Essentials of Biomolecules: Nucleic acids, Peptides and Carbohydrates
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Lecture- 30
Purification Techniques-II and Characterization Techniques of Biomolecules

Hello, everybody and welcome back to biomolecules. So, today, we are actually discussing and some of the modern techniques used in Chemical Biology. And in the last lecture, we have seen some of the purification techniques such as HPLC to start with and how you can use the high performance liquid chromatography in order to separate it in the larger molecules, especially the biomolecules in high purity.

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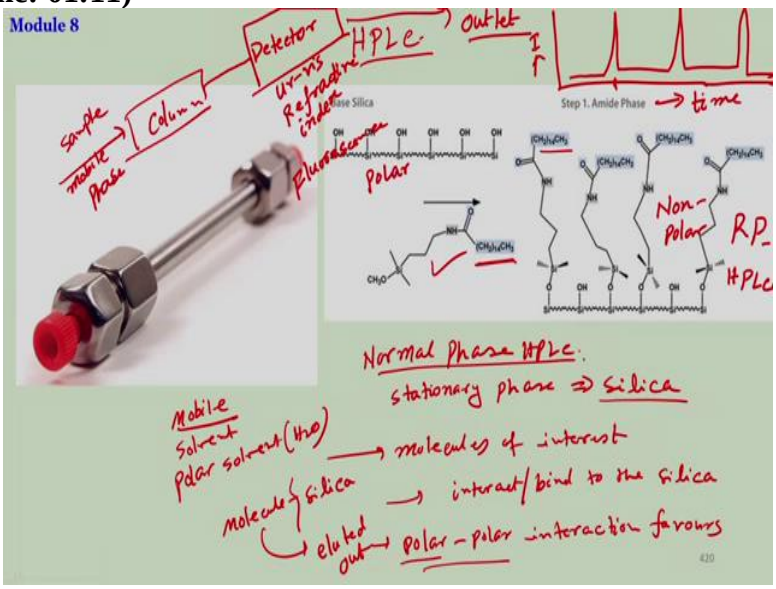

And we have talked about, so, this is your HPLC that such kind of column is being used, which is usually filled up with stationary phase in this case is silica. So, I have talked about reversed phase in the last lecture. So, there are many kinds of HPLC columns, it is based on different types of physical principles depending upon their physical properties, what of the material that is inside the column.

So, such as you have the normal phase HPLC you have reversed phase HPLC you have ion exchange HPLC and so on. Affinity chromatography also is there and all of these they vary in their binding properties. So, reversed phase, if you talk about I have not talked about normal phase I have not talked about will just give you a brief intro on the normal phase HPLC. So, normal phase HPLC has the stationary phase which is the silica.

So, I have said that an HPLC the basic working principle of chromatography is that, it will have one stationary phase that will not move and then it will have on that stationary phase you will inject your sample, the sample will go and be bound to the stationary phase by different physical interactions such as adsorption such as ionic interactions and so on polar – polar interactions and so on. So, your molecules of interest will be bound to the stationary phase.

And then depending upon the physical properties, they are binding affinity or the binding constant would be different for different kinds of molecule. So, therefore, you can have a different set of bindings with your molecules if you have impurities that will bind in one aspect or that will have sometimes the weak binding property if you have longer biopolymers that might have stronger binding property.

And now in order to get out your components or get out your samples, you have to inject a mobile phase. So, mainly 2 phases one is stationary phase and other is mobile phase, mobile phase is a solvent that will break the interactions that were present between your sample and the stationary phase. So, and again, depending upon their binding constant, the breaking of the interactions will also occur.

So, molecules will come out ultimately at different time intervals and therefore, you can purify. So, for normal phase HPLC your stationary phase is silica, which is this and obviously, you can see silica is polar in nature does hydroxyl groups. So, if you send the material here molecules of interest it can be by bio-molecule or it can be other organic molecules as well.

Then, they will go interact or bind to the silica your molecules will go inside the column and will be bound with the silica. Now, since this is polar, obviously, if you have more polar molecule that will bind stronger with silica. So polar-polar interaction favors more polar molecule will interact strongly with polar silica. So, if you have a set of more number of molecules with different polarity then if you inject in the normal face HPLC column.

Then according to their polarity difference, they are binding affinity or they are binding constant with the silica would be different. So, after that now sent. So, since this is a polar-polar interaction, what can the mobile phase you will be need? So, mobile phase is basically a

solvent whose job is to break the interactions. So, if you want to break a polar-polar interaction, what do you need, you need a very strong polar solvent such as water.

So, in this case you need polar solvent. So, if you inject now, polar solvent that is more polar compared to this interaction. So, it will break the molecule to silica interaction would be broken because silica will now interact with the solvent, the solvent is more polar compared to your molecule and naturally organic molecules are not as much polar as water. So, this interaction will be broken new interaction will happen.

So, that will release the molecule will be eluted out of color and you can quantify it. So, that is the basic principle of normal face HPLC. Now, one thing I have not talked about the design of HPLC. So, I will just mention here in that how do you detect. So, you have a column. This is this, you are injecting your sample you are injecting mobile phase molecules are coming out of the column according to their interactions at different time interval.

Now, how do you know that your molecules are coming out? So, you have to detect the concentration of the molecules or to detect the concentration of whatever material that is coming out of the column. So, after the column you need a detector. So, that is the basic design of HPLC. So, column after the column, whatever is coming out the capillary has to go through a detector.

Detector can be of various kinds. There are many kinds of detectors that you can choose actually, if your molecule is a homophone many molecules are homophone molecules, so, you can use UV visible detector. So, that you can see the absorption and from there you can estimate how much compound you have the concentration using the Lambert beer's law. If your molecules are not homophonic, you can simply use the refractive index.

Refractive index detection is another method which basically measures anything that comes out how much of the quantity it is, you can have fluorescence detection is very effective and highly sensitive that will show you even a presence of a tiny bit of compound if a molecule has a fluorescence tag. So, these are the detector after it comes out of the column, the channel the capillary which contains the eluted material is going through a detector and then it will come out and go to the waste.

