Introduction to Proteomics
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Lecture - 24
Mass Spectrometry: Ionization Sources

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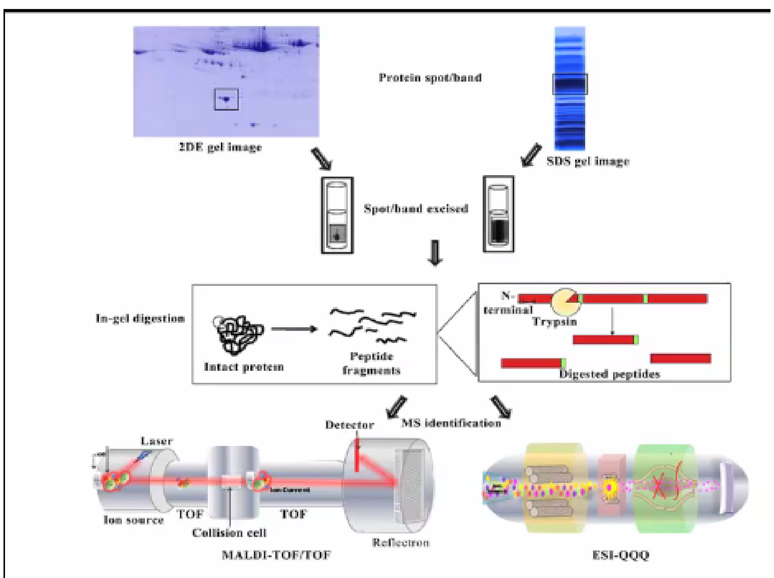

This slide gives you an overview of In-gel digestion process. We will talk in more details about each of these steps as they go along in the next lecture talking more specifically about specific type of Mass Spectrometry technique. But today I am giving you sort of an overview various type of concepts and various steps involved in performing the mass spectrometry experiments.

So In-gel digestion regardless of you have done the gel based proteomic or you want to perform the gel free proteomic you can resolve the proteins on the gel simplify the proteome axis either the Spot or the bends of your interest and then subject those to the In-gel digestion or in solution digestion so that proteins can liquid into the small peptides and these can be then further analyzed by using ionization source and mass analyzers.

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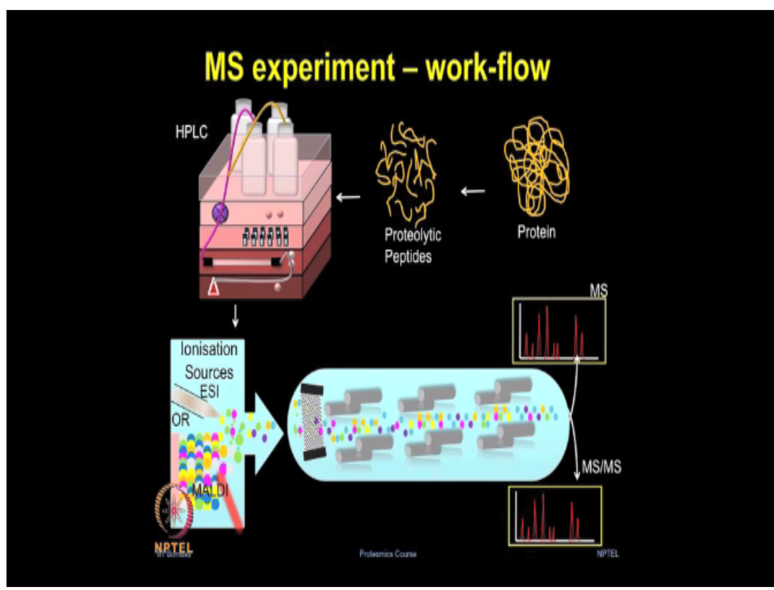

This slide shows you an overview of workflow involved in performing in MS phase experiment. First of all, you can pre-fractionate your sample by using liquid chromatography or one can also try different methods of doing the pre-fractionation and then applying that for doing the MS analysis. After pre-fractionation then In-gel digestion or Proteolytic cleavage can be performed by using various In-lines such as Trypsin.

