacuum (around 10^{-3} torr to 10^{-6} torr pressure) to prevent any undesired molecular interactions between the ions and the atmosphere¹.

Table 1: Comparison of mass analyzers (de Hoffmann & Stroobant, 2007)

	Quadrupole	Ion trap	TOF	TOF reflectron	Magnetic	FTICR	Orbitrap
Mass limit	4000 Th	6000 Th	> 1000 000 Th	10 000 Th	20 000 Th	30 000 Th	50 000 Th
Resolution	2000	4000	5000	20 000	100 000	500 000	100 000
FWHM (m/z 1000)							
Accuracy	100 ppm	100 ppm	200 ppm	10 ppm	<10 ppm	<5 ppm	<5 ppm
Ion sampling	Continuous	Pulsed	Pulsed	Pulsed	Continuous	Pulsed	Pulsed
Pressure	10^{-5} Torr	10^{-3} Torr	10^{-6} Torr	10^{-6} Torr	10^{-6} Torr	10^{-10} Torr	10^{-10} Torr
Tandem mass spectrometry	Triple quadrupoles	—	—	PSD or TOF/TOF	Consecutive sectors	—	—
	MS/MS	MS ⁿ	—	MS/MS	MS/MS	MS ⁿ	—
	fragments	fragments	—	fragments	fragments	fragments	—
	precursors	—	—	—	precursors	—	—
	neutral loss	—	—	—	neutral loss	—	—
	Low-energy collision	Low-energy collision	—	Low- or high-energy collision	High-energy collision	Low-energy collision	—

Five main characteristics for the measuring performance of a mass analyzer are the mass range limit, analysis speed, transmission, mass accuracy, and resolution⁵. According to de Hoffmann and Stroobant, mass range is the mass-to-charge (m/z) limit the mass analyzer can measure ions, expressed in units of Th (Thomson), or u (unified atomic mass unit) for ions with an elementary charge ($z=1$), where $\{1 \text{ Th} = 1 \text{ u/e} = 1 \text{ Da/e} = 1.036426 \cdot 10^{-8} \text{ kg/C}\}$ ⁵. Analysis speed (scan speed) is the rate the analyzer measures over a particular mass range, expressed in mass units per second (u/s) or in milliseconds (u/ms)⁵. Transmission is the ratio between the number of ions reaching the detector and number of ions entering the mass analyzer, including ion losses from other sections of the mass analyzer, such as the electric lenses⁵. Mass accuracy, as it sounds, is the accuracy of the m/z provided by the mass analyzer⁵. It is the difference between the theoretical m/z ($m_{\text{theoretical}}$) and measured m/z (m_{measured}), expressed in millimass units (mmu) or parts per million (ppm)⁵. This parameter is closely related to the stability and resolution of the analyzer, for example, a low-resolution instrument cannot provide high accuracy⁵. The resolution or the ability of the analyzer to yield distinct signals from two ions with similar to small differences in m/z can be expressed as the following in Equation (4)⁵.

$$R = \frac{m}{\Delta m} \quad (6.3.4)$$

Where m is the mass of the first peak, divided by the difference, Δm , between the neighboring peaks². The data is improved with more resolution (higher R)⁵.

Detectors

A common type of detector used in conjunction with a quadrupole mass analyzer for example, is an electron multiplier (EM) detector⁵. In this type of detector electrons are accelerated to a high velocity, enhancing detection efficiency⁵. To achieve this, an electrode called a conversion dynode is held at a high potential from + 3 kV to + 30 kV, opposite to the ions' charges⁵. Once ions strike the conversion dynode, several secondary particles are emitted⁵. Secondary particles are typically negative and positive ions, electrons, and neutral particles⁵. For example, if positive ions strike the conversion dynode, negative ions and electrons are the secondary particles of interest, likewise, if negative ions strike the conversion dynode the secondary particles are positive ions⁵. The secondary particles are then amplified in a cascade effect to produce a current⁵.

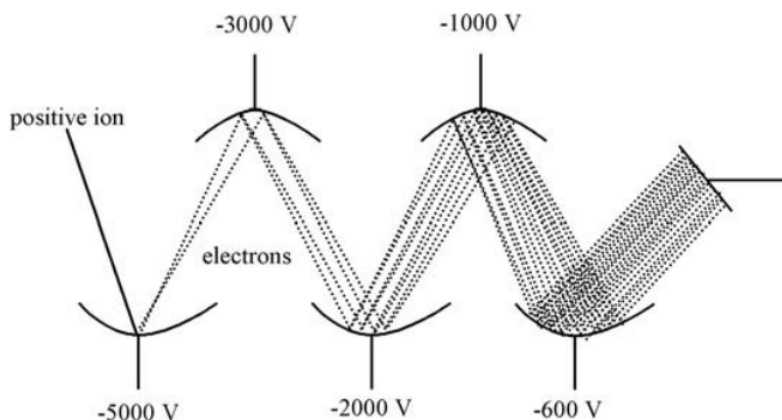


Figure 6.3.8: Discrete dynode electron multiplier schematic, the first dynode (at -5000 V) converts ions into electrons (de Hoffmann & Stroobant, 2007)

Figure 6.3.8 shows a schematic for a discrete dynode electron multiplier detector⁵. The first dynode starts at a higher magnitude potential (but negative), causing the secondary particles to emit electrons⁵. The secondary particles and electrons accelerate to each subsequent dynode (due to the lower potential) until the electrons reach the output of the electron multiplier, which is held at ground potential⁵. A cascade of electrons are created at the end, and the current produced and amplified by conventional electronic amplification⁵.