So, here is the main important thing that will tell you how much material is coming out and in what time and waste basically outlet I would say not waste it is definitely not waste. This is outlet you can collect, because that is what you want you want purified samples. So, it comes out in the outlet and then you collect. And if you have for example, if you have a UV visible detector or a fluorescence detector or refractive index detectors, what you can see in the computer.

That it will give you peaks something like this, where this is your intensity in general, I will tell you will visible this is your observance for refractive index, this is refractive index calculator for fluorescence, this is your intensity and this is your time at which time they are eluting out. So, in terms of minutes for example, so, this component will come out first. If you are talking about the polar-polar interactions, which means this molecule is less polar.

This molecule is more polar, this molecule is farther more polar, because they are eluting out later. So, the interaction was very strong. And depending upon the nature of the molecules, they will be looted out at different time intervals and you can collect them. So, you will have a separated component that is all, the basic principle of HPLC works and after normal phase HPLC.

I have talked about the reverse phase HPLC. So, this is for reverse phase RP, HPLC. So, for the reverse phase HPLC you have to modify the stationary phase which was silica by changing its polarity, so it was polar. Now you are making this surface on nonpolar. So, that is why you are changing you are reversing the phase and that is done by reacting the silica with this compound at the end it has a long carbon chain.

So, C-14 C -15 C -16 total 18 carbon are here, carbons are here carbons are here a long carbon chains. So, they are very much non-polar obviously, and they are hydrophobic in nature. So, once they are conjugated with your stationary phase, now, your stationary phase is not polar like here, your stationary phase becomes non-polar this was polar this is non-polar. So, once you have now non-polar material, now things become somebody different. And then I have talked about the details of the reverse phase HPLC working principle in the last lecture.

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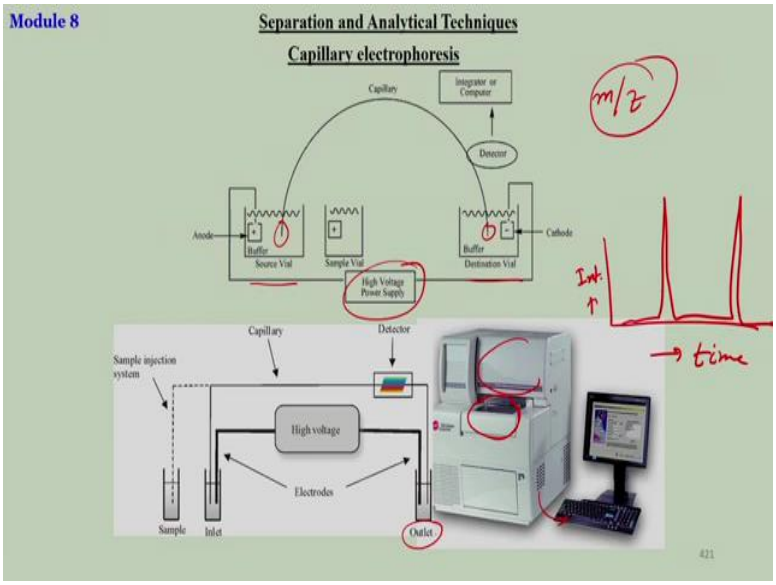

Now, I will also talk a little bit on the capillary electrophoresis that I have mentioned a study in the last lecture the capillary electrophoresis is based on separation of charge. Charge separation, if your molecules have different charge, then those can be easily separated using the capillary electrophoresis. And usually the capillary electrophoresis is used for a very small amount of material, nano molar quantity sometimes very small amount of materials you cannot use large amount of materials for separation in capillary electrophoresis.

So, HPLC can be used both for purification as well as for analytical purposes, capillary electrophoresis also can be used for purification as well as for analytical measurements, but mostly we use capillary electrophoresis for analytical purposes, because the amount of material is very, very small. So, purification does not make much that does not help much because you do not get much compound there.

So, mostly it is used for analytical measurements. So, yeah, so, this is a typical picture, one of the capillary electrophoresis machines, this is from brakeman culture. And here in this box, your sample chamber is there. And here inside, there is this capillary and that is connected with a laser. Usually, it can be connected with a detector, it has to be connected with the detector. That detector can be close laser that counts fluorescence.

It can be UV visible detector; it can be other detectors also. And then that is connected with the computer here in your sample chamber also you have the buffers and your capillary contents at the end of the capillary the content electrodes, those electrodes would be dipped in buffer first, then your capillary, if you send the voltage across the capillary then your buffer

would be moving across the capillary. So, first your capillary would be filled up with the buffer. Buffer means salt.

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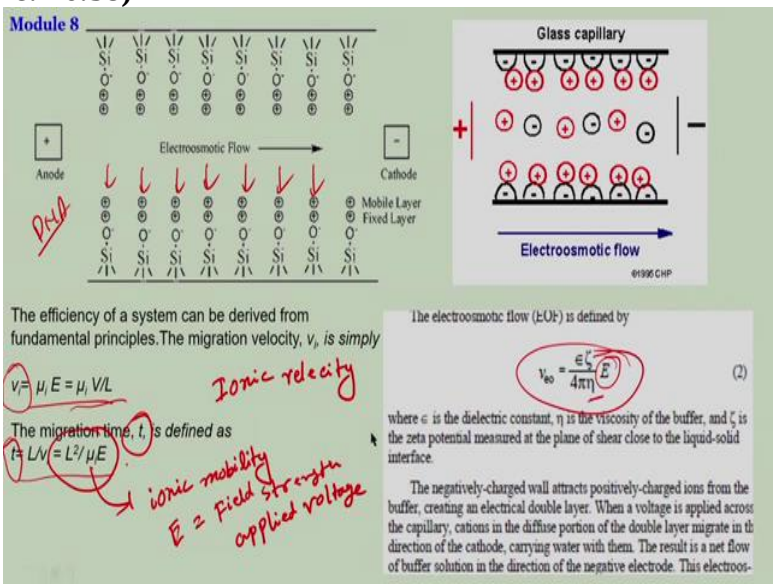

So, silica capillary I have shown you, the capillary is basically the coated the coating material on the capillary is usually silica which has negatively charged and then if you fill the capillary with your buffer that has salt, so, the positive charge will interact here. So, that will basically give you a positive charge environment and then you send your samples to it. So, according to the polarity of the samples according to the charge of the samples, the electro osmotic flow and the molecules will move accordingly.