And then the sample can be injected into the ionization source whether it can be ESI or MALDI most commonly use for proteomics as well as other type of ionization sources and then these ions can be further resolved into the Mass analyzers. Again there are various type of mass analyzers and then data can be analyzed in MS or MS/MS mode.

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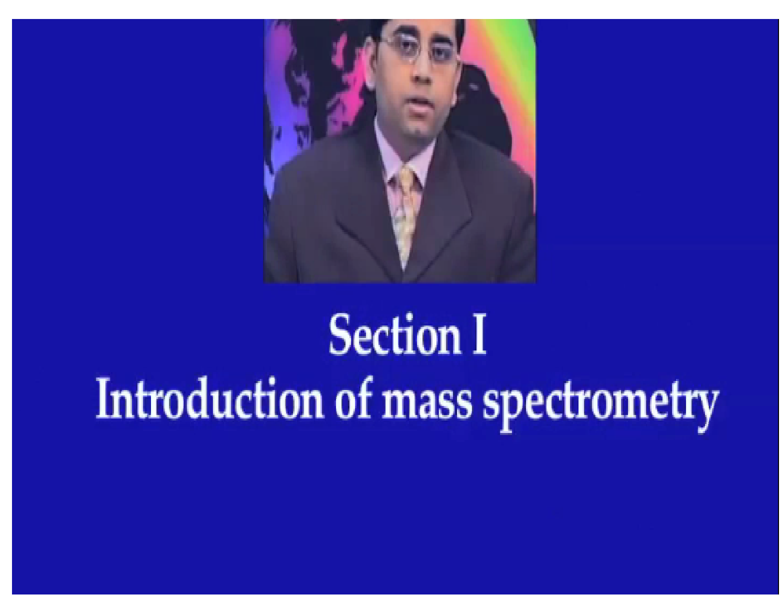

Now let us talk about different parts of the mass spectrometry.

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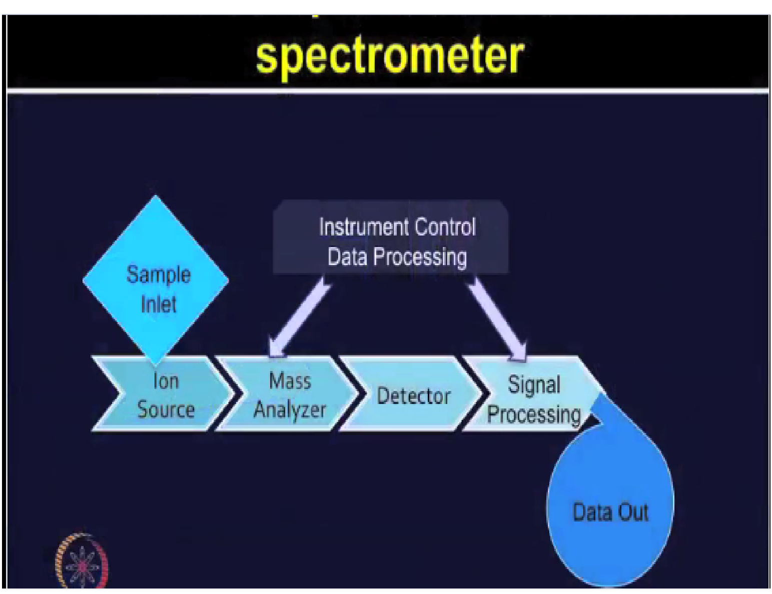

So major components include the sample inlet, ion source, mass analyzer, detector, signal processing components and data output. Let us look at each of these components in little more details. Sample inlet it transfers a sample into the ionization source. The ion source or ionization source it converge neutral sample molecules into the gas phase ions. Mass analyzers it separates an analysis mass of the ionic species.

There is various type of mass analyzer available which we will discuss in more detail during the subsequent part of the lecture. Now one need to maintain the vacuum condition, a very low

pressure is maintained inside mass spectrometer. Detector (()) (03:52) and amplifying ion current of mass resolved ions. And then we need electronics to control the operation of various units. The data system it records processes, stores and help to display the data output.