Electrospray Ionization on Membrane Proteins

Membrane proteins are of particular interest because of their importance in cell signaling, transport, adhesion⁶, and intercellular interactions. Some advantages of mass spectrometry methods in studying membrane proteins are that freezing or crystallization (as in X-ray crystallography) are not required, and that it is possible to study these proteins in their native (unfolded) state⁶. Utilization of electrospray ionization (ESI) mass spectrometry (MS) on membrane proteins typically falls under two categories: native MS and labeling MS⁶. Native MS involves maintaining non-covalent interactions, which preserves tertiary and quaternary structures, and is carried out *in vacuo*⁶. Labeling MS methods involve chemical crosslinking, hydrogen-deuterium exchange, and hydroxyl radical footprinting (HRFP) to name a few⁶. For each method, proteins are labeled in solution (peptides reacted with chemical labels), undergo proteolysis, and are subsequently measured/quantified via MS method⁶ (see Figure 6.3.9 below).

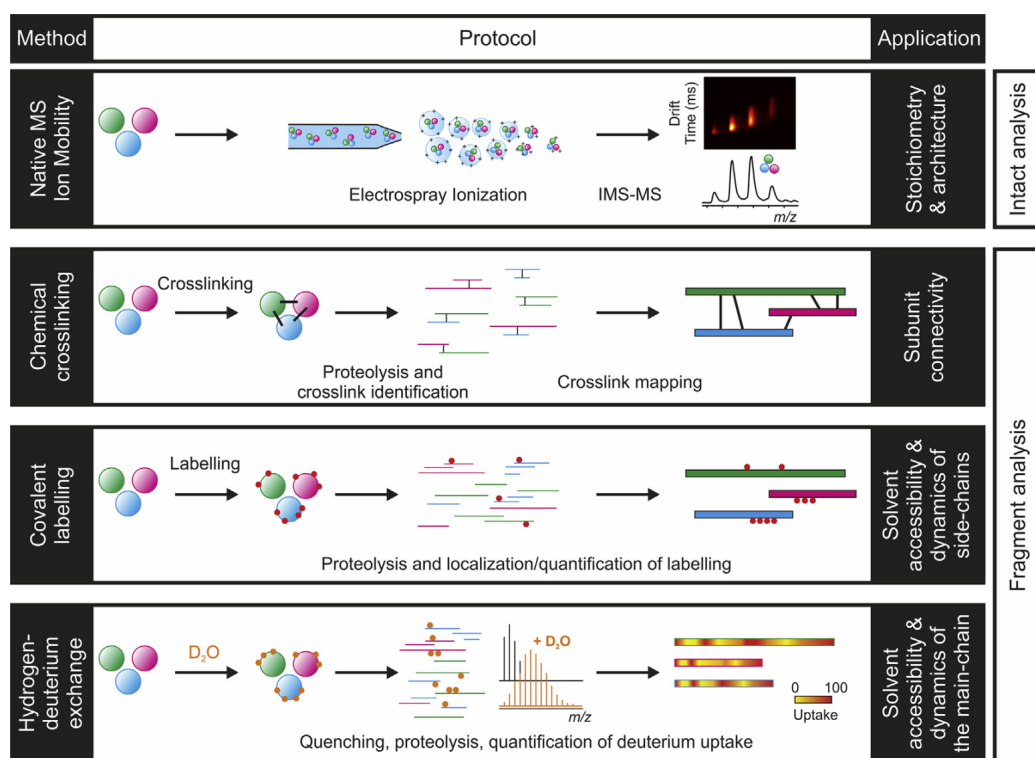


Figure 6.3.9 Summary of Native (Intact) and Labeling (Fragment) Mass Spectrometry (MS) methods. Intact/Native MS involves retaining tertiary and quaternary structures for analysis, whereas fragment/labeling MS methods involve chemical crosslinking, exchanging, or tagging of peptides followed by proteolysis. Labeling MS methods then study fragmentation patterns of labeled peptide segments (Calabrese and Radford 2018).