And the velocity of movement, so, molecules with less charge or if you are, if you are talking about here the positive charge then if your molecules have negative charge, so, molecules with high negative charge will move slowly, molecules with low negative charge will move fast. So, speed matters here and that is how the equation is the velocity of the ion I it is called the ionic velocity. Ionic velocity is measured at the end.

And then ionic velocity has been converted into the migration time t. So t is defined as this L square by mu i into E, L is the length of the capillary, mu i is ionic mobility. E is the applied voltage or electric field, field strength which depends upon the applied voltage, the voltage that you have applied across the capillary and that is given here. So, basically that will be this, here is the sample, sample injection happens in the capillary and your electrodes are dipped here, voltage is applied here across the capillary these are the electrodes.

This is one electrode, this is another electrode. So, your sample is going through the capillary and depending upon the interactions, it will move in different speeds, it will reach the detector here. Now, after that it will go to the outlet. So, once it goes to the detector, you can see again the nature how they are eluted out. So, same time because you have converted the speed in terms of the migration time and this is the intensity it will show you again the similar different if you have 2 components with different charge, it will they will appear at different time intervals.

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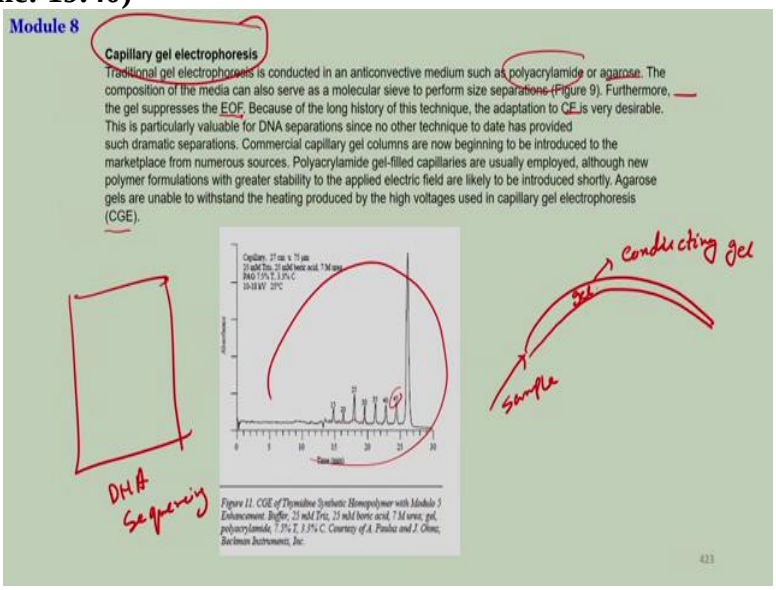

The capillary gel electrophoresis I have talked about that, in this case, you fill up the capillary with the gel. If this is your capillary, then capillary is filled up with the gel. Non-conductive gel of course conducting gel, so if you will now inject your sample across the capillary, it has to go through the gel. And obviously, if the sample has charged, gel has charged, so they will happen.

It is exactly similar like the electro radio gram I had shown during DNA sequencing the gel electrophoresis the gel pattern did if you have remembered that I had shown during DNA sequencing, saying that is method how different length of the DNA were appearing in the gel electrophoresis pattern. So that is called electro radiography. So, same interaction happens here. So accordingly, the samples will move.

And here of course, since you are using the gel which with a very high viscosity, so, the speed of the sample would be less. So, it is to give you a little bit of better separation and to give you a little bit of more time. So, in this case time of separation would be a little bit

longer, but very fine separations. So, traditional gel electrophoresis is conducted in an anti-conductive medium such as poly acrylamide.

Poly acrylamide is a kind of a material conducting material that is used for gel or agarose. Agarose we have used for the gel electrophoresis here the composition of the media can also serve as a molecular sieve to perform size separations. So, sometimes you can use that for as well as size exclusion, separation also, because they will have different pore sizes and obviously the molecules will get into the pores.

Furthermore, the gel suppresses the EOF electro osmotic flow as I was saying since the gel has high viscosity it will return the flow of the samples the free flowing of the samples the free electro osmotic flow of the sample would be retarded by the presence of the gel. EOF means electro osmotic flow. Because of the long history of this technique, the adaptation to see capillary electrophoresis is very desirable.

This is particularly valuable for DNA separations. Since no other technique to date has provided such dramatic separations, very fine separation actually. Commercial capillary gel columns are now beginning to be introduced to the marketplace from numerous sources. Poly acrylamide gel filled capillaries are usually employed although new polymer formulations with greater stability to the applied electric field are likely to be introduced shortly.

Agarose gels are unable to withstand the hitting produced by the high voltages used in the capillary gel electrophoresis that is called the CGE capillary gel electrophoresis. So, agarose gel has the disadvantage is that it cannot withstand the high heat that is generated when you provide a large voltage across the capillary. So, sometimes the gel might be broken that is the disadvantage of using agarose as the gel inside electrophoresis but poly acrylamide can work fine.

And here you can see the separations very short or differences change is 5. Nucleus change in very long DNA sequence you can actually identify up to 45 more base pair sequences. They are all T poly T sequences actually.

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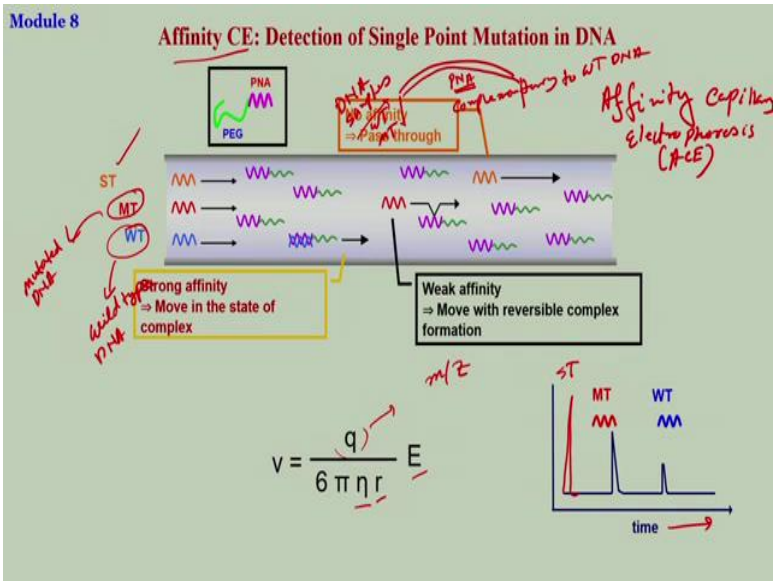

Now, like gel, we had worked on a different kind of capillary electrophoresis that is known as affinity capillary electrophoresis. Affinity capillary electrophoresis is ACE. Affinity capillary electrophoresis works on a principle that you have the capillary was electrode here electrode there. So, in the normal capillary you had buffer inside the capillary, before injecting the sample. In the gel, you had gel inside the capillary; they were interacting with your sample.

Affinity capillary electrophoresis is that the capillary is filled up with different compound that we have synthesized, or with different compound that has high affinity. In this case, we have used the PNA. PNA I will talk about later, just after this. The next module PNA is peptide nucleic acid. It is basically a probe. So the capillary is now filled up with a probe. PNA is just like DNA basically. So let us say it is a DNA.

I will keep it PNA to make it different. So it is a probe. It is just like DNA. And then you inject your sample such as DNA samples. So, if you inject your DNA sample here, what will happen? This is also a probe which hybridizes with DNA. Now, if you have a wild type DNA, which means the perfect DNA and if you have a mutant MT means for mutant. This is means the wild type.

Wild type means basically that normal DNA or normal gene. MT means the mutant mutated. So, both are DNA. Single point mutation if you have only up point mutation in the whole long DNA sequence, so, presence of one mismatch, how do you detect. That is very important in order to detect the cancer cells or even other there are a lot of mutations that are going on and those are responsible for several kinds of diseases.

So, finding out or identifying point mutations or single point mutations are very, very important. Affinity capillary electrophoresis we have used to detect such mutated DNA. So, now, you have 2 samples basically a wild type and muted. This probe is designed so, the sequence of this probe is such that it will have perfect complementary sequence with the wild type the normal DNA because normal DNA sequence is known.

We know our own sequences, but what mutation has happened? We did not know we had to find that out. So, this probe is complementary. 2 wild type DNA. So, now we have injected 2 samples. Wild type and mutated sample then what will happen? This PNA will hybridize perfectly with the wild type and it will form a complex the overall size or the overall molecular weight of the complex would be higher. On the other hand mutated DNA will not be complimentary, because it has one base mismatch.

So, hybridization will not be stronger with this probe, weak hybridization. So, if this has a strong hybridization, it will have more charge because this has even this is neutral, at least the size will be different and we are separating in terms of poisson ratio so, mass by charge. So, even the mass, if you can change the mass that itself also will have an impact. So in that case, because of the large size of the complex hybridized complex in this case now.

Their movement will be slow compared to the mutant, so mutant will appear first, then will come the wild type. So, that I will show you here. So, particularly what we have done that we had a DNA and that was attached with a polymer called PEG, it is a biopolymer long chain polymer to give it more size or to give it to make it a large weight. So, if you have a large size, high molecular weight, then the speed becomes much less.

ST is a scrambled sequence. Scrambled sequence means it is not at all complimentary with the probe, so it will move it will have no interaction at all. So this will have no interaction with your probe. This will have week interaction and the wild type will have the stronger interaction. Now let us see how they moves. So these are this is ST mutant and wild type. That is how they are moving. So this is wild type wild type forms.

So this is my probe PNA with PEG. Wild type is connected is perfectly hybridized and you have a bulky polymer attached to it. So it is now a complex and the whole complex is moving. Obviously, the movement would be slow because it has high size. Mutant interacts

also with this, but weak interactions. So it will interact and then open up break it again because weak interaction is equilibrium will interact will break up again will interact will break up again.

And that is how it will move. So it does not form a stable complex. So it moves with weak interactions open, weak interactions open. So its movement is hindered, but not as much as the wild type. Scramble on the other hand, which has no complimentary sequences with your probe, they do not interact at all and go straight are hindered. I will show it again. Here, complex moving, this is week interactions. Coming and then moving out this has no introduction.

It will go straight without any blockage. So, strong affinity move in the state of complex, weak affinity move with reversible complex formations, so forming and then unwinding, forming and unwinding. That is how it is going. And this is the velocity of migration, v is the velocity is q is the charge q basically comes can be calculated from m / z follow, mass by charge. So, you are concentrating mass as well as the charge q / 6 pi eta r into E. Eta is the viscosity of the buffer of the medium r is the distance that E is the applied electric field.

This is how we do look like. So first your mutant ST is not here. ST to come here at the very beginning, which would be ST. This is the time. So, scramble sequence would appear at the very beginning, unhindered then comes the mutant then will be your wild type DNA. So, we have separate between your wild type DNA and if there is a mutation present in your sample that.

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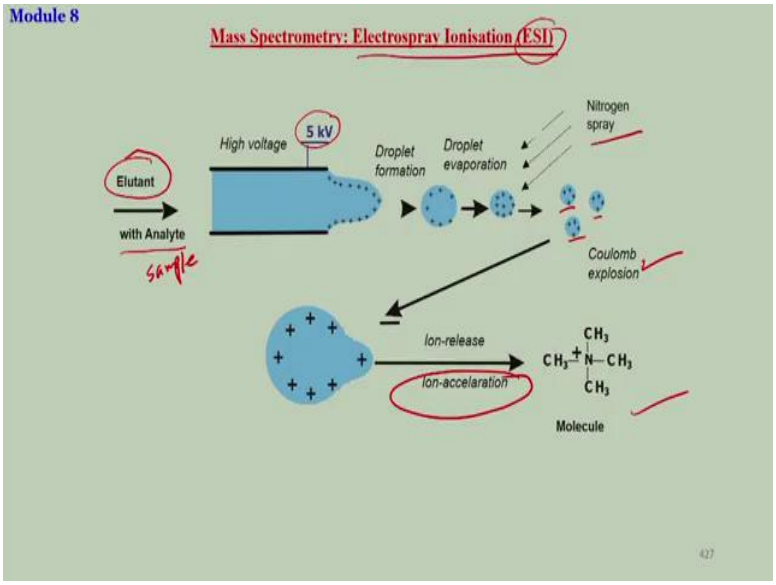

We have actually used that to separate very long 60 molar DNA base pair sequence which has only one mismatch in it that I will show later when we will be talking about the molecular probes. Now, separation technique is over. Now, coming to one characterization technique, very, very important characterization technique, that is, mass spectrometry used for chemistry used in biology.

Of course, for the biomolecules; in order to characterize the presence of the biomolecules or the presence of any molecules actually. So, this is the basic principles for the electrospray ionization. Mass spectrometry has many different variations. Electrospray ionization is the most commonly used mass spec technique. In short, it is called ESI electrospray ionization. Obviously, the name suggests that you are forming ionized particles by spraying electrons. So, you have your sample in a liquid form here with analyte.

Elutant means the solvent with analyte is your sample compound that you want to analyze that is mixed up with the solvent and that is placed inside the mass spec chamber which has a high voltage in it around 5 kilo volt. So, if it goes through a high voltage your molecules will be charged. So, if you depending you can make it positively charged you can make it negatively charged depending upon your voltage plus voltage.

When you apply you basically make positive charges of your molecules and then it will move slowly and then forms a droplet. So, droplet contains the solvent as well as your molecules that are positively charged. So, this is basically electrospray because you are making a

positive charge by spraying electrons by spraying the voltage. Then the droplet was big we have to do evaporation.

Droplet gets smaller the nitrogen spray dry nitrogen is sprayed on the droplet, it will evaporate the solvents that you have used, if the solvent evaporated, the droplets would be even smaller sizes. And now, by doing so, the size of the droplets would be so small gradually that at one point of time, the positive charge density would be so high that it will burst or it will explode.

This is known as Coulomb explosion. Once the coulomb explosion is done then these droplets would be divided into very fine droplets like this. I have drawn it larger but this is the very fine droplets that are created from this after explosion and then it has to go through ion-acceleration chamber. So, there it will interact your charge that is moving, if you have multiple components here, so, according to their charge, they will move through the ion accelerator and obviously, they will interact with accelerator, because accelerator has also the charge and then they will ultimately reach the detector.

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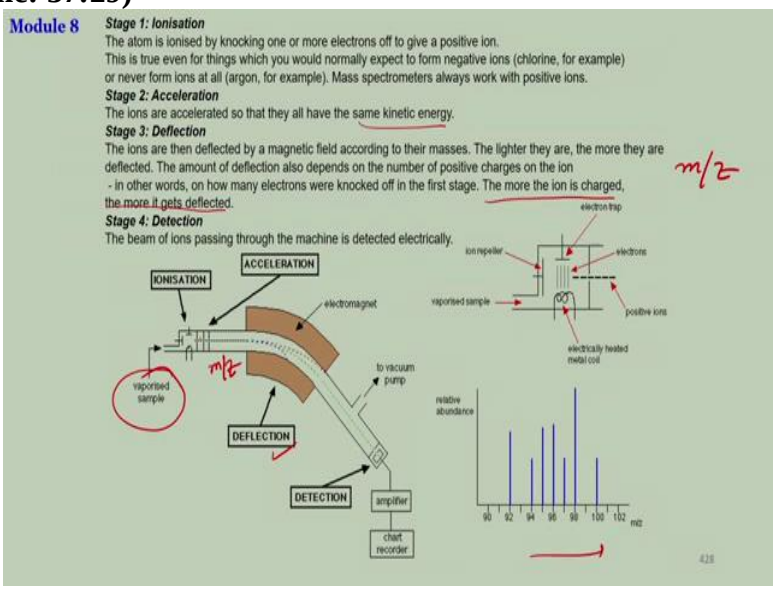

So, stage 1 is the ionization, the atom is ionized by knocking over one or more electrodes have to give a positive bias. This is true even for things which you would normally expect to form negative ions or never form ions at all. Mass Spectrometer always work with positive winds. Stage 2 acceleration the ions are accelerated. So, that they all have the same kinetic energy and then comes the deflection.

The ions are then deflected by a magnetic field. So, I have made a mistake is ion-accelerator is the chamber, where all ions will have equal velocity, same kinetic energy. Then, comes the deflection. The ions are deflected by magnetic field, according to their masses. The lighter they are, the more they are deflected. The amount of deflection also depends on the number of positive charges on the ion.

So, it is basically again a by m/z separation, mass by charge. In other words, on how many electrons were knocked off in the first stage, the ion is charged, the more it gets deflected. Stage 4 final stage is the detection the beam of ions passing through the machine is detected electrically. So, it will basically detect electrical signals. This is the design. This is the vaporized sample.

Here comes the ionization which forms the ions and then ions then droplets then spray the coulomb explosions and finally, these charged ions. So, that is the job of the ionization and then the charged ions are sent to the accelerator where they will be given the same kinetic energy. So, same speed. Now, they are moving, all ions are moving in the same speed. But they have different m by charge m/z different charge different mass and here is the electromagnetic chamber where the deflection happens.

So, according to their m/z, they will interact with the magnetic field electromagnetic field and then they will deflect. So, here the more ion is charged the more it gets deflected and then they will move. So, the ones which are deflected will come out later the one which is not deflected will come straight. So, this is the detection, which will detect the electric field and then amplified and then it will record.

So, this would be the m/z here, the more you go higher will be m/z which means the higher the m/z that will be deflected more will come out later and it You will see the lines at different masses. So, whatever mass or whatever components you have, you will see it here. At the same time, your molecule will also undergo chemical destructions. And those will also appear if your molecule is decomposed and that is the basis of mass spectrometry also that your molecule will be decomposed.

But in a different pattern depending upon the chemical nature of your molecule and then you will get the fragments of the molecule those will also appear in the mass spec and by

analyzing the fragments, you can actually characterize the structure of the whole molecule also.

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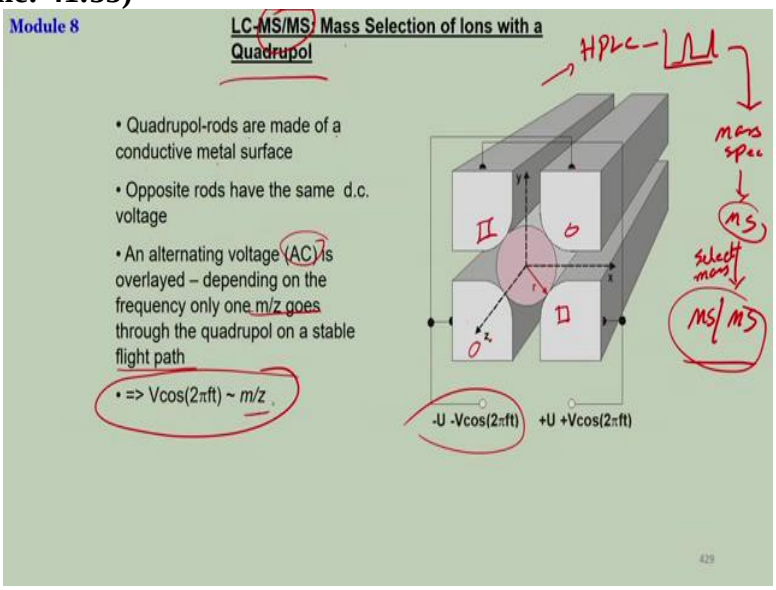

So, one of the best technique that is used by chemists and biologists variation of that, so far I have talked about was only about the normal mass spec. Now, the modifications or the apart from the normal mass spectrometry, there are many variations that nowadays are used very highly modern techniques actually for better separations for better analysis of your compound that is called MS MS mass mass.

LC is liquid chromatography LC MS MS means liquid chromatography mass mass. So, in this case this basically means you have HPLC you send your molecule through an HPLC. HPLC will separate the molecule and then that is the same it is together that after the HPLC separation the eluted samples are coming through our mass spec and you measure the mass. That is normal LC MS MS. is that you have mass MS.

And then this mass is fragmented go through a different kind of detector, where this mass would be fragmented and be analyzed. So, that is called the MS MS. MS MS is mass selection of ions with the quadrupol. Mass selection means, here you when you measure the normal mass, you have seen that you can see many spectrum or many lines, each of them has a different mass.

So, from here sometimes it is very hard to analyze, what is the nature of your molecule or what is the structure of your molecule. If you have a predetermined mass if you know that

what is the mass you are supposed to see, then the MS MS is what you can do, you can select a particular mass. For example 98 after you get the normal mass which contains all the buses that you have in your sample.

Now, here you can select a mass select mass, in this case, you can select for example 98 and then that particular mass can be fragmented farther to see what is more into it. So, this has sometimes has higher but is that if you have isomeric molecules, they have the same mass and what if they are present in a mixture. Both of them will show you a single peak here, but you do not know which isomer because it can be this or the other one both have the same mass you cannot fine tune what is the structure.

So, you can pick up that pick up 98 because you will know that that molecule will exist here, you select that mass here and then that line will further be fragmented. So, from here you can find out whether you have the desired isomer that you are looking for. So, this is the technique is based on a quadrupol. I will talk very in a very simple sense quadrupol basically has 4 rods made out of conductive metal surface.

Opposite rods have same DC voltage. So, this rod and this rod this and this they are connected with the same DC voltage Well, this is the equation, this is important cell energy this important cell that applied potent cell. So, these 2 have the same voltage, these 2 the same voltage. Now, you apply AC current alternating current is overlaid on it. Now, depending on the frequency only one m/z that I was saying you can select only one m/z it goes through the quadrupol or a stable flight path. This is the flight path. V cos 2 pi ft which is almost proportional to m / z value and then this happens.

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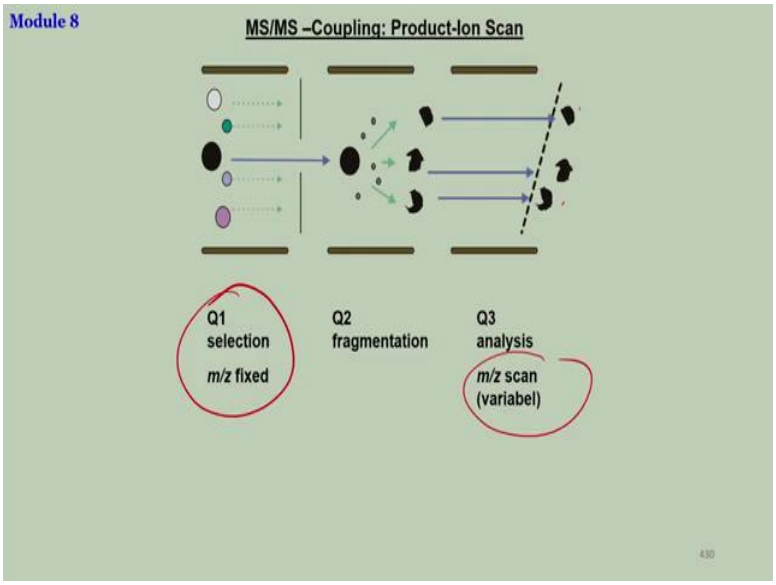

So, you have set of mass 1 2 3 4 5 for example. So, 4 quadruples are there. First quadrupol you fix a definite invoice it. So, only this is selected to go to that next quadrupol and here it will be fragmented. This is the fragmentation pattern of this particular mass. Remember all these mass is are actually a fragmented pattern from your earlier sample. So, this is the next phase MS MS phase we are talking about.

And from there, it will analyze the fragmented patterns that is what basically it. So, this is the 3 quadrupol fourth is the detector. MS MS coupling product ion scanned. This is high throughput technique that we use for biomolecules, if you want to see DNA fragmented patterns if you want to see proteins is especially used for proteomics, because protein is a very large molecule and you want to know which avoided acids are present, you want to know what is the proper characterization techniques.

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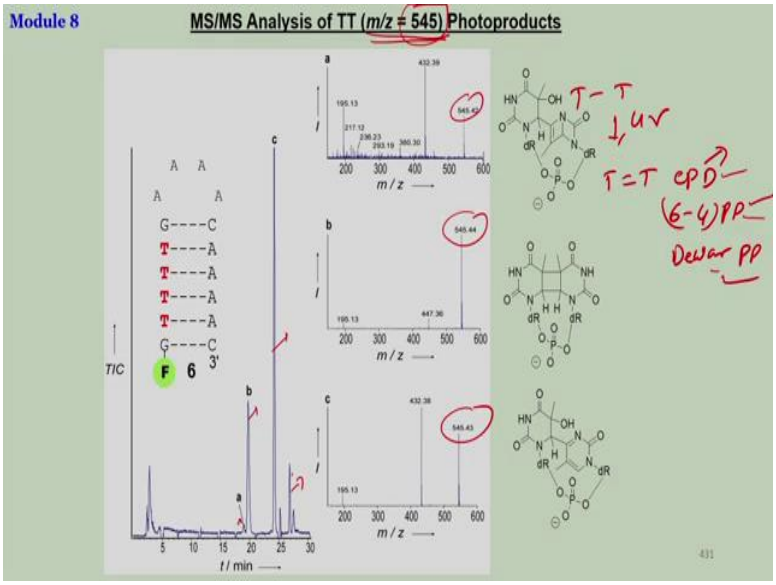

I will give you one example of how we have used it. So, you have a DNA and remember I have talked about the UV induced linear analysis that if you have TT sequence then if you irradiate with UV light then you will have thiamine-thiamine CPD dimer. Thiamine-thiamine is 6-4 photo product and thiamine-thiamine Dewar valence isomer Dewar valence photo product. There are different types of CPD CCM, transit that we have seen have the same mass 6-4 and Dewar valance photo products have the same mass.

So now the question is, how would you know that whether this is the molecule that you have isolated is 6 - 4 photo product or Dewar one. How would you know; whether this is transit CPD or the CCM CPD that we have analyzed using mass mass MS MS technique. And here we have selected 545 as the mass selected mass after you get the this is the HPLC diagram, which is connected for the to the mass.

So, all these peaks will be going individually will be going through the mass spec to analyze what is the mass of this? What is the fragmented pattern and the next, the next the next ABCD something like that ABC basically. This is the MS MS pattern for the E this will be here, this is the pattern for b this is the pattern for C, this is the pattern, all of them have the mass 545 and these are the fragmentations of these mass.

So, obviously, if you see the 3 peaks, if you see the 3 fragmentations all of them are 545 but their patterns of fragmentations are very different, which basically means they are not the same molecules. They are isomer. Maybe CPD maybe 6-4 maybe T1. So, from here we have analyzed which one is which that this is actually the Dewar valence. This is actually the CPD

cyclo pyrimidine dimer. This is actually the 6-4 photo product, but they are all isomers have the same mass. So, it is some very advantages and used a lot in Chemical Biology today especially in proteomics.

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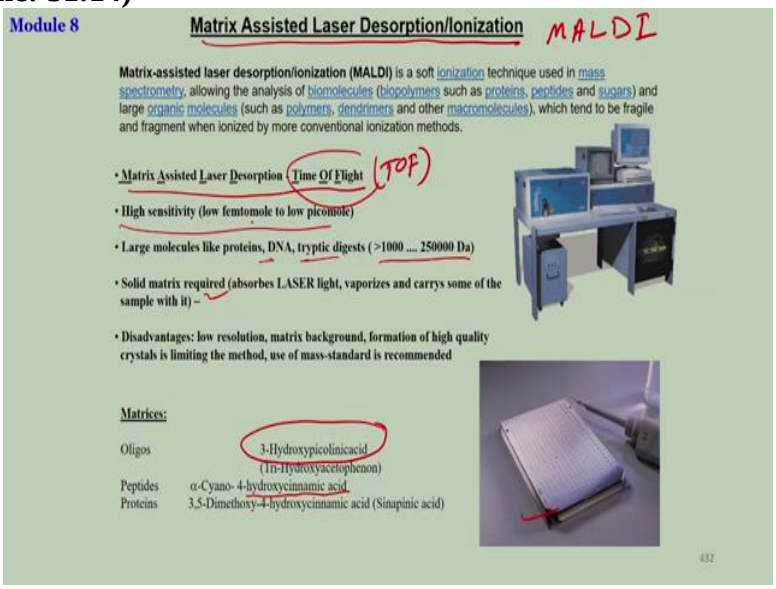

And now, another type of mass spectrometry which is especially designed for polymeric molecules and for poly biomolecules, long DNA long proteins and so on. Proteomics is now it is used if you remember I had talked about during protein sequencing that nowadays a high end mass spectrometry techniques have been used to determine the sequence of proteins. So, MALDI as well as mass spec, other mass spec has are very popular, very very popularly used to study the proteins.

So, matrix assisted laser desorption or ionization is called the MALDI. Matrix assisted laser desorption or ionization. MALDI is a mass z technique. It works on time of flight TOF. Time of flight. So the time it requires to fly. So very sensitive technic low femtomole to low picomole quality you can measure. You can detect large molecules like proteins DNA, tryptic digests.

MALDI is usually for large molecular weights more than 1000 up to maybe 250,000 Dalton. It requires a matrix. That is why it is called matrix assisted. This is kind of a chamber or which you can see the small dots are there. Those are matrix on those dot matrix those are spots on that you put your matrix. So, solid matrix required. You need a matrix which is basically an organic molecule, organic molecule is deposited on these spots, that are called the matrix.

You can use matrix, if you are studying DNA, 3- hydroxypicolinicacid is what kind of matrix that is used for if you want to analyze oligo samples. If you want to analyze protein samples, then you can use 4-hydroxycinnamic acid. So, those organic molecules are deposited in liquid form with a solvent are deposited on this spots, and then they get solidified and you put your samples, if it is DNA, you put your DNA on those matrices.

So if you now have a combination of matrix and your sample, maybe a DNA maybe a protein and they gets crystallized at the beginning and that chamber is placed inside the mass spec machine. Now, what happens?

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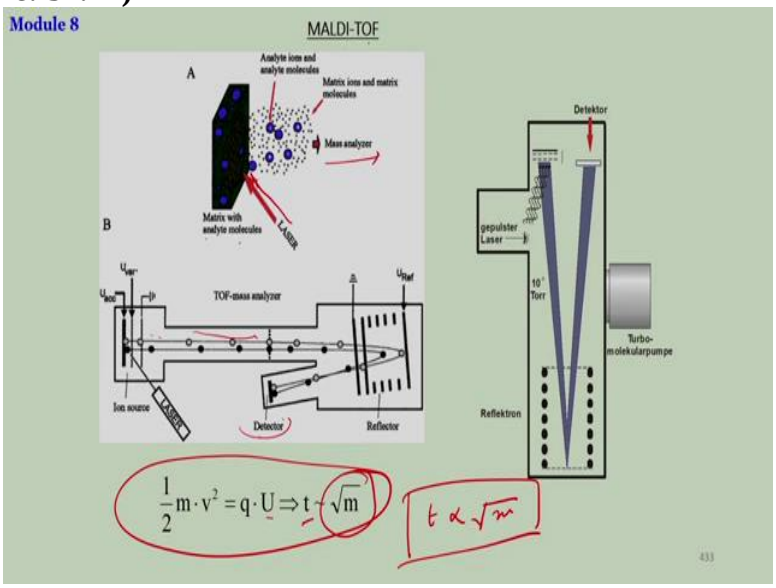

So, basically now, your material is present in a solid crystalline form, why do we use the matrix because normally DNA would crystallize protein crystallize they would go to the solid phase. So, in order to help that, you have to use the matrix and then in the matrix what happens. That has been popped with the hydrogenation parameters. And then, on the matrix, the ions are thrown off from the matrix.

If you have the molecules here, if you have the samples DNA or protein, so, here it has been some hydrogenation source laser actually we use the laser. Laser would be pumped in injected in here and it will break the crystal parameters, it will force the ions that are present here to move flight that is what is called flight and depending upon their m/z their type of flight would be different that is the basis of it.

So, matrix with analyte molecule you side the laser here, a laser will dissolve all the ions that are there, analyte ions and analyte molecules are coming out. And these are the matrix that will also come out what matrix would matter they have a pretty small mass and those ions According to their time of flight will come at different time to come out and then you can analyze their mass.

So, this is the laser, TOF is type of flight mass analyzer, this is a detector. So, if this is coming out first this will have more speed it will go here deflection detector. So, it will reach the detector early this will reach the detector later. So, it based on that time of flight, this is the equation of the movement of the velocity time of flight half m v square is the kinetic energy that is equals to q is the charge multiplied by U that is equivalent to the time t is the time of flight that is roughly proportional to root over of m.

Depends on the mass. So, time is proportional to the root over of root of mass. This is the equation for MALDI TOF, we called it MALDI TOF. MALDI TOF time of flight.

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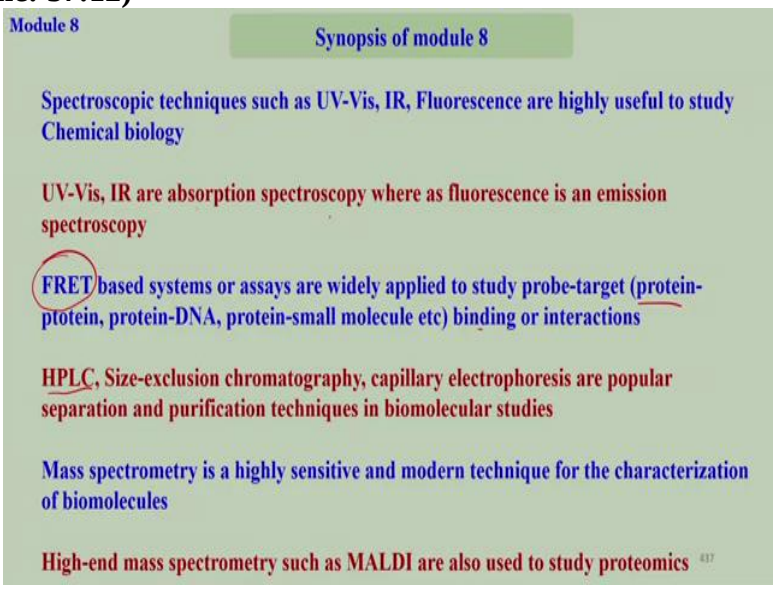

So, you can go through more of the modern methods on. These are the some of the techniques that I have talked about that are being used in Chemical Biology, for characterization, in what I have not talked about, of course, because it is the vast subject. For characterization, for purification and for analysis. So, with that, I will conclude the synopsis. This module, which is module 8.

In this module, we have talked about spectroscopic techniques, such as UV visible infrared spectroscopy, fluorescence, that is highly useful to study chemical biology. UV – visible IR absorption spectroscopy, because they are working principle is going from the ground electronic state to the higher excited electronic states. So, they absorbed radiation. So, that is why they are called absorption spectroscopy.

Whereas, fluorescence is an emission spectroscopy because in fluorescence it comes down from the excited state to the ground state by emitting radiation. FRET fluorescence resonance energy transfer FRET based systems or acids are widely applied to study probe-target interactions. What kind of interactions may happen between your probe and your target molecule that you want to study such as protein-protein interaction, protein-DNA interaction, protein-small molecule interaction.

Those bindings or interests you want to study are heavily used in Chemical Biology. Then comes, the purification HPLC, size-exclusion chromatography, capillary electrophoresis they are very popular, separation and purification techniques in bio-molecular studies. Mass spectrometry is a highly sensitive and modern technique for the characterization of biomolecules.

High-end mass spectrometry such as MALDI is also used to study proteomics, which means to study the sequence of proteins and to study also the chemistry of the proteins. Nowadays, there is a field called genomics, which is also studied a lot using the mass spec techniques. Thank you