So although there are three major components involved Ionization source, Mass Analyzer and Detector. But then there are some necessary components which are also equally important for doing the mass spectrometry based experiment.

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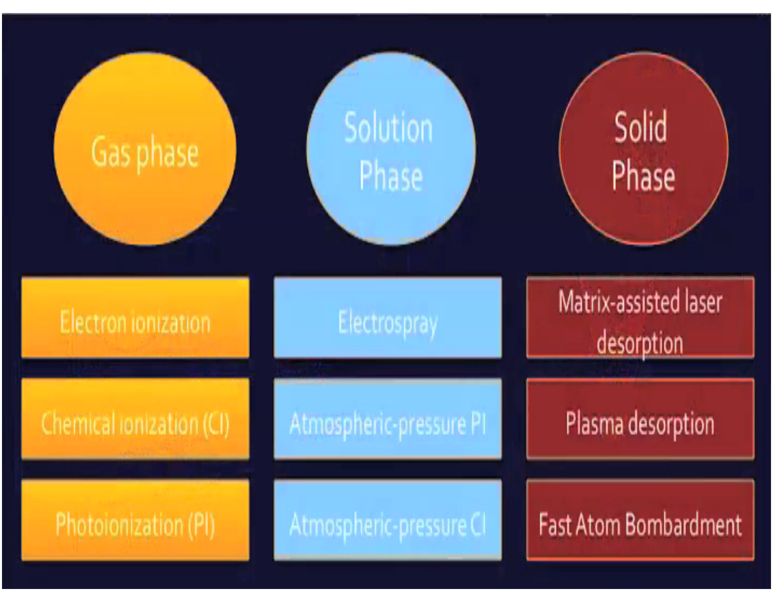

So what are different types of ionization sources involved in the MS analysis? So success of mass spectrometry experiment lies in efficiency of converting a neutral compound to a gas phase ionic species. So you have various type of options currently available. You can select what type of ionization source you want for your specific application. So the choice of particular ionization source is dictated largely by the nature of sample which one wants to investigate.

The gas phase electron ionization, chemical ionization and Photoionization these are the more commonly used ionization sources. What solution phase, Electrospray, Atmospheric pressure and Atmospheric-pressure CI these are the more commonly used ionization sources. The Solid Phase MALDI or Matrix-assisted laser desorption, ionization, Plasma desorption are the more commonly used solid phase ionization sources.

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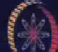
Need for soft ionization methods

- In MS due to ionization generally large molecules are broken into several random fragments
- Non-selective fragmentation
- Very difficult to interpret
- Therefore, need for soft ionization methods



The traditional ionization sources use for the small molecule chemical application relied on the chemical or electrical ionization. But these processes are too (()) (06:10) to ionize intact large bio-molecules and the lead to the unpredictable analyze decomposition. So for proteomic application there was need for the soft ionization methods in mass spectrometry. These are non-selective fragmentation. The hard ionization is very difficult to predict.

So therefore it led to the need of soft ionization methods in proteomics. What are different properties of ionization source? The main function of an ion source is to convert sample molecules or atoms into the gas phase ion species.

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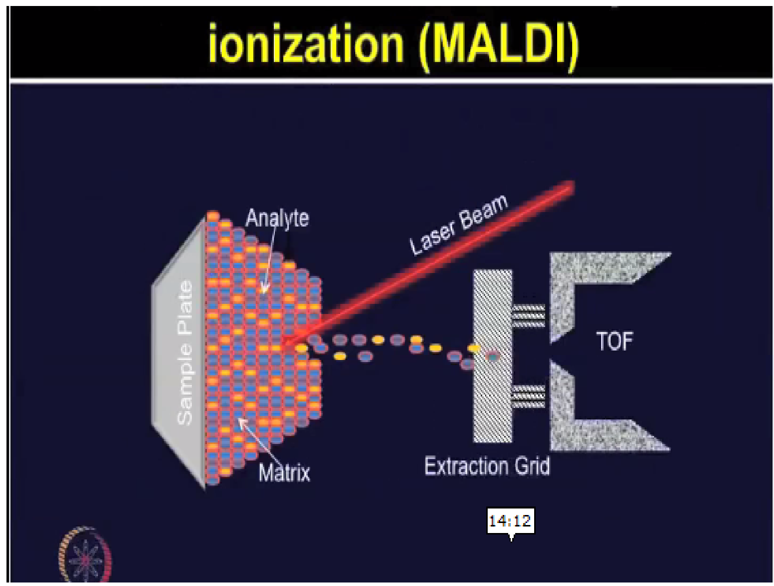

The ionization source is responsible for converting analyte molecules into gas phase ions in vacuum. This has been made possible by the development of soft ionization techniques which ensures that the non-volatile protein sample is ionized without completely fragmenting it. Most commonly used ionization sources are MALDI and ESI. Additionally, there are other ionization sources such as Fast Atom Bombardment FAB, Laser desorption LD, Plasma desorption PD. Let discuss the two most commonly used soft ionization technique MALDI and ESI in more detail.

So MALDI is an efficient process for generating gas phase ions of peptides and proteins for mass spectrometry detection. MALDI is one of the most widely used ionization technique currently applicable in the proteomic area. These ionization methods were independently developed by two scientists Koichi Tanaka and Helen Camp. Tanaka also receives the noble prize for his novel contribution into soft ionization technique such as MALDI.

Let us just talk about Matrix assisted, Laser desorption ionization or MALDI. So Analyte or the proteins of interest are mix with the matrix which is really an aromatic compound. The various type of matrix is available which we will talk in more detail when we come to the sample preparation and matrix selection.

But just for your reference we can use 2/5 dhyhydro acid benzoic acid, we can use Sinapinic acid and there are several other choices. Once you selected a matrix for the experiment then analyte and matrix can be dissolved in the organic solvent after which then it can be placed on the metallic target. As you can see in the slide the first left section shows you how to place the analyte and matrix together on the sample plate.

Now, once you have placed the matrix and analyte on the target plate you can put that in the vacuum chamber and apply high voltage. Now these crystals are targeted with the short laser beams as you can see in the slide then rapid sublimation can convert analyte into the gas phase ions. Now these ions once generated they can accelerate away from the target plate through the mass analyzer which is time of flight TOF tube and they can reach towards the detector.

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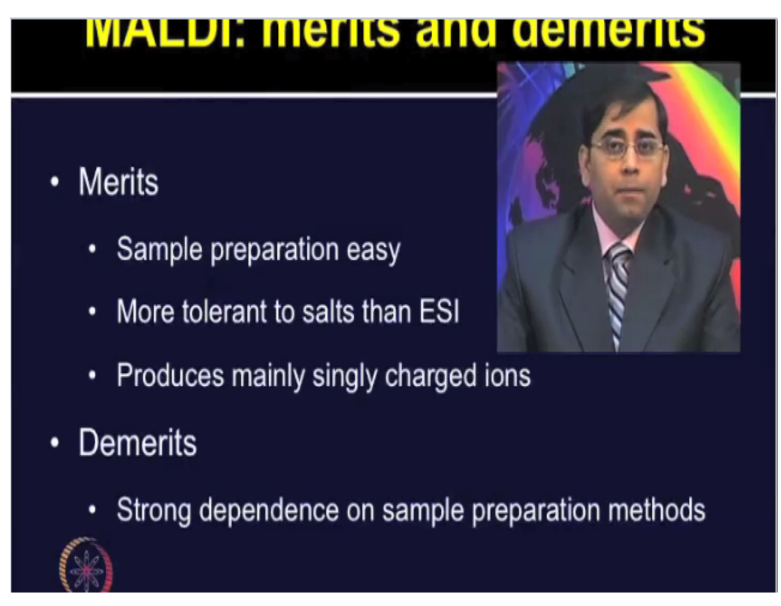

The various advantages and these advantages of using multi ionization source. The sample preparation is very easy. The MALDI provides high tolerance to salt as compare to the Electrospray ionization methods. The MALDI produces single charge species. Most analyte can accept the single photon. The single charged electrics can result in some molecules having large master charge values.

So therefore the MALDI is typically integrated with the TOF Mass Analyzer which can provide the M/Z range for the large ions as well. Now these are various merits of using MALDI, obviously it has to be collected with the TOF. Now, the various demerits of using the system. There is strong dependences on how to prepare good sample for the-- this analysis. So sample preparation methods heavily influence the spectrum generated from these experiments.

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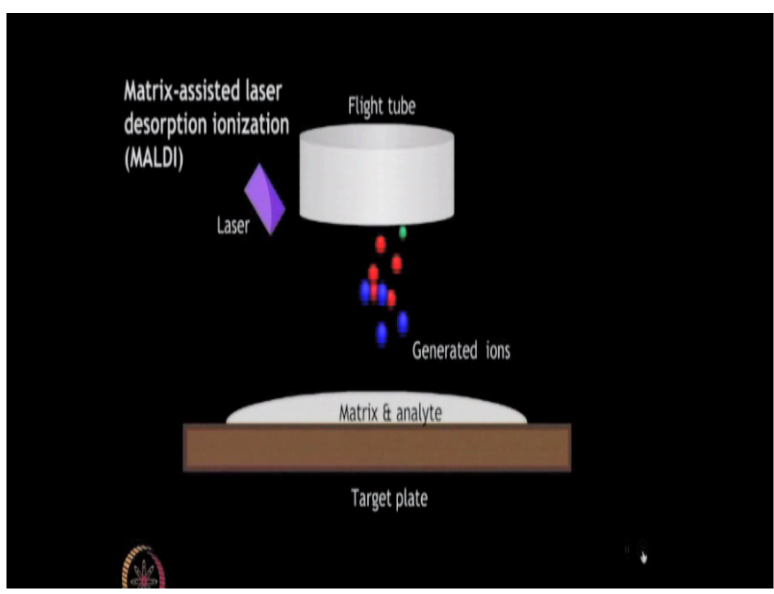

In MALDI, the analyte of interest is mixed with an aromatic matrix compound such as alpha-Cyano four hydro acid ceramic acid or Sinapinic acid. This is dissolved in an organic solvent and placed on a metallic sample plate. The evaporation of solvent leaves the analyte embedded in the matrix. Target plate is placed in a vacuum chamber with high voltage and short laser pulses are applied.

The laser energy gets observe by the matrix and it transfers to the analyte molecules which undergo rapid sublimation resulting in gas phase ions. These ions then accelerate towards the mass analyzer based on their mass to charge issues.

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Electrospray Ionization (ESI)

- ESI requires sample of interest to be in solution
- To ionize samples high voltage is applied to high conductively coated needle
- Distinguishing feature of ESI
  - its ability to produce multiply charged ions

So ESI, it requires sample of interest to be in solution. To ionize the samples high voltages applied to high conductively coated needle, so this voltage results into the sample becoming charged either positive or negative. The positive ions are primarily used for the analysis of proteins. The distinguish features of Electrospray ionization includes its ability to produce multiple charged ions.

The number of charges that can be accepted by a particular molecule depends on its basicity and its size.

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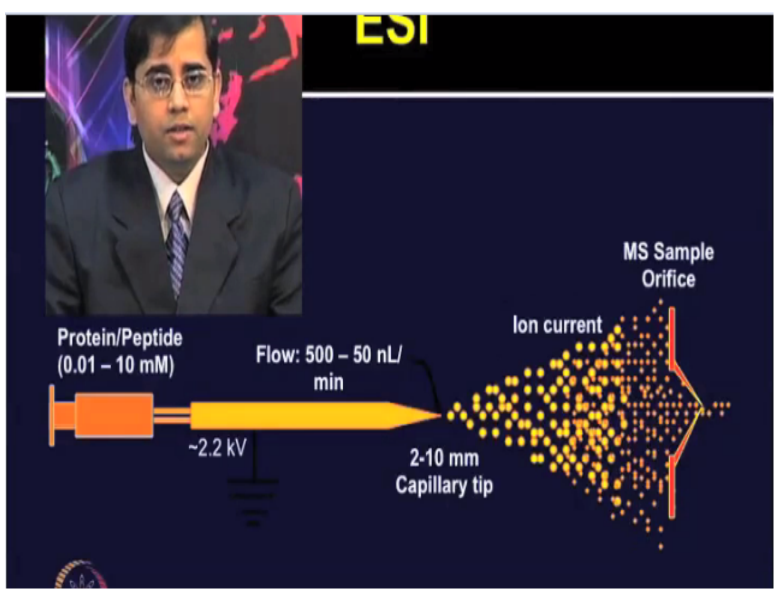

Now here you can get an overview of the process involved in the electrospray ionization. The small droplet of solutions is generated by the Taylor cone which contain the peptide analyte, protons from the acidic solution provides droplets the positive charge, so that it can move from the needle to the negatively charge instrument.

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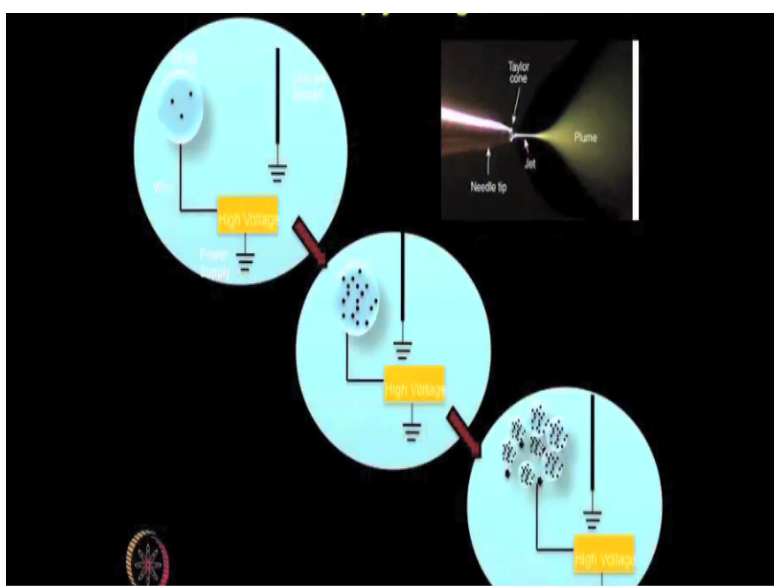

You can see this process in much with much clarity in this slide. The top panel is showing the Taylor cone generation and the center it is shown that the production of multiple charged ions, usually it is coupled with the MS via real-time liquid separation.

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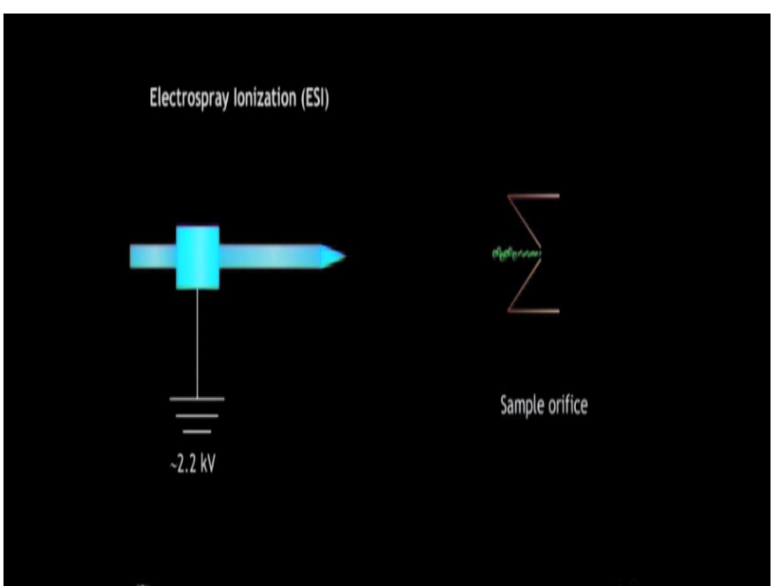

In Electrospray ionization, the sample is present in the liquid form and ions are created by spraying a dilute solution of the analyte at atmospheric pressure from the tip of a fine metal capillary creating a mix of droplets. The droplets are formed in a very high electric field and become highly charged. As the solvent evaporates the peptides and protein molecules in the droplet pick up one or more proton from the solvent to form charged ions.

These ions are then accelerated toward the mass analyzer depending upon their mass in charge.

The Agilent HPLC chip integrates enrichment and analytical columns, (()) (16:23) all connections and (()) (16:25) nano electrospray tip on an inlet multi layer polyimide system and smaller than the credit card. The compact architecture of the Agilent HPLC chip reduces peak desperation and combines all steps from sample loading through compound ionization for a seamless operation.

A closer look at the HPLC chips reviews the sample enrichment and separation columns of the Nanoflow system are integrated with the integrate connections and Nano Electrospray tip for compound ionization in mass spectrometry. This eliminates 50% of the traditional fittings and connections typically required in the Nanoflow LC/MC system which dramatically reduces the possibility of leaks and dead values and significantly improves each of use sensitivity, productivity and reliability.

The HPLC chip also incorporates all electrical contacts with the Nano Electrospray tip and features and embedded radio frequency ID tag which attracts the usage and operating parameter parameters of the chip. The chip cube includes an electrospray ion source with optic or spray resolution. HPLC chip loading and injection mechanism. Nano LC connection and Micro valve switching. The HPLC chip loading mechanism precisely and optimize positions, the electrospray tip or (()) (17:54) MS in that for maximum sensitivity and robustness; day end and day out.

With the Agilent series 1200 series Nano LC system including Microvalve plate auto sample and loading pump connected directly to the chip queue and HPLC chip is loaded and leak tank fluid are established automatically by sandwiching the tip between the Rotor and the Stator of the built-in Microvalve. The Rotor and Stator dock onto the chip and the (()) (18:25) Nano LC to port in the chip service.

Fast movement of the Rotor is reliable switching between sample loading and sample analysis positions on the HPLC chip. Replacement of the HPLC is simple and can be completed in a few seconds. Let us look at how the Agilent 1200 series HPLC chip MS system can be applied to a

typical protein identification analysis. The Agilent Microvalve plate auto sampler loads the digested proteins, a solvent flow moves the peptides into the traveling column, the Microvalve changes the flow path. The gradient flow from the Nanoflow pump takes the enriched sample traveling column to the separation column. The peptide just separated just like on a conventional Nanoflow column reduce peak dispersion, yield better separation and efficiency and sensitivity.

The integrated Nanospray tip ensures reproducible (()) (19:24) right over optimum ionization of compounds and the best results. Proven Nanoflow LC/MS technology and the new and exciting capabilities of the Microfluidics combine deform the system and it easy to setup.

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Comparing Soft Ionization Techniques

	MALDI	ESI
Sample analysis	Simple peptide mixtures (e.g. peptides derived from a single spot from 2D gel)	Analysis of complex sample
Bias	Polar/charged peptides	Nonpolar peptides
Effect of salt	Salt tolerant	Salt sensitive
LC	Offline	Online, analysis can be coupled to LC
Sequence coverage	Less	More

Let us have a comparison between MALDI and Electrospray Ionization and discuss their pros and cons which can be used for the analysis of different types of protein samples. In MALDI the sample analysis is for the simple peptide mixture whereas ESI it can be use for the analysis of sample. There is a bias toward the polar or charged peptides in MALDI whereas it is for the non-polar peptides in ESI.

MALDI is more salt tolerant whereas ESI is more salt sensitive. Liquid chromatography can be performed offline whereas in ESI it is online and analysis can be coupled to the liquid chromatography. For the proteomic application the sequence coverage is less in MALDI as

compare to the electrospray ionization. Both MALDI and ESI development where awarded with the noble prize.