Considerations for ESI on Membrane Proteins

Careful consideration in optimizing the parameters are absolutely necessary when analyzing biological materials in mass spectrometric methods. Two examples of these parameters are the collision voltage and selection of detergent⁶. The collision voltage is that which is applied to molecular ions, accelerating them into the collision cell with an inert gas⁵. Optimization of collision voltage involves selecting a voltage that enables fragment ions to be observed, but also well resolved. This voltage goes hand-in-hand with careful consideration of the buffer/detergent. Ideally, the buffer/detergent needs to be able to efficiently solubilize the protein, and also be easily removed to allow the protein to be properly desolvated (see Figures 3 and 4)⁶. Detergents are used for membrane proteins because of their amphiphilic nature, similar to the membrane proteins themselves⁶. Where these two parameters come together are when the membrane protein-detergent complex transfers into the gas phase: the collision voltage

must be high enough to desolvate the membrane protein from the detergent, and the detergent must not strongly solvate the membrane protein⁶. Strong solvating detergents require higher energies which risk destabilizing the protein prior to detection in the mass analyzer.

If the membrane protein is not liberated from the detergent, perhaps due to low collision voltage, the membrane proteins' signals may be suppressed by noise from the remaining detergent (see Figure 6.3.10b and 10f). Figure 6.3.10 below also demonstrates the concepts of selecting proper collision voltage combined with an appropriate detergent.

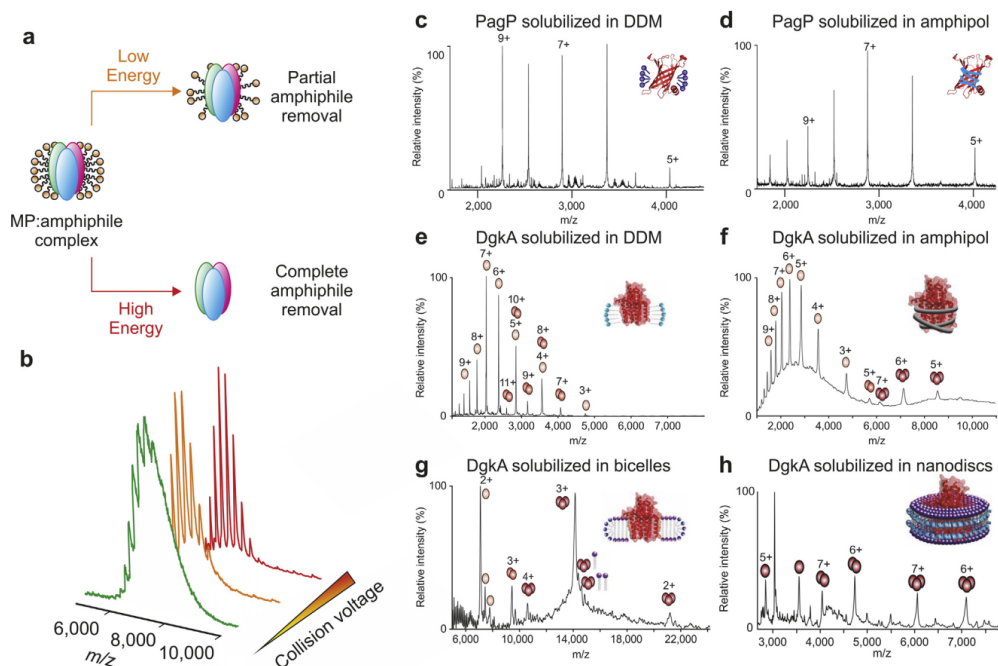


Figure 6.3.10: Examples from native mass spectrometry of membrane proteins (Calabrese and Radford 2018). (a) Demonstrates that low collision voltage/energy results in partial removal of detergent/amphiphile, resulting in unresolved peaks. Conversely, sufficiently high collision voltage results in complete detergent/amphiphile removal, resulting in resolved peaks. (b) echoes concepts from 10a demonstrating a theoretical mass spectra from low collision voltage (green/front) to high collision voltage (red/back). (c-d) Demonstrates differences in using detergents DDM (c) and amphipol (d) on PagP, suggesting two conformations are present. (e-f) Shows DgkA protein mass spectra differences using DDM (e) vs. amphipol (f). Use of amphipol for DgkA demonstrates its strong solvation of the protein, resulting in higher noise. Careful selection of detergents for the membrane protein of interest is necessary as a single detergent may not be appropriate for all membrane proteins. (g-h) Shows the resulting mass spectra when solubilizing DgkA in bicelles and nanodiscs (Calabrese and Radford 2018).

Summary

- Electrospray (ESI) is a soft-ionization technique that has gained popularity in biological applications as it is capable of maintaining non-covalent interactions of proteins.
 - Use of appropriate buffers/detergents/optimized instrument conditions renders ESI-mass spectrometry (MS) to be a powerful and sensitive tool to elucidate membrane protein structures.
- ESI can be coupled with a variety of mass analyzers (see Table 1), providing users of mass spectrometry flexibility in analysis.
- ESI can also be coupled downstream from a variety of chromatographic techniques such as HPLC to enhance mass spectrometry-based studies on complex molecular structures.

References